

FELLOWSHIP FINAL REPORT

Adaptive strategies of forest trees to climate changes:
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ABSTRACT

Larix decidua, the European larch, is an excellent model to evaluate the association between genetic and phenotypic variation with environmental gradients in forest species. In the present work we evaluated the genetic variation of neutral and selective SNP markers together with the variation of eight quantitative traits along an altitudinal gradient in a natural population of this species located in the Provence-Alpes-Côte d'Azur Region (France). Four samples of about 200 trees each were obtained respectively from plots situated at 1350, 1700, 2000, and 2300 m above sea level. In each sampled individual four tree ring variables and four plasticity variables were evaluated. The molecular dataset consisted of the individual patterns of 46388 SNP loci. The joint analysis of molecular and quantitative trait data allowed evaluating population structure, detecting presumptively selective loci, and demonstrating the adaptive significance of the quantitative variables considered.

1- Introduction

Climate change is increasingly impacting forest ecosystems, raising important questions about the adaptive capacity of tree species. Understanding the respective roles of genetic variation and phenotypic plasticity is therefore essential.

Forest trees growing along altitudinal gradients provide valuable models to study adaptation to environmental variation. In this context, the European larch (*Larix decidua*) is particularly suitable due to its wide distribution across elevation ranges.

This study aims to investigate genetic and phenotypic variation along an altitudinal gradient by combining SNP markers and quantitative trait analyses, in order to assess

population structure and identify signatures of adaptive variation.

2- Experimental details

1. Identification of selective loci. Outlier loci were identified with the software Bayescan 2.1 (Foll and Gaggiotti 2008). This method estimates F_{ST} for each SNP locus, establishes the general trend for neutral loci and identifies outliers which may correspond to loci under diversifying (highest F_{ST}) or stabilizing selection (lowest F_{ST}).

2. Association between quantitative traits and SNP loci (GWAS). The association between SNP loci and quantitative trait loci was evaluated using two different packages of the R software (ver. 4.5.1) (R Core Team 2025) : *NAM* (Xavier et al. 2015) and *rrBLUP* (Endelman 2011). *NAM* allows user to take into account prior information regarding population

stratification (sampling sites) to relax the linkage phase assumption. The function *GWAS* of *rrBLUP* performs genome-wide association analysis based on the mixed model applying ridge regression approach.

3. Neutrality test for quantitative trait differentiation. The sampling design involved a hierarchical system, that allows the partition of total variance of phenotypic traits in two **observable components** corresponding to the variances between and within sites. A kinship matrix between individuals estimated from the SNP data allowed to obtain the corresponding **causal components** (within and between sampling sites) of genetic variance. From these components, the genetic structure for quantitative traits (Q_{ST}) were obtained according to Spitze (1993). The neutrality test consisted of the comparison between the Q_{ST} of quantitative traits and the F_{ST} obtained for neutral SNP loci. Two approaches were applied to conduct this analysis, (1) univariate tests involving trait by trait comparison of Q_{ST} with the F_{ST} , and (2) a multivariate dual test (Martin et al. 2008), based on the proportionality (ρ) between the among-population (D) and within-population (G) genetic covariance matrices of phenotypic traits, where $\rho = D/G$ is expected to be equal $2F_{ST}/(1-F_{ST})$. The matrices D and G were estimated using the R package *MCMCglmm* (Hadfield 2010). The proportionality between D and G was evaluated by a maximum likelihood test with the function *prop.vcv.test* of the package *vcvComp* (Le Maitre and Mitteroecker, 2019), and ρ was obtained with the function *scaling.BW* of the same package.

3- Results and discussion

Molecular data consisted of 46388 SNP loci studied in four samples of about 200 trees each obtained respectively from plots situated at 1350, 1700, 2000, and 2300 m above sea level. The analysis conducted with Bayescan showed a general trend to a low but highly significant genetic structure with an average $F_{ST} = 0.004$. Only 197 SNPs (0.4%) showed a significant departure from this trend, with high F_{ST} estimates ranging from 0.02 to 0.05, suggesting the occurrence of diversifying selection on genome regions linked to these loci.

The analysis of association between SNP loci and quantitative traits (GWAS) was conducted by two methods, respectively with the R packages *NAM* and *rrBLUP*. The results from these approaches were only partially consistent. Only a few SNPs showed significant association with the quantitative traits evaluated. According to the outcome from *NAM* package the trait associated with the lowest number (2) of SNPs was the plasticity of early wood density, whereas plasticity of late wood density was the trait with highest number (81) of associated SNPs. In the case of the *rrBLUP* approach the number of SNPs associated with each trait ranged from 13 (late wood density) to 30 (plasticity of early wood density). The consistency between both approaches was in average 14% (range= 3 – 21%), and those SNPs that were significantly associated with each trait in both analysis are considered as candidate markers for future studies. The number of candidate loci ranged from 1 (plasticity of early wood density) to 13 (ring wood density).

Both univariate and multivariate neutrality tests based on the $F_{ST} - Q_{ST}$ comparison yielded consistent results supporting the view that the distribution of the quantitative traits evaluated cannot be explained solely on the basis of interaction between gene flow and genetic drift. In all cases the genetic structure of quantitative traits was much higher than the expectation for a neutral model ($Q_{ST} \gg F_{ST}$). This difference was much higher for the multivariate analysis. The proportionality coefficient (ρ) between the matrices of genetic variance between and within sampling sites retrieved from the multivariate analysis was also much higher than the expected from the F_{ST} estimate also rejecting the neutrality hypothesis. In conclusion both univariate and multivariate Q_{ST} estimates were significantly higher than the F_{ST} coefficient, rejecting the hypothesis of neutrality for the phenotypic differentiation among sampling sites.

4- Conclusion

European larch is a key forest tree species all over the Alps and in certain regions of Central Europe. As one of the very few deciduous conifer species, it confers a very distinctive appearance to the Alpine landscapes (Garbarino et al 2013). In the southwestern part of its

natural area, Larch is distributed along elevation gradients starting as low as 1200 m and culminating as high as 2500 m. Elevational gradients are the most powerful natural experiments to test ecological responses to geophysical influences, such as temperature (Körner 2007).

The larch altitudinal gradient is an essential project of the INRAe Val de Loire. For more than 10 years, the INRAe team and collaborators have gathered a unique database of complex phenotypic and genotypic data of high scientific interest.

The joint analysis of the distribution of variability at quantitative traits and molecular data along environmental gradients is a powerful approach to evaluate the nature of genetic polymorphisms in forest species even in cases where the matrix of additive relationships between individuals is absent. In the present project dendroecology, genomic ecology, quantitative and population genetics have been associated. The results attained so far identify candidate genome regions associated to quantitative traits of adaptive relevance. The $F_{ST} - Q_{ST}$ comparison produced strong evidence for the adaptive significance of the quantitative trait variation.

These results should be confirmed on a cross transplantation trial throughout an elevation gradient installed in 2013 and 2014 where family information is available.

5- Perspectives of future collaborations with the host laboratory

A cross transplantation trial throughout an elevation gradient installed in 2013 and 2014 constitutes one of the two experimental supports of the research proposal. Adult larch trees have been sampled and geo-referenced (GPS records) on a mountain slope near Briançon, Hautes-Alpes, at four elevation levels (plots) (1350, 1700, 2000, and 2300 m asl respectively). A sample of 200 trees were identified and measured at each elevation for dendroecological and genetic studies. Increment cores were collected and microdensity profiles have been already obtained. Microdensitometry profiles retrospectively describe the history of tree response to environmental changes. These

microdensity profiles have been already obtained by Dr. Philippe Rozenberg's team. A collaborative analysis of this trial would provide a test for the predictions derived from the research conducted within the frame of the present project.

Currently, the research teams of UMR 0588 BIOFORA, ONF, INRAE, Orléans, France and Departamento de Ecología, Genética y Evolución, Universidad de Buenos Aires are working together in the redaction of papers reporting the results attained.

6- Articles published in the framework of the fellowship

Manuscripts in preparation

Vilardi, JC ; Benoit, V ; Rogier, O ; Saidman, BO ; Pâques, LE ; Pometti, C ; Scotti, I ; Rozenberg, P. Landscape genomic analysis of a European larch population along an elevation gradient. Manuscript in preparation

Vilardi, JC; Benoit, V; Rogier, O; Saidman, BO; Pâques, LE; Pometti, C; Rozenberg, P. Evidence of local adaptation of wood traits in a structured population of the European larch (*Larix decidua*). Manuscript in preparation

Oral communications

Vilardi, JC. Strategies to identify adaptive trait variation in forest tree populations. Studium Thursdays. Orleans, France, May 5, 2022

Vilardi, JC. Eco-Genetic Studies in Woody Species of the Genera *Prosopis* and *Acacia* in the Ecoregions of Chaco and Monte. Internationalisation des recherches sur la forêt en région Centre-Val de Loire. Le Studium Workshop, Orleans, France, June 9, 2022.

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