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State of the Mediterranean Forest genetic resources in a climate change context

Master in Mediterranean Forestry and
Natural resources management

Thesis

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“The mind that opens to a new idea never returns to its original size.”

ALBERT EINSTEIN

Abstract

Forest Genetic Resources (FGR) known as genetic variability found within and between species that can be profitable by human use, is the result of an evolutionary and demographic process and it is the fundamental basis for species evolution and adaptation. In this work, I performed a systematic review to infer about the current state of the FGR. For that, I did a comprehensive meta-analysis using observed and expected heterogeneity of simple sequence repeat (SSR) and isozyme markers of the retrieved data for 25 species and 381 studies. In order to infer about the population structure and we tested statically the coefficient of inbreeding (Fis). Populations of the Eastern Mediterranean countries seem to be more affected by the inbreeding. In order to manage the forest in a sustainable way it is crucial that FGR data is available and can be used by policy-makers. By identified gaps and pitfalls in existent data, this work give an important contrition to better understand of the current states of FGR and their potentiality to face climate change.

Key words: Forest genetic Resources, Mediterranean, Meta-analysis, Climate change.

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Introduction

1.1 Mediterranean biodiversity and its importance

The Mediterranean basin is considered one of the richest biological zone on Earth (Myers *et al.*, 2000). There are around 25 000 plant species which are native to the region and more than half of them are endemics. This remarkable level of endemisms can be explained by several bio-physical factors. The Mediterranean has a complex topography resulting from the Tertiary geological movements of the Eurasian and African tectonic plates, where steep mountain ranges and flat terrains can be found. The geological composition varies significantly, from most widespread limestone soils of marine origin to the less frequent siliceous and volcanic soils (Pinborg *et al.,* 2002). Mediterranean climate follows a bimodal regime, with hot and dry summers and colder and humid winter. One of the most relevant characteristics of this region is the absence of precipitation for at least 2 months each year, during the hottest season (summer in the northern hemisphere), leading to drought. Under such climate conditions vegetation is more prone to fire. In order to cope with such disturbances species developed, along their evolutionary process, well adapt biological mechanisms (Blondel *et al.,* 2010).

The myriad forms of living species found in Mediterranean are not only result of climate and soil structure but also presence of anthropogenic impact. In fact, humans have been profoundly modifying the Mediterranean landscape for millennia. The use and transformation of primary forest by the first civilizations led to alternating periods of reduction and expansion of the forest area and contributed to the reallocation and change in the dynamics of species and communities, over time and space. The current distribution of many Mediterranean species such as chestnut (*Castania sativa*), stone pine (*Pinus pinea*) and cork oak (*Quercus suber*) are probably linked more to human management than to ecological factors. These socio-economical species were not only explored for timber (used in all types of construction and furniture making industries), but also for energy (especially in the poorest northern Africa and eastern Mediterranean countries) (Blondel *et al.,* 2010).

They also provide valuable ecosystem services and non-wood forest products. Cork oak forest, for instance, is characterized by a mix of different forest types, pasture and extensive agriculture and provides a innumerable goods and services for local communities such as cork, fuel, pasture, forage, aromatic herbs, mushrooms, beekeeping, water regulation, soil protection, CO_2 fixation, nature tourism and leisure activities associated with rural areas. Thus, this type of forest has a significant impact at environmental and socio-economic context (Allard *et al.,* 2013).

1.2 The role of Forest Genetic Resources (FGR)

The wide range of good and service provided by the forest results in large part from the genetic variability found in forest plant species (FAO, 2014). The genetic diversity, known as the variation at level of individual genes, occurs within and between species and it is the result of an evolutionary process of incessant interaction between and among biological systems and their surrounding environment (Mondini *et al.,* 2009).

The variation in alleles (heterogeneity) is the crucial basis for species evolution and plays an important role in their mitigation and adaptation to changing and adverse environmental conditions (FAO, 2004). Besides, it can be the basis for germplasm artificial selection and breeding programmes and thus enhancing their productivity. The "genetic variation in trees of potential or present benefit to humans" is known as forest genetic resources (FAO, 2004). In the current work, we studied the genetic status of the forest resources in order to infer about their capability to face one of the most important threats in the Mediterranean – climate change (Allard *et al.,* 2013).

1.3 Current issues in the Mediterranean Basin

The current model of development results in a tremendous pressure on forest genetic resources, with different impacts on the north and south of the Mediterranean. In the north, there is an expansion of woodlands favored by the humid/ temperate climate of the region and by the massive abandonment of agriculture and livestock husbandry (rural exodus), led to loss of open habitats (mostly grasslands). On the other hand, in the south and east, the anthropogenic pressure (due to population increase in urban and coastal areas), land use change for agriculture, tourism together with low precipitation regimes and high temperatures lead to a serious issues of habitat/biodiversity loss and land degradation (soil erosion, salinization, loss of organic matter, acidification and desertification) (Pinborg *et al.,* 2002).

The exploration and consumption of forest genetic resources resulted not only to land use transformation and deforestation but also participated in the emission of CO_2 to the atmosphere, contributing significantly to climate change. The Mediterranean has been identified as one of the areas most affected by change in temperatures and rainfall regimes in the world (Giorgi, 2006). The most recent climate models for the Mediterranean project drier and hotter summers (due to an increase of the overall temperature) and more intense and short period of rainfall, especially in the eastern and southern countries. This could increase, even more, to the risk of fire and flooding as well as enlarge the already existent pressure on water resources. The projections show an expansion of arid zones and the contraction of the humid-ones. The displacement of species will be not only in latitude but also in altitude. In the Mediterranean, it is estimated that an increase of 1ºC in temperature could provoke the migration of certain plants approximately 180 Km to the north or 150 m in altitude (Blue Plan, 2009). This puts at risk two main groups of plants: cold adapted (mountains) and endemic species. In France, for instance it is predicted that mountain forest species will disappear by 2100 (Oesterheld et al., 2000)

The redistribution of the species and communities could also alter the distribution of their pathogens, their behavior and interaction as well as on the timing of the plant flowering and their pollinators. The reduction of water availability during long dry periods and the increase of the atmospheric temperature could lead to an increase of the evapotranspiration, modifications in the photosynthetic rate (and their ability of store carbon) and thus threat their survival (Allard et al., 2013).

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1.4 Study context: Aim and objectives

Under this scenario, will forest resources, particularly those of tree and shrub species, be able to cope with the present human and environmental pressures? How is genetic diversity geographically structured in the Mediterranean? Are there sites with higher genetic diversity than other in the Mediterranean, potentially more capable to face or mitigate the effects of the climate change?

The current work is part of an international project which aims to "maximize the production of goods and services of Mediterranean forest ecosystems in the context of climate change". My job was to compile and summarize the information available about forest genetic resources. The aim was to study the genetic diversity status of different forest populations across the Mediterranean, since they could be involved in an environmental adaptation process to climate change (FAO, 2004). For that, I carried out an exhaustive literature search following a systematic review approach. I was able to collect population genetic data from 34 years of published data of some of the most important trees and shrubs occurring in the Mediterranean. My specific objectives within this context were to 1) localize highest and lowest forest genetic diversity areas; 1) identify potential trends of genetic diversity across the Mediterranean 2) identify potential trends of genetic diversity across the Mediterranean and 3) identify gaps and pitfalls resulted from the literature analysis.

2

Materials and Methods

2.1 Project description

The international cooperation resulted from the emergent need of understanding the capability of Mediterranean forest genetic resources to cope with the ever-increasing human pressure (overgrazing, land conversion, deforestation, pollution and wildfires) and understand the effects of the climate change on the population dynamics. The project, funded by the French Global environment facility (FFEM), counted with international collaboration at different levels: the FAO committee on Mediterranean forests (Silva Mediterranea), the Collaborative Partnership for Mediterranean forests (PCFM) and the Blue Plan. The aim was to promote sustainable management of forest ecosystems by optimizing the production of goods and services (FAO, 2014).

2.2 Characterization of the study area

2.2.1 Area and distribution

The study area is the Mediterranean Basin localized between 27° to 47°N and 10°W to 7°E. The region was divided 3 main divisions: southern European, eastern and North African Mediterranean countries, to facilitate the posterior analysis (fig. 1). For the implementation of the project seven pilot sites were selected distributed by five Mediterranean Basin countries (tab.1). The pilot sites cover a forest area about 19 million hectares. The selection criteria were the following: 1) being a region representative of the Mediterranean ecosystems/bioclimatic; 2) where exist historical climate data and 3) show signs of species declining being related with the effects of climate change. The vulnerability of the pilots sites are measured according to the level of 1) threats to survival; 2) change of distribution range and 3) impacts on ecosystem functioning (goods and services provided) (FFEM, 2014).

Thus, the idea behind the sites selection was to identify that could be a model from which climatic/ species relationship could be extrapolated and give us information of the possible impact of the climatic change on the capability of species to adapt.

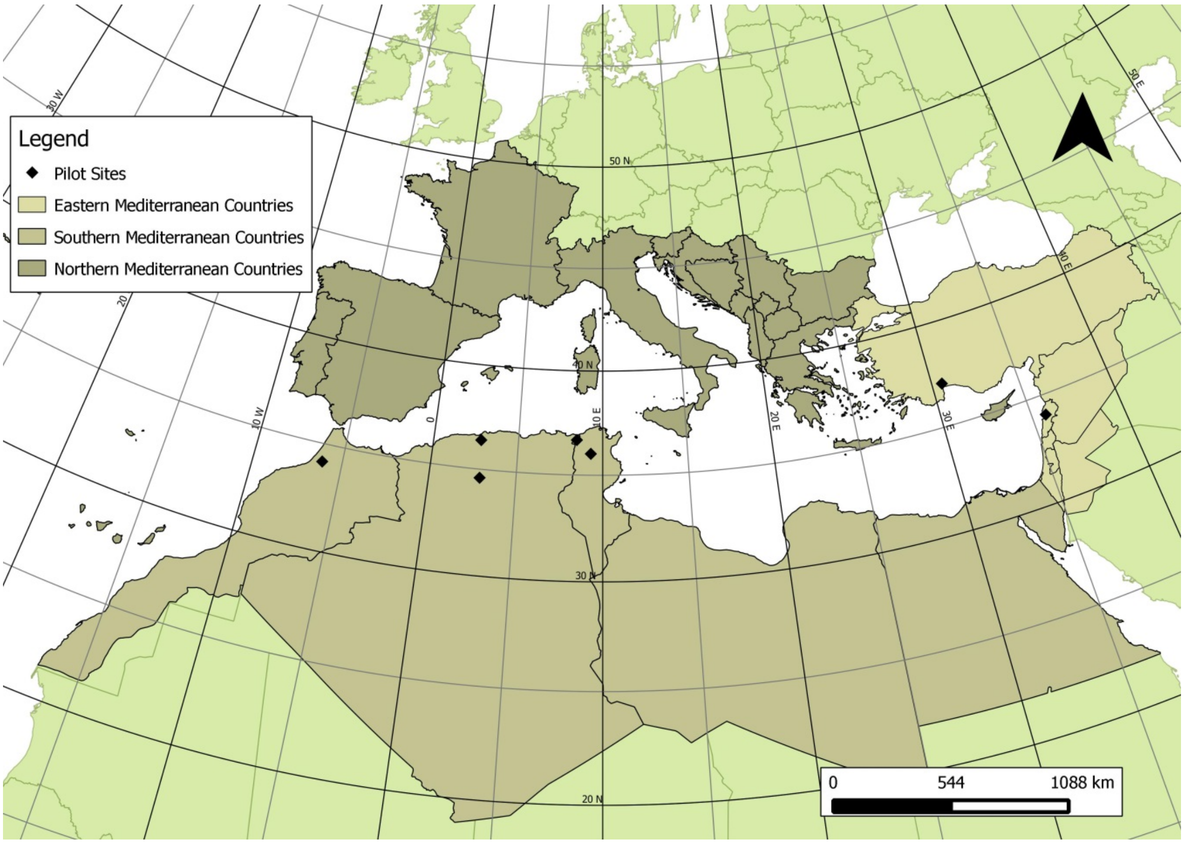

Figure 1 – Delimitation of the study area and respectively sub-division (eastern, southern and Northern Mediterranean countries), following the FAO Mediterranean countries list. The geographical position of the pilot sites are represented by the black circle (Nadine Wazen, 2014).

2.3 Species selection

For each of the seven pilot sites, it was selected the most important species - species that contribute significantly to 1) social; 2) economic and 3) ecological status of the region and being representative of Mediterranean climate (e.g. *Pinus nigra*). A total of 24 species were selected according to the criterias mentioned above (tab.1).

Table 1 - List of the 24 species most representative of the Mediterranean climate and are present among the7 pilot sites. The only exception is *Pinus nigra* which was added to the list, although it was not identify at any site (data provided by Nadine Wazen).

Pilot sites / Species name	DUZLERCAMI TURKEY	DJELFA ALGERIA	CHREA ALGERIA	JABAL MOUSSA LEBANON	MAAMORA MOROCCO	BARBARA TUNISIA	SILIANA TUNISIA
Acer hyrcanum subsp. tauricolum (Boiss. & Balansa) Yalt.				X			
Arbutus unedo L.			X				X
Cedrus atlantica (Endl.) Manetti ex Carriere			X				
Cedrus libani A. Rich.	X						
Chamaerops humilis L.			X		X		
Ilex aquifolium L.			X				
Juniperus drupacea Labill.				X			
Juniperus excelsa M.-Bieb.	X						

Juniperus oxycedrus L.		X	X				
Juniperus phoenicea L.		X					
Laurus nobilis L.			X				
Pinus brutia Ten.	X			X			
Pinus halepensis Mill.	X	X	X			X	X
Pinus nigra J.F. Arnold							
Pinus pinea L.	X					X	
Pistacia lentiscus		X	X		X	X	X
Platanus orientalis L.	X			X			
[1]Quercus coccifera L. **(sin. Q. calliprinos Webb)**				X			
Quercus cerris L.				X			
Quercus ilex L.		X	X				X
[2]Quercus canariensis Willd. **(sin. Q. mirbeckii Durieu)**			X			X	
Quercus suber L.			X		X	X	X
Taxus baccata L.			X				
Tetraclinis articulata (Vahl) Mast.			X				

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[1] [2] for these species the search were performed using both the original name and synonym.

2.4 Methods

2.4.1 Systematic Review

There are thousands of genetic diversity studies on Mediterranean plants. In fact, it is reported 66,780 worldwide and 1150 in the Mediterranean published data on the web of science, from 1900 to 2014. However, few are known about the overall state of the forest genetic resources in the Mediterranean Basin. In order to compile all existing data about the topic, it was performed a systematic review. The systematic reviews were developed early in the 80's due to the need of the medical trials update as well as retrieve high quality assessment data from specific topics (PRISMA, 2009). It differs from traditional reviews since it is based on a 1) clearly formulated question, 2) identifies relevant studies following several criterias (established according to the research topic), 3) select and critically appraisal their quality and 4) summarize the data from individual studies. It not only provides a synthesis of specific topic (reported in traditional reviews) but also describes the methodology used, reducing potential bias (Akobeng, 2005).

Thus, by synthesizing and critically appraisal all the past and on-going studies in a specific topic, reduces the time, costs and accessibility to reliable information by the interested public (e.g. researchers, consumers or policy makers). Also, it can be used to identify gaps in the available literature. The issues related with the collection and analysis of significant amount of data is that: different studies might use different methodology for the same outcome proposes. Systematic review required by the person charged to do it, significant amount of time and sometimes the collection of data can be comprised by the no existence of data or no reliable of the existent ones (Grag *et al.,* 2008).

2.4.2 Data collection

The 24 species selected previously were submitted to a comprehensive bibliography search in the Web of Science^{TM}. The aim was to get quantitative population genetic information about the current FGR status in the Mediterranean.

The search was confined to the following years: from 2009 to 2014 and the key-words used for each of the 24 species were the following: "species name" AND "genetic diversity" AND "Mediterranean".

All the data retrieved using the systematic review approach, where compiled in a database file. In here, it was possible to retrieved information about: 1) genetic diversity index used (described above); 2) molecular markers; 3) sample size; 4) geographical site locations (latitude, longitude and altitude); 5) study origin (author, year); 6) taxonomic features of the species (family, genera and species), 7) occurrence (Mediterrranean/ Eurasian), 8) lifeform, lifespan, vegetation altitudinal belts (infra, thermo, meso, supra, mountain and oro-mediterranean) and 9) polen and seeds dispersal agent. The present dataset was added to a previous database which had incorporated measurements of within population genetic diversity for 34 years, from 1980 to 2009 (Conord *et al.,* 2012).

2.4.3 Measurements and selection criteria

In order to quantitatively assess genetic variation within populations, data from two diversity estimators were used: observed and expected heterogeneity (He and Ho). The observed heterozygosity is defined as the observed number of individuals heterozygous per locus while the expected heterozygosity (or gene diversity) is obtained from the individual alleles frequencies under random mating. The values in both estimators vary between 0 and 1. The high and low genetic diversity spots were localized in maps done using QGis software version1.9.

In order to understand genetic structure of the populations, it was calculated the coefficient of inbreeding (Fis) using the raw data of He and Ho (Fig. 2). I have only used Fis due to its statistical properties (varies from -1 to +1) and the significance of its values. The negative values indicates an excess of heterozygotes while the positive ones an excess of homozygotes in comparison to what would be expected under random mating (Hardy-Weinberg equilibrium). The output values were statically tested using the Meta-analysis for Ecology and Evolutionary Biology (*OpenMEE*) software (Dietz *et al.,* 2014).

$$Fis = 1 - \frac{Ho}{He}$$

Figure 2 - Coefficient of inbreeding formula.

2.4.4 The statistical software

The open MEE software relies on statistical meta-regression so that it tries to know how well an outcome variable can be explained by one or more explanatory variables (or covariates). In our study case, we wanted to know if the genetic heterogeneity of the Mediterranean trees and shrubs species was an effect of their habitat location.

The software relies on a meta-analysis approach. Thus, it allowed us to combines and compares quantitative data across the several studies about the same subject.

The software analyzes the data by looking for an effect size. In other words, it takes in consideration the sample size of the measure estimated (e.g. observed heterozygosity) within and/or between studies by clustered them in groups. The groups are identified according to the studies ID. There are three distinct statistical models that could be selected to run the test: fixed, random or mixed effects model. The fixed effect model will treat all data as coming from the same study, whereas in the random effect the data is treated as a compilation of studies that were conducted independently about the same subject. The mixed-effect model compares the data between and within studies (groups), giving us the differences and the respectively variability. Thus, the software calculated not only the effect size (Zr), but also its associated variance (Var(Zr)).

By doing this, the analysis will give greater weight to the results from studies which give us more information (higher sample size and lower variation), giving more statistical power to those. The weights are calculated using the inverse of the variance (the square of the standard error) of the size effects, which relates closely to sample size. From the statistical operation, the variance or degree of variability of the data between studies is estimated through Tau Squared (τ^2).

2.4.5 Data analysis

In the OpenMee software I conducted two main statistical tests. Firstly, it was test the potential influence of the moderators (latitude and longitude) in the inbreeding effect (Fis). The goal was to understand if the inbreeding was 1) related with the spatial distribution of the populations and 2) whether it could result in pattern at spatial level. For that, it was performed a meta-regression analysis. The variables input in the software were the 1) coefficient of inbreeding measurements; 2) sample size; 3) geographic coordinates (latitude and longitude) and 4) study ID. The test was run as mixed-effect model, using the restricted maximum likelihood method for a level of significance 95%. The residuals were tested under a continuous random-effect model, using the DL: DerSimonian-Laird method (DerSimonian 1986), at a level of significance 95%.

In our last test, we tried to identify the more critical sites (those that the inbreeding effect in the populations are significant high). The test was run using the sub-groups method in order to compare differences between groups. Once the groups are selected and identified as a categorical variable (e.g. countries), the software analyses the different molecular markers and highlight the differences between then. The test was performed using a random effect model following the DerSimonian-Laird method (Laird & DerSimonian, 1986). Here, the input variables were the 1) coefficient of inbreeding measurements; 2) sample size; 3) geographic coordinates (latitude and longitude); 4) study ID and 5) country name.

3

Results

3.1 Systematic Review

From the online search of literature in the Web of Science^{TM}, it was found 180 papers, from a period between 2009 and 2014 and using the key-words: 1) "species name" AND 2) "genetic diversity" AND 3) "Mediterranean". From the analysis of the papers above just 22 were retrieved since they reported measurements of genetic diversity (e.g. observed heterogeneity, H0) within populations.

In the search, the highest number of studies was conducted in *Pinus halenpensis* and *Quercus ilex* while no data was found for 5 selected forest species *Acer tauricolum*, *Chamaerops humilis, Ilex aquifolium, Juniperus drupacea* and *Quercus mirbeckii* (*sin. Q.canarensis*) (fig.3).

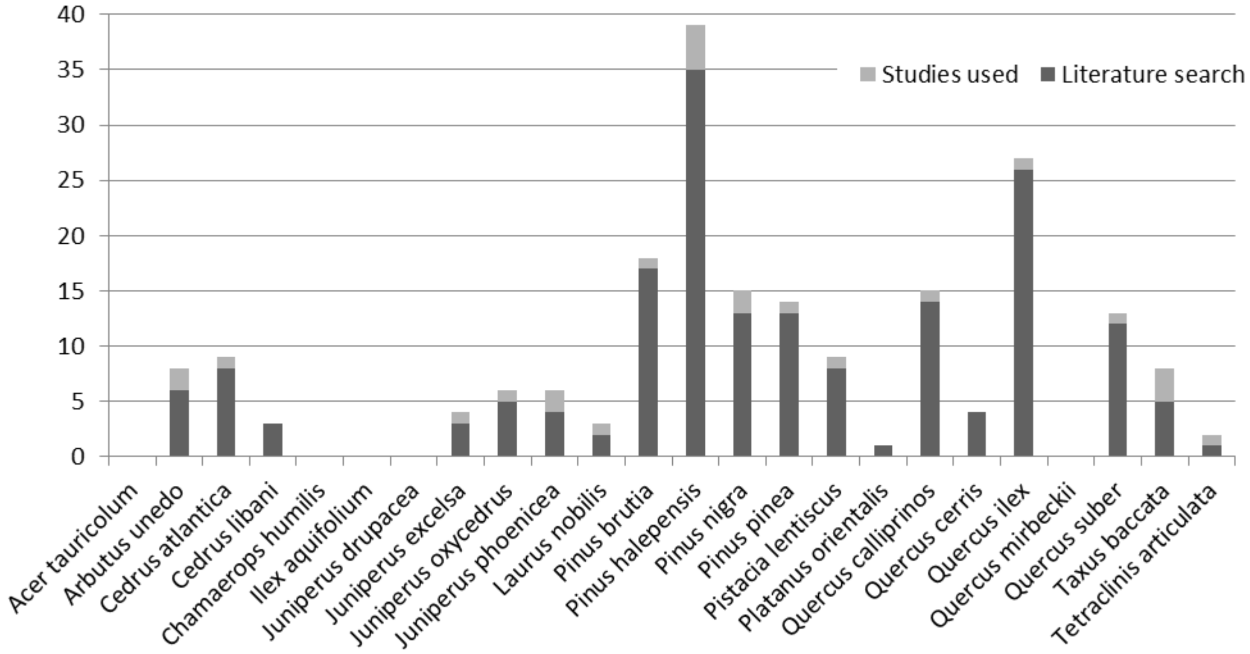


Figure 3 - Total number of studies for each of the 24 pilot site species found in the ISI Web of knowledge ™.

3.2 The Database

The data above was added to a previous database (Conord *et al.,* 2012), composed by quantitative information about genetic diversity Mediterranean trees and shrubs species from 1980 to 2009. In total, it was analyzed 280 studies, for 52 Mediterranean species which were distributed by 25 countries (see annex I). Most of studies were performed in the Northern Mediterranean Countries (166) while the Southern and Eastern Countries share approximately the same number of studies (49 and 45, respectively) (fig. 4).

Table 2 – Number of studies conducted by the different Mediterranean Regions.

Mediterranean Regions	Studies No.	Total No. Populations	Mean sample size
Southern Mediterranean Countries (SMC)	45	255	25
Eastern Mediterranean Countries (EMC's)	49	410	31
Northern Mediterranean Countries (NMC's)	166	1310	32

3.3 Measurements and Maps

The selection of the 2 diversity estimator (Ho and He) resulted in 2 types of molecular markers retrieved: SSR and isozymes of 381 populations. The output of the raw data for both estimators using the QGis software is illustrated in the following maps (fig.5 e 6).

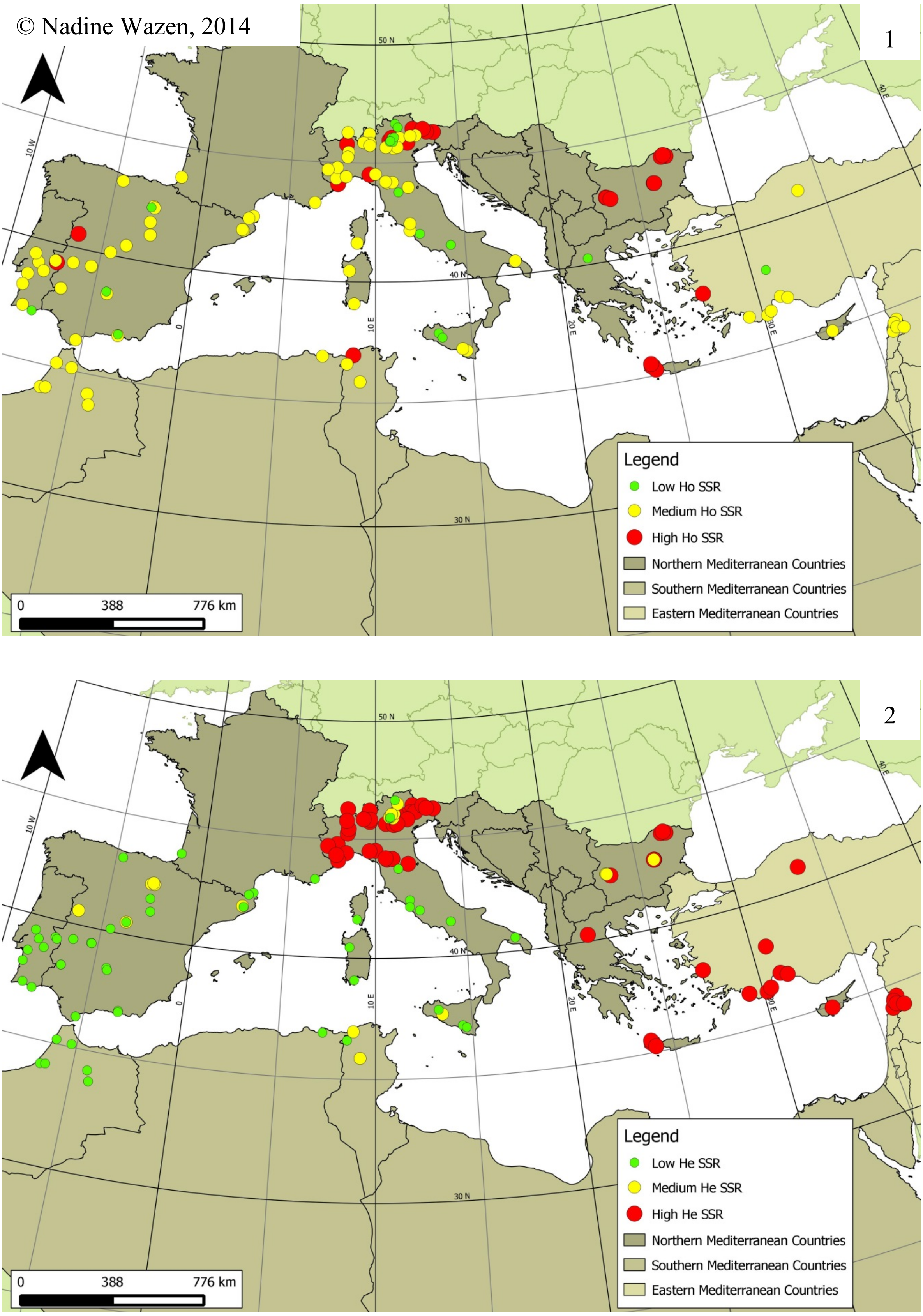


Figure 4 - Genetic diversity for SSRs molecular marker: 1) observed heterogeneity and 2) expected heterogeneity.

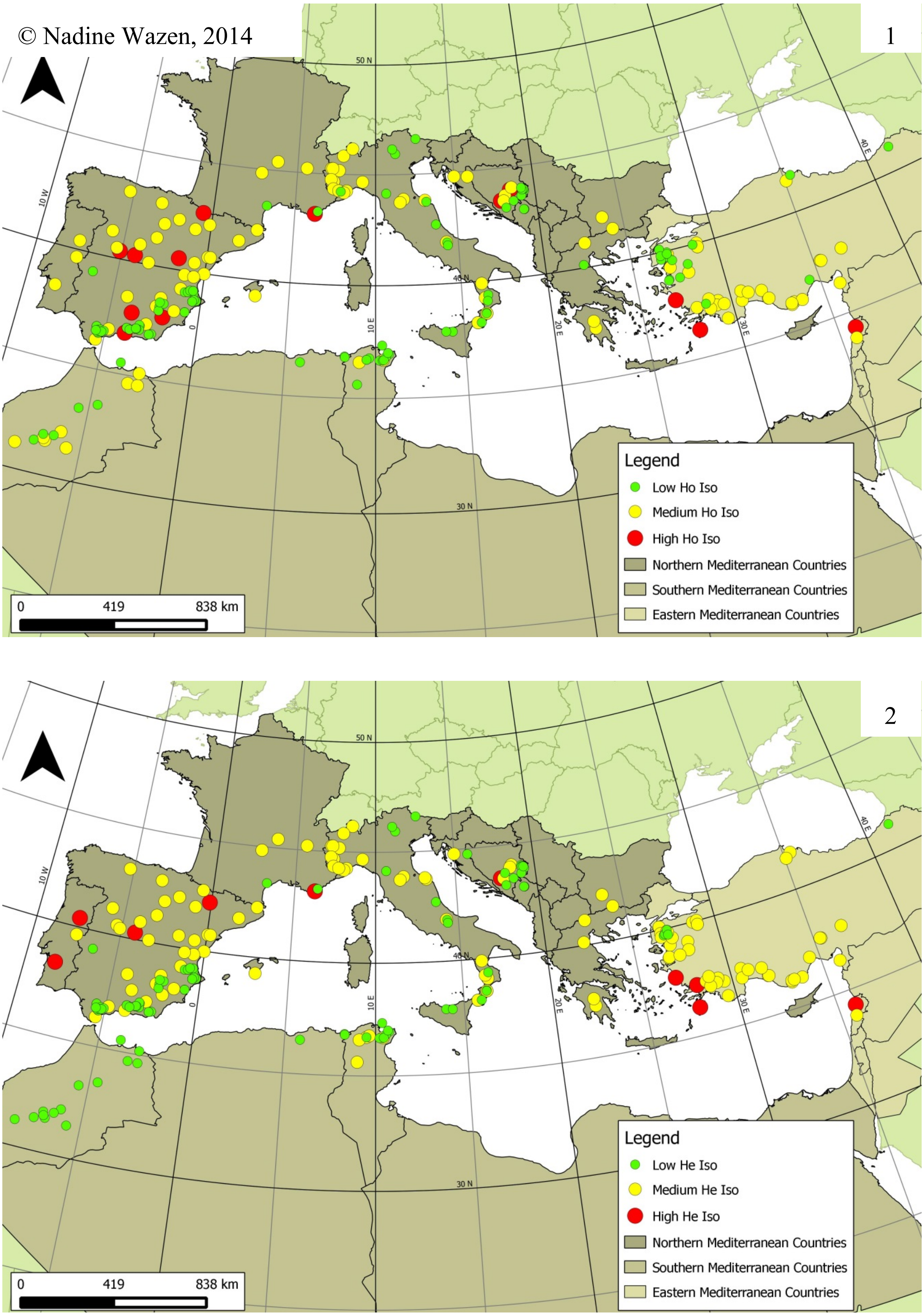


Figure 5 - Genetic diversity for isozymes molecular marker: 1) observed heterogeneity and 2) expected heterogeneity.

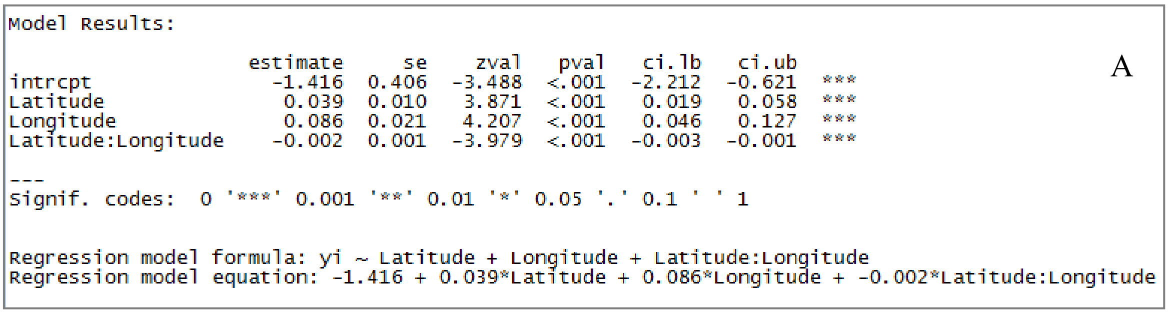
3.4 Statistical tests : Meta-Regression

The tests of the effect of latitude and longitude in the inbreeding coefficient (Fis) show a positive and significant effect of the longitude (in both SSR and Isozymes) at level of significance 0.05 (tab.2). The latitude studied independently for SSR (126 studies) show at high a positive effect (p-value = 0.001), while the interaction between the 2 moderators show slightly negative values. The interaction using isozymes (255 studies) show also a negative effect but at lower p-value (0.1).

The Fis residuals were tested using the funnel-plot methods (fig.7). The inverse funnel shape indicates the area and form that the residuals distribution should follow. Thus, population with high number of samples have a small variation (lower standard error) and are closely located to the y axis, while population with small sample size show a significant variability and are widely disperse on the bottom of the graphic (higher standard error). The graphic indicates that SSRs have a better distribution of the residuals comparing with the isozymes which the bias results are located for standard errors between 0.10 and 0.20.

By grouping the countries it seems that Italy, Turkey and Lebanon contribute significant and positively for the increase of F in the eastwards (Tab.3). On the other hand, Morocco has the most significant and negative value for F.

Tabela 3 - Statistical test for the effect of the moderators (latitude and longitude) in the FIS using molecular information of SSR (A) and isozymes (B).



```
Model Results:

                      estimate     se     zval    pval    ci.lb    ci.ub
intrcpt                 -1.416  0.406   -3.488   <.001   -2.212   -0.621   ***
Latitude                 0.039  0.010    3.871   <.001    0.019    0.058   ***
Longitude                0.086  0.021    4.207   <.001    0.046    0.127   ***
Latitude:Longitude      -0.002  0.001   -3.979   <.001   -0.003   -0.001   ***

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Regression model formula: yi ~ Latitude + Longitude + Latitude:Longitude
Regression model equation: -1.416 + 0.039*Latitude + 0.086*Longitude + -0.002*Latitude:Longitude
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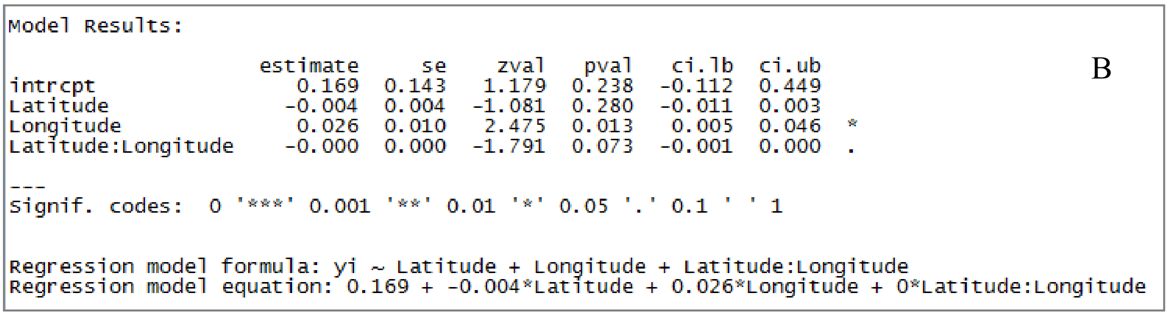

```
Model Results:

                      estimate     se     zval    pval    ci.lb    ci.ub
intrcpt                  0.169  0.143    1.179   0.238   -0.112    0.449
Latitude                -0.004  0.004   -1.081   0.280   -0.011    0.003
Longitude                0.026  0.010    2.475   0.013    0.005    0.046   *
Latitude:Longitude      -0.000  0.000   -1.791   0.073   -0.001    0.000   .

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Regression model formula: yi ~ Latitude + Longitude + Latitude:Longitude
Regression model equation: 0.169 + -0.004*Latitude + 0.026*Longitude + 0*Latitude:Longitude
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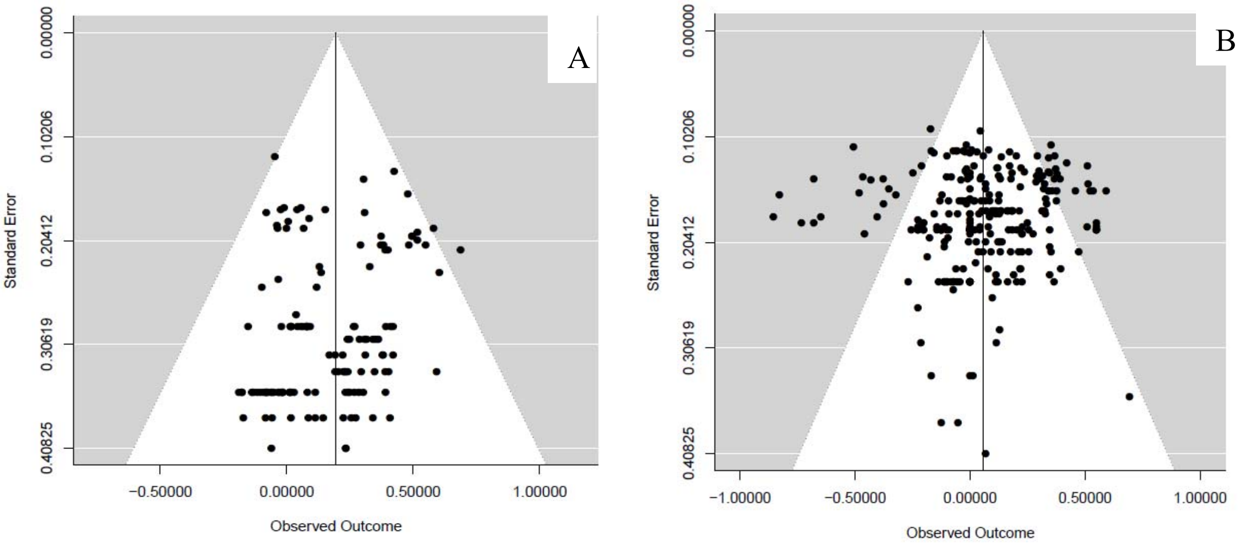
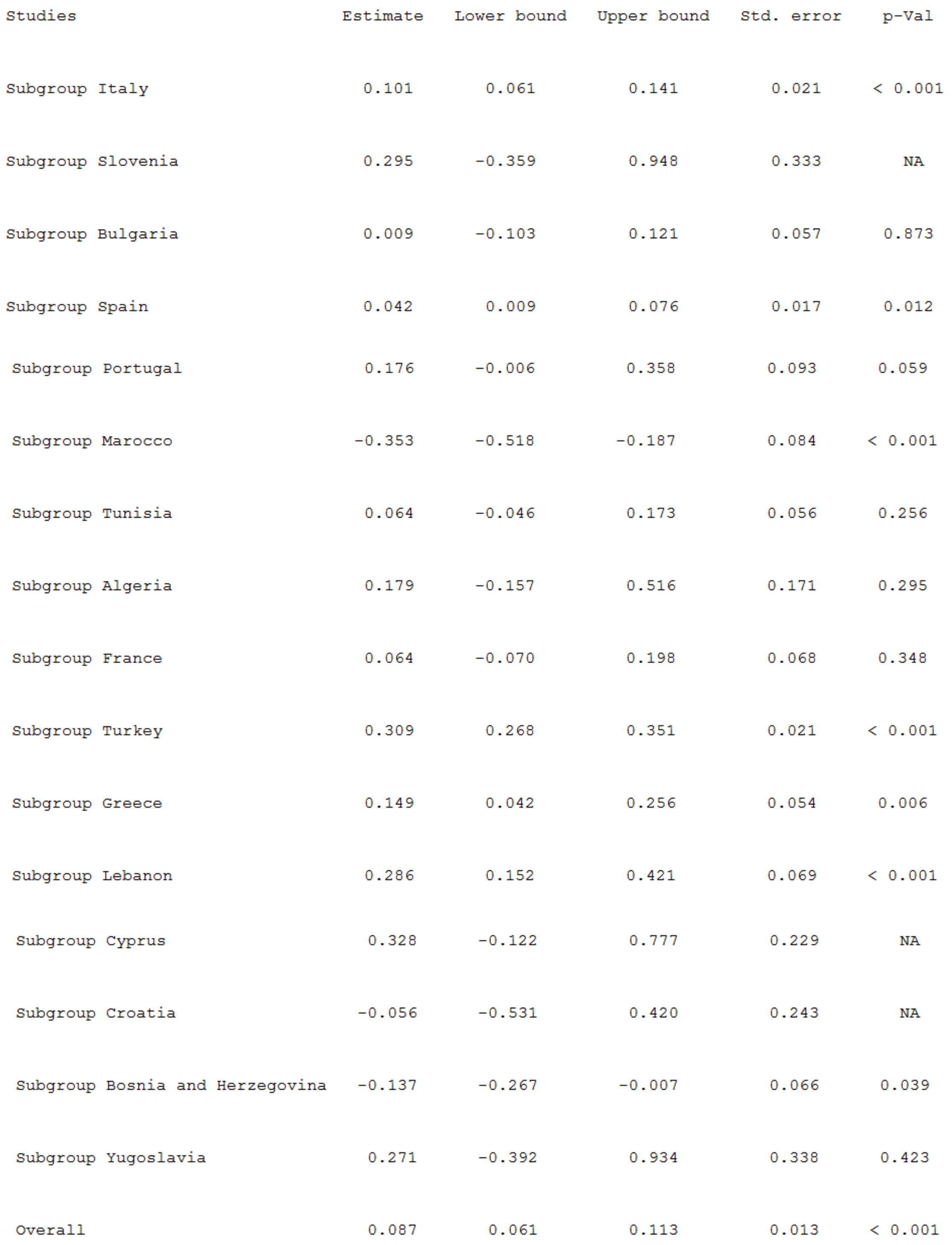


Figure 6 - Residuals test for Fis, using the funnel-plot method from OpenMee software. The black dots represent the residuals for Populations studied, using SSR (A) and isozymes (B).

Tabela 4 - Continuous random effect model for Fis values per country.



Studies	Estimate	Lower bound	Upper bound	Std. error	p-Val
Subgroup Italy	0.101	0.061	0.141	0.021	< 0.001
Subgroup Slovenia	0.295	-0.359	0.948	0.333	NA
Subgroup Bulgaria	0.009	-0.103	0.121	0.057	0.873
Subgroup Spain	0.042	0.009	0.076	0.017	0.012
Subgroup Portugal	0.176	-0.006	0.358	0.093	0.059
Subgroup Marocco	-0.353	-0.518	-0.187	0.084	< 0.001
Subgroup Tunisia	0.064	-0.046	0.173	0.056	0.256
Subgroup Algeria	0.179	-0.157	0.516	0.171	0.295
Subgroup France	0.064	-0.070	0.198	0.068	0.348
Subgroup Turkey	0.309	0.268	0.351	0.021	< 0.001
Subgroup Greece	0.149	0.042	0.256	0.054	0.006
Subgroup Lebanon	0.286	0.152	0.421	0.069	< 0.001
Subgroup Cyprus	0.328	-0.122	0.777	0.229	NA
Subgroup Croatia	-0.056	-0.531	0.420	0.243	NA
Subgroup Bosnia and Herzegovina	-0.137	-0.267	-0.007	0.066	0.039
Subgroup Yugoslavia	0.271	-0.392	0.934	0.338	0.423
Overall	0.087	0.061	0.113	0.013	< 0.001

4

Discussion

Our results from the assessment of the genetic variability using the two diversity estimators (He and Ho) reveals a positive and significant correlation for F values increasing from west to east when longitude is considered. The coefficient of inbreeding (Fis) gives us direct insight from the ratio between Ho and He. Thus, the positive estimation means that the expected heterogeneity is higher and the observed heterogeneity or there is less heterogeneity than that was expected. For this result, there is a significant contribution of 2 eastern Mediterranean countries (EMC's): Turkey and Lebanon, which for both cases showed significant and positively correlation, for a p-value of 0.001 at level of significance 0.05. Several factors could explain the previous results. Populations from the EMC's could be affected by 1) selection (either natural or antropogenical) contributing to fragmented or patchy populations; 3) limitation to gene flow (for instance from natural geographic barrier in mountain ranges), leading to habitat isolation); 5) lack of pollinators or low capacity of dispersal of the species; 6) inbreeding or 7) any other factor that could interfere in the natural random mating system of the species. (Alrababah *et al.,* 2009; Aparicio *et al.,* 2012; Boratynski *et al.,* 2009; Dzialuk *et al.,* 2011; Douaihy *et al.,* 2012; Gonzalez- Martínez *et al.*, 2010; Jaramilo-Correa *et al.,* 2010; Kurt *et al.,* 2011; Kurt *et al,* 2012; Lopes *et al.,* 2012; Marzouki *et al.,* 2009; Marzouki et al., 2009, Sanchez-Gomez *et al.,* 2013; Takrouni *et al.,* 2010; Vernesi *et al.,* 2012)

Different scenario, can be found for instance in Morroco, where there is a significant and negative correlation. It could be explained due to the fact that the species here studied (P. halenpensis and Q. suber) are closer to their last glacial *refugia* in the eastern part of the Iberian Peninsula (Médail *et al.,* 2009). Other factors can be identified such as the lack of evolution time to the inbreeding effect is genetically detected or the fact that samples could come from habitat that facilitates the pollen and seed dispersal (well-connected habitats) (Dubreuil *et al.,* 2010; Troeber *et al.,* 2011).

It was not clear the existence of south-north trend as well as the effect of the interaction when isozymes were applied. Significant results were only obtained for SSRs since most of the studies using this marker was conducted in the North Mediterranean countries (NMC’s).

The data above can also be explained by the fact that the 2 countries that more contribute to the positive Fis values, most of the studies for 207 populations used the isozymes as markers against 34 populations for SSR. On the other hand, the studies conducted in Morocco have higher number of populations using SSR in comparision with isozymes (64 and 35, respectively). Isozymes is a allelic variant of enzymes so that it is present at low rates in the organisms and the number of polymorphism per loci that can be detected is also limited, while SSR (Simple Sequences Repeats) are very abundant and allow the identification of many alleles at single locus (Mondini *et al.,* 2009). Thus, higher number of studies using SSR will have more change to show higher genetic variability comparing with isozymes. In our results, it seems SSR give us more unbiased genetic information than isozymes showed in the residuals test and in its significance and higher estimate values.

Not only the number of populations but also the sample size for each population and the species studied can have a significant impact in the results obtain. For instance, in our case the 3 countries belong to the SMC and EMC group. Here the numbers of populations as well as the average of the sample sizes for each population are similar. However, if we analysed the species that are in studies we able to identify that 2 major groups: high and low diversity species. For instance, Morocco studies’ has high diversity species such as *Quercus suber, Cedrus atlantica* and *Taxus baccata* while Turkey and Lebanon present in their studies low diversity species as *Pinus pinea* (Pinzauti *et al.,* 2012; Cheddadi *et al.,* 2009; Ramirez-Valiente *et al.,* 2009; Juan *et al.,* 2012; Rubio-Moraga *et al*, 2012; Salim *et al.,* 2010; Wahid *et al.,* 2010).

5

Conclusion

The quantitative assessment to the forest genetic resource in the Mediterranean shows very diverse patterns across the Region. The eastern Mediterranean populations seems undergo a inbreeding process or having a more structure sub-population either because of selection/ habitat isolation, few sample size and populations or due to the use of marker selected. SSR seems to be a promising molecular marker for the use in genetic diversity studies. The majority number of studies was carried out by the NMC which could explain the higher genetic diversity observed in the area. However, more studies are needed, especially in Southern and Eastern Mediterranean Countries.

The capability to adapt to the climate change depends not only on their inherence genetic diversity and demographic history but also on the socio-economic factors that could dictate the extensive forest management or its absence. In order to have a sustainable management of the forest it is needed a "dynamic conservation approach". This should promote silviculture pratices that preserve or enhance the genetic pool of forest resource to mitigate or allow the species adaptation to environment changes. My work gives an important contribution towards better managing of the forest, by identifying the hotspots of genetic diversity of Mediterranean populations and give insights of their vulnerability to face climate change.

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Index I

The pilot sites and respectively countries selected for the implementation of the study.

Country	Pilot Site	Geographic coordinates
Algeria	Chrea	36º23’10”N; 2º47’31”E
	Djelfa/Senalba	34º35’31”N; 2º54’43”E
Lebanon	Jabal Moussa	34º 3’ 47” N; 35º46’ 43” E
Marocco	Maamora	34º 8’ 59” N; -7º 51’ 55” E
Tunisia	Barbara	36º 39’ 60” N; 8º 27’ 40”E
	Siliana	36º0’59”N; 9º17’59”E
Turkey	Duzlercami	37º2’4” N; 30º35’ 45” E

Index II

Number of studies per species in the Northern Mediterranean countries (NMC's).

Gen	Species	Albania (4)	Andorra (2)	Bosnia (5)	Bulgaria (4)	Croacia (5)	Cyprus (7)	France (23)	Greece (25)	Italy (36)	Macedonia	Malta (1)	Portugal	Slovenia	Spain (39)
Fagus	sylvatica	1		1	1	1			1	1	1			1	
Pinus	leucodermis	2								2	2				
Pinus	halepensis	1						3	3	3					5
Juniperus	phoenicea		**2**					2							2
Pinus	nigra			1	1	1	1	3	1	2	1			1	3
Abies	alba			1				2		6					

Picea	omorika			1											
Taxus	baccata			1				1		1					2
Picea	abies				1	1		1	1						
Fraxinus	excelsio				1					1				1	
Carpinus	betulus					1			1	1					1
Myrtus	communis					1		1	1	2			1		1
Cupressus	sempervirens						2	1	4	1					
Pinus	brutia						1		2						
Cedrus	brevifolia						2								
Juniperus	excelsea						1		1						
Olea	europaea							2	1	3					3

Pinus	sylvestris	1					2
Pinus	pinea	1				1	2
Cedrus	atlantica	1					1
Pinus	pinaster	1				1	1
Juniperus	thurifera	1		1			1
Quercus	suber	1		2		2	4
Laurus	nobilis	1					
Cistus	salvifolius		1			1	1
Abies	cephalonica		4		1		
Abies	borisii_regis		3		1		
Carpinus	orientalis		1	1			

Acer	pseudoplatanus								1					
Abies	nebrodensis								2					
Quercus	petraea								1					
Quercus	cerris								1					
Quercus	robur								1					
Quercus	pubescens								1					
Quercus	ilex								1					
Castanea	sativa								1					
Tetraclinis	articulata										**1**			1
Cistus	albidus													1
Arbutus	unedo											1		
Pinus	canariensis													1

Abies	pinsapo														1
Phlomis	lychnitis														1
Phlomis	crinita_subsp._Crinita														1
Juniperus	oxycedrus														1
Quercus	coccifera														1
Phlomis	crinita_subsp._Malacitana														1

Number of studies per species in the Southern Mediterranean countries (SMC's).

Gen	Sps	Morocco (24)	Algeria (11)	Tunisia (8)	Lybia (2)
Olea	europaea	2	1	1	1
Cedrus	atlantica	4	2		
Abies	numidica		2		
Pinus	halepensis	6	2	1	1
Juniperus	thurifera	1	1		
Quercus	suber	2	1	1	
Laurus	nobilis		1	1	
Tetraclinis	articulata	1	1	1	
Cupressus	sempervirens	1		2	
Arbutus	unedo			1	
Atriplex	halimus	1			
Pinus	pinaster	1			
Pinus	nigra	2			
Abies	pinsapo	1			
Cupressus	atlantica	1			
Taxus	baccata	1			

Number of studies per species in the Eastern Mediterranean countries (EMC's).

Gen	Sps	Turkey (32)	Israel (4)	Jordan (4)	Lebanon (7)	Syria (3)
Cedrus	libani	3			3	1
Abies	equi-trojani	3				
Abies	bornmuelleriana	3				
Pinus	nigra	3				
Fagus	sylvatica	1				1
Cupressus	sempervirens	4		1		
Pinus	brutia	4			1	
Pinus	halepensis	3	2	2		
Pinus	pinea	1			2	
Abies	nordmanniana	1				
Olea	europaea	1	1	1		1
Abies	cilicica	1				
Castanea	sativa	1				
Juniperus	excelsea	1			1	
Laurus	nobilis	1				
Myrtus	communis		1			

Index III

Middle term Report: Characterization of the Mediterranean Basin

Mediterranean landscape is represented by important mountains range (e.g. Atlas, Pyrenees, Alps, Taurus mountains of Anatolia) altered by flat plains (e.g. the lowland desert in Egypt and Libya). The steepest mountains can reach 4000 m of altitude, while most of plateaus lie between 200 and 500 m above the sea level (Pinborg *et al.,* 2002).

Mediterranean climate is characterized by a bi-modal regime: cold and wet winters and with hot and dry summers, which varies from 2 months in the western part and 6 in the eastern Mediterranean (Bondel *et al.,* 2010). Temperatures can reach easily 30ºC in some regions (south and eastern countries) or -10ºC in mountain areas. The average annual rainfall varies between 600 and 1200 mm/year. However, it could reach more than 1500 mm at coastal mountain ranges or low than 100mm/year in desert or semi-desert regions (Pinborg *et al.,* 2002).

Mediterranean soils composition are not only linked with the parental rock (mainly limestone origin), but also significant affected by the climate regime of the sites). Thus, in the north as climate is more humid, calcareous soils presents higher amount of organic matter while the mineral content are generally lower. On the other hand, in the south where the temperature are significantly higher and the availability of water is lower, there is an accumulation of oxidized minerals leading to, in some cases, ferruginous red soils formation (known as "terra rossa"). Soil composition varies also according to the granulometry and use of the substrate. Less usually, is the presence of volcanic soils which can be found locally (e.g. Italy) (Blue Plan, 2003).

Wind is also important element since it is responsible for the spread of the pollen and seeds as well as it dramatically increase the evapotranspiration rates, and thus exacerbating the drought on plants, during the summer. The most important winds that cross the Mediterranean are the hot winds come from the Sahara desert (sirocco) and the extreme cold winds from the north (mistral and transmontane in the southern of France; bora in the Adriatic Sea and gregal in the Gulf of Genoa). Moreover, wind is not only an agent of dispersal but it can also influence the migration of other agents (birds and insects) that contributes for seeds and pollen dispersal (Bondel *et al.,* 2010).

Forest distribution and composition

Forests and woodlands covers an area of approximately 73 million ha, representing about 8,5 % of the total Mediterranean area. More than half of this forest is distributed in the north countries of the basin, with poor representative portion in the east and south due to the arid climate and poor soils. However, the total stock of biomass has (forest area) increased by about 2 million tonnes within a period of ten years (1990-2010), mainly explained by the abandonment of the rural ad forest areas from southern European countries. The highest values of growing stock were found in Bulgaria, France, Italy, Spain and Turkey while the lowest were in Israel, Jordan, Libya, Lebanon, Tunisia and Morocco (Allard *et al.,* 2013).

Most of the primary untouched forest does no longer exist. Today, about 11% of the Mediterranean forest area consists on plantations mostly of pines and eucalyptus. The forest was explotation or management for different proposes: livingstock raising, coppicing or woodcutting, and some prescribed fire, led to a progressive replacement of deciduous broadleaves species (e.g. *Castanea sp.* or *Fraxinus sp.*) to evergreen sclerophyllous shrubs and small trees communities (e.g. *Quercus sp., Olea sp. or Pistacia sp.*), by the enlargement of open woodlands (Bondel *et al.,* 2009).

Wood and scrublands occupy the thermo-meso Mediterranean belt, at low altitude. They are composed mainly by sclerophyllous evergreen species such as holm oak (*Quercus ilex*), cork oak (*Quercus suber*) and kermes oak (*Quercus* *coccifera*). However, the most diverse broadleaves forests are composed yet by the remaining species of the tropical family *Lauraceae* from the Terciary (e.g *Laurus sp., Myrica sp., and Arbutus* sp.). At higher elevations (supra to oro- Mediterranean zones) predominates deciduous species such as *Cedrus spp, Castanea sativa, Fagus sylvatica, Pinus sylvestris, Abies alba* and *Acer spp*. While, along the fluvial margins where the availability of water is not a constraint, forest of *Quercus robur, Platanus orientalis, Fraxinus species, Populus alba* and *Pinus nigra* can be found. There are some species such as *Pinus halepensis* and *Juniperus spp*, (which are adapted to fire and dry conditions) that cover a wide range from coastal to high mountains areas (Quézel, 2005).

However, the most widespread vegetation form under Mediterranean climate is *macchia*, which is considered the first state of forest degradation (resulted from fire or orvergrazing activities). *Macchias* formation includes "any sclerophyllous evergreen dense vegetation type, capable of attaining 4-6 m in height". It occurs in acidic, silicious soils and is composed by dominant genres such as *Quercus spp., Ceratonia spp. or Pistacia* spp. In opposition to *macchias, garrigues* proliferate in alkaline, limestone soils and are composed by all of species associated to holm oak (*Quercus ilex*) (Bondel *et al.,* 2009; Quézel, 2005).

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