**Strengthening conservation:
a key issue for adaptation of marginal/peripheral populations
of forest trees to climate change in Europe (MaP-FGR)**

SHORT TERM SCIENTIFIC MISSION REPORT

Analysis of the genetic diversity of wild populations of Pistacia lentiscus

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FPS COST Action FP1202Strengthening conservation: a key issue for adaptation of marginal/peripheral populations of forest trees to climate change in Europe (MaP-FGR)

COST

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Abstract (max 150 words, spacing included)

The main objective of this STSM was the study of the genetic diversity of wild populations of *Pistacia lentiscus* from Tunisia. This plant is one of the most important ecologically and economically important forests genetic resources which is part of a sustainable development plan in Tunisia involving local rural communities.

The basis of genetic data of this plant has already been developed and the work conducted under this STSM was focused on analyzing the data, with a particular emphasis on detecting the differentiation that might exist between central, marginal and disjunct populations using demographic scenarios. The use of an approximate Bayesian computation (ABC) allowed finding the relationship between marginal and central populations of *P. lentiscus* and establishing their evolutionary history over time. This STSM was an opportunity to master new software and to provide more information on the populations of *P. lentiscus*.

1. Introduction

This document reports the main scientific outcome of the short term scientific mission (STSM) undertaken by **Faten Mezni** to the National Institute for Agronomic Research in Avignon (INRA Avignon) (COST Action FP1202, 3rd February to 14th February 2016). The host in the INRA Avignon was Dr Bruno Fady. The mission focused on analyzes of the genetic diversity of populations of *Pistacia lentiscus* L. from Tunisia. This shrub belonging to the *Anacardiaceae* family and is widely distributed in the Mediterranean region. It is considered as an important economic and ecological forest resource in Tunisia (Rejeb et al., 2006; Mezghani, 1992).

2. Objective of the STSM

The goal of this STSM is to analyze the genetic diversity of the ecologically and economically important forest genetic resources of *Pistacia lentiscus* from Tunisia, which is part of a sustainable development plan in Tunisia involving local rural communities. Genetic data were already available and the STSM focused on analyzing the data, with a particular emphasis on detecting the differentiation that might exist between central, marginal and disjunct populations, and between Tunisian and other Mediterranean populations.
The STSM was an occasion to run demographic scenarios (DIY ABC) so as to explain the structure of diversity found in the dataset.

This work has already been initiated through the support of COST Action. A scientific paper has already been drafted and the results found in this short term scientific mission will be used to improve the content of this article using the Bayesian approach.

3. Links with Cost Action FP1202 MaP FGR

Marginal populations of *P. lentiscus* are an important floristic element in the vegetation and an economically important plant that requires the adequate measures of conservation and sustainable management programs. In Tunisia, this forest resource is increasingly exploited and it is subjected to overexploitation. It is also influenced by ongoing climate change in addition to the arising of human activities. To maintain and improve this local genetic

resource, there is a need to maintain its genetic diversity, which should be based on comprehensive information regarding the structure of the populations.

4. Material and methods

The work was conducted on 44 Tunisian populations of *Pistacia lentiscus*.

Pistacia lentiscus leaves were sampled from 8-10 trees in each of natural population. The DNA extraction, PCR and Microsatellite genotyping were already conducted, prior to the STSM, and the work was done on available genetic data.

4.1. Statistical analysis

During this STSM, statistical analysis were conducted using several software; GenAlEx-Genetic Analysis in Excel (offers analysis of codominant, haploid and binary genetic loci and DNA sequences and distance-base and other genetic parameters), STRUCTURE (detects the underlying genetic population among a set of individuals genotyped at multiple markers), POPULATION (allows the determination of distances between individuals and between populations), TREEVIEW (bulding of the phylogenetic trees).

4.2. DIY ABC

DIY ABC v 2.0 software was used to run demographic scenarios and to explain the structure of diversity found in the dataset.

DIYABC allows considering complex population histories involving any combination of population divergences, admixtures and population size changes, with population samples potentially collected at different times. DIYABC can be used to compare competing evolutionary scenarios and quantify their relative support, and estimate parameters for one or more scenarios. Eventually, it provides a way to evaluate the amount of confidence that can be put into the various estimations and to achieve model checking computation within an approximate Bayesian computation (ABC) framework. The version 2.0 of the program implements a number of new features and analytical methods allowing extensive analyses of large molecular datasets, including single nucleotide polymorphism (SNP) data.

5. Results

This STSM with the collaboration with INRA Avignon was successful and productive. All the planned points were carried out successfully. In addition to the genetic analysis conducted during this training, I reviewed literature and different articles disposed by the Host Institute. This part of the work was important in order to get acquainted with the DIY ABC software and the Bayesian approach. The obtained results allowed improving the content of a scientific paper already drafted and which will be submitted to a scientific journal.

During my visit to INRA Avignon, I was acquainted with the new genetic software DIY ABC. We tested several possible scenarios to define the most confident one describing the relationship between the 44 Tunisian populations of *P. lentiscus*. The most important results are summarized below:

Results from STRUCTURE revealed the presence of three homogeneous and one admixed groups of populations. The 44 populations studied were devided to 4 groups as shown in figure 1:
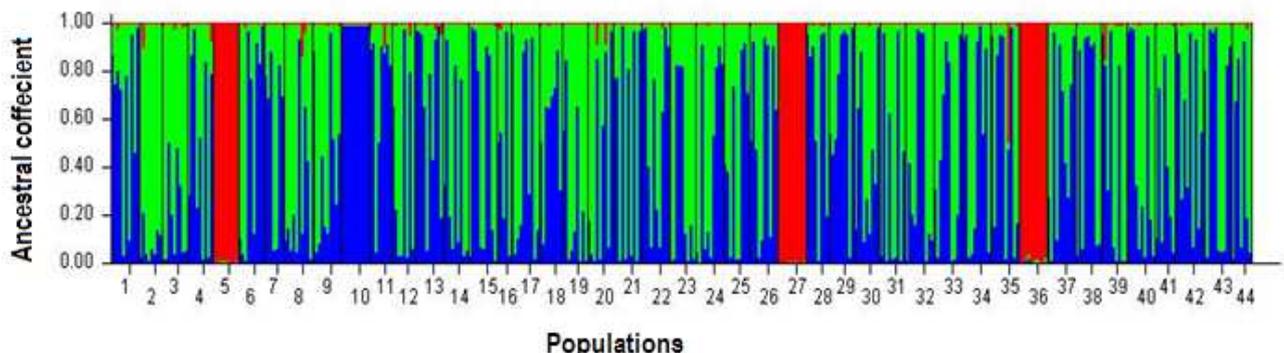

Figure 1. Grouping *Pistacia lentiscus* populations using STRUCTURE software

- **Group 1:** population 2; green color (90% of individuals are green)
- **Group 2:** populations 5, 27 and 36; red color (99% of individuals are red)
- **Group 3:** population 10; blue color (99% of individuals are blue)
- **Group 4:** other populations; green/blue color

The matrix of the fixation index (Fst), elaborated with GenAlEx, showed that the highest values were recorded between populations of the red group (5, 27 and 36) and the population of the blue group (10). The values varied from 0.197 to 0.23 indicating a high

genetic difference between the populations of the two groups. A phylogenetic tree was elaborated using POPULATION and TREEVIEW. The results showed the same grouping determined by STRUCTURE.

For each group, the Bayesian approach was conducted. All populations showed a reduction of the population size when compared to an earlier time suggesting an ancient bottleneck in the species' history. Several parameters were determined using DIYABC software such as actual number of populations (N_0), number of populations in earlier time (N_1), time of size variation (t_0), size variation (Var) and Mean Garza Williamson (MGW) (figure 2).
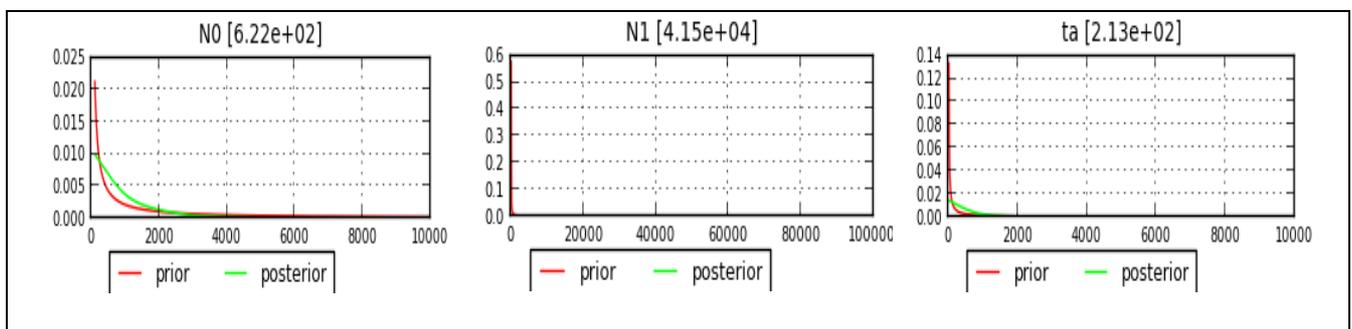

Figure 2. Pre-evaluate scenario prior combinations and estimation of posterior distribution of parameters

Several scenarios were also tested referring to STRUCTURE results to elaborate the relationship between the different groups. Group 1, 2 and 3 were shown as separate ancestral homogenous populations. Group 4 was the result of the admixture of both group 1 and 3.

6. Discussion

Tunisian populations of *P. lentiscus* showed a sharp reduction of its size when compared with its size in the past. This variation in size could be related to several reasons. Reduction in the size of a population can be due to environmental events (such as earthquakes, floods, fires, disease, or droughts) or human activities (Ambrose, 1998). Such events can reduce the variation in the gene pool of a population; thereafter, a smaller population with a correspondingly smaller genetic diversity remains to pass on genes to future generations of offspring through sexual reproduction. Genetic diversity remains lower, only slowly increasing with time as random mutations occur (William and Catton, 2009).

In consequence of such population size reductions and the loss of genetic variation, the robustness of the population is reduced and its ability to survive selecting environmental changes, like climate change or a shift in available resources, is reduced (Hufbauer et al., 2004).

7. Conclusions

The STSM allowed deepening the present investigation that demonstrates the importance of population history for understanding present-day genetic diversity within natural populations of *Pistacia lentiscus* in Tunisia, as well as for conservation biology. The reconstruction of recent historical population sizes allowed us to investigate the influence of random evolutionary processes on present-day genetic diversity in populations of this forest resource.

8. References

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