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ARTICLE

Spatial and genetic structure of the lodgepole × jack pine hybrid zone[1]

Ian Burns, Patrick M.A. James, David W. Coltman, and Catherine I. Cullingham

Abstract: In north-central Alberta, lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia*) and jack pine (*Pinus banksiana* Lamb.) form a mosaic hybrid zone, the spatial extent of which remains poorly defined. We sought to refine the genetic and geographic distribution of this hybrid zone in western North America to provide information important in predicting future risk of mountain pine beetle (*Dendroctonus ponderosae* Hopkins) outbreaks. We used 29 single nucleotide polymorphism (SNP) markers to discriminate lodgepole pine, jack pine, and their hybrids. We compared and contrasted spatial patterns of hybridization in northern and southern forest zones based on the colonization history of the two species. We found that patterns of introgression were more similar between the zones than expected by chance, but there were significant differences between these regions at specific loci. Using logistic regression, we created a robust predictive model to distinguish among lodgepole pine, jack pine, and their hybrids using a combination of geographic and environmental predictors. Using model selection based on Akaike information criterion, we found that location, elevation, and moisture are important predictors for species class. Quantification of the genetic differences between these two regions, combined with an accurate model for predicting the spatial distribution of lodgepole pine, jack pine, and their hybrids, provides essential information for continued effective management of forest resources.

Key words: jack pine, lodgepole pine, mountain pine beetle, hybridization, distribution modelling.

Résumé : Dans le centre-nord de l'Alberta, le pin tordu latifolié (*Pinus contorta* Dougl. ex Loud. var. *latifolia*) et le pin gris (*Pinus banksiana* Lamb.) forment une zone caractérisée par une mosaïque d'hybrides dont l'étendue spatiale reste mal définie. Nous avons cherché à raffiner la répartition génétique et géographique de cette zone d'hybrides située dans l'ouest de l'Amérique du Nord afin de fournir des informations importantes pour prédire le risque futur d'épidémie de dendroctone du pin ponderosa (*Dendroctonus ponderosae* Hopkins). Nous avons utilisé 29 marqueurs SNP pour distinguer le pin tordu latifolié, le pin gris et leurs hybrides. Nous avons comparé et distingué les patrons spatiaux d'hybridation dans les zones forestières septentrionale et méridionale à partir de l'histoire de la colonisation des deux espèces. Les patrons d'introgression entre les zones étaient plus similaires que prévu par le hasard, mais il y avait des différences significatives entre ces régions pour certains loci. À l'aide de la régression logistique, nous avons construit un modèle prédictif robuste pour distinguer le pin tordu latifolié, le pin gris et leurs hybrides en utilisant une combinaison de variables prédictives de nature géographique et environnementale. Grâce à une sélection de modèles basés sur le critère d'information d'Akaike (AIC), nous avons constaté que la localisation, l'altitude et l'humidité sont des prédicteurs importants pour la classe d'espèce. La quantification des différences génétiques entre ces deux régions, associée à un modèle précis de prédiction de la répartition spatiale du pin tordu latifolié, du pin gris et de leurs hybrides, permet de fournir une information essentielle pour que la gestion des ressources forestières demeure efficace. [Traduit par la Rédaction]

Mots-clés : pin gris, pin tordu latifolié, dendroctone du pin ponderosa, hybridation, modélisation de la répartition.

Introduction

The Canadian boreal forest extends from the Yukon and northern British Columbia through to the east coast of Canada (Critchfield 1985). It comprises Canada's primary source of timber (Brandt et al. 2013) and includes multiple species of pine, including lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia*) and jack pine (*Pinus banksiana* Lamb.). Lodgepole and jack pine differ in their ecological preferences, varying across elevation, climate (Carlson et al. 1999), soil, and shade tolerance (McLeod and MacDonald 1997). Lodgepole and jack pine are sister species (Wheeler et al. 1983; Eckert and Hall 2006) and their divergence time has been estimated to be within the Pleistocene at ~500 000 years BP (Dancik and Yeh 1983), with some data suggesting pre-Pleistocene divergence (Eckert and Hall 2006). The current spatial distributions of these two species can be explained by their respective recolonization routes following retreat of the glacial ice (~12 000 years B.P.). Lodgepole pine migrated northward into British Columbia (MacDonald and Cwynar 1985), east into Alberta ~10 000 years B.P., and eventually northwest into northern British

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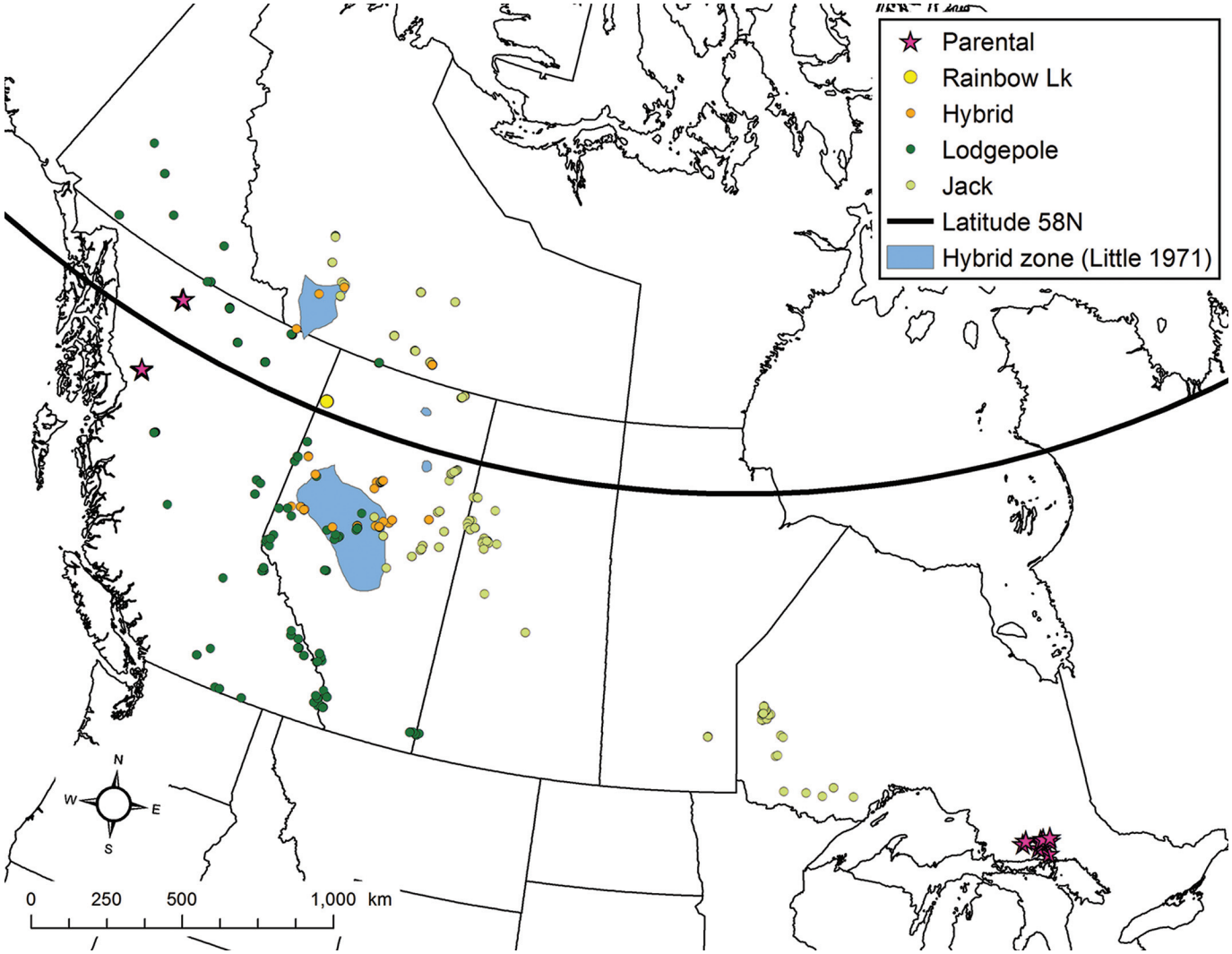
Fig. 1. Distribution of lodgepole pine, jack pine, and hybrid samples. The potential northern and southern hybrid zones predicted by the Little (1971) range maps are included for perspective. The dividing line of 58°N, which was used to test for different patterns of introgression between the two regions of contact between lodgepole pine and jack pine, is indicated. Individuals used to estimate parental allele frequencies for this analysis are indicated by star symbols.



Columbia and the Yukon. Jack pine migrated westward from Quebec and the western Appalachians, eventually reaching Alberta ~8000 years B.P. (Godbout et al. 2005), and slowly migrated northward reaching current latitudes by ~4000 years B.P. Evidence from the fossil record would suggest that two distinct contact zones formed in succession as these species expanded their ranges (McLeod and MacDonald 1997), first in central Alberta and then in northern Alberta and the Northwest Territories.

The most-complete distribution map of lodgepole and jack pine is from Little (1971). This map was created from a combination of site surveys of morphological traits and predictions. While Little (1971) acknowledges the existence of a hybrid zone between lodgepole and jack pine and shows an overlap in their species ranges in central Alberta and southeastern Northwest Territories, the spatial extent of the hybrid zone remains poorly defined. Since 1971, mitochondria (mt) and chloroplast DNA markers have been used to investigate this hybrid zone (Dong and Wagner 1993; Godbout et al. 2012), revealing evidence for hybridization in central Alberta and lodgepole pine introgression in Saskatchewan. Cullingham et al. (2012) investigated the hybrid zone more thoroughly using microsatellite resources. They developed a distribution model using the genetic data and found that the hybrid zone was more extensive than Little (1971) predicted but did not find any evidence of lodgepole pine introgression in eastern Alberta (Saskatchewan was not sampled extensively enough to test for introgression). Their sampling, similar to Dong and Wagner (1993) and Godbout et al. (2012), did not extend into the approximated northern hybrid zone identified in the Little (1971) range maps (Fig. 1), and information on the distributions of lodgepole and jack pine in this northern region remains limited. Improved characterization of the species distributions and the lodgepole × jack pine hybrid zone is essential to understanding future mountain pine beetle (MPB; *Dendroctonus ponderosae* Hopkins) epidemic spread risk and for guiding proactive management and silvicultural interventions aimed at reducing this risk.

Mountain pine beetle population dynamics are characterized by quasi-cyclic episodic outbreaks followed by endemic periods of low population density (Safranyik and Carroll 2006). Epidemic phases result in landscape-level mortality of host trees and historically have been limited in spatial scale, with localized economic impacts (Taylor and Carroll 2003; Fettig et al. 2014). The spatial extent and impact of the most recent epidemic phase of MPB growth and range expansion have been substantially greater than those previously recorded. From 1999 to 2014, MPB range ex-

panded from southern British Columbia across the Rocky Mountains into the lowlands of Alberta and has continued north and east towards the Northwest Territories and Saskatchewan, respectively (Cullingham et al. 2011; Dhar et al. 2016; Cooke and Carroll 2017). This progression was well publicized as it resulted in the loss of over 17 million hectares of lodgepole pine forest in Canada (Walton 2012; Corbett et al. 2016). With demonstrated successful colonization of jack pine, MPB could now be considered a native invasive species (Cullingham et al. 2011). The presence of a hybrid zone between lodgepole and jack pine in Alberta may have facilitated this range expansion via mechanisms proposed in the "hybrid bridge hypothesis" (Floate and Whitham 1993). This hypothesis suggests that hybrids fill the morphological or genetic gaps between the pure species. This hypothesis has been invoked to describe host–pest interactions between gall aphids and *Populus* spp. in which pests were able to transfer to backcrossed hybrids of their host species (Floate and Whitham 1993; Floate et al. 2016). These dynamics have also been described in plant–pathogen complexes (reviewed by Stukenbrock 2016) in which hybridization of plant hosts can promote the transfer of fungal pathogens. Although in situ examples of host-range expansion of plant pests via a hybrid bridge are rare (Pilson 1999), historically and spatially extensive hybridization of lodgepole and jack pine would suggest that range expansion of the MPB could be a consequence of a hybrid bridge of these two allopatric pine species.

In this study, we sought to fully characterize the current hybrid zone between lodgepole and jack pine using genetic data. To do so, we first examine the genetic characteristics of the hybrid zone to determine if there are differences in genetic introgression between the two hybrid zones, which we will define as north and south. Next, we use spatial information and climatic predictors to model genetic ancestry using a spatial logistic regression model. Using this fitted model, we predict the fine-scale distribution of lodgepole pine, jack pine, and their hybrids across much of their Canadian distribution, including the northern extent of their range, which has not been examined previously. This work is part of the TRIA-Net project (http://tria-net.srv.ualberta.ca/), and the overarching goals of the project are to better understand the MPB system given exposure to a novel environment and develop tools for industry and government to improve forest management. The development of a distribution model for lodgepole pine, jack pine, and their hybrids will provide essential information regarding the processes and patterns that determine lodgepole and jack pine species occurrence in western Canada and will allow for improved management of these important forest resources under threat of MPB spread.

Methods

Sample collection

We collected pine needles from individual pine trees in a total of 61 pine stands in British Columbia (18 stands, $n = 240$), Alberta (20 stands, $n = 426$), Saskatchewan (2 stands, $n = 41$), Manitoba (1 stand, $n = 10$), Ontario (4 stands, $n = 46$), Yukon (7 stands, $n = 43$), and Northwest Territories (9 stands, $n = 151$) for a total of 957 samples (Fig. 1). Samples collected from Wood Buffalo National Park were completed under permit WB-2015-19658. For a summary of sample collection see Supplementary Table S1[2]. DNA was extracted from 783 individuals for this study; 174 had been previously genotyped (Cullingham et al. 2013*a*, 2013*b*). Geographic locations of all sampled trees were recorded using Garmin GPS units (Garmin International, Olathe, Kansas, USA). Needles were stored on ice in coolers and then transferred to storage at –20 °C or –80 °C until DNA extraction was performed.

Of those 61 stands, we extracted DNA for six Yukon stands from lab-grown seedlings (23 individuals). Seeds were obtained from the National Tree Seed Centre (Natural Resources Canada) from bulk seed lots (multiple trees). Seeds were sterilized using Tween-20 and 20% bleach before stratification in autoclaved seedbeds built from 10 μL micropipette tip boxes and Kimpads. Twenty seeds were germinated in each seedbed for a 12 h light – 12 h dark cycle at 25 °C and 75% humidity. Seedlings were harvested when the megagametophyte could be removed easily from the seedling. Seedlings were manually ground with a pestle in individual 1.2 mL tubes of 96-well extraction plates.

DNA extraction

To prepare pine needle tissue for extraction, needles were chopped and ground into a fine powder using a Retsch MM301 mixer mill. We extracted DNA from pine needle and pine seedling tissues using a hexadecyltrimethylammonium bromide (CTAB) method optimized for pine by Roe et al. (2010). We modified Roe et al.'s (2010) method in three ways: (*i*) we expanded the procedure to permit use of 96-well collection microtube plates for higher throughput; (*ii*) samples were inverted every 30 min for the 2 h, 65 °C incubation; and (*iii*) all centrifugation steps were performed at 6000*g*. We re-suspended pellets in 100 μL of nuclease-free water. DNA was quantified using a NanoDrop 2000 (Thermo Scientific). Only samples with a concentration greater than 20 ng·μL^{-1} and 260/280 ratio values of 1.7 to ≤2.1 were retained for genotyping.

Single nucleotide polymorphism (SNP) selection and characterization

Samples were typed at 29 single nucleotide polymorphism (SNP) loci (Table 1) previously determined to be completely discriminating (9 SNPs) or highly differentiated (20 SNPs) between lodgepole and jack pine (Cullingham et al. 2013*a*, 2013*b*). Annotation was performed by Cullingham et al. (2014) through comparison with multiple databases: NCBI's non-redundant protein filtered for plant taxa; TAIR9 (Lamesch et al. 2012); and the Arborea white spruce gene catalogue (Rigault et al. 2011). SNP typing was performed at Delta Genomics using the Sequenom system (Gabriel et al. 2009).

We calculated diversity measures with GenAlEx ver. 6.51 (Peakall and Smouse 2012), which uses χ^2 tests for allelic diversity measures. Lodgepole pine, jack pine, and their hybrids were assessed separately, once defined, for the following characteristics: unbiased estimate of expected heterozygosity (H_E), observed heterozygosity (H_O), fixation index (F), and the Hardy–Weinberg equilibrium (HWE). We assessed linkage disequilibrium (LD) at each locus for lodgepole pine, jack pine, and their hybrids separately using composite measure of LD in GENEPOP version 4.2 (http://genepop.curtin.edu.au), with a Markov chain dememorization of 10 000 and 10 000 bootstrapping repetitions for significance testing (Raymond and Rousset 1995). We assessed significance with Benjamini–Hochberg False Discovery Rate (BH FDR) corrected alpha values (Benjamini and Hochberg 1995).

Hybrid identification

To identify hybrid ancestry, STRUCTURE ver. 2.3.4 (Pritchard et al. 2000) was used. This program implements a Bayesian admixture model to estimate Q values or admixture proportions for individuals from K number of populations, where K is defined by the user. We ran STRUCTURE for $K = 2$ using the following parameters: burn-in of 50 000, 500 000 MCMC steps for data collection, admixture, and using a correlated allele frequency model (which assumes independence of all samples, a conservative model). This analysis was performed first for all samples to identify "pure" lodgepole pine ($Q > 0.9$) or jack pine ($Q < 0.1$), with intermediates being considered

[2]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfr-2018-0428.

Table 1. SNP loci, their annotations as determined from transcriptome sequencing, previously published in Cullingham et al. (2014), and patterns of introgression.

Locus	Annotation	All samples	Northern	Southern
C17954-P346	Photosynthetic electron transfer A (chloroplast)	NA	NA	NA
C26372-P562	Calcium-dependent lipid binding (CaLB domain) family protein	Neutral	Neutral	Neutral
C35213-P325	Eukaryotic aspartyl protease family protein	Neutral	Neutral	Neutral
C39371-P429	**Protein of unknown function (DUF3353)**	***High**	***High**	***High**
C52254-P578	Photosystem I PsaA/PsaB protein (chloroplast)	*No hets	*No hets	*No hets
C54523-P103	Translation protein SH3-like family	Neutral	Neutral	Neutral
C55350-P439	Chaperone protein DnaJ-related	*Lodge	*Lodge	Neutral
C55378-P723	Transcription factor jumonji domain-containing protein	Neutral	Neutral	Neutral
C55401-P415	Transcribed locus	Neutral	Neutral	Neutral
C63961-P710	Manganese transport protein MntH	*High/Lodge	*High/Lodge	*High/Lodge
C64907-P190	Thioredoxin superfamily protein	Neutral	Neutral	Neutral
C66807-P512	Beta-amylase/glycosyl hydrolase family 14	*Lodge	Neutral	*Lodge
C84852-P331	CRAL/TRIO domain/Sep14p-like phosphatidylinositol transfer family protein	*Jack	Neutral	*Jack
C85320-P102	DEK domain-containing chromatin associated protein	*Low/Jack	Neutral	*Low/Jack
C85506-P364	Transcribed locus	*High/Lodge	Neutral	*High/Lodge
JpLpc04112p131	SSXT family protein	*Jack	Neutral	Neutral
JpLpc36252p1327	Histone chaperone/global transcription factor C	Neutral	Neutral	Neutral
JpLpc39993p867	Uncharacterized conserved protein (DUF2358)/SnoaL-like domain	Neutral	Neutral	*Lodge
JpLpc41319p340	Uncharacterized BCR, YbaB family COG0718	Neutral	Neutral	Neutral
JpLpc44782p470	KNOX1/2 domain/KNOTTED-like	Neutral	Neutral	Neutral
JpLpc45225p571	B-cell receptor-associated protein 31-like	Neutral	Neutral	Neutral
JpLpc47089p1831	Dof-type zinc finger DNA-binding family protein	*High/Lodge	Neutral	*High/Lodge
JpLpc47778p1036	Chlorophyll A–B binding family protein	*Low/Jack	*Low/Jack	*Low/Jack
JpLpc50195p453	Complex I subunit	*Low/Jack	Neutral	*Low/Jack
JpLpc66545p1207	**Transcribed locus**	**Neutral**	***Low/Jack**	**Neutral**
JpLpc86157p398	RNA recognition motif/SC35-like splicing factor 28	*Jack	*Jack	*High
Lp-C45579-P117	Myb-like HTH transcriptional regulator family protein	Neutral	Neutral	Neutral
Lp_c00150p459	Circadian clock associated 1	Neutral	Neutral	Neutral
Lp_c12025p1415	Core-2/I-branching beta-1,6-*N*-acetylglucosaminyltransferase family protein	*Low	*Low/Jack	*Low

Note: Three analyses were completed: "All samples" includes the study wide patterns; "Northern" includes only those sampling regions north of 58° latitude; "Southern" includes all study areas south of 58° latitude including Rainbow Lake. For the patterns, "High" and "Low" indicate heterozygotes are over- or under-represented, respectively. An asterisk (*) indicates significant deviation from neutral introgression ($p < 0.001$). Loci in boldface type indicate significant differences between northern and southern genomic clines. We did not test introgression patterns for chloroplast loci because there are only homozygotes. "No hets" indicates no heterozygous individuals were identified because they are chloroplast markers.

as hybrids (Cullingham et al. 2011), and then again for each group separately using the same set of parameters as above. Separate STRUCTURE analyses were performed to rule out conflicting admixture from inclusion of shore pine (*Pinus contorta* Dougl. ex Loud. var. *contorta*), a subspecies of lodgepole pine coincident with lodgepole pine stands in western British Columbia, and to rule out possible substructuring in jack pine across the large species distribution. Jack pine stands are located over a wide range of environments, and though it has not been examined specifically, substructure may be possible due to the large geographic range included.

We used parental allele frequencies from the pure species to identify hybrid classes for sampled individuals to discern the age and dynamics of the hybrid zone. We used the program NEWHYBRIDS (Anderson and Thompson 2002) to assign individuals to three generations of crosses: first- and second-generation hybrids (F1 and F2, respectively) and hybrids backcrossed with their parental species, including F1 × lodgepole pine, F1 × jack pine (B1L and B1J, respectively), B1L × lodgepole pine, B1J × jack pine (B2L and B2J, respectively), B1L × F2 and B1J × F2, and B2L × lodgepole pine and B2J × jack pine. NEWHYBRIDS uses a Bayesian method similar to STRUCTURE to generate genetic heritage proportions. Samples were run with a burn-in of 50 000 and a data collection of 500 000. We then compared the composition of hybrids between the northern and southern hybrid zones.

Introgression analysis

We estimated introgression for each marker using the program INTROGRESS (Gompert and Buerkle 2010). Hybrid index values were calculated for every individual at each of the 29 loci (Supplementary Fig. S1[2]). INTROGRESS requires a priori parental populations to estimate a hybrid index for each individual (Buerkle 2005). Fifteen individuals each from two sites in British Columbia and 30 individuals from Ontario (Temiscaming and Algonquin) were selected to cover the parental ranges of lodgepole and jack pine, respectively (Fig. 1). Two loci had ~20% missing data, as SNP typing on previous samples was performed at 27 of 29 loci. The two loci with no hybrid individuals (C17954-P346 and C52254-P578) are chloroplast loci and only one was included in the introgression analysis as they are linked.

INTROGRESS also estimates genomic clines for each locus using multinomial regressions, which estimate the effect of a given genotype at each locus on genome-wide admixture (Gompert and Buerkle 2009). Deviations likely indicate selective forces acting on the locus or closely linked regions (Gompert and Buerkle 2009). Genomic clines and hybrid indices were calculated twice: first jointly to investigate introgression across the entire hybrid zone, and then in latitude-separated groups to investigate the potential differences between the two contact zones. For this, samples were divided by 58° latitude into northern and southern groups (Fig. 1). Rainbow Lake (north of 58°) is not continuous with the northern hybrid zone, so sensitivity analysis was used to determine if a change in grouping would result in a change in hybrid index values. There was no significant difference in hybrid indices; therefore, Rainbow Lake was included as a southern stand. For each analysis, genomic clines were estimated for 1000 permutations using the parametric approach.

To determine if genomic clines of the northern and southern hybrid zones were significantly different overall, we used the "compare.clines" function in INTROGRESS to determine the cline similarity across loci between the two hybrid zones. We then used a

binomial test to determine if our observations were different from that expected at random, where we would expect the probability (p) of a locus as having the same cline between the two regions as 0.5. The observed number of loci showing the same cline (k) was compared with the probability of observing the same or fewer loci with the same cline. This was estimated from the cumulative probability distribution with a success rate of p over n loci.

Distribution modelling

We sought to build a spatial predictive model for the occurrence of lodgepole pine, jack pine, and their hybrids using logistic regression. In this model, Q values derived from STRUCTURE for all individuals were used as the response variable. Potential predictors included 25 spatial and annual climate variables, including those related to elevation, moisture, temperature, and precipitation (Supplementary Table S3[2]). All climate data were derived from ClimateNA v5.10 (Wang et al. 2016), available at http://tinyurl.com/ClimateNa, and from Environment Canada (Supplementary Table S3[2]). Environmental variables were selected for their demonstrated relationship with pine growth in provenance studies (Yeatman and Teich 1969).

Prior to fitting our model, we examined the Pearson correlation between all pairs of predictors. When a correlation greater than 0.7 was identified, the variable in the pair with the greatest variance inflation factor (VIF) was removed. The final set of potential predictors represented a subset of 10 variables, all of which exhibited correlation less than 0.7. We then sought to identify the "best" model describing pine species occurrence based on minimization of Akaike information criterion (AIC) through stepwise variable selection. Once we had identified a candidate model on the basis of AIC, we examined the VIF associated with each retained predictor and iteratively removed predictors with a VIF > 5 (Zurr et al. 2010). High VIFs can indicate multicollinearity between predictors and can result in overfit models and biased coefficient estimates. VIFs were calculated in R using the "car" package version 2.1-6 (Fox and Weisberg 2011).

Once the model was identified based on our AIC and VIF criteria, we assessed model performance using the receiver operator curve (ROC) and the associated area under the curve (AUC) statistics. ROC is a method of k-fold cross-validation that assesses the predictive capacity of a model using new data. ROC analysis was undertaken using a split of 60 training to 40 testing with 1000 bootstrapped replicates. The resulting "confusion matrix" of true and false positives, as well as true and false negatives, were used to generate the ROC plot of the true positive rate vs. the false positive rate for varying threshold levels of classification for a true positive. We generated an ROC curve for each bootstrap replicate and calculated an average AUC for all ROCs associated with a single model. ROC analysis was undertaken using the package "ROCR" version 1.0-7 (Sing et al. 2005) in R.

We used the selected model to spatially predict species distribution over the extent of our study area. We extracted climate data for model predictors in ArcMap10.5 using the centroids of a 10 km grid across the study area and our model-generated pine predictions for each location. We classified predicted probabilities output from our selected regression model such that predicted values <0.1 indicated the presence of jack pine and values >0.9 indicated the presence of lodgepole pine; intermediate values indicated the presence of hybrids (Cullingham et al. 2011). The resulting predictive layer was masked to include only those regions where pine is found using pine distribution data from Yemshanov et al. (2012) and R. Legare (Energy Mines and Resources, Yukon) using the Spatial Analyst toolbox in ArcMap10.5.

Results

Genotyping

We genotyped 783 new samples using Sequenom with >90% sequencing success. These new data were combined with 174 previously genotyped samples (Cullingham et al. 2013*a*, 2013*b*) for a total of 957 pine samples. Five loci each for lodgepole and jack pine were out of HWE with significant heterozygote deficit, except for one locus in jack pine with heterozygote excess. Significant LD was present at 11 locus pairs in lodgepole pine, six locus pairs in jack pine, and almost all locus pairs for hybrid individuals. A summary of diversity measures at each locus for all individuals and for lodgepole and jack pine separately can be seen in Table 2.

Hybrid identification

Preliminary analyses using STRUCTURE identified two distinct groups with a range of admixture proportions at $K = 2$. No additional structure was identified when lodgepole or jack pine individuals were analyzed separately. Of 957 individuals, 379 were assigned as jack pine ($Q > 0.9$), 436 as lodgepole pine ($Q < 0.1$), and 142 as hybrids ($0.1 \leq Q \leq 0.9$). Assignments from NEWHYBRIDS were similar to our structure results, with a greater number of hybrids assigned (347 jack pine, 393 lodgepole pine, and 217 hybrids); the mismatches between the two programs were all individuals with split assignment across backcrossed categories. Based on the proportion of ancestry for each of the hybrid classes (Table 3), there were no early generation hybrids identified, and there were no differences between the northern and southern hybrid zones.

Introgression analysis

Genomic clines were estimated with INTROGRESS using multinomial regressions; of the 28 loci examined, 14 showed significant deviations from neutral expectations across the entire dataset (Table 1; Supplementary Fig. S2[2]). Patterns of introgression were similar across all three datasets (all, north, and south). When comparing the northern and southern zones, two loci had significantly different patterns of introgression ($p < 0.05$). This is significantly less than you would expect if the two zones were independent ($p < 0.001$). If the two zones were independent, you would expect 19 or more loci to be significantly different. The two loci were C39371-P429 and JpLpc66545p1207, both proteins of unknown function (Table 1).

Distribution modeling

Our final selected model of pine ancestry included the physical variables of elevation, latitude, and longitude, as well as the climatic variables climate moisture index (CMI) and summer heat moisture (SHM) (Table 4). In evaluating model performance using ROC, we determined that our model had a high degree of predictive accuracy (mean AUC = 0.94; Fig. 2). Species prediction with the model coincided well with previous estimates of pure distributions, while the range of hybrids is greater than previously determined by Little (1971) (Fig. 3).

Discussion

This study aimed to characterize the pine hybrid zone in western Canada and expand upon research into the distribution of pine species based on improved sampling across the range, especially in the northern and eastern ranges of lodgepole and jack pine distributions. We found evidence of the northern and southern hybrid zone described in Little (1971) and that they are more similar than expected by chance, which suggests that parallel selection may be acting across the hybrid zone in western Canada. While the zones have similar patterns of introgression overall, they are not identical on a locus by locus basis, and significant differences in clines at two loci suggest that individuals in either zone may be responding differently to selective pressures, which

Table 2. Diversity measures for 29 SNP loci across all samples of lodgepole pine, jack pine, and their hybrids ("All"), as well as estimates for the pure species.

	All				Jack pine				Lodgepole pine			
Locus	*N*	H_O	H_E	*F*	*N*	H_O	H_E	*F*	*N*	H_O	H_E	*F*
C17954-p346*	954	0.000	0.496	1.000	378	0.000	0.000	NA	436	0.000	0.000	NA
C26372-P562	954	0.127	0.494	0.743	378	0.114	0.112	–0.016	434	0.035	0.034	–0.018
C35213-P325	952	0.112	0.499	0.775	373	0.067	0.070	0.039	436	0.087	0.083	–0.046
C39371-P429	905	0.382	0.463	0.174	356	**0.612**	**0.428**	**–0.434**	417	0.113	0.106	–0.060
C52254-P578*	953	0.000	0.496	1.000	375	0.000	0.000	NA	436	0.000	0.000	NA
C54523-P103	956	0.086	0.499	0.828	378	0.040	0.039	–0.020	435	**0.034**	**0.034**	**–0.018**
C55350-P439	956	0.131	0.489	0.733	377	0.178	0.166	–0.069	436	0.023	0.023	–0.012
C55378-P723	955	0.108	0.498	0.783	378	0.050	0.054	0.069	434	0.060	0.058	–0.031
C55401-P415	957	0.119	0.498	0.761	378	0.063	0.067	0.044	436	0.067	0.064	–0.034
C63961-P710	956	0.159	0.495	0.679	378	0.159	0.160	0.004	436	0.071	0.077	0.077
C64907-P190	957	0.104	0.494	0.788	378	0.114	0.112	–0.016	436	0.011	0.011	–0.006
C66807-P512	957	0.103	0.491	0.789	378	0.122	0.119	–0.023	436	0.014	0.014	–0.007
C84852-P331	947	0.079	0.498	0.841	372	0.051	0.050	–0.026	432	0.019	0.018	–0.009
C85320-P102	956	0.060	0.499	0.880	378	0.026	0.026	–0.013	435	**0.005**	**0.014**	**0.664**
C85506-P364	764	0.136	0.500	0.728	351	**0.128**	**0.149**	**0.141**	308	0.023	0.023	–0.011
JpLpc04112p131	957	0.089	0.499	0.822	378	0.034	0.034	–0.017	436	0.046	0.045	–0.023
JpLpc36252p1327	953	0.118	0.499	0.764	377	0.064	0.062	–0.033	435	0.064	0.062	–0.033
JpLpc39993p867	957	0.159	0.493	0.678	378	0.161	0.179	0.097	436	0.050	0.049	–0.026
JpLpc41319p340	956	0.100	0.499	0.799	377	**0.056**	**0.059**	**0.058**	436	**0.039**	**0.051**	**0.241**
JpLpc44782p470	953	0.185	0.478	0.614	378	0.288	0.304	0.050	432	0.019	0.018	–0.009
JpLpc45225p571	957	0.146	0.493	0.703	378	0.153	0.177	0.131	436	0.041	0.045	0.079
JpLpc47089p1831	956	0.152	0.485	0.687	378	0.190	0.198	0.036	435	0.014	0.014	–0.007
JpLpc47778p1036	745	0.068	0.475	0.856	374	0.005	0.005	–0.003	269	**0.074**	**0.119**	**0.372**
JpLpc50195p453	864	0.109	0.500	0.782	351	**0.034**	**0.039**	**0.125**	403	0.087	0.097	0.101
JpLpc66545p1207	949	0.095	0.498	0.809	374	0.048	0.047	–0.025	434	0.028	0.027	–0.014
JpLpc86157p398	953	0.137	0.500	0.725	378	0.053	0.052	–0.027	432	0.120	0.129	0.069
Lp-C45579-P117	957	0.138	0.488	0.717	378	0.188	0.187	–0.004	436	**0.016**	**0.020**	**0.214**
Lp_c00150p459	948	0.162	0.476	0.658	371	0.261	0.281	0.067	435	0.018	0.018	–0.009
Lp_c12025p1415	955	0.177	0.497	0.644	378	**0.040**	**0.044**	**0.097**	434	0.242	0.237	–0.023

Note: Two chloroplast loci with no heterozygotes are marked with an asterisk (*). *N*, number of individuals sampled at each locus; H_O, observed heterozygosity; H_E, unbiased estimate of expected heterozygosity; *F*, the fixation index. Measures were calculated in GenAlEx 6.51 (Peakall and Smouse 2012). Numbers in boldface type indicate loci out of Hardy–Weinberg equilibrium (HWE); note that all loci were out of HWE when "all" samples were included. NA, estimate cannot be obtained because no heterozygotes were identified (chloroplast markers).

Table 3. Proportion of ancestry assigned by NEWHYBRIDS to each class.

	Proportion of class		
Hybrid class	All	North	South
L	35.65	38.67	34.08
J	40.95	36.13	43.46
F1	0.00	0.00	0.00
F2	0.19	0.14	0.21
B1L	0.02	0.02	0.02
B1J	0.01	0.02	0.01
B2L	6.64	6.44	6.75
B2J	4.92	7.03	3.82
B1LF2	1.45	1.72	1.32
B1JF2	3.99	3.86	4.06
B3L	2.90	3.74	2.46
B3J	3.27	2.24	3.81

Note: The proportions for the entire dataset (All), as well as the northern (North) and southern (South) hybrid zones, are included. Classes: pure lodgepole pine (L), pure jack pine (J), and their hybrids (F1, F2), and backcrossed generations: F1 × lodgepole pine and F1 × jack pine (B1L and B1J, respectively); B1L × lodgepole pine and B1J × jack pine (B2L and B2J, respectively); B1L × F2 and B1J × F2 (B1LF2 and B1JF2, respectively); and B2L × lodgepole pine and B2J × jack pine (B3L and B3J, respectively).

could indicate important adaptive differences between the naïve northern and attacked southern hybrid zones regarding MPB exposure. We did not find any evidence of lodgepole pine introgression in eastern Alberta or Saskatchewan, which has been described previously for mtDNA data (Dong and Wagner 1993; Godbout et al. 2012). Additionally, we have used spatial environmental data to accurately predict pine species class across the landscape and create a new, fine-scale distribution map of lodgepole and jack pine. This new predictive map extends previous genomics-based models of pine species ranges (Cullingham et al. 2012) into the previously unexamined northern distribution. Taken together, we now have a better understanding of the overall structure of the hybrid zone.

Introgression analysis

The process of introgression between species can result in novel assemblages of genes that can promote adaptation, reduce fitness, or any combination between these two extremes (Harrison and Larson 2014). When analyzed as one population, all loci were significantly out of HWE (Table 2), showed heterozygote deficit, and had high fixation indices. This is to be expected as the loci were selected to be species discriminating, but the observed deviations from neutral introgression may indicate that selection is acting at these loci (Gompert and Buerkle 2009). Selection at SNP loci can lead to differential survival of individuals and inheritance of genes (Krehenwinkel and Tautz 2013). Genes of selective benefit are more likely to be passed between hybridizing species, which may result in differential introgression between individuals (Harrison and Larson 2014).

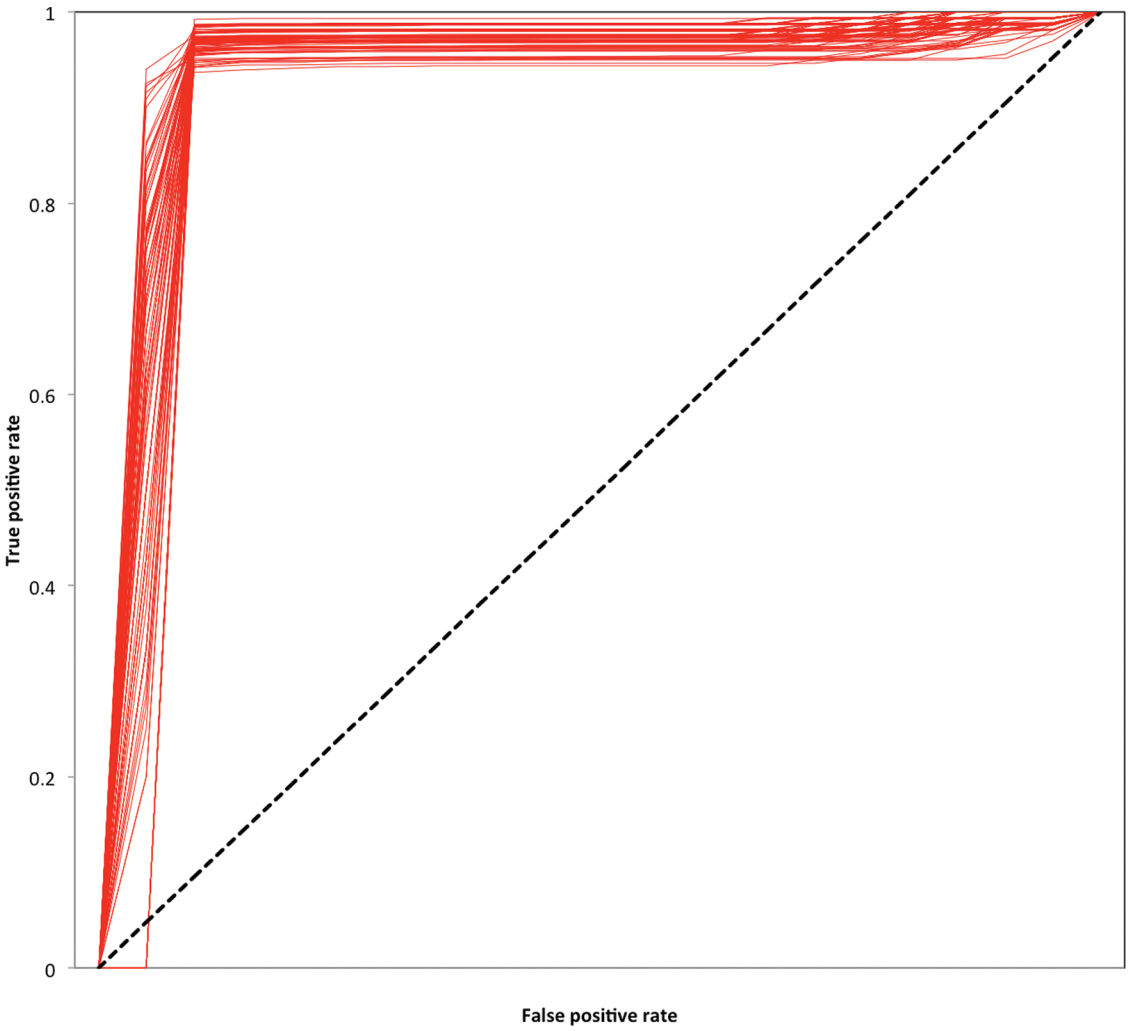
To test the hypothesis that lodgepole and jack pine have formed two genetically distinct hybrid zones, we compared patterns of introgression between the northern and southern regions. We found that the northern and southern hybrid zones were more similar than would be expected if they were independent. This may indicate selection acting in a parallel manner on the two

Table 4. Summary of final logistic model of pine ancestry chosen using a minimization of AIC; *p* values were calculated using a likelihood ratio test.

Predictor	Coefficient	±SE	*p* value	Effect on *p*(Lp)	VIF
Elevation	0.022	0.0009	<0.001	+	4.051
Latitude	−0.414	0.0707	0.025	+	2.591
Longitude	−0.803	0.0163	<0.001	−	2.853
Summer heat moisture	−0.031	0.0093	0.048	+	3.118
Climate moisture	0.105	0.0230	0.005	+	1.004

Note: VIF, the variance inflation factor, which measures correlation between predictors. Effect on *p*(Lp), the direction of the effect on probability of lodgepole pine; the minus sign refers to the probability of jack pine. All predictors listed were significant.

Fig. 2. Receiver operator characteristic (ROC) curves for cross-validation tests of model predictive success using 60% training and 40% testing datasets. One-hundred bootstrapped replicates are shown out of a total of 1000 replicates each. Average area under the curve (AUC) was calculated for the ROC curves. [Colour online.]

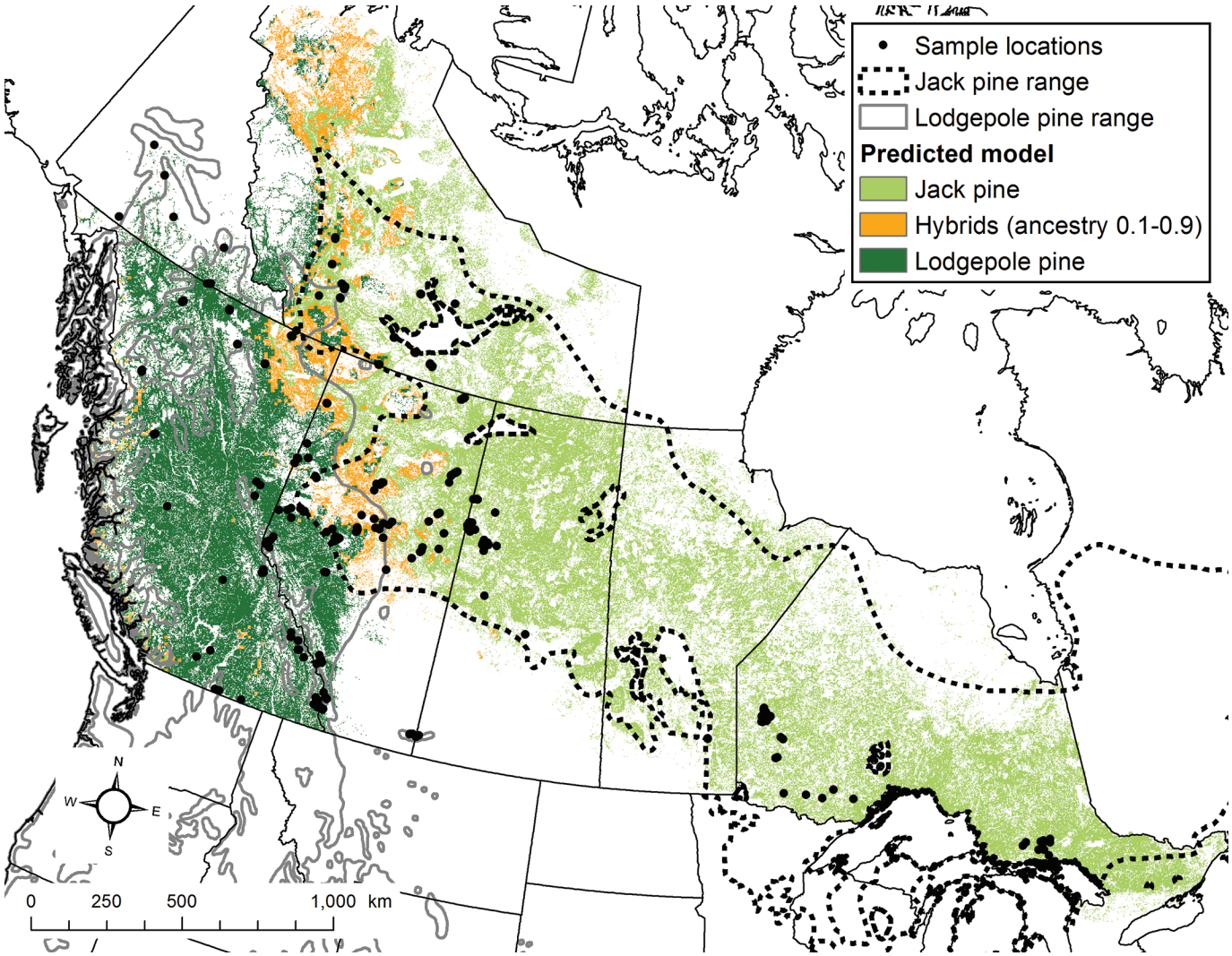


zones despite environmental and landscape differences, with average temperatures being lower in the northern region. There were loci that had significantly different patterns of introgression between the two regions; however, these loci are in proteins of unidentified function.

Distribution modeling

The predictive model that we developed contained two main environmental variables, elevation and climate moisture index (CMI). Elevation is highly correlated with many environmental variables, especially those derived from moisture and temperature indices (Körner and Diemer 1987; Guisan and Zimmermann 2000; Körner 2007). Despite these known correlations, we are confident in the independent and relevant contributions of all retained variables as their VIF values are all less than 5. That is, there is no risk of multicollinearity biasing our final model selection. Elevation and, similarly, longitude are indirect variables with gradients that greatly influence physiologically relevant variables for plants (water, heat, nutrients). Climate moisture index is the difference between annual precipitation and potential evapotranspiration (Hogg 1997) and is positively related to the distribution of lodgepole pine and negatively related to the jack pine distribution. This is consistent with known growth conditions, as lodge-

Fig. 3. Predictive map for lodgepole pine, jack pine, and their hybrids based on the spatial model, which includes geographic location. Predicted *Q* values are indicated by the species class colour at each geographic location, with dark green representing lodgepole pine, light green representing jack pine, and blue indicating hybrid pine. Yellow circles indicate sample sites included in this study. Grey lines and dotted black lines indicate the proposed distributions of lodgepole pine and jack pine, respectively, from Little (1971).



pole pine proliferates in a higher moisture environment than jack pine (Yeatman and Teich 1969; Rweyongeza et al. 2007).

The model that we developed revealed that the distribution of hybrids is greater in extent and width of range than that estimated previously by Little (1971) or Cullingham et al. (2012). We found hybrid individuals ranging from central Alberta north through to the Northwest Territories and northeastern British Columbia to the Alberta–Saskatchewan border. Notable changes from Little's (1971) assessments include the presence of individuals of hybrid ancestry in the Northwest Territories and eastern Alberta; however, individuals with hybrid ancestry also cover most of northern Alberta and parts of the British Columbia – Alberta border. Despite improved sampling of both pine species for this study, the distribution of samples in northern Alberta is still limited and the hybrid zone in this area requires further sampling for finer scale resolution of the hybrid map. Sampling here is challenging because of limited road access to the stands in this region. Some hybridization was predicted by the model in central British Columbia. Predictions in this case are likely due to the influence of environmental variables on the model, combined with the absence of samples in those regions. Our work here has made great strides towards characterizing the extent and structure of the hybrid zone and species distributions; however, there is still room for improvement with more extensive sampling.

Hybrid zone structure

In general, there are three types of hybrid zones, which can be distinguished based on the fitness of hybrids and the effect of environment. Clinal or tension hybrid zones (Key 1968) may result from strong gene flow in the presence of selection against hybrids. This results in a narrow hybrid zone maintained by continuous hybridization between parental species and smooth clines, which are spatially correlated (May et al. 1975). Bounded hybrid superiority zones are the opposite: selection causes hybrids to exhibit superior fitness to parental species in intermediate habitats (Moore 1977). Hybrids also show reduced fitness in parental habitats. The third type of hybrid zone is a mosaic hybrid zone, characterized by multiple generations of hybrids with variable introgression and patchy distributions of hybrids across the landscape. Mosaic hybrid zones are formed by a combination of superior, inferior, and variable hybrid fitness caused by variable environmental selection across the regions where parental species meet. This leads to differential selection among genotypes

(May et al. 1975) and pockets of pure species and hybrids across the landscape (Harrison 1986).

Based on the data that we have presented here, we propose that the lodgepole × jack pine hybrid zone represents a mosaic zone, consistent with a previous assessment of the southern hybrid zone (Cullingham et al. 2012). Three lines of evidence support this idea. First, results from NEWHYBRIDS assignments opposed a tension character for the hybrid zone. In Table 3, first-generation hybrids and backcrosses are assigned at low to negligible frequencies, whereas the majority are late-generation hybrids. This would suggest that the hybrid zone is both self-sustaining and variable. If the lodgepole × jack pine hybrid zone were a tension zone, hybrid individuals would experience a selective disadvantage (Key 1968), and we would therefore see few late-generation hybrids (F2 and F3). Additionally, the absence of F1 hybrids does not necessarily indicate that hybrids are selected against (which would suggest a tension zone), as incomplete sampling will greatly influence the representation of hybrid generations, especially across such an expansive distribution. Second, we observed differential introgression between the northern and southern zones at two genes. This is consistent with characteristics of a mosaic hybrid zone, where a selection gradient often leads to differential selection among genotypes (May et al. 1975). Finally, discontinuous selection gradients of a mosaic hybrid zone, caused by strong gene flow in the presence of selection, often results in patchy, or discontinuous, spatial distributions across the landscape (Gompert et al. 2017). This can be seen in the predicted distribution map (Fig. 3) where pure lodgepole and jack pine individuals can be found in multiple stands in Alberta and the Northwest Territories within the described hybrid zone. Practically, the presence of a mosaic hybrid zone translates into a patchy distribution of preferred habitat for MPB. Such patchy distribution can reduce population movement among patches and may help prevent the establishment of novel epidemic populations of MPB in hybrid and (or) jack pine stands (Bone et al. 2013).

Implications for management of MPB

Improving our understanding of the distribution and genetics of pine allows for further assessments of the role that hybrid zones play in host-shift dynamics. Currently, MPB has not reached the northern limit of lodgepole or hybrid pine. Establishment of MPB in the northern hybrid zone may be limited by temperature, elevation, and the patchy distribution of hosts (Safranyik et al. 2010). It has been shown that naïve lodgepole pines are more susceptible, resulting in greater reproductive success of MPB than in trees in epidemic areas (Cudmore et al. 2010). This is concerning given that the northern hybrid zone and the entire boreal forest comprise naïve trees potentially susceptible to MPB establishment. Also, hybrid trees likely represent an intermediate phenotypic environment in which levels of chemical production by hybrid hosts are intermediate to lodgepole pine and jack pine (Lusebrink et al. 2013; Erbilgin et al. 2014). This transitional environment could promote MPB transfer by providing a progressive change in host environment across the landscape; however, the more recent establishment of the northern zone would suggest that introgressive hybridization has occurred less frequently. Also, significant differences exist between the affected southern hybrid zone and the naïve northern hybrid zone in specific genes. A more comprehensive genome analysis would better identify differences between these zones and determine whether the environment is playing a role in the differences.

Accurate pine species distribution data are essential to industry and government for resource allocation, reforestation of attacked stands, and MPB control and spread prediction. As part of the TRIA-Net project, the resource that we have created will allow for the prediction of at-risk pine stands and aid in the implementation of appropriate conservation measures to manage the potential spread of MPB through the boreal forest. Beyond the goals of the TRIA-Net project, the model may also be of use for predictive modeling of future distributions of suitable pine habitat under climate change, which will ensure that appropriate seed stock is deployed for future forest sustainability.

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