Triploid Birch Hybrids

Fluorescence Imaging of Birch Mitosis and Meiosis

The objective of this paper is to demonstrate the importance of light and fluorescence microscopy in the study of plant genecology and population-based genetics. Analyzing mitotic chromosomes of birch tree species has made it possible to detect interspecific hybridisation in natural woodlands, the process that drives gene flow across species boundaries. Capturing meiotic chromosomes in division, which occurs one week once a year, has confirmed the role of hybrids in mediating the gene flow.

Birch woodland is an integral component of the tundra biome. Arctic tundra is located in the northern hemisphere, encircling the North Pole and extending south to the coniferous forests of the taiga. In geographical Europe, this includes, from east to west, northern Russia, Fennoscandia (northern Finland and Scandinavia), the Svalbard archipelago and Iceland. Tundra vegetation is mostly herbaceous, consisting of a mixture of mosses and lichens, grasses and forbs, along with dwarf shrubs and trees. Birch (*Betula* L.) is generally the most dominant woodland plant (fig. 1). In Iceland, woodland area has diminished significantly since the first settlement in the ninth century, due to the use of birch as firewood, sheep grazing and recurrent periods of climate cooling. Understanding the ecology and genetics of woodland birch can therefore help manage the ongoing conservation and regeneration of natural vegetation effectively.

Birch Species Hybridisation in Natural Woodlands in Iceland

Two species of *Betula* co-exist in Iceland: diploid dwarf birch (*B. nana*) and tetraploid tree birch or downy birch (*B. pubescens*), and they hybridize in their natural habitats [1]. Both species are found together in most areas, although downy birch occupies lower elevations and a drier habitat compared with dwarf birch, which is more dominant on highlands and near waterfalls and rivers. *Betula nana*, a circumpolar species, is a prostrate shrub up to 1 m in height, whereas *B. pubescens*, a European species, may grow up to 25 m tall. However, in Iceland and Fennoscandia a shrub-like subspecies *tortuosa* (mountain birch) is prevalent.
This type of *B. pubescens* birch is believed to be the result of introgressive hybridisation with *B. nana*, via their hybrids [1-4]. Introgressive hybridisation, or hybrid introgression, is a process by which hybridisation leads to gene flow between species through backcrossing of the hybrid with its parental species. The process allows for the transfer of neutral or adaptive traits from one species to another and can increase genetic polymorphism in one or both species. Hybridizing birch dominates woodlands in Iceland (fig. 1). Based on the botanical, cytogenetic, molecular and palynological studies on Icelandic birch [3-7], Iceland is a birch hybrid zone harbouring genetic variation that may be advantageous in the ecological context.

Only a few partially fertile hybrids are sufficient for introgression to occur, and therefore these hybrids may not have been detected. Identifying birch hybrids by morphology is known to be difficult, due to the fact that hybrid introgression has resulted in a huge phenotypic variation in both species [4]. It is nevertheless quite easy to differentiate the two parental species, presumably because each parental type is highly adaptive in its own habitat, but to identify an interspecific hybrid by morphology it is only 49% possible [5]. On the other hand, there is no variation in chromosome number within ploidy groups [3], thus the most reliable means is to identify birch hybrid by determining chromosome number under a microscope. Hybridisation between diploid (2n=2x=28) *B. nana* and tetraploid (2n=4x=56) *B. pubescens* produces triploid (2n=3x=42) hybrids (fig. 2a-c). An efficient method of chromosome isolation from birch shoot-tips collected directly in the field has been developed [8]. Chromosomes on microscopic slides are stained with DAPI (4,6-Diamidino-2-phenylindole), the fluorochrome that binds specifically to chromosomes (fig. 2) and not to cytoplasmic artefacts. DAPI is excited with ultraviolet light and when bound to double-stranded DNA its absorption maximum is at 358 nm and its emission maximum is at 461 nm (blue). In addition, the technique of fluorescence *in situ* hybridisation (FISH), which has been optimized for mapping of the major 45S ribosomal genes on birch chromosomes [9], can further confirm the identity of
hybrid and species (fig. 2b,d).

Fluorescence microscopy has made it possible to conduct a large-scale, population-based genetics of tree species. Hundreds of Betula plant samples were collected from woodlands all around Iceland and the results of chromosome counting showed that triploid hybrids were more common than previously thought [5]. Of the 461 plants examined, 9.5% were found to be triploid hybrids. However, the occurrence was variable among locations, from being undetectable up to 20%. Qualitative and quantitative analyses of morphological features of individual plants revealed bidirectional introgression and geographical structure. Shrub-like birch with intermediate or hybrid-like morphology is more common in the woodlands experiencing cold summers (often associated with glacial sites or the interior highlands), compared to birch in the woodlands in lowland areas.

**Fertility of Triploid Birch Hybrids**
Interspecific triploid hybrids, if they are not totally sterile, can mediate gene flow between the parental species via backcrossing of the hybrids. To demonstrate that a triploid birch hybrid is able to produce fertile gametes, chromosome behavior at meiosis in male catkins has been investigated and the results are shown here for the first time (fig. 3). In a normal meiosis, homologous chromosomes pair completely during meiosis I (fig. 3a-b), thus forming bivalents at metaphase, 28 in tetraploid *B. pubescens* (fig. 3c) and 14 in diploid *B. nana* (fig. 3d). Segregation in the following stages proceeds normally (fig. 3e-f) and pollen produced would be normal and viable. On the other hand, the genome of a triploid birch comprises one haploid set of chromosomes from each parental species, therefore the pairing is either incomplete or no pairing at all, i.e. forming 42 univalents. The segregation at later stages is chaotic, often showing chromosome lagging, anaphase bridges, if multivalents exist, and micronuclei (fig. 3g-i). Individual univalents are randomly included in the two daughter nuclei, resulting in some having too many and others having too few chromosomes. Abnormalities in the segregation at meiotic anaphase are common among plant hybrids [10]. But in a few fortunate cases, the whole haploid sets of univalents may separate from each other forming large (presumably *B. pubescens*) and small (*B. nana*) microspores, which later become pollen grains (fig. 3i-l). Based on our palynological study [11], the pattern of pollen size distribution within plants indicates that triploid hybrids produce two sizes of triporate pollen grains, but the small *B. nana* size is far more prevalent than the larger *B. pubescens* size. Regardless of size, viable pollen grains show normal triporate morphology (pollen with 3 pores), whereas aberrant grains tend to be non-triporate [11]. A few pollen grains produced by triploid plant can be triporate and are viable (e.g. fig. 3l).

The meiotic results presented here are the final proof that triploid birch hybrid is fertile. The results support our hypothesis [3] that progenies (descendants) of backcrosses of triploid hybrids with parental species could be diploid, triploid or tetraploid, depending on whether a hybrid gamete is the small (n=14) or the large (n=28) types. Triploid descendants are therefore second-generation hybrids, and the cycle can go on producing later-generation hybrids. Our population-based introgression study [5] has revealed that triploid hybrids are evidently more than just the F1 generation. The release of a hidden, non-additive genetic variation may be of adaptive significance in the fluctuating environment, for the segregating hybrids as well as for the two species via backcrossing.

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References
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