Gene conservation and breeding of tree species in the Azores islands

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Abstract

Since no forest tree gene conservation activities were carried in the Azores islands with its unique flora, it is urgent to develop a strategy for management of the Azorean forest tree genetic resources. To safeguard the potential for adaptation was identified as the prime objective for gene conservation of tree species in Azores. Three species were identified as of potential interest for tree improvement. *Prunus lusitanica* was selected since it is rarely occurring and close to extinction. *Piconia azorica* and *Juniperus brevifolia* were selected based on their good wood quality and the interest in these trees by the Azorean Forest Service. Combined gene conservation and breeding was suggested for them. The breeding of all of them will follow the Multiple Population Breeding System concept. Open-pollinated seed will be collected for establishment of seedling seed orchards for each ecogeographic zone (cf Fig. 5). Culling of trees with undesired characteristics will be carried out before seed collection. Seeds will be used for establishment of new progeny plantations. For *Prunus lusitanica* only 1-2 seedling seed orchards are suggested. For the two other species several seedling seed orchards are suggested. For tree species not included in the breeding, *in situ* subpopulations are recommended. They should cover the entire range of distribution of these tree species. Whenever two or more species coexist, combined gene resource populations could be used to reduce the cost for conservation. Promotion of flowering by cutting of competing tree species should be carried out to guarantee regeneration of the gene resource populations. Supportive research for the management program is urgently needed.

Germplasm coinservation, tree breeding, Azores islands

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Introduction

General information about the Azores islands

Location

Azores is an archipelago with nine islands divided in three groups in the North Atlantic Ocean between the latitudes 36º 55' and 39º 42' N, and longitude 25º and 31º 30' W of Greenwich. Flores and Corvo compose the western group, in the central group there is Faial, Pico, São Jorge, Graciosa and Terceira, in the closest group (eastern) to the mainland with a distance of 1480 Km it is the islands of São Miguel and Santa Maria. All islands are inhabited and the size of the population was 237.795 inhabitants in 1991, although it has been more densely populated before.

Figure 1: map of Azores islands and their location in the globe.

The total area of Azores islands is 2.333 km^2, the biggest island is São Miguel with 747 km^2, the smallest Corvo with 17 km^2 and Pico the highest mountain peak of Portugal with 2.351 meters. All the islands are of volcanic origin, resulting in a big variation of hills and valleys.

Climate

The islands have a quite similar oceanic climate although with a considerable increase of precipitation as we move from São Miguel (751 mm) to Flores (1592 mm) at sea level, it also increases 25% each 100 m of altitude (Sjögren 2001). The temperature varies from an annual minimum of 14ºC to a maximum of 24.8ºC with high level of humidity (77% average/yr.).

Flora

The problem in Azores, like in many places in the world, is that it is not natural selection that is responsible for endangering some of our autochthonous tree species. An abusive use by man of our forests for over five centuries has lead to an abnormal rate of threat.

Nowadays the most probable problem is the small size of the scattered stands/individuals that may lead to genetic drift. There is a need to search for populations that are scattered in the 9 islands, as the degree of genetic drift may already be of big concern. Most of the autochthones species exist in the four archipelagos that constitute Açores, Cabo Verde, Canarias and Madeira, although there are several species endemic only to Azores islands.

The autochthonous *Laurisilva* forest of the Azores islands is composed of a wide diversity of species such as cedar (*Juniperus brevifolia*), English holly (*Ilex perado ssp.azorica*), sheepberry (*Viburnum tinus, ssp.subcordatum*), Scots heather (*Daboecia azorica*), heath (*Erica scoparia, ssp.azorica*), wildberry (*Vaccinium cylindraceum*), spurge-flax Daphne (*Euphorbia stygiana*), "Pau Branco" (*Picconia azorica*), "Faia" (*Myrica faia*), morello wild cherry (*Prunus lusitanica ssp.azorica*), several of them facing serious threats.

The main economical activity is farming with the raising of dairy cows for obtaining high quality beef. Even some of the steepest slopes are used for grazing all year round. This leads to a destruction of the native forest to install pastures in all the 9 islands, with a strong impact in some of them; Terceira, Pico and Faial are the islands with the widest natural forest (Sjögren 2001). The construction of roads enabled the planting of large areas with the imported species, *Cryptomeria japonica*, that is now the most abundant species in Azores. There is also some production of *Eucalyptus globulus*, but this species has probably not a big future as the cellulose companies gave up their production in Azores. There are species that were introduced as ornamental trees and now are invading and competing with the natural forest, like the *Acacia melanoxylon* and the *Pittosporum undulatum*, that are probably the most devastating with a very fast growth and regeneration combined with their ability to grow in very poor and rocky soils.

There are many reasons why we should invest time and money in our autochthones species, one important reason being the risk that it is to bring foreign species like those mentioned above. The wood of some of these autochthones species is quite valuable as is the case with *Juniperus brevifolia, Prunus lusitanica L. ssp. Azorica, Persea indica, Piconia azorica*. These species are already adapted to the Azores climate, so we will not have problems with maladapted species.

One of the questions that may be raised is "For which market will we be producing this wood"? The answer is that it will be for the local market in Azores, but we can also export it to the mainland and overseas. Contrary to the *Cryptomeria* or *Eucalyptus* that have a not very valuable wood, some selected autochthonous tree species produce quality timber much appreciated for furniture. Instead of shipping, a volume of wood that has a low price we can export the same volume (the same cost of transportation), but with a much higher price and consequently bigger income, so we are optimising resources.

The autochthonous species probably will not grow as fast as some foreign species, but still the income is expected to be bigger than compared with fast growing species. There is also another economical factor favouring the autochthonous species, they have priority to be supported by funding through EU. Particularly now with the new CAP (Common Agricultural Policy) measures that subsidise by the area and not occupation that previously was favouring an intensive farming. So this will be a help to attract forest owners to invest, but with the kind of forest use that is going on now it will not be enough. Therefore, it is urgent that the state increases the awareness of the value of the existing forest resource of the Azores islands. For the time being the way to run a forest in Azores, in most cases, is just to cut the existing forest and leave it without any special measures for regeneration, and if regeneration is done there is not a big concern about the quality of the material used.

If we are preserving our forest patrimony it will attract more tourists interested in ecology and natural flora to come and see our endemic forest, something that we will not do with an island covered with a tree from Japan or Australia. Tourism will be very important as a source of income to the islands and so justifying the importance of a project concerning the autochthonous species and their conservation.

Looking into a wider and more global point of view, by producing our own hardwoods we are also helping to preserve the tropical forests where some of the currently used hardwoods come from. This means that this production of hardwood will be economically more important with the restraints that will be put in wood coming from unmanaged tropical forests (Eriksson, 2001).

As mentioned above the breeding project will be complemented with a low cost gene conservation part. These two should be running side by side, combining these two goals in the best and cheapest way.

Aims of the present study

To develop a strategy for forest tree gene conservation in the Azores islands.

To develop a combined conservation and breeding program for a few tree species in the Azores islands

Evolutionary factors

Different authors interpret the terms adaptability, adaptation, and adaptedness differently. The definitions used in this paper are given in Box 1 taken from Eriksson and Ekberg (2001).

Box 1

Adaptation = the process that leads to a better adaptedness in a specific environment
Adaptedness = is the degree to which an organism is able to live and reproduce in a given set of environments

Closely related to adaptedness according to the above definition is the **fitness** concept. Fitness is an expression for an individual's contribution to the next generation in relation to the other individuals in the same population.

Adaptability = the ability of a population to respond genetically or phenothypically to changed environmental conditions. The amplitude of a trait of a genotype studied in at least two different environments is called phenotypic plasticity. The term *reaction norm* is used to describe the trait value change of a genotype studied along an environmental gradient.
Eriksson and Ekberg (2001)

Evolution of species is a continuously ongoing process. They will never reach a climax, this means they will never reach a perfect adaptedness, because the environment surrounding them is in a continuous change. As we have seen by paleontological evidences of a succession of glacial and warmer times, and now approaching another warmer global climatic change that will require a large adaptability. In order to survive the existing species have to cope with these changes and this is achieved by maintaining a genetic diversity that makes an adaptation possible for species in the case of environmental changes, so a good adaptability is needed.

Evolution is influenced by four main factors, natural selection, genetic drift, gene flow, and mutations. The definitions of these four factors are given in Box 2 taken from Eriksson and Ekberg (2001).

Box 2

Mutations = change of genes

Natural selection = differential transfer of alleles to the next generation resulting in increased fitness

Random genetic drift = random loss of alleles in small populations

Gene flow = migration to a recipient population from another population with a different allele frequency

Phenotypic plasticity = the amplitude of a trait of a genotype studied in at least two different environmental conditions

Eriksson and Ekberg (2001)

Natural selection will only take place if there are differences in fitness and these differences are genetically conditioned. If all the individuals have the same adaptedness it is not possible to favour one over the others. Only the most fit phenotypic forms, expression of a good genotype, will contribute more than others to the progeny of the next generation. Natural selection varies from strong to weak (Endler 1986). Sometimes it can also lead to maladaptation in the long term if it reduces the genetic diversity.

Many genes regulate most traits of significance for adaptation with a minor effect on the trait. These traits mostly show normal distribution with a bell-shaped curve (Fig. 2).

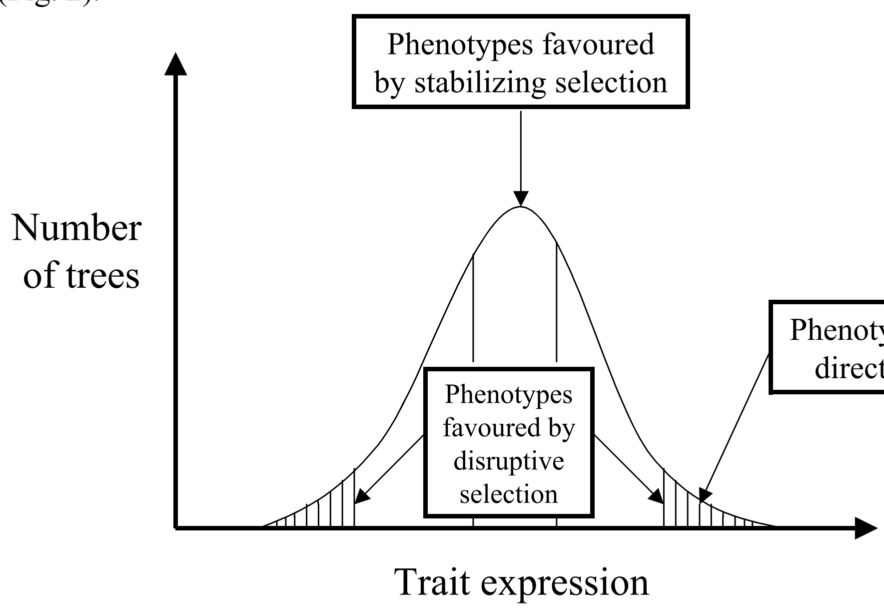

Fig 2. Bell shaped curve representing the normal distribution of traits. The arrows indicate which individuals in the curve will be favoured in each of the three most relevant kinds of natural selection.

Three main types of natural selection can be distinguished, as can be seen in the above figure. Stabilizing selection in which individuals in both tails of the distribution are selected against consequently favouring the ones in the middle, directional selection when individuals in one of the tails of the curve are favoured by the existing conditions, and disruptive selection when individuals in both tails of the curve have the highest adaptedness for the existing conditions.

Recombination allows the emergence of new allele combinations. It does not change the gene frequency directly, only indirectly, creating new options for natural selection to act on and so having a very important role.

Genetic drift is of greatest significance in small populations, meaning few individuals crossing each other. This will consequently lead to inbreeding and homozygozity by loss or fixation of alleles. This factor is of great relevance and has to be controlled in a breeding or gene conservation project, but should not be very relevant for wind pollinated species with large populations. Genetic drift will act randomly and so it might not favour the fittest genotype, and if isolation is maintained for a long time this may lead to extinction. Populations with a similar gene pool may diverge owing to a strong genetic drift, leading to populations with quite different phenotypes, as can be seen from figure 3 it is a promoter of differentiation.

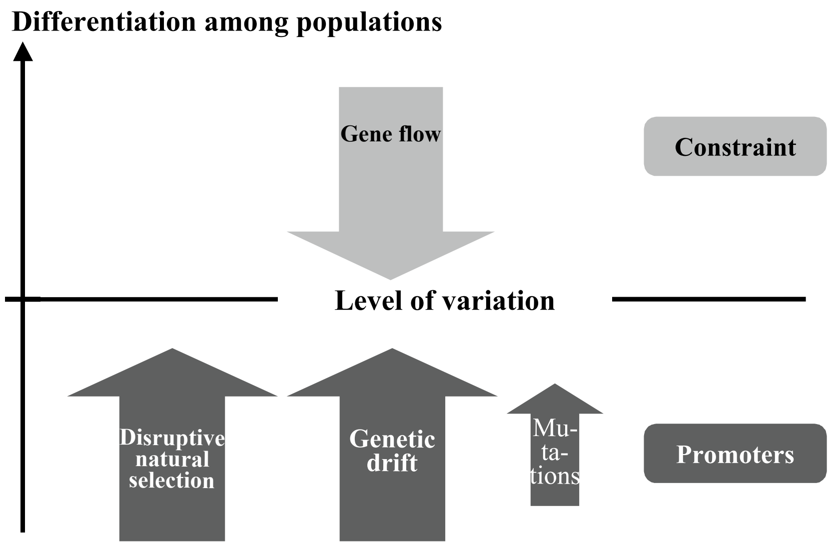

Figure 3. The influence of the four evolutionary factors on differentiation among populations. Arrows pointing upwards increase the differentiation and thus are promoters of differentiation while the downward pointing arrow reduces the differentiation among populations and thus it is a constraint.

Mutations are errors that occur during the process of DNA replication, or induced physically or chemically by mutagens at a very low rate 1:10 thousand to 1:1 million, so this is not the main factor in population differentiation (Eriksson and Ekberg, 2001). Most of these errors are very big with reduced fitness leading to poor survival of the individual carrying the mutation, but if the case is that we have a small error that allows the individual to survive to the next generation then we might have a possible evolution, or at least a wider genetic variation. The genome should have a low rate of mutations and gene replication must be conservative, there is a need for stability (Varela and Eriksson 1995). A high rate of mutations would lead to a chaotic situation without a possibility for adaptation.

Mutations occur at a very low rate, and the chance of the same mutation occurring in two different populations is practically impossible, so the populations will differ from each other in relation to mutations, this is the reason why we can see it as a promoter in the above chart.

The speciation is conducted by the separation of populations by physical barriers (rivers, mountains, etc.) or, not so often, by genetic differences. Gene flow among several populations as opposite to the other factors (natural selection, genetic drift and mutations) referred to before is a constraint to speciation and homogenizes the variation among populations, if there is a different gene pool between those populations. Gene flow can be carried out by pollen transfers (most important for wind pollinated species), seeds, fruits, nuts or vegetative propagules. Migration of one gamete per generation is enough to prevent the fixation of neutral alleles (Slatkin 1987).

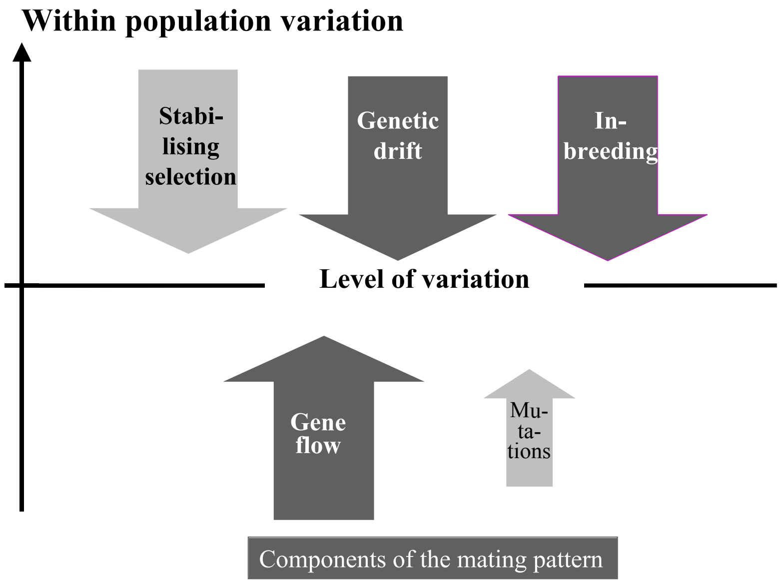

Figure 4. The influence of the four evolutionary factors on within-population variation. Arrows pointing upwards increase the variability and thus are promoters of differentiation while the downward pointing arrow reduces the variability within the population and thus is a constraint.

As we can see from Fig. 4, when it comes to within-population variation it is almost the contrary to the previous scheme. Now gene flow and mutations lead to a higher genetic variation, while stabilizing natural selection, genetic drift and inbreeding will repress it. Gene flow may bring new alleles from other populations with different gene frequencies, which increases the variation. The same happens with mutations that will generate new alleles. Stabilizing selection will reduce variation through the favouring of the existing genotypes at the centre of the distribution curve, genetic drift and inbreeding are known to reduce variability and leading to homozygozity.

Sometimes phenotypic plasticity is treated as an evolutionary factor (Bradshaw 1965). Phenotypic plasticity allows plants with the same genotype to occupy different environments expressing a different phenotype, and allows it to overcome some changes during their long life period presumed that they have a large phenotypic plasticity. This can be a problem for natural selection or even artificial selection, because it “masks” the genotype.

General information about forest tree gene conservation

Objectives

One should not start a gene conservation project before establishing the objectives. There are several gene conservation objectives, depending on what kind of programme is being developed. In my point of view the fundamental role of gene conservation is not to be static (preservation), but to promote fitness to the future and allow future adaptation when changes in climate or habitat arise (Soulé and Mills1992). This means "*to safeguard the potential for adaptation of a species",* and allow the species to assure their existence in a future with different conditions from present time (Eriksson 2001).

One important factor for many gene conservation projects is the financial aspect; it must be of low costs since only low cost projects will be implemented.

The concept of genotypes conservation is too limiting, in this kind of conservation a big importance is given to the genotype as a whole and not just to the different alleles. As a result of cross-pollination each tree has a unique genotype, unless they are clones, this makes it impossible to save all genotypes (Eriksson and Ekberg, 2001). One reason for this impossibility is of course the economical one, with an enormous amount of money being needed.

A more realistic project for gene conservation will be focused on alleles. This way the most common genotypes must be represented in our population, the most rare alleles are not so important. The cost of including rare alleles is high and they do not contribute to the additive variance, some of them are mutations that have no importance at all in conservation, or they are rare just because they do not contribute to fitness of their carriers.

An objective that will be easier to carry out because it has a bigger economical support is the one that combines conservation and breeding. The populations used for breeding must also be representative of the existing genotypes in order for a careful selection to be made; so these populations of the earlier stages can be used as a gene pool for the conservation part of the project.

A very important objective is to save populations threatened by land use. It is very common for the time being, with the increasing impact of men, to have populations that need to be conserved.

Gene conservation of associated species is taken care of if we are caring for more than one species; there is no separation between the keystone species and the ones sharing the same habitat. They will be occupying the same large natural area and management is done caring about the forest as a whole and not as isolated species, still having in mind that all species must have the widest genetic variability possible.

Box 3

Target species = A species given priority in gene conservation for scientific reason, threat, charisma, or economic reasons.

Associated species = A species dependent on another species for its existence.

Eriksson and Ekberg (2001)

Methods

There are several gene conservation methods, the most developed ones will involve sampling, plantation, management and regeneration on a wide span of site conditions; this will favor the genetic variation among populations (Varela and Eriksson 1995).

The Multiple Population Breeding system (MPBS), which was suggested by Namkoong et al. (1980) for breeding is a conservation method that has been widely accepted for gene conservation as well (Namkoong 1984, Graudal et al. 1997). MPBS means that the gene resource population is split into approximately 20 subpopulations each with an effective population size of 50. MPBS has several good qualities, by having several small sub-populations we may have a different evolution in each one, they can be exposed to several types of conditions (edaphic, climatic, altitudes, sunlight exposition), this may lead to an increase of different rare alleles in the different populations. With MPBS, enough variability is supported in a way that it is possible to have gene conservation in intensive breeding projects (Eriksson et al 1993). One disadvantage maybe the cost of it, because it requires a big area and the increase of costs that several scattered subpopulations will have.

These small equivalent subpopulations of about 50 individuals have a faster response to selection than bigger populations and still are able to save genes with frequencies ≥ 0.01 during many generations (Varela and Eriksson 1995). Inbreeding will be on an acceptable rate because of effective population size (N_e) = 50, the coefficient of inbreeding $F=1/(2N_e) = 0.01$ (Varela and Eriksson 1995).

Another system that encompasses breeding and gene conservation simultaneously is the Hierarchial Open Ended (HOPE) method. This system will continuously incorporate new genetic material at any stage of the project. In the meanwhile the mentioned system is of low relevance for forest trees because of the long generations implied and usually used in short rotation species (Eriksson et al 1993).

The Gene Resource Populations (GRPs) can be *in situ* or *ex situ*; these concepts will depend on the author, the definitions used in this paper are, *in situ* = already existing self regenerated natural forests and *ex situ* = man made forests even if it is the same natural species in the same normal habitat.

Conservation *in situ* is advantageous because it keeps the target species in their natural habitat what will also benefit other species living in that habitat. In the meanwhile for the target species a bigger variability would be needed. With the conservation *ex situ* we are focusing on the target species, so other species will not benefit much from that, but a larger variability for the target species will or should be present in this kind of conservation.

Any method must be classified according to how well it represents the existing genetic constitution and its capacity to promote adaptation; the one referred above is one of the most elaborate (Varela and Eriksson 1995). A similar method can be managed without planting, but with a careful selection.

Provenance and progeny trials can also be a good part of gene conservation if they represent the existing variation in the species, although evolution is not promoted. Another method uses seedling seed orchards, but they are mostly not representative with their limited number of selected individuals/families, the same is the case with clone archives. Some static methods will conserve pollen, seed and tissue banks. The limited space and the low cost of these methods are advantageous. In the methods that use simple population we have large natural populations that are mostly important for the conservation of species cohabiting with the target one; unmanaged natural reserves are not fulfilling our aims, because they are not promoting adaptation in the way it is needed, unless preserving a structure of existing endemic species (habitat), and the botanical gardens have just few individuals, so there is almost no value at all (Varela and Eriksson 1995).

Gene conservation of rarely occurring species consists mainly in reducing the risk of genetic drift by increasing the effective population size (Ne), there are two ways with different cost to do this. The more expensive way produces grafts with the purpose of creating clonal archives or seed orchards, not mixing different ecogeographic zones (uses MPBS method). The raised seeds obtained at this point are used to recolonize forests and will be submitted to natural selection (Kleinschmit 1994). The second and cheapest way prevents the rare existing individuals to be cut, may also favor them by a careful silviculture and delivery of trees from that ecogeographic zone to be planted by private forest owners (Eriksson 2001).

Suggested gene conservation methods for the Azores islands

The objective of this project is to develop a strategy for conservation of genetic material of the tree species in the Azores, at the same time as we have some income from the breeding of these same species. The role of gene conservation in Azores islands is to assure that the existing species will not become extinct in a few decades, so evolution will be favored in this project. It will consist of individuals from several populations to facilitate evolution that will enable these species to cope with future changes in climate or habitat.

The method to be used in this project must insure that a wide genetic variability is included and that the most important objective, to safeguard the potential for adaptation, will be fulfilled. These species are suffering a reduction in their natural populations, so it is up to man to provide them with the conditions that will promote evolution. These conditions consist of new carefully planned artificial stands.

Many measures need to be taken for gene conservation in Azores, one very important part is that stronger protection measures must be applied to protect the still existing stands/individuals of native species and their habitats. This means that in some cases the foreign species will have to be controlled to reduce the threat they constitute. A study must be done to determine what, if any, other threats are endangering these species. For example it has been mentioned to me that *Armilaria* disease is attacking some stands of *Persea indica* in Graciosa island (Jorge Belerique pers comm.). For the time being no studies have been made, that I am aware of.

The funding resources available for this gene conservation will hopefully come from EU programs, the regional government, and also from breeding of the selected species as it has been explained previously. With the new CAP policies there will be less productive lands to be set aside, so these can be used as new forest areas and will receive strong financial supports from the EU. The regional government will also benefit by investing in our natural patrimony, as tourism will turn out to have a bigger role than it has had before.

In Table 1 in the appendix the autochthonous tree species from Azores were ranked according to several characteristics. The species are classified by their occurrence in the islands, abundance, preferred climatic conditions, edaphic conditions, and then by height, estimated guesses of their annual ring, which lead to an estimated rotation cycle, value of tree and finally the breeding objectives to the chosen species.

In the column referring to commonly occurring species we state which islands the species have been observed according to Sjögren (2001), the referred species usually occur in all islands except for Graciosa. Abundance is classified by three levels, **very rare** being the case of only one species, **few individuals/stands** because some of the species occur isolated and others in stands, and finally **well dispersed** meaning that most likely there is no threat to these species. As regards climatic conditions the main factor is the altitude and sunlight exposition, because most of the possible differences in climate within an island are controlled by these factors. Edaphic conditions are also a very important factor and the classification was done in a very simple way with only three types; **not demanding** meaning that these trees might even grow in very rocky places with a small amount of soil, **medium** will need a better and deeper soil, **very demanding** does not occur and would be the case of trees only growing in agricultural soils. A column for tree height is included since it is of importance because the size of wood pieces to be taken is economically relevant. Estimated annual ring was obtained from some data available in a project done by Dias (1999) in which he refers to the annual ring size of some species, the others were estimated by comparison of the species development. Based on this column another one was prepared with a possible rotation cycle just to give a general idea, being this the age by the time a tree would reach 40 cm diameter. The column that shows the flowering age, very important when planning a breeding project, is not completely filed due to the lack of studies and consequently only very recently some data made it possible to realize for some species. The value of tree was again estimated and kept simple with only four classes, **low** = **usually not commercialized, medium** = **with low quality, high** = **good quality, very high** = **good quality and highly appreciated** because being most of these species protected they cannot be commercialized and the existing market is not ruled by any organization that controls prices. Breeding objectives were planned for the target species of this project.

The species were grouped by abundance, being the most rare the ones with greatest importance and the second criterion for grouping was their economic value, with the highest commercial value on top.

Some species are facing a big risk of extinction, like *Prunus lusitanica L. ssp. Azorica*. To save this species will be a top priority. It is also important to choose the species with bigger economical value for the breeding project and these are the ones that can provide good wood to be used in furniture.

To start the project we need to know the abundance of the selected species, but just a common census is not enough, this will give the total number of existing trees. For this project it is particularly important to know the number of existing trees that can reproduce. The census number must be at least three times larger than the effective population size required since all trees do not flower.

One way to make this project a little easier to carry out is if a grouping of species is done. I decided to group species according to their abundance, which will also group them by a very important factor for adaptation, additive variance (Eriksson, 2001). The next factor would be the value of the tree, but in our case all the selected species are quite valuable. *Prunus lusitanica L. ssp. azorica* will constitute the first group with very rare species, and the second group of the selected species will include *Piconia Azorica* and *Juniperus brevifolia* that are somewhat more common than the previous.

In my opinion *Prunus lusitanica L. ssp. azorica,* should have an intensive gene conservation project to give this species a more certain future and avoid the risk of extinction that it is facing for the time being. This will be elaborated in the following paragraphs. To the second group of species there is not a such major need to implement a very intensive gene conservation, because the risk of extinction is not so big, so these species will have their gene conservation taken care in the breeding part of this project.

For the commonly occurring species we suggest the use of *in situ* MPBS with low-intensity management. We will opt for the gene resource population *ex situ* if it does not require much higher cost, or if the species is included in breeding.

Depending on the number of existing individuals and islands that the species currently occupies the project should use approximately 20 subpopulations (Multiple Population Breeding System, MPBS), that must be distributed in a broad span of possible conditions, at Azores islands this means a range of longitudes, altitudes, sunlight expositions and soils. A part of this can be perceived from Fig. 5, in which, each dot represents a sub-population to each species of our project. The lowest altitudes (<300 m) are mostly occupied with agriculture; as a result we must include higher altitudes. Because of the costs involved and in an attempt to reduce them it is wise to mix species in the subpopulations; that is what the shaded area indicates. Instead of having 20 subpopulations of each species, we will have two or more species whenever possible in the same subpopulation (represented by a dot or square).

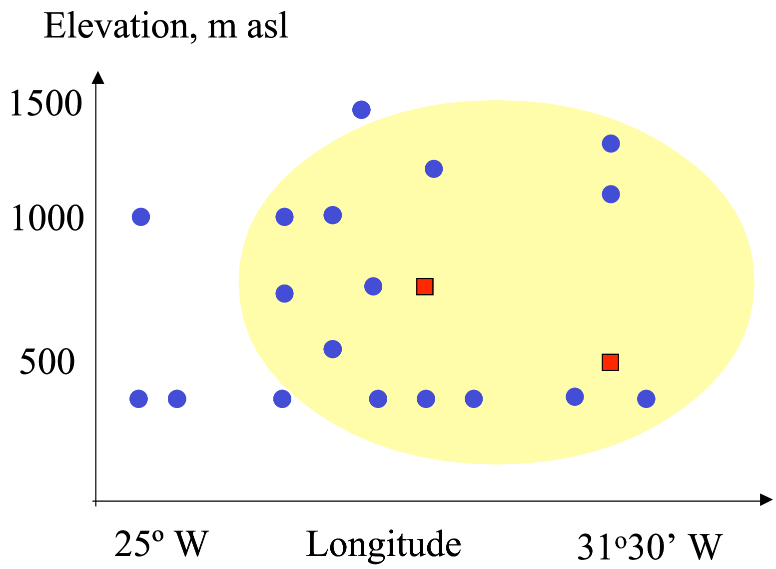

Figure 5. Principle of how the gene conservation of tree species may be carried out in the Azores islands. Each dot represents a subpopulation in a gradient of longitude and altitude, the shaded area indicates that two species coexist in this area and joint gene conservation of the two species may occur. The potential location for two progeny trials for *Prunus lusitanica* is indicated by squares.

These populations should not occupy a large area and 20 is the maximum number of subpopulations to be considered We will probably need and use a lower number of each species, a suggestion with too large number of subpopulations may risk any implementation of this project. In the meantime the subpopulations must be representative of the existing adaptedness. With the MPBS we are able to have a good number of trees and bring together non-related individuals reducing genetic drift and increasing additive variance. It is not of interest to include alleles with frequencies lower than 1%, since these alleles do not contribute to additive variance (Falconer and Mackay, 1996).

To prevent or diminish the risk of genetic drift in the populations used in this project we may also include some populations from Madeira as well as from the Canary Islands, this way it will be possible to have a wider gene pool to start with in a breeding and gene conservation project in the case of the most endangered species, with not that many individuals available in nature. This would be possible to some species due to the common background of the flora, the *Laurisilva* forest, as was mentioned under Flora in the Introduction.

To the species under consideration it is of great interest to figure out if there is any natural stand with a considerable number of individuals. Such an area should be turned into an *in situ* gene resource population. Turning this area into a “reserve” is not enough, it must be managed in order to preserve the existing individuals and promote the emergence of new flowers and seedlings (Rotach 1999). All other species that will outcompete our target species must be controlled by thinning.

To the *ex situ* gene resource population it has been mentioned above the use of the MPBS, and these populations must have the widest gene pool possible of alleles with frequencies higher than 1%. In the meanwhile crosses with low-vitality genotypes, that could have been included, will be outcompeted and eliminated during the growth of the seedlings (Eriksson, 2001). This implies bringing individuals from all the islands to the same subpopulations, because we are lacking genetic knowledge on population differentiation the selection of individuals/alleles to be included in the MPBS will be based on educated guesses. When a species is facing extinction an extra effort can be done by carrying out artificial crosses within the clone archives or seed orchards (Eriksson, 2001).

Breeding project

The following tree species were selected for breeding:

Prunus lusitanica L. ssp. Azorica
Piconia azorica,
Juniperus brevifolia,

Prunus lusitanica was selected because it is one of the most endangered species in the Azorean flora and it has a valuable wood with a fast growth. *Piconia azorica* and *Juniperus brevifolia* were selected based on their good wood quality, *Persea indica* may not be autochthonous and was not selected for breeding for this reason. Although one of the most important characteristics for a breeding project is to know as much as possible about flowering, there is limited data available about this matter.

The breeding objective for the mentioned species is to produce high value wood and at the same time have a part in conservation. The wood of these trees must be of very good quality to be used in “noble ends”, like highly valuable furniture. None of the selected species reaches very high sizes, being sometimes regarded as shrubs or small trees, in the meanwhile the average size they have now is enough to produce profitable products; these sizes are reported in nature with no silviculture practices at all, so it is to expect that they can have an increment with adequate silvicultural practices and one of the aims of this project is also to increase the average size in these tree species. In the long term the investment that has been made in this breeding project has to have a return, so it is important that these species are profitable in some way (fast growth or very valuable wood). This must be a long log, thick, good density, free of knots, meaning few small branches, and branch angles as close to 90º as possible, low spiral-grain angle, and finally try to combine good quality with fast growth. One other trait that does not concern the direct quality of the wood but is also of big relevance is the resistance to the *Armilaria* disease, which is affecting some of our natural tree species like *Persea Indica* in the island of Graciosa. We should not choose too many traits to be improved because that will complicate breeding. The more traits we have the more individuals will be needed, and this number will be even bigger if the traits are not correlated (Eriksson and Ekberg, 2001). For each trait we must determine the genetic variation, the mode of inheritance and estimate additive variance (Eriksson and Ekberg, 2001).

As it was mentioned above there are no deep studies about the selected species, so a study must be carried out to determine some very important information about the selected tree species, because there is nothing or very few data available. Information on the flowering and rotation age, type of flowers, pollen dispersal and possibility of self-incompatibility are mostly unknown. These characteristics are essential information to decide if it is profitable to invest in such a breeding project. The same is also valid for the heritability that must be estimated. The kind of pollination that takes place is also of interest, because we want to promote cross-pollination among the selected individuals but not with others. We must know if they are wind or insect pollinated, these are the two options at the islands since there are no other animals acting as pollen vector.

As a strategy for this project we will use the MPBS that will allow us to have a combined breeding and gene conservation project, in breeding less subpopulations will be used. By having these subpopulations separated we can be aiming for the same or different traits in each subpopulation what would not be possible with just one big population. It is easier and faster to lead a small population to one goal or even to change goals, and have a more intense selection. A change of goals might be needed, obvious if we look to the long rotation age of tree species (50 to 150 years), because climatic changes may arise as well has changes in the wood industry practices and their needs (Eriksson and Ekberg, 2001).

To start the breeding project an inventory about the remains of the selected species populations must be done for the archipelago of Azores, so we can know the real dimension of it. A special care must be given to determine the size of the effective population (1/3 of total may be considered), to know the size of our gene pool and determine the degree of relatedness among and within populations.

Now we must start doing a selection of the plus trees with the desired phenotypic characteristics among the natural populations. From these selected plus trees we must collect seeds or scions to propagate them; scions will be used for cutting propagation if this is possible and easily done. Since grafting is a very expensive technique for the project it will probably not be used. The collected material will be planted at young age in order to allow the plants to have a good root system and choose the strongest and more competitive. It is highly likely that seedlings will be used in these plantations. In such a case the plantations will be designed as progeny trials and will be converted to seedling seed orchards when flowering starts. It is of interest to have some competition among our young trees. This will facilitate the selection of parents for the next generation. We want to promote competition among the trees. Competition may sharpen the differences among the various families and thus facilitate the selection of new parents (cf Franklin 1979). Much time and care must be devoted to be sure that we have reliable results. Naturally regenerated plants must be culled. When the trees have reached a size of a few meters assessments should be taken to enable estimation of genetic parameters for the selected traits.

If possible the subpopulations of two or three different species will be installed close together, this will allow the costs to be reduced and at the same time it also increases biodiversity, what will be important for ecological reasons and will attract more tourists.

The location of the subpopulations was explained previously, in a general way it must be representative of the possible existing conditions, mainly edaphic, altitude and sunlight exposure. Altitude must be within limits imposed by the lower lands intensively agricultured and the highest altitudes with too much wind, within this range we must also diversify between slopes and different sunlight exposures. The areas of each subpopulation are supposed to be enough to produce a quantity of seeds that will enable us to do some reforestation, but these areas can not be very big as the project is to be realized in islands and it would not be realistic to have very wide areas. Several factors must be considered, like the amount of seeds produced by each tree, germination rate, the vigor of the young plants, rate of survival in the first years, the variation of seed production from year to year. Very recent studies have germination rate values for *Juniperus brevifolia* with a mean average of 15%, but with a big variability among individuals sometimes reaching 40%, vegetative propagation can reach 60% of success (Jorge Belerique pers. comm.).

After the installation of the stands we expect open-pollination to take place, or else we have no breeding project because this is the essence of it; we should try to isolate the seed orchards from pollen contamination of other trees outside the selected ones further on this project, one idea is to have the stands in the middle of mature *Cryptomeria* stands occupying relatively big areas. It would be of interest to have some controlled pollination, but this is a very expensive technique which turns out to be impossible to carry out, unless for some extraordinary exceptions.

As we move further on the breeding project we should avoid bringing genetic material from natural stands, because this will have a regression in gains. Only in the case of very low genetic variability this should be done, and in this case as was said before we can select individuals from several populations in Macaronesia, so we can have a diverse gene pool. Individuals must be selected in several populations according to the desirable traits.

There must be an evaluation of the progenies from this first trial, so we can select the best parents for the second generation seed orchards, when this second generation is already producing material for regeneration the first generation stands will be kept as gene resource populations. The evaluation of an individual can be achieved by the mean value of its progeny, this is general combining ability (GCA) (Eriksson and Ekberg, 2001). It is not possible to wait until the end of the rotation age, so by one third of that time there must be an evaluation of the progeny and then choose the individuals with best results.

On these second generation subpopulations we should have big gains in our genetic improvement goals, by having the best individuals with cross pollination among each others and not having the trees with undesired phenotypic characteristics to lower the standard.

During this process young trees should be supplied to private forest owners to help reforestation with the autochthonous species and so fulfill a part of the goals in this project; that is to protect our natural tree species and also get some income from them. A big campaign should be done to alert the forest owners of the advantages of choosing these autochthonous species, ecologically and financially, at the same time this must become one of the slogans to promote Azores as a place that respects nature and still has much to show to tourists.

As stated at several places above a supportive research program is supposed to take place along the entire project. Above all it is important to determine important information about flowering and the biology of these species that is still unknown. Estimates of genetic parameters for important traits are also required for a genetically sound breeding program.

Conclusions

For five centuries the landscape and flora composition of the Azores islands was affected by human activities. This led to endangering of some of the existing autochthonous tree species. Presently it has become more important to save those species for ecological, economical, as well as moral reasons.

Like everything else there is a limit of investment that can be done without revenue, so conservation will be achieved in a way that produces income through a breeding program, and so increasing the possibilities of success in this project. It is of great importance that the selected species will have conditions matching the prime objective in gene conservation *to safeguard the potential for adaptation*.

Only three species were selected for combined breeding and gene conservation not because they were the only ones that could be used but because more than that would be unrealistic. One of the main factors in the selected species is that they must produce valuable wood in order to have the refunding that will support part of the project.

The species *Prunus lusitanica L. ssp. Azorica* was selected for that reason and because it is one of the most threatened species in Azores, for this reason a more intense conservation project was designed for it to try to reduce the effect of genetic drift that may be significant in this species. The method to be used is based on the multiple populations breeding system (MPBS) with several subpopulations, but much less than the usual 20 because of the rarity of the species and its natural occurrence in only five out of the nine islands.

For the two other species there is also a need for conservation, but not as strong as for *Prunus lusitanica L. ssp. Azorica*. The conservation of *Piconia azorica and Juniperus brevifolia* will be taken care of in the breeding part of the project. There is no need for an intense project for their conservation, in the meantime the breeding project must be careful enough to include all alleles in frequencies over 1%.

For the rest of the autochthonous tree species low cost *in situ* MPBS gene conservation is suggested with subpopulations distributed over the entire range of distribution. Whenever species coexist combined gene conservation can be carried out, promoting biodiversity and making it cheaper. The associated species will benefit from the conservation of our autochthonous tree species. A management of these areas must be planned, reduction of exotic species will also be very important for the conservation program; presently exotic species constitute a threat to our natural flora.

The designed project needs to be flexible to be able to adapt to new conditions that may arise, and new knowledge about these species that may come further on in the project.

Supportive research must be carried out to improve biological and genetic knowledge of the selected species; this will be a very important part during the entire process. No such studies were carried out so far. An inventory must be done before starting this project, so we can identify stands or individuals of the threatened species and decide if it will be needed to bring genetic material from the other archipelagos of Macaronesia.

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Web sites:
http://www.drtacores.pt/
http://www.destinazores.com/

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