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Multiple Pleistocene refugia for Arctic Bell-Heather revealed with genomic analyses of modern and historic plants

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Abstract

Aim: Arctic plants survived the Pleistocene glacial cycles, but the number, ages, and locations of these refugia remain unknown. Using genomic data from a large number of populations, we aim to evaluate the biogeography of this species and determine if independent refugia or a single refugium in the Arctic exist.

Location: Circumpolar Arctic and Coastal British Columbia.

Taxon: *Cassiope tetragona* L., *C. saxatilis* (L.) DC., and *C. mertensiana* (Ericaceae).

Methods: We built a reference genome for *C. tetragona* and sampled tissue from 36 Arctic populations, including those collected under glacial ice on Ellesmere Island. We used this reference to call variants. Population structure was inferred from PCA, ADMIXTURE, fastsimcoal2, and other population genomics statistics.

Results: Population structure analyses identified three distinct geographic locations. Nucleotide diversity was highest in Alaska and lowest in the Arctic. Demographic coalescent analyses suggest that *C. tetragona* and *C. saxatilis* diverged ~1.4 Mya, and *C. mertensiana* diverged ~0.4 Mya.

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For affiliations refer to page 1973.

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and Greenland (Nylander et al. 2010). Northern Canada populations during the current interglacial (7–9 kya). Alaska appear more diverse than the European and Greenland populations appear to be founded during the last glacial period. Conclusions: Population and demographic analyses suggest that the Arctic flora of Alaska, Europe and Greenland were founded by independent populations appear to be founded during the last glacial period. A analysis of other Arctic species with high resolution genomic markers and demographic analyses will provide a better understanding of the role of the Arctic flora and other circumpolar glacial refugia.

KEYWORDS

climate change, empirical population genetics, glacial refugia, glaciations, historic DNA, ITEX, range expansion

1 | INTRODUCTION

The Arctic flora has a complex history shaped by repeated glacial cycles causing extinctions throughout the Pleistocene (Dyke & Brown 2008; Hu 1987). While complex biogeographic patterns in the Arctic can be difficult to deduce from fossil records, a means to infer refugia, their routes and estimates of the time of origin have great potential in the Arctic (Went 1949). Demography and predict future climate change (Dyke & Brown 2008). Few studies have tested the plant refugia using genomic data (Dyke & Brown 2008) and there is still much uncertainty about the refugia formed for various species (Dyke & Brown 2008). Sampling of genomic markers (Dyke & Brown 2008) can begin to infer the age of Arctic plant populations (Dyke & Brown 2008) and gene flow in Arctic plant populations (Dyke & Brown 2008). Beringia (the region between eastern Siberia and Alaska) has been proposed as an unglaciated area during the last glacial period (Dyke & Brown 2008). Genetic diversity is generally high in Beringia (Dyke & Brown 2008) suggesting Beringia lineages are old (Dyke & Brown 2008). However, details for individual lineages are scarce (Dyke & Brown 2008). Distribution models suggest that the genus originated in the mountains in North America (Dyke & Brown 2008). Central Asia (Dyke & Brown 2008) and the Himalayas (Dyke & Brown 2008) are potential refugia where Arctic/alpine species may have persisted during the last glacial period (Dyke & Brown 2008). Fossils and pollen records support the Beringian refugia hypothesis (Dyke & Brown 2008). Many High Arctic plant species have been found in the tundra (Dyke & Brown 2008) (millions of years ago) (Dyke & Brown 2008). The presence

not be ruled out due to limited genotyping (preparation of a 265 AFLP marker library from 56 populations) that may have obscured some of the patterns of genetic diversity. The results of the AFLP analysis are consistent with the results of the microsatellite analysis and enzymatic depletion of the AFLP marker library.

We investigated the *C. tetragona* of range (Auerbach et al. 2017), enriched libraries for 37 extant Arctic populations to 500-fold depth (2,500x) including 10 of 12 historic populations sampled from under glacial till in the samples. Chondrichthys, H. glacialis, Arctic and *A. mertensiana* ranging up to 26,250 bp. Single libraries were sequenced for nucleotide polymorphisms (SNPs). Sequencing was done on the Illumina HiSeq 2500. The current circulator *C. tetragona* depositions lead to genome assembly 571 million 150 bp paired-end cent expansion out of a 8.2 Gb genome (Eidesen et al. 2007) and a refuge. Whether *C. tetragona* populations could have persisted in several other glacial refugia during the 2P. In the Arctic, the glacial refugia. Specifically, we estimate the ages of genetic clusters to see if they are younger or older than the Last Glacial Maximum (LGM) (18,000 years ago). We also investigated the age and population history of the *C. tetragona* (Purcell et al. 2014). The ends of read coverage

2 | MATERIALS AND METHODS

2.1 Sample collection and

[illegible]

DNA was extracted from 20 mg of *C. tetragona* leaves using a modified 3% CTAB protocol (Zeng et al. 2002) that was modified for high-throughput sequencing. A modified 3% CTAB protocol was also used to extract DNA from *C. tetragona* leaves. DNA was obtained from 38 samples, which were subsequently prepared for sequencing. reads to the de novo reference genome.

2.2 Sequencing libraries

Genotyping-by-sequencing (GBS) was selected as the most cost-effective way to obtain enough SNPs to differentiate and population structure analysis geographically expanded regions. GBS strategy was kept only 60% of the total DNA digested conducted using New England Biolabs MspI and SbfI restriction enzymes following a modified protocol of Elshire et al. (2011). Genotyping by sequencing the population statistics were performed using PLINK.

parameters. The best run of each 3mpd RIE S W L T S elected based on the maximum estimated likelihood and used for extracting the values for each parameter. Site frequency. 1 p S N T R a c w e i p i g t t e d for the raw data in python 3.10 via [dadi](#) (Gutenkunst et

2. § Historic samples

To determine the ages of the his m o d e l l i n g s b e c a u s e u s e d C r e t a c e o u s i n g f e d t h i c e , leaf/stem tissue from 14 i d e a t o f t h e g e n o t y p e w a s s a m p l e s o w a s a d y o f using Accelerator Mass Spectrometry (AMS) at the AMS Laboratory, University of Ottawa.

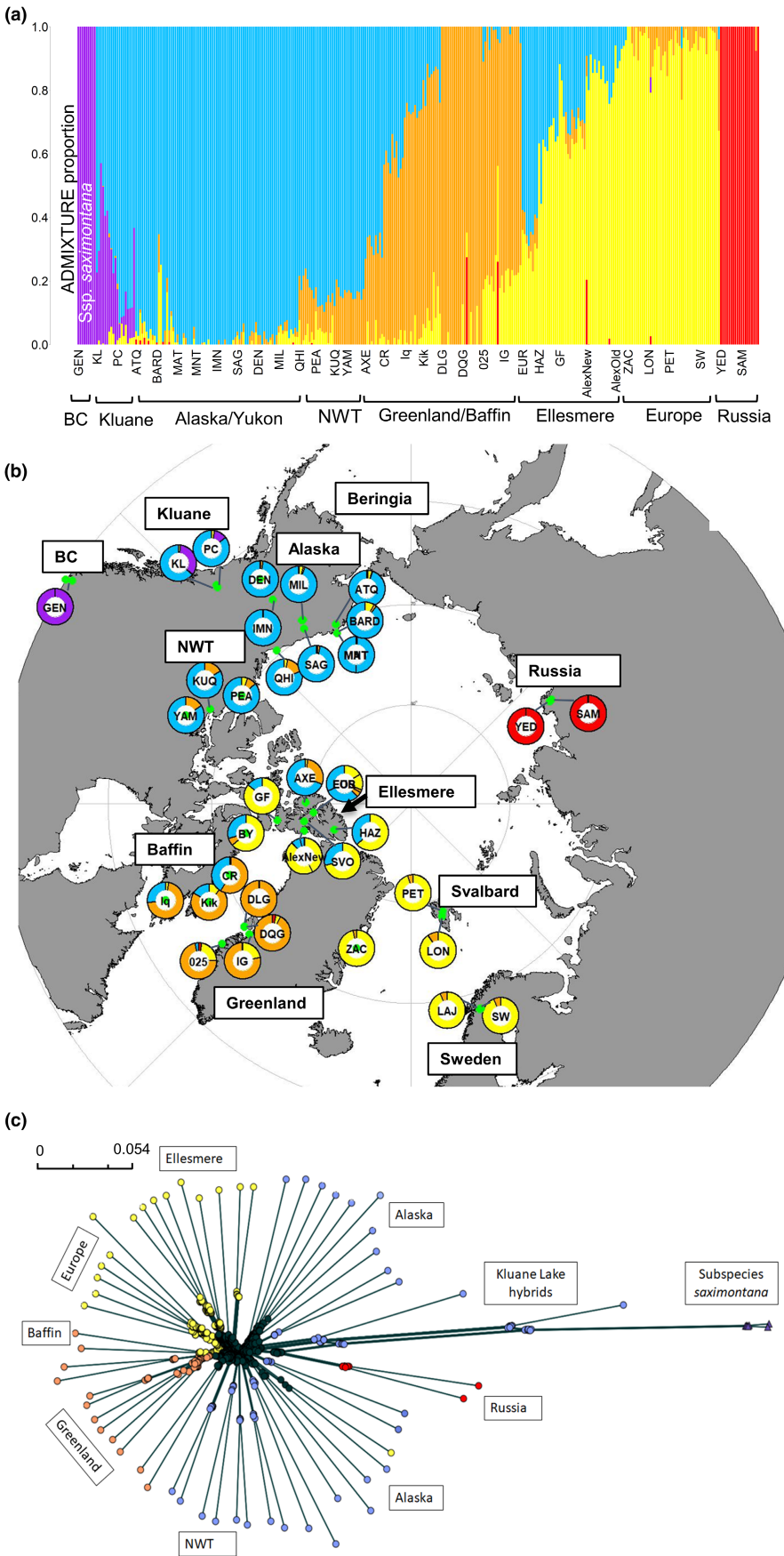
To test recent admixture between two major genetic clusters, p l a y s a m d - h i s t o r i c p o p u l a t i o n s R o p a u l e a n d o r n a s t o r u c t u r e were used. We e m p l o y e d A B B A n D s u i t e (Durand et al. [2011](#); Green [2016](#); Malina [2021](#)) e a c r o s s P o p u l a t i o n s t r u c t u r e a n a l y s e s w e r e p o p u l a t i o n s : P 1 , P 2 , P 3 a n d t h e i n d i g n i t u p a l (O) a f t e r l a t e d t o y i n t h e p h y l o g e n y (((P 1 , P 2) , P 3) , O) . S N P s a f r o m t h i s w a s a b s o r b e d w i t h e r e d t h a v e a s m a n y v a r i a n t s a s p o s s i b l e s e p a r a t o r a c t o i n g a t o s p o t w e l e n t e t r o p o d i f y i g d a p e a l s f r o m t h e o u t g r o u p t e n s a v e n i t h a n t a b l e 6 § . K l u a n e L a k e , Y u k o n (b l u e) n a d m i x t u r e) , A l e x O l d a n d A l e x N e w s a m p l e b e t w e e n S N P s t h e r e w o f i s l u t h e s p e e d c i e s . A o u t i f t h e y : h a d o a w e a e i t y d i e l e r t i o n o c c u r s a l o n g P C 2 s e p a r a t i n g P S N P s a n d c a l l e d i n l e s s t h a n - 9 5 % h o f A r i o n d i o v i d u a l e r s e (a p p r o x i m a t e o u t a f e r g r o u p , A l e x N e w a n d A l e x O l d) . T h e (a r l a n g l e) c a u r n i d e b y w e r e R u s s i a n g r o u p e d) w a s d e s i g n a t e d a s t h e a n c e s t r a l D a e l s p e i t e (A) , l a w d a r b e n d e r o f v e a d r a l t i e r e w a s d e s i g n a t e d a s B . U n d e r t h e P C 4 I (t y p e 3 %) e s P E 3 w e p i a h a s s u n l e s s k a a n o g e n e f l o w a f t e r P 1 a n d P 2 s p i d e n t a r e d A (B r B e A d / y B e l s h a v e d a b y e P 2 a n d P a n d P 3) a n d B A B A (B s h a r e d b y P 1 a n d E P 3 o p e a t y e l r h o w a r i e n e x p e c t i e d r o t o o c c u r w i t h e q u a l f r e q u e n c y - d w e a b s o n c a m p l e e = P 5 A n a m i d e 1 2 F a d g m i e f i n g . A s i g n i f i c a n t i n c r e a s e i n A B B A f o u n d A B A e i s a n c o n s l i s t e n t i n g i t h a t t e i n t r o g r e s s i o n b e t w e e n R 3 B A B A , - e r i d a t e P 1 n d A B B A t i d e v a l u e s) o r P 2 B A (A B B A s a t u v e s) . I A D M A X T U R E c l u s t e r i n g 1 4 r e a s u e l t c o n f o r m s t e s c e n a r i o , P 1 a n d P 2 w e r e r e p r e s e n t e d b y K e n t o b o w i o n t i e n t (A y l e s h o l w d i) n g t h a n d p r e s e n t (A l e x N e w) s a m p l e s , r e s p e c t i v e l y . K a n t i , a S N P I 3 X , T U R E r e s u l t s s u s e d m u l t i p l e p o p u l a t i o n s f r o m A l a s k a r s A T C u r r e a r d y D E N , B C I , M N R , u s s i a , M I L , M N T a n d S A G) , a s w e l l a s a p o p u l a t i o n i s a l i n p o p u l a t i o n C a n a d a (N V T b y 10 i n d i v i d u a l s f r o m t h e A l a s k a r g e n e t h a v e c h u s e d r a (a d m i x t u r e f r o m A l a m e m b e r s o h . i 9 p 9) .

2. § Flow cytometry

Flow cytometry w a s t e t r a g o n ' s t o g e r s o t m e n a s 3 § a p o p u l a t i o n s t a t i s t i c s (N u c l e o t i d e c o n t e n t , p l o i d y a n d g e n e t i c d i s t a n c e) I s l a n d , G r e e n l a n d . W e c u t u p 6 . t e t r a g o n a n d S o l a n e d l y c o p e r s i c u m (- g e n o m e s i z e s t a n d a r d v a r i e t y ' S t u p i l c k e w p r o d o w s a s e d ' s a p t e r o n a c h r e o s m f o e r d c i f r o m t h e D o l e z e a [2022](#)) (T e m s c h i s s t e i d i e n d o i u v r e r n s u i t y g a v e c o m p a r a b l e r e s u c l e i e x t r a c t i o n b u f f e r , w a s h e d a m d 2 6 i , 3 5 0 S N P s a n d i 3 3 0 e f i n d e i v d i d i a g s w i t h p r o p i d i u m i o d i d e . F l o w c y t o m e s t r y w a s r e u a t o n a h C y t o w a s e x o f o h a w b o t c y t o m e t e r i n t h e U B C B i o m e d i c a l a r n e o s u e n a t r c o h f G e i n t e e r s . i t O y u b o d e t a w i l e n d - i n c u f l o w c y t o m e t r y p r o t o c o l 6 a n - t e l o d i o n a n t a n a A p p e n d i x a n d w a x i m o n t a n t (v i c i n e c y t o m e t r y .

$n = 3 \times 10^4$. $p = 10^{-10}$. $R^2 = 0.98$. This relation

FIGURE 1 Population structure. (a) Relationship between ADMIXTURE results based on 330 individuals, ordered by population genetic cluster. Color proportion of that in associated with *K=5* (h_{CV} = 0.262). (b) Geographic collection sites. Each by a pie chart depicting ADMIXTURE *K=5* proportions for information about (PlotSvalbard 2020) age (c) Splits tree built with the uncorrected using two individuals from every population. Colours based on *K=5* ADMIXTURE groups. Net 25 572 SNPs and 60 in shows the uncorrected



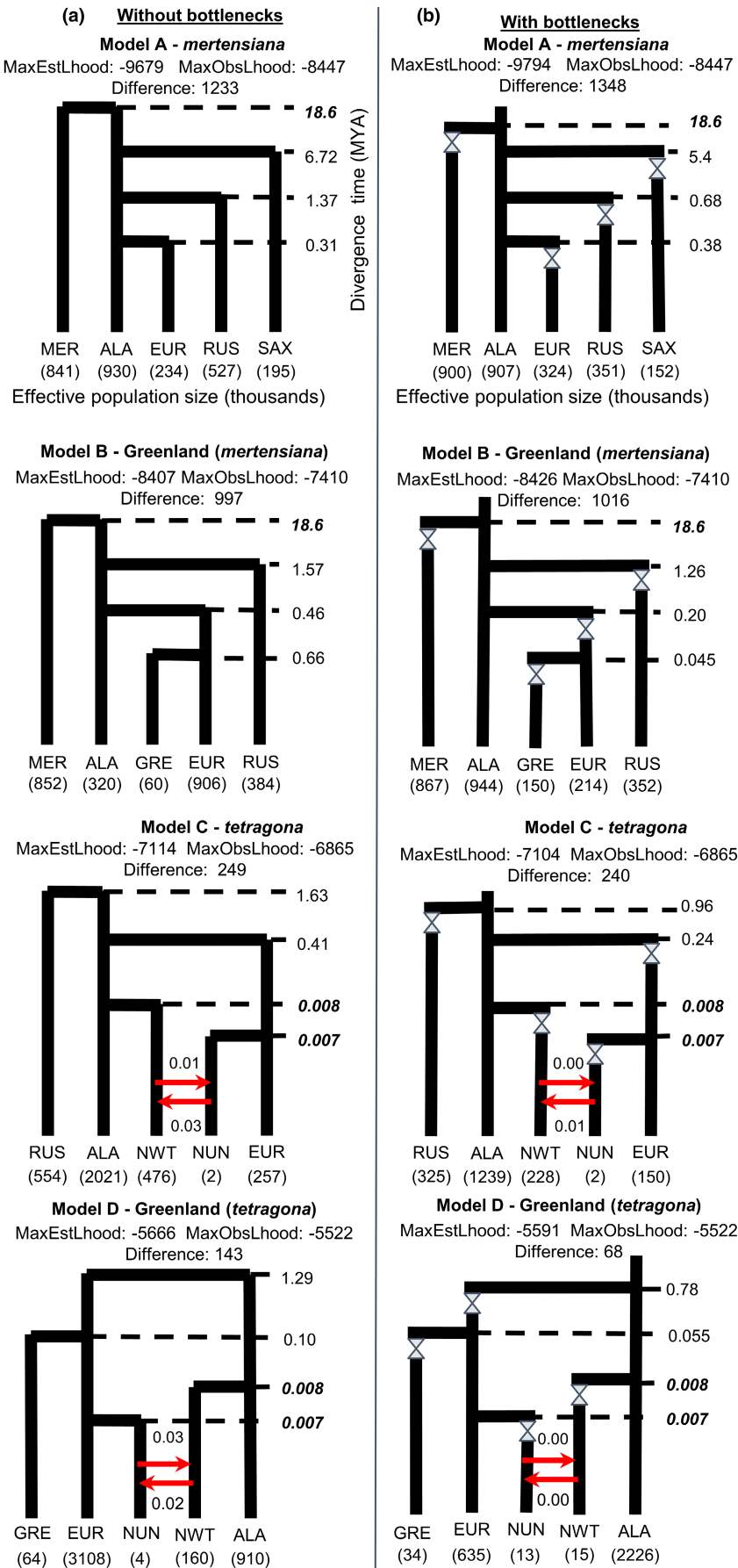
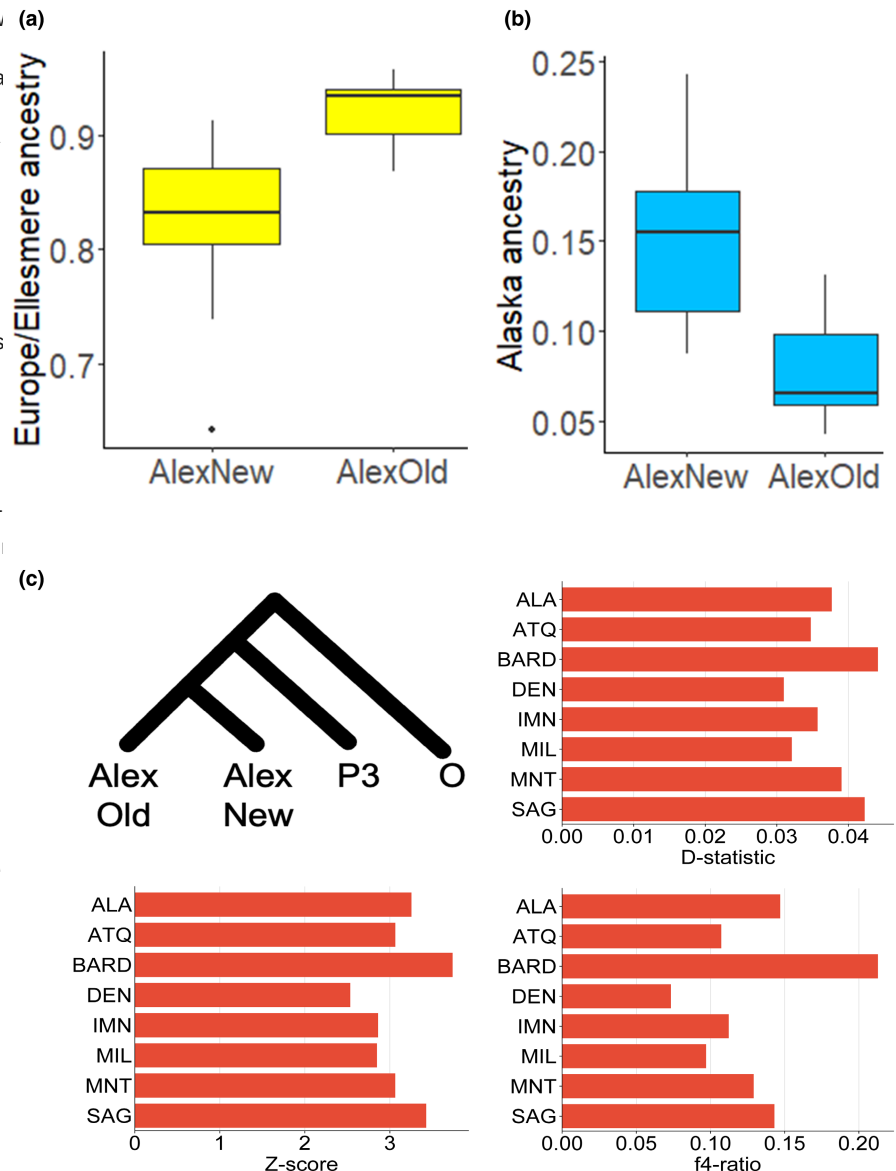


FIGURE C02a Lescent demographic analysis results from four models that were further shown with the best fit including (a) and including during the population bottleneck. The four models (A, B, C, and D) are not compared to each other. Models A and B use the fixed time for the split between *Cassiope tetragona* and *C. mertensiana* (MER). Models C and D use the fixed time of the split from northern Canada (Hurd and the Northwest Territories) to be 87-thousand years ago. Models C do not include Greenland. Models B and D remove one of the models A and C to incorporate

FIGURE 5 Comparison between (a) historic and modern samples. Sample *C. tetragona* from AlexNew, Ellesmere Island living individuals (AlexNew) and the retreating Twin G of ADMIXTURE ancestry for the 23 individual Fiord (based on 9285 330 individuals; Fig. 5a). (b) Relative proportions of ancestry observed in and historic Wshaitnileys-Wilcox on the 0.023). (c) proportions of Alaska observed in modern samples with a Wilcoxon T $p = 0.0046$ (Z-score = 3.1), a ratio for admixture in the ABBA test. The phylogenetic relation scenario, P1 and P2 were by the historic and modern respectively, and as populations from Alaska, DEN, IMN, MIL, MNT, as an artificial population from the (ALA; admixture = 0.10) in the



driven by the higher diversity among geographic populations (Bartington et al. 2010) because when samples from Beringia were reintroduced to the friarclians, the relationship was no longer (0.30 for 0.20 for 0.50) (zero in the majority of populations were) (Fig. 5a, Table S3).

Generally, values of F_{ST} (near zero) indicate a large degree of genetic distance among populations, while 'vauluoopse cllousetre rtso' (near zero) indicate a low degree of genetic distance among populations. F_{ST} was calculated for all the geographic populations (Fig. 5b), and the D-statistic was calculated for all the geographic populations (Table S3). The D-statistic was calculated for the regional ADMIXTURE (Fig. 5c) and for western Ellesmere and from expected, the values were found with the same pattern in the *C. tetragona* in the Arctic (AlexNew and AlexOld) in BC.

(GEN) (OF Fig. 5b). With the Russian populations (SAM and YES) (AlexNew and AlexOld) in the D-statistic analysis (for pairwise comparisons followed by the Disko Island in Greenland (Fig. 0.2; Table S3, dark green). The D-statistic for the Arctic (AlexNew and AlexOld) compared to others (Alaska, Yukon, NWT, Nunavut, and Northwest Territories) and to Sweden) all showed a significant difference (Fig. 5f) when compared with the major genetic groups (i.e.,

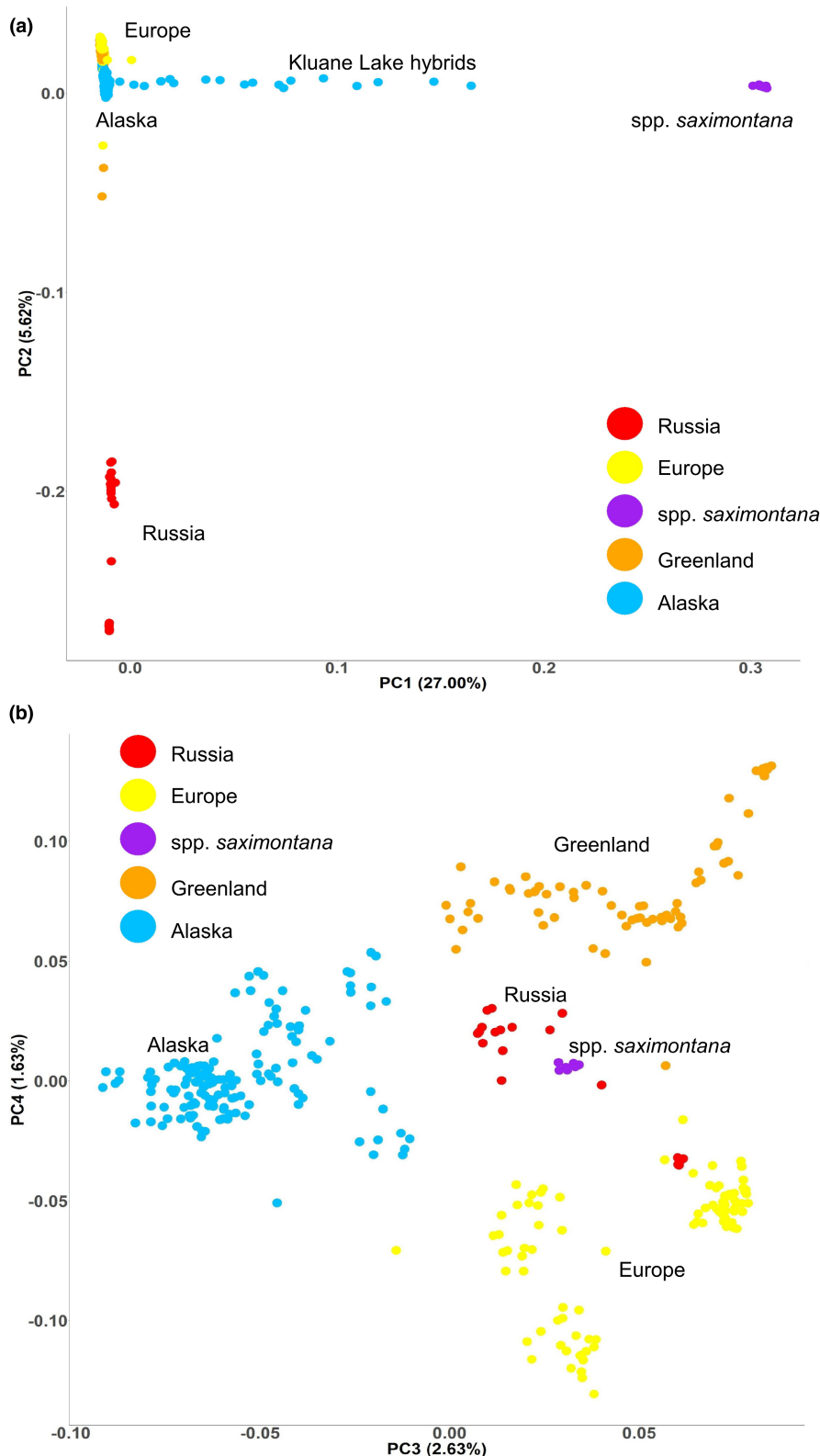
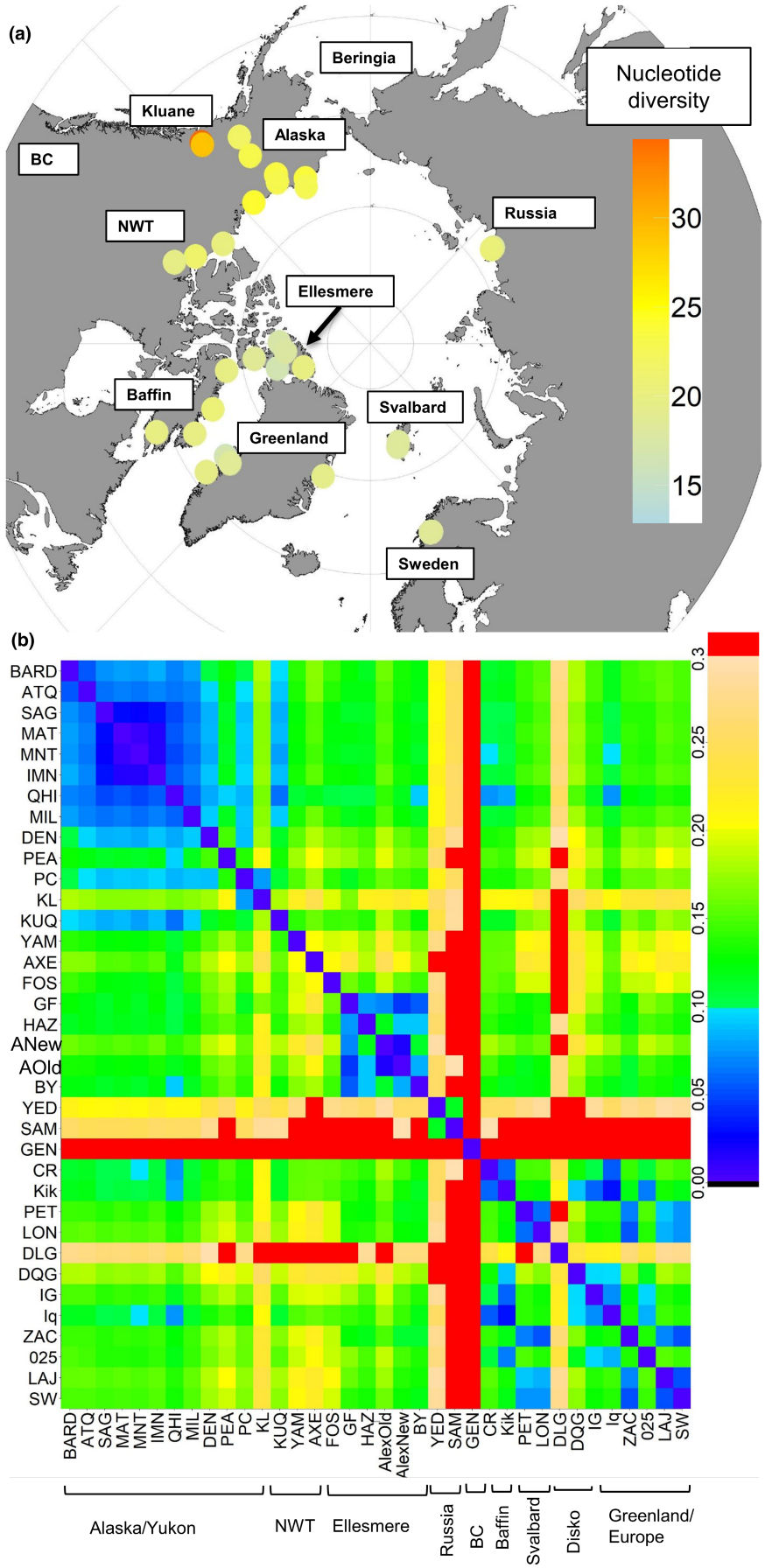


FIGURE 4 Principal component analysis (PCA) run on 349 *Cassiope tetragona* individuals. Individuals are colored according to their ADMIXTURE group (outgroup *C. mertensiana* is excluded). Percentage of variance accounted by each component shown in label. (a) The second principal component (PC2) versus PC1. (b) PC4

interglacial and interglacial (Fig. 2a, b, S1), supporting the 1p. 10e-1 = 60 m2a), (Followed by Europe and Alaska, followed by Russia, Europe and Greenland. Divergence of 0.10 ± 0.01 (Fig. 2b, S1). The lower consistent with genetic differentiation between Europe and Alaska may (F). In our *saximontana* dispersed first from Alaska through the Canadian *saximontana* about 5.4 ± 0.7 Ma. (Russia divergence times between the four models

FIGURE 1 Population status (sum of within-nucleotide diversity) mapped for each population (Table S3). See names for location information (PlotSvalbard page (b)). Values for all populations (ordered by Barrow, Alaska to Svalbard, Alaska to Svalbard) are shown in red (Bristolmontana population).



[illegible]

3.5 Flow cytometry (genome size) (kya). Moreover, using historical

We determined the genome size of *ibosttptbpu\$aread\$ingouand\$onx\$pa\$isking\$e* Island, Greenland (DLG and DQG) *dis\$ct\$et\$y\$wh\$e\$th\$e\$rh\$ag\$e\$if\$pre\$po\$et\$ion\$of* ploidy was caused by the *ble\$it\$ve\$E\$th\$e\$sp\$re\$cy\$A\$e\$e\$ad\$ra\$Fi\$ord\$pop\$ulation\$of* populations. Flow [Table S11](#) *h\$ow\$C\$e\$sh\$e\$e\$qu\$ivalent\$historic\$pop\$ulations\$fo\$* *tragona* has a haploid genome size of roughly 1.6 Gbp and there were no clear genome size differences between the two populations on Disko Island.

3.6 Historic samples

[illegible]

4 | DISCUSSION

Using GBS data from *Cassiope tetragona* and AFLPs, some studies have used historic samples to detect whether there has been a single event or multiple introductions. For example, *Salix herbacea* was found to have four *tetragona* lineages that diverged from one another in the last 1000–1500 kya (Russia) and 60–600 kya (Greenland) (2017). *Salix herbacea* is a common species in the Arctic region, and it is a potential refugia for other species (2015; 2012; 2012). The strong evidence of a single event between Europe and North America (2008; 2008; 2013). A preserved between Europe and North America (2007) using AFLP markers to identify the point of divergence. The resolution of the *tetragona* population was most successful. This supports previous evidence that after the LGM from a Alaskan/Ber Alaskan *Salix herbacea* found evidence supporting the presence of a single event.

$K = 5$ and 10, most of these recent mutations have originated in Alaska and are not widespread in the other populations. The age present in northern populations is consistent with the fact that extant populations carry more Alaskan ancestry than do other populations. Possible explanations for this include (1) dominant wind currents are still present from Alaska (Houlahan & MacIsaac 2017; MacIsaac 2021), (2) glacial retreat continues to provide corridors for gene flow, (3) establish (Al 2021), (4) sexual reproduction may have been less frequent during colder growing seasons, and (4) to allow foreign genotypes to enter the system (Bir 1966; Malca 2002; McG 1985). In addition, increased Alaskan ancestry in Alexandria, the last valley, suggests that the genotype from Alaska is spreading to other genotypes, gradually replacing them.

To determine whether missing data caused this difference between historical samples, we re-analysed the data and re-estimated any individuals with missing data. More than 10% of SNPs (Res Table S4). We observed slightly higher homozygosity in the two historic populations, which could be a result of allele dropout due to DNA degradation and more variation at restriction enzyme sites preventing DNA amplification (Hov 2016). It seems unlikely to completely explain the difference between Alaskan SNPs in the historic samples.

5 | CONCLUSIONS

We provide strong evidence for the presence of a unique lineage in central/western North America, possibly a secondary, younger refuge. Beringia was a refugium for North America's unique lineage that also survived the glacialiations in the Arctic biome. The Arctic biome is logistically challenging, and many of the genomic resources available are limited to a few species. With the Arctic climate rapidly shifting as well as to herbivores in lower latitudes, it is important to establish a network of genomic studies, such as the International Genomic Network (IGN), to allow widespread sampling and data collection for genomic studies, reducing some of the logistical constraints.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Sequence data are available in the GenBank database under accession numbers: 1222726 and 1222727. Detailed data processing notes and scripts are available at <https://github.com/Elphinstone/ArcticGenomics>.

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