

Cost action number: COST Action FP1202

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### 1. Purpose of the STSM;

The main purpose of the Short Term Scientific Mission (STSM) was to deepen the knowledge about the spatial genetic structure, the extent of the introgression between *Pinus sylvestris* and *Pinus uncinata* in two marginal populations in Spain, and how introgression may pose a threat to the conservation of marginal *P. uncinata* stands.

### 2. Description of the work carried out during the STSM;

Data from seven paternally inherited cpSSRs (Vendramin et al., 1996) were analyzed in a total of 797 individuals. Those individuals belonged to two different population of *P. uncinata* mixed with *P. sylvestris*: Sierra Cebollera (*P. uncinata* in Sierra Cebollera (Vpu): n=117 individuals, *P. sylvestris* in Sierra Cebollera (Vps): n = 157) and Sierra de Gudar (*P. uncinata* in Sierra de Gudar (Gpu): n=170, *P. sylvestris* in Sierra de Gudar (Gps): n=157, regeneration in Sierra de Gudar (undetermined species, Gr): n= 102). Additionally, two allopatric *P. sylvestris* populations were sampled 15 km south (Nogueruelas (Nps): n=47) and 12 km northwest (Allepuz (Aps): n=47) of the Sierra de Gudar *P. uncinata* population.

We carried out a genetic diversity analysis considering the following parameters: the polymorphisms in each population, the number of population-specific haplotypes (i.e. private), the rarefied allelic richness and rarefied private allelic richness, the average distance  $D_{sh}^2(i,j)$  between all pairs of individuals (Echt et al., 1998; Heuertz et al., 2010), the haplotypic diversity ( $H_e$ ) corrected for the sample size, and pairwise genetic differentiations between populations based on unordered ( $F_{ST}$ ,  $G_{ST}$ ) and ordered ( $N_{ST}$ ) alleles (Hardy et al., 2003, Pons and Petit, 1996). Spatial genetic structure (SGS) within populations was assessed following Vekemans and Hardy (2004). All the calculations were performed using the programs SPAGeDi (Hardy & Vekemans 2002) and HP Rare (Kalinowski, 2005). The Bayesian genetic clustering program BAPS v6.0 was used to undertake a non-spatial genetic mixture analysis (Corander et al., 2008) in order to cluster our different groups of individuals based on their haplotype similarities.

### 3. Description of the main results obtained:

#### **-Haplotype analysis among populations:**

Populations of *P. sylvestris* were genetically similar to each other and shared many haplotypes with the regeneration in Sierra de Gudar, while populations of *P. uncinata* were very different from all con- or allospecific populations (Fig. 1 and 2), indicating that possible introgression from *P. sylvestris* is not strong yet.

In Sierra Cebollera, three out of the four most abundant haplotypes in *P. uncinata* were shared with *P. sylvestris* and their frequencies were much lower in *P. sylvestris* (Fig 2A). In Sierra de Gudar, *P. uncinata* presented haplotypes shared with the regeneration and also with *P. sylvestris* (Fig 2B). Those haplotypes included the more

common haplotypes of *P. uncinata* but also three haplotypes that were at least four times more common in *P. sylvestris* than in *P. uncinata*. These results suggest that there is introgression between *P. sylvestris* and *P. uncinata*, although the direction seems to be mainly from *P. uncinata* to *P. sylvestris* in Sierra Cebollera, while in Sierra de Gudar introgression seems to be bilateral between both species.

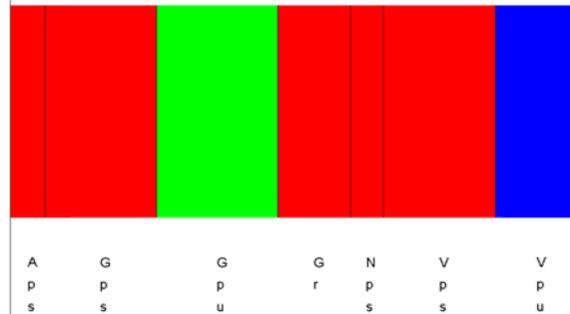


Fig 1. Bayesian genetic clustering of *P. sylvestris* and *P. uncinata* populations.

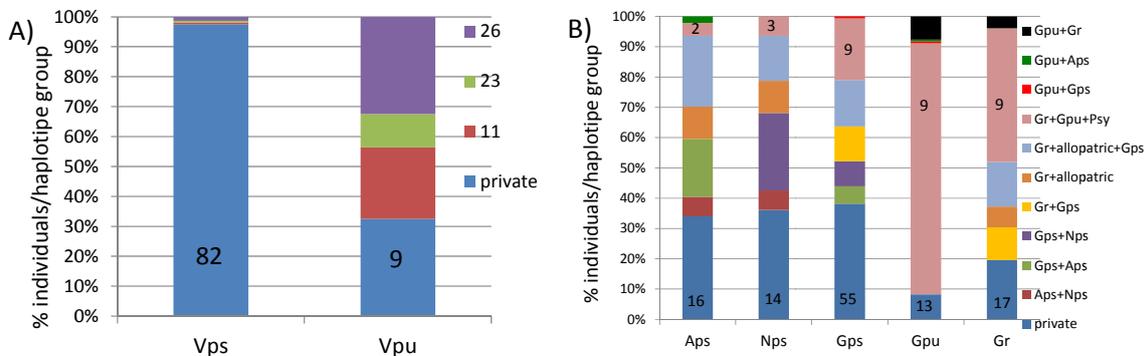


Fig 2. Proportion of the different haplotype groups in each population. A) In Sierra Cebollera. B) In Sierra de Gudar. The groups represent either the number of haplotype, or the group of population sharing the same haplotypes. The numbers in the bars represent the quantity of different haplotypes within the group.

#### -Diversity and differentiation statistics among populations:

Populations of *P. uncinata* presented the lowest allelic richness, private allelic richness, the lowest haplotypic diversity and they were also the most differentiated. They showed the highest number of significant pairwise differentiation ( $F_{ST}$ ) tests, together with the regeneration in Sierra de Gudar. Isolation of those populations (Heuertz *et al.*, 2010) and therefore, pronounced genetic drift could have caused this pattern. Populations of *P. sylvestris* were quite homogeneous, presenting similar composition of haplotypes.

Only the regeneration showed spatial genetic structure within the Sierra de Gudar population (Gr:  $S_p = -0.0072$ ,  $p < 0.01$ ) and also presented the highest average genetic distance, possibly due to the mixed regeneration in groups of *P. uncinata* and *P. sylvestris*, as we found haplotypes of both species.

#### 4. Future collaboration with the host institution:

The support of Dr Myriam Heuertz from the University of Fribourg (Switzerland) has been fundamental for the development of the analyses of *Pinus uncinata*. This collaboration will continue in the future in order to finish the current work but also to

continue working in another tree species complex (the *Symphonia* genus) in the framework of my PhD thesis.

5. Foreseen publications/articles resulting from the STSM:

The results of the analyses will be used in a scientific publication that compiles the work done during the Short-Term Scientific Mission, as soon as the review of the literature will be completed.

6. Confirmation by the host institution of the successful execution of the STSM:

See the other attachment.

**References cited:**

- Corander J, Sirén J, Arjas E (2008). Bayesian spatial modeling of genetic population structure. *Comput Stat* **23**: 111–129.
- Echt CS, De verno L.L., Anzidei M, Vendramin GG (1998). Chloroplast microsatellites reveal population genetic diversity in red pine , *Pinus resinosa* Ait . *Mol Ecol* **7**: 307–316.
- Hardy OJ, Charbonnel N, Fréville H, Heuertz M (2003). Microsatellite allele sizes: a simple test to assess their significance on genetic differentiation. *Genetics* **163**: 1467–82.
- Heuertz M, Teufel J, González-Martínez SC, Soto A, Fady B, Alía R, *et al.* (2010). Geography determines genetic relationships between species of mountain pine ( *Pinus mugo* complex) in western Europe. *J Biogeogr* **37**: 541–556.
- Kalinowski ST (2005). Hp-Rare 1.0: a Computer Program for Performing Rarefaction on Measures of Allelic Richness. *Mol Ecol Notes* **5**: 187–189.
- Pons O, Petit JR (1996). Measuring and Testing Genetic Differentiation With Ordered Versus Unordered Alleles. *Genetics* **144**: 1237–1245.
- Vekemans X, Hardy OJ (2004). New insights from fine-scale spatial genetic structure analyses in plant populations. *Mol Ecol* **13**: 921–935.
- Vendramin, GG, Lelli L, Rossi P, Morgante M (1996). A set of primers for the amplification of 20 chloroplast microsatellites in *Pinaceae*. *Mol Ecol*, **5**: 595–598.