Plant Syst Evol
DOI 10.1007/s00606-014-1072-2

ORIGINAL ARTICLE

Sexual reproduction as a source of ploidy level variation in the model agamic complex of *Pilosella bauhini* and *P. officinarum* (Asteraceae: Lactuceae)

Radka Rosenbaumová · František Krahulec

Received: 13 January 2014 / Accepted: 27 April 2014

Abstract We studied the significance of sexual reproduction as a source of ploidy level variation in a model system consisting of hexaploid, facultatively apomictic *Pilosella bauhini* and tetraploid, sexual *P. officinarum*. As the maternal parent, apomictic *P. bauhini* generated higher ploidy level variation than sexual *P. officinarum*. Ploidy levels of its progeny ranged from triploid to octoploid under experimental conditions and even to decaploid in the field. This progeny diversity resulted from the breeding system in *P. bauhini*, which included haploid parthenogenesis and sexual reproduction through both reduced and unreduced gametes besides prevailing apomixis; these particular reproductive pathways have been quantified. Sexual *P. officinarum*, on the other hand, reproduced exclusively through fusion of reduced gametes and produced only pentaploid hybrids or tetraploid progeny from autogamy, allogamy or both. Surprisingly, sexual *P. officinarum* was also the species showing stronger reproductive isolation, especially under the field conditions where intra-specific fertilization was highly favoured, most probably through competition between conspecific and heterospecific pollen. Apomictic *P. bauhini* thus appeared to be a significant source of ploidy level variation in the model population even though most of its progeny was formed clonally through apomixis. Only part of this variation was manifested in the field.

Keywords Facultative apomixis · Haploid parthenogenesis · Hybridization · *Pilosella* · Residual sexuality · Unreduced gametes

Introduction

Apomixis is asexual reproduction through seeds in flowering plants (Nogler 1984; Asker and Jerling 1992; Koltunow 1993; Bicknell and Koltunow 2004; van Dijk and Vijverberg 2005). Meiosis and fusion of gametes are both omitted during apomictic reproduction. Apomictically derived progeny are therefore genetically identical to their maternal parent (Koltunow 1993). Formerly, this fact led to the widespread belief that apomixis reduces the evolutionary potential of the respective taxonomic group and causes its evolutionary death (e.g., Darlington 1939; Stebbins 1950). Nevertheless, plants reproducing exclusively through apomixis (obligate apomicts) are quite rare in nature (Nogler 1984; Asker and Jerling 1992; Koltunow 1993; Naumova 2008). Most apomictic plants are facultative apomicts retaining a certain level of sexuality (residual sexuality) and participating in sexual reproduction as both maternal and paternal parents (Richards 2003; Bicknell and Koltunow 2004; Whitton et al. 2008).

Facultative apomicts produce a mixture of apomictically and sexually derived progeny, taking advantage of both of these modes of reproduction (Grossniklaus et al. 2001). Apomixis fixes the current maternal genotype and transmits it again and again to the next generations; an apomictic genome can remain constant for ages and spread

Dedicated to the memory of Franz Schuhwerk (07.08.1948–11.12.2013), who stimulated our work by questions and ideas.

R. Rosenbaumová (✉)
Department of Botany, National Museum, Cirkusová 1740,
193 00 Prague 9 - Horní Počernice, Czech Republic
e-mail: radka.rosenbaumova@seznam.cz

F. Krahulec
Institute of Botany, Academy of Sciences, Zámek 1,
252 43 Průhonice, Czech Republic

over a large geographical area (Nogler 1984; Koltunow 1993; Grossniklaus 2001; Loomis and Fishman 2009). Residual sexuality disrupts the constancy fixed by apomixis and leads to the formation of genetically diverse progeny in apomictic plants (Nogler 1984; Koltunow 1993; Koltunow et al. 1995; Grossniklaus 2001; Grossniklaus et al. 2001). Fusing gametes can be reduced (n) or unreduced ($2n$) and can come from individuals with the same or different ploidy level and belonging to the same or different species (Asker and Jerling 1992; Bicknell et al. 2003; Krahulcová et al. 2004). Moreover, haploid parthenogenesis, parthenogenetic development of reduced female gametes, can occur in apomictic plants, resulting in the formation of polyhaploid ($n + 0$) progeny (Asker and Jerling 1992; progeny terminology according to Harlan and de Wet 1975). Taken together, residual sexuality can lead to enormous diversity in progeny of apomictic plants. It is no wonder that it is now considered to be the most important source of evolutionary variation in populations of apomictic plants (Asker and Jerling 1992; Richards 2003).

Apomixis associated with sexual reproduction frequently leads to the formation of intricately structured polyploid agamic complexes that are characterized by exceptional diversity in morphology, ploidy level, mode of reproduction, degree of hybridity and age of particular forms they consist. Taxonomically, agamic complexes represent some of the most challenging groups of flowering plants; illustrative examples are the agamic complexes of *Taraxacum*, *Alchemilla*, *Crataegus*, *Hieracium*, *Pilosella* and *Rubus* (Dickinson 1998; Hörandl 1998). To improve our understanding of the evolutionary significance of the diversity generated by sexual reproduction in populations of apomictic plants, it is necessary to understand processes involved in the formation of this diversity. Young developing agamic complexes, such as the agamic complex of the genus *Pilosella*, represent suitable model systems for such studies.

The genus *Pilosella* Vaill. (Asteraceae Martinov: Lactuceae Cass.) ranks among the most important model systems in research of apomixis in dicotyledonous plants, along with *Taraxacum*, *Erigeron*, *Antennaria*, *Hypericum perforatum* and *Boechera holboellii* (Noyes and Rieseberg 2000; Naumova et al. 2001; Sharbel and Mitchell-Olds 2001; Matzk et al. 2001, 2003; van Dijk et al. 2003; Bayer and Chandler 2007). Apomixis in *Pilosella* is gametophytic (embryogenesis is preceded by formation of unreduced megagametophyte), aposporic (unreduced megagametophyte origins from ovular cell/s other than the megaspore mother cell), and autonomous (formation of both embryo and endosperm is independent of fertilization) (for review of individual types of apomixis see Asker and Jerling 1992; Koltunow 1993). In addition, sexual and apomictic reproduction can co-occur not only within one maternal plant/capitulum (Koltunow et al. 1998; Krahulcová et al. 2004), but also within one ovule (polyembryony; Bicknell et al. 2003).

For many years *Pilosella* was considered as a subgenus of *Hieracium* L. especially in central Europe. In most recent treatments, however, *Pilosella* is accepted as a separate genus (cf. Bräutigam and Greuter 2007). Main difference between *Pilosella* and *Hieracium* consists in type of apomixis; apomixis in *Hieracium* is diplosporic (unreduced megagametophyte origins from the megaspore mother cell) and pseudogamous (fertilization of the central cell is required for endosperm development). In our study, we followed recent trend and treated *Pilosella* as a separate genus.

Taxonomic structure of the genus *Pilosella* is intriguing, shaped by polyploidy (chromosome base number $x = 9$), frequent spontaneous inter-specific and/or inter-cytotype hybridizations, facultative apomixis, and clonal growth (reviewed in Krahulcová et al. 2000; Fehrer et al. 2007). Studies of morphological and cytotype variation in the genus *Pilosella* have an over 100 years long tradition and have led to the description of a huge number of forms, including hybrids and hybridogenous species (e.g., Nägeli and Peter 1885; Zahn 1922–1930; Bräutigam and Greuter 2007). More recently, researchers started to study the biological background of this variation, especially with the help of hybridization experiments, detailed progeny analysis, and molecular tools. Due to numerous hybridization experiments, we now have rather thorough knowledge of the manifestations of residual sexuality in apomictic members of the genus *Pilosella* under experimental conditions (Skalińska 1971a, b, c, 1973, 1976; Gadella 1982, 1984, 1987, 1988, 1991, 1992; Krahulcová and Krahulec 2000; Bicknell et al. 2003; Krahulcová et al. 2004, 2011; Rosenbaumová et al. 2012).

The significance of residual sexuality was also confirmed in natural populations of apomictic plants. Chapman et al. (2000) suggested residual sexuality as a source of genetic variation in *Pilosella officinarum* in New Zealand, where only polyploid apomicts were introduced late in the nineteenth century. In Europe, genetic variation of apomictic *Pilosella* varies from near clonality across large geographic distances in some basic species and stabilized hybridogenous species to substantial genetic variation and multiple origins in recent hybrids (Krahulec et al. 2004; Fehrer et al. 2005). From studies on breeding behaviour also followed that some recent hybrids showed lower penetrance of apomixis and produced more polyhaploid and hybrid progeny than stabilized hybridogenous taxa of the same origin (Krahulec et al. 2008; Krahulcová et al. 2011). Studies of chloroplast DNA haplotypes showed that apomicts served as a maternal parent during origin of almost 50 % of analyzed hybridogenous taxa and recent hybrids (Krahulec et al. 2004, 2008; Fehrer et al. 2005). But only a small number of studies have looked into the manifestation of residual sexuality in the field (e.g., Krahulcová et al. 2009, 2012, 2014; Urfus et al. 2014). More field studies are needed to understand how residual sexuality impacts genetic variation in nature.

In the present study, we attempted to evaluate the significance of residual sexuality as a source of ploidy level variation in a model system consisting of hexaploid, facultatively apomictic *Pilosella bauhini* (Schult.) Arv.-Touv. ($2n = 6x = 54$) and tetraploid, sexual *P. officinarum* Vaill. ($2n = 4x = 36$). Our study followed previous field investigations on hybrid swarms between *P. bauhini* and sexual *P. officinarum* made by Krahulcová et al. (2009, 2014). Their results show that apomictic biotypes might play a substantial role in the formation of population structure because they produced higher ploidy level variation than sexual biotypes. To assess the broader validity of this observation, we decided to study another population consisting of hexaploid *P. bauhini* and tetraploid *P. officinarum*. Our study aimed to address the following questions: (1) What amount of ploidy level variation could be potentially formed in the model system consisting of *P. bauhini* and *P. officinarum* under conditions of experimental crosses? (2) What amount of ploidy level variation is formed under the field conditions at the model locality, and how does this variation differ from that obtained in experimental crosses? (3) Does the ability to form ploidy level variation differ between apomictic and sexual parental species? (4) Are there any cytotypes that are produced by *P. bauhini* or *P. officinarum* which are, however, absent at the locality as adult plants or vice versa?

Materials and methods

Model locality and plant sampling

All plants used in the present study were collected at the locality Valov where recent natural hybridizations between *P. bauhini* and *P. officinarum* take place. The locality is situated in the westernmost part of a warm area of NW Bohemia north of the village of Valov, just on the western periphery of a forest complex. The exact localization of the model population is: 0.99 km WNW of the village of Valov, 2.12 km SES of the town of Podbořany, NW Bohemia, Czech Republic (50°12′34.6″N, 13°24′53″E; about 350 m.a.s.l.). The stand is partly an old orchard and partly an old field with a scattered invasion of young trees of *Pinus sylvestris*.

At the model locality, *P. bauhini* and *P. officinarum* grow intermingled with each other, and plants of hybrid origin are scattered among them. Hybrid plants are easily distinguishable in the population because the parental species, *P. bauhini* and *P. officinarum*, differ in a number of morphological characters and because hybrid plants combine the morphological characters of both of them. *P. officinarum* is characterized by low stature, an unbranched stem and the presence of stellate trichomes on the abaxial side of leaves. *P. bauhini* is usually taller than 0.5 m with many small capitula in cymose inflorescences and lacks stellate trichomes on leaves.

Preliminary sampling at the locality Valov was made in 1998 (Rotreklová et al. 2002). Chromosome counting and analysis of mode of reproduction confirmed presence of facultatively apomictic, hexaploid *P. bauhini* with 54 chromosomes ($x = 9$) and tetraploid *P. officinarum* with 36 chromosomes (Rotreklová et al. 2002). Genome size ($2C$ value) of hexaploid *P. bauhini* was determined to be 11.268 pg, monoploid genome size (C_x value) being 1.879 pg (Suda et al. 2007).

During the years 2002 and 2003, we collected a set of 40 plants morphologically representing the parental species *P. bauhini* and *P. officinarum* together with all morphologically discernible hybrid types. Sampling was repeated in two successive years to ensure that we covered as much as possible of the morphological variation present at the locality. The flowering time of parental species overlaps almost completely but individual plants did not flower at the same time. During a single visit, the non-flowering plants, especially different hybrid types, could be easily overlooked.

In total, 14 plants of *P. bauhini,* 18 plants of *P. officinarum* and 8 plants of hybrid types were collected and transplanted to the Experimental garden of the Institute of Botany, Průhonice, Czech Republic (49°59′40″N, 14°34′01″E).

Plant analyses

The ploidy level of each collected plant was determined using flow cytometry (see below). The genome size was determined in selected plants (for method see Suda et al. 2007). The mode of reproduction (apomixis, sexuality and sterility) was also scored for each plant collected. The potential for apomixis was defined as the ability of the plant to set viable seeds after emasculation: this was done by decapitation of the upper part of an immature capitulum (Ostenfeld 1906; Koltunow et al. 1995). Plants that formed some seeds after emasculation were scored as apomictic. Plants that did not form any seeds after emasculation, but formed them in open-pollinated capitula were scored as sexual. Finally, plants that did not form seeds either after emasculation or after open pollination were scored as sterile. At least three emasculated and three open-pollinated capitula were scored for each plant.

Clone identity was determined in all collected plants of *P. bauhini*, all hybrids and a selection of plants of *P. officinarum* by multilocus DNA fingerprinting (for method, see Fehrer et al. 2005; Krahulcová et al. 2009). The chloroplast DNA (cpDNA) haplotype was assessed for each clone detected by DNA fingerprinting (for method, see Fehrer et al. 2005; Krahulcová et al. 2009). Chloroplasts are inherited only maternally in the genus *Pilosella* (Fehrer et al. 2005). We used cpDNA haplotype analysis to determine whether *P. bauhini* or *P. officinarum* served as the maternal parent of hybrids established at the model locality. This approach was used successfully several times in the past in the genus *Pilosella*.

To quantify ploidy level variation extant in the field, ripe achenes were collected from representative plants of the parental species *P. bauhini* and *P. officinarum* at the Valov locality in 2005. Well developed achenes were germinated in pots filled with sterilized garden soil in a glasshouse. The ploidy level of maternal plants and cultivated seedlings was determined using flow cytometry (see below).

Voucher specimens are preserved in herbarium PR.

Experimental hybridizations

Reciprocal crosses between *P. bauhini* and *P. officinarum* were conducted to quantify their potential to generate ploidy level variation under experimental conditions. For experimental hybridizations, one accession of *P. bauhini* (126/637 BA) and two accessions of *P. officinarum* (625 PI and 632 PI) were chosen as apomictic and sexual parents, respectively; each accession represented a different clone according to DNA fingerprinting. These accessions were chosen because they represented potential crossing partners as they grew in close proximity at the model locality. The accession of *P. bauhini* was the same as that used for chromosome counting and genome size estimation (Rotreklová et al. 2002; Suda et al. 2007).

The accession of *P. bauhini* was reciprocally crossed with each of the two accessions of *P. officinarum*; four crosses were performed in total: 126/637 BA × 625 PI, 126/637 BA × 632 PI, 625 PI × 126/637 BA, and 632 PI × 126/637 BA (the first symbol represents the maternal parent). The design of reciprocal experimental crosses and ploidy levels of progeny that might arise in these crosses is detailed in Fig. 1.

Inflorescences of both maternal and paternal plants were enclosed in fine nylon mesh bags before anthesis to prevent contamination of crosses. Pollination was performed by thoroughly rubbing whole parental capitula together. As individual flowers in the capitulum open successively from the margin to the centre, pollination of each maternal capitulum was repeated on two or three consecutive days, each time with a new capitulum of the same pollen donor. Ripe achenes were harvested 2 weeks later, and the proportion of well developed achenes (checked by tweezers) was assessed. Well developed achenes were germinated in pots filled with sterilized garden soil in a glasshouse. The ploidy level of germinated seedlings was determined using flow cytometry (see below). The design of experimental crosses allowed us to deduce the origin of individual seedlings (fusion of reduced and/or unreduced gametes, haploid parthenogenesis and/or apomixis) based on their ploidy levels (Fig. 1). The only exception was progeny resulting from autogamy and apomixis in hexaploid *P. bauhini*, as they both are hexaploid and thus indistinguishable from each other (see Fig. 1).

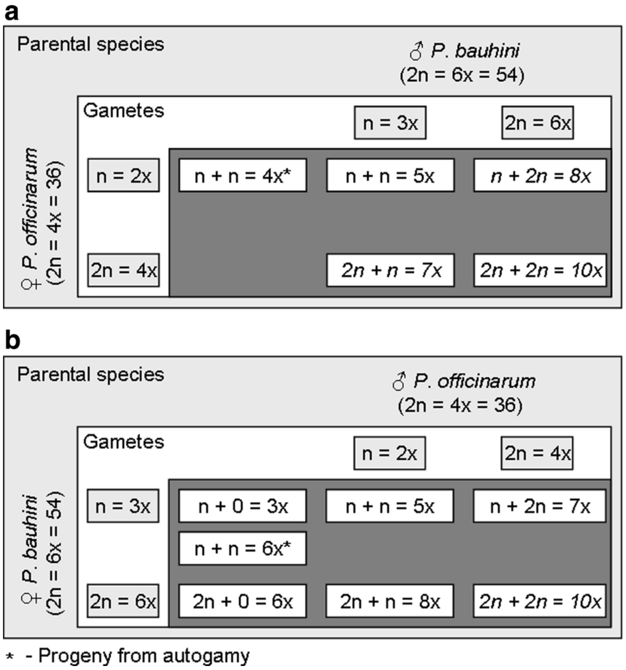

Fig. 1 Ploidy level of individual progeny types potentially arising in the reciprocal crosses between hexaploid, facultatively apomictic *Pilosella bauhini* (accession 126/637 BA) and tetraploid, sexual *P.officinarum* (accession 625 PI and 632 PI). **a** Cross with *P. officinarum* as the maternal parent. **b** Cross with *Pilosella bauhini* as the maternal parent. Progeny types that could theoretically arise but were not detected in our reciprocal crosses are given in *italics*

The crossing experiments were performed in an unheated glasshouse during the years 2004 and 2005.

Ploidy level estimation

We used flow cytometry to determine the ploidy level of all plants collected at the Valov locality, the progeny from crossing experiments and the progeny germinated from achenes collected at the same locality. A Partec PA II flow cytometer (Partec GmbH., Münster, Germany) was used with the modified two-step procedure originally described by Otto (1990)—cf. Rosenbaumová et al. (2012). For each plant analysed, approximately 0.5 cm^2 of a fresh young leaf was chopped together with the leaf tissue of *Bellis perenis* L. (Asteraceae; reference standard; $2C = 2.96$ pg according to Leong-Škorničková et al. 2007) with a sharp razor blade in 0.5 ml of ice-cold Otto I buffer (0.1 M citric acid, 0.5 % Tween 20). The suspension was filtered through a 42 μm nylon mesh. After 20 min incubation (room temperature with occasional shaking), 1 ml of Otto II buffer (0.4 M $Na_2HPO_4 \cdot 12H_2O$) with 4 μg/ml of the AT-selective fluorochrome 4′,6-diamidino-2-phenylindole (DAPI) and 2 μg/ml β-mercaptoethanol was added. 1–2 min were allowed to attain full staining. The fluorescence of at least 5,000 nuclei was recorded for each sample. Only measurements with coefficients of variance (CV) lower than 5 % were taken into account; measurements with CV exceeding 5 % were discarded, and the samples were re-analysed.

Results

Composition of population

Table 1 presents a summary of overall characteristics (ploidy level, genome size, clonal identity, cpDNA haplotype and mode of reproduction) of all plants collected at the model locality. Ploidy level estimations reveal that the model population consists of hexaploid (6*x*) and pentaploid (5*x*) *P. bauhini*, tetraploid (4*x*) *P. officinarum* and various hybrid types at the pentaploid (5*x*), heptaploid (7*x*) and octoploid (8*x*) ploidy level. Absolute genome size of all measured plants fell within the range for corresponding ploidy level/species published in previous study on genome size variation in *Pilosella* (Suda et al. 2007); no intraspecific variation was observed. Hexaploid *P. bauhini* was represented by at least two different clones at the model locality according to the DNA fingerprinting analysis; both clones were assessed as apomictic. Pentaploid *P. bauhini* was represented by one apomictic clone. Each of all ten plants of *P. officinarum* that were put through the DNA fingerprinting analysis belonged to different clones. This result is in accordance with the expectation for sexual species; all 18 collected plants of *P. officinarum* were found to be sexual. Pentaploid hybrids were represented by three clones. Two of them were found to be sexual, and one was nearly sterile. The heptaploid hybrid plant was found to be apomictic. Unfortunately, the octoploid hybrid plant died soon after transplantation before any characteristic could be determined. Most plants from the Valov locality, parental species as well as hybrids, carried the II/7 cpDNA haplotype (haplotype terminology according to Fehrer et al. 2005). Pentaploid *P. bauhini*, one pentaploid hybrid clone (the sterile one) and the heptaploid hybrid carried the II/11 cpDNA haplotype.

Crossing experiments

Crosses with tetraploid, sexual *P. officinarum* as the maternal parent had a rather low yield. Only 22.2 % of all harvested achenes were developed well. A total of 321 F_1 seedlings were recovered from these crosses (Table 2). Most of them, 84.7 %, were represented by pentaploid $n + n$ hybrids with *P. bauhini* (hybrid terminology according to Harlan and de Wet 1975). The remaining 15.3 % were represented by tetraploid $n + n$ progeny from autogamy (Table 2; Fig. 2). In crosses with hexaploid apomictic *P. bauhini* as the maternal parent, on average 82.4 % of all harvested achenes were well developed. A total of 1,058 F_1 seedlings were recovered from these crosses (Table 2). They showed substantially higher variation in ploidy level than progeny from the reciprocal cross; their ploidy levels ranged from triploid to octoploid (Table 2; Figs. 2, 3). The most abundant progeny class was represented by hexaploids (93.4 %). They were most probably of apomictic origin ($2n + 0$ progeny), but some of them could also represent $n + n$ progeny from autogamy (Fig. 1). Hybrids with *P. officinarum* were represented by pentaploid $n + n$ hybrids (4.6 %), heptaploid $n + 2n$ hybrids (0.1 %) and octoploid $2n + n$ hybrids (0.8 %). The last progeny class was represented by trihaploid $n + 0$ progeny originated from parthenogenetic development of reduced female gametes of apomictic *P. bauhini* (1.1 %).

Progeny cultivated from the seeds collected in the field

A total of 790 seedlings were recovered from achenes collected at the Valov locality; their ploidy levels are summarized in Table 3. From the achenes that were collected from tetraploid sexual *P. officinarum*, 318 seedlings were recovered. Most of them (98.7 %) were represented by tetraploids and corresponded morphologically to the maternal *P. officinarum*, most probably representing $n + n$ progeny from allogamy or autogamy in *P. officinarum*. The remaining 1.3 % of seedlings were represented by pentaploid $n + n$ hybrids (Table 3; Fig. 2). From the achenes that were collected from hexaploid apomictic *P. bauhini*, 472 seedlings were recovered. Most of them (92.4 %) were represented by hexaploids originated from apomixis or, less likely, from autogamy. In addition, diverse types of hybrids ranging from pentaploids to decaploids and trihaploid progeny from haploid parthenogenesis were detected (Table 3; Fig. 2).

Table 1 List of plants (species and hybrids) collected at the model locality Valov with data on their ploidy level, genome size, clonal structure, haplotypes, and mode of reproduction

Species	No. of plants	Ploidy level (no. of plants)	Genome size 2*C* value/C_x value (pg DNA)[a]	No. of clones/no. of analysed plants	Haplotype	Mode of reproduction
P. officinarum	18	$4x$ (18)	6.96/1.74 (8)[b]	10/10	II/7 (10)[b]	Sexual (18)[b]
P. bauhini	14	$6x$ (11)	11.28/1.88[c]	2/11	II/7	Apomictic
		$5x$ (3)	10.00/2.00	1/3	II/11	Apomictic
Hybrid plants	8	$5x$ (6)	9.10/1.82	2/5	II/7	Sexual
			–[d]	1/1	II/11	Sterile
		$7x$ (1)	13.30/1.90	1/1	II/11	Apomictic
		$8x$ (1)[e]	–	–	–	–

[a] 2*C* value stands for holoploid genome size, C_x value stands for monoploid genome size (2*C* value/ploidy level)

[b] No. of plants analysed

[c] Published in Suda et al. (2007)

[d] The pentaploid hybrid died before genome size could be measured

[e] The octoploid hybrid died soon after transplantation before any characteristic could be determined

Table 2 Progeny from reciprocal experimental crosses between hexaploid, facultatively apomictic *P. bauhini* and tetraploid, sexual *P. officinarum*

Progeny ploidy	Maternal parent			
	P. officinarum		*P. bauhini*	
	No. of plants	Percentage	No. of plants	Percentage
$3x$	–	–	12	1.1
$4x$	49[a]	15.3	–	–
$5x$	272	84.7	49	4.6
$6x$	–	–	988[b]	93.4
$7x$	–	–	2	0.1
$8x$	–	–	8	0.8
$10x$	–	–	–	–
Sum	321	100.0	1,058	100.0

[a] Progeny from autogamy

[b] Progeny from apomixis (including rare progeny from autogamy)

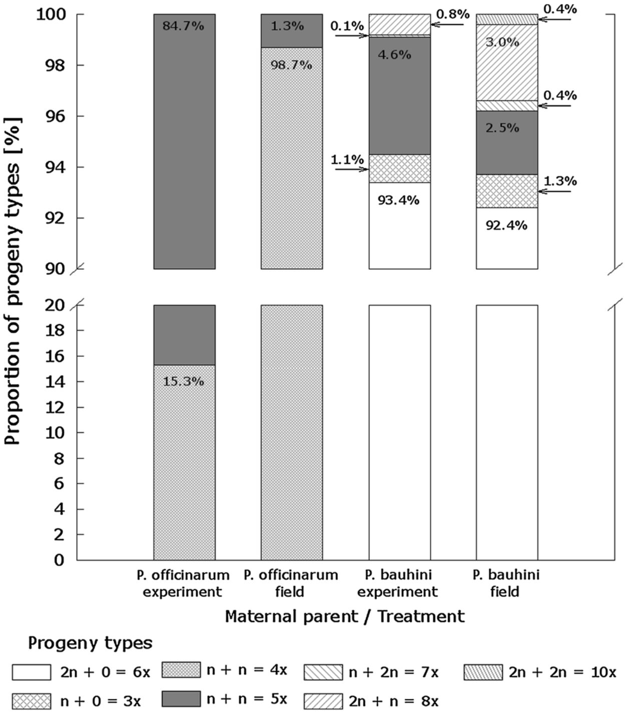

Fig. 2 Proportion of individual progeny types from reciprocal experimental crosses and from the field. Note the *y axis* break and different scale in the *upper part* of the graph. They both serve to highlight minority non-apomictic progeny of facultatively apomictic *P. bauhini*

Discussion

Pilosella officinarum as the maternal parent

The seed sets were rather low in experimental crosses with sexual *P. officinarum* as the maternal parent. On average, well developed achenes formed only 22.2 % of all harvested achenes. This is interesting because it points to some kind of partial reproductive isolation between the parental species *P. officinarum* and *P. bauhini*. However, hybridization barriers are assumed to be weak or virtually non-existent in the genus *Pilosella* (Fehrer et al. 2007; Urfus et al. 2014). Only two other cases of reproductive isolation have been published in the genus *Pilosella*; Fehrer et al. (2007) described total failure of experimental crosses between sexual *P. officinarum* (maternal parent) and apomictic *P. floribunda*, and Krahulcová and Krahulec (2000) found the yield of experimental crosses between sexual *P. officinarum* (maternal parent) and sexual *P. lactucella* to be almost ten times lower than the yield of reciprocal crosses.

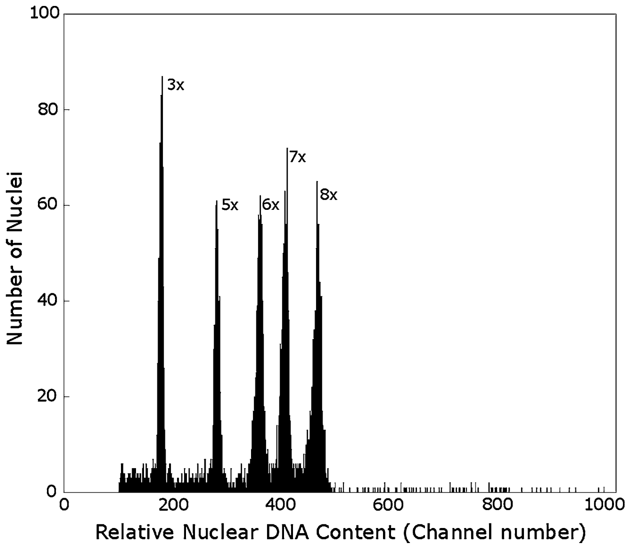

Fig. 3 Histogram of simultaneous cytometric analysis of progeny from experimental hybridization between hexaploid, facultatively apomictic *P. bauhini* (maternal parent) and tetraploid, sexual *P. officinarum*—trihaploid progeny, pentaploid, heptaploid and octoploid hybrid and hexaploid progeny from apomixis

Table 3 Progeny cultivated from seeds collected in the field; seeds were sampled from sexual, tetraploid *P. officinarum* and facultatively apomictic, hexaploid *P. bauhini*

Progeny ploidy	Maternal parent			
	P. officinarum		*P. bauhini*	
	No. of plants	Percentage	No. of plants	Percentage
3*x*	–	–	6	1.3
4*x*	314[a]	98.7	–	–
5*x*	4	1.3	12	2.5
6*x*	–	–	436[b]	92.4
7*x*	–	–	2	0.4
8*x*	–	–	14	3.0
10*x*	–	–	2	0.4
Sum	318	100.0	472	100.0

[a] Progeny from allogamy and/or autogamy in *P. officinarum*

[b] Progeny from apomixis (including rare progeny from autogamy)

In the other experimental crosses with sexual *P. officinarum* as the maternal parent, no traces of reproductive isolation were observed. Reduced pollen fertility in apomictic *P. bauhini* could also be responsible for reduced seed sets in experimental crosses. Nevertheless, apomictic members of the genus *Pilosella* usually produce fertile pollen, including semi-sterile F1 hybrids (Krahulcová et al. 2000; Rotreklová 2008). Further investigations are thus needed to shed more light on the question of reproductive isolation in *P. officinarum* or other members of the genus *Pilosella*.

Diversity of progeny obtained from experimental crosses with sexual *P. officinarum* as the maternal parent was rather low (Table 2; Fig. 2). Only two progeny types were obtained, namely pentaploid $n + n$ hybrids with *P. bauhini* and tetraploid $n + n$ progeny from autogamy. Sexual members of the genus *Pilosella* including *P. officinarum* are generally considered to be self-incompatible under field conditions (Gadella 1984, 1987). Nevertheless, a mixture of self-pollen and foreign pollen can break down self-incompatibility and allow self-fertilization (Krahulcová et al. 1999). This autogamy induced by foreign pollen is known as the mentor effects (reviewed in de Nettancourt 2001). It has been hypothesized several times that mentor effects may play some role in reproductive isolation through promotion of self-fertilization at the expense of hybridization (Rieseberg et al. 1998; Desroches and Rieseberg 1998; Wolf et al. 2001; Mráz 2003; Brock 2004). High levels of induced autogamy (almost 90 %) were detected in some experimental crosses within the genus *Taraxacum* (Morita et al. 1990; Tas and van Dijk 1999). However, generally the level of induced autogamy is not so significant, and the impact of mentor effects on hybridization rates is considered to be rather low. In our experimental crosses, the proportion of autogamously formed progeny reached 15.3 % (Table 2; Fig. 2). A moderate impact of mentor effects on the rate of hybridization can thus be proposed for *P. officinarum*. Nevertheless, without further investigations, particularly at the population level, it is difficult to draw any decisive conclusion about the role of mentor effects as a reproductive barrier in the genus *Pilosella*.

Under field conditions, the hybridization rate was even lower than in the experimental crosses (Table 3; Fig. 2). Pentaploid hybrids formed only 1.3 % of seedlings that were recovered from seeds collected from *P. officinarum* at the model locality Valov. Similar phenomenon was observed in *Taraxacum* where diploid sexuals produced fewer hybrids in mixed populations with triploid apomicts than in experimental hybridizations (Verduijn et al. 2004; Mártonfiová 2006). Reduced hybridization rate in sexual diploids was also observed when they were pollinated with a mixture of pollen from sexual diploids and apomictic triploids (Mártonfiová 2006).

Our field investigations thus show that interspecific hybridizations with *P. officinarum* as the maternal parent occurred at the model locality in a lower frequency than was expected on the basis of the crossing experiments (Tables 2, 3; Fig. 2). Some further mechanisms strengthened reproductive isolation of *P. officinarum* in the field. The main difference between the experimental crosses and the model locality resided in the origin of available pollen. In the experimental crosses, only self-pollen and heterospecific pollen (from *P. bauhini*) was available, allowing only hybridization or induced autogamy. At the model locality, however, *P. officinarum* formed genetically diverse population, as revealed by our multilocus DNA fingerprinting analysis (Table 1). This means that there was an abundance of conspecific pollen donors and competition between conspecific and heterospecific pollen may play substantial role as an interspecific reproductive barrier. The significance of mixed pollination as an important mechanism for preventing hybridization has been confirmed, for example, in *Helianthus*, *Taraxacum* and *Centaurea* (Rieseberg et al. 1995; Verduijn et al. 2004; Mártonfiová 2006; Koutecký et al. 2011).

To a lesser extent, other mechanisms could also contribute to reproductive isolation in the field. We observed that the flowering time of parental species *P. officinarum* and *P. bauhini* overlaps almost completely. Nevertheless, there was a small phenological shift (*P. officinarum* started to flower few days earlier), which could contribute to the reduced rate of interspecific hybridizations. Also, the difference in stature (*P. officinarum* is smaller than *P. bauhini*) could contribute to the reduction of the hybridization rate because it could affect the visiting pattern of pollinators. In addition, possible floral constancy of pollinators may also play some role in promoting reproductive isolation of *P. officinarum*. Behaviour of pollinators represents interesting topic and deserves further study.

Pilosella bauhini as the maternal parent

The seed-sets were rather high in experimental crosses with hexaploid apomictic *P. bauhini* as the maternal parent. On average, well developed achenes formed 82.4 % of all harvested achenes. However, most of the progeny was produced through apomixis without any involvement of the sexual parent *P. officinarum* (Table 2; Fig. 2). The high seed sets thus seemed to be rather a consequence of successful apomixis than a sign of better compatibility in this direction of crosses.

Although most of the progeny was formed through apomixis, its overall diversity was huge in the experimental crosses with apomictic *P. bauhini* as the maternal parent (Table 2; Figs. 2, 3). Ploidy levels of individual progeny types ranged from triploid to octoploid. The observed cytotype diversity seemed to be caused by a highly diverse breeding system in apomictic *P. bauhini*, especially by the participation of unreduced gametes and haploid parthenogenesis, the reproductive pathways that were not detected in *P. officinarum* during the reciprocal crosses. Experimental crosses thus showed that facultatively apomictic *P. bauhini* is able to generate more ploidy level variation than sexual *P. officinarum* under experimental conditions. A similar phenomenon, i.e., more diverse progeny from apomictic than from sexual mothers, was observed before in the genus *Pilosella* in experimental crosses between hexaploid facultatively apomictic *P. rubra* and tetraploid sexual *P. officinarum*. In these crosses, ploidy levels of progeny ranged also from triploid to octoploid when apomictic *P. rubra* served as the maternal parent, but only pentaploid hybrids, one heptaploid hybrid and tetraploids from autogamy were formed in reciprocal crosses with sexual *P. officinarum* as the maternal parent (Krahulcová and Krahulec 2000; Krahulcová et al. 2004, 2011; Rosenbaumová et al. 2012). Moreover, previous field studies on hybrid swarms between *P. bauhini* and *P. officinarum* identified apomictic biotypes as the main sources of ploidy level variation (Krahulcová et al. 2009, 2014).

During our field investigations, we found that at the model locality *P. bauhini* generated even higher ploidy level variation than in the experimental crosses (Table 3; Fig. 2). A new cytotype was detected that was represented by two decaploid plants. Heptaploid and octoploid hybrids were formed at the model locality at a higher frequency than in experimental crosses (Tables 2, 3; Fig. 2). Pentaploid hybrids, on the other hand, were formed at the model locality at a lower frequency than in experimental crosses. As a result, octoploids represented the most frequent type of sexually derived seed progeny of *P. bauhini* at the model locality instead of pentaploid $n + n$ hybrids, which was the case in experimental crosses.

The composition of the model population might be a main cause of the difference between the results of experimental crosses and hybridizations at the model locality. The model population consisted not only of parental species, which were used in experimental crosses, but also of established hybrids (Table 1). Pollen of established hybrids might also participate in hybridizations at the model locality and change the overall diversity of progeny and proportions of individual progeny types. For example, decaploid hybrids could arise as $2n + 2n$ hybrids between the parental species *P. bauhini* and *P. officinarum*, representing a low probability feature that was not detected during experimental crosses (Fig. 1). However, they could also represent $2n + n$ hybrids between hexaploid *P. bauhini* and heptaploid or octoploid hybrids that grow at the model locality (Table 1). The same holds for the rest of the hybrids originated at the model locality.

The genetic variation of parental species might also play some role at the model locality. In experimental crosses, we used only two accessions of *P. officinarum*, but at the model locality many different clones (and thus potential pollen donors) were presented. Possible difference in compatibility between *P. bauhini* and different clones of *P. officinarum* might contribute to observed difference between the results of experimental crosses and hybridizations at the model locality.

Population structure

Experimental crosses and field investigations confirmed that hybridizations between *P. officinarum* and *P. bauhini* were a significant source of ploidy level variation; especially, when *P. bauhini* served as the maternal parent (Tables 2, 3; Figs. 2, 3). However, the ploidy level variation occurring at the model locality was lower than that obtained from experimental crosses and detected among progeny germinated from seeds collected at the model locality (Table 1). Neither decaploid hybrids nor trihaploids were detected in the field as adult plants. It seems as if some disadvantage impeded the establishment of high polyploids and polyhaploids at the model locality. Krahulcová and Krahulec (2000) have already pointed out that the range of progeny types obtained from experimental crosses within the genus *Pilosella* is usually more extensive than the range of plants found in natural populations.

High polyploids

High polyploids (cytotypes above the hexaploid level) are rare in nature despite the fact that they are regularly produced in experimental crosses between members of the genus *Pilosella* (Gadella 1988; Skalińska 1973, 1976; Bicknell et al. 2003; Bicknell and Koltunow 2004; Krahulcová et al. 2004). These hybrids arise through participation of unreduced gametes; more frequently unreduced female gametes participate in hybrid formation than male ones (Gadella 1988; Krahulcová et al. 2004, 2011). In our study, the frequency of progeny from unreduced female gametes was approximately eight times higher than that of unreduced male gametes when *P. bauhini* served as the maternal parent (Tables 2, 3; Fig. 2). Interestingly, in reciprocal crosses, no unreduced gametes participated at all. This fact indicates that sexual *P. officinarum* has a more regular meiosis than apomictic *P. bauhini*.

Octoploids represent the highest ploidy level ever found in the field at the adult stage, and all octoploids published to date come from hybrid swarms between *P. bauhini* and *P. officinarum* (Krahulcová and Krahulec 2000; Rotreklová et al. 2002). Higher ploidy levels are known only from experimental crosses (Skalińska 1976; Gadella 1988). Our decaploid hybrids represent the first documented origin of this ploidy level in the field (Table 3). Nevertheless, we did not found any decaploid adult at the model locality. It is thus questionable whether they would be able to survive in nature.

Why are high polyploids rare or not present in the field? What impedes their successful establishment and perpetuation? It is hypothesized that high polyploidy entails lower ability to survive (Gadella 1988). Recent studies on reproductive behaviour of high-polyploid hybrids from crosses between apomictic and sexual parent offer another possible explanation (Krahulec et al. 2008; Krahulcová et al. 2009, 2011). The authors of these studies observed that the genome of newly formed $2n + n$ hybrids tended to be “unstable”. Despite the fact that these hybrids contained the whole ($2n$) genome of the apomictic mother, they displayed variable progeny and mostly low penetrance of apomixis. Instead of apomictically, they preferably reproduced either through $n + n$ mating or through haploid parthenogenesis and formed mostly progeny with a lower ploidy level than is their own. This fact may contribute to the rarity of high polyploids in nature because without a sufficient level of apomixis the newly formed high polyploid hybrids cannot effectively perpetuate and spread.

A hint of reduced penetrance of apomixis was observed also in the heptaploid hybrid from the model locality Valov (unpublished results). Only half of the progeny from open-pollinated capitula were formed apomictically. Remaining progeny originated from sexual reproduction. We were unable to ascertain the mode of reproduction in the octoploid hybrid from the model locality Valov because it was rather small and weak, so it perished almost immediately after transplantation. Its capitula, however, contained 53 mature achenes at the time of sampling. Only four seedlings germinated from these achenes, each having a different ploidy level: hexaploid, octoploid, decaploid and dodecaploid (unpublished results). The low germination rate and high diversity of progeny testified for low fertility and a tendency for instability in this octoploid hybrid, too. Nevertheless, these observations are preliminary, and further investigations are needed to draw any significant conclusion regarding reproductive behaviour in high polyploids from the model locality Valov.

Polyhaploids

Polyhaploids are well known from hybridization and emasculation experiments with apomictic members of the genus *Pilosella*, but their occurrence in nature is elusive (Krahulcová et al. 2004; Krahulec et al. 2006). To date only one occurrence of a polyhaploid has been published from nature (Šumava Mts., Czech Republic; Krahulec et al. 2008). The polyhaploid was triploid and grew in the neighbourhood of a recently arisen unstable hexaploid hybrid between *P. aurantiaca* and *P. officinarum*. The hybrid was characterized by formation of a high proportion of trihaploid progeny under experimental conditions (39.0 %; Krahulec et al. 2008; Krahulcová et al. 2011). In the genus *Pilosella*, there are plants that form even higher proportions of polyhaploid progeny exceeding 80 %, but the production of polyhaploids is usually lower and does not exceed 5 % (Krahulec et al. 2011). *P. bauhini* from the model locality Valov formed 1.1 and 1.3 % of polyhaploids in experimental crosses and in the field, respectively (Tables 2, 3).

Experimentally produced polyhaploids are usually weak and sometimes even sterile (Krahulcová and Krahulec 2000; Krahulec et al. 2011). Trihaploids obtained from our experimental crosses between *P. bauhini* and *P. officinarum* were no exception. In addition, Krahulec et al. (2006) observed strong selection against polyhaploids during germination and early seedling establishment in progeny of apomictic hexaploid *P. rubra*. Low production of trihaploids and their low viability could explain why we did not detect any triploid plant at the model locality (Table 1). Trihaploids thus do not seem to have played any significant role in the evolution of the model population.

The origin of established hybrids in the field

Experimental crosses and population investigations revealed that facultatively apomictic *P. bauhini* hybridizes more readily and formed more diverse progeny than sexual *P. officinarum* (Tables 2, 3; Fig. 2). Published analyses show that facultative apomicts serves as the maternal parent of numerous natural hybrids and hybridogenous species, e.g., *P. glomerata*, *P. iserana*, *P. rubra*, hexaploid *P. stoloniflora*, and part of tetra- and hexaploid *P. piloselliflora* (Krahulec et al. 2004, 2008; Fehrer et al. 2005, 2007). In previously analysed hybrid swarms between *P. bauhini* and *P. officinarum*, apomicts participated as maternal parents in twice as many hybrids as sexual plants (Krahulcová et al. 2009, 2013).

In our model population, hexploid *P. bauhini* belonged to the same cpDNA haplotype as *P. officinarum* (haplotype II/7; Table 1). Pentaploid *P. bauhini*, however, carried a different cpDNA haplotype (haplotype II/11; Table 1). Because one pentaploid hybrid and the heptaploid hybrid carried also cpDNA haplotype II/11 (Table 1), it is justified to suggest that pentaploid *P. bauhini* was involved as the maternal parent during their origin. In contrast, the remaining two pentaploid hybrid clones carried cpDNA haplotype II/7, and thus it was impossible to determine whether hexaploid *P. bauhini* or *P. officinarum* served as the maternal parent during their origin.

Monoploid genome size (C_x value) differed between parental species, being 1.74, 1.88 and 2.00 pg in *P. officinarum*, hexaploid *P. bauhini* and pentaploid *P. bauhini*, respectively (Table 1; for information on genome size variation in genus *Pilosella* see Suda et al. 2007). Genome size of hybrid plants thus could be used as supportive character to infer their origin (Suda et al. 2007). Genome size of pentaploid hybrids with haplotype II/7 corresponded to theoretical value for $n + n$ hybrids between hexaploid *P. bauhini* and tetraploid *P. officinarum* almost completely (9.10 vs. 9.12 pg). Genome size of heptaploid hybrid corresponded best to theoretical value for $2n + n$ hybrids between pentaploid *P. bauhini* and tetraploid *P. officinarum* (13.30 vs. 13.48 pg). Unfortunately, pentaploid hybrid with haplotype II/11 died before genome size could be measured.

Conclusions

Our investigations show that it is not always possible to extrapolate results of simplified experimental crosses to complex situations in the field, where many factors influence the actual breeding pattern. We have confirmed the results of previous studies (Krahulcová et al. 2009, 2012, 2013) that residual sexuality represents a significant source of ploidy level variation in populations consisting of facultatively apomictic *P. bauhini* and sexual *P. officinarum*. Surprisingly, the species showing stronger reproductive isolation in the field was sexual *P. officinarum*. The question of reproductive isolation of sexual *P. officinarum* is interesting and deserves more attention, as sexual tetraploid *P. officinarum* is the central species of many hybrid swarms in central Europe.

Acknowledgments We thank to Anna Krahulcová for many critical and useful comments. This work was financially supported by the Ministry of Culture of the Czech Republic (DKRVO 2013/09, National Museum, 00023272 to RR) and by the long-term institutional research plan No. AVOZ60050516 from the Academy of Sciences of the Czech Republic (to FK).

References

Asker SE, Jerling L (1992) Apomixis in plants. CRC Press, Boca Raton

Bayer RJ, Chandler GT (2007) Evolution of polyploid agamic complexes: a case study using the *Catipes* group of *Antennaria*, including the *A. rosea* complex (Asteraceae: Gnaphalieae). In: Grossniklaus U, Hörandl E, Sharbel T, van Dijk P (eds) Apomixis: evolution, mechanisms and perspectives. Regnum Vegetabile 147, Koelz Scientific Books, Koenigstein, pp 317–336

Bicknell RA, Koltunow AM (2004) Understanding apomixis: recent advances and remaining conundrums. Plant Cell 16:228–245

Bicknell RA, Lambie SC, Butler RC (2003) Quantification of progeny classes in two facultatively apomictic accessions of *Hieracium*. Hereditas 138:11–20

Bräutigam S, Greuter W (2007) A new treatment of *Pilosella* for the Euro-Mediterranean flora [Notulae ad floram euro-mediterraneam pertinentes 24]. Willdenowia 37(1):123–137

Brock MT (2004) The potential for genetic assimilation of a native dandelion species, *Taraxacum ceratophorum* (Asteraceae), by the exotic congener *T. officinale*. Amer J Bot 91:656–663

Chapman HM, Parh D, Oraguzie N (2000) Genetic structure and colonizing success of a clonal, weedy species, *Pilosella officinarum* (Asteraceae). Heredity 84:401–409

Darlington CD (1939) The evolution of genetic systems. Cambridge University Press, Cambridge de Nettancourt D (2001) Incompatibility and incongruity in wild and cultivated plants, 2nd edn. Springer, Berlin

Desroches AM, Rieseberg LH (1998) Mentor effects in wild species of *Helianthus* (Asteraceae). Amer J Bot 85:770–775

Dickinson TA (1998) Taxonomy of agamic complexes in plants: a role for metapopulation thinking. Folia Geobot 33:327–332

Fehrer J, Šimek R, Krahulcová A, Krahulec F, Chrtek J, Bräutigam E, Bräutigam S (2005) Evolution, hybridisation, and clonal distribution of apo- and amphimictic species of *Hieracium* subgen. *Pilosella* (Asteraceae, Lactuceae) in a Central European mountain range. In: Bakker FT, Chatrou LW, Gravendeel B, Pelser PB (eds) Plant species-level systematics: new perspectives on pattern and process. Regnum Vegetabile 143, Koelz Scientific Books, Koenigstein, pp 175–201

Fehrer J, Krahulcová A, Krahulec F, Chrtek J Jr, Rosenbaumová R, Bräutigam S (2007) Evolutionary aspects in *Hieracium* subgenus *Pilosella*. In: Grossniklaus U, Hörandl E, Sharbel T, van Dijk P (eds) Apomixis: evolution, mechanisms and perspectives. Regnum Vegetabile 147, Koelz Scientific Books, Koenigstein, pp 359–390

Gadella ThWJ (1982) Cytology and reproduction of *Hieracium pilosella* L. and some related diploid species. Acta Bot Neerl 31:140–141

Gadella ThWJ (1984) Cytology and the mode of reproduction of some taxa of *Hieracium* subgenus *Pilosella*. Proc K Ned Akad Wet 87:387–399

Gadella ThWJ (1987) Sexual tetraploid and apomictic pentaploid populations of *Hieracium pilosella* (Compositae). Plant Syst Evol 157:219–246

Gadella ThWJ (1988) Some notes on the origin of polyploidy in *Hieracium pilosella* aggr. Acta Bot Neerl 37:515–522

Gadella ThWJ (1991) Variation, hybridization and reproductive biology of *Hieracium pilosella* L. Proc K Ned Akad Wet 94(4):455–488

Gadella ThWJ (1992) Notes of some triple and inter-sectional hybrids in *Hieracium* L. subgenus *Pilosella* (Hill) S. F. Gray. Proc K Ned Akad Wet 95:51–63

Grossniklaus U (2001) From sexuality to apomixis: molecular and genetic approaches. In: Savidan Y, Carman JG, Dresselhaus T (eds) The flowering of apomixis: from mechanisms to genetic engineering. CIMMYT, IRD, EC, Mexico, pp 168–211

Grossniklaus U, Nogler GA, van Dijk PJ (2001) How to avoid sex: the genetic control of gametophytic apomixis. The Plant Cell 13(7):1491–1497

Harlan JR, de Wet JMJ (1975) On Ö. Winge and a prayer: the origins of polyploidy. Bot Rev 41:361–390

Hörandl E (1998) Species concepts in agamic complexes: applications in the *Ranunculus auricomus* complex and general perspectives. Folia Geobot 33:335–348

Koltunow AM (1993) Apomixis: embryo sacs and embryos formed without meiosis or fertilization in ovules. The Plant Cell 5:1425–1437

Koltunow AM, Bicknell RA, Chaudhury AM (1995) Apomixis: molecular strategies for the generation of genetically identical seeds without fertilization. Plant Physiol 108:1345–1352

Koltunow AM, Johnson SD, Bicknell RA (1998) Sexual and apomictic development in Hieracium. Sex Plant Reprod 11:213–230

Koutecký P, Bad'urová T, Štech M, Košiar J, Karásek J (2011) Hybridization between diploid *Centaurea pseudophrygia* and tetraploid *C. jacea* (Asteraceae): the role of mixed pollination, unreduced gametes, and mentor effects. Biol J Linn Soc 104:93–106

Krahulcová A, Krahulec F (2000) Offspring diversity in *Hieracium* subgen. *Pilosella* (Asteraceae): new cytotypes from hybridization experiments and from open pollination. Fragm Florist Geobot 45(1–2):239–255

Krahulcová A, Chrtek J, Krahulec F (1999) Autogamy in *Hieracium* subgen. Pilosella Folia Geobot 34:373–376

Krahulcová A, Krahulec F, Chapman HM (2000) Variation in *Hieracium* subgen. *Pilosella* (Asteraceae): what do we know about its sources? Folia Geobot 35:319–338

Krahulcová A, Papoušková S, Krahulec F (2004) Reproduction mode in the allopolyploid facultatively apomictic hawkweed *Hieracium rubrum* (Asteraceae, *H.* subgen. *Pilosella*). Hereditas 141:19–30

Krahulcová A, Rotreklová O, Krahulec F, Rosenbaumová R, Plačková I (2009) Enriching ploidy level diversity: the role of apomictic and sexual biotypes of *Hieracium* subgen. *Pilosella* (Asteraceae) that coexist in polyploid populations. Folia Geobot 44:281–306

Krahulcová A, Krahulec F, Rosenbaumová R (2011) Expressivity of apomixis in 2n + n hybrids from an apomictic and a sexual parent: insights into variation detected in *Pilosella* (Asteraceae: Lactuceae). Sex Plant Reprod 24:63–74

Krahulcová A, Raabe U, Krahulec F (2012) Prozesse innerhalb hybridisierender *Pilosella*-Populationen: *P. aurantiaca* und *P. officinarum* in Hagen (Nordrhein-Westfalen). Kochia 6:123–142

Krahulcová A, Rotreklová O, Krahulec F (2014) The detection, rate and manifestation of residual sexuality in apomictic populations of *Pilosella* (Asteraceae, Lactuceae). Folia Geobot 49(2). doi:10.1007/s12224-013-9166-0

Krahulec F, Krahulcová A, Fehrer J, Bräutigam S, Plačková I, Chrtek J Jr (2004) The Sudetic group of *Hieracium* subgen. *Pilosella* from the Krkonoše Mts: a synthetic view. Preslia 76:223–243

Krahulec F, Krahulcová A, Papoušková S (2006) Ploidy level selection during germination and early stages of seedling growth in the progeny of allohexaploid facultative apomict, *Hieracium rubrum* (Asteraceae). Folia Geobot 41:407–416

Krahulec F, Krahulcová A, Fehrer J, Bräutigam S, Schuhwerk F (2008) The structure of the agamic complex of *Hieracium* subgen. *Pilosella* in the Šumava Mts and its comparison with other regions in Central Europe. Preslia 80:1–26

Krahulec F, Krahulcová A, Rosenbaumová R, Plačková I (2011) Production of polyhaploids by facultatively apomictic *Pilosella* can result in formation of new genotypes via genome doubling. Preslia 83:471–490

Leong-Škorničková J, Šída O, Jarolímová V et al (2007) Chromosome numbers and genome size variation in Indian species of *Curcuma* (Zingiberaceae). Ann of Bot 100:505–526

Loomis ES, Fishman L (2009) A continent wide clone: population genetic variation of the invasive plant *Hieracium aurantiacum* (orange hawkweed; Asteraceae) in North America. Int J Plant Sci 170(6):759–765

Mártonfiová L (2006) Possible pathways of the gene flow in *Taraxacum* Sect. *Ruderalia*. Folia Geobot 41:183–201

Matzk F, Meister A, Brutovska R, Schubert I (2001) Reconstruction of reproductive diversity in *Hypericum perforatum* L. opens novel strategies to manage apomixis. Plant J 26:275–282

Matzk F, Hammer K, Schubert I (2003) Co-evolution of apomixis and genome size within the genus *Hypericum*. Sex Plant Reprod 16:51–58

Morita T, Menken SBJ, Sterk AA (1990) Hybridization between European and Asian dandelions (*Taraxacum* section *Ruderalia* and section *Mongolica*). 1. Crossability and breakdown of self-incompatibility. New Phytol 114:519–529

Mráz P (2003) Mentor effects in the genus *Hieracium* s.str. (Compositae, Lactuceae). Folia Geobot 38(3):345–350

Nägeli C, Peter A (1885) Die Hieracien Mittel–Europas. Monographische Bearbeitung der Piloselloiden mit besonderer Berücksichtigung der mitteleuropäischen Sippen. R. Oldenbourg, München

Naumova TN (2008) Apomixis and amphimixis in flowering plants. Cytol Genet 42:51–53

Naumova TN, van der Laak J, Osadtchiy J, Matzk F, Kravtchenko A, Bergervoet J, Ramulu KS, Boutilier K (2001) Reproductive development in apomictic populations of *Arabis holboellii* (Brassicaceae). Sex Plant Reprod 14:195–200

Nogler GA (1984) Gametophytic apomixis. In: Johri BM (ed) Embryology of angiosperms. Springer, Berlin, pp 475–518

Noyes RD, Rieseberg LH (2000) Two independent loci control agamospermy (apomixis) in the triploid flowering plant *Erigeron annuus*. Genet 155:379–390

Ostenfeld CH (1906) Experimental and cytological studies in the Hieracia. I. Castration and hybridization experiments with some species of Hieracia. Bot Tidsskr 27:225–248

Otto F (1990) DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA. Methods in Cell Biol 33:105–110

Richards AJ (2003) Apomixis in flowering plants: an overview. Philos Trans R Soc Lond Series B Biological Sciences 358:1085–1093

Rieseberg LH, Desrochers AM, Youn SJ (1995) Interspecific pollen competition as a reproductive barrier between sympatric species of *Helianthus* (Asteraceae). Amer J Bot 82(4):515–519

Rieseberg LH, Baird SJE, Desrochers AM (1998) Patterns of mating in wild sunflower hybrid zones. Evolution 52(3):713–726

Rosenbaumová R, Krahulcová A, Krahulec F (2012) The intriguing complexity of parthenogenesis inheritance in *Pilosella rubra* (Asteraceae, Lactuceae). Sex Plant Reprod 25(3):185–196

Rotreklová O (2008) *Hieracium* subgen. *Pilosella*: pollen stainability in sexual, apomictic and sterile plants. Biol 63(1):61–66

Rotreklová O, Krahulcová A, Vaňková D, Peckert T, Mráz P (2002) Chromosome numbers and breeding systems in some species of *Hieracium* subgen. *Pilosella* from Central Europe. Preslia 74:27–44

Sharbel TF, Mitchell-Olds T (2001) Recurrent polyploid origins and chloroplast phylogeography in the *Arabis holboellii* complex (Brassicaceae). Heredity 87:59–68

Skalińska M (1971a) Experimental and embryological studies in *Hieracium aurantiacum* L. Acta Biol Cracov Ser Bot 14:139–159

Skalińska M (1971b) Further studies in apomixis of *Hieracium aurantiacum* L. Genet Pol 12:209–212

Skalińska M (1971c) Further studies in twins of *Hieracium aurantiacum* L. Acta Biol Cracov Ser Bot 14:43–53

Skalińska M (1973) Further studies in facultative apomixis of *Hieracium aurantiacum* L. Acta Biol Cracov Ser Bot 16:121–133

Skalińska M (1976) Cytological diversity in the progeny of octoploid facultative apomicts of *Hieracium aurantiacum*. Acta Biol Cracov Ser Bot 19:39–46

Stebbins GL (1950) Variation and evolution in plants. Columbia University Press, New York

Suda J, Krahulcová A, Trávníček P, Rosenbaumová R, Peckert T, Krahulec F (2007) Genome size variation and species relationships in *Hieracium* sub-genus *Pilosella* (Asteraceae) as inferred by flow cytometry. Ann of Bot 100:1323–1335

Tas ICQ, van Dijk PJ (1999) Crosses between sexual and apomictic dandelions (*Taraxacum*). I. The inheritance of apomixis. Heredity 83:707–714

Urfus T, Krahulec F, Krahulcová A. (2014) Hybridization within a *Pilosella* population: a morphometric analysis. Folia Geobot 49(2). doi:10.1007/s12224-013-9179-8

van Dijk PJ, Vijverberg K (2005) The significance of apomixis in the evolution of the angiosperms: a reappraisal. In: Bakker FT, Chatrou LW, Gravendeel B, Pelser PB (eds) Plant species-level systematics: new perspectives on pattern and process. Regnum Vegetabile 143, Koelz Scientific Books, Koenigstein, pp 175–201

van Dijk PJ, van Baarlen P, de Jong JH (2003) The occurrence of phenotypically complementary apomixis-recombinants in crosses between sexual and apomictic dandelions (*Taraxacum officinale*). Sex Plant Reprod 16:71–76

Verduijn MH, Van Dijk PJ, Van Damme JMM (2004) The role of tetraploids in the sexual–asexual cycle in dandelions (*Taraxacum*). Heredity 93:390–398

Whitton J, Sears CJ, Baack EJ, Otto SP (2008) The dynamic nature of apomixis in the angiosperms. Int J Plant Sci 169(1):169–182

Wolf DE, Takebayashi N, Rieseberg LH (2001) Predicting the risk of extinction through hybridization. Conserv Biol 15(4):1039–1053

Zahn KH (1922–1930) *Hieracium*. In: Ascherson P, Graebner P (eds) Synopsis der mitteleuropäischen Flora 12(1). Gebrüder Bornträger, Leipzig, pp 1–492