1 **Using long-term data to reveal the geographical variation in timing and quantity of pollen and**

2 **seed production in silver and pubescent birch in Finland: implications for gene flow,**

3 **hybridization and responses to climate warming**

4

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13

14 **Abstract**

15 Silver (*Betula pendula*) and pubescent birch (*B. pubescens*) are the two main broad-leaved tree

16 species in boreal forests and Subarctic areas, with great significance for both northern societies and

17 ecosystems. Silver birch has more economical importance as it grows taller, but pubescent birch

18 reaches much further North. The adaptability and genetic diversity of Subarctic birch populations

19 are assumed to derive from inter- and intraspecific hybridization. Southern pollen clouds could in

20 turn increase the adaptability of northern populations to warming climate. In the boreal forest zone

21 of warmer climate, incompatibility reactions may prevent interspecific hybridization and much

22 depends on the synchrony of flowering. Direct in situ observations are, however, mostly lacking

23 and earlier results concerning the spatial and temporal match of flowering phenology between the

24 species are contradictory. Conclusions based on pollen catches may also be biased as the pollen of

25 silver and pubescent birch are notoriously difficult to sort out and the geographical origin of pollen

26 is virtually impossible to determine. Here we employ direct flowering observations and reanalyze

27 old pollen and seed production data, collected along a South-North gradient in Finland, to shed

28 more light on these issues. Our results suggest that interspecific hybridization is an unlikely

29 mechanism of adaptation in silver and pubescent birch as there is no significant overlap in

30 flowering either near Subarctic or in more southern boreal areas (covering latitudes 60– 68° N).

31 Long-distance southern gene flow also unlikely has importance in the adaptation of northern

32 populations to a warming climate as heat sum requirements for flowering in northern and southern

33 populations are equal and northern birches are therefore not receptive at the time of southern

34 flowering. Long-term data of pollen and seed production in turn suggest that pubescent birch is

35 more effective in seed production through the whole South-North gradient, but increasingly so

36 towards the North. However, it appears that this difference is not due to silver birch flowering and

37 regeneration being more sensitive to interannual variation as earlier suggested. Although there are

38 more factors than reproduction alone that can affect species distributions, these two findings

39 indicate that climate warming may not significantly alter the relative abundances of silver and

40 pubescent birch in Subarctic Fennoscandia.

41

42 keywords: Betula pendula, Betula pubescens, adaptability, global warming, gene flow, hybridiza-

43 tion, pollen

44 **Introduction**

45 Gene flow is one of the key processes that shape the genetic composition of tree populations.

46 Addition of alien genes to populations of trees can lead to unfavorable, neutral or favorable changes

47 depending on the source population (Lenormad 2002, North et al. 2010). Under climate warming,

48 intraspecific gene flow from South to North can be important in adaptation to warming

49 temperatures in the North, as suggested by the modeling work with Scots pine (*Pinus sylvestris*;

50 Savolainen et al. 2007). Also, it has been suggested that interspecific gene flow between sister

51 species and introgression support adaptation to extreme environments in many taxa (Lewontin &

52 Birch 1966, Baskett & Gomulkiewicz 2011), including birches at their northern limits (Kallio et al.

53 1983, Salojärvi et al. 2017).

54 Silver birch (*Betula pendula*) and pubescent birch (*Betula pubescens*), hereafter referred to as white

55 birches, are the main broad-leaved tree species in European and Asian boreal forests, where their

56 responses to environmental changes seem to closely accompany general ecosystem level responses.

57 Their geographical distributions are mostly overlapping and both species occur at lower altitudes in

58 most parts of boreal Eurasia (Atkinson 1992). Pubescent birch extends to more northern and higher

59 sites (Hultén 1971), while silver birch is only sporadically found in Fennoscandia and Russia north

60 of 67–68° N. Both species thrive on moraine and sandy soils, but silver birch often dominates dryer

61 soils while pubescent birch is more common in organic soils and moist habitats. White birches are

62 monoecious, with male and female flowers in different catkins. Silver birch is diploid (2n=28) and

63 pubescent birch tetraploid (2n=56). The origin of pubescent birch is still open; it may be an

64 autotetraploid of silver birch, or an allotetraploid with silver birch as one and possibly, *B. humilis* as

65 another parent (Howland et al. 1995).

66 White birches are known for their capacity to produce enormous amounts of light pollen, which in

67 turn is one of the furthest transported pollen grains (Hjelmroos 1991, Sofiev et al. 2006). Long

68 distance transport covering several hundred kilometers is common. For instance, in northern

69 Finland catches of birch pollen before local flowering can exceed local pollen production (Hicks et

70 al. 1994, Oikonen et al. 2005). However, maximum distances of effective gene flow are difficult to

71 verify for tree species with such dense populations and wide distribution as white birches. While

72 some studies suggest that gene flow can effectively reduce variation among birch populations

73 (Rusanen et al. 2003; Järvinen 2004), others conclude that efficient movement of pollen clouds can

74 range some tens or hundreds of meters only (Sarvas 1952, 1956, Whitehead 1969, Andersen 1970,

75 Koening & Ashley 2003, for review see Ashley 2010). The gradual decrease of silver birch

76 abundance towards the North could be expected to lead to pollen limitation, but it appears that

77 pollen limitation is actually more common in mountainous populations of pubescent birch (Holm

78 1994). Northern seed production, on the other hand, appears to be more limited by resources in

79 silver compared to pubescent birch (Holm 1994).

80 Apparently, gene flow largely depends on the flowering phenology of populations. In white birches,

81 spring heat accumulation (temperature sum) determines the phenology of bud burst and flowering

82 in spring, which in white birches occurs almost simultaneously (Rousi & Heinonen 2007, Rousi et

83 al. 2011). However, many tree species also show fine-scale adaptation to the length of the frost free

84 growing season (Frewen et al. 2000); southern populations of *Betula alleghaniensis*, *B. lenta* and *B.

85 pendula* in common garden experiments tend to flush later compared to northern populations

86 (Pauley and Perry 1954, Sharik & Barnes 1976, Junttila et al. 2003, Li et al. 2002). Smaller heat

87 sum requirement of flowering in northern birches could enlarge the phenological window for

88 southern pollen and thus enhance their adaptability to a warming climate. To confirm this, however,

89 more research is needed concerning the match of flowering phenology in southern and northern

90 areas.

91 Interspecific hybridization of birches was thought to be rare and to take place at the northern limits

92 of their distribution only, since studies suggested synchronous flowering only above the Arctic

93 Circle (Kallio et al. 1983). However, more recent results have contradicted the idea of a

94 phenological barrier and the timing of pollen anthesis in white birches has been suggested to be

95 largely synchronous also in southern latitudes of 60–67 °N (Luomajoki 1999, Linkosalo et al.

96 2010). Supporting these results, studies of birch chloroplast DNA suggest that large-scale

97 interspecific hybridization of birches took place during postglacial colonization (Palmé et al. 2004).

98 And while some results suggest that interspecific hybridization is prevented by incompatibility

99 reactions (Hagman 1971), cold subarctic weather has been suggested to suppress such reactions

100 (Kallio et al. 1983)

101 Studies of duration and intensity of flowering in white birch populations have commonly been

102 based on data from pollen samplers (Sarvas 1952, Luomajoki 1999, Linkosalo et al. 2010). Early

103 studies by Sarvas (1952) suggest that pubescent birch flowers on average one week later compared

104 to silver birch, whereas more recent work by Luomajoki (1999) and Linkosalo et al. (2010) suggest

105 longer duration and a near complete overlap of the pollen season for the two species. These later

106 studies also, rather unexpectedly, show a tendency of increasing heat sum requirement for spring

107 phenology in silver birch when moving towards the North.

108 A difficulty and a potential source of error in studies with white birch pollen is that pollen cannot be

109 identified to species-level, let alone to origin (Clausen 1960, Prentice 1981, Birks 1986, Mäkelä

110 1996); apparently a serious obstacle with species, which have easily transported pollen. Here, we

111 suggest that this dilemma could be resolved by defining a window for ex situ pollen from sampler

112 data using temperature data (i.e. heat sum accumulation or temperature sum), as the timing of silver

113 birch pollen anthesis can be reliably predicted using spring heat sum accumulation (Rousi et al.

114 2011). Using this approach, we take another look at the data presented by Luomajoki (1999) and

115 Linkosalo et al. (2010).

116   We hypothesize that this re-analysis will show that (1) birches growing in southern Finland always

117   flower earlier than those growing in the North. Nonetheless, because northern populations require a

118   smaller heat sum and there is large interannual variation in temperatures, gene flow from southern

119   to northern locations, or vice versa, might be possible in some years. Following the early results,

120   which showed virtually no interspecific overlap in local flowering schedules (Sarvas 1952), we

121   further hypothesize that (2) silver and pubescent birch have different flowering schedules, largely

122   preventing local interspecific hybridization. As a potential explanation for the gradual increase of

123   pubescent birch dominated stands towards the North, we also predict that (3) there is no difference

124   in reproductive investment (amount of pollen and seeds produced) between the two species in

125   southern Finland, whereas in northern Finland, pubescent birch is more efficient. Finally, we utilize

126   the data to test the hypothesis that (4) the proportion of silver birch is lower in the North because

127   reproduction of silver birch is more sensitive to environmental variation (Holm 1994). If supported,

128   the latter two hypotheses would suggest that a warming climate could increase the relative

129   proportion of silver birch in the North.

Material and methods

131   The pollen anthesis, flowering and seed production data sets used here were collected by late

132   professor Risto Sarvas, whose untimely death did not allow him to publish the data. The long-term

133   measurements of the quantity of flowering and seed crop were available in Koski and Tallqvist

134   (1978), while the calculations of pollen anthesis are based on the original data. Since spring

135   phenology of trees, silver birch in particular (Rousi and Heinonen 2007, Rousi et al. 2011, Basler &

136   Körner 2012), is driven by temperature (e.g. Hänninen & Tanino 2011), we calculated heat sum as

137   growing degree days (dd) using a 5 °C threshold. This value is commonly used to evaluate

138   phenology, also in silver birch (e.g. Hunter et al. 1992, Rousi et al. 2011). Temperature sum is the

139   daily mean temperature above a threshold value (here +5°C), summed over a year, expressed in

140   degree days (dd)). In Finland, heat sum accumulation may in a warm spring day be more than 15

141   dd, but cold periods of 1–2 weeks without heat sum development are also possible.

142   *Silver birch pollen data*

143   For silver birch, the pollen was collected in 1964–1973 in nine tree stands using one to three

144   rotating, tree-top level pollen samplers (Sarvas–Vilska) at each stand (Sarvas data). The two

145   southernmost stands were by the Gulf of Finland in Hammarland and Bromarv (60°02'–60°14' N),

146   four stands in southern Finland in Hartola, Punkaharju, Kerimäki and Vilppula (61°45'–62°04' N),

147   two northern stands at the Arctic Circle in Rovaniemi (XXI and XXVIII; 66°21' N) and the

148   northernmost stand in Kittilä in the world's northernmost natural silver birch stand (67°44' N). In

149   Punkaharju, pollen was collected every year, while at other stands the time series cover 5–9 years.

150   One data point in Hammarland and three in Rovaniemi were excluded from the analyses because of

151   minimal pollen production. Unlike Luomajoki (1999), we also excluded the 10-year time series

152   available for a curly birch (*B. pendula* var. carelica) stand in Punkaharju (stand LXIII). Also, the

153   original data indicate large and seemingly random, interannual variation in the onset and

154   termination of pollen collection both in terms of days and temperature sums. For instance, in the

155   southern locations recordings of anthesis started at 1–30 dd and terminated at 68–163 dd. In the

156   North, observation periods had less interannual variation and recordings terminated mostly at 90–

157   100 dd.

158   Pollen sampler data can include both in situ and ex situ conspecific and heterospecific pollen.

159   Conspecific ex-situ pollen is generally impossible to distinguish and in the case of silver birch and

160   pubescent birch even heterospecific pollen cannot be reliably sorted out in large pollen samples. To

161   our best knowledge, so far no effort has been made to sort out species in the present material. In a

162   recent long-term data set of daily phenology observations for 30 silver birches, the lowest heat sum

163   for the onset of anthesis (for any tree) was 32 dd and the highest heat sum for the termination of

164   anthesis 82 dd (Rousi et al. 2011). To exclude heterospecific local pollen and long distance

165   conspecific pollen from the silver birch pollen data, we therefore delimited the 1964–73 pollen

166   catches to a heat sum area of 30–90 dd and additionally shortened the tails of the 30–90 dd heat sum

167   area by omitting dates when <5% and >95% of pollen were obtained (for shortening the tails see

168   Dahl & Strandhede 1996, Ranta et al. 2006, Ranta & Satri 2007).

169   *Timing of anthesis and pollen catch for both species*

170   For pubescent birch, the timing of anthesis was visually observed for two phenotypes growing in

171   the same stand, for the same years (1998–2004), using the same methodology (our own direct daily

172   observations of the canopy) as in Rousi et al. (2011). This allows direct comparisons with

173   concurrent silver birch phenology. A 10-year data set (1964-73) of daily pollen catch was also

174   available for both species from two stands growing on mineral soils in Punkaharju less than 2 km

175   apart.

176   *Quantity of pollen and seed production for both species*

177   Quantity of flowering and seed crop was measured in 1955-1973 using metal funnels (opening 0.05

178   m^2) set up in pure, mature birch stands (6–10 funnels in each stand) in different parts of Finland

179   (61–68° N). Cloth bags, attached to the base of each funnel, were emptied once a month and the

180   remains of male flowers were sorted out of the accumulated litter, dried at 105 ℃ and weighed. The

181   quantity of shed pollen was then estimated for both species by multiplying the mass of male flower

182   residue by 0.27 (Sarvas 1962).

183   To study the magnitude of interannual variation in anther and seed production in silver and

184   pubescent birch populations at the same geographical locations, we utilized the long-term data sets

185   available in Koski and Tallqvist (1978): i.e. a 13–year data set for southern Finland (Punkaharju), a

186   17–year data set for northern Finland (Artic Circle, Rovaniemi) and a 10–year data set for the

187   location of northernmost silver birch stand (Kittilä).

188

189 **Results**

190 *Delimitation of 1964-1973 silver birch pollen records*

191 Delimiting silver birch pollen catches using the heat sum range 30–90 dd rejected part of catches in

192 21% of the 120 observations. In Punkaharju, for example, the interannual variation of starting

193 pollen collection was large (0 dd in 1967 and 1973, 27 dd in 1965), and the same is true for ending

194 the recordings (73 dd in 1965, 138 dd in 1973). In coastal Bromarv, 55% and 16% of the total

195 pollen catch was rejected in 1967 and 1971, respectively. Although the quantity of pollen rejected

196 was generally very small, the impact of delimitation on the length of the pollen period was

197 considerable. In Bromarv, for example, the time range of first and last pollen observation during the

198 7-year observation period was reduced from 5 weeks (Luomajoki 1999) to two and half weeks by

199 omitting those pollen catches, which based on current knowledge concerning the drivers of white

200 birch phenology are unlikely to have originated from local silver birch stands. Shortening the tails,

201 i.e. omitting periods of <5% and >95% of the cumulative pollen catch, had only a minimal impact

202 on the duration of pollen period or the quantity of pollen collected. Also, shortening the tails had

203 virtually no impact on the day when ≥50% of the pollen was caught: in only 1.6% of cases the time

204 was adjusted by one day.

205 *Phenological variation among and within silver birch populations across Finland*

206 Differences between coastal (~ 60° N) and southern (~ 62° N) silver birch populations in their

207 interannual variation of onset and termination of pollen anthesis were small (Fig. 1). The range over

208 years was April 30 –May 28 for coastal and May 10–28 for southern populations (Fig. 1). Peak

209 pollen catch and the mean (50%) of anthesis took place May 17, on average (Fig.1, Table 1).

210 Northern populations (67° N) had their anthesis about three weeks later, the range being May 29 –

211 June 17 (Fig. 1). Both peak pollen catch and the mean of anthesis in these populations was June 4,

212 on average (Fig. 1, Table 1). As a result, in every year the pollen was collected, the anthesis in the

213 North started after pollen production in the South had terminated (Fig. 1). In the North, no pollen

214   was caught before 30 dd, thus only pollen caught after 90 dd was rejected. Dates of maximum

215   pollen catch and 50% anthesis were the same in 64% of cases, the mean deviation between these

216   two incidents being 0.7 d. The heat sum requirement for flowering was lower in the coastal stands

217   (on average 50 dd) than in all other stands (on average 57 dd), but the southern inland and northern

218   stands did not seem to differ (Table 1).

219

220   **Table 1.** Date and heat sum (dd) requirement of mean (50%) anthesis in nine silver birch stands

221   ranging from Coastal (Bromarv and Hammarland), South (Hartola, Punkaharju, Kerimäki and

222   Vilppula) to North (Rovaniemi and Kittilä) Finland.

223

Birch stand	Latitude	Date	dd	Observation years
Bromarv	60° 02'	May 16	49	7
Hammarland	60° 14'	May 18	50	4
Hartola	61° 45'	May 18	56	5
Punkaharju	61° 49'	May 17	57	10
Kerimäki	61° 50'	May 18	58	9
Vilppula	62° 04'	May 16	56	5
Rovaniemi XXI	66° 21'	June 3	58	7
Rovaniemi XXVIII	66° 21'	June 4	63	7
Kittilä	67° 44'	June 4	54	6
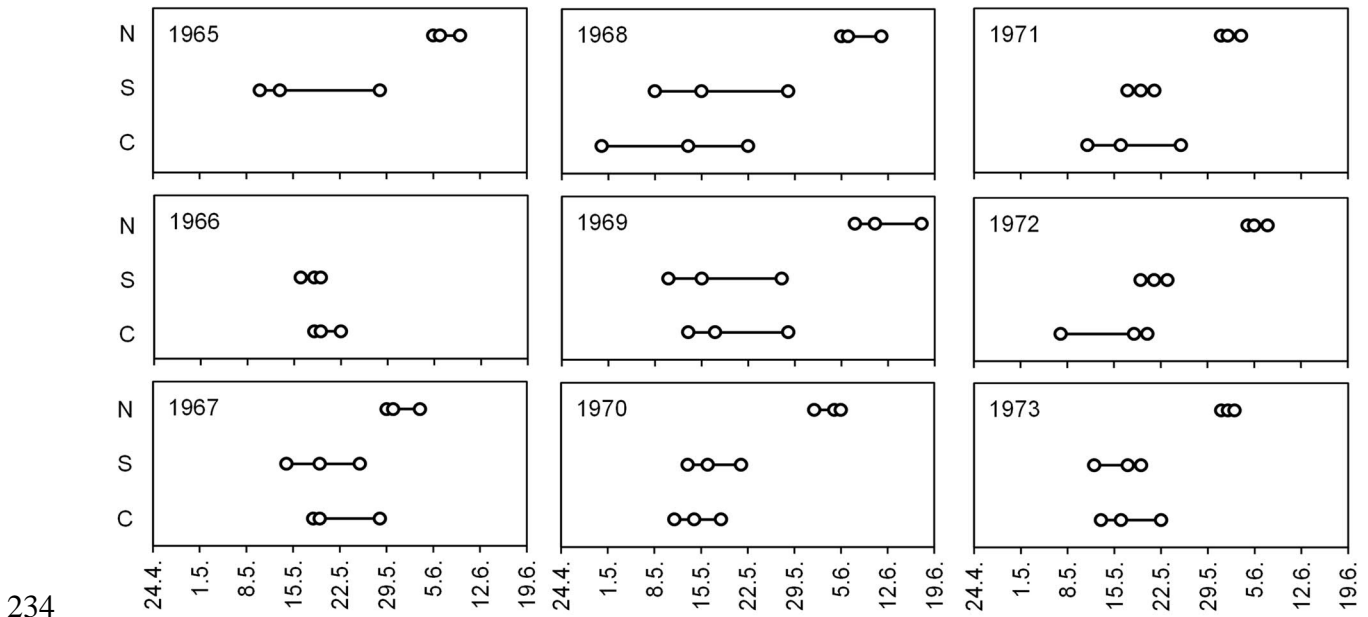
Figure 1. Duration of pollen period in coastal (C, 60°N), South (S, 61–62°N) and North Finland (N,
66–68°N) based on pollen catch data (the three dots denote the onset of pollen period, the day of
peak pollen catch and the termination of pollen period).

Phenological differences between silver birch and pubescent birch in southern Finland

Based on the visual 6–year in situ observations in Punkaharju (southern Finland), silver birch was
on average 5 d earlier in the onset and termination of anthesis compared to pubescent birch, with a
heat sum difference of 25-30 dd (Table 2). In 2003, there was no overlap in pollen production
between the species, while in other years, some of the latest flowering silver birches had a 1-d
overlap with earliest pubescent birches.

246 **Table 2.** Interannual variation of date and heat sum (dd) for the onset and termination of pollen

247 anthesis in silver birch and pubescent birch growing in the same forest stand in Punkaharju, South

248 Finland (data for silver birch from Rousi et al. 2011; n.m. = not measured).

249

	Onset				Termination			
	Silver birch		Pubescent birch		Silver birch		Pubescent birch	
	Date	dd	Date	dd	Date	dd	Date	dd
1998	May 11	48	May 18	89	n.m.		n.m.	
2000	May 1	42	May 10	69	May 5	49	May 12	72
2002	May 2	48	May 4	67	May 3	58	May 5	69
2003	May 12	41	May 15	69	May 14	61	May 23	125
2004	May 5	41	May 8	71	May 7	60	May 10	84
2005	May 21	47	May 23	69	May 24	82	May 25	94
Mean	May 8	45	May 13	72	May 10	62	May 15	88

260

261 According to pollen captured in 1964-1973, the heat sum requirement for mean (50%) anthesis was

262 on average 57 dd for silver birch and 75 dd for pubescent birch, which corresponds to an average of

263 a 6–d difference (Table 3). The length of overlap of pollen periods between the two species had,

264 however, large interannual variation (Table 3). For instance, a rapid accumulation of heat sum

265 apparently led to a small difference in days, but not in heat sum requirement (e.g. years 1966, 1972

266 in Table 3), and in the year of exceptionally late flowering for silver birch (1971), a cool spell

267 before main flowering evidently delayed silver birch, but not pubescent birch, leading to

268 simultaneous flowering of the two species. In contrast, there was no overlap in flowering in 1965

269 and the difference between the species in mean anthesis was almost three weeks (Table 3). The day

270 of mean anthesis seemed to reliably reflect the timing of in situ flowering: in most years, the days of

271 peak pollen catch and the days of mean anthesis coincided and the mean difference of these two

272 observations was 0.5 and 0.7 d for silver birch and pubescent birch, respectively.

273

274 **Table 3.** Heat sum (dd) needed for mean anthesis (50% pollen production) for silver birch and

275 pubescent birch in Punkaharju, South Finland in 1964-73, difference of mean anthesis between the

276 species in terms of heat sum (dd) and days, and the proportion of pubescent birch pollen produced

277 by mean silver birch anthesis.

278

279		Mean anthesis (dd)		Difference		Pubescent birch pollen produced
280	Year	Silver birch	Pubescent birch	dd	days	by mean silver birch anthesis (%)
281	1964	55	67	12	3	18
282	1965	56	82	26	19	0
283	1966	61	74	13	1	23
284	1967	48	63	15	3	12
285	1968	52	77	25	11	13
286	1969	57	83	26	13	10
287	1970	55	74	19	5	22
288	1971	75	76	1	2	34
289	1972	65	73	8	1	41
290	1973	50	81	31	4	11
291	Mean	57	75	20	6.2	18

292

293

294   The quantity of silver birch pollen that we rejected based on the 30-90 dd heat sum range was very

295   small, on average 3% of total pollen (Table 4). This was mostly because small amounts of pollen

296   were detected after 90 dd, and only in one year (1967) a larger amount of pollen (11%) was

297   observed earlier than 30 dd (Table 4). On average 89% of pubescent birch pollen was produced

298   before 90 dd, but in 1964 and 1971 almost one third of pollen was caught after 90 dd (Table 4).

299

300   **Table 4.** Interannual variation in the proportion of silver birch pollen caught within the heat sum

301   range 30–90 dd, and the proportion of pubescent birch pollen caught after 90 dd and after silver

302   birch pollination in forest stands growing in Punkaharju, South Finland.

303

|	% of silver birch pollen caught	% of pubescent birch pollen caught	
Year	within 30–90 dd	after 90 dd	after silver birch pollination
1964	100	27	27
1965	100	11	60
1966	98	4	4
1967	88	2	2
1968	98	14	14
1969	100	14	35
1970	100	8	15
1971	93	27	2
1972	98	12	7
1973	96	18	18
Mean	97	11	18

317

318

319   *Pollen and seed production of silver and pubescent birch across Finland*

320   Interannual variation (n = 10 years) of pollen production correlated strongly and positively with

321   interannual variation of anther residual production in silver birch populations in Punkaharju (r =

322   0.87) and Rovaniemi (r = 0.91) and in a pubescent birch population in Punkaharju (r = 0.95).

323   Variation of anther residual production was further positively correlated, not only among stands of

324   the same species, but among stands of different species, both in the North (r = 0.87–0.94, n = 17 for

325   two pubescent birch stands and one silver birch stand in Rovaniemi; r = 0.73, n = 10 for one stand

326   of both species in Kittilä) and in the South (r = 0.87–0.95, n = 13 for two pubescent birch stands and

327   one silver birch stand in Punkaharju). The corresponding correlations for seed production were even

328   stronger (r = 0.96–0.97). Finally, the interannual variation of anther residual production was

329   strongly correlated with the variation of seed production for both species both in the South and the

330   North (Fig. 2). In the South, the anomaly of relatively low seed production following the highest

331   production of anther residuals is explained by June and July precipitation, which in Punkaharju in

332   1964 covered one third of the 1961-1991 climate normal only (i.e. drought). In the North, the silver

333   birch stand in Kittilä showed poorer anther residual–seed correlation than the stands in general (Fig.

334   2).
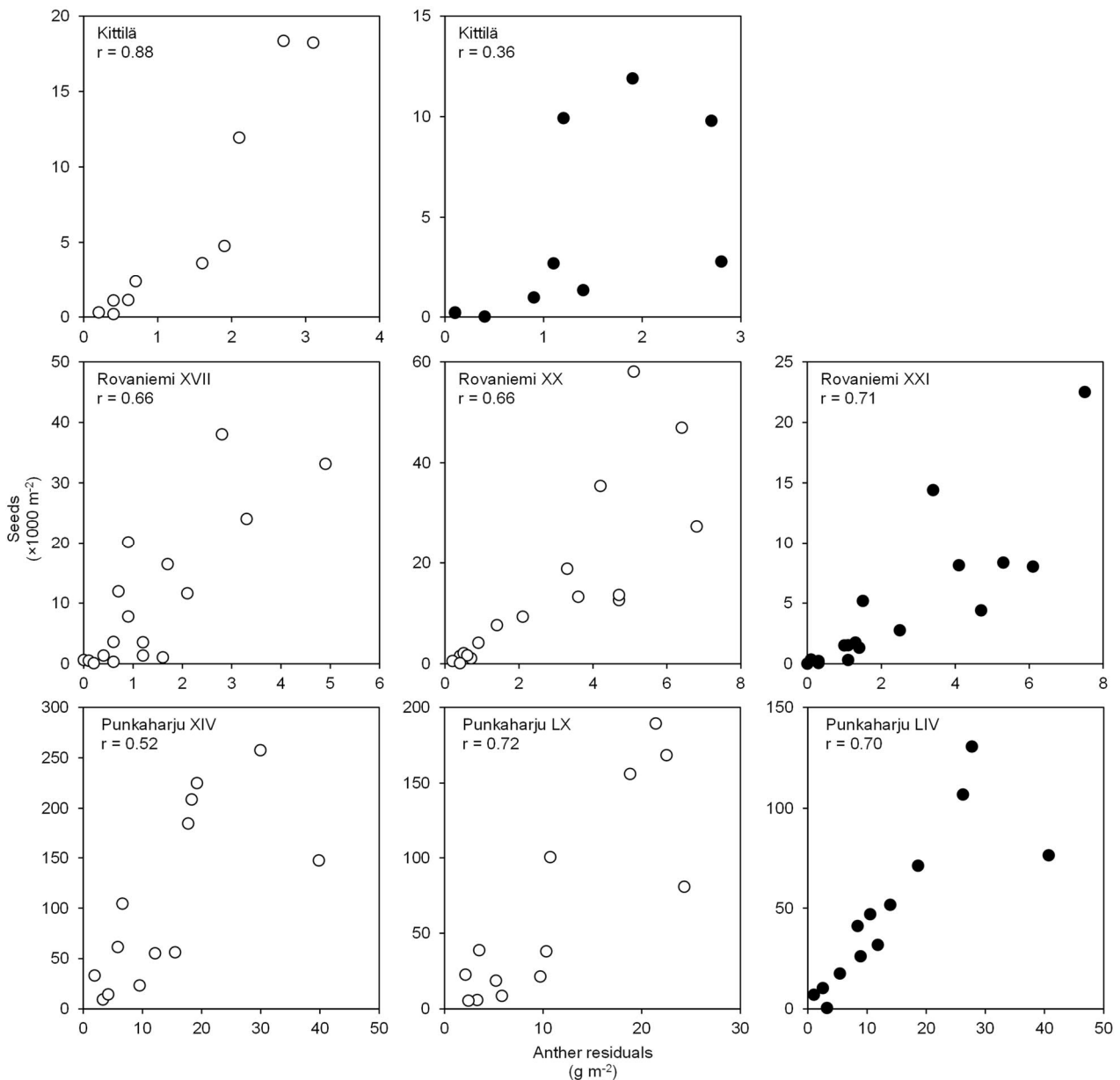
Figure 2. Correlation of anther residual and seed production across years in silver birch (black dots) and pubescent birch (white dots) stands (identified by roman numerals when applicable) in South (Punkaharju, n = 13 years) and North Finland (Rovaniemi, n = 17; Kittilä, n= 10). Data is from Koski and Tallqvist (1978).

For both species, the pollen (estimated using the mass of collected anther residuals) and seed production were 6-10 times higher in southern Finland than near the Arctic Circle (Table 5, Fig. 3).

343 However, interannual variation (measured as coefficient of variation among the years) was large in

344 all stands and for both anther residual and seed production (Table 5, Fig. 3). The variation was

345 generally larger in northern compared to southern stands except for the production of anther

346 residuals, which did not vary more in Kittilä than in Punkaharju (Table 5). Intraspecific differences

347 among stands were also significant; e.g. production of anther residuals was 98% and production of

348 seeds 45% higher in pubescent birch stand XVII than in stand XX, although both are situated in

349 Rovaniemi (Table 5). It appears that the two species did not generally differ in pollen production,

350 but pubescent birch produced more seeds than silver birch across Finland and was therefore always

351 more effective in seed production (i.e. in the number of seeds produced per unit of pollen) (Table 5,

352 Fig. 3).

353

354 **Table 5.** Mean production (with the coefficient of variation, CV of yearly records in brackets) of

355 anther residuals (g m^{-2}) and seeds (number m^{-2}) and the mean reproduction efficiency (number of

356 seeds produced per g anther residuals) in pubescent and silver birch forest stands in Punkaharju,

357 South Finland (a record of 13 years), and Rovaniemi (17 years) and Kittilä (10 years), North Fin-

358 land.

359

	Pubescent birch			Silver birch		
	Anther	Seeds	Eff	Anther	Seeds	Eff
Punkaharju XIV	14.1 (79)	106000 (83)	7500	13.8 (85)	48000 (83)	3500
Punkaharju LXII	10.8 (76)	66000 (101)	6100			
Rovaniemi XX	1.37 (95)	10400 (117)	7600	2.45 (106)	4800 (128)	1900
Rovaniemi XVII	2.71 (84)	15000 (116)	5500			
Kittilä	1.37 (77)	6200 (116)	4500	1.26 (77)	4000 (116)	3200
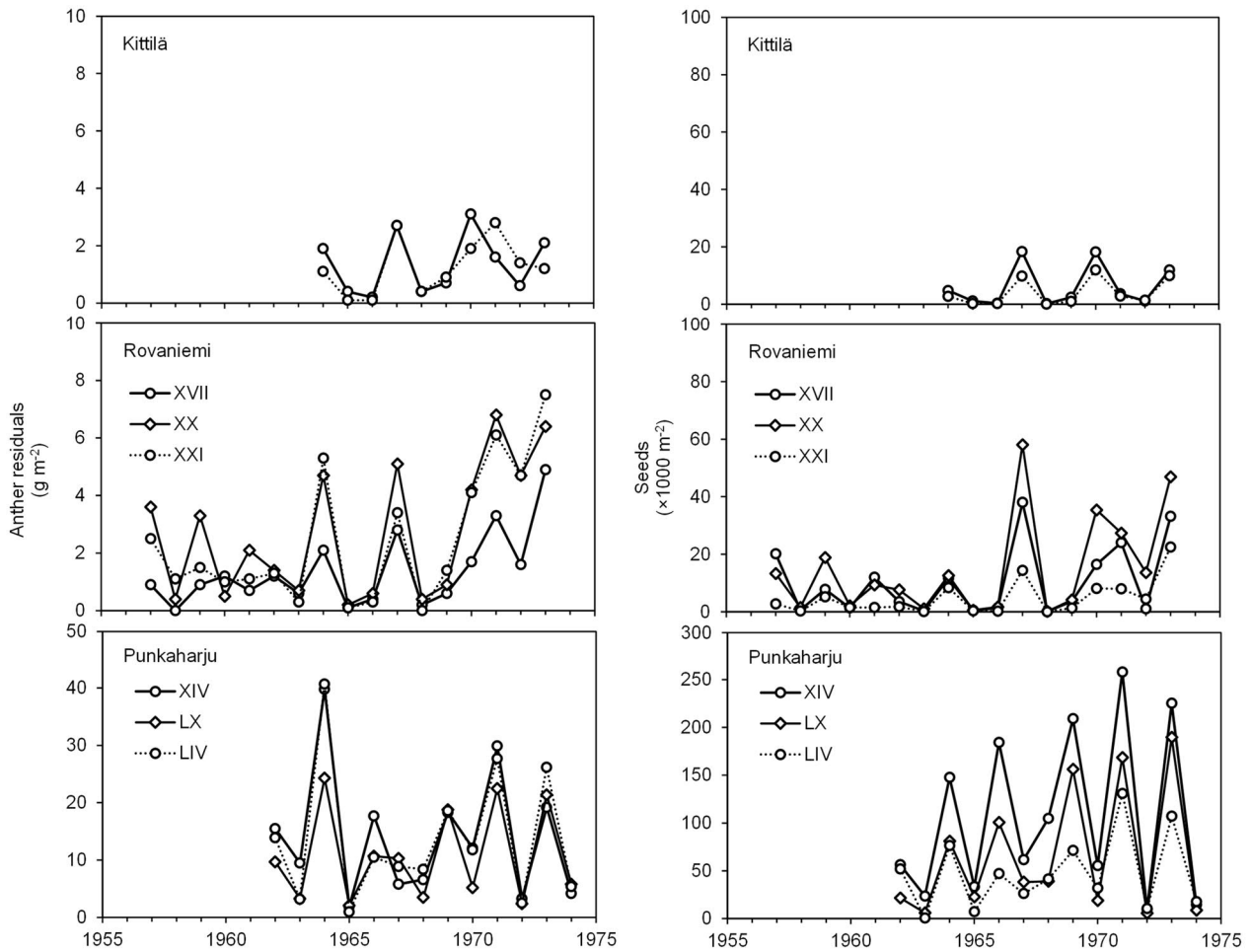
Figure 3. Interannual variation of anther residual and seed production for silver birch (dashed line) and pubescent birch (continuous line) stands (identified by roman numerals when applicable) in South (Punkaharju) and North Finland (Rovaniemi and Kittilä). Data is from Koski and Tallqvist (1978); note the different scales in South and North Finland graphs.

Discussion

As a drastic environmental change, the ongoing climate warming requires high adaptability of the boreal and Arctic tree species. In this study, we examined if the adaptation of white birches – i.e. silver birch and pubescent birch – in the North could be enhanced by gene flow from the South or interspecific hybridization, and if the current dominance of pubescent birch in the North is due to the reproduction of silver birch being more adversely affected by northern conditions. Our data

378 suggest that white birches growing in southern Finland always flower earlier than those growing in

379 the North. Also there appears to be no significant overlap in local flowering schedules among the

380 boreal populations of the two species. These results suggest that gene flow from the South and

381 interspecific hybridization likely play a minor role in the adaptation of white birches to the present

382 climate warming. Contrary to our expectations, we found that pubescent birch produces

383 significantly more seeds and has a higher reproduction efficiency than silver birch, not only in the

384 North, but also in southern Finland. Also, based on the interannual variation in pollen and seed

385 production among years, the susceptibility of the two species to interannual weather variation does

386 not significantly differ. Together, this suggests that the current dominance of pubescent birch in the

387 far North is not due to the reproduction of silver birch being more adversely affected by the

388 northern climate. This in turn indicates that climate warming may not significantly alter the relative

389 abundances of silver and pubescent birch in Subarctic Fennoscandia.

390 *Timing of anthesis in southern and northern silver birch populations*

391 Long distance gene flow is often thought to increase the ability of anemophilious species, such as

392 birches, to adapt to global change. Our data suggest that long distance (100–200 km) gene flow

393 among silver birch populations is possible in southern Finland as not only the onset of pollen

394 season, but also the timing of main pollen production of southern inland and coastal stands showed

395 a good match. However, contrary to earlier results with birch (Sharik & Barnes 1976, Junttila et al.

396 2003, Li et al. 2002) and other species (Beuker 1994), it appears that heat sums needed for silver

397 birch flowering are equal all over Finland, which means that there is no phenological overlap

398 between southern and northern birch populations. This suggests that, at least for birches, gene flow

399 between the southern (60–62º N) and northern (66–67º N) populations is unlikely. On the other

400 hand, the regional phenological patterns that occur in present climatic conditions may change under

401 climate warming (Ruosteenoja et al. 2011) and subsequently, adjust the present interpopulation

402 flowering synchrony.

403

404 Apart from the lack of phenological overlap in flowering between the southern and northern birch

405 populations, other reasons also speak against effective south-north gene flow. First, movement of

406 pollen clouds depends on complex dynamics of atmosphere (Solomon 2002) and pollen quantities

407 usually fall exponentially with distance (Sarvas 1952, 1956). Second, the frequency of male and

408 female catkins is -at least in silver birch- strongly and positively correlated (Rousi et al. 2011). As a

409 result, in good flowering years, prolific local pollen production hinders the possibilities of ex situ

410 pollen, whereas in years of low flowering, the success of ex situ pollen is reduced by pollen dilution

411 and low frequency of female flowers. Third, very little is known of the tolerance of pollen to high

412 altitude climatic conditions, but it appears that fertility of birch pollen deteriorates easily because of

413 unfavorable abiotic conditions and even prolonged flowering can reduce germinability (Saarnijoki

414 1941, Friedman & Barret 2009).

415 *Flowering phenology of co-occurring silver and pubescent birch populations*

416 The pollen catch data that we used suggest that the mean difference of flowering time between

417 silver and pubescent birch in warm springs is only a few days and that in most years pubescent

418 birch produces a substantial amount of pollen at the end of silver birch flowering. In situ

419 observations of catkins, in contrast, show a minor overlap in flowering phenology even when the

420 species grow at the same site and therefore, suggest low likelihood for silver and pubescent birch

421 hybridization in present climatic conditions in Finland. When pondering the reliability of the pollen

422 catch data, it is good to note that we used very conservative heat sum delimitation (i.e. the largest

423 observed heat sum for silver birch anthesis with a safety margin). As a consequence almost all

424 (90%) pollen collected in the southern pubescent birch stand remained in the data. Nevertheless,

425 even if we had used the mean heat sum for the onset of pubescent birch flowering (72 dd, Table 2),

426 still only 50% of pubescent birch pollen (yearly variation 28–79%) would have been omitted.

427 The discrepancy between the shorter duration of flowering in direct observations of silver birch

428 catkins and the longer duration estimated from pollen catch data suggests that pollen catch data can

429 have risks when concluding the duration of birch pollen period. The pollen recordings in southern

430 Finland suggest occasional days of pollen sharing, which are mostly late pollen peaks in silver birch

431 stands accompanied by simultaneous abundant pollen production in a nearby pubescent birch stand.

432 Exceptionally late pollen recordings in southern stands may, however, be due to drifting pollen

433 from the North in years of exceptionally prolific pollen production all over Finland (e.g. 1964 and

434 1971). Earlier studies did not make an effort to distinguish heterospecific and conspecific ex-situ

435 and in-situ pollen. This is a likely explanation for the longer estimate of 15 d for the duration of

436 silver birch pollen period in Linkosalo et al. (2010) in comparison to the estimate of 7 d in the

437 present study and 6 d in visual in-situ observations (Rousi et al. 2011). Drifting pollen (see Sarvas

438 1952) may also be the main reason why silver birch and pubescent birch flowering periods were

439 suggested to be almost identical by Luomajoki (1999) and Linkosalo et al. (2010). This suggestion

440 is not supported by our findings, and while the explanation for this discrepancy may largely be

441 pollen mixing, it could also partly originate from the different proportion of northern populations

442 among the examined silver birch and pubescent birch populations (Linkosalo et al 2010). All in all,

443 it appears that because pollen movements are unpredictable and no reliable methods, despite recent

444 advances in pollen analyses (Heidmann et al. 2016), are available for separating the pollen of the

445 two birch species on a practical scale (Jenyts-Szaferowa 1928, Mäkelä 1996 and references therein),

446 pollen catch data should not be used to estimate silver birch and pubescent birch flowering

447 phenology. Direct observations of flowering phenology are more reliable and not hindered by

448 methodological issues.

449 *Reproduction of silver and pubescent birch in the North*

450 We speculated that the dominance of pubescent birch over silver birch in the Subarctic

451 Fennoscandian forests might be explained by its more effective reproduction in the far North.

452   However, it appears that pubescent birch produces more seeds and has a higher efficiency of

453   reproduction (with respect to seeds produced per unit pollen) than silver birch already in southern

454   boreal forests. Therefore, although there is a trend of difference in the efficiency of reproduction

455   between the species growing towards the North, other factors are clearly needed to explain the

456   northern dominance of pubescent birch. Long-term, over 40-year old tree line common gardens near

457   the Kevo Subarctic field station of the University of Turku (70° N) show that transplanted silver

458   birches of more southern origin can, not only acclimate, but also be superior in growth compared to

459   other white birches (pers. obs.). During centuries, this may have led to selective cutting, thus

460   diminishing silver birch occurrence. Silver birch regeneration is also effectively prevented by

461   reindeer grazing in the North as illustrated by reindeer exclosures at sites suitable for silver birch

462   (pers. obs.). Generally, the efficient reproduction may though explain why pubescent birch, and not

463   silver birch, was the first to invade Northern Europe after the ice age.

464   Our data show how intrapopulation variations in reproduction can be large (compare the two

465   pubescent birch populations in Punkaharju and Rovaniemi, Table 5) in spite of no apparent

466   difference between the sites (same dominant tree height and age) and climatic conditions

467   (measurements carried out in common years). This is clearly a caveat in species comparisons, which

468   are based on a few tree stands, and cannot be compensated by temporally extensive data sets. For

469   instance, the interspecific difference in reproduction efficiency (as measured by seed production) at

470   the very northern site in Kittilä is surprisingly small when compared to the difference in Rovaniemi.

471   This is likely to be explained by an unrepresentative Kittilä pubescent birch stand, which produced

472   a significantly smaller seed set by using double amount of pollen than another, nearby stand (Koski

473   and Tallqvist 1978). We did not measure the quality of seeds, but interspecific differences may not

474   be large (Sarvas 1955, Holm 1994) although Sarvas (1952) suggested that seed germinability

475   increases exponentially with pollen quantity. Relating to this observation, it could be that the

476 relatively larger pollen production of silver birch in the North is needed to maintain seed

477 germinability in places, where birch stands are dominated by pubescent birch.

478 We expected the interannual variation in heat sum to have the strongest effect on reproduction at the

479 tree limit of silver birch – the average heat sum in Kittilä is only half that in Punkaharju – but found

480 the main leap in interannual variation in pollen and seed production between Punkaharju and

481 Rovaniemi and no further increase between Rovaniemi and Kittilä. The reason could be that many

482 environmental parameters, over and above heat sum, have significant effects in the marginal areas

483 of species distributions as recently found for Scots pine survival and growth (Rousi et al. 2018).

484 Based on the conclusions by Holm (1994), we further expected that in the far North, silver birch is

485 more reliant on favorable circumstances for large seed production than pubescent birch. However,

486 our material suggests that there is no difference between the species in this respect; i.e. there is no

487 difference in coefficients of variation of yearly records in anther and seed production in Kittilä. If

488 anything, it appears that the interannual variation of reproduction is higher for silver than pubescent

489 birch at the Arctic Circle. While these results need to be treated with caution as the comparisons are

490 based on one or two stands only, they seem to suggest that in the far North silver birch reproduction

491 is not more vulnerable to the interannual weather variation than the reproduction of pubescent birch.

492 As a result, the ongoing climate change may not significantly affect the relative abundance and

493 distribution of silver and pubescent birch in the North through altering the differences in their

494 reproductive output.

495

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501 dominant Betula species, spectromics and genomics" (decision #285030).

502 **References**

503 Andersen, S.T. 1970. The relative pollen productivity and pollen representation of north European

504 trees, and correction factors for tree pollen spectra, Danmarks geologiske Undersøgelse, Series II

505 96: 1–99.

506 Ashley, M. V. 2010 Plant Parentage, Pollination, and Dispersal: How DNA Microsatellites Have

507 Altered the Landscape Critical Reviews in Plant Sciences 29:148–161. DOI:

508 10.1080/07352689.2010.481167

509 Baskett, M. L., Gomulkiewicz, R. 2011. Introgressive hybridization as a mechanism for species

510 rescue. Theoretical Ecology 4: 223–239.

511 Basler, D., Körner, C. 2012. Photoperiod sensitivity of bud burst in 14 temperate forest tree species.

512 Agricultural and Forest Meteorology 165: 73–81. https://doi.org/10.1016/j.agrformet.2012.06.001

513 Beuker, E. 1994. Adaptation to climatic changes of the timing of bud burst in populations of Pinus

514 sylvestris L. and Picea abies (L.) Karst. Tree Physiology, 14, Issue 7-8-9961–970.

515 https://doi.org/10.1093/treephys/14.7-8-9.961

516 Birks, H.J.B. 1968. The identification of Betula nana pollen. New Phytologist 67: 309-314.

517 Clausen, K.E. 1960. A survey of variation in pollen size within individual catkins of three taxa of

518 Betula. Pollen et Spores 2: 299-304.

519 Dahl A., Strandhede S.O. 1996. Predicting the intensity of the birch pollen season. Aerobiologia 12:

520 97–106.

521   Frewen, B. E., Chen, T. H. H., Howe, G. T., Davis, J.. Rohde, A., Boerjan, W., Bradshaw, H. D. Jr.

522   2000. Quantitative trait loci and candidate gene mapping of bud set and bud flush in Populus.

523   Genetics 154: 837-845.

524   Friedman, J., Barret, S. C. H. 2009. Wind of change: new insights on the ecology and evolution of

525   pollination and mating in wind-pollinated plants. Annals of Botany 103: 1515-1527.

526   Hagman, M. 1971. On self- and cross-icompatibility shown by Betula verrucosa Ehrh. and Betula

527   pubescens Ehrh. Communications Instituti Forestalia Fenniae 73: 1-125.

528   Hänninen H., Tanino K. 2011.Tree seasonality in a warming climate. Trends in Plant Science 16:

529   412–416

530   Heidmann, I., Schade-Kampmann, G., Lambalk, J., Ottiger, M., Di Berardino, M. 2016. Impedance

531   Flow Cytometry: A Novel Technique in Pollen Analysis.

532   https://doi.org/10.1371/journal.pone.0165531

533   Hicks, S., Helander, M., Heino, S. 1994. Birch pollen production, transport and depositionthe

534   period 1984-1993 at Kevo, northernmost Finland. Aerobiologia. 10: 183 – 191.

535   Hjelmroos. M. 1991. Evidence of long-distance transport of birch pollen. Grana 30: 215-228.

536   Holm, S-O. 1994. Reproductive variability and pollen limitation in three Betula taxa in northern

537   Sweden. Ecography 17: 73-81. https://doi.org/10.1111/j.1600-0587.1994.tb00078.x

538   Howland, D.E, Oliver, R. R., Davy A.J. 1995. Morphological and molecular variation in natural

539   populations of Betula. New Phytologist 130: 117–124.

540   Hultén, E. 1971. The circumpolar plants. II. Dicotyledons. Kungliga Svenska

541   Vetenskapsakademiens Handlingar, Fjärde Serien, 13: 1– 463.

542   Hunter, A.F. and M.J. Lechowicz. 1992. Predicting the timing of budburst in temperate trees.

543   Journal of Applied Ecology 29: 597–604.

544   Järvinen, P. 2004. Nucleotide variation of birch (Betula L.) species: population structure and

545   phylogenetic relationships. PhD dissertation University of Joensuu, 34.

546   Jenyts-Szaferowa, J. (I928). La structure des membranes du pollen de Corylus, de Myrica et des

547   especes europeenes de Betula et leur determination a l'etat fossile. Bull. int. Acad. pol. Sci. Lett.,

548   Ser. B, 68, i.

549   Junttila, O., Nilsen, J., Igeland, B. 2003. Effect of temperature in the induction of bud dormancy in

550   various ecotypes of Betula pubescens and B. pendula. Scandinavian Journal of Forest Research 18:

551   208–217.

552   Kallio, P., Niemi, S., Sulkinoja, M.,Valanne, T. 1983. The Fennoscandian birch and its evolution in

553   the marginal forest zone. Nordicana 47: 101-110.

554   Koenig, W. D., Ashley, M. V. 2003. Is pollen limited? The answer is blowin' in the wind. Trends in

555   Ecology and Evolution 18: 157–159.

556   Koski, V. & Sievänen, R. 1985. Timing of growth cessation in relation to the variations in the

557   growing season. In: Crop physiology of forest trees (eds. Tigerstedt, P. M. A., Puttonen, P. &

558   Koski, V.). Helsinki University Press, 167–193.

559   Koski, V., Tallqvist, R. 1978. Results of long-time measurements of the quantity of flowering and

560   seed crop of forest trees (in Finnish with summary in English). Folia Forestalia 364. 60 pp.

561   Lenormand, T. 2002. Gene flow and the limits to natural selection. Trends in

562   Ecology and Evolution 17: 183–189.

563 Lewontin, R. C., Birch, L. C. 1966. Hybridization as a source of variation for adaptation to new

564 environments. Evolution. 20: 315–336.

565 Li, C., Puhakainen, T., Welling, A., Viherä-Aarnio, A., Ernstsen, A., Junttila, 0., Heino, P., PaIva,

566 E. T. 2002. Cold acclimation in silver birch (Betula pendula Roth). Development of freezing

567 tolerance in different tissues and climatic ecotypes. Physiologia Plantarrum 116: 478-488.

568 Linkosalo, T., Ranta, H., Oksanen, A., Siljamo, P., Luomajoki, A., Kukkonen, J., Sofiev, M. 2010.

569 A double-threshold temperature sum model for predicting the flowering duration and relative

570 intensity of Betula pendula and B. pubescens. Agricultural and Forest Meteorology 150: 1579-1584.

571 Luomajoki, A. 1999. Differences in the Climatic Adaptation of Silver Birch (Betula pendula) and

572 Downy Birch (Betula pubescens) in Finland Based on Maler Flowering Phenology. Acta Forestalia

573 Fennica 263, 35pp.

574 Mäkelä, E. M. 1996. Size distinctions between Betula pollen types- a review. Grana 35: 248-256.

575 North, A., Pennanen, J., Ovaskainen, O., Laine, A.-L. 2010. Local adaptation in a changing world:

576 the roles of gene-flow, mutation, and sexual reproduction. Evolution 65-1: 79-89.

577 Oikonen M, Hicks S, Heino S, Rantio-Lehtimäki A. 2005. Separation of long distance transported

578 (LDT) pollen from local mountain birch pollen at the Kevo subarctic monitoring station, Finland.

579 Grana 44: 181-186.

580 Palmé A. E., Qiao, S., Palsson, S., Lascoux, M. 2004. Extensive sharing of chloroplast haplotypes

581 among European birches indicates hybridisation among *Betula pendula*, *B. pubescens* and *B. nana*.

582 Molecular Ecology 13: 167-178.

583 Pauley, S. S., Perry, T. O. 1954. Ecotypic variation of the photoperiodic response in Populus. J.

584 Arnold Arbor. Harv. Univ. 35: 167-188.

585   Prentice, I. C. 1981. Quantitative birch (Betula L.) pollen separation by analysis of size frequency

586   data. New Phytologist 89: 145-157.

587   Ranta, H., Kubin, E., Siljamo, P., Sofiev, M., Linkosalo, T., Oksanen, A., Bondestam, K. 2006.

588   Long distance pollen transport cause proplems for determining the timing of birch pollen season in

589   Fennoscandia by using phenological observations. Grana 45: 297-304.

590   Ranta H., Satri P. 2007. Synchronized inter-annual fluctuation of flowering intensity affects the

591   exposure to allergenic tree pollen in North Europe. Grana, 46: 274 - 284.

592   Rousi, M., Heinonen, J. 2007. Temperature sum accumulation effects on within-population

593   variation and long-term trends in date of bud burst of European white birch (Betula pendula). Tree

594   Physiology 27: 1019–1025. https://doi.org/10.1093/treephys/27.7.1019

595   Rousi, M., Heinonen, J., Neuvonen, S. 2011. Intrapopulation variation in flowering phenology and

596   fecundity of silver birch, implications for adaptability to changing climate. Forest Ecology and

597   Management 262: 2378–2385. https://doi.org/10.1016/j.foreco.2011.08.038

598   Rousi, M., Possen, B. J. M. H., Ruotsalainen, S., Silfver, T., Mikola, J. 2018. Temperature and soil

599   fertility as regulators of tree line Scots pine growth and survival—implications for the acclimation

600   capacity of northern populations. Global Change Biology 24:e545–e559.

601   https://doi.org/10.1111/gcb.13956

602   Ruosteenoja, K., Räisänen, J., Pirinen, P. 2010. Projected changes in thermal seasons and growing

603   seasons in Finland. International Journal of Climatology 31:1473-1487. doi: 10.1002/joc.2171

604   Rusanen, M., Vakkari, P., Blom, A. 2003. Genetic structure of Acer platanoides and Betula pendula

605   in Northern Europe. Canadian Journal of Forest Research 33: 1110–1115.

606 Saarnijoki, S. 1941. Verssuche uber die Keimung von Waldbaumpollen. Communicationes Instituti

607 Forestalis Fenniae 29, 17 pp.

608 Salojärvi,J., Smolander, O-P…Jaakko Kangasjärvi, J. 2017. Genome sequencing and population

609 genomic analyses provide insights into the adaptive landscape of silver birch. Nature Genetics

610 volume 49: 904–912.

611 Sarvas, R. 1952. On the flowering of birch and the quality of seed crop. Communications Instituti

612 Forestalia Fenniae 40(7), 38 pp. URN:NBN:fi-metla-201207171072

613 Sarvas, R. 1955. Investigations into the flowering and seed quality of forest trees. Communications

614 Instituti Forestalia Fenniae 45(7), 37pp.

615 Sarvas, R. 1956. Investigations into the dispersal of birch pollen with a particular view to the

616 isolation of seed source plantations. Communications Instituti Forestalia Fenniae 46, 19pp.

617 Sarvas, R. 1962. The development of the tree species composition of the forests of southern Finland

618 during the past two thousand years. Communications Instituti Forestalia Fenniae Comm Inst For

619 Fenn 55, 14 pp. URN:NBN:fi-metla-201207171087

620 Savolainen, O., Pyhäjärvi, T., Knürr, T. 2007. Gene flow and local adaptation in trees. Annual

621 Review of Ecology, Evolution and Systematics. 38, 595–619.

622 https://doi.org/10.1146/annurev.ecolsys.38.091206.095646

623 Sharik, T.L., Barnes B.V. 1976. Phenology of shoot growth among diverse populations of yellow

624 birch (Betula alleghaniensis) and sweet birch (B. lenta). Canadian Journal of Botany 54: 2122-2129.

625 Sofiev, M., Siljamo, P., Ranta, H., Rantio-Lehtimäki, A. 2006. Towards numerical forecasting of

626 long-range air transport of birch pollen: theoretical considerations and a feasibility study.

627 International Journal of Biometeorology 50: 392-402.

628 Solomon, W.R., 2002. Airborne pollen: a brief life. Current reviews of allergy and clincal

629 immunology. 109: 895–900

630 Whitehead, D. R. 1969. Wind pollination in the Angiosperms: Evolutionary and Environmental

631 considerations. Evolution 23: 28-35.