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Review paper

# Genetic resources and forestry in the Mediterranean region in relation to global change

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Received 28/09/2015 - Accepted 24/11/2015 - Published online 15/12/2015

**Abstract** - The purpose of this review is to examine a few aspects of global change effects on forest genetic resources and their interaction. Genetic resources can provide many opportunities for the development of adaptive forest management in the Mediterranean region. At the same time, forestry and its various disciplines can offer manifold chances to develop methods and techniques for the *in situ* and *ex situ* protection, as well as for the correct management of species and populations at risk because of climate change. Among these aspects, the studies on the Marker Assisted Selection are particularly taken into consideration, as well as the phenotypic plasticity and the different types of assisted migration. A special emphasis is given to genetic resources growing at marginal peripheral populations, which need to be safeguarded as possible containers of adaptive diversity. They are subjected, in fact, to an extreme climatic stress more than others.

**Keywords** - Forest genetic resources, forest reproductive materials, genetics, adaptation, assisted migration, marginal peripheral populations, Mediterranean area, global change, silviculture.

### Introduction

This paper aims to examine some key interactions between environmental factors and the management of forest genetic resources (FGR) in a context of rapid global changes.

The Mediterranean region is surely one of the major genetic hotspots for natural resources, closely interacting with human populations and cultures (Cavalli-Sforza 1991, Cavalli-Sforza et al. 1994) and it needs special care from both the scientific community and the policy makers and the public opinion.

Combining genetic, breeding and FGR management experiences, the Author provides some examples and considerations in order to draw attention to the complexity of managing Forest Genetic Resources in the context of the already evident changes occurring in the Mediterranean region.

### **Climate factors and Forest Trees**

Over million years of evolution, trees have developed different behaviors in order to optimize their adaptive traits. Adaptation occurs at different levels and following ways (Larcher 2003).

- Modulative adaptation, it is important in sites

where wide abrupt fluctuations of ecological factors occur (i.e. the circadian rhythms, as the opening and closing of stomata and chloroplast movements in relation to the minor or major light intensity fall here);

- *Modificative adaptation*, it takes place during morphogenesis in response to the environment and it is generally irreversible. The formation of light-leaves and shade-leaves in many species (e.g. silver fir) is one example. It could be regarded as a synonym of phenotypic plasticity;
- *Evolutionary adaptation*, it is fixed in the genotype according to habitat characteristics, preference of the species or its temperament. The distinction between shade-tolerant and light-demanding trees is an example, as well as between xero-tollerant or mesophyte trees or those adapted or not to acid soils.

Light, temperature and water availability are ultimately the main climatic factors interacting with forest trees, those that can significantly affect tree life and ecosystems where they live, with regard to the changes taking place.

Photosynthesis is the first adaptation process that biological forms invented to store energy

<sup>1</sup> Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Forestry Research Centre (CREA-SEL), Arezzo, Italy \* fulvio.ducci@entecra.it from the sun. This biological process has radically changed Earth's environment, triggering ecological processes and enabling the evolution, which is nothing but the search for species of the right position to take advantage of a trickle flow of energy flowing through the ecosystem (Larcher 2003).

Among the different ecological factors, temperature is one of the most important and can significantly address species distribution. In Mediterranean contexts, temperature, on the ground, up to  $70^{\circ}$ C. at Aleppo pinewoods in southern Italy were usually measured (Magini 1955).

Clear evidence exists of the adaptive meaning of temperatures influencing the photosynthetic function in trees, in relation to habitats (Taylor et al. 2012). Seedlings collected along an elevation gradient, from 730 to 1460 m above the sea level, showed a photosynthetic optimum temperature reduced to about 4.3° C per 500 m in altitude. These values are closely related to the negative elevation gradient of the measured air temperature that equals to  $\pm 3.9$  C per 500 m on average.

Under these conditions, the pressure of natural selection towards a greater photosynthetic rate is very heavy.

A plant is under water stress when the water requirement necessary for transpiration exceeds the amount provided by the roots. The trees react by increasing their own stomatal resistance, i.e. by closing stomata and reducing the  $CO^2$  and water flow.

### Climate modifications expected in the future

Since 1980s, global change has started to show its evidence and has been acknowledged as seriously affecting all of Earth ecosystems. Under this framework, forests, biodiversity and genetic resources are under threat, as well as people depending on goods and services (WMO 2007).

Diversity is the main tool for fragmenting risks in the Biosphere and also forest diversity contains adaptive traits which can be useful for the adaptation of species to the changing environment and for supplying the variation to be used in silvicultural activities to develop adaption and mitigation strategies of management.

The effects of global changes, with special regard to climate parameters can endanger forest tree populations because of the persistent and intense modification of patterns within main climatic factors (i.e. intensity, seasonal and geographical distribution).

Water availability, together with air temperature, will change seasonal patterns too rapidly and trees may not be able to adapt to the changes. The repeated drought stress over several years and its cumulative effects can at times be quantified only when the damage is already nearly definitive.

The Mediterranean region, already subjected to highly variable climate regimes, is more and more characterized by unpredictability and by an increasing frequency of extreme events. In this area, the effects of global change and especially of climate are likely to be even more sensitive. It therefore raises the need to manage and preserve the Mediterranean forest tree resources because differentiated and characterized by the presence of adaptive traits possibly useful to develop strategies for adaptation (Ducci et al. 2011 in Besacier et al. 2011, Ducci 2014).

The historical occurrence of many civilizations that significantly influenced the life of ecosystems and their genetic resources is a typical attribute of the Mediterranean region. People have been using and taking advantage of this biodiversity for millennia. Although it resulted in the rise of original habitats and human-designed landscape structures, in fact, the interaction between humans and biodiversity has sometimes led to the decline and disappearance of species and populations (Blondel and Aronson 1999).

Nowadays, climate change effects pose other formidable challenges to biodiversity in this region. Most valuable forest ecosystems and species in the Mediterranean region experienced and survived the most recent post-glaciation climate warming thank to the presence of mountain ranges where suitable ecological conditions were found at different altitudes. But the changes since last glacial periods were not so rapid as the changes predicted today. At least five cold periods have been recorded in Europe over the last 8000 years; their duration was between 100 and 300 years on average and they influenced both the life standard of civilizations and the diffusion-extension and type of forest ecosystems (Pinna 1977).

More recently, the IPCC (Intergovernmental Panel on the Climate Change 2001, 2012) predicted an average temperature increment over the next 50 years in Europe of  $2 \div 4^{\circ}$ C, according to the different regions. This change means the upward shifting of climatic and ecological areas in the mountains. For a number of reasons however, this "escape" will not always be sufficient to solve the problem. In fact, the on-going climate modification and its effects will be more complex than we can imagine and many factors will change the patterns and the related feedbacks and interactions.

### Changes in the main patterns

The actual changes in climate seem to be very rapid as compared to past alternate warming, gla-

 Table 1 Scenarios for minimum temperature changes estimated according to different scenarios and in different regions of Italy (courtesy of Perini, Salvati et al. 2007). Legenda: IS: Initial Situation; S0: lower intensity scenario; S1: averaged intensity scenario; S2: higher intensity scenario.

	Minimum temperatures (°C.)															
Geografic Region (Italy)	Winter				Spring			Summer				Autumn				
	IS	<b>S</b> 0	<b>S</b> 1	S2	IS	S0	<b>S</b> 1	S2	IS	<b>S</b> 0	<b>S</b> 1	S2	IS	<b>S</b> 0	<b>S</b> 1	S2
Nord	2.4	1.1	1.7	1.1	3.8	3.7	3.7	4.6	12.1	12.1	12.4	13.1	5.8	5.7	5.9	6.1
Central	2.3	3.5	3.4	3.8	6.8	6.7	6.9	7.5	15.3	15.3	15.7	16.3	9.8	9.7	10.0	10.4
South	5.7	7.0	7.0	7.2	9.4	9.4	9.5	9.9	18.2	18.2	18.5	19.2	13.0	12.9	13.2	13.5
Sicily	7.8	9.2	9.3	9.5	10.6	10.6	10.5	11.1	19.3	19.2	19.7	20.2	15.2	15.0	15.5	15.8
Sardinia	6.4	7.6	7.7	7.6	9.2	9.2	9.3	9.7	17.7	17.6	18.1	18.5	13.3	13.1	13.5	13.7

cial and micro-glacial periods. But the occurrence of climate change is not new and climate warming started earlier than admitted by media and popular press. Such change was recorded by climatologists since the second half of the XIX century.

At the end of 1800, many glaciers were lost or widely reduced in area on the Alps and other mountain ranges. In 1935, the sea mean temperature, at higher latitudes, increased of 1 to 3.5°C (Pinna 1977). Many similar forecasts and scenarios are recorded in the literature on this topic. Concerning Italy, for instance, Perini and Salvati (2007) produced some scenarios with major details. In particular, three different cases (different intensities of change) were considered by the authors, named respectively SO (ΔT (°C): 0.1 Tm 13.0° C.), S1 (ΔT (°C): 0.2 Tm 13.4° C.) and S2 ( $\Delta$ T (°C): 0.8 Tm 14.0° C.) over the reference period 1960 - 1990). Climatic patterns change mainly with latitude, but also they vary according to orographic variation, local morphology and distance from the sea.

The mean annual temperature change is actually forecasted to increase from  $13^{\circ}$ C to 13.4 and  $14.8^{\circ}$ C respectively, with a maximum increment of  $0.8^{\circ}$  C.

This perspective of the authors is relatively moderate as compared with current ICCP scenarios (2012).

*Minimum temperatures* will probably change with different patterns and intensities in different geographical areas of Italy; the South of Italy and the larger Mediterranean islands will be especially affected (Table 1).

The changed patterns are even more pronounced when *maximum temperatures* are considered (Table 2). Predicted change in *rainfall amount* would be also significantly heavy. About 60% of the annual precipitation could be lost in some areas, especially in winter and spring. The autumn rainfall would predictably be more stable, but both intensity and concentration of precipitation could become a problem due to the increased occurrence of floods. Their *geographical distribution* would also predictably change and, according to the most optimistic scenario (S0), Sicily and Sardinia would reduce total rainfall amounts between 17 and 21%, whilst reductions would amount to about 5% - 9% in the continental regions of Italy.

If both of the two worst scenarios (S1 and S2) are taken into account, the decrease of precipitation would predictably be 20% in northern, 17% in central Italy, 25-26% in the South and Sardinia, and 12% in Sicily (Table 3). An increasing drought towards a 200 kilometres higher latitude would affect Italian central regions.

### Main traits of forest genetic resources under climate driving forces

According to Thompson (2005), the climate changes in the Mediterranean region, the most recent ones being occurred during the Quaternary (Huntley and Birks 1983), together with the salinity crisis in late the Messinian Tertiary, have driven the shaping of diversity among and within Mediterranean species.

Together with Balkans and the Iberian Peninsula, the Mediterranean region is indeed considered among the major and complex refuge zones in the Pleistocene. As a result, plant diversity is relatively high in this area (Hampe and Petit 2005, Fady and Conord 2010).

 Table 2 Scenarios for maximum temperature changes in different regions of Italy and in different seasons (courtesy of Perini, Salvati et al. 2007). Legenda: IS: Initial Situation; S0: lower intensity scenario; S1: averaged intensity scenario; S2: higher intensity scenario.

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		Maximum temperatures (°C.)														
Geografic Region (Italy)	Winter				Spring			Summer					Autumn			
	IS	S0	S1	S2	IS	S0	S1	S2	IS	S0	<b>S</b> 1	S2	IS	S0	<b>S</b> 1	S2
Nord	4.6	5.3	5.3	5.8	13.2	13.0	13.5	14.8	22.8	228.8	23.1	24.0	14.5	14.2	14.5	14.6
Central	10.0	11.1	11.2	11.6	16.8	16.6	17.1	18.0	27.1	27.1	27.4	28.4	19.3	19.0	19.4	19.7
South	12.7	14.0	14.2	14.5	18.2	18.1	18.4	19.2	28.3	28.1	28.7	29.7	21.4	21.1	21.7	22.1
Sicily	14.6	15.9	16.0	16.3	18.9	18.8	19.2	20.0	28.8	28.5	29.3	30.0	23.0	22.7	23.2	23.7
Sardinia	13.8	15.0	15.1	15.3	18.5	18.2	18.9	19.8	28.7	28.5	29.2	30.2	22.1	21.8	22.4	22.8

 Table 3 Scenarios for rainfall changes in different regions of Italy in different seasons (courtesy of Perini, Salvati et al. 2007). Legenda: IS:

 Initial Situation; S0: lower intensity scenario; S1: averaged intensity scenario; S2: higher intensity scenario.

		Precipitation (mm)														
Geografic Region (Italy)	Winter				Spring			Summer				Autumn				
	IS	S0	S1	S2	IS	<b>S</b> 0	S1	S2	IS	S0	S1	S2	IS	S0	<b>S</b> 1	S2
Nord	220	221	152	179	264	250	220	184	263	242	238	204	282	262	212	267
Central	278	270	236	239	199	178	161	163	140	122	120	96	282	277	238	269
South	286	255	241	237	166	148	152	119	78	73	65	47	248	231	226	203
Sicily	223	203	226	247	132	109	113	88	34	21	19	26	186	177	202	191
Sardinia	241	180	198	190	126	124	90	92	34	24	21	19	201	146	177	169

As above mentioned, the Mediterranean region is one of the world's biodiversity hotspots for its outstandingly high plant species richness and endemism (Médail and Diadema 2009). In this area, the presence of genetically valuable populations of forest species belonging to central and northern ranges will be seriously affected and, in a few cases, endangered. These populations are in most cases characterized by high levels of differentiation and are frequently typically marginal.

It is thus likely that forest genetic resources originated in the southern latitudes of Europe have often developed their major traits under difficult environments and survived well under unsuitable ecological conditions because of unusual adaptive traits. They are valuable sources of variation for expanding as well as retreating forests under the climate pressure and for developing adaptive management, silviculture and mitigation initiatives.

The changing climatic patterns may have significant influences on the adaptive traits of species, and any rapid change can cause disequilibrium in growth, physiology and reproductive systems.

In Mediterranean climate areas, forest species adapted their living cycles to the rainfall time; autumn and winter represent for most of them the growing period and provide the best conditions for seed germination.

Besides, most of trees, especially those growing in temperate areas in mountain sites and belonging to mature ecosystems, such as many hardwoods, can activate their annual cycles only strictly following the seasonal climatic change. Thus, growth and reproductive steps can occur only in suitable conditions and/or when adverse conditions can be mitigated or avoided.

As above mentioned, the isotherms will probably shift very rapidly because of the increasing average temperatures. With regard to the ability of tree populations to migrate under the influence of isotherm shifts, Màtyàs (2007) proposed a scenario where mean temperature would increase by about  $2^{\circ}$ C in 35 years, an estimate more cautious than the Italian evaluations. According to his standpoint, in Europe the south thermal gradient would shift towards the north at the speed of 3 km/year and of 11.5 m in elevation (Màtyàs 2007).

In northern America, the shift is estimated 6-15 km/year in the plains and, in any case, the estimated speed would exceed the potential migration rate of most of forest trees (Aitken et al. 2008).

This change is very rapid, as most of the species have migration rates which can vary between 100 and 400 m/year in the plains (Màtyàs, personal communication), according to seed weight, reproductive system, habitat, ground morphology and other ecological and biological factors at local level. Iverson et al. (2004) estimated this rate at 100 - 200 m/year for northern America.

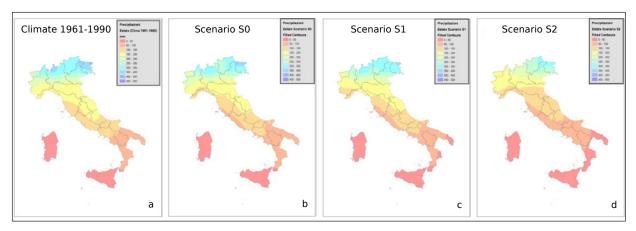


Figure 1 - Maps showing the peculiar situation of decreased summer mean rainfalls in most of central and southern Italy which will be only partially offset by the presence of the Apennines. a) 1961-1990; b) IPCC lower intensity scenario S0; c) IPCC lower average scenario S1; d) IPCC higher intensity scenario S2.

With special regard to the southern European and the Mediterranean area, the mountain area is widespread and could probably contribute to mitigate changes with the elevation and aspect effects. There, tree species have to migrate upward following the shift of their environmental envelope and niche to survive. Migration towards mountain tops is made more difficult because of gravity. Moreover, mountains are not always so wide and high enough to allow the migration of an adequate gene pool to sufficiently high elevations.

### Possible FGR responses to climate change

Tree species and their ecosystems in the Mediterranean region are adapted to several climatic constraints, such as summer drought, late spring frost and severe winter frost, depending on site.

Even the lower range of climate change scenarios predicts a worsening of these conditions, with increases in mean temperature and lengthening of summer drought (Màtyàs 2007, Màtyàs et al. 2009) and frequency/intensity of extreme events (IPCC 2007).

Already in 1937, De Philippis proposed an effective and realistic scheme of relationships between the main climatic and edaphic characteristics of the Mediterranean region and the corresponding change of main forest ecosystems (Fig. 2). In this work, the Author defined the limits of the Mediterranean area and the vegetation type replacing the holm oak forest as the mature forest ecosystem, highlighting, moreover, the possible main trends according to changing patterns.

According to the State of Mediterranean Forests 2013 (Besacier et al. 2013, COFO-FAO 2014), Forest Genetic Resources (FGRs) may respond in various ways to environmental change, including migration to track the geographical shift of areas providing suitable environmental conditions (Parmesan and Yohe 2003):

- 1. acclimation through phenotypic plasticity (i.e. the change in functional traits expressed by an individual in response to environment change across its lifetime; Chevin et al. 2010);
- 2. evolutionary adaptation (i.e. a change in gene frequency from one generation to the next resulting in a change in fitness; Kawecki and Ebert 2004);
- 3. changes in the forest community (van der Putten et al. 2010).

It is often accounted that migration was the dominant factor in shaping genetic diversity during the Pleistocene (Petit et al. 2003). However, despite past glacial and post-glacial migrations of many taxa (inferred from fossil pollen records and genetic data), which suggest a robust capacity for range shifts, today the migration potential of several species is considered insufficient to keep pace with the projected rapid future climatic change (Loarie et al. 2009).

Evolutionary adaptation can also be very rapid: major shifts have been demonstrated over only a few generations, such as increased drought resistance and growth in *Cedrus atlantica* (Lefèvre 2004) and epigenetic-based shifts in bud break phenology in *Picea abies* (Yakovlev et al. 2012). There are examples in the Mediterranean region of local adaptation in trees (although they are mostly phenological), especially from 'common gardens' and ex situ experiments (Savolainen et al. 2007, Vitasse et al. 2009). Phenotypic plasticity has been demonstrated to be an efficient response mechanism to change (e.g. *Cedrus atlantica* in Fallour-Rubio et al. 2009).

In nature, migration is an important way to reestablish populations in suitable conditions (Pitelka et al. 1997). The possibility to migrate or find local refuges exists principally in the northern and eastern sides of the Mediterranean region; migration would be a problem, however, for forest tree species in the southern part of the Mediterranean area, where the only chance of natural migration is towards the mountain top.

Another important limiting factor is drought. In general, it would affect the southern border of natural tree species ranges and its effects would probably be more tangible in the Mediterranean region than elsewhere in central and northern Europe. This region is characterized by several traits which make it an important hotspot of adaptive variation:

- 1. it is the xeric southern limit of many northern and continental European tree species (mostly in the northern side of the Mediterranean sea);
- 2. there are typical Mediterranean species of conifers and hardwoods, adapted to the Mediterranean climate;
- 3. there are also Mediterranean temperate forests, which are very sensitive to change;
- 4. there are remnants of former climatic changes (colder periods), found in refuges on the mountains (mostly on the southern side of the Mediterranean).

Concerning the above categories, the following short predictions can be formulated according to Pitelka (1997):

case 1) the southern populations of northern species, in many cases fragmented and isolated would be reduced progressively in size

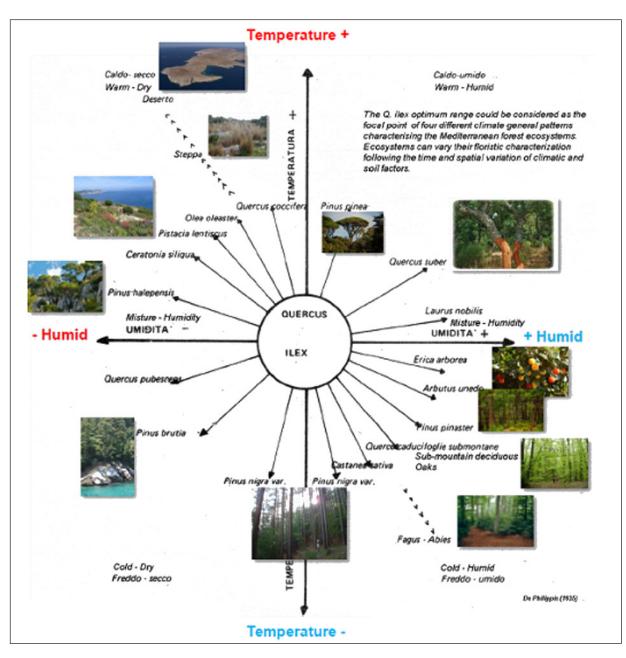


Figure 2 - Studying holm oak and cork oak (*Quercus ilex* and *Quercus suber*) in Mediterranean arboreal vegetation, Pavari highlighted the climatic and edaphic parameters influencing the distribution of these two species, then defined their needs and drew attention to the consequences of heavy and prolonged human action on their distribution (De Philippis 1937 in Pavari 1959, modified).

and eroded and, somewhere, populations would disappear. Indeed, the isotherm shift towards north would probably leaves smaller populations growing at higher elevations. These would remain isolated and genetically eroded. In the case, these populations were differentiated for adaptive traits and their disappearing will determine the loss of important amounts of genetic information. In any case, this information would be really endangered.

case 2) the Mediterranean forests and ecosystems (i.e. Mediterranean Pines, evergreen oaks and shrubs communities) could be initially the less endangered during the first steps of climate change. Most of them are



The marginal population of Pinus nigra v. Villetta Barrea in centralsouthern Italy (M. Marchi and A. Teani - CREA SEL). characterized by extended and/or continuous populations and, in principle, this species should be more adaptable to drought. Despite this, even the toughest forest trees in the Mediterranean climate, need a minimum amount of water availability to survive. Their resources could be probably endangered in the same way of point (1) due to the progressive drying of climate, to weather extremes and also to related causes (i.e. forest fires and migration of human activities as agriculture and grazing).

- case 3) the deciduous trees forests of hardwoods or pines and other conifers covering the mountain sites at average elevations. Their water and temperature requirements make them really sensitive to the changes expected in the Mediterranean region. Adaptation and migration towards higher elevations should be consequently implemented using natural (silviculture) and artificial methods. The search of local suitable condition spots for survival would be a good strategy.
- case 4) *several relict populations as Cedars and Mediterranean firs and others*, grow in isolated and endangered populations at the higher elevations of all the Mediterranean contour. Most of them are small and/or under genetic erosion since long time. Climate change would probably put them in the first category of priorities for common action for their rescue.

Throughout the Mediterranean region, the selection pressure caused by drought has been always significant over the past millennia and its effects will probably increase with the ongoing climate change. In some areas, however, tree species have reached an equilibrium and/or found microclimates able to supply a refuge for them. The progressive further effects of drought will be probably initially seen in the decline of tree species in many areas and by mortality related to weather extremes. A recent evidence is the outcome of prolonged summer droughts of 2003, 2007, 2010 and 2011.

The prolonged drought decade since the 1980s, resulted in recurrent and frequent pullulations of pests and diseases for many species. Symptoms were initially mistaken as primary causes of mortality, whilst the former stress was probably the predisposing factor.

*Temperature, drought and day length,* are the most pronounced driving factors for phenological processes (eco-dormancy) in northern areas, and

temperature is, among them, the most important and variable factor (Richardson et al. 1974, Linvill 1990). Temperatures can thus be seen as the limiting factor for the northern borders of the natural range of forest species in the area. Dormancy is an important function for tree phenology and for seed germination, related to temperature effect.

*Dormancy.* which is also related to phenology and seed germination (Walck et al. 2011) can be seriously influenced in many areas. Actually, changes in the "*Chilling Units*" (CU) amount can lead to disequilibrium in phenology for growth, flowering and seed germination, which are crucially important for a successful natural regeneration (Fig. 3). As an example, damages due to late spring frost occurrence are increasing in frequency (Hodkinson et al. 2011).

Each species, or *taxon*, requires a specific average number of CU to activate physiological processes and to break endo-dormancy.

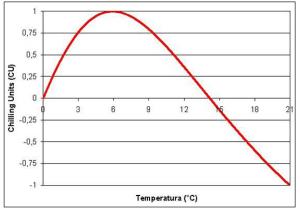
The absence of early lower temperature signals in autumn concluding hardening and too early warmish weeks at the end of winter and in early spring can induce breaking of dormancy too early in many species. In such cases, these species will be not able to avoid damage by late frosts.

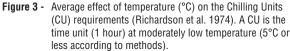
### **Genetic adaptation**

Species were so far generally successful to adapt to climate changes (Eriksson et al. 1993).

The mechanisms used to confront such changes have proven effective in a long series of earlier climatic events and changes, even in relatively recent times. Earlier evolution did not account however the most dangerous environmental factor (i.e. humankind and its explosive (and polluting) demographic and industrial development).

Until now, trees have reacted relatively well to the change, and it is commonly thought that genetic variation is still enough to supply materials for the





adaptation of the populations and to sustain their dynamics (Eriksson 1996 and 1998, Hamrick 2004).

Moreover, even phenotypic plasticity (Schlichting 1986, Pigliucci 2001) and long life cycles of forest trees can support natural regeneration over the decades, in spite of environmental fluctuations. Micro-environmental variability can play an important role in the process of conserving variability and in some cases microclimate refuges can be maintained. Adaptation is estimated to be relatively rapid: 2 o 3 generations can be sufficient for a forest tree population to modify its adaptive patterns.

However, as above mentioned, the speed of environmental change is increasing and there is a general fear that tree species and populations may not be able to adapt rapidly enough.

In the Mediterranean Region, a genetic hotspot region (Fady 2005), forest tree populations are in general small and genetically eroded; they are also generally isolated, and gene flows and gene exchanges between them are often limited.

Most species are distributed by meta-populations, which probably exchanged genes in past times, when the climate allowed their expansion. Nowadays, human activities, at least in Europe, have often interrupted the chance of gene flows definitively, and probably for longer times than similar interruptions in natural conditions.

Thus, in addition to natural factors which may influence the response of trees to environmental change, *social* and *civilization aspects* should be considered as factors which play a role in the spontaneous migration of forest species in places where agricultural areas, industrial trees, roads and human settlements establish insuperable barriers.

In the past, a lot of genetic information was used by forest populations and tree species to adapt to environmental change; the present, rapid climatic changes may exceed the tolerance limits of many species. We have to expect a dramatic loss of variation for populations in the Mediterranean area and, in some cases, we should expect an extinction of the species or part of them, especially in the most extreme conditions of their natural range.

Together with a genetic response, a wide part of the interest of the research community is focused on the assessment of the *quantitative response to change*. In fact, knowing rapidly and experimentally those responses is fundamental to support decision making and the actions aimed at conserving variation.

Conditions deserving special attention are both frequency and intensity of extreme events (frost, drought, high temperatures etc). These extreme events are important factors for the selection within populations and species. It is well known that species response to natural selection is not monolithic, as variation in responses to change can be clearly observed for many species (as many comparative provenance and progeny or clonal tests have widely shown).

Two main complex characteristics are used by forest tree species to be adapted.

*Resistance/Tolerance* and *Phenotypic plasticity* are the two faces of adaptation and are the basic traits which can be used to study the effects of changes on forest tree populations and their response:

- *Tolerance:* it is the ability of a genotype to preserve its fitness under the pressure of a damage factor. It is genetically settled and fixed by the evolutionary force; it allows each species to occupy a given ecological niche in a given habitat.
- *Phenotypic plasticity:* it is the asymmetric response of genotypes to extreme events. It can be defined as the property of a given genotype to produce different phenotypes in response to distinct environmental conditions.

Both of these properties are presently considered as a key for adaptation to climatic changes.

Among them the role played by evolution in shaping phenotypic plasticity remains still poorly understood (Pigliucci 2005). This property of many phenotypic traits can be used by organisms to start adaptation, which can become the first step of an evolutive process, producing fixation and then divergence and differentiation among populations.

Chambel et al. (2005) discussed this concept as a tool to understand the adaptive processes in forest species.

In a recent review Healy and Schulte (2015) supported the idea that the variety of patterns observed in the gene expression evolution in some species could evidence that a set of traits can occur in organisms, and that each of them can respond to environmental variation through phenotypic plasticity and genetic divergence in different ways. This aspect can influence genetic divergence between species and populations and finally their adaptive potential.

In this field, Santos-del-Blanco et al. (2013) heightened this concept by studying 52 Mediterranean pine populations. They found that pines can represent a model to learn about the adaptive value of allocation to reproduction vs. vegetative growth. That happens thanks to their higher differentiation among-population, from the adaptive point of view, and their ability to cope with environmentally dry and harsh contexts.

Their results followed theoretical predictions and support the idea that phenotypic plasticity for reproduction can be considered as adaptive under stressful environments. Phenotypic plasticity, together with natural selection on reproductive traits, can therefore play a relevant role in the future adaptation of forest tree species in an increasing selective context as in the Mediterranean area. Aranda et al. (2010) and Climent et al. (2009a, 2011, 2013) findings contributed to confirm these concepts as for Mediterranean and Asian pines. This is also important in introduced species adaptation, even in cases where genetic variability seems to be low after their introduction. For instance, Fady et al. (2003) reported about Juglans regia, a tree introduced in western Europe by different human migration waves (Pollegioni et al. 2014) and characterized by low genetic variation. They found that, for adaptation, the selection pressure operated by human uses is still high and bud break ranking is significantly correlated with its European provenances. As a practical consequence, southern European early bud break plant materials should not be planted under most middle European conditions. Moreover, as in these areas late spring frost can be expected, damages on the apical buds caused by frost are closely correlated to architectural traits (and thus wood quality).

A debate is still ongoing about the real ability of species to adapt in view of so rapid changes, but, pragmatically, strategies and possibly common actions plans have to be established in case they will be in danger of genetic impoverishment or extinction.

Whilst related information on adaptive characteristics of boreal species has gradually been collected over the past years, the lack of knowledge about the Mediterranean species in this regard is still wide. As above mentioned, other influences and pressures due to global change effects as pests and diseases, can affect tree and plant species.

On the sidelines of this work, primarily focused on adaptation to the physical factors of the environment, it is necessary to refer shortly to the complexity of ecosystems hit by the changes. Global change is actually increasing the biodiversity patterns in Europe and in other regions because of the rapid migration of new pathogenic or invasive organisms.

Over their long life-span, forest trees have to develop not only a demographic interaction with individuals of their own and other species, but also with biotic factors migrated or introduced in their natural range, as pathogens, parasites etc. Several case can be recorded where trees have to cope with many new problems.

Many palm species, for instance, are heavily endangered by the attacks of *Rhyncophorus ferrugineus* (*Coleoptera*) in the Region (Gomez and Ferry 1998).

Recently, the economy of Stone Pine (*Pinus pinea* L.) coastal pinewoods has been seriously

damaged by fungal pathogens, among them *Diplodia pinea* (Waterman 1943) and the western conifer seed bug *Leptoglossus occidentalis* (Tescari 2001), which have contributed to important losses of stone pine nuts.

Again, most of the chestnut (*Castanea* spp.) production in Europe is severely affected by *Dryocosmus kuriphilus* (Yasumatsu 1951) gall wasp known as chestnut gall wasp, Oriental chestnut gall wasp or Asian chestnut gall wasp. This pest is native to China and it is rapidly spreading to other world regions where competitors are lacking (Graziolin and Santi 2008).

### Challenges for management and policy making

Climate change could have implications for the forest ecosystem services needed for human wellbeing, such as water cycling, carbon sequestration and the production of numerous wood and non-wood products (Millennium Ecosystem Assessment 2005).

FGRs also face other challenges: Sala et al. (2000), for example, showed that land-use change and biological invasions remain key drivers of biodiversity change in Mediterranean biomes.

Nevertheless, all forest management decisions should now take climate change into consideration, but how to take the uncertainty associated with climate change into account in management plans is a formidable challenge.

In a region with the attributes of the Mediterranean, where fragmentation is high because of geomorphology and the history of human activities is long, it is unlikely that the migration of plant species and forest types will be fully able to reduce the impacts of climate change on forests. Even where migration is possible, societies may be unwilling to accept massive forest dieback in some areas and the subsequent natural selection of more suitable genotypes, and may demand intervention.

Societies may also be unwilling to accept a substantial reduction in the productivity of high-yield forests as a consequence of phenotypic plasticity.

Some of the challenges that forest managers will face in developing strategies under the uncertainties of climate change are listed below. For each challenge, research can already provide management options.

### Possible actions to be undertaken

Assuming that climatic change is ongoing and that its gravity is increasing, the possibility to undertake initiatives for the mitigation of climate change effects and the conservation of forest genetic resources (FGRs) in the Mediterranean region, should be considered.

In agreement with Sala et al. (2000), integrated efforts by climatologists, ecologists, social scientists, and policy makers will be required to get realistic scenarios of future changes in the Earth system, especially the Mediterranean.

These scenarios should be based on quantitative analyses and have to consider studies of the interactions among factors to which local biodiversity is most sensitive in each biome.

The knowledge of the expected effects on biodiversity will help to develop management practices according to the biological, social, and economic characteristics of the area where you are working on.

#### **Choice of priorities**

Research initiatives should be prioritized in order to save time and resources.

A first useful approach to apply priorities might be based on a list of *tree species which are presently considered to be seriously endangered.* 

A method for prioritizing actions could be based on choosing *model species able to represent different climatic situations or altitude ranges.* 

A third way could be based on choosing *model* species which represent a number of genera.

Màtyàs (2007) reported on a valuable comparison carried out among forest tree populations, according to their main characteristics and the structure of populations, and the corresponding urgency levels for action to be undertaken; see Table 4 below.

Mediterranean pines, evergreen oaks and other tree species belonging to Mediterranean habitats may be initially less endangered than central northern temperate habitat species, actually they are generally better adapted to drought.

However, with the progressive drying of climate, the increase in weather extremes and related events such as forest fires, and shifts in human activities such as agriculture and grazing (particularly in the southern Mediterranean region), also the habitats in this area will come under increasing pressure.

Isolated populations of many mountain Mediterranean species (e.g. *Alnus cordata, Pinus nigra* v.

Table 4 -Comparison between species/population structures and their<br/>urgency requirements (Màtyàs 2007, modified).

A. Species/population structures requiring <b>low urgency</b> for initiatives	str	Species/population ructures requiring <b>gh urgency</b> for initiatives
<ol> <li>Continuous distribution</li> <li>High density</li> <li>Naturally regenerated</li> <li>Effective gene flows</li> <li>Spontaneously spreading</li> <li>Extensive, zonal habitats</li> <li>Reproduction unaffected</li> </ol>	3. 4. 5. 6.	Fragmented or isolated distribution Small or scattered Artificially regenerated Limited replenishment of the gene pool Low dispersal capacity Extreme sites as habitat or small size Disturbances in flowering and seeding

*laricio* and *P. heldreichii*, Cedars and Mediterranean firs) growing at their ecological or geographical margins will be endangered. Most of these populations are small and scattered and have been affected by genetic erosion in the past.

Other isolated populations of widely distributed central and northern European species should receive special attention. This may be the case in southern areas of natural ranges in the Mediterranean area. Here, isolated populations growing on southern aspects of mountain sites could not have space to migrate in altitude to follow future climatic isotherm shifts. All these populations are likely to be already seriously suffering the effects of the present climate change, and they cannot migrate as a response to such pressure.

In some case, these populations are accounted and classified as Marginal Peripheral populations, also known as MaPs (Yeh and Layton 1979, Hampe and Petit 2005, Eckert et al. 2008).

Furthermore, it is to be considered that particular attention and therefore priority should be given to species or populations which are isolated and growing under extreme conditions. Very often, these species and populations, important for specific uses or endemic, are really endangered.

In these populations, the gene flow is generally reduced, as well as the ecosystem below a critical mass, and there are problems related to the low dispersal capacity and the genetic erosion.

This is the typical situation where the habitat has been severely reduced and even small additional disturbances can compromise the survival of the population. Consistent examples are *Cedrus libani*, *Abies nebrodensis*, *Cupressus dupreziana* and a number of other spp.

### In situ conservation

Foresters should be aware that conserving and managing the genetic variability of forest trees *in situ*, following a dynamic approach, is the basis for selection and for developing adaptive management. It is also important to perpetuate the ecosystem functions and services as well as to ensure more resilience to the ecosystem.

*In situ*, conservationists and improvers can interact in order to select adaptable basic materials within Marginal populations, allowing the production of adapted offspring. Thus, the reaction of basic materials can be tested *in situ*, being exposed to the temporal and spatial variation of micro-environmental conditions. It is evident that *in situ* selection should be carried out mostly on highly heritable adaptive traits, mainly eco-physiological (drought and frost resistance/tolerance) and phenological.

McKown et al. (2014) studied this topic on *Populus trichocarpa* where they found that environmental gradients can shape both the phenotypic adaptive trait variation and the genetic structure.

Gene flow, genetic drift, selection, recombination, and the reproductive system characteristics are factors that act in determining the genetic structure of a population, but the value of the genetic resources of a stand is also strictly determined by the way foresters manage them.

In general, silviculturists do not seem to be aware of these concepts and they also do not seem to be used to account the genetic value of each single tree in the forest and their possible contribution to the structure of the species population they belong to.

Individual trees, through the variation of their fitness and by means of sexual interactions, determine the genetic structure of a population and, together with mutations, they produce variation and are the basis of the evolutive processes, adaptation included.

In silviculture and forest management, as for the *in situ* management of valuable populations, the knowledge of the spatial genetic structure of forests should be a common and shared approach.

A first simple model of structure in a stand is the subdivision of a population in sub-populations or *demes*. According to this model, if a plant population is dense enough, it is likely that pollen and seeds are dispersed not far from the trees that produce them (Sagnard et al. 2011), regardless of the type of dispersion. In this way, the exchange of genes in a population is very slow between groups of trees which are more distant and relatively quicker and easier within these and the entire population can be accounted for a number of sub-populations.

Because of their small size and isolation, isolated demes can differentiate themselves from others due to genetic drift. If their structure is stable for several generations, within demes you may have a certain degree of consanguinity between neighboring trees.

Gene flow between demes counteracts the effect of differentiation. The structuring pattern increases with number of generations and gradually, during that population is consolidating, until a balance between drift and gene flow is reached.

Managers and silviculturists should take in account the genetic structure of populations, as above described, in order to preserve genetic variation and they should therefore have in mind the need to have a good knowledge of the genetic structure and the spatial distribution of the genetic information of the forest they are managing (Lindner et al. 2011, Bolte et al. 2015). This would be necessary to preserve biodiversity and sustainability, as well as to maintain the availability of the selected materials to be used as a source for planting programmes.

As an example, an explorative study (Ducci et al. 2004) showed that in *Quercus cerris* coppices with standards, most of the genetic variation can be found in the left untouched or cut, depending on the practice in progress purpose, dominated stumps. Therefore, new forms of coppice management should be tested in order to preserve an amount of dominated or apparently not useful stumps as a stock of the genetic information belonging to the local gene pool.

Two cases regarding pedunculate oak (*Quercus robur*) and silver fir marginal populations (*Abies alba*) in the Po Valley and in central Italy, respectively, are reported in boxes A and B.

Silviculturists should account the right way to preserve this variation which was in the quoted case study neutral but, at the same time, an indicator of the genetic structure of the populations of concern.

#### A - A case-study.

### The in situ management of marginal pedunculate oak populations in the Po Valley.

Most Italian populations of pedunculate oak, fractionated and usually not extended, are made up of a mature layer, consisting of a few trees per unit area and a nearly total absence of regeneration established.

The consistency of the mature layer decreases progressively, due to the natural ageing of individuals or to heavy meteo events, these leading to the collapse of mature trees. Not being available a continuous monitoring over time of the genetic structure, one can only assume the occurrence of an ongoing process of progressive genetic erosion. This aspect may help to explain the very low observed heterozygosity (Ho), despite the good potential expressed by high values of expected heterozygosity (He) and the high rate of inbreeding.

To address the population to a higher dynamic equilibrium is therefore necessary to carry out silvicultural operations which promote natural regeneration and, more generally, the dynamics of populations. It is necessary to try to encourage the mixing within the population as much as possible, both through improved pollen exchanges between trees and with the active management promoting the occurrence of dissemination and regeneration establishment (internal transfer of seeds and seedlings).

This pro-active practice will influence the following generations, increasing the levels of diversity and interchanges between related groups (demes made by families of siblings or half-siblings), especially within and among peripheral strips of the population, affected by intense genetic erosion.

A well-suited example is given by the small and isolated oak population of Capriano del Colle in the province of Brescia, Po Valley, already described by Ducci (2007). In relatively recent times gene flows in both pedunculate and sessile oak populations growing in the Po Valley (northern Italy) were interrupted. The main changes occurred during the Renaissance age, when wood exploitation and the use of soil for agriculture had a dramatic rise following the population increase and the diffusion of settlements. Later, the industrial and urban development (XIX and XX century) made the original widespread oak forest covering the plains which were progressively reduced to small-sized, isolated remnants.

The forest of Capriano del Colle is small but relatively large as compared to the forest cover in this area of the Po valley. It has quite a favorable genetic potential useful to restore a dynamic population, now subjected to a high risk of genetic erosion, because of its state of maturity and for the absence of a natural regeneration already established.

Its genetic structure is articulated and clearly identifiable on the ground by clusters (Fig. 4) and characterized by a variation core surrounded by peripheral areas of lower variability. The low mobility of seeds, the ground morphology and the microenvironmental conditions drive the distribution of genetic clusters, following the shape of a small river valley. The site is also characterized by a relatively dry climate as compared to other populations and this may have influenced the genetic parameters, favoring, for instance, certain genotypes rather than others, and negatively affecting the viability of seed and the regeneration establishment in some years.

This attribute should be taken into account in planning any collection activities of reproductive materials.

Seed collections should be performed from trees located in different patches of the population, together with the silvicultural management needed to start population dynamics again and to provide, by targeted practices, sufficient brightness for the development and success of seedlings cohorts under the mature canopy trees. That should be done mainly within the variation core, in order to collect seedlings to be planted in clear areas and in the peripheral population, with lower genetic richness.

Seedlings originated from different areas of the forest, will improve the within population gene flows in the future generations and increase levels of diversity in the most peripheral and genetically eroded population. That will help to preserve the gene pool dynamism, as well as to reduce the likelihood of random rare alleles loss.

As for the *in situ* conservation of forest tree species aimed at **mitigating the effects of climate change**, there is no reliable scientific information available for the Mediterranean area. Many studies have been carried out on the genetics of forest tree populations and on quantitative productive traits in multi-site comparative trials. Such quantitative traits can be considered as indirect indicators for adapt-

ability, but no specific research has been systematically carried out on the effects of climate change.

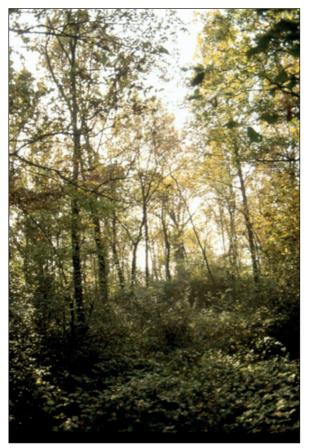
It is time to start studies on the role of the *in situ* conservation, including analyses on silviculture and tending effects on the genetic structure and therefore on management sustainability.

Konnert and Hosious (2010) discussed on these topics showing the importance of genetic aspects for developing a sustainable forest practice both for silviculture targeted to natural regeneration and for implementing artificial regeneration.

Special attention should be given to mixtures of tree species, and to the control of microclimate, through canopy cover and crown density regulation, to reduce susceptibility to drought.



The central area containing the variation core of the forest of Capriano del Colle (BS).



A peripheral strip of the oak population in Capriano del Colle (BS).

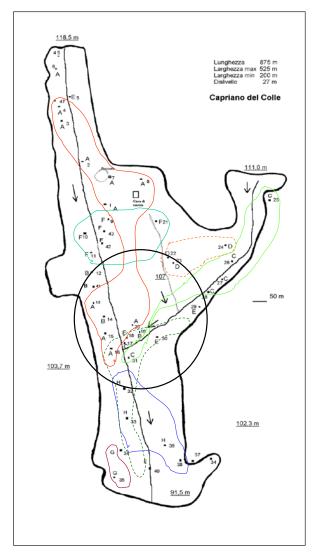


Figure 4 - Distribution of the genetic clusters at the Capriano del Colle (Bs) forest. The central core of genetic variation is circled (Ducci 2007).

Tree spacing and tree density can be managed to control light and then susceptibility to drought. Managers can also change the composition of species diversity to reduce the vulnerability of forests to disturbances as fire, drought, wind, insects or pathogens and find out and choose tree specific compositions better suited to a changed climatic regime.

Changes in tree density will also affect genetic diversity within species (Sagnard et al. 2011). Applying a not extensive silviculture, based on small management areas, will produce patchy alternatives where natural selection occurs. This can also allow managers to maintain the same tree species composition.

## Monitoring *in situ*: genetic diversity and adaptability

Diversity is essential for the adaptation of tree populations and monitoring the genetic dynamics of trees is fundamental for developing long-term strategies. Namkoong et al. (1996, 2002) and Eriksson et al. (1993, 1996, 1998) proposed different methods for monitoring the impact of forest management on genetic diversity by using genetic and demographic indicators to evaluate the efficiency of management actions which drive genetic processes, such as genetic drift, migration and selection, useful to maintain the existing levels of genetic diversity.

The concept of monitoring can be applied at several stages and in different fields of forestry, from the management of forest reproductive materials to plantations and natural populations.

### *B* - The case of Abies alba MaP populations in central Italy (after the LIFE project RESILFOR)

In the framework of the Life Nature project entitled "Resilfor - Restoring Silver-fir Forest" (Miozzo et al. 2014) a few permanent plots were established to monitor changes in stand structures and also adaptive and genetic characteristics. These plots were established in northern Apennines, forest of La Verna (Arezzo) and in the forests of Mount Amiata, an ancient volcano (Southwestern Tuscany).

1) **Forest of La Verna** (community of Chiusi della Verna, Arezzo, Italy)

- Plot A: Lat. 43°42'30,22N, Long. 11°55'54.34"E, 1 ha, aspect NO, alt. 1166 m;

- Plot B: Lat. 43°42'31.80"N, Long. 11°56'01.42"E , 1 ha, aspect NE, alt. 1188 m.

2) **Natural reserve of Pigelleto** (community of Piancastagnaio, Siena, Italy):

- Plot A: Lat. 42°48'17.30"N, Long. 11°38'46.69"E, 1 ha, aspect NE , alt. 780 m [very low elevation as for the Mediterranean area];

### Forest structure

The silver fir in the Apennines is undergoing a slow and steady contraction of its range, in favor of broadleaf trees (mainly beech).

Going into detail about each area, the forest of La Verna is substantially made up of fir - beech even-aged forest, with the sporadic presence of other species.

At Pigelleto, vice versa, chestnut first, along with elm, ash and hornbeam are commonly associated in the chestnut phytoclimatic belt.

The structure of fir populations, included in the permanent monitoring plots, revealed a tendency to form an even-aged groups, even if very irregular structure, probably resulting from not well-planned cuts of the past (Pigelleto), or influenced by natural disasters (storms) such as those occurred in La Verna.

### Genetic structure

Genetic data, and especially data of the regeneration, fully confirm these features. The populations still contain relatively high levels of genetic variability useful to support the evolutionary dynamics for future and new challenges. The analysis, both within and among adult/ mature layers and on natural regeneration, showed good levels of panmittic balance in the different demographic classes. A trend to homozygosity is relatively usual within forest species especially those where self-pollination is frequent.

The clustered spatial distribution of diversity on the plot, at the ground level, showed to be lightly structured. This feature confirms what has been observed in small populations of other species such as *Abies nebrodensis* in Sicily and pedunculate oak (*Quercus robur*) in the Po Valley (Ducci et al. 1999, Ducci 2007, see box A): diversity is highest at the center of the population, where genetic clusters tend to overlap. This distribution can be driven by forces such as gravity or direction of local winds, which disperse the seeds in the direction of the slope or in the more sheltered inner valleys.

At La Verna and at Pigelleto, the number of genotypic clusters decreases towards the margins of the populations, while outward the number of individual genotypes increases and clusters are fewer.

In the case of small populations, this phenomenon can be generated by genetic erosion at the edges, as well as determined by local ecological factors.

In the case of larger populations, the same occurrence may depend on trees spreading pollen and seeds in the tested plot area from more distant parts or unknown *demes* of the forest, not covered by the analysis and topographically more distant.

Concerning the natural regeneration pattern, the mixture determined by wind dispersion of seeds and pollen is high. This is confirmed by the spatial overlapping of clusters and by the high variability.

### Adaptation

To better characterize the populations from the adaptive and plasticity potentials viewpoint, the phenological-cambial activity has been monitored. This technology is very precise and data reliability is high.

High intra-population variability were highlighted, with respect to the earliness and tardiness (dates of the beginning and end of the vegetative phase) of firs. Some fir trees were able to grow until December under warmer conditions, while others have growth periods much shorter. There are a few trees which are highly sensitive to the availability of summer rains, constantly producing false rings, while others are very regular.

A high plasticity of the species was also observed. The phenotypic plasticity is defined as the ability of a genotype. In this case, the species (i.e. the relationship between the Tyrrhenian fir populations) change the phenotypic behavior, in relation to different environmental factors. In fact, the firs of La Verna and Pigelleto revealed to be enough "plastic" to cope with many different environmental conditions. The phenological monitoring allowed to observe both the plasticity at the spatial scale (the two sites), and at the time scale (the years of monitoring) and to observe the behavior of silver in two environments as diverse as La Verna (cold, relatively dry and internal) and Pigelleto (much more temperate and with a longer growing season).

At Pigelleto, the warmer weather until late December in 2011, resulted in phenological cambial stages, typical of spring and summer time.

The results clearly showed how fir copes with different environmental conditions. Its variation in adaptation and plasticity at an individual and population level will be a source of diversity, useful to preserve the species in these areas and to develop adaptive silvicultural strategies.

### Guiding principles for managing these populations

It is fundamental that genetic monitoring has been repeated for several years to pick up different reactions in relation to different climatic and biological seasons and trends.

While plots should be managed with the highest standards of 'close to nature' silviculture, or at least continuing to proceed as made so far [given the function of monitoring areas], on the other hand, it is basic to manage in reducing the influence of beech where this species tends to be invasive. Anyway, this has to be carried out in a controlled way, by maintaining edge micro climate 'ocean like' conditions, because this marginal/cline conditions provide protection to fir offspring in the early stages of development.

Silviculturists have to manage the overripe layers of tree population, isolating adult trees vigorous enough to produce and bear fruiting.

The quantitative assessments of molecular genetic variation at either the neutral or the adaptive level can be therefore used.

Advances are being made in genomics and with bio-statistical tools to improve the efficiency and cost-effectiveness of genetic monitoring and the inference on demographic relationships within populations obtained good results (Schwartz et al. 2006, Chybicki and Burczyk 2010).

Recent advances in forest trees were made about association studies between single nucleotide polymorphism (SNP) and adaptive traits (Holliday et al. 2010, Eckert et al. 2009).

### The importance of preserving marginal and/or peripheral populations (MaP)

As above introduced, Marginal Populations

(MaP) and the genetic information they contain are especially vulnerable.

With this definition, the populations growing at the edges of the natural range of forest species are determined. Margins can be identified at both lower and higher latitudes and similar situations can be generated by other factors, in relation to the elevation effects on temperature and water availability.

Even those populations having a margin context determined by an environmental factor may be marginal, regardless of location, it includes those produced by human influences at the local level.

It is meaningful that in southern areas of many tree species range, most of the present genetic variation is concentrated at the rear edge populations, concentrated in three main hot spots, respectively located in the Iberian peninsula, in Italy and in the Balkans. That is a clear heritage of the Pleistocene glacial refuges.

Marginal populations, which generally survive in less favorable environmental contexts, are typically isolated (geographically separated) from the central continuous range and can be small and at risk of extinction.

Among them, we can identify:

1) populations of the geographic edge (*leading* edge and rear edge) that may also coincide with the marginal ecological requirements of the species,

2) marginal populations, from an ecological viewpoint, living in the central range of species but in ecologically marginal conditions as compared with the species requirements,

3) populations growing at the altitudinal margins.

The pioneer populations in the *leading edge*,

migrating along the shifting of climate zones, leave behind the central population (*core population*) in its optimum. They are followed, in turn, by progressively more and more rarefied populations which remain isolated from the central core (*rear edge*, or marginal populations).

The MaP populations, growing at the geographical edges, may be divergent from the genetic viewpoint due to the joint effect of genetic drift and natural selection, but they can also fill the role of containers of genetic diversity among species due to the outcomes of the spatial-temporal environmental variation at the edges and to the consequent dynamics being induced (Hampe and Petit 2005).

The *leading edge* populations can be characterized by dispersion over long distances and probably the founder effect occurrence is frequent. The *rear edge* populations can be subject to extinction whilst the natural range can undergo latitudinal displacement (*trailing edge*) and sometimes leave small populations surviving in relatively suitable environmental conditions, but with reduced extension (*stable edge*).

The same occurrence takes place when the upland forest populations are being accounted. Here, leading or rear fronts can be detected within the limits of high and lower altitude (Ettinger et al. 2011, Batllori et al. 2012).

According to climatic oscillations, the fronts were reversed several times in past ages, and probably, at each time, populations have shuffled or swapped their genetic information.

The importance of these marginal populations resides in the fact that they may contain adaptive traits of special interest for the species because of

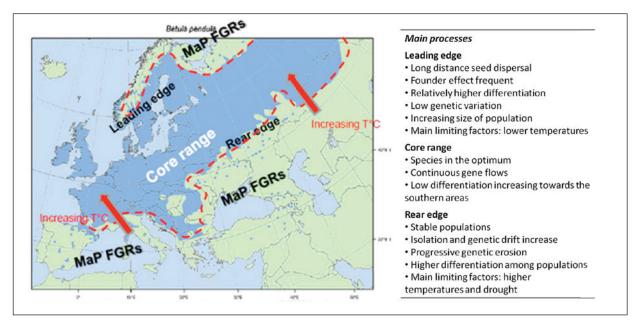


Figure 5 - Variation of different genetic parameters of the species in relation to the stucture of their range and according to the type of edge in advancement or withdrawal (Ducci 2015, after Hampe and Petit 2005).

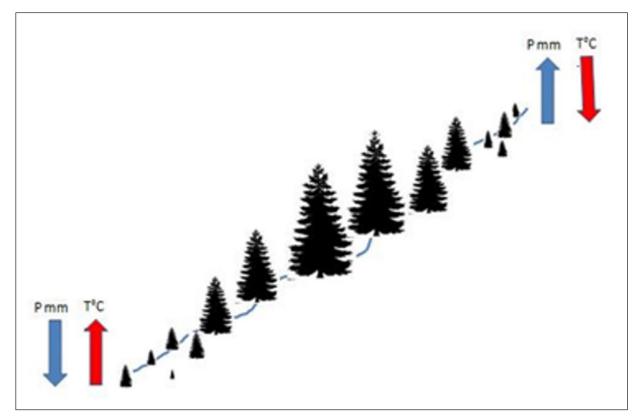


Figure 6 - Representation of the optimum and of upper and lower margins in the sense of the altitude (Ducci 2015, elaborated after Ettinger et al. 2011).

more marked and variable evolutionary factors at margin areas.

Limiting factors or otherwise factors able of exerting selective pressures, typical of southern marginal areas or low altitude, are related to the effects of high temperatures and aridity.

In northern and higher-altitude areas, the main driving factors are connected to lower temperatures.

While, for boreal species, especially in the northern districts of their distribution areas, the information on the genetic structure of populations is wide, for those having southern and Mediterranean range it is still inadequate.

### Ex situ conservation

Without scientific experimental information on adaptive indicators, it is difficult to manage genetic resources and strategies for their conservation. For this purpose, it is extremely important to profit from the existence of several *ex situ* experimental networks and collections established in the Mediterranean area.

IUFRO, FAO *Silva Mediterranea* and other important international networks established, in the past, wide international multi-site tests; these concerned mainly conifers (4 genera and some 20 species) and *Quercus suber*. This genetic stock, just recently inventoried in the framework of *Silva*  *Mediterranea* (Pichot 2011, in Besacier et al. 2011) and European projects as TreeBreedex and Trees-4Future, will be a sound base on which future actions may be developed.

Traits to be accounted for the future activities will reflect the present trends in forest and tree breeding research, as such research has proved to be efficient (formerly *Silva Mediterranea* itself, IU-FRO, EUFORGEN networks, EU TREEBREEDEX, Tree4Future etc.).

Adaptive characters and *phenotypic plasticity* have to be initially investigated as simply phenotypic quantitative traits; in the following steps, the genetic aspects of these traits should also be investigated as a basis for breeding, improvement and conservation, and for genetic mapping (i.e. QTLs, QTNs, SNPs association techniques etc.). The relationship between variation in complex traits and molecular diversity of genes can be studied following a genomic approach, but the identification of genes responsible for variation remains a slow and time-consuming process, especially in long-lived organisms such as forest trees (Vendramin and Morgante 2005).

New strategies have to be identified, tested and adopted according to a common agreement, to reduce the negative impacts of climate change on tree species and populations and the loss of the genetic information they represent. Moreover, the basic concept to be taken into consideration is that *ex situ* conservation should target, where and when possible, dynamic traits.

### **Reproductive materials and legislation**

*Ex situ* conservation can be carried out all levels: regional, national and international. The possibility for entering international agreements for safeguarding national genetic resources in another country should be reviewed and better developed. This concept is not new. International field trials and common field experiments can be, partially, considered as forms of conservation abroad of genetic resources.

In the case of the Mediterranean region, seriously endangered and valuable forest tree populations or species should be identified and conserved with the support of the international community.

Efforts should be shared among countries and framed within the networks created to host genetic materials in suitable environments and managing them according to common plans. This point is very important and it should stimulate cooperation.

Conceivably, laws and regulations on management *in situ*, nursery systems and trade in forest reproductive materials, should be modified and integrated into the overall forest management, thus contributing to the mitigation of negative climate change effects.

Furthermore, in relation to the above statements, the Revision of *seed zones* and *provenance regions delineation* should be considered, and the present concepts about relatively static Provenance regions systems should be modified following a more dynamic vision, while also the rapid modifications of climate and phytoecological parameters should be taken into account (Ying and Yanchuk 2006).

Almost universally, forest reproductive materials (FRMs) are used in forest plantation projects according to guidelines written under the assumption that local soil and climatic conditions will remain stable. The organization for Economic Co-operation and Development (OECD) is the main reference for FRM certification and standardization in the Mediterranean region, but the European Directive 1999/105/CE also provides general criteria and guidelines for FRM trade within the EU. According to most climate models, climatic conditions in the region will not remain stable in the next decades and there is need to revise the rules on the delineation of the provenance of the species and the transfer of seeds and other reproductive materials (Konnert et al. 2015).

Under this framework, the recent Survey of World Forest Genetic Resources (2014) with the contribution of FAO *Silva Mediterranea* for Forest Genetic Resources in the Mediterranean region should be accounted. Already in the 90's, Topak (1997) inventoried the FRMs used for reforestation in 17 FAO *Silva Mediterranea* countries adopting the OECD standards.

Moreover, the FAO *Silva Mediterranea* database lists national and international forest tree common gardens<sup>1</sup> in the Mediterranean region. Such basic information is essential for rethinking seed zone delineation and provenance selection in the face of climate change.

The recent establishment of the Nagoya Protocol (2014), focused on the Access to Genetic Resources and Equitable Sharing of Benefits arising from their use. This international instrument adopted by the Conference of Parties to the CBD (Convention on Biological Biodiversity) at its X Meeting held in October 2010 in Nagoya, Japan, was opened for signature on Feb. 2, 2011.

The aim of the Protocol is the fair and equitable sharing of benefits arising from the utilization of genetic resources, including the appropriate access to genetic resources and appropriate transfer of relevant technologies, taking into account all rights to those resources and those technologies and appropriate funding thereby contributing to the conservation of biological diversity and to the sustainable use of its components.

The Protocol contains provisions ruling both the access to genetic resources and the equitable sharing of benefits arising from the use of them. A user who wants to access a genetic resource of another country (e.g. a medicinal plant for studying the active ingredient, or to produce a medication) must follow the provided procedure in the country access, providing that resource.

In addition, a contract must be drawn up providing for the equitable sharing of benefits, between user and provider, arising from the use of the resource in question (e.g. profits, technology, knowledge, and so on). Genetic resources are often associated with the traditional knowledge of indigenous and local communities. Therefore some provisions of the Protocol rule the access to such knowledge and the equitable sharing of benefits procured by their application.

<sup>&</sup>lt;sup>1</sup> 'Common garden': field test in which many individuals (clones, families, populations) of a given plant species sampled from an identified geographic area are grown in a common environment, making it possible to infer genetic information from the observation of phenotypic differences. These networks were early known as "multisite comparative trials".

### Assisted migration

The above mentioned legislative tools can help to develop actions aimed at preserving really endangered genetic resources, when no other possibilities can be considered for their rescue. Assisted Migration is an option still to be studied more in depth.

Examples of artificial migrations or translocation in forest trees are spread in Europe since long time. We can mention agriculture crop species as well as fruit crop trees following mankind in his migrations.

Among forest trees, several historical case studies can be found in areas where ancient civilizations passed and left signs of successful translocations. Some of them are millennial. Genetic evidences of the translocation of walnut along the Silk Road were found by Pollegioni et al. (2014), but we can also record widespread examples as chestnut in western Europe mountain ranges, cypress introduced in Tuscany by Etruscans and Greeks, *Pinus pinea* spread by Phoenician and Greeks in all the Mediterranean, and more recently Greek fir, Black pine and finally Douglas fir as one of the most important and successful recent intercontinental translocations.

Despite trees are used to migrate in response to changing climate eras, the present climate projections show the real impossibility of forest tree species to migrate so rapidly. In most cases, probably species will be able to find out adaptation within their gene pools.

Aitken et al. (2011) discussed this topic concerning boreal populations trees. These populations are characterized by adaptive variation patterns and - as above said - they show moderate to heavy clines in phenology and growth along temperature gradients. These adaptive traits appear to be the result of small effects of many genes, and may facilitate rapid local adaptation despite the high gene flow in the northwestern American area. On this way, the gene flow with alleles linked to adaptive traits to warmer climate conditions may promote adaptation and migration at the leading edge, while populations at the rear will likely face extirpation.

In the opinion of the Authors 'widespread species with large populations and high fecundity are likely to persist and adapt, but will likely suffer adaptation lag for a few generations. As all tree species will be suffering lags, interspecific competition may weaken, facilitating persistence under suboptimal conditions'.

Species with small populations, fragmented ranges, low fecundity, or suffering declines due to introduced insects or diseases should be candidates for facilitated migration.

So, the idea that most of the widespread species own resources and variation are sufficient conditions for adaptation to climate changes is widely diffused. But, in some cases, the natural tools will not be enough to preserve species and populations and human actions would be probably oriented towards the adoption of Assisted migration methods. In this case, transfer guidelines have to be developed rapidly, possibly based on experimental results or already existing cases.

Assisted migration, implies some human interventions to help a species to migrate. This basic concept can be also extended to single endangered populations. Several variants/synonyms can be found in literature of this definition as assisted population migration, translocation, reinforcement, assisted population/range expansion, assisted long-distance migration (Ste-Marie et al. 2011). As a very complex development of these variants, Seddon (2010) introduced the concept of migration of groups of species defined as community construction.

In the present experiences on assisted migration, a prudential "mimic" approach is generally used. Indeed, Authors highlight the increasing risks of unforeseen influences and consequences when very wide migration distances are being considered (Vitt et al. 20110). For this reason, the within range relocation is mainly diffused.

Anyway, in some extreme cases, a more determined approach should be considered. In Europe and especially in southern areas, an early human activity has left examples of forest tree species relocated and, in a few cases, this presence is reported since several hundred years (i.e. Cypress, Stone pine, Aleppo pine, Chestnut, Firs, Cedars). This artificial old population could be taken into account to test the possible consequences of the long geographic and ecological distances of the past translocations, even from the genetic structure and adaptive viewpoint.

Another basic topic concerns the trade of forest reproductive materials. The present rules concerning forest reproductive materials do not take care of the procedures related to future climate conditions and the associated risks (Williams and Dumrose 2013).

Several techniques, as Assisted gene flow (AGF) between populations and Assisted migration, can help to mitigate any maladaptation due to the climate change effects (Aitken and Whitlock 2013). Several initiatives were undertaken in Canada, where species have access to wide free lands where they can carry out migration strategies.

On the contrary, in Europe situations related to a fragmented landscape are more frequently diffused. In this landscape also the range of many species is fragmented and a huge number of species and

populations may be unable to migrate to suitable habitats and get locally extinct.

McLachlan et al. (2007) proposed several examples, comments and considerations about this topic, on how relevant policy analysis and decisions about the opportunity to adopt this approach and at what intensity have to be undertaken.

The authors posed some important and basic questions about the developing of informed policies on Assisted Migration strategies. The first is aimed to identify the demographic trait that should trigger the implementation of assisted migration; a second one is aimed to know how many or which species or other taxa should be prioritized as candidates for translocation, and eventually to know how to manage populations in order to minimize adverse ecological effects.

Human land use may also create an impediment to gene flow among populations. In situations where trees are prevented from migration, human intervention may be necessary to prevent extinction. This action, can take the form of "assisted migration" or "managed relocation", a set of frequently considered controversial techniques useful to ensure the maintenance of (forest tree) populations in a changing global environment through the intentional creation of populations beyond the boundaries of their current presence (Ducci 2011).

The tendency to consider the opportunity to give rise to assisted migration in these cases already exists. As we have seen in the context of the risks for the species and forest populations induced by climate change, migration is an important strategy. Species will be successful in their perpetuation purpose only if able to move and adapt in environments and places where historically they did not previously exist.

In addition to the geographical barriers and the limits imposed by the areal distribution of ecological factors, the effect of human activities on the landscape fragmentation has been the crucial additional factor over the last centuries. It took place with the clearing of the large forest cover and of natural areas.

Nowadays, we have to face an almost permanent discontinuation of possible migration routes which could be useful to establish and/or restore the gene flow by tracking the motion of local climate. This interruption is mainly due to human settlements and agriculture.

In these cases, therefore, there is the need to provide forms of assisted migration, which aims to implement the physical transfer of populations, which are especially recognized for their adaptive or biological value, in areas outside of their natural range. The main purpose of assisted migration is to preserve the genetic information contained in the original gene pool and restart the evolutionary dynamics along with these demographic - structural patterns as well as to recreate an *ex situ* secondary ecosystem, where they can start dynamics again.

Several definitions of Assisted migration can be found in the literature regarding this topic. Vitt et al. (2009) and S.te Marie et al. (2011) used different key words and definitions to define the concepts of Assisted migration, Assisted colonization, Managed relocation.

A few examples can be cited about species or population translocations: concerning *Pinus albicaulis*, McLane and Aitken (2012) reported their ongoing experiments in Northwestern America to test a model for establishing translocations. Furthermore, in Canada, Gray et al. (2011), tested assisted migration methods on *Populus tremuloides* populations. According to their experimental findings, model projections for this area seem restricted to a short 20-years planning horizon for prescribing seed movement in reforestation programs.

They also found that a safe and realistic climate change adaptation strategy has to be based on a holistic approach to obtain information. Some uncertainty is associated with recommendations for assisted migration, due to the rapid evolution of scenarios.

About Europe, we can cite the experimental case study of *Abies nebrodensis*, the Sicilian fir.

Various inventories and numerous research trials (Morandini 1969, Morandini 1986, Morandini et al. 1994, Virgilio et al. 2000) have shown that this species, although reduced to a small-sized population almost totally unable of originating a viable and dynamic regeneration in situ, still contains sufficient variability to enable a rescue attempt (Vendramin 1997, Ducci et al. 1999). This fir was chosen as a model to develop and then to implement an experimental program of assisted migration. Within this program, the residual individuals were transferred in the northern Apennines, in the form of grafted trees, at the beginning of the '90s (Ducci 2011). Two seed orchards were created with these grafts and the obtained seeds made possible to produce siblings that, year after year, are being transplanted into two areas away from any source of pollen contamination. Thus, they will form the first generation in the new environment.

Each of the two areas (Ducci 2014), respecting the climatic requirements of the species, has similar physical environmental characteristics. They are differentiated only with regard to the former vegetation cover, it being in the first case an ancient mixed chestnut and sporadic beech forest with hornbeam, yew and holly. In the second area, an abandoned field ground surrounded by the forest, former conditions ranged from semi-forest cover to full light conditions.

The purpose is to trigger more driving forces, give rise to different dynamics and allow more genetic characteristics to be preserved and to become clear.

In Sicily, Raimondo and Schicchi (2005) carried out a similar programme, both at local and regional level, in the framework of a LIFE Natura project, dedicated to *Abies nebrodensis in situ* and *ex situ* conservation, between 2001 and 2005.

Assisted migration has far-reaching consequences, far beyond the technical problems of physical translocation, all the manifold dimensions of community ecology, conservation and socio-economy being concerned (Richardson et al. 2009). These would need to be addressed also by forest managers and policymakers.

Garzon and Fernandez (2015) tested tools and scenarios for evaluating the sensitivity of target sites and identifying potential sources not yet developed. They used the Spanish and French National Forest Inventories to design scenarios for AM on *Pinus halepensis* Miller and *Pinus pinaster* Aiton, following a projection to 2050. Results suggest that volume and mortality trends are not always correlated with seed sources and targets, that projected provenances mortality do not always follow a southern to northern pattern and that refugia may be useful for compensating for the effects of climate change only in a subset of provenances.

### Conclusions

Several indicators confirm that the Mediterranean region is being strongly affected by the climate change.

Over the last decades, temperatures have increased along with the frequency of deep and prolonged drought episodes, while rainfalls reduced by up to 20 % in the Italian peninsula as well as in other regions of the Mediterranean.

By 2100, temperatures are expected to increase from 2°C to 4°C on average, while precipitations could decrease from 4% to 30% and might interact with the already typical vulnerability of Mediterranean countries related to the general environmental degradation which is due to the relatively diffused high human pressure.

These direct and indirect effects of the global change may lead to huge human, social and economic losses.

Concerning forest resources, especially the genetic ones, the observed and predicted effects of

climate change, specifically the increased extreme events, bring new threats forth and risk exacerbating the existing pressures, the environmental degradation and the vulnerability of Mediterranean ecosystems, particularly the valuable genetic resources contained within.

Together with climate change effects, several cohorts of natural hazards such as pests, diseases and also the increased frequency and intensity of forest fires, are endangering our resources.

Major, sometimes irreversible, changes are affecting the most vulnerable forest ecosystems where marginal populations of mesic species are growing and have probably developed some valuable adaptive traits (e.g. the rear edges).

The main challenge is thus connected to the opposition to the climate change acceleration, which makes difficult also any adaptation strategy. Only a scientific approach conveying a more in-depth knowledge would be useful to rethink and prepare any kind of strategy. This means that part of the already existing possibly useful data must be reorganized and, again, inferred under a new vision and updated approaches. This requires time and the tools must be adapted to the new needs, and there must be the production of new climatic and phytoclimatic models. Details in mapping have to be improved using smaller scales as well as new software is required for the inference of models and traits related to genomics in adaptation.

The present concept tools (including, modeling, silviculture, nursery legislation, policies etc.) and research networks are still based on a '*static*' or too long-term vision of the environment, whilst a major and rapid dynamism is nowadays perceived (Kerr and Dobrowski 2013).

The generally diffused current low level of silvicultural management of our forests constitutes a real danger for the maintenance of these specific populations, which need to be carefully managed *in situ* with proper and well-focused adaptive cultivation techniques, aimed at preserving their diversity and demographic structure, as well.

Strategies for the management of Mediterranean mountain forests should carefully take into account the issues to strengthen their natural resilience and to equally distribute resources. In this context, urgent initiatives are strongly required to mitigate the impact of climate change on our Mediterranean forest ecosystems and other woodlands.

### Acknowledgements

The author wishes to acknowledge the valuable inputs received from the National project funded by the Ministry for Agriculture, Food and Forestry Policies (MiPAAF) "RGV FAO - International Treaty", the EU projects TreeBreedex and Trees4Future, the Cost Action FP1202 'Strengthening conservation: a key issue for adaptation of marginal/ peripheral populations of forest tree to climate change in Europe (MaP-FGR)' (http://www.cost. eu/domains\_actions/fps/Actions/FP1202, http:// map-fgr.entecra.it/ ) along with the preparation of the proposal documents and from Colleagues of FAO Silva Mediterranea WG4 on 'Forest Genetic Resources in the Mediterranean Region' (http:// www.fao.org/forestry/24287/en/ ).

The author wants to thank the anonymous reviewers for their helpful comments.

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