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High congruence of intraspecific variability in floral scent and genetic patterns in *Gentianella bohemica* Skalický (Gentianaceae)

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ABSTRACT

Gentianella bohemica Skalický (Gentianaceae) is a critically endangered species endemic to the Bohemian Massif in the border region of Germany, Czechia and Austria. It consists of a restricted number of extremely scattered populations which are known to form distinct genetic groups. The objective of this work was to test for differences in the floral scent between *Gentianella bohemica* and *Gentianella germanica* and within these two species among populations, and to test for a correlation of scent and genetic similarity among the populations of *G. bohemica*. Floral scent was collected from the inflorescences/plants of eight flowering populations of *G. bohemica* and three populations of *G. germanica* using dynamic headspace methods, followed by GC/MS analyses. Both species emitted several aromatic and terpenoid compounds and multivariate analyses revealed differences in scent between the two species and within species among *G. bohemica* populations. Volatile components overlapped as expected for closely related species but floral scent was taxon-specific. Floral scent differentiation among *G. bohemica* populations was in high congruence with the genetic differentiation suggesting that scent differences among populations have a genetic basis and showing that scent is a suitable chemotaxonomic marker in this species.

1. Introduction

The Bohemian Gentian (*Gentianella bohemica* Skalický) was common in the tri-border region Germany/Czechia/Austria until the beginning of the 20th century (Königer et al., 2012). Since then the species has shown a dramatic decline, presumably due to land-use abandonment, intensified agriculture and afforestation. It now is a critically endangered species endemic to the Bohemian Massif and listed as priority species on Annex II of the Habitus Directive (IUCN, 2012). Today *G. bohemica* is reported to have only 60 populations left in the tri-border region that are most often isolated several kilometres from each other with frequent transfer of pollen or diaspores between populations being unlikely (Dolek et al., 2010; Königer et al., 2012). With the

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exception of some Czech and Austrian populations with thousands of individuals, populations are often very small (Brabec, 2005; Engleder, 2006; Königer et al., 2012) with considerable fluctuations (Dolek et al., 2010; Engleder, 2012; Königer et al., 2012). Research on the genetic structure within and among populations of *G. bohemica* in Bavaria, Bohemia and the Mühlviertel using amplified fragment length polymorphisms (AFLP) fingerprint data revealed that the German populations are genetically strongly isolated from each other and from the Czech and Austrian populations that show a lower differentiation (Königer et al., 2012). Reasons for the noticeable population differentiation might be strong physical barriers and historic constraints that facilitated isolation of the Bavarian populations and panmixis of the Austrian and Czech populations in the former empire of Habsburg (Königer et al., 2012). Genetic diversity within the populations is significantly correlated with the effective population size pointing at a serious impact of demographic bottleneck events (Königer et al., 2012).

G. bohemica is a member of *G. germanica* agg. (Greimler et al., 2004) which is characterised by large pentamerous flowers with mostly stalked ovary. Systematics of the genus *Gentianella* is not trivial due to hybridisation, reticulate evolution and introgression; moreover, morphology of the taxa does often not reflect the genetic relationships (Jang et al., 2005). In AFLP analyses *G. bohemica* is closest related to alpine *G. germanica* both forming a monophyletic group, while *G. germanica* from the lowlands is placed in another group (Greimler et al., 2004). Uncertainties in the treatment of the taxon exist due to seasonal dimorphism and ecotypic polymorphisms in morphology. The variations of morphological characters correlate with flowering time and result in the distinction of aestival and autumnal forms (Greimler et al., 2004; Rothmaler, 2005). Recently, Plenk et al. (2016) found genetic independency of the annual cohorts.

Besides land use changes and among other factors low reproductive success might also contribute to the rarity of *G. bohemica*. Plenk et al. (2016) have observed a mixed mating system with self-pollination and pollination by insects such as bumblebees, honeybees, hoverflies and moths. Attraction and direction of pollinators is achieved by floral scents together with visual signals (Raguso and Willis, 2002; Dötterl et al., 2011). Well beyond their ecological importance, floral fragrances have been recognised as highly valuable chemotaxonomic markers among species in plant taxa as Orchidaceae (Barkman, 2001), Nyctaginaceae (Levin et al., 2003), and in the apomictic genera *Hieracium* and *Sorbus* (Feulner et al., 2011, 2014). Scent is also known to differ among populations within species (e.g., Dötterl et al., 2005, Giuliani et al., 2016), however, it is not known whether differences in floral scents among populations also correlate with genetic differences among populations.

G. bohemica is an ideal model to study infraspecific scent differentiation since it has a strongly fragmented distribution area consisting of populations far from each other. Additionally, processes such as genetic drift may influence floral scent and strengthen scent variation and population differentiation (Königer et al., 2012).

In the study presented, we analysed inflorescence scents in eight different populations of *G. bohemica* and three populations of *G. germanica*. We tested for differences in scent between the two species and within both species among populations, and tested for a correlation of scent and genetic similarity in *G. bohemica*. We expect that the scent differs between the two *Gentianella* species and also among populations of *G. bohemica*. We suggest that scent differences correlate with genetic data, based on data obtained in other plants, and floral scent therefore serves a suitable chemotaxonomic marker in *G. bohemica*.

2. Materials and methods

2.1. Study species

Gentianella bohemica Skalický (*G. praecox* A. and J. Kerner supsp. *bohemica* [Skalický] Holub, Gentianaceae) is a herbaceous biennial hemicryptophyte. Flowering individuals reach a height of 5–50 cm, with inflorescences ranging from sparsely flowered to ones rich in flowers. The red to violet or blue petals are 20–35 mm long and are partially fused to form a bell-shaped radially-symmetric corolla tube. Hairy coronal scales within the corolla throat produce the typical 'bearded' appearance. Five alternating sepals form a partly united distinctly aliferous calyx tube. The points of the calyx are triangular to linear with U– to V-shaped sinuses between them (Engleder and Zimmerhackl, 2002; Dolek et al., 2010). In contrast, *Gentianella germanica* (WILLD.) Börner has wider points of the calyx with sharp V-shaped sinuses (Engleder and Zimmerhackl, 2002; Dolek et al., 2010). Short-conical papillae on the calyx tube that are typical for *G. germanica* are also frequent in *G. bohemica*. Flowering time of *G. germanica* is July to August, that of *G. bohemica* a few weeks later except the one early flowering population in 'Sonnen'.

In Bavaria, *G. bohemica* grows mainly on siliceous substrates in submontane and montane *Nardus* grasslands (Nardion) which have been traditionally mown or grazed. In parts of Bohemia and the Mühlviertel *G. bohemica* can also be found in mesic *Arrhenatherum* meadows (Arrhenatherion), *Cynosurus* pastures (Cynosurion), intermittently wet *Molinia* meadows (Molinion), dry calcareous grasslands (Bromion erecti and Koelerio-Phleion phleoidis) and forest fringe vegetation (Brabec, 2005; IUCN, 2012; Königer et al., 2012).

G. germanica occurs in a range of habitats from alpine calcareous dry grasslands (Seslerion variae), traditionally mown or grazed grassland such as nutrient-poor meadows on calcareous (Bromion erecti) and silicious substrates (Nardetalia strictae) (Haeupler and Muer, 2007).

2.2. Study sites

Eight populations of *G. bohemica* and three populations of *G. germanica* were chosen to obtain floral scents *in situ* (Fig. S1). The sampled populations of *G. bohemica* were situated in the Bavarian Forest in Germany, the Mühlviertel in Austria and the