Triploid birch hybrids from natural woodlands in Iceland

Kesara Anamthawat-Jónsson

Institute of Life and Environmental Sciences, University of Iceland, Askja – Sturlugata 7, Reykjavík, IS-102, Iceland
Corresponding author: kesara@hi.is

Birch (*Betula* L.) is the most dominant woodland plant in Iceland, the birch woodland which today covers 1.5% of the land area. In these woodlands, two birch species coexist: the diploid dwarf birch (*B. nana* L.) and the tetraploid downy birch (*B. pubescens* Ehrh.) Both species are found together in most areas, although downy birch occupies lower elevations and drier habitats, whereas dwarf birch is more prevalent in the interior highlands and at colder sites. *Betula nana*, a circumpolar species, is a prostrate shrub up to 1 m in height, whereas the European *B. pubescens* may grow up to 25 m tall; however, in Iceland this birch is mostly shrub-like often less than 2 m tall, often called mountain birch.

These two birch species hybridize in their natural habitats [1]. Such hybridization forms triploid interspecific hybrid, and the hybrid serves as a bridge to gene flow between the two species via back-crossing, the process known as introgressive hybridization, or introgression [1]. This introgression is thought to be the main drive behind the existence of shrub-like mountain birch. Introgression is the process that allows for the transfer of neutral or adaptive traits from one species to another, and can increase genetic polymorphism in one or both species. Our botanical and molecular studies have confirmed the presence of triploid hybrids in natural woodlands all around the country, with ca. 10% occurrence, and have demonstrated the presence of bi-directional introgression via triploid hybrids in all woodlands [2-3]. For the introgressive hybridization to occur, triploid hybrids must be fertile, at least partially.

The aim of the present presentation is to summarize our studies of birch fertility from all three ploidy groups, i.e., diploid, triploid and tetraploid, both on the paternal side by examining male meiosis and evaluating pollen viability and germination, and on the maternal side by measuring seed germination [4-5].

The microscopic analysis of male catkins formed in early autumn showed normal meiotic division in the diploid and tetraploid plants, but revealed numerous meiotic abnormalities in the triploid plants, especially in chromosome pairing behavior, resulting in aborted pollen grains. Then in the spring, pollen samples were collected from woodlands throughout Iceland, from 99 plants, including 22 triploid hybrids. The germination of pollen from the triploid hybrids was about one third that of the diploid or tetraploid species. Most triploid plants had a pollen germination of less than 2%; however, some showed exceptionally high pollen fertility. Microscopic examination revealed normal pollen tube growth and elongation in the diploid (**Figure 1**) and tetraploid plants, as well as in the triploid hybrids with low germination. Other hybrids showed abnormal tube growth, such as pollen tubes with bent, curled or folded tips. Fluorescence microscopy indicated pollen viability in all ploidy groups, with green autofluorescence from the cytoplasmic contents and red fluorescence from the exine wall. The Aniline test of pollen viability showed that grains with three pores (normal, triporate grains) stained positively, but non-triporate grains were unstained and empty. This study indicates the presence of a pre-zygotic (before fertilization) reproductive barrier that may prevent triploid populations from increasing in size, displacing the parental species.

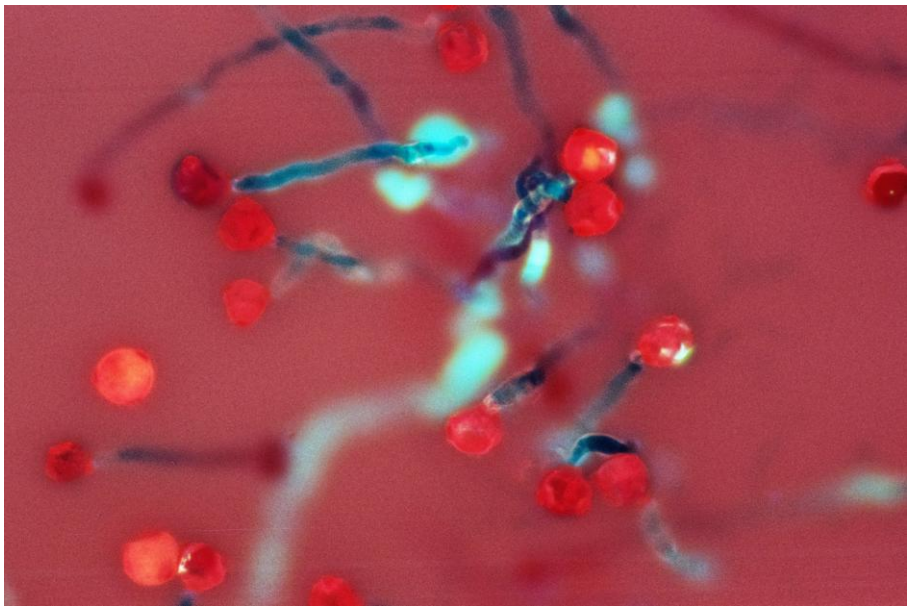

Figure 1. *Normal pollen germination and pollen tube growth in* Betula nana *(diploid plant D-22 from Ásbyrgi, NE Iceland, lat./long. 66.01/-16.50). This plant had 71% pollen germination, which is among the highest percentages from the present study. The pollen was stained with Aniline Blue and visualized in the epifluorescence microscope Nikon Eclipse 800 through the triple band filter-set. Red is autofluorescence of the pollen exine wall. Turquoise blue is an autofluorescence from the tip of pollen tube, possibly from pectin or actin. Aniline Blue stains the cytoplasmic content of pollen tube. The pollen grains are about 17-22 um in size.*

Germination tests were performed on seeds collected in late autumn in birch woodlands throughout Iceland, from 246 plants, including 21 triploid hybrids. The germination percentage of seeds from triploid hybrid plants was about 20 times lower than that of the diploid or the tetraploid species. The result indicates that pre-zygotic reproductive barriers are also apparent on the maternal side, but confirms that triploid hybrids do produce viable seeds.

Triploid birch hybrids are not sterile. Both the paternal and maternal fertility of the hybrids should be sufficient to facilitate gene flow via backcrossing with the parental species.

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[1] Anamthawat-Jónsson K, Science Asia **45**, 203-211 (2019).
[2] Thórsson ÆTh et al., Annals of Botany **99**, 1183-1193 (2007).
[3] Thórsson ÆTh et al., Journal of Biogeography 37, 2098-2110 (2010).
[4] Anamthawat-Jónsson K, Imaging Microscopy **20**, 18-21 (2018).
[5] Anamthawat-Jónsson K et al., New Forests online first (2020).