



Grana

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/sgra20>

Differentiating pollen of *Betula* species from Iceland

Lilja Karlsdóttir^a, Ægir Th. Thórsson^a, Margrét Hallsdóttir^b, Adalsteinn Sigurgeirsson^c, Thröstur Eysteinnsson^d & Kesara Anamthawat-Jónsson^a

^a Institute of Biology, University of Iceland, Reykjavik, Iceland

^b Icelandic Institute of Natural History, Reykjavik, Iceland

^c Icelandic Forest Research (Research Division of the Icelandic Forest Service), Mógilsá, Reykjavik, Iceland

^d Iceland Forest Service, Egilsstaðir, Iceland

Available online: 31 May 2007

To cite this article: Lilja Karlsdóttir, Ægir Th. Thórsson, Margrét Hallsdóttir, Adalsteinn Sigurgeirsson, Thröstur Eysteinnsson & Kesara Anamthawat-Jónsson (2007): Differentiating pollen of *Betula* species from Iceland, *Grana*, 46:2, 78-84

To link to this article: <http://dx.doi.org/10.1080/00173130701237832>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.tandfonline.com/page/terms-and-conditions>

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Differentiating pollen of *Betula* species from Iceland

LILJA KARLSDÓTTIR¹, ÆGIR TH. THÓRSSON¹, MARGRÉT HALLSDÓTTIR²,
ADALSTEINN SIGURGEIRSSON³, THRÖSTUR EYSTEINSSON⁴ & KESARA
ANAMTHAWAT-JÓNSSON¹

¹Institute of Biology, University of Iceland, Reykjavik, Iceland, ²Icelandic Institute of Natural History, Reykjavik, Iceland,

³Icelandic Forest Research (Research Division of the Icelandic Forest Service), Mógilsá, Reykjavik, Iceland, and ⁴Iceland Forest Service, Egilsstaðir, 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

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.

Material 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, based on growth form, habit and leaf morphology), 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 preparation by standard methods (Fægri & Iversen, 1989) including sequential treatment in hot solutions of 10% NaOH for 15 min, 10% HCl for 5–10 min, 40% HF for 5 min and 10% HCl for 10 min, followed by acetolysis and dehydration in ethanol and TBA buffer. Individual pollen grains were examined under $400\times$ magnification with 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 $0.3\ \mu\text{m}$ (Micrometer 2 mm 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. When pollen shape was unbalanced, the largest diameter and pore depth were used. 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 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. See also Figure 4. The altitude is shown as the average level for the woodland. Note: *Only *Betula pubescens* was found in this area.

Location	Label	Latitude (°N)	Longitude (°W)	Altitude (m)
Brekuskógur	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

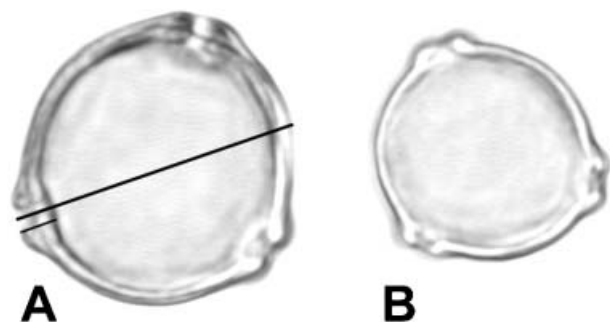


Figure 1. Microscope images of *Betula* pollen under the same magnification: (A) *B. pubescens* and (B) *B. nana*. Black lines show grain diameter (24 µm) and pore depth (3.6 µm).

Results

Pollen diameters (D), pore depths (P) and D/P ratios were obtained from a total of 9 021 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). 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 (Poisson-type) 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 µm for *B. nana* and *B. pubescens* respectively, and the cut-point between the two species distribution curves was at 22.1 µm (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 µm 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 µm for *B. nana* and *B. pubescens* respectively, and the cut-point between the two species was approximately at 2.5 µm (Figure 2B, Table II). The

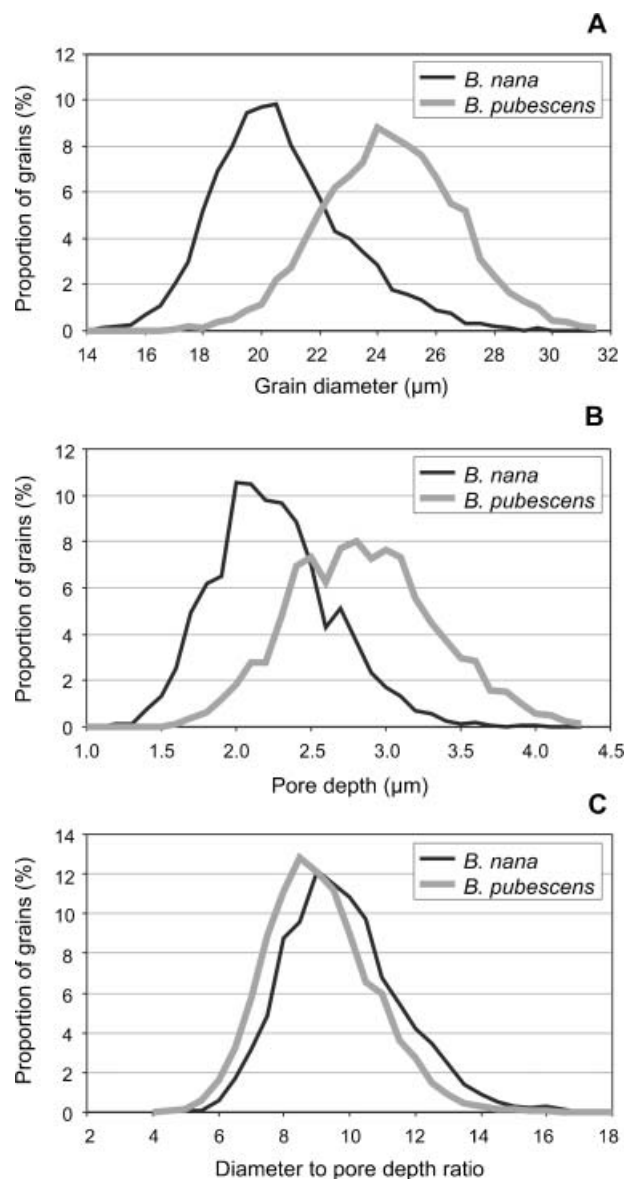


Figure 2. Cumulative distributions by species of (A) pollen grain diameter, (B) pore depth and (C) ratio of diameter to pore depth.

species-specific 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. However, 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 (84%) to make this parameter useful for species differentiation (Figure 2C). Neither pore depths nor D/P ratios were analysed further statistically in this study.

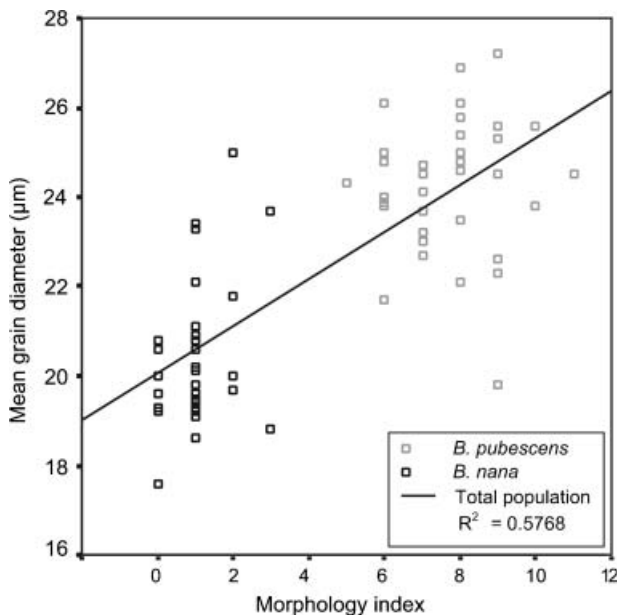
Positive correlation was found between mean pollen size (i.e. grain diameter) and plant morphology index of individual trees/shrubs (Figure 3).

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

	<i>B. nana</i> number of plants=31 number of pollen grains=3998				<i>B. pubescens</i> number of plants =39 number of pollen grains =5023			
	mean	SD	min	max	mean	SD	min	max
Grain diameter (D, μm)	20.42	2.29	14.3	30.4	24.20	2.36	14.3	33.9
Pore depth (P, μm)	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			

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*.

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). However, 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/sites (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 difference in pollen diameter among different sites was further analysed using Tukey's HSD test to compare the mean values. The test divided the mean pollen diameters into six groups for each species (groups a–f), from the smallest to the largest means of pollen diameter, and at each site the means could fit into one to two groups but they were significantly different from the rest (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 *Þökulsá í Lóni*, eastern Iceland). However, *B. nana* pollen grains from most (six 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

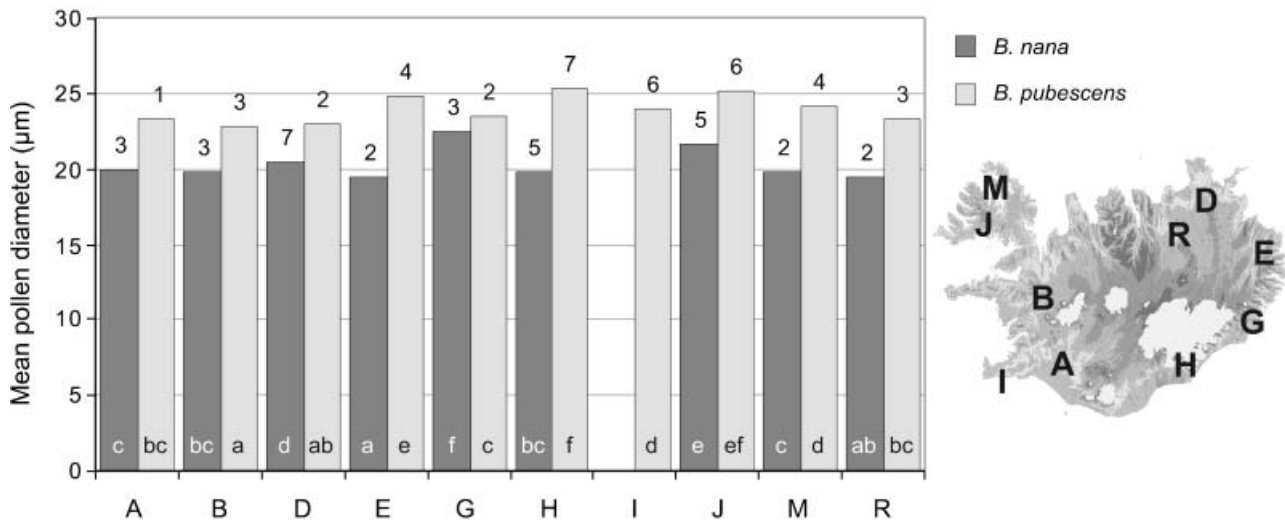


Figure 4. Mean grain diameters of *Betula* pollen by species and sites. Numbers above the columns indicate number of individual trees/shrubs per site. Small letters at the bottom of columns show results of Tukey's HSD test^{a,b,c} for each species separately, columns with different letters differ significantly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

($r=0.02$) 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.

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 sig-

nificance 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 18.5% if calculated from the mean values (20.42 µm for *B. nana* and 24.20 µm for *B. pubescens*), whereas the size difference deduced from other studies is in the range of 25–45% (Prentice, 1981; Mäkelä, 1996; Caseldine, 2001), larger than that in the present study. The mean grain diameters of *B. nana* pollen from other studies (Birks, 1968; Mäkelä, 1999;

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

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

Caseldine, 2001) are 15.50–19.23 μm , 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 μm . 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, several explanations are possible. 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 explanation 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 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 made 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 (Rannis: grant no. 040238021).

References

- Anamthawat-Jónsson, K. (2003a). Hybrid introgression in *Betula*. In A. K. Sharma & A. Sharma (Eds), *Plant genome – Biodiversity and evolution*, Vol. 1, *Phanerogams* (pp. 249–265). Enfield, Plymouth: Sci. Publ. Inc.
- Anamthawat-Jónsson, K. (2003b). Preparation of chromosomes from plant leaf meristems for karyotype analysis and *in situ* hybridisation. *Methods Cell Sci.*, 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. *Can. J. For. Res.*, 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. *J. Ecol.*, 80, 837–870.
- Birks, H. J. B. (1968). The identification of *Betula nana* pollen. *N. Phytol.*, 67, 309–314.
- Blackmore, S., Steinmann, J. A. J., Hoen, P. P. & Punt, W. (2003). The Northwest European Pollen Flora 65: Betulaceae and Corylaceae. *Rev. Palaeobot. Palynol.*, 123, 71–98.
- Caseldine, C. (2001). Changes in *Betula* in the Holocene record from Iceland – a palaeoclimatic record or evidence for early Holocene hybridisation? *Rev. Palaeobot. Palynol.*, 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.
- Fægri, K. & Iversen, J. (1989) *Textbook of pollen analysis* (4th rev. ed. K. Fægri, P. E. Kaland & K. Krzywinski). Chichester, New York, Brisbane, Toronto, Singapore: J. Wiley & Sons.
- Fredskild, B. (1991). The genus *Betula* in Greenland – Holocene history, present distribution and synecology. *Nord. J. Bot.*, 11, 393–412.
- Hallsdóttir, M. (1990). Studies in the vegetational history of North Iceland. A radiocarbon-dated pollen diagram from Flateyrdalur. *Jökull*, 40, 67–81.
- Hultén, E. & Fries, M. (1986). *Atlas of North European Vascular Plants*. Köningstein: Koeltz Sci. Books.
- Leitch, I. J. & Bennett, M. D. (2004). Genome downsizing in polyploidy plants. *Biol. J. Linn. Soc.*, 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*. Helsinki: Dept Geol. Div. Geol. & Palaeontol. Univ. Helsinki.
- Prentice, I. C. (1981). Quantitative birch (*Betula* L) pollen separation by analysis of size frequency data. *N. Phytol.*, 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. *J. Hered.*, 92, 404–408.
- Vaarama, A. & Valanne, T. (1973). On the taxonomy, biology and origin of *Betula tortuosa* Ledeb. *Rep. Kevo Subarct. Res. St.*, 10, 70–84.
- Walters, S. M. (1964). Betulaceae. In T. G. Tutin, V. H. Heywood, N. A. Burges, D. H. Valentine, S. M. Walters & D. A. Webb (Eds), *Flora Europaea*, vol. I (pp. 57–59). Cambridge: Univ. Press.