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PCR-RFLP studies of non-coding chloroplast DNA in European *Hieracium* subgen. *Pilosella*

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As part of an ongoing project about the biodiversity, genetic structure and differentiation of *Hieracium*, subgenus *Pilosella*, the relationships of the species occurring in the borderland of Saxony (Germany), the Czech Republic and Poland have been investigated by PCR-RFLP analysis of the chloroplast *trnT-trnF* spacer region. As outgroups, species of *Hieracium* subgen. *Hieracium* and *Chionoracium* as well as of the closely related genus *Andryala* were included.

The target region was PCR-amplified using the universal primers described by TABERLET et al. (1991. *Plant Mol. Biol.* 17: 1105-1109). Amplification products were submitted to restriction enzyme digestions. Out of 15 enzymes tested, only *AluI*, *MboI*, *BclI*, *BglII* and *EcoRI* yielded differences between the species and were therefore used for further analysis.

No changes in restriction sites, but only length variations were observed. Fragment sizes were determined. Identical haplotypes were observed for representatives of the following species: *H. cymosum*/*H. glomeratum*, *H. pilosella*/*H. piloselliflorum*, *H. bauhini*/*H. leptophyton*, *H. lactucella*/*H. floribundum*/*H. aurantiacum*, the two subspecies of *H. caespitosum* (*madarum* and *caespitosum*), *H. piloselloides* ssp. *obscurum*/*H. echioides*, *H. sabaudum*/*H. umbellatum*, *H. fritzei* (*schneiderianum*)/*H. alpinum*. In three cases (*H. glomeratum*, *H. piloselliflorum*, *H. leptophyton*), the species have previously been considered as presumed early generation hybrids based on their morphology, occurrence, DNA content and ploidy levels. Because of the maternal inheritance of the chloroplast DNA, evidence for the seed parent is now suggested. The result is in accordance with the assumed modes of reproduction. *H. floribundum* – although sometimes being considered as a ‘main’ species in the sense of NÄGELI & PETER – was suggested to be a hybrid between *H. lactucella* and *H. caespitosum* (KRAHULCOVÁ, A. & KRAHULEC, F. 1999. *Preslia* 71: 217-234). Usually, the few sexual species (*H. pilosella* – tetraploid, *H. cymosum* and *H. lactucella* – diploid) served as the seed parent. In the case of *H. bauhini*, however, an unreduced chromosome set of this pentaploid apomict might have been pollinated by diploid (reduced) pollen of *H. pilosella*, yielding the heptaploid hybrid *H. leptophyton*. For the remaining haplotypes, no direct evidence from other sources is as yet available to explain the patterns. More data are needed to elucidate these relationships, and homology of the length variations has to be established by DNA sequencing.

Generally, the *Hieracium* species of all subgenera as well as of closely related *Andryala* showed only little sequence variation as judged by the missing RFLPs. Insertions/deletions seem to account for most variation as has also been shown recently for other Asteraceae of the *Lactuca* tribe (VIJVERBERG, K. & BACHMANN, K. 1999. *Amer. J. Bot.* 86: 1448-1463). However, most variation was found between *trnT-trnL* in our case, but not in the *trnL-trnF* part of the amplified fragment, as indicated by a restriction site map and partial DNA sequencing.

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