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Flow cytometry measurements of ploidy level in British *Hieracium* section *Foliosa* and section *Prenanthoidea* (Asteraceae)

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Abstract
Ploidy level has been estimated in all extant British members of *Hieracium* section *Foliosa* (Fr.) Arv.-Touv. and section *Prenanthoidea* W. D. J. Koch (Asteraceae) from seed samples using flow cytometry. *Hieracium bakerianum, H. drummondii, H. latobrigorum, H. maritimum, H. melanoglochin, H. reayense, H. reticulatiforme, H. strictiforme, H. subcrocatum, H. subumbellatiforme, H. tavense* and *H. prenanthoides* were all triploid.

Keywords: chromosome count; triploid.

Introduction
As work towards a revision of British *Hieracium* section *Foliosa* (Fr.) Arv.-Touv. and section *Prenanthoidea* W. D. J. Koch (Asteraceae) by T.C.G. Rich, we have collected seeds of all extant members of the two sections and determined the ploidy level using flow cytometry. The ploidy results are briefly summarised below; further details and analysis will be published elsewhere (Mráz *et al.*, in prep.).

Methods
Seeds were collected by T.C.G. Rich, Ian Green or Linda Robinson from plants in the wild in Britain in 2019, or in a few cases from material cultivated in Cardiff by T. Rich. Vouchers were verified by D. McCosh and are deposited in **PRC** or **herb. T. Rich**.

The flow cytometry method is set out in full in Mráz & Zdvořák (2019) and is briefly summarised below. Batches of five *Hieracium* seeds were co-chopped with leaves of *Bellis perennis* as an internal standard. 4′,6-diamino-2-phenylindole (DAPI) was used as a fluorescent stain. A Partec Cyflow instrument equipped with a HBO lamp was used for ploidy level analysis. Histograms were accumulated at a flow rate of *c.*10–30 particles s^{-1} for a total count of 3000-6000 particles. The ploidy level of embryos was inferred as the relative position of the sample G1 peaks (corresponding to embryos) relative to that of the G1 peak of *Bellis* internal standard, which had previously been calibrated against *Hieracium* plants of known ploidy level. The coefficients of variation of fluorescent intensities (=peaks) of measured samples varied between 1.09% and 6.14 %, with a mean of 3.29 %, and those of internal standard varied between 1.57 % and 4.66%, with a mean of 3.2%. Between 5 and

45 seeds of each species were analysed (Table 1), i.e. from one to nine analyses were done per accession. Because of obligate sexuality in diploids, obligate or almost obligate apomixis in polyploids, and extremely rare incidence of between-ploidy gene flow, the ploidy of maternal plant can be easily deduced from the ploidy of seed progeny it produces (Mráz & Zdvořák, 2019).

Results

The estimated ploidy levels of 11 species of section *Foliosa* and one species of section *Prenanthoidea* are shown in Table 1; all ploidy levels were found to be triploid.

Discussion

These are the first estimates of ploidy level for nine of the section *Foliosa* species in Britain. The only previous two chromosome counts for the section *Foliosa* are 2n=27 (3x triploid) for *H. subcrocatum* from Clova (v.c.90) (Morton, 1974) and an unlocalised British count of 2n=27 (3x triploid) by R. A. Finch for *H. latobrigorum* (Moore, 1982). In addition, Tyler & Jönsson (2009) using flow cytometry ascertained a triploid level for one section *Foliosa* accession from Scandinavia. Our estimates of ploidy level are thus in agreement with these previous counts / flow cytometric estimates.

There are no previous estimates of ploidy level for *H. prenanthoides* s.l. from Britain, and the four populations estimated are all triploid. There are many counts for *H. prenanthoides* s.l. from Europe; diploids (2n=18) occur solely in the SW Alps in France and Italy and they belong to *H. prenanthoides* Vill. (s.str.), whereas triploids (2n=27) are widespread in Europe and the Carpathians, and tetraploids (2n=36) have been recorded from Central Europe and Iceland (Chrtek et al., 2004, 2007, 2020; Ilnicki & Szeląg, 2011; Kocián, 2013; Mráz & Zdvořák 2019). The British triploids thus belong to the most widespread cytotype.

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Table 1. Ploidy levels in extant British members of *Hieracium* section *Foliosa* and section *Prenanthoidea.* Nomenclature follows Sell & Murrell (2006). The number after slash is the relative position (averaged value if several samples per accessions were run) of the G1 peak of endosperm relative to the G1 peak of the internal standard *Bellis perennis.*

Species	Locality	Ploidy/mean (embryo/standard)	No. seeds analysed	T.C.G. Rich's collecting number
H. bakerianum Pugsley	above High Force (v.c.65)	3x / 3.24	5	2019-169
H. drummondii Pugsley	Crinan (v.c.101)	3x / 3.24	20	2019-106
H. latobrigorum (Zahn) Roffey	Ormsary Water (v.c.101)	3x / 3.21	20	2019-105
H. latobrigorum (Zahn) Roffey	Grandtully (v.c.88)	3x / 3.27	20	2019-116
H. maritimum (F. Hanb.) F. Hanb.	Melvich (v.c.109)	3x / 3.31	45	2019-061
H. maritimum (F. Hanb.) F. Hanb.	Crear Burn (v.c.101)	3x / 3.36	25	2019-102
H. melanoglochin (E.F. Linton) P.D. Sell	Acharn, Killin (v.c.88)	3x / 3.21	20	2019-109
H. reayense (Pugsley) P.D. Sell	Invervar (v.c.88)	3x / 3.24	20	2019-113
H. reayense (Pugsley) P.D. Sell	Sandside, Reay (v.c.109)	3x / 3.27	20	2019-137
H. reticulatiforme P.D. Sell	Nant y Llyn (v.c.42)	3x / 3.15	20	2019-099
H. strictiforme (Zahn) Roffey	Loch Sgioport (v.c.101)	3x / 3.25	30	2019-172
H. strictiforme (Zahn) Roffey	Glenernie, Divie (v.c.95)	3x / 3.27	20	2019-130
H. subcrocatum (E.F. Linton) Roffey	Cwm-porth (v.c.42)	3x / 3.15	20	2019-067
H. subcrocatum (E.F. Linton) Roffey	Glenernie, A940 (v.c.95)	3x / 3.27	20	2019-131
H. subumbellatiforme (Zahn) Roffey	Boat of Garten (v.c.95)	3x / 3.27	20	2019-129
H. subumbellatiforme (Zahn) Roffey	Dowally, Roar (v.c.89)	3x / 3.29	20	2019-159
H. tavense (Ley) Ley	Nant-y-Llyn (v.c.42)	3x / 3.27	10	2019-160
H. prenanthoides Vill. s.l.	Tarren-yr-Esgob (v.c.42)	3x / 2.97	20	2019-064
H. prenanthoides Vill. s.l.	Braemar (v.c.92)	3x / 3.01	10	2019-065
H. prenanthoides Vill. s.l.	Invervar (v.c.88)	3x / 3.06	5	2019-112
H. prenanthoides Vill. s.l.	Auchallater, Braemar (v.c.92)	3x / 3.09	20	2019-123

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