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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

Bohemian Forest in Czechia and were exactly the same localities as in Königer et al. (2012) - with the exception of 'Chvalšiny' - to be able to compare scent with genetic data. *G. germanica* was sampled as reference for the dissimilarity of floral scents among *G. bohemica* populations and between *Gentianella* species. The three populations of *G. germanica* were situated in the surroundings of Bayreuth (Northeast Bavaria, Germany) in the German ordnance maps TK-6032 (Scheβlitz), TK-6033 (Hollfeld) and TK-6137 (Kemnath). The exact coordinates of the populations will not be published due to reasons of species protection.

2.3. Scent collection and analysis

Five flowering and two vegetative individuals per population of *G. bohemica* and *G. germanica* were sampled using the dynamic headspace method described by Dötterl et al. (2005) and Dötterl and Jürgens (2005). The sampled individuals were chosen randomly within the populations. All scent samples were collected between 10:00 a.m. and 3:00 p.m. on generally sunny, although partly cloudy, days in August and September 2012. Samples were collected from inflorescences (number of flowers was noted), whereas vegetative scents were collected from inflorescences with closed flower buds or dried up flowers. Floral or vegetative parts were enclosed within a polyethylene oven bag (Toppits® Bratschlauch, Melitta GmbH & Co. KG) for an accumulation time of 30 min. The bags were of different sizes (10–15 × 20–25 cm) to fit the plant and inflorescence sizes. To trap the emitted volatiles, a small hole was cut into the top of the bag into which the absorbent tube (fixed on a silicone tube) was inserted. The air enriched with volatiles was sucked through the absorbent tube for 5 min using a membrane pump (G12/01 EB, ASF Rietschle-Thomas, Puchheim, Germany) at a constant flow rate of 200 ml min^{-1} adjusted by a flow meter (LPM-Meter, Cole-Parmer Instrument Company, IL, USA). ChromatoProbe™ quartz microvials (15 mm in length, 2 mm inner diameter; Varian Inc., Palo Alto, CA, USA) were filled with a 1:1 mixture of Tenax TA® (mesh 60–80) and Carbotrap B® (mesh 20–40; both Supelco Analytical Inc., Bellefonte, PA, USA), which were fixed with silanized glass wool (Supelco) at both ends, and used as absorbent tubes. Ambient air samples served as negative controls.

Chemical analysis of the samples was performed with the method as described in Dötterl et al. (2005) and Dötterl and Jürgens (2005) by using a Varian Saturn 2000 mass spectrometer (MS) coupled with a Varian 3800 gas chromatograph (GC). The GC was equipped with a 1079 injector fitted with the ChromatoProbe™ kit.

2.4. Data analysis

The obtained GC/MS data were processed with the Saturn software package SaturnView™ version 5.2.1. Component identification was carried out with the NIST 02 mass spectral database and the database available in MassFinder 3 and confirmed by comparison of both mass spectrum and retention time with published data (Adams, 2007; El Sayed, 2012). Structures of several compounds were confirmed by comparing mass spectra and retention times with those of synthetic reference samples available in the reference collection of SD.

Inflorescence scent, vegetative samples and negative control samples were compared to identify flower-specific compounds and green leaf volatiles, respectively. Compounds that were only found in the inflorescence scent samples were regarded as flower-specific and noted as floral volatiles (FV). Compounds that were found in both inflorescence scent and vegetative samples but not in the negative controls were treated as green leaf volatiles (GLV).

Total scent emission was estimated by injecting known amounts of monoterpenoids, aromatics, and aliphatics (added to the adsorbent tubes). The mean response of these compounds (mean peak area) was used to determine the total amount of each compound extracted from the small adsorbent tubes (Dötterl et al., 2005).

2.5. Statistical analysis

To test for differences in semiquantitative (using percentage amounts of single compounds to total peak area in a sample) and qualitative (presence/absence of compounds) patterns in flower-specific scents between species and among populations, we performed PERMANOVA analyses (10,000 permutations) based on pairwise Bray-Curtis and Jaccard similarities, respectively (Primer 7.0.11; Clarke and Gorley, 2015). We used *species* and *population* (*nested in species*) as fixed and random factors, respectively, in these analyses. Following these global analyses, we used PERMANOVA as post hoc analyses to determine which populations (within species; population as fixed factor) differ among each other. PERMANOVA is a technique for testing the simultaneous response of one or more variables to one or more factors in an ANOVA experimental design on the basis of a (dis)similarity (distance) matrix with permutation methods (Anderson et al., 2008). To test whether samples of *G. bohemica* and *G. germanica,* and samples of the different populations within the two species differ in variability (dispersion), and if these differences might be responsible for the significant species effect found in the PERMANOVA analyses, we performed permutational analysis of multivariate dispersions (PERMDISP) in Primer.

Non-linear multidimensional scaling (NMDS) was used to detect meaningful underlying dimensions and to visualise similarities and dissimilarities between the individual scent samples with respect to the semiquantitative scent patterns of floral volatiles.

Mantel tests were used for a correlation analysis between the qualitative floral scent data of eight populations of *G. bohemica*, AFLP data and effective population sizes (N_e) extracted from Königer et al. (2012). Similarity matrices based on

Jaccard similarity coefficient were calculated from floral scent data and effective population sizes. Mantel tests were calculated with the method RELATE (Spearman Rank correlation, 10.000 permutations).

NMDS was performed using the software environment R (version 3.1.2 (Pumpkin Helmet), R Development Core Team, 2014) supported by the add-on packages vegan (version 2.2–1, Oksanen et al., 2013). All other analyses were conducted with the software Primer7 (version 7.0.11) (PRIMER E-Limited, Plymouth Routines in Multivariate Ecological Research, Plymouth, UK).

3. Results

3.1. Quantitative and qualitative differences between species and among populations

Median emission rates per flower and 30 min were comparably low and ranged from 0.31 ng ('Tirschenreuth') to 0.48 ng ('Welkendorf') in *G. germanica*, and 0.17 ng ('Sonnen') to 1.2 ng ('Mauth') in *G. bohemica*.

Altogether, 18 flower-specific volatiles (FV), mostly terpenoids and a few aromatic compounds, and five vegetative scents ((Z)-3-hexen-1-ol, α-pinene, β-myrcene, (*Z*)-3-hexen-1-ol acetate and (Z)-ß-ocimene) were found in the scent samples of *G. bohemica* and *G. germanica*.

Both gentian species had most of the flower-specific compounds in common, however, the sesquiterpenes β-caryophyllene and elemol were exclusively detected in *G. bohemica* but none was exclusively found in *G. germanica* (Table 1, Fig. 1).

The two species *G. bohemica* and *G. germanica* (Pseudo-$F_{df=1,43} = 7.69$; $P = 0.007$) and nested in species also different populations (Pseudo-$F_{df=9,43} = 5.68$; $P < 0.001$) emitted different sets of compounds (Table 1). The species effect and the population effect in *G. bohemica* cannot be explained by differences in dispersion between the species ($F_{df=1,52} = 0.02$; $P = 0.91$) and among the populations ($F_{df=7,31} = 2.60$; $P = 0.09$). In *G. germanica*, however, we found a significant effect of dispersion among populations ($F_{df=1,12} = 7.27$; $P = 0.03$) - it was highest in the population 'Welkendorf' (Table 2). For *G. bohemica*, post-hoc PERMANOVA analyses revealed that 18 of the 28 pairwise comparisons among populations were significant. In *G. germanica*, differences could not be localised in the post-hoc analyses (all $P > 0.05$; 'Tirschenreuth' versus 'Welkendorf': $P = 0.055$).

In *G. bohemica*, both 'Finsterau' and 'Aigen' differed from all other populations ($P < 0.05$). 'Mauth' did not show significant differences in floral scent composition to Czech or Austrian populations ($P > 0.5$) with the exception of Aigen ($P = 0.008$). The *G. bohemica* population Sonnen is highly differentiated from all other populations of the Bohemian gentian (pairwise R between 0.868 and 0.998; $P = 0.008$) and also to *G. germanica* (pairwise R between 0.546 and 0.894; $P = 0.008$). Responsible for this is a low number of floral volatile compounds and the absence of nepetalactone, its derivates and elemol in the floral scent.

The average dissimilarity in floral scents between 'Mauth' and populations from Czechia or Austria ranged between 10.5% ('Mauth' & 'Onšovice) and 15.5% ('Mauth' & 'Leopoldschlag').

3.2. Semi-quantitative differences between species and among populations

In most populations of *G. bohemica* scent is dominated by β-elemene, linalool and nepetalactone (Table 1), whereas in most populations of *G. germanica* scent is dominated by (E)-ß-ocimene, linalool and 4-oxoisophorone (Table 1).

The two species (Pseudo-$F_{df=1,53} = 6.72$; $P < 0.001$) and also the populations (nested in species) (Pseudo-$F_{df=9,53} = 2.47$; $P < 0.001$) emitted also different relative amounts of scents and neither species nor population effects can be explained by differences in dispersion between species/among populations (PERMDISP analyses; $P > 0.26$). Post-hoc PERMANOVA analyses revealed that 11 of the 28 pairwise comparisons among populations in *G. bohemica* were significant, whereas there is one significant difference in scent among populations within *G. germanica* ('Tirschenreuth' - 'Welkendorf'; $P = 0.03$). The semi-quantitative differences of the composition in floral volatiles between individuals of *G. bohemica* and *G. germanica* are visualised in Fig. 2.

3.3. Correlation of floral scent data with AFLP data and population size of G. bohemica

Floral scent data correlated with AFLP data extracted from Königer et al. (2012) (RELATE: $R = 0.796$, $P = 0.003$), but not with the effective population sizes gained form Königer et al. (2012) (RELATE: $R = 0.328$, $P = 0.15$).

4. Discussion

Most of the 18 flower-specific scent compounds of *G. bohemica* are well-known floral odours (Knudsen, 2006). The compounds (E)-ß-ocimene, linalool and 2-phenylethanol are even among the most widespread floral scents (Knudsen, 2006). Moreover, these compounds are known to elicit physiological and/or behavioural responses in bumblebees, honey bees, and hoverflies (Henning et al., 1992; Verheggen et al., 2008; Jürgens et al., 2014), which are the most important pollinators of *G. bohemica* (Dolek et al., 2010; Königer et al., 2012), and thus seem to be involved in pollinator

Table 1
Chemical composition of the floral scent of the populations of *G. bohemica* (eight sampled populations) and *G. germanica* (three sampled populations): relative amount [%] of each compound classified as floral volatile, compounds listed within classes according to *Scan Number* (Scan No.), all percentages > 5% are printed in bold (for acronyms, see Fig. 2).

	Gentianella bohemica									
	So		Ma		Fi		Le		Ai	
Number of samples [n]	$n = 5$		$n = 5$		$n = 4$		$n = 5$		$n = 5$	
Mean total number of compounds	4		10		7		8		13	
Median emission rate [ng flower^{-1} 30 min^{-1}]	0.17		1.19		0.53		0.52		0.37	
Relative Amount [%]	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max
Aromatics										
2-Phenylethanol*	**8.3**	1.0–**35.3**	**22.1**	**5.2–92.5**	**38.0**	2.2–**72**	4.0	1.4–**29.1**	3.2	0.5–**9.8**
1,4-Dimethoxybenzene*	–	–	–	–	–	–	0	0–4.7	–	–
Monoterpenes										
(E)-ß-Ocimene*	**20.1**	**6.0–51.3**	3.1	1.1–**8.3**	**12.4**	3.0–**19.7**	2.8	1.5–**7.1**	4.2	0–**34.5**
(E)-Linalool oxide (furanoid)*	–	–	3.4	1.1–**5.2**	3.8	1.1–**11.1**	1.0	0–**8.9**	1.9	0–3.5
Linalool*	**26.4**	**11.4–39.4**	4.7	0.6–**10.1**	**14.9**	0–**30.5**	**10.9**	**5.2–27.5**	**17.4**	4.2–**32.4**
allo-Ocimene*	–	–	0.5	0.3–2.9	0.8	0–**10.9**	0	0–**7.1**	0.2	0–**8.3**
neoallo-Ocimene*	–	–	–	–	–	–	–	–	0	0–0.6
Unk m/z: 91, 39, 79, 65, 107	–	–	–	–	0	0–1.2	–	–	0	0–1.9
Citronellal*	–	–	–	–	–	–	–	–	0	0–0.9
Nepetalactone isomer*	–	–	3.5	1.1–**35.8**	**18.6**	2.7–**49.4**	1.0	0.5–**31.6**	**18.7**	**10.2–35.6**
Nepetalactone isomer*	–	–	0	0–0.6	0	0–0.3	0	0–0.4	0.2	0.1–0.3
Dihydronepetalactone	–	–	0.6	0–2.9	2	1.2–2.9	0.6	0–3.5	4.1	0.8–**13.3**
Unk m/z: 159, 131, 117, 145, 105	–	–	0.1	0–0.6	0	0–1.1	0	0–0.5	0.2	0.1–0.3
Sesquiterpenes										
ß-Elemene	**35.7**	0–**43.7**	**5.8**	1.6–**44.8**	0.9	0–**5.6**	**43.8**	**28.1–52.5**	**30.1**	**15.9–53.0**
ß-Caryophyllene*	0	0–3.8	0	0–0.3	0	0–0.8	0.3	0–0.5	0.2	0–0.7
Elemol	0	0–**19.1**	1.1	0–3.2	0	0–1.6	**5.2**	2.7–**7**	2.3	0.6–3.8
Irregular Terpenes										
Dihydrooxoisophorone*	–	–	–	–	–	–	–	–	0.5	0.1–1.4
4-Oxoisophorone*	**5.3**	0–**13.0**	**9.9**	0.2–**37.7**	–	–	0.7	0–**44.4**	1.1	0.1–3.2

	G. bohemica						*G. germanica*					
	Po		On		Ho		T		S		W	
Number of samples [n]	$n = 5$		$n = 5$		$n = 5$		$n = 5$		$n = 5$		$n = 5$	
Mean total number of compounds	12		11		12		9		8		10	
Median emission rate [ng flower^{-1} 30 min^{-1}]	0.62		0.29		0.42		0.31		0.32		0.48	
Relative Amount [%]	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max
Aromatics												
2-Phenylethanol*	2.6	2.3–**9.1**	3.9	1.1–**8.5**	**8.5**	1.5–**19.1**	1.9	0.7–**15**	1.4	0–**6.9**	2.1	1–**20.9**
1,4-Dimethoxybenzene*	–	–	–	–	–	–	3.7	0–**10.9**	0	0–4.2	0	0–2.4
Monoterpenes												
(E)-ß-Ocimene*	**35.4**	4.1–**43.7**	2.8	2.5–3.5	**9.5**	2.6–**23.4**	4.2	1–7	0.7	0–**28.9**	**39.4**	3.2–**61.7**
(E)-Linalool oxide (furanoid)*	0.9	0.7–**6.6**	1.7	0.4–2.3	2.3	0.9–3.8	**7**	4.7–**14.9**	**5.7**	0–**9.3**	0.3	0–4.6
Linalool*	**6.8**	4.3–**13.2**	**26.8**	4.5–**68.3**	**17.3**	**9.6–43.1**	**53.7**	**25.3–74.2**	**66.5**	**12.3–89.3**	**21.8**	3.4–**84.5**
allo-Ocimene*	**6.3**	0.4–**10.3**	0.1	0–0.9	0.7	0–**5.2**	0.2	0–1.3	0	0–1.3	0	0–2.4
neoallo-Ocimene*	0.2	0–4.9	–	–	0	0–0.3	–	–	–	–	0	0–2.1
Unk m/z: 91, 39, 79, 65, 107	0.3	0–**9**	–	–	1.3	0–2.9	–	–	0	0–**18.8**	**6.1**	0–**25.1**
Citronellal*	–	–	–	–	0	0–0	**5.9**	1.9–**31.3**	0.9	0–**7.5**	0	0–**20.9**
Nepetalactone isomer*	1.1	0.3–**16.7**	**17.5**	0.4–**44.1**	**16.1**	3.0–**56.5**	0	0–0.2	–	–	0	0–0.2
Nepetalactone isomer*	0	0–0.1	0	0–0.2	0.1	0–0.2	–	–	–	–	0	0–0.1
Dihydronepetalactone	0.2	0–1.9	1.6	0–**7.5**	0.9	0–**6.1**	–	–	–	–	0	0–0.1
Unk m/z: 159, 131, 117, 145, 105	0.2	0–2.8	0.1	0–0.2	0.1	0–0.1	–	–	–	–	0	0–0.2
Sesquiterpenes												
ß-Elemene	**22.4**	8–**52.4**	**17.3**	**12.2–50.1**	**15.4**	4.9–**28.4**	2.5	0.4–4.5	0.1	0–1.1	1.4	0–**6.7**
ß-Caryophyllene*	0	0–0.2	0.1	0–0.6	0.3	0–1.1	–	–	–	–	–	–
Elemol	3.7	0.4–**11.4**	2.4	1.4–4.8	2.2	0.2–3.1	–	–	–	–	–	–
Irregular Terpenes												
Dihydrooxoisophorone*	–	–	0	0–0.3	–	–	0.2	0–4.8	1.5	0.7–1.9	1.2	0–**5**
4-Oxoisophorone*	**6.8**	1.6–**15.6**	2.6	0.7–**27.1**	0.7	0–**20.5**	**19**	0.2–**24.9**	**10.2**	0.1–**83.3**	0.4	0–**6.2**

*Identity of compounds marked with an asterisk was verified by authentic standards.

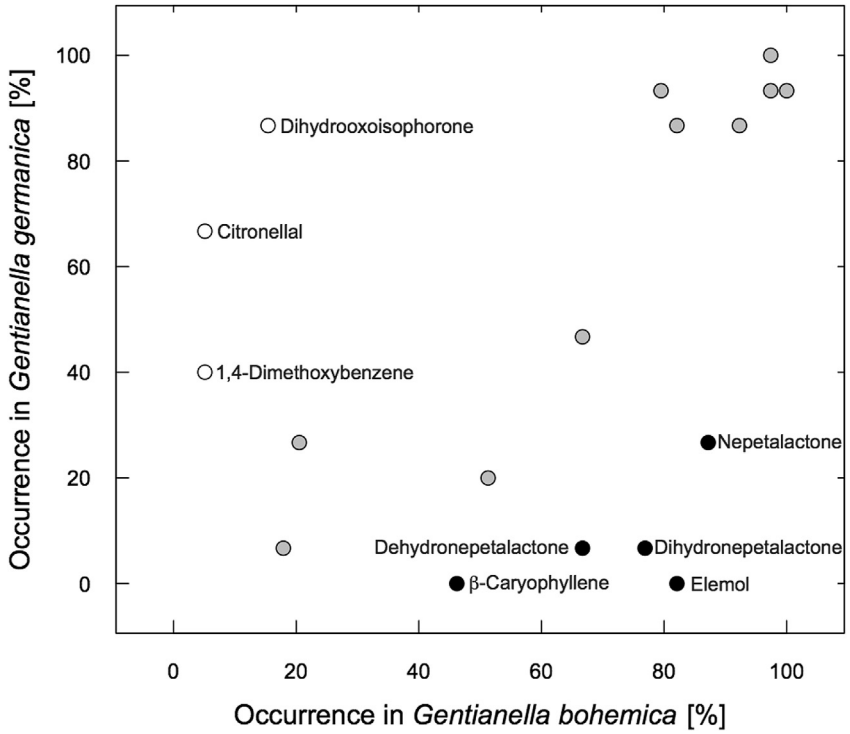

Fig. 1. Occurrence (in %) of individual substances in the floral scent profiles of *G. bohemica* and *G. germanica* (Table 1). Open circles: substances typical for *G. germanica* (>40% occurrence); black circles: substances typical for *G. bohemica* (>40% occurrence); grey circles: other substances shared between both *Gentianella* species.

Table 2
Scent dispersion in the populations of *G. bohemica* and *G. germanica* (mean distance to centroid, Jaccard coefficient). *n*: sample size, SE: standard error; different indices indicate significant differences between the populations; for acronyms see Fig. 2.

Population	*n*	Average dispersion	SE
So	5	21.3 ^{a}	3.8
Ma	5	11.8 ^{ab}	2.5
Fi	4	26.4 ^{bc}	1.3
Le	5	20.8 ^{bcd}	2.2
Ai	5	12 ^{c}	4
Po	5	10.7 ^{c}	2.9
On	5	13.1 ^{d}	1.5
Ho	5	14.2 $^{n.s.}$	4.3
T	5	12.2 ^{cd}	2.3
S	5	14 $^{n.s.}$	5.8
W	5	31.4 ^{abcd}	1.6

attraction in *G. bohemica*. The emission rates of floral scents in *G. bohemica* are comparably low (compare with Burger et al., 2012; Schäffler et al., 2012; Milet-Pinheiro et al., 2013).

Analysis of the floral scent data revealed that all volatile compounds except for the sesquiterpenes β-caryophyllene and elemol are shared between the two gentian species *G. bohemica* and *G. germanica* (Fig. 1). In general, overlapping volatile components in the floral scent could be expected in closely related species, especially of genera such as *Gentiana*, where hybridisation, reticulate evolution and large scale introgression plays an important role in speciation (Whitehead and Peakall, 2009; Jang et al., 2005). Nevertheless, the analysis of the chemical composition of the floral scent of *G. bohemica* and *G. germanica* revealed population specificity in the presented study.

Mainly samples of population 'Sonnen' differed from samples of the other populations: It is characterised by (1) a low number of floral volatile compounds and (2) by the absence of a variety of compounds such as nepetalactone, its derivates and elemol and is thus distinguished from the other populations of *G. bohemica*. This floral scent profile makes the 'Sonnen'-population resemble more the populations of *G. germanica* than of *G. bohemica*. This trait is congruent with observations that the population usually flowers up to three weeks earlier than all other populations of *G. bohemica* (Zipp, pers. comment). Our

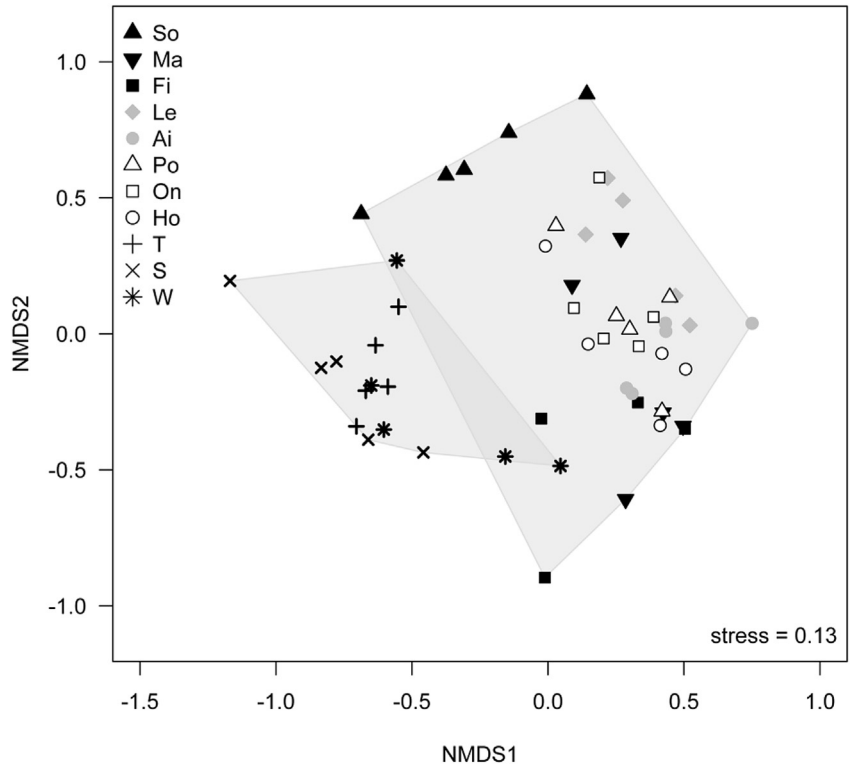

Fig. 2. NMDS of the semiquantitative floral scent profile of 39 samples of *G. bohemica* and 15 samples of *G. germanica* based on Bray-Curtis similarities; stress = 0.13; *G. bohemica*: black (Germany/Bavaria; So = 'Sonnen', Ma = 'Mauth', Fi = 'Finsterau'), grey (Czechia/Bohemian Forest; Po = 'Polná', On = 'Onšovice', Ho = 'Hroby') and open symbols (Austria/Mühlviertel; Le = 'Leopoldschlag', Ai = 'Aigen'); *G. germanica*: plus (T = 'Tirschenreuth'), cross (S = 'Scheβlitz') and asterisk (W = 'Welkendorf').

results are congruent with the findings by Plenk et al. (2016) who confirmed genetic independency of the early-flowering morphs by AFLP analyses. Forming a phenological group and because of the geographical distance to other populations 'Sonnen' is isolated from the other studied populations of *G. bohemica* (Fig. S1).

The iridoid monoterpene nepetalactone, discovered as essential oil component of the catnip *Nepeta cataria* (L.) (Bates and Sigel, 1963), has on the one hand repellent properties against several families of insects (Schultz et al., 2004), and on the other hand it is together with derivatives thereof a sex pheromone in various aphids (Boo et al., 2000 and references therein). In *G. bohemica*, it could explain pollinator differences between late, releasing this component, and early flowering populations, which lack it. Plenk et al. (2016) found no pollinators in early but a wide range of pollinators in late flowering *G. bohemica*. More studies are needed to test the importance of floral scents and single components thereof (including nepetalactone) in interactions of *G. bohemica* with flowers visitors/pollinators.

In this study the floral scent profiles of *Gentianella bohemica* and *G. germanica* have proven to be taxon-specific as they isolate the two gentian species from each other. Feulner et al. (2014) have shown that congruency of floral scents and genetic data is high in plant complexes where hybrid speciation and asexual reproduction play a strong role such as in the genus *Sorbus* (Rosaceae). In a previous survey on *G. bohemica*, genetic analyses through (AFLP) fingerprint data revealed three genetically isolated groups: (1) the German populations 'Finsterau' and 'Mauth', (2) the strongly isolated 'Sonnen'-population and (3) the Czech and Austrian populations (Königer et al., 2012). In this study, the correlation between the AFLP and the floral scent data confirmed a surprisingly high genetic differentiation for this sexually reproducing species with nearly 80% of the variation in floral scent data being explained by genetic distances between the populations. Flower-specific scents of *G. bohemica* populations extensively reflect the genetic population differentiation. Consequently, floral scent is a suitable chemotaxonomic marker in *G. bohemica*. To our knowledge, this is the first study to have shown differentiation of floral volatiles on the population level and its correlation with genetic data.

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Author contribution

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Conflict of interest

There are no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2017.01.004.

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