

FPS COST Action FP1202

**Strengthening conservation:
a key issue for adaptation of marginal/peripheral populations
of forest trees to climate change in Europe (MaP-FGR)**

SHORT TERM SCIENTIFIC MISSION REPORT

***Insights into the evolutionary dynamics affecting populations from
the rear edge of *Picea* sp. European distribution***

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STSM Start/End: 09.10.2015/08.11.2015

Abstract

The purpose of my visit to the Dr. Vendramin's lab was to carry out molecular analyses in their well-equipped molecular labs in order to compare the genetic features of remnant, highly fragmented populations of *Picea omorika*, a coniferous species endemic to the Balkans, with those of marginal populations of *Picea abies* (L.) Karst. from the same area in the Balkans, and to analyze molecularly individuals from a single, newly discovered population, which cannot unambiguously be assigned to *P. omorika* nor to *P. abies*. The samples of *P. omorika* comprised 735 individuals from 19 populations, and those of *P. abies* 241 individuals from 20 populations. Molecular tools comprised 9 nuclear microsatellites, 3 chloroplast microsatellites and a mitochondrial locus. Insights into genetic patterns in these populations are required for ensuring their long-term survival in terms of global climate change.

1. Introduction

Genetic structure of extant populations of forest tree and other species is determined by range and population dynamics driven by various factors including Quaternary climate changes (Cheddadi et al. 2006; Magri et al. 2006; Tollefsrud et al. 2009), and evolutionary factors such as natural selection and adaptation to local environments, genetic drift and gene flow (Savolainen et al. 2007). The effective gene flow, which may be expected in conifers due to the rather high dispersal ability of their pollen and seeds, is likely to constrain divergence through homogenizing allele frequencies across space especially at local scales. However, gene flow is usually more limited in fragmented landscapes (Young et al. 1996) typical for marginal populations at the limits of the species' range distributions that are more susceptible to genetic drift and are usually found in less favourable environmental conditions and subjected to different regimes of natural selection as compared to central populations (Savolainen et al. 2007; Eckert et al. 2008). Such populations as well as populations of species with a narrow distribution are expected to be most severely affected by the global climate change (Alberto et al. 2013).

2. Objective of the STSM

Two species of the genus *Picea* (Pinaceae) are present within the refugial Balkan region – southern Norway spruce, *Picea abies* (L.) Karst., having a wider central- and southern-European distribution with fragmented marginal populations confined to the Balkans, and *Picea omorika* (Panč.) Purk. endemic to this region and represented with c. 30 remnant populations scattered within an area of c. 10.000 km². My goal is to compare the genetic features of fragmented populations of these two species with contrasting ranges which are both found within the same region. Although the sizes of their pollen and seeds are comparable suggesting similar dispersal abilities, previous studies on *P. abies* populations revealed weak genetic structuring at nuclear genome at micro-geographic scales (Unger et al. 2011; Scalfi et al. 2014), while surprisingly high genetic differentiation and almost complete lack of pollen flow was observed in *P. omorika* based on EST-SSR variability (Aleksić et al. 2009; Aleksić and Geburek 2014) as well as seed flow based on variability at the maternally inherited mitochondrial locus (Aleksić and Geburek 2010, 2014). The opposite findings, however, were observed at allozyme (Ballian et al. 2006) and chloroplast microsatellite loci in *P. omorika* (Nasri et al. 2008). These data as well as inference on populations' history are essential for defining suitable conservation measures which would enable long-term survival of populations of these species in terms of a global climate change (Alberto et al. 2013).

In addition, individuals from a single newly discovered population, which cannot unambiguously be assigned to *P. omorika* nor to *P. abies*, were analyzed as well in order to resolve their currently unknown taxonomic status. The finding of such

individuals further supports the view that the Balkan region, which is a well-known European centre of speciation and diversification (Griffiths et al. 2004), is not only genetically understudied but also botanically unexplored even with respect to coniferous species.

3. Links with Cost Action FP1202 MaP FGR

In accordance with the main goal of the Cost Action FP1202 MaP FGR – Strengthening conservation: a key issue for adaptation of marginal/ peripheral populations of forest trees to climate change in Europe (MaP-FGR), populations of two *Picea* species with contrasting natural ranges - *Picea omorika* (a coniferous species with extremely limited natural range within the Balkans) and southern *Picea abies* (having marginal populations confined to the Balkans), were analysed at the molecular level in order to provide scientifically based guidelines for their conservation and long-term survival in terms of changing environment. Suitable conservation measures would also be applied to the single population comprising individuals of currently unknown taxonomic status.

4. Materials and methods

The sample of *P. omorika* comprised 499 individuals from 10 populations (50 individuals per population) which were used for previous work and were already analyzed with 5 EST-SSRs and a mitochondrial locus (*nad1* intron2) (Aleksić and Geburek 2010, 2014), 203 individuals from 8 populations sampled during 2013, and 33 individuals from one population sampled during 2014 (735 individuals in total from 19 populations). The sample of *P. abies* comprised 241 individual from 20 populations (nine to 19 individuals per population) scattered throughout the Balkans which were sampled during 2014 and 2015. Eleven individuals from a single population, comprising individuals of unknown taxonomic status, were used as well.

Molecular tools comprised 9 nuclear microsatellites (EST-SSR loci WS0019.F22, WS0022.B15, WS0023.B03, WS0053.K16, WS0073.H08, WS00111.K13, WS00716.F13 and WS0092.A19 of Rungis et al. 2004, and a locus SpaGG03 of Pfeiffer et al. 1997). Chloroplast microsatellites comprised 3 loci (Pt71936, Pt26081 and Pt63718) of Vendramin et al. (1996), while mitochondrial marker was the second intron of the *nad1* gene (*nad1* intron 2) amplified with primers of Sperisen et al. 2001. *P. omorika* samples already analysed with 5 EST-SSRs of Rungis et al. (2004) were additionally analysed with four new nuclear microsatellites and three chloroplast microsatellites, while the remaining samples of this species were analysed with all nine nuclear microsatellites, chloroplast and mitochondrial markers. Selected samples of *P. abies*

and individuals of unknown taxonomic status were analysed with all markers. Altogether, more than 9000 data accessions were generated.

DNA extractions from plant tissue (silica-gel dried needles) homogenized with TissueLyzer (QIAGEN, Valencia, CA, USA) was performed using Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). Multiplex PCR reactions, performed using Type-it Microsatellite PCR kit (Qiagen, GmbH, Hilden, Germany) and following manufacturer instructions, were performed with a PTC100 thermal cycler (MJ Research, San Francisco, CA, USA) and were optimized separately for *P. omorika* and *P. abies*. Fragment analyses were carried out with a 3500 Genetic Analyzer (Applied Biosystems, Inc., Foster City, USA). Scoring of alleles was performed with GeneMapper ver. 4.0 (Applied Biosystems, Foster City, USA).

Data analyses - assessment of levels of genetic diversity, genetic structuring and gene flow with standard population genetics softwares (e.g. Arlequin 3.11, Excoffier et al. 2005, GENEPOP 4.0, Rousset 2008), Bayesian approaches (e.g. STRUCTURE, Pritchard et al. 2000, InSTRUCT, Gao et al. 2007) and Approximate Bayesian computation (ABC, Beaumont et al. 2002) for inferring population histories are in progress.

5. Results

In progress.

As planned jointly with Dr. Vendramin, the most of the lab work regarding data generation on *P. omorika* was done during this one-month visit, and more than 9000 data accessions were obtained. They have to be checked, harmonized and assembled with already available data set on this species, and used for data analyses which will be carried out in December.

The remaining *P. abies* individuals will be analyzed later, most likely with a help of a PhD student from Dr. Vendramin's group. They will be used to compare the genetic features of fragmented populations of *P. omorika* and *P. abies* which have contrasting ranges but are both found within the same region. In addition, these data will be assembled with already available data set on *P. abies* populations from Europe in order to compare the genetic patterns in populations of this species from the main range and rear-edge. These results will be presented in at least 2 scientific publications.

Finally, we found that individuals of unknown taxonomic status (11 individuals from a single location) most likely represent *P. abies* because they share alleles at nuclear and chloroplast genomes not with *P. omorika* but with *P. abies*. These findings provide a basis for a potential characterization of these individuals as an infraspecific

taxon of *P. abies*, which would be accompanied with their characterization at morphological and anatomical levels.

6. Discussion

In progress.

As discussed with Dr. Vendramin, data set on *P. omorika* generated during this one-month visit is sufficient for preparation of the first manuscript on this endangered species with a very limited range which is highly threatened by the global climate change. Based on field observations, drying of trees caused most likely by the climate warming is becoming more common at several localities, and urgent actions on *ex situ* conservation of this species (via seed storage), establishment of seed orchards and/or enlargement of number of individuals grown in botanical gardens throughout Europe are required. The present work is essential for depicting the source populations and formulating sample sizes for such actions because previous work on this species revealed 2 deeply divergent gene pools at the mitochondrial level (Aleksić and Geburek 2010), and exceptionally high genetic differentiation of populations at the nuclear genome along with almost complete lack of pollen and seed flow (Aleksić and Geburek 2014).

Data set on *P. abies*, which will be completed during the next year, is important because marginal populations of this species originating from the Balkans have rarely been studied genetically. The sample size may even be increased during the next year in case that the planned field trips with the members of Dr. Vendramin's team take place. This data set will provide new insights into genetic patterns in marginal populations of this species from the Balkans, and will be used for comparisons with genetic patterns observed in *P. omorika* and thus, it would be possible to compare evolutionary dynamics in the rear-edge populations of *Picea* sp. European distribution.

Finally, further work on morphological and anatomical characterization of individuals from a single population, which most likely represent a specific form of *P. abies*, will provide new insights into genetic, morphological and anatomical variability in this species.

7. Conclusions

As a result of the one-month visit, a large amount of data was generated, and they represent a part of a broader working plan which will be finalized during the next year or two. I am very pleased for having the opportunity to visit Dr. Vendramin's lab,

to work in well-equipped labs, and to discuss on-going and future projects with Dr. Vendramin and his team. This represents a sound foundation for future joint EU projects and collaboration on other forest tree species and their marginal populations.

8. References

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