Extensive Long-Distance Pollen Dispersal in a Fragmented Landscape Maintains Genetic Diversity in White Spruce

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Abstract

Conifers are among the most genetically diverse plants but show the lowest levels of genetic differentiation, even among geographically distant populations. High gene flow among populations may be one of the most important factors in maintaining these genetic patterns. Here, we provide empirical evidence for extensive pollen-mediated gene dispersal between natural stands of a widespread northern temperate/boreal conifer, Picea glauca. We used 6 polymorphic allozyme loci to quantify the proportion of seeds sired by pollen originating from different sources in a landscape fragmented by agriculture in North Central Ontario, Canada. In 7 stands, a small proportion of seeds were sired by self-pollen or neighboring trees but 87.1% (±1.7% standard error [SE]) of seeds were sired by pollen from at least 250 to 3000 m away. In 4 single isolated trees, self-fertilization rates were low and more than 96% (±1.3% SE) of seeds were sired by immigrant pollen. The average minimum pollen dispersal distance in outcrossed matings was 619 m. These results provide strong evidence that extensive long-distance pollen dispersal plays a primary role in maintaining low genetic differentiation among natural populations of P. glauca and helps maintain genetic diversity and minimize inbreeding in small stands in a fragmented landscape.
composed for the most part of white spruce, eastern white zone wind-pollinated trees. Residual tree stands are mediated gene flow in isolated stands of temperate/boreal by agriculture making it an ideal landscape to study pollen-

White spruce (*Picea glauca* [Moench] Voss) is a well-suited species for quantifying pollen movement in northern conifers. It is wind pollinated and has a transcontinental range across the boreal forest of North America (Nienstaedt and Zasada 1990). Like most conifers, white spruce shows conifers. It is wind pollinated and has a transcontinental species for quantifying pollen movement in northern populations (C176 Nipissing in North Central Ontario, Canada (46°20’N, 80°10’W; Figure 1). This area has been heavily fragmented by agriculture making it an ideal landscape to study pollen-mediated gene flow in isolated stands of temperate/boreal zone wind-pollinated trees. Residual tree stands are composed for white spruce, eastern white pine (*Pinus strobus* L.), white birch (*Betula papyrifera* Marsh.), and trembling aspen (*Populus tremuloides* Michx.). Tree stands remain on rocky outcrops and are surrounded by larger tracts of agricultural fields. Small stands also occur on islands in Lake Nipissing (Figure 2). In the surrounding tracts of contiguous forest, white spruce makes up less than 10% of the trees.

Sample Collection and Genotyping

Cones were collected from 32 white spruce trees from 11 small stands in August and September 1994 within a 378-km² area (O’Connell et al. 2006b). Seeds from an additional 72 trees from 12 larger stands were sampled to obtain background allele frequencies (O’Connell et al. 2006a). All sampled stands were separated by 250–3000 m from the nearest adjacent white spruce stand. Most of these stands were located on rocky outcrops surrounded by agricultural fields, but 4 stands were located on islands in Lake Nipissing (stands A, B, C, and D; Figure 1). The average distance to the nearest conspecific tree within a stand was 20 m. All reproductive white spruce trees were sampled in each of the 11 small stands. Four stands had a single isolated tree, and 7 stands had 3–8 reproductive trees per stand (Table 1). We genotyped a total of 2967 seeds (96 per tree, except for 2 trees in stand C where only 31 and 56 seeds were genotyped) at 6 polymorphic allozyme loci (*Fum1, Idh1, Lap1, Lap2, Pgm1, and Pgm2*) from 4 enzyme systems. We detected 3–5 alleles per locus, with a total of 24 alleles at these loci (O’Connell et al. 2006a). Megagametophytes were also separately assayed for 10% of seeds. Assaying the haploid megagametophyte, which has the same genetic constitution as the female gamete, identifies the maternal allele passed on to the embryo. Although highly variable markers, such as microsatellites, improve the power to discriminate between individual pollen parents, allozymes are less vulnerable to genotyping error or new mutations. Even a small genotyping error can greatly inflate gene flow rates (Burczyk and Chybicki 2004). We obtained an exclusion probability of 63% based on the allele frequencies of the pollen pool at the 6 loci (O’Connell et al. 2006a; calculated following Jamieson 1994). Because of the low exclusion probability of isozymes, we sampled a large number of seeds per tree (i.e., 96 seeds per tree) to reduce experimental error for the mating system parameter estimates.

Mating System and Gene Flow Analysis

We estimated mating system parameters in the 11 small stands of white spruce using a combination of the neighborhood (Adams 1992; Burczyk et al. 2002) and mixed-mating models (Ritland 2002). To identify immigrant pollen, all trees within a stand were genotyped at all 6 allozyme loci. Knowledge of background allele frequencies, although not necessary, yields a more accurate estimate of cryptic gene flow (Burczyk and Chybicki 2004), and these data were available (O’Connell et al. 2006a). The back-

Materials and Methods

Study Area

The study area was located around the western arm of Lake Nipissing in North Central Ontario, Canada (46°20’N, 80°10’W; Figure 1). This area has been heavily fragmented by agriculture making it an ideal landscape to study pollen-mediated gene flow in isolated stands of temperate/boreal zone wind-pollinated trees. Residual tree stands are composed for white spruce, eastern white pine (*Pinus strobus* L.), white birch (*Betula papyrifera* Marsh.), and trembling aspen (*Populus tremuloides* Michx.). Tree stands remain on rocky outcrops and are surrounded by larger tracts of agricultural fields. Small stands also occur on islands in Lake Nipissing (Figure 2). In the surrounding tracts of contiguous forest, white spruce makes up less than 10% of the trees.

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the 23 pooled stands (O’Connell et al. 2006a) using Multilocus Mating System Program MLTR version 3.0 (Ritland 2002) and fixing the outcrossing rates at 94%. We detected 26 different alleles in the background pollen pool. In single trees, selfing ($s$) was estimated with MLTR using the Newton–Raphson method, where $s = 1 - t_m$ and $t_m$ is the multilocus outcrossing rate. Standard errors (SEs) were obtained from 300 bootstraps of the data with resampling of individuals within families. For single isolated trees, all seeds that are not self-fertilized are pollinated by immigrant pollen ($m$) so that $m = t_m$. To quantify the amount of pollen immigration into stands with 3–8 reproductive trees, we used the program NEIGHBOR version 2.0 (Buczcyk et al. 2002). This program simultaneously estimates selfing rates ($s$) and the proportion of seeds sired by immigrant pollen ($m$) using the Newton–Raphson method. SEs are derived from the Hessian (variance–covariance) matrix (Buczcyk et al. 2002). The proportion of seeds sired by trees within a stand (neighborhood) was calculated as $1 - s - m$. The percentage of directly observable immigrants ($b$) is the number of embryos with allele combinations that cannot be produced by fathers within a stand divided by the total number of seeds analyzed and was obtained with NEIGHBOR. The proportion of cryptic immigrants is equal to $m - b$. MLTR was used to estimate the proportion of related neighbor matings (i.e., biparental inbreeding) calculated as $(t_m - t_s)$, where $t_s$ is the mean single-locus outcrossing rate. The proportion of unrelated neighbor matings was calculated as $1 - s - m - (t_m - t_s)$.

The location of 23 sampled white spruce stands near the western arm of Lake Nipissing, Ontario. The location of the study site is indicated on the inset map by an open square. Mating system parameters were estimated for small stands only in this study (A–K, represented by triangles; stands with single trees include B, E, G, and I). Medium-sized stands (L–Q) and large stands (R–W), respectively, represented by circles and squares, were sampled to obtain background allele frequencies. Not all white spruce stands are indicated on the figure, only sampled stands. This figure is reproduced from O’Connell et al. (2006b) with the permission of the National Research Council of Canada Research Press.

Figure 1. The location of 23 sampled white spruce stands near the western arm of Lake Nipissing, Ontario. The location of the study site is indicated on the inset map by an open square. Mating system parameters were estimated for small stands only in this study (A–K, represented by triangles; stands with single trees include B, E, G, and I). Medium-sized stands (L–Q) and large stands (R–W), respectively, represented by circles and squares, were sampled to obtain background allele frequencies. Not all white spruce stands are indicated on the figure, only sampled stands. This figure is reproduced from O’Connell et al. (2006b) with the permission of the National Research Council of Canada Research Press.

Figure 2. Isolated tree stands on islands in Lake Nipissing.
Table 1. The percentage of pollen from different sources siring seeds in 11 small stands of white spruce in North Central Ontario

<table>
<thead>
<tr>
<th>Stand</th>
<th>c</th>
<th>N/n</th>
<th>s (SE)</th>
<th>95% CI</th>
<th>m (SE)</th>
<th>95% CI</th>
<th>l – s – m</th>
<th>t_m – t_b (SE)</th>
<th>95% CI</th>
<th>b</th>
</tr>
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<tr>
<td>B</td>
<td>2000</td>
<td>1/96</td>
<td>6.4 (4.0)</td>
<td>93.6</td>
<td></td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>300</td>
<td>1/96</td>
<td>5.9 (3.6)</td>
<td>66.0 (6.4)</td>
<td>28.7</td>
<td>3.1 (3.4)</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>700</td>
<td>1/96</td>
<td>5.3 (3.6)</td>
<td>66.0 (6.4)</td>
<td>28.7</td>
<td>3.1 (3.4)</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>250</td>
<td>1/96</td>
<td>25.6 (7.1)</td>
<td>74.4</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single trees</td>
<td>4/384</td>
<td>3.8 (1.3)</td>
<td>1.7–5.9</td>
<td>96.2</td>
<td>93.7–98.7</td>
<td>49</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>400</td>
<td>3/288</td>
<td>12.9 (3.6)</td>
<td>68.5 (5.0)</td>
<td>18.4</td>
<td>5.4 (2.7)</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>3000</td>
<td>3/183</td>
<td>0.04 (1.1)</td>
<td>112.9 (1.7)</td>
<td>-13.2</td>
<td>3.1 (4.3)</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1500</td>
<td>3/288</td>
<td>5.3 (3.6)</td>
<td>66.0 (6.4)</td>
<td>28.7</td>
<td>3.1 (3.4)</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>300</td>
<td>4/384</td>
<td>4.9 (2.6)</td>
<td>82.2 (4.6)</td>
<td>12.9</td>
<td>-0.1 (2.5)</td>
<td>12</td>
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<td>H</td>
<td>500</td>
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<td>1.7</td>
<td>4.3 (2.6)</td>
<td>34</td>
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<tr>
<td>J</td>
<td>300</td>
<td>8/768</td>
<td>15.3 (2.7)</td>
<td>108.8 (5.9)</td>
<td>-24.1</td>
<td>6.5 (1.6)</td>
<td>15</td>
<td></td>
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<tr>
<td>K</td>
<td>250</td>
<td>4/384</td>
<td>0.8 (2.1)</td>
<td>95.7 (2.9)</td>
<td>3.5</td>
<td>-1.8 (2.3)</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3–8 trees</td>
<td>28/2583</td>
<td>6.0 (1.0)</td>
<td>4.0–8.0</td>
<td>87.1 (1.7)</td>
<td>83.8–90.4</td>
<td>6.9</td>
<td>3.7 (0.9)</td>
<td>1.9–5.5</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

\(c\) distance to the closest neighboring white spruce stand in meters; \(N\), the number of reproductive trees in a stand; \(n\), the number of seeds sampled; \(s\), the percentage of self-fertilized matings; CI, confidence interval; \(m\), the percentage of immigrant pollen; \(l – s – m\), the percentage of cross-fertilized matings within a stand (neighbor); \(t_m – t_b\), the percentage of biparental inbreeding (related neighbor matings); \(b\), the percentage of directly observable immigrants.

* Estimates with a dash failed to converge because of the small sample size (i.e., one tree per stand).

**Mating system parameters are not means but are estimated simultaneously for the group.

### Pollen Dispersal Distance

The minimum pollen dispersal distance was estimated for outcrossed matings only. The mean minimum dispersal distances of pollen siring seeds in each small stand were estimated as

\[
\left(1 - s - m\right)sd + mnc \over (1 - s)n
\]

where, for each stand, \(s\) is the selfing rate, \(m\) is the immigration rate, \(n\) is the number of seeds sampled in a stand, \(d\) is the mean distance to all other trees within a stand, and \(c\) is the distance to the closest neighboring stand. The overall mean dispersal distance was weighted by the number of seeds sampled in each stand.

### Results

At the stand level, selfing rates ranged from 0.04% to 25.6%, but all stands showed high levels of immigrant pollen (66–100%; Table 1). Estimates of \(m > 100\%\), and \(1 - s - m\) and \(t_m – t_b < 0\%\) are statistical artifacts. The biological range for these parameters is 0–100%. Thus, the biological estimates corresponding to the statistical estimates \(> 100\%\) and \(< 0\%\) are \(\leq 100\%\) and \(\geq 0\%\), respectively. In the 4 single isolated trees, 96.2% of seeds were sired by outcrossed, immigrant pollen (Table 1). About half of the outcrossed seeds were sired by directly observable immigrant pollen and the remainder by cryptic immigrants (Figure 3). In stands with 3–8 trees, 87.1% of seeds were sired by outcrossed, immigrant pollen (Table 1). Matings within the stands were divided between inbred matings (6.0% selfed and 3.7% related neighbor) and unrelated neighbor matings \([l – s – m – (t_m – t_b) = 3.2\%]\) (Figure 3). Only 16% of the seeds in stands with 3–8 trees were pollinated by directly observable immigrant pollen (Figure 3).

Although the overall power to directly detect immigrant pollen was low, we obtained small SEs for the mating estimates over all single trees (SE = 1.3) and overall stands with 3–8 trees (SE < 1.7; Table 1). We did not observe any relationship between the size of a stand (\(N\) or the distance to the closest stand (\(c\)) and any of the mating system parameters. We estimated that the minimum pollen dispersal distance in outcrossed matings was 619 m for all the trees and 654 m when single isolated trees were excluded.

### Discussion

The extensive pollen-mediated gene flow we observed into small isolated stands of white spruce indicates that there is high connectivity among these fragments at the landscape scale. The estimates of pollen immigration into these small white spruce stands are higher than the observed levels of around 50% in conifer seed orchards (Adams and Birkes 1991; Xie and Knowles 1994; Harju and Nikkanen 1996; Adams et al. 1997). There is increasing evidence that fragmentation may increase gene flow in forest trees (Young et al. 1996; Dyer and Sork 2001; White et al. 2002; Smouse and Sork 2004). We have indeed found that in small fragmented stands, the majority of seeds were sired by pollen from outside the stands and few matings occurred between neighbors (6.9%). However, we do not have any evidence that these gene flow rates are higher than in larger stands in this landscape. Nevertheless, in large stands, given the same amount of background pollen, the additional pollen must come from within the stand, thus lowering the proportion of immigrant pollen, that is, gene flow rate. Selfing and biparental inbreeding levels in 6 medium-sized (23–56 trees) and 6 large stands (≥100 trees) were previously estimated and did not significantly differ from levels in the 11 small stands in this study (O’Connell et al. 2006a). On the other hand, we previously observed lower...
fertilization rates in these small stands due to pollen limitation. Although fragmentation negatively affects the fertilization rates, it does not seem to decrease genetic diversity or increase inbreeding levels in resulting fully-developed seeds.

In this study, mating system parameters were obtained from filled seeds so that the realized gene flow does not necessarily represent the pollen flow. In most conifers, most inbred embryos are eliminated due to inbreeding depression before they are genotyped at the seed stage. We have previously found that trees in stands with 3–56 trees have a higher proportion of empty seeds (47%) than trees in larger stands (≥100 trees; 35% empty seeds), suggesting higher levels of primary selfing in the former stands (O’Connell et al. 2006a). The elimination of inbred embryos can increase the genetic heterozygosity of the resultant seed and contribute to the maintenance of genetic diversity. In contrast, the low proportion of aborted seeds (30%) in the 4 single isolated trees was comparable with that in the larger continuous stands, suggesting low primary rates of self-fertilization in single trees. Although strong selection against inbred seeds through early abortion can contribute to the maintenance of genetic diversity in offspring, the primary mechanism contributing to the high genetic diversity of seed produced in these isolated white spruce populations seems to be extensive pollen-mediated gene flow.

The large pollen dispersal distances we observed in white spruce may partly be due to the scale of the study. The scale of this study and the mean pollen dispersal distances that we observed are an order of magnitude greater than previous studies in wind-pollinated trees (Burczyk et al. 2004; Bacles et al. 2005). Another explanation for our observation of such extensive gene dispersal in this agricultural landscape is that it is surrounded by relatively contiguous forest, albeit with a white spruce component of <10%. Pollen from single trees or small groups of trees may not be competitive with pollen produced in the surrounding forest. Most of the local pollen in small stands may be carried away by strong winds. Other studies estimating pollen-mediated gene dispersal distances in conifers were conducted on relict (Robledo-Arnuncio and Gil 2004), seed orchard, or extremely isolated populations (Schuster and Mitton 2000) in which less than 10% of seeds were sired by pollen from outside the stands.

Rather than observing the occasional long-distance dispersal event, we have shown that mating between trees separated by several hundred meters is common in the small geographically isolated white spruce stands in this study. We have provided empirical evidence of extensive gene flow through long-distance pollen dispersal in a widespread, temperate and boreal zone conifer at the landscape level. These results may be representative of pollen-mediated gene dispersal patterns between conifer populations, which, like white spruce, show high genetic diversity and low differentiation among populations (Hamrick et al. 1992). Long-distance gene flow also maintains genetic diversity in small stands so that trees and stands separated by several hundred meters from large contiguous tracts of forest are not genetically isolated. Thus, forest fragmentation does not necessarily affect the levels of genetic diversity of the seed produced by isolated white spruce. Extensive, long-distance, pollen-mediated gene flow seems to be the primary mechanism for maintaining connectivity and genetic diversity among these populations. This empirical evidence of extensive gene flow in a temperate/boreal conifer may also explain how conifer populations maintain genetic connectivity despite a geological history of massive range contractions and expansion during recurring glacial cycles.

**Funding**

Natural Sciences and Engineering Research Council of Canada (L.M.O.); Canada Research Chair Program (CRC...
Acknowledgments

We thank J. Burczyk for providing the program NEIGHBOR version 2.0; I. DeMerchant and R. Simpson for producing the map of the study site; B.Arsenault and L. Deverno for help in the laboratory; L. Dubrich for locating and collecting samples; and P. Smouse, R. Latta, J. Burczyk, K. Percy, and Y.-S. Park for comments on the manuscript. O.P.R. holds the Senior Canada Research Chair in Forest and Conservation Genomics and Biotechnology.

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Received December 3, 2006
Accepted September 26, 2007

Corresponding Editor: James Hamrick