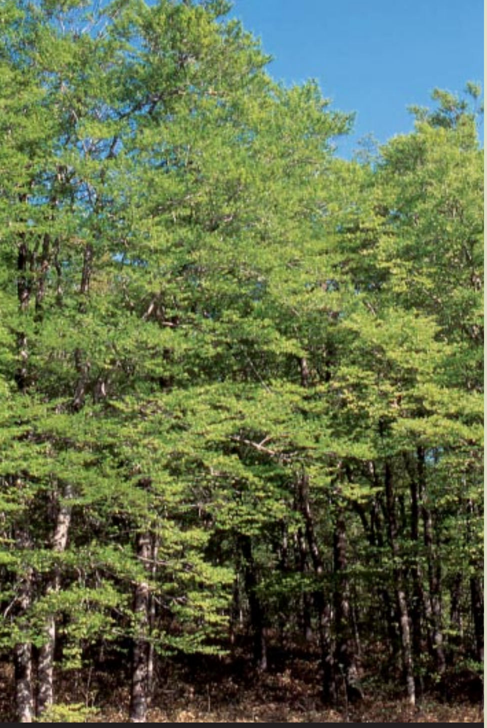

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PREFACE

The 8th IUFRO (International Union of Forest Research Organizations) Beech Symposium was held during September 8 – 13th, 2008, at the Onuma Seminar House in Nanae town located at the southern part of Hokkaido, northern Japan, including two field excursions to the northern edge of natural distribution of *Fagus crenata* and the pristine beech forests in the UNESCO World Heritage Site "Shirakami-Sanchi".

The aims of this scientific meeting were to exchange and share state-of-the-art knowledge on the ecology and silviculture of beech (*Fagus*) forests among researchers and forest managers worldwide, and to reflect the current scientific achievements on the future directions for sustainable management of beech forests in changing social needs and global climate.

In addition, this conference was the first-ever IUFRO Beech Symposium held in East Asia which holds an important part of the entire distribution range of *Fagus* and a number of beech species. Thus, the conference provided excellent opportunities for the participants to discuss both similarities and differences in ecology, genetics and management among *Fagus* species.

Ninety-one participants from 16 countries attended the conference, including young researchers and students. In the 3-day technical sessions, 6 keynotes, 35 volunteer oral papers and 26 posters were presented. Through an array of relevant presentations and discussion, the participants shared the current scientific progress and future challenges in understanding beech populations from a broader perspective in terms of both time and space. On the time axis, the presented topics ranged from the migration history of beech populations into the Holocene landscape to their potential responses to future climate changes predicted over 100 years. The technical sessions also covered wide range of topics on the spatial scale: experimental, modeling and field studies of an individual tree and on a stand/landscape level, population dynamics of beech on regional or continental scale, as well as genetic structure and diversity of beech populations revealed by field trials and analyses of genetic markers.

These proceedings were published as a collection of the extended abstracts of papers presented at the conference. The proceedings include all of the papers which had been approved for presentation by the Scientific Committee in order to appreciate the significant contributions of the authors, although some papers were unfortunately not presented at the conference on site due to justified absence of the authors.

Last but not least, we thank all of the people who participated in the conference, with our special gratitude to the members of the Scientific Committee, keynote speakers and moderators of the technical sessions, and the local hosts of the field excursions. We also express our sincere thanks to all of the people and organizations who sponsored and supported the conference.

Editors

Kazuhiko Terazawa
Palle Madsen
Khosro Sagheb-Talebi

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Extended abstracts

Authors' names denoted by the following symbols indicate,

* : corresponding authors,

__ (underline), presenters at the conference.

THE HOLOCENE HISTORY OF *FAGUS*

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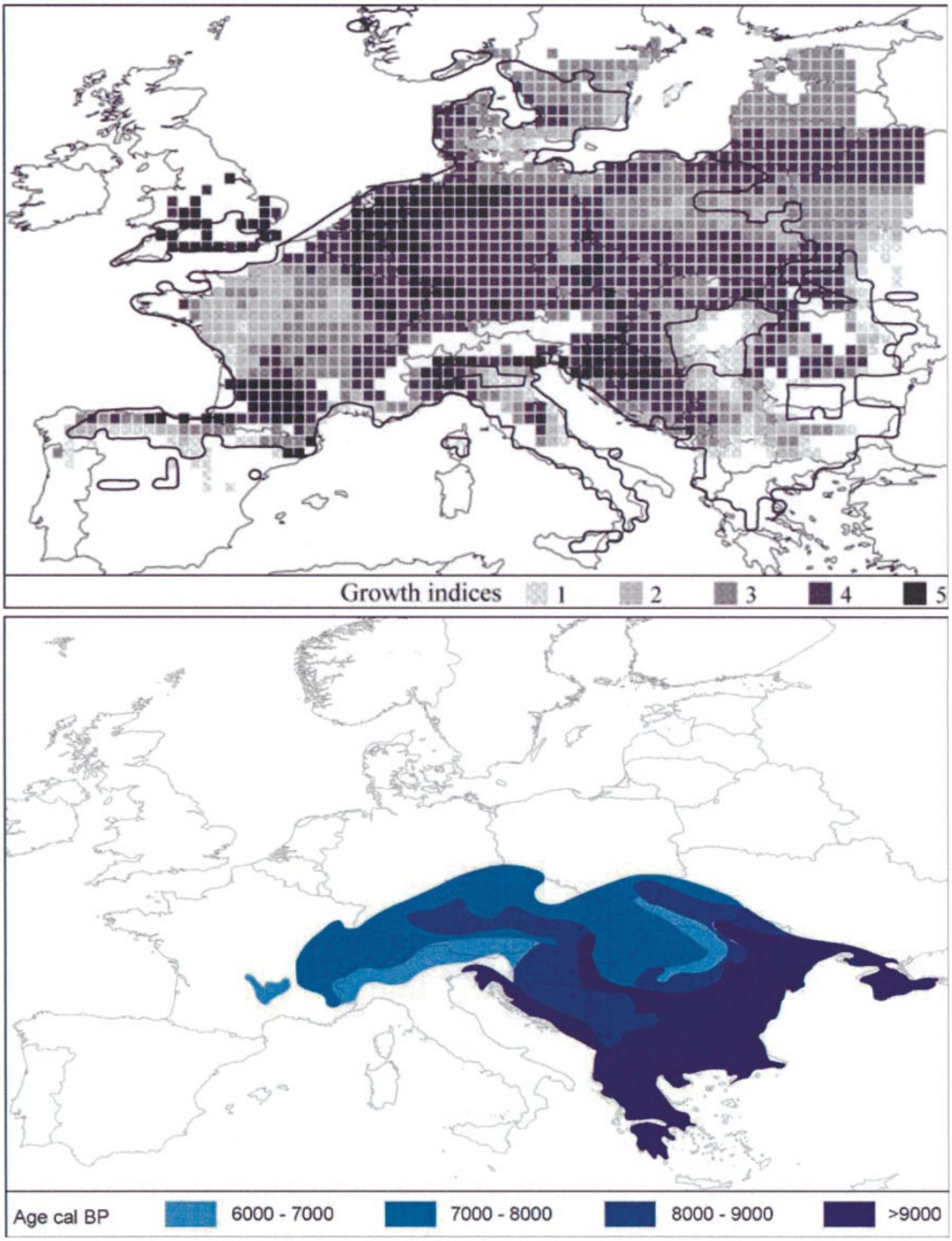
Fagus are widespread trees of widespread ecological importance in N.E. USA, Europe, China and Japan. Following the last ice age, pollen data suggest that *Fagus* populations spread northwards in all three geographic regions in response to changing climate. It can be assumed that nearly all *Fagus* pollen from European Holocene deposits belongs to *F. sylvatica* and its spread can be reconstructed from the comparison of pollen records. The tree has a significant presence in the catchment of a lake after the onset of the continuous pollen curve, while macrofossils of *F. sylvatica* have been found coincident with the first pollen records from small forest hollows in southern Sweden. Although the detection of small populations is difficult using pollen analyses and depends on site characteristics and the number of pollen grains counted, pollen analysis serves as a robust tool to reconstruct the population expansion of *Fagus*. European-scale reconstructions of the Holocene spread of *F. sylvatica* have been derived from more than 1700 pollen diagrams by Gliemeroth (1995). Regional mapping the threshold of 1% total terrestrial pollen indicates the time when small populations of *F. sylvatica* expanded and coalesced, although the local establishment of the tree may have occurred hundreds or thousands of years earlier. Compared with other major European trees, *F. sylvatica* is the last to start a Holocene spread at about 9000 years ago. This spread did not occur in a continuous manner in all directions, but showed 1000-year long delays in different regions and at different times. A prominent feature of the *Fagus* curve in many pollen diagrams is the long tail with a regular but often discontinuous occurrence of pollen grains before a sustained rise to great abundance. Discontinuous tails can often be traced back to about 8000 years ago suggesting that small isolated populations could have occurred long before the tree became abundant. This suggests that the initial spread occurred at low population densities. The later population expansion occurred in a regionally-organized manner. Populations expanded first at south-eastern and northern sites in the Alps, beginning in the mountains of Slovenia. Populations in the south-western Alps generally expanded a few thousand years later and often only after 6000 years ago. During the second half of the Holocene the population expansion in the northern lowlands of central Europe seems to follow a wave-like pattern, while the populations in the Bulgarian and Romanian mountains increased from small populations during the same time period. The late population expansion in the Bulgarian mountains is of special interest as the tree is thought to have occurred there during the late glacial period.

The European distribution of *Fagus* 6000 years ago was simulated using a physiologically-based bioclimatic model driven by three different atmospheric general circulation model (AGCM) outputs. The three simulations generally showed *F. sylvatica* to have potentially been as widespread 6000 years ago as it is today, which gave a profound mismatch with pollen-based reconstructions of the *F. sylvatica* distribution at that time. The mismatch between the modelled and reconstructed European distributions of *F. sylvatica* at 6000 years ago has a number of possible explanations.

Figure 1: a Simulation of the *Fagus sylvatica* distribution based on the ECHAM3 AGCM output of climate parameters 6000 years ago. The modern distribution of *F. sylvatica* according to the Atlas Florae Europaeae is shown for comparison (bold black line). b Interpretation of the spread of *F. sylvatica* based on the time that the 1% threshold was reached in selected pollen diagrams (after Gliemeroth 1995).



Our analysis of 6000 years ago has relevance for the entire Holocene history of *F. sylvatica* and shows that no single driving force or climatic parameter can adequately account for the observed changes in abundance and distributional limits. The established sensitivity of the distributional limits to drought and late frosts suggest that more explicit modelling of these climatic parameters would improve data–model matches. It is likely that both the mean and the variability of climate parameters were different 6000 years ago. Therefore, scenarios including different frequencies of weather events, such as late frosts or droughts, might improve our ability to model past vegetation.

In North America, *Fagus grandifolia* apparently spread from refugia in the southeast beginning around 14000 years ago: much earlier than in Europe. Most of its present range was attained by about 7000 years ago, although there was limited further expansion to the north-east during the late Holocene. Detailed studies in Michigan have suggested spreading rates of 200 m/yr with no evidence for local disturbance of existing vegetation to facilitate establishment. This is in contrast to stand-scale studies in northern Europe where local establishment was often associated with disturbance, including fire. The northward expansion of *Fagus crenata* in Japan also began early in the Holocene, apparently spreading from northern Honshu to Hokkaido, although distinguishing initial spread from expansion of populations that had established earlier is difficult. *Fagus* populations at their northern limits on Hokkaido were only established a few hundred years ago and continue to spread very slowly northwards. *Fagus* establishment is currently hindered by dense understories of dwarf bamboo at northern sites. There is growing evidence from both North America and Europe for cryptic northern refugia that act as centres for subsequent population expansions and the same probably applies to Japan.

Study of the Holocene history of *Fagus* dynamics on three continents has yielded insight into how forest communities develop and the nature of their relationships to climatic change. Stand-scale palaeoecological studies in Europe have indicated the importance of disturbance in the late Holocene spread of *Fagus* that is not yet documented from North America or Japan. It could be that human activities have had considerable impact on European *Fagus* forests and significantly altered their ecology. Future studies in Japan and China would be useful to develop our understanding of long-term *Fagus* forest ecology.

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HISTORICAL AND CONTEMPORARY FACTORS SHAPING GENETIC VARIATION IN *FAGUS*: AN OVERVIEW

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Forest trees represent excellent models for population, evolutionary and ecological genomic studies. They have often large native populations, are typically outcrossed and have high levels of genetic variation at adaptive traits, and hence are suitable organisms to unveil the molecular basis of adaptive divergence in nature.

Understanding the interplay of evolutionary factors, demography and population structure that, together, shape genetic variation and adaptation in tree species is an important goal in population genetics and evolutionary biology.

Fagus (beech, Fagaceae) is a small genus of 10 monoecious tree species in the northern hemisphere. It is the most abundant broadleaved forest tree in Europe and western Asia and forms an important component of mixed broadleaved evergreen–deciduous forests in North America and East Asia.

We briefly describe population genomic tools and approaches applied to *Fagus* populations, and how they might be useful for understanding patterns of neutral and adaptive variation in beech populations. In addition we describe demographic processes in beech and discuss how their effect on the genetic structure may provide insights into the relative roles that gene dispersal, mating system, drift, and natural selection play in shaping spatial and genetic patterns within and among beech populations. A summary of the phylogeographic studies in *Fagus* will also be presented. Studies of glacial refugia and postglacial recolonization provide a view on how species evolve and diverge through time.

BEECH REGENERATION RESEARCH: ECOLOGICAL AND SILVICULTURAL ASPECTS

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Introduction

In this paper beech is presented as a "model genus" to show how silvicultural techniques may be derived from detailed research findings about species ecology. *Fagus* includes ecologically similar species, all found in the northern hemisphere (Peters, 1997). Many epitomize shade-tolerant, heavy-seeded climax species. On first glance it seems possible to apply a uniform *Fagus* silviculture around the globe. However, besides differentiating concerns of individual landowners, an ecological approach must account for any silvical differences between *Fagus* species, as well as between environmental conditions on different continents. Thus, an investigation of *Fagus* ecology should look at the relation between the plant's life cycle and the environment.

We refer to regeneration research for four important beech species of the northern hemisphere, i.e. *Fagus crenata* Blume, abbreviated "*F.c.*", *Fagus grandifolia* Ehrh., "*F.g.*", *Fagus orientalis* Lipsky, "*F.o.*", *Fagus sylvatica* L., "*F.s.*".

Pattern of forest disturbance, natural regeneration and silviculture

Commonly "disturbance" in temperate regions is equated with creation of openings in the overstory canopy. Some investigations regarding gap size frequency distributions in beech dominated forests show that small gaps, i.e. ≤ 200m², prevail. This corresponds well with the size of old beech trees crowns, and indicates a single-tree mortality pattern. In natural forests, gaps cover between 5 and 25% of the area, with Japanese forests having the highest proportion of area in gaps (Nakashizuka, 1987).

Based on the multi-age and irregular structure of virgin *F.o.* stands in Caspian forests of northern Iran (Sagheb-Talebi and Schütz, 2002), it seems important to recognize the development stages within natural virgin stands, and how the pattern of disturbance and regeneration differs among developmental stages. Also, "disturbance" includes other alterations of the forests structure besides degree of canopy closure. In *F.c.* ecosystems, the dominance and synchronous death of dwarf bamboos (*Sasa* spp.) on the forest floor must be taken into account (Nakashizuka, 1987). In addition, disturbances to shallow tree roots from natural and other causes, e.g. beech bark disease, or by logging operations, are important in promoting root suckers of *F.g.* in North America.

Flowering and seed production of beech species does not begin before the age of 40 in *F.s.* and *F.g.*. The amount of seed produced by individual trees is correlated with tree diameter and differs markedly between years, showing typical "masting" events in *F.c.*, *F.g.*, and *F.s.*. Yet

seed production in *F.g.* has decreased measurably as beech bark disease initially progressed in trees > 25-30 cm dbh, reducing with beechnut abundance by two-thirds to three-fifths.
Most often dispersal is by barochory, up to 20m from the tree. This is utilized by forest managers in designing cutting treatments for mixed and pure stands, and often in connection with masting events. Zoochory by birds and mammals is not commonly considered by forest managers.
As northern temperate plants, *Fagus* species have a tolerance to winter climate which inhibits growth and development. While dormant on the forest soil, the heavy fruits are subject to loss by manifold pests, e.g. fungi, birds, voles, mice, and other mammals. In *F.g.* many birds and mammals eat beechnuts, and both animal reproduction rates and their survival during harsh winters may depend on beechnut abundance (Jensen, 1985). The nuts fall from the tree just prior to autumn leaf fall. This buries the nuts, affording some protection against predators and desiccation.
Soil preparation dramatically improves the wintering of beech nuts and the sprouting of seedlings the first spring after seedfall. Following sprouting the seedlings are extremely vulnerable to birds, rodents, deer and other herbivores (Olesen and Madsen, 2008). For mechanized direct seeding, beechnuts are sown in rills or spots. But the seed is subject to predation and other losses before germination, and the emerging seedlings are often eaten by small herbivores like mice. Other problems with direct seeding include determining the best time for sowing, and how to handle the bulky fruits.

In general, beech seedlings become established under a wide range of light conditions and degrees of root competition from old trees (Sagheb-Talebi and Schütz, 2002, Collet and Chenost, 2006; Wagner et al., 2009), and commonly develop into a persistent seedling bank beneath even a closed canopy (Collet and Le Moguedec, 2007). Advance *F.s.* seedlings have a "stop and go" feature which enables them to develop in abundance as advance regeneration and increase in growth following release. That is also observed in some stands of *F.g.*. And although height growth of small *F.g.* may be slow after a first release, the rate increases after a second and third release. *F.c.* natural regeneration is most often concentrated or even limited in gaps (Nakashizuka, 1982). Yet saplings grown under a closed canopy for less than 10 years eventually respond to release after gap formation.
Fagus seedlings and saplings are sensitive to frost and drought, as well as wetness. They are often damaged or destroyed by mice and voles, but are robust against most weeds. However, when *Sasa* ssp. interferes with *F.c.*, it prevents establishment of a seedling bank.
Planting is the most common method of artificial regeneration in *F.s.* Direct seeding offers higher stock densities at a lower cost; but is also less reliable. Standard bare root seedlings, containerized seedlings and wildlings are three alternative stock types for planting.

F.c., *F.g.*, *F.o.* and *F.s.* are all capable of vegetative sprouting. However, *F.g.* is the only species that frequently produces root suckers (Nyland et al., 2006). Despite an abundance of small *F.g.* seedlings at some sites, root suckers tend to dominate the tall advance beech regeneration (>0.6 meters) (Nyland, 2008). Evidence suggests that root suckers have become increasingly abundant in unmanaged stands where beech bark disease has weakened or killed large *F.g.* trees.
Coppice systems have been developed for *F.c.* and *F.s.*, but are now mostly not used.

Competition and predation in the establishment phase – impact to mixtures
Under closed canopies, growth of *F.g.* and *F.s.* seedlings and saplings exceeds that of associated species, increasing chances for development of a dense beech understorey that will interfere with other species. Compared to their associated species, *F.s.* and *F.g.* are less often

browsed, and are relatively tolerant to competition from neighbouring vegetation. Although many growth curves have been developed for *F.s.* and its associated species at young stages, little data are available for the stage when it is necessary to act to ensure the maintenance of species diversity, e.g. cleaning. Also, little quantitative data are available to describe the dynamics of the competitiveness of *F.s.* towards its associated species. However, practitioners understand the good competitive ability of *F.s.* compared to its associated species. Site preparation to control understory *F.g.* will prove essential for promoting regeneration of other species and converting stands infected by beech bark disease to a more productive status.

Silvicultural systems and trends in silviculture

The tasks/aims in beech silviculture for *F.c.*, *F.o.*, and *F.s.* are to insure a high density of beech regeneration and/or seedlings of high quality, to make target diameter cuttings before red heart forms in a tree, and to secure the development of associated species. However, beech bark disease has killed large (>20 cm) *F.g* trees across northeastern North America, making long-term management of that species impractical.

In an attempt to capitalize on masting events, beech silviculture in the past has favoured the shelterwood system for *F.s.* and *F.c.*. However, because the vigorous growth of dwarf bamboo and shrub species after logging inhibits tree regeneration, *F.c.* regeneration has rarely occurred following shelterwood method seed cutting. Theory predicts that the shelterwood system with long regeneration periods would favour beech as a climax species. This has been observed with *F.s.*. Due to root suckering of *F.g.* it seems that this species would also dominate stands treated by the shelterwood system (Nyland et al., 2006). In *F.c.*, heavy shelterwood cutting has not adequately ensured the regeneration of *F.c.* trees, but has been a superior option for maintaining plant species diversity (Nagaike et al., 1999).

In more recent years, single-tree selection and group selection silviculture have been proposed for *F.c.*, *F.g.*, *F.o.*, and *F.s.*. In *F.c.* this should be done in conjunction with site preparation during masting years. These recommendations are consistent with the climax character of beech species, and with the natural disturbance regime of beech ecosystems (Sagheb-Talebi et al., 2005). However, the abundance of other species will probably decrease with selection system silviculture.

Contrasting silvicultural results/experiences with species having similar autecological characteristics as *Fagus* suggest that there is no single "system" by which all aims can be fulfilled simultaneously. Instead, an adaptive management approach should take alternate measures into account to address different management objectives.

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CROP-TREE AND WHOLE-STAND SILVICULTURE OF EUROPEAN BEECH: TREE GROWTH, WOOD QUALITY AND ECONOMIC YIELD

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Among the large variety of silvicultural systems for the management of European beech (*Fagus sylvatica* L.) applied across Europe, the two most widely used are single-tree or crop-tree silviculture and whole-stand silviculture. Crop-tree silviculture promotes the growth and yield of a few, selected crop trees, whereas whole-stand silviculture comprises interventions that apply uniformly to all trees in the whole stand.

The process of crop-tree silviculture in European beech is two-fold: firstly, a heavy thinning, secondly, the selection of crop trees at an early age during stand rotation. The earliest experiments on the impact of heavy thinnings on growth and yield of beech were conducted by Seebach in the 1840s, followed by Michaelis in 1910, who promoted heavy thinning from above to speed up diameter and value growth of the best trees. Only in 1934 the idea of crop-tree selection was defined more specifically by Schädelin (1934), who described two distinct and consecutive phases: first tending, then crown thinning to favour selected trees. Today, modern crop-tree thinning combines both ideas: heavy thinning (i.e., schematic, from above or crown thinning) and selection of crop trees (typically between 50 and 100 per ha). Recent and complete descriptions date from the late 1990s (Skovsgaard 1998, Wilhelm *et al.* 1999a,b,c).

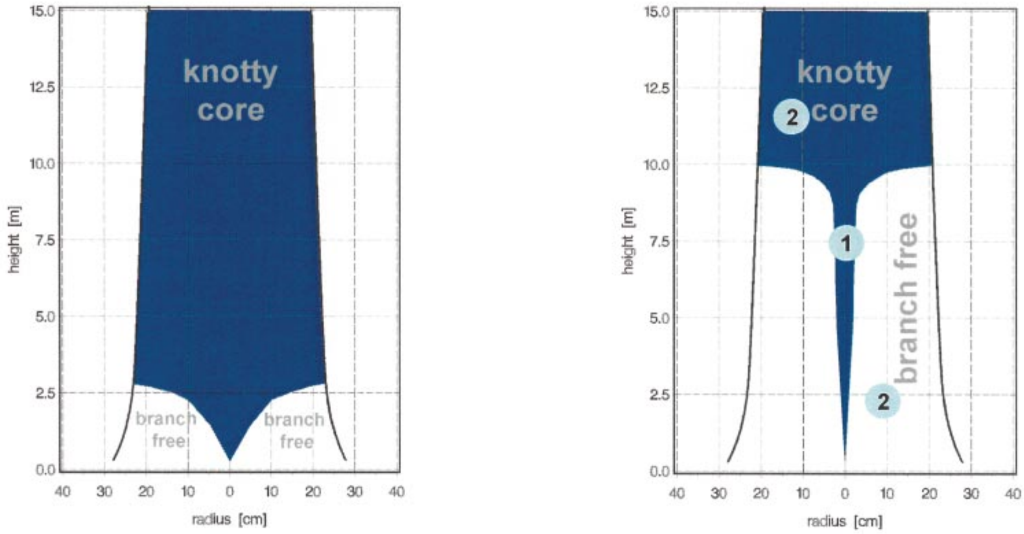

Figure 1: *Longitudinal section through a beech trunk depicting its knottiness. Left: at free growth (open grown, no competition). Right: at low annual radial increment (2-3mm/y) during the first (1) phase of a crop-tree system and free growth during the second (2) phase (stop of crown recession).*

This contribution analyses crop-tree selection and its effects on tree and stand growth, stem quality, and wood quality traits such as branchiness and red heartwood. We derived our insights mainly from two unique series of long-term experimental plots on growth and yield in Denmark (70 yrs of observation, cf. Henriksen 1951, 1956, Bryndum 1980, 1987, 1988, Jørgensen & Skovsgaard 1995, Henriksen et al. 1996) and Germany (35 yrs of observation, cf, Altherr 1970, 1981, Klädtke 1997, Hein *et al.* 2007). Both series focus on crop-tree silviculture and heavy thinnings from above. They have been analysed for their impact on wood quality, timber assortment and value production.

The analysis of round wood prices of beech of reveals the essential role of log dimension (log length and diameter) and quality traits (Knoke *et al.* 2006). Important quality traits include stem curvature, spiral grain, roughness of bark, growth stresses and, especially, red heartwood. The presence and extent of red heartwood relates directly to branch characteristics (Wernsdörfer *et al.* 2005, 2006, literature review: Knoke 2003) as branches which are not yet occluded allow oxygen to enter the inner parts of the stem. At later stages of the expansion this leads to considerable loss of value and even wood decay. However, the key process of branch development and occlusion can be controlled (Hein 2008). Three significant factors alter the time of branch occlusion: (positive) branch diameter, dead branch portion and (negative) the ring width of the trunk during the time of branch occlusion. In order to control knottiness (i.e. branchiness inside the trunk) the following so called two-phase system with crop-tree selection can be recommended: The first phase aims for low stem diameter growth, small branches, high branch mortality, and a small knotty core (Figure 1, left). The second phase aims for speeding up diameter growth of a few selected crop trees (fast branch occlusion and rapid stem diameter growth), forming of large crowns on crop trees and development of primary branches that remain alive through permanent crown release (*Figure 1*, right).

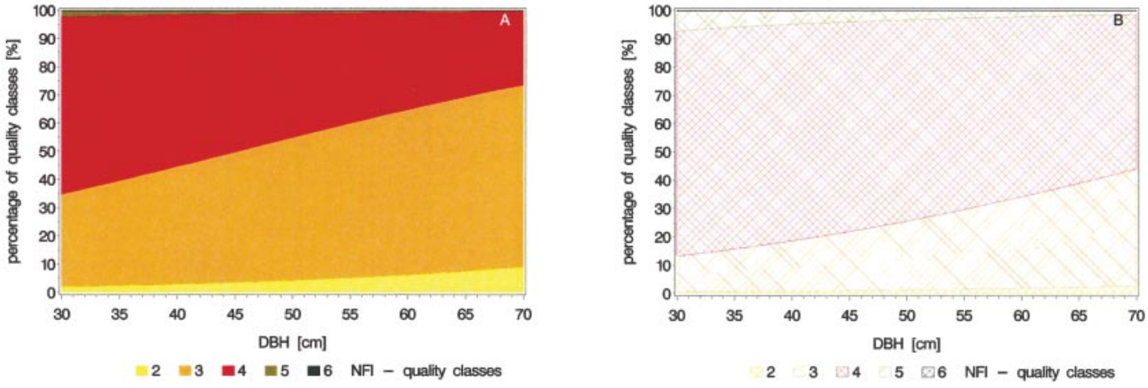

Figure 2: *Simulated proportions of the standing tree quality classes (according to the second German National Forest Inventory (NFI)) for (A) crop trees and (B) the non-crop trees over tree diameter at breast height.*

The impact of crop-tree silviculture and a two-phase treatment on quality classes has been evaluated by Hein et al. (2007) based upon growth and yield plots in south-western Germany. The assessment was done according to the criteria of the second German National Forest Inventory for standing trees. The classification was done to six classes (1-3 = above average; 4-6 = below average). The outcome of the ordered logistic mixed model showed that on the long-term experimental plots, crop trees had a higher above-average stem quality compared to the non-crop trees (Figure 2). In addition, the crop trees from the crop-tree thinning had, compared to their equivalents from the stand-level thinnings, a better stem quality. This reflects the higher quality requirements of crop-tree selection as well as the higher stem diameters of the dominant trees after 35 years.

Furthermore, crop-tree thinning led to two important observations concerning the yield in timber assortment from the plots (Hein *et al.* 2007). The crop-tree plots yielded larger shares of large-dimension, thinned trees compared to the plots treated with whole-stand thinning from below (these shares would have been even higher, if the second phase had started earlier). Moreover, these shares were provided earlier compared to the whole-stand plots (Figure 3). Apparently, the trees remaining after the first thinnings took advantage of the free crown space and grew faster in diameter (Klädtke 1997, 2002). Similarly, at the last measurements, the timber assortment of the remaining trees of the crop-tree thinned plots had higher shares of large-dimension timber compared to stand-level thinning (Figure 3, right).

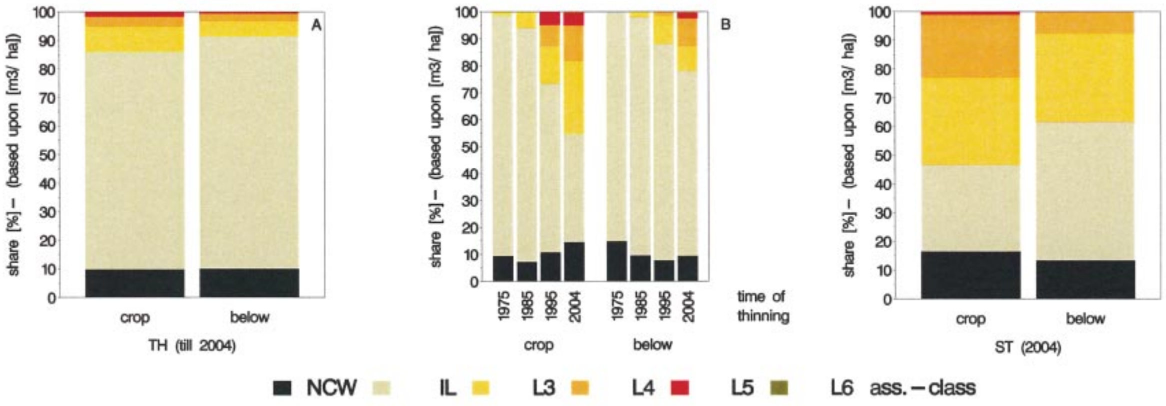

Figure 3: *Left: Mean of the plot-wise timber assortments of removed trees ("TH") from the CROP-tree-plots and the plots thinned from BELOW. Centre: Temporal development of timber assortments from the CROP-tree-plots and the plots thinned from BELOW. Right: Mean relative timber assortments (remaining stand "ST") from the plots under thinning regimes according to the CROP-tree concept and the plots thinned from BELOW at the measurement in 2004. Legend: NCW = non commercial wood, IL = industrial wood log, L3 – L6 roundwood timber diameter classes 3 to 6.*

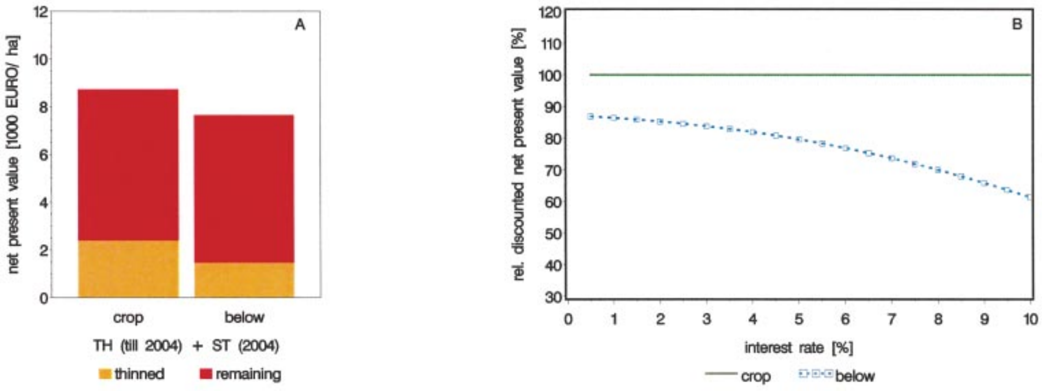

Figure 4: *Left: Mean net present value of removed and remaining trees ("TH+ST") for the plots of the experiments thinned according to the CROP-tree concept and the plots thinned from BELOW. Right: Mean net present value, discounted to the start of the experiment (removed and remaining trees) of the thinning experiment plots thinned from BELOW, relative to the values of the CROP-tree thinned plots (= 100 %).*

The production value of plots, where crop-tree thinning was applied, was higher than for plots, where thinning from below was applied (Figure 4). When additionally considering the temporal occurrence of the harvested timber, through the introduction of a range of interest rates between 0.5 and 10%, the crop-tree thinning was 12 to 40 % more profitable (discounted net present value) than thinning from below.

Even though crop-tree thinning in a two-phase silviculture looks profitable and is applied on large forest areas (e.g., von Teuffel & Hein 2005) there are still many lessons to be learned. They may be summarised by a set of questions and comments: Is the impact of such a two-phase system sufficiently integrated into our reflections on quality? How should we balance an early start of the second phase and a late beginning? What are the positive and negative effects on natural pruning, clear bole length, red heartwood, and changes in rotation length? Furthermore, the impact of crop-tree selection on biodiversity, nutrient cycling, carbon sequestration, production risks (e.g., drought stress) and regeneration potential still needs further consideration (Jørgensen & Skovsgaard 1995, Henriksen et al. 1996, Skovsgaard & Henriksen 2007).

Finally, European beech is the most important broadleaved tree species in Europe and still none of the growth and yield simulators has sufficiently integrated wood quality aspects such as branchiness or red heartwood in the model system.

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HOW DO MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS DETERMINE THE CHARACTERISTICS OF INDIVIDUAL BEECH TREES?

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Introduction

Trees, including beech and many other species, have important functions that make their own lives possible. Trees have a conspicuous characteristic three-dimensional branching structure within which their functions take place. Both function and structure are important for understanding the ecology of trees. However, researchers have often picked up only one trait when studying trees, and interpret the results as if the traits work independently of other traits. Recently, functional-structural tree models have been developed that represent both the three-dimensional structures and functions of trees (e.g., Sterck et al. 2005). Behind the development of functional-structural tree models lies the fact that the functions and structures of trees are tightly interrelated and they cannot be understood without the interactions among them. For example, light interception and photosynthesis by leaves in the crown are determined by the structure of the crown (mutual shading), and the development of crown structure depends largely on the amount of photosynthesis in the crown. The functional-structural tree models should reproduce the realistic behavior of trees in computer simulations and predict their performance under various environmental conditions. They should also increase our understanding of the ecology of individual trees. We can conduct various "what if" experiments by modifying certain components of tree functions and structures in the models and evaluate the relative importance of the traits in question.

In this presentation, we present a new functional-structural models developed for beech saplings. Using this model, we evaluated the relative importance of shoot structure, foliar phenology, and their interaction in determining photosynthesis under open and shaded conditions.

Functional-Structural Model for Beech

The new model simulates a beech sapling to calculate annual photosynthesis (Fig. 1). This model used shoots (stem that emerges from one bud and elongates in a growing season with leaves attached to it) as basic units of 3D structure. The arrangement of leaves along a current-year stem and the spatial relationship between current-year shoots and their parent shoot (one-year-old shoot) were modeled based on measurements of 3D structure of aerial parts of beech sapling. The model simulated phonological change in shoot structure (emergence and fall of leaves and elongation of stems).

The modeled functions were the interception of light and photosynthesis. Using a detailed model for light environment, we calculated hourly intensity of direct and diffuse light. The ratio of diffuse radiation to the total radiation was determined with a cloudiness index (the ratio of incident radiation to the radiation above the atmosphere). We used measured data of cloudiness index as a model input. To calculate the light intensity on the surface of each leaf, we used ray-tracing method. We determined whether diffuse light coming from each sector of the hemisphere and direct light coming from the solar disc are intercepted by obstacles or reach the leaf. In this calculation, we consider the shading effect by canopy and leaves of the same individual (self shading). We used hemispherical photographs to determine the location of obstacles (leaves and stems) in the canopy.

Photosynthesis of a leaf was determined by the age of the leaf and the intensity of the light intercepted by the leaf. The light-saturated photosynthetic rate (A_{max}) was assumed to decrease linearly with leaf age. The light response curve of photosynthesis was expressed by a non-rectangular hyperbolic function.

We calculated hourly photosynthetic rate of each leaf during a growing season, and summed up the photosynthetic rates to obtain annual photosynthetic production of a simulated sapling.

"What if" experiments

We assume that saplings have alternative options to invest a given amount of biomass in new shoots. Saplings largely differ in the orientation of new shoots and in the timing of producing and shedding leaves. We expect these traits to influence carbon gain strongly. Therefore, we might expect that natural selection strongly influences these traits for a species adapted to certain conditions. Beech often has slanting current-year shoots whereas some other tree species (e.g., *Alnus*) have more upright current-year shoots (Hallé et al. 1978). Beech flushes current-year shoots within a very short period in spring and sheds all leaves almost simultaneously at the end of the growing season whereas some other tree species (e.g., *Alnus*) have succeeding leafing phenology whereby trees produce leaves for a long period at the top of the shoot and start to shed leaves from the middle of a growing season (Kikuzawa, 1983). Therefore, we simulated a sapling with slanting current-year shoots and flush leafing phenology (baseline simulation for beech) and saplings with other traits: a sapling with upright current-year shoots and flush leafing phenology (upright shoot), a sapling with slanting current-year shoots and succeeding leafing phenology (succeeding leafing), and a sapling with slanting current-year shoots and succeeding leafing phenology (upright shoot + succeeding leafing), and made a interspecific comparison between the carbon gains by these saplings. We conducted the simulations under two light conditions (moderately shaded and open).

Results and Discussion

Hourly photosynthetic rate of a simulated sapling changed corresponding to the diurnal change in light intensity (Fig. 2A). As the intensity of light was affected by whether condition (cloudiness), the photosynthetic rate differed from day to day.

The photosynthetic rate also changed seasonally due to the decrease in A_{max}. This effect was obvious for saplings with the flush leafing phenology (Fig. 2B). For saplings with succeeding leafing phenology, the change in the number of leaves was also important for determining the photosynthetic rate (Fig. 2C). Because the number of leaves peaked in summer for them, the photosynthetic rate also peaked in summer.

Under both light conditions (moderately shaded and open), the annual photosynthetic production was largest for the sapling with slanting current-year shoots and flush leafing phenology (Fig. 3). This indicated that beech has the set of traits with which it can conduct photosynthesis very efficiently at the individual level. By flushing all leaves simultaneously at the beginning of the growing season and shedding all leaves at the end of the season, saplings can utilize the full growing season to conduct photosynthesis. Slanting shoots can increase the efficiency of light interception by avoiding the self shading.

It is interesting that succeeding leafing phenology only slightly reduced the total photosynthetic production (Fig. 3) although the number of leaves at a given time for a sapling for this type was less than that for a sapling with flush leafing phenology. This is because young leaves with high A_{max} are always located at the top of a shoot with the succeeding leafing phenology whereas many young leaves are shaded in lower positions with flush leafing phenology. Under shaded conditions, the photosynthetic production was larger for the sapling with upright shoots and succeeding leafing phenology than for the sapling with upright shoots. It indicated that succeeding leafing phenology mitigated the adverse effect of upright shoots.

In the present simulations, we did not consider the vertical gradient of light intensity. This seems to be a major reason why the sapling with upright shoots performed badly. If there is a vertical gradient in light intensity, upright shoots can place their leaves in higher positions and receive more light. Moreover, if the gradient shifts upward, as often found in developing young plant communities, upright shoots are an important growth strategy to keep up with the competition for light.

In summary, "what if" experiments using a functional-structural tree model revealed that the traits of beech in terms of foliar phenology and shoot architecture were strategies for efficient photosynthesis at the individual level under uniform and stable light environment.

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Figure 1: An example of simulated saplings.

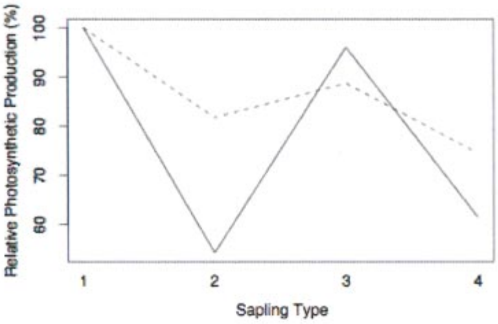

Figure 3: Relative annual photosynthetic production. Relative value was calculated with the baseline simulation as the reference. 1: baseline, 2 upright shoot, 3 succeeding leafing, and 4 upright shoot + succeeding leafing. Solid line: simulations under moderately shaded condition, Broken line: simulations under open condition.

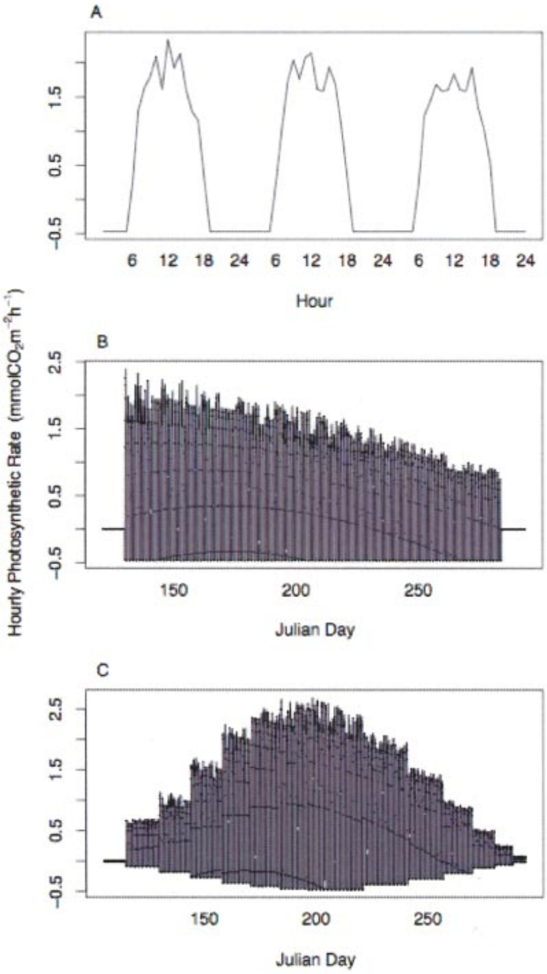

Figure 2: Change in photosynthetic rate of a simulated sapling. A) diurnal change, B) seasonal change for the baseline simulation, C) seasonal change for a sapling with succeeding leafing phenology.

HOW WILL *FAGUS SYLVATICA* L. RESPOND TO CLIMATE CHANGE? MODELLING ITS GEOGRAPHIC DISTRIBUTION AND ADAPTIVE POTENTIAL

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Profound changes in the geographic distribution of European beech (*Fagus sylvatica* L.) are projected both by advanced statistical and process-based modelling techniques. However, some authors argue that tree have high phenotypic plasticity that allows them to withstand large environmental fluctuations during their life time (Rehfeldt *et al.* 2002). In addition, from a genetic point of view it is argued that adaptation to climate change is possible because European beech has high within population genetic diversity (Buiteveld *et al.* 2007) (Leonardi and Menozzi 1995) and because gene flow of pollen is found to take place over large distances thereby exchanging favorable genetic variants between isolated stands (Petit and Hampe 2006). Other authors argue on the contrary that: trees are unable to adapt because the rate of climate change is high relative to the longevity of individual trees (Davis and Shaw 2001); trees may not have adequate genetic diversity to adapt to the changing environmental conditions (Davis and Kabinski 1992); and trees may not be able to disperse to newly available habitat to match the rate of global change as the landscape trees have to cross is highly fragmented (Jump and Penuelas 2005).

In this review we present an overview of species-area models that take either a process-based or a statistical approach. We analysed climate change scenarios in combination of the modelled features of European beech that result in the predicted changes in its distribution. The main factors that determine a species' area in both the process-based and the statistical models are: water limitation, late spring frosts, and duration of the growing season (Pearson and Dawson 2003) (Sykes *et al.* 1996). In case of the process-based model, also conditions for establishment of seedlings and competition for resources are included. Species-specific thresholds in these abiotic factors and resource availability then determine the predicted future species area.

In addition, from a genetic point of view we present a modelling analysis of the adaptitive potential of beech to respond to climate change. This means in particular the potential to change in the population by means of selection the thresholds in the above mentioned abiotic factors. We thereby focussed on traits related to the duration of the growing season, i.e. phenology and traits related to water use.

An example of climate change impacts on the geographic distribution of European beech based on the process model LPJ-GUESS (Sykes *et al.* 1996) (Smith *et al.* 2001) is presented in Figure 1. This figure shows that the climate change impacts are most severe in the southern/south-eastern limit of the distribution of beech, indicating a strong effect of increased water limitation in a future climate. In addition is the extension of the northern boundary of the distribution rather restricted, suggesting limitations based on the start of the growing season in combination with late spring frosts. Figure 2 presents a similar example, but then based on aggregated results of a suite of statistical niche-based models (Thuiller 2003, 2004). These results show a northward shift of the southern limit of the distribution of beech, in agreement with the process-based model results. However, the statistical models do indicate a northward extension of the northern limit, not forecasted in the process model.

An example of adaptive potential of the start of the growing season to climate change is presented in Figure 3, based on the model ForGEM(Kramer *et al.* 2008). In this example are temperature, forest management, and the extend of external pollen input varied for a 2-ha beech stand. There is a strong effect of temperature on the average day of budburst in the simulated population. This is partly a phenotypic plastic response as the phenological submodel is temperature dependent. However, some selection did take place as the chilling requirements somewhat reduced with increased temperature (Figure 4). The consequences of these adaptive responses on the distribution of beech will be presented, as well as such consequences of adaptive responses of traits related to water use.

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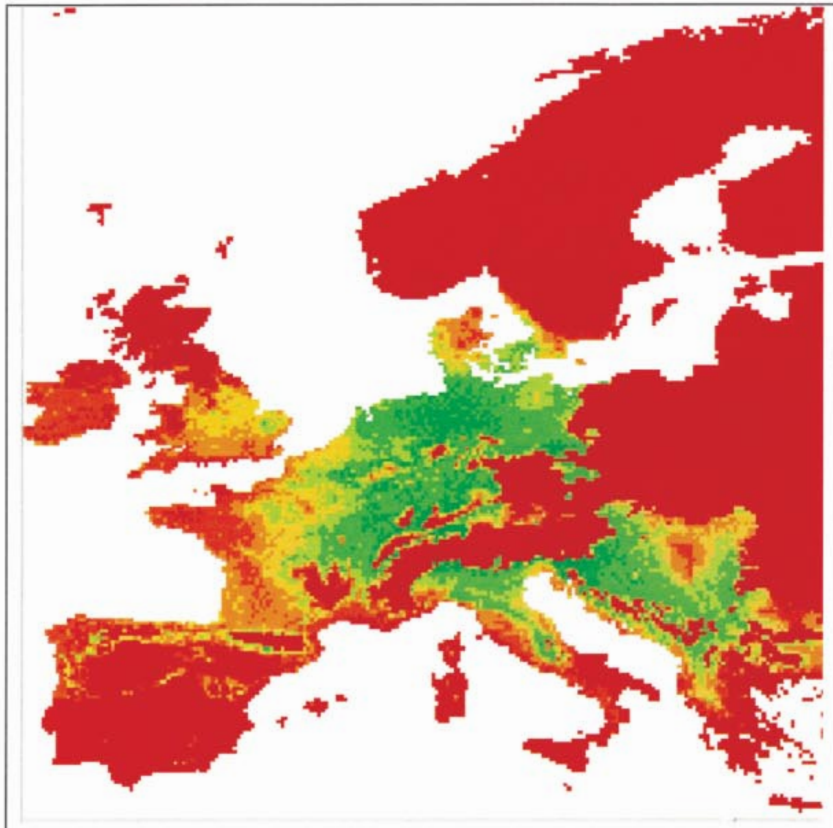


Figure 1A. Predicted LAI of *Fagus sylvatica* under current climate based on LPJ-GUESS model. Average of 1971-2000.

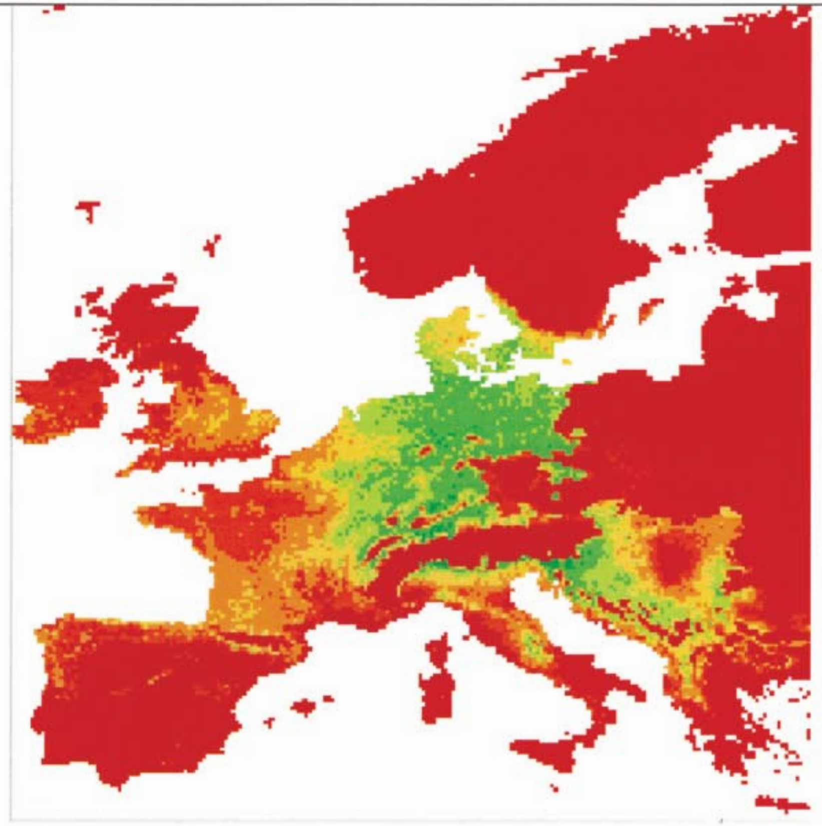
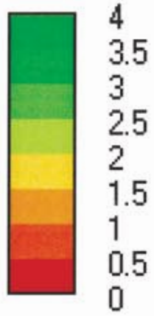


Figure 1B. Predicted LAI of *Fagus sylvatica* under Hadley A2 scenario based on LPJ-GUESS model. Average of 2051-2180.



Legend (m m-2)

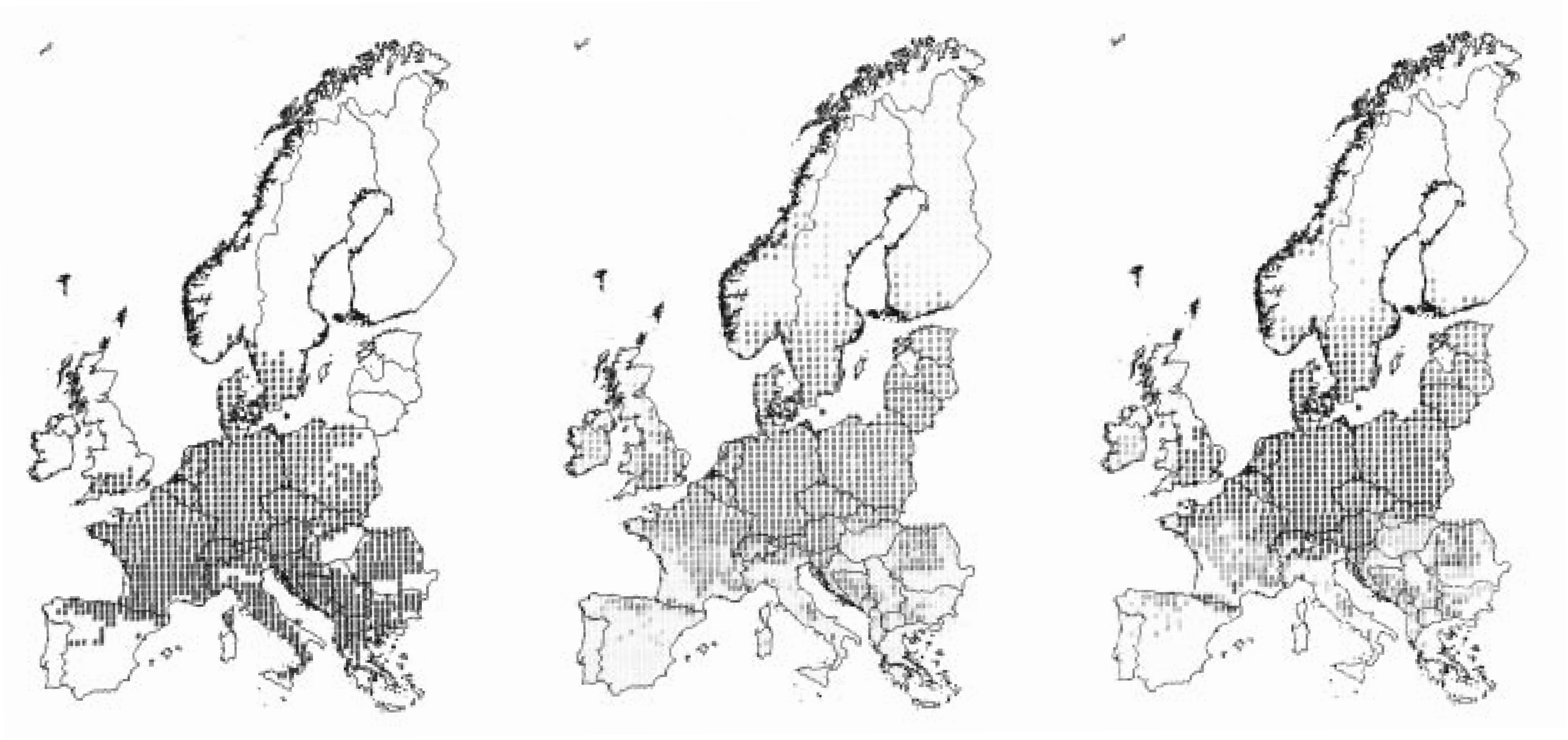


Figure 2. Current and future (scenario A2 and B1) potential distributions of Beech in Europe using statistical niche-based models. The maps indicate the average presence value across models, evaluation methods and scenarios (A2 & B1), weighted by the models' evaluation scores: 1 (black) = suitable, 0 (light grey) = unsuitable according to all models.

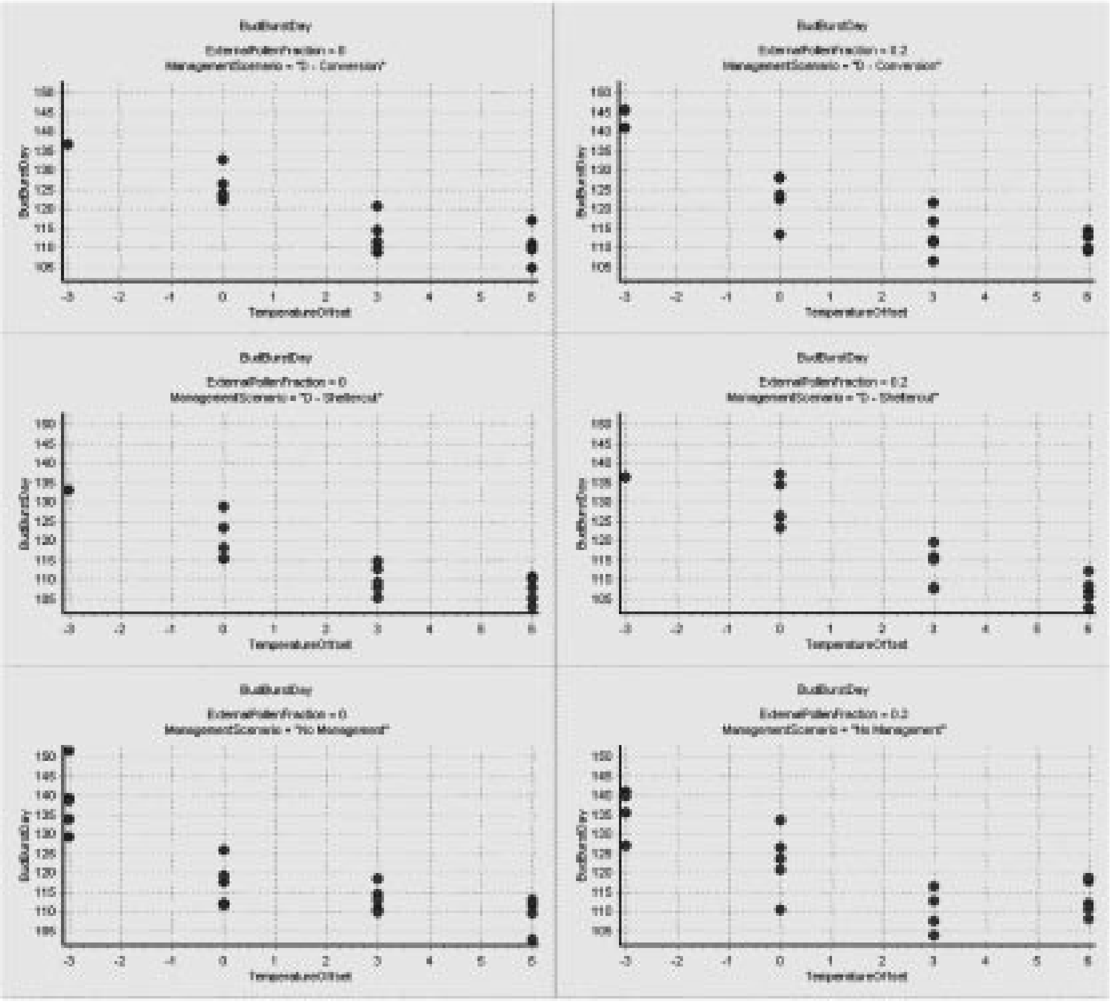


Figure 3. Average budburst day (day of year) after 300 yr of simulation under 4 temperature change scenarios), 3 forest management scenarios and 2 levels of external pollen input on a ca. 2-ha European beech stand. Multiple dots at the same temperature offset indicate replicates. In the -3C temperature scenario in several instances no trees survived after 300yr of simulation.

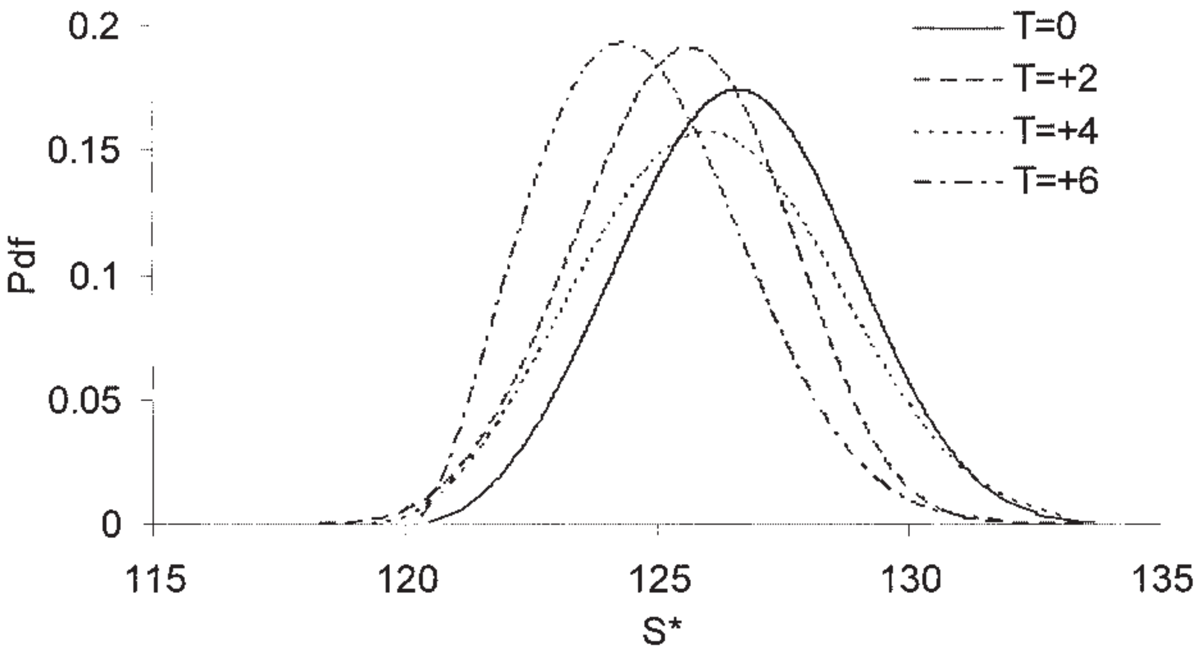


Figure 4. Adaptive response of chilling requirement, S*, of a *Fagus sylvatica* to elevated temperature scenarios. Pdf indicates the probability density function of the Weibull distribution fitted throughout the relative frequency of S* in the simulated beech population.

GROWING EVIDENCE FOR LOCAL POST-GLACIAL DEVELOPMENT OF EUROPEAN BEECH POPULATIONS IN THE SOUTHEASTERN ALPS

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Introduction

The genus *Fagus* L. spread from Asia to Europe in the Tertiary by the late Early Oligocene (ca. 30 mya) as the species *F. castaneifolia* Unger, and was distributed continuously in Europe in the Late Oligocene (Denk et al. 2005). During the Neogene two polymorphic and geographically clearly distinct species, *F. gussonii* Massalongo and *F. haidingeri* Kovats, were present in Europe, the latter resembling a number of modern species including European beech (*Fagus sylvatica* L.) (Denk 2004). The evolution of European beech is not well known, but Pleistocene glacial and interglacial periods are considered as key periods for its present distribution in Europe. European beech populations survived the last glacial period in several refuge areas and for a long time south-European peninsulas were considered as major refugia from where beech spread to the rest of Europe in the Holocene. It is known that beech has repeatedly been present in southern Europe during most of the last 500 kyr (Tzedakis et al. 1997). Until recently, the southeastern Alps and partly the northwesternmost part of Dinaric mountains, mainly the area of present-day Slovenia, has not been considered as an important area of beech glacial refugia. However, there is growing evidence that this was one of the very important European beech refuge areas.

The aim of the present work was to review and evaluate all the available data and present different types of evidence to support the hypothesis on the continuous survival and local development of European beech populations in the southeastern Alps since the Pleistocene.

Materials and Methods

Available paleovegetational studies of the Tertiary flora were surveyed to establish the presence of beech in the studied area in the Tertiary. Numerous published pollen diagrams and palynological research as well as reports of macroscopic plant remains, mainly charcoal from Palaeolithic sites, were analysed to examine the presence of European beech in the late Pleistocene and Holocene. With the use of isozyme gene markers (9 isozyme systems, 12 loci) 130 beech populations from central and southeastern Europe were analysed to investigate their genetic structure and differentiation. Additionally, the distribution of several taxonomically rather isolated herbaceous and insect species, characteristic for mesophilous beech associations from the studied area, were analysed.

Results and Discussion

Fossil remains, mainly leaves, found in Oligocene marl layers in several Slovenian coal mines, including Zagorje, Trbovlje and Novi Dol clearly prove the presence of beech in this territory in the Oligocene (ca. 34-23 mya) even if they are not very abundant. Most samples from these locations were assigned to the rather polymorphic species *Fagus feroniae* Unger (Ettingshausen 1872), while the samples from geographically close Croatian locations (Planina, Podsused, Sv. Nedelja, Radoboj) were identified as *Fagus pristina* Saporta (Pilar 1883). After the taxonomic revision of *Fagus* from the Cenozoic of Europe (Denk 2004) these samples would probably be included in some other Cenozoic species.

There are no reliable records of the presence of beech in the southeastern Alps and northwestern part of Dinaric mountains in the Lower and Middle Pleistocene. However, it reappeared sporadically in sediments as soon as the end of the Riss-Würm interglacial, attaining relatively high values in the Würm interstadial Brörup in the Ljubljana Moor (Culiberg 1991). In the Bölling interstadial (12.500–13.300 years BP) high concentrations of beech pollen were recorded in several locations in the Ljubljana Moor (Šercelj 1963). These findings suggest the presence of small isolated populations that must have survived in protected locations in the surrounding mountainous landscape.

However, the sporadic appearance of beech pollen in Würm sediments, though found in many places, may throw doubt upon the actual presence of beech at the precise location where the pollen was found since the presence might be interpreted as a result of long-distance transport or as a redeposit. But this can not be the case with macroscopic plant remains. In numerous Palaeolithic sites in Slovenia, charcoal of different tree species was found. Beech charcoal of exceptional age was determined in several sites: Lukenjska jama (12.580 years BP) and Koprivška luknja (16.830 years BP) (Osole 1983) and Šandalja (Istria) (27.000 years BP) (Culiberg, Šercelj 1995). The most recent analyses of charcoal and pollen excavated in the Palaeolithic site Divje babe I in the mountains of western Slovenia revealed unambiguously a permanent presence of forest as early as the Middle Glacial (Middle Würm) in the entire period from approximately 80.000 to 40.000 years BP. In addition to different conifers, various deciduous species including beech grew in the vicinity of the site (Culiberg 2007). The presence of beech is restricted mainly to the warmer intervals of this period, but it is clear that it had survived cold periods in close vicinity to the site.

Isozyme analyses revealed some rare alleles, such as *Mdh-2/B,* present almost exclusively in the southeastern Alps and central Europe and almost absent in the Balkan peninsula, and several alleles (e.g. *Mdh-1/A, Mdh-1/B, Mdh-1/D, Mdh-1/E, Pgi-2/A'*) which are exclusively or predominantly present in the central and eastern Balkan peninsula. In the studied area of central and southeastern Europe the clinal course of genetic variation becomes clearly apparent in the direction from northwest to southeast. Based on genetic distances, populations roughly merge into two distinct groups: the first and larger group comprises areas in the northwestern part (southeastern Alps and northwestern Dinarics) including central Europe, while the second group comprises areas in the central and eastern part of the Balkan peninsula, which to some extent, coincides with the area of the putative taxon Balkan beech (Brus 1999, Gömöry et al. 1999, Brus et al. 2000). These findings also indicate possible local development of beech populations in the southeastern Alps and northwestern Dinarics.

Of special interest, although widely overlooked, is a wide range of so-called illyricoid species, forest herbs for the most part, which are taxonomically rather isolated and endemic to the area of southeastern Alps and northwestern Dinarics. Trinajstič (1992) lists 26 species with such distribution, among them *Anemone trifolia* L., *Aposeris foetida* (L.) Less., *Aremonia agrimonioides* (L.) DC., *Cardamine enneaphyllos* (L.) Crantz., *Cardamine trifolia* L., *Cyclamen purpurascens* Miller, *Epimedium alpinum* L., *Hacquetia epipactis* (Scop.) DC., *Lamium orvala* L., *Omphalodes verna* Moench. and *Scopolia carniolica* Jacq. They all belong to characteristic species of recent mesophilous beech associations. Their taxonomical and biogeographical statuses indicate their probable Tertiary origin (Turill 1929) as well as the possibility that in the area of their present range, they continuously survived in microrefugia as a component of mesophilous beech forests.

A similar geographical distribution pattern is observed in some insect species which are typically bound to beech forests. *Miramella irena* (Frugsdorfer) and *M. carinthiaca*

(Obenberger) (Acrididae) occur mainly in the south-eastern part of the Alps (Baur and Coray, 2004), while other species of the genus, even if distributed in other parts of central Europe too, indicate their possible survival in local beech forests in this area.

In conclusion, a large number of different plant remnants prove the permanent presence of beech in the southeastern Alps and the northwesternmost part of the Dinaric mountains, as well as its fast reappearance and local spread after every glaciation. Furthermore, the genetic structure of beech populations and the phytogeographical characteristics of several herbaceous and insect species highly support the continuous presence of beech in this area and the possibility that some microrefugia had indeed been inhabited by beech uninterruptedly since the Tertiary. They also highly support the local development of beech forests after the glacial periods. Even more, it seems that these populations have also played an important role in the colonisation of central and western Europe in the Holocene. Earlier it was believed that in the postglacial beech had spread to the part of central Europe from the Balkan peninsula. However, taking into account the geomorphology of the region, the migration flow along the Dinaric mountain chain was certainly possible, but could not have been very expansive. In the area of Velika Kapela, where the Dinaric mountains are situated between the Pannonian plain (with a continental climate and very likely marshiness at that time) and today's Adriatic, there was probably a bottleneck for massive southeast-northwest migration. But as suggested earlier (Brus 1999, Brus et al. 2000), even if spread in this direction was possible, the area would already have been occupied with locally developed beech populations. Results of isozyme analyses even indicate the possibility that beech has spread towards the southeast to some extent. And finally, the evidence presented above support the conclusions in a recent study which combined genetic and fossil data across a whole species range (Magri et al. 2005).

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VARIATION AMONG *FAGUS SYLVATICA* AND *FAGUS ORIENTALIS* PROVENANCES IN YOUNG INTERNATIONAL FIELD TRIALS

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Introduction

Three series of beech (*Fagus sylvatica* L.) provenance trials were planted 1986, 1987 and 1988 (International Beech Provenance Trials 1983/86, 1984/87, 1985/88), with the aim to test a wide scope of provenances in order to perceive much of the natural variation of the species (von Wuehlisch, 2007). The three trial series were planted on 14 sites, one in Denmark, The Netherlands, Belgium, two in France, and nine in Germany (Muhs, 1985, 1991, von Wuehlisch and Muhs, 1992; von Wuehlisch et al. 1998). On these sites, height growth, stem diameter, mortality, leaf flushing in spring as well as stem form were recorded up to the age of fifteen years.

Materials and Methods

In total, 188 provenances were planted on the trials sites at the age of three years. The provenances originate from Austria, Belgium, Croatia, Czech Republic, Denmark, France, Germany, United Kingdom, Hungary, The Netherlands, Poland, Romania, Slovakia, Slovenia, and Switzerland. Additionally, eight *Fagus orientalis* provenances from northern Turkey were included. The seeds originated mostly from registered stands assumed to be autochthonous. Due to limitations in seedlings and trial area, the trials are unbalanced with incomplete replications. The plots were planted in five rows with 20 plants each resulting in plots of 10 by 10 m and with planting distances of 1 m in the rows and 2 m between the rows resulting in plant densities of 10,000 plants per ha.

The Danish trial is located on the Island of Falster (Orenaes, Bu3), the Netherlands trial in Horsterwold (Bu7) and the Belgian at Paliseul (Bu12). Two trial sites are located in north-west France: Vascoeuil (Bu8 and Bu11). The nine trials in Germany are located in the north: Malente (Bu6), Dodau (Bu13 and Bu17); in the west: Attendorn (Bu5 and Bu10); in the centre: Buedingen (Bu4 and Bu14), and in the south-west: Wildbad (Bu9 and Bu15). The first series contains the trials Bu3, Bu4 and Bu5. The second series contains Bu6, Bu7, Bu8, Bu9 and Bu10 and the third series consists of Bu11, Bu12, Bu13, Bu14, Bu15, and Bu17. All sites were former forest stands, except for those at Malente (Bu6), Dodau (Bu13) and Dodau (Bu17), which were planted on former arable land.

At age of 15 years the natural mortality rate (before thinning took place) as well as tree height and stem diameter in 1.3 m above ground (dbh) was commonly measured for most trials. The number of trees to be measured was reduced to measuring only the second and fourth row of the five-row-plots. Stem form was assessed on the trees which were measured for height focussing on stem straightness and on forking of the stem. Leaf flushing was recorded in earlier years repeatedly.

Results and Discussion

The mortality for all trials reached 39 % at age fifteen, which corresponds to a remaining mean density of 6.087 trees per ha. The mean mortality rates for the sites range from 13 % at Dodau, (Bu17) on former arable land to 63 % at Attendorn (Bu5), a former Norway spruce site with soil low in pH. Also among the provenances the mortality varies highly. It ranges from 0 % provenances Dierdorf/Germany at Vascoeuil (Bu8) and Spiez/Switzerland at Vascoeuil (Bu11) to 92% provenance Eawy/France at Attendorn (Bu5). A clear pattern could not be found and it is remarkable that the provenances from the regions where the trials are located do mostly not belong to the provenances with the lowest mortality. The *F. orientalis* provenances from Turkey tend to have a relative high mortality and reduced growth performance. However, provenances of neither species failed totally at any of the sites, which demonstrates a degree of plasticity and ability to adapt to differing sites.

The mean height for all sites is 465 cm and the mean diameter is 4.1 cm (at 1.3 m). Like the mortality rates, height and stem diameter varies considerably among provenances. Also, between the trial sites these differences are pronounced. However, in the five cases, where two trials are located close to each other the traits have similar magnitude. The site conditions seem to be a decisive factor for most traits that were recorded. For survival and growth performance the site effect is based on the different climate and soil conditions, given by the geo- and topographical parameters. An altitudinal cline was observed with sites at lower altitudes having higher trees. However, also precipitation and especially soil nutrient availability influenced growth. Accordingly, the provenances reacted differently to the site conditions resulting in low or no correlations between the different trials at the provenance level and pronounced genotype by site interactions. Even among provenances from one region large variation was found. Usually the local provenance was not the best performing and the provenance of distant origin not performing poorest. Therefore it is difficult to predict the performance of a certain provenance at a certain site. The results are partly comparable to results from earlier trials established by Krahl-Urban (Kleinschmit and Svolba, 1996).

For stem form the impact by the site is strong. This may be due to site conditions with more favourable conditions causing poorer stem forms. Stem form and frequency of forks were found to differ between provenances. However, there are no provenances which seem to be best or worst on all the sites. Rather, the distribution of the assessment grades varies from site to site. On the whole, provenances from Romania and Slovakia from intermediate to higher elevations seem to show better stem forms and fewer forks. The *F. orientalis* provenances from Turkey also seem to be more favourable for both traits. Stem form was found to be under genetic control (Richter, 1999; Geburek and Thurner, 1993; Kleinschmit, 1985; Kleinschmit and Svolba, 1996; Larsen, 1985).

The time of flushing of the leaves in spring differed significantly between the provenances and sites. It proved to be a trait, which is influenced by climatic conditions (correlated to accumulated temperature sums) and stays stable over all sites and series. A late flushing provenance always tends to be late, and an early one early. Flushing was found to be under strong hereditary control. A cline from south-eastern to north-western and Western Europe can be observed, with flushing time moving from early to late flushing provenances (Liesebach 2000, Liesebach et al. 1999, von Wuehlisch, 1995*a*, *b*). This was found to hold true also for the *F. orientalis* provenances from Turkey when comparing them to the *F. sylvatica* provenances. The time of flushing is a clear adaptation to late frost occurrence, which obviously exerts a strong selection pressure, probably, because late frost damages not only the flushing leaves but also the flowers, which emerge synchronously with the leaves.

Generally, the variation within the provenances is high and not as pronounced between the provenances. Except for flushing time, a general geographic trend to explain the variation between the provenances could not be found. The provenances reacted differently to the sites resulting in pronounced genotype by site interactions. It is not easy to find provenances that are suitable for a large range of sites. This demonstrates an adaptedness of beech provenances to the local sites. At the same time, an ability to adapt to differing sites can be assumed, because many provenances can cope with site conditions, which are different from those at their origin.

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GENETIC VARIATION OF FLUSHING AND WINTER LEAF RETENTION IN EUROPEAN BEECH PROVENANCE TEST IN CROATIA

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Introduction

Several studies of various sets of European beech (*Fagus sylvatica* L.) provenances have shown that traits connected to flushing phenology are highly heritable and important adaptive traits. Some authors reported clinal geographic variation regarding those traits (Muhs 1985, Teissier du Cros et al. 1988, von Wuehlisch et al 1995), while others revealed ecotypic differentiation (Chmura and Rozkowski 2002, Jazbec et al. 2007).

Winter leaf retention is well known phenomenon within genera *Quercus* and *Fagus*, but precise explanation of its causes is still an open question. Some authors tried to explain it as a juvenile physiological peculiarity (Dunberg 1982), others as an adaptation to various ecological conditions such as infertile sites, frost, dry wind or interspecific competition (Nilsson 1983, Escudero and Arco 1987). While several studies have dealt with environmental causes of winter leaf retention in oaks and beeches, we have found only one paper which deals with genetic causes of the phenomenon and that one consider oaks (Herzog and Krabel 1996).

The aim of this study was to determine amount and pattern of genetic variation of flushing and winter leaf retention in beech, as well as to discuss its possible causes.

Material and Methods

Seeds were collected during years 2002, 2003 and 2004 within mother stands of eight countries, mostly from central and south-eastern Europe. Plants were raised in nursery of the Institute for Forest Genetics in Grosshansdorf, Germany. The provenance test (N 45°53'5,6"; E 15°55'13,77") was laid in March 2007, according to randomized complete block design with three replications (blocks). Fifty plants per replication of each of twenty one provenances were planted in spacing 1.0 × 1.0 m. Dead leaf retention was assessed on April 23th 2008 by two step scale (0 – plant without dead leaves and 1 – plant with retaining dead leaves). Flushing phases were recorded twice (on April 23rd and April 30th) on 1-6 scale (1- dormant buds, 2- buds swell and get round, 3- bud burst, 4- leaves are visible but coiled, 5- leaves more visible but still not fully expanded, 6- fully expanded leaves).

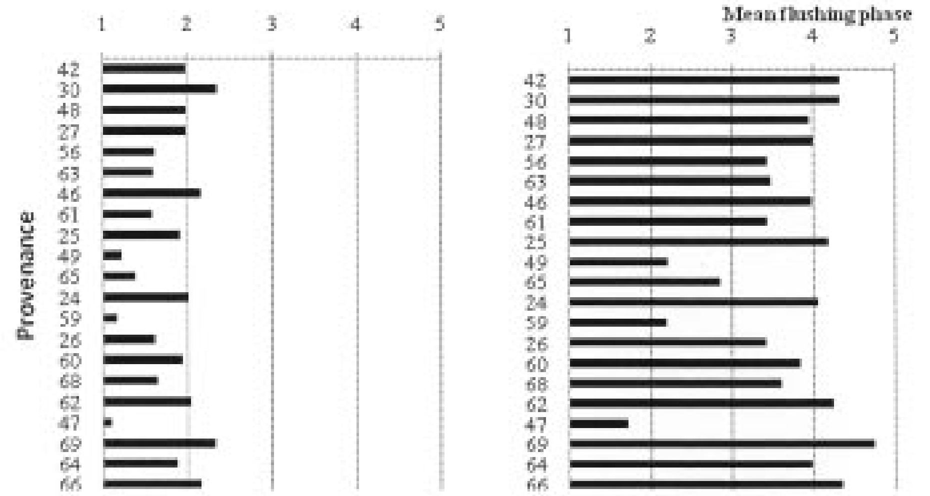
All statistical analysis was done with SAS software version 8.2. The descriptive statistical parameters were calculated by the MEANS procedure. Analysis of variance was conducted by the MIXED procedure in order to determine the significance of variances caused by provenances, blocks and provenance by block interaction. The MIXED procedures were done according to the linear model (SAS 2000): $y_{ijk} = \mu + P_j + B_k + PB_{jk} + e_{ijk}$;

where: Y_{ijk} - individual tree observation; μ - overall mean; Pj - fixed effect of the *j-th* provenance ($j = 1,2,3....21$); B_k – fixed effect of the *k-th* replication (block) ($k = 1,2,3$); PB_{jk} – random effect of provenance by block interaction; ε_{ijk} - the random error effect. All studied effects were considered normally distributed.

Differences between studied provenances have been analyzed by the Tukey test. The REG and CORR procedures were conducted to analyze relationships between studied traits as well as between studied traits and ecological variables of the provenance mother stands (SAS 2000).

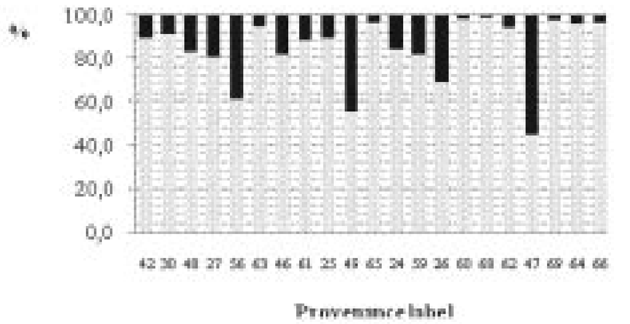
Results and Discussion

Figure 1. Mean flushing phases of studied provenances on two dates (on the left: flushing on April 23rd; on the right: flushing on April 30th)



Differences in flushing phenology between studied provenances (fig.1) were statistically highly significant (p<0.0001). Provenances 47, 49 and 59 were late flushing and provenances 25, 30, 42, 62, 66 and 69 were early flushers, while others could be described as intermediate. Although clinal genetic differentiation was revealed by several authors studying beech flushing phenology as well as enzyme markers (von Wuehlisch et al. 1995, Nielsen and Jorgensen 2003, Gomory et al. 2007), Tukey test of provenance mean differences did not show clinal geographical pattern. Different geographic pattern in this study might be attributed to smaller number of studied provenances and their more restricted distribution range (mostly to the Balkan region), which do not represents well much broader distribution range of the species.

Figure. 2. Percentage of trees which retained dead leaves until flushing (marked black)



However, obtained results from this study supports ecotypic pattern of differentiation as were reported in some other papers (Comps et al. 1991, Paule 1995, Gomory et al. 1998, Chmura and Rozkowski 2002, Jazbec et al. 2007). Ecotypic variation also supports previously reported higher level of genetic variability of beech populations from south-eastern Europe (Paule 1995).

Differences between studied provenances in winter leaf retention were also statistically highly significant (p<0.0001). Several provenances (namely: 47, 49, 56 and 26) showed significantly higher proportion of plants which retained dead leaves until flushing (fig. 2). It can be noticed that e.g. provenance no. 47 had more than 50% of trees with leaf retention, while some provenances (such as 60 and 68) had very low percentage of plants with the trait. These results strongly support genetic determination of the trait as was reported for *Quercus robur* (Herzog and Krabel 1996).

Statistically significant, negative correlation between leaf retention and yearly mean temperature was found, though it explained low percentage of variation (-0.11, $p<0.0001$, $R^2=0.013$). Also, quite high negative correlation coefficient was found between flushing time and winter leaf retention (-0.66). Although not ultimately clear, those results indicate that observed genetic differentiation could has been driven by natural selection conditioned by frosts and low winter temperatures, as hypothesized by Nilsson (1983). However, ecological parameters that we were using, had limited value as they originate from nearest weather stations and not exactly from the mother stands. Observed genetic differentiation could have been driven by genetic drift or by combined influences of natural selection and genetic drift as stated by Herzog and Krabel (1996). Further studies of the mother stands might put more light on causes of observed genetic differentiation pattern.

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ASSESSMENT OF GENETIC STRUCTURE WITHIN AND AMONG IRANIAN POPULATIONS OF BEECH (*FAGUS ORIENTALIS* LIPSKY): IMPLICATIONS FOR IN SITU GENE CONSERVATION

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Introduction

Oriental beech (*Fagus orientalis* Lipsky) is a widespread monoecious and wind-pollinated tree species. Beech forests are located on the northern slopes of Alborz Mountains, within an altitude of about 600-2000 m above sea level. It is one of the major components of the Iranian forests and it is of both ecological and economical importance. The conservation and protection of this tree species is most promising *in situ*, within the frame of forestry management, with the aim of combining gene conservation efforts and the production of high quality timber. Information on crucial life-history characteristics of forest tree species (e.g. mating system, pollen and seed dispersal) and their patterns of genetic variation is still lacking. In this context, the application of molecular genetic techniques to the conservation of forest tree species is anticipated to bring valuable data that could be used to extract relevant biological information, to document hotspots of genetic diversity, and to infer their phylogeography (Newton et al. 1999). In this study, we assess general pattern of genetic variation and the level of genetic differentiation in *F. orientalis* from Hyrcanian forests at three different geographical scales (among individuals within populations, among populations within natural regions, and between regions) using six nuclear microsatellite loci. The implications of the results of this study for *in situ* conservation of genetic resources of Hyrcanian beech forests are also discussed.

Material and Methods

Bud material was sampled in 13 natural beech populations covering a large part of the distribution range of oriental beech (*Fagus orientalis* Lipsky) in North of Iran. In all collection sites, 40 trees were randomly chosen (separated by at least 30 m) and genotyped using 6 nuclear SSRs (Pastorelli et al. 2003).

For the codominant SSR loci, homozygous and heterozygous genotypes were inferred from the banding patterns. Standard population genetic estimates of allele frequency, number of alleles, observed heterozygosity Ho, expected heterozygosity He and the fixation index Fis were calculated directly from these genotypes. The 95% confidence intervals about the estimates of Fis were determined by bootstrap resampling of individuals as recommended by Van Dongen (1995).

We used the analysis of molecular variance (AMOVA) framework, to perform hierarchical analysis of genetic differentiation with tests of significance via random permutation, as per Peakall *et al.* (1995). Total genetic variation was partitioned into three levels: among regions, among populations within regions and among individuals within populations with variation summarized both as the proportion of the total variance and as *F*-statistics (*F*-statistic analogues). All genetic analyses were performed using a modified version of our own software package, GenAlEx6 (Peakall & Smouse 2006) with bootstrap resampling and permutational tests performed 1000 times.

The mantel test (Mantel 1967) available in software Spagedi (Hardy and Vekemans, 2002) was used to test for isolation by distance. As input the two matrices, Fst values and the natural logarithm of distance in Km between samples were used. A simulated annealing procedure implemented in the spatial analysis of molecular variance (SAMOVA) algorithm (Dupanloup et al. 2002) was used to define groups of populations that are geographically homogeneous and maximally differentiated from each other.

Results and Discussion

Whithin population diversity

The results of this study revealed significant differences in genetic diversity measures among the populations studied (Table 1). In almost all populations a significant excess of homozygotes was found, which is in agreement with previous isozyme (Salehi Shanjani et al. 2002) and SSR (Buiteveld et al. 2007) publications.

Overall, populations of *F. orientalis* are slightly, but significantly inbred (Fis=0.060, Fit=0.115). For microsatellite, the most commonly reported cause of positive values of the inbreeding coefficient are heterozygote deficiency, self-fertilization and biparental inbreeding, i.e. inbreeding caused by mating between related individuals. Evidence for the occurrence of biparental inbreeding comes from the analysis of genetic structure within populations. Indeed, we detected an association between genetic relatedness and spatial position of individuals, in agreement with the concept of isolation by distance (Hardy and Vekemans, 2002) in all populations.

A low, but significant, differentiation among all populations in aggriment with *F. Sylvatica* (Buiteveld et al. 2007) was found (Fst = 0.058), even if, as expected, less pronounced (based on AMOVA, 4% and 4% of total nSSR variance is due to differences among regions and among populations within regions, respectively). A very strong and significant correlation between genetic (using nSSR data) and geographic distances (R^2=0.522, P=0.01) was estimated, thus showing an isolation by distant effect.

Geographic Diversity and *In Situ* Gene Conservation

A new type of *in situ* gene conservation programs is developing the Gene Management Zone (GMZ) consept, allowing evolutionary processes to take place in populations of plant and animal species while protecting genetic resources of important tree species as well as wild relatives of species. Protection of all studied beech populations in the Hyrcainan forests may not be economically feasible. The number and size of Gene Management Zone (GMZ) as an in situ reserve could not be determined by the genetic data presented here alone since there will be other biological, economical and adminstrative issues which sould be considered during decision making. However based on present genetic data, we could point out some potential populations as GMZ sites throuout distribution range of beech forests to help forest managers and conservation biologists in their decision making.

We applied SAMOVA for our data, which defined 4 groups of populations in Hyrcanian zone that are geographically homogeneous and maximally differentiated from each other (fig. 1). The SAMOVA approach also suggests the different groups in very close populations, e.g. populations from different elevation of region Gorgan or two populations in two sides of a valley (populations Kelardasht and Kheirud). To explain it, the presence of a very efficient barrier to gene flow, this would have prevented short-range migrations between populations from these regions.

In Beech forests, considering the genetic distances between populations and grouping pattern and genetic diversity statistics of populations (heterozygosity and mean number of alleles per locus), G-1900, K-1200, C-1300 and A-1200 could be suggested as potential GMZ populations.

However, for the final decision on Gene Management Zone selection in beech forests as in situ reserve, genetic diversity of a species is not the unique criterion. Other factors such as presence of other economically important species and degree of biological diversity in the site, accessibility and suitability of the area for in situ preservation and protection should be also taken into consideration.

Acknowledgements

This project was supported by the International Plant Genetic Resource Institute, IPGRI (project D06C Fellowships). Parvin Salehi Shanjani received Vavilov-Frankle Fellowship (2003) from IPGRI.

Table 1 Genetic variability at six microsatellite loci in 13 beech populations (sample size (N), mean number of alleles per locus (A), observed (Ho) and expected (He) heterozygosities and fixation index (Fis)).

Pop	Region	Altitude (m)	Latitude (N)	Longitude (E)	N	A (St. Er.)	Ho	He	Fis
G-1900	Gorgan	2000	36° 45'	54° 07'	33.5	7.67±2	0.55	0.624	0.063
G-1400	"	1400	36° 41'	54° 05'	60.0	8.50±2	0.57	0.58	-0.035
G-600	"	600	36° 42'	54° 06'	46.8	9.83±1.8	0.67	0.69	-0.006
N-1400	Neka	1400	36° 22'	53° 33'	46.2	11.33±2.5	0.72	0.68	-0.077
N-900	"	900	36° 29'	53° 27'	45.0	10.50±2.5	0.61	0.67	0.043
S-1900	Sangdeh	1900	36° 00'	53° 12'	36.3	11.67±3.2	0.67	0.64	-0.028
S-1400	"	1400	36° 03'	53° 14'	40.2	10.83±2.7	0.59	0.66	0.059
S-900	"	900	36° 06'	53° 16'	47.5	11.67±2.3	0.59	0.70	0.164
K-1200	Kheirud	1200	36° 32'	51° 39'	45.0	11.33±2.6	0.66	0.72	0.047
K-600	"	600	36° 35'	51° 33'	35.8	10.33±2.6	0.58	0.69	0.113
C-1300	Clardasht	1300	36° 40'	51° 10'	23.0	11±2.2	0.57	0.72	0.225
A-1200	Asalem	1200	37° 38'	48° 48'	44.8	11.3±2.4	0.59	0.65	0.028
A-600	“	600	37° 41'	48° 48'	36.8	9±1.4	0.48	0.65	0.204
mean						10.38	0.60	0.67	0.061

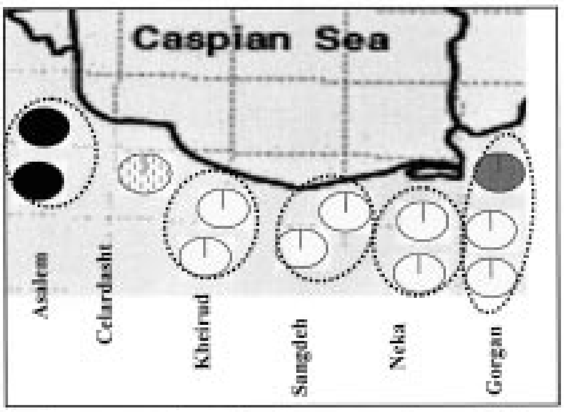

Figure 1 Presentation the map of population grouping, which defined by nSSR data .

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GEOGRAPHICAL TRENDS IN GENETIC DIVERSITY WITHIN AND AMONG POPULATIONS OF JAPANESE BEECH SPECIES, *FAGUS CRENATA* AND *FAGUS JAPONICA*

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Introduction

Two endemic beech species occur in Japan, *Fagus crenata* Blume and *F. Japonica* Maxim. *F. crenata* is widely distributed from the southern part of Hokkaido to Kyushu Island along the Japan Sea and Pacific sides. On the other hand, the distribution of *F. japonica* is restrictedly distributed from the northeastern part of Honshu Island (Iwate Prefecture) to Kyushu Island mostly on the Pacific side (Horikawa 1972). We have studied genetic diversity and structure in the two beech species by analyzing allozymes and nuclear microsatellites (SSR, simple sequence repeat) in populations distributed across most of their species' ranges. Here, we present a short review of findings from the studies, with comparing the results of the two species [Tomaru et al. (1997) and our unpublished studies] and with referring to their phylogeographic structures revealed by organelle DNA studies of *F. crenata* [Tomaru et al. (1998), Fujii et al. (2002), Okaura and Harada (2002), and an unpublished study (Takahashi, et al.)]. In the review, we discuss how the species evolve and diverse through time. Finally, we briefly describe a perspective of studies on adaptive variation in the species.

Materials and Methods

We sampled winter buds and leaves from populations distributed across most of the species' ranges of the two species. The total numbers of sampled trees and populations of *F. crenata* were 1640 trees from 23 populations for allozyme analysis and 798 trees from 23 populations for SSR analysis, while the corresponding numbers of *F. japonica* were 511 trees from 15 populations for allozyme analysis and 545 trees from 16 populations for SSR analysis.

Allozyme analysis: Enzyme extraction, electrophoresis and staining methods were similar to those described by Tsumura and Ohba (1993). The *F. crenata* trees were analyzed for nine enzyme systems and their genotypes were scored at 11 loci. On the other hand, genotypes of the *F. japonica* trees were scored at ten loci controlling a total of eight enzyme systems.

SSR analysis: Total genomic DNA was extracted from leaves and buds using the hexadecyltrimethylammonium bromide method with minor modifications. Genotypes of the *F. crenata* trees were determined at 14 loci: *mfc5, mfc11, mfc12* (Tanaka et al. 1999) and *sfc7-2, sfc18, sfc36, sfc109, sfc161, sfc195-2, sfc305, sfc378, sfc1063, sfc1105* and *sfc1143* (Asuka et al. 2004), while those of the *F. japonica* trees were determined at 13 loci excluding *sfc161*. DNA at the SSR loci was amplified by PCR. The PCR products were separated and genotypes were scored using an ABI 3100 Genetic Analyzer.

Data analysis: The population genetics analyses to assess genetic diversity within and among populations were separately conducted between the two sets of genetic markers (allozymes and SSRs) and between the two species. Results were then compared between the two species.

Results and Discussion

Allozyme variation: In both the species, within-population genetic diversity was high, whereas population differentiation was very low (Table 1). However, the comparisons in genetic diversity between the two species revealed that within-population genetic diversity was much higher for *F. japonica* than for *F. crenata*. We observed the geographical trends in genetic diversity of *F. crenata*, i.e., southwestern populations tended to have higher within- and among-population genetic diversity than northeastern populations, suggesting that the genetic diversity of *F. crenata* is largely influenced by historical shifts in distribution caused by climatic changes. In contrast, no geographical trends in genetic diversity were detected in *F. japonica*. Contrary to general expectations, the populations of *F. crenata* were not distinguished into two genetic clusters, one along the Japan Sea and one along the Pacific side, as found in the cpDNA analyses (Fujii et al. 2002).

Table 1 Genetic diversity measures estimated within and among populations of *F. crenata* and *F. japonica*

	TA[a]	H_T	H_S	H_O	F_{IS}	F_{ST}	R_{ST}	$\overline{D}_m$[b]	G'_{ST}[c]
Allozyme									
F. crenata	4.0	0.194	0.187	0.170	0.103	0.037	-	0.007	0.047
F. japonica	4.7	0.330	0.321	0.313	0.027	0.029	-	0.010	0.042
SSR									
F. crenata	27.5	0.862	0.839	0.792	0.053	0.027	0.041	0.024	0.168
F. japonica	14.9	0.673	0.659	0.641	0.026	0.023	0.025	0.017	0.072

[a] Average total number of alleles per locus

[b] Measure of absolute genetic differentiation among populations (Nei 1973)

[c] Standardized measure of relative genetic differentiation among populations (Hedrick 2005)

SSR variation: In both the species, population differentiation was very low (Table 1), in accordance with data acquired in the allozyme studies. However, for *F. crenata*, both neighboring-joining tree clustering analysis based on D_A distances and Bayesian clustering analyses with multilocus genotypes provided clear evidence of genetic divergence between populations along the Japan Sea (Japan Sea lineage) and Pacific (Pacific lineage) sides, which the allozyme study failed to detect. It suggests that physical barriers to migration and gene flow, notably the mountain ranges separating the populations along the Japan Sea and Pacific sides, have promoted genetic divergence between the populations. The two lineages of the nuclear genome are generally consistent with those of the chloroplast genome detected in the cpDNA analyses (Fujii et al. 2002). However, there were discrepancies between the two genomes in several populations, probably due to intraspecific invasion at the gene level through historical contacts followed by pollen swamping from one lineage to the other. We also observed significant genetic structure among populations of *F. japonica*, by the same analyses as of *F. crenata*, which reflected well the geographical positions of the populations.

Within-population genetic diversity was generally very high in the both species. Genetic diversity within populations of *F. crenata* decreased in a clinal fashion from southwest to northeast, as observed by the allozyme study. However, we found that the geographical trend across the species' range was largely due to the trend across populations of the Japan Sea lineage. This geographical gradient may have resulted from the postglacial recolonization to the northeast, which led to a loss of within-population genetic diversity due to cumulative founder effects. On the contrast, no geographical trends in within-population genetic diversity were detected in *F. japonica*.

A perspective of studies on adaptive variation: In *F. crenata*, the strong concordance was found between the distributions of the two lineages of its nuclear and organelle genomes and two types of beech forests ('Japan Sea type' and 'Pacific type', classified by species composition and the relative dominance and morphology of the species), which reflect the contrast in environments (especially differences in the amount of snowfall) between the Japan Sea and Pacific sides of the Japanese islands. It suggests that the differences in the lineages affect the fitness of the populations and thus may have profound evolutionary significance, presenting further challenges to address adaptation in future studies of the species.

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PHYLOGEOGRAPHY OF *FAGUS CRENATA* REVEALED BY CHLOROPLAST SNPS

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Introduction

Fagus crenata is a keystone species in cool temperate forest ecosystems in Japan and its timber is valuable. Phylogeographic studies enable evolutionary and geographic changes within species to be described in terms of geological time scales. Using this method, Okaura and Harada (2002) and Fujii et al. (2002) detected genetic differences between *F. crenata* populations. Although the genetic resources of this species has been depleted by logging, seeds are planted extensively for reforestation. As no seed transfer zones have been delineated, transfer of seeds from one region to another may result in mixing of gene pools and have unfavorable consequences. To conserve genetic resources of *F. crenata*, we determined its phylogeography using a large number of samples, which enabled us to identify appropriate seed transfer zones.

Materials and Methods

Samples (leaves or winter buds) were collected from 2525 trees in 421 locations (mean, six samples per population; range, 1–35 samples per population), comprising almost the entire distribution of *F. crenata* in Japan, which extends from Mt. Takakuma in Kyushu to Kuromatsunai Lowland in Hokkaido. DNA was extracted using a modified CTAB procedure. Fujii et al. (2002) investigated chloroplast sequence variations of the intergenic spacer between the *trn*L(UAA) 5′-exon and *trn*F(GAA) (LF) and the entire *trn*K intron (including *mat*K; K) and identified 13 chloroplast haplotypes. To identify chloroplast haplotypes, we analyzed 19 single nucleotide polymorphisms in three regions, the LF and K regions and the recently sequenced intergenic spacer between *trn*Q(UUG) and *trn*S(GCU) (QS), using SNaPshot (ABI Incorp.) and then subjected them to electrophoresis using a 3100 Genetic Analyzer (ABI Incorp.). Because of the large number of samples, only SNPs were investigated. Two haplotypes (H and J) were indistinguishable because they differed from the F and I haplotypes by a single indel. A haplotype network was constructed using Network 4.5.0.0 and 19 SNPs to examine relationships among the chloroplast haplotypes. Phylogenetic relationships among haplotypes were inferred using the neighbor-joining (NJ) method. The NJ tree was constructed using MEGA version 4 (Tamura et al., 2007) with the option of the two-parameter model of Kimura (1980).

Results and Discussion

Haplotypes: Fourteen haplotypes were detected; 11 corresponded to those reported by Fujii et al. (2002) and three (B2, F2, and N) were novel. The B2 and F2 haplotypes were differentiated from the B (B1) and F (F1) haplotypes on the basis of SNPs at the QS.

Relationships among haplotypes: The haplotype network (Fig. 1) consisted of three distinct groups, one intermediate group (D, E), and one outlier (I). The structure of the NJ tree was similar to that of the network, but the location of the branch between F2 and I differed, probably because of homoplasy and/or insufficient variation.

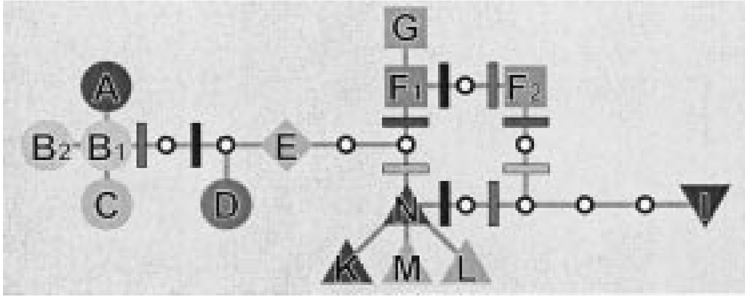

Figure 1 Haplotype network of 14 *F. crenata* chloroplast haplotypes constructed using 19 SNPs and Network 4.5.0.0.
Each node denotes a single mutation and colored boxes denote different homoplasic mutations.

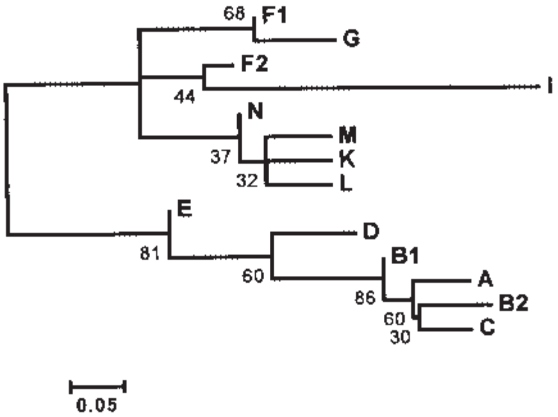

Figure 2 Neighbor-joining tree for *F. crenata* chloroplast haplotypes constructed using 19 SNPs and the two-parameter model of Kimura (1980).
Numerals at branches denote bootstrap percentages based on 1000 replicates.

Geographic distribution of haplotypes: Fourteen chloroplast haplotypes had phylogeographically structured distributions. Haplotypes A to C were distributed from the Japanese Sea region of northern Honshu to Hokkaido, haplotypes D and E occurred in central Honshu, haplotypes F1 to G were distributed along the Pacific Ocean region of Honshu, haplotype I occurred along the Japanese Sea region of southwestern Japan, and haplotypes K to L were distributed along the Pacific Ocean region of southwestern Japan. Haplotype N was scattered along the Pacific Ocean side of central Honshu.

Implications for conservation: In terms of chloroplast phylogeny, *F. crenata* populations are genetically differentiated (G_{ST} = 0.789). We observed a few chloroplast genealogies throughout the distribution range of *F. crenata*. Hufford and Mazer (2003) discussed the potential risks of introducing foreign genotypes into genetically differentiated populations. Koyama et al. (unpublished data) and Kanno et al. (unpublished data) found that the performance of nonlocal progeny is inferior to that of local progeny. The results of this phylogeographic study will be useful for establishing appropriate management guidelines for seed transfer.

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COMPARISON OF DIRECT AND INDIRECT GENETIC METHODS FOR ESTIMATING SEED AND POLLEN DISPERSAL IN OLD-GROWN AND EXPANDING POPULATIONS OF *FAGUS SYLVATICA*

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Introduction-The study of dispersal both at ecological and evolutionary timescales has gained increasing importance, due to the crucial role of dispersal in the response of populations to fragmentation and global change (Bullock and Nathan, 2008; Ronce, 2007). In tree species as in many other plants, dispersal is mainly achieved through the movements of seeds and pollen. Population expansion and persistence in heterogeneous environment depends on seed dispersal, in particular at long distance. Whereas pollen flow does not allow the colonisation of new habitats, it has been shown to widely homogenise the genetic composition of already established tree populations (Smouse and Sork, 2004).

The recent development of highly polymorphic molecular markers and of refined statistical techniques provides ecologists with efficient approaches to estimate pollen and seed dispersal from genotype data (Smouse and Sork, 2004). First, historical levels of gene flow can be inferred from spatial genetic structure (SGS). For instance, in continuous populations, the decay rate of genetic relatedness between individuals with distance has been shown to be inversely proportional to σ^2_e, the mean-squared gene dispersal distance (Rousset, 2000). Alternatively, patterns of contemporary gene flow between parental and offspring cohort can be addressed by using genetic fingerprint and parentage analyses in order to assign seedlings to their parent trees, and to infer the pollen and seed dispersal curves (Oddou-Muratorio and Klein, 2008).

Here, we investigate seed and pollen dispersal abilities of *Fagus sylvatica* in expanding and old-grown populations. Within and among sites, we compare historical estimates of gene flow using SGS, and contemporary estimates of gene flow using parentage assignment.

Material and methods-We studied three Intensive Study Plots (ISP) within two different locations. Two ISP (S1 and S2) are located in Mont Ventoux, South-Eastern France, where an expanding population of *F. sylvatica* mixed with *Abies alba* is re-colonising a Pine forest. The third ISP S3 is located in old-grown Haye Forest, North-Eastern France. Within each plot, adult trees (i.e. with diameter > 10 cm or producing seeds) were exhaustively mapped (S1: 96 trees; S2: 50 trees; S3: 350 trees), their diameter was measured (Fig. 1). Young and old seedlings were sampled and mapped (S1: 256; S2: 356; S3:221 seedlings). All individuals were genotyped at a set of either 5 (S3) or 11 (S1 and S2) highly variable microsatellite markers (Asuka *et al.*, 2004; Pastorelli *et al.*, 2003; Tanaka *et al.*, 1999).

SGS and historical estimates of gene flow- SGS was characterised by mean of autocorrelation of genetic relatedness, as measured by the kinship coefficient (F_{ij}) of Loiselle *et al.* (1995), against logarithm of geographic distance d_{ij} between every pairs of individuals *i* and *j*. An indirect estimate of the product $d_e\sigma^2_e$, where d_e is the effective population density, was obtained through: $d_e\sigma^2_e = -(1-F_N)/4\Pi b_r$, where b_r is the restricted regression slope of F_{ij} on $\ln(d_{ij})$ in the range $\sigma e > d_{ij} > 20\sigma e$, and where F_N is the kinship coefficient between neighbouring individuals (Rousset, 2000). All computations were performed with SPAGEDI (Hardy and Vekemans, 2002).

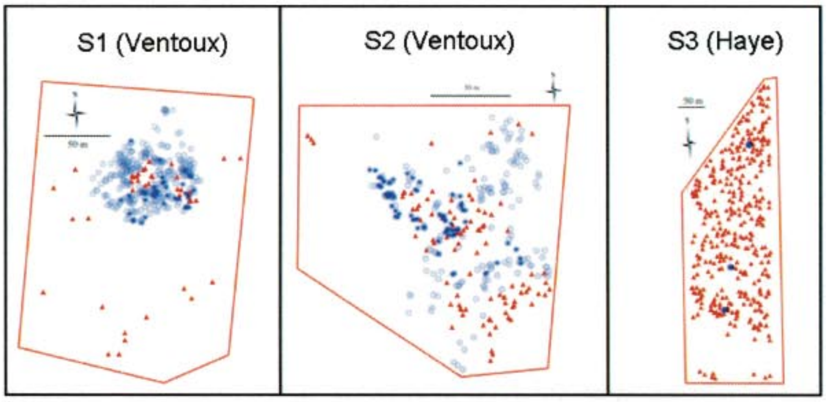

Figure 1: Sampling design within each ISP. Adult trees (▲) were exhaustively mapped within the ISP. Old seedlings (○age >4) and/or young seedlings (◉age <4) were sampled preferentially in the central part of ISP.

Neighbourhood model and contemporary estimates of gene flow- We used the neighbourhood mating model extensively described in Oddou-Muratorio and Klein (2008). We considered that each sampled seedling *i* can be mothered either (1) by a mother tree located outside the ISP due to seed immigration (with probability m_s) or (2) by a local mother tree located within the ISP (with probability (1- m_s)). In the latter case, we considered that offspring *i* may be the result either from self-pollination (with probability *s*), or from pollen flow from outside the ISP (with probability m_p), or from pollen from a sampled male (with probability $1\text{-}s\text{-}m_p$). Additionally, the seed rain contributed by each mother tree at the location of seedlings *i* is a function of the seed dispersal function θ_s, and of factors affecting female fecundity (diameter, seed production). Similarly, the proportion of pollen from a given male in the pollen cloud of a given female is a function of the pollen dispersal kernel θ_p, and of factors affecting male fecundity (diameter). To model seed and pollen dispersal kernel, we used exponential functions of parameter δ, the mean dispersal distance. All parameters were estimated simultaneously by maximising the likelihood of the whole set of seedlings genotypes (Oddou-Muratorio and Klein, 2008).

Results and Discussion

SGS and historical estimates-SGS was significant in the three ISPs, with adults/seedlings more related than expected by chance for distance up to 30-40 metres (Figure 2).

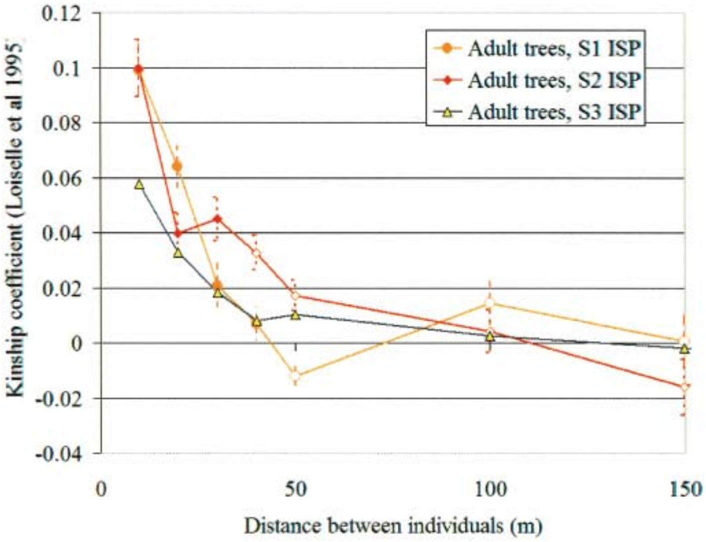

Figure 2: Correlograms of kinship coefficient for adult trees in the three ISPs. Abscise values correspond to the upper limit of the distance intervals. Filled symbols represent values significantly different from the expected value under a random distribution of genotypes (95% confidence level). Confidence intervals around each F_{ij}-value were obtained through a jackknife procedure over loci.

Using observed density d_{obs} to estimate d_e, SGS converted into axial variance of gene dispersal (σ_e) between 25 m (S1) and 45 m (S3). Fixing $d_e = d_{obs}/10$, σ_e increased to 80 m (S1) and 145 m (S3). These estimates were consistent among cohorts (i.e. between adult and seedlings on the same site).

Neighbourhood model and contemporary estimates- Direct estimates of mating and dispersal parameters are summarised in Table 1. First, important seed migration (m_s = 16% on average for young seedlings) and very high pollen migration (70% on average) from outside the ISP were detected. These results can be explained by long-distance dispersal, in particular for pollen in this anemophilous species (seeds could also be dispersed at long distance by birds). However, the high m-values are also probably contributed by different experimental biases: (1) mortality/logging of adults within the ISP could result in false migration events, as supported by high m_s-values observed in the old seedlings (m_s= 48% against 16% for young seedlings); (2) genotyping error and null alleles could also result in false parentage exclusion and overestimated migration rates.

Table 1: Selfing rate, seed and pollen migration rate and seed and pollen dispersal parameters estimated by the direct approach in the different ISP.

ISP	Pollen dispersal			Seed dispersal			
				Young seedlings		*Old seedlings*	
	δ_p	m_p	s	δ_s	m_s	δ_{s2}	m_{s2}
S1-Ventoux	8.26	65.6%	0.036	7.68	16.9%	5.64	56.2%
S2-Ventoux	28.88	69.0%	0.010	12.66	13.0%	18.1	40.0%
S3-Haye	∞	75.7%	0.040	11.55	18.3%	-	-

Regarding dispersal patterns within the ISP, seed dispersal distances were consistent across ISP, with a mean δ_s –value of 10.6 m for young seedlings. These results reflect preferential dispersal at short distance, consistently with dispersal by gravity and with secondary dispersal by small mammals. Pollen dispersal distances δ_p should be considered with caution, as they were estimated with only few seedlings finding at least one compatible parent pair within the ISP (δ_p actually could not be estimated in S3).

Comparison- Overall, contemporary estimates of total gene dispersal (σ_c) ranged from 8.3 (S1) to 26.1 m (S2). These are probably underestimates, as they do not account for migration rates. Interestingly though, contemporary (σ_c) and historical (σ_e) estimates of gene flow fell within the same range of magnitude (few tens of meters). The lower SGS and conversely higher σ_e estimated in S3 as compared to S1 and S2 could result from higher range of pollen dispersal distance, and/or from higher adult tree density, ensuring a better mixing of seed rain.

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