Chromosome numbers in *Hieracium* and *Pilosella* species (Asteraceae) from the Iberian Peninsula and the Balearic Islands

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Mitotic chromosome numbers are reported from 16 *Hieracium* and two *Pilosella* species from the Iberian Peninsula and the Balearic Islands that are little known cytogenetically. The chromosome numbers of *Hieracium aragonense* Scheele (2n = 27, 36), *H. compositum* Lapeyr. (2n = 27), *H. murcandidum* G. Mateo (2n = 27), *H. spathulatum* Scheele (2n = 27), *H. securae* Mateo (2n = 27), *H. teruelanum* Mateo (2n = 27), *H. valentinum* Pau (2n = 27), *Pilosella pseudovahlii* (De Retz) Mateo (2n = 18), and *P. tardans* (Peter) Sójak (2n = 36) were determined for the first time. New cytotypes were detected in *H. cordifolium* Lapeyr. (2n = 27) and *H. loscosianum* Scheele (2n = 36). The karyotype of the studied species was similar in overall morphology and comprised metacentric and submetacentric chromosomes; secondary constrictions (up to two) were detected in the chromosome complements of some species. © 2007 The Linnean Society of London, Botanical Journal of the Linnean Society, 2007, 153, 311–320.

ADDITIONAL KEYWORDS: apomicts – endemic species – intraspecific polyploidy – Mediterranean flora.

INTRODUCTION

*Hieracium* L. and the closely related *Pilosella* Hill. (usually included within the former as a subgenus) are taxonomically intricate groups comprising mainly herbs distributed in the Northern Hemisphere. The breeding system in hawkweeds is related to a large extent to the ploidy level. Thus, seeds of sexual origin are produced mainly by diploid plants (2n = 18; but see Gadella, 1987). Moreover, asexual seed production through diplospory is widespread and is usually linked to polyploidy. Facultative apomixis has been indicated to be present in some polyploid species (Chapman & Bicknell, 2000; Houlston & Chapman, 2001), and its occurrence in *Pilosella* and *Hieracium* could be more widespread than is indicated by the few existing records.

The number of polyploid species (mainly triploid) recorded in *Hieracium* and *Pilosella* clearly outweighs the number of diploid sexual species so far known. Of course, this could be a result of the common practice of taxonomically splitting and formally naming apomict variants that differ by few morphological features (microspecies). However, this ploidal asymmetry could have a biological basis, as it has been suggested for a long time that agamospermous polyploid species have originated through reticulate evolution (Zahn, 1921–23). If this hypothesis is true, a large number of undetected diploids could have been involved in past hybridization events. Cytogenetic knowledge of *Hieracium* and *Pilosella* species is satisfactory in several regional territories in Europe, including Great Britain (Stace et al., 1995) and the central western Carpathians and surrounding areas (Chrték, Mráz & Severa, 2004). However, many Mediterranean species are unknown or barely known from a cytogenetic point of view. Given the presence of relict angiosperm lineages in three Mediterranean glacial refuges (the Iberian Peninsula, Italian Peninsula, and the Balkans; Taberlet et al., 1998), the search for diploid *Hieracium* and *Pilosella* cytotypes in these areas is strongly recommended.

About 120 species of *Hieracium* and 30 species of *Pilosella* are native to the Iberian Peninsula and the
Balearic Islands (G. Mateo, unpubl. data). Of these, about 40 are narrow endemics, restricted to these territories. Unfortunately, published chromosome counts in Iberian and Balearic species of *Hieracium* and *Pilosella* are few, with only about 20 species known karyologically (Martín, 1991, and references cited therein; Schuwerk, 1996). A knowledge of the ploidy level in these genera is imperative to assess their patterns of microevolution and diversification, and to substantiate the hypothesis that diploid species have found refuge in selected areas of the Mediterranean basin.

We have started a research project aimed at assessing the karyological diversity of the western Mediterranean taxa of *Hieracium* and *Pilosella*. In this work, the chromosome numbers are provided for 43 accessions of *Hieracium* from 16 species and of three samples of *Pilosella* belonging to two species.

**MATERIAL AND METHODS**

**PLANT MATERIAL**

Plants and fruits were collected from field populations in the Iberian Peninsula and Balearic Islands. In addition, mature fruits were selected from herbarium specimens collected between 1992 and 1995 and preserved at VAL (Herbarium of the Botanical Garden of Valencia University). The plants were cultivated at the Botanical Garden of Valencia University. Cypselas were either pretreated at low temperatures (0–4 °C) for 3–4 days to increase germination rates and germinated on moist filter paper in Petri dishes at room temperature, or germinated on solid agar in Petri dishes at a constant temperature of 20 °C and 12 h of white light daily. Voucher specimens are deposited at VAL.

**CHROMOSOME PREPARATION**

Root tips were pretreated with 0.05% colchicine solution at room temperature for 2–3 h, or with 0.002 M 8-hydroxyquinoline solution for 2 h at 4 °C and 2 h at room temperature. Other pretreatments have been reported (α-paradichlorobenzene, Chrtek, 1994; 0.002 M 8-hydroxyquinoline, Vogt & Oberprieler, 1994; distilled water at 0 °C, Pajarón, 1986), but did not increase the number of metaphases. Subsequently, the material was washed with distilled water, fixed in fresh Carnoy I solution overnight, and stored in 70% ethanol at 4 °C until use.

Meristems were hydrolysed for 5–10 min in 1 M HCl at 60 °C, washed, and stained in aceto-orcein for 4–6 h. Squashing of the stained tips was performed in a drop of 45% acetic acid, and preparations were sealed by mounting in Canada balsam.

**KARYOTYPE ANALYSIS**

Cells with well-spread metaphases were photographed with an Olympus Camedia C-2000-Z digital camera and processed with Adobe Photoshop 7.0. At least five well-spread metaphases of each accession were analysed to determine the chromosome number by direct observation and from the photomicrographs. For the estimated numerical parameters of the karyotypes, the lengths of the short (S) and long (L) arms of the chromosomes of each metaphase plate were measured from digital images using the processing image software ImageTool 5.0. For centromere position, the nomenclature of Levan, Fredga & Sandberg (1964) was followed.

**RESULTS**

*Hieracium amplexicaule* L.


Accessions of this widespread species showed two ploidy levels (Figs 1, 2). The chromosome complement of the diploid cytotype had three metacentric and six submetacentric pairs. In contrast, the triploid cytotype showed mainly submetacentric chromosomes.

**Figures 1–8.** Mitotic metaphase plates of *Hieracium* species. Fig. 1. *H. amplexicaule* (Lleida, Josa del Cadi), 2n = 18. Fig. 2. *H. amplexicaule* (Soria, Vinuesa), 2n = 27. Fig. 3. *H. aragonense* (Cuenca, Tragacete), 2n = 27. Fig. 4. *H. aragonense* (Cuenca, Zafrrilla), 2n = 36. Fig. 5. *H. compositum* (Castellón, Villamalur), 2n = 27. Fig. 6. *H. cordifolium* (Barcelona, Montseny), 2n = 27. Fig. 7. Tetraploid cytotype of *H. glaucinum* (Teruel, Noguera de Albarracín), 2n = 36. Fig. 8. Triploid cytotype of *H. murcandidum* (Andorra, Canillo), 2n = 27. Scale bars, 10 μm.
The chromosomes were small in length (2–3 µm) in both cytotypes, and two secondary constrictions were detected in a submetacentric chromosome pair. Most of the determined ploidy levels for this species were triploid (for example, Galland, 1988; Schuhwerk & Lippert, 1998) or tetraploid (Gadella & Kliphuis, 1968). Previously, only the triploid cytotype was known from the Iberian Peninsula (Cueto & Blanca, 1987).

**Hieracium aragonense Scheele**


Balearic Islands. Mallorca, Puig Major de Son Torrella (31SDS20), 1400 m, ix.2004, J.A. Rosselló. No voucher. Living plant cultivated at the Botanical Garden of Valencia. 2n = 36.

The triploid level was detected in continental accessions of this species, which is restricted to the north-east mountains of the Iberian Peninsula and the Balearic Islands (Fig. 3). However, five individuals from Zafrilla, obtained from germinating cypselas, were tetraploid (Fig. 4). Metaphase chromosomes were small in size in both cytotypes (2–3 µm). The triploid cytotype showed a karyotype of nine metacentric and 18 submetacentric chromosomes, whereas the tetraploid plants from the Iberian Peninsula showed 12 metacentric and six submetacentric pairs. In contrast, the karyotype of the Balearic accession differed slightly and showed six metacentric and 12 submetacentric pairs. Secondary constrictions were not observed in any studied individual. To our knowledge, this is the first chromosome count for this species, which is restricted to the Pyrenees and Iberian mountains.

**Hieracium cordifolium La Peyr.**


Diploid and triploid cytotypes were determined in this south-western European species (Fig. 6). Metaphase chromosomes were small (2–3 µm) in both cytotypes. The karyotype of the diploid plants showed six metacentric and three submetacentric chromosome pairs, and the triploid accession showed a similar karyotype with 18 metacentric and nine submetacentric chromosomes. Previously, only the diploid cytotype was known for this species (Schuhwerk & Lippert, 1998).

**Hieracium elisaeanum Arv.-Touv. ex Willk.**


The studied accession of this restricted Iberian species was triploid, with nine metacentric and 18 submetacentric chromosomes. Previous counts, with similar karyotype morphology, were reported by Blanca & Cueto (1984) from southern Iberian populations.

**Hieracium glaucinum Jord.**


Most of the analysed accessions were triploids, but a single sample was tetraploid (Fig. 7). The metaphase chromosome length ranged from 4 to 8 µm. Triploid accessions showed a karyotype with 12 metacentric and 15 submetacentric chromosomes. A secondary constriction was observed in a submetacentric chromosome pair. The tetraploid accession showed eight metacentric and ten submetacentric pairs. Satellites were observed in two putatively nonhomologous chromosomes (one metacentric and one submetacentric). Diploid (Natarajan, 1981, 1988), triploid (Mills & Stace, 1974; Natarajan, 1981, 1988), and tetraploid cytotypes (Natarajan, 1981, 1988) have been recorded in the H. glaucinum aggregate.

HIERACIUM LANIFERUM Cav.

The karyotype of this species showed small chromosomes (1.5–3 µm), with seven metacentric and two submetacentric pairs. No secondary constrictions were observed. Our results agree with previous chromosome counts showing the diploid level for this endemic species of north-east Spain (Merxmüller, 1975).

HIERACIUM LOSCOSIANUM Scheele
Balearic Islands. Mallorca, Puig Major de Son Torrella (31SDE80), 1400 m, ix.2004, J.A. Rosselló. No voucher. Living plant cultivated at the Botanical Garden of Valencia. 2n = 27.

Triploid and tetraploid cytotypes were detected in this south-west European species. The metaphase chromosome size ranged from 1.5 to 6 µm. Karyotype heterogeneity was observed in the studied samples. Thus, the karyotype of the triploid accessions from the Iberian Peninsula showed 15 metacentric and 12 submetacentric chromosomes, whereas the Balearic accession showed six metacentric and 21 submetacentric chromosomes. The tetraploid cytotype showed a karyotype with 20 metacentric and 16 submetacentric chromosomes. Secondary constrictions were not observed in either cytotype. The triploid cytotype has been reported previously by Luque & Díaz Lifante (1991) in Iberian samples, but the tetraploid level was reported for the first time here.

HIERACIUM MURCANDIDUM Mateo

This narrowly distributed species (endemic to the northern Iberian Peninsula) showed a triploid cytotype of medium-length chromosomes (4.5–8 µm), with 15 metacentric and 12 submetacentric chromosomes (Fig. 8). Secondary constrictions were visible in a submetacentric chromosome pair. This is the first karyological report for this species.

HIERACIUM MURORUM L.

All studied Iberian populations had a triploid cytotype. The chromosome complement ranged from 3.5 to 8 µm and showed nine metacentric and 18 submetacentric chromosomes. Secondary constrictions were visible in a submetacentric chromosome pair. Triploid and tetraploid cytotypes have been reported for this widespread species (for example, Gadella & Kliphuis, 1972; Lavrenko, Serditov & Ulle, 1990), but no previous karyological knowledge from Iberian plants was available.

HIERACIUM PRENANTHOIDES Vill.
The triploid cytotype was determined for the studied individuals of this widespread European species. The karyotype of the triploid accession showed medium chromosomes (3–6.5 µm), with nine metacentric and 18 submetacentric chromosomes. Diploid, triploid, and tetraploid cytotypes are known for this species (Christoff & Popoff, 1983; Favarger, 1969; Nazarova, 1984; Lavrenko et al., 1990), but this is the first karyological report for Iberian populations.

HIERACIUM SCHMIDTII TAUSCH


We detected two ploidy levels in the studied accesions. The karyotype in both cytotypes of this widespread European species showed small chromosomes (1.5–3 µm), mainly metacentric. Our results agree with previous chromosome counts reporting triploid and tetraploid populations (Gentscheff, 1937; Moore, 1982; Mesícek & Jarolímová, 1992).

HIERACIUM SEGURAE MATEO


This narrowly distributed north-eastern Iberian species showed small chromosomes (1.5–3 µm), with a karyotype of 18 metacentric and nine submetacentric chromosomes (Fig. 9). No secondary constrictions were visible in our material. This is the first karyological record for the species.

HIERACIUM SPATHULATUM SCHEELE


The karyotype of this Iberian endemic species showed small chromosomes (2–3 µm), with 18 metacentrics and nine submetacentrics (Fig. 10). No secondary constrictions were observed. This is the first karyological report for the species.

HIERACIUM TERUELANUM MATEO


The two populations of this Iberian endemic species showed a triploid cytotype (Fig. 11). The karyotype of both accessions had 18 metacentric and nine submetacentric small chromosomes (1.5–3 µm). No secondary constrictions were observed. This is the first karyological record for the species.

HIERACIUM VALENTINUM PAU


The karyotype of this Iberian endemic species showed small chromosomes (1.5–3.5 µm), mainly metacentric (Fig. 12). No visible secondary constrictions were observed. This is the first chromosome count reported for this species.

PILOSELLA PSEUDOVALLII (DE RETZ) MATEO


The chromosome number of the studied accessions of this restricted Iberian species was diploid (Fig. 13). Metaphase plates showed medium chromosomes ranging in size from 3 to 5.5 µm. The karyotype was composed of three metacentric and six submetacentric chromosome pairs. A submetacentric chromosome pair showed a visible satellite. This is the first chromosome count reported for this species. P. vahlii, which is closely related to P. pseudovallii, also shows the same diploid chromosome number (Merxmüller, 1975).

PILOSELLA TARDANS (PETER) SÓJAK


This taxon showed medium chromosomes (2–5 µm), with metacentric and some submetacentric pairs (Fig. 14). This is the first tetraploid count reported for this species.
Figures 9–14. Mitotic metaphase plates of *Hieracium* and *Pilosella* species. Fig. 9. *H. segurae* (Zaragoza, Jaraba), $2n = 27$. Fig. 10. *H. spathulatum* (Valencia, Buñol), $2n = 27$. Fig. 11. *H. teruelanum* (Guadalajara, Poveda de la Sierra), $2n = 27$. Fig. 12. *H. valentinum* (Zaragoza, Calamarza), $2n = 27$. Fig. 13. *P. pseudovahlii* (Teruel, Bronchales), $2n = 18$. Fig. 14. *P. tardans* (Teruel, Valdelinares), $2n = 36$. Scale bars, 10 µm.
DISCUSSION

*Hieracium* comprises an immense taxonomic complex with a basic chromosome number of \(x = 9\) (Chrték, 1994). Reported ploidy levels have ranged from diploids \((2n = 18)\) to heptaploids \((2n = 63)\), although triploids \((2n = 27)\) and tetraploids \((2n = 36)\) are by far the most predominant cytotypes in the genus. Thus, triploid and tetraploid levels representing about 70–80% of the investigated taxa have been detected in the Balkan Peninsula, 90% in Central Europe, and 97% in the western Carpathians (Schuhwerk & Lippert, 1998, 1999).

The success of triploid species in this genus is intimately linked to their agamospermy mode of reproduction, as they are often more vigorous vegetatively than diploids or tetraploids, and the disadvantage of their sexual sterility is cancelled by their apomictic reproduction. The apomixis involves apospory (the formation and development of unreduced embryo sacs following the *Antennaria* type of diplospory; Nogler, 1984), or haploid parthenogenesis coupled with autonomous embryo and endosperm formation (Asker & Jerling, 1992). Further, facultative apomictic polyploids, acting as seed parents, can generate polyploid progeny of hybrid origin, and are capable of being pollen donors (involving reduced or unreduced gametes) to give rise to new polyploid hybrid combinations that, if viable, should perpetuate by apomictic reproduction (Krahulcová, Papousková & Krahulec, 2004; Morgan-Richards *et al.*, 2004).

Diploid species are very rare and have been reported mostly from south-west Europe, the eastern and southern Carpathians, the Balkan Peninsula, and in the Asian part of Russia (Chrték *et al.*, 2004).

Our results agree with these observations (Table 1), and show a prevalence of triploid (76.2%) over diploid (14.3%) and tetraploid (9.5%) populations. The finding of tetraploid cytotypes in four species (*H. schmidtii*, *H. glaucinum*, *H. loscosianum*, and *H. aragonense*) is of cyto-biogeographical interest, as only diploid and triploid cytotypes were previously known to occur in the Iberian Peninsula (Mráz & Szelał, 2004).

Although nine of the 16 studied *Hieracium* species were exclusively triploid and only one (*H. laniferum*) was represented by diploid populations, six species showed different ploidy levels in the area. Intraspecific polymorphism concerning the ploidy level has been reported previously for geographically widespread *Hieracium* species, and different cytotypes have even been reported to coexist within populations of a species (Chrték, 1994; Rotreková, 2004).

Most of the ploidy levels reported in these widespread and karyologically differentiated species, namely *H. amplexicaule* (triploid and tetraploid), *H. schmidtii* (triploid and tetraploid), *H. glaucinum* (diploid, triploid, and tetraploid), *H. mororum* (triploid and tetraploid), and *H. prenanthoides* (diploid, triploid, and tetraploid), were found in the accessions studied here. Only the tetraploid cytotypes of *H. amplexicaule* and *H. mororum* and the diploid and tetraploid cytotypes of *H. prenanthoides* are apparently missing, or remain undetected, in the Iberian Peninsula.

Interestingly, the previously unknown diploid cytotype of *H. amplexicaule*, which is otherwise polyploid, was detected in a single Pyrenean locality. As this diploid population is assumed to be an ancestor of triploid and tetraploid *H. amplexicaule*, its occurrence in the Iberian Peninsula gives additional support, together with the presence of the diploids *H. cordatum* Scheele (Auquier & Renard, 1979), *H. cordifolium* (Schuhwerk & Lippert, 1998; this study), *H. hispanicum* Arv.-Touv. (Merxmüller, 1975), *H. laniferum* (Merxmüller, 1975; this study), and *H. vayredianum* Arv.-Touv. (Auquier & Renard, 1979), for the location in this area of a refuge for diploid hawkweeds.

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<tr>
<th>Taxon</th>
<th>No. of accessions at ploidy level</th>
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<td><em>H. amplexicaule</em> L.</td>
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<td>Sect. Oreadea Fr.</td>
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<td><em>H. schmidtii</em> Tausch</td>
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<td><em>H. glaucinum</em> Jord.</td>
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<td><em>H. mororum</em> L.</td>
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<td><em>H. prenanthoides</em> Vill.</td>
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<td>Sect. Italica Fr.</td>
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<td>Sect. Cerintheoidea Koch</td>
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<td><em>H. laniferum</em> Cav.</td>
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<td><em>H. segurae</em> Mateo</td>
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<td>Pilosella</td>
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<td><em>P. pseudovahlii</em> (De Retz) Mateo</td>
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<td><em>P. tardans</em> (Peter) Sójak</td>
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Hieracium amplexicaule has traditionally been considered as a basic species (species principales; Zahn, 1921–23), in contrast with the intermediate species (species intermediae; Zahn, 1921–23) that are believed to be of hybrid origin. It is a key species belonging to sect. Amplexicaulia Fr. that has been proposed to be one of the parental species involved in the origin of many restricted Iberian Hieracium belonging to sect.


It is unknown whether this large number of Iberian endemic microspecies has originated through somatic mutations of polyploid H. amplexicaule or through hybridization events between this species and other extant sexual or facultative apomictic species. The presence of diploid H. amplexicaule in the Iberian Peninsula is compatible with the hypothesis that this cytotype could be involved in the origin of some of these local Iberian species.

Our results suggest that cytotype variation is not necessarily greater in widely distributed taxa of Hieracium, because two cytotypes were detected in H. loscosianum (triploid and tetraploid), H. cordifolium (diploid and triploid), and H. aragonense (triploid and tetraploid), three restricted Iberian species. In H. aragonense, we found two mixed ploidy levels in seedlings derived from a single capitulum. Four individuals were triploid and five were tetraploid. On a theoretical basis, the tetraploid progeny could be the result of a hybridization event between a tetraploid and a diploid plant. The tetraploid seedlings, in turn, could be produced by a fertile unreduced triploid gamete from a triploid individual, which fused with a haploid gamete from a diploid or triploid plant. As the fruits were selected from herbarium specimens preserved at VAL, basic information concerning the ploidy level of the mother plant and the presence of additional congeneric species growing in the locality (if any) is entirely lacking. We speculate on the basis of limited and circumstantial evidence that the triploid progeny originated from a 3x H. aragonense through apomixis, and that the tetraploids arose from the fusion of an unreduced female gamete (3x) and a reduced (1x) pollen grain from individuals of the same or another triploid species. We believe that the latter is the more probable hypothesis for the following reasons: (1) tetraploid cytotypes have rarely been detected in Iberian Hieracium species and only the Balearic species have shown uniformly tetraploid populations (Table 1); (2) diploid species are not known in Zafrilla and the surrounding areas; and (3) facultative apomicts can serve as pollen donors and as seed parents (Skalinska, 1968, 1971; Chapman & Bicknell, 2000; Morgan-Richards et al., 2004).

The karyotypes of the studied Hieracium species are rather similar and differ only in their metacentric/submetacentric chromosome ratios and the number of satellite chromosomes. Unfortunately, no valuable information on relationships or evolutionary trends can be deduced from these observations. In addition, no substantial knowledge of evolutionary interest has been inferred in other studies (for example, Chrtek et al., 2004) using conventional karyological methods, other than the chromosome number. This is by contrast with the related Pilosella, where a single long chromosome marker, occurring in a single copy in the chromosome sets in apomictic tetraploid and pentaploid members of some complexes (Krahulcová & Krahulec, 1999; Krahulec et al., 2004), has been used to infer useful evolutionary relationships.

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