

Variation in Ploidy Level Among Birch Taxa

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Significance to Industry: Information on ploidy levels is extremely valuable for use in plant breeding programs. Fertility, crossability, gene dose, and expression of traits are all influenced by ploidy levels. This research provides information on ploidy levels for specific taxa of birch and will allow for more systematic and efficient progress in the development of improved hybrids.

Nature of Work: Ploidy level (number of complete sets of chromosomes) can vary substantially among species of birch (*Betula* spp. L.) ranging from $2n=2x=28$ to $2n=8x=112$ (3,6). Intraspecific ploidy differences can also occur. For example, different varieties and cultivars of *B. papyrifera* Marsh. can be 4x, 5x, or 6x (6). Furthermore, most species of birch can readily hybridize, both between ploidy levels and by fertilization from reduced or unreduced gametes, resulting in offspring with a tremendous range of ploidy levels (5). Although ploidy level can be accurately determined by counting chromosomes, flow cytometry provides a reliable and much faster means for determining relative nuclear DNA content and associated ploidy level (1). The objective of this study was to determine the approximate genome size and estimated ploidy level of a diverse collection of birch.

Leaf samples were collected from trees growing in established, unreplicated, field plots. Approximately 0.5 cm² each of sample and standard tissues were chopped with a razor blade in a Petri dish containing 0.5 mL of extraction buffer (CyStain UV Precise P Nuclei Extraction Buffer, Partec, Münster, Germany). The suspension was filtered through a 50 Φ M filter and nuclei were stained with 1.5 mL of 4',6-diamidino-2-phenylindole (DAPI) buffer (CyStain UV Precise P Staining Buffer, Partec). The suspension was analyzed using a flow cytometer (PARTEC PA-I, Partec) to determine the mean sample nuclei fluorescence relative to that of the internal standard. Approximately 5,000 nuclei were measured per sample. Genome sizes were calculated as nuclear DNA content for unreduced tissue (2C) as: $2C \text{ DNA content of sample} = (\text{mean fluorescence value of sample} \times 2C \text{ DNA content of standard}) / \text{mean fluorescence value of the standard}$. *Malus hupehensis* (Pamp.) Rehd. R3T3-SF, with a known genome size of $2C = 3.46$ pg (2), was used as a standard.

Results and Discussion: There was considerable variation in approximate genome size among the taxa surveyed (Table 1). Some variation in ploidy levels among *Betula* taxa has been previously documented (3,6). Most of the species

reported as diploids, including *B. fontanalis* Sarg., *B. lenta* L., *B. nigra* L., *B. pendula* Roth., and *B. populifolia* Marshall, had very similar 2C genome sizes ranging from 1.1–1.2 pg. Using this genome size range as an approximate reference for 2x, we also identified putative 3x (1.8–2.2 pg), 4x (2.2–2.6 pg), 5x (2.8 pg), 6x (3.1–3.5 pg), and 8x (5.4 pg) taxa. In most cases, these estimates are consistent with prior reports, with some exceptions. *Betula utilis* var. *prattii* Burkill has been reported to be a diploid (6), while our accessions (different varieties) of *B. utilis* var. *jacquemontii* (Spach) H.J.P. Winkl. and *B. utilis* var. *occidentalis* (Kitam.) all appeared to be tetraploids, suggesting intraspecific variation among varieties. *Betula costata* Trautv. and *B. turkestanica* Litvin have been reported as diploids (4), while our accessions were estimated to be tetraploids, possibly suggesting intraspecific variation or hybridization. *Betula ermanii* Cham. has been reported as a tetraploid (3). One of our accessions of *B. ermanii* was estimated to be tetraploid while the other was octaploid. *Betula maximowicziana* Reg. has been reported as a diploid (6). One of our accessions of *B. maximowicziana* was estimated to be diploid while the other was triploid, suggesting that it is probably a hybrid. The *B. nigra* H1997-007 through H1997-018 were treated with the mitotic inhibitor oryzalin as seedlings yielding tetraploids and one mixoploid (cytochimera), $2n=2x+4x$. The *B. nigra*, H2002-036-003 through H2002-038-004, were derived from open pollinated seeds from these 4x *B. nigra* (planted near 2x cultivars) and yielded triploids (the result of interploidy hybridization). The potential for hybridization and intraspecific variation in ploidy levels among birch taxa, emphasizes the need for this type of cultivar specific data.

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Table 1. Approximate genome size and estimated ploidy level of *Betula* taxa determined from flow cytometry analysis of isolated nuclei.

Taxa	Approximate genome Size (pg)	Estimated ploidy level
<i>Betula albosinensis</i> Burkill.	1.1	2x
<i>Betula nigra</i> L 'Little King' Fox Valley®	1.2	2x
<i>Betula nigra</i> 'Cully' Heritage®	1.1	2x
<i>Betula nigra</i> 'BNMTF' Dura-Heat™	1.2	2x
<i>Betula fontinalis</i> Sarg. (syn. <i>B. occidentalis</i>)	1.2	2x
<i>Betula platyphylla</i> Sukachev., Kamchatka source	1.1	2x
<i>Betula szechuanica</i> (C.Schneid.) Jansson.	1.1	2x
<i>Betula pendula</i> Roth. 'Dalecarlica'	1.2	2x
<i>Betula pendula</i> 'Dark Prince'	1.2	2x
<i>Betula pendula</i> 'Purpurea'	1.1	2x
<i>Betula</i> 'Crimson Frost'	1.2	2x
<i>Betula</i> 'Penci-2' Royal Frost®	1.2	2x
<i>Betula lenta</i> L.	1.2	2x
<i>Betula populifolia</i> Marshall 'Whitespire'	1.2	2x
<i>Betula maximowicziana</i> Reg. (2002-179)	1.2	2x
<i>Betula nigra</i> (H2002-036-003)	1.6	3x
<i>Betula nigra</i> (H2002-036-004)	1.6	3x
<i>Betula nigra</i> (H2002-036-005)	1.5	3x
<i>Betula nigra</i> (H2002-036-006)	1.6	3x
<i>Betula nigra</i> (H2002-036-007)	1.6	3x
<i>Betula nigra</i> (H2002-036-008)	1.7	3x
<i>Betula nigra</i> (H2002-036-009)	1.6	3x
<i>Betula nigra</i> (H2002-036-010)	1.7	3x
<i>Betula nigra</i> (H2002-036-011)	1.6	3x
<i>Betula nigra</i> (H2002-036-012)	1.6	3x
<i>Betula nigra</i> (H2002-036-013)	1.7	3x
<i>Betula nigra</i> (H2002-036-014)	1.7	3x
<i>Betula nigra</i> (H2002-036-015)	1.6	3x
<i>Betula nigra</i> (H2002-036-016)	1.6	3x
<i>Betula nigra</i> (H2002-036-017)	1.6	3x
<i>Betula nigra</i> (H2002-036-018)	1.6	3x
<i>Betula nigra</i> (H2002-036-019)	1.6	3x
<i>Betula nigra</i> (H2002-036-020)	1.6	3x
<i>Betula nigra</i> (H2002-036-021)	1.6	3x
<i>Betula nigra</i> (H2002-036-022)	1.6	3x
<i>Betula nigra</i> (H2002-036-023)	1.6	3x
<i>Betula nigra</i> (H2002-037-001)	1.5	3x
<i>Betula nigra</i> (H2002-037-002)	1.6	3x
<i>Betula nigra</i> (H2002-037-003)	1.6	3x
<i>Betula nigra</i> (H2002-037-004)	1.6	3x

Taxa	Approximate genome Size (pg)	Estimated ploidy level
<i>Betula nigra</i> (H2002-037-005)	1.5	3x
<i>Betula nigra</i> (H2002-038-001)	1.6	3x
<i>Betula nigra</i> (H2002-038-002)	1.6	3x
<i>Betula nigra</i> (H2002-038-003)	1.6	3x
<i>Betula nigra</i> (H2002-038-004)	1.6	3x
<i>Betula maximowicziana</i> (1998-297)	1.8	3x
<i>Betula turkestanica</i> Litvin	2.2	4x
<i>Betula tianschanica</i> Rupr.	2.2	4x
<i>Betula costata</i> Trautv.	2.5	4x
<i>Betula ermanii</i> Cham. 'Grayswood Hill'	2.5	4x
<i>Betula utilis</i> var. <i>jacquemontii</i> (Spach) Winkl.		
'Grayswood Ghost'	2.6	4x
'Doorenbos'	2.3	4x
'Fascination'	2.5	4x
'Silver Shadow'	2.5	4x
'Ramdana River'	2.4	4x
'Snow Queen'	2.4	4x
'Polar Bear'	2.5	4x
<i>Betula utilis</i> var. <i>occidentalis</i> (Kitam.)		
Ashburner & Schilling 'Kashmir White'	2.5	4x
<i>Betula nigra</i> (H1997-007)	1.1+2.1	2x+4x
<i>Betula nigra</i> (H1997-009)	2.3	4x
<i>Betula nigra</i> (H1997-011)	2.2	4x
<i>Betula nigra</i> (H1997-014)	2.1	4x
<i>Betula nigra</i> (H1997-016)	2.3	4x
<i>Betula nigra</i> (H1997-018)	2.3	4x
<i>Betula</i> (473)	2.8	5x
<i>Betula papyrifera</i> Marsh. (1997-094)	3.1	6x
<i>Betula papyrifera</i> (1998-564)	3.5	6x
<i>Betula</i> (254)	3.5	6x
<i>Betula</i> (270)	3.4	6x
<i>Betula ermanii</i>	5.4	8x

²Numbers in parentheses are accession numbers.

[^]Values are means, n=2.