



# **Hybridisation of Icelandic birch in the Holocene reflected in pollen**

**Lilja Karlsdóttir**





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Lilja Karlsdóttir

**Dissertation submitted in partial fulfillment of a  
*Philosophiae Doctor* degree in biology**

## **Advisor**

Kesara Anamthawat-Jónsson

## **PhD Committee**

Margrét Hallsdóttir

Ægir Þór Þórsson

Ólafur Eggertsson

Ása Aradóttir

## **Opponents**

Christopher J Caseldine

Guðrún Gísladóttir

Faculty of Life and Environmental Sciences  
School of Engineering and Natural Sciences  
University of Iceland  
Reykjavik, March 2014

Title: Hybridisation of Icelandic birch in the Holocene reflected in pollen

Icelandic title: Kynblöndun ilmbjarkar og fjalldrapa á nútíma lesin af frjókornum

Short title: Hybridisation of Icelandic birch in the Holocene

Dissertation submitted in partial fulfillment of a *Philosophiae Doctor* degree in biology

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Faculty of Life and Environmental Sciences

School of Engineering and Natural Sciences

University of Iceland

Askja - Sturlugata 7

101, Reykjavík

Iceland

Telephone: 525 4000

Bibliographic information:

Lilja Karlsdóttir, 2014 *Hybridisation of Icelandic birch in the Holocene reflected in pollen*, PhD dissertation, Faculty of Life and Environmental Sciences, University of Iceland, 116 pp.

ISBN 978-9935-9146-3-7

Printing: Háskólaprent ehf.

Reykjavík, Iceland, March 2014

*Dedication*

*To all the hikers of Icelandic wilderness  
marvelling at the sight of a treelike growth  
in the barren landscape*



## Abstract

The introgressive hybridisation between downy birch, *Betula pubescens* Ehrh., and dwarf birch, *B. nana* L., has been confirmed in Iceland but limited knowledge on the extent or timing of such hybridisation exists. The present study focuses on hybridisation in the Holocene, its frequency and scope, and the environmental factors initiating hybridisation. The history of *Betula* in Iceland is reviewed; possible means of dispersion from NW-Europe and development of woodlands in the Holocene are discussed.

This work is centred on three studies published in attached papers III, IV and V which all rely on the results from papers I and II. The first two papers (I and II) describe the size and shape of *Betula* pollen, sampled from *B. nana*, *B. pubescens* and triploid hybrids in ten existing woodlands. The main study (III, IV and V) was conducted on subfossil pollen sampled from peat at three locations in Iceland. Pollen was prepared with heated NaOH, HCl, HF and acetolysis with conventional methods. The pollen samples were analysed with special emphasis on *Betula* pollen, which was measured for size, and any deviations from normal structure were noted for identification of hybrid pollen. Species proportions were calculated from size distributions.

The three sites revealed different climatic conditions as reflected in the abundance of *Betula* species pollen, other pollen and spores, as well as in meteorological data existing from the 20<sup>th</sup> century. Proportion of *Betula* species fluctuated with climate changes and periods of hybridisation were detected at all three sites, especially connected to warming climate and tree birch advances near the Holocene thermal maximum.





## Útdráttur

Kynblöndun ilmbjarkar, *Betula pubescens* Ehrh., og fjalldrapa, *B. nana* L., og genaflæði milli tegundanna er þekkt en lítið er vitað um umfang blöndunarinnar né hvenær hún hefur orðið. Þessi rannsókn beinist að tegundablöndun birkis á nútíma, þ.e. síðustu tíu þúsund árum, tíðni blöndunar og umfangi jafnframt því sem leitað er að svörum við því hvers konar aðstæður ýta undir blöndunina. Saga birkis á Íslandi er rakin, mögulegar dreifingarleiðir birkis frá norðvestur Evrópu og þróun birkiskóglendis á nútíma rædd.

Verkið snýst að mestu um þrjár rannsóknir sem birtar eru í meðfylgjandi greinum, merktum III, IV og V en þær byggja allar á niðurstöðum rannsókna sem lýst er í greinum I og II. Tvær elstu greinarnar lýsa stærð og lögun birkifrjókorna sem safnað var af ilmbjörk, fjalldrapa og þrílitna blendingum í tíu skóglendum sem enn eru til. Aðalefnið eru rannsóknir sem voru gerðar á frjókornum úr mó sem tekinn var á þremur stöðum á landinu. Frjósýni voru undirbúin með hefðbundnum aðferðum, hituð í NaOH, HCl, HF og brennisteinssýrulausn og síðan skoðuð með sérstakri áherslu á birkifrjókorn. Þau voru mæld og frávik frá eðlilegu útliti skráð til þess að greina blendingafrjókorn. Hlutföll birkitegunda voru reiknuð frá stærðardreifingu.

Mismunandi veðurfarsaðstæður voru á rannsóknarstöðunum þremur sem birtist í magni birkitegundanna, öðrum frjókornum og gróum eins og einnig er hægt að sjá í veðurgögnum frá síðustu öld. Hlutföll ilmbjarkar og fjalldrapa sveifluðust með breytilegu veðurfari og merki um hrinur tegundablöndunar fundust frá öllum stöðunum, einkum í tengslum við hlýnandi veðráttu og útbreiðslu ilmbjarkar nálægt hlýjasta skeiði nútíma.



## Preface

The objectives of these studies were to explore the history of the two *Betula* species native to Iceland and their interaction in the past ten millennia, in order to gain a better understanding of the birch woodlands, so precious in contemporary Icelandic landscape.

For that purpose, subfossil pollen was analysed to reveal firstly, the proportion of each species at different times by measuring and calculating sizes, and secondly, indications of hybridisation of the two species by searching for hybrid pollen.

This was done by utilizing the results of previous research on *Betula* pollen gathered in ten different woodlands and published in papers I and II included in this thesis.

Armed with the results from paper I and II, we approached the objectives by selecting sites in different regions of Iceland, one in the middle north, one in the southwest and the third in the northeast. The third one should preferably have been in the east or southeast, but in that part of the country, proximity to glaciers has left few sites from the earliest Holocene vegetation untouched, thus the northeast was chosen as an alternative site.

In each of these three studies, published in papers III, IV and V, the objectives were met by measuring *Betula* pollen for calculation of species proportions from diameter-based size distribution and by noting and counting non-triporate pollen which is more frequent in hybrids than in the parent species.

The results were interpreted in connection to other vegetation characteristics seen in the pollen samples and what is known of the palaeoclimate for the area and period in question.



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- I. Karlsdóttir L, Thórsson ÆTh, Hallsdóttir M, Sigurgeirsson A, Eysteinnsson Th and Anamthawat-Jónsson K (2007). Differentiating pollen of *Betula* species from Iceland. *Grana* 46: 78-84.
- II. Karlsdóttir L, Hallsdóttir M, Thórsson ÆTh and Anamthawat-Jónsson K (2008). Characteristics of pollen from natural triploid *Betula* hybrids. *Grana* 47: 52-59.
- III. Karlsdóttir L, Hallsdóttir M, Thórsson ÆTh and Anamthawat-Jónsson K (2009). Evidence of hybridisation between *Betula pubescens* and *B. nana* in Iceland during the early Holocene. *Review of Palaeobotany and Palynology* 156: 350-357.
- IV. Karlsdóttir L, Hallsdóttir M, Thórsson ÆTh and Anamthawat-Jónsson K (2012). Early Holocene hybridisation between *Betula pubescens* and *B. nana* in relation to birch vegetation in Southwest Iceland. *Review of Palaeobotany and Palynology* 181: 1-10.
- V. Karlsdóttir L, Hallsdóttir M, Eggertsson Ó, Thórsson ÆTh and Anamthawat-Jónsson K (submitted). Birch hybridization in Thistilfjörður, Northeast Iceland during the Holocene. The paper has been submitted to a peer-reviewed journal.

*On grounds of copyright issues the version of the papers in this online edition are Preprint versions or Author's Accepted Manuscripts of the articles in questions. The final recitable form is available at the websites referred to in each papers heading.*

## Abbreviations and glossary

In most of this text the Icelandic letters Þ/þ have been replaced with Th/th and the letters Ð/ð with D/d. This was done to coordinate spelling with the published papers enclosed, and because the aforementioned letters have sometimes been troublesome when moving from one computer software to another. I apologise to the people who love the Icelandic language and the Icelandic persons whose names I have changed in this way.

<sup>14</sup>C BP – Carbon dating, carbon years before 1950

Aneuploidy - An abnormal number of chromosomes within a cell.

b2k – Ice core years before 2000.

Birch (Icelandic: birki) is here used for any species of the genus *Betula*.

Cal ka BP – Calibrated kilo annos Before Present, thousand years before 1950

Cal years BP – Calibrated years Before Present, number of calendar years before 1950

HTM – Holocene Thermal Maximum, the warmest part of the last 12000 years.

LDD - Long Distance Dispersal

LIA – Little Ice Age, cold period roughly from the 16<sup>th</sup> to the 19<sup>th</sup> century.

LOI – Loss On Ignition, weight loss by burning carbon from soil, roughly equal to OC.

Ma – million years ago

m a.s.l. – meters above sea level

MAT - mean annual temperature

MWP – Medieval Warm Period, mild climate around 1000 cal years BP

OC – Organic Carbon

PAR – Pollen Accumulation Rate, number of pollen deposited on each square cm per year

Ploidy – number of sets of chromosomes in a cell: n refers to the gametic set (haploid) and 2n refers to the somatic complement; the 2n numbers can be diploid containing two sets of chromosomes ( $2n=2x$ , where x is base number of a given group of species), triploid containing three sets ( $2n=3x$ ), tetraploid with four sets ( $2n=4x$ ) and so on so forth.

RWP – Roman Warm Period, mild climate around 2000 cal years BP

TLP – Total Land Pollen, all pollen from terrestrial plant species.

## Acknowledgements

I owe a lot to my advisor and my PhD committee and want to express my thanks to them:

Kesara Anamthawat-Jónsson, for without her none of this would have been done.

Ægir Þór Þórsson, who guided me into research of the birch and gave me access to his results, helped with every equipment, joined in for field sessions and always created an environment of patience and good humour.

Margrét Hallsdóttir, who guided me into the magic world of pollen studies.

Ólafur Eggertsson, who knows all about time, age and trees, and gave a lot of his time, knowledge and labour to retrieve samples and get them dated.

Ása Aradóttir, who inspired my interest in birch, knows all about statistics and no nonsense.

I also want to thank people who have helped me along in various ways:

Sigurður Halldór Árnason, who gave days of work to help with field work.

Magnús Jóhannsson, who was the first to work on Icelandic *Betula* pollen fertility.

Egill Erlendsson, who freely gave advice and access to equipment when needed.

Guðrún Larsen, who is the godmother of all tephra studies.

Bryndís G. Róbertsdóttir, who also knows a thing or two about tephra and sites to sample.

Last of all, I thank my good husband Snorri Páll Snorrason for patience, encouragement and advice, among so many other things that they could never be listed.

The project was supported by the University of Iceland (Doctoral grant 2010–2013) and the University Research Fund.



# 1 Introduction

This work is based on several studies on pollen from the two species of birch native to Iceland, the downy birch, *Betula pubescens* Ehrh. and the dwarf birch *Betula nana* L. The results of studies on pollen from existing woodlands were used to interpret and draw conclusions from studies on subfossil pollen on the growth, interactions and hybridisation of the two species through the Holocene.

## 1.1 The oldest known floras of Iceland

The history of Iceland as a distinct geological entity covers around 60 million years (Graham et al. 1998). On the other hand the very nature of an island, located on an oceanic ridge mantle plume, is a continuous regeneration of surface above sea level. Therefore, the oldest known rock in Iceland is less than 16 million years old (McDougall et al. 1984) so traces of older vegetation are probably lost forever. Fossils from the oldest parts of Iceland have revealed a fascinating Miocene flora quite unlike the present. Among the species found were several types of *Betula*, among them the endemic and now-extinct *Betula islandica* (Denk et al. 2005). The oldest known Icelandic flora was a broad-leaved deciduous forest 15 million years ago (Ma) with conifers on mountain slopes, prevailing in this temperate to warm-temperate climate for millions of years, described by Grímsson (2007). In this flora, *Fagus* has been most studied but examples of other taxa were *Sequoia*, *Magnolia*, *Platanus*, *Ulmus* and *Aesculus*. The estimated maximum mean annual temperature (MAT) was around 13.5°C at 12 Ma. From that time MAT decreased to around 10°C at 8 Ma and near the end of the Miocene at 6-7 Ma the MAT was somewhere between 5.4 and 9.4°C (Grímsson 2007). The cooling of climate continued in the Pliocene epoch (Buchardt and Símonarson 2003) although still considerably warmer than late 20<sup>th</sup> century climate. Verhoeven et al. (2013) estimate the climate from pollen assemblage as “Csb” in Köppen climate classification or Oceanic Mediterranean – comparative to modern Portugal or San Francisco. *Betula* pollen grains were seen amongst a diverse array of other species, both trees and herbs, throughout the Pliocene and into the Pleistocene epoch.

Then, the climate got a lot colder. Marine sediments from the Hatton plateau, approximately 1000 km south of Iceland and west of Ireland, show evidence of gradual cooling from 2.9 Ma and a severe drop in temperature at 2.4 Ma, derived from benthic foraminifers oxygen isotope balance. At 2.4 Ma, first ice-rafted debris of Greenland origin was found (Zimmerman et al. 1984). The Pleistocene had arrived.

The oldest glacial deposits found in Iceland are estimated 3.8-4.0 and 3.4 Ma, inland in the NE-Iceland. They are thought to have been local in a limited area. The first major ice sheet is dated 2.9 Ma. Glaciations intensified at 2.7 Ma and glacial deposits dated 2.5-2.4 Ma have been found in all parts of Iceland (reviewed in Geirsdóttir 2004; 2011). The flora changed as the climate did; conifers and the temperate deciduous forest disappeared, but *Alnus*, *Betula* and *Salix* were among the last of the Pliocene flora (Einarsson and Albertsson 1988).

The Pleistocene flora in Iceland is only known from few locations where plant-bearing sediments with limited extensions have been found. Four of them are fairly well studied, the oldest one dated 2.4-2.1 Ma and the others from interglacials dated 1.7, 1.1 and 0.8 Ma. The interglacial floras found were similar to the modern Icelandic flora and birch was found in all of them (Denk et al. 2011b).

Based on the insolation forcing and oxygen isotopes from Pleistocene deposits the glacial-interglacial cycles of the last 2 Ma may have been 40-50 (Huybers 2006). In Iceland, evidence of 13-23 glaciations are known, thereof at least 12 boasted icecaps that reached sea level (Einarsson and Albertsson 1988).

Geirsdóttir et al. (2009) have reviewed the deglaciation in Iceland from around 15 cal ka, when the Icelandic ice sheet broke up as relative sea levels rose 60-150 m. Rapid deglaciation combined with volcanic eruptions created numerous catastrophic jökulhlaup (outburst floods). During the Early Bölling and through Alleröd, the coastal lowlands of present-day Iceland were probably ice-free, but submerged.

## 1.2 The Holocene flora of Iceland

From the beginning of Holocene, the Icelandic history of vegetation has been continuous. The same habitat types as are seen today; similar or the same species have alternatively gained or lost ground in harmony with climatic changes. Knowledge of Holocene vegetation in Iceland is reasonably more detailed than that of previous geological epochs and stems mostly from palynological research on deposits. Hallsdóttir and Caseldine (2005) listed 31 references for 62 pollen sites in Iceland in the period 1944-2005. Since 2005, at least 16 papers describing Icelandic pollen sites have been published. Three of these focus on Miocene and Pliocene-Pleistocene pollen but the remaining 13 show various periods of the Holocene (Figure 1). A list of Icelandic Holocene pollen sites, based on Hallsdóttir (1995) and Hallsdóttir and Caseldine (2005) with modifications is shown in Appendix A.

Thorleifur Einarsson, one of the pioneers of Icelandic geology, worked on many of the first pollen analyses done in Iceland (Appendix A). His interest within geology was broad but his focus was particularly on vegetation history. He divided the vegetation history of Iceland into phases beginning in the Late glacial, 18-11.5 cal ka BP, with pioneer vegetation. Six phases within the

Holocene were designated; (1) Pioneer vegetation, 11.5-10 cal ka BP, mostly without birch, except in the NE; (2) The older birch period, 10-7.5 cal ka BP; (3) The older bog period, 7.5-5.5 cal ka BP; (4) The younger birch period 5.5-2.5 cal ka BP; (5) The younger bog period, after 2.5 cal ka BP and lastly (6) Historical time, after 870 AD (Einarsson 1962, 1968; timing approximate using Intcal09, Reimer et al. 2009). This summary of vegetation history has aged well and, in spite of numerous exceptions and variations found in different sites, the big picture has not changed much. More detailed and updated summaries are found in Hallsdóttir (1995) and Hallsdóttir and Caseldine (2005).

Birch is always at the centre of interpretation of palaeontological data in Iceland, being the only tree species forming continuous woodlands. For the most part, the Holocene climate in Iceland has been near the margin of the requirements of tree birch (see section 1.3.2). This makes the presence, absence and quantity of birch pollen a useful tool to estimate climate variation through time and among sites.

Several attempts to reconstruct Holocene climate history from palynological data, and data on glacial retreats and advances, have been made, especially for the middle north Iceland from where most data exist (Gudmundsson 1997; Stötter et al. 1999; Wastl et al. 2001). They show a more detailed climate history than the Einarsson (1962; 1968) model but still the overall picture remains. The warm period with tree birch after 10 cal ka and before 7 cal ka was not continuous, but ruptured by cold spells before the decline in temperature and birch ca. 7-5 cal ka. There were also cooler events during the mild period and birch advance ca. 5-3 cal ka and after 3 cal ka birch seems to have retreated stepwise in a cooler climate broken by the Roman Warm Period (RWP) and Medieval Warm Period (MWP)

### 1.3 The origin of the Holocene flora of Iceland

Opinions have varied on the origin of the present biota of Iceland, including birch, whether plant survival in ice-free refugia through the Weichselian played an important role (reviewed by Ægisdóttir and Thórhallsdóttir 2004) and on the mechanisms of plant dispersal to Iceland in the early Holocene (reviewed by Buckland and Panagiotakopulu 2010).

Compared to Scandinavia and Britain, the Icelandic flora is species poor. Still, the current estimate is 489 species of vascular plants, 606 mosses, 755 lichens, 2100 fungi and 1660 algae species (Kristinsson, H. Flóra Íslands, <http://www.flora.islands.is/> Last viewed October 15<sup>th</sup> 2013).

Genetically, the Icelandic flora, like the rest of the biota, is mostly related to European (Scandinavian or British) species (Abbott and Brockmann 2003; Brockmann et al. 2003). Examples of species studied are the plants *Cerastium arcticum* Lange (Hagen et al. 2001), *Juniperus communis* L. (Adams et al. 2003),

*Vaccinium uliginosum* L. (Eidesen et al. 2007), *Carex bigelowii* Torr. ex Schwein. (Schönswetter et al. 2008), *Sagina caespitosa* (J. Vahl) Lange (Westergaard et al. 2011) and *Betula* (Maliouchenko et al. 2007; Thórsson et al. 2010). Other organisms also show close relations to NW European populations, like the marine bivalve mollusc *Arctica islandica* Linné (Dahlgren et al. 2000).

Half of the vascular plant species found in Iceland have circumpolar distribution; the rest are either European or Amphi-Atlantic. Only about 2% of the native vascular plant species are definitely of North American origin and only one or two (if any) are endemic (Einarsson, E. 1961). The poverty of endemics indicates that the Icelandic flora has not been isolated for the whole of the Pleistocene so considerable influx from abroad in interglacial periods is certain (reviewed by Brochmann et al. 2003). Whether the entire present flora has immigrated in the Holocene or whether some species survived the last glaciation has been a matter of debate.

### **1.3.1 Theories on origin of the postglacial flora and means of migration**

When dealing with questions on how an organism came to live in its present habitat the possibilities are numerous and the answers are often provided by inductive reasoning. Various theories have been set forth regarding origin of the Icelandic flora. Some have been more popular than others, at least for a period in time, but all have contributed something to our understanding; however with a growing body of knowledge, theories should be reevaluated from time to time. Seven of the most discussed explanations are:

- i. Land bridge
- ii. Local refugia
- iii. Wind dispersal
- iv. Sea borne propagules
- v. Rafting on driftwood or ice
- vi. Animals (mostly birds)
- vii. Human introduction

#### **1.3.1.1 Land bridge**

Within continuous dry land, or closely spaced islands, plants and animals would have dispersed without difficulty. In the early 20<sup>th</sup> century a land bridge was thought to explain relationship in the present biota of N Europe and N America (e.g. Scharff 1909). The idea was mostly abandoned around 1960 when tectonics and sea-floor structure became better understood. The so-called Thulean land bridge, connecting Europe to Greenland via Scotland, Faeroes and Iceland is still believed to have existed in the Tertiary (Nilsen 1978; Milne 2006; Denk et al. 2011a). Biotic connections across the North Atlantic land bridge were gradually



lost. Continuous passageway for vertebrates disappeared after the Early Eocene (~40 Ma) but a closely spaced island chain may have persisted through the Middle Miocene (~14 Ma) or even longer (Denk et al. 2010). At last, these stepping stones disappeared and only Faeroes and Iceland were left between Europe and North America. The exact timing is not known but scientists agree on a time in the Tertiary. The present flora of Iceland must therefore have immigrated by other means.

### ***1.3.1.2 Refugia***

The idea of ice-free areas, where plants survived the Ice Age, originated in the late 19<sup>th</sup> century and has persisted with varying popularity to the present day (reviewed by Brochman et al. 2003 and Ægisdóttir and Thórhallsdóttir 2004). Little is doubted about the existence of greater refugia, like Beringia, from which plants distributed after the retreatment of continental ice sheets (Milne 2006; Eidesen et al. 2013). The role of smaller local refugia or nunataks is not as clear. Around the middle of the 20<sup>th</sup> century, up to half of the Icelandic flora was believed to have survived the glaciations (Einarsson 1968) but the emphasis on such refugia has diminished in recent years with better understanding of how harsh the environment must have been. Additional explanations on present plant distribution have yet to be found. Still, some recent molecular studies of arctic-alpine plants support the theory of local refugia (e.g. Westergaard et al. 2011; Parducci et al. 2012).

There is perhaps a reason to mention seed survival in frozen soil as an alternative to the refugia theory. It has to be considered theoretically possible after the germination of Pleistocene seeds found in a frozen lemming burrow in Alaska (Porsild et al. 1967) and the regeneration of a plant from 30 000 year old fruit in Siberia (Yashina et al. 2012). Though unlikely to be a major source for vegetation recovery, old seed and bryophyte spores might occasionally have germinated when conditions amended after cold periods.

### ***1.3.1.3 Wind dispersal***

When the popularity of the land bridge- and the local refugia theories dwindled, scientists had to face the idea of long-distance dispersal as the main source of the Holocene flora of Iceland. For terrestrial plants and freshwater fauna, a minimum distance of 430 km (Iceland – Faeroes) over the open Atlantic Ocean seems hard to overcome.

Dispersal by wind is a great way to spread for organisms with very small propagules. In any pollen sample taken from Icelandic soil, there are likely to be several pollen of foreign plant species, such as pine. The spores of bryophytes and ferns are of a comparable size and may thus have arrived by air at any time in

history. Wind should therefore be considered the main transport mode of the terrestrial spore-producing flora of Iceland.

Seeds are 1-4 orders of magnitude larger than spores and do not travel as easily on wind. In papers dealing with wind-dispersed seeds, the term long-distance dispersal (LDD) is used for any distance over 100 m (Greene and Johnson 1989; Tackenberg 2003). Direct tests of wind dispersed seeds give maximum values less than 1000 m (Augspurger 1986; Matlack 1992) while some wind dispersal models for plant seed reach up to 10 km (Cain et al. 1998).

There is no good way to estimate the probability of a seed arriving in Iceland from Europe by wind. Still, we can play with numbers to get a feeling for the probability. If we use a simple method to extend a modelled dispersal curve with high percentage of LDD, a species with effective wind-dispersal method and favourable weather conditions (modified Greene and Johnson model from Tackenberg (2003), Table 3, experiment 9, seeds of *Cirsium arvense* (L.) Scop.), we find that about one in ten thousand seeds lands at a distance between 430 and 830 km from the source. That is the distance a seed from Faeroes would travel to land in Iceland. How often the weather conditions and the wind direction would be right is still not accounted for, nor are the chances for a seed to establish itself after landing. Although extension of models on this scale may not be realistic, the possibility of successful dispersal exists, while probability is low.

For birch seeds the probabilities of reaching Iceland from Europe by wind would be considerably lower because birch seed dispersal by wind is not as effective as the pappus of the thistle, *Cirsium arvense*. The falling velocity for birch seed is estimated 0.3-1.6 m sek<sup>-1</sup> (Tackenberg 2001) compared to 0.15 m sek<sup>-1</sup> for the thistle seed (Tackenberg 2003).

The rare events of dispersal may be the most important, but the extremely rare occurrence of a seed blown from Scandinavia or Britain to Iceland, seems unlikely to have contributed much to the Icelandic flora. A few taxa with very aerial seeds like the thistle may have entered Iceland by wind; these include *Salix*, *Epilobium*, *Erigeron*, *Eriophorum*, *Hieracium*, *Populus*, *Senecio*, *Taraxacum* and *Tussilago*, all of which have falling velocity of 0.2 m sec<sup>-1</sup> or less (Tackenberg 2001).

#### **1.3.1.4 Sea borne propagules**

Coastal algae may have had better chances of surviving the Pleistocene than terrestrial plants and could probably reestablish by sea currents any time in the Holocene. Some seaside growing vascular plants have propagules adapted to floating and can disperse by sea. Koutstaal et al. (1987) did experiments on some coastal plant propagules and found several species well adapted for long-term floating without damage to germination ability. Those included three species of the existing Icelandic flora; *Tripleurospermum maritimum* (L.) W. D. J. Koch,

*Plantago maritima* L. and *Triglochin maritima* L. and two species with Icelandic relatives; *Atriplex littoralis* L. and *Rumex crispus* L. On the volcanic island Surtsey, that emerged from sea 30 km away from the southern coast of Iceland in 1963, 69 species of vascular plants had colonized by 2008; of these 9%, or six species, are believed to have dispersed by sea; *Cakile arctica* Pobed., *Leymus arenarius* Hochst., *Honckenya peploides* (L.) Ehrh., *Mertensia maritima* (L.) Gray., *Angelica archangelica* L. and *Atriplex glabriuscula* Edmondston (Magnússon et al. 2009). Even if dispersal over several hundred km of the Atlantic Ocean is riskier and may take considerable time, these species and probably several more could have immigrated to Iceland with free floating propagules.

Birch seeds have a good floating ability (Anderson et al. 2000, Appendix I), but the salt tolerance of birch seed and seedlings is limited (Dragsted and Kubin 1990; Robichaud and Bégin 1997; Fostad 2000; **Forestry Commission England 2011**). This mode of birch dispersal from Europe to Iceland is therefore not unthinkable but rather unlikely.

#### **1.3.1.5 Rafting on driftwood or ice**

For plant species not well adapted to floating, rafting over sea on driftwood or ice is an option. A constant supply of driftwood arrives at the coasts of North Iceland. Although the greater part seems to have gotten lost from timber floating, some of the logs have intact root systems and have presumably been eroded from river banks by natural causes. There are reasons to assume that naturally felled trees have been drifting ashore on the islands around the Arctic and in Iceland for most of the Holocene (Tremblay et al. 1997). Eggertsson (1993) studied the origin and age of driftwood on Icelandic shores and found that it originated from Russia and Siberia. The logs may have been several years in the sea, probably frozen in sea ice most part of the time, which makes this way of plant dispersal challenging though not impossible. As the Icelandic biota shares most species with Scandinavia and Britain, rather than Russia and Siberia (e.g. Eidesen et al. 2013), this cannot be the main origin of the Icelandic flora but neither can it be completely ruled out as an alternative route.

An interesting hypothesis of an event that could have brought plant propagules to the Atlantic islands has been connected to the catastrophic draining of the Baltic Ice Lake (Buckland and Dugmore 1991). Around 12 cal ka BP when ice melted in southern Sweden, approximately 9400 km<sup>3</sup> of fresh water was released into the North Atlantic, triggering a short-lived disturbance of the North Atlantic circulation (Andrén et al. 2002; Lampe 2005). The exact timing of the drainage and volume of freshwater have been debated, but little doubt remains of the existence of the lake and a major drainage taking place (Jacobson et al. 2007). This event could tie hypothesis of long distance dispersion by ice-

rafting to the relationship of Icelandic and Scandinavian flora. This probably happened too early to apply to the tree birch, as suitable habitats were unlikely to be ready and July temperatures still low (Geirsdóttir et al. 2009).

### **1.3.1.6 Dispersion by animals**

The only terrestrial mammal in pre-settlement Iceland was the arctic fox. Even if predators have sometimes been shown to act as secondary dispersal vehicles for plant propagules (Nogales et al. 1996; 2002), the arctic fox is not a likely to have contributed to the Icelandic flora. On the other hand, birds are.

The role of birds in the dispersion of plants to the North-Atlantic islands has been part of the discussion for decades but somehow without the interest it truly deserves. Bennike (1999, 2000) is an exception, emphasizing the role of bird dispersal in postglacial Greenland flora. For example, Rundgren and Ingólfsson (1999), Johansen and Hytteborn (2001) and Buckland and Panagiotakopulu (2010), all mention bird-dispersed seed as feasible while concentrating on other methods of dispersion. The failure to recognise the importance of dispersal by birds probably lies in the focus on bird migration routes. Migrating birds mostly move north in spring and south in autumn, when seeds are mature and are thus not likely to bring seeds from Scandinavia or Britain to Iceland. Buckland and Panagiotakopulu (2010: p. 194) also add that "Until the high productivity of Arctic island ecosystems had been established by the prior immigration of terrestrial and freshwater biota, there could be no migration".

But migration is not the only reason for birds to cross the sea and end up in Iceland. According to The Icelandic Institute of Natural History (<http://en.ni.is/zooology/birds/> observed 1.11.13), a total of 75 bird species nest in Iceland regularly but sighted species are around 370. Average wind speed at the south coast of Iceland is around 11 m sec<sup>-1</sup> or 40 km hour<sup>-1</sup> (The Icelandic Meteorological Office: [http://www.vedur.is/Medaltalstoflur-txt/Stod\\_815\\_Stor\\_hofdi.Ars\\_Medal.txt](http://www.vedur.is/Medaltalstoflur-txt/Stod_815_Stor_hofdi.Ars_Medal.txt) observed 5.11.13) and average observed flight speed of birds is about the same (Pennycuick 1997). The flight to Iceland from Scotland or Norway thus only takes 10-12 hours when wind direction is right and possibly less in a strong favourable wind.

Every year hundreds of vagrant birds are reported in Iceland, most of them in autumn (Icelandic Birding Pages, <https://not.endur.hi.is/~yannk/birdnews.html> and Ebird <http://ebird.org/content/ebird/>; both observed 1.11.13). Those do not include birds of species nesting in Iceland such as snow buntings that might have erred in their migrating from Scandinavia to Scotland. Why any bird flies from Europe to Iceland in the autumn is hard to say but some might be exploring juveniles, some might have been caught in storms and born far out to sea, and some might just be lost or disorientated. What happens to them after they arrive is probably also different; some

might find their way back home, some could survive a mild Icelandic winter and some die. These autumn visits probably continued for the whole of the Holocene, giving European plant seeds a lot of opportunities to hitch a ride.

Another argument against seed dispersion by birds has been the birds' cleanliness (Buckland and Panagiotakou 2010). Searches of birds' feathers and feet have seldom revealed attached plant seeds, but still some observations and tests show that the preening of birds is not effective enough to eliminate all propagules (Carlquist, S. 1981; Weisbrod and Johnson 1989; Figuerola and Green 2002). The most important method of long-distance dispersal by birds is probably seeds swallowed and carried internally (Brochet et al 2009).

Perhaps the strongest argument of bird dispersal as an important vector in the vegetative succession in Iceland in the Holocene is from Surtsey, the volcanic island that emerged from sea south of Iceland in 1963 and has been protected for research since 1965. The island lies about 5 km from the nearest small island, 20 km from a larger inhabited island and approximately 30 km from the Icelandic southern coast. The first vascular plant was found in 1965 and colonization and survival of plants on the island has been recorded since then. In 1986, 26 species of vascular plants had been found growing on the island and the majority, or 64%, had probably been transported there by birds (Fridriksson 1987). After gulls formed a breeding colony on the island in 1986, the number of vascular plant species grew fast and in 2008, 69 species had been found; a vast majority, or 75%, were thought to have been carried there by birds (Magnússon et al. 2009). In spring 1967, 97 birds were caught on the island for inspection and searched for organisms externally and internally. Snow buntings, *Plectrophenax nivalis*, migrating from Scotland, proved to carry viable seeds in their gizzards (Fridriksson and Sigurdsson 1968).

Birds are a feasible method of long-distance dispersal. The seeds are an important source of food for the snow buntings, redpolls and other birds that might carry them internally. The genetic variability of birch in Iceland (Thórsson et al. 2010) supports a hypothesis of multiple colonisations rather than a single event, making bird dispersal a more attractive method than others, e.g. rafting.

#### 1.3.1.7 Human introduction

In the last 1100 years, humans have added to the flora of Iceland both deliberately and unintended. Undoubtedly, both crops and weeds immigrated with the first settlers, but gardening for pleasure has only about a 100-year history, and for the last 100 years the number of imported species has multiplied.

In addition to the native growing *Betula* species, *B. nana* and *B. pubescens*, both *B. pendula* Roth. and *B. ermanii* Cham. are widely grown as ornamental trees and botanical gardens grow at least nine other *Betula* species: *B. alleghaniensis* Britton,

*B. costata* Trautv., *B. divaricata* Ledeb., *B. lenta* L., *B. neolaskana* Sarg., *B. occidentalis* Hook., *B. populifolia* Marshall and *B. pumila* L.

### 1.3.2 Distribution of Icelandic birch in the Holocene

Some birch pollen is found in most Icelandic Holocene pollen sites. Rundgren (1995) reported scattered *B. nana* pollen from Lake Torfadalsvatn, Skagafjörður ca. 13.2-12.7 cal ka BP, which probably is the oldest Holocene *Betula* pollen found in Iceland. Still, *B. nana* did not become a significant part of the vegetation until after 12.7 cal ka BP. This was in the early part of the Younger-Dryas cold period, a time not expected to be favourable for vegetation in general. Additional research from the same district shows comparable results (Rundgren 1998). Several papers report the presence of *B. nana* from the early Boreal in the central northern part of Iceland followed by *B. pubescens* in late Boreal or around 9 cal ka (Vasari and Vasari 1990; Hallsdóttir 1990, 1995; Hallsdóttir and Caseldine 2005). Fewer sites from the southern part of Iceland show such early distribution of *Betula*, but the establishment of the species seems to have followed a comparable pattern. At Lómatjörn, S-Iceland *B. nana* appeared ca 9.2 cal ka, a little later than in the north, but *B. pubescens* around the same time as in north Iceland, 9 cal ka, although pollen numbers were still low (Vasari and Vasari 1990). In western Iceland, few sites reach back to the earliest Holocene floras. Caseldine et al. (2003) found *Betula* pollen older than 11 cal ka at Efstadalsvatn in NW Iceland but presumably not derived locally, and was possibly reworked older interstadial pollen. Local *B. nana* was found around 9 cal ka and *B. pubescens* not until 7.6 cal ka. Efstadalsvatn is located at an altitude of 123 m a.s.l. and is not in the warmer parts of Iceland so delay in woodland formation might be local.

Once birch was established anywhere in Iceland, wind dispersal and secondary wind dispersal by seeds on snow (Greene and Johnson 1997) could account for spreading through the whole country in a few hundred years, supposing maximum rate of spread 1 km year<sup>-1</sup> (Clark 1998) and the maximum distance within Iceland around 500 km. Dispersal by seed-eating birds like the rock ptarmigan and snow buntings could reduce the time by at least an order of magnitude. Therefore, the earliest reported finds of pollen may, or may not, reflect where the first seeds germinated after long-distance dispersal from overseas. Rather, it tells where the postglacial environment first suited the species in question. In that regard, summer temperatures are important as is stable soil surface. Therefore, it is not surprising that the first dated tree birch pollen has been found in sheltered valleys in the north rather than in the more oceanic climate of South Iceland, where glacial floods and shifting sands were more common.

Those who have studied tree lines have had a hard time defining the exact environmental factors needed to support the growth of trees. The species in question, soil, biotic impact like browsing animals and local topography have their effect, but climate is usually the main factor. Climate includes wind, precipitation, solar radiation, length of growing season and temperature (Holtmeier and Broll 2005). It is the summer temperature that most often is used to predict changes in tree lines and in the northern temperate zone it is conventional to connect the tree line with 10°C average temperature for July (e.g. Seppä and Birks 2001; Grace et al. 2002). Odland (1996) found best correlation to the average maximum temperature of June-September set at 13.2°C but Wielgolaski (2005) estimates this number to be nearer to 8°C. There is reason to believe that soil temperature may predict natural tree line better than air temperature (Weih and Karlsson 2001) and that average root zone temperature of 6-7°C in the whole of the growing season marks the tree line best (Körner and Paulsen 2004), but the simplicity and availability of air temperature data outweighs the benefits of other measurements. Shrubs do not necessarily have the same requirements for temperature as trees and the dwarf birch, *B. nana*, can manage down to 6°C July temperatures (De Groot et al. 1997).

Research on tree lines in Iceland is limited, but the highest tree lines in the Holocene are estimated between 400 and 450 m a.s.l. in the north (Wastl et al. 2001) and near 600 m a.s.l. in the southern part of Iceland (Fridriksson 1963). In the research by Wastl et al (2001) the tree line exceeded 400 m a.s.l. several times but the peak near 8-7 cal ka coincided with the highest PAR values for *B. pubescens* pollen seen in the research. This is in accordance with other research (Hallsdóttir 1995).

Wöll (2008) found that the limits for *B. pubescens* are now mostly between an altitude of 300 and 500 m, although trees growing above 400 m rarely reach 2 m in height. Those tree lines were mostly between July temperatures of 8 and 9°C. By also taking effects of oceanic climate into account, Wöll (2008) estimates the area climatically available for *B. pubescens* growth in modern Iceland, and gets a figure of over 40 thousand km<sup>2</sup>, thereof 25 thousand km<sup>2</sup> where trees could reach 2 m in height. That corresponds to 40% and 24% of Iceland, respectively. The lower figure is near the estimate commonly made on the extent of birch woodlands at the time of settlement (874-930 AD) but the higher figure is as good a guess as any on the maximum extent of birch woodlands in the Holocene.

## 1.4 The present woodlands in Iceland

Both *Betula nana* and *B. pubescens* are found in all parts of the country but still there is a considerable difference in the distribution (Figure 2). *B. nana* grows further up in the highlands but is not abundant in the southern part. Frequently,



both species grow together forming woodlands/scrublands where only scattered stands of trees reach over 2 m height.

Typical flowering times are May-June for *B. nana* and June for *B. pubescens* (Kristinsson 2010), frequently overlapping.

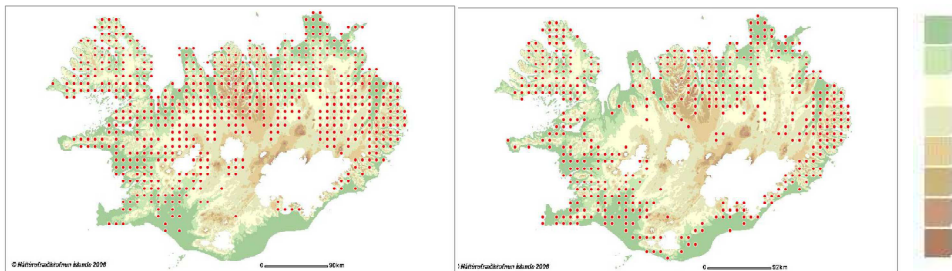


Figure 1. Distribution of *Betula* in Iceland. Left *B. nana*, right *B. pubescens*. Dots show presence of the species in 10x10 km squares. Source: The Icelandic Institute of Natural History, Plöntuvefsjá.

Downy birch in Iceland and Fennoscandia often has a low-lying growth form and is polycormic. This type of birch has been called mountain birch and has sometimes been given scientific names as a subspecies or variety of *Betula pubescens*. According to the Kew plant list, the accepted name of this type of birch now is *Betula pubescens* var. *pumila* (L.) Govaerts; there are 12 synonyms, including *Betula czerepanovii* N.I. Orlova and *Betula tortuosa* Ledeb. In this work, Icelandic tree birch with chromosome number  $2n=56$  is referred to simply as *B. pubescens* (sensu lato), regardless of growth form.

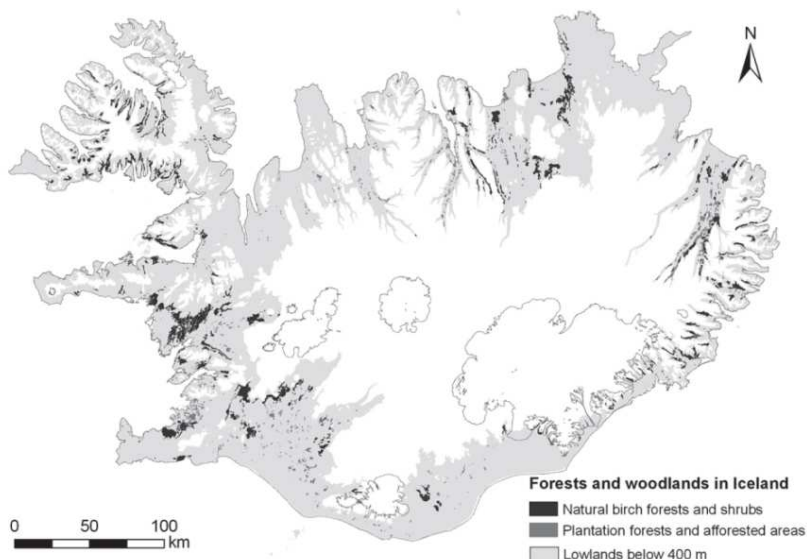


Figure 2. Woodlands in Iceland. Source: Traustason and Snorrason (2008).



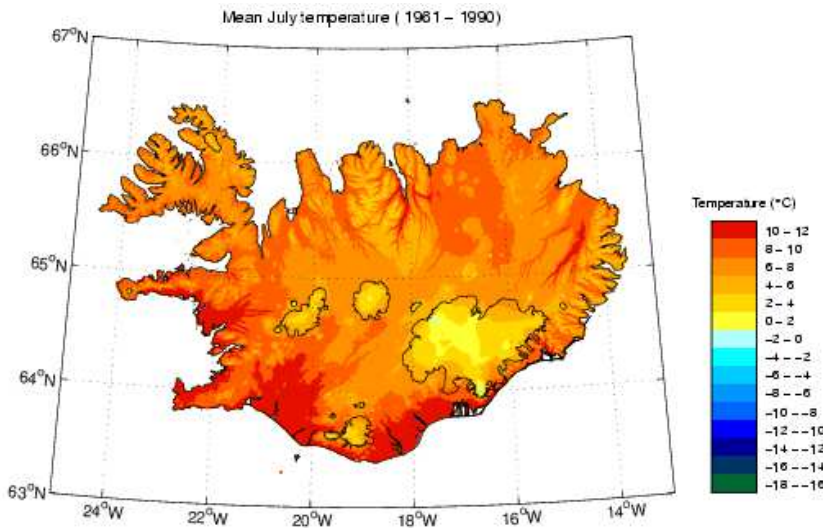


Figure 3. Mean July temperature in Iceland 1961-1990. Source: *The Icelandic Meteorological Office*.

Before the settlement, 874-930 AD, woodlands are thought to have covered at least 25% of the land area (Arnalds 1987) but have been reduced through unsustainable land use and cooling climate to merely 1.2% (Ministry for the Environment and Natural Resources 2007; Figure 3). In the last decades, examples of expanding natural woodlands have been observed in areas free from sheep grazing, probably as a result of combined effects of preservation and warming climate.

The Icelandic Meteorological Office (<http://www.vedur.is/>) keeps records of temperature measurement from a few weather stations from around the mid-20<sup>th</sup> century. Since about 1960, weather stations have covered the country fairly well. Figure 4 shows mean July temperatures for the period 1961-1990. Data for the period 1991-2013 show a rise in temperature of 0.5-1.0°C from the means of the previous 30 years. The red and dark red areas should therefore meet the temperature requirements for tree birch growth. On the other hand, some of the warmer areas, especially in the southeast, are outset for glacial floods and do not offer safe habitats for trees. Still, there is a great gap between the potential and actual woodlands.

Human impact on Icelandic vegetation, especially birch woodlands, has been drastic (e.g. Dugmore et al., 2009). The deforestation was driven by grazing and cutting but cooling climate from the Medieval Warm Period and into the Little Ice Age doubtless played an important role. In Iceland, the effects on birch woodlands by the warming climate in recent decades may already be considerable but obscured by changing grazing patterns. In Scandinavia, changes

in tree line and dwarf shrub cover above tree line are already showing (e.g. Kullman 2002; Truong et al. 2007).

## 2 Pollen from modern woodlands

In the years 2003-2005, Ægir Thór Thórsson sampled 14 natural woodlands in Iceland (Figure 5), representing a diversity of natural habitats sustaining birch (Thórsson 2008). The samples included leaves, leaf buds, emerging male catkins, seed and mature male catkins containing pollen. Leaves were used for morphological estimate, leaf buds and emerging catkins for cytogenetic research (Thórsson 2008), pollen and seeds for evaluation of fertility (unpublished) and the size of pollen was measured (papers I and II).

The purpose of the pollen study was to establish frames of reference to differentiate pollen from downy birch and dwarf birch and to explore the hybrid pollen in search of characteristics usable for identification. The results are published in Papers I and II included in this thesis and form a base for the studies described in Papers III, IV and V which are the main content of the thesis.



Figure 4. Fieldwork in the woodland Jafnaskardsskógur, near Bifröst.

Pollen from ten woodlands, a total of 92 ploidy-determined individual trees or shrubs, was used. These were 31 diploid individuals, representing *B. nana*; 22 triploids, representing hybrids; and 39 tetraploids or *B. pubescens*.

## 2.1 *Betula* pollen differentiation - Paper I

The pollen produced by different species of *Betula* is similar in structure and in size although each species has some typical characteristics. Pollen of *B. nana* and *B. pubescens* differs in size, thickness of the pollen wall and size and shape of structures surrounding the pores (Blackmore et al. 2003). While the different features are conspicuous when looking at a pollen grain typical for its species, other grains will always show features that are in between the defined margins (Figure 6).

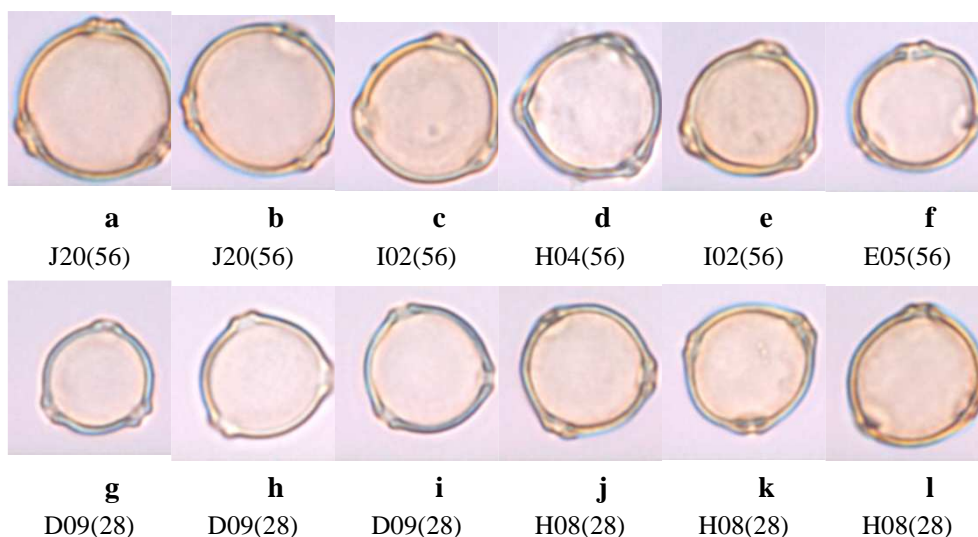


Figure 5. *Betula pubescens* (a-f) and *B. nana* pollen (g-l). Sample name of tree and chromosome number below picture. Size of frames: width 29  $\mu\text{m}$ , height 27  $\mu\text{m}$ .

Several attempts have been made to find a reliable way to differentiate species in mixed pollen samples and the diameter has proven to be the safest choice (reviewed by Mäkelä 1996), although some authors have effectively differentiated *Betula* pollen on the basis of their overall characteristics (see section 3.2.1).

In our study, the pollen from modern trees was prepared as if it was a soil sample, using NaOH, HCl, HF and acetolysis, to make pollen sizes comparable to subfossil material. Two measurements were made on a digital micrograph of each pollen grain: the diameter and the pore depth. Around 120 grains from each tree were measured, a total of more than 13 thousand grains.

As expected, the results showed different means for *B. nana* and *B. pubescens* diameters and pores as well as the ratio of these two. The distribution of measurements of the species did overlap but distribution of diameters was separated well enough to allow calculation of the two normal curves out of a mixed pool of both species (Figure 7).

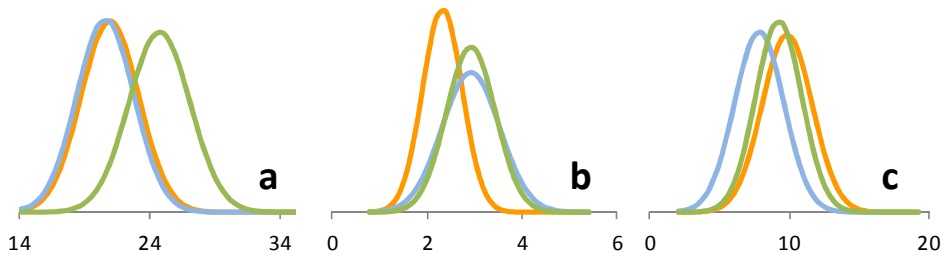


Figure 6. Normal curves drawn from means and standard deviations of measured pollen of diploid *B. nana* (orange), tetraploid *B. pubescens* (green) and triploid hybrids (blue). a) Diameter ( $\mu\text{m}$ ) b) Pore depth ( $\mu\text{m}$ ) c) Ratio of diameter to pore depth.

## 2.2 *Betula* hybrids and their pollen - Paper II

Chromosome numbers in the two *Betula* species differ. The dwarf birch *B. nana* is diploid with  $2n=2x=28$ , while the downy birch *B. pubescens* is tetraploid with  $2n=4x=56$ .

By the mid 20<sup>th</sup> century, theories attributing the growth form of mountain birch to introgressive hybridisation of the downy birch and dwarf birch surfaced (Elkington 1968). At first, a few were sceptical of the idea, pointing out that hybrids of *B. nana* and *B. pubescens* would be triploid and hence sterile (Áskell Löve, personal communications with K. Anamthawat-Jónsson). In fact, the partial fertility of triploid hybrids has been known for a hundred years (e.g. Gates and Thomas 1914, Lutz 1917), making introgression possible. The possibility of introgressive hybridisation in Icelandic birch was finally confirmed by Anamthawat-Jónsson and Tómasson (1990) with backcrosses. Triploid *Betula* hybrids were fertilised with *B. pubescens* pollen, producing nine progeny whereof four had the 56 chromosomes typical of *B. pubescens* and had; thereby, the potential to carry genes back to the tree birch. Since then, the questions on birch hybridisation have not been *if*, but rather *when* and *how much*. Caseldine (2001) raised such questions in the context of palynological data from northern Iceland.

Although a triploid hybrid is not completely sterile, the fertility is greatly reduced due to the inevitable meiotic irregularities which arise when a genome of three parts is divided in two (or rather two times three parts divided to four cells). Ramsey and Schemske (1998) have reviewed research literatures on ploidy and found that most reported pollen produced by triploids were aneuploid. Still, the average viability of pollen was over 30%. The frequency of (viable) unreduced gamete formation can increase in certain environmental conditions, e.g. cold climates (Otto and Whitton 2000).

When it came to measuring the hybrid *Betula* pollen and comparing it to the pollen of parent species, the outcome was not foreseen. The mean diameter of

hybrid *Betula* pollen was the same as found in *B. nana*, while the mean pore depth was almost identical to *B. pubescens* (Figure 7).

Theoretically, it is possible to calculate the proportion of hybrid pollen in a mixed sample by comparing results of diameter- and pore depth measurements. The hybrid pollen would count with *B. nana* when diameter was used and with *B. pubescens* when pore depth was used. The difference of the two results would be an index of hybrid pollen. In practice, this method is not feasible as errors would probably be greater than the hybrid proportions. A different approach was needed to detect the presence of hybrid pollen and luckily another method presented itself in the study.

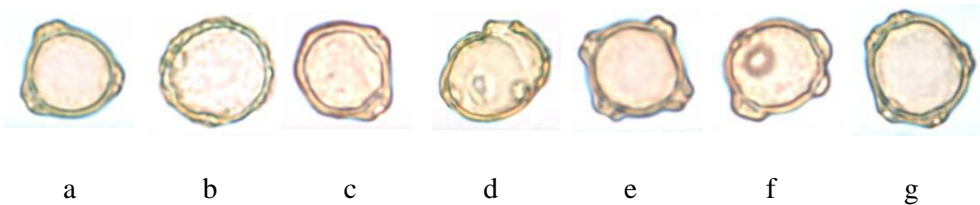


Figure 7. *Betula* hybrid pollen. a) Typical *Betula* hybrid pollen, diameter 18.1  $\mu\text{m}$ . b-g) Abnormal hybrid pollen.

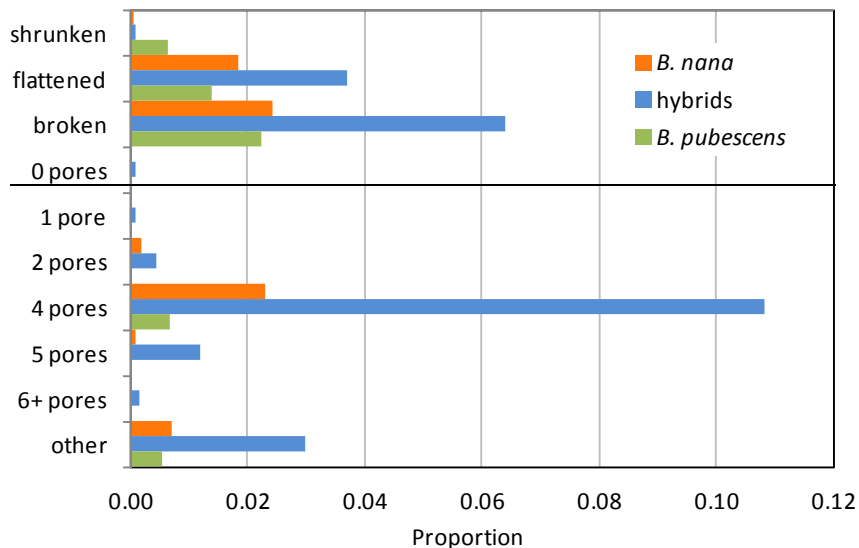


Figure 8. Frequency of damaged and deformed *Betula* pollen grains from diploid, triploid and tetraploid trees or shrubs.

While measuring pollen of the triploid hybrids, we noticed that aberrant pollen grains were frequent among them, probably caused by aneuploid gametes (Figure 8). Only normal triporate pollen was measured but the slides were scanned again for abnormal pollen. For each individual, diploid, triploid or tetraploid, a thousand grains were counted and several anomalies were noted.

Thirteen types of deviations were found of which three were presumably caused by environmental factors or during preparation of samples; shrunken, flattened or broken grains. The types of deformities associated with the innate structure of the grain were 0, 1, 2, 4, 5, 6 or more pores and four types that have been combined in Figure 9 under "other" in which grains with uneven or lumpy walls were the most numerous.

Both damaged and deformed grains were more frequent among the triploid *Betula* hybrids than the parent species, but working with subfossil pollen, non-triporate pollen would be the easiest to identify, especially pollen with four or more pores. Such pollen makes up 12-13% of the hybrid pollen but less than 2% of other *Betula* pollen. A quantity of non-triporate *Betula* pollen in a subfossil sample would therefore indicate the presence of *Betula* hybrids at the time of deposit.







## **3 Subfossil pollen and birch hybrids**

### **3.1 Three studies on Holocene birch pollen, papers III, IV and V**

The aims of this project were to explore the extent of past hybridisation and consequent introgression by studies of sub-fossil pollen from Holocene sediments or peat. We set out to sample materials from different time periods and geographical regions.

Paper III describes the first attempt made to find evidence on past hybridisation by looking for non-triporate *Betula* pollen in pollen samples prepared from peat, sampled in N-Iceland and dated from ten to seven thousand years before present.

Paper IV reports results of a study of subfossil pollen in peat sampled in SW-Iceland, dated from almost the same period as in paper III. As no previous study from this site existed, all pollen and spores were counted for a pollen diagram describing vegetation development in the area.

Paper V describes results from a pollen site in NE-Iceland that covers the last ten thousand years or all the history of Holocene birch in the area.

### **3.2 Materials and methods**

The materials for studies in papers III, IV and V are all retrieved from peat, sampled from drainage ditches in agricultural landscapes in different regions in Iceland (Table 1; Figure 10). They were: Hella in Eyjafjörður, N-Iceland (Paper III), Eyvík in Grímsnes SW-Iceland (Paper IV) and Ytra-Áland in Thistilfjörður NE-Iceland (Paper V). The sites are all named after the nearest farms.

The peat was sampled in 20 cm long aluminium boxes, hammered into the cut face of peat in ditches. The easily recognised Saksunarvatn tephra, 10.3 cal ka, was used as a landmark near the base of each monolith. The Hella monolith was sampled by Margrét Hallsdóttir and Halldór G. Pétursson in 1996. The peat monoliths were extracted from Eyvík in 2006 and Ytra-Áland in 2011.

Pollen preparation followed conventional methods. Five mm thick samples, one or two ml, were cut from the monoliths at two to three cm intervals, heated in NaOH, sieved and cleaned with HCl, HF, acetolysis and ethanol. The Hella samples were mounted in glycerol, Eyvík and Ytra-Áland in silicon oil. Sieve residues were scanned for macrofossils. A test of four samples from Hella was prepared and mounted in silicon oil to compare to the glycerine samples. Samples were dated partly from known tephra layers and partly by carbon dating.

Table 1. Sampling sites.

| Name   | Hella           | Eyvík           | Ytra-Áland                   |
|--|-----------------|-----------------|------------------------------|
| Latitude   | 65°56'35'' N    | 64°03'17''N     | 66°12'29''N                  |
| Longitude  | 18°24'20'' W    | 20°41'37''W     | 15°33'19''W                  |
| Altitude   | 15 m a.s.l.     | 65 m a.s.l.     | 34 m a.s.l.                  |
| Monolith length                                  | 200 cm          | 108 cm          | 108 cm                       |
|  |                 | 7.6-10.3 cal    |                              |
| Period covered                                   | 7.1-11.4 cal ka | ka              | 0 -10.3 cal ka               |
| Mean annual temp 1930-1960*                      | 3.2°C           | 3.6°C           | 2.8°C                        |
| Mean July temp 1930-1960*                        | 10.5°C          | 10.6°C          | 8.5°C                        |
| Number of samples                                | 39              | 44              | 47                           |
|  |                 | 2 tephras, 2    |                              |
| Dating method                                    | 2 tephras       | <sup>14</sup> C | 5 tephras, 2 <sup>14</sup> C |
| Average deposition rate (mm year <sup>-1</sup> ) | 0.5             | 0.4             | 0.1                          |

\*Data from nearby weather station (Hella: Akureyri, Eyvík: Hæll, Ytra-Áland: Thorvaldsstadir). Source: Icelandic Meteorological Office.

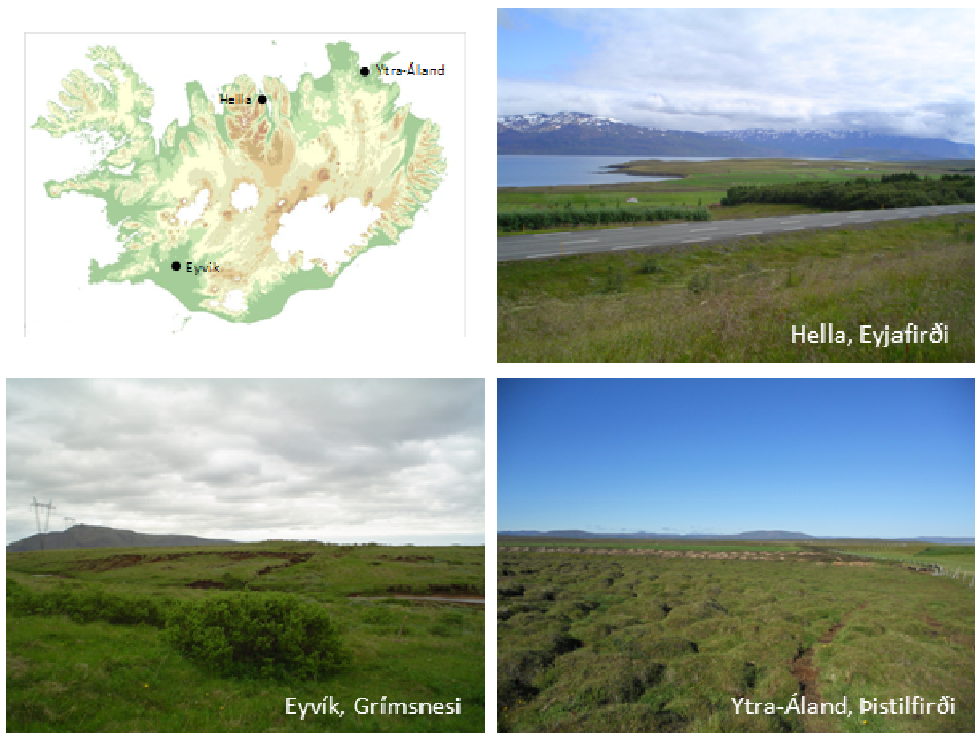


Figure 9. Overview of sampling sites in studies published in papers III, IV and V.

A pollen analysis for Hella, by Margrét Hallsdóttir, existed before the current study began, as shown in the diagram published in Hallsdóttir and Caseldine (2005). Therefore samples from Hella were only scanned for *Betula* pollen. Pollen and spores from Eyvík and Ytra-Áland were counted and analysed for background on vegetation history before the samples were scanned again for *Betula* pollen only. All *Betula* pollen grains were digitally photographed and measured if possible. Non-triporate *Betula* pollen was counted not measured.

A modified version of Bhattacharya's method for the separation of components within distributions (Bhattacharya 1967; Pauli and Caddy 1985) was used to obtain means and standard deviations of size distributions within each sample. The results were refined and proportions of species were calculated with iterations in the Bmod program (Morgan 2005) and Járαι–Komlódi's formula (Prentice 1981) to obtain the best fitting results (Figure 11).

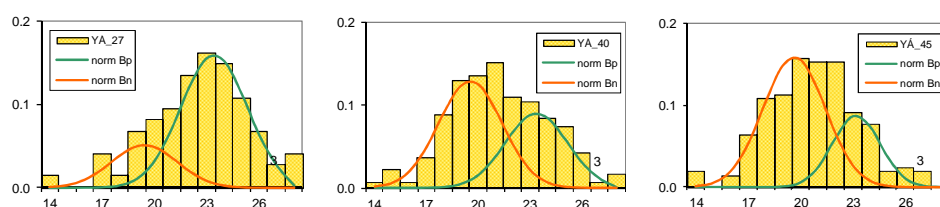


Figure 10. Examples of actual size distribution of *Betula* pollen within samples (columns) and calculated best fitting normal curves of the distribution.



## 4 Results and discussion

### 4.1 *Betula* pollen sizes, species and media

The small test on the Hella pollen in glycerol and in silicon oil showed that pollen sizes changed not only with mounting media but also with depth. Diameters increased with depth when pollen was in glycerol but decreased with depth in silicon oil.

This could not have happened unless time and environment affected pollen sizes as well as the mounting media. As a consequence, no comparison of size is possible unless the pollen has been exposed to the exactly same physical and chemical environment for the same length of time. Therefore, comparison of *Betula* pollen sizes between samples cannot be a reliable index on species proportions. For that reason, the size distribution within each sample is used to determine species proportions, as demonstrated in Figure 11.

In spite of the possibility of misleading pollen sizes, there is a connection between average pollen sizes and species proportions in different samples within sites. Thus, there was a strong correlation between calculated *B. pubescens* proportion and mean pollen diameter in the Ytra-Áland samples,  $r^2 = 0.91$ ,  $P < .0001$ . In Hella and Eyvík, the correlation was much poorer, or  $r^2 = 0.29$ ,  $P = 0.001$  and  $r^2 = 0.23$ ,  $P = 0.021$ , respectively. The lack of correlation may be caused by different conditions in the peat, affecting pollen size within each monolith but some of the samples were small so standard errors in calculations may be partly responsible.

### 4.2 Pollen analysis

The pollen analysis of the Hella samples had been done before (Hallisdóttir and Caseldine 2005) and it is not a part of this thesis. It shows the initial vegetative succession of the area from hardy pioneer species through stages of succession characterized by *Salix*, then *Juniperus* and *Betula* which began with *B. nana* but rose to becoming woodlands with abundant *B. pubescens*. The woodlands gave way for heath with *Ericales* and then rose again near the upper limits of 7 cal ka. The monolith is dated by tephra layers at top and bottom and the rate of deposition is assumed to be constant in Figures 12 and 13. An inconsistency to climate index from the Greenland ice core project is seen in Figure 12, suggesting faster deposition in the earlier part (discussion in Paper III, 4.2 p. 356).

The pollen analyses of Eyvík and Ytra-Áland peats are not as detailed as that from Hella and are meant more as crude background information on vegetation

for the study on birch hybridisation. They show that the trends in vegetation were comparable to Hella, with some similarities and notable differences.

The Eyvík monolith spans a slightly shorter period than Hella. The vegetation prior to the Saksunarvatn shows a stage characterized by increasing *Salix* and dwarf shrubs, then *Juniperus* until *Betula* started expanding, in a sequence which is very similar to that of Hella. The main difference was that the juniper stage lasted longer and the *Betula* did not expand until approximately 500 years later. The tree birch followed the dwarf birch faster and was only about 300 years later than in Hella. In Hella, a drastic decline in *Betula* pollen was seen in connection to the 8.2 cal ka cold event. In Eyvík, the woodlands were not as dominant as at Hella (Figure 12), therefore, the woodland decline was not as prominent.

The Ytra-Áland monolith covers the last ten thousand years, three times the time span of the other two. One sample retrieved beneath the Saksunarvatn tephra displayed *Salix*, Ericales and Caryophyllaceae, comparable to the earliest stages in Hella and Eyvík. This type of vegetation continued above Saksunarvatn tephra with added Poaceae and *B. nana* in low quantities until around 8.8 cal ka when Ericales pollen became dominant. Tree birch did not spread in the area until after the 8.2 cal ka cold event when *B. pubescens* was seen spreading rapidly for a short period ending abruptly around 7 cal ka. The fast advance of tree birch is partly masked in the pollen diagram (Paper V) and in Figure 12 by the large amount of other pollen released in this period, seen in pollen accumulation rates (PAR) values on the pollen diagram. This indicates a local Holocene Thermal Maximum (HTM). A cold and very wet period followed but woodland expansion was repeated around 6 cal ka. The woodlands reached their maximum around 5 cal ka and slowly declined thereafter with setbacks and advances up to the present. As seen in species proportions, dwarf birch was a considerable part of the woodlands and other vegetation, especially Ericales, increased gradually. Near 3 cal ka, the decline of woodlands accelerated and by the time of human settlement eleven hundred years ago, little was left in Thistilsjördur area but heather with dwarf birch.

### **4.3 *Betula* pollen measurements and calculation of species proportions**

We were able to test our method of species calculation on the Hella samples (Karlsdóttir et al. 2009, paper III). A pollen analysis of the same samples had already been done by Margrét Hallsdóttir, using visual characteristics of the *Betula* pollen to differentiate the species (results published in Hallsdóttir and Caseldine 2005). This was before the pollen was measured and species proportion calculated in the present study. There was little difference in the

results regarding the relative proportions of *B. nana* and *B. pubescens*. This could be due to the normal triporate hybrid pollen, which looks like *B. pubescens* pollen with a thick wall and deep pores but has small diameter and thus adds to the *B. nana* pollen pool when calculated.

In all the studies, *Betula* pollen was found in nearly all samples but only part of the samples yielded sufficient quantities for calculation of species proportions. Both *B. nana* and *B. pubescens* pollen was present for some period within the time span of samples at all sites. In all cases, the oldest samples had very few if any *Betula* pollen. Small *Betula* pollen, in quantities indicating the presence of *B. nana* locally, was first detected at Hella around 10 cal ka, in Eyvík around 9.5 cal ka and in Ytra-Áland around 8.7 cal ka. A rapid increase in pollen numbers and size, indicating *B. pubescens* locally, occurred in Hella around 9.6 cal ka, at Eyvík around 9.3 cal ka and at Ytra-Áland around 8.0 cal ka, always approximately a hundred years after *B. nana*.

The great difference between the initial tree birch woodlands at Hella and at Ytra-Áland emphasizes the role of environment rather than availability of seed as a prime factor in birch growth in Iceland. Later development of the established woodlands confirms the environmental differences of the sites.

Looking at the calculated proportions of *Betula pubescens* pollen in all terrestrial pollen (Figure 12) shows the woodlands in the Hella area were more like real birch forests compared to the scattered shrub- and woodlands seen at Eyvík and especially Ytra-Áland.

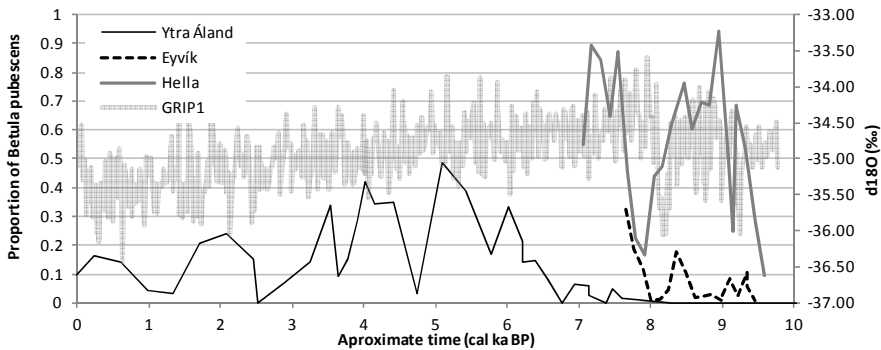


Figure 11. Calculated proportion of *B. pubescens* pollen in all pollen and Greenland ice core (GRIP1, Vinther et al 2006) index on climate. Hella (dark grey line), Eyvík (dotted line) and Ytra-Áland (solid black line).

#### 4.4 Phases of hybridisation

Non-triporate *Betula* pollen was found in nearly half of the samples but usually just one or two grains, which is well within the 1-2% expected from modern *B.*

*nana* or *B. pubescens*. In 15 samples, the non-triporate pollen was over 5%, indicating presence of triploid hybrids. Eight of those were Hella samples, two were at Eyvík and five were at Ytra-Áland. At all three sites, a raised proportion of non-triporate pollen was found near the time when *B. pubescens* started to colonize the area, in Hella around 9 cal ka, in Eyvík a little after 9 cal ka and in Ytra-Áland at 7.5 cal ka. Additional samples with raised numbers of non-triporate pollen were detected later, at Hella around 7.3 cal ka, At Eyvík near 7.8 cal ka and at Ytra-Áland near 3.3 cal ka.

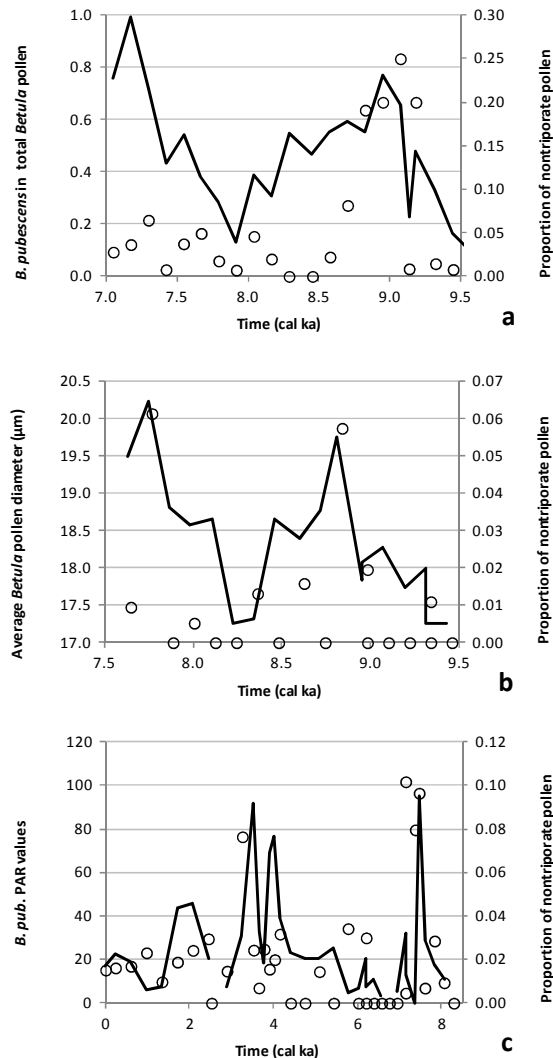


Figure 12. Nontriporate pollen (open circles) compared with other variables (lines). a) Proportion of *B. pubescens* in total *Betula* pollen at Hella. b) Average *Betula* pollen diameter at Eyvík. c) Calculated *Betula* pollen PAR values at Ytra-Áland.



Our method of counting non-triporate pollen only detects phases of intense hybridisation because triploid hybrids probably produce less pollen than diploid or tetraploid individuals and most hybrids pollen is triporate and looks normal. Therefore, low levels of hybridisation can go unnoticed and probably did in our study at some level.

At Hella, the peaks in non-triporate pollen were quite high; the proportion in *Betula* pollen reaching 25%. This is remarkable, as an average modern hybrid only produces half as much or 12-13% non-triporate pollen. There was, however, a large variation among individual triploid hybrids, whereby individuals were seen to have higher proportion, up to 53%. It therefore seems likely that such individuals were growing close to the sampling site.

A connection of non-triporate peaks to increasing *B. pubescens* was evident but it is not clear which variables would best predict a phase of hybridisation. In Hella, we saw a connection to peaks in *B. pubescens* proportion of terrestrial pollen. In Eyvík, peaks of non-triporate pollen and pollen diameters coincided, but at Ytra-Áland peaks of non-triporates coincided with peaks in calculated *B. pubescence* PAR (Figure 13).

In modern woodlands, triploid hybrids are frequent (Thórsson 2008), and the possibility to connect the present global warming to a new phase of birch hybridisation is tempting.

## 4.5 Birch hybridisation in the Holocene

The results of the present study show us that special environmental conditions facilitate the production of hybrids. It seems reasonable to conclude that changes of climate between temperatures favourable for tree birch and dwarf birch drive the hybridisation. A climate close to the lower margins of tree birch requirements seems to have prevailed in Iceland for long periods within the Holocene so small changes in temperature have repeatedly moved the natural boundaries between tree and dwarf shrub back and forth. This has made considerable parts of Iceland into a hybrid zone. The repeated increase in the downy birch and possible hybridisation, followed by retreat that gives ground to the dwarf birch again, sets the stage and the frequent presence of hybrids invites introgression.

Elkington (1968) constructed a hybrid index for birch, based on morphological traits and used it to compare individual trees and shrubs sampled in north-western Iceland, England and Scotland. His results showed much more hybrid traits in Iceland than in Britain, especially in the *B. pubescens*.

Methods, comparable to what Elkington (1968) used for his hybrid index, were used by Thórsson et al. (2007) to evaluate the morphology of ploidy determined Icelandic birch. The score of the triploid hybrids showed great

variation but they tend to resemble *B. nana* more than *B. pubescens*. The diploid and tetraploid plants, i.e. *B. nana* and *B. pubescens*, also had continuous variation in morphology, showing the hybrid traits Elkington (1968) found. This strongly suggests past introgression via triploid hybrids that has been proven possible by controlled backcrosses (Ananthawat-Jónsson and Tómasson 1990) and confirmed by statistical (Thórsson et al. 2007) and molecular methods (Thórsson et al. 2010).

#### **4.5.1 The role of hybridisation**

In evolution, speciation is achieved by reproduction barriers, allowing the new species to optimise fitness within a given habitat (Rieseberg and Wills 2007). The opposite procedure, hybridisation of taxa by breaking down barriers and allowing gene flow between species sacrifices the specialisation for increased genetic variation that might promote the development of new adaptations (Andersson and Stebbins 1954; Rieseberg et al. 2003). It also stands to reason as Barton (2001) pointed out, that even though an average recombination of two well-adapted genotypes is likely to be less fit than the parents, some of the hybrid genotypes might be even fitter. More importantly, in the context of evolution, hybrids, regardless of their fitness, serve as a bridge for gene transfer between species, as is the case of birch introgressive hybridisation in Iceland (Ananthawat-Jónsson 2003, 2012).

The history of birch in Europe in the Pleistocene and Holocene seems to be complicated and involve intensive hybridisation. The three *Betula* species, *B. pendula* Roth, *B. pubescens* and *B. nana*, all share genetic material extensively and greater genetic variation is seen among geographical regions than among sympatric species (Palme et al. 2004).

It has been reasoned that hybridisation might contribute to the colonization of novel habitats, range expansions, and invasiveness of species (Ellstrand and Schierenbeck 2000; Rieseberg et al. 2007). In the extreme climate changes of the Pleistocene, with alternating glaciations and interglacials, *B. pubescens* could have existed with either *B. pendula* or *B. nana* in mobile hybrid zones, while vegetation advanced or retreated. This might have played an important part in revegetation both during Pleistocene interglacials and in the Holocene. Rieseberg et al. (2007) describe how hybridisation can increase a species ability to expand its range and even become invasive. Their example is the common sunflower, *Helianthus annuus* L. in Texas. The hybridisation of *B. nana* and *B. pubescens*, with subsequent introgression, led to the establishment of mountain birch and might have increased the abilities of both species to expand in interglacials and the Holocene.

## 4.6 The future of Icelandic woodlands

In Iceland, woodlands retreated in the latter half of the Holocene. Their maximum cover may have been around 40 thousand km<sup>2</sup>, reduced almost by half before the settlement around 900 AD and by 1990 they were 1200 km<sup>2</sup> (sections 1.3 and 1.4). Near the beginning of the 20<sup>th</sup> century, a growing concern for the fate of the remaining woodlands led to the establishment of the Iceland Forest Service in 1908. Protection of woodland remnants and afforestation began slowly, but gained momentum in the later part of the century. One of the main hindrances in the regeneration of woodlands has been the uncontrolled sheep grazing, but woodlands enclosed by protective fences have grown considerably in the later years (Eysteinnsson 2013).

Icelandic woodlands are likely to benefit from the present climate changes threatening wildlife and human economy worldwide. A rise in annual mean temperature of around 5°C is predicted in the Arctic during the 21<sup>st</sup> century but summer temperatures are supposed to rise less, around 2°C (Christensen et al. 2007). Vegetation has increased in recent years and birch forest boundaries have moved further up into the highlands (Björnsson et al. 2008). Further changes would lead to an establishment of birch woodlands in vast areas, now defined as heathland, fellfields, or tundra, if land use allowed. Both *B. nana* and *B. pubescens* would expand to higher altitudes. In the process, a new wave of triploid *Betula* hybrids could be expected when *B. nana* flowers are pollinated by *B. pubescens*. The hybrids would gradually give way for the taller-growing and more fertile *B. pubescens*. In the meantime, some of them could backcross to the parent species, adding new combinations of genes for evolution to work with.

## 4.7 Conclusions

The distribution of birch in Iceland after the retreat of the Pleistocene ice sheet seems to have relied more on availability of suitable habitats than seed. Birds may have played an important role in birch dispersion to and within Iceland in early Holocene. Local conditions, especially summer temperatures, but also stability of soil surface, could have determined the time and place of birch establishment.

Environment, as well as treatment of pollen, affects the pollen size. Subfossil pollen sampled at different depths in the same monolith may have been exposed to different conditions and their size alone may not reflect species proportion directly. A comparison of sizes within each sample is more secure. Species differentiation on the basis of visual identification can also give a reliable result.

The proportion of non-triporate *Betula* pollen can be used as an index of birch hybridisation. The hybridisation between *B. pubescens* and *B. nana* occurs

in waves rather than continuously, presumably when several conditions are met simultaneously. Such waves of hybridisations have occurred a few times during the Holocene.

There is a connection between waves of hybridisation and the advance of *B. pubescens*. As *B. pubescens* requires warmer summers than *B. nana* does, the waves connect to warming climate. The frequency of triploid hybrids in modern woodlands indicates a contemporary hybridisation wave.

## References

- Abbott RJ and Brochmann C (2003). History and evolution of the arctic flora: in the footsteps of Eric Hultén. *Molecular Ecology* 12: 299-313.
- Adams RP, Pandey RN, Leverenz JW, Dignard N, Hoegh K, and Thorfinnsson T. (2003). Pan-Arctic variation in *Juniperus communis*: historical biogeography based on DNA fingerprinting. *Biochemical Systematics and Ecology* 31: 181-192.
- Anamthawat-Jónsson K (2003). Hybrid introgression in *Betula*. *Plant genome: biodiversity and evolution* 1: 249-265.
- Anamthawat-Jónsson K (2012). Hybridisation, introgression and phylogeography of Icelandic birch. In: Current Topics in Phylogenetics and Phylogeography of Terrestrial and Aquatic Systems (Anamthawat-Jónsson, ed.). InTech – Open Access Publisher, Croatia, pp. 117-144, ISBN 978-953-51-0217-5
- Anamthawat-Jónsson K and Tómasson Th (1990). Cytogenetics of hybrid introgression in Icelandic birch. *Hereditas* 112: 65-70.
- Anderson E and Stebbins JrGL (1954). Hybridization as an evolutionary stimulus. *Evolution* 8: 378-388.
- Andersson E, Nilsson C and Johansson ME (2000). Plant dispersal in boreal rivers and its relation to the diversity of riparian flora. *Journal of Biogeography* 27: 1095-1106.
- Andrén T, Lindeberg G and Andren E (2002). Evidence of the final drainage of the Baltic Ice Lake and the brackish phase of the Yoldia Sea in glacial varves from the Baltic Sea. *Boreas* 31: 226-238.
- Arnalds A (1987). Ecosystem disturbance in Iceland. *Arctic and Alpine Research* 19: 508-513.
- Augspurger CK (1986). Morphology and dispersal potential of wind-dispersed diaspores of neotropical trees. *American journal of Botany* 73: 353-363.
- Barton NH (2001). The role of hybridization in evolution. *Molecular Ecology* 10: 551-568.

- Bennike O (1999). Colonisation of Greenland by plants and animals after the last ice age: a review. *Polar Record* 35: 323-336.
- Bennike O (2000). Palaeoecological studies of Holocene lake sediments from west Greenland. *Palaeogeography, Palaeoclimatology, Palaeoecology* 155: 285-304.
- Bhattacharya CG (1967). A simple method of resolution of a distribution into Gaussian components. *Biometrics* 23: 115–135.
- Björnsson H, Sveinbjörnsdóttir ÁE, Daníelsdóttir AK, Snorrason Á., Sigurdsson BD, Sveinbjörnsson E, Viggósson G, Sigurjónsson J, Baldursson S, Thorvaldsdóttir S and Jónsson T (2008). Hnattrænar loftslagsbreytingar og áhrif þeirra á Íslandi – Skýrsla vísindanefndar um loftslagsbreytingar. Ministry for the Environment and Natural Resources, Reykjavík. [In Icelandic].
- Blackmore S, Steinmann JA, Hoen PP and Punt W (2003). Betulaceae and Corylaceae. *Review of Palaeobotany and Palynology* 123: 71-98.
- Brochet AL, Guillemain M, Fritz H, Gauthier-Clerc M and Green AJ (2009). The role of migratory ducks in the long-distance dispersal of native plants and the spread of exotic plants in Europe. *Ecography* 32: 919-928.
- Brochmann C, Gabrielsen TM, Nordal I, Landvik JY and Elven R (2003). Glacial survival or tabula rasa?: The history of North Atlantic biota revisited. *Taxon* 52: 417-450.
- Buchardt B and Símonarson LA (2003). Isotope palaeotemperatures from the Tjörnes beds in Iceland: evidence of Pliocene cooling. *Palaeogeography, Palaeoclimatology, Palaeoecology* 189: 71-95.
- Buckland PC and Dugmore, AJ (1991). If this is a refugium, why are my feet so bloody cold? The origins of the Icelandic biota in the light of a recent research. In: Maizels JK and Caseldine C (eds.). *Environmental change in Iceland: past and present*: 107-126. Kluwer. Dordrecht.
- Buckland PC and Panagiotakopulu E (2010). Reflections on North Atlantic Island biogeography: a quaternary entomological view. In: Bengtson SA, Buckland PC, Enckell, PH and Fosaa AM (eds.): *Dorete – Her Book:- Being a Tribute to Dorete Bloch and to Faroese Nature. Annales Societatis Scientiarum Færoensis Supplementum* 52: 187–215.
- Cain ML, Damman H, and Muir A (1998). Seed dispersal and the Holocene migration of woodland herbs. *Ecological Monographs* 68: 325-347.

- Carlquist S (1981). Chance Dispersal: Long-distance dispersal of organisms, widely accepted as a major cause of distribution patterns, poses challenging problems of analysis. *American Scientist* 69: 509-516.
- Caseldine C (2001). Changes in *Betula* in the Holocene record from Iceland - a palaeoclimatic record or evidence for early Holocene hybridisation? *Review of Palaeobotany and Palynology* 117: 139-152.
- Caseldine C, Geirsdóttir Á and Langdon P (2003). Efstadalsvatn—a multi-proxy study of a Holocene lacustrine sequence from NW Iceland. *Journal of Paleolimnology* 30: 55-73.
- Christensen JH, Hewitson B, Busuioc, Chen A, Gao X, Held I, Jones R, Kolli RK, Kwon W-T, Laprise R, Magana Rueda V, Mearns L, Menendez CG, Räisänen J, Rinke A, Sarr A and Whetton P (2007). Regional climate projections. In: Solomon S, Qin D., Manning M, Chen Z, Marquis M, Averyt KB, Tignor M and Miller HL (eds.). *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. pp 847-940.
- Clark JS (1998). Why trees migrate so fast: confronting theory with dispersal biology and the paleorecord. *The American Naturalist* 152: 204-224.
- Dahlgren TG, Weinberg JR and Halanich KM (2000). Phylogeography of the ocean quahog (*Arctica islandica*): influences of paleoclimate on genetic diversity and species range. *Marine Biology* 137: 487-495.
- De Groot WJ, Thomas PA and Wein R. W (1997). *Betula nana* L. and *Betula glandulosa* Michx. *Journal of Ecology* 85: 241-264.
- Denk T, Grímsson F and Kvaček Z (2005). The Miocene floras of Iceland and their significance for late Cainozoic North Atlantic biogeography. *Botanical Journal of the Linnean Society* 149: 369-417.
- Denk T, Grímsson F and Zetter R (2010). Episodic migration of oaks to Iceland: Evidence for a North Atlantic “land bridge” in the latest Miocene. *American Journal of Botany* 97: 276-287.
- Denk T, Grímsson F, Zetter R and Símonarson LA (2011a). The biogeographic history of Iceland—the North Atlantic land bridge revisited. In: *Late Cainozoic Floras of Iceland* pp 647–668). Springer Netherlands.

- Denk T, Grímsson F, Zetter R and Símonarson LA (2011b). The Pleistocene Floras (2.4–0.8 Ma)–Shaping the Modern Vegetation of Iceland. In: *Late Cainozoic Floras of Iceland* (pp. 555–645). Springer Netherlands.
- Dragsted J and Kubin E (1990). Some results from Danish investigations in salt stress on trees. In *Proceedings of the SNS Seminar on Stress in Nature*, Oulanka, Finland, September 11–14, 1989. *Aquilo. Ser. Botanica* 29: 21–23.
- Dugmore AJ, Gísladóttir G, Simpson IA and Newton A (2009). Conceptual models of 1200 years of Icelandic soil erosion reconstructed using tephrochronology. *Journal of the North Atlantic* 2: 1–18.
- Eggertsson Ó (1993). Origin of the driftwood on the coasts of Iceland; a dendrochronological study. *Jökull* 43: 15–32.
- Eidesen PB, Alsos IG, Popp M, Stensrud, Ø, Suda J and Brochmann C (2007). Nuclear vs. plastid data: complex Pleistocene history of a circumpolar key species. *Molecular Ecology* 16: 3902–3925.
- Eidesen PB, Ehrich D, Bakkestuen V, Alsos IG, Gilg O, Taberlet P, and Brochmann C (2013). Genetic roadmap of the Arctic: plant dispersal highways, traffic barriers and capitals of diversity. *New Phytologist* 200: 898–910.
- Einarsson E (1961) Grös og gróður. In Thórarinnsson S. (ed.) *Náttúra Íslands*. Reykjavík, Almenna Bókafélagid. pp 267–294. [In Icelandic]
- Einarsson Th and Albertsson KJ (1988). The glacial history of Iceland during the past three million years. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences* 318: 637–644.
- Einarsson Th (1962). Vitnisburður frjógreiningar um gróður, veðurfar og landnám á Íslandi. *Saga: Tímarit sögufélagsins* 24: 442–469. [In Icelandic, English summary].
- Einarsson Th (1968). *Jardfræði, saga bergs og lands*. Mál og Menning. Reykjavík. pp 335. [In Icelandic].
- Elkington TT (1968). Introgressive hybridization between *Betula nana* L. and *B. pubescens* Ehrh. in North-West Iceland. *New Phytologist* 67: 109–118.
- Ellstrand NC and Schierenbeck KA (2000). Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences* 97: 7043–7050.



- Eysteinnsson Th (2013). Retrieved from Forestry in a treeless land. <http://www.skogur.is/english/forestry-in-a-treeless-land/> on 16 January 2014).
- Figuerola J and Green AJ (2002). How frequent is external transport of seeds and invertebrate eggs by waterbirds? A study in Donana, SW Spain. *Archiv für Hydrobiologie* 155: 557-565.
- Forestry Commission England (2011). Pathology Advisory Note (No. 11):** De-icing salt damage to trees. *Forest Research* 2011.
- Fostad O and Pedersen PA (2000). Container-grown tree seedling responses to sodium chloride applications in different substrates. *Environmental Pollution* 109: 203-210.
- Fridriksson S (1963). Þættir úr gróðursögu hálendisins sunnan jökla. *Náttúrufræðingurinn* 33: 1-48. [In Icelandic, English summary].
- Fridriksson S (1987). Plant colonization of a volcanic island, Surtsey, Iceland. *Arctic and Alpine Research* 19: 425-431.
- Fridriksson S and Sigurdsson H (1968). Dispersal of seed by snow buntings to Surtsey in 1967. *Surtsey Research Progress Report* 4: 43-49.
- Gates RR and Thomas N (1914). Memoirs: A Cytological Study of *Ænothera* mut. *lata* and *Æ. mut. Semilata* in Relation to Mutation. *Quarterly Journal of Microscopical Science* 2: 523-571.
- Geirsdóttir Á (2004). Extent and chronology of glaciations in Iceland; a brief overview of the glacial history. In: Ehlers J and Gibbard P (eds): *Quaternary glaciations extent and chronology. Part I: Europe*: 175-182. Elsevier, Amsterdam.
- Geirsdóttir Á (2011). Pliocene and Pleistocene glaciations of Iceland: a brief overview of the glacial history. In: Ehlers J, Gibbard PL, Hughes PD, (eds). *Developments in Quaternary Science* 15. Amsterdam: Elsevier: 199–210.
- Geirsdóttir Á, Miller GH, Axford Y and Ólafsdóttir S (2009). Holocene and latest Pleistocene climate and glacier fluctuations in Iceland. *Quaternary Science Reviews* 28: 2107-2118.
- Grace J, Berninger F and Nagy L (2002). Impacts of climate change on the tree line. *Annals of Botany* 90: 537-544.

- Graham DW, Larsen LM, Hanan BB, Storey M, Pedersen AK and Lupton JE (1998). Helium isotope composition of the early Iceland mantle plume inferred from the Tertiary picrites of West Greenland. *Earth and Planetary Science Letters* 160: 241-255.
- Greene DF and Johnson EA (1989). A model of wind dispersal of winged or plumed seeds. *Ecology* 70: 339-347.
- Greene DF and Johnson EA (1997). Secondary dispersal of tree seeds on snow. *Journal of Ecology* 85: 329-340.
- Grímsson F (2007). The Miocene floras of Iceland: *Origin and evolution of fossil floras from North-West and Western Iceland 15 to 6 Ma*. Doctoral dissertation, University of Iceland, Institute of Earth Sciences, Reykjavík.
- Gudmundsson HJ (1997). A review of the Holocene environmental history of Iceland. *Quaternary Science Reviews* 16: 81-92.
- Hagen AR, Giese H and Brochmann C (2001). Trans-Atlantic dispersal and phylogeography of *Cerastium arcticum* (Caryophyllaceae) inferred from RAPD and SCAR markers. *American Journal of Botany* 88: 103-112.
- Hallsdóttir M (1990). Studies in the vegetational history of North Iceland. A radiocarbon-dated pollen diagram from Flateyjaralur. *Jökull* 40: 67-82.
- Hallsdóttir M (1995) On the pre-settlement history of Icelandic vegetation. *Búvísindi* 9: 17-29.
- Hallsdóttir M and Caseldine CJ. (2005). The Holocene vegetation history of Iceland, state-of-the-art and future research. In: Caseldine CJ, Russell A, Hardardóttir J and Knudsen OF (eds): *Iceland: Modern Processes and Past Environments*: pp 319-332. Elsevier, Amsterdam.
- Holtmeier FK and Broll G (2005). Sensitivity and response of northern hemisphere altitudinal and polar treelines to environmental change at landscape and local scales. *Global Ecology and Biogeography* 14: 395-410.
- Howland DE, Oliver RP and Davy AJ (1995). Morphological and molecular variation in natural populations of *Betula*. *New Phytologist*, 130: 117-124.
- Huybers P (2006). Early Pleistocene glacial cycles and the integrated summer insolation forcing. *Science* 313: 508-511.

- Jakobsson M, Björck S, Alm G, Andrén T, Lindeberg G, and Svensson NO (2007). Reconstructing the Younger Dryas ice dammed lake in the Baltic Basin: Bathymetry, area and volume. *Global and Planetary Change* 57: 355-370.
- Johansen S and Hytteborn H (2001). A contribution to the discussion of biota dispersal with drift ice and driftwood in the North Atlantic. *Journal of Biogeography* 28: 105-115.
- Karlsdóttir L, Hallsdóttir M, Þórsson ÆTh, Anamthawat-Jónsson K (2009) Evidence of hybridisation between *Betula pubescens* and *B. nana* in Iceland during the early Holocene. *Review of Palaeobotany and Palynology* 156: 350-357.
- Koutstaal BP, Markusse MM and De Munck W (1987). Aspects of seed dispersal by tidal movements. In: Huiskes AH, Blom CWPM and Rozema (eds) *Vegetation between land and sea* pp. 226-235. Junk Publishers, Netherlands.
- Kristinsson H (2010). *Íslenska plöntuhandbókin: Blómplöntur og byrkningar*, 3<sup>rd</sup> ed. Mál og menning. Reykjavík. [In Icelandic].
- Kullman L (2002). Rapid recent range-margin rise of tree and shrub species in the Swedish Scandes. *Journal of Ecology* 90: 68-77.
- Körner C and Paulsen J (2004). A worldwide study of high altitude treeline temperatures. *Journal of Biogeography* 31: 713-732.
- Lampe R (2005). Lateglacial and Holocene water-level variations along the NE German Baltic Sea coast: review and new results. *Quaternary International* 133: 121-136.
- Lutz AM (1917). Characters indicative of the number of somatic chromosomes present in *Oenothera* mutants and hybrids. *The American Naturalist* 51: 375-377.
- Magnússon B, Magnússon SH and Fridriksson S (2009) Developments in plant colonization and succession on Surtsey during 1999–2008. *Surtsey Research* 12: 57–76.
- Mäkelä EM. (1996). Size distinctions between *Betula* pollen types—a review. *Grana* 35: 248-256.
- Maliouchenko O, Palmé AE, Buonamici A, Vendramin GG and Lascoux M (2007). Comparative phylogeography and population structure of European *Betula* species, with particular focus on *B. pendula* and *B. pubescens*. *Journal of Biogeography* 34: 1601-1610.

- Matlack GR (1992). Influence of fruit size and weight on wind dispersal in *Betula lenta*, a gap-colonizing tree species. *American Midland Naturalist* 128: 30-39.
- McDougall I, Kristjansson L and Sæmundsson K (1984). Magnetostratigraphy and geochronology of northwest Iceland. *Journal of Geophysical Research: Solid Earth (1978–2012)* 89: 7029-7060.
- Milne RI (2006). Northern Hemisphere plant disjunctions: a window on tertiary land bridges and climate change? *Annals of Botany* 98: 465-472.
- Ministry for the Environment and Natural Resources (2007). Vernd og endurheimt íslenskra birkiskóga – skýrsla og tillögur nefndar. Ministry for the Environment and Natural Resources, Reykjavík. [In Icelandic].
- Morgan JH (2005). A computer method for resolving mixed normal distributions. *Ringings and Migration* 22: 145-152.
- Nilsen TH (1978). Lower Tertiary laterite on the Iceland-Faeroe Ridge and the Thulean land bridge. *Nature* 274: 786-788.
- Nogales M, Medina FM and Valido A (1996). Indirect seed dispersal by the feral cats *Felis catus* in island ecosystems (Canary Islands). *Ecography* 19: 3-6.
- Nogales M, Quilis V, Medina FM, Mora JL and Trigo LS (2002). Are predatory birds effective secondary seed dispersers? *Biological Journal of the Linnean Society* 75: 345-352.
- Odland A (1996). Differences in the vertical distribution pattern of *Betula pubescens* in Norway and its ecological significance. *Paläoklimaforschung* 20: 43-59.
- Otto S.P and Whitton J (2000). Polyploid incidence and evolution. *Annual Review of Genetics* 34: 401-437.
- Palme AE, Su Q, Palsson S and Lascoux M (2004). Extensive sharing of chloroplast haplotypes among European birches indicates hybridization among *Betula pendula*, *B. pubescens* and *B. nana*. *Molecular Ecology* 13: 167-178.
- Parducci L, Jørgensen T, Tollefsrud MM, Elverland E, Alm T, Fontana SL, Bennett KD, Haile J, Matetovici I, Suyama Y, Edwards ME, Andersen K, Rasmussen M, Boessenkool S, Coissac E, Brochmann C, Taberlet P, Houmark-Nielsen M, Larsen NK, Orlando L, Gilbert MTP, Kjær KH, Alsos IG and Willerslev E (2012). Glacial survival of boreal trees in Northern Scandinavia. *Science*, 335: 1083-1086.

- Pauly D and Caddy JF 1985. *A modification of Bhattacharya's method for the analysis of mixtures of normal distributions*. FAO Fisheries Circular, vol. 781. FAO, Rome.
- Pennycuik C (1997). Actual and optimum flight speeds: field data reassessed. *Journal of Experimental Biology* 200: 2355-2361.
- Porsild AE, Harington CR and Mulligan GA (1967). *Lupinus arcticus* Wats. grown from seeds of Pleistocene age. *Science* 158: 113-114.
- Prentice IC (1981) Quantitative birch (*Betula* L.) pollen separation by analysis of size frequency data. *New Phytologist* 89: 145-157.
- Ramsey J and Schemske DW (1998). Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* 29: 467-501.
- Reimer PJ, Baillie MG, Bard E, Bayliss A, Beck JW, Blackwell PG, Ramsey CB, Buck CE, Burr GS, Edwards RL, Friedrich M, Grootes PM, Guilderson TP, Hajdas I, Heaton TJ, Hogg AG, Hughen KA, Kaiser KF, Kromer B, McCormac FG, Manning SW, Reimer RW, Richards DA, Southon JR, Talamo S, Turney CSM, van der Plicht J and Weyhenmeyer CE (2009). IntCal09 and Marine09 radiocarbon age calibration curves, 0–50,000 years cal BP. *Radiocarbon* 51: 1111-1150.
- Rieseberg LH and Willis JH (2007). Plant speciation. *Science* 317: 910-914.
- Rieseberg LH, Raymond O, Rosenthal DM, Lai Z, Livingstone K, Nakazat T, Durphy JL, Schwarzbach AE, Donovan LA and Lexer C (2003). Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* 301: 1211–1216.
- Rieseberg LH, Kim SC, Randell RA, Whitney KD, Gross BL, Lexer C, and Clay K (2007). Hybridization and the colonization of novel habitats by annual sunflowers. *Genetica* 129: 149-165.
- Robichaud A and Bégin Y (1997). The effects of storms and sea-level rise on a coastal forest margin in New Brunswick, eastern Canada. *Journal of Coastal Research* 13: 429-439.
- Rundgren M (1995). Biostratigraphic evidence of the Allerød-Younger Dryas-Preboreal oscillation in Northern Iceland. *Quaternary Research* 44: 405-416.

- Rundgren M (1998). Early-Holocene vegetation of northern Iceland: pollen and plant macrofossil evidence from the Skagi peninsula. *The Holocene* 8: 553-564.
- Rundgren M and Ingólfsson O (1999). Plant survival in Iceland during periods of glaciation? *Journal of Biogeography* 26: 387-396.
- Scharff RF (1909). On the evidences of a former land-bridge between northern Europe and North America. In: *Proceedings of the Royal Irish Academy. Section B: Biological, Geological, and Chemical Science* (Vol. 28, pp. 1-28). Royal Irish Academy.
- Schönswetter P, Elven R and Brochmann C (2008). Trans-Atlantic dispersal and large-scale lack of genetic structure in the circumpolar, arctic-alpine sedge *Carex bigelowii* s.l. (Cyperaceae). *American Journal of Botany*, 95: 1006-1014.
- Seppä H and Birks HJB (2001). July mean temperature and annual precipitation trends during the Holocene in the Fennoscandian tree-line area: pollen-based climate reconstructions. *The Holocene* 11: 527-539.
- Stötter J, Wastl M, Caseldine C, and Häberle T. (1999). Holocene palaeoclimatic reconstruction in northern Iceland: approaches and results. *Quaternary Science Reviews* 18: 457-474.
- Tackenberg O (2001). *Methoden zur Bewertung gradueller Unterschiede des Ausbreitungspotentials von Pflanzenarten*. Doctoral dissertation, Universitätsbibliothek Marburg.
- Tackenberg O (2003). Modeling long-distance dispersal of plant diaspores by wind. *Ecological Monographs* 73: 173-189.
- Thórsson ÆTh 2008. *Genecology, introgressive hybridisation and phylogeography of betula species in Iceland*. Doctoral dissertation, University of Iceland, Faculty of Science. Reykjavík.
- Thórsson ÆTh, Pálsson S, Sigurgeirsson A, Anamthawat-Jónsson K (2007). Morphological variation among *Betula nana* (diploid), *B. pubescens* (tetraploid) and their triploid hybrids in Iceland. *Annals of Botany* 99: 1183-1193.
- Thórsson ÆTh, Pálsson S, Lascoux M and Anamthawat-Jónsson K (2010). Introgression and phylogeography of *Betula nana* (diploid), *B. pubescens* (tetraploid) and their triploid hybrids in Iceland inferred from cpDNA haplotype variation. *Journal of Biogeography* 37: 2098-2110.

- Traustason B and Snorrason A (2008). Spatial distribution of forests and woodlands in Iceland in accordance with the CORINE land cover classification. *Icelandic Agricultural Sciences* 21: 39-47.
- Tremblay LB, Mysak LA and Dyke AS (1997). Evidence from driftwood records for century-to-millennial scale variations of the high latitude atmospheric circulation during the Holocene. *Geophysical Research Letters*, 24: 2027-2030.
- Truong C, Palmé AE and Felber F (2007). Recent invasion of the mountain birch *Betula pubescens* ssp. *tortuosa* above the treeline due to climate change: genetic and ecological study in northern Sweden. *Journal of Evolutionary Biology* 20: 369-380.
- Vasari Y and Vasari A (1990). L'histoire Holocène des lacs Islandais. In: Malaurie, Jean (ed.), *102 témoignages en hommage à quarante ans d'études arctiques*, pp 279-293. Paris: Éditions Plon. [In French].
- Verhoeven K, Louwye S and Eiríksson J (2013). Plio-Pleistocene landscape and vegetation reconstruction of the coastal area of the Tjörnes Peninsula, Northern Iceland. *Boreas* 42: 108-122.
- Vinther BM, Clausen HB, Johnsen SJ, Rasmussen SO, Andersen KK, Buchardt SL, Dahl-Jensen D, Seierstad IK, Siggaard-Andersen ML, Steffensen JP, Svensson AM, Olsen J and Heinemeier J (2006). A synchronized dating of three Greenland ice cores throughout the Holocene. *Journal of Geophysical Research* 111, D13102, doi:10.1029/2005JD006921
- Wastl M, Stötter J, and Caseldine C. (2001). Reconstruction of Holocene variations of the upper limit of tree or shrub birch growth in northern Iceland based on evidence from Vesturárdalur-Skíðadalur, Tröllaskagi. *Arctic, Antarctic, and Alpine Research* 33: 191-203.
- Weih M and Karlsson PS (2001). Growth response of Mountain birch to air and soil temperature: is increasing leaf-nitrogen content an acclimation to lower air temperature? *New Phytologist* 150: 147-155.
- Weisbrod AR and Johnson RC (1989). Lyme disease and migrating birds in the Saint Croix River Valley. *Applied and Environmental Microbiology* 55: 1921-1924.
- Westergaard KB, Alsos IG, Popp M, Engelskjøn T, Flatberg KI and Brochmann C (2011). Glacial survival may matter after all: nunatak signatures in the rare European populations of two west-arctic species. *Molecular Ecology* 20: 376-393.

- Wielgolaski FE (2005). History and environment of the Nordic mountain birch. In: *Plant Ecology, Herbivory, and Human Impact in Nordic Mountain Birch Forests*, pp. 3-18. Springer, Berlin Heidelberg.
- Wöll C. (2008). Treeline of mountain birch (*Betula pubescens* Ehrh.) in Iceland and its relationship to temperature. Department of Forestry: Technical University Dresden. p. 125.
- Yashina S, Gubin S, Maksimovich S, Yashina A, Gakhova E and Gilichinsky D (2012). Regeneration of whole fertile plants from 30,000-y-old fruit tissue buried in Siberian permafrost. *Proceedings of the National Academy of Sciences* 109: 4008-4013.
- Zimmerman HB, Shackleton NJ, Backman J, Kent DV, Baldauf JG, Kaltenback AJ and Morton AC (1984). History of Plio-Pleistocene climate in the northeastern Atlantic, deep sea drilling project Hole 552A. *Initial Reports of the Deep Sea Drilling Project* 81: 861-876.
- Ægisdóttir HH and Thórhallsdóttir, ThE. (2004). Theories on migration and history of the North-Atlantic flora: a review. *Jökull* 54: 1-16.



# PAPER I

## **Differentiating pollen of *Betula* species from Iceland**

Karlsdóttir L, Thórsson ÆTh, Hallsdóttir M, Sigurgeirsson A, Eysteinnsson Th and Anamthawat-Jónsson K (2007)

*Grana* 46: 78-84.

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*<http://www.tandfonline.com/doi/full/10.1080/00173130701237832>*

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# Differentiating pollen of *Betula* species from Iceland

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L. Karlsdóttir<sup>1</sup>, Æ. Th. Thórsson<sup>1</sup>, M. Hallsdóttir<sup>2</sup>, A. Sigurgeirsson<sup>3</sup>, Th. Eysteinnsson<sup>4</sup>, & K. Ananthawat-Jónsson<sup>1</sup>

<sup>1</sup>Institute of Biology, University of Iceland, Askja - Sturlugata 7, Reykjavik, IS-101, Iceland, <sup>2</sup>Icelandic Institute of Natural History, Box 5320, Reykjavik, IS-125, Iceland, <sup>3</sup>Icelandic Forest Research (Research Division of the Icelandic Forest Service), Mógilsá, Reykjavik, IS-116, Iceland, <sup>4</sup>Iceland Forest Service, Box 98, Egilsstaðir, IS-700, Iceland.

## Abstract

Subfossil pollen from two co-existing *Betula* species in Iceland, *B. nana* and *B. pubescens*, is frequently found in sediments and peat. Interpretation of the findings often depends on the ability to differentiate between the two species according to pollen size and structure. Fresh pollen samples were prepared from 70 individual trees/shrubs which had been identified to species by chromosome number. Grain diameters and pore depths were measured and ratios of grain diameter to pore depth (D/P ratios) were calculated. The mean grain diameters of pollen from diploid *B. nana* and tetraploid *B. pubescens* were 20.42 and 24.20 µm, whereas mean pore depths were 2.20 and 2.81 µm respectively. Mean D/P ratios were therefore 9.55 for *B. nana* and 8.85 for *B. pubescens*. The difference between species was statistically significant for all three pollen parameters. Grain diameter appeared to be the most useful parameter, as only about 20% of the samples were in the overlapping region of the species distributions. Pollen size (grain diameter) was also positively correlated to tree morphology, which was evaluated using species-specific botanical characters. Pollen samples from different locations/populations in Iceland varied slightly in mean size and ratio. The size difference between pollen of *B. nana* and *B. pubescens* in this study is less than other papers have reported, which may be due to the effect of introgressive hybridisation between the two birch species in Iceland.

**Keywords:** *Betula nana*, *Betula pubescens*, pollen size, pollen morphology, polyploidy

## Introduction

Two birch species are found in Iceland: arctic dwarf birch (*Betula nana* Linnaeus 1753) and downy birch (*Betula pubescens* Ehrhart 1790). *Betula nana* is a prostrate shrub up to one metre in height and has its main distribution in the sub-arctic and alpine regions of the Northern Hemisphere. The species is represented by subspecies *nana* (Sukaczew) Hultén in Europe (including Iceland) and western Asia, and subspecies *exilis* (Sukaczew) Hultén in North America and central and eastern Asia (Hultén & Fries 1986). *Betula pubescens* is a European species, represented by subspecies *pubescens* Ehrhart, which may grow up to 25 m tall with single or many stems, and also by subspecies *tortuosa* (Ledebour) Nyman, which is a shrub or low-growing tree found in the mountain regions of northern Europe (Walters 1964). The latter subspecies, so-called mountain birch, is believed to be the result of introgressive hybridisation with *B. nana* (reviewed in Atkinson 1992). Due to extensive and continuous morphological variation of birch in Iceland (Thórsson et al. 2001; Anamthawat-Jónsson 2003a), this tree birch species is not divided into subspecies in this study but is treated as one, *Betula pubescens* sensu lato.

*Betula nana* and *B. pubescens* are wind pollinated and both species produce large quantities of pollen, which can frequently be found in sediments and peat proximate to their habitat. Their pollen morphology is very similar. But since the species represent different ecosystems, it is important to be able to differentiate the pollen, especially when reconstruction of vegetation history is based on subfossil pollen. Pollen size measurements on different species of *Betula* have been published in several papers, for example Birks (1968), Mäkelä (1999) and Caseldine (2001). Pollen samples from trees/shrubs, which are identified to species based on morphological features, are normally used as a reference for distinguishing subfossil pollen. However, even if mean sizes differ markedly; size frequency curves tend to overlap so much that species separation becomes difficult. Species differentiation by pollen structure is also possible (Blackmore et al. 2003): for example by comparing pollen pore depths and ratios of grain diameter to pore depth (Birks 1968; Mäkelä 1996; Clegg et al. 2005). Species differentiation of subfossil pollen is best accomplished using methods based on combined parameters, together with appropriate statistical analyses.

The aim of this paper was to establish a reliable way of differentiating pollen of *B. nana* and *B. pubescens* using samples of birch plants that were identified to species based on cytotaxonomy rather than morphology alone. *Betula* in Iceland has been shown to be chromosomally stable – the genus comprises three ploidy groups, i.e. diploid ( $2n = 2x = 28$ ) *B. nana*, tetraploid ( $2n = 4x = 56$ ) *B. pubescens* and a triploid ( $2n = 3x = 42$ ) hybrid between the two birch species (Anamthawat-Jónsson & Tómasson 1990; Thórsson et al. 2001). On the other

hand, all three groups are extremely variable morphologically, due to bidirectional introgression between the two species via triploid intermediates (Thórsson et al. 2001; Anamthawat-Jónsson 2003a). Such morphological variation has often made species identification very difficult, and as a consequence species references for pollen differentiation can not be standardized. In this work we made pollen measurements on samples from field plants with known chromosome number, i.e. diploid *B. nana* and tetraploid *B. pubescens*. These measurements should allow us to estimate *Betula* species composition in subfossil pollen samples more reliably.

## Materials and Methods

Pollen samples were collected in the spring of 2004 and 2005 from individual plants (trees or shrubs) that had been verified by chromosome number and morphological analysis. The method of chromosome preparation from leaf buds followed Anamthawat-Jónsson (2003b). Diploid birch plants (with 28 chromosomes) were classified as *Betula nana*, whereas tetraploid plants (with 56 chromosomes) were treated as *B. pubescens*. The diploid group also had morphological characteristics typical of *B. nana* (low scores of morphology index), whereas most tetraploid plants had *B. pubescens* morphology (high scores, see the scale in Thórsson et al. 2001). The plants were selected from ten different sites representing all major woodlands in Iceland (Table I). Male catkins were either collected directly in the field or from branches that were taken indoors to allow fresh anthesis.

Samples of pollen were mounted in silicon oil after boiling in NaOH, HCl and HF, followed by acetolysis (Fægri & Iversen 1989). Individual pollen grains were examined under 400 x magnifications in a Nikon Eclipse-800 microscope and photographed using Nikon DXM1200F digital camera through a frame size of 640×480 pixels. The digital image of each pollen grain was measured using ImageJ software (<http://rsb.info.nih.gov/ij/>) and the measurement was checked against the microscopic scale which allowed precision of about 0.3 µm (Micrometer 2mm 200Teile, Leitz Wetzlar). From each individual tree, 120 – 130 pollen grains were photographed and measured. Only triporate pollen grains (normal grains with three pores) lying in polar view were used. Two lines, grain diameter and pore depth, were measured from each pollen grain (Figure 1). Grain diameter (D) was defined as the distance from the outside tip of the pore to the outer margin of the facing wall, whereas pore depth (P) was defined as the distance from the outside tip of the pore to the inner margin of the nexine through the vestibulum. The D/P ratio was then calculated for each pollen grain. Nested ANOVA, Tukey's HSD test and Pearson's

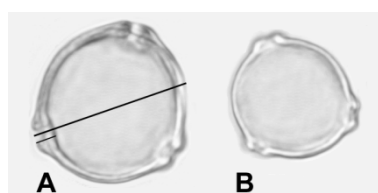
correlation coefficient were performed using the SPSS 10.0.5 software (©SPSS Inc., 1989).

**Table I**

Location of birch woodlands in Iceland where individual trees were studied and their pollen measured. The altitude is shown as average level for the woodland.

Note: \*Only *Betula pubescens* was found in this area.

| Location       | Label | Latitude (°N) | Longitude (°W) | Altitude (m) |
|----------------|-------|---------------|----------------|--------------|
| Brekkuskógur   | A     | 64.27         | 20.51          | 129          |
| Bifröst        | B     | 64.76         | 21.59          | 62           |
| Ásbyrgi        | D     | 66.01         | 16.50          | 41           |
| Eidar          | E     | 65.32         | 14.36          | 49           |
| Jökulsá í Lóni | G     | 64.43         | 14.90          | 36           |
| Skaftafell     | H     | 64.03         | 16.98          | 267          |
| Reykjanes      | I*    | 63.89         | 21.72          | 20           |
| Kjálkafjörður  | J     | 65.62         | 22.94          | 14           |
| Kaldalón       | M     | 66.10         | 22.39          | 36           |
| Mývatn         | R     | 65.58         | 16.85          | 304          |



**Figure 1:** Microscopic images of *Betula* pollen under the same magnification: (A) *B. pubescens* and (B) *B. nana*. Black lines show grain diameter (24  $\mu\text{m}$ ) and pore depth (3.6  $\mu\text{m}$ ).

## Results

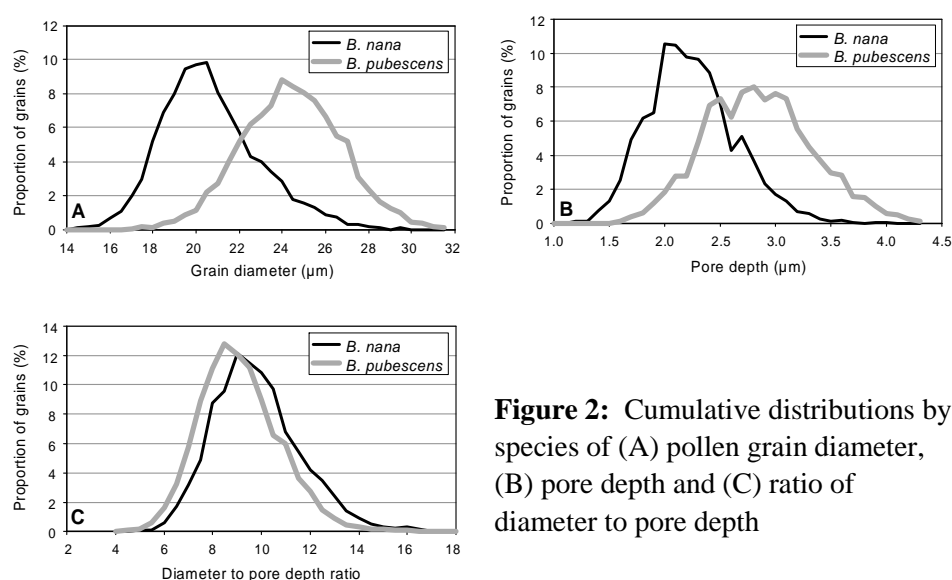
Pollen diameters (D), pore depths (P) and D/P ratios were obtained from a total of 9021 pollen grains from 70 *Betula* plants in ten locations in Iceland. Among these birch plants, 31 were confirmed by chromosome counting as being diploid *B. nana* (3998 pollen grains measured) and 39 were tetraploid *B. pubescens* (5023 grains measured). In general, pollen of the diploid *B. nana* was smaller, with a thinner wall and smaller pore (vestibulum) than pollen of the tetraploid *B. pubescens* (Figure 1). The measurements showed that most of the *B. nana* pollen grains had a smaller grain diameter and pore depth than those of *B. pubescens* (Figure 2, Table II). The statistical analysis by nested ANOVA

(Table III) showed significant ( $P < 0.001$ ) difference between the two birch species and among individuals within species in all three pollen parameters (D, P and D/P ratio). Among these parameters, grain diameter could differentiate the two species more effectively than pore depth (higher F values, Table III), which in turn was better than the D/P ratio.

Each *Betula* species showed a bell-shaped distribution curve with a distinctive peak by all three parameters, and for each parameter the two species distribution curves were connected with an overlapping region (Figure 2). The mean pollen diameters were 20.42 and 24.20  $\mu\text{m}$  for *B. nana* and *B. pubescens* respectively, and the cut-point between the two species distribution curves was at 22.1  $\mu\text{m}$  (Figure 2a, Table II). Only about 20% of the pollen grains fell within the overlapping region – most (79%) of *B. nana* pollen grains were smaller than 22.1  $\mu\text{m}$  and 81% of *B. pubescens* pollen grains were bigger than this cut-point size. Pollen pore depth was greater in *B. pubescens* compared to *B. nana*: the mean pore depths were 2.20 and 2.81  $\mu\text{m}$  for *B. nana* and *B. pubescens* respectively, and the cut-point between the two species was approximately at 2.5  $\mu\text{m}$  (Figure 2b, Table II). The species distribution curves for this parameter were somewhat similar to those for grain diameter, i.e. about the same proportion (ca. 80%) of *B. nana* pollen grains had pore depth less than the cut-point, although a smaller proportion (less than 70%) of pollen grains from *B. pubescens* pollen had deeper pores. The mean grain D/P ratio of *B. nana* was also significantly different from that of *B. pubescens* (Table III), but the overlapping region was too large to make this parameter useful for species differentiation (Figure 2c). Neither pore depths nor D/P ratios were analysed further statistically in this study.

Positive correlation was found between mean pollen size (i.e. grain diameter) and plant morphology index of individual trees/shrubs (Figure 3). Low-lying shrubs that had relatively rounded leaves with single toothed margin and short petiole (*B. nana* type, low morphology index) often produced smaller pollen grains than those from birch trees which had more pointed and ovate-shaped leaves with dentate margin and long petiole (*B. pubescens* type, high morphology index). The correlation was statistically significant when combining the two birch species ( $r = 0.759$ ;  $P < 0.001$ ;  $n = 70$ ), but when the species were analysed separately the significance existed only in *B. nana* ( $r = 0.359$ ;  $P = 0.024$ ;  $n = 31$ ), not in *B. pubescens* ( $r = 0.035$ ;  $P = 0.417$ ;  $n = 39$ ). Diploid *B. nana* plants with a low morphology index (Figure 3: scores 0 – 1) clearly had smaller pollen than those which had a higher morphology index (scores 1 – 2). But tetraploid *B. pubescens* plants, which had a wider range of morphology scores (5 – 11) compared to *B. nana* (scores 0 – 3), appeared to have similarly large pollen grains (Figure 3).

Differences in pollen size were found among locations (Figure 4). The nested analysis of variance (Table IV) showed significant difference ( $P < 0.001$ ) in pollen grain diameter among sites in each species, as well as among individual trees/shrubs within sites. The Tukey's HSD test divided the sites into six different size groups in each species (groups a – f), from the smallest to the largest means of pollen diameter (Figure 4). The largest *B. pubescens* pollen, with a mean diameter of  $25.3 \mu\text{m}$  was found in the woodland *Skaftafell* (site H, south-eastern Iceland), but the smallest pollen grains of this species, with a mean diameter of  $22.7 \mu\text{m}$ , were from the woodland *Bifröst* (site B, western Iceland). Interestingly, the smallest *B. pubescens* pollen was similar in size to the largest *B. nana* pollen, which had a mean diameter of  $22.4 \mu\text{m}$  (site G at *Jökulsá í Lóni*, eastern Iceland). However, *B. nana* pollen grains from most (six out of nine) woodlands under study had small pollen with mean grain diameters of  $19.4 - 20.0 \mu\text{m}$ . There is no indication of geographical pattern regarding pollen size. The pollen size ratios between the two species (*B. pubescens* to *B. nana*) among all sites ranged from 1.05 to 1.28, while the ratio derived from all locations combined was 1.19. No correlation ( $r = 0.02$ ) was found between pollen size of *B. nana* and *B. pubescens* within the same site, meaning that local conditions do not seem to affect pollen size.



**Figure 2:** Cumulative distributions by species of (A) pollen grain diameter, (B) pore depth and (C) ratio of diameter to pore depth

**Table II**

Differentiation of *Betula nana* and *B. pubescens* pollen by grain diameter, pore depth and ratio of diameter to pore depth.



|                                    | <b><i>B. nana</i></b>   |      |      |      | <b><i>B. pubescens</i></b> |      |      |      |
|------------------------------------|-------------------------|------|------|------|----------------------------|------|------|------|
|                                    | number of plants = 31   |      |      |      | number of plants = 39      |      |      |      |
|                                    | number of pollen = 3998 |      |      |      | number of pollen = 5023    |      |      |      |
|                                    | mean                    | SD   | min  | max  | mean                       | SD   | min  | max  |
| Grain diameter (D, $\mu\text{m}$ ) | 20.42                   | 2.29 | 14.3 | 30.4 | 24.20                      | 2.36 | 14.3 | 33.9 |
| Pore depth (P, $\mu\text{m}$ )     | 2.20                    | 0.40 | 1.2  | 4.4  | 2.81                       | 0.50 | 1.4  | 5.2  |
| D/P ratio                          | 9.55                    | 1.80 | 4.7  | 17.9 | 8.85                       | 1.63 | 4.3  | 18.2 |

**Table III**

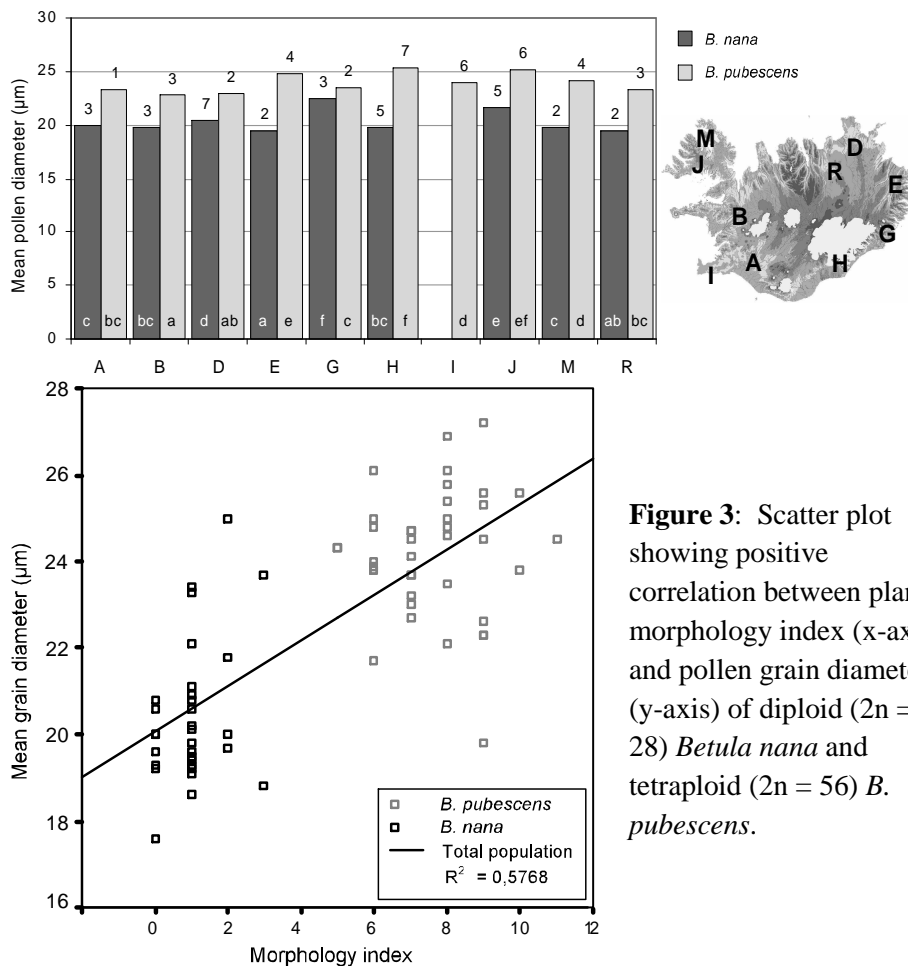
ANOVA test of species and individual effects on pollen diameter, pore depth and their ratio.

| Source                           | Dependent variable | SS    | df   | MS      | F     | P <  |
|----------------------------------|--------------------|-------|------|---------|-------|------|
| Between species                  | Diameter           | 31762 | 1    | 31761.8 | 10134 | .000 |
|                                  | Pore depth         | 843   | 1    | 842.7   | 6041  | .000 |
|                                  | D/P ratio          | 1115  | 1    | 1115.2  | 512   | .000 |
| Among individuals within species | Diameter           | 21029 | 68   | 309.3   | 99    | .000 |
|                                  | Pore depth         | 667   | 68   | 9.8     | 70    | .000 |
|                                  | D/P ratio          | 6739  | 68   | 99.1    | 45    | .000 |
| Corrected total                  | Diameter           | 80843 | 9020 |         |       |      |
|                                  | Pore depth         | 2753  | 9020 |         |       |      |
|                                  | D/P ratio          | 27333 | 9020 |         |       |      |

**Table IV**

ANOVA test of site (location) effect on pollen diameter for each *Betula* species.

| Species                    | Source                        | SS    | df   | MS    | F   | P <  |
|----------------------------|-------------------------------|-------|------|-------|-----|------|
| <b><i>B. nana</i></b>      | Among sites                   | 4186  | 8    | 523.3 | 196 | .000 |
|                            | Among individuals within site | 6080  | 23   | 264.3 | 99  | .000 |
|                            | Corrected total               | 20990 | 3997 |       |     |      |
| <b><i>B. pubescens</i></b> | Among sites                   | 4330  | 9    | 481.0 | 137 | .000 |
|                            | Among individuals within site | 6320  | 29   | 217.9 | 62  | .000 |
|                            | Corrected total               | 28094 | 5022 |       |     |      |



**Figure 3:** Scatter plot showing positive correlation between plant morphology index (x-axis) and pollen grain diameter (y-axis) of diploid ( $2n = 28$ ) *Betula nana* and tetraploid ( $2n = 56$ ) *B. pubescens*.

**Figure 4:** Mean grain diameters of *Betula* pollen by species and sites. Numbers over the columns indicate number of individual trees/shrubs per site. Letters at the bottom of columns show results of Tukey's HDS test<sup>a,b,c</sup> for each species separately, columns with different letters differ significantly ( $P < 0.05$ ). The map on the right shows location of the sites (see also Table I).

## Discussion

Pollen size (grain diameter) is a better and more reliable parameter than pollen structure (as represented by pore depth) for differentiating *Betula nana* and *B. pubescens* in this study. However, pollen pore depth and D/P ratio could be used to verify the differentiation and help resolve the uncertainty if grain diameter falls within the overlapping region between the two species. The

difference in mean pollen size between these two birch species may be the consequence of substantial difference in genome size and DNA content, which is normally accompanied by differences in cell size. Statistical significance was also obtained when comparing the size of pollen of North American birch species, i.e. between diploid dwarf birch (*B. nana* and *B. glandulosa*) and polyploid tree birch such as *B. papyrifera* (Clegg et al. 2005). In the same study, no statistical significance existed in pollen size among species having the same ploidy level. Another example came from Greenland (Fredskild 1991) where pollen of the diploid species *B. glandulosa* was smaller than pollen of the European tetraploid tree birch species *B. pubescens*.

In *Betula*, the total chromosome complement length for tetraploids was estimated to be approximately double that for diploids, but the increase was less than proportional as the chromosome number increased (Taper & Grant 1973). The difference in genome size (nuclear DNA amount) between diploid and tetraploid species of the same genus is thought to be reducing significantly over evolutionary time (Leitch & Bennett 2004). Although chromosomes of the diploid species *B. nana* are, on average, bigger (longer) than chromosomes of the tetraploid *B. pubescens* (Anamthawat-Jónsson & Heslop-Harrison 1995), genome size difference between species having different ploidy should be large enough to account for significant differences in cell size as well as pollen size.

The pollen size difference between diploid *B. nana* and tetraploid *B. pubescens* in this study is about 18% if calculated from the mean values (20.42  $\mu\text{m}$  for *B. nana* and 24.20  $\mu\text{m}$  for *B. pubescens*), whereas the size difference deduced from other studies is in the range of 25 – 45% (Prentice 1981; Mäkelä 1996; Caseldine 2001), more than that in the present study. The mean grain diameters of *B. nana* pollen from other studies (Birks 1968; Mäkelä 1999; Caseldine 2001) are 15.50 – 19.23  $\mu\text{m}$ , after adjustment for the pollen preparation using  $1.2^{-1}$  reduction for samples mounted in glycerol to samples mounted in silicon oil according to Fægri and Iversen (1989). The mean grain diameters for *B. pubescens* (combined subspecies) from these studies are 18.86 – 26.92  $\mu\text{m}$ . The pollen of *B. nana* in the present study is clearly bigger than pollen of the same birch species from Scandinavia and Britain, whereas the pollen size of *B. pubescens* is within the range of these previous measurements.

If pollen size of the Icelandic *Betula nana* is in fact different from pollen sizes of the same species elsewhere as the data suggests, there could be several possible explanations. For example, this might be the chance result of homogeneity and isolation of the population (founder effect), or conditions might have favoured some genetic traits with this phenotype (selection). However, the more likely possibility is that conditions in Iceland have favoured hybridisation of the two species, leading to genetic modification of the species

via introgression. Considerable evidence has indeed supported this introgressive hybridisation, which is believed to be the cause of morphological diversity in both species (Thórsson et al. 2001). In that study, genetic material from *B. pubescens* was evident in the genome of Icelandic *B. nana*, whereas a *B. nana* sample from southern Finland was more pure. Furthermore, the morphology of Icelandic *B. nana* was modified by *B. pubescens* (through introgression), as also shown in the present study. The mean scores of morphology index for *B. nana* from nine woodlands in Iceland range from 0.65 to 2.00, while taxonomically the species should have the lowest score (zero). Also in the present study, pollen size of *B. nana* correlates positively to plant morphology such that *B. nana* plants with lower morphological scores produce smaller pollen than the more modified (introgressed) *B. nana* plants. Such correlations support that introgressive hybridisation is the most likely cause of large pollen in the Icelandic *B. nana*, hence the smaller difference in pollen size between the two birch species compared to data from other studies.

Measurements of *Betula* pollen recovered from sediments in northern Iceland and dated from early Holocene (Hallsdóttir 1990) showed smaller *B. nana* pollen than that of the present study. A possible interpretation is that *B. nana* pollen in Iceland has grown larger with time because of introgression from *B. pubescens*. On the other hand, the pollen size of *B. pubescens* could predictably be reversed, i.e. become smaller, due to the introgression being bidirectional. This change in pollen size requires further investigations. Most interestingly, measurements of *B. pubescens* pollen grains from different areas of Fennoscandia (Mäkelä 1996) showed smaller pollen size in mountain birch (subspecies *tortuosa*) by about 16% when compared to pollen from the more southern *B. pubescens*. As this mountain birch had many features from *B. nana*, it was suggested to be due to introgressive hybridisation (Vaarama & Valanne 1973). Therefore the introgressed (*tortuosa*) type of *B. pubescens* should supposedly produce small pollen, the same way as the introgressed type of *B. nana* produces bigger pollen. This correlation is in fact statistically significant, i.e. plants that have lower scores of morphology index (due to introgression) produce smaller pollen than those having higher morphological scores. We are currently studying pollen characteristics including size of pollen grains produced by triploid hybrids (between *B. nana* and *B. pubescens*) and have found that the majority of these triploid plants have intermediate or hybrid-like morphology. Their pollen grains are small (unpublished results). Pollen size as a species parameter – in conjunction with multivariate species-specific traits, especially in the leaf morphology – may be used to create a reliable introgression scale for analysing subfossil pollen.

## Conclusions

Pollen samples collected from ten birch woodlands in Iceland were measured for grain diameter and pore depth. The plants were identified to species based on chromosome number, and species-specific characters, especially in the leaf morphology, were scored for each plant. Identification of plants by ploidy determination eliminates the involvement of triploid hybrids, which makes the species delineation unclear. Pollen of tetraploid *B. pubescens* was statistically larger than pollen of diploid *B. nana* and this could be related to the difference in ploidy level, i.e. the tetraploid genome being larger than the diploid genome of the same genus. The difference in pollen size between the two species is less than in most other reports, and this is believed to be due to the bidirectional introgressive hybridisation that has presumably brought the plant genetic differences closer. As pollen size was significantly correlated with plant morphology, it should be possible to establish an introgression scale suitable for studying subfossil pollen. The significance of the present study is two-fold: firstly, pollen size can be used to separate *B. nana* pollen from that of *B. pubescens* more effectively; and secondly, pollen size can be used to estimate the extent of past introgression that may have played a major role in birch vegetation history in Iceland since the early Holocene.

## Acknowledgements

The project was supported by the Icelandic Research Centre (Rannís: grant no. 040238021).

## References

- Anamthawat-Jónsson, K. (2003a). Hybrid introgression in *Betula*. In A. K. Sharma, & A. Sharma (Ed.), *Plant Genome – Biodiversity and Evolution*, vol. 1, *Phanerogams* (pp. 249-265). Enfield, Plymouth: Science Publishers Inc.
- Anamthawat-Jónsson, K. (2003b). Preparation of chromosomes from plant leaf meristems for karyotype analysis and *in situ* hybridisation. *Methods in Cell Science*, 25, 91-95.
- Anamthawat-Jónsson, K., & Heslop-Harrison, J. S. (1995). Molecular cytogenetics of Icelandic birch species: physical mapping by *in situ* hybridisation and rDNA polymorphism. *Canadian Journal of Forest Research*, 25, 101-108.
- Anamthawat-Jónsson, K., & Tómasson, T. (1990). Cytogenetics of hybrid introgression in Icelandic birch. *Hereditas*, 112, 65-70.

- Atkinson, M. D. (1992). Biological flora of the British Isles: *Betula pendula* Roth (*B. verrucosa* Ehrh.) and *B. pubescens* Ehrh. *Journal of Ecology*, 80, 837-870.
- Birks, H. J. B. (1968). The identification of *Betula nana* pollen. *New Phytologist*, 67, 309-314.
- Blackmore, S., Steinmann, J.A.J., Hoen, P.P., & Punt, W. (2003). The Northwest European Pollen Flora 65: Betulaceae and Corylaceae. *Review of Palaeobotany and Palynology*, 123, 71-98.
- Caseldine, C. (2001). Changes in *Betula* in the Holocene record from Iceland – a palaeoclimatic record or evidence for early Holocene hybridisation? *Review of Palaeobotany and Palynology*, 117, 139-152.
- Clegg, B. J., Tinner, W., Gavin, D. G., & Hu, F. S. (2005). Morphological differentiation of *Betula* (birch) pollen in northwest North America and its palaeoecological application. *The Holocene*, 15, 229-237.
- Fredskild, B. (1991). The genus *Betula* in Greenland – Holocene history, present distribution and synecology. *Nordic Journal of Botany*, 11, 393-412.
- Fægri, K., & Iversen, J. (1989). *Textbook of pollen analysis*. (4<sup>th</sup> ed). Revised edition by K. Fægri, P. E. Kaland, & K. Krzywinski. Chichester, New York, Brisbane, Toronto, Singapore: J. Wiley & Sons.
- Hallsdóttir, M. (1990). Studies in the vegetational history of North Iceland. A radiocarbon-dated pollen diagram from Flateyjarðalur. *Jökull*, 40, 67-81.
- Hultén, E., & Fries, M. (1986). *Atlas of North European Vascular Plants*. Köningstein, Germany: Koeltz Scientific Books.
- Leitch, I. J., & Bennett, M. D. (2004). Genome downsizing in polyploidy plants. *Biological Journal of the Linnean Society* 82, 651-663.
- Mäkelä, E. M. (1996). Size distinctions between *Betula* pollen types – A review. *Grana*, 35, 248-256.
- Mäkelä, E. M. (1999). *The Holocene history of birch in north-eastern Fennoscandia - an interpretation based on fossil birch pollen measurements*. University of Helsinki, Department of Geology, Division of Geology and Palaeontology, Helsinki.
- Prentice, I. C. (1981). Quantitative birch (*Betula* L) pollen separation by analysis of size frequency data. *New Phytologist*, 89, 145-157.
- Taper, L. J., & Grant, W. J. (1973). The relationship between chromosome size and DNA content in birch (*Betula*) species. *Caryologia*, 26, 263-273.
- Thórsson, A. T., Salmela E., Anamthawat-Jonsson, K. (2001). Morphological, cytogenetic, and molecular evidence for introgressive hybridisation in birch. *Journal of Heredity* 92, 404-408.
- Vaarama, A., & Valanne, T. (1973). On the taxonomy, biology and origin of *Betula tortuosa* Ledeb. *Reports of the Kevo Subarctic Research Station*, 10, 70-84.
- Walters, S. M. (1964). Betulaceae. In T. G. Tutin et al. (Ed.), *Flora Europaea*, vol. 1 (pp. 57-59). Cambridge: Cambridge at the University Press.

# PAPER II

## **Characteristics of pollen from natural triploid *Betula* hybrids**

Karlsdóttir L, Hallsdóttir M, Thórsson ÆTh and Anamthawat-Jónsson K (2008)

*Grana* 47: 52-59.

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# Characteristics of pollen from natural triploid *Betula* hybrids

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Lilja Karlsdóttir<sup>1</sup>, Margret Hallsdóttir<sup>2</sup>, Aegir Th. Thórsson<sup>1</sup> & Kesara Ananthawat-Jónsson<sup>1</sup>

<sup>1</sup>Institute of Biology, University of Iceland, Sturlugötu 7, Reykjavik, Iceland &

<sup>2</sup>Icelandic Institute of Natural History, Hlemmi 3, Reykjavik, Iceland

## Abstract

Birch has a key role in the Holocene vegetation history of northern Europe and in sub-arctic climates dwarf birch and tree birch still co-exist. In Iceland, triploid hybrids between diploid *Betula nana* (dwarf birch) and tetraploid *B. pubescens* (downy birch) are common and contribute to pollen influx. Pollen from 22 triploid trees/shrubs from ten woodlands in Iceland was examined and its size and shape compared with pollen from the parental species. The mean diameter of pollen grains from the triploid hybrids was not statistically different from that of *B. nana* pollen, but it was significantly smaller than the mean value of *B. pubescens* pollen. On the contrary, the size of the vestibulum was similar to that of *B. pubescens*, which was significantly greater than that of *B. nana*, and therefore the diameter-pore depth ratio was lower than the values from either species. The size of triporate pollen grains from the triploid birch was highly variable among individual plants. The pattern of size distribution within plants indicated that triploid hybrids might have produced two sizes of pollen grains, but the small *B. nana* size was far more prevalent than the larger *B. pubescens* size. Several anomalies in pollen structure were common among the hybrid pollen grains: four or more pores were the most frequent type of deformity. Characteristics of the pollen of triploid *Betula* hybrids, especially structural anomalies, may provide a means to reveal periods of interspecific hybridisation in the analysis of sub-fossil pollen.

**Keywords:** *Birch*, *Betula nana*, *Betula pubescens*, grain diameter, hybrid pollen, pollen pore depth, pollen size, pollen structure, triploid hybrid

## Introduction

Two birch species have co-existed in Iceland since the early Holocene: dwarf birch *Betula nana* L. (diploid,  $2n = 28$ ) and downy birch *B. pubescens* Ehrh. (tetraploid,  $2n = 56$ ). Triploid plants, derived from hybridisation between the two species, are common in Iceland – they are found at an average of about 10% of birch plants when sampling at random (Thorsson et al., 2001, 2007). In controlled crosses (Anamthawat-Jónsson & Tómasson, 1990) and from open-pollinated *B. nana* plants (Anamthawat-Jónsson & Tómasson, 1999), the proportion of triploid hybrids among the progeny is much higher and the triploid plants are not sterile. Such interspecific hybrids can mediate gene flow between the parental species via backcrossing of the hybrids. This process, so-called introgressive hybridisation or introgression, has been shown to occur in natural birch woodlands in Iceland (see review in Anamthawat-Jónsson 2003a). The multivariate data analysis of species-specific morphological characters (Thórsson et al., 2007) has confirmed the occurrence of bidirectional introgression and indicated that the triploid group may have played an important role in maintaining genetic variation of the woodland birch species. Such variation is likely to be advantageous in cold climates and the environments found in Iceland and in other sub-arctic regions.

In northern Europe, birch has played a key role in vegetation succession as glaciers retreated at the beginning of the Holocene, and for the northernmost parts, such as Iceland and Fennoscandia, birch woodlands remain important features of the vegetation. As different birch species represent different ecosystems, several attempts have been made to differentiate sub-fossil pollen of *Betula*, especially between *B. pubescens* and *B. nana* (see Caseldine 2001). Pollen of *B. pubescens* is on average larger than pollen of *B. nana*, has more protruding apertures and a larger vestibulum (Birks 1968). However, size frequency curves overlap significantly and interpretation of sub-fossil data has to rely on mean sizes rather than individual grains.

Pollen grains of *Betula* are triporate with a distinct vestibulum and a grain diameter of usually 17 – 27  $\mu\text{m}$  (e.g. Mäkelä 1996; Caseldine 2001). No clear characteristics of pollen separate species within the genus *Betula*, but differences in mean size, the relative size of vestibulum and the minor structure of pores make identification on species level just about possible (Blackmore et al., 2003). In our recent study (Karlsdóttir et al., 2007), pollen size was measured and compared between samples of *B. nana* and *B. pubescens* that were classified based on chromosome number. Due to the introgressive hybridisation described above, morphological variation in Icelandic *Betula* is so great that species identification based on morphology is rendered difficult. But the chromosome number of Icelandic birch plants is stable: only three groups of plants have been identified (Thórsson et al., 2001, 2007). Pollen of diploid *B. nana* was therefore compared with that of tetraploid *B. pubescens*; the results showed significant difference

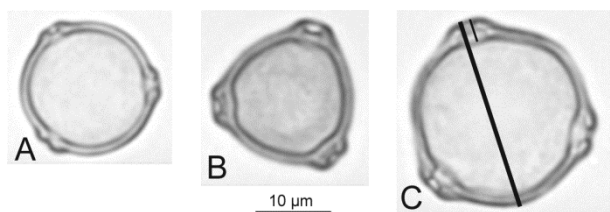
in the mean size of pollen grains of these two species, regardless of the large overlap in plant morphology.

As triploid birch hybrids are quite common in natural woodlands in Iceland, it is important to know to what extent pollen from the triploid hybrids has contributed to the pollen pool and how it has affected the species differentiation of birch pollen in general. This should have implications in interpreting data from sub-fossil pollen and hence the vegetation history. Sub-fossil *Betula* pollen is found in Icelandic sediments from the early Holocene (Hallsdóttir 1990; Rundgren 1998; Caseldine 2001; Caseldine et al., 2003). If we could identify specific characteristics of pollen from triploid birch, it would be possible to study the history of hybridisation and introgression in Iceland using sub-fossil pollen. The work plan of this study was to compare pollen of triploid birch plants with pollen of diploid *B. nana* and tetraploid *B. pubescens* from the same woodlands in Iceland.

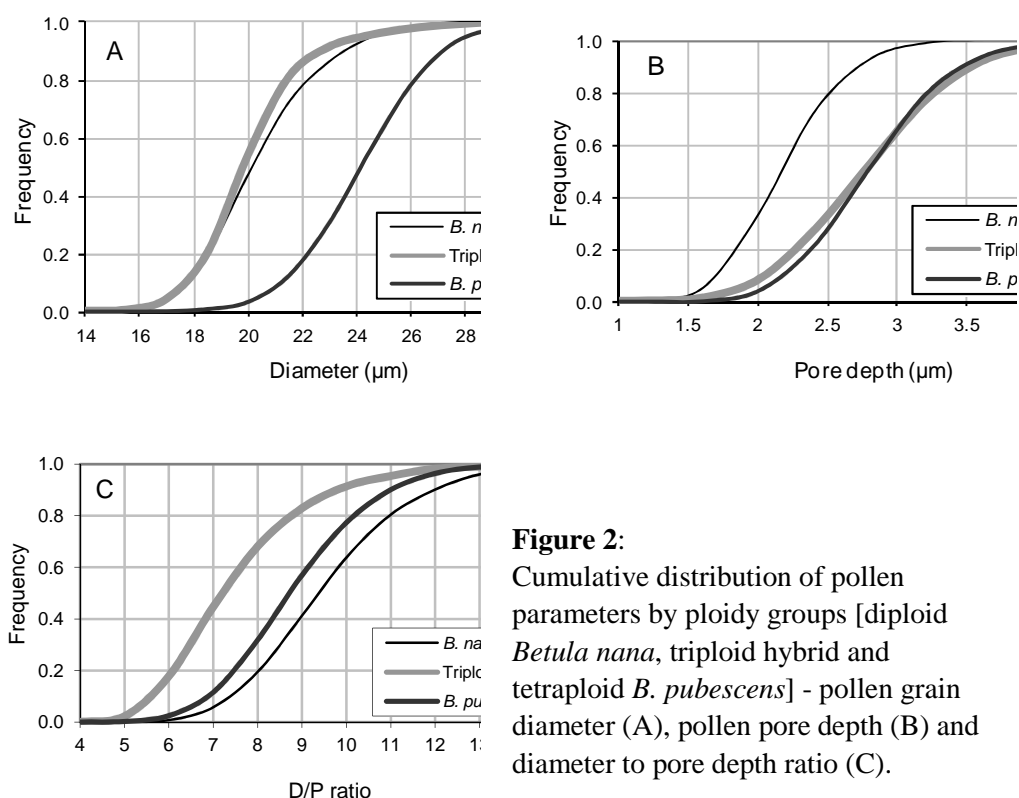
**Table I:** Mean pollen size by ploidy groups.

| Species and chromosome number | No. of trees |    | Diameter (µm) |     |     | Pore depth (µm) |     |      | Diameter/Pore depth ratio |     |      |
|-------------------------------|--------------|----|---------------|-----|-----|-----------------|-----|------|---------------------------|-----|------|
|                               | n            |    | mean          | SD* | SE* | mean            | SD* | SE*  | mean                      | SD* | SE*  |
| <i>B. nana</i>                | 28           | 31 | 20.4          | 1.6 | 0.3 | 2.2             | 0.2 | 0.04 | 9.5                       | 0.9 | 0.16 |
| Hybrid                        | 42           | 22 | 20.1          | 1.3 | 0.3 | 2.8             | 0.4 | 0.08 | 7.5                       | 1.0 | 0.22 |
| <i>B. pubescens</i>           | 56           | 39 | 24.2          | 1.5 | 0.2 | 2.8             | 0.3 | 0.05 | 8.8                       | 0.9 | 0.14 |

\*among individual trees



**Figure 1:** Pollen from diploid *Betula nana* (A), triploid hybrid (B) and tetraploid *B. pubescens* (C) showing grain diameter (27.4 µm) and pore depth (2.9 µm). The 10 µm scale bar applies to all pollen grains in this figure.



## Material and methods

Pollen samples were collected from 92 individual trees/shrubs in ten different sites in Iceland: Brekkuskógur, Bifröst, Ásbyrgi, Eidar, Jökulsá í Lóni, Skaftafell, Reykjanes, Kjálkafjörður, Kaldalón and Mývatn (for description of the sites and map, see Thorsson et al., 2007). All of these plants had been identified beforehand by chromosome number, which was obtained from direct counting of metaphase chromosomes from leaf bud cells as in Ananthawat-Jónsson (2003b). The counting yielded three groups of plants (Table I): diploid *Betula nana* ( $2n = 28$ ), tetraploid *B. pubescens* ( $2n = 56$ ) and triploid hybrids ( $2n = 42$ ). Male catkins were either collected whole at the place of origin or allowed to mature on cut branches indoors. All samples were collected and prepared during the same time period and under the same conditions. The analysis of pollen from the diploid *B. nana* and the tetraploid *B. pubescens* was reported in Karlsdóttir et al. (2007), and as a continuation of that work the analysis of pollen from the triploid plants is presented here in comparison with pollen of the two parental species.

The pollen samples were prepared and mounted in silicon oil as in Karlsdóttir et al. (2007). Images of 120-130 pollen grains from each individual tree/shrub were taken using a Nikon digital camera DXM1200F on a Nikon eclipse E800 microscope with 400 x microscopic magnification. Grain diameter (D) and pore depth (P) were defined and measured for each pollen grain as in Karlsdóttir et al. (2007). Only triporate pollen grains (normal grains with three pores) lying in polar view were measured. The grain diameter (representing pollen size) was measured digitally with an accuracy of approximately 0.3  $\mu\text{m}$ . The measurement of pore-depth was less accurate, owing to varying visibility of the inner wall and a relatively greater effect of the visual angle.

Abnormal pollen grains from the same preparations as used for the size measurement were examined. Approximately 1000 grains per individual plant were counted and all types of abnormalities (anomalies or deviations from normal appearance) were noted. In eleven cases, pollen from an individual tree/shrub was sampled in two consecutive years and the average outcome was used in the results. These were five *B. nana*, four hybrids and two *B. pubescens* plants.

ANOVA and Tukey's HSD were performed to test pollen size differences among the ploidy (chromosome number) classes, one-sample Kolmogorov-Smirnov goodness-of-fit test was used for comparison to normal distribution and Pearson's correlation coefficient was also performed, all using SPSS software version 10.0.5 (© SPSS Inc. 1989-1999).

## Results

### Triporate pollen

In this study and in Karlsdóttir et al. (2007), all measurements were made on pollen grains having three pores (triporate), which was considered to be the normal type in *Betula*. Mean pollen diameter (D), mean pollen pore depth (P) and the D/P ratio differed significantly (ANOVA:  $P < 0.001$ ) among ploidy groups, i.e. diploid *B. nana*, tetraploid *B. pubescens* and triploid hybrid between the two species.

Triporate pollen grains from the triploid plants were often similar in size to *B. nana* pollen, but smaller than pollen of *B. pubescens* (Figure 1, Figure 2A). The mean grain diameters of the diploid, triploid and tetraploid groups were 20.5, 20.1 and 24.3  $\mu\text{m}$ , respectively (Table I). The Tukey HSD test showed a significant difference ( $P < 0.001$ ) in pollen size between the triploid group and *B. pubescens*, but not between the triploid group and *B. nana*. Based on these mean grain diameters, pollen grains of *B. nana* and the hybrid were similar in size but smaller than *B. pubescens* pollen. However, when looking at size distribution within plants, pollen grains from the triploid hybrids were not uniformly small compared to size distribution within individuals of *B. nana*. Pollen sizes were normally distributed within each individual of *B. pubescens* and *B. nana* (as

shown by the K-S goodness-of-fit test). Of the 22 triploid hybrids, four showed significant diversion from normal size distribution and in one case a distinct bimodal distribution. Of the 18 triploid hybrids that showed the normal size distribution, ten individuals had *B. nana* type (Figure 3A); the most common sizes clustered around the mean value for the diploid species while the largest diameters were seldom above 24  $\mu\text{m}$ . Eight triploid plants showed similar size distribution to Figure 3A, but the majority of the grains were bigger than the *B. nana* average and some (5 – 10%) were even bigger than the average *B. pubescens* size (above 24  $\mu\text{m}$ ). Of the four plants that showed significant deviation from the normal distribution curve, one triploid tree produced mainly large pollen grains with the same mean diameter as *B. pubescens* (Figure 3B), whereas another tree showed two peaks of size distribution: small and large pollen grains (Figure 3C). None of the triploid plants showed a clear case of hybrid peak, i.e. containing pollen grains mainly of intermediate size.

The mean depth of pollen pore (P) also differed significantly among the ploidy groups, but regarding this parameter pollen of the triploid group differed significantly from *B. nana*, yet not from *B. pubescens* (Figure 1, Figure 2B). The mean pore depths in the diploid, triploid and tetraploid groups were 2.2, 2.8 and 2.8  $\mu\text{m}$ , respectively (Table I). As the triploid hybrids produced mostly small pollen like *B. nana* and deep pores like *B. pubescens*, their D/P ratio became the lowest compared to the ratios obtained from the two species (Table I, Figure 2C). Differences in D/P ratio were statistically significant among all three groups (Tukey's HSD:  $P < 0.01$  for *B. nana* vs. triploid hybrid;  $P < 0.01$  for *B. pubescens* vs. hybrid; and  $P < 0.05$  for *B. nana* vs. *B. pubescens*).

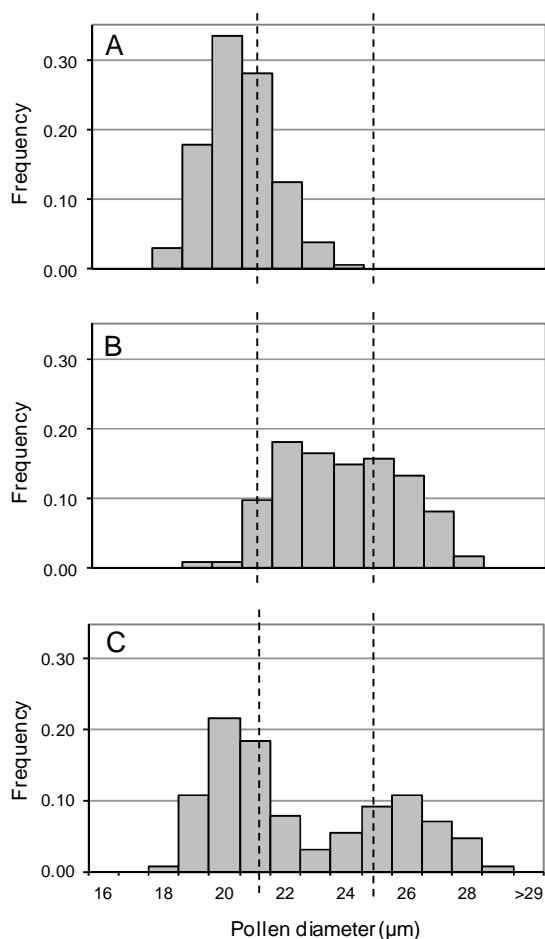


Figure 3: Examples of distribution of pollen grain diameters within individual triploid hybrid: tree numbers J11 (A), A13 (B) and J23 (C), based on 128, 121 and 130 triporate grains measured, respectively. Two vertical dotted lines represent mean grain diameters of *Betula nana* (20.4 μm) and *B. pubescens* (24.2 μm).

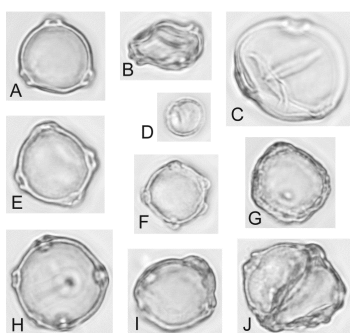
## Abnormal pollen

Eleven classes of structural abnormalities were recorded (Figure 4): three classes were presumably caused by damage to normal grains (shrunken, flattened or broken grains, presumably caused by environmental factors or during preparation of samples) and eight classes included deformed grains (0, 1, 2, 4, 5, 6 or more pores, fused grains, and grains with uneven pollen wall or other anomalies). Both groups, damaged and deformed, were significantly more frequent among pollen samples from the triploid hybrids than those from *B. pubescens* or *B. nana* (ANOVA:  $P < 0.001$ ; Tukey's HSD:  $P < 0.01$ ) (Figure 5). The difference between *B. pubescens* and *B. nana* pollen was not statistically significant. Within ploidy groups, the proportion of abnormal pollen varied considerably among individual plants, as reflected in the large standard deviation and

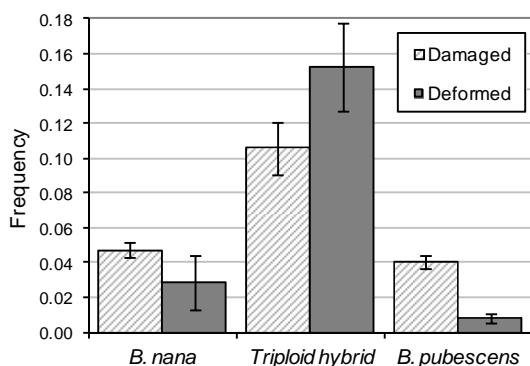
standard error (Figure 5). In some triploid hybrids, half of the pollen grains were deformed. Eleven out of 22 hybrid individuals had more than 10% deformed pollen grains. This was rarely found among samples from the two species, occurring in only two out of 31 *B. nana* individuals and one of the 39 *B. pubescens* trees.

The most frequent type of deformity in pollen structure was pollen with four pores instead of the normal three. Pollen with four pores was found in 2.3% of *B. nana* pollen examined, 0.7% of *B. pubescens* pollen, but 10.8% in the hybrid pollen. Other deviations from three pores were rare. Non-triporate pollen grains combined were found in 2.4% and 0.7% of *B. nana* and *B. pubescens*, respectively, but 12.2% in the triploid hybrids.

In the cases where pollen was sampled from the same individuals in two consecutive years, no significant difference in the frequency of abnormal pollen grains was found between years. Furthermore, the proportion of deformed pollen was significantly correlated between years ( $r = 0.85$ ;  $P < 0.001$ ), as well as the proportion of non-triporate pollen ( $r = 0.84$ ;  $P < 0.001$ ). The correlation in damaged pollen was not significant.



**Figure 4:** Examples of deformed and damaged pollen grains found frequently among pollen samples from triploid hybrids, compared to the normal triporate type (A): damaged grains (B: shrunk, C: flattened) and deformed grains (D: dwarf size with one pore, E: four pores, F: five pores, G: abnormal and uneven exine, H: multiple pores, I: uneven exine, and J: fused grains).



**Figure 5:** Frequencies of abnormal pollen by ploidy groups [diploid *Betula nana*, triploid hybrid and tetraploid *B. pubescens*]. Vertical bars represent standard errors.



## Discussion

Pollen of diploid *B. nana* and tetraploid *B. pubescens* from Iceland differs in average size and shape, although frequency curves overlap on grain diameters (D), pore depths (P) and D/P ratios (Karlsdóttir et al., 2007). Pollen of the triploid hybrids between *B. nana* and *B. pubescens*, as shown in the present study, differs from pollen of the parental species and several anomalies in its structure are common. Hybrid pollen is not intermediate in size and shape, but possesses characters from both or either parental species. The majority of pollen grains from the triploid hybrids have the small size of *B. nana* pollen whereas the deep pollen pore is like *B. pubescens*, which gives some of the hybrid pollen a distinguished triangular look. This finding may provide a means to detect the presence of hybrid pollen in a mixed sample, by its unique morphology, and to calculate proportions of hybrid pollen according to distribution curves, especially those with low D/P ratios. By this means, it may be possible to identify interspecific hybridisation in sub-fossil pollen samples. Furthermore, anomalies from normal pollen structure are very frequent among pollen from triploid hybrids. As comparable anomalies are rare in the diploid and tetraploid species, this might prove to be a true indicator of hybridisation if abnormal pollen grains are frequent in a mixed sample. The proportion of non-triporate pollen grains is of special interest in this context, as they can easily be recognized in sub-fossil pollen. Repeated sampling shows that the rate of non-triporate pollen formation does not fluctuate much between growing seasons.

High frequencies of structural abnormalities in pollen of the triploid hybrids are not unexpected, due largely to meiotic irregularities occurring in PMCs (pollen mother cells) of interspecific hybrids. This has been observed in a number of *Betula* natural hybrids (e.g. Woodworth 1929; Johnsson 1949; Brown & William, 1984). As a consequence of pairing problems during the first meiotic division, especially between the homoeologous genomes, a huge number of variants among the microspores (and pollen) are often formed. Woodworth (1929) reported that pollen grains from a triploid hybrid between *B. pendula* and *B. pubescens* from northern Sweden were variable in size, and the sizes among pollen grains of a triploid hybrid between *B. lenta* and *B. pumila* varied from being microcytes (micronuclei) to more or less normal-sized grains, to large double-sized grains. Pollen of a presumed triploid birch hybrid from Poland (Szwabowicz 1976) contained empty grains (devoid of cellular content), deformed grains with a thicker wall and a greater number of pores (4, 5 up to 8 pores), and viable grains which varied greatly in size. The present study shows a similar range of deformity among hybrid pollen.

These deformed pollen grains are not likely to be viable, as selection would act against meiotic configurations that generate unbalanced gametes. Previous studies

involving triploid F<sub>1</sub> hybrids from *B. nana* x *B. pubescens* crosses and backcrossed progeny of the triploid hybrids, essentially with *B. pubescens* (Anamthawat-Jónsson & Tómasson, 1990), indicated that the triploid hybrids produced viable euploid gametes containing a full genome complement, i.e. having 14 (1x) or 28 (2x) chromosomes. All other numbers must have been eliminated, either via gametic or sporophytic selection, as only backcrossed progeny having diploid, triploid and tetraploid chromosome numbers were recovered. No aneuploid was found among these backcrossed progeny, nor has any aneuploid ever been found in Icelandic woodlands (Anamthawat-Jónsson & Thórsson, 2003). Unreduced gametes (3x) might have been formed among the hybrid pollen, but no 5x or 6x birch plants have ever been discovered. However, unusually large, presumably unreduced, pollen grains have often been observed in birch hybrids (Woodworth 1929). In the present study, unusually large grains were found among those having more than three pores, but non-triporate pollen grains were not size-measured. Unreduced gametes from triploid hybrids may have a critical role in polyploid formation, especially tetraploid fixation in diploid or mixoploid populations of horticultural plants (Ramsey & Schemske, 1998, 2002; Husband 2004).

Within a given taxonomic or phylogenetic framework, ploidy level (chromosome number) appears to correlate positively with genome size and cell size (Beaulieu et al., 2007; Gregory et al., 2007). In *Betula*, 1C DNA value (genome size) tends to be directly related to ploidy and is considered to be close to the theoretical expected value for the 42, 56 and 70-euploid levels (Taper & Grant 1973). Pollen of diploid dwarf birch species (*B. nana* and *B. glandulosa*) is considerably smaller than that of polyploid tree birch species examined, such as *B. pubescens* and *B. papyrifera* (Caseldine 2001; Clegg et al., 2005; Karlsdóttir et al., 2007). However, in relation to the diploid and tetraploid species in the present study, the mean size of pollen from the triploid individuals is not different from that of the diploid species *B. nana*.

If chromosome number was a dominating factor in determining pollen size, one would expect pollen produced by the triploid *Betula* hybrids to be larger on average than *B. nana* pollen. Clearly there has been selection in favour of the small size of *B. nana* (presumably having 14 chromosomes) among triporate pollen grains from the triploid hybrids. Eighteen (out of 22) triploid hybrids produced small pollen grains similar in size to the diploid species *B. nana*. However, a small proportion of hybrid pollen consists of large pollen grains similar in size to those of *B. pubescens*, and in some samples the large size is prominent. No intermediate size of triporate pollen grains is clearly observed. This supports the hypothesis proposed initially in the crossing study of Anamthawat-Jónsson and Tómasson (1990), i.e. that triploid hybrids produce viable euploid gametes, hence gametic selection. Szwabowicz (1976) observed that in meiosis of the (triploid) hybrid birch there was a tendency towards reduction of the number of chromosomes to  $n = 14$  (by chromosome elimination) in both the first anaphase and

telophase stages and also in the second meiotic division. Preferential chromosome elimination is known to occur in synthesized hybrids from interspecific crosses, for example in wheat and barley (Laurie & Bennett, 1988; Anamthawat-Jónsson et al., 1993). Preferential transmission of chromosomes via (triploid) hybrids has also been shown to be genome- or species specific, for example in *Brassica* whereby the effect of the chromosome number on fertilization fitness is more pronounced in some hybrids, as they show a stronger tendency in keeping their gametes toward euploid numbers (Lu & Kato, 2001; Leflon et al. 2006). Aneuploidy, in both plants and animals, may disturb gene expression and, as a consequence, the phenotypic characteristics (Birchler et al., 2001). It has been suggested that the aneuploid effects result from altered stoichiometry of transcriptional regulators and that there is a tendency for genes to exhibit a “per cell” expression that is directly correlated with ploidy. In a study with maize aneuploids (Guo & Birchler, 1994), monosomics and trisomics are often less vigorous than euploid normals. Natural plants that thrive in extreme environments, like sub-arctic *Betula* in this study, can not afford aneuploidy.

In northern Europe, birch has a key role in the early Holocene vegetation history. When the history is reconstructed using sub-fossil pollen from sediments as a main source, emphasis is usually made on differentiating the tree-birch pollen of *B. pubescens* from the pollen of dwarf-shrub birch *B. nana* (e.g. Birks 1968; Mäkelä 1999; Caseldine 2001). The results of the present study suggest that the hybridisation of the two species, which has presumably led to the establishment of mountain birch (*B. pubescens* ssp. *tortuosa*) near the tree-line, could be traced back by observing sub-fossil birch pollen, both in terms of the structural changes and the occurrence of deformed pollen grains.

## Conclusions

Triploid *Betula* hybrids produced pollen with distinct characteristics which may be used to detect interspecific hybridisation in sub-fossil samples. Large and protruding pores tended to distinguish hybrid from *B. nana* pollen, which was of equal size but smaller than pollen of *B. pubescens*. Several anomalies in pollen structure, including unusual numbers of pores, were frequent in pollen samples from the triploid hybrids but were very rare among pollen grains from the parental species.

## Acknowledgements

The project was supported by the Icelandic Research Centre (Rannís: grant no. 040238021).

## References

- Anamthawat-Jónsson, K. (2003a). Hybrid introgression in *Betula*. In A. K. Sharma & A. Sharma (Ed.), *Plant Genome – Biodiversity and Evolution*, vol. 1, *Phanerogams* (pp. 249-265). Enfield, Plymouth: Science Publishers Inc.
- Anamthawat-Jónsson, K. (2003b). Preparation of chromosomes from plant leaf meristems for karyotype analysis and *in situ* hybridisation. *Methods in Cell Science*, 25, 91-95.
- Anamthawat-Jonsson, K., & Thorsson, A. T. (2003). Natural hybridisation in birch: triploid hybrids between *Betula nana* and *B. pubescens*. *Plant Cell, Tissue and Organ Culture*, 75, 99–107.
- Anamthawat-Jónsson, K., & Tómasson, T. (1990). Cytogenetics of hybrid introgression in Icelandic birch. *Hereditas*, 112, 65-70.
- Anamthawat-Jónsson, K., & Tómasson, T. (1999). High frequency of triploid birch hybrid by *Betula nana* seed parent. *Hereditas*, 130, 191-193.
- Anamthawat-Jónsson, K., Schwarzacher, T., & Heslop-Harrison, J. S. (1993). Behavior of parental genomes in the hybrid *Hordeum vulgare* x *H. bulbosum*. *The Journal of Heredity*, 84, 78-81.
- Beaulieu, J. M., Moles, A. T., Leitch, I. J., Bennett, M. D., Dickie, J. B., & Knight, C. A. (2007). Correlated evolution of genome size and seed mass. *New Phytologist*, 173, 422-437.
- Birchler, J. A., Bhadra, U., Bhadra, M. P., & Auger, D. L. (2001). Dosage-dependent gene regulation in multicellular eukaryotes: implications for dosage compensation, aneuploid syndromes, and quantitative traits. *Developmental Biology* 234, 275–288.
- Birks, H. J. B. (1968). The identification of *Betula nana* pollen. *New Phytologist*, 67, 309-314.
- Blackmore, S., Steinmann, J. A. J., Hoen, P. P., & Punt, W. (2003). The Northwest European Pollen Flora 65: Betulaceae and Corylaceae. *Review of Palaeobotany and Palynology*, 123, 71-98.
- Brown I. R., & William D. A. (1984). Cytology of the *Betula alba* L. complex. *Proceedings of the Royal Society of Edinburgh*, 85B, 49-64.
- Caseldine, C. (2001). Changes in *Betula* in the Holocene record from Iceland – a palaeoclimatic record or evidence for early Holocene hybridisation? *Review of Palaeobotany and Palynology*, 117, 139-152.

- Caseldine, C., Geirsdottir, A. & Langdon, P., (2003). Efstadalssvatn – a multi-proxy study of a Holocene lacustrine sequence from NW Iceland. *Journal of Paleolimnology*, 30, 55–73.
- Clegg, B. J., Tinner, W., Gavin, D. G., & Hu, F. S. (2005). Morphological differentiation of *Betula* (birch) pollen in northwest North America and its palaeoecological application. *The Holocene*, 15, 229-237.
- Gregory, T. R., Nicol, J. A., Tamm, H., Kullman, B., Kullman, K., Leitch, I. J., Murray, B. G., Kapraun, D. F., Greihuber, J., & Bennett, M. D. (2007). Eukaryotic genome size databases. *Nucleic Acids Research*, 35, D332-338.
- Guo, M., & Birchler, J. A. (1994). Trans-acting dosage effects on expression of model gene systems in maize aneuploids. *Science*, 266, 1999-2000.
- Hallsdóttir M. (1990). Studies in the vegetational history of North Iceland. A radiocarbon-dated pollen diagram from Flateyjaralur. *Jökull*, 40, 67-81.
- Husband, B. C. (2004). The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations. *Biological Journal of the Linnean Society*, 82, 537-546.
- Johnsson, H. (1949). Hereditary precocious flowering in *Betula verrucosa* and *B. pubescens*. *Hereditas*, 35, 112-114.
- Karlsdóttir, L., Thórsson, Æ. Th., Hallsdóttir, M., Sigurgeirsson, A., Eysteinnsson, Th. & Ananthawat-Jónsson, K. (2007). Differentiating pollen of *Betula* species from Iceland. *Grana*, in press.
- Laurie, D. A., & Bennett, M. D. (1988). The production of haploid wheat plants from wheat x maize crosses. *Theoretical and Applied Genetics*, 76, 393-397.
- Leflon, M., Eber, F., Letanneur, J. C., Chelysheva, L. Coriton, O., Huteau, V., Ryder, C. D., Barker, G., Jenczewski, E., & Chèvre, A. M. (2006). Pairing and recombination at meiosis *Brassica rapa* (AA) x *Brassica napus* (AACC) hybrids. *Theoretical and Applied Genetics*, 113, 1467-1480.
- Lu, C., & Kato, M. (2001). Fertilization fitness and relation to chromosome number in interspecific progeny between *Brassica napus* and *B. rapa*: a comparative study using natural and resynthesized *B. napus*. *Breeding Science*, 51, 73-81.
- Mäkelä, E. M. (1996). Size distinctions between *Betula* pollen types – A review. *Grana*, 35, 248-256.
- Mäkelä, E. M. (1999). *The Holocene history of birch in northeastern Fennoscandia - an interpretation based on fossil birch pollen measurements.*

- University of Helsinki, Department of Geology, Division of Geology and Palaeontology, Helsinki, Finland.
- Ramsey, J., & Schemske, D. W. (1998). Pathways, mechanisms and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics*, 29, 467-501
- Ramsey, J., & Schemske, D. W. (2002). Neopolyploidy in flowering plants. *Annual Review of Ecology and Systematics*, 33, 589-639.
- Rundgren, M. (1998). Early-Holocene vegetation of northern Iceland: pollen and plant macrofossil evidence from the Skagi peninsula. *The Holocene*, 8, 553-564.
- Szwabowicz, A. (1976). Microsporogenesis of *Betula oycoviensis* Besser and of its progeny. *Acta Societatis Botanicorum Poloniae*, 45, 169-187.
- Taper, L. J., & Grant, W. J. (1973). The relationship between chromosome size and DNA content in birch (*Betula*) species. *Caryologia*, 26, 263-273.
- Thorsson, A. T., Salmela, E., Anamthawat-Jonsson, K. (2001). Morphological, cytogenetic, and molecular evidence for introgressive hybridization in birch. *Journal of heredity*, 92: 404-408.
- Thorsson, Æ. Th., Pálsson, S., Sigurgeirsson, A., Anamthawat-Jónsson, K. (2007). Morphological variation among *Betula nana* (diploid), *B. pubescens* (tetraploid) and their triploid hybrids in Iceland. *Annals of Botany*, in press.
- Woodworth, R. H. (1929) Cytological studies in the Betulaceae I. *Betula*. *Botanical Gazette*, 137: 331-363.

# PAPER III

## **Evidence of hybridisation between *Betula pubescens* and *B. nana* in Iceland during the early Holocene**

Karlsdóttir L, Hallsdóttir M, Thórsson ÆTh and Anamthawat-Jónsson K  
(2009)

*Review of Palaeobotany and Palynology* 156: 350-357.

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Review of Palaeobotany and Palynology, Volume 156, Issues 3–4,  
September 2009, Pages 350–357, available online:  
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# Evidence of hybridisation between *Betula pubescens* and *B. nana* in Iceland during the early Holocene

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Lilja Karlsdóttir<sup>1</sup>, Margrét Hallsdóttir<sup>2</sup>, Ægir Th. Thórsson<sup>1</sup>, Kesara Ananthawat-Jónsson<sup>1</sup>

<sup>1</sup>Institute of Biology, University of Iceland, Sturlugata 7, Reykjavik, IS-101, Iceland  
Tel: +354 525 4620; Fax: +354 525 4069; E-mail: kesara@hi.is

<sup>2</sup>Icelandic Institute of Natural History, Hlemmur 3, Reykjavik, IS-105, Iceland

## ABSTRACT

The aim of this study was to find evidence of Holocene hybridisation between downy birch (*Betula pubescens*) and dwarf birch (*B. nana*) in Iceland. We measured *Betula* pollen from an early Holocene peat profile from Hella in Eyjafjörður, mid-northern Iceland, with 39 samples taken at ca. 100-yr intervals between ca. 10.3 and 7.0 cal. ka BP based on known tephra layers. Species proportions were estimated and compared with data on present-day birch pollen. We found that *Betula* pollen from old samples prepared in glycerol was larger than pollen in parallel samples mounted in silicon oil by a factor of 1.2 to 1.6, depending on the age of the peat. Therefore the analysis of Hella peat profile was entirely based on size frequency distribution within samples. The size frequency distribution of *Betula* pollen changed throughout the profile. In all samples, two normal curves with different means gave good fit to the observed frequency distribution, which denoted small pollen of *B. nana* and large pollen of *B. pubescens*. A low proportion of *B. pubescens* pollen was found in the oldest peat samples and again around 7.8 cal. ka BP, when *B. nana* predominated. The proportion of *B. pubescens* pollen peaked approximately at 8.7 and 7.2 cal. ka BP. Evidence of *Betula* hybrids was found in several samples, especially simultaneously with the earlier *B. pubescens* peak. Pollen with low D/P ratios (hybrid pollen) was found at different frequencies throughout the profile. Non-triporate *Betula* pollen grains, which are frequent among present-day

triploid hybrids, were observed in most samples. The frequency of non-triporate pollen in a period between 9.2 and 8.7 cal. ka BP far exceeded the average level produced by the present-day triploid hybrids. Climatic and ecological conditions may have favoured hybridisation of birch species during the expansion of downy birch over dwarf birch colonies in warm periods.

**Keywords:** Birch, *Betula*, Iceland, Holocene, hybridisation, pollen.

## 1 INTRODUCTION

Two species of birch are found in natural habitats in Iceland: dwarf birch (*Betula nana* L., diploid with  $2n = 2x = 28$ ) and downy birch (*B. pubescens* Ehrh., tetraploid with  $2n = 4x = 56$ ). *Betula nana*, a circumpolar species (de Groot et al., 1997), grows up to one metre in height with procumbent, wide-spreading stems and a strong branching habit. It has orbicular or obovate-orbicular leaves which are regularly crenate and are rounded at both leaf tip and base. *Betula pubescens* is a European species (Atkinson, 1992), represented by the subspecies *pubescens* Ehrh., which may grow up to 25 m tall with single or many stems (monocormic or polycormic type), and subspecies *tortuosa* (Ledeb.) Nyman, which is a shrub or low tree found in the mountain regions of northern Europe and is therefore called “mountain birch”. The birch in Iceland (*B. pubescens*) has in some occasions been referred to as mountain birch, due to its low stature and scrub-like growth form (e.g. Jónsson, 2004). Elkington (1968) compared birch from Iceland and Scotland and suggested that the morphological variability and the shrub-like appearance in Icelandic birch were due to genetic introgression from *B. nana* into *B. pubescens* via triploid hybrids between the two species. This was confirmed with both crossing experiments and population-based studies (Anamthawat-Jónsson and Tómasson, 1990; Anamthawat-Jónsson and Thórsson, 2003). Furthermore, these studies revealed that the introgression occurred in both directions, i.e. also from the tetraploid to the diploid birch species, resulting in a high degree of variation in both species.

Birch species are common in sub-arctic vegetation and as birch is wind-pollinated, it produces a large quantity of pollen which can be found in sediments and peat. In Iceland, downy birch is the only tree species known to have formed forests and woodlands in the Holocene and is therefore a key ecological species in the history of vegetation. An assessment of several morphological traits of present-day Icelandic birch, focused upon leaf shape and size, has revealed continuous variation from a typical diploid *B. nana* appearance to tetraploid trees with *B. pubescens* traits (Thórsson et al., 2007). The triploid hybrids also varied widely in their appearance but were often intermediate. Measurements of pollen from these trees and shrubs, which were differentiated by ploidy levels, showed small but significant differences in the average

size and shape between the diploid and the tetraploid species (Karlsdóttir et al., 2007). Although abnormal pollen grains were found to be prevalent among the triploid hybrids, normal triporate pollen was also produced (Karlsdóttir et al., 2008). This triporate pollen was not intermediate in its appearance, but was on average as small as *B. nana* pollen while its vestibulum was as large as *B. pubescens* pollen. This type of pollen is presumably rare in the *Betula* pollen pool, but it may be viable and could therefore take part in the introgressive hybridisation process. Triploid plants (hybrids between diploid *B. nana* and tetraploid *B. pubescens*) make up about 10% of individual birch trees/shrubs in natural woodlands in Iceland today (Thorsson et al., 2007). The presence of triploid hybrids in Icelandic birch populations indicates active hybridisation, which is likely to result in gene introgression if the triploid hybrids are not sterile. Anamthawat-Jónsson and Tómasson (1990) showed that seed progeny of open-pollinated triploid plants consisted of diploid, triploid and tetraploid individuals, confirming the role of triploid hybrids as seed parents in the introgressive hybridisation process. There is no study to date showing that triploid plants contribute directly to this process as pollen parents. On the other hand, the characterization of pollen produced by triploid plants has provided a reliable means for identification of introgressive hybridisation between the two birch species in the past.

There have been questions about the origin of Icelandic birch. The dwarf birch (*B. nana*) may have survived some cold periods of the late Pleistocene (Rundgren and Ingolfsson, 1999) but more likely it was a Holocene immigrant coming from Europe, like *B. nana* in north-eastern Greenland (Bennike et al., 1999). Even for the high-Arctic species like *Campanula uniflora*, one of the most likely glacial survival candidates in the Icelandic flora, there is no molecular evidence for glacial survival (Ægisdóttir and Thórhallsdóttir, 2004). Downy birch (*B. pubescens*), on the other hand, is believed to have dispersed from Europe (western Europe and/or Scandinavia) to Iceland when the climate improved. The question remains whether it was the introgressed mountain birch (*B. pubescens* ssp. *tortuosa*) that colonized Iceland in the early Holocene, or if the original tree birch was *B. pubescens* ssp. *pubescens* and the introgressive hybridisation occurred locally. If local hybridisation is the case, there is still a question about the extent of past hybridisation, i.e. whether it has been a continuous process for the last 10,000 years or restricted to specific conditions of the climate and vegetation succession.

The early Holocene vegetation history of Iceland based on several palynological records has been described (Hallsdóttir, 1995; Erlendsson, 2007). In most of these records, the concentration of *Betula* pollen was very low before ca. 9.8 cal. ka BP. Although pollen profiles from different sites are not uniform, there is an overall tendency towards an increase in *Betula* pollen starting at 9.0 - 9.5 cal. ka BP, reaching a maximum shortly before the deposition of the Hekla-5 tephra at 7.0 - 7.1 cal. ka BP,

sometimes with a distinct decline and recovery somewhere in the period of 7.5 - 8.5 cal. ka BP. In the present study, we examined an early Holocene peat profile from the period when birch was being established after the retreat of glaciers and into the first known birch maximum. We used the morphometric methods and results obtained with present-day *Betula* pollen (Karlsdóttir et al., 2007, 2008) to analyse the composition of birch species in this period and to search for evidence of past hybridisation.

Our aims were (1) to test if size comparison of subfossil pollen with present-day pollen would be possible, (2) to separate subfossil pollen of *B. pubescens* from that of *B. nana* on the basis of coefficients obtained for pollen diameter (D), pore depth (P) and D/P ratio, (3) to identify pollen from triploid hybrids which would be a clear indication of past hybridisation, and (4) to reveal periods of intense hybridisation if these existed and to analyse the nature of such periods in terms of species composition and climatic characteristics.

## 2 MATERIALS AND METHODS

### 2.1. Location

The sampling site is a drained lowland hayfield belonging to the farm “Hella” in Eyjafjörður, mid-northern Iceland, at 65°56'35'' N/ 18°24'20'' W and approximately 15 m a.s.l. (Fig. 1). The drainage ditch exposed the peat, which was sampled by hammering 5x5x20 cm<sup>3</sup> metal boxes into the vertical peat profile. The boxes were cut free without disturbing the peat inside, after which the peat was wrapped in plastic and stored in a cool place.

### 2.2. Materials

The profile, which was extracted in 1996, contained 2 m of peat with 18 tephra layers. A total of 39 samples for pollen analysis was taken between the two best-dated tephra layers, Saksunarvatn and H5, 10 ml each and with 5 cm spacing; of these, 29 samples were used in the present study. For comparison of mounting media, an additional 10 ml was retrieved from the same boxes in 2006, parallel to the original samples at four different depths. The Saksunarvatn tephra has been dated to 10.3 cal. ka BP or 10347±89 years before A.D. 2000 (b2k) according to Greenland Ice Core Chronology 2005 (Rasmussen et al., 2006, 2007). The Hekla tephra H5 has been dated to 6185±100 <sup>14</sup>C yr BP (Thórarinnsson, 1971) or 6070±120 <sup>14</sup>C yr BP (Vilmundardóttir and Kaldal, 1982), which together roughly corresponds to 7000 – 7100 cal. yr BP. The average time interval among the pollen samples was therefore just over 100 years, given the average rate of sedimentation of approximately 0.5 mm per year.

### 2.3. Preparation protocol

The samples were treated with the conventional NaOH, hot HF and acetolysis (Fægri and Iversen, 1989) and mounted in glycerol. A pollen diagram has been generated from these samples (Hallsdóttir and Caseldine, 2005). The excess samples, which were stored in closed glass tubes for twelve years, were retrieved for the present study. Four new samples were taken for size-comparison from the original metal boxes, at the same levels as the older samples at selected profile depths of 5, 25, 75 and 110 cm. They were treated using the same procedure as the old samples, but dehydrated with tert-butyl alcohol and mounted in silicon oil.

### 2.4. Pollen measurements

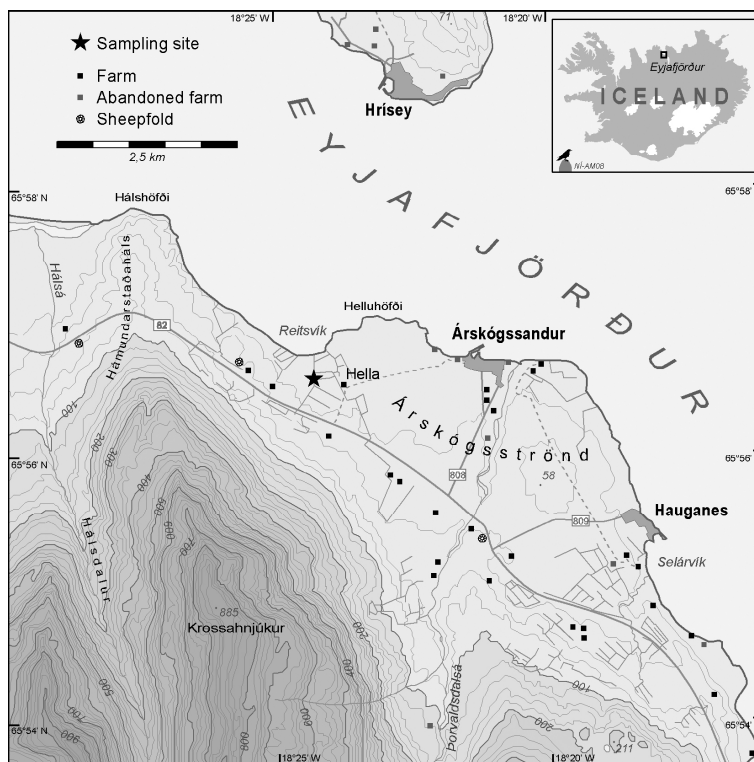
The samples were examined in a Leica Phase Contrast microscope DM2500 under x400 magnification. Any *Betula* pollen found was digitally photographed with a Leica DFC320R2 camera. About 67% of the pollen images showed measurable pollen grains, while the remaining pollen was damaged, partly obscured by debris or the angle of view unusable. The pollen was measured as in Karlsdóttir et al. (2007), either in polar view or semi-equatorial view with one pore protruding (Fig. 2a, b). Grain diameter (D) was defined as the distance from the outside tip of the pore to the outer margin of the facing wall, whereas pore depth (P) was the distance from the outside tip of the pore to the inner margin of the nexine through the vestibulum (Birks, 1968). The precision of measurement was approximately 0.3  $\mu\text{m}$ , but the accuracy depended also on visibility and angle of view. This was especially the case with the measurement of pore depth when the position of the inner margin was not always clear (Fig. 2c). In the situation where pollen shape was unbalanced in the polar view, the largest diameter and pore were used. In semi-equatorial view no such choice was possible. To test the effect of semi-equatorial pollen on the overall results, we statistically compared polar and semi-equatorial types from over 100 pollen grains in a single mount. Although the grains photographed in polar view measured on the average 1 % larger and their pores 3.7 % deeper than the semi-equatorial ones, the difference was not significant (one tailed t-test;  $P \sim 0.3$ ). Non-triporate pollen grains were noted and counted but not measured for size.

### 2.5. Data analysis and calculations

As pollen sizes of the two *Betula* species overlap considerably (Karlsdóttir et al., 2007), analysis of size distribution of diameters and pore depths within samples was performed using Bhattacharya's method (Bhattacharya, 1967). This method utilizes the fact that the logarithms of size-class ratios of normally distributed data form a straight line when plotted against midpoints of size classes. If the data contains more than one normally

distributed component, the curve will be S-shaped with a straight line corresponding to each subset. The point on the x-axis that intercepts the line drawn through the straight section gives the mean of the underlying normal distribution and the slope corresponds to the standard deviation. A modification of the method (Pauly and Caddy, 1985) proposed that such lines could be defined using three (or more) points and the straightness can be tested by the degree of negative correlation. Intercepts and slopes can then be derived using the linear regression technique.

Means and standard deviations of subsets within samples (presumably *B. nana* vs. *B. pubescens*) were calculated from three or more points when the correlation coefficient was under - 0.95. The plotting was repeated with varying size-class widths between 1.0 and 2.0  $\mu\text{m}$ , and the best results were refined with the Bmod program (Morgan, 2005) using the least Kolmogorov-Smirnov D (KS-D) statistic as the measure of fit. The best-fitted proportions of each group were then calculated using Járαι-Komlódi's formula (Prentice, 1981).



**Figure 1**  
Map of Iceland showing location of the sample site at Hella in Eyjafjörður, northern Iceland. Source: National Land Survey of Iceland.



### 3. RESULTS

#### 3.1. Comparison of pollen mounted in glycerol and silicon oil

Four pairs of samples were used to compare pollen in different mounting media (Table 1). Pollen from samples prepared in silicon oil was considerably smaller than pollen in samples mounted in glycerol, both in terms of grain diameter (D) and pore depth (P). As the mounting media had a little less effect on pore size than on grain diameter, the resulting D/P ratios were approximately 10% higher when pollen was mounted in glycerol than in silicon oil.

For each depth of the peat profile, the difference due to mounting media (as inferred from glycerol/silicon oil, or G/S ratio) was about the same with the D and P parameters (Table 1). However, the G/S ratio increased down the peat profile, from the lowest ratio of 1.3 in the uppermost samples at 5 cm depth to the highest ratios of 1.5 and 1.6 in the oldest samples at 110 cm profile depth. This is mainly due to the increase of pollen size down the profile in samples mounted in glycerol, whereas the reverse was observed among samples mounted in silicon oil. Regression analysis of the data with R linear model ANCOVA (Analysis of covariance) confirmed the significant ( $P < 0.001$ ) effect of mounting media, estimated age of peat and the media-age interaction.

As the data clearly indicated that either the type or age of the peat affected final pollen sizes and that the effects were different depending on the mounting media, any comparison of pollen sizes between samples would be unreliable. Therefore the analysis in the present study was entirely based on size frequency distribution within samples.

**Table 1:** Mean pollen diameter (D) and pore depth (P) of *Betula* pollen from the Hella profile, from samples mounted in glycerol (G) and silicon-oil (S).

| Depth<br>(cm) | Glycerol |                                       |                         |                                   |                         |             |     | Silicon-oil |                                       |                         |                                   |                         |             |         | G/S ratio |     |     |
|---------------|----------|---------------------------------------|-------------------------|-----------------------------------|-------------------------|-------------|-----|-------------|---------------------------------------|-------------------------|-----------------------------------|-------------------------|-------------|---------|-----------|-----|-----|
|               | <i>n</i> | Diameter<br>mean<br>( $\mu\text{m}$ ) | SD<br>( $\mu\text{m}$ ) | Pore<br>mean<br>( $\mu\text{m}$ ) | SD<br>( $\mu\text{m}$ ) | D/P<br>mean | SD  | <i>n</i>    | Diameter<br>mean<br>( $\mu\text{m}$ ) | SD<br>( $\mu\text{m}$ ) | Pore<br>mean<br>( $\mu\text{m}$ ) | SD<br>( $\mu\text{m}$ ) | D/P<br>mean | D<br>SD | P         | D/P |     |
|               |          |                                       |                         |                                   |                         |             |     |             |                                       |                         |                                   |                         |             |         |           |     |     |
| 0             | 218      | 25.6                                  | 3.0                     | 2.9                               | 0.7                     | 9.1         | 1.7 | 261         | 20.0                                  | 2.5                     | 2.3                               | 0.45                    | 8.8         | 1.54    | 1.3       | 1.3 | 1.1 |
| 24            | 188      | 27.1                                  | 2.4                     | 2.7                               | 0.5                     | 10.3        | 1.9 | 166         | 20.6                                  | 1.9                     | 2.3                               | 0.40                    | 9.1         | 1.42    | 1.3       | 1.2 | 1.1 |
| 86            | 281      | 28.0                                  | 2.7                     | 3.4                               | 0.6                     | 8.5         | 1.5 | 174         | 19.1                                  | 1.8                     | 2.3                               | 0.46                    | 8.4         | 1.71    | 1.5       | 1.4 | 1.0 |
| 116           | 211      | 29.8                                  | 3.4                     | 3.1                               | 0.6                     | 9.8         | 1.7 | 198         | 18.2                                  | 1.4                     | 2.1                               | 0.33                    | 8.9         | 1.52    | 1.6       | 1.5 | 1.1 |

#### 3.2. Pollen size

The size frequency distribution of *Betula* pollen from Hella (Table 2) showed changes in pollen diameter throughout the period represented in the profile, i.e. between the Saksunarvatn (10.3 cal. ka BP) and Hekla-H5 (7.0 – 7.1 cal. ka BP) tephra layers. The first five samples above the Saksunarvatn tephra contained little *Betula* pollen and were therefore pooled for further analysis. The smallest pollen found in this study had a mean

grain diameter of 23.0  $\mu\text{m}$ , whereas the largest measured 30.2  $\mu\text{m}$ . The mean pollen pore depth ranged from 2.4 to 3.5  $\mu\text{m}$ .

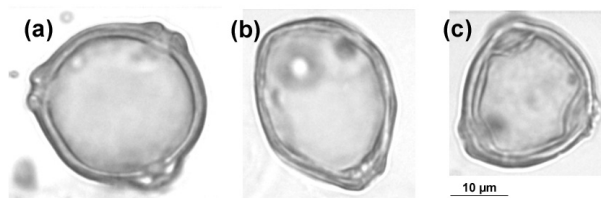
**Table 2.** Pollen size distribution, mean sizes and proportion of non-triporate *Betula* pollen per sample from Hella.

| Depth<br>(cm) | Approx<br>age (cal.<br>ka BP) | Number of<br>pollen<br>measured <i>n</i> | Size distribution of <i>Betula</i><br>pollen | Mean<br>pollen<br>diameter<br>( $\mu\text{m}$ ) | SD<br>( $\mu\text{m}$ ) | Mean<br>pore<br>( $\mu\text{m}$ ) | SD<br>( $\mu\text{m}$ ) | Mean<br>D/P<br>ratio | SD  | Non<br>triporate<br>proportion |
|---------------|-------------------------------|--|--|---|-------------------------|-----------------------------------|-------------------------|----------------------|-----|--------------------------------|
| 0             | 7.05                          | 218                                      |  | 25.6  | 3.0                     | 2.9                               | 0.7                     | 9.1                  | 1.7 | 0.03                           |
| 6             | 7.2                           | 190                                      |  | 30.2  | 3.0                     | 3.4                               | 0.5                     | 9.0                  | 1.3 | 0.04                           |
| 12            | 7.3                           | 281                                      |  | 26.5  | 2.7                     | 3.2                               | 0.6                     | 8.8                  | 2.4 | 0.06                           |
| 18            | 7.4                           | 68                                       |  | 26.0  | 2.4                     | 2.8                               | 0.6                     | 9.5                  | 1.6 | 0.01                           |
| 24            | 7.5                           | 188                                      |  | 27.1  | 2.4                     | 2.7                               | 0.5                     | 10.3                 | 1.9 | 0.03                           |
| 30            | 7.7                           | 48                                       |  | 27.9  | 2.0                     | 3.1                               | 0.6                     | 9.4                  | 1.8 | 0.05                           |
| 36            | 7.8                           | 78                                       |  | 27.4  | 2.7                     | 3.0                               | 0.6                     | 9.4                  | 1.6 | 0.02                           |
| 42            | 7.9                           | 110                                      |  | 23.0  | 2.0                     | 2.8                               | 0.4                     | 8.3                  | 1.3 | 0.01                           |
| 48            | 8.0                           | 58                                       |  | 25.2  | 3.2                     | 2.8                               | 0.5                     | 9.2                  | 1.4 | 0.05                           |
| 54            | 8.2                           | 64                                       |  | 24.2  | 2.9                     | 2.8                               | 0.5                     | 8.8                  | 1.5 | 0.02                           |
| 60            | 8.3                           | 65                                       |  | 26.9  | 2.5                     | 3.2                               | 0.6                     | 8.6                  | 1.4 | 0.00                           |
| 68            | 8.5                           | 62                                       |  | 26.6  | 3.4                     | 3.0                               | 0.5                     | 9.1                  | 1.4 | 0.00                           |
| 74            | 8.6                           | 134                                      |  | 26.5  | 2.8                     | 3.0                               | 0.6                     | 9.0                  | 1.4 | 0.02                           |
| 80            | 8.7                           | 75                                       |  | 29.3  | 2.9                     | 3.5                               | 0.7                     | 8.6                  | 1.5 | 0.08                           |
| 86            | 8.8                           | 281                                      |  | 28.0  | 2.7                     | 3.4                               | 0.6                     | 8.5                  | 1.5 | 0.19                           |
| 92            | 8.9                           | 63                                       |  | 28.0  | 2.5                     | 3.4                               | 0.7                     | 8.5                  | 1.6 | 0.20                           |
| 98            | 9.1                           | 92                                       |  | 28.3  | 2.0                     | 3.3                               | 0.7                     | 9.0                  | 1.8 | 0.25                           |
| 101           | 9.1                           | 154                                      |  | 24.6  | 2.6                     | 2.8                               | 0.5                     | 8.8                  | 1.4 | 0.01                           |
| 103.3         | 9.2                           | 119                                      |  | 27.1  | 2.1                     | 3.2                               | 0.6                     | 8.8                  | 1.8 | 0.20                           |
| 110           | 9.3                           | 151                                      |  | 28.0  | 3.9                     | 3.3                               | 0.7                     | 8.6                  | 1.4 | 0.01                           |
| 116           | 9.4                           | 211                                      |  | 29.8  | 3.4                     | 3.1                               | 0.6                     | 9.8                  | 1.7 | 0.01                           |
| 123.3         | 9.6                           | 228                                      |  | 24.0  | 2.1                     | 2.7                               | 0.5                     | 9.1                  | 1.6 | 0.01                           |
| 129.3         | 9.7                           | 82                                       |  | 26.2  | 2.1                     | 2.7                               | 0.5                     | 9.9                  | 1.7 | 0.02                           |
| 135.3         | 9.8                           | 68                                       |  | 24.8  | 4.0                     | 2.4                               | 0.5                     | 10.7                 | 2.2 | 0.01                           |
| 142-158       | 10.0-10.3                     | 78                                       |  | 23.1  | 2.6                     | 2.5                               | 0.5                     | 9.4                  | 1.9 | 0.01                           |

Bhattacharya graphs of size distribution throughout the period represented in the peat profile (Table 2) indicated more than one normal curve for grain diameters in most samples. In all 25 samples, two normal curves representing different mean diameters gave closer fit to the observed size distribution than one curve and in ten of these samples the difference was statistically significant (G-test in Bmod). In 7 out of 25 cases



of size distribution of pollen pore depth, two normal curves fitted significantly closer to the observed distribution than one normal curve. D/P ratios for the three different ploidy groups (as in Karlsdóttir et al., 2008) could not be detected with adequate accuracy using the current sample sizes.



**Figure 2**

Examples of measurable triporate *Betula* pollen grains from the Hella peat profile: (a), polar view; (b), semi-equatorial view; and (c), grain with ambiguous margins of nexine.

### 3.3. *Betula* species

The relative proportions of *B. pubescens* and *B. nana* pollen were calculated assuming that the upper means of grain diameter and pore depth represented *B. pubescens* and the lower means represented *B. nana*. Pollen from triploid hybrids would be included in lower means of diameter and in upper means of pore depth (based on Karlsdóttir et al., 2008). The ratios of upper/lower diameter means were on average 1.13 (range: 1.06 – 1.21) and for the means of pore depth they were on average 1.25 (range: 1.14 – 1.54). Neither of these ratios changed significantly over time.

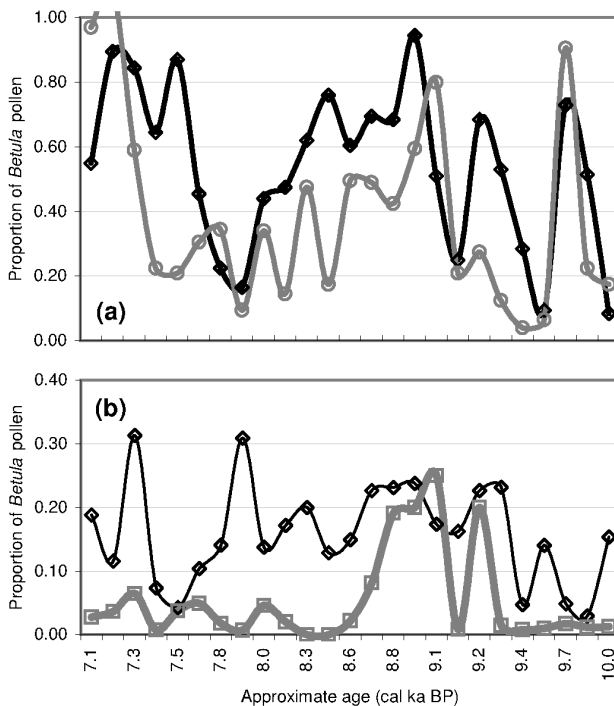
Proportions of *Betula* species calculated from pollen diameters and pore depths differed but were relatively correlated ( $r^2 = 0.28$ ). Both approaches showed that the original population of *Betula* at the onset of early Holocene vegetation (from the oldest samples in the peat profile) consisted almost entirely of *B. nana*. This was overtaken by *B. pubescens* in the first few hundred years after the Saksunarvatn tephra fall. The proportion of *B. pubescens* then declined for a period of time, but rose again and remained high in the middle part of the profile. It then declined slowly but rose sharply again towards the end of the period represented by the peat profile (Fig. 3a). The calculated proportions of *B. pubescens* throughout the profile are also correlated ( $r^2 = 0.25$ ) to the overall frequency of *Betula* pollen in the previous analysis by Hallsdóttir and Caseldine (2005). As the quantity of *B. nana* pollen (identification visually based on morphology) was low but relatively steady throughout the profile, the fluctuation in total *Betula* pollen represented mostly *B. pubescens*. Two periods with large quantities of *B. pubescens* pollen can be seen (Fig. 4): the earlier period from approximately 9.3 to 8.3 cal. ka BP and the later from approximately 7.3 cal. ka BP, with a peak at 7.2 cal. ka BP, but continuing to the end of the profile.

### 3.4. Hybridisation

Our previous study on the present-day *Betula* pollen in Iceland (Karlsdóttir et al., 2008) showed that the proportion of abnormal pollen grains, especially non-triporate pollen, was high among pollen from triploid hybrids or 12%, compared to 2.4% for *B. nana* and 0.7% for *B. pubescens*. Pollen with four or more pores was the most frequent type of structural abnormality. In the present investigation of the Hella peat profile, non-triporate *Betula* pollen grains were found in 23 out of 25 samples (Table 2; Fig. 3b). In ten of these, non-triporate pollen was found in over 3% of total *Betula* pollen, indicating the presence of hybrids. Non-triporate pollen was most prevalent (8 – 25%) in the early Holocene period between approximately 9.2 and 8.7 cal. ka BP.

The proportion of pollen from hybrids between *B. nana* and *B. pubescens* can also be estimated from the size and shape of pollen grains. Average triporate pollen from triploid hybrids has approximately the same grain diameter (D) as *B. nana* pollen but similar pore depth (P) to pollen of *B. pubescens*, leading to a low D/P ratio for hybrid pollen (Karlsdóttir et al., 2008). In the Hella samples, *Betula* pollen with low D/P ratio ( $< 7.5$ ) was found at different frequencies throughout the profile (Fig. 3b).

The correlation between proportions of non-triporate pollen and pollen with low D/P ratio (Fig. 3b) was low but statistically significant, ( $r = 0.37$ ;  $P = 0.036$ ). If the plots were split up, correlation in the lower part of the profile (approximately between 9.4 and 8.5 cal. ka) was considerably higher ( $r$  for the first 14 samples = 0.59;  $P = 0.014$ ) than in the upper part of the profile. A significant positive correlation was also found between total *Betula* pollen originally counted from this same Hella peat profile (Hallsdóttir and Caseldine, 2005; Fig. 4) and both parameters of hybrid pollen, i.e. the proportion of non-triporate pollen ( $r = 0.69$ ;  $P < .0001$ ) and the proportion of pollen with low D/P ratio ( $r = 0.60$ ;  $P < 0.01$ ).

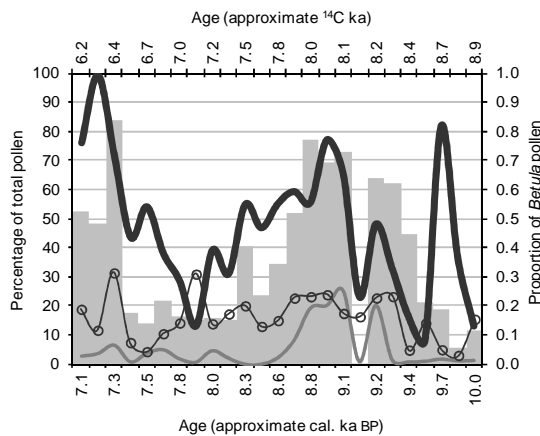


**Figure 3**

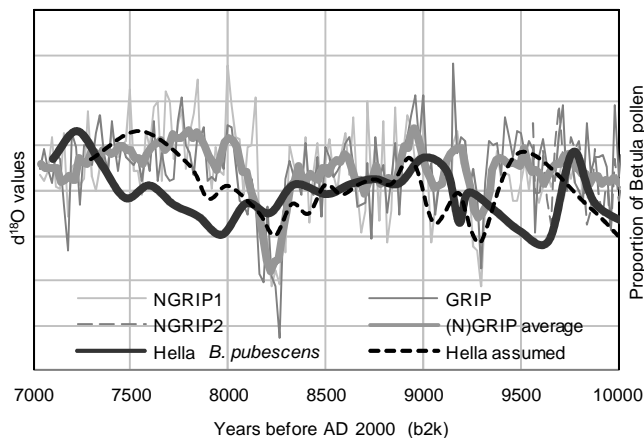
Distribution of *Betula* pollen from the Hella peat profile throughout the early Holocene period approximately between 10 and 7 cal. ka BP: (a) Proportion of *B. pubescens* pollen in total *Betula* pollen of the profile calculated from pollen diameters (black line) and pollen pore depth (grey line). (b) Proportion of non-triporate pollen (heavy grey line) and proportion of pollen with low (< 7.5) D/P ratios (thin black line). Notice different scales in (a) and (b).

### 3.5. Connection to climatic data

Changes in the calculated proportions of *Betula* species from the present study were compared to the existing climatic data obtained from the evaluation of changes in oxygen isotopes in the Greenland ice core (Rasmussen et al., 2006; Vinther et al., 2006). The calculated proportion of *B. pubescens* was not significantly correlated with the  $\delta^{18}\text{O}$  values ( $r = 0.14$ ;  $P = 0.25$ ) when accepting a constant sedimentation rate of approximately  $0.5 \text{ mm year}^{-1}$  as in Table 2, Fig. 3 and Fig. 4. However, when varying sedimentation rates and manually changing proposed sample ages, a correlation coefficient as high as 0.69 was possible (Fig. 5). The sedimentation rates necessary to provide such results varied between  $0.2$  and  $2.0 \text{ mm year}^{-1}$ , with highest values of  $1 - 2 \text{ mm year}^{-1}$  in cold periods and lower values of  $0.2 - 0.6 \text{ mm year}^{-1}$  in warmer periods.



**Figure 4.** Distribution of *Betula* pollen from the Hella peat profile throughout the early Holocene period approximately between 10 and 7 cal. ka BP. Left axis - Percentages of *Betula* pollen in total pollen found in the profile (grey columns) derived from the pollen analysis published in Hallsdóttir & Caseldine (2005). Right axis – Calculated proportions of *B. pubescens* pollen to total *Betula* pollen (heavy black line), proportions of pollen grains with low D/P ratios (thin black line) and non-triporate *Betula* pollen grains (grey line) in the present study.



**Figure 5.** Average calculated proportions of *Betula pubescens* pollen from the Hella peat profile based on constant sedimentation rates (black line) and varying rates (0.2 – 2.0 mm/year) of sedimentation (dashed black line) superimposed on the 20 years moving averages of  $\delta^{18}\text{O}$  values from the Greenland ice core project (grey lines) for the same period of time. Source of the climate data: Centre for Ice and Climate, Copenhagen University, <http://www.iceandclimate.nbi.ku.dk/data/>, accessed April 18<sup>th</sup>, 2008 and explained in Rasmussen *et al.* (2006) and Vinther *et al.* (2006)

## 4. DISCUSSION

### 4.1. Effect of mounting media and age of peat on pollen size

The comparison of *Betula* pollen from the Hella peat profile mounted in glycerol or silicon oil produced surprising results. We found that *Betula* pollen from samples prepared in glycerol was larger than pollen in parallel samples mounted in silicon oil by a factor of 1.2 to 1.6, depending on the profile depth (age of peat). Firstly, we expected similar differences in pollen size between samples mounted in glycerol and in silicon oil from all sample pairs. Each series of samples was treated simultaneously in a single batch so there was no reason to suspect size difference as a result of different handling of the materials. Secondly, the difference in terms of glycerol/silicon oil (G/S) ratio is clearly not random but increases with the age of the peat.

It has long been known that treatment methods and mounting media may affect pollen size (reviewed by Reitsma, 1969). Fægri and Iversen (1989) quoted G/S ratios of 1.1 – 1.3 for pollen diameter and 1.1 – 1.5 for pore size (diameter of pore opening). When we measured present-day *Betula* pollen, obtained directly from identified plants and prepared in silicon oil in exactly the same way as with samples in the present study, we found mean diameters of 20.4  $\mu\text{m}$  and 24.2  $\mu\text{m}$  for *B. nana* and *B. pubescens* respectively (Karlsdóttir et al., 2007). We therefore expected diameter means from the Hella samples in silicon oil to fall within or close to these figures, but the means have turned out to be 18.2 – 20.0  $\mu\text{m}$  for total *Betula* pollen. Caseldine (2001) reported somewhat similar results when comparing Holocene *Betula* to present-day pollen and interpreted this as higher mean diameters for present-day tree birch pollen but no change in diameter means for *B. nana*.

If present-day birch pollen was generally larger than Holocene pollen (as suggested by Caseldine, 2001), we should have found the same trend whichever mounting medium was used for the Hella samples. But our results show a decrease of pollen size with age in samples mounted in silicon oil, whereas pollen size increases with age among samples mounted in glycerol. This leaves us with a paradox which can only be solved by taking the effects of sedimentary conditions into account and assuming a connection between the effects of peat conditions and mounting media. Conditions in peat and sediments are known to influence size of pollen preserved (Prentice, 1981; Paus, 2000; Paus et al., 2003; Vorren, 2007). The Hella pollen might have shrunk according to the length of time in the soil, thus the oldest pollen being smallest when mounted in silicon oil, but it may have expanded in glycerol relative to how much shrinkage had already occurred.

## 4.2. Pollen sizes and *Betula* species

As the present data indicate that some effects of time and sedimentary conditions affect pollen size, more focus has been given to size distribution within samples rather than size differences between samples. The size distribution of pollen within samples is statistically not uniform and is best explained by assuming two or more species with different mean pollen diameters instead of one species with normally distributed pollen size. *Betula* species in the Hella profile have therefore been differentiated on basis of pollen size distribution. Since there are only two *Betula* species in Iceland, diploid *B. nana* and tetraploid *B. pubescens* (sensu lato), we expected the bulk of pollen to originate from either one or both. We did in fact find two groups of pollen, small pollen presumably belonging to *B. nana* and large pollen corresponding to *B. pubescens*. There could also be pollen of other *Betula* species in sediments and peat, coming from outside Iceland. The possibility of an occasional influx of pollen from Europe exists, similar to what happened in early May 2006 when airborne *Betula* pollen in Reykjavik exceeded average whole-season quantities (<http://www.ni.is/eldri-frettir/2006/nr/839>; <http://www.eumetsat.int/Home/Main/Media/News/005954?1=en>). Such pollen is likely to originate from both *B. pubescens* and *B. pendula*, which dominate birch forests in most of Europe. But pollen of these two tree birch species is not readily distinguishable by size (Mäkelä, 1996).

The calculated mean size of pollen in the Hella samples varied considerably between samples and therefore their species ratio was not uniform. The average ratio of *B. pubescens*/*B. nana* pollen diameter among present-day Icelandic modern birch is 1.19 while the average ratio of pore depth is 1.27 (Karlsdóttir et al., 2007). Therefore the average ratio of 1.13 for pollen diameter in the calculated groups of *Betula* pollen from Hella is lower than expected. This may have been due to some kind of systematic error associated with small sample size, such as false splitting of one normal distribution into two components. But there could be a genetic reason. If the level of introgression (i.e. genetic modification after hybridisation of the two birch species) had been accumulating over time, a decreasing ratio of upper and lower means would be expected, but no such trend could be detected. Pollen size variation in present-day *Betula* species in Iceland has been found to be positively correlated to the level of hybrid introgression measured by plant morphological variables (Karlsdóttir et al., 2007). If the introgression was a continuous process and had been going on since the early Holocene, an event such as bidirectional introgression, as shown among present-day *Betula* species in Iceland (Thórsson et al., 2001, 2007), would have reduced the gap between the two species with respect to morphological variables and pollen size. Although such a trend is not observed in the present study, the occurrence of past introgression should not be ruled out. Detection of past introgression is a challenge because the process is known to be highly complex. As shown in other plants, the species integrity can be maintained even

in the face of extensive gene flow over a prolonged period (Strasburg and Rieseberg, 2008). The frequency of hybridising species may overestimate the rate of introgression (Baack and Rieseberg, 2007), due to both internal (i.e. genomic changes) and external environments such as ecological and climatic conditions.

Climatic changes during the early Holocene are likely to have significantly influenced birch vegetation in Iceland. On the whole we would expect the proportion of *B. pubescens* and the frequency of *Betula* pollen to rise with warming climates and decline in colder periods. The data on Holocene climate from the Greenland ice core project reflects main climate trends in the North Atlantic, including Iceland (Bond et al., 1997). In particular, the cold period recorded at approximately 8.2 cal. ka BP has left its mark on most of the Northern hemisphere (von Grafenstein et al., 1998) and a cool period between 9.0 and 9.5 cal. ka BP has been recorded for some sites (Andresen and Björck, 2005; Hoek and Bos, 2007). As the profile at Hella has not been dated between the known tephra layers at top and bottom, a direct correlation of the data can not be made. Nevertheless, both the frequency of *Betula* pollen (Hallsdóttir and Caseldine, 2005) and the calculated proportion of *B. pubescens* at Hella (the present study) have some resemblance to  $\delta^{18}\text{O}$  values from the Greenland ice core for the same period of time. The association is even stronger when relevant factors such as sedimentation rates are taken into consideration. The Hella profile currently assumes constant rates of sedimentation for the whole period, but changes in deposition rates initiated by volcanic eruptions and wind-blown material due to erosion in connection to climate changes are possible. Rapid sedimentation possibly occurred in cold periods as a result of slower decomposition of plant remains, together with an increase in the aeolian component due to erosion. If rapid sedimentation is assumed in the cold periods and slow sedimentation in warmer periods, the *B. pubescens* proportion of *Betula* pollen would oscillate with the  $\delta^{18}\text{O}$  values (as shown in Fig. 5).

### 4.3. Early Holocene hybridisation

We found evidence of interspecific hybridisation in distinct periods throughout the early Holocene in Iceland. In nearly half of the samples from the Hella profile, a frequency of non-triporate type of pollen grains (especially those having abnormal number of pores) exceeded the expected levels of 0.7 – 2.6% in the present-day pollen of *B. nana* and *B. pubescens*. In some of these samples, the proportion of non-triporate pollen was about 19 – 25% of the total pollen, well exceeding the 12% mean for present-day triploid hybrids. This clearly indicates an existence of triploid hybrids in the vicinity of Hella during the periods in question, i.e. around 9.1 – 8.7 cal. ka BP. This is the first evidence of Holocene birch hybridisation in Iceland.



Fredskild (1991) noted abnormal numbers of pores in pollen samples from present-day birch in Greenland and connected the finding to species hybridisation. Such a connection may not always be obvious or noted. As an example, some quantities of small and deformed *Betula* pollen were observed in several Pleistocene samples from Lugovskoe in the central West Siberian Plain, Russia (Leshchinskiy et al., 2006). It is not clear if the pollen grains mentioned were just badly conserved in the soil or if the abnormal pollen possibly indicated Pleistocene hybridisation.

Another factor characterising pollen from present-day triploid hybrids is the low D/P ratios. In the present study, the correlation between the proportion of non-triporate pollen and the frequency of low D/P ratios in the Hella samples was generally significant, as expected. But there were severe discrepancies between these variables, especially in the upper part of the profile or in the period between 8.2 and 7.1 cal. ka BP (Fig. 3b). In the study of pollen from present-day triploid hybrids, there is a large variance between individual trees/shrubs with both of these variables. The frequencies of non-triporate pollen varied between 0 and 0.53% and the mean D/P ratio from 6.0 to 10.2 (unpublished data). If a high percentage of the pollen in each sample stems from a few trees/shrubs in the immediate vicinity of the site, the inconsistency of these two variables could be explained by individual differences. As the proportions of non-triporate pollen grains are positively correlated to the *B. pubescens* proportion of pollen, the early Holocene birch hybridisation in Iceland was likely to occur when *B. pubescens* was expanding its distribution. Pollen rain from *B. pubescens* probably reached *B. nana* habitats in an open landscape, and then the percentage of hybrids increased with the rising proportion of *B. pubescens* pollen, but declined when woodlands dominated by *B. pubescens* became densely colonized.

## Acknowledgements

The authors thank Dr Adalsteinn Sigurgeirsson from the Icelandic Forest Research and Dr Thröstur Eysteinnsson from the Iceland Forest Service, both of whom were actively involved in our research on present-day birch pollen. We also thank geologist Halldór G. Pétursson from the Icelandic Institute of Natural History, who originally drew attention to the site and assisted with the sample collection; Dr Gudrun Larsen of the Institute of Earth Sciences, University of Iceland, for advice on the dating of tephra layers; and Ms Anette Theresia Meier for her work with the site map. The project was supported by the Icelandic Research Centre (Rannís: grant no. 060266021).



## REFERENCES

- Anamthawat-Jónsson, K., Tómasson, Th., 1990. Cytogenetics of hybrid introgression in Icelandic birch. *Hereditas* 112, 65–70.
- Anamthawat-Jonsson, K., Thórsson, Æ.Th., 2003. Natural hybridisation in birch: triploid hybrids between *Betula nana* and *B. pubescens*. *Plant Cell, Tissue and Organ Culture* 75, 99–107.
- Andresen, C.S., Björck, S, 2005. Holocene climate variability in the Denmark Strait region – A land–sea correlation of new and existing climate proxy records. *Geografiska Annaler* 87, 159–174.
- Atkinson, M.D., 1992. Biological flora of the British Isles, No. 175: *Betula pendula* Roth. (*B. verrucosa* Ehrh.) and *B. pubescens* Ehrh. *Journal of Ecology* 80, 837–870.
- Ægisdóttir, H.H., Thórhallsdóttir, Th.E., 2004. Theories on migration and history of the North-Atlantic flora: a review. *Jökull* 54, 1–16.
- Baack, E.J., Rieseberg, L.H., 2007. A genomic view of introgression and hybrid speciation. *Current Opinion in Genetics & Development* 17, 513–518.
- Bennike, O., Björck, S., Böcher, J., Hansen, L., Heinemeier, J., Worlfarth, B., 1999. Early Holocene plant and animal remains from North-east Greenland. *Journal of Biogeography* 26, 667–677.
- Birks, H. J. B., 1968. The identification of *Betula nana* pollen. *New Phytologist* 67, 309–314.
- Bhattacharya, C.G., 1967. A simple method of resolution of a distribution into Gaussian components. *Biometrics* 23, 115–135.
- Bond, G., Showers, W., Cheseby, M., Lotti, R., Almasi, P., Demenocal, P., Priore, P., Cullen, H., Hajdas, I., Bonani, G., 1997. A Pervasive Millennial-Scale Cycle in North Atlantic Holocene and Glacial Climates. *Science* 275, 1233–1236.
- Caseldine, C., 2001. Changes in *Betula* in the Holocene record from Iceland – a palaeoclimatic record or evidence for early Holocene hybridisation? *Review of Palaeobotany and Palynology* 117, 139–152.
- de Groot, W.J., Thomas, P.A., Wein, R.W., 1997. *Betula nana* L and *Betula glandulosa* Michx. *Journal of Ecology* 85, 241–264.
- Elkington, T.T., 1968. Introgressive hybridisation between *Betula nana* L. and *B. pubescens* Ehrh. in North-West Iceland. *New Phytologist* 67, 109–118.
- Erlendsson, E., 2007. Environmental change around the time of the Norse settlement of Iceland. PhD Thesis, College of Physical Sciences of the University of Aberdeen.
- Fægri, K., Iversen, J., 1989. Textbook of pollen analysis, 4<sup>th</sup> edition. John Wiley & Sons, Chichester.
- Fredskild, B., 1991. The genus *Betula* in Greenland-Holocene history, present distribution and synecology. *Nordic Journal of Botany* 11, 393–412.

- Hallsdóttir, M., 1995. On the pre-settlement history of Icelandic vegetation. *Búvísindi [Icelandic Agricultural Sciences]* 9, 17-29.
- Hallsdóttir, M., Caseldine, C., 2005. The Holocene vegetation history of Iceland, state-of-the-art and future research. In: Caseldine, C., Russel, A., Hardardóttir, J., Knudsen, Ó. (Eds.), *Iceland - Modern Processes and Past Environments*. Elsevier, Amsterdam, pp. 319-334..
- Hoek, W.Z., Bos, J.A.A., 2007. Early Holocene climate oscillations - causes and consequences. *Quaternary Science Reviews* 26, 1901-1906.
- Jónsson, T.H., 2004. Stature of sub-arctic birch in relation to growth rate, lifespan and tree form. *Annals of Botany* 94, 753-762.
- Karlsdóttir, L., Thórsson, Æ.Th., Hallsdóttir, M., Sigurgeirsson, A., Eysteinnsson, Th., Anamthawat-Jónsson, K., 2007. Differentiating pollen of *Betula* species from Iceland. *Grana* 46, 78-84.
- Karlsdóttir, L., Hallsdóttir, M., Thórsson, Æ.Th., Anamthawat-Jónsson, K., 2008. Characteristics of pollen from natural triploid *Betula* hybrids. *Grana* 47, 52-59.
- Leshchinskiy, S.V., Maschenko, E.N., Ponomareva, E.A., Orlova, L.A., Burkanova, E.M., Konovalova, V.A., Teterina, I.I., Gevlya, K.M., 2006. Multidisciplinary paleontological and stratigraphic studies at Lugovskoe (2002-2004). *Archaeology, Ethnology & Anthropology of Eurasia* 25, 54-69.
- Mäkelä, E., 1996. Size distinctions between *Betula* pollen types – a review. *Grana* 35, 248-56.
- Morgan, J.H., 2005. A computer method for resolving mixed normal distributions. *Ringed & Migration* 22, 145-152.
- Pauly, D., Caddy, J.F., 1985. A modification of Bhattacharya's method for the analysis of mixtures of normal distributions. *FAO Fisheries Circular* 781. FAO, Rome.
- Paus, A.A., 2000. Interpretative problems and sources of error related to pollen-analytical studies of the Holocene on the Timan ridge, western Pechora Basin, northern Russia. *Archaeological Museum, Stavanger, Norway. AmS-Skrifter* 16, pp. 111-126.
- Paus, A.A., Svendsen, J.I., Matioushkov, A., 2003. Late weichselian (valdaian) and Holocene vegetation and environmental history of the Timan ridge, European arctic Russia. *Quaternary Science Reviews* 22, 2285-2302.
- Prentice, I.C., 1981. Quantitative birch (*Betula* L.) pollen separation by analysis of size frequency data. *New Phytologist* 89, 145-157.
- Rasmussen, S.O., Vinther, B.M., Clausen, H.B., Andersen, K.K., 2007. Early Holocene climate 10 oscillations recorded in three Greenland ice cores. *Quaternary Science Reviews* 26, 1907-1914.

- Rasmussen, S.O., Andersen, K.K., Svensson, A.M., Steffensen, J.P., Vinther, B.M., Clausen, H.B., Siggaard-Andersen, M.-L., Johnsen, S.J., Larsen, L.B., Dahl-Jensen, D., Bigler, M., Röthlisberger, R., Fischer, H., Goto-Azuma, K., Hansson, M.E., Ruth, U., 2006. A new Greenland ice core chronology for the last glacial termination. *Journal of Geophysical Research* 111, D06102, doi:10.1029/2005JD006079.
- Reitsma, T.J., 1969. Size modification of recent pollen grains under different treatments. *Review of Palaeobotany and Palynology* 9, 175-202.
- Rundgren, M., Ingolfsson O., 1999. Plant survival in Iceland during periods of glaciation? *Journal of Biogeography* 26, 387-396.
- Strasburg, J. L., Rieseberg, L. H., 2008. Molecular demographic history of the annual sunflowers *Helianthus annuus* and *H. petiolaris* – large effective population sizes and rates of long-term gene flow. *Evolution* 62, 1936-1950.
- Thórarinnsson, S., 1971. Aldur ljósu gjóskulaganna úr Heklu samkvæmt leiðréttu geislakolstímatali [The age of the light Hekla tephra layers according to corrected C<sup>14</sup> dating]. *Náttúrufræðingurinn* 41, 99-105. [In Icelandic, English abstract].
- Thórsson, Æ.Th., Salmela E., Anamthawat-Jónsson, K., 2001. Morphological, cytogenetic, and molecular evidence for introgressive hybridisation in birch. *Journal of Heredity* 92, 404-408.
- Thórsson, Æ.Th., Pálsson, S., Sigurgeirsson, A., Anamthawat-Jónsson, K., 2007. Morphological variation among *Betula nana* (diploid), *B. pubescens* (tetraploid) and their triploid hybrids in Iceland. *Annals of Botany* 99, 1183-1193.
- Vilmundardóttir, E. G., Kaldal, I., 1982. Holocene sedimentary sequence at Trjáviðarlækur basin, Þjórsárdalur, southern Iceland. *Jökull* 32, 49-59.
- Vinther, B.M., Clausen, H.B., Johnsen, S.J., Rasmussen, S.O., Andersen, K.K., Buchardt, S.L., Dahl-Jensen, D., Seierstad, I.K., Siggaard-Andersen, M.-L., Steffensen, J.P., Svensson, A.M., Olsen, J., Heinemeier, J., 2006. A synchronized dating of three Greenland ice cores throughout the Holocene. *Journal of Geophysical Research* 111, D13102, doi:10.1029/2005JD006921.
- Von Grafenstein, U., Erlenkeuser, H., Müller, J., Jouzel, J., Johnsen, S., 1998. The cold event 8200 years ago documented in oxygen isotope records of precipitation in Europe and Greenland. *Climate Dynamics* 14, 73-81.
- Vorren, K.D., Blaauw, M., Wastegård, S., Van Der Plicht, J., Jensen, C., 2007. High-resolution stratigraphy of the northernmost concentric raised bog in Europe: Sellevollmyra, Andøya, northern Norway. *Boreas* 36, 253-277.



# PAPER IV

## **Early Holocene hybridisation between *Betula pubescens* and *B. nana* in relation to birch vegetation in Southwest Iceland**

Karlsdóttir L, Hallsdóttir M, Thórrsson ÆTh and Anamthawat-Jónsson K (2012)

Review of Palaeobotany and Palynology 181: 1-10.

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# Early Holocene hybridisation between *Betula pubescens* and *B. nana* in relation to birch vegetation in Southwest Iceland

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Lilja Karlsdóttir<sup>1\*</sup>, Margrét Hallsdóttir<sup>2</sup>, Ægir Th. Thórsson<sup>1</sup>, Kesara Anamthawat-Jónsson<sup>1</sup>

<sup>1</sup>Institute of Life and Environmental Sciences, University of Iceland, Sturlugata 7, Reykjavík, IS-101 Iceland

<sup>2</sup>Icelandic Institute of Natural History, Urridaholtsstræti 6-8, Gardabær, IS-212 Iceland

## ABSTRACT

The aims of the present research were to gain better insight into the early Holocene vegetation history of *Betula* in South Iceland and to investigate whether hybridisation between downy tree-birch (*B. pubescens*) and dwarf birch (*B. nana*) could be discovered in this region, similar to that previously found in North Iceland. A peat monolith spanning the period from about 10.3 to 7.6 cal ka BP from Eyvík, Southwest Iceland, provided 44 samples for pollen analysis. The samples were dated by known tephra layers, Saksunarvatn and Seydishólar tephtras, and two <sup>14</sup>C datings. The macrofossil and spore/pollen results showed several progressive phases of vegetation development in the area until birch woodland became established around 9.5 cal ka BP. The timing of birch colonization is in good agreement with results from other sites in Iceland. From these Eyvík samples, all morphologically normal *Betula* pollen grains were size-measured and abnormal pollen grains recorded. Species proportions within samples were estimated statistically on the basis of pollen size. As birch woodland in this area became established rather late, statistical analysis of *Betula* pollen size was only possible in the uppermost 18 samples, approximately spanning the period between 7.6 and 9.5 cal ka BP. The frequency distribution of pollen size indicated populations of *B. nana* predating those of *B. pubescens*. The proportion of *B. pubescens* pollen was oscillating but increased with time. In two samples at the top of the peat monolith, more than half of the measured *Betula* pollen grains belonged to *B. pubescens*. Evidence of hybridisation

based on high frequencies of non-triporate *Betula* pollen was found in two samples dated at about 8.8 and 7.7 cal ka BP. Conditions appeared to be comparable to the corresponding hybridisation events previously reported for North Iceland, i.e. in warm periods when downy birch was expanding near dwarf birch habitats.

**Keywords:** Birch, *Betula*, Iceland, Holocene, hybridisation, pollen.

## 1. INTRODUCTION

Downy birch (*Betula pubescens* Ehrh., tetraploid with  $2n=4x=56$ ) has its main distribution in central and northern Europe (Atkinson, 1992), whereas dwarf birch (*Betula nana* L., diploid with  $2n=2x=28$ ) has a circumpolar distribution (de Groot et al., 1997). Distributions of the two species overlap in most of northern Europe and Iceland (Hultén and Fries, 1986). In the zones where the two species coexist, they cross-pollinate and produce triploid hybrids (Thórsson et al., 2001). Although the fertility of triploid hybrids is severely reduced, due to meiotic pairing abnormalities, the plants are not totally sterile and can produce viable diploid or tetraploid gametes (Ramsey and Schemske, 1998). This is the case with birch, as interpreted from crossing experiments (Anamthawat-Jónsson and Tómasson, 1990), and with birch in natural woodlands (Anamthawat-Jonsson and Thórsson, 2003). Triploids which produce viable gametes can facilitate gene flow between the parental species via back-crossing of the gametes – this is the most likely mechanism driving the bidirectional introgression observed between *B. pubescens* and *B. nana* in Iceland (Thórsson et al., 2007).

Birch is wind pollinated and produces ample quantities of pollen found in sediments and peat. The need to accurately differentiate pollen from different birch species, particularly when it is to be used for tracing different vegetation histories, past ecological and climatic requirements, has led to further research on the differences in pollen size and shape based on pollen from living plants (Mäkelä, 1996; Karlsdóttir et al., 2007). These studies showed not only that there is significant difference in the relative size of *B. pubescens* and *B. nana* pollen, but that triploid hybrids also have their own morphology that can be recognized and, more importantly, they produce large numbers of abnormal pollen grains (Karlsdóttir et al., 2008).

These results were utilized in our analysis of an early Holocene peat section from Hella in Eyjafjörður, mid-northern Iceland (Karlsdóttir et al., 2009). In the process of measuring *Betula* pollen from Hella, we found that type or age of the peat affected final pollen size. We therefore avoided comparison of pollen sizes between samples and focused on analysis of pollen size frequencies within each sample. Utilizing methods of Bhattacharaya (1967) and Járaí-Komlody (Prentice, 1981), we calculated an estimate for the proportion of each species's pollen in total *Betula* pollen within each sample and thereby obtained an overview of the ratios between birch species over time. The



counting and classification of abnormal pollen grains showed peaks of interspecific hybridisation i.e. the presence of triploid hybrids. From the study of Hella peat section, we concluded that hybridisation was likely to have occurred when *B. pubescens* was expanding its distribution in habitats where *B. nana* had predominated. These results, even though strongly suggestive, were limited to a study representative of only one geographical region. We therefore repeated the procedure in the present study with a peat section from a different region covering roughly the same period as before, or the first few thousand years of the Holocene. We aim to answer questions regarding the history of birch in the area of study, i.e. the south-western region, its colonization and the interaction of the two birch species. For the period covered by the present study, very little is known about vegetation history of this area as most studies to date are about regions in North Iceland. Of special interest to us is the question of hybridisation, and if we can see comparable indications to that in the North. Apart from the search for hybrid pollen, we compared periods of each species progression in order to find out if the timing would have been synchronous between North and South Iceland and also looked for comparable environmental factors associated with the dynamics of birch ecology and hybridisation. As far as our data allow, we try to link the birch pollen curve to known climatic variations of the period.

## 2. MATERIALS AND METHODS

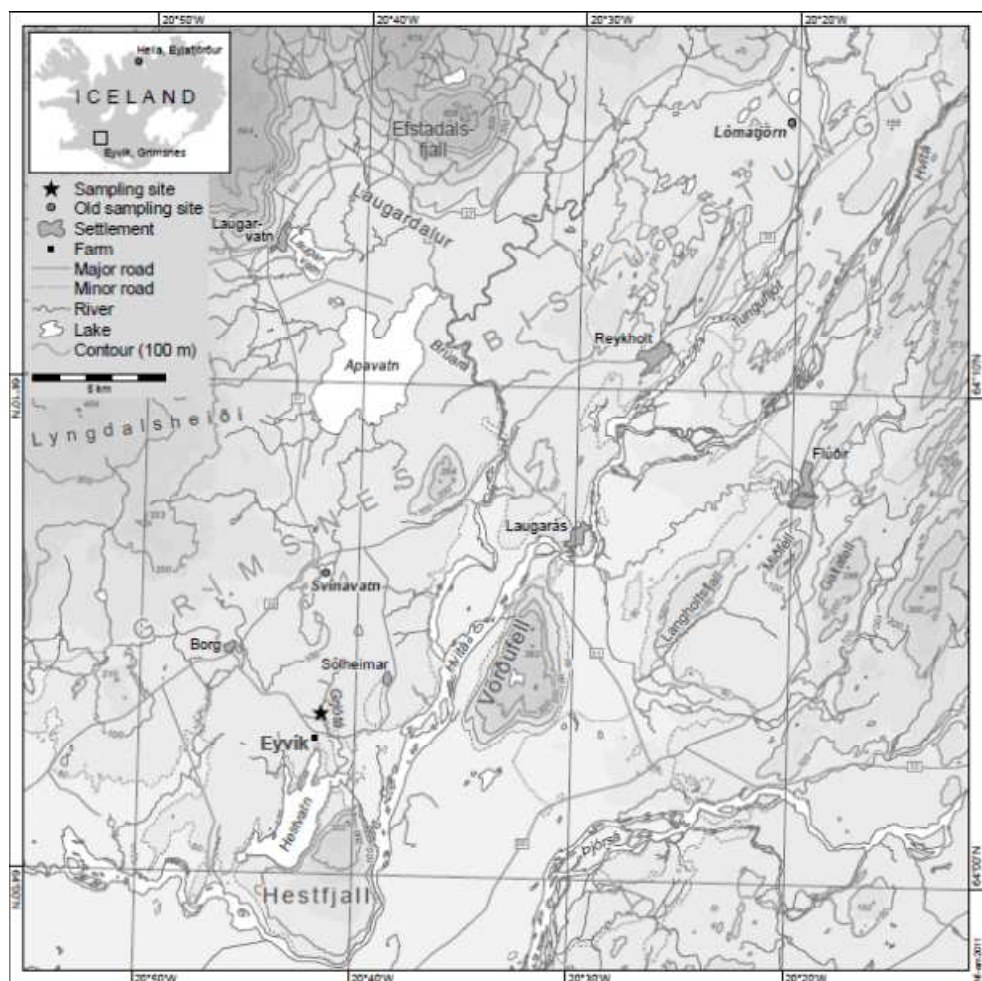
### 2.1 Location

The sampling site was a pasture near the farm Eyvík in Southwest Iceland, at 64°03.3' N, 20°41.6' W and approximately 65 m asl (Fig. 1). A drainage ditch which opens into a small river, called Grjóta, had exposed a peat section and provided easy access to the peat stratigraphy. The landscape of the area is rather flat, with the exception of the low mountains of Hestfjall (322 m) and Vörðufell (392 m) at a distance of 4 and 6 km from Eyvík respectively. The site is now surrounded by an agricultural landscape where hayfields and pasture are intersected by a network of drainage ditches. The area is practically treeless, probably as a consequence of grazing, although there are a few willow shrubs in a fenced-off area (Fig. 2). A few dwarf birch plants can be found near Grjóta river.

### 2.2 Materials

The eastern bank of the drainage ditch was cut vertically and cleaned, then peat was sampled by hammering 5×5×20 cm metal boxes into the section and cutting them free without disturbing the peat inside. The peat monolith consisted of six boxes, the

lowermost four in a single column and the two uppermost approximately 25 cm to the left of the first column, with a 12 cm vertical overlap. The unbroken section measured 108 cm, mostly consisting of peat with several bands of tephra. The thick Saksunarvatn tephra near the bottom of the peat section and the red, coarsely grained Seydishólar tephra 20 cm higher up (Fig. 2, inset), were tephra layers that were already known and dated.



**Figure 1.** Map of Iceland showing location of the sampling site at Eyvík (indicated with a star) in Grímsnes, Southwest Iceland. Source: National Land Survey of Iceland.

Forty-four samples for pollen preparation, 1 ml each, were taken from the monolith. Most of these were taken at 25 mm intervals, but there were a few exceptions due to tephra layers and the overlap of boxes. Each sample was 5 mm high and represented 5 to 25 years. Of the 44 samples, two were repetitions from the same depth (61.5 and 69 cm)

in overlapping boxes. For easier reading of the pollen diagram, 0.5 cm was subtracted and the repeated samples plotted as 61 and 68.5 cm.



**Figure 2.** Left: Overview of the sampling site, showing the ditch opening into a small river, Grjóta, in the middle of the picture. Mount Hestfjall, 322 m high, is 4 km away on the left; a low shrub of willow *Salix phylicifolia* is in the foreground. The buildings at the farm Eyvík, one km away, can be seen against the skyline above the ditches. Right: The lowest part of the peat section, with two 20 cm aluminium sample boxes. The dated tephra layers, Seyðishólar (faint reddish) and Saksunarvatn (coal black), are indicated.

## 2.3 Pollen sample preparation protocol

One tablet with *Lycopodium* spores (Lund University, Department of Quaternary Geology, Batch 124961, with  $12,542 \pm 415$  spores/tablet) was added to each sample. Samples were prepared using the conventional method of Fægri and Iversen (1989), including heating in 10% NaOH, sieving, hot HF, acetolysis and dehydration with TBA, after which they were mounted in silicon oil. The coarse peat remains on the nylon sieve were washed with distilled water and examined under a stereo microscope. Plant macrofossils were identified according to Nilsson (1952).

## 2.4 Pollen counting and analysis

Counting was performed under a Leica DM 2500 light microscope using x400 or x630 magnification. A minimum of 100 land pollen grains were counted from at least half a slide and up to ten slides for each sample. Pollen was identified to family or genus, seldom to species. The number of land pollen taxa was 35. Pteridophyta spores were

identified to class. Bryophyte spores were not differentiated except for *Sphagnum*. A pollen diagram was plotted using the program PSIMPOLL 4.27 (Bennett, 2009).

## 2.5 Radiocarbon dating

Two samples were sent to the Radiocarbon Dating Laboratory at Lund University for  $^{14}\text{C}$  dating. One of these was from the depth of 73.5 – 74 cm (sample ID: LuS 9415), which consisted of several *Menyanthes* seeds. The other sample was from the depth of 41.5 – 42 cm (LuS 9414), consisting of *Betula nana* leaves. The obtained age was based on the radiocarbon half-life of 5568 years. The  $^{14}\text{C}$  ages were corrected for the deviation from the agreed standard value of  $^{13}\text{C}/^{12}\text{C}$  ratio. Weighted mean age and standard deviation of dated samples are calculated with the Radiocarbon Calibration Program IntCal09 (Reimer et al., 2009). These two carbon-datings and two previously dated tephra layers were used for the time-depth model with linear interpolation between the four points (Table 1).

**Table 1.** Dating of the Eyvík peat monolith.

|                       | Reference              | Depth (cm) | $^{14}\text{C}$ years BP | b2k        | Cal yr BP <sup>a</sup> |
|-----------------------|------------------------|------------|--------------------------|------------|------------------------|
| LuS 9414 <sup>b</sup> | The present paper      | 41.5-42    | 7245 ± 55                |            | ~8030                  |
| LuS 9415 <sup>b</sup> | The present paper      | 73.5-74    | 8600 ± 60                |            | ~9550                  |
| Seydishólar tephra    | Sinton et al., 2005    | 114-117    | 8880 ± 50                |            | ~10040                 |
| Saksunarvatn tephra   | Rasmussen et al., 2007 | 134-130    |                          | 10347 ± 89 | ~10.297                |

<sup>a</sup>Midpoint of calibration for  $^{14}\text{C}$  years with uncertainty. Calibration with IntCal09 (Reimer et al., 2009).

<sup>b</sup>Lab number from Radiocarbon Dating Laboratory, Lunds Universitet.

## 2.6 Betula pollen measurements

Slides were scanned separately for *Betula* pollen and every *Betula* pollen grain found was digitally photographed. About 72% of the pollen images showed measurable pollen grains, while the remaining pollen was damaged, partly obscured by debris or the angle of view was not usable. The pollen was measured as in Karlsdóttir et al. (2007), either in polar view or semi-equatorial view with one measurable pore. Grain diameter (D) was defined as the distance from the outside tip of the pore to the outer margin of the facing wall, whereas pore depth (P) was the distance from the outside tip of the pore to the inner margin of the nexine through the vestibulum. Non-triporate pollen grains were noted and counted but not measured.

## 2.7 Data analysis and calculations

Analysis of size distribution of diameters and pore depths within samples was performed as described in Karlsdóttir et al. (2009) using Bhattacharya's method (Bhattacharya, 1967; Pauly and Caddy, 1985). Means and standard deviations of subsets within samples (presumably *B. nana* vs. *B. pubescens*) were calculated from three or more points when the correlation coefficient was under -0.95. The plotting was repeated with varying size-class widths between 1.0 and 2.0  $\mu\text{m}$ , and the best results were refined with the Bmod program (Morgan, 2005) using the least Kolmogorov-Smirnov D (KS-D) statistic as the measure of fit. The best-fitted proportions for each group were then calculated using Járαι-Komlódi's formula (Prentice, 1981).

## 3. RESULTS

### 3.1 Dating of samples

Dating of the Eyvík peat monolith at specific depths is shown in Table 1. The peat monolith spans 108 cm, from a depth of 140 cm up to 34 cm. Known tephra layers are Saksunarvatn, dated as 10.3 cal ka BP (Rasmussen et al., 2006, 2007), and the Seydishólar tephra from 9.4 to 10.1 cal ka BP (Sinton et al., 2005), both are shown in Fig. 2 (inset). Based on these two tephra layers, the rate of sedimentation/deposition near the bottom of the peat section was therefore ca. 0.7 mm per year. Two samples from 73 and 42 cm depth were dated by radiocarbon, samples LuS 9415 and LuS 9414 respectively (Table 1). The dating results in  $^{14}\text{C}$  years BP were converted to 9531 – 9556 and 8005 – 8065 cal BP, or 9.5 and 8.0 cal ka BP respectively (IntCal09; Reimer et al., 2009). According to this dating, the average deposition of peat was approximately 0.9 mm per year for 10.2 – 9.5 cal ka BP (depth of 131 – 73 cm) but was much slower for 9.5 – 8.0 cal ka BP (depth of 73 – 42 cm), or about 0.2 mm per year. These averages were used for the calculations of PAR values (pollen accumulation rates) of *Betula*.

### 3.2 Macrofossil samples from peat

A lithology column constructed from the Eyvík peat monolith is shown in Fig. 3. Macrofossils were examined in 44 samples extracted from the monolith.

Three samples were taken from peat underneath the Saksunarvatn tephra, at a depth of 140 – 135 cm. The samples consisted mostly of brown mosses (*Amblystegium* group) with considerable amount of decomposed material. The uppermost sample was sandy. The Saksunarvatn tephra, 5 – 7 cm thick, lay below a mixture of sand and peat.



Nine samples were taken from a depth of 129 – 112 cm. The peat consisted of mosses with rootlets of both Cyperaceae (sedges) and *Equisetum*. The red layer from Seydishólar tephra was found in this section. Little decomposition had occurred in the organic matter, and some remains of leaves and invertebrates (e.g. *Acarina*) were found. The moss leaves were identified as *Scorpidium scorpioides* (Hedw.) Limpr. at the Icelandic Institute of Natural History.

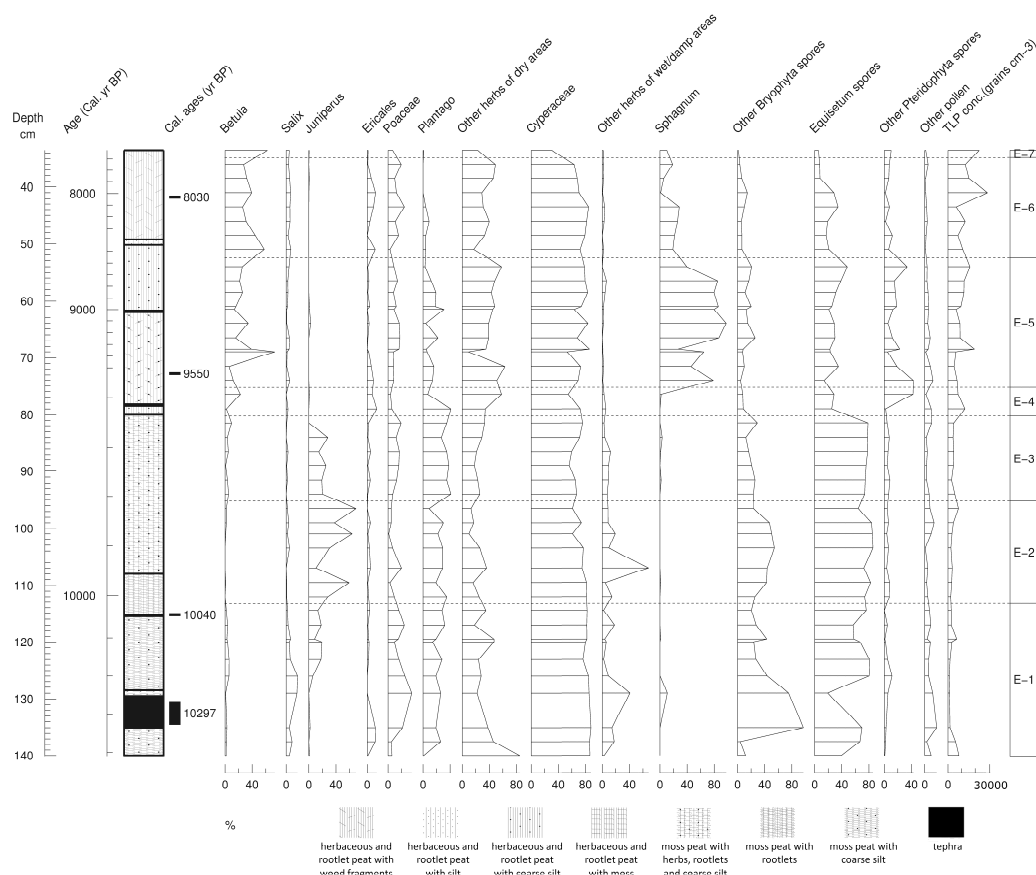
Eleven samples were taken from the depth of 110 – 80 cm. Cyperaceae and *Equisetum* rootlets were more abundant in these samples and moss leaves from some new species, including *Sphagnum*, were detected.

Fourteen samples were taken from the depth of 80 – 50 cm. The peat consisted mostly of Cyperaceae rootlets. *Equisetum* rootlets were found together with *Sphagnum* leaves. Decomposed organic matter was abundant. The samples included seeds of the wetland plant *Menyanthes* and epidermal tissue of dicotyledonous leaves.

Seven samples were taken from the depth of 50 – 34 cm. Cyperaceae rootlets were the most abundant, but remains of dicots were evident, including leaf fragments of *Betula nana*. This is the first time period in the Eyvík peat section where macrofossil remains of *Betula* could be identified.

### 3.3 Pollen analysis

Pollen concentrations were low to moderate (950-30000 land pollen grains cm<sup>-3</sup>), but increased with time up the section. Peaks in pollen concentration followed peaks of *Betula* pollen percentage ( $r = 0.64$ ;  $P < 0.0001$ ). Calculated accumulation rates of total land pollen ranged from 50 to 1100 grains cm<sup>-2</sup> yr<sup>-1</sup> with a peak at 79 cm depth, around the time when *Betula* pollen first became a considerable proportion of the pollen sum. However, at a similar depth a change in the rate of deposition was detected (see section 3.1) and this makes it difficult to determine if this peak was genuine.



**Figure 3.** Pollen and spore diagram from the Eyvík peat monolith together with a lithology column showing sediment types and the datings. Percentages are based on total land pollen (Cyperaceae and spores excluded).

The main results of the pollen/spore analysis show several changes of vegetation, both in the wetland flora at the sampling site itself and in the dry land flora of the surrounding areas. We therefore divided the Eyvík peat monolith into seven zones, based on the pollen data and fossil assemblages (Table 2; Fig. 3).

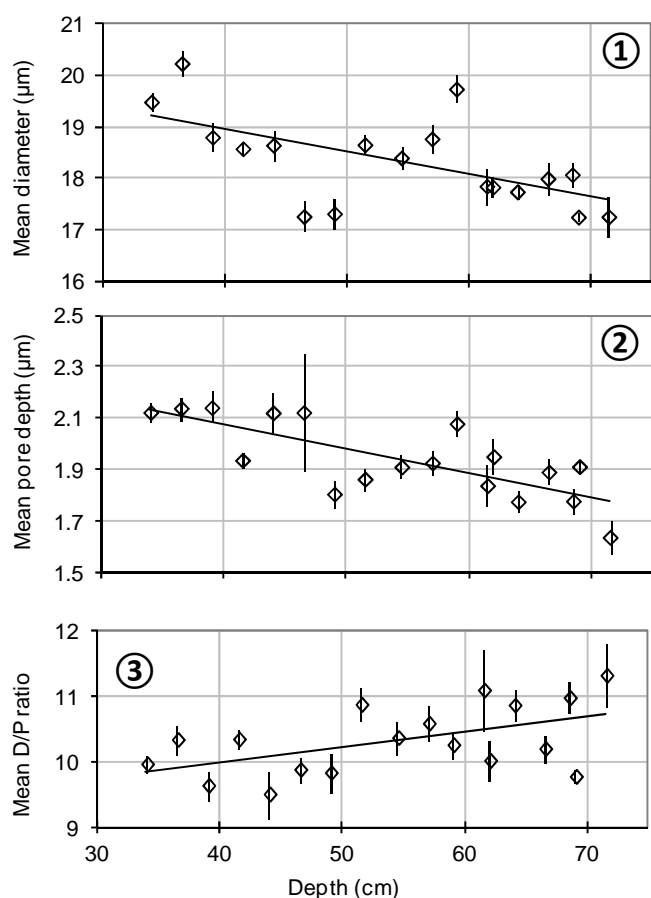
**Table 2.** Zonation of the Eyvík peat monolith

| Zone | Depth (cm) | No. of samples. | Pollen  | <i>Betula</i> PAR (grains cm <sup>-3</sup> yr <sup>-1</sup> ) | Sediment type  | Comments  |
|------|------------|-----------------|---|---|--|---|
| E-7  | 34         | 1               | <i>Betula</i> 62%, with <i>Salix</i> , <i>Galium</i> , Poaceae, <i>Thalictrum</i> and <i>Filipendula</i> .                | 302   | Herbaceous peat with fragments of leaves and branches.                 |   |
| E-6  | 36-51      | 7               | <i>Betula</i> 26 – 58% with <i>Salix</i> , Ericaceae, <i>Galium</i> , Poaceae, <i>Thalictrum</i> and <i>Filipendula</i> . | 33-241  | Herbaceous peat with fragments of leaves and branches.                 |   |
| E-5  | 54-74      | 11              | <i>Betula</i> 6-73% with Poaceae, Ericaceae, <i>Galium</i> and <i>Plantago</i> .  | 6-303   | Herbaceous and rootlet peat with moss, upper part with wood fragments. | <i>Sphagnum</i> spores abundant. Unknown tephra at 62 cm. |
| E-4  | 76.5-79    | 2               | <i>Betula</i> 2-23% with Ericaceae, <i>Galium</i> and <i>Plantago</i> .   | 23-147  | Herbaceous and rootlet peat with moss and silt.                        | Unknown tephra at 78.5 cm.                                |
| E-3  | 81.5-94    | 6               | <i>Plantago</i> most abundant, with <i>Thalictrum</i> and Poaceae. <i>Betula</i> 1-10%.                                   | 3-36  | Herbaceous and rootlet peat with moss and silt.                        |   |
| E-2  | 96.5-112   | 7               | <i>Juniperus</i> 11-75% with <i>Plantago</i> , Poaceae and <i>Thalictrum</i> . <i>Betula</i> sporadic.                    | 0-12  | Moss peat with rootlets and herbs and rootlets with moss.              |   |
| E-1  | 114.5-140  | 10              | Main pollen groups were <i>Plantago</i> , Poaceae, Rosaceae and <i>Thalictrum</i> . <i>Betula</i> 2-6%.                   | 1-9   | Moss peat, some with herbs and rootlets, sometimes with silt.          | Saksunarvatn and Seydishólar tephra inside the zone.      |

### 3.4 *Betula* pollen measurement

The amount of *Betula* pollen necessary for a statistical estimate of species was not found below 70 cm depth. In samples from the depth of 70 cm up to the top of the peat section at 34 cm depth, the number of measured pollen grains ranged from 20 to 261 per sample. From these, a small but significant change in mean sizes was discovered – the diameter (D) and pore depth (P) increased up the peat section while the diameter/pore ratio (D/P) decreased (Fig. 4). The regression slopes in  $\mu\text{m}\cdot\text{cm}^{-1}$  were D: -0.043; P: -0.008; and D/P: 0.023 ( $P_{\text{two tailed}}$  = D: 0.008; P: <0.002; D/P: 0.031). On the average *B. pubescens* pollen grains have greater diameter and lower D/P ratio than *B. nana* pollen grains so this is the trend expected for a growing proportion of *B. pubescens* pollen vs. *B. nana*.



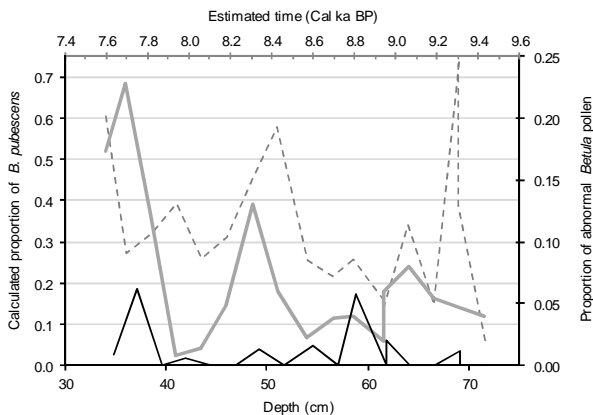


**Figure 4.** Mean size of *Betula* pollen in each of the 18 uppermost samples from the Eyvík peat monolith: 1) diameter, 2) pore depth and 3) diameter-pore depth ratio. Vertical bars represent standard deviation of the mean, whereas straight black lines show trends.

### 3.5 Calculated proportions of *Betula* species

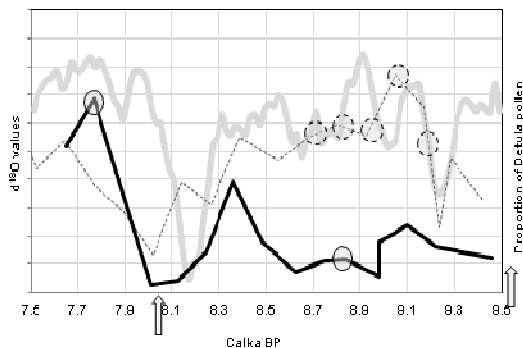
Proportions of *Betula* species were calculated from measurements of pollen diameter only, assuming two normal curves with different means within each sample, i.e. larger *B. pubescens* pollen versus smaller *B. nana* pollen. However, due to small sample sizes, the total distribution of pollen size within samples never differed significantly from normal distribution. As should be expected, a model assuming two normal curves always fitted better to the observed data than a single curve. Due to low numbers of *Betula* pollen in the present study, the likelihood ratio test in BMod (Morgan 2005) only confirmed significant better fit of the two-component model in six cases out of the 18 tested. In 11 of the 12 remaining cases, this statistic could not be computed due to lack of degrees of freedom and one case fell just short of being significant ( $P = 0.052$ ). Assuming both species were present, the results revealed a low proportion of *B.*

*pubescens* at 70 cm depth (Fig.5), which increased up the peat section with varying frequencies between 6% and 24% until a sudden peak of 39% was reached at 49 cm depth (ca. 8.3 cal ka BP). After that, the proportion dwindled to a minimum value of 2% before rising again to a maximum of 68% at 36.5 cm or around 7.7 cal ka BP (Fig. 5).



**Figure 5.** The proportion of *Betula* pollen in the uppermost part of the Eyvík peat monolith, from about 9.5 to 7.6 cal ka BP, showing calculated proportion of *Betula* pollen in total land pollen (dashed grey line, left vertical axis), calculated proportion of *B. pubescens* pollen in total *Betula* pollen (thick grey line, left vertical axis), and proportion of abnormal pollen grains in total *Betula* pollen (thin black line, right vertical axis).

The changes in *Betula* species proportions in the Eyvík section under study were similar to the pattern obtained from the Hella peat section from North Iceland (Karlsdóttir et al., 2009), and therefore correspond partly to climatic data of the time period in the early Holocene (Fig. 6). Although the timing of birch woodland expansion at both regions was similar, as reflected by the *Betula* species proportions, the downy tree-birch (*B. pubescens*) never really formed dense birch woodland in the Eyvík area during the first phase of birch colonization in ca. 9.3 – 8.3 cal ka BP (Fig. 6). The low PAR values for *Betula* in Eyvík correspond better to values from open arctic–alpine heath in Fennoscandia than to birch forests (Seppä and Hicks 2006). In both Eyvík and Hella there was a drastic recession of birch pollen around 8.2 cal ka BP, corresponding to the cold event inferred from the Greenland ice core data (Fig. 6).



**Figure 6.** The proportion of *B. pubescens* pollen in total *Betula* pollen from the Eyvík peat monolith under study (thick black line) compared to the *B. pubescens* proportion from Hella peat section, North Iceland (dashed line), previously published in Karlsdóttir et al. (2009). An indicator of temperatures (thick grey line) is from the 20 years moving averages of  $\delta^{18}O$  values in NGRIP1, NGRIP2 and GRIP from the Greenland ice core projects (Rasmussen et al., 2006; Vinther et al., 2006). Events of significant hybridisation are shown by circles. Radiocarbon-dates from the present study are indicated with arrows.

### 3.6 Abnormal *Betula* pollen

Of 1917 *Betula* pollen grains counted above the depth of 70 cm, 22 grains (slightly more than 1%) had four or more pores. The abnormal pollen grains were, however, not evenly distributed as most of them were found in only a few samples (Fig. 5). Non-triporate *Betula* pollen grains were found in 8 of the 18 uppermost samples of the Eyvík peat monolith, although in most cases only one or two grains were detected. In two samples in particular, at the depths of 59 and 36.5 cm, pollen with more than three pores comprised 6% of all *Betula* pollen in each sample, strongly indicating the presence of triploid hybrids in Eyvík at these two time points (Fig. 5). When tested using Box Plot (<http://www.physics.csbsju.edu/stats/box2.html>), the two peaks with 6.1% and 5.7% abnormal *Betula* pollen are definitely outliers, i.e. more than 1.5 interquartile range above the third quartile. In the KS-D test, the significant difference from normal distribution is over 99%. Among pollen grains from the living birch plants of the two species (tetraploid downy birch and diploid dwarf birch), only one to two percents of the pollen would be expected to have more than three pores, whereas triploid birch plants produce a significantly larger number of abnormal pollen (Karlsdóttir et al., 2008). Although this evidence of hybridisation is not as substantial as that previously found in Hella, North Iceland (Karlsdóttir et al., 2009), the timing of the earlier hybridisation event in the present study, i.e. at 59 cm depth or about 8.8 cal ka BP, coincides with the Hella data (Fig. 6).

## 4. DISCUSSION

### 4.1 The sampling site

The site was chosen to reflect the environment typical of South Iceland, in contrast to the site in North Iceland that we have previously studied (Karlsdóttir et al., 2009). The geological history and palaeoenvironment of this region during the early Holocene has been summarized by Geirsdóttir et al. (2000), who also conducted a lithological analysis of sediment cores from Lake Hestvatn. At the end of the Pleistocene and early Holocene, relative sea level changes were substantial, as reviewed by Norddahl and Pétursson (2005). In the area of our site, relative sea levels probably varied from 65 m higher than present in Younger Dryas to 40 m lower than present, before starting to rise again towards the present level. The great Thjórsárhraun lava flow, from around 8.6 cal ka BP, reached a sea level that was 15 m lower than present. Lake Hestvatn lies approximately 1.5 km south of our sampling site, Eyvík, and the lake's present surface level is 10 – 12 m lower than the sampling site. This lowland area was eroded by the Weichselian glacier, and later on great sandur plains were deposited by glacial rivers in front of the retreating glacier at the beginning of the Holocene. The maximum sea level in the area, dated around 12.1 – 11.2 cal ka BP, was 65 – 110 m higher than at present. With the glacio-isostatic uplift of the land, the shoreline retreated; in Lake Hestvatn, the change from sea- to freshwater probably happened at 10.2 – 9.6 cal ka BP (Geirsdóttir et al., 2000).

*Plantago maritima* is an herbaceous perennial plant, known to prefer coastal habitats. As we found increasing proportion of its pollen in samples from 140 – 79 cm depth (Zones E1 – E3, estimated from 10.3 to 9.6 cal ka BP) and lower proportions thereafter, we conclude that the site was not far from the shoreline at the time. This coincides perfectly with the change from marine to freshwater environment in Lake Hestvatn as estimated by Geirsdóttir et al. (2000), but indicates a somewhat slower drop in relative sea levels than Norddahl and Pétursson (2005) had assumed for South- and Southwest Iceland as a whole.

### 4.2 Time and climate

The Holocene Thermal Maximum (HTM) in terrestrial Iceland has been estimated between 10.3 and 5.6 cal ka BP based on results from six sites covered in five papers by various authors (reviewed in Kaufman et al., 2004). The peak appears to be between 8.5 and 6.5 cal ka BP in South Iceland (Geirsdóttir et al., 2009) with the summer temperatures at least 1.5 °C above the averages of the 20<sup>th</sup> century (Wastl et al., 2001; Caseldine et al., 2006). The period covered by our Eyvík peat section, ca. 10.2 – 7.7 cal

ka BP, spans more than half of the HTM but does not include the last and probably warmest part. Nevertheless, the period is typified by climatic fluctuations, the most severe being the 8.2 cal ka BP cold event which seems to have had serious effects on the newly established birch population in the area (Fig. 6). An earlier cold event seen in the Greenland ice-cores at 9.2 cal ka BP clearly had a retrogressive effect on the north-Icelandic Hella birch population, whereas the sparsely distributed birch at the southern site, Eyvík, did not seem to be affected. Relatively cool periods at 9.0 and 8.7 – 8.6 cal ka BP as seen in the NGRIP records (Rasmussen et al., 2006) affected tree-birch pollen, hence disturbing the balance between birch species both at Hella and Eyvík.

Connecting the depth of a sample with age was rather problematic. Three points in time gave us two sets of average deposition with a very large difference, i.e. 0.9 mm per year in the lower part and 0.2 mm per year above 74 cm. The reasons for this difference may include volcanic activity early in the Holocene, aeolian deposition from areas disturbed by tephra, and slow decomposition of bryophytes that were typical of the earliest part of the section. As we lack further information on the changes of deposition rates, we use the simple averages as links between time and depth.

### 4.3 The peat stratigraphy

Extended mires and wetland flora were to be expected in this region as the climate was known to be humid, and as described by Geirsdóttir et al. (2000) the area was subjected to glacial floods and catastrophic meltwater events throughout the beginning of the early Holocene, resulting in extended plains of sand and silt.

The macrofossils seen in the Eyvík peat samples revealed vegetation dynamics comprising a wetland succession from a mineral-rich fen with brown mosses to a poorer fen with sedges, *Equisetum* and *Sphagnum*, and onto a shrub-dominated fen with sedges. Before 10.3 cal ka BP, organic matter had been accumulating on wet mineral soil for some time. Since the brown moss *S. scorpioides* was prevailing at the time, high pH values and only slight oceanic effects may be presumed, as the species is known to have low tolerance to salinity (Vitt et al., 1993). The development of fen with brown mosses was reinitiated after the deposition of the Saksunarvatn tephra. Sedges and *Equisetum* gradually became more abundant while *S. scorpioides* was replaced by other brown mosses and *Sphagnum*, indicating changes in water supply towards a fen relatively poorer in minerals and richer in nitrogen and phosphorus (Gignac and Vitt, 1994; Kooijman and Bakker, 1995). A number of *B. nana* leaves found in the upper part of the Eyvík section suggests a succession towards shrub heath around 8.5 cal ka BP.

#### 4.4 The pollen record

Pollen records for the period covered by the present study exist from several Icelandic sites: Lómatjörn, the only other site in Southwest Iceland (Vasari and Vasari, 1990), Efstadalvatn in Northwest Iceland (Caseldine et al., 2003), but most of them are from North Iceland including Hafratjörn (Vasari and Vasari, 1990), Krosshólsmyri (Hallsdóttir, 1990), Vatnskotsvatn (Hallsdóttir, 1995), Torfadalsvatn (Rundgren, 1998), Vesturdalur 2 (Wasl et al., 2001), Hámundarstadaháls and Vatnamýri (Caseldine et al., 2006). There is a good correspondence in the vegetative succession revealed from these records. After the retreat of glaciers pioneer plants became established but later gave way to arctic heath with Ericales, *Salix* and *Juniperus* followed by an increase in *Betula* pollen (see review by Hallsdóttir, 1995). In all cases some *Betula* pollen was found from the earliest samples and onwards. In general, these records indicate that the earliest birch woodlands were established in the eastern part of North Iceland around 9.5 cal ka BP, somewhat later in the western part of North Iceland and in Southwest Iceland or around 8.5 cal ka BP, and even later in the north-western region. Results from the present study are congruent with the overall picture of birch vegetation in Iceland. No comparable records exist for locations from East- or Southeast Iceland.

The pollen and spore diagram from Eyvík (Fig. 3) has provided us with important background information on vegetation that is necessary to set the local history of birch in perspective. The diagram outlines main trends in both dry- and wetland flora. At about 10.2 cal ka BP, the pioneering dry land vegetation of *Salix* and Ericales gave way to *Juniperus* heath. This *Juniperus* phase continued for five hundred years until it came to an end at approximately 9.6 cal ka BP, when birch became evident. At the same time, the wetland flora changed dramatically when *Equisetum* and brown mosses gave way to *Sphagnum*. This phase continued until around 8.6 cal ka BP when shrub woodlands of both *B. pubescens* and *B. nana* had become established. Along with the birch woodlands, Ericales, Poaceae and herbs covered dry areas, while wetland flora consisted mostly of Cyperaceae. As far as can be interpreted from the limited pollen data available for South Iceland (Hallsdóttir, 1995), a similarly prolonged stage of shrub heath prevailed in the beginning when *Juniperus* and *Salix* accounted for the greater part of the heathland vegetation, with *Betula nana* appearing later on.

#### 4.5 *Betula*

Few pollen records of peat or lake sediments from South Iceland include the beginning of the Holocene. The existing pollen diagram from Lake Hestvatn (Hallsdóttir, 1995), which is situated near our sampling site or about 1.5 km south of Eyvík, only covers a vegetation history of about 7300 cal. years, or from the period above our Eyvík peat monolith. On the other hand, the lake sediment core from Lake Lómatjörn, 21 km

northeast of Eyvík, includes the period of about 9.5 – 1.9 cal ka BP and is the only one record from this region supported by radiocarbon dating (Vasari 1972; Vasari and Vasari 1990). The oldest zone of the Lómatjörn pollen diagram showed typical pioneer species and a few grains of birch pollen were found as early as 9.4 cal ka BP. Birch (*B. pubescens*) woodlands were present from about 8.2 cal ka BP.

The peat section at Eyvík reveals a similar pattern, with sporadic *Betula* pollen grains from the beginning, around 10.3 cal ka BP. An established population of *B. nana* appeared around 9.6 cal ka BP, with a low input of *B. pubescens* pollen rain, either from distant woodlands or smaller populations. Birch woodlands near Eyvík were established much later or somewhere around 8.5 cal. ka BP, possibly at higher ground near Mount Hestfjall. Around 8.3 cal ka BP, the proportion of *B. pubescens* pollen increased from around 10% to almost 40% of total *Betula* pollen. At the same time, total *Betula* pollen in total land pollen increased from around 25% to more than 50%. This could be explained by the expansion of downy birch woodlands not far from our sampling site. These first woodlands declined in the cold event of 8.2 cal ka BP but were recovering and expanding from 8.0 cal ka BP towards the upper limit of our section, at approximately 7.6 cal ka BP. Pollen data from Lake Hestvatn (Hallsdóttir, 1995) showed a continuation of birch woodland expansion in this region into the middle Holocene. This expansion persisted until falling drastically around the time of settlement in the ninth century (Hallsdóttir, 1987).

The repeated retrogressions seen in the lowland birch woodlands during the early Holocene, due to climatic and geological environment typifying southern and south-western Iceland, may have acted as genetic bottlenecks in the post-glacial birch evolution in Iceland. Our molecular and cytological study of the present-day Icelandic birch (Thórsson et al., 2010) showed an east-west separation of maternally inherited chloroplast haplotypes in both *Betula* species via introgressive hybridisation (reviewed in Anamthawat-Jónsson, 2012). Birch in western and south-western regions possesses a different (and younger) set of haplotypes compared to birch in northern and north-eastern part of Iceland. Furthermore, this western set appears to be similar to that found in North Scotland and South Greenland, whereas the eastern set is similar to that found in dense woodlands and birch forests in northern Europe and Scandinavia. At this stage of research it is not possible to explain this pattern. Western and south-western birch may have originated from a later colonization of *B. pubescens* via a different and more southern migration route across the Atlantic from Europe, or the birch simply regenerated locally through bottlenecks inferred by palynological studies including the present one. Certain haplotypes, neutral or adaptive, may be enriched during woodland regenerations, and unique or rare haplotypes may also be generated. A similar study of *Betula maximowicziana* endemic to Japan (Tsuda and Ide, 2010) revealed a north-south



phylogeographical separation of chloroplast DNA haplotypes, indicating multiple colonization events or asymmetrical gene flow, via pollen, between ancient lineages.

#### 4.6 Evidence of hybridisation

In our previous work (Karlsdóttir et al., 2008), we have shown that triploid *Betula* hybrids produce significantly high numbers of abnormal pollen, especially pollen grains having more than three pores. The elevated frequencies of non-triporate *Betula* pollen observed in the Eyvík peat section can therefore be interpreted as an indicator of hybridisation between *B. nana* and *B. pubescens*. There are two peaks in the proportion of abnormal *Betula* pollen, the first one around 8.8 cal ka BP and the second one around 7.7 cal ka BP (Fig 5). These peaks, about 6 % each, are clearly (and tested significantly) above the base line 1 – 3% of the present-day diploid *B. nana* and the tetraploid *B. pubescens* (Karlsdóttir et al., 2008). We have previously reported high peaks in the production of abnormal *Betula* pollen associated with expanding *B. pubescens* populations at Hella, North Iceland (Karlsdóttir et al., 2009). The peaks in our present Eyvík data fall into the same category; the later peak in particular is a clear example of such an event. The earlier peak of hybridisation (around 8.8 cal ka BP) occurred within the large time period of the first woodland expansion in the North (Fig. 6), but dense birch woodland had not established during that time in the region represented by the Eyvík pollen data. The concurrent timing of *B. pubescens* woodland expansion and hybridisation is to be expected. The positive correlation between elevated proportions of non-triporate pollen grains and the *B. pubescens* proportion of pollen at both Hella and Eyvík sites suggests that the early Holocene birch hybridisation most likely occurred when *B. pubescens* was expanding its distribution. Pollen rain from *B. pubescens* probably reached *B. nana* habitats in an open landscape, and then the percentage of hybrids increased with the rising proportion of *B. pubescens* pollen, but declined when woodlands dominated by *B. pubescens* became densely colonized such that there was no marginal space for the hybrids to establish themselves. Artificial crosses between *Betula* species were found to be more successful when a low-ploid species (in this case diploid *B. nana*) was the female parent and a high-ploid species (tetraploid *B. pubescens*) was the pollen donor than during reciprocal crosses (Eriksson and Jonsson, 1986). Seeds collected from open-pollinated *B. nana* plants from natural woodlands in South and Southwest Iceland were found to germinate mostly into triploid plants (Anamthawat-Jónsson and Tómasson, 1999). Newly formed birch hybrids are likely to be able to establish themselves in natural habitats, especially at the beginning of the woodland expansion. Aradóttir (1991) has shown that birch seedlings establish themselves more easily in open or disturbed soil than in a dense area of birch woodland. This could explain the formation and existence of triploid birch concurrently with birch woodland expansion during the Holocene.



Although we found significant evidence of early Holocene hybridisation in Icelandic birch from both sites studied so far (Eyvík – the south-western site in the present study and Hella – the northern site reported in Karlsdóttir et al., 2009), there is a clear difference in the extent of hybridisation during the same time periods. At the earlier peak around 8.8 cal ka BP, where the proportion of abnormal pollen grains in total *Betula* pollen is significantly higher than the relevant base line, such hybridisation is estimated to be about 6% at Eyvík, but from Hella non-triporate pollen was most prevalent (8 – 25%) over a period between approximately 9.2 and 8.7 cal. ka BP. This difference is likely due to the local environment, especially geographical and climatic. As described earlier in Section 4.1, the geological history and palaeoenvironment of this south-western region during the early Holocene is typified by glacial flood and dramatic changes of the sea level, whereas in the north and north-eastern valleys of Skagafjörður and Eyjafjörður the paleoenvironment might have been more favourable to both birch colonization and subsequent hybridisation as a result of different deglaciation patterns in that area together with early-Holocene warming in North Iceland (Norrdahl, 1991; Rundgren, 1998; Caseldine et al., 2006). The success of hybridisation in *Betula* is not only determined by the plants' genetic makeup but is also known to be dependent on several environmental factors including prevailing weather conditions and temperatures. In general, significant correlation may be found between temperatures (within a given range) and rates of growth and development in both male and female parents (reviewed in Hedhly et al., 2009). For example in *Betula*, an acceleration of pollen tube growth under high temperatures, hence greater seed-siring success, and a slowing-down under low temperatures seem to be a general phenomenon (Pasonen et al., 2002). An increase in spring thermal sum is known to initiate bud burst, hence early onset of growth, in woody plant species of northern latitudes like *Betula* (Linkosalo et al., 2010; Rousi et al., 2011). Good growth is prerequisite to the plant's fertility and fecundity. In the case of early Holocene birch in Iceland, hybridisation (intra- or interspecific) could therefore be expected to be more common at sites offering optimal climatic conditions such as those in North Iceland.

In the studies of present-day birch by Þórsson et al. (2007, 2010), both the occurrence of triploid plants (i.e. interspecific hybridisation) and the extent of introgressive hybridisation (via triploid hybrids) have the same geographical structure. Populations that are least introgressed are those woodlands experiencing cold summer, often associated with glacial sites or the interior highlands, whereas the woodlands in optimal climatic environments harbour a great deal of gene flow (reviewed in Anamthawat-Jónsson, 2012). Difference in the success of woodland establishment in the early Holocene might have been critically dependent on the extent of gene flow (i.e. introgressive hybridisation) between the two species. Birches are pioneer tree species, often rapidly colonizing opened areas, marginal and disturbed habitats such as forest clearings and heathlands with their tiny wind-blown seeds. Birches establish most

effectively on bare soils and very poorly in even the lowest vegetation (Kinnaird, 1974; Aradóttir, 1991), presumably due to the lack of affinity for any particular soil type, their ability to grow on nutrient-poor soils and their intolerance of shade. Certain characteristics of introgressed birch may be advantageous especially in the early stage of woodland establishment, for example the plant's ability to spread vegetatively by branching near or below ground, its scrub-like growth form and several other hybrid-like features. In the northern part of the Urals and Western Siberia, in the region of forest tundra-taiga, changes in leaf parameters in *B. pubescens* including shape and complexity were found to correlate with climatic conditions such as long-term average temperatures (Migalina et al., 2010) and this may have physiological advantages especially in the photosynthesis. Such morphological differentiation is likely to be driven by the introgressive hybridisation process, if the introgressant types are more adaptable (or more tolerant) to environmental pressure and habitats such as those found in Iceland and elsewhere in the subarctic regions.

## 5. CONCLUSIONS

There were no dense birch woodlands or forests near our sampling site at Eyvík in the period covered by the present study, ca. 10.3 – 7.6 cal ka BP, although both *B. nana* and *B. pubescens* were present in the vicinity from about 9.6 cal ka BP. Our previous conclusion, that the presence of abnormal *Betula* pollen grains at elevated frequencies can be used as an indicator of hybridisation periods in birch vegetation history in Iceland, is supported by the present results from a different region. The effect of palaeoclimate on the balance of *Betula* species can be seen. There is also certain conformity between the development of birch woodlands in North and South Iceland regarding the balance of birch species and the time of hybridisation events.

## 6. ACKNOWLEDGEMENTS

The authors thank Bryndís G. Róbertsdóttir, geologist at the National Energy Authority of Iceland, who guided us to the sampling site at Eyvík and assisted us with the recognition of tephra layers. We also thank Gróa Valgerður Ingimundardóttir, biologist at the Icelandic Institute of Natural History, for help with identification of moss remains, and Ólafur Eggertsson, geologist at the Iceland Forest Research at Mógilsá, for advice regarding the radiocarbon dating. This project was supported by the Icelandic Research Centre (Rannís grant no. 060266021-23) and the University of Iceland (Doctoral grant 2010 – 2012).

## 7. REFERENCES

- Anamthawat-Jónsson, K., 2012. Hybridisation, introgression and phylogeography of Icelandic birch. In: Anamthawat-Jónsson, K. (Ed.), *Current Topics in Phylogenetics and Phylogeography of Terrestrial and Aquatic Systems*, pp. 117–144. InTech – Open Access Publisher, Croatia. ISBN 978-953-51-0217-5.
- Anamthawat-Jonsson, K., Thórsson, Æ.Th., 2003. Natural hybridisation in birch: triploid hybrids between *Betula nana* and *B. pubescens*. *Plant Cell, Tissue and Organ Culture* 75, 99–107.
- Anamthawat-Jónsson, K., Tómasson, Th., 1990. Cytogenetics of hybrid introgression in Icelandic birch. *Hereditas* 112, 65–70.
- Anamthawat-Jónsson, K., Tómasson, T., 1999. High frequency of triploid birch hybrid by *Betula nana* seed parent. *Hereditas* 130, 191–193.
- Aradóttir, Á.L., 1991. Population biology and stand development of birch (*Betula pubescens* Ehrh.) on disturbed sites in Iceland. PhD Dissertation. Texas A&M University, College Station.
- Atkinson, M.D., 1992. Biological flora of the British Isles, No. 175: *Betula pendula* Roth. (*B. verrucosa* Ehrh.) and *B. pubescens* Ehrh. *Journal of Ecology* 80, 837–870.
- Bennett, K.D., 2009. Psimpoll 4.27: C program for plotting pollen diagrams and analysing pollen data. Available online from Queen's University of Belfast, Department of Archaeology and Palaeoecology. Last accessed 6 December 2010, <http://www.chrono.qub.ac.uk/psimpoll/psimpoll.html>
- Bhattacharya, C.G., 1967. A simple method of resolution of a distribution into Gaussian components. *Biometrics* 23, 115–135.
- Caseldine, C., Geirsdóttir, Á., Langdon, P., 2003. Efstadalsvatn — a multi-proxy study of a Holocene lacustrine sequence from NW Iceland. *Journal of Paleolimnology* 30, 55–73.
- Caseldine, C.J., Langdon, P.G., Holmes, N., 2006. Early Holocene climate variability and the timing and extent of the Holocene thermal maximum (HTM) in northern Iceland. *Quaternary Science Reviews* 25, 2314–2331.
- de Groot, W.J., Thomas, P.A., Wein, R.W., 1997. *Betula nana* L and *Betula glandulosa* Michx. *Journal of Ecology* 85, 241–264.
- Eriksson, G., Jonsson, A., 1986. A review of the genetics of *Betula*. *Scandinavian Journal of Forest Research* 1, 421–434.
- Fægri, K., Iversen, J., 1989. Textbook of pollen analysis, 4th edn. John Wiley & Sons, Chichester.

- Geirsdóttir, Á., Hardardóttir, J., Sveinbjörndóttir, Á.E., 2000. Glacial extent and catastrophic meltwater events during the deglaciation of Southern Iceland. *Quaternary Science Reviews* 19, 1749–1761.
- Geirsdóttir, Á., Miller, G.H., Axford, Y., Ólafsdóttir, S., 2009. Holocene and latest Pleistocene climate and glacier fluctuations in Iceland. *Quaternary Science Reviews* 28, 2107–2118.
- Gignac, L.D., Vitt, D.H., 1994. Responses of northern peatlands to climate change: Effects on bryophytes. *Journal of the Hattori Botanical Laboratory* 75, 119–132.
- Hallsdóttir, M., 1987. Pollen analytical studies of human influence on vegetation in relation to the Landnám Tephra layer in Southwest Iceland. LUNDQUA thesis 18, Lund University, Department of Quaternary Geology: 46 pp.
- Hallsdóttir, M., 1990. Studies in the vegetational history of north Iceland, a radiocarbon-dated pollen diagram from Flateyjarðalur. *Jökull* 40, 67–81.
- Hallsdóttir, M., 1995. On the pre-settlement history of Icelandic vegetation. *Icelandic Agricultural Sciences* 9, 17–29.
- Hedhly, A., Hormaza, J.I., Herrero, M., 2009. Global warming and sexual plant reproduction. *Trends in Plant Science* 14, 30–36.
- Hultén, E., Fries, M., 1986. *Atlas of North European Vascular Plants*. Köningstein: Koeltz Scientific Books.
- Karlsdóttir, L., Thórsson, Æ.Th., Hallsdóttir, M., Sigurgeirsson, A., Eysteinnsson, Th., Anamthawat-Jónsson, K., 2007. Differentiating pollen of *Betula* species from Iceland. *Grana* 46, 78–84.
- Karlsdóttir, L., Hallsdóttir, M., Thórsson, Æ.Th., Anamthawat-Jónsson, K., 2008. Characteristics of pollen from natural triploid *Betula* hybrids. *Grana* 47, 52–59.
- Karlsdóttir, L., Hallsdóttir, M., Thórsson, Æ.Th., Anamthawat-Jónsson, K., 2009. Evidence of hybridisation between *Betula pubescens* and *B. nana* in Iceland during the early Holocene. *Review of Palaeobotany and Palynology* 156, 350–357.
- Kaufman, D.S., Ager, T.A., Anderson, N.J., Anderson, P.M., Andrews, J.T., Bartlein, P.J., Brubaker, L.B., Coats, L.L., Cwynar, L.C., Duvall, M.L., Dyke, A.S., Edwards, M.E., Eisner, W.R., Gajewski, K., Geirsdóttir, A., Hu, F.S., Jennings, A.E., Kaplan, M.R., Kerwin, M.W., Lozhkin, A.V., MacDonald, G.M., Miller, G.H., Mock, C.J., Oswald, W.W., Otto-Bliesner, B.L., Porinchu, D.F., Ruüland, K., Smol, J.P., Steig, E. J., Wolfe, B.B., 2004. Holocene thermal maximum in the western Arctic (0–180°W). *Quaternary Science Reviews* 23, 529–560.
- Kinnaird, J.W., 1974. Effects of site conditions on the regeneration of birch (*Betula pendula* Roth and *B. pubescens* Ehrh.). *Journal of Ecology* 62, 467–473.

- Kooijman, A.M., Bakker, C., 1995. Species replacement in the bryophyte layer in mires: The role of water type, nutrient supply and interspecific interactions. *Journal of Ecology*, 83, 1-8.
- Linkosalo, T., Ranta, H., Oksanen, A., Siljamo, P., Luomajoki, A., Kukkonen, J., Sofiev, M., 2010. A double-threshold temperature sum model for predicting the flowering duration and relative intensity of *Betula pendula* and *B. pubescens*. *Agricultural and Forest Meteorology* 150, 1579-1584.
- Mäkelä, E., 1996. Size distinctions between *Betula* pollen types — a review. *Grana* 35, 248–256.
- Migalina, S.V., Ivanova, L.A., Makhnev, A.K., 2010. Changes of leaf morphology in *Betula pendula* Roth and *B. pubescens* Ehrh. along a zonal-climatic transect in the Urals and Western Siberia. *Russian Journal of Ecology* 41, 293-301.
- Morgan, J.H., 2005. A computer method for resolving mixed normal distributions. *Ringed & Migration* 22, 145–152.
- Nilsson, T., 1952. Kvartärpaleontologi och Kvartärpaleontologiska undersökningsmetoder. Lunds Universitet, 2 volumes, 238 pp. + 63 Plates.
- Norddahl, H., 1991. A review of the glaciation maximum concept and the deglaciation of Eyjafjörður, North Iceland. In: Maizels, J.K., Caseldine, C. (Eds.), *Environmental change in Iceland: past and present*, pp. 31-47. Kluwer Academic Publishers, Dordrecht.
- Norddahl, H., Pétursson, H.G., 2005. Relative sea-level changes in Iceland; new aspects of the Weichselian deglaciation of Iceland. In: Caseldine, C., Russel, A., Hardardottir, J., Knudsen, O. (Eds.), *Iceland – Modern processes and past environments*, pp. 25-78. Elsevier, Amsterdam.
- Pasonen, H.L., Pulkkinen, P., Kärkkäinen, K., 2002. Genotype-environment interactions in pollen competitive ability in an anemophilous tree, *Betula pendula* Roth. *Theoretical and Applied Genetics* 105, 465-473.
- Pauly, D., Caddy, J.F., 1985. A modification of Bhattacharya's method for the analysis of mixtures of normal distributions. *FAO Fisheries Circular* 781. FAO, Rome.
- Prentice, I.C., 1981. Quantitative birch (*Betula* L.) pollen separation by analysis of size frequency data. *New Phytologist* 89, 145–157.
- Ramsey, J., Schemske, D.W., 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* 29, 467-501.
- Rasmussen, S.O., Andersen, K.K., Svensson, A.M., Steffensen, J.P., Vinther, B.M., Clausen, H.B., Siggaard-Andersen, M.-L., Johnsen, S.J., Larsen, L.B., Dahl-Jensen,

- D., Bigler, M., Röthlisberger, R., Fischer, H., Goto-Azuma, K., Hansson, M.E. & Ruth, U., 2006. A new Greenland ice core chronology for the last glacial termination. *Journal of Geophysical Research*, 111, D06102, doi:10.1029/2005JD006079.
- Rasmussen, S.O., Vinther, B.M., Clausen, H.B. & Andersen, K.K., 2007. Early Holocene climate 10 oscillations recorded in three Greenland ice cores. *Quaternary Science Reviews* 26, 1907–1914.
- Reimer, P.J., Baillie, M.G.L., Bard, E., Bayliss, A., Beck, J.W., Blackwell, P.G., Bronk Ramsey, C., Buck, C.E., Burr, G.S., Edwards, R.L., Friedrich, M., Grootes, P.M., Guilderson, T.P., Hajdas, I., Heaton, T.J., Hogg, A.G., Hughen, K.A., Kaiser, K.F., Kromer, B., McCormac, F.G., Manning, S.W., Reimer, R.W., Richards, D.A., Southon, J.R., Talamo, S., Turney, C.S.M., van der Plicht, J. and Weyhenmeyer, C.E., 2009. INTCAL 09 and MARINE09 radiocarbon age calibration curves, 0–50,000 years Cal BP. *Radiocarbon* 51, 1111–1150. ISSN 0033-8222.
- Rousi, M., Heinonen, J., Neuvonen, S., 2011. Intrapopulation variation in flowering phenology and fecundity of silver birch, implications for adaptability to changing climate. *Forest Ecology and Management* 262, 2378–2385.
- Rundgren, M., 1998. Early-Holocene vegetation of northern Iceland: pollen and plant macrofossil evidence from the Skagi peninsula. *The Holocene* 8, 553–564.
- Seppä, H., Hicks, S., 2006. Integration of modern and past pollen accumulation rate (PAR) records across the arctic tree line: a method for more precise vegetation reconstructions. *Quaternary Science Reviews* 25, 1501–1516.
- Sinton, J.M., Grönvold, K., Sæmundsson, K., 2005. Postglacial eruptive history of the Western Volcanic Zone, Iceland, *Geochemistry, Geophysics, Geosystems* (G3). Q12009 10.1029/2005GC001021.
- Thórsson, Æ.Th., Salmela, E., Anamthawat-Jónsson, K., 2001. Morphological, cytogenetic, and molecular evidence for introgressive hybridisation in birch. *Journal of Heredity* 92, 404–408.
- Thórsson, Æ.Th., Pálsson, S., Lascoux, M., Anamthawat-Jónsson, K., 2010. Introgression and phylogeography of *Betula nana* (diploid), *B. pubescens* (tetraploid) and their triploid hybrids in Iceland inferred from cp-DNA haplotype variation. *Journal of Biogeography* 37, 2098–2110.
- Thórsson, Æ.Th., Pálsson, S., Sigurgeirsson, A., Anamthawat-Jónsson, K., 2007. Morphological variation among *Betula nana* (diploid), *B. pubescens* (tetraploid) and their triploid hybrids in Iceland. *Annals of Botany* 99, 1183–1193.
- Tsuda, Y., Ide, Y., 2010. Chloroplast DNA phylogeography of *Beutla maximowicziana*, a long-lived pioneering tree species and noble hardwood in Japan. *Journal of Plant Research* 123, 343–353.

- Vasari, Y., 1972. The history of the vegetation in Iceland during the Holocene. In Vasari, Y., Hyvärinen, H. and Hicks, S. (eds.) Climatic changes in Arctic areas during the last ten-thousand years, 239-252. University of Oulu. Oulu.
- Vasari, Y., Vasari, A., 1990. L'histoire Holocène des lacs Islandais. In Jean Malaurie (ed.) 102 témoignages en hommage á quarante ans d'études arctiques, 279-293. Éditions Plon. Paris (in French).
- Vitt, D.H., Van Wirdum, G., Halsey, L., Zoltai, S., 1993. The Effects of Water Chemistry on the Growth of *Scorpidium scorpioides* in Canada and The Netherlands. The Bryologist 96, 106-111.
- Vinther, B.M., Clausen, H.B., Johnsen, S.J., Rasmussen, S.O., Andersen, K.K., Buchardt, S.L., Dahl-Jensen, D., Seierstad, I.K., Siggaard-Andersen, M.-L., Steffensen, J.P., Svensson, A., Olsen, J., Heinemeier, J. 2006. A synchronized dating of three Greenland ice cores throughout the Holocene. Journal of Geophysical Research 111, D13102, doi:10.1029/2005JD006921.
- Wastl, M., Stötter, J., Caseldine, C., 2001. Reconstruction of Holocene variations of the upper limit of tree or shrub birch growth in northern Iceland based on evidence from Vesturárdalur-Skiðadalur, Tröllaskagi. Arctic, Antarctic, and Alpine Research 33, 191–203.





# PAPER V

## **Birch hybridization in Thistilfjörður, Northeast Iceland during the Holocene**

Karlsdóttir L, Hallsdóttir M, Eggertsson Ó, Thórsson ÆTh and Anamthawat-Jónsson K.

The paper has been submitted to a peer-reviewed journal.

*This is an Author's Revised Manuscript of an article considered for publication in Icelandic Agricultural Sciences (IAS)  
<http://www.ias.is/landbunadur/wgsamvef.nsf/key2/ias>  
Changes may have been made to this work since it was submitted for publication.*



# Birch hybridization in Thistilfjörður, Northeast Iceland during the Holocene

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Lilja Karlsdóttir<sup>1</sup>, Margrét Hallsdóttir<sup>2</sup>, Ólafur Eggertsson<sup>3</sup>, Ægir Th. Thórsson<sup>1,4</sup> and Kesara Anamthawat-Jónsson<sup>1</sup>

<sup>1</sup> Institute of Life and Environmental Sciences, University of Iceland, Sturlugata 7, IS-101 Reykjavík, Iceland, liljaka@hi.is (corresponding author\*), kesara@hi.is

<sup>2</sup> Icelandic Institute of Natural History, Urridaholtsstræti 6–8, Gardabær, IS-212 Iceland, m.hallsd@gmail.com

<sup>3</sup> Icelandic Forest Research, Mógilsá, IS-116 Reykjavík, Iceland, olie@skogur.is

<sup>4</sup> The Farmers Association of Iceland, Bændahöllinni by Hagatorg, IS-107 Reykjavík, Iceland, aegir@bondi.is

## Abstract

Past episodes of birch hybridization in Iceland were studied by pollen analysis. The low stature and polycormic form of downy birch *B. pubescens* in the subarctic probably results from such hybridization, with the dwarf birch *B. nana*. Two previous studies in different regions in Iceland revealed events of hybridization connected to early *B. pubescens* expansion. The present study examined a peat monolith from Thistilfjörður, Northeast Iceland, covering the last ten thousand years. *Betula* pollen was measured, proportions of *B. nana* and *B. pubescens* calculated and the presence of hybrids estimated. *Betula pubescens* expansion started around 7.2 cal ka BP with a peak in non-triporate pollen, indicating hybrids. Low *Betula* pollen concentrations followed. A second period of considerable hybridization is indicated around 3.3 cal ka BP. Both peaks were associated with *B. pubescens* expansion.

## Yfirlit

Tegundablöndun ilmbjarkar og fjalldrapa var rannsökuð í frjókornum. Lágar og margstofna ilmbjarkir á norðlægum slóðum eru líklega afleiðing slíkrar blöndunar. Tvær

eldri rannsóknir frá mismunandi landshlutum sýndu hrinur blöndunar snemma á Nútíma sem tengdust framrás ilmbjarkar. Í þessari rannsókn voru sýni tekin úr mósniði í Þistilfirði, sem spannar síðustu tíu þúsund ár. Birkifrjókorn voru mæld, hlutföll tegundanna reiknuð og leitað ummerkja um blöndunina. Ilmbjörk tók að breiðast út fyrir um 7200 árum og fram kom toppur afbrigðilegra frjókorna sem gefa til kynna fjölda blendingstrjáa. Tímabil með litlu birki fylgdi í kjölfarið en önnur hrina erfðablöndunar varð fyrir um 3300 árum. Báðar hrinurnar tengdust aukinni útbreiðslu ilmbjarkar.

Keywords: *Betula nana*, *Betula pubescens*, birch hybrid, Holocene climate, Iceland, pollen

## Introduction

Downy birch (*Betula pubescens* Ehrh.) is the most important native tree in Iceland as it is the only tree species forming continuous natural woodlands. Birch woodland is an integral component of the subarctic ecosystem. In Iceland as well as in the higher parts of northern Scandinavia, downy birch, which is often referred to as mountain birch, has lower stature and more frequently displays polycormic growth than in most other parts of Europe. This may be the result of introgressive hybridization with dwarf birch (*B. nana* L.), facilitated by the downy birch's adaption to harsh climatic conditions. Theories attributing the growth form of the subarctic birch to introgressive hybridization began to evolve in the mid-twentieth century (Elkington 1968) and were confirmed around the turn of the century (Anamthawat-Jónsson & Thórrsson 2003). However, the extent of the hybridization, its frequency through time and circumstances promoting it were still unclear. It is therefore our objective to investigate past hybridization events in Iceland through the Holocene period.

For several years now, we have been studying birch hybridization and introgression, both past and present. This paper is the third in a series on past hybridization in Iceland based on subfossil pollen records spanning the period from the early Holocene to the present. In our studies, we relied on the previously established fact that hybrids of the two *Betula* species present in Iceland, downy birch and dwarf birch, produce a considerable quantity of easily recognisable abnormal pollen (Karlsdóttir et al. 2008). As the dwarf birch *Betula nana* is diploid with chromosome number  $2n=2x=28$  and the downy birch *B. pubescens* is tetraploid with  $2n=4x=56$ , their hybrids are triploid with  $2n=3x=42$  (Anamthawat-Jónsson & Tómasson 1990). Triploid plants are viable although their fertility is drastically reduced as meiosis cannot proceed in the normal fashion. A considerable proportion of the gametes will be aneuploid (Ramsey & Schemske 1998) and may suffer from genetic imbalance (Birchler & Veitia 2007) or from disturbances in gene expression during the process of microsporogenesis and

pollen development (McCormick 1993). The consequences may include morphological variation in the pollen produced by triploids, the most common anomaly being four or more pores instead of the normal three (non-triporate vs. triporate). Triploids in natural woodlands in Iceland were found to be relatively common (Thórsson et al. 2007). In a further study (Karlsdóttir et al. 2008), pollen from several triploid trees/shrubs was compared to pollen from diploid and tetraploid individuals and the results revealed high frequency of non-triporate pollen from the triploids, around 12% compared with one or two per cent among tetraploid and diploid individuals.

The frequency of abnormal birch pollen in relation to hybridization had been noted before without being studied further. Fredskild (1991) studied birch in Greenland, especially downy birch (*B. pubescens*) and the local dwarf birch (*B. glandulosa* Michx.), which is a relative of *B. nana* and is also diploid. He compared pollen retrieved from several individuals of each species and their hybrids, identified by morphology. The results showed non-triporate pollen, around 7% within species and 13% from hybrids. Since then it has been shown that triploid birch hybrids cannot be identified by plant morphology alone (Thórsson et al. 2007) and this probably explains the difference in the results from Greenland and Iceland. From our results (Karlsdóttir et al. 2008), it is clear that significant occurrence of non-triporate pollen indicates the presence of triploid birch hybrids.

We have published findings of periods of intensive hybridization in the vegetation succession following the glacial retreat of the Weichselian ice sheet in North Iceland (Karlsdóttir et al. 2009) and Southwest Iceland (Karlsdóttir et al. 2012). These hybridization periods were attributed to climate warming, when *B. pubescens* expanded over areas previously covered with heath, including *B. nana*. To complete the picture of birch hybridization in Iceland during the Holocene a further study was needed. The most urgent question was if the hybridization was limited to the original establishment of tree birch in Iceland or occurred also in the latter half of the Holocene. The interpretation of the current frequency of triploid hybrids in present-day woodlands (Thórsson et al. 2007) depends on the answer. A study from a third region within Iceland was also needed to confirm that the trends observed are general and not reflecting local conditions. Based on the established picture of Holocene vegetation development in Iceland (e.g. Hallsdóttir and Caseldine 2005), a progression of birch woodland establishment occurred first in the northern valleys and inner fjords, but in South Iceland the development was delayed. Very little is known about palaeovegetation in the eastern and north-eastern part of Iceland. Therefore, we decided to investigate vegetation history in Thistilfjörður, and aimed for a peat monolith covering the entire Holocene history of birch. This was necessary, not only to gather new information about birch woodland/forest establishment and development in this part of Iceland, but also to gain a better insight into changes in birch species composition, and possible hybridization

through time. Our previous studies provided us with the methods to differentiate *Betula* pollen at the species level and to recognize the presence of hybrids (Karlsdóttir et al. 2007, 2008) and based on these, we were able to calculate the proportions of *B. nana* and *B. pubescens* in total *Betula* pollen in palynological samples (Karlsdóttir et al. 2009, 2012).

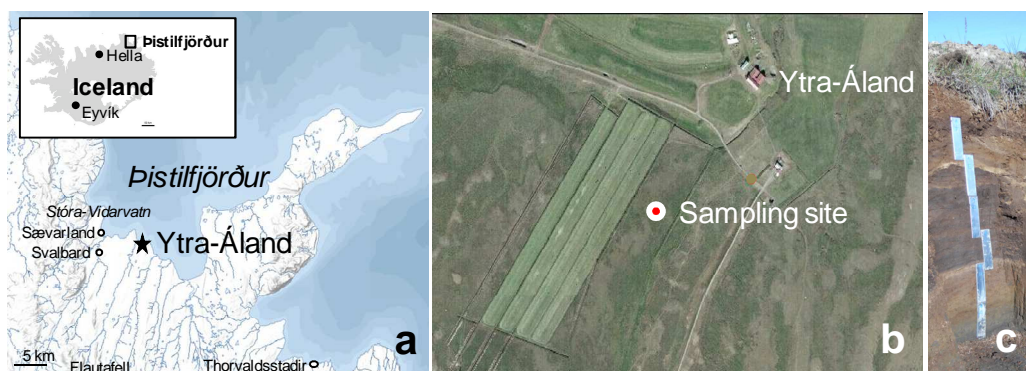
## Materials and methods

### Study area

Peat samples were taken from a draining ditch in a pasture at the farm Ytra-Áland in Thistilfjörður, Northeast Iceland (Figure 1), at the coordinates of 66.2076 °N/15.556 °W and the altitude of 34 m. Coastal areas in Thistilfjörður were deglaciated and transgressed by sea in early Bölling and shorelines, presumably formed at that time, are now at an altitude of around 65 m (Norddahl & Pétursson 2005). The Younger Dryas glaciation covered the area again around 12 cal ka, but lowland areas became ice-free in the Preboreal around 11.2 cal ka (Norddahl et al. 2008).

The area of Thistilfjörður was settled by Ketill Thistill during the Icelandic period of settlement 874-930 AD. The earliest written source of information about the farm Áland is a document from the year 1378 (Óbyggðanefnd 2005).

The site is approximately 600 m from the shoreline and is surrounded by sparsely vegetated hills and pasture land. The nearest hill is 92 m a.s.l. but higher mountains are to the west; the nearest, Flautafell, is approximately 13 km from the site. The weather station at Thorvaldsstadir, located 30 km southeast of Ytra-Áland, shows a 30 year (1961-1990) annual mean temperature of 2.8°C, a mean July temperature of 8.5°C, an average maximum July temperature of 11.2 °C and a four month average maximum temperature in June-September of 10.1°C.



**Figure 1.** Sampling site. a) Map, sample site indicated by star. b) Aerial photo showing farmhouses and draining ditches. c) Photo showing sampling in process.

## Sampling and stratigraphic description

A section in the northern bank of the drainage ditch was cut and scraped clean. Samples were taken by hammering 5×5×20 cm metal boxes into the peat and cutting them free without disturbing their contents. The monolith consisted of six boxes, more or less in a single column but the third and sixth boxes were shifted a few centimetres to one side horizontally with a vertical overlap to adjacent boxes. This was necessary to obtain undisturbed peat (Figure 1c). The unbroken monolith measured 108 cm, spanning the depth interval 17-125 cm, mostly consisting of peat with several bands of tephra. At the bottom of the monolith, the Saksunarvatn tephra (Gudrún Larsen, personal communication) was 17-20 cm thick and underlying it was a light coloured silted soil, which was sampled separately.

Loss on ignition (LOI) was tested to acquire an estimate on the proportion of soil organic matter. Parallel to each pollen sample, approximately 3 g of (wet) peat was sampled for this purpose. The samples were dried overnight at 110°C and weighed to the nearest mg. They were then heated to 550°C for 4 hours, cooled down to 110°C and weighing repeated.

The stratigraphy of the section was described based on field observation, peat samples in boxes and sieve residues with regard to the Troel-Smith (1955) system as described by Faegri and Iversen (1989) and Kershaw (1997).

## Chronology

Ten distinct tephtras were noted in the peat monolith and more were suspected. Five previously dated tephtras, recognized in the sampled peat section, were used for the age-depth model. Four samples were extracted from the peat and sent for AMS radiocarbon dating to Lund University Radiocarbon Dating Laboratory. From the upper half of the monolith, twigs and bark were used. No good material for dating was found in the lower half, so *Carex* rootlets were used and this produced inadequate results. Carbon dates of the rootlets were lower than from the samples above, presumably because roots had grown down through the peat into the samples. Therefore, only two radiocarbon datings, i.e. at depths of 43 and 70 cm, were used for the chronology (Table 1). Radiocarbon ages were calibrated using the Intcal09 calibration dataset (Reimer et al. 2009) Age model was based on linear interpolation between mid points of calibrated age ranges.

Five tephra layers and two radiocarbon datings were used for the chronology of the Ytra-Áland peat section (Table 1). On average, each pollen sample represented approximately 50 years, varying from 25 to 74 years. Mean peat deposition rate was 0.11 mm year<sup>-1</sup>, varying from 0.07 to 0.20 mm year<sup>-1</sup>.

Throughout the paper cal ka and cal years refer to calibrated time before present (BP), “present” meaning 1950 AD.

**Table 1.** Chronological data for the Ytra-Áland peat sequence.

**Tephra:**

| Depth (cm) | Name             | Cal yr BP | Source                  |
|------------|------------------|-----------|-------------------------|
| 22.5-22.7  | Veidivötn 1477AD | 473       | Larsen, (1984)          |
| 39.0-40.5  | Hekla 3          | ~3000     | Dugmore et al., (1995). |
| 57.0-58.0  | Hekla 4          | ~4200     | Dugmore et al., (1995)  |
| 85.0-86.0  | Hekla 5          | ~7050     | Thorarinsson (1971)     |
| 123-139 cm | Saksunarvatn     | ~10300    | Rasmussen et al. (2007) |

**<sup>14</sup>C datings:**

| Depth (cm) | Lab no    | Code   | Material      | Dry weight (mg) | <sup>14</sup> C age ± 1σ ( <sup>14</sup> C yr BP) | Calibrated age interval (cal yr BP) |
|------------|-----------|--------|---------------|-----------------|---|-------------------------------------|
| 43         | LuS 10601 | YA-43  | twig and bark | 4               | 3270 ± 50   | 3445-3560                           |
| 70         | LuS 10602 | YA-70  | twig and bark | 12              | 5120 ± 50   | 5885-5920                           |
| 89*        | LuS 10603 | YA-89  | rootlets      | 8               | 4555 ± 55   | not used                            |
| 104*       | LuS 10604 | YA-104 | rootlets      | 5               | 4515 ± 50   | not used                            |

\* These samples consisted of *Carex* roots which probably have grown deep down through the peat.

## Pollen preparation

From the monolith, 47 samples were taken for pollen preparation. Forty-six were from the peat in the boxes and one sample from the soil underlying the Saksunarvatn tephra. All samples were 1 ml, 5 mm thick and taken at approximately 25 mm intervals. One tablet of *Lycopodium* spores (Lund University, Department of Quaternary Geology, batch 1031, with  $20848 \pm 1546$  spores per tablet) was added to each sample to enable estimation of pollen accumulation rates (PAR). The sample preparation was adapted from Fægri and Iversen (1989), including 10% NaOH, sieving, 10% HCl, 46% HF and acetolysis.

## Pollen analysis

Counting was performed under a Leica DM 2500 light microscope using x400 or x630 magnification. A minimum of 300 land pollen grains per sample were counted except for seven samples at depths 106-121 cm, where pollen concentration was very low (149-439 grains ml<sup>-1</sup>). In those samples, only 15-60 land pollen grains were counted. Pollen was identified to family or genus, seldom to species (Nilsson 1961, Fægri & Iversen 1989, Kapp et al. 2000). The number of land pollen taxa recovered was 29.



## Betula pollen measurements

Slides were scanned separately for *Betula* pollen and every grain found was digitally photographed. About 90% of the pollen images showed measurable pollen grains. The pollen diameter was measured as in Karlsdóttir et al. (2007) as the distance from the outside tip of pore to the outer margin of the facing wall. Non-triporate pollen grains (identified as in Karlsdóttir et al., 2008) were noted and counted but not measured for size.

## Data analysis and presentation

Analysis of size distribution of *Betula* pollen diameters within samples was performed as described in Karlsdóttir et al. (2009), using Bhattacharya's method (Bhattacharya, 1967, Pauly and Caddy 1985). For successful calculation of species proportions, an effort was made to include 100-200 grains per sample. Due to varying *Betula* pollen concentrations, this was only possible in 38 out of 47 samples.

Means and standard deviations of subsets within samples (presumably *B. nana* vs. *B. pubescens*) were calculated from three or more points when the correlation coefficient was below -0.95. Results were refined with the Bmod program (Morgan 2005) and the best-fitted proportions calculated using Járαι-Komlódi's formula (Prentice 1981).

Pollen stratigraphic, lithostratigraphic (including LOI) and chronological data were plotted using the PSIMPOLL 4.27 software (Bennett 2009). Pollen data is presented as percentage of total terrestrial pollen (TLP), while zonation of the pollen diagram (Table 2; Figure 2) using optimal splitting, implemented in PSIMPOLL, was based on all pollen and spore taxa.

## Results

### Lithological description

The whole monolith consisted of brown herbaceous peat and *Carex* rootlets were abundant in sieve residues from all samples (Table 2). Fragments of wood were found in the upper half of the monolith. Ten layers of tephra were noted but grains of tephra were found in various quantities throughout the monolith (Figure 2). On the whole, loss on ignition was around 34% but varied from 6-71% (Figure 2), with the lowest values near the Saksunarvatn tephra layer.

In Table 2, zones from the pollen diagram (Figure 2) are shown. The Preboreal light silt (YA-1) was buried under the thick Saksunarvatn tephra at 10.3 cal ka (Rasmussen et al. 2007). Organic matter was low, and sand in the soil indicates frequent wind

deposition of loose tephra (YA-2). Gradually, the soil stabilized and the organic part of the soil increased while soil at the sampling site remained wet. This phase (YA-3) ended around 7.7 cal ka BP. In the earlier part of the next phase (YA-4), organic content increased. Wetter conditions were detected in the later part of the zone by increased presence of mud. The H5 tephra deposited at 7050 cal years, marks a colour shift in the peat from light to much darker brown (Figure 1c), possibly as a result of increased degradation of organic matter. A period of very wet conditions followed (YA-5), when the sampling site was practically under water as indicated by the presence of algae and quantities of presumed *Isoetes* spores. Organic matter and mud in the peat increased. Ponds dried out and became fens (YA-6) and small woody fragments were found in the peat. The H4 tephra (4 cal ka) occurs within this section. Near 3.5 cal ka, wood remains start to get a slightly less common. The H3 tephra (3 cal ka) is found at the bottom of the zone YA-7 and the Veidivötn AD 1477 is in its upper part.

**Table 2.** Lithostratigraphy and pollen stratigraphy for the Ytra-Áland peat sequence and inferred site development.

| Depth (cm) | Age (Cal ka) | Pollen zones | Samples (n) | PAR TLP | PAR <i>Betula</i> | Main components of sieve residue   | Implied site conditions             | Implied conditions of pollen source area                |
|------------|--------------|--------------|-------------|---------|-------------------|--|-------------------------------------|---|
| 17-41      | ~0-3.1       | YA-7         | 10          | 72-230  | 27-113            | Brown herbaceous peat with grains of fine sand and small woody fragments | Mire with sedges                    | Shrub heath with stands of downy birch                  |
| 41-73      | ~3.1-6.1     | YA-6         | 13          | 19-633  | 10-604            | Brown herbaceous peat with grains of fine sand and small woody fragments | Mire with sedges                    | Woodlands and shrub heath. Birch hybrids at 3.3 cal ka  |
| 73-84      | ~6.1-6.8     | YA-5         | 5           | 45-277  | 8-39              | Brown herbaceous peat and mud with algae                                 | Shallow pond                        | Woodlands and shrub heath                               |
| 84-94      | ~6.8-7.7     | YA-4         | 6           | 78-2204 | 9-964             | Light brown herbaceous peat and mud with algae                           | Mire with sedges and ponds          | Tree birch advancing. Birch hybrids                     |
| 94-105     | ~7.7-8.8     | YA-3         | 5           | 17-2368 | 1-326             | Light brown herbaceous peat and mud with algae                           | Mire with sedges and ponds          | Shrub heath with Ericales and dwarf birch               |
| 105-121    | ~8.8-10.3    | YA-2         | 7           | 2-5     | 0                 | Light brown herbaceous peat, sand and mud                                | Mire with sedges and seasonal ponds | Sparsely vegetated land with willows, grasses and herbs |
| 121-139    | 10.3         | -            | -           | -       | -                 | Saksunarvatn tephra  |                                     |   |
| 140        | ~10.4        | YA-1         | 1           | -       | -                 | Coarse silt with light herbaceous soil                                   | Wetland with sedges                 | Tundra/Arctic heath with <i>Salix</i>                   |

## Pollen record and birch vegetation

The Holocene pollen record of Ytra-Áland, Thistilfjörður, was divided into seven zones (Figure 2). In this diagram, *Betula* pollen was not differentiated in the pollen count.

In Figure 2, the first zone YA-1 at the depth of 140 cm, containing a single sample taken just below the Saksunarvatn tephra, where *Salix* pollen was the most abundant, together with *Silene* type of Caryophyllaceae and *Empetrum* indicated arctic heath vegetation. Pollen of *Koenigia* a hardy annual arctic plant was present, pointing to a tundra-like ecosystem. Pollen concentration was 4500 land pollen grains  $\text{cm}^{-3}$ . Two *Betula* pollen grains were found, which is too few to conclude on species or the distance to the source.

Zone YA-2 covered 10.3~8.8 cal ka and included seven samples taken above the thick Saksunarvatn tephra. Pollen concentrations were extremely low, or 149-439 land pollen grains  $\text{cm}^{-3}$  and pollen accumulation rate (PAR) was approximately 2-5 grains  $\text{year}^{-1} \text{cm}^{-2}$ . Total number of land pollen counted in all seven samples was only 253. The pollen taxa indicated sparsely vegetated land with shrubs, Ericales and grasses. *Betula* pollen was 6% of land pollen while calculations of species proportions from combined samples suggested only *B. nana* was present.

Zone YA-3, ~ 8.8-7.7 cal ka, was marked by a peak in Ericales pollen, together with high pollen concentration, the highest being more than 200000 grains  $\text{cm}^{-3}$  with PAR values of nearly 2400 land pollen grains  $\text{cm}^{-2} \text{year}^{-1}$ . *Betula* pollen concentrations were rising at the end of the period, from 100 to 30000 grains  $\text{cm}^{-3}$ . A few grains from aquatic plants, i.e. *Isoetes* spores and *Myriophyllum* pollen, were found.

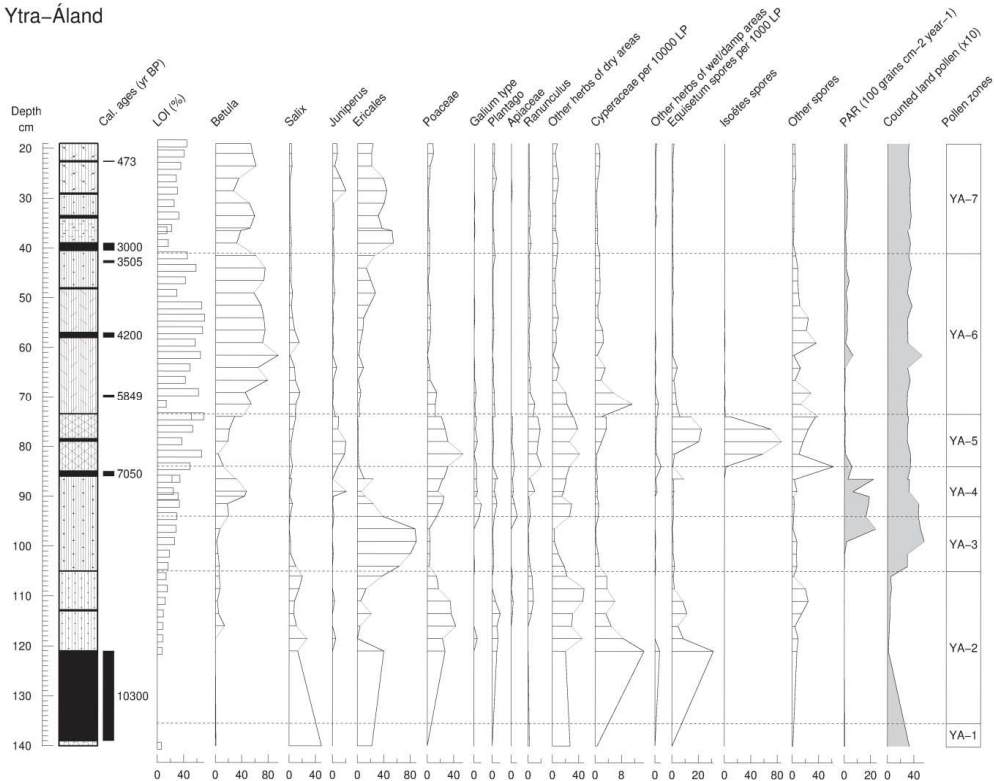
Zone YA-4, ~ 7.7-6.8 cal ka, showed a peak in *Betula* pollen percentage and an increasing number of *Juniperus* pollen towards the end and a decline of Ericales. Pollen concentrations were still high or 50-200 thousand grains  $\text{cm}^{-3}$ . *Betula* pollen concentrations were high but falling sharply by the end of the period. The *Betula* pollen was mostly *B. nana* but with a fair amount of *B. pubescens* suggesting arctic-alpine heath with stands of downy birch.

Zone YA-5, ~ 6.8-6.1 cal ka, revealed a wet period with birch almost absent but an increase in grasses, *Ranunculus* and Apiaceae. The *Ranunculus* was probably mostly *Ranunculus aquatilis* L., an aquatic plant, although counted as terrestrial, as the pollen could not be identified to species. The Apiaceae were probably *Angelica*. There was also a marked peak in *Isoetes* spores. Land pollen concentrations were 3-20 thousand  $\text{cm}^{-3}$ .

In zone YA-6, which covers the long period of ~ 6.1-3.1 cal ka, mixed birch wood- and shrublands were re-established and became dominant. Land pollen concentration increased from 1500 to 18000 grains  $\text{cm}^{-3}$ , of which approximately two-thirds were *Betula* pollen and in turn half of the *Betula* pollen was *B. pubescens*.

Zone YA-7 covers the last 3000 years of the Holocene. No changes in vegetation connected to the human settlement in the area 1100 years ago were obvious, although sampling at higher resolution might have detected small-scale changes. Birch continued to decline and there was a slight increase in Poaceae, however neither change was drastic. Land pollen concentrations were 10-30 thousand grains  $\text{cm}^{-3}$ , about half of them *Betula*.

Ytra-Áland

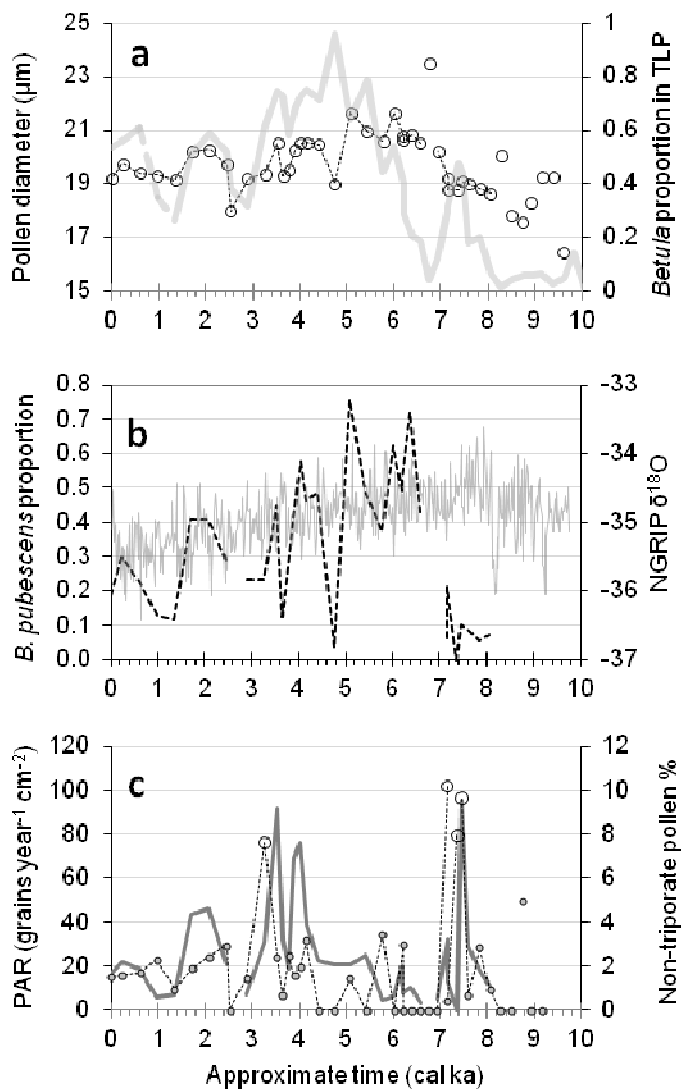


**Figure 2.** Pollen and spore diagram for the peat monolith from Ytra-Áland, Thistilfjörður, Northeast Iceland. Lithostratigraphy, loss-on-ignition (LOI) data and chronological data on the left. Pollen percentages are based on total land pollen (TLP), Cyperaceae and spores excluded. Pollen accumulation rates, number of counted land pollen and local pollen zones on the right. Note different scales on Cyperaceae, Equisetum, pollen accumulation rate (PAR), LOI and counted land pollen. Calibrated ages of carbon dated samples at 43 and 74 cm are 3445-3560 and 5885-5920 cal years respectively.

## Betula pollen size and species balance

The average size of all measured *Betula* pollen was 19.6  $\mu\text{m}$ . Average sizes within samples increased between 10 cal ka and 5 cal ka but decreased slightly from 5 cal ka to

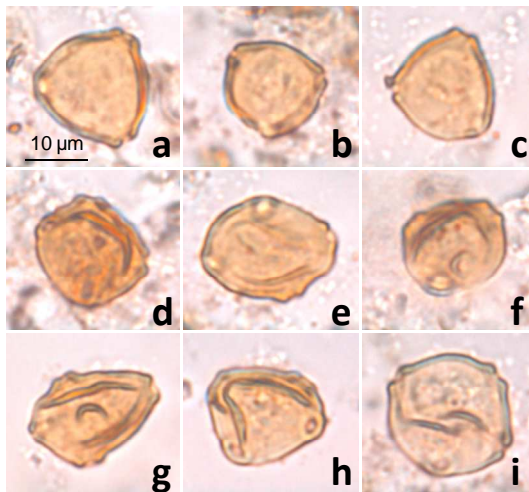
the present (Figure 3a). Correlation between mean size of *Betula* pollen and the proportion of *Betula* pollen in TLP within each sample was weak but statistically significant ( $r = 0.30$ ;  $P_{\text{one-tailed}} = 0.027$ ; Figure 3a). Calculated proportions of *Betula* species within each sample revealed mean *B. nana* pollen sizes around  $19 \mu\text{m}$ , ranging from  $18.7$  to  $19.5 \mu\text{m}$  and *B. pubescens* mean pollen size around  $23 \mu\text{m}$ , ranging from  $22.6$  to  $23.2 \mu\text{m}$ . The results from calculations, which were possible for 35 of the 47 samples, showed great fluctuations in species balance, suggesting unstable climate (Figure 3b). Calculated PAR values for *B. pubescens* pollen in particular never reached  $100 \text{ grains year}^{-1} \text{ cm}^{-2}$  but peaked at  $95 \text{ grains year}^{-1} \text{ cm}^{-2}$  at  $\sim 7.5 \text{ cal ka}$  and again at  $92 \text{ grains year}^{-1} \text{ cm}^{-2}$  at  $\sim 3.5 \text{ cal ka}$  (Figure 3c).



**Figure 3.** a) Mean pollen diameter (circles and dashed line, data points with  $n$  less than 20 not connected) and proportion of *Betula* pollen in total land pollen (grey line). b) Calculated proportion of *B. pubescens* pollen in all *Betula* pollen (dashed line) and stable oxygen isotope ( $\delta^{18}\text{O}$ ) data from the NGRIP Greenland ice core project (fine grey line) primarily indicating temperature changes during the Holocene (Vinther et al., 2006). c) Calculated PAR values of *B. pubescens* (grey line) and proportion of abnormal *Betula* pollen (circles and black dotted line, data points over 5% large circles, data points with  $n < 20$  not connected)

## Abnormal *Betula* pollen

Non-triporate *Betula* pollen grains are indicative of the presence of triploid *Betula* hybrids when they occur in a significant amount (Karlsdóttir et al. 2008). A total of 156 non-triporate *Betula* pollen grains from the Ytra-Áland monolith were noted (examples shown in Figure 4). Of these, 82 were found in just 4 samples at depths of 90, 89, 86.5 and 41.5 cm, corresponding to two peak periods, ~ 7.5-7.1 cal ka and ~ 3.3 cal ka (Figure 3c). The percentage of non-triporate pollen in those samples was 8-10%. To confirm that these samples were indeed statistically different from other samples, Tukey's boxplot method was used, confirming the data points as suspected outliers. The abnormal pollen is unlikely to be normally distributed (KS-test  $P < 0.01$ ). Two additional samples with raised percentage of non-triporate *Betula* pollen, at depths of 111 (out of scale in Figure 3c) and 104 cm (~8.8 cal ka), had too few *Betula* pollen grains to be regarded as evidence of hybridization (Figure 3c).



**Figure 4.** Examples of *Betula* pollen from Ytra-Áland samples. a-c) Normal pollen. d-i) Non-triporate pollen.

## Discussion

### Characteristics of the research area in comparison to previous sites investigated

The present study on the relative abundance and hybridization of the two *Betula* species in Iceland, based on subfossil pollen, spans the period from the first Holocene colonization of birch to the present. We have previously used the same approach on samples from two other sites; Hella in Eyjafjörður, North Iceland (Karlsdóttir et al. 2009) and Eyvík in Grímsnes, Southwest Iceland (Karlsdóttir et al. 2012), but both

studies only cover a few thousand years in the early Holocene from ~10.3 cal ka and onwards. Because of the extended time span, the present study not only strengthens our previous results from the earlier half of the Holocene, but it also reveals for the first time birch hybridization in the late Holocene. Furthermore, the addition of Thistilfjörður makes it reasonable to consider Holocene birch hybridization to be applicable to Iceland as a whole.

Besides being situated in different regions of Iceland, the three sites differ in other respects. The Hella site in Eyjafjörður lies on the western side of a major fjord with steep mountain slopes to the west. In Akureyri, a nearby weather station, the mean annual temperature from 1961-1990 was 3.2 °C and the mean July temperature was 10.5 °C. The Eyvík site in Grímsnes lies in a relatively flat landscape. It is now around 65 m a.s.l. and 30 km inland but was close to the shoreline during the early Holocene. At Hæll, a nearby weather station, mean annual temperature from 1961-1990 was 3.6 °C and the mean July temperature was 10.6 °C. Ytra-Áland is neither far from the shore nor is it sheltered by steep mountain slopes. In comparison to the two other sites Ytra-Áland has the least favourable climate for downy birch. At the weather station Thorvaldsstadir, mean annual temperature from 1961-1990 was only 2.8 °C and the mean July temperature was 8.5 °C. Mean wind velocity is also a little higher near Ytra-Áland or approximately 4.4 m sec<sup>-1</sup> compared to 4.0 and 4.1 m sec<sup>-1</sup> near Hella and Eyvík respectively (The Icelandic Meteorological office 2013).

## Soil development

The parent materials of Icelandic soils are mixtures of tephra layers and eolian sediments consisting mostly of volcanic glass (Arnalds 2004). In Icelandic soils, there are more than 800 Holocene tephra layers (Larsen & Eiríksson 2008). They were generated by various volcanoes and the distribution of each tephra depends mainly on the size of the eruption and wind directions during the first fierce eruption days. Gudmundsdóttir et al. (2012) listed 85 tephras aged 10300-70 cal years, recognized from the core MD99-2275 on the North Icelandic shelf 100 km northwest from the sampling site at Ytra-Áland. Five of the most easily recognized of those tephras have been used to date the samples in this study but most of the others are likely to be present too, adding bulk material to the sequence and depressing LOI values.

The low pollen concentration and LOI values of the samples just above the Saksunarvatn tephra indicate frequent sandstorms but the duration of this period is unknown as we did not find suitable material for radiocarbon dating in the lower part of the monolith. Hallsdóttir and Caseldine (2005) inferred several decades or even centuries of unstable environment as a consequence of the huge eruption that produced the Saksunarvatn tephra. After this period, LOI values increased slowly until around 7



cal ka, near the boundaries of zones YA-4 and YA-5. At first, the low values were probably due to erosion in the sparsely vegetated environment where tephra was abundant but later warmer and dryer climate may have kept the rate of organic matter accumulation low through faster decomposition. The peak of *Betula* pollen in zone YA-4 (around 7.5-7.0 cal ka), with its high values of *B. pubescens* PAR, may well represent the Holocene thermal maximum in the area. In zone YA-5 (from 7 cal ka) the LOI values increased rapidly as the conditions became wetter. Even though the ponds gave way to fens in zone YA-6 (from ~6.2 cal ka), LOI values remained high for the most part until around 3 cal ka, when the great eruption of Hekla deposited the H3 tephra. After that, LOI values were low for a while, but then started to grow slowly again and kept growing in spite of human settlement from around 900 AD. Volcanic eruptions thus seem to have been a major factor in determining carbon content of the soil at Ytra-Áland, although water levels, controlled by climate, also played their part.

In general the main changes in Icelandic soils related to human settlement are a decrease in woodlands mostly caused by winter grazing and wood cutting for iron processing (Hallsdóttir 1987), followed by a marked increase in deposition rate caused by wind erosion (Thorarinnsson 1961). Climate cooling has doubtless enhanced these effects. In remote areas without birch woodlands, these changes are less conspicuous. Zutter (1997) studied the anthropogenic impact on vegetation in Thistilfjörður and published two pollen records for the period ~7.3 cal ka to the present, obtained at the Svalbard and Sævarland farms, 7-8 km from Ytra-Áland. She found a marked increase in deposition rates at the Svalbard site but not at Sævarland, showing that these effects can be very local.

## **Birch in Thistilfjörður through the Holocene**

### **Changing equilibrium of *Betula* species – the rise and fall of tree-birch**

Size of pollen differs between *Betula* species but is also affected by conditions during development and continues to change as a response to environmental factors after being shed (Dunbar & Rowley 1984, Edwards et al. 1991). Chemical treatment has been shown to alter pollen sizes (e.g. Reitsma 1969) and there are reasons to suspect size differences in relation to soil conditions of subfossil pollen (Karlsdóttir et al. 2009). We therefore do not put any emphasis on absolute difference in pollen size between samples, but concentrate on size distribution within each sample when calculating the relative number of different species. When these data were used to calculate PAR values for *B. pubescens* pollen we obtained the maximum value of 95 grains cm<sup>-2</sup> year<sup>-1</sup> at around 7.5 cal ka. This is far below the PAR values seen in birch woodlands in other studies including Eyvík, Southwest Iceland (Karlsdóttir et al. 2012) and in Scandinavian



forests (Seppa & Hicks 2006, Hättstrand et al. 2008). Based on this, birch woodlands in the Thistilfjörður region were never dense during the Holocene, but rather a shrubland with dwarf birch and scattered birch trees. This conclusion was strengthened by the observation of birch remains, up to 10 cm in diameter, protruding from the peat in a nearby ditch. No such wood remains were found at the sampling site.

The dwarf birch seems to have found its way to Northeast Iceland sometime around 9 cal ka. The pollen concentration was, however, still low until after ~8.2 cal ka when rapid colonization took place. The downy birch became established in Thistilfjörður around that time, 8.2-7.6 cal ka. That is later than seen in some other parts of Iceland where the first birch woodlands appeared ca. 9.5-8.2 cal ka (Vasari & Vasari 1990, Hallsdóttir 1995, Hallsdóttir & Caseldine 2005), but may be comparable with data from lake Efstadalvatn in Northwest Iceland at 123 m a.s.l., where conditions for tree birch were also limited (Caseldine et al. 2003). Where tree birch had been established early, a decline of birch vegetation is seen during the cold event of 8.2 cal ka (Thomas et al. 2007), followed by a rapid recovery and a new expansion when conditions improved. The peak of the birch period, both in North and South Iceland, was between 8.0 and 6.8 cal ka (Vasari & Vasari 1990, Hallsdóttir 1995, Hallsdóttir & Caseldine 2005). At Ytra-Áland the peak in *B. pubescens* at ~7.5-7.1 cal ka was short-lived (Figure 3c). Both *Betula* species retreated, probably in response to cold and wet conditions. The dwarf birch recovered faster but the downy birch took a thousand years to begin its recovery and did not reach another peak until around 4 cal ka. The peak in combined *Betula* pollen around 4.7 cal ka (Figure 2) mainly reflects *B. nana* pollen. This is consistent with Zutter's (1997) results on birch woodlands in Thistilfjörður where periods of birch expansion and decline with wetter conditions were found at similar times. Our results show that from 4 cal ka until the present, birch has been declining. Downy birch almost disappeared from the area around 3 cal ka, recovered for a while in the Roman warm period, but was retreating even before settlement at 1.1 cal ka. The dwarf birch, on the other hand, prevailed and seemed to have expanded rather than decreased. The human settlement in the area had little or limited effect on the already declining tree birch vegetation.

## **Holocene climate in Thistilfjörður**

A temperature of 10°C in the warmest month has usually been considered a minimum for birch forest limits in the northern temperate belt (Odland 1996). Attempts to define the climatic requirements for mountain birch have proved difficult. Other figures for July temperature have been suggested (reviewed by Odland 1996; in Iceland measured by Wöhl 2008), and temperature during the entire growing season, either air (Odland 1996) or soil (Körner & Paulsen 2004) has been found to predict tree line altitudes somewhat better, but the results are never far from the 10 °C July mean temperature.

Using this conventional figure, we see that in the Thistilfjörður area, a climate warmer than in the late twentieth century would have been needed for tree-birch to thrive there. A minimum July temperature for *B. nana*, on the other hand, is estimated closer to 6°C (De Groot et al. 1997), which is well below the present-day conditions in the area. Therefore we would expect *B. nana* to be the dominant source of *Betula* pollen except during the Holocene Thermal Maximum (HTM) when temperatures may have been around 1.5 °C higher than present (Kaufman et al. 2004). Even at that time, the tree birch would have been struggling near the lower limits of its temperature requirements. If some local meteorological condition reduced or delayed HTM, it would have further limited the tree birch to scattered stands of trees in favourable places rather than continuous woodlands.

Zutter (1997) concluded, mostly from *Betula* pollen, that conditions in Thistilfjörður had been warmer and dryer than present in the period 5.1-2.1 cal ka. Later Axford et al. (2007) studied subfossil midges in Thistilfjörður throughout the entire Holocene and came to the conclusion that in Northeast Iceland HTM occurred several thousand years later than expected or around 5.0-2.5 cal ka, compared with HTM between 10.3-5.6 cal ka as reflected by five studies on terrestrial sites in Iceland (Kaufman et al. 2004). The reasons for this are far from clear as summer insolation peaked in the early Holocene. The authors suggest modulation by sea ice, enhanced by salinity stratification of sea waters. Our records of *Betula* pollen proportion and *Betula* species balance suggest a period of warmer climate than present between 8 and 7 cal ka, followed by a cold and wet period 7.0-5.5 cal ka. It is therefore debatable if the peak in *B. pubescens* PAR at 7.5-7.1 cal ka represents the HTM in the area or if HTM was delayed until 5.4-3.5 cal ka when *Betula* in TLP was highest. Anomalies in pollen concentrations in the earlier half of the Holocene and variation in *Betula* species balance, support theories of an unstable climate in the far north-eastern Iceland during the early and mid Holocene; something that might be attributed to variations in drift ice or periods of persisting northerly winds.

## Holocene birch hybridization

In the present study, we detected two significant peaks of non-triporate *Betula* pollen indicating the presence of hybrids in the Ytra-Áland peat; an earlier peak at 7.5-7.1 cal ka and a later one around 3.3 cal ka. Both peaks are associated with peaks in *B. pubescens* PAR values, and this association is in concordance with the results we have published before from North and Southwest Iceland, where periods of hybridization were also linked to *B. pubescens* pollen influx values higher than those characteristic for established *B. nana* populations. This is the first time birch hybridization is detected in the Late Holocene.

The results of the present study, along with results from two previous studies on Holocene birch hybridization, indicate that the hybridization followed expansion of *B. pubescens* dominating woodlands, presumably during regional warming of climate. An increase in annual mean temperature of around 5°C is predicted in the Arctic in the 21<sup>st</sup> century but summer temperatures are supposed to rise less, or around 2°C (Christensen et al. 2007). Such a change of climate would benefit vegetation and lead to establishment or expansion of birch woodland, given there is available space for recruitments. In the process, a new wave of birch hybrids may be expected.

## Conclusions

Periods of *Betula* hybridization were identified, based on pollen data from Thistilfjörður in Northeast Iceland, supporting previous results from North and Southwest Iceland. As found in the earlier studies, periods of hybridization were connected to the expansion of local *B. pubescens* populations. Our new data confirms period of hybridization occurred in the earlier half of the Holocene. Comparable periods of hybridizations are also reported here in connection with birch expansion, in the later half of Holocene. Conditions in the oceanic Northeast Iceland were near the lower limits of the temperature requirements of downy birch for most of the Holocene, resulting in fluctuating *Betula* species balance. The effects of human settlement on birch in the area were small compared to climatic effects. Theories on late HTM in Thistilfjörður, compared to other parts of Iceland are partly supported, as birch woodlands peaked from 5.4-3.3 cal ka. Still a short period of warm climate, seen as a peak in PAR values for *B. pubescens* and land pollen in general, was detected near 7.5 cal ka and might represent the local HTM,

## Acknowledgements

The authors would like to thank Sigurdur H. Árnason for help with the fieldwork; the residents at Ytra-Áland for access to the sampling site; Egill Erlendsson for his help and access to equipment for the loss on ignition test; Guðrún Larsen for advice on tephra identification and an anonymous reviewer for useful comments and suggestions. The project was supported by the University of Iceland (Doctoral grant 2010–2013) and the University of Iceland Research Fund.

## References

- Anamthawat-Jónsson K & Thórsson ÆTh 2003.. Natural hybridisation in birch: triploid hybrids between *Betula nana* and *B. pubescens*. *Plant cell, tissue and organ culture* 75, 99-107.
- Anamthawat-Jónsson K and Tómasson T 1990. Cytogenetics of hybrid introgression in Icelandic birch. *Hereditas* 112, 65-70.
- Arnalds O 2004. Volcanic soils of Iceland. *Catena* 56, 3-20.
- Axford Y, Miller GH, Geirsdóttir Á & Langdon PG 2007. Holocene temperature history of northern Iceland inferred from subfossil midges. *Quaternary Science Reviews* 26, 3344-3358.
- Bhattacharya CG 1967. A simple method of resolution of a distribution into Gaussian components. *Biometrics* 23, 115–135.
- Bennett KD 2009. Psimpoll 4.27: C program for plotting pollen diagrams and analysing pollen data. Available online from Queen's University of Belfast, Department of Archaeology and Palaeoecology. Accessed 2 May 2013 at [www.chrono.qub.ac.uk/psimpoll/psimpoll.html](http://www.chrono.qub.ac.uk/psimpoll/psimpoll.html) ().
- Birchler JA & Veitia RA 2007. The gene balance hypothesis: from classical genetics to modern genomics. *The Plant Cell Online* 19, 395-402.
- Caseldine C, Geirsdóttir Á & Langdon P 2003. Efstadalsvatn—a multi-proxy study of a Holocene lacustrine sequence from NW Iceland. *Journal of Paleolimnology* 30, 55-73.
- Christensen JH, Hewitson B, Busuioc A, Chen A, Gao X, Held R et al. 2007. *Regional climate projections. Climate Change, 2007: The Physical Science Basis.* Contribution of Working group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge: University Press, pp 847-940.
- De Groot WJ, Thomas PA & Wein RW 1997. *Betula nana* L. and *Betula glandulosa* Michx. *Journal of Ecology* 85, 241-264.
- Dugmore AJ, Cook GT, Shore JS, Newton AJ, Edwards KJ & Larsen G 1995. Radiocarbon dating tephra layers in Britain and Iceland. *Radiocarbon* 37, 379-388.
- Dunbar A & Rowley JR 1984. *Betula* pollen development before and after dormancy: exine and intine. *Pollen Spores* 26, 299-338.
- Edwards ME, Dawe JC & Armbruster WS 1991. Pollen size of *Betula* in northern Alaska and the interpretation of late Quaternary vegetation records. *Canadian journal of botany* 69, 1666-1672.
- Elkington TT 1968. Introgressive hybridization between *Betula nana* L. and *B. pubescens* Ehrh. in North-West Iceland. *New Phytologist* 67, 109-118.

- Fredskild B 1991. The genus *Betula* in Greenland-Holocene history, present distribution and synecology. *Nordic journal of botany* 11, 393-412.
- Fægri K & Iversen J 1989. *Textbook of pollen analysis*, 4th edn. Chichester: John Wiley & Sons.
- Gudmundsdóttir ER, Larsen G & Eiríksson J 2012. Tephra stratigraphy on the North Icelandic shelf: extending tephrochronology into marine sediments off North Iceland. *Boreas* 41, 719-734.
- Hallsdóttir M 1987. *Pollen analytical studies of human influence on vegetation in relation to the Landnám tephra layer in southwest Iceland*. Ph.D. thesis. LUNDQUA thesis 18, Lund University, Department of Quaternary Geology, 45 p.
- Hallsdóttir M 1995. On the pre-settlement history of Icelandic vegetation. *Búvísindi* 9, 17-29.
- Hallsdóttir M & Caseldine CJ 2005. The Holocene vegetation history of Iceland, state-of-the-art and future research. In: C. Caseldine, A. Russell, J. Hardardóttir, Ó. Knudsen (eds.) *Iceland: Modern Processes and Past Environments*. Amsterdam: Elsevier, pp. 319-334.
- Hättestrand M, Jensen C, Hallsdóttir M & Vorren KD 2008. Modern pollen accumulation rates at the north-western fringe of the European boreal forest. *Review of Palaeobotany and Palynology* 151, 90-109.
- Kapp RO, King JE & Davis OK 2000. *Ronald O. Kapp's pollen and spores*. 2nd ed. American Association of Stratigraphic Palynologists Foundation Publication, Texas, 279 p.
- Karlsdóttir L, Thórsson ÆTh, Hallsdóttir M, Sigurgeirsson, Eysteinnsson Th & Anamthawat-Jónsson K 2007. Differentiating pollen of *Betula* species from Iceland. *Grana* 46, 78-84.
- Karlsdóttir L, Hallsdóttir M, Thórsson ÆTh & Anamthawat-Jónsson K 2008. Characteristics of pollen from natural triploid *Betula* hybrids. *Grana* 47, 52-59.
- Karlsdóttir L, Hallsdóttir M, Thórsson ÆTh, Anamthawat-Jónsson K 2009. Evidence of hybridisation between *Betula pubescens* and *B. nana* in Iceland during the early Holocene. *Review of Palaeobotany and Palynology* 156, 350-357.
- Karlsdóttir L, Hallsdóttir M, Thórsson ÆTh & Anamthawat-Jónsson K 2012. Early Holocene hybridisation between *Betula pubescens* and *B. nana* in relation to birch vegetation in Southwest Iceland. *Review of Palaeobotany and Palynology* 181, 1-10.
- Kaufman DS, Ager TA, Anderson NJ, Anderson PM, Andrews JT, Bartlein PJ et al. 2004. Holocene thermal maximum in the western Arctic (0–180 W). *Quaternary Science Reviews* 23, 529-560.

- Kershaw AP 1997. A modification of the Troels-Smith system of sediment description and portrayal. *Quaternary Australasia* 15, 63-68.
- Körner C & Paulsen J 2004.. A worldwide study of high altitude treeline temperatures. *Journal of Biogeography* 31, 713-732.
- Larsen G 1984. Recent volcanic history of the Veidivötn fissure swarm, southern Iceland – an approach to volcanic risk assessment. *Journal of Volcanology and Geothermal Research* 22, 33-58.
- Larsen G & Eiríksson J 2008. Late Quaternary terrestrial tephrochronology of Iceland—Frequency of explosive eruptions, type and volume of tephra deposits. *Journal of Quaternary Science*, 23, 109-120.
- McCormick S 1993. Male gametophyte development. *The Plant Cell* 5, 1265-1275.
- Morgan JH 2005. A computer method for resolving mixed normal distributions. *Ringings & Migration* 22, 145-152.
- Nilsson T 1961. *Kompendium i kvartärpaleontologi och kvartärpaleontologiska undersökningsmetoder* [Compendium in *Quarter paleontology and Quarter paleontological research methods*]. University of Lund, Lund, 2 volumes, 238 pp. + 63 plates.
- Norrdahl H & Pétursson HG 2005. Relative Sea-Level Changes in Iceland; New Aspects of the Weichselian Deglaciation of Iceland. In: Caseldine C, Russel A, Hardardóttir J & Knudsen O (eds.) *Iceland – Modern Processes and Past Environments*, pp. 25-78. Elsevier, Amsterdam.
- Norrdahl H, Ingólfsson Ó, Pétursson HG & Hallsdóttir M 2008. Late Weichselian and Holocene environmental history of Iceland. *Jökull*, 58, 343-364.
- Óbyggðanefnd 2005. *Úrskurður Óbyggðanefndar mál nr. 4/2005, Svalbarðshreppur, Þórshafnarhreppur og Skeggjastaðahreppur*. [Verdict of The Wilderness Committee, Case nr. 4/2005 Svalbarðshreppur, Þórshafnarhreppur and Skeggjastaðahreppur], 241 p. Reykjavík, Óbyggðanefnd. [In Icelandic].
- Odland A 1996. Differences in the vertical distribution pattern of *Betula pubescens* in Norway and its ecological significance. In: Frenzel, B. (editor). *Holocene treeline oscillations, dendrochronology and palaeoclimate*; p. 43-59. Stuttgart: Gustav Fischer, Pauly D & Caddy JF 1985. *A modification of Bhattacharya's method for the analysis of mixtures of normal distributions*. FAO Fisheries Circular 781. Rome: FAO, 16 p.
- Pétursson HG 1991. The Weichselian glacial history of West Melrakkaslétta, Northeastern Iceland. In *Environmental Change in Iceland: Past and Present* (pp. 49-65). Springer Netherlands.
- Prentice IC 1981. Quantitative birch (*Betula* L.) pollen separation by analysis of size frequency data. *New Phytologist* 89, 145-157.



- Ramsey J & Schemske DW 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* 29, 467-501.
- Rasmussen SO, Vinther BM, Clausen HB & Andersen KK 2007. Early Holocene climate 10 oscillations recorded in three Greenland ice cores. *Quaternary Science Reviews* 26, 1907-1914.
- Reimer PJ, Baillie MGL, Bard E, Bayliss A, Beck JW, Blackwell PG, Bronk Ramsey C, Buck CE, Burr GS, Edwards RL, Friedrich M, Grootes PM, Guilderson TP, Hajdas I, Heaton TJ, Hogg AG, Hughen KA, Kaiser KF, Kromer B, McCormac FG, Manning SW, Reimer RW, Richards DA, Southon JR, Talamo S, Turney CSM, van der Plicht J & Weyhenmeyer CE 2009. IntCal09 and Marine09 radiocarbon age calibration curves, 0–50,000 years cal BP. *Radiocarbon* 51, 1111-1150.
- Reitsma TJ 1969. Size modification of recent pollen grains under different treatments. *Review of Palaeobotany and Palynology* 9, 175-202.
- Seppä H & Hicks S 2006. Integration of modern and past pollen accumulation rate (PAR) records across the arctic tree-line: a method for more precise vegetation reconstructions. *Quaternary Science Reviews* 25, 1501-1516.
- The Icelandic Meteorological office 2013. Tímaraðir fyrir valdar veðurstöðvar [Time series for selected weather stations]. Accessed 2 July 2013 at [www.vedur.is/vedur/vedurfar/medaltalstoflur](http://www.vedur.is/vedur/vedurfar/medaltalstoflur)
- Thomas ER, Wolff EW, Mulvaney R, Steffensen JP, Johnsen SJ, Arrowsmith C, Whited JWC, Vaughn B & Popp T 2007. The 8.2 ka event from Greenland ice cores. *Quaternary Science Reviews* 26, 70-81.
- Thorarinsson S 1961. Uppblástur á Íslandi í ljósi öskulagarannsóknna. [Soil erosion in Iceland in the light of tephra research]. *Ársrit Skógræktarfélags Íslands 1960–61*, 17-54 [In Icelandic, English summary]
- Thorarinsson S 1971. Aldur ljósu gjóskulaganna úr Heklu samkvæmt leidrétu geislakolstímatáli. [The age of the light Hekla tephra layers according to corrected C<sup>14</sup>-datings]. *Náttúrufræðingurinn* 41, 99-105 [In Icelandic].
- Thórsson ÆTh, Pálsson S, Sigurgeirsson A & Anamthawat-Jónsson K 2007. Morphological variation among *Betula nana* (diploid), *B. pubescens* (tetraploid) and their triploid hybrids in Iceland. *Annals of botany* 99, 1183-1193.
- Troels-Smith J 1955. Characterization of unconsolidated sediments. *Danmarks Geologiske Undersøgelse IV* 3, 1-73.
- Vasari Y & Vasari A 1990. L'histoire Holocène des lacs Islandais [The Holocene history of Icelandic lakes]. In: Devers, S. (Ed.), *Pour Jean Malaurie*. Editions Plon, Paris, pp. 277–293 [In French].

- Vinther BM, Clausen HB, Johnsen SJ, Rasmussen SO, Andersen KK, Buchardt SL, Dahl-Jensen D, Seierstad IK, Siggaard-Andersen M-L, Steffensen JP, Svensson AM, Olsen J & Heinemeier J 2006. A synchronized dating of three Greenland ice cores throughout the Holocene *Journal of Geophysical Research* 111, 3102. DOI: 10.1029/2005JD006921
- Wöll C 2008. Treeline of mountain birch (*Betula pubescens* Ehrh.) in Iceland and its relationship to temperature. Department of Forestry: Technical University Dresden. p 125.
- Zutter C 1997. *The Cultural Landscape of Iceland: A Millennium of Human Transformation and Environmental Change*. Doctoral dissertation, University of Alberta USA, 239 p.



# Appendix A



# APPENDIX A Pollen sites in Iceland

*Based on Hallsdóttir (1995) and Hallsdóttir and Caseldine (2005) with adaptations*

| Site name           | Locality      | Municipality                  | Iceland quarter |   | ref no | Essence from Title                               | Period 14C ka | Cal ka  | Author            |
|---------------------|---------------|-------------------------------|-----------------|---|--------|--|---------------|---------|-------------------|
| Naustamýri          | Akureyri      | Akureyri                      | NW              | N | 1      | Nákuðungslögin við Húnaflóa                      | 0-(8-10)      | 0-11    | Thorarinsson 1955 |
| Hoffell             | Nesjum        | Hornafjörður                  | SE              | E | 2      | Glacial drift in Iceland                         | 0-(8-10)      | 0-11    | Okko 1956         |
| Laugardalur         | Reykjavík     | Reykjavík                     | SW              | W | 2      | Glacial drift in Iceland                         | 0-(8-10)      | 0-11    | Okko 1956         |
| Héðinsvík           | Húsavík       | Tjörneshreppur                | NE              | N | 3      | ...Moorprofiles aus Nord-Island                  | 0-4.5         | 0-5.1   | Straka 1956       |
| Sogamýri            | Reykjavík     | Reykjavík                     | SW              | W | 4      | Tvö frjólínurit úr íslenskum mómýrum             | 0-(8-10)      | 0-11    | Einarsson 1957    |
| Undir Ingólfsfjalli | Ölfus         | Ölfus                         | SW              | S | 4      | Tvö frjólínurit úr íslenskum mómýrum             | 0-(8-10)      | 0-11    | Einarsson 1957    |
| Seltjörn            | Reykjavík     | Reykjavík                     | SW              | W | 5      | ..spat-und postglazialen Klimageschichte Islands | 0-9           | 0-10    | Einarsson 1961    |
| Borgarmýri          | Reykjavík     | Reykjavík                     | SW              | W | 5      | ..spat-und postglazialen Klimageschichte Islands | 0-<9          | 0-<10   | Einarsson 1961    |
| Villingavatn        | Í Grafningi   | Grímsnes- og Grafningshreppur | SW              | S | 5      | ..spat-und postglazialen Klimageschichte Islands | 0-(8-10)      | 0-11    | Einarsson 1961    |
| Ölkelda             | Staðarsveit   | Snæfellsbær                   | SW              | W | 5      | ..spat-und postglazialen Klimageschichte Islands | 0-(8-10)      | 0-11    | Einarsson 1961    |
| Torfalækur          | Blönduós      | Húnavatnshreppur              | NW              | N | 5      | ..spat-und postglazialen Klimageschichte Islands | 0- <7         | 0-<8    | Einarsson 1961    |
| Varmahlíð           | Skagafjörður  | Skagafjörður                  | NW              | N | 5      | ..spat-und postglazialen Klimageschichte Islands | 0- <6         | 0-<7    | Einarsson 1961    |
| Sólheimagerði       | Skagafjörður  | Akrahreppur                   | NW              | N | 5      | ..spat-und postglazialen Klimageschichte Islands | 0-7           | 0-8     | Einarsson 1961    |
| Moldhaugar          | Eyjafjörður   | Hörgársveit                   | NE              | N | 5      | ..spat-und postglazialen Klimageschichte Islands | 0-(8-10)      | 0-11    | Einarsson 1961    |
| Hallormsstaður      | Hérað         | Fljótadalshérað               | NE              | E | 5      | ..spat-und postglazialen Klimageschichte Islands | 0-(8-10)      | 0-11    | Einarsson 1961    |
| Lómatjörn           | Biskupstungur | Bláskógabyggð                 | SW              | S | 6      | L'histoire Holocène des lacs Islandais.          | 2-8.4         | 2-9.5   | Vasari 1972       |
| Hafratjörn          | Blönduós      | Húnavatnshreppur              | NW              | N | 6      | L'histoire Holocène des lacs Islandais.          | 4-8.6         | 4.5-9.5 | Vasari 1972       |

|                      |               |                               |    |   |       |   |          |           |                                |
|----------------------|---------------|-------------------------------|----|---|-------|---|----------|-----------|--------------------------------|
| Ytri Bægisá          | Hörgárdalur   | Hörgársveit                   | NE | N | 7     | ...peat deposits at Ytri Baegisa                    | 0-8.8    | 0-9.9     | Bartley 1973                   |
| Tjarnarver           | Þjórsárver    | Skeiða- og Gnúpverjahreppur   | C  | C | 8     | Frjógreining á jarðvegi                             | 0-4      | 0-4.2     | Fridriksdóttir 1973            |
| Sóleyjarhöfði        | Þjórsárver    | Ásahreppur                    | C  | C | 8     | Frjógreining á jarðvegi                             | 0-6      | 0-7.0     | Fridriksdóttir 1973            |
| Garðskagi            | Reykjanes     | Garður                        | SW | W | 9     | Um tvö frjólínurit af Romshvalanesi                 | 4-7.5    | 4.2-8.3   | Skaftadóttir 1974              |
| Miðnesheiði          | Reykjanes     | Reykjanesbær                  | SW | W | 9     | Um tvö frjólínurit af Romshvalanesi                 | 0-1      | 0-1       | Skaftadóttir 1974              |
| Búrfellshraun        | Hafnarfjörður | Hafnarfjörður                 | SW | W | 10    | Frjógreining úr mó undan Búrfellshrauni             | 7.2-8.8  | 8.0-9.9-  | Sighórsdóttir 1976             |
| Laugarvatn           | Laugardal     | Bláskógabyggð                 | SW | S | 11    | Moorkundliche Untersuchungen am Laugarvatn          | ?- (8-9) | ?-(9-10)  | Schwar 1978                    |
| Svínavatn            | Grímsnes      | Grímsnes- og Grafningshreppur | SW | S | 12    | ...human influence on vegetation                    | 0->6.2   | 0->7.1    | Hallsdóttir 1987               |
| Krosshólmýri         | Flateyjaralur | Þingeyjarsveit                | NE | N | 13    | Studies in the vegetation history of North-Iceland  | 6.8-9.7  | 7.7-11.2  | Hallsdóttir 1991               |
| Hvítahlíð            | Bitrufjörður  | Strandabyggð                  | NW | N | 14    | Sea-Level Change in Vestfirðir                      | 8.8-6.9  | 9.8-7.7   | Hansom and Briggs 1991         |
| Vatnaskotsvatn       | Skagafjörður  | Skagafjörður                  | NW | N | 15-16 | Synthesis of the Holocene history of vegetation     | 0-9      | 0-10.2    | Hallsdóttir 1995, 1996         |
| Torfadalsvatn        | Skagi         | Skagabyggð                    | NW | N | 17    | ... last glacial-interglacial environmental changes | 8-10.6   | 9-12.6    | Björck et al. 1992             |
| Krosshóll            | Skíðadalur    | Dalvíkurbyggð                 | NE | N | 18    | Interpretation of Holocene climate change           | 0-6.3    | 0-7.3     | Caseldine and Hatton 1994      |
| Hestvatn             | Grímsnes      | Grímsnes- og Grafningshreppur | SW | S | 15    | ...pre-settlement history of Icelandic vegetation   | 0-6.2-   | 0-7.2     | Hallsdóttir 1995               |
| Hraunsvatn           | Skagi         | Skagafjörður                  | NW | N | 21    | Dynamic sea level change .. last deglaciation       | 9-9.6    | 10.2-10.9 | Rundgren 1997                  |
| Geitakarlsvötn       | Skagi         | Skagabyggð                    | NW | N | 21    | Dynamic sea level change .. last deglaciation       | 9-9.9    | 10.2-11.3 | Rundgren 1997                  |
| Kollúsátursvatn      | Skagi         | Skagafjörður                  | NW | N | 21    | Dynamic sea level change .. last deglaciation       | 9-9.6    | 10.2-10.9 | Rundgren 1997                  |
| Neðstavatn           | Skagi         | Skagafjörður                  | NW | N | 21    | Dynamic sea level change .. last deglaciation       | 9-9.9    | 10.2-11.3 | Rundgren 1997                  |
| Torfadalsvatn        | Skagi         | Skagabyggð                    | NW | N | 19    | Allerød-Younger Dryas-Preboreal oscillation         | 9-11.3   | 10.2-13.0 | Rundgren 1995                  |
| Torfadalsvatn        | Skagi         | Skagabyggð                    | NW | N | 20/21 | Early-Holocene vegetation of northern Iceland       | 9.6-9.9  | 8.6-11.4  | Rundgren 1998                  |
| Geitakarlsvötn       | Skagi         | Skagabyggð                    | NW | N | 20    | Early-Holocene vegetation of northern Iceland       | 9-9.9    | 10.2-11.3 | Rundgren 1998                  |
| Vestra Gíslholtsvatn | Holt          | Rangárþing yra                | SW | S | 31    | The Holocene vegetation history of Iceland          | 0-5.6    | 0-6.4     | Hallsdóttir and Caseldine 2005 |

|               |                |                               |    |   |       |  |           |          |                                |
|---------------|----------------|-------------------------------|----|---|-------|--|-----------|----------|--------------------------------|
| Vesturárdalur | Skíðadalur     | Dalvíkurbyggð                 | NE | N | 22    | ...Holocene variations of the upper limit of tree or shrub birch | 0-9.2     | 0-10.4   | Wastl et al. 2001              |
| Álftárbakki   | Mýrar          | Borgarbyggð                   | SW | W | -     | (Without title: Vegetation history; ~1985)                       | 0-(8-10)  | 0-11.4   | Hallsdóttir unpubl.            |
| Nykurtjörn    | Fjörður        | Grýtubakkahr.                 | NE | N | -     | (Without title: Vegetation history)                              | >2.5-7    | >2.7-7.8 | Hallsdóttir unpubl.            |
| Gíslholtsmýri | Holt           | Rangárþing ytra               | SW | S | -     | (Without title: Vegetation history)                              | 0-(8-10)  | 0-11.4   | Hallsdóttir unpubl.            |
| Hella         | Árskógsströnd  | Dalvíkurbyggð                 | NE | N | 31    | The Holocene vegetation history of Iceland                       | 6.2- > 10 | 7.1-11.4 | Hallsdóttir and Caseldine 2005 |
| Skallakot     | Þjórsárdalur   | Skeiða- og Gnúpverjahr.       | SW | S | 23    | Tefrokronologiska studier på Island                              | 0.8-1.5   | 0.7-1.4  | Thórarinnsson 1944             |
| Stöng         | Þjórsárdalur   | Skeiða- og Gnúpverjahr.       | SW | S | 23    | Tefrokronologiska studier på Island                              | 0.8-1.5   | 0.7-1.4  | Thórarinnsson 1944             |
| Skálholt      | Biskupstungur  | Bláskógabyggð                 | SW | S | 24    | ... vegetation and climate history of Iceland...                 | 0-4       | 0-4.4    | Einarsson 1963                 |
| Borgarmýri    | Reykjavík      | Reykjavík                     | SW | W | 24    | ... vegetation and climate history of Iceland...                 | 0-<9      | 0-<10.2  | Einarsson 1963                 |
| Lágafell      | Landeyjar      | Rangárþing eystra             | SW | S | 25    | A pollen analytical study on a peat deposit                      | 0.4-2.5   | 0.5-2.5  | Páhlsson 1981                  |
| Vaðlamýri     | Hrafnkelsdalur | Fljótaldshérað                | NE | E | 26    | Frjógreining tveggja jarðvegssniða úr Hrafnkelsdal               | 0.5-1.5   | 0.5-1.4  | Hallsdóttir 1982               |
| Vaðbrekka     | Hrafnkelsdalur | Fljótaldshérað                | NE | E | 26    | Frjógreining tveggja jarðvegssniða ...                           | 0.5-1.5   | 0.5-1.4  | Hallsdóttir 1982               |
| Herjólfsdalur | Heimaey        | Vestmannaeyjar                | SW | S | 27    | Frjógreining tveggja jarðvegssniða á Heimaey                     | 1-1.5     | 0.9-1.4  | Hallsdóttir 1984               |
| Torfmyri      | Heimaey        | Vestmannaeyjar                | SW | S | 27    | Frjógreining tveggja jarðvegssniða á Heimaey                     | 1-1.5     | 0.9-1.4  | Hallsdóttir 1984               |
| Vatnsmýri     | Reykjavík      | Reykjavík                     | SW | W | 12    | ... human influence on vegetation...                             | 0-2       | 0-2.5    | Hallsdóttir 1987               |
| Mosfell       | Grímsnesi      | Grímsnes- og Grafningshreppur | SW | S | 12    | ... human influence on vegetation...                             | 0-1.5     | 0-0.9    | Hallsdóttir 1987               |
| Þrándarholt   | Hreppum        | Skeiða- og Gnúpverjahreppur   | SW | S | 12    | ... human influence on vegetation...                             | 0-3       | 0-2.5    | Hallsdóttir 1987               |
| Tjörnin       | Reykjavík      | Reykjavík                     | SW | W | 28    | Saga lands og gróðurs  | 0.2-1.1   | 0.3-1.0  | Hallsdóttir 1992               |
| Viðey         | Reykjavík      | Reykjavík                     | SW | W | 29    | Frjókornarannsókn á mósniðum úr Viðey                            | 0-1.2     | 0-1.1    | Hallsdóttir 1993               |
| Foss á Síðu   | Síða           | Skaftárhreppur                | SE | S | unpl. | Cultivation history  | 0.7-1.5   | 0.7-1.4  | Hallsdóttir, M. unpublished    |
| Svalbarð      | Þistilfjörður  | Svalbarðshreppur              | NE | N | 30    | The Cultural Landscape of Iceland                                | 0-6.3     | 0-6.4    | Zutter 1997                    |
| Sævarland     | Þistilfjörður  | Svalbarðshreppur              | NE | N | 30    | The Cultural Landscape of Iceland                                | 0-6.3     | 0-7.3    | Zutter 1997                    |

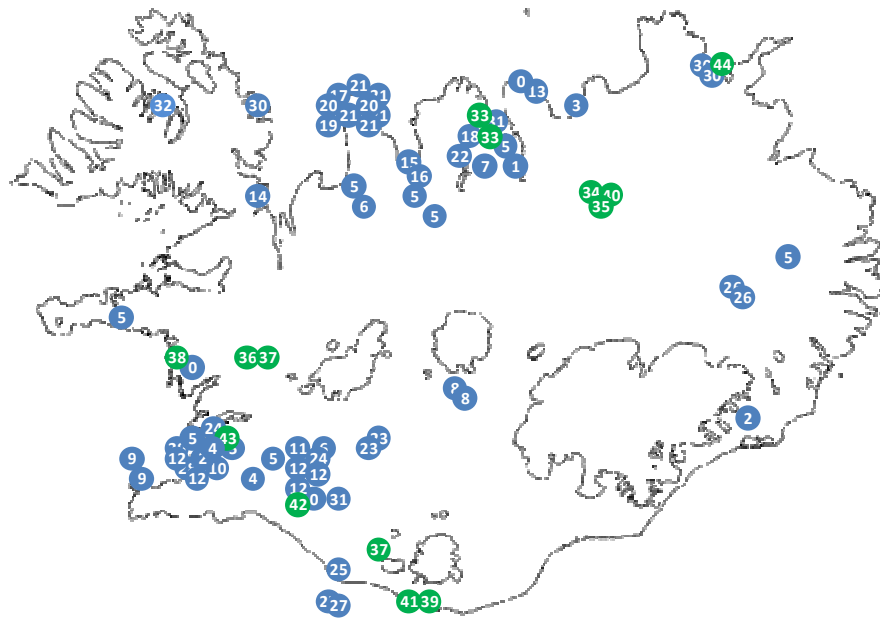
|                       |                      |                                  |    |          |    |  |         |          |                                |
|-----------------------|----------------------|----------------------------------|----|----------|----|--|---------|----------|--------------------------------|
| Gjögurvatn            | Strandir             | Árneshreppur                     | NW | N        | 30 | The Cultural Landscape of Iceland  | 0-6     | 0-6.9    | Zutter 1997                    |
| Efstadalsvatn         | Ísafjarðardjúp       | Súðavíkurhreppur                 | NW | W<br>(N) | 32 | Vegetation history   | 3.5-10  | 4-11     | Caseldine et al. 2003          |
| Hámundarstaða<br>háls | Eyjafirði            | Dalvíkurbyggð                    | NE | N        | 33 | Vegetation history   | 6-9     | 7-10.5   | Caseldine et al. 2006          |
| Vatnamýri             | Eyjafirði            | Dalvíkurbyggð                    | NE | N        | 33 | Vegetation history   | 6-9     | 7-10.5   | Caseldine et al. 2006          |
| Helluvaðstjörn        | Mývatnssveit         | Skútustaðahreppur                | NE | N        | 34 | Landscapes of settlement in northern Iceland   | 0-2.9   | 0-3      | McGovern et al. 2007           |
| Helluvaðstjörn        | Mývatnssveit         | Skútustaðahreppur                | NE | N        | 35 | Environmental impacts of the Norse settlement  | 0-3.3   | 0-3.5    | Lawson et al.                  |
| Reykholt              | Borgarfjörður        | Borgarbyggð                      | SW | W        | 36 | The palaeoecology of a high status Icelandic farm                                      | 0.1-0.9 | 0.1-0.9  | Sveinbjarnardóttir et al. 2007 |
| Stóra Mörk            | Undir<br>Eyjafjöllum | Rangárþing eystra                | SW | S        | 37 | The timing and causes of the final pre-settlement expansion of <i>Betula pubescens</i> | 1.2-1.6 | 1-1.5    | Erlendsson and Edwards 2009    |
| Reykholtsdalur        | Borgarfjörður        | Borgarbyggð                      | SW | W        | 37 | The timing and causes of the final pre-settlement expansion of <i>Betula pubescens</i> | 0.9-13  | 0.9-1.2  | Erlendsson and Edwards 2009    |
| Breiðavatn,           | Borgarfjörður        | Borgarbyggð                      | SW | W        | 38 | Lake sediment evidence for late Holocene climate change                                | 0-2.9   | 0-3      | Gathorne-Hardy et al. 2009     |
| Ketilsstaðir          | Mýrdalur             | Mýrdalshreppur                   | SW | S        | 39 | Vegetational response to human colonisation  | 0.5-1.3 | 0.5-1.2  | Erlendsson et al.              |
| Grænavatn             | Mývatnssveit         | Skútustaðahreppur                | NE | N        | 40 | Historical Resilience of Landscapes to Cultural and Natural Stresses                   | 0.2-1.2 | 0.2-1.1  | Colquhoun et al. 2010          |
| Stóra Mörk            | Undir<br>Eyjafjöllum | Rangárþing eystra                | SW | S        | 41 | 1000 years of environmental change and human impact                                    | 0.4-2.1 | 0.5-2    | Vickers et al. 2011            |
| Eyvík                 | Grímsnes             | Grímsnes- og<br>Grafningshreppur | SW | S        | 42 | ...hybridisation between <i>Betula pubescens</i> and <i>B. nana</i>                    | 6.8-9   | 7.6-10.3 | Karlsdóttir et al. 2012        |
| Hrísbú                | Mosfellsdalur        | Mosfellsbær                      | SW | W        | 43 | Feasting in Viking Age Iceland   | 0.5-1.3 | 0.5-1.2  | Zori et al. 2013               |
| Ytra-Áland            | Þistilfjörður        | Svalbarðshreppur                 | NE | N        | 44 | Birch hybridization in Thistilfjörður...   | 0-9     | 0-10.3   | Karlsdóttir et al. (submitted) |

Numbers of references follow Hallsdóttir and Caseldine (2005) with addaptions and additions. Sites with same name and reference have been combined, even if more than one profile were analyzed.

The period covered in each study is sometimes only a rough estimate.

The Miocene pollen research has been excluded.

In the table pollen sites are 77 and references 43.



*Figure 1 from main text. Icelandic Holocene pollen sites, based on list from Hallsdóttir and Caseldine (2005) with adaption. Blue points show sites studied up to 2005, green points after 2005. Point locations are approximate and some have been intentionally dislocated to enhance visibility. Numbers refer to reference numbers in Appendix A. Sites with unpublished results marked 0. Two sites, with results published in Hallsdóttir and Caseldine (2005), have the reference number 31 in blue.*

## References cited in Appendix A

- 1 Thorarinsson S (1944). *Tefrokronologiska studier på Island: Þjórsárdalur och dess förödelse*. Doctoral dissertation, Stockholms Högskola. pp. 217.
- 2 Okko VOT (1956). Glacial drift in Iceland: its origin and morphology. *Bull. Commission Geologique de Finlande* 170: 1-133.
- 3 Straka H (1956). Pollenanalytische Untersuchungen eines Moorprofiles aus Nord-Island. *Neues Jahrbuch für Geologie und Palaentologie* 6: 262-272.
- 4 Einarsson Th. (1957). Tvö frjólínurit úr íslenskum mómýrum. *Ársrit Skógræktarfélags Íslands 1957*: 89-97.
- 5 Einarsson Th (1961). *Pollenanalytische Untersuchungen zur spat-und postglazialen Klimageschichte Islands*. Sonder-veröff. Geologische Institut der Universität Köln 6: 52 pp.
- 6 Vasari Y and Vasari A (1990). L'histoire Holocène des lacs Islandais. In: Devers S (ed.), *Pour Jean Malaurie. 102 té-moignages en hommage à quarante ans d'études arctiques*. Paris: Éditions Plon: 277-293. [in French].
- 7 Bartley DD (1973). The stratigraphy and pollen analysis of peat deposits at Ytri Baegisa near Akureyri, Iceland. *GFF* 95: 410-414.
- 8 Friðriksdóttir SP (1973). *Frjógreining á jarðvegi úr Tjarnarveri og Sóleyjarhöfða*. BSc. thesis. University of Iceland. Verkfræði- og raunvísindadeild. Reykjavík. [In Icelandic].
- 9 Skaftadóttir Th (1974). *Um tvö frjólínurit af Romshvalanesi og ágrip af sögu þess*. BSc. thesis (Mimeograph). University of Iceland. Verkfræði- og raunvísindadeild. Reykjavík. [In Icelandic].
- 10 Sigthórsdóttir M (1976). *Frjógreining úr mó undan Búrfellshrauni*. Research Report. [In Icelandic].
- 11 Schwaar J (1978). *Moorkundliche Untersuchungen am Laugarvatn (Südwest-Island)*. Berichte aus der forschungsstelle Neðri-Ás, Hveragerði 29: 1-19.
- 12 Hallsdóttir M (1987). *Pollen analytical studies of human influence on vegetation in relation to the Landnám tephra layer in southwest Iceland*. LUNDQUA thesis 18, Lund University, Department of Quaternary Geology, 45 pages.
- 13 Hallsdóttir M (1991). Studies in the vegetation history of North-Iceland. A radiocarbon-dated pollen diagram from Flateyjarðalur. *Jökull* 40: 67-81.



- 14 Hansom JD and Briggs DJ (1991). Sea-Level Change in Vestfirðir, North West Iceland. In: *Environmental Change in Iceland: Past and Present* (pp. 79-91). Springer Netherlands.
- 15 Hallsdóttir M (1995). On the pre-settlement history of Icelandic vegetation. *Búvísindi* 9: 17-29.
- 16 Hallsdóttir M (1996). Synthesis of the Holocene history of vegetation in northern Iceland. *Palaeoklimaforschung* 20: 203-214.
- 17 Björck S, Ingólfsson Ó, Hafliðason H, Hallsdóttir M and Anderson NJ (1992). Lake Torfadalsvatn: a high resolution record of the North Atlantic ash zone I and the last glacial-interglacial environmental changes in Iceland. *Boreas* 21: 15-22.
- 18 Caseldine C and Hatton J (1994). Interpretation of Holocene climate change from the Eyjafjörður area of northern Iceland from pollen-analytical data: comments and preliminary results. *Münchener Geographische Abhandlungen* B12: 41-62.
- 19 Rundgren M (1995). Biostratigraphic evidence of the Allerød-Younger Dryas-Preboreal oscillation in Northern Iceland. *Quaternary research* 44, 405-416.
- 20 Rundgren M (1998). Early-Holocene vegetation of northern Iceland: pollen and plant macrofossil evidence from the Skagi peninsula. *The Holocene* 8: 553-564.
- 21 Rundgren M, Ingólfsson Ó, Björck S, Jiang H and Hafliðason H (1997). Dynamic sea-level change during the last deglaciation of northern Iceland. *Boreas* 26: 201-215.
- 22 Wastl M, Stötter J and Caseldine C (2001). Reconstruction of Holocene variations of the upper limit of tree or shrub birch growth in northern Iceland based on evidence from Vesturárdalur-Skíðadalur, Tröllaskagi. *Arctic, Antarctic, and Alpine Research* 33: 191-203.
- 23 Thórarinnsson S (1944). *Tefrokronologiska studier på Island*. Munksgaard, Copenhagen: 217 pp.
- 24 Einarsson Th (1963). Pollen analytical studies on the vegetation and climate history of Iceland in late and post-glacial times. In: Löve Á and Löve D (eds.) *North Atlantic biota and their history*, 355-365. Pergamon Press. Oxford.
- 25 Páhlsson I (1981). A pollen analytical study on a peat deposit at Lágafell, Southern Iceland. In: H. Haraldsson (Ed.), *The Markarfljót Sandur Area, Southern Iceland: Sedimentological, Petrological and Stratigraphical Studies*, *Stria* 15: 60-64
- 26 Hallsdóttir M (1982). Frjógreining tveggja jarðvegssniða úr Hrafnkelsdal. In: Thórarinsdóttir H, Óskarsson ÓH, Steinthórsson S and

- Einarsson Th (eds.). Eldur er í norðri. Reykjavík, Sögufélag. [In Icelandic].
- 27 Hallsdóttir M (1984). Frjógreining tveggja jarðvegssniða á Heimaey. *Árbók hins Íslenska forleifafélags* 1983, 48-68. [In Icelandic].
  - 28 Hallsdóttir M (1992). Saga lands og gróðurs. In: Nielsen ÓK (ed.) *Tjörnin, saga og lífríki*, 11-17. City of Reykjavík, Reykjavík.
  - 29 Hallsdóttir M (1993). *Frjórkornarannsókn á mósniðum úr Viðey. RH-08-93*. Raunvísindastofnun Háskólans and Árbæjarsafn. Reykjavík. [In Icelandic].
  - 30 Zutter C (1997). *The Cultural Landscape of Iceland: A Millennium of Human Transformation and Environmental Change*. Doctoral dissertation, University of Alberta. Edmonton. Canada.
  - 31 Hallsdóttir M and Caseldine CJ. (2005). The Holocene vegetation history of Iceland, state-of-the-art and future research. In: Caseldine CJ, Russell A, Hardardóttir J and Knudsen OF (eds.): *Iceland: Modern Processes and Past Environments*: pp 319-332. Elsevier, Amsterdam.
  - 32 Caseldine C, Geirsdóttir Á and Langdon P (2003). Efstadalsvatn – a multi-proxy study of a Holocene lacustrine sequence from NW Iceland. *Journal of Paleolimnology* 30: 55-73.
  - 33 Caseldine C, Langdon P and Holmes N (2006). Early Holocene climate variability and the timing and extent of the Holocene thermal maximum (HTM) in northern Iceland. *Quaternary Science Reviews* 25: 2314-2331.
  - 34 McGovern TH, Vésteinsson O, Fridriksson A, Church MJ, Lawson IT, Simpson IA, Einarsson A, Dugmore AJ, Cook GT, Perdikaris S, Edwards KJ, Thomson AM, Adderley WP, Newton AJ, Lucas G, Edvardsson R, Aldred O and Dunbar E (2007). Landscapes of settlement in northern Iceland: historical ecology of human impact and climate fluctuation on the millennial scale. *American anthropologist* 109: 27-51.
  - 35 Lawson IT, Gathorne-Hardy FJ, Church MJ, Newton AJ, Edwards KJ, Dugmore AJ and Einarsson A (2007). Environmental impacts of the Norse settlement: palaeoenvironmental data from Myvatnssveit, northern Iceland. *Boreas* 36: 1-19.
  - 36 Sveinbjarnardóttir G, Erlendsson E, Vickers K, McGovern TH, Milek KB, Edwards KJ, Simpson IA and Cook, G. (2007). The palaeoecology of a high status Icelandic farm. *Environmental Archaeology* 12: 187-206.
  - 37 Erlendsson E and Edwards KJ (2009). The timing and causes of the final pre-settlement expansion of *Betula pubescens* in Iceland. *The Holocene* 19: 1083-1091.

- 38 Gathorne-Hardy FJ, Erlendsson E, Langdon PG and Edwards KJ (2009). Lake sediment evidence for late Holocene climate change and landscape erosion in western Iceland. *Journal of Paleolimnology* 42: 413-426.
- 39 Erlendsson E, Edwards KJ and Buckland PC (2009). Vegetational response to human colonisation of the coastal and volcanic environments of Ketilsstaðir, southern Iceland. *Quaternary Research* 72: 174-187.
- 40 Colquhoun L, Tisdall E, Smith H and Simpson I (2010). *Historical Resilience of Landscapes to Cultural and Natural Stresses: Grænavatn farm estate, Mývatnssveit north-east Iceland*. Report to NABO-NSF, School of GeoSciences at the University of Edinburgh.
- 41 Vickers K, Erlendsson E, Church MJ, Edwards KJ and Bending J (2011). 1000 years of environmental change and human impact at Stóra-Mörk, southern Iceland: a multiproxy study of a dynamic and vulnerable landscape. *The Holocene* 21: 979-995.
- 42 Karlsdóttir L, Hallsdóttir M, Þórsson ÆTh and Anamthawat-Jónsson K (2012). Early Holocene hybridisation between *Betula pubescens* and *B. nana* in relation to birch vegetation in Southwest Iceland. *Review of Palaeobotany and Palynology* 181: 1-10.
- 43 Zori D, Byock J, Erlendsson E, Martin S, Wake T and Edwards KJ (2013). Feasting in Viking Age Iceland: sustaining a chiefly political economy in a marginal environment. *Antiquity* 87: 150-165.
- 44 Karlsdóttir L, Hallsdóttir M, Eggertsson Ó, Þórsson ÆTh and Anamthawat-Jónsson K (submitted). Birch hybridization in Thistilfjörður, Northeast Iceland during the Holocene. The paper has been submitted to the journal *IAS*