Chapter 9

Evolution, hybridisation, and clonal distribution of apo- and amphimictic species of *Hieracium* subgen. *Pilosella* (Asteraceae, Lactuceae) in a Central European mountain range

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Hieracium subgenus *Pilosella* is an extremely difficult group taxonomicaly, as it combines apomixis with extensive hybridisation. The resultant vast number of morphological forms and cytotypes, which are extremely difficult to distinguish, has in the past deterred study of their microevolutionary relationships. We present here a multidisciplinary approach combining molecular techniques (DNA fingerprinting and the analysis of chloroplast DNA) with morphological-taxonomic studies, cultivation, experimental hybridisation, analysis of ploidy level and mode of reproduction.

In a selected area, a transsect in the three-border land of Germany, the Czech Republic and Poland, eight species of a hybridogenous complex representing two morphological series were studied in detail at the clone, population and species level. Proper assignment of individual plants and populations to apomictic clones was achieved and provided the basis for further analysis. Different levels of variability were found in the apomictic species: from near uniformity across the study area to variability within the locality. These differences were related to the different ages and histories of the respective taxa. A partial biogeographic isolation between two mountain regions was suggested by the distribution of widespread clones.

The chloroplast haplotypes formed two major groups that showed no correlation to geographic distribution, but matched the species' delimitation except in the case of a recent hybrid which was obviously produced by reciprocal crosses between parental species of different haplotype groups. Apart from that, each species including the intermediates possessed one haplotype indicating unidirectional transmission of the chloroplast DNA, despite multiple origins of most apomicts. Moreover, in the majority of the cases examined, the facultative apomict rather than the sexual species acted

as seed parent. Thus, the residual sexuality of the apomicts seems to play a larger role in the speciation of this group than hitherto assumed.
This study leads to a much better understanding of a variety of aspects of the group and will serve as a basis for future analyses.

KEYWORDS: Apomixis, Asteraceae, cpDNA, DNA fingerprinting, *Hieracium* subgen. *Pilosella*, hybridisation, polyploidy, *trnT-trnL.*

INTRODUCTION

Hieracium L. s.l. (hawkweeds) is one of the world's largest plant genera (850–>10,000 species depending on species concept). All plants are herbaceous perennials which can reproduce sexually or apomictically, i.e., by producing seeds without fertilisation. Three subgenera are recognised: the American subgenus Chionoracium and the two Eurasian subgenera *Hieracium* and *Pilosella* whose centres of diversity are in the European mountains (Bräutigam & Hilbig, 1980; Gottschlich, 1996). The subgenera differ in their mode of reproduction: as far as known, *Chionoracium* species are all sexual diploids, *Hieracium* (s.str.) species are either polyploid obligate apomicts of the diplosporous type or diploid sexuals, and subgenus *Pilosella* (which is the focus of this study) is characterized by a mixture of sexual and facultatively apomictic taxa of the aposporous type (for reviews see Koltunow & al., 1995; Krahulcová & al., 2000; also see Van Dijk & Vijverberg, this volume, chapter 5). In their native environment *H.* subg. *Pilosella* species are weak competitors, often colonising open and disturbed habitats. Hybridisation and introgression events are common, which, in combination with the apomictic mode of reproduction, lead to a rapid fixation of new genotypes (Krahulcová & al., 2000; Krahulec & al., 2004).

The chromosome base number in the genus *Hieracium* is x = 9. In subgen. *Pilosella*, different ploidy levels are common for most of the species (Schuhwerk & Lippert, 1997). Diploids do exist, but tetra- and pentaploids are predominating; hexa-, tri-, hepta- and octoploids occur with decreasing frequency (e.g., Gadella, 1984; Schuhwerk, 1996; Schuhwerk & Lippert, 2002). Aneuploids are extremely rare under natural conditions. While autogamy can sometimes be stimulated by foreign pollen (Krahulcová & al., 1999), the plants are generally self-incompatible (e.g. Gadella, 1984). Allopolyploidy is considered to be the usual mechanism for the formation of polyploids. The (facultatively) apomictic plants usually produce pollen and can therefore regularly take part in hybridisations and backcrosses; reduced pollen of, e.g., pentaploids being diploid, triploid or anything in between (Gadella, 1987; 1991; Krahulcová & Krahulec, 2000). The facultative apomicts have residual sexuality and can therefore also act as seed parents; the frequency of sexually derived progeny has been estimated in hexaploid *H. rubrum* to occur at a rate of up to 8% under experimental conditions (Krahulcová et al., 2004). The same

plant can not only reproduce apomictically or sexually during its lifetime, but is able to do both simultaneously, even within a single capitulum (Skalińska, 1971; Krahulcová & Krahulec, 2000). According to experimental crosses, the egg cells and pollen of sexuals as well as apomicts can be either reduced or unreduced in the production of hybrids (Gadella, 1988; Skalinska, 1971; 1976; Krahulcová & Krahulec, 2000) so that an enormous variety of cytotypes can be produced. The combination of these processes has lead to a heavily reticulate taxonomic structure in this group, and the vast number of morphological forms resulting from it makes species delimitation an extremely difficult task. These well-known principal difficulties have often resulted in a marked reluctance of researchers to study such groups at all. Our study is the first molecular approach shedding some light onto natural populations of taxa from *Hieracium* subgen. *Pilosella* and the processes that might have produced them.

In the two important monographs on *Hieracium* (Nägeli & Peter, 1885; Zahn 1921–23), a distinction is made between 'basic' species (Hauptarten/species principales) and intermediate species (Zwischenarten/species intermediae). Most of the basic species comprise diploid plants whereas the intermediates are generally restricted to higher ploidy levels. In this definition, intermediate species show a combination of the morphological characters of at least two basic species. Their origin can be due to (i) recent or ancient hybridisation, the latter eventually accompanied by (at least local) extinction of one or both parents or (ii) split of apomictic lineages due to somatic mutations and/or a combination of these factors.

In order to elucidate these processes, we selected two morphological series that consist of basic and intermediate species representing a continuity of morphological forms (Fig. 1a). As a study area, mountain ranges and adjacent regions of the three-border land of Germany, Poland and the Czech Republic were chosen, because the distribution areas of the investigated taxa overlap here (Fig. 1b).

Due to the limitations of the morphological and cytological approaches used so far, we investigated the potential of molecular markers for species delimitation and for better understanding of hybridisation/introgression processes that may have led to the present situation in *H.* subg. *Pilosella*. The current study consists of two parts: (i) the determination of apomictic clones by classical DNA fingerprinting, which was used in combination with morphological and taxonomic studies as a sorting tool in order to promote a proper taxonomic delimitation. A further purpose of this part of our study was to investigate single versus multiple origins of apomictic lineages and their geographic distribution; (ii) the analysis of maternal lineages based on PCR-RFLPs and sequencing of a chloroplast DNA intergenic spacer region. Based on the fingerprinting results, we provide new insights into hybridisation/introgression processes and seed dispersal in *H.* subg. *Pilosella*.

MATERIALS AND METHODS

Taxon sampling and cultivation. — In a transsect from the Erz-

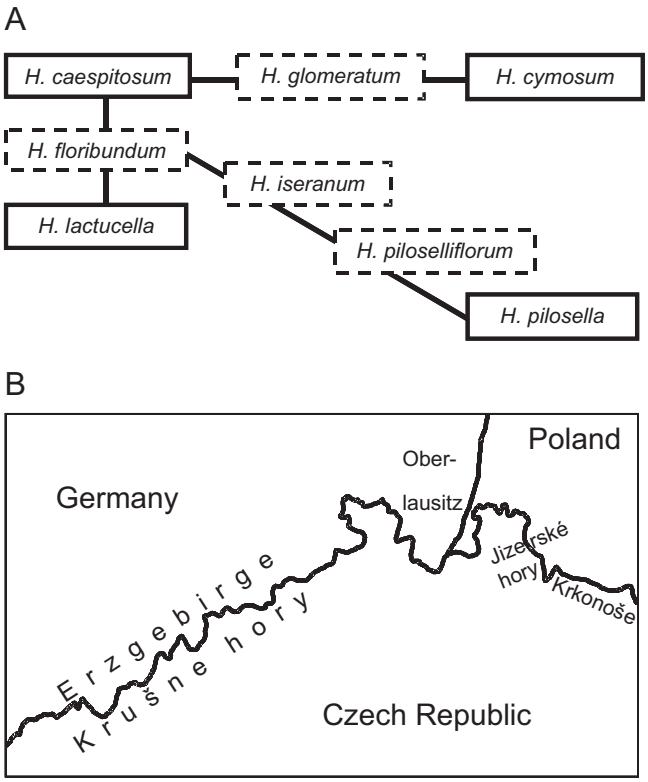

Fig. 1. Taxa studied and investigation area. (a) Two interconnected morphological series (*H. caespitosum–H. glomeratum–H. cymosum* and *H. floribundum–H. iseranum–H. piloselliflorum–H. pilosella*). Relationships are after Nägeli & Peter (1885). Basic species are framed by solid, intermediates by dashed borders. As *H. floribundum* is itself an intermediate, *H. lactucella* as a putative parent was also included; (b) The three-border land is outlined; names of landscapes and mountain ranges mentioned in the text are given for overview; the Jizerské hory and Krkonoše both belong to the Sudetes.

gebirge/Krušné hory via the hill country and mountains of the Oberlausitz to the Jizerské hory/Isergebirge and Krkonoše/Riesengebirge (the latter two being part of the Sudetes), plants were sampled at about 50 localities (Table 1), which were chosen in such a way that they were both as evenly distributed as feasible under natural conditions as well as included different types of habitat. Preference was further given to localities where more than one of the species of interest occurred together (up to seven species). In general, six plants per population were sampled from the following species (see Table 1, also Fig. 1a): *H. caespitosum* Dum., *H. glomeratum* Froel., *H. floribundum* Wimm. & Grab., *H. iseranum* (Uechtr.) Zahn ssp. *iseranum* and ssp. *confinium* (Naeg. & Peter) Zahn, *H. piloselliflorum* Naeg. & Peter (including *H. apatelium* Naeg. & Peter), *H. pilosella* L., and *H. lactucella*

Table 1. Taxon sampling: localities and species. Column 1 refers to the positions of quarter quadrants of Central European topographic maps 1: 25,000 ("Meßtischblatt"). Localities along the transsect (all but the last four) are roughly sorted from west to east.

Position	Locality (Abbreviation)	Species sampled
5542/32	D, Johanngeorgenstadt, south (JgS)	*caespitosum*, *floribundum*
5542/41	D, Johanngeorgenstadt, east (öJG/JgsO)	*floribundum*
5542/32	D, Johanngeorgenstadt, center (JMi)	*caespitosum*, *glomeratum*
5542/32	D, Johanngeorgenstadt, north (JgN)	*floribundum*, *pilosella*, *piloselliflorum*
5542/42	D, Halbemeile (Hme)	*iseranum*, *pilosella*
5543/13	D, Zweibach, slagheap (Zwh)	*caespitosum*, *dubium*
5543/13	D, Zweibach, west (ZwW)	*lactucella*, *floribundum*, *piloselliflorum*, *pilosella*
5543/44	D, Oberwiesenthal (Obw)	*caespitosum*, *floribundum*
5644/11	CZ, Klínovec, Meluzína (Wirbelsteine, Keilberg) (Wbs/57)	*caespitosum*, *cymosum* ssp. *cymigerum*
5443/22	D, Frohnau/Annaberg (Fro)	*floribundum*
5445/31	D, Hirtstein, Satzung (Hir/GR)	*lactucella*, *caespitosum*
5445/14	D, Reitzenhain (Rei)	*caespitosum*
5344/43	D, Großrückerswalde (Grw)	*floribundum*
5345/23	D, Ansprung (Ans)	*floribundum*
5247/33	D, Neuwernsdorf (Neu)	*lactucella*, *caespitosum*
5247/42	D, Hermsdorf (Her)	*iseranum*, *piloselliflorum*, *pilosella*
5248/11	D, Schellerhau, hotel (Hot)	*lactucella*, *iseranum*, *piloselliflorum*, *pilosella*, *floribundum*
5248/23	D, Altenberg, Goethestraße (Goe)	*iseranum*
5248/14	D, Altenberg, former biathlon area (Bia)	*aurantiacum*, *caespitosum*, *iseranum*
5248/21	D, Geisingwiesen (Gei)	*iseranum*, *pilosella*
5248/23	D, Altenberg, railway station (AlB)	*aurantiacum*, *floribundum*
5248/24	D, pasture betw. Geising & Fürstenau (Fuer)	*caespitosum*
5149/41	D, Gottleuba, dam (Got)	*bauhini*, *caespitosum*
4949/33	D, Dresden-Pillnitz, park of castle (Pil)	*pilosella*
4746/41	D, Diesbar, Göhrisch (Goer)	*echioides*
5153/22	D, Großschönau (GSc)	*glomeratum*, *iseranum*, *pilosella*
5153/24	D, Waltersdorf/Butterberg (But)	*caespitosum*, *pilosella*
5154/32	D, Jonsdorf, border (Jon)	*lactucella*, *piloselliflorum*, *pilosella*
5154/31	D, Jonsdorf, parking lot (Jon)	*iseranum*
5154/41	D, Lückendorf (Lüc)	*piloselliflorum*
5054/22	D, Großhennersdorf (Gro)	*iseranum*
4955/14	D, Schönau-Berzdorf, edge of slagheap (AM)	*aurantiacum, bauhini, piloselloides* ssp. *obscurum, glomeratum, pilosella*
4955/14	D, Schönau-Berzdorf/Pließnitz (Pli)	*caespitosum*
4955/23	D, Tauchritz, near power station (Tau)	*aurantiacum*, *iseranum*
4855/24	D, Görlitz, cemetery (GFr)	*glomeratum*
4855/42	D, Görlitz, southern town (GRG)	*piloselloides* ssp. *praealtum*
4753/34	D, street to Purschwitz (Pur)	*caespitosum*
4753/41	D, Baruth/Schafberg (mäh)	*glomeratum*
5255/23	CZ, Kryštofovo údolí (Christophsgrund) (Cri)	*glomeratum*
5158/13	D, Groß Iser, former village (GIO)	*iseranum, pilosella, glomeratum, piloselliflorum*, *caespitosum*
5158/32	PL, Orle/Izera (Karlstal/Groß Iser) (Gr2)	*iseranum* ssp. *confinium*, *floribundum*
5158/34	PL, Orle/Jakuszyce (Karlstal/ Jakobstal) (Jac)	*lactucella*, *caespitosum*, *floribundum*, *glomeratum*, *iseranum*, *piloselliflorum*, *pilosella*
5158/43	PL, Jakuszyce (Jakobstal), tower (Tur)	*floribundum*
5259/23	CZ, Š. Mlýn, Davidovy Boudy (Davidbaude) (Dba)	*glomeratum*

Table 1. (continued).

Position	Locality (Abbreviation)	Species sampled
5259/24	CZ, Špindlerovka (Spindlerbaude) (Spb)	*aurantiacum*
5259/23	CZ, Š. Mlýn, Petrovka (Peterbaude) (Pba)	*caespitosum*, *iseranum*
5260/42	CZ, Pomezní Boudy (Grenzbauden) (Gba)	*piloselliflorum*, *pilosella*, *iseranum*, *lactucella*, *floribundum*
5260/44	CZ, Dolní Malá Úpa (Klein Aupa) (Kau)	*piloselliflorum*
5360/22	CZ, Pec, Velká Úpa (Groß Aupa) (Gau)	*caespitosum*, *glomeratum*, *pilosella*, *floribundum*, *iseranum*, *rubrum*
5360/12	CZ, Pec, Velká Úpa (Groß Aupa) west (GAW)	*glomeratum*, *floribundum*
5360/22	CZ, Pec, bouda Jana (Jonaboden) (Jbo)	*onegense*, *glomeratum*, *piloselliflorum*, *pilosella, lactucella, schultesii, tubulascens*
5360/31	CZ, Cerný Důl (Schwarzental) (Mar)	*glomeratum*
5258/42	CZ, Víchovska Lhota (Víc)	*glomeratum*
5548/44	CZ, Louny, Oblík (12)	*cymosum* ssp. *cymosum*
5852/32	CZ, Prague-Bohnice, Zámka (99/104.3)	*echioides*
9044/13	A, Alps, Upper Egger-Alm (34 hop)	*hoppeanum*
7276/43	SK, Nitrica (137 mac)	*macranthum*

Wallr. Of the rare *H. cymosum* L. ssp. *cymosum*, no populations were found in the transsect but one of the subspecies *cymigerum* (Reichb.) Peter was. Material was completed by plants from Bohemia and the adjacent south-eastern regions (26 populations of *H. cymosum* ssp. *cymosum* and an additional three of ssp. *cymigerum* (Šimek, 2001)—a list of these localities is available upon request from the corresponding author, only the *H. cymosum* specimens used for sequencing were included in Table 1). Additionally, further *Pilosella* basic species occurring in the same or adjacent regions were included (*H. onegense* (Norrl.) Norrl., *H. aurantiacum* L., *H. piloselloides* Vill. (ssp. *obscurum* (Reichb.) Zahn, ssp. *praealtum* (Vill.) Zahn), *H. bauhini* Bess., *H. echioides* Lumnitz.) as well as two further diploids (*H. hoppeanum* Schult. s.str., *H. macranthum* (Tenore) Tenore). Finally, the following four intermediate taxa from the investigation area were included: *H. tubulascens* (Norrl.) Norrl., *H. rubrum* Peter, *H. dubium* L., and *H. schultesii* F.Schultz.

All taxa were sampled in the same way, i.e., by taking rosettes from patches of about 20 square meters, while trying to avoid picking clones established by stolon growth (in order to enable comparison of variability between different taxa). For each population and taxon, at least two voucher specimens were deposited in the Görlitz herbarium (GLM). *H. cymosum* plants of Šimek (2001) were deposited in the Pruhonice herbarium (PRA). A major proportion of plants was cultivated under uniform growth conditions. Once the influence of habitat on morphological characters was assessed, several misidentifications were corrected. From about 180 plants (either representatives of individual clones or all cultivated plants from taxa with intraspecific variability at the locality level), specimens were prepared for each individual plant and deposited in the herbarium GLM. Plants were determined by S. B.

Cytology and mode of reproduction. — Ploidy levels were determined by flow cytometry as described previously (Bräutigam & Bräutigam, 1996;

Krahulcová & al., 2004) for about 250 plants from 70 populations. Chromosome counting was done for some individual plants and most *H. cymosum* (Šimek, 2001); results from both approaches corresponded to each other.

Breeding systems were studied using a combination of isolating capitula, open pollination and emasculation in order to decide between allogamy, sterility, autogamy and apomixis as described before (e.g., Gadella, 1984; 1987; Richards, 1997). Only amphimictic and apomictic modes of reproduction were found among the investigated taxa.

Molecular methods. — DNA isolations were done as previously described (Štorchová & al., 2000), but fresh material was used whenever possible.

Multilocus fingerprinting. Classical Southern hybridisation was used to determine apomictic clones. Minisatellite sequences were used, because they usually give individual-specific patterns (Bruford & Saccheri, 1998). After testing several combinations of minisatellite probes and restriction enzymes, a combination of the human minisatellite 33.15 (Jeffreys & al., 1990) with *Taq* I-digested DNA was chosen. The human minisatellites have been shown to also occur in plants (Dallas, 1988) and have been used before to distinguish between apomictic clones in *Rubus* (Nybom & Kraft, 1995).

3.5 µg of genomic DNA was digested and separated on a 0.8% agarose gel at 1 V/cm overnight in 2x TTE buffer (taurine replacing boric acid for its glycerol tolerance). Blotting was performed by standard methods and the DNA was cross-linked to Hybond-N^+ nylon membranes (Amersham). Hybridisation with the 33.15 probe (directly labelled with alkaline phosphatase) and chemiluminescent detection using CDP-Star™ as a substrate were carried out according to the manufacturer's instructions (NICE oligo kit, Cellmark Diagnostics/Tepnel Lifesciences Plc., Abington/Maryland).

The combination of *Taq* I with the 33.15 probe produced very complex and highly polymorphic patterns. Their individual specificity was confirmed by comparison of 20 sexual and four apomictic populations of *H. cymosum* ssp. *cymosum* and *H. c*. ssp. *cymigerum* (64 and 19 plants, respectively). Only in exceptional cases were identical patterns found among sexual plants, which were attributed to vegetative propagation. Apart from that, plants of sexual populations differed considerably from each other while apomictic plants gave identical patterns (Fig. 2). These marked differences allowed straightforward qualitative identification of clonal lineages, even across different blots, by visual inspection. In doubtful cases, the particular samples were blotted again in adjacent lines for confirmation, and patterns were always found to be reproducible.

DNA fingerprinting was applied to the apomictic species *H. caespitosum*, *H. glomeratum*, *H. iseranum* and *H. floribundum*. For *H. piloselliflorum*, a highly variable species with respect to morphology, ploidy levels and mode of reproduction, subsets of the populations were analysed by fingerprinting, and partly by isozyme analysis using enzymes known to be polymorphic in *Hieracium* (AAT(GOT), SHDH, PGM, LAP) and following protocols as described previously by Štorchová & al. (2002). This analysis was carried out in order to account for

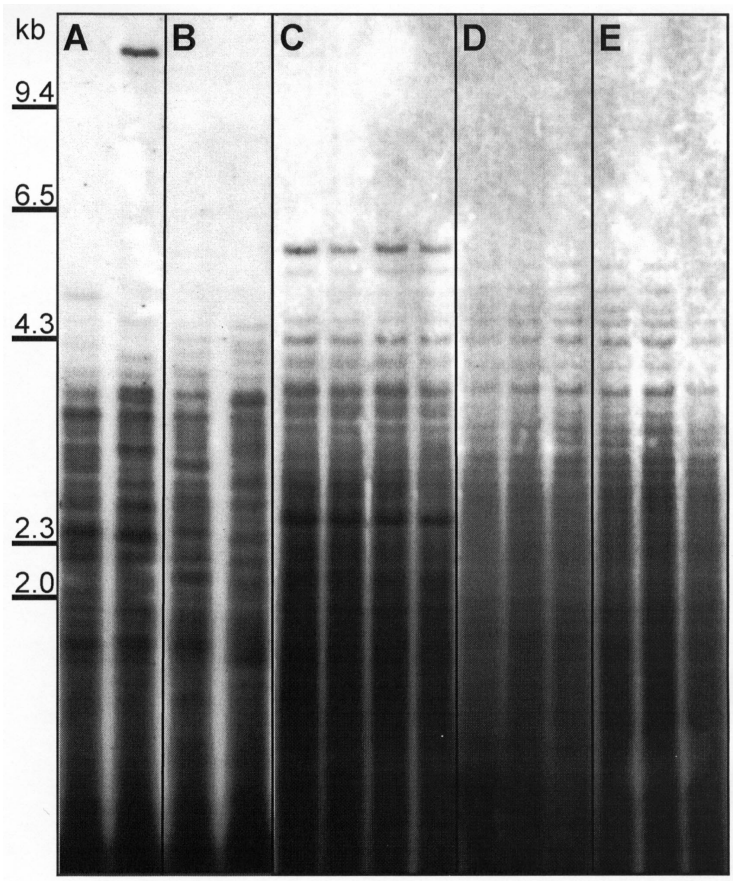

Fig. 2. Individual- and clone-specific fingerprints. A, B, two sexual populations of *H. cymosum* ssp. *cymosum*; C–E, three apomictic populations of *H. cymosum* ssp. *cymigerum*; D, E, (20 km apart from each other, both tetraploid) were addressed as the same clone. C was a pentaploid that according to similarity could have originated from a backcross of the tetraploid clone in D and E (unreduced gamete) with a reduced gamete of a diploid plant of ssp. *cymosum* (see also their distribution in Fig. 5). For the purpose of this study, C is simply addressed as being qualitatively different from D and E. Other clones of intermediate species usually differed much more from each other, comparable to the examples in A and B, suggesting that recombination was involved in their production.

between- and within-population differences. The variability of the combined isozyme patterns of the polyploids corresponded approximately to the level of resolution seen in a single DNA fingerprint.

PCR-RFLPs and DNA Sequencing. Based on previous results from PCR-RFLPs of the *trnT–trnF* fragment of cpDNA (Fitze, 2000; Fitze & Fehrer, 2000), the *trnT–trnL* intergenic spacer was chosen for further analysis, as it showed the

highest variability in our species. PCR reactions were performed according to Taberlet & al. (1991), using their primers a and b (a/b or a/f for the PCR-RFLPs). Both strands were sequenced (GATC Biotech AG, Konstanz, Germany); resulting ABI tracer files were obtained and proof-read manually. Alignment was done using CLUSTAL X (Thompson & al., 1997) with manual adjustment of regions containing indels. Sequences were deposited in GenBank (accession numbers AY192646–83). Based on the alignment a haplotype network was constructed by hand, which was straightforward since observed changes were free of homoplasy except in a poly-A region described below. At least one representative of each taxon was sequenced; additionally, representatives of all apomictic clones found by fingerprinting were either sequenced or typed by PCR-RFLP. For sexual taxa and *H. piloselliflorum*, at least one representative of each population was typed by PCR-RFLP to check for within-species variability. Length variations of 5 bp and higher were detectable by restriction digestion of *trnT–trnL* or *trnT–trnF* fragments with *Eco*RI, and separation of the resulting fragments on high-resolution agarose. In this way, many haplotypes were identifiable without further sequencing, providing a helpful tool for the assignment of all investigated populations to the major haplotypes.

Maternal inheritance of chloroplast DNA was ascertained by performing restriction analysis on artificial hybrids from reciprocal crosses of two species pairs (*H. lactucella*/*onegense* and *H. lactucella*/*pilosella*), the parental species of which are known to belong to different chloroplast haplotype groups (see haplotypes in Table 2 and Fig. 4).

RESULTS

Identification, clonal structure and distribution of apomicts. — In this study a combination of cultivation, detailed taxonomic analysis and DNA fingerprinting either confirmed or rejected the provisional field identifications made, and enabled us to reliably allocate plants to individual clones/species. Sometimes individual plants of a population, sometimes entire populations, proved to belong to a taxon differing from that assumed at the time of collection. The main reason for this was that often rosettes were sampled in the field (i.e., without inflorescences), because (i) they provided better material for analyses and cultivation, and (ii) not enough plants were flowering at the same time. Except in one case where taxonomic assignment remained contradictory (and was thereafter removed from the study), we found a perfect correlation between fingerprint pattern and morphology, i.e., evidence from both was mutually supporting. In every case, morphologically detectable differences corresponded to a unique fingerprint, the reverse not always being the case, however. As different species/clones often grew intermingled even when this was not obvious in the field, it proved essential to determine each plant individually and compare it to its respective fingerprint. In this way, the proper assignment of each individual to the correct taxon (as a prerequisite for further

Table 2. Sequenced taxa. Basic species are given in boldface. Identifiers contain locality information (compare Table 1). Haplotypes correspond to Fig. 4. For the taxa of the morphological series, fingerprint analyses are indicated for the apomictic clones and *H. cymosum* ssp. *cymosum*; for the sexual taxa and those clones not sequenced, PCR-RFLPs were performed to identify the haplotype group. For *H. piloselliflorum*, eight populations (28 plants) were additionally analysed for their isozyme patterns. All populations were covered in one and/or the other way so that clones (for apomicts) and haplotypes (for all populations) were fully resolved. * presumably a hybrid *H. lactucella* x *H. onegense*, see Fig. 3d.

Species	Identifier	Haplotype group/ number	Ploidy level	PCR-RFLPs populations/ plants	Fingerprinted populations/ plants
H. aurantiacum	aur.Spb.1	I/ 3	4*x*		
H. bauhini	bau.AM.1	II/11	5*x*		
H. bauhini	bau.Got.1	II/11	5*x*		
H. caespitosum	glo.Fuer.4	II*/13	5*x*	3/5	6/19
H. caespitosum	glo.Gau.1	II/ 7	4*x*	8/13	13/38
H. cymosum* ssp. *cymosum	cym 12/4	II*/14	2*x*	8/8	20/64
H. cymosum* ssp. *cymigerum	cymi.Wbs.	II*/12	4*x*	2/2	4/19
H. dubium	flo.Zwh.1	I/ 5	4*x*		
H. echioides	ech.Goer.1	II/ 9	2*x*		
H. echioides	ech 99/104.3	II/ 8	2*x*		
"*H. floribundum*"*	flo.FL	I/ 1	2*x*		
H. floribundum	flo.Fro.2	I/ 1	n.d.		
H. floribundum	flo.Jac.2	I/ 1	4*x*	10/12	16/41
H. floribundum	flo1.JgS.1	I/ 5	4*x*		
H. floribundum	flo.ZwW.1	I/ 5	4*x*		
H. glomeratum	glo.Dba.1	II/ 7	5*x*	1/1	1/4
H. glomeratum	glo.Jac.2	II/ 7	5*x*	5/10	12/36
H. glomeratum	glo.JMi.1	II/ 7	4*x*		1/5
***H. hoppeanum* s.str.**	34 hop	II/ 7	2*x*		
H. iseranum ssp. *iseranum*	iser GSc.1	I/ 1	4*x*	13/17	15/53
H. iseranum ssp. *confinium*	ise.Gr2.2	I/ 1	4*x*	1/1	1/3
H. lactucella	lac.Jac.1	I/ 1	2*x*		
H. lactucella	lac.Jon.1	I/ 1	2*x*	8/8	
H. lactucella	la.GR.1	I/ 4	2*x*		
H. macranthum	137 mac	II/ 6	2*x*		
H. onegense	caeb.Jbo.2	II/ 7	2*x*		
H. pilosella	pla.Gba.1	II/ 7	4*x*		
H. pilosella	pla.Jbo.2	II/ 7	4*x*	16/21	
H. pilosella	pla.Jbo.1	II/10	4*x*		
H. piloselliflorum	pfl.Jbo.2	I/ 1	4*x*		
H. piloselliflorum	pfl.Jbo.3	I/ 1	4*x*	13/33	8/29
H. piloselliflorum	pfl.Kau.5	I/ 4	4*x*		
H. piloselliflorum	pfl.Kau.4	II/ 7	6*x*		
H. piloselloides* ssp. *obscurum	poio.AM.1	II/ 7	4*x*		
H. piloselloides* ssp. *praealtum	poip.GRG.5	I/ 3	5*x*		
H. rubrum	rub.Krk.1	I/ 2	6*x*		
H. schultesii	scu.Jbo.1	II/ 7	4*x*		
H. tubulascens	tub.Jbo.1	I/ 1	4*x*		

study) could be achieved. Table 1 shows the corrected list of taxa.

Furthermore, here we were able to resolve the taxonomic confusion concerning the infraspecific structure of *H. caespitosum* and *H. glomeratum* in the study

area (see below). More detailed morphological analysis and taxonomic results of this study will be presented elsewhere (Bräutigam & al., in prep.).

Hieracium caespitosum. The existence of infraspecific taxa for this species as described previously (Zahn, 1922–1930) was not supported in our investigation area. Only two major clones were detected, corresponding to tetraploid and pentaploid plants, respectively (Fig. 3a). The tetraploid clone was found all over the study site and corresponded to *H. caespitosum* ssp. *caespitosum*; the pentaploid seemed to be the result of an introgression from *H. cymosum* (see below) and was restricted to the Erzgebirge region. The distinction between *Hieracium caespitosum* ssp. *caespitosum* and ssp. *colliniforme* (Peter) P. D. Sell proved to be artificial as the morphological differences disappeared in cultivation and the respective populations could be assigned to one or the other clone (Fig. 3a).

Another taxon originally described both as *H. caespitosum* ssp. *madarum* (Naeg. & Peter) S. Bräutigam and as *H. glomeratum* ssp. *cymigeriforme* Naeg. & Peter was confirmed to belong to *H. glomeratum* due to its identity with the most abundant clone (Fig. 3b). A rare diploid Eastern European/Siberian taxon, which had only recently been rediscovered in our region and considered as *H. caespitosum* ssp. *brevipilum* (Naeg. & Peter) P. D. Sell in, e.g., *Flora Europaea* (Sell & West, 1976), was treated as a separate basic species according to morphological and cytological evidence and is referred to here as *H. onegense* (Norrl.) Norrl. (Schneider, 1889; Krahulcová & al., 2001; Šimek, 2001; Rotreklová & al., 2002). Furthermore, an origin resulting purely from a reduction of the tetraploid to a dihaploid, a process demonstrated previously for other *Pilosella* species (Skalińska, 1971; 1976; Bicknell, 1997; Krahulcová & Krahulec, 2000), could be ruled out by the *H. onegense* fingerprint pattern: it was entirely different from the tetraploid instead of just showing a subset of bands, which would have been expected given the tetraploid → dihaploid scenario.

After our attention had been focused on the existence of two widespread apomictic clones of *H. caespitosum*, we were able to find some corresponding morphological differences between them which had previously gone undetected, e.g., a slightly different leaf colour, which is not usually apparent in herbarium specimens, and is difficult to detect in the field if no plants are available for direct comparison.

Hieracium glomeratum. Three clones of this species occurred in the study area (Fig. 3B), a widespread one which was pentaploid and two local ones which differed morphologically in having markedly longer stolons and flagellae in the pentaploid Krkonoše population (Dba), and very short trichomes and either missing or extremely short stolons in the tetraploid Erzgebirge population (JMi). The Oberlausitz populations of the most abundant clone correspond to the questionable taxon *H. caespitosum* ssp. *madarum* discussed in the previous paragraph. Apart from the occurrence of a single tetraploid clone in the west, we did not find *H. glomeratum* in the Erzgebirge region; two other populations from there, initially resembling *H. glomeratum*, turned out later to be identical to the pentaploid clone of *H. caespitosum*. This similarity which was even more pronounced than the gen-

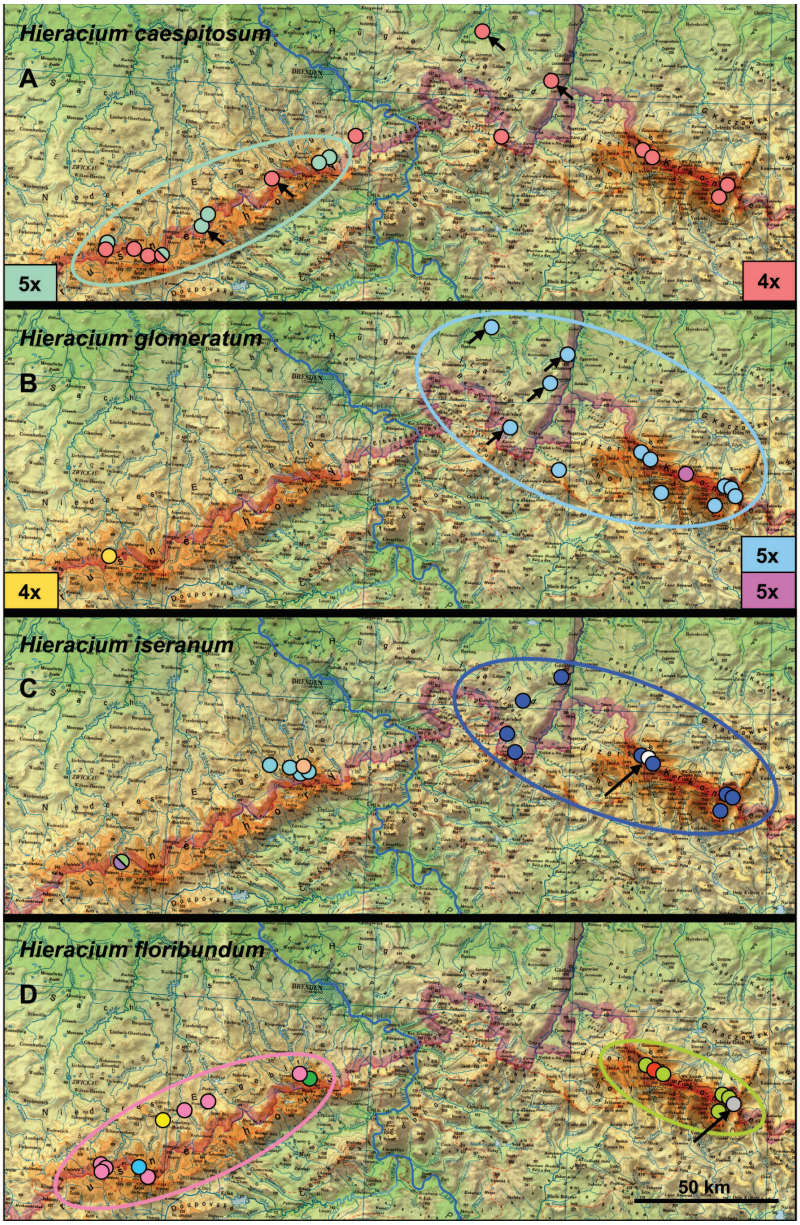

Fig. 3. Distribution of apomictic clones, with identical clones indicated by colouring. (a) arrows indicate populations originally assigned to *H. caespitosum* ssp. *collinifforme*. (b) arrows indicate populations originally assigned to *H. caespitosum* ssp. *madarum*. (c) arrow indicates ssp. confinium; all plants were tetraploid. (d) arrow indicates a diploid *floribundum*-like population presumably representing a hybrid between *H. lactucella* and *H. onegense*; the others were tetraploid (flo.Fro, yellow, n.d.). Ovals mark widespread clones restricted to different mountain ranges.

erally close resemblance of *H. caespitosum* and *H. glomeratum* will be dealt with below (see discussion).

Hieracium iseranum. For this species one widespread clone with a distribution similar to that of the abundant *H. glomeratum* clone was found (Fig. 3c, indicated in blue). It has its biogeographic centre in the Jizerské hory (Isergebirge) after which the species is named. More clones were detected in the Erzgebirge, in one case two at the same locality. No differences in morphology and ploidy level were detected among these clones (all included here were tetraploid) and we therefore treat them all as *H. iseranum* ssp. *iseranum*. Only a single population from the Jizerské hory could be distinguished morphologically (ssp. *confinium*) and represented a different clone as well (Fig. 3c, white).

Hieracium floribundum. This species showed many independent origins of its clones (Fig. 3d). Two clones were more widespread, one occurring in the Erzgebirge (Fig. 3d, in pink) and one in the Sudetes (Fig. 3d, in green). No morphological differences were detected between clones within the same mountain range. Differences between clones from different mountains, e.g., a tendency of the Erzgebirge populations towards hairyness and stronger formation of stolons and flagellae, became less pronounced in culture. Plants from the type locality in Poland did not correspond to any of our clones (not shown). These results fit the assumption of a hybridogenous species of repeated (including recent) origin. Apart from a rare diploid resembling *H. floribundum* and presumably representing a recent hybrid between the diploids *H. lactucella* and *H. onegense* (see above), all investigated plants were tetraploid.

Hieracium piloselliflorum. In this extremely polymorphic taxon, almost each population appeared to have independent origins, as only two could not be distinguished by their isozyme patterns. In about half of the investigated populations, additional within-population variability was found, sometimes accompanied by different ploidy levels as well (4*x*/5*x*, 4*x*/6*x*). As only 2–8 plants per population were analysed, this variability is remarkable in comparison to the other taxa included in this study. Some of the tetraploids of this species are known to be sexual, other tetraploids and plants with higher ploidy level are facultative apomicts (Krahulcová & Krahulec, 1999; Krahulcová & al., 2001). In addition to the large genuine morphological variability, a strong influence of phenotypic modification was found as well, i.e., individual plants of *H. piloselliflorum* were able to change their growth form according to season. Other observations, such as the disjunct distribution and the frequent co-occurrence of the presumed parental species (*H. floribundum*/*iseranum* and *H. pilosella*, respectively) suggest that *H. piloselliflorum* is a recent hybrid whose phenotype can be produced in different ways (see also the chloroplast haplotype study below).

Maternal lineages. — In this study the *trnT–trnL* intergenic spacer of maternally inherited chloroplast DNA (amplified as a 609–659 bp fragment) was sequenced for 38 taxa/clones. Most of the length variation was either due to duplications of adjacent parts (6–33 bp) or to different numbers of A's in a central hypervariable poly-A stretch (A/T content of the entire region was about 70%).

Substitutions occurred throughout the sequence, but variability was relatively low, i.e., max. 2.5% sequence divergence. Fig. 4 shows a haplotype network based on the aligned sequences; the corresponding taxa are listed in Table 2.

The haplotypes fell into two well-distinguished groups referred to as I and II, which were separated by a number of substitutions (half of them transversions) and a large indel. Sequence divergence between group I and II was 1.2%.

Within each haplotype group, one particular sequence (1 of group I and 7 of group II) was shared by several species. On the other hand, several species were slightly polymorphic, usually due to variation in the hypervariable poly-A region, which is, therefore, likely to be subject to homoplasy. The only example of a species comprising plants from both haplotype groups was the recent hybrid *H. piloselliflorum* for which we found that type I occurred in three populations, type II in 8, and type I or II in 2 populations. This indicates that hybridisation occurred in both directions, involving parents of the different haplotype groups. No clear correspondence was found between haplotype groups and ploidy level. However, most tetraploids of *H. piloselliflorum* showed type I, most penta- and hexaploids type II, further indicating that this taxon was formed in different ways.

In contrast to this recent hybrid, the two intermediate species *H. iseranum* and *H. floribundum* showed only haplotypes of group I although these species also originated 6–8 times independently, based on the observed number of distinct clones (see Fig. 3c, d). The same holds for the intermediate *H. glomeratum*: the three clones we found in our investigation area (see Fig. 3b) all had identical sequence (II/7) so that they could have obtained their cpDNA only from tetraploid *H. caespitosum*, but not from *H. cymosum* (see below).

Concerning *H. caespitosum*, a distinction between the maternal origin of the tetraploid and the pentaploid clone became necessary. The latter showed a somewhat different haplotype (II*) which was characterized by a 6 bp-insert in the poly-A region (Fig. 5a) and also present in the two *H. cymosum* taxa. While the insert showed slight variation, it is nevertheless considered to have originated from a single insertion event, since no further inserts were found while gains and losses of single nucleotides in the hypervariable region are common. Using this diagnostic indel as a tool, more populations of *H. cymosum* ssp. *cymosum* and ssp. *cymigerum* were tested for length difference between types II and II* by PCR-RFLP. All of them showed the same pattern as the pentaploid *H. caespitosum*. A (preliminary) distribution map for these three taxa (Fig. 5b) indicates that introgression of *H. cymosum* ssp. *cymosum* cpDNA into ssp. *cymigerum* and/or into *H. caespitosum* may well have been possible. In addition, the morphology of these three taxa does not contradict such a scenario. In the highest mountain of the range (locality Wbs, Table 1), tetraploid *H. cymosum* ssp. *cymigerum* as well as both cytotypes of *H. caespitosum* were found to grow intermingled. *H. cymosum* ssp. *cymosum* is probably extinct in our investigation area and also has different habitat preferences to the rest of the taxa studied which makes a direct introgression of its cpDNA in the Erzgebirge less likely. However, no investigation into the distribution of the pentaploid *H. caespitosum* clone on the southern side of the Erzgebirge, where it might

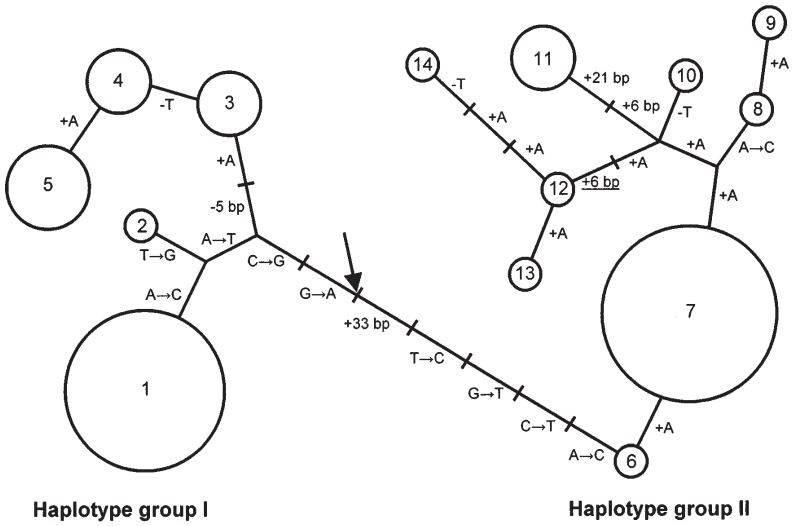

Fig. 4. Haplotype network of the *Pilosella trnT–trnL* intergenic spacer of chloroplast DNA. Bullet sizes correspond to the number of individuals with identical sequences; numbers refer to Table 2. The direction of substitutions and indels was assumed from the insert point (arrow) of outgroup taxa (not shown). Two major haplotype groups (I and II) were separated by several substitutions and a large indel; within-group variability was mostly due to length variations (+A, -T) at a mutational hotspot. A 6 bp-insert characterizing a subset of haplotype group II, "*H. cymosum* group" (II*) comprising sequences 12–14, is underlined (see text and Fig. 5 for details).

occur as well, has been carried out. The formation of pentaploid *H. caespitosum* could have occurred via a reduced egg cell of diploid *H. cymosum* ssp. *cymosum* plus unreduced pollen of tetraploid *H. caespitosum*, which would also account for the high similarity between the two *H. caespitosum* clones. Alternatively, contribution of *H. cymosum* ssp. *cymigerum* instead of ssp. *cymosum* cannot as easily be explained cytologically, but is somewhat suggestive with regard to the mentioned locality. However, more work will have to be done before firm conclusions about this aspect can be drawn.

Apart from this example, no correlation between haplotype and geographical distribution was found in our study. With the exception of *H. piloselliflorum*, every species, even the 'intermediates', always possessed either one or the other haplotype, despite their sympatric occurrence as well as being members of a hybridogenous complex. Also, in contrast with the DNA fingerprint results, no correlation between haplotype group and ploidy level or haplotype group and morphological characters could be detected.

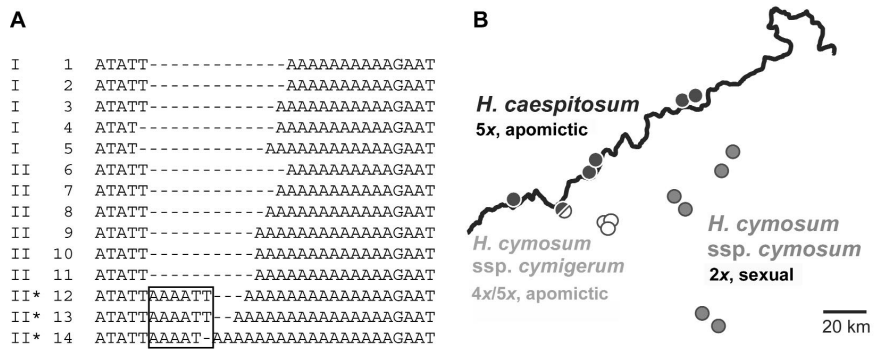

Fig. 5. "*H. cymosum group*" (A) Alignment of the hypervariable poly-A part of the *trnT–trnL* intergenic spacer. The box indicates the insert characterizing *H. cymosum* ssp. *cymosum*, *H. cymosum* ssp. *cymigerum* and the pentaploid clone of *H. caespitosum* (haplotype group II*). Numbers and haplotype groups are as in Table 2. (B) Location of samples of the "*H. cymosum* group". The border between Germany and the Czech Republic is outlined. The Klínovec (Wbs) locality where the two apomictic taxa occurred together is the highest mountain of this range (Erzgebirge/Krušné Hory). Two more populations of *H. cymosum* ssp. *cymosum* some 80 km south of this map's area also have group II* haplotype. For discussion, see text.

DISCUSSION

Delimitation of facultative apomicts. — The correct determination and delimitation of the apomictic taxa proved to be even more difficult than previously assumed, even for a specialist familiar with the group. However, in the end, the problem could be solved satisfactorily for the taxa in our study area by a combination of morphological analysis, cultivation and the use of clone-specific molecular markers. Whether these results will be transferable to other regions (e.g., the Alps), which are known to have different species composition and an apparently different history of speciation processes, remains to be seen. The recognition of an apomictic taxon, especially if it belongs to an intermediate species, is inevitably dependent on the conceptual background as well as on the specific knowledge of the particular region. Even different specialists for a group such as *Hieracium* may often hold different opinions about the same plant. The situation in many herbaria is even worse, because the specimens deposited there have often not been identified by any specialist at all so that, e.g., *H. floribundum* or *H. glomeratum* from other sources cannot automatically be assumed to represent the same taxa as, e.g., those of the same name included in this study. A comparable problem appeared to occur in *Rubus*, where presumably different species had identical or almost identical fingerprints (Nybom & Kraft, 1995). In our opinion, taxonomic uncertainties are the most likely explanation for such findings in apomictic groups, especially if the plants have been identified by different people. Apart from this taxonomic point of view, our study showed that what is now called a species in *Pilosella* consists of

morphologically similar types which have arisen several times independently (see also Van Dijk & Vijverberg, this volume, Chapter 5).

Regarding the fact that evidence from morphological and molecular data is often incongruent (e.g., Dubuisson & al., 1998; Shaw & al., 2003), we found the almost perfect correspondence between taxa suggested by DNA fingerprinting and/or morphology, respectively, rather surprising. It suggests that the human minisatellite probe detected sequences at many different loci, which may be scattered across the different chromosomes (Bruford & Saccheri, 1998), providing a good overall representation of the organism's genome.

Variation at different scales: what makes up a species? — The apomictic taxa analysed in detail (species in Fig. 3 and *H. piloselliflorum*) showed quite different levels of genetic variability on the same geographic scale:

The least variable proved to be the 'basic' species *H. caespitosum* apparently consisting of a single clone. One can easily imagine a much wider distribution of the tetraploid (*H. caespitosum* ssp. *caespitosum*) occurring throughout the investigation area. A similar situation was found in apomictic *Rubus* species that also consisted of a few widespread genotypes only (Nybom & Kraft, 1995), and in the agamospecies *Hieracium holosericeum* which was found to consist of only a single genotype (Shi & al., 1996).

Compared with *H. caespitosum* the intermediate species *H. glomeratum*, *H. iseranum* and *H. floribundum* showed a higher number of clones, but always including widespread ones. Different clones of the same species were morphologically quite similar to each other, and in many cases indistinguishable. The three species occupy distribution areas up to half the size of Europe and are often abundant so that their treatment as species seems to be justified because of their biological and ecological significance. The differences in biogeographic distribution among these three species possibly reflect the different ages of the clones of which they consist. For instance, due to the apomictic mode of reproduction, a genotype newly formed by hybridisation would immediately become fixed, and the more advantageous will have spread over larger distances than others as wind dispersal is likely to be efficient in hawkweeds, due to their fruit morphology (hairy pappus). In this way one could perhaps regard widespread clones simply as older because having had more chance to spread.

While most plants of the extremely variable *H. piloselliflorum* were of different (hybridogenous) origin, this species nevertheless represents a more or less recognizable taxon. Thus, a high variability, whether morphological (e.g., in *H. piloselliflorum* and also in the sexual 'basic' species *H. pilosella*) or genetic (e.g., in *H. piloselliflorum* and generally in sexual species) does in itself not constitute a good species definition in apomictic complexes. On the other hand, the low variability found in the apomictic 'basic' species and in fixed intermediate taxa does reflect a biological reality. However, if consequently applied, it would lead to a microspecies concept in which every apomictic lineage of single origin had to be classified as a separate taxon (Bachmann, 1998). Thus, there simply is not a single concept, not even theoretically, that would do justice to the biological situation in

all its aspects in the different species. Additionally, it is evident that any classification determined amidst ongoing processes, i.e., taking a snapshot of species that are just undergoing different stages in their process of speciation, will be preliminary. At present, it therefore seems most appropriate as well as practical to adhere to a wide morphological species definition, keeping in mind that the term "species" subsumes quite different underlying processes. This is especially true at larger geographic scales: a general correlation between latitude and degree of apomixis has been suggested with a tendency towards the apomictic mode of reproduction in Northern Europe (Asker & Jerling, 1992). In *Hieracium*, these differences have even led to different species concepts for the Central European and the North-Eastern European region, respectively (reviewed in Merxmüller, 1975). Further work including the detailed analysis of other geographical regions and covering the entire distribution area of the respective taxa are needed to obtain a more representative picture.

Habitats, dispersal and time. — The geographic distribution of the widespread clones (Fig. 3) revealed a partial isolation between the two mountain ranges. This barrier did apparently not affect the tetraploid *H. caespitosum* which occurs throughout the investigation area, consistent with its status as basic species of supposedly relatively older age than the intermediates. The latter are likely to have originated from basic species only after the typical habitats in the respective regions became available.

In the Erzgebirge, originally a completely wooded area, suitable habitats for *Pilosella* were first created by agricultural activities (pastures, forest fringes, road margins), later by the onset of mining activities up to the highest altitudes (slagheaps, industrial areas, large clearings, etc.), so that the present pattern of forests and open habitats in principle dates back to the 16th century (Bernhardt & al., 1986). About the same time frame holds for the Sudetes (Lokvenc, 1978; Speranza & al., 2000), where most of Pillosellae occur in secondary grasslands in the forest (montane) zone. Although the probably oldest suitable habitats for *Pilosella* existed in the hill country of the Oberlausitz (Bernhardt & al., 1986), our specimens were found exclusively in very recent man-made habitats (e.g., parking spaces, cemeteries, slagheaps, and roadsides). We assume that these localities have been colonised from the Sudetes because of a concentration of the intermediate species on the eastern part of the Oberlausitz and their absence in its northern and western parts. *H. iseranum* especially belongs ecologically to an eastern-montane distribution type mainly reflecting the ecophysiological constitution of the species. We therefore consider the alternative scenario of early intermediate species formation in the hill country and subsequent colonisation of the Sudetes to be unlikely. Thus, the intermediate taxa in our study area are presumably no older than c. 500 years.

Biogeographic separation between the Erzgebirge and the other regions may have been promoted by a lack of habitats suitable for the *Pilosella* species investigated here in the area between them, i.e., in the Elbsandsteingebirge, a mountain area flanking the Elbe (Labe) river valley. More evidence for this partial isolation

comes from a recent study on the variability of isozyme patterns in *Abies alba* Mil. (Gómez & Braun, 1995) in the western part of our investigation area: clear differences between the Erzgebirge and Elbsandsteingebirge populations were demonstrated. Thus, the influence of Sudetic (eastern) genotypes in *Abies* ended in the Elbsandsteingebirge, in *Hieracium* already in the Oberlausitz due to habitat availability, but in both cases, the Erzgebirge was not colonised from the east.

Maternal lineages and species relationships. — The separation of the *Pilosella* species into two well distinguished haplotype groups (Fig. 4) was unexpected as it did not correspond to any discriminating biological features. Group I contained less species, among them three 'basic' species of which only one (*H. lactucella*) was diploid (Table 2). Group II comprised the majority of the species analysed, among them nine basic species of which five were diploid (the total number of *Pilosella* basic species is about 25). This suggests that separation of the major haplotype groups predated speciation in *Pilosella*. Very little divergence of the cpDNA lineages occurred after their separation into the two major lineages, because within each group, most species shared the most abundant haplotype or were very similar to it. In the case of the basic species, this might be explained by persistence and differential sorting of ancient polymorphisms (Schaal & al., 1998). In the case of the intermediates it is more likely due to unidirectional introgression, at least in most cases (see also below).

Hybridogenous types and apomictic basic species are assumed to have originated from diploid progenitors, eventually via several subsequent events. We consider gene flow from polyploid intermediates towards diploids as unlikely and therefore ignore this scenario in the further discussion.

In a hybridogenous complex, one could expect to find the intermediate species "jumping" between the haplotype groups if they were produced by reciprocal crosses of parental species of one or the other type or, if one of the parents was an apomict, they would exhibit the cpDNA of the sexual (seed) parent. Our findings, however, were mostly contrary to both expectations:

H. piloselliflorum and H. iseranum. Of the four intermediate species analysed in detail, only *H. piloselliflorum* showed both types of cpDNA. While the sexual *H. pilosella* was expected to be the seed parent, five out of 13 populations had exclusively or partly the "wrong" haplotype. According to morphology and species distribution, only *H. floribundum* or *H. iseranum* could have acted as crossing partners together with *H. pilosella* (Fig. 6). Given the maternal transmission of the chloroplast genome which we confirmed in reciprocal experimental hybridizations for our species complex (see methods), we conclude that in the observed cases, the apomicts underwent sexual reproduction and repeatedly acted as seed parents (seven times independently in *H. piloselliflorum* in our restricted data set).

The situation was even more pronounced in *H. iseranum*. Not a single plant with the haplotype of the sexual *H. pilosella* was found (Fig. 6) although this species should have contributed to the formation of *H. iseranum*. This is because the size and inflorescence of the intermediate can only be explained by the contribution of a small, single-stemmed species with large capitulum and red-striped

ligulae of which *H. pilosella* is the only candidate occurring in that region. Furthermore, two other basic species with similar phenotypes to *H. pilosella*, *H. hoppeanum* and *H. macranthum*, also belong to haplotype group II. This means that the facultative apomict *H. floribundum* remains the only probable seed parent.

Experimental crosses support these findings: using *H. pilosella* as seed parent and *H. iseranum* as pollen donor, a large variety of plants with *H. piloselliflorum* phenotype was produced (Krahulcová, unpubl. results). However, all attempts to use *H. floribundum* instead of *H. iseranum* as pollen donor failed to produce any progeny at all. It can be concluded that only part of the possible *H. piloselliflorum*-like types were realised in these crosses (namely those showing the *H. pilosella* haplotype), whereas an *H. iseranum*-like phenotype was never produced, because for some unknown reason, it also did not seem to exist in nature. But when we, inspired by the chloroplast data, tried the reciprocal cross and used the facultatively apomictic *H. floribundum* as mother plant and pollen from the sexual *H. pilosella*, two hybrids of *H. iseranum* phenotype were obtained. Similarly, the cross between apomictic *H. iseranum* as female parent and *H. pilosella* produced progeny of *H. piloselliflorum* phenotype.

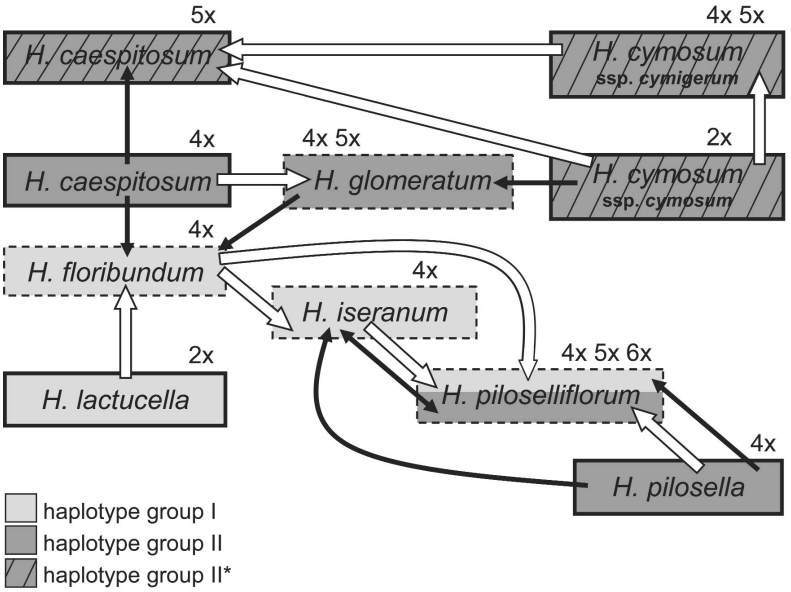

Fig. 6. New working hypothesis of relationships. Suggested relationships between species were based on cpDNA haplotypes, morphology, locality information (e.g., *H. piloselliflorum*, "*H. cymosum* group") and experimental hybridisations. The white arrows indicate maternal lineages/seed flow, the black ones represent assumed pollen donors. For details see text. In addition to the diploids, all *H. pilosella* and part of tetraploid *H. piloselliflorum* are sexual. More ploidy levels are reported for the respective taxa, but only those actually occurring in the investigated plants are shown.

H. floribundum. All *H. floribundum* clones uniformly showed haplotype group I, this time meeting the expectation of having obtained their cpDNA from diploid sexual *H. lactucella*. Both species are slightly polymorphic in their haplotypes (Table 2). Apart from morphology, evidence for *H. caespitosum* as the second parental species again came from experimental crosses, in which a *H. floribundum* phenotype was obtained in crosses between *H. lactucella* and tetraploid *H. caespitosum*. However, these hybrids were triploid, a cytotype not found in *Pilosella* in the studied region and rarely found in general (Schuhwerk, 1996; Schuhwerk & Lippert, 1997; 2002). The triploid progeny was morphologically indistinguishable from tetraploid *H. floribundum*. Even if no tetraploid cytotype was produced in these crosses, it might still arise from the same parents in nature, either as a more than one-step process or as the product of an unreduced diploid egg cell fertilised by reduced pollen of the tetraploid.

Experimental crosses suggested a further possibility for the production of a *H. floribundum* phenotype: hybridisations between *H. lactucella* and pentaploid *H. glomeratum* resulted partly in tetraploid *H. floribundum*-like progeny as well as in triploids not distinguishable from the triploids produced experimentally by *H. lactucella* and tetraploid *H. caespitosum*. While this might be an alternative way (Fig. 6) that could also be realised in nature, it does not explain the origin of *H. floribundum* in the Erzgebirge as we did not find pentaploid *H. glomeratum* there, and *H. floribundum* is partly of recent origin judged from the distribution of the local clones found. No attempts have as yet been made to use the pentaploid clone of *H. caespitosum* as pollen donor. From its distribution range (Fig. 3A) and cytotype, it could theoretically be imagined to have participated in the rise of the Erzgebirge clones of *H. floribundum*, however, from its morphology, we consider this to be less likely.

H. glomeratum. The existence of the *H. cymosum*-like subgroup of haplotypes (II*, see Fig. 6) rules out *H. cymosum* as a seed parent for *H. glomeratum* as this subgroup is not found in the latter species. This leaves tetraploid *H. caespitosum* as the only probable candidate as, for morphological reasons, other species of haplotype group II can be disregarded. This presents another example of a facultative apomict rather than a sexual species acting as seed parent. Having traced the potential pathways of the maternal lineages, the striking similarity between *H. glomeratum* and the pentaploid *H. caespitosum* which had first led to serious difficulties in their determination, becomes more understandable. They appear to represent reciprocal crosses between tetraploid *H. caespitosum* and diploid *H. cymosum* ssp. *cymosum*. Their much stronger resemblance to *H. caespitosum* ssp. *caespitosum* can be explained by assuming that they either received a higher number of chromosome sets from it than from *H. cymosum* and/or that they result from backcrosses to *H. caespitosum*.

Formation of intermediate taxa. — Although it cannot be categorically ruled out that some of the intermediate taxa are products of an ancient divergence of apomictic lineages due to somatic mutations, this possibility seems to be less likely in the taxa investigated here for several reasons: (i) Even the oldest and most

widespread clones had almost invariant fingerprints whereas the differences between clones were so pronounced that recombination events are the much likelier explanation for their formation. Even the fingerprints of the same species presumably produced by the same combination of parental species were nevertheless so different from each other that assigning them to one or another clone or else defining them as obviously different was straightforward. One identical genotype of the apomict could even have contributed several times independently to the formation of a hybrid taxon (see *H. floribundum* as an example). (ii) As all habitats were quite young (i.e., less than c. 500 years) and of anthropogenic origin, somatic mutations alone could hardly have produced the differences observed in the DNA fingerprints within such a short time frame. Colonisation by taxa which might have already existed long before the habitats became available did not correspond well to the geographic distribution of the clones and would apply at best to part of the widespread ones (but see discussion above).

On the other hand, ample evidence favouring hybridisations/allopolyploidy as the most probable mechanisms for the origin of the intermediate species is presented in this study. The different relative ages of the hybridogenous taxa are demonstrated by differences in the frequency and distribution of clones as well as by field observations, i.e., whether both, one or none of the parental species occurred together with the intermediate.

Distribution of chloroplast haplotypes. — In sexual species of angiosperms, there is a general tendency for cpDNA to exhibit considerable spatial structure such that the majority of genetic variation is distributed among, rather than within, geographic populations (McCauley, 1995). In some cases, cpDNA haplotypes even transgress species boundaries, reflecting the geographic distribution of the sampled taxa rather than being indicative of individual species (e.g., Whittemore & Schaal, 1991; Wolf & al., 1997). However, in *Pilosella*, with the exception of the '*H. cymosum* group', no geographic structure of the haplotypes was detected. Haplotype groups were remarkably conservative for any given taxon with the exception of *H. piloselliflorum*. In a group representing a hybridogenous complex, a much more confusing, scattered distribution of cpDNA might be expected. Especially in three cases of apomictic intermediate taxa which were all of multiple origin (*H. glomeratum*, *H. floribundum*, *H. iseranum*), it was striking that they seemed to have been produced in a rather similar way with respect to the seed parent. The possibility of an incompatibility between chloroplast and nuclear genome, which has been demonstrated in hybrids of Lousiana irises (e.g., Burke & al., 1998), could apply in our case insofar as attempts to produce an experimental hybrid between *H. pilosella* and *H. floribundum* using the sexual as seed parent regularily failed, a surprising finding in a group whose ability for hybrid formation seems to be virtually unlimited. As the separation of the two major haplotype groups is probably rather old (see above), incompatibilities might indeed arise and account for the observed one-way road, but can presently not be fully understood. In any case, it cannot be a general feature, as in most crosses, the problem is not apparent.

In many cases of introgression and chloroplast capture in plants (summarised e.g., in Rieseberg & Wendel, 1993), there is also evidence for unidirectional gene flow. It should be kept in mind, however, that most examples described so far refer to sexual taxa. It is therefore questionable whether the underlying mechanisms and driving forces follow a general pattern or are rather case-specific. A convincing explanation of the haplotype uniformity that we found in the older (apomictic) intermediate species is therefore still missing. Whether our examples will turn out to be typical for *Pilosella* or other groups with similar modes of reproduction remains to be demonstrated by additional studies.

Most striking was the discovery that in the majority of cases investigated in this study, the apomict served as seed parent rather than the sexual taxon. It shows that the role of residual sexuality is evolutionarily important and might be greater than expected, the apomicts not only serving as pollen donors, but even as maternal plants (see also Van Dijk & Vijverberg, this volume, chapter 5). This aspect is currently being explored by us in an ongoing project focusing on residual sexuality of facultative apomicts in *Pilosella* in which the frequency of residual sexuality will be compared with the frequency of hybridogenous types in the field and be correlated to ploidy level.

Future prospects. — While many new results presented here contribute to a better understanding of species relationships and ongoing processes in this complicated apomictic group, we are still only beginning to understand what is happening, and much further work has to be done. For instance, Fig. 6 is still oversimplified by subsuming many cytotypes and the ways in which they were established under the label of a single species name. Also, the concrete formation of the cytotypes still remains unclear in many cases, because there is no way to access the actual plants producing them in natural populations, especially in the case of older and widespread clones. As cytotypes often differ markedly between natural populations and experimentally produced hybrids, especially concerning the frequency of triploids and aneuploids (Krahulcová & al., 2000; Krahulcová & Krahulec, 2000), only a combined analysis following both approaches might help to gain a better understanding of the underlying processes. Experimentally produced cytotypes might give an idea of the initial stages in the formation of new taxa that may be produced by similar processes in nature, but then, only a selection of them will be successful in the establishment of stable populations and will spread subsequently. A somewhat intermediate situation is currently observed in New Zealand (Chapman & al., 2000) where *Pilosella* is represented by a number of invasive taxa causing severe problems presumably due to the lack of their natural competitors. *Pilosella* species were introduced to New Zealand from Europe in the late 19th century. Already by the 1940's, the *Pilosella*s were considered common weeds there. This timescale of little more than 100 years since their introduction also mediates between experimental conditions (present situation) and the presumed age of the intermediate taxa of our investigation area.

The combination of approaches applied in this study certainly helped to avoid many misleading conclusions and resulted in a much more detailed picture of spe-

ciation in *Pilosella*, but our knowledge of the group is still very incomplete. For instance, a (backbone) molecular phylogeny for the entire subgenus is not far off (Fehrer, in prep.) which confirms haplotype grouping within a wider phylogenetic framework. Additional techniques such as chromosome painting or the use of single-copy nuclear markers could be used to identify the different genomes which have been combined by allopolyploidy in the past.

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