

The use of microsatellites markers in molecular characteristics of hybrids between *Pulsatilla* species

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Introduction

Hybridization is widespread in plants. The genus *Pulsatilla* contains more than 30 species of herbaceous perennials. It is characterized by a relatively high hybridization rate. Spontaneous hybridization between *P. patens* and *P. pratensis* occurs in the natural habitats, most often in sites together with parental forms. Completed plastid genome provide molecular evidence for the hybrid origin of *Pulsatilla ×hackelii* [1]. *P. ×hackelii* is morphologically intermediate between the pure *P. patens* and *P. pratensis*. The distinction between hybrid individuals and pure *Pulsatilla* species requires a whole set of morphological features. In our study we present the genotypic characteristics of *P. ×hackelii* based on microsatellites markers developed for *P. vulgaris* [2]. Some of loci can crossamplified only for one species from *P. patens* and *P. pratensis*.



Pulsatilla ×hackelii leaf and flower. Fot. G. Łazarski

Methods and results

A hybrid specimen was found in the Świętokrzyskie Mountains, near Bocheniec village (southern Poland), in the vicinity of a forest road in a fresh mixed coniferous forest (*Quercus robur*-*Pinetum*). We amplified a set of ten microsatellites in two multiplex PCR reactions. From the ten analyzed loci, we successfully amplified eight. The analyzed microsatellites in population of three *Pulsatilla* species showed some species specific alleles. In case of the *P. ×hackelii* sample, we obtained PCR products in the case of five loci developed for *P. vulgaris*, but successfully crossamplified in *P. patens* and *P. pratensis*. Additionally, we obtained results for two loci, which crossamplified only for *P. patens* and only one for *P. pratensis* (Figure 1). Two of the analyzed loci were polymorphic (PV32 and PV65).

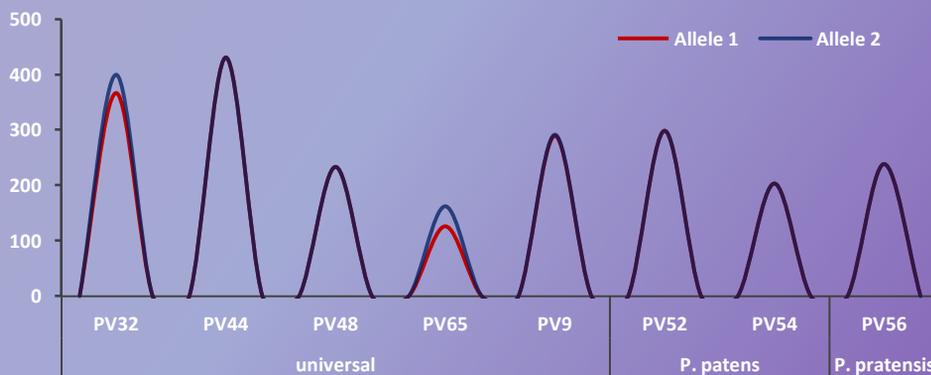


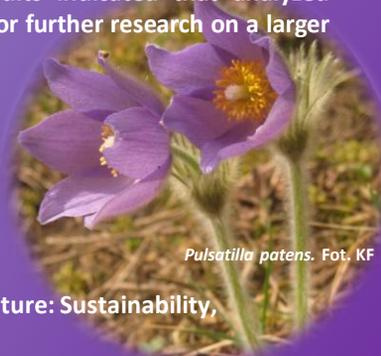
Figure 1. Genotypic characteristics of *Pulsatilla ×hackelii* based on microsatellites markers

Discussion and conclusions

Among the investigated markers, two were monomorphic (PV44 and PV48) and the remaining six were polymorphic. Markers PV32, PV65 and PV9 are highly polymorphic, what making them highly informative in population studies of pasque-flower species as *P. patens*, *P. pratensis* and *P. vernalis*. The obtained results indicated that analyzed microsatellites markers can be used for hybrids identification, although there is a need for further research on a larger number of hybridized individuals.

References

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2. Dileo, M.F.; Graf, R.; Holderegger, R.; Rico, Y.; Wagner, H.H. Highly polymorphic microsatellite markers in *Pulsatilla vulgaris* (Ranunculaceae) using next-generation sequencing. Applications in Plant Sciences 2015 3 (7): 1500031. doi:10.3732/apps.1500031.



Pulsatilla patens. Fot. KF