

# Nuclear DNA content and genetic relationships based on AFLP data in the genus *Helleborus*

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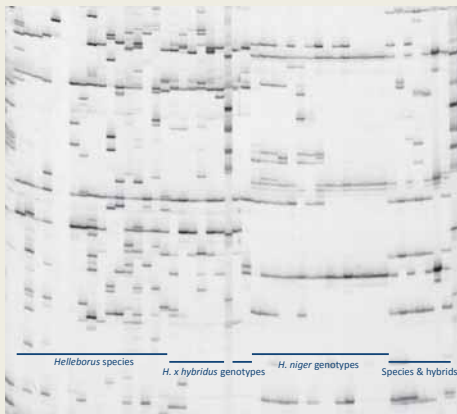
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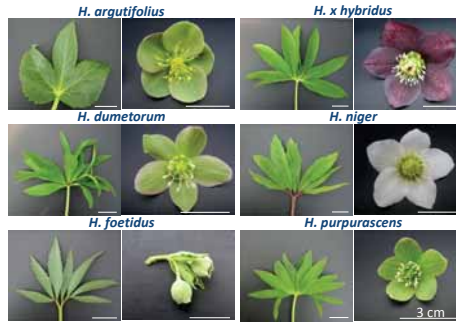
## Background

*Helleborus* is a genus of herbaceous perennials belonging to the family *Ranunculaceae*. Within this genus six sections with a total of 22 species are found which are distributed to different parts of Europe as well as East Asia. The largest section *Helleborastrum* contains 16 species for which genetic relationships are still unclear.

In order to evaluate the genetic relationships within the genus *Helleborus*, species were analysed using multilocus and genome-wide distributed amplified fragment length polymorphism (AFLP) markers (Vos et al. 1995). Furthermore nuclear DNA contents were determined by flow cytometry.



Example of an AFLP banding pattern of different *Helleborus* species and genotypes.



Leaves and flowers of six different *Helleborus* species and hybrids.

## Plant material

### Outgroup

*Pulsatilla vulgaris* 'Violet'

### Section *Helleborastrum*

*H. abruzzicus*

*H. atrorubens*

*H. bocconei*

*H. croaticus*

*H. cyclophyllus*

*H. dumetorum*

*H. hercegovinus*

*H. istriacus*

*H. liguricus*

*H. multifidus*

*H. odorus*

*H. orientalis/x hybridus*

*H. purpurascens*

*H. torquatus*

*H. viridis*

### Section *Syncarpus*

*H. vesicarius*

### Section *Griphopus*

*H. foetidus*

### Section *Chenopus*

*H. argutifolius*

*H. lividus*

### Section *Helleborus*

*H. niger*

### Section *Dicarpon*

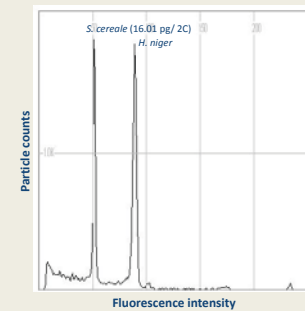
*H. thibetanus*

## Methods

### Flow cytometry

#### ► Determination of genome size

- Sample: 0.6 cm<sup>2</sup> of young leaf tissue
- Internal reference standard: *Secale cereale* subsp. *cereale* (16.01 pg/2C)
- Staining of isolated nuclei with propidium iodide
- Analysis with CyFlow Ploidy Analyser



	<i>S. cereale</i>	<i>H. niger</i>
Counts	4419	6271
Mean	53.6	91.3
CV	1.8	1.8

### AFLP analysis

#### ► DNA Extraction:

- CTAB method

#### ► AFLP reactions:

- *Pst*I and *Mse*I were used for restriction digestion
- Selective *Pst*I primers were labelled with fluorescein

#### ► Data analysis:

- Each reaction was repeated once. Fragment analysis was done by visual evaluation
- Dendrograms were calculated using PHYLIP 3.69 software

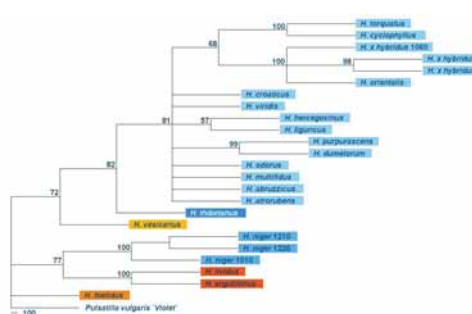
### Histogram of flow cytometric analysis of *H. niger*

- The internal reference standard was stained and measured simultaneously with the sample
- 2C DNA content of the sample = mean of sample peak divided / mean of standard peak \* the amount of DNA of the standard

## Nuclear DNA content of 21 *Helleborus* species

Section	Species	Nuclear DNA content [pg/2C]	
		Mean*	St dev*
Syncarpus	<i>H. vesicarius</i>	27.2	0.05
	<i>H. foetidus</i>	22.0	0.14
Griphopus	<i>H. foetidus</i>	18.3	0.5
	<i>H. lividus</i>	19.5	0.3
Helleborus	<i>H. niger</i>	26.8	0.21
	<i>H. abruzzicus</i>	27.0	0.44
	<i>H. atrorubens</i>	29.1	0.22
	<i>H. bocconei</i>	30.5	0.27
	<i>H. croaticus</i>	30.2	0.43
	<i>H. cyclophyllus</i>	28.6	0.22
	<i>H. dumetorum</i>	29.6	0.27
	<i>H. hercegovinus</i>	27.4	0.12
	<i>H. istriacus</i>	29.2	0.35
	<i>H. liguricus</i>	29.9	0.22
	<i>H. multifidus</i>	29.1	0.26
	<i>H. odorus</i>	28.6	0.04
	<i>H. orientalis/H. x hybridus</i>	28.7	0.29
	<i>H. purpurascens</i>	31.3	0.04
	<i>H. torquatus</i>	28.2	0.22
	<i>H. viridis</i>	28.7	0.37
Dicarpon	<i>H. thibetanus</i>	32.7	0.13

\*mean and standard deviation of three measurements of each plant



Majority rule consensus tree of 1000 Neighbor joining trees based on Nei and Li (1979) similarity indices computed from 1109 AFLP markers for 19 *Helleborus* species and *P. vulgaris* 'Violet' as outgroup. PHYLIP 3.69 for windows was used to generate the distance matrix for tree construction. Numbers above branches correspond to bootstrap values.

## Results

### Genome sizes

The nuclear DNA contents varied between 18 and 33 pg DNA/2C. Similar genome sizes were found for species within sections *Helleborus*, *Helleborastrum* and *Syncarpus*.

### Genetic relationships

In total 1109 polymorphic fragments were obtained. On average 111 markers were produced per primer pair.

The tree supports the division of the genus *Helleborus* into six sections. The two newly described species *H. abruzzicus* and *H. liguricus* were allocated to section *Helleborastrum*.