

Genetic relationships based on AFLP data in the genus *Helleborus*



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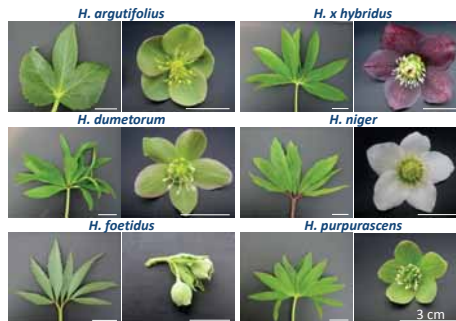
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Background

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 in 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* 19 out of 22 *Helleborus* species and were analysed using amplified fragment length polymorphisms (AFLP) (Vos et al. 1995).



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

Plants used in this study

- Pulsatilla vulgaris* 'Violet'
- H. atrorubens*
- H. croaticus*
- H. cyclophyllus*
- H. dumetorum*
- H. foetidus*
- H. multifidus*
- H. odorus*
- H. purpurascens*
- H. thibetanus*
- H. torquatus*
- H. vesicarius*
- H. viridis*
- H. hercegovinus*
- H. liguricus*
- H. abruzzicus*
- H. orientalis* / *H. x hybridus* (8 genotypes)
- H. niger* (13 genotypes)
- H. argutifolius*
- H. lividus*

AFLP analysis

DNA Extraction:

- ▶ CTAB method

Restriction digestion and ligation at once:

- ▶ 250 ng DNA
- ▶ Enzymes: six-base cutter (2.5 U) & four-base cutter (1 U)
- ▶ Adapters in concentrations of 2.5 pmol & 25 pmol
- ▶ 1 U T4-DNA-ligase
- ▶ Digestion at 37 °C for 3 h then ligation at 16 °C overnight

Preamplification:

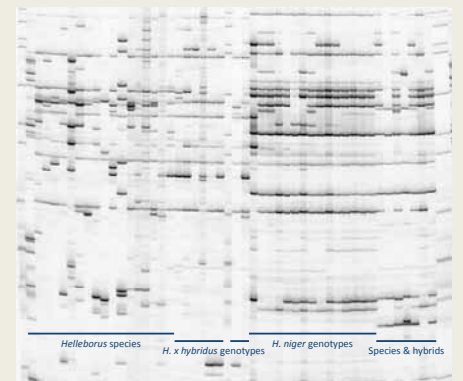
- ▶ 72 °C 2 min.
 - ▶ 94 °C 30 sec.
 - ▶ 60 °C 1 min.
 - ▶ 72 °C 2 min.
- 20x

Selective amplification:

- ▶ One primer was labelled with fluorescein at the 5'end
 - ▶ 10 AFLP primer combinations were used
 - ▶ PCR program:
 - ▶ 94 °C 2 min.
 - ▶ 94 °C 30 sec.
 - ▶ 65 - 1 °C/cycle 30 sec.
 - ▶ 72 °C 2 min.
 - ▶ 94 °C 30 sec.
 - ▶ 56 °C 30 sec.
 - ▶ 72 °C 2 min.
- 9x
23x

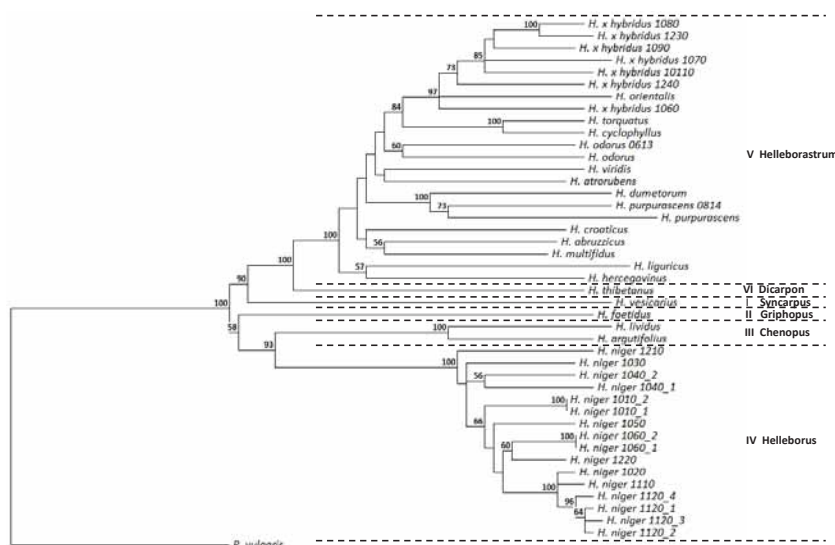
Electrophoresis and gel analysis:

- ▶ 5 % denaturing polyacrylamide gel
- ▶ Fragment detection was done with a fluorescence scanner



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

Each reaction was repeated once. Fragment analysis was done by visual evaluation.



Neighbor joining phenogram of 19 species and the outgroup based on an AFLP analysis with ten primer pair combinations. The Nei & Li (1979) index of similarity was used to generate the distance matrix for tree construction with treecron for windows. Horizontal branch lengths correspond to genetic distances. Numbers above branches correspond to bootstrap values.

Results

In total 1109 evaluable polymorphic bands were obtained. On average 111 bands were produced per primer pair.

Number of scored bands per primer combination.

Primer combination	Scored bands
1	144
2	143
3	122
4	99
5	140
6	70
7	55
8	85
9	96
10	155

The tree supports the division of the genus *Helleborus* into six sections as they are indicated in the dendrogram. The two new described species *H. abruzzicus* and *H. liguricus* belong to section V *Helleborastrum*.