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Michael S. Bobola
University of New Hampshire - Main Campus

Kimberly A. Hillenberg
University of New Hampshire - Main Campus

Steve B. Gendreau
University of New Hampshire - Main Campus

Robert T. Eckert
University of New Hampshire, Robert.Eckert@unh.edu

Anita S. Klein
University of New Hampshire, Anita.Klein@unh.edu

See next page for additional authors

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Hybridization between *Picea rubens* and *Picea mariana*: differences observed between montane and coastal island populations

Michael S. Bobola, Robert T. Eckert, Anita S. Klein, Karen Stapelfeldt, Kimberly A. Hillenberg, and Steve B. Gendreau

Abstract: Foliage was collected from natural stands of montane and island red spruce (*Picea rubens* Sarg.) and black spruce (*Picea mariana* (Mill.) BSP) to examine within- and among-population genetic variation. Samples were scored for frequencies of nuclear ribosomal DNA (rDNA) alleles, and mitochondrial and chloroplast haplotypes. Samples were classified as red spruce, black spruce, or hybrid using two molecular methods: a three-character discriminant function based on molecular markers or a three-character molecular index. These results were found to be highly congruent with classification based upon a discriminant function using morphological traits. Among montane populations, hybridization and introgression between red and black spruce did not appear to be a major factor in the observed patterns of variation on elevational transects on Mount Washington and Mount Lafayette, N.H. However, extensive hybridization and introgression were detected among populations on Isle au Haut, Maine. The Mount Lafayette population displayed low variation in rDNA alleles compared with populations on Mount Washington and a range-wide provenance test in Stewartstown, N.H.


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M.S. Bobola, K.A. Hillenberg, and S.B. Gendreau. Department of Biochemistry and Molecular Biology, University of New Hampshire, Durham NH 03824, U.S.A.

R.T. Eckert. Program in Genetics and Department of Natural Resources, University of New Hampshire, Durham, NH 03824, U.S.A.

A.S. Klein. Department of Biochemistry and Molecular Biology, and Program in Genetics, University of New Hampshire, Durham, NH 03824, U.S.A.

K. Stapelfeldt. Department of Natural Resources, University of New Hampshire, Durham, NH 03824, U.S.A.

1 Scientific contribution 1862 of the New Hampshire Agricultural Experiment Station, Durham, N.H.

2 Author to whom all correspondence should be addressed.

Introduction

Decreased growth and reproductive rates and increased mortality have been reported (Scott et al. 1984; Vogelmann et al. 1985) for certain high-elevation red spruce (*Picea rubens* Sarg.) stands in northeastern U.S. forests (summarized in Eagar and Adams 1992). Similar declines have not been reported for black spruce (*Picea mariana* (Mill.) BSP) or white spruce (*Picea glauca* (Moench) Voss), the only other spruce species sympatric with red spruce. Symptoms of red spruce decline appear to be associated with winter injury (Friedland et al. 1984) and decreased cold tolerance (DeHayes et al. 1990; DeHayes 1992), both of which are attributed to acidic cloud deposition (DeHayes et al. 1991; McLaughlin and Kohut 1992).

Observation of recent decline in red spruce without concomitant decline in sympatric white or black spruce raises...
questions about the history and health of red spruce. We focus here on questions of genetic variation of red spruce and its potential for hybridization with black spruce. Investigations of allozyme variability have confirmed that red spruce has approximately one-half the genetic heterozygosity, fewer alleles per locus, and one-half the polymorphic loci of the other northeastern spruces (DeHayes and Hawley 1988; Eckert 1988). Low genetic diversity in red spruce may be a contributing factor in red spruce decline. Early reports (Morgenstern 1969; Roche 1969) suggested that putative hybrid and introgressed\(^3\) spruce show less winter drying than red spruce. Khalil (1987) analyzed red spruce provenances from New Brunswick, Nova Scotia, Maine, and West Virginia. His findings indicated that red spruce provenances from the lowlands of New Brunswick, containing putative natural hybrids of red and black spruce, exhibited positive heterosis for height growth. When Fowler et al. (1988) removed these heterotic provenances from their analysis of the same set of provenances to allow analysis of pure red spruce, the tallest trees were removed. This reduced the level of variation in growth characteristics attributable to provenance for red spruce. However, Manley and Ledig (1979) observed negative heterosis for photosynthesis in controlled-cross hybrids. Manley and Ledig (1979) and Gordon (1976) provide the most comprehensive discussions of the dynamics of hybridization between these two species. The potential effects of hybridization of red with black spruce are not clear, and gene flow between these two species has not been thoroughly examined in natural populations.

Early morphological indices used to separate species and identify possible hybrids are controversial. Persistence of hybrids has been the subject of considerable debate, ranging from a low of 0.4% in Ontario (Gordon 1976) to large, numerous hybrids swarms (Manley 1971, 1972). Later, Manley and Ledig (1979) came independently to the same conclusions as Gordon (1976), which indicate that generally hybrids will be uncommon unless sites are disturbed and competition from parental species is low.

Berlyn et al. (1990) described changes in nuclear DNA content of spruce along elevational transects on three northeastern mountains. They reported that the low-elevation red spruce had twice the genomic DNA content of the high-elevation black spruce. Also, for Mount Washington, they reported that the DNA content decreased as elevation increased \((R^2 = 0.828)\). Berlyn et al. (1990) concluded that the decrease in nuclear DNA content was due to hybridization and introgression, and that the degree of introgression was related to elevation.

**Geographic variation in biochemical markers of *Picea rubens***

Geographic trends in genetic variability of isozymes (Eckert 1988) and rDNA (Bobola et al. 1992) have been observed for red spruce. Canonical analysis of isozyme frequencies from 16 sample-plot locations in northern New England and New York revealed a significant \((p \leq 0.01)\) relationship between isozyme variability and elevation (Eckert 1988). Analysis of samples from a range-wide red spruce provenance test in Stewartstown, N.H., showed significant variation in rDNA alleles according to geographic origin, with latitude being the most important variable (Bobola et al. 1992). Comparison of geographic trends in red and black spruce rDNA variation suggested that introgression was not a strong factor in within-species differences in rDNA allelic frequencies (Bobola et al. 1992).

**Identification of *Picea* species**

The significance of hybridization has been extensively reviewed (Arnold 1992). Molecular methods to evaluate hybridization of red and black spruce have recently been developed (Bobola et al. 1996; Perras et al. 1995). The organellar inheritance patterns observed in red and black spruce are useful in determining the direction of hybridization (black \(\times\) red vs. red \(\times\) black) and can be used in conjunction with nuclear RFLPs (restriction fragment length polymorphisms) to quantify frequency of hybrids present in natural populations. Chloroplast inheritance is predominantly maternal in the Pinaceae, whereas mitochondria are maternally inherited (Neale and Sederoff 1988). By examining RFLP variation within a population, past hybridization can be detected using a three-character molecular index (Bobola et al. 1996).

We used samples of red spruce collected from a range-wide provenance test located at Coleman State Forest, Stewartstown, N.H., and samples of black spruce from a provenance test of the eastern black spruce complex, located on the Massabesic Experimental Forest, Alfred, Maine, to develop our discrimination models for these two species. Controlled-cross hybrids were supplied by A. Gordon, Ontario Forest Research Institute, Sault Ste. Marie, and the late J. Hanover, Michigan State University, East Lansing, for analysis and development of discrimination techniques. These sources and techniques are described more fully in Bobola et al. (1996).

We were interested in evaluating natural populations that we thought might contain \((i)\) differing frequencies of hybrids and \((ii)\) hybrid frequencies that followed an environmental gradient. We sampled from an island in the Gulf of Maine and from elevational transects on two mountains in the Presidential Range in New Hampshire. Nuclear rDNA markers were examined for significant variation related to elevation and for differences between populations on these mountains. Foliar samples were classified according to species using a morphological index, a discriminant model, and the molecular index recently developed in our laboratory (Bobola et al. 1996). Samples from the island were classified using only the molecular index. Substantial differences were found among montane and coastal island populations.

**Materials and methods**

**Foliation collections**

Foliation was collected from populations on Mount Washington and Mount Lafayette, in the Presidential Range of New Hampshire, and four sites on Isle au Haut, midcoast Maine.
Table 1. Unstandardized discriminant coefficients used for calculating individual tree scores for morphological data from Mount Washington foliage samples.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Function 1, black</th>
<th>Function 2, hybrid</th>
<th>Function 3, red</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-289.9654</td>
<td>-338.9731</td>
<td>-406.1044</td>
</tr>
<tr>
<td>Hue</td>
<td>14.8231</td>
<td>15.0532</td>
<td>15.5026</td>
</tr>
<tr>
<td>Chroma</td>
<td>12.2985</td>
<td>14.5304</td>
<td>19.6747</td>
</tr>
<tr>
<td>Descriptive color</td>
<td>7.4490</td>
<td>7.4855</td>
<td>8.2529</td>
</tr>
<tr>
<td>Bud color</td>
<td>0.3307</td>
<td>9.5760</td>
<td>12.2484</td>
</tr>
<tr>
<td>Ridge shape</td>
<td>-1.9181</td>
<td>1.5674</td>
<td>4.3593</td>
</tr>
</tbody>
</table>

Note: Discriminant function derived was based upon known sources as follows: red spruce, provenance test located in Stewardstown, N.H.; black spruce, provenance test located in Alfred, Maine. The formula for the first function is \( f_m = -289.9654 + 14.8231 \times \text{(hue value)} + 12.2985 \times \text{(chroma value)} + 7.4490 \times \text{(descriptive color value)} + 0.3307 \times \text{(bud color value)} - 1.9181 \times \text{(ridge shape value)} \), where \( f_m \) denotes the discriminant score for case \( m \) on function 1. The discriminant model correctly reclassified red and black spruce provenance test samples more than 95% of the time, and correctly reclassified controlled-cross hybrids more than 64% of the time.

Fig. 1. Collection locations near Head Harbor, Isle au Haut, Maine. Site 1, between bog and upland site \((n = 26)\); Site 2, bog \((n = 26)\); Site 3, between bog and ocean \((n = 18)\); Site 4, upland and inland of the bog \((n = 17)\). Map scale 1:24,000; grid 1000-m Universal Transverse Mercator; Maine, East Zone. Note that 50 ft \(= 15.2\) m and 100 ft \(= 30.5\) m.

Eighty spruce trees were sampled along an elevational transect on the eastern slope of Mount Washington during March 1991. Samples were taken from approximately the same locations as those of Berlyn et al. (1990), and at several lower elevations. Foliage samples were collected at eight elevations: 530 m (1740 ft, \(n = 10\)); 610 m (2000 ft, \(n = 10\)); 670 m (2200 ft, \(n = 10\)); 790 m (2600 ft, \(n = 10\)); 880 m (2900 ft, \(n = 15\)); 1060 m (3500 ft, \(n = 8\)); 1220 m (4000 ft, \(n = 8\)); and 1435 m (4700 ft, \(n = 8\)). Branches for morphological scoring were kept at 4°C in Ziploc™ plastic bags until evaluation three days after collection. On Mount Lafayette, samples were collected from 94 individuals on the northwestern slope along the Scookumchuck trail at three elevations: 710 m (2320 ft, \(n = 29\)); 1190 m (3900 ft, \(n = 39\)); and 1430 m (4685 ft, \(n = 26\)).

Foliage samples were collected during August 1990 from 87 spruce trees just west of Head Harbor on Isle au Haut, Maine. Collections were made at four locations (Fig. 1): site 1, east of and adjacent to bog \((n = 26)\); site 2, bog \((n = 26)\); site 3, south and east of bog \((n = 18)\); and site 4, north of bog \((n = 17)\). Sampled trees were at least 5 m apart to cover each stand as evenly as possible.

Morphological classification
Foliage color, bud color, and twig ridge shape were identified by Gordon (1976) to be reliable variables for differentiating red and black spruce and their possible hybrids. We used Gordon's approach to identify red and black spruce, and possible hybrids, on Mount Washington. Five variables (Table 1) were used: hue and chroma of adaxial surfaces of the needles, which were assigned numerical values using Munsell plant color plates; overall color of foliage, according to a subjective scale developed by Gordon; bud color as a numerical value according to whether they were shiny red or dull gray; and numerical values for twig ridges based on their cross-sectional shapes (Gordon 1976). Foliage color and twig ridge shape were scored on second-year growth, the former at reading distance and the latter using a binocular dissecting microscope (8X). Branches from high elevations were damaged by their harsh environment, affecting needle color, bud color, and twig ridge shape. Therefore, it was not possible to morphologically score three samples from 1430 m because the twigs had only stunted, discolored current-year foliage, and the twig ridges and buds were moribund.

Morphological data from 69 specimens collected on Mount Washington were evaluated with a parametric discriminant model developed from provenance test material from Stewardstown, N.H. (72 red spruce individuals), and Alfred, Maine (60 black spruce individuals). Data from five black \(\times\) red and eight red \(\times\) black controlled-cross hybrids from Ontario were included in the discriminant analysis. Thus the discriminant model was based on three classes of known material and five morphological variables. A classification criterion was developed based on the pooled covariance matrix and prior probabilities of membership in the known classes, proportional to class size. Once the discriminant function was derived, individual tree scores were calculated from the Mount Washington morphological data (see Table 1).
Table 2. Distribution of chloroplast haplotypes over the test sites on Isle au Haut.

<table>
<thead>
<tr>
<th>Test site</th>
<th>cp-haplotype a</th>
<th>cp-haplotype b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>17</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>73</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>24</td>
</tr>
</tbody>
</table>

Molecular methods
Samples were analyzed as described in Bobola et al. (1996). Total genomic DNAs were restricted and used to prepare Southern blots. These blots were probed with radiolabelled rDNA fragment, mitochondrial, or chloroplast probes. The identities of the nuclear rDNA markers are given in Bobola et al. (1996). Autoradiography and densitometry were used to measure the relative proportions of each rDNA fragment. Since several forms of the rDNA repeat exist within individuals, each are referred to as alleles of the rDNA multigene family. Mitochondrial and chloroplast haplotypes were also scored. These data were analyzed with the three-character molecular index that distinguishes red spruce, hybrid and introgressed, and black spruce, and (or) with a discriminant model (Bobola et al. 1996).

Analysis of molecular data
The molecular discriminant model and the three-character index were applied as described in Bobola et al. (1996). Box-plot comparisons of medians and ranges of ribosomal ITS marker EMW 4.35 frequencies for each sampling elevation were made to contrast rDNA allelic frequencies for different elevations on Mount Washington. This approach was chosen to avoid difficulties associated with small sample size at each elevation. Box plots were calculated using DataDesk™ 3.0 (Velleman 1989). Canonical discriminant analysis was used to explore differences in the mitochondrial, chloroplast, and nuclear DNA marker frequencies of red spruce on Mount Lafayette, Mount Washington, and in the provenance test. For this analysis, observations from all elevations on each mountain were combined to provide an acceptable number of observations. Canonical analysis was calculated using the CANDISC and DISCRIM procedures of SAS version 6.1 (SAS Institute Inc. 1990). Post hoc pairwise comparisons of rDNA marker frequencies for red spruce from the mountain sites, provenance test, and Isle au Haut were made using Tukey’s method for multiple comparisons (Guenter 1964) with SYSTAT (Wilkinson 1989).

Results
Distribution of organelle markers
The chloroplast and mitochondrial haplotypes were scored for all samples from the mountain populations. The chloroplast haplotype (cp-haplotype a), associated with black spruce (Bobola et al. 1996), was observed on Mount Lafayette in only one individual, from 708 m; all others had the red spruce associated cp-haplotype b. On Mount Washington, 12 individuals had cp-haplotype a: one at 793 m, three at 1220 m, and eight at 1433 m. Only one sample, from 1430 m on Mount Lafayette, had the black spruce associated mitochondrial haplotype (mt-haplotype b), while all other samples displayed the mt-haplotype a associated with red spruce. On Mount Washington all individuals had the mt-haplotype a.

The frequency of chloroplast haplotypes among Isle au Haut samples was 0.47 for cp-haplotype a and 0.53 for cp-haplotype b. The distribution of chloroplast haplotypes varied among sites (Table 2). Chloroplast haplotype was observed previously to be a strong indicator of paternal lineage in hybrids of red and black spruce (Bobola et al. 1996). Interestingly, all samples from Isle au Haut displayed the red spruce associated mt-haplotype a.

Classification results
Samples from both Mount Washington (79 individuals) and Mount Lafayette (94 individuals) were classified as to species using the molecular RFLP models developed by Bobola et al. (1996). The molecular discriminant model (D), based on the organelle haplotypes and the individual allele ratio of one nuclear rDNA marker, SITS, is useful when separating the species and black spruce × red spruce F₁ hybrids; however, it is not sensitive to red spruce × black spruce hybrids. The three-character molecular index (I), also based on the internal frequency of SITS and the organelle haplotypes, is effective for classifying individuals and identifying hybridization in both directions.

No black spruce were detected among the samples from Mount Lafayette. Using D, we identified one hybrid on Mount Lafayette. Applying I to the Lafayette samples, three trees displayed RFLP patterns that are suggestive of hybridization (Table 3). We speculate that black spruce...
Table 3. Spruce from Mount Washington (W) or Mount Lafayette (L) classified as hybrid or introgressed by the three-character molecular index (I).

<table>
<thead>
<tr>
<th>Mountain</th>
<th>Tree</th>
<th>Elevation (m)</th>
<th>SITS</th>
<th>cp-haplotype</th>
<th>mt-haplotype</th>
<th>Direction of cross</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>1150</td>
<td>710</td>
<td>53</td>
<td>a</td>
<td>a</td>
<td>Red × black</td>
</tr>
<tr>
<td>L</td>
<td>1102</td>
<td>1190</td>
<td>99</td>
<td>b</td>
<td>a</td>
<td>Black × red</td>
</tr>
<tr>
<td>L</td>
<td>1123</td>
<td>1430</td>
<td>65</td>
<td>b</td>
<td>b</td>
<td>Black × red</td>
</tr>
<tr>
<td>W</td>
<td>31</td>
<td>790</td>
<td>100</td>
<td>b</td>
<td>a</td>
<td>Black × red</td>
</tr>
<tr>
<td>W</td>
<td>46</td>
<td>790</td>
<td>98</td>
<td>a</td>
<td>a</td>
<td>Red × black</td>
</tr>
<tr>
<td>W</td>
<td>77</td>
<td>1220</td>
<td>98</td>
<td>b</td>
<td>a</td>
<td>Black × red</td>
</tr>
<tr>
<td>W</td>
<td>79</td>
<td>1220</td>
<td>98</td>
<td>a</td>
<td>a</td>
<td>Red × black</td>
</tr>
<tr>
<td>W</td>
<td>80</td>
<td>1220</td>
<td>100</td>
<td>b</td>
<td>a</td>
<td>Red × black</td>
</tr>
<tr>
<td>W</td>
<td>89</td>
<td>1220</td>
<td>84</td>
<td>a</td>
<td>a</td>
<td>Red × black</td>
</tr>
<tr>
<td>W</td>
<td>95</td>
<td>1435</td>
<td>75</td>
<td>a</td>
<td>a</td>
<td>Red × black</td>
</tr>
</tbody>
</table>

Note: Direction of cross refers to paternal and maternal spruce lineage but does not necessarily indicate that the individual is an F1 hybrid.

Table 4. Comparisons among the morphological and molecular indexes applied to the Mount Washington samples where disagreements occurred among the analyses.

<table>
<thead>
<tr>
<th>Tree</th>
<th>Three-character molecular index (I), direction of cross</th>
<th>Morphological discriminant model, posterior probabilities</th>
<th>Molecular discriminant model (D), posterior probabilities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Black</td>
<td>Hybrid</td>
<td>Red</td>
</tr>
<tr>
<td>46</td>
<td>Red × black</td>
<td><em>b</em></td>
<td><em>b</em></td>
</tr>
<tr>
<td>77</td>
<td>Black × red</td>
<td>0.0000</td>
<td>0.0002</td>
</tr>
<tr>
<td>81</td>
<td>Black</td>
<td>0.0000</td>
<td>1.0000</td>
</tr>
<tr>
<td>87</td>
<td>Black</td>
<td>0.0000</td>
<td>1.0000</td>
</tr>
<tr>
<td>89</td>
<td>Red × black</td>
<td>0.0000</td>
<td>0.9972</td>
</tr>
<tr>
<td>95</td>
<td>Red × black</td>
<td><em>b</em></td>
<td><em>b</em></td>
</tr>
</tbody>
</table>

Note: The three-character molecular discriminant model is insensitive to red spruce × black spruce hybrids, classifying these hybrids as black spruce.

*Posterior probability is the probability that an individual belongs to a group based on the discriminant function (Klecka 1980).

*Missing observation due to poor morphological condition of the sample.

occurs there only infrequently and these trees are probably introgressed and not F1 hybrids, since no black spruce were found in our samples from Mount Lafayette. Two of the three individuals identified as hybrids by the ratio of markers in the nucleus displayed a strong red spruce influence.

Using the morphological discriminant model, classification analysis was applied to samples collected from 69 individuals along the elevation transect on Mount Washington. Branches from 55 of these trees were also used in the DNA analyses. Results from the morphological discriminant analysis indicated that 64 (93%) were red spruce, 4 (6%) were hybrids, and 1 (1%) was black spruce. All four that were classified as hybrids were at approximately 1220 m, and the one black spruce was at 1435 m.

The I values generated species classifications similar to those of the morphological discriminant model: 64 (80%) as red spruce, 7 (9%) as hybrids, and 9 (11%) as black spruce. Classification using D was similar to that of the morphological model and of I, except that D classified the red spruce × black spruce hybrids as black spruce. We found that D classified 65 (81%) as red spruce, 4 (5%) as hybrid, and 11 (14%) as black spruce.

A comparison of the disagreements among these three methods is presented in Table 4. The morphological discriminant function, based on 55 trees in common with the molecular analyses, showed only a four-tree difference in classification results. Morphologically, two trees (Nos. 81 and 87) were classified as hybrid; these had been classified as black spruce by both molecular methods. Tree 77 was classified as red spruce by the morphological function, and as a hybrid by D and by I. Tree 89 was classified as hybrid by both the morphological function and I, but as black spruce by D. The morphological function may have a small bias toward classification of black spruce as hybrids in comparison with the molecular methods.

A total of 80 trees were analyzed using both I and D. Only three trees (Nos. 46, 89, and 95) were classified differently by these two methods. All three were red spruce × black spruce hybrids according to I, which D cannot
detect, and classified as black spruce. We believe that of the three methods we developed for discrimination, 1 provides the most accurate assessment of black spruce, red spruce, and hybrids in our samples.

Individuals from Isle au Haut were classified as to species using only 1 (Table 5). Overall, 36 individuals (41%) were classified as hybrid or introgressed, 28 (32%) as black spruce, and 23 (26%) as red spruce. Thirteen of the hybrid or introgressed type (15% of the all samples) displayed RFLP patterns suggestive of red spruce × black spruce hybridization, and 23 (26% of the total) displayed RFLP patterns suggestive of black spruce × red spruce hybridization. Hybridization was unevenly distributed among the sites, with greater levels of hybridization occurring at site 1 (50% were hybrid) and site 3 (67% were hybrid) (Table 5). Site 1 (east of the bog) and site 2 (the bog) contained the highest percentage of black spruce, 35% and 65%, respectively. Site 4 contained 58% red spruce, the highest percentage of all the sites. This site is north of the bog and at the base of an upland slope.

Variation in nuclear rDNA alleles among spruce from Isle au Haut
Three nuclear rDNA markers, HMW 1.6, SITS, and EMW 4.35, were examined for variation among red and black spruce and putative hybrids on Isle au Haut. Table 6 displays the mean frequencies for the nuclear rDNA markers and compares them with provenance test material. Standard deviations for EMW 4.35 and HMW 1.6 are large, but are comparable with values found previously (Bobola et al. 1992). Mean values for SITS were similar to those observed in the provenance tests, while variation in the other two nuclear markers was dissimilar. Analysis of variance of Isle au Haut samples detected significant variation (F = 61.5, p ≤ 0.01) among black spruce, red spruce, and hybrid or introgressed groups only for SITS.

Comparisons among red spruce populations on Mount Washington, Mount Lafayette, and the Stewartstown, N.H., provenance test
Multivariate and univariate analyses were carried out to examine among-population differences in red spruce for three nuclear rDNA markers: HMW 1.6, SITS, and EMW 4.35. Canonical analysis yielded one significant vector useful in separating the populations (p ≤ 0.01, Wilks' λ = 0.632). Loadings on the first canonical variate in the total canonical structure matrix were HMW 1.6 (0.270), SITS (0.500), and EMW 4.35 (0.970). A plot of frequencies of canonical scores for individual trees (Fig. 2) along the significant canonical variate for the two mountain populations and the provenance test revealed that the range in scores is roughly equivalent for the Mount Washington
Table 7. Tukey post hoc pairwise comparisons of red spruce rDNA allele marker frequencies for populations on Mount Washington and Mount Lafayette (N.H.), Isle au Haut (Maine), and a red spruce range-wide provenance test in Stewartstown, N.H.

<table>
<thead>
<tr>
<th>Marker and location</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Provenances</th>
<th>Isle au Haut</th>
<th>Lafayette</th>
<th>Washington</th>
</tr>
</thead>
<tbody>
<tr>
<td>SITS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Provenances</td>
<td>49</td>
<td>0.77</td>
<td>0.11</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isle au Haut</td>
<td>25</td>
<td>0.75</td>
<td>0.13</td>
<td>0.98</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mt. Lafayette</td>
<td>88</td>
<td>0.71</td>
<td>0.12</td>
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Fig. 3. Box plots of EMW 4.35 allelic frequencies by elevation on Mount Washington, N.H. Dark areas indicate 95% confidence intervals for comparison of median values; asterisk indicates an outlier. Elevation classes are (1) 530 m; (2) 610 m; (3) 670 m; (4) 790 m; (5) 880 m; (6) 1060 m; (7) 1220 m.

population and the provenance test, while scores for the Mount Lafayette population exhibit less range and a more clustered appearance. The Mount Washington population displayed more variable internal frequencies in nuclear rDNA alleles, particularly for the EcoRI polymorphism EMW 4.35. Although only three elevations were sampled on Mount Lafayette, they are low, intermediate, and high, and thus include elevation extremes. Median marker frequencies differed ($p \leq 0.05$) according to elevation only for EMW 4.35 on Mount Washington (Fig. 3). Frequencies at 670, 790, and 1220 m were significantly ($p \leq 0.05$) higher than those at 1060 m. While this pattern suggests a non-linear relationship between EMW 4.35 frequencies and elevation, confirmation requires additional sampling.

Univariate post hoc comparisons of red spruce rDNA allele marker frequencies from the natural populations and the Stewartstown provenance test revealed significant differences for each marker and support the canonical analysis for the mountain populations (Table 7). Isle au Haut populations differed from the mountain populations for the HMW 1.6 and EMW 4.35 markers. The Stewartstown provenance test differed from all three natural populations for HMW 1.6 and from Isle au Haut and Mount Lafayette for EMW 4.35.

Discussion

We have examined red and black spruce in natural populations to address the question of whether hybrids between these species persist in the two mountain populations and on Isle au Haut. We compared the frequency of molecular markers with those of provenance tests of red and black spruce. We examined the results of a discriminant model based on needle and twig morphology and compared these results with those of two different molecular characterization models. The results of the different methods were generally congruent. We found low levels of hybridization in mountain populations and evidence for extensive hybridization in the coastal island population.

The morphological discriminant model was robust. It classified groupings similar to $I$ except for the cases noted in Table 4, where it classified two black spruce identified by $I$ as hybrids, and one high-elevation hybrid identified...
by I as a red spruce. The model was less effective at high elevations, since the quality of morphological samples became problematic for those collected above 1220 m on Mount Washington.

Berlyn et al. (1990) used nuclear genome content to separate red and black spruce, and to assess hybridization and introgression. In their study, samples were collected from elevation transects on Mount Washington (N.H.), Camels Hump (Vt.), and Whiteface Mountain (N.Y.). Using nuclear genome size and (or) a morphological index modified from Manley (1971, as described in Berlyn et al. 1990), all individuals sampled from Whiteface Mountain were classified as pure red spruce. Trees from Camels Hump were all classified as introgressed, with no pure black or red spruce found. On Mount Washington, Berlyn et al. (1990) reported introgression occurring along an elevation gradient, with pure black spruce above 1220 m, hybrids between 1000 and 1220 m, and relatively pure red spruce at lower elevations. They concluded that hybridization between red and black spruce was frequent and that the degree of introgression was related to elevation.

Our transect of Mount Washington covered the same elevations and aspect as Berlyn et al. (1990). We did not detect the high level of introgression or the strong relationship between elevation and introgression on Mount Washington reported by Berlyn et al. (1990). We found evidence for hybridization and (or) potential introgression in only 11% of our samples from Mount Washington. It seems likely that the correlation between nuclear genome content and elevation observed by Berlyn et al. (1990) was not due to introgression.

Although we detected five hybrid or introgressed individuals at high elevation (above 1220 m) on Mount Washington, frequent hybridization seems unlikely. It would be improbable to find red spruce persisting in the harsh conditions at high elevations, and flowering in black spruce may be a rare event. We do not have a large enough sample to distinguish between the occurrence of hybrid or introgressed individuals and selection for within-species genetic variation (see Bobola et al. 1992; Strauss and Tsai 1988). Nor do we know the extent to which the current pattern of organelle markers reflects species distribution under historic climatic conditions. Movement of red spruce pollen upslope would be required to fertilize receptive black spruce females. The presence of individuals from introgressed or hybrid seed produced downslope seems unlikely under the climatic conditions that prevail today.

Nuclear rDNA restriction site frequencies were examined for elevation site differences. On Mount Lafayette, no significant relationships were detected between rDNA alleles and elevation. Among the red spruce samples from Mount Washington, however, the median frequency of EMW 4.35 at 1060 m differed from those at 670, 790, and 1220 m. The difference does not appear to be due to hybridization and (or) introgression with black spruce; EMW 4.35 is not diagnostic for species differences (Bobola et al. 1992). In the analysis of the red spruce provenance test, EMW 4.35 showed the strongest relationship with latitude of seed source (Bobola et al. 1992). Variation in the marker EMW 4.35 on Mount Washington and in the red spruce provenance test may be due to ecogeographic selection similar to that which has been observed for specific rDNA alleles in barley (Sagheri Maroof et al. 1990). An alternative hypothesis is that differences are due to genetic drift. Frequencies of the other nuclear rDNA markers (SITS and HMW 1.6) were not related to either latitude of provenance or elevation on Mount Washington, and appear not to be subject to the same potential selection pressures as EMW 4.35. We infer that the frequencies of the different rDNA allelic markers are independent of one another in these mountain populations, as was previously reported for rDNA frequencies between the provenance tests (Bobola et al. 1992).

Based on the frequency distribution of canonical scores of rDNA markers for individual trees (Fig. 3), the range of variation on Mount Washington was similar to that of the red spruce provenance test, and approximately twice as great as that on Mount Lafayette. Variation in frequency of the EMW 4.35 rDNA marker contributed most strongly to this pattern. The populations on Mount Lafayette are of reduced variability for this polymorphism.

This finding suggests that population history may be important in determining current levels of variation in rDNA markers for these two mountains. Leak and Graber (1974) indicate that Mount Washington was logged on slopes lower than 914 m until the late 1800s. Logging in this area was opportunistic, in that sites of easiest access were logged and those more difficult to reach were left undisturbed (W. Winturri, personal communication). Logging left a patchy mix of conifers below 914 m, and a natural disturbance pattern above that elevation. In contrast, Mount Lafayette was severely burned in 1903 by a fire that removed almost all vegetative cover (D. Govatski, personal communication). These differing stand histories may contribute to the patterns of genetic variation we observed in red spruce on the two mountains. The observed genetic differences could also be due to ecogeographic variation or genetic drift, including founder effects.

Using the marker SITS, which we determined to be the best indicator of hybridization (Bobola et al. 1996), we detected a substantial proportion of potentially hybrid and introgressed trees on Isle au Haut. We also observed significant differences between Isle au Haut and mountain populations for the markers HMW 1.6 and EMW 4.35. Previously we had detected a relationship between geographic origin of red spruce provenances and frequencies of the several rDNA markers (Bobola et al. 1992). The correlation was strongest between EMW 4.35 and latitude and longitude of provenance. We speculate that the difference in persistence of hybrids and of rDNA allele frequencies between the coastal island and mountain populations may be due to climatic factors and stand history. However, these propositions must be investigated further.

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References


