



Research Networking Programmes

Short Visit Grant or Exchange Visit Grant

(please tick the relevant box)

Scientific Report

The scientific report (WORD or PDF file – maximum of eight A4 pages) should be submitted online within one month of the event. It will be published on the ESF website.

Proposal Title: Assessing diversity patterns of potential adaptive value during the development of *Silene ciliata* (Caryophyllaceae) seedlings.

Application Reference N°: 4794

1) Purpose of the visit

Adaptation plays a critical role in the responses of species to new environmental conditions imposed by climate change. It has been argued that populations that occur at the range limits may have great adaptive value as they are found under environmental conditions that are limiting for the species (Barton 2001; Sexton 2011). Nevertheless, this is a controversial statement because it is also foreseeable that populations inhabiting marginal environments at the range limit may present low genetic diversity on which natural selection can barely operate (Brown 1984; Leimu & Fischer 2008). Consequently, it is critical to assess the adaptive genetic variation in marginal populations in order to determine their potential to adapt to ongoing climate warming. This knowledge will support decision making when developing conservation strategies.

High-mountain environments are ideal focus of studies on the potential evolutionary responses of plant populations to future climate change. Altitude acts as a surrogate of environmental change, which is an exceptional tool for evaluating species responses to oncoming warming and for corroborating evolutionary hypotheses (Körner 2007). *Silene ciliata* Pour. (Caryophyllaceae) is a Mediterranean high-mountain plant threatened by global warming. Seedling stage of the species is subjected to a large selective pressure showing great mortality during the first year of life. In this sense, previous studies have shown local adaptation patterns in seed germination and seedling performance in

marginal populations situated in the periphery of its range at Central Spain (Giménez-Benavides et al. 2007). Consequently, *S. ciliata* is a good model organism to evaluating mountain species responses to oncoming warming and for corroborating evolutionary hypotheses.

Taking *Silene ciliata* as study species, the purpose of the visit was to identify genes expressed during the development of *S. ciliata* seedlings, examine the diversity patterns of potential adaptive value in central and peripheral populations of the species and identify loci that may be involved in local adaptation processes. With this purpose, a massive sequencing of the transcriptome of seedlings of this species and their functional annotation were carried out.

REFERENCES.

- Barton N, 2001. In: Integrating Ecology and Evolution in a Spatial Context, (eds Silvertown J, Antonovics J), pp. 365–392. Blackwell, London.
- Brown, JH, 1984. *American Naturalist* 124: 255–279.
- Giménez-Benavides L et al. 2007. *Annals of Botany* 99: 723-734.
- Körner C. 2007. *Trends in Ecology & Evolution* 22: 569-574.
- Leimu R & Fischer M, 2008. *PLoS ONE* 3 : e4010.
- Sexton JP et al., 2011. *PNAS* 108: 11704-11709.

2) Description of the work carried out during the visit

For the transcriptome analysis we used RNeasy Plant Mini Kit (QIAGEN) to extract and isolate RNA from 6 seedlings grown under controlled conditions (one for each of the 6 study population: 1 central and 1 peripheral in three mountains). “Central” populations represent the environmental optimum for the species and are located at high elevations, whereas “peripheral” populations represent marginal environmental conditions (warmer and drier) and are located at low elevations. One sequencing run was carried out in an Illumina platform through 100 bp paired-end reads.

RNA-seq reads were aligned to the genome of *Silene latifolia* using a reference-based transcriptome assembly strategy and program BWA (Griffith et al. 2010). Polymorphisms in candidate genes between low and high populations (i.e., outlier SNPs) were identified using Reads2snp (Gayrial et al. 2013). The program package VCFtools (Danecek et al. 2011) was used to estimate the genetic variation at the whole genome scale and to assess the hypothesis in which low populations have lower genetic diversity than higher populations.

Contigs containing outlier SNPs (hereafter candidate genes) were aligned to protein databases such as Swissprot, KEGG or NCBI Nr protein using BLASTX. We retrieved proteins with the highest sequence similarity with the given candidate gene. All candidate genes were gene ontology (GO)-annotated using Blast2GO (Conesa et al. 2005). GO term enrichment analyses were performed using Fisher's exact tests as implemented in Blast2GO. P-values were corrected for multiple testing using the Benjamini & Hochberg false discovery rate.

REFERENCES.

- Conesa A. et al. 2005. *Bioinformatics* 21: 3674-3676.
Danecek P. et al. 2001. *Bioinformatics* 27: 2156-2158.
Gayrial P. et al. 2013. *Plos Genetics* 9: e1003457.
Griffith M. et al. 2010. *Nature Methods* 7, 843-847.

3) Description of the main results obtained

The genetic diversity within each population and elevation was similar (mean heterozygosities \pm SD, 0.22 ± 0.08 and 0.28 ± 0.02 for high and low elevations respectively). After several filtering process we identified a total of 147 118 SNPs distributed throughout 12 688 contigs (mean =13.7). On the basis of the allele frequency differences, 163 candidate genes containing polymorphisms strongly associated with elevation were selected. Gene ontology searches were successful for 143 candidate genes, and these were enriched for response to stress (n =59) and to abiotic stimulus (n = 53). These candidate SNPs and associated genes to stress responses will facilitate subsequent steps to confirm ecological adaptation at marginal populations (i.e. low) situated in the periphery of *S. ciliata* range at Central Spain.

4) Future collaboration with host institution (if applicable)

The stay has been very useful to gain practical experience in NGS data analysis and to strengthen the collaboration between the Area of Biodiversity and Conservation of Rey Juan Carlos University and the Plant Ecological Genetics group of ETH Zurich. Both at the personal and team levels, future collaboration with the genomic studies on *Silene ciliata* is expected. To validate the accuracy of SNP calling and allele frequency estimates from deep RNA sequencing, the genotyping of the selected SNPs and associated candidate genes will be extended to a larger number of individuals of each of our study populations (e.g., 20 individuals x 6 populations).

5) Projected publications / articles resulting or to result from the grant (*ESF must be acknowledged in publications resulting from the grantee's work in relation with the grant*)

The results of the stay will be presented at the 13th European Ecological Federation (EEF) conference that will take place in Rome from 21st - 25th September 2015. As long as SNPs accuracy is validated we expect our results will be published as a research article in journals such as PLoS ONE, *Molecular Ecology* or *BMC-Journals*. ESF and Research Networking Programme ConGenOmics will be acknowledged in all future publications that might result from this collaboration.

6) Other comments (if any)

Due to calendar impediments with the technician helping in the lab work a change in the dates of the visit was authorized by ESF. Final dates were from 19th January 2015 to

20th of April 2015 instead of the initially proposed dates (starting date: 3rd November 2014).