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Pollen-limited production of viable seeds in an endemic dwarf birch, *Betula apoiensis*, and incomplete reproductive barriers to a sympatric congener, *B. ermanii*

Teruyoshi Nagamitsu[a,*], Takayuki Kawahara[a], Ayako Kanazashi[b]

[a]Hokkaido Research Center, Forestry and Forest Products Research Institute (FFPRI), Hitsujigaoka 7, Toyohira, Sapporo 062-8516, Japan
[b]Forestry and Forest Products Research Institute (FFPRI), Tsukuba 305-8687, Japan

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ABSTRACT

An endemic dwarf birch, *Betula apoiensis*, is critically endangered, and two populations of this species are restricted to the ridges of Mt. Apoi in Hokkaido, Japan. We observed the flowering phenology, pollen dispersal, and viable seed production and conducted pollination experiments in order to examine pollen limitation and hybridization with a sympatric congener, *B. ermanii*. *B. apoiensis* flowered earlier than *B. ermanii* but had a more variable flowering time among trees than *B. ermanii*. The female flowering of *B. apoiensis* temporally overlapped with the male flowering of *B. ermanii* as well as with that of *B. apoiensis*. Pollination experiments demonstrated that seed set and seed germination were higher in female flowers outcrossed than in those that were non-pollinated, selfed, hybridized with *B. ermanii* pollen, or pollinated naturally. A few selfed or hybrid seeds were filled and germinated, which indicates that self-incompatibility and reproductive barriers are not complete. Logistic regressions of local density of conspecific trees on natural seed set and seed germination were significantly positive. These results suggest that *B. apoiensis* is pollen-limited.

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1. Introduction

Small populations are more likely to be prone to extinction due to stochastic changes in environment and demography (Shaffer, 1981) as well as systematic declines in suitable environment (Thomas, 1994). In addition, small populations may suffer from an Allee effect that reduces survival and/or fertility at population densities lower than a critical threshold (Allee, 1938). In plants, the Allee effect on reproductive success has received much consideration (Hackney and McGraw, 2001; Forsyth, 2003) because declines in population density diminish the opportunities of mating, particularly in sessile organisms. The Allee effect may reduce reproductive success due to not only a decrease in conspecific pollination with compatible mates but also increase in interspecific pollina-

tion with related species. The former occurs in plant populations in which reproduction is pollen-limited. The latter happens in plant taxa in which reproductive barriers are incomplete. Both processes threaten small relict species and island endemics that should be conserved (Rieseberg et al., 1989).

Wind-pollinated plants usually produce abundant pollen that can travel long distances. The quantity of pollen did not limit fertilization of *Staberoha banksii* and that wind was highly effective as a pollen vector (Honig et al., 1992). However, an Allee effect due to pollen limitation was demonstrated in isolated patches of *Spartina alterniflora* at the invasion front (Davis et al., 2004). Pollination efficiency tends to increase as flowering tree density increases (Kelly and Sullivan, 1997), which is regarded as one of the selective advantages of mast

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seeding (Kelly et al., 2001). Reproduction limited by the availability of pollen from near-neighbor mates may impact the regeneration of threatened populations in which habitat is fragmented and density is reduced (Knapp et al., 2001).

Hybridization resulting from interspecific pollination may reduce reproductive success in rare plant species (Levin et al., 1996). Hybrid seeds, whether they are viable or not, may be produced at the expense of conspecific seeds. Even if introgressive hybridization between species is bi-directional, a numerically inferior species is likely to produce a larger proportion of hybrid seeds than an abundant species due to pollen swamping. The weaker the reproductive barriers, the greater the impact on seed production and, ultimately, on population viability (Wolf et al., 2001). If hybrid progeny are viable and fertile, they may displace pure conspecifics, a process known as genetic assimilation.

A dwarf birch, *Betula apoiensis* Nakai, is critically endangered and is restricted to the ridges of Mt. Apoi in Hokkaido, Japan (Environment Agency of Japan, 2000). The alpine vegetation of Mt. Apoi is unique due to ultra-basic rock made of peridotite and strong winds blowing from the sea. Taxonomists recognized at least three species endemic to this mountain, one of which is *B. apoiensis* (Tatewaki, 1963). Some observers reported that the number of *B. apoiensis* trees was less than a hundred (Environment Agency of Japan, 2000). Such small populations may lead to the Allee effect on seed production in *B. apoiensis*. In addition, *B. apoiensis* is mixed with a more abundant congener, *B. ermanii*, that has recently been increasing (Watanabe, 2001). Birches have been known to hybridize artificially or naturally between congeneric species (Thorsson et al., 2001). If reproductive barriers between *B. apoiensis* and *B. ermanii* are weak, hybridization may cause pollen swamping and/or genetic assimilation. Knowledge of reproductive features is regarded as fundamental for conservation of threatened plants but has not been clarified in *B. apoiensis*.

In this study, we investigated whether production of viable seeds is pollen-limited in *B. apoiensis* and whether reproductive barriers are complete between *B. apoiensis* and *B. ermanii*. In order to examine pollen limitation, we compared viable seed production between artificially outcrossed and naturally pollinated flowers and among trees from populations with different local densities. In order to examine reproductive barriers, we observed flowering phenology and temporal changes in airborne pollen abundance of *B. apoiensis* and *B. ermanii* and compared viable seed production in *B. apoiensis* between treatments pollinated by conspecific pollen and by *B. ermanii* pollen.

2.   Materials and methods

2.1.   Study site and species

This study was conducted on the western and southern ridges of Mt. Apoi in Hokkaido, northern Japan (42°6′N, 142°1′E, altitude 580–810 m) (Fig. 1). In Hokkaido, ultra-basic rock areas are distributed along the central tectonic line (Horie et al., 2000). Mt. Apoi is located at the southernmost part of the ultra-basic rock areas and is characterized by high contents of peridotites. In addition to this exceptional substrate, strong
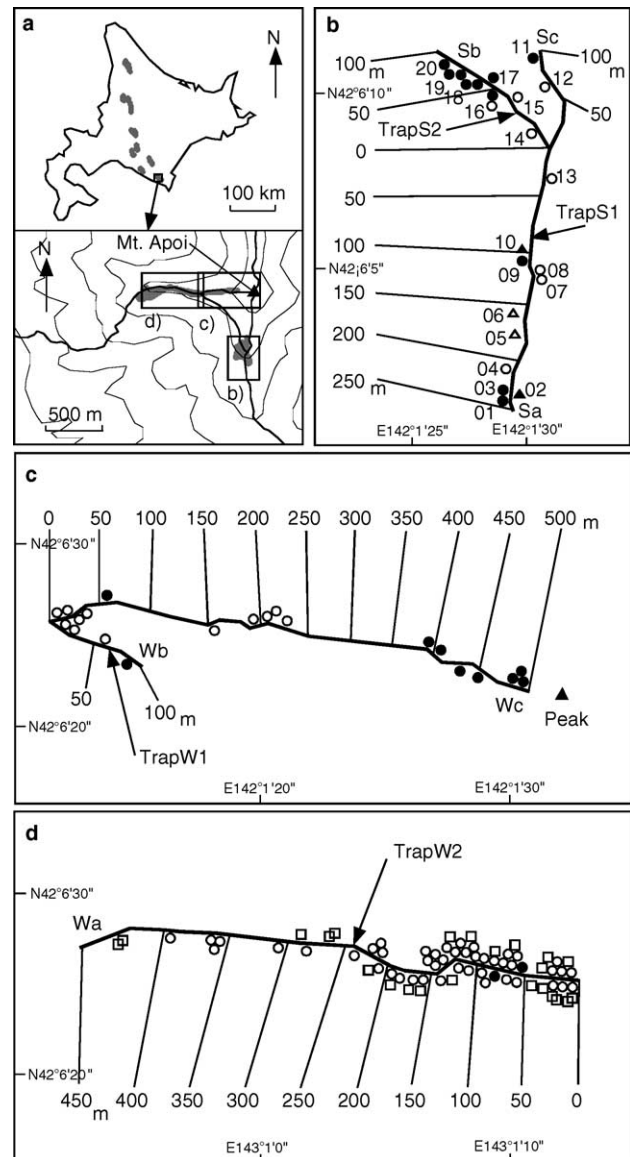

Fig. 1 – Location of the study sites, transects, and trees of *Betula apoiensis* and *B. ermanii*. (a) Study sites on the southern and western ridges on Mt. Apoi in Hokkaido. Shaded areas show ultra-basic rock areas in Hokkaido and alpine vegetation areas on Mt. Apoi. Contour lines are 100 m intervals. (b–d) Six transects (Sa, Sb, Sc, Wa, Wb, and Wc) and trees on the southern ridge (b), the upper (c), and lower (d) parts of the western ridge. Open and closed circles show fruiting trees of *B. apoiensis* and *B. ermanii*, respectively, in the transects. Open squares show *B. apoiensis* trees in which pollination treatments were conducted next to transect Wa. Open and closed triangles show trees of *B. apoiensis* and *B. ermanii*, respectively, in which the flowering phenology was observed next to transect Sa. The tree number corresponds to Fig. 3. Positions of four Durham traps (S1, S2, W1, and W2) to collect airborne pollen are also shown.

winds blowing from the Pacific Ocean makes the environmental conditions unique, which may promote endemism in the alpine flora on this mountain.

B. apoiensis Nakai is a prostrate shrub up to 1.5 m in height that is endemic to Mt. Apoi. Although this species is thought to be taxonomically close to a dwarf birch, *B. ovalifolia*, and a tree birch, *B. ermanii*, the chromosome number and phylogenetic origin of *B. apoiensis* are not known (Hara et al., 1989). *B. apoiensis* is listed as a critically endangered species (Category IA) in the Red Data Book for Threatened Wildlife (vascular plants) in Japan (Environment Agency of Japan, 2000). Our preliminary survey showed that *B. apoiensis* was found only on the western and southern ridges of Mt. Apoi (unpublished data). The habitats of *B. apoiensis* have been restricted due to the succession of alpine vegetation and growth of creeping pines, but the number of *B. apoiensis* has been stable during the last 50 years probably because illegal plant collectors have ignored this species (Watanabe, 2001). *B. ermanii* Cham. is a sympatric congener on Mt. Apoi. This species is distributed on the slopes between the ridges and has been increasing and growing around the mountain peak for the last 50 years (Watanabe, 2001).

2.2. Population survey

We counted the number of trees of *B. apoiensis* and *B. ermanii* in six transects with 4 m width along trails in 2002 (Fig. 1). *B. apoiensis* forms clusters of shoot(s) growing from the same base. We regarded each cluster as a tree. Because the trails went through the alpine vegetation on Mt. Apoi, the transects seemed to represent the habitats of *B. apoiensis*. Three transects, Sa, Sb, and Sc, were on the southern ridge, and the other three, Wa, Wb, and Wc, were on the western ridge. We recorded the locations of trees and the presence of fruits on each tree.

We distinguished *B. apoiensis* and *B. ermanii* based on leaf morphology. We performed a principal component analysis for seven leaf characters of individual trees (length of apical and basal halves, width, petiole length, base angle, the numbers of veins, and the number of teeth between the first and second veins) using JMP software (SAS Institute, 2001) (Fig. 2). We recognized two groups of trees on the coordinates of the principal components. We determined thresholds of a few morphological characters to discriminate the two groups that should correspond to *B. apoiensis* and *B. ermanii*.

2.3. Flowering phenology

We observed the flowering phenology of 10 trees of *B. apoiensis* and 10 trees of *B. ermanii* on the southern ridge every week from 7 May to 2 June in 2003 (Fig. 1). Some trees were observed next to transect Sa. Because the two species were mixed more closely on the southern ridge than on the western ridge, the observed trees seemed to share more similar environmental conditions in their habitats. We tagged three branches with flower buds of each tree and then counted the number of male and female inflorescences of which most flowers had prominent, open anthers or exposed, reddish stigmas, which indicated that sexual function was active. We observed an average of 23.5 (range: 6–46) male and 9.5 (1–34) female inflorescences from each tree.

The proportion P_{it} of flowering inflorescences was calculated for each tree i on each date t.
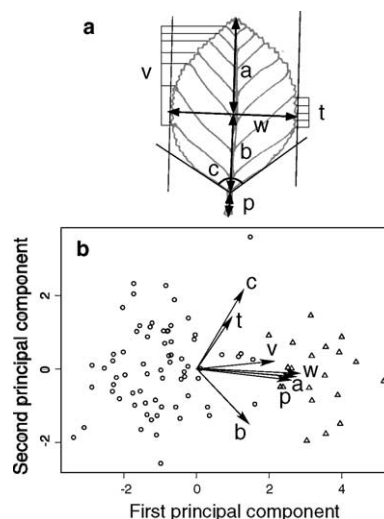

Fig. 2 – Analysis of leaf morphology in *Betula apoiensis* and *B. ermanii*. (a) Measurements of leaf morphology. a: apical length, b: basal length, w: width, c: base angle, v: the number of veins, and t: the number of teeth between the first and second veins. (b) Principal component analysis based on the seven measurements. Individual trees identified as *B. apoiensis* (circles) and *B. ermanii* (triangles) are plotted on the coordinates of the first and second principal components. Arrows show vectors of coefficients of the measurements for the first and second principal components.

$$P_{it} = N_{it}/\Sigma_t N_{it}, (\Sigma_t P_{it} = 1),$$

where N_{it} is the observed number of flowering inflorescences of tree i on date t. The index of temporal overlap O_{ij} of flowering was calculated between female flowers of a tree and male flowers of another tree of the same species or of a different species. This overlap index is usually called percentage similarity, which ranges between 0 (no overlap) and 1 (complete overlap) (Whittaker, 1952).

$$O_{ij} = 1 - 0.5\Sigma_t|P_{it} - P'_{jt}|,$$

where P_{it} is the proportion of flowering male inflorescences of tree i on date t and P'_{jt} is that of flowering female inflorescences of tree j on date t. Variations in O_{ij} between conspecific and interspecific sexes were compared in each of the *B. apoiensis* and *B. ermanii* trees.

2.4. Pollen dispersal

Four Durham pollen traps (DK-1SA, Nishi-Seiki Co., Ltd.) were placed on the rocks at about 1 m height above the ground on the southern and western ridges (Fig. 1). Every week from 10 May to 6 June in 2002 and from 2 May to 11 June in 2003, slides covered with white vaseline on the upper surface were set in the traps at noon. The slides were collected at noon on the next day. A day with good weather was selected for trapping. The genera of pollen grains that had been trapped were identified under a light microscope. The grains of each genus were counted in $288 \, mm^{-2}$ areas ($24 \times 12 \, mm$ squares) on the slides.

A description of the pollen morphology indicated that the diameter of pollen grains was different between *B. apoiensis* (20.1–28.8 μm, $n = 50$) and *B. ermanii* (30.6–39.6 μm, $n = 50$) (Nakamura, 1980). In order to confirm the difference in pollen size, the diameters of pollen grains collected from male inflorescences of *B. apoiensis* and *B. ermanii* on Mt. Apoi were measured. Male inflorescences from more than five trees of each species were sampled in 2002, and the pollen grains in these samples from each species were mixed. The pollen grains were deposited on slides covered with white vaseline on the upper surface, and the diameter of the pollen grains was measured under a light microscope.

The temporal changes in frequency distributions of the diameter of airborne pollen grains of birches were investigated in 2002 and 2003. The diameters of birch pollen grains collected in the four traps on each of the three dates when they were abundant were measured. Pollen grains were sampled in the unit area on the slides from each of the four traps. Differences in the frequency distributions of the diameter of pollen grains were tested using the Kolmogorov–Smirnov test.

2.5. Pollination experiment

Pollination treatments were conducted for female flowers of *B. apoiensis* along transect Wa in 2002 and 2003 (Fig. 1). Before the flowers opened, all male inflorescences on selected branches of a tree were collected. The male inflorescences collected from these branches of *B. apoiensis* and from *B. ermanii* in the six transects were stored at 4 °C and used as pollen donors. All female inflorescences on these branches were enclosed with paper bags for pollination (19.5 × 40 cm, Japan Forest Tree Breeding Association). In the bagged treatment, the flowers remained in this condition until flowering ended. The bagged treatment was conducted for 24 inflorescences of seven trees in 2002 and 21 of six in 2003. The male inflorescences were dried and warmed to open anthers a few days before the pollination treatments. In the selfed treatment, pollen from the same tree was injected into the bags using pollen guns (Japan Forest Tree Breeding Association) on 15 and 22 May in 2002 and on 21 and 28 May in 2003. The selfed treatment was conducted for 29 inflorescences of nine trees in 2002 and 23 of six in 2003. In the other two treatments, flowers were pollinated in the same way as in the selfed treatment. Pollen of male inflorescences collected from trees in the bagged treatment was used in the outcross treatment (seven trees in 2002 and six in 2003), and pollen from the same number of *B. ermanii* trees in the each year was used in the hybridized treatment. The outcross treatment was conducted for 24 inflorescences of eight trees in 2002 and 30 of 11 in 2003, and the hybridized treatment was conducted for 25 inflorescences of eight trees in 2002 and 20 of six in 2003. After flowering ended, the bags were removed in all treatments.

Fruits on the branches in the four treatments were collected on 28 and 29 August 2002 and on 8 and 9 September 2003. On these dates, 90 fruits of *B. apoiensis* that had been pollinated naturally were also collected from 30 trees in 2002, and 87 fruits were collected from 29 trees in 2003 (Fig. 1). These trees were randomly sampled in the six transects.

2.6. Viable seed production

Seed walls always develop even in the absence of pollination and embryo development in birches (Atkinson, 1992; DeGroot et al., 1997). Thus, the number of seeds, regardless of whether they are filled or empty, is equal to that of female flowers. A soft-X ray photography and germination experiment was conducted to determine whether the seeds were viable or not.

Soft-X ray photographs of the seeds collected in 2002 and 2003 were taken with a Soft X-Ray Apparatus SR-3010 (Yutaka Electric Mfg. Co., Ltd.). The seeds were exposed to an X ray at 16 kV and 14 mA for 220 s. The seeds in which embryos filled the inside of the seed walls were counted.

Seeds collected in 2002 and 2003 were sown on a moistened filter paper and a 2-mm thick sponge disc in a 9-cm Petri dish. The seeds were incubated at 15–20 °C in a 14:10-hour day–night cycle after a treatment at 4 °C in darkness for one week. The seeds that had germinated were counted after the incubation for two weeks.

The probability that the seeds were filled or germinated was analyzed separately using logistic regression. The logistic model included year (2002 and 2003), pollination treatment (bagged, selfed, outcross, hybridized, and natural), and their interaction.

The effect of local density of *B. apoiensis* trees on the probability that seeds pollinated naturally were filled or germinated was also evaluated using logistic regression. The model consisted of year (2002 and 2003), local tree density around a mother tree within each of eight distances (10, 30, 60, 100, 150, 210, 280 and 360 m) along the transects, and their interaction. The models with the densities within different distances were compared based on log likelihood. The model with the highest log likelihood value was selected as the best fitting model (Agresti, 1996).

The significance of the factors and interactions in the selected model was tested by Wald χ^2 values. Statistical analysis was conducted by JMP software (SAS Institute, 2001).

3. Results

3.1. Population structure

The principal component analysis of leaf morphology indicated two groups of trees, which were identified as *B. apoiensis* and *B. ermanii* (Fig. 2). The first and second principal components contributed 61% and 16%, respectively, of the total variation in the seven characters. Based on the coefficients of these characters for the principal components, petiole length, leaf width, and length of the apical half of a leaf mainly discriminated the two species (Fig. 2). A tree with a petiole longer than 14 mm, a leaf wider than 40 mm, and an apical half of a leaf longer than 30 mm was identified as *B. ermanii*. The other trees were identified as *B. apoiensis*.

Six transects on the southern and western ridges of Mt. Apoi were 1500 m long in total (Fig. 1). In these transects, 306 trees of *B. apoiensis* and 379 trees of *B. ermanii* were found. Among them, 66 of *B. apoiensis* and 22 of *B. ermanii* produced fruits in 2002 (Fig. 1b–d). These two species were mixed, but their spatial distributions were likely to differ. *B. apoiensis*

was abundant at the middle of the western ridge (Fig. 1(d)), whereas *B. ermanii* was abundant at the southwestern slope (Fig. 1(b)) and around the peak (Fig. 1(c)).

3.2. Flowering phenology

In both *B. apoiensis* and *B. ermanii*, male inflorescences tended to flower earlier than female ones in the same tree, but the flowering time overlapped between males and females within a tree (Fig. 3). At the population level, male and female flowering overlapped more than at the individual tree level due to variation in flowering time among trees. In both male and female inflorescences, *B. apoiensis* trees flowered earlier, longer, and with more variance in the time of flowering than *B. ermanii* trees (Fig. 3).
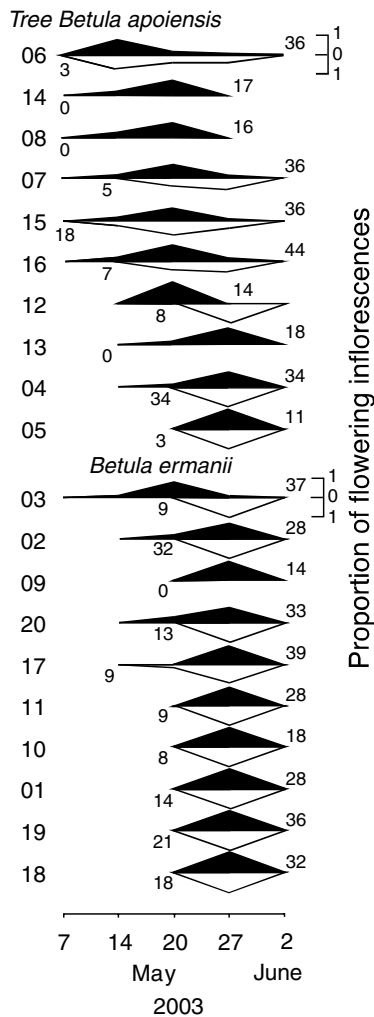
The index of temporal overlap between the female flowering of *B. apoiensis* trees and the male flowering of conspecific trees was 0.43 [0.07–0.59], $n = 63$ (median [25–75 percentiles], the number of combinations of trees), and that between *B. apoiensis* female and *B. ermanii* male flowering was 0.57 [0.36–1.00], $n = 70$. The overlap index between *B. ermanii* female and conspecific male flowering was 1.00 [0.83–1.00], $n = 81$, and that between *B. ermanii* female and *B. apoiensis* male flowering was 0.07 [0.00–0.71], $n = 90$.

3.3. Pollen dispersal

Pollen dispersal of birches peaked in the middle of May on Mt. Apoi (Fig. 4). The temporal changes in the number of trapped pollen grains of birches were different between 2002 and 2003. In 2003, pollen dispersal had two peaks, and the early peak was absent in 2002. Birch pollen grains seemed to be more abundant in 2003 than in 2002.

The frequency distributions of pollen diameter were not significantly different from normal in *B. apoiensis* (Pearson's χ^2 normality test: $P = 0.075$) and *B. ermanii* ($P = 0.305$). The mean diameter of pollen grains significantly differed between *B. apoiensis* and *B. ermanii* (*t*-test: $P \leqslant 0.001$). The pollen diameter was 29.8 ± 2.0 μm (mean ± SD, $n = 100$) in *B. apoiensis* and 31.7 ± 1.9 μm ($n = 100$) in *B. ermanii*. In spite of the difference in the mean diameter, the ranges of the diameter overlapped between the two species.

In 2002, the frequency distributions of the diameter of airborne pollen grains of birches significantly differed between 10 and 16 May (Kolmogorov–Smirnov test: $P \leqslant 0.001$), but they did not significantly differ between 16 and 23 May ($P = 0.228$) (Fig. 5). In 2003, those were significantly different not only between 9 and 15 May ($P \leqslant 0.001$) but also between 15 and 21 May ($P \leqslant 0.001$). In 2003, the frequency distribution was unimodal with a small mode (28.2 μm) on 9 May, bimodal with small (26.6 μm) and large (34.6 μm) modes on 15 May, and unimodal with a large mode (34.6 μm) on 21 May (Fig. 5). In 2002, the frequency distributions changed from bimodal with small (29.8 μm) and large (33.8 μm) modes to unimodal with a large mode (33.0 μm). Thus, the cumulative frequency of small pollen grains was lower in 2002 than in 2003. These small and large modes of pollen sizes were likely to correspond to the mean pollen sizes in *B. apoiensis* and *B. ermanii*, respectively.

Fig. 3 – Flowering phenology of male and female inflorescences of *B. apoiensis* and *B. ermanii* trees. The tree number corresponds to Fig. 1. Closed (male) and open (female) diagrams show the proportions: (the number of flowering inflorescences on each date)/(the total number of flowering inflorescences observed during the whole period). Numbers beside the area show the number of male (upper right) and female (lower left) inflorescences observed.
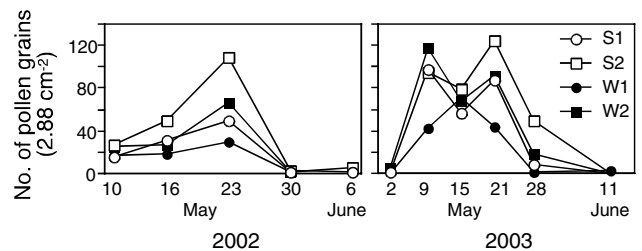

Fig. 4 – Temporal changes in the number of birch pollen grains collected by four Durham traps (S1, S2, W1, and W2) shown in Fig. 1. Pollen grains trapped in white vaseline in a 2.88 cm^{-2} area on a slide were counted.
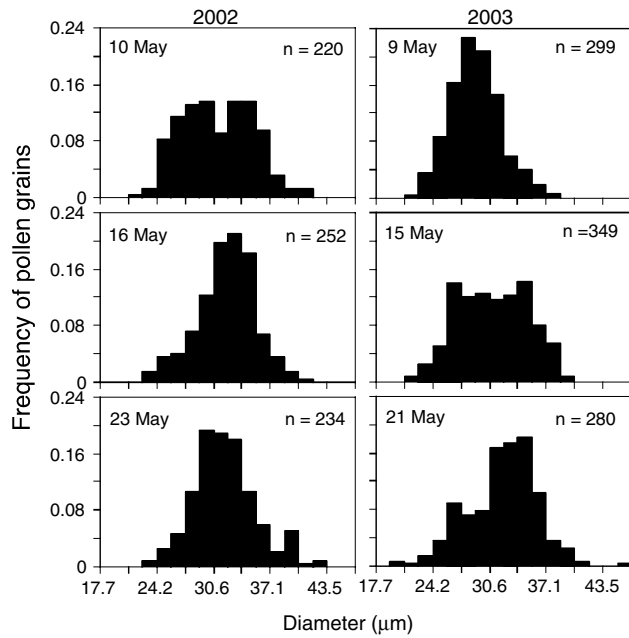
Fig. 5 – Temporal changes in frequency distributions of the diameter (μm) of birch pollen grains collected by four Durham traps. Pollen grains in unit areas on slides from the four traps on each trapping date were pooled.

3.4. Viable seed production

The probability that seeds were filled (seed set) differed significantly among the five pollination treatments ($P \leqslant 0.001$) (Table 1). The probability that seeds germinated (seed germination) showed the same result. Both seed set and seed germination were high in the outcross treatment, low in the selfed and hybridized treatments as well as in the natural pollination treatment (Fig. 6). The bagged treatment resulted in very low seed set and seed germination (Fig. 6). The interaction between the year and pollination treatment was significant in seed set ($P = 0.032$) and seed germination ($P \leqslant 0.001$) (Table 1), indicating that effects of the pollination treatments varied between the years (Fig. 6).

The log likelihood value of a logistic model with the local tree density within 60 m was highest (Table 2). In this model,
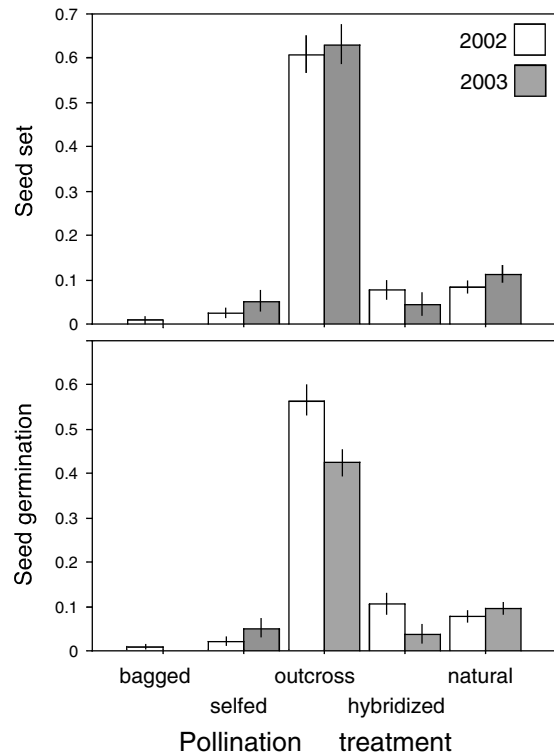

Fig. 6 – Probability that seeds were filled (seed set) or germinated (seed germination) in five pollination treatments in 2002 and 2003. Error bars show 95% confidential intervals.

the local tree density had a significantly positive effect on both seed set and seed germination ($P \leqslant 0.001$) (Table 3 and Fig. 7). The effect of the density on seed set significantly varied between years ($P \leqslant 0.001$) (Table 3). In 2002, seed set gradually increased as the local tree density increased and peaked at the highest density class (Fig. 7). In 2003, however, seed set varied among density classes, and a systematic increase was not observed.

4. Discussion

4.1. Pollen limitation

Our survey demonstrates that *B. apoiensis* was fragmented into two populations on the western and southern ridges of Mt. Apoi. One population on the western ridge had a single core habitat, and the tree density decreased at the peripheries of the habitat. The tree density was lower on the southern ridge than on the western ridge. This pattern of distribution produces a wide variation in the local tree density of *B. apoiensis*.

Our pollination experiments show that artificially outcrossed flowers produced 4–6-fold more viable seeds than naturally pollinated flowers. This result supports the pollen limitation of viable seed production. The abundance of airborne pollen of birches was higher in 2003 than in 2002, particularly in small grain size and in the early flowering time, in which pollen may belong to *B. apoiensis*. Viable seed production in the natural condition was also higher in 2003 than in

Table 1 – Effects of year, pollination treatment, and their interaction on the probability that seeds were filled (seed set) or germinated (seed germination) in logistic models

Factor	df	Wald χ^2 value
Seed set		
Year (Y)	1	0.27
Pollination treatments (P)	4	1307.66***
Y × P	4	10.55*
Seed germination		
Year (Y)	1	0.67
Pollination treatments (P)	4	1604.12***
Y × P	4	47.12***

* $P \leqslant 0.05$.
*** $P \leqslant 0.001$.

Table 2 – Log likelihood values of logistic models with local tree densities within different distances

Distance (m)	Log-likelihood	
	Seed set	Seed germination
10	−968.12	−1738.02
30	−955.26	−1733.77
60	−941.13	−1719.25
100	−953.92	−1724.41
150	−965.09	−1724.43
210	−972.58	−1729.56
280	−979.10	−1731.38
360	−981.28	−1736.02

Dependent variable of the model is the probability that seeds were filled (seed set) or germinated (seed germination). Independent variables of the models are year, local tree density, and their interaction.

Table 3 – Effects of year, local tree density within 60 m, and their interaction on the probability that seeds were filled (seed set) or germinated (seed germination) in logistic models

Factor	df	Wald χ^2 value
Seed set		
Year (Y)	1	17.63***
Density within 60 m (D)	1	78.99***
Y × D	1	20.87***
Seed germination		
Year (Y)	1	7.29**
Density within 60 m (D)	1	61.19***
Y × D	1	0.81

** $P \leqslant 0.01$.
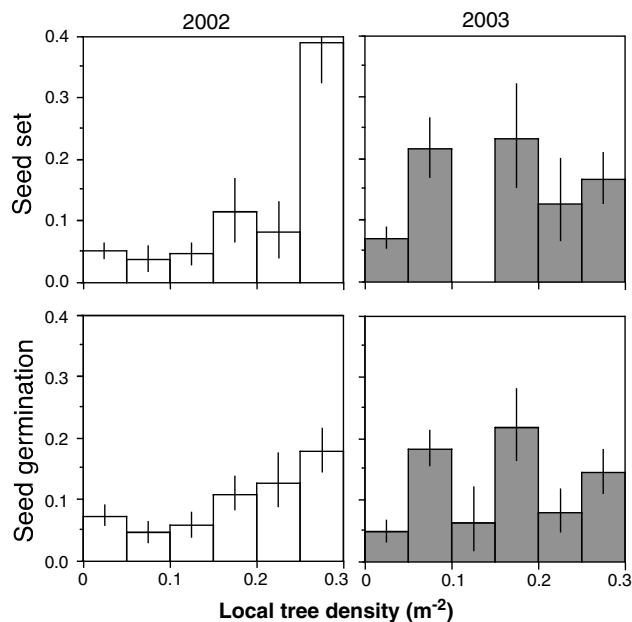*** $P \leqslant 0.001$.

Fig. 7 – Probability that seeds were filled (seed set) or germinated (seed germination) in six classes of local tree density within 60 m in 2002 and 2003. Error bars show 95% confidential intervals.

2002, and thus viable seed production is likely to depend on the abundance of conspecific pollen.

Supplement of pollen increased seed production in several birch species growing at high elevations (Holm, 1994), which suggests that pollen abundance is presumably limited due to spatial patchiness and unfavorable weather conditions. An arctic dwarf birch, *B. glandulosa*, produces few viable seeds and reproduces clonally (Hermanutz et al., 1989), which implies that adverse climatic conditions hampered sexual reproduction. In addition to it, the dominance of a few large clones was likely to increase geitonogamous pollination, which reduced viable seed production in the self-incompatible mating system in a mechanism identical to pollen limitation. Clonal reproduction of *B. apoiensis* has not been clarified although a closely related species, *B. ovalifolia*, rarely reproduces clonally (Nagamitsu et al., 2004).

Although wind-pollinated trees produce abundant pollen that can travel long distances in the air, acorn production of *Quercus douglasii* was limited by the abundance of pollen from a short distance as little as 60 m (Knapp et al., 2001). Genetic analysis using TWOGENER model demonstrated that the average effective distance of pollen movement was only 65 m in *Q. lobata* (Sork et al., 2002). Similarly, the local tree

density within a 60 m distance positively affected the viability of *B. apoiensis* seeds pollinated naturally in 2002 when pollen limitation seemed to be severe. This range with radius of 60 m is similar to the size (about 150 m length) of the core habitat of *B. apoiensis* on the western ridge. This aggregation of trees is likely to be a main pollen source, and pollen diffuses around this source. Thus, trees in the core habitat may receive more compatible pollen than trees in the periphery and outside of it.

Selfed flowers also produced much fewer viable seeds than outcrossed flowers, which indicates partial self-incompatibility and/or inbreeding depression during embryo development. In other birch species, self-incompatibility is common, but it is not always complete (Atkinson, 1992; DeGroot et al., 1997). In *B. apoiensis* trees that receive little pollen from other trees, inbred progeny are potentially produced due to partial self-compatibility. In *B. pendula*, inbreeding depression in survival was 58% in the first generation of selfed trees (Wang et al., 1999). If inbreeding depression is common in birches, inbred progeny of *B. apoiensis* may also suffer from reduced viability during tree growth. However, selfing may contribute population persistence by means of ensuring seed production under pollen limitation. Selfing can be an option to regenerate *B. apoiensis* if both outcrossing and selfing have occurred its populations, which should be confirmed by genetic studies.

4.2. Reproductive barriers

A sympatric congener, *B. ermanii*, not only formed woodlands around the mountain peak and slopes between the ridges (Watanabe, 2001) but also occurred within the habitat of *B. apoiensis*. Sympatric birch species potentially hybridize

regardless of differences in ploidy levels and taxonomic groups (Erikkson and Jonsson, 1986). Introgressive hybridization among European birches was confirmed in some sympatric habitats (Thorsson et al., 2001; Palme et al., 2004). If *B. apoiensis* and *B. ermanii* can hybridize, hybridization between the two species is subject to conservation issues of *B. apoiensis* because hybridization may lead to the extinction of less-competitive, rare populations when reproductive barriers are weak (Wolf et al., 2001). In this study, reproductive barriers were investigated in three processes: flowering, pollen dispersal, and viable seed production.

Female flowering of *B. apoiensis* trees temporally overlapped with male flowering of conspecific and interspecific trees in a similar intensity. Female flowering of *B. ermanii* trees, however, overlapped with male flowering of conspecific trees more than that of interspecific trees. These results suggest that pollination from *B. ermanii* to *B. apoiensis* is more frequent than that in the opposite direction. Thus, most hybrids may derive from a combination of *B. apoiensis* mothers and *B. ermanii* fathers, and *B. apoiensis* genomes inherited maternally may be preserved in the hybrids. This asymmetric pattern is mainly due to more synchronized flowering among trees in *B. ermanii* than in *B. apoiensis*. Our observation was conducted in a restricted area with homogeneous climatic conditions. The whole range of distributions of the two species on Mt. Apoi, however, varies in climatic conditions depending on topographic factors, such as the altitude and direction of slopes. Because the various climatic conditions cause variation in flowering phenology within each species, temporal overlaps between the two species may be larger than the observed overlaps.

Pollen can be distinguished between a dwarf birch *B. nana* and a tree birch *B. pubescens* based on the size of pollen grains (Prentice, 1981). In this study, pollen size was smaller in *B. apoiensis* than in *B. ermanii*, although the range largely overlapped between the two species. Temporal changes in the size distributions of airborne pollen reflected flowering phenology that overlapped between the earlier flowering of *B. apoiensis* and the later flowering of *B. ermanii*. If airborne pollen is randomly deposited on stigmas, reproductive barriers are weak in the pollination process.

The female flowers of *B. apoiensis* hybridized with pollen of *B. ermanii* produced fewer viable seeds than those outcrossed with conspecific pollen. This result supports the presence of incomplete reproductive barriers in fertilization and/or embryo development processes. Similar results were reported from a survey of interspecific crosses in other birches (Erikkson and Jonsson, 1986). Hybridization between *B. ermanii* and each of four species in the dwarf birch taxon, the subsection *Nanae*, demonstrated that two out of the four species produced viable seeds. These lines of evidence suggest that introgressive hybridization has already occurred, at least in the direction from *B. ermanii* to *B. apoiensis*.

Phenotypic and genetic markers that discriminate pure and hybrid progeny are necessary to monitor hybrids between *B. apoiensis* and *B. ermanii*. Since morphological characteristics do not always discriminate hybrids from their parental species in birches (Thorsson et al., 2001), molecular markers are useful for this monitoring. In order to assess an extinction risk, it is necessary to compare the frequency of hybrids between adults in the present population and progeny reproduced by these adults using these markers (Wolf et al., 2001). If the frequency of hybrids is higher in progeny than in adults, *B. apoiensis* may be at risk due to introgressive hybridization.

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