1. General introduction

1.1 Genetic diversity in foundation species

Genetic diversity is important for the evolutionary changes and adaptation (Amos and Harwood, 1998; Lundqvist *et al.*, 2008). This becomes even more important for the survival and maintenance of the populations and species considering environmental change (Boshier and Amaral, 2004). Populations identical in genetic composition may not survive if drastic change in the environment occurs. Genetic diversity ensures the population fitness and minimizes the risk of extinction (Vellend and Geber, 2005). Populations and species with low genetic diversity are more susceptible to infection and severe disease outbreaks, ultimately put them in the verge of extinction (Elton, 1958; Schmid-Hempel, 1998). Therefore, the retention of high levels of genetic diversity is an essential element for the stability of populations and ecosystems.

A high level of genetic diversity in a population may increase the productivity and many other economic benefits. Higher level of productivity and resistance against the adverse environment was reported in genetically diverse populations (Hughes and Stachowicz, 2004; Reusch *et al.*, 2005; Crutsinger *et al.*, 2006). Further, genetic diversity is the basis for tree improvement and breeding (Lundqvist *et al.*, 2008). Genetic variability containing the alleles responsible for important traits enables to develop new varieties of economic importance. Conservation of genetic diversity is essential to open new windows for many possible future uses (Boshier and Amaral, 2004).

The maintenance of genetic diversity of foundation species is even more important (Glaubitz *et al.*, 2002; Wimp *et al.*, 2004). Foundation species are locally abundant and regionally common which provides habitat for a large number of associated species by maintaining important ecosystem processes (Ellison *et al.*, 2005; Ellison *et al.*, 2010). Therefore, the genetic structure and diversity of the foundation species has community consequences and important roles for maintaining biodiversity (Wimp *et al.*, 2004; Bangert *et al.*, 2005; Bangert *et al.*, 2008). Genetic variation in a foundation tree species may produce habitat variation for dependent communities which in turn can assist increasing species richness (Vellend, 2005; Vellend and Geber, 2005; Shuster *et al.*, 2006). For instance, Wimp *et al.* (2004) reported that plant genetic diversity in cottonwood (*Populus* spp.) accounted for nearly 60% of the arthropod diversity. Some arthropods and herbivores are restricted to a subset of genotypes in host plant

(Bangert *et al.*, 2005; Wimp *et al.*, 2005). Therefore, the genetic diversity within the foundation species can be considered analogous to species diversity within a functional group (Hughes and Stachowicz, 2004).

The genetic variation of dominant tree species in temperate and boreal regions is more important than in other regions of the world because of the monospecific dominance in large areas (Gömöry *et al.*, 2010). European beech (*Fagus sylvatica*) is the foundation tree species of deciduous temperate forest in Germany and in other Central European countries. Therefore, the conservation of the genetic diversity and important genes / alleles in this species is not only crucial for its adaptation and survival but also has positive community consequences.

1.2 Factors responsible for genetic variation

Various evolutionary factors and their interactions shape the genetic variation within and among populations (Lacy, 1987; Hamrick *et al.*, 1992). Gene flow, mutation, population size of the species and their spatial distribution can influence the genetic variability of the population and the species (Amos and Harwood, 1998). Gene flow via seeds and pollen and mutation increase the genetic variability within population (Amos and Harwood, 1998). However, the mutations are rare events and estimated to be in the range of 10^{-4} to 10^{-8} for most of the genes in higher organisms (Hartl and Clark, 1989; Drake *et al.*, 1998; Finkeldey and Hattemer, 2007). Therefore, its role to influence the genetic diversity and differentiation is not extensive in general. Random genetic drifts due to very small population size and natural selection decrease the within population variation but increase genetic differentiation among populations (Lacy, 1987; Amos and Harwood, 1998). Environmental factors precipitation, temperature, humidity, wind flow as well as site-specific soil characteristics and management activities etc. influence the mating systems, gene flow, adaptation and natural selection, which ultimately may affect the genetic diversity, differentiation and spatial genetic structure of the stands. Detail knowledge about the genetic diversity and spatial genetic structure provide the opportunity to understand the evolutionary factors operating in the populations and species (Hamrick *et al.*, 1992; Valbuena-Carabana *et al.*, 2007).

1.3 Management impact on genetic diversity and structure

Forest management is the deliberate human intervention, in general through the implementation of various silvicultural practices in order to improve the productivity of forest goods and services to achieve the objectives of the owner and society (Bettinger *et al.*, 2009). Thinning and harvesting activities followed by the regeneration of the harvested stands may a) reduce the number of seeders, b) enhance or decrease reproductive isolation, c) facilitate migration of genetic material and d) alter site-specific environment (Finkeldey and Ziehe, 2004; Buiteveld *et al.*, 2007). All of these factors, individually or collectively, may influence the genetic diversity and the structure of a stand (Hosius *et al.*, 2006; Buiteveld *et al.*, 2007; Konnert and Hosius, 2010). Many studies found management impacts on reducing genetic diversity and spatial genetic structure of the stands (Young *et al.*, 1996; Buchert *et al.*, 1997; Hussendörfer and Konnert, 2000; Takahashi *et al.*, 2000; Dounavi *et al.*, 2002; Jolivet and Degen, 2011). Hussendörfer and Konnert (2000) reported that selective felling have reduced the genetic variation in beech populations in Germany. Similarly, based on isozyme study Dounavi *et al.* (2002) found the reduction of average number of alleles per locus, genetic diversity and differentiation in the thinned compared to untreated beech plots. Contrarily, there are other studies reporting the absence of any management impact on the genetic diversity (Wickneswari *et al.*, 2004; Buiteveld *et al.*, 2007; Pospíšková and Dostálek, 2009). Buiteveld *et al.* (2007) investigated the beech stands with four microsatellite markers and reported no reduction in any of the genetic diversity parameters such as observed and expected heterozygosity, allelic richness, number of rare alleles and effective number of alleles due to the shelterwood forest management. However, most of the aforementioned studies constrained with the small number of sample sizes and genetic markers and the lack of the sufficient replication. This study with the large number of sample size using microsatellite and AFLP markers and enough replications definitely provides better understanding about the management impacts on the genetic diversity and structure of the beech stands. The information about the management impacts on the genetic diversity and structure of the stands provides better opportunities to plan and implement sustainable utilization of the forests and genetic resources.

1.3.1 Forest management systems in Germany

Forest management system has long tradition in Germany to improve the productivity and to assure sustainability (Konnert and Hosius, 2010). Until the 19th century, German forests were generally managed as coppice forests and coppice with standards in order to produce mainly fuelwood and small timber (Häusler and Scherer-Lorenzen, 2001; Wäldchen *et al.*, 2011). Rotation periods were short (20 to 30 years) for fuelwood and relatively long (~100 years) for timber production (Häusler and Scherer-Lorenzen, 2001; Hessenmöller *et al.*, 2011). However, these systems were replaced long time ago by high forest management systems (Häusler and Scherer-Lorenzen, 2001). For instance, the conversion of coppice with standards forests into age class and selection forests were made in Central Germany during the 19th century in order to increase the timber production (Wäldchen *et al.*, 2011).

The government imposes very strict legislations for the sustainable and multifunctional management of the forests (Hagner, 1999; Häusler and Scherer-Lorenzen, 2001). Clear cutting is restricted by law (Häusler and Scherer-Lorenzen, 2001). The production, marketing, import export and proper identification of forest reproductive materials are well regulated by the law in Germany.

Management activities and anthropogenic disturbance might influence the genetic structure of the forests but the impact of forest management and human disturbances on the genetic diversity and structure has not been sufficiently studied despite its implicit role in sustainable and most productive management of the forest and forestry resources.

1.3.1.1 Age class forests

Age class forests are the predominant types of managed beech forests in Germany. Shelterwood system is the most frequent silvicultural system to establish age class beech forests. In this system, the regeneration is initiated and supported by the removal of mature trees in two or more successive cuttings, at the rotation periods of 100 to 140 years (Mund, 2004; Fischer *et al.*, 2010; Hessenmöller *et al.*, 2011). The harvested stands used to be established by natural regeneration from a single or few mast years promoting the even aged structure of the compartment (Müller, 1990). Forests managed by this system provide a high economic return but may lose many natural characteristics (Hagner, 1999).

Age class beech forests are regularly thinned every 5 to 10 years from the early stage after its establishment (Mund, 2004). About 50% of the forest growth used to be extracted during thinning operations from beech stands (Hessenmöller *et al.*, 2011) which may influence the genetic diversity and spatial genetic structure of the current stands and future generations.

1.3.1.2 Selection cutting forests (Plenterwald)

About 1.4% of German forests are managed under selection cutting system (Anonymous, 1996). The main objective of this management system is to create, conserve and utilize the forest stands of uneven ages and high structural diversity (Hagner, 1999). In selection cutting forest, individual trees or small groups of trees are harvested when they attain the target diameter size (TMLFUN, 2004). For instance, the state department of Thuringia, Central Germany has fixed the target diameter (≥60 cm dbh) for the beech stands. The selection cutting cycle is about 5-10 years in Hainich region (Mund, 2004).

This system can be applied in forest stands containing shade tolerant tree species, but cannot be used as such for light demanding pioneer tree species (Hagner, 1999). These forests are close to unmanaged forests and retain high structural diversity, composed of different heights, diameter classes and ages of the trees growing together in a small area of less than 0.1 ha (Hagner, 1999; Boncina, 2011). In this management type, genetic variability and structure were not much affected due to the removal of a limited number of old trees (Müller, 1990). However, it exhibits more pronounced family structure and preferential mating among relatives compared to age class forest (Finkeldey, 2002; Finkeldey and Ziehe, 2004).

1.3.1.3 Unmanaged / natural forests

Most of the European forests have been highly influenced by human activities in the course of time. Only about 0.3 million hectare forest, 0.4% of the total forest area, is estimated to be virgin in Europe (Parviainen, 2005). It is very difficult to find untouched or virgin forest. Therefore, the forest stands which have not been harvested or carried out any silvicultural operations for more than last 60 years were categorized as currently unmanaged forests (Fischer *et al.*, 2010) and taken as the reference in this study in order to investigate management impacts. Most of these unmanaged forests are located in national parks and biosphere reserves.

Unmanaged forests are natural old growth uneven-aged forest (0-250 years), characterized by the occurrence of a large number of aged trees (over 100 years), multiple height strata and the presence of dead and fallen trees. In natural forests, the genetic diversity and structure are mainly the result of long-term evolutionary processes, population histories and natural selection.

1.4 Genetic variation and DNA markers

DNA markers have been widely used for the assessment of genetic diversity in various plant and animal species with the aim to identify their status, risk and utilization possibilities, to design conservation strategies, management practices and marker-assisted breeding programs (Hattemer, 1995; Geburek, 1997). Based on their inherent characteristics, DNA markers are different owing to the investment of time and costs, amount of required DNA, levels of polymorphism and their statistical power (Garcia *et al.*, 2004; Nybom, 2004).

There are two types of markers namely neutral and adaptive markers. Neutral and anonymous markers such as microsatellite (genomic SSR) and Amplified Fragment Length Polymorphisms (AFLP) and potentially adaptive, genic SSR (EST-SSR) markers were combined in this study. To analyze the management impacts on the genetic diversity and spatial genetic structure independent of marker system, AFLP markers were used additionally to a subset of experimental plots and trees. The genetic diversity assessed with neutral genetic markers, is in general not directly influenced with fitness and adaptation (Gomez-Mestre and Tejedo, 2004; Holderegger et al., 2006). The relationship between 'neutral' genetic diversity, diversity in genes of adaptive significance, and variation patterns at adaptive traits are not simple. However, the neutral genetic markers has the great potential to infere stochastic processes such as to estimate the gene flow, migration and dispersal (Holderegger et al., 2006). The important inherent properties of the different marker systems have been described in following sections.

1.4.1 Microsatellite markers

1.4.1.1 Neutral microsatellite markers

Microsatellites or simple sequence repeats (SSR) are short (1-6 bp) stretches of tandemly repeated DNA sequence motifs present in all prokaryote and eukaryote genomes (Field and Wills, 1996; Hancock, 1999; Weising *et al.*, 2005). They are highly polymorphic, abundant, codominantly inherited and locus-specific (Powell *et al.*, 1996; Li *et al.*, 2004; Schlötterer, 2004). Due to these properties, microsatellite markers have been one of the most popular genetic markers in paternity analysis, genetic mapping, population genetics and forensics (Powell *et al.*, 1996; Goldstein and Schlötterer, 1999).

Despite many advantageous properties, microsatellite markers have also some disadvantages, for instance the presence of null alleles, stutter bands, high mutation rates and homoplasy (Schlötterer, 2004). *De novo* development of microsatellite markers requires sequence information of the species and is expensive and time-consuming (Gerber *et al.*, 2000; Pandey *et al.*, 2004; Mottura *et al.*, 2005; Gasic *et al.*, 2009). The cross-transferability of microsatellite markers is limited to closely related species (Ekue *et al.*, 2009; Pandey and Geburek, 2009).

1.4.1.2 EST-SSR markers

Expressed sequence tags (ESTs) are short; usually 300-500 bp, single pass complementary DNA sequences generated from randomly selected library clones (Liu *et al.*, 1999; NCBI, 2011). EST-SSRs are derived from genic regions, and thus might be affected by selection and are very often linked to a known function (Liu *et al.*, 1999; Woodhead *et al.*, 2005; Pashley *et al.*, 2006). Only a small fraction of ESTs (2%- 5% in plant) contains SSRs (Kantety *et al.*, 2002; Pashley *et al.*, 2006). About 70 million EST sequences from over 2200 taxa are already available in the NCBI database (NCBI, 2011). The utilization of public databases demands low investment and less time to develop EST primers (Squirrell *et al.*, 2003; Thiel *et al.*, 2003; Ellis and Burke, 2007). Therefore, it provides immense opportunities to execute genetic studies even for understudied taxa.

These markers are highly transferable across species within genera, among genera and sometimes among families (Ellis and Burke, 2007; Gasic *et al.*, 2009; Durand *et al.*, 2010). Pashley *et al.* (2006) reported that EST-SSRs are three times more transferable across species compared to SSRs. For instance, the transferability of neutral microsatellite markers between *Castanea*, *Fagus* and *Quercus*, the most studied genera in the Fagaceae family, was low whereas it was comparatively high in EST-SSR markers (Kremer *et al.*, 2007; Durand *et al.*, 2010). Fewer amounts of null alleles, stutter bands and homoplasy were reported with EST-SSRs compared to SSRs (Rungis *et al.*, 2004; Woodhead *et al.*, 2005). A few disadvantages of the markers are also reported. EST-SSRs are located within genes, conserved regions, and are hence less polymorphic than SSRs (Thiel *et al.*, 2003; Pashley *et al.*, 2006). Selection in these loci may affect the estimation of genetic diversity (Pashley *et al.*, 2006).

1.4.2 Amplified fragment length polymorphism (AFLP)

AFLP is a multilocus DNA fingerprinting technique (Vos *et al.*, 1995; Krauss, 2000) which can be used to analyze the DNA of any origin and complexity (Blears *et al.*, 1998). A large number of loci randomly distributed within the entire genome can be screened in individuals to prepare a robust, reliable and highly reproducible DNA fingerprint (Vos *et al.*, 1995; Mariette *et al.*, 2002; Weising *et al.*, 2005; Meudt and Clarke, 2007). AFLP markers are comparatively easy and inexpensive to produce as they do not require previous sequence information (Blears *et al.*, 1998; Weising *et al.*, 2005). A large number of markers can be prepared by simply changing the restriction enzyme and the selective nucleotides (Blears *et al.*, 1998).

Dominant and biallelic characteristics, low polymorphisms and cumbersome tasks to score fragments are some of the limitations in the AFLP technique. Because of a large number of polymorphic loci covering the entire genome, AFLP markers may counterbalance the information loss due to the dominant characteristics of the markers (Gerber *et al.*, 2000).

The AFLP technique has been used to estimate genetic variability (Harada *et al.*, 2000; Papageorgiou *et al.*, 2008), spatial genetic structure (Jump and Peñuelas, 2007), phylogeny (Gailing and von Wuehlisch, 2004) and genetic mapping (Scalfi *et al.*, 2004) of *Fagus* spp. The applications of AFLP markers is constantly growing (Schlötterer, 2004) and has been used in number of applications (Chauhan *et al.*, 2004; Hu *et al.*, 2005; Nuroniah *et al.*, 2010).

1.5 Objectives and research hypotheses

The overall aim of this study was to assess the spatial dynamics of intraspecific genetic variation of European beech (*Fagus sylvatica* L.) foussing on the genetic differentiation at varied spatial scales. Large-scale patterns of genetic differentiation among plots from different regions allow to understand the post-glacial colonization history of the populations and local adaptation. Medium-scale patterns of genetic differentiation among plots within exploratory reflect about the site-specific selection and differences in management types and intensities. Fine-scale spatial genetic structures within plot provide information about the microenvironment selection, demography and management activities.

Forest management, notably the thinning and harvesting activities, is often criticized by nature conservationists. The key concerns are related to biodiversity issues and genetics. Thus, a negative impact of silvicultural measures on genetic diversity is suspected. However, only few studies are available which have comprehensively tested this supposition. In this context, the main objective of the study was to assess the natural and human impacts on the genetic diversity and structure of the adult beech stands at different spatial scales. Following specific objectives and hypotheses were set for this study.

1.5.1 Objectives

Specific objectives of the study were:

- To assess the genetic diversity and differentiation of *F. sylvatica* at the different spatial scales,
- To estimate the levels and magnitudes of the fine-scale spatial genetic structure in beech stands,
- To assess the impact of forest management on the genetic diversity and fine-scale spatial genetic structures of *F. sylvatica* independent of the marker systems,
- To compare microsatellite and AFLP markers to assess the genetic diversity and genetic structures of European beech.

1.5.2 Hypotheses

Following fundamental hypotheses were tested in this study:

- The recolonization history of *Fagus sylvatica* in the study area goes back to the beech populations that sheltered in the refugial areas mainly in the Eastern Alp-Slovenia-Istria during the climatic extremes of last glaciations (Magri *et al.*, 2006; Magri, 2008). The absence of significant genetic differentiation among beech stands and among widely separated geographic regions (exploratories) was expected due to the common origin of the post-glacial remigration of beech populations,
- Studied beech stands were very heterogeneous in their demographic and ecological characteristics, which may have direct influence on gene flow and mating system affecting the spatial genetic structures within populations. Restricted seed dispersal and the varied demographic characteristics of the beech stands allow us to expect the presence of significant spatial genetic structure within studied beech stands and the occurrence of highly variable fine-scale genetic structure across beech stands,
- Absence of the effect of forest management activities on the genetic diversity, differentiation, and fine-scale genetic structure of the studied beech stands.