

Genetic and environmental factors affecting the allergenicity of birch (*Betula pubescens* ssp. *czerepanovii* [Orl.] Hämet-Ahti) pollen

J. U. AHLHOLM, M. L. HELANDER and J. SAVOLAINEN*

Unit of Aerobiology and Mycological Ecology, Department of Biology and *Medicity Research Laboratory, Department of Pulmonary Diseases and Clinical Allergology, University of Turku, Turku, Finland

Summary

Background Environmental variation, such as an increase of mean temperature due to the greenhouse effect, as well as the genetic factors may affect the allergenicity of pollen and thus, the prevalence of allergies. The connection between these factors and the allergen content of pollen is poorly understood.

Objectives To evaluate the role of environmental and genetic factors on the allergenicity of birch pollen.

Methods Mountain birch (*Betula pubescens* ssp. *czerepanovii* (Orl.) Hämet-Ahti) pollen was studied using SDS-PAGE and IgE-immunoblotting. Pollen samples were collected from the trees of 10 half-sib families. The study trees from each family were reared in two tree line gardens where the daily mean temperatures were different during the growing season.

Results The quantitative analysis of band intensities suggested that the responses of the major birch pollen allergen, Bet v 1, were stronger in the samples collected from the garden with higher daily mean temperature. Half-sib families and individual trees differed in their Bet v 1 content.

Conclusions Our results show that both genetic and environmental factors have an effect on the amount of Bet v 1. This suggests that breeding for trees low in allergen content may be possible.

Keywords: allergen, genetic factors, *Betula*, birch, immunoblotting pollen, temperature

Clinical and Experimental Allergy, Vol. 28, pp. 1384–1388. Submitted 3 October 1997; revised 3 December 1997; accepted 8 May 1998.

Introduction

Birch (*Betula* sp.) pollen is the main cause of seasonal allergy in northern European countries. The results of some studies on birch (*Betula pendula* Roth. syn. *Betula verrucosa* Ehrh.) trees have shown that environmental factors, such as air pollution [1,2], light intensity or temperature [3,4], may affect the allergenicity of birch pollen. According to Hjelmroos *et al.* [3], the variation of allergens in pollen was probably due to higher light intensity or temperature on the south sides of trees. This was seen by the higher number

of allergens in pollen collected from the south and west sides of birch trees than other sides of trees.

In addition to variation within the individual trees, temperature differences between growing sites could also produce allergenic variation of pollen between the birch trees. Thus, the mean temperatures of the growing season could be an important factor affecting the allergenicity of birch pollen. In that case, a possible rise of mean temperature, caused by the greenhouse effect, could lead to changes in allergen content of pollen. This could affect the prevalence of birch pollen allergies, especially near the northern distribution limit of birch trees where temperature is possibly a significant factor limiting the growth [5,6].

Correspondence: J. U. Ahlholm, Department of Biology, University of Turku, FIN-20014 Turku, Finland.

Genetic factors of trees may have an effect on the allergenicity of pollen. For example, the number of different allergens or the mean level of allergens in pollen can be inherited from the parent trees. Between growing sites, the inheritance of allergenicity could lead to the formation of regional differences in the allergenicity of birch pollen. This is because the seeds of a single mother tree mainly spread in the vicinity of the mother tree, although, for example, wind velocity, the release heights of seeds and local vegetation affect to the dispersal of seeds [7–10].

One of the purposes of the present study was to clarify the role of temperature with respect to the allergenicity of birch pollen. The pollen samples were collected from two tree line gardens located at two different altitudes above sea level. During the growing season, the mean temperatures are higher in the garden located at the lower altitude. The objective of this study was also to compare the allergen content of pollen between different progenies of birch trees and evaluate the possible effects of genetic factors on the allergenicity of mountain birch pollen.

Materials and methods

Study trees, area and sampling

In Fennoscandia the most common species of the genus *Betula* are *B. pubescens* Ehrh., *B. pendula* Roth. and *B. nana* L. Mountain birch (*Betula pubescens* ssp. *czerepanovii* (Orl.) Hämet-Ahti), the species studied in this research, is one of the subspecies of *B. pubescens* Ehrh. whose range extends into northern most Finland.

The mountain birch pollen samples were collected at Kevo Subarctic Research station (69°45'N, 27°01'E) in June 1996. The study area was situated close to the arctic tree line in northern Finland. The trees studied were grown in two tree line gardens called TLG1 and TLG2. TLG1 is located in the bottom of Utsjoki river valley (90 m above sea level) and TLG2 on Skallovaara hill (270 m asl). During the growing season, the daily mean temperatures are on average 1.0–2.5°C lower and the growing seasons are shorter in TLG2 compared with that in TLG1. The year before the sampling (1995), the difference in the daily mean temperatures was on average 1.1°C (June–August) between the gardens. In 1996, the birch pollen season started approximately 2 weeks later in TLG2 than in TLG1. The distance between these two gardens is 5 km.

The study trees were saplings of 10 different mother trees forming 10 progeny groups (A–J). The trees of each progeny were reared in both tree line gardens, except for progeny F which were grown only in TLG1. The mother trees were from different locations in northern Finland and they were open-pollinated and therefore the individuals of a

certain progeny are most likely half-sibs. In 1996, the range of heights of these 20-year-old trees was 1.5–4 m.

Pollen samples were collected from 53 mountain birches (*Betula pubescens* ssp. *czerepanovii* Orl.). Thirty-eight of them were situated in TLG1 and 15 in TLG2. Semi-permeable pollination bags were put over each catkin and they were checked daily. Immediately after anthesis, the bags with released pollen were taken to the laboratory for the pollen to be weighed. The pollen samples, stored in liquid nitrogen, were transferred to Turku, southern Finland, for biochemical analyses.

Pollen extracts

Pollen was extracted 1:10 (w/v) in 0.125 mol/L ammonium carbonate with 0.015 mol/L sodium azide for 3 h at 4°C. The volume (mL) of each extract was directly proportional to raw material. Extracts were centrifuged at 12 000 g for 30 min and supernatants collected and stored at –70°C.

Sera

Sera were obtained from 24 birch allergic patients and five healthy persons. All the sera were individually tested with the international standard of birch pollen extract (ALK, Copenhagen, Denmark) by IgE-immunoblotting. A patient serum pool was prepared from equal volumes of eight patient sera. Three healthy sera were chosen for the negative control serum pool. The same patient sera pool was used in all experiments.

SDS-PAGE and electrophoretic blotting

Pollen antigens and molecular weight markers were separated with SDS-PAGE in 5–17.5% gradient gel according to Laemmli [11]. After SDS-PAGE, proteins were transferred to nitrocellulose membrane (NCM). For the quantitative analysis of band intensities, internal standard samples were used in each gel in order to standardize the differences in the transfer of proteins. IgE-binding to antigens was studied according to Bengtson *et al.* [12], but instead of X-ray films, the radioactively labelled NCMs were visualized by a sensitive phosphor imager system (Bio Rad Laboratories, CA, USA). In order to confirm that different exposures were comparable, ¹²⁵I-conjugated anti-IgE antibodies reference disks were used as described by Aukurst *et al.* [13].

NCMs were incubated in PBS containing 0.5% Tween to block unoccupied protein binding sites. After blocking, each NCM was incubated for 12 h in serum pool and washed in 0.9% NaCl containing 0.5% Tween. NCMs were incubated in ¹²⁵I anti-IgE (cap RAST-RIA, Pharmacia) solution

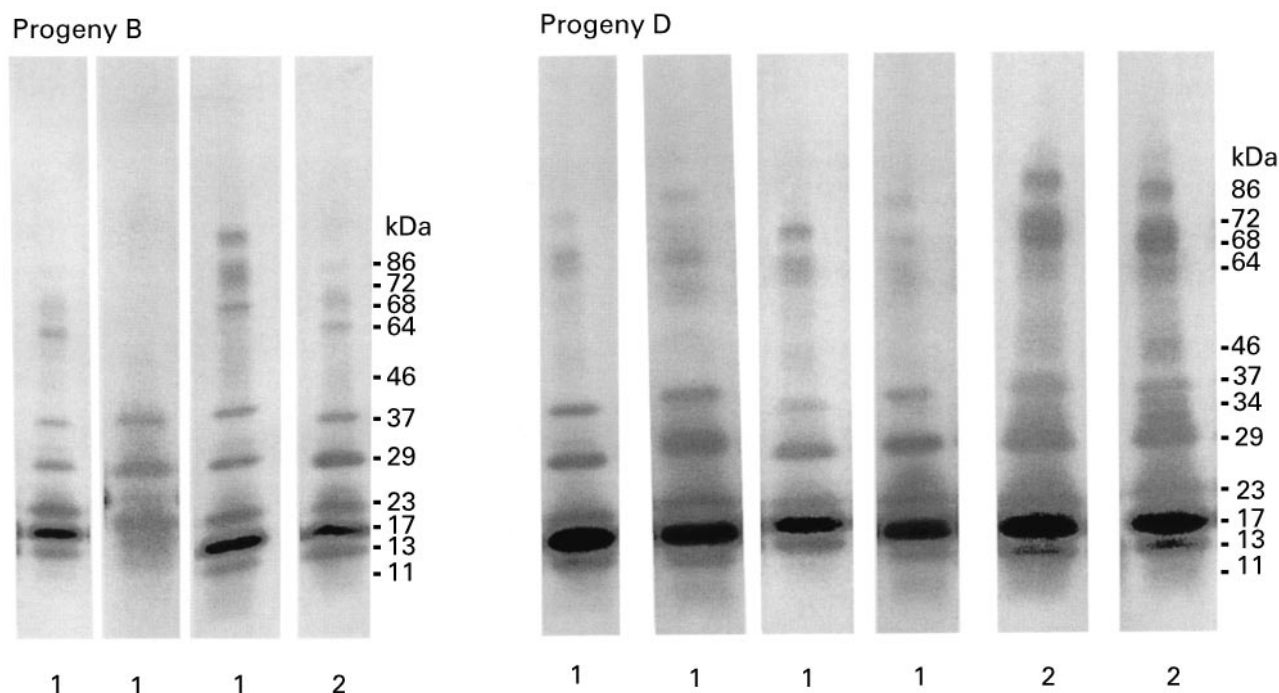


Fig. 1. Immunoblotting analysis of the reactivity of IgE antibodies to components of the birch pollen extracts in two different progenies (B and D). Tree line gardens are indicated in both progeny as follows: TLG1, 1 and TLG2, 2.

diluted to 1:10 in PBS-Tween for 4 h at room temperature. NCMs were washed in NaCl-Tween and distilled water. Radioactively labelled nitrocellulose sheets were exposed to molecular imaging screens (Bio Rad). The bands were visualized and the intensities of bands

were measured with molecular analysis 1.1 software (Bio Rad). Since the transfer efficient was slightly different between the immunoblots, the band intensities of the samples were normalized to the bands in the standard samples and expressed as percentages (band/standard band*100).

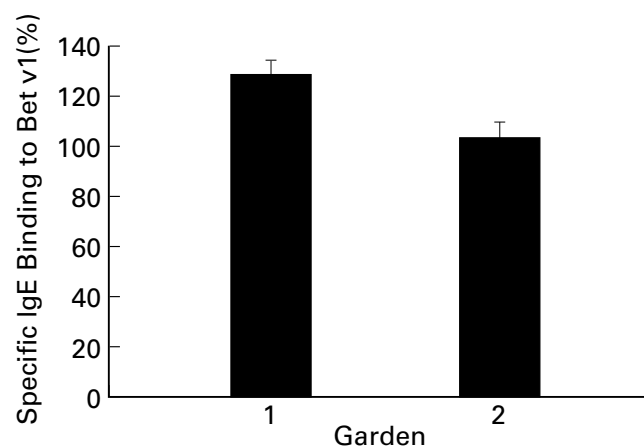


Fig. 2. The means and the standard errors of the band intensities of Bet v 1 in pollen samples collected from the tree line gardens. Values are proportional to standard bands and expressed as percentages. The bars were significantly different (Tukey's test, $P < 0.05$).

Statistical analysis

In the statistical data analysis, analysis of variance (ANOVA) was used, with descendant group and location of garden as factors. The statistical tests were performed with the GLM-procedure (SAS Institute Inc [14]). ANOVA was followed by pair-wise comparisons with Tukey's test.

Results

The pooled sera contained IgE-antibodies against proteins with the molecular weights of 11, 13, 17, 23, 29, 34, 37, 46, 64, 68, 72 and 86 kDa (Fig. 1). All the bands, except the band of 34 kDa, were detected in all of the progenies and in the pollen extracts from both tree line gardens. In the quantitative analysis of band intensities, the responses to Bet v 1 (17 kDa) were significantly stronger in the samples collected from TLG1 than TLG2 (Fig. 2, Table 1). The main effect of progeny was also significant. In pair-wise comparison, the mean peak area

of Bet v 1 in the progeny group B was significantly lower than in groups C, D, F, G, H and J (Fig. 3). For the proteins of 13, 23, 29 and 37 kDa none of the sources of variation was significant.

The total number of bands did not vary significantly in relation to half-sib family or to location of garden (Table 2), although minor differences between individual birch trees were found. For example, the band of 34 kDa was only detected in a single pollen extract from a tree in descendant group D, and birch major allergen Bet v 1 (17 kDa) was nearly absent in a pollen extract of an individual tree in half-sib family B (Fig. 3).

Discussion

The results of the present study clearly suggest that both genetic and environmental factors affect the level of the synthesis of Bet v 1. The responses to Bet v 1 were clearly stronger in the samples collected from TLG1 than TLG2. This indicates that the amount of Bet v 1 was higher in the garden in which the daily mean temperatures are higher during the growing season. Thus, temperature seems to especially affect the allergen content of birch pollen. This effect could have been originated during the previous growing season [3], when the development of pollen begins, or during the following spring when pollen development ends [15] and Bet v 1 is synthesized [16].

The cumulative temperature sum seemed to be a trigger for anthesis, at the beginning of flowering, at 48 °C for both gardens. The snow melted almost 2 weeks before the first flowering trees in TLG1, in contrast, in TLG2, ~30% of the garden was covered by snow at the onset of anthesis. Based on these observations, the soil temperature was probably lower in TLG2 than TLG1 before and during anthesis.

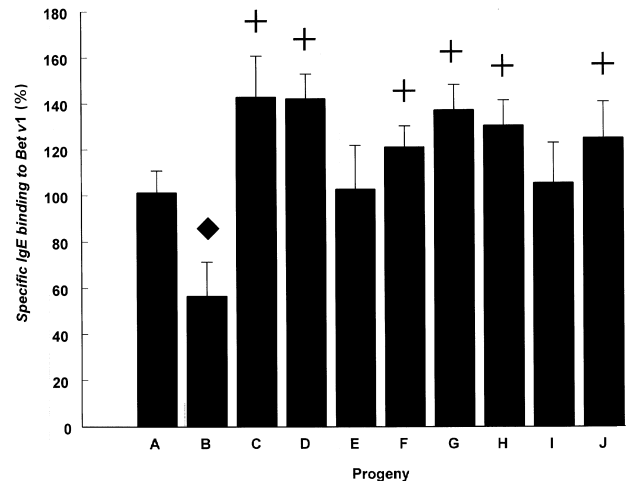


Fig. 3. The means and standard errors of the band intensities of Bet v 1 in the different progenies (A–J). The values are expressed as percentages in relation to the standard bands. The bars with different symbols were significantly different (Tukey's test, $P < 0.05$).

The low soil temperature is probably a critical factor limiting the nutrient uptake and the growth of mountain birch in the arctic regions [6]. Root nitrogen uptake, plant nitrogen concentrations and the growth rate of the trees strongly increase at soil temperatures between 5 and 10 °C. Thus, low soil temperatures could also be a factor affecting the rate of allergen synthesis in pollen. Bet v 1 is synthesized just prior to the release of pollen during the spring, when the diurnal temperatures are low [16]. At that time, even small changes in soil temperature may cause changes in the allergen synthesis of pollen. Thus, the variation of the amount of Bet v 1 between the gardens suggest that possible future climatic changes, such as increasing mean

Table 1. ANOVA table for differences in band intensities of Bet v 1 in relation to progeny and the location of tree lane gardens (TLG)

Source of variance	df	SS	MS	F	P
TLG	1	10999.39	10999.39	14.40	0.0006***
Progeny	9	30328.74	3369.86	4.41	0.0007***
TLG*Progeny	7	2557.68	365.86	0.48	0.8437
Error	35	26741.37	764.04		

Table 2. ANOVA table for the number of bands in relation to progeny and location of tree lane garden (TLG)

Source of variance	df	SS	MS	F	P
TLG	1	0.17	0.17	0.12	0.7344
Progeny	9	10.46	1.16	0.79	0.6243
TLG*Progeny	7	2.00	0.28	0.20	0.9845
Error	35	51.25	1.46		

temperatures and changes in precipitation (snow), may cause changes in the allergenicity of birch pollen and, thus, affect the prevalence of hay fever.

The IgE-binding profiles of the samples were very similar to each other and the number of allergens was invariable between the different progeny groups. Thus, it seems evident that the genomes of mother trees do not cause significant variation in the number of allergens of mountain birch pollen. Further, the results imply that male genomes do not have a significant influence on the number of allergens in pollen. It is obvious that the mother trees were pollinated by different father trees, since mother trees were grown in different locations in northern Finland and birch trees are cross-pollinated. If the genomes of father trees caused variation in the number of allergens, immunoblot analysis should reveal more differences in the number of allergens between the half-sib families and, perhaps, between the study trees within the descendant groups. The number of the allergens of pollen appears to be quite invariable between the individual mountain birches.

In studies focused on the interactions of different environmental factors and the allergenicity of pollen, both genetic and environmental factors have to be taken into consideration when planning the pollen sampling. These factors seem to have an effect, at least on birch trees, on the amount of allergens in pollen. If pollen samples are collected from a restricted area, the genomes of study trees and especially temperature differences between the sampling areas can diminish the reliability of the results of other studied factors, e.g. air pollution. Further research is needed to confirm these results in other allergologically important species.

Acknowledgements

We thank Kaija Peltola for valuable help in the field. For comments on the text and fruitful discussion, we would like to express thanks to Auli Rantio-Lehtimäki and Eija Yli-Panula.

References

- 1 Breitenbach M, Swoboda I, Scheiner O, Heberle-Bors E, Kraft D. Tree pollen allergens: functional and environmental aspects. In Proceedings of the International Workshop-Symposium on Molecular Biology and Immunology of Allergens. CRC Press, Vienna, 1992.
- 2 Jilek A, Swoboda I, Breiteneder H et al. Biological functions isoforms and environmental control in the Bet v I gene family. In: Kraft D, Sehon A, eds Molecular biology and immunology of allergens. Boca Raton, Florida: CRC Press. 1993.
- 3 Hjelmroos M, Schumacher MJ, Van Hage-Hamsten M. Heterogeneity of pollen proteins within individual. *Betula pendula* trees. *Int Arch Allergy Appl Immunol* 1995 108:368–76.
- 4 Helander ML, Savolainen J, Ahlholm J. Effects of air pollution and other environmental factors on birch pollen allergens. *Allergy* 1997 52:1207–14.
- 5 Nordell KO, Karlsson PS. Resorption of nitrogen and dry matter prior to leaf abscission: Variation among individuals, sites and years in the mountain birch. *Func Ecol* 1995 9:326–33.
- 6 Karlsson PS, Nordell KO. Effects of soil temperature on the nitrogen economy and growth of mountain birch seedlings near its presumed low temperature distribution limit. *Ecoscience* 1996 3:183–9.
- 7 Greene DF, Johnson EA. A model of wind dispersal winged or plumed seeds. *Ecology* 1989 70:339–47.
- 8 Houle G, Payette S. Seed dynamics of *Betula alleghaniensis* in a deciduous forest of north-eastern North America. *J Ecol* 1990 78:677–90.
- 9 Houle G. Spatial relationship between seed and seedling abundance and mortality in a deciduous forest of northeastern North America. *J Ecol* 1992 80:99–108.
- 10 Matlack GR. Influence of fruit size and weight on wind dispersal in. *Betula lenta*, a gap-colonizing tree species. *Am Midland Naturalist* 1992 128:30–9.
- 11 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227:680–5.
- 12 Bengtson A, Rolfsen W, Einarsson R. Characterization of allergens and patient sera by a nitrocellulose immunoprint technique. *Int Arch Allergy Appl Immunol* 1985 78: 139–44.
- 13 Aukurst L, Aas KA. reference system in crossed radio-immunoelectrophoresis. *Scand J Immunol* 1977 6:1093–9.
- 14 SAS Institute Inc. SAS/STAT User's Guide, Version 6, Fourth Edn, Volume 2, Cary, NC, USA, 1989.
- 15 Dunbar A, Rowley JR. *Betula* pollen development before and after dormancy: Exine and intine. *Pollen Spores* 1984 26:299–338.
- 16 Swoboda I, Dang TCH, Heberle-Bors E, Vicente O. Expression of Bet v 1, the major birch pollen allergen, during anther development. An in situ hybridization study. *Protoplasma* 1995 187:103–10.