New insights into the history of *Fagus sylvatica* L. in European forest stands during the Holocene

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The current distribution of *Fagus sylvatica L.* (European Beech) spans across Europe from southern Scandinavia into the Balkan, Italian and Iberian peninsulas. At the beginning of the Holocene its geographical distribution was limited to south of the continent. Its migration north, compared with other deciduous species, was relatively late. How the species migrated into and became established in the forests of Europe during the Holocene has been subject to an intense research debate. This thesis provides further information on the species history. Palaeoecological studies were carried out using predominantly forest hollow sites to provide stand-scale data. Traditional pollen studies were carried out as well as two novel investigations. The first was a data-model comparison which examined the species migration. The second examined the potential use of ancient DNA.

The data-model comparison study is used to investigate the colonisation of both *F. sylvatica* and *Picea abies* in southern Scandinavia during the late Holocene. The output of the model is compared to the actual arrival times taken from stand-scale fossil pollen diagrams. Using a combination of long distance dispersal events and local dispersal, the model was able to generate quite comparable patterns to those observed from the pollen data. By applying several different parameter combinations, a range of possible values for the speed and mode of migrations were observed where the deviations from the observed arrival times are minimal. The study suggests that migration speeds of 100 m/yr for *F. sylvatica* and 250 m/yr for *P. abies* best fit the data. Dispersal alone can explain the wavelike spread of *P. abies* but fails to explain the scattered pattern found for *F. sylvatica* in Scandinavia.

This scattered pattern is likely an result of the strong anthropogenic activity, particularly disturbance, on the species establishment. The second part of this thesis examines if this observation is unique to Scandinavia or prevalent in other areas. Data from northern and eastern Germany was collected to assess what role disturbance plays in the species establishment of these regions at the stand-scale. Pollen and charcoal analyses are presented from one forest hollow in the Schlaubetal area of East Brandenburg and from a second hollow close to Neustrelitz in Mecklenburg-Vorpommern. The second hollow has an unusually long record for stand-scale sites. Disturbance is shown to be present immediately before establishment of *F. sylvatica* at both sites. Furthermore, comparisons to regional reconstructions illustrate the insights gained from vegetation reconstructions at different spatial scales within the same study area.

Finally, attempts were made to extract ancient DNA from plant macrofossils at three sites using microsatellite markers. Studies of ancient DNA raise several new possibilities for improving our understanding of past population processes in plants. It would make direct testing of hypothesis regarding postglacial tree migrations of individual species possible. The work presented here has helped to setup future research projects.

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aDNA	Ancient DNA
AMS	Accelerator mass spectrometry
CA	Cellular automata
cpDNA	Chloroplast DNA
HCl	Hydrochloric acid
HF	Hydrofluoric acid
LDD	Long distance dispersal
LGM	Last Glacial Maximum
LOI	Loss on ignition
NaOH	Sodium hydroxide
nDNA	Nuclear DNA
NPP	Non-pollen palynomorph
PAR	Pollen accumulation rate
PCA	Principal component analysis
PCR	Polymerase chain reaction
SD	Standard deviation
TBA	Tertiary butyl alcohol

Chapter One – Introduction

1.1 Overview

Studies into the migration and establishment of European tree species during the Holocene have taken place since the early 20th century (von Post 1916). Throughout this period, analysis of fossil pollen has been the major methodology used by palaeoecologists to reconstruct the vegetation history of Europe. In parallel, studies of plant macrofossil remains have provided important information into the location of different tree species in the past. More recently, a fusing together of these two traditional palaeoecological techniques with results from genetic marker studies are now appearing in the literature and are growing in number (Petit *et al.,* 2002; Magri *et al.,* 2006; Tollefsrud *et al.,* 2008). It is becoming apparent that both traditional and genetic studies will be needed to help solve some of the unanswered questions relating to how and why tree species migrated and established in new areas in the current interglacial. Understanding these past changes are important as they can inform current predictions into future changes and inform conservation options (Petit *et al.,* 2008; Hu *et al.,* 2009).

An appreciation that different depositional environments represent different spatial scales in a pollen diagram has allowed a greater understanding of what a diagram actually represents in terms of vegetation on the landscape (Jacobson and Bradshaw 1981; Sugita 1994; Davies 2000). The spatial scale represented in any pollen diagram is largely dependent on two factors. The first is the size of the basin from which the sediment core is extracted (Jacobson and Bradshaw 1981; Sugita 1994). The second is the type and pattern of the vegetation around the site (Sugita 1994; Bunting *et al.,* 2004). The majority of pollen diagrams in existence have been produced from various sizes of lakes and bogs. These sites typically represent vegetation assemblages on a regional scale. In contrast, only a relatively small number of stand-scale pollen diagrams exist. These stand-scale sites, small peaty hollows in forests, reconstruct the vegetation immediately surrounding the sedimentary basin (Bradshaw 1988). They are particularly useful at examining local processes that have affected long-term vegetation dynamics. The information contained in these sites can be useful to both the plant ecologist and palaeoecologist (Bradshaw 2007).

A single stand-scale site is able to record the vegetation changes that have taken place immediately surrounding the hollow over periods of thousands of years. Combined with charcoal analysis (Higuera *et al.*, 2005) and plant-macrofossil records (Hannon *et al.*, 2000) these sites can reconstruct the history of individual forest stands in detail. They have be used to examine drivers of past vegetation change (Bradshaw and Hannon 1992), to inform conservation debates (Lindbladh *et al.*, 2007), compared with forest stand models (Cowling *et al.*, 2001) and combined with archaeological data (Hannon *et al.*, 2008). Although the main purpose of these sites is to examine the history of individual forest stands they can be collated to be used in examining regional patterns (Bradshaw and Lindbladh 2005; Lindbladh and Foster 2010).

The collation of multiple pollen diagrams, at various spatial scales, to produce synthesis has allowed patterns in space and time to be assessed on regional and continental scales (Godwin 1975; Huntley and Birks 1983; Lang 1994). However, traditional pollen sites may miss populations at low abundance. Regional sites often show long 'tails' in the diagram for spreading pollen types, which may be pollen from small localised populations, but could also be from larger populations outside the region of interest with the pollen in the diagram arriving to the site via long distance dispersal (LDD). Therefore stand-scale sites, with their smaller pollen source area and higher concentrations of pollen are likely to contain higher proportions of local pollen. Therefore they can be used with more confidence when examining local founding populations as opposed to regional sites. Although it would represent a novel approach to the problem, the ability of stand-scale sites to detect founding populations, with more confidence, is potentially important in the migration debate and therefore should be considered in migration studies.

Furthermore, the quality of macrofossil preservation often found in stand-scale sites means these depositional environments could be potentially very good for studies involving ancient DNA (aDNA). Information from this new technique is potentially very important in improving understanding of Holocene tree migrations. Traditional ideas that most tree species survived the last glaciations in southern refugial areas have come under increasing scrutiny (Stewart and Lister 2001; McLachlan *et al.,* 2005; Anderson *et al.,* 2006). Growing evidence suggests that species survived in more northern locations, where suitable microclimates existed. Genetic data should allow assessment to whether these hypothesised northern refugia are genetically distinct from populations in the south or are similar, in which case, the most likely scenario is that these populations are a result of rapid migration after deglaciation. McLachlan *et al.,* (2005) used molecular markers to examine the present geographic location of different tree populations within the same species to make inferences regarding refugial location. These types of synthesis have now been combined with traditional palaeoecological data to help further refine understanding of past migration patterns for individual species. In Europe, these types of synthesis now exist for *Quercus* (Petit *et al.,* 2002), *Picea* (Tollefsrud *et al.,* 2008) and *Fagus* (Magri *et al.,* 2006). Ancient DNA can also help test the various migration scenarios presented in these new combined synthesis by pinpointing the different molecular markers in time. Ancient DNA studies using sedimentary basins in temperate areas are rare, even though these basins provide plenty of material in which aDNA could potentially be preserved. In temperate environments the extraction success of aDNA has often been low (c.2% from pollen samples). Considerable progress has been made in recent years in human and animal studies using aDNA, while less success has been reported with plant material. Published studies include DNA extractions from wood (Dumolin-Lapègue *et al.,* 1999; Liepelt *et al.,* 2006), pollen (Parducci *et al.,* 2005; Paffetti *et al.,* 2007), plant remains (Pollmann *et al.,* 2005; Suyama *et al.,* 2008), bulk sediment from permafrost areas (Willerslev *et al.,* 2003), silty sediment found at the base of a Greenland ice core (Willerslev *et al.,* 2007) and arctic lake sediments (Willerslev and Brochmann pers. comm.). In theory forest hollows should allow for rapid burial of macrofossils, as trees fall directly into the sedimentary basin, which would be better for greater preservation quality as the macrofossil is preserved intact in saturated anaerobic conditions.

This thesis uses stand-scale sites to provide further information about migration and expansion of trees in the Holocene. Stand-scale sites are pollen diagrams produced from organic sediment found in small hollows in forested areas. There is a focus on one particular species, *Fagus* sylvatica L. (European Beech). The species is a deciduous tree which forms a dense canopy. It is a stress-tolerant competitor which is able to regenerate under dense canopy (Grime *et al.,* 1988). The species has a trans-continental distribution (Bohn *et al.,* 2000). It's northern distribution extends to southern England and southern Scandinavia. To the south it extends into the Iberian, Italian and Balkan Peninsulas (Figure 1.1). It is a tree of important economic importance and is often regarded as being of being representative of 'natural' forest cover. At present it is one of the dominant tree species in the forests of

Europe, and with its history regarded as being controlled by a complex interplay of natural and anthropogenic forcing (Giesecke *et al.*, 2007; Bradshaw *et al.*, 2010).

Figure 1.1 Location of potentially dominating *F. sylvatica* forests in Europe (Bohn *et al.*, 2000).

This tree species was chosen for study because its history has been and still is currently subject to an intense research effort (Huntley *et al.*, 1989; Pott 2000; Magri *et al.*, 2006; Tinner and Lotter 2006; Giesecke *et al.*, 2007; Magri 2008; Bradshaw *et al.*, 2010). Furthermore the species has been subjected to numerous stand-scale studies that have focused on the northern limit of its distribution (Bradshaw and Lindbladh 2005). Only investigations focussing on *Picea abies* (Bradshaw and Lindbladh 2005) and *Quercus* (Lindbladh and Foster 2010) have a comparable number of stand-scale palaeoecological studies in Europe. Finally, the tree species has been subject to numerous genetic studies examining the current distribution of genetically distinct populations (Demesure *et al.*, 1996; Gailing and von

Wuehlisch 2004; Vettori *et al.,* 2004). Magri *et al.,* (2006) produced a Europe wide synthesis of modern haplotype distributions and synthesis of the pollen and plant macrofossil data. These studies have laid the foundation for aDNA attempts using the species.

The remainder of this chapter reviews the Holocene history of *F. sylvatica* by giving an overview of the present knowledge of the species spread and expansion. Finally, the last part of the chapter identifies research challenges for the species and sets of the aims and rationale of the questions being tested in this thesis.

1.2 Holocene history of *Fagus sylvatica* L.

One of the most debated changes in the Holocene vegetation history of Europe is the appearance and expansion of the species *Fagus sylvatica* L. (European Beech). In northern Central Europe, the main expansion took place in the mid to late Holocene after the species had migrated from its glacial refugial areas in more southerly latitudes (Magri *et al.,* 2006). The spread occurs much later than that of other deciduous species such as *Quercus*, *Tilia* and *Ulmus* and it is debated as to whether its spread was that of a migrating front or disjointed and mediated by local disturbance of the forest ecosystem (Küster 1997; Giesecke *et al.,* 2007).

The understanding of the species history on a local, regional and continental scale has undergone a number of recent research advances, firstly in collection and syntheses of pollen data (Gliemeroth 1995; Brewer 2002; Magri *et al.,* 2006), secondly through the incorporation of modern day genetic patterns with these syntheses (Brewer 2002; Magri *et al.,* 2006) and thirdly by attempts at understanding past dynamics at various spatial scales using data-model comparisons (Sykes *et al.,* 1996; Cowling *et al.,* 2001; Giesecke *et al.,* 2007; Magri 2008). Aside from these studies, major reviews of the species history have been carried out by Pott (2000) and Bradshaw *et al.,* (2010). On a regional scale, recent reviews have been carried out for the Iberian Range (Lopez-Merino *et al.,* 2008), Rhône Region (Delhon and Thiebault 2005), southern pre-Alps (Valsecchi *et al.,* 2008), Alps (van der Knaap *et al.,* 2005), southern Central Europe (Tinner and Lotter 2006) and southern Scandinavia (Bradshaw and Lindbladh 2005).

The species history can be particularly well resolved as the pollen grains found in sediment can be ascribed with confidence to solely to *F. sylvatica.* Although the pollen grain of the *F. sylvatica* is identical to that of *Fagus orientalis,* in Europe the present ranges of the two species only overlap in the southern Balkans. However it has never been suggested that *F. orientalis* was present in Europe except the Balkans during the Holocene. Therefore any *Fagus* pollen grains found in sediments almost certainly originate from *F. sylvatica.*

1.3 The northward migration of *F. sylvatica*

The Oxford English Dictionary defines migration has the movement of one entity from one locality to settle in another. In tree studies, migration can be defined as the spread of a species into a novel area, whilst the parent population remains static. Studying initial migrations with pollen analysis is difficult, the work of Bennett (1988) shows that populations of low abundances may not be detected in pollen diagrams. It is entirely possible that small outlying populations exist but are cryptic in the data (Woods and Davis 1989). Therefore this section deals when the species is first detected in sediment record and can be confidently tied to a population in close proximity. This interpretation accepts that the actual first arrival may be much earlier than the date given. This problem is discussed in detail in chapter five of this thesis.

1.3.1 Movement

The collection of a number of different pollen diagrams means it is possible to help track how the species spreads. In the English language the first major review is Godwin (1956; 1975), who quotes the work of Firbas (1949) who stated that the species appeared in the Böhmerwald (southern Germany) about 5000 to 6000 BC and was at the Baltic coast around 2000 BC. Godwin (1975) also summarizes that *F. sylvatica* was likely to be present in isolated stands over a very large area before it achieved its general dominance.

The pollen atlas of Huntley and Birks (1983) provided the first complete synthesis on a European scale of the species history (Figure 1.2). This synthesis suggested that the species migration started deep in the Balkans. The species moved north and eastward entering central Europe around 4050 BC and reaching the Baltic by 2050 BC. At 50 BC the species had migrated into Sweden.

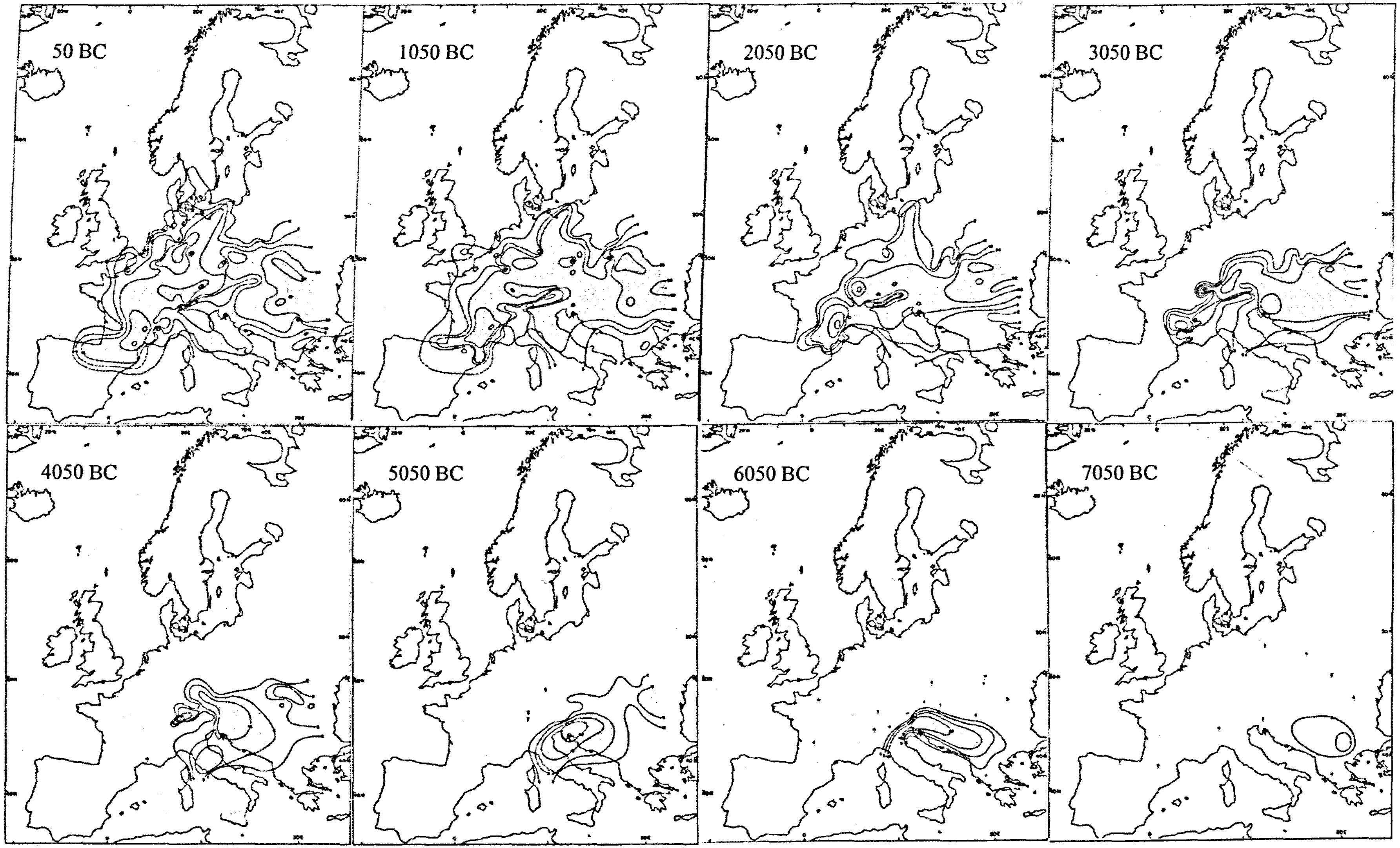

Figure 1.2 Location of glacial refugia and Holocene spreading patterns (after Huntley and Birks 1983)

Gliemeroth (1995) produced a study based on 1700 pollen diagrams. The data-model comparisons of Giesecke *et al.,* (2007) used this study, they highlighted that the threshold of 1% total terrestrial *Fagus* pollen indicated when small populations expanded, although the initial establishment may have occurred hundreds or thousands of years earlier (Figure 1.3). The Gliemeroth study shows the spread did not occur in continuous manner in all directions, but shows 1000 year long delays in different regions and at different times (Giesecke *et al.,* 2007, citing Gliemeroth 1995).

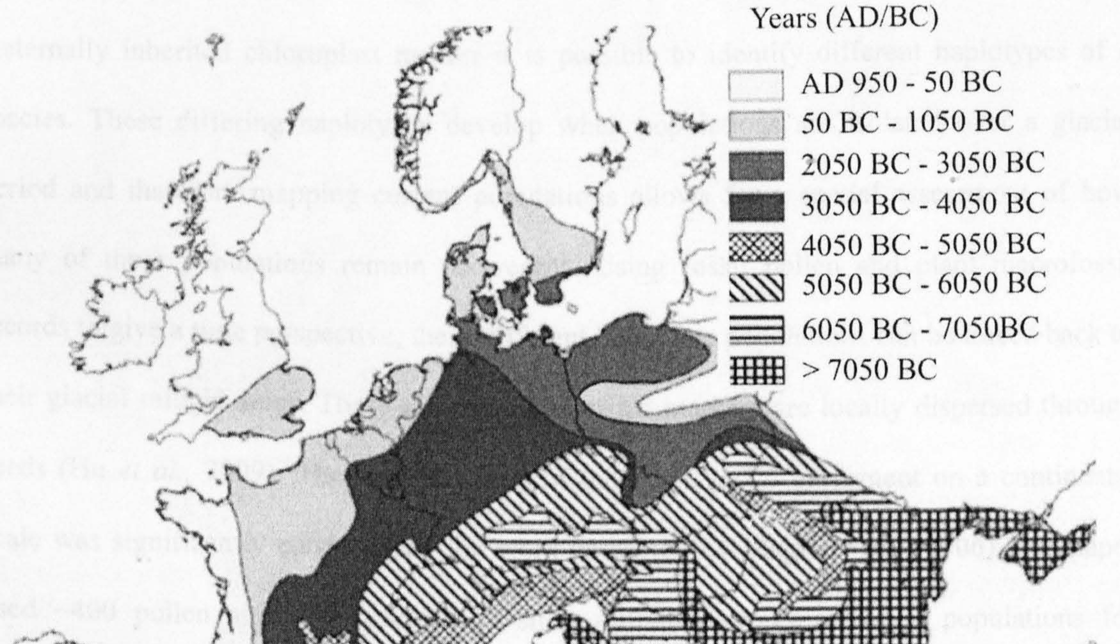

Figure 1.3 Interpretation of the spread of *F. sylvatica* based on the time the 1% threshold was reached in selected pollen diagrams based on data from Gliemeroth (1995). Note that this presentation emphasizes spread on the European scale; shaded areas may also cover regions where *F. sylvatica* never occurred, (after Gieseke *et al.,* 2007).

Using only the results of fossil pollen sites (Magri *et al.,* 2006) state that the postglacial spread of the species was continuous without any evidence of significant retreats and re-advances. This dataset it is also argued supports the idea that modern *F. sylvatica* populations are the direct descendants of the first settlers in the area. This is an important assumption for the combined fossil pollen and modern genetic marker studies (see below).

Genetic analysis has added a new dimension to migration work because past vegetation changes often leave a distinct imprint on current populations (Hu *et al.,* 2009). Using the maternally inherited chloroplast marker it is possible to identify different haplotypes of a species. These differing haplotypes develop when populations are isolated over a glacial period and therefore mapping current populations allows for a spatial assessment of how many of these populations remain at present. Using fossil pollen and plant macrofossil records to give a time perspective, these different haplotype populations can be traced back to their glacial refugia areas. These maternally inherited markers are locally dispersed through seeds (Hu *et al.,* 2009). The understanding of the *F. sylvatica* movement on a continental scale was significantly enhanced by the work presented by Magri *et al.,* (2006).This paper used ~400 pollen sites, 80 macrofossil sites, 450 modern *F. sylvatica* populations for chloroplast markers and 600 modern *F. sylvatica* populations for nuclear markers. The main conclusions about spreading history given by Magri *et al.,* (2006) were that *F. sylvatica* survived the last glaciation in multiple refugia areas, the central European and Mediterranean refugia were separate and the Mediterranean refugia did not contribute to the colonisation of central and northern Europe. This combined approach significantly changed the perception that *F. sylvatica* spread into central Europe using two routes. The first from the Balkans to the northwest migrating through the Dinaric Alps, eastern Alps, Danube valley, Bohemian central highlands into northern Germany and the Polish lowlands before reaching southern

Sweden. The second route came from the southern Apennine peninsula into the western Alps and through northwest France reaching its limit in southern England (Pott 1997).

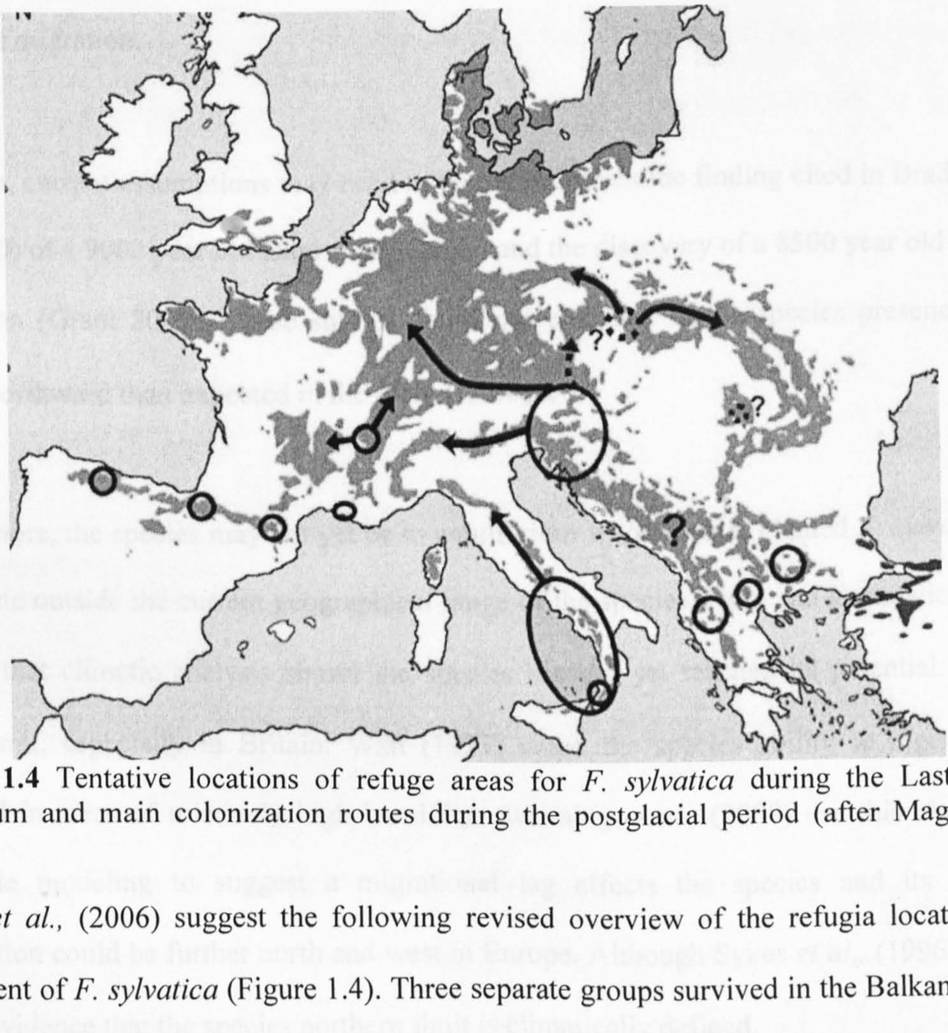

Figure 1.4 Tentative locations of refuge areas for *F. sylvatica* during the Last Glacial Maximum and main colonization routes during the postglacial period (after Magri *et al.*, 2006).

Magri *et al.*, (2006) suggest the following revised overview of the refugia locations and movement of *F. sylvatica* (Figure 1.4). Three separate groups survived in the Balkans but did not spread. A population that survived in southern Italy spread north but did not cross the Po Valley in northern-central Italy. A population survived in the French Mediterranean but although it spread early it stayed local. Three areas existed in the northern Iberian Peninsula supported by macrofossil evidence and three different isozymes markers. These populations stayed in this area. A population in the western Alps was responsible for the colonisation of southern France. The population based in the eastern Alps is shown that have colonised the Alps, central Europe, northern France, England and Scandinavia.

The pattern of spread would depend on the type of movement (disjointed, retreat and advances, wave-like front). Considering the studies of Gliemeroth (1995) and Magri *et al.*, (2006) a disjointed migration northward with no known major retreats seems the most likely pattern of migration.

Although, current assumptions may need to be revised given the finding cited in Bradshaw *et al.*, (2010) of a 9000 year old Danish population and the discovery of a 8500 year old English population (Grant 2005). These studies give clear evidence of the species presence much further northward than expected in the early Holocene.

Furthermore, the species may not yet be in equilibrium with climate. Planted *F. sylvatica* can regenerate outside the current geographical range of the species. Fang and Lechowicz (2006) suggest that climatic analysis shows the species has not yet reached its potential northern range limit, especially in Britain. Watt (1923) noted the species ability to regenerate in Scotland in areas of relatively high humidity. Svenning *et al.*, (2008) use this observation alongside modeling to suggest a migrational lag affects the species and its potential distribution could be further north and west in Europe. Although Sykes *et al.*, (1996) provide strong evidence that the species northern limit is climatically defined.

1.3.2 Spreading rates

The speed at which a tree species migrates can be measured in two ways. Firstly, present day observations can take place. Secondly inferred migration rates can be made if the distance measured from the known location of glacial refuge populations and the current limits are calculated and then divided by the time taken. Nearly always these two calculations disagree significantly in what is known as Reid's Paradox of rapid plant migration (Clark *et al.*, 1998).

In respect of *F. sylvatica*, studies on a continental scale (Huntley and Birks 1983; Huntley *et al.*, 1989) and a regional scale (Brewer 2002; Bradshaw and Lindbladh 2005) have used pollen data to infer the rate at which *F. sylvatica* has spread during the Holocene. The rates for *F. sylvatica* range from 100 m/yr (Brewer 2002; Bradshaw and Lindbladh 2005) to 300 m/yr (Huntley and Birks 1983; Huntley *et al.*, 1989). These figures are influenced by study area, data set and time period.

Study	***Rate (m/yr)***
Huntley and Birks (1983)	250-300 (Mid Holocene)
Huntley and Birks (1983)	200-300 (Northern Europe)
Huntley, Bartlein and Prentice (1989)	150-300
Brewer (2002)	100-150
Bradshaw and Lindbladh (2005)	100

Table 1.1 Summary of spreading rates quoted in the literature for *F. sylvatica.*

Integration of pollen analysis with population genetic studies has helped to redefine understanding of the main refugial locations and the movement of species. The northern European *F. sylvatica* populations, which are genetically distinct from populations in Spain and the Balkans, were most likely present in Slovenia during the Last Glacial Maximum (LGM) (Magri *et al.*, 2006). Therefore working out the rate at which *F. sylvatica* expanded from Slovenia and into central Europe and eventually southern Scandinavia is probably the best estimate of migration rate for this haplotype of *F. sylvatica.* Although Bradshaw *et al.*, (2010) argue that given the potential of scattered, founder populations of the species, the most reliable estimates of spreading rates might come from observed, active regions of spreading for the tree in forested landscapes such as southern Sweden. In this case migration rates may be much lower than 100 m/yr.

1.3.3 Mechanisms and controls

The mechanisms by which the species spread are a combination of biological and physical processes. The distance of seed dispersal will follow a highly skewed pattern towards a high number of short dispersals, with longer dispersals being much rarer in frequency. Short dispersals are likely to be caused by wind and seed fall. For longer dispersals, Küster (1997) suggests the spread of the fruit is possible by mammals and birds, which would have been attracted to abandoned farmland which would have had nutritional sources over and above those of undisturbed woodland. Pott (1997) states that bears can carry the capulae of *F. sylvatica* when nuts get caught in their fur. Various birds and mammals could disperse nuts, with birds being the most effective long distance dispersal (LDD) agency. However only birds that routinely cache nuts in the ground in favourable habitats for the tree (e.g. Blue Jay, North America) to make the dispersal effective (Johnson and Webb III 1989) can be considered important migration vectors. Long distance spreading via rivers cannot be overlooked because the fruit and seeds of *F. sylvatica* are able to float (Pott 1997). Horn *et al.,* (2001) noted that all seeds within wind-dispersed species maybe viable candidates for long-distance dispersal, therefore it must be assumed that the species is certainly possible of LDD by wind

For the entire European range (Giesecke *et al.,* 2007) concluded that no single driving force could account for the observed distributional limits 6000 years ago, or the pattern of spread during the Holocene. Giesecke *et al.,* (2007) present simulations which suggest the species should have been as widespread 6000 years ago as it is at present. One suggestion for the data-model mismatch is that the pollen data can only show that the species was not abundant 6000 years, but it is difficult to resolve small populations from pollen data. The most recent review of the Holocene history of *F. sylvatica* was carried out by Bradshaw *et al.,* (2010). This study focused on species in Europe, North America and Japan. The three species (*F.crenata*, *F.grandifolia* and *F. sylvatica*) were shown that have similar climatic controls on their spreading history but that the spread was also strongly influenced by the location of glacial refugia and the establishment of outlying founder populations in the early Holocene. The study also argues that climatic factors alone cannot explain fully the Holocene history. Other processes such as spreading biology, population dynamics and disturbance need to be investigated (Bradshaw *et al.,* 2010).

Magri (2008) argues that the conformity of the increase in the distribution of the species to the classical logistic model of population growth supports the notion that, given a non-limiting climate, a biological process was the main factor shaping the post-glacial expansion in Europe. Magri (2008) suggests that this pattern was induced by multiplicative biological factors but fails to describe these factors in detail. Globally Fang and Lechowicz (2006) suggest that thermal climate is the most important climatic control on the species distribution, with moisture the second most important factor.

1.4 Expansion of the species

Expansion is defined as the increase of a population in a particular area. Expansion can take place at a number of different spatial scales, e.g. within a forest, on a landscape and on a larger scale regional size. These stand-scale data can be used to look at how expansion takes place on local scale, e.g. within a forest stand. At a landscape level, pollen diagrams produced from lakes can help examine what controls expansion. On a regional scale a number of pollen diagrams can be analysed together to look for synchronicity. On a regional scale recent reviews (Bradshaw and Lindbladh 2005; Tinner and Lotter 2006) have been carried out and a pattern emerges of differing controls on expansion in different geographic areas. It is unlikely that one dominant factor could explain the expansion for the entire range of the species.

Examination of the numerous regional syntheses reveals the complexity in understanding the species history. In southern Europe and southern central Europe multiple factors, such as climate, fire regime and anthropogenic activity, are put forward to explain the species distribution, but it is suggested that anthropogenic activity had only a limited role (Tinner and Lotter 2006; Valsecchi *et al.,* 2008). This is in contrast to work in northern central Europe and Scandinavia, where the species appears to have experienced a strong anthropogenic control on its expansion (Godwin 1956; Iversen 1973; Behre 1988; Küster 1997; Bradshaw and Lindbladh 2005; Bradshaw *et al.,* 2010)

1.4.1 Southern Europe and Southern Central Europe

Tinner and Lotter (2006) examined the evidence for southern Central Europe using two sites on the Swiss Plateau and one on the German Alpine foreland. Using cross-correlations between pairs of pollen types, and pollen and charcoal they suggest that neither disturbance of the forest caused by anthropogenic processes or fire substantially aided the expansion. Instead Tinner and Lotter suggest that population expansion occurred in response to climatic cooling and increases in precipitation suggesting that the main forcing factor of the species expansion was climate. Pott (1997) states that the reduction in mixed forests, comprising of *Tilia*, *Ulmus*, *Quercus* and *Corylus*, and the invasion of *F. sylvatica* in Central Europe was very probably a result of natural succession processes related to climatic trends in the Atlantic period (~ 6950 to 3750 BC). However, these natural processes were probably accelerated and influenced by anthropogenic impact prior to the onset of this period of time.

Tinner and Lotter (2006) suggest that the tree expanded stepwise across the continent during climatic periods characterised by wetter and cooler conditions (e.g. 1850-1450 and 750-350 BC). Evidence for this climatic control is argued for by the timing of the species expansion being synchronous over a spatial range of approximately 500km (Tinner and Lotter 2006). Valsecchi *et al.*, (2008) used two lakes in the southern pre-Alps in Italy to investigate the species expansion. This study concluded that the expansion was triggered by more than one factor. It found that the expansion of *F. sylvatica* at both sites was co-incident with a cold and wet climatic phase and also anthropogenic indicators that increased at the same. This study also suggested that decrease in fire helped facilitate expansion.

1.4.2 Northern Central Europe

In northern Central Europe and southern Scandinavia there are numerous studies that link the establishment of the species to disturbance, especially of anthropogenic origin (felling, land-use changes etc). However this point is still contested (Gardner and Willis 1999). Magri *et al.*, (2006) showed that the species major expansion into northern central Europe occured around 5000 cal yr BP, with the species expanding in southern Scandinavia from AD 400 to present (Bradshaw and Lindbladh 2005). New studies suggest the possibility that small scattered populations existed before this period. Given the limitations of pollen analysis in detecting low abundance populations (see discussion of Bennett 1988 regarding *F. grandifolia* in Canada) it is entirely likely that small outpost population may have existed long before the main expanding front in this area.

Tinner and Lotter (2006) suggest that in addition to climatic change, human impact influenced the expansion of *F. sylvatica* in northern Central Europe. Tinner and Lotter (2006) cite the work of Ralska-Jasiewiczowa *et al.,* (2003) to suggest a climatic control on the species expansion. The northern Central European regional expansion occurred synchronously and in a period of 8.2 cal yr BP type events (1850-1450 and 750-350 BC) similar to that of southern Central Europe.

In northern central Europe, particularly Germany, Küster (1997) rules out climate and suggests the species replaces *Quercus* and other species. Küster suggests that from 5000 BC, *Quercus* dominated woodlands were cleared by prehistoric farmers to establish settlements and perform farming. Once these settlements and farmland were abandoned during shifting cultivation, a secondary phase of woodland succession could begin. The establishing species would include *Betula* and *Populus,* and then *Quercus* and *F. sylvatica* dominated woodland became established. Küster's (1997) argument is that as secondary woodland succession was a feature of semi-natural vegetation development over a long period, this had the overall effect of favouring *F. sylvatica*. Odgaard (1994) highlighted that the history of the species in Eastern Denmark shows it expands primarily in periods of low disturbance, following intervals of perturbation. A regional synthesis for southern Scandinavia (Bradshaw and Lindbladh 2005) used an approach which examined both regional and stand-scale pollen diagrams and showed that different forces acted on the species, dependent on the spatial scale examined. The study highlighted the importance of stand-scale diagrams in complementing regional pollen data when examining a species history. It was demonstrated that the establishment of *F. sylvatica* benefited greatly from anthropogenic disturbance in the region.

Gardner and Willis (1999) critiqued the argument of Küster (1997) that expansion was heavily influenced by land-use practices. Gardner and Willis argued that interplay between *F. sylvatica* and transition to agriculture is because of the rate of expansion from refugia rather than anthropogenic disturbance. Although the Gardner and Willis critique is based on four sites in Slovenia and Hungary that lie east of the main part of Küster's study region. Figure two of Küster (1997) also suggests that in montane environments the establishment was 'probably natural'.

At the northern limits of the species distribution Björkman (1996) suggests that climate cannot be the direct cause of the establishment at the local scale and that the establishment was associated with a phase of woodland clearance and the former system of land-use. However, Björkman (1996) cites the need for a temporary reduction in anthropogenic activity. Lindbladh *et al.,* (2000) presented a rate of change analysis spanning the previous 3000 years showing the changes in forest composition over the past 150 years are the most significant but the transformation was initiated 850 years ago.

As well has aiding establishment, anthropogenic activities may also have restricted the establishment if the amount of anthropogenic activity was too high. Odgaard (1994) proposed that the establishment of *F. sylvatica* in West Jutland was limited mainly by strong and continuous human impact rather than by climatic or edaphic mechanisms.

In summary the literature suggests that distinct geographical patterns exist in the pattern of the species expansion in the Holocene. Using regional pollen diagrams, anthropogenic disturbance does not appear linked to the species expansion in southern Europe and southern Central Europe. However the evidence in northern Central Europe suggests the anthropogenic land-use patterns and activities do play a significant role in the species expansion. In Denmark and southern Sweden anthropogenic disturbance has been explicitly linked to the species expansion at the stand-scale. Yet testing this idea outside this area has yet to be done because of a lack of investigations at stand-scale palaeoecological sites.

1.5 Ecological aspects of the species history

(Grime *et al.*, 1988) describes *F. sylvatica* has a deciduous tree with broad crown and smooth bark. It forms a dense canopy (Figure 1.5). It is a stress-tolerant competitor and can regenerate under dense canopy. *F. sylvatica* reproduces by seed which germinate in spring. Seed set is between September and October and the tree flowers between April and May. Bolte *et al.*, (2007) summarise the temperature and water constraints on the species occurrence in the literature. Minimum requirements are given as, precipitation rates of 500mm per year, or 250mm between May and September; July mean temperature lower than 19°C; January mean temperature above -3°C; Fewer than 141 frost days (daily minimum temperature below 0°C) and more than 217 days with a daily mean temperature of 7°C or above (Bolte *et al.*, 2007).

Figure 1.5 Photograph of *F. sylvatica* forest near Neustraliz in northern Germany. This photograph was taken on a sunny day and highlights the ability of the tree to cast intense shade beneath the canopy.

Future changes in climate may have an adverse effect on the species as it is known the species dislikes waterlogging and drought. In central Europe (Geßler *et al.*, 2007) suggest that growth may be impacted by drought that occurs in the growing season. This would cause both seedlings and mature trees to suffer from xylem embolism and consequently restricted nutrient uptake and reduced growth (Geßler *et al.*, 2007). Marginal beech populations may exhibit a better drought adaptation because of higher seed mass enabling seedling survival in the first year of drought and also root morphology and/or root growth phenology better adapted to more frequent summer droughts at the range margin (Rose *et al.*, 2009).

1.6 Summary of research challenges for *F. sylvatica*

F. sylvatica is one of the most thoroughly investigated forest trees because of its wide distribution and its economic importance (Magri 2008). There is now a wealth of ecological, palaeoecological and genetic data available in the literature. Yet there remain a number of questions that are either unanswered or answered unsatisfactorily.

Pollen diagrams from stand-scale sites in Denmark and Southern Sweden show that a disturbance event occurs prior to the onset in rise of the pollen percentages of *F. sylvatica* (Bradshaw and Lindbladh 2005). It is not known if this observation is limited to this geographic area. Bradshaw *et al.,* (2010) state that comparable observations have not been made throughout its range, because high resolution stand-scale sites have only been studied in northern Europe. Therefore there is a clear need for new stand-scale sites in new areas.

The understanding of the species migration history is also unsatisfactory. There is disagreement as to how the species moved across the continent, and whether there were any outlying founding populations. If so, to what extent and what was their location? In this instance because of the inability to study outlying populations, except by chance, data-model exercises may be useful in testing different scenarios.

The synthesis of Magri *et al.,* (2006) now means the species is primed to have aDNA studies conducted on it. The technique is largely untried in temperate areas for plant species. Methodology needs developing. Also it is not known what age the technique will work to or which sites would preserve the DNA best. Also no studies have yet taken place on the issue of contamination or the role of leaching in aDNA studies. Developing the technique so that it can aid migration studies would be a major breakthrough. Giesecke *et al.*, (2007) state the need for new insights into the role of rapid genetic adaptation.

It was after taking into consideration these findings that the research program for this thesis was established. This thesis aims to use three approaches. It will examine the controls on the stand-scale establishment of the species in the area south of Southern Scandinavia. It will also examine the species migration in two ways. A data model comparison exercise will examine seed dispersal characteristics and migration rates and compare the output with stand-scale pollen diagrams. The potential of aDNA to be used as a migration marker will be assessed.

1.7 Questions and rationale

a) What role has seed dispersal characteristics and postglacial migration rate had in the shaping of the current distribution of *F. sylvatica* and *Picea abies* in Denmark and southern Sweden?

Denmark and Southern Sweden have a unique coverage of stand-scale pollen diagrams in Europe (Bradshaw 2007). During the late Holocene the area has witnessed two invasions of new tree species. *F. sylvatica* migrated into the area from the south in a scattered pattern whilst *P. abies* migrated into the area from the north in a wave-like pattern. It has been suggested that anthropogenic activity is responsible for the spread of *F. sylvatica* as information from the individual pollen diagrams suggest disturbance is significant. However both species are known to have different dispersal characteristics and postglacial migration rates. To examine what effects these characteristics and rates have on the current distribution, a data-model comparison exercise will be performed. A cellular automata model (adapted from Bialozyt *et al.,* 2006) will be set up to simulate the spread of the two species on the landscape under natural conditions. The model output will then be compared with expansion time taken from stand-scale pollen data.

b) What role does disturbance play in the stand-scale establishment of *F. sylvatica* in Northern and Eastern Germany?

Bradshaw and Lindbladh (2005) review stand-scale sites in Denmark and southern Sweden and conclude that anthropogenic activities facilitate the expansion of *F. sylvatica* in the forest stands of this region. The question to whether this is a process unique to that area or one that occurs elsewhere requires the study of well-dated stand-scale pollen sites in other areas of Europe. A good place to start testing this question is in northern and eastern Germany as these regions are immediately south of the sites reviewed by Bradshaw and Lindbladh. A literature search of stand-scale sites show this area to be lacking in coverage compared to southern Scandinavia, although exceptions occur (Spandenburg 2008). This section of the thesis therefore will set out to gather data to find an answer to the question of whether disturbance facilitates the species expansion in these regions.

c) The use of aDNA analysis in helping understand the history of *F. sylvatica* in the Holocene.

Studies of aDNA raise several new possibilities for improving our understanding of past population processes in plants (Gugerli *et al.,* 2005). Direct testing of hypotheses regarding postglacial tree migrations and the genetic consequences of the spreading histories requires aDNA. The aim of this section was to develop a methodology that would yield higher success rates of authenticated aDNA from sites known to collect plant macrofossils over time spans stretching back into the early Holocene. Target sites were small forest hollows. It is hypothesised that these sites allow for rapid burial of macrofossils which would be better for greater preservation quality as the macrofossil is preserved intact and is denied oxygen. The initial aims were to screen the DNA for the different haplotypes of the species, which would allow the scenarios presented by Magri *et al.*, (2006) to be tested.

Chapter Two - Review of methods

2.1 Stand-scale sites

2.1.1 Overview

Small depressions situated in forests have the potential to accumulate sediments for thousands of years. If these depositional environments are not significantly disturbed and do not dry out, they will often contain a high concentration of fossil pollen grains and abundant macrofossil remains. These types of palaeoecological sites are referred to in the literature as forest hollows, small forest hollows and stand-scale sites. The hollow will receive its pollen grains from both the regional and local area (Parshall and Calcote 2001). However the small canopy gap at these types of site means much more pollen is deposited from the trees surrounding the basin and therefore the proportion of regional pollen, which the basin receives, is reduced. The macrofossils present, except in exceptional circumstances, will be sourced from the vegetation overhanging the hollow or growing on the site. Therefore these sites enable the reconstruction of the changes in forests at the stand-scale over thousands of years. Stand-scale palaeoecological analyses from small forest hollows yield detail about the invasion process as a new tree species becomes established (Bradshaw 2007). Other ecological processes and properties that can be more easily studied using stand-scale sites rather than conventional palynology include the effects of disturbance on vegetation (Bradshaw and Hannon 1992), vegetation structure (Bradshaw and Hannon 2004) and openness (Mitchell and Cole 1998). These types of sites have been reviewed previously by Bradshaw (1988; 2007).

In Europe, the majority of pollen diagrams from forest hollows are in southern Scandinavia (Bradshaw *et al.,* 2010). Denmark and Southern Sweden have now been subject to numerous stand-scale studies (Aaby 1983, Abrahamsson 1996, Andersen 1988, Andersson 1996, Axelsson 1996, Björkman 1996, Björkman 1997a, Björkman 1997b, Björkman 2001, Björkman and Bradshaw 1996, Björkman and Sjögren 2003, Eriksson 1996, Hannon *et al.,* 2000, Hultberg 2008, Lindbladh 1999, Lindbladh and Bradshaw 1998, Lindbladh *et al.,* 2003, Lindbladh *et al.,* 2007, Lindbladh *et al.,* 2007b and Valdemardotter 2001). Elsewhere, other examples in Europe include, Mitchell and Cole (1998) who used two forest hollows from Bialowieza Forest, Poland to investigate old-growth temperate woodland. Forest hollows have also been investigated in England (Bradshaw 1981, Jones pers com, Grant 2005), Finland (Clear per coms), Norway (Molinari *et al.,* 2005, Bjune *et al.,* 2009) and further north in Sweden (Bradshaw and Hannon 1992). Field surveys for forest hollows in Bulgaria found hollows but were unsuitable for study. A search in the Apennine Mountains, Italy found no hollows but sites have been found in the Dolomites (Bradshaw pers. comm.). In Alpine areas, van der Knapp (pers. comm.) has produced diagrams from small mires, surrounded by trees, and although springs feed these sites, the source area for the majority of pollen must be stand-scale. Despite a long tradition of pollen analytical work, few forest hollow records exist in Germany. A recent search by Spandenburg (2008) revealed very few (< 10) sites in the Mecklenburg-Vorporuum and Brandenburg regions.

2.1.2 Spatial Scale

The reason for using these types of site is to reconstruct stand-scale forest dynamics. With the exception of mor humus and soil profiles, no other type of depositional environment is able to do this because, in general terms, the larger the surface area of the depositional environment, the larger proportion of pollen grains derived from the regional area, thus increasing the effective pollen source area (sensu Sugita 1994) of the site and its associated pollen diagram. Understanding the source area of pollen grains has long been a problem for the subject (see review of Davis 2000). A summary of estimates of pollen source area up until 2004 is provided by Davies and Tipping (2004). At the stand-scale it is the general consensus that the site samples vegetation at a different spatial scale to that of more conventional pollen sites (Bradshaw 2007). Early work examining source area by Andersen (1970) and Bradshaw (1981) suggested a source area of approximately 20 to 30m. Calcote (1995) using a method of multiple hollows to provide information on site to site variation argued that half or more of the pollen deposited on the surface is from the regional background. Lindbladh *et al.,* (2000), in southern Sweden, showed that stand-scale sites in the region capture both the regional trend and yield site specific details about forest composition. The argument of Calcote (1995) that a large dataset of modern reference samples would improve interpretations of fossil pollen assemblages from hollows and that many more forest hollow surface samples from a wider geographic range and from different forest communities are needed appears valid.

2.1.3 Sedimentary basin

The sedimentary basins range from round to elongate in shape. Usually the slopes surrounding the basins are not too steep and any slope that does exist will be likely stabilised by the trees growing on the slopes. The basins tend to have vegetation coverage of grasses and aquatic plant species. Small trees can grow on the surface. Wetness of hollows ranges from being damp to having standing water in certain areas. The sediments in forest hollows may be mud or peat (Bradshaw 2007). The sites are often in areas of conservation so the coring process cannot be too damaging. Also too much activity on a hollow would make any future studies more difficult. The loose sediment surface and thick layers of wood that are often present can make retrieval of sediments at depth difficult.

Forest hollows, unlike lakes, do not have a protective layer of water to stop humans and animals from walking and mixing the surface layers of sediment. Anderson (1984) noted deer bathing in hollows in Denmark. Trees surrounding the basin are liable to fall into the sediments, which is good for preservation of macrofossils but bad for a consistent sedimentary deposition. The removal of sediments by peat cutting is an issue in certain areas. Hiatuses can be rather common (Bradshaw 2007). It must be concluded that in theory at least these types of sites are far from ideal sedimentary environments for palaeoecological analysis. Sadly, there is little way of knowing that the site has been interfered with before analysis begins. Instead, interrogation of the pollen diagram and age depth models should pick out any hiatus or major sediment mixing episodes. It must be noted though that despite these theoretical problems, the majority of forest hollows records have excellent sedimentary sequences and usually poor sites can be identified early during the analytical process.

2.1.4 Dating forest hollows

The first role of dating a sedimentary sequence from a hollow must be to check the integrity of the sequence. Once this has been established the dates can be used to work out sedimentation rates and give events in the pollen diagram a chronology. The most common form of dating palaeoecological records in forest hollows is using accelerator mass spectrometry (AMS) dating. Although some forest hollows are excellent at preserving macrofossils which can be used for AMS, other hollows are not. In this case, bulk sediment has to be used. Where bulk sediment has been used previously, two separate fractions have been identified, both possible to date (pers comms Beta Analytic). The first is fibrous degraded plant remains (> 180 microns). The second was silty peat (< 180 microns). The silty peat fraction may contain mobilized humic acids which could bias the age. Forest hollows often have plants growing on them so root penetration can also be a problem. For younger sediments, Higuera *et al.,* (2005) highlighted the use ^{210}Pb. They suggest that their overall interpretations should be robust to few or minor dating errors but did discuss the problems of this dating technique for these sedimentary environments. Specifically they suggest the drying of sediment in the summer months may facilitate downcore movement of ^{210}Pb and lead to a wrong young date. Work by Aaby (1983) in Draved forest, Denmark used forest records to correlate events in the pollen record to a time. Some useful sites are undated but can still be used to examine processes.

2.1.5 Testing for disturbance in the ecosystem

The role of disturbance is an important concept in forest history. A single disturbance event may be able to perturb a system into an alternative stable state, e.g. *Tilia* forest into *Fagus* forest. Bradshaw and Lindbladh (2005) showed that disturbance is a very important process in stand-scale forest dynamics of *F. sylvatica* in southern Scandinavia. Previous work has used anthropogenic indicator species to examine different types of anthropogenic activity (Behre 1981; Gaillard *et al.,* 1992). Charcoal and charred fossil remains can be used as a proxy of fire. Significant changes in forest composition after a spike in anthropogenic indicators or change in fire regime would be a good indication that disturbance has affected the ecosystem. Analysis can be aided by the method presented by Tinner and Lotter (2006) that used cross correlation to test the effect of fire and anthropogenic activity on the establishment of *F. sylvatica*. Confirmation of disturbance and their localness could be aided by the study of non-pollen palynomorphs (NPP) (Blackford pers comms).

Like pollen it is assumed that charcoal remains in forest hollows have a more local source area. However like pollen studies, charcoal source area calibrations would depend on the ecosystems, climatic regimes and anthropogenic impacts on the region. Higuera *et al.,* (2005) indicate that hollows accurately record high-severity fires but miss many moderate- and low-severity fires, at least in *Pinus*, *Pseudotsuga* and *Tsuga* forest. Higuera *et al.,* (2005) indicate that small hollow charcoal records would be most useful in forests where fires are large and intense and fire frequency is low. Examination of charcoal source areas for different basins in different types of ecosystem would be very useful information.

2.2 Data-model exercises

2.2.1 Overview

Studies that test ideas gathered from palaeoenvironmental data with models are increasing in number (see review of Anderson *et al.,* 2006). This thesis uses stand-scale sites, which have been used in a number of applications (see 1.1; 2.1), to address a variety of questions. Perhaps their least utilised application, at present, is their use in data-model comparisons. The exception is the study of Cowling *et al.,* (2001) who used a stand-scale model to assess the role of climate on Draved forest in Denmark and compared its output with data from a small forest hollow. The results of this study suggested that the dominance of *F. sylvatica* is a likely reflection of anthropogenic influence on the ecosystem from around the AD 1600's. The ability to examine the influence of anthropogenic activity alongside climate and ecosystems, particularly at the small spatial and temporal scale, is an application that models should be able to simulate (Anderson *et al.,* 2006), with these ideas being able to be tested against the palynological record at various spatial scales (Bradshaw 2008). Considering the suggestion that anthropogenic impact has had important impact on the species, models could potentially provide useful information to help tease out the relative impact of anthropogenic activity compared to a solely climatic control.

A growing number of data model comparisons exist for *F. sylvatica* at various spatial scales (Sykes *et al.,* 1996; Cowling *et al.,* 2001; Svenning and Skov 2004; Giesecke *et al.,* 2007; Magri 2007; Svenning and Skov 2007; Pearman *et al.,* 2008; Svenning *et al.,* 2008), although the majority of these studies do include the effect of anthropogenic activity. A recent review by Bradshaw (2008) discusses data-model exercises involving *F. sylvatica* in detail and discusses the potential in identifying anthropogenic control on the species.

A specific aim of this thesis is to examine what role has seed dispersal characteristics and postglacial migration rate had in the shaping of the current distribution of *F. sylvatica* and *Picea abies* in Denmark and southern Sweden. A cellular automata dispersal model (adapted from Bialozyt *et al.,* 2006) will be used to simulate the spread of the two species on the landscape under natural conditions. The model output will then be compared with expansion time taken from numerous stand-scale pollen sites (Aaby 1983; Andersen 1988; Bradshaw and Hannon 1992; Abrahamsson 1996; Axelsson 1996; Björkman 1996; Björkman and Bradshaw 1996; Eriksson 1996; Andersson 1997; Björkman 1997a; Björkman 1997b; Lindbladh and Bradshaw 1998; Lindbladh 1999; Hannon *et al.,* 2000; Björkman 2001; Valdemardotter 2001; Björkman and Sjögren 2003; Lindbladh *et al.,* 2003; Lindbladh *et al.,* 2007a; Lindbladh *et al.,* 2007b; Hultberg 2008; Eriksson unpublished; Hannon unpublished).

Within this region, information from Sykes *et al.,* (1996) who used a bioclimatic model to examine the entire European range to show that the current northern limits of *F. sylvatica* can be accounted for by a small number of physiologically well-defined characteristics is important. In particular, the parameter of an experimentally determined high chilling requirement was shown to be crucial. This finding should not be ignored as the model that will be used does not account for climate and is not dispersal limited.

The remaining parts of the thesis do not use models but information gathered by them could be of significant relevance. Pollen diagrams will be created to examine the establishment of populations (Section 1.7, aim 2) using sites that should be sensitive to early populations. Giesecke *et al.,* (2007) present simulations which suggest the species should have been as widespread 6000 years ago as it is today. One suggestion for the data-model mismatch is that the pollen data can only show that the species was not abundant 6000 years and are not able to resolve small populations. For the entire European range (Giesecke *et al.,* 2007) concluded that no single driving force could account for the observed distributional limits 6000 years ago, or the pattern of spread during the Holocene. Modeling of species dispersal would also be useful for the aDNA work. The work aims to develop the technique in migration studies. The results from the aDNA can help refine ideas about how fast a species spread by better understanding its migration route. Conversely, the potential of ultra-long distance dispersal can be examined using models to see what scenarios are potentially possible. At present testing different scenarios with a dispersal model and comparing the results with pollen data is lacking in the literature.

2.2.2 Spreading studies

Data-model exercises that examine how *F. sylvatica* spread throughout Europe in the Holocene are few in the number, which is surprising given that the migration capacity of forest species is currently a 'hot' topic (Pearson 2006), as future distributions are likely to differ from present under altered climatic conditions.

Studies that have used models to test ideas relating to the dominant control on species migration (Svenning and Skov 2004; Svenning *et al.*, 2008) compare output with distributions at present day without giving consideration to the palaeoecological record. Svenning and Skov (2004) used a model to show that European tree species (including *F. sylvatica*) have not filled their potential climatic range and that this is likely caused by dispersal limitation, although this study did not include competition between the differing tree species. Svenning and Skov (2007) argue that the large scale distribution is limited by dispersal rather than climate and that this should remain an important research focus. Svenning *et al.*, (2008) present results that they argue show a need to reconsider the equilibrium postulated as a basis for calibrating predictive species distribution models. This proposed equilibrium with climate is something that Sykes *et al.*, (1996) assume but argue the results from their study and the data of Huntley *et al.*, (1989) provide good evidence that this assumption is largely correct. Magri (2008) used a comprehensive summary of palaeoecological data to identify regions of Europe where *F. sylvatica* populations had exponentially increased their areal coverage for almost 10,000 years, until all available habitats were occupied, in a process that corresponds to a simple logistic model of population growth. At the landscape level, Collingham and Huntley (2000) showed other factors affect migration by demonstrating a significant relationship between habitat availability and migration rate, with the rate of migration being reduced in landscape composed of fewer suitable patches that were larger and more isolated from neighbouring patches.

In any data model comparison exercise examining migration of tree species, accurate information is required about where trees were first present on a landscape to compare with the model output. Examining first arrival is very difficult with palaeoecological data. Bennett (1983) using pollen accumulation rates (PAR) showed that pollen values often rise as an exponential population increase and that species probably have a period of low population before this rise begins. Bennett (1988) showed that small populations may go undetected in the pollen record. This means that cryptic populations that exist on the actual landscape are missed in pollen diagrams. This is important given the suggestion that tree species, at least in small numbers existed much further north than previously believed (Stewart and Lister 2001). In Europe, Pearson (2006) argues that more northerly populations in the glacial period may show that species can live in small pockets within regionally unsuitable climate. In Europe this has led to the idea that trees were much more northerly distributed than previously thought in the glacial in what Svenning *et al.,* (2008) term the 'northern refugia hypothesis'. These northern populations would mean that migration rates have been overestimated and may be part of the solution to paradox of rapid plant migration (McLachlan *et al.,* 2005; Clark *et al.,* 1998). At present there is no known study that compares the output of a spreading model and stand-scale pollen data.

2.3 Palaeo-genetics and forest history

2.3.1 Overview

Willerslev and Cooper (2005) begin their introduction of a review paper examining Ancient DNA by stating that, 'in the past two decades, ancient DNA research has progressed from the retrieval of small fragments of mitochondrial DNA from a few late Holocene specimens, to large-scale studies of ancient populations, phenotypically important nuclear loci, and even whole mitochondrial genome sequences of extinct species'. Gugerli *et al.,* (2005) reviewed specifically the field of ancient plant DNA and showed the subject to be still in its infancy but growing in terms of publications per year. The two contrasting reviews highlight that the study of ancient DNA in plants is still trailing far behind the study of animals and humans. Since these reviews, there have been numerous landmark papers, especially using ancient human DNA (e.g., Green *et al.,* 2010; Rasmussen *et al.,* 2010). Yet the field of ancient plant DNA, in temperate areas, is still at the stage of the retrieval of small fragments of chloroplast DNA from late Holocene species.

Obtaining the information that these small fragments of DNA contain would be very important for the subject of Holocene tree migration. Traditionally, migration studies have focussed on a series of palaeoecological sites and examined when a species first arrived. Genetic markers have also been used to examine the genetic structure of populations and infer their past distributions (McLachlan *et al.,* 2005; Provan and Bennett 2008). This information allows the construction of the kinship between populations throughout Europe (Giesecke 2007). These two types of study have now been combined for a number of tree species in Europe (Petit *et al.,* 2002; Magri *et al.,* 2006; Tollefsrud *et al.,* 2008) to better refine tree migrations. However, to test hypotheses in these studies, information about the location of different markers (haplotypes) in the past is required and therefore the technique of aDNA needs to be used. Numerous reviews of aDNA exist (Hofreiter *et al.,* 2001; Gugerli *et al.,* 2005; Willerslev and Cooper, 2005)

Obtaining useful, authentic results is difficult. Any DNA preserved would have been subjected to post mortem degradation, through the processess of hydrolysis and oxidation (Gugerli *et al.,* 2005) and attacked by fungi and bacteria (Parducci and Bennett 2005). This means that when a DNA extraction is multiplied by polymerase chain reaction (PCR) any DNA that contaminates the ancient samples will be preferably amplified leading to false positive results. This process of obtaining false positive results is very simple and therefore strict laboratory criteria to remove contamination now exist. Yet as Willerslev and Cooper (2005) note, that even when these criteria are met the results may potentially still not be authentic. Gilbert *et al.,* (2005) note that a researcher needs to be critical of any results obtained in their research.

2.3.2 aDNA and European tree species

Temperate areas must be considered far from optimal for preservation of DNA and the majority of studies have taken place in permafrost areas or very dry areas. Studies using sedimentary basins in temperate areas are rare, yet these basins provide plenty of material in which ancient DNA could potentially be preserved. This abundance of material though must be weighed against the low chance of preservation. Basic information is still required, such as the temperature that fossils experience in depositional basins and how much water is present. Published studies include aDNA extractions from wood (Dumolin-Lapègue *et al.,* 1999;

Liepelt *et al.,* 2006), pollen (Parducci *et al.,* 2005; Paffetti *et al.,* 2007) and plant remains (Pollmann *et al.,* 2005; Suyama *et al.,* 2008). The majority of the studies have focussed on developing the technique and therefore the results have been at species identification level. The exception is the study of Parducci *et al.,* (2005) who identified haplotypes of *Picea* using pollen grains from a Swedish lake. The study of Liepelt *et al.,* (2006) is the most rigorous of these studies and showed a maximum age for wood, preserved in clay, of 1000 years.

Paper	**Species**	**Material**	**Depositional Environment**	**Age**
a	*Quercus*	Dry wood from bridge pile	Alluvial deposits	AD 1203
a	*Quercus*	Dry grey wood from foundation structure	Alluvial deposits	AD 1700-1734
a	*Quercus*	Dry wood from habitation structure	Clay	AD 1715
a	*Quercus*	Dry wood from boat fragment	Lakeside sediment	AD 1550
a	*Quercus*	Dry wood from pile	Clay	AD 260
b	*Pinus sylvestris*	Pollen grains	Small Lake	AD 1850
b	*Pinus sylvestris*	Pollen grains	Small Lake	8050 BC
b	*Picea abies*	Pollen grains	Small Lake	AD 1850
b	*Pinus sylvestris*	Pollen grains	Small Lake	AD 1850
b	*Pinus sylvestris*	Pollen grains	Small Lake	8050 BC
c	*Fagus orientalis*	Pollen grains	50m in a marine record	43 050 BC
d	*Abies*	Wood	Ice cave	AD 1550
d	*Fagus sylvatica*	Wood	Clay Sediment	AD 950
d	*Abies*	Wood	Waterlogged	AD 1650
e	*Prunus*	Fruit stones	Waterlogged	~ AD 1 - 300
e	*Prunus*	Fruit stones	Waterlogged	~ AD 1 - 300

Table 2.1 Overview of authentic sequences, from temperate areas, published at the beginning of the PhD research. (a) Deguilloux *et al.*, (2006); (b) Parducci *et al.*, (2005); (c) Paffetti *et al.*, (2007); (d) Liepelt *et al.*, (2006) and (e) Pollmann *et al.*, (2005).

In temperate areas of central Europe any tree with that has its modern population structure mapped could act as a target species for study. Before ancient DNA can be utilised it is important that a species' present day genetic distribution is relatively well understood. This is important for two reasons. Firstly to generate data to highlight where migration is still poorly understood and therefore pinpoint where ancient DNA can be utilised. Secondly the primers that search for specific parts of the DNA are developed in modern samples.

Brewer (2002) states the need for a genetic marker which reflects the history of the taxon, for example the maternally inherited chloroplast DNA (cpDNA) in deciduous oaks. cpDNA and mtDNA are preferred targets over nuclear DNA (nDNA) because they contain multiple copies (Gugerli *et al.,* 2005). It is these markers that have been used to examine the migration of *F. sylvatica* (Magri *et al.,* 2006). The comparative rarity of the changes in the cpDNA structure means that the cpDNA will only differ in populations that have been isolated during a sufficiently long period (Brewer 2002).

Target depositional environments could be potentially any that are used in current palaeoecological studies. At present it is not possible to suggest if terrestrial, lacustrine or marine environments would have more optimal preservation environments. The presence of water is theoretically bad which makes the finding reported by Pollmann *et al.,* (2005) of aDNA in fruit stones in waterlogged conditions surprising. Although this may highlight the species specific conditions that maybe required as the *Prunus* fruit stones used in this study contain hydrophobic lignified tissue that stops microbial attack. Also the pH was slightly alkaline (Pollmann *et al.,* 2005).

Three target materials could be used to potentially extract DNA. These are sediment, pollen and plant macrofossils. Gugerli *et al.,* (2005) highlight that plant DNA can come from wood, tissue of fruits or seeds, herbarium specimens, fossilised plant remains, in faeces as secondary components or even absorbed to sediment after leakage from their original cellular source. Haile *et al.*, (2007) summarise that providing methodological challenges can be overcome, aDNA from non-frozen environments could prove to be a powerful tool in the reconstruction of past environments. Success with extractions from pollen grains have been reported (Suyama *et al.*, 1996; Parducci *et al.*, 2005). Theoretically the pollen grain is a good idea as the walls are extremely resistant to chemical or physical attack (Parducci *et al.*, 2005) but the threat of contamination is high (Gugerli *et al.*, 2005). Parducci *et al.*, (2005) report success with 10,000 year old pollen grains in Boreal lake sediment but with a success rate of less than 2%. Paffetti *et al.*, (2007) report success from a 45,000 year old pollen sample taken from marine sediment. With a few exceptions, most aDNA studies have used wood. Liepelt *et al.*, (2006) argue that wood should be the preferred material as it can be large enough to be split to send to more than one laboratory. Deguilloux *et al.*, (2006) report a success rate around 10% with wood, although this work did not take place in two separate laboratories. The maximum age of recovery was AD 260. Dumolin-Lapègue *et al.*, (1999) report success with a 600 year old wood remain. It should be considered that the longevity of certain tree species may mean that DNA of present populations may be much more useful, and easier to obtain, than very recent aDNA sequences.

2.4 Summary

In summary, forest hollows are able to reconstruct the history of forest stands over time scales of thousands of years. Providing the sedimentary sequence is not disturbed too greatly these environments provide excellent preservation for fossil pollen, plant macrofossils and charcoal. These sites are ideally suited to test ideas regarding the impact of disturbance on forest ecosystems (Question 2). Their local pollen signal means they should be able to detect the first arrival of a tree species, close to the site, better than more traditional sites. In Europe a very good network of sites documenting the history of *F. sylvatica* exist in Denmark and southern Sweden and this region can be used to test ideas about migration (Question 1). The excellent preservation of macrofossils in an anoxic environment means they are potentially good targets to obtain ancient DNA sequences from (Question 3).

Chapter Three - Project overview and methods

This chapter has two purposes. The first is to give an overview of the specifics of the research and introduce the different sites used in the thesis. Secondly, as the result chapters in this thesis are written in a concise manner for publication, this chapter also explains the basic methodologies employed in this thesis. The exception to this is the data-model comparison exercise (chapter four) which contains a lengthy methods section.

3.1 Project overview and site selection

All sites (Figure 3.1), including those used in the data-model exercise, were selected because they can be defined as stand-scale. The only deviation from this is Lago Pratigano. This site was used in the aDNA analysis because it has a very good macrofossil record for *F. sylvatica* (Hannon pers. comm.), in a geographically important region for the species (Vettori *et al.,* 2004) with regard to the European genetic structure (Magri *et al.,* 2006). Lago Pratigano has an elongated basin and the topography surrounding the basin means trees are likely to fall directly into the basin. Detailed site descriptions for each site are provided in the chapters in which the sites are used.

The research was designed so that all sites were to contribute macrofossils to the ancient DNA project. However, for new sites (Carlshof, Dammendorf and Pine Hollows), this was dependent on the quality of the record. All three sites produced good pollen, charcoal and loss on ignition (LOI) records but no *F. sylvatica* macrofossils. Therefore macrofossils for the aDNA project were only collected from Suserup, Muritz and Lago Pratigano. An attempt was made to use a small forest hollow in Bulgaria but was abandoned because the stratigraphic integrity of the site could not be confirmed.

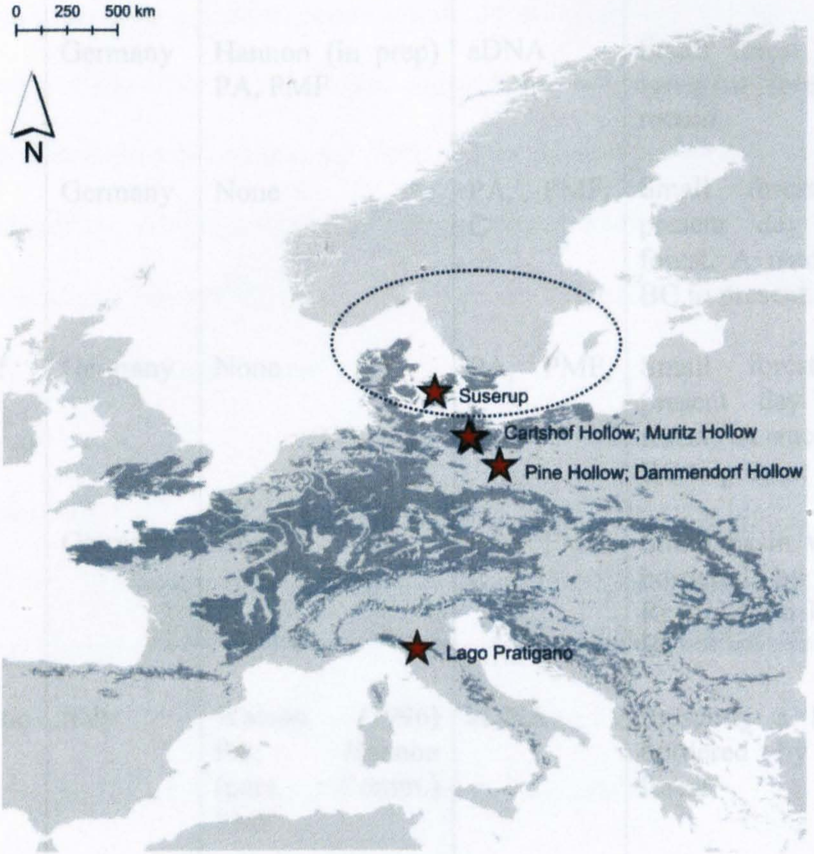

Figure 3.1 Sites used in this thesis. The ellipse indicates the area where the data-model comparison project was performed. Dark grey shading indicates areas where of *F. sylvatica* would be dominant under natural conditions in Europe (Bohn *et al.*, 2000).

Chapters five and six report new forest hollow records. Before fieldwork, potential new areas were assessed using Google Earth and maps. The new sites were found after field survey. Pine hollow and Dammendorf hollow were discovered in August 2006. Carlshof hollow was discovered in September 2007. The previously worked sites were revisited and re-sampled; these have been subjected to various palaeoecological investigations by other authors (Table 3.1).

Site	Location	Previous work	Work in this thesis	Description of site
Suserup	Denmark	Hannon *et al.*, 2000. PA, PMF, C	aDNA	Small forest hollow in *F. sylvatica* forest. 6000 year record
Muritz	Germany	Hannon (in prep) PA, PMF	aDNA	Small forest hollow in *F. sylvatica* forest. 3000 year record.
Carlshof Hollow	Germany	None	PA, PMF, C	Small forest hollow in present day *F. sylvatica* forest. A record from 9000 BC to present.
Dammendorf Hollow	Germany	None	PA, PMF, C	Small forest hollow in present day *F. sylvatica* forest. A record from 500 BC to present
Pine Hollow	Germany	None	PA, PMF, C	Small basin which is today bordered by *F. sylvatica.* Record from 11, 000 to 7000 BC
Lago Pratigano	Italy	Watson (1996) PA; Hannon (pers. Comm.) PMF	aDNA	Presently a lake which is bordered by *F. sylvatica* forest

Table 3.1 Overview of primary data collection sites used in this thesis. aDNA – ancient DNA; C – Charcoal analysis; PA – Pollen analysis; PMF – Plant macrofossil analysis.

3.2 Methods

3.2.1 Coring

The exact location of all core sites in the sedimentary basin was determined by preliminary cores to determine where the deepest record was and also by where it was physically possible to take a core. Further consideration had to be given not to disturb the basin and sedimentary environment too greatly. This was especially important at Muritz which is in a sensitive nature reserve.

All sites were cored using a Russian corer (Jowsey 1966). Type and diameter were dependent on the sediments at the different sites. Basic sedimentary descriptions were performed in the field. The cores were then placed in drain pipes and wrapped in plastic bags for transportation back to the laboratory. All cores were frozen on return to the laboratory with the exception of Carlshof which was stored at 7°C. The primary reason for freezing cores was to help preserve any DNA that might have been present.

3.2.2 Sub-sampling

The three new sites were cleaned after removal from storage before being sliced into 1cm thick contiguous samples. Whilst they were worked on they were stored at 7°C. Before pollen and charcoal preparation began a known volume of sediment was taken. Measurement was made using volumetric displacement.

The three sites that were used for the aDNA analysis remained frozen until the day they were analysed for macrofossil content. After they defrosted they were cleaned by removing the sediments on the outside.

3.2.3 Pollen analysis

Pollen preparation followed standard protocol (Berglund & Ralska-Jasiewiczowa 1986; Moore *et al.,* 1991). A known volume of sediment was spiked with *Lycopodium* spores

(Stockmarr 1971) in heated water. Then a treatment of 10% Hydrochloric acid (HCl), followed by 10% Sodium Hydroxide (NaOH) was performed before the sample was passed through a 100μm sieve. The remains too large to pass through the mesh were retained for inspection under a binocular microscope. The finer residue was collected and then treated with 10% Sodium Pyrophosphate and 40% Hydrofluoric Acid (HF) before acetolysis was performed to remove soluble cellulose. Samples were returned to neutral pH before being treated with Ethanol and subsequently dehydrated with Tertiary Butyl Alcohol (TBA). All samples were suspended in silicone oil.

Pollen counts were made using magnification x400 – 1000. Analysis was guided by the identification key in Moore *et al.,* (1991), and pollen photographs in Reille (1992). A Scandinavian pollen guide was also consulted (Erdtman *et al.,* 1961). The University of Liverpool's pollen reference collection was also used.

Pollen counts were made to a minimum pollen sum of 400 grains. The pollen sum at the sites excludes *Lycopodium annotinum*, *Polypodium*, monolete spores, *Sphagnum*, *Typha*, *Potamogeton* and *Pteridium*.

In the late Holocene both natural and cultivated species belonging to the Poaceae family are theoretically likely in the pollen rain. The guidelines for differentiation proposed by Andersen (1979) are adopted. Poaceae is therefore divided into four groups based on annulus diameter, mean pollen size (largest diameter + diameter 90° to largest/2) and sculpturing. The guidelines are based on silicone oil mounts and therefore no conversion is needed as the fossil pollen in this study also were mounted in silicone oil.

	Annulus Diameter	Mean Pore Size	Sculpture
Wild Grass Group	<8um	<37um	Scabrate or verrucate
Hordeum Group	8-10um	32-45um	Scabrate
Avena-Triticum Group	>10um	>40um	Verrucate
Secale cereale	8-10um	large oblong	Scabrate

Table 3.2 Details of criteria for differentiation of Poaceae pollen (adapted from Andersen 1979)

The location of the study area also means that the occurrence of *Zea mais* is a possibility. Fægri and Iversen (1989) quote a value for mean grain size of >60µm but this is based on slides prepared in glycerol. Faegri and Iversen also quote a conversion factor from silicone to glycerol of 1.2, conversely a grain of any size > 50µm must be considered and checked against reference material.

3.2.4 aDNA extraction

Before plant macrofossils were taken from the sediment cores, work areas were treated with bleach before any laboratory worked was conducted. The cores were then visually inspected and visible macrofossils were picked. Sections of sediment were then sieved using only distilled water. Each separate macrofossil was placed in an air tight container then frozen.

All extractions took place in the ancient DNA laboratory at Copenhagen. All the protocols to reduce and eliminate laboratory contamination of the samples were observed. Extractions took place outside the flowering period of *F. sylvatica*. Frozen macrofossils were cleaned by scraping the edges off using a disposable scalpel. They were then placed in a fast prep soil tube. Extraction of any DNA from the macrofossil used the Sergey Bulat extraction protocol (Bulat *et al.*, 2000). After the addition of Qiagen PB buffer, 10% of the solution was removed and archived at -20 °C to allow re-extraction in case of errors/contamination at later stages of the analysis. Specific PCR conditions and primers used are detailed in results chapter. Cloning was performed on successful PCR amplifications. After sequencing results were compared against published results in GenBank (http://www.ncbi.nlm.nih.gov/Genbank/).

3.2.5 Charcoal analysis

Charcoal analysis at Dammendorf Hollow, Pine Hollow and Carlshof Hollow (sections 330-430cm) were carried out on each contiguous 1cm sample. After measurement by volumetric displacement samples were sieved at 300µm. Before sieving the Pine Hollow samples the sediment was soaked in hot NaOH for 10 minutes to aid sediment disaggregation. Particles larger than 300µm were retained and added to 80ml water. This was mixed using a mechanical stirrer before a 20ml sample was taken by pipette for analysis. The tip of the pipette was enlarged so that no size bias was observed. Samples were counted in a Petri dish and all charcoal particles were counted. The sample was analysed under a binocular microscope using magnification x10. Charcoal was defined as black objects with angular sides with the larger pieces appearing crystalline. Examination of macro-charcoal (>300 µm) alone was performed

3.2.6 Loss on ignition

Loss on ignition (LOI) analysis was performed on every 1cm contiguous sample at Pine, Dammendorf and Carlshof Hollow. Weighed samples were heated at 450°C for 4 hours. Twenty samples at Carlshof were inspected for carbonate. These were heated at 850°C for 30 minutes.

3.2.7 Dating

All dates presented in this thesis were produced by Accelerator Mass Spectrometry (AMS). Before shipment, samples were cleaned and dried at 95°C for 5 hours except samples sent to the NERC laboratory. All radiocarbon ages were calibrated in Bcal (Buck *et al.,* 1999; http://bcal.sheffield.ac.uk) using the 2004 atmospheric curve (Reimer *et al.,* 2004). Age-depth models were constructed in PSIMPOLL v4.26 (Bennett 2007). Specific details of pretreatment methods for each date are given in chapter 5 for Dammendorf Hollow and chapter 6 for Carlshof small forest hollow.

3.2.8 Data presentation and analysis

PSIMPOLL v4.26 (Bennett 2007) was used to display stratigraphic data. PSIMPOLL was also used to provide zonation to the pollen diagrams. Canoco 4.5 (ter Braak & Smilaver, 2002) was used to perform principal component analysis (PCA) on the data. Arc GIS was used to produce maps in the thesis. All diagrams and maps were improved visually using Coral Draw X4.

Chapter Four - Modelling the spread of *Fagus sylvatica* and *Picea abies* in southern Scandinavia during the late Holocene

4.1 Introduction

Migration and range adjustment have been convincingly proposed as the response of tree species to climatic change since the Last Glacial Maximum in Europe and North America (Huntley and Birks 1983; Williams *et al.,* 2004). Yet concern has been expressed as to whether inferred potential migration rates for forest trees are sufficiently rapid to track forecasted climatic changes during the present century (Davis and Shaw 2001; Hu *et al.,* 2009). There is also continued debate over whether current species distributions are in quasi-equilibrium with climate (Webb 1986; Huntley and Webb 1989; Sykes *et al.,* 1996), or lag behind changing climates because of relatively slow biological spreading rates (Davies *et al.,* 1986; Woods and Davies 1989; Higgins and Richardson 1999; Svenning and Skov 2004; 2007). Improved understanding of the rate and process of species spread would be an important contribution to this debate.

Past migration rates for trees ranging from hundreds to thousands of metres per year have been estimated from palaeoecological data (Huntley and Birks 1983; Delcourt and Delcourt 1991). The validity of these migration rates has been questioned for two major reasons. Firstly, the incorporation of genetic studies using molecular markers, with palaeoecological data, has led to significant reinterpretation of both refugia location and migration pathways previously derived solely from palaeoecological data (Petit *et al.,* 2002; McLachlan *et al.,* 2005; Anderson *et al.,* 2006; Magri *et al.,* 2006; Provan and Bennett 2008; Tollefsrud *et al.,* 2008). Secondly, the collection of new fossil evidence has shown that refugia existed in areas previously thought to be devoid of tree species (Kullman 1998; 2008; Willis *et al.,* 2000). The combination of these two factors has led to a significant downscaling in the estimates of how fast species have spread since the start of the Holocene.

However these revised estimates are still faster than modern day observations of spreading rates; a situation described as Reid's Paradox (Clark *et al.,* 1998). Long distance dispersal (LDD) of seeds has been suggested to play an important role in resolving Reid's Paradox (Clark *et al.,* 1998). Clark *et al.,* (2001; 2003) demonstrated that altering the frequency and distance of LDD events had a major influence on calculated migration rate. Le Corre *et al.*, (1997) systematically tested the influence of the frequency and extent of LDD events on the speed of colonisation, but a rapid spreading rate was still needed to resolve Reid's Paradox. Clark *et al.*, (2001) warned that spreading velocities predicted from mean dispersal distances (i.e. diffusion) were qualitatively and quantitatively inaccurate.

The understanding of past vegetation dynamics has been improved by data-model comparisons (Austerlitz and Garnier-Gere 2003; Bialozyt *et al.,* 2006). Modelling different parameters controlling tree spreading and comparing them through time with palaeoecological data would allow further insights into the validity of migration rates and the role of LDD. Pollen and plant macrofossil analyses provide the appropriate data about past tree migration. However pollen data from lakes and peat deposits may not sensitively detect small outlying populations (Bennett 1988). Small forest hollows have been shown by both theory and observation to be more sensitive to local vegetation than the traditionally studied sedimentary basins and such sites should be able to record small, founding populations (Bradshaw 2007).

In Denmark and southern Sweden there is now a comprehensive palaeoecological data set derived from small forest hollows (Björkman 1999; Lindbladh *et al.,* 2000; Bradshaw and Lindbladh 2005). The majority of these hollows have a record for immigration of *Fagus sylvatica* and *Picea abies.*

During the late Holocene, *F. sylvatica* spread into southern Scandinavia from the south, whereas *P. abies* spread from the north (Bradshaw and Lindbladh 2005). The current dynamics of the northern European margins of the range of *F. sylvatica* are predominantly controlled by anthropogenic activity (Bradshaw *et al.,* 2010); however the timing and establishment pattern of the founding populations are still debated (Giesecke *et al.,* 2007). Magri (2008) used a comprehensive summary of palaeoecological data to identify regions of Europe where *F. sylvatica* populations had exponentially increased their areal coverage for almost 10,000 years, until all available habitat was occupied. The author argued that biological processes dominated the species' spreading history with climate and anthropogenic activity being of secondary importance. *P. abies* spread in a wave-like manner into western Fennoscandia and is believed to have been largely uninfluenced by anthropogenic activity, expect for recent planting (Giesecke and Bennett 2004; Giesecke 2005; Seppä *et al.,* 2009).

In this paper we test the hypothesis that the spread of *F. sylvatica* and *P. abies* into southern Scandinavia during the late Holocene can be accounted for by a simple diffusion process. We compare stand-scale pollen data with the output of a modified diffusion model incorporating LDD dispersal events (Bialozyt *et al.,* 2006). We examine the spreading parameters that best explain the observed immigration of *F. sylvatica* and *P. abies.* We critically evaluate how the species spread in the area and discuss implications for future migrations of the two species.

4.2 Material and Methods

4.2.1 Pollen Data

Selection of pollen sites followed two criteria. The sites should be of stand-scale (Bradshaw 2007) and independently dated. Where possible the original pollen count data were examined. We used the empirical limits to estimate arrival time of each species at a site (Smith and Pilcher 1973). The empirical limit for a taxon is defined as the point in time when its pollen first becomes recorded in every consecutive sample regardless of the percentages it exhibits. Macrofossil studies have shown that *F. sylvatica* and *P. abies* may be present even when the pollen curve is discontinuous, so the empirical limit at a stand-scale site may underestimate first arrival time (Hannon *et al.,* 2010).

4.2.2 Model

A modified version of the two-dimensional cellular automata (CA) model presented by Bialozyt *et al.,* (2006) is used. The model was organised as a regularly spaced grid representing the landscape (Figure 1). The extent of the simulation area was 940x730 km with each cell having an area of 5x5 km. All grid cells were equal in respect to their state space, neighbourhood template and transition function. The state space of a cell was defined by the demographic processes of the occupying populations, either of *F. sylvatica*, *P. abies* or both species simultaneously. Each cell had a defined population size (carrying capacity (k)). The cells changed their states according to the transition function, which was dependent on their own state and the state of the cells in the neighbourhood defined by dispersal parameters. The population dynamic within any one cell has been modelled using a logistic growth process of both species with a joint carrying capacity (Equation 1),

$$dN_S = r_S * N_S * \left(1 - \frac{N_S + N_B}{K}\right) + I_S$$

EQUATION ONE

$$dN_B = r_B * N_B * \left(1 - \frac{N_S + N_B}{K}\right) + I_B$$

where, N_B and N_S were the population size of *F. sylvatica* and *P. abies* respectively, r_B and r_S the population based growth rates of *F. sylvatica* and *P. abies* and K is the joint carrying capacity within one cell. The parameters I_S and I_B are the immigrant seeds from outside the cell.

Both a localized and a long distance dispersal (LDD) component were incorporated into the model for each species at each time-step to model the species spread. We used a combination of two normal distributions with two very different standard deviations according to Ibrahim *et al.,* (1996). The classical Moore neighbourhood (Adamatzky 1994; Wolfram 1994), in which only the eight adjacent cells derive seeds from the central cell was used to model local dispersal. The values of the dispersal matrix for local dispersal were obtained using a small standard deviation (SD1). The proportion of SD1 to the size of the grid yielded the probability of dispersing seeds into the neighbouring cells.

It was necessary to use an individual-based approach to be able to follow the fate of each separate seed to model LDD. For this component, the neighbourhood was enlarged to include distances up to 100 cells away depending on the value of a second standard deviation (SD2). Dispersal probabilities were obtained for all cells whose cumulative probability represented at least 99% of the distribution. Four look-up tables were obtained, one for each type of dispersal and species.

The parameters of all simulations are listed in table 4.1. One time step corresponds to the time needed to reach sexual maturity, which was assumed to be 50 years for both species.

Parameters	*F. sylvatica*	*P. abies*
Time step/generation time (years)	50	50
Colonisation speed (m/yr)	50, 100, 200, 300	150, 250, 350, 500
SD2 of LDD (km)	50, 100, 150, 200, 250	50, 100, 150, 200, 250
Doubling time (years)	200	150
Population growth rate	1.19	1.26
Maximum age (years)	300	400
Death rate	0.1	0.1

Table 4.1 The main parameters used in the simulation. Several values for the speed and the distance of LDD events (SD2) are given, because all these combinations were used for simulation.

The carrying capacity (K) was set to 1000 when most of the grid cell was covered by land and 0 otherwise. To mimic the mountains of Norway, the region was assigned a carrying capacity of 1 which meant only a very limited amount of dispersal could occur into these areas.

Some initial assumptions were made in order to generate the simplest possible model. Inter-specific competition was modelled by competition for space only; the model simulated overlapping generations; time was discrete and one time step corresponded to the time needed to reach sexual maturity; population growth within each cell was density-regulated; the death rate was constant and independent of age and species, hence there was no senescence and every seed produced in this simulation could potentially become a mature tree, except those which leave the simulation area across the border. Hence neither specific germination rates nor seedling mortality rates were considered.

The population dynamics within each cell depended on population growth rate and size as well as on local seed production, seed dispersal and immigration of seeds (Equation 1). Therefore the dynamics were regulated in each cell by five processes: (a) within a cell, trees died according to a uniform, random Markovian process, in which only the current state of the population was taken into account; (b) population growth per cell was deterministic and modelled by a logistic function (characterized by the carrying capacity K and the growth rate r). Applying this function yields the number of seeds produced. Seeds that move to a new cell, either because of local dispersal or LDD, are removed from the total of those produced locally; (c) for local dispersal, seeds were collected from the Moore neighbourhood corresponding to the local population sizes; (d) the number of seeds that were dispersed through LDD was calculated according to parameter (a) and the local population size of that species only. The individual seeds were added to a new cell within the CA according to the LDD look-up table of that species; (e) within one cell, the number of seeds originating from the two dispersal functions were added together. These seeds became the new trees within the next time step. Hence, a new cell could be colonised in two ways. Either a seed produced in the directly adjacent cell entered the cell through local dispersal or a seed colonises the cell through a LDD event.

The border of the CA is defined empty. This means that no seeds came from outside the simulation area. In our specific landscape most borders lie on water and there were no seeds entering the area across these borders. Additional seed sources only lay to the north-east and south and these seeds would only affect dynamics at the beginning of the simulation. To start the simulation, *F. sylvatica* needed to be present at the southern margin of the modelled area and *P. abies* present in the north. Data for location and time were taken from regional pollen studies (Giesecke and Bennett 2004; Magri *et al.,* 2006). These cells were completely filled with trees at carrying capacity. The remaining cells of the simulation area were empty.

A variety of migration speeds were investigated. Values between 50 to 300 m/yr were used for *F. sylvatica* and values between 150 to 500 m/yr were used for *P. abies.* In order to find the appropriate parameters for a given colonisation speed, a rectangular simulation area of size 270x6500 km^2 with all cells as habitat (k=1000) was used. At the start, the first two lines of cells at the northern limit of this area were occupied by trees. We then followed the colonisation of this area and calculated the speed of colonization at each time step. If the speed settled into a roughly constant value, we used this for determination of our model parameters. Because two parameters are responsible for the final coloniation speed i.e. the distance and the frequency of the LDD events, we used different distances for LDD (Table 4.1) and adjusted the frequency to obtain the desired speed. Several speeds have been tested (Table 4.1) in order to find the best solution for each species alone and for both in common (Figure 4.2).

4.2.3 Data-model Comparison Method

During the simulation we recorded the first arrival of the species at each of our 24 sites. These data were stored in specific files for later analysis using the software 'R' (R Development Core Team

2009). We calculated the mean squared distance from each simulated arrival time of a species to the arrival time of that species estimated from the pollen data to obtain estimates of the quality of the 150 simulations for each parameter combination (distance and frequency of LDD) displayed as box plots. The average from all sites (divided by a constant) gives a figure for the accuracy of each simulation in replicating the observed pattern. A value of zero would indicate a perfect match.

4.3 Results

4.3.1 Species arrival dates

Twenty-four sites were selected for comparison with the model (Table 4.2). Three of those sites are located in Denmark and the remaining twenty-one are distributed across southern Sweden (Figure 4. 1). Eighteen sites have a record of immigration for both species; three have a record for just *F. sylvatica* and three a record for just *P. abies.* Storasjö records both species but *P. abies* extends to the base of the profile in very small quantities and therefore it is likely have a slightly older colonization date then listed in table 4.2.

The dates for *F. sylvatica* show a scattered pattern of arrival. The arrival at Draved is dated to AD 1650, much later than at the two other Danish sites (Glyceria and Suserup). The species arrives especially early at Eriksberg on the south-eastern Swedish coast, about 1050 BC according to our criteria. The variability in the arrival of this species is best highlighted at sites Ekenäs and Skärsgölarna where 1750 years separates its detection, even though these sites are only 10km apart.

The dates presented for *P. abies* in contrast show a less scattered pattern of arrival with a general trend of a later arrival date the further south the site (Bradshaw and Lindbladh 2005). Sites that are anomalous to this pattern are Ekenäs which has a late arrival time, Asa and Osaby, which have very early arrival times and are geographically central in the study area.

Figure 4.1 Location of the stand-scale sites in Denmark and Sweden used in this study. Distributional limits for *Fagus* and *Picea* forests according to Lindquist (1931, 1959) (after Björkman 1996*)*.

ID	Site Name	Reference	Location	*F. sylvatica*	*P. abies*
1	Draved section 365:2	Aaby (1983)	55° 01'N 08 58'E	AD 1650	AD 1950
2	Glyceria (Naesbyholm)	Andersen (1988)	55° 21'N 11 36'E	1100 BC	none
3	Suserup	Hannon *et al.*, (2000)	55° 22'N 11 34'E	1300 BC	none
4	Torup forest	Hultberg *et al.* (2010)	55°56’N 13°21’E	200 BC	AD 1700
5	Fulltofta	Eriksson (unpublished)	55°43'N 13°38'E	650 BC	AD 1550
6	Häggenäs	Lindbladh *et al.*, (2007)	55°53'N 13°36'E	AD 1050	AD 1500
7	Kyllingahus	Lindbladh *et al.*, (2007)	55°53'N 13°39'E	AD 200	AD 1850
8	Kullaberg	Björkman (2001)	56°18'N 12°30'E	AD 600	AD 1950
9	Eriksberg	Hannon (unpublished)	56°11'N 15°00'E	1050 BC	None
10	Västragylet Peat	Björkman and Sjögren (2003)	56°21'N 14°53'E	AD 400	AD 1550
11	Siggaboda	Björkman and Bradshaw (1996)	56° 28'N 14°34'E	110 BC	AD 1200
12	Djäknabygd	Lindbladh and Bradshaw (1998)	56°37'N 14°12'E	AD 650	AD 1500
13	Kalvaberget	Lindbladh *et al.*, (2008)	56°48'N 12°54'E	AD 400	AD 950
14	Osaby out-field	Lindbladh (1999)	56°46'N 14°47'E	300 BC	1000 BC
15	Bocksten a	Björkman (1997a)	57°07'N 12°34'E	AD 50	AD 700
16	Flahult	Björkman (1997b)	56°58'N 13°50'E	AD 1150	AD 1250
17	Storasjö	Eriksson (1996)	56°55'N 15°17'E	AD 800	AD 700
18	Ekenäs	Valdemardotter (2001)	56°57'N 16°01'E	AD 1800	AD 1200
19	Skärsgölarna	Lindbladh *et al.*, (2003)	57°01'N 16°07'E	AD 50	AD 600
20	Asa	Andersson (1996)	57°08'N 14°47'E	150 BC	AD 50
21	Bohult	Axelsson (1996)	57°14'N 16°10'E	none	AD 350
22	Mattarp	Björkman (1996)	57°29'N 14°37'E	AD 700	AD 750
23	Ryfors	Abrahamsson (1996)	57°55'N 13°50'E	none	AD 800
24	Fiby	Bradshaw and Hannon (1992)	59°53'N 17°21'E	none	650 BC

Table 4.2 Site details and species arrival times estimated from pollen data using the empirical limit (see text for details).

4.3.2 Varying colonisation speed of the two species

Altering the average spreading rate had a significant impact on the quality of the data-model comparison (Figure 4.2). Variation in the deviation statistic for *F. sylvatica* arrival times ranged between 78 and 254 and although a better data-model match was achieved for *P. abies* (52.5), the variation in the range is much larger with values between 52.5 and 368.

The data-model comparison for *F. sylvatica* arrival times showed a general pattern of an increase in deviation as the spreading speed for *F. sylvatica* declines (Figure 4.2). The results are largely stable until an apparent threshold is met at 100 m/yr. Speeds below this value show a sharp increase in the deviation statistic. Altering the spreading speed of *P. abies* has little effect on the deviation except when *F. sylvatica* is set below 100 m/yr, when a reduction in the *P. abies* speed reduces the deviation, although it is still high compared to values above the 100 m/yr threshold for *F. sylvatica.*

The same pattern in the results is exhibited for matching the *P. abies* arrival times, only with a greater range in the deviation. Figure 4.2 shows a much steeper increase in the gradient of the deviations as the spreading rate of *P. abies* declines. *F. sylvatica* migration speeds have less of an effect on the deviation than *P. abies* migration speed, although they exert a stronger influence on the overall results compared with *P. abies* on the *F. sylvatica* arrival times. *F. sylvatica* values below 250 m/yr keep the overall deviation low except when *P. abies* values are low, although once *P. abies* drops below 250 m/yr the deviation increases sharply regardless of the speed for *F. sylvatica.*

A clear optimum to match the arrival times exists at migration rates of 100 m/yr for *F. sylvatica* and 250 m/yr for *P. abies.* Although a possibility exists to obtain a better result for *P. abies* alone, changing the spreading speeds in either direction results in a clear deterioration in the simulation results when both species are analysed.

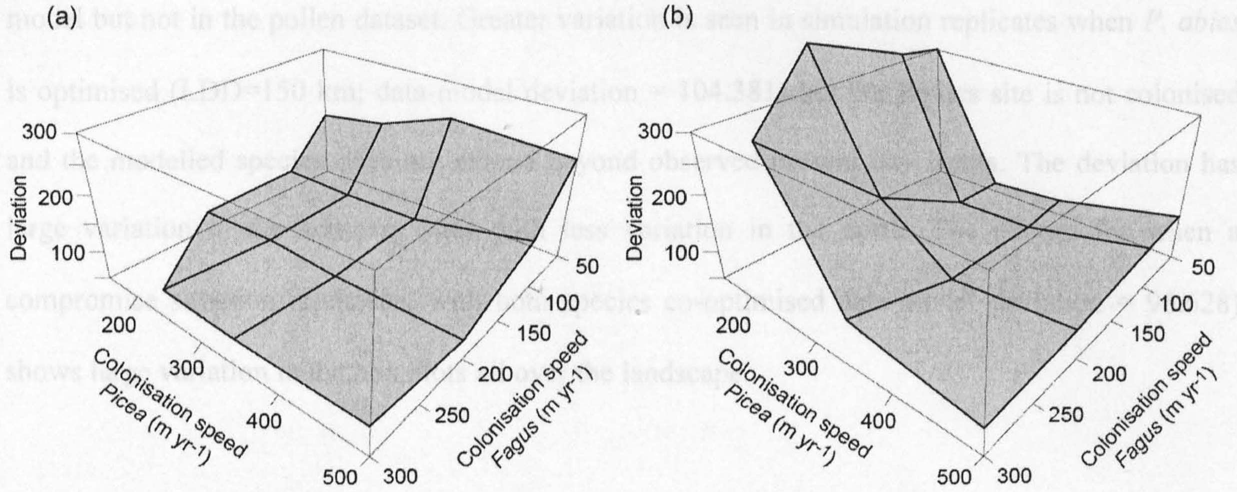

Figure 4.2 Deviation of the simulation results from the palaeoecological record. Every point in the surface represents the average over all tested parameter combinations for the chosen speeds for *F. sylvatica* and *P. abies* respectively.

4.3.3 Varying parameters of LDD

For this part of the analysis spreading rates were set at the optimum values of 100 m yr^{-1} for *F. sylvatica* and 250 m/yr for *P. abies*. The general pattern shown by the surface plots differs between species. The *F. sylvatica* slope rises as the LDD component increases (Figure 4.3) whilst the *P. abies* data-model deviations increase as the LDD component decreases (Figure 4.4). Each species deviation is largely determined by the LDD component of that species and small LDD variations cause a response in data-model deviation for both species (Figure 4.3).

For *F. sylvatica* arrival times the range of the deviation is between 77.1 and 147.7 (Figure 4.3). The surface shows a smooth rise in deviation as LDD of *F. sylvatica* increases. Altering the LDD component of *P. abies* has little effect on the overall surface plot except for slight variation when the values of *F. sylvatica* are high. The model fails to match the scattered arrival dates regardless of which species is optimised. Optimising *F. sylvatica* (LDD=50 km) produces box plots that are narrow (data-model deviation = 77.084), however the Bohult and Ryfors sites are colonised in the model but not in the pollen dataset. Greater variation is seen in simulation replicates when *P. abies* is optimised (LDD=150 km; data-model deviation = 104.381) but the Ryfors site is not colonised and the modelled species does not extend beyond observed present day limits. The deviation has large variation in the southern sites with less variation in the north. The pattern for when a compromise situation is chosen, with both species co-optimised data-model deviation = 96.628) shows large variation in the box plots all over the landscape.

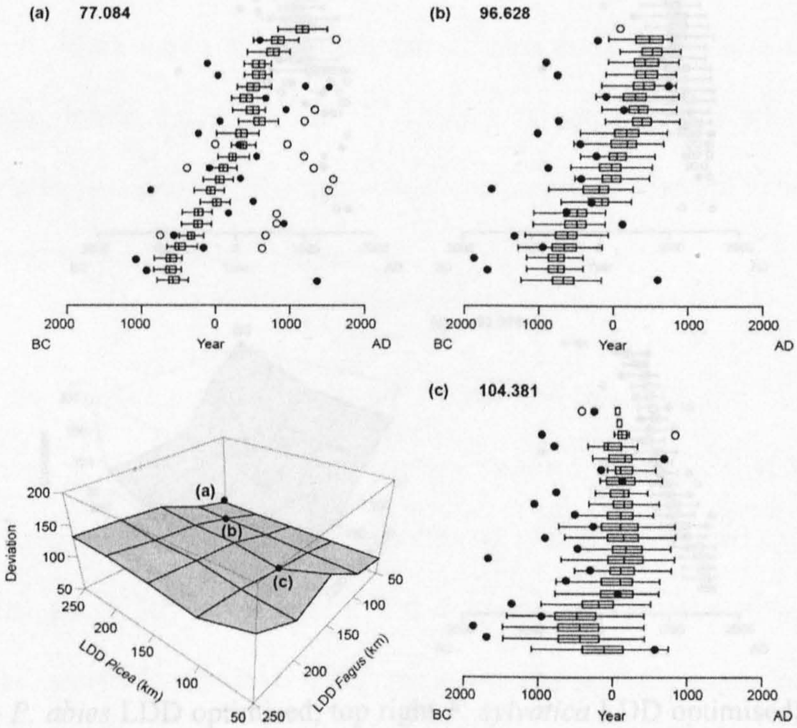

Figure 4.3 Fitting landscape and individual site results for *F. sylvatica*. The boxplots represent the variation within the 150 repeated simulations. The filled dots indicate the species arrival time estimated from the pollen data. The sites are arranged in the order of table 4.2 with site number 1 at the base. The letters in the surface plot indicate the values of the boxplots. The surface shows the average value obtained for all possible parameter combinations.

The range of the data-model deviation is reduced to values between 53.1 and 206.7 for *P. abies* arrival times (Figure 4.4). Even with LDD optimised for *P. abies* alone (LDD = 100 km), the smallest deviation is only 16 units lower than when both species are co-optimised. Deviation is minimal when *F. sylvatica* LDD ranges between 150 to 250 and *P. abies* LDD ranges from 50 to 200. The three box plots show the model to be a good match to the data when *P. abies* and both species are optimised but less successful when just *F. sylvatica* is optimised (LDD = 50 km; data-model deviation = 206.733). In the model *P. abies* is able to migrate to all sites except when *F. sylvatica* is optimised, in which case modelled *P. abies* fails to colonise half of the southern sites.

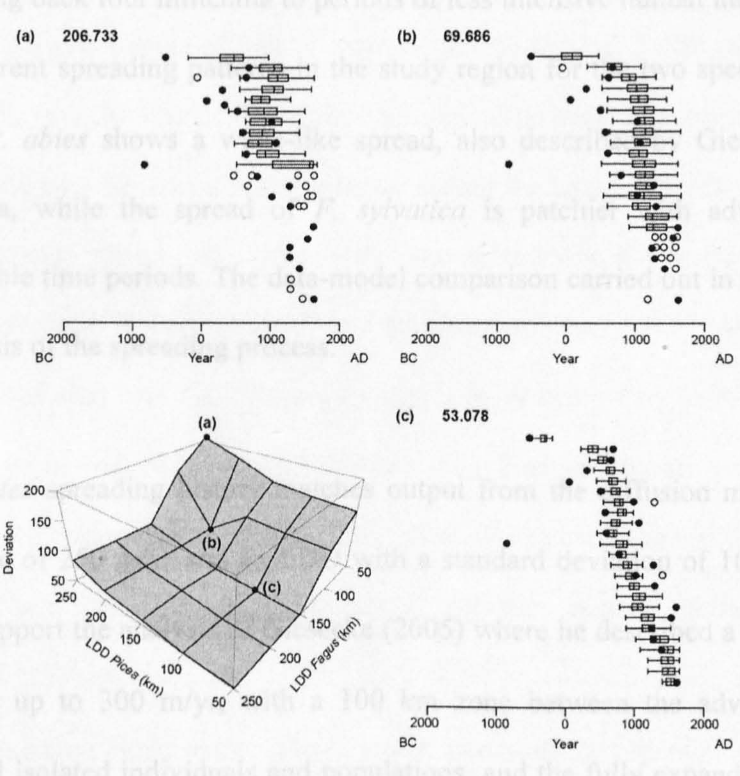

Figure 4.4 Left – *P. abies* LDD optimised; top right *F. sylvatica* LDD optimised; lower right, both species LDD co-optimised.

4.4 Discussion

The present distributions of *F. sylvatica* and *P. abies* in southern Scandinavia are influenced by anthropogenic activities, which complicate their interpretation in terms of current migration processes or relationships with climate change. *F. sylvatica* has most likely experienced recent range reduction due to historical exploitation (Björkman and Bradshaw 1996), while *P. abies* has expanded its range, particularly in Denmark through plantation (Bradshaw *et al.,* 2000). The palaeoecological data from the small hollows however have documented the past spread of these two species extending back four millennia to periods of less intensive human intervention. The data have indicated different spreading patterns in the study region for the two species (Bradshaw and Lindbladh 2005). *P. abies* shows a wave-like spread, also described by Giesecke (2005) using regional pollen data, while the spread of *F. sylvatica* is patchier with advanced populations establishing at variable time periods. The data-model comparison carried out in this study permits a more detailed analysis of the spreading process.

The observed *P. abies* spreading history matches output from the diffusion model rather closely with a spreading rate of 250 m/yr and an LDD with a standard deviation of 100 km (Figure 4.4). These parameters support the analysis of Giesecke (2005) where he described a wave of expanding populations moving up to 300 m/yr, with a 100 km zone between the advancing wave-front, represented by small isolated individuals and populations, and the fully expanded population zone covering all suitable habitat. Giesecke (2005) studied four sites in central Sweden north of the present study area, but the spread of *P. abies* appears to have continued to the south in a very similar manner. In both these data-model comparison exercises there was no need to invoke background climatic change or altered life cycle characteristics to model the observations, which covered a 4000 year period. This suggests that long-term, significant changes in *P. abies* distribution have occurred independently of climate change, which has important implications for forecasting the future dynamics of *P. abies* distributions using climate-driven vegetation models (e.g. Bradshaw *et al.,* 2000; Koca *et al.,* 2006).

The data-model match for *F. sylvatica* is poor compared with *P. Abies,* with the majority of the observed arrival dates lying outside the range of the modelled boxplots even when the dispersal parameters are optimised for *F. sylvatica* (Figure 4.3). The observed arrival times are both older and younger than the model results, suggesting that the major factors controlling arrival and population expansion are not related to simple dispersal properties. Magri (2008) reported that the area occupied by *F. sylvatica* throughout Europe exponentially increased for 10 000 years after the last glaciation and then showed a reduced rate of increase during the last 3 500 years. She interpreted this sequence as indicative of "the multiplicative biological process of population increase" of *F. sylvatica* until suitable sites became a limiting factor during the last 3 500 years (Magri 2008). Our analysis covers this latter period and confirms that the spread was not in the form of a "diffuse moving front" as described by Magri (2008) and suitable sites may have been limiting. Previous studies have suggested that site disturbance through fire or temporary cultivation are possible mechanisms that have helped *F. sylvatica* become established in northern parts of its European range (Küster 1997; Bradshaw and Lindbladh 2005). It would be useful to test the Magri hypothesis further by applying our model to more southern *F. sylvatica* regions earlier in the Holocene.

The data-model comparison exercise also allows an evaluation of the potential interactions between these two dominant species in determining spreading rates. One could hypothesise that when these two species begin to overlap in distribution the slower moving species (*F. sylvatica*) will be disadvantaged. The simulations do not suggest this to be the case as the modelled spreading rates are most sensitive to the spreading rate of the species under study (Figure 4.2) and the modelled *F. sylvatica* populations often increase their rate of site colonisation with time (Figure 4.3), although the fit to the data is poor. There is a greater effect with the LDD component where model output for

P. abies changes significantly when *F. sylvatica* LDD falls below 100 km while remaining insensitive to alterations in *P. abies* LDD (Figure 4.4). *F. sylvatica* spreading, conversely, is sensitive to variation in its own LDD (Figure 4.3). Thus *P. abies* spread is most sensitive to rate of movement, while *F. sylvatica* is more sensitive to its ability to establish colonies in advance of the main migrating front. The overall analyses however indicate little change in spreading properties as the species' ranges overlap, suggesting little direct competition between *P. abies* and *F. sylvatica.* This novel and possibly unexpected conclusion is one benefit of data-model comparison. Individual pollen diagrams and dynamic vegetation simulations have been inconclusive over the outcome of *P. abies* - *F. sylvatica* interactions (Björkman and Bradshaw 1996; Sykes and Prentice 1996)

The model suggests an effective migration rate of 250m/yr for *P. abies* which is broadly consistent with Giesecke (2005) but is only half of the 500m/yr proposed by Huntley and Birks (1983) for Fennoscandia calculated from the distance covered from presumed glacial refugia. The location and nature of glacial refugia (Kullman 2008; Provan and Bennett 2008), 'cryptic' refugia inferred from modern genetic structure (Petit *et al., 2002*; McLachlan 2005; Hu *et al.,* 2009) and the importance of long distance founding events in the early Holocene (M Petersen unpublished data) all influence apparent migration rates.

It is important to model potential inter-specific interactions and incorporate several species into estimation of colonisation abilities and rates. In some cases the establishment of one species is necessary prior to the colonisation of another species, as seen in the example of the fire-induced colonisation of *F.sylvatica* (Björkman 1997; Bradshaw 2004). Our findings indicate that an estimation of dispersal abilities of one species on the basis of fossil remains, like pollen or macrofossils, is only valid in a tabula-rasa scenario. However species did sequentially colonise northern Europe, such that the pattern of a colonising species may be well shaped by the distribution of the already established species (Seppä *et al.,* 2009).

4.5 Conclusions

Our analyses suggest that the late Holocene spread of *P. abies* in Scandinavia was rather rapid and was only limited by biological processes of dispersal, while that of *F. sylvatica* was limited by other factors most likely controlled by site properties. *P. abies* has maintained a rapid and constant rate of spread throughout the last 4000 years at least, despite significant changes in climate. There is uncertainty about the precise relationships between *P. abies* and climate in Scandinavia (Miller *et al.,* 2008), so future conditions are not easy to forecast. For *F. sylvatica* in Scandinavia, site quality has been a limiting factor, so future land use is more likely to dictate its distribution dynamics than at least moderate climatic change.

Note

Significant parts of this manuscript will be submitted for peer-review publication in a paper led by Ronald Bialozyt. Ronald led the modelling section presented in this manuscript and drafted the model section of the material and methods.

Chapter Five - Examining *Fagus sylvatica* forest dynamics at different spatial scales in the Schlaubetal Valley, Brandenburg, Germany

5.1 Introduction

Detailed studies of forest dynamics on long timescales can be made at different spatial scales using pollen analysis. The use of a combined approach with investigation at both regional and stand scales in the same area generates a greater insight into vegetation history (Berglund 1986). The larger site can highlight the history of the wider landscape cover and the forces that drive vegetation change. The larger site also gives a context to the smaller sites, which allow insights into the local factors that drive forest dynamics at the stand-scale. Previous studies have used this approach to decipher fine scale differences in landscape changes through time (Bradshaw 1981; Heide 1984; Björkman and Sjögren 2003). Another approach is to examine more than one stand-scale site in the same area. This allows insights into how different forest stands in close proximity behave (Björkman 1997; Sugita *et al.,* 2006; Lindbladh *et al.*, 2008). The time-consuming nature of pollen analysis means the potential for studies combining both approaches in close proximity (<10km) is limited, although see de Klerk (2002).

The spatial scale represented in any pollen diagram is largely dependent on two factors. The first is the size of the basin from which the sediment core is extracted (Jacobson and Bradshaw 1981; Sugita 1994). The second is the type and pattern of the vegetation around the site (Sugita 1994; Bunting *et al.,* 2004). In a forested landscape a pollen diagram produced from a small to medium sized lake will be dominated by pollen coming from within a few kilometres of the site. The lake surface although receiving pollen from vegetation close to the edge of the lake receives proportionally more pollen grains from the vegetation of the region. In contrast palaeoecological work from closed canopy forest hollows provides records of vegetation history at the forest stand-scale (Bradshaw 1988; Sugita 1994). The hollow, like the lake, will receive pollen grains from both the regional and local area (Parshall and Calcote 2001). However the small canopy gap at these types of site means much more pollen is deposited from the trees surrounding the basin and therefore the regional signal is suppressed. When combined with local records of fire history (Higuera *et al.*, 2005) and macrofossil analysis (e.g. Hannon *et al.*, 2000) these types of site record the history of a forest stand in detail. This scale of study provides insights into the mechanisms of vegetation change such as succession and disturbance.

The history of the species *Fagus sylvatica* L. has been studied at a variety of spatial scales (Bradshaw *et al.*, 2010). The species at present has a trans-European distribution (Jalas and Suominen 1972-99). It is a shade tolerant competitor on a wide range of soil types but avoids extreme dry and wet areas (Ellenberg 1996). Moisture availability is important for germination and seedlings are easily killed by drought or late frost (Watt 1923). The species history is of particular interest as it established relatively late in many parts of Europe to become the dominant species over a large area (Magri *et al.*, 2006). The reasons for this late establishment are still much debated (Huntley *et al.*, 1989; Pott 2000; Tinner and Lotter 2006; Giesecke *et al.*, 2007; Magri 2008). As a consequence of its late expansion the species invaded a landscape already covered by forest. A large number of studies conducted at the stand-scale in southern Sweden and Denmark (Björkman 1999; Bradshaw and Lindbladh 2005) have shown that the rise in *F. sylvatica* pollen, and thus the trees abundance, occurred immediately after a disturbance event. It has been argued that anthropogenic activity has heavily influenced the species throughout north Central Europe (Küster 1997). In particular *F. sylvatica* benefited from the clearance of *Tilia* (Behre 1988). In this situation the species was able to become dominant once a cultivated area had been abandoned (Turner 1962).

The relationship between anthropogenic activities and the establishment of *F. sylvatica* is not always clear however, especially in southern Europe (Valsecchi *et al.,* 2008). The species also expanded late in areas such as Slovenia and Hungary even though a significant history of anthropogenic activity is known for these areas before the expansion (Gardner and Willis 1999). Further stand-scale records in different areas are needed to help decipher the role of disturbance in the species history.

In southern Sweden, where *F. sylvatica* is at the edge of its distribution the species populations expanded during the last 2000 years ago (Bradshaw and Lindbladh 2005). Here the species loses its dominance and may be near its physiological limits (Björkman 1996). It is of interest to see if the same processes take place in areas where the species is also restricted, but with a different combination of limiting climate parameters and a longer history. The Schlaubetal in south-east Brandenburg is an area where the species is not dominant but has a history of around 5000 years (Giesecke 2001).

A pollen diagram from a forest hollow is presented in this paper and is used to investigate stand-scale forest dynamics in the Schlaubetal (Figure 5.1). The new diagram is then compared to a diagram from a medium sized lake (Giesecke, 2001). We examine how different vegetation changes are sensed at the contrasting sites and investigate the controls on the establishment of *F. sylvatica* at the stand-scale. Palynomorph nomenclature follows the rules of the European Pollen Database. Radiocarbon ages are presented as calibrated calendar (BC/AD) years to aid comparison with historical documents.

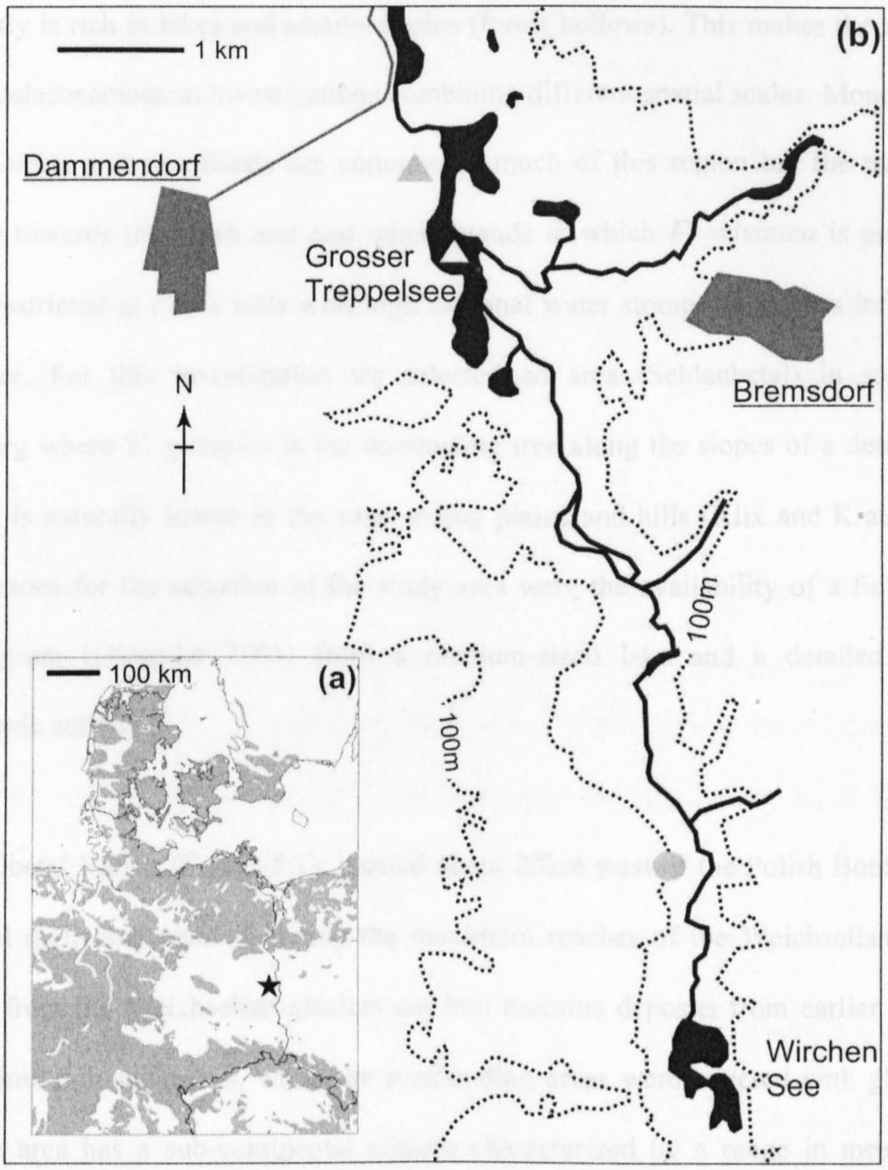

Figure 5.1 Location and details of the study area. (a) North-eastern Germany and bordering countries; grey shading highlights the location of potentially dominating *F. sylvatica* forests (Bohn *et al.,* 2000); black star - study area. (b) Overview of Schlaube Valley. Solid black - water. Solid grey - settlement. Dashed line - 100m contour line. Underscored text - settlement names. Unformatted text - water bodies. White triangle - core location from Grosser Treppelsee. Grey triangle - Dammendorf Hollow. Grey circle - Pine Hollow.

5.1.1. Regional overview and site selection

North-eastern Germany was covered by continental ice during the last glaciation and consequently is rich in lakes and smaller basins (forest hollows). This makes the area ideally suited for palaeoecological investigations combining different spatial scales. Monospecific *F. sylvatica* forests and woodlands are common in much of this region but the tree loses its dominance towards the south and east where stands in which *F. sylvatica* is prevalent are generally restricted to richer soils with high seasonal water storage or to sites influenced by groundwater. For this investigation we selected an area (Schlaubetal) in south-eastern Brandenburg where *F. sylvatica* is the dominating tree along the slopes of a deeply-incised valley, but is naturally scarce in the surrounding plains and hills (Klix and Krausch 1958). Further reasons for the selection of the study area were the availability of a full Holocene pollen diagram (Giesecke 2001) from a medium-sized lake and a detailed record of anthropogenic activity.

The Schlaubetal Valley (Figure 5.1), located about 20km west of the Polish Border, formed as a glacial meltwater channel during the maximum reaches of the Weichselian ice sheet. Meltwater from the Weichselian glaciers cut into moraine deposits from earlier glaciations exposing lime-rich sediments, while the surrounding areas were covered with glaciofluvial sands. The area has a sub-continental climate characterized by a range in mean monthly temperature of about 20°C whilst the mean annual precipitation recorded at surrounding weather stations varies between 507 and 568 mm (Meteorologischer Dienst der DDR 1978). The potential natural vegetation within 30 km of the study area is a species-poor, mixed *Quercus* forest that is locally co-dominated by *Pinus sylvestris* with an admixture of *Carpinus betulus* and *Tilia cordata* (Scamoni 1960; Krausch 1966). The present landscape is characterized by *Pinus* dominated woodlands and forests in between extensive fields, while the Schlaubetal itself is a nature reserve and almost completely forest covered.

Using detailed topographical maps and local knowledge, a number of potential small basins were visited within the valley and two sites were selected for coring. Dammendorf Hollow (52°09N 14°26E, 83 m a.s.l) is a small basin (20 x 20m) and at the time of core extraction was a closed canopy site. It is situated about 200m from the eastern shore of the north basin of Grosser Treppelsee. It is currently surrounded by a mature forest dominated by *F. sylvatica*, which gives way to *Quercus*, *Carpinus* and *Pinus* along the rising slope east of the hollow.

A full Holocene pollen diagram was constructed from Grosser Treppelsee (Giesecke 2001). This lake with three aligned basins is situated in the middle of the Schlaube Valley and the pollen diagram was obtained from a sediment core extracted from the middle basin. The Schlaube, the major stream in the valley, flows through the lake and the basin also receives surface water run-off through the Planfliess draining the area east of the lake. Thus unlike the forest hollow, the lake receives additional pollen through the transport by water. The middle basin of Grosser Treppelsee has a diameter of 500m and its pollen diagram thus portrays vegetation change of the wider area.

5.2 Material and methods

5.2.1 Sampling and sediment analyses

Sediments from Dammendorf Hollow were collected using a Russian corer (Jowsey 1966) in August 2006. The core was cleaned to remove possible contamination from the field extraction process before being visually described. It was sliced into 1cm thick segments and stored at 7°C prior to sub-sampling. Loss on ignition was carried out on each contiguous sample from the core to help determine organic matter content. At each depth approximately 1 gram of sediment was heated to 450°C for 4 hours.

A 27.80m long core was obtained from the deepest part of the middle basin of Grosser Treppelsee in April 1998 using a rod-operated piston corer (Usinger corer, Mingram *et al.*, 2007). Detailed core descriptions and results of sediment analysis have been presented elsewhere (Schonfelder *et al.,* 2000; Giesecke 2001).

Sample preparation for pollen analyses at all three sites followed standard methods (Berglund and Ralska-Jasiewiczowa 1986; Moore *et al.*, 1991) and counts were made using magnification 400 – 1000x. Analysis was guided by the identification key in Moore *et al.*, (1991), and pollen photographs in Reille (1992). The University of Liverpool's pollen reference collection was also used for the Dammendorf site. At Dammendorf Hollow the differentiation of the Poaceae family into natural and cultivated species follows Andersen (1979). Pollen proportions were expressed as the sum of terrestrial pollen and spores excluding *Sphagnum* and aquatics. *Lycopodium* tablets were added to a known volume of each sample in the pollen preparation to calculate fossil pollen concentration (Stockmarr

1971). Macrofossil analysis of the forest hollow core focused on depths of interest for dating, but also included material encountered during the pollen preparation procedure.

Charcoal analysis on each contiguous samples for material from Dammendorf was adapted from a method presented in Molinari *et al.,* (2005) using approximately 2cm^3 of sample measured by volumetric displacement. Sediments from Dammendorf Hollow easily disintegrated without chemical treatment. The samples were sieved at 300μm and the fragments that remained on the mesh were added to 80ml water. After stirring, a 20ml subsample was taken by pipette for analysis in a Petri dish. The sample was analysed under a binocular microscope using magnification 10x. Charcoal was defined as black objects with angular sides with the larger pieces appearing crystalline. Examination of macro-charcoal alone was performed to investigate the occurrence of local fire.

5.2.2 Sediment age determination

At Dammendorf Hollow, four levels were submitted for radiocarbon age determination by accelerator mass spectrometry (AMS). At Grosser Treppelsee terrestrial plant remains from six levels were dated using the AMS technique. All radiocarbon ages were calibrated in Bcal (Buck *et al.,* 1999; http://bcal.sheffield.ac.uk) using the 2004 atmospheric curve (Reimer *et al.,* 2004). Pre-treatment of the Dammendorf Hollow samples at the AMS laboratory analysis included a hot acid wash before treatment with alkali and acid to remove both carbonates and humic acids. The removal of rootlets was also carried out on the sediment samples.

Age-depth models were constructed in PSIMPOLL v4.26 (Bennett 2007) using the weighted averages of the calibrated probability distributions. At the hollow, the age-depth model was constructed using linear interpolation between dates assuming an age of AD 2006 for the core top (Figure 2). The age depth relationship for Grosser Treppelsee presented in Schonfelder *et al.*, (2000) uses a four term polynominal with an age of AD 1998 for the sediment-water interface.

5.2.3 Numerical analysis

Numerical analyses were carried out in order to assist in the visual interpretation and to detect and quantify differences between pollen diagrams from the forest hollow and the lake. To assess temporal changes at individual sites, pollen diagrams were statistically zoned in PSIMPOLL v4.26 (Bennett 2007) using optimal splitting by information content after square root transformation. Difference diagrams were constructed to visualise the similarities and differences in pollen percentages from the forest hollow compared with the percentages from the lake diagram. The stand-scale sites were subtracted from Grosser Treppelsee so positive percentages indicate a greater presence at the regional site and negative percentages indicate a greater presence at the forest hollow. Confidence intervals were computed on individual datasets in PSIMPOLL v4.26 (Bennett 2007) using the modification of Maher's lognormal distributions to assess the effect of errors on the calculation of the differences.

5.3 Results

5.3.1 Sediment analysis and chronologies

Dammendorf Hollow spans the most recent 2500 years, the sediments showed no signs of mixing and the full length of the core was used in the analysis. The dates obtained from the site were in stratigraphic order. The age-depth model (Figure 5.2) from the site shows an increase in sedimentation rate towards the top of the profile.

Depth (cm from the surface)	Lab Number	Material Dated	Reported ^{14}C age BP	Calibrated age (2 sigma range)
20 -21	Beta-243687	Bulk sediment	220 +/- 40	AD 1654 - 1732
36 -37	Beta-243688	Bulk sediment	460 +/- 40	AD 1428 - 1464
49 -50	Beta-243689	Bulk sediment	1250 +/- 40	AD 735 - 793
75 -76	Beta-238149	Unidentified wood	2110 +/- 40	167 - 99 BC

Table 5.1 Radiocarbon dates from Dammendorf Hollow.

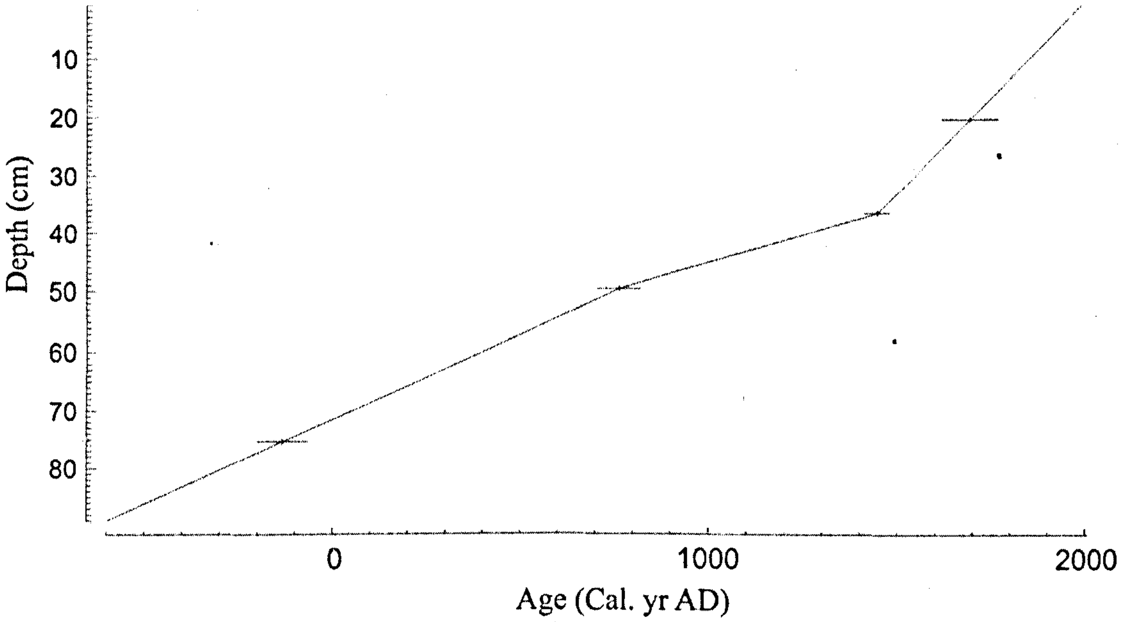

Figure 5.2 Age-depth model from Dammendorf Hollow

The sediment sequence analysed from Dammendorf Hollow extends from around 600 - 700 BC to present, although below 100 BC the age-depth model is not constrained. Macrofossil preservation is very poor throughout the core. Visual inspection of the core showed it to be composed of homogeneous organic sediment; however LOI analysis revealed systematic variation. From the base of the profile to AD 400, organic matter accounts for less than 30% of the analysed samples. Residue from sieving during pollen preparation suggests that sand deposition is responsible for the low LOI values. Charcoal is present in most samples between AD 400 – 1600 with a peak around AD 1200. The most recent 400 years of the profile are characterised by lower pollen and charcoal concentrations.

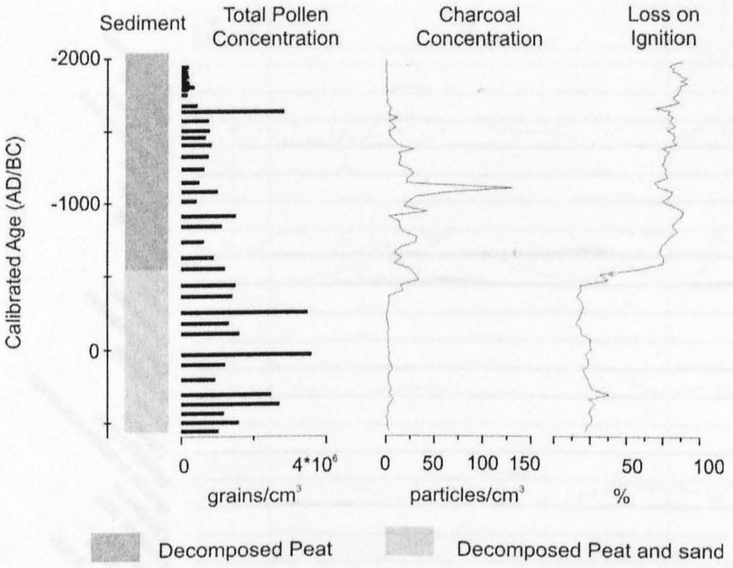

Figure 5.3 Sediment descriptions, total pollen and charcoal concentrations and loss on ignition from Dammendorf Hollow.

Dammendorf Hollow

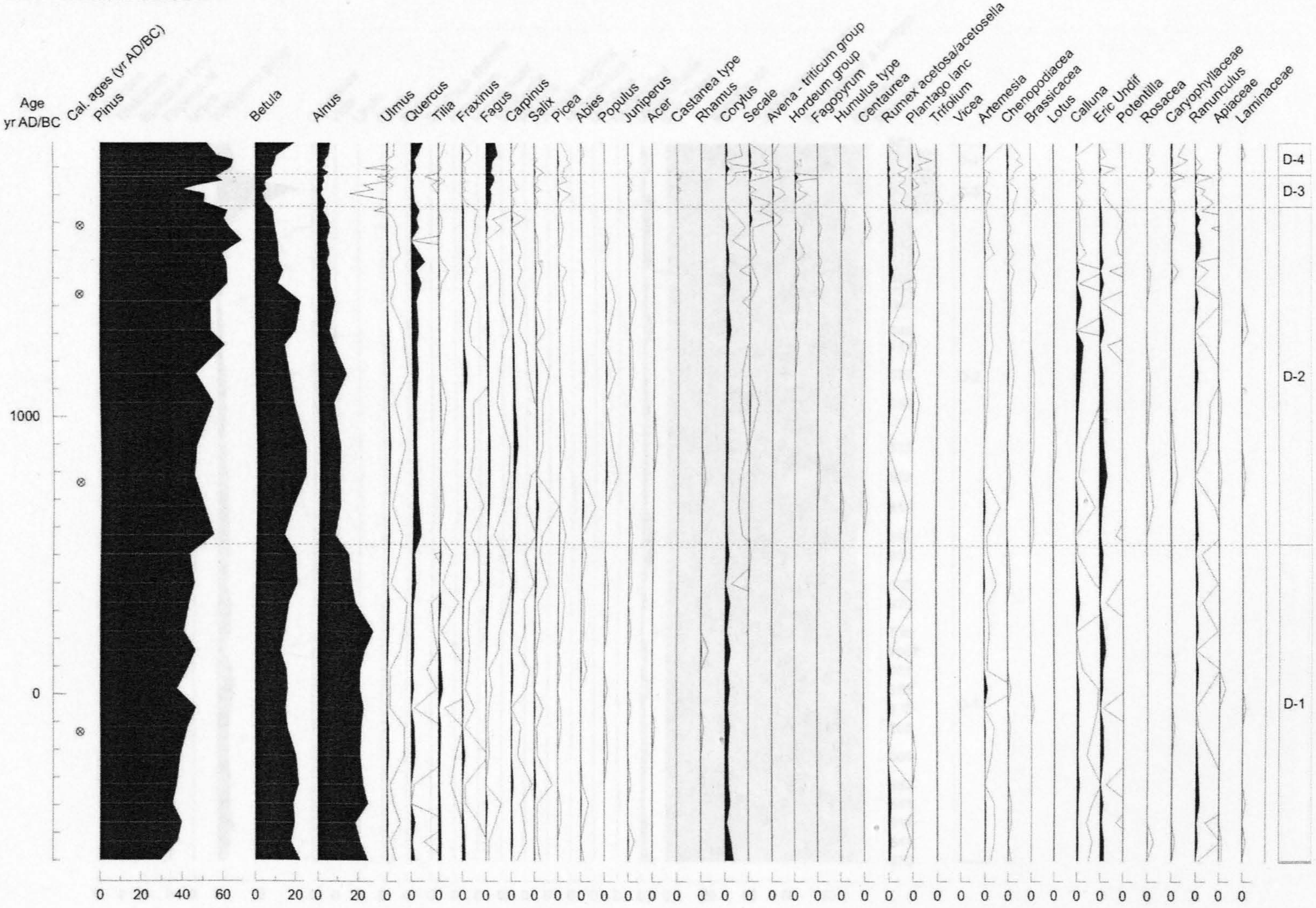

Figure 5.4 Pollen Percentage Diagram from Dammendorf Hollow

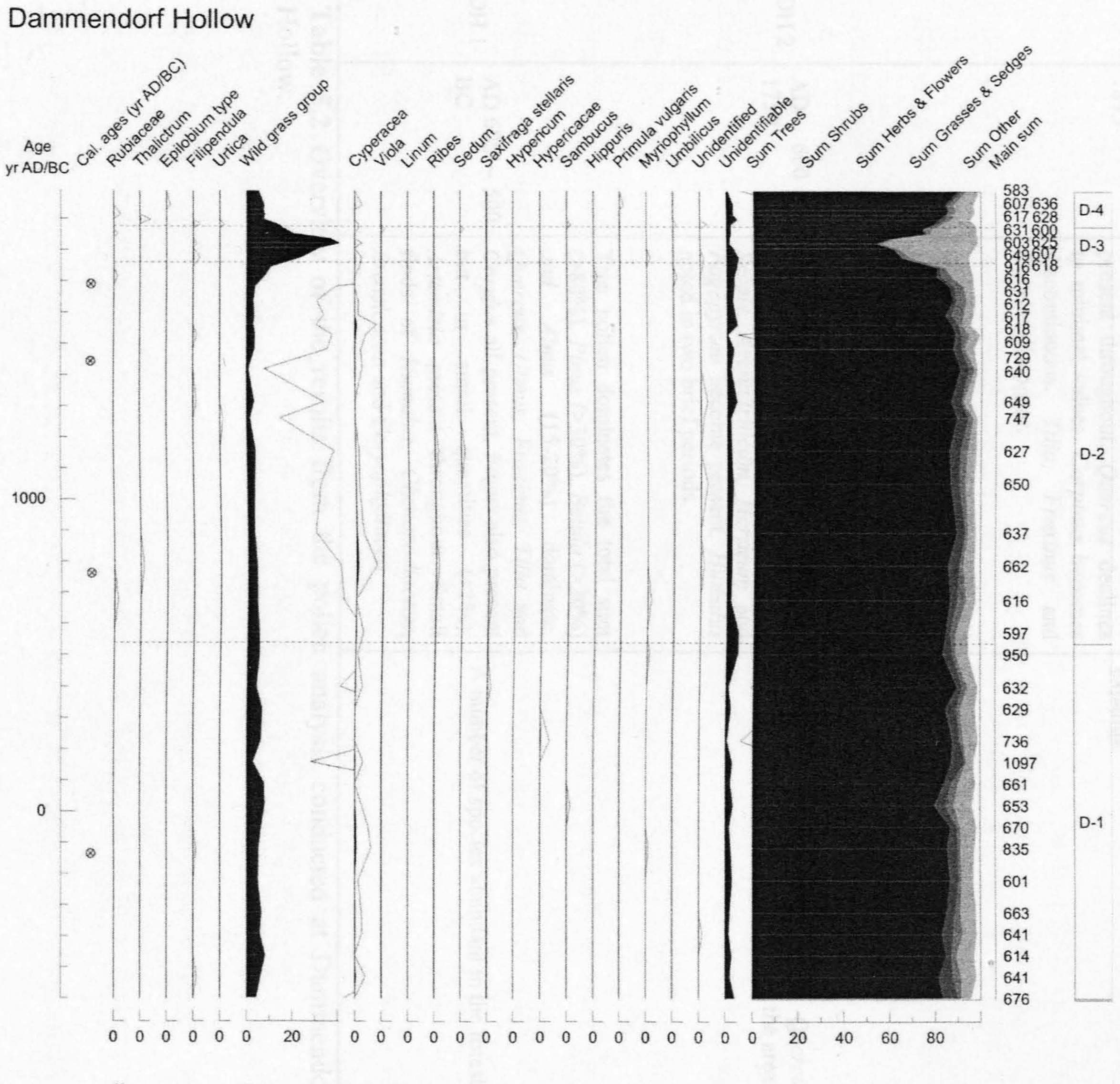

Figure 5.4 (continued)

Zone	AD/BC	Results	Interpretation
DH 4	AD 1875 – Present	Tree pollen rises from 75 to 90% of total sum. *Pinus* dominant. *Betula* increases by 10%. *Fagus* continues to rise to 5%. *Quercus* rises gently. *Tilia* and *Corylus* return after absence. Poaceae returns to pre-peak levels. Anthropogenic indicators present throughout, notably *Secale*, *Plantago lanceolata*, *Chenopodiaceae* and *Caryophyllace*.	*Fagus* now an important constituent of the forest around the hollow but *Pinus* is most abundant species in the area. Other trees present but in less abundance than previously.
DH 3	AD 1750 – 1875	*Fagus* percentages begin to rise. Rapid expansion to maximum levels of Poaceae followed by sharp decline. *Filipendula*, *Secale*, *Avena-triticum*, *Hordeum*, *Fagopyrum*, *Plantago lanceloata*, *Chenopodiacea*, *Caryophyllacea* and *Brassicacea* present throughout. *Quercus* declines to minimal values. *Carpinus* becomes discontinuous. *Tilia*, *Fraxinus* and *Corylus* disappear.	Clearance event close to the hollow. *Fagus* expands.
DH 2	AD 600 – 1750	Tree pollen dominates the total sum (>80%). *Pinus* and *Betula* most abundant. *Alnus* values slowly decline to around 10% and *Quercus* values rise to 2%. *Fagus* and *Ulmus* present in small quantities <1%. *Tilia* becomes discontinuous. Between 850-750 *Secale, Avena/triticum, Hordeum* and *Fagopyrum* become present. *Humulus* noted in two brief periods.	*Pinus* forest with *Betula* and *Quercus* present. Anthropogenic activity in the area.
DH 1	AD 600 – 500 BC	Tree pollen dominates the total sum (>80%). *Pinus* (>30%), *Betula* (>20%) *and Alnus* (15-20%) dominate. *Quercus, Ulmus, Fraxinus, Tilia* and *Corylus* all present. *Fagus* also present but in small quantities (<1%). *Artemisia* present throughout. Small finds of *Humulus*, *Chenopodiaceae*, *Brassicacea* and *Caryophyllacea*.	A number of species abundant in the forest.

Table 5.2 Overview of the results from the pollen analysis conducted at Dammendorf Hollow.

5.3.2 Vegetation history of Dammendorf Hollow and comparison with Grosser Treppelsee

At Dammendorf Hollow the pollen diagram (Figure 5.5) shows a vegetation history (Table 5.2) principally dominated by forest. Zonation indicates four significantly different pollen zones. Two are approximately 1000 years in age and two, closest to the top of the profile, are periods of less than 200 years. The main changes in this diagram are a decline in *Alnus*, a brief period of Poaceae dominance and a late rise in values of *F. sylvatica.* Applying the same technique to the samples over the same time period at Grosser Treppelsee yielded only two major divisions of the pollen sequence separated by a boundary around 1300AD. At this time there is a reduction in the amount of tree pollen and an increase in Poaceae and anthropogenic indicators (sensu Behre 1981). The pollen diagram from Dammendorf Hollow does not show any dramatic change around AD 1300.

Over the last 3000 years the pollen diagram from Grosser Treppelsee has a comparably high time resolution as Dammendorf Hollow and comparisons are based solely on AMS chronology, which is corroborated for Grosser Treppelsee by historical events. Visual analysis of Dammendorf Hollow and Grosser Treppelsee show both sites to be dominated by the tree taxa *Pinus*, *Betula* and *Alnus* (Figure 5.4). *Pinus* has greater percentages at the lake in the early part of the profile. From AD 900 to present the species has much higher percentages at the hollow. *Betula* is always more abundant at the hollow and conversely *Quercus* at the lake. *Alnus* percentages gradually become greater at the lake through time. *Quercus* has always higher values at Grosser Treppelsee compared to Dammendorf Hollow (Figure 5.6). Both sites show a major reduction in tree percentages but these occur at different times and have different characteristics. In the early part of the profile, *Tilia* has higher proportions at the hollow. *F. sylvatica* attains higher pollen percentages at the hollow relatively recently, compared to the lake, when the pollen percentages increase at the hollow.

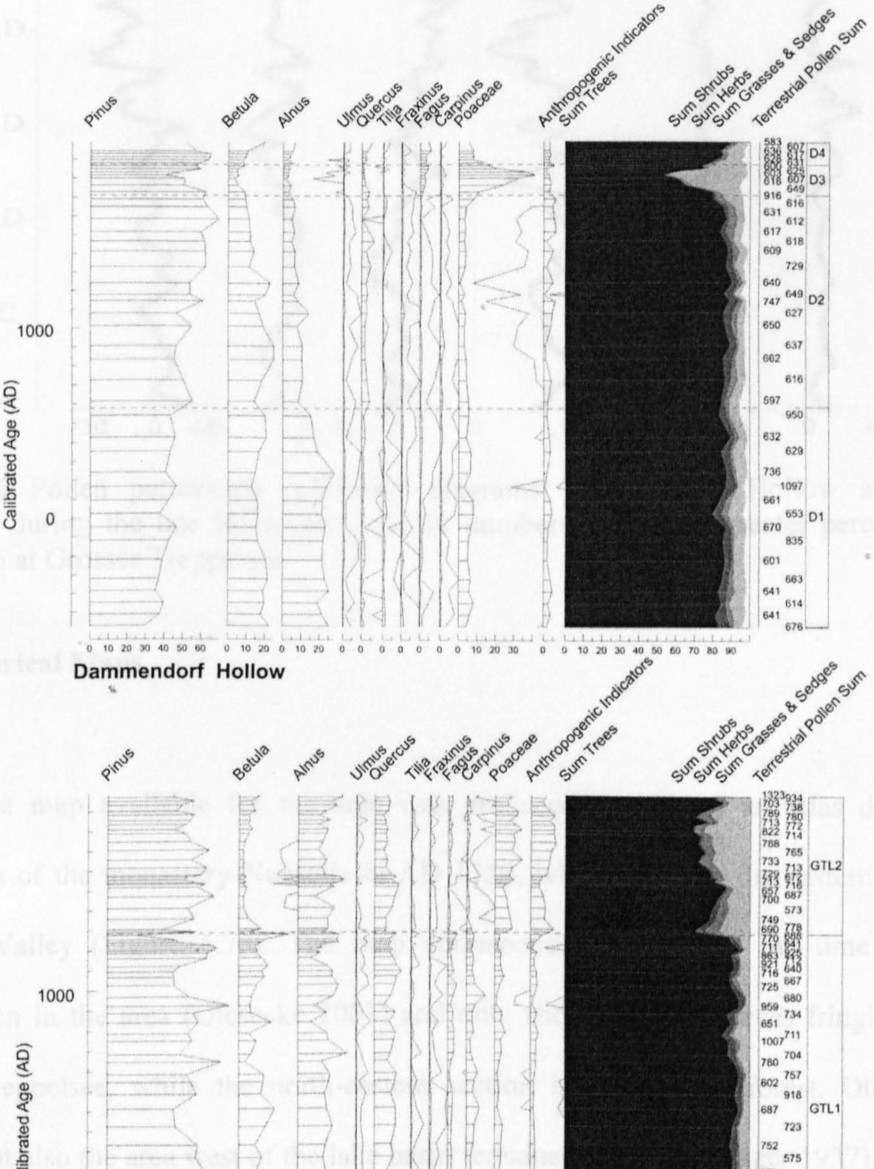

Figure 5.5 Summary pollen diagram showing selected taxa from Dammendorf Hollow and comparison with the pollen stratigraphy from Grosser Treppelsee for the same time period. Anthropogenic indicators defined according to Behre (1981).

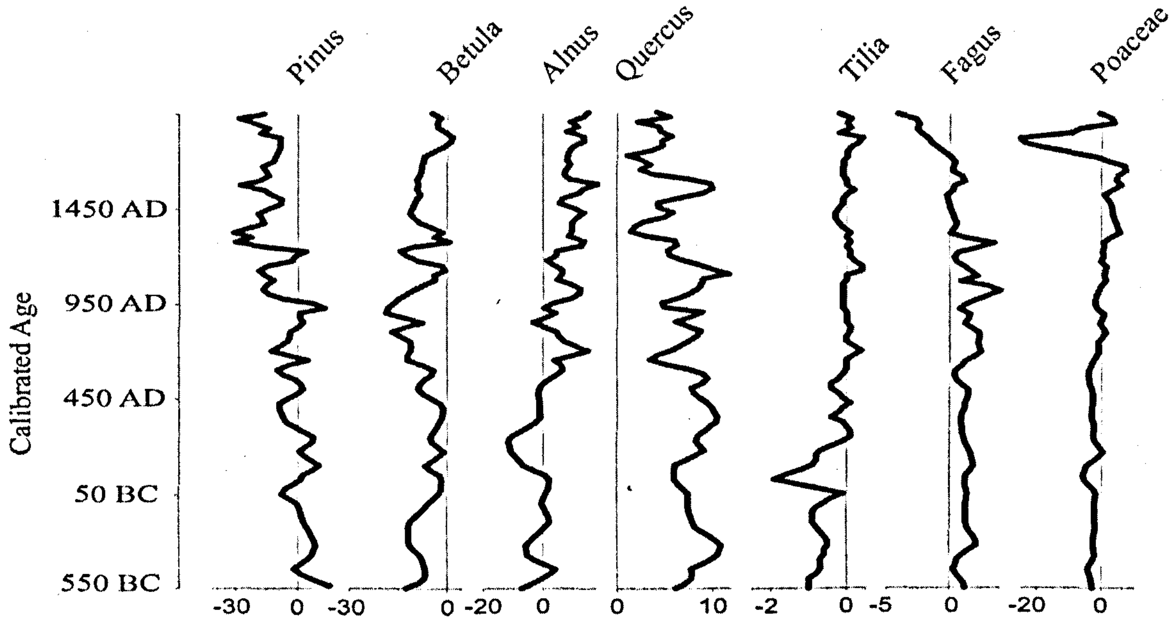

Figure 5.6 Pollen percentage difference diagrams Dammendorf Hollow and Grosser Treppelsee during the late Holocene. Positive numbers indicate a greater percentage of a given taxon at Grosser Treppelsee.

5.3.3 Historical Maps

The earliest map available for the area was produced as part of an atlas detailing the possessions of the monastery Neuzelle in AD 1759, which includes the eastern side of the Schlaube Valley (Figure 5.7a). The map corresponds probably to the time of highest deforestation in the area (Giesecke 2001) and only shows a line of trees fringing the lake Grosser Treppelsee, while the north-eastern section is covered by forest. Other sources indicate that also the area west of the lake basin remained forested (Krüger 1937). The slopes on the eastern shore of the lake towards Bremsdorf were cultivated and the small stream entering the middle basin was running through them. Undoubtedly the stream transported pollen from the cultivated land into the lake basin from which the sediment core for pollen analysis was extracted. For this reason the pollen diagram from Grosser Treppelsee especially mirrors land use changes on the eastern side of the lake.

The first topographical map available from the area dates to AD 1844 (Figure 5.7b), it shows a greater forest cover than the AD 1759 map on the eastern shore of Grosser Treppelsee and indicates a continuous mature forest on the western side. However, the reduction of fields on the eastern shore of the lake is finding only a small if any representation in the pollen percentages in the diagram from Grosser Treppelsee.

A map from AD 1934 (Figure 5.7c) shows the continued increase in forest cover on the eastern shore of the lake. The pollen diagram from the lake shows a rise in total tree pollen consistent with this observation. There is still continued dominance of forest around the hollow site. A map from the present day (Figure 5.7d) shows the narrow band of *F. sylvatica* running along the western shore. An old forest inventory map as well as the age structure of the trees indicates that most of these sites have been occupied by *F. sylvatica* for at least the past 250 years. The dominating tree on the eastern shore is *Pinus* with a small *Alnus* population next to the southern inflow. A recent rise in *Alnus* percentages in the lake pollen diagram is observed.

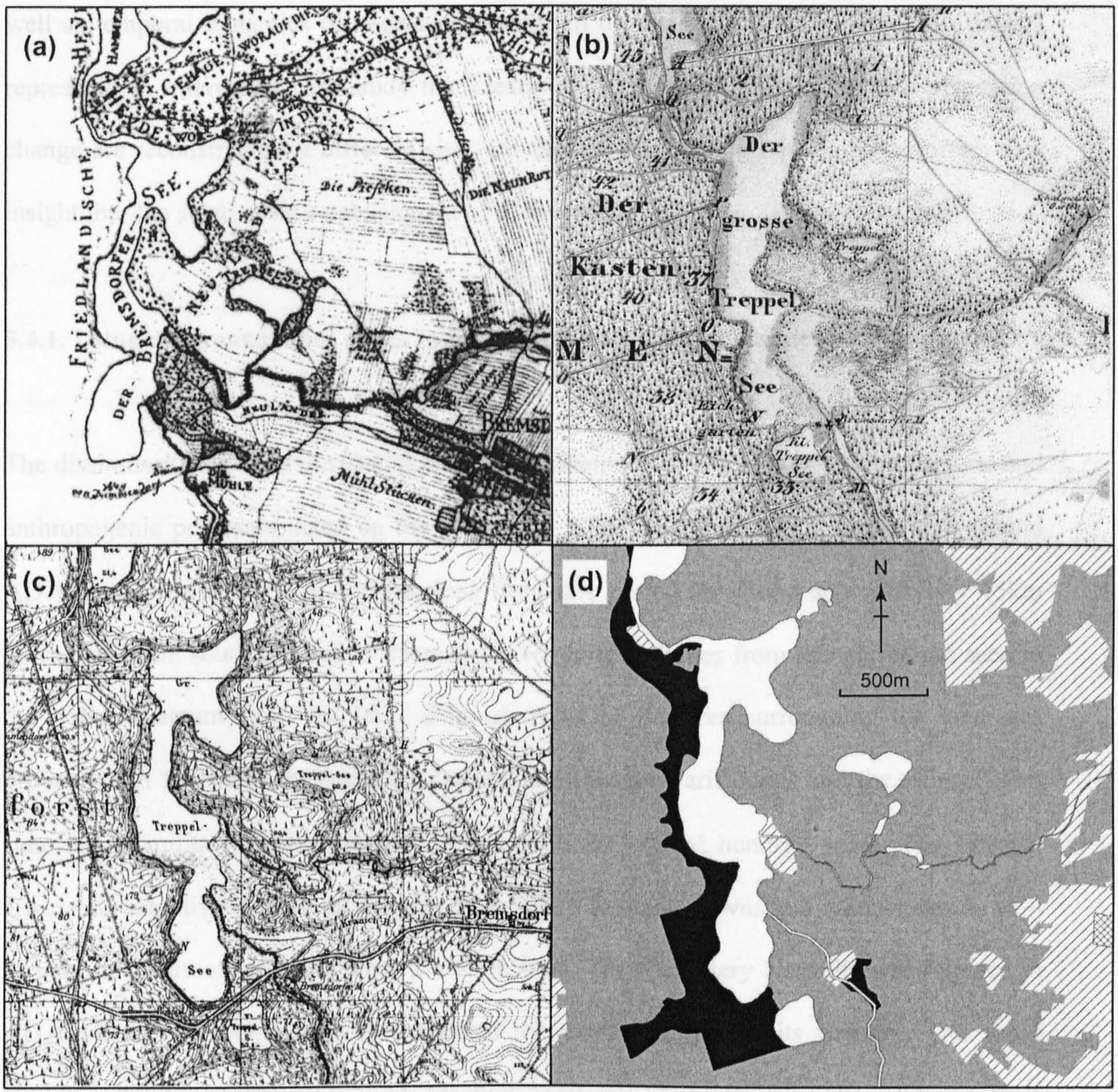

Figure 5.7 Maps around Grosser Treppelsee (a-d) and Dammendorf Hollow (b-d). A) Map from 1759 detailing the eastern side of the lake. B) Map of 1844 showing significant reforestation on the eastern shore and forest presence on western shore. Note the tracks present in the western forest C) Map of 1934 showing continued forest dominance around the lake. D) Land use at present. Black – *F. sylvatica* forest. Grey – other forest. Diagonal line – open land. Cross pattern - settlement.

5.4 Discussion

The pollen analyses of sediments from the forest hollow complement the full Holocene pollen diagram from Grosser Treppelsee (Giesecke 2001) and give insight into the spatial as well as temporal dynamics of vegetation change in the Schlaube Valley. Thus, this dataset represents an interesting case study that clearly shows how different aspects of vegetation change are reconstructed if different sites are chosen. Dammendorf Hollow also offers new insight into the stand-scale establishment of *F. sylvatica.*

5.4.1. Using different spatial scales to investigate past forest dynamics

The dissimilarity between the spatial scales is apparent in the late Holocene when natural and anthropogenic processes acted on the vegetation. Slavonic tribes settled in the area around AD 900 and the remains of fortifications from this period are found approximately 15km north and 14km south of Grosser Treppelsee. Farming activities from this period are seen in the pollen diagram from the lake, although most of the area surrounding the lake was probably still forested. The eastward expansion of German aristocrats and the rising Polish state led to military conflict in the wider region for several hundred years. The German aristocrats established their sovereignty in areas by founding towns and monasteries as well as importing farmers and craftsmen from the west. The monastery Neuzelle was founded in AD 1268 and the Schlaube Valley marked the western border of its territory. This rapid change in land management around AD 1300 is clearly visible in the pollen diagram from Grosser Treppelsee with a sharp rise in anthropogenic impact pollen indicators. The historical map (Figure 5.7a) gives an impression of the extent of the fields at the time of most extensive land use.

The pollen diagram from Dammendorf Hollow, located close to the western shore of the lake, does not show any significant changes in land use before AD 1700. This suggests that the western side of the lake was not cleared during the 400 year period (AD 1300-1700) of intense anthropogenic activity that occurred in the wider area. The view is supported by historical information and by the first topographical map showing both sides of the Schlaube Valley from AD 1844 (Figure 5.7b). A dramatic rise in Poaceae pollen around AD 1800 shows that the vicinity of the hollow was cleared. The peak in the Poaceae pollen is not followed by pollen from pasture weeds or arable fields. This suggests that the felling of trees was not carried out to obtain farmland but rather to supply the charcoal production or lime kilns in the area. The area around the hollow is marked as forest on the AD 1844 map suggesting that the deforestation was brief and that the forest was allowed to regenerate immediately after the clearance.

5.4.2. Regional and stand-scale establishment of *F. sylvatica*

The pollen diagram from Grosser Treppelsee indicates that *F. sylvatica* has grown in the area since about 3700 BC, where currently it occupies the valley slopes. This is in keeping with the timing of expansion in north central Europe (Magri *et al.*, 2006). The pollen diagram from Grosser Treppelsee shows the beginning of the continuous *F. sylvatica* curve before increased indication of anthropogenic activity. An initial small rise in the curve does coincide with an increase in pollen indicators of anthropogenic impact. Pollen diagrams from the wider region also show situations where the continuous or rising *F. sylvatica* curve is preceded or followed by anthropogenic pollen indicators (Wolters 1999; Jahns 2000, 2001; Brande 2007). Elsewhere in the distribution area of *F. sylvatica*, the tree expands before any anthropogenic impact is detectable (Gardner and Willis 1999; Tinner and Lotter 2006), although other disturbance mechanisms may also facilitate the expansion.

Although the initial timing and establishment of the species in the area cannot be determined at the stand-scale as the tail in its pollen curve extends to the base of the profile, we are able to examine the expansion phase of the species at this spatial scale. It is shown that the tail persists at very low values for over 2000 years. Percentage values only increase around AD 1700 indicating that the present stand of *F. sylvatica* trees around the site is not older than two tree generations. This raises the question to why it took the tree so long to occupy this apparently suitable area of the valley. The diagram from Dammendorf Hollow clearly shows that the expansion of *F. sylvatica* occurred at the same time as local clearance of the forest. Thus, the relative stability of forest cover around the hollow before AD 1700 may have prevented the local expansion of *F. sylvatica*. The increase of *F. sylvatica* and Poaceae pollen in the pollen diagram from Dammendorf Hollow occurs at the same time as a decrease in *Quercus* and *Pinus* pollen. This indicates that the hollow, prior to the Poaceae peak, was surrounded by these two species probably accompanied by *Fraxinus*, *Ulmus* and *Betula*. *Quercus* was a highly valued wood for construction and ship building and local farmers were generally not allowed to cut it. As *Quercus* was the more economically valuable tree it is unlikely that *F. sylvatica* was actively planted after the removal of *Quercus*. Thus, it is conceivable that *F. sylvatica* seized the chance of new gaps in the canopy and established locally around the hollow. In southern Scandinavia, near the northern limit of the species, its local establishment always followed an opening of the forest (Björkman 1996; Björkman and Bradshaw 1996; Bradshaw and Lindbladh 2005). Anthropogenic control for the species expansion was also proposed elsewhere in northern Central Europe (Küster 1997), but contradicts the competitive advantage that this shade tolerant tree should demonstrate (Ellenberg 1996).

In the Schlaube Valley, *F. sylvatica* was present in the area but locally absent from the vicinity of the forest hollow. In the current vegetation on the western slopes behind the forest hollow, *F. sylvatica* gives way to *Quercus, Carpinus* and *Pinus*. If this local vegetation change is determined by water availability than it could be argued that the *F. sylvatica* trees growing around the hollow today occupy a location that is marginal for the growth of the tree. This could be the reason why *F. sylvatica* was not able to out-compete its competitors before they were removed by anthropogenic activity. This may also be the reason why the link between anthropogenic impact and the expansion of *F. sylvatica* is so clearly seen near its northern margins in Scandinavia.

Charcoal is present throughout the period AD 400 to 1500. Before AD 400 the LOI data and inspection of the sieving residue showed that sand was a major component of the sediment matrix. It is possible that even if charcoal was produced it was not preserved in this period. Charcoal values rise after AD 400 but it is impossible to distinguish between natural and anthropogenic causes of fire. There is little or no charcoal found after AD 1500 and there is no sedimentary or LOI change at this point. This suggests that fire was beginning to be suppressed in this period. The earlier evidence for fire is another possible explanation as to why *F. sylvatica* did not expand earlier at this site. The species may have had to wait for a tolerable fire regime before taking advantage of a disturbance event.

5.5 Conclusion

The use of pollen diagrams with different spatial scales has demonstrated that major forest compositional changes in the Schlaube Valley are not parallel. This finding suggests a mosaic of forest stands with different dynamics. In the late Holocene, anthropogenic activities are shown to heavily influence the vegetation changes. The two pollen diagrams suggest that in peripheral areas, the species *F. sylvatica* requires disturbance for expansion at the forest stand-scale, in agreement with Björkman (1996).

Note

Thomas Giesecke provided the regional dataset and the information from the sources written in German. The manuscript has benefited from the comments of four anonymous reviewers.

Citation

Bradley LR, Giesecke T and Bradshaw RHW (in review). Examining *F. sylvatica* forest dynamics at different spatial scales in the Schlaubetal Valley, Brandenburg, Germany.

Chapter Six – Stand-scale forest dynamics in Peutscher Forest, Mecklenburg-Vorpommern, northern Germany

6.1 Introduction

Fagus sylvatica L. (European Beech) is one of the dominant tree species in the forests of Europe and it is an important natural and economic resource. This importance has led to the species having a long research history, including numerous studies examining its long term history. Studies examining the species migration (Gliemeroth 1995; Brewer 2002; Magri *et al.,* 2006; Magri 2008) and expansion (Godwin 1956; Iversen 1973; Behre 1988; Küster 1997; Bradshaw and Lindbladh 2005; Tinner and Lotter 2006; Giesecke *et al.,* 2007;. Valsecchi *et al.,* 2008; Bradshaw *et al.,* 2010) have argued for a number of different controls on the species Holocene history.

In northern Central Europe and southern Scandinavia, anthropogenic activity has been shown to have a strong impact on the species expansion (Behre 1988; Küster 1997; Bradshaw and Lindbladh 2005). This activity can be in the form of felling, land-use changes and alterations to fire regimes. The disturbance to the forest ecosystem benefits any small population of *F. sylvatica* that maybe present. The population would have likely migrated late into forest stands compared to other species, with the exception of *Carpinus* and *Picea.* A possible mechanism for its spread in northern Europe is that a disturbance event clears an area of the formerly dense forest and the population of *F. sylvatica* is then able to expand into the gap created. The species' ability to regenerate under the dense shade that its canopy creates allows the species to out-compete rivals and dominate the forest. However, Ralska-Jasiewiczowa *et al.,* (2003) argue that shifts to wetter and cooler periods of climate are responsible for the species expansion in the northern section of the species distribution. Also, in areas further south in Europe, anthropogenic impact appears less important compared with climate (Tinner and Lotter 2005) or migrational lag (Gardner and Willis 1999).

The link between expansion and disturbance is nowhere more explicit that in southern Scandinavia. The species has expanded in the region over the last 2000 years. Odgaard (1994) showed in eastern Denmark that the species expanded primarily in periods of low disturbance, following intervals of perturbation. A regional synthesis for southern Scandinavia (Bradshaw and Lindbladh 2005) used an approach which examined both regional and stand-scale pollen diagrams and showed that different forces acted on the species, dependent on the spatial scale examined. The study highlighted the importance of stand-scale diagrams in complementing regional pollen data when examining a species history. It was demonstrated that the establishment of *F. sylvatica* benefited greatly from anthropogenic disturbance in the region.

In central Europe, particularly Germany, Küster (1997) also argues for anthropogenic activity to exert critical influence on the species expansion with *F. sylvatica* replacing *Quercus* and other species. Küster suggests that from 5000 BC, *Quercus* dominated woodlands were cleared by prehistoric farmers to establish settlements and perform farming. After these settlements and farmlands were abandoned, during periods of shifting cultivation, a secondary phase of woodland succession began with *Betula* and *Populus* establishing before *Quercus* and *F. sylvatica* dominated woodland became established. Küster's (1997) argument is that secondary woodland succession was a feature of semi-natural vegetation development over a long period, which had the overall effect of favouring *F. sylvatica*. Tinner & Lotter (2006) suggest that although anthropogenic activity is a factor, climate change is the greatest influence on the expansion of *F. sylvatica* in northern Central Europe. Tinner and Lotter (2006) cite the work of Ralska-Jasiewiczowa *et al.,* (2003) to suggest a climatic control on the species expansion. It is suggested that the northern Central European regional expansion occurred synchronously and in a period of '8.2' type events (1850-1450 BC and 750-350 BC) similar to that of southern Central Europe.

Gardner and Willis (1999) critique Küster's (1997) argument that expansion was heavily influenced by land-use practices. Gardner and Willis argue that interplay between *F. sylvatica* and transition to agriculture is because of the rate of expansion from refugia rather than anthropogenic disturbance. The critique of Gardner and Willis (1999) is based on four sites in Slovenia and Hungary, which are located to the east of the main region covered by Küster's argument. Figure two of Küster (1997) also suggests that in montane environments, *F. sylvatica* was 'probably natural'. Pott (1997) suggests that in southern Central Europe, climate is likely the dominant control. This view is supported by the study of Tinner and Lotter (2006) that argued for a climatic control in this area. Tinner and Lotter show that rapid population expansion took place in response to an increase in precipitation and temperature cooling. This expansion was synchronous over a spatial range of approximately 500km (Tinner and Lotter 2006).

The literature suggests that distinct geographical patterns exist in the Holocene species expansion of *F. sylvatica.* Using regional pollen diagrams, anthropogenic disturbance cannot be linked to the species expansion in southern Europe and southern Central Europe. However the evidence in northern Central Europe suggests that anthropogenic land-use patterns and activities do play a significant role in the species expansion. In Denmark and southern Sweden anthropogenic disturbance has been explicitly linked to the species expansion at the stand-scale. Yet testing this idea outside this area has yet to be done because of a lack of stand-scale sites (Bradshaw *et al.,* 2010). Also long records of forest history at the stand-scale are rare outside of Scandinavia. Therefore, the problem exists that for continental comparisons, stand-scale sites in the north are being compared with regional sites in the south.

Despite a long tradition of pollen analytical work, few forest hollow records exist in Germany. Those that do are often undated and largely inaccessible. A recent search by Spandenburg (2008) revealed very few sites in the areas of Mecklenburg and Brandenburg. The lack of published stand-scale sites and the dominant presence of *F. sylvatica* in the forests of these regions make these areas ideally suited for testing ideas relating to the influence of disturbance on the species expansion outside southern Scandinavia.

This chapter has the following aims,

1) Produce a stand-scale site examining the initial colonisation and subsequent expansion of the species *F. sylvatica* in northern central Europe

2) Examine the relationship between anthropogenic indicators and/or fire and the establishment of *F. sylvatica.*

3) Examine if the expansion coincides with a cooler and wetter phase as proposed by Tinner and Lotter (2006).

Dates are presented in calibrated calendar ages to ease comparison with other sections of the thesis. Palynomorph nomenclature follows guidelines of the European Pollen Database.

6.2 Material and methods

6.2.1 Study Area and site

The region of Mecklenburg-Vorpommern in northern Germany was chosen for this study, specifically areas around Muritz and Serrahn, an area where *F. sylvatica* is dominant in the forests at present (Figure 6.1a). The maximum extent of the Weichselian glacier margins reached into northern Germany. This glacial history means it is full of lakes and smaller basins thus making it ideal for pollen studies. At present over 50% of the Muritz national park is covered by forest, predominantly *F. sylvatica* and *Pinus*. Planted stands of *Picea* are also present in areas. The historical centre at Hohenzieritz is within the area. After examining Google Earth, local maps and making detailed field observations, an area was scouted in detail for potential basins around the town of Neustrelitz in Mecklenburg-Vorpommern. This area is largely composed of forest, woven in between areas of cultivated land. Neustrelitz is the major town in the area with numerous smaller satellite villages also on the landscape (Figure 6.1b). The climatic averages for the period 1961 to 1990 (www.dwd.de), show a yearly mean temperature of 8°C. Lowest mean temperatures are record at -1.1°C in January; highest mean temperature is 16.9°C in July. Average sunshine hours per year are 1669.6, December records the lowest values with a mean of 38 hours, and the highest mean is in May with 235 hours. Precipitation ranges from a low in February of 31.2mm to a high in June of 68.2mm. Mean annual total precipitation is 584.2mm.

A number of small basins were discovered in the study area. The depth of each of these basins and the sediment composition was examined. A final site, named Carlshof Small Forest Hollow, (53°25'N 13°04'E, 60m.a.s.l) was selected in the north-east section of Peutscher forest, between the hamlets of Carlshof and Weisden. The forest has a railway line dissecting it in half and has numerous forest tracks present (Figure 6.1b). The forest is dominated by *F. sylvatica* but also has *Picea abies* plantations. One of these stands is located approximately 200 to 300m from the site, on the slopes that descend towards the basin. The site (Figure 6.1c) is a classic small forest hollow which today is surrounded by regenerated *F. sylvatica* and *Quercus*. At the time of sampling, one edge of the hollow was submerged below 5-10cm of water. This site represented the best hollow that could be found in the area which had a long sediment record. The site is also situated close to a small lake (200m x 200m) and therefore the area could be used in future work to compare the palaeoecological records of a stand-scale site and a small enclosed lake.

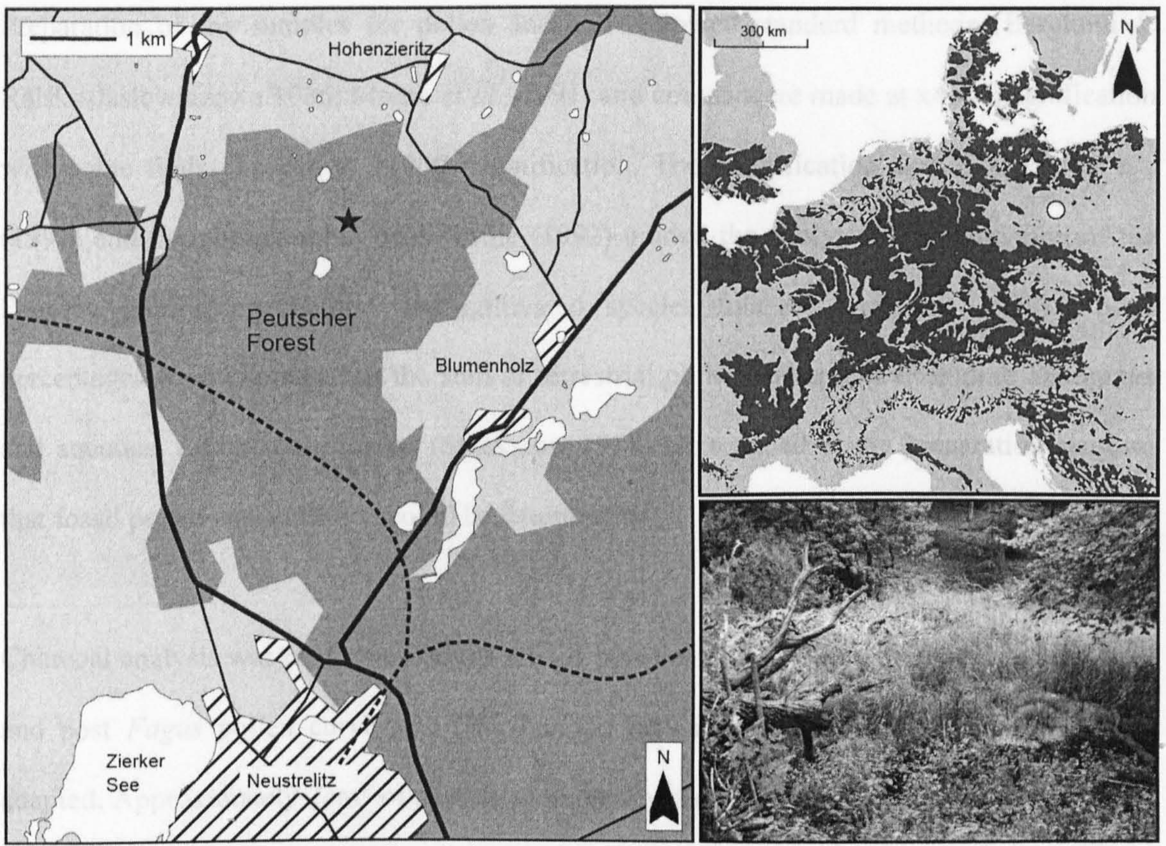

Figure 6.1 Location and details of the study area (a) Continental Europe; Green shading indicated the location of potentially dominating *F. sylvatica* forests (Bohn *et al.*, 2000); Red Star – Carlshof Small Forest Hollow; Blue Circle – Dammendorf Hollow (chapter five). (b) Overview of land-use and settlements around Neustrelitz. (c) Photograph of the site.

6.2.2 Sampling and sediment analysis

Sediment from Carlshof Hollow was collected using a Russian corer (Jowsey 1966) in September 2007. The core was cleaned to remove any contamination caused by a result of the extraction from the ground. It was then sliced into 1cm thick segments and stored at 6°C. The core 0-1m was damaged in storage before being sliced and therefore only has preliminary pollen work carried out on it. Loss on ignition (LOI) was carried out on all samples from 1.00 – 7.87m to help determine organic matter content. Each sample was oven dried at 105°C for 12 hours to assess moisture content before being heated at 450°C for 4 hours to determine the LOI value. Twenty samples were examined to determine calcium carbonate content before samples were sent for dating.

Preparation of the samples for pollen analyses followed standard methods (Berglund & Ralska-Jasiewiczowa 1986; Moore *et al.,* 1991) and counts were made at x400 magnification with some finds checked at x1000 magnification. The identification key in Moore *et al.,* (1991) and the photographic book Reille (1992) guided the analysis. Differentiation of the Poaceae family into natural and cultivated species follows Andersen (1979). Pollen percentages were expressed as the sum of terrestrial pollen and spores excluding *Sphagnum* and aquatics. *Lycopodium* tablets (Stockmarr 1971) were added in the preparation stage so that fossil pollen concentration could be determined.

Charcoal analysis was performed on the depths between 330-430cm which represents the pre and post *Fagus* pollen curve rise. The method presented in Molinari *et al.,* (2005) was adapted. Approximately 2cm^3 of sample measured by volumetric displacement was analysed from every sample. The samples were sieved at 300μm and the remains added to 80ml of water. This was then stirred vigorously and a 20ml subsample was taken by pipette for analysis in a Petri dish. The sample was analysed under a binocular microscope using magnification x10. Charcoal was defined as black objects with angular sides with large pieces appearing crystalline.

6.2.3 Sediment age determination

Thirteen levels from the core were submitted for radiocarbon age determination by accelerator mass spectrometry (AMS). Twelve of the samples were bulk sediment samples and one sample was a *Corylus avellana* nut. The samples analysed at the SUERC laboratory were all bulk peat. The pre-treatment included hot acid and hot alkali washed to remove humics and mineral acid. The samples from Beta followed an acid/alkali/acid treatment to remove carbonate and humic acid.

All radiocarbon ages were calibrated in Bcal (Buck *et al.,* 1999; http://bcal.sheffield.ac.uk) using the 2004 atmospheric curve (Reimer *et al.,* 2004). An age-depth model was constructed in PSIMPOLL v4.26 (Bennett 2007) using the weighted averages of the calibrated probability distributions. A liner interpolation between the dates was assumed. A date of AD 2007 was presumed for core top.

6.2.4 Numerical analysis

To assess temporal changes at the site, the pollen diagram was statistically zoned in PSIMPOLL v4.26 (Bennett 2007) using optimal splitting by information content after square root transformation.

6.3 Results

6.3.1 Sediment analysis and chronology

The sediment sequence at this site is 787cm in length. It is likely that the basin was deeper but retrieval proved impossible below this depth as the sediment became increasing composed of clay. Numerous sedimentary changes were observed in the sediment sequence (Table 6.1; Figure 6.3). The most striking sediment change was a band of mineral rich clay from 328 to 333cm.

Depth (cm)	Age (cal AD/BC)	Sediment Description
0-240	AD 300 to present	Woody Peat
240- 324	200 BC to AD 300	Sphagnum peat
324-328	300 to 200 BC	*Substantia humosa* + few plant remains
328-333	500 to 300 BC	Clay with mineral particles
333-369	1000 to 500 BC	*Substantia humosa* + sphagnum remains
369-386	1500 to 1000 BC	*Substantia humosa* + mineral particles
387-392	1600 to 1500 BC	*Substantia humosa* + mineral particles + plant remains
392-396	1650 to 1600 BC	*Substantia humosa*
396-400	1700 to 1650 BC	*Substantia humosa* + plant remains
400-636	6200 to 1700 BC	*Substantia humosa*
636 -737	6500 to 6200 BC	Sphagnum peat
737-754	7050 to 6500 BC	Sphagnum peat becoming more decomposed
754-767	7900 to 7050 BC	*Substantia humosa*
767-787	~9200 to 7900 BC	*Substantia humosa* + increasing composed of clay

Table 6.1 Sediment analysis

The radiocarbon dating was performed on bulk sediment because of the lack of macrofossils found throughout the core. One exception was a *Corylus* nut found at a depth of 364 to 365cm. The results of the AMS dating (Table 6.2) report twelve of the thirteen dates to be in stratigraphic order. One reversal is noted at a depth of 188-189cm.

Depth (cm from the surface)	Lab Number	Material Dated	Reported ^{14}C age BP	Calibrated age (2 sigma range)
115-116	SUERC-28851	Bulk Sediment	1026+/-37	AD 982 – 1028
167-168	SUERC-28850	Bulk Sediment	1215+/-37	AD 776 – 834
188-189	BETA-261653	Bulk Sediment	3690+/-40	
313-314	SUERC-28849	Bulk Sediment	2219+/-37	366 – 311 BC
364-365	LUS-8458	*Corylus* Nut	2735+/-50	913 – 862 BC
383-384	SUERC-28848	Bulk Sediment	3135+/-37	1431 – 1387 BC
450-451	SUERC-28847	Bulk Sediment	4044+/-38	2622 – 2537 BC
517-518	SUERC-28846	Bulk Sediment	5398+/-36	4290 – 4230 BC
537-538	BETA-261654	Bulk Sediment	5890+/-40	4785 – 4739 BC
623-624	SUERC-28845	Bulk Sediment	7222+/-39	6125 – 6067 BC
671-672	SUERC-28842	Bulk Sediment	7566+/-37	6442 – 6416 BC
745-746	SUERC-28841	Bulk Sediment	7676+/-40	6500 – 6542 BC
768-769	SUERC-28840	Bulk Sediment	9024+/-43	8272 – 8211 BC

Table 6.2 Radiocarbon dates

The age-depth model (Figure 6.2) suggests that the basin slowly filled during the period 9000 to 6500 BC. At 6500 BC there is a sharp rise in sedimentation rate which decreases around 6000 BC. The age-depth model then suggests a gradual filling of the basin with an gradual increasing of the rate of sedimentation around 500 BC to present.

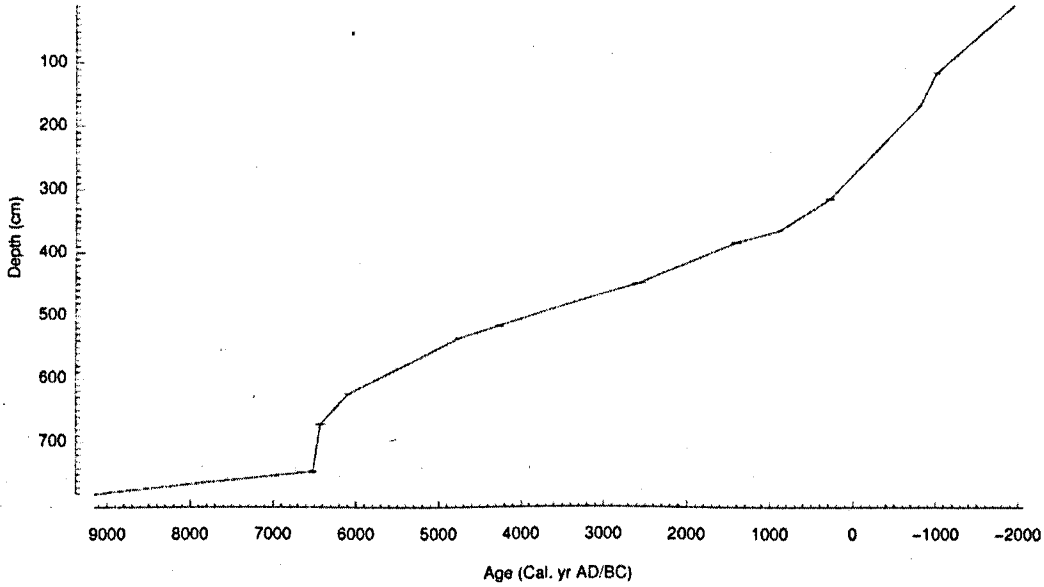

Figure 6.2 Age depth model from Carlshof Small Forest Hollow

The LOI data (Figure 6.3) suggest that the majority of the sediments are largely organic with values above 90%. A few notable exceptions occur. The oldest part of the profile from ~9400 to 8700 BC had much lower LOI values. A reduction in LOI values is also observed around 1300 to 1000 BC. A sharp, but brief, drop is also observed at approximately 500 BC. Between AD 1000 and present there are no LOI measurements.

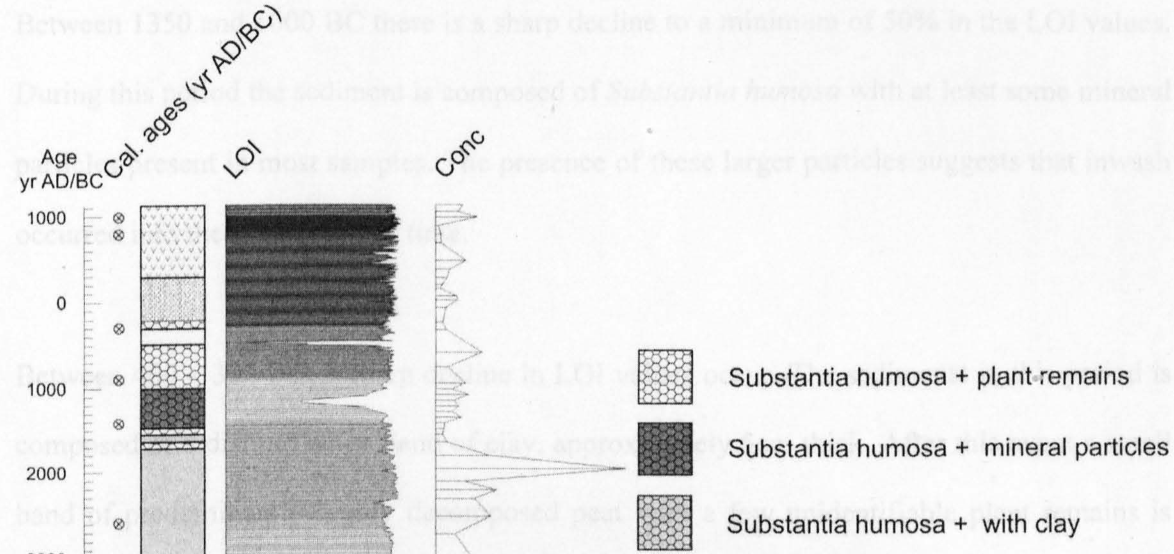

Figure 6.3 Sediment profile, LOI analysis and pollen concentration values. No values recorded between AD 900 and present due to accidental damage of the top of the sedimentary core.

The rapid increase in sedimentation rate between 6500 - 6100 BC is consistent with sediments changing from *Substantia humosa* with clay component to *Sphagnum* peat. The pollen concentration values are also very low in the period, relative to the rest of the profile, which also indicates rapid sedimentation.

Between 1350 and 1000 BC there is a sharp decline to a minimum of 50% in the LOI values. During this period the sediment is composed of *Substantia humosa* with at least some mineral particles present in most samples. The presence of these larger particles suggests that inwash occurred into the system at this time.

Between 400 – 300 BC, a sharp decline in LOI values occur. The sediments in this period is composed of a distinct white band of clay, approximately 5cm thick. After this event a small band of predominantly highly decomposed peat with a few unidentifiable plant remains is present until 200 BC. It is during this phase that the one anomalous AMS is reported (188-189cm, Beta-261653). Numerous explanations are possible, including sediment inwash of reworked older deposits from outside the basin being incorporated into the sediment. Significant disturbance to the basin though it unlikely as the other dates suggests are fairly consistent sedimentary environment.

No LOI data are available after AD 900 but there is consistent stratigraphy from AD 300 to core top. The uniform stratigraphy and consistent but low pollen concentrations supports the age-model throughout this period. The small peak in *P. abies* pollen during the most recent 100 years of the pollen diagram supports the conclusion that the surface sediment is dated to the present day. At present, a stand of managed *P. abies* forest is approximately 150-250m from the hollow.

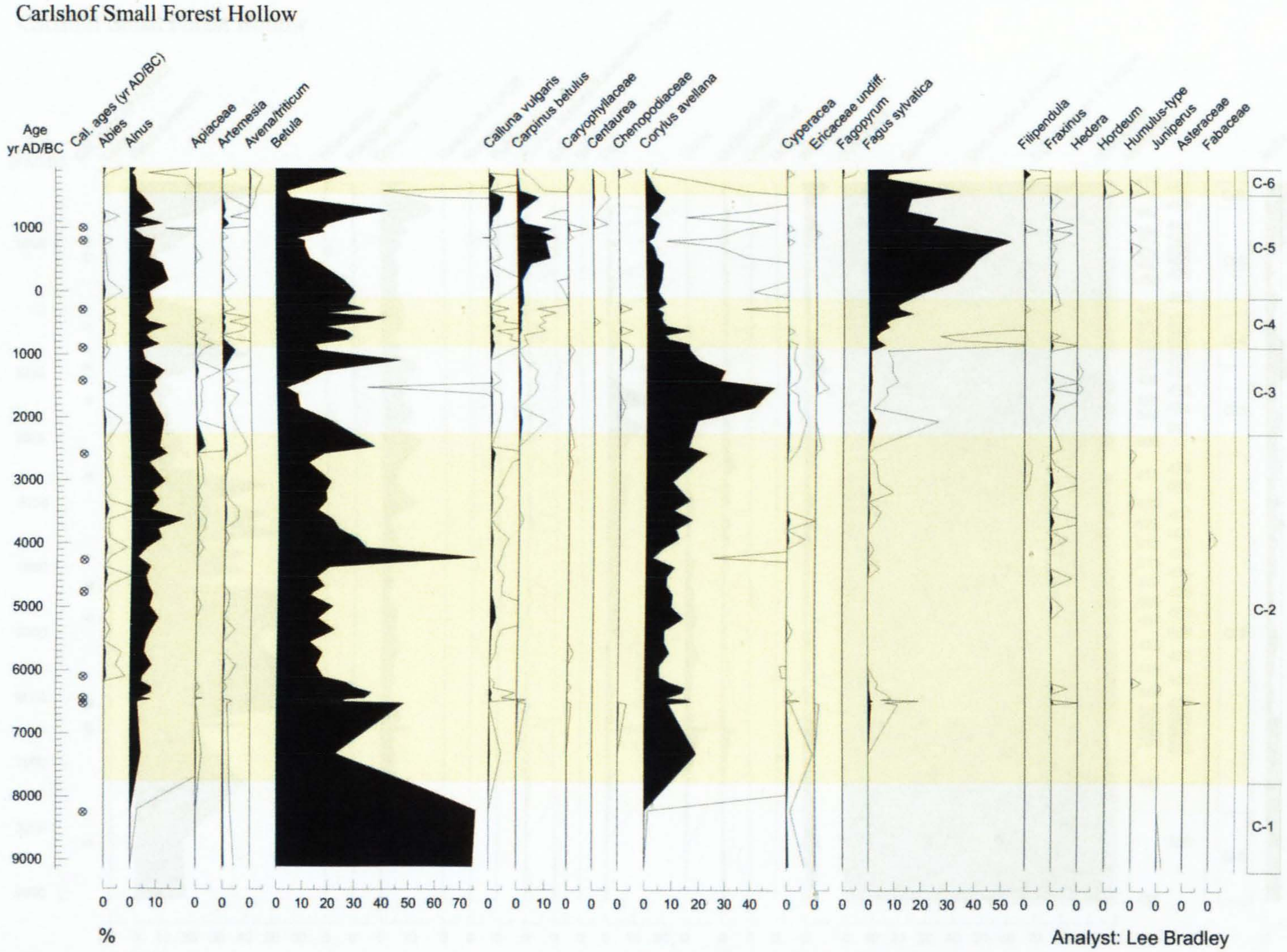

Figure 6.4 Pollen diagram from Carlshof Small Forest Hollow. Solid black blocks indicate percentages. Hollow blocks indicate 10x exaggeration. Yellow banding indicate different pollen zones. Anthropogenic indicators sensu Behre 1981.

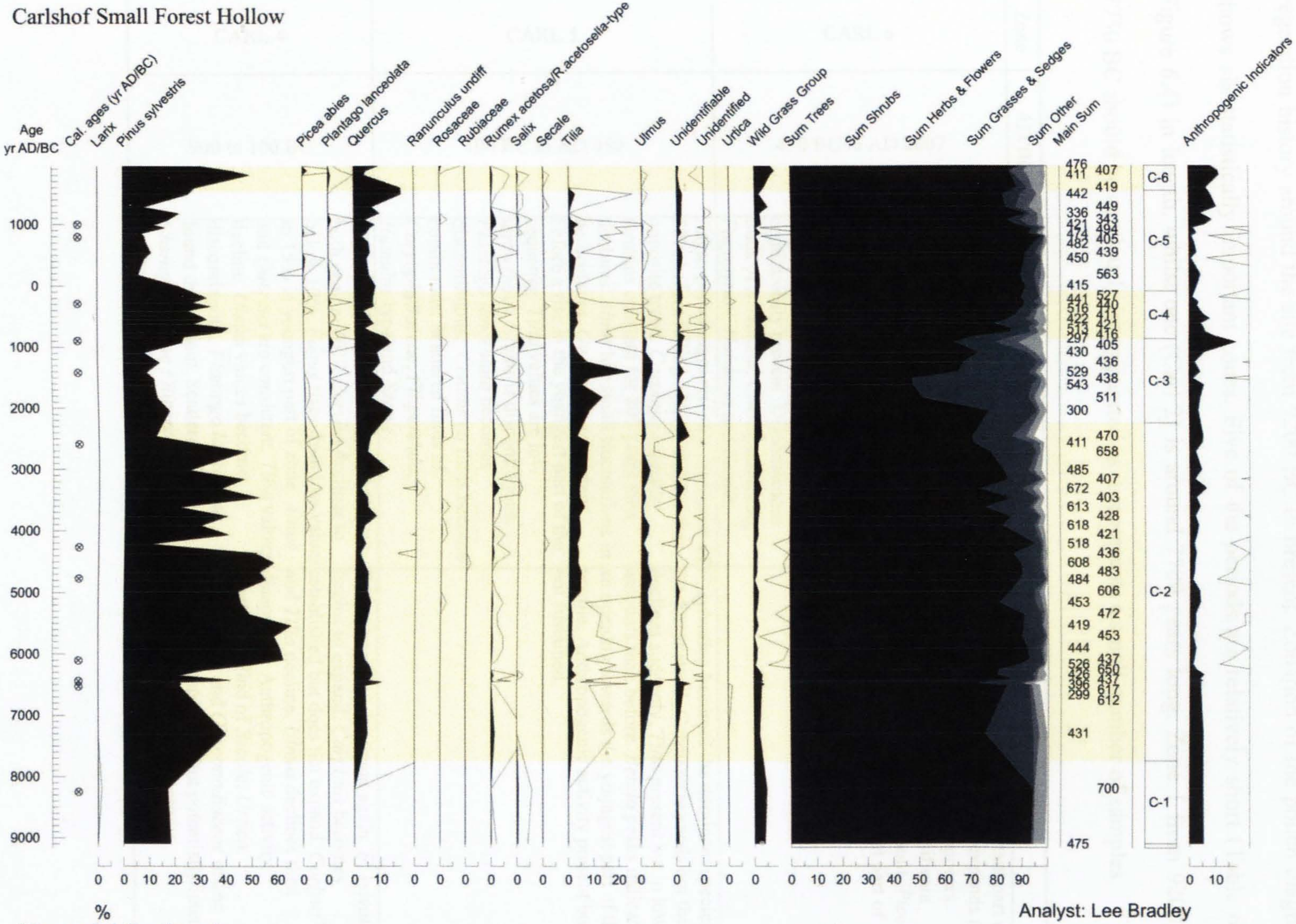

Figure 6.4 Continued

The summary of the pollen assemblages (Table 6.3) shows the significant changes in the vegetation history around the site from 9200 BC to present. Zonation of the pollen diagram shows six statistically important zones. Five of the periods are relatively short (Table 6.3; Figure 6.4) in length, whilst one (Carl 2) is around 7700 years long. Zone 1 from 9200 to 7750 BC should be cautiously interoperated as it represents a small number of samples.

Zone	AD/BC	Results	Interpretation
CARL 6	450 BC to AD 2007	*Betula* and *Pinus* values rise sharply. *Fagus* values oscillate. These three taxa dominate the pollen assemblages. *Alnus*, *Carpinus* and *Quercus* values fall. *Tilia* disappears. *Picea* present in the youngest sediment sections. Poaceae values increase. *Avena/Triticum*, *Caryopyllaceae*, *Centaurea*, *Filipendula*, *Plantago lanceolata* continuously present. Discontinuous finds of *Artemisia*, *Chenopodiaceae*, *Fagopyrum*, *Hordeum* and *Secale*.	*Fagus* declines but still an important part of the forest composition. *Quercus* expands as does *Betula* and *Pinus*. *Tilia* disappears. Poaceae values rise slightly. Significant anthropogenic indicators throughout. *Picea* rise linked to planting in youngest part of profile.
CARL 5	100 BC to AD 450	*Fagus* percentages rise to a maximum of 50% before falling. *Betula* follows the opposite trend. *Carpinus* expands, remains constant for 800 years then declines. *Alnus* has small fluctuations in percentages. *Quercus* is consistent before a rise in the youngest part of the sequence. *Tilia* values are low throughout. *Ulmus* is discontinuous. *Plantago lanceolata* becomes discontinuous. *Centaurea* finds become continuous. Scattered finds of *Caryophyllaceae*, *Filipendula*, *Humulus*-type and *Secale*.	*F. sylvatica* becomes the dominant species in the forest stand. *Carpinus* expands but then declines suddenly. *Tilia* present but in lower amounts than before. *Betula* peaks indicate an opening towards the youngest part of the profile. Anthropogenic activity present but not sustained.
CARL 4	900 to 100 BC	*Pinus* and *Betula* have highest values; both peak at 40%. *Corylus* declines to below 10%. *Fagus* rises from low values to 15% in youngest part of zone. *Alnus* and *Quercus* are consistent. *Tilia* values decline. *Ulmus* values become discontinuous. *Plantago lanceolata* found throughout. Scattered finds of *Chenopodiaceae* *Filipendula*, *Plantago lanceolata*, *Secale* pollen grains found.	Forest becomes dominant again. *F. sylvatica* begins to expand. *Carpinus* becomes established but does not expand. *Corlyus* and *Tilia* decline. *Ulmus* declines then disappears. Anthropogenic activity sustained. Find of *Secale*, *Urtica*, *Filipendula* and *Chenopodiaceae* in one period suggests small but potentially intense period of anthropogenic activity.

CARL 3	2250 to 900 BC	*Corylus* values dominate the zone. Values rise from 15% to 45% before dropping to 20%. *Betula* values decline except for one peak between 1350 to 1000BC. *Tilia* expands to a peak of 25% before declining to lower than 5%. *Pinus* lower than 20%. *Quercus* lower than 10%. *Ulmus* lower than 5%. A small Poaceae peak in the youngest part of the zone. *Fagus* and *Carpinus* become continuous but only in small values. *Plantago lanceolata* and *Rumex* present throughout but discontinuous.	First period of sustained anthropogenic activity indicated by the *Plantago lanceolata* record. The vegetation structure is more open, indicated by high values of *Corylus*. Peak in *Betula* and increase in Poaceae suggest a more open structure around 1300 BC. *Fagus* becomes continuous but does not expand.
CARL 2	7750 to 2250 BC	*Pinus* values between 20-65%. *Betula* values between 15-50%, except one notable peak (70%) around 4200 to 4300 BC. *Corylus* values between 5-25%. *Alnus* values below 15% but gently increase throughout the period. *Quercus* continuous, values between 3-15%. *Tilia* has low values which rise at 5200 BC. *Ulmus* has low values throughout. Small but continuous values for *Fagus* in period 7250 to 5800 BC. Also in this period finds of *Carpinus*. No *Fagus* or from 5800 to 4500 BC After 4500 BC *Fagus* values low and discontinuous. *Carpinus* reappears 2500 BC. *Abies*, *Fraxinus* and *Picea* present but discontinuous. Poaceae values low but continuous. *Rumex* and *Calluna* present throughout but discontinuous. Scattered finds of *Caryophyllaceae*, *Chenopodiaceae*, *Humulus*-type, *Asteraceae*, *Fabaceae*, *Plantago lanceolata*. One isolated find of *Secale*.	*Pinus* and *Betula* significant. Expansion of *Alnus*, *Quercus*, *Tilia* and *Ulmus*. *Tilia* prevalent especially after 5200 BC. A small population of *Fagus* in the period 7250 to 5800 BC but this population disappears. *Fagus* present in small quantities but never significant after 4500 BC. *Abies* and *Fraxinus* are present. Small scale anthropogenic activity but not sustained or significant. One significant opening of the forest indicated by a shape spike in *Betula* at 4300 BC.
CARL 1	9200 to 7750 BC	*Betula* dominates (75%). *Pinus* (20%). Poaceae values are consistently around 5%. *Artemisia, Cyperaceae, Rumex* and *Salix* continually present. *Juniperus*, *Larix* in present in certain samples. Small values of *Corylus* (<2%) before rising at 8200 BC. Small, but continuous, values for *Quercus, Tilia* and *Ulmus* .	Forest dominated by *Betula* with a presence of *Pinus*. *Juniperus* and *Larix* present. *Salix, Artemisia,* Poaceae and *Cyperaceae* presence suggest the structure of the landscape to be open forest. *Corylus* expands. Deciduous species present but not expanding.

Table 6.3 Summary of the results from the pollen analysis and interpretation

6.3.3 Charcoal Analysis

The pollen diagram showed that *F. sylvatica* expanded in the period 2000 to 500 BC (Figure 6.4; Figure 6.5). Charcoal analysis in this period shows a period of greater fire between 1350 BC to 750 BC with a peak at 1050 BC and a large peak at 830 to 840 BC (Figure 6.6).

6.4 Discussion

The pollen diagram obtained from Carlshof Small Forest Hollow provides an uninterrupted record of vegetation history at the stand-scale from approximately 9000 BC to AD 2007. This site allows for a detailed examination of the stand-scale record of the establishment of *F. sylvatica* and the influence on the species of climate, anthropogenic activity and its interaction with fire. It is one of the few stand-scale sites in Mecklenburg-Vorpommern.

The pollen diagram shows that two populations of *F. sylvatica* have existed in the north-eastern section of Peutscher forest during the Holocene. The first in the period 7300 to 5900 BC was characterised by low pollen percentages (<5%) throughout, which never expanded. This suggests only a small population of *F. sylvatica* was close to the hollow during this period. The second population existed from 4500 BC to present. This population also had low pollen percentages for approximately 3600 years highlighted by a long extended tail in the pollen diagram from 4500 to 900 BC. This suggests that during this period *F. sylvatica* was present in the forest but never in significant quantities. This pollen curve starts expanding around 950 to 900 BC, before reaching a peak in pollen percentages around AD 900. During this period *F. sylvatica* is the likely dominant species of the forest stand. After AD 900 the values in the *Fagus* pollen curve subsequently declines with some fluctuations. The youngest sample counted (~AD 1950) has a percentage of 27% in a forest co-dominated by regenerated *F. sylvatica.*

Godwin (1956) citing the work of Firbas (1949) discusses the migration of *F. sylvatica* across Germany. It is discussed that the species is present in the Böhmerwald in southern Germany around 5000 to 6000 BC and arrives at the Baltic Coast around 2000 BC. Although refined by subsequent research, the timing is approximately correct. The maps presented by Magri *et al.,* (2006) suggest that *F. sylvatica* spread into northern Germany, near the study area, around 3750 BC. This makes the first *Fagus* population around the hollow, which dates in the period 7300 to 5900 BC, an anomaly. Traditional interpretation would be that this pollen is of long-distance origin. However this view must be rejected for two reasons. Firstly the site type is stand-scale and therefore receives the majority of its pollen grains from trees surrounding the hollow, making these types of sites less susceptible to pollen of non-local origin. Although there is a chance of sporadic long-distance pollen making its way into the pollen rain, the observation that the *Fagus* pollen curve is continuous in this period, argues against this being chance long-distance pollen. Secondly there is growing acceptance that small outlying populations of differing tree species existed away from the main expansion wave of the parent population in Holocene tree migrations. Bennett (1988) and Woods and Davies (1989) discuss the possibility of small populations existing on a landscape, long before larger pollen sites can detect them, thus making the population cryptic. McLachlan *et al.,* (2005) provide evidence for this scenario using a genetic marker study. In respect of *F. sylvatica,* two other recent sites also show early populations, long before traditional interpretations suggest they migrate into the area. Bradshaw *et al.,* (2010) cite the finding of a 9000 year old Danish population whilst Grant (2005) discusses the discovery of an 8500 year old English population. These findings suggest the assumed migration rates for the species migration are overestimated.

Between 5900 BC and 4500 BC the species is not detected in the pollen record at Carlshof. The suggestion that the tree is present but masked by other high pollen producers must be rejected as *Tilia*, a much lower pollen producer, is present throughout this period. During this period the pollen diagram suggests that *Pinus* and *Tilia* are significant species in the forest composition. Also, *Betula* values are low, anthropogenic indicators are missing and the charcoal record has no peaks suggesting little disturbance in the forest ecosystem in this period. The failure of this first *F. sylvatica* population to expand contradicts the competitive advantage that this shade tolerant tree should demonstrate once the species migrates into an area (Ellenberg 1996), suggesting other forces must be the dominant control on its long term expansion history.

Fagus pollen grains reappear in the diagram from around 4500 BC. Whether this 2nd population of *F. sylvatica* is a direct descendent of the 1st would require information only obtainable by genetic analysis. Behre (1988) discusses that 4500 to 3800 BC (Behre 5500 to 5000 years uncalibrated BP) is the period which witnesses the real advance of farming cultures into the North German lowlands. At Carlshof, the reappearance of *Fagus* pollen coincides also with a find of *Secale* pollen and a very large spike in *Betula* values. This presents a potential argument that *F. sylvatica* was reintroduced, probably unintentionally, by farmers. Charcoal data shows that preceding 4500 BC there was a higher occurrence of peaks in the charcoal record. This increase in charcoal indicates that fire was more prevalent in this period. It could be argued that during this period, the fire regime was unsuitable for the species.

After the reappearance of *F. sylvatica*, its pollen curve is discontinuous until 3800 BC, after which it appears in every sample for the remainder of the profile. It however has low pollen percentages, exhibited in a long tail in the diagram until 900 BC. During this 3000 year period the pollen diagram shows *Alnus*, *Betula*, *Fraxinus*, *Pinus*, *Quercus* and *Tilia* to be tree species present in the forest. *Corylus* is the main dominant pollen taxon and suggests the shrub was an important component of the vegetation assemblages in an open forested landscape around the hollow. The view of Ralska-Jasiewiczowa *et al.*, (2003) that in a battle for colonisation between *F. sylvatica* and *Carpinus betulus,* which both spread late into the area, *F. sylvatica* can more easily colonise *Corylus* shrubbery than *Carpinus,* is difficult to interpret in this pollen diagram. Both *F. sylvatica* and *Carpinus* co-exist with *Corylus* for a long period of time suggesting other species were blocking them from expanding.

Although it appears disturbance facilitates the re-introduction of the species it asks the question to why the species does not expand at 4200 BC, in the period of low disturbance after the clearance event? The disturbance appears to have no effect on the *Tilia* pollen percentages suggesting that potentially competitive species were not disadvantaged. Also in the period 3800 to 2400 BC there are a number of charcoal peaks. It may be the case that the fire regime may have been unsuitable for the species to expand.

The species begins its delayed expansion between 950 – 900 BC (Figure 6.5). This time interval coincides with a shift to wetter and cooler conditions in the climate of the region (Ralska-Jasiewiczowa *et al.*, 2003). Evidence for this is found in the pollen record of the hollow itself. *Sphagnum* values rise sharply and grains of *Potamogeton* are found around 850 - 800 BC until around 450 BC. Considering the associated errors with the radiocarbon dates and age-depth models this period is almost synchronous with the period 750 to 350 BC (2700-2300 cal BP), a period of wetter/cooler condition purposed by Van Geel et al., (1996) and discussed in the context of *F .sylvatica* dynamics by Ralska-Jasiewiczowa *et al.*, (2003). The period of expansion also coincides with lower amounts of charcoal being present in the sediment. However, this combination of factors is also present in the period 1850 to 1450 BC and *F. sylvatica* does not expand. During this early period, *Tilia* percentages suggest the species is a significant part of the forest and percentages of anthropogenic indicators are low.

Around 1300 BC, *Tilia* values begin to decline and there is an increase in *Betula* values and charcoal particles. Then at 1100 BC, there is an increase in anthropogenic indicators exemplified by the wild grass group, *Artemisia*, *Caryophyllaceae*, *Chenopodiaceae* and *Plantago lanceolata*. Interpreting this assemblage after Behre (1988) suggests that the land around the site was abandoned after the disturbance initiated around 1300 BC. It is immediately after this abandonment that the *Fagus* pollen curve begins to rise. A large peak in charcoal after the initial establishment does not appear to impede the rise in the curve but instead causes *Pinus* to decline, potentially aiding *F. sylvatica*. This sequence of events argues that disturbance, in the form of clearance and removal of *Tilia*, is the most important driver for *F. sylvatica* expansion but that the correct climate conditions and fire regime at the time of establishment are important precursors to help facilitate the expansion.

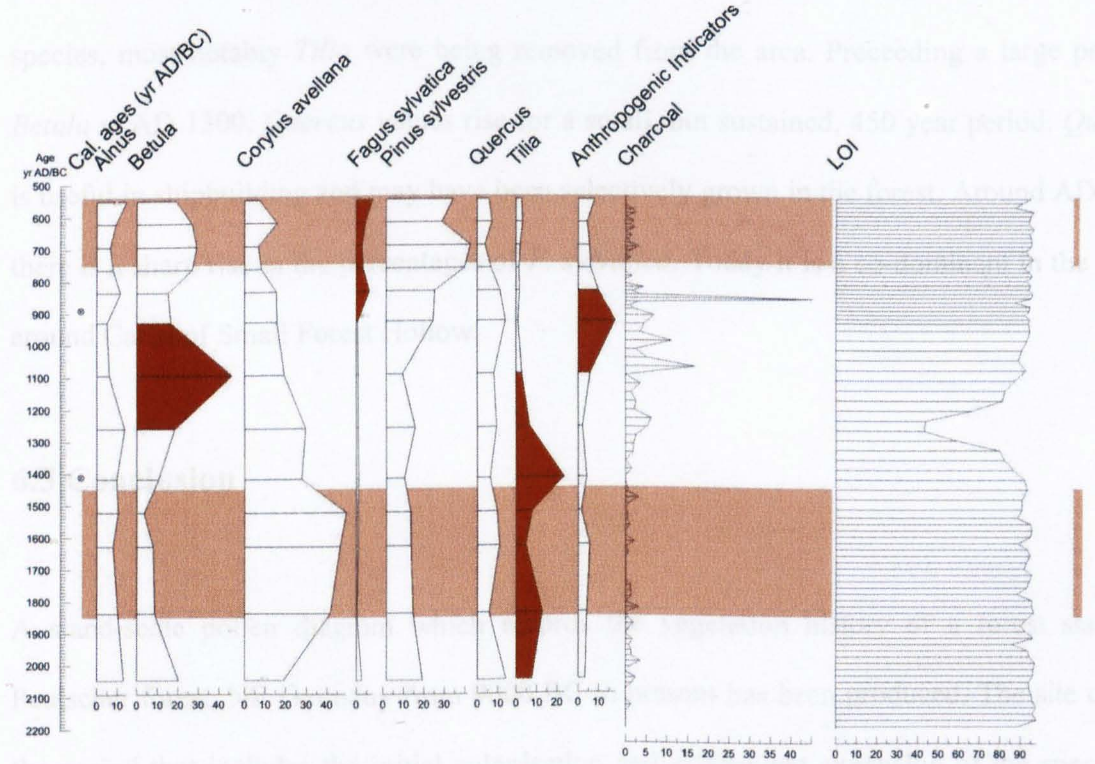

Figure 6.5 Summary of key pollen taxa, anthropogenic indicators (sensu Behre 1981), charcoal and LOI values over the period of initial *F. sylvatica* expansion, 2100 to 500BC. Pink zones highlight wetter and cooler periods discussed by Ralska-Jasiewiczowa *et al.*, (2003) and Tinner and Lotter (2006).

After this initial expansion, *Fagus* pollen percentages rise sharply until approximately AD 800, when they reach their highest values of the entire profile at 50%. The observation of uninterrupted dominance of the forest fits the competitive advantage that this shade tolerant tree should demonstrate once the species migrates into an area (Ellenberg 1996). It also coincides with fewer disturbances in the forest, in a similar observation to that of Odgaard (1994) in Denmark.

Between AD 800 and AD 2007 the *Fagus* pollen curve declines, but is still a dominant component of the pollen assemblage and therefore the forest around the site. This decline coincides with an increase in anthropogenic indicators. The presence of *Avena/Triticum*, *Centaurea*, *Hordeum* and *Humulus*-type suggest that cereals were being grown close to the site. However, the major change is an increase in *Betula* and *Pinus* suggesting the other tree species, most notably *Tilia* were being removed from the area. Preceeding a large peak in *Betula* at AD 1300, *Quercus* values rise for a small, but sustained, 450 year period. *Quercus* is useful in shipbuilding and may have been selectively grown in the forest. Around AD 1850 there is a sharp rise in the percentages of *F. sylvatica.* Today it is a co-dominant in the forest around Carlshof Small Forest Hollow.

6.5 Conclusion

A stand-scale pollen diagram which records the vegetation history of a forest stand in Peutscher forest, NE Germany from 9000 BC to present has been produced. The site covers the period that includes the initial colonisation and subsequent expansion of the species *F. sylvatica* in the forest. This allows this site to be put in context with other findings in northern central Europe. The site records a small population of *Fagus* around the hollow, long before regional pollen data suggest it should be present. This finding adds to the growing volume of evidence (Grant 2005; Bradshaw *et al.,* 2010) that the species had a number of outlying populations away from the main migrating front. This has important consequences for the interpretation of how the species spread across the continent of Europe in the Holocene.

The pollen curve of *F. sylvatica* rises around 950-900 BC, after a 3600 year period of low abundance in the forest. This expansion appears facilitated by a clearance event, including the reduction in the amount of *Tilia* in the forest. The success of the species in taking advantage of this disturbance was improved by the climate being in a wetter and cooler phase and the low frequency and intensity of the fire regime. In historical times, *F. sylvatica* appears disadvantaged by anthropogenic activities, mainly farming and clearance, in the area.

Chapter Seven – Studying *Fagus sylvatica* using ancient DNA

7.1 Overview

The literature review for *Fagus sylvatica* (chapter one) showed that the species' modern haplotype population structure is relatively well understood at the continental scale (Demesure *et al.,* 1996; Magri *et al.,* 2006). These continental studies have been supplemented by studies at the regional scale (Vettori *et al.,* 2004) as well as studies into different microsatellite markers (Pastorelli *et al.,* 2003; Gailing & von Wuehlisch 2004). These modern structures combined with fossil pollen and macrofossil data have allowed inferences to be made into both the direction and timing of how the species migrated during the Holocene (Brewer 2002; Magri *et al.,* 2006). To test these ideas directly, information is needed about the location of different haplotypes during different time periods. This information can only be obtained by retrieving DNA from the past.

The review of ancient DNA (chapter two) showed that the research field is very advanced in certain areas and for a number of different depositional environments. These advances though, have been mainly with human and animal species and the depositional environments have concentrated on permafrost or dry climate areas. In plant migration studies, outside these areas, the aDNA research field is very much in its infancy. To help better understand Holocene tree migrations in Europe, the extraction of aDNA from fossil material would have to be carried out focussing on depositional environments in temperate areas. This is far from optimal as DNA preserves better in cold and dry environments, it's no coincidence that the vast-majority of studies that have been carried out, so far, has done so with material preserved in these areas.

In temperate areas of Europe, potential aDNA projects could take place targeting any tree species whose modern geographic DNA structure is relatively well known. Target depositional environments could be potentially any that are used in current palaeoecological studies. Three target materials for extraction potentially exist; these are sediment, pollen and plant macrofossils. There is a pressing need for studies assessing DNA preservation and authentication issues using these different combinations of study materials and sites.

For this study, *F. sylvatica* was chosen as a target species because its modern DNA distribution pattern is well understood (Magri *et al.,* 2006). Furthermore, the existence of numerous, well preserved, macrofossil records for the species is known. Macrofossils were chosen as they provided a much greater sample volume than pollen grains. The macrofossils can be split and therefore full replication of the original sample can take place. This cannot happen with a pollen grain. Macrofossils are also, in theory, less susceptible to leaching out of DNA than raw sediment. Stand-scale sites were chosen as target depositional environments, as these sites allow for rapid burial of macrofossils, which it was hypothesised allow for greater preservation quality as the macrofossil is preserved intact and is denied oxygen. In this project the target sites were small basins bordered by *F. sylvatica* trees. No previous studies using ancient DNA had been performed using the species *F. sylvatica* or from materials found in forest hollow sediment.

The initial research aim was to test if aDNA was preserved in macrofossils from these types of environments (study I). Secondly, the potential of any preserved material to provide useful information to the palaeoecologist (study II & III) was examined. This chapter discusses the findings of work performed in the EVOLTREE project (www.evoltree.eu) from August 2006 to July 2009.

7.2 Project design

The original plan was to use sites developed in chapter three, alongside known macrofossil records from Suserup (Hannon *et al.,* 2000) and Muritz (Hannon in prep). Two other sites in the southern range for the species were also considered, a site in Bulgaria and a site in northern Italy. The sites were all stand-scale except the northern Italian site (Lago Pratigano). This site is a larger palaeoecological site but was included in the study as it matched two specific criteria. Firstly, it preserved macrofossils in a very good condition, and secondly it had a record of the species history from the early Holocene (Watson 1996). Also this site should, in theory, highlight different haplotypes from the hollows further north (Figure 7.1).

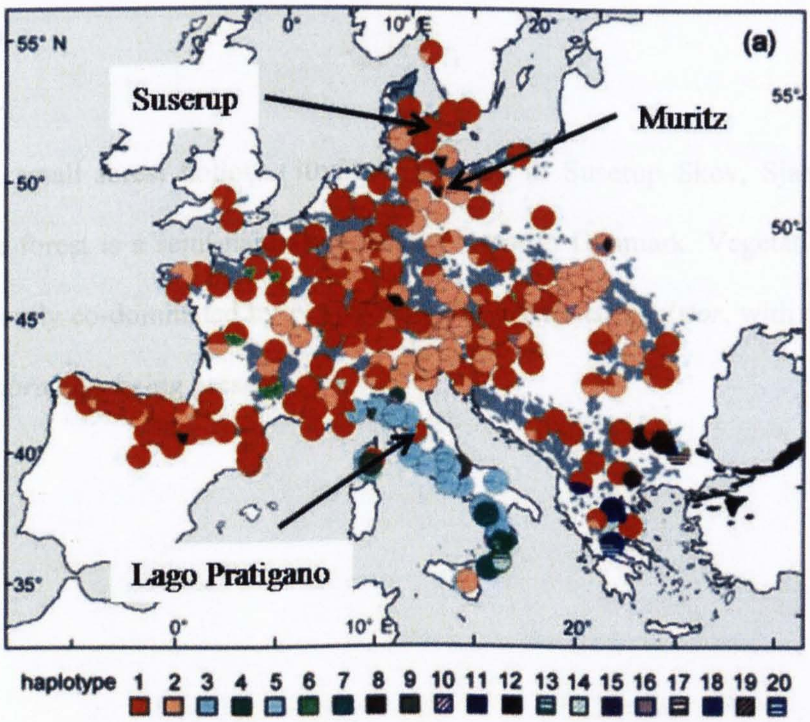

Figure 7.1 Location of sites and haplotype distribution after Magri *et al.,* (2006)

The work requires the use of isolated, specialised clean laboratories to minimise the risk of contamination. The ancient DNA laboratory at the University of Copenhagen was used in this study.

7.3 Sites

Three sites were used in this study. Suserup is a small forest hollow in Denmark, Muritz is a small forest hollow in Germany, Lago Pratigano is a larger peat basin in Italy. The sites presented in chapters 5 and 6 were examined for suitability in this study. At each site the macrofossil content was not sufficient to proceed with aDNA studies. Also a forest hollow located in Bulgaria (42°02"N 22°53"E) was examined but judged unsuitable for any palaeoecological study.

7.3.1 Suserup

This site is a small forest hollow (30x20m) situated in Suserup Skov, Sjaelland, eastern Denmark. The forest is a semi-natural deciduous forest in Denmark. Vegetation around the hollow is currently co-dominated by *F. sylvatica* and *Fraxinus excelsior*, with *Quercus robur* and *Ulmus glabra* also being present.

Figure 7.2 Photograph of Suserup small forest hollow

The forest has a strong research history of direct observation summarised in Hahn *et al.*, (2007). This is complemented by the detailed palaeoecological study of Hannon *et al.*, (2000) which investigated the history of the forest using pollen, macrofossil and charcoal analyses from the forest hollow. Hannon's study showed that *F. sylvatica* replaced *Tilia* in process catalysed by anthropogenic activity. This process commenced around 1000 BC and lasted till around AD 1000. From this period, macrofossils of *F. sylvatica* are found in significant quantity. This study also showed that stand-scale sites can preserve a large number of well preserved macrofossils

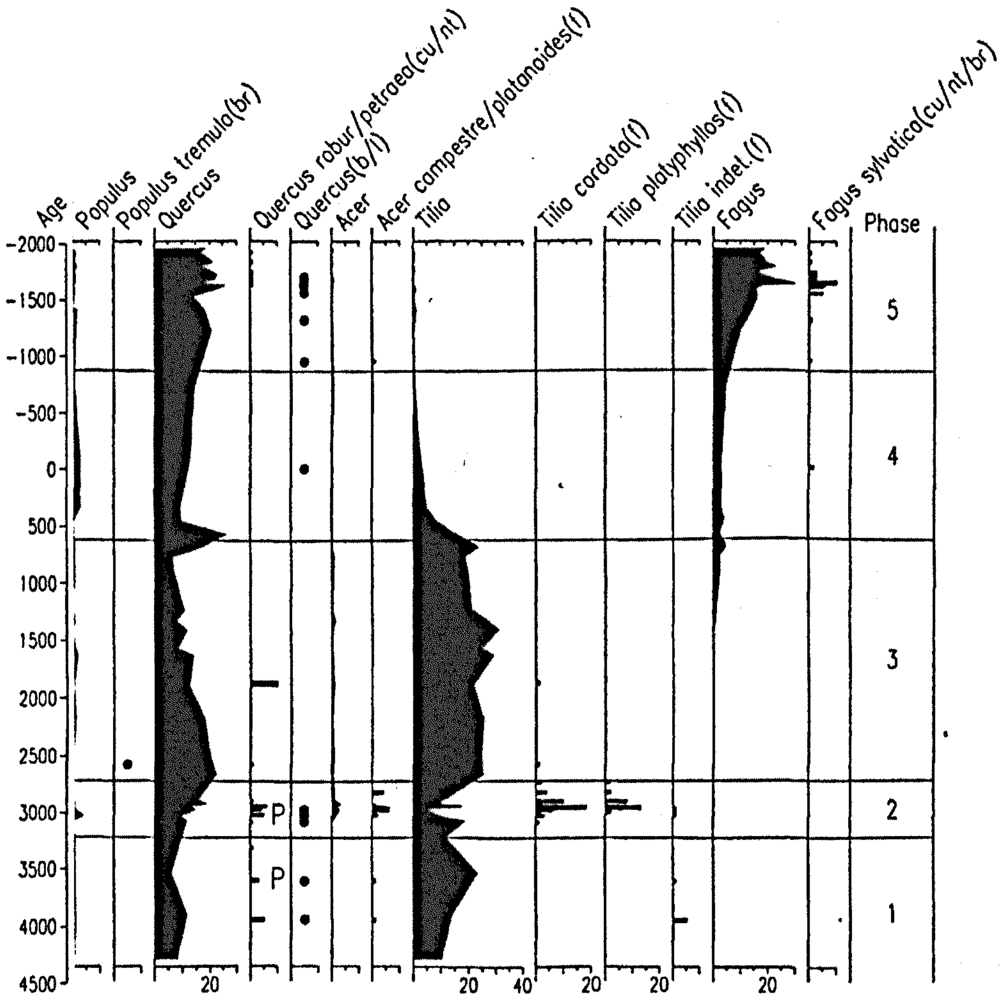

Figure 7.3 Summary pollen and plant macrofossil diagram from Hannon *et al.*, (2000).

7.3.2 Muritz

This site is a small forest hollow that is rectangular in shape. The site is situated in Muritz national park in a nature conservation area. Water accumulation at the basin has been observed to a depth of at least 1m. Hannon (in prep) showed the site to have an excellent macrofossil record for the period 3750 to 250 BC over a 140cm sedimentary profile. The majority of finds were from the species *Betula*, *Alnus* and *Cyperaceae*. Macrofossils of *Populus*, *Quercus*, *Tilia* were also common. Leaves and bracts for *F. sylvatica* were found after 1250 BC but never in large quantities.

Figure 7.4 Photograph of Muritz forest hollow

7.3.3 Lago Pratigano

This site is elongated basin containing both lake and bog sediment. It is situated in the Apennine Mountains, Italy. The eastern side of the lake contains *F. sylvatica* forest whilst the western side is dominated by *Abies* (Figure 7.5)

Figure 7.5 Photograph of Lago Pratigano.

The site was investigated using fossil pollen by Watson (1996) who reports a rise in *Fagus* pollen around 3750 BC (Figure 7.6).

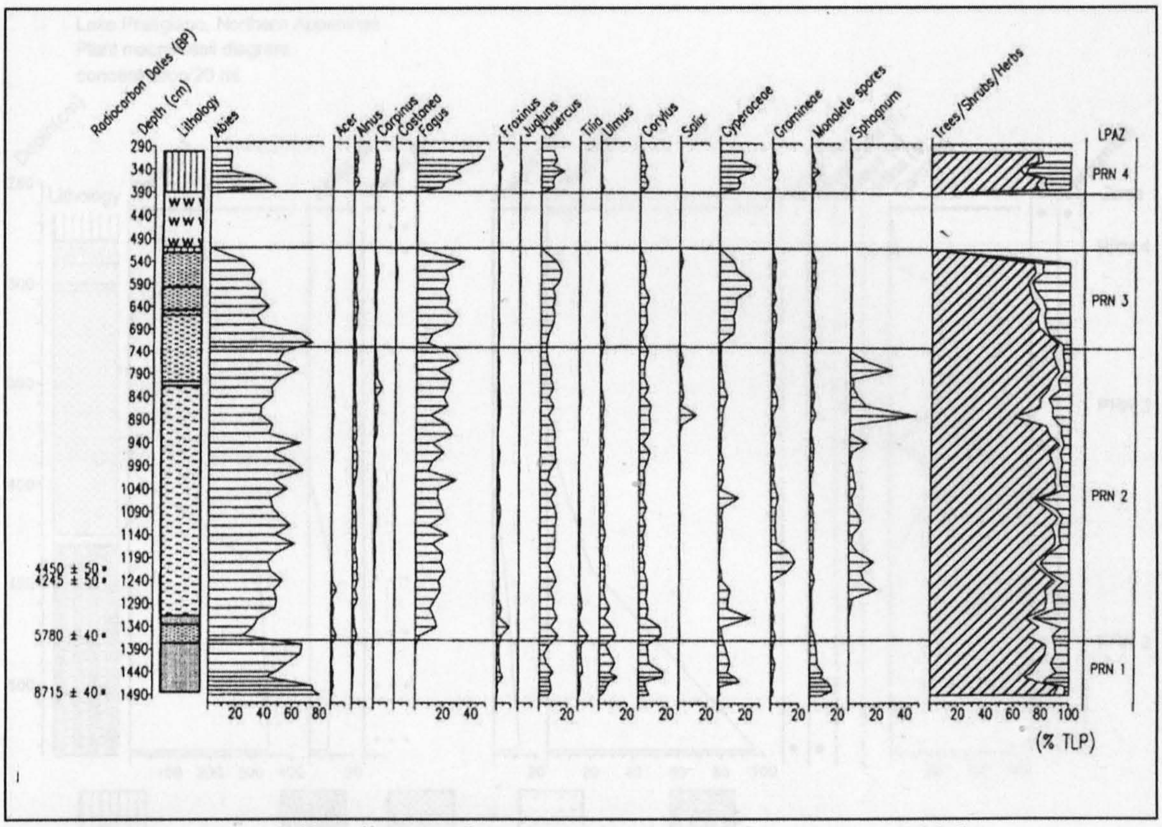

Figure 7.6 Summary pollen diagram from Lago Pratigano after Watson (1996)

A study into the macrofossil content of the sediments was performed by Hannon (pers. comm.). The site was found to be abundant in macrofossils of *F. sylvatica* (Figure 7.7) from a core located close to the edge of the basin.

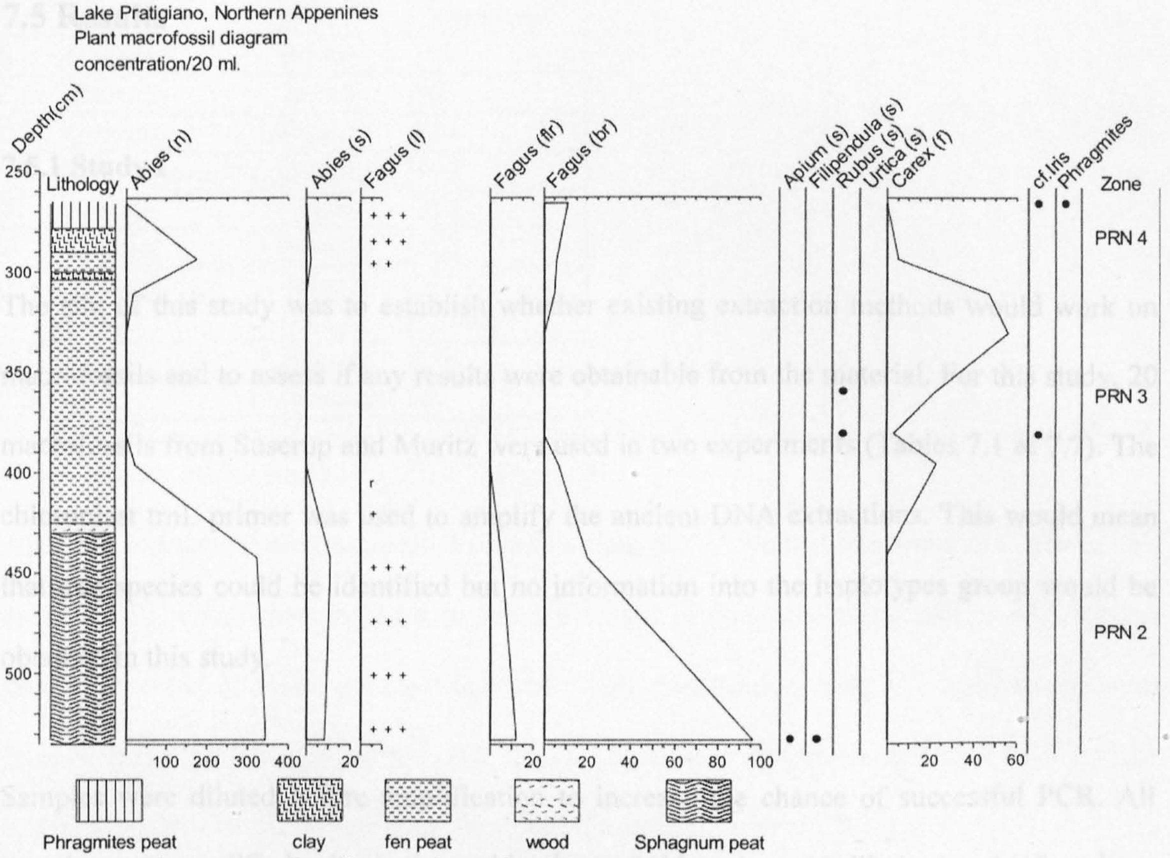

Figure 7.7 Plant macrofossil overview after Hannon (pers. comm.).

7.4 Methods

Chapter three discusses how the fieldwork, macrofossil analysis and aDNA extraction were performed. Specific details of each Polymerase chain reaction (PCR run) for the three studies are explained in the results sections of each study.

7.5 Results

7.5.1 Study I

The aim of this study was to establish whether existing extraction methods would work on macrofossils and to assess if any results were obtainable from the material. For this study, 20 macrofossils from Suserup and Muritz were used in two experiments (Tables 7.1 & 7.7). The chloroplast trnL primer was used to amplify the ancient DNA extractions. This would mean that tree species could be identified but no information into the haplotypes group would be obtained in this study.

Samples were diluted before amplification to increase the chance of successful PCR. All samples were amplified using both combinations of (45 cycles x 10 dilution) and (45 cycles x 50 dilution). One experiment comprising 60 cycles was also performed. During extraction, laboratory and PCR setup controls were also processed to assess contamination caused by the laboratory process.

Study I – Experiment One

The first experiment focused on macrofossils from predominantly the Muritz site (Table 7.1). Comparisons with the dated macrofossil record of Hannon (in prep) suggest the oldest ages are approximately 2200 BC at Murtiz and AD 1000 at Suserup. Alongside these samples, one extraction control and five PCR controls were processed to detect contamination in the laboratory.

ID	Species	Site	Depth (cm)
1	*Quercus*	Muritz	120
2	*Quercus*	Suserup	96
3	*Quercus*	Muritz	124
4	*Fagus sylvatica*	Muritz	107
5	*Populus*	Muritz	250
6	*Populus*	Muritz	220
7	*Populus*	Muritz	151
8	*Tilia*	Muritz	167-170
9	*Tilia*	Muritz	220
10	*Tilia*	Suserup	96

Table 7.1 Overview of samples used in extraction A. Analysts Lee Bradley, Tina Brand, Gina Hannon. Performed 13th and 14th March 2007.

After the extraction protocol was finished, all the aDNA extractions were run through 60 cycles of PCR and then assessed to see the length of fragment using gels. Amplification products were checked on gel electrophoresis with visualisation using UV light. Bands were promising for the *Populus* samples (ID 5,6,7). There were also bands in both a PCR control and a lab control confirming that contamination is present in the PCR runs.

ID	Species	Site	Depth (cm)	Bands
1	*Quercus*	Muritz	120	
2	*Quercus*	Suserup	96	
3	*Quercus*	Muritz	124	
4	*Fagus sylvatica*	Muritz	107	
5	*Populus*	Muritz	250	Yes
6	*Populus*	Muritz	220	Yes
7	*Populus*	Muritz	151	Yes
8	*Tilia*	Muritz	167-170	
9	*Tilia*	Muritz	220	
10	*Tilia*	Suserup	96	

Table 7.2 Overview of first PCR run (60 cycles)

A second PCR was run with a reduced number of PCR cycles (Table 7.3). Strong bands were discovered for ID's 3 4 6 7 8. Bands also discovered in extraction control and 3 of the PCR controls.

ID	Species	Site	Depth (cm)	Bands
1	*Quercus*	Muritz	120	
2	*Quercus*	Suserup	96	
3	*Quercus*	Muritz	124	Yes
4	*Fagus sylvatica*	Muritz	107	Yes
5	*Populus*	Muritz	250	
6	*Populus*	Muritz	220	Yes
7	*Populus*	Muritz	151	Yes
8	*Tilia*	Muritz	167-170	Yes
9	*Tilia*	Muritz	220	
10	*Tilia*	Suserup	96	

Table 7.3 Overview of second PCR run (45 cycles + diluted x10)

A third PCR was then performed on this extraction (Table 7.4), this time the extractions were more dilute. Strong bands were discovered for samples 4, 6, 7 and 9. Bands were also found in the extraction control and in two PCR controls.

ID	Species	Site	Depth (cm)	Bands
1	*Quercus*	Muritz	120	
2	*Quercus*	Suserup	96	
3	*Quercus*	Muritz	124	
4	*Fagus sylvatica*	Muritz	107	Yes
5	*Populus*	Muritz	250	
6	*Populus*	Muritz	220	Yes
7	*Populus*	Muritz	151	Yes
8	*Tilia*	Muritz	167-170	
9	*Tilia*	Muritz	220	Yes
10	*Tilia*	Suserup	96	

Table 7.4 Overview of third PCR run (45 cycles + diluted x50)

A variety of PCR products were chosen for cloning (Table 7.5) after the result of all three PCR's were analysed.

ID	Species	Site	Depth	PCR Run
A	*Populus*	Muritz	107	1
B	*Populus*	Muritz	151	1
C	Extraction control			1
D	*PCR control*			2
E	*Quercus*	Muritz	124	2
F	*Populus*	Muritz	220	2
G	*Tilia*	Muritz	167 – 170	2
H	*Fagus sylvatica*	Muritz	107	3
I	*Tilia*	Muritz	220	3
J	PCR control			3

Table 7.5 PCR products chosen for cloning

The results of the cloning showed the majority of products did produce clones but in relatively small numbers (Table 7.6)

ID	Attempt 1	Attempt 2	Further PCR
A			
B		x18	
C		x11	
D		x8	
E			
F	x10	x7	Yes
G	x7	x7	
H	x10		
I	x16		Yes
J			

Table 7.6 Successful clones from individual samples

Study I – Experiment Two

This experiment focused on a different set of macrofossils (Table 7.7). This time, the majority of macrofossils were from Suserup. Correlation with the age-depth model of Hannon *et al.,* (2000) suggested the oldest macrofossils from this site could potentially date to AD 800.

Two extraction controls and four PCR controls were also used.

ID	Species	Site	Depth (cm)
1.2	*Tilia*	Suserup	95
2.2	*Tilia*	Muritz	167-170
3.2	*Populus*	Muritz	250
4.2	*Populus*	Muritz	260
5.2	*F. sylvatica*	Suserup (Core 2)	19-21
6.2	*F. sylvatica*	Suserup (Core 2)	23-25
7.2	*F. sylvatica*	Suserup (Core 2)	37-39
8.2	*F. sylvatica*	Suserup (Core 2)	39-41
9.2	*F. sylvatica*	Suserup (Core 2)	41-43
10.2	*F. sylvatica*	Suserup (Core 2)	43-45

Table 7.7 Overview of extraction two. Analyst Lee Bradley. 20th-21st March 2007. Suserup (Core 2) was stored and processed in GEUS

The same PCR procedures as experiment one was followed except that the experiment with 60 cycles of PCR was not performed. Results from the 1st PCR run (Table 7.8) showed bands for four *F. sylvatica* samples (ID's 5.2, 7.2, 8.2 and 9.2). Both controls used in this experiment were clear indicating no contamination at the laboratory stage of the process.

ID	Species	Site	Depth (cm)	Bands
1.2	*Tilia*	Suserup	95	
2.2	*Tilia*	Muritz	167-170	
3.2	*Populus*	Muritz	250	
4.2	*Populus*	Muritz	260	
5.2	*F. sylvatica*	Suserup (Core 2)	19-21	Yes
6.2	*F. sylvatica*	Suserup (Core 2)	23-25	
7.2	*F. sylvatica*	Suserup (Core 2)	37-39	Yes
8.2	*F. sylvatica*	Suserup (Core 2)	39-41	Yes
9.2	*F. sylvatica*	Suserup (Core 2)	41-43	Yes
10.2	*F. sylvatica*	Suserup (Core 2)	43-45	

Table 7.8 1st PCR run (45 cycles + diluted x10)

A second PCR run was performed (Table 7.9) with the original extractions being more dilute. Bands were found in five samples (ID's 5.2, 7.2, 8.2, 9.2 and 10.2). The two extraction controls and four PCR controls were all negative.

ID	Species	Site	Depth (cm)	Bands
1.2	*Tilia*	Suserup	95	
2.2	*Tilia*	Muritz	167-170	
3.2	*Populus*	Muritz	250	
4.2	*Populus*	Muritz	260	
5.2	*F. sylvatica*	Suserup (Core 2)	19-21	Yes
6.2	*F. sylvatica*	Suserup (Core 2)	23-25	
7.2	*F. sylvatica*	Suserup (Core 2)	37-39	Yes
8.2	*F. sylvatica*	Suserup (Core 2)	39-41	Yes
9.2	*F. sylvatica*	Suserup (Core 2)	41-43	Yes
10.2	*F. sylvatica*	Suserup (Core 2)	43-45	Yes

Table 7.9 2nd PCR run (45 cycles + diluted x50)

After both PCR results had been analysed, five different macrofossil extractions were chosen for cloning (Table 7.10).

ID	Species	Site	Depth (cm)	PCR Run
A.2	*F. sylvatica*	Suserup	19-21	1
B.2	*F. sylvatica*	Suserup	37-39	1
C.2	*F. sylvatica*	Suserup	39-41	1
D.2	*F. sylvatica*	Suserup	41-43	1
E.2	*F. sylvatica*	Suserup	43-45	2

Table 7.10 Samples chosen for cloning from experiment two

Four of the samples were successfully cloned (Table 7.11). These four sequences were sent for sequencing.

ID	Species	Site	Depth (cm)	PCR Run	Successful clones
A.2	*F. sylvatica*	Suserup	19-21	1	14
B.2	*F. sylvatica*	Suserup	37-39	1	23 (16)
C.2	*F. sylvatica*	Suserup	39-41	1	
D.2	*F. sylvatica*	Suserup	41-43	1	14
E.2	*F. sylvatica*	Suserup	43-45	2	32 (16)

Table 7.11 Result of cloning

Following PCR amplification and cloning of samples, there was sufficient DNA in five *F. sylvatica* samples from Suserup and three Populus samples from Muritz for sequencing. Only the *F. sylvatica* extractions could be positively identified as *F. sylvatica* by comparison with

GenBank sequences (http://www.ncbi.nlm.nih.gov/Genbank). These *F. sylvatica* sequences are listed in table 7.12. The oldest inferred ages are approximately AD 800.

Depth (cm)	Inferred age (AD)	Sequence	Result (% match to Genbank for *F. Sylvatica)*
19-21	1500	ATCCTATTTTCCGAAAACAAATAAGGGTTGAGAAGA AAGCAAGAATAAAATAAAAAAAAAAAAGG	100
		ATCCTATTTTCCGAAAACAAATAAGGGTTGAGAAGA AAGCAAGAATAAAATAAAAAAAAAAAAGG	100
		ATCCTATTTTCCGAAAACAAATAAGGGTTGAGAAGA AAGCAAGAATAAAATAAAAAAAAAAAGGG	100
		ATCCTATTTTCCGAAAACAAATAAGGGTTGAGAAGA AAGCAAGAATAAAATAAAAAAAAAAAANG	96
		ATCCTATTTTCCGAAAACAAATAAGGGTTGAGAAGA AAGCAAGAATAAAATAAAAAAAAAAAAGGG	100
37-39	1200	CTTTTTTTTTTTTATTTTATNCTTGCTTTCTTCTCAACC CTTATTNGTTTTCGGAAAATAGGAT	No Hit
		ATCCTATTTTCCGAAAACAAATAAGGGTTGAGAAGA AAGCAAGAATAAAATAAAAAAAAAAANG	96
		CTTTTTTTTTTTTATTTTATTCTGGCTTTCTTCTCAACC CTTATTTGTTTTCGGAAAATAGTAT	95
41-43	1000	ATCCTATTTTCCGAAAACAAATAAGGGTTGAGAAGA AAGCAAGAATAAAATAAAAAAAAAAANG	96
		ATCCTATTTTCCGAAAACAAATAAGGGTTGAGAAGA AAGCAAGAATAAAATAAAAAAAAAAAAG	100
		ATCCTATTTTCCGAAAACAAATAAGGGTTGAGAAGA AAGCAAGAATAAAATAAAAAAAAAAAA	100
		ATCCTATTTTCCGAAAACAAATAAGGGTTGAGAAGA AAGCAAGAATAAAATAAAAAAAAAAAAG	100
		CTTTTTTTTTTTNATTTNATNCTGGCTTNCTNCCCAAC CCTTATTNGTTTTCGNAAAATAGNNT	No Hit
		CTTTTTTTTTTTNATTTNATNCTGGCTTNCTNCCCAAC CCTTATTNGTTTTCGNAAAATAGNNT	No Hit
		CTTTTTTTTTTTNATTTNATNCTGGCTTNCTNCCCAAC CCTTATTNGTTTTCGNAAAATAGNNT	No Hit
		ATCCTATTTTCCGAAAACAAATAAGGGTTGAGAAGA AAGCAAGAATAAAATAAAAAAAAAAAAG	100
		ATCCTATTTTCCGAAAACAAATAAGGGTTGAGAAGA AAGCAAGAATAAAATAAAAAAAAAAAANG	96
43-45	800	ATCCTATTTTCCGAAAACAAATAAGGGTTGAGAAGA AAGCAAGAATAAAATAAAAAAAAAAAANG	96
		ATCCTATTTTCCGAAAACAAATAAGGGTTGAGAAGA AAGCAAGAATAAAATAAAAAAAAAAANG	96
		ATCCTATTTTCCGAAAACAAATAAGGGTTGAGAAGA AAGCAAGAATAAAATAAAAAAAAAAANG	96
		ATCCTATTTTCCGAAAACAAATAAGGGTTGAGAAGA AAGCAAGAATAAAATAAAAAAAAAAAANG	96
		ATCCTATTTTCCGAAAACAAATAAGGGTTGAGAAGA AAGCAAGAATAAAATAAAAAAAAAAAANG	96

Table 7.12 DNA fragments recovered from *F. sylvatica* macrofossils in Suserup Skov, Denmark. Inferred ages come from the age-depth model presented in Hannon *et al.*, (2000).

7.5.2 Study II

Following the success of study I a number of questions were immediately raised. Could this study be replicated? Could older samples be analysed? Could the different haplotypes identified by Magri *et al.,* (2006) be distinguished in macrofossils?

Study II used 15 macrofossils from Lago Pratigano (Table 7.13). All samples were *F. sylvatica.*

ID	Depth (m)	Type
1	7-7.02	Leafs and bracts
2	9.94-9.96	Leaves
3	9.94-9.96	Leaves
4	9.96-9.98	Leaves
5	9.98-10	Leaves
6	9.98-10	Leaves
7	10-10.02	Leaves
8	10-10.02	Bracts
9	10-10.02	Leaves
10	10.02-10.04	Bracks
11	10.02-10.04	Bracks
12	10.04-10.06	Flowers and leaves
13	10.04-10.06	Leaves
14	10.04-10.06	Leaves
15	10.08-10.10	Leaves

Table 7.13 Macrofossils used. A mixture of leaves and bracts were found. Analysts Lee Bradley, Gina Hannon and Christoph Sperisen.

The first study used two different primers (Table 7.14). Both showed bands of varying strength. Bands were also present in the extraction control and PCR control.

ID	Depth (m)	rbcl	trnl
1	7-7.02		Band
2	9.94-9.96		
3	9.94-9.96		
4	9.96-9.98	Band	
5	9.98-10	Band	Faint Band
6	9.98-10		
7	10-10.02	Band	Band
8	10-10.02		
9	10-10.02		Faint Band
10	10.02-10.04		
11	10.02-10.04	Faint Band	Faint Band
12	10.04-10.06	Band	Band
13	10.04-10.06	Faint Band	Faint Band
14	10.04-10.06		
15	10.08-10.10	Band	
16	Ex control		Band
17	PCR control		
18	PCR control	Band	
19	PCR control		

Table 7.14 Results of Extraction A. Date 22nd October 2008. (PCR 45 cycles, diluted 10x. PFU polymerase)

A second extraction (Table 7.15) was performed with slightly different protocol. The trnl primer failed to produce any bands.

ID	Depth (m)	rbcl	trnl
1	7-7.02	Band	
2	9.94-9.96	Faint Band	
3	9.94-9.96	Band	
4	9.96-9.98	Band	
5	9.98-10	Band	
6	9.98-10	Band	
7	10-10.02		
8	10-10.02	Band	
9	10-10.02		
10	10.02-10.04		
11	10.02-10.04		
12	10.04-10.06	Band	
13	10.04-10.06	Faint Band	
14	10.04-10.06	Band	
15	10.08-10.10	Band	
16	Ex control		
17	PCR control	Band	
18	PCR control		
19	PCR control		

Table 7.15 Results of Extraction B. Date 24th October 2008. (PCR 45 cycles, diluted 10x. HiFi polymerase.

After the extractions were performed it was decided not to sequence the products.

7.5.3 Study III

This study revisited the extractions made in study II. Seven primers were designed to detect the 14 potential haplotypes shown for the region in Vettori *et al.*, (2004). These primers were OA754 (112bp), OA642 (118bp), CCMP7 (108bp), CCMP4 (107bp), CDR (74bp), OA830 (91bp) and CCMP6 (60bp). Their design is as follows,

Dt-CDR1 F (213)
GATAATCAAATCAATCTTTCAATG
DT-CDR1 R (286)
AAGGAAAAGGACCAAATAAAG

Fagus_OA642_F
TGAAACTGAAAAGATTGAATGC
Fagus_OA642_R_117
AAAAAGAACCATCGATTGACAAA

Fagus_OA754_F
ACGATTGGGACGCTTATCAC
Fagus_OA754_R_112
GCATAGTCCGGGAATAATTGA

Fagus_OA830_F
ATTCCCGGACTATGCTATGG
Fagus_OA830_R_91
TTCGGATTTTGAAACGAAAGA

Fagus_ccmp4_52_F
TGCTGAATCGATGACCTACG
Fagus_ccmp4_52_R_107
GGGAGGACTCTTCTGACCAA

Fagus_ccmp6_32_F
CATTACGTGCGACTATCTCCA
Fagus_ccmp6_32_R_60
AgCgGaTtTgCtCtTtCtTt

Fagus_ccmp7_54_F
CCACTGTCAAGAGTGAATTTCTT
Fagus_ccmp7_54_R_108
GcAaCcCaAtCcTtGtTtTt3

The extractions detailed in study II were revisited. One sample from study II was removed so controls and extractions could fit on the same plate. Bands were discovered after PCR with four primers (Table 7.16). Three of the primers (CDR1, OA830 and CCMP6) appeared not to work. Neither the extraction control nor PCR were clean, suggesting laboratory contamination.

ID	Depth (cm)	OA 754	OA 642	CCMP7	CCMP4	CDR1	OA830	CCMP6
1	700 - 702	Band	Band	Band	Band			
2	994 - 996		Band		Band			
3	994 - 996		Band	Band	Band			
4	996 - 998			Band				
5	998 - 1000		Band		Band			
6	998 - 1000		Band					
7	1000 - 1002			Band				
8	1000 - 1002			Band				
9	1000 - 1002		Band	Band	Band			
10	1002 - 1004		Band	Band				
11	1002 - 1004		Band	Band	Band			
12	1004 - 1006		Band		Band			
13	1004 - 1006							
14	1008 - 1012							
15	Ex control	Band			Band			
16	PCR control			Band				

Table 7.16 PCR results. Analysts Gina Hannon, Christoph Sperisen, March/April 2009

7.6 Discussion

7.6.1 Overview

This chapter investigated the possible utilization of ancient DNA to help test hypotheses generated in the combined modern genetic pattern, fossil pollen and plant macrofossil record papers that are appearing in the literature (Petit *et al.,* 2002; Magri *et al.,* 2006; Tollefsrud *et al.,* 2008). The chapter had two aims with the analysis split into three studies. The first study tested the first aim and examined if DNA was preserved in macrofossils in forest hollow sediment. The second and third study tested the second aim and hoped to show that information needed in the migration debate was possible to find. This would mean finding modern haplotypes identified by Vetori *et al.,* (2004) and Magri *et al.,* (2006), in the past.

The problem of contamination and the ease of obtaining false positive identification means there are now strict criteria to be met before any reports of ancient DNA finds are accepted as authentic (Willerslev and Cooper 2005). Gilbert *et al.,* (2005) argue that a researcher should give enough, detailed information as to how to data were obtained and why the data should believed to be authentic.

Study I was the first attempt at extracting ancient DNA from macrofossils preserved in forest hollow sediment. In study I, four of twenty original samples produced sequences that could be matched to known genetic markers for the species. This represents a 20% success rate eve when the extraction protocol was by no means optimal. No systematic study yet exists to examine optimal techniques for plant macrofossils. All the successful sequences were from extraction B which also had clean extraction and PCR controls. This suggests the only contamination source could be from the macrofossil being contaminated before analysis. All samples were sieved in Liverpool with non sterile water which is a possible source of contamination. Field contamination is unlikely as the macrofossil were imbedded in the sediment matrix. Appropriate molecular behaviour experiments would help to authenticate the results. The extraction should contain more short fragments compared to longer fragments. This information can be found using quantitative PCR. The analysis of sequence results was also essentially a blind test. The matches of the sequences produced in the laboratory with the GenBank sequences were performed by someone outside the project who had no idea which macrofossils we were analysing.

Study II used a different set of macrofossils. These were all from one site in the Apennine mountains. This site is in an area where confusion over the history of *F. sylvatica* is possible. Sitting south of the Po Valley it is unlikely that the tree migrated from the Alps with the most likely scenario being that species migrated from southern Italy. The results from study II and III were also promising but the extraction controls and PCR controls showed signs of contamination. Unlike study I though, sterile water was used throughout.

Study III revisited the extractions made in study II. This time specific primers had been designed and used on the extractions. After PCR, bands in the extraction controls and PCR controls were present. Also three of the seven primers did not work.

The three studies suggest there is reason to be optimistic that macrofossils from forest hollows and small lakes can be used in aDNA studies. Extraction B from study I was the most successful of the experiments for the simple reason that processed controls were blank. Although, there is a need for further tests to be performed before full authentication can be claimed. The results reported in this chapter did not satisfy the strict criteria for full authentication of aDNA. Therefore this study can report promising results from the sites but further research is required to make the argument for authentication stronger. However, this study has given valuable information that can be used in future research design for aDNA studies of *F. sylvatica*.

7.6.2 aDNA from macrofossils from small palaeoecological sites

These types of studies offer the opportunity to test hypotheses relating to plant movement. A basic requirement though is the need for sites in key areas. This should not be a problem given the large number of palaeoecological sites now analysed in Europe.

The length of record and abundance of macrofossil material may be a problem as it is required that there is enough material, around the time of first establishment, of the target species. Liepelt *et al.,* (2006) argue that wood should be the preferred material as it can be large enough to be spilt and sent to more than one laboratory. However we argue that macrofossils also provide the same criteria and provide enough material for independent replication. This study has shown that half an individual macrofossil can provide enough material for a single DNA extraction. Both wood and macrofossils provide a greater amount of working material than pollen grains.

Macrofossils may also provide more chance of positive amplification. Deguilloux *et al.,* (2006), whose study included 51 wood samples, indicate that inhibition could be the major cause of negative amplification in samples extracted from wood. Deguilloux *et al.,* (2006) report a success rate around 10%, although the work did not take place in two separate laboratories. The maximum age of recovery was AD 260. Wood is also found at our sites but was avoided in this study.

Water at the sites could be a major problem in preservation, as hydrological processes damage the DNA molecule. Although Deguilloux *et al.,* (2006) note the surprising finding that aDNA appears to preserve in some waterlogged surroundings, probably attributable to the anaerobic conditions. Haile *et al.,* (2007) tested the vertical migration of aDNA and showed reconstruction to be possible but urged that caution is exercised because genetic material can migrate downwards. The study of Haile *et al.,* (2007) used cave sediment that contained a variable amount of clay. Haile *et al.,* (2007) argued that as clay binds the sediment, it makes it firm and cohesive and lends stability to vertical sections. This means that macroscopic particles cannot migrate through or between the different sedimentary layers. Given the probability of the sediments housing the macrofossil being of critical importance, studies that examine different sites need to be performed. Although, generalizations with so few sites analysed maybe difficult. For example, in this study, Muritz is essentially the same character as Suserup yet one site produced data, and the other did not. The majority of contamination can be controlled. Coring equipment can be stained with known DNA, which if present in extractions, indicates contamination. Samples for DNA extraction are best retrieved in a clean-air room under elevated pressure, and care should be taken to avoid collecting material from the sample surface (Gugerli *et al.,* 2005). Effective demonstrations of the technique, with no contamination, are still required. At present the study of Liepelt *et al.,* (2006) remains the standard at which ancient plant DNA specific to trees in temperate areas of Europe needs to carried out.

Further work investigating modern genetic structures may be just as useful. The idea that early populations have a greater impact on current population structures, thus 'fossilising' the structure which is little affected by more recent immigrants (Hu *et al.,* 2009) may mean that modern DNA patterns should not be underestimated when looking at initial colonization. More detail and filling in of gaps has shown finer scale haplotype patterns exist. Sperisen and Van der Knapp (in prep) have shown in the Swiss Alps that a unique population of *Picea* exists. This led to a re-examination of the pollen data and a new conclusion that the species migrated from southern Germany. This contradicts the previous hypothesis of an East-West traverse of the Alps for the species. With regard to *F. sylvatica*, future work will also benefit from the efforts of the EVOLTREE program. During this research project a large number of genetic markers have been developed and the full genome of the species sequenced for the first time (Vendramin pers. comms.).

Note

The fieldwork was assisted by Richard Bradshaw, Claire Jones, Thomas Giesecke and Jan Bloemendal. The extraction work was a shared effort alongside Gina Hannon and Christoph Sperisen. Primer design was carried out by Christoph Sperisen and Morten Rasmussen, and checked by Beppe Vendramin. James Hartwell, Susanne Boxhall and Pim van der Knapp are thanked for useful discussions. Tina Brand is thanked for training and patience in the aDNA laboratory.

Chapter Eight – Discussion

8.1 Overview

This body of work was designed to provide information that could help to further understand the Holocene history of the tree species *Fagus sylvatica* L. (European Beech). The introduction to this thesis and recent papers (Magri 2006; Tinner & Lotter 2006; Giesecke 2007; Magri 2008; Bradshaw et al., 2010) have examined various aspects of the Holocene spread and expansion of *F. sylvatica* and revealed that a number of issues are still to be resolved in understanding the species history. Despite recent work in northern Germany (Spandenburg 2008), and further work in southern Scandinavia (Lindbladh *et al.,* 2008; Hannon *et al.,* 2010; Hultberg *et al.,* 2010), there was, and still is, a clear need for new stand-scale sites, containing *Fagus* records, in different areas of Europe. The main aim of these sites would be to examine the relationship between disturbance and the species establishment. The overarching theme of this thesis was therefore to collect information on the species at the forest stand-scale. The thesis attempted to look at both spread and expansion of the species and took a broad approach with three separate projects. The results obtained in each project have made a small contribution to better understanding the species' past.

The work produced in this thesis comprises two new stand-scale pollen and charcoal diagrams from Germany. One of these diagrams are from a site located in Brandenburg. This has been compared with the regional diagram of Giesecke (2001) and when the data are combined, they provide a detailed examination of analysing forest dynamics, including *F. sylvatica,* at different spatial scales in the Schlaubetal Valley. The primary finding of this study was that the rise in *Fagus* pollen percentages occurs immediately after a disturbance event in the forest ecosystem. This study also highlights the contribution that historical maps can make to stand-scale studies. The third new palaeoecological dataset was collected from Mecklenburg-Vorpommern. The record at this site is approximately 9000 years old, which is unusually long for a stand-scale site. This site has a particularly interesting *F. sylvatica* record. Firstly, it provides evidence of an outpost population of *F. sylvatica* before the main population expanded. Secondly it provides a stand-scale viewpoint of the first major establishment of the species in the forest.

The data-model comparison exercise was performed using an adapted cellular automata model with its output being compared with published stand-scale pollen diagrams from Denmark and southern Sweden (Aaby 1983; Andersen 1988; Bradshaw and Hannon 1992; Abrahamsson 1996; Axelsson 1996; Björkman 1996; Björkman and Bradshaw 1996; Eriksson 1996; Andersson 1997; Björkman 1997a; Björkman 1997b; Lindbladh and Bradshaw 1998; Lindbladh 1999; Hannon *et al.,* 2000; Björkman 2001; Valdemardotter 2001; Björkman and Sjögren 2003; Lindbladh *et al.,* 2003; Lindbladh *et al.,* 2007a; Lindbladh *et al.,* 2007b; Hultberg 2010; Eriksson unpublished; Hannon (unpublished)). This work was performed with the aim of testing hypotheses related to spreading dynamics of *F. sylvatica* and its interaction with the species *Picea abies*. The analysis and simulations covers the late Holocene and confirms that the spread of *F. sylvatica* was not in the form of a diffuse moving front as described by Magri (2008) but a more scattered migration northward. Finally, attempts were also made to retrieve ancient DNA from macrofossils of various ages from small forest hollows and a small overgrown lake. Results from this study are promising. The data collected in the thesis allow for the three questions raised in chapter one to be answered.

8.2 What role has seed dispersal characteristics and postglacial migration rate had in the shaping of the current distribution of *F. sylvatica* and *P. abies* in Denmark and southern Sweden?

The fossil pollen data indicate that *F. sylvatica* and *P. abies* have different spreading patterns in southern Scandinavia. *F. sylvatica* is patchier with advanced populations establishing at variable time periods. This is a similar finding to other studies on a larger spatial scale. Gliemeroth (1995) showed that *F. sylvatica* spread across Europe in a stepwise fashion. It would be interesting to upscale the study presented in this thesis and run the model simulation across Europe. Of particular interest on a European scale would be the distribution and the number of outlying populations which exist under different scenarios given the findings of Giesecke *et al.,* (2007) who showed that climate 6000 years ago was suitable for the species over a large area of its current distribution. Also recent stand-scale work, including work in this thesis, has indicated that early Holocene outliers existed in northern Germany, southern England (Grant 2005), Denmark (Mette Overbolle-Petersen *et al.*, unpublished.) and southern Sweden (Hannon *et al.,* 2010). These outliers must exist either by extreme LDD events in the early Holocene or the species surviving further north, during glacial times, than is currently thought. Extreme LDD could be from original refugia or in a series of LDD events across the continent that occurred in quick succession. These different possibilities need examining. The idea of more northerly refugia fits with the idea of cryptic northern refugia (Stewart and Lister 2001) and also recent molecular work in North America (McLachlan *et al.,* 2005). These more northerly refugia locations have clear implications for the calculations of migration rates and could help, in part, solve Reid's Paradox of rapid plant migration (Clark *et al.,* 1997).

Overall the diffusion model is very poor at explaining the distribution of *F. sylvatica* in southern Scandinavia. Observed arrival times are both older and younger than simulated by the model. Therefore we reject that the dispersal characteristics are responsible for its spread. This finding suggests that another factor or multiple factors are important in explaining the species spread. Anthropogenic impact is important in the species expansion in this area (Bradshaw and Lindbladh 2005). It could also be important in the species migration if human activity acts as a vector of migration. The most likely anthropogenic influence on the species migration is the clearing of suitable areas for the species to move into. The study concluded that *F. sylvatica* is sensitive to its ability to establish colonies in advance of the main migrating front. However this study cannot rule out microclimate or soil as important factors as they were not incorporated into the model.

8.3 What role does disturbance play in the stand-scale establishment of *F. sylvatica* in Northern and Eastern Germany?

Southern Scandinavia has numerous stand-scale pollen diagrams containing records of *F. sylvatica.* Elsewhere in Europe stand-scale coverage is poor, yet it is in these areas where there is still intense debate into the controls on the species establishment. Field surveys conducted as part of this thesis and other surveys have shown the regions of Mecklenburg and Brandenburg to have plenty of hollows that could be studied. In the eastern German field area (Figure 8.1), a total of seven sedimentary basins were discovered. However, only Dammendorf could be defined as explicitly stand-scale, the others being more peatland or temporary small lakes. In the northern German field area (Figure 8.1), a number of basins were discovered that were closed canopy. Both areas have the potential to have numerous stand-scale studies conducted in the same area. The Carlshof site was chosen, in part, because of a small lake situated close to the hollow.

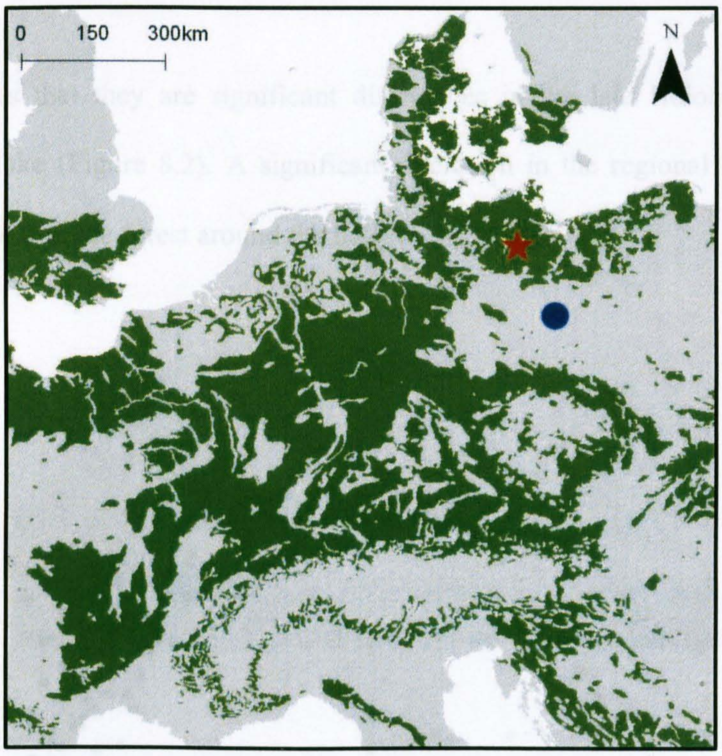

Figure 8.1 Field locations used in Germany. Red star - Carlshof Small Forest Hollow; Blue circle - Dammendorf and Pine Hollow.

In the eastern German field area, at the regional site (Giesecke 2001) the *F. sylvatica* tail begins its rise before an increase in anthropogenic indicators. In other diagrams from the region this relationship is confused, some sites have indicators before expansion, others afterwards. No stand-scale diagram exists in the mid-Holocene to examine this relationship at a better defined spatial scale.

At Dammendorf Hollow there is a persistent pollen tail for approximately 2000 years. The pollen percentages only begin their increase after AD 1700. This lack of expansion, but presence, rejects the competitive edge that the tree's present ecology suggests (Ellenberg 1996). Expansion appears to be prevented by stability in the forest stand. It is only after a clearance event that expansion takes place.

PCA work shows that they are significant differences in the late Holocene between the hollow and the lake (Figure 8.2). A significant alteration in the regional landscape at AD 1300 does not occur in the forest around the hollow.

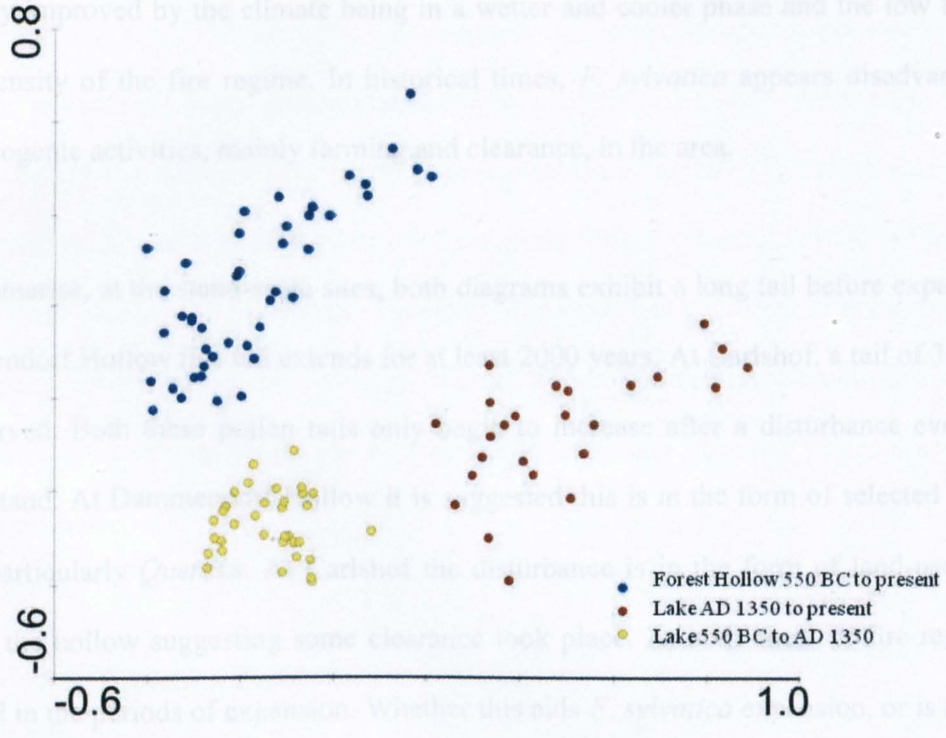

Figure 8.2 PCA Analysis of late Holocene pollen percentage dataset

This study highlights that predicting future changes is especially difficult in areas where the landscape has been fragmented by anthropogenic activity. In the 'semi natural' forest stands of northern Central Europe, multi-scaled reconstructions and stand-scale work are important to understand the histories of forest stands. Diagrams from larger sites may misrepresent the behaviour and history of the remaining forest fragments, especially during the late Holocene in periods of intense anthropogenic activity on the landscape.

In northern Germany, at Carlshof, the pollen curve of *F. sylvatica* rises around 950-900 BC, after a 3600 year period of low abundance in the forest. This expansion appears facilitated by a clearance event, including the reduction in the amount of *Tilia* in the forest. This switch from *Tilia* to *F. sylvatica* is a classical observation in pollen diagrams in northern Central Europe (Behre 1988). The success of the species in taking advantage of this disturbance was probably improved by the climate being in a wetter and cooler phase and the low frequency and intensity of the fire regime. In historical times, *F. sylvatica* appears disadvantaged by anthropogenic activities, mainly farming and clearance, in the area.

To summarise, at the stand-scale sites, both diagrams exhibit a long tail before expansion. At Dammendorf Hollow this tail extends for at least 2000 years. At Carlshof, a tail of 3600 years is observed. Both these pollen tails only begin to increase after a disturbance event in the forest stand. At Dammendorf Hollow it is suggested this is in the form of selected felling of trees, particularly *Quercus*. At Carlshof the disturbance is in the form of land-use changes around the hollow suggesting some clearance took place. At both sites the fire regimes are reduced in the periods of expansion. Whether this aids *F. sylvatica* expansion, or is a result of it, remains unclear. Testing these ideas with cross-correlation analysis would be beneficial. Climate may also be important. At Carlshof, the expansion takes place during a cooler and wetter period, agreeing with the ideas of Ralska-Jasiewiczowa *et al.*, (2003) and Tinner and Lotter (2006). At Dammendorf, this climatic control may not be so important, although the expansion does begin around the time of the Little Ice Age (LIA) climatic event. This observation needs further investigation.

8.4 Can aDNA analysis be useful in helping understand the history of *F.sylvatica* in the Holocene?

The three studies suggest there is reason to be optimistic that macrofossils from forest hollows and small lakes can be used in aDNA studies. Study I was the first attempt at extracting ancient DNA from macrofossils preserved in forest hollow sediment. In study I, four of twenty original samples produced sequences that could be matched to known genetic markers for the species. All the successful sequences were from extraction B which also had clean extraction and PCR controls. This suggests the only contamination source could be from the macrofossil being contaminated before analysis. The results reported in this thesis did not satisfy the strict criteria for full authentication of aDNA. Therefore this study can report promising results from the sites but conclude further research is required to make an argument for authentication stronger. However, this study has given valuable information that has already been used by other studies and can be used in future research design. Studies using aDNA are now set up for the species *F. sylvatica* with regard to protocol and species-specific primers.

8.5 Further findings from data

In addition to providing answers to the three questions set out in the beginning of the thesis, a number of other unexpected results were obtained in the analyses performed. Perhaps one of the most striking and unexpected findings of the pollen analytical work was the detection of a population of *F. sylvatica* in Peutscher forest during the period 7300 to 5900 BC. This population was presumed to be small as it was represented in the pollen diagram by low pollen percentages (<5%) throughout the profile. At no time did this population expand.

The lack of macrofossils from the sediment cores from Carlshof and Dammendorf Hollow meant that the aDNA work relied on revisiting sites that had previously been studied. This allowed for the ability to replicate macrofossil records to be tested. At the three sites where this was performed, Muritz Hollow, Suserup and the larger site Lago Pratigano, excellent correlation between the cores used for the aDNA analysis and the original records were observed, although quantifying this observation was not made as the macrofossils in the aDNA had to processed quickly. This observation is maybe of little surprise in the small basins of Muritz and Suserup but gives confidence to the idea that basins can be resampled.

8.6 Potential Future Research

The research presented during this thesis has contributed, in small part, to the knowledge about the stand-scale history of *F. sylvatica.* Another positive was that throughout work on the three aims it became clear that there is still much to do in researching the species history. Already discussed in this thesis is the need to upscale to data-model work, although the up-scaling would not be possible using stand-scale sites alone. Instead all pollen sites available should be used for inter-comparisons. aDNA studies examining authentication of results and preservation potential of basins are also of critical importance if the technique is to be utilised in migration studies in temperate areas.

Although the work in this thesis highlights the need for data-model comparisons and the fusing together of palaeoecological studies with genetic studies, it also highlights that using fossil pollen grains is still the most effective way to understand forest dynamics in the past. At the stand-scale, there is a very good network of sites documenting the history of *F.sylvatica* in Denmark and southern Sweden. The question as to whether the establishment process is unique to that area or occurs elsewhere has started to be answered by sites in this thesis and others (Spandenburg 2008). There is still a need for the study of well-dated stand-scale pollen sites in other areas of Europe to address this question fully.

Furthermore, the field areas studied in this thesis could be revisited for more detailed work. Studies (Calcote 1995; Björkman 1997; Sugita *et al.,* 2006 and Lindbladh *et al.,* 2008) have showed the benefits of studying multiple hollows in the same area. The topography and forest cover of northern and eastern Germany means there is great potential for this to happen here. Other basins in the Schlaubetal area would allow assessment of fine-scale vegetation patterns and whether changes in assemblages are mirrored at regional and stand-scale. A stand-scale record older than 5000 years in the Schlaubetal would be ideal to compare with the lake record of Giesecke (2001). In this region it is noted that the explicit relationship between anthropogenic activities exists in certain diagrams but not in others. In Mecklenburg, the reverse problem exists. Here Carlshof Small Forest Hollow provides a long history of stand-scale forest dynamics but lacks a comparison with a regional dataset. Potential to expand the study of *F. sylvatica* dynamics, viewed at different spatial scales, exists. During the fieldwork in Peutcher forest a small lake was also investigated (Figure 8.3). Around 3m of sediment was retrieved from this enclosed lake just by using a rubber boat as a platform. Returning to the lake with the correct equipment should allow for a retrieval of the sedimentary sequence.

Figure 8.3 Unnamed lake situated close to Carlshof Small Forest Hollow

Also at individual stand-scale sites, investigations can be further refined. Charcoal records can be better calibrated to fully understand source areas in different ecosystems. The incorporation of non-pollen palynomorphs (NPP) studies would aid fire and anthropogenic disturbance arguments, which are critical in the species establishment, at least in the northern half of its current distribution.

8.7 Summary

Attempts to better understand how the forest trees of Europe have come to arrive at their current location has been an academic discipline for around 100 years. The long-held idea that *F. sylvatica* spread from the peninsulas of southern Europe onto the plains of northern Central Europe and then into southern Scandinavia and southern England is in need of revision. The work of Magri *et al.,* (2006) showed that the northern European population were located to the east of the Alps during the glacial periods. However, how quickly these species moved northwards is not fully understood. The Carlshof site records a small population around the hollow, long before regional pollen data suggests it should be present. This finding adds to the growing volume of evidence (Grant 2005; Bradshaw *et al.,* 2010) that the species had a number of outlying populations away from the main migrating front. This has important consequences for the interpretation of how the species spread and at what rate across the continent of Europe in the Holocene. Stand-scale pollen diagrams suggest that disturbance, in the form of land-use changes, is a very important factor in the species establishment. The time between first arrival and establishment can be of the order of 1000s of years.

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