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HIGHLY POLYMORPHIC MICROSATELLITE MARKERS IN *PULSATILLA VULGARIS* (RANUNCULACEAE) USING NEXT-GENERATION SEQUENCING

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**Premise of the study:** We developed novel microsatellite markers for the perennial plant *Pulsatilla vulgaris* (Ranunculaceae) to investigate the effects of fragmentation on gene flow in this imperiled species.

**Methods and Results:** We identified microsatellites and developed primers based on 454 shotgun sequences. We identified 14 markers that were polymorphic and produced clean bands. Of these, eight could be analyzed as diploids. Genotyping of 97 individuals across two populations revealed these markers to be highly polymorphic with seven to 17 alleles per locus and observed heterozygosity from 0.41 to 0.83.

**Conclusions:** The markers are highly informative and will be used to test if the reintroduction of shepherding in southern Germany improves genetic connectivity among fragmented populations of *P. vulgaris*. The combination of diploid and tetraploid markers presented here will be useful in resolving the polyploidization history of this and related species.

**Key words:** 454 sequencing; microsatellites; *Pulsatilla vulgaris*; Ranunculaceae; simple sequence repeat (SSR) markers.

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*Pulsatilla vulgaris* Mill. (Ranunculaceae) is an early-flowering perennial herb of conservation concern and a flagship and specialist species of calcareous grasslands across central Europe, ranging from France in the south to Sweden at its northern limit (Wells and Barling, 1971; Pfeifer et al., 2002). Over the last century, *P. vulgaris* has witnessed rapid decline and local extirpation across its range, and is listed as “near threatened” by the International Union for Conservation of Nature (IUCN, 2014). Its decline is linked to the abandonment of traditional grazing practices, which has resulted in the severe loss and fragmentation of calcareous grasslands following afforestation (Butaye et al., 2005), and increased above-ground competition from coarse grasses (Walker and Pinches, 2011). Knowledge of the landscape-scale distribution of genetic variation is required to create effective management plans for fragmented populations, and evaluations of genetic diversity and inbreeding will allow the identification of populations that are at highest risk of extirpation. This, however, requires genetic markers with high resolution such as microsatellites. No such markers are yet available for *P. vulgaris*.

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These new microsatellite markers are highly informative and will be used to quantify gene flow across fragmented populations of *Pulsatilla vulgaris*. The markers were developed de novo, with 18 loci developed in *P. vulgaris* and one in *P. pratensis* that were selected for additional accuracy, as well as for compatibility with existing markers in the *Pulsatillaceae*. The results from these markers will be used to understand the genetic structure and dynamics of the species, and to inform conservation and management efforts. The study highlights the importance of developing new markers for species with limited genetic data, particularly those with fragmented populations.
populations of *P. vulgaris* in southern Germany. We will test whether the reintroduction of shepherding is a suitable conservation measure to improve genetic connectivity among populations of this species. The combination of diploid and tetraploid markers presented here will be useful in clarifying the polyploidization history of this and related species.

**LITERATURE CITED**


