



Research Networking Programmes

Short Visit Grant or Exchange Visit Grant

(please tick the relevant box)

Scientific Report

Scientific report (one single document in WORD or PDF file) should be submitted online within one month of the event. It should not exceed eight A4 pages.

Proposal Title: Local adaptation and genetic structure in natural *Pinus pinaster* stands

Application Reference N°: 4390

1) Purpose of the visit

In this ConGenOmics project, I pursued two aims:

1) Investigate the strength of the spatial genetic structure (SGS) caused by micro-environmental heterogeneity within populations of *Pinus pinaster* and the likely selective drivers underlying SGS at functional SNP markers. Previous to the exchange visit I conducted an intense sampling (over 450 trees) in three stands and characterized abiotic (e.g. elevation, aspect, slope) and biotic (e.g. vegetation cover, species richness) factors around each sampled tree, as well as individual phenotypes in adaptive traits (wood density and water use efficiency, WUE (measured as carbon isotope ratio, $\delta^{13}C$), growth of tree rings). For the exchange visit a dataset of genotypes derived from a 384-SNP assay (Illumina VeraCode® technology) enriched with adaptive genes in forest trees was available.

2) Evaluate the genome-wide genetic response of trees to contrasted drought conditions. To accomplish this goal, pools of individuals in a paired design (25 individuals per pool; three pools in dry, south-facing slopes, versus three pools from shady, north-facing slopes) were planned to be included in a RAD sequencing experiment. Genome-wide SNP frequency data would allow us to study divergent selection between contrasted habitats at short spatial scales.

2) Description of the work carried out during the visit

During the exchange visit at WSL in Birmensdorf, I carried out the data analyses to accomplish the first goal. During discussions mainly with Dr Felix Gugerli but also

with Dr Rolf Holderegger and Dr Christian Rellstab, the most adequate analytical methods were chosen to correlate genetic structure of *Pinus pinaster* and phenotypes with environmental factors. Specifically, partial Mantel tests (Smouse et al. 1986), multiple regression of distance matrices (MRDM, Legendre et al. 1994) and a Redundancy Analysis (RDA, Legendre & Legendre 1998) were applied to identify environmental factors that shape the genetic structure at short spatial scales. In order to explicitly address isolation by adaptation (IBA) and exclude the effect of pure isolation by distance (IBD) due to limited dispersal, I controlled for the effect of geographic distance in each analysis. Multiple linear regressions were used to investigate the relationships of phenotypes and environmental factors. Finally, Latent Factor Mixed Models (LFMM, Fricot et al. 2013) were conducted to correlate allele frequencies of individual SNPs with the most important environmental factors. However, this analysis did not reveal any SNP significantly associated to elevation in Eslida which was previously identified to be the most important environmental gradient of those studied.

With respect to the second aim of the exchange visit, the projects FLAG (ANR, France) / TipTree (BiodivERsA ERAnet), within which the genetic data were foreseen to be produced, had to change strategy. This has delayed the process of generating the genomic data. Recent publications pointed to problems and low reliability of RAD-sequencing. Gautier et al. (2013) and Arnold et al. (2013) showed that allele drop out, i.e. the failure to detect existing alleles, caused by mutations within the restriction sites, generates a bias in allele frequency estimation. Therefore, the samples which had already been collected will be genotyped by an exome capture approach at RAPID genomics, which will yield ~10,000 SNPs per sample. Compared to RAD-sequencing this approach allows the genotyping of individuals (pooling of samples from the same population is not necessary) and it is more cost efficient.

3) Description of the main results obtained

In summary, the only environmental factor identified to shape the genetic structure consistently by all methods (Mantel tests, MRDM and RDA) was altitude and only in the population of Eslida, which had the steepest altitudinal gradient. Variance partitioning (using partial RDA) revealed ~ 30% of the explainable variance to be attributed to altitude, ~ 66% to geographic position and ~3 % to both due to collinearity.

We hypothesized that micro-environmental topographic variation, probably associated with water availability, might shape the genetic structure within natural stands of *Pinus pinaster*, hence inducing a pattern of isolation by adaptation. However, we did not find any combination of the environmental variables measured to be relevant to shape pine population genetic structure. Only altitude in Eslida was consistently associated with the observed genotypic pattern. Altitudinal gradients are known to shape the population genetic structure of plants (reviewed in Ohsawa & Ide 2008). However, population structure due to elevation at such a small spatial scale in a species with high gene flow, even when weak, is remarkable.

A number of environmental factors could co-vary along the altitudinal gradient in Eslida, which could lead to divergent selection between upper and lower parts of slopes, e.g. a decrease in total atmospheric pressure, temperature reduction, increasing solar radiation, and increasing UV-B radiation with altitude (Körner 2007). Assuming a lapse rate of - 0.6 °C per 100 m altitude denotes a temperature difference of ~1.8°C between low and high altitudes in Eslida. Furthermore, soil depth and associated water and nutrient availability probably increase with distance to hilltop in the study area. Abiotic

conditions shape biotic responses. Especially temperature is an important factor triggering the flowering phenology in pine species (Jackson & Sweet 1972), which influences the mating system and the spatial genetic structure. Gauzere et al. (2013) found differences in the mating system within and among populations along a ~300 m altitudinal gradient of *Fagus sylvatica*, another wind-pollinated species, with seeds at high altitudes being more often the product of immigrating pollen than at low altitudes. Furthermore, they found lags in the flowering phenology of several days between trees at different altitudes. Lags in the flowering phenology might lead to temporally conditioned assortative mating (Gérard et al. 2006), which could shape population genetic structure along environmental gradients in the absence of divergent selection (Soularue & Kremer 2012). However, also selection at genes related to flowering phenology has been described along altitudinal gradients (Alberto et al. 2013). Therefore, both adaptive and/or plastic responses in the flowering phenology could lead to assortative mating and the observed population genetic structure in *Pinus pinaster*.

Furthermore, *P. pinaster* in Eslida grows in a region with a high frequency of stand-replacing fires and exhibits serotinous cones (Hernández-Serrano et al. 2013; Budde et al. 2014). This cone type remains closed and forms a canopy seed bank until high temperatures (e.g. during a fire event) trigger the opening and seed release (Lamont et al. 1991). Synchronized seed release after fire events might facilitate selection acting on a high diversity of genotypes covering the ground in each micro-environmental patch. Selection is strong on early life stages in these populations (Vizcaíno-Palomar et al. submitted). Soil depth, water and nutrient availability, and solar radiation are important factors in early life stages and determine the successful establishment of seedlings in micro-sites (Gómez-Aparicio 2008). Hence, we would expect stronger selection at high altitudes and stony slopes due to harsher conditions for early life stages.

No correlation between genetic structure and environmental factors was found in Serra Calderona and Sinarcas, the other two populations studied. We assume that environmental gradients in these populations were not sufficiently pronounced to generate a detectable pattern. Hence, gene flow might exceed the migration–selection equilibrium in these stands, or the relevant environmental variables have not been measured in this study.

Altitude also significantly influenced the growth of tree rings in *P. pinaster* in Eslida, while $\delta^{13}\text{C}$ was significantly related to slope in this stand. Growth of tree rings was positively affected by altitude and might reflect less stressful conditions at least for adult life stages in upper elevations. Water use efficiency (as evaluated by $\delta^{13}\text{C}$) was positively correlated with slope which indicates a more efficient use of water at steeper slopes.

In Serra Calderona, slope also significantly affected wood density. As it had to be expected, trees at shallow slopes exhibited significantly less wood density while trees at steeper slopes had higher wood density due, at least in part, to slower growth. The phenotypic relationship to environmental factors is probably a plastic response to different environmental conditions, although given our results based on potentially functional markers, genetic adaptation cannot be fully excluded.

4) Future collaboration with host institution (if applicable)

My visit to WSL has served to reinforce the collaboration of this institution with INIA-CIFOR, my home research centre. We are planning to continue collaborating in the

framework of a new recently submitted project. The proposal entitled "Future trees for future climate? Adaptive genetic variation in *Pinus cembra* in response to environmental gradients across the Alpine timberline ecotone (PiCadapt)" has been submitted to the Swiss National Science Foundation and it is planned to apply similar methodologies, as those used during my visit, to *Pinus cembra*, an Alpine species. INIA-CIFOR participates in this project as "External collaborator".

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*)

Budde KB, Heuertz M, Gugerli F, Hernández-Serrano A, Pausas JG, Verdú M, González-Martínez SC (in preparation) Local-scale genetics — genetic structure in natural *Pinus pinaster* populations at short spatial scales.

6) Other comments (if any)

Both, Katharina Budde and her supervisor Dr Santiago C. González-Martínez, held seminar talks at WSL and participated in discussions concerning the research of the spatial genetic structure of trees at short spatial scales and the implication of environmental factors as driving forces shaping the genetic patterns.

References

Alberto FJ, Derory J, Boury C et al. (2013) Imprints of natural selection along environmental gradients in phenology-related genes of *Quercus petraea*. *Genetics*, 195, 495–512.

Arnold B, Corbett-Detig RB, Hartl D, Bomblies K (2013) RADseq underestimates diversity and introduces genealogical biases due to nonrandom haplotype sampling. *Molecular Ecology*, 22, 3179–3190.

Budde KB, Heuertz M, Hernández-Serrano A et al. (2014) In situ genetic association for serotiny, a fire-related trait, in Mediterranean maritime pine (*Pinus pinaster*). *New Phytologist*, 201, 230–241.

Frichot E, Schoville SD, Bouchard G, François O (2013) Testing for associations between loci and environmental gradients using Latent Factor Mixed Models. *Molecular Biology and Evolution*, 30, 1687–1699.

Gautier M, Gharbi K, Cezard T et al. (2013) The effect of RAD allele dropout on the estimation of genetic variation within and between populations. *Molecular Ecology*, 22, 3165–3178.

Gauzere J, Klein EK, Oddou-Muratorio S (2013) Ecological determinants of mating system within and between three *Fagus sylvatica* populations along an elevational gradient. *Molecular Ecology*, 22, 5001–5015.

Gérard PR, Klein EK, Austerlitz F, Fernández-Manjarrés JF, Frascaria-Lacoste N (2006) Assortative mating and differential male mating success in an ash hybrid zone population. *BMC Evolutionary Biology*, 6, 96.

Gómez-Aparicio L (2008) Spatial patterns of recruitment in Mediterranean plant species: linking the fate of seeds, seedlings and saplings in heterogeneous landscapes at different scales. *Journal of Ecology*, 96, 1128–1140.

Hernández-Serrano A, Verdú M, González-Martínez SC, Pausas JG (2013) Fire structures pine serotiny at different scales. *American Journal of Botany*, 100, 2349–2356.

Jackson D, Sweet G (1972) Flower initiation in temperate woody plants. *Horticultural Abstracts*, 42, 9–24.

Körner C (2007) The use of “altitude” in ecological research. *Trends in Ecology & Evolution*, 22, 569–574.

Lamont B, Maitre D Le, Cowling RM, Enright NJ (1991) Canopy seed storage in woody plants. *The Botanical Review*, 5, 277–317.

Legendre P, Legendre L (1998) *Numerical Ecology*. Elsevier, Amsterdam, Netherlands.

Legendre P, Lapointe F, Casgrain P (1994) Modeling brain evolution from behavior: a permutational regression approach. *Evolution*, 48, 1487–1499.

Ohsawa T, Ide Y (2008) Global patterns of genetic variation in plant species along vertical and horizontal gradients on mountains. *Global Ecology and Biogeography*, 17, 152–163.

Smouse P, Long J, Sokal R (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology*, 35, 627–632.

Soularue J-P, Kremer A (2012) Assortative mating and gene flow generate clinal phenological variation in trees. *BMC Evolutionary Biology*, 12, 79.

Vizcaíno-Palomar N, Revuelta-Eugercios B, Zavala MA, Alía R, González-Martínez SC (submitted) Early survival in maritime pine: the role of local adaptation and microenvironmental variation.