NRC Research Press

ARTICLE

Environment drives spatiotemporal patterns of clonality in white spruce (*Picea glauca*) in Alaska

David G. Würth, Pascal Eusemann, Mario Trouillier, Allan Buras, Andreas Burger, Martin Wilmking, Carl A. Roland, Glenn P. Juday, and Martin Schnittler

Can. J. For. Res. Downloaded from cdnsciencepub.com by UNIVERSITY OF ALASKA-FAIRBANKS on 11/19/20
For personal use only.

Abstract: Many plant species reproduce by cloning if environmental conditions are unfavorable for sexual reproduction. To test the alternative hypotheses, whether cloning is an "exit strategy" or caused by selection, clonal growth in white spruce (*Picea glauca* (Moench) Voss) was investigated in three stands in Alaska, each consisting of a core (closed forest) plot and an edge (tree-line) plot. In total, 2571 trees were mapped and genotyped with 11 single sequence repeat (SSR) markers. The proportion of clonal trees follows a moisture gradient and was lowest in the dry Interior basin (4.5%), followed by the sites at the Alaska Range (9.0%) and the Brooks Range (21.7%). At the two latter sites, clonal growth was more frequent in the edge plot. A comparison among 960 aged trees revealed that clonal growth becomes more likely with increasing age and continues over the life span of a tree. Genetic data do not indicate any genetic predisposition for cloning. Clonal growth in white spruce most likely takes place via layering and depends on environmental conditions. Because performance of the trees, and therefore likely plant reproductive success, is lower in plots with a high proportion of clones, selection for clonal growth seems to be highly unlikely.

Key words: boreal forest, climate change, clonal growth, microsatellites, *Picea glauca*.

Résumé : Plusieurs espèces végétales se reproduisent par clonage lorsque les conditions environnementales ne sont pas favorables à la reproduction sexuée. Pour tester les hypothèses alternatives selon lesquelles le clonage est une « stratégie de sortie » ou est provoqué par la sélection, la croissance clonale de l'épinette blanche (*Picea glauca* (Moench) Voss) a été étudiée dans trois peuplements de l'Alaska, dans chacun desquels on a établi une placette d'intérieur (forêt fermée) et une placette de bordure (limite des arbres). Au total, 2571 arbres ont été cartographiés et génotypés à l'aide de 11 marqueurs SSR. La proportion d'arbres clonaux suit un gradient d'humidité et était la plus faible dans le bassin intérieur sec (4,5 %), suivi par les stations de la chaîne de l'Alaska (9,0 %) et de la chaîne de Brooks (21,7 %). Dans ces deux dernières stations, la croissance clonale était plus fréquente dans la placette de bordure. Une comparaison parmi 960 vieux arbres a révélé que la croissance clonale devient plus probable avec l'âge et se poursuit tout au long de la vie d'un arbre. Les données génétiques n'indiquent aucune prédisposition génétique au clonage. La croissance clonale de l'épinette blanche se fait fort probablement par marcottage et dépend des conditions environnementales. Étant donné que la performance des arbres, et donc probablement leur succès de reproduction, est plus faible dans les placettes ayant une proportion élevée d'arbres clonaux, la sélection pour la croissance clonale semble être hautement improbable. [Traduit par la Rédaction]

Mots-dés : forêt boréale, changements climatiques, croissance clonale, microsatellites, *Picea glauca*.

Introduction

The majority of vascular plant species employs two reproductive options, sexually via pollen and seeds and vegetatively by cloning. Clonal reproduction can be achieved through plant structures fragmenting mechanically or by decay (stolons, rhizomes, root suckers), through vegetative diaspores (e.g., bulbils, turions), or through agamospermy via seeds with vegetatively derived embryos (Hörandl and Paun 2007). Although very different in terms of achieved dispersal distances, all of these mechanisms result in genetically identical individual plants. Cloning is a common strategy among many plants (e.g., in two-thirds of all central European vascular plant species; Klimešová and De Bello 2009) due to several major advantages.

First, cloning is a backup strategy for harsh environments where sexual reproduction is at risk (Nichols 1976; Bostock 1980). Second, spatial expansion by clonal growth can increase sexual fitness if it brings pollen within reach of other genotypes (Matsuo et al. 2014), which depends on the dispersal features of pollen and the distances bridged by clonal growth (stands for all of these trees originating from vegetative reproduction) and on the degree of intermingling between clones (Somme et al. 2014). Model calculations (Van Drunen et al. 2015) demonstrated a fitness gain for higher clonality in cases of spatially restricted dispersal of pollen and especially seeds; however, mating interference (Vallejo-Marín et al. 2010) may lead to inbred offspring. Similarly, disruption of sexual polymorphisms can lead to unbiased ratios of mating groups (Barrett 2015). Both processes should decrease sexual fitness.

Received 1 June 2018. Accepted 5 September 2018.

D.G. Würth, M. Trouillier, A. Burger, M. Wilmking, and M. Schnittler. Institute of Botany und Landscape Ecology, Ernst-Moritz-Arndt University Greifswald, Soldmannstr. 15, 17489 Greifswald, Germany.
P. Eusemann. Institute of Forest Genetics, Thuenen Institute, Eberswalder Chaussee 3a, 15377 Waldsieversdorf, Germany.
A. Buras. Chair of Ecoclimatology, TU Munich, Hans-Carl-von-Carlowitz Platz 2, 85354 Freising, Germany.
C.A. Roland. Denali National Park and Preserve, P.O. Box 9, Denali Park, AK 99755, USA.
G.P. Juday. Forest Sciences Department, University of Alaska Fairbanks, Fairbanks, AK 99775, USA.
Corresponding author: David G. Würth (email: david.wuerth@uni-greifswald.de).
Copyright remains with the author(s) or their institution(s). Permission for reuse (free in most cases) can be obtained from RightsLink.

Can. J. For. Res. Downloaded from cdnsciencepub.com by UNIVERSITY OF ALASKA-FAIRBANKS on 11/19/20
For personal use only.

For taxa that have lost the capability for sexual reproduction, cloning is the only mode of reproduction (Pfeiffer et al. 2012; James and McDougall 2014). In regions where natural disturbances are likely to severely damage trees, cloning may help to regenerate them. If conditions for pollinators are unfavorable, cloning helps because no pollination is needed. This might explain why clonality often increases towards the margin of a species range (Silvertown 2008; Klimešová and Doležal 2011) where the environment may be harsher compared with the optimum conditions for a species.

Selection for clonal growth should occur as long as a clone produces more offspring or persists longer than its singleton peer (Pan and Price 2002); however, a trade-off appears to occur because clonal growth diverts resources away from sexual reproduction (Van Drunen and Dorken 2012), which can be compensated for only by enhanced resource capture. If increased sexual fitness indeed selects for increased rates of cloning, selective effects should occur especially within the main range of a species where the conditions for sexual reproduction are optimum. In contrast, if cloning serves as a backup strategy to increase survival or replaces missing sexual reproduction, it should be more advantageous at the margin of the range.

To test these hypotheses, we use data from a massive genotyping effort in Alaskan white spruce (*Picea glauca* (Moench) Voss). As with black spruce (*Picea mariana* (Mill.) B.S.P. (Légère and Payette 1981; Payette et al. 1994; Laberge et al. 2001; Viktora et al. 2011), white spruce has been shown to grow clonally in certain circumstances (Walker et al. 2012; Wilmking et al. 2017), most likely to endure periods unfavorable for sexual reproduction. We compared edge and core populations at latitudinal, elevational, and drought-controlled tree lines to answer the following questions. Is cloning ubiquitous throughout the range of white spruce in Alaska? Does it occur in all life stages of a tree? Is cloning more common in more extreme habitats? What are the drivers of cloning in white spruce?

Material and methods

Study species

White spruce is a common tree-line species and one of the most widely distributed conifers in North American boreal forests, occurring across the continent from Newfoundland and Labrador to Alaska. It reaches ca. 69°N at its northernmost stands in the Northwest Territories, Canada; the southern edge of the contiguous distribution is marked by the Great Lakes at about 44°N. Its elevational distribution ranges from sea level to 1520 m (Burns and Honkala 1990). While the tree line in eastern North America is mainly formed by black spruce (Lavoie and Payette 1992; Gamache and Payette 2004), white spruce takes over as the primary tree-line-forming species in western North America (Payette and Filion 1985; Lloyd et al. 2005). This species is widely favored for timber production in Canada and the United States and is one of the most important commercial species in the boreal forest (Burns and Honkala 1990).

Study sites

We established three study sites: (1) at the elevational tree line in the Alaska Range; (2) at a moisture-limited tree line on a south-facing bluff near Fairbanks in Interior Alaska, and (3) at the latitudinal tree line in the Brooks Range. All study areas were located on south-facing slopes, and each plot was laid out parallel to the slope. In each study area, two plots with at least 300 trees were selected for sampling. Initially, the plots were designed for an area of 1 ha (100 × 100 m), but if less than 300 trees were found in this plot size, we increased the area. A core plot was established in a closed canopy forest below the tree line, and an edge plot was established at the tree line (Table 1). In the Alaska Range, a large saddle separated the core and edge plots, which were about 1.3 km apart. In the Brooks Range, core and edge plots were situated at a steep slope and are only about 30 m apart. The drought-controlled tree-line site in the Interior basin consisted of the upper slope of a south-exposed bluff of the Tanana River (edge) and a mature, closed canopy forest site (core). Both plots are part of the Bonanza Creek LTER site and about 7 km apart (Viereck et al. 1986; Juday 2012). Monthly climate data were obtained from the Scenarios Network for Alaska + Arctic planning (SNAP; https://www.snap.uaf.edu/) as gridded data for each of the three sites.

Within each plot, all trees were mapped using a Trimble R3 differential GPS device (Trimble) attaining a mean precision of 0.48 m in floating mode. Needles for DNA extraction were collected from all living trees and dried and stored on silica gel. Tree height and, if applicable, diameter at breast height (DBH) were recorded using a Suunto PM-5 clinometer and a measuring tape, respectively. The basal diameter of the crown and the height of its lowermost living branch above the ground were measured for each tree (see https://figshare.com).

Age of the trees was determined by three methods: (1) coring; >96% of the older trees with a DBH exceeding 5 cm were cored at 20–50 cm height for age determination; the age derived from these cores was corrected for (*a*) deviation from pith and (*b*) height of coring (this was not possible for the Interior basin core plot as we did not have permission to core trees within the LTER); (2) a height–age relationship was established for young trees below 1.4 m (breast height); 20–40 trees just outside of each plot were cut at ground level and aged (see Table 1), and the resulting relationship was used to estimate the age of young trees in the plots (due to National Park regulations, we could not cut trees in the Denali National Park (Alaska Range), so we assumed seedling height growth per year as the mean between the Brooks Range and Interior basin sites; (3) for the remaining medium-sized tress (usually taller than 1.4 m but less than 5 cm DBH), we estimated the age from the relationship of DBH to age obtained by a linear regression of a scatterplot including the first two cohorts. These methods were applied for 65.2% (method 1), 18.5% (method 2), and 16.3% (method 3) of all trees. In addition, we calculated the height–age ratio for each tree with available age data to obtain a comparable proxy for the growth rate.

DNA extraction and microsatellite analysis

About 70 mg of needle tissue was homogenized using a Retsch ball mill MM301 (Retsch). DNA was extracted using the Invisorb Spin Plant Mini Kit (Stratec) following the manufacturer's protocol. We analyzed 11 microsatellite loci developed by Hodgetts et al. (2001) and Rajora et al. (2001). For the Alaska Range and the Brooks Range, these primers were combined into three multiplex assays amplifying several loci simultaneously, developed by Eusemann et al. (2014). For the Bluff plot, the three multiplex systems were combined into two multiplex systems (Supplementary Table S1).[1] PCRs were performed on Eppendorf Mastercycler thermocyclers (Eppendorf) using the Qiagen Multiplex PCR Plus Kit (Qiagen) and a modified protocol in a total volume of 10 μL. Each reaction contained 1× Multiplex PCR Plus buffer (Qiagen), 0.2 μM of each primer, and 20 ng DNA. For each assay, a primer mix containing 2 μM of all primers used within the respective assay was prepared. An equimolar concentration of 0.2 μM produced balanced signals for all loci within an assay. PCR conditions were as follows: a cooling step of 5 min at 4 °C while the lid of the thermocycler heats up, a denaturing and polymerase activation step of 5 min at 95 °C, followed by 30 cycles of 95 °C for 30 s, annealing at 60 °C for 90 s, elongation at 72 °C for 30 s, and a singular final extension at

[1]Supplementary Table S1 is available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfr-2018-0234.

Can. J. For. Res. Downloaded from cdnsciencepub.com by UNIVERSITY OF ALASKA-FAIRBANKS on 11/19/20
For personal use only.

Table 1. Characteristics of the six mapped and genotyped stands of *Picea glauca*.

	Alaska Range		Interior basin		Brooks Range	
Region	Core	Edge	Core	Edge	Core	Edge
Longitude (dd.ddddd°W)	149.01000	149.00972	148.28077	148.30889	149.74615	149.75306
Latitude (dd.ddddd°N)	63.72472	63.73667	64.76661	64.70361	67.94564	67.94639
UTM Easting	6W 400880	6W 400680	6W 439080	6W 437660	6W 384960	6W 384440
UTM Northing	7067650	7069040	7183060	7176070	7539360	7539940
Elevation (m a.s.l.)	802	1008	406	180	876	924
Exposition	South	South	South	South	South	South
Precipitation (mm)	38.7		24.9		36.3	
Temperature (°C)	–3.04		–2.71		–7.75	
No. of trees sampled	380	313	677	584	470	358
No. of trees genotyped	352	303	640	529	419	328
No. of trees in clones	20	39	32	20	79	83
Size of largest clone	2	13	2	2	7	14
No. of clones	10	10	16	10	27	23
Mean clone size	2.00±0.00	2.97±4.60	2.00±0.00	2.00±0.00	3.20±2.18	5.62±4.91
Proportion of clonal trees	0.06	0.13	0.05	0.04	0.19	0.25
Clonality (C)	0.03	0.10	0.03	0.02	0.12	0.18
AMOVA (%)	1.2	0.0	1.2	0.3	3.2	0.3
No. of trees cored and aged	175	165	5	299	324	123
No. of trees with estimated age	196	150	495	257	142	215
Average DBH ± SD (cm)[a]	15.5±14.1	5.6±5.9	8.5±15.1	9.7±9.1	8.8±8.7	4.8±6.4
DBH of largest tree (cm)	68	29	59	37	45	29
Height–age ratio	0.071	0.055	n.d.	0.114	0050	0.047
Average age ± SD (years)	106±71	55±39	n.d.	55±35	94±73	60±54
Age of oldest tree (years)	353	188	n.d.	129	444	254
Mean age of clonal trees (years)	65±63	61±40	n.d.	53±32	164±70	123±56
Mean age of singleton trees (years)	106±71	54±38	n.d.	58±33	86±71	40±34
Height correction for age (years per cm height)	3.00	3.00	n.d.	3.40	3.40	2.39
Lowermost twig: height above the ground (m)	0.69±0.55	0.23±0.24	n.d.	0.93±1.34	0.62±0.74	0.29±0.46

Note: Coordinates (WGS84) of the lower left edge are given for each plot. Precipitation and temperature are the monthly means for the period 1901–2009; results for analysis of molecular variance (AMOVA) depict the percent genetic diversity between clonal and singleton trees; a.s.l., above sea level; DBH, diameter at breast height; SD, standard deviation; n.d., not determined.

[a]Only trees above 1.5 m height considered.

68 °C for 10 min. PCR products were diluted 1:5 in ddH_2O. Fragment analysis was carried out on a 3130×l Genetic Analyzer (Life Technologies) using 1 μL of diluted PCR product, 0.15 μL of 500 GeneScan LIZ size standard, and 8.85 μL HiDi Formamide (both Life Technologies). Fragment size determination and binning were performed using Peak Scanner software (Life Technologies), Genemapper 5.0 (Applied Biosystems), and Allelogram (Morin et al. 2009).

Genotyping and population genetic analyses

Clones were determined by identification of identical multilocus genotypes (MLG) using GENALEX 6 (Peakall and Smouse 2006). To account for genotyping errors, we additionally used the algorithm described in Schnittler and Eusemann (2010). This allowed variable thresholds to be set for genotyping errors (i.e., MLGs with 0, 1, 2,… deviating alleles assigned to a clone) and a histogram to be constructed for 1 to *n* deviating loci for all pairwise combinations of trees to derive the optimum value for this threshold. For all analyses based on microsatellite data, only trees showing not more than two loci with null alleles or genotyping errors were considered (counted as successfully genotyped).

As clonal diversity measures, we calculated $R = (G - 1)/(N - 1)$, with G being the number of genotypes and N being the number of sampled trees, and clonality (C), its opposite parameter, being $1 - R$ (Dorken and Eckert 2001), as well as the proportion of trees belonging to clones within the plot. Probability of identity (PID) was calculated using GENALEX 6, and null allele frequencies were calculated using GENEPOP 4.0 (Rousset 2008). The number of trees per clone and tree age were compared between core and edge plots using the Wilcoxon test. To test for genetic predisposition of clonal growth, a PCA for all microsatellite genotypes was carried out separately for the three study regions (Brooks Range, Interior basin, Alaska Range) using PC-ORD (McCune 1986), and the genotypes belonging to trees with clonal growth were mapped over the plot of sample scores. For this procedure, each clone was considered only once, and loci with null alleles were replaced by the most frequent allele at the respective locus. In addition, a second PCA was performed with only individuals showing no null alleles or genotyping errors at any locus.

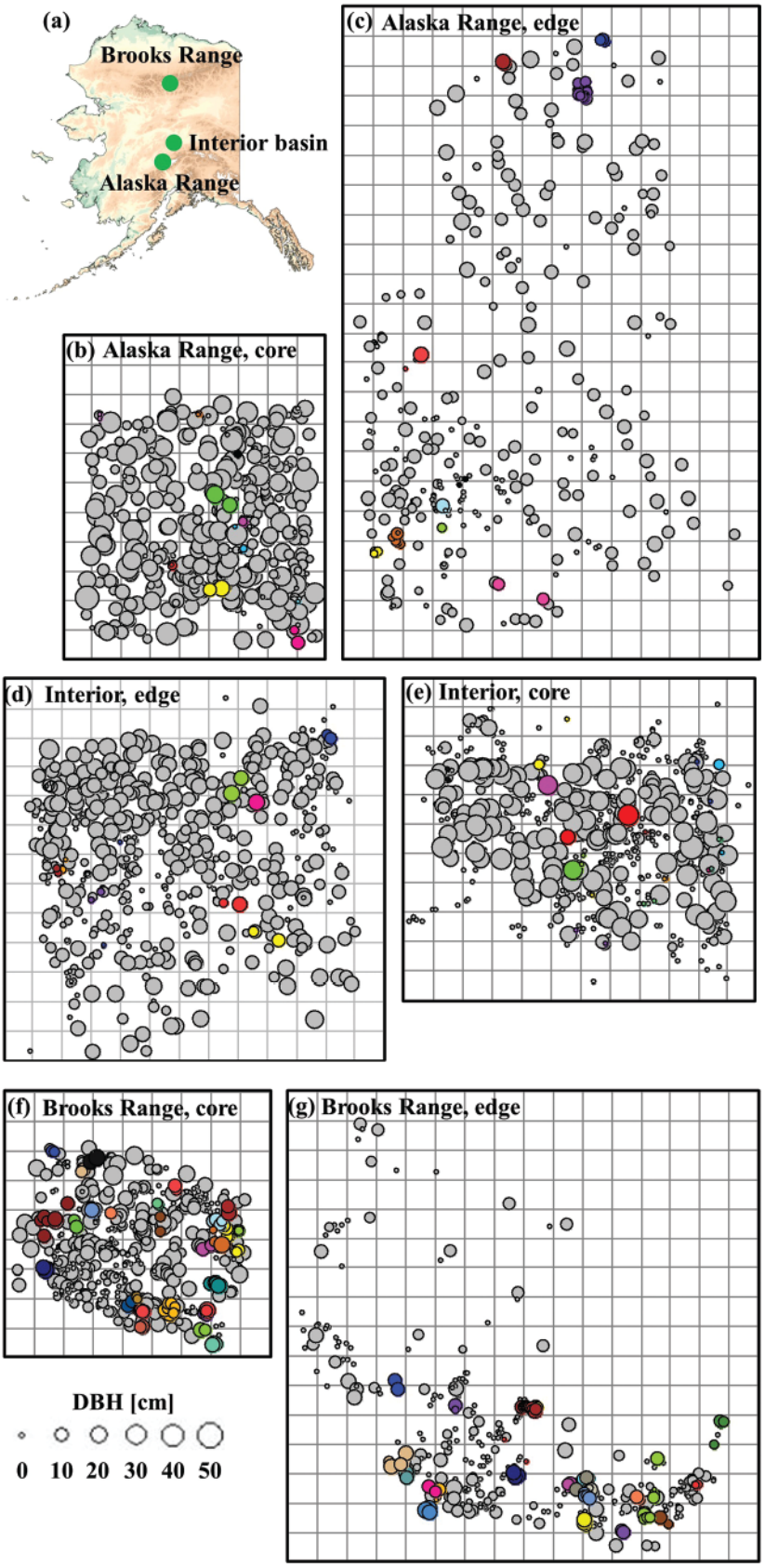
To test for genetic clustering of clonal trees among all genotypes, the difference of the centroids for the first three axes between nonclonal genotypes and clonal genotypes was calculated. A Mantel test, selecting randomly 999 times the same number of clonal genotypes as recorded for the study region, was used to calculate confidence intervals (CI) for the average distance between centroids and compared with the figure calculated from actual clonal genotypes. In addition, the calculation of F_{ST} (Wright 1949) and Dest (Jost 2008) values and an analysis of molecular variance (AMOVA) using GENALEX 6 were carried out, treating singleton and clonal trees as two separate populations, allowing a calculation of the proportion of genetic diversity (*i*) between plots and (*ii*) between trees belonging to a clone and those growing as singletons. A Wilcoxon test was performed to test for age differences between clonal and singleton trees and for differences in the proportion of clonal trees between sites because our data followed non-normal distributions. Tests were performed for all plots separately using R 3.4.0 (R Core Team 2017).

Results

Genetic diversity and plot structure

In total, 2782 trees in three paired plots (see Fig. 1*a*) were mapped; 92% of these were successfully genotyped (the remaining 8% failed due to poor sample quality not allowing successful

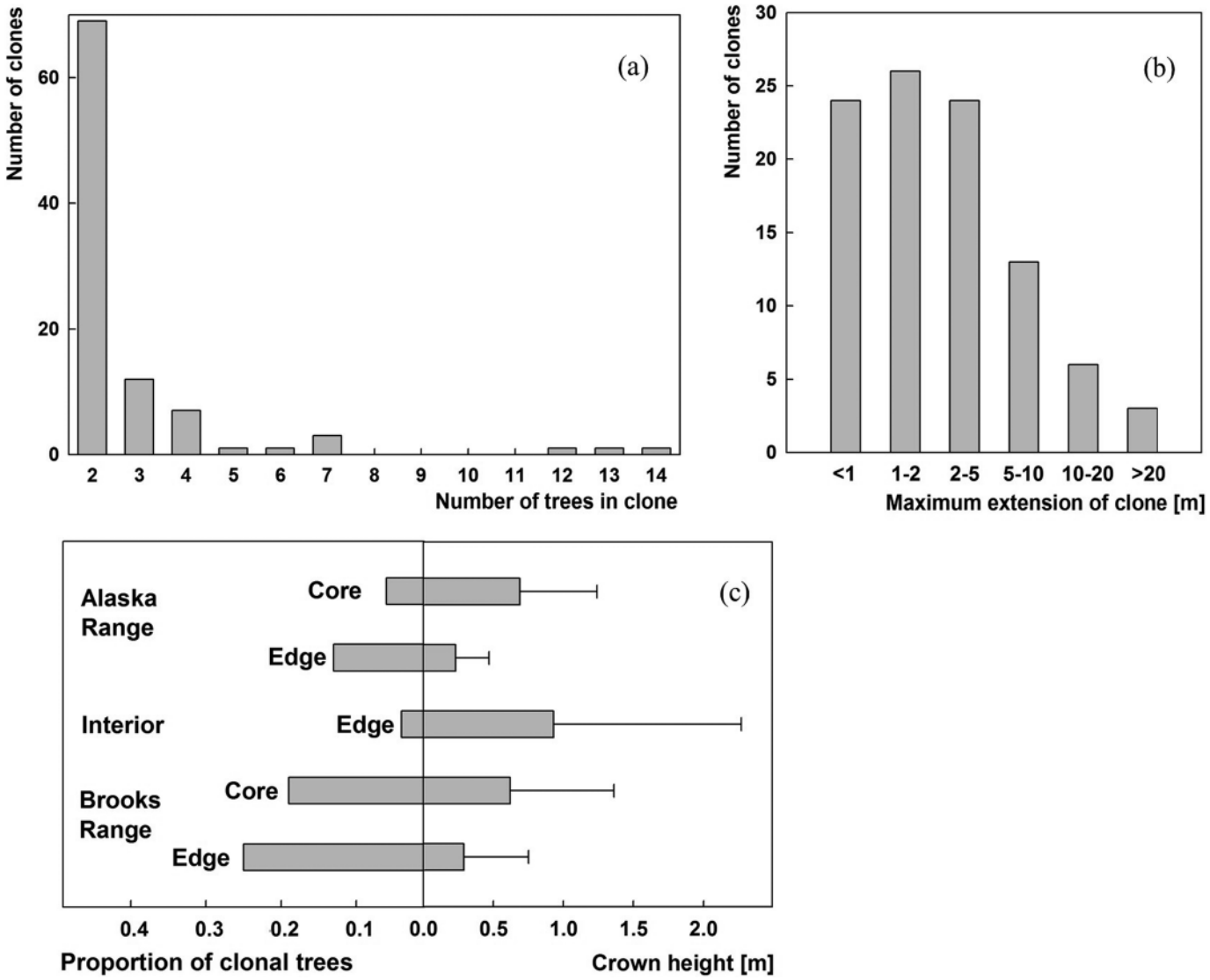
Fig. 1. (*a*) Map of Alaska (U.S. Geological Survey 2016) showing location of the investigated stands (dots). (*b*–*g*) Maps of the six investigated stands drawn to scale (one grid cell = 10 × 10 m), showing trees belonging to clones (differently colored circles) and singleton trees (light gray circles). The size of the circle reflects the tree diameter at breast height (DBH) (see scale).



Can. J. For. Res. Downloaded from cdnsciencepub.com by UNIVERSITY OF ALASKA-FAIRBANKS on 11/19/20
For personal use only.

Can. J. For. Res. Downloaded from cdnsciencepub.com by UNIVERSITY OF ALASKA-FAIRBANKS on 11/19/20
For personal use only.

Fig. 2. Histograms of (*a*) the number of trees in a clone and (*b*) the maximum extension of clones (maximum distance of the pairwise comparison of all members in clone). (*c*) Mean crown height (distance of lowermost twigs to the ground) of trees in relation to the proportion of clones in a plot (no data are available for the Interior basin core plot).



extraction of DNA). This resulted in 2571 successfully genotyped trees and a total of 339 alleles detected across the 11 microsatellite loci. We repeated about 10% of all samples to check for genotyping errors, but obtained an error rate of zero (the multilocus genotypes were identical). Allele numbers ranged from 8 to 68 per locus, with a mean of 30.8. This high variability resulted in high exclusion probabilities with a PID computed over all loci of 1.34×10^{-15}. We identified a total of 96 clones (Fig. 2) using a threshold of two scoring errors (a maximum of two alleles that did not match). The clonal identity of 223 trees was unambiguously identified (all 11 microsatellite loci are identical for the clone), whereas 40 trees differed in one locus, and 10 trees differed in two. Of the 50 mismatches (a tree deviated at one or two loci from the genotype of the clone), 70% involved a homozygous locus (which may contain a hidden null allele) or a visible null allele.

The investigated plots are genetically diverse but not genetically differentiated: even between the plots in the Alaska Range and the Brooks Range, separated over a distance of ca. 800 km, F_{ST} and Dest values do not exceed 0.061 and 0.234, respectively (Table 2*a*). When comparing clonally growing and singleton trees with each other, F_{ST}, G_{ST}, and Dest values show no differentiation between the two groups (Table 2*b*). The AMOVA resulted in a maximum value of 3.2% for the genetic diversity found between clone members and singleton trees (Table 1).

Clones in white spruce

In the six plots, we identified a total of 96 clones, including 273 trees among 2571 genotypes; on average, 3.8% of all trees formed clones. Clonal growth occurred in all investigated plots but in different proportions. Cloning was more prevalent in edge plots than in core plots (Table 1). For the regions, the number of trees belonging to clones was highest in the Brooks Range, followed by the Alaska Range, and lowest in the Interior basin (Table 1; Figs. 1*b*–1*g*). Table 1 shows the most important climatic parameters (period 1901–2009) for the three regions (mean temperature, calculated from monthly means, and mean precipitation).

Most of the clones comprise only two or three trees (Fig. 2*a*). The three clones with more than 10 trees are all from edge plots (one in Alaska Range and two in Brooks Range). Clones were of limited size: 95% of the clones did not exceed 13.8 m between the two most distant trees; 90% were below 8.4 m. The PCA of the 11 microsatellite loci revealed a distance different from zero for all three

Can. J. For. Res. Downloaded from cdnsciencepub.com by UNIVERSITY OF ALASKA-FAIRBANKS on 11/19/20
For personal use only.

Table 2. (*a*) F_{ST} values (lower left) and Dest values (upper right) between the six different stands (see Table 1 for sample sizes); (*b*) F_{ST}, G_{ST} and Dest values between clonal growing trees and their singleton counterparts.

(*a*) F_{ST} values (lower left) and Dest values (upper right) between the six different stands.

	Alaska Range		Interior basin		Brooks Range	
	Core	Edge	Core	Edge	Core	Edge
Alaska Range						
Core	—	0.050	0.066	0.083	0.232	0.186
Edge	0.016	—	0.069	0.068	0.190	0.136
Interior basin						
Core	0.023	0.021	—	0.034	0.232	0.170
Edge	0.029	0.021	0.008	—	0.234	0.173
Brooks Range						
Core	0.061	0.047	0.059	0.061	—	0.051
Edge	0.054	0.037	0.047	0.049	0.013	—

(*b*) F_{ST}, G_{ST}, and Dest values between clonal growing trees and their singleton counterparts.

	Alaska Range		Interior basin		Brooks Range	
	Core	Edge	Core	Edge	Core	Edge
F_{ST}	0.025	0.015	0.012	0.014	0.016	0.007
G_{ST}	0.007	–0.003	0.000	–0.004	0.009	–0.001
Dest	0.035	–0.019	0.000	–0.025	0.076	–0.010

regions between the centroids of the sample scores for singleton and clonal trees, but this was nearly always within the confidence interval for the bootstrapped distances (Alaska Range, observed 0.541, bootstrapped CI 5% 0.178, mean 0.452, CI 95% 0.798; Interior basin, observed 0.569, bootstrapped CI 5% 0.119, mean 0.309, CI 95% 0.552; Brooks Range, observed 0.477, bootstrapped CI 5% 0.127, mean 0.337, CI 95% 0.587). Neither PCA nor AMOVA (Table 1) showed significant differences between MLGs of clonal and singleton trees.

Tree traits and clonality

Trees differed strongly in shape and age between plots. In the dry Interior basin, the lowermost branches of a tree occur, on average, ca. 0.9 m (data only for the edge plot available) above the ground. In the two mountain ranges, lowermost branches were ca. 0.6 m above the ground in the core plots, but only 0.3 m above the ground in the edge plots (Table 1). The proportion of clonal trees was inversely correlated with the height of the lowermost branches ($r = -0.71$; not significant), although we can only call this a trend due to the lack of significance. Age as a covariable had no effect ($r = 0.01$) on this correlation: the lower the branches are, the more likely cloning becomes (Fig. 2*c*). As shown in Table 1, trees in the core plots are, on average, nearly two times older than those in the edge plots, and a similar pattern occurs for the oldest trees in the plots.

The age distribution of the trees (Fig. 3) shows pronounced peaks in recruitment. Judging by the slope of the height to age ratio (Table 1), trees grow fastest in the dry Interior basin (data only available for the edge plot), followed by the Alaska Range and then the Brooks Range. In the two mountain ranges, growth at the edge appears to be slower than in the core plots (which are both at lower elevations compared with the respective edge plots). Clones are older compared with singleton trees, especially in the Brooks Range, where the proportion of clonal trees is generally highest (Fig. 3).

Because nearly all living trees in a plot were genotyped, most of the young clone members were included. Age of the trees within a clone is very unevenly distributed: from the 80 clones with age data, the oldest and the youngest member differed by less than 20 years in 26 trees, by less than 40 years in 45 trees, and by less than 80 years in 53 trees, whereas in the remaining third, the maximum age differences reach up to 200 years. This holds true even more for the maximum age of the clone members, which ranges from 3 to 444 years, without any recognizable age peak.

For the five plots for which age data are available, there is a negative correlation ($r = -0.43$; $p < 0.05$) between the mean yearly increment (height to age ratio) and the percentage of clones within each plot. Clonal trees show significant differences ($p < 0.05$) in age between the Brooks Range plots and the Alaska Range plots, as well as between the Brooks Range plots and the Interior basin plots. There is no significant difference ($p > 0.05$) between the plots from the Alaska Range and between them and the Interior basin edge plots (Table 1). Within all plots except the Alaska Range edge plot and the Interior basin edge plot, age of clonal trees differs significantly from singleton tree age (Table 1). For the Interior basin core plot, no age data are available except for five trees, but the distribution of DBH data suggests two very pronounced age cohorts: an old-growth cohort (mean DBH 32.3 ± 10.7 cm, range 10.1–59.2 cm, $n = 165$) and a much younger cohort (mean DBH 1.0 ± 0.4, range 0.3–3.4 cm, $n = 45$, with additional 432 trees below 1.5 m height where a DBH could not be measured).

Discussion

White spruce appears to be capable of self-cloning at almost every age: the youngest clone found involved two trees less than 1.5 m in height estimated to be 21 and 22 years old, and the oldest clone comprised two trees of 424 and 262 years. It is worth noting, however, that the mean age of clonal trees was significantly higher than that of singleton trees (Table 1).

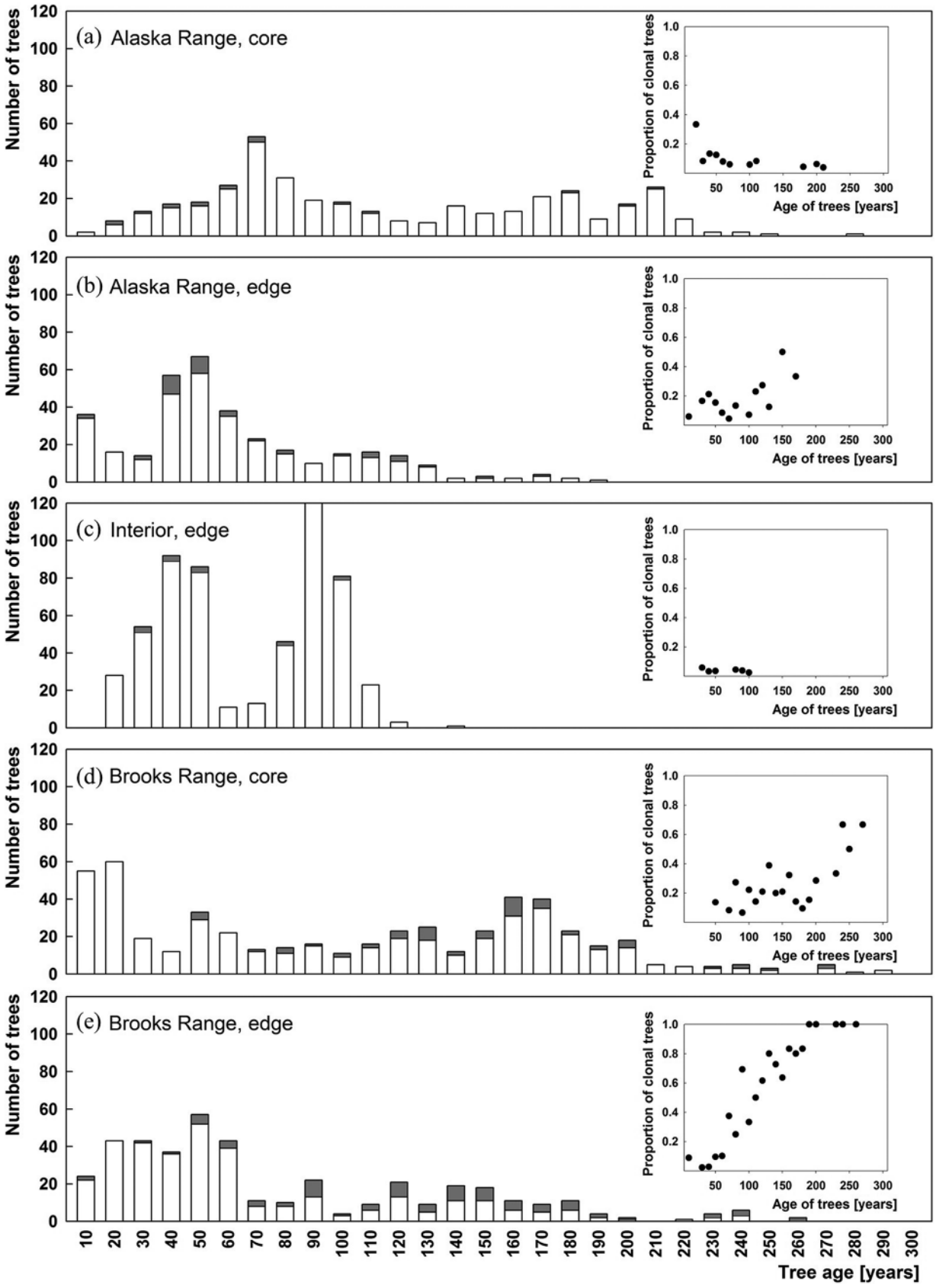
Potential mechanisms of clonal growth in white spruce

The proportion of clonal trees showed a negative correlation with the height of the lowermost living branches (measured as crown height above the ground; $r = -0.71$; not significant) in our sites. This result suggests that layering is the common mechanism generating clones. According to a study in the Canadian arctic, this mechanism is known for white spruce (Caccianiga and Payette 2006). Other possible mechanisms such as resprouting, root suckering (many Rosaceae and Salicaceae; Wiehle et al. 2009), or rooting of broken twigs (*Salix fragilis*; Beismann et al. 1997) seem to be fairly unlikely. A second possible mechanism includes fallen trees that may root with their apical branches; however, we noted only a very few fallen trees in the plots. Even the majority of the dead trees were still standing — most likely because the extremely narrow crown of white spruce is less vulnerable to toppling from snow or wind forces.

Colonization of tree lines in Alaska and age distribution

Both clonal and singleton trees are significantly younger in the edge plots at the Alaska Range and Brooks Range if compared with the respective core plots (Table 1). Although age data for the Interior basin core plot are not available, DBH data suggest a similarly pronounced age difference between forest and edge plots in the Interior basin. With trees showing maximum ages of 188 years (Alaska Range), 129 years (Interior basin), and 254 years (Brooks Range) within the edge plots, our data indicate that initial treeline advance does not date to recent decades and that complete re-establishment after disturbance did not occur following initial tree establishment. However, the medians of 47 years (Alaska Range), 71 years (Interior basin), and 40 years (Brooks Range) suggest that the majority of the tree colonization did indeed happen only recently, consistent with a recent process of "thickening" or increased stand density. It appears, however, that microclimate limits the successful establishment of seedlings, which, for its part, limits forest expansion.

Fig. 3. (*a*–*e*) Histograms of age distribution of all trees in (*a*) Alaska Range core, (*b*) Alaska Range edge, (*c*) Interior basin edge, (*d*) Brooks Range core, and (*e*) Brooks Range edge, with age data (classes of 10 years). Shaded portion of each bar indicates trees belonging to clones. Inset: proportion of clonal trees for the respective age classes.



Can. J. For. Res. Downloaded from cdnsciencepub.com by UNIVERSITY OF ALASKA-FAIRBANKS on 11/19/20
For personal use only.

Can. J. For. Res. Downloaded from cdnsciencepub.com by UNIVERSITY OF ALASKA-FAIRBANKS on 11/19/20
For personal use only.

Potential benefits of clonal growth for trees

Clonality, especially at high elevations and in harsh environments, seems most important as a factor to enhance an individual's survival chances (Kimura et al. 2013). Additionally, in the relatively harsh conditions extant in tree-line situations, facilitation effects may be operative: tree islands can have positive effects for survival and growth rates of their members (Körner 2012). Co-occurrence of high levels of facilitation and clonality in arctic and alpine environments is not uncommon, and clonality likely plays a role in regulating facilitation processes (Brooker 2017). Our data fit well into this pattern: the higher proportion of clonal trees in edge plots compared with core plots may indicate a shift from facilitation to competition from tree line to forest for tree stem recruitment and early survival, as postulated by Körner (2012). For white spruce in Alaska, the main factor limiting clonal growth seems to be the climate (see Table 1): cool and wet conditions favor clonal recruitment in contrast to drought-controlled sites. The Interior basin edge plot, situated at the edge of a bluff, has certainly the warmest and driest microclimate and shows the lowest proportion of clonal trees (Table 1). For individual growth of already established trees with adequate rooting depth, the warmer and drier conditions in the core plots (forest) appear to be more favorable than at the edge (tree line): in both Alaska Range and Brooks Range trees, the core plots, situated at lower elevations and more sheltered sites than edge plots, sustain greater radial growth. The negative correlation ($r = -0.43$; $p < 0.05$) between average increment (calculated as height to age ratio) and the percentage of clones within a plot indicates that the better the individual growth of the trees within a plot is, the less likely cloning becomes.

Clones may enjoy increased fitness by (*i*) increasing the lifetime of the genotype, (*ii*) producing more stems, allowing a higher seed output, and (*iii*) bridging larger distances for pollination. If clonal growth increases the persistence of a genotype, thus ensuring future seed production, a fitness advantage should be realized for such a genet (Fischer and Van Kleunen 2001; Pan and Price 2002; Douhovnikoff et al. 2004) as fitness is often estimated as lifetime reproductive success (Antonovics and Ellstrand 1984). If a multistemmed clonal individual of a tree produces more seeds, this may as well translate into a fitness advantage. Although we cannot assume that tree performance is correlated with reproductive output, our data suggest that a fitness advantage of clones, if it exists, is small. Increased pollination distances are unlikely to be of any importance, as boreal forest trees are usually wind-pollinated gymnosperms that can realize large distances for pollination (O'Connell et al. 2007), and this is strongly suggested by the low F_{ST} and Dest values in our data (Table 2*a*). In addition, the small average radius (3.9 ± 4.8 m) of the clones found in white spruce makes a fitness gain by increased pollination distances unlikely, especially when compared with data on pollen dispersal in closely related Norway spruce (*Picea abies* (L.) Karst.; Burczyk et al. 2004).

In contrast, clonal fitness may suffer from (*i*) resources diverted from sexual reproduction to clonal growth, (*ii*) competition between trees of a clone, and (*iii*) mating interference that leads to more selfed offspring, which appears to have fitness disadvantages (Charlesworth and Charlesworth 1987; Husband and Schemske 1996).

For trees, root suckers, which occur in different species, can bridge large distances (up to 40 m in *Populus euphratica* Oliv.; Wiehle et al. 2009). In contrast, branch layering or resprouting of fallen trunks, which seems to be the mechanism for white spruce, leads to much smaller clonal spread distances, because the crown radius of even the 25% quantile of the strongest trees (highest DBH values) is below 1.5 m and their height is below 12.7 m. Therefore, individual trees in white spruce clones are close together: we measured a mean distance between a member of a clone and its nearest clonal neighbor of 2.9 ± 5.2 m.

Ecological importance of cloning

For persistence of the species in a changing climate in Alaska through space and time, cloning can be of high ecological importance. There are several studies indicating that clones have survived since and even during the last glaciation (Kemperman and Barnes 1976; May et al. 2009).

Spruce has adapted to survive severe climate and can persist for hundreds of years by vegetative propagation (de Vernal and Hillaire-Marcel 2008); presumably this ability has been relatively more important during periodic unfavorable intervals in the past such as the "Little Ice Age" and other times when range contraction may have occurred in white spruce (Caccianiga and Payette 2006). The ability to reproduce by clones in combination with its facilitation effects should be beneficial for persistence, especially in marginal habitats. Snow accumulation could play an additional role in facilitation as shown for white spruce by Scott et al. (1993) and for shrubs by Sturm et al. (2001). One hypothesis put forward by MacDonald (1984) is that the apparently explosive surge of white spruce populations along the western interior "corridor" after the glacial retreat was initiated from small populations that persisted vegetatively in isolated localities with particularly favorable microclimates. Clonal growth might be the reason why white spruce trees, although in low densities, survived in glacial refugia in East Beringia (Brubaker et al. 2005) but also in Alaska (Anderson et al. 2006), as suggested by only trace amounts of pollen in lake sediments. The production of viable seeds in white spruce is extremely episodic, particularly in marginal tree-line habitats (Roland et al. 2014), with large cone and seed crops synchronized in time ensuring maximum seed output, especially after large-scale wildfires (Juday et al. 2003). Thus the ability to reproduce vegetatively is an important "stop-gap" that would allow persistence (and perhaps even encourage expansion) during extended intervals of low sexual reproductive output and thus prevent the decay of marginal populations (Caccianiga and Payette 2006).

Selection pressure for clonality in white spruce?

While clonality benefits a genotype directly, it is less suited as a long-distance colonizing strategy for trees, as both root suckering and layering is limited to the immediate vicinity of a tree. Rare exceptions in trees include broken branches in riparian species of willows and poplars, which may be washed away by floods and root far away from the mother tree (Densmore and Zasada 1978; Asaeda et al. 2011). In gymnosperms, clones always seem to be capable of only limited spatial expansion, and this certainly holds true for white spruce (Fig. 2*b*). Because of the small spatial scale of clones compared with distances bridged by pollen, detrimental consequences of mating interference should be negligible. Furthermore, this limits potential positive effects such as siring more offspring by increased pollination distances. Therefore, increased sexual fitness of clones, if it occurs at all, is most likely to be associated with the potential larger reproductive (seed) output of clones. The lifetime reproductive output of trees cannot be assessed easily, especially with the high fluctuations in seed output among years known for white spruce. If DBH is taken as a rough proxy for reproductive output, however, a significantly higher seed output of clones compared with a similar area of singletons seems to be unlikely. The last theoretical possibility for increased sexual fitness is a longer lifetime reproductive success, i.e., a longer persistence of clones compared with singletons. This is difficult to assess from our data, as the plots at the tree line are naturally younger than those in the forests (Table 1).

The white spruce stands investigated in this study, however, show an important feature that makes selection for cloning unlikely: trees grow best in plots where the proportion of clonal trees is lowest. Judged by the height–age relations, trees in the Interior basin edge plot grow 2.4 times faster than those at the edge plot in the Brooks Range, but judged from the proportion of clonal genotypes to all genotypes, trees at the Brooks Range tree

Can. J. For. Res. Downloaded from cdnsciencepub.com by UNIVERSITY OF ALASKA-FAIRBANKS on 11/19/20
For personal use only.

line have a 4.1-fold higher probability of belonging to a clone. This argues against selective pressure for cloning, as this mechanism works best where white spruce is at the range margin. The generally low F_{ST}, G_{ST}, and Dest values between the clones and their singleton counterparts (Table 2*b*) provide further evidence against selection. In addition, the low F_{ST} and Dest values between the six investigated plots do not indicate a genetic differentiation (Table 2*a*), in spite of the significant differences in the proportion of clonal trees. We therefore assume that the occurrence of clones is mainly determined by the environment.

It should be noted, however, that the microsatellite loci used in our study are neutral markers, which limits their suitability to investigate adaptive processes (Kirk and Freeland 2011). The ultimate proof for a selective advantage of clonal growth can only be demonstrated by monitoring reproductive success, i.e., fitness, of clones compared with singletons, and markers from sequences that are known to be under selection should reveal a differentiation between clonal and singleton trees.

Summarizing, we can state that clonality seems to be widespread in Alaskan white spruce populations, especially in tree-line populations, but it does not constitute the primary mode of reproduction. Clonality seems to be triggered by particular environmental conditions that favor layering. A genetic predisposition or selection for cloning is unlikely, as (*i*) the genetic differentiation of populations throughout Alaska is low, (*ii*) clones are not genetically distinct from singleton trees, and (*iii*) trees grow best and thus likely have the highest reproductive output where the proportion of clones is lowest; however, facilitation effects may be invoked to explain white spruce clonal growth, especially at the tree line. Environmentally induced cloning is not necessarily more common in harsh environments. For instance, clonal plants are not consistently more frequent in cold environments (Klimešová and Doležal 2011). The drivers for cloning most likely result from a mixture of phylogenetic constraints (the mechanism of cloning determines clone extension and the degree of intermingling of clones) and environmental conditions (advantages of cloning for plant regeneration and persistence) and are slightly different for each plant species.

Acknowledgements

This research was funded by the DFG Research Training Group RESPONSE (DFG GRK2010) and DFG Wi2680/8-1. The authors thank Carlos A. Martínez-Muñoz who contributed with preparation of needle samples and sequencing. The authors declare no conflict of interest. Author's contributions: M.W., D.G.W., and M.S. designed the study; D.G.W., P.E., M.T., A.B., M.W., and M.S. carried out the fieldwork; D.G.W. and P.E. performed the microsatellite analyses; D.G.W., P.E., and M.S. analyzed the data; G.P.J. supplied data for the Interior basin core plot; M.W., C.A.R., and G.P.J. contributed critically to the drafts. All authors gave the final approval for publication.

References

Anderson, L.L., Hu, F.S., Nelson, D.M., Petit, R.J., and Paige, K.N. 2006. Ice-age endurance: DNA evidence of a white spruce refugium in Alaska. Proc. Natl. Acad. Sci. U.S.A. 103(33): 12447–12450. doi:10.1073/pnas.0605310103. PMID: 16894151.

Antonovics, J., and Ellstrand, N.C. 1984. Experimental studies of the evolutionary significance of sexual reproduction. I. A test of the frequency-dependent selection hypothesis. Evolution, 38(1): 103–115. doi:10.1111/j.1558-5646.1984.tb00263.x. PMID:28556083.

Asaeda, T., Gomes, P.I.A., Sakamoto, K., and Rashid, M.H. 2011. Tree colonization trends on a sediment bar after a major flood. River Res. Appl. 27(8): 976–984. doi:10.1002/rra.1372.

Barrett, S.C.H. 2015. Influences of clonality on plant sexual reproduction. Proc. Natl. Acad. Sci. U.S.A. 112(29): 8859–8866. doi:10.1073/pnas.1501712112. PMID: 26195747.

Beismann, H., Barker, J.H.A., Karp, A., and Speck, T. 1997. AFLP analysis sheds light on distribution of two *Salix* species and their hybrid along a natural gradient. Mol. Ecol. 6(10): 989–993. doi:10.1046/j.1365-294X.1997.00273.x.

Bostock, S.J. 1980. Variation in reproductive allocation in *Tussilago farfara*. Oikos, 34(3): 359–363. doi:10.2307/3544296.

Brooker, R.W. 2017. Clonal plants and facilitation research: bridging the gap. Folia Geobot. 52(3–4): 295–302. doi:10.1007/s12224-016-9267-7.

Brubaker, L.B., Anderson, P.M., Edwards, M.E., and Lozhkin, A.V. 2005. Beringia as a glacial refugium for boreal trees and shrubs: new perspectives from mapped pollen data. J. Biogeogr. 32(5): 833–848. doi:10.1111/j.1365-2699.2004.01203.x.

Burczyk, J., Lewandowski, A., and Chalupka, W. 2004. Local pollen dispersal and distant gene flow in Norway spruce (*Picea abies* [L.] Karst.). For. Ecol. Manage. 197(1–3): 39–48. doi:10.1016/j.foreco.2004.05.003.

Burns, R.M., and Honkala, B.H. 1990. Agriculture handbook. *In* Agriculture handbook. Vol. 1: Conifers. U.S. Department of Agriculture, Washington, D.C.

Caccianiga, M., and Payette, S. 2006. Recent advance of white spruce (*Picea glauca*) in the coastal tundra of the eastern shore of Hudson Bay (Québec, Canada). J. Biogeogr. 33(12): 2120–2135. doi:10.1111/j.1365-2699.2006.01563.x.

Charlesworth, D., and Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. Annu. Rev. Ecol. Syst. 18(1): 237–268. doi:10.1146/annurev.es.18.110187.001321.

de Vernal, A., and Hillaire-Marcel, C. 2008. Natural variability of Greenland climate, vegetation, and ice volume during the past million years. Science, 320(5883): 1622–1625. doi:10.1126/science.1153929. PMID:18566284.

Densmore, R., and Zasada, J.C. 1978. Rooting potential of Alaskan willow cuttings. Can. J. For. Res. 8(4): 477–479. doi:10.1139/x78-070.

Dorken, M.E., and Eckert, C.G. 2001. Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). J. Ecol. 89(3): 339–350. doi:10.1046/j.1365-2745.2001.00558.x.

Douhovnikoff, V., Cheng, A.M., and Dodd, R.S. 2004. Incidence, size and spatial structure of clones in second-growth stands of coast redwood, *Sequoia sempervirens* (Cupressaceae). Am. J. Bot. 91(7): 1140–1146. doi:10.3732/ajb.91.7.1140. PMID:21653469.

Eusemann, P., Herzig, P., Kieß, M., Ahlgrimm, S., Herrmann, P., Wilmking, M., and Schnittler, M. 2014. Three microsatellite multiplex PCR assays allowing high resolution genotyping of white spruce, *Picea glauca*. Silvae Genet. 63(1–6): 230–233. doi:10.1515/sg-2014-0029.

Fischer, M., and Van Kleunen, M. 2001. On the evolution of clonal plant life histories. Evol. Ecol. 15(4–6): 565–582. doi:10.1023/A:1016013721469.

Gamache, I., and Payette, S. 2004. Height growth response of tree line black spruce to recent climate warming across the forest–tundra of eastern Canada. J. Ecol. 92(5): 835–845. doi:10.1111/j.0022-0477.2004.00913.x.

Hodgetts, R.B., Aleksiuk, M.A., Brown, A., Clarke, C., Macdonald, E., Nadeem, S., Khasa, D., and Macdonald, E. 2001. Development of microsatellite markers for white spruce (*Picea glauca*) and related species. Theor. Appl. Genet. 102(8): 1252–1258. doi:10.1007/s00122-001-0546-0.

Hörandl, E., and Paun, O. 2007. Patterns and sources of genetic diversity in apomictic plants: implications for evolutionary potentials. *In* Apomixis: evolution, mechanisms and perspectives. *Edited by* E. Hörandl, U. Grossniklaus, P. Van Dijk, and T. Sharbel. ARG Gantner Verlag KG, Lichtenstein. pp. 169–194.

Husband, B.C., and Schemske, D.W. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. Evolution, 50(1): 54–70. doi:10.1111/j.1558-5646.1996.tb04472.x. PMID:28568860.

James, E.A., and McDougall, K.L. 2014. Spatial genetic structure reflects extensive clonality, low genotypic diversity and habitat fragmentation in *Grevillea renwickiana* (Proteaceae), a rare, sterile shrub from south-eastern Australia. Ann. Bot. 114(2): 413–423. doi:10.1093/aob/mcu049. PMID:24737718.

Jost, L. 2008. G_{ST} and its relatives do not measure differentiation. Mol. Ecol. 17(18): 4015–4026. doi:10.1111/j.1365-294X.2008.03887.x. PMID:19238703.

Juday, G.P. 2012. Monitoring hectare-scale forest reference stands at Bonanza Creek Experimental Forest LTER. *In* Long-term silvicultural and ecological studies: results for science and management. Vol. 2. Global Institute for Sustainable Forestry Research Paper 013. *Edited by* A.E. Camp, L.C. Irland, and C.J.W. Carroll. Yale University, School of Forestry and Environmental Studies. pp. 31–48.

Juday, G.P., Barber, V., Zasada, J., Rupp, S., and Wilmking, M. 2003. A 200-year perspective of climate variability and the response of white spruce in interior Alaska. *In* Climate variability and ecosystem response at long-term ecological research sites. *Edited by* D. Greenland, D.G. Goodin, and R.C. Smith. Oxford University Press. pp. 226–250.

Kemperman, J.A., and Barnes, B.V. 1976. Clone size in American aspens. Can. J. Bot. 54(22): 2603–2607. doi:10.1139/b76-280.

Kimura, M.K., Kabeya, D., Saito, T., Moriguchi, Y., Uchiyama, K., Migita, C., Chiba, Y., and Tsumura, Y. 2013. Effects of genetic and environmental factors on clonal reproduction in old-growth natural populations of *Cryptomeria japonica*. For. Ecol. Manage. 304: 10–19. doi:10.1016/j.foreco.2013.04.030.

Kirk, H., and Freeland, J.R. 2011. Applications and implications of neutral versus non-neutral markers in molecular ecology. Int. J. Mol. Sci. 12(6): 3966–3988. doi:10.3390/ijms12063966. PMID:21747718.

Klimešová, J., and De Bello, F. 2009. CLO-PLA: the database of clonal and bud bank traits of Central European flora. J. Veg. Sci. 20(3): 511–516. doi:10.1111/j.1654-1103.2009.01050.x.

Klimešová, J., and Doležal, J. 2011. Are clonal plants more frequent in cold

environments than elsewhere? Plant Ecol. Divers. 4(4): 373–378. doi:10.1080/17550874.2011.586734.
Körner, C. 2012. High elevation treelines. *In* Alpine treelines. Springer, Basel. pp. 1–10. doi:10.1007/978-3-0348-0396-0_1.
Laberge, M.-J., Payette, S., and Pitre, N. 2001. Development of stunted black spruce (*Picea mariana*) clones in the subarctic environment: a dendroarchitectural analysis. Écoscience, 8(4): 489–498. doi:10.1080/11956860.2001.11682679.
Lavoie, C., and Payette, S. 1992. Black spruce growth forms as a record of a changing winter environment at treeline, Quebec, Canada. Arct. Alp. Res. 24(1): 40–49. doi:10.2307/1551318.
Légère, A., and Payette, S. 1981. Ecology of a black spruce (*Picea mariana*) clonal population in the hemiarctic zone, northern Quebec: population dynamics and spatial development. Arct. Alp. Res. 13(3): 261–276. doi:10.2307/1551033.
Lloyd, A.H., Wilson, A.E., Fastie, C.L., and Landis, R.M. 2005. Population dynamics of black spruce and white spruce near the arctic tree line in the southern Brooks Range, Alaska. Can. J. For. Res. 35(9): 2073–2081. doi:10.1139/x05-119.
MacDonald, G. 1984. Postglacial plant migration and vegetation development in the western Canadian boreal forest. Ph.D. thesis, University of Toronto, Toronto, Ont.
Matsuo, A., Tomimatsu, H., Suzuki, J.-I., Saitoh, T., Shibata, S., Makita, A., and Suyama, Y. 2014. Female and male fitness consequences of clonal growth in a dwarf bamboo population with a high degree of clonal intermingling. Ann. Bot. 114(5): 1035–1041. doi:10.1093/aob/mcu176. PMID:25228034.
May, M.R., Provance, M.C., Sanders, A.C., Ellstrand, N.C., and Ross-Ibarra, J. 2009. A Pleistocene clone of Palmer's Oak persisting in southern California. PLoS One, 4(12): e8346. doi:10.1371/journal.pone.0008346. PMID:20041136.
McCune, B. 1986. PC-ORD: an integrated system for multivariate analysis of ecological data. Abstracta Botanica, 10(2): 221–225.
Morin, P.A., Manaster, C., Mesnick, S.L., and Holland, R. 2009. Normalization and binning of historical and multi-source microsatellite data: overcoming the problems of allele size shift with allelogram. Mol. Ecol. Resour. 9(6): 1451–1455. doi:10.1111/j.1755-0998.2009.02672.x. PMID:21564931.
Nichols, H. 1976. Historical aspects of the Northern Canadian treeline. Arctic, 29(1): 38–47. doi:10.14430/arctic2786.
O'Connell, L.M., Mosseler, A., and Rajora, O.P. 2007. Extensive long-distance pollen dispersal in a fragmented landscape maintains genetic diversity in white spruce. J. Hered. 98(7): 640–645. doi:10.1093/jhered/esm089. PMID:17981919.
Pan, J.J., and Price, J.S. 2002. Fitness and evolution in clonal plants: the impact of clonal growth. *In* Ecology and evolutionary biology of clonal plants. *Edited by* J.F. Stuefer, B. Erschbamer, H. Huber, and J.-I. Suzuki. Springer, Dordrecht, Netherlands. pp. 361–378. doi:10.1007/978-94-017-1345-0_20.
Payette, S., and Filion, L. 1985. White spruce expansion at the tree line and recent climatic change. Can. J. For. Res. 15(1): 241–251. doi:10.1139/x85-042.
Payette, S., Delwaide, A., Morneau, C., and Lavoie, C. 1994. Stem analysis of a long-lived black spruce clone at treeline. Arct. Alp. Res. 26(1): 56–59. doi:10.2307/1551877.
Peakall, R., and Smouse, P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes, 6(1): 288–295. doi:10.1111/j.1471-8286.2005.01155.x.
Pfeiffer, T., Klahr, A., Peterson, A., Levichev, I.G., and Schnittler, M. 2012. No sex at all? Extremely low genetic diversity in *Gagea spathacea* (Liliaceae) across Europe. Flora, 207(5): 372–378. doi:10.1016/j.flora.2012.03.002.
R Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
Rajora, O.P., Rahman, M.H., Dayanandan, S., and Mosseler, A. 2001. Isolation, characterization, inheritance and linkage of microsatellite DNA markers in white spruce (*Picea glauca*) and their usefulness in other spruce species. Mol. Gen. Genet. 264(6): 871–882. doi:10.1007/s004380000377. PMID:11254135.
Roland, C.A., Schmidt, J.H., and Johnstone, J.F. 2014. Climate sensitivity of reproduction in a mast-seeding boreal conifer across its distributional range from lowland to treeline forests. Oecologia, 174(3): 665–677. doi:10.1007/s00442-013-2821-6. PMID:24213628.
Rousset, F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol. Ecol. Resour. 8(1): 103–106. doi:10.1111/j.1471-8286.2007.01931.x. PMID:21585727.
Schnittler, M., and Eusemann, P. 2010. Consequences of genotyping errors for estimation of clonality: a case study on *Populus euphratica* Oliv. (Salicaceae). Evol. Ecol. 24(6): 1417–1432. doi:10.1007/s10682-010-9389-y.
Scott, P.A., Hansell, R.I.C., and Erickson, W.R. 1993. Influences of wind and snow on northern tree-line environments at Churchill, Manitoba, Canada. Arctic, 46(4): 316–323. doi:10.14430/arctic1359.
Silvertown, J. 2008. The evolutionary maintenance of sexual reproduction: evidence from the ecological distribution of asexual reproduction in clonal plants. Int. J. Plant Sci. 169(1): 157–168. doi:10.1086/523357.
Somme, L., Mayer, C., Raspé, O., and Jacquemart, A.-L. 2014. Influence of spatial distribution and size of clones on the realized outcrossing rate of the marsh cinquefoil (*Comarum palustre*). Ann. Bot. 113(3): 477–487. doi:10.1093/aob/mct280. PMID:24284813.
Sturm, M., Holmgren, J., McFadden, J.P., Liston, G.E., Chapin, F.S., Racine, C.H., Sturm, M., Holmgren, J., McFadden, J.P., Liston, G.E., Chapin, F.S., III, and Racine, C.H. 2001. Snow–shrub interactions in Arctic tundra: a hypothesis with climatic implications. J. Clim. 14(3): 336–344. doi:10.1175/1520-0442(2001)014<0336:SSIIAT>2.0.CO;2.
U.S. Geological Survey (USGS). 2016. Alaska digital elevation model. Available from https://agdc.usgs.gov/data/usgs/erosafo/300m/300m.html [accessed 23 May 2017].
Vallejo-Marín, M., Dorken, M.E., and Barrett, S.C.H. 2010. The ecological and evolutionary consequences of clonality for plant mating. Annu. Rev. Ecol. Evol. Syst. 41(1): 193–213. doi:10.1146/annurev.ecolsys.110308.120258.
Van Drunen, W.E., and Dorken, M.E. 2012. Trade-offs between clonal and sexual reproduction in *Sagittaria latifolia* (Alismataceae) scale up to affect the fitness of entire clones. New Phytol. 196(2): 606–616. doi:10.1111/j.1469-8137.2012.04260.x. PMID:22897332.
Van Drunen, W.E., Van Kleunen, M., and Dorken, M.E. 2015. Consequences of clonality for sexual fitness: clonal expansion enhances fitness under spatially restricted dispersal. Proc. Natl. Acad. Sci. U.S.A. 112(29): 8929–8936. doi:10.1073/pnas.1501720112. PMID:26195748.
Viereck, L.A., Van Cleve, K., and Dyrness, C.T. 1986. Forest ecosystem distribution in the taiga environment. *In* Forest ecosystems in the Alaskan taiga. *Edited by* K. Van Cleve, F.S. Chapin, P.W. Flanagan, L.A. Viereck, and C.T. Dyrness. Springer, New York. pp. 22–43. doi:10.1007/978-1-4612-4902-3_3.
Viktora, M., Savidge, R.A., and Rajora, O.P. 2011. Clonal and nonclonal genetic structure of subarctic black spruce (*Picea mariana*) populations in Yukon territory. Botany, 89(2): 133–140. doi:10.1139/B11-002.
Walker, X., Henry, G.H.R., McLeod, K., and Hofgaard, A. 2012. Reproduction and seedling establishment of *Picea glauca* across the northernmost forest–tundra region in Canada. Global Change Biol. 18(10): 3202–3211. doi:10.1111/j.1365-2486.2012.02769.x.
Wiehle, M., Eusemann, P., Thevs, N., and Schnittler, M. 2009. Root suckering patterns in *Populus euphratica* (Euphrates poplar, Salicaceae). Trees, 23(5): 991–1001. doi:10.1007/s00468-009-0341-0.
Wilmking, M., Buras, A., Eusemann, P., Schnittler, M., Trouillier, M., Würth, D., Lange, J., van der Maaten-Theunissen, M., and Juday, G.P. 2017. High frequency growth variability of white spruce clones does not differ from non-clonal trees at Alaskan treelines. Dendrochronologia, 44: 187–192. doi:10.1016/j.dendro.2017.05.005.
Wright, S. 1949. The genetical structure of populations. Ann. Eugen. 15(1): 323–354. doi:10.1111/j.1469-1809.1949.tb02451.x. PMID:24540312.

Can. J. For. Res. Downloaded from cdnsciencepub.com by UNIVERSITY OF ALASKA-FAIRBANKS on 11/19/20
For personal use only.