Chapter XIX

Evolutionary aspects in *Hieracium* subgenus *Pilosella*

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The hawkweed *Hieracium* subgenus *Pilosella* (Cichoriae, Asteraceae) is known for its notoriously complicated taxonomic structure due to ongoing reticulate evolution, combined with a facultative apomictic mode of reproduction and allopolyploidy. Recently, molecular approaches at the clone, population and species level have begun to shed some light on these processes. Gene flow across ploidy levels is common, and parental species of hybrid taxa often include apomicts, even as seed parents. Sexual taxa (diploid or polyploid) usually show high genetic variability. Apomicts vary from near clonality across large geographic distances to multiple origins on a small scale. Selection plays an important role in the establishment and fixation of particular cytotypes/genotypes in the field. A broad range of reproductive strategies and frequent hybridizations, combined with good colonization properties under low-competition conditions in their native Eurasian environment, provide an enormous evolutionary potential, which is also reflected by the group's strong invasive behavior on other continents. Phylogenetic analyses reveal that *Pilosella* chloroplast haplotypes form two major groups with no correlation to morphology and taxonomic grouping whereas nuclear DNA sequences reflect species relationships. Incongruence between molecular markers implies two ancient hybridization events predating most of the speciation observed in the subtribe Hieraciinae: one between the *Hieracium/Chionoracium* subgenera ancestor and partly differentiated *Pilosella*, and a subsequent event between this introgressed *Pilosella* lineage and the closely related *Andryala* genus ancestor. Distribution areas and numbers of *Pilosella* species belonging to one or the other haplotype group as well as the extinction of intermediate haplotypes suggest their differentiation in different glacial refuges. The introgressed *Pilosella* lineage gave rise to the majority of recent species which show an increased ecological amplitude. Secondary contact generated a large geographic overlap of haplotype groups with no apparent reproductive isolation between species. Phylogenetic, developmental genetic, biogeographic, and mechanistic aspects of the origin of polyploidy and apomixis in *Pilosella* are discussed, and guidelines for dealing with natural populations of apomictic groups are suggested. A comprehensive list of adventive *Pilosella* species, an updated map of their native range, and a preliminary map of the distribution of *Andryala* are provided.

KEYWORDS: apomixis, Asteraceae, hybridization, polyploidy, residual sexuality, speciation.

INTRODUCTION

Among various strategies for studying apomixis, developing an understanding of natural apomicts with respect to their clonal population structure, reproductive pathways, speciation and evolutionary history, plays an important role. We present here an overview on *Pilosella* hawkweeds, a group with intricate taxonomic structure due to a combination of sexuality, facultative apomixis, polyploidy, and frequent hybridizations.

Hieracium L. subgenus *Pilosella* (Hill) S.F. Gray is an ongoing taxonomic and genetic riddle that has both puzzled and intrigued botanists for well over 150 years. The first hybridization experiments go back to Friedrich W. Schultz (1848) and Gregor Mendel (1869). Mendel tried to verify the results he had obtained in diploid peas by crosses of *Hieracium* subgen. *Pilosella* species. Much to his disappointment, this second system did not confirm his previous conclusions. While F_1 hybrids were not intermediate and segregated extensively, most F_2 plants proved to be uniform. Only decades later, the phenomenon of apomixis (asexual seed production) was described in *Hieracium* (Ostenfeld & Raunkiaer, 1903; Ostenfeld, 1906). This provided an explanation for Mendel's unexpected findings as most species he had used for his crosses were apomicts. Parallel detailed embryological studies in *Pilosella* provided the first information about aposporous apomixis in angiosperms (Rosenberg, 1906, 1907).

Hieracium subgenus *Pilosella* is sometimes treated as a separate genus (Sell & West, 1975; Bremer, 1994). Due to the existence of different species concepts even within the group (for an overview see Krahulec & Krahulcová, 2006), we shall briefly outline the taxonomic approach we are adopting in this paper. Following important monographs on *Hieracium* (Nägeli & Peter, 1885; Zahn, 1921–1923; 1922–1930), we are using a macrospecies concept, distinguishing "basic" ("Hauptarten", "species principales") and "intermediate" ("Zwischenarten", "species intermediae") species. Basic species are characterized by unique morphological characters, whereas intermediate species are defined by a combination of morphological characters of two or more basic species. The latter are supposed to be of hybrid origin, which has in many cases been inferred from early crossing experiments (Peter, 1884). Thus, this species concept is already implying potentially underlying evolutionary processes. Approximately 25 of the ~200 *Pilosella* macrospecies belong to the group of basic species. Some basic species consist of—or at least comprise—diploids, whereas others are only recorded as polyploids (Schuhwerk, 1996; for schematic representations of relationships between particular basic and intermediate species see Schuhwerk & Fischer, 2003). Intermediate species are usually polyploid and fill the morphological space between different basic species, possibly indicative of extensive hybridizations and backcrosses. In more than half of all species, different ploidy levels have been found (e.g., Schuhwerk & Lippert, 1997; Krahulcová & al., 2000; Rotreklová, 2004; Rotreklová & al., 2005), and it is likely that there are still many undescribed cytotypes.

Cytotypes found in the field range from diploid to octoploid with $x = 9$ as the base chromosome number. Tetra- and pentaploids predominate, triploids are occasionally found and are apomictic or often sterile (reviewed by Krahulcová & al., 2000), but can occasionally also be sexual (Peckert & Chrtek, 2006). Although aneuploids seem to occur more frequently in some hybrid swarms where progeny are generated by heteroploid crosses (O. Rotreklová, A. Krahulcová, F. Krahulec, unpubl.), they are generally rare in natural populations in Europe (Bräutigam & Bräutigam, 1996; Krahulec & al., 2004b). In contrast, a considerable frequency of aneuploids has been recorded in New Zealand (Chapman & Lambie, 1999), but it is not clear if these chromosome numbers refer to plants from the field or to germinated seeds (see below).

Apomixis is in this group not strictly linked to polyploidy. Diploid (i.e., dihaploid by origin) apomicts have been synthesized (Bicknell, 1997; Koltunow & al., 2000; Krahulcová & Krahulec, 2000, unpubl.), although they have never been recorded in nature. Conversely, polyploids, although predominantly apomictic, can be sexual, mostly at even ploidy levels (Gadella, 1984; Krahulcová & al., 2000; Rotreklová, 2004), but sometimes also at odd ones (Pogan & Wcisło, 1995; Houliston & Chapman, 2001; Rotreklová & al., 2002, 2005). The group is characterized by gametophytic apomixis of the aposporous type with autonomous endosperm development (Koltunow & al., 1998). Mechanisms, developmental pathways, and what is presently known about the genetic basis have been recently reviewed by Bicknell & Koltunow (2004).

As in most apomictic plant groups, *Pilosella* apomicts usually produce fertile pollen (Skalińska, 1971a; Gadella, 1982; Chapman & Bicknell, 2000; Krahulcová & Krahulec, 2000) and as such can readily act as the paternal parent in hybridizations. Additionally, (facultative) apomicts can sometimes hybridize as seed parents (Houliston & Chapman, 2004; Krahulec & al., 2004b; Fehrer & al., 2005). Both modes can be present even within a single capitulum (Skalińska, 1971a; Krahulcová & Krahulec, 2000). Maternal as well as paternal gametes can be reduced or unreduced (Skalińska, 1971a, b; Gadella, 1988; Bicknell & al., 2003; Krahulcová & al., 2004), which explains the multitude of cytotypes, part of which are "addition hybrids" in the sense of Harlan & de Wet (1975) ($n + 2n$, $2n + n$, or $2n + 2n$) with increased ploidy level. Unreduced female gametes are more frequent than unreduced pollen in both sexuals (Peckert & Chrtek, 2006) and apomicts (Bicknell & Koltunow, 2004, unpubl.). Another, albeit rarer possibility is the rise of polyhaploids: $2x$, $3x$ or $4x$ plants produced by parthenogenesis of meiotic egg cells of $4x$, $6x$, or $8x$ individuals (Skalińska, 1971a, 1976; Bicknell, 1997; Krahulcová & Krahulec, 2000; Bicknell & al., 2003; Krahulcová & al., 2004). Although foreign pollen can in experiments induce autogamy (mentor effect) in *Pilosella* (Krahulcová & al., 1999; Bicknell & al., 2003), species are usually considered as self-incompatible (Gadella, 1984). The role of autogamous reproduction in the field is unknown, because plants in mixed populations are usually exposed to a mixture of self and foreign pollen. The multitude of processes, cytotypes, and modes of reproduction underlying the tremendous variation of forms has been reviewed by Krahulcová & al. (2000).

Like many apomictic groups, *Pilosella* hawkweeds are perennials, with species often being highly polymorphic morphologically. Apart from sexual and apomictic seed production, many species also propagate vegetatively by stolons. Some diploids rather occur in natural or even relict habitats whereas polyploids are often found to occupy disturbed areas like roadsides, railway tracks, slagheaps, and fallow land, but also grow on unfertilized meadows. *Pilosella* hawkweeds generally avoid eutrophic habitats, and as oligotrophic habitats (at least in central Europe) are currently scarce at lower altitudes, species are often found above 500 m, and some reach up to 3000 m (Bräutigam, 1992). Despite their being weak competitors in their native Eurasian distribution area (Fig. 1), they have a strong tendency to become invasive in other parts of the world such as New Zealand (Jenkins, 1992; Chapman & al., 2000) or North America (Wilson & al., 1997, 2006). Many species have similar habitat preferences, and wherever they come into contact, they hybridize frequently (Lepage, 1967; Gadella, 1987; Krahulec & al., 2004b; Morgan-Richards & al., 2004; Trewick & al., 2004).

Pilosella provides an exceptionally good model for the study of apomixis and its influence on speciation. The group has been studied extensively for more than a hundred years with various different methods: detailed embryological investigations (e.g., Rosenberg, Skalińska, Koltunow), cytology, experimental hybridization and study of breeding systems (e.g., Ostenfeld, Skalińska, Gadella, Krahulcová, Rotreklová), molecular and developmental genetics (e.g., Bicknell, Koltunow, Tucker) and, more recently, molecular ecology, population genetics, and molecular systematics (e.g., Chapman, Houliston, Krahulec, Morgan-Richards, Trewick, Fehrer). As an essential prerequisite with regard to the continuous formation and maintenance of numerous morphologically distinct, but interfertile forms, the best taxonomic background currently available for this group is being provided by

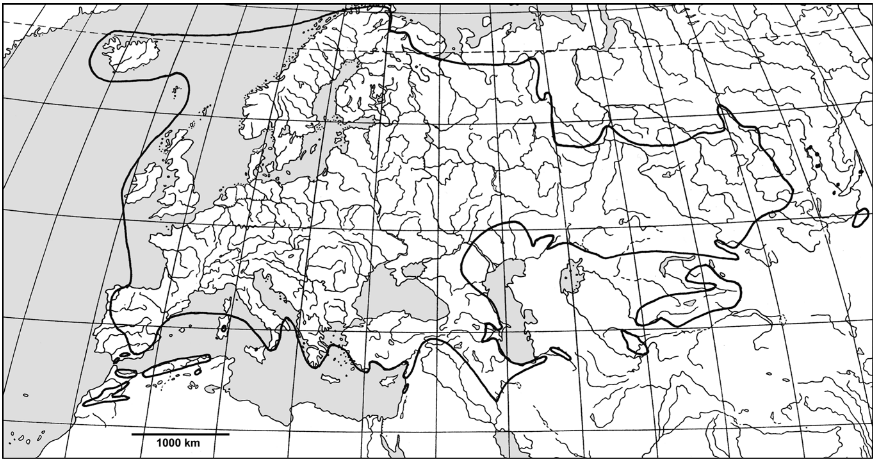

Fig. 1. Native distribution of *Hieracium* subgen. *Pilosella* (following Bräutigam, 1992; updated after Tupicyna [1997]).

international *Hieracium* workshops taking place almost annually since 1997 in different central European countries (http://www.ibot.cas.cz/hieracium/ study-group/workshops.html).

HOW TO STUDY NATURAL POPULATIONS OF APOMICTIC GROUPS?

Of utmost importance in dealing with apomictic groups is the correct identification of the material. The taxonomy of agamic complexes is notoriously difficult (Dickinson, 1998; Hörandl, 1998). Incorrectly determined plants will easily confound interpretations of population patterns and evolutionary processes. In the course of our first study on the genetic structure of natural populations of *Pilosella* species (Fehrer & al., 2005) and from ongoing experience, we have developed a set of guidelines that might prove useful not only for *Pilosella*, but also more generally for similar studies dealing with apomictic complexes.

(1) Populations comprised of hybridizing plants should be treated as one entity, i.e., representative sampling of all types/taxa at a particular locality is important to elucidate the interactions at that site. Experience has shown that extrapolating across sites is of limited utility, as interactions vary over spatial scales.

(2) All determinations should be done by at least one person having long-term experience with the respective taxa. For a particular study, (re-)determination of the material by the same expert can enhance consistency of treatment.

(3) Determinations should be done for all individual plants to be included in a study, and vouchers of all specimens, or at least for two individuals of a proven clone from each population (see 7), should be deposited in herbaria for possible reassessment of characters.

(4) In groups in which phenotypic plasticity is known or assumed, plants should be cultivated under uniform growth conditions to ensure comparability.

(5) Ploidy levels should be routinely assessed. If flow cytometry is used, results should at least occasionally be confirmed by chromosome counts to account for technical problems, variations in DNA content, and for identifying aneuploids.

(6) All analyses (morphological, cytological, molecular etc.) should be done on the same individual plant or on vegetatively propagated progeny, because a fraction of the progeny from germinated seeds of a facultative apomict may not be true to the maternal type due to hybridization or haploid parthenogenesis (see below).

(7) Clonal structure should be investigated by (near-)neutral, co-dominant molecular markers with the power to resolve genotypes at the individual-specific level (e.g., DNA fingerprinting techniques, isozyme phenotypes).

(8) At the population level, examination of chloroplast DNA (cpDNA) haplotypes can sometimes be useful to correctly determine rare morphotypes that were introgressed by other species and give a hint to their potential maternal origin.

(9) Results from the above approaches should be compiled and checked for contradictions to identify potential erroneously assigned taxa or individuals.

(10) Care needs to be taken to avoid circular reasoning, and individuals whose identity remains contradictory shall either be excluded from the study or more thoroughly investigated by additional criteria.

In *Pilosella*, species and cytotypes rather frequently grow intermingled within a locality (Gadella, 1982; Bräutigam & Bräutigam, 1996; Krahulcová & al., 2001; Fehrer & al., 2005), and reproductive isolation between species is virtually absent, even across different cytotypes. This is in contrast to other groups where the distribution of cytotypes is mostly parapatric, and only a few intercytotype hybrids are normally recorded (van Dijk & Bakx-Schotman, 1997; Husband & Schemske, 1998; Segraves & al., 1999; Baack, 2004). By combining the above approaches, we were able (i) to identify incorrectly determined plants and populations, (ii) to account for phenotypic variability of the same genotype (e.g., lowland/mountain plants, different soil conditions), (iii) to detect occasional contamination of garden cultures by other *Pilosella* genotypes, and (iv) to infer the altering effect of a potential parasite infection on the morphology of a single clone. While even these precautions still do not guarantee that all potential sources of error are identified, we consider their application as an important step forward towards establishing a sound basis for dealing with natural populations of apomicts, especially for *Pilosella*. As other systems might be easier to study in some respects (e.g., parapatric cytotypes, reproductive isolation, different ecological preferences), different subsets of these guidelines may be useful for most population studies of apomicts.

PATTERNS OF VARIABILITY IN SEXUALS AND FACULTATIVE APOMICTS

Clonal and population level. — Sexual diploid and tetraploid *H. cymosum* L. and sexual tetraploid *H. pilosella* populations show high intra- and interpopulation genetic variability based on multilocus minisatellite fingerprinting and isozyme analysis (Fehrer & al., 2005, unpubl.). This is in concordance with the generally outcrossing behavior of *Pilosella* species (Gadella, 1984). Apart from occasional clones most probably resulting from vegetative propagation, individual sexual plants typically show unique genotypes reflecting recombination.

In apomicts, there is a wide range of genetic population structure. Some species that by all evidence are recent hybrids often show cytotypic and genetic variability even at local scales (Krahulec & al., 2004a, b; Fehrer & al., 2005, unpubl.). Similarly, Chapman & al. (2000) found levels of genotypic variation in recently founded, predominantly apomictic populations of *H. pilosella* in New Zealand that were almost equivalent to those of outcrossing species. On the other extreme, single apomictic genotypes can form stable, widespread populations (Fehrer & al., 2005) such as seen in tetraploid genotypes of the basic species *H. aurantiacum* and *H. caespitosum*. One *H. aurantiacum* clone was detected in all central European populations we studied so far (western Sudetes, Oberlausitz, Erzgebirge/Krušné hory Mts., Böhmerwald/Šumava Mts.; Fehrer & al., unpubl.). One *H. caespitosum* clone occurs also in these regions and was additionally detected in western Germany and in Pacific North America (Wilson & al., 2006). The same genotype of tetraploid *H. echioides* Lumn. (subsp. *echioides* var. *tauscheri* Nägeli & Peter) occurs in several geographically isolated populations in the Danube basin, indicative of a more widespread distribution in former times (Peckert & al., 2005). The intermediate species *H. floribundum* and *H. glomeratum* also comprise widespread genotypes. While they are most probably of hybrid origin, they can form well-established populations with predominating clones that sometimes show distinguishing morphological features (Fehrer & al., 2005; Wilson & al., 2006). Signs of differentiation, large native distribution areas, invasive behavior on other continents, and their involvement in the formation of further intermediate taxa, some of which are partly already stabilized (see below), suggest advanced stages of hybrid speciation. Most *Pilosella* apomicts, basic as well as intermediate species, show a mixture of widespread and local genotypes, highlighting that most of them are of recurrent origin and comprise lineages of different age (Fehrer & al., 2005, unpubl.).

Population and species level. — Studies of chloroplast DNA haplotypes contribute to elucidating hybridization, introgression, and maternal gene flow in natural populations. Maternal transmission of cpDNA in *Pilosella* was ascertained by reciprocal crosses of sexual taxa of different major haplotype groups (*H. onegense* Nym. × *H. lactucella*; *H. pilosella* × *H. lactucella*; Fehrer & al., 2005).

Pilosella species are divided into two well-distinguished haplotype groups (*Pilosella* I and II) that can be easily screened by PCR-RFLP due to a characteristic 33 bp indel (Fehrer & al., 2005), composed of two independent duplications of 17 bp and 16 bp length. Within each of these groups, further subtypes can be distinguished: a 6 bp insert is characteristic of *H. cymosum* (*Pilosella* II group), a 5 bp deletion occurs, e.g., in *H. aurantiacum*, and a loss of a restriction site characterizes some *H. rubrum* Peter genotypes (both *Pilosella* I group, Krahulec & al., 2004b). Figure 2 shows a compilation of interrelationships in an area from the Erzgebirge/Krušné hory Mts. along the northern border between Germany and the Czech Republic to the western Sudetes (Riesengebirge/Krkonoše Mts.). Despite the complexity of interactions between species, the network is not yet comprehensive for this relatively small area. Strict proof for the contribution of both parental genomes is still missing due to a lack of appropriate markers. As the species are all very closely related (see below), it is not an easy task to develop specific markers for evolutionarily young taxa (see also Stace & al., 1997), but we plan to develop new approaches for this level of relatedness in the future.

In population-level studies of *Pilosella* in this area (Krahulec & al., 2004b; Fehrer & al., 2005), most species show only one of the major haplotype groups (Fig. 2). Thus, basic species, at least at the diploid and tetraploid level, seem to be remarkably uniform with respect to their chloroplast DNA (but see below). In *H. cymosum* and *H. aurantiacum*, the higher ploidy levels may be derived from diploid individuals (still present in *H. cymosum*, but lacking in *H. aurantiacum*) via unreduced gametes within the same taxon. This is suggested by a lack of morphological evidence for the influence of any other species. Many of the stabilized inter-

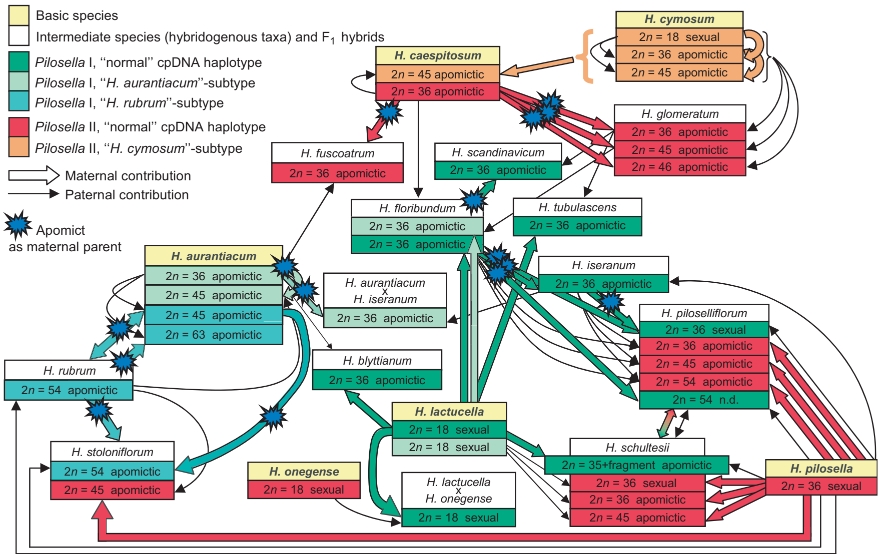

Fig. 2. Interactions of *Pilosella* species in a central European area. These data allow to derive testable hypotheses about parental taxa, their cytotypes, reduced or unreduced condition of gametes involved, and about residual sexuality of apomicts. Putative relationships follow Zahn (1921–23) based on intermediate morphology and phytogeographic data. Chloroplast haplotypes are based on sequencing and RFLPs of the *trnT-trnL* intergenic spacer. Involvement of particular cytotypes (e.g., *H. cymosum*) and direction of cpDNA introgression (e.g., *H. rubrum*) are not always clear. The origin of the "*H. rubrum*-subtype" that requires 2–3 independent mutations from any other group I sequence variant (see also Fig. 4) is unknown. Introgression between *H. piloselliflorum* and *H. schultesii* may be responsible for the apomictic *H. schultesii* cytotypes. Data are compiled from Krahulec & al. (2004b), Fehrer & al. (2005), and additional unpublished results from the same areas.

mediate species also have only one haplotype despite recurrent formation (as inferred from their clonal structure). This suggests that they either were not produced by reciprocal crosses or that only lineages with a particular maternal parent survived. In contrast, cpDNA of some recent hybrid taxa (*H. piloselliflorum*, *H. schultesii* F. Schultz, *H. stoloniflorum*) indicates reciprocal hybridization resulting in the same phenotype. Other recent hybrids [e.g., *H. tubulascens* (Norrl.) Norrl., *H. fuscoatrum*, *H. scandinavicum* Dahlst.] and F_1 hybrids may have also resulted from reciprocal hybridization, but as they are not sufficiently widespread in our study areas, we cannot assess their total variability. Apart from the latter, most of the species in Fig. 2 are represented by many populations and individuals so that the levels of cytotype, haplotype and reproduction mode variation are comparable between species. The same holds for the relative number of clones (see above). The much higher variability at the genotype level adds to the diversity indicated in Fig. 2 and shows the same general trends.

There appears to be a progression from very diverse young hybrid taxa to less variable intermediate species to more uniform basic species. The older and more established an apomictic species becomes, the more consistent it seems to become in morphology, clonal structure, major cpDNA haplotype group, cytotype and reproductive mode. Early generation hybrids/allopolyploids are often highly variable in these respects. Those that dispersed and formed stable populations through apomixis reduced their variation in many of these traits to the point that they are classified as "stabilized hybrid taxa". The most successful of such lineages underwent further stabilization over time, and the subsequent reduction in variation may have lead to them being classified as well-recognizable apomictic "basic species", major recent evolutionary units whose putative diploid (or polyploid) predecessors might no longer be known or became extinct.

Crossing experiments. — A similar trend of decreasing polymorphism as species become more "fixed" can be derived from artificial hybridizations (Krahulcová & al., 2004, unpubl.). Results from several hexaploid facultatively apomictic plants used as seed parents in crosses with tetraploid sexual *H. pilosella* (Table 1) allowed the assessment of variation in the progeny and was related to different levels of stabilization. This reflects to the ability of a respective maternal parent to form ecologically stable populations in the field, or, as expressed above, to the degree of "species specificity" it has already reached. The diversity of progeny ploidy allows the determination of reproduction pathways operating in respective maternal plants and the estimation of their rates (Bicknell & al., 2003; Krahulcová & al., 2004). The frequency of apospory ($2n + 0$ progeny in Table 1), which conserves the maternal genotype, decreases from stabilized basic species (*H.*

Table 1. Progeny of hexaploid facultative apomicts hybridized with tetraploid sexual *H. pilosella* 69/72PI (625/632PI in case of *H. bauhini*).

Seed parents	Progeny frequency (%)						
Process of origin	**Apomixis**		**Hybridization**				
Ploidy	$6x$	$3x$	$5x$	$7x$	$8x$	$10x$	**No. of plants**
Female + male gametes[1]	$2n + 0$[2]	$n + 0$[3]	$n + n$	$n + 2n$	$2n + n$	$2n + 2n$	
H. pilosella	94.8	-	1.4	0.5	2.8	0.5	214
H. bauhini[4]	93.5	1.2	4.5	0.1	0.7	-	849
H. piloselliflorum 224/1	94.7	3.0	2.3	-	-	-	132
H. piloselliflorum 548	15.0	82.1	2.9	-	-	-	140
H. rubrum 11/97RU	89.8	5.3	3.7	-	1.2	-	244
H. rubrum 549	3.4	39.0	57.6	-	-	-	118
H. rubrum 756	28.3	66.7	5.0	-	-	-	60

[1] $2n$ specifies unreduced gametes, n reduced ones (after Harlan & de Wet, 1975).

[2] Apospory (maternal).

[3] Haploid parthenogenesis.

[4] *H. bauhini* was pollinated with different genotypes of *H. pilosella*. As the paternal genotype can have an influence on progeny classes under some environmental conditions (Krahulcová & al., 2004), data from this species are not strictly comparable to the other seed parents that have been exclusively pollinated with the same *H. pilosella* genotype. However, similar offspring proportions from stabilized triploid, aneuploid, and pentaploid seed parents in New Zealand belonging to different *Pilosella* species (Houliston & Chapman, 2001; 2004; Bicknell & al., 2003) suggest that the influence of the maternal genotype may play a much larger role than the paternal parent in determining the variation present in reproductive pathways.

pilosella, *H. bauhini*) across a stabilized hybrid species (*H. rubrum* Peter 11/97RU) towards recent hybrids (*H. rubrum* 549 and 756, *H. piloselliflorum* 548). The polymorphic hybrid taxon *H. piloselliflorum* as a whole comprises an assemblage of geno-, haplo- and cytotypes which suggests it is of recent origin (Krahulec & al., 2004b; Fehrer & al., 2005). However, the high frequency of apospory recorded in one particular hexaploid clone from the Krkonoše Mts. (*H. piloselliflorum* 224/1) seems to reflect a more advanced stabilization of this local clone, which might eventually lead to its successive fixation and spread in the field. Apart from an apparent correlation between progeny classes and different degrees of stabilization/speciation of the maternal parent, there are also large differences between particular genotypes. For example, *H. rubrum* 11/97RU from the Krkonoše Mts./Riesengebirge is a stabilized hybrid species, which has most likely originated as a $2n + n$ hybrid between *H. aurantiacum* and *H. pilosella* (Krahulcová & al., 2004; Krahulec & al., 2004b). In contrast, *H. rubrum* accessions 549 and 756 are rare distinct clones from the Šumava Mts./Böhmerwald (a mountain range along the southern border between Germany/Austria and the Czech Republic). These appear to be recent hybrids that originated from the same parental combination. In morphology, ploidy (Krahulec & al., 2004a), and proportion of parental genomes (Suda & al., unpubl.), they correspond to stabilized *H. rubrum* 11/97RU. *Hieracium rubrum* 549 and 756 share a different chloroplast haplotype and have considerably reduced seed set (A. Krahulcová, F. Krahulec, unpubl.). In concordance with the aforementioned trend, the stabilized type produces a very high amount of offspring by apospory ($2n + 0$). The percentage of this progeny class in the two recent hybrids is considerably lower than in the stabilized clone, and differs almost tenfold between the two accessions. In addition, plant 549 preferentially produces hybrids, whereas plant 756 produces mostly trihaploids (Table 1). The differences can be attributed to different maternal genotypes, as the same *H. pilosella* genotype was used for pollination. Krahulcová & al. (2004) have also shown that different accessions belonging to the same maternal clone have offspring proportions that do not differ significantly, even under different environmental conditions. While a high proportion of maternal offspring seems to be linked to stabilization and speciation, recent facultatively apomictic hybrids show a much higher potential to generate variable progeny. Some of these offspring genotypes may eventually stabilize. For example, trihaploids produced by unstable *H. rubrum* 549 and 756 have higher vigor than those produced by their stabilized counterpart 11/97RU (F. Krahulec, A. Krahulcová, unpubl.).

While more data on the different reproductive behavior of stabilized and recent biotypes, and also among particular genotypes of recent hybrids, are needed to infer more general patterns, two evolutionary consequences can be deduced from our work: (i) stabilizing potential through a high proportion of apomictic offspring is apparent, and (ii) variable progeny of unstabilized genotypes might provide raw material for selection under changing or particular environmental conditions.

THE ROLE OF SELECTION

In the comparably rare sexual populations, there is no discernible difference in reproductive mode between artificial and field conditions, as would be expected due to the obligate nature of this trait. However, hybridization experiments involving apomicts show that many different reproductive pathways can be expressed in the progeny (i.e., addition hybrids, aneuploids, polyhaploids) with significant frequency, revealing an enormous potential of possible variation in this group. In contrast, the occurrence of many of these types in the field is unknown or rare. In consequence, comparably few intermediate species actually establish and form stabilized populations in the field. This suggests that selection and maybe other factors are acting at the recruitment level and influencing the resulting composition of ecologically stable entities. For example:

1. Aneuploids. — Aneuploids generated by experimental heteroploid crosses involving apomicts are rather common (Krahulcová & Krahulec, 2000), but their occurrence in stabilized natural populations is very rare (Krahulec & al., 2004b). Some recent hybrid swarms (*H. bauhini* × *H. pilosella*) show an intermediate proportion of aneuploids (O. Rotreklová, A. Krahulcová, F. Krahulec, unpubl.). This suggests that surplus chromosomes may get lost with ongoing cell divisions and/or purifying selection is purging aneuploid cytotypes over time. Aneuploid plants cannot be recognized as such in the field as they do not differ from other cytotypes in vigor and morphology (F. Krahulec, A. Krahulcová, unpubl.). While their fitness has not yet been investigated, environments with low competition and high establishment probability may allow for their persistence (but this is a general tendency with *Pilosella* species). In heteroploid crosses involving odd ploidy level sexual parents, aneuploids were not observed at all (Peckert & Chrtek, 2006). This suggests that selective and/or other mechanisms may act already at the pre- and postzygotic level in sexual individuals.

2. Triploids. — Triploids of hybrid origin (for trihaploids, see next paragraph) occur regularly in experimental crosses between diploids and tetraploids and, less frequently, in crosses between diploids and pentaploids (Gadella, 1992; Krahulcová & Krahulec, 2000), but are rare in natural populations. In areas investigated in detail, triploids were either not found among many hundreds of plants analyzed (Krahulec & al., 2004b; Fehrer & al., 2005) or at very low frequency (e.g., some sterile *H. schultesii* (*H. lactucella*-*H. pilosella*) plants in the Šumava Mts./Böhmerwald; Krahulec & al., 2004a, unpubl.). This is not due to a lack of contact between the species as, for example, diploid *H. lactucella* often occur together with various (sexual and apomictic) tetraploids. A more likely explanation is that the often observed seed sterility of these plants may establish a kind of triploid block (Marks, 1966; Thompson & Lumaret, 1992).

3. Polyhaploids. — They can be generated by polyploid facultative apomicts (see introduction and Table 1), in emasculation experiments as well as—in higher proportion—in crossing experiments (Krahulcová & al., 2004, Krahulec & al., 2006). While their detection in the field is complicated by the absence of unequivocal criteria for their recognition, there are some indications that they exist. We collected two trihaploid individuals (probably descendants of *H. rubrum* in the Šumava Mts./Böhmerwald) as young rosettes, however, there are no published records of polyhaploids occurring in field populations of *Pilosella*.

Dihaploids resulting from crossing experiments are rare and usually have low vigor. They have either low pollen fertility or are male-sterile, and have sterile as well as apomictic flowers. The germination rate of apomictic seeds typically is only about 5–10% of their tetraploid parents, but they can occasionally set a high percentage of asexual seed with good germination rates (R. A. Bicknell, pers. comm.). We have found a single individual dihaploid *H. aurantiacum*, which regained the tetraploid state in seed progeny from emasculated flowers, and two further such seedlings of *H. caespitosum* were recently identified (R. A. Bicknell, personal observation). In these cases, outcrossing or selfing can be ruled out, meaning that they may have arisen from the fusing of aposporous initials, a process observed in histological studies (Koltunow & al., 1998). Experimentally produced trihaploids are mostly weak, but some genotypes have sufficient vigor to survive under field conditions. The range of variation in their pollen and seed production is comparable to dihaploids, but average fertility of trihaploids may be somewhat higher (R. Rosenbaumová, unpubl.). Similarly, tetrahaploids can have either low or normal vigor; they usually have better seed production than lower-level polyhaploids.

In experiments distinguishing between the frequencies of different ploidy levels in seeds (embryos) versus seedlings, a significant decrease in the proportion of trihaploid progeny between seeds and germination/seedling establishment was found (Krahulec & al., 2006). Here, selection acts at least at two levels: between seed and seedling, and between the mature plant and its establishment in natural populations.

These findings make it unlikely that polyhaploids in *Pilosella* represent evolutionarily relevant intermediate stages of polyhaploid-polyploid cycles as they are observed in other facultative apomicts (Asker & Jerling, 1992; Richards, 1997).

4. High-polyploids. — Octoploids are regularly produced in crossing experiments (Skalińska, 1976; Krahulcová & al., 2004), but are extremely rare in the field (Rotreklová & al., 2002). Experimentally produced octoploids are vigorous plants that produce mainly tetrahaploid offspring (R. Rosenbaumová, unpubl.). Thus, if polyploid-polyhaploid cycles of evolutionary significance exist at all in *Pilosella*, they probably occur at the 8*x*-4*x* level, either as a possible means of purging the genome of deleterious mutations, or because high-polyploids might be unstable. We plan to investigate the fate and progeny of these tetrahaploids in the near future. Decaploids are not known at all from natural populations, but have been produced in artificial hybridizations (Skalińska, 1976; Gadella, 1988; a single plant among thousands of progeny seedlings, Table 1). Nothing is known at present about their reproductive behavior. High-polyploids might be rare in the field as their progeny, if any, are usually of lower ploidy, reducing the time window in which they can be detected.

5. Hybrids produced from a facultatively apomictic mother. — Experimental hybridizations between a facultatively apomictic maternal parent and a sexual paternal parent usually produce a low proportion of hybrid offspring (around 5%, Table 1). This would suggest that a similarly low frequency of hybrids in the field is produced in this way. We would therefore expect hybrids produced from a sexual seed parent and an apomictic pollen donor to be more numerous, however, the opposite is found in natural populations (Fig. 2, and F. Krahulec, J. Fehrer & al., unpubl.). Hybrid progeny can usually be obtained from reciprocal experimental crosses. Except for a single case of apparent pre-zygotic isolation (see below), we did not find evidence for cytoplasmic or other potential incompatibility that would prevent the reciprocal cross with the sexual partner as seed parent. It is unclear why hybrid genotypes with facultatively apomitic maternal parents (produced via *n* or 2*n* female gametes) seem to be favored by selection over hybrids from sexual maternal parents in natural populations.

Apomicts are often considered as organisms that lack sufficient variation for selection to act upon (Asker & Jerling, 1992). Our findings are in strong contrast to this assumption. A large variety of possible reproductive pathways and species interactions produce ample raw material for selection in *Pilosella* hawkweeds. Selection processes during germination and seedling establishment are generally considered as an important filter during life history (Fenner, 1987). However, the only example from our data concerns a highly significant decrease of trihaploids (Krahulec & al., 2006). Thus, in *Pilosella*, mechanisms that reduce the variation found among plants in experimental cultivation to that found in natural populations seem to be more important. As most findings from crossing experiments are based on adult plants that have already passed the germination/seedling filter, further processes, random and/or selective, have to be invoked to explain the observed differences between experimental and field conditions.

IMPORTANCE OF RESIDUAL SEXUALITY IN THE *PILOSELLA* APOMICTIC COMPLEX

The term residual sexuality is used here to describe the frequency of offspring produced by fertilization of reduced or unreduced *female* gametes of facultative apomicts. While the latter avoids meiosis, which is considered as one of the substantial processes of sexual reproduction (Asker & Jerling, 1992; Czapik, 1994), hybridization via unreduced gametes can legitimately be regarded as a sexual process (Harlan & de Wet, 1975; Asker & Jerling, 1992). We neither consider haploid parthenogenesis (sometimes included in broad definitions of apomixis; Czapik, 1994; Johri & Srivastava, 2001; Table 1) nor pollen production (i.e., male fertility), although both are results of meiosis. Pollen production is irrelevant to our question as there is no difference between sexuals and apomicts in this respect. While haploid parthenogenesis produces autonomously derived progeny, the resulting plants are products of meiosis and thus differ from the maternal parent.

As there is at the moment no unequivocal definition of apomixis (or sexuality), we emphasize here fertilization (female fertility) as the decisive criterion for (residual) sexuality as it requires contributions from two individuals. Similarly, Houliston & Chapman (2001, 2004) use the frequency of hybrid progeny as a measure for sexual reproduction in *Pilosella*.

Our studies show for the first time the relevance and relative abundance of sexually reproducing facultative apomicts in natural populations: Fig. 2 indicates many cases in which a facultative apomict must have been the seed parent according to cpDNA evidence. The same holds for the Šumava Mts./Böhmerwald comprising a similar set of basic species, but with a different composition and frequency of intermediate species, cyto- and haplotypes (Krahulec & al., 2004a, unpubl.). In crosses between two apomictic taxa, one of them needs to have been reproducing sexually. But it is intriguing that there are several cases of hybrid species in which, contrary to expectation (see above), a sexual parent was involved as pollen donor, whereas the facultative apomict was the seed parent. Examples are hexaploid *H. stoloniflorum*, part of tetra- and hexaploid *H. piloselliflorum*, *H. rubrum*, probably *H. glomeratum*, and *H. iseranum* Uechtr. (Fig. 2). In the latter, six different genotypes (clones), all tetraploid, had an apomictic *H. floribundum* mother and a *H. pilosella* pollen donor (Fehrer & al., 2005). In this combination only, reciprocal experimental crosses with a sexual *H. pilosella* mother failed, indicating an incompatibility that is highly unusual in the subgenus. These results show that facultative apomicts, although maintained by apomixis, also play an unexpectedly important role as seed parents in the establishment of novel genotypes in the field.

In natural populations in New Zealand, where *Pilosella* colonized approximately 110 years ago, the potential for sexual reproduction of pentaploid facultatively apomictic *Hieracium pilosella* ranged from 0.2 to 2.7% (Houliston & Chapman, 2001, 2004). As this was estimated from seeds collected from hand-pollinated plants in the field, these results are approximately comparable to those of our experimental crosses in which stabilized types were used as maternal parents (Table 1). While this does not allow conclusions about the fate of the hybrid progeny and their evolutionary potential, the European situation where populations and genotypes are older on average may be regarded as an extrapolation of a potential future development to occur in adventive situations. The importance of residual sexuality can also be deduced from sexual *H. pilosella* cytotypes that repeatedly originated from purely apomictic populations in New Zealand (Chapman & al., 2003).

Sexual reproduction does not only play an important role in aposporous apomicts as shown here, but to some extent also in diplosporous taxa such as *Taraxacum* (e.g., van der Hulst & al., 2000; van Baarlen & al., 2002; Verduijn & al., 2004) and the *Boechera holboellii* complex (Sharbel & Mitchell-Olds, 2001; Sharbel & al., 2005). These findings challenge long-held views that considered apomicts as dead ends of evolution (Stebbins, 1950; Burt, 2000). They also demonstrate that the "twofold cost of sex" (Maynard Smith, 1978; Richards, 1997) needs

Table 2. Neophytic distribution of *Hieracium* subgenus *Pilosella* species[1].

Species	Region	Reference
H. arvicola Nägeli & Peter	E North America (New Brunswick)	Wilson & al., 2006
H. aurantiacum L.	Siberia	Tupicyna, 1997
	Russian Far East	Charkevic, 1992
	Japan (Hokkaido)	Iwatsuki & al., 1995
	SE Australia, Tasmania	CRC Weed management, 2003
	New Zealand	Webb & al., 1988
	North America	Wilson & al., 2006
	South America (e.g., Falkland Islands, Tierra del Fuego)	Bräutigam, 1992; Zuloaga & Morrone, 1999
H. bauhini Schult. (*H. praealtum* auct. p.p.)	New Zealand	Webb & al., 1988
	North America	Wilson & al., 2006
	South America (Argentina, Chile)[2]	Zuloaga & Morrone, 1999
H. brachiatum DC.	E North America	Wilson & al., 2006
H. caespitosum Dumort. (*H. pratense* Tausch)	Japan	http://www.env.go.jp/en/topic/as/041110.pdf[3]
	New Zealand	Webb & al., 1988
	North America	Wilson & al., 2006
H. derubellum Gottschl. & Schuhw. (*H. atramentarium* auct.)	E North America	Wilson & al., 2006
H. dorei Lepage	E North America	Lepage, 1967
H. flagellare Willd.	North America	Wilson & al., 2006
	South America (Tierra del Fuego)	Moore, 1983
H. floribundum Wimm. & Grab.	Siberia[4]	Tupicyna, 1997
	Russian Far East	Charkevic, 1992
	Japan (Hokkaido)	leg. K. Marhold 2004, unpubl. (herbaria PRA, GLM)
	North America	Wilson & al., 2006
H. fuscoatrum Nägeli & Peter	Siberia	Tupicyna, 1997
	E North America	Wilson & al., 2006
H. glomeratum Froel.	W North America	Wilson & al., 2006
H. lactucella Wallr. (*H. auricula* auct.)	E North America (Nova Scotia)	Wilson & al., 2006
H. pilosella L.	Tasmania	Rudman & Goninon, 2002
	New Zealand	Webb & al., 1988
	North America	Wilson & al., 2006
	South America (Tierra del Fuego; Patagonia)	Moore, 1983; Domínguez & al., 2004
H. piloselliflorum Nägeli & Peter (incl. *H. apatelium* Nägeli & Peter)	E North America	Wilson & al., 2006
H. piloselloides Vill. (*H. praealtum* auct. p.p.)	North America	Wilson & al., 2006
H. stoloniflorum Waldst. & Kit.	New Zealand	Webb & al., 1988
	E North America	Wilson & al., 2006

[1] Only neophytic occurrences outside of Europe are summarized. Some species are known to grow outside of their natural distribution areas within Europe (e.g., *H. aurantiacum*, *H. caespitosum*, and *H. bauhini*), but neophytic status is difficult to assess.

[2] Given as *H. praealtum*; unclear whether *H. bauhini* or *H. piloselloides* are referred to in the reference.

[3] "Alien species recognized to be established in Japan or found in the Japan wild" 2004.

[4] *Pilosella czerepninii* Tupitzina probably corresponds to *H. floribundum*; we are assuming neophytic status.

to be reconsidered with respect to the evolutionary strategy of these plants (Houliston & al., 2006).

COLONIZATION BEHAVIOR AND INVASIVE POTENTIAL

In their native European distribution area (Fig. 1), species of subgenus *Pilosella* are growing on unproductive habitats that are subject to different degrees of disturbance (Grime & al., 1988). Their preference for poor soils and their low competitiveness make them good colonizers of pioneer stands. These features also contribute to their enormous success as invaders in other parts of the world (North and South America, New Zealand, NE Asia). A list of adventive *Hieracium* subgen. *Pilosella* species, most of which are invasive, is given in Table 2.

Their reproductive versatility is evidently a main factor of their success. Apomictic reproduction together with clonal growth allows the establishment and rapid spread of a new population from a single seed. Wilson & al. (2006) give an example of a new invasive species in North America that probably originated from a single introduction. The presence of hybridization in this group has most likely facilitated the success of highly invasive *Pilosella* biotypes in New Zealand (Chapman & al., 2000, 2003). Thus, the presence of facultative sexuality in predominantly apomictic individuals plays an important evolutionary role in the *in situ* formation of hybrids in secondary distribution areas (e.g., *H. dorei*, *H. flagellare*, Table 2). It also facilitates rapid invasion, with both intra- and interspecific hybridization playing a role (Lepage, 1967; Houliston & Chapman, 2004; Trewick & al., 2004). The versatile mode of reproduction and extensive hybridization in *Hieracium* subgen. *Pilosella* add to other characteristics frequently observed in invasive plant species: perennial habit, (allo)polyploidy, and fixed heterozygosity (e.g., Stebbins, 1985; Soltis & Soltis, 2000). All invasive *Pilosella* species are polyploid. The only diploid that managed local establishment on other continents (Nova Scotia) is *H. lactucella* (Scoggan, 1979), but it has neither been reported as an aggressive weed nor as a parent of in situ originated hybrids. However, given the sympatry with many species, with which it hybridizes in Europe, it may eventually contribute to the production of new invasive types in Nova Scotia. The high evolutionary potential of this subgenus as shown by the continuous generation of novel successful genotypes in its native range may be amplified when released from its ancestral environment and competitors in adventive areas.

MOLECULAR PHYLOGENY, ANCIENT HYBRIDIZATIONS AND INTROGRESSIONS

Hybridization, an important feature in present-day *Pilosella*, appears to have already played a role in this group prior to the first appearance of polyploidy and apomixis. Evolutionary relationships of diploid *Pilosella* species, as revealed by

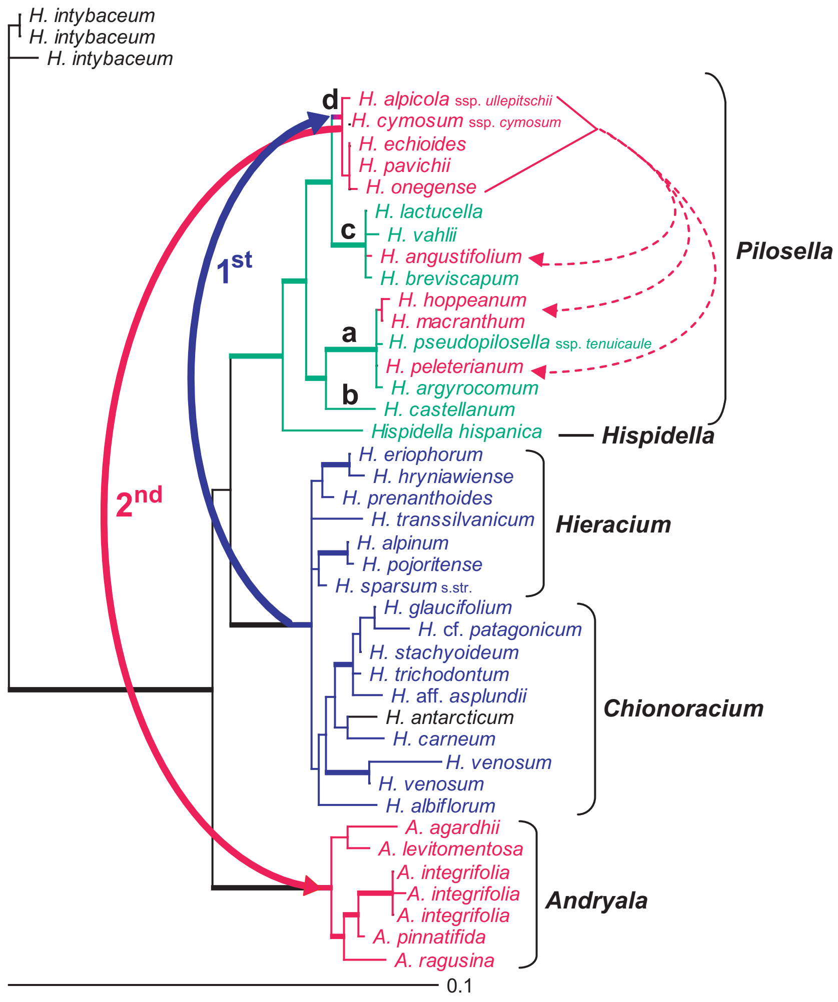

Fig. 3. Chloroplast capture events by ancient hybridization and introgression, shown on a maximum likelihood tree based on ITS sequence data of diploid species; internal branches with at least 70% bootstrap support are given in bold (modified after Fehrer & al., 2007). Major chloroplast haplotype groups are mapped on the species phylogeny (green: *Pilosella* I/*Hispidella*, red: *Pilosella* II/*Andryala*, blue: *Hieracium/Chionoracium*). Major *Pilosella* lineages are designated a–d. Chloroplast transfer from the ancestor of the *Hieracium/Chionoracium* clade into *Pilosella* lineage d (blue arrow, "1st") is postulated, where the transmitted haplotype acquired additional mutations found in all *Pilosella* II and *Andryala* species (see also Fig. 4). Subsequent transfer from the introgressed lineage to the *Andryala* progenitor is shown by a red arrow ("2nd"). More recent transfers within *Pilosella* are indicated by dashed red arrows.

nuclear ribosomal internal transcribed spacer (ITS) sequences, correspond to taxonomic expectation, morphology and other evidence, and apparently reflect speciation (Fehrer & al., 2007). According to ITS, *Pilosella* is monophyletic with the annual monotypic genus *Hispidella* Lam. as sister taxon (Fig. 3). The other two *Hieracium* subgenera, *Hieracium* and *Chionoracium* Dumort (= *Hieracium* sensu Bremer, 1994) except *H. intybaceum* All. (for discussion see Fehrer & al., 2007), constitute a second major lineage, and the closely related genus *Andryala* L. represents a third monophyletic clade.

Within *Pilosella*, there are four major lineages (Fig. 3, a–d). One of these ("a") comprises a clade of section *Pilosellina*, species characterized by an unbranched stem and a single large capitulum (*H. argyrocomum* Zahn and allied taxa), with *H. castellanum* Arv.-Touv. ("b") as sister taxon. The other major branch comprises a clade consisting of species of the closely related sections *Auriculina* and *Alpicolina* (*H. breviscapum* DC. and allies) ("c"), while the second lineage contains exclusively species with chloroplast haplotype group II (*H. echioides* and allies) ("d").

Trees based on chloroplast DNA markers (*trnT-trnL*, *matK*) are incongruent with all other evidence in two major aspects (Fehrer & al., 2007). As found for selected species in a restricted area (Fehrer & al., 2005; see also Fig. 2), cpDNA split all *Pilosella* basic species into very distinct haplotype groups I and II, with haplotypes of subgenera *Hieracium* and *Chionoracium* derived from intermediate extinct ones (Fig. 4). Additionally, a variant of haplotype group I is also present in *Hispidella*, and *Pilosella* haplotype group II and its derivatives also occur in all studied species of genus *Andryala*. These major discrepancies revealed the occurrence of two ancient hybridization events by detailed analysis of *trnT-trnL* character state evolution along the species phylogeny (Fehrer & al., 2007). A first hybridization occurred between the *Hieracium*/*Chionoracium* ancestor and *Pilosella* lineage d, involving an extinct chloroplast haplotype (see also Fig. 4). A subsequent hybridization occurred between this introgressed lineage and the *Andryala* ancestor (Fig. 3). This is thought to be a later event as features of the *trnT-trnL* cpDNA region suggest that *Pilosella* II haplotype group divergence is more recent (Fehrer & al., 2007), and *Andryala* cpDNA is derived from the most widespread ancestral *Pilosella* II type (Fig. 4).

Despite extensive hybridization and the apparent ease with which the plastid genome can be transferred among *Pilosella* species (Fig. 2), basic species and stabilized intermediates show a surprisingly high intraspecific uniformity of major chloroplast haplotypes. In basic species, this may partly reveal underlying phylogenetic pattern, and partly be due to restricted gene flow among diploids or towards diploids and tetraploids. In stabilized intermediates, the most probable reason is selection for types from a particular direction of hybridization that eventually leads to unidirectional transmission of cpDNA (see above). Nevertheless, there are also examples of cpDNA major haplotype group exchange among diploid basic species (Fig. 3, dashed arrows). The degree of differentiation of cpDNA haplotypes can be used to distinguish between original and "captured" chloroplast genomes (Soltis & Kuzoff, 1995). This allowed us to infer introgression of haplotype II (red) into

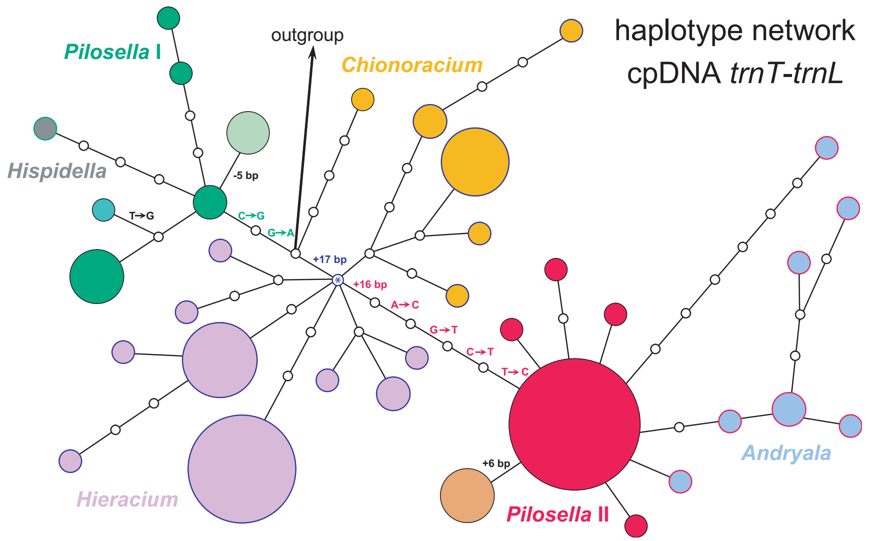

Fig. 4. Haplotype network of chloroplast *trnT-trnL* sequences, comprising *Hispidella hispanica*, all basic and several intermediate species of subgenus *Pilosella*, a selection of species of genus *Andryala* plus of subgenera *Hieracium* and *Chionoracium*. Haplotypes are indicated as colored circles, sizes correspond to the number of species sharing that particular haplotype. Mutations characterizing the *Pilosella* I and II haplotype groups are indicated in green and red, respectively. A 17 bp insert is present in most *Hieracium/Chionoracium*; together with a 16 bp insert, it distinguishes the major *Pilosella* haplotype groups by RFLP. Further mutations used for haplotype screening are indicated in black; colors of haplotype subtypes are the same as in Fig. 2. Extinct haplotypes are shown as small open circles; the extinct haplotype introgressing *Pilosella* is indicated in blue, marked by an asterisk.

species with original haplotype I (green) in all 3(–4) cases indicated in Fig. 3 (Fehrer & al., 2007). The same kind of introgression was recently detected in two individuals of *H. lactucella* from the Šumava Mts./Böhmerwald (F. Krahulec, J. Fehrer & al., unpubl.), of which only haplotype group I cpDNA was known previously. This suggests that more complex patterns may be found within each species, and that sampling of many individuals from across the species distribution areas will be necessary to obtain a more representative picture. Nevertheless, haplotype distribution among species appears too structured to obscure the more general patterns.

BIOGEOGRAPHY AND RELATIVE TIME SCALES

Unlike other plant groups (Wolf & al., 1997; Gutiérrez Larena & al., 2002; Trewick & al., 2002), *Pilosella* chloroplast DNA hardly shows any geographical pattern in our central European study areas. However, at a larger scale including all

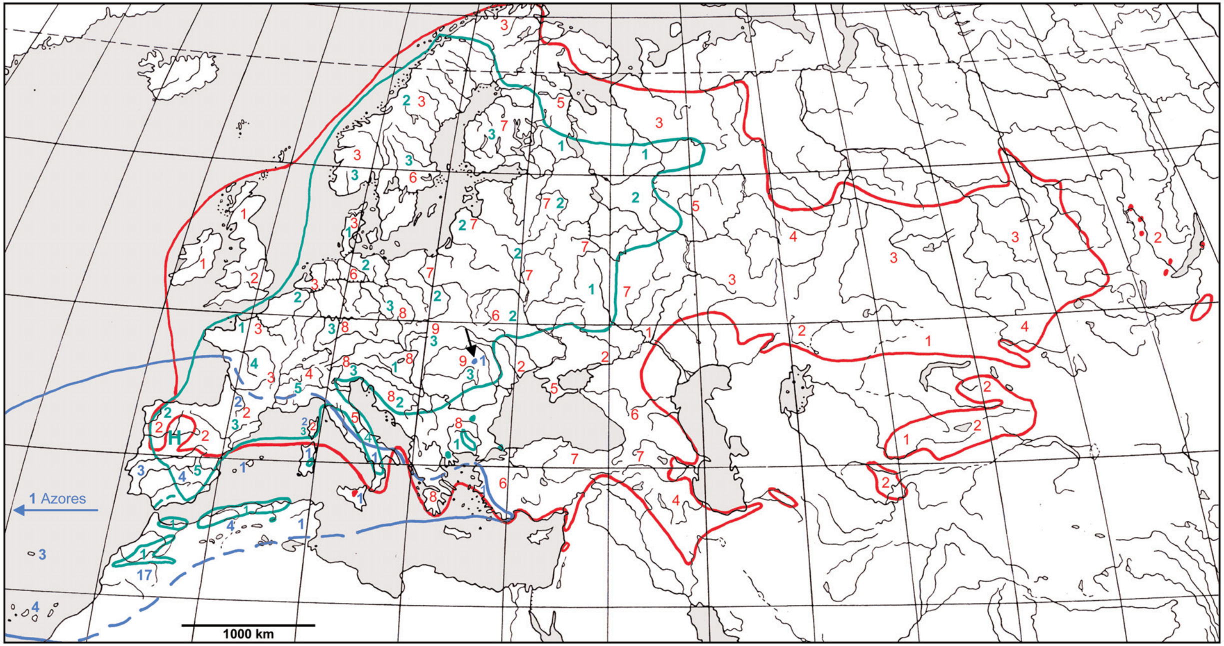

Fig. 5. Distribution maps of *Pilosella*, *Andryala*, and *Hispidella*. Dashed lines indicate uncertainty in range limits; regional species numbers are given. *Pilosella* species (basic species only) are divided according to their chloroplast haplotype group (green: *Pilosella* I, red: *Pilosella* II). In addition to the diploids (Fig. 3), polyploid basic species are included: *H. aurantiacum*, *H. saussureoides* (Arv.-Touv.) St.-Lag., and *H. piloselloides* ssp. *praealtum* (Vill.) Zahn (*Pilosella* I); *H. caespitosum*, *H. bauhini*, *H. caucasicum* Nägeli & Peter, *H. pilosella*, *H. procerum* Fr., *H. verruculatum* LINK, and *H. piloselloides* ssp. *obscurum* (Reichb.) Zahn (*Pilosella* II). Areas are restricted to native range, ignoring neophytic occurrences (see Table 2). *Hispidella hispanica* Lam. is represented by the green "H" on the Iberian peninsula. *Andryala* is shown in blue; one species occurs on the Azores outside the area of the map, another (*A. levitomentosa*, indicated by a black arrow) in the Romanian Carpathians.

Pilosella basic species, a discernable differentiation appears. Figure 5 shows the distribution areas of *Pilosella* species according to their respective chloroplast haplotype groups. Species numbers show that the majority of *Pilosella* I species, especially endemics, and its sister *Hispidella* (indicated by a green "H" within the *Pilosella* I range) occur in SW Europe, whereas most *Pilosella* II species, especially diploids, occur in central and eastern Europe. This pattern seems robust even if species with presumably captured *Pilosella* II chloroplast genome are transferred to *Pilosella* I. In this case, the western border of *Pilosella* II type species (red) shifts further eastwards, excluding Portugal, Spain, SW-France, Britain and Ireland; and the *Pilosella* I type extends further south-east into the Balkan Peninsula, Anatolia and Caucasus. Thus, cpDNA reveals an ecogeographic aspect that is neither obvious from morphology nor from the nuclear ITS data. *Pilosella* I type species do not reach the continental woodland and steppe regions of Russia and central Asia whereas *Pilosella* II type species have a much broader ecological amplitude as reflected by their larger distribution area. It may be possible to speculate that the apparent evolutionary success of *Pilosella* II type species, comprising the majority of present-day basic species (albeit fewer diploids than *Pilosella* I), is at least partly due to the inferred introgression via wide hybridization with a divergent lineage.

If we consider the evidence from nuclear ITS sequences, chloroplast haplotyping, geographic distribution, and inferred intergeneric hybridizations, the following hypothetical scenario can be sketched: The earliest events, i.e., the split of the three main lineages (*Pilosella*/*Hispidella*, *Hieracium*/*Chionoracium*, and *Andryala*) remain rather obscure. For our purpose, we only need to postulate that their distribution areas showed sufficient overlap when the first introgression occurred (which is probable). The presence of an insert in the chloroplast *trnT-L* region, characteristic for all but one species of *Hieracium*/*Chionoracium* (Fig. 3, blue color; Fig. 4, "+17 bp"), which also occurs in the introgressed *Pilosella* lineage, indicates that this event predates the radiation of *Hieracium* and *Chionoracium*. Subgenus *Hieracium* is holarctic, includes both New and Old World taxa, and comprises primarily diplosporous apomicts with very few sexuals (Bräutigam, 1992; Chrtek & al., 2004). *Chionoracium* is a New World subgenus consisting of about 150 obligatory sexual diploid species (Beaman, 1990; and references therein). *Chionoracium* habits comprise the spectrum of *Pilosella* and *Hieracium* species, which may imply introgression by a representative of the later *Chionoracium* group. Dating of the first introgression is not feasible. The earliest Asteraceae macrofossils are dated to the Oligocene/Miocene border (Wagenitz, 1976), and pollen uniformity among Cichorieae does not allow accurate determination of genera (Tomb, 1975). However, a tentative temporal assignment can be attempted from the *Pilosella* and *Chionoracium* geographic distribution by taking into account that the introgression event predated speciation and dispersal of *Chionoracium* in the New World. Two routes between Eurasia and North America are possible; one via the Bering land bridge, which has played an important role for migrations of many genera (Wen, 1999), and a second via the North Atlantic. We consider the eastern route as unlikely, because traces of relict taxa in Asia are entirely missing. Asteraceae diaspores are well-adapted for long-distance dispersal by wind or birds (Jäger, 1992). Colonization of Greenland by *H. alpinum* L. (subgen. *Hieracium*) shows that this distance can be bridged (Bräutigam, 1992). While in the present floristic migrations of temperate species are unlikely to occur between northern Europe and America, climatic conditions up until the end of the Tertiary could have allowed such transfers. Therefore, the first interlineage chloroplast transfer can probably be narrowed down to before the end of the Tertiary. While climatic conditions during interglacials may have been suitable for migrations to North America, the time scale for *Chionoracium* speciation with its current center of diversity in South America might be too short in this case. The geographical region in Europe where the first introgression has occurred can not be determined from the data. What we can deduce is that it was followed by chloroplast haplotype evolution leading to an extant ancestral *Pilosella* II type (large red circle in Fig. 4) of which all intermediate haplotypes are now extinct. It is possible that the development and/or maintenance of the *Pilosella* II haplotype and subsequent speciation has taken place under allopatric conditions with *Pilosella* I type species. Given the lack of reproductive isolation between them, geographic and/or ecological barriers may have kept them apart during this period of their evolutionary history. The current *Pilosella* I and II species distributions (Fig. 5) support the idea of such a separation. While the *Pilosella* II haplotype may have evolved in grasslands of the periglacial steppe region and in the south-eastern (e.g., Pannonian-Balkans and Caucasian-Anatolian) glacial refugia, it is more likely that *Pilosella* I type species survived in Iberian refugia due to the presence of four endemic species plus *Hispidella*.

As all *Andryala* species studied show derivatives of the *Pilosella* II haplotype (Fig. 4), the second hybridization resulting in chloroplast capture must have taken place after the ancestral *Pilosella* II haplotype arose. Present-day distribution areas of *Pilosella* II and *Andryala* show little overlap, but the relictual occurrence of *Andryala levitomentosa* (Nyár.) P.D. Sell (Fig. 5, black arrow) suggests a broader distribution of the genus in former times. Besides the more important SE glacial refugia (Taberlet & al., 1998; Thompson, 1998), smaller refuges also occurred in some parts of the eastern Carpathians (Zhang & al., 2001; Petit & al., 2003). This also holds for the Mţii. Bistriţei, where the last remaining population of *A. levitomentosa* was found. Subsequent migration from east Carpathian and Balkan refuges to the west has been reported for many animal and plant species (Hewitt, 1996; Taberlet & al., 1998; Petit & al., 2003). The onset of *Andryala* speciation occurred after the second introgression (Fig. 3) which in turn occurred after presumed separation of *Pilosella* I and II type species, possibly due to glacial conditions. As *A. agardhii* DC. (confined to the Sierra Nevada, Spain) and *A. levitomentosa* are glacial relict species, the majority of *Andryala* speciation should have occurred before the end of the Pleistocene.

Probably after the Pleistocene, the group II haplotype introgressed into several *Pilosella* I species (Fig. 3, dashed arrows) after the two haplotype groups came into secondary contact. The lack of geographic structuring that we initially observed

(Fehrer & al., 2005) can be easily explained by our study areas being situated in the middle of the present-day overlap zone of *Pilosella* I and *Pilosella* II distributions.

Figure 5 presents the distribution of species and their chloroplast haplotype groups. The data are either based on one or a few individuals per species or on many from a small geographic area in central Europe. Although this is likely a gross simplification of the actual distributions, the relative uniformity of cpDNA haplotypes found in basic species in the overlap zone seems to justify this extrapolation to a certain extent and can be viewed as a testable hypothesis that awaits further data. Our expectation is that a broader analysis of chloroplast haplotypes across regions and species will demonstrate a more marked east-west differentiation than the current data.

ORIGIN OF POLYPLOIDY AND APOMIXIS IN *PILOSELLA*

Molecular phylogenetics. — Our analysis of nuclear ITS sequences concentrated on diploid species to provide a frame of basal relationships (Fig. 3). Early divergence of *Pilosella* lineages (clades a–d) is better resolved than relationships within *Hieracium*/ *Chionoracium* and *Andryala*, but most of their speciation is very recent as indicated by their low genetic variability and the largely unresolved topology of clades a, c, and d (Fig. 3). As diploids are supposed to be ancestral to polyploids, the origin of apomixis is even more recent than these most recent speciation events. The subgenera *Pilosella* and *Hieracium* are phylogenetically embedded in the wholly diploid sexual (sub)genera (*Chionoracium*, *Andryala*, *Hispidella*). Both have substantially more polyploids and apomicts than sexual diploids. Additionally, different forms of apomixis (apospory in *Pilosella* versus diplospory in *Hieracium*) have developed in these very closely related taxa. Repeated switches to the apomictic reproductive mode are also known in *Erigeron* (Noyes, 2000). It has been suggested that certain groups might have a predisposition to the apomictic phenotype (Grimanelli & al., 2001; Quarin & al., 2001; Sharbel & Mitchell-Olds, 2001).

Developmental genetics. — Little is known about the molecular events that regulate apomictic development (Rodrigues & Koltunow, 2005). In *Pilosella*, apomixis can be understood as a deregulation and modification of sexual developmental programs in time and space that lead to cell fate changes and the omission of critical steps in the sexual process. Comparative analyses of gene expression patterns show that sexual and apomictic pathways are closely interrelated and share regulatory regimes at the molecular level (Tucker & al., 2003). Furthermore, epigenetic mechanisms (e.g., DNA methylation patterns) might lead to complete maternal control of reproductive development (Rodrigues & Koltunow, 2005). Apomixis in *Pilosella* might therefore be understood as an optional regulatory program that has been initially switched on under particular conditions.

Ecogeography. — Compatible with our biogeographical model (see above), the apomictic mode of reproduction in *Pilosella* was possibly a response to the virgin habitat revealed by the retreat of Pleistocene glaciers and periglacial conditions. Pleistocene scenarios for the origin of polyploidy and apomixis have also been suggested in other plant groups like *Taraxacum* sect. *Palustria* (Kirschner & Štepánek, 1998) and European *Rubus* (Weber, 1996). Establishment of the successful natural allopolyploid *Arabidopsis suecica* O.E. Schulz might have been favored by marked environmental changes that are associated with glaciation (Sall & al., 2003). Frequent polyploid formation, rapid adaptation and efficient invasion of newly deglaciated areas are known from arctic flora (Brochmann & al., 2004), including examples of polyploids formed after the last glaciation and throughout the Quaternary. Environmental factors such as photoperiod and temperature are able to influence apomictic reproduction (Asker & Jerling, 1992; Koltunow, 1993; Richards, 1997) as well as the production of unreduced gametes, a prerequisite for the formation of polyploids (reviewed in Ramsey & Schemske, 1998).

Mechanistic aspect. — Early studies suggested that apomixis results from hybridization events, because apomicts were found to be polyploid and highly heterozygous (Ernst, 1918). Almost a century later, while polyploidization is considered as an important evolutionary force (Comai, 2005), processes and mechanisms responsible for the origin of polyploidy (and apomixis) are still not entirely understood. In *Pilosella*, the ability of diploids to produce unreduced gametes has been shown only once, at a frequency of 0.5% (Gadella, 1988). Despite a failure to detect unreduced gametes in a large-scale crossing experiment with sexual cytotypes of *H. echioides*, the absence of exclusively tetraploid populations suggested the origin of tetraploids in diploid populations (Peckert & Chrtek, 2006). Our studies of natural populations, comprising mixed sexual and apomictic taxa and cytotypes, imply the potential production of unreduced diploid gametes in *H. lactucella*, resulting in *H. floribundum*, *H. schultesii*, *H. blyttianum* Fr., and *H. tubulascens* (Fig. 2). Even if these were produced in a more than one-step process via triploids, which is possible as they can be pollen fertile and rarely also seed fertile (A. Krahulcová, unpubl.), initial production of unreduced diploid gametes needs to be postulated.

The origin of apomixis by hybridization of related species (Ernst, 1918), resulting in polyploidization and altered gene regulatory and expression patterns, is still thought to hold for many gametophytic apomicts (Carman, 1997, 2001). In addition, particular environmental conditions and genetic predisposition are probably important factors. More research is needed to gain a better understanding of the underlying processes and mechanisms.

OUTLOOK

This chapter summarizes our present state of knowledge about evolutionary aspects in *Hieracium* subgenus *Pilosella*. While much has been achieved, we are only beginning to untangle the complex patterns and processes involved in their speciation. Based on our current understanding, there are a number of topics worthy of further study:

(1) An inventory of diploid species will be necessary. It is highly probable that within a large number of taxa described earlier, some unknown diploids of evolutionary significance are present, e.g., in the *H. bauhini* group. Special attention should be paid to Mediterranean and Sub-Mediterranean areas.

(2) Clarifying diploid species relationships, using genetic markers with greater resolution and including several accessions from across their respective distribution areas, is desirable to provide a solid framework for dealing with polyploids.

(3) A better overview of clonal structure, morpho- and cytotypes in key regions (evolutionary hotspots) is needed to elucidate the role of ecological and environmental factors on diversification and to untangle taxonomically intricate species complexes.

(4) Detailed studies of the distribution of chloroplast haplotypes across the whole *Pilosella* range is needed in order to establish a high-resolution phylogeography.

(5) A combination of molecular (e.g., co-dominant markers, single-copy genes) and karyological (e.g., GISH) techniques is needed to trace differential contributions of diploid parental genomes to polyploid basic species and hybrids and to elucidate their origin.

(6) Further case studies on species interactions and breeding systems in natural (source and neophytic) populations can help to shed light on the influence of genotype, reproductive mode, genetic variability, and environment on the production of novel, and the establishment of stabilized, biotypes. This will help elucidate which factors are driving selection and which may have application in bio-control programs.

(7) The genetic basis of apospory should be studied in a broader spectrum of species to determine if apomixis evolved repeatedly in different lineages, or if the same mechanism is found throughout *Pilosella*. Comparison with the subgenus *Hieracium* (apomixis of the diplosporous type) may provide insights into the potential predisposition for the evolution of apomixis in both groups, potentially detecting similarities in regulatory pathways and gene expression for apomixis. This would also tell us whether the major differences in the type of apomixis in the two subgenera could lie in inheritable differential expression of the trait with respect to the embryo-forming cell type.

Recent diploid species diversification within most *Pilosella* lineages, reticulate evolution, and the presence of facultative apomixis and vegetative propagation present obvious difficulties for taxonomic treatment and species concepts. A better understanding of the evolutionary processes underlying the variation of forms will be needed before a theoretically sound and yet practicable species concept can be formulated which is applicable to all *Pilosella* species, independent of geographic region, ploidy, mode of reproduction, and population structure. To attempt solving this problem is one of the long-term goals of our interdisciplinary collaboration. (http://www.ibot.cas.cz/*hieracium*/studygroup/).

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