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Evolutionary and conservation consequences of interspecific hybridization in rare plant species

Evoluční a ochranářské důsledky mezidruhové hybridizace u vzácných druhů rostlin

PhD Thesis

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Content

Declaration

I hereby declare that I wrote this thesis independently using the mentioned references. I did not submit or present any part of this thesis for any other degree or diploma.

Průhonice, 13. 5. 2014

Author contribution statement

I declare that I have contributed to all involved papers substantially and my contribution to particular papers are as follows:

I. **Vít P**., Wolfová K., Urfus T., Tájek P. and Suda J. (2014): Interspecific hybridization between rare and common plant congeners inferred from genome size data: assessing the threat to the Czech serpentine endemic *Cerastium alsinifolium* (Caryophyllaceae). – Preslia 86: 95-117.
Study design, field sampling, lab work, data analyses and manuscript preparation

II. **Vít P**., Lepší M. and Lepší P. (2012): There is no diploid apomict among Czech *Sorbus* species: a biosystematic revision of *S. eximia* and discovery of *S. barrandienica*. – Preslia 84: 71-96.
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III. Kabátová K., **Vít P.** and Suda J. (2014): Species boundaries and hybridization in Central-European water lilies as inferred from genome size and morphometric data. – Preslia 86: 131 - 154.
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IV. Hanušová K., Ekrt L., **Vít P.**, Kolář F. and Urfus T. (2014): Continuous morphological variation correlated with genome size indicates frequent introgressive hybridization among *Diphasiastrum* species (Lycopodiaceae) in Central Europe. – Plos One (**accepted**)
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Summary

Hybridization plays an important role in the evolution of vascular plants. It can have both positive and negative consequences, ranging from the origin of new species on the one hand to the extinction of taxa through introgression on the other. These effects may be pronounced in geographically restricted or rare species. The core of this thesis are three case studies addressing interspecific hybridization involving rare angiosperm species. Finally, the thesis is completed with a study considering hybridization as a source of variation and new species. The coexistence of frequent primary hybrids with their parental taxa was revealed in the system comprising the rare species *Cerastium alsinifolium* and its widespread counterpart *C. arvense*. The spatial distribution of the endemic species and its habitat preferences were elucidated. In contrast, comparatively rare hybridization events were found in the *Nymphaea alba – N. candida* complex. Although it has been assumed that water lilies hybridize freely, our karyological data do not support this hypothesis. Hybrids therefore do not present a serious risk to either of these rare species. The third study describes interspecific hybridization in the spore-bearing genus *Diphasiastrum*. Traditionally, three basic and three hybridogenous species are recognized in Central Europe. However, species boundaries are blurred through frequent introgressive hybridization. Introgression has been catalysed by human activities (disturbances), which facilitate spatial contact between originally partly allopatric species and subsequent interspecific hybridization. The origin of a new agamospermous lineage through interspecific hybridization was described in the genus *Sorbus*. Apomictic triploids most likely originated via hybridization between diploid and tetraploid taxa. Their mode of reproduction shifted from sexual to apomictic, which assured their long-term persistence.

Souhrn

Hybridizace hraje významnou roli v evoluci cévnatých rostlin. Obecně ale může mít jak pozitivní tak negativní důsledky, sahající od vzniku nových taxonů až po možné vyhynutí druhu skrze introgresi. Tyto důsledky mohou být znásobeny obzvláště u taxonů s omezeným geografickým rozšířením nebo u vzácných taxonů. Základem předkládané dizertační práce jsou tři studie zabývající se mezidruhovou hybridizací s účastí vzácného taxonu. Práce je doplněna studií, ve které vystupuje hybridizace jako zdroj variability a nového taxonu. Koexistence početných primárních hybridů s rodičovskými taxony byla odhalena u endemického rožce Slavkovského lesa (*Cerastium alsinifolium*) a jeho široce rozšířeným protějškem (*C. arvense*). Naproti tomu, hybridizace nativních leknínů (*Nymphaea alba*, *N. candida*) je velmi vzácná. Předpokládalo se, že oba taxony mohou volně hybridizovat, avšak naše karyologická data tento předpoklad nepotvrdila. Hybridizace v obou případech není vážným ohrožením vzácných taxonů. Třetí studie osvětluje důsledky mezidruhové hybridizace v rámci rodu *Diphasiastrum*. Obecně jsou ve střední Evropě rozlišovány tři základní a tři hybridogenní taxony. Vymezení jednotlivých taxonů je však nejednoznačné a díky introgresivní hybridizaci existují přechody (jak v morfologii, tak ve velikosti genomu) mezi taxony. Tato introgrese je z velké části umožněna lidskými aktivitami (m.j. disturbance), které způsobily kontakt mezi původně prostorově izolovanými taxony a následnou hybridizaci. Původ nové agamospermické linie v rodu *Sorbus* byl popsán v poslední studii. Obdobné linie vznikají ve střední Evropě opakovaně díky mezidruhové hybridizaci. Nejčastěji vznikají agamospermičtí triploidi hybridizací diploiního (*S. torminalis*) a tetraploidního taxonu (např. *S. danubialis*, *S. graeca*). Dlouhodobá existence těchto linií je umožněna přechodem k agamospermickému způsobu reprodukce.

Introduction

1) Interspecific hybridization in a nutshell

Hybridization[1] plays an important role in the evolution of living organisms. It is the basic mechanism of processes such as introgression of diverse phenotypic traits between diverged taxa or hybrid speciation. Most flowering plants and ferns originated through (allo-)polyploidization (= hybridization followed by genome duplication), which is supposed to be the most powerful "engine" of plant evolution (Soltis et Soltis 2009). Hybridization plays a striking role in human "nutrition evolution". Although the first written evidence is dated back to the early 18th century, hybridization has been important to humans since the Neolithic era (Rieseberg et Carney 1998). When domestication and breeding of plants and animals was in its infancy, hybridization events happened mostly accidentally and inadvertently. Later, by selecting crops (or breeds) carrying required characters, hybridization of closely related species or local races became intentional and increased in intensity.

In early studies devoted to hybridization, several incorrect presumptions had been made. Hybridization had been considered a "blind alley" of evolution because of assumed hybrid sterility (Grant 1981, Rieseberg et Carney 1998, Ouyang et al. 2010). The evolutionary impact of hybridization was once enormously underestimated due to presumed rarity of this phenomenon (Knobloch 1972). Although botanists have paid considerable attention to hybridization (Rhymer et Simberloff 1996), it has been proved in the last decades that hybridization plays an inestimable role not only in plant, but also in animal evolution (Dowling et Secor 1997, Hegarty et Hiscock 2005, Wissemann 2007, Soltis et Soltis 2009).

Hybridization is of paramount importance in the conservation of many rare species. Hybridization is essential for generating new evolutionarily independent lineages (which may ultimately develop into new species); on the other hand, it can have a detrimental effect on populations of rare species. These may suffer from hybridization with common congeners, resulting in blurring of boundaries between taxa and threatening species´ genetic integrity. Low abundance of individuals, low number of populations, marginal populations and discontinuous range of distribution are the main "natural" reasons rare species are under threat. During the last century, human influence considerably increased the risk of rare species becoming extinct, eg. by landscape fragmentation, changes in traditional landscape management, habitat loss and degradation, etc. Rare species represent an important component of both local and global biodiversity and are often regarded as indicators of biodiversity richness (Heywood et Iriondo 2003). They often have narrow ecological niches, which are patchily distributed in the modern landscape. Populations of these specialists therefore often consist of a low number of individuals. Spatially-limited species include endemics of particular geographic regions (Kaplan 2012). The present thesis addresses questions concerning cases of interspecific hybridization that involve at least one rare species, with an emphasis on conservation consequences including a risk assessment.

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[1]   Hybridization as a term has several different meanings – from the most strict : "every fusion of two gametes", through "fusion of two gametes from two individuals from different populations of the same species", to the widely accepted "cross-fertilization between two individuals of different (and isolated) species" (eg. Rhymer et Simberloff 1996, Arnold 1997, Soltis et Soltis 2009). The latter meaning is used throughout this thesis, unless specified otherwise.

2) Evolutionary aspects of hybridization

Hybridization is not uniformly distributed across the plant kingdom, but unevenly across different taxonomic groups. Some taxonomic groups hybridize freely whereas many others do not (Rieseberg et Carney 1998). Some of the most "promiscuous" temperate species are members of the families Asteraceae, Rosaceae, Poaceae, Scrophulariaceae, Brassicaceae and Salicaceae (Ellstrand et al. 1996). Attempts to analyse and compare local floras may run into problems when operating with different taxonomic concepts, especially when agamospermy occurs (Stace 1975, Ellstrand et al. 1996, Danihelka et al. 2012). The so-called narrow taxonomic concept is characterized by an increased number of described taxa (microspecies; see eg. Tyler 2011, Schischkin et Bobrov 2002). The so-called broad taxonomic concept does not distinguish between hybrids and hybridogenous species, so the number of regular taxa is generally lower (Bräutigam et Greuter 2007, Aldasoro et al. 1998). Although the narrow concept reflects evolutionary patterns, numerous microspecies are impractical for non-expert botanists (Dickinson 1998, Hörandl 1998, Kirschner 1998, Stace 1998). The number of recognized taxa is frequently connected with so-called taxonomic favouritism (Bickford et al. 2007) and popularity of specific groups of taxa. There is, for example, a considerably higher proportion of hybridogenous *Sorbus* species in Germany and the British isles compared to the rest of Europe (especially Balkan Peninsula). It is open to argument whether low or high numbers of taxa reflect true biological diversity in nature and whether high numbers of recognized taxa merely indicate taxonomical bias. In spite of the mentioned difficulties, proportions of hybrid species in national floras are comparable and range from 10 to 20% (British Isles 10–20%, Czech Republic 14%, Scandinavia 10%; Stace 1975, Ellstrand et al. 1996, Danihelka et al. 2012). Hybridization is generally a widespread phenomenon in vascular plants. Approximately 25% of species are known to hybridize, but this proportion may be underestimated because hybrid origins are often difficult to prove (Wagner 1969, Mallet 2007).

Advantages and disadvantages of hybridization

Hybridization has both evolutionarily positive and negative consequences, which all stem from the fact that two different genomes are combined. Hybridization increases genetic diversity and enables gene flow between previously isolated taxa. If backcrossing occurs, some beneficial alleles may be transferred from one species to another. Interspecific hybrids are highly variable in fertility and vigour – especially F1 hybrids (eg. between geographic races or closely related taxa) tend to exceed their parents in vegetative vigour or robustness (heterosis; Grant 1975). This phenomenon is frequently utilized in crop breeding to reach specific characters increasing yield. Heterosis may also partially explain the success of allopolyploids and many clonal hybrid lineages (eg. agamic complexes in the genera *Sorbus*, *Rubus* or *Taraxacum*). Hybrids may also possess characters, which were suppressed or inexpressive in parental generations (Rieseberg and Carney 1998), or exhibit novel or extreme characters (transgressive segregation; Rieseberg 1997, Rieseberg et al. 2003, Seehausen 2004). These characters can enable hybrids to reach novel niches and allow selection to act in favour of their establishment (Rieseberg and Carney 1998). Generally, hybrids have a broader adaptation ability (frequently combining that of their parents; Abbott 1992, Buerkle et al. 2000, Rieseberg et al. 2003). Hybridization allows genetic novelties to accumulate faster than through mutations alone (Martinsen et al. 2001). If a newly arisen hybrid is capable of independent reproduction and is reproductively isolated from its parental species, it may act as a separate species.

The main disadvantage of hybridization is the breakdown of the genetic integrity of parental taxa. Recurrent hybridization may lead to the emergence of the third group of plants (F1 hybrids) in the habitat. This model of hybridization is not harmful provided that hybridization events are rare or F1 hybrids are sterile, precluding the formation of complex hybrid swarms. If hybrids are fertile or if reproductive barriers towards (at least one) parental species is broken, backcrossing may occur, and

differences between species may get blurred, possibly leading to genetic swamping [2]of parental species (Rhymer & Simberloff 1996). Taxa with low abundance may become extinct if strong introgression occurs (Levin et al. 1996). Hybrids, even if completely sterile, present a significant burden for parental species with regard to competition (Wolf et al. 2001, Prentis et al. 2007). Plant hybrids often share the same habitat and compete with their parents for resources (nutrients, water and radiation) or may dramatically decrease the number of suitable breeding partners (Buerkle et al. 2000, Bleeker 2007). Hybrids can ultimately replace their parental species altogether (demographic swamping[3]; Wolf et al. 2001).

Reproductive isolation mechanisms

To avoid hybridization (and its evolutionary consequences), several breeding barriers in plants have developed. These reproductive isolation mechanisms may be classified into two major categories – prezygotic and postzygotic, referring to the ontogenetic stage in which they take effect (before or after fertilization). Prezygotic barriers include habitat, temporal (different growth period and flowering time) and behavioural isolation (pollinator fidelity, morphological adaptation avoiding pollination), gametic competition (pollen tube competition) or some kind of incompatibility (preventing pollen grains to germinate). Prezygotic mechanisms seem to represent the most efficient reproductive barriers against hybridization, although some of them may be easily overcome (eg. mentor effect in avoidance of pollen germination; Richards 1997, Krahulcová et al. 1999, Mráz 2003). Postzygotic barriers include zygote mortality, reduced hybrid vigour (hybrids fail to develop or do not reach maturity), reduced fertility (hybrids fail to produce gametes, eg. due to irregular chromosome pairing) and hybrid breakdown (descendants following the F1 generation are of various fitness, often inviable). Reproductive isolating mechanism does not work as a rigorous barrier, but more likely as permeable filters (Mallet 2007). In cases of closely related species (and species recently originated or diverged), isolation mechanisms are often weak or are not yet established. On the other hand, postzygotic barriers may decrease the fitness of parental taxa and thus are often replaced by prezygotic mechanisms of some kind.

Hybridization is frequently initiated by the breakdown of spatial reproductive isolation barriers (either due to natural drivers or human activity). Another possible trigger of hybridization is the breakdown of ecological mechanisms isolating two potentially hybridizing taxa; in this case human activities play a major role (eg. transport of goods, changes in habitats, disturbances, new types of secondary habitats; Abbott 1992, Krahulcová et Krahulec 1999, Hanfling et Kollmann 2003, Krahulec et al. 2004).

Although hybridization had been believed to be predominantly bidirectional, asymmetric patterns have often been found (Rieseberg et Carney 1998, Lepais et al. 2009, Ma et al. 2010). Unidirectional crossing is more common when hybridizing species are of different ploidy levels (ie. one ploidy level is the donor of pollen and second is the acceptor; Krahulec et al. 2004, Ludwig et al. 2013).

Outcomes of hybridization

When reproductive barriers between two species are broken, hybridization can follow several different evolutionary trajectories, including the formation of a hybrid zone, hybrid swarm and genesis of a new species. A hybrid zone is formed where two genetically distinct groups meet and

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[2] The process when genes from a larger population dominate over the genes in a small population. Genetic diversity in the small population is thus significantly reduced.

[3] If hybrids are sterile or display reduced fitness, the population growth rate of the hybridizing taxa may decrease below that required for replacement of one or both parental species.

hybridize (Barton et Hewitt 1985). The existence of selection against hybrids stabilizes the hybrid zone and complicates later hybridizations. Hybrid zones are often formed at boundaries between different habitats – if each habitat favours one parental taxon, hybrids are selected against in both habitats (Anderson 1948, Harrison 1993), and hybridization is restricted to a relatively narrow area where the two species are in contact. More or less stable hybrid zones have been observed, for example, in *Arctium* (Repplinger et al. 2007), *Senecio* (Prentis et al. 2007) or *Cardamine* (Marhold et al. 2002, Lihová et al. 2007b); selection against hybrids has been documented in most hybrid zones studied (Arnold 1994, Allendorf et al. 2001, Seehausen 2004). An extreme example of hybrid zones are hybrid swarms, which result from introgressive hybridization. When newly arisen hybrids are (inter)fertile and backcross with their parents, the integrity of parental species is progressively blurred. After several generations of introgression, hybrids merge with their parental species, and a morphological and genetic continuum originates (plants with different proportions of parental genomes). Introgression either affects both parental taxa or may be unidirectional (backcrossing with one parental taxon only; eg. *Rhododendron* – Ma et al. 2010, *Quercus* – Lepais et al. 2009). A special and confounding product of introgression is chloroplast capture. During such an introgressive hybridization event, the cytoplasm of one species is replaced by that of another species (Rieseberg et Soltis 1991). To avoid introgression, reproductive barriers in introgressed population are often reinforced through selection for assortative mating (Arnold 1992). Introgression allows expansion into new habitats due to the production of new genotypes that may be better adapted than parental species (Arnold 1992, Rhymer et Simberloff 1996). Introgression is notoriously difficult to prove, which explains the lack of biosystematic studies dealing with it. Introgressive hybridization has been documented, for example, in the genera *Viola* (Krahulcová et al. 1996), *Populus* (Martinsen et al. 2001), *Cardamine* (Lihová et al. 2007b), *Rhododendron* (Ma et al. 2010) or *Diphasiastrum* (Hanušová et al. 2014). Rare hybridization events between two parapatric species may lead to the formation of a contact zone. Hybrids in contact zones have decreased fitness and are often sterile. As hybridization occurs at low frequency, introgression does not play a major role. A mosaic or tension contact zone may develop (Petit et al. 1999). In a mosaic zone, parental species are distributed patchily depending on ecological conditions. Where an ecological cline occurs, a tension contact zone may form. Studies of contact zones are frequently focused on the coexistence of different ploidy levels (Castro et al. 2012, Krejčíková et al. 2013), while the coexistence of parental taxa and their hybrids is often neglected.

A hybrid (or hybridogenous) taxon may originate if some hybrids become independent of their parental species and are able to reproduce themselves. Single hybridization events usually do not lead to the emergence of hybrid species; series of hybridization events are most likely needed. Different asexual or modified sexual modes of reproduction often evolve to overcome the influence of parental species (eg. autogamy, agamospermy and clonal growth). Shifts in the reproductive mode accompanied by polyploidization play a crucial role in the establishment of agamic complexes (eg. *Sorbus* – Nelson-Jones et al. 2002, *Pilosella* – Krahulcová et al. 2000, *Rubus* – Krahulcová et al. 2013, *Crataegus* – Campbell et al. 1991).

Hybrid speciation and polyploid formation

Hybrid speciation is more common in plants than in animals, where other speciation modes prevail (Otto et Whitton 2000). Indeterminate growth, longevity, clonality, hermaphroditism with selfing potential and limited gene flow are the main differences which favour the formation of plant hybrid species (Mallet 2007). From a speciation point of view, hybridization is a process which allows faster accumulation of genetic novelties than random evolutionary events (eg. mutation, genetic drift; Martinsen et al. 2001). The presence of transgressive characters in hybrids supports the statement that hybridization is the main source of variation upon which selection can act (Rieseberg et Ellstrand 1998). Reproductive isolation between parental and hybrid species is essential for their long-term existence, hybrid species must remain distinct even if they get in contact with their parents

secondarily (Mallet 2007). The formation of hybrid species is risky because newly arisen species always suffer (similarly to a newly formed cytotype) from processes analogous to the minority cytotype disadvantage (Levin 1975, Mallet 2007). Hybridization and hybrid speciation allow rapid evolutionary changes by generating novel gene combinations, which may lead to increased genetic variation and fitness, and to the adaptation to new environments (Ellstrand 1992). That is why hybrid speciation is common among rapidly radiating groups (Seehausen 2004, Mallet 2007, Fehrer et al. 2009). Reproductive success is crucial for newly originated hybrids. Complete or partial sterility has been detected in many homoploid hybrids (*Cirsium* – Bureš et al. 2010; *Cerastium* – Vít et al. 2014) as well as in heteroploid hybrids (*Viola* – Krahulcová et al. 1996; *Cardamine* – Lihová et al. 2007a, *Cirsium* – Bureš et al. 2010; *Sorbus* – Rich 2009; *Nymphaea* – Kabátová et al. 2014). Analogously, reduced fertility has been observed in hybrid pteridophytes (spore abortion; *Dryopteris* – Ekrt et al. 2009, *Diphasiastrum* – Hanušová et al. 2014). Reproductive success is often reduced in homoploid hybrids too, because they often face problems with chromosome pairing during meiosis (Grant 1981). After meiosis, gametes carry an unbalanced number of chromosomes because somatic cells of the hybrid contain only one chromosome set from each parent. This often results in aneuploid somatic chromosome numbers (Ramsey and Schemske 1998). However, hybrids may overcome this "blind end" through polyploidization when all chromosomes are duplicated and then undergo regular meiosis. Alternatively, hybrids can switch their reproductive system to clonal growth (which is, however, not possible for all plants, eg. annuals) or apomixis (Asker et Jerling 1992). Many plant groups are predisposed to apomixis (Asker et Jerling 1992, Catanach et al. 2006), and its occurrence generally correlates with hybridization and polyploidization (eg. in *Sorbus* – Nelson-Jones 2002, *Crataegus* – Campbell et al. 1991, *Pilosella* – Krahulcová et al. 2000, *Taraxacum* – Richards 1997).

Recent studies indicate that most angiosperms are of ancient polyploid origin (Soltis et Soltis 2009). Hybridization accompanied by chromosome doubling is thus essential for generating contemporary species diversity (Grant 1981, Soltis et Soltis 1993). Polyploidy is highly correlated with asexual modes of reproduction (apomixis, haploid parthenogenesis), selfing and longevity in plants as well as in animals (Mallet 2007, Otto et Whitton 2000). Two types of polyploidy are recognized from a genetic point of view – autopolyploids arise within a single population or between ecotypes of a single species whereas allopolyploids are derived from interspecific hybrids (Ramsey et Schemske 1998). Polyploids originate in different ways depending on the particular mechanism of chromosome doubling: 1) autopolyploidization of diploids, 2) triploid bridge (fusion of a reduced and an unreduced gamete), 3) fusion of two unreduced gametes. Unreduced gametes are rarely formed in diploids and non-hybrid taxa (mean frequency around 0,5%), but are about fifty times more frequent in hybrids (frequency around 25%; Ramsey et Schemske 1998). High numbers of aneuploid and probably also unreduced gametes seem to originate from polyploids with odd chromosome numbers (Krahulcová et al. 2000). When a polyploid successfully overcomes the phase of formation, other problems usually emerge (eg. demographic establishment of new a new cytotype facing the minority cytotype disadvantage; Ramsey et Schemske 1998). Polyploids are often reproductively independent of their diploid parents, but when they backcross, progeny with odd numbers of chromosomes occurs. Although these offspring may be viable, they frequently produce sterile gametes or gametes with aneuploid chromosome counts (Grant 1981, Ramsey et Schemske 1998). On the other hand, this triploid bridge is essential for most novel cytotype formations. The origins, distribution and spreading of many recently formed allopolyploids is well documented (eg. *Senecio cambrensis* – Abbott et Lowe 2004, *Spartina anglica* – Ainouche et al. 2004, *Tragopogon mirus* and *T. miscellus* – Soltis et al. 2004, *Cardamine schultzii* – Urbanska et al. 1997, Mandáková et al. 2013, Zozomová-Lihová et al. 2014). Moreover, the evolutionary history of allopolyploid crops selected for transgressively high yields is also well described (Anderson et Stebbins 1954, Grant 1981, Soltis et Soltis 2009).

3) Human-triggered hybridization involving rare species

In cases of rare species, the consequences of hybridization may be even stronger because their populations are often small or occur at the margins of the species´ distribution areas. Microevolutionary processes (eg. speciation, inbreeding depression, bottleneck effect, genetic drift) act more readily in small populations than in large populations (Rhymer et Simberloff 1996). Consequently, both beneficial and harmful consequences of hybridization (genesis of new evolutionary units vs. potential extinction of populations) are more striking (Rieseberg and Ellstrand 1993). Many rare species originated from widely distributed relatives (textbook examples from the Czech flora are *Minuartia smejkali* – Dvořáková 1988; *M. corcontica* – Dvořáková 1999; *Cerastium alsinifolium* – Novák 1960), and are therefore more prone to hybridize with their progenitors. Several other taxonomically complex groups (Ennos et al. 2006) comprise rare taxa originating (eg. via allopolyploidy) from their widely distributed counterparts.

Hybrid genotypes often vary considerably in their fitness (Rieseberg et Carney 1998). Although hybrids from early-generations are on average less vigorous than parental taxa, individuals with transgressive characters originate regularly. If reproductive isolation mechanisms are not established, further hybridization events may follow soon. Repeated rounds of hybridization, possibly leading to the establishment of hybrid swarms can dramatically jeopardize the genetic integrity of rare species. This process can ultimately result in genetic swamping of the rare species by hybrids. Parental species are more likely to get replaced by hybrids through genetic swamping than due to higher average fitness of hybrids (Rieseberg et Carney 1998). An analogous situation occurs in insular-like specialists (eg. serpentine or mountain relicts), whose distribution and gene pool is limited due to long-term isolation to specific ecological conditions, but are surrounded by many related and potentially crossable genotypes (eg. *Knautia* – Kolář et al. 2009, *Cerastium alsinifolium* – Vít et al. 2014).

One still overlooked phenomenon is so-called anthropohybridization (Wójcicki 1991), which refers to hybridization processes with the participation of cultivated or human-introduced species. Such species might have detrimental effects on related native plants. Dilution of their gene pool and gene transfer from crops/aliens are of the most important consequences of anthropohybridization (Abbott 1992, Bleeker et al. 2007, Campbell et al. 2009). Recent examples in which anthropohybridization has been recorded are *Prunus fruticosa* (Musilová 2013) or *Malus sylvestris* (Cornille et al. 2013). Anthropohybridization is largely facilitated by the absence of reproductive isolation mechanisms between crops/aliens and rare species. Crop cultivars often originated in different parts of the world. Reproductive isolation mechanisms are therefore often missing; when cultivars and native taxa come into contact, they may hybridize freely (Ellstrand et al. 1999). Contact between introduced and native taxa is facilitated by three main human activities: plant introduction, landscape fragmentation and habitat modification (Allendorf et al. 2001). Determining whether hybridization is of natural or anthropogenic origin is crucial for conservationists, whose task is to set up appropriate management plans and to take necessary actions. Human-induced changes in habitats may lead to secondary contact of previously separated species, promoting their hybridization (Rhymer et Simberloff 1996). It has been documented, for example, in *Viola lutea* subsp. *sudetica × V. tricolor* (Krahulcová *et al.* 1996), *Senecio hercynicus × S. ovatus* (Raudnitschka *et al.* 2007), *Arctium lappa / A. tomentosum × A. minus* (Repplinger *et al.* 2007), *Cerastium alsinifolium × C. arvense* (Vít et al. 2014) or *Diphasiastrum* species (Hanušová et al. 2014). A classic example of when human-induced hybridization can take place are mountain meadows in the Krkonoše Mts, where native montane and introduced lowland species meet due to long-term human activities. Hybridization between alpine and lowland *Hieracium* subg. *Pilosella* species has led to the origin of hybridogenous species and lineages restricted to these habitats (eg. *Hieracium iseranum*; Krahulec et al. 2004). The role of habitat disturbance in hybridization has been a subject of discussion since Anderson (1948). He argued that disturbances create open niches which may host a wide diversity of hybrid genotypes. Disturbances may also support the breakdown of established reproductive

isolation mechanisms. Levin et al. (1996) consider disturbances as corridors promoting movement of species and leading to sympatry (and hybridization) between allo- or parapatric species. Expansion of one species to the geographical range of another may also be prompted by habitat modification (Rhymer et Simberloff 1996). A recurrent issue in conservation biology is whether populations with hybridizing rare species should receive the same conservation effort as non-hybridizing populations (eg. Thompson et al. 2009). Efforts should be targeted at maintaining remaining pure populations rather than at trying to save population already affected by high degrees of hybridization (eg. removal of non-native species and hybrids or restoration of habitats; Allendorf et al. 2001).

Reciprocal transplant experiments are essential for obtaining environment-dependent fitness data for parental and hybrid individuals (Rieseberg and Carney 1998), but may endanger rare species populations. In a similar way, replanting experiments are often used by conservationists for strengthening population numbers or reintroducing rare species from distant (genetically different) populations. In stable populations of rare species, reproductive isolation mechanisms against closely related species may exist. Introducing "alien" individuals from distinct populations (which might be adapted to another environment) may lead to loss of local adaptation (eg. reproductive isolation mechanisms) and decrease (often substantially) the average fitness of targeted populations (Barton et Hewitt 1985, Ellstrand 1992). Moreover, the resulting outbreeding depression[4] often promotes hybridization. When hybridization occurs, well intentioned reintroduction projects can be counterproductive if hybrids become fertile (Rhymer et Simberloff 1996). Rieseberg et Carney (1998) therefore advise to avoid transplantation experiments or to manage them under special conditions (removing anthers during the flowering period and harvesting seeds before they disperse). However, such measures are time-consuming and costly.

Generally, biosystematic studies of species complexes with rare or endangered taxa threatened by hybridization with common relatives or intricate agamic complexes are highly appreciated by conservationists. Such studies allow them to better evaluate the risks of hybridization based on knowledge of reproductive modes, hybrid frequency and other important facts. The key question surrounding all conservation efforts undoubtedly is "How do we recognize hybrids?".

4) Current methods for hybrid identification

Hybrids have been recognized and studied at least since the times of **Linnaeus** (Rieseberg and Carney 1998), although "products of hybridization" were observed much earlier, at the dawn of agriculture (eg. descendants of cereal breeding and ancient agricultural selection of the most productive plants; Feuillet et al. 2007). As in many other areas, the sensitivity and resolution of research methods have changed rapidly over the last decades. Detection of hybridization (or ancient hybridization events) is much easier nowadays thanks to the development of highly sensitive approaches. Methods widely used for detecting hybridization and ongoing processes in populations of hybridizing taxa are discussed further below.

Phenetic methods

Phenetic methods have been for a long time the sole technique for hybrid detection. Intermediate appearance or a combination of morphological characters of parental taxa is a classic indication of hybrid origin. However, many hybrids have eluded detection by this approach (eg. hybrid swarms; Rhymer et Simberloff 1996, Allendorf et al. 2001). Transgressive or novel characters are not an exception, but occur regularly among hybrids and become more frequent in later hybrid generations (ca 10% in F1 hybrids; Rieseberg and Ellstrand 1993). A meta-analysis of 46 hybridization studies made by Rieseberg and Ellstrand (1993) found significant deviations from presumed intermediacy.

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[4] Crosses between genetically distant sources produce offspring with lowered fitness

Hybrids from the first generation were characterized by a mosaic of parental and intermediate characters, rather than possessing exclusively intermediate ones (Rieseberg 1995). Differences between particular morphological characters and their inheritance in hybrids can be explained by differences in their genetic control and interactions with the environment. Rieseberg and Ellstrand (1993) suppose, that morphological characters under multigenic control tend to attain intermediate values, while characters under simple control usually manifest as parental or intermediate appearance in the first hybrid generation. However not all morphological characters have a genetic basis (Allendorf et al. 2001) and hybrid characters constitute a mosaic of parental phenotypes. It is difficult to determine based on morphology alone whether a particular hybrid represents the first or a later generation or backcross (Allendorf et al. 2001). This knowledge is nevertheless crucial for conservationists whose job is to evaluate the risk of hybridization for rare species. Last but not least, it is necessary to take into account the quantity and potential correlation of morphometric characters when evaluating complex hybrids. Functional or developmental correlations highly reduce the informative content of each morphological character (Rieseberg and Ellstrand 1993). Only scoring of a considerable amount of characters may bring valuable results that truly indicate hybridization and backcrossing.

On the other hand individuals with intermediate morphologies are often interpreted as interspecific hybrids (eg. in the genus *Nymphaea*; Heslop-Harrison 1955, Ejankowski & Małysz 2011) even though their hybrid status is not supported by other methods (karyology or molecular markers). Many recent studies have not confirmed hypotheses about the hybrid origins of such individuals (eg. in the genus *Nymphaea*; Kabátová et al. 2014). Analogously, several putative hybrids have finally turned out to be ecomorphoses, or individuals damaged during early developmental stages or altered by suboptimal conditions (eg. occurrence of frequent hybridization in the genus *Chenopodium* was disproved using karyological techniques; Mandák et al. 2012).

Karyology

Detection of hybrids using conventional karyology is relatively easy and straightforward when differences in chromosome number or karyotype exist. Special attention is necessary when chromosome deviations (eg. aneuploidy or chromosome rearrangements) are expected. This phenomenon is more common in hybrids (eg. higher frequency of aneuploidy; Ramsey et Schemske 1998). The need of mitotically active tissue, huge laboriousness and the need of an experienced karyologist to analyse material are the main disadvantages of this method. Advanced karyological methods based on in situ hybridization (GISH, FISH) are suitable for detection of homoploid hybrids as well as for tracking the origin of allopolyploids. Nevertheless, exact chromosome counts or ploidy determination using karyological techniques are essential for the calibration of flow-cytometric analyses (Doležel et al. 2007).

Flow cytometry

Flow cytometry (FCM) is a fast and effective method for analysing optical characteristics of isolated particles. Estimation of genome size and detection of DNA ploidy level are routine applications of FCM in plant biology. Estimation of DNA ploidy level is much faster and easier than using conventional karyological techniques (Doležel et al. 2007). If differences in genome size or ploidy level between parental taxa exist, flow cytometry can easily be used for detecting both homoploid and heteroploid hybrids (Kron et al. 2007, Loureiro et al. 2010). Most hybridization events are not connected with changes in nuclear DNA content, and genome size of hybrids can be straightforwardly inferred from values of their putative parents (Kron et al. 2007, Loureiro et al. 2010). Several obstacles may arise, however – for example, when taxa with the same or similar genome size have different chromosome numbers (eventually holocentric chromosomes may occur; eg. Bozek et al. 2012, Pazy & Plitmann 1995, Hipp et al. 2009). The use of flow cytometry for detecting hybrids of rare species might be beneficial because it requires only small amounts of tissue.

Moreover, some benchtop flow cytometers are mobile, and samples may be analysed directly in the field. Compared to karyology, flow cytometry is not dependent on mitotically active tissues, and samples may be prepared from various types of tissue (from roots to flowers). Nevertheless, each newly detected DNA ploidy level should be confirmed by chromosome counting (Suda et al. 2006).

Molecular methods

Data obtained from molecular markers have several advantages compared to other types of data (eg. karyological, morphological). Molecular markers are universal (they may be used to study morphologically or karyologically distant taxa and allow their direct comparison). Moreover, the number of characters obtained by molecular markers is many times higher compared to morphological ones. Molecular characters are also well defined (4 nucleotides in DNA sequences) and discrete. Nowadays, a wide spectrum of PCR-based methods is available and commonly used in studies of hybridization. As more and more sensitive methods are routinely used for hybrids detection, many new hybridization events are discovered. Ancient hybridization (eg. Grimm et Denk 2008, Fehrer et al. 2009) and cryptic hybrids (eg. Jasińska et al. 2010, Nicole et al. 2007, Paule et al. 2012) represent previously overlooked phenomena that were often for the first time unravelled using modern molecular techniques in the last decade. Hence, the selection of suitable genetic markers for studying hybridization is essential. It always depends on the required resolution and relationships of the studied taxa.

Sequences of nuclear genes are useful for detecting hybrids because of their biparental inheritance. A sequence (or restriction profile) of a targeted gene is transferred from both parents equally to hybrids. However, because of the variation in inheritance patterns, recombinations and linkages, results must be interpreted with caution. Markers from nuclear ribosomal DNA (nrDNA; ITS region) are the most widely used to detect hybrids (eg. Fuertes Aguilar et al. 1999, Lihová et al. 2007a). In cases of both ancient and recent hybridization events, potential consequences of concerted evolution (eg. homogenization of sequences) must be taken into account (Alvarez et Wendel 2003). The use of genes which are resistant to concerted evolution (eg. low copy genes) strikingly increased in the last decade (eg. Shimizu-Inatsugi et al. 2009, Krak et al. 2013, Ramadugu et al. 2013, Schneider et al. 2013). However, their application has also brought numerous difficulties, stemming mainly from population genetic processes such as incomplete lineage sorting, genetic drift or natural selection (Sang 2002, Small et al. 2004, Linder & Rieseberg 2004). Low-copy or nrDNA markers often suffer from insufficient variation, which causes problems in studies focused on the population level (eg. detection of hybridization rates, assessment of reproductive modes). More sensitive markers should therefore be adopted – traditionally, microsatellites or AFLPs are used in population based studies (Meudt et Clark 2007). For instance, SNPs[5] derived from high-throughput sequencing techniques (Rad-seq, GBS) are becoming an important source of molecular data useful for elucidating hybrid origin (Hohenlohe et al 2011, 2013, Wagner et al. 2013).

Organellar DNA (chloroplast DNA, mitochondrial DNA) can also be highly informative when detecting plant hybridization thanks to its predominantly uniparental inheritance in plants (Harris et Ingram 1991). Several aspects of microevolutionary processes may be tracked, including the direction of hybridization or introgression. Sufficient variation between the hybridizing taxa is the main prerequisite, despite the plethora of cpDNA markers (Taberlet et al. 1991, Demesure et al. 1995, Shaw et al. 2005, Shaw et al. 2007). The use of cpDNA seems to be easy, but one must bear in mind that cpDNA reflects only one genetic line (parental species), while the second remains hidden. It is therefore appropriate to use cpDNA markers in combination with nuclear markers to enable the reconstruction of relationships between both parental species and hybrids.

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[5] Single nucleotide polymorphisms

5) Species under study

Cerastium alsinifolium

Cerastium alsinifolium Tausch (Smejkal 1990, Kaplan 2012) is an outcrossing serpentine endemic of western Bohemia (Slavkovský les Mts). The total area occupied by this species does not exceed 15 km^2, with all sites situated within the Protected Landscape Area Slavkovský les (Tájek et al. 2012). *Cerastium alsinifolium* is a critically endangered plant in the Czech flora (Grulich 2012, Kaplan 2012). Currently, *C. alsinifolium* is reported from two rather contrasting types of habitat on serpentine bedrock, namely dry open grassland on rocky outcrops, and (semi)shaded springs and seeps in coniferous forests (Melichar 2005, Tájek et al. 2012). At several sites in the Slavkovský les Mts, it co-occurs with another perennial large flowered species, *C. arvense*, which is widely distributed in Europe and usually inhabits dry grasslands or semiruderal sites (Smejkal 1990). *Cerastium arvense* is relatively tolerant of soils with a high heavy metal content (Levine & Greller 2004), and in the Slavkovský les Mts it occasionally grows on outcrops of serpentine or in their immediate vicinity.

Potential hybridization between serpentine endemic *Cerastium alsinifolium* and its widespread counterpart *C. arvense* in the Slavkovský les Mts has been suspected for a long time (Smejkal 1990, Hrouda 2002, Rybka et al. 2004). It has, however, not been proved by biosystematic approaches. Possible evolutionary consequences of the hybridization also remain unknown.

Sorbus

In the genus *Sorbus*, taxonomic difficulties stem from recognizing and describing new species originating through hybridization of diploid sexual [*S. torminalis* (L.) Crantz, *S. aucuparia* L. and *S. chamaemespillus* (L.) Crantz] and tetraploid [taxa from the group of *Sorbus aria* (L.) Crantz] species. Primary hybrids occur spontaneously and are of the same ploidy level as their parents (Meyer et al. 2005). Hybrid lineages and stabilized hybridogenous species (or so-called microspecies) have higher ploidy levels, indicating their formation through unreduced gametes or through hybridization of polyploids. Their evolutionary success is connected to agamospermy (apospory), which can be accompanied by residual sexuality (Proctor et Groenhof 1992, Robertson 2004). Newly originated lineages can persist *in situ* for many years and further shape the population structure of parental species (eg. as pollen donors; Ludwig et al. 2013). Microspecies may originate recurrently from the same parental combination and exhibit highly similar morphology with negligible differences due to distinct parent genotypes. Most discovered stabilized lineages are subsequently described as new species (see the number of *Sorbus* taxa in the flora of the Czech Republic; Kaplan 2012). This approach may, however, spoil the taxonomy, especially when each and every local lineage is formally described as a separate species. *Sorbus quernea*, a formerly recognized endemic rowan from Prague, is an example of an apomictic taxon that was sunk into synonymy after a thorough taxonomic revision (Lepší et al. 2013).

The study of agamic complexes in the frame of conservation efforts is a very difficult task. With respect to taxonomy, agamic complexes are a "dynamic system" of newly described and rejected taxa (see Lepší et al. 2013). This approach is, however, often difficult to digest for conservationists. Conservation of higher taxonomic units (eg. at the subgeneric level; Pellicer et al. 2012) or the conservation of evolutionary units that generate taxonomic diversity seems to be an alternative (Rhymer et Simberloff 1996). The questions remain: What should be protected (the product or the speciation trigger), and which phenomenon is more valuable – endemism or speciation? One may argue that hybridogenous agamic species are "blind alleys" of evolution that do not deserve protection due to their asexual mode of reproduction. This is not the case, however, as they may still enter further hybridizations as pollen donors (production of viable pollen grains is relatively high; Rich 2009). Hybridization is a significant evolutionary process, and hybridizing populations of parental species and hybrids alike are extremely important from several standpoints. To protect taxonomically

complex groups and processes taking place within them, it is crucial to conserve these "engines" of evolution (Stace 1998, Ennos et al. 2006).

Biosystematic evaluation of *Sorbus eximia* is presented in the second study. It was a textbook example of diploid-tetraploid taxon with agamospermous mode of reproduction (including agamospermy at the diploid level; Jankun et Kovanda 1988). This exceptional mode of reproduction was several times cited (Nelson-Jones 2002, Talent et Dickinson 2006, Dobeš et al. 2013), but never been reliably confirmed.

Nymphaea

Two native *Nymphaea* species occur in Central Europe – *N. alba* and *N. candida*. Species boundaries between them are blurred by overall morphological similarities, high phenotypic plasticity and possible interspecific hybridization. The situation is further complicated by the occurrence of many garden cultivars. Morphological similarities are at least partly caused by close evolutionary relationships between *N. alba* and *N. candida* (Volkova et al. 2010). Individuals with intermediate morphologies have often been interpreted as interspecific hybrids (Heslop-Harrison 1955, Ejankowski & Małysz 2011) although their hybrid status has only rarely been evidenced. The few exceptions include crosses between *N. alba* and *N. candida* (= *N. × borealis* Camus) from several sites in Germany and Sweden, confirmed by AFLP fingerprints (Werner & Hellwig 2006, Nierbauer et al. 2014). Natural interspecific hybridization in *Nymphaea* seems to be quite extensive, as indicated by the great number of horticultural crosses (Slocum 2005). Garden cultivars have been repeatedly introduced, be it accidentally or intentionally, into natural habitats, where they can survive for long periods and potentially interact (compete or mate) with native plants. Reliable discrimination between escaped white-flowered cultivars and native species on the basis of morphological traits is difficult, if not impossible.

Diphasiastrum

Six diploid *Diphasiastrum* taxa are traditionally recognized in Europe: three (basic) species and three morphologically intermediate hybrids traditionally treated as species. Mixed populations frequently occur in Central Europe and often form apparent hybrid swarms. Species determination is quite problematic in mixed populations and especially in hybrid swarms occurring in man made habitats (eg. ski slopes, deforested strips). A number of factors complicate investigations of hybridization patterns in *Diphasiastrum*: simple morphology with few characters suitable for evaluation, high phenotypic plasticity and impossibility to accomplish hybridization experiments due to mycorrhizal gametophytes (Wilce 1961, 1965, Whittier 1977, Vogel et Rumsey 1999). The patterns of hybridization in *Diphasiastrum* have recently been addressed using two types of markers: low-copy nuclear genes and genome size. Sequences of three regions of the nuclear genome confirmed the hybrid status of *D. ×issleri*, *D. ×oellgaardii* and *D. ×zeilleri* (Aagaard et al. 2009a, 2009b). This study also indicates certain levels of recent hybridization and backcrossing within European *Diphasiastrum*. Its frequency and variation patterns in natural populations remain unknown, however. On the contrary, discrete variation in genome size in several parts of Europe indicates only primary hybridization with no hint of backcrossing (except for a few rare triploid hybrids) or introgression (Bennert et al. 2011).

Aims of the thesis

1) To evaluate the risk of interspecific hybridization in selected rare species native to the Czech Republic

2) To assess the value of different methodological approaches (incl. karyological, phenetic and molecular techniques) for hybrid identification

3) To elucidate the human impact on the hybridization of rare plant species under investigation

Conclusions and future directions

Although much attention has been paid to the conservation of rare species during the last decades, most studies have not taken a complex biosystematic approach. The conservation and biosystematic points of view have rarely been integrated in a single study. Fortunately, recent years have seen significant progress, which is manifested by the publication of research papers at the interface between conservation and biosystematics (eg. Hedrén et al. 2012, Moreira et al. 2013).

This thesis presents three cases of interspecific hybridization involving rare species. Hybrids coexist with parental taxa in the **Cerastium** *alsinifolium/C. arvense* system. The Czech serpentine endemic *C. alsinifolium* is threatened by competition from hybrids over both abiotic and biotic resources (light, nutrients and pollinators). Considering the absence of backcrosses, hybridization does not seem to severely affect the gene pool of the endemic species. Its genetic integrity will thus most likely be preserved. Nevertheless, in open sites, hybrids usually dominate over *C. alsinifolium* and may possibly outcompete it. Interspecific hybridization is much less pronounced in forest sites, which host core populations of the endemic and are therefore a conservation priority.

Flow-cytometric measurements revealed a ca 45% difference in genome sizes between **Nymphaea** *alba* and *N. candida*. Moreover, the genome sizes of *Nymphaea* cultivars were considerably lower than those of native species. Statistical analyses of morphological characters allowed reliable phenotypic delimitation of both *Nymphaea* species and garden cultivars. Although morphotypes with intermediate values of characters and/or a mosaic-like combination of characters have often been interpreted as interspecific hybrids, our results indicate that interspecific hybridization under natural condition is quite rare (at least in the Czech Republic), and a hybrid origin was confirmed in only eleven out of 612 analysed plants (ca 1.8%). Native *Nymphaea* species are thus not directly threatened by interspecific hybridization. An important finding is the frequent occurrence of accidentally or intentionally introduced *Nymphaea* cultivars in more or less natural habitats in the Czech Republic. It is likely that white-flowered cultivars have previously often been confused with indigenous species.

The frequency of interspecific hybridization among **Diphasiastrum** species and its consequences were evaluated using genome size analysis, and numerical and geometric morphometrics. Although genome sizes of basic taxa tend to differ, hybrids often form phenotypic continua. The most intricate genome size values were found in *D. ×issleri* and *D. ×oellgaardii.* The genome sizes of these hybridogenous species completely overlap even though they originated from different parental combinations. Very low genome size variation was detected in single-taxon populations. The highest variation was found in several populations that consisted of all six species, and in mixed populations comprising both *D. alpinum* and *D. tristachyum*. A similar pattern of variation was subsequently observed in both numerical and geometric morphometrics.

The origin of the hybridogenous species **Sorbus** *eximia* was elucidated, and a new species (*S. barrandienica*) was recognized during the biosystematic revision of the *Sorbus eximia* group in the

Bohemian Karst. Flow cytometry did not confirm the existence of two ploidy levels (di- and tetraploid) and reported agamospermy at the diploid level (Jankun et Kovanda 1988). All accessions of *S. eximia* and *S. barrandienica* turned out to be triploid. The genetic variation of both investigated species was extremely low, indicating their single origins. Long-term persistence of their populations was most likely supported by their agamospermous mode of origin.

The last decade has seen several attractive research directions in the study of hybridizing rare plant species. Although they might be methodologically challenging, they offer opportunities for gaining deeper insights into the patterns and processes of interspecific hybridization, ultimately leading to the identification of "common patterns".

Agamic complexes present a particularly promising group for the study of several microevolutionary processes. Although taxonomic studies (often resulting in the recognition of new agamospermous lineages) clearly prevailed in the last few decades (eg. in the genera *Sorbus* – Kovanda 1961, 1996, Lepší et al. 2008; *Rubus* – Lepší et Lepší 2009, Trávníček et Žíla 2011; *Taraxacum* – Trávníček et al. 2008), more recent works address ecological and microevolutionary questions (eg. in the genus *Sorbus*; Vít et al. 2012, Lepší et al. 2013, Ludwig et al. 2013). Many recent studies attempt to reveal microevolutionary mechanisms responsible for the genesis of hybridogenous species. Other attractive topics are detection of the mode of reproduction (using DNA flow cytometry and microsatellites), identification of parental taxa (microsatellites), elucidation of the direction of hybridization (chloroplast markers) and susceptibility of each parent to hybridization (using hybridization experiments).

The spread of introduced (and possibly invasive) plant taxa has been well documented. Occasionally, they can hybridize with their native counterparts, and these systems offer unique opportunities to study hybridization at its initial stages. Future studies should clarify the evolutionary consequences of hybridization on populations of native species, using a multi-method approach involving, among others, detailed ecological studies, sophisticated spatial models (Phillips et al. 2006) and historical data coupled with molecular and cytogenetic techniques. Such studies will paint a holistic picture of the patterns, processes and dynamics of interspecific hybridization.

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Publications

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Popularization papers

Tájek P., Klaudisová A. & Vít P. (2012): Vývoj populace křížence rožce kuřičkolistého (*Cerastium alsinifolium*) a rožce rolního (*C. arvense*) v NPP Křížky v letech 1984-2012. – Sborník Muzea Karlovarského Kraje 20: 173-194.

Urfus T. & Vít P. (2009): Plavuníky – tajemní návštěvníci z pravěku. – Živa 57/6: 251-253.

Kolář F. & Vít P. (2008): Endemické rostliny českých hadců 1. Zvláštnosti hadcových ostrovů. – Živa 56/1: 14-17.

Kolář F. & Vít P. (2008): Endemické rostliny českých hadců 2. Chrastavec, mochna, hvozdík a trávnička. – Živa 56/2: 67-69.

Kolář F. & Vít P. (2008): Endemické rostliny českých hadců 3. Rožec kuřičkolistý a kuřička Smejkalova. – Živa 56/3: 111-113.

Vít P. & Suda J. (2006): Endemické hybridogenní jeřáby ČR. – Živa 54/4: 251-255.

Conference presentations

Koltunow A., Johnson S. D., Okada T., Oelkers K., Vít P., Krahulcová A., Chrtek J. and Fehrer J. (2012): Apomixis in polyploid *Hieracium* species. - International Conference on Polyploidy, Hybridization and Biodiversity. Průhonice, CZ.

Suda J., Krejčíková J., Lučanová M., Sudová R., Trávníček P., Urfus T., Vít P., Dreyer L., Oberlander K. and Weiss-Schneeweiss A. (2012): Understanding the whole genome processes in a world biodiversity hotspot: Flow cytometric investigations of the Cape flora. - International Conference on Polyploidy, Hybridization and Biodiversity. Průhonice, CZ.

Chudáčková H., Urfus T., Vít P. (2009): Morphological and ecological differentiation within *Glyceria fluitans* -group. - Analytical cytometry V. Olomouc, CZ.

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Suda J., Trávníček P., Vít P. (2007): Flow cytometry as a powerfull tool in plant conservation biology. - Analytical cytometry IV, Brno, CZ.

Conference posters

Kolář F., Lučanová M., Píšová S., Fér T., Vít P., Urfus T., Chrtek J., Koutecký P. and Suda J. (2012): Evolutionary history of *Galium pumilum* agg. polyploid complex in deglaciated Europe. - International Conference on Polyploidy, Hybridization and Biodiversity. Průhonice 7-10.5.2012.

Kabátová K., Vít P. and Suda J. (2012): Interspecific hybridization between Central-Europaean species of the genus *Nymphaea* - insight from flow cytometric, molecular, and phenotypic data. - International Conference on Polyploidy, Hybridization and Biodiversity. Průhonice 7-10.5.2012.

Urfus T., Ekrt L., Dvořáková K. and Vít P. (2012): Biosystematic study of Central Europaean *Diphasiastrum* species. - International Conference on Polyploidy, Hybridization and Biodiversity. Průhonice 7-10.5.2012.

Vít P., Krahulcová A., Fehrer J. and Koltunow A. (2012): Distribution of apomixis-related markers in sexual and apomictic *Hieracium* subg. *Pilosella* accessions. - International Conference on Polyploidy, Hybridization and Biodiversity. Průhonice 7-10.5.2012.

Lepší M. Lepší P. and Vít P. (2012): Hybridogenous polyploid species *Sorbus quernea* – a taxonomic confusion raised by naturalisation of an alien species and revealing of introgression in *S. mougeotii*. - International Conference on Polyploidy, Hybridization and Biodiversity. Průhonice 7-10.5.2012.

Kalůsková J., Vít P. and Suda J. (2012): Assessing the threat from interspecific hybridization to the rare endemic *Dianthus arenarius* subsp. *bohemicus* (Caryophyllaceae). - International Conference on Polyploidy, Hybridization and Biodiversity. Průhonice 7-10.5.2012.

Dvořáková K., Urfus T., Krak K. & Vít P. (2010): Hybridization and microevolutionary relationships among Central European *Diphasiastrum* species. - 19th International Symposium "Biodiversity and Evolutionary Biology" of the German Botanical Society (DBG). September 16th – 19th 2010.

Kubešová M., Loureiro J., Trávníček P., Urfus T., Vít P. & Suda J. (2009): Patterns and dynamics of genome size variation in Taraxacum stenocephalum (Asteraceae). International Conference on Polyploidy, Hybridization and Biodiversity. Saint Malo, France. 17-20.5.2009.

Urfus T., Krahulec F., Vit P. & Kubešová M. (2009): Variation in Pilosella officinarum F. W. Schultz et Sch. Bip. in Central Europe: ploidy levels and their corelation with morphology. International Conference on Polyploidy, Hybridization and Biodiversity. Saint Malo, France. 17-20.5.2009.

Vít P., Suda J., Seifertová K., Kubešová M., Urfus T. (2009): Hybridization of *Cerastium alsinifolium* – caryological and morphological variation of serpentine endemic species. - International Conference on Polyploidy, Hybridization and Biodiversity. Saint Malo, France. 17-20.5.2009.

Slovák M., Vít P., Urfus T. & Suda J. (2008): Intraspecific genome size variation in *Picris hieracioides* L. - X[th] Symposium of the International Organisation of Plant Biosystematists. Vysoke Tatry, Slovakia. 2-4.7.2008.

Vít P. & Suda J. (2008): Threatened plants and management approaches – could flow cytometry help conservation programmes? - ISAC XXIV International Congress. Budapest, Hungary. 17-21.5.2008.

Grant projects

2007-2009: Významný český endemitní rožec *Cerastium alsinifolium* - evoluční historie, reprodukční úspěšnost a důsledky křížení s *Cerastium arvense*. (GAAV, KJB601110709, project leader).

2007-2009: Introgresivní hybridizace u rožce kuřičkolistého (*Cerastium alsinifolium* Tausch): hrozí eroze genofondu význačného hadcového endemita? (GAUK, 29507/200, project leader).

Awards

2010: Cena Josefa Hlávky

2009: Cena Živy za nejlepší článek v kategorii 26-30 let - Endemické rostliny českých hadců 1.- 3

2007: "Purkyňova cena" – Cena časopisu Živa za nejlepší popularizační článek (kategorie nad 30 let): Endemické jeřáby – perly mezi českými dřevinami.

Organization membership

since 2003: Czech botanical society