

# Genetic diversity of silver fir (*Abies alba*) and European beech (*Fagus sylvatica*) populations from the south-eastern limits of their natural distribution<sup>†</sup>

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**Abstract:** Observed (Ho) and expected (He) heterozygosity, as well as allelic richness (AR) were calculated using SSR genotyping for two important European keystone forest tree species. *Abies alba* and *Fagus sylvatica* are under pressure in a large part of their distribution due to climate change. Since Greece is their south-eastern limits, climatic pressure is even higher. As rear edge/ peripheral populations, it is expected to harbor valuable and well adapted germplasm in conditions such as those expected in central Europe by the end of the century. Comparison of their genetic variation amongst the Greek marginal populations and population from the SW, S or main range of their distribution was performed, finding no statistical differences.

**Keywords:** Silver fir; European beech; SSRs; allelic richness; heterozygosity; rear edge; peripheral

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## 1. Introduction

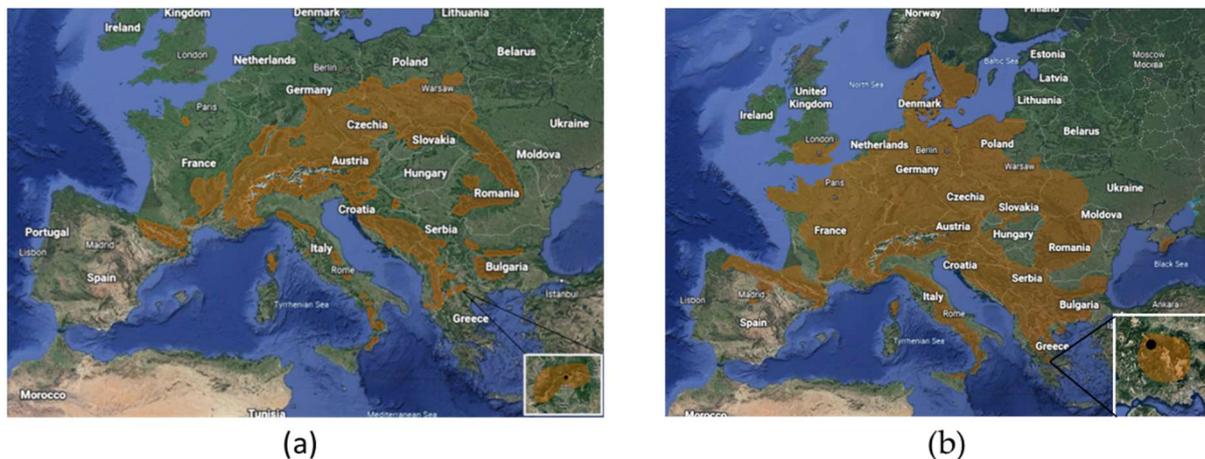
Greece, as part of the Mediterranean Basin, is considered to be one of the twenty-five global biodiversity hotspots [1]. It ranks third in Europe and fourth in the Mediterranean Basin in terms of species richness [2]. The expected increase of temperature and drought categorize Greece, and similarly the whole Mediterranean Basin, among the most vulnerable areas of Europe [3]. According to most species' distribution models, two of the most important keystone European tree forest species, *Abies alba* (silver fir) and *Fagus sylvatica* (European beech) are expected to face survival issues in parts of their distribution range due to the upcoming change of the climate [4-7]. In the long term, the persistence of the marginal Greek populations that constitute the species' south-eastern limit of natural expansion [8,9], is not certain. Moreover, such populations are highly important as a genetic reserve well adapted to xerothermic conditions [10].

*Abies alba* has been showing a declining trend of its growth at its south-western limit that is related to increasing temperatures [8], and in general in its distribution range [5]. *Fagus sylvatica* populations have shown to shift north-east and to higher altitudes due to the climate change, where climatological conditions are more favorable [4,11] with a potential habitudinal loss at the southern edge of its distribution [11,12]. In fact, according to a study, if no measures are taken by 2100, the forest cover of Greece will shrink, including areas of *Abies alba* and *Fagus sylvatica* [13]. Therefore, it is of high interest to evaluate the genetic variation of the most south-eastern marginal populations of these valuable tree species.

## 2. Materials and Methods

### 2.1. Sampling, DNA extraction, SSR Genotyping and Data analysis

Plant material was collected from the most south-eastern population for each of the species; (1) *Abies alba* (Figure 1a) (Mt Pinovo; 41° 07.209' N, 22° 04.346' E; altitude: 1266m asl) and (2) *Fagus sylvatica* (Figure 1b) (Mt Oxia; 38° 46.601' N, 21° 58.618' E; altitude 1686m asl). Selected trees were spatially distributed (minimum distance 30 m), in order to avoid sampling related individuals. The material was stored at – 80°C until processed for DNA extraction using the Nucleospin Plant II kit (Macherey-Nagel, Düren, Germany).



**Figure 1.** Distribution maps of (a) *Abies alba* and (b) *Fagus sylvatica*. Black circles in the inset of the maps indicate selected Greek populations.

Genetic diversity was assessed for *Abies alba* using twelve microsatellite loci in two combinations [14–16] (Combination A: Aat11, Aat15, SF1, NFF7, NFH3, NFF3; Combination B: Aat01, Aat04, Aat06, Aag01, SFb4 and NFH15) and for *Fagus sylvatica* using fifteen microsatellite loci in three combinations [17] (Combination A: sfc0036, csolfagus31, sfc\_1143, FS1\_15; Combination B: csolfagus19, csolfagus\_05, csolfagus\_29, csolfagus\_06; Combination C: DES576\_A0F, EEU75\_A0, DUKCT\_A0, EJV8T\_A0, EMILY\_A0, ERHBI\_A0, contact14\_A0).

PCR products were run on an ABI Prism 3730xl (Applied Biosystems) sequencer with GS500LIZ\_3730 as the internal size standard. Allelic profiles were scored by automatic binning and visual checking using the GeneMapper Software v4.1 (Life Technologies). Observed and Expected heterozygosity were calculated using GeneAlex ver. 6.5 [18] and HP-Rare for allelic richness [19].

### 2.2. Comparing populations

Results on a per species basis were compared to those of populations from the main distribution range. To this end, a journal literature search was conducted for studies using SSRs that included common loci.

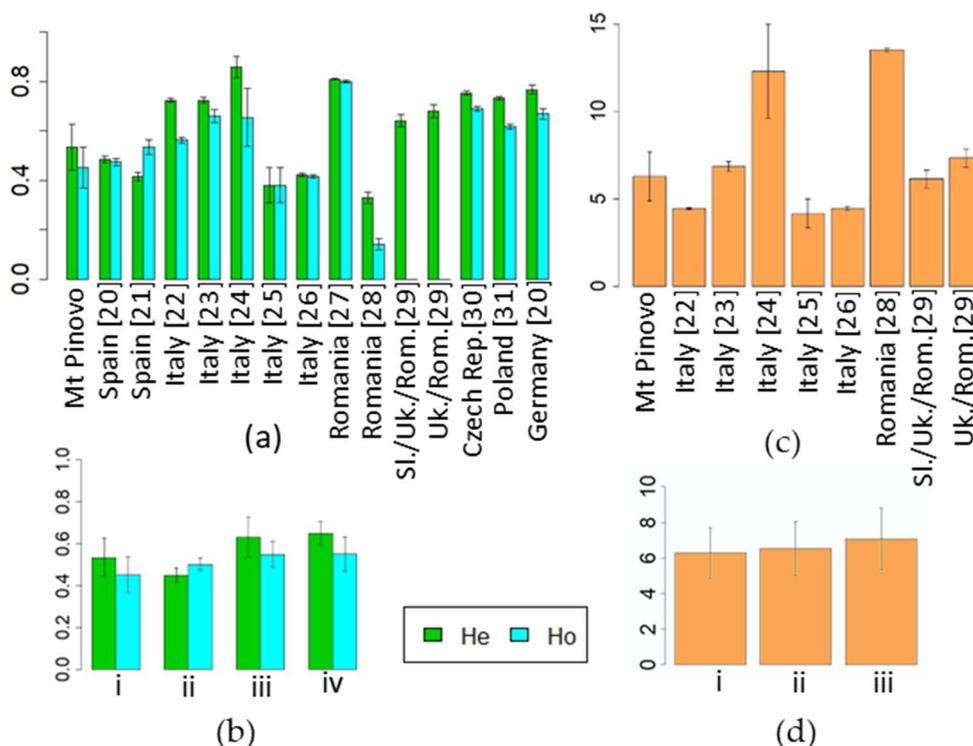
Analysis of Variance (ANOVA) was performed for each of the species using the R 3.5.0 package to detect if differences exist amongst the Greek marginal populations and populations from the main range or from Spanish, French or Italian parts of their distribution.

## 3. Results and Discussion

### 3.1. Comparison of the *Abies alba* marginal population with populations from others parts of their distribution

The observed heterozygosity of the Greek population at Mt Pinovo averaged 0.452, the expected heterozygosity averaged 0.534 whereas allelic richness averaged 6.29. The

statistical analysis showed no significant differences of Ho, He (Figure 2a-b) and AR (Figure 2c-d) amongst (i) Mt Pinovo (SE margin) (ii) Spain (SW part of the distribution) [20,21] (iii) Italy (S part of the distribution) [22-26] and (iv) main range of the distribution of *A. alba* [22,26-31].



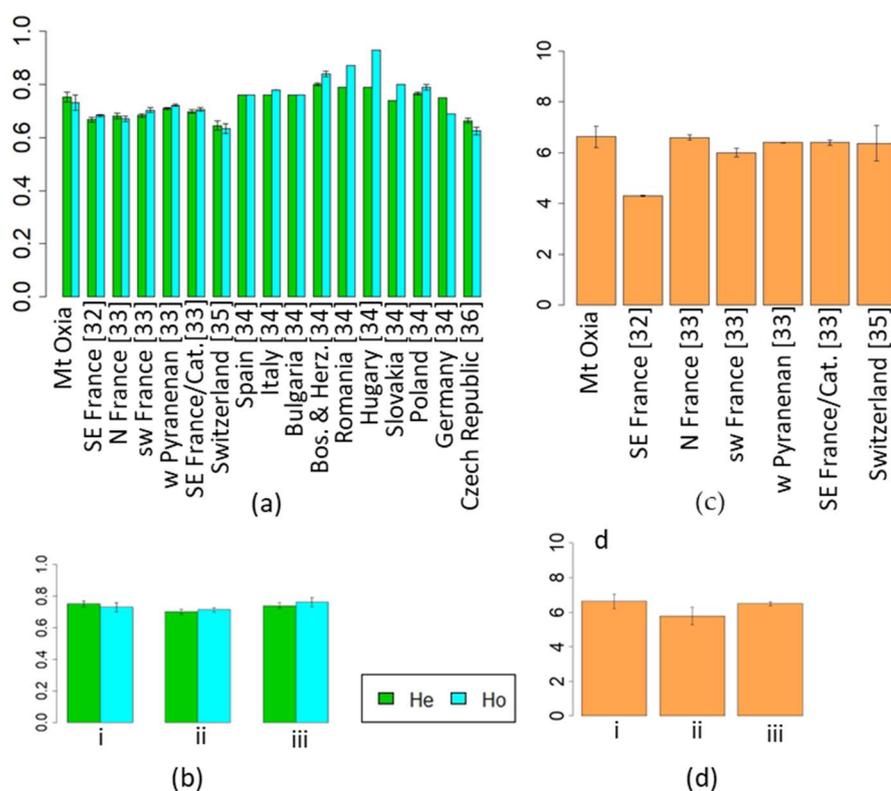
**Figure 2.** Comparing: (a-b) Observed and Expected Heterozygosity (c-d) Allelic Richness of Mt. Pinovo with populations from SW, S part of the region and the main region of *Abies alba* distribution. Ho from Slovakia (Sl.), Ukraine (Uk.) and Romania (Rom.) were not available. No AR was listed for populations from Spain. i: Mt Pinovo, ii: Spain [20-21], iii: Italy [22-26], iv: Main range [22, 26-31]. Numbers in brackets are the references used.

The absolute averages of Ho and He from Mt Pinovo showed to be similar to populations from Spain [20,21], but lower from populations in Italy [22-24], from Central Europe such as the Czech Republic [30], Germany [20], Poland [31] and the Balkans [29]. Studies that assessed the Ho and He from the Romanian Carpathians showed to have either much higher [27] or much lower [28] averages. Some populations from Italy [25,26] showed to have lower values compared to this study.

As for the absolute average of allelic richness, Mt Pinovo was higher than a number of Italian populations [22,25,26] as well as from Slovakia and Ukraine [29]. On the other hand, populations from Italy [23,24] and also from Central and northern Balkans showed to have much higher AR than Mt Pinovo [27,29].

### 3.2. Comparison of the *Fagus sylvatica* marginal population with populations from others parts of their distribution

The observed heterozygosity in the Greek population of the Mt. Oxa population averaged 0.732, and the expected 0.753. Furthermore, allelic richness averaged 6.63. No statistical differences were found in all three genetic diversity parameters (Figure 3a-d; Ho, He and AR) amongst (i) Mt Oxa (SE margin) (ii) Spain and France [32-34] (SW part of the distribution) and (iii) core area of the distribution of *F. sylvatica* [33-36].



**Figure 3.** Comparing: (a-b) Expected and Observed Heterozygosity and (c-d) Allelic Richness of Mt Oxia with populations from SW and the main region of *Fagus sylvatica* distribution. i: Mt Oxia, ii: SW Part [32-34], iii: Main range [33-36]. Numbers in brackets are the references used. Cat.: Catalonia, Bos.: Bosnia; Herz.: Herzegovina.

In absolute values  $H_o$  and  $H_e$  of Mt Oxia are lower than those of populations from western Europe (France) [32,33], Central Europe (Switzerland and Czech Republic) [35,36] and Northern Balkan countries [34]. Populations from Spain, Italy, and Bulgaria [34] have shown comparable values.

Furthermore, Mt Oxia’s AR was higher than South Eastern French populations [32] and comparable to the values of populations from other parts of France [33] and Switzerland [35].

### 3. Conclusions

The genetic variation ( $H_o$ ,  $H_e$  and AR) of the Greek marginal populations, when compared with populations from the southwest or the south parts of their distribution or with their main range, showed no statistical differences. Based on several species’ distribution models, the upcoming change of the climate might lead to habitual losses for a number of European species, including *Abies alba* and *Fagus sylvatica*. Greek populations are well adapted to xerothermic conditions, and therefore can provide valuable genetic material to European populations that may face survival issues. Consequently, the conservation of their genetic resources is of high priority.

**Author Contributions:** E.D. is responsible for the formal analysis, data curation, writing – original draft preparation and visualization; N.T is responsible for the data curation; F.A.A. is responsible for conceptualization, methodology, validation, funding acquisition, writing—review and editing and supervision. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The raw data supporting the findings of this study are available from the corresponding author upon reasonable request.

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