STSM Reference number: **COST-STSM-FP1202-14453**

COST Action number: **FP1202**

Grantee´s name: **Ana Isabel de Lucas Herguedas**

1. Purpose of the STSM

The goal of the Short Term Scientific Mission has been to deepen the knowledge about the fire influence on genetic diversity and mating systems of peripheral and marginal populations of Mediterranean maritime pine combining for it existing genetic data (i.e. bibliographic and databases) with some experimental genetic analysis.

2. Description of the work carried out during the STSM

During this STSM we have been able to carry out all tasks proposed in the initial scientific project. Specifically:

- A comprehensive review of the literature on the fire influence on the diversity populations and mating systems has been made, proving that there are not many studies in this regard and there is very little (and old) works done on conifers, with short temporal series in its serotinous cones (ej. Cheliak et al. 1985. *Genetics*; Perry and Dancik 1985. *Silvae Genetica* or Lamont et al 1991. *The Botanical Review*).

- The already available DNAs from Sierra Bermeja peripheral population have been analyzed with new six nuclear microsatellite markers in the laboratory of Dr. GG Vendramin in the Institute of Plant Genetic in Firenze (Table 1). This task was made the first week of the STSM.

- These genetic data were added to those already existing (previously obtained for the applicant in LDG laboratory of University of Valladolid and those kindly provided by Dr. Santiago C. González-Martínez from INIA, Spain) and were refined for subsequent statistical analysis.

Table 1. Molecular data summary used in this study

Molecular marker	Laboratory
6 nuSSRs: NZPR544, A6F03, Epi3, Epi5, gPp14 and 2669	CNR-IGV Plant Genetic Institute Firenze (Italy)
4 nuSSRs: NZPR413, NZPR1078, Frpp94, Itph4516	INIA (Spain)
3 nuSSRs: Ctg4363, Ctg275 and Rptest11	LDG ITAGRA.CT-University of Valladolid (Spain)
42 polymorphic **SNPs**	INIA (Spain)

- The statistical analysis performed over our genetic data can be structured in the following three sections:

   o ***Mating systems and pollen gene pools description***

   To calculate the estimators and parameters describing the genetic diversity (H_e, A, A^P and F_{ST}) and the mating system (AS%, t_m, t_s, t_m-t_s and r_p) have been used the following programs: SpaGeDi 1.2 (Hardy et al. 2002); MLTR 3.4 (Ritland 2002); Hp-Rare (Kalinowski 2005).

- o ***Time trends and detection of singular events***

  - ▪ An **aridity index (AI)**, which encapsulates water availability as a function of temperature and precipitation, was calculated according to Eckert et al. (2010) using the climatic data from the nearest meteorological station (Malaga airport) Subsequently we have searched for significant **correlations** of this index with genetic diversity estimates and mating system parameters calculated previously.

  - ▪ The detection of **atypical data** (true outliers) were performed by Dixon´s Q-test (for detection of a single outlier) and by Mandel´s h and k statistics (to estimate the relative deviation from the mean value and the precision of the values compared to the pooled standard deviation across all groups).

  - ▪ To identify possible **trends** in the temporal data series was used the Mann-Kendall test.

- o ***Aerial seed banks: accumulation of diversity and germination rates***

  To describe the accumulation of diversity over time we have used CONTRIB 1.02 software (Petit et al. 1998). This software provides a measure of the contribution of each population to total diversity (measured the expected heterozygosity or the allelic richness). This contribution is splitted in two components: one due to the diversity of the population, the other due to its differentiation from the remaining populations.

3. Description of the main results obtained

- ***Mating systems and pollen gene pools description***

Multilocus outcrossing rates (t_m) are uniformly high among stands (=mothers) indicating near random mating. Differences between multilocus and single locus estimates (t_m-t_s) are small indicating a low level of biparental inbreeding in the three stands studied. Pairwise F_{ST} estimates among the stands are quite high showing three pollen pools genetically well differentiated although the sampled trees belong to the same population and distances are not large (ej. Figure 1).

Figure 1. Temporal series of biparental inbreeding (t_m-t_s) and correlated paternity (r_p) for tree C.
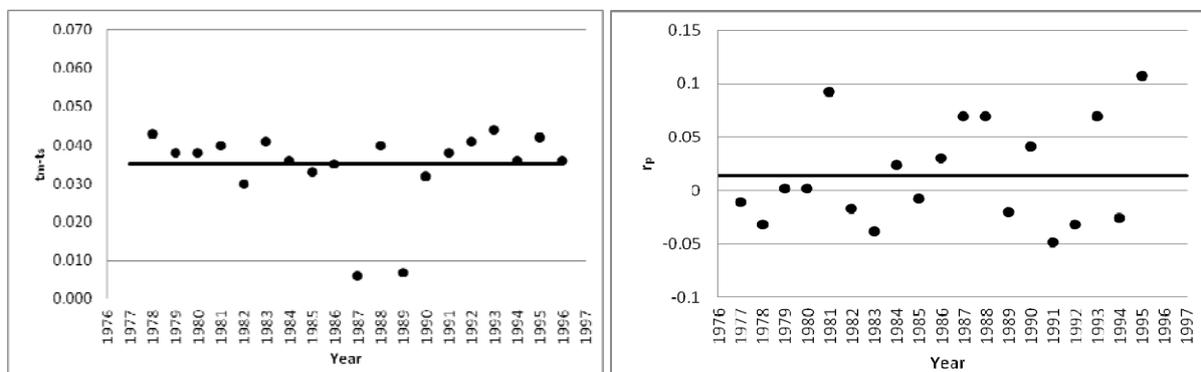
Levels of correlated paternity (r_p) among trees for both markers are next to zero supporting pairwise F_{ST} data (i.e. three pollen pools well differentiated). Moreover, correlated paternity

values within trees and averaged within cones, although higher than among trees, are low indicating that the tree crowns receive pollen from multiple male parents (Ne=1/rp ranking between 40 and 145).

- ***Time trends and detection of singular events***

Time trends

- Tree C shows a weak but significant increasing in the number of alleles over time (S=67; p=0.020)

- Tree D shows a significant decreasing in the heterozygosity level over time (S=-44; p=0.002)

- Tree D shows a significant increasing in correlated mating over time (S=38; p=0.009)

Figure 2. Significant trends in the temporal series according to Mann-Kendall test.
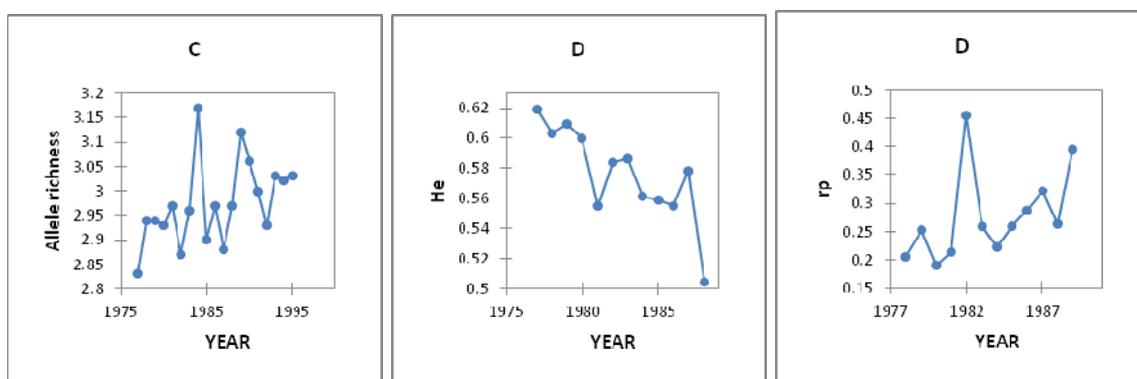

Singular events

According to Dixon´s test for outliers, there are not any statistical significant atypical data (i.e. true outliers) in the temporal series for genetic diversity estimates and mating system parameters.

On the other hand, disturbances (e.g. fires, aridity) do not seem to have a great impact on the levels of genetic diversity and mating system parameters of our population from *Pinus pinaster*. In stand B seems to be a decrease in the levels of genetic diversity (heterozygosity) and an increase in correlated paternity after the fire of 1992, but these changes in the temporal series trend are not significant. On the other hand, there was no significant correlations between AI (Aridity Index) and any of the both genetic diversity and mating systems parameters calculated.

- ***Aerial seed banks: accumulation of diversity and germination rates***

The ranges of variation of the annual contribution to the total diversity are highly variable among trees (especially for allelic richness). Furthermore, the allelic richness of trees C and D does not show the part of contribution due to differentiation.

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Figure 3. Year contributions of cones to global diversity (heterozygosity and allelic richness) for tree B following Petit et al. (1998). The contribution is represented by two components: its own diversity and its own divergence.
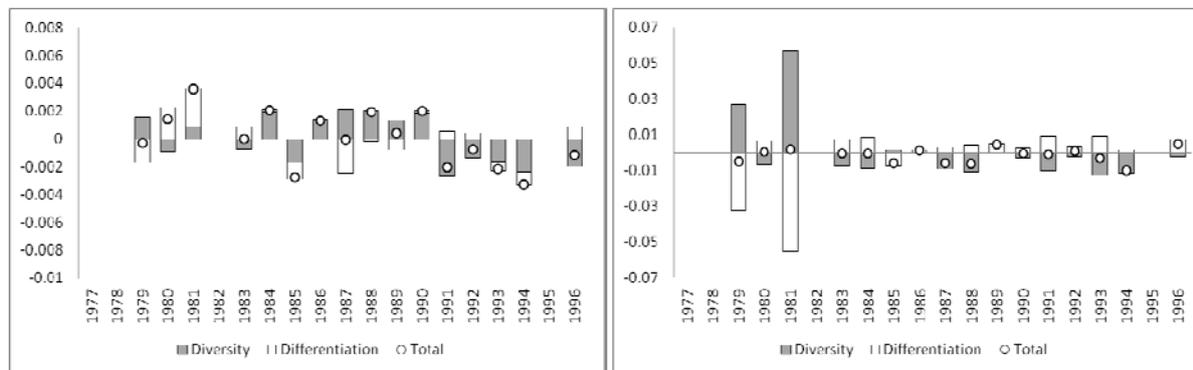

In general, and except concrete cones crops (e.g. year 1989 for trees C and D and 1980 for tree B), we have obtained high levels of germination in the seed collected (above 88%). The average number of days to germinate ranged from 0 to 26.5, with the average from trees shown in the Table 2.

Tabla 2. Germination estimates in three Mediterranean maritime pine seed pools

	% Germination	SE	Average Number of days to germinate	SE
B	91	19	14.335	4.837
C	91	22	11.609	3.228
D	88	27	17.820	2.746

In relation to time trends, tree C shows a weak but significant decreasing of viability of seeds (i.e. germination rate) with age of cones (S=63; p=0.09). This trend is also maintained when the outlier from the series (year 1989) is removed. Moreover, the mean number of days to germinate in tree B decreased significantly with seed age (S= -62; p=0.005) (Figure 4).

Figure 4. Proportion of viable seeds over time for tree C (left) and mean number of days to germinate in tree B (right)

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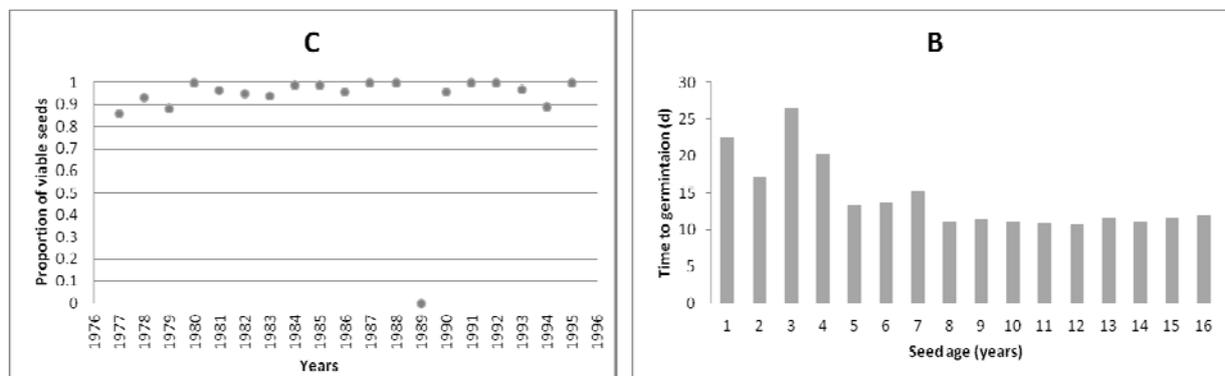
Finally, and according to Dixon´s text for outliers, there are the following atypical data (i.e. true outliers) in the temporal series.

- **Year 1980**. Significant outlier for % germination for tree B
  Year 1989. Significant outliers for % germination and average number of days to germinate for trees C and D.

- ***References* cited**

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4. Foreseen publication/articles resulting or to result from the STSM

All tasks outlined in the STSM proposal have been performed, so we have obtained enough data and results to start writing a scientific article which will be entitled and authored:

"Temporal variation of mating system and pollen gene pools in pine stands under recurrent fire. de-Lucas AI, González-Martínez SC, Vendramin GG"

5. Confirmation by the host institution of the successful execution of the STSM

See the other attachment.

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