

Fingerprinting of genetic diversity and patterns of spatial genetic variation in the endemic tree *Cedrus brevifolia* (Hook f.) Henry from Cyprus: implications for its conservation

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1. INTRODUCTION

1.1 Genetic diversity and differentiation

Genetic variation is basis for evolutionary adaptation of a species or a population to environmental change (Nei 1987) and is the raw material upon which natural selection acts. The absence of genetic variation may result in a species lacking in adaptive capacity and/or in a species demonstrating reduced reproductive response towards environmental challenges, thus ultimately being in danger of extinction.

Furthermore, diversity at the intraspecific level is a critical component of biodiversity because it allows the species to evolve over time and in space and thus plays a key role for its long-term survival (Ledig 1986). Genetic variation is continually being created by mutation and at the same time eroded by selection and drift, and, it is a prerequisite for evolutionary adaptability, but not for physiological traits (Finkeldey & Mátyás 1999). In fact genetic variation within a species is a fundamental concept for ecological genetics, and consists of three fundamental components: (i) genetic diversity -which is the amount of genetic variation within a population, (ii) genetic differentiation -which conveys the distribution of genetic variation among populations and (iii) genetic distance -which characterises the amount of genetic variation between pairs of populations (Lowe et al. 2005).

Most forest tree species possess considerable genetic variation, which is distributed among and within populations across the landscape. The high levels of genetic diversity typically found in forest tree populations are influenced by: (i) characteristics of the organism and its environment (i.e. *Population size, Gene flow, Reproduction system, Natural selection*) and (ii) genome consideration (*Mutation, Polyploidy, Linkage*) (Hamrick et al. 1992; Lowe et al. 2005; White et al. 2007). The non-continued distribution of the species within an area is the main parameter for the formation of genetically different cohorts of individuals (populations). The patterns and the degree of the genetic differentiation among populations are reflecting both the effects of past evolutionary forces and ongoing evolution in current environments. The main evolutionary forces influencing the shape and amount of genetic differentiation observed among populations are: gene flow or migration, natural selection and genetic drift (Hedrick 2005; White et al. 2007).

The evolutionary factors and their genetic variation and differentiation among populations are illustrated in Fig.1.1 from Eriksson (2005). Based on this concept, natural selection within a population is stabilising, hence resulting in increasing differentiation among populations at the species levels; while gene flow, genetic drift and inbreeding constitute the factors making up the mating pattern, thus changing the within-population differentiation.

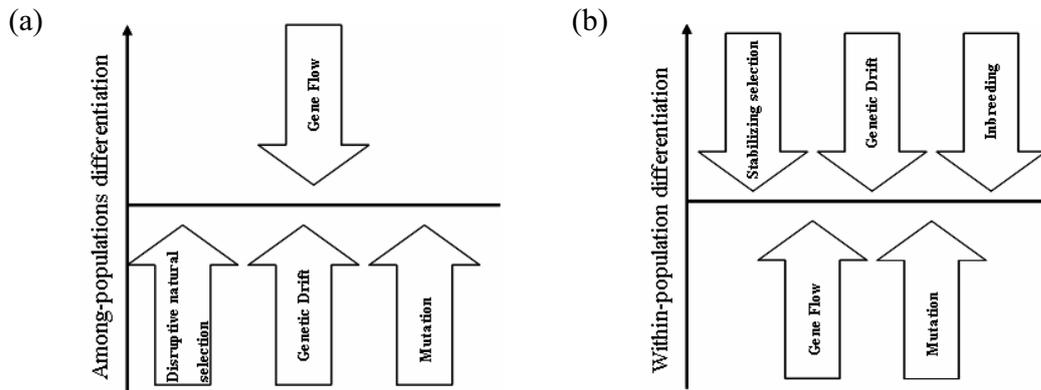


Figure 1.1: Schematic illustration of the evolutionary factors influencing (a) the genetic differentiation among populations and (b) the genetic variation within population (Eriksson 2005).

The ultimate goal of population genetics is to describe patterns of genetic diversity and to understand their origin, maintenance and evolutionary significance (Allendorf & Luikart 2007). Thus population genetics provides the framework for an understanding of the dynamics of genetic structures and/or evolution. The assessment of within-population genetic variation involves the determination of genetic multiplicity (i.e. proportion of polymorphic loci, number of alleles per locus and allelic richness) and genetic diversity (i.e. effective number of alleles, expected and observed heterozygosity) (Finkeldey & Hattermer 2007).

Population genetics is not only fundamental for understanding the evolution and adaptation of forest trees in their native habitats, but also for understanding the genetic changes caused by forest management, including breeding (Finkeldey & Hattermer 2007). In order to understand the role of selection, inbreeding, genetic drift, gene flow and mutation in population genetics, one must first be able to describe and quantify the amount of genetic variation in a population and the pattern of genetic variation among populations (Hedrick 2005). The genetic diversity of endemic species are usually credited congeners (Gitzendanner & Soltis 2000), because random genetic drift is more likely to happen in small populations following demographic bottlenecks (Young et al. 1994).

During recent years numerous molecular markers have been developed, enabling the assessment of patterns of genetic variation within and among species. Nowadays there exists a vast array of *DNA-based* genetic markers, which can be classified into two general types: (i) Those based on *DNA-DNA hybridisation* and (ii) those based on amplification of *DNA sequences* using the polymerase chain reaction (PCR) (White et al. 2007). However, molecular markers for neutral loci only reveal one aspect of a species' genetic variation, that is, the neutral variation. The other important part of genetic diversity is adaptive variation, which is currently difficult to estimate using molecular markers, although much progress has been made in environmental and functional genomics dealing with polymorphisms at adaptive loci (Allendorf & Luikart 2007; White

et al. 2007). The application of a rapidly increasing number of types of genetic markers to forest tree species confirms the insight that most long-living woody plant species are genetically variable and that the spatial and temporal dynamics of genetic information are complex (Namkoong 1989).

1.2 Spatial genetic structure

In plants, two very distinct vehicles mediate the dispersal function: the male gametophyte (*pollen*) and the young sporophyte (*seed*) (Bensch & Åkesson 2005). The distribution of genotypes over space (spatial genetic structure) and over age classes (temporal genetic structure) within a stand influences the mating system and the propensity of populations to become subdivided due to genetic drift or selection (Levin & Kerster 1974; Epperson 1993). Spatial genetic patterns can be observed within and among populations. In both cases spatial genetic patterns can be interpreted as the result of interdependence between many genetic processes of populations (i.e. microenvironmental selection, clinal selection) or capture the cumulative effects of forces acting over many generations and many populations exhibiting strong spatial patterns of genetic variation, usually in the form of positive spatial autocorrelations (Epperson 2003). Furthermore, the form and variants of the landscape mosaic and the meteorological factors (i.e. wind direction, raining season) should be taken into account when studying spatial genetic structure, since gene migration or dispersal may be more frequent along the direction created by these factors.

At the heart of spatial and space-time analyses of population genetics is the connection between observed spatial patterns and the space-time processes that generate them. The most significant key of spatial-temporal processes is genetic isolation by distance.

Spatial genetic distributions can be divided in spatial distribution of individual genotypes within a large, more or less continuously distributed population, and in spatial distribution of gene frequencies among discrete, well-delineated populations (Epperson 2003). Spatial genetic structure within a population is intimately connected with the mating system (Van Staaden et al. 1996), as well as with the measures of dispersal distances effective from the individuals' density (Wright 1943; Slatkin & Barton 1989). On the other hand, the spatial scale of geographic patterns of genetic variation among populations depends on distances of migration among populations, which in most of the cases is relative to the density of populations per unit area. Moreover, spatial scale affects data-based contrasts between patterns of selectively neutral loci and other types, such as clinal selection (Epperson 2003).

Even though the spatial genetic structure has been investigated in several organisms, it is more common in plants, since plants are immobile organisms, often showing moderate to strong spatial restriction in their dispersal of seed and/or pollen (Vekemans & Hardy

2004). Contrary to the theoretical expectation, the spatial structure in plant populations is rarely consistent across loci or sites and, when found, may be very weak (Smouse & Peakall 1999).

Spatial genetic structure can be categorised by Isolation-By-Distance (*IBD*) and spatial autocorrelation. Isolation-By-Distance (*IBD*), in the context of population genetics, is the process by which geographically restricted gene flow generates a genetic structure, because random genetic drift occurs locally: it occurs in subdivided populations, when subpopulations exchange genes at a rate dependent upon the distance, or within a continuously distributed population, when dispersal of gametes and/or zygotes is spatially restricted. Spatial autocorrelation analysis consists of a set of statistics describing how variable is autocorrelation through space. Interestingly, this method of population genetics was first illustrated by numerical simulation of a population subject to Isolation-By-Distance (*IBD*). This is a statistical procedure that has been used to identify correlations among the genotypes of mapped individuals (Heywood 1991).

Investigation of spatial population genetic structure is very important in order to understand gene transfer within the population, which may also contribute towards the understanding of the way in which populations respond to selection (Epperson 1989). Particularly, the knowledge on the genetic diversity of an endangered plant species is essential for a correct diagnosis of the status, threats, and viability of populations (Ritland 1985; Williams & Hamrick 1996). The lack of knowledge about the genetic structure of a population can lead to a biased assessment of other biological phenomena within plant populations. Furthermore, genetic structure can be important for conservation genetics since it suggests ways to maximize genetic diversity in sampled *ex situ* or conserved *in situ* populations. Observed spatial patterns may also be used to infer ancient events such as the geographical origination of genetic variants, population contractions, species range expansions, as well as recent population admixture. Ancient events may leave signatures in spatial or spatial-temporal patterns of genetic variation: such signatures may be long-lasting, but they are ultimately transient, in contrast to the stable patterns produced by selection, genetic drift and migration (Epperson 2003).

1.3 Genetic conservation

Conservation of genetic resources makes use of genetic theory and techniques in order to reduce the risk of extinction of any species or group of species with ecological and/or economical interests. The long-term goal of genetic conservation is to preserve species as dynamic entities capable of coping with environmental change. It is derived from evolutionary genetics and from the quantitative genetic theory that underlies selection breeding of domesticated plants and animals (Frankham et al. 2002). Additionally, genetic conservation is used towards the conservation of general biodiversity, as well as

towards the diversity preservation of existing population systems that are degraded by exploitation. Thus genetic conservation aims to restore damaged biosystems, while also creating new systems in appropriate areas (Altukhov 2006).

Genetic resources can be preserved through *in situ* or *ex situ* approaches. For the majority of tree species, gene conservation is achieved by *in situ* methods, in which genetic variation is maintained in populations growing in their place of origin (National Researches Council 1991). Such gene conservation typically involves managing native forests as nature reserves, although other stands produced by local seed or remnant stands may also be used to protect genetic resources (White et al. 2007). *In situ* conservation is considered “dynamic”, since it allows or even promotes adaptive changes of genetic structure, as a reaction to newly experienced or modified environmental conditions (Finkeldey & Hattermer 2007). Contrary to this, *ex situ* conservation includes all methods requiring the transfer of reproductive material to preservation sites outside the natural environment of the species. It involves holding germplasm in cold storage or *Gene Banks* (i.e. as seed, pollen, or vegetative tissues) and growing trees in plantations outside their native location. For forest trees *ex situ* conservation is conducted only when natural populations are in danger and cannot be sufficiently protected (White et al. 2007). For the preservation of *ex situ* populations, the “dynamic” conservation of genetic resources is compatible with breeding. Breeding and propagation populations are selected for their own superior phenotype or that of their progeny; they should not be considered genetic resources for the preservation of the evolutionary adaptability of a species (Finkeldey & Hattermer 2007).

During the procedure of both *in situ* and the *ex situ* conservation, a specific genetic conservation strategy should be applied. In order to lead to effective conservation, such strategy must include defining priorities, identifying clear objectives, selecting genetic resources, choosing method of physical preservation of genetic information and regenerating the resources (Finkeldey & Hattermer 2007).

1.4 The genus *Cedrus*

As cited by Christou (1997) “The exceptional ability of *Cedrus* to grow on a wide range of soils, to withstand extreme climatic conditions and its resistance to pests have been reported by a number of authors. In addition, the hardness and quality of its wood and its aesthetic value make it one of the most promising genera for the rehabilitation of non productive or degraded forests in the mountainous Mediterranean basin”.

The genus *Cedrus* is one of the eleven commonly accepted genera in *Pinaceae* (Farjón 2001), with a wide but discontinuous distribution in the old world during pre-Quaternary time, and a highly geographic disjunctive distribution in the circum-Mediterranean and west Himalayan (Farjón 1990, 2001; Pons 1998). The cedar is represented by four closely