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INTERACTIONS BETWEEN NATURAL AND ANTHROPOGENIC IMPACTS ON THE GENETIC DIVERSITY AND POPULATION STRUCTURE OF EUROPEAN BEECH FORESTS

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Dedicated to

Merly, Leif, and Tom

THESIS SUMMARY

The accurate assessment of forest persistence under environmental change is dependent on the fundamental understanding of the genetic consequences of human intervention and its comparison to that of natural processes, as declines in genetic diversity and changes in its structuring can compromise the adaptive ability of a population. The European beech, *Fagus sylvatica*, has experienced prolonged human impact over its 14 million ha range with contemporary forests harbouring high ecological, economic, and cultural value.

Historical traditional management practices, such as coppicing and pollarding, have impacted a large portion of Europe's forests. This form of management encouraged vegetative regeneration, prolonging the longevity of individual trees. In several cases, the structure and function of managed trees and their associated ecosystems were significantly altered. Specifically, coppiced beech forests in Europe displayed significantly larger extents of spatial genetic structuring compared to their natural counterparts, revealing a change in the genetic composition of the population due to decades of management.

Humans have also aided in the dispersal of beech within and outside of its natural range. In Great Britain, the putative native range retained signals of past colonisation dynamics. However, these signals were obscured by the wide-spread translocation of the species throughout the country. Evidence of post-glacial colonisation dynamics can be found in Sweden as well. In contrast to Britain, the structure of this natural leading range edge displays a gradual reduction in population size where isolation was found to have acted as an effective barrier to gene flow reducing the genetic diversity of populations.

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INTRODUCTION

1.1 The importance of forests and genetic diversity

Forests extend over 31% (i.e. 4,033 million hectares) of global land area (FRA 2010) and are fundamental to ecosystem processes, biodiversity, and human livelihoods. This enormous distribution extends its influence into biogeophysical, hydrological, and atmospheric processes, presenting a potential resource that can be used in the mitigation of climate change (Bonan 2008). The recognition of forests as vital carbon stocks, storing an estimated total of 289 gigatonnes of carbon in biomass (FRA 2010), has prompted a move towards sustainable forestry practices aiming to reduce deforestation. Since 2000, there has been a reduction in the rate of deforestation, although it remains high with a loss of 13 million hectares per year (FRA 2010). Anthropogenic impacts remain the leading cause of deforestation, with changes in land use being the primary driver. The increasingly appreciated value of forests has led to a drive to understand how to encourage ecosystem resilience and ensure their persistence under expected climate change.

Conserving forest genetic resources is a key strategy in improving forest resilience to environmental change. The International Union for Conservation of Nature (IUCN) recognised genetic diversity as one of three forms of biodiversity (McNeely et al. 1990). Genetic diversity and its spatial distribution are primarily influenced by gene flow, genetic drift, and selection, which differ in strength according to effective population size (Loveless and Hamrick 1984). A reduction in genetic diversity can lead to inbreeding and a reduction in fitness associated with the loss of adaptive potential (Keller and Waller 2002, Jump et al. 2009). Inbreeding depression can manifest as reduced growth and higher extinction rates in plant populations (Ellstrand and Elam 1993, Keller and Waller 2002). Effective genetic resource management

requires an understanding of the factors influencing genetic diversity to identify circumstances that can lead to negative consequences and how these might be avoided or alleviated. It is essential to consider the spatial distribution of genetic diversity, as complex familial structuring driven by pollen and seed dispersal (Sokal et al. 1989) will influence the suitability of management strategies (this will be discussed further in Chapter 3).

Recent advancements in molecular technology has seen an increase in processing power allowing the cost-effective analysis of molecular markers such as microsatellites (*viz.* simple sequence repeats (SSRs)) (Selkoe and Toonen 2006). Polymorphic, selectively neutral, nuclear microsatellites typically display a large amount of variation that can be used to answer questions on parentage and population history (Beaumont and Bruford 1999, Hancock 1999). Due to the lack of recombination in the chloroplast genome, chloroplast microsatellites are less polymorphic, with haplotypic distributions often reflecting ancient colonisation processes derived from seed dispersal (Provan et al. 2001) in angiosperms, due to its maternal inheritance (Reboud and Zeyl 1994). The highly polymorphic nuclear markers can provide information on contemporary phylogeographic events. Advancements in technology have increased the potential of population genetic studies with research applied worldwide on several tree species. The next section focuses on the study species, the beech tree, *Fagus sylvatica*, and its range in Europe which has been under human influence since its Holocene migration after the last glacial maximum.

1.2 An overview of the European beech - *Fagus sylvatica*

The European beech, *F. sylvatica*, is a monoecious, deciduous broadleaf tree in the Fagaceae family. There are currently 11 recognised species in the *Fagus* genus. The exact number of species in the genus has been debated, primarily due to the low number of unique alleles in some alleged species (Shen 1992, Denk et al. 2002). *Fagus* is distributed throughout the

northern hemisphere with species within the genus displaying similar ecological traits such as shade-tolerance, masting, and sensitivity to frost and drought (Wagner et al. 2010). Three common species in the genus include *Fagus crenata*, native to Japan, *Fagus grandifolia*, native to North America, and *Fagus orientalis*, native to eastern Europe and Western Asia. Where the distribution of *F. sylvatica* and *F. orientalis* meet in the Rhodope Mountains, extensive hybridisation zones exist. However, as range overlap is limited introgression rarely occurs in the core range.

Fagus sylvatica is a keystone species defining a habitat known for its high floral and faunal diversity. It has a typical lifespan of 300 years, which can be extended through management (Read 2006). Beech is primarily outcrossing, with flowering and seed, also known as beech mast, produced after 40 years of age, typical for wind-pollinated trees (Wagner et al. 2010), and following an inherent biennial masting rhythm with intervals of up to 15 years (Hilton and Packham 2003, Packham et al. 2012). Successful cross-pollination leading to fruit set is dependent on the density of reproductive trees, as small stands of beech have been found to produce a higher proportion of empty seed coats. This varies between mast and non-mast years, with the latter showing a lower rate of successful fruit set (Nilsson and Wästljung 1987). The masting phenomenon in Beech is essential for regeneration as it satiates seed predators (Watt 1925) and controls predator abundance through starvation in non-mast years (Silvertown 1980). Predators of beech mast, such as the European Jay (*Garrulus glandarius*) (Nilsson 1985) and the nuthatch (*Sitta europaea*) (Perea et al. 2011), are also important seed dispersers.

The species distribution extends over an estimated 14 million ha (Figure 1.1). In its core range, beech is a strong competitor, being highly shade tolerant and growing on well-drained soils ranging from acidic to calcareous substrates (Packham et al. 2012). However, at the southern

range edge, its sensitivity to drought poses a risk for its persistence under predicted climate change (Jump et al. 2006, Geßler et al. 2007). *F. sylvatica* is considered a valuable model to use for examining patterns of post-glacial migration (Widmer and Lexer 2001). It has a wealth of paleobotanical evidence describing the Quaternary history of the species and its closest relative, *Fagus orientalis*, occurs in Asia Minor with minimal overlap between the species' ranges, minimising introgression.

Magri et al. (2006) presents the benchmark study for the Holocene history of beech in Europe and its migration route into the contemporary range for the species. Significant beech refugia and post-glacial spread are evaluated using a combination of paleobotanical and phylogeographical evidence. Magri et al. (2006) used a combination of nuclear isozyme

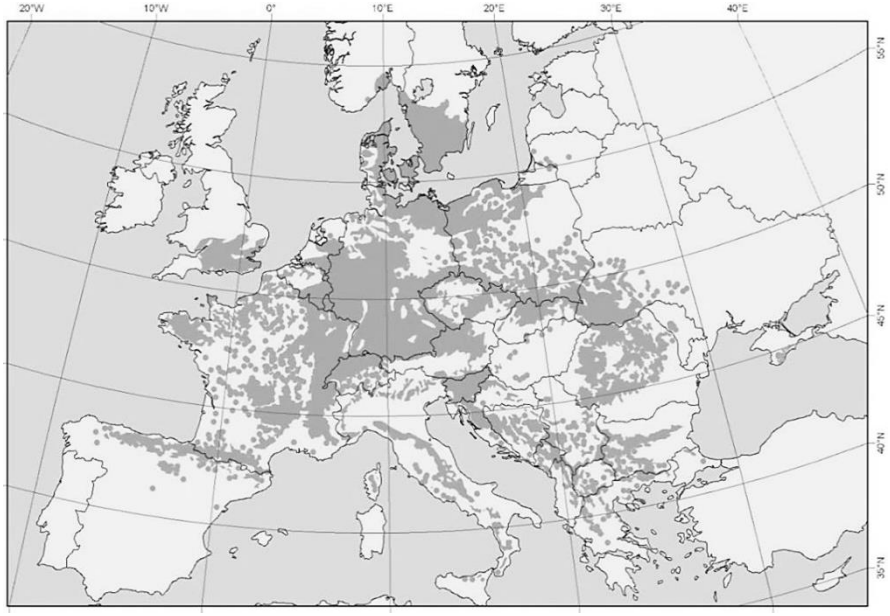

Fig. 1.1 EUFORGEN (2009) natural distribution of beech. Shaded areas show natural distribution of beech.

markers, chloroplast microsatellite markers, and chloroplast restriction fragment length markers to map genetic variation in Europe, confirming the presence of glacial refugia. Beech did not experience major range contractions during its Holocene spread, and therefore

contemporary forests are likely to be direct descendants of the populations that first colonised those areas. Paleobotanical data indicate that most of Europe was colonised from principal refugia in southern France, eastern Alps-Slovenia-Isteria, with potential refugia in Moravia and southern Bohemia. With beech in the classic southern refugia (i.e. the Iberian, Italian and Balkan peninsulas) expanding relatively late and thus not contributing to the colonisation of central and northern Europe. Southern and western populations displayed a complex distribution of genetic variation, indicating the presence of genetically differentiated populations in southern France and in the Iberian, Italian, and Balkan Peninsulas. The remaining beech distribution in central, eastern, and northern Europe were relatively homogenous in genetic structure. Contrary to several plant and animal species (Taberlet et al. 1998), mountain ranges did not present a significant barrier to the post-glacial migration of beech, which instead expanded along slopes of prominent mountain chains, such as the Alps. Recent palynological evidence suggests that small populations of temperate trees, including beech, established ahead of the main colonisation front through long distance dispersal events tracking suitable climatic conditions and habitats (Overballe-Petersen et al. 2013).

The range of beech at a regional scale is believed to be under broad climatic control (Huntley et al. 1989), with sensitivity to drought and late frosts generally limiting the southern and northern distribution, respectively. However, due to the late arrival of beech in Great Britain and its translocation throughout the country, the extent of its native range remains uncertain (this will be discussed further in chapter 4). Birks (1989) presents the most comprehensive palynological work on temperate tree migrations into the British Isles during the Holocene. Palynological data indicate that beech arrived in Great Britain relatively late, about 3000 BP, and the width of the isochrones indicate that it maintained a constant rate of spread. Its widespread natural regeneration throughout the country has led to speculations that it may not have reached its climatic limit before human intervention (Packham et al. 2012). Pollen

records have found an association with beech colonisation and human activity which may be influencing the patchy dynamic colonisation front seen in some regions, such as southern Sweden (Björkman 1997, Küster 1997, Bradshaw and Lindbladh 2005) (this will be discussed further in chapter 5). However, it has been argued that patterns of colonisation dynamics may primarily be a product of the ecological traits of this late successional tree, displaying inherent slow migration and establishment rates, which has resulted in its spread coinciding with anthropogenic activity (Gardner and Willis 1999, Giesecke et al. 2007).

Fig. 1.2 Examples of different forms of beech forest. LEFT: Semi-natural beech forest - Spessart Mountain Range, Germany. MIDDLE: Ancient Beech Coppice - Montage de Lure, France. RIGHT: Wood pasturage with Pollards - New Forest, United Kingdom. [Photographs by M. J. Sjölund]

The majority of forests in Europe today are classed as semi-natural (FOREST EUROPE and UN/ECE-FAO 2011) with many experiencing prolonged traditional management (an in-depth review on these practices is given in Chapter 2). Traditional management practices include coppicing and pollarding, both of which exploit the vegetative regeneration of an individual tree through the repeated cutting of stems (Evans 1992). Managed trees could be found in cultural landscapes such as wood pasture, where farm animals grazed and fed on the beech mast crop (Figure 1.2). Long-term management has often resulted in habitats with high structural complexity that have been recognised for their conservation value (Fuller and Warren 1993). Beech trees were widely coppiced throughout Europe in the past, maintaining populations primarily through vegetative regeneration and using harvested stems as a source

of fuel and building materials (Nocentini 2009, Read et al. 2010, Packham et al. 2012) (the genetic consequences of coppicing will be discussed further in Chapter 3).

1.3 Thesis outline

Improving future beech forest persistence rests on having an understanding of how past and current forest management practices affect the genetic diversity and the spatial genetic structuring at stand to regional levels, as a loss in genetic diversity and changes in genetic structure can compromise the ability of beech to persist under changing environmental conditions. Natural systems can provide insight into studies on anthropogenic impacts as they act as controls, providing useful comparisons to managed systems. The research presented in this thesis forms part of a larger, interdisciplinary, EU-wide project called Beech Forests for the Future (BEFOFU) that aims to synthesise information on the ecological, economic, and policy aspects of beech forest protection in Europe. BEFOFU focuses on beech forests which form part of a network of protected areas, the Natura 2000 network, designated under the EU Habitats and Birds directives. Chapters 2 to 5 present research undertaken in this thesis, which encompass various spatial scales, exploring stands with natural and human influenced histories to understand the factors shaping the forests in Europe, with specific focus on the population genetics of beech.

I will now present an outline for each chapter. Details of each chapter and a summary of research outcomes and implications are given in Table 1.1 at the end of the introduction. Chapters are presented in manuscript format; chapters 2 and 3 are published in scientific journals, with the intention to publish chapters 4 and 5 in the near future.

1.3.1 An in-depth review of traditional management in Europe

Chapter 2 gives a review of the literature on traditional forest management practices that exploit vegetative regeneration, with particular focus on European forests. Practices, such as coppicing and pollarding (see section 1.2) were historically common, suffering declines in the nineteenth century due to socio-economic changes, such as decreases in the demand of fuelwood commonly produced through coppicing (Evans 1992). Coppiced trees were cut at ground level or head height to produce shoots harvested for several uses (Harmer and Howe 2003). The habitats created from traditional management are now recognised as areas of high conservation value being rich in biodiversity as a result of their high structural complexity (Rackham 2008). Research on these widespread historical forest systems was surprisingly sparse and the review presents the first in-depth study considering the benefits and implications of these historically pervasive practices. Information from molecular to ecosystem level was synthesised, drawing on research and historical knowledge to assess the viability of vegetative regeneration as a tool for improving forest persistence in unfavourable environmental conditions. Management recommendations and suggestions for future research are given.

1.3.2 Genetic impacts of maintaining vegetative regeneration

Chapter 3 reveals the effects of coppicing on the spatial genetic structure and diversity in beech. Despite the widespread management of beech forests as coppices in Europe, research exploring the consequences of maintaining these forests through prolonged vegetative regeneration was severely lacking. As coppicing alters the primary regeneration pathway within a stand, it is expected to alter the level and structuring of genetic diversity within populations. This research differed from other studies in the past as it employed pairwise comparisons, isolating the effects of coppice management by comparison with nearby unmanaged stands. Coppice beech forests were found to be as rich in genetic diversity as their

high forest counterparts. However, spatial genetic structure extended up to 10m - 20m further in coppiced stands. While relatively small in magnitude, these differences indicate that local-scale patterns of geneflow were significantly altered by generations of forest management. The outcome of this research has implications for genetic resource management on a spatial scale and provides information that is particularly useful to those who manage the large fraction of previously coppiced semi-natural forests in Europe.

1.3.3 Exploring the impact of historic anthropogenic translocations

Chapter 4 reveals the genetic impacts of historic translocations of beech throughout Great Britain. British beech forests are particularly interesting as they grow further north than their putative native range, which is commonly thought to be restricted to the south-east of Britain (Figure 1.1). Genetic studies on the regional range of beech in Britain are lacking and the genealogical histories of contemporary populations are generally uncertain. By grouping sites using *a priori* stand origins (based on pollen data and written forest history), potential native sites revealed the persistence of cryptic signals of population colonisation such as the presence of isolation-by-distance and high haplotype richness in the south-east. Extensive planting and movement of plant material had diminished this signal in the non-native range. Gene flow between sites has resulted in cryptic genetic structuring following a distinct regional trend that approximately adheres to isochrone borders. South-westerly populations displayed similar clustering patterns to the native range, indicating significant gene flow between these regions. The genetic aspect of this research complements palynological evidence for the Holocene migration of beech into Britain (Birks 1989) and historical evidence indicating the existence of potential native forests (Rackham 1980). The research suggests that boundaries between the native and non-native range are not as clear as previously thought, having several implications for the future management of beech.

1.3.4 Long-term isolation at the range edge of the species

Chapter 5 reveals the interacting effects of colonisation dynamics and persistent isolation on beech forests. Southern Sweden presents a natural gradient of isolation at the northern range edge of the species. Several studies on fragmentation and isolation lack biologically meaningful measurements of isolation. An accurate index of isolation was obtained for this study by georeferencing an exceptionally detailed historical map of Swedish beech distributions (Lindquist 1931), allowing the isolation levels of different study sites to be defined using both area- and distance-based measures. Past colonisation dynamics, as well as founder effects and genetic drift in small, isolated, marginal populations led to a general decrease in genetic diversity and distinct clustering of populations. A south-westerly cluster was found to be concomitant with the initial expansion of beech into Sweden as defined by palynological evidence (Bradshaw and Lindbladh 2005). Further sub-structuring was found in the north-eastern clusters which were colonised later, compared to the south-westerly population, and displayed higher levels of isolation. A reduction in genetic diversity and gene flow between isolated sites was supported by the reduced levels of external pollen dispersal in isolated populations, which we suggest is due to lower densities of surrounding forest patches and hence lower density pollen clouds surrounding isolated sites. The results agree with theoretical predictions of the effects of isolation on genetic diversity and builds upon palynological evidence for Holocene beech migration into Sweden.

1.4 Summary

Many European forests have been shaped by humans for prolonged periods of time. The European beech tree has been a part of human livelihoods since its post-glacial migration during the Holocene due to its many uses and its subsequent frequency throughout the continent, creating a species rich in cultural, economic, and ecological value. The semi-natural range of beech presents several possibilities to study the impacts of natural processes and

human activities on population genetics. These impacts shape genetic variation at local to regional scales. The thesis is structured to present local scale effects first, focussing on stand management, followed by regional trends in beech forest. The next chapter will explore the long history of traditional management of forests, providing information on how historic silvicultural practices could be used to reduce the impacts of increased drought at the southern-range edge of a species.

Table 1.1 Summary of research output of chapters 2 to 5

Chapter	Style	Publication details	Scale	Region	Plots #	Loci #	Sample #	Research Findings and Implications
2	Review paper	Forestry (2013) 86 (5): 503-513. DOI: 10.1093/forestry/cpt030	Molecular to ecosystem	Europe	-	-	-	• Historic management shaped structure and function of forests from a genetic to ecosystem level. • Effects of management vary with species/site specific traits. • Systems in drought-prone areas may benefit from the use of traditional practices to promote canopy and soil cover and hence regeneration.
3	Research article	Forest Ecology and Management (2015) 336, 65-71. DOI: 10.1016/j.foreco.2014.10.015	Local	Italy France Germany	6	11	812	• Coppice forests are as rich as natural forests in genetic diversity. • Familial structures extend further in coppices. • Highlights importance of considering spatial aspect in resource management.
4	Research article	Will be submitted to Molecular Ecology	Regional	Great Britain	42	10 N 3 Cp	840	• Cryptic regional genetic structure occurs throughout Britain • *A priori* knowledge on stand origin allows signatures of the post-glacial colonisation in natural range to be detected. • Sites outside putative native range are connected with natural populations through gene flow blurring boundaries between ranges.
5	Research article	Will be submitted to Molecular Ecology	Regional	Sweden	14	11 N	1400	• Colonisation dynamics in beech persist in genetic structure. • Isolation decreases genetic diversity, through a reduction in gene flow with particular effect on pollen dispersal. • Confirms palynological data on beech. • Identifies consequences of isolation and value of using combination of area- and distance-based indices of isolation.
Total	**-**	**-**	**-**	**Europe**	**62**	**11 N 3 Cp**	**3052**	

Descriptions of research chapters are given, including the number of plots, loci (indicated as 'N' for nuclear and 'Cp' for chloroplast loci), and samples. Published papers are indicated with relevant DOI numbers. Research findings and implications are given for each chapter.

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Chapter 2

THE BENEFITS AND HAZARDS OF EXPLOITING VEGETATIVE REGENERATION FOR FOREST CONSERVATION MANAGEMENT IN A WARMING WORLD

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2.1 Abstract

Forest management practices in European temperate and Mediterranean regions have frequently exploited coppicing and pollarding - two silvicultural techniques that promote vegetative regeneration. These practices were historically very common with trees being cut at ground level or above the level of browsing to produce shoots that were harvested for a variety of uses. Many habitats created from such traditional management are now recognised as areas of high conservation value, being rich in biodiversity. Yet their persistence has been under threat after these practices suffered a decline in the 19th century. The focus of this review is to synthesise information on coppicing and pollarding from the ecosystem to the molecular level and to highlight characteristics that may help or hinder climate adaptation. Understanding the benefits and hazards of exploiting vegetative regeneration is the first step in assessing whether promoting this means of reproduction could be exploited for conservation by increasing forest persistence in unfavourable future climate conditions. Practical management recommendations are given and suggestions are made for future research.

2.2 Introduction

Climate change brings about a new impetus to understanding the consequences of different forest management practices for forest persistence (IPCC 2001, Millar et al. 2007). Promoting forest persistence, in this review, is defined as encouraging the presence of an adaptable forested system and avoiding major changes in species composition instigated by climate change. There is a need to encourage forest resilience both for benefit of reliable resource provision and for conservation and society (Bonan 2008, Allen et al. 2010). Knowledge on the ways in which management affects forests from the molecular level to the ecosystem level is essential to allow reliable risk assessment and to plan appropriate adaptation strategies. This is of particular importance to European forests, many of which have been subjected to profound and long-term anthropogenic intervention (Bradshaw 2004) inevitably altering the ecological and genetic composition of forests and creating a cultural landscape, rich in heritage value. In the temperate and Mediterranean regions, past forest management has been highly reliant on coppicing and pollarding, two forest systems dependent on vegetative regeneration.

Repeatedly coppicing or pollarding a tree, if performed correctly, can result in trees having a significantly longer lifespan than their naturally grown counterparts as trees are kept in a partially juvenile state (Blake 1980) (Table 2.1). For example, Rackham (1986) described ancient *Fraxinus excelsior* L. (European ash) coppice stools (the regenerating stump which gives rise to shoots) occurring on waterlogged sites which were found to be thousands of years old, in contrast to the normal lifespan of *F. excelsior* which is around 200 years. Whilst, Read (2006) reported the presence of pollarded *Fagus sylvatica* L. (European beech) over 500 years, living twice as long as maiden trees that have a lifespan of 200-250 years. These forms of management provided materials which were suited to past social and economic needs but also encouraged the coexistence of early and late successional species. Long-rotation coppice

systems and pollarding in wood-pasture (an open forest structure with a grazed understory) were historically common and shoots were harvested on a rotational basis for uses including fuel wood, animal fodder, crafts, and building materials (Read 2000). Although there are still areas, mainly in Southern Europe, where these traditions remain alive, many have suffered a decline during the 19th century, primarily due to changes in market demand for forestry products posing a threat to the existence of these habitats (Agnoletti and Paci 1998, Watkins and Kirby 1998, Bürgi 1999, Harmer and Howe 2003, Petit and Watkins 2003, Hopkins and Kirby 2007, Rackham 2008). When actively managed, a variety of associated species often benefit from the high level of habitat heterogeneity arising from the contiguous panels (sections of a coppice that differ in their stages of succession), age since last cutting, and vegetation height (Evans and Barkham 1992, Fuller and Warren 1993). Consequently, many coppiced and pollarded habitats have been recognised as areas of high conservation value, prompting a call for the revival of traditional management today (Rackham 1980, Peterken 1992, 1993, Harmer and Howe 2003). The value of cultural landscapes in terms of their biodiversity, genetic resource value, historical, and aesthetic value needs to be identified before they are lost, considering that the economic implications of re-introducing traditional management is a major barrier to their conservation, which may only be achievable for high priority populations where costs can be subsidised (Jump et al. 2010).

Considerable evidence links contemporary climate change to recent range shifts in species distributions (Parmesan and Yohe 2003). While warmer temperatures are allowing expansion of tree populations upwards in mountain regions and towards the poles, higher temperatures and increased drought stress can lead to the disappearance of low-latitude, rear-edge populations (Allen et al. 2010). Populations which persist at the rear-edge include relict populations and those which occurred in or around glacial refugia (Bennett et al. 1991). These populations are highly important reservoirs of intraspecific diversity, often being unique in

Table 2.1 Summary of traditional forest management systems and their regeneration mechanisms

System	Management Description	Regeneration mechanism
Coppice with standards	Two-storey forest structure; coppice underwood with standard trees of maiden or coppice origin. Standards retained for 3-8 cycles. Can be worked on a long rotation of approx. 10 - 20. Single species or different species of coppice and standards (Evans 1992).	• Vegetative by off-shoot growth or root-suckering. Natural regeneration limited by length of rotation and contribution to gene pool skewed by cutting of age classes. Reproductive age and size will significantly limit seed contribution to regeneration. Natural regeneration from standards is mainly limited by competition with vegetative shoots.
Simple coppice	Single or mixed species crop, worked on same cycle (even-aged) (Evans 1992).	• Mainly vegetative by off-shoot growth or root-suckering. Period of natural regeneration limited by length of rotation. Reproductive age and size limits seed contribution to regeneration.
Short-rotation coppice (SRC)	Worked on <10 year rotation. Provided material for rural crafts but recent surge of interest in high intensity production for energy biomass using fast-growing species (Evans 1992).	• Similar to simple coppice, however reproduction is further limited by shorter rotation length.
Coppice selection system	Worked on 8-12 year rotation, usually three age classes, with shoots of different age/diameter on same stool. At each rotation, larger shoots harvested and remaining shoots lightly thinned, maintaining canopy cover at all times (Coppini and Hermanin 2007).	• Similar to simple coppice, although the creation of gaps for natural regeneration reduced by maintaining canopy.
Stored coppice	Lapsed cutting cycle resulting in over-mature and/or abandoned coppice. Might contain standards depending on prior system. Structure resembles that of high forest (Evans 1992).	• Similar to simple coppice, with vegetative or natural establishment comparable to that in natural high forest gap dynamics.
Wood pasture	Stem cut well above ground level at a point that prevents animals from browsing shoots. Mostly pollards present. Standards sometimes present at low densities. Heavy browsing of understorey creates distinct browse-lines (Evans 1992).	• Natural regeneration heavily limited by browsing but not in competition with vegetative sprouts. Unlike coppice, horizontal clonal spreading is limited. Cutting limits contribution to gene pool.

terms of genotypic composition and/or diversity (Petit et al. 2003, Hewitt 2004, Hampe and Petit 2005, Magri et al. 2006, Hampe and Jump 2011).

Increased drought in the low latitude range edge of a species has been shown to have a negative effect on seedling survival as well as adult growth (e.g. in *Quercus ilex* L. (holm oak) (Perez-Ramos et al. 2010), *F. sylvatica* (Jump et al. 2006, Silva et al. 2012), and *Phillyrea latifolia* L. (Lloret et al. 2004)) further threatening the persistence of such populations under climate change (Jump et al. 2010). A significant proportion of these range edge populations have experienced prolonged and widespread coppicing in the past (Nocentini 2009). The 2000 Global Forest Resource Assessment reported approximately 25 million hectares (14% of total forested area) of coppice in Europe (excluding the Russian Federation) (UN/ECE-FAO 2000). This estimate includes all forests composed of stool-shoots or root suckers with or without standard trees. However, the State of Europe's Forest 2011 reported a much lower area of approximately 2.9 million hectares (FOREST EUROPE2011). It should be noted that this latter figure refers to the forest undergoing active coppice regeneration, and therefore does not include areas of historically managed forest that are no longer under active coppice management that are included in the earlier estimate. Both reports agree that coppice is most common in Southern and Central Europe. Research into the persistence of stable rear-edge populations through means other than recruitment, such as through longevity, clonal growth, and persistent seed banks is essential for their conservation (Hampe and Petit 2005). The long-term survival of plant populations can be greatly determined by their ability to employ traits conferring longevity and/or vegetative reproduction in unfavourable environments (Bond and Midgley 2003). For example, *Populus tremula* L. (common aspen) has been observed regenerating through purely vegetative means on the Dutch Wadden island of Treschelling where harsh sea winds inhibits vertical growth and germination (Koop 1987). This response to disturbance and site productivity has been subsequently found in several other forest systems

(Kennard et al. 2002, García and Zamora 2003, Bellingham and Sparrow 2009, Papalexandris and Milios 2010, Nzunda and Lawes 2011).

Vegetative regeneration presents an alternative regeneration pathway that can be used to maintain existing trees in a forest, facilitating the adaptation of associated species by avoiding substantial changes in species composition, and therefore promoting current forest persistence. Traditional management techniques, such as coppicing, can be used to increase forest persistence in unfavourable climatic conditions, where mature trees are being lost faster than they can be replaced by natural regeneration, as forest cover can be maintained, thereby maximising the chance that new individuals can establish before existing adult trees are lost. It should be stressed that the suitability of a given management strategy will differ with local climate. For example, in areas which are more likely to be affected by drought, keeping an overstorey of high forest and promoting a coppice understorey would provide canopy cover and soil cover. Consequently, the coppice selection system (Table 2.1) was developed in Southern Europe since it maintains canopy cover and hence protects the soil from excessive heat exposure (Coppini and Hermanin 2007), whereas rotational coppice systems which would open up large areas of the canopy and hence expose the soil would be less suitable.

Vegetative sprouts have competitive advantages over seedlings originating from seed mainly due to their access to an established root system and hence greater water and nutrient availability (Lloret et al. 2004, Zhu et al. 2012). The survival benefits of sprouting in trees has been increasingly recognised in natural systems (Pigott 1992, Bond and Midgley 2001, Lawes and Clarke 2011, Zywiec and Holeksa 2012, Clarke et al. 2013) but research exploring the contribution of vegetative reproduction in traditional management is still scarce (see Milios (2010) for information on exploiting sprouting in traditional shelterwood systems). In cultural

landscapes, sprouting ability is maintained through regular management; therefore the dynamics of vegetative regeneration are different to that of a natural unmanaged population of the same species.

The focus of this review is to synthesise available information on coppicing and pollarding and to explore how traditional forest management practices in Europe might be exploited for conservation purposes in a changing environment. Understanding the benefits and hazards of exploiting vegetative regeneration is the first step in assessing whether promoting this means of reproduction could be used to increase forest persistence in less favourable future climate conditions. The effects of forest management systems that exploit vegetative reproduction are considered from the ecosystem to the molecular level and characteristics that may help or hinder climate adaptation are highlighted.

2.3 Biodiversity in traditionally managed forests

Habitat heterogeneity in coppice and pollard systems is a key factor promoting high floral and faunal diversity. However, the success of an associated species can vary depending on the characteristics of the site including the tree species and management system. Coppice or pollard management of individual trees increases the structural complexity of trees and hence shapes the structure of the habitat as a whole. The biodiversity of coppice and pollard systems has been well-studied and numerous taxa, including small mammals, breeding birds, understorey plants, saproxylic invertebrates and epiphytes, have been reported to benefit from the abundance of microhabitats arising from the multi-stemmed growth form of the trees and the continuity of ages within an area (Mitchell 1992).

Many wood-pasture habitats are known for their diversity of plants, lichens, birds, beetles, and snails (Tucker and Evans 1997, Bergmeier et al. 2010). Slow-colonizing epiphytes are able

to exploit the minute changes in aspect and moisture gradients on the aged bark surface of pollards (Moe and Botnen 1997, Fay 2004). Microhabitats in the rotten heartwood of ancient pollards support several saproxylic beetles (Kirby et al. 1995, Desender et al. 1999, Taboada et al. 2006, Dubois et al. 2009). In contrast, coppice systems have a lower volume of deadwood and here, late successional species will benefit from more mature trees and deadwood as found in abandoned coppice (Greatorex-Davies and Marrs 1992). However, it should be noted that it can take several decades to develop the continuity of deadwood required for a rich deadwood fauna (Kirby 1992, Peterken 1992).

Regular but low-level disturbance in coppice and pollard systems, such as the creation of gaps in the forest structure and soil disturbance from harvesting stems, generate potential colonization sites (Evans and Barkham 1992) and prevent the dominance of a few shade-tolerant plant species which would otherwise dominate under non-intervention (Barkham 1992, Baeten et al. 2010). In regions prone to drought, however, heat exposure in canopy gaps and hence an increase of temperature at ground level can be detrimental to plant germination. Management systems, such as selective coppicing, exist which have been adapted to this climate (Coppini and Hermanin 2007). Jacquemyn et al. (2009) found that canopy closure and changes in disturbance regime after the conversion of coppice to high forest can affect the genetic diversity of an associated species, *Orchis mascula* L. (early-purple orchid), by reducing population sizes and increasing fragmentation.

The diversity of ground flora in coppice is mainly driven by the rotational cutting of stands which provide cyclic variations in light, moisture, and nutrient content (Kirby 1990). A rich mosaic of stem age classes in actively managed coppice can benefit early, as well as late, successional species. Butterflies generally prefer the clearings provided by young coppice panels and rides where larval-food plants are able to grow. *Melitaea athalia* Rott. (heath

fritillary butterfly) in Britain has become highly associated with coppice after centuries of management and population declines have coincided with the decline in coppicing (Warren and Thomas 1992, Hopkins and Kirby 2007). Rotation length has been found to influence bird and small mammal diversity by altering structural habitat components such as undergrowth development in coppice (Fuller 1992, Fuller and Henderson 1992, Gurnell et al. 1992, Fuller and Green 1998). However, structural components which increase certain bird species can differ according to region (Quine et al. 2007). Fruit and seed from standard trees are likely to be an important source of food for birds and small mammals, and can therefore influence species richness and diversity between different coppice management systems (Gurnell et al. 1992).

2.4 Impacts on tree growth and survival

Determining what changes occur in the physiology, anatomy, and morphology of managed trees can shed light on their potential to endure environmental change. Changes in tree growth due to a response to management can be seen at two stages after coppicing or pollarding; (1) shortly after cutting, where changes are observed in the shoot regrowth; or (2) after a prolonged period post-cutting, where the regrowth has aged considerably, in which case the combination of management followed by abandonment brings about growth changes.

Direct impacts on tree physiology (changes occurring shortly after cutting) have been detected in *in situ* and *ex situ* experiments on coppice. An experimental study which simulated a 15% reduction in rainfall in thinned and unmanaged Mediterranean mixed coppice of *Q. ilex* and *Quercus cerrioides* Willk. & Costa in Spain, found species specific differences in response to drought (Cotillas et al. 2009). Thinning essentially involved the re-introduction of the coppice selection system, which was historically common in the area. Only the deciduous *Q. cerrioides*

suffered decreases in relative height growth rate with reduced rainfall. This was mainly attributed to differences in leaf habit allowing sclerophyllous evergreen oaks such as *Q. ilex* to reduce overall water losses. Other studies on *Q. ilex* have also reported the benefits of thinning on stem and stool survival as competition is reduced, hence improving growth (Ducrey and Toth 1992, Mayor and Rodà 1993, Cañellas et al. 2004). Cotillas et al. (2009) also found that thinned plots had higher soil moisture levels compared to unmanaged plots, possibly due to diminished rain interception and canopy transpiration. Although this study revealed many positive effects of thinning on stools, the magnitude of these effects diminished over the three-year experimental period due to increasing competition for below-ground resources resulting from vigorous re-sprouting of shoots after thinning. Observations that soil water content increased after coppicing, in a coppice-with-standards system, were reported by Salisbury (1924). A similar interaction between drought and coppicing on soil water content was found in Swanton Great Wood, an ancient forest in the UK dominated by *Tilia cordata* Mill. (small leaved lime) and *Corylus avellana* L. (European hazel) historically managed as a coppice-with-standards (Cummings and Cook 1992). Cummings and Cook's (1992) study revealed similar results to that of Cotillas et al. (2009); only in dry years was soil water content significantly higher under recently coppiced plots (cut 3 to 5 years prior to the study) compared to older plots (cut 9 to 11 years before). In addition, surface soil water content depleted faster under higher densities of stools (approx. < 2000 stools/ha) before canopy closure. In systems where water is limited, thinning out the stems on stools could be used as a conservation measure to improve the survival rate of coppiced trees. However, long-term benefits can diminish in species which are vigorous sprouters and occur in high density populations. Potentially, controlled grazing may be able to substitute the effects of thinning as it limits inter-stem competition (Tanentzap et al. 2012). While tree species' characteristics and forest structure will affect how a particular forest responds to management, economic factors such as the demand for coppice products, viability of grazing, and availability of skilled labour

will have a major influence on whether re-introducing traditional management will be sustainable in the long term.

Several studies conducted on traditional coppice systems provide an insight into the changes in structure and function of over-mature coppice (where management has ceased and the coppice has been left to mature beyond its regular cycle) (see 'Stored Coppice' in Table 2.1). Observations that droughts in the Mediterranean basin seem to affect over-mature, abandoned coppice stands disproportionately has sparked research into the effects of climatic stress on these forests (Corcuera et al. 2006, Di Filippo et al. 2010). Age dependent responses to climatic stress which altered the wood anatomy and survival of *Quercus pyrenaica* Willd. (Pyrenean oak) coppiced trees in the Mediterranean were found by Corcuera et al. (2006). Stems of ageing coppiced trees had tree rings with proportionally more earlywood and consequently less latewood compared to that of unmanaged trees. The coppice stems produced very narrow tree rings, typically composed of earlywood vessels which were a single cell thick. Their production increased exponentially with age, reducing radial growth and leading to a minimal increase in stem perimeter. Over-mature coppice was therefore more vulnerable to climatic stress and xylem cavitation due to the lower proportion of latewood vessels, known to be less vulnerable to embolism. This greater drought susceptibility is reflected in the reports of *P. deltoides* - SRC's grown under elevated CO_2 conditions which were also more susceptible to xylem cavitation, displaying lower wood densities coupled with high stomatal densities (Bobich et al. 2010). However, it should be noted that the intensive management of SRCs differs significantly to that of traditional coppice (for more information on SRCs see Oliver et al. (2009)).

Further studies on the interaction of elevated CO_2 with stand management in species such as *Q. pyrenaica* are crucial, especially if stands have a predisposition to increased xylem

cavitation due to their maturity (Corcuera et al. 2006, Bobich et al. 2010). As earlywood vessels are linked to hydraulic conductivity, a decrease in their number will reduce carbon assimilation and hence growth. Since earlywood vessels form around the perimeter of the previous tree ring this decrease results in a reduction in latewood width and tree ring perimeter in over-mature trees, which are subsequently unable to augment the number of earlywood vessels produced during the growing season (Corcuera et al. 2006). Over-mature coppice stems, like those in younger coppice (Cotillas et al. 2009), have been shown to have increased radial growth after thinning (Ciancio et al. 2006, Corcuera et al. 2006). However, this growth increase was found to be dependent on site differences with changes being more pronounced in the mesic site where thinned trees formed more latewood and multiseriate tree-rings than over-mature trees. Tanentzap et al. (2012) found that the growth and survival of *C. avellana*, *Crataegus laevigata* Poir. (midland hawthorn), and *Crataegus monogyna* Jacq. (common hawthorn) increased in multi-stem growth forms. However, under conditions of high inter-specific competition, i.e. after a reduction in grazing pressure, stem survival declined as the number of stems increased within a multi-stemmed tree due to high intra-stem competition for resources, indicating that resources do not increase linearly with stem number. Continued management or thinning out the stems of stools could be used as a means to decrease over-mature coppice or pollard susceptibility to drought. However, the age and species of the stand should be carefully assessed as some stands may contain trees which will not respond positively to resuming management. If the time since the last cut is unknown, a trial cut should be performed to assess responsiveness to management re-introduction.

Continued management of coppiced and pollarded trees is essential to ensure their long-term survival. Panaïotis et al. (1997) found age-related trends in survival in over-mature *Q.ilex* coppice where the senescence of the original stump led to the death of the whole tree. The rate of re-sprouting arising naturally, not from management, was found to be too low

compared to the rate of stool deterioration to guarantee the persistence of the forest without a re-introduction in management. Furthermore, many naturally arising stems had well developed rot in the heartwood. The area where sprouts originate from on a stool determines whether the shoots will suffer from heart rot. Re-introducing management and cutting close to the ground layer will encourage re-sprouting at the collar of the stool, thus reducing heart rot and stem loss (Tredici 2001, Harmer and Howe 2003). Bacilieri et al. (1994) have reported that present day *Q.ilex* coppice in Southern France is also under threat of disappearing without management re-introduction, as they are being recolonized by the naturally occurring Q*uercus pubescens* Willd. (downy oak). Abandoned coppice stands were characterised by improved germination rates for Q*. pubescens*, which indicated the start of succession towards naturally dominant Q*. pubescens* forest. In these areas, reproducing past anthropogenic activities, such as clear-cutting and fires increased the resilience of *Q.ilex* coppice.

Leaving a coppiced tree or pollard to over-mature is generally known to be detrimental to their health and a decrease in survival rate as time since management increases has been reported in the literature (Mountford et al. 1999, Read 2000, Fay 2002, Harmer and Howe 2003). With the establishment of restoration programs for abandoned coppice and pollards, it has become increasingly recognised that re-sprouting ability of an abandoned tree declines with age since the last cutting (Read 2000, Coppini and Hermanin 2007). Tree species and site fertility may influence re-sprouting ability; however empirical evidence which identifies the physiological or anatomical reasons for this decline in responsiveness to management is scarce. Unmanaged pollards are more vulnerable to crown collapse and crown die back which is likely due to the excessive weight from the overgrown poles and can lead to tree death (Fay 2002, Rozas 2004, Rozas 2005, Read 2006, Read et al. 2010). In Britain, Burnham Beeches - a site of high conservation value derived from its wealth of ancient pollards, pollards were being lost at a rate of 10 trees per year, which would have rendered the population of 574 pollards

extinct in 57 years without intervention (Read et al. 2010). Rates of tree loss from mechanical failure in other forests in Britain have been reported at 5-10% per annum (Fay 2002). Significant damage to pollards by the invasive *Sciurus carolinensis* Gmelin 1788 (grey squirrel) has become a problem in certain forests of high conservation value in Britain (Mountford et al. 1999, Read et al. 2010). It has been suggested that pollards are less susceptible to pathogenic agents or decay fungi as the production of multiple branches and swelling around the top of the bolling (the ageing main stem which remains after cutting) limit the spread of infection (Read 2000). Since the tradition of pollarding has largely declined, so have studies on the effects of pollarding, making it difficult to determine any causal relationships. Responses to a re-introduction of pollarding will likely differ between species and sites, therefore information from research trials is essential for the construction of effective management guidelines.

Pressures that limit germination and regeneration in coppice and pollard systems include intense grazing and canopy closure (Ratcliffe 1992). Rozas (2004) found that a combination of these two factors significantly decreased regeneration in abandoned pollarded *Quercus robur* L. (pedunculate oak) forests in La Isla park of Tragamón in Northern Spain . Decreased reproduction was attributed to a lack of temporal and spatial variation of grazing pressures. A similar reduction in regeneration due to heavy grazing and browsing has been reported in the New Forest in Britain by Mountford et al. (1999). Whilst damage by the invasive *Muntiacus reevesi* Ogilby, 1839 (muntjac deer) to coppice regrowth in Monks Wood in Britain have been reported by Cooke and Lakhani (1996).

2.5 Impacts on population genetic structure and diversity

Inter or intra specific diversity can potentially have profound effects on ecosystem resilience and health as it can alter the functional diversity which influences ecosystem processes (Peterson et al. 1998, Chapin III et al. 2000). As with maintaining high levels of species and

functional diversity, maintaining high levels of genetic diversity can maximise the potential for a population to adapt and persist during periods of environmental change and improve long-term population health (Schaberg et al. 2008). When considered from the viewpoint of community genetics, the genotypic diversity of structurally important organisms such as trees, can also influence ecosystem function through interactions with species survival, which in turn shape biodiversity and hence functional diversity (Booy et al. 2000, Whitham et al. 2010).

There is evidence that in some species-poor ecosystems, high genetic diversity can augment low species diversity, buffering the effect of environmental perturbations (Reusch et al. 2005). In addition to selection, genotypic diversity does not only allow populations to endure environmental stress by ensuring the survival of some individuals but can act through forces such as facilitation or niche differentiation where all genotypes, rather than one robust genotype, provide a collective benefit to the community. This is of particular relevance to European temperate forests, many of which have just one or two dominant tree species and may, therefore, be more susceptible to substantial reductions in genetic diversity in the dominant species.

Genetic diversity can be measured in several ways including the number of alleles in a population, the frequency of those alleles in a population, and the number of rare or unique alleles to a population (private alleles). The benefits of avoiding a reduction in the genetic diversity of populations will be influenced by its spatial distribution, which is fundamentally affected by local patterns of geneflow (Sokal et al. 1989). Seed and pollen flow, together with selection and genetic drift, are the driving forces behind the formation of spatial genetic structure in populations (SGS) (Loveless and Hamrick 1984). Environmental and demographic events which alter gene flow, particularly via seed and pollen dispersal, can lead to significant alteration of genetic structuring (Heuertz et al. 2003, Vekemans and Hardy 2004). Given that

management practices alter these processes it is likely that practices will differ in their genetic consequences.

Practices which encourage vegetative regeneration can alter genetic diversity and structure by modifying plant breeding systems and promoting clonal expansion. These two factors can affect geneflow, selection, and drift and can directly limit the effective population size. Although management which alters the regeneration mechanism in a stand is expected to have genetic consequences, there is little research in this area. To date, only five studies consider the genetic consequences of coppicing with only four (Aravanopoulos et al. 2001, Mattioni et al. 2008, Valbuena-Carabaña et al. 2008, Dostálek et al. 2011) of those studies designed to explicitly explore this issue.

2.5.1 Altering plant breeding systems

An adequate level of gene flow is essential for maintaining genetic diversity within a stand. Limiting breeding systems through management can affect gene flow via pollen and seed and could therefore alter genetic diversity and its spatial distribution within a stand. These limitations are relevant to areas where natural regeneration is encouraged as it will affect the gene pool of the next generation of seedlings arising from sexual reproduction.

Depending on the management system, the ability of a coppice to regenerate, vegetatively or by seed, can be compromised by management cessation. *Q. pyrenaica* in Spain is commonly worked on a simple coppice (Table 2.1) (Serrada et al. 1994). Núñez et al. (2012) examined an abandoned coppice stand of *Q. pyrenaica* protected for biodiversity conservation. Its persistence was threatened by a lack of sexual reproduction due to the age structure of the forest. The area had been intensively managed until the 1980s, after which fuelwood extraction was halted but grazing continued. Because the trees had been managed as a simple

coppice, there was no variation in the age of stems and there was an absence of acorns since stems had not reached a sufficient age for fruiting. Heavy grazing also reduced the amount of natural regeneration. As stems are reverted back to a juvenile state when coppicing (Blake 1980), it can take decades for fruiting to occur, and in an abandoned simple coppice the lack of age variation can undermine the continuity of the habitat and threaten the persistence of the forests under non-intervention. Observations in another *Q. pyrenaica* coppice forest have noted a general lack of acorns, possibly due to the high shoot densities that intensify competition for light, water and nutrients and reduce allocation of resources to flowering and fruiting (Serrada et al. 2008). The genetic diversity of establishing seedlings will be heavily influenced by the number and identity of those individuals with the highest fecundity, which will be influenced by environmental factors and the management system. Changes to pollen and seed production are known to alter gene flow and therefore significant genetic structuring is likely to occur in the next generation (Vekemans and Hardy 2004). Another example of a limitation to gene flow in managed coppice is the harvesting of stems before fruiting is achieved (Cottrell et al. 2003).

2.5.2 Altering clonal expansion

Exploiting vegetative reproduction of individuals in a population can essentially 'fix' its genetic characteristics in time. Cottrell et al. (2003) found higher allelic diversity in an abandoned mixed coppice of *Quercus petraea* (Matt.) Liebl. (sessile oak) and *Q. robur,* in Britain when compared with a natural stand, in France from a previous study (Streiff et al. 1998). Although the abandoned coppice population harboured high genetic diversity, it displayed an unexpected excess of homozygotes, usually a sign of inbreeding. Here it was suggested that coppicing had 'fixed' genetic variation in the past and that the lack of genetic equilibrium in the abandoned coppice was likely a remnant of past colonisation dynamics instead of resulting from inbreeding (Cottrell et al. 2003). The time period in which the forest had been managed

as a coppice was 300 years, approximately 6% of the time the forest has been in existence given its establishment some 5000 years BP. This would suggest that the genetic consequences of coppicing may be more significant for populations which have been under traditional management regimes for longer periods of time. In this case, 'fixing' the genetic pattern of a coppice population in time made the abandoned coppice population distinct from surrounding unmanaged forests. Genetic divergence may be increased between coppice and unmanaged natural forests that are undergoing processes of drift and selection, thereby creating unique populations that are valuable candidates for gene reserve forests. However, research on the genetic resource value of coppice and pollard systems is lacking and there is currently no evidence of how genotypes retained through coppicing might impact a population's response to environmental change.

Further consequences of the 'fixing' of population genetic variation over time in coppiced stands was found by Mattioni et al. (2008). The study, which examined orchards, coppice, and naturalized stands of Castanea sativa Mill. (sweet chestnut), suggested that the decay of linkage disequilibrium (overall allelic correlations between loci) was reduced in stands that were maintained through clonal reproduction. Since coppicing exploits vegetative regeneration, sexual reproduction and hence recombination ceases in coppiced stands and the genetically effective population size is reduced (Hill 1981). Mattioni et al. (2008) identified that sexual reproduction in the naturalized stand led to the decay of linkage disequilibrium, such that linkage disequilibrium in naturally regenerated stands was significantly lower than in coppiced stands.

During the establishment phase, seedlings may have to compete with vegetative regrowth from stools. Species specific vegetative traits, such as primarily producing root-suckers (e.g. in *Q. pyrenaica* (Valbuena-Carabaña et al. 2008)) or shoots, (e.g. in *F. sylvatica* (Coppini and

Hermanin 2007)), will affect the clonal expansion abilities in genets (a group of genetically identical individuals). Depending on the extent of clonal expansion, it is intuitive to think that genetic diversity could be affected by the reduction of newly established individuals within a given area. However, studies on coppice systems differ in their results for their measures of genetic diversity (e.g. allelic richness, allelic frequency, clonal diversity) and certain studies find increases in genetic diversity (e.g. Cottrell et al. 2003, Valbuena-Carabaña et al. 2008) whilst others do not differ significantly from levels in natural populations (Aravanopoulos et al. 2001, Mattioni et al. 2008, Dostálek et al. 2011).

Interestingly, Valbuena-Carabaña et al. (2008) reported lower clonality levels in *Q. pyrenaica* coppice compared to a nearby natural stand as a result of high shoot competition in the coppice (Valbuena-Carabaña et al. 2008). Genetic diversity was preserved in small clonal assemblies which were 4.6-fold smaller than the mean extension covered by a genet in the natural forest (52.4 m^2) where shoot competition was lower due to the absence of profuse sprouting after cutting. Research on the clonality levels in *Q.ilex* by Ortego et al. (2010) suggests that clonality levels increase in more open habitats where competition is reduced. *Q.ilex* stools growing in habitats with different degrees of fragmentation; 1) a natural *Q.ilex* forest, 2) a pasture with some scattered trees, 3) an extensively cultivated area with highly isolated trees. It was found that clonal expansion, measured in distances between ramets (individual units of a clone – e.g. individual stems all belonging to the same genetic individual) generally increased with higher levels of fragmentation. The high clonality levels in trees growing in extensively cultivated areas were thought to be due to their coppice-like management which encouraged elevated shoot production. This management form, together with the lack of competition is likely to have promoted extensive clonal propagation. The importance of competition should be considered in coppice forests with species, which display vigorous sprouting ability as reducing the density of the stand by indiscriminate thinning may

actually remove small but unique clonal assemblies and decrease overall genetic diversity, potentially increasing disease susceptibility (Valbuena-Carabaña et al. 2008).

It has been argued that plants with natural sprouting ability are able to buffer the detrimental genetic effects of small population size and a reduction in pollination or dispersal due to the increased longevity of individual genets (Bond and Midgley 2001). A study by Acosta et al. (2012) suggested that this could be the case for a naturally sprouting species, *Nothofagus antarctica* Forst. (Antarctic beech), which displayed higher levels of genet diversity than their non-sprouting counterpart *Nothofagus pumilio* Poepp. & Endl. (lenga beech). A previous study by Premoli and Steinke (2008) also suggested that there were genetic benefits to sprouting in *N. Antarctica*, which occurs in high disturbance, fire-prone, environments. It should be noted that in traditionally managed stands, trees are artificially induced to sprout, and therefore species sprouting traits will be influenced by management technique and may differ in their genetic composition when compared to their natural, unmanaged counterpart.

Observations from a management re-introduction programme in Burnham Beeches forest in Britain indicate that *F. sylvatica* trees displaying epicormic growth responded better to pollarding in terms of shoot production (Read et al. 2010). Phenotypic observations in the same stand suggested the presence of genotypes adapted to coppice or pollard management. Previous studies have suggested that epicormic shoot formation may be partly genetically determined in some species (Ward 1966, Bryan and Lanner 1981, Jensen 2000, Nicolini et al. 2001) although research investigating the genetic basis of epicormic shoot formation in traditionally managed stands is absent. The ability to identify individuals which are most responsive to coppicing or pollarding would be invaluable for conservation plans which aim to re-introduce management in abandoned systems.

2.6 Recommendations for management and future research

2.6.1 Considerations for future genetic research

Although the majority of studies on coppices have reported little or no differences in population genetic parameters when compared to natural forest, observations such as the restriction of sexual reproduction make these results counter-intuitive and debate is still ongoing. Our understanding of the genetic consequences of exploiting vegetative reproduction using traditional practices is limited and mainly based on a few studies which report small but significant differences in other genetic parameters, such as the extent of linkage disequilibrium (Mattioni et al. 2008) and the fixation index (Cottrell et al. 2003). Further research is required to determine whether genetic differences found between managed and unmanaged stands are widespread. It is therefore essential that future research employs a level of resolution strong enough, in terms of the numbers of molecular markers or samples, to detect small but significant differences. The few studies which focus on genetic effects have primarily been conducted on coppice systems and comparability is limited by differences in study species, molecular markers, and sampling design. The applicability of the research, in terms of geographical scale, varies, with only one study covering a European-wide scale (i.e. Mattioni et al. (2008)). Future studies should exploit highly polymorphic co-dominant molecular markers in order to adequately estimate population genetic parameters in these systems (Nybom 2004).

2.6.2 Management re-introduction

One of the key findings of this review is that continued management of coppice forests and pollards in Europe is essential to ensure their long-term survival. Over-mature coppiced trees and pollards suffer from detrimental physiological changes, a reduction in re-sprouting ability, and increased mechanical failure which substantially decreases their longevity. Further research investigating the causes of this degeneration and the effects of management

reintroduction is needed to ensure the success of future conservation programs. It should be noted that while this review focuses mainly on Europe, much of the information here is relevant to other regions with similar climatic conditions. However, it essential that management at the site scale is based on a thorough consideration of local environmental conditions.

Shoot-density dependent effects will be more likely in species, such as *Q. pyrenaica* (Valbuena-Carabaña et al. 2008), compared to species which do not produce root suckers and have relatively lower shoot production. Thinning the stems on stools can be an effective method to reduce the negative effects experienced by some abandoned coppice, such as the increased risk of xylem cavitation (Corcuera et al. 2006) and reduction in soil moisture levels (Cotillas et al. 2009). In some areas, thinning out stems or stools can reduce drought stress on trees by weakening competition for resources (Ruiz-Benito et al. 2013). However, the long lasting benefits of this management intervention are dependent on species sprouting ability, economic support, and labour availability as species which re-sprout vigorously will need frequent and continuous management (Valbuena-Carabaña et al. 2008, Cotillas et al. 2009). Nevertheless, thinning could be a valuable tool for improving stool survival rate in abandoned coppice forests consisting of species with a relatively low sprouting ability, such as *F. sylvatica*.

Reducing the density of stools can reduce clonal reproduction in over-mature coppiced trees, promoting opportunities for sexual reproduction and hence promoting the decay of linkage disequilibrium (Mattioni et al. 2008). Thinning out the stems on a stool or controlled grazing can also be used to promote stem production and stool survival (Cotillas et al. 2009, Núñez et al. 2012, Tanentzap et al. 2012). Though grazing is likely to be negative for seedling establishment if temporal and spatial variation in grazing pressures are absent (Rozas 2004). The success of these methods will again be dependent on species sprouting ability and

indiscriminate thinning should be avoided as it could be detrimental to genetic diversity in certain stands (Valbuena-Carabaña et al. 2008). Leaving log piles derived from thinning would be an ideal method for encouraging more dead wood production for conservation purposes as it would promote the diversity of both early and late successional invertebrate species (Kirby 1992).When considering the conversion of coppice to high forest, the amount of flowering and fruiting should be examined. In some stands, coppicing may be linked to a reduction in flowering and fruiting (Cañellas et al. 2004, Núñez et al. 2012). Trees which are reproductively active should be protected from heavy thinning, to increase the number of individuals which can contribute natural regeneration.

Responses may differ by genotype and species (Montes et al. 2004, Cotillas et al. 2009), and conservation management would benefit if individuals which are likely to respond positively to coppicing or pollarding can be distinguished beforehand by genetic or morphological assessment. Stands which have been managed for longer time periods may harbour unique genotypes or population characteristics that differ significantly from the natural population (Streiff et al. 1998, Mattioni et al. 2008, Dostálek et al. 2011).

2.6.3 Management of biodiversity

The effects of traditional management on biodiversity have been widely recognised and exploiting vegetative regeneration has become a valuable conservation tool, essentially providing more flexibility to managers in manipulating microclimates. Certain variables including rotation length, size and distribution of panels, density of stools and standards, and species composition can be altered to improve habitat heterogeneity and hence biodiversity. However, species requirements will differ and may conflict with each other and therefore management objectives need to be made explicit beforehand.

Previous work has identified the need for multiple age classes in coppice forests and that a combination of short and long rotation management can create a rich diversity of habitats (Fuller 1992, Mitchell 1992, Fuller and Warren 1993). In coppice, the rides between panels can provide a linear feature within the habitat which promote connectivity and allow the dispersal of certain taxa, such as butterflies (Warren and Thomas 1992). Whilst standard trees and log piles can be used to create microhabitats associated with deadwood and aged bark surfaces which coppice forests often lack but are abundant in pollarded habitats (Kirby et al. 1995, Moe and Botnen 1997, Desender et al. 1999, Fay 2004, Taboada et al. 2006, Dubois et al. 2009). In general, improving habitat heterogeneity will improve species diversity as early, as well as late successional species, will be supported.

2.7 Conclusion

Habitat heterogeneity in forests historically shaped by coppicing and pollarding can significantly affect the structure and function of populations from the genotype to the ecosystem. It should be noted that management impacts on biodiversity, tree physiology, and genetic diversity are not mutually exclusive. For example, genetics can influence tree physiology, forest structure and even associated biodiversity (Booy et al. 2000, Whitham et al. 2010), and therefore a holistic approach should be considered when constructing management plans. The cultural landscapes found in Europe that have been created from centuries of traditional management are becoming increasingly recognised as having high conservation value, yet their current conservation value often derives from an interaction of their past management and present neglect.

Whether traditional management techniques could be used as a tool to improve forest persistence under climate change remains unexplored and future research into this subject is required to discern its practicality. However, the increasing awareness of the decline in growth

and natural regeneration in high forests due to increased drought is highlighting the need for novel adaptive strategies, which in some cases could draw upon knowledge from historic management practices. Practices developed in regions prone to drought have been designed to deal with the problem of soil exposure from management. These locally adapted management systems should be carefully considered if vegetative regeneration is to be exploited to improve forest persistence. Considering species and site specific characteristics is necessary to maximise the success of management which, together with future research, should enable vegetative regeneration to become a valuable tool for improving forest persistence under climate change.

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Chapter 3

COPPICE MANAGEMENT OF FORESTS IMPACTS SPATIAL GENETIC STRUCTURE BUT NOT GENETIC DIVERSITY IN EUROPEAN BEECH (*FAGUS SYLVATICA* L.)

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3.1 Abstract

Coppice management of forests was historically common in Europe. Actively managed coppice persists through vegetative regeneration prolonging the lifespan of trees and reducing flowering, seed production, and establishment. As coppicing alters the primary regeneration pathway within a stand, it is expected to alter the level and structuring of genetic diversity within populations. The study species, European beech (*Fagus sylvatica* L.), has historically experienced widespread coppicing throughout the range of the species. Genetic material was obtained from paired coppiced and high forest stands, in each of three study sites across Europe located in Germany, France, and Italy. Trees were genotyped at 11 microsatellite loci. Estimates of genetic diversity were found to be equally high as those found in natural forests. Significant spatial genetic structure of coppice stands extended 10m to 20m further than their paired high forest indicating that local-scale patterns of gene flow have been significantly altered by generations of forest management in the coppice stands. Understanding the implications of such changes for the structure and level of diversity within traditionally managed populations can assist with management planning for conservation and resource use into the future.

3.2 Introduction

Much of Europe's forest has been subject to human intervention for millennia, with approximately 70% of all forests in Europe being classed as semi-natural (FOREST FOREST EUROPE and UN/ECE-FAO 2011). Prolonged management has shaped their distributions and changed the pattern of genetic diversity within and amongst populations (Bradshaw 2004, Schaberg et al. 2008, Piotti et al. 2013, Sjölund and Jump 2013). Maintaining genetic diversity can retain the adaptive potential of a population in response to environmental change (Jump et al. 2009). Furthermore, levels of genetic diversity in dominant species can profoundly influence ecosystem functioning (Christensen et al. 1996, Peterson et al. 1998, Booy et al. 2000, Reusch et al. 2005, Whitham et al. 2010). This effect is particularly relevant to many European forests which are often comprised of a few dominant tree species (EEA 2007). Therefore the adaptive management of Europe's semi-natural forests is dependent on understanding how prolonged management has shaped forest genetic resources (Lefèvre 2004).

Traditional coppice management was historically common in Europe and was sustained by the demand for shoots and poles which were used for fuelwood, animal fodder, crafts, and building materials (Read 2000). Coppice products were derived by cutting the main stem of a tree at ground level leaving a stump, called a stool, which subsequently produces a re-growth of shoots that are harvested at different intervals (Evans 1992, Harmer and Howe 2003). At least 25 million ha of forested areas in Europe (excluding the Russian Federation) have been managed as coppice in the past (UN/ECE-FAO 2000), with only 2.9 million ha remaining under active coppice regeneration in 2011 (FOREST EUROPE and UN/ECE-FAO 2011).

Continued coppice management often increases the longevity of the tree allowing it to persist as long as vegetative regeneration is exploited (Blake 1980). One of the oldest coppice stools

found was a European Ash (*Fraxinus excelsior* L.) and was thought to be thousands of years old, much older than their unmanaged counterparts, which have a typical lifespan of ~200 years (Rackham 1986). The resulting microhabitat complexity supports a wide range of species and creates cultural landscapes that are recognised for their heritage and ecological value (Rackham 1980, Peterken 1992, Fuller and Warren 1993, Peterken 1993, Harmer and Howe 2003). Traditional coppice practices suffered a decline during the nineteenth century primarily due to socio-economic changes. The ecological value and persistence of many previously coppiced forests has declined owing to cessation of management or the conversion of coppice to high forest for timber production (Bacilieri et al. 1994, Panaïotis et al. 1997, Watkins and Kirby 1998, Harmer and Howe 2003, Nocentini 2009).

Forest management practices, such as coppicing, which alter the primary regeneration pathway within a stand, are expected to have significant effects on the structuring of genetic diversity within populations (Loveless and Hamrick 1984, Heuertz et al. 2003, Vekemans and Hardy 2004). Appropriate management of forest genetic resources requires an understanding of the spatial structuring of genetic diversity within populations. Significant structuring within a population can influence local breeding and evolution (Smouse and Peakall 1999). Gene flow, genetic drift, and selection are the main processes that shape spatial genetic structure (SGS) (Loveless and Hamrick 1984). In plant populations, the effects of gene flow on SGS are largely driven by pollen and seed dispersal (Sokal et al. 1989), but can also be influenced by clonal propagation depending on the regeneration pathway, i.e. natural vs. vegetative regeneration (Sjölund and Jump 2013). Coppicing limits the effective population size by reducing flowering and encouraging clonal expansion that can restrict gene flow. Such changes influence the structuring of genetic diversity within a population. It is therefore necessary to assess whether coppicing, a management practice which was historically widespread and long-standing, has altered the genetic diversity and structure of these semi-natural forests.

This study focuses on the European beech (*Fagus sylvatica* L.) which forms the dominant forest type over much of Western and Central Europe and extends into the Mediterranean at higher altitudes. Coppice management was historically widespread throughout the range of the species despite the fact that beech rarely reproduces vegetatively under natural conditions and is therefore one of the less responsive species to coppice management (Packham et al. 2012). A variety of systems have been used, including the coppice-with-standards system, common in the northern and core range of beech and the coppice selection system, which maintains canopy cover and thus is widespread in the drought prone southern range edge (Harmer and Howe 2003, Coppini and Hermanin 2007, Nocentini 2009, Wagner et al. 2010). In addition, trees were sometimes coppiced in silvopastoral systems (Read 2006, Read et al. 2010). Traditional coppice systems were managed on long rotation cycles that led to a substantial increase in the longevity of individual plants but reduced opportunities for establishment from seed when compared with their high forest counterparts.

Research on the genetic effects of coppicing has been carried out on a few species, (e.g. Beech (Paffetti et al. 2012, Piotti et al. 2013), Pyrenean oak (*Quercus pyrenaica* Willd. (Pyrenean oak) (Valbuena-Carabaña et al. 2008), pedunculate oak (*Q. robur* L.)(Cottrell et al. 2003), sessile oak (*Q. petraea* Matt. Liebl.) (Cottrell et al. 2003, Dostálek et al. 2011), and sweet chestnut (*Castanea sativa* Mill.) (Aravanopoulos et al. 2001, Mattioni et al. 2008)). However, it is difficult to draw general conclusions from these studies due to the lack of paired plots, their limited geographic spread, and the low number of molecular markers used in some studies. Our study differs from previous studies as it employs extensive sampling within paired stands, focusing on the effects of coppice management by comparing those stands with nearby, unmanaged stands in the same forest. In the present work, we were able to determine the effects of promoting vegetative regeneration through traditional coppice management on the

amount and structuring of genetic diversity within populations of European beech using a paired plot design in three regions. We hypothesised that prolonged vegetative reproduction should decrease genetic diversity and increase spatial genetic structure due to the reduced probability of establishment from seed. Such information will be useful for the managers of the large fraction of semi-natural forests that have experienced coppicing in the past. Furthermore, understanding the spatial genetic structure of populations will have consequences for genetic resource management on a spatial scale, for example the collection of seed for gene banks or silviculture.

3.3 Materials and methods

3.3.1 Study species

The wind-pollinated European beech is a broadleaved, monoecious tree that is highly outcrossing, with large seeds (beech mast) that are mainly dispersed by animals and gravity (Packham et al. 2012). With a range of roughly 14 million ha, it commonly forms near monospecific stands but is also a major component of many mixed forests. The lifespan of unmanaged beech is typically between 150 and 300 years and rarely exceeds 300 (Packham et al. 2012). Traditional management has been reported to increase the longevity of trees due, in part, to their persistence in a partially juvenile state (Blake 1980), although coppicing success is variable (Harmer and Howe 2003). Beech has a shallow root system which makes it particularly vulnerable to wind-throw and drought. All parts of the tree and seedlings are susceptible to frost. Flowering can begin between the age of 40 to 80 years depending on the density of the stand, however coppice management can restrict flowering as stems are not allowed to reach maturity (Blake 1980).

3.3.2 Study sites

Three study sites were selected across Europe (Germany, France, and Italy) to attain broad

coverage of the species range (Table 3.1). In each site, two paired plots were sampled, a coppice and a high forest stand. Paired stands were no further than 10km apart to maintain comparable colonisation history. High forest stands were defined as having little or no historic or contemporary management and originated from seed primarily through natural regeneration. Coppiced stands were defined as stands with either a history of coppice management which has ceased, or is currently under active coppice management. The primary regeneration pathway is natural in the former and vegetative in the latter. Both stand types originate from native forest with a continuous history. Stand codes are used to refer to stands in this paper, and were derived from the first letter of the country (G = Germany, F = France, I = Italy) and the management history of the stand (H = high forest stand, C = coppice stand).

Table 3.1 Details of study sites

Country	Site	Stand code	Stand management	N	Latitude Longitude	Elevation
Germany	Spessart	GH	High forest	168	N50.0412 E9.5521	495
		GC	Converted coppice	170	N49.9600 E9.5451	486
France	Mt Lure	FH	High forest	112	N44.1246 E5.8257	1307
		FC	Abandoned coppice	170	N44.1224 E5.8340	1177
Italy	Mt Gelbison	IH	High forest	100	N40.2167 E15.3383	1521
		IC	Abandoned coppice	170	N40.2078 E15.3494	1352

Stand codes were derived from the first letter of the country (G = Germany, F = France, I = Italy) and the management history of the stand (H = high forest stand, C = coppice stand). Latitude and longitude are presented in decimal degrees and elevation in metres.

Sampling was carried out on the original coppiced trees which were the dominant form in the stands and could be easily identified. GC was managed as a simple coppice, after which it was converted to high forest (pers. comm. R. Herrmann). FC is a neglected coppice that occurs in an area of Montagne de Lure which has a history of coppicing dating back at least to the beginning of the 19th century with beech coppice managed on a long rotation coppice system (Simon et al. 2007). IC was managed in the past as a coppice-with-standards system (pers.

comm. F. Bottalico), which now experiences low-level harvesting of stems by local residents (pers. obs.). It should be noted that the German high forest (GH) was managed as a shelterwood system up until 1988 (pers. comm. R. Herrmann). Although there has been intermittent low intensity harvesting of trees for timber in each of the high forest stands, the three high forest stands differ from the coppice stands in terms of the primary regeneration pathway.

3.3.3 Sample collection and microsatellite analysis

To account for short distance classes and hence allow the detection of fine-scale SGS, trees were sampled on a grid (approximately 150m x150m in size) with points at every ~10m. An additional 20 trees were sampled along a 100m transect extending out of the grid to extend the spatial range covered (not implemented in IH site as it was not possible due to topographic restrictions) (see supplementary material S3.1 for diagram of sampling design). Sample size ranged from 100 to 170 samples (Table 3.1). Geographic coordinates were recorded for each tree sampled using a GARMIN 62s handheld GPS. As beech typically produces shoots originating from the stool, instead of roots in response to coppicing (Coppini and Hermanin 2007), individuals can be easily distinguished and the sampling of clones avoided and confirmed from genetic data.

Genomic DNA was obtained from leaf or cambium samples (Colpaert et al. 2005). Samples were dried in silica gel and DNA was isolated using BIOLINE Isolate Plant Kit and QIAGEN 96 Plant Kit according to the manufacturer's instructions. A total of 812 individuals (Table 3.1) were genotyped at 13 polymorphic SSRs (fs1-03, fs1-15, fs3-04, fs4-46, fcm5 (Pastorelli et al. 2003), mfc7 (Tanaka et al. 1999), mfs11 (Vornam et al. 2004), sfc0007-2, sfc0018, sfc0036,sfc1143, sfc1061, sfc1063 (Asuka et al. 2004)) in three multiplexes designed for this study; FSNplex1, FSNplex2, and FSNplex3. Multiplex PCR was carried out using 10ng of

template DNA and the QIAGEN Type-it Microsatellite PCR Kit with the following combinations for primer mixes. FSNplex1 consisted of primers fs3-04, sfc1143, mfc7, and fs4-46 at concentrations of 1µM, 3µM, 1µM, and 2µM respectively. FSNplex2 consisted of primers sfc0007-2, fs1-15, sfc1063, sfc1061, fcm5 at a concentration of 0.5µM, 1µM, 2µM, 0.5µM, and 3µM respectively. FSNplex3 consisted of primers sfc0036, sfc0018, fs1-03, mfs11 at a concentration of 3µM, 1µM, 1µM, and 2µM. Annealing temperature for each multiplex was 60°C, 58°C, and 60°C respectively. The total PCR reaction volume was 10µl. Fragment analysis was performed using an ABI 3730 DNA Analyzer (Applied Biosystems).

The presence of genotyping errors and null alleles were checked using MICRO-CHECKER (Van Oosterhout et al. 2004). Repeated sampling of null genotypes and significant deviations from Hardy-Weinberg equilibrium suggested that there was a significant proportion of null alleles in fs4-46 and fcm5 in more than half of the stands in this study. Analyses presented exclude fs4-46 and fcm5 and use a total of 11 loci. However, similar results in genetic diversity estimates and SGS were obtained when performing analysis on all 13 loci (data not shown). Pairs of loci were checked for gametic disequilibrium. Analysis was performed using FSTAT 2.9.3.2 (Goudet 1995), with significant associations identified by randomly associating genotypes at pairs of loci 1100 times and using a 5% nominal level after Bonferonni correction.

3.3.4 Genetic diversity and spatial genetic structure

We obtained general multilocus estimates of genetic diversity within stands on SPAGeDi 1.4b (Hardy and Vekemans 2002). We used ADZE 1.0 to obtain mean private allelic richness (A_P) (Szpiech et al. 2008). Because of the definition of private alleles, i.e. unique to a single population, analysis was performed within sites to compare differences between treatments. The minimum number of gene copies used for allelic richness and private allelic richness was 198. We tested differences in allelic richness (A_R), unbiased gene diversity (H_S), and the

inbreeding coefficient (F_{IS}) among groups of coppiced stands and high forest stands using FSTAT 2.9.3.2 (Goudet 1995). Groups are compared, by calculating the average of the desired estimator (x) over all samples and loci for each group to obtain an observed statistic (OS_x). OS_x is obtained from the difference between the estimators of the two groups, $OS_x = x1 - x2$. 10000 permutations were performed between the groups to obtain a randomised dataset from which the statistic S_x can be calculated. *P*-values for the tests are interpreted as the proportion of randomised datasets with $S_x > OS_x$.

Analysis of fine-scale SGS was performed in SPAGeDi 1.4b (Hardy and Vekemans 2002). Pairwise comparisons between individuals within each stand were used to compute a codominant estimator of the kinship coefficient (F_{ij}) as reported by Loiselle *et al.* (1995). The kinship coefficient can be described as $F_{ij} = (Q_{ij} - Q_m)/(1 - Q_m)$, where Q_{ij} is the probability of identity by state for random genes coming from two individuals i and j, and Q_m is the average probability of identity by state for gene copies coming from a reference population of random individuals (Hardy and Vekemans 2002). SPAGeDi 1.4b performs a Mantel test to test for statistically significant structuring within a stand. The observed regression slope, b_F, of F_{ij} on the natural logarithm of the distance, $ln(r_{ij})$, was compared to the expected estimate after permuting locations among individuals 10000 times, also used to attain upper and lower 95% confidence intervals. Standard errors and mean multilocus F_{ij} estimates within each distance class, $F_{(d)}$, were obtained through jackknifing over loci following Sokal and Rohlf (1995). Analyses were performed using 17 even distance classes of 10m, ranging from 0 to 170m.

To allow comparisons in the intensity of SGS between stands we used the *Sp* statistic (Vekemans and Hardy 2004, Piotti et al. 2013). The *Sp* statistic quantifies SGS by the ratio $-b_F/(1 - F_{(1)})$, where b_F is the regression slope of F_{ij} on the natural logarithm of the distance, r, between individuals i and j, $ln(r_{ij})$, and $F_{(1)}$ is the mean F_{ij} belonging to the individuals of the

first distance class (0-10m) which includes all pairs of neighbours. The variability of the *Sp* statistic is expressed in the standard error of b_F, which is calculated by jackknifing over loci (Hardy et al. 2006).

Table 3.2 Summary of forest inventory plots within each stand

	GH	GC	FH	FC	IH	IC
Proportion of multi-stemmed trees	0.000	0.565***	0.241	0.446***	0.056	0.346**
Mean largest stem DBH [Range] (cm)	32	35	7	9**	28	22
Density adults/ha	35.0	28.6	316.3	218.8	97.5	45.0
Density saplings/ha	85.0	2.5	120.0	93.8	21.3	0.0

Significant *P*-values for differences between the proportion of multi-stemmed trees and the mean largest DBH in high forest and coppice stands (i.e. GH vs. GC; FH vs. FC; and IH vs. IC) are indicated next to the coppice stand values as *P <0.05; **P<0.01; ***P<0.001.

Summary forest inventory data were recorded in two 20m x 20m plots of each site (Table 3.2). Data from both plots were combined to give a summary in Table 3.2. The diameter at breast height (DBH) for all species of adult trees (i.e. height > 140cm) was recorded. All saplings, defined as trees between 10cm and 140cm in height, were counted. A chi-square test for independence was used to determine the differences between paired stands in the proportions of multi-stemmed vs. single stemmed trees. Differences in the largest stem DBH between paired stands were tested using Welch's t-test.

3.4 Results

Across the 11 loci investigated here, the maximum number of alleles ranged from 6 to 40 per locus, with a multilocus average of 17.91 in all populations combined. All pairs of microsatellite loci were in gametic equilibrium considering a 5% nominal level after Bonferroni correction. Multilocus estimates of allelic richness, A_R, were high, ranging from 9.58 to 14.34, with little difference in allelic richness between paired stands. For unbiased gene diversity, H_S, multilocus estimates ranged from 0.695 to 0.788. Positive F_{IS} values indicated a significant

departure from Hardy-Weinberg genotypic proportions in three stands GC, IH, and IC presenting an excess of homozygotes (Table 3.3). Permutation tests on genetic estimators revealed no significant differences in A_R, H_S, and F_{IS} when stands of coppice and stands of high forest were analysed as groups; A_R: High Forest 11.38, Coppice 11.40 (P = 1.00), H_S: High Forest 0.72, Coppice 0.74 (P = 0.50), and F_{IS}: High Forest 0.024, Coppice 0.043 (P = 0.47). No consistent pattern in private allelic richness, A_P, was found between coppice and high forest stands (Table 3.3).

Table 3.3 Summary of multilocus genetic diversity estimators and SGS coefficients

	Genetic diversity estimators				**SGS parameters**		
Stand code	A_R	A_P	H_S	F_{IS}	$F_{(1)}$	SGS_{MAX} (m)	$Sp \pm$ SE
GH	10.12	1.51	0.695	0.019	0.0277***	20	0.0037 ± 0.0008
GC	10.45	1.94	0.722	0.044***	0.0122*	30	0.0032 ± 0.0014
FH	9.69	1.34	0.704	0.022	0.0231*	40	0.0088 ± 0.0019
FC	9.58	1.28	0.731	0.013	0.0563***	60	0.0114 ± 0.0019
IH	14.34	2.36	0.788	0.034**	0.0127	30	0.0062 ± 0.0018
IC	14.17	1.95	0.780	0.071***	0.0186**	40	0.0040 ± 0.0013

Terms for genetic diversity estimators are as follows; A_R, allelic richness (Petit et al. 1998); A_P, private allelic richness (Szpiech et al. 2008); H_S, unbiased gene diversity (Nei 1978); F_{IS}, inbreeding coefficient (Weir and Cockerham 1984). The minimum number of gene copies (k) used for rarefication analysis of A_R and A_P is 198. P-values for F_{IS} are obtained after 10000 permutations of gene copies within individuals of each stand. Terms for SGS parameters are as follows; $F_{(1)}$, kinship coefficient for first distance class (i.e. 0-10m); SGS_{MAX}, the greatest distance at which the mean kinship coefficient within a given distance class, $F_{(d)}$, becomes significant to $P < 0.05$; $Sp \pm$ SE, Sp statistic ± standard error. Significant P-values are indicated as *P <0.05; **P <0.01; *** P<0.001. 2-sided P-values are presented for F_{IS} with 1-sided P-values presented for $F_{(1)}$ and SGS_{MAX}.

We found differences in the fine-scale spatial genetic structure between paired high forest and coppice stands. SGS_{MAX}, defined by Jump *et al.* (2012) as the greatest distance at which the mean kinship coefficient within a given distance class, $F_{(d)}$, becomes significant to $P < 0.05$, revealed significant structuring in coppices that consistently extended 10-20m further than in its high forest counterpart (Figure 3.1 and Table 3.3). This relationship between the extent of SGS and management was not reflected in the maximum intensity of SGS by the Sp statistic,

which showed little difference within sites (Table 3.3). Notably, spatial genetic structuring extended up to a maximum distance of 60m in the coppice stand of the French site, FC. This stand also exhibited the strongest kinship coefficient in the first distance class, $F_{(1)}$, as well as *Sp* statistic (Table 3.3). $F_{(1)}$ for IH was not statistically significant partly because of the reduced number of pairs of neighbours (N = 61) within that distance class which also contributed to the large standard errors. The remaining stands had a minimum number of 89 pairs for each distance class, with the exception of FH where N = 60 in the first distance class.

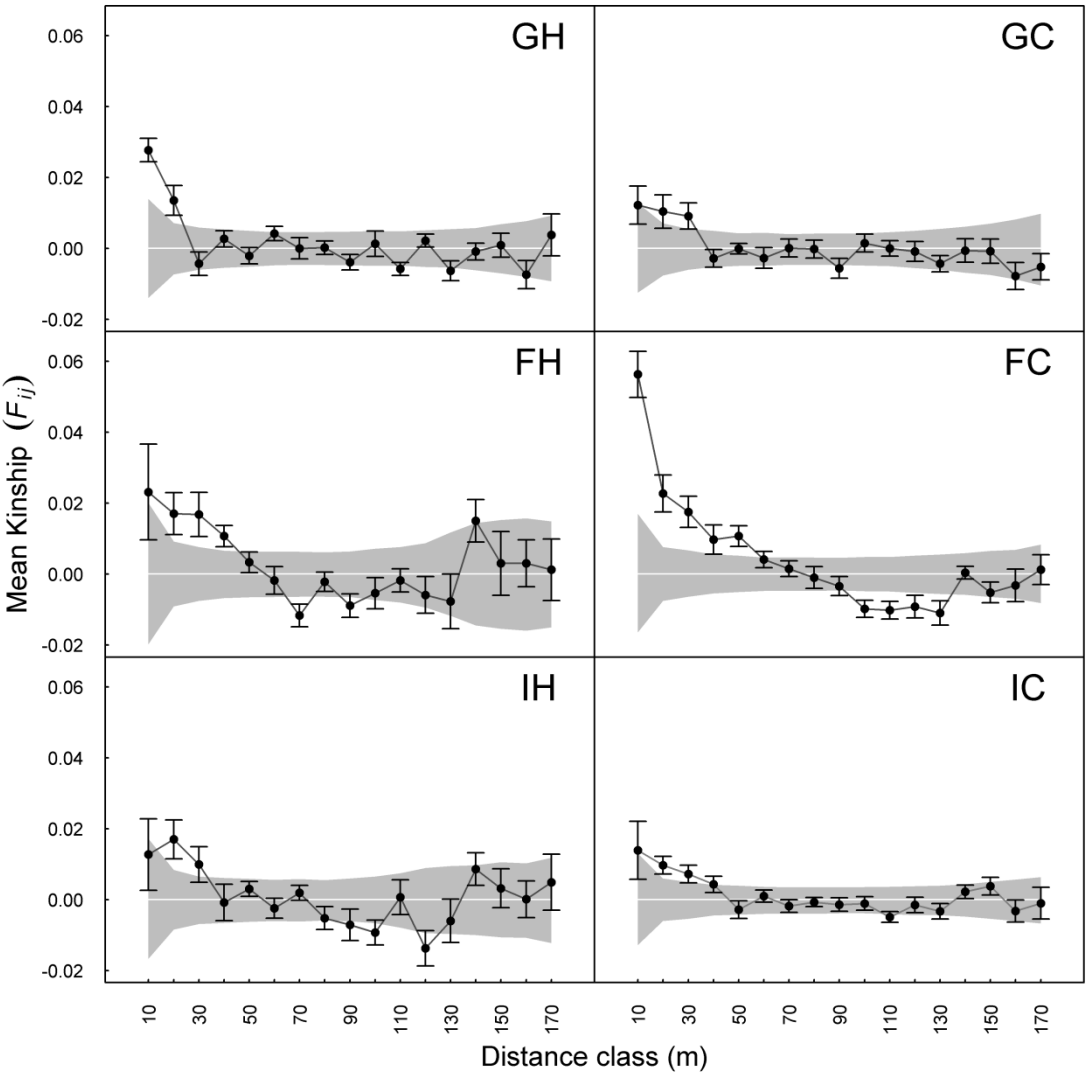

Fig. 3.1 Spatial autocorrelograms for each stand using the kinship coefficient (F_{ij}) as described in Loiselle *et al.* (1995) and consecutive 10m distance classes. Upper and lower 95% confident intervals derived from 10000 location permutations are indicated by shaded areas. Black bars around mean F_{ij} values represent standard errors obtained through jackknifing over loci following Sokal and Rohlf (1995) to obtain multilocus estimates.

Descriptive data obtained from the forest inventory plots revealed a high proportion of multi-stemmed trees in coppice stands, with a significantly higher proportion of multi-stemmed trees in the coppice plots when compared to their high forest counterpart (Germany X^2 (2, N = 51) = 18.37, P>0.001; France X^2 (2, N = 428) = 19.65, P>0.001; Italy X^2 (2, N = 114) = 9.49, P>0.01) (Table 3.2). A significantly higher largest stem DBH ($t_{(361)}$ = 2.99, P>0.01) was found in FC compared to FH. However, no significant differences were found between the stands in the German site ($t_{(44)}$ = 0.78, P=0.44) and the Italian site ($t_{(43)}$ = 1.41, P=0.17) (Table 3.2). Higher densities of adult trees and saplings were found in the high forest stands than in the coppice stands (Table 3.2).

3.5 Discussion

There were no statistically significant differences in genetic diversity between coppice and high forest stands. However, consistent differences in the spatial structuring of genetic diversity were found between paired stands. An increase of 10-20m in SGS_{MAX} was found in coppice stands when compared to their paired high forest stand. Beech coppices experience a reduction in sexual reproduction which is evident by the lower sapling densities found in the coppice stands. The increase in SGS_{MAX} might be the reflection of extended seed shadows that can result from rare establishment events, which occur over the long generation times experienced in coppices. As management removes trees from the breeding population through the cutting of stems, the dispersal of pollen and seed, two vectors that shape genetic structure, become less frequent in coppices. The long generation times coupled with rare establishment events in coppice stands, differ from the more frequent establishment of seedlings under high competition pressures in unmanaged populations that can lead to the break-down of spatial genetic structure (Loveless and Hamrick 1984).

The *Sp* statistic ranged from 0.0032 to 0.0114, which is within the range for that found in the

literature for beech (Jump and Peñuelas 2007, Chybicki et al. 2009, Jump et al. 2012, Piotti et al. 2013) and is typical for other outcrossing, gravity dispersed, and wind pollinated trees (Vekemans and Hardy 2004). Extensive spatial genetic structure was found in the French coppice site (SGS_{MAX} = 60m, Sp = 0.0114) with an SGS_{MAX} that exceeded the generally accepted maximum of 30-40m for European beech in the literature, when obtained from SSR markers (Vornam et al. 2004, Chybicki et al. 2009, Oddou-Muratorio et al. 2010, Piotti et al. 2013). The remaining stands in our study display clustering of related individuals up to a typical distance of 40m found with SSR markers. Jump *et al.* (2007) compare differences in SGS_{MAX} using varying numbers of SSR markers (N_{MAX} = 6) and samples (N_{MAX} = 200) and caution against using less than 6 SSR markers to detect SGS. The greater number of SSR markers used in this study (N = 11) could have contributed to the finding of an SGS_{MAX} of 60m in the French coppice stand. However, as the SGS_{MAX} of the remaining sites did not extend over the commonly reported SGS_{MAX} of 40m, it could be argued that this unusually high value for the French coppice stand is a reflection of site characteristics as opposed to the power of our markers.

Previous studies have found limited differences in genetic diversity between coppice and unmanaged stands (Aravanopoulos et al. 2001, Mattioni et al. 2008, Dostálek et al. 2011). However, some report trends found in coppices that are absent in natural stands, such as an increased level of linkage disequilibrium (Mattioni et al. 2008) and a higher fixation index (Cottrell et al. 2003). Increases in clonal diversity has been reported by Valbuena-Carabaña et al. (2008). Genotypic diversity was maintained by coppice management as it promoted the persistence of small clonal assemblages owing to the high shoot competition in coppices, which limited the spatial spread of clones. A two-fold increase in the spatial extent of clones was reported in nearby open oak woodland managed as high forest. The effect of coppicing on genetic diversity will be largely influenced by the primary regeneration strategy of the managed species. Valbuena-Carabaña et al. (2008) investigated Pyrenean oak (*Q. pyrenaica*) -

a highly clonal tree that naturally spreads through root-suckers. Therefore it is likely that the impact of coppicing on clonal diversity is reduced in species, such as beech, which primarily regenerates naturally and does not produce root-suckers (Coppini and Hermanin 2007). Clonal plant populations can have a similar level of genetic diversity to that found in outcrossing species (Hamrick and Godt 1996). The maintenance of genetic diversity in clonal populations is promoted by their longevity (Booy et al. 2000). Since coppice populations display similar traits to clonal populations, genetic diversity could be maintained though similar mechanisms, as genotypes and their alleles persist in the population for longer, therefore increasing their potential to spread through infrequent events of natural regeneration. Cottrell *et al.* (2003) examined the genetic diversity in mixed forest of pedunculate oak (*Quercus robur*) and sessile oak (*Q. petraea*), both species with similar pollen and seed dispersal mechanisms to beech. The site had been coppiced for at least 300 years and little difference was found in the spatial structuring of genetic diversity when comparing the site to an unmanaged native forest. However, the coppiced site had higher levels of genetic diversity as well as a significant heterozygote deficit. The authors hypothesise that the significant heterozygote deficit was thought to be a remnant of past population dynamics. The site occurred at the range edge where heterozygote deficits are likely to occur due to the mixing of populations from different refugia causing a Wahlund effect which has persisted as genetic variation has become fixed in time through management.

Historic coppice management can alter the structuring of genetic diversity but have no effect on the amount of genetic diversity within an area (Paffetti et al. 2012, Piotti et al. 2013). In contrast to our study, Paffetti *et al.* (2012) and Piotti *et al.* (2013) found a decrease in structuring in stands that have historically been under coppice management. However, it should be noted that the coppice stand examined in both studies had been converted to shelterwood systems by regeneration felling. Work by Rajendra *et al.* (2014) comparing

unmanaged beech stands to stands under various management systems in Germany found similar results to Paffetti *et al.*(2012) and Piotti *et al.* (2013)., although it is not clear if coppiced stands were included in this study. The reduction in the maximum extent of SGS (SGS_{MAX}) in managed stands was attributed to the removal of trees, through practices such as thinning, leading to the break-down of familial structures that would otherwise arise through the mating of adjacent, related individuals and the ineffective dispersal of beech mast. Although trees are removed from the reproductive cohort in coppices, they are not physically removed from the population, thereby preserving the familial structures that have developed prior to management. Such familial structuring can thus be extended when rare establishment events occur, leading to a consequent increase in SGS extent. In contrast, re-establishing thinning and logging in order to convert coppices to other management systems, such as the conversion to shelterwood in Paffetti *et al.*(2012) and Piotti *et al.* (2013), could rapidly reduce the extent of SGS by breaking up established family structures. Spatial genetic structure in beech stands is, therefore, likely to be particularly sensitive to the management type in practice.

3.6 Conclusion

This study demonstrates the importance of considering the spatial component of genetic diversity and the findings have wide reaching implications as many beech forests in Europe have experienced coppice management in the past. Coppice forests can be as rich in genetic diversity as natural forests. However, consistent differences in the extent of spatial genetic structuring in these populations, while relatively small in their magnitude, indicate that local-scale patterns of geneflow have been significantly altered by generations of forest management in the coppice stands. Understanding the implications of such changes for the structure and level of diversity within traditionally managed populations can assist with management planning for conservation and resource use into the future.

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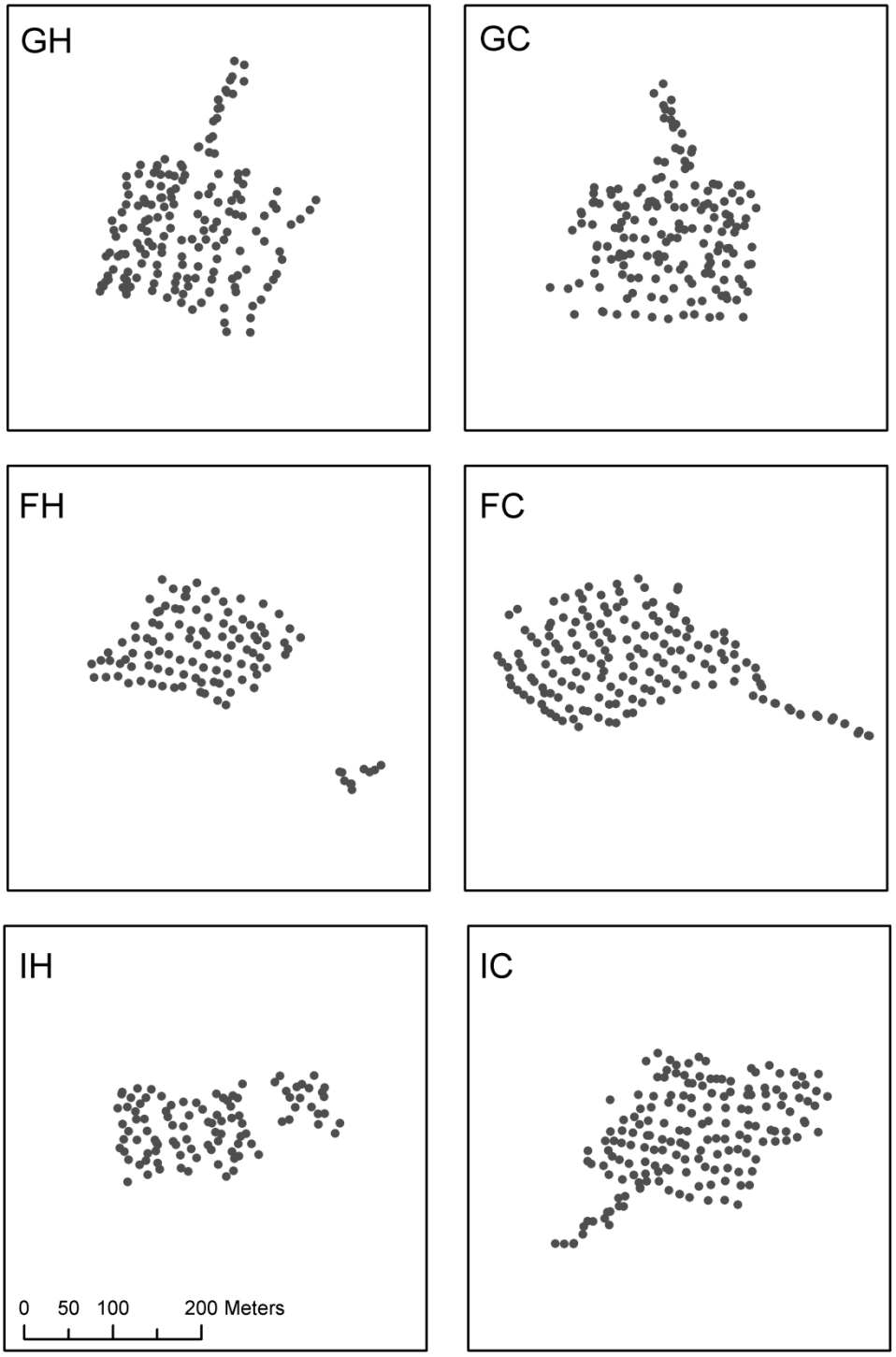
3.9 Supplementary Material



S3.1 Map of sampling design at each site. Trees (grey circles) are sampled on a grid, with transects projecting from the main sampling area. Exceptions include FH that did not have a continuous transect, and IH that did not include a transect, due to access limitations dictated by topography.

Chapter 4

CRYPTIC GENETIC STRUCTURE PERSISTS IN BRITAIN'S NATIVE *FAGUS SYLVATICA* L. KUHN FOREST DESPITE A PROLONGED HISTORY OF HUMAN TRANSLOCATIONS

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4.1 Abstract

Species ranges have been shaped by human impacts throughout the Holocene. The late arrival of beech in Britain coupled with historic translocations of plant material by humans has blurred the boundaries between the native and non-native range. Using a combination of nuclear and chloroplast microsatellite markers, genetic patterns driven by natural colonisation were found to persist in putative native sites, with higher nuclear gene diversity within groups of native sites. This pattern was not reflected in rarefied allelic richness which, unlike gene diversity, was lower than continental estimates, potentially decreasing the differences between the ranges. Chloroplast diversity revealed high haplotypic diversity near the purported entry point of beech into Britain. When considered country-wide, genetic variation was found to be structured regionally, driven by high gene flow between sites, diminishing the boundary of the putative native and non-native range of beech in Britain.

4.2 Introduction

An understanding of the factors that shape species distributions is needed to accurately estimate past species ranges and gain insight into their potential future distributions. The beginning of the Holocene marked the start of the migration of species into their contemporary ranges (Taberlet et al. 1998) and coincided with growing human impact (Kalis et al. 2003). Historic range limits have been examined from an ecological perspective and

post-glacial plant migrations from a phylogeographic perspective (Comes and Kadereit 1998, Hewitt 2000, Jump et al. 2010, Magri 2010). However, many natural systems have been under profound and persistent anthropogenic influence, shaping species distributions and the genetic composition of their component populations (Bradshaw 2004, Alessa and Chapin Iii 2008, Schaberg et al. 2008).

The influence of forest management, through the selective removal of genotypes and the translocation of plant material, can impact genetic diversity and its spatial distribution within populations and across regions (Savolainen and Kärkkäinen 1992, Bradshaw 2004, Schaberg et al. 2008). The translocation of locally adapted material by humans to areas within and outside of species ranges is likely to have an influence on the organisms' response to environmental change (Savolainen et al. 2007). The impacts of translocations have long been studied through the use of provenance trials which monitor adaptive responses of trees to varying climatic conditions (Konnert and Ruetz 2001, Hubert and Cundall 2006). Using information from provenance trials, tree species and provenances that may best adapt to specific environmental conditions can be identified (Bolte et al. 2009). Recent concerns on the mitigation of climate change impacts have called for the planting of species outside of historic ranges to assist species migrations, a proposal that has been a topic of much debate (Ricciardi and Simberloff 2009, Schlaepfer et al. 2009, Vitt et al. 2010, Hewitt et al. 2011). Arguments against assisted migration are centred on the difficulty of assessing potential risks of species introductions over time, which can ultimately result in the damage and extinction of co-occurring native species (Ricciardi and Simberloff 2009). The European beech, *Fagus sylvatica,* provides a valuable case study to assess potential long term impacts of assisted migration as it has experienced wide-scale translocations outside of its native range in the past.

Having many uses, including timber, fuel and fodder, the European beech experienced prolonged traditional management in the past (Nocentini 2009, Read et al. 2010, Packham et al. 2012). In Britain, there is a current uncertainty surrounding the limits of the natural range of beech, as it was planted extensively throughout the country. There has been some anecdotal historical evidence and palynological evidence that the native range of beech was limited to south-east England (see Rackham (1980), Pott (2000), Packham (2012)). Despite the commonly held view that beech is native to south-east England, it is classified as native all over the country in the *New Atlas of the British & Irish Flora*, which argued that there was insufficient evidence to define an exact native range in Britain (Preston et al. 2002). At the regional scale, the species range is believed to be under broad climatic control (Huntley et al. 1989). However, the distribution of beech in Great Britain is unique because it grows further north than its presumed native range in the south-east of the country and has become naturalised in areas where it has been planted (Watt 1931a, Dierschke 1985). It also grows at higher latitudes in Sweden (Lindquist 1931) when compared with Great Britain, indicating that the historic limits of beech in Britain might not be climatically determined. There have been suggestions that human intervention might have manipulated the species range before it reached its climatic range limit (Watt 1931b, Packham et al. 2012).

Previous paleoecological research has suggested a link between anthropogenic disturbance and beech establishment (Küster 1997, Bradshaw and Lindbladh 2005, Bolte et al. 2007). Although, Gardner and Willis (1999) argue that ecological traits of beech, such as its inherent slow migration and establishment rates, may be the main drivers of migration which occurred parallel to anthropogenic activity. Despite anthropogenic influences, many native forests are expected to retain genetic signals arising from natural processes from the natural regeneration of local stock (Bradshaw 2004) allowing the detection of natural ranges. Therefore, the indistinct native and non-native range of beech in Great Britain could be genetically

distinguished, providing insight into the effects of anthropogenic influence on the genetic structure and diversity of a species.

Paleobotanical and genetic data indicate central and northern European populations were colonised from source populations in southern France, eastern Alps-Slovenia-Isteria and potentially Moravia and southern Bohemia (Magri et al. 2006). Residual populations in classic southern refugia (i.e. the Iberian, Italian and Balkan peninsulas) expanded relatively late and did not significantly contribute to the colonisation of central and northern Europe. Recent evidence found in Denmark suggests that post-glacial colonisation was aided by occasional long-distance dispersal events, leading to the establishment of beech and other temperate tree species ahead of their main colonisation fronts (Overballe-Petersen et al. 2013) and might have contributed significantly to the observed rates of spread of the species (Feurdean et al. 2013). Pollen records from Great Britain indicate that beech migrated into the south-east, with its first establishment in Kent just before 3000 BP and maintained a steady rate of spread of 100-200 m per year, whereas the majority of other tree species displayed a decrease in the rate of spread. The relatively constant rate of spread suggests that beech had not reached its natural climatic limit by 1000 BP (Birks 1989). Historical evidence has suggested the existence of potential native populations which occur further north (Rackham 1980) than the predicted range as suggested by pollen evidence. Confirmation of species presence in the pollen record does not directly translate to the confirmation of the forests which exists today as native. Therefore, the genetic aspect of the study aims to add genetic information of contemporary populations to build upon current palynological and historical information.

We sought to determine the interacting effects of past anthropogenic impacts and past migration on the current genetic structure and diversity of beech by identifying the phylogeographic signal of natural colonisation, which may persist in its native range. We used

a combination of highly variable nuclear markers and conservative, maternally-inherited chloroplast markers (Reboud and Zeyl 1994, Magri et al. 2006) to explore regional trends in genetic variation. Extensive sampling was carried out in both the native and non-native range across Great Britain. Population structure and admixture levels of beech throughout the country were analysed using Bayesian assignment methods. The output of this research provides direct information on extant forests, reconciling the genetic, paleoecological, and historical evidence of the native range of beech in Britain. This information can be used to optimise conservation plans and can indicate avenues for future research, for example, in examining the impacts of translocation on local adaptation through the assessment of phenotypic variation.

4.3 Materials and methods

4.3.1 Study species

The broadleaf tree species, *F. sylvatica,* covers approximately 14 million ha forming the dominant forest type in much of mainland Europe. With the exception of Great Britain, the distribution of beech is primarily climatically limited owing to the species' drought susceptibility (Peterken and Mountford 1996) and frost sensitivity (Watt 1923). Beech trees can reach approximately 300 years of age with flowering beginning between 40 to 80 years, depending on the density of the stand (Firbas and Losert 1949). Beech is a monoecious, primarily outcrossing species, with pollen dispersed by wind and seeds dispersed by gravity and animals (Wagner et al. 2010, Packham et al. 2012).

4.3.2 Study sites

A total of 42 populations were sampled across Great Britain, covering the putative native and the non-native range of beech (Table 4.1; Figure 4.1). Using a combination of historical records, palynological, and anecdotal evidence, study sites were designated *a priori* stand

origins of native or non-native (see supplementary material S4.1, S4.2, and S4.3 for summary of evidence). In each site, leaf samples were collected from 20 mature trees within a 10ha area, preferentially sampling the oldest trees determined by using diameter at breast height (DBH) as a proxy for age. Trees were sampled no closer than 10m to each other to avoid sampling possible ramets and all samples were geo-referenced using a GARMIN 62s handheld GPS.

4.3.3 Molecular analysis

DNA was obtained from the leaf samples, dried in silica gel. DNA isolation was performed using the QIAGEN DNeasy 96 Plant Kit (QIAGEN, Netherlands) according to manufacturers' instructions. Out of a total of 840 samples, 837 individuals were successfully genotyped using 13 nuclear, microsatellite (*viz.* simple-sequence repeats (SSR)) markers (fs1-03, fs1-15, fs3-04, fs4-46, fcm5 (Pastorelli et al. 2003), mfc7 (Tanaka et al. 1999), mfs11 (Vornam et al. 2004), sfc0007-2, sfc0018, sfc0036,sfc1143, sfc1061, sfc1063 (Asuka et al. 2004)) processed in three multiplexes as detailed in Sjölund and Jump (2015). 802 individuals were successfully genotyped using four Chloroplast DNA (CpDNA) SSR markers (ccmp4, ccmp7 (Weising and Gardner 1999), cmcs3, and cmcs12 (Sebastiani et al. 2004)). Chloroplast SSRs were combined in one PCR multiplex, FSCplex, using 10ng of template DNA and the QIAGEN Type-it Microsatellite PCR Kit with the following primer concentrations, ccmp4 at 0.5 µM, ccmp7 at 0.5µM, cmcs3 at 3µM, and cmcs12 at 3µM. Annealing temperature was set to 55°C, with a total PCR reaction volume of 10µl. Fragment analysis was performed on an ABI 3730 (Applied Biosystems) and allele scoring on GENEMARKER 2.4.0 (SoftGenetics).

Scoring errors and null alleles in nuclear loci were checked using MICRO-CHECKER (Van Oosterhout et al. 2004). Null alleles were identified by repeated sampling of null genotypes and significant deviations from Hardy-Weinberg equilibrium. Significant proportions of null

Table 4.1 The 42 study sites in Great Britain grouped by potential stand origins

Code	Site name	Latitude Longitude
Native		
BED	Bedford Purleius	N52.5899 W0.4646
BLE	Blean Woods	N51.3068 E1.0284
BUC	Buckholt Wood	N51.8166 W2.1560
BUR	Burnham Beeches	N51.5604 W0.6238
CWe	Cwm Clydach (east)	N51.8060 W3.1280
CWw	Cwm Clydach (west)	N51.7403 W3.9094
DEN	Denny Wood	N50.8563 W1.5250
FEL	Felbrigg Great Wood	N52.9138 E1.2612
FRI	Friary Wood	N51.3302 W2.3124
GRE	Greenfield Copse	N51.6247 W0.9764
LAD	Lady Park Wood	N51.8254 W2.6541
LUL	Lullington Country Park	N51.3545 E0.1817
MON	Monk Wood	N51.6632 E0.0547
SAV	Savernake Forest	N51.4121 W1.7163
SEC	Seckley Wood	N52.4033 W2.3413
WEA	Wealden Edge Hangars	N51.0504 W0.9637
WYC	Wychwood (Conbury Park)	N51.8590 W1.5131
Non-native		
APP	Applecross Wood	N57.4328 W5.8105
BAR	Baron's Haugh	N55.7741 W3.9716
BEE	Beech Hill Wood	N54.3225 W2.9390
BRI	Bridford Wood	N50.6792 W3.7009
CAR	Carstramon Wood	N54.9113 W4.1974
CLE	Clerkhill Wood	N57.1983 W2.1566
CRA	Craig Wood	N57.5777 W4.1435
DEV	Devachoys Wood	N50.1966 W5.1229
DRU	Drumneil House	N56.5480 W5.4003
DUN	Dunnottar Wood	N56.9601 W2.2197
ECC	Ecclesall Woods	N53.3366 W1.5167
GEL	Gelt Wood	N54.9089 W2.7311
GOL	Golitha Wood	N50.4931 W4.4997
HEM	Hembury Wood	N50.5002 W3.8046
KIN	Kinnoul Hill Woodland Park	N56.3891 W3.3993
MAB	Mabie Forest	N55.0226 W3.6461
PLO	Plora Wood	N55.6191 W3.0342
STR	Strid Wood	N54.0030 W1.9037
TAL	Talhenbont	N52.9304 W4.2910
TAN	Tan-y-Coed	N52.6323 W3.8415
TON	Tongue Wood	N58.4989 W4.4091
TWO	Two Mile Bottom	N52.4576 E0.7191
WAL	Wallington East Woods	N55.1533 W1.9506
WYT	Wytham Wood	N51.7720 W1.3368
YEL	Yellowcraig Wood	N56.0617 W2.7793

Sites are grouped into potential native and non-native sites and ordered alphabetically within group. Stand codes are derived from the first three letters of the site name, with the exception of Cwm Clydach east, denoted CWe, and Cwm Clydach west, denoted CWw.

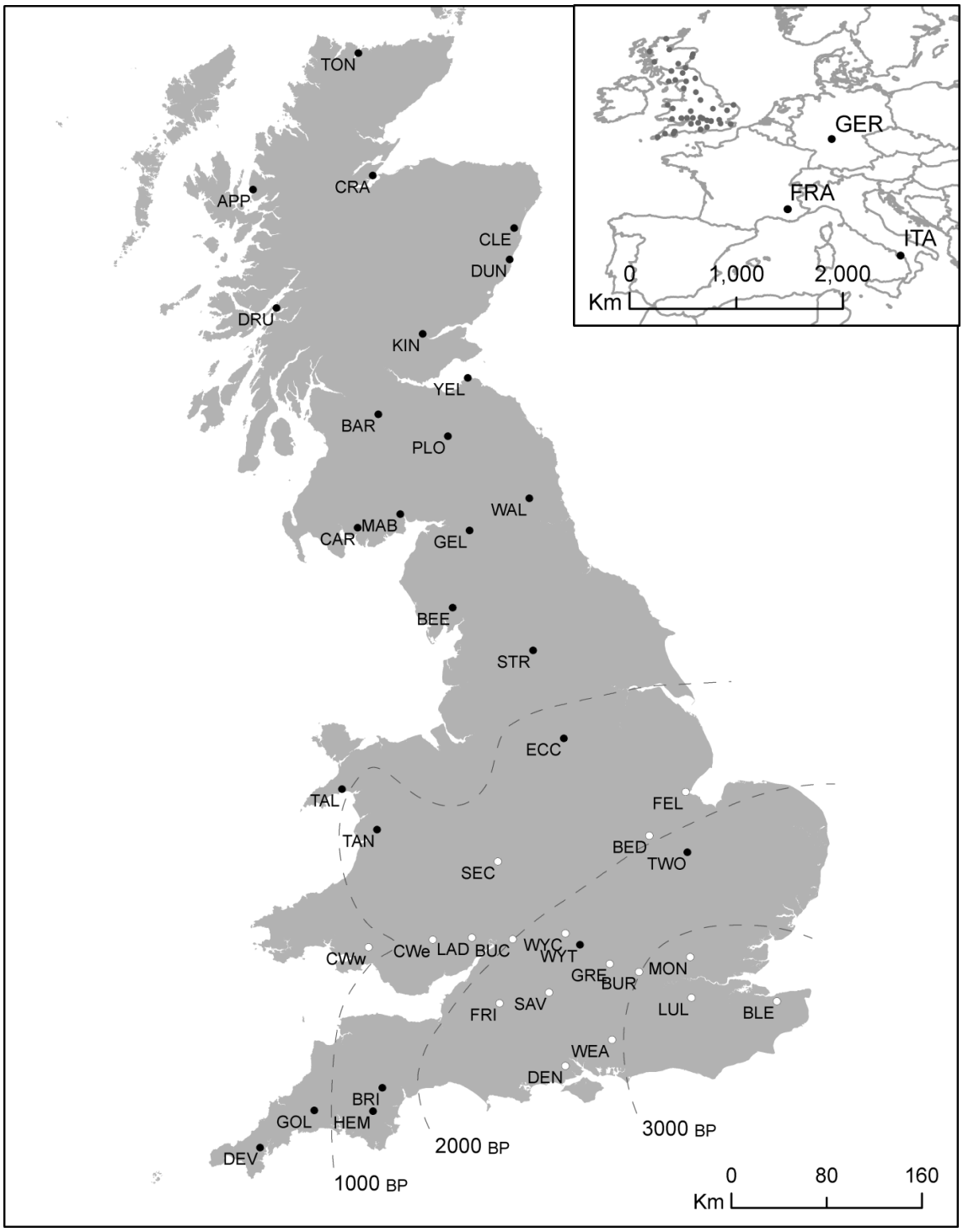

Fig. 4.1 Map of study sites in relation to Birks' (1989) isochrones including continental sites. Sites are labelled with site codes and possible native sites are indicated by white circles. Birks' (1989) isochrones for *F. sylvatica* have been redrawn on the map, labelled in years BP. The location of continental sites in relation to sites in Britain are indicated by black circles in the top right hand corner.

alleles were found in fs4-46, fcm5, and fs1-15. Analyses presented exclude fs4-46, fcm5, and fs1-15 and use a total of 10 nuclear SSR loci. A total of three chloroplast SSR loci were used, excluding cmc12 as it was monomorphic. We tested for gametic disequilibrium between nuclear loci pairs on FSTAT 2.9.3.2 (Goudet 1995), identifying significant associations between loci by randomly associating genotypes at pairs of loci 1100 times, using a 5% nominal level after Bonferonni correction. The multilocus average error rates were 0.4% for the 10 nuclear loci included in analysis, and 0.0% for the 3 chloroplast loci. The error rate per locus was calculated as the number of erroneously assigned loci over 45 repeated samples.

4.3.4 Analysing population clusters

Individual-based Bayesian assignment methods were performed using data from nuclear loci in STRUCTURE 2.3.4.(Pritchard et al. 2000). No stand origin was included *a priori* in cluster analysis. To examine relationships between British samples and source populations in Europe, we included a subset of 150 samples collected from native beech forests in France, Germany and Italy as detailed in Sjölund and Jump (2015). Analysis without continental samples revealed a similar structure in Britain to that found with continental samples, therefore we present the data including continental samples to set the results in context. The STRUCTURE model employed the correlated allele frequency model (Falush et al. 2003) and the admixture ancestry model. We included the site location *a priori* (LOCPRIOR option) to improve the detection of weak population structure (Hubisz et al. 2009). *K* was set from 1 to 20, with 10 runs performed for each number of *K*. Runs consisted of 500,000 Markov Chain Monte Carlo (MCMC) iterations with a burn-in period of 100,000. To determine the number of clusters in the data, we plotted the log probability of the data (LnP(D)), identifying the point where log likelihood values ceased to converge. Individual Q-matrices were computed in CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007), with graphics created in DISTRUCT 1.1 (Rosenberg 2004). Q-matrices presented are ordered according to Birks' isochrones following an approximate

geographical gradient. As results indicated cryptic genetic structure in the native range (see section 4.4.3), we performed a subsequent analysis on a subset of the 17 potential native sites (i.e. using the stand origin *a priori*) under the same conditions as the main model and excluding continental samples to test for further population sub-structure.

We present data in supplementary material S4.4 using a second cluster assignment program to explore the samples further as recommended by Guillot et al. (2009). Partitioning of the data into clusters was performed using discriminant analysis of principle components (DAPC), a multivariate statistics based method executed in the *adegenet* (Jombart 2008) package in R 3.0.2 (R Development Core Team 2012).

4.3.5 Measuring genetic diversity and structure using nuclear and chloroplast markers

To distinguish estimates of genetic diversity we have prefixed estimators with '*n*' for nuclear and '*c*' for chloroplast. Nuclear genetic diversity was measured in rarefied allelic richness (nA_R) (Petit et al. 1998), gene diversity corrected for sample size (nH_S) (Nei 1978), and the inbreeding coefficient (nF_{IS}) (Weir and Cockerham 1984), calculated in SPAGeDi 1.4b (Hardy and Vekemans 2002), and rarefied private allelic richness (nA_P) calculated in ADZE 1.0 (Szpiech et al. 2008). For the purpose of mapping genetic differentiation, for each site we calculated the percentage of total sites that it was significantly differentiated to (i.e. percentage of differentiated sites, *nDS (%)*), based on nF_{ST} values (Weir and Cockerham 1984). nF_{ST} values were obtained from pairwise tests of genetic differentiation not assuming Hardy-Weinberg , with significances determined for a 5% nominal level after Bonferonni correction in FSTAT 2.9.3.2 (Goudet 1995). Chloroplast haplotypic diversity was measured as the number of haplotypes (cH_N) and the number of private haplotypes (cH_P). Multilocus estimates of genotypic and haplotypic diversity were mapped in ARCMAP 10 (ESRI software) against the redrawn isochrones lines from Birks' (1989) Holocene isochrone map for the rational limit of beech

pollen.

To test for differences of nuclear-based measurements, nA_R, nH_S, nF_{IS} and nF_{ST} among groups of native and non-native sites, we performed permutation tests using FSTAT 2.9.3.2 (Goudet 1995). The desired estimator (x) over all sites and loci for groups of native and non-native sites was calculated to obtain an observed statistic (OS_x), which is the difference between the estimators of the two groups, i.e. $OS_x = x1 - x2$. Individuals were permuted 10,000 times between groups to obtain a randomised dataset from which the statistic S_x is calculated. P-values signify the proportion of randomised datasets where $S_x > OS_x$. The difference between native and non-native groups in the remaining estimators nA_P and cN_H were tested using the non-parametric Mann-Whitney U test. We tested for genetic structure amongst native and non-native sites, using both chloroplast and nuclear markers in a hierarchical analysis of molecular variance (AMOVA) performed in ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010).

4.3.6 Measuring geographic patterns in genetic structure

Subsets of sites with potential native and non-native origin were tested for isolation-by-distance (IBD) in the British dataset using nuclear markers. IBD was analysed with and without the continental datasets to check whether a geographical cline in allelic frequencies could be influencing clustering (Guillot et al. 2009). Following Rousset (1997), we used the $F_{ST}/(1- F_{ST})$ ratio as a measure of genetic distance as it is expected to vary linearly with the natural log of the geographical distance. The significance of IBD was determined by permuting site locations among individuals 10,000 times. To test for geographic gradients in genetic diversity we performed non-parametric corrected Spearman's Rank tests on all genetic diversity estimators and all sites, including separate tests on subsets of native and non-native sites.

Table 4.2 Genetic diversity estimates obtained from nuclear (*n*) and chloroplast (*c*) markers

Sites	*nN*	nA_R	nA_P	nH_S	nF_{IS}	*nDS (%)*	*cN*	cH_N
Native	340	6.22±0.13	0.053±0.13	0.707±0.006*	0.022±0.010	46.1±6.4	321	1.5±0.2
BED	20	5.95	0.025	0.722	0.059	17.1	18	1
BLE	20	6.82	0.027	0.721	0.030	24.4	18	4†
BUC	20	5.33	0.000	0.682	0.010	87.8	20	1
BUR	20	6.80	0.109	0.713	-0.003	22.0	19	2
CWe	20	6.64	0.146	0.750	0.055	85.4	20	1
CWw	20	6.00	0.000	0.710	-0.037	85.4	20	1
DEN	20	5.97	0.024	0.706	0.031	58.5	20	1
FEL	20	5.17	0.095	0.651	0.057	75.6	17	2
FRI	20	6.51	0.087	0.679	0.007	26.8	20	1
GRE	20	5.98	0.004	0.712	0.038	26.8	20	1
LAD	20	6.72	0.110	0.736	0.033	22.0	20	1
LUL	20	6.09	0.020	0.727	-0.054	29.3	17	3
MON	20	6.71	0.156	0.716	-0.013	56.1	19	1
SAV	20	6.09	0.001	0.687	0.041	43.9	16	1
SEC	20	6.06	0.008	0.717	-0.040	56.1	17	1
WEA	20	5.86	0.007	0.703	0.070	56.1	20	2
WYC	20	7.12	0.077	0.694	0.088*	9.8	20	1
Non-native	497	6.25±0.11	0.037±0.008	0.690±0.006*	-0.006±0.010	32.9±4.5	481	1.4±0.1
APP	19	6.68	0.065	0.682	0.004	14.6	19	2
BAR	19	6.50	0.001	0.702	-0.012	17.1	19	2
BEE	20	4.58	0.000	0.606	-0.152***	95.1	19	2
BRI	20	6.21	0.080	0.663	-0.065	58.5	20	1
CAR	19	5.92	0.000	0.685	0.057	26.8	18	1
CLE	20	6.30	0.014	0.695	0.014	17.1	20	1
CRA	20	5.29	0.000	0.706	-0.086*	75.6	20	1
DEV	20	6.30	0.038	0.691	-0.066	22.0	16	2†
DRU	20	6.44	0.042	0.692	-0.012	39.0	20	1
DUN	20	7.16	0.103	0.684	0.005	24.4	19	2
ECC	20	6.32	0.025	0.700	0.036	56.1	20	2
GEL	20	6.32	0.013	0.700	0.014	17.1	20	1
GOL	20	5.49	0.048	0.672	0.034	36.6	20	1
HEM	20	5.86	0.001	0.672	-0.042	70.7	20	1
KIN	20	5.87	0.013	0.675	-0.005	34.1	20	1
MAB	20	6.14	0.011	0.701	0.089*	9.8	20	1
PLO	20	6.04	0.013	0.693	0.019	24.4	17	2
STR	20	6.67	0.099	0.692	-0.012	12.2	20	1
TAL	20	6.28	0.122	0.667	0.018	17.1	15	2
TAN	20	7.19	0.077	0.747	0.065	51.2	20	1
TON	20	6.23	0.000	0.680	0.023	24.4	20	1
TWO	20	6.33	0.014	0.717	0.003	39.0	20	1
WAL	20	6.52	0.033	0.708	-0.069	7.3	20	1
WYT	20	6.79	0.103	0.700	-0.005	4.9	20	2
YEL	20	6.84	0.000	0.722	-0.012	26.8	19	1
Total	837	6.25±0.08	0.044±0.007	0.696±0.004	0.004±0.008	38.7±3.8	802	1.4±0.1

Table 4.2 (*continued*):

Prefixes of 'n' for nuclear markers and 'c' for chloroplast marker are given to distinguish the markers used per estimate. Terms are as follows; *nN,* number of samples used in nuclear analysis; nA_R, nuclear rarefied allelic richness; nA_P, nuclear rarefied private allelic richness; nH_S, nuclear gene diversity; nF_{IS}, nuclear inbreeding coefficient; *nDS%,* percentage of significantly differentiated sites*; cN,* number of samples used in chloroplast analysis; cH_N, number of haplotypes with † indicating the occurrence of two individuals with a private haplotype (cH_P) in separate sites. The minimum number of gene copies (*k*) used for rarefication analysis of nA_R and nA_P is 38.

For each group (*Native, Non-native)* and all sites (*Total*), the mean ± standard error are given for genetic diversity estimators, with the sum for the number of samples (*nN, nC*). *P*-values for F_{IS} within sites are obtained after 10,000 permutations of gene copies within individuals per site. Significant *P*-values for groups of native and non-native sites represent results from permutation tests for nA_R, nH_S, nF_{IS}, and for *nDS%*(using nF_{ST}), and a Mann-Whitney U test for nA_P, cN_H. Significant *P*-values are indicated as * $P < 0.05$, *** $P < 0.001$.

4.4 Results

4.4.1 General estimates of nuclear genetic diversity

Multilocus estimates of genetic diversity were obtained for 10 nuclear loci, with an average number of 13.3 alleles, and a maximum number of 5 to 30 alleles depending on the locus (Table 4.2 and Figure 4.2). Rarefied allelic richness (nA_R) varied from 4.58 to 7.19 with rarefied private allelic richness (nA_P) ranging from 0 to 0.156. Gene diversity estimates ranged from 0.606 to 0.750. Potential native site WYC and non-native MAB displayed a significant homozygote excess (WYC $nF_{IS} = 0.088$, $P < 0.05$; MAB $nF_{IS} = 0.089$, $P < 0.05$), whilst a heterozygote excess was found in sites BEE and CRA, both non-native sites (BEE $nF_{IS} = -0.152$, $P < 0.001$; CRA $nF_{IS} = -0.086$, $P < 0.05$). Genetic differentiation varied greatly between sites, with percentage differentiation per site ranging from 4.9% to 95.1% of all sites. All nuclear loci were under gametic equilibrium considering a 5% nominal level after Bonferroni correction.

4.4.2 Detection of cryptic population structure

Mean log-likelihood values for each of the STRUCTURE runs on samples from Britain including the continental samples, gradually ceased to converge after $K = 2$, after which they began to plateau (Figure 4.3). Examination of Q-matrices indicated that $K = 3$ provided meaningful

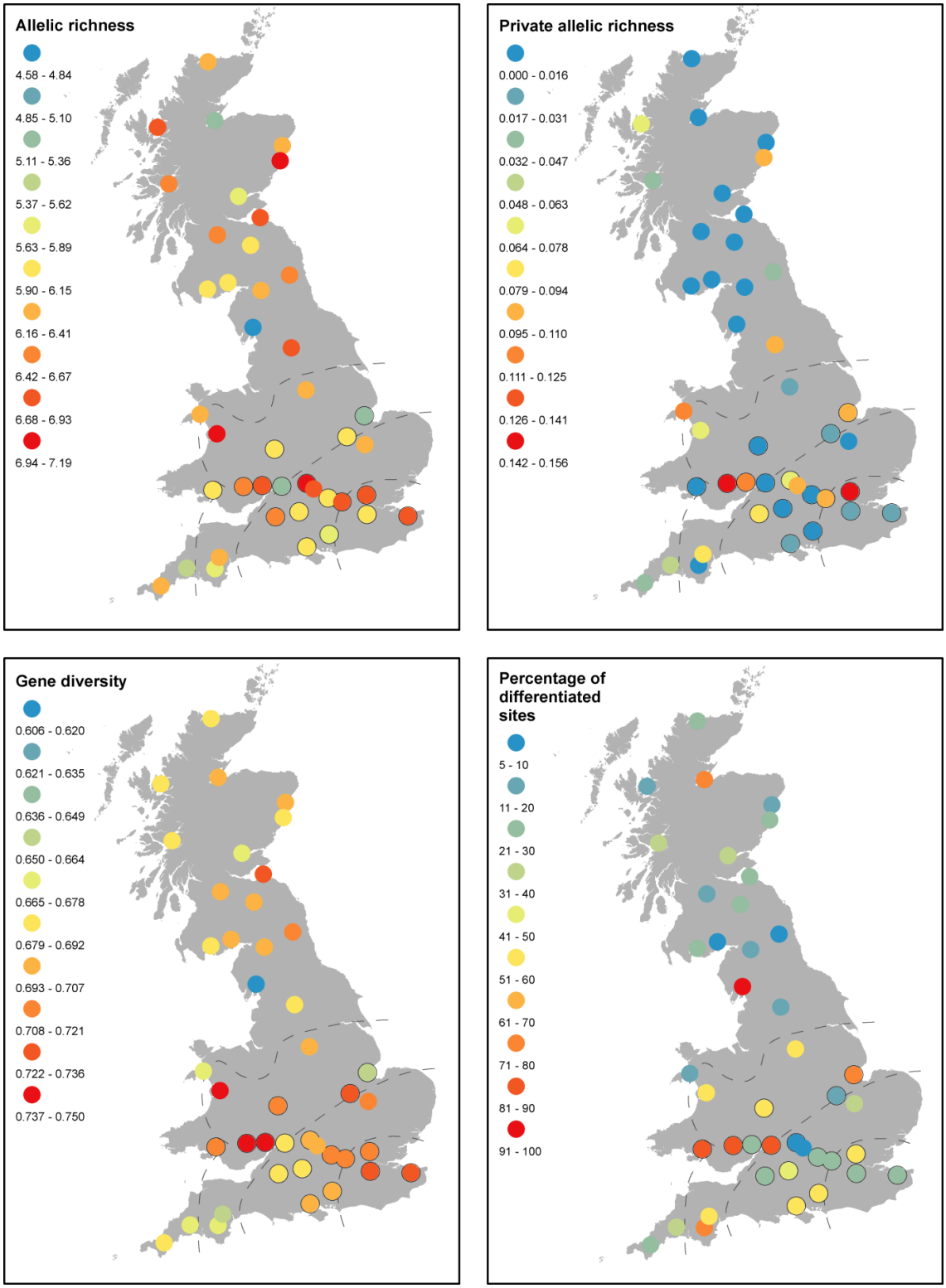

Fig. 4.2 Maps of estimators of nuclear genetic diversity. Estimators include rarefied allelic richness (nA_R), rarefied private allelic richness (nA_P), gene diversity (nH_S), and the inbreeding coefficient (nF_{IS})(Weir and Cockerham 1984)(Weir and Cockerham 1984)(Weir and Cockerham 1984)(Weir and Cockerham 1984). For every given site we present the percentage of significantly differentiated sites (i.e. *nDS%*), based on F_{ST} values that were obtained from pairwise tests of genetic differentiation not assuming Hardy-Weinberg. Sites with possible native origin are outlined. Birks' (1989) isochrones for *F. sylvatica* have been redrawn.

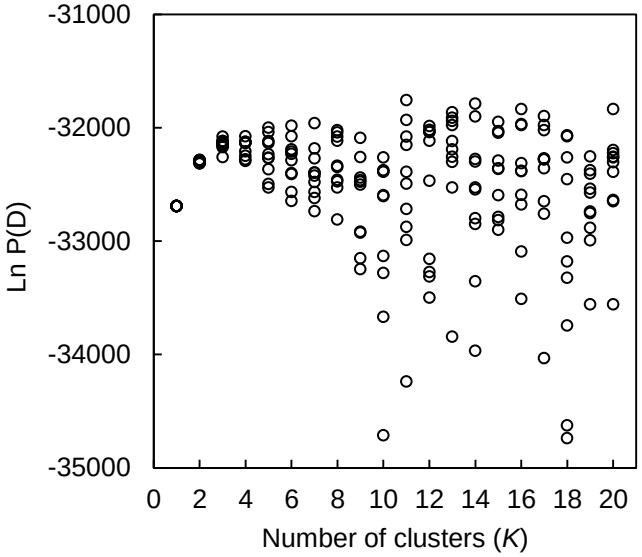

Fig. 4.3 Log-likelihood values for the number of clusters in the data. The number of clusters (*K*) is plotted against the log probability of the data (Ln P(D)) for each of the 10 runs per *K* value.

biological clusters that followed a regional distribution (Figure 4.4). Although values of Ln P(D) for *K* = 3 varied more than *K* = 2, assignment of individuals to clusters were congruent between runs (see supplementary material S4.5 for Q-matrices for per run).

Several sites throughout Britain contained highly admixed individuals. Individuals from continental sites displayed homogenous levels of admixture within site. Organising the Q-matrix according to Birks' (1989) isochrones in approximate geographic order revealed some consistency in cluster assignment between neighbouring sites (Figure 4.4). The predominant cluster in the continental subset changed from the blue cluster in ITA, to the grey cluster in FRA and GER, with the introduction of the red cluster in GER. Individuals from Great Britain were predominantly assigned to the grey and red clusters, with the red cluster being relatively distinct to the country. There were no clear differences between the sites with potential native and non-native origins. However, the red cluster appeared to be associated with sites within the putative native range and the non-native sites in the south-west (DEV, GOL, HEM, and BRI). Similar patterns were observed for *K* = 2 and *K* = 3 when analysing a subset of British samples alone and a subset of potential native sites, both revealing no further population sub-structuring (see supplementary material S4.3 and S4.7). Population structure

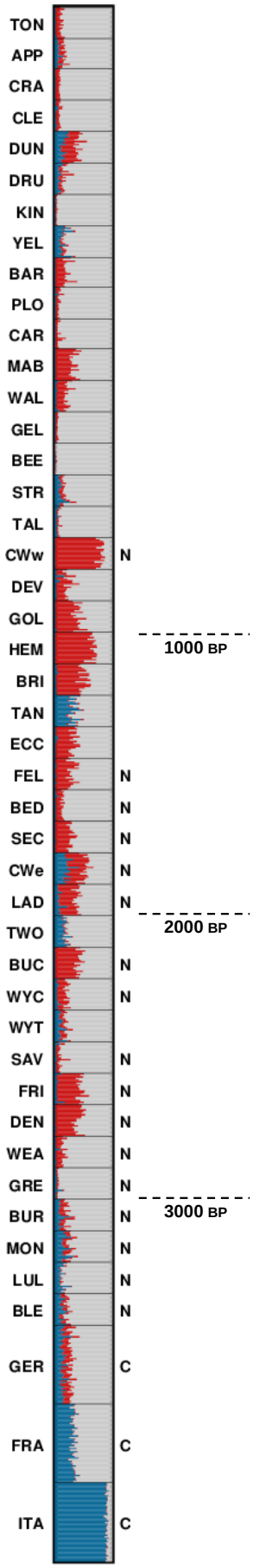

Fig. 4.4 Regional genetic structure in Britain. Three clusters are shown in blue, red, and grey. Each horizontal bar represents an individual with the proportions of its genetic make-up assigned probabilistically to each of the three clusters. The STRUCTURE Q-matrix is calculated using the average assignment probabilities over 10 consecutive runs. Sites are ordered on an approximate geographical gradient by ordering sites following Birks' (1989) isochrones to reflect the migration route of Beech into Britain. Continental samples are based at the bottom of the graph, with a general northward trend to the top of the graph. Stand history of the sites are indicated on the right of the Q-matrix, with continental sites labelled C, native sites N, and non-native sites left blank. Approximate borders of the isochrones are indicated by a dashed line with years in BP, with site codes on the left.

was less differentiated in DAPC with a weaker regional signal compared to STRUCTURE. Increasing the number of clusters indicated complex and biologically uninformative structuring.

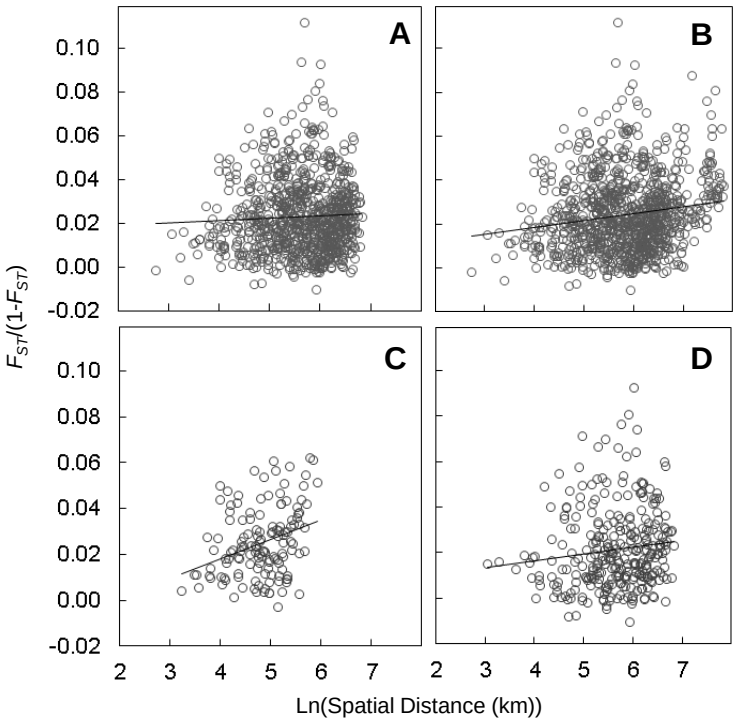

Fig. 4.5 Comparison of isolation-by-distance analyses. Black lines represent the slope of the regression of the natural log of the linear spatial distance (Ln(Spatial Distance (km)) against $F_{ST}/(1-F_{ST})$ after 10,000 permutations of sites among locations. Sites included in the analysis are as follows; A) British sites only (slope=0.0011, R^2<0.01); B) British and continental sites (slope=0.0032, R^2=0.03); C) native sites (slope=0.0085*, R^2=0.10)); D) non-native sites (slope= 0.0030, R^2=0.02).

4.4.3 Geographic trends in genetic diversity between native and non-native sites

Significant IBD was found in British sites with potential native history (refer to Figure 4.5C: slope = 0.0085, R^2 = 0.10, P < 0.05). IBD was not significant in sites with non-native stand origins, nor was it significant in all sites in Britain with and without the continental subset (refer to Figure 4.5, A: British sites only (slope = 0.0011, R^2 < 0.01, P = 0.223), B: British and continental sites (slope = 0.0032, R^2 = 0.03, P = 0.056), D: non-native sites (slope = 0.0030, R^2 = 0.02, P = 0.088). Within the native sites, there was a significant reduction in haplotype number following an east to west gradient (rho = 0.70, P < 0.01; Figure 6). This effect disappeared when all sites were analysed together (rho = 0.18, P = 0.241), and was not found in non-native sites alone (rho = -0.02, P = 0.900). It should be noted that some differences between native and non-native groups could be due to geographical distribution as native sites occur further

east and non-native sites occur further west (U(40) = 358, Z = 3.72, $P < 0.001$).

Significantly higher levels of gene diversity (nH_S) were found in native sites, compared to non-native sites; H_S: Native 0.708, Non-native 0.690 ($P < 0.05$) (Table 4.2). The general trend of lower gene diversity in sites outside of the native range can be seen in the map for gene diversity in Figure 4.2. No significant differences were found for other estimators; nA_R: Native 6.225, Non-native 6.252 ($P = 0.874$), nA_P: Native 0.053, Non-native 0.037 (U(40) = 245, Z = 0.84, $P = 0.411$), nF_{IS}: Native 0.022, Non-native -0.005 ($P = 0.073$), nF_{ST}: Native 0.024, Non-native 0.021($P = 0.5919$), and cN_H: Native 1.5, Non-native 1.4 (U(40) = 208, Z = -0.155, $P = 0.89$). No significant correlations were found in the nuclear genetic diversity estimators and geographic variables, latitude and longitude, overall sites and within subsets of native and non-native sites (data not shown).

Three variants were detected for each locus, ccmp4, ccmp7 and cmcs3. The number of haplotypes (cH_N) within sites ranged from 1 to 4, with a total of 7 haplotypes recorded (Table 4.2, Figure 4.6). One haplotype (A) was present in all sites and was the dominant haplotype within sites. Haplotype diversity was highest in site BLE ($cH_N = 5$), the most south-easterly British site, with its neighbouring site attaining the second highest measure of diversity ($cH_N = 3$). Both BLE and LUL have potential native origins. Two private haplotypes, F and G, were present in sites BLE and DEV, respectively. The AMOVA revealed that there was significant genetic structuring in chloroplast and nuclear allelic variation between sites, although no significant difference was found between groups, with the remaining variation present within individuals (Table 4.4).

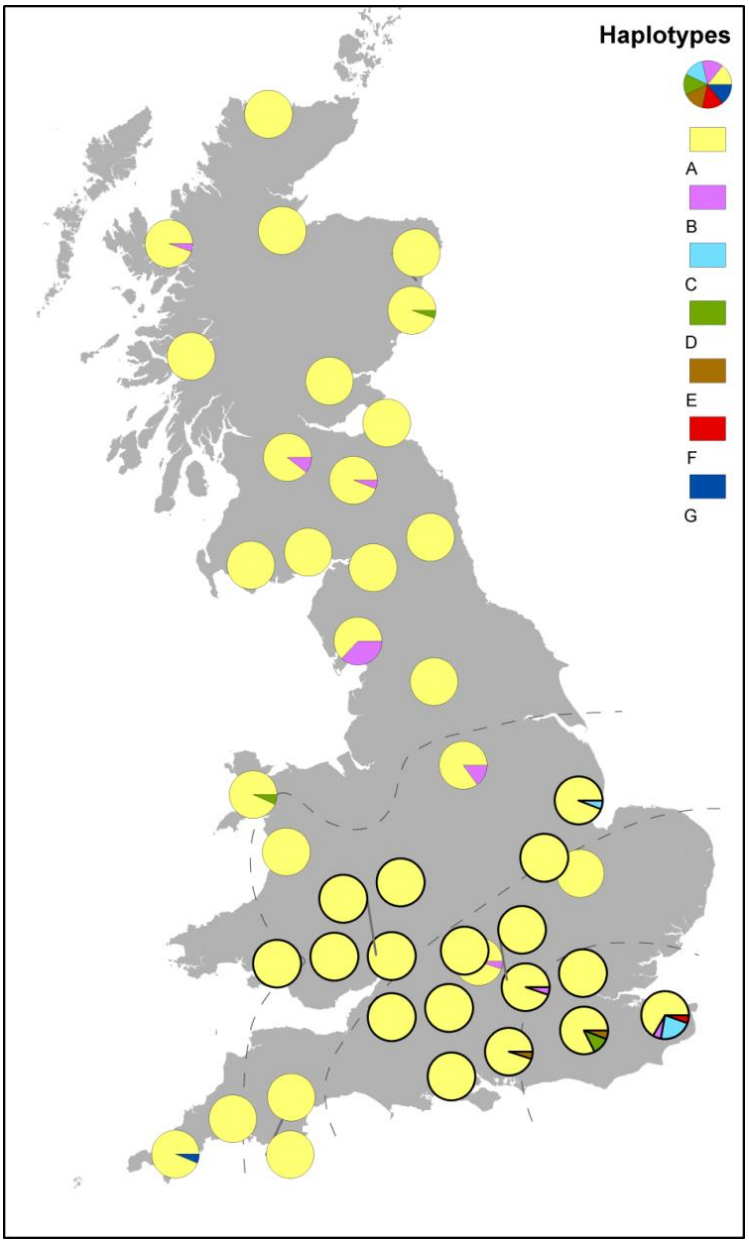

Fig. 4.6 Map of chloroplast haplotypic diversity. A total of seven haplotypes are displayed. Birks' (1989) isochrones have been redrawn on the map. Sites with possible native origin are outlined in bold.

Table 4.3 Allelic composition for each haplotype (Hap) at three loci (ccmp4, ccmp7, and cmcs3)

Hap	ccmp4	ccmp7	cmcs3
A	115	144	170
B	115	145	170
C	115	144	171
D	115	143	170
E	114	144	170
F	115	143	171
G	115	144	169

Table 4.4 Hierarchical analysis of molecular variance (AMOVA) for chloroplast and nuclear markers

	Chloroplast			***Nuclear***		
Levels	***df***	**Variation (%)**	***F*-statistic**	***df***	**Variation (%)**	***F*-statistic**
Among groups	1	0.00	0.000	1	0.01	0.000
Among sites within groups	40	12.59	0.124***	40	2.25	0.022***
Within sites	760	87.63	0.126***	795	97.74	0.023***

Sites are grouped into potential stand origin, native or non-native. The degrees of freedom (*df*), percentage of variation explained by each level (Variation (%)), and the relevant F-statistic are presented with significant *P*-values indicated as *** $P < 0.001$.

4.5 Discussion

4.5.1 Genetic diversity of beech in Britain

Nuclear markers indicated average levels of rarefied allelic richness in Britain (nA_R = 6.25±0.08) were lower than those reported in studies using some of the same microsatellite markers, approximately ranging from 8.2 to 18.2 in other studies (Jump and Peñuelas 2006, Buiteveld et al. 2007, Sjölund and Jump 2015). Sites in Britain also displayed lower levels of rarefied private allelic richness (nA_P = 0.044±0.007) compared to Sjölund and Jump (2015) where values for nA_P ranged between 1.51 and 2.36. Other studies on beech do not employ rarefied private allelic richness and therefore cannot be compared. Overall levels of gene diversity were similar to that found in other studies (nH_s = 0.696±0.004) (Jump and Peñuelas 2006, Buiteveld et al. 2007, Oddou-Muratorio et al. 2009, Sjölund and Jump 2015).

We found high levels of chloroplast diversity for the three microsatellite loci with a total of seven haplotypes (cH_N) (Table 4.3), although all sites were dominated by one haplotype (A). The high number of haplotypes is in contrast to that found by Magri et al. (2006) who report one haplotype throughout Britain. However, restricting analysis to two of the three loci used by Magri et al. (2006), ccmp4 and ccmp7, gives a total of four haplotypes with no more than 3 haplotypes in any one site (data not shown). The regional trend using a subset of two loci closely matches the trend seen with all three loci (Figure 4.6), with sites LUL and BLE again displaying the highest number of haplotypes. It is possible that the larger sample size used in our study significantly increased the potential of detecting rarer haplotypes as the AMOVA on chloroplast allelic variation revealed significant partitioning between sites (Table 4.4). The large haplotype diversity found in our study is likely a consequence of polymorphism in locus cmcs3, the use of a large number samples and sites, and the inclusion of several planted sites that could have originated from continental stock and thus may harbour rarer haplotypes.

4.5.2 Regional patterns of postglacial migration of beech into Britain

Within Britain, neighbouring sites displayed congruent levels of admixture with lower levels of admixture in northern, non-native sites (Figure 4.4). A gradient of admixture marked the transition of continental regions to Britain, with continental sites assigned predominantly to the blue and grey cluster. Individuals from the south of Britain displayed a relatively higher probability of assignment to the red cluster, which was generally associated with the native range, in addition to the south-west region, including non-native sites DEV, GOL, HEM, and BRI. This suggests significant gene flow between sites in the native range and sites in the south-western peninsula. DEV also displayed a private haplotype adding to the distinctiveness of the south-westerly beech forests.

There appeared to be a transition between the assignment of individuals to the red and grey cluster which occurred in proximity to the border for the 1000 BP isochrone, with a tendency towards assigning individuals to the red cluster in regions pre-1000 BP. Only one potential native site, CWw, occurred outside of the border of the 1000 BP limit and was predominantly assigned to the red cluster. CWw is less than 15km away from the 1000 BP isochrone and borders can only be determined in relation to the original study sites used in Birks (1989) of which there were relatively few with reliable records for beech. Therefore small errors in the geographical position of boundaries are probable and CWw may still be within regional palynological boundaries.

Population structure identified using DAPC was weaker and did not display as strong regional trends as those found in STRUCTURE. DAPC is based on fewer assumptions than STRUCTURE (Jombart et al. 2010). The detection of cryptic population structure in Britain was likely aided by the use of sample group information as location priors, and the incorporation of assumptions on admixture and allele frequencies in STRUCTURE (Hubisz et al. 2009). This is in

agreement with preliminary analysis, as the detection of structure in Britain was found to be sensitive to changes in model assumptions, as no patterns of genetic structure was found when using the independent allele frequency model in STRUCTURE (data not shown). STRUCTURE has been successfully used to detect genetic structure at low F_{ST} levels (Latch et al. 2006). Although the regional trend was not detected in DAPC, it was reproducible when using the same model parameters in STRUCTURE for a subset of sites with possible native origin and a subset of sites in Britain, highlighting the existence of a repeatable trend.

The distribution of chloroplast haplotypes in Britain matches the expected phylogeographic signal of postglacial colonisation with the highest number of haplotypes found in south-eastern sites, LUL and BLE, (Figure 4.6) in close proximity to the purported entry point of beech migration into Britain (Birks 1989). Site BLE also displayed one of the two private haplotypes found in Britain. The chloroplast genome in beech is maternally inherited (Magri et al. 2006), therefore, patterns of initial beech migration into Britain were driven by significant seed movement into the south-east as high diversity exists in these sites. The gradual loss of haplotype diversity may be a result of a founder effect induced by the progressive movement of the migration front in the native range (Excoffier et al. 2009), and the artificial creation of stands through sowing or planting by humans in the non-native range (Lefèvre 2004).

4.5.3 Genetic variation between groups of different *a priori* stand origins

Although Britain's semi-natural forests have been influenced by humans for prolonged periods of time (Rackham 1980), historic patterns of genetic variation may persist in populations that arise from the natural regeneration of local stock (Bradshaw 2004). Isolation-by-distance (IBD) occurs when the genetic differentiation between individuals or populations increases with geographic distance (Wright 1940). In plants, this is primarily a consequence of restricted gene flow via seed or pollen (Loveless and Hamrick 1984). Although beech is assumed to show high

levels of gene flow as a wind-pollinated tree, it has been found to display significant structuring at local (Chybicki et al. 2009, Jump et al. 2012, Piotti et al. 2013, Sjölund and Jump 2015) and regional scales (Jump and Peñuelas 2006, de Lafontaine et al. 2013). In agreement with the significant genetic structuring found in natural populations of beech, the potential native sites in Britain displayed a weak but significant trend of IBD. This pattern was not found in a subset of sites with non-native origins and their inclusion in the analysis using all sites obscured the IBD signal of the native range (Figure 4.5). A study on populations of beech in France with relatively recent colonisation histories displayed stronger IBD compared to southern refugial populations in France (de Lafontaine et al. 2013). As beech only arrived in Britain around 3000 BP (Birks 1989), IBD in the native range is likely driven by relatively recent colonisation dynamics. In contrast, widespread translocations are likely to have prevented the development of IBD between non-native populations due to the anthropic movement of plant material throughout the country. Similar effects on IBD were found in the winter annual, *Arabidopsis thaliana*, which displayed weaker IBD in the introduced range in Europe compared to populations within its native range in Asia (Beck et al. 2008). Human-mediated dispersal was suggested as a potential cause of reduced IBD as it promoted long distance dispersal in a species with fairly restricted natural dispersal. Contrary to beech, the spread of *A. thaliana* was not driven by economic interests and human-mediated dispersal was unintentional (Beck et al. 2008), suggesting that a significant amount of dispersal within its introduced range was through subsequent natural dispersal, therefore enabling a weak signal of IBD to develop. Whereas beech is of high economic value, and was extensively translocated throughout Britain by humans (Dierschke 1985).

Studies that aim to determine the 'native status' of a species in a particular region have hypothesised that introduced populations are genetically depauperate as they originate from a limited amount of source propagules (Fuentes-Utrilla et al. (Stone and Sunnucks 1993,

Fuentes-Utrilla et al. 2014). We found a significant decrease in gene diversity (nH_S) in non-native sites, suggesting a reduction in genetic diversity due to founder effects. However, no significant difference was found for rarefied allelic richness between sites of different origins. Allelic richness is expected to be more sensitive to reductions in effective population sizes, as rare alleles, which do not contribute considerably to gene diversity, are more likely to be lost first (Nei et al. 1975). Low levels of allelic richness throughout Britain (see section 4.5.1) suggest a significant proportion was lost during post-glacial colonisation, probably due to a founder effect. The lack of a pattern in allelic richness between native and non-native sites may be due to a lack of sensitivity of the analysis arising from the comparison of two already limited gene pools. In agreement with theoretical predictions, gene diversity throughout Britain displayed similarly high levels to that found on the continent (Jump and Peñuelas 2006, Buiteveld et al. 2007, Oddou-Muratorio et al. 2009, Sjölund and Jump 2015).

There are incongruences in some sites between levels of gene diversity and allelic richness. Outliers such as the planted site, DUN, which has one of the highest levels of allelic richness (nA_R = 7.16) but a below average gene diversity (H_S = 0.684) might have contributed to increasing overall allelic richness in the non-native range (Table 4.2). Non-native sites with high genetic diversity can arise from the planting of seeds from differentiated stocks or specific management methods such as the equalisation of parental contributions to seed typically employed in seed orchards (Lefèvre 2004). Site BEE is a particularly interesting outlier, displaying low nuclear genetic diversity and high differentiation. The minority haplotype (B) reaches 35% which is the highest proportion for any minority haplotype. The high ratio of minority haplotypes may indicate that this stand originated from stock taken from a limited number of individuals. The collection of several seeds from an individual for planting would result in all seeds displaying the same maternal haplotype, which could skew the proportion of haplotypes within a site. Although native sites displayed the highest average

private allelic richness (nA_P), this difference was not statistically significant.

4.5.4 A note on assigning a native status to beech stands in Great Britain

The dynamic nature of species ranges makes the justification for assigning a putative native range to beech within Britain seem overly simplistic and ecologically flawed (Brown 1997, Warren 2007). As the species naturally regenerates throughout Great Britain, it is likely that without human intervention, beech would have eventually spread throughout the country. Northern beech stands in Britain have also been found to develop typical plant communities that are valued for conservation in the south-eastern beech stands (Wesche et al. 2006).

Although this study uses the terms native and non-native to assign sites for analytical purposes and clarity, it is inadvisable to take it as precedent. When considering the individual assignment of sites as native or non-native, it should be noted that even though the sites allocated as native display differences as a group and display the genetic signal of natural expansion, it does not imply the precise assignment of sites as native since the inclusion of a few wrongly assigned sites might not be enough to diminish the genetic signal of the natural range. Therefore, our results should be interpreted as evidence for the persistence of signals of the natural colonisation of beech in Britain despite wide-scale translocations. Overall, the current species range has experienced high gene flow between sites, leading to the creation of a regional trend in Britain with significant gene flow between native and non-native sites.

In the light of climate change, and species range shifts, growing conditions for beech in Britain are expected to decline at southerly latitudes, and improve in northerly regions (Broadmeadow et al. 2005, Kramer et al. 2010). Future research on phenotypic variation unique to the native range may identify potential local provenances. However, the high level of gene flow throughout the country suggests that any locally adapted genes from the native

range are likely to spread naturally into northerly stands over time.

4.6 Conclusion

With knowledge of palynology and history, we were able to identify the signature of post-glacial migration of the species, contributing information on contemporary beech forests in Britain that builds upon current palynological and historical evidence. Although cryptic genetic signals of population expansion remain in the native range of beech, we caution against using this as a means to classify stand origins, as gene flow between neighbouring regions essentially blur the borders of the native range. There is evidence of gene flow between native and non-native regions, in particular non-native sites in the south-west peninsula, which may be leading to the 'naturalisation' of non-native populations. The natural range of beech presents a source of genetic diversity and potential locally adapted genotypes. It is paramount that climate-induced range shifts are considered in management plans, as many northerly non-native populations may be more productive than those in the native range in the future. Overall, our research shows that although patterns of past colonisation dynamics persist in the native range, high gene flow between native and non-native populations are blurring the boundaries between them, agreeing with *New atlas of the British & Irish flora* (Preston et al. 2002) classification of beech as native throughout Britain.

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4.9 Supplementary material

S4.1 Key references used for stand origins of native sites

Code	Isochrone range	Historic range	Other sources
BED	1000	In	Defined as native (Rackham 1980).
BLE	3000	In	Defined as native woodland [1,2].
BUC	2000	In	Part of Cotswold, defined as wildwood remnant (NATURAL ENGLAND 2012).
BUR	3000	In	Unknown origins (pers. comm. H. Read), but exists at core of putative native range.
CWe	1000	In	Defined as native [3].
CWw	0	In	Unknown origins (pers. comm. D. Anning) but exists on boundary of putative native range, near to native site CWw.
DEN	2000	In	Defined as native (Rackham 1980).
FEL	1000	In	Defined as native (Rackham 1980). Designated SSSI described as having potential native origins [4].
FRI	2000	In	Unknown origins, exists within putative native range, several ancient beech trees present (pers. obs.).
GRE	2000	In	Part of Chilterns, defined as native [5].
LAD	1000	In	Ancient woodland (Peterken and Jones 1987, 1989, Peterken and Mountford 1996).
LUL	3000	In	Part of medieval deer park [6](KENT COUNTY COUNCIL 2010).
MON	3000	In	Designated SSSI described as defined as remnant native [7].
SAV	2000	In	Relicts of ancient pasture woodland with some planting of beech during 18th, 19th, and 20th centuries [8]. Attempted to sample veteran trees, likely to have originated from the ancient wood pasture, therefore considered to harbour potential native beech.
SEC	1000	Out	Part of Wyre Forest, defined as native woodland remnant [9].
WEA	2000	In	Designated SSSI, defined as natural chalk beech woodland [10].
WYC	2000	In	Part of royal hunting forest, evidence of presence up to Roman times [11].

Key references for palynological, historical, and anecdotal evidence used to assign *a priori* stand histories to sites are given. These include the site's position on Birks' (1989) Holocene isochrone map (*Isochrone range*), with positions given in years BP. The approximate position based on Rackham's (1980) range (*Historical range*) is indicated. Sites are identified as *in* or *out* of an approximate boundary surrounding areas with potential surviving native populations, including outlier populations, and where beech was recorded but was presumed extinct during the middle-ages. Other forms of evidence are summarised (*Other sources*) with appropriate references listed in S4.3.

S4.2 Key references used for stand origins of non-native sites

Code	Isochrone range	Historic range	Other sources
APP	0	Out	Planted beech woodlands [12].
BAR	0	Out	Extensive landscaping and planting of ornamental gardens during 1700 [13], sampled beech grew amongst gardens [pers. obs.].
BEE	0	Out	Beech defined as non-native in management plan (WOODLAND TRUST 2010a).
BRI	1000	Out	Unknown origins (pers. comm. M. Jones), exists on border of putative native range, but near planted site HEM.
CAR	0	Out	Unknown origins (WOODLAND TRUST 2008) but occurs far north of putative native range.
CLE	0	Out	Unknown origins, exists far north of putative native range.
CRA	0	Out	Planted mid 1800s (pers. comm. M. Carter).
DEV	0	Out	Beech described as non-native on-site signage (pers. obs) and website [14]. Spatial organisation of sampled beech in rows suggests it was planted (pers. obs).
DRU	0	Out	Spatial organisation suggests it was planted (pers. obs), exists far north of putative native range.
DUN	0	Out	Woods created in the 1800s [15].
ECC	1000	Out	Beech planted in mid 1800s (Jones and Jones 2008, SHEFFIELD CITY COUNCIL 2012).
GEL	0	Out	Designated SSSI report states beech was planted [16].
GOL	0	Out	Spatial organisation, avenue formation, suggests it was planted (pers. obs).
HEM	1000	Out	Planted beech (pers. comm. M. Jones). The sampled beech trees near the river Dart were reported as planted for ornamental purposes in 1800s [17].
KIN	0	Out	History of planting as arboretum [18].
MAB	0	Out	Planted, beech described as non-native (Norman 2009).
PLO	0	Out	Beech planted in mid 1800s (WOODLAND TRUST 2010b).
STR	0	Out	Beech originated from planted and natural regeneration [19].
TAL	0	Out	Unknown origins, structure of forest suggests landscaped and planted (pers. obs.).
TAN	1000	Out	Planted 1944 (pers. comm. D. Farmery).
TON	0	Out	Occurs far north of putative native range.
TWO	2000	In	Structure strongly indicative of plantation (pers. obs.).
WAL	0	Out	Woods planted during mid 1700s [20].
WYT	2000	In	Beech planted in the 1800s (Butt et al. 2009).
YEL	0	Out	Woods planted in 1800s [21].

Key references for palynological, historical, and anecdotal evidence used to assign *a priori* stand histories to sites are given. These include the site's position on Birks' (1989) Holocene isochrone map (*Isochrone range*), with positions given in years BP. The approximate position based on Rackham's (1980) range (*Historical range*) is indicated. Sites are identified as *in* or *out* of an approximate boundary surrounding areas with potential surviving native populations, including outlier populations, and where beech was recorded but was presumed extinct during the middle-ages. Other forms of evidence are summarised (*Other sources*) with appropriate references listed in S4.3.

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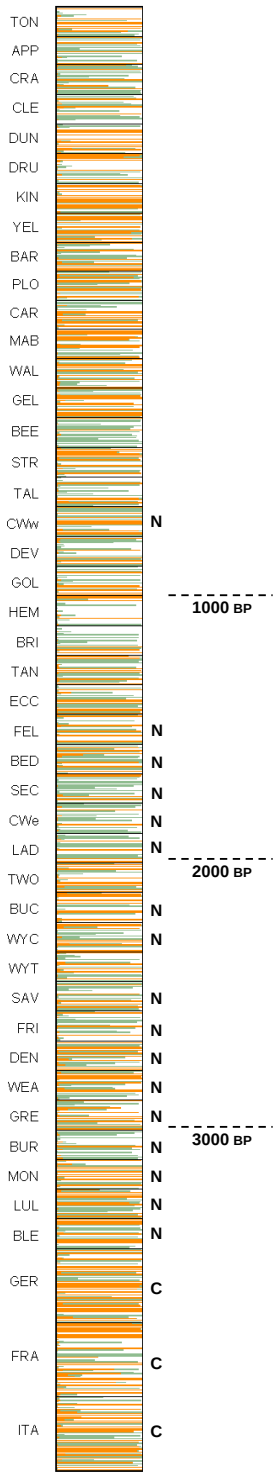
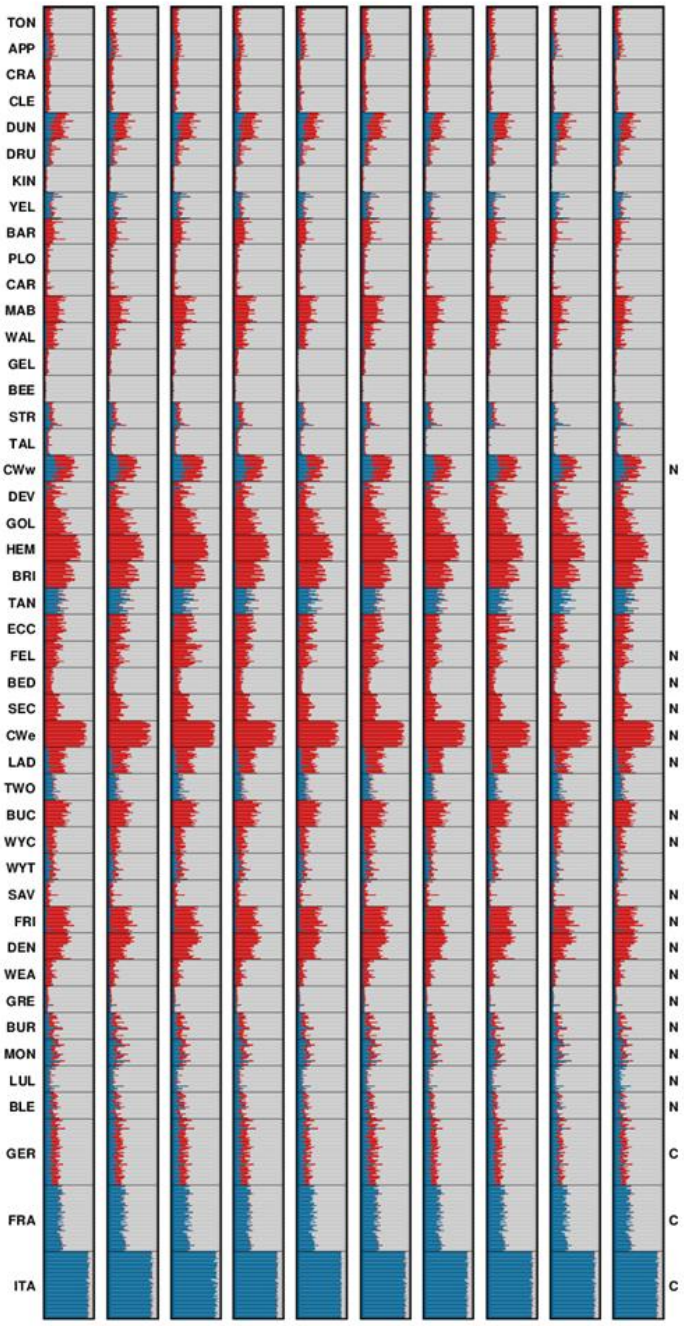
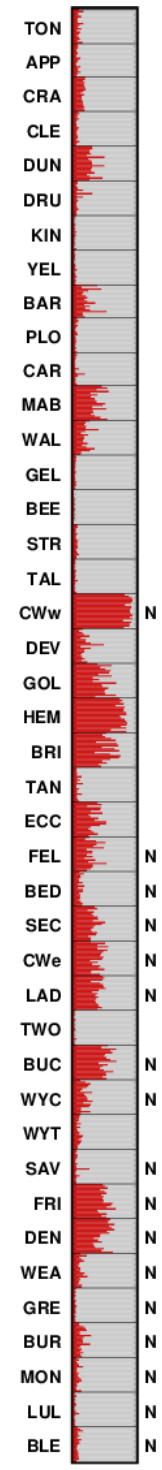


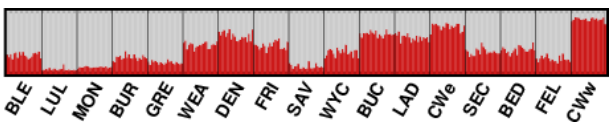
S4.4 DAPC output for *K* = 3. The DAPC output of membership probabilities of each individual for each cluster, shown in orange, green, and white, which is based on the retained discriminant functions. Although this plot is not the equivalent of the STRUCTURE ancestry coefficients, it can be interpreted as the proximity of individuals to each cluster. Site order and labelling corresponds to that in the STRUCTURE Q-matrix in Figure 4.4.



S4.5 Individual Q-matrices for 10 conservative runs in STRUCTURE. Three clusters are shown in blue, red, and grey. Site order and labelling corresponds to that in the STRUCTURE Q-matrix in Figure 4.4.



S4.6 Individual Q-matrix for solely British samples using STRUCTURE for *K* = 2. Site order and labelling corresponds to that in the STRUCTURE Q-matrix in Figure 4.4, excluding continental sites.



S4.7 Individual Q-matrix for a subset of samples with potential native origins. Mean Q-matrices are calculated for nine runs, exlcuding one run as it was an outlier for log-likelihood values. Sites are ordered on an approximate geographical gradient by ordering sites following Birks' (1989) isochrones.

Chapter 5

GENE FLOW AT THE LEADING RANGE EDGE - THE LONG-TERM CONSEQUENCES OF ISOLATION IN EUROPEAN BEECH (*FAGUS SYLVATICA* L. KUHN)

Authors: M. Jennifer Sjölund, Jose J. Moreno-Villena, & Alistair S. Jump

5.1 Abstract

Theory predicts a negative impact of isolation on populations due to a reduction in effective population size and gene flow, which exacerbate the effects of genetic drift. Beech in southern Sweden presents a gradient of isolation towards the leading range edge of the species. Using historical sources, we attained area- and distance-based measures of isolation. Long-term isolation generally had a negative impact on genetic diversity. Bayesian cluster analysis revealed that isolation was also acting as a barrier to gene flow in the north-eastern distribution of beech. Results are discussed in light of palynological evidence of post-glacial migration of beech into Sweden.

5.2 Introduction

The isolation of predominantly outcrossing plant species can lead to a reduction in external gene flow and the loss of genetic diversity through inbreeding and genetic drift (Willi and Fischer 2005, Jump and Peñuelas 2006, Pickup and Young 2007). Isolation can be the result of contemporary anthropogenic impacts, such as deforestation, or colonisation dynamics that occur at the range edge of a species distribution (Hamrick 2004, Hampe and Petit 2005). Populations at the leading edge are important for species migration, population growth, and persistence, considering that current range shifts are driven by contemporary climate change (Walther et al. 2002, Parmesan and Yohe 2003).

Peripheral populations frequently display decreasing density as environmental conditions depart from the optimum (Brown 1984) and hence the range edge of a species often presents a matrix of increasingly naturally isolated populations the further they exist from the core species distribution. Fundamental processes shaping the genetic structure of leading edge populations include founder events, long distance dispersal, population growth and migration (Hampe and Petit 2005). Highly fragmented migration fronts consisting of isolated populations are shaped by founder effects, which can produce correlative reductions in allelic number and heterozygosity that can result in lower genetic diversity and genetic bottlenecks (Nei et al. 1975, Young et al. 1996, Comps et al. 2001, Eckert et al. 2008). These processes generally have greater consequences in small, isolated populations, which are vulnerable to the stochastic process of genetic drift (Ellstrand and Elam 1993). The effects of genetic drift increase temporally if migration and hence gene flow is persistently reduced, increasing the risk of inbreeding depression within populations, in addition to creating highly differentiated outlier populations (Ellstrand and Elam 1993, Ouborg et al. 2006, Eckert et al. 2008). A reduction in gene flow and genetic diversity can compromise the adaptability of a species and the resilience of populations to environmental change (Jump and Peñuelas 2005, Willi and Fischer 2005). Outcrossing trees may be disproportionately sensitive to a reduction in pollen-mediated gene flow owing to their often high levels of heterozygosity that may mask deleterious recessive alleles, which if expressed can lead to a reduction in fitness (Bacles and Jump 2011). In contrast, a reduction in gene flow may be beneficial to marginal populations and promote the expansion of range edges, as it can prevent a drop in the adaptive potential of outlier populations due to swamping gene flow, defined as pollen or seed dispersal from maladapted individuals from the core range (Kirkpatrick and Barton 1997, Bridle and Vines 2007, Savolainen et al. 2007).

Although isolation caused by the sequential colonisation of a habitat, and isolation caused by fragmentation, have certain fundamental processes which differ, such as the presence of colonisation dynamics in the former and lack of in the latter; natural isolation gradients found at the leading range edge of tree species can shed light on the potential long-term effects of contemporary anthropogenic impacts, such as deforestation. An estimated loss of 13 million hectares of forests per year occurred in the past decade (FRA 2010) leading to chronic population fragmentation and hence a reduction in their genetic connectivity. It can take several generations for the impacts of disturbance to manifest in the genetic structure, essentially delaying the effects of fragmentation (Mona et al. 2014). Organisms with long generation times, such as trees, may experience an effective lag to recent fragmentation events (Aguilar et al. 2008, Bacles and Jump 2011), which is reflected in the inconsistent empirical evidence existing for the effects of fragmentation on tree populations, coined the *paradox of forest fragmentation genetics* (Kramer et al. 2008).

Tree species may have the potential to buffer the effects of genetic drift in fragmented populations through high gene flow rates via seed and pollen dispersal (Hamrick 2004, Sork and Smouse 2006). However, chronic fragmentation and persistent isolation can lead to reduction in gene flow, even in wind pollinated species. Studies that have found significant effects of isolation and small population size on the genetic variability of wind pollinated tree species include Jump and Peñuelas (2006), Leonardi et al.(2012) (*Fagus sylvatica),* Provan et al. (2008) (*Juniperus communis*), Aizawa et al. (2009) (*Picea jezoensis*), Liepelt et al.(2009) (*Abies alba*), Hensen et al. (2012) (*Polylepis incana*), with effects on pollen-mediated gene flow found in Wang et al. (2010) (*Pinus tabulaeformis*), Vranckx et al. (2014) (*Quercus robur*). In contrast, various studies have found no effect of isolation, including Schuster and Mitton (2000) (*Pinus flexilis),* (Muir et al. 2004) (*Quercus petraea*), Bacles et al. (2005), Bacles et al. (2006) (*Fraxinus excelsior*), Buschbom et al. (2011) (*Q. robur*), Ortego et al. (2014) (*Quercus*

ilex).

We used a gradient of increasingly isolated forest patches found at the northern range edge of the European beech, *Fagus sylvatica*, in Sweden to determine the impact of relatively long-term isolation on genetic variation. As the isolation gradient coincides with the post-glacial migration front in beech, we measured historic gene flow and contemporary pollen-mediated dispersal rates to gain insight into the contributions of past colonisation dynamics and current isolation on the genetic structure of beech in Sweden. Beech is a predominantly outcrossing (Merzeau et al. 1994), wind-pollinated tree species, with seeds that are dispersed by gravity and animals (Wagner et al. 2010, Packham et al. 2012). Generation times range from 150 to 300 years, with flowering beginning at 40 to 60 years of age. Its 14 million ha range in Europe is predominantly climatically limited (Huntley et al. 1989), with the establishment of populations in Sweden further limited by anthropogenic impacts, such as the clearance of deciduous forests preferentially colonised by beech (Bradshaw and Lindbladh 2005) and intensive land use (Björkman 1996). The present-day distribution suggests a discontinuous migration front with outlying populations which promote colonisation (Björkman 1999). The Swedish distribution of beech during 1927-30 was extensively mapped by Lindquist (1931) using aerial reconnaissance techniques, charting stands of pure/beech-dominated forests, mixed stands with beech, and solitary trees. The map provided a comprehensive resource to derive both area- and distance-based indices of isolation, which have been shown to differ in their effectiveness as a measure of connectivity (Moilanen and Nieminen 2002).

5.3 Materials and methods

5.3.1 Sample collection, site selection and study sites

Sites were selected based on their historic level of isolation, which was determined using a Lindquist's (1931) map (Figure 5.1). A total of 14 sites were sampled (Table 5.1) towards the

northern edge of the native range of beech in southern Sweden (Figure 5.2). Within each site, a plot of 50 adults was established, sampling all adult trees within the plot. Samples were collected from a cohort of 50 seedlings that germinated during the year of sampling (2012), towards the centre of the plot of adults to improve the probability of capturing the mother tree for parentage analysis. As beech has no persistent seed bank (Packham et al. 2012), seedlings were the result of pollen-mediated gene flow during the previous year (2011), also a mast year.

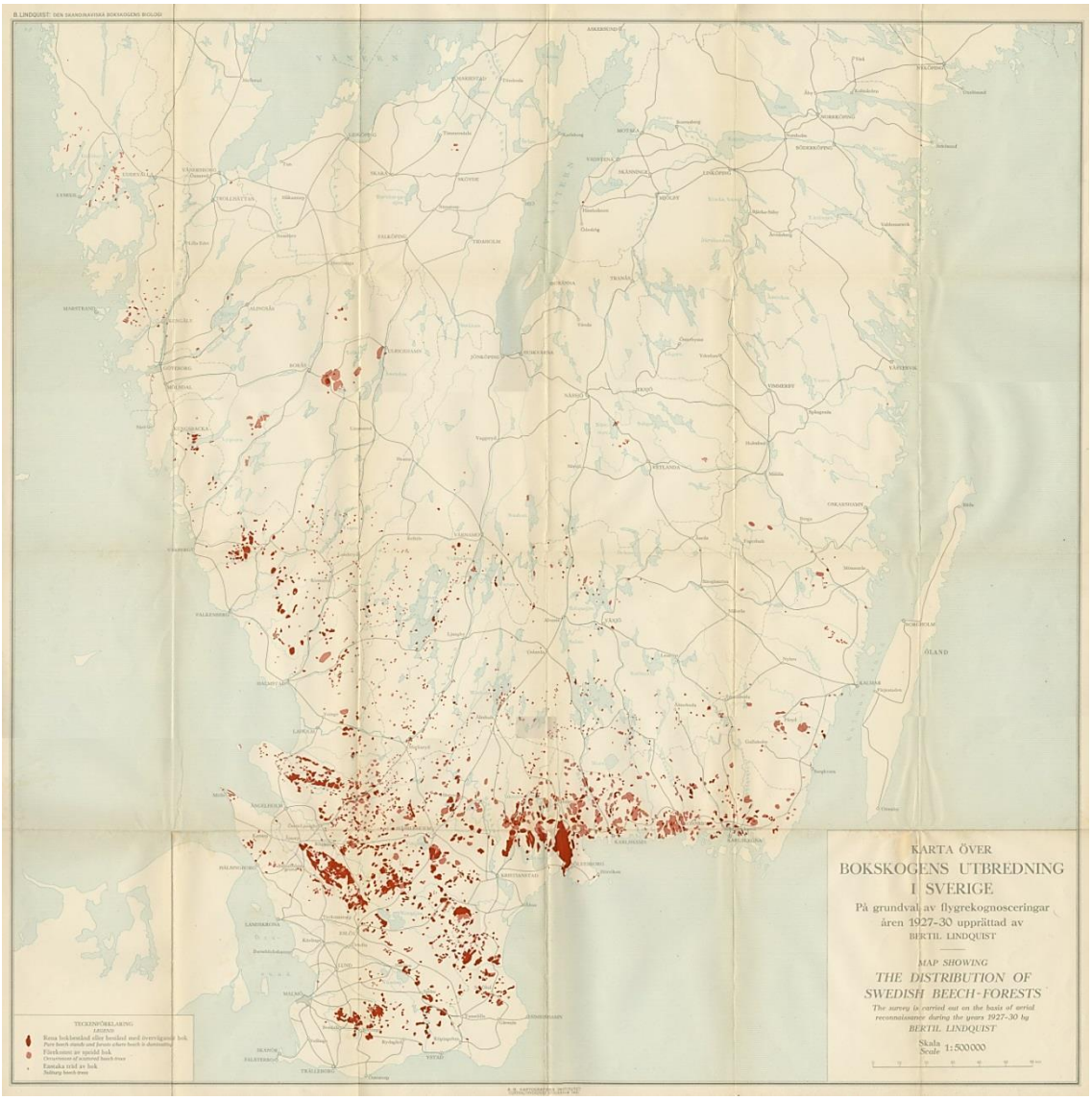


Fig. 5.1 Distribution of beech in Sweden taken from Lindquist (1931). Beech forest in Sweden mapped between 1927 to 1930 using aerial reconnaissance techniques. Dark red areas represent pure beech stands, or forests where beech was dominant, and hashed areas indicate the occurrence of scattered beech trees. Red open circles represent single beech trees.

Table 5.1 Details of study sites and isolation indices

				Area (ha)				Distance (km)	
Site name	**Code**	**Lat. Long.**	**Elev. (m)**	**5km**	**10km**	**15km**	**Site boundary**	**CB**	**BB**
Söderåsen	SOD	N56.0264 E13.2235	321	3649.95	5650.5	8152.86	6181.65	3.758	0.067
Ryssberget	RYS	N56.0674 E14.5903	73	2861.01	3810.39	5593.13	8396.37	4.451	0.338
Häckeberga Sjön	HAC	N55.5733 E13.4131	16	2342.50	2858.21	2628.66	1651.73	1.657	0.083
Osbecks Bokskoggar	OSB	N56.4119 E12.9794	47	906.21	2952.2	2859.55	319.90	1.747	0.065
Tromtö	TRO	N56.1684 E15.4698	31	575.27	1656.88	3423.32	190.37	1.180	0.053
Biskorpstorp	BIS	N56.8023 E12.8940	151	727.13	1069.22	2792.19	182.91	2.285	1.561
Flahult	FLA	N56.9738 E13.8226	109	244.19	306.35	918.71	55.92	1.401	1.070
Gullmarsberg	GUL	N58.3745 E11.6514	149	432.07	674.18	360.48	96.71	0.654	0.217
Stoms Ås	STO	N57.5509 E12.5560	195	511.56	744.54	0.01	213.50	3.076	1.873
Måråsa	MAR	N57.0382 E13.2366	156	239.51	223.73	540.27	65.25	1.186	0.827
Hornsö Ekopark	HOR	N57.0338 E16.1402	148	623.69	84.54	8.44	361.28	1.465	0.377
Garpäror	GAR	N58.4955 E13.8352	103	119.92	65.86	0.00	117.60	1.596	1.139
Mattarp	MAT	N57.4927 E14.6146	96	75.38	0.00	3.98	60.31	4.864	4.290
Omberg Ekopark	OMB	N58.2976 E14.6473	169	32.94	0.00	0.00	32.94	50.804	50.429

The first three letters of the site names were abbreviated to give a site code (*Code*). Geographic coordinates (*Lat. Long.*) are presented in decimal degrees with elevation (*Elev.*) in metres. The sites are ordered in terms of the sum area of beech within all the buffer zones. The number of genotyped samples is given for each adult cohort (*Adult (N)*) and seedling cohort (*Seedling (N)*). Area-based measurements of beech in 5km, 10km, 15km exclusive buffer zones, and site boundary are grouped under *Area (ha)*, with the centre to boundary (*CB*) and boundary to boundary (*BB*) distance-based measures grouped under *Distance (km)*.

5.3.2 Measuring the multiple dimensions of isolation

Lindquist's (1931) map was geo-referenced using ArcMap 10 (Esri software) allowing us to measure the approximate area of beech present during the fertilisation of the sampled adult trees. To attain different measures of isolation, circular buffer zones with a radius of 5km, 10km, and 15km were created around the group of samples at each site. Within each buffer zone, polygons were created for all beech stands on Lindquist's (1931) map (see supplementary material S5.1 for examples). Mapped single beech trees were counted and given an arbitrary value of 78.54m^2, an estimate of the circular crown area of a mature beech tree with a radius of 5m, which was added to the area of polygons, giving a total area of beech forest within each buffer zone. Area measurements in each of the exclusive buffer zones (i.e. centre to 5km, 5km to 10km, and 10km to 15km) were used to account for the additive effect of including each buffer zone.

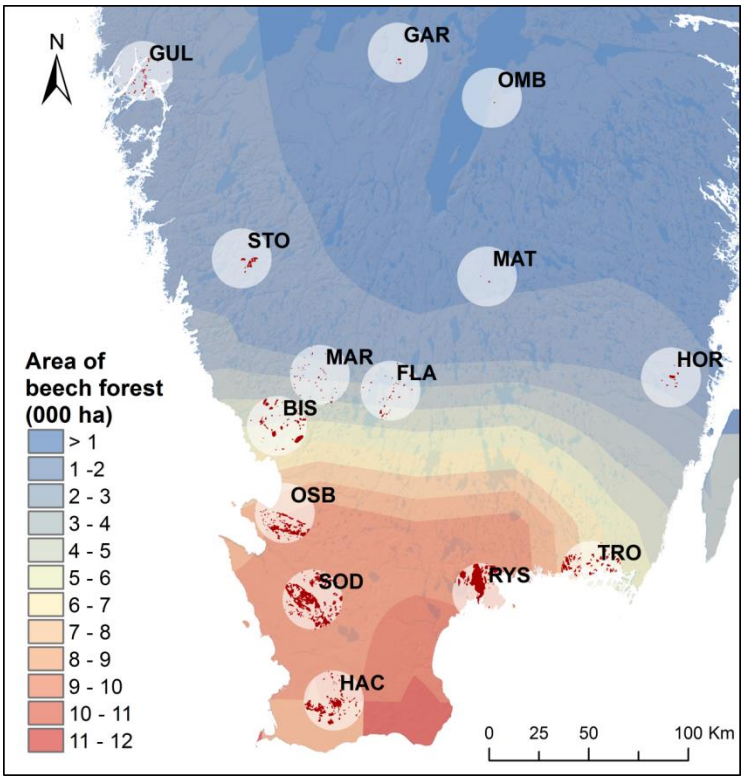


Fig. 5.2 Study sites on an interpolated map of the area of beech in 15km buffer zones. Labelled sampling sites are surrounded by 15km *inclusive* buffer boundaries, shown as light circles, with geo-referenced beech forest from Lindquist's (1931) map in dark red. The map is overlaid on a basemap of water bodies that indicate the positioning of the two largest lakes, Vänern (west) and Vättern (east), near sites GUL, GAR, and OMB.

The area of beech within the site boundary was also measured, as it is sometimes used as a proxy of population size (e.g. Jump and Peñuelas (2006), Wang et al. (2010), and Hensen et al.

(2012)), although in practice determining a boundary for a forest can be difficult and dependant on tree density at the forest edge. Distance measures, commonly used to establish isolation levels (e.g. Jump and Peñuelas (2006) and Leonardi et al. (2012)), provide a comparison with area-based measurements. Measurements of distance included the shortest distance from the centre of the sampled forest to the neighbouring forest boundary (abbreviated CB), and the shortest distance from the boundary of the sampled forest to the neighbouring forest boundary (abbreviated BB). We used two measurements, as their explanatory capacity was dependant on the heterogeneity in the distribution and structure of the surrounding forest patches. Both methods work best when working with small, scattered populations. However, larger forest patches with uneven, indistinct boundaries can result in dissimilar measurements for a given site.

5.3.3 DNA isolation and microsatellite analysis

Genomic DNA was extracted from dried leaf and cambium samples (Colpaert et al. 2005) dried in silica gel, using the BIOLINE Isolate Plant Kit and the QIAGEN 96 Dneasy Plant Kit according to manufacturer's instructions. A total of 1376 individuals were genotyped at 12 polymorphic SSRs (fs1-03, fs1-15, fs3-04, fcm5 (Pastorelli et al. 2003), mfc7 (Tanaka et al. 1999), mfs11 (Vornam et al. 2004), sfc0007-2, sfc0018, sfc0036,sfc1143, sfc1061, sfc1063 (Asuka et al. 2004)) in three multiplexes as detailed in Sjölund and Jump (2015). Fragment analysis was performed on an ABI 3730 DNA Analyzer (Applied Biosystems) with scoring on GeneMarker 2.4.0 (SoftGenetics).

Possible null alleles were identified in Micro-checker (Van Oosterhout et al. 2004) as loci showing significant deviations from Hardy-Weinberg equilibrium with excess homozygosity occurring in all allele size classes. 11 out of 14 sites had a significant proportion of null alleles in fcm5. Analyses presented exclude fcm5 and use a total of 11 loci. Two further loci, sfc0018

and fs3-04, were removed from parentage analysis, as CERVUS 3.0.6 (Kalinowski et al. 2007) detected possible null alleles close to 10% and the accuracy of parentage assignment is particularly sensitive to null alleles. Gametic disequilibrium between loci pairs were tested using FSTAT 2.9.3.2 (Goudet 1995), which identifies significant associations by the random association of genotypes at pairs of loci 1,100 times, using a 5% nominal level after Bonferonni correction. The multilocus average error rates were 0.5% for the 11 loci used in population genetic structure analyses, and 0.6% for the 9 loci used in parentage analyses. The error rate per locus was calculated as the number of erroneously assigned loci over 80 repeated samples.

5.3.4 Measuring and visualising genetic diversity and pollen dispersal

We used inverse distance weight methods available on the spatial analyst interpolation tool on ARCGIS (ESRI) to map multilocus estimates of genetic diversity for the adults and seedlings using 11 loci, and the percentage of external pollen-mediated gene flow attained from parentage analysis using nine loci.

Rarefied allelic richness (A_R) and rarefied private allelic richness (A_P) were obtained using ADZE 1.0 (Szpiech et al. 2008). Private alleles were defined as those unique to a single site within either the adult or seedling cohort. Estimates of gene diversity corrected for samples size (H_S) and the inbreeding coefficient (F_{IS}) were obtained using SPAGEDI 1.4b (Hardy and Vekemans 2002). The difference between adult and seedling cohorts for each diversity estimator was tested using the non-parametric Mann-Whitney U test.

Parentage analysis was performed within each site to quantify the proportion of seedlings arising due to pollen-mediated gene flow from adult trees existing outside the sampled plot. Maximum-likelihood based methods were used to identify potential parents using CERVUS (Kalinowski et al. 2007). A combined exclusion probability of >99.99% for parent pairs was

obtained for nine loci.

Three relative measures of external pollen-mediated gene flow were determined using a variation of the methods employed by Buschbom et al. (2011). Within each site, seedlings were primarily assigned a maternal tree at 90% confidence intervals (CI) using the LOD score, obtained from the natural log of the overall likelihood ratio. A maternal tree was assigned to a seedling if it had the highest LOD score at 90% CI. Seedlings with no reconstructed parents at 90% CI were excluded from further analysis. Out of the seedlings which were assigned a maternal tree, a random subset of 15 seedlings (i.e. the lowest number of maternal trees assigned for any given site) was used for parentage analysis to standardise the sample size per site and eliminate potential bias. For the purpose of maternal tree assignment, seedlings were assumed to be primarily dispersed by gravity (Wagner et al. 2010), which is likely as the seedlings originated from a mast year, known to satiate primary predators, including animals involved in seed dispersal.

Seedlings originating from external pollen dispersal were determined using three measures derived from parent pair analysis, paternity analysis, and simple counts of foreign alleles. For parent pair analysis, seedlings were considered a result of external pollen dispersal if analysis of parent pairs with unknown sexes yielded no local parent pair for the seedling at 95% CI. For paternity analysis, external pollen dispersal was considered when a local pollen donor could not be assigned at a 95% CI using known mothers. For the counts of foreign alleles, seedlings that had one or more foreign alleles were considered to be the result of external pollen dispersal. Critical LOD scores and Delta (the difference in LOD scores between the most likely candidate parent and the second most likely candidate parent) for parent pairs (unknown sexes) and paternity (known mothers) analyses were obtained through simulating 100,000 offspring using the following simulation settings: all adult trees were included as candidate

parents and represented 100% of potential parents, the proportion of loci typed varied from 0.998 to 1, the proportion of loci mistyped was set to 0.01. The number of foreign alleles was calculated for each seedling per site, and was defined as an allele present in the seedling cohort but not present in the adult cohort. The three measures are not considered absolute, but were instead relative estimates of parentage that were analysed separately to find congruent trends when tested against isolation levels.

5.3.5 Modelling the effects of isolation on genetic diversity and pollen-mediated gene flow

We performed a partial least squares regression (PLSR) using the R package PLSDEPOT 0.1.17 to compare the effects of isolation on each of the genetic diversity estimators and the measures of external pollen-mediated gene flow. PLSR is relatively robust to small sample sizes compared to multiple regression and has the added benefit of allowing easy visualisation of data consisting of correlated predictor variables (Carrascal et al. 2009). PLSR deals with the lack of independence among predictor variables by grouping them into one or more orthogonal, linear gradients of covariation whilst maximising the explained variance in the response variable (Palomino and Carrascal 2007).

We tested three variables describing genetic diversity in adults and seedlings; allelic richness (A_R); private allelic richness (A_P); and gene diversity (H_S). We did not perform tests on F_{IS} as patterns are confounded by the significance of the F_{IS} values. For parentage analysis, we tested the three continuous measures of external gene flow, the number of seedlings with; no local parent pairs at 95% CI; no local fathers 95% CI; and foreign alleles (*FA*). The predictor variables used were the six measures of isolation derived from Lindquist's (1931) map which included the four area-based (5km buffer, 10km buffer, 15km buffer, site boundary) and two distance-based (CB, BB) measurements. Latitude and longitude were included as predictors to account for geographical variation. Predictor variables were log(x+1) transformed.

Components which displayed significant correlations with the response variables and explained more than 10% of the original variation were tested using a cross-validation procedure splitting the data into 10 segments, using nine segments to predict observations in the remaining test segment. Two response variables, adult allelic richness (A_R) and seedling gene diversity (H_S) did not pass cross-validation. However, as the procedure is compromised by small datasets (N = 14), we still present the results from significant components of adult A_R and seedling H_S and interpret them together with maps of genetic diversity to identify clear trends. In models with two significant components, the secondary components were found to be redundant, revealing similar trends as the main component and are therefore not presented. We present weights of significant predictor variables, which indicate the trend and the importance of the relationship with the component.

To explain the trends between isolation and genetic diversity, we performed preliminary analysis for recent bottlenecks in the adult and seedling cohort using 11 loci. We used the program BOTTLENECK 1.2.02 tested under the Two-phase model (TPM), allowing 95% single-step mutations and 5% multi-step mutations with a variance of 12 among multi-step mutations under 1000 simulation iterations, as recommended for microsatellites by Piry et al. (1999). Recent reduction in population effective sizes display a correlative reduction in allelic richness and heterozygosity (H_e, analogous to Nei's (1987) gene diversity (H_S)), where allelic richness is reduced faster than H_e leading to a larger heterozygosity than expected under mutation-drift equilibrium (H_{eq}). Significance tests for $H_e > H_{eq}$ were performed using the Wilcoxon's test.

5.3.6 Identifying regional population structure at the leading edge

individual-based assignment methods were performed on the adult cohort using GENELAND 4.0.4 (Guillot et al. 2005) - a spatially explicit Bayesian clustering model. Seedlings were

excluded from cluster analysis as significant deviations from Hardy-Weinberg equilibrium were found in six out of the 14 sites, as well as significant isolation-by-distance in seedlings (slope 0.02649, $p < 0.01$; data not shown) deviating from model assumptions for both clustering programs (STRUCTURE methods described below). Although isolation-by-distance was present, it was weaker in adults (slope of 0.0124, $p < 0.05$; data not shown). Site GAR was removed from analysis as it was exclusively assigned to a cluster which displayed significant deviations from Hardy-Weinberg equilibrium and linkage disequilibrium (data not shown). Results presented exclude site GAR from analysis as there was a noticeable improvement in the precision of the number of clusters (*K*) between individual runs after its removal.

Runs were performed in GENELAND for 500,000 Markov Chain Monte-Carlo (MCMC) iterations with a thinning of 500, and a burn-in of 200. To determine the initial number of *K*, the uncorrelated allele frequency model with a spatial prior was used with *K* varying from 1 to 13. Using a spatial prior allows the identification of genetic discontinuities associated with barriers to gene flow and potentially isolation (Francois & Durand 2010). Since primers for *F. sylvatica* are known to have null alleles (Chybicki and Burczyk 2009), the null allele model was implemented as recommended by Guillot et al. (2008). Runs were performed 10 times for each model to compare average posterior probabilities for each value of *K*. To check compliance of inferred clusters with modelling assumptions (Guillot et al. 2009), we performed tests for gametic disequilibrium within the three inferred clusters and genetic differentiation between pairs of clusters in FSTAT 2.9.3.2 (Goudet 1995).

To refine cluster membership we used the correlated allele frequency model with *K* fixed at the value obtained from the uncorrelated allele frequency model. Setting *K* as a variable in the correlated model can lead to its overestimation (Guillot et al. 2014) as larger values are not sufficiently penalised, resulting in the inference of spurious sub-populations, which occurred in

preliminary tests (data not shown). The correlated model is better at detecting low differentiation from recent ecological events, although it is more sensitive to departures from model assumptions (Guillot 2012). Post-processing analysis was performed on the correlated allele model output to assess the level of admixture using 500,000 iterations with a burn-in of 200. Admixture and substructure within subsets of the westerly and easterly clusters were analysed further using the same protocol as above.

To validate our results, we analysed the data using a second Bayesian clustering program, STRUCTURE 2.3.4 (Pritchard et al. 2000), as recommended by Guillot et al. (2009). Repeats of 10 runs were performed for each *K* value, set from 1 to 10, with each run consisting of 500,000 MCMC iterations, with a burn-in period of 100,000, using the correlated allele frequency model (Falush et al. 2003) and the admixture ancestry model. Unlike GENELAND, georeferencing information cannot be implemented as a spatial prior in this program. We examined the mean log-likelihood values for each *K* to identify their convergence and the true number of clusters in the data. The value of *K* was validated using the method of Evanno et al. (2005) on STRUCTURE HARVESTER 0.6.94 (Earl and vonHoldt 2012), which measures Δ*K*, a statistic related to the second-order rate of change in the log probability of the data. Post-processing of Q-matrices was performed in CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007) with graphics created in DISTRUCT 1.1 (Rosenberg 2004).

5.4 Results

5.4.1 Isolation indices

The level of isolation obtained by area-based measurements ranged from 8396.37ha to 32.94ha of surrounding forest. Distance measures of isolation ranged from 0.065km to 50.429km (Table 5.1). Mapping the total area of beech within a 15km *inclusive* buffer zone around each site revealed a north easterly trend of increasing isolation (Figure 5.2), which

Table 5.2 Multilocus estimates of genetic diversity and differentiation

Site	Adults (N)	Seedlings (N)	A_R		A_P		H_S		F_{IS}	
			Adult	Seedling	Adult	Seedling	Adult	Seedling	Adult	Seedling
SOD	49	50	6.38 ± 0.58	6.11 ± 0.48	0.08 ± 0.04	0.20 ± 0.13	0.620	0.611	0.016	-0.054*
RYS	49	50	7.07 ± 0.68	6.16 ± 0.63	0.27 ± 0.14	0.12 ± 0.06	0.678	0.673	0.018	-0.016
HAC	50	49	6.86 ± 0.73	6.11 ± 0.55	0.39 ± 0.22	0.53 ± 0.36	0.681	0.666	-0.029	-0.083***
OSB	50	50	6.30 ± 0.62	5.77 ± 0.57	0.13 ± 0.05	0.14 ± 0.08	0.667	0.659	-0.028	-0.032
TRO	50	47	5.85 ± 0.45	4.98 ± 0.36	0.03 ± 0.02	0.00 ± 0.00	0.679	0.643	0.002	-0.088***
BIS	49	50	6.52 ± 0.61	5.82 ± 0.48	0.05 ± 0.03	0.12 ± 0.06	0.646	0.636	-0.007	-0.003
FLA	50	50	5.89 ± 0.61	5.76 ± 0.43	0.02 ± 0.02	0.19 ± 0.12	0.677	0.683	-0.010	-0.044
GUL	49	48	6.07 ± 0.62	5.34 ± 0.56	0.12 ± 0.06	0.15 ± 0.09	0.667	0.603	0.027	-0.053*
STO	47	47	5.61 ± 0.46	5.16 ± 0.46	0.20 ± 0.10	0.13 ± 0.06	0.601	0.534	-0.011	-0.043
MAR	49	50	6.53 ± 0.69	6.17 ± 0.67	0.21 ± 0.10	0.40 ± 0.12	0.699	0.687	0.000	-0.043
HOR	50	49	5.74 ± 0.57	5.55 ± 0.60	0.19 ± 0.12	0.36 ± 0.20	0.639	0.648	-0.047	-0.006
GAR	47	50	5.06 ± 0.38	4.50 ± 0.34	0.13 ± 0.09	0.18 ± 0.12	0.644	0.627	-0.104***	-0.061*
MAT	49	48	5.27 ± 0.57	4.91 ± 0.57	0.11 ± 0.08	0.08 ± 0.08	0.685	0.645	-0.011	-0.001
OMB	50	50	7.17 ± 0.66	5.76 ± 0.48	0.30 ± 0.14	0.11 ± 0.05	0.703	0.688	0.002	-0.066**
		Mean ± SE	6.17 ± 0.17*	5.58 ± 0.14*	0.16 ± 0.03	0.19 ± 0.04	0.663 ± 0.008	0.648 ± 0.011	-0.013 ± 0.009***	-0.042 ± 0.008***

Terms for genetic diversity estimators are as follows; A_R, allelic richness (Petit et al. 1998); A_P, private allelic richness (Szpiech et al. 2008); H_S, gene diversity corrected for sample size (Nei 1978); F_{IS}, inbreeding coefficient (Weir and Cockerham 1984). The minimum number of gene copies (k) used for rarefication analysis of A_R and A_P is 94 with standard errors of the rarefaction procedure provided. P-values for F_{IS} are obtained after 10,000 permutations of gene copies within adult or seedling individuals of each site. *P*-values for mean genetic diversity estimates indicate significant differences between adults and seedlings. Significant 2-sided *P*-values are indicated as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The sites are ordered in terms of total area of beech within the 15km *inclusive* buffer zone. Multilocus estimates of genetic diversity were attained from 11 loci.

appeared to reflect the distribution in the original map (Figure 5.1). There were significant correlations between variables within area and distance-based groups ($P < 0.001$), although variables from different groups did not display significant correlations with each other (data not shown).

5.4.2 Estimates of genetic diversity and external pollen-mediated gene flow

The 11 loci used in this study were found to be in gametic equilibrium considering a 5% nominal level after Bonferroni correction. The maximum number of alleles for all samples had a multilocus average of 13.36, ranging from 4 to 28 alleles per locus. Rarefied allelic richness (A_R) in adults ranged from of 5.06 to 7.17, and 4.50 to 6.17 in seedlings, with adults having a significantly higher level of allelic richness compared to seedlings (U(12) = 147, Z = 2.25, $P < 0.05$) (Table 5.2). Adult rarefied private allelic richness (A_P) ranged from 0.02$\pm$0.02 to 0.39$\pm$0.22, with seedlings displaying levels ranging from 0.00 to 0.53$\pm$0.36.There were no significant differences between levels of private allelic richness in adults and seedlings (U(12) = 87, Z = -0.51, $P = 0.63$), although the highest levels of A_P were consistently found in the most southerly site (HAC) in both adults and seedlings (Figure 5.3). Gene diversity (H_S) estimates ranged from 0.601 to 0.703 in adults, and in seedlings ranged from 0.534 to 0.688, with no significant differences found between adults and seedlings (U(12) = 126, Z = 1.28, $P = 0.21$). No evidence of homozygote excess was found in either cohort. However, significantly negative inbreeding coefficients (F_{IS}) indicated a heterozygote excess in one site for the adult cohort and in six sites for the seedling cohort (Table 5.2, Figure 5.3), with significantly lower values of F_{IS} in seedlings (U(12) = 154, Z = 2.57, $P < 0.01$).

The variation in the sensitivity of the three methods used to quantify the proportion of seedlings resulting from pollen derived from adults outside the plot was reflected in the range of estimates attained per method (Figure 5.4). The largest estimate was found using parent

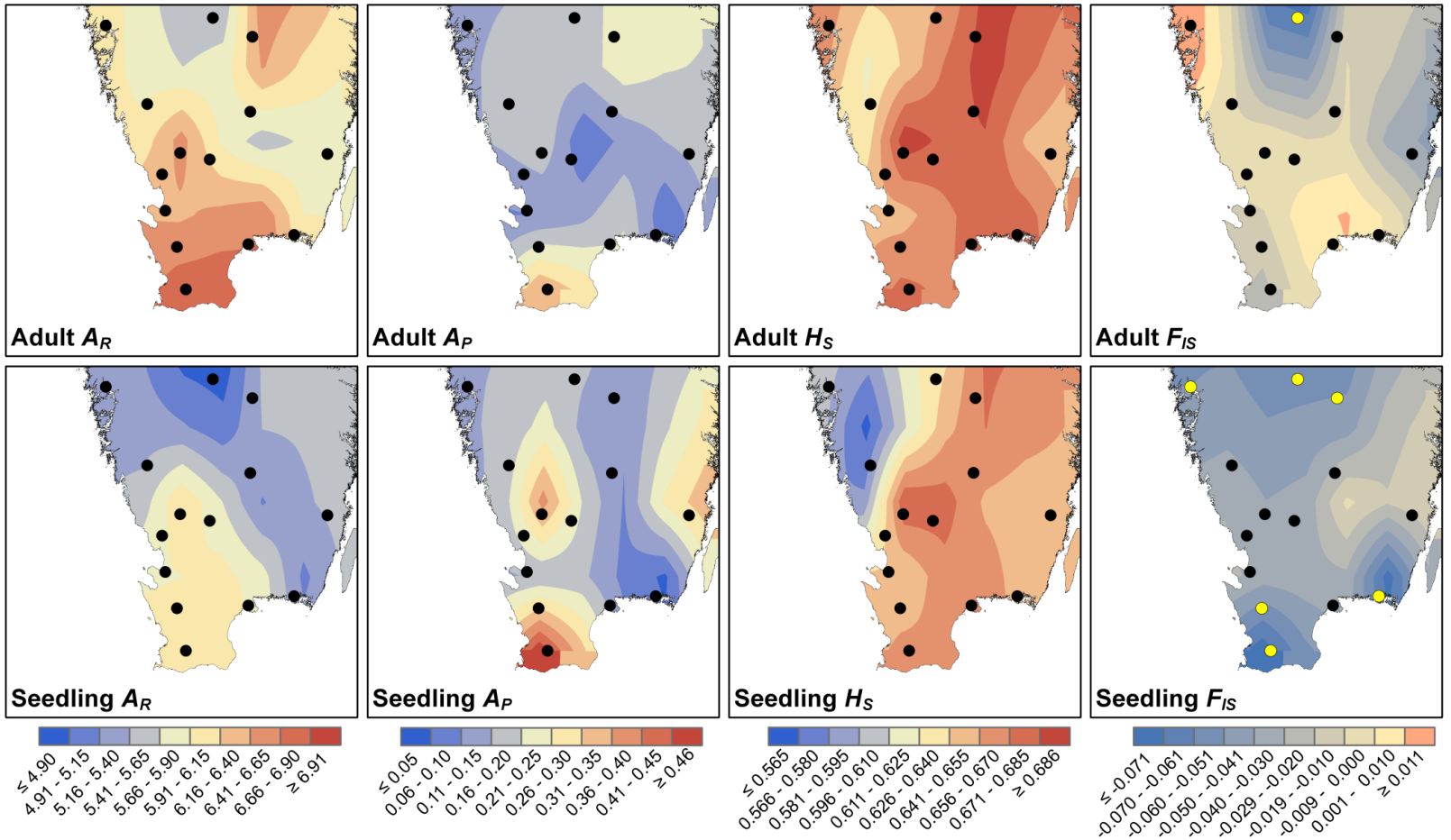


Fig. 5.3 Distribution of genetic diversity in adults and seedlings. Multilocus genetic diversity estimators using 11 loci are abbreviated as follows; rarefied allelic richness (A_R); rarefied private allelic richness (A_P); gene diversity corrected for sample size (H_S); and, the inbreeding coefficient (F_{IS}). *P*-values for F_{IS} are obtained after 10,000 permutations of gene copies within adult or seedling individuals of each site. Significant 2-sided *P*-values less than 0.05 are indicated by yellow circles.

pair analysis, which identified a range of 13.3% to 86.7% of seedlings with no local parent pairs at 95% CI. Paternity analysis identified 0% to 46.7% of seedlings with no local father at 95% CI with the counts of seedlings with foreign alleles identifying from 0% to 26.7% of external dispersal events per site.

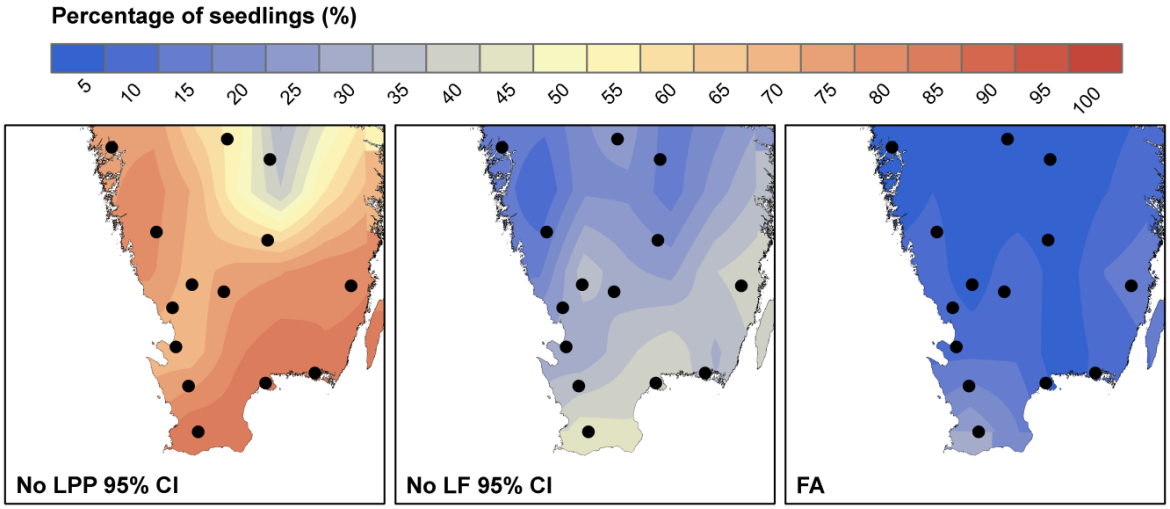


Fig. 5.4 Percentage of seedlings resulting from external pollen dispersal. Interpolated maps of the three methods of parentage analysis using nine loci. Maps depict the percentage of seedlings (%) with no local parent pair at 95% CI (No LPP 95% CI), with no local father at 95% CI (No LF 95% CI) and with foreign alleles (*FA*). Percentages are calculated from a subset of 15 seedlings per site.

5.4.3 PLSR models of genetic diversity and external pollen-mediated gene flow

Significant PLSR components were found for rarefied allelic richness (A_R) and gene diversity (H_S) in adults and seedlings, as well as for all three variables used for parentage analysis (Table 5.3). No significant relationships between components and response variables were found for private allelic richness (A_P) in adults or seedlings (data not shown). It should be noted that significant correlations between buffer-based isolation measures and latitude were found (Pearson's *r* and significances, 5km: $r = -0.713$, $P < 0.01$; 10km: $r = -0.732$, $P < 0.01$; 15km: $r = -0.733$, $P < 0.01$; Site boundary: $r = -0.520$, $P = 0.06$; CB: $r = 0.373$, $P = 0.19$; BB: $r = 0.424$, $P = 0.130$). No correlations were found between isolation indices and longitude (5km: $r = -0.068$, $P = 0.82$; 10km: $r = -0.150$, $P = 0.60$; 15km: $r = -0.032$, $P = 0.91$; Site boundary: $r = 0.071$, $P = 0.81$; CB: $r = 0.219$, $P = 0.45$; BB: $r = 0.209$, $P = 0.47$). There was no evidence for recent genetic

bottlenecks in any of the 14 sites in the adult or seedling cohort (data not shown).

For genetic diversity estimators, the strongest relationship between predictor variables and response was found in seedling A_R (R^2 = 58.3%, $P < 0.05$), which was significantly negatively related to isolation. The remaining genetic diversity response variables in order of the original variance explained were seedling H_S (R^2 = 33.8%, $P < 0.05$), seedling A_R (R^2 = 30.1%, $P < 0.05$), and adult H_S (R^2 = 26.2%, $P < 0.05$).

An increase in A_R in adults was associated with southern sites, with a high area of surrounding beech forest (Table 5.3, Figure 5.3). Latitude explained the largest variation in adult A_R (R^2 = 7.3%, $P < 0.001$), with site boundary explaining the most variation out of the area-based measurements (R^2 = 5.8%, $P < 0.001$). There was an unexpected significant, positive relationship of increasing distance and increasing adult A_R. However, this explained <0.1% of R^2 ($P < 0.05$) and therefore was not considered biologically relevant. Seedling A_R revealed a similar trend to that found in adults, although isolation was the primary driver of variation in the response, instead of latitude. Increased A_R in seedlings was associated with a high area of surrounding forests and low distances between forests in southern latitudes. The area-based measure of beech in the 15km buffer zone was the primary contributor to variation in the response (R^2 = 8.3%, $P < 0.001$) with a comparable amount of variance explained by the distance measure, boundary to boundary (BB) (R^2 = 8.2%, $P < 0.001$). Latitude only explained 0.4% of the total variation in the response ($P < 0.001$).

Adult H_S was primarily related to increased area of surrounding beech, specifically associated with the 15km buffer zone (R^2 = 14.9%, $P < 0.001$). As with adult allelic richness, southern sites also displayed higher levels of H_S. However, in the maps of genetic diversity (Figure 5.3), the trend for adult H_S was not as clear as that displayed by adult A_R. Seedling H_S was the only

response significantly influenced by longitude, with H_S increasing on a west to east gradient, explaining the largest amount of variation in the response (R^2 = 14.7%, $P < 0.001$) (Table 5.3, Figure 5.3). The relationship with isolation was contradictory to that found in the other genetic diversity response variables, as higher levels of seedling H_S were associated with a decrease in surrounding beech area (10km: R^2 = 4.3%, $P < 0.01$) and lower distances between forests (CB: R^2 = 2.7%, $P < 0.05$).

Table 5.3 Significant PLSR models with predictor weights and their contributions to R^2

	Genetic diversity				**External pollen-mediated gene flow**		
	Adult A_R	**Adult H_S**	**Seedling A_R**	**Seedling H_S**	**No LPP 95% CI**	**No LF 95% CI**	***FA***
Cross-validation	NS	SIG	SIG	NS	SIG	SIG	SIG
Response R^2	30.1*	26.2*	58.3*	33.8*	58.2**	41.4*	29.4*
R^2 contributions							
5km	4.5		6.9		10.1	5.8	6.9
10km	0.4		2.3	4.3	8.7	3.7	3.3
15km	4.6	14.9	8.3		7.9	7.0	3.2
Site boundary	5.8		5.7		5.7	5.4	4.4
CB				2.7	10.2		
BB	<0.1		8.2		9.0	4.7	3.2
Latitude	7.3	7.0	0.4		6.5	9.1	7.8
Longitude				14.7			
Predictor weights							
5km	0.386***		0.450***		0.417***	0.375***	0.482***
10km	0.113*		0.262**	-0.358**	0.387***	0.298***	0.336***
15km	0.393**	0.755**	0.495***		0.367***	0.412***	0.329***
Site boundary	0.439***		0.410***		0.313**	0.363***	0.388***
CB				0.282*	-0.419*		
BB	0.004*		-0.162***		-0.394***	-0.337***	-0.331***
Latitude	-0.492***	-0.518**	-0.511***		-0.334***	-0.468***	-0.516***
Longitude				0.660***			

Only response variables which had significant relationships to components were included in the table. All predictor variables include four area-based measures (m^2), the 5km, 10km, and 15km buffer zones, and site boundary; two distance-based measures (m), the centre to boundary (CB), and boundary to boundary (BB); and two geographic measures, latitude and longitude. Predictor weights and their contribution to R^2 are given for those significantly related to the component. Model cross-validation outcomes are indicated as significant (SIG) or non-significant (NS). Significant *P*-values are indicated as, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Again, the varying sensitivities of the methods used to estimate the proportion of seedlings resulting from external pollen dispersal were reflected in the relationship between the main component and the response variables. The strongest relationship between component and response was found for the number of seedlings with no local parent pairs at 95% CI (R^2 = 58.2%; $P < 0.01$), followed by the number of seedlings with no local fathers at 95% CI (R^2 = 41.4%; $P < 0.05$), and the number of seedlings with foreign alleles (*FA*) (R^2 = 29.4%; $P < 0.05$). The number of seedlings with no local parent pairs at 95% CI increased in sites with lower distances between forests, higher areas of surrounding beech, following a north to south gradient. The contribution of significant isolation variables and geographic variables were similar, with distance-based variable, BB, explaining 10.2% ($P < 0.001$) of the variation within the response, the area of beech within the 5km buffer zone explaining 10.1% ($P < 0.001$), and latitude explaining 9.0% ($P < 0.001$). The number of seedlings with no local fathers at 95% CI and the number of seedlings with foreign alleles, revealed a similar pattern to that found using parent pair analysis (No LPP 95% CI), except that latitude was the primary contributor to R^2 in the two former methods (No LF 95% CI: R^2 = 9.1%, $P < 0.001$; *FA*: R^2 = 7.8%, $P < 0.001$), although isolation variables were still important contributors to variation in the response (Table 5.3).

When considering all models for both genetic diversity and external pollen-mediated gene flow, area-based measurements significantly contributed to the explanation of the response for all presented response variables, whereas distance-based measures failed to explain significant variation in the response in one model, adult H_S. Concerning area-based measures, significant contributions were made by the addition of each buffer zone in all response variables except for H_S, which in adults only related to the 15km buffer, and in seedlings, the 10km buffer. The boundary-based distance measure, BB, which was significant in most models, did not significantly explain variation in adult or seedling H_S.

5.4.4 Regional genetic structure at the leading range edge

Three clusters were identified using individual-based assignment methods in 8 out of 10 runs using the uncorrelated model, with consistent results in 9 out of 10 runs with the subsequent correlated model. The spatially explicit models in GENELAND presented the highest average posterior probabilities for the clusters that extended over three regions; 1) the west; 2) the south-east; and, 3) the north-east (Figure 5.5; see supplementary material S5.2 for maps of cluster posterior probabilities), reflecting the north-easterly gradient of increasing isolation (Figure 5.2). Admixture analysis in the spatially explicit program, GENELAND, revealed distinct clusters with low admixture (Figure 5.6). Further substructuring was found in the south-eastern and north-eastern cluster, 2 and 3, when analysed separately, with each of the four populations clustered individually with relatively low admixture levels within each (Figure 5.5 and 5.7; see supplementary material S5.3 for maps of cluster posterior probabilities). No further substructuring was found in the western cluster 1 (data not shown). All inferred clusters using 13 sites were found to be significantly differentiated and in gametic equilibrium considering a 5% nominal level after Bonferroni correction, except for site OMB, which showed significant disequilibrium at one pair of loci.

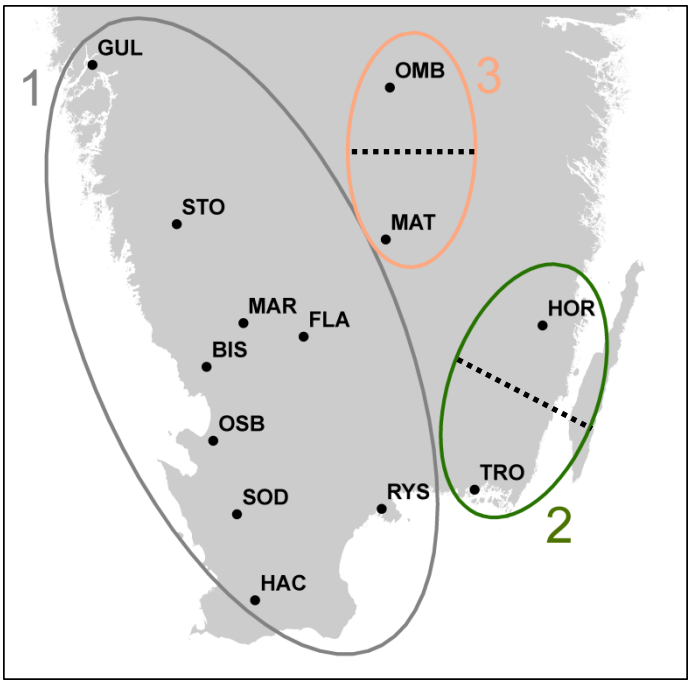


Fig. 5.5 Inference of genetic clusters in the adult cohort over 13 sites. Sites are displayed as small black circles, with large open circles indicating the grouping of the three inferred population clusters; the western cluster 1 (grey); the south eastern cluster 2 (green); and the north eastern cluster 3 (orange). The dotted lines indicate further substructuring found in further analysis of the subset of sites in cluster 2 and 3. 11 loci were used in analysis.

Analysis with STRUCTURE was concurrent with GENELAND and indicated a presence of 3 clusters in the data (see supplementary material S5.4 for the log probability of the data and Δ*K*). The levels of admixture were higher in the non-spatial STRUCTURE model compared to the spatially explicit GENELAND model, with a trend of decreasing admixture as isolation (i.e. area of beech forest in the 15km buffer zone) increased (Figure 5.8).

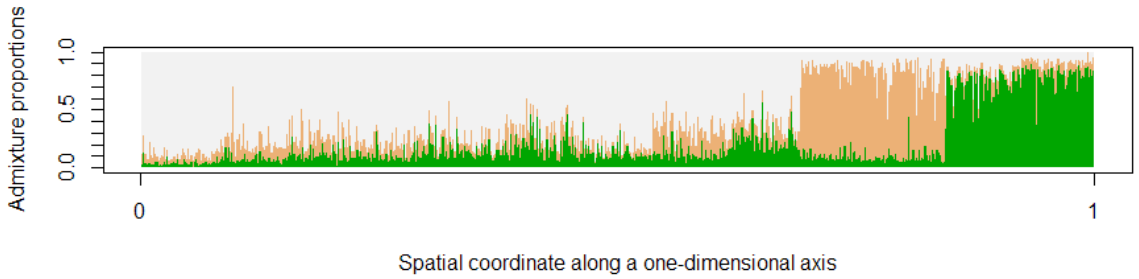


Fig. 5.6 Inference of posterior proportions of admixture from GENELAND. Spatial coordinates represent a west to east gradient. Colours of clusters are equivalent to those presented in Figure 5.5 with the western cluster 1 (grey); the south eastern cluster 2 (green); and the north eastern cluster 3 (orange). Analysis was conducted on the run with the highest posterior probability.

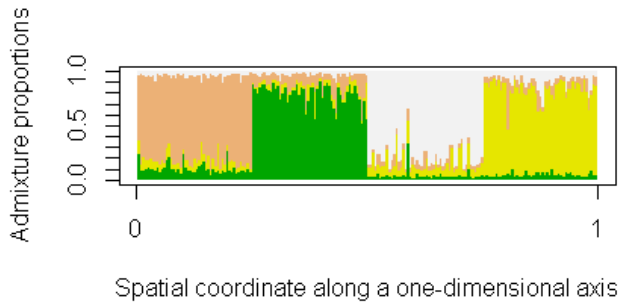


Fig. 5.7 Posterior proportions of admixture in south-eastern (2) and north-eastern (3) clusters from GENELAND. Spatial coordinates represent a west to east gradient. Colours of clusters are TRO (white) and HOR (yellow) which comprise the north eastern cluster (2), and MAT (orange) and OMB (green) comprising the south eastern cluster (3). Analysis was conducted on the run with the highest posterior probability.

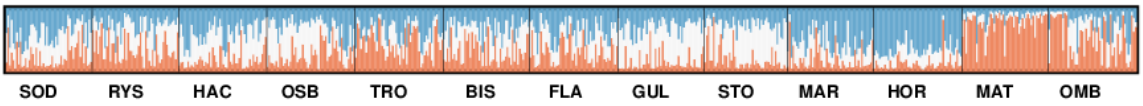


Fig. 5.8 Individual assignment to each cluster using STRUCTURE. The Q-matrix presents the average assignment probabilities over 10 consecutive runs for *K* = 3. Site codes are indicated below, and are ordered by increasing isolation from left to right obtained from estimates for the area of beech (ha) in the 15km *inclusive* buffer zone. A total of 11 loci were used in analysis.

5.5 Discussion

5.5.1 Founder events and isolation shape genetic diversity

We found evidence of reduced rarefied allelic richness (A_R) in both adults and seedlings in isolated sites, with latitude also significantly explaining a large proportion of variation in adults (Table 5.3). Adult gene diversity (H_S) revealed a similar trend to that found in allelic richness. The observed pattern in genetic diversity also resembled the *southern richness and northern purity* paradigm, coined by Hewitt (1999), and was based on the observed reduction in population size through founder events at the leading edge, away from refugia, resulting in a loss of alleles through genetic drift (Nei et al. 1975, Lande 1988, Excoffier et al. 2009). Vucetich and Waite (2003) predicted that this trend was also caused by low migration between isolated populations. Although beech in Sweden is far from its post glacial refugia, southern populations are in proximity to the entry point in Sweden (Bradshaw and Lindbladh 2005), therefore displaying a similar movement away from a source population as post-glacial migration progressed. As rare alleles are at risk of disappearing first, a reduction in population size can affect A_R disproportionally more than H_S (Piry et al. 1999, Comps et al. 2001, Jump and Peñuelas 2006). This is reflected in both adults and seedlings, which display a higher amount of variation explained by predictor variables for A_R compared to H_S. Additionally, the most southerly site, HAC, located near the entry point of beech into Sweden displayed the highest level of rarefied private allelic richness (A_P) in adults and seedlings (Figure 5.3), although PLSR models for A_P were not significant.

The interacting effects of latitude and isolation on A_R in adults suggest that marginal, isolated populations are subject to strong genetic drift, possibly due to the combination of founder events and persistent small population sizes that lead to the loss of alleles over time. The relatively weaker effect of isolation on A_R in adults, compared to latitude, may also be influenced by an outlier, site OMB, which displayed relatively high levels of allelic richness

(Figure 5.3). Although OMB is not the most northerly site, it is the most isolated in terms of surrounding area and the distance to the nearest neighbouring forest (Table 5.1), influencing the trend found with isolation indices. Seedling A_R was strongly affected by isolation, with very little contribution of latitude. This stronger negative impact of isolation on seedlings compared to adults (Table 5.2, Figure 5.3) reflects temporal effects of genetic drift on small populations, which intensify over time (Ellstrand and Elam 1993, Aguilar et al. 2008). This is in agreement with the observed adult to seedling relative reduction of A_R in site OMB, compared to remaining sites (Figure 5.3). As the lifespan of beech is between 150 to 300 years, the oldest populations, having established approximately at 3000 BP (Bradshaw and Lindbladh 2005), have experienced at least 10 to 20 generations (not considering overlapping generations). This is just within the lower bounds of the number of generations determined by simulations, for isolation to affect genetic diversity at the leading range edge, with more than 100 generations being the optimal (Mona et al. 2014), suggesting a relatively strong effect of isolation on genetic diversity in these populations.

The observed reduction in A_R and H_S in the adult cohort with isolation and latitude suggest the existence of founder effects (Nei 1978, Ellstrand and Elam 1993, Young et al. 1996). However, the founder effects were not associated with recent genetic bottlenecks. Aizawa et al. (2009) similarly found no bottlenecks in isolated, range edge populations of *Pinus jezoensis* in Japan, which also displayed the low levels of allelic richness associated with isolation. The authors suggest that it was possible that populations recovered from past bottlenecks, aided by the high mutation rate in microsatellites promptly returning the population to mutation-drift equilibrium (Cornuet and Luikart 1996). Wind pollinated trees, such as beech, typically have delayed reproduction, which can dampen founder effects and may prevent bottlenecks, as several migrants can colonise the site before reproduction begins (Austerlitz et al. 2000, Widmer and Lexer 2001).

The trend of increased H_S in seedlings in easterly and isolated sites might have arisen due to rare gene flow events between genetically divergent populations, as further clusters and hence population substructure was found in easterly sites (Figure 5.5 and 5.7). This is supported by the negative F_{IS} values, which are significantly lower in seedlings compared to adults (Table 5.2) with more sites in seedlings displaying a significant heterozygote excess (Figure 5.3). Genetic bottlenecks have been attributed to similar trends indicating negative correlations between A_R and H_S found at a regional level for beech (Comps et al. 2001). However, we did not find any significant negative correlations between A_R and H_S in seedlings (data not shown). There is a possibility that this trend is driven by complex colonisation dynamics as it reflects the easterly spread of beech after 2000 BP (Bradshaw and Lindbladh 2005). However, it may not be the sole driver, as there were no significant correlations between longitude and the isolation indices (data not shown), suggesting that isolation may be independently contributing to the variation. Interestingly, the Swedish population in Comps et al. (2001), contrasted with the majority of northern populations in their study as it displayed a higher than average allelic richness and lower gene diversity with no significant evidence of a bottleneck. Recent palynological evidence suggests the establishment of a small outlying population of beech and other temperate tree species in Denmark as early as 10,000 BP formed as a result of long distance dispersal events ahead of the main migration front (Overballe-Petersen et al. 2013) that might have contributed to the genetic diversity of populations in Scandinavia, potentially preventing strong bottlenecks.

5.5.2 Post-glacial colonisation is reflected in regional genetic structure

The large western cluster 1 identified by GENELAND in the adult cohort (Figure 5.5), excluding site GAR, is concurrent with palynological evidence on the initial colonisation of beech in Sweden around 3000 BP (Bradshaw and Lindbladh 2005). Bradshaw and Lindbladh (2005)

found that regional palynological data, using a threshold of 0.2% of total tree pollen, indicated an initial expansion resulting in a westerly distribution in southern Sweden at 3000 BP, that subsequently expanded eastward with the establishment of outlier populations in areas further north than the current range. The distribution at 1000 BP reflects the contemporary range of beech in Sweden (Björkman 1996). Bradshaw and Lindbladh (2005) describe the migration front as patchy and dynamic due to the colonisation of an already fragmented, cultural landscape where beech preferentially colonised areas dominated by deciduous forest, a habitat that suffered a decline after 2000 BP. Keller (2010) found that genetic clusters reflected similar post-glacial colonisation patterns in populations of *Populus balsamifera* in North America. A large central cluster was identified near the primary refuge, and hence the source of the population during post-glacial migration. North-easterly populations displayed higher levels of divergence, lower genetic diversity, and received migrants from the central population.

Further population substructure observed for clusters 2 and 3 (Figure 5.5 and 5.7) are likely to have arisen from genetic drift in marginal populations (Excoffier et al. 2009) created by the patchy north-eastward colonisation front after the initial south-westerly expansion (Bradshaw and Lindbladh 2005). Gradual regional changes in allelic frequencies caused by isolation-by-distance can lead to the false assignment of clusters using GENELAND. Although we found isolation-by-distance in the adult cohort, it appears that paired sites for each geographic distance displayed a greater genetic distance between them, if sites originated from different, instead of the same cluster, suggesting the existence of genuine barriers to gene flow (McRae et al. 2005, Rosenberg et al. 2005, Fontaine et al. 2007) (see supplementary material S5.5 for plot of geographic and genetic distance within and between clusters). Clustering results using STRUCTURE revealed a trend of increased admixture in south-westerly sites with increased homogeneity among individuals between north-eastern clusters (Figure 5.8). A similar trend

has been found in leading-edge populations of *Acer campestre* in Poland, which also display further genetic structuring and less admixture with latitude (Chybicki et al. 2014).

The only discernible geographical barriers which have the potential to reduce gene flow are the two large lakes, Vänern and Vättern, as the topography of southern Sweden is relatively flat (Figure 5.2). However, Vättern is the only lake which is directly situated between any two sites, specifically sites GAR and OMB, and as GENELAND analysis did not include GAR, it is unlikely that the major geographical entities acted as barriers shaping the structure found between the 13 remaining sites. Therefore, isolation is likely to be acting as a barrier to gene flow at the range edge in Sweden, which is supported by the reduction in external pollen-mediated gene flow with increased isolation (Figure 5.4). As pollen grains in beech are relatively large (Andrew 1984) and dispersal distances limited (Oddou-Muratorio et al. 2010, Poska and Pidek 2010, Soepboer et al. 2010), isolation could restrict gene flow more than expected for a wind-pollinated tree. Negative impacts of fragmentation have been found in the southern range edge of beech (Jump and Peñuelas 2006). It should be noted that Lindquist's (1931) map reveals a general trend of smaller forest patches towards the north-east which is not fully captured by our isolation variables as it does not reflect the matrix of forest patches between sites.

In terms of adaptation and expansion at the range edge (Kirkpatrick and Barton 1997, Bridle and Vines 2007), the presence of barriers to gene flow in the north-east implies that swamping gene flow may not be a major factor limiting adaptation and hence range expansion. This is in agreement with palynological and model predictions for beech range expansion (Kramer et al. 2010). Local-scale palynological evidence for one of the isolated stands in used our study, MAT, revealed a steady increase in pollen influx values for beech from the time of establishment, suggesting the continued expansion of the stand which was

thought to be primarily limited by the lack of surrounding suitable habitat due to human activity (Björkman 1996). However, migration of maladapted genotypes into high latitude, isolated populations, situated within the westerly cluster could lead to swamping gene flow, preventing northwards expansion in this region.

5.5.3 Pollen dispersal and its relation to forest density

The directional relationships observed for pollen dispersal with isolation and latitude were consistent between models, even though there were differences in the amount of variation explained in each of the response variables. As our study plot exists within a forest fragment, measures of external-mediated pollen dispersal reflect the density of the pollen cloud produced by surrounding forest as opposed to strict long-distance pollen flow. As pollen production is related to the number of reproductive trees, it is likely that pollen production is higher in continuous populations compared to isolated population. Therefore, the probability of fertilisation by pollen grains from external trees decreases with isolation. This is in agreement with previous studies that have found an increase in pollen dispersal with tree density (Wang et al. 2010, Vranckx et al. 2014). Although the isolation indices used in our study do not incorporate density in terms of tree numbers, the buffer measures do incorporate the density of forest fragments. The reduction in allelic richness between adult and seedling cohorts suggests that the pollen donor diversity is not large enough to safeguard against the effects of genetic drift under small population sizes (Table 5.2, Figure 5.3). As seedlings were the result of pollen dispersal during a mast year, we would expect a stronger negative impact of isolation on pollen dispersal during non-mast years as flowering and pollination success are significantly lower in non-mast years (Lindquist 1931, Nilsson and Wästljung 1987, Hilton and Packham 1997).

5.5.4 Effective measurements of isolation

A combination of isolation variables was effective at explaining variation in genetic diversity and pollen dispersal. Area-based measurements using buffer zones present a standardised measure for the surrounding area of forest. We found a general additive effect of increasing buffer zone size, implying a sensitivity to buffer size and also the importance of local and regional isolation levels on genetic diversity and pollen dispersal of beech. Buffer-based measurements were particularly effective predictor variables in our study, as at least one buffer zone was significant in all presented PLSR models. Site boundary did not explain any variance in gene diversity (H_S) and is compromised by its definition, as mapped boundaries may not be biologically relevant, especially for wind-pollinated tree species. This has implications for the definition of population size, as site boundaries may not be biologically relevant in structurally complex range edges with high variability in the spatial structuring of forest fragments. The boundary to boundary (BB) distance measure did not explain any variation in H_S either. The centre to boundary (CB) measure was the most ineffective measure of isolation, although CB did explain variation in seedling H_S that was not described by BB.

In a meta-analysis by Moilanen and Nieminen (2002), buffer-based measurements were found to be superior to distance-based measurements in defining isolation. However, buffer-based measures were also sensitive to the size of the buffer used and Moilanen and Nieminen (2002) concluded that a measure of site boundary combined with distance to all source populations was the best measure of isolation. Measuring distances to all source populations would be very labour intensive for a high gene flow species, such as beech, which, in Sweden, also displays a heterogeneous species distribution structure with thousands of forest fragments (Figure 5.1). Therefore, buffer-based measurements provide a relatively easy and quick way of measuring isolation that, in our study, has performed better than commonly used site boundary and distance measures.

5.6 Conclusion

The interaction between colonisation dynamics and isolation at the leading range edge of beech in Sweden has created gradients of historical and contemporary gene flow within the species. Isolation has a negative impact on allelic richness, which is exacerbated over time, further affecting progeny. In adult populations, gene diversity follows a similar trend to allelic richness. The current genetic structure of beech in the south-west consolidates palynological evidence describing post-glacial colonisation routes into Sweden. North-eastern populations appear to be shaped by barriers to gene flow imposed by isolation, as opposed to geographical features. This study highlights the long-term cumulative effects of isolation on beech forests and its negative impacts on genetic diversity and gene flow, which can lead to inbreeding depression and higher extinction risk as genetic variability is reduced. However, negative consequences are balanced by the potential for adaptation at the range edge that may not be an issue for north-eastern populations, but may limit northward range expansion of western populations.

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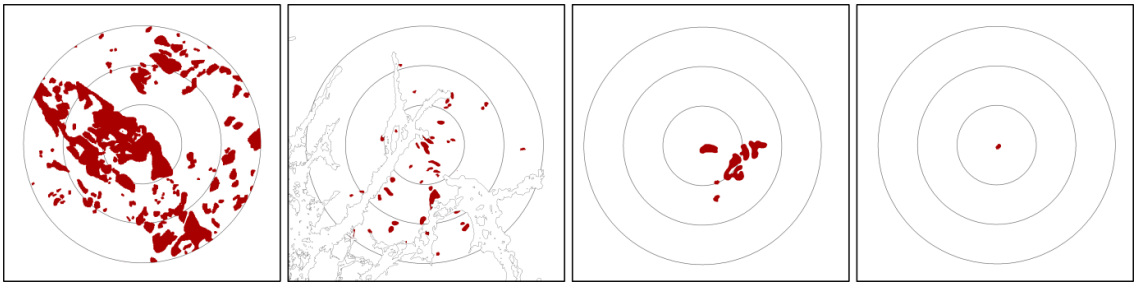
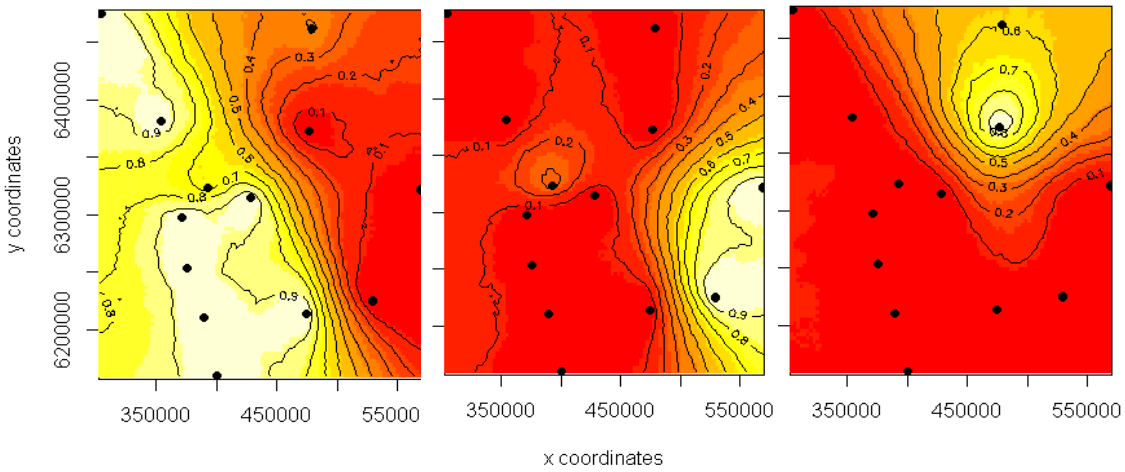
5.9 Supplementary material

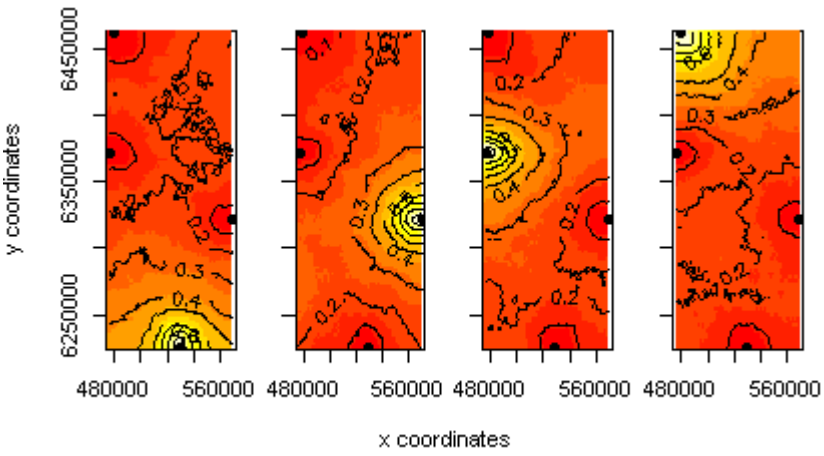


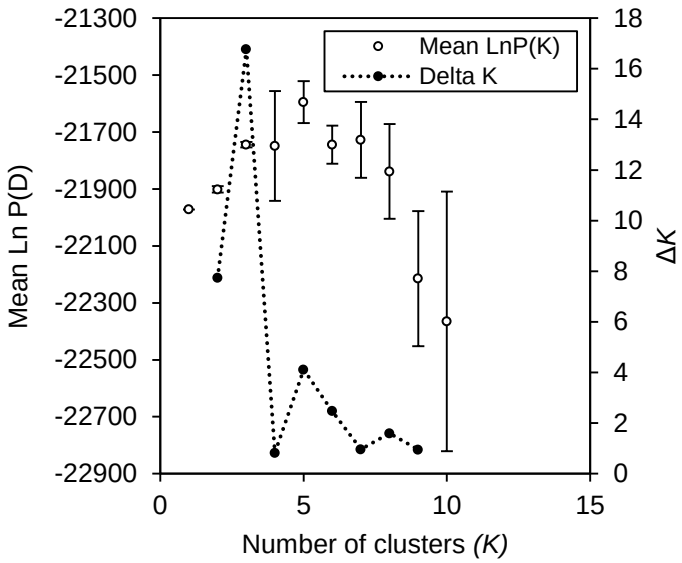
S5.1 Examples of buffer zones. Sites, from left to right with decreasing beech area (ha), are SOD, GUL, STO, and OMB.



S5.2 Maps of posterior probability of cluster membership for each population in GENELAND. Contour lines represent posterior probabilities of belonging to cluster 1, 2, and 3, in order from left to right.

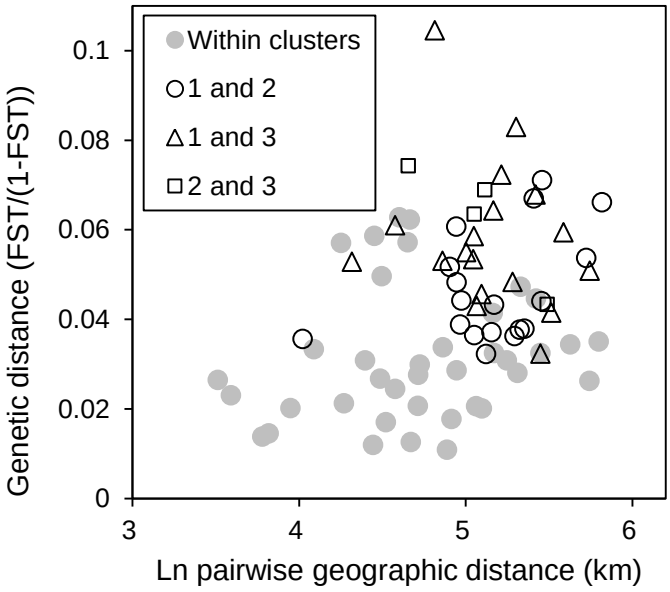
S5.3 Maps of posterior probability of cluster membership for the subset of clusters 2 and 3 in GENELAND. The subset includes sites TRO and HOR from the north-eastern cluster (2), and MAT and OMB from the south-eastern cluster (3). Contour lines represent posterior probabilities of belonging to the four clusters.





S5.4 Identifying the number of *K* in the data from analysis in STRUCTURE. We assessed the convergence of mean values for the log probability of the data (Ln P(D)) and used the Evanno et al. (2005) method to determine the number of clusters. Data consists of the adult cohort at 13 sites.

S5.5 The relationship between geographic and genetic distance partitioned into comparisons within and between GENELAND clusters. Grey circles are comparisons of sites within the same cluster. Open symbols represent comparison of sites in different clusters, i.e. between cluster 1 and 2 (open circles), 1 and 3 (open triangles), and 2 and 3 (open squares).



Chapter 6

GENERAL DISCUSSION

6.1 Research purpose

Forests in Europe have long been influenced by humans through silvicultural management, the harvesting of timber, the grazing of animals, the planting of stands, and the clearing of forests for land-use change. These practises shape forest populations at local spatial scales through their influence on regeneration and establishment, and at regional spatial scales through their influence on migration and colonisation. By examining forests that have been heavily managed and their natural counterparts, we can uncover factors that limit gene flow and impact genetic diversity. The research presented in this thesis focused on the ecologically and economically important tree species, the European beech, as its extensive range exhibits a mosaic of forests, ranging from natural to heavily managed stands. Several spatial scales are explored from fine-scale effects on genetic variation, to larger, regional patterns in genetic variation.

6.2 Altering regeneration and establishment at the local scale

Many European forests have experienced historic traditional management practices, such as coppicing and pollarding, which alter the fundamental mechanism of regeneration within a stand, reduce the rate of establishment through grazing (Rozas 2004), and change canopy-cover dynamics (Coppini and Hermanin 2007). Converting from a primarily naturally regenerated system to one which regenerates primarily vegetatively is likely to have consequences for the distribution of genetic diversity, the physiology of trees, the associated species diversity, the resulting habitat structure, and ecosystem functioning, which is intensified over time.

The review in chapter 2 highlighted the effects of exploiting vegetative regeneration for several decades, identifying some significant negative consequences of subsequently halting management, such as an increased vulnerability of neglected trees to climatic stress (Corcuera et al. 2006, Bobich et al. 2010, Di Filippo et al. 2010), age-related deterioration (Panaïotis et al. 1997), and mechanical failure (Fay 2002, Read et al. 2010), which together with a lack in establishment (Ratcliffe 1992, Rozas 2004) may compromise forest persistence. The review presents the first comprehensive study of the literature concerning traditional management and its effect on the managed trees and their associated species. Its outcomes can be applied to management using the information given under section 2.6 *Recommendations for management and future research*, providing a resource of research-based recommendations for forest managers, whether it is for timber or conservation management of traditionally managed forest.

It can be argued that the genetic consequences of traditional management is greater for species that primarily regenerate naturally, such as beech (Coppini and Hermanin 2007). The review in chapter 2 highlighted the state of knowledge concerning the genetic consequences of traditional management. Only a handful of studies explored the genetic consequences of coppicing on tree species (Aravanopoulos et al. 2001, Cottrell et al. 2003, Mattioni et al. 2008, Valbuena-Carabaña et al. 2008, Dostálek et al. 2011), with only two considering these effects on beech (Paffetti et al. 2012, Piotti et al. 2012). The review highlighted the importance of species-specific management, and identified a lack in knowledge on the effects of traditional management on genetic variation in beech, one of the most important tree species in Europe.

Chapter 3 presents genetic research to fill this gap in knowledge, and is unique amongst other genetic studies on traditionally coppiced beech forest, in its use of pairwise comparisons with nearby stands, maintained primarily through natural regeneration, to isolate the impacts of

coppice management on genetic variation. This is the most in-depth study to date on the genetic effects of coppicing in a temperate tree species, employing a high number of molecular markers, samples, and replicates of paired sites. An exception is the study of Mattioni et al.(2008), who use more paired plots but only sample 26 trees within each, therefore reducing the power to detect fine-scale spatial genetic structure.

The research in chapter 3 identified differences in the fine-scale spatial genetic structure between paired sites, employing 112 to 170 individuals that were genotyped at 11 loci. A high number of samples and markers improved the resolution of our study, allowing the detection of increased structure extending 10m - 20m further in coppiced stands, compared to natural stands, despite no difference in the genetic diversity between paired plots. While relatively small in their magnitude, the trend was consistently found over all sites, which occur under diverse abiotic conditions and have different population histories, suggesting that prolonged management had changed genetic structuring in coppiced forest.

Using more than three paired site replicates occurring in neighbouring regions in the experiment in chapter 3 may have been more informative and would have allowed us to see if the change in genetic structure remained consistent. However, the cost-benefit trade-off between having an in-depth sampling scheme and having more sites was a barrier to the inclusion of more study sites. An average of more than 150 samples has been recommended to detect fine-scale spatial genetic structure in high gene flow species, such as wind-pollinated trees (Cavers et al. 2005, Jump and Peñuelas 2007). Previous research has found that genotyping up to 200 individuals in 6 SSR loci may not provide adequate power to detect fine-scale genetic structure (Jump and Peñuelas 2007). Previous research was used to optimise the number of samples and loci employed in chapter 3, allowing the detection of subtle changes in spatial genetic structure between three paired sites.

6.3 Migration and colonisation shape regional genetic variation

Consolidating the genetic evidence derived from this thesis, with paleoecological evidence (Hu et al. 2008) has developed our understanding of the dynamics of beech migration and colonisation during the Holocene. The regional-scale work in chapters 3 and 4 present the most comprehensive research of beech population genetics in both countries. Both studies employ the use of historical sources (e.g. sources from Rackham (1980) for Great Britain, and Lindquist (1931) for Sweden) which, together with paleoecological data, add a novel dimension to genetic research.

Artificial long-distance dispersal has led to the homogenisation of genetic variation in Britain, creating a population range typified by high gene flow and little spatial genetic structuring (chapter 4). However, cryptic signals driven by natural migration, such as isolation-by-distance and gradients of haplotype diversity, remain in the putative native range. The palynological evidence of the Holocene post-glacial spread of beech in Britain (Birks 1989) can be described as having identified primeval beech forests, whilst the current research on the genetic component suggests that there exists descendants of primeval forests, thereby reconciling a temporal gap in the history of beech in Great Britain.

The research in chapter 4 prompts a change in the view of beech as native to a fraction of Great Britain, echoing a previous call to move away from the restrictive and ecologically unfounded labelling of native woodland in Britain (Brown 1997). Results revealed that Britain's forests had been impacted so drastically by humans that there exists little basis to assign a native range for beech in the country, therefore agreeing with Preston et al. (2002) in assigning beech as native throughout. This finding frames beech in Britain as an ideal case study to examine potential impacts of translocations to shed light on the implications of

assisted migrations, a practice which has the potential to mitigate some climate change impacts on biodiversity, but that has been hindered by uncertainty surrounding the potential effects of introductions on local biodiversity (Ricciardi and Simberloff 2009, Vitt et al. 2010, Hewitt et al. 2011). The introduction of beech ahead of its leading range in Britain bears some similarities to assisted migrations, albeit far less regulated. Monitoring naturalised populations may provide information on the effects of translocated beech on associated species diversity and the long-term consequences of assisted migrations. The existing literature on Britain's northern beech forests (Watt 1931a, b) may already yield insights into potential consequences of assisted migrations through examining the literature from an alternative standpoint.

The distribution of beech in Sweden contrasts that in Britain, as the range is primarily shaped by natural processes, such as migration, colonisation, and abiotic conditions (Lindquist 1931). Chapter 5 presents the first study in beech exploring the leading range edge of the species. It highlights issues and provides solutions to measuring isolation of wind-pollinated tree species, where visible boundaries between forest patches may not translate to biological boundaries. Founder effects and prolonged isolation had a negative impact on genetic diversity and led to increased genetic differentiation between forests, which is congruent with theoretical predictions (Ellstrand and Elam 1993, Ouborg et al. 2006, Eckert et al. 2008).

The research in chapter 5 benefitted from access to a key piece of historical work, Lindquist's (1931) map of beech distribution in Sweden, which allows us to estimate the surrounding area of beech forest at each site. Regional maps with this level of detail are understandably uncommon because of the sheer effort involved in their creation and the suitability of certain species to mapping techniques. For example, using aerial reconnaissance techniques, beech in Sweden could only be mapped during a short window in May when its unique foliage colour and time of budburst allowed it to be distinguished from other co-occurring tree species

(Lindquist 1931) . Nevertheless, future studies on isolation may benefit from employing buffer based measures that can be attained for a fraction of the surrounding area without the need for a regional scale map.

The pattern of genetic clustering in Swedish beech populations reflected palynological patterns revealing its colonisation route into the country during the Holocene (Bradshaw and Lindbladh 2005). Eastern, isolated sites were more genetically differentiated than western sites. This pattern was thought to be driven primarily by past-colonisation dynamics coupled with the long-term effects of isolation. The isolation indices used in chapter 5 may have been improved by including a measurement of the amount of beech forests between sites, as the regional distribution appears to follow a south-west to north-east isolation gradient that was not fully captured by the isolation measurements used. This could be attained through pairwise measurements of beech forest patches between sites or the use of resistance matrices (McRae 2006).

Isolation can occur as a result of migration and colonisation of sub-optimal habitat, or as a consequence of anthropogenic habitat fragmentation through deforestation. The reduction in genetic diversity and increased genetic differentiation found in isolated populations in Sweden, can lend insights into the long-term consequences of anthropogenic forest fragmentation. It should be noted that fragmented populations situated in the core range lack the relatively strong influence of colonisation dynamics, compared to range-edge populations. However, founder events during colonisation can have some similar consequences on genetic variation to that of genetic bottlenecks created by a reduction in population size as a result of fragmentation, such as the loss of rare alleles, the reduction in genetic diversity, and the reduction in gene flow between patches (Young et al. 1996). The effects of relatively recent forest fragmentation on genetic diversity may experience a functional lag due to the long

generation time of trees (Bacles and Jump 2011). Improving the measurements of isolation used in fragmentation studies by including a variety of informative indices may improve accuracy and hence analysis power, allowing the detection of small, but significant changes in the genetic diversity of populations.

6.4 Future research and conclusions

The extensive data sets collected for this thesis provide a valuable resource for several avenues of future research. Results from research in chapter 3 on coppiced forests can provide information that may improve the parameterisation of population models, which aim to determine genetic variation of future beech populations (Kramer et al. 2008, Kramer et al. 2010). Studies using simulations can be used to provide greater detail of past processes that have shaped populations, and also provide future scenarios of population dynamics. Scenarios of alternative colonisation histories can be tested against each other using modelling methods, such as approximate Bayesian computation (ABC) (Cornuet et al. 2008). Next generation sequencing can be used to generate the summary statistics used for ABC analysis. Cost effective forms of next generation sequencing, such as RADseq, allow the study of genomics at a population level (Davey and Blaxter 2010). These methods can yield thousands of potential markers, overcoming issues of low marker numbers experienced in past studies, such as those exploring the genetic effects of coppicing, as discussed in section 2.6.1.

The movement of beech through Europe has been largely driven by natural processes. However, long-standing human impacts have changed local and regional patterns of genetic variation. Widespread traditional management, which promotes vegetative regeneration and reduces establishment, has the potential to fundamentally alter familial neighbourhood sizes in stands. Colonisation dynamics have shaped the leading range-edge of the species, creating

distinct patterns of genetic clusters, with founder effects and genetic drift in isolated populations influencing their genetic diversity and differentiation. In Great Britain, the genetic signal created by the natural migration of the species has been concealed by the artificial dispersal of the species within and outside of its historic natural range. Humans have played an integral part in shaping contemporary forests and examining both natural and anthropogenic processes can shed light on novel strategies that can be used to increase forest persistence in the future.

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