

## Enriching Ploidy Level Diversity: the Role of Apomictic and Sexual Biotypes of *Hieracium* subgen. *Pilosella* (Asteraceae) that Coexist in Polyploid Populations

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**Abstract** The capacity to generate variation in ploidy and reproductive mode was compared in facultatively apomictic versus sexual maternal plants that coexist in two model populations. The population structure was studied in polyploid hybrid swarms comprised of *Hieracium pilosella* (usually sexual, less commonly apomictic), *H. bauhini* (apomictic), and their hybrids (sexual, apomictic, or sterile). Relationships among established biotypes were proposed on the basis of their DNA ploidy level/chromosome number, reproductive mode and morphology. Isozyme phenotypes and chloroplast DNA haplotypes were assayed in the population that was richer in hybrids. The reproductive origin of seed progeny was identified in both sexual and apomictic mothers, using alternative methods: the karyological, morphological and reproductive characters of the cultivated progeny were compared with those of respective mothers, or flow cytometric seed screening was used. In both populations, the progeny of sexual mothers mainly retained a rather narrow range of ploidy level/chromosome number, while the progeny of facultatively apomictic mothers was more variable. The high-polyploid hybrids, which had arisen from the fertilization of unreduced egg cells of apomicts, mainly produced aberrant non-maternal progeny (either sexually and/or via haploid parthenogenesis). Apparently, such versatile reproduction resulted in genomic instability of the recently formed high-polyploid

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hybrids. While the progeny produced by both true apomictic and sexual mothers mostly maintained the maternal reproductive mode, the progeny of those ‘versatile’ mothers was mainly sexual. Herein, we argue that polyploid facultative apomicts can considerably increase population diversity.

**Keywords** Facultative apomixis · Genome instability · Haploid parthenogenesis · Hybrid swarms · Residual sexuality · Unreduced gametes

## Introduction

Sexual reproduction connected with outcrossing enables a free recombination of parental genomes that generates variation (Richards 1997). Conversely, apomixis, defined in flowering plants as the asexual formation of seed, avoids the processes of meiosis and fertilization and thus replicates the maternal genome in the progeny (Koltunow 1993; Bicknell and Koltunow 2004; van Dijk and Vijverberg 2005). Facultative apomixis, deriving benefits from both reproductive systems, combines the usually dominant asexual seed formation with occasional (residual) sexuality. Thus, aberrant (i.e., non-maternal) progeny are produced beside the true apomictic (mother-replicating) progeny (Asker and Jerling 1992; Czapik 1994; Savidan et al. 2001). The advantages of reproducing by both apomixis (fixation and spread of adapted genotypes) and residual sexuality (the increase of progeny variation by recombination and segregation) often help facultatively apomictic biotypes invade new habitats (Asker and Jerling 1992; Richards 2003; Wilson et al. 2006). Differences in *i*) the timing of development between sexual and apomictic embryo sacs and *ii*) the associated timing of pollination are among the crucial mechanisms that determine the proportion of sexual/apomictic reproduction in facultative apomicts (Savidan et al. 2001; Bicknell and Koltunow 2004). However, the results of some pollination experiments do not confirm the essential relevancy of this effect (Krahulcová et al. 2004). The actual expression of apomixis can be modified in some species by the environment and/or influenced by the respective fertilizing pollen donor (Nogler 1984; Asker and Jerling 1992; Bengtsson and Ceplitis 2000).

Many apomicts retain their sexual function as pollen donors, producing viable reduced pollen much like the sexuals (Richards 2003; Bicknell and Koltunow 2004; Whitton et al. 2008). Sexual reproduction in facultatively apomictic maternal plants can generate progeny resulting from the fusion of reduced ( $n$ ) and/or unreduced ( $2n$ ) gametes (Asker and Jerling 1992; Bicknell et al. 2003; Krahulcová et al. 2004). Thus, four different types of sexually derived progeny can arise:  $n+n$ ,  $n+2n$ ,  $2n+n$  and  $2n+2n$ , where the first term represents the female gamete (Harlan and de Wet 1975). Due to the participation of unreduced gametes in matings, some of the individual progeny may have ploidy that exceeds that of their parents. Moreover, a fraction of the progeny of many facultative apomicts, which are almost exclusively polyploid, can be formed by haploid parthenogenesis, i.e., by a parthenogenetic development of a reduced egg cell ( $n+0$ , polyhaploid progeny), in addition to the formation of the usually dominant true apomictic progeny ( $2n+0$ ) and sexual

progeny (Asker and Jerling 1992). Haploid parthenogenesis is the pathway that generates variation: the progeny, formed via meiotic recombination have only one half of the maternal genome. In spite of the production of such aberrant progeny, haploid parthenogenesis is sometimes included in the broadly defined apomixis, because it avoids fertilization (for citations and discussion see Krahulcová et al. 2004; Fehrer et al. 2007b). We also accept this classification in the present paper.

The actual mode of progeny origin in facultatively apomictic mothers can be revealed at different developmental stages of reproduction, from the embryonic stage (by Flow Cytometric Seed Screen, FCSS: Matzk et al. 2000) to mature progeny. Screening in mature progeny is based on the combination of distinct parental morphological characters and different ploidy levels (e.g., Houlston and Chapman 2004; Krahulcová et al. 2004). Interbreeding of sexual and facultatively apomictic biotypes that have different ploidy and coexist in the field provides a system suitable for evaluating their role in progeny diversification, especially with respect to ploidy level and reproductive mode.

The taxonomic group of *Hieracium* subgen. *Pilosella* (Hill) Gray fulfills the criteria for such studies. Overlapping effects of polyploidy (the basic chromosome number  $x=9$ ), hybridization, facultative apomixis and clonal growth cause variation in natural populations of this polyploid agamic complex (Krahulcová et al. 2000; Fehrer et al. 2007b). Predominantly outcrossing species are considered as self-incompatible (Gadella 1984), but to some extent foreign pollen can induce autogamy (Krahulcová et al. 1999; Bicknell et al. 2003). In apomictic biotypes (always polyploid in the field), progeny can be generated independently of fertilization due to autonomous apospory (Koltunow et al. 1998). Frequent spontaneous hybridization (e.g., Gadella 1987; Krahulec et al. 2004; Morgan-Richards et al. 2004) results in the common occurrence of recent hybrids, beside the already stabilized hybridogenous taxa. The barriers for interspecific and inter-cytotype crosses are rather weak and the ecological demands of many of these species are similar. Thus, the co-occurring sexual and apomictic biotypes commonly form hybrid swarms under conditions suitable for seed reproduction. Although the apomicts usually produce fertile pollen (Krahulcová et al. 2000; Rotreklová 2008) with which they can take part in mating, they also serve as seed parents in the formation of numerous natural hybrids (Krahulec et al. 2004, 2008; Fehrer et al. 2005, 2007b). Thus, their residual sexuality appears to considerably contribute to the formation of new biotypes in hybridizing populations. For example, in a secondary distribution area, the formation of new *Hieracium* subgen. *Pilosella* hybrids has been recorded (Lepage 1967; Houlston and Chapman 2004; Morgan-Richards et al. 2004; Trewick et al. 2004). The presence of hybridization, even in apomicts, most likely facilitated the success of a number of highly invasive biotypes (Chapman et al. 2003; Wilson et al. 2006). Nevertheless, the recent hybridization in the secondary area may not be a necessary condition for the successful invasion of apomictic *Hieracium* subgen. *Pilosella* species (e.g., *Hieracium aurantiacum* L. in USA – Loomis 2007).

The present study in *Hieracium* subgen. *Pilosella* examines the role of sexual versus facultatively apomictic maternal biotypes (both polyploid) in the field, especially their contribution to ploidy and reproductive mode diversity in mixed populations. Two hybrid swarms in which both homoploid and heteroploid

spontaneous crosses could occur were chosen as a model system. Both populations of coexisting facultatively apomictic and sexual biotypes were comprised of two morphologically distinct parental species, *H. pilosella* L. and *H. bauhini* Besser, and their hybrids. Morphological characters distinguish the parental species, especially the height and branching of the stem, the absence/presence of stellate hairs on the leaf underside and the size of the inflorescences (Chrtek 2004). The composition of the populations was compared to the progeny produced by the respective maternal plants. To supplement these findings from the field, garden pollination experiments were carried out to simulate the possibilities of progeny formation.

## Material and Methods

### Study Sites

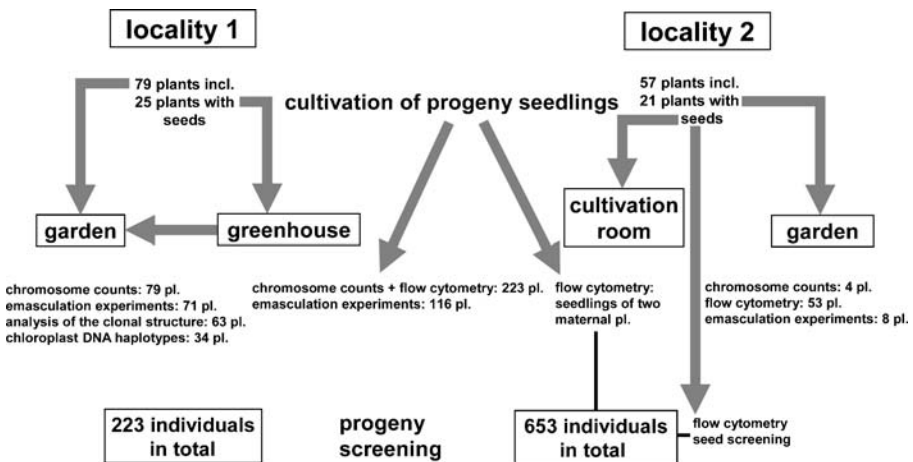
The first hybrid swarm studied occurred in Prague, Czech Republic above the railway line close to the railway station Praha-Vysočany (locality 1). The approximately 500 m long zone was delimited by the coordinates 50°06'38" N, 14°29'00" E and 50°06'44" N, 14°29'22" E; the altitude is about 210 m. The habitat was the lower part of a 30–40° steep slope with southern orientation, sparsely overgrown by trees and shrubs. The substrate was a fine gravel of moderately acidic and soft schists. Occasional slides of the steep slope provided sites stripped of dense vegetation where the establishment of seedlings might be facilitated. Among several microlocalities there, the stand of *Hieracium* subgen. *Pilosella* plants formed a dominant vegetation cover. This population, rich in species of *H. pilosella*, *H. bauhini* and in various morphotypes of their hybrids, was discovered in 1999 (J. Chrtek, pers. comm.). Although the actual age of this hybrid swarm is unknown, the habitat in its present form probably originated as early as 25 years ago, when the railway line at the foot of the slope was reconstructed.

The second hybrid swarm studied was found at the northwestern periphery of the city of Brno, Czech Republic, on Kamenný kopec hill (locality 2). The plants were sampled in two different habitats situated ca. 1 km apart. The first one was on a southwest-facing slope above a road close to the crossroads of the Chironova and Nad Pisárkami streets (49°11'02" N, 16°32'38" E) at an altitude of 350 m. This granite rocky slope was covered with poor vegetation that enabled the establishment of seedlings. Although large populations of *H. bauhini* and *H. pilosella* had been observed at this locality since 1999, the first hybrid morphotypes were found as late as 2004. The second habitat was situated along Travní Street (49°10'47" N, 16°33'18" E) at an altitude of 360 m. The hawkweeds grew there in a secondary dry grassland on shallow soil on siliceous bedrock (granite and diorite). Although the parental taxa were collected from this site during the last century (specimens in herbarium BRNU) the first hybrid morphotypes were found in 1998. Because the vegetation cover there was dense, open microhabitats suitable for the establishment of seedlings were only scattered. At both collection sites of locality 2, the parental morphotypes dominated, while the hybrids were rare.

**Plant Sampling and Progeny Cultivation**

Plants from locality 1 were sampled in late spring (years 2000, 2001, 2002 and 2005) when most of the *Hieracium* subgen. *Pilosella* biotypes were still in bloom and the seed progeny could also be acquired from the fruiting plants. In total, 79 plants were chosen for outdoor garden cultivation to cover the whole morphological variation observed at the locality (for sampling design see Fig. 1). The spectrum of morphotypes collected included parental species, *H. pilosella* and *H. bauhini*, as well as hybrid morphotypes (*H. brachiatum* DC. = *H. bauhini* < *H. pilosella*, *H. leptophyton* Nägeli & Peter = *H. bauhini* > *H. pilosella*). The hybrid formulae indicating the position of hybrids between the parental species follow Zahn (1922–30), because they are commonly used for the floras of continental Europe. The sampled hybrids differed in proportions of parental morphological characters and also included morphotypes more or less intermediate between the parents. From the total set of 79 plants, 25 were fruiting, comprising representative morphotypes found at this locality. Seeds (achenes) were collected from these particular maternal plants either in the field or at latest 10 days after their transfer to the garden, to ensure that the labeled maturing inflorescences had been pollinated at the locality. After harvest in summer, the seeds were sown in pots with sterilized garden soil, and germinated seedlings were cultivated under greenhouse conditions (Fig. 1). When the seedlings were of sufficient size (usually next spring), their leaf tissue was analyzed using flow cytometry and/or their root-tips were collected for conventional chromosome counting. A total of 223 progeny individuals were analyzed. Afterwards, the mature progeny were transferred to outdoor beds for further cultivation in the experimental garden of the Institute of Botany, Průhonice, Czech Republic.

In total, 57 plants were sampled at locality 2 (years 2002, 2004, 2005 and 2006). Among them were plants morphologically representing both parental species, *H. pilosella* and *H. bauhini*, the hybrid morphotype *H. brachiatum* and one plant with a transitional morphotype between *H. brachiatum* and *H. pilosella* (Fig. 1). Plants



**Fig. 1** Experimental design for sampling and processing plant material of *Hieracium* subgen. *Pilosella* collected in the field (pl. – plant individual)

from the field were transferred to the experimental garden of the Faculty of Education, Masaryk University, Brno-Kejby and to the experimental plot of the Department of Botany and Zoology, Brno-Řečkovice, both the Czech Republic. From the whole plant set the mature seeds of 21 fruiting plants were available and collected at locality 2 in late spring (Fig. 1). A portion of the seeds of two sexual maternal plants was sown immediately. The seedlings were grown in a cultivation room under a 16 h light/8 h dark light regime and were analyzed using flow cytometry about three months after seed sowing (Fig. 1). All remaining seeds from the field were analyzed using the FCSS method (Fig. 1). In total 653 progeny individuals were analyzed.

For the purpose of this study, all plants sampled at both localities whose morphology combined the characters of the two parental species were classified as hybrids without any detailed taxonomic evaluation (Table 1). Voucher specimens of mature plants from the field and of progeny cultivated from seeds were deposited in the herbaria PRA (material from locality 1) and BRNU (material from locality 2).

### ***Chromosome Counts***

Chromosomes of all 79 plants from locality 1 and of four plants from locality 2 were counted in root-tip meristems stained by lacto-propionic orcein as described previously (Krahulcová and Krahulec 1999, for demonstration of experimental design see Fig. 1). The chromosome numbers were also determined in each of cultivated progeny plants originating from sexual mothers from locality 1 (for the detection of the reproductive mode see below). With respect to the occurrence of the odd-ploid putative parents at this locality, aneuploids were supposed to occur rather frequently among sexually derived progenies. The ploidy level of cultivated progeny produced by facultatively apomictic mothers from locality 1 was at first determined using flow cytometry (see below). To confirm the accuracy of this cytometric progeny screening, the chromosomes were counted in representatives of those progeny classes, the ploidy of which deviated from that of their apomictic maternal parent. In addition, the chromosome numbers were determined in all aberrant progeny individuals produced by apomictic heptaploid plants in the field.

### ***Flow Cytometry***

The DNA ploidy level (Suda et al. 2006) was assessed in 53 plants sampled at locality 2, in the cultivated progeny of two sexual plants from locality 2, and in all cultivated progeny of the facultatively apomictic plants from locality 1 (Fig. 1). The variation in DNA ploidy level/chromosome number was also evaluated in selected seed progeny obtained from pollination experiments (see below). In analyzing the aberrant progeny of apomictic mothers, the DNA ploidy ratio between the maternal plant (standard) and a progeny individual with a proven chromosome number was used as a clue for cytometric analyses of other half-siblings produced by the respective mothers. For the flow cytometry, the DAPI staining method (Otto 1990) was used with two cytometers, Partec PA I and II (for details of the procedure see Krahulcová et al. 2004).

**Table 1** Population structure in the two studied hybrid swarms of *Hieracium* subgen. *Pilosella*

Locality 1			Locality 2
Plants assigned to	Ploidy/RS (nr. of plants)	Number of genotypes (nr. of plants analyzed)	Ploidy/RS (nr. of plants)
<i>H. pilosella</i>	4x/sex (17)	14 (16)	
	5x/sex (3)	2 (3)	
<i>H. bauhini</i>			6x/sex (15)
			6x/apo (7)
			6x/ster (5)
	4x/apo (3)	2 (3)	
	5x/apo (6)	4 (7)	5x/apo (15)
	5x/? (1 n.s.)		
	6x/? (3 n.s.)		6x/apo (4)
	7x/var* (1)	1	
Hybrids	4x/sex (4)	3 (4)	4x/sex (1)
	4x/apo (3)	3 (3)	
	4x/? (1 n.s.)		
	5x/sex (1)	1	5x/ster (5)
	5x/apo (5)	2 (5)	
	6x/sex (2)	1	6x/apo (1)
			6x/ster (1)
	7x/var* (8)	9 (13)	
	7x/ apo** (4)		
	7x/? (2 n.s.)		
	8x/ var* (3)	1 (3)	8x/var* (2)
			8x/apo** (1)
	Aneuploids:	11 (12)	
2n=37, 38, 39, 48, 49, 58/sex (10)			
2n=40/apo (1)			
2n=50/? (1 n.s.)			
Total nr. of plants: 79	Total nr. of genotypes: 54 out of 72 plants	Total nr. of plants: 57	

## Abbreviations and explanatory notes:

RS – reproductive system; apo – apomictic; sex – sexual; var – variable; ster – seed-sterile; n.s. – reproductive system not studied; \* – facultatively apomictic plants producing prominent aberrant progeny, i.e., plenty of polyhaploids after emasculation and/or polyhaploids and  $n+n$  hybrids after open pollination; \*\* – facultatively apomictic plants producing progeny parthenogenetically (viable seed-set after emasculation), while the path leading to progeny origin was not studied

Most seed progeny sampled at locality 2 had already been analyzed at the embryonic stage using flow cytometric seed screening (FCSS method, Fig. 1). This screening is based on the different embryo/endosperm ploidy ratio in ripe seeds of sexual versus apomictic origin (Matzk et al. 2000). Two fresh achenes were chopped in nuclei-isolating buffer with a razor blade together with 0.05 cm<sup>2</sup> of leaf tissue of reference

standard; the suspension was filtered and incubated for 10 min at room temperature. Further steps of the procedure (except centrifugation) followed the DAPI staining method described in Krahulcová et al. (2004). As the long-term storage of achenes under dry and warm conditions results in a reduction of endosperm in *Hieracium* (Krahulcová and Suda 2006), the fresh achenes were stored in a refrigerator and analyzed by FCSS no more than three months after harvest to detect a sufficient amount of endosperm nuclei. Accessions of tetraploid ( $2n=36$ ) and hexaploid ( $2n=54$ ) *Hieracium bauhini* and of octoploid ( $2n=72$ ) *H. pilosellinum* F. W. Schultz were used as internal reference standards.

### ***Study of the Reproductive System***

Routine recognition of the reproductive mode (sexual versus apomictic) has been done by comparing the seed-set in emasculated and open pollinated capitula under garden conditions (Gadella 1987; Krahulcová and Krahulec 1999). The test (Fig. 1) involved 71 maternal plants from locality 1, eight maternal plants from locality 2 (that had no seeds from the field available for FCSS analysis), and 116 individuals of the cultivated progeny. The reproductive mode in the other respective maternal plants from locality 2 was detected using flow cytometric screening of their seeds (Fig. 1) that had been collected either at the locality or/and in the garden (FCSS method, see above). Plants that did not produce any seeds after open pollination were considered to be sterile.

### ***Detecting the Reproductive Pathways in Experimental Plants***

Using both open pollination and emasculation experiments, the progeny origins were evaluated in six apomictic hepta- and octoploid maternal plants, which formed many polyhaploid and sexually derived progeny in the field. These high-polyploid plants, which represented different genotypes, were exposed to open pollination in the greenhouse without protection from pollinating insects. In addition, the progeny obtained from the same set of emasculated (see above) plants were analyzed for their origin. Reciprocal crosses in the greenhouse (the method according to Krahulcová et al. 2004) involved another set of six parental plants, simulating the hybridization between *H. bauhini* (both pentaploid and hexaploid) and the hexaploid *H. pilosella* at locality 2. The origin of the seed progeny obtained after all treatments was traced *i*) at the embryonic stage using either the FCSS method described above or its modification (Krahulcová and Suda 2006), or *ii*) at the seedling stage. In the latter case, the seedling progeny were cultivated, and their ploidy variation was assessed using flow cytometry with the maternal plant used as an internal standard (Krahulcová et al. 2004).

### ***Assignment of the Clonal Structure and Analysis of Maternal Lineages***

To identify particular clones within distinct groups of morphotypes and cytotypes at locality 1, isozyme analysis and multilocus fingerprinting were used. The minimum number of individual genotypes (clones) within each group was inferred from a unique pattern of isozyme phenotypes derived from a combination of four enzymes tested (AAT, EST, LAP, and PGM). It was proved that this system had a sufficient resolution



efficiency in the *Hieracium* subgen. *Pilosella* (Krahulec et al. 2004). In multilocus fingerprinting, as alternative genetic markers for distinguishing the genotypes, human minisatellite probe 33.15 (Jeffreys et al. 1990) and a (GATA)<sub>5</sub> microsatellite were used. DNA isolation, restriction digests of genomic DNA, Southern blotting, and minisatellite fingerprinting were done as described in Fehrer et al. (2005), and microsatellite fingerprinting was performed according to Wilson et al. (2006). Out of 63 plants from the hybrid-rich locality 1 that were analyzed for clonal structure (Fig. 1), 34 plants were processed using isozymes, five through fingerprinting and 24 plants using both methods. The resulting number of distinguished genotypes (Table 1) represents a minimum number of genotypes actually occurring at the locality.

The chloroplast DNA (cpDNA) haplotypes were studied in 34 plants from hybrid-rich locality 1 that had distinct genotypes as determined by isozymes and fingerprinting (Fig. 1). As the matroclinal inheritance of cpDNA was verified for *Hieracium* subgen. *Pilosella* (Fehrer et al. 2005), the haplotypes were analyzed to reveal the direction of the crosses in which the interspecific hybrids may have originated. The chloroplast *trnT-trnL* intergenic spacer allows to distinguish individual sub-haplotypes within two main haplotype groups (called I and II) in the *Hieracium* subgen. *Pilosella* (Fehrer et al. 2005). Haplotype group II involves several sub-haplotypes, among them a more widespread sub-haplotype II/7 (in this paper referred to as ‘normal’) shared by *H. pilosella* and some biotypes of *H. bauhini*, and the sub-haplotype II/11 found previously (Fehrer et al. 2005) in some biotypes of *H. bauhini* (in this paper called ‘bauhini’). The latter type is characterized by two inserts (GenBank accession numbers AY192672-4, AY192647-8) that can be detected either by restriction digestion with *EcoRI* as variation in length of restriction fragments, or by restriction digestion with *DraI* as an additional restriction site.

DNA was isolated according to Štorchová et al. (2000) using fresh material. PCR amplifications of the *trnT-trnL* intergenic spacer were done as described by Fehrer et al. (2007a). For PCR-RFLPs, about 50–100 ng of amplified product were digested overnight with 20 units of *EcoRI* or *DraI* according to the manufacturer’s instructions (Fermentas), separated on 2.5% agarose gels and visualized under UV light after staining with ethidium bromide.

## Results

### *Population Structure at Locality 1*

At locality 1, 79 plants were collected: 20 plants of *Hieracium pilosella*, 14 plants of *H. bauhini* and 45 hybrid plants. Chromosome numbers recorded among them ranged from the tetraploid ( $2n=36$ ) to the octoploid level ( $2n=72$ , Table 1). Irrespective of the clonal structure, the dominating tetraploid cytotype was recorded in 28 plants (35%); the other euploid cytotypes were pentaploid (16 plants), hexaploid (five plants), heptaploid (15 plants), and octoploid (three plants). Altogether 12 plants were aneuploid (15% of all plants collected); all aneuploids were classified as hybrids (Table 1).

Emasculation experiments showed an almost equal frequency of sexual and apomictic plants (37 sexual and 34 apomictic plants, respectively, Table 1). The two parental species differed in reproductive system: whereas *H. pilosella*, both

tetraploid and pentaploid, was exclusively sexual, all tetraploid and pentaploid plants of *H. bauhini* were apomictic (Table 1). One additional heptaploid accession of *H. bauhini* had a specific reproductive mode (see below). The reproductive system of the rare hexaploid *H. bauhini* could not be studied (Table 1) as the cultivated plants perished before they flowered, but they may also be apomictic like other hexaploid accessions of this species (Rotreklová 2004). The hybrids were sexual or apomictic with a highly variable ploidy level (Table 1). In addition, ploidy screening of the seed progeny of apomictic mothers from this locality revealed 12 apomictic plants (nine heptaploid, three octoploid), which later formed considerable amounts of  $n+n$  progeny after open pollination and/or plenty of polyhaploids after emasculation. Thus, these maternal plants (11 hybrids and one plant of *H. bauhini*) displayed a high frequency of aberrant progeny despite their apomictic nature (Fig. 4), and their reproductive mode was indicated as ‘variable’ (Table 1). We have already used this term in a previous paper (Krahulec et al. 2008).

The clonal structure indicates a richness of genotypes, not only among the sexual plants, but also among the facultative apomicts, both *H. bauhini* and the hybrids (Table 1). Each sexual plant analyzed did not display its own unique genotype, which is not surprising for plants growing clonally by means of stolons. The chloroplast haplotypes of 34 plants representing the distinct genotypes (Table 2) belonged to major haplotype group II as expected; only the haplotype of one hybrid plant belonged to major haplotype group I (Fehrer et al. 2005, 2007a). All of the four genotypes of *H. pilosella* examined (two tetraploid and two pentaploid) had the ‘normal’ variant of major haplotype group II previously found in the tetraploid populations of this species (Krahulec et al. 2004; Fehrer et al. 2005, 2007b). As in *H. bauhini* both sub-haplotypes were also found in hybrids at this locality, both the ‘normal’ one (shared with *H. pilosella* and other species) and the particular ‘bauhini’ type (Table 2). Among the hybrids, the ‘bauhini’ sub-haplotype was recorded twice as frequently as the ‘normal’ variant (Table 2).

### Population Structure at Locality 2

At locality 2, 57 plants were collected: 27 plants of *H. pilosella*, 19 plants of *H. bauhini* and 11 hybrid plants. Among them, the hexaploids and pentaploids prevailed (together 93% of the total, Table 1). Although *H. pilosella* was invariably hexaploid there, it was comprised of apomictic, sexual and seed-sterile biotypes. Sexual *H. pilosella* was the most frequent type sampled at this locality (56% of plants sampled). *H. bauhini* was apomictic and was comprised of the predominant pentaploid and the rare hexaploid cytotype (Table 1). The ploidy of rather rarely occurring hybrids ranged from the tetraploid to the octoploid level (Table 1). Because the flow cytometry used for ploidy estimation of plants from the field did not reveal any inter-ploid peaks, no such aneuploids that would have major differences in DNA content from the euploid biotypes were expected to occur there.

The hybrid plants examined for reproductive mode were either apomictic or seed-sterile (Table 1). The pollen viability of these seed-sterile plants (predominantly pentaploid) was examined in a separate study, using the stainability test (Rotreklová 2008). These plants showed a frequency of stainable pollen that did not exceed 30%. The octoploids displayed a high frequency of aberrant progeny, analogous to the

**Table 2** Haplotype structure (according to Fehrer et al. 2005, 2007b) detected in the hybrid swarm of *Hieracium* subgen. *Pilosella* at the locality Praha-Vysočany (locality 1), detected for the 34 distinct genotypes

cpDNA haplotype group	Plants assigned to					
	<i>H. pilosella</i>		<i>H. bauhini</i>		Hybrids	
	ploidy/RS	nr. of genotypes	ploidy/RS	nr. of genotypes	ploidy/RS	nr. of genotypes
II sub-type 'normal'	4x/sex	2	4x/apo	2	4x/apo	1
	5x/sex	2	5x/apo	1	4x/n.s.	1
					5x/sex	1
					6x/sex	1
					8x/var*	1
					aneuploid/sex	1
					aneuploid/apo	1
II sub-type 'bauhini'			5x/apo	2	4x/sex	2
			5x/n.s.	1	5x/apo	1
			7x/var*	1	7x/var*	5
					7x/apo**	1
					7x/n.s.	1
					aneuploid/sex	5
I					4x/sex	1

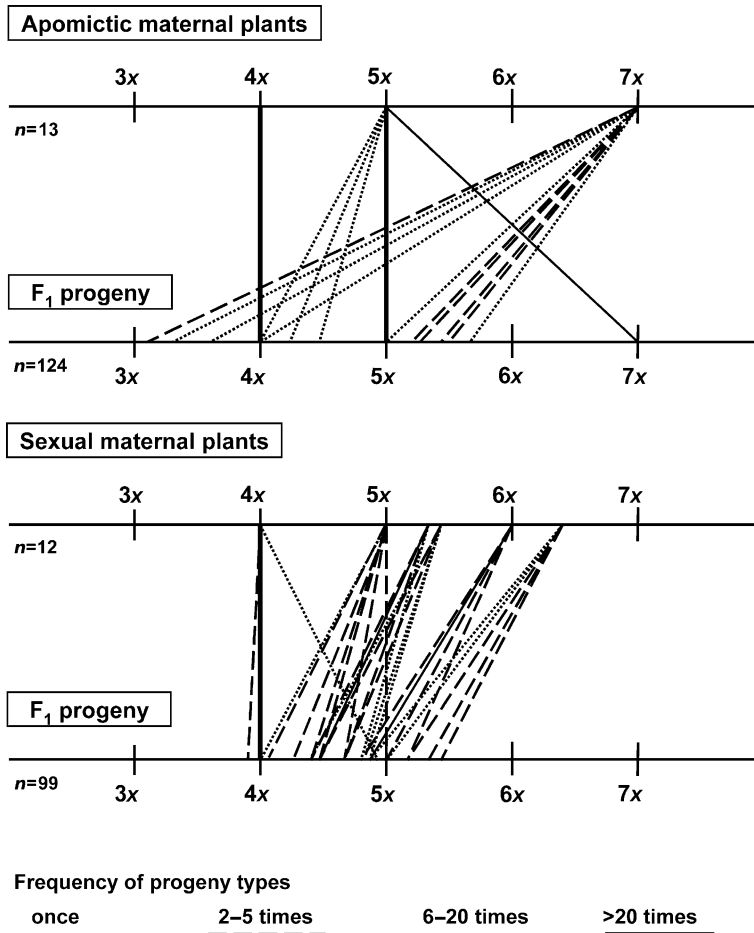
For the genotype structure, symbols and abbreviations see Table 1. The shaded field delimits the plant genotypes sharing the 'bauhini' sub-haplotype.

heptaploid and octoploid plants from locality 1 (see above and Table 1). The only exception among the otherwise either apomictic or seed-sterile hybrids was one plant that was intermediate in morphology between *H. pilosella* and *H. brachiatum*; it was tetraploid and sexual.

### ***Progeny of the Sexual Versus Facultatively Apomictic Mothers***

#### *Ploidy Variation and Origin*

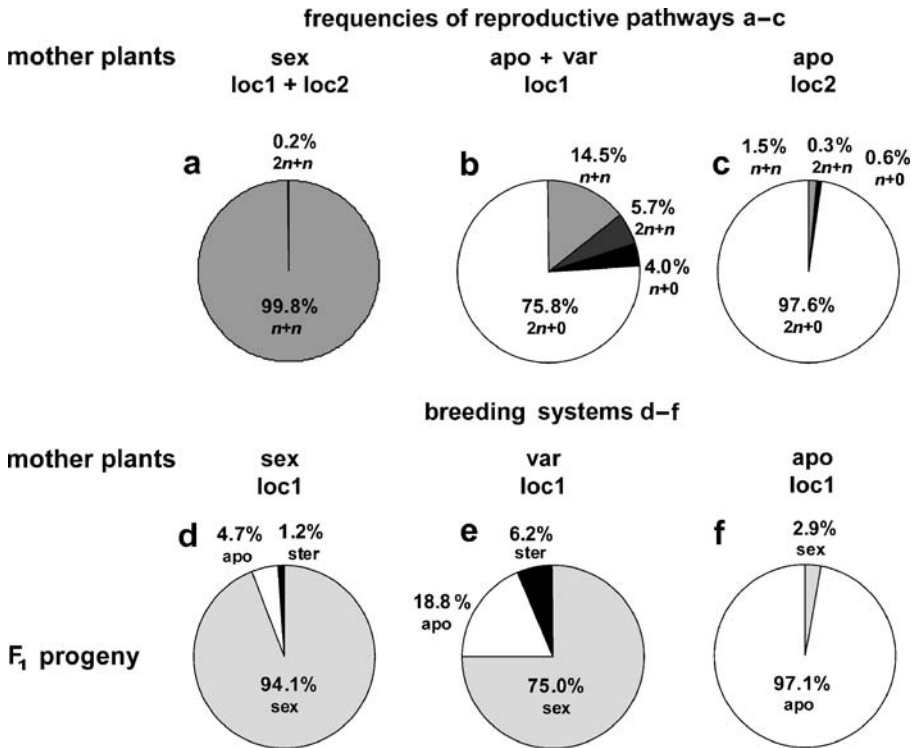
Comparing the total range of ploidy variation among maternal plants and their progeny as a whole, the difference between sexual and facultatively apomictic mothers is obvious (Fig. 2, Appendix). While the range of chromosome number variation at locality 1 was higher in apomictic mothers towards their progeny (maternal range  $2n=36-63$ , progeny range  $2n=28-63$ ), the corresponding variation in the progeny of sexual mothers was lower (maternal range  $2n=36-58$ , progeny range  $2n=35-48/49$ ). A principal difference in the mode of seed formation in the field between the sexual and facultatively apomictic mothers consisted in the diversity of their reproductive pathways (Fig. 3a-c). While the progeny of sexual mothers was formed almost exclusively by mating via a fusion of reduced gametes



**Fig. 2** Chromosome number and ploidy variation in selected maternal plants and in their F<sub>1</sub> seed progeny, at locality 1. The mother-progeny relationship was independently evaluated for 13 apomictic (i.e., in terms of the production of viable seeds in emasculated capitula) and 12 sexual maternal plants to demonstrate the range of variation of the progeny of a particular plant. For the source data see the [Appendix](#)

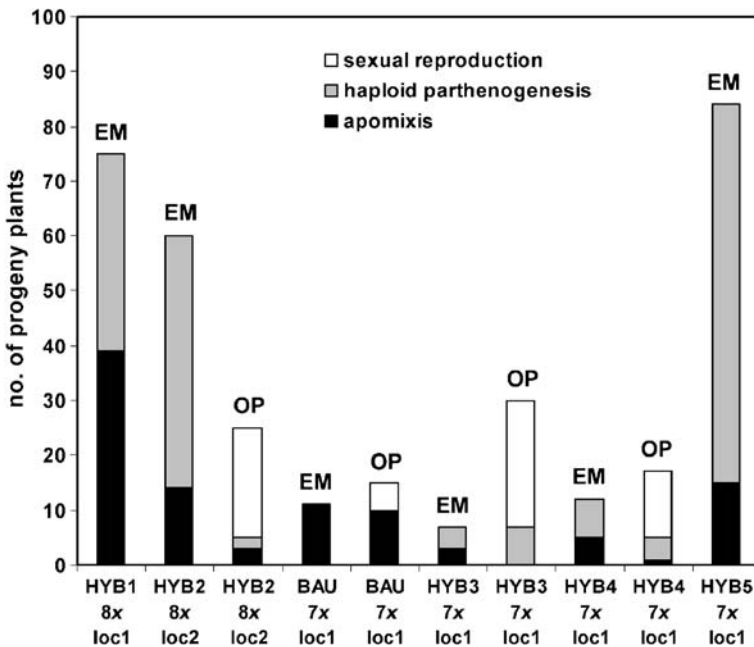
as expected ( $n+n$  progeny, Fig. 3a), the progeny of facultatively apomictic mothers originated via four different pathways (Fig. 3b,c), i.e., apomixis (both the somatic parthenogenesis,  $2n+0$ , and haploid parthenogenesis,  $n+0$ ), and sexual reproduction via fusion of either reduced ( $n+n$ ) or unreduced ( $2n+n$ ) gametes. Seven individuals of polyhaploid origin ( $n+0$ ) were detected in total among the progeny of the heptaploid (locality 1) and pentaploid (locality 2) apomictic mothers (see [Appendix](#)). However, those progeny categories referred to as true apomictic reproduction ( $2n+0$ ) might involve a small fraction of progeny that originated sexually via selfing (Bicknell et al. 2003; Krahulcová et al. 2004), because foreign pollen capable of inducing autogamy was available in the field.

If we compare the frequencies of aberrant progeny of facultatively apomictic mothers in the two model populations (Fig. 3b,c), it is 10 times lower at locality 2



**Fig. 3** Reproductive pathways operating in sexual/apomictic maternal plants and breeding systems recorded in F<sub>1</sub> seed-progeny that spontaneously arose at the localities Praha-Vysočany (loc1) and Brno-Kamenný kopec (loc2). The source data (for a–c, see Appendix) have been combined for sexual mothers (sex) and facultatively apomictic mothers (apo) including those having variable reproduction (var, see below), irrespective of maternal ploidy level/chromosome number. In the case of identical ploidy level and strong morphological similarity between the apomictic mother and its cultivated progeny, an apomictic origin of such progeny was postulated and maintenance of the maternal apomictic reproductive system was supposed. Progeny origins (upper row) – a 12 maternal plants, 99 progeny individuals/locality 1, and nine maternal plants, 317 progeny individuals/locality 2; b 13 maternal plants, 124 progeny individuals; c 12 maternal plants, 336 progeny individuals. Reproductive systems (apo – apomictic, sex – sexual, ster – seed sterile, lower row) were detected in the F<sub>1</sub> progeny generated by sexual mothers (d 12 maternal plants, 85 progeny individuals), apomictic mothers having variable reproduction (var), producing a lot of polyploid and/or sexually derived offspring beside true apomictic progeny (e three maternal plants, 16 progeny individuals), and apomictic mothers predominantly producing true apomictic progeny (f nine maternal plants, 104 progeny individuals)

than at locality 1. Nevertheless, the diversity of minority reproductive pathways among facultatively apomictic mothers from locality 2 is still higher than the reproductive diversity recorded among sexual mothers (Fig. 3a,c). The respective apomictic maternal plants from locality 2 are comprised of both even-ploid (hexaploid) and odd-ploid (pentaploid) cytotypes (Appendix). Although the total frequency of aberrant progeny formed here by hexaploid and pentaploid apomicts was only 2.4% (Fig. 3c), all of this variation was exclusively produced by the apomictic pentaploids (Appendix). Interestingly, the odd number of chromosome sets does not appear to hinder meiosis and thus the formation of reduced gametes, either in pentaploids or in heptaploids (Appendix, Fig. 4).



**Fig. 4** Progeny origins in emasculated (EM)/open-pollinated (OP) maternal plants (7x – heptaploid, 8x – octoploid), inferred from comparing the maternal and the progeny ploidy levels. Abbreviations: HYB – hybrid; BAU – *H. bauhini*. Origin of the experimental maternal plants: loc1 – locality Praha-Vyšocany; loc2 – locality Brno-Kamenný kopec. The different plant labels refer to different genotypes

Pollination experiments on apomictic maternal plants under greenhouse conditions confirmed the substantial potential of the high-polyloid hybrids (heptaploids, octoploids) to generate progeny that vary considerably, as was recorded in the field (Fig. 4). Conversely, the results of crosses between selected pentaploid/hexaploid parental biotypes of *H. bauhini* and the hexaploid *H. pilosella* from locality 2 suggested a rather low hybridization rate when apomictic *H. bauhini* served as the maternal parent. Using the FCSS method, only one hybrid pentaploid embryo was recorded among 227 progeny seeds analyzed in total. However, those plants of *H. bauhini* that produced some hybrid progeny in the field (Appendix) were not available later on to be used as the maternal plants in crosses.

### Reproductive System

The inheritance of the apomictic/sexual reproductive system was traced among the progeny of 24 maternal plants in total (12 sexual and 12 apomictic) that had been open-pollinated at locality 1. The reproductive system was tested in a total of 85 progeny individuals of sexual mothers (Fig. 3d) and 31 progeny individuals of apomictic mothers. The reproductive system in an additional 89 progeny individuals from apomictic mothers was inferred from their supposed apomictic origin (Fig. 3e,f). As a whole, the maternal reproductive mode was maintained in most progeny (Fig. 3d,f). Departure from this rule was the progeny of those apomictic mothers having the ‘variable’ system of reproduction, which was mainly sexual (Fig. 3e).

### *Origin of Hybrids in the Field*

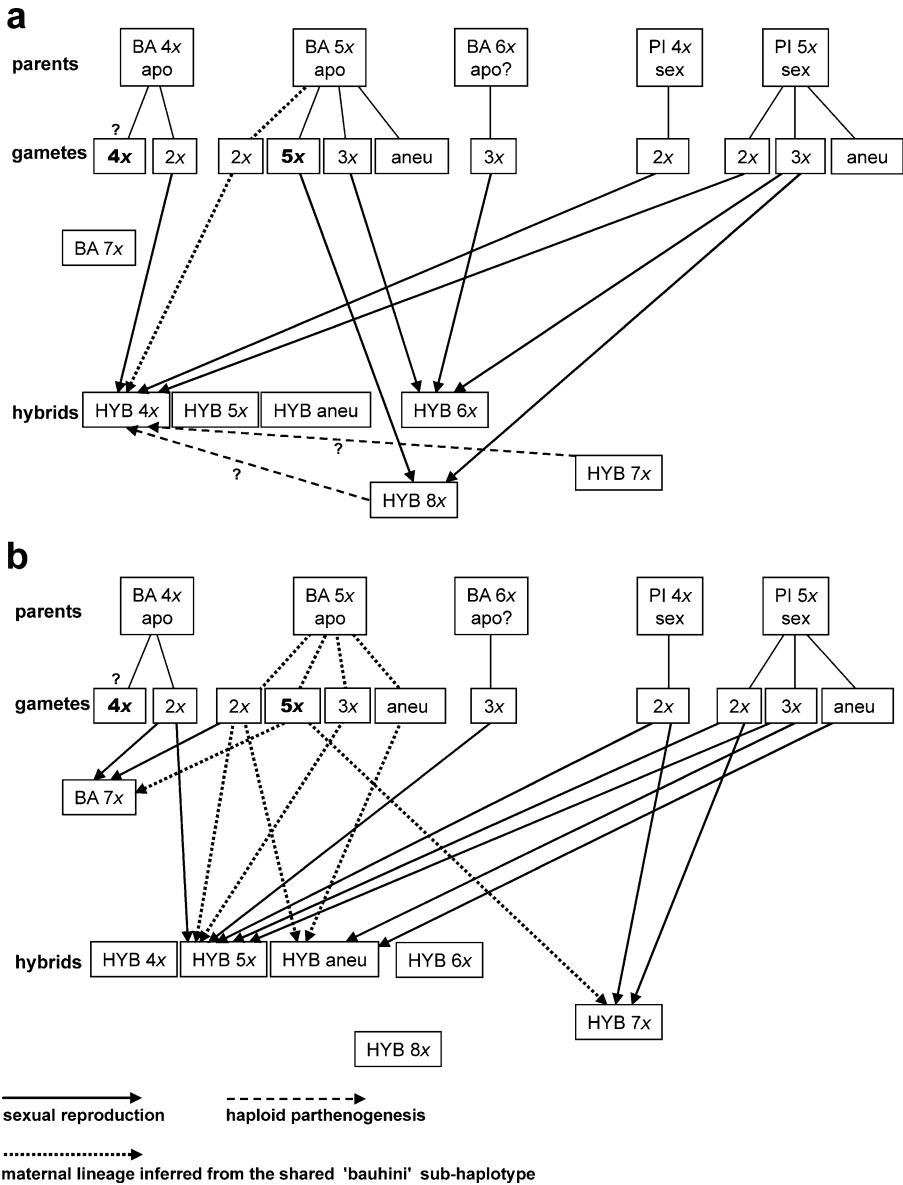
The clonal and cpDNA haplotype structure of the plants from locality 1 suggests the recurrent mating of apomictic mothers. The sub-haplotype ‘bauhini’, maternally transmitted from some parental biotypes of *H. bauhini* to their hybrids, was frequently recorded in hybrid plants having different ploidy levels, both euploid and aneuploid (Table 2). The assumed origin of established hybrids, inferred from maternal plant-seed progeny relationships (Appendix) and from the clonal and haplotype structure of the hybrid swarm (Tables 1, 2), is illustrated in Fig. 5a,b. While the origin of the heptaploids from the  $2n+n$  mating of pentaploid mothers appears clear (Table 2, Fig. 5b), the origin of the octoploid hybrid clone is not so obvious, as its ‘normal’ sub-haplotype could have been obtained from either maternal parent (Table 2, Fig. 5a). However, the origin via multistep crosses is not excluded either in the heptaploid, or in the octoploid hybrids. The population structure at locality 2 (Table 1) shows a rarity of established interspecific hybrids, many of which were found to be seed-sterile. Therefore, a relevant role of such plants as seed parents in recurrent hybridizations or in backcrosses is improbable. Nevertheless, in spite of their reduced pollen viability, they still might have some potential to serve as pollen donors in crosses, and thus could also shape the population structure (Fig. 6).

The origins of many corresponding cytotypes recorded among already established plants at both localities (Table 1) can be inferred from the origins of the seed progeny produced by particular maternal plants (Appendix). This assumption holds for the rare octoploid  $2n+n$  hybrids from locality 2 (Fig. 6), as well as for the heptaploid ( $2n+n$ ) and the aneuploid ( $n+n$ ) hybrids from locality 1 (Fig. 5b). The pentaploid  $n+n$  embryos, rarely recorded among the seed progeny of the pentaploid *H. bauhini* from locality 2 (Appendix), may suggest the genesis of the seed-sterile pentaploid hybrid growing there (Fig. 6), but they might also be a product of either an intraspecific cross or of autogamy in *H. bauhini*.

## **Discussion**

### ***Diversity in the Model Populations and Origins of the Progeny***

The co-occurrence of the same two parental species resulted in different population structures at the two localities studied (Table 1, Figs. 5a,b, 6). However, the different ploidy structure of *Hieracium pilosella* between the two localities may already reflect the distinctness in cytotype distribution in the study area (Rotreklová et al. 2002, 2005; Mráz et al. 2008). It appears that the hybrid-rich structure of the population at locality 1 is more shaped by sexual reproduction, including the residual sexuality of apomicts, than the population at locality 2. This difference between the two studied systems, comprising both sexual and facultatively apomictic biotypes, may be caused in part by differing compatibility between cytotypes/genotypes of the putative parental species. Also influencing the structure of hybridizing populations are the conditions that enable the formation and establishment of hybrids. In this respect, locality 1 appears to be more suitable

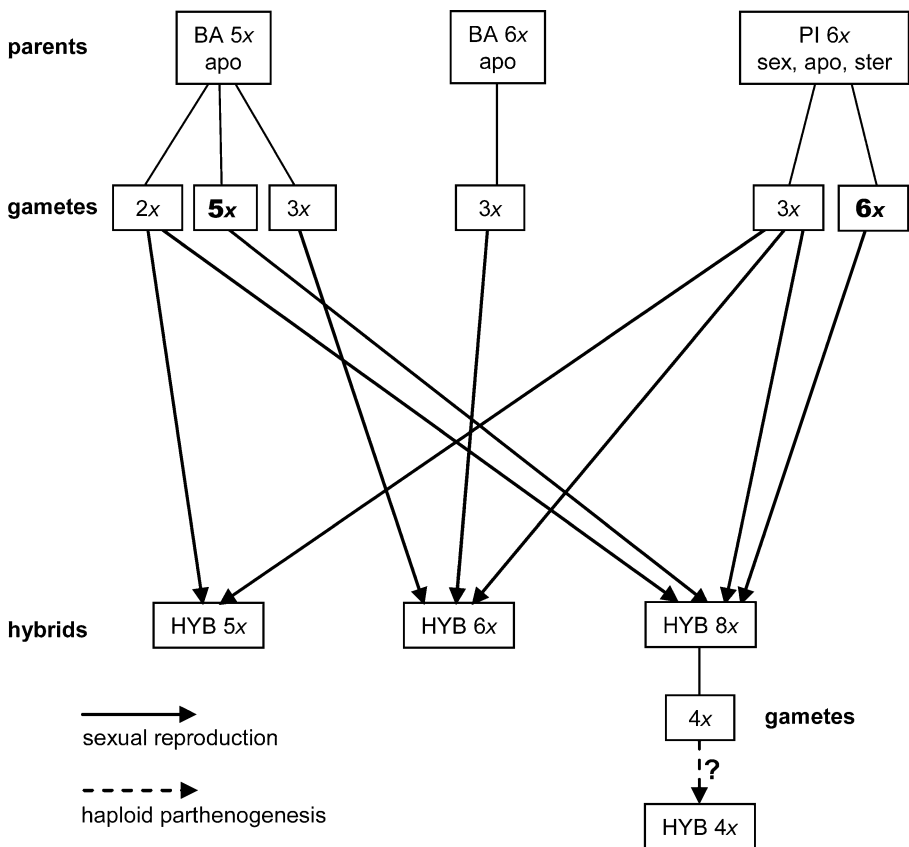


**Fig. 5** Hypothetical relationships among the plants established in the hybrid swarm at locality 1. Origins of hybrids are inferred *i*) from their ploidy and haplotype structure compared with the putative parents (Tables 1, 2), and *ii*) from the corresponding ploidy structure of the respective seed progenies collected in the field (Appendix). For simplicity, only one-step hybridizations are taken into account. Due to the complex structure of the population at locality 1, the plant origins for the even-ploid (a) and odd-ploid/aneuploid hybrids (b) are presented separately. Abbreviations and symbols: BA – *H. bauhini*; PI – *H. pilosella*; HYB – hybrid; aneu – aneuploid; apo – apomictic; sex – sexual. A question mark represents an uncertain pathway. The symbols of unreduced gametes are printed in bold



for recurrent interspecific and intercytotype crosses, which can lead to the establishment of an advanced hybrid swarm (Fig. 5a,b). This situation might also be influenced by the potentially different ages of suitable habitats at the two respective localities. Our knowledge of the (intraspecific) variation among the cpDNA haplotypes is still incomplete in *Hieracium* subgen. *Pilosella* as a whole; thus, the infiltration of the ‘foreign’ haplotype of major haplotype group I (Table 2) might be due to several reasons, among them, a possible past hybridization with other species.

A complex population structure such as that seen in the population at locality 1 (Table 1) appears to depend on the presence of facultatively apomictic species, which have a versatile reproductive system and co-occur with sexual species, as well as on the specific ecological conditions. The detailed investigation of the



**Fig. 6** Hypothetical relationships among the plants established in the hybrid swarm of the studied *Hieracium* subgen. *Pilosella* species at locality 2. Origins of hybrids are inferred *i*) from their ploidy compared with the putative parents (Table 1) and *ii*) from the corresponding ploidy structure of the respective seed progenies collected in the field (Appendix). For simplicity, only one-step hybridizations are taken into account. Abbreviations and symbols: BA – *H. bauhini*; PI – *H. pilosella*; HYB – hybrid; apo – apomictic; sex – sexual; ster – seed sterile. A question mark represents an uncertain pathway. The symbols of unreduced gametes are printed in bold

population structure at this locality alone revealed several novelties in the whole agamic complex of *Hieracium* subgen. *Pilosella*: *i*) the first record of an octoploid cytotype in nature, *ii*) fully sexual reproduction in pentaploids (both already published by us in Rotreklová et al. 2002), *iii*) a rather frequent aneuploidy among plants in the field (for the rarity of aneuploids in Europe thus far see the review by Krahulcová et al. 2000), *iv*) the fully sexual reproduction of aneuploids, even those with a high chromosome number, and *v*) the first detection of those high-polyploid (here the hepta- and octoploid) apomictic genotypes in the field, which frequently produce hybrids and polyhaploids. We recently described the last phenomenon in hexaploid plants from the Šumava Mts in the Czech Republic (Krahulec et al. 2008).

The genotypes of all of the analyzed heptaploid plants from locality 1 were characterized by the ‘bauhini’ sub-haplotype (Table 2). Thus, the unreduced egg cells of the pentaploid *H. bauhini* and/or the hybrid sharing the same haplotype, fertilized by the reduced pollen of a tetraploid donor, are suggested as the most probable path of their origin (Fig. 5b). Based on this haplotype structure, the same origin of the corresponding heptaploid hybrid was supposed in another hybrid swarm in Germany (Fitze and Fehrer 2000). No cytotypes below the tetraploid level were found among the plants sampled in both populations studied here. This suggests the absence of polyhaploid plants in the field, especially triploid or aneuploid ones, which might be generated by the heptaploid mothers at locality 1. This idea is in agreement with the selection of specific progeny classes during germination and the early development of seedlings, which have been proved experimentally (Krahulec et al. 2006). The origin of the established tetraploid hybrids at both localities, however, is controversial (Figs. 5a, 6). Two alternatives are possible, namely the polyhaploid (from both heptaploid and octoploid mothers) or hybrid origin, multi-step hybridization included. In fact, there is no rigid test to confirm the polyhaploid nature of a respective plant. We can prove the putative polyhaploid origin only if the maternal plant is known for certain and if it is compared with its progeny.

### ***Facultative Apomicts as the Source of Population Diversity***

Ploidy variation generated by sexual versus facultatively apomictic seed parents in the mixed but purely polyploid populations was previously unexplored in any polyploid apomictic complex of angiosperms. Evidently, facultative apomicts do contribute to generating and maintaining diversity in the mixed populations, acting both as pollen and seed parents in crosses (Whitton et al. 2008). For instance, in comparing the reproductive success of the dominant versus the minority cytotype (both polyploid and facultatively apomictic) in populations of *Arnica cordifolia* Hook., apomixis was shown to promote the coexistence of both in the field (Kao 2007). Several studies in *Taraxacum* sect. *Ruderalia* Kirschner, H. Øllgaard et Štěpánek confirmed gene exchange among the sexual diploids and apomictic triploids and tetraploids (e.g., Menken et al. 1995; Meirmans et al. 2003; Verduijn et al. 2004). Similarly, an increase of karyological and reproductive diversity due to self-fertilization ( $2n+n$ ) and outcrossing ( $n+n$ ) was studied in populations of the tetraploid facultative apomictic grass *Hypar-*

*rhenia diplandra* (Hack.) Stapf. In this case, as opposed to our finding, only 1% of the seeds originated via those rare reproductive pathways (Durand et al. 2000). The effect of reproductive diversity was also confirmed in the natural populations of *Hypericum perforatum* L. Using flow cytometry, varying degrees of apomixis and sexuality were found by analyzing seeds in different populations of this facultative apomict (Barcaccia et al. 2006).

Irrespective of rather scarce data describing processes in the field in detail, the substantial existing information about the ability of facultative apomicts to hybridize and to generate new cytotypes is based on crossing experiments (e.g., Bicknell et al. 2003; Krahulcová et al. 2004; Schranz et al. 2005; Mártonfiová 2006; Mártonfiová et al. 2007). Our present data confirm for the first time the reproductive versatility of the facultative apomicts in *Hieracium* subgen. *Pilosella* under field conditions. We demonstrate the structure of established plants in two natural hybrid swarms and their respective seed progenies, suggesting that the facultatively apomictic mothers generate more variation in ploidy than the sexual mothers coexisting in the mixed populations (Fig. 2). This effect is due to the diverse reproductive modes operating in addition to true apomixis, i.e., haploid parthenogenesis (which we consider, in a broad sense, to be an apomictic reproductive pathway) and sexual reproduction via both reduced and unreduced gametes (Fig. 3a–c). The ploidy level among the progeny in the two model populations was detected using two different methods that aim at different developmental stages of progeny individuals. These two methods are not fully comparable due to selection acting on specific progeny classes (ploidy categories) during germination and early seedling development (Krahulec et al. 2006). Taking this possible bias into account, the ploidy variation among the progeny from locality 2 (analyzed at the embryonic stage) should be expected to be higher than among progeny from locality 1 (analyzed at the seedling stage). Because, however, the opposite result was found, the application of the different methods does not affect the principle results in this case, probably because of the much higher diversity of the population at locality 1. The particular morphological variation within the progeny of apomictic versus sexual maternal plants from this locality is being examined in an ongoing study.

Our results confirm an important role of facultatively apomictic mothers in the formation of hybrids and hybridogenous species in the agamic complex of *Hieracium* subgen. *Pilosella*. The involvement of other facultatively apomictic species as maternal parents in hybridizations has been inferred from the ploidy and haplotype structure of field populations (Krahulec et al. 2004, 2008; Fehrer et al. 2005, 2007b). Our study also shows an indispensable role of apomicts having a highly versatile reproduction in the generation of fully sexual progeny (compare Fig. 3e and f). This finding is consistent with the expected reduced transmission of the apomictic trait in those apomicts that acted as maternal parents of sexually produced progeny (Whitton et al. 2008). Such apomicts are represented in our model population by heptaploid hybrids displaying considerably high rates of residual sexuality and haploid parthenogenesis. Thus, the classification of the reproductive system in such plants is disputable. Although they do form a number of viable seed progeny autonomously via parthenogenesis as do true apomicts, a considerable (even the predominant) number of their progeny are different from the maternal parent. Many of these high-polyploid plants could be derived from

apomictic mothers as primary  $2n+n$  hybrids (Appendix, Figs. 5a,b, 6) probably being subjected to selection; a parallel process was demonstrated in *Taraxacum* (de Kovel and de Jong 2000). Irrespective to obvious genomic instability, such hybrids could still serve as important sources of new genotypes and cytotypes, as has been demonstrated in our present study. Not until recently have such hybrid biotypes of *Hieracium* subgen. *Pilosella* with unstable genomes at the hexaploid level been described from the field (Krahulec et al. 2008; for discussions on high-polyploids see also Fehrer et al. 2007b). Although they behave like apomicts in emasculation experiments, producing progeny independently of fertilization, the analysis of their progeny from either experimental or field origin implies a high level of residual sexuality and/or haploid parthenogenesis. The tetraploid biotypes with the same variable reproductive behaviour were also found during hybridization experiments in *Panicum maximum* Jacq. (Savidan and Pernès 1982) and in dandelions (*Taraxacum* sect. *Ruderalia*, Verduijn et al. 2004). The search for such temporarily persisting biotypes in other polyploid apomictic complexes may reveal new sources of population diversity. In light of the novelties described above, the role of facultative apomicts in the generation of population variation is still undervalued.

### ***Taxonomic Implications***

Based on the results presented here we suggest that two categories of hybrids in *Hieracium* subgen. *Pilosella* should be distinguished according to their reproductive behaviour: *i*) the stabilized, predominantly apomictic hybridogenous taxa maintaining their genome via dominating, true apomixis and *ii*) the recent hybrids. These hybrids are either sexual or facultatively apomictic, but in the latter case usually have a high level of residual sexuality and/or haploid parthenogenesis. At present, this hybrid genome instability has been especially demonstrated at the hexaploid and higher ploidy levels. The reproductive system of such facultatively apomictic hybrids is referred to as 'variable' in this study, as well as in our previous paper (Krahulec et al. 2008). Evidently, the recent hybrids are repeatedly formed, even at the same locality. Because of a low production of identical progeny by (facultative) apomixis, they usually do not spread outside the place of their origin. The stabilized hybridogenous taxa, however, behave as species at least at the landscape level. Fehrer et al. (2007b) and Krahulec et al. (2008) have already discussed these arguments. Hence, the discrimination between the recent and stabilized hybrids reflects the different life history of both categories that have a different impact on the structure of the agamic complex.

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**Appendix**

Overview of chromosome numbers, ploidy levels and genotypes of maternal plants (sexual and apomictic) of the studied *Hieracium* subgen. *Pilosella* and their seed progeny, spontaneously arisen at localities Praha-Vyšochany (locality 1) and Brno-Kamenný kopec (locality 2). The labels (BA – *Hieracium batulini*, PI – *H. pilosella*, HYB – hybrid) refer to individual maternal plants and genotypes symbolized by lowercase letters. The supposed origin of the progeny was inferred *i*) by comparing their own and the maternal ploidy level, and from the morphology of cultivated progeny plants (all progeny from locality 1, part of the progeny of plants 2-3763 PI and 2-3734 PI from locality 2), or *ii*) by FCSS analysis (the other progeny from locality 2). Symbols of progeny origins follow Harlan and de Wet (1975).

Apomictic maternal plants			Progeny			Sexual maternal plants			Progeny		
Cytotype	Plant label	Genotype	2n / ploidy (x=9)	Nr. of individuals	Origin	Cytotype	Plant	Genotype	2n / ploidy (x=9)	Nr. of individuals	Origin
2n=4x=36	1-656 BA	BA b	4x	23	2n+0	2n=4x=36	1-296 PI		36	1	n+n
2n=5x=45	1-292 BA	BA c	5x	6	2n+0		1-505 PI	PI i	36	6	n+n
	1-644 BA	BA d	2n=39	1	n+n				44	1	n+n
			5x	15	2n+0		1-511 PI	PI l	36	7	n+n
	1-649 BA	BA d	5x	2	2n+0		1-646 PI	PI m	35	2	n+n
			2n=7x=63	1	2n+n				36	6	n+n
	1-652 BA	BA d	5x	10	2n+0		1-654 PI	PI n	36	5	n+n
			2n=7x=63	1	2n+n		1-643 HYB	HYB c	36	5	n+n
	1-655 BA	BA e	5x	5	2n+0		1-642 HYB	HYB c	36	1	n+n
			2n=7x=63	1	2n+n	2n=5x=45	1-506 PI	PI p	36	1	n+n
	1-647 HYB	HYB l	5x	7	2n+0				37	2	n+n
	1-661 HYB	HYB l	2n=41	1	n+n				39	2	n+n
			5x	19	2n+0				40	2	n+n
			2n=7x=63	2	2n+n				41	2	n+n

**Locality 1:** 13 individuals

**Locality 1:** 12 individuals



1-653 HYB	HYB l	4x	1	$n+n$		42	2	$n+n$
		5x	7	$2n+0$		45	2	$n+n$
		$2n=7x=63$	2	$2n+n$	1-660 HYB	43	2	$n+n$
$2n=7x=63$	1-508 HYB	$2n=28$	1	$n+0$		44	6	$n+n$
	1-513 HYB	$2n=29$	1	$n+0$		45	3	$n+n$
		$2n=45$	1	$n+n$		46	3	$n+n$
		$2n=48-50$	1	$n+n$	$2n=48$ (aneuploid)	40	3	$n+n$
	1-515 HYB	$2n=32$	1	$n+0$	1-295 HYB	41	8	$n+n$
		$2n=36$	1	$n+0$		42	4	$n+n$
		$2n=47$	5	$n+n$		43	1	$n+n$
		$2n=48$	1	$n+n$		44	1	$n+n$
		$2n=49$	2	$n+n$	$2n=49$ (aneuploid)	40	1	$n+n$
		$2n=50$	2	$n+n$		41	5	$n+n$
	1-651 HYB	$2n=28$	1	$n+0$		42	2	$n+n$
		$2n=46$	1	$n+n$		43	1	$n+n$
		$2n=48$	1	$n+n$	1-641 HYB	44	1	$n+n$
		$2n=51$	1	$n+n$		45	1	$n+n$
	Total		124			46	5	$n+n$
						46/47	1	$n+n$
						47	2	$n+n$
						48	1	$n+n$
						48/49	1	$n+n$
						Total	99	
<b>Locality 2: 12 individuals</b>					<b>Locality 2: 9 individuals</b>			
$2n=5x$	2-3672 BA	5x	9	$2n+0$	$2n=6x$	6x	15	$n+n$
		5x	1	$n+n$		6x	2	$n+n$
	2-3701 BA	5x	32	$2n+0$		6x	17	$n+n$

(continued)

Apomictic maternal plants			Progeny			Sexual maternal plants			Progeny		
Cytotype	Plant label	Genotype	$2n$ / ploidy ( $x=9$ )	Nr. of individuals	Origin	Cytotype	Plant	Genotype	$2n$ / ploidy ( $x=9$ )	Nr. of individuals	Origin
<b>Locality 2: 12 individuals</b>											
$2n=5x$	2-3706 BA		5x	12	$2n+0$	$2n=6x$	2-3763 PI		6x	6	$n+n$
			5x	1	$n+n$				8x	1	$2n+n$
	2-3707 BA		5x	29	$2n+0$		2-3734 PI		6x	64	$n+n$
	2-3703 BA		5x	3	$2n+0$		2-3735 PI		6x	30	$n+n$
			5x	2	$n+n$		2-3772 PI		6x	71	$n+n$
	2-3782 BA		5x	8	$2n+0$		2-3774 PI		6x	62	$n+n$
			5x	1	$n+n$		2-3775 PI		6x	49	$n+n$
			8x	1	$2n+n$				Total	317	
$2n=6x$	2-3784 BA		5x	33	$2n+0$						
			3x	2	$n+0$						
	2-3669 BA		6x	77	$2n+0$						
	2-3702 BA		6x	15	$2n+0$						
	2-3730 PI		6x	14	$2n+0$						
	2-3733 PI		6x	38	$2n+0$						
	2-3737 PI		6x	58	$2n+0$						
			Total	336							