Rating Introgression between Lodgepole and Jack Pine at the Individual Tree Level Using Morphological Traits

Katherine P. Bleiker and Allan L. Carroll

ABSTRACT

Introgressive hybridization between species generates novel gene combinations and phenotypes. We required an accessible, objective method of rating introgression between lodgepole pine (*Pinus contorta* var. latifolia [Engelm.] Critchfield) and jack pine (*Pinus banksiana* Lamb.) for individual trees where their ranges overlap in Canada for use in another study on host species effects on resistance to an eruptive herbivore that has recently expanded its range. We adapted, simplified, and fully quantified a morphological index developed to rate introgression of pine populations and applied it to individual trees. In addition to principal component analysis (PCA), we also used discriminant function analysis (DFA), a potentially more powerful method given a priori knowledge of parent taxa, to generate introgression ratings. Among-tree variation in morphological traits and introgression was high at sites within the hybrid zone but very low at pure parent sites. PCA and DFA produced similar introgression ratings at the stand level, but ratings differed substantially for some individual trees. Certain morphological traits may be omitted from both PCA and DFA with little impact on stand-level ratings. The discriminant functions presented here are based on easy-to-measure, fully quantifiable morphological traits and can be used by other researchers to produce relative introgression ratings for lodgepole and jack pine. The approach may also be applied to other plant hybrid systems.

Keywords: *Pinus contorta*, *Pinus banksiana*, hybridization, introgressive hybridization

P lant hybridization and the introgression of genes from one species into another produce genetic admixtures and various phenotypes that may affect host resistance to diseases or herbivory (e.g., Whitham 1989, Fritz et al. 1994). Lodgepole pine (*Pinus contorta* var. latifolia [Engelm.] Critchfield) and jack pine (*Pinus banksiana* Lamb.) hybridize and backcross in northern Alberta, where their ranges overlap (e.g., Moss 1949, Critchfield 1985). A clinal gradient in the susceptibility of pine populations to certain pests across the hybrid zone in northern Alberta suggests that introgressed jack pine genes may confer some resistance to pests in lodgepole pine (Wu et al. 1996, Wu and Ying 1998). However, sampling host resistance to insects and diseases across a cline requires that the organisms be distributed across the cline and that sampling be sufficient to capture the gradient as the pine hybrid zone is characterized by mosaics rather than clinal patterns of variation (Wagner et al. 1987, Wheeler and Guries 1987).

As an alternative to clinal sampling, other studies have examined disease resistance of stands classified as hybrid, lodgepole, or jack pine within the hybrid zone. Yang et al. (1997, 1999) found that trees at hybrid sites were just as susceptible to western gall rust (*Endocronartium harknessii* [J.P. Moore] Y. Hiratsuka) as trees at lodgepole sites (Yang et al. 1997, 1999). Rice et al. (2007b) found evidence of transgressive hybrid inferiority after inoculating trees at a hybrid site and two parent sites with a phytopathogenic fungus associated with the mountain pine beetle (*Dendroctonus ponderosae*

Hopk.); however, between-year differences in results led the authors to posit that high genetic variation among hybrid trees within their hybrid site may have contributed to this difference (Rice et al. 2007a). In addition to high among-tree variation within hybrid stands, site effects (e.g., climate, edaphic characteristics) may confound results, as lodgepole, jack, and hybrid pines have different site preferences and niches (Rudolph and Yeatman 1982, Critchfield 1985). Quantifying introgression at the individual tree level, instead of the stand level, may facilitate the identification of host species effects because site effects could be removed and high among-tree variation considered. Tree-level ratings would also aid studies on introgression and host species effects on resistance to pests that are not currently distributed across the cline (e.g., invading pests).

Lodgepole and jack pine are easily distinguished morphologically (Wheeler and Guries 1982). Using transitional morphological traits, Wheeler and Guries (1987) created two aggregate introgression indices to classify hybrid pine populations. The first index follows Anderson's (1949) classic method: traits are assigned a value of 0, 1, or 2 on the basis of whether they are judged to be representative of lodgepole, hybrid, or jack pine, respectively, and the means of all traits are used as the index. With no cutoff values or guidelines reported for the variables, this highly subjective method is difficult to replicate. The second index uses principal component analysis (PCA) of morphological traits, including some qualitative traits that are given a number for analysis. These methods assign all characters
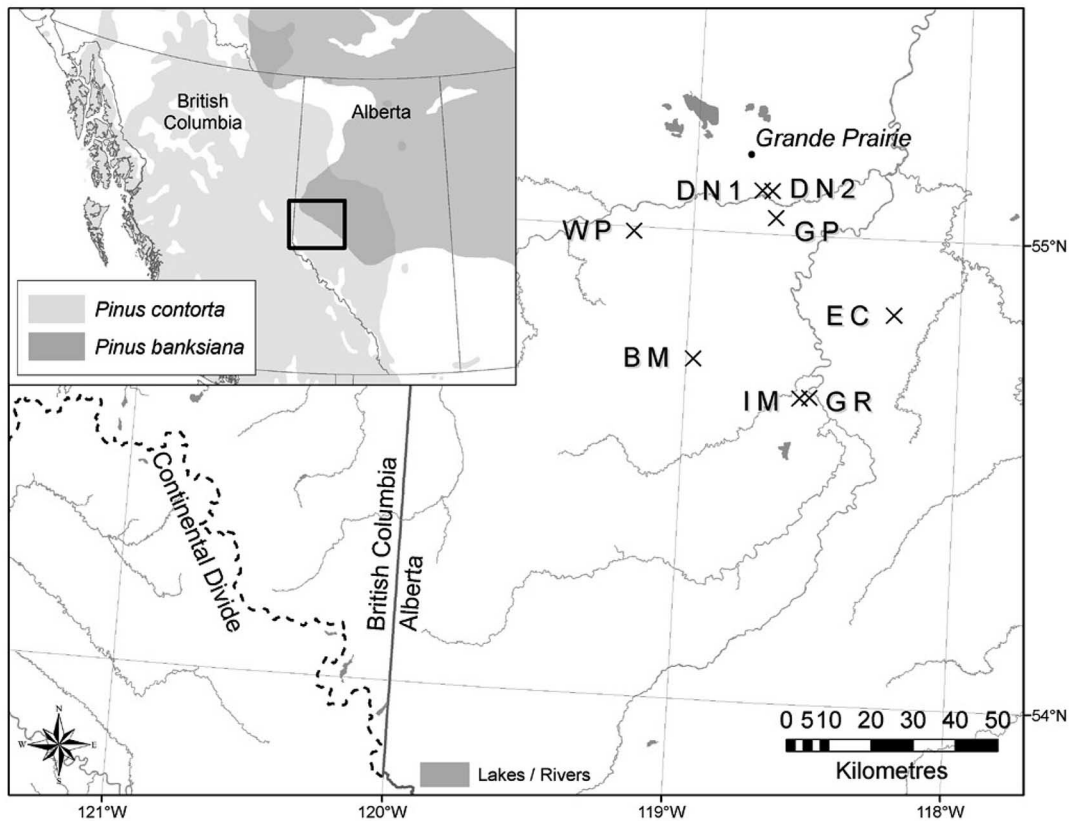
Figure 1. Location of sites sampled in northern Alberta, where the ranges of lodgepole pine and jack pine overlap and the two species hybridize. IM, Imperial; EC, Economy Creek; BM, Bald Mountain; GR, Greas; WP, Wapiti; DN1, Dunes 1; DN2, Dunes 2.

equal weighting; however, given a priori knowledge of parent taxa, discriminant function analysis (DFA) may be more powerful because it maximizes the ratio of between-group to within-group differences (Adams 1982, McGarigal et al. 2000, Quinn and Keough 2002).

We required an accessible method of rating lodgepole-jack pine introgression for use in another study on host resistance to the mountain pine beetle, which is an eruptive herbivore. The historic range of the mountain pine beetle is west of the Rocky Mountains in northern and central British Columbia, where its primary host was lodgepole pine (Safranyik and Carroll 2006). However, during the recent, unprecedented epidemic in British Columbia, significant numbers of beetles breached the continental divide in northern British Columbia and are now successfully reproducing within the lodgepole-jack pine hybrid zone in northwestern Alberta. The hybrid zone in northern Alberta bridges the ranges of lodgepole pine on the west and jack pine on the east and provides a potential corridor by which the beetle may invade jack pine in Canada's vast boreal forest (Figure 1). To assess beetle behavior and success in the new host trees—lodgepole-jack pine hybrids and backcrosses—we needed an objective, accessible, and economically feasible method for ranking introgression for specific trees: those selected and attacked by the mountain pine beetle and unattacked control trees. The objective of this study was to build on previous work to develop such a rating that did not require genetic analysis capability, and an associated sampling methodology that is effective at the tree level. In addition, we wanted to assess introgression ratings produced using PCA, a data reduction technique, and DFA, a classification and prediction technique (McGarigal et al. 2000), using different mor-

phological traits to determine the most effective and efficient combination of variables and method of analysis. The simple method and individual-tree level approach presented here may be used in other applied contexts and systems and may aid in unraveling the potentially confounding effects of site on the organisms involved in plant-pest interactions.

Materials and Methods

Sample Trees and Sites

Cones (seed cones) and needles (produced in pairs) were collected from 11 to 16 apparently healthy pine trees at each of eight putatively hybrid sites outside Grande Prairie in northwestern Alberta (Figure 1). At each site, four prism plots (basal area factor 5) located 50 m apart were established, and at least three dominant or codominant pine trees were sampled in each plot. If plots did not contain three trees with enough cones, the cones were unreachable with a 14-m sectional pole pruner, or the trees could not be felled safely, neighboring pine trees were sampled. Only closed cones were sampled (cones of both species are predominantly serotinous and often remain closed on the trees for years [Farrar 1995]). A total of 10 cones and 15 pairs of needles were sampled per tree (measurements described below). Five branches per tree were sampled, and two cones and three pairs of needles were sampled per branch. If branches only had one suitable cone, additional branches were sampled. Similarly, we also obtained cone and needle samples from putatively pure lodgepole and jack pine populations near Lac le Jeune, British Columbia, and Shellbrook, Saskatchewan, respectively.
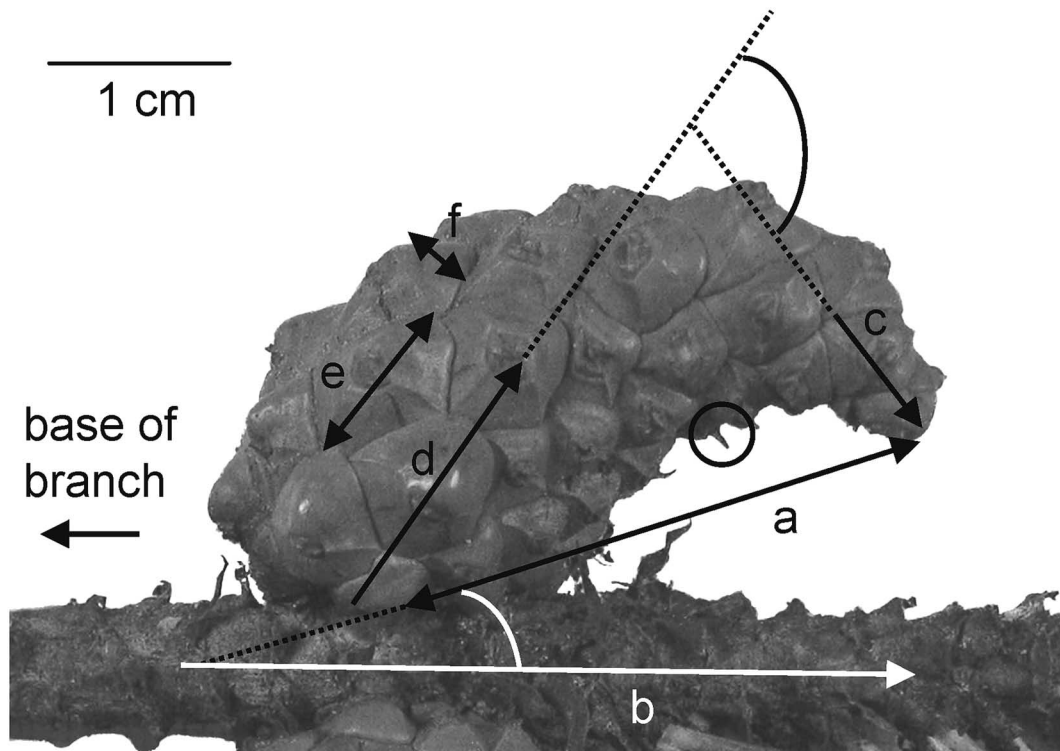
Figure 2. Seed-cone of a lodgepole-jack pine hybrid or backcross from northern Alberta showing the following cone traits: cone length (the distance indicated by double-headed arrow a), cone angle (the angle between arrows a and b), cone curvature (the angle between the extensions of arrows c [distal direction of cone growth] and d [basal direction of cone growth]), apophysis length (the distance shown by double-headed arrow e), apophysis and umbo height (the distance shown by double-headed arrow f), and prickle (circle).

Cone and Needle Measurements

Sections of branches with mature closed cones (2 years old or older, as cones mature in their second year) and needles were placed in plastic bags and stored at 3°C until the following traits were measured.

Cone Length

Length to the nearest millimeter was measured in a straight line from the cone's tip to its attachment point on the branch (Figure 2).

Cone Angle

The angle between the cone length axis and the center line of the branch was measured to the nearest degree with the vertex proximate to the base of the branch (Figure 2). Cone angles were positive if the cone tip was on the same side of the branch as the attachment point and negative if the cone tip crossed the center line of the branch so that the tip was on the opposite side of the branch from the attachment point. Cone angles were always less than 180°.

Cone Curvature

The initial, basal axis of cone growth was visually estimated by determining the most prominent direction of cone growth, independently of attachment point on the branch (Figure 2). Similarly, the most prominent direction of the distal part of the cone was visually estimated. Cone curvature was the absolute value of the angle to the nearest degree between the distal and basal axes of cone growth with the vertex located toward the cone's base (Figure 2).

Apophysis Length and Apophysis and Umbo Height

Digital calipers with a depth rod were used to measure apophysis length and apophysis and umbo height to the nearest millimeter on three cone scales on each cone. Length was the distance between the Vs formed by subjacent and superjacent cone-scale tip edges (Figure 2). Height was measured from the lowest point on the apophysis surface to the height of the bulge of the umbo (Figure 2). Apophysis size varies greatly within a cone; therefore, we consistently selected three cone scales in one helical row along the outside arc of the cone distal to the branch (Figure 2). We selected the third, fourth, and fifth apophyses up from the bottom of the cone, starting with the first apophysis that was apparent to the naked eye (some apophyses are obscured by the branch).

Prickle

The sharp spike or prickle was rated on a subjective scale from 0 to 6, with 0 being no prickle and 6 being well-armed (Figure 2).

Needle-Pair Length and Spread

The length of each pair of needles (needles in a pair are the same length) and the distance between the tips of the needles were measured to the nearest millimeter.

Needle Serrations

We counted the number of serrations that occurred in 1 cm along one side of one needle from each pair using a dissecting microscope at ×40 magnification. The 1-cm-long sample zone was centered midneedle.

Table 1. Mean (SD) seed-cone and needle traits measured for jack pine from Saskatchewan (SK), lodgepole pine from British Columbia (BC), and putative hybrid pines from Alberta (AB).

Seed-cone and needle traits	t-tests, lodgepole versus jack pine		Mean (SD)		
	t value	P	Jack pine (SK)	Lodgepole pine (BC)	Putative hybrids[a] (AB)
Cone length (mm)	$t_8 = -1.1$	0.29	38.0 (4.2)[b]	41.1 (4.5)[b]	39.3 (4.7)
Cone angle (degrees)	$t_4 = -7.8$	<0.001	−23.6 (8.1)[b]	109.0 (37.0)[c]	95.7 (43.7)
Cone curvature (degrees)	$t_8 = 7.2$	<0.001	70.6 (13.8)[b]	9.3 (13.0)[c]	16.7 (19.0)
Apophysis length (mm)	$t_6 = 8.4$	<0.001	9.9 (1.0)[b]	5.9 (0.5)[c]	6.6 (0.9)
Apophysis and umbo height (mm)	$t_8 = -3.3$	0.01	2.2 (0.4)[b]	3.0 (0.5)[c]	2.6 (0.9)
Prickle (rated 0 to 6)	$t_4 = -29.5$	<0.001	0.2 (0.5)[b]	6.0 (0.0)[c]	3.5 (1.3)
Needle-pair length (mm)	$t_5 = -10.5$	<0.001	36.0 (2.4)[b]	69.3 (6.7)[c]	55.9 (10.5)
Needle-pair spread (mm)	$t_5 = -1.9$	0.11	10.7 (3.6)[b]	21.0 (11.3)[b]	13.8 (4.6)
Ratio of needle length to spread	$t_8 = -0.3$	0.74	4.2 (2.1)[b]	4.7 (2.5)[b]	5.6 (2.8)
No. of needle serrations in 1 cm	$t_6 = 9.0$	<0.001	68.7 (6.1)[b]	41.3 (3.1)[c]	49.8 (6.6)

[a] Also includes backcrosses and potentially trees of pure parental stock.
[b,c] Means of a trait followed by the same letter are not significantly different between jack and lodgepole pine (Student's t-test, $P > 0.05$).

Data Analysis

Cone and needle traits were averaged by branch, and the mean of the branch averages was calculated for each tree. Student's t-tests were used to identify traits that varied between lodgepole and jack pines sampled in British Columbia and Saskatchewan. Six of the seven traits that differed were selected for further analysis (see Results). We used mixed-model analyses of variance (ANOVAs) to examine trait variation at the site, tree, and branch levels within the hybrid zone in northern Alberta. The ANOVAs included three factors: site (fixed), tree nested under site (random), and branch nested under tree and site (random). The model was fitted using the restricted maximum likelihood method, which is appropriate for random factors (JMP IN 5.1, SAS Institute, Cary, NC). All subsequent analyses were conducted on tree averages, which were calculated as described above.

The six selected traits that differed between lodgepole pine and jack pine were used to generate a discriminant function. The function was applied to the trees sampled in the hybrid zone, and the discriminant scores were used to create an introgression rating for each tree. The introgression rating, expressed as a proportion, was standardized against the mean lodgepole pine score as follows: (Individual tree score − Mean score for known lodgepole pine trees)/(Mean score for known jack pine trees − Mean score for known lodgepole pine trees). Thus, the lower the introgression rating for a tree or stand the more lodgepole pine-like traits it possesses compared with trees or stands with higher ratings that possess more jack pine-like characteristics. Because we did not extensively sample pure lodgepole and jack pine stands, our introgression ratings should be considered relative, not absolute, ratings. That is, a tree with a rating of 0.3 possesses more lodgepole pine-like characteristics than a tree with a rating of 0.8. Users should *not* interpret a tree with a rating of 0.3 as being 70% lodgepole and 30% jack pine in genetic composition. Introgression ratings less than 0 and greater than 1.0 were reported as 0 and 1, respectively. Introgression ratings for lodgepole pine from British Columbia and jack pine from Saskatchewan are included in the results for comparison purposes.

We also created introgression ratings from discriminant functions from three reduced trait combinations to determine whether a more efficient sample procedure could be used to rate trees. The reduced trait combinations excluded the variable with the lowest discriminating power (apophysis and umbo height; see Results), the variable that was the most time-consuming to measure (number of needle serrations), or both variables. We used paired t-tests to determine whether ratings for trees from the reduced trait combinations varied significantly from the full complement of traits. Similarly, we created introgression ratings using PCA for the same complements of variables and used paired t-tests to assess differences between full- and reduced-trait combinations, as well as from corresponding ratings produced using DFA. All principal components with eigenvalues greater than 1.0 were included in the introgression ratings (PCA scores).

Data met the required assumptions for PCA and DFA outlined in McGarigal et al. (2000). Mixed-model ANOVAs and PCA were conducted using JMP IN 5.1 (SAS Institute); all other analyses were conducted using SYSTAT 7.0 (SPSS, Inc., Chicago, IL). Significance was set at $P \leq 0.05$.

Results

Variables and Trait Combinations

Seven of the 10 cone and needle traits differed between lodgepole and jack pines: cone angle, cone curvature, apophysis length, apophysis and umbo height, prickle, needle-pair length, and number of needle serrations in 1 cm (Table 1). Prickle rating, the only subjective variable, was not included in the analysis because prickles wear off cones over time (prickle was highly correlated with cone age for cones greater than 5 years old; data not shown). Our goal was to rate any selected (specific) tree, but many trees lack sufficient numbers of younger cones with intact prickles. However, most trees held adequate numbers of old cones to meet the sampling protocol.

Five of the six selected cone and needle traits that differed between the putatively pure lodgepole and jack pine sites varied among the eight putatively hybrid sites in northern Alberta (Table 2). Only apophysis length did not vary significantly among the sites in the hybrid zone (Table 2). Variation among trees within a site was high for all six traits: 61–92% of the total variation for the cone traits, 40% of the total variation for needle-pair length, and 54% of the total variation for the number of serrations (Table 2). In contrast, among-branch variation within a tree was low for most traits, accounting for only 3–15% of the total variation in a trait, with the exception of needle-pair length, where among-branch variation accounted for 50% of the total variation (Table 2).

Four combinations of traits were used to create introgression ratings (Table 3). Combination 1 included all six traits identified by the t-tests as potential differentiators of lodgepole and jack pines: cone angle, cone curvature, apophysis length, apophysis and umbo

Table 2. Variation in seed-cone and needle traits attributed to site, tree, and branch effects for eight sites in the lodgepole-jack pine hybrid zone in northern Alberta.

| | | Total variation attributed to random factors | | |
	Fixed factor effect: site	Tree nested within site	Branch nested with tree and site	Residual
		. . . .(%).		
Cone angle	$F_{7,94} = 4.9$, $P < 0.0001$	92	3	5
Cone curvature	$F_{7,94} = 7.5$, $P < 0.0001$	72	3	25
Apophysis length	$F_{7,94} = 1.8$, $P = 0.10$	61	15	24
Apophysis and umbo height	$F_{7,94} = 2.1$, $P = 0.05$	76	5	19
Needle-pair length	$F_{7,93} = 11.4$, $P < 0.0001$	40	50	10
Needle serrations in 1 cm	$F_{7,93} = 5.3$, $P < 0.0001$	54	14	32

Table 3. Discriminant coefficients (eigenvectors) for seed-cone and needle traits used to create four discriminant functions (DF) to classify lodgepole and jack pine trees.

| | DF constants and coefficients | | | |
	DF1	DF2	DF3	DF4
Function constant	30.634	32.837	−11.692	−7.304
Cone angle	0.039	0.032	0.080	0.050
Cone curvature	−0.124	−0.134	0.031	$<0.0001^a$
Apophysis length	−2.375	−2.426	−0.935	−1.055
Apophysis and umbo height	−0.467		−2.415	
Needle-pair length	0.343	0.314	0.393	0.256
Needle serrations in 1 cm	−0.462	−0.477		

^{a} This variable was excluded from the function because the coefficient was negligible.

height, needle-pair length, and number of serrations. Combination 2 was the same as combination 1 except that apophysis and umbo height was excluded because it had the lowest t value and its standardized coefficient was considerably lower than the other variables (−0.19, compared with absolute values greater than 1 for the other variables). Combinations 3 and 4 were the same as combinations 1 and 2, respectively, except that the most laborious variable to measure, number of serrations, was excluded.

DFA

The discriminant functions produced from all four trait combinations successfully discriminated between lodgepole and jack pine trees (approximate F values for Wilks's lambda: combination 1, $F_6 = 330.8$, $P = 0.0003$; combination 2, $F_5 = 523.1$, $P < 0.0001$; combination 3, $F_5 = 138.8$, $P = 0.0001$; combination 4, $F_4 = 146.1$, $P < 0.0001$). Overall, introgression ratings did not differ between the full complement of traits and the reduced trait combinations (combination 1 versus 2, $t_{111} = −1.2$, $P = 0.23$; combination 1 versus 3, $t_{111} = −0.50$, $P = 0.62$; combination 1 versus 4, $t_{111} = −0.92$, $P = 0.36$). The mean difference in introgression ratings between the full complement of traits and the reduced combinations was minimal (≤ 0.008). Introgression ratings generated from the four combinations of traits ranked the sites in a similar order on the basis of the mean of each site (Figure 3). However, different functions produced substantially different introgression ratings for some individual trees. The absolute maximum change in an introgression rating for a tree between the full complement of traits, combination 1, and the reduced combinations was only 0.03 for combination 2, but it was 0.36 for combination 3 and 0.26 for combination 4. For combinations 1 and 2, 12% of the trees received introgression ratings less than 0, compared with 18% for combinations 3 and 4. Few trees received values greater than 1. Some values outside the 0–1 range were expected, because 0 and 1 represent the means for lodgepole and jack pine, respectively.

PCA

Introgression ratings generated for the sites using PCA were comparable for the four trait combinations (Figure 3); however, overall, tree ratings produced by the reduced combinations differed from the full complement of traits (combination 1 versus 2, $t_{111} = −4.6$, $P < 0.001$; combination 1 versus 3, $t_{111} = −3.8$, $P < 0.001$; combination 1 versus 4, $t_{111} = −3.9$, $P < 0.001$). The mean overall absolute difference in introgression ratings between combination 1 and the reduced trait combinations was 0.01 for combination 2 and 0.02 for combinations 3 and 4. These are minor differences, but they are likely statistically significant because changes were mostly in the same direction. The maximum change in an individual tree rating generated using the full complement of traits, combination 1, and the reduced combinations was 0.07 for combination 2, 0.15 for combination 3, and 0.21 for combination 4. For each combination, 30–40% of the trees received ratings less than 0 using PCA; only a few trees received ratings greater than 1.

DFA versus PCA Ratings

Overall, for a given combination of traits, DFA produced higher introgression ratings than PCA (combination 1, $t_{111} = −5.5$, $P < 0.001$; combination 2, $t_{111} = −4.7$, $P < 0.001$; combination 3, $t_{111} = −4.4$, $P < 0.001$; combination 4, $t_{111} = −4.8$, $P < 0.001$). Mean differences in introgression ratings between DFA and PCA were 0.06 for combination 4, 0.07 for combinations 2 and 3, and 0.08 for combination 1. The rank order of the sites based on introgression ratings produced by DFA and PCA varied for the most lodgepole-like sites (Figure 3). Variation in the introgression ratings for sites was greater with PCA than with DFA (Figure 3).

Range of Ratings

Excluding the number of needle serrations, the most labor-intensive variable measured, from the recommended combination 2 increased the range of introgression ratings generated by DFA and
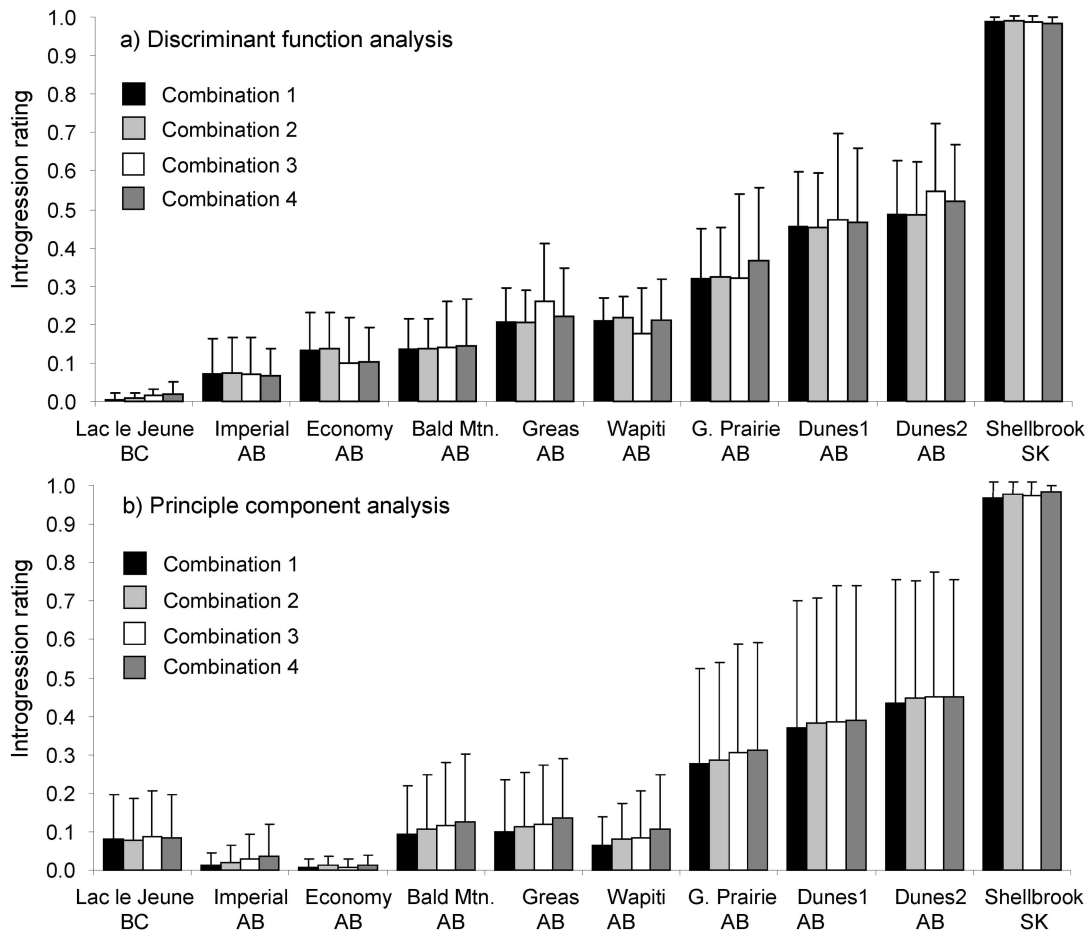
Figure 3. Mean (+SD) lodgepole-jack pine introgression ratings for 10 sites produced by discriminant function analysis (a) or principal component analysis (b) of four different combinations of cone and needle traits. Introgression ratings range from 0 (mean of known lodgepole pine from Lac le Jeune, British Columbia [BC]) to 1 (mean of known jack pine from Shellbrook, Saskatchewan [SK]). Sites in Alberta (AB) contained putative hybrids and could also contain pure parental stock. Cone and needle traits included in each analysis were as follows: combination 1, cone angle, cone curvature, apophysis length, apophysis and umbo height, needle-pair length, and number of needle serrations; combination 2, same as combination 1 with the exception of apophysis and umbo height; combination 3, same as combination 1 with the exception of needle serrations; and combination 4, same as combination 1 with the exception of apophysis and umbo height and needle serrations. The numbers of trees sampled at each site, in the order presented on the x-axis from left to right, were 5, 12, 16, 11, 12, 12, 14, 13, 12, and 5.

PCA at most of the sites (Table 4). However, the differences in the overall average ranges between combinations 2 and 4 was relatively small (0.09 for DFA and 0.02 for PCA) (Table 4).

Discussion

High among-tree variation in introgression ratings within hybrid sites (Figure 3, Table 4) and high variation among hybrid sites indicates that lodgepole-jack pine introgression is common in the study area (Figure 3). Low among-branch variation in traits within a tree indicates that our protocol of sampling five branches per tree is sufficient for tree-level inferences (Table 2). Extensive introgression between the species is likely due to differences in their habitat preferences and the wide range of habitat niches available in the area. Jack pine has a patchy distribution in the hybrid zone growing predominantly on xerophytic sandy soils, whereas lodgepole pine has a more continuous distribution and prefers relatively richer mesophytic sites (Rudolph and Yeatman 1982, Critchfield 1985). Stand-level introgression ratings were congruent with general site quality observations (e.g., the Dunes sites had well-drained, sandy

soils and were the most jack pine-like, whereas Economy Creek and Bald Mountain were richer, moister sites and were the most lodgepole pine-like) (Figure 3). These observations support Anderson's (1949) argument that the success of backcrosses is related to the range of habitats and ecological conditions available. Variation in ratings within and among sites in the region highlights the need for rating introgression at the tree level.

Introgression ratings produced from PCA were similar to those produced by DFA at the stand level; however, PCA and DFA ratings for some individual trees differed substantially (data not shown). In addition, variation was lower with DFA. PCA is an exploratory data analysis technique that aims to reduce the number of variables that explain the data by creating uncorrelated, independent linear combinations of the original variables (components) (Quinn and Keough 2002). Group differences are not considered in PCA, and each character is assigned equal weight. In contrast, discriminant functions (components) are constructed to maximize the ratio of between-group to within-group variation. Given a priori knowledge of parent taxa, DFA may be the more powerful and appropriate tool

Table 4. Range of introgression ratings produced using discriminant function analysis (DFA) and principal components analysis (PCA) of four combinations of seed-cone and needle traits for lodgepole pine sampled in British Columbia (BC), jack pine sampled in Saskatchewan (SK), and eight sites in the hybrid zone in northern Alberta (AB). The range was calculated by subtracting the minimum rating from the maximum rating for each site.

Site name[a]	DFA		PCA	
	Combination 2[b]	Combination 4[b]	Combination 2[b]	Combination 4[b]
Lac le Jeune, BC (5)	0.03	0.07	0.22	0.22
Imperial, AB (12)	0.28	0.18	0.12	0.23
Economy Creek, AB (16)	0.37	0.36	0.07	0.07
Bald Mountain, AB (11)	0.21	0.29	0.30	0.38
Greas, AB (12)	0.25	0.45	0.38	0.43
Wapiti, AB (12)	0.17	0.37	0.27	0.34
Grande Prairie, AB (14)	0.45	0.65	0.67	0.71
Dunes 1, AB (13)	0.40	0.67	0.89	0.90
Dunes 2, AB (12)	0.38	0.42	0.77	0.72
Shellbrook, SK (5)	0.02	0.03	0.08	0.04
Average range	0.26	0.35	0.38	0.40

[a] Numbers in parentheses indicate the number of trees sampled at each site.
[b] Seed-cone and needle traits included in each analysis were as follows: combination 2, cone angle, cone curvature, apophysis length, needle-pair length, and number of needle serrations; combination 4, same as combination 2 with the exception of needle serrations.

because it maximizes between-group differences and minimizes within-group differences for the purpose of classification (Adams 1982, McGarigal et al. 2000, Quinn and Keough 2002).

The cone and needle traits identified here could be used to rate introgression (Table 2). To increase efficiency, cone curvature, apophysis and umbo height, and the number of needle serrations could be excluded from stand-level introgression ratings because all trait combinations tested produced similar introgression scores for the sites using both PCA and DFA (Table 3, Figure 3). At the tree level, apophysis and umbo height could be omitted, as it had little effect on introgression ratings (maximum 0.03 and 0.07 change for DFA and PCA, respectively, for an individual tree). Excluding needle serrations, the most laborious trait to measure, changed some individual tree ratings substantially (e.g., 0.36 and 0.15 for DFA and PCA, respectively). Therefore, omitting this trait when tree-level comparisons are the goal should be considered only when samples sizes are large enough to negate the potential impact of a small percentage of the trees.

The constants and coefficients from the DFA presented in Table 3 can be used by other researchers to produce introgression ratings for individual trees that are lodgepole-jack pine hybrids or back-crosses. For example, using discrimination function 1 as an example, other users could calculate an introgression rating (IR) by inserting their observed values into the following equation: IR = 30.634 + 0.039 (Observed cone angle) − 0.124 (Observed cone curvature) − 2.375 (Observed apophysis length) − 0.467 (Apophysis and umbo height) + 0.343 (Observed needle-pair length) − 0.462 (Observed number of needle serrations). Note that ratings can be standardized against a specific population (e.g., known pure parent population) if desired, as described in the Methods.

The ratings will be relative to the two end points used in this study: lodgepole pine from Lac le Jeune, British Columbia, and jack pine from Shellbrook, Saskatchewan. Given the great morphological differences between the pine species, these endpoints, or any selected allopatric endpoints, should be sufficient to produce relative ratings. We standardized the ratings against the mean for lodgepole pine to facilitate interpretation; however, the ratings are not absolute measures or proportions of genetic composition. A tree with a rating of 0.60 should be interpreted as having more jack pine-like characteristics than a tree with a rating of 0.30. To increase effi-ciency, reduced trait combinations may be used depending on whether the goal is tree- or stand-level ratings (Table 3).

Seed-cone and needle traits are a diagnostic and practical method for identification of pine hybrid populations (Rweyongeza et al. 2007). In addition to providing a relative rating of introgression, a morphological index may be more feasible and accessible for some researchers than genetic analysis. Introgressive hybridization may influence interactions between host trees and other organisms, such as insects and diseases. A method for rating introgression at the individual tree level will facilitate studies on species interactions and aid in unraveling the potentially confounding effects of site on the organisms involved in these interactions. Our next step will be to use this approach to examine host species and introgression effects on the behavior and success of the mountain pine beetle in northern Alberta, where it has recently invaded the lodgepole-jack pine hybrid zone.

Literature Cited

ADAMS, R.P. 1982. A comparison of multivariate methods for the detection of hybridization. *Taxon* 31:646–661.

ANDERSON, E. 1949. *Introgressive hybridization.* John Wiley and Sons, Inc., New York, NY. 109 p.

CRITCHFIELD, W.B. 1985. The late Quaternary history of lodgepole and jack pines. *Can. J. For. Res.* 15:749–772.

FARRAR, J.L. 1995. *Trees in Canada.* Fitzhenry & Whiteside Ltd. and Natural Resources Canada, Ottawa, ON, Canada. 502 p.

FRITZ, R.S., C.M. NICHOLS-ORIANS, AND S.J. BRUNSFELD. 1994. Interspecific hybridization of plants and resistance to herbivores: Hypotheses, genetics, and variable responses in a diverse herbivore community. *Oecologia* 97:106–117.

MCGARIGAL, K., S. CUSHMAN, S., AND S. STAFFORD. 2000. *Multivariate statistics for wildlife and ecology research.* Springer, New York, NY. 283 p.

MOSS, E.H. 1949. Natural pine hybrids in Alberta. *Can. J. Res. Sect. C* 27:218–229.

QUINN, G.P., AND M.J. KEOUGH. 2002. *Experimental design and data analysis for biologists.* Cambridge University Press, New York, NY. 537 p.

RICE, A.V., M.N. THORMANN, AND D.W. LANGOR. 2007a. Mountain pine beetle associated blue-stain fungi cause lesions on jack pine, lodgepole pine, and lodgepole × jack pine hybrids in Alberta. *Can. J. Bot.* 85:307–315.

RICE, A.V., M.N. THORMANN, AND D.W. LANGOR. 2007b. Virulence of, and interactions among, mountain pine beetle associated blue-stain fungi on two pine species and their hybrids in Alberta. *Can. J. Bot.* 85:316–323.

RUDOLPH, T.D., AND C.W. YEATMAN. 1982. *Genetics of jack pine.* US For. Serv. Res. Pap. WO-38. 60 p.

RWEYONGEZA, D.M., N.K. DHIR, L.K. BARNHARDT, C. HANSEN, AND R.C. YANG. 2007. Population differentiation of the lodgepole pine (*Pinus contorta*) and jack pine (*Pinus banksiana*) complex in Alberta: Growth, survival, and responses to climate. *Can. J. Bot.* 85:545–556.

SAFRANYIK, L., AND A.L. CARROLL. 2006. The biology and epidemiology of the mountain pine beetle in lodgepole pine forests. P. 3–66 in *The mountain pine beetle: A synthesis of biology, management and impacts on lodgepole pine*, Safranyik, L., and B. Wilson (eds.). Natural Resources Canada, Victoria, BC, Canada. 304 p.

WAGNER, D.B., G.R. FURNIER, M.A. SAGHAI-MAROOF, S.M. WILLIAMS, B.P. DANCIK, AND R.W. ALLARD. 1987. Chloroplast DNA polymorphisms in lodgepole and jack pines and their hybrids. *Proc. Natl. Acad. Sci. U.S.A.* 84:2097–2100.

WHEELER, N.C., AND R.P. GURIES. 1982. Population structure, genetic diversity and morphological variation in *Pinus contorta* Dougl. *Can. J. For. Res.* 12:595–606.

WHEELER, N.C., AND R.P. GURIES. 1987. A quantitative measure of introgression between lodgepole and jack pines. *Can. J. Bot.* 65:1876–1885.

WHITHAM, T.G. 1989. Plant hybrid zones as sinks for pests. *Science* 244:1490–1493.

WU, H.X., AND C.C. YING. 1998. Stability of resistance to western gall rust and needle cast in lodgepole pine provenances. *Can. J. For. Res.* 28:439–449.

WU, H.X., C.C. YING, AND J.A. MUIR. 1996. Effect of geographic variation and jack pine introgression on disease and insect resistance in lodgepole pine. *Can. J. For. Res.* 26:711–726.

YANG, R.C., Z. YE, AND Y. HIRATSUKA. 1999. Susceptibility of *Pinus contorta–Pinus banksiana* complex to *Endocronartium harknessii*: Host–pathogen interactions. *Can. J. Bot.* 77:1035–1043.

YANG, R.C., N.K. DHIR, F.C. YEH, AND Y. HIRATSUKA. 1997. Geographic variability in susceptibility of Alberta lodgepole pine to western gall rust. *Can. J. For. Res.* 27:1398–1405.