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The Dynamics of a Changing Lutz Spruce (*Picea* x *Lutzii)* Hybrid Zone on the Kenai Peninsula, Alaska

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ABSTRACT

We investigated the genetic makeup of Lutz spruce, a natural hybrid between white and Sitka spruce on the Kenai Peninsula, Alaska. Microsatellites indicate 72% of individuals sampled had predominantly white spruce ancestry whereas 14% had predominantly Sitka spruce ancestry; some individuals classified as white spruce had Sitka spruce plastid genotypes. As *Picea* mitochondria are maternally inherited and plastids are paternally inherited, it appears that white spruce was the ancestral seed parent of nearly all spruce on the western peninsula, whereas Sitka spruce alleles originated from pollen. Pollen records show that white spruce colonized the western peninsula ~8,500 YBP from glacial refugium, whereas

Sitka spruce arrived on the eastern peninsula ~4,000 YBP after migrating up the Pacific coast. Our data suggest that Sitka spruce migration onto the peninsula may have occurred not via seed dispersal, but by long distance transport of wind-borne pollen and subsequent hybridization with established white spruce populations. Hybridization was an important mechanism that allowed Sitka spruce to expand the leading edge of its range in response to historical climate change. As the climate continues to warm, climate envelope modeling suggests Lutz spruce may ultimately displace white spruce on the western peninsula even as Sitka spruce is constrained to the eastern peninsula where it will continue to hybridize.

KEYWORDS Alaska, amplicon sequencing, climate change, climate envelope model, hybridization, Kenai Peninsula, Lutz, microsatellite, migration, mitochondria capture, *nad5*, *Picea glauca*, *Picea* X *Lutzii*, *Picea sitchensis*, range expansion, *trnT-trnL*

INTRODUCTION

In response to a rapidly changing climate, Aitken et al. (2008) suggested that tree populations have three fates: adaptation, migration, or extirpation. Interspecific hybridization facilitates adaptation and may indeed have quite a varied role in the evolution of tree species including (1) extinction of one of the parental species, (2) reinforcement of species boundaries, (3) creation of a third, hybrid species, (4) formation of a stable hybrid zone, or (5) partial introgression between the two hybridizing lineages (Chunco 2014). With up to 25% of plant species thought to be of hybrid origin, hybridization is increasingly recognized as an important mechanism involved in generating novel genetic recombinants (Janes and Hamilton 2017). Interspecific hybridization may be particularly valuable in long-lived forest species, where generation length and reduced mutation rates limit accumulation of novel genetic variation (Janes and Hamilton 2017).

Many ecologically and economically important genera in temperate and boreal regions contain species pairs capable of interspecific hybridization (Aitken et al. 2008). This possibility is of particular interest in western North America, where several pairs of maritime and more continental taxa have distributions largely separated by major north–south mountain ranges, but have some opportunity for genetic contact through east–west river valleys or over the crest of lower parts of these ranges (Aitken et al. 2008).

Lutz spruce (*Picea* x *Lutzii* Little) is a natural hybrid between white (*P. glauca* (Moench) Voss) and Sitka spruce (*P. sitchensis* (Bong.) Carr.) that occurs in northern British Columbia, Canada, and on the Kenai Peninsula in southcentral Alaska. It was named after Yale University forestry professor, Harold J. Lutz, who collected the type specimen at Jerome Lake (60°32'48"N, –149°34'39"W) in 1950 on the Kenai

Peninsula, where it grew adjacent to both parental species (Little 1953). The two parent species are phylogenetically distinct (Ran et al. 2006) and they are widely thought to be ecologically divergent with Lutz spruce filling the intergrade of the divergent zones (Auders and Spicer 2012). What are the dynamics of this hybrid zone on the Kenai Peninsula?

The native range of Sitka spruce spans a narrow strip of maritime climate on the north Pacific coast of North America, extending for 2,900 km from 61°N latitude on the Kenai Peninsula in south-central Alaska to 39°N in northern California (Harris 1990). In contrast, white spruce spans the boreal forest from 52°N to 69°N. It grows in a predominantly continental climate with a correspondingly transcontinental range, from Newfoundland and Labrador west for 6,000 km across Canada along the northern limit of trees to the Bering Sea at Norton Bay and the Gulf of Alaska at Cook Inlet. In British Columbia, white spruce occurs ≤ 100 km from the Pacific Ocean in the Skeena Valley where it overlaps with Sitka spruce (Nienstaedt and Zasada 1990).

Lutz spruce genetics has been well studied in the hybrid zone in British Columbia (Hamilton and Aitken 2013, Hamilton et al. 2013a, Hamilton et al. 2013b). Pollen records from eastern British Columbia suggest that Sitka spruce arrived first, followed by white spruce; Sitka spruce rapidly expanded its range northward along the Pacific coast 15,000 YBP (Hamilton et al. 2013a) whereas white spruce arrived there from the east 9,000 YBP (Pisaric et al. 2003), contributing to asymmetrical patterns of introgression with Sitka spruce dominating the hybrid zone (Currat et al. 2008; Petit & Excoffier 2009; Hamilton et al. 2013a). Indeed, Hamilton and Aitken (2013) concluded that established Sitka spruce individuals were the likely seed parents of early hybrid generations, receptive to pollen flow from later-colonizing white spruce.

In contrast, pollen records show white spruce colonized the western Kenai Peninsula first (8,500 YBP; Anderson et al. 2006b) with Sitka spruce arriving on the eastern Kenai peninsula more recently (circa 4,000 YBP) as it continued to migrate northward along the Pacific coastline from British Columbia (Ager et al. 2010). Ager (2000) suggests that where Sitka spruce came into proximity with white spruce, hybridization occurred, ultimately influencing the spruce forests over large areas of the Kenai Lowland. Here, the combination of prevailing southeast winds from the Gulf of Alaska, which both blow spruce pollen westward and create a rain shadow in the lee of the Kenai Mountains that separates rain forest from boreal forest, and the historical arrival of white spruce before Sitka spruce, suggests that introgression of Sitka spruce into white spruce may be more likely.

Lutz spruce also has an ecological significance that may be unique to the Kenai Peninsula. Continuous stands of mostly mature Lutz spruce in Caribou Hills on the southwestern peninsula were the epicenter of an unprecedented spruce bark beetle (*Dendroctonus rufipennis* Kirby) outbreak from the late 1980s through the 1990s that thinned 1.2 million ha of spruce forest in south-central Alaska (Berg et al. 2006). The Caribou Hills area experienced the most substantial reductions in spruce volume, size class and stand structure (van Hees 2005, Boucher and Mead 2006). Although the presence of relatively high densities of mature large diameter host trees and regional drought-induced stress of these trees may have amplified the outbreak (Berg et al. 2006, Boggs et al. 2008), Lutz spruce is also more susceptible to spruce bark beetle damage and mortality than either white or Sitka spruce (Holsten and Werner 1990). In the aftermath of this outbreak, the release of *Calamagrostis canadensis* (Michx.) from the understory and subsequent spring wildfires have transformed much of the epicenter previously dominated by mature Lutz spruce into a novel grassland (Bowser et al. 2017, Magness and Morton 2018). It is not clear, however, whether this ongoing deforestation is the result of properties unique to this hybrid spruce or the effects of a rapidly changing climate on the southwestern Kenai Peninsula.

In this paper, we address three objectives: (1) explore the Lutz spruce genome makeup on the Kenai Peninsula, using mitochondrial and plastid DNA and nuclear microsatellites to assess introgression by Sitka versus white spruce (Sutton et al. 1991); (2) better define the spatial distribution of the hybrid zone and associated climate within deforested areas of the Caribou Hills; and (3) model the spatial distribution of Lutz spruce on the Kenai Peninsula.

MATERIALS AND METHODS

Study area

The 24,300 km^2 Kenai Peninsula juts into the Gulf of Alaska between Cook Inlet and Prince William Sound, connected to the mainland by a 16-km wide isthmus. The Kenai Mountains form a rugged spine that hosts three icefields (Harding, Sargent, and the Grewingk-Yalik complex), with elevations up to 2,015 m that essentially form the capstone between two biomes: the northern fringe of the Sitka spruce-dominated coastal rainforest on the eastern peninsula, and the western-most reach of North American boreal forest composed of white and black spruce (*P. mariana* Mill.) on the western Kenai Lowland. The Kenai Lowland forms a broad plateau 15–100 m in elevation, extending along the Cook Inlet from Turnagain Arm in the north to Kachemak Bay in the south. Kachemak Bay and the Fox River separate the Caribou Hills as a discrete mountain range from the Kenai Mountains, reaching 870 m at Ptarmigan Head (Figure 1). Elevational treeline in the Kenai Mountains varies from 370–500 m (Reger et al. 2007).

Ager (2001) suggested that spruce distribution coincides with the regional microclimates on the Kenai Peninsula, reflecting the influences of maritime climates on the outer coast and precipitation shadow effects of the Kenai Mountains in the Kenai Lowland. Along the coast, the climate in recent decades ranges from truly maritime with a mean annual temperature (MAT) of 4.4°C and mean annual precipitation (MAP) of 499 cm at Whittier on Prince William Sound to MAT of 2.7°C and MAP of 112 cm at Seward on Resurrection Bay to more moderate maritime at the City of Kenai on Cook Inlet with MAT of 1.9°C and MAP of 56 cm. From the Cook Inlet, the climate grades eastward to drier and more continental conditions in the rain shadow of the Kenai Mountains (Berg and Anderson 2006). An intermediate climate occurs on the southern tip of the peninsula, where MAT is 3.2°C and MAP is 55 cm at Homer (WRCC; https://wrcc.dri.edu/, accessed 11/6/2022).

The distribution of Lutz spruce on the Kenai Peninsula is generally known but has not been mapped explicitly because of the difficulty in distinguishing it from its two parental species based on remote-sensed imagery. Berg and Anderson (2006) referred to "a hybrid zone of Lutz spruce extended north from Kachemak Bay to the Kenai River on the central Kenai Peninsula", an area coincident with the eastern Caribou Hills and western Kenai Mountains. However, others (Werner and Holsten 1983; Reynolds and Holsten 1994) studied Lutz spruce stands further north in the Kenai Mountains along Resurrection Creek, Skilak Lake, Russian Lakes and Summit Lake, all ≤ 40 km from where Harold Lutz collected the original type specimens. Lutz spruce may be even more prevalent in the Kenai Mountains north of the Harding Icefield and west of the Sargent Icefield extending into Turnagain Arm (Hollingsworth et al. 2017).

Sampling frame and sample locations

Initially, we were primarily interested in confirming the taxonomy of the spruce that occurred on the southern Kenai Peninsula prior to deforestation due to spruce bark beetle and wildfire. We acquired two Landsat TM images from USGS Earth Explorer website (http://earthexplorer.usgs.gov/), dated 12 Sep 1986 (Landsat 5) and 25 Sep 2014 (Landsat 8). We used ENVI to pre-process the imagery to normalize the data (Radiometric Calibration Tool to calculate reflectance) and remove atmospheric effects (Dark Subtraction). We imported the pre-processed multi-spectral image into ERDAS Imagine. We conducted an unsupervised classification for each time-step and then visually identified the classes that related to spruce forests. Pixels that were spruce forest in 1986 but not in 2014 were classified as deforested. We converted the deforested pixels into a shapefile and dissolved "donut holes" ≤ 0.4 ha. Polygons were buffered and joined to adjacent polygons, excluding the 2014 Funny River Fire. Our focal area for genetics sampling was a 37,790-ha union of major fire polygons south of Tustumena Lake on the southern Kenai Peninsula (1994 Windy Point Fire, 1996 Crooked Creek Fire, 2005 Fox Creek Fire, and 2007 Caribou Hills Fire). Within this area, our sample frame became the centroids of the 250-m pixels from the Alaska eMODIS product (Jenkerson et al. 2010), selecting every 12th pixel in both north-to-south and east-to-west axes, making a grid of 58 points spaced at 3-km intervals. This grid ensured we had a representative sample of spruce growing on the southern Kenai Peninsula.

Plant samples

We collected spruce needles for genetic analysis from a total of 446 spruce seedlings and adult trees from 56 sites within the sample frame. Two additional sites outside the sample frame were intended to serve as reference populations for parental genomes: white spruce at Silver Lake Trail on the western peninsula (N = 75) and Sitka spruce at Lost Lake Trail on the eastern peninsula (N = 61)(Figure 1). Needles were frozen and stored at -80°C until DNA was extracted.

We also sampled 532 spruce trees distributed elsewhere on the Kenai Peninsula for morphological analysis. We applied the introgression scoring, developed by John Alden (1995), to cone and needle characteristics in which 1 is pure white spruce, 7 is pure Sitka spruce, and scores in between assess the degree of hybridization. These samples were included with genetic samples for the purpose of modeling Lutz spruce distribution on the Kenai Peninsula.

Molecular methods

DNA was extracted from two spruce needles per individual using the Gentra PureGene Tissue Kit. Prior to extraction, needles were dried for 24 hours in a lyophilizer, and ground to a fine powder in a bead beater. We screened 24 microsatellite primer pairs, selecting those that reliably amplified and produced the most scorable bands. Thirteen microsatellite loci, using 12 primer pairs, were amplified from all individuals. One locus proved too difficult to score, so analyses are based on 12 loci from 11 primer pairs (Table 1). All forward primers had an added M13 tail (CACGACGTTGTAAAAC), which allowed priming of a third, universal M13 primer that was fluorescently labeled, and used for visualization of PCR products (Oetting et al. 1995). We performed microsatellite polymerase chain reaction (PCR) amplification in 10 µL volume reactions containing 4 ng genomic DNA, 1 U of Kapa3G Plant DNA polymerase, KAPA Plant PCR Buffer, 0.3 µM of each locus-specific primer, and 0.15 µM of the fluorescently labeled M13 primer. PCR amplification conditions were: 1 cycle of 95°C for 3 minutes; 10 cycles of 98°C for 20 seconds, a locus-specific annealing temperature (Table 1) for 30 seconds, and 72°C for 30 seconds; 28 cycles of 98°C for 20 seconds, 55C for 15 seconds, and 72°C for 30 seconds; a final extension of 72°C for 15 minutes. PCR products were sized on an ABI 3730xl genetic analyzer at the Cornell University Institute of Biotechnology.

In the genus *Picea*, mitochondria are maternally inherited, while plastid are paternally inherited (Sutton et al. 1991). To determine which parental species mitochondrial and plastid genomes were inherited from, we sequenced DNA from one mitochondrial locus (nad5a; Jaramillo-Correa and Bousquet 2003) and one plastid locus (the *trnT-L* intergenic space; Taberlet et al. 1991) using the Illumina miSeq. PCR primers were developed by aligning GenBank sequences from all spruce species on the Kenai Peninsula (*P. glauca*, *P. mariana*, *P. sitchensis*) to ensure that the region amplified encompassed regions where the species differed. We used Primer3plus (Untergasser et al. 2012) to identify good priming sites that did not vary between species, and that were less than 500bp apart, ensuring overlap between the 300bp forward and reverse miSeq sequence reads. Primers contained iTru tails that allowed the addition of Illumina adapters containing P5 and P7 sequencing primer sites and Adapeterama II indices used to demultiplex individual samples after sequencing (Glenn et al. 2016, 2019). The *trnT-L* PCR amplifications were carried out in one reaction per sample that included both locus-specific primers, and the indexed iTru primers. However, a single PCR reaction produced too much primer-dimer with *nad5a* primers, so we did two reactions per sample. The first reaction used the tailed locus-specific primers, and the second used the index primers. Samples were cleaned with Solid Phase Reversible Immobilization (SPRI) beads to remove primers and primer dimer prior to the second PCR reaction. After the second PCR, all samples were pooled, cleaned with SPRI beads, and sent to the University of Alaska Core lab for sequencing on the Illumina miSeq.

Locus-specific primer sequences for *trnT-L* (including iTru tails) were *iTru_trnT_3f*: 5'-ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT AGC TAA GCA GGC TCA ATG GA-3' and *iTru_trnT_3r*: 5'-GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC TTA CTC CCC TTC TCT CGC CAT-3' and primers for *nad5a* were *iTru_nad5_1f*: 5'-ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT GAA GGA AGA AGG GGC CCA AG -3'

and *iTru_nad5_1r*: 5'-GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC TCG AGC TCT GTT ACC CTT GCA-3'. PCR for *trnT-L* was carried out in 10µl volume with 4ng genomic DNA, 5 µl KAPA HiFi Readymix, and 0.3 µM of each primer (4 primers total). PCR amplification conditions were: 1 cycle of 95°C for 3 minutes; 25 cycles of 98°C for 20 seconds, 57°C for 15 seconds, and 72°C for 15 seconds; 8 cycles of 98°C for 20 seconds, 60C for 15 seconds, and 72°C for 15 seconds; a final extension of 72°C for 1 minute. The first PCR for *nad5a* was carried out in 10µl volume with 4 ng genomic DNA, 5 µl KAPA HiFi Readymix, and 0.3 µM of each primer. PCR amplification conditions were: 1 cycle of 95°C for 3 minutes; 30 cycles of 98°C for 20 seconds, 57°C for 15 seconds, 72°C for 15 seconds, and a final extension of 72°C for 1 minute. Cleaned PCR products were used as templates in the second PCR with the same conditions, but only 8 cycles, and an annealing temperature of 60°C.

DNA data analyses

Microsatellite peaks were scored in STRand 2.2.30 (Locke et al. 2007), and binned in MSatAllele 1.05 (Alberto 2009). Peaks and bins were manually checked and corrected as necessary. The fraction of ancestry from each parental species for each individual was estimated from microsatellite data using the admixture model in the Bayesian clustering software STRUCTURE 2.3.4 (Pritchard et al. 2000; Falush et al. 2007). Allele frequencies were assumed to be uncorrelated among clusters, and no prior population information was used in clustering. It was assumed that null alleles could be present, but PCR reactions that produced no peaks were assumed to be missing data. Dirichlet parameters (□) for degree of admixture were estimated and different clusters were allowed to have different values (POPALPHAS=1), allowing for asymmetric admixture, following recommendations by Wang (2017). The burn-in for the Markov Chain was set to 100,000 steps, and we collected data from 100,000 subsequent steps. To identify the value of K (the number of ancestral populations or clusters) with the highest probability of

explaining the data, we compared the mean likelihood of models with different values of K, ranging from K=1 to 10. Each model was repeated 10 times. The best value of K was chosen as the smallest value of K where the mean log likelihood of the model plateaued, as suggested by the STRUCTURE manual and by Wang (2017). We also evaluated K using *delta K* method of Evanno et al. (2005). Results were visualized with DISTRUCT (Rosenberg 2004) and PHYLOGEOVIZ (Tsai 2011).

DNA sequence data from *trnT-L* and *nad5a* DNA was analyzed using the SeekDeep pipeline version 2.6.2 (Hathaway et al. 2017), intended for analysis of targeted amplicon sequencing (TAS), where high-throughput sequencing is used with targeted genes amplified by polymerase chain reactions. The reads of amplicons shorter than 150 bp were discarded at the filtering step of SeekDeep. Similarly, we discarded reads of amplicons longer than 478bp and 550bp for *trnT-L* and *nad5a*, respectively, on the assumption that these were off-target sequences. We used additional arguments "--converge --leaveOutSinglets" for the Qluster step, "--converge --illumina --pop-noErrors --collapseLowFreqOneOffs" for the processClusters step. The option “--converge” causes the clustering to iterate until there is no more collapsing. These extra options remove the singlet clusters and unreliable and/or low-frequency one-off haplotypes. The extra clean-up options for the processClusters step were recommended for the cases where no PCR replicates are available to help correct for PCR noise. After haplotypes of all individuals were determined, we used fastq_masker of FASTX-Toolkit version 0.0.14 (Hannon 2009) to convert bases with Phred Quality Score less than 20 to ambiguous nucleotides (N). Each sequence read was assigned to a haplotype in SeekDeep, and haplotypes were assigned to White, Black or Sitka spruce based on their match to GenBank sequences. Spatially explicit maps of sampling sites were created using R (version 4.2.2) and packages maps (3.4.1), mapdata (2.3.1) and plotrix (3.8.2) (Becker and Wilks 1995, Lemon 2006, R Core Team 2022).

277

278 Spatial modeling of climate

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280 We used 938 spruce trees to build a climate envelope model of the white-Lutz-Stika hybrid zones. Trees
281 were scored from 1 to 7 based on phenotype (n = 530) or genotype (n = 408). The trees scored by
282 phenotype were based on Alden (1995) in which 1 was classed as white spruce, 7 as Sitka spruce, and
283 2–6 showed varying degrees of introgression based on cone scale characteristics and needle shape. The
284 trees scored by genotype were a subset of the 446 trees sampled in the microSatellite analysis. Of these,
285 15 trees did not produce valid genetic results and 23 were excluded because they were black spruce
286 hybrids (ancestry coefficient for black spruce > 0.1); the proportion of white spruce detected in the
287 remaining 408 trees ranged from 0.17–0.991. To convert genetic output to the Lutz spruce scale, we
288 divided the 0–1 proportion range equally into 7 parts. We did not score trees by phenotype that were
289 used in the genetic analysis.

290

291 We related trees to downscaled climate data by spatially grouping trees within each 1-km^2 climate grid
292 cell. A total of 303 cells contained sampled spruce, but we excluded six cells that were missing climate
293 information due to proximity to the coast. We used the average Lutz scale in each cell to build a
294 predictive climate envelope model using climate normals for 18 standard bioclimatic variables
295 (1961–1990 period; Wang et al. 2016). A correlation matrix indicated six biologically relevant variables
296 were independent (< 0.7, Table 2) and so were used in subsequent modeling: Julian date on which the
297 frost-free period begins (BFFP), extreme maximum temperature in °C over 30 years (EXT), mean annual
298 precipitation in mm (MAP), mean annual solar radiation in MJ m-2 d-1 (MAP), mean annual temperature
299 in °C (MAT), and the difference between mean temperatures (°C) of coldest and warmest months (TD).

300

We used the RandomForest package in Program R (vers. 4.6-14; Liaw 2002) to build a climate envelope model using the average Lutz hybrid score of 297 grid cells with the 6 climate variables as predictors. We used Random Forest to build a regression model (mtry = 2, ntrees = 500). We applied the model to predict across the 20,286 climate cells that comprise the Kenai Peninsula using the 1960-1990 historical climate layers and to forecast for 2025, 2055, and 2085. We forecast based on the Coupled Model Intercomparison Project Phase 5 (CMIP 5) scenarios (Wang et al. 2016). For Alaska, all 15 models forecast warmer and wetter conditions. We forecast using data from the model with the least climate change exposure (INM-CM4), the highest exposure (GFDL-CM3), and the 15-model average (Jenness et al. 2013).

RESULTS

Microsatellites

We obtained high quality microsatellite data from 431 individuals at 12 microsatellite loci. Banding patterns were consistent with diploid, nuclear inheritance (1-2 alleles/individual for all loci). Individuals with > 4 missing loci were discarded from further analysis (15/446 individuals). We estimated the number of ancestral populations (K) using the approaches of Wang (2017) and Evanno et al. (2005). Both methods supported the same value of K=3 (Figures 2a, 2b). These groups appear to correspond to white spruce, Sitka spruce, and black spruce (*Picea mariana* (Miller) Britton, Sterns & Poggenburg). Although black spruce was not a focus of our study, it occurs in small numbers in the study area. Since we collected spruce samples without regard for their appearance, some black spruce was in our sample.

STRUCTURE analysis of the microsatellite data (Figures 3a, 4a) indicates that the majority of individuals had predominantly white spruce ancestry (68% had at least 80% of their genome assigned to white spruce), while some had predominantly Sitka spruce ancestry (13%), and some appear to be intermediate (14%; 20-80% white spruce in their genomes). Less than 5% of the samples were assigned to black spruce.

F_1 hybrids should inherit 50% of their genome from each parental species, therefore the estimates of ancestry (admixture) coefficients should be close to 50%. Although at least 13% of individuals appear to be hybrids between white and Sitka spruce, very few individuals appear to be F_1s, if we assume that F_1s would be estimated to have between 40—60% of their genome assigned to Sitka spruce. Rather, most hybrids inherited the majority of their genomes from either white or Sitka spruce, suggesting that they were likely late-generation hybrids rather than F1s.

The white spruce reference population at Silver Lake Trail consisted mostly of white spruce individuals as expected. That sampling site also contained a few black spruce and some white x Sitka hybrids with varying fractions of each species in their genome, including a few that had mostly Sitka alleles. In the Sitka spruce reference population at Lost Lake, as well as nearby Divide Ski Area, both east of the Harding Icefield, nearly all individuals appear to be white x Sitka hybrids deriving the majority of their genomes from Sitka spruce. Fortunately, STRUCTURE does not require reference populations, so the fact that our “reference populations” were not pure white or Sitka spruce does not pose a problem for our analysis.

West of the Harding Icefield, most individuals appear to be white spruce or white x Sitka hybrids that are derived primarily from white spruce. Hybrids with a high fraction of their genome coming from Sitka spruce seem to be most common in the southern part of the sampled region (Figures 3a and 4a).

Plastid sequence (*trnT-L*): paternally inherited

We obtained high-quality sequences from the intergenic spacer between plastid *trnT* and *trnL* genes (*trnT-L*) from all 446 individuals with an average of 16,440 (median=11,942) merged paired-end sequence reads per sample. We obtained a total of 6 haplotypes. Four of the haplotypes had 100% sequence identity to sequences in GenBank (1 black spruce, 1 Sitka spruce and 2 white spruce). Three rare haplotypes were most similar to white spruce but differed by a single base substitution; these were assigned to white spruce. Surprisingly, most individuals had two or more plastid variants in a cell (i.e., heteroplasmy), with a mean of 2.50 (StDev = 1.08) haplotypes per individual. We deposited the six haplotypes into GenBank (accession numbers: OP091380-6).

As expected, individuals in the Sitka spruce reference population at Lost Lake contained mostly Sitka spruce haplotypes, although a few individuals (10%) also produced a substantial number (>10%) of sequence reads assigned to Sitka spruce. Individuals from nearby Divide Ski Area also had mostly Sitka haplotypes, with 25% of individuals producing a substantial number of white spruce sequence reads. Most individuals from the white spruce reference population at Silver Lake Trail contained primarily white spruce haplotypes as expected, but again, a few individuals (3%) contained a substantial fraction of reads assigned to both white and Sitka spruce. Further, some individuals contained primarily Sitka or black spruce haplotypes (16% Sitka, 5% black). Outside of these regions, most sampling sites contained both white and Sitka haplotypes, with Sitka haplotypes being more common in the south.

Overall, 53% of individuals had primarily white spruce plastid haplotypes (≥ 90% of sequence reads were assigned to that species), 31% had primarily Sitka haplotypes, and 3.8% had black spruce haplotypes. Ten percent of individuals had a substantial number (>10%) of reads assigned to both white and Sitka spruce (Figures 3b, 4b).

Mitochondrial sequence (*nad5a*): maternally inherited

We obtained high quality sequence data from the *nad5a* mitochondrial gene from 440 of 446 individuals, with an average of 11,092 (median=10,428) merged paired-end sequence reads per sample. We observed 4 primary haplotypes, which could be assigned to species based on their match to sequences on GenBank. We observed another 25 very rare haplotypes that rarely made up more than 2% of sequence reads per individual. Most individuals (99.3%) had only one of the primary haplotypes in >10% frequency of sequence reads. Haplotypes that made > 5% of reads in at least one individual with > 1,000 read pairs were deposited to GenBank (accession number: OP019372-9).

In stark contrast to microsatellite and plastid datasets, almost all individuals (94.5%) had white spruce mitochondrial haplotypes, including the Sitka reference population (Figures 3b, 4b). Only 2 individuals had the Sitka spruce haplotypes. These 2 individuals occurred in sampling sites with relatively high Sitka assignments in the microsatellite analysis (Lost Lake and Maritime Helicopters). Microsatellite analysis classified one of these individuals as Sitka spruce and the other as a White x Sitka hybrid. Both individuals had Sitka plastid DNA. Five percent of individuals had the black spruce mitochondrial haplotype. These individuals were also classified as black spruce in the microsatellite analysis and the majority of plastid sequences in all of these individuals was classified as black spruce.

394

395 Comparison among genetic datasets

396

397 The plastid results are generally consistent with the microsatellite results, but mitochondrial results
398 were very different from the other two genomes (Figures 3a-c, 4a-c). A large number of individuals
399 appear to have primarily white spruce assignments in all three genomes, suggesting that those
400 individuals do not have a hybrid ancestry. By contrast, almost all individuals with any Sitka
401 microsatellite alleles also have Sitka plastid haplotypes but white spruce mitochondrial haplotypes.
402 These individuals clearly have a hybrid ancestry. Further, in these individuals, the fraction assigned to
403 Sitka spruce was higher for the plastid genome than for the microsatellites. Since spruce plastids are
404 paternally inherited and mitochondria are maternally inherited, the ancestral hybrids must have been
405 produced by Sitka spruce pollen and white spruce ovules. Only one individual has Sitka spruce in all
406 three genomes: an individual from the Sitka spruce reference population.

407

408 All individuals classified as black spruce by the microsatellite analysis also primarily contained plastid
409 and mitochondrial haplotypes assigned to black spruce. For black spruce, the correlation coefficient
410 between microsatellite and plastid datasets was very high (Pearson's r=0.98), as was the correlation
411 between microsatellite and mitochondrial datasets (Pearson's r=0.99.6). This high correlation is likely
412 due to little if any hybridization between black spruce and the other spruce species, expected from
413 individuals without hybrid ancestry.

414

415 Climate envelope models

416

417 Sample sizes per 1-km^2 grid cell ranged from 1–69 spruce trees (Figure 5a). Mean TD, a measure of
418 continentality, was highest for white spruce (21.3) and lowest (19.7) for Sitka spruce. The means of five
419 of the six bioclimatic variables for the cells (n = 149) in which Lutz spruce occurred were intermediate
420 between the cells that contained white (n = 96) and Sitka (n = 96) spruce (Table 2); MAT, however, was
421 lower for Lutz than for either white or Sitka. Overall, the average Lutz spruce cell (783 mm/yr) was
422 intermediate in MAP between white (565 mm/yr) and Sitka spruce (1332 mm/yr), but tended to be
423 cooler (1.8°C) than areas occupied by either parental species (2.2–2.5°C).
424
425 The predicted composite distribution of Sitka, white and Lutz spruce in 1960–1990 (Figure 5b) is
426 reasonable based on a supervised classification of satellite imagery from 2002 (Magness and Morton
427 2018). Figure 5b is significant as it is the first time Lutz spruce distribution has been modeled or mapped
428 on the Kenai Peninsula, with the RandomForest model explaining 63% of the variance in its distribution.
429 Both GCMs and the 15-model ensemble model show similar changes in pattern over the three time
430 periods. As the future climate becomes wetter and warmer, conditions for white spruce are forecasted
431 to decline by 2055, with Lutz spruce replacing white spruce on the western peninsula by 2085 (Figures
432 6a–c). Based on predicted values for exposure, climate conditions favorable for Sitka spruce become
433 increasingly constrained to the eastern peninsula, diminishing in its distribution through 2085.
434
435 **DISCUSSION**

436

437 In response to a changing climate, Aitken et al. (2008) suggested that tree species could adapt, migrate
438 or be extirpated. On the Kenai Peninsula, we see elements of all three happening to white and Sitka
439 spruce, albeit at different times, with migration taking precedence during previous millennia,
440 hybridization in more contemporary times, and extirpation as a possible future outcome.

441

442 Migration played an important role in colonizing deglaciated areas with Sitka and white spruce during
443 the Holocene. With the exceptions of the Caribou Hills and an area in the northwestern Kenai
444 Mountains, the Kenai Peninsula was almost entirely glaciated during the late Wisconsin glacial
445 maximum, and glaciers receded 10000-13000 YBP (Reger et al. 2007). White spruce was first to reach
446 the western peninsula 8500 YBP (Anderson et al. 2006b), presumably from Beringia and other scattered
447 refugia to the north and west (Anderson et al. 2006a, Anderson et al. 2017), and then spread slowly
448 southward on the Kenai Lowland at 0.03 km/yr (Ager 2000). Black spruce arrived 4600 YBP (Anderson et
449 al. 2006b) but has remained constrained in distribution to the Kenai Lowland, mostly in the central and
450 northern portions where kettle moraines and peatlands are common. Sitka spruce migrated up the
451 Pacific coast, showing up in pollen records near the isthmus, shortly after 3400 YBP (Ager et al. 2010)
452 but not reaching its southwestern extreme on the peninsula near Kachemak Bay until 2000–1600 YBP
453 (Ager 2000). These two locales, the peninsula isthmus and southernmost tip, are effectively topographic
454 breaks in the climatic barrier created by the Kenai and adjacent Chugach Mountains; combined, they
455 help form the leading edge of the northwestern distribution of Sitka spruce (Elleouet and Aitken 2018).
456 As Ager (2001) pointed out, coastal forests appear to still be migrating west along the coast of south-
457 central Alaska, but their spread northward is being limited by drier, colder winter climates in the lee of
458 the Kenai and Chugach Mountains.

459

Hybridization, which can facilitate adaptation, appears to be an important mechanism that has allowed Sitka spruce to continue expanding its range up the coast via pollen transport despite the topographic barriers which limit seed dispersal. Indeed, our microsatellite data show that nearly all Sitka spruce individuals on the Kenai Peninsula have a history of hybridization, although we identified some pure white spruce individuals. Almost all (98%) of the individuals classified as hybrids or as Sitka spruce using microsatellites have white spruce mitochondria and Sitka spruce plastid genotypes. Also, some individuals classified as white spruce using microsatellites have Sitka spruce plastid haplotypes. As spruce mitochondria are maternally inherited and plastids are paternally inherited, it appears that white spruce were the ancestral seed parents of nearly all spruce trees on the western Kenai Lowland, whereas Sitka spruce alleles originated almost entirely from pollen.

Our data suggest that spread of Sitka spruce onto the Kenai Lowland and/or up the Pacific coast may have occurred not via seed dispersal, but by long distance transport of windborne Sitka pollen and subsequent hybridization with established white spruce populations. During April through July, when most spruce pollen is released, prevailing winds are generally from the east and east-southeast in Prince William Sound and eastern Turnagain Arm (Ager et al. 2010), effectively transporting Sitka spruce pollen towards the western Kenai Peninsula and into resident white spruce populations. In autumn and winter, storms spawned by low pressure systems in the Gulf of Alaska and northeast Pacific Ocean generate high wind speeds that favor westward dispersal of seeds along the southern coast (Ager et al. 2010). In contrast to findings in the British Columbia hybrid zone (Hamilton and Aiken 2013), our mitochondrial and plastid data strongly suggest introgression towards white spruce, consistent with the colonization history of the Kenai Peninsula by *Picea* species and prevailing winds over the Kenai Mountains.

The modeled current distribution of Lutz spruce on the Kenai Peninsula (Figure 5b) is a consequence of this colonization history, with prevailing winds and topographic barriers as mediating factors. This hybrid zone forms the broad ecotone between the coastal rainforest and boreal biomes. Climate continentality (TD) and MAP for Lutz spruce were intermediate between areas occupied by the two parental species, but MAT was slightly cooler where Lutz spruce occurred. Indeed, Hamilton et al. (2013a) found that hybrid genotypes exhibited greater cold hardiness on average than either parental species at -8°C, but at lower temperatures (-18°C) the difference was negligible. Furthermore, this distribution coincides well with what others have described anecdotally in the literature for the Kenai Peninsula (see study area description); i.e., Lutz spruce occurs on the Kenai Lowland, particularly along the Cook Inlet coastline of the southwestern peninsula, in the Caribou Hills, and in a rain-shadow band along the western flank of the Kenai Mountains. The distributions of poplar hybrids (*Populus balsamifera* L. ssp.; Viereck and Foote 1970) and birch species (*Betula* spp.; Packee 2004) on the Kenai Peninsula also generally coincide with this spruce delineation (Viereck and Foote 1970), suggesting a strong climate signal. Indeed, hybrid zones are valuable sources of genetic variation across a shifting landscape (Janes and Hamilton 2017).

Climate envelope models suggest that white spruce may ultimately be extirpated from the Kenai Peninsula, a consequence of increasingly favorable climate conditions for Lutz spruce. However, this future climate is based on downscaled GCMs that call for higher temperatures and more precipitation. While this has proven true, it has been manifested somewhat unevenly across the landscape, with the eastern peninsula warming 1.7°C on average and annual precipitation increasing 16.5% during 1969–2018, whereas the western peninsula has increased 1.9°C but only 3.4%, respectively (Thoman and Walsh 2019). Because temperatures are increasing faster than precipitation, mean annual available water (precipitation minus potential evapotranspiration) in 2021 has decreased 69% since 1968 in Kenai and 38% since 1988 in Homer, based on community airport weather records (Berg et al. 2009; E. Berg,

unpubl. data). This continued warming and drying of the western peninsula over the past several decades has set the stage for perennial thinning of spruce forests by spruce bark beetle as soon as trees reach susceptible size (Berg et al. 2006), while reducing wildfire return intervals (Berg and Anderson 2006) and lowering the water table in extensive wetlands (Berg et al. 2009).

Our modeling also does not account for competition with other tree species or the possibility of deforestation. In the former case, extant deciduous tree species such as birch (*Betula* spp.) and aspen (*Populus tremuloides* Michx.) may become more competitive and thus more widely distributed on the peninsula in a warmer climate (Magness and Morton 2018). In the latter case, Lutz spruce stands may be degraded because it is more susceptible to spruce bark beetle mortality than either parental species (Holsten and Werner 1990). This attribute may be a manifestation of transgressive segregation, whereby hybrids can have phenotypes outside of the range of parental species due to backcrossing or further introgression (Aitken et al. 2008). Evidence of this susceptibility can be seen in the fact that the Caribou Hills was the epicenter of the spruce bark beetle outbreak that ultimately infested 1.2 million ha of forest in southcentral Alaska during 1987–2000. There, the forest was primarily contiguous, very mature white-Lutz spruce that had neither burned for centuries (Berg and Anderson 2006) nor had a significant beetle outbreak since the 1870–1880s (Berg et al. 2006). Beetle-induced mortality during the more recent outbreak in the Caribou Hills was substantial (87% reduction in basal area of white-Lutz spruce >12.7 cm diameter-at-breast height), changing much of the forested area to woodlands and herbaceous types, and was linked to both *Calamagrostis* expansion and low tree seedling densities (Boucher and Mead 2005). In contrast, white-Lutz spruce forests further north in the Kenai Mountains experienced only moderate mortality (46% basal area reduction; Boucher and Mead 2005).

Despite high tree mortality and the competitive effect of released *Calamagrostis*, Boggs et al. (2008) argued that the forest would eventually regenerate because of spruce seedlings germinating in downed and heavily decayed logs and stumps (i.e., "nurse" trees) scattered across a denuded landscape. That outcome might have been realized had the fire regime retained its historical attributes of being canopy carried every few centuries. However, the 2005 Tracy Avenue Fire on the southern peninsula was a harbinger of a changing fire regime as the first spring grassland fire (albeit human caused) in modern times, so novel that the Alaska Division of Forestry changed the start of its official fire season from May 1 to April 1. This game-changing event was followed by the human-caused 2007 Caribou Hills Fire that burned 24,000 ha of primarily *Calamagrostis*, eventually culminating in the first lightning-caused spring grassland fires on the Kenai Peninsula in 2019. These short fire return intervals are killing spruce seedlings that would have otherwise been the source of forest regeneration (e.g., Knapp et al. 2018, Whitman et al. 2019).

Overall, Lutz spruce seems to have a competitive advantage over white and Sitka spruce in that it occupies areas on the Kenai Peninsula with historically slightly cooler MAT, perhaps because of its greater cold hardiness at a temperature that falls between maritime and boreal winters. Its greater intolerance to spruce bark beetle attack than either parental species is not a significantly detrimental attribute in a climate envelope that usually is wetter than that occupied by white spruce. However, it is clear that the Kenai Peninsula has warmed and dried in the last half century, perhaps passing a climatic tipping point in the 1990s with an unprecedented spruce bark beetle outbreak. While hybridization has allowed Sitka spruce to partially colonize the western peninsula in recent centuries despite the topographic and climatic barrier created by the Kenai Mountains, we believe a unique combination of factors may derail the ecological trajectory forecasted by climate envelope modeling; i.e., expansion of Lutz spruce and extirpation of white spruce in the near term. The increasing frequency and intensity of

554 spruce bark beetle outbreaks and wildfire in a warming and drying climate, despite slightly more
555 precipitation, may drive both Lutz and white spruce to near extirpation on the peninsula; in that
556 scenario, they would likely to be replaced by more hardwoods and grasslands (Magness and Morton
557 2018). The future of Lutz spruce on the Kenai Peninsula may be dictated by whether or not the
558 forecasted increase in precipitation ultimately reverses the decreasing trend in annual available water.
559
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562
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811

TABLE HEADINGS

Table 1. Microsatellite loci.

FOOTNOTE:
[a](A'Hara and Cottrell 2004)
[b](Hodgetts et al. 2001)
[c](Rungis et al. 2004)

Table 2. Six uncorrelated (< 0.7) bioclimatic variables used to model the climate envelope of Lutz spruce. Climate variable means were averaged for the Kenai Peninsula (KP) using the 20,686 1-km^2 grid cells; means for the white (n = 96), Lutz (n = 149), and Sitka (n = 96) were generated from the 297 cells in which spruce were sampled and classified based on genotype or phenotype (see text for details).

FIGURE CAPTIONS

Figure 1. Kenai Peninsula study area in southcentral Alaska, USA (see inset). Jerome Lake is the collection site for the original type specimen of Lutz spruce (Little 1953). Lost Lake and Silver Lake are the reference sites for Sitka and white spruce, respectively, used in this study. The Kenai Mountains create a rain shadow over the Kenai Lowland.

Figures 2a, 2b. Two methods for estimating the best value of K, the number of ancestral populations or clusters, indicate there are likely K = 3 clusters: (a) Mean Log Likelihood of STRUCTURE models assuming different values of K, and (b) delta K for different values of K (see Evanno et al. 2005).

Figures 3a, 3b, 3c. Hybrid index of each individual. Each vertical line represents the genome(s) of one individual. Colors indicate the fraction of the genome(s) originating from each parental species (grey= black spruce, white = white spruce, orange = Sitka spruce): (a) nuclear genome in which Individual Q-values are estimated by STRUCTURE from 12 microsatellite loci (n = 431); (b) plastid haplotypes based on the fraction of *trnT-L* sequence reads from each parental species (n = 446); and (c) mitochondrial haplotypes based on the fraction of *nad5* sequence reads from each parental species (n = 440).

Figures 4a, 4b, 4c. Maps of all sampling sites showing average genome composition (black = black spruce, white = white spruce, orange = Sitka spruce): (a) nuclear genome in which population Q-values are estimated by STRUCTURE from 12 microsatellite loci; (b) mitochondrial haplotypes based on the fraction of *nad5* sequence reads from each parental species averaged across individuals; and (c) plastid haplotypes based on the fraction of *trnT-L* sequence reads from each parental species averaged across individuals. Spatially explicit maps of sampling sites were created using R (version 4.2.2) and packages maps (3.4.1), mapdata (2.3.1) and plotrix (3.8.2) (Becker and Wilks 1995, Lemon 2006, R Core Team 2022).

Figures 5a, 5b. Sample sizes used (5a, left) to generate data for the climate envelope model of 1960-1990 baseline distribution of Sitka, white and Lutz spruce on the Kenai Peninsula (5b, right). Lutz spruce (variations of mostly green) tends to occur in the rain shadow of the western Kenai Mountains. We used the RandomForest package in Program R (vers. 4.6-14; Liaw 2002).

Figures 6a, 6b, 6c. Future distributions of white, Sitka and Lutz spruce on the Kenai Peninsula, Alaska, in 2025 (a), 2055 (b), and 2085 (c) based on least climate change exposure (INM-CM4), highest exposure (GFDL-CM3), and the 15-model average. We used the RandomForest package in Program R (vers. 4.6-14; Liaw 2002).

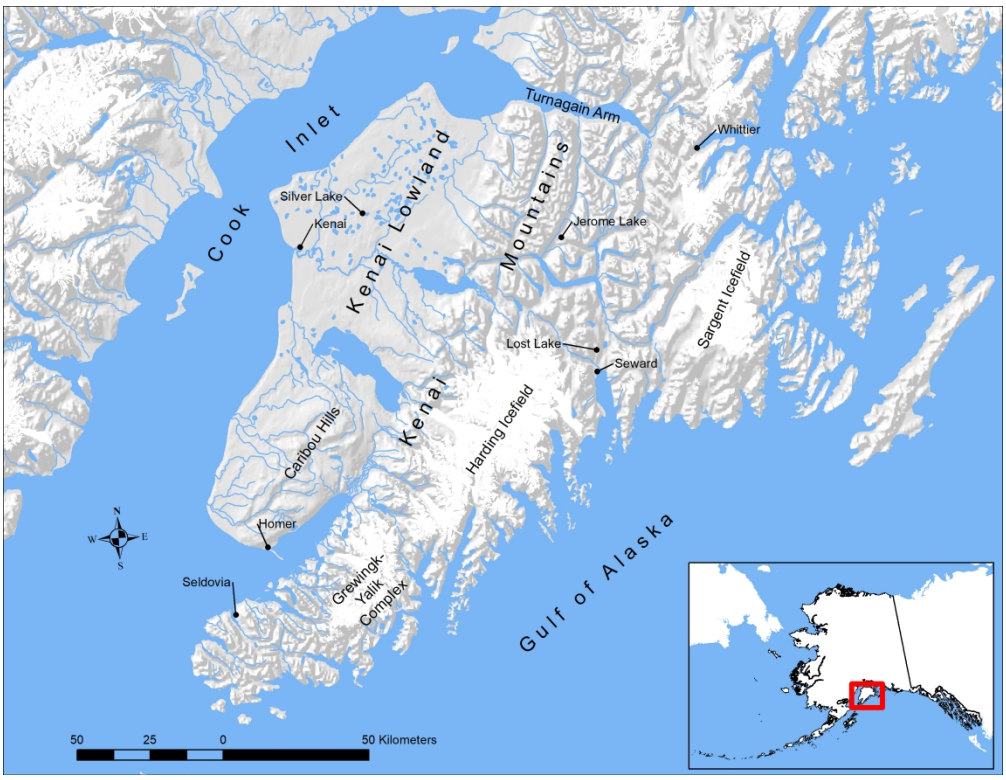
Table 1. Microsatellite loci.

Locus	Annealing temp (°C)	Repeat length (bp)	Fragment size range (bp)	Number alleles
SS63[a]	57	2	129-242	45
SS69[a]	57	2	224-342	49
SS70[a]	57	2	135-169	14
SS75[a]	57	2	166-214	26
SS76[a]	57	2	176-292	72
UAPgAG150[b]	60	2	131-159	12
UAPgAG150-2[b]	60	2	159-195	19
WS0022.B15[c]	53	2	190-228	18
WS0046.M11[c]	53	3	225-254	7
WS00716.F13[c]	53	2	210-248	21
WS0092.A19[c]	53	2	236-304	7
WS0092.M15[c]	53	3	209-240	8

[a](A'Hara and Cottrell 2004)
[b](Hodgetts et al. 2001)
[c](Rungis et al. 2004)

Table 2. Six uncorrelated (< 0.7) bioclimatic variables used to model the climate envelope of Lutz spruce. Climate variable means were averaged for the Kenai Peninsula (KP) using the 20,686 1-km^2 grid cells; means for the white (n = 96), Lutz (n = 149), and Sitka (n = 96) were generated from the 297 cells in which spruce were sampled and classified based on genotype or phenotype (see text for details).

Bioclimatic variable	KP mean (sd)	White mean (sd)	Lutz mean (sd)	Sitka mean (sd)
BFFP = Julian date on which the frost-free period begins	144.1 (9.0)	149.1 (5.8)	149.0 (5.7)	141.5 (4.1)
EXT = extreme maximum temperature over 30 years (°C)	28.4 (1.6)	29.8 (0.9)	28.8 (0.7)	28.6 (0.6)
MAP = mean annual precipitation (mm)	1725.5 (1223.8)	564.6 (134.7)	782.7 (298.3)	1332.1 (641.6)
MAR = mean annual solar radiation (MJ m-2 d-1)	12.1 (0.8)	11.5 (0.3)	11.6 (0.6)	11.9(0.6)
MAT = mean annual temperature (°C)	1.9 (1.2)	2.2 (0.6)	1.8 (0.6)	2.5(0.6)
TD = difference between mean temperatures (°C) of coldest and warmest months (a measure of continentality)	20.0 (2.4)	21.3 (2.1)	20.6 (2.0)	19.7 (1.3)



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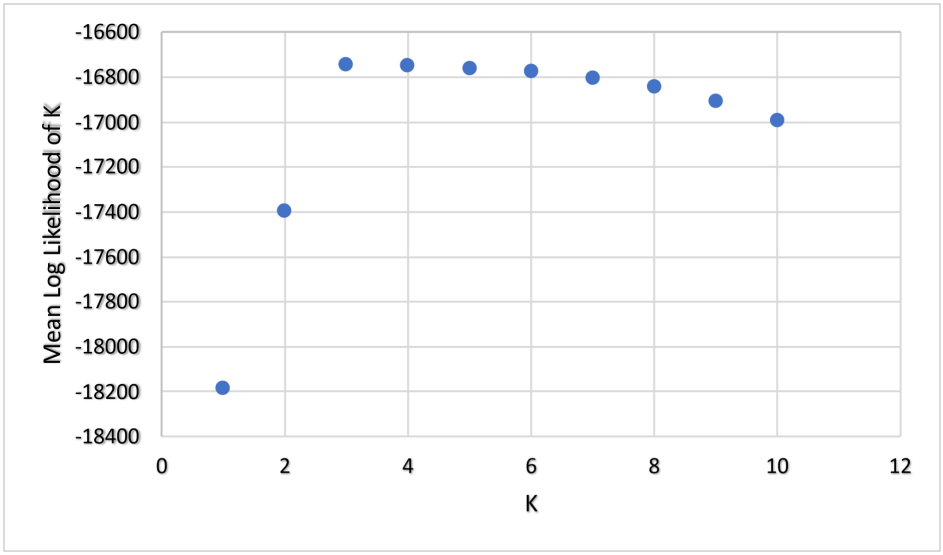
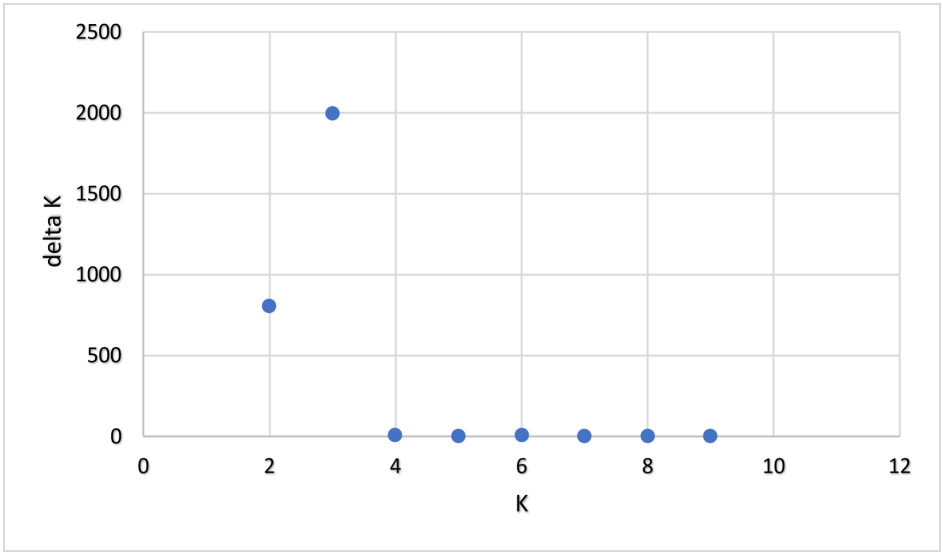
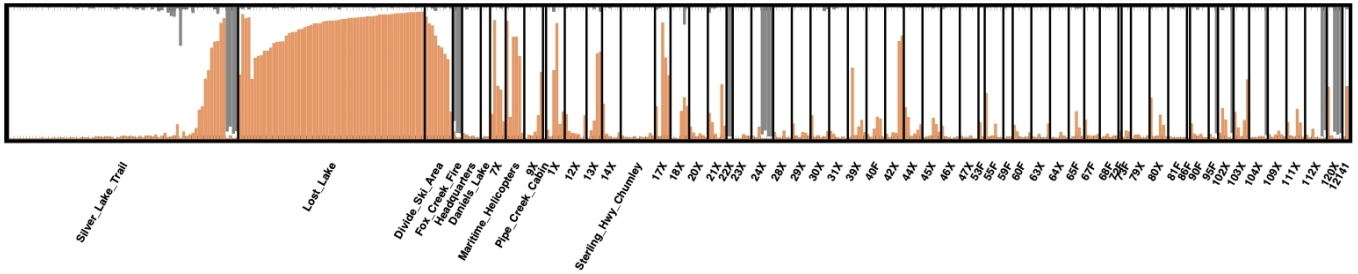
707x544mm (118 x 118 DPI)

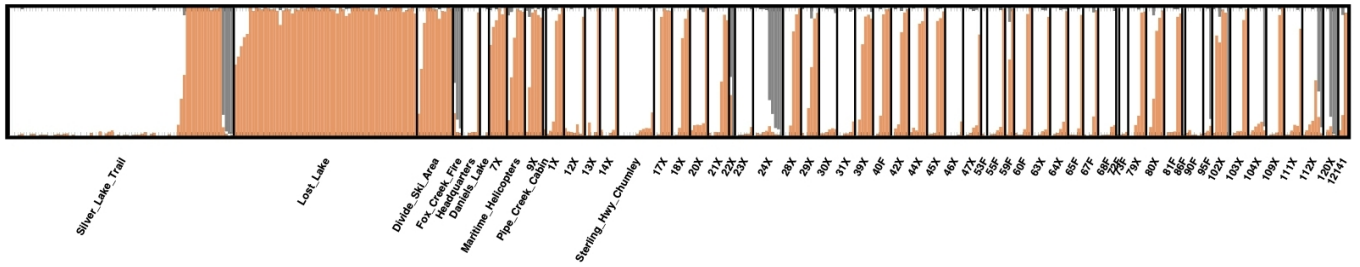




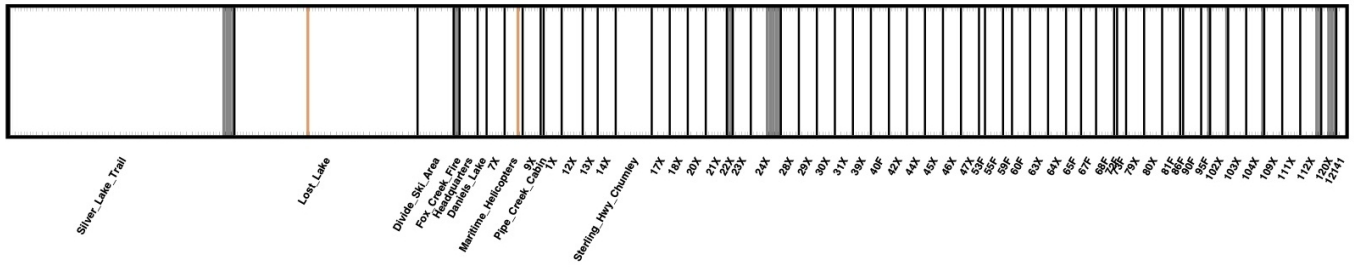
a. microsatellites

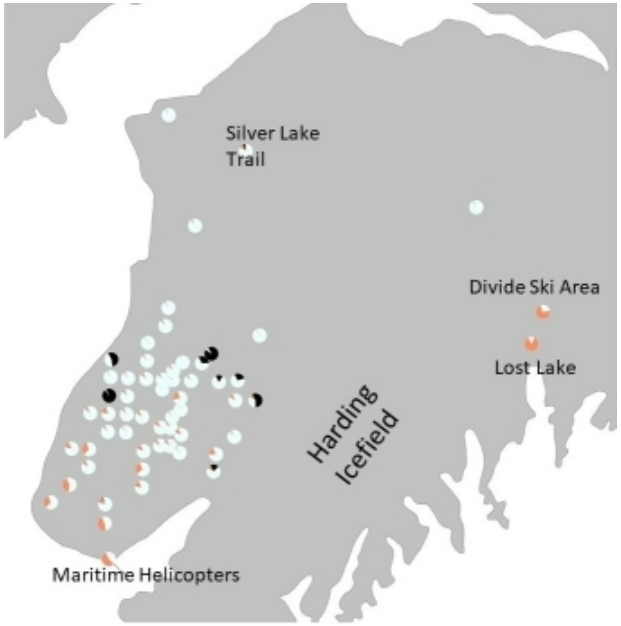


b. *trnT-L* (chloroplast)

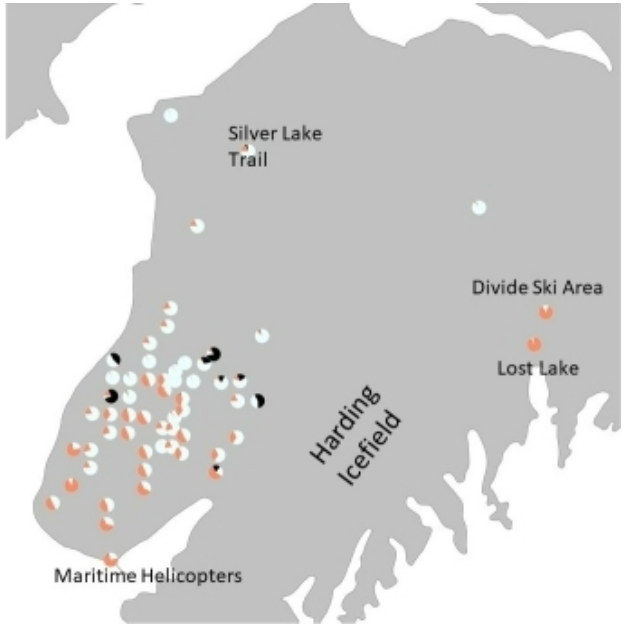


c. *Nad5* (mitochondria)

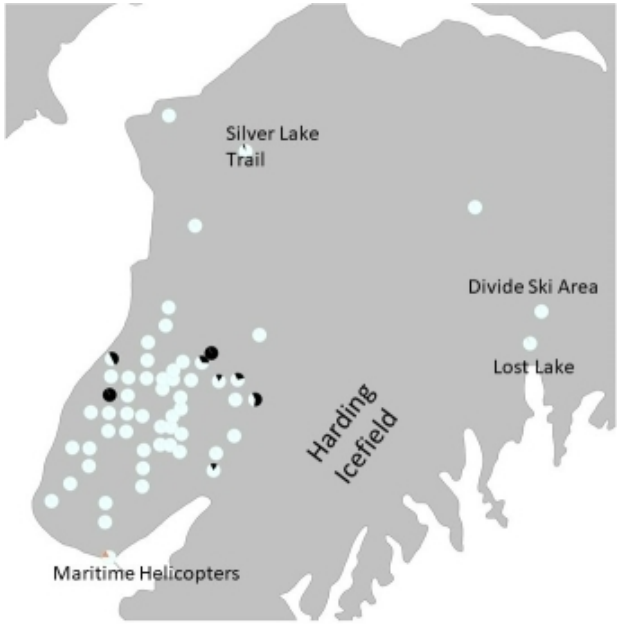




a. microsatellites



b. *trnT-L* (chloroplast)



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