



# Conserving on the edge: genetic variation and structure in northern populations of the endangered plant *Dracocephalum ruyschiana* L. (Lamiaceae)

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## Abstract

Loss of biodiversity is accelerating, including the loss of genetic diversity. Conservation of small, isolated populations may be important, as they can provide valuable contributions to overall genetic variation and long-term viability of species. Furthermore, such populations may play an essential role in adaptation to new environments following changes in e.g. land-use and climate. *Dracocephalum ruyschiana* is a threatened plant species throughout its European distribution, but 25% of the European populations are situated within Norway. Therefore, the species has its own action plan in Norway, which includes demographic monitoring. However, this monitoring does not cover genetic variation nor is the selection of monitored populations based on genetic differentiation, therefore this fundamental level of biodiversity is overlooked. We analyzed 43 sites using 96 SNPs developed for *D. ruyschiana*, to investigate whether the monitored populations cover the genetic variation and differentiation found within the Norwegian distribution. The results show structuring and differentiation between populations and indicate that there are at least four distinct genetic groups, of which only two are covered extensively by current demographic monitoring. We suggest that two sites representing the two other genetic groups should be included in the national monitoring program to better conserve the genetic variation found in the Norwegian population of *D. ruyschiana*. Overall, our results highlight the importance of an integrated, interdisciplinary framework to better monitor and conserve biodiversity at several levels.

**Keywords** Conservation genetics · Genetic monitoring · Genetic structure · Management · Norway · Peripheral populations

## Introduction

The loss of biodiversity is accelerating with negative consequences for populations, species, communities and ecosystems (Hughes et al. 2008; Ceballos et al. 2015). The main threat to biodiversity loss is land-use change (IPBES 2019), which causes population fragmentation that may lead to loss

of genetic diversity (Aguilar et al. 2008). Genetic variation is the smallest unit of biodiversity, and thus crucial for conserving biodiversity at higher levels, such as species and ecosystems (Bruford et al. 2017). Conserving genetic diversity provides genotypes for selection in a changing environment (Barrett and Schluter 2008), contributes to faster recovery of populations after disturbance (Reusch et al. 2005), and increases the stability of ecosystem processes (Crutsinger et al. 2006; Des Roches et al. 2018).

An important international political document for halting biodiversity loss is the United Nations' Convention on Biological Diversity (CBD; [www.cbd.int](http://www.cbd.int)), which also gives an explicit goal to protect genetic diversity. The fundamental idea is that conservation should aim to conserve evolutionary processes, adaptive potential, and not just current species (Bowen 1999). However, very limited action has yet been taken on a global scale to fulfil this goal (Laikre 2010;

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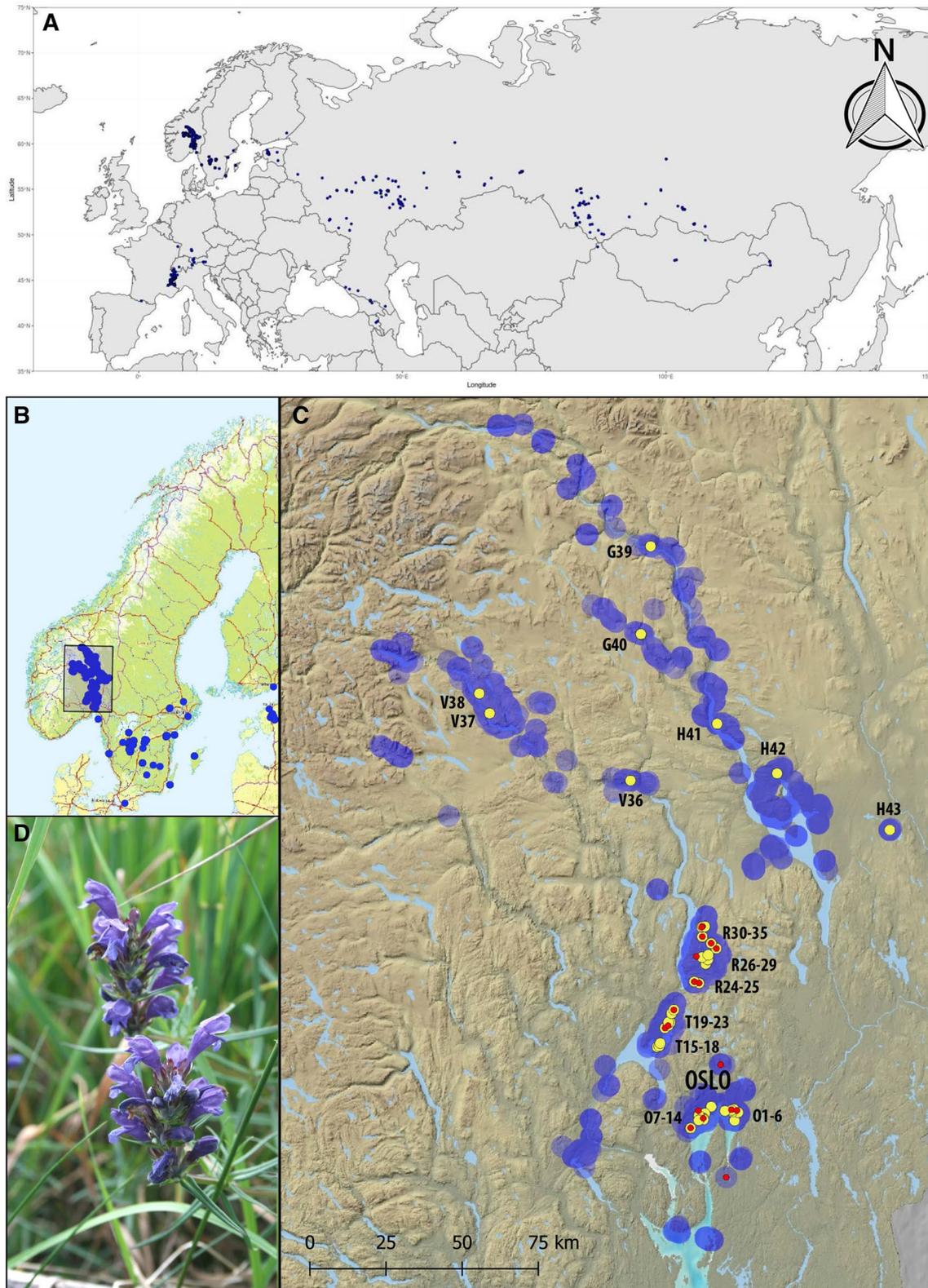
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Laikre et al. 2010). Furthermore, the IUCN Red List does not consider genetic variation (Rivers et al. 2014).

Populations of threatened species are typically small and isolated. Such populations may exhibit lower genetic variation than larger populations, potentially reducing their

**Fig. 1** **a** The global distribution of *Dracocephalum ruyschiana* based on data from GBIF Secretariat (2017, see Lazarević et al. 2009 for a more detailed map). **b** The distribution of *D. ruyschiana* in Fennoscandia based on data collected after 1975 (GBIF Secretariat 2017). **c** Norwegian distribution of *D. ruyschiana* (blue dots, data from Norwegian Biodiversity Information Centre, artsdatabanken.no), the collected sites for this study (yellow dots) and sites that are currently included in demographic monitoring (red dots). The site ID indicated in the map follows Table 1 and the assigned geographical regions are indicated by the letter in the ID (O-Oslofjorden, T-Tyri-fjorden, R-Randsfjorden, V-Valdres, G-Gudbrandsdalen, H-Hedmark). The Norwegian populations of *D. ruyschiana* are situated in the southeastern parts of Norway. **D**: Flowering stand of *D. ruyschiana* (photo: Anders Endrestøl)

abilities to adapt to changing environmental conditions (Keller and Waller 2002; Barrett and Schluter 2008). Moreover, small populations are more prone to genetic drift and inbreeding than larger populations, further lowering their genetic diversity (for example, Wagner et al. 2012). Regardless of levels of genetic variation, populations at the distribution margin may play an important role in the range shift of species following climate change (Razgour et al. 2013). Furthermore, such populations can also hold unique ecological and genetic variation (Osborne et al. 2012) and potentially enhance within-species genetic diversity (García-Ramos and Kirkpatrick 1997). Thus, conserving these populations may be especially important, particularly if the species is threatened across its entire distribution.

To conserve genetic diversity and guide practical conservation strategies, it is crucial to identify population-genetic structuring, such as genetic distinctiveness and diversity, and ideally to couple this information with knowledge on geographical barriers and divergent local ecological adaptation. In Norway, only a few rare and endangered plant species have been investigated genetically with the intention to guide conservation plans (Westergaard et al. 2011a, b; Birkeland et al. 2017; Westergaard et al. 2019).

Here we use the vascular plant ‘northern dragonhead’ *Dracocephalum ruyschiana* L. as a case to investigate how analyses of genetic variation can be used to guide decision makers and management when conserving biodiversity nationally. The populations of *D. ruyschiana* are rapidly declining in Europe: the species is listed under Appendix 1 of the Bern Convention (<http://conventions.coe.int/Treaty/FR/Treaties/Html/104-2.htm>) as well as on many national and regional Red Lists (e.g. Gärdenfors 2005; Király 2007; Witkowski et al. 2003), including the Norwegian Red List, in which it is listed as vulnerable (VU) (Henriksen and Hilmo 2015). Habitat loss due to urbanization or decreasing management of agricultural land, is considered the main threat to the species in Norway (Solstad et al. 2015), as well as in several European countries (Käsermann and Moser 1999; Lazarevic et al. 2009). Despite the documented decline of *D. ruyschiana*, knowledge of genetic variability, structure

and evolutionary history of this species is still lacking for Norwegian and global populations.

*Dracocephalum ruyschiana* (Lamiaceae,  $2n = 2x = 14$ ) is a long-lived, insect-pollinated herb with a continental, fragmented distribution in Eurasia (Lid and Lid 2005; Lazarevic et al. 2009). It is a typical steppe plant, and grows on exposed, well-drained, shallow calcareous soils in dry meadows and rocky outcrops (Lid and Lid 2005; Lazarevic et al. 2009). In Europe (excluding Russia), more than 25% of the remaining populations are found in Norway (Norwegian Directorate for Nature Management 2010). The species reaches its European northwestern limit in Norway, where it has a southeastern distribution through the country (Fægri and Danielsen 1996, Lazarevic et al. 2009). The Norwegian habitats of *D. ruyschiana* include dry calcareous meadows, rocky outcrops along roads, and extensively managed agricultural lands (Norwegian Directorate for Nature Management 2010). Mature plants have several stems normally approximately 30 cm long, arising from a short, branched rhizome (Lid and Lid 2005). The flowering period is typically in the middle of June, and the flowers are blue, 2–2.5 cm long, and gathered in a spike-like inflorescence with 50–60 flowers (Fig. 1d). The flowers are mainly pollinated by long-tongued insects such as bumblebees *Bombus* ssp. (Milberg and Bertilsson 1997). The fruits are typical for the family Lamiaceae, resulting in four dry one-seeded nutlets, without obvious adaptations for long-distance dispersal. The species is most likely self-compatible (Milberg and Bertilsson 1997), as self-incompatibility systems are not known from the Lamiaceae (Owens and Ubera-Jiménez 1992; Allen and Hiscock 2008).

In Norway, *D. ruyschiana* is one of only three vascular plants regulated to have priority for conservation (Lovdata 2011). As a large part of the European population is located in Norway, it is crucial to conserve the species nationally. Thus, an action plan for conservation has been prepared (Norwegian Directorate for Nature Management 2010) and a monitoring program for Norwegian populations of *D. ruyschiana* developed (Evju et al. 2016), aiming to collect data on population size and structure to inform managers on status and trends for the species at the national level. The monitoring protocol has so far been tested on 18 populations in and around the city of Oslo (Evju pers. comm.). The selection of populations was not based on genetic variation or structuring in the species, but on geographic clustering (regions). Furthermore, the action plan includes no actions to conserve genetic diversity.

Here we use single nucleotide polymorphisms (SNPs) developed for *D. ruyschiana* (Kleven et al. 2019) to investigate genetic diversity and structure within Norway. In particular, we aim at identifying genetic groups within its Norwegian distribution to evaluate whether established national monitoring sites sufficiently cover the observed

genetic variation and structuring observed. Our results may help guide future practical conservation management of *D. ruyschiana*, and serve as an example of combining genetic analyses with traditional demographic monitoring of plant species.

## Methods

### Specimen sampling, DNA extraction and SNP dataset

With collection permits from local authorities (see Acknowledgements), leaf material of *D. ruyschiana* was sampled from 43 sites across the Norwegian distribution range (Table 1, Fig. 1), with relatively sparser coverage of the distribution margins. Samples were taken in 2012–2014, prior to the development and establishment of the monitoring program in 2016 and 2017, respectively, therefore there is not complete overlap between our sites and the monitored sites. Two different sampling schemes were used when collecting leaves. In the first scheme, ten 1 m<sup>2</sup>-plots were randomly placed across a site, after first identifying all flowering clusters of *D. ruyschiana*. Leaves were then sampled from the plant closest to the center of the plot. In total, 20 sites were sampled using this scheme, and later several of these sites were included in the monitoring initiated in 2017. For the second sampling scheme in the remaining 23 sites, plants were sampled randomly. Where possible, 10 individuals were sampled from each site, however, in sites with fewer than 10 clusters of individuals (likely belonging to the same clone), leaves from one plant representing each cluster were collected.

We delimited six regions based on geographic proximity and topographic barriers, and assigned all sampled sites to a region: Oslofjorden (14), Tyrifjorden (nine), Randsfjorden (12), Valdres (three) Gudbrandsdalen (three) and Hedmark (three).

Genomic DNA was extracted from the silica dried leaves using either the NucleoSpin Plant II extraction kit (Macherey–Nagel) or DNeasyPlant Mini Kit (Qiagen), following the manufacturer’s protocols. The amount of extracted DNA was quantified on a Qubit 2.0 using the HS Assay kit (Thermo Fisher Scientific). All samples were genotyped at 96 presumably non-coding SNPs applying a SNP-typing assay recently developed specifically for the purpose of facilitating monitoring of the northern dragonhead (see Kleven et al. 2019 for details). Briefly, SNPs were genotyped on a 96.96 Dynamic Array using the Fluidigm EP1 instrument according to the manufacturer’s protocol and scored using the Fluidigm SNP genotyping analysis software (<https://www.fluidigm.com/software>). Deviation from Hardy–Weinberg equilibrium for each SNP in each population was

estimated using Arlequin ver. 3.5.1.2 (Excoffier and Lischer 2010). As none of the 96 SNPs deviated significantly from Hardy–Weinberg equilibrium consistently across populations, we included all SNPs in the downstream analyses.

### Genetic diversity and structure

Genetic diversities in each *D. ruyschiana* site were explored by calculating mean number of alleles ( $N_A$ ), observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity, the inbreeding coefficient ( $F$ ) and percentage of heterozygous loci in GenAlEx 6.5 software (Peakall and Smouse 2006, 2012). Furthermore, structuring of genetic variation within and between sites, and between the six geographic regions was estimated in analyses of molecular variances (AMOVA), and population differentiation at the regional level was estimated using the  $F_{ST}$ -value. We performed one more analyses in GenAlEx 6.5: the assignment test by using the “leave one out” option to explore the origin of individuals based on their genotypes. We also tested whether genetic distance was correlated with geographical distance, using the Mantel test (mantel.rand-test) with 999 permutations with the R package ADE4 v1.7-13 (Dray and Dufour 2007) in R v.3.2.3 in RSTUDIO v. 1.0.44 (RStudio Team 2015).

Genetic structuring was explored using MULTISPATI-PCA (Dray et al. 2008) performed with the R package ADE4 v1.7-13 (Dray and Dufour 2007) and SPDEP v1.1-2 (Bivand et al. 2008). This MULTISPATI-PCA is an extension of the principal component analysis that also takes the spatial covariability among variables into consideration. To further explore the number of genetically homogeneous groups ( $K$ ) and overall structuring in the dataset, we ran genetic cluster algorithms in STRUCTURE v2.3.4 (Pritchard et al. 2000; Falush et al. 2003, 2007; Hubisz et al. 2009). In the initial analysis, the admixture and correlated allele frequencies models were applied with 50,000 runs as burn-in followed by 150,000 runs. The analysis was run 10 times for each  $K$  from 1 to 15. The probability of obtaining the genotype data  $X$  given  $K$  (Mean LnP( $K$ )) increased with the number of  $K$ s, however, the increase was only marginal from  $K = 10$  (not shown). Using the  $\Delta K$  statistics of Evanno et al. (2005), the most likely number of groups from the preliminary run was 2. However, using the default setting of  $\alpha$  may lead to inaccurate estimates of  $K$  and assignments probabilities when population sizes are uneven and under the assumption that populations are descendants of recent ancestral populations (Wang 2017). Thus, we reran the analyses using 50,000 runs as burn-in followed by 150,000 runs, but with adjusted  $\alpha$ -values based on the initial analyses ( $\alpha = 1/\text{assumed optimal } K$ ;  $\alpha = 1/10$  and  $\alpha = 1/2$ ), applying the admixture and correlated frequencies models.

The number of likely genetic groups was explored using calculations of Mean LnP( $K$ ) (Pritchard et al. 2000) and  $\Delta K$

**Table 1** Collection information for the 43 investigated sites of *Dracocephalum ruyshiana* in Norway

ID	Site name	Geographical region	Latitude	Longitude	Year	Collector(s) <sup>a</sup>
O1	Malmøya	Oslofjorden	59,862	10,751	2014	AE
<b>O2</b>	<b>Ekebergskråningen</b>	Oslofjorden	59,887	10,768	2014	AE
O3	Bleikøya	Oslofjorden	59,888	10,735	2014	AE
O4	Hovedøya 1	Oslofjorden	59,896	10,741	2014	AE
<b>O5</b>	<b>Hovedøya 2</b>	Oslofjorden	59,892	10,729	2014	AE
O6	Nakholmen	Oslofjorden	59,889	10,691	2013	AE
O7	Trekantenga, Fornebu	Oslofjorden	59,899	10,609	2014	AE
O8	Klovodden, Ostøya	Oslofjorden	59,877	10,575	2014	AE
<b>O9</b>	<b>Møllerenga</b>	Oslofjorden	59,863	10,567	2014	OS
O10	Hesthagebukta	Oslofjorden	59,861	10,570	2014	OS
O11	Lille Ousttangen	Oslofjorden	59,859	10,568	2014	OS
O12	Nesøya	Oslofjorden	59,870	10,540	2014	AE
O13	Brønnøya	Oslofjorden	59,856	10,541	2014	AE
<b>O14</b>	<b>Spireodden</b>	Oslofjorden	59,831	10,497	2014	ME, OS, MKB
T15	Bjørketangen	Tyri fjorden	60,065	10,268	2014	OS, MKB
T16	Fekjær	Tyri fjorden	60,068	10,278	2014	AE
T17	Vik	Tyri fjorden	60,077	10,284	2014	AE
T18	Åserud	Tyri fjorden	60,123	10,305	2014	OS, MKB
T19	Åsa	Tyri fjorden	60,131	10,323	2014	OS, MKB
T20	Nordby	Tyri fjorden	60,141	10,334	2014	OS, MKB
T21	Ulltvedt	Tyri fjorden	60,141	10,323	2014	OS, MKB
T22	Haug	Tyri fjorden	60,168	10,338	2014	AE
T23	Bølgen	Tyri fjorden	60,179	10,350	2014	OS, MKB
<b>R24</b>	<b>Aslaksrud</b>	Randsfjorden	60,263	10,488	2014	OS, MKB
<b>R25</b>	<b>Klingenberg</b>	Randsfjorden	60,267	10,459	2014	OS, MKB
R26	Narverud	Randsfjorden	60,322	10,519	2014	OS, MKB
R27	Vien Nedre	Randsfjorden	60,335	10,530	2012	OS
<b>R28</b>	<b>Falang</b>	Randsfjorden	60,341	10,495	2014	OS, MKB
R29	Lyngstad	Randsfjorden	60,349	10,531	2014	OS, MKB
<b>R30</b>	<b>Skjervum</b>	Randsfjorden	60,370	10,576	2014	OS, MKB
<b>R31</b>	<b>Gjefsen</b>	Randsfjorden	60,384	10,543	2014	OS, MKB
<b>R32</b>	<b>Grindaker</b>	Randsfjorden	60,402	10,488	2014	OS, MKB
<b>R33</b>	<b>Nedre Røykenvik</b>	Randsfjorden	60,430	10,480	2014	OS, MKB
<b>R34</b>	<b>Øvre Røykenvik</b>	Randsfjorden	60,432	10,487	2014	OS, MKB
R35	Eggelinna	Randsfjorden	60,433	10,499	2012	OS
V36	Thomle	Valdres	60,851	9990	2012	OS
V37	Øygaard	Valdres	61,015	9105	2012	OS
V38	Ringerud	Valdres	61,071	9031	2012	OS
G39	Steberg	Gudbrandsdalen	61,550	10,002	2013	AE
G40	Sørmo	Gudbrandsdalen	61,286	9986	2012	OS
H41	Bergseng	Hedmark	61,038	10,488	2012	OS
H42	Veldre	Hedmark	60,902	10,871	2012	OS
H43	Solberg	Hedmark	60,753	11,571	2013	AE

Site identification (ID, the letter indicating geographical region), site name, geographical region, latitude and longitude of the sampling site, collection year (Year), and collectors are given in the table. Sites included in the monitoring program (Evju et al. 2016) are marked in bold

<sup>a</sup>AE A. Endrestøl, OS O. Stabbetorp, MKB M. K. Brandrud, ME M. Evju

(Evanno et al. 2005) in Structure Harvester (Earl and vonHoldt 2012). To compare Structure results at multiple values of  $K$ ,

we aligned and visualized bar plots using the Clumpak (Cluster Markov Packager across  $K$ ) web server (Kopelman et al. 2015).

## Results

A dataset of 355 individuals collected from 43 sites was genotyped for 96 SNPs. The number of individuals sampled from each site varied between two and 13 (Table 2). Eight sites sampled five or fewer individuals, due to small total population sizes, 11 sites had between six and nine individuals, 23 sites had ten individuals, while one had 13. The level of missing SNP data was 0.003%. Genetic variation is summarized in Table 2, showing only small differences between sites. The total mean expected heterozygosity ( $H_E$ ) for all individuals was 0.27, with the lowest level found in site V37 Øygaard ( $H_E=0.20$ , two individuals) followed by site G40 Sørmo ( $H_E=0.21$ , three individuals) and site T17 Vik ( $H_E=0.21$ , ten individuals), while site R35 Eggelinna had highest  $H_E$  (0.32, six individuals). The total mean number of different alleles for all individuals was 1.79, ranging from 1.49 (site V37 Øygaard) to 1.94 (site G39 Steberg) among sites. The total mean percentage of polymorphic loci over all individuals was 79%. The lowest percentage of polymorphic loci (56%) was estimated for site T17 Vik, while the highest (94%) was found in site G39 Steberg. There were no signs of inbreeding as most inbreeding coefficient values ( $F$ ) were slightly lower than 0 (Table 2).

The AMOVA results (Table 3) showed that most of the genetic variation was found within sites (87%), while 8% and 5% of variation was found among sites and geographical regions, respectively. The total  $F_{ST}$  value was 0.134 ( $p<0.001$ ), while all values estimated between regions were lower than 0.10 (Table 4). The lowest  $F_{ST}$  value was found between Oslofjorden and Randsfjorden ( $F_{ST}=0.027$ ) and the highest between Valdres and Hedmark ( $F_{ST}=0.081$ ). The Mantel test revealed a positive correlation between genetic distance and geographical distance ( $R=0.56$ ,  $p=0.001$ , Fig. 2).

The estimated likely number of  $K$  was not consistent across analyses, however, the different runs yielded the same results across sites, with only small variations in resolution among genetic clusters. Figure 3 shows the major mode of runs in Structure of  $K=2-4$  (10/10 runs) and  $K=11$  (9/10 runs) using the admixture and correlated frequencies models and  $\alpha=0.1$ . The Mean  $\text{LnP}(K)$  increased as the number of  $K$  increased and had not reached an optimum at  $K=15$  (Online Resource 1). The  $\Delta K$  estimates indicated that  $K=2$  was the most likely number of genetic groups for most analyses (Online Resource 1). A combined interpretation of the results obtained for increasing  $K$  values showed a hierarchical resolution of genetically homogeneous groups (cf. Meirmans 2015). Individuals from sites in Oslofjorden and site H43 Solberg clustered together, while the rest of the individuals belonged to another genetic group at  $K=2$  (Fig. 3). In the latter group,

more individuals were admixed between the two groups than were individuals in the former group. When  $K=3$ , site T17 Vik splits off in a separate group (Fig. 3). There were no admixed individuals in this site and it stayed homogeneous as  $K$  increased. The nearby sites consisted of individuals admixed with the “purple” Vik-cluster and mainly the “blue” genetic cluster, but most of the sites split off to separate genetic groups as  $K$  increased (see  $K=11$  in Fig. 3). At  $K=4$  (Fig. 3), sites R24 Aslaksrud, H41 Bergseng and H43 Solberg separated into a fourth homogeneous group. These sites split into separate groups as  $K$  increased (see Fig. 3,  $K=11$ ). Other sites also split off in distinct groups as  $K$  increased, and there was a slight tendency that the sites in the Oslofjord region separated in two; an eastern group and a western group (see  $K=11$  in Fig. 3).

The Structure results were supported by the MULTIS-PATI-PCA result, showing that individuals from sites in the Oslo region group together, while individuals from sites in Randsfjorden group together (Fig. 4). Furthermore, individuals from Tyrifjorden group together, with site T17 Vik at a distance from the other sites. Site H43 Solberg was intermediate of sites from Oslofjorden and Randsfjorden regions, and forms its own group. This is in accordance with the Structure results for  $K=4$  (Fig. 3). The genetic variation observed among sites from Oslofjorden and Randsfjorden is thus covered in the monitoring program; these two regions represent the “blue” and “orange” genetic clusters identified by Structure (Fig. 3).

The admixture of genetic groups within sites recognized by Structure was also evident from the results of the assignment test. The test showed that most individuals originated within their sampled region, but within the regions there seemed to be a higher exchange of individuals between sites (Online Resource 2). In sites with no or low numbers of admixed individuals, such as T17 Vik, R24 Aslaksrud, and H43 Solberg, no individuals were assigned to other sites.

## Discussion

Our results indicate the existence of at least four distinct genetic groups of *Dracocephalum ruyschiana* in Norway, of which only two groups are covered by the current monitoring program. To better conserve the genetic variation present in Norway, and thus a substantial portion of the European genetic variation, we suggest that the monitoring program should include sites with genetic groups not yet covered and downscale coverage of other sites.

**Table 2** Genetic variation within each of 43 sampling sites in Norway of *Dracocephalum ruyschiana*

ID	Site name	# of individuals	PPL	$N_A$	$H_E$	$H_O$	$F$
O1	Malmøya	9	83	1.83 ± 0.04	0.33 ± 0.02	0.30 ± 0.02	- 0.12 ± 0.03
<b>O2</b>	<b>Ekebergskrånningen</b>	10	85	1.85 ± 0.04	0.32 ± 0.02	0.28 ± 0.02	- 0.11 ± 0.03
O3	Bleikøya	10	82	1.82 ± 0.04	0.31 ± 0.02	0.29 ± 0.02	- 0.08 ± 0.03
O4	Hovedøya 1	5	68	1.68 ± 0.05	0.29 ± 0.03	0.26 ± 0.02	- 0.12 ± 0.04
<b>O5</b>	<b>Hovedøya 2</b>	10	83	1.83 ± 0.04	0.29 ± 0.02	0.28 ± 0.02	- 0.05 ± 0.03
O6	Nakholmen	9	85	1.85 ± 0.04	0.30 ± 0.02	0.27 ± 0.02	- 0.10 ± 0.03
O7	Trekantenga, Fornebu	13	93	1.93 ± 0.03	0.32 ± 0.02	0.30 ± 0.02	- 0.04 ± 0.03
O8	Klovodden, Ostøya	10	81	1.81 ± 0.04	0.29 ± 0.02	0.28 ± 0.02	- 0.04 ± 0.04
<b>O9</b>	<b>Møllerenga</b>	10	82	1.82 ± 0.04	0.32 ± 0.02	0.30 ± 0.02	- 0.07 ± 0.03
O10	Hesthagebukta	8	79	1.79 ± 0.04	0.28 ± 0.02	0.27 ± 0.02	- 0.02 ± 0.04
O11	Lille Ousttangen	10	80	1.80 ± 0.04	0.30 ± 0.02	0.27 ± 0.02	- 0.11 ± 0.03
O12	Nesøya	8	81	1.81 ± 0.04	0.30 ± 0.02	0.28 ± 0.02	- 0.07 ± 0.03
O13	Brønnøya	10	90	1.90 ± 0.03	0.34 ± 0.02	0.31 ± 0.02	- 0.12 ± 0.03
<b>O14</b>	<b>Spireodden</b>	10	88	1.88 ± 0.03	0.29 ± 0.02	0.29 ± 0.02	- 0.02 ± 0.03
	<i>Oslo region</i>	132	100	2.00 ± 0.00	0.32 ± 0.02	0.31 ± 0.02	0.04 ± 0.01
T15	Bjørketangen	10	82	1.82 ± 0.04	0.26 ± 0.02	0.27 ± 0.02	0.03 ± 0.03
T16	Fekjær	10	82	1.82 ± 0.04	0.26 ± 0.02	0.27 ± 0.02	- 0.01 ± 0.03
T17	Vik	10	56	1.56 ± 0.05	0.25 ± 0.03	0.21 ± 0.02	- 0.19 ± 0.03
T18	Åserud	8	69	1.69 ± 0.05	0.28 ± 0.03	0.24 ± 0.02	- 0.13 ± 0.03
T19	Åsa	10	84	1.84 ± 0.04	0.30 ± 0.02	0.28 ± 0.02	- 0.06 ± 0.03
T20	Nordby	3	59	1.59 ± 0.05	0.33 ± 0.03	0.23 ± 0.02	- 0.38 ± 0.03
T21	Ulltvedt	9	86	1.87 ± 0.04	0.29 ± 0.02	0.27 ± 0.02	- 0.09 ± 0.03
T22	Haug	10	86	1.87 ± 0.04	0.32 ± 0.02	0.30 ± 0.02	- 0.06 ± 0.03
T23	Bølgen	3	61	1.61 ± 0.05	0.29 ± 0.03	0.23 ± 0.02	- 0.25 ± 0.04
	<i>Tyrifjorden region</i>	73	98	1.98 ± 0.02	0.30 ± 0.02	0.28 ± 0.02	0.06 ± 0.01
<b>R24</b>	<b>Aslaksrud</b>	10	73	1.73 ± 0.05	0.28 ± 0.02	0.26 ± 0.02	- 0.11 ± 0.03
<b>R25</b>	<b>Klingenberg</b>	10	89	1.89 ± 0.03	0.28 ± 0.02	0.28 ± 0.02	0.00 ± 0.03
R26	Narverud	10	81	1.81 ± 0.04	0.28 ± 0.02	0.28 ± 0.02	- 0.01 ± 0.03
R27	Vien Nedre	9	90	1.90 ± 0.03	0.31 ± 0.02	0.28 ± 0.02	- 0.10 ± 0.03
<b>R28</b>	<b>Falang</b>	10	88	1.88 ± 0.03	0.32 ± 0.02	0.29 ± 0.02	- 0.10 ± 0.03
R29	Lyngstad	10	84	1.84 ± 0.04	0.29 ± 0.02	0.28 ± 0.02	- 0.04 ± 0.03
<b>R30</b>	<b>Skjervum</b>	10	77	1.77 ± 0.04	0.31 ± 0.03	0.25 ± 0.02	- 0.19 ± 0.03
<b>R31</b>	<b>Gjefsen</b>	7	79	1.79 ± 0.04	0.28 ± 0.02	0.26 ± 0.02	- 0.09 ± 0.03
<b>R32</b>	<b>Grindaker</b>	10	83	1.83 ± 0.04	0.29 ± 0.02	0.26 ± 0.02	- 0.09 ± 0.03
<b>R33</b>	<b>Nedre Røykenvik</b>	10	91	1.91 ± 0.03	0.30 ± 0.02	0.29 ± 0.02	- 0.04 ± 0.03
<b>R34</b>	<b>Øvre Røykenvik</b>	6	78	1.78 ± 0.04	0.30 ± 0.03	0.26 ± 0.02	- 0.14 ± 0.03
R35	Eggelinna	6	93	1.93 ± 0.03	0.33 ± 0.02	0.32 ± 0.02	- 0.03 ± 0.04
	<i>Randsfjorden region</i>	108	100	2.00 ± 0.00	0.31 ± 0.01	0.30 ± 0.01	0.06 ± 0.01
V36	Thomle	6	85	1.85 ± 0.04	0.34 ± 0.02	0.31 ± 0.02	- 0.11 ± 0.03
V37	Øygard	2	49	1.49 ± 0.05	0.32 ± 0.04	0.20 ± 0.02	- 0.53 ± 0.03
V38	Ringerud	4	57	1.57 ± 0.05	0.23 ± 0.03	0.22 ± 0.02	- 0.08 ± 0.05
	<i>Valdres region</i>	12	90	1.90 ± 0.03	0.31 ± 0.02	0.30 ± 0.02	- 0.01 ± 0.03
G39	Steberg	10	94	1.94 ± 0.03	0.34 ± 0.02	0.31 ± 0.02	- 0.10 ± 0.03
G40	Sørmo	3	55	1.55 ± 0.05	0.26 ± 0.03	0.21 ± 0.02	- 0.27 ± 0.04
	<i>Gudbrandsdalen region</i>	13	96	1.96 ± 0.02	0.33 ± 0.02	0.32 ± 0.02	0.00 ± 0.03
H41	Bergseng	5	70	1.70 ± 0.05	0.33 ± 0.03	0.26 ± 0.02	- 0.24 ± 0.03
H42	Veldre	2	57	1.57 ± 0.05	0.32 ± 0.03	0.24 ± 0.02	- 0.35 ± 0.05
H43	Solberg	10	79	1.80 ± 0.04	0.26 ± 0.02	0.25 ± 0.02	- 0.01 ± 0.03
	<i>Hedmark region</i>	17	92	1.92 ± 0.03	0.29 ± 0.02	0.28 ± 0.02	0.04 ± 0.03
	<i>Total mean</i>	355	79 ± 2	1.79 ± 0.01	0.30 ± 0.00	0.27 ± 0.00	- 0.10 ± 0.01

The number of individuals sampled per site (# of individuals), percentage of polymorphic loci (PPL; %), average number of alleles at each locus ( $N_A \pm SE$ ), expected heterozygosity ( $H_E \pm SE$ ), observed heterozygosity ( $H_O \pm SE$ ) and the fixation index (inbreeding coefficient,  $F \pm SE$ ) are shown in the table. Sites included in the monitoring program (Evju et al. 2016) are marked in bold

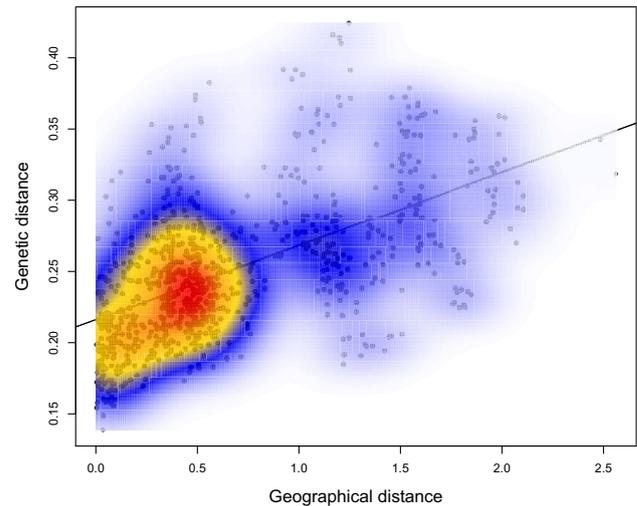
**Table 3** Partitioning of genetic variation among 43 sites in Norway of *Dracocephalum ruyschiana* using analyses of molecular variance (AMOVA)

	df	Variance component	Variance (%)
Among regions	5	0.86	5
Among sites within regions	37	1.33	8
Within sites	667	14.10	87
Total	709	16.29	100

### Genetic diversity and structuring

Genetic variation in Norwegian populations of *D. ruyschiana* was estimated by expected heterozygosity (total  $H_E = 0.27$ ) and the levels found were relatively similar among populations (ranging from 0.20 to 0.32). Compared with other threatened plants (e.g. Tero et al. 2005; Dostálek et al. 2010; but see Minasiewicz et al. 2018), the genetic variation in the studied *D. ruyschiana* sites seem to be moderate. As different genetic markers have been applied in these studies, the interpretation of genetic variation should be treated cautiously. There was no sign of inbreeding, as the inbreeding coefficient was close to 0 for most sites. Thus, outcrossing seems to be maintained by pollinating insects (Milberg and Bertilsson 1997). Sites with few individuals have high negative inbreeding coefficients, but are not considered as this is likely an artefact of low sample size. Most of the genetic variation was found within sites (87%), indicating low differentiation between sites ( $F_{ST} = 0.134$ ). This is comparable to what was found in the close relative *D. austriacum* in the Czech and Slovak Republics; high genetic diversity within populations (80%) and relatively low differentiation among populations both within and between regions (Dostálek et al. 2010).

In *D. ruyschiana*, isolation by distance was observed which may bias the Structure results (see Perez et al. 2018 and references therein). However, the spatial multivariate analysis (MULTISPATI-PCA) takes spatial data into account and the result obtained is very similar to the Structure result. Therefore, we use the two analyses combined to explore the genetic structuring of Norwegian *D. ruyschiana*. The PCA-result show that sites in the Oslofjorden region, Randsfjorden

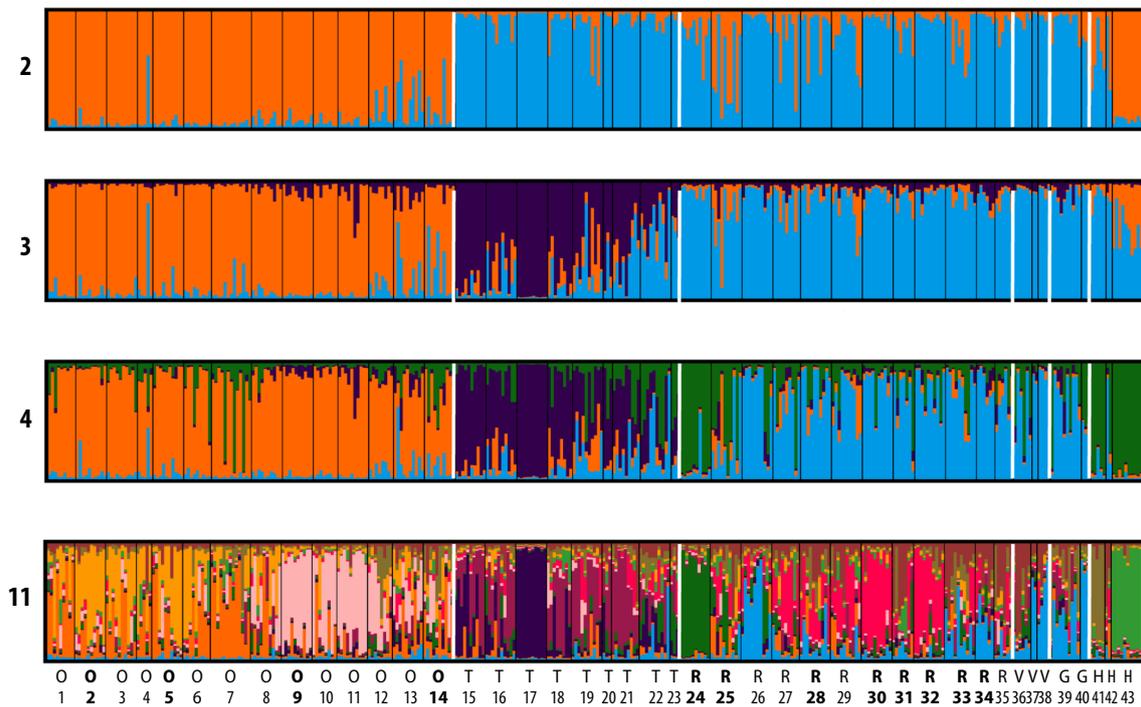
**Fig. 2** Relationship between genetic and geographic distance among 43 *Dracocephalum ruyschiana* sampling sites

region and Tyrifjorden region, form three separate clusters. This finding is supported by the Structure analyses ( $K = 3$ ) showing that sites in the Oslofjorden region belong to the same genetic group ('orange' group, Fig. 3), including site H43 Solberg (Hedmark region) and only a few individuals from other sites. All other sites form a separate group at  $K = 2$  ('blue' group, Fig. 3), but at  $K = 3$ , a 'purple' group is revealed in Tyrifjorden region. As the number of  $K$  increases, more sites splits off in new genetic groups. At  $K = 4$ , H43 Solberg splits off in a 'green' group and also forms its own cluster in the PCA. Hence, genetic variation is geographically structured within the Norwegian distribution of *D. ruyschiana*.

The Structure results indicate that there is admixture between genetic groups, as two or more genetic groups are presented in single individuals (Fig. 3). Furthermore, individuals from two genetic groups are often found within one site. The assignment test also estimated that several individuals originated from other sites. However, between regions there seems to be limited exchange of individuals. This pattern may reflect gene flow within regions, which is likely as this is an outbreeding species dependent on insects such as *Bombus* species, for pollination (Milberg and Bertilsson

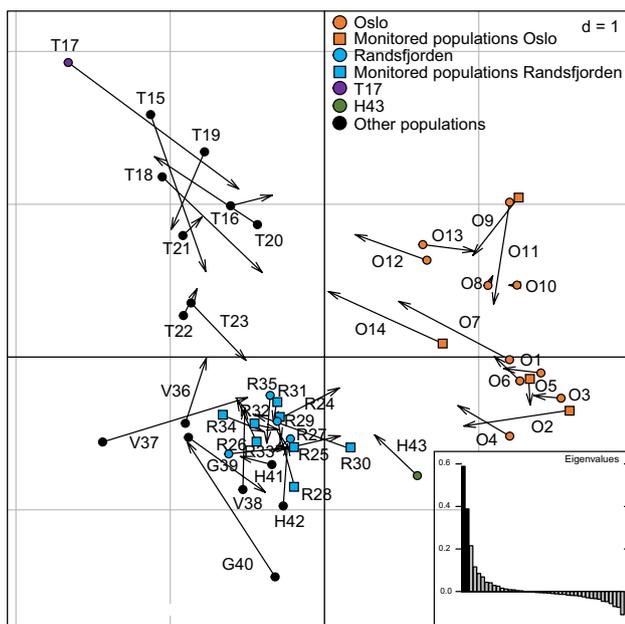
**Table 4** Pairwise  $F_{ST}$  values among six geographic regions in Norway of *Dracocephalum ruyschiana*

	Oslofjorden	Tyrifjorden	Randsfjorden	Valdres	Gudbrandsdalen	Hedmark
Oslofjorden						
Tyrifjorden	0.044					
Randsfjorden	0.027	0.029				
Valdres	0.059	0.053	0.034			
Gudbrandsdalen	0.058	0.053	0.038	0.050		
Hedmark	0.049	0.070	0.045	0.081	0.077	



**Fig. 3** Genetic structuring in *Dracocephalum ruyschiana* was investigated using Structure 2.3.4. The barplots show how 355 individuals from 43 sites using 96 SNPs were assigned to different genetic groups ( $K$ ), when  $K=2, 3, 4$ , and  $11$  (indicated to the left of the barplots), using admixture and correlated frequencies models and  $\alpha=0.1$ . For  $K=2-4$ , the barplots summarize 10/10 runs, while for  $K=10$  the

major mode 9/10 runs are shown. The site ID follows Table 1 and Fig. 1 and the assigned geographical regions are indicated by the letter in the ID (O-Oslofjorden, T-Tyri-fjorden, R-Randsfjorden, V-Valdres, G-Gudbrandsdalen, H-Hedmark). Sites are separated by black lines, while regions are separated by white lines. The sites that are currently included in demographic monitoring are marked in bold



**Fig. 4** The PCA shows a spatial multivariate analysis of *Dracocephalum ruyschiana*. The boxes each represent one site and the site ID follow Table 1 and Fig. 3. Short arrows reveal a local spatial similarity, while a long arrows reveal a spatial discrepancy. The colours indicate the genetic group identified by Structure at  $K=4$

1997), but over larger distances pollen dispersal becomes more limited. Moreover, the large seeds are not adapted to long distance dispersal (Dostálek et al. 2010). This could explain the observed isolation by distance. However,  $F_{ST}$  values between regions are very low and might reflect a historically larger meta-population. Land-use change and degradation have lately led to a more fragmented distribution of *D. ruyschiana* habitat (Evju et al. 2014), with an assessed 30-50% reduction in population size (Henriksen and Hilmo 2015). If the current distribution range of *D. ruyschiana* becomes even more fragmented, a population decline can be expected, potentially leading to a decline in genetic variation and increased genetic isolation (Rubidge et al. 2012). A combination of isolated populations and decreasing numbers of pollinators (Hallmann et al. 2017) could further restrict future outcrossing within and between populations of *D. ruyschiana*. Reduced outcrossing rates may affect fitness, such as seed production (Milberg and Bertilsson 1997; Castro et al. 2015).

Review studies show a general positive relationship between population size, genetic variation and fitness, meaning larger populations tend to have higher levels of genetic variation and higher fitness (Leimu et al. 2006). Indeed, higher seed production was found in larger, and more

genetically diverse populations of *D. austriacum*, suggesting that genetic diversity might directly affect plant fitness and populations within this genus (Dostálek et al. 2010, but see Plenk et al. 2019). This finding could also be expected for the close relative *D. ruyschiana*, though further studies will be necessary to confirm this.

### Implications for conservation in Norway

Mimura et al. (2017) highlight the importance of conserving genetic variation and suggest monitoring of intraspecific genetic variation at global, regional, and local scales. *Dracocephalum ruyschiana* is threatened both at regional scales (for example Henriksen and Hilmo 2015) and continental scales (Europe; Bern Convention list I). This is the first genetic study of the species, and the first aimed at guiding monitoring and conservation plans. To prioritize among populations in conservation genetics, the delimitation of management units (MUs), defined as populations with different allele frequencies, has been suggested as a suitable conservation approach (Moritz 1994). Based on an interpretation of genetic structure and diversity in Norwegian populations and regions of *D. ruyschiana*, we presently recognize at least four management units corresponding to the groups recognized at  $K=4$  (“orange”, “blue”, “purple”, and “green”) by Structure (Fig. 3). The Structure result showed that as the number of  $K$  increases, more sites seem to split off in separate genetic groups. Nevertheless, the four groups cover all regions and genetic extremes (Fig. 4) studied so far. However, spatial genetic structuring may also be present even at a local scale (Minasiewicz et al. 2018; Tero et al. 2005) and regions such as the valleys stretching north-westwards, represented by few sites and/or individuals, should be further explored for undetected genetic variation in *D. ruyschiana*.

Current demographic monitoring of *D. ruyschiana* is concentrated on sites in the regions Oslofjorden and Randsfjorden, which together cover the two main genetic groups recognized (“orange” and “blue”, respectively). The southernmost sites in Tyrifjorden seem to be the most genetically distinct sites in this region, with site T17 Vik being the most distinctive (Fig. 4); however, none of these sites are covered by ongoing monitoring. Hence, as a minimum, site T17 Vik should be included in the current monitoring program. Sites from the Hedmark region are also distinct in the analyses, and inclusion of site H43 Solberg in the monitoring program would increase the coverage of genetic variation monitored in Norway. At the same time, monitoring is unduly intense in some areas and some of the sites could be excluded based on genetic similarities, especially from Randsfjorden (Fig. 4). Criteria for exclusion should be based on representativity of habitat types and population states.

The SNP markers used were developed to guide monitoring of genetic variation within Norway and are based on individuals collected throughout the Norwegian distribution (Kleven et al. 2019). If genetic monitoring of the species is implemented in Norway, the markers are well suited for the purpose, as they can easily be applied to additional sites and/or used to monitor the species through time as the results are reproduceable. However, applying other genomic tools could likely aid in identifying locally adaptive genetic variation, revealing other management units (Funk et al. 2012). Genetic analyses of *D. austriacum* using AFLP markers, demonstrated that neutral and adaptive genetic variation was not correlated (Bonin et al. 2007). Thus, considering both neutral and adaptive genetic variation may be necessary to best conserve the evolutionary potential of a species (Razgour et al. 2018; Mable 2019). Furthermore, increasing our understanding of genetic structure and adaptive variation in *D. ruyschiana* on both regional and global scales, requires the application of modern genomic tools as well as phylogeographic analyses on a global dataset.

In 2009, Norway updated its laws on management of biodiversity (The Biodiversity Act (Naturmangfoldloven 2009)). The new act aims to give the authorities the possibility to halt the loss of biodiversity, including genetic diversity, through protection and sustainable use of natural resources. *Dracocephalum ruyschiana* is one species protected by this law. However, studies show a positive correlation between human population density and the loss of rare species (Thompson and Jones 1999), and as *D. ruyschiana*'s core distribution overlaps with the most densely populated areas of Norway, the species is still under pressure of habitat loss and fragmentation (Evju et al. 2014). Loss of populations may have negative consequences for future communities and ecosystems (Des Roches et al. 2018). Therefore, active measures must be applied to prevent further fragmentation and loss of populations of *D. ruyschiana*. To better protect the species from becoming locally extinct and conserve its evolutionary potential throughout its distribution range, ecologists, geneticists, and conservation managers should work together to develop an integrated, interdisciplinary framework to better inform monitoring programs and conservation actions (Flanagan et al. 2017; Razgour et al. 2018).

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