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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*

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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.

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

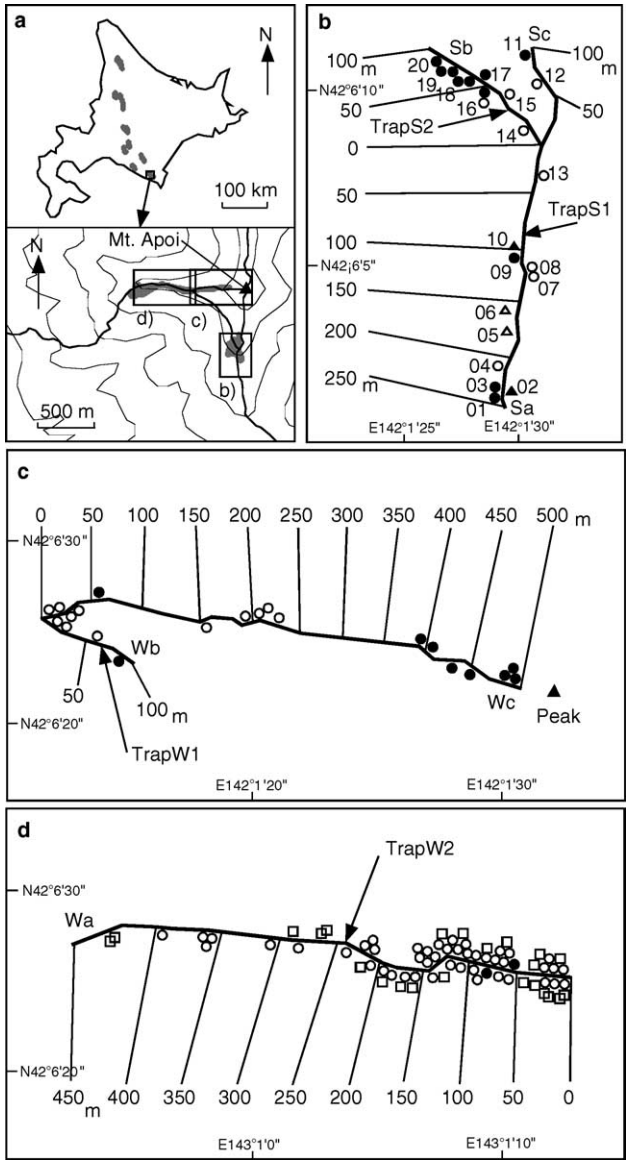

Fig. 1 – Location of the study sites, transects, and trees of *Betula apoiensis* and *B. ermanii*. (a) Study sites on the southern and western ridges on Mt. Apoi in Hokkaido. Shaded areas show ultra-basic rock areas in Hokkaido and alpine vegetation areas on Mt. Apoi. Contour lines are 100 m intervals. (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.