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Natural regeneration of *Fagus sylvatica* L. adapts with maturation to warmer and drier microclimatic conditions

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ABSTRACT

Due to its drought sensitivity, the performance and competitiveness of beech as a favoured species of forest management in Central Europe is likely to be negatively affected by the prognosticated climate change, leading to major impacts on the vulnerability of managed forest ecosystems. We studied the genetic differentiation between two populations from a relatively cold and wet northeast (representing the current climate of the majority of beech forests in Central Europe) and a relatively warm and dry southwest facing slope (representing the future climate of an increasing area covered by beech forests in Central Europe) at the same forest site to investigate the adaptation processes in these two populations under different microclimatic conditions. For this purpose, two different techniques, *i.e.*, nuclear microsatellites (neutral) and isozyme markers (adaptive), were applied to adult trees and natural regeneration at both slopes. Although microsatellites are considered to be neutral markers, they have been shown in several studies to give signals of selectively-driven changes. In our study, two of the five microsatellites behaved as "outlier loci", exhibiting directional selection. Our results show independent of the technique applied that natural regeneration of the southwest slope and the natural regeneration and adult trees of the northeast slope were genetically closer than the adult trees from the southwest slope. Thus, we conclude that natural selection and potential adaptation account for genetic changes and different genetic structures among the two adult populations in this case study.

1. Introduction

European beech (Fag*us sylvatica* L.) constitutes the dominant tree species of the potential natural vegetation in moist to moderately dry areas of the sub-mountainous altitude range in Central Europe under the current climate (Ellenberg, 1996), and is only rarely found in habitats with warm and dry climatic conditions. As a tree species sensitive to strong and prolonged periods of drought (Backes and Leuschner, 2000; Rennenberg et al., 2004; Gessler et al., 2007), the performance and competitiveness of beech could be negatively affected (*e.g.* Fotelli et al., 2003; Gessler et al., 2007) by the prognosticated climate changes (*i.e.*, a rise in temperature as well as an increase in frequency and duration of summer droughts (IPCC, 2007)).

In Central Europe, forest management practices have been altered by forest practitioners and governments during the past decades from the preference of conifer monocultures to nowadays supporting mixed species stands, promoting the natural regeneration of deciduous tree species in general (Tarp et al., 2000; Petritan et al., 2009; Simon et al., 2010), and of beech in particular. However, as a consequence of climate change, the anticipated regional warming and reduced water availability are predicted to impose considerable impact on the ecological performance and fitness of forest ecosystems in Central Europe (IPCC, 2007), especially on tree growth and development. Thus, major reorganisation of managed forest ecosystems appears to be required in this part of Europe to ensure sustainable forest growth and development. For this purpose, suitable populations have to be identified and selected in tree breeding programmes for use in future afforestation.

Previous isozyme studies have shown significant genetic differences among beech populations of different elevations (Lochelt and Franke, 1995; Sander et al., 2000). These results were confirmed in studies of several tree species by using molecular genetic

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markers (Ohsawa and Ide, 2008). In this context, allele frequency differences at microsatellite loci revealed divergent selection in cork oak populations along a temperature cline (Ramirez-Valiente et al., 2010). Given that elevation gradients present many different site conditions, the impact of environmental factors on genetic structures is not evident. On the other hand, under the assumption of the same origin, a high genetic differentiation between populations from southwest and northeast exposed aspects at the same site would be an indication of different microclimatic effects and adaptation processes among the two populations. In this case, the influence of drought on the genetic structures of south-east exposed populations will provide important selection markers for future afforestation in the face of global climate change. Therefore, this study aimed to investigate the genetic structure of two natural populations of beech including adult trees and their natural regeneration at two opposite-facing slopes in a case study in southern Germany. The relatively cold and wet northeast represents the current climate of the majority of beech forests in Central Europe, whereas the relatively warm and dry southwest facing slope was considered to represent the future climate of an increasing area covered by beech forests in Central Europe (Gessler et al., 2007).

2. Materials and methods

2.1. Study area

The study area is located in a low mountain range in southwestern Germany, the Swabian Alb. Beech populations including adult trees and natural regeneration from two opposite-exposed slopes (NE and SW aspect) of a narrow valley were included in this study at the research station in Tuttlingen-Möhringen (47°58′43″N, 08°44′53″E). Elevation is 800 m and 740–780 m a.s.l. for the NE and SW aspect, respectively, both with a slope of 23–30°. At both sites, the soil type is a rendzic leptosol (IUSS Working Group, 2006) with a yearly N deposition below 10 kg ha^{-1} (see Gessler et al., 2005 for further detail on the field sites). *F. sylvatica* L. is the dominant species (>90% of total basal area of adult trees) with an average age of 77–87 years at both sites (Gessler et al., 2005). Each site was equipped with two forest meteorological research towers (1.5 hg). Air temperature data were measured above the canopy (1.3 hg) by use of Vaisala humicaps (HMP 45D, Vaisala, Helsinki, Finland). Precipitation was continuously recorded by the use of tipping buckets (ARG100, Campbell Sci., Shepshed, GB) on the top of two forest meteorological research towers. All data were registered every 30 s by Campbell data logggers (CR 23x, Campbell Sci., Shepshed, GB) from 2001 to 2009. Mean air temperature (Jan.–Dec.) was with 6.9 °C considerably lower at the NE slope, compared to 7.7 °C at the SW slope. However, mean air temperature during the vegetation period did not differ between the two aspects (May–Sep.: 14.4 °C). Total annual precipitation (Jan.–Dec.) as well as precipitation during the vegetation period (May–Sep.) was higher at the SW slope (796/386 mm at the NE slope compared to 1013/538 mm at the SW slope, respectively). These differences in above canopy precipitation sums are mostly caused by the orographic situation, with the SW aspect being oriented windwards during the prevailing wind direction and the NE aspect lying in the wind shade of the slope. At the canopy level, water supply was lower at the SW slope compared to the NW slope despite higher annual precipitation. This was due to the difference in radiation interception between the two aspects resulting in higher air and soil temperatures as well as a lower water supply for the forest stands on the SW slope (Gessler et al., 2001, 2005; Keitel et al., 2003). Retrospective analysis of meteorological data as well as retrospective analyses of the water status and growth of adult beech trees showed a continuously lower water availability and higher air temperature at the SW site compared to the NE site for several decades (Gessler et al., 2001, 2005).

2.2. Sample collection

To avoid sampling members of the same family, trees were selected randomly with a minimum distance of at least 50 m to next tree individuals in each subpopulation at both slopes. Since gene flow in beech populations via seed is mainly limited to a distance of 50 m (Müller-Starck, 1996), distinct family structures should be observed due to restricted pollen and seed dispersal (Dounavi et al., 2010). For each slope, fresh leaves were collected from 80 adult trees and 100 seedlings of the natural regeneration and used for microsatellite analysis. In addition, winter buds collected from the same number of individuals per population and tree age were used for isozyme analysis.

2.3. Isozyme study

To determine the genetic structure, 10 enzyme systems (*i.e.*, AAT = aspartate-aminotransferase, ACO = aconitase, IDH = isocitrate dehydrogenase, MNR = menadione reductase, MDH = malate dehydrogenase, PGI = phosphoglucose isomerise, PGM = phosphoglucomutase, PEX = peroxidase, 6PGDH = 6-phosphogluconate dehydrogenase, SKDH = Shikimate dehydrogenase), with 16 gene loci were investigated: AAT-A, AAT-B, ACO-A, ACO-B, IDH-A, MNR-A, MDH-A, MDH-B, MDH-C, PGI-B, PGM-A, PEX-B, 6-PGDH-A, 6-PGDH-B; 6-PGDH-C, SKDH-A. The technical procedure and the genetic interpretation of zymograms followed Müller-Starck and Starke (1993) as well as Müller-Starck (1993). The degree of genetic variation within and between populations was quantified using various genetic parameters (*e.g.* Hattemer, 1991) such as genetic multiplicity (A/L), genetic diversity (n_e), hypothetical multilocus diversity (v_{gam}) as a measure of the potential to create different gametes within a population (Gregorius, 1978), heterozygosity (H_o), F_{IS}-value (Wright, 1978), genetic distance (D) (Gregorius, 1974) and subpopulation differentiation (D_j) (Gregorius and Roberds, 1986). The differentiation among populations was calculated by subpopulation differentiation measures (D_j) as the genetic distance between each population and its complement (all other populations). The computation was carried out with the assistance of the specific SAS macro MACGEN (Stauber and Hertel, 1997, 1999: personal communication). The significance of observed differences was tested using the Chi^2-test and the exact test according to Fisher ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). These classical measures were used in order to compare our study with other isozyme studies in beech (Konnert, 1995; Lochelt and Franke, 1995; Sander et al., 2000; Konnert and Ruetz, 2001). In addition, to compare the isozyme results with the microsatellite data, the software GenAlEx 6.1 (Peakall and Smouse, 2006) and FSTAT (Goudet, 1995) were used to calculate genetic diversity and genetic distance measures (Tables 3 and 4).

2.4. Nuclear microsatellite analysis

Genomic DNA was extracted from fresh leaves using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Five microsatellite loci described by Pastorelli et al. (2003) and Tanaka et al. (1999) were chosen for this study: FS1_25, FS1_03, FS3_04, FS4_46, and FCM_5. The Mendelian inheritance was confirmed for all five microsatellites. Furthermore, they showed high levels of polymorphism in various *Fagus* sp. (Pastorelli et al., 2003). Multiplex PCR amplifications were performed using fluorescent labelled primers in a multiplex mixture containing 2 μL of pure DNA, 5 μL of Mastermix (2×), 1 μL Primermix (2 μM from each primer) and 2 μL distilled H_2O. One Multiplex PCR reaction contained the microsatellites FS1_25, FS3_04, and FS1_03 with an annealing temperature of 65 °C, a second multiplex comprised the microsatellites FCM_5 and FS4_46 with an annealing temperature of 60 °C. After an initial