



The influence of hybridization on epidermal properties of birch species and the consequences for palaeoclimatic interpretations

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Abstract

The Fennoscandian birch population primarily consists of *Betula nana*, *B. pendula* and *B. pubescens* ssp. *czerepanovii*, the Mountain birch. Frequent hybridization between the Mountain birch and *B. nana* generates a wide range of genotypic and phenotypic plasticity in the subarctic birch zone of Fennoscandia. Phases of subarctic conditions prevailed during the Late Glacial in large parts of NW Europe, and palynological as well as macrofossil analysis provide some evidence for the occurrence of birch hybrids during these intervals. Leaves from genetically controlled specimens of *Betula pendula*, *B. pubescens* ssp. *czerepanovii*, *B. nana* and the hybrids *B. pubescens* ssp. *czerepanovii* × *nana* and *B. nana* × *pubescens* ssp. *czerepanovii* are investigated for their specific characteristics of the epidermis morphology. Frequency and size of epidermal cells and stomata reveal a close affinity of both hybrids to *B. nana* and allow a differentiation of the intermediate forms between *B. nana* and the Mountain birch. With respect to palaeoatmospheric CO₂ reconstructions based on stomatal index, epidermal analysis shows that a possible occurrence of hybrids in fossil leaf assemblages has no profound consequences for combined species records. However, the significant differences observed in *B. nana* demand the separation of this species. A comparison of the cuticle properties of *B. pendula* and *B. pubescens* from Finnish Lapland and leaf material from The Netherlands reveals a divergence of the stomatal index that may be due to differences in day light length.

Introduction

The Fennoscandian birch assemblage primarily consists of three species, namely *Betula pendula*, *B. pubescens* (ssp. *pubescens* as well as ssp. *czerepanovii*, the Mountain birch) and *B. nana*. In general, these species are separated by an effective incompatibility system based on length of the growing season and temperature (Stern 1963; Hagman 1971). However, in the northernmost areas, hybridization and introgression between the species generate a wide range of genotypic and phenotypic variability in the birch population. The occurrence of hybrids is due to an adaptive response to the specific environmental factors prevailing within the subarctic realm (Sulkinoja 1990). Shortening of the growing season, in combination with the reduction of the thermal sum, induces the synchronous anthesis of the three birch species that

enables hybridization (Kallio et al. 1983). The resulting hybrids constitute a common element in the birch zone north of the coniferous forest line. Characterized by intermediate morphological and genetical features, frequent hybrids arise from crossing of Mountain birch and *B. nana*.

The present occurrence of hybrids is likely to be related to the afforestation of Fennoscandia after the last deglaciation (Kallio et al. 1983). The specific subarctic environmental conditions that provoke the occurrence of hybrids between Mountain birch and *B. nana* are today restricted to the area extending from the northern part of the Kola Peninsula, through the mountain region of Fennoscandia, to Iceland and South Greenland. In the Late Glacial, episodes of low mean summer temperatures characterized large areas of the cool-temperate zone of the Northern Hemisphere. The past environmental conditions must have been closely

comparable to those that nowadays support hybridization of the different *Betula* species. Macrofossil and pollen analysis provides some insight in the spatial and temporal distribution patterns of the Mountain birch and hybrids in the geological past. Evidence for intermediate birch forms (*B. cf nana* × *pubescens*; Van Geel et al. 1989) in The Netherlands is available from catkin scales concentrated around the Older Dryas, a short cooling pulse during the Bølling–Allerød Interstadial. The statistical analysis of size distribution in fossil birch pollen from sites in Finnish Lapland has recently indicated that phases of extensive hybridization are likely to have taken place in periods of climatic deterioration during the Holocene (Mäkelä 1998). Unfortunately, the analytical differentiation of the pure birch species and the hybrid pollen is rather problematic, since the pollen grain size is strongly influenced by differential effects of preservation of fossil material as well as by preparation (Mäkelä 1996; Mäkelä 1998).

Epidermal properties of deciduous tree leaves are currently the subject of intensive investigations, because the stomatal frequency is negatively correlated to changing atmospheric CO₂ levels (e.g. Woodward 1987; Peñuelas and Matamala 1990; Beerling 1992; Wagner et al. 1996; Kürschner et al. 1997). This relation is increasingly applied as a palaeobotanical proxy for changes in palaeoatmospheric CO₂ concentrations (Van der Burgh et al. 1993; Beerling et al. 1995; Wagner et al. 1999). However, the rate of phenotypic stomatal frequency response to changing CO₂ is specific at genus or even species level (Kürschner et al. 1997). *B. pendula* and *B. pubescens* are highly sensitive to CO₂ changes and their widespread occurrence in late Quaternary leaf assemblages make them excellent taxa for palaeoclimatic studies (Wagner et al. 1996; Kürschner et al. 1997; Wagner et al. 1999).

Because stomatal frequency is applied as proxy for palaeoatmospheric CO₂ reconstructions, and birch hybrids may be present in the fossil record, it is important to test the influence of hybridization on stomatal properties.

In the present study, a variety of epidermal properties of leaves from genetically controlled birch species and hybrids are analyzed in order to recognize specific morphological characteristics for the pure species and the degree of alteration caused by hybrid forming.

Epidermal characteristics of modern leaves may provide an actualistic background for studying past distribution patterns of the Mountain birch and its hybrids on the basis of fossil leaf material. Cell fre-

quency measurements are expected to reveal whether the occurrence of hybrid leaves in fossil assemblages causes an increased scatter in the stomatal density and stomatal index, the relevant parameters for palaeoatmospheric CO₂ reconstructions.

Material and methods

Birches were studied near the Kevo subarctic Research Station (69°45' N, 27° E), Utsjoki, Finnish Lapland (Figure 1). Genetically controlled specimens of *Betula pendula* (*B.pe*), *B. pubescens* ssp. *czerepanovii* (Olova) Hämet-Athi (1987) (*B.pb*), *B. nana* (*B.na*) as well as the hybrids *B. pubescens* ssp. *czerepanovii* × *nana* (*B.pb* × *na*) and *B. nana* × *pubescens* ssp. *czerepanovii* (*B.na* × *pb*) were sampled in September 1997 at experimental field I, the Rassejohka garden. *B. pendula* and *B. pubescens* were additionally sampled at experimental field II, in the Skallovarri (for more information about the experimental gardens, see Elamo et al. 1999). Birch leaves were taken from SW-exposed short shoots at about 1.5 m height; the age of the trees was 21–23 years at the time of sampling. One leaf per tree was analyzed according to the sampling strategy recommended by Tuomisto and Neuvonen (1993). Pieces of 0.5 × 0.5 cm² from the upper third of each leaf were bleached in sodium hypochlorite and the lower cuticle was embedded in glycerine jelly for microscopic analysis. Epidermal cell properties were determined on a Leica Quantimet 500 C Image Analysis System. The following parameters were analyzed: Stomatal density [n/mm²] (SD), Epidermal cell density [n/mm²] (ED), Stomatal Index [%] (SI=(SD/(SD+ED))×100 according to Salisbury 1927), Epidermal cell area [μm²] (CA) and circumference [μm] (CC), Undulation Index (UI = CC/((√(CA/π))2π) [dimensionless]; according to Kürschner 1996), Stomatal length [μm] (SL) and Pore length [μm] (PL). The measurements were restricted to intercostal areas. SD and ED measurements are based on 7 digital images per leaf (magnification × 640, field area 0.035 mm² optimized on average intercostal area size in birch). The PL and SL as well as CA, CC, and UI are averages of 50 measured stomata/epidermis cells per leaf, stomata dimensions and cell dimensions were measured on the same digital images used for SD and ED. For the taxa and hybrids, as well as for the epidermal parameters, the abbreviations in brackets will be used in the further text and figures.

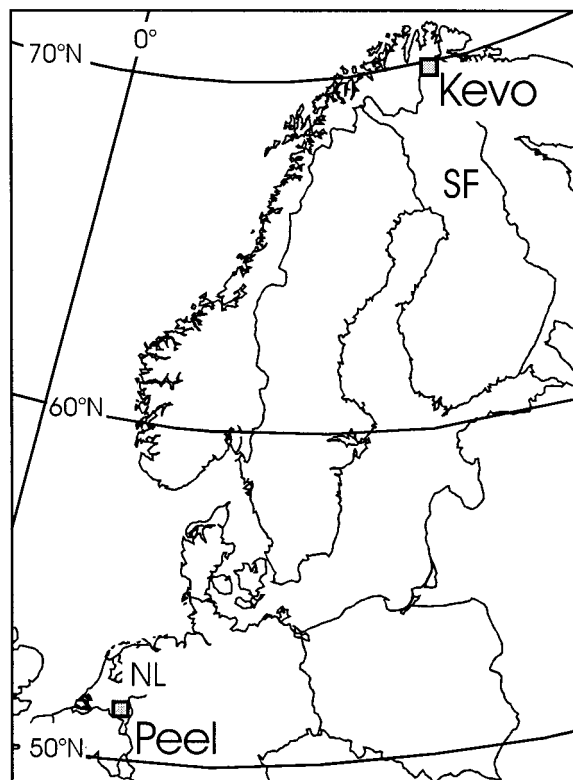


Figure 1. Location map of sampling sites. □ Kevo: Forest Line Arboretum, University of Turku, at the Subarctic Research Station in Kevo, Finnish Lapland (SF); □ Peel: Mariapeel Nature Reserve, The Netherlands (NL) for cited Dutch material.

For the statistical analyses, tree specific mean values were used. Log_e -transformation was used with SL and CA to normalize the error distributions. The analyses of variance were done with PROC GLM in SASv6. Owin software by using Type III Sums of Squares. The differences between birch taxa and hybrids were tested on specimens growing at field I. Pairwise comparisons of means were done with Bonferroni T test (at $\text{Alpha} = 0.05$) which controls the type I experiment-wise error rate. In addition, environmental effects on *B. pe* and *B. pb* and species*garden interactions were tested with 2*2-factorial ANOVA: we studied 9–10 individuals from both species growing at two altitudes: at 90 m a.s.l (field I; Rassejohkagarden) and at 270 m a.s.l. (field II; Skallovarri garden).

Results

Table 1 summarizes the mean epidermal cell parameters.

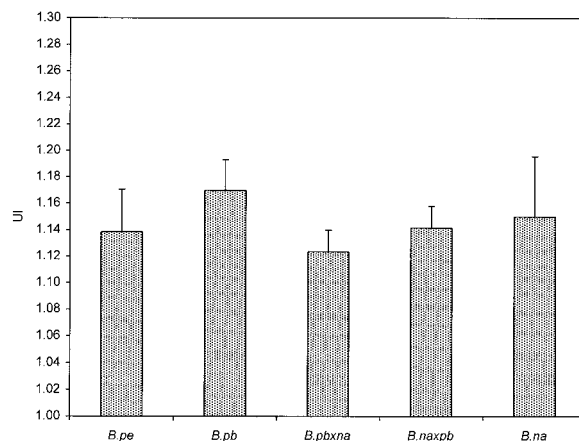


Figure 2. Mean values and standard deviations of the calculated undulation index (UI) for the studied species. For abbreviations of species names see Material and methods.

B. pe develops the smallest epidermal cells as indicated by the CA and CC. However, the difference in CC between *B. pe* and *B. pb* seems to be smaller in the upper than in the lower garden (difference $37 \mu\text{m}$ vs. $26 \mu\text{m}$, respectively; species*garden-interaction: $F_{1,34} = 4.95$, $p = 0.0328$). CA and CC are highest in *B. pb*, significantly higher than in the hybrids as well as *B. na*, which do not differ significantly from each other.

The UI (Figure 2) shows rather complicated behavior: it is higher in *B. pb* than in *B. pe* in the lower garden but the difference is almost nonexistent (and in the other direction, although not significantly) in the upper garden (species*garden-interaction: $F_{1,34} = 13.26$, $p < 0.001$). Furthermore, in the lower garden the UI of the hybrids is significantly lower than that of *B. pb* but the UI of *B. na* is between these.

The SL and PL (Figure 3) of *B. pe* are about 25% lower than in *B. pb* and differ significantly from those in all other taxa. The highest values are in *B. pb* and in *B. na* and those of both hybrid types are slightly lower than in the pure species (*B. na*×*pb* even differing significantly from *B. pb*). SL and PL in *B. pb* and *B. pe* are slightly but significantly higher in the upper than in the lower garden ($F_{1,34} = 7.97$ and 12.72 , $p = 0.0079$ and 0.0011 , respectively).

The mean SI values (Figure 4) of *B. pb* are significantly higher than those of *B. pe* and *B. na*, which do not differ significantly from each other. The SI of hybrids is between that of the pure species. There are no significant differences in SI between *B. pe*, *B. na*, *B. pu*×*na*, and *B. na*×*pu*. The SI is not affected by garden ($F_{1,34} = 0.05$, $p > 0.8$) nor by

Table 1. Epidermal cell parameters in different birch taxa in the Lower Garden at Kevo, Northernmost Finland, as mean values and some test statistics. SI = Stomatal Index; SD = Stomatal Density; ED = Epidermal Cell Density; PL = Pore Length; SL = Stomatal Length; CA = Epidermal Cell Area; CC = Epidermal Cell Circumference; UI = Undulation Index; n: number of trees sampled; In each row means with the same letter are not significantly different (Bonferroni T tests for each variable, Alpha= 0.05); MSD: Minimum Significant Difference; F: test statistic from ANOVA, df = 4, 40; * : $p < 0.001$.

	<i>B. pe</i>	<i>B. pb</i>	<i>B. pb</i> × <i>na</i>	<i>B. na</i> × <i>pb</i>	<i>B. na</i>	MSD	F
n=	9	10	8	8	10		
SI	8.4 ^b	9.6 ^a	8.9 ^{ab}	8.4 ^b	8.0 ^b	1.08	6.49*
SD	258 ^a	174 ^b	206 ^b	202 ^b	174 ^b	45.53	9.99*
ED	2758 ^a	1619 ^c	210 ^b	2180 ^b	2052 ^b	399.2	19.41*
PL	14.6 ^c	20.8 ^a	18.7 ^{ab}	18.0 ^b	21.2 ^a	2.74	17.20*
SL [#]	27.8 ^c	37.1 ^a	34.2 ^{ab}	33.2 ^b	34.1 ^{ab}	2.8–3.4 [†]	22.02*
CA [#]	398 ^c	801 ^a	554 ^b	536 ^b	513 ^b	10–20 [†]	24.00*
CC	80.5 ^c	117.6 ^a	94.3 ^b	94.1 ^b	92.7 ^b	10.7	29.94*
UI	1.136 ^{bc}	1.169 ^a	1.124 ^c	1.142 ^{bc}	1.155 ^{ab}	0.024	10.10*

[#]ANOVA with \log_e -transformation. Means shown are back-transformed from logarithms;

[†]Note that in the arithmetic scale the back-transformed MSDs are smaller in the lower than in the higher part of the range of a variable.

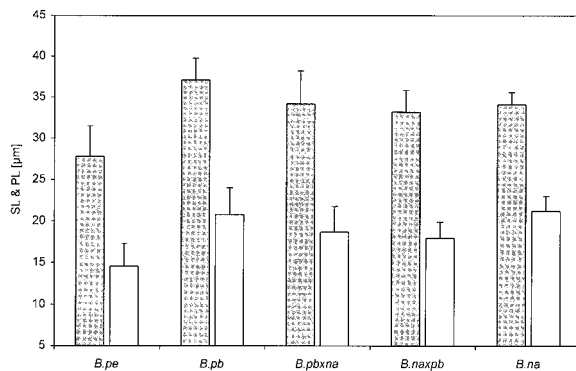


Figure 3. Mean values and standard deviations of the measured stomatal dimensions from the studied species from the Kevo arboretum. Shaded columns are stomatal length measurements (SL), white columns are pore length measurements (PL). For species abbreviations see material and methods.

species* garden-interaction ($F_{1,34} = 0.21$, $p > 0.6$). The SD of *B. pe* is significantly higher than in the other taxa which do not differ significantly from each other. *B. pe* also has significantly higher ED than all the other taxa. *B. na* and the hybrids do not differ in the ED, but all three have significantly higher ED than *B. pb*.

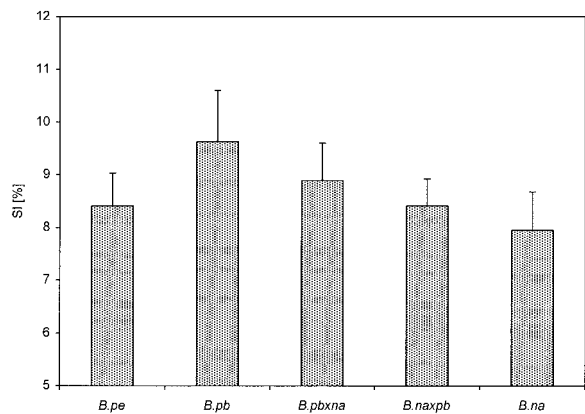


Figure 4. Mean values and standard deviations of the calculated stomatal index (SI) of the studied species. For abbreviations of species names see material and methods.

Discussion

Stomatal dimensions

The most distinct differences in the measured epidermal parameters occur within the SL (Figure 3). With a mean of 37.1 μm , *B. pb* develops by far the largest stomata. 27.8 μm mean SL is measured in *B. pe*, which are 25% smaller than in *B. pb*. *B. na* stomata are intermediate; with 34.1 μm they are *c.*10% shorter than in *B. pb* and *c.*20% larger than in *B. pe*. The characteristic size relation has been reported in earlier studies for *B. pe* and *B. pb* and is suggested to have po-

tential in discriminating the two tree birch species (cf. Kujala 1946; Atkinson 1992). In Kujala's (1946) measurements on Finnish leaf material, the mean $41.1 \mu\text{m}$ SL in *B. pb* strongly exceeds the mean $30.9 \mu\text{m}$ SL in *B. pe*. In leaf material from Great Britain (Atkinson 1992) the $30.9 \mu\text{m}$ mean SL for *B. pe* and $39.9 \mu\text{m}$ mean SL in *B. pb* revealed similar size relations. The present observations are consistent with these reports and the size differentiation of *B. pe* and *B. pb* may help to determinate *Betula* leaf remains at a species level. Although less clear, the differences between the average SL (Figure 3) of *B. na* and the tree birches may allow in fossil material a further determination based on this parameter for all three species.

The enlargement of stomatal dimensions frequently results from increasing ploidy levels in plants (Speckman et al. 1965; Chia & Brun 1978; Mishra et al. 1991; Masterson 1994; Mishra 1997). The observed size differentiation of the subsection *Albae* representatives may therefore be linked to the different ploidy levels of *B. pe* and *B. pb* with chromosome number $2n = 28$ and $2n = 56$, respectively (Helms & Jørgensen 1925; Brown & Al-Dawoody 1979).

The average SL for the hybrids (Figure 3) is with $34.2 \mu\text{m}$ for *B. pb* × *na* and $33.2 \mu\text{m}$ for *B. na* × *pb* significantly lower than in *B. pb*. No significant differences are noted between *B. na* and the hybrids, independent whether *B. na* holds the female or male counterpart. This may suggest that the stomatal size of the hybrids is mainly controlled by *B. na*.

A further SL based differentiation of *B. pb* and the hybrids with Mountain birch is theoretically possible. However, since *B. na* and the hybrids reveal analogous stomatal dimensions in modern material, certain problems may occur in fossil leaf assemblages composed of *B. pb*, *B. na* and hybrids.

PL measurements (Figure 3) show a comparable size distribution pattern. However, there is certain evidence, at least for *B. pe* (Wagner et al. 1996) that PL can be influenced by environmental factors. It is uncertain, therefore, to what extent this parameter can be used for species identification.

The relevance of the stomatal dimensions for species determination has been demonstrated for *B. pe* and *B. pb* in Holocene peat deposits from The Netherlands (Wagner, work in progress). The determination on species level for tree birches holds valuable information on the local environment but is often difficult to perform on fragmentary preserved leaf remains in fossil assemblages. The simple method of stomatal size

differentiation might therefore be included in standard macrofossil analysis.

Epidermal cell shape

In the discussion on the taxonomy of the fennoscandian Mountain birch as separate species (*Betula tortuosa* Lebed.) or as a subspecies within the *Betula pubescens* complex, Vaarama & Valanne (1973) considered intense sinuosity of the epidermal cell walls in *B. pb* as parameter to distinguish this species from *B. tortuosa* Ledeb.. Their analysis of epidermal cells show a distinct undulation in *B. pb* leaves collected in S-Finland, which is absent in *B. tortuosa* leaves from Kevo (N-Finland). The intense sinuosity of the epidermis cell walls has been introduced as parameter of taxonomical value (Vaarama & Valanne 1973). In contradiction to their observations, the genetically controlled species and hybrids examined in the present study reveal no consistent differences in the epidermal cell sinuosity, quantified here as the undulation index [UI] (Table 1, Figure 2, Plate 1). Accordingly, field studies on the influence of different light intensities on the leaf morphology in *B. pendula* and *B. pubescens* from the Netherlands have demonstrated that the capacity of shade adaptation, inclusive of the typical development of epidermal cell wall undulation under shade conditions, is limited in tree birches (Wagner 1998). The increase in leaf area under low light intensities in *B. pubescens* coincides with an increase of epidermal cell area and proportional cell wall expansion, leading to larger, straight walled epidermal cells.

The leaves sampled by Vaarama & Valanne (1973) are from 2.5-year-old *B. tortuosa* saplings collected near Kevo (70°N) and 3.5-year-old *B. pb* material Punkaharju ($61^\circ43'\text{N}$), whereas the *B. pb* leaves in the Netherlands were sampled at $51^\circ28'\text{N}$. This difference in latitude causes major changes in the temperature and light regimes to which local vegetation is exposed during the growth season. Highly significant alteration of leaf size development has been demonstrated to be depending on length of the photoperiod in *B. pubescens* population from Ringebu, Norway (61°N) and Alta, Norway (70°N), and the average leaves from the southern location are about four times larger than those at the northern site (Håbjørg 1972). The long photoperiod and the relative long growth season in S-Finland is likely to affect epidermal cell growth through regulation of the gibberellin metabolism. It has been suggested that gibberellin may

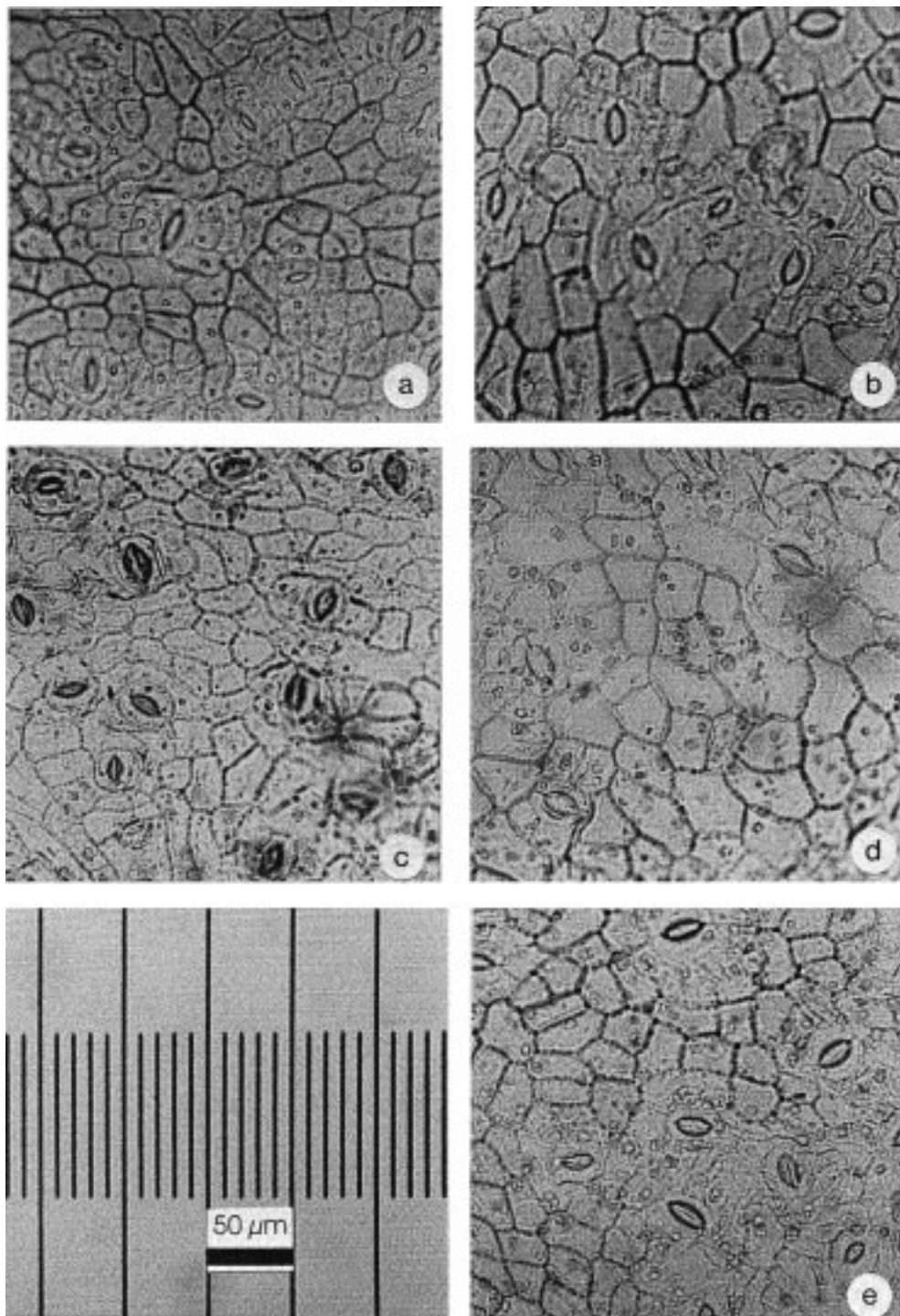


Plate I. Cuticle photographs of the studies species and hybrids from Finnish Lapland at 20×3.2 magnification. (a) *B. pendula*, (b) *B. pubescens*, (c) *B. nana*, (d) *B. pubescens* \times *nana*, (e) *B. nana* \times *pubescens*.

enhance cell growth by preventing reactions which otherwise would cause cell wall stiffening (Taiz & Zeiger 1998), which may have induced also the intensive undulation of the cell walls as described for *B. pb* by Vaarama & Valanne (1973). In spite of an even longer photoperiod in N-Finland the thermal sum is considerably lower and the growth season shortened, which again may result in a suppression of cell undulation. A positive correlation between the degree of undulation in *B. pb* leaves and growth temperature under long daylight conditions (18 hours) has recently been demonstrated in growth experiments (Wagner 1998). The undulation of epidermal cell walls can therefore not be used for taxonomic purposes, since environmental conditions are likely to be the driving force for the leaf morphological development.

Epidermal cell wall undulation is also a common feature of leaves which grew under low light conditions, where similar to the processes in long-day plants described above, the cell wall and cuticle hardens much less rapidly than under high light conditions (Watson 1942; Metcalfe & Chalk 1979). The UI has originally been introduced to describe the light depending variability of the leaf epidermal cell shape in order to distinguish sun- and shade- morphotypes in modern and fossil leaf assemblages (Kürschner 1997), but it may also be a helpful tool revealing the effects of a palaeo-photoperiodism in fossil floras.

Stomatal frequency

The sensitivity of stomatal frequency in *B. pb* and *B. pe* to changing CO₂ regimes has been demonstrated in herbarium studies and in records of annual leaf shedding in peat deposits in The Netherlands, where both species show comparable SI adaptation rates (Wagner et al. 1996; Kürschner et al. 1997; Wagner et al. 1999). Since the resulting response curves act as calibration curves for palaeoatmospheric interpretations of fossil leaf assemblages, it has to be tested on modern leaf material to what extent extreme, subarctic growth conditions influence the stomatal frequency. In general, the mean SI is the more constant parameter and should therefore preferably be used for palaeoatmospheric CO₂ reconstructions (Kürschner 1997; Wagner et al. 1996). Apart from the response to changing CO₂, the mean SD is also strongly affected by environmental factors other than CO₂. In the present study, the SD as well as the ED for *B. pe* (258 and 2758, respectively), are both significantly higher than for *B. pb* (174 and 1619; Table 1). Such a characteristic de-

crease in stomatal frequency as well as epidermal cell frequency with increasing ploidy level has also been described for polyploid species in the genus *Coffea* (Mishra 1997). These observations are in good agreement with the present results. The genotypic variance of the two *Betula* species may be the cause for the distinct differentiation of the general epidermal cell frequency and stomatal apparatus dimensions.

The present study further reveals some significant differences in the mean SI values for the species from the Kevo arboretum (Figure 4). The largest discrepancy occurs between *B. pb* and *B. na*; the SI for *B. pe* is intermediate between the former species. The mean SI values for the hybrids (Figure 4) fall within the range from *B. pb* to *B. pe* and *B. na*. In relation to palaeoatmospheric CO₂ reconstructions, these results imply that the occurrence of hybrids in fossil leaf assemblages will not broaden the band of scatter in leaf assemblages containing *B. pe* and *B. pb*. In herbarium material and subfossil leaves, the rate of CO₂ responsiveness in *B. pb* and *B. pe* is closely comparable (Kürschner et al. 1997; Wagner 1998; Wagner et al. 1999). In mixed Holocene leaf assemblages the two species can therefore be treated as a single group in the way they respond to CO₂.

The close comparability of the SI values in the hybrids and *B. na* might enable a combined analysis of those taxa in fossil leaf assemblages. However, the overall adaptation pattern for *B. na* needs to be intensively tested in advance. Until herbarium studies and analysis of well dated subfossil leaf sequences have confirmed the comparability of the shrub and tree birches, *B. na* should not be combined with the tree birches for palaeoatmospheric CO₂ reconstructions.

Comparing the mean SI values of *B. pb* and *B. pe* with modern material from The Netherlands, the SI from the Finnish site exhibits higher values with $8.4 \pm 0.83\%$ SI versus $6.64 \pm 0.65\%$ SI for *B. pe* and $9.6 \pm 1.18\%$ SI versus $7.09 \pm 0.51\%$ SI for *B. pb* (Kürschner et al. 1997; Wagner 1998; Wagner et al. 1999). For several crop species, an enhanced stomatal initiation rate has been described as one of the effects of elongated photoperiod, independent of irradiation intensity (Schürmann 1959). It may be hypothesized that a comparable mechanism causes the differences in the SI values for *Betula* observed under the latitudinally contrasting regimes of northern Finland and The Netherlands. Due to the significant SI discrepancies between tree birches from high and low latitudes, a direct comparison of data sets from various sites requires extra care and calibration curves for the higher

latitudes are needed. The proposed influence on the stomatal index as well as on the epidermal cell undulation of the different light and temperature regimes has to be evaluated and is presently under investigation (Wagner, work in progress).

Concluding remarks

- The results of this study demonstrate that epidermal cell undulation for taxonomical purposes within the genus *Betula* cannot be considered valid.
- Determination of tree birches on species level can be performed by stomatal length measurements.
- The possible occurrence of hybrids in fossil leaf assemblages will not cause significant scatter in palaeoatmospheric CO₂ reconstructions based on the mean SI calculations for the *B. pendula/pubescens* group.
- Due to the significant discrepancies between tree birches from high and low latitudes, a direct comparison of data sets from various localities requires extra care. The proposed influence of the different photoperiods and temperature regimes on epidermal properties should be part of further investigations.

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