

Hybridization within a *Pilosella* Population: a Morphometric Analysis

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Abstract We traced hybridization processes taking place within a mixed population of *Pilosella piloselloides* subsp. *bauhini* and *P. officinarum* by means of a morphometric analysis of plants sampled in the field. Our results show that hybridization is frequent between the two taxa as well as between their two stabilized hybrids (*P. brachiata* and *P. leptophyton*). Plants utilizing three different modes of reproduction (sexual, facultatively apomictic and variable) participated in these hybridizations, *Pilosella brachiata* being the most important player. We identified several trends in progeny morphology, which evidently reflect different reproductive pathways, namely sexuality, apomixis and haploid parthenogenesis, occurring within the population under study. Introgression into sexual *P. officinarum* is commonplace.

Keywords Hybridization · Introgression · Multivariate morphometrics · *Pilosella*

Introduction

Enormous variation is a key feature of the genus *Pilosella* Vaill. (*Hieracium* subgen. *Pilosella* (Hill) Gray). This variation has several sources: hybridization accompanied by polyploidization (detected cytotypes: $2x$, $3x$, $4x$, $5x$, $6x$, $7x$, $8x$; $x=9$), variation in reproductive mode (apomixis combined with sexuality, often within a single capitulum)

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and vegetative reproduction via stolons (Gadella 1987, 1991; Krahulcová et al. 2000). These sources of variation are accompanied by the production of fertile pollen, even in the case of apomicts and completely sterile plants such as F1 triploids, and the occurrence of haploid parthenogenesis or autogamy via the mentor effect (Krahulcová et al. 1999, 2000; Krahulcová and Krahulec 2001). Moreover, all of these phenomena are often combined in intricate ways. Apomixis in apomictic lineages is facultative, so plants can hybridize with other cytotypes or taxa, giving rise to an enormous number of different morphological lineages and cytotypes (Krahulcová and Krahulec 2001). Several additional important sources of variation have not been studied exhaustively. The frequency of unreduced gametes, for example, has only rarely been assessed (e.g., Krahulcová et al. 2004). Last but not least, nothing is known about the importance of subsequent genome duplication in polyhaploids, which frequently results in the formation of highly distinct lineages (Krahulec et al. 2011).

Seminal studies of *Pilosella* hybrids were carried out at the dawn of modern genetics (Mendel 1869) and only shortly after the discovery of apomixis (Ostenfeld and Raunkiaer 1903; Ostenfeld 1906). Hybridogenous species form not only between basic species but also between individual hybridogenous species. New types therefore arise as a result of both one-step and multi-step hybridization events. This finding has led to the creation of a rather complicated taxonomic system (e.g. Nägeli and Peter 1885; Schuhwerk and Fischer 2003; for a contrasting approach, see Tyler 2001). The ease of detecting hybrids had prompted many researchers to limit their work to merely describing even more of them. In geographically limited areas, molecular markers such as chloroplast haplotypes have been used to reveal the maternal parent. Unexpectedly, apomictic plants turned out to be the mother plants of about half of the hybrids and hybridogenous species (Krahulec et al. 2004, 2008). There are surprisingly few studies on topics such as hybridization and related processes including introgression at the population level (Krahulcová et al. 2009, 2012). In a detailed study of two model populations, Krahulcová et al. (2009) show that apomictic maternal plants produce more variation with respect to ploidy level than sexual maternal plants. Because that study does not consider different morphological types of hybrids in detail, we investigated one of these populations in hope that we could demonstrate the directions of hybridization by examining morphological characters. These processes are related to the reproductive mode of maternal plants, which produce seed progeny in the field.

This state of knowledge has inspired us to ask and attempt to answer the following questions: *i*) To what extent are microevolutionary processes such as polyploidy, hybridization and introgression reflected in morphology? *ii*) Does the progeny of plants with different reproduction modes differ in morphology? *iii*) What is the pattern of variation in morphology of the most frequent progeny of *Pilosella brachiata* (DC.) F.W. Schultz & Sch. Bip.?

Material and Methods

Population under Study

The study population (Praha-Vysočany, see Krahulcová et al. 2009) comprised two basic species, *Pilosella officinarum* Vaill. and *P. piloselloides* subsp. *bauhini* (Schult.) S.

Bräutigam & Greuter (hereafter referred to as *P. *bauhini*; taxonomic treatment according to Bräutigam and Greuter 2007). These species markedly differ in their morphology. *Pilosella officinarum* has one large capitulum and a dense cover of stellate trichomes underleaf. *Pilosella *bauhini* is a tall plant with many small capitula in its cymose inflorescence and without any stellate trichomes on its leaves. These two species hybridize freely and frequently, but their morphological characters allow easy identification of the resulting hybrids. Based on morphology, they form two types of hybrids. (1) *Pilosella brachiata* ($P. *bauhini \leq P. officinarum$; symbols "<, >, ≤, ≥" at hybrids express the degree of morphological similarity to parental species) is characterized by a range of scattered to abundant stellate trichomes underleaf, relatively few capitula and a short, deeply branched stem (e.g. Chrtek 2004). It corresponds to primary $n + n$ hybrids or to different products of backcrossing with *P. officinarum*. (2) *Pilosella leptophyton* (Nägeli & Peter) P.D. Sell & C. West ($P. *bauhini > P. officinarum$) has more abundant but smaller capitula in a cymose inflorescence and scattered stellate trichomes underleaf. *Pilosella leptophyton* results from backcrossing of *P. brachiata* with *P. *bauhini* or gets produced directly as a $2n + n$ hybrid (from unreduced gametes of *P. *bauhini*).

Pilosella officinarum at the locality under study (see Krahulcová et al. 2009) was mostly tetraploid and sexual, although several plants were found to be sexual pentaploids. *Pilosella *bauhini* was determined to be tetra-, penta-, hexa- and heptaploid at this locality, and its individuals of all ploidy levels were apomictic. A complex pattern emerged in the ploidy composition and reproductive modes of hybrids. There were many aneuploids and combinations that exhibited different reproductive modes, which was especially apparent in *P. brachiata*. A combination of sexual and apomictic reproduction can be viewed as a third mode of reproduction, the so-called variable mode, which is predominantly sexual but partly involves haploid parthenogenesis and apomixis. Apomictic plants usually retain a level of residual sexuality of up to 10%. Specimens with the variable mode, which were usually $2n + n$ hybrids that resulted from an apomictic and a sexual parent, retained up to 80% of their sexuality. Such hybrids therefore produce more variation compared to their apomictic parent because they form a low proportion of progeny that is identical to the mother plant. They also often form a high proportion of polyhaploid and/or hybrid progeny.

Plant Material

We used herbarium specimens (deposited in PRA) that originated from the hybrid population in Prague (Praha-Vysočany; Krahulcová et al. 2009). We studied both mature plants that were established at the locality and their seed progeny. This progeny was cultivated from seeds, and its morphological characters were evaluated together with their maternal parent. The population was formed by *P. officinarum*, *P. bauhini* and various morphotypes of their hybrids ($P. brachiata = P. bauhini \leq P. officinarum$, $P. leptophyton = P. bauhini > P. officinarum$). The following two sets of plants, which corresponded to the material that was sampled and processed in a previous study (Krahulcová et al. 2009: locality 1 in Fig. 1), were analyzed: (1) The maternal dataset consisted of 86 plants collected in the field and chosen for subsequent cultivation in the experimental garden of the Institute of Botany, ASCR Průhonice. The chromosomes of all 86 plants were counted. The DNA ploidy level of the progeny was determined using flow cytometry, and the chromosomes of the progeny class representatives were counted to confirm the accuracy

of the flow cytometry (see Table 1). The reproductive mode (parthenogenetic *versus* sexual; see Table 1) of all plants was confirmed using a routine emasculation test (Gadella 1987; Krahulcová and Krahulec 1999). A karyological analysis of the progeny (detection of ploidy and chromosome number variation) allowed us to distinguish between the variable and the apomictic reproductive mode in plants which produced some parthenogenetic seeds in the emasculation test (Gadella 1987; Krahulcová and Krahulec 1999). (2) The filial dataset (progeny) was obtained from seeds from the maternal plants collected 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. Seedlings were cultivated to the rosette stage. The ploidy (or chromosome number) was determined to distinguish between the apomictic and the variable reproductive mode of corresponding maternal plants (see Table 1). In total, 224 plants were cultivated to maturity and collected for the herbarium. These plants were characterized by the taxonomic identity of their maternal plant, their reproductive mode and their chromosome number.

Morphometric Methods

In total, we measured or scored 21 morphological characters (quantitative, semiquantitative or binary) on 310 herbarium specimens (Table 2, Fig. 1). These characters include traits widely used for determining species in the group. For each character, we computed basic statistical parameters such as the mean, standard deviation, and the 1, 5, 95 and 99 percentiles. To avoid distortion of the multivariate analysis by high levels of correlation among characters, we calculated Pearson's correlation coefficient and the non-parametric Spearman's rank coefficient and excluded characters which were correlated above the threshold of 0.95. As a result, the character the number of capitula was not included because it was highly negatively correlated with the density of stellate trichomes on the adaxial side of the five largest leaves.

To reduce the multidimensional nature of the character space, we employed principal component analysis (PCA, Krzanowski 1990) based on a correlation matrix and ascertained the variation explained by the first three PCA axes. Then we performed canonical discriminant analyses (CDA) and classificatory discriminant analyses following the methodology provided by Klecka (1980). Prior to these

Table 1 Basic biosystematic features of the taxa studied (according to Gadella 1984, 1987; Krahulcová and Krahulec 1999; Rotreklová 2004; Rotreklová et al. 2005; Mráz et al. 2008; Krahulcová et al. 2009)

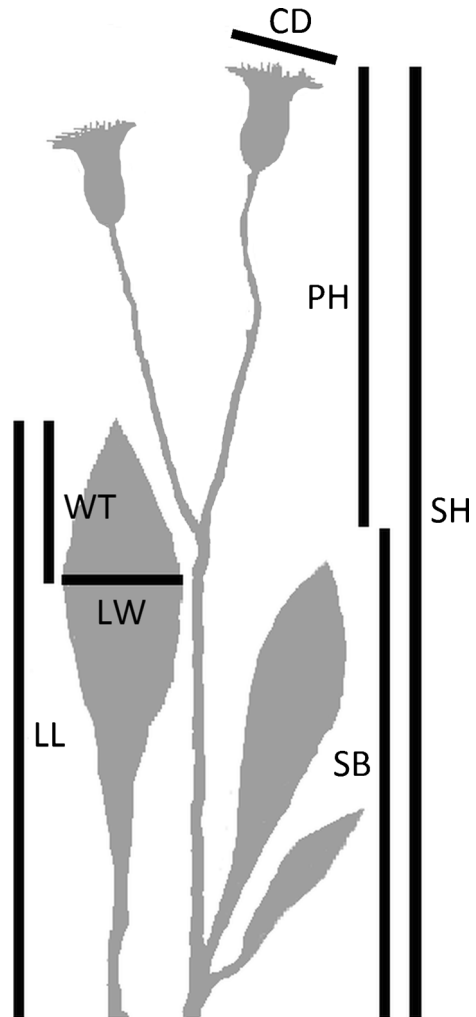
Species	Ploidy level		Reproductive mode	
	Generally known	Studied population	Generally known	Studied population
<i>P. officinarum</i>	2x, 4x, 5x, 6x, 7x	4x, 5x	sexual, apomictic	sexual
<i>P. *bauhini</i>	4x, 5x, 6x, 7x	4x, 5x, 6x, 7x	sexual, apomictic, variable	apomictic, variable
<i>P. brachiata</i>	4x, 5x, 6x, 7x	4x, 5x, 7x, 8x	sexual, apomictic, variable, sterile	sexual, apomictic, variable
<i>P. leptophyton</i>	4x, 5x	4x, 5x	sexual, apomictic	apomictic

Table 2 List of morphological characters used (for selected characters see Fig. 1)

Continuous quantitative and semiquantitative characters	
the largest leaf length (mm)	LL
the largest leaf width (mm)	LW
distance between the widest point of the largest leaf to the tip (mm)	WT
shape of the largest leaf apex (1 – sharp, 2 – obtuse, 3 – rounded)	TA
density of stellate trichomes on adaxial surface of the five largest leaves (1 – trichomes absent, 2 – sparse trichomes, 3 – dense trichomes)	SA
density of single trichomes on abaxial surface of the five largest leaves (1 – trichomes absent, 2 – sparse trichomes, 3 – dense trichomes)	SL
stem height (up to the terminal capitulum; mm)	SH
stem height (up to the first branching; mm)	SB
length of the terminal capitulum peduncle (mm)	PH
density of single trichomes on stem under involucre (1 – trichomes absent, 2 – sparse trichomes, 3 – dense trichomes)	SS
density of stellate trichomes on stem under involucre (1 – trichomes absent, 2 – sparse trichomes, 3 – dense trichomes)	STS
density of eglandulate trichomes on stem under involucre (1 – trichomes absent, 2 – sparse trichomes, 3 – dense trichomes)	ES
average length of five involucre bracts (mm)	BL
average width of five involucre bracts (mm)	BW
terminal capitulum diameter (mm)	CD
rate of dark colour of single trichomes on involucre (1 – bright trichomes, 2 – trichomes with dark bases, 3 – dark trichomes)	TD
density of simple trichomes on involucre (1 – trichomes absent, 2 – sparse trichomes, 3 – dense trichomes)	SI
density of stellate trichomes on involucre (1 – trichomes absent, 2 – sparse trichomes, 3 – dense trichomes)	STI
density of eglandulate trichomes on involucre (1 – trichomes absent, 2 – sparse trichomes, 3 – dense trichomes)	EI
Binary characters	
stolon occurrence (1 – present, 0 – absent)	SO
flagella occurrence (1 – present, 0 – absent)	FO

discriminant analyses, we defined groups corresponding to the four maternal taxa identified using key morphological and biosystematic characters, e.g., the number of capitula per stem (not used further due to high correlation with the density of stellate trichomes) supported by information about the plants' ploidy level and mode of reproduction (e.g., *P. officinarum* characterized by a single capitulum stem, 4x or 5x, and sexual reproduction). Individual plants were used as operational taxonomic units (OTUs). We then tested the ability of the discriminant function obtained from the maternal dataset to classify progeny individuals into these *a priori* defined groups. Finally, a non-parametric k-nearest neighbour classificatory discriminant analysis

Fig. 1 Illustration of selected morphological characters included in morphometric analyses (see also Table 2): *LL* the largest leaf length, *LW* the largest leaf width, *WT* distance between the widest point of the largest leaf to the tip, *SH* stem height (up to the terminal capitulum), *SB* stem height (up to the first branching), *PH* length of the terminal capitulum peduncle, *CD* terminal capitulum diameter



allowed us to determine the percentage of progeny individuals from respective taxonomic groups. These multivariate analyses were performed using SAS 9.1.3 (SAS Institute, Cary, NC) and PAST (Hammer et al. 2001).

Results

Principal Component Analyses

The results for the maternal dataset are rather straightforward (Fig. 2). The first axis corresponds to the gradient between *P. *bauhini* (and its morphologically closer hybrid *P. leptophyton*) on one side and *P. officinarum* on the other. The most common hybrid, *P. brachiata*, occupies most of the space and overlaps with the parental taxa on both sides. No overlap between the parental taxa occurs in the plane of the first

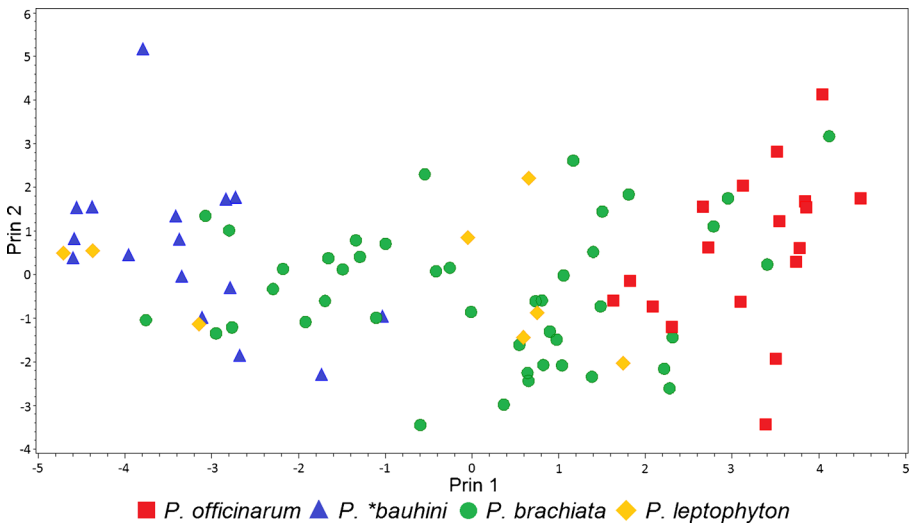


Fig. 2 Principal component analysis (PCA) ordination diagram (maternal dataset of 86 individuals of *Pilosella officinarum*, *P. *bauhini*, *P. brachiata* and *P. leptophyton* based on 21 characters). The first component axis explains 33.5 % of the total variation; the second explains 12.3 % of the total variation

two axes. The same analysis carried out for the progeny of maternal specimens (Fig. 3) showed some differences, however. The progeny of both of the maternal species involved in the reciprocal hybridizations (*P. *bauhini* and *P. officinarum*) overlap to a greater degree with the progeny of *P. brachiata*. This finding indicates the presence of two processes that can act simultaneously. At least part of the progeny of the maternal species was of hybrid origin, and *P. brachiata* was pollinated by both parental species, so a portion of its progeny were products of backcrosses. It seems

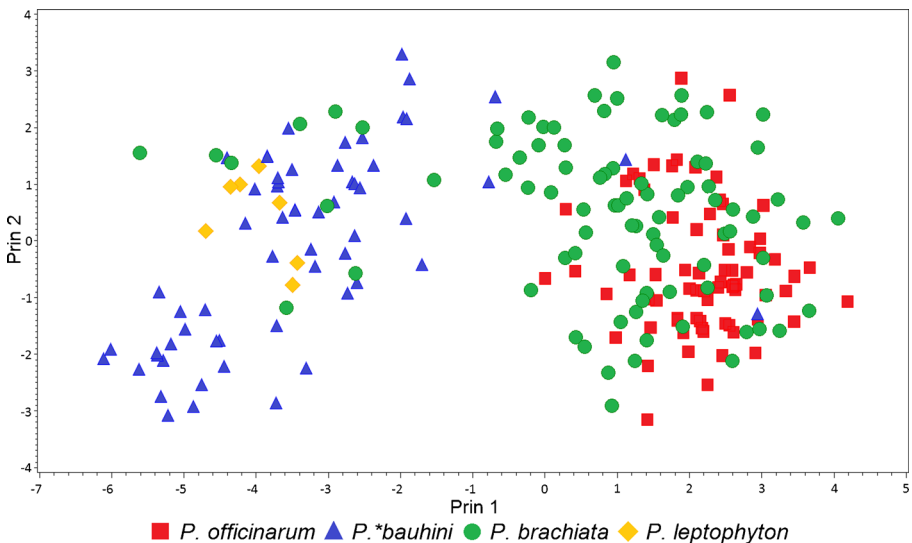


Fig. 3 Principal component analysis (PCA) ordination diagram (filial dataset of 224 progeny plants of *Pilosella officinarum*, *P. *bauhini*, *P. brachiata* and *P. leptophyton* based on 21 characters). The first component axis explains 36.4 % of the total variation; the second explains 10 % of the total variation

that *P. brachiata*, which is partly sexual and partly variable in its reproductive system, produced hybrids which were closer to *P. officinarum*; however, apomictic plants produced plants which were closer to *P. *bauhini*. This result was even more pronounced when we treated the progeny of *P. brachiata* separately with respect to the reproductive mode of the mother plant (Fig. 4). The primary gradient correlates with the sexual and apomictic parents, but the progeny of plants with the variable reproductive system is scattered between the parents and shows a greater overlap with the progeny of sexual maternal plants.

Discriminant Analyses

Our discriminant analysis (Fig. 5) of the maternal dataset revealed a clear separation of the individual taxa. The first canonical axis separates the two parental species (*P. *bauhini* and *P. officinarum*); the second axis partially separates the two hybridogenous species (*P. leptophyton* and *P. brachiata*) in addition to *P. officinarum* and *P. brachiata*. These results are not surprising because the characters used were selected based on their ability to differentiate between these species. The filial dataset that was based on the discriminant function trained on maternal specimens (Fig. 6) showed a complete overlap between the progeny of *P. *bauhini* and *P. leptophyton* and the penetration of the progeny of *P. brachiata* into the progeny of *P. officinarum* and partially also into *P. *bauhini*.

The percentages of the progeny assigned by the discriminant function to different types of maternal plants speak about the processes taking place within the population (Table 3). *Pilosella officinarum* produced a high percentage of *officinarum*-type progeny, followed by *brachiata*-type progeny, which corresponds to $n + n$ hybrids. Surprisingly, some of the progeny was of the *leptophyton* type. The apomictic *P. *bauhini* primarily produced plants of the *bauhini* type, but it also produced both types

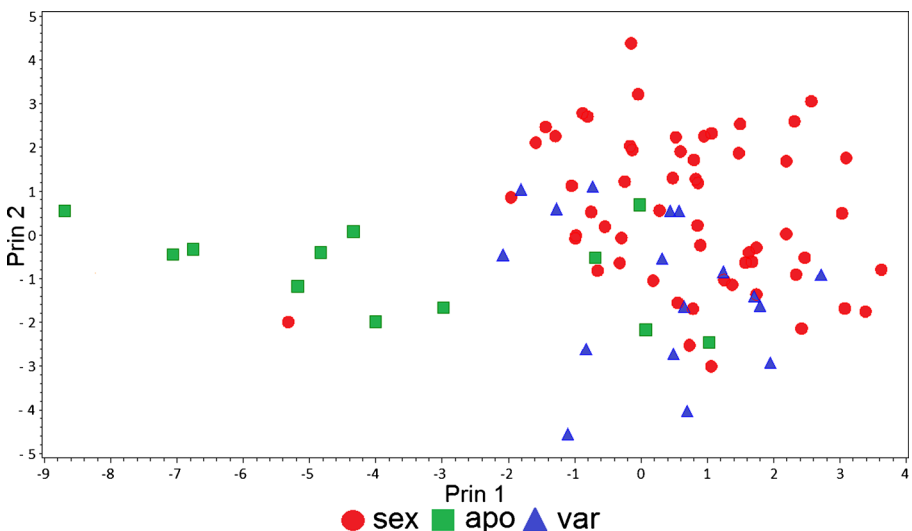


Fig. 4 Principal component analysis (PCA) ordination diagram (the progeny of the *P. brachiata* dataset comprising 104 individuals, based on 21 characters). The first component axis explains 27.1 % of the total variation, and the second explains 14.6 % of the total variation

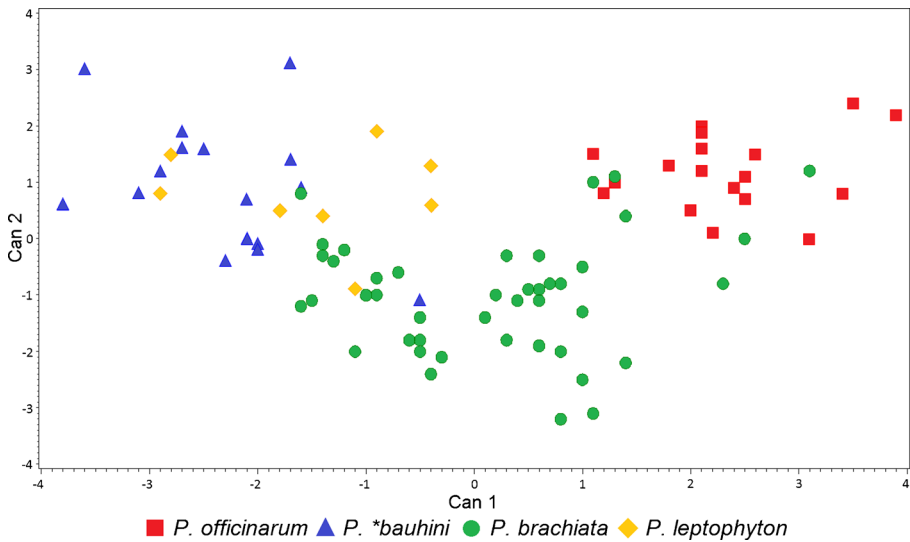


Fig. 5 Canonical discriminant analysis (CDA) diagram (a maternal dataset of 86 individuals of *Pilosella officinarum*, *P. *bauhini*, *P. brachiata* and *P. leptophyton* based on 21 characters). The first canonical axis expresses 62.3 % of the total variation, and the second expresses 30.7 % of the total variation

of hybrids. Most of the *P. brachiata* progeny were classified as *P. officinarum*. This result suggests that *P. brachiata* participates in backcrossing and introgression. Some of the progeny were also of the *P. leptophyton* or *P. *bauhini* type, which also suggests that backcrosses with the second parent took place. Most of the *P. leptophyton* progeny got identified as *P. *bauhini*. The filial generation displayed different proportions with respect to its origin. Its numbers (not percentages) are presented in Table 3.

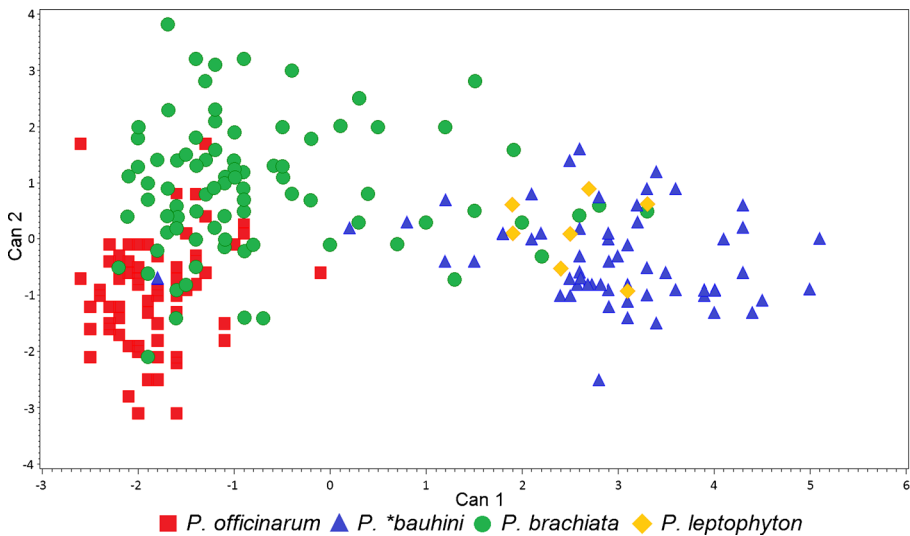


Fig. 6 Canonical discriminant analysis (CDA) diagram (the filial dataset of 224 individuals comprising the progeny of *Pilosella officinarum*, *P. *bauhini*, *P. brachiata* and *P. leptophyton*, based on 21 characters). The first canonical axis expresses 80.7 % of the total variation, and the second expresses 14.8 % of the total variation

Table 3 Number of filial generation specimens with respect to the determination of mother plants (according to CDA, 21 characters used). Total – total number of individuals analyzed

Progeny of	Determined as				Total
	<i>P. officinarum</i>	<i>P. brachiata</i>	<i>P. leptophyton</i>	<i>P. *bauhini</i>	
<i>P. officinarum</i>	45 (62.5%)	22 (30.6%)	5 (6.9%)	0	72
<i>P. brachiata</i>	42 (48.3%)	36 (41.4%)	2 (2.3%)	7 (8%)	87
<i>P. leptophyton</i>	0	1 (14.3%)	0	6 (85.7%)	7
<i>P. *bauhini</i>	0	2 (3.5%)	10 (17.5%)	45 (78.9%)	57

The most diverse reproductive mode of the maternal generation was that of *P. brachiata*, which displayed all three types of reproduction (i.e. the sexual, apomictic and variable mode). The progeny of mother plants differed with respect to their reproductive mode (Table 4). Surprisingly, a greater proportion of the progeny that were classified as identical to the mother plant was found among the progeny of plants with the variable reproduction mode than among that of apomictic plants. The plants with a variable mode produced more progeny classified as *P. officinarum* than apomictic plants; the latter produced a high proportion classified as *P. *bauhini*.

Evaluation of Characters

Table 5 presents data (eigenvectors, total canonical structure) pertaining to the degree of correlation of the morphological characters with particular axes in the PCA and

Table 4 Composition of filial generation with respect to the reproductive mode of mother plants (according to CDA, 21 characters used). Total – total number of individuals analyzed

Mother plant	Progeny determined as				Total
	<i>P. officinarum</i>	<i>P. brachiata</i>	<i>P. leptophyton</i>	<i>P. *bauhini</i>	
<i>P. officinarum</i>					
- sexual	44 (62%)	22 (31%)	5 (7%)	0	71
- apomictic	1 (100%)	0	0	0	1
<i>P. brachiata</i>					
- sexual	33 (57.9%)	22 (38.6%)	1 (1.75%)	1 (1.75%)	57
- apomictic	2 (14.3%)	5 (35.7%)	1 (7.1%)	6 (42.9%)	14
- variable	7 (43.75%)	9 (56.25%)		0	16
<i>P. leptophyton</i>					
- apomictic	0	1 (14.3%)	0	6 (85.7%)	7
<i>P. *bauhini</i>					
- apomictic	0	2 (3.5%)	10 (17.5%)	45 (79%)	57
					223

Table 5 Eigenvectors (PCA) and total canonical structure (CDA) values expressing the degree of correlations of the morphological characters with the particular axes in the PCA and CDA, respectively. The highest eigenvectors and total canonical structure values are given in bold. For character abbreviations, see Table 2

Character	PCA – maternal dataset			CDA – maternal dataset	
	Prin 1	Prin 2	Prin 3	Can 1	Can 2
LL	-0.293	0.274	0.235	-0.730	0.112
LW	-0.097	0.485	0.344	-0.102	0.170
WT	-0.270	0.255	0.298	-0.568	0.041
TA	0.235	0.063	0.047	0.368	0.547
SA	0.315	-0.095	0.103	0.783	0.116
SL	0.165	0.135	-0.146	0.357	0.230
SH	-0.322	0.154	0.096	-0.827	0.004
SB	-0.304	-0.044	0.117	-0.774	-0.009
PH	0.233	0.171	0.280	0.592	0.294
SS	-0.002	0.223	-0.417	-0.077	-0.103
STS	0.186	-0.083	0.076	0.391	0.355
ES	0.170	0.126	0.007	0.479	0.242
BL	0.293	0.233	0.198	0.698	0.309
BW	0.271	0.229	0.006	0.711	0.171
CD	0.271	0.235	0.119	0.714	0.399
TD	-0.035	0.037	-0.055	-0.255	-0.084
SI	0.006	0.363	-0.447	0.105	-0.251
STI	0.237	-0.052	0.187	0.374	0.561
EI	0.123	-0.205	0.272	0.209	-0.237
SO	0.172	0.212	-0.169	0.351	0.065
FO	-0.049	0.286	-0.171	-0.078	-0.130

CDA. Characters which significantly correlated with the first axis of the PCA were leaf length (positively correlated with *P. *bauhini*) and the density of stellate trichomes underleaf (a character of *P. officinarum*). The second axis correlated with leaf width and the density of simple trichomes on the involucrem. The combination of characters that could be used to discriminate among the parental species and the hybrids within the maternal dataset were, again, the leaf length, the length of the leaf from its widest point to the tip, stem height and the height to the first branch (all of the characters positively correlated with *P. *bauhini*). Characters that positively correlated with *P. officinarum* were the length of the terminal capitulum peduncle (in *P. officinarum* this was identical with the stem height), the length and width of the involucrel bracts and the diameter of the terminal capitulum. The density of stellate trichomes underleaf and the shape of the leaf apex correlated with the second canonical axis, and these characters can, in fact, discriminate between the two hybrid types.

Discussion

The study of hybridization in *Pilosella* has a long tradition, which has produced two types of studies: descriptive and experimental. Most common are studies dealing with descriptions of new hybrids or subspecific categories. This type of research has led to the proliferation of an enormous system of intraspecific units, which are catalogued in monographs (Nägeli and Peter 1885; Zahn 1921–1923) and numerous local floras. The second type of study revolves around experimental hybridization. One early example is a study by Mendel (1869), which was inspired by Nägeli. Experimental hybridizations carried out by Nägeli and Peter produced many of the hybrids described in their monograph (Nägeli and Peter 1885). Skalińska (1976) studied the progeny of crosses in the *P. aurantiaca* group and found great variation in ploidy. Recent studies of *Hieracium* hybrids tend to focus on the genetics of apomixis (e.g. Bicknell et al. 2000; Catanach et al. 2006; Koltunow et al. 2011; Krahulcová et al. 2011; Rosenbaumová et al. 2012).

Only a limited number of authors have conducted field studies of ploidy levels and breeding systems (e.g. Gadella 1982, 1984, 1991; Turesson and Turesson 1960, 1963). Gadella (1987) performed a small-scale population study of *P. officinarum*. The last decade has seen the publication of a series of papers about populations in selected geographic regions with respect to their ploidy, breeding systems and detailed distribution (Fehrer et al. 2005; Krahulec et al. 2004, 2008; Trávníček et al. 2011). Only two studies, however, compare mother plants with their progeny and at the same time directly deal with hybridization in the field, ploidy levels and breeding systems (Krahulcová et al. 2009, 2012). These studies on hybridization also employed chloroplast haplotypes and other molecular markers. Our present study aims to follow up on one of these studies (Krahulcová et al. 2009) by adding results concerning morphological characters useful for delimiting species and products of hybridization. Comparison of maternal plants with their progeny allowed us to demonstrate the direction of hybridization for particular members of a hybrid swarm.

The PCA of the maternal dataset revealed a morphological gradient spanning from one parental species to the other with both the hybrid taxa in the middle. Overlaps were nevertheless present. The PCA of the filial dataset revealed the same gradient, but there were greater overlaps between the progeny of individual maternal specimens. This finding can be explained by the formation of new hybrids between the parental species followed by backcrossing and segregation of hybrids. Like the PCA, the classificatory discriminant analysis revealed an overlap between progeny and maternal taxa. The percentage of progeny plants identified differently than their maternal plants (“misidentifications”) was high in all groups, which suggests that hybridizations and/or segregation took place among the progeny of hybrids.

The progeny of sexual *P. officinarum* was primarily of the *officinarum* type, but a minority were hybrids of both types, of which the *brachiata* type, which corresponds to $n + n$ hybrids, prevailed. This result suggests that about one third of the progeny was derived from a cross with *P. *bauhini* or one of its interspecific hybrids. The progeny of *P. brachiata* was diverse. The largest part of it was surprisingly identified as *P. officinarum*. This result suggests the common presence of backcrosses with *P. officinarum* and introgression into this species. This hybridization may have been the source of the pentaploid sexual type of *P. officinarum* observed at this locality (Krahulcová et al. 2000, 2009). A small proportion of the progeny was identified as *P. *bauhini*, pointing to

possible introgression into *P. *bauhini*, only at a ten-fold lower frequency. It seems that the rare hybrids produced by apomictic plants were more similar to *P. *bauhini* (Figs. 3 and 4, Table 4). Conversely, the progeny of sexual plants was more similar to *P. officinarum*. With respect to the space along the first PCA axis (Fig. 3), the progeny of apomictic plants was more variable than the progeny of sexual plants, which corroborates the results of our previous study on ploidy levels (Krahulcová et al. 2009). We are nonetheless aware that the number of progeny of apomictic plants was rather low because of their low residual sexuality. The composition of the progeny of the second hybrid, *P. leptophyton*, also suggests introgression from a closely related parental species, specifically *P. *bauhini*, but the low number of analyzed plants weakens this conclusion. The progeny of the apomictic maternal *P. *bauhini* comprised a relatively high proportion of hybrids (over 20 %). All of the results show that interspecific hybridization is very common and that it involves both the parental taxa and their hybrids including apomicts.

The only morphotype that exhibited all three types of reproductive mode (i.e., sexual, apomictic and variable) was *P. brachiata*. It is not easy to explain the composition of individual plants with their various reproductive modes in the progeny. It is evidently necessary to analyze a greater number of mother plants before a definite conclusion can be reached. The frequency of hybrids and the high variation in their chromosome numbers, including aneuploids (Krahulcová et al. 2009), suggests common hybridization and subsequent introgression into the sexual parent, at least at the locality under study. The data presented in this paper indicate that hybridization takes place in almost all directions despite the fact that one of the parental species (*P. *bauhini*) and a portion of the hybrids are facultatively apomictic, serving as both parents. The high proportion of both hybrids and aneuploids was detected not only in seeds but also among plants growing in the field, which confirms that the rates of both hybridization and establishment are high. This finding agrees with our field observations that open soil with fine gravel and sand on the surface facilitates the growth of most seedlings provided that there are good moisture conditions.

Rather different results were found in other apomictic groups. Rates of sexual reproduction found in other facultatively apomictic taxa range from a few percent to almost half of the progeny (Savidan et al. 2001; Hörandl and Paun 2007). Apomicts are usually presumed to be pollen donors when they hybridize with sexual taxa (Hörandl and Paun 2007; Mogie et al. 2007). Hybridization between sexuals and apomicts is rare in several groups such as in the *Ranunculus cassubicus* complex or the *Antennaria rosea* complex (reviewed in Hörandl and Paun 2007) and *Taraxacum* sect. *Erythrosperma* (Mártonfiová et al. 2010). In *Taraxacum* sect. *Ruderalia*, hybridization contributes to the genotypic variation of facultative apomicts (Hörandl and Paun 2007; Mártonfiová 2011). In *Rubus*, common production of hybrids by apomictic mother plants was found at least in some populations (Šarhanová et al. 2012). In our study, we found that hybridization occurs in both directions and that it is more frequent in the direction of the sexual parent. The same has been found in a hybridizing population of *Pilosella officinarum* and *P. aurantiaca* in Germany (Krahulcová et al. 2012). How usual this process is in general remains to be investigated.

Several of the characters that were found to be important for distinguishing the species (especially size parameters) are not commonly used in keys. Characters such as the shape of the leaf tip and the position of the widest part of the leaf are easily observable and can be used in the field.

One problem associated with frequent hybridization in *Pilosella* is that two different types of plants which share the same hybrid origin may occur in the field: (1) hybridogenous species reproducing apomictically, which behave independently of their parents; and (2) recent hybrids, which can be sterile, sexual or partly apomictic whilst retaining some degree of sexuality and haploid parthenogenesis. This phenomenon is herein referred to as the variable reproductive system. Both these types share the same morphology and origin. Whether they are recent hybrids or hybridogenous species can only be detected through an analysis of their progeny. In the literature, they carry binomial names as if they were regular species even though some of them are recent hybrids.

The whole problem is even more complicated, however. There are several categories of hybrids of the same origin ($n + n$, $2n + n$, $n + 2n$). Hybrids can be produced in a single hybridization event and still differ in morphology, ploidy, etc. Moreover, hybrids can be of both types described above. In such cases, products of a single hybridization event may be given different names.

Conclusions

i) Morphometric analyses (PCA, CDA) of maternal plants confirm the relationships among the taxa under study, including the hybrid origins of *P. brachiata* and *P. leptophyton*. By contrast, the analyses of the filial dataset show a less structured pattern in the data with obvious shifts towards parental taxa compared to the maternal dataset. Especially the *P. officinarum* and *P. brachiata* groups markedly overlap, which is a sign of backcrossing. On the other hand, the *P. *bauhini* group does not remain separated either. *ii*) Reproductive pathways are markedly manifested in morphology. The progeny of sexual plants tends to be closer to *P. officinarum*, suggesting introgression. The progeny of apomicts, by contrast, leans towards *P. *bauhini*. Progeny of plants with the so-called variable mode of reproduction (i.e., one which combines sexual reproduction, haploid parthenogenesis and apomixis) is scattered throughout the dataset; in other words, variable reproduction produces the most variable progeny. *iii*) The most frequent and most variable progeny of *P. brachiata* was analyzed separately. The results of these analyses illustrate the crucial role of the parental reproductive mode. CDA identified *P. brachiata* as the most important entity taking part in hybridization within the population under study.

Acknowledgements We thank Siegfried Bräutigam and Jindřich Chrtek for their help with plant determination and Frederick Rooks for doing a language revision of our manuscript. This study was supported by the Czech Science Foundation (project No. 206/08/0890) and by the long-term institutional research plan No. AVOZ60050516 from the Academy of Sciences of the Czech Republic.

References

- Bicknell RA, Borst NK, Koltunow AM (2000) Monogenic inheritance of apomixis in two *Hieracium* species with distinct developmental mechanisms. *Heredity* 84:228–237
- Bräutigam S, Greuter W (2007) A new treatment of *Pilosella* for the Euro-Mediterranean flora (Notulae ad floram euro-mediterraneam pertinentes No. 24). *Willdenowia* 37:123–137

- Catanach AS, Erasmuson SK, Podivinsky E, Jordan BR, Bicknell R (2006) Deletion mapping of genetic regions associated with apomixis in *Hieracium*. *Proc Natl Acad Sci USA* 103:18650–18655
- Chrtek J (2004) *Hieracium* L. – jestřábník. In Slavík B, Štěpánková J (eds) *Květena České republiky (Flora of the Czech Republic)* 7. Academia, Praha, pp 540–701
- Fehrer J, Šimek R, Krahulcová A, Krahulec F, Chrtek J Jr, 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, Pelsner P (eds) *Plant species-level systematics: new perspectives on pattern & process*. Regnum Veg 143, A.R.G. Gantner Verlag, Rugell, pp 175–201
- Gadella TWJ (1982) Cytology and reproduction of *Hieracium pilosella* L. and some related diploid species. *Acta Bot Neerl* 31:140–141
- Gadella TWJ (1984) Cytology and the mode of reproduction of some taxa of the *Hieracium* subgenus *Pilosella*. *Proc Kon Ned Acad Wetensch C* 87:387–399
- Gadella TWJ (1987) Sexual tetraploid and apomictic pentaploid populations of *Hieracium pilosella* (Compositae). *Plant Syst Evol* 157:219–245
- Gadella TWJ (1991) Reproduction, variation and interspecific hybridization of three species of *Hieracium* sect. *Pilosellina* (Compositae). *Polish Biol Stud* 2:85–103
- Hammer Ø, Harper DAT, Ryan P D (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontol Electronica* 4:1–9
- Hörandl E, Paun O (2007) Patterns and sources of genetic diversity in apomictic plants: implications for evolutionary potentials. In Hörandl E, Grossniklaus U, Sharbel TF, (eds) *Apomixis: evolution, mechanisms and perspectives*. Regnum Veg 147, A.R.G. Gantner Verlag, Rugell, pp 169–194
- Klecka WR (1980) *Discriminant Analysis*. Sage University Papers, Series: Quantitative application in the social sciences, no. 19, Sage, Beverly Hills and London
- Koltunow AMG, Johnson SD, Rodrigues JCM, Okada T, Hu Y, Tsuchiya T, Wilson S, Fletcher P, Ito K, Suzuki G, Mukai Y, Fehrer J, Bicknell RA (2011) Sexual reproduction is the default mode in apomictic *Hieracium* subgenus *Pilosella*, in which two dominant loci function to enable apomixis. *Plant J* 66:890–902
- Krahulcová A, Krahulec F (1999) Chromosome numbers and reproductive systems in selected representatives of *Hieracium* subgen. *Pilosella* in the Krkonoše Mts (the Sudeten Mts). *Preslia* 71:217–376
- Krahulcová A, Krahulec F (2001) Offspring diversity in *Hieracium* subgen. *Pilosella* (Asteraceae): new cytotypes from hybridization experiments and from open pollination. *Fragmenta Flor Geobot* 41:239–255
- 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, Roteklová 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
- 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, 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
- Krzanowski WJ (1990) *Principles of multivariate analysis*. Clarendon Press, Oxford
- Mártonfióvá L (2011) Comparison of breeding behaviour of *Taraxacum* sect. *Ruderalia* and *Taraxacum* sect. *Erythrosperma* (Asteraceae). *Thaiszia* 21:177–184
- Mártonfióvá L, Mártonfi P, Šuvada R (2010) Breeding behavior and its possible consequences for gene flow in *Taraxacum* sect. *Erythrosperma* (H. Lindb.) Dahlst. *Pl Spec Biol* 25:93–102

- Mendel G (1869) Über einige aus künstlicher Befruchtung entnommenen *Hieracium*-Bastarde. *Verh Naturf Vereins Brünn* 8:26–31
- Mogie M, Britton NF, Stewart-Cox JA (2007) Asexuality, polyploidy and the male function. In Hörandl E, Grossniklaus U, Sharbel TF (eds) *Apomixis: evolution, mechanisms and perspectives*. Regnum Veg 147, A.R.G. Gantner Verlag, Rugell, pp 195–214
- Mráz P, Šingliarová B, Urfus T, Krahulec F (2008) Cytogeography of *Pilosella officinarum* (Compositae): Altitudinal and longitudinal differences in ploidy level distribution in the Czech republic and Slovakia and the general pattern in Europe. *Ann Bot (Oxford)* 101:59–71
- Nägeli C, Peter A (1885) *Die Hieracien Mittel-Europas, Piloselloiden*. R. Oldenbourg, München
- 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
- Ostenfeld CH, Raunkiaer C (1903) Kastreringsforsog med *Hieracium* og andre Cichorieae (Castration experiments with some species of *Hieracium* and other Cichorieae). *Bot Tidsskr* 25:409–413
- Rosenbaumová R, Krahulcová R, Krahulec F (2012) The intriguing complexity of parthenogenesis inheritance in *Pilosella rubra* (Asteraceae, Lactuceae). *Sexual Pl Reprod* 25:185–196
- Rotreklová O (2004) *Hieracium bauhini* group in Central Europe: chromosome numbers and breeding systems. *Preslia* 76:313–330
- Rotreklová O, Krahulcová A, Mráz P, Mrázová V, Mártonfiová L, Peckert T, Šingliarová B (2005) Chromosome numbers and breeding systems of some European species of *Hieracium* subgen. *Pilosella*. *Preslia* 77:177–195
- Šarhanová P, Vašut RJ, Dančák M, Bureš P, Trávníček B (2012) New insights into the variability of reproduction modes in European populations of *Rubus* subgen. *Rubus*: how sexual are polyploid brambles? *Sexual Pl Reprod* 25:319–335
- Savidan Y, Carman JG, Dresselhaus T (eds) (2001) *The flowering of apomixis: from mechanisms to genetic engineering*. CIMMYT, IRD, European Commission DG VI (FAIR), Mexico, D.F.
- Schuhwerk F, Fischer MA (2003) Bestimmungsschlüssel der Untergattung *Hieracium* subgen. *Pilosella* in Österreich und Südtirol. *Neireichia* 2–3:13–58
- Skalińska M (1976) Cytological diversity in the progeny of octoploid facultative apomicts of *Hieracium aurantiacum* L. *Acta Biol Cracov, Ser Bot* 19:39–46
- Trávníček P, Dočkalová Z, Rosenbaumová R, Kubátová B, Szelag Z, Chrtek J Jr (2011) Bridging global and microregional scales: ploidy distribution in *Pilosella echioides* (Asteraceae) in central Europe. *Ann Bot (Oxford)* 107:443–454
- Turesson G, Turesson B (1960) Experimental studies in *Hieracium pilosella* L. I. Reproduction, chromosome number and distribution. *Hereditas* 46:717–736
- Turesson G, Turesson B (1963) Observations on chromosome number and reproduction in some Piloselloids. *Bot Not* 116:157–160
- Tyler T (2001) The genus *Pilosella* in the Nordic countries. *Svensk Bot Tidskr* 95:39–67
- Zahn KH (1921–1923) *Hieracium*. In Engler A (ed) *Das Pflanzenreich* 4(280). Wilhelm Engelmann, Leipzig, pp 1–1705

Received: 19 January 2012 / 16 May 2013 / Accepted: 10 June 2013 /

Published online: 3 December 2013