

## Genome size in *Hieracium* subgenus *Hieracium* (Asteraceae) is strongly correlated with major phylogenetic groups

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• **Background and Aims** *Hieracium* subgenus *Hieracium* is one of the taxonomically most intricate groups of vascular plants, due to polyploidy and a diversity of breeding systems (sexuality vs. apomixis). The aim of the present study was to analyse nuclear genome size in a phylogenetic framework and to assess relationships between genome size and ploidy, breeding system and selected ecogeographic features.

• **Methods** Holoploid and monoploid genome sizes (C- and Cx-values) of 215 cultivated plants from 89 field populations of 42 so-called ‘basic’ *Hieracium* species were determined using propidium iodide flow cytometry. Chromosome counts were available for all analysed plants, and all plants were tested experimentally for their mode of reproduction (sexuality vs. apomixis). For constructing molecular phylogenetic trees, the external transcribed spacer region of nuclear ribosomal DNA was used.

• **Key Results** The mean 2C values differed up to 2.37-fold among different species (from 7.03 pg in diploid to 16.67 in tetraploid accessions). The 1Cx values varied 1.22-fold (between 3.51 and 4.34 pg). Variation in 1Cx values between conspecific (species in a broad sense) accessions ranged from 0.24% to 7.2%. Little variation (not exceeding the approximate measurement inaccuracy threshold of 3.5%) was found in 33 species, whereas variation higher than 3.5% was detected in seven species. Most of the latter may have a polytopic origin. Mean 1Cx values of the three cytotypes (2n, 3n and 4n) differed significantly (average of 3.93 pg in diploids, 3.82 pg in triploids and 3.78 pg in tetraploids) indicating downsizing of genomes in polyploids. The pattern of genome size variation correlated well with two major phylogenetic clades which were composed of species with western or eastern European origin. The monoploid genome size in the ‘western’ species was significantly lower than in the ‘eastern’ ones. Correlation of genome size with latitude, altitude and selected ecological characters (light and temperature) was not significant. A longitudinal component was only apparent for the whole data set, but absent within the major lineages.

• **Conclusions** Phylogeny was the most important factor explaining the pattern of genome size variation in *Hieracium sensu stricto*, species of western European origin having significantly lower genome size in comparison with those of eastern European origin. Any correlation with ecogeographic variables, including longitude, was outweighed by the divergence of the genus into two major phylogenetic lineages.

**Key words:** Apomixis, chromosome numbers, Compositae, genome size, hawkweeds, *Hieracium* subgenus *Hieracium*, mode of reproduction, nuclear DNA content, phylogeny, polyploidy.

### INTRODUCTION

Genome size has become a widely studied phenomenon, beginning in the 1950s when large differences in the nuclear content of different organisms were detected (e.g. Swift, 1950; Laurie and Bennett, 1985; Bennett and Leitch, 1995). Large differences in DNA content can be caused by several mechanisms. It has been found that nuclear DNA content is primarily influenced by the proportion of non-genic repetitive DNA, much of which is generated by transposable elements (Flavell *et al.*, 1977; Barakat *et al.*, 1997). In particular, it has been found that retrotransposon copy number can vary among genomes (Arumuganathan and Earle, 1991; Vicient *et al.*, 1999; Kalendar *et al.*, 2000; Piegu *et al.*, 2006; Wicker and Keller, 2007; Hawkins *et al.*, 2008). Decrease in genome size can result from a higher overall rate of deletions than insertions, selection against transposable elements, unequal crossing over and illegitimate recombination

(Morgan, 2001; Petrov, 2002; Wendel *et al.*, 2002; Ma *et al.*, 2004; Bennetzen *et al.*, 2005).

Correlations between genome size and specific life traits, most importantly life history and breeding systems, have been documented. Selfers were found to have smaller Cx-values than related outcrossers (Labani and Elkington, 1987; Govindaraju and Cullis, 1991; Albach and Greilhuber, 2004). Annuals, especially weedy species, tend to have lower genome size in comparison with related perennials (Bennett, 1972; Rejmanek and Richardson, 1996; Bennett *et al.*, 1998; Garnatje *et al.*, 2004; Grotkopp *et al.*, 2004), probably due to an association of annual life history with selfing (e.g. Albach and Greilhuber, 2004). Relationships between genome size and ecological factors are less clear (see, for example, Knight *et al.*, 2005). Correlations between genome size and frost resistance in the British flora (MacGillivray and Grime, 1995), elevation in some groups of *Centaurea* (Bancheva and Greilhuber, 2006), *Veronica* (Albach and Greilhuber, 2004), *Dactylis* (Reeves *et al.*, 1998) and

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*Berberis* (Bottini *et al.*, 2000) and continentality and habitat conditions (moisture) in *Cirsium* (Bureš *et al.*, 2004) have already been documented. There are also more or less close associations between genome size and cell size and leaf anatomical traits (Bennett, 1972; Edwards and Endrizzi, 1975; Knight *et al.*, 2005; Sugiyama, 2005; Beaulieu *et al.*, 2008), cell cycle duration (e.g. Rees *et al.*, 1966; Bennett *et al.*, 1983; Lawrence, 1985), seed mass (e.g. Knight *et al.*, 2005; Beaulieu *et al.*, 2007) and photosynthetic rate (e.g. Knight *et al.*, 2005).

Polyploids often have smaller Cx-values than their diploid relatives (e.g. Leitch and Bennett, 2004; Weiss-Schneeweiss *et al.*, 2006). These decreases correlate with a mutational bias towards deletion over insertions (Petrov, 2002), and illegitimate recombination has been shown to eliminate retrotransposon sequences (Bennetzen, 2002; Devos *et al.*, 2002; Ma *et al.*, 2004). However, exceptions of this downsizing pattern have been found, e.g. in the genus *Orobancha* (tetraploid *O. transcaucasica*). It was hypothesized that such polyploids are relatively young and that there was not enough time for a substantial reduction in nuclear DNA content (Weiss-Schneeweiss *et al.*, 2006).

Genome size alone is of little value as a phylogenetic indicator at higher taxonomic levels, but can be helpful in infragenetic classification assessments, species delimitation or hybrid identification (Keller *et al.*, 1996; Buitendijk *et al.*, 1997; Morgan *et al.*, 1998; Thalmann *et al.*, 2000; Zonneveld, 2001; Šiško *et al.*, 2003; Bureš *et al.*, 2004; Baack *et al.*, 2005; Závěský *et al.*, 2005; Suda *et al.*, 2007). An important issue that is still largely neglected in the literature, mostly due to a lack of comparative analyses between DNA sequence and genome size data sets, is the understanding of how genome size variation is linked with species evolution (but see Wendel *et al.*, 2002; Albach and Greilhuber, 2004; Grotkopp *et al.*, 2004; Jakob *et al.*, 2004; Weiss-Schneeweiss *et al.*, 2006). Species relationships were therefore assessed based on the external transcribed spacer (ETS) of nuclear ribosomal DNA in order to relate genome size variation to their evolutionary history.

*Hieracium* subgenus *Hieracium* is distributed in temperate regions of Europe, Asia, Mediterranean Africa and North America and has been introduced to several other regions, e.g. New Zealand. The genus is suitable for the study of genome size variation due its remarkable diversity in ploidy (coupled with breeding systems), habitat preferences and distribution of particular species. Polyploid (triploid, tetraploid and rarely pentaploid,  $x=9$ ) taxa with asexual reproduction through parthenogenetic development of the unreduced egg cell (*Antennaria*-type diplospory) prevail in this group, i.e. they are (near-)obligate apomicts (e.g. Nogler, 1984). Sexual reproduction is rather rare and restricted to diploid species (Schuhwerk, 1996; Chrtek *et al.*, 2004). The species occupy forests, forest margins, various grasslands and rocks from the lowlands to the alpine belt.

Species concepts in *Hieracium* have long been a matter of discussion (e.g. Schuhwerk, 2003). The Central European school of 'hieraciology' (founded by Nägeli and Peter in the 19th century) accepts a broad species definition (species are then divided into subspecies, varieties, etc.), whereas Scandinavian, British and Russian botanists follow a narrow

species concept, i.e. nearly all morphologically recognizable forms are treated at species rank ('microspecies'). We follow the Central European concept because, in our opinion, it better reflects the situation across the whole distribution area, especially in central and southern Europe where most diploids occur, from which the apomictic polyploids are thought to be derived. According to this concept, approx. 500 species (in the broad sense) are accepted (Zahn, 1921–1923; and species described since that time), being either so-called 'basic' or 'intermediate' taxa. The latter share morphological characters of two or more basic species and are supposed to be of hybridogenous origin (hybrids stabilized by agamospermy). Basic species (about 45, including diploids and polyploids) are tentatively considered as main units of species evolution in *Hieracium*.

Here, a nuclear DNA content analysis of 42 basic species of *Hieracium* subgenus *Hieracium* (*sensu* Zahn, 1921–1923, with a few exceptions, see Materials and methods) is reported. The following questions were addressed: (a) how does the level of intraspecific variation in holoploid and monoploid genome sizes relate to the circumscription of species *sensu* Zahn? (b) how does monoploid genome size (Cx) relate to ploidy (diploids, triploids and tetraploids), i.e. is there evidence for downsizing of genomes in polyploids? (c) is there any congruence between the phylogenetic structure and the pattern of genome size variation? and (d) how does nuclear genome size relate to selected ecogeographic features (latitude, longitude, altitude, temperature and light)?

## MATERIALS AND METHODS

### *Plant material*

Two hundred and fifteen samples from 89 populations of 42 *Hieracium* species were collected in the field (or grown from seeds in a few cases) throughout Europe and transferred to the experimental garden of the Institute of Botany in Průhonice (Table 1; for details of sample localities see Supplementary Data, available online). Taxon sampling was restricted to so-called 'basic', supposedly non-hybridogenous species, generally following Zahn (1921–1923) with a few exceptions. The species concept of section *Cerinthoidea* followed Mateo (2005), *H. plumulosum* (*H. waldsteinii sensu lato*) was treated as a separate species, and two newly described diploid Balkan species (*H. kittanae* and *H. petrovae*; Vladimirov, 2003; Vladimirov and Szélag, 2006) were included. Complete analysis covering all recognized and mostly (allo)polyploid hybridogenous species (approx. 500 'broad' species) was not feasible, and interpretation of estimated genome sizes would be extremely complicated due to the often unknown origin of polyploids and the reticulate patterns of variation.

For diploid, sexually reproducing species and for agamospermous polyploids with a rather small distribution area, one or two populations were chosen. For sexual diploids with large geographic areas and for more widely distributed agamospermous polyploids, two to six populations were selected. The number of plants analysed per population varied from two in agamospermous species with likely clonal population structure (e.g. Shi *et al.*, 1996; Mráz *et al.*, 2001; Storchová *et al.*,

TABLE 1. Accession origin and genome size

Species	Locality (no. of plants) (S): plant cultivated from seed	2n	2C (pg) mean $\pm$ s.e.	2C (pg) range	1Cx (pg)	1Cx (pg) species mean $\pm$ s.e.	1Cx (pg) species range	1Cx species variation (%)	ETS clade <sup>†</sup>
<i>H. alpinum</i> L.	Ukraine: Chornohora (4)	18	7.90 $\pm$ 0.01	7.88–7.92	3.95	3.95 $\pm$ 0.01	3.94–3.97	0.76	E
	France: Hautes Alpes (1)	27	11.87	–	3.96				n.d.
<i>H. amplexicaule</i> L.	Austria: Hohe Tauern, Fragant (2)	27*	10.70 $\pm$ 0.01	10.7–10.71	3.57	3.60 $\pm$ 0.02	3.54–3.68	3.95	X(W)
	Austria: Hohe Tauern, Mallnitz (1)	27*	10.74	–	3.58				n.d.
	Spain: prov. Gerona (1)	27*	10.8	–	3.6				n.d.
	France: Hautes Alpes (1)	27	10.62	–	3.54				n.d.
	Italy: Rhaetian Alps (2)	36*	14.66 $\pm$ 0.07	14.59–14.72	3.67				n.d.
<i>H. bifidum</i> Kit.	Slovakia: Roháče (1)	27	10.67	–	3.56	3.53 $\pm$ 0.01	3.51–3.56	1.42	W
	Czech Rep.: distr. Beroun	27	10.52 $\pm$ 0.01	10.52–10.53	3.51				n.d.
	Czech Rep.: Krkonoše Mts.	27	10.62 $\pm$ 0.01	10.61–10.63	3.54				n.d.
<i>H. bracteolatum</i> Sibth. & Sm.	Greece: Pilion (2) (S)	27	12.39 $\pm$ 0.08	12.31–12.47	4.13	4.13 $\pm$ 0.03	4.10–4.16	1.46	X
<i>H. bupleuroides</i> C.C.Gmel. I.	Slovakia: Biele Karpaty (2)	27*	11.95 $\pm$ 0.05	11.91–12.01	3.99				E(H)
<i>H. bupleuroides</i> C.C.Gmel. II.	Slovakia: Chočské vrchy (2)	27*	12.00 $\pm$ 0.02	11.96–12.00	3.99				n.d.
	Slovakia: Roháče (2)	27	11.73	–	3.91				E
	Austria: Dachstein massif (1)	27*	11.61 $\pm$ 0.05	11.56–11.66	3.87				n.d.
	Slovakia: Slovenský raj (1)	27*	12.03	–	4.01				n.d.
	Austria: Allgäuer Alpen (1)	27	11.63	–	3.88				n.d.
	<i>H. bupleuroides</i> s.l. mean					3.95 $\pm$ 0.06	3.85–4.01	4.20	
<i>H. caesium</i> (Fr.) Fr.	Sweden: prov. Gotland (3)	36*	14.66 $\pm$ 0.12	14.52–14.89	3.67	3.68 $\pm$ 0.01	3.64–3.72	2.20	X(W)
	Sweden: prov. Gästrikland (3)	36*	14.75 $\pm$ 0.05	14.66–14.83	3.69				n.d.
<i>H. cerinthoides</i> L.	Spain: Pyrenees (2)	27*	10.68 $\pm$ 0.03	10.66–10.71	3.56	3.56 $\pm$ 0.01	3.55–3.57	0.56	W(H)
<i>H. cordifolium</i> Lapeyr.	Andorra: Pyrenees (6)	18*	7.18 $\pm$ 0.02	7.11–7.22	3.59	3.59 $\pm$ 0.01	3.56–3.61	1.40	W(H)
<i>H. eriophorum</i> St.-Amans	France: Landes, Labenne (6) (S)	18*	8.51 $\pm$ 0.03	8.45–8.61	4.25	4.27 $\pm$ 0.01	4.22–4.31	2.13	E
	France: Landes, Vieux-Boucau-les-Bains (6) (S)	18*	8.55 $\pm$ 0.03	8.44–8.61	4.27				n.d.
<i>H. glaucum</i> All.	Slovenia: Julijske Alpe, Podklanec (2)	27*	11.31 $\pm$ 0.03	11.29–11.34	3.77	3.79 $\pm$ 0.01	3.76–3.81	1.33	n.d.
	Slovenia: Julijske Alpe, Zadnjica (2)	27*	11.42 $\pm$ 0.00	11.42–11.42	3.81				n.d.
	Slovenia: Julijske Alpe, izvir Soče (2)	27	11.39 $\pm$ 0.05	11.34–11.44	3.8				X
<i>H. gouani</i> Arv.-Touv.	Spain: prov. Gerona (8)	18*	7.10 $\pm$ 0.01	7.07–7.12	3.55	3.55 $\pm$ 0.00	3.54–3.56	0.56	X(W)
<i>H. gymnocephalum</i> Griseb. ex Pant.	Albania: Jezerce (2)	18	8.44 $\pm$ 0.01	8.43–8.45	4.22	4.23 $\pm$ 0.01	4.22–4.23	0.24	X
<i>H. gymnocerinthae</i> Arv.-Touv. & G.Gaut.	Spain: Serra del Cadí (2)	27*	10.6 $\pm$ 0.02	10.58–10.61	3.53	3.54 $\pm$ 0.01	3.53–3.54	0.28	W(H)
<i>H. heterogynum</i> (Froel.) Guterm.	Montenegro: Lovčen (2) (S)	27	12.52 $\pm$ 0.02	12.52–12.55	4.17	4.18 $\pm$ 0.01	4.17–4.18	0.24	X
<i>H. humile</i> Jacq.	Austria: Dachstein massif (2)	36*	14.25 $\pm$ 0.01	14.24–14.26	3.56	3.55 $\pm$ 0.01	3.54–3.57	0.85	W
	France: Corbières (2)	27	10.64 $\pm$ 0.01	10.61–10.66	3.55				W
<i>H. kittanae</i> Vladimirov	Bulgaria: Rodopi (3)	18*	8.41 $\pm$ 0.04	8.38–8.46	4.21	4.21 $\pm$ 0.01	4.19–4.23	0.95	E
<i>H. lachenalii</i> Suter	Czech Rep.: Křivoklátsko (2)	27*	11.25 $\pm$ 0.01	11.27–11.29	3.76	3.75 $\pm$ 0.01	3.73–3.76	0.80	n.d.
	Czech Rep.: distr. Znojmo (2)	27*	11.22 $\pm$ 0.03	11.19–11.24	3.74				X(W)
	Czech Rep.: distr. Praha-east (2)	27	11.24 $\pm$ 0.06	11.20–11.32	3.75				n.d.
<i>H. laevigatum</i> Willd.	Czech Rep.: Brdý (2)	27*	12.05 $\pm$ 0.02	12.01–12.08	4.02	4.05 $\pm$ 0.02	3.97–4.14	4.28	X
	Czech Rep.: Hradec Králové (2)	27*	12.00 $\pm$ 0.08	11.94–12.10	4				n.d.
	Germany: Kamenz (2) (S)	27	12.41 $\pm$ 0.01	12.40–12.42	4.14				n.d.
<i>H. lawsonii</i> Vill.	France: Corbières (2)	27	10.76 $\pm$ 0.02	10.74–10.78	3.59	3.62 $\pm$ 0.03	3.58–3.68	2.79	W
	France: Briançon (1)	36	14.71	–	3.68				n.d.
<i>H. murorum</i> L.	Czech Rep.: Plzeň (2)	27*	10.62 $\pm$ 0.06	10.55–10.68	3.54	3.56 $\pm$ 0.01	3.52–3.59	1.99	W
	Czech Rep.: Doupovské hory (2)	27*	10.67 $\pm$ 0.05	10.63–10.72	3.56				n.d.
	Czech Rep.: Český kras (2)	27*	10.74 $\pm$ 0.02	10.72–10.76	3.58				n.d.
<i>H. naegelianum</i> Pančić	Montenegro: Durmitor Mts (2)	27*	10.89 $\pm$ 0.08	10.81–10.97	3.63	3.63 $\pm$ 0.03	3.60–3.66	1.67	E
<i>H. olympicum</i> Boiss.	Bulgaria: Kaloferska Planina (2)	27*	12.27 $\pm$ 0.05	12.22–12.33	4.09	4.05 $\pm$ 0.01	4.04–4.06	0.50	X

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Continued

TABLE 1. *Continued*

Species	Locality (no. of plants) (S): plant cultivated from seed	2n	2C (pg) mean ± s.e.	2C (pg) range	1Cx (pg)	1Cx (pg) species mean ± s.e.	1Cx (pg) species range	1Cx species variation (%)	ETS clade <sup>†</sup>
<i>H. pannosum</i> Boiss. I	Bulgaria: Trojanska Planina (2)	27*	11.71 ± 0.05	11.66–11.77	3.9				E
<i>H. pannosum</i> Boiss. II	Greece: Peloponnesos (2) (S)	36	16.67 ± 0.01	16.66–16.67	4.17				n.d.
	<i>H. pannosum</i> s.l. mean					4.04 ± 0.08	3.89–4.17	7.20	
<i>H. petrovae</i> Vladimirov & Szelağ	Bulgaria: Rodopi (1)	18*	7.9	–	3.95				E
<i>H. pictum</i> Pers.	France: Montegenèvre (2)	27	10.78 ± 0.27	10.5–11.05	3.59	3.59 ± 0.04	3.50–3.68	5.14	n.d.
	France: Briançon (2)	27	10.75 ± 0.13	10.62–10.88	3.58				W
<i>H. piliferum</i> Hoppe	Austria: Gurktaler Alpen (2)	36	15.57 ± 0.04	15.54–15.60	3.89	3.91 ± 0.02	3.86–4.01	3.89	E-int.
	Austria: Reisseck-Gruppe (1)	27	11.58	–	3.86				
	Italy: Alps, Spluga (1)	27	11.58	–	3.86				
	France: Hautes Alpes (2)	27	11.95 ± 0.07	11.89–12.02	3.99				
<i>H. pilosum</i> Schleich. ex Froel. I.	Slovenia: Julijske Alpe (1)	27	11.57		3.86				E
<i>H. pilosum</i> Schleich. ex Froel. II.	Slovenia: Julijske Alpe (1)	27	11.8		3.93				X
	<i>H. pilosum</i> s.l. mean					3.90 ± 0.04	3.86–3.93	1.81	
<i>H. plumulosum</i> A.Kern.	Montenegro: Mrtvica canyon (1)	18*	8.59	–	4.29				X(E)
<i>H. porrifolium</i> L.	Austria: Karawanken (6)	18*	7.76 ± 0.01	7.7–7.79	3.88	3.89 ± 0.01	3.85–3.93	2.08	E
	Austria: Karawanken (1)	18	7.7	–	3.85				n.d.
	Slovenia: Julijske Alpe (6)	18*	7.82 ± 0.02	7.74–7.85	3.91				n.d.
<i>H. prenanthoides</i> Vill. I.	Poland: Karkonosze (2)	27	10.82 ± 0.03	10.78–10.85	3.61				X(W)
	France: Hautes Alpes, La Grave (1)	18	7.11	–	3.55				X(W)
	France: Hautes Alpes, Briançon (2)	18	7.29 ± 0.01	7.24–7.24	3.64				n.d.
<i>H. prenanthoides</i> Vill. II.	Andorra: Canillo (2)	27*	11.41 ± 0.02	11.40–11.43	3.8				X(W)
	<i>H. prenanthoides</i> s.l. mean					3.67 ± 0.04	3.56–3.81	7.02	
<i>H. racemosum</i> Waldst. & Kit. ex Willd.	Czech Rep.: distr. Znojmo (2)	27	12.41 ± 0.02	12.39–12.44	4.14	4.11 ± 0.06	4.08–4.15	1.72	X
	Czech Rep.: Ústí nad Orlicí (2)	27	12.26 ± 0.01	12.26–12.26	4.09				n.d.
	Slovakia: Gemer (1) (S)	27	12.24	–	4.08				n.d.
<i>H. ramondii</i> Griseb.	Andorra: Encamp (2)	27*	10.63 ± 0.07	10.56–10.7	3.54	3.54 ± 0.03	3.51–3.57	1.71	W
<i>H. recoderi</i> De Retz	Spain: prov. Barcelona (8)	18*	7.09 ± 0.01	7.00–7.09	3.53	3.53 ± 0.01	3.50–3.68	1.43	W
<i>H. sabaudum</i> L.	Czech Rep.: Praha (2)	27	12.51 ± 0.05	12.47–12.56	4.17	4.17 ± 0.02	4.12–4.23	2.67	n.d.
	Germany: Oberlausitz (2)	27	12.65 ± 0.03	12.62–12.68	4.22				X
	Czech Rep.: České středohoří (2)	27	12.36 ± 0.01	12.35–12.37	4.12				n.d.
<i>H. schmidtii</i> Tausch	Czech Rep.: České středohoří (2)	27*	10.64 ± 0.05	10.59–10.69	3.55	3.54 ± 0.01	3.52–3.56	1.14	n.d.
	Czech Rep.: České středohoří (2)	27*	10.58 ± 0.00	10.61–10.61	3.54				W
	Czech Rep.: Křivoklátsko (2)	27	10.60 ± 0.01	10.60–10.61	3.53				n.d.
<i>H. sparsum</i> Friv.	Bulgaria: Vitoša (1)	18	8.15	–	4.08	4.03 ± 0.03	3.99–4.08	2.56	E(H)
	Bulgaria: Pirin (2)	18	8.01 ± 0.03	7.98–8.04	4.01				
<i>H. stelligerum</i> Froel.	France: Ardèche (3)	18*	7.03 ± 0.06	6.91–7.14	3.51	3.51 ± 0.03	3.47–3.57	2.89	W
<i>H. tomentosum</i> L.	France: Alpes Maritimes (8)	18*	7.48 ± 0.02	7.41–7.58	3.74	3.75 ± 0.01	3.71–3.79	2.16	W
	France: Briançon (1)	27	11.27	–	3.76				n.d.
	France: Hautes Alpes (2)	27	11.25 ± 0.08	11.17–11.33	3.78				n.d.
<i>H. transylvanicum</i> Heuff.	Ukraine: Marmaros'ki Al'py (8)	18*	8.56 ± 0.01	8.52–8.59	4.28	4.28 ± 0.00	4.26–4.30	0.94	W
<i>H. umbellatum</i> L.	Poland: Baltic coast, Jantar (1)	18	8.34	–	4.17	4.26 ± 0.01	4.17–4.30	3.12	E
	Czech Rep.: Praha (8)	18*	8.54 ± 0.01	8.48–8.59	4.27				n.d.
	Germany: Nordfriesland (2) (S)	18	8.48 ± 0.03	8.45–8.52	4.24				n.d.
<i>H. villosum</i> Jacq. I.	Slovakia: Strážovské vrchy (2)	36	15.71 ± 0.04	15.66–15.75	3.93				E
<i>H. villosum</i> Jacq. II.	France: Hautes Alpes (1)	27	11.6	–	3.87				X(E)
	<i>H. villosum</i> s.l. mean					3.91 ± 0.02	3.87–3.94	1.81	
<i>H. viosum</i> Pall.	Russia: Rostov-na-Donu (2) (S)	27	13.06 ± 0.03	13.02–13.09	4.35	4.34 ± 0.00	4.33–4.36	0.69	E
	Russia: Altajskij kraj (3) (S)	27	13.00 ± 0.01	12.98–13.03	4.34				E
' <i>Hieracium</i> ' <i>intybaceum</i> All.	Italy: Rhaetian Alps (1)	18	7.5	–	3.75	3.76 ± 0.01	3.74–3.79	1.34	



<i>Pilosella lactucella</i> (Wallr.) P.D.Sell & C.West	Italy: Alpi Orobie (2)	18	7.53 ± 0.07	7.48–7.58	3.77
<i>Andryala integrifolia</i> L.	Austria: Ötztaler Alpen (2)	18	7.53 ± 0.07	7.52–7.54	3.77
<i>Andryala levitomentosa</i> (Nýár.) P.D.Sell	Germany: Erzgebirge (1)	18	4.07 <sup>‡</sup>		2.04
<i>Hispidella hispanica</i> Barnades ex Lam.	Spain: Andalusia (1)	n.d.	n.d.		
	Romania: Pietrosul Bogolini (1)	18	5.31 <sup>§</sup>		2.66
	Spain: Sierra de Guadarrama (1)	18 <sup>#</sup>	~4.00		~2.00

\* From Chrtek et al. (2007).

<sup>†</sup> E, 'eastern' clade; W: 'western' clade; X: interclade hybrid; X(E) and X(W), interclade hybrids with predominant 'eastern' or 'western' sequence variant; E(H) and W(H), hybrids within 'eastern' or 'western' clade; hybrid origin of *H. sparsum* and *H. lachenalii* inferred from plastid DNA (J. Fehrer et al., unpubl. res.). E-int. indicates ETS character additivity between the 'eastern' clade and '*Hieracium*' *intybaceum* indicative of hybrid origin.

<sup>‡</sup> See also Suda et al. (2007).

<sup>§</sup> With *Pilosella lactucella* standard.

<sup>#</sup> Luque (1981); Elena-Rosselló et al. (1985).

2002) to eight in supposedly genetically variable populations of sexual diploids. Two species, *H. petrovae* and *H. plumulosum*, were represented by a single plant due to their rarity in the field or because of cultivation problems. When two or more ploidies have been reported for a species, this diversity was covered as far as possible. Voucher specimens of all samples are deposited in the herbarium PRA.

#### Chromosome numbers and breeding system

At least two plants per population were checked for their chromosome number using the method described in Chrtek et al. (2007); counts for selected accessions have been published (Chrtek et al., 2007). The mode of reproduction was also tested, generally following Gadella (1987) and Krahulcová and Krahulec (1999). In diploids (in which sexual reproduction was expected), randomly selected capitula were bagged at the bud stage and tested for late-acting autogamy (in the absence of active pollination); results were compared with control capitula from the same plant in open pollination treatments. In polyploids (in which agamospermy was expected), the upper part of the capitulum was cut off at the bud stage (emasculatation) and the number of 'full' achenes was counted as a measure of seed set and compared with the number of achenes from untreated capitula of the same plant. Percentages of 'full' achenes after emasculatation in particular plants/species are available upon request.

#### Genome size estimation

Genome size was determined by flow cytometry using a Partec CyFlow cytometer equipped with a green (532 nm) solid-state laser. *Zea mays* 'CE-777' (2C = 5.48 pg; Lysák and Doležel, 1998) and *Pisum sativum* 'Ctirad' (2C = 8.85 pg; Doležel et al., 1994; Suda et al., 2007) were used as internal standards for diploid and polyploid species. The modified two step-procedure described by Otto (1990) was employed for sample preparation. Intact leaf tissue (approx. 1 cm<sup>2</sup>) of the analysed species and an appropriate quantity of the internal standard were co-chopped with a sharp razor blade in a plastic Petri dish with 1 mL of ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween 20) as the nuclear isolating solution. The suspension was filtered through a 42-µm nylon filter and centrifuged at 15 g for 5 min. The supernatant was discarded, and the pellet was resuspended in 100 µL fresh Otto I buffer. Samples were incubated for at least 10 min at room temperature and mixed with 1 mL Otto II buffer (0.4 M Na<sub>2</sub>HPO<sub>4</sub>) supplemented with propidium iodide as the fluorochrome, RNase IIA (both at a concentration of 50 µg mL<sup>-1</sup>) and β-mercaptoethanol (2 µL mL<sup>-1</sup>). Samples were stained for 5 min at room temperature before measurement. Usually, 5000 nuclei were analysed for each sample. Nuclear genome size was calculated as a linear relationship between the ratio of 2C peaks of sample and standard. Each plant was measured at least three times on different days by the same operator to eliminate potential artefacts. If the difference between the three measurements exceeded 2%, the value was discarded, and the sample was re-analysed. The coefficients of variation (CVs) of G<sub>0</sub>/G<sub>1</sub> peaks did not exceed 5% (with two exceptions in *Hieracium* samples).

### Molecular methods

A representative subset of 49 *Hieracium* accessions was selected for phylogenetic analysis. As the outgroup, species of the most closely related genera *Andryala*, *Hispidella* and *Pilosella* (sometimes treated as a subgenus of *Hieracium*) and '*Hieracium*' *intybaceum* were chosen according to previous results (Fehrer *et al.*, 2007). Although the latter species was traditionally placed in *Hieracium* subgenus *Hieracium*, molecular data (ITS sequences) suggested it belongs to an older isolated lineage clearly separated from a cluster formed by *Hieracium s.l.* and its closely related genera *Andryala* and *Hispidella* (Fehrer *et al.*, 2007), which is also supported by the present data based on the ETS region.

Total genomic DNA was extracted from fresh or CTAB-preserved material using a sorbitol extraction method (Štorchová *et al.*, 2000). The ETS region of the nuclear ribosomal DNA was amplified using the primers Ast-8 and 18 S (Noyes, 2006). PCR amplifications were done in 25- $\mu$ L reactions containing 2.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 0.5  $\mu$ M of each primer, 0.5 unit of *Taq* DNA polymerase (Fermentas, Ontario, Canada), 1  $\times$  *Taq* buffer with KCl (Fermentas) and a few nanograms of genomic DNA. An initial denaturation step at 94°C for 3 min was followed by 35 cycles of denaturation (94°C for 30 s), annealing (55°C for 30 s) and extension (72°C for 40 s) steps, and a final extension at 72°C for 10 min. The PCR products were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced (GATC Biotech, Konstanz, Germany). Both strands were sequenced using the PCR primers. For one accession, *Pilosella lactucella*, direct sequencing was not successful and, therefore, the amplified fragment was subcloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions, but downscaled to half reactions. Three clones of this sample were sequenced (GATC Biotech), using the primer Ast-8.

### Molecular data analyses

Sequence chromatograms were inspected by eye. In many accessions intra-individual polymorphism, i.e. more than one allele of the ETS region, was detected. Polymorphic sites were represented by the NC-IUB ambiguity symbols (e.g. R for A or G).

Initial sequence alignment was done with Clustal X (Thompson *et al.*, 1997) and further edited manually in Bioedit (Hall, 1999). It was unambiguous due to low overall variation. Visual inspection of the alignment revealed the existence of two major groups within *Hieracium s.s.*, and a proportion of accessions were identified as hybrids among these groups according to the additive pattern of polymorphic sites (these are referred to as 'interclade hybrids'). Furthermore, the *Hieracium piliferum* accession analysed was identified as a hybrid between one of the major groups and '*Hieracium*' *intybaceum*. Sequences were submitted to GenBank (EU821362–EU821419).

Bayesian (MrBayes V3.1.2; Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) and maximum likelihood (RAxML V7.0.3; Stamatakis, 2006) analyses were performed (a) on the complete data set and (b) on a modified

data set excluding interclade hybrids and *H. piliferum*. Bayesian analyses were run with two nucleotide substitution rates and gamma distribution. This corresponds to a HKY + G model found in hierarchical Likelihood Ratio Tests as the model of molecular evolution best fitting to the data as implemented in Modeltest V3.5 (Posada and Crandall, 1998). Two parallel runs with four chains each were used for both analyses, sampling every 1000th tree. The analysis of the complete data set was computed for 2 million generations until convergence. The first 500 trees per run were discarded as burn-in and the remaining 1501 trees per run were summarized. For the modified data set, 1 million generations were sufficient for reaching convergence, the first 250 trees per run were discarded as burn-in and the remaining 751 trees per run were summarized. Maximum likelihood analyses were done using the rapid BS algorithm in combination with a maximum likelihood search, using the GTR model of nucleotide substitutions with a gamma model of rate heterogeneity and 1000 bootstrap replicates.

### Statistical analyses of genome size

Intraspecific variation in morphologically defined species *sensu* Zahn was assessed and species with variation exceeding the approximate inaccuracy threshold of 3.5% [following Suda *et al.* (2007) in *Hieracium* subgenus *Pilosella*] were marked (*H. amplexicaule*, *H. bupleuroides*, *H. laevigatum*, *H. pannosum*, *H. pictum*, *H. piliferum* and *H. prenanthoides*). In *H. bupleuroides* and *H. prenanthoides*, differences in ETS sequences among accessions corresponding with the differences in genome size were found (J. Fehrer *et al.*, unpubl. res.) which were caused by hybridization with other species. These heterogeneous species were therefore split into more natural units (accessions or groups of accessions) according to genome size and treated separately in the following analyses. The same was done for *H. pannosum*, in which the accessions differed distinctly in genome size (and in ploidy), and for *H. pilosum* and *H. villosum*, in which differences in ETS sequences due to introgression were found between accessions (although genome size variation was rather low in these cases). On the other hand, morphologically homogeneous species with high intrapopulation variation in genome size (*H. pictum*) and species with unclear patterns of genome size variation (*H. amplexicaule*, *H. laevigatum* and *H. piliferum*) were not split. The units after splitting held the name of the broad species and were numbered (I, II) (Table 1). For convenience, they are referred to as species in the following paragraphs. Forty six taxa were recognized after the split (Table 1). To test the correlation between monoploid DNA amount (1Cx) and chromosome number, the Spearman rank order correlation coefficient and the one-way ANOVA were used with a matrix of all samples ( $2n = 18, 27$  and 36).

Evolution of genome size was investigated on a sample of trees using the generalized least-squares method implemented in BayesTraits (Pagel and Meade, 2007). For this purpose, the last 751 trees from each run of the Bayesian analysis of the modified data set (without hybrids) were sampled and merged into one file. All 1502 trees were rooted manually with the outgroup (*Andryala integrifolia*, the taxon used as

outgroup in the Bayesian analysis), using the program Dendroscope (Huson *et al.*, 2007). Analyses were conducted on three different data sets: (1) on a complete set of these species; (2) separately for species of the western clade; and (3) separately for species of the eastern clade. All genome size data were  $\log_{10}$  transformed prior to analyses. Two models of trait evolution were compared, using likelihood ratio statistics (Huelsenbeck and Rannala, 1997) or BayesFactor. Model A (drift model) corresponds to the standard constant-variance random-walk model, and model B is a directional random-walk model (Pagel, 1999, 2004). The scaling parameters  $\lambda$  ( $\lambda$ ),  $\kappa$  ( $\kappa$ ) and  $\delta$  ( $\delta$ ) were optimized for 1Cx-values.  $\lambda$  assesses the contribution of the phylogeny to a character,  $\kappa$  scales branch lengths and can be used to test punctual vs. gradual modes of trait evolution, and  $\delta$  scales the overall path length in the phylogeny. Values of 1.0 correspond to the null model (tree topology and branch lengths accurately describe models A and B). To test whether a model with estimated scaling parameters is a better fit than the null model where all scaling parameters are set to 1 (i.e. a strict Brownian motion model) the likelihood ratio test or BayesFactor was used. Two different methods of analysis were used, namely maximum likelihood and Monte Carlo Markov chain (MCMC).

How the variation in genome size matched the two major lineages of *Hieracium* (named 'western' and 'eastern') suggested by molecular phylogenetic analyses of the ETS region was tested further. In addition, genome sizes of inter-clade hybrid accessions were analysed. Five informal groups were recognized and used for these analyses, restricting the genome size data set to the accessions for which sequence data were available (Table 1): (a) 'western', corresponding to the phylogenetically distinguished 'western' group and containing 'pure' accessions as well as hybrid/hybridogenous accessions within the 'western' group [W and W(H)]; (b) 'eastern', corresponding to the phylogenetically distinguished 'eastern' group and containing 'pure' accessions and hybrid/hybridogenous accessions within the 'eastern' group [E and E(H)]; (c) hybrid/hybridogenous accessions between 'western' and 'eastern' clade species with about equal contribution of ETS variants from each parent (X); (d) interclade hybrid accessions with strongly dominating 'western' ETS sequence type [X(W)]; and (e) interclade hybrid accessions with strongly dominating 'eastern' group ETS [X(E)] (Table 1). For the purpose of this analysis, no distinction was made between 'pure' and intraclade hybrids (a, b) because of the low sequence variation within each clade and because their genome sizes did not differ. Two comparisons were performed: (1) the 'western' and 'eastern' groups with a group comprising all E–W hybrids; and (2) the 'western' and 'eastern' groups with the three different groups of hybrids (c–e) specified above. More details about ETS sequence features and the identification of particular hybrids will be presented elsewhere (J. Fehrer *et al.*, unpubl. res.). Both comparisons were conducted separately with and without *H. transylvanicum* (which fell into the phylogenetically defined western lineage, but has a genome size and geographic range congruent with the 'eastern' group; see Discussion). The correlation between genome size and phylogenetic pattern (five groups, see above) was tested.

The Spearman non-parametric rank order correlation coefficient was used in testing whether DNA amounts correlated with selected Ellenberg's indicator values, namely for light and temperature (Ellenberg *et al.*, 1992). Mean 1Cx values for species *sensu* Zahn were used for this analysis; only a subset of central European species (for which these values are available) was chosen. Genome size variation was also tested against altitudinal and geographical position (longitude and latitude) for (a) the complete set of accessions (mean accessions' 1Cx values were used), and (b) excluding accessions of widely distributed species (*H. bifidum*, *H. lachenalii*, *H. laevigatum*, *H. murorum*, *H. sabaudum* and *H. umbellatum*) for which the results are strongly affected by the collection site of the samples.

The only significant correlations of genome size variation with other parameters concerned phylogeny and longitude. In order to distinguish between these two factors, three correlation tests concerning longitude were performed for each pairwise comparison, constrained to accessions for which sequence data were available: (1) across all accessions from the 'western' and 'eastern' clades; (2) within the 'western' clade only; and (3) within the 'eastern' clade only. If the correlation is significant across all species, but not significant within either of the two clades analysed separately, this would argue for a connection between phylogenetic relationships and genome size variation. If, however, significant correlations are found for each of these tests, a relationship of genome size to longitude independent of species relationships would be supported. Data were analysed using the statistical package 'Statistica for Windows 6.0' (StatSoft, 1984–2002).

## RESULTS

### *Chromosome counts and mode of reproduction*

Chromosome numbers for plants of 43 populations analysed in the present paper belonging to 28 species were published elsewhere (Chrtek *et al.*, 2007), and counts for the remaining 46 populations are presented here (Table 1). A new ploidy is reported for *H. gymnocephalum* ( $2n = 18$ ). Other counts confirmed previously published chromosome numbers. All diploids studied were found to be sexual and allogamous, and all polyploids ( $3x$ ,  $4x$ ) were agamosperous (data not provided).

### *Flow cytometry*

Flow cytometric analyses yielded high-resolution histograms with CVs of  $G_0/G_1$  peaks for *Hieracium* samples ranging from 0.83 to 5.76% (mean 2.28%), the values for internal reference standards were 0.97 to 5.0% (mean 2.19%). Generally, CVs of *Pisum sativum* were lower than those of *Zea mays*.

### *Nuclear DNA content: within-species variation*

Intraspecific variation was assessed in 40 of 42 species *sensu* Zahn. Variation within accessions (populations) was generally low (Table 1). Mean values with standard errors (ranges for 2C and means for 1Cx genome sizes) for each population and



mean values with standard errors and ranges for 1Cx for each species are summarized in Table 1. Variation in 1Cx values between conspecific accessions (in both homo- and multiploid species) ranged from 0.24% in *H. gymnocephalum* and *H. heterogynum* to 7.2% in *H. pannosum*. Variation exceeding the approximate measurement inaccuracy threshold of 3.5% was detected in seven species, namely *H. amplexicaule*, *H. bupleuroides*, *H. laevigatum*, *H. pannosum*, *H. pictum*, *H. piliferum* and *H. prenanthoides*.

However, variation in the more naturally delimited (without heterogeneity in ETS sequences and inter-population genome size; see Materials and methods) *H. bupleuroides* was below the threshold of 3.5% variation. Variation within the separately treated populations of morphologically heterogeneous *H. pannosum* was also below 3.5% (Table 1). In further paragraphs, these ‘narrower’ taxa (a total of 46 taxa) are used.

#### C-values in the total set of ‘basic’ species

The mean 2C values differed up to 2.37-fold among different species (from 7.03 pg in diploid *H. stelligerum* to 16.67 in a tetraploid accession of *H. pannosum*). The 1Cx values varied 1.22-fold between 3.51 pg in *H. stelligerum* and 4.34 pg in *H. viosum* (mean 1Cx value of 3.87, s.d. 0.27; Fig. 1). The 1Cx values of diploids (including diploid accessions of multiploid species, means for species/cytodemes) varied 1.22-fold between 3.51 pg in *H. stelligerum* and 4.29 pg in *H. plumulosum* (mean 1Cx value 3.92 pg, s.d. 0.30), in triploids [including triploid accessions of multiploid species (means for species/cytodemes)] 1.23-fold between 3.53 pg in *H. bifidum* and 4.35 pg in *H. viosum* (mean 1Cx value of 3.81 pg, s.d. 0.25), and in tetraploids 1.17-fold between 3.56 pg in *H. humile* and 4.17 pg in *H. pannosum* II (mean 1Cx value of 3.79, s.d. 0.19).

#### Correlation between genome size, ploidy and breeding system

Diploids differed significantly in their 1Cx values from both triploids ( $t = 2.71$ , d.f. = 196,  $P = 0.007$ ) and tetraploids ( $t = 2.01$ , d.f. = 109,  $P = 0.047$ ), but triploids did not differ significantly from tetraploids ( $t = 0.72$ , d.f. = 119,  $P = 0.476$ ) (Fig. 2). The value of the Spearman non-parametric rank order correlation coefficient was  $r = -0.179$ ,  $P = 0.009$ . The

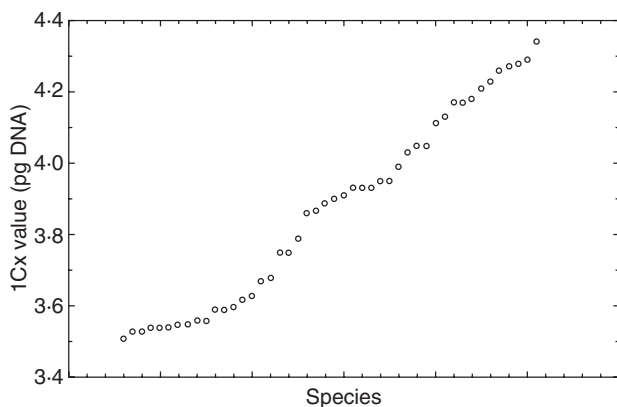


FIG. 1. 1Cx-value variation in 46 taxa of *Hieracium* subgenus *Hieracium*.

mean 1Cx value was 3.93 pg in diploids, 3.82 pg in triploids and 3.78 pg in tetraploids, suggesting a trend towards smaller genome size with increasing ploidy.

In multiploid species, there was no general trend to either genome downsizing or upsizing. In *H. prenanthoides* 2x/3x there was 2.47% upsizing, in *H. villosum* 3x/4x 0.06% downsizing, in *H. tomentosum* 2x/3x 0.36% upsizing, in *H. humile* 3x/4x 0.36% upsizing and in *H. alpinum* 2x/3x 0.17% upsizing.

Comparison between 1Cx values of sexually reproducing plants (i.e. all diploids; polyploids were exclusively apomictic) and apomicts (triploids and tetraploids) revealed significant differences at  $\alpha = 0.01$  ( $t$ -test,  $t = 3.04$ , d.f. = 213,  $P = 0.003$ ); the mean 1Cx value in sexuals was 3.93 pg, whereas in apomicts it was 3.82 pg, corresponding to the value for triploids due to the low number of tetraploid accessions.

#### Molecular phylogenetics of *Hieracium*

Analysis of ETS data including all sequenced accessions resulted in the same tree with both methods. It indicates monophyly of *Hieracium* subgenus *Hieracium*, but species relationships remained completely unresolved as reflected by a large polytomy with only two small subclusters that received low support (Fig. 3). However, as two major species groups could be identified by visual inspection of the alignment and many sequences showed additive patterns indicative of hybridization involving both groups, these accessions were deleted from subsequent analysis, because reticulation is known to collapse branches (Feliner *et al.*, 2001; Soltis *et al.*, 2008). With the reduced data set, a clear separation into two major clades with strong statistical support was found with both methods (Fig. 4). These lineages were designated ‘eastern’ and ‘western’ clade because they contained species of predominantly eastern or western European origin. A large number of accessions (18) showed ETS variants of both clades in either equal proportion or with the ‘eastern’ or ‘western’ sequence type dominating as indicated in Fig. 4. Details of these analyses will be given in a parallel paper (J. Fehrer *et al.*, unpubl. res.).

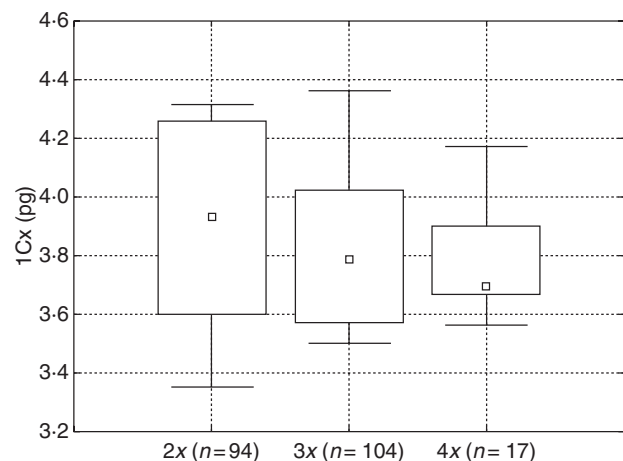


FIG. 2. Variation of genome size among diploids, triploids and tetraploids (all samples). 1Cx values of all accessions are shown. Differences between diploids and triploids, and between diploids and tetraploids are significant. The box indicates the interquartile (25–75%) range, the small square within the box is the median. The whiskers indicate minimum and maximum values.



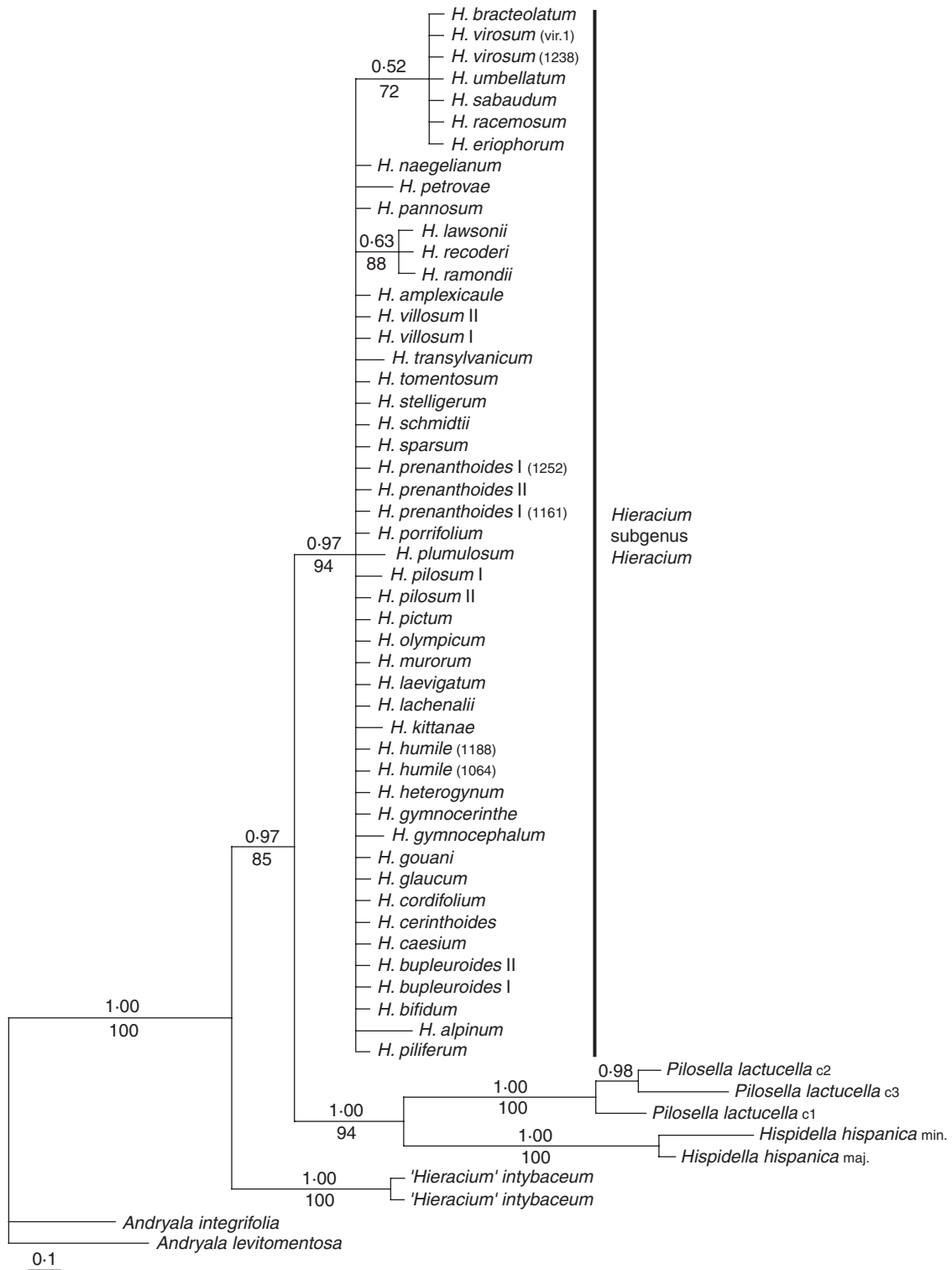


FIG. 3. Phylogenetic analysis of ETS sequences based on all accessions. A Bayesian consensus tree of 3002 saved trees is shown with posterior probabilities above branches. The maximum likelihood tree has the same topology; bootstrap values are indicated below branches. *Hieracium* subgenus *Hieracium* (= *Hieracium sensu stricto*) is monophyletic, but species relationships are completely unresolved when hybrids are included in the analysis. Support for the two subclusters is low.

*Correlation of genome size with phylogenetic signal*

The ‘western’ clade included 15 accessions: 2C values ranged from 7.03 pg in diploid *H. stelligerum* to 14.25 pg in

a tetraploid accession of *H. humile*; 1Cx values ranged from 3.51 pg in *H. stelligerum* to 4.28 pg in *H. transylvanicum* (mean ± s.d.: 3.61 ± 0.19 pg; with *H. transylvanicum*

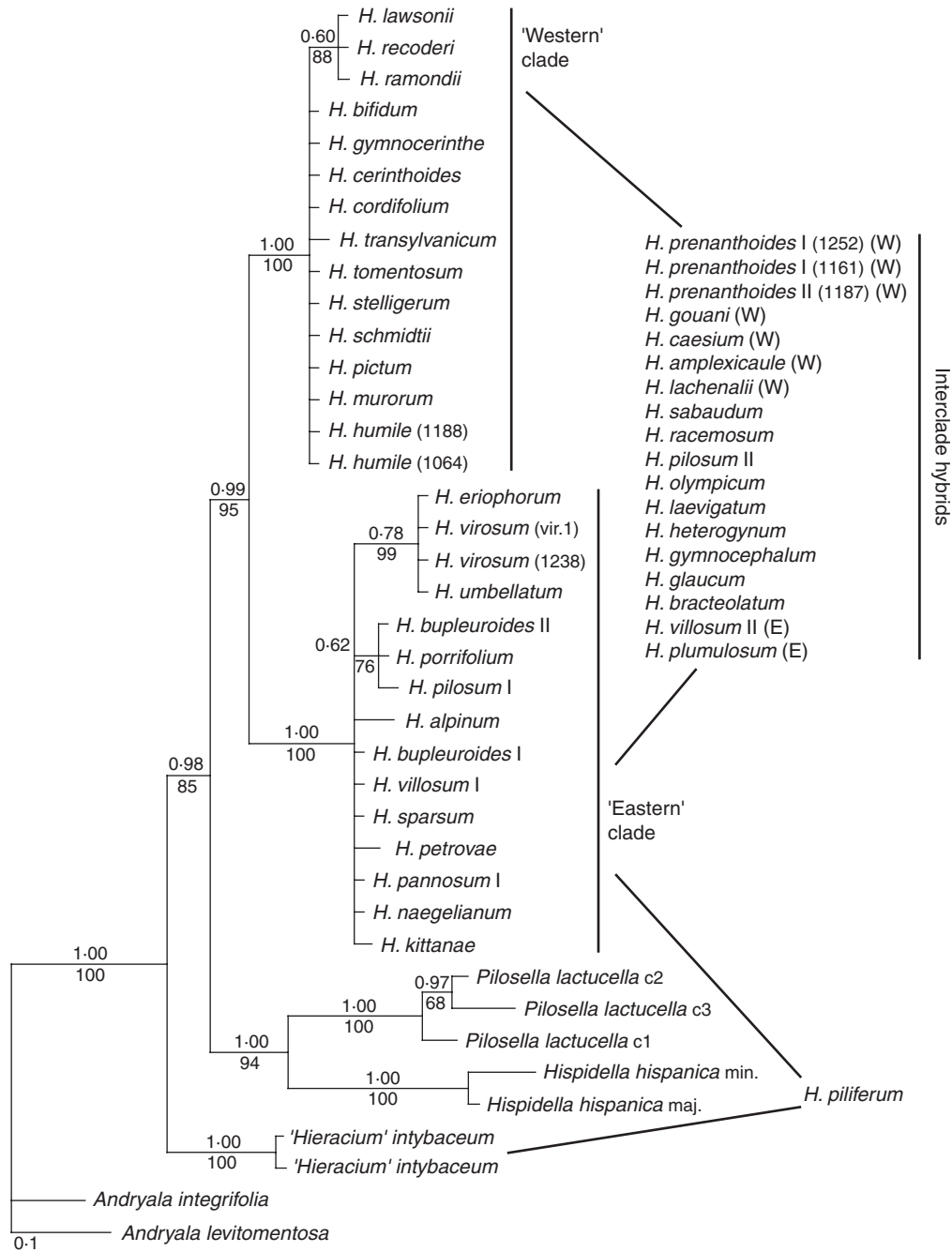


FIG. 4. Phylogenetic analysis of ETS sequences excluding interclade hybrid accessions. A Bayesian consensus tree of 1502 saved trees is shown with posterior probabilities above branches. The maximum likelihood tree has the same topology; bootstrap values are indicated below branches. After exclusion of hybrids based on character additivity, two major groups are resolved (referred to as 'eastern' and 'western' clades). Hybrid accessions composed of parents from both clades (interclade hybrids) are listed to the right. (W), interclade hybrids with predominant 'western' ETS type; (E), interclade hybrids with predominant 'eastern' ETS type. *Hieracium piliferum* is a hybrid between '*Hieracium*' *intybaceum* and an 'eastern' clade taxon. For details about accessions, see Table 1 and Supplementary Data, available online.

excluded: up to 3.74 pg in *H. tomentosum*,  $3.57 \pm 0.06$  pg). The 'eastern' clade also comprised 15 accessions: 2C values ranged from 7.78 pg in diploid *H. porrifolium* to 15.71 pg in a tetraploid accession of *H. villosum*; 1Cx values ranged from 3.63 pg in *H. naegelianum* to 4.35 pg in *H. virosus* ( $4.02 \pm 0.20$  pg). Significant differences in 1Cx values were found between the clades at  $\alpha = 0.001$  (Student's *t*-test),

with ( $t = -5.71$ , d.f. = 28,  $P < 0.001$ ) and without ( $t = -8.23$ , d.f. = 27,  $P < 0.001$ ) *H. transylvanicum*.

Differences in 1Cx values between accessions of the 'western' (W) and 'eastern' (E) clades and of interclade hybrid accessions (X) are significant, independent of the inclusion of *H. transylvanicum* ( $F = 13.79$ , d.f. = 45,  $P < 0.001$  with *H. transylvanicum*,  $F = 20.87$ , d.f. = 44,

$P < 0.001$  without *H. transylvanicum*; Fig. 5A). However, *post hoc* comparison (Scheffé test) revealed only two groups at  $\alpha = 0.05$ , the first comprising all ‘western’ accessions, and the second embracing ‘eastern’ and ‘hybrid’ accessions. Thus, ‘eastern’ and ‘hybrid’ accessions do not differ significantly from each other. Significant differences were also found between five groups, i.e. after splitting the bulk of hybrids into three groups, namely hybrids with intermediate position (X) and hybrids with strongly dominating ‘western’ [X(W)] or ‘eastern’ [X(E)] ETS sequences ( $F = 17.07$ , d.f. = 43,  $P < 0.001$  with *H. transylvanicum*,  $F = 28.86$ , d.f. = 42,  $P < 0.001$  without *H. transylvanicum*). The Scheffé test revealed only two groups at  $\alpha = 0.05$ , the first including W and X(W) accessions, the second X, X(E) and E accessions (Fig. 5B). A significant correlation (Spearman rank coefficient  $r = 0.705$ ,  $P < 0.001$ ) between phylogenetic signal and hybrid origin [all five groups – W, E, X, X(W) and X(E)] and the pattern of genome size variation was found. *Hieracium piliferum* (1Cx = 3.9) occupies an isolated position, and it was identified as a hybrid between an ‘eastern’ clade taxon and ‘*Hieracium*’ *intybaseum* (1Cx = 3.76).

*Evolution of genome size*

*Maximum likelihood method.* For the complete data set (all species), a directional model of evolution (model B) did not result in significantly higher likelihood scores than the drift model of evolution (model A; 74.856 vs. 75.746), indicating that there is no general trend to either genome size increase or decrease. Scaling parameters leading to the highest likelihood for 1Cx values were  $\lambda = 0.908$ ,  $\delta = 0.819$  and  $\kappa = 1.035$  in model A and  $\lambda = 0.701$ ,  $\delta = 0.637$  and  $\kappa = 1.201$  in model B, respectively. For both models, the values of scaling parameters did not differ significantly from 1 (the null expectation, LR test), indicating that the phylogenetic tree correctly predicts the pattern of covariance among species on the trait (1Cx) and that there is no evidence of accelerated evolution.

For the western clade, likelihood scores of models A and B did not differ significantly (44.061 vs. 44.790). The maximum

likelihood values for  $\lambda$  ( $< 1$ ; 0.550 in model A, 0.523 in model B) show a role of adaptive response to some external factors. Values of  $\delta$  and  $\kappa$  are  $> 1$  in both models (not shown) indicating that longer paths contribute more to 1Cx evolution (accelerated evolution as time progresses) and that longer branches contribute more to the trait evolution. Likelihoods of the null model (with scaling parameters set to 1.0) are significantly lower in both models, indicating that scaling parameters improve the fit of the data to the models.

For the eastern clade, likelihood scores of models A and B also did not differ significantly (40.915 vs. 42.141). Scaling parameters are not significantly different from 1 (the null expectation, Brownian motion, data not shown) indicating that the phylogenetic tree correctly predicts the pattern of covariance among species and that there is no evidence of accelerated evolution.

*MCMC method.* For the complete data set, comparison of harmonic means of log maximum likelihoods of models A and B showed a somewhat higher value in the latter (82.544 vs. 84.670). The model with estimated scaling parameters is a better fit than the null model (with scaling parameters set to 1) for both models A and B, showing that the scaling parameters improve the fit of the data to the model. The values of  $\lambda$  did not differ significantly from 1, and relatively high values of  $\kappa$  (3.379 and 3.606, respectively) indicate that longer branches contribute more to genome size evolution.

For the western clade only, the harmonic mean of model B is also higher than that of model A (49.888 vs. 45.494) and the harmonic mean of the null model is significantly lower than that for the model with estimated scaling parameters. Scaling parameters are similar to those found with the maximum likelihood method, i.e.  $\lambda$  and  $\delta < 1$ ,  $\kappa > 1$  (for interpretation see above).

For the eastern clade, harmonic means of models A and B do not differ significantly. Values of  $\lambda$  and  $\delta$  do not differ from 1, higher values of  $\kappa$  (1.988 in model A and 2.212 in model B) again indicate accelerated rates of evolution within long branches.

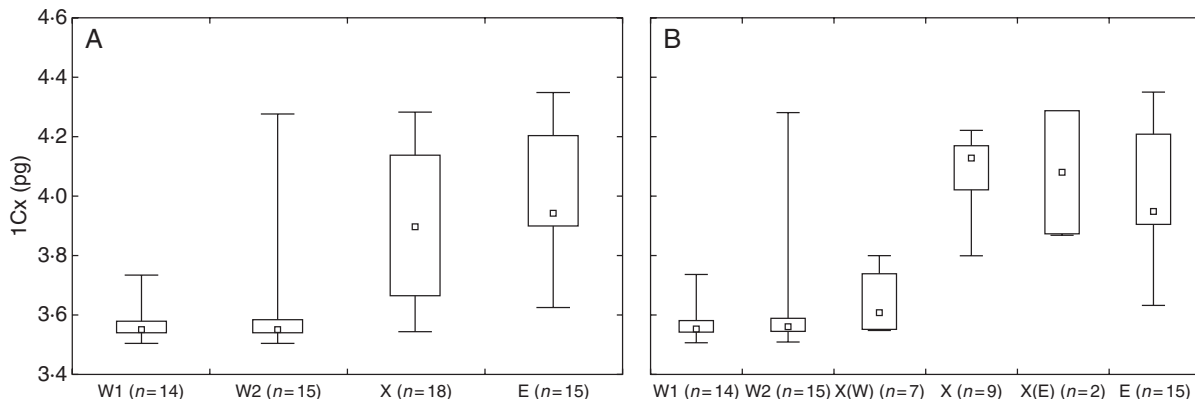


FIG. 5. Correlation of 1Cx values with phylogeny. Only accessions for which sequence data were available are included. (A) W1, ‘western’ clade accessions without *H. transylvanicum*; W2, ‘western’ clade accessions including *H. transylvanicum*; X, interclade hybrid accessions; E, accessions of the ‘eastern’ clade. (B) W1, W2 and E as before, hybrids divided into those with predominant ‘western’ [X(W)], equal (X) and predominant ‘eastern’ [X(E)] ETS sequence composition (see also Table 1). The box indicates the interquartile (25–75%) range, the small square within the box is the median. The whiskers indicate minimum and maximum values.

### Correlation between genome size and ecogeographic features

The genome size of particular accessions was significantly correlated with their geographic position (longitude) in a west–east direction, both in the complete set of accessions (Spearman rank coefficient  $r = 0.562$ ,  $P < 0.001$ ; Fig. 6A) and after exclusion of widely distributed species ( $r = 0.617$ ,  $P < 0.001$ ; i.e. without *H. bifidum*, *H. lachenalii*, *H. laevigatum*, *H. murorum*, *H. sabaudum* and *H. umbellatum*; Fig. 6B). The correlation was stronger in the second case due to the strong dependence on the part of the geographic area from which the target plants of widespread species were sampled.

No correlation between genome size and latitude ( $r = 0.049$ ,  $P = 0.646$ ) or genome size and altitude ( $r = -0.224$ ,  $P = 0.034$ ) was found (complete set of accessions, results not shown). Also, no significant correlation was found between genome size and selected ecological parameters (Ellenberg's indicator values), namely temperature ( $r = 0.194$ ,  $P = 0.427$ ) and light ( $r = -0.236$ ,  $P = 0.331$ ) in a subset of species occurring in central Europe (results not shown).

### Distinction between longitudinal and phylogenetic correlation

In order to determine whether the increase in genome size towards the east/'eastern' clade is based on geographic distribution or on species relationships, the longitudinal correlation

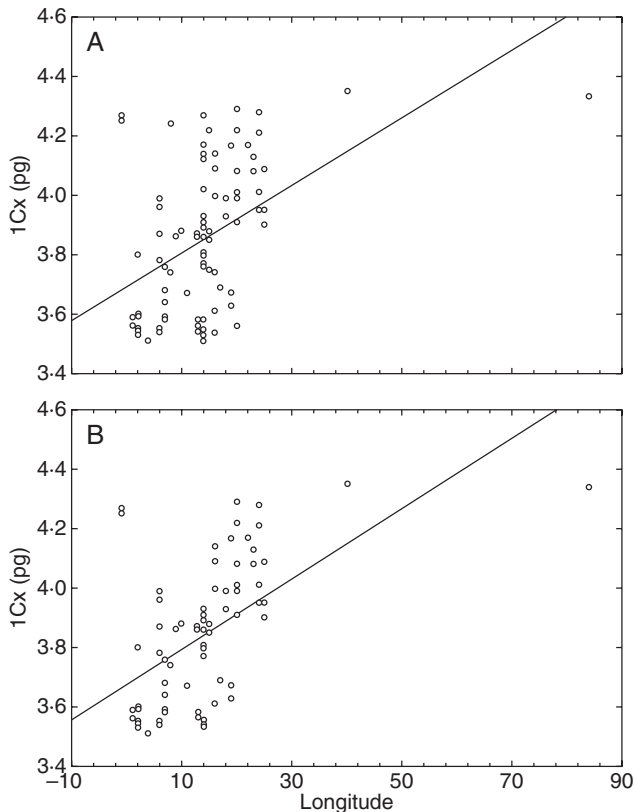


FIG. 6. Distribution of 1Cx values versus longitudinal position of collection sites: (A) based on the complete set of accessions/populations (Spearman rank coefficient  $r = 0.562$ ,  $P < 0.001$ ); (B) based on a subset after excluding accessions of widely distributed species (*H. bifidum*, *H. lachenalii*, *H. laevigatum*, *H. murorum*, *H. sabaudum* and *H. umbellatum*;  $r = 0.617$ ,  $P < 0.001$ ).

was re-analysed for those accessions for which molecular data were available. Even stronger correlation was found between longitude and genome size in a set of accessions with known ETS sequences independent of including ( $r = 0.656$ ,  $P < 0.001$ ) or excluding ( $r = 0.688$ ,  $P < 0.001$ ) accessions of widely distributed species (Fig. 7A, B). In contrast, no significant correlation was found either among species of the 'western' ( $r = 0.161$ ,  $P = 0.567$ ) or among species of the 'eastern' ( $r = 0.394$ ,  $P = 0.245$ ) clade when tested separately (Fig. 7C and D). These results reveal that the evolutionary history due to eastern or western origin of the species is the dominant parameter affecting genome size in *Hieracium* rather than longitude.

## DISCUSSION

### Chromosome numbers and mode of reproduction

Chromosome numbers for plants from 46 populations belonging to 26 species are published here for the first time, and counts for the remaining accessions have been published elsewhere (Chrtek *et al.*, 2007). Among the new data, a new ploidy (diploid) is reported for *H. gymnocephalum* (*s.l.*); previously reported counts (Niketić *et al.*, 2006) refer only to triploids ( $2n = 3x = 27$ ). After *H. petrovae*, this is the second diploid count within section *Pannosa*. Also worth mentioning is the diploid ( $2n = 2x = 18$ ) count for *H. prenanthoides* from the western Alps. Although this number had been reported from the same area in the 1960s (Favarger, 1969; Löve, 1969), it has not been confirmed until now. The remaining chromosome numbers correspond to previously published counts for the target species [cf. Schuhwerk (1996) and other standard reference manuals]. Analysis of the mode of reproduction confirmed the pattern already published for selected species and suggested it to be generally valid throughout the genus: diploid species reproduce sexually and are allogamous whereas polyploids are agamosperous.

### Intraspecific genome size variation

Variation beyond arbitrary fluctuation (3.5%) was found in seven species *sensu* Zahn, namely *H. piliferum* (3.89%), *H. amplexicaule* (3.95%), *H. bupleuroides* (4.2%), *H. laevigatum* (4.28%), *H. pictum* (5.14%), *H. prenanthoides* (7.02%) and *H. pannosum* (7.2%). All are agamosperous polyploids, two of them including two cytodesmes (*H. amplexicaule*,  $3x/4x$ ; *H. prenanthoides*,  $2x/3x$ ). As only a subset of populations was used for phylogenetic analysis, these results need to be interpreted with caution. In several cases in which sequence data were obtained for more than one accession, multiple origins of a given taxon, namely in *H. bupleuroides*, *H. pilosum*, *H. prenanthoides* and *H. villosum*, were found, which allows a plausible explanation of genome size variation at least for *H. prenanthoides*. In this species, sequences of three accessions, one being diploid and two triploid, were analysed. All three showed identical signatures of an ancient interclade hybridization already affecting the diploid (J. Feher *et al.*, unpubl. res.). The triploids resulted from different subsequent hybridizations, one involving a 'western' and one an 'eastern' lineage. This fits well with



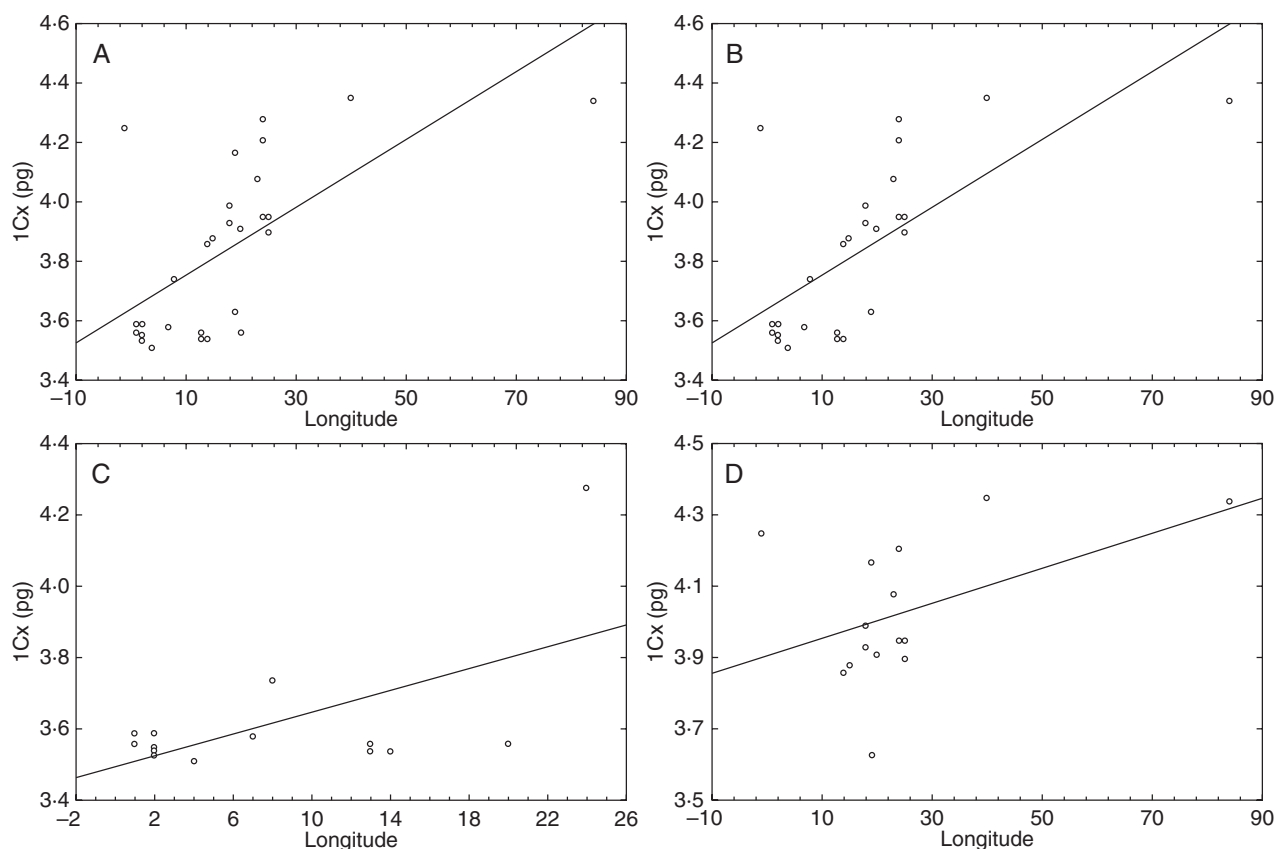


FIG. 7. Longitudinal component of genome size variation for accessions of known phylogenetic origin: (A) based on a complete set of 'western' and 'eastern' accessions/populations analysed by molecular methods (excluding interclade hybrid accessions; Spearman rank coefficient  $r = 0.656$ ,  $P < 0.001$ ); (B) based on a subset after excluding accessions of widely distributed species (*H. bifidum*, *H. murorum* and *H. umbellatum*;  $r = 0.688$ ,  $P < 0.001$ ); (C) based on a subset of accessions with 'western' ETS type ( $r = 0.161$ ,  $P = 0.567$ ); (D) based on subset of accessions with 'eastern' ETS type ( $r = 0.394$ ,  $P = 0.245$ ).

the 1Cx values: whereas values of the diploid and 'western' introgressed triploid populations ranged from 3.56 pg to 3.67 pg, the 1Cx value of the 'eastern' introgressed triploid was higher (3.81 pg). *Hieracium laevigatum*, *H. amplexicaule* and *H. piliferum* are, according to the ETS sequences, hybrids/hybridogenous types (at least the analysed accessions), and higher intraspecific variation might reflect recurrent polytopic origins. High inter-population variation in *H. pannosum* could also be related to multiple origins as suggested by the different ploidies of the analysed accessions. The high variation in *H. pictum* cannot be explained by the present data. Broader sampling for molecular analysis in these species might reveal hybrid accessions that have not yet been discovered. Thus, unequivocal evidence for intraspecific genome size variation was not found in 'good' species of subgenus *Hieracium*.

Similar results were obtained for *Hieracium* subgenus *Pilosella* in which the majority of wild species/cytotypes possess constant nuclear DNA amounts (variation in fluorescence intensity lower than 3.5%). Nevertheless, higher divergence was observed in six cytotypes belonging to three 'intermediate' species (the same terms as in subgenus *Hieracium*, i.e. hybrids/hybridogenous species) and among genetically variable  $F_1$  offspring of experimental crosses between hexaploid *H. rubrum* and tetraploid *H. pilosella* (Suda *et al.*, 2007).

Intraspecific genome size variation in 'non-hybridogenous' species recently became a matter of debate (Murray, 2005). Whereas many of the examples of variation have been shown to be artefacts of measurement methods (e.g. Teoh and Rees, 1976; Greilhuber, 1998, 2005), there are some reports documenting C-value variation where appropriate controls and standards have been used (Reeves *et al.*, 1998; Hall *et al.*, 2000; Moscone *et al.*, 2003; Pecinka *et al.*, 2006).

#### Interspecific genome size variation

The present estimates of nuclear DNA content in *Hieracium* subgenus *Hieracium* are the first to be published for this group; all other data on *Hieracium* available so far refer to species of (subgenus) *Pilosella* (Bennett and Leitch, 2005), genomes of which are considerably smaller than those of subgenus *Hieracium* (see below). Holoploid (2C) genome sizes in *Hieracium* species included in the present study ranged 2.37-fold from 7.03 pg to 16.67 pg (mean 2C value 10.16 pg, median 10.61 pg). As almost all so-called 'basic' species were investigated, the present results should cover the genome size variation within the subgenus. A few rare pentaploid hybridogenous (i.e. not 'basic') taxa exist which were not analysed, and thus the upper limit could be higher. Variation in Cx values among species is relatively high (up to about 20%), but more or less continuous.

In *Hieracium* subgenus *Pilosella* with the same basic chromosome number ( $n = 9$ ), holoploid (2C) genome size differs 4.33-fold and ranges from 3.53 pg to 15.30 pg (Suda *et al.*, 2007). However, *Pilosella* has more extensive variation in ploidy, ranging from diploid to octoploid. Monoploid genome sizes (1Cx values) in subgenus *Hieracium* ranged 1.22-fold from 3.51 pg to 4.29 pg (mean 2C value 3.86 pg, median 3.85 pg), whereas genome size in *Pilosella* is distinctly lower (it varies 1.23-fold from 1.72 pg to 2.16 pg). Thus, *Pilosella* has consistently about half the DNA content compared with *Hieracium*. The reasons for these large differences among closely related groups (Fehrer *et al.*, 2007) are unclear at the moment. Chromosomes of subgenus *Hieracium* are distinctly larger than those of *Pilosella* (no quantitative assessments available). Accumulation of repetitive sequence elements as in other plant groups might be one of the causes, but insights into *Hieracium* genomes are still lacking.

#### Genome size and ploidy

Diploid hawkweeds differ significantly in their 1Cx values from both triploids and tetraploids, but the latter do not differ from each other. This might indicate general downsizing of genomes in polyploid hawkweeds. However, there is no general trend to either downsizing or upsizing within multiploid species. Their origin remains to be elucidated in many cases and could involve autopolyploid origin as well as participation of another taxon (introgression which cannot always be detected by morphology). In Asteraceae, a similar situation was documented in the genus *Centaurea s.l.* in four multiploid (consisting of diploid and tetraploid cytotypes) species: downsizing was found in two species, upsizing in one species and equal monoploid genome size in one species (Bancheva and Greilhuber, 2006). However, downsizing of the genome after polyploidization is widely supposed to be a general trend in angiosperms (Kellogg and Bennetzen, 2004; Leitch and Bennett, 2004; Weiss-Schneeweiss *et al.*, 2006), as seems to be the case for *Hieracium*. The present data also suggest that species of autopolyploid origin might have more uniform genome size (and morphology) than allopolyploids of multiple (hybrid) origin.

#### Genome size and phylogeny

Genome size distribution basically matches two phylogenetically defined major lineages, i.e. a 'western' and an 'eastern' group (Figs 4 and 5). It thus reveals a strong correlation of nuclear DNA content with the basal evolutionary divergence of *Hieracium* subgenus *Hieracium*. Both clades include sexual diploids, agamosperous triploids and rare tetraploid apomicts. A similar pattern has also been observed for the geographic ranges. Both groups comprise local endemics (e.g. *H. stelligerum* and species of section *Cerinthoidea* in the 'western' group and *H. kittanae*, *H. petrovae* and *H. eriophorum* in the 'eastern' group) and widely distributed species (e.g. *H. murorum* and *H. bifidum* in the 'western' and *H. umbellatum* in the 'eastern' group). Differences of genome size between the two clades were also significant when only diploid or triploid accessions were compared

(despite indication for some genome downsizing in polyploids), and thus all cytodesmes were analysed together.

As mentioned above, *H. transylvanicum* falls into the western lineage but has a genome size and geographic range congruent with the 'eastern' group. Two alternative scenarios for its origin can be proposed: (1) the species has an eastern origin as suggested by its current distribution and DNA content and shows some ancient introgression from western species, some of which are widespread. Its ETS sequence then became completely homogenized towards the western type by concerted evolution (Arnheim, 1983); and (2) the species originated in western Europe, spread towards the east, the original populations became extinct probably during the Ice Ages and only the eastern populations survived in an eastern glacial refuge like the Carpathian basin. In this case, the high DNA content may be due to other reasons than phylogenetic relationships. Another accession of a 'western' clade species, *H. lachenalii*, has plastid DNA matching some 'eastern' clade species which suggests ancient introgression (J. Fehrer *et al.*, unpubl. res.). Correspondingly, its DNA content is also slightly higher than that of most other species of the 'western' clade. *Hieracium eriophorum*, a local endemic of the Atlantic coast near Arcachon in western France, is another species with an incongruent geographic range and position in the phylogenetic tree. Despite its western European distribution, it is most likely derived from widespread *H. umbellatum*, a species belonging to the same 'eastern' clade (Fig. 4). The morphology of *H. eriophorum* could therefore be interpreted as a local adaptation to sand dunes along the sea coast. The lowest genome size within the 'eastern' clade (2C = 10.89 pg, 1Cx = 3.63 pg) was detected in triploid *H. naegelianum*. The distribution of this species fits well with other 'eastern' species as it occurs in the Balkan Peninsula and in the Abruzzi Mountains in central Italy, mostly in refugial areas. With respect to morphology, it is the only species of *Hieracium* subgenus *Hieracium* with long underground stolons, which enable the plant to spread vegetatively. Although no evidence of introgression from a 'western' species is apparent from the present molecular data, its occurrence in Italian glacial refuges could be indicative of past contacts and introgression from which only an unusually small genome size is left. Its plastid DNA is unique, and its relationships with other 'eastern' clade species are unresolved.

#### Hybrid (hybridogenous) 'basic' species

Fourteen 'basic' species were found to be of hybrid origin between 'eastern' and 'western' clade species (Fig. 4): *H. amplexicaule*, *H. bracteolatum*, *H. caesium*, *H. glaucum*, *H. gouanii*, *H. gymnocephalum*, *H. heterogynum*, *H. lachenalii*, *H. laevigatum*, *H. olympicum*, *H. plumulosum*, *H. prenanthoides*, *H. racemosum* and *H. sabaudum*. In addition, individual accessions of *H. pilosum* and *H. villosum* also had this type of hybrid origin. Based on the known mean 1Cx values for the 'western' and 'eastern' groups (3.61 and 4.02 pg, respectively), intermediate genome sizes of the previously mentioned species might be expected. However, hybrid accessions were more similar to the 'eastern' species group and significantly different from the

‘western’ group. The median 1Cx values of intermediate hybrid taxa and those with dominating ‘eastern’ ETS sequence were even higher than those for both clades (Fig. 5B). Potential interpretations could be to assume extinct parents with higher genome sizes or, more likely, an increase in DNA content in species of hybrid origin in comparison to their parents, as has been documented in *Helianthus* by Baack *et al.* (2005). The present results also show that hybrids/hybridogenous types with strongly dominating ‘western’ type ETS (e.g. *H. amplexicaule*, *H. caesium*, *H. gouani*, *H. lachenalii* and *H. prenanthoides*) have similar DNA content in comparison with ‘western’ species, which is significantly different from hybrids with equal contribution of ‘eastern’ and ‘western’ parents. This might suggest repeated backcrossing towards ‘western’ species at the diploid level before genomes became fixed by apomixis. Intermediate hybrids with dominant ‘eastern’ ETS, as expected, did not differ significantly from ‘intermediate’ hybrids or ‘eastern’ species.

One accession of *H. piliferum*, a basic species distributed in European mountains was found to be a hybrid between an unidentified ‘eastern’ taxon and ‘*H. intybaceum*’ by character additivity in ETS sequences. Comparing 1Cx values of ‘*H. intybaceum*’ (3.76 pg, Table 1), *H. piliferum* (3.91 pg) and members of the two (‘western’ and ‘eastern’) major clades, past hybridization between ‘*H. intybaceum*’ and an ‘eastern’ species might be expected, which is also congruent with the results of the molecular analyses. Intraspecific genome size variation beyond the arbitrary fluctuation in *H. piliferum* could be indicative of multiple origins.

#### Evolution of genome size

Two different approaches implemented in BayesTraits for continuous data were applied, namely the maximum likelihood method and the MCMC approach, and two models of evolution (A, drift model; B, directional model) were compared. In most comparisons, our data fit (higher log-likelihoods and harmonic means) model B better, indicating that there is some but no strong trend to genome size increase.

For the complete data set and the maximum likelihood method, the values of scaling parameters did not depart significantly from the default values (1.0) suggesting that tree topology and branch lengths accurately describe the constant variance random-walk model A or B. Thus, genome size is evolving, as expected, given the tree topology, fitting well with the basal split into the two clades. Using the MCMC method, more changes on longer branches were indicated (longer branches contribute more to genome size evolution).

The tempo and mode of evolution differ between the western and eastern clade. In the western clade, values of scaling parameters depart from the default value (1.0) indicating that genome size evolution has not followed the topology or the branch lengths. Phylogenetic history has lower impact ( $\lambda < 1$ , presumed adaptive response to some external pressures) in this case which may also be reflected by the almost complete lack of resolution of species within the western clade (Fig. 4). Nevertheless, longer paths and branches contribute more to 1Cx evolution (accelerated evolution as time progresses). In contrast, for the eastern clade, the maximum

likelihood method revealed that topology and branch lengths accurately describe the constant variance random-walk model A or B. However, using the MCMC method, accelerated rate of evolution in long branches is indicated.

Studies using a phylogenetic approach to evaluate the directionality of genome size evolution revealed both DNA decrease and increase and often different tendencies in genome size diversification in different phylogenetic lineages (e.g. Wendel *et al.*, 2002; Jakob *et al.*, 2004; Caetano-Anollés, 2005; Price *et al.*, 2005). Ancient genome size enlargement followed by more or less drastic parallel reduction in the main phylogenetic lineages was found in *Festuca* (Šmarda *et al.*, 2008).

#### Genome size and ecogeographic features

In order to identify further components of genome size variation for *Hieracium*, correlations were tested with a number of other factors. A significant, positive correlation was found between 1Cx value and longitude of sampling sites, both in the complete set of accessions and in a restricted set without species with large distribution areas for which the results depend strongly on sampling (Fig. 6). Restriction of these analyses to accessions analysed by molecular data showed that these correlations were even stronger when accessions of ambiguous origin were excluded (Fig. 7A, B). As no significant correlation was found within either of the two clades (‘western’ and ‘eastern’; Fig. 7C, D), it can be concluded that the basal divergence into two phylogenetic lineages is most likely the determining factor of genome size variation in *Hieracium* (or vice versa) rather than longitudinal distribution.

In *Hieracium* subgenus *Pilosella* (Suda *et al.*, 2007), a longitudinal component of genome size distribution was also found: the highest 1Cx values were detected in *H. echioides*, a species distributed mainly in steppic habitats in Asia and eastern Europe (and well differentiated from the remaining species by the absence of a basal leaf rosette at flowering time). However, no comparison with species relationships is available. An opposite relationship between genome size distribution and geographic ranges has been observed in the genus *Cirsium* (Bureš *et al.*, 2004). At the intraspecific level, a geographically correlated variation in DNA content with an increase towards the east has been documented, e.g. in several taxa of *Koeleria* (Pecinka *et al.*, 2006), but no correlation was found in *Sesleria albicans* (Lysák *et al.*, 2000). Thus, there does not seem to be a general trend in genome size variation in relation to longitude. The same holds for a relationship between genome size and latitude, where positive, negative, or non-significant correlations with genome size have been found (reviewed in Knight *et al.*, 2005).

Altitude was also studied. The correlation between this parameter and genome size has been a matter of debate in the past years, and divergent results have been obtained. In subgenus *Hieracium*, genome size variation does not depend on altitude. However, the present data had to be based on altitudes of the sampling sites and are therefore biased by this selection, especially in species occurring across a large range of different altitudes (e.g. *H. bifidum*). Similarly, no correlation between genome size and altitude in Asteraceae was found in *Cirsium*



(Bureš *et al.*, 2004) or *Artemisia* and *Tripleurospermum* (García *et al.*, 2004, 2005). In other families, no or even a negative correlation between genome size and altitude was found by Creber *et al.* (1994), Reeves *et al.* (1998) and Vilhar *et al.* (2002) (all on intraspecific variation in *Dactylis glomerata*). On the other hand, an increase in genome size with higher altitude was found in *Centaurea s.s.* (Bancheva and Greilhuber, 2006) and in some groups of grasses (Bennett, 1976; Laurie and Bennett, 1985; Rayburn and Auger, 1990). Thus, altitudinal genome size variation also seems to be dependent on the particular plant group analysed (Knight *et al.*, 2005). In *Hieracium*, there are specifically montane or alpine taxa, but they are distributed in the Pyrenees, the Alps or the Balkan mountains, i.e. in western, central and eastern European regions, and therefore, the geographic origin of the species strongly dominates any altitudinal genome size variation that might be found.

Also no correlation was found between Cx values and two selected approximate ecological parameters published for German plant species by Ellenberg *et al.* (1992), namely light and temperature. However, the use of Ellenberg's indicator values for *Hieracium* is ambiguous. Many *Hieracium* species have large ecological amplitudes and therefore these approximate values could indeed be useful indicators, but these values are only available for central European species, and therefore species confined to either western or eastern Europe had to be excluded.

Many published correlations between ecogeographical factors (and others such as life form, etc.) and genome size must be interpreted with caution, as phylogenetic information is lacking. Albach and Greilhuber (2004) showed quite different correlations between selected factors (habitat, life history and breeding system) and genome size and DNA C-values in the genus *Veronica* if using a simple statistical test without phylogenetic information or more sophisticated methods incorporating phylogeny (independent contrast, GLSM). Furthermore, the use of linear regression analysis could obscure patterns in relationships if they are not linear. Knight and Ackerly (2002), Knight *et al.* (2005) and Beaulieu *et al.* (2007) used quantile regression analysis and showed that, although the relationship between genome size and a particular parameter was poor for species with small genomes, as genome size increased, the relationship became increasingly significant.

### Conclusions

Genome size variation in *Hieracium* subgenus *Hieracium* is congruent with the phylogenetic pattern, with species of putative western European origin having significantly lower genome size than those of eastern European origin. Consequently, a significant longitudinal correlation can also be inferred. Separate analyses of closely related species (i.e. within each phylogenetic clade) clearly show that despite considerably overlapping scales, no significant geographic component is apparent. Thus, in *Hieracium*, any correlation of genome size with longitude, and with other ecogeographic variables such as latitude, altitude, light and temperature, is outweighed by the basal phylogenetic divergence into species of eastern or western European origin.

### SUPPLEMENTARY DATA

Supplementary data are available online at [www.aob.oxfordjournals.org/](http://www.aob.oxfordjournals.org/) and provide detailed information about the sample localities.

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