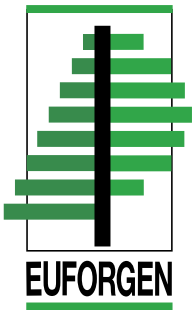


# Genetic conservation and management of *Quercus suber*

**Gösta Eriksson, Maria Carolina  
Varela, Roselyne Lumaret, Luis Gil**



European Forest Genetic Resources Programme (EUFORGEN)



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**Citation:** Eriksson G., Varela M.C., Lumaret R., Gil L. 2017. Genetic conservation and management of *Quercus suber*. Technical Bulletin. European Forest Genetic Resources Programme (EUFORGEN), Bioversity International, Rome, Italy. 43 p.

Cover photo: Fotolia/Springfield Gallery

ISBN 978-92-9255-062-2

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## 1. Summary

This publication presents technical recommendations for the implementation of a long-term conservation and management strategy for cork oak (*Quercus suber* L.) genetic resources within the species' distribution area. It is intended to assist those who cherish the valuable cork oak genepool and its inheritance, either in the conservation of valuable seed sources or through its use in practical forestry and tree improvement. The focus of this Technical Bulletin is on conserving the genetic diversity of the species at the European scale. The recommendations provided should be regarded as a commonly agreed basis for implementation and further development in local, national or regional contexts. The recommended genetic conservation strategy is based on scientific knowledge available in 2015 and on widely accepted methods for the conservation of forest genetic resources.

Cork oak is an ancient species in the Mediterranean basin, with fossil remnants dating back to the Tertiary period. The species is of major ecological importance in its distribution range. Furthermore, cork oak is the only producer of quality cork and, therefore, of utmost importance for the production of cork stoppers and for the cork industry as a whole. The economic use of cork gave rise to the development of a particular type of silvicultural management that maintains its forests, ensuring the regeneration and sustainable use of the species over large areas. However, at the same time, as human activity has transformed a major part of the woodlands into agricultural land and destroyed or dramatically changed the original ecosystem, a large portion of cork oak stands are now park-like landscapes managed through agroforestry systems. Lack of regeneration, largely caused by grazing, is one of the main resulting problems.

In Portugal, cork oak is benefiting from rural abandonment. Due to its resistance to fire and its sprouting ability from stump and epicormic buds, the species may regenerate profusely in untended, burned pine forests.

Existing cork oak genetic knowledge should guide the development of the sound genetic conservation of cork oak. This Bulletin contains a summary of such knowledge. Genetic results available in 2015 are compiled in a separate paper (Eriksson 2017).

It is suggested that the genetic conservation of cork oak be conducted according to the Pan-European strategy for genetic conservation of forest trees, recently developed by the European Forest Genetic Resources Programme (EUFORGEN) (de Vries *et al.* 2015). The strategy is based on the establishment of a core network of dynamic conservation units in all countries within the distribution range of cork oak and with emphasis on natural evolutionary processes. Each of these units should contain at least 50 flowering trees. Details of this strategy are presented in Section 5.2. At least a few of the genetic resource populations should be large enough (200-300 hectares) to also ensure

the conservation of so-called associated species, meaning those dependent on cork oak for their survival and well-being. It is also recommended that some marginal and isolated populations be included in the conservation programme.

In case of unsatisfactory regeneration, sowing or planting is necessary. In such instances, reforestation material should be selected from localities with similar abiotic site conditions.

## 2. Introduction

Cork oak thrives in humid and warm climates, with an optimum range of average annual temperature between 13° and 18°C. Low temperature is an important limiting factor in terms of distribution of this species, as it does not survive when the minimum temperature falls below -10 °C. Cork oak resists summer droughts but needs an annual precipitation of 600 to 1,000 mm. It grows around the western Mediterranean, under a range of natural conditions, and is found in both small, fragmented natural populations and relatively large plantations managed for cork production. The species' largest distribution area is on the Iberian Peninsula, followed by Algeria and Morocco.

Cork oak is commonly grown in agroforestry systems, known as *montado* in Portugal and *dehesa* in Spain. These are open woods with low tree density (50–300 trees/ha). In these systems, forage species are commonly grown under the trees and grazed by cattle.

Cork oak is not endangered, and no threats are foreseen at the species level as long as cork has economic value. However, marginal populations, often growing in small and scattered stands and in restricted habitats, are at risk of disappearing. In such populations, the number of reproducing trees may be too small to maintain enough genetic diversity for future generations. This is exacerbated if the trees produce limited amounts of acorns. Swamping of pollen from other flowering oaks overlapping in some years, such as *Quercus ilex*, *Q. faginea*, *Q. pyrenaica*, *Q. coccifera*, may play a major role in the evolution of such populations. Acting together, these factors limit natural regeneration in small populations. The threat becomes critical when such populations are grazed. Even when the population size is large enough to maintain viable genetic progenies, regeneration may be insufficient to keep the population size. Acorns are extremely attractive to many animals, resulting in a large portion being eaten, especially if the population is located far from stands of other species bearing attractive forage seeds.

The primary use of cork oak is as a source of cork. Cork is obtained by peeling the bark away from the trunk, leaving a thin layer of new cork still covering the functional secondary phloem on the trunk. Cork oak was a neglected species before manufacturers began to systematically use cork as raw material for bottle stoppers. For more than three centuries, production

of stoppers has been the driving force for sustainable management of cork oak forests. Stopper production uses about 20% of the cork, while generating more than 80% added value. The industrial use of cork has been a positive development in mitigating the decline of cork oak populations observed during the past century. The economic use of cork gave rise to the development of a particular type of silvicultural management that maintains forests, ensuring the regeneration and sustainable use of the species over large areas. As in any forest management situation, it is critical to ensure the conditions necessary to maintain genetic variation of populations are compatible with the long-term survival and economic use of the species. The key aspect to achieve this is the management of the reproductive process.

In the face of potential environmental changes in the Mediterranean, the role of cork oak as a key species in these ecosystems has brought the conservation of the species' genetic resources to the forefront.

The essence of forest tree gene conservation is to expose genetic resource populations to existing environmental conditions and to allow the resource populations to adapt to such conditions. Genetic conservation is thus dynamic and allows the genetic resource populations to cope with any changes in the ambient conditions. Dynamic genetic conservation means active population management to promote natural selection is an integrated part of the genetic conservation in order to promote natural selection. The requirement for a dynamic genetic conservation approach has been discussed in many recent papers related to EUFORGEN (Collin *et al.* 2004; Koskela *et al.* 2013; Collin and Bozzano 2015; de Vries *et al.* 2015).

Genetic variation within any species is a basic requirement for natural evolution that has a remarkable set of mechanisms for maintaining diversity. Thus, processes to avoid inbreeding or to increase seed dispersal are examples of natural evolutionary processes promoting genetic variation.

Besides natural evolutionary processes, conservation of genetic resources must take into account human interference contributing to the maintenance of genetic variation. Conservation of genetic resources of forest trees is compatible with human management of forest stands, and this type of management is desirable in many cases to ensure adaptation of the species.

Section 3 on *Distribution and ecology* provides the reader with background information about cork oak, based on current knowledge. Every genetic conservation programme should be based on the genetic knowledge available for the species under consideration. Annex 1 explains basic principles of evolutionary population genetics, and thus provides complementary reading to the section on long-term genetic conservation. A selected bibliography on cork oak genetic resources and a glossary of terms (Annex 2) are included.

Section 5 on *Long-term genetic conservation and management strategy* provides recommendations considered to be the minimum requirements for the maintenance of cork oak genetic diversity. The adoption and implementation of

these recommendations is strongly encouraged in each country within the cork oak distribution area. In parallel to the *in situ* and *ex situ* conservation measures in designated genetic conservation populations or units, as described in this Bulletin, it is necessary to conduct more research to better understand cork oak genetic processes, especially those associated with drought and cold tolerance. This publication is the result of close cooperation among the members and collaborators of the EUFORGEN *Quercus suber* and Mediterranean Oaks Networks. The *Quercus suber* Network was established in 1995, and its scope was later expanded to include promotion of the minimum requirements for genetic conservation for all Mediterranean oaks. From 1996–2000, a European Union project involving six European and three North African countries was implemented within the EC FAIR Programme (FAIR 1 CT95-0202). The objective was to promote the establishment of simultaneous provenance and progeny trials using a standard methodology. Cork oak material representative of the entire geographic distribution area of the species was included in six trials across six cork oak countries. A handbook was published (Varela 2003) summarizing the main objectives of and methodologies used throughout the phases of the project. Recently, results from the trials were published by Varela *et al.* (2015).

The aim of this Bulletin is to contribute to the improved conservation and sustainable use of cork oak, a species of extreme socio-economic and cultural importance for the Mediterranean region and beyond.

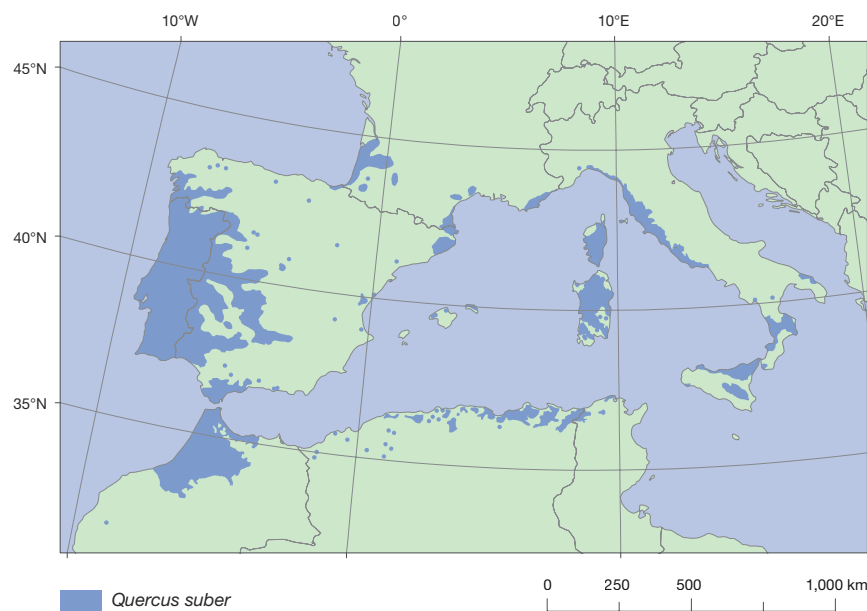
### 3. Distribution and ecology

#### 3.1 Distribution

Cork oak is one of the most ancient species in the Mediterranean basin, with fossil remnants dating back to the Tertiary period. Glacial and interglacial oscillations during the Quaternary period fragmented the species' original area and shaped its present distribution. Cork oak forests are currently located in the western part of the Mediterranean; however, the species' restriction to the western Mediterranean was recently challenged by Schirone *et al.* (2015). The species thrives in humid and warm climates, with an optimum range of average annual temperature of between 13° and 18°C.

Low temperature is an important limiting factor in terms of distribution of this species, since it does not survive when the minimum temperature falls below -10 °C. Cork oak resists summer droughts, but needs abundant rainfall throughout the rest of the year. It is found under a wide range of annual precipitation, ranging from 479 mm in Mamora (Morocco) to 2,400 mm in some north-western areas of Portugal and southern Spain. The optimum annual precipitation is 600-1,000 mm. Cork oak grows in acid or non-calcareous soils.





**Figure 3.0.** Distribution range of cork oak.

Cork oak mainly occurs in sub-humid, thermo-Mediterranean sclerophyllous forests, where it competes with other sclerophyllous species, such as holm oak (*Q. ilex L.*) and, especially in more arid sites, with wild olive. If soils are undeveloped and nutrient-poor, it mixes with Mediterranean pines, predominantly *Pinus pinea* and *P. pinaster*. In more humid environments, cork oak appears with deciduous and semi-deciduous oaks, such as *Q. canariensis*, *Q. cerris*, *Q. faginea*, *Q. pyrenaica*, *Q. humilis* and *Q. robur*. Human activity has transformed a major part of the woodlands into agricultural land, destroying or dramatically changing the original ecosystem, resulting in a large portion of cork oak stands now resembling park-like landscapes managed through agroforestry systems. Lack of regeneration, largely due to grazing, is one of the main resulting problems.

Two core geographic areas can be distinguished in its distribution range. The main area is located in the Atlantic region of Morocco and the Iberian Peninsula and extends north to Landes, France. The second distribution area includes stands around the Tyrrhenian Sea, in Italy, France, Spain, Algeria and Tunisia (see Fig. 3.0). Discontinuous and isolated populations are found throughout the second core area, with stands on the Italian Adriatic coast being the easternmost populations. The natural occurrence of the species in the eastern Mediterranean is doubtful. Former reports of the species in the Balkans seem to correspond to *Q. crenata* Lam. (= *Q. pseudo-suber* Santi), which has been identified as a hybrid between *Q. suber* and *Q. cerris* (Schwarz 1964, Pignatti 1982).

Cork oak woodlands occupy 2,195,000 ha. Fig 3.1 reveals its main distribution area is in Portugal, followed by Spain and Algeria. Portugal is the dominating producer of cork, followed by Spain (Fig. 3.2). The characteristics of cork oak resources are described in more detail below for each country, according to the area of importance and production of cork.

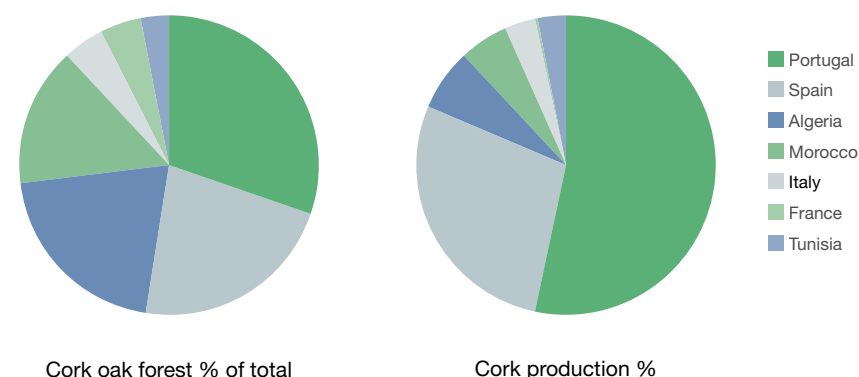
### 3.2 Biology and reproduction

Cork oak (*Quercus suber L.*) is an evergreen broad-leaved tree, which thrives in siliceous soils. The species is long-lived (200–250 years), commonly growing to a height of 15–20 m, but can reach 25 m under ideal conditions.

Stem diameter at breast height can reach more than 300 cm. The tree’s bark is up to 20 cm thick, porous and furrowed, with deep longitudinal fissures. The thick dermal system protects the trees from forest fires.

Leaves are alternate, simple and with the margin entire or with 4–7 pairs of acute teeth. They fall during the second year (13–23 months after leaf flush).

Cork oak is wind pollinated and has predominantly separate male and female flowers on the same tree. Male inflorescences are long, pedunculate and arise from the axillary buds of the previous year’s branches. Female flowers appear on the axils of new leaves. Climate is the principal factor governing the growth period of the tree. Cork oak is a sub-continuous growing species with initiation of the growth cycle in April or May, dependent on weather conditions.



**Figure 3.1.** The cork oak forest area as the percentage of the total area in seven cork oak countries. Total cork oak area = 2,195,000 hectares.

**Figure 3.2.** The percentage of contribution to cork production by country. Total production = 306,000 tons/year.

After spring, flushing may occur at any time, depending on weather conditions, contrary to the seasonality of many temperate forest species. Flowering occurs in the same period and pollinated flowers cease to grow in periods of decreased water availability. Fertilization takes place in late August and acorn development is complete during the autumn. Occasionally, these autumn flushes lead to a secondary flowering event, but not to the production of acorns. In cold climates, leaf burst and flowering may be delayed until June or July. When pollination takes place at that time, fertilization will be delayed until the following spring, and acorns will only develop during the second autumn after female flowers have appeared; the result is a biennial cycle. Some reports (e.g. Elena Rosello *et al.* 1993 and Diaz-Fernandez *et al.* 2004) revealed annual patterns in southern populations and a biennial cycle in northern ones (e.g. Elena Rosello *et al.* 1993 and Diaz-Fernandez *et al.* 2004). In intermediate areas, both patterns are present, even within the same tree, depending on the individual and the weather conditions of the particular year. The difference between southern and northern populations was not observed in Portugal (Varela pers. comm.).

### 3.3 Country characteristics

#### 3.3.1 Portugal

Portugal is the main cork producing country in the world and has the largest area of cork oak. According to the 6th forestry inventory, the area covered by cork oak is estimated at 737,000 ha, with a production of 162,000 tons/year (<http://www.icnf.pt/portal/florestas/ifn/resource/ficheiros/ifn/ifn6-res-prelimv1-1>). Cork oak grows throughout country, except for in mountainous areas or locations with inadequate, poorly drained and calcareous soils. Today, the most important forests are in the southern part of the country. Previously, cork oak was more important in the north; however, an extensive expansion of maritime pine (*Pinus pinaster*) until 1960, followed by afforestation with *Eucalyptus*, resulted in the present-day fragmentation of cork oak stands in northern and central Portugal. Cork revalorization and effective protection of the species have made it possible to recover outstanding areas in the north of the country, such as Mirandela or Tua, among others (Vieira Natividade 1950). Cork oak is also benefiting from rural abandonment. Due to its resistance to fire and its sprouting ability from stump and epicormic buds, the species regenerates profusely in untended, burned pine forests.

Cork oak shares its area with most other Portuguese forest species. In the north, it is often a secondary species forming small stands surrounded by deciduous oaks and maritime pine. In the south, there are extensive stands where cork oak is normally the dominant species. In the inland *montados* (open forests) of the Alentejo, it occurs together with holm oak (*Q. ilex*) and, in the

Algarve, with carob tree (*Ceratonia siliqua*) and stone pine (*Pinus pinea*).

Cork oak forests face challenges mainly related to poor natural regeneration, largely attributed to the destruction and grazing of seedlings by excess cattle, as well as mismanagement. There is a severe shortage of young cork oak trees, with a skewed age distribution in favour of old stands. Cork oak has profuse natural regeneration - up to thousands of seedlings per hectare under adequate management. Thanks to the economic importance of cork extraction, the risk for decline of this species in Portugal is lower than in other countries.

#### 3.3.2 Spain

The area covered by cork oak is estimated to range between 365,000 and 478,000 ha, depending on the classification criteria of the stands, and the cork production at 85,000 tons/year (Montero 1987).

The species is divided into two population groups, the larger being in the southwestern part of the country and the smaller in Catalonia. Interestingly, small populations appear dispersed in other areas of the country under quite different ecological conditions from the core area (Díaz Fernández *et al.* 1996) located in the Balearic Islands (López de Heredia *et al.* 2005), where Minorca Island represents the most relevant population. With only 67 trees located at 12 sites, the Minorca population constitutes a relict zone revealed as the major genetic diversity hotspot for the species (Lorenzo *et al.* 2009). In the southwestern core area, the most important stands are found in western Andalusia, where cork oak is the dominant species. Further to the north, the species appears mainly in Extremadura and along the siliceous mountains near the central plateau. The Catalan core area is found between the coastal mountain range and the Mediterranean and in the eastern foothills of the Pyrenees. The populations growing in the rare siliceous soils from Eastern Spain (a mainly calcareous area), were observed to have a chloroplast characteristic of *Q. ilex* (Jiménez *et al.* 2004).

In Spain, cork oak has suffered a historical reduction due to land cultivation and grazing impeding regeneration. Also, human activity has favoured other species, such as holm oak for its valuable acorns. Nowadays, due to greater public awareness, this trend has stopped and even reversed (González-Doncel and Gil 2013).

#### 3.3.3 Algeria

Cork oak occurs on a coastal strip less than 100 km wide, with a few small, isolated forests found in other parts of the country, indicating a more extensive historical distribution range. The woodlands are fragmented throughout the coastal regions, with three principal core areas, the main one being near Constantina in the east of the country, constituting 80% of the species' distribution area in Algeria. In this region, cork oak grows in mixed stands

with *Q. canariensis* and the endemic North African species, *Quercus afares* (Messaoudène and Merouani 2009). This last species was shown to originate from hybridization between *Q. suber* and *Q. canariensis* (Mir *et al.* 2006).

In the last century, Algeria's cork oak forests suffered considerable reduction due to fire, massive tree felling and overgrazing. In 1950, Vieira Natividade (1950) estimated the area covered by the species in Algeria was only two-fifths of that in the 19th century. Today, the cork oak crisis is characterized by the same causes which led to its historical decline (Doufene 1988; Messaoudene *et al.* 1998). Cork production is low: with an area similar to that of Spain (440,000 ha), Algeria produces only one-fourth as much as in Spain (20,000 tons/year). This is mainly due to the ageing of the stands and difficulties in cultivation (Doufene 1988).

### 3.3.4 Morocco

Stands extend between the Atlas Mountains and the Atlantic, from approximately Casablanca to Tangier. Like in Algeria, the species has suffered an accelerated reduction in the last century in Morocco. Relic forests indicate the species extended all the way to Marrakech in the past (M'hirit *et al.* 1997). The altitude range is extensive, with stands from sea level to 2,000 masl in the Atlas Mountains, and occasionally reaching 2,400 masl (Sauvage 1961), the maximum altitude at which this species is found.

Two large groups of cork oak woodlands are recognized in Morocco: the plain stands that represent half of the total area, and the mountain stands that represent the other half (Benzyane 1998). The main cork oak areas are those of the Atlantic forests (128,000 ha), the Rif (80,000 ha), the central plateau (78,000 ha) and the Middle Atlas (13,000 ha). Together with scattered stands throughout the rest of the country which cover another 20,000 ha, total cork production is estimated to be 16,000 tons/year (M'Hirit *et al.* 1997).

Morocco's forests display a great diversity of types. In the Atlantic plains, pure stands are predominant, while in the mountains they tend to form mixed stands. Most of the populations growing in the Middle Atlas and all those collected in the High Atlas regions were found to possess chloroplast DNA characteristic of *Q. ilex* (Belhabib *et al.* 2001). As in the other Maghreb countries, cork oak forests are declining, mainly due to fires, tree felling and limited natural regeneration (Benzyane 1998). The evolution of the Maamora forest is an example of this. At the beginning of the 1900s, the species covered 130,000 ha; it has been reduced to less than 70,000 ha (Benzyane 1998).

### 3.3.5 Italy

Cork oak occurs widely in Italy, although it does not occupy large areas. It occurs along the entire west coast, but the main forests are found in the

southern part of the country. On the Adriatic coast, the species appears in Apulia, constituting the eastern limit of the species. It is much more abundant on the islands of Sicily and Sardinia. More than 50% of the distribution area in Italy is found in Sardinia (Mercurio and Saba 1998). Pure cork oak stands are rare and are also found mostly in Sardinia. It is more common to find a mixture of cork oak with other evergreen and deciduous oaks, chestnut and pines. The presence of oak species belonging to the same section (subgenus *Cerris*) (Pignatti 1982) explains the existence of numerous hybrid individuals between *Q. suber* L., *Q. cerris* L., *Q. macrolepis* Kotschy and *Q. trojana* Webb. The Italian *Q. suber* populations possess exclusively chloroplast haplotypes shared with the other three oak species (Simeone *et al.* 2013) and not observed in the other parts of the cork oak range. The debated *Q. crenata* Lam. (= *Q. pseudo-suber* Santi), which occurs all along the Italian peninsula and the Balkans, is, for many authors, a taxon originating from *Q. cerris* × *Q. suber* crossing (Schwarz 1964; Pignatti 1982).

With respect to area and production, currently about 100,000 ha produce around 10,000 tons of cork per year. Generally, the sources consulted indicate strong regression during the 20<sup>th</sup> Century. For example, Apulian cork oak forests currently cover 45 ha, which is 230 ha less than in 1958 (Bellarosa and Schirone 1997).

### 3.3.6 Tunisia

Cork oak woodlands occupy a north-facing strip between the coastal mountains and the Mediterranean Sea. The species is found from sea level up to 600–700 m. Its main core area is located in the northeast, between the Algerian border and the city of Sjnane. Cork oak is also found further east, near Bizerte, with minor stands in Zaghouan, approximately 50 km from the capital and next to Cap Bon, the easternmost population in the country. Cork oak shares its territory with maritime pine in drier habitats and with *Q. canariensis* and *Q. afares* in moist sites.

Tunisia's cork oak forest has been greatly reduced over the last century and is currently facing the same problems as those in Algeria: fires, overgrazing, illegal woodcutting, ploughing of forests for cultivation, etc. (Hasnaoui 1998). Data from 1950 reported an area of 127,000 ha (Vieira Natividade 1950), while the last forest inventory (1995) recorded only 63,000 ha producing around 9,000 tons of cork per year (Hasnaoui 1998).

### 3.3.7 France

In France, cork oak reaches the northernmost limit of its distribution area. The decline of the species in France since the 19<sup>th</sup> Century has been considerable, due



to the collapse of the raw cork market in the middle of the 20<sup>th</sup> Century, followed by a global regression of cork oak forests' management. The coverage has dropped from 200,000 ha to the current 56,500 ha (Santelli 1998). The distribution area can be divided into three groups: the Mediterranean coast, the Landes on the mainland, and the island of Corsica. The stands in Corsica occupy 15,000 ha (Vieira Natividade 1950). Scattered trees appear practically all over the island, more so in the south and along the east coast (Porto Vecchio area). On the mainland's Mediterranean coast, the principal core area is in the Department of Var (massif des Maures), with 33,000 ha (Santelli 1998) - a distressing figure considering that Var cork oak forest covered 113,000 ha at the beginning of the 20<sup>th</sup> Century (Vieira Natividade 1950). Less outstanding are the core areas along the coastal Alps and Provence (Esterel), and in the eastern Pyrenees, where the forest of Roussillon (massifs of Aspres and Albera) includes pure stands considered to be the northward continuation of the Catalan occurrences of the species. The most important stands in the Landes are in Soustons (Marensin region). There are also small stands elsewhere in the region (mainly in the Department of Lot-et-Garonne), mixed with maritime pine and deciduous oaks. Heading inland, cork oak trees are found along the Garonne and its tributaries, even past the city of Toulouse. Here, cork oak grows in a humid, Atlantic climate, and low temperatures limit its extension towards the north and the interior. The long rotation for commercial cork extraction - 12 to 15 years - indicates the marginal environment where the species grows in this region (Vieira Natividade 1950).

### 3.3.8 Summary

An analysis of the evolution of the area occupied by cork oak confirms a strong trend towards global regression. Cork oak grows in a narrow range of environmental conditions: siliceous soils in a warm and humid Mediterranean climate. The specific habitat demands make the species very sensitive to changes in the environment.

The same causes reducing the cork oak distribution area in northern Africa in the first half of the 20<sup>th</sup> Century will influence its future as well. In Europe, the reduction of cork oak forests in France and Italy is noticeable, while the future is still unclear in the Iberian Peninsula, despite the stabilization of the area covered by the species. The problems of ageing stands, damage by pests, drought and regeneration limitations have been the subject of numerous meetings and specific congresses. Concerns over threats to cork oak led to the establishment and activities of the EUFORGEN *Quercus suber* Network in 1995.

## 4. Genetic variation and hybridization

This is a brief summary of relevant knowledge for the genetic conservation of cork oak. It is important to note that selection of genetic resource populations must be based on differentiation of adaptive traits such as survival, growth, fecundity and tolerance against pests and diseases. Non-adaptive markers give useful information on pollen flow and, as such, information on security distances for pollen flow.

Before the 1990s, little was known about genetic variation in cork oak. Field trials established after this date revealed substantial population differentiation for several traits, such as growth and mortality. In the most comprehensive study by Varela *et al.* (2015), Moroccan populations showed good growth and survival over a wide range of site conditions. Generally, southern transfers of populations resulted in below average growth and above average mortality.

Other markers (fragments of DNA in cell nucleus or cell organelles) showed variable population differentiation. Studies on isozymes revealed that pollination occurs over wide geographic areas (*e.g.* Toumi and Lumaret 1998).

The hybridization of cork oak with other species occurs spontaneously. A detailed phenological study of flowering in cork oak and holm oak revealed low possibility for hybridization between these two species (Varela *et al.* 2008). Based on nuclear microsatellite polymorphism, hybridization frequency was estimated to be low, even in mixed stands (Burgarella *et al.* 2009; Lumaret and Jabbour-Zahab 2009). In addition, artificial crosses resulted in low percentages of hybrid individuals. The difference in flowering time between cork oak and holm oak, characteristic for certain areas, constitutes a constraint to hybridization between these two species. In addition, post-pollination processes leading to embryo abortion contributes to low hybridization rates (Varela *et al.* 2008). However, under rapidly changing environmental conditions species hybridization might be of significance for future evolution.

Based on cytoplasmic polymorphism, approximately 20% of cork oak populations are derived from multiple, original hybridization events between cork oak (predominantly as paternal parental species) and holm oak. In addition, Magri *et al.* (2007) considered that the Italian cork oak populations (approximately 10% of the entire population) may be derived from hybridization between cork oak and *Q. cerris* or another species of the *Cerris* group (see above). Between cork oak and holm oak, nuclear introgression is low and bidirectional. It occurs in contact areas, specifically in mixed stands, even in the absence of cytoplasmic introgression, and shows a pattern suggestive of gene filtering (Burgarella *et al.* 2009; Lumaret and Jabbour-Zahab 2009). Ramírez-Valiente *et al.* (2014) showed cork oak saplings originating from the whole species range and studied at the common garden trial located in western Spain exhibited differences in plant architecture associated with cpDNA lineage. Particularly, the Italian cork oak populations (considered as derived from cork oak hybridization with species of the *Cerris* group, see above) differed significantly from the other populations by lower apical dominance and higher values for branchiness. Promising results were also obtained from preliminary local analyses comparing cork oak seedlings or adult trees derived or not from hybridization with holm oak (*e.g.* Staudt *et al.* 2004).

## 5. Long-term genetic conservation and management strategy

### 5.1 Objectives of the strategy

Genetic conservation methods should always match specific conservation objectives, taking into consideration the available genetic and other relevant knowledge. Therefore, this section first presents the objectives, followed by the methods and, lastly, recommendations for implementation.

During the last 100,000 years, species have coped with continuously changing climatic conditions. There are three ways in which this has occurred: through migration; through evolution; or through a combination of the two. It is anticipated that, due to global climate change, the changes may be more rapid than ever before. It is therefore widely agreed that continued adaptation of species should be facilitated across genetic conservation measures. Consequently, the **key objective** of genetic conservation is **safeguarding the potential for adaptation**. Other objectives include:

- Breeding to improve the quality and quantity of products for all commercially important tree species. As cork oak is of great social, ecological and economic importance in several countries, a combined breeding and gene conservation objective is fully justified.
- Endangered populations may carry specific genes or genotypes, which calls for a conservation.
- Finally, many so-called associated species are dependent on cork oak for their existence. An additional objective for cork oak gene conservation programmes is therefore to ensure the maintenance of associated species.

Naturally, the first objective has the highest priority, and, given that the species should be regarded as a whole, the overall goal is formulated as follows: *Identify minimum standards for safeguarding the potential for adaptation of cork oak in its distribution area.*

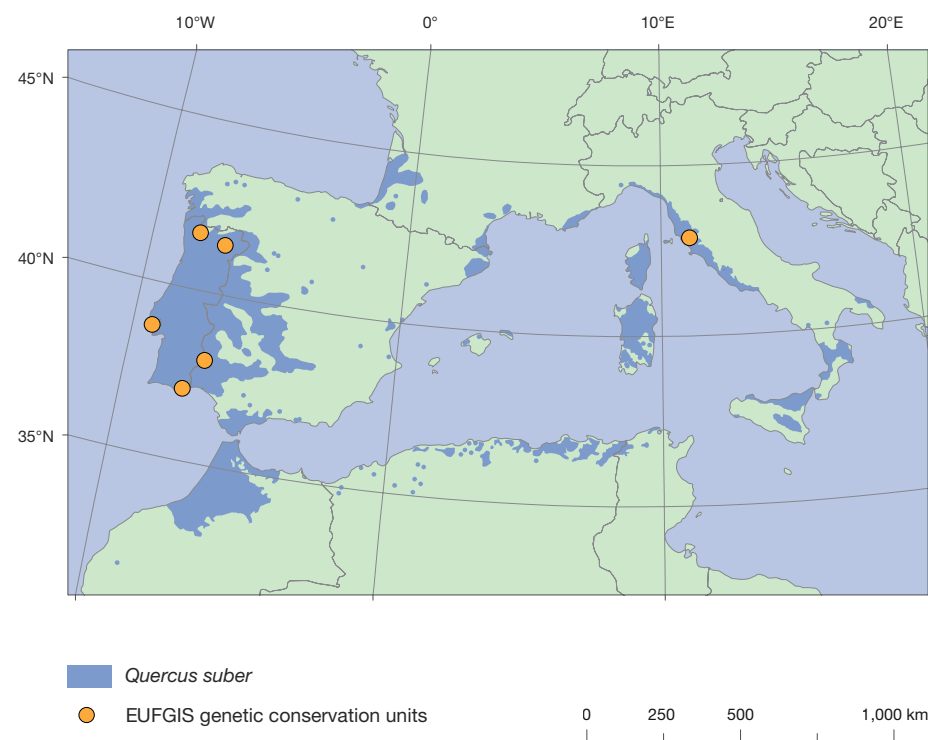
### 5.2 Dynamic conservation

To cope with the changes in the environment, genetic resource populations should be exposed to natural or artificial selection. Thus, genetic conservation should be a dynamic process in which species benefit from natural or artificial selection. (For a detailed discussion on dynamic genetic conservation see Eriksson *et al.* 1993.)

Since the environmental conditions in the present distribution area of cork oak vary considerably, it is expected and observed that adaptation has taken place, probably leading to differentiation in adaptive traits among populations. The total existing variation should be included in genetic conservation efforts. The focus should be on adaptive variation, but neutral variation should also be considered. The large variation observed for growth and survival in the international series of provenance trials (Varela *et al.* 2015) indicates it requires several populations to encompass the adaptive variation in cork oak. Moreover, in these provenance trials, the sources MA27 (from Morocco) and TU33 (from Tunisia) showed surprising and striking ability to survive and maintain good growth under precipitation reduction of 50% and 60%. These results are important as they unveil populations of cork oak with unexpected adaptive flexibility.

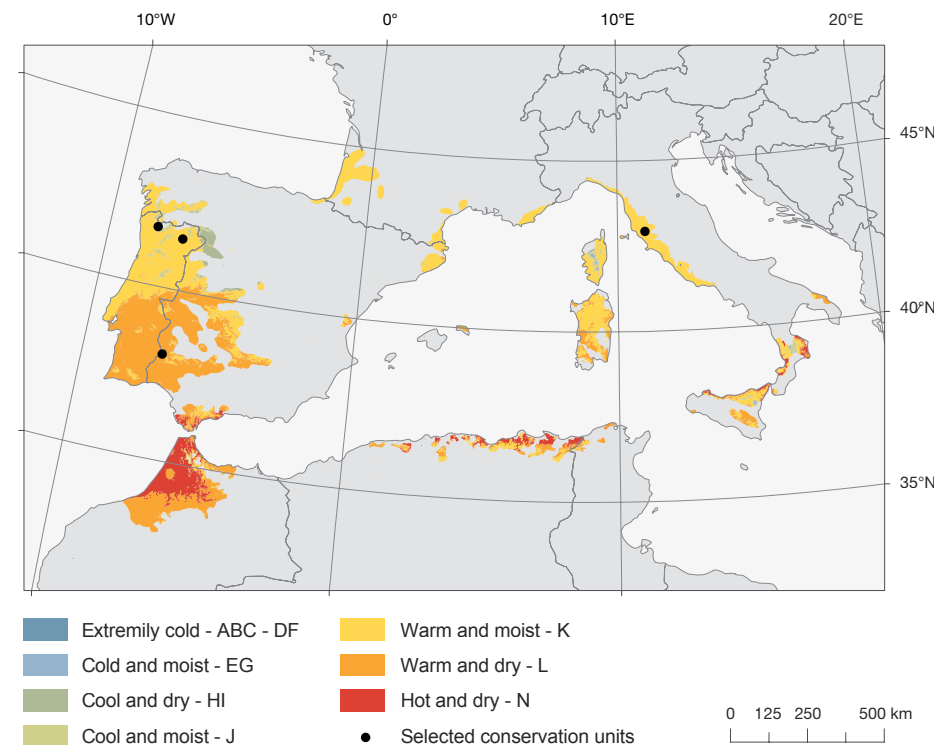
These results are strongly supported by findings in other field trials. The selection of genetic resource populations in the paper by Varela and Eriksson (1995) is still valid. The lower differentiation revealed by some markers does not call for any other strategy. *In conclusion, cork oak conservation units should represent as many as possible of the site conditions found within the entire distribution area of cork oak.* Therefore, it is recommended that the pan-European strategy for genetic conservation of forest trees recently developed by EUFORGEN be applied (de Vries *et al.* 2015), in line with genetic conservation according to the Multiple Population Breeding System (MPBS) (Namkoong 1976, Eriksson *et al.* 1993, Varela and Eriksson 1995). The features of the Pan-European strategy for forest tree genetic conservation are outlined in the next paragraph.

The pan-European strategy for forest tree genetic conservation recommends that a core network of dynamic conservation units be established. These units should be appointed in all countries within the distribution range of cork oak. Within each country, at least one unit per climate zone should be appointed, with climate zoning used as a proxy for characterizing the differences in adaptive genetic variation of the species. This strategy was created under the scope of the EUFORGEN programme to conserve both adaptive and neutral genetic diversity of forest trees, though it was decided to give priority to adaptive diversity. Consequently, the Pan-European conservation strategy outlined in that report aims at conserving the adaptive diversity of forest trees throughout their distribution ranges. De Vries *et al.* (2015) developed a draft report based on the environmental zones of Europe, as identified by Metzger *et al.* (2005) and then prepared the final report based on the new environmental stratification developed by Metzger *et al.* (2013). Subsequently, gaps in conservation efforts have been identified according to country borders and climatic zones within each country. Furthermore, a systematic approach was applied to select the most valuable genetic conservation units at the Pan-European level for the establishment of a core network of dynamic conservation units for several pilot tree species. The selection was done using the EUFGIS



**Figure 5.2.** The six genetic conservation units of cork oak entered in the EUFGIS information system as of December 2016.

database (<http://portal.eufgis.org>) and each species' core network should ultimately cover all countries and climatic zones within the distribution range of the given species. To test this approach, 14 pilot tree species representing stand-forming and scattered species with wide and limited distribution ranges were used (de Vries *et al.* 2015). The Pan-European minimum requirements for the genetic conservation units are presented in a recent paper by Koskela *et al.* (2013). These minimum requirements are based on the dynamic conservation approach, i.e. long-term conservation of evolutionary processes within tree populations to maintain their adaptive potential. The units can be located in natural or man-made tree populations specifically managed for genetic conservation. Each unit should have a designated status and management plan, and contain one or more of the tree species recognized as target species for genetic conservation. The units should contain a minimum of 500, 50 or 15 reproducing individuals depending on tree species and conservation objectives. Furthermore, silvicultural interventions intended to promote adaptation of tree populations should be allowed and, ideally, field inventories should be carried



**Figure 5.3.** The four conservation units selected for the cork oak core network. De Vries *et al.* 2015.

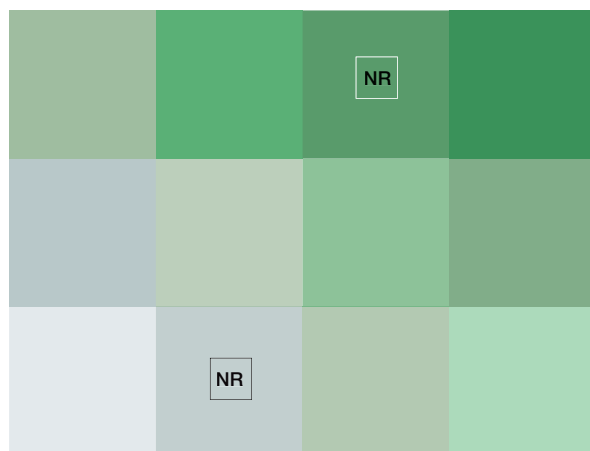
out every five or ten years to monitor regeneration and population size. In line with these minimum requirements, European countries have entered data on more than 3200 genetic conservation units which harvest more than 4000 tree populations of about 100 tree species into the EUFGIS Portal (<http://portal.eufgis.org/> as of August 2016). For cork oak, there are currently six genetic conservation units appointed in the entire distribution range (see Fig. 5.2), of which four are selected in two countries for the core network (3 in Portugal and 1 in Italy, Fig. 5.3). Since the total number of possible combinations of *environmental zone x countries* for cork oak is 41, there are gaps in 37 *environmental zone x countries* possibilities for cork oak in its total range of distribution.

Varela and Eriksson (1995) discussed how to incorporate genetic conservation of cork oak's numerous associated species into the genetic conservation of cork oak. An enlargement of a few of the core units to cover an area of 200–300 ha of cork oak forest was suggested. Within the individual units chosen for combined genetic conservation of cork oak and its associated species, as many site conditions as possible should be targeted, as illustrated

in Fig. 5.4. This will address differences among the localities with respect to flora and fauna. A few areas should be set aside as nature reserves to permit evolution without human impact. Moreover, the associated flora and fauna in nature reserves usually differ from that in managed populations. If an area of 200–300 ha of cork oak forest is impossible to find, then three or four smaller areas within the same climatic region might be sufficient.

### 5.3 Static conservation

Regarding so-called static conservation, whole plants/trees or parts of them, such as seeds, pollen, or cuttings and grafts, can be kept and stored for longer periods without any regeneration and thus without any selection. This means the genetic composition will remain the same over time. Seed banks are frequently used for genetic preservation of agricultural crops, with the main purpose of introducing individual genes from the genetic resource population into a highly bred variety via repeated back crossings. The long generation time of forest tree species renders repeated back crossing unfeasible. Moreover, most adaptive traits in long-lived forest trees are believed to be quantitative, *i.e.* they are regulated by many genes, each with a small effect on the quantitative trait. This means introduction of individual genes is of low importance. Currently, cork oak does not have any highly bred cultivars. Moreover, long-time storage of acorns is not yet possible.



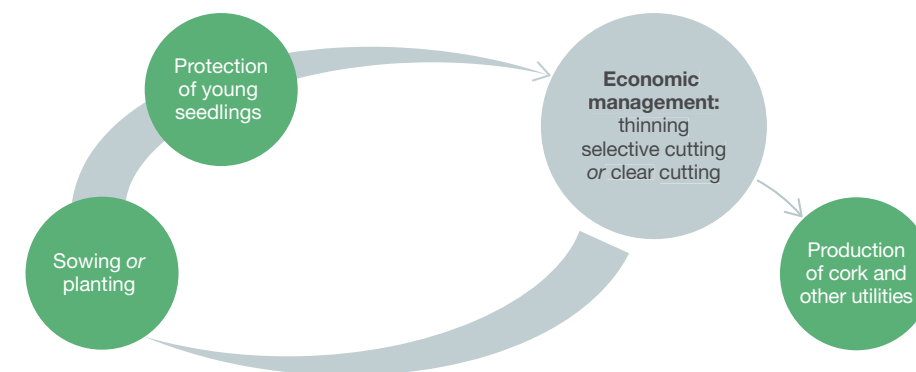
**Figure 5.4.** Model for combining simple breeding and conservation of cork oak genetic resources while maintaining associated species, under natural regeneration and multipurpose use of cork oak forests. The twelve squares with different colors symbolize twelve areas with varying site conditions. When the conservation objective of a target species is extended to include conservation of associated species, it is recommended to include as many site conditions as possible in the conservation effort. It is also recommended to select a few of the areas as nature reserves. Modified from Eriksson *et al.* 2013.

### 5.4 Natural regeneration

Whenever site conditions are favourable, natural regeneration of autochthonous populations is the best way to maintain genetic resources of cork oak as the reproductive material comes from an adapted seed source. However, virgin European cork oak forests are rare. One advantage with natural regeneration is it allows for plants from more than one year to be present in the progeny generation. Data available from reproductive studies show individual trees change their reproductive behaviour and the intensity of such behaviour over time. Thus, regeneration accomplished over a series of years might result in increased genetic diversity of the new stand.

If planted seedlings are exposed to severe drought they will suffer more than naturally regenerated seedlings. The latter ones benefit from an early establishment of a root system.

It is essential to take active measures that facilitate natural regeneration in each genetic conservation population. This does not mean that traditional use of cork oak stands, such as grazing in an agroforestry type of management, is precluded to any great extent. Active measures, such as fencing or protective tubes, or both, to prevent cattle from feeding on acorns and young seedlings, may be necessary with this type of agroforestry management. As outlined in Fig. 5.5, genetic conservation is a cyclic process that does not usually interfere with economic use of the genetic conservation populations.



**Figure 5.5.** This figure illustrates that genetic conservation can be seen as a cyclic process with sowing or planting of seedlings and management. Management aims to guarantee regeneration and improvement of the cork quality and to provide the land owner with revenue from the gene resource population. Such a process will take place in each of the genetic resource subpopulations.

To combine successful regeneration – a prerequisite for the conservation of genetic resources – with grazing and other production activities under cork oak canopy in a given population, the area submitted to natural regeneration must be managed under a long-term plan of subdivision into fenced plots or units.

- **Area.** To maintain genetic variation and avoid inbreeding, the population of the unit to be closed should contain  $\geq 50$  fructifying trees. Studies of reproductive behaviour of cork oak showed that, on an average, only 1/3 of the trees in a stand is fructifying. Therefore, to ensure 50 fructifying trees, it is recommended that the area include 200–250 mature trees. Depending on the conditions of the cork oak stands, this number is usually equal to an area of between 2 and 10 ha. The observation that there is a spatial relationship between close neighbours means the distance between fructifying trees should be larger than 100 meters.
- **Management.** During the regeneration process, management should focus on thinning and pruning measures, together with shrub control to minimize risks for fire. Unhealthy and poorly growing trees should be culled. This is especially important in populations with the dual purpose of breeding and genetic conservation. In these populations, trees with inferior cork quality should be culled to improve cork quality in subsequent generations.
- **Assisted natural regeneration.** If natural regeneration does not occur successfully in some of the units, then supplemental planting with seedlings from appropriate sources might be carried out (see section on artificial regeneration, below).

Beyond the genetic and physiological advantages of natural regeneration, there are also practical consequences related to site preparation. Soil scarification associated with planting (artificial regeneration) should be kept at a minimum as soil disturbances usually have negative consequences in the Mediterranean forest ecosystem. This is of particular importance in the case of poor and shallow soils.

Whenever natural regeneration is unsatisfactory, sowing and/or planting must be carried out. The seed source must be selected in such a way that the objective of maintaining a genetic conservation population is not compromised. Caution must be used when considering commercial seed lots and seed lots from years with poor acorn production as commercial seed lots may be a blend of several provenances, particularly in years with a poor acorn crop.

In conclusion, it is worth reiterating that human intervention is frequently needed for the genetic conservation of cork oak, as well as for its associated species.

## 5.5 Artificial regeneration in large, commercial stands

Owing to potential damage by rodents and other wildlife, planting is a method that attracts many cork oak managers or owners. Special attention must be paid to the selection of reproductive material for either sowing or planting, otherwise serious failures may occur. However, genetic selection of plants is not enough. Plantation technique is essential to ensure the growth of the root system is deep before summer. Although it is more expensive, as it requires a deeper hole, planting technique of “buried collar” provides the highest success rates. (Amandier and Varela pers. comm.). When artificial regeneration is carried out according to the principles of genetic conservation, the following requirements for the use of reproductive material must be observed:

- Preference should always be given to local material, unless results from provenance trials point to inferior quality or growth characteristics in the local population. Local material usually guarantees retention of the evolutionary and adaptive characteristics that have developed at a given site under specific conditions over generations. Lack of adaptedness may lead to serious failures at any stage of the long lifespan of cork oak and other forest tree species.
- If there is no local material available or if there are signs of inbreeding, then restoration may rely on the introduction of material from outside. Material from localities sharing the site conditions with the regeneration site are preferred.
- The number of flowering and seeding trees provides a practical approach to assess the effective size of a given population. As stated above, at least 50 fructifying and unrelated trees are required to keep genetic variation at a satisfactory level within a population. Therefore, information about the number of female flowering trees should always accompany the material description of a given lot.
- The number of trees participating in the reproductive process is highest during mast years. Therefore, it is highly desirable that artificial planting uses the acorns from mast years. Reproductive material collected during very poor seed years should be avoided whenever possible.

## 5.6 Small and marginal populations

Marginal populations growing in extreme environmental conditions may carry particular genetic characteristics making them potentially valuable for the overall species evolution. They deserve to be designated as genetic conservation populations/units.

Small and marginal populations often have poor natural regeneration. In such circumstances, it is recommended that all seedlings in a population be protected.

For areas in which the species is rare, supplementary planting with seedlings from the same region is a useful alternative. If the germination of acorns is poor, it is recommended to try to improve the germination rate before sowing in the nursery. If there is no acorn production at all, acorns from populations growing under similar site conditions should be used. Grafting of trees from the marginal populations for acorn production and future seeding or planting inside the small populations is recommended. This will increase the population size.

Small populations may require different conservation approaches depending on the census number of trees:

- For marginal populations with more than 50 fructifying trees, emphasis should be on conserving and managing the population by means of natural regeneration.
- For populations with less than 50 fructifying trees, the size of the population needs to be increased. All available acorns should be collected to raise seedlings for planting.
- If the population is not fructifying, grafting should be used to ensure future enhancement of the population.
- Use of reproductive material from external sources may possibly be used, but only if the requirement of ecological similarities is fulfilled (as discussed above).
- Fencing is critical if cattle are a threat. Special management must also be provided for populations under threat of exotic invading species.

## 5.7 Nature reserves

Strictly protected nature reserves do not necessarily ensure or support the conservation of genetic resources of cork oak and other forest tree species. If the area suffers from serious disturbance, then the absence of management may mean withdrawing the only positive human interference still at hand. All land is affected by human disturbances, either past or present, with which the ecosystem itself cannot necessarily cope, including the long-term effects of air pollution. After irreversible changes in the ecosystem, human intervention may become critical to preserve the genetic resources of the species.

## 5.8 The role of field trials as genetic conservation populations/units

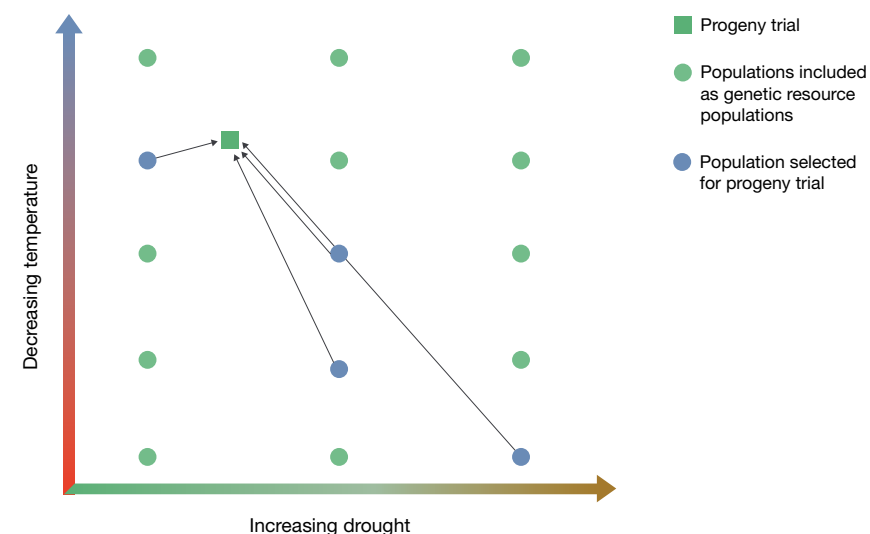
The provenance and progeny trials of cork oak established during 1997–98 (Varela *et al.* 2003), as well as any older, smaller trials established in various countries, constitute an important complement to *in situ* genetic conservation. However, the trials must be regarded as static conservation units that do not

contribute to regeneration owing to provenance hybridization that might occur within these trials.

They fulfil the requirement of preservation of the present genetic constitution (*ex situ* conservation) as long as there is no way to effect long-term storage of cork oak acorns.

Breeding programmes were initiated both in Portugal and Spain (d'Alpuim and Roldao 1993; Garcia-Valdecantos and Catalan 1993). In 2014, the GENOSUBER project was launched for production of the first full-sib population in cork oak (Varela pers. comm.). The breeding populations constitute components in genetic conservation of cork oak. Field trials and clonal archives may be used for crosses to generate material for future genetic resource populations.

The good performance of the Moroccan populations in the Portuguese trials supports the suggestion for combined provenance and progeny trials outlined in Fig. 5.6 (from Eriksson *et al.* 2013). This is also a schematic example of how to mitigate global warming through the establishment of combined provenance and progeny trials. Four of the 12 populations are represented in the combined trial, with around 20 families each, while the other eight constitute a mix of families from each population. The four former populations are selected to represent the local conditions as well as conditions of increasing temperature and drought. Such a design provides information both on population differentiation and on breeding potentials in parts of the populations. This approach would be useful whenever resources are available for establishment of combined provenance and progeny trials.



**Figure 5.6.** The principle for selection of populations for progeny trials in case of an expected increase of temperature and drought. For further information see text. Eriksson *et al.* 2013.



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## Acknowledgement

The preparation of this Technical Bulletin for genetic conservation of cork oak was initiated by Jozef Turok. Pedro M. Diaz-Fernandez contributed to the original manuscript. Sven de Vries scrutinized the draft and contributed the integration of the Pan-European strategy for forest tree genetic conservation into the suggested conservation strategy. In 2015, Michele Bozzano invited the senior author to finalize the Technical Bulletin together with the co-authors and provided support throughout its development. We are most grateful to all persons mentioned. Thanks are due to Nicole Hoagland for revision of the English text.

## Annex 1

### Basic genetics of significance for genetic conservation

Prepared by Gösta Eriksson, Department of Plant Biology, Swedish University of Agricultural Sciences (SLU) BioCenter, Uppsala, Sweden

#### Most traits of evolutionary significance show a continuous variation

When Mendel detected the laws of inheritance, he used distinct traits that could easily be classified, such as the green or yellow colour of peas. Many traits – such as growth and number of flowers – do not show these distinct classes. Mostly, there is a continuous distribution, for tree height, for example. If we turn to man, we find that for many human traits we cannot identify distinctive classes. However, we can see that human children resemble their parents more than any randomly selected population of human beings. Such a resemblance between related trees has also been revealed for a large number of traits in forest trees. Thus, it is evident that heredity plays a role for these types of traits. Since such traits lack classes, they are frequently referred to as quantitative, or sometimes as metric, since variation can only be revealed by measurement. Quantitative traits are characterized by:

- more-or-less continuous variation of the trait;
- influence on the trait by many genes;
- small (infinitesimal) influence by each gene;
- similar influence on the trait by many genes; and
- environmental influence on the trait.

Genes regulating quantitative traits follow the laws of inheritance detected by Mendel; however, the effect of an individual gene can only be revealed with great difficulty since it has a small effect on the trait.

The overwhelming majority of traits of significance for the continued existence of a species are quantitative. The first point above (more-or-less continuous variation of the trait) does not hold true for traits like survival and occurrence of disease infection; although, even such traits are quantitative. We must assume that different genotypes differ in potential for survival or disease resistance. For genetic conservation, point four above (similar influence on the trait by many genes) is reassuring as it implies that a loss of one gene involved in the regulation of a quantitative trait is not catastrophic since its effect on that trait can be substituted by another gene with the same influence on the trait.

### Breeding value

To reveal how strong the genetic regulation of a trait is and the extent of environmental influence on that trait, progeny trials are established. In progeny trials, the genetic value of a tree is estimated following measurements or recordings of the offspring from that tree. Progeny trials contain offspring from all trees that should be tested, and the genetic value of a tree derived in progeny trial(s) is called breeding value.

The breeding value is the double deviation of the mean value of the progeny of a particular tree from the mean value of all progenies.

An example will be taken from Table A1.1 to illustrate how the breeding value is calculated. Let us assume that the 10 values in the first column are mean values of progenies of 10 trees obtained from crosses with other trees. The global mean value is 12, the breeding value of the first tree is  $2 \times (-6) = -12$ , and the breeding value of the last tree is  $2 \times (+6) = +12$ . The breeding values of the other 8 trees vary between -8 and +8. The reason we multiply the deviation by 2 is that only half of the genes in the offspring of a particular tree comes from the tree itself, the other half comes from other trees.

Statisticians use different estimates of variation. One is the variance that is the sum of the squared deviations from the mean divided by the number of observations. The sum of the squared deviations from the mean is = 122 for the values in Table A1.1.

**Table A1.1.** Values, their deviations from the mean value, and their squared deviations.

| Tree No. | value           | $x_i - \bar{x}$                 | $(x_i - \bar{x})^2$ |
|----------|-----------------|---------------------------------|---------------------|
| 1        | 6               | -6                              | 36                  |
| 2        | 8               | -4                              | 16                  |
| 3        | 9               | -3                              | 9                   |
| 4        | 11              | -1                              | 1                   |
| 5        | 12              | 0                               | 0                   |
| 6        | 12              | 0                               | 0                   |
| 7        | 14              | +2                              | 4                   |
| 8        | 14              | +2                              | 4                   |
| 9        | 16              | +4                              | 16                  |
| 10       | 18              | +6                              | 36                  |
|          | <b>x = 12.0</b> | <b><math>\bar{x} = 0</math></b> | <b>122</b>          |

To obtain the variance for the values in the table, the sum (122) should be divided by the number of observations, which is 10. This division leads to an estimated variance of 12.2 for this example.

### Additive variance, the fuel in the evolutionary process

The variance of the breeding values is called the additive variance. The amount of additive variance determines the potential for progress via natural or artificial selection. Without additive variance, no evolution is possible either in nature or breeding. Additive variance is the numerator in the ratio constituting the heritability. The phenotypic variance is the denominator in this ratio. The phenotypic variance is the total variance in a progeny trial, while the additive variance is the genetic aspect natural selection or breeders can exploit. The heritability of a trait is an estimate of the resemblance among related individuals for that trait.

It seems self-evident that the stronger the selection, the larger the progress will be in a selection process. However, if there is no additive variance for the trait under selection, there will be no progress - no matter how strong the selection might be.

### Genetic gain

The progress via selection is sometimes referred to as genetic gain which is = heritability  $\times$  strength of selection. This formula is valid for both natural and artificial selection.

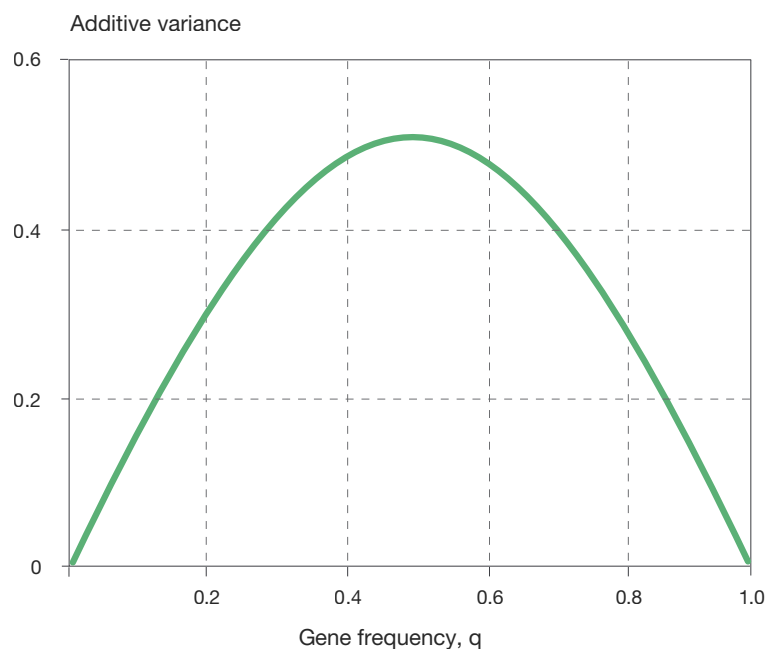
### Gene frequencies

Since the additive variance is dependent on gene frequencies, we have to explain the meaning of the term 'gene frequencies'. Let us assume that we have a population of 50 individuals with the following genotypes in a locus on a chromosome:

- 30 AA;
- 10 Aa;
- 10 aa;

in which **A** and **a** designate two genes at this locus. To find the gene frequencies of genes **A** and **a**, we need to analyse the frequency of gametes formed in this population. We assume that the individuals contribute gametes in relation to their number in the population. If this is the case, the 30 AA trees will contribute  $30 \times 2 = 60$  **A** gametes, the 10 Aa will contribute 10 gametes of each type **A** and **a**, while the 10 aa will contribute  $10 \times 2 = 20$  **a** gametes. To summarize: we will have 70 **A** gametes and 30 **a** gametes. The gene frequencies in this population is thus 70% **A** and 30% **a**.

The relationship between gene frequency and the additive variance in the case of complete additive gene action is illustrated in Fig. A1.1. Complete additive gene action means the value of  $Aa$  is exactly intermediate to the values of  $AA$  and  $aa$ . From Fig. A1.1, it is seen that genes at low frequencies (<5%) and high frequencies (>95%) do not contribute much to the additive variance. This means it is almost impossible to raise the frequencies of genes at percentages <1% by artificial or natural selection. Their existence in a population will solely be dependent on random events. Similarly, random events will be responsible for increment of genes at frequencies >99%. Once a gene that brings an advantage to its carrier has reached selectable frequencies owing to chance, it will slowly increase in frequency during the first generations. The changes will gradually be faster and the maximum speed is reached at a gene frequency of 50%. Then, its increase will gradually become slower and slower, to cease at very high frequencies when random events again will be responsible for any increase or decrease. The genes that occur in intermediate frequencies, 20–80%, are the genes that contribute most to a response to selection, either in nature or in breeding.

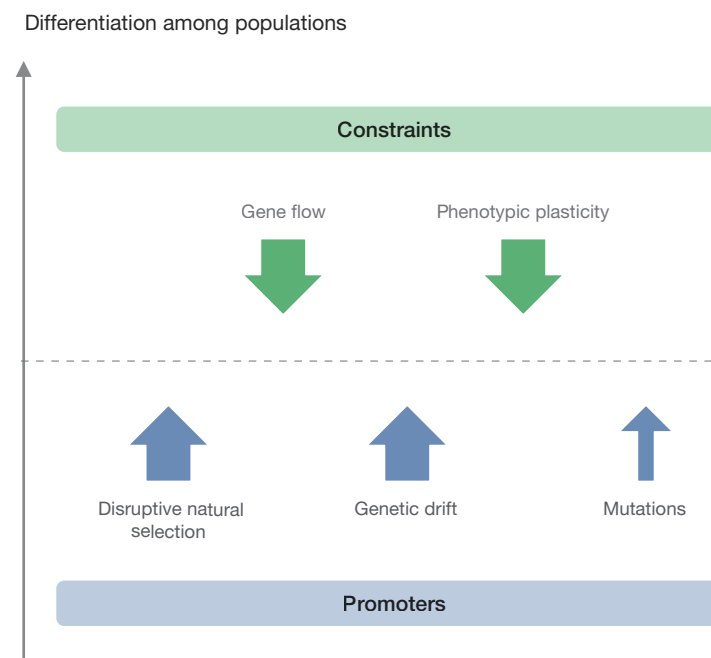


**Figure A1.1.** The relationship between gene frequency and additive variance with complete additive gene action. After Eriksson *et al.* 2013.

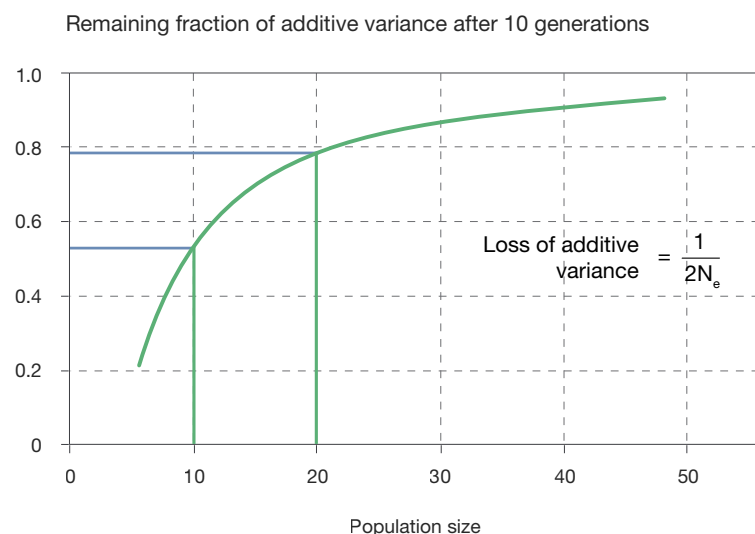
### Evolution

First, it ought to be stressed that evolution is a change in the genetic structure of a population. Evolution may or may not lead to improved adaptedness. Adaptedness, adaptation, adaptability and fitness are concepts that are used in evolutionary discussions (see Glossary of terms in Annex 2).

Obviously, adaptedness and fitness are closely related. For genetic conservation purposes, we are most interested in evolution in the form of differentiation among populations. The reason is that we want to include as much genetic variation as there may exist in a species. Fig. A1.2 illustrates that natural selection, genetic drift and mutations cause differentiation. Of these three causes, only natural selection leads to improved adaptedness. Although natural selection frequently is described as an active force, such a description might be misleading, since natural selection is merely the result of varying success of transfer of genes to the next generation. Genetic changes caused by natural selection were originally thought to be rather small, but it has been shown that large changes may also occur.



**Figure A1.2.** Schematic illustration of evolutionary factors promoting and preventing population differentiation. The arrows pointing upwards increase the differentiation while strong gene flow and phenotypic plasticity are constraints to differentiation between populations. Mutations have a limited impact on differentiation. After Eriksson *et al.* 2013.



**Figure A1.3.** Remaining fraction of additive variance after 10 generations as a consequence of genetic drift. It is assumed that the effective population size is constant during these 10 generations.  $N_e$  = the effective population size. After Eriksson *et al.* 2013.

Genetic drift is of importance in small populations and it leads to random losses of genes, whether the genes contribute to fitness or not. The loss of genes leads to a decrease in additive variance. Therefore, it is of significance to know how large the loss of additive variance is for different population sizes. In many textbooks, the loss of additive variance is shown for hundreds of generations, which is of limited interest for forest trees with their long generation times. Therefore, the loss of additive variance after 10 generations is shown in Fig. A1.3. The curve in the figure illustrates that, at population sizes of less than 20, genetic drift is a strong cause of genetic change. The reason why genetic drift causes an increase of the among-population differentiation is simply that different genes are lost in different populations. In small populations, genetic drift can cause considerable among-population differentiation.

Mutations are the source of genetic variation. The rate of change in an individual locus (position on a chromosome where alternative genes influencing one trait are located) is low. Estimates vary roughly from 1 per ten thousand to 1 per million per generation. The reason mutations cause among-population differentiation is that with the low rate of change it is unlikely the same mutation will appear in two different populations. The thin arrow in Fig. A1.2 indicates that mutations will not cause much differentiation between populations.

When populations adapted to different site conditions exchange pollen or seeds, geneticists talk about gene flow. If two adjacent populations are adapted to their site conditions, it means they vary with respect to their genetic structure. If gene flow occurs between the two populations, this gene flow tends to level the differences in genetic structure between the two populations. In this way, gene flow is a constraint to evolution and may be a strong constraint for wind-pollinated species.

Phenotypic plasticity of a genetic entry (entry stands for an individual, progeny or population) is defined as the maximum amplitude of a trait studied under at least two environmental conditions. Plants, especially, with their non-deterministic growth, can show large phenotypic plasticity. Different traits will have different degrees of phenotypic plasticity. Thus, floral structures have less phenotypic plasticity than tree height. Phenotypic plasticity can be regarded as a disguise of the genotype (all genes in an individual). This also means there is less conformity between the genotype and the phenotype (the traits that are visible). Let us, for a moment, forget that natural selection is not an active force. If so, we could say natural selection at high phenotypic plasticity of a trait would only identify the genotype of an individual with difficulty. In this way, phenotypic plasticity is a constraint to evolution. Long generation times and large among-population gene flow are two conditions favouring development of large phenotypic plasticity.

Among lay persons, there is a popular belief that natural selection has produced perfect genotypes for different site conditions. This is a misconception. To obtain perfect adaptedness, all traits must be independent of each other, and no environmental change is permitted.

The fitness of an individual is an average fitness of all the traits this individual possesses, which means one trait might be improved by selection while there is a regression in another trait if there is a negative correlation between the two traits. Environmental conditions are never stable. Thus, neither of these two conditions for perfection is fulfilled.

As is evident from the discussion above, natural selection is not the only cause of evolution. We also have the constraints in the form of gene flow and phenotypic plasticity to consider as mediators of imperfection in adaptedness. In nature, the five factors (Fig. A1.2) interact in a complex way, such that a perfect adaptedness will never be obtained. Moreover, many genotypes may give rise to the same phenotype, which means the existing genetic structure of a population is only one out of many and it is transient.

For genetic conservation, this means the existing genetic structure is not what we should have as our goal for conservation, but it is useful as starting material in a dynamic genetic conservation approach.



### The magic number of 500

One frequently raised question is: 'How many trees do we need for a satisfactory capture of the genetic variation?' Again, it is the amount of additive variance that remains in the population after the selection of trees for the genetic conservation population that is of importance. If this selection is random, the rate of loss of additive variance in percentage terms is equal to  $100/2N$ , in which  $N$  is the number of trees mating to give rise to the next generation. Therefore, the remaining percentage will be equal to  $100 - (100/2N)$ . If we assume that the number of trees is equal to 10 ( $N = 10$ ), we find that the value in the parenthesis is 5, and a subtraction of 5 from 100 means even after selection of a small population of only 10 trees, the remaining additive variance is 95% of the total variance. If we sample 500 trees, the value in the parenthesis will be 0.1%. Thus, after a random selection of 500 trees, 99.9% of the total additive variance is kept. A random selection of another 500 trees gives a value of 0.05%. This means such a large increase in the population only gives an additional contribution of 0.05% to the additive variance in the selected population. Thus, we expect 99.95% of the total variance is kept if 1000 trees are selected. An increase in the genetic conservation population from 500 to 1000 does not seem worthwhile. A prerequisite for this is that all trees participate in the mating process, resulting in a new generation.

Studies on the mutation rate in quantitative traits suggest this rate might be equal to what is lost in a sample of 500 trees. In other words, mutations that increase the additive variance match the losses from sampling just 500 trees.

This knowledge about loss and gain of additive variance is the reason why some population geneticists talk about the magic number of 500. This figure has recently been challenged, since some of the additive variance might reduce the fitness of its carrier. To overcome this, 5000 trees would be a better estimate of the number needed. At the same time, directional selection over 100 generations in much smaller populations than 500 has resulted in a continuous response to this kind of selection. This indicates sufficient additive variance was available during all these generations. If one prefers a conservative approach to sampling, the larger number is needed. However, this means enough trees will seldom be available for genetic conservation of rare and less common tree species.

### Genetic pollution

Gene flow is sometimes referred to as genetic pollution. This is because of the belief that the adaptedness is perfect and any gene flow will reduce the adaptedness of the recipient population, and the belief that any gene flow will break a fine-tuned genetic structure of the recipient population, leading to drastic reduction in fitness.

The latter belief requires that a specific adaptation to particular site conditions has taken place, such that the activities of many genes are dependent on the presence of many other genes. Once this specific combination of genes

is broken up by crosses with alien pollen, the adaptedness would be drastically reduced. Such situations may exist, but for cork oak, which is a wind-pollinated species, such a situation is less likely. All genetic evidence also indicates that there is fairly free exchange of pollen among cork oak populations, which would be a great constraint to specific adaptedness.

It must be assumed that the differences among cork oak populations are due to differences in gene frequencies rather than different sets of genes in different populations. Differences in gene frequencies among populations are a result of natural selection. Therefore, there is, in principle, no difference between gene flow and natural selection; both cause a change in gene frequency. It is, therefore, inappropriate to equate gene flow with pollution. However, it must be admitted that gene flow will, in most cases, reduce the adaptedness of the recipient population.

### Why is the local population outgrown by transferred populations in provenance trials?

While this assertion may not be true for every provenance trial, there is an overwhelming number of provenance trials worldwide in which the local population is outgrown by transferred populations. Intuitively, one would imagine that the local population should be better adapted to its local conditions than other populations originating from other environmental conditions. However, it must be remembered that a provenance trial is different from a natural regeneration of a stand. This means the conditions offered to the plants at plantation may be quite different from the ones at natural regeneration. For a plantation, there is a requirement that at least 80% of the plants should survive, while under natural conditions, the requirement is that each mature tree be substituted by one new plant to maintain population size. Since Scots pine trees may produce one million seeds, it is evident that Scots pine wastes energy on seed production. Even if a cork oak tree does not produce one million acorns during its lifetime, it is obvious that the number of acorns from one cork oak tree greatly exceeds the number required to maintain the cork oak population.

It is, therefore, important to distinguish between what is useful in nature and in plantations. To do so, two types of fitness - Darwinian and domestic - are defined. Darwinian fitness is the ability of a population to transfer its genes to the progeny population. Domestic fitness is the ability of a population to produce utilities demanded by man. These two types of fitness are rarely identical.

A population aimed for production of utilities for human use (= production population) does not need to have as large an additive variance as a genetic conservation population. Rather, part of the variation in a genetic conservation population might reduce the domestic fitness of that population. In conclusion, genetic conservation populations and breeding populations need to have a large additive variance. Production populations only need the amount of genetic variation satisfactory for production of the utilities required.

## Summary

Most traits of adaptive significance are quantitative, which means no distinctive classes are segregating in the progeny. Quantitative traits are regulated by many genes; most of them have a small effect on the trait. The effect of one gene may be substituted by another gene with identical effect. Therefore, there is no need to conserve rare genes in genetic conservation. Presence of additive variance for a trait is a prerequisite for change via natural selection. Additive variance is the variance of breeding values. The breeding value is the double deviation of the mean value of the progeny of a particular tree from the mean value of all progenies. The continued existence of rare genes, <1% in a population, is dependent on chance events, whether the gene contributes to fitness or not. Evolution is the change of genetic structure of a population. Natural selection, genetic drift, and mutations cause evolution while gene flow and phenotypic plasticity are constraints to evolution. All of these may act simultaneously, leading to a very dynamic situation. The present genetic structure is one out of many possible and it is transient. Therefore, it should not be the target in a dynamic genetic conservation approach. A random selection of 500 trees in a population captures almost all the genetic variation of importance for natural selection.

It is important to distinguish between what is useful for a population for natural regeneration and what is demanded for production of human utilities. The former is designated as Darwinian fitness, the latter as domestic fitness. Genetic conservation populations and breeding populations need a large additive variance. The amount of genetic variation that is satisfactory for production of human utilities is usually much less than the genetic variation required for the genetic resource populations.

## Annex 2

### Glossary of terms

|                                   |  |
|-----------------------------------|--|
| <b>Adaptability</b>               | The ability to respond genetically or phenotypically to changed environmental conditions   |
| <b>Adaptation</b>                 | The process of genetic change of a population caused by natural selection, which results in better adaptedness in a specific environment.  |
| <b>Adaptedness</b>                | The state of being adapted that allows a population to survive, reproduce and permanently exist in certain conditions of the environment.  |
| <b>Allele</b>                     | An alternative form of a gene. Alleles are located on corresponding loci of homologous chromosomes.  |
| <b>Anemogamous</b>                | Wind-pollinated.   |
| <b>Associated species</b>         | Species dependent on other species for their well-being and survival.  |
| <b>Autochthonous (population)</b> | A population that has been continuously regenerated by natural regeneration.   |
| <b>Base population</b>            | The population of trees from which selection of reproductive material is made for the next generation of breeding.   |
| <b>Breeding population</b>        | A set of trees from a base population that is selected for their desirable characters to serve as parents for the next generation of breeding.   |
| <b>Conservation stand</b>         | See: genetic conservation stand or population.   |
| <b>Distribution area</b>          | The geographical occurrence and arrangement of a species, or a population; usually refers to the natural extension of the area occupied by a species.  |
| <b>Drift (genetic drift)</b>      | Random loss of genetic variants due to stochastic processes.   |
| <b>Ecosystem</b>                  | The ecological complex of, for example, a forest community, including the non-living components of the environment, functioning together as a system in which exchange of nutrients follows a circular path. |
| <b>Effective population size</b>  | In a broad sense, the number of individuals in a population successfully involved in reproduction in a given generation.   |

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| <b>Ex situ conservation</b>                             | Conservation of genetic resources that entails removal of individuals or reproductive material from its site of natural (original) occurrence, i.e. conservation 'off site'.  |
| <b>Fitness</b>  | The propensity of an individual to transfer its genes to the next generation.   |
| <b>Gene (or genetic) conservation</b>                   | All activities, including collection, maintenance, storage, management, protection and regeneration, aimed at ensuring the continued existence, evolution and availability of genetic resources, both <i>in situ</i> and <i>ex situ</i> .                     |
| <b>Genetic conservation stand or population ex situ</b> | Population established with the specific objective of genetic conservation using basic material collected by random sampling in the target genetic conservation unit and grown or stored elsewhere.   |
| <b>Genetic conservation stand or population in situ</b> | Forest stand in which appropriate management is carried out to ensure the conservation of genetic resources of target species.  |
| <b>Genetic conservation unit</b>                        | A common term for all units in which genetic resources are maintained, including gene reserves; <i>in situ</i> and <i>ex situ</i> genetic conservation stands or populations; seed lots stored in gene banks; clone collections; seed orchards; and arboreta. |
| <b>Genetic diversity</b>                                | A measure of genetic variation present in a population as a consequence of its evolution.   |
| <b>Gene frequency</b>                                   | The frequency of the occurrence of alternative forms of genes (alleles) in relation to the frequency of all the alleles at a particular locus in a given population.  |
| <b>Gene flow</b>  | Migration through the dispersal of either gametes (pollen) or zygotes (seeds) to a recipient population from another population with a different gene frequency.  |
| <b>Gene pool</b>  | The sum of all genetic information encoded in genes and their alternative forms (alleles) present in a population at a given time.  |
| <b>Genetic reserve</b>                                  | See: [in situ] genetic conservation stand or population.  |
| <b>Genetic resources</b>                                | The biological material containing useful genetic information of actual or potential value.   |
| <b>Genetic variability</b>                              | The ability of a population to produce individuals carrying different genetic variants (alleles, genes or genotypes); the capability of a population to generate genetic variation.   |
| <b>Genetic variance</b>                                 | A statistical measure of genetic variation.   |
| <b>Genetic variation</b>                                | The occurrence of genetic variants (alleles, genes or genotypes). Genetic variation is brought about by a change in genes, as distinct from differences due to environmental factors.   |

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| <b>Genotype</b>                                   | Genetic constitution of an individual tree possessing a particular set of alleles (i.e. different forms of genes that may occupy the same position on a chromosome).  |
| <b>Hybridization</b>                              | The formation of a diploid organism, mostly by sexual reproduction between individuals of dissimilar genetic constitution.  |
| <b>Inbreeding</b>                                 | Selfing or mating between related individuals.  |
| <b>In situ conservation</b>                       | Conservation of genetic resources 'on site' – in the natural and original population, on the site formerly occupied by that population, or on the site where genetic resources of a particular population developed their distinctive properties. Although usually applied to stands regenerated naturally, <i>in situ</i> conservation may include artificial regeneration whenever planting or sowing is done without conscious selection and in the same area where the reproductive material was collected. |
| <b>Inheritance</b>                                | The transmission of genetic information from parents to progeny.  |
| <b>Isozyme or allozyme</b>                        | Enzymes existing in different molecular forms but with function similar in character.   |
| <b>Mast year(s)</b>                               | Years at certain intervals in which most tree species produce large crops of seeds as part of their biological and ecological strategy.   |
| <b>Mating system</b>                              | System whereby individuals of opposite sexual type are paired to produce progeny. There are two major types: wind pollination and animal pollination; the latter type can be pollination by insects, birds, or bats.  |
| <b>Metapopulation</b>                             | Set of local populations within some larger area, where migration from one local population to at least some other patches is typically possible  |
| <b>Multiple population breeding system (MPBS)</b> | Split of the breeding or gene resource population into approximately 20 subpopulations growing under different environmental conditions and thus exposed to different selection criteria.   |
| <b>Open pollination</b>                           | Natural, or random pollination, when the transfer of pollen from an anther to a stigma is freely exposed to pollen flow without any human interference in seed formation.   |
| <b>Origin</b>                                     | For an autochthonous stand of trees, the place in which the trees are growing; for a non-autochthonous stand, the place from which the seeds or plants were originally introduced.  |
| <b>Outbreeding</b>                                | The mating system in which mating events occur successfully between individuals less closely related than average pairs chosen from the population at random. It is the most common mode of sexual reproduction in forest trees.  |
| <b>Outcrossing</b>                                | Mating between unrelated individuals.   |
| <b>Phenotype</b>                                  | The observable (structural and functional) characters of an individual resulting from interaction of the genotype with the environment.   |

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|---|---|
| <b>Population</b>   | A [Mendelian] population is defined as a unit present under certain [environmental] conditions, composed of biological organisms able to reproduce sexually and where every pair of individuals is allowed to have common ancestry over generations.  |
| <b>Population genetics</b>                                  | Studies of gene frequencies in populations and their changes.   |
| <b>Production population</b>                                | A population used strictly to produce seeds or vegetative material for afforestation or reforestation purposes.   |
| <b>Progeny offspring</b>                                    | Descendants of a particular mating event or of a particular mate.   |
| <b>Provenance.</b>  | The place in which any stand of trees is growing. The stand may be autochthonous or non-autochthonous (see: origin).  |
| <b>Regeneration</b>   | The spontaneous (natural) or artificial creation of a next generation of a population. In the case of natural regeneration, the regeneration stock originates from mating in the respective population.   |
| <b>Reproduction</b>   | The process of forming new individuals of a species by sexual or asexual means. Sexual reproduction involves the union of gametes that are typically haploid and of two kinds (male and female). The production of new individuals by detachment of some part of the current individual is called vegetative reproduction. Sometimes, this term is used synonymously with asexual reproduction, in which case it includes all forms of reproduction in which daughter individuals are produced without the sexual process of gamete and zygote formation. |
| <b>Reproductive age</b>                                     | The age at which the tree produces its first flowers and seed crop.   |
| <b>Reproductive material (forest reproductive material)</b> | Seeds (cones, fruits and seeds) and vegetative parts of trees intended for the production of plants, as well as plants raised by means of seeds or vegetative fragments; also includes natural regeneration.  |
| <b>Sampling</b>   | The selection of populations and trees within populations from which seeds or other material is collected.  |
| <b>Seed [collection] stand</b>                              | A stand of trees superior to the accepted mean for the prevailing ecological conditions when judged by a standard set of phenotypic criteria and which may be treated for the production of seeds.  |
| <b>Seed source</b>  | Trees within an area (stand or seed orchard) from which seed is collected.  |
| <b>Selection</b>  | Any non-stochastic process, natural or artificial, that permits a change in the genetic structure of populations in succeeding generations.   |
| <b>Natural selection</b>                                    | Improvement of adaptedness via differential transfer of alleles to the next generation. It requires that there is a genetically conditioned phenotypic variation causing variable fitness.  |
| <b>Stand (forest stand)</b>                                 | A population (natural or planted) of trees possessing sufficient uniformity in composition, constitution and arrangement to be distinguishable from adjacent populations. 'Stand' is the conventional unit for forestry management and is used interchangeably with the term 'population' (see also: population).   |

ISBN: 978-92-9255-062-2

Cover photo: Fotolia/Springfield Gallery