

Proceedings

The Use of Microsatellites Markers in Molecular Characteristics of Hybrids between *Pulsatilla* Species[†]

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Abstract: *Pulsatilla ×hackelii* Pohl. is the most common hybrid between *Pulsatilla patens* and *Pulsatilla pratensis*. It occurs in the natural environment, most often in sites together with parental forms. *Pulsatilla ×hackelii* is morphologically intermediate between the pure *Pulsatilla* species. Separation of hybrid individuals from pure *Pulsatilla* species requires a whole set of morphological features. Completed plastid genome provide molecular evidence for the hybrid origin of *Pulsatilla ×hackelii*. In our study we presents the – genotypic characteristics of *Pulsatilla ×hackelii* based on microsatellites markers developed for *Pulsatilla vulgaris*. A specimen of the hybrid was found in the Świętokrzyskie Mountains, near Bocheniec village, in the vicinity of a forest road in a fresh mixed coniferous forest (*Quercus roboris*-*Pinetum* (W.Mat. 1981) J.Mat. 1988). We amplified a set of 10 microsatellites in two multiplex PCR reactions. From the 10 analyzed loci, we successfully amplified eight. The analyzed microsatellites in population studies of three *Pulsatilla* species showed some species specific alleles. In case of the *Pulsatilla ×hackelii* sample, we obtained PCR products in the case of 5 loci developed for *P. vulgaris*, but successfully crossamplified in *P. patens* and *P. pratensis*. Additionally we obtained results for 2 loci, which crossamplified only for *Pulsatilla patens* and 1 only for *Pulsatilla pratensis*. Among the investigated markers, 2 were monomorphic and the remaining six were polymorphic, what making them highly informative in population studies for pasque-flower species as *Pulsatilla 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.

Keywords: *Pulsatilla*; microsatellites markers; natural hybrid; PCR

1. 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*.

2. Materials 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 roboris-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).

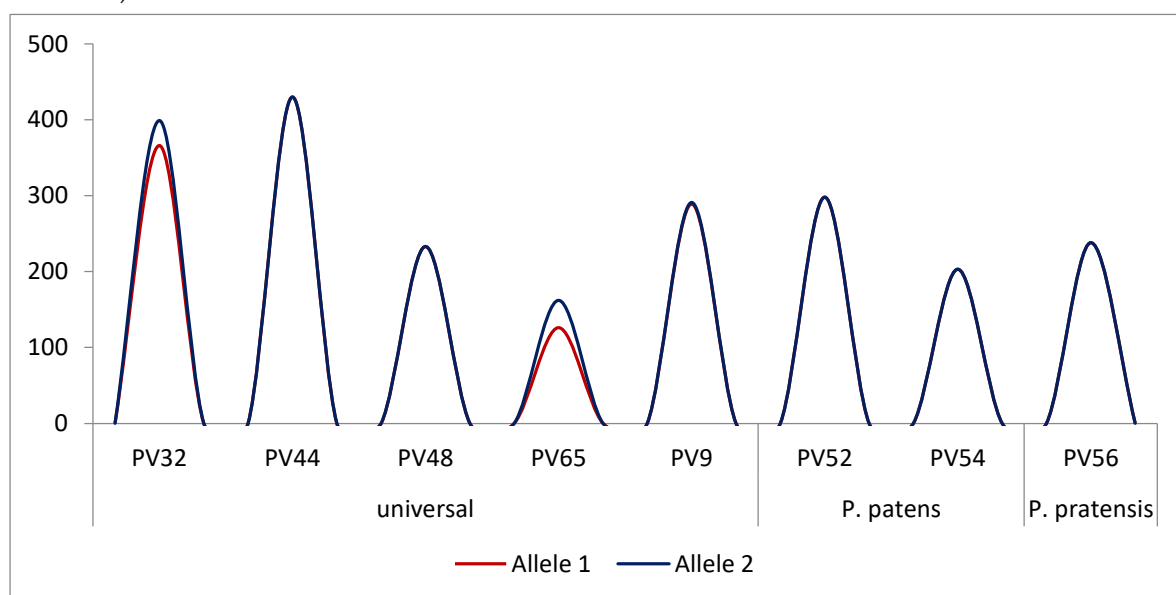


Figure 1. Genotypic characteristics of *Pulsatilla ×hackelii* based on microsatellites markers.

3. 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.

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