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Joint final conference on

**“Marginal and peripheral tree populations:  
a key genetic resource for European forests”**



*Forestry Research Centre (CREA SEL)  
Arezzo, Italy, 26-29 September 2016*

# Book of abstracts

# Plenary meeting of Cost Action FP1202

## Working Group 1

### **“Integrating geography and environment in a standardized procedure to assess marginality and its effects on genetic patterns”**

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Genetic patterns in marginal populations have been largely studied, as these populations may harbour unique genetic combinations due to their location in the rear/leading edge of species distribution. However, according to the abundant-centre hypothesis, they may have low genetic diversity due to patchy distributions and small population sizes. Until the date, empirical studies are controversial, the main reason being that we lack standardized definition of marginality, which would require considering processes affecting gene flow, such as isolation by distance (IBD) and isolation by environment (IBE). Here, we provide a consistent approach to define marginality integrating these two processes, by separately accounting for geographic and environmental factors respectively. We based our methodology on a set of indices (both geography and environment related) that can be directly integrated, in a subsequent step, with genetic diversity indices, to further understand the relationship among the three elements. To assess geographic marginality, we used four different indices namely, core size, distance to the border of the core, cost-distances to the centroid of the distribution and distance to the nearest large patch, characterizing the position of populations within their core patches and their position in respect to the entire distribution of the species. To assess environmental marginality, we employed the probability of environmental suitability obtained by fitting species distribution models (SDMs) for each species, characterizing each location with its probability of environmental suitability. We illustrated our approach with seven European conifers (four of them Mediterranean), namely, *Pinus halepensis* Mill., *Pinus pinaster* Ait., *Pinus nigra* Arnold, *Pinus pinea* L., *Abies alba* Mill., *Pinus sylvestris* L., and *Picea abies* L., all them genetically characterized, in order to perform a second stage analysis, in which we will assess the consequences of marginality on genetic patterns.

# WG meeting of Cost Action FP1202

**“Relationship among marginality and variation of quantitative traits: first results of a case study for Aleppo and maritime pine”**

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**Keywords:** adaptive traits, phenotypic/genetic variation, phenotypic plasticity, marginality indexes  
Marginal populations of forest tree species in the Mediterranean basin are target populations for application of conservation strategies and their level of significance has been mainly determined by their expected divergence from core populations.. However, we are still lacking information on the relationship among marginality of populations and parameters indicating the adaptive potential and the plasticity of species, i.e: levels of standing genetic variation and phenotypic plasticity. Multi-site provenance tests of Aleppo pine (*P. halepensis* Mill.) and Maritime pine (*P. pinaster*) were used to estimate intra-population phenotypic variation, considered as a proxy to genetic variation, and phenotypic plasticity for quantitative traits of adaptive significance. For the populations tested in the experimental trials five marginality indexes were computed, namely the Environmental Marginality Index, the distance of the populations to the border, the size of the core of the distribution, the distance to the core of the distribution and the distance to the distribution centroid, based on the niche model of each species and its distribution range. The phenotypic traits were linearly regressed against the marginality indexes to establish the relationship among phenotypic variation and marginality. The first results will be presented from a case study including two Mediterranean pine species and the obtained preliminary results will be discussed for further studies.

**Session 1**  
**The stakeholder perspective on MaP-FGR**

*“Genetic Resources for Food and Agriculture in a Changing Climate”*

**Yousry El-Kassaby**

University of British Columbia, Canada - Science Director DIV 2 IUFRO

*“Recent developments on climate change adaptation and mitigation strategies of the forest based sector in Europe”*

**Giuseppe Scarascia Mugnozza**

University of Viterbo, Italy - President of the EFI Board

*“Monitoring the implementation of the Global Plan of Action on forest genetic resources”*

**Jarkko Koskela**

FAO Forestry Department, Forestry Officer

**Luc Emile Paques**

INRA-Orléans, Trees4Future general Coordinator

*“Relevance and future development of the work generated by the Action in the framework of the EUFORGEN Programme”*

**Michele Bozzano**

Biodiversity International, Euforgen/Eufgis Coordinator

**Session 2**  
**Marginal population genetics,  
detection and monitoring**

## **“Genetic and epigenetic variation at the European south-eastern rear edge margins of some forest trees”**

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**Keywords:** rear edge, epigenetics, population genetics, forest trees

The population genetic and epigenetic diversity was studied in some forest trees situated in the European south-eastern rear edge margins. In particular, the genetic diversity of Greek populations of two woody perennial angiosperms (*Prunus avium* and *Platanus orientalis*) and two conifers (*Pinus nigra* and *Cupressus sempervirens*) was estimated using molecular genetic markers (SSR, ISSR, AFLP). In addition, epigenetic diversity (in the form of differential DNA methylation) was evaluated by the MSAP technique in the same populations. Most of the genetic as well as the epigenetic variation resided within populations. Epigenetic and genetic diversity did not differ significantly and were not significantly correlated, but genetic diversity values were usually numerically higher. Epigenetic variation, assessed in the same populations and individuals, was not congruent to genetic variation and in general the insofar results support the thesis that epigenetic variation is uncoupled from genetic variation in these marginal populations of the south-eastern European edge. Our study presents a first insight into the partitioning of epigenetic diversity within and among natural forest tree marginal populations.

## **“Biogeography of *Juniperus* taxa in the eastern Mediterranean region”**

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**Keywords:** Nuclear microsatellites, *Juniperus excelsa*, East Mediterranean, Genetic diversity, distribution.

*Juniperus excelsa* is a major mountain forest element in the East Mediterranean Basin and sub-Mediterranean region. It colonizes sites that vary from sub-humid to the adjacent semi-arid steppe zone of the Mediterranean region. The altitudinal range of *J. excelsa* is very wide. In the Anatolian peninsula, it is mainly found at elevations between 1000 and 1300 m, and in Lebanon between 1600 and 1800 m in the western and eastern slope of Mount Lebanon. It forms the tree line in the East Mediterranean Basin with old, sparse populations reaching elevations of 2100m in Greece, and some individuals can be found at elevations of 2700– 2800 m in the Taurus. Although Junipers are still common in Mediterranean basin, some species suffer serious degradation processes and isolation. Genetics tools help shedding light on the genetic diversity of these species and therefore on their ability to adapt to climate change. These junipers have high ecological value and are more and more demanded for forest ecological restoration. NrDNA, cpDNA markers and genome size evaluation by flow cytometry were used to investigate genetic variation in *Juniperus* spp. in the East Mediterranean region. These tools allowed the distinction of the different species and interestingly, allowed to report for the first time the occurrence of *J. polycarpus* in Lebanon. Indeed, two *Juniperus* populations from Aarsal and Wadi El Njass, were found to be *J. polycarpus*. On the other hand, morphologically very similar trees to *J. excelsa* were found to be in reality *J. foetidissima*. The genome size helps differentiate these two taxa. The biogeography and

diversification history of Junipers and distribution mapping of the isolated populations per species and country in the eastern Mediterranean region will be discussed in this communication. Special insight is given to leading and rear edge populations.

### **“Where north meets south – marginal populations in a benign climate”**

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**Keywords:** *Arbutus unedo*; *Salix phylicifolia*; Ireland; Genetic diversity

Climatically Ireland is wet and mild with few extremes except for occasional storms. Despite this, or possibly as a result of this benign climate the flora of Ireland consists of some enigmatic species distributions, such as the co-occurrence of arctic and Mediterranean species. While most of the tree flora consists of pan-European species, there are exceptions that represent populations at the margin of their range. The predominantly arctic species *Salix phylicifolia* is close to its southern limit in Ireland and is restricted to 3 small populations. In contrast to this the Mediterranean /Atlantic species *Arbutus unedo* is at its northern limit in Ireland and thrives in a limited geographic area. The limits on these species tend not to be physical or abiotic, although the populations tend to be limited in size. The limitations are more likely to be related to ecological competition with surrounding species. Thus, these unusual distributions can be used to investigate the importance of ecology on range expansions or survival in marginal populations. For example, the *Arbutus unedo* populations can help understand a potential future expansion north of this species. Little is known about these populations although preliminary results indicate they have limited genetic diversity. They may represent unique genotypes at the edge of their range or stochastic distribution events such as founder populations or refugial populations following from the retreat of the ice-sheets. The genetic provenance of these populations is currently being investigated and will hopefully shed light on their continued survival.

### **“Tree populations in Scotland: characteristics of forests at Europe’s north-western periphery”**

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At the extreme north-western edge of Europe tree populations reach their limits in a predominantly very oceanic, but highly variable environment. All of these populations have established post-glacially, from distant or more proximal refugial populations, meaning that much of the dynamics of colonisation – including bottlenecks, dispersal-related genetic structure and hybridisation – may still be in progress. However, most tree species are capable of highly effective gene dispersal over large distances and so even apparently isolated populations may still retain contact with a larger gene pool. With these factors in mind, we consider what these peripheral populations represent in the wider context of European genetic diversity, and what the future holds for them



## “Genetic differentiation between marginal and core populations of common beech (*Fagus sylvatica*) in Romania”

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**Keywords:** *Fagus sylvatica*, marginal populations, core populations, microsatellites

The average temperature has increased by nearly 1 °C over the last two decades in the lowlands of southeastern Romania and will continue to rise under most climate change scenarios. Common beech (*Fagus sylvatica*) is the predominant tree species in the Carpathian Mountains but scattered marginal beech populations also occur in the lowlands. The aim of this study was to identify and characterize marginal beech populations in comparison to core mountainous populations by means of microsatellite genetic markers. We found that marginal beech populations differentiate from core populations and cluster together. Our results suggest that genetic diversity is lower in marginal populations. This might be explained by genetic drift effects and adaptation to extreme conditions. Our findings support the hypothesis that marginal beech populations possess specific genetic structures. Their conservation should be an important component of sustainable forest management in the context of climate change.

## “Maritime beech forest in Croatia as marginal beech population – vegetational approach”

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**Keywords:** marginal beech population, Croatia

European beech (*Fagus sylvatica*) is one of the major forest trees in mountain regions of Europe with extremely broad climatic and edaphic amplitude. Spreading of beech above the current range of distribution is limited mainly by climatic conditions. Too short growing season, low winter temperatures and late frost in spring limit beech distribution at its northern and eastern boundaries and high altitudes (Bolte et al. 2007). On the opposite, water deficiency limits beech distribution to the southern Europe and Mediterranean.

Relatively small Croatian territory is spreading through four of the main European biogeographical regions (Continental, Pannonian, Alpine and Mediterranean) where beech forest types characterized by very high plant diversity are common. Following the altitude, beech forms climazonal forest communities like subalpine beech forests (*Ranunculo platanifolii-Fagetum sylvaticae*) in Alpine region, beech-fir forests (*Omphalodo-Fagetum sylvaticae*) and submountainous beech forests (*Lamio orvale-Fagetum sylvaticae*) in Continental region and maritime beech forests (*Seslerio - Fagetum sylvaticae*) in Mediterranean region. Some of these beech communities (ex. *Seslerio-Fagetum sylvaticae*) reach actually a rear edge of their distribution area.

Along the Adriatic littoral belt (600-1300 m) there is a very sharp transition into warm and dry Mediterranean climate exceeding xeric limits for beech. This boundary region, where thermophilous maritime beech forests dominate, form a sublittoral belt, stretching from Istria to Albania, and characterized by the massive occurrence of *Sesleria autumnalis* and the presence of many Mediterranean and sub-Mediterranean species, including *Fraxinus ornus*, *Ostrya carpinifolia*, *Acer obtusatum*, *Sorbus aria*. Also, *Corylus avellana*, *Euphorbia amygdaloides*, *Rubus hirtus*,

*Cyclamen purpurascens*, *Anemone nemorosa*, *Lathyrus vernus*, *Mycelis muralis*, *Dentaria enneaphyllos*, *Calamintha grandiflora*, *Lamium orvala*, and some other mesophilous species are common in this community. Such species pool (mixture of Fagetalia species and thermophilous Mediterranean species) can easily help to recognize marginal beech populations, which are already partly adapted to Mediterranean climate, hot and dry summers and mild and wet winters.

### **“Monitoring Apennine *Abies alba* geographically marginal populations: an integrated research approach”**

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**Keywords:** *Abies alba*, adaptive management, marginal populations, monitoring methods

Preserving and enhancing the Apennine Silver fir in priority areas and habitats of the “Natura 2000” – LIFE+ Project RESILFOR network, through an interdisciplinary approach. The project aimed to preserve the autochthonous central Apennines Silver fir population. A multidisciplinary approach: genetic, ecological, structural and silvicultural characterization of the studied habitats.

Aims: comparing the *Abies alba* growth trends in two study sites where climate conditions are relatively different;

Monitoring the genetic structure and adaptation (cambial phenology and xylogenesis) of Silver-fir to stress and external influences:

Developing an adaptive silviculture to re-start succession dynamics in order to spread/preserve the Silver-fir gene pool in the context of the climate change effects (isotherm shift in primis).

Conclusion

- The length of growth season is really different between the two sites and also within the same site (La Verna) according to the aspect.
- It has been interesting that in the Pigelleto population trees started to grow again with cellular stages typical of the spring season in autumn. Because of the mild 2011 climate.
- The species seems to be plastic and that will be useful for the future challenges for developing an adaptive management.
- The genetic parameters show still good variation genetic levels and exchanges within the populations layers.
- The permanent plot system will be an useful tool to monitor the plasticity either on a geographic, either on a temporal way.

### **“Genetic diversity of autochthonous marginal and peripheral Norway spruce populations in comparison to populations in their optimum in Slovenia: case studies based on isoenzyme and nuclear SSR markers”**

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**Keywords:** marginal and peripheral populations, *Picea abies* (L.) Karst., genetic diversity, Slovenia

Norway spruce is one of the most valuable forest tree species with significant economic and ecological role in Slovenia. Autochthonous populations can be primarily found at the altitudinal leading edge, on edaphically marginal sites and vegetation inversion sites in Karst sinkholes. The research objects were chosen in different marginal and peripheral as well as in optimal Norway spruce sites in the Alpine and Dinaric phytogeographical regions. Each sample constituted of vital, randomly chosen dominant or co-dominant trees. In the presented work we combine results obtained with isoenzyme and SSR markers to describe genetic diversity of autochthonous Norway spruce populations in their ecologically marginal and peripheral environments and compare it to genetic diversity found in native populations growing in their optimum.

### **“The use of national forest inventories for inventorying marginal populations: an application to the Italian database”**

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**Keywords:** INFC 2005; Species distribution modelling; EUFGIS; Climate change

The National Forest Inventories (NFIs) can be classified among the most important and informative sources for research purposes on forest systems. Indeed, a detailed and up-to-date knowledge about forest genetic resources (FGR) is fundamental for developing adaptation strategies to cope with the possible effects of Global Change and the management of valuable genetic sources such as Marginal forest populations (MaPs).

The last Italian NFI (INFC 2005) has been recently published and, in this work, an application of the Species Distribution Modelling approach (SDM) is proposed with the aim to study the future scenarios for the Italian gene-conservation-units included into the EUFGIS database. Among the twenty forest categories defined by this inventory, three forest types were selected, corresponding to the range of two dominant species (*Fagus sylvatica*, *Abies alba*) plus a more general forest type (*Quercus* spp.). The same target species were derived from the EUFGIS database and compared with model's predictions. The 1981-2010 normal period was used to represent the current climatic conditions while two future climatic scenarios, the RCP4.5 and RCP8.5 for the 2050s, were adopted as future conditions. Soil characteristics were derived from the JRC database and the modelling procedure was performed using the biomod2 package of R CRAN programming language with 250m of spatial resolution.

NFIs represents the highest level of geo-referenced information. Anyway this source may become inadequate when a species-level information is required. In this view the forest-type concept should be re-considered either to pick up forest areas where small marginal populations grow either to include geo-referenced genetic information in new forest types. An adequate knowledge about the existing genetic potentials in each European Country could be fundamental to balance the future strategies (e.g. gene pools transferability or enrichment, assisted migration).

**Session 3**  
**Evolutionary factors and adaptation mechanisms**  
**in MaP-FGR**

## **“Epigenetic response to climate conditions during the reproductive phase; an evolutionary factor of northern Norway spruce populations?”**

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**Keywords:** *Picea abies*, climatic adaptation, reproductive environment, epigenetic regulation

We have repeatedly shown that Norway spruce (*Picea abies*) can rapidly adjust its adaptive performance as a response to the temperature and (probably) photoperiod from the time of its embryo development. The temperature conditions during seed development, in particular, influence the phenotypes of the offspring; seedlings from seeds produced under warm conditions have later terminal bud set and reduced autumn frost hardiness than those from seed produced under colder conditions, and thus perform like a more southern provenance. Comparisons of the performance of seedlings from seeds collected in the same provenance regions or seed orchards in years with different temperature conditions show that seedlings from years with high temperature sums during the period of seed development consistently produce taller seedlings with a later terminal bud set and later bud flush. The effect of the reproductive environment has been shown to persist for years. It mimics the variation among provenances from different latitudes and altitudes and may explain much of the observed variability in bud flush, bud set and early height growth among natural populations of Norway spruce. The observed phenomenon suggests the presence of an epigenetic memory effect in the developing embryo that senses environmental signals such as temperature and influences adaptive traits. The molecular basis of the epigenetic memory effect has been investigated and studies show transcriptional and microRNA differences among epitypes. We will discuss the implications of this epigenetic phenomenon for the interpretation of the genetic diversity among and within northern Norway spruce populations, for tree breeding and for its role in adaptation to climate change.

## **“Genetic variation of two contrasting for environmental marginality hellenic sweet chestnut populations and their plastic responses to interannual climatic variation”**

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**Keywords:** genetic markers, adaptation to xerothermic environments, climatic differences

Hellenic sweet chestnut populations (*Castanea sativa* Mill.) growing in two ecologically contrasting environments (optimal vs xerothermic), were studied for their genetic differentiation by using genetic markers, adaptive traits of fruits, seedlings and progenies tested in a provenance-progeny test. The genetic material was sampled from individuals growing in differently managed populations within each area (old growth natural and coppice). Pronounced differences were demonstrated between the populations growing in environmentally marginal conditions and those growing under optimal environmental conditions regarding species requirements for both genetic and adaptive variation. Genetic analysis was carried out using 10 polymorphic isoenzyme loci (21 alleles). The populations of the marginal xerothermic environment presented higher values in their genetic diversity parameters, such as observed and expected heterozygosity. Differences in the respective values between the populations of the two sites were statistically significant according to

a t-test. Furthermore, the populations growing in the marginal xerothermic environment reflected a high adaptation to the local conditions and presented a high genetic control over the adaptive traits of nuts, seedlings and saplings growing in the experimental trial. The opposite result was obtained for the populations growing in the most favorable environment, a fact that indicates a higher plasticity potential for the adaptive traits studied. The differential plasticity was also revealed for fruit traits and from population response when studied under contrasting annual climatic conditions (normal vs xerothermic year). During the xerothermic year, the environmentally marginal populations exhibited stability for their fruit traits, while those growing in the optimal environment had a pronounced plastic response, by allocating less resources to fruit formation that led to significantly reduced fruit size and weight. Moreover, the progenies originating from the environmentally marginal population, when compared to those originating from the optimal environment, presented phenotypes that can secure their survival in the seedling phase, as they were characterized by significantly smaller leaves, lower above ground height and more extensive root system, indicating the high adaptation to the marginal environment, while their saplings tested in the progeny test were significantly shorter in height, but with higher root collar diameter, indicating higher storage of carbon in the root system and the stem. The environmentally marginal population progenies flushed buds later and exhibited a longer flushing duration than those of the population growing in optimal conditions. Multivariate analysis demonstrated the high differentiation among the populations of the two environmentally contrasting areas and revealed the traits contributing more to their differences. The role of marginal populations in the face of climate change, their use for forestations and breeding and the undeniable need for their genetic monitoring and conservation, is discussed.

### **“Climate-growth relationships of *Quercus gussonei* (Borzi) Brullo in the Mediterranean region: adaptive traits and water use efficiency”**

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**Keywords:** carbon isotope discrimination, dendroecology, forest dieback, peripheral population in Sicily

Due to their marginal distribution, peripheral tree populations are highly vulnerable and are more likely to be influenced by extreme climate conditions. This occurrence is known to cause dieback in many species at their Mediterranean margins. *Quercus gussonei* (Borzi) Brullo is a deciduous oak endemic of Sicily. It is a thermophilous, peripheral form of *Quercus cerris* L. which is showing an incipient but ongoing decline. A deeper ecophysiological knowledge is urgently needed on this species in order to plan proper conservation actions and reduce the risk of its extinction.

In the aim to understand at what extent changes in environmental conditions could be responsible for *Q. gussonei* decline, we carried out dendrochronological and water-use efficiency investigations. Annual ring widths, earlywood and latewood analyses were accomplished in order to assess the climatic factors influencing the growth of *Q. gussonei*. In addition, we determined the carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) in tree rings, allowing to evaluate WUE variations and the expression of possible plant adaptive traits. Climate-growth relationships were analyzed using response functions.

Our findings highlight significant relationships between tree-rings width and climate data. The dendrochronological analysis of *Q. gussonei* showed a rather good adaptation of this species to the typical Mediterranean climate and oak trees seem to retain a certain resilience to efficiently face

less favorable environmental conditions. Furthermore  $\Delta^{13}\text{C}$  indicates a long-term adjustment in water-use efficiency and the increase of WUE could be interpreted as a fair adaptation of this species to drought-prone environments.

### **“Comparison of genetic parameters between optimal and marginal populations of Oriental sweet gum”**

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Genetic parameters of 9 oriental sweetgum (*Liquidambar orientalis* Mill) populations were estimated at a common garden test. Open pollinated seeds were collected from 16-27 families for each population. The common garden test was established using randomized complete block design in 25 blocks with single tree plot in 2009. Each block included 223 families. Breast height diameter, height and crown diameter were measured at the age five.

The study revealed significant variability in all traits evaluated. In variance components, variation of populations was three times to variation of families. Individual heritabilities for breast height diameter, height and crown diameter in pooled data from all populations were  $0.21\pm 0.04$ ,  $0.27\pm 0.04$  and  $0.11\pm 0.03$  and additive genetic coefficient of variations were 13.4%, 9.1% and 7.1%, respectively. For all traits, optimal and marginal populations appeared in different groups in Tukey test. Individual heritabilities for breast height diameter, height and crown diameter in marginal and optimal populations were  $0.27\pm 0.10$ ,  $0.19\pm 0.08$  and  $0.19\pm 0.08$  and  $0.19\pm 0.04$ ,  $0.29\pm 0.05$  and  $0.09\pm 0.03$ , respectively. Additive genetic coefficient of variations for breast height diameter, height and crown diameter in marginal and optimal populations were 16.7%, 8.3% and 10.8% and 12.8%, 9.1% and 6.2%, respectively. While breast height diameter and crown diameter were more heritable for marginal populations, height was more heritable for optimal populations.

### **“Molecular evidence of autochthony of wild cherry (*Prunus avium* L.) populations based on its obligate allogamy”**

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**Keywords:** wild cherry, autochthony, adaptation, self-incompatibility

Wild cherry (*Prunus avium* L.) occurs naturally in much of Eurasia, showing a vast distribution area stretching from Ireland to the Caspian sea and from southern Scandinavia to North Africa. Within this area, the species generally occurs as individual trees or small clusters scattered throughout mixed hardwood stands. As a result of its high site requirements, wild cherry tends to form marginal populations whenever site conditions (soil, climate) are less favourable. The degree of adaptation can be determined through assessment of autochthony. Strictly speaking, a population is considered autochthonous when it settled and regenerated naturally after the last Ice Age. In practice, the degree of autochthony is assessed on the basis of ancient forest maps and the occurrence of typical shrub and herbaceous vegetation. However, for wild cherry a more objective and molecular tool is available. Wild cherry is allogamous. This trait is governed by a single multiallelic S locus with

gametophytic action which controls self-incompatibility and cross-incompatibility between some individuals. Therefore S genes are subject to a negative frequency-dependent selection (i.e. balancing selection). Wild cherry trees carrying rare S genes are advantaged to participate in the reproductive process within a population as they can fecundate the majority of the other individuals. Thus, in consecutive generations, the frequency of these rare S genes will rise, whereas the initially frequent S alleles will occur ever less. Therefore, it can be expected that in populations which have rejuvenated during several generations on the same site, all S genes will be more or less equally present. This assumption was tested in a mixed hardwood forest (Bertembos) where the wild cherry population is believed to be autochthonous based on the “traditional“ criteria. The distribution of the S genes in this population is compared to the distribution in an artificial stand planted in the same forest complex.

### **“Shift in a plant community composition under climate change simulation: investigating the plant traits responsible for species success and decline”**

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**Keywords:** climate change, drought stress, two-phases resources dynamic, changes in species composition

In arid and semiarid areas, drought stress represents the main constraint to plant performance: extending the aridity period, climate change can differentially affect the fitness of co-occurring species, leading to changes in plant community composition. To investigate the impact of the forecast reduction of rainfall input, we applied for 11 years a reduced rainfall regime to a Mediterranean garrigue: the study was focused on the three main shrub species occurring at experimental site, that showed in natural conditions contrasting changes in their relative abundance (increase, stability, decrease). To investigate the processes underlying these changes we also monitored the morphological, phenological and physiological traits of these species, following a conceptual framework based on the two-phase resource dynamic model: we tested the hypothesis that the fitness of an individual depends on an appropriate combination of traits allowing: i) to compete for resource under optimal conditions, and ii) to survive under stressful conditions. Two parameters related to these abilities were derived: i) the plant level photosynthetic index at best resource availability (PPI<sub>max</sub>, positively related to competition ability), and ii) the length of the periods with low photosynthetic rates (days<sup>1/3</sup>, negatively related to plant survival). The ratio (defined PPI<sub>red</sub>) between these two factors summarizes the trade-off between the traits determining the competition and the survival ability, and it is expected to be related to the persistence probability of an individual within the community.

A positive relationship was found between PPI<sub>red</sub> and the variation rate of the species abundance in both natural and manipulated conditions. This relationship shows that the different combinations of competition and survival capacities could explain the changes in the species abundance within the monitored community; also, it suggests that a trait based approach combining physiologic, morphologic and phenological traits can contribute to the analysis of vegetation dynamics under climate change.



## **“Physiological and genetic mechanisms underlying drought stress response in *Pinus halepensis*”**

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Climate change, which is leading to increased mean temperatures and, in the Mediterranean region, to decreases in annual precipitation, may be too rapid to allow adaptation of long-lived forest trees to the new environment. Assisted migration of genetic material is one of several strategies for genetic conservation and forest reproduction. Afforestation of the semi-arid area in Israel with *Pinus halepensis* is an example for assisted migration of a species outside its natural and optimal conditions. The 3,000 ha planted forest is now 50 years old and provides a good opportunity to examine the physiological and molecular responses of *P. halepensis* to prolonged drought conditions.

Using rooted cuttings from specific trees, we have achieved high-throughput physiological measurements and found that tree stem diameter correlates with water use efficiency. In addition, we found that *P. halepensis* from xeric provenances resisted drought-induced embolisms due to smaller pit apertures.

Analysing the molecular response, we found that the reactive oxygen species (ROS) scavenging machinery is activated at the outset of drought stress, followed by osmotic adjustment and ABA synthesis in the needles. While the photosynthetic gene expression stayed stable, terpene biosynthesis related genes were downregulated. Candidate genes from this analysis can be tested on different phenotypes to identify molecular markers for drought resistance.

**Special Session for best  
Short Term Scientific Missions (STSM)**

## **“Intraspecific variation of below-ground traits in Scots pine”**

**Patricia Gonzalez Diaz**

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Natural tree populations consist on individuals that exhibit intra- and inter-specific variation at different geographic scales, as a result of the balance between gene flow and selection. Roots constitute important traits for water and nutrient acquisition and their architecture could be highly influenced by the local environment. The extent and magnitude of below-ground variation will influence the adaptive capacity of trees under forthcoming changing environmental conditions. Scots pine in Scotland is the iconic species of the remnant Caledonian forest and appear in a relatively narrow geographic area with a steep East-West environmental gradient, increasing in rainfall and temperature. We hypothesized that western populations could be better adapted to warmer conditions regarding below-ground traits and, as a consequence, could perform differently to predicted increased temperature. We performed an experiment under strictly-controlled conditions with a nested hierarchical design including region (west of east), population and families. We used two temperature scenarios, current temperature and increased temperature, and analysed germination and growth of below-ground traits. The largest amount of variation occurred at the family level. Nevertheless, regional and population differences were detected, where eastern populations invested more in roots. In addition, increased temperature had strong effects on early growth similar in the two regions, with advanced germination, enhanced growth and biomass about 10 times, but it was not accompanied by shifts in biomass partitioning. Despite the intra-specific variation found in Scots pine populations, our results reveal consistent effects of increased temperature on growth and germination. Natural tree populations consist on individuals that exhibit intra- and inter-specific variation at different geographic scales, as a result of the balance between gene flow and selection. Roots constitute important traits for water and nutrient acquisition and their architecture could be highly influenced by the local environment. The extent and magnitude of below-ground variation will influence the adaptive capacity of trees under forthcoming changing environmental conditions. Scots pine in Scotland is the iconic species of the remnant Caledonian forest and appear in a relatively narrow geographic area with a steep East-West environmental gradient, increasing in rainfall and temperature. We hypothesized that western populations could be better adapted to warmer conditions regarding below-ground traits and, as a consequence, could perform differently to predicted increased temperature. We performed an experiment under strictly-controlled conditions with a nested hierarchical design including region (west of east), population and families. We used two temperature scenarios, current temperature and increased temperature, and analyzed germination and growth of below-ground traits. The largest amount of variation occurred at the family level. Nevertheless, regional and population differences were detected, where eastern populations invested more in roots. In addition, increased temperature had strong effects on early growth similar in the two regions, with advanced germination, enhanced growth and biomass about 10 times, but it was not accompanied by shifts in biomass partitioning. Despite the intra-specific variation found in Scots pine populations, our results reveal consistent effects of increased temperature on growth and germination.

## **“Genetic erosion in European crab apple (*Malus sylvestris* L.) populations”**

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**Keywords:** *Malus sylvestris* L., *Malus × domestica*, microsatellites, gene diversity, conservation

European crab apple (*Malus sylvestris* L.) is the only wild *Malus* species native to Europe and is growing on edges of forests very often as isolated trees; farmland hedges and marginal sites. Living in scattered meta-populations, it is exposed to genetic erosion due to underutilization, hybridization and habitat modification.

By reviewing the studies of *Malus sylvestris* L. and other species with similar biology we collected results describing gene diversity dynamics obtained from microsatellite markers. Our aim was to perform comparative analysis of values of observed and expected heterozygosity in order to shape the view about genetic erosion in *Malus sylvestris* L.

The erosion of genetic diversity was rather expected in cultivated apple relative (*Malus × domestica* Borkh.) due to the bottlenecks during improvement processes. Comparative analysis among *Malus sylvestris* L. and *Malus × domestica* Borkh. showed opposite state: gene diversity in *Malus sylvestris* L. was somewhat lower (0.741) than in *Malus × domestica* Borkh. (0.791). This is in accordance with *Malus sylvestris* L. being an endangered species and the most threatened species are having reduced genetic diversity when compared to taxonomically related species that are not in threat. On the other hand, relatively high gene diversity is still maintained within *Malus sylvestris* L. when compared to e.g. wild grapevine (*Vitis sylvestris* L.) (0.625). This is partly ensured by the biology of the species itself: it is predetermined to collect high levels of variability being self-incompatible thus readily hybridizing with other *Malus* species.

The conclusions from these contemplations are not different from the already existing thoughts: conservation programs must ensure improvement of gene diversity state by direct interfering of humans. During conservation processes interspecific hybridization should be drowned to the reasonable level in order to preserve species authenticity.

## **“Does phylogeographic structure relate to climatic niche divergence? A test using maritime pine (*Pinus pinaster* Ait.)”**

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This work is the result of a Short Term Scientific Mission funded by COST ACTION FP 1202 in 2013. We aimed at (i) disentangling the effects of environmental and geographical processes driving phylogenetic distances among clades of maritime pine (*Pinus pinaster* Ait) and (ii) to assess the implications for conservation management of combining molecular information with species distribution models (SDMs).

We undertook two cluster analyses for eight genetically-defined pine clades based on climatic niche and genetic similarities. We assessed niche similarity by means of a Principal Component Analysis and Schoener's D metric. To calculate genetic similarity, we used the Unweighted Pair Group Method with Arithmetic Mean based on Nei's distance using 266 SNPs. We then assessed the contribution of environmental and geographic distances to phylogenetic distance by means of Mantel regression with variance partitioning. Finally, we compared the projection obtained from SDMs both fitted from the species level (SDMsp), and composed from the eight clade-level models (SDMcm).

Genetically- and environmentally-defined clusters were identical. Environmental and geographic distances explained 12.6 % of the phylogenetic distance variation and overall, geographic and environmental overlap among clades was low. Large differences were detected between SDM<sub>sp</sub> and SDM<sub>cm</sub> (57.75 % of disagreement in areas predicted as suitable).

We concluded that the genetic structure within the maritime pine subspecies complex is primarily a consequence of its demographic history, as seen by the high proportion of unexplained variation in phylogenetic distances. Nevertheless, our results highlight the contribution of local environmental adaptation in shaping the lower-order, phylogeographic distribution patterns and spatial genetic structure of maritime pine as: i) genetically- and environmentally-defined clusters are consistent, and ii) environment, rather than geography, explained a higher proportion of variation in phylogenetic distance.

SDMs, key tools in conservation management, characterize the fundamental niche of the species better when including molecular information.

### **“Genetic structure of *Betula pendula* (Roth) marginal populations in their Southern European limits”**

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**Keywords:** birch, molecular markers, rear edge

During the last glacial age, in Southern Europe tree populations moved in the southern most latitudes on the Italian Apennines and Southern Balkans and colonized lower altitudes as well. All major biomes in Europe contain Marginal/Peripheral (MaP) populations of forest tree species, whose persistence is threatened by climate change. Under these circumstances, tree populations are moving northwards and at higher elevations, but species ranges are limited by biotic and abiotic constraints, that can differently affect the isolation of the populations.

One of the species facing this kind of threat is silver birch (*Betula pendula* Roth), whose distribution covers uniformly almost all Europe, but in the western and southern parts of the distribution range, like in the Iberian peninsula, Greece and Italy, its presence is patchy and confined at the higher altitudes only.

The genetic pattern of silver birch in the main distribution area is well studied, whilst very little is known about the amount and organization of genetic variation in the southern marginal areas. Refugial areas, as those cited above, are theoretically expected to show higher genetic variability compared with surrounding recolonized regions.

Herein, parameters of genetic diversity and structure (such as A, H<sub>o</sub>, H<sub>e</sub>, FIS FST) are presented for different MaP populations from Italy and Greece. The analysis of the genetic structure of these populations points towards the existence of diverse gene pools exhibiting differential genetic variability. The influence of environmental complexity in the genetic differentiation observed is also addressed. The potential peculiarities of marginal birch populations compared to core population diversity are discussed.

# Posters

## “Genetic variability of selected marginal populations of Scots pine in the Czech Republic”

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**Keywords:** Scots pine, microsatellites, polymorphism, marginal populations

Understanding the genetic structure and diversity of edge populations can shed light on the role of peripheral populations and their relevance for conservation strategies (SCALFI et al. 2009). The genetic diversity of six populations of Scots pine was studied by DNA analyses using the Simple Sequence Repeats (SSR) method. Total genomic DNA was extracted by DNA Plant Mini Kit (Qiagen) from needles taken from three marginal populations of Scots pine located on serpentines, from one population growing at high altitude (Šumava mountains) and from two core populations of Scots pine from Czech Republic. PCR was optimized for the tested primers that have been scanned in publications (SEBASTIANI et al. 2012; SORANZO et al. 1998; AUCKLAND et al. 2002). Twelve polymorphic nuclear microsatellite markers were selected. Measurement of the size of amplification products was carried out on the genetic analyzer Applied Biosystems 3500. The obtained data were analysed by means the statistical program GenAlEx 6.5 (PEAKALL, SMOUSE 2012) and Cervus (KALINOWSKI et al. 2007). There were detected 146 different alleles at 12 loci in the 179 Scots pine individuals. The expected heterozygosity ( $H_e$ ) for the total group of 179 individuals was highest at locus SPAG 7.4 (0.928). The population frequency of private alleles was ranged between 0.083 and 0.583. Genetic distances among populations were calculated based on Nei's standard genetic distance (NEI 1972) and the marginal populations located on serpentines was clearly separated by the others. Pairwise  $F_{ST}$  values (the inbreeding coefficient) obtained from the study populations ranged from 0.008 to 0.017. Most of the genetic diversity was found within populations, while small amount of the variability occurred among populations. The ten selected genetic loci were verified as highly polymorphic and could be further used for comparative genetic analyses of Scots pine populations.

## “Polymorfism and differentiation in populations of *Pinus Sylvestris* in the Ukrainian Carpathians”

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**Keywords:** *Pinus sylvestris*, Ukrainian Carpathians, polimorphism, differentiation

The analysis and synthesis of the data on allozyme structure, polymorphism and degree of genogeographic differentiation of isolated mountain populations of Scotch pine in the Ukrainian Carpathians showed considerable genetic variability of foothill and mountain populations of *Pinus sylvestris* L., as well as the average genetic distance between these groups of populations. Small mountain populations of *Pinus sylvestris* L. in the Ukrainian Carpathians, long isolated from each other by mountain and mechanical and phenological (at least distant) reproductive isolation, are characterized by reduced heterozygosity, 2.5 times higher genetic subdivision ( $F_{st} = 7.2\%$ ) in comparison to the foothills populations, as well as by a deficit of heterozygotes, which indicates their inbreeding. Due to differences in reproductive isolation of mountain populations, genetic distances between them are highly variable (0.008-0.036), averaging  $0.021 \pm 0.005$ , which is three

times larger than between foothills populations ( $0.007\pm 0.001$ ). Cluster analysis showed distinct genogeographical division of Carpathian populations of *Pinus sylvestris* into two groups: mountain (at an altitude of 630–1100 m a. s. l.) and foothills (at an altitude of 420–480 m a. s. l.), the border between which can conditionally be drawn at an altitude of 500 m a. s. l. The average genetic distance between the groups of mountain and foothills populations is  $0.017\pm 0.004$ , which is 1.5–2 times greater than between foothills populations in the Ukrainian Carpathians. The differentiation between these groups of populations isolated by the ridges corresponds to the rank of geographic groups of populations. On the basis of our results there are sufficient grounds to separate the Carpathian mountain geographical race of *Pinus sylvestris* L., - var. *carpatica* Klika.

### “Altitudinal patterns of genetic structure of *Pinus brutia* Ten. within the island of Cyprus.”

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**keywords:** Elevation, Island, Mediterranean

*Pinus brutia* is a coniferous tree species, widespread in the eastern Mediterranean Basin. In Cyprus the thermophilous pine forests with *P. brutia* are the most extensive and widespread, occurring in all mountainous areas from dry to sub-humid climates (0–1.400m), occupying 66% of island-wide forest land. This study evaluated genetic patterns and local adaptation along altitudinal gradients in this species within a single forest in an island ecosystem. To achieve this, sampling of 250 trees from three different altitude levels (400m, 800m and 1.200m) within Paphos forest was carried out. Isoenzyme analysis (10 loci) was performed on the seed maternal tissue (megagametophyte). Also, 20 morphoanatomical traits were measured in order to assess their differentiation among the altitude range. Analysis showed a mean genetic diversity of  $HE=0.210$ , while the middle elevation samples (800m) have the highest genetic diversity ( $HE=0.237$ ) among the three altitudinal gradients. Such results may have been caused by altitudinal range fluctuations amplified by the local topography during Pleistocene glaciations and interglaciations, as was typical for Euro-Mediterranean ecosystems. In such case, an admixture zone (in the middle range) could have led to the maintenance of high genetic diversity in this zone. Also, low but significant genetic structure was revealed among the three altitude zones ( $\Phi_{PT}=0.034$ ,  $P<0.05$ ) while  $K$  was equal to 3 (STRUCTURE analysis). Similar results were detected for morphoanatomical traits, since slight differentiation was observed at different altitude ranges. Such outcomes may be linked to local adaptation, since a specific haploid seems to be absent in the different altitude zones, while the morphoanatomical traits have different values at different elevations. Besides, assessing genetic diversity patterns within species altitudinal gradients is likely to detect the effect of demographic and local adaptation on genetic diversity patterns.



## “Small and ecologically marginal populations of pedunculate oak (*Quercus robur* L.) on Dinaric Mountains in Bosnia and Herzegovina”

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**Keywords:** *Quercus robur*, marginal populations

Seven pedunculate oak (*Quercus robur*, L.) provenances in bosnian provenance test have been analysed using isozyme markers. The selected provenance originating from marginal, isolated, small populations on Dinaric mountains in Bosnia and Herzegovina.

Ten enzyme systems have been used, with total 14 genetic loci with 54 alleles. Out of 14 analysed enzyme systems, ten of them are polymorphic, while others are relatively monomorphic. A high level of polymorphism is detected in AAP-A, PGM-B, IDH-A, IDH-B and IDH-C genetic loci.

The smallest average number of alleles per locus is 2,3572 detected in Olovo provenance, and the highest is 3,0714, detected in Miljevina and Sokolac. The greatest number of polymorphic loci is detected in provenances Miljevina and Sokolac (10).

The analysis has proven that there exist massive variabilities within the populations. The lowest value of observed heterozygosity is present in Mrkonjić Grad provenance (0,2102), while the greatest value is present in Olovo (0,2907). The lowest theoretic heterozygosity is present in Mrkonjić Grad provenance (0,2264), and the greatest is in Drvar provenance (0,2605). The lowest value of fixation index has been detected in Olovo provenance (-0,1269), while the greatest index has been found in Drvar provenance (0,0927).

The lowest multilocus divergence has been detected between Miljevina and Ključ, amounting to 0,1428 while the greatest divergence has been found between Olovo and Miljevina, amounting to 0,2975.

The lowest mutual divergence of genetic pool is present in Miljevin and Ključ provenance, amounting to 0,0732 while the greatest divergence is found between Olovo and Bugojno (0,163).

## “Structural characterization and diversity of *Betula pendula* Roth marginal (MaP) populations along an altitudinal gradient in Central Italy”

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**Keywords:** structural indexes, diversity indexes, *Betula pendula*, marginal populations, genetic diversity

The birch (*Betula pendula* Roth) is a typical tree of central and northern European biomes and Mediterranean areas represent the edges of its range. Birch populations in central and southern Italy can be considered marginal characterized by genetic and adaptive diversity due to limiting conditions to survival. It is known that marginal and peripheral (MaP) populations of a species are much more sensitive to the effects of climate change, thus studying adaptive processes in MaP populations is fundamental to develop sustainable strategies for conservation. In the context of a national project (RGV-FAO), birch MaP populations in central and Southern Italy were identified and characterized at structural and adaptive genetic level. In this framework, three different birch

central Italy MaP populations are studied along an altitudinal transect from 1080 to 260 m a.s.l. The three sites are respectively characterized by widely different soils: sandstone rock, peat lands and acid volcanic soils with hydrothermal activities. The birchs' population characteristics were assessed by means of plots which the surfaces ranged from 1695 to 1257 m<sup>2</sup>. The following parameters were recorded: climate, soil, species' composition, social position, diameter and height. The main structural and diversity indexes were calculated. The structure and diversity differences were analyzed in relation to altitudinal and pedo-climatic characteristics. The analysis and comparison of these indexes showed a variability among the populations in terms of structure and diversity, from more complex mixed structures at high and middle altitudes (Pratomagno - FI and Belagaio - GR) to simple monospecific structures at lower altitudes with more constraining soil conditions (Caldara di Manziana - RM). The results will be discussed considering also the genetic diversity of the birch populations.

### **“Decrease of photosynthetic performance and thermostability of photosystem II in European beech (*Fagus sylvatica* L.) towards range periphery”**

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**Keywords:** heat stress, chlorophyll fluorescence, provenance trial

Heat stress is one of the factors strongly influencing growth and survivability of forests. We assessed geographical patterns of photosynthetic performance and the PSII thermostability of European beech and intraspecific differences associated with the climate of origin. Two provenance trials of the international experiment with beech coordinated by von Thuenen Institute, Grossahansdorf, with rather contrasting climate were used for the study: Tále, Slovakia (a colder plot, 18 provenances included) and Zbraslav, Czech republic (a warmer plot, 10 provenances). At non-stressing temperature, the performance of PSII was significantly better at the warmer plot. The populations close to the Slovenian refugium, as well as closer to the site of plantation, showed higher photosynthetic performance. In contrast, thermostability parameters were not significantly correlated with the distance from refugia or trial plot. The severity of thermal stress (R) and the thermal limit of PSII resistance (T15) significantly differed between the plots. The effect of heat stress was much stronger at Tále compared to Zbraslav. Despite the fact that the origin of provenances partly explained the variation among provenances, acclimation driven by the climate of the plantation site plays a major role in response to heat.

## **“The Significance of Gene Conservation of Peripheral Tree Species, Examples for *Corylus colurna* and *Fagus sylvatica*”**

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Keywords: *Corylus colurna*, *Fagus sylvatica*, climate change, peripheral population

Species diversification is viewed as one of the major ways to stabilize forests against threats associated with climate change, e.g. wind storms, floods, drought, etc. Diversification can be achieved by introduction of species or by upgrading existing species by admixing selected provenances. The term “assisted migration” is being used to demonstrate the intention of moving plant material in the form of their genetic resource from presently inhabited areas into areas considered to be suited in future times, the usual direction of movement being northward or higher in altitude. In the anticipated scenarios of climate change, numerous target species and provenances are anticipated to be able contribute to stabilization of forests and at the same time provide valuable goods and services. An example of introducing a species might be Turkish filbert (*Corylus colurna* L.), which has proved its suitability to withstand adverse conditions in numerous civic environments as ornamental tree, where it demonstrates impressively resistance to drought, high temperatures, intensive radiation, pollution etc. An example of an outstanding provenance of a widely grown species might be the beech (*Fagus sylvatica* L.) provenance from Gotze Delchev at the south side of the Pirin Mountains, Bulgaria. This provenance has proved its suitability in numerous common garden sites of the International Beech Provenance Trials throughout Europe established by the Thuenen-Institute of Forest Genetics, Grossshansdorf, Germany and is considered to be able to withstand threats associated with climate change (Eilmann et al. 2014) as it originates from the southern periphery of the distribution range of the species. It is crucial to conserve peripheral forest stands for general reasons of conservation but especially as a valuable genetic resource for future use.

## **“Life for forest genetic monitoring : an opportunity to monitor marginal forest tree populations in Greece”**

Filippos Aravanopoulos, Evangelia Avramidou\*, Ermioni Malliarou, Nikos Tourvas, Ioannis Ganopoulos, Paraskevi Alizoti, Evangelos Barbas, Pavlos Bekiaroglou, Pavlos Hasilidis, Georgios Roussakis, Fotis Kiourtsis, Nikitas Fragiskakis, Marjanna Westergren, Barbara Fussi, Monika Konnert, Hojka Kraigher

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**keywords:** LIFE GENMON, genetic monitoring, Greece

There is now abundant evidence that modern climate change is reestablishing the geographic distributions of forest plant species world-wide. Special attention must be given to geographically peripheral-marginal populations, which are found at the edge of distribution areas and facing already significant changes due to climate change, pollution, human direct and indirect impacts, land use changes etc. As genetic variation is the key component which enables species to adapt

sufficiently to new conditions, the adaptability and maintenance of those important marginal populations in the future depend on their extant genetic diversity and conservation.. Tracking and quantifying temporal changes in populations' genetic variation and structure, "genetic monitoring" can serve as an early warning mechanism for changes that on higher levels (species/ecosystem levels) could only be seen later on. Through the project "LIFE FOR EUROPEAN FOREST GENETIC MONITORING SYSTEM" (LIFEGENMON) which started in July 2014 and will last until 2020, Forest Genetic Monitoring (FGM) will be performed for monitoring areas for two key selected species (*Fagus sylvatica*, *Abies alba* / *Abies borisii-regis*) among three partners, Slovenia, Greece and Germany. The project aims to develop a system for FGM in order to implement in an applied setting and derivation of detailed guidelines for the realization of genetic monitoring on a European level will be produced. Greece is allocated in the southern part of Europe, in the edge of many species distribution range and is characterized by a disproportionately high plant biodiversity, given the landmass of the country. A large portion of this biodiversity, consists of ecologically and especially geographically marginal populations of a series of important ecologically keystone species (*Fagus sylvatica*, *Populus nigra*, *Fraxinus excelsior*, *Abies alba/Abies borisii-regis* complex, *Pinus nigra*, *Prunus avium*, *Quercus petraea/robur* complex etc) that are existing in the geographic area of Greece and are expected to be affected due to climatic change. An implementation of an FGM strategy which will be produced by LIFEGENMON will help to evaluate forest genetic resources and can improve the sustainability of applied forest management practices and direct further research for those important marginal species which are hot-spot of genetic diversity. A case study example for developing baseline data for the FGM of *Abies borisii-regis* is presented.