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RESEARCH ARTICLE

Coping with environmental constraints: Geographically divergent adaptive evolution and germination plasticity in the transcontinental *Populus tremuloides*

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Societal Impact Statement

Syntheses clearly show that global warming is affecting ecosystems and biodiversity around the world. New methods and measures are needed to predict the climate resilience of plant species critical to ecosystem stability, to improve ecological management and to support habitat restoration and human well-being. Widespread keystone species such as aspen are important targets in the study of resilience to future climate conditions because they play a crucial role in maintaining various ecosystem functions and may contain genetic material with untapped adaptive potential. Here, we present a new framework in support of climate-resilient revegetation based on comprehensively understood patterns of genetic variation in aspen.

Summary

- Elucidating species' genetic makeup and seed germination plasticity is essential to inform tree conservation efforts in the face of climate change. *Populus tremuloides* Michx. (aspen) occurs across diverse landscapes and reaches from Alaska to central Mexico, thus representing an early-successional model for ecological genomics. Within drought-affected regions, aspen shows ploidy changes and/or shifts

from sexual to clonal reproduction, and reduced diversity and dieback have already been observed.

- We genotyped over 1000 individuals, covering aspen's entire range, for approximately 44,000 single-nucleotide polymorphisms (SNPs) to assess large-scale and fine-scale genetic structure, variability in reproductive type (sexual/clonal), polyploidy and genomic regions under selection. We developed and implemented a rapid and reliable analysis pipeline (FastPloidy) to assess the presence of polyploidy. To gain insights into plastic responses, we contrasted seed germination from western US and eastern Canadian natural populations under elevated temperature and water stress.
- Four major genetic clusters were identified range wide; a preponderance of triploids and clonemates was found within western and southern North American regions, respectively. Genomic regions involving approximately 1000 SNPs under selection were identified with association to temperature and precipitation variation. Under drought stress, western US genotypes exhibited significantly lower germination rates compared with those from eastern North America, a finding that was unrelated to differences in mutation load (ploidy).
- This study provided new insights into the adaptive evolution of a key indicator tree that provisions crucial ecosystem services across North America, but whose presence is steadily declining within its western distribution. We uncovered untapped adaptive potential across the species' range which can form the basis for climate-resilient revegetation.

KEYWORDS
clonal richness, genetic diversity, keystone species, local adaptation, maternal effects, ploidy, quaking aspen

1 | INTRODUCTION

Woody angiosperms can persist on the landscape for a very long time, either as long-lived single individual stems (e.g., oak: Plomion et al., 2018) or as massive clones/genets (e.g., the *Populus tremuloides* Michx. Pando clone) displaying thousands of ramets (Dewoody et al., 2008; Kemperman & Barnes, 1976). Among angiosperms, polyploidy is frequent (more than 50%: Weiss-Schneeweiss et al., 2013) including intraspecific variation in ploidy levels, which make angiosperms distinct from gymnosperms where polyploidy is rare (less than 10%: Ohri, 2021; but see Scott et al., 2016).

Dioecy, whereby male and female reproductive organs are on separate plants, is less common in angiosperms (only 6%; Dorken & Van Drunen, 2018) than in gymnosperms (65% according to newest statistics; Walas et al., 2018) for all extant species, and it also occurs mostly in perennials (Dorken & Van Drunen, 2018). In the Salicaceae family (including *Populus*, *Salix*), however, dioecy is the predominant breeding system (Cronk et al., 2015), and willows and poplars are among the best studied forest trees in terms of life histories such as breeding system and sexual dimorphism, ploidy level and reproductive type (sexual or vegetative). The combination of dioecy and clonality in plants has also been suggested to lead to the loss of sex in extreme cases where vegetative propagation is the naturally dominant reproductive form. Clonality may also lead to variation in sex ratios within populations (Barrett, 2015). An accumulation of somatic mutations in aging clones can furthermore negatively affect fitness, as shown for *P. tremuloides* (Ally et al., 2010), but may also contribute to the build-up of genetic diversity in predominantly sterile clonal populations (Barrett, 2015).

The effects of dioecy, clonality and polyploidy have been connected to local adaptation of plant populations in several ways. First, males and females may differ in resource allocation, causing their spatial segregation within heterogeneous environments (Barrett & Hough, 2013). Second, clonal populations may display local adaptation based on somatic mutations, epigenetic variation and phenotypic plasticity (Barrett, 2015). Third, ploidy level can influence plant physiology, as evidenced for *P. tremuloides* (Blonder et al., 2020; Greer et al., 2018), and polyploidy has been shown in many other plant species (van de Peer et al., 2021). Environmental fluctuations can lead to adaptive responses in eukaryotes via the formation of polyploids (van de Peer et al., 2017), especially in plants in connection with stress (van de Peer et al., 2021), which in turn can help in species range

expansion (Weiss-Schneeweiss et al., 2013). Species' potential to colonize deglaciated regions (*ibidem*) and persist under climate oscillations (Dynesius & Jansson, 2000) was attributed to the higher genetic diversity of polyploids.

P. tremuloides (quaking aspen) can produce autopolyploids and mostly triploids (3n) (Mock et al., 2012, and this study) which arise through mechanisms involving unreduced gametes (Comai, 2005; van de Peer et al., 2017). Phenotypic consequences of polyploidization can be attributed to (1) a higher number of mutations for the entire organism, based on the increase in the total DNA amount in a polyploid, which generates higher standing genetic variation for that organism (van de Peer et al., 2017); (2) an effective buffering of recessive deleterious mutations that can otherwise represent an adaptive solution in an epistatic context with other genetic variants (van de Peer et al., 2017); and (3) effects of increased gene dosage, that is, gene copy numbers. The phenotypic variation may include delayed flowering as well as ecological adaptation (Doyle & Coate, 2019). *P. tremuloides*, which reproduces clonally and/or sexually, can occur in mixed-ploidy stands, with a preponderance of polyploid clones in geographic areas affected by drought (Mock et al., 2012).

The occurrence of clonal aspen forests has also been linked to drought stress ecology (Blonder et al., 2020; Greer et al., 2018). *P. tremuloides*' distribution is wide ranging (Rogers et al., 2020), across Canada's boreal forest and encompassing the entire western North America (NA) reaching south to central Mexico. This tree species can thrive within diverse and sometimes extreme ecological settings such as the mountainous regions of the Sierra Madre Occidental in Mexico and areas experiencing severe droughts (Ding et al., 2017; Rogers et al., 2020). It occupies ecologically sensitive areas such as riparian habitats and relies on natural disturbances (e.g., fire and pests) to colonize new areas or to rejuvenate existing stands (Swift & Ran, 2012). However, sexual regeneration through aspen seedlings is more common in eastern NA including Canada than in the western United States (Peterson & Peterson, 1992), potentially because seed germination and seedling growth are extremely sensitive to soil moisture availability (Landhäusser et al., 2019; McDonough, 1979).

Taken together, *P. tremuloides* represents an ecological keystone species (Rogers et al., 2020), thereby an important indicator plant for climate change impacts on valuable ecosystems it forms, and also an important model to study angiosperm evolution (ploidy, clonality, and dioecy). Aspen is already experiencing drought-induced dieback (Worrall et al., 2013) and should be one of the prioritized species to be considered for targeted forest management (Paine, 1995). Resilience in forest management is required to maintain biodiversity and ecosystem services, which includes all the benefits that ecosystems provide including for human well-being (Haines-Young & Potschin, 2018). However, intraspecific genetic makeup is often overlooked in forest management, while it would help anticipate potential threats to a species, and thus inform mitigation management. The main purpose of our present study was to conclusively elucidate genetic diversity of neutral and adaptive nature, clonality and ploidy across the entire range of this most widely distributed North American early-successional tree species, for the first time, including populations representative of its Mexican distribution, and a much broader sampling strategy for eastern NA than previously reported (Bagley et al., 2020; Latutrie et al., 2019).

2 | MATERIALS AND METHODS

A detailed description of the methods can be found in File S1. These methods are presented briefly here. Referenced tables and figures are in File S2 (Figures S1–S5, S16, S23–S28, Tables S1 and S2 and Dataset S1).

2.1 | Plant material and genomic data collection

Leaf samples from geo-referenced *P. tremuloides* individuals were gathered across NA. First, samples came from a base collection at the Canadian Forest Service (CFS, Laurentian Forestry Centre, Quebec City) encompassing genotypes from across Canada and the United States ($n = 396$). Second, additional leaf samples were added from Mexico ($n = 656$), Utah/Idaho ($n = 30$) and Saskatchewan ($n = 71$). The sampled southern populations encompassed around 1/4 of the entire *P. tremuloides* stands present within Mexico. Third, DNA samples from two previously published studies by Callahan et al. (2013) ($n = 198$) and Bagley et al. (2020) ($n = 24$) were included, for a grand total of 1375 individuals. The original spatial extend of populations ranged from 0 to 120 km (determined by sample providers). For 105 samples from the Callahan collection, ploidy was already known (98 diploids along with seven triploids, based on flow cytometry and microsatellites, or on microsatellites alone) (Mock et al., 2012). We also included 59 *Populus grandidentata* leaf samples (also provided by CFS), as *P. tremuloides* may hybridize with this species within *P. tremuloides*' north-eastern distribution.

Collected leaf samples were air dried (CFS, Mexico, Saskatchewan) or dried on silica gel (Utah/Idaho from collaborator K. Mock) and stored at ambient room temperature. DNA was extracted using the Nucleospin 96 Plant II kit (Macherey-Nagel, Bethlehem, PA, USA) following the manufacturer's protocol but with modifications regarding the cell lysis step (PL2 buffer was heated for 2 h at 65°C instead of 30 min). Details about GbS library preparation, sequencing and treatment of sequencing data (single-nucleotide polymorphism [SNP] calling) can be found in File S1.

2.2 | Filtering of sequencing data

We assessed variant depth, variant missingness, minor allele frequency (MAF), individual depth and individual missingness in the raw VCF using VCFtools (Danecek et al., 2011). The raw Stacks VCF

encompassed 589,751 SNPs with an average depth of 16.7 per SNP. The average depth per individual was 16.8. The VCF was filtered with the https://github.com/enormandeau/stacks_workflow/tree/master/00-scripts/05_filter_vcf_fast.py script considering all samples within the same group based on criteria 8 80 0 34 (File S1). Filtering without the relatedness filter resulted in 44,140 SNPs among 1072 *P. tremuloides* samples with mixed ploidy (see ploidy determination below). Samples filtered for a relatedness cut-off (detected with –relatedness in VCFtools) were marked and removed; this step was skipped for ploidy and clonality determination at subsequent stages of analyses. We chose 0.3 and 0.85 as relatedness cut-offs, leading to two datasets termed REL0.3 (37,519 SNPs; 609 genotypes) and REL0.85 (39,862 SNPs; 762 genotypes), respectively. The rationale for using the 0.85 cut-off was to include all samples before the peak of self-relatedness of samples. The 0.85 relatedness cut-off allowed to incorporate more samples from western United States in the analysis. Both datasets (REL0.3/REL0.85) were used in the genetic structure and outlier analyses.

2.3 | Genetic clustering assessment in aspen

For an overall picture of the population structure, we performed principal component analyses (PCAs) with plink v1.90b5.3 (Purcell et al., 2007) and visualized the results in R using ggplot2. To assess detailed structure, we used the program Mycorrhiza 0.0.28, a machine learning approach that utilizes phylogenetic networks to identify features that encode evolutionary relationships among samples that are then supplied to a Random Forest classifier to identify structure (Georges-Filteau et al., 2020). The program requires pre-identified groups as input to assess the accuracy of samples assigned to a group. We used our PCA results to define four groups that are also coherent with previous evidence (Bagley et al., 2020; Callahan et al., 2013). Mycorrhiza was first run with 800 SNPs (selected based on highest mutual information among groups) as input to identify the number of SNPs needed for highest accuracy. Hereafter, using the REL0.3 dataset, samples were reassigned to clusters for better fitting based on resulting *Q* values, and the program was run again with 800 SNPs. We used the accuracy plot produced by Mycorrhiza to choose the optimal number of SNPs and reran the program with those SNPs.

2.4 | Ploidy determination using GbS data

Ploidy was assessed with the custom-made script FastPloidy.R (available at https://github.com/RGoess/Ploidy_detection). FastPloidy reads a VCF in R and works as follows: (i) It extracts the allele depth for reference and alternative allele (minimum depth of 16); (ii) the depth of the reference allele is divided by the total depth to obtain the allelic ratio of the reference allele and only heterozygous SNPs are kept (allelic ratio within 0.1–0.9); (iii) each SNP is then assigned to a class depending on the value of the allelic ratio (A: 0.273–0.393, B: 0.44–0.56, C: 0.607–0.727, 'Other' would refer to a value anywhere outside the range). These allelic ratio ranges were chosen because we expect diploids to have mostly a ratio around 0.5 (thus equal depth for reference and alternative alleles), while for triploids, we expect a ratio of 0.33 or 0.67 because of their three chromosome copies. Hereafter, we determined the ploidy ratio per individual as *Number SNPs in Class A* + *Number SNPs in Class C* ÷ *Number SNPs in Class B*. Triploids should harbour a higher ploidy ratio, as most SNPs should be found within classes A and C, while diploids should harbour most SNPs in class B and thus a lower ratio. We visualized results as a stacked dot plot (count of ploidy ratio vs. ploidy ratio) or a dot plot (number total SNPs vs. ploidy ratio). We plotted the correlation of the ratio of triploids per population against annual heat–moisture index (AHM) and summer heat–moisture index (SHM) extracted from the ClimateNA database (Wang et al., 2016). Dry and warm environments have a high index, while cold and wet environments have a low index.

2.5 | Clonality and genetic diversity assessment

To assess clonality among mixed-ploidy individuals with the Genodive program (Meirmans, 2020), it was required to obtain exact SNP calls for triploid individuals, thus for each chromosomal copy. Therefore, we had to reperform SNP calling as STACKS only outputs SNPs in a diploid model. First, the original bam files generated by bwa were sorted with samtools (Li et al., 2009) and tagged with Picard (http://broadinstitute.github.io/picard/). Then, we called variants separately for diploid and triploid individuals with the reference genome using the GATK HaplotypeCaller (Poplin et al., 2018) that allows for any ploidy (used options: –ploidy 2 or 3). The individual VCFs were merged into one VCF file with a custom R script and only SNPs overlapping the STACKS VCF were kept. We filtered for a minimum median depth of 12 and maximum missingness of 20%. The resulting file with 3886 SNPs was analysed in Genodive version 3.04, a software that can analyse mixed-ploidy data (Meirmans, 2020). We assessed clonality among mixed ploidy per cluster using the infinite allele model and considered missing data as one mutation step. We used thresholds 2287 (NENA and NWNA clusters), 1472 (WUS cluster) and 1092 (MX cluster); see Section 3 for information about these genetic clusters.

Diploid clones were extracted from the STACKS VCF (without relatedness filter) and observed heterozygosity and inbreeding coefficient *F* were calculated per individual in comparison to the whole sample size using the –het function from vcftools (Danecek et al., 2011). Moreover, pairwise Weir and Cockerham's (1984) F_{ST} between clusters and statistics per cluster (observed heterozygosity [H_{OBS}], within-population gene diversities [H_S] and inbreeding coefficients [F_{IS}]) were calculated using Hierfstat (Goudet, 2005). Nucleotide diversity (π) was calculated with the populations step in STACKS2 (Catchen et al., 2013). We performed an AMOVA within each cluster using poppr (Kamvar et al., 2014) function poppr.amova with ade4 as the method. Significance of the AMOVAs was

determined using the randtest() function from R package Adegenet (Jombart et al., 2008) with 999 permutations.

2.6 | Spatial autocorrelation analysis

Within each genomic cluster determined by Mycorrhiza, we performed a spatial principal component analysis (sPCA) using the R package Adegenet with 5000 randomly selected SNPs. We performed this analysis to explore cryptic spatial genetic patterns within clusters and to aid defining new populations with adequate size (number of individuals >2, radius <500 km) within our clusters for follow-up analyses. We used the REL0.3 dataset for north-east NA, north-west NA and Mexico clusters and used REL0.85 for the western US cluster to obtain a wider sample representation in this case.

2.7 | F_{ST} outlier analysis and SNP annotation

Bayescan analysis was performed to identify divergent loci based on F_{ST} (Foll & Gaggiotti, 2008) using the REL0.3 and REL0.85 datasets. Triploids and very isolated individuals that encompassed populations with less than two individuals were excluded from the analysis. Outlier SNPs were annotated with snpEff (Cingolani et al., 2012) and with the available blast files on popgenie.org (v1.1). We performed GO-term enrichment analysis for outlier SNPs within each genetic cluster (FDR < 0.05) using *Populus trichocarpa* identifiers and *Arabidopsis thaliana* identifiers (blastp results available at popgenie.org) in AgriGO (Tian et al., 2017).

2.8 | Redundancy analysis (RDA)

To assess which environmental variables influence adaptive genetic variation over the whole range of aspen, we performed RDA analysis using the rda() function in the R package vegan (Oksanen et al., 2020). The following variables were used: temperature difference (TD), mean annual precipitation (MAP), May–September precipitation (MSP), precipitation as snow (PAS), extreme maximum temperature over 30 years (EXT), relative humidity (RH) and degree days between 10°C and 40°C (DD1040).

2.9 | Seed germination

We tested different temperature and water stress regimes to evaluate germination plasticity in *P. tremuloides* by contrasting two geographical regions, that is, Quebec ($n = 24$) and Utah ($n = 30$). We expected seeds to respond differently between those two regions (isolated Mexican populations rarely produce seeds; therefore, this region could not be included here). We tested effects of temperature (20°C–28°C–36°C daytime temperature; 16°C night-time temperature) and water stress (15% PEG vs. H_2O [control]) under 16 h light and 40% RH. In total, 1152 Quebec seeds versus 1440 Utah seeds per treatment combination were compared in the analyses.

3 | RESULTS

3.1 | Genetic structure and neutral genetic diversity in *P. tremuloides*

PCA and Mycorrhiza software were used to examine genetic structure for filtered data sets REL0.3 and REL0.85, respectively. Based on the PCAs and then Mycorrhiza (averaged *Q* values per population are visualized in Figure 1), we detected four main genetic groups: north-east NA (all provinces east of Manitoba; NENA), north-west NA (incl. north-west of Saskatchewan and British Columbia; NWNA), western United States (all provinces in the US west of Montana; WUS) and Mexico (MX). Based on the run with the REL0.3 dataset and 800 SNPs, we reassigned all samples from Baja California (Mexico) to the 'western US' group. Groupings between both relatedness cut-offs were highly similar, except more populations were represented in the WUS cluster with the REL0.85 dataset.

Based on the plots with sPCA eigenvalues, decomposed components, local and global tests, we concluded that none of the clusters displayed significant local eigenvalues. The NENA and WUS clusters showed one extreme global eigenvalue and the MX cluster showed two extreme global eigenvalues (File S2). The NWNA cluster did not show any extreme eigenvalue indicating that neither global nor local substructure is present. The NENA cluster encompassed a clear north–south gradient (Figure 2a,b), the WUS cluster a gradient from the coastal region towards the southeast (Figure 2c,d). The MX cluster showed differentiation north-east versus west (Figure 2e,f), while the second global eigenvalue for the MX cluster showed differentiation north versus south. Based on the values of the first PC, we created new larger populations for follow-up analyses (Dataset S1, column H).

Hereafter, we extracted the 639 diploid clones (see C + D; Table 1) and calculated observed heterozygosity (H_{OBS}) and the inbreeding coefficient *F* per cluster on an individual basis. Lowest H_{OBS} was found in the MX cluster while highest H_{OBS} was found in the two northern clusters (covering Canada) (Table 2). Pairwise F_{ST} calculation showed that the NENA and NWNA clusters only slightly diverged ($F_{ST} = 0.008$). The WUS cluster is more diverged from the two northern clusters ($F_{ST} = 0.05$). Finally, the MX cluster had highest divergence from the two northern clusters ($F_{ST} = 0.17$ in both cases) and appreciable divergence from the WUS cluster ($F_{ST} = 0.14$). On a cluster basis, highest inbreeding (F_{IS}) was observed within the WUS cluster, while the two northern clusters and the MX cluster had similar inbreeding levels (Table 2). Inbreeding (*F*) on an individual level showed highest values for both the MX and WUS cluster. AMOVA results (Table 2) indicated that most variance could be explained within samples (ranging between 85.7% and 92.6% for all clusters),

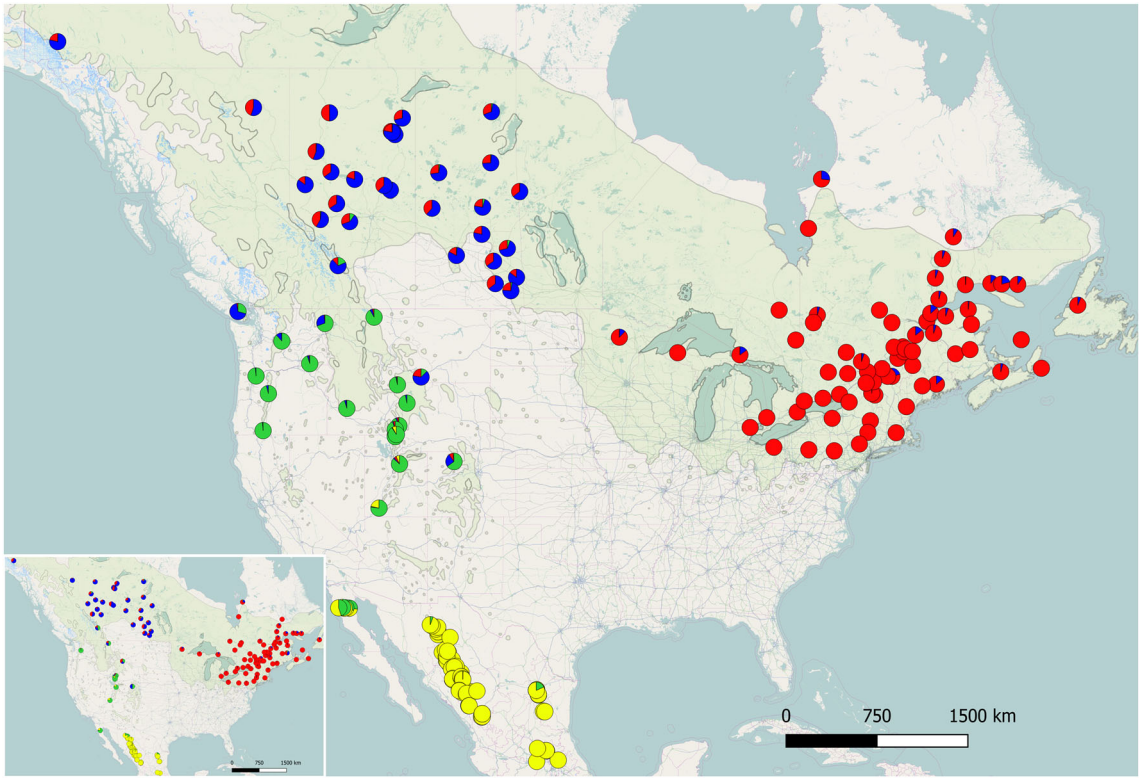

FIGURE 1 Averaged *Q* values (Mycorrhiza) per *Populus tremuloides* population shown as pie charts on the map. The main figure indicates results with 0.85 relatedness cut-off. Pie-chart colours indicate the averaged *Q* values per population for the genetic clusters, that is, north-east North America (red), north-west North America (blue), western United States (green) and Mexico (yellow). The inset figure indicates results with 0.3 relatedness cut-off. Light green area depicts the species' range (Little, 1971). See Figure S1 in File S2 for entire distribution of *P. tremuloides*

hereafter, within populations (ranging between 3.6% and 7.4%), finally, between populations (ranging between 0.1% and 8.5%). All AMOVA components were significant (*P* value <.01).

3.2 | Ploidy, clonality and adaptive genetic variation in *P. tremuloides*

The FastPloidy.R script identified 959 diploids and 113 triploids (Dataset S1, Table 1 and Figure 3). Our custom script detected all samples except one triploid (MXQ-16) matching flow cytometry + microsatellite data (total $n = 97$, triploids $n = 7$, diploids $n = 90$) (see Figure 3). We acknowledge that the outlying sample (GUTO-9) could potentially be a tetraploid; however, we kept it in our analyses together with triploids. Individuals with a higher number of heterozygous SNPs showed clearer separation between diploid and triploids (Figure 3).

In total, 685 unique genotypes among the 1072 individuals were identified by Genodive (Table 1). By far, the highest number of clonemates was found in Mexico (corresponding to lowest %C in Table 1). The highest number of triploids (T) among unique genotypes (%C + T) was found for western United States, while the lowest for NENA. Significant correlations between the rate of triploids per population (clones/unique genotypes only) and AHM (0.36) and SHM (0.3) were observed (Figure 4a–d).

Bayescan detected 7 to 410 F_{ST} outliers per cluster (dependent on region and relatedness cut-off) under a 0.05 FDR (Table 3). Information on the number of overlapping outliers among the different genetic clusters and relatedness datasets (REL0.3; REL0.85) is accessible in File S2. Most of these SNPs were located within or close to annotated genes (>85%) with significant blastx hits for >80% (Table 3). These annotated genes were found to be functionally diverse but did not reveal any enriched GO terms. Detailed annotation, blastx, blastp, GO term and PFAM results for outlier loci, most prevalent GO term categorized PFAMs among outliers, most abundantly present categorized GO terms within these PFAMS related to biological process, and those related to molecular function are presented in File S2. The allelic distribution for three outliers of interest was plotted, that is, *Potrs017598g19854* (Figure 5a), *Potrs000450g00618* (Figure 5b) and *Potrs001211g02063* (Figure 5c), identified, respectively, within the NENA, WUS and MX genetic clusters. For the NWNA cluster, a relatively low number of outliers was detected, coinciding with less variation in environmental variables (Figure 5d).

To assess climate association with genetic variation, we undertook RDA for four precipitation and three temperature-related variables. RDA1 and RDA2 jointly explained 37% of the variance; RDA1

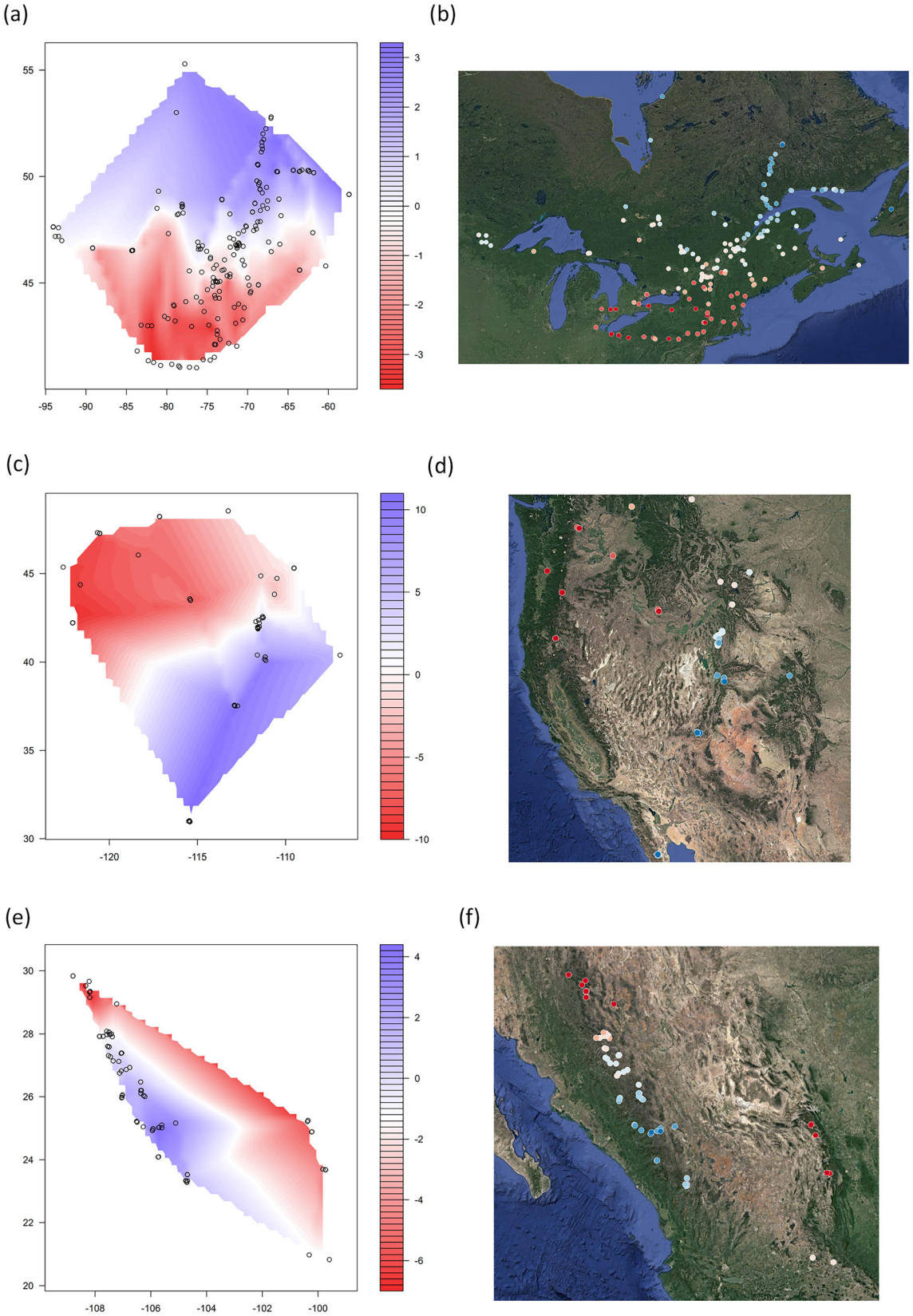

FIGURE 2 (a–f) Values of the first principal component (PC) for three major genetic cluster within *Populus tremuloides* that showed significant global structure in spatial principal component analysis (sPCA). Interpolated lagged values shown in (a), (c) and (e). PC values on the map shown in (b), (d) and (f). (a,b) North-east North America cluster using the REL0.3 dataset, (c,d) Western US cluster with REL0.85 dataset and (e,f) Mexico cluster with REL0.3 dataset

TABLE 1 Summary table for clonality and ploidy present in the *Populus tremuloides* sample

Genetic cluster	N	D	T	C	C + D	C + T	%T	%C	%C + T
North-east NA	291	289	2	276	274	2	0.7	94.8	0.7
North-west NA	181	162	19	168	154	14	10.5	92.8	8.3
Western United States	104	81	23	87	73	14	22.1	83.6	16.1
Mexico	496	427	69	154	138	16	13.9	31.0	10.4

Note: A clone represents as per definition a unique genotype. N: total number of individuals. D: total number of diploids, T: total number of triploids. C: total number of clones. C + D: clone and diploid. C + T: clone and triploid. %T: percentage of triploids in the total number of individuals. %C: percentage of clones in the total number of individuals. %C + T: percentage of triploid clones among the total number of clones. NA indicates North America.

TABLE 2 Population statistics and analysis of molecular variance (AMOVA) for diploid clones in *Populus tremuloides* on a genetic cluster basis

Genetic cluster	H_{OBS}	H_S	F_{IS}	π	Between population (%)	Between samples within population (%)	Within samples (%)
North-east NA	0.1665	0.1818	0.0845	0.1825	0.3	7.4	92.3
North-west NA	0.1666	0.1815	0.0820	0.1823	0.1	7.3	92.6
Western United States	0.1552	0.1824	0.1491	0.1832	8.5	5.8	85.7
Mexico	0.1474	0.1609	0.0840	0.1621	4.4	3.6	92.0

Note: H_{OBS}: observed heterozygosity. H_S: within-population genetic diversity. F_{IS}: inbreeding coefficient. π: nucleotide diversity. The last three columns indicate components of variance in percentage. NA indicates North America.

alone explained 19% and was mostly correlated to temperature. We identified in total 2989 and 7005 RDA outliers for REL0.3 and REL0.85 datasets, respectively, with 1715 overlapping between both datasets. Several RDA outliers matched F_{ST} outliers within clusters (see analyses above), respectively, for REL0.3 and REL0.85 datasets, 110 and 54 for NENA, 4 and 10 for NWNA, 53 and 127 for WUS and 97 and 169 for MX clusters. Predictors for adaptive genetic variation were mostly found for temperature based on highest correlation of each candidate SNP with the respective environmental predictors.

3.3 | Seed germination plasticity under employed water and temperature stress

Under 20°C and 28°C temperatures and without any water stress, all populations showed a germination rate close to 100%, indicating that seeds were viable (Figure 6). Model Germ_region showed that application of PEG (15%), a commonly used inducer of drought stress in plant physiology studies (He et al., 2014), alone and in combination with higher temperatures of 28°C and 36°C, significantly reduced ($P < .05$) normal seed germination rates compared with control conditions (Figure 6). Most strikingly, the germination rates for Utah genotypes were significantly more affected by drought stress compared with Quebec genotypes (Figure 6). Furthermore, the Germ_pop model identified a significantly negative interactive effect of elevated temperature (36°C vs. 20°C) and 15% PEG (vs. water control) on normal germination. Under the Germ_pop model, several populations, most of which from Utah (i.e., CMR, FBR, GTC, SBR and TGT), showed significantly reduced germination rates under drought stress induced by 15% PEG, compared with the reference population AMOS (Quebec). Four triploids were among our mother trees, but no significant effect on germination rates was found.

Models Hypo_region, Hypo_pop identified a significantly negative effect of high temperature (36°C vs. 20°C) and 15% PEG (vs. water) on the average postgerminative hypocotyl and radicle growth. Significantly negative interaction of drought stress (15% PEG) with Utah genotypes was uncovered (Hypo_region); more specifically, the Hypo_pop model identified significantly negative interactions for two Utah populations (SBR; CMR) with 15% PEG application. Again, no effect of ploidy was found; but for 75% of triploid mother trees, we did observe a substantially higher percentage of germinants with three or four cotyledons compared with the diploid mothers within the same populations.

An extended presentation of the results can be found in File S2 (Figures S6–S29, Tables S3–S12 and Datasets S1–S6). These results were presented in a concise form here.

4 | DISCUSSION

Our sampling strategy encompassed the entire natural distribution of *P. tremuloides* across a myriad of climate and edaphic gradients. It thus enabled a general appreciation of neutral and adaptive genetic makeup (Figures 1 and 5) in relation to ploidy and reproduction (dioecy; clonal and sexual reproduction) in this important keystone species.

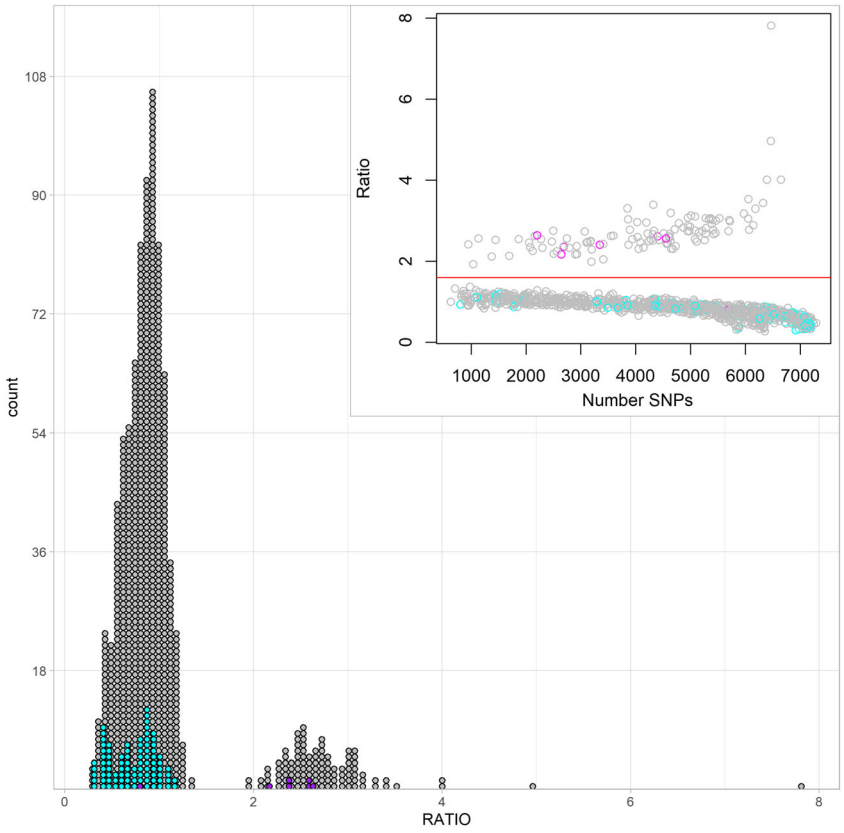

FIGURE 3 Stacked dot plot for the number of individuals per calculated ratio in ploidy assessments of *Populus tremuloides*. SNPs count in 0.607–0.727 + 0.273–0.393 ranges divided by the SNPs number in 0.440–0.560 range (see Methods). Inset shows the ratio per individual versus number of SNPs included in the ratio calculation. Grey dots indicate samples that were tested by FastPloidy only; cyan dots indicate diploids as determined by flow cytometry + microsatellites or microsatellites only (see Dataset S1 in File S2) (Mock et al., 2012) and FastPloidy; purple dots indicate triploids as determined by flow cytometry + microsatellites or microsatellites only (see Dataset S1 in File S2) (Mock et al., 2012) and FastPloidy. The horizontal line in the inset figure gives an arbitrary threshold of 1.6 that could be used to differentiate between diploids and triploids

Our genetic assessment of *P. tremuloides* populations brought to light several important findings: (1) contrary to previous population genetics studies in *P. tremuloides* (Bagley et al., 2020; Callahan et al., 2013), and due to much higher sampling density here, within the species' most northern distribution, two large genetic clusters (NWNA and NENA) emerged that were clearly distinguishable, albeit slightly differentiated with lowest pairwise F_{ST} among all comparisons; (2) the low between-cluster F_{ST} points to the possibility that these two genetic clusters emerged through distinct postglacial recolonization routes from a common refugium; one candidate for such refugium could be the region south of the Great Lakes (Jaramillo-Correa et al., 2009); indeed, a palaeoclimatic habitat reconstruction study suggested that *P. tremuloides* recolonized boreal Canada from southern refugia in the east NA after the last glacial retreat (Ding et al., 2017); (3) within the NENA cluster, we identified important genetic discontinuities following a north/south gradient that could be explained by the presence of multiple glacial refugia in east NA (Godbout et al., 2005; Jaramillo-Correa et al., 2009); (4) a novel major cluster encompassing Mexico was uncovered and high pairwise F_{ST} with all other three clusters indicated that the MX cluster has been isolated for a long period of time and could represent relict populations, similarly as for Mexican populations of the largely distributed *Pseudostuga menziesii* (Wei et al., 2011). Indeed, palaeoclimatic habitat reconstructions suggested the presence of stable habitats in the Sierra Madre mountain range (Ding et al., 2017); specifically, spatial genetic differentiation between the north-eastern and the south-western part corresponds to Sierra Madre Occidental, Sierra Madre Oriental and the Transverse Vulcanic Belt separation, while the second level of structure indicated a north–south gradient corresponding to the Sierra Madre Occidental separation; these patterns are corresponding to the phylogeography of multiple local species, such as *Picea chihuahuana* and *Pinus strobiformis* (Jaramillo-Correa et al., 2009); and finally (5), the presence of a WUS cluster substructure (i.e., the gradient from south, including Baja California [Mexico], and east of the Sierra Nevada towards the Pacific coastal region) that confirms previous results (Bagley et al., 2020; Callahan et al., 2013) and the lack of any substructure for the NWNA cluster confirms previous results (Bagley et al., 2020; Latutrie et al., 2016). While the identification of the two separate NENA and NWNA clusters could be the result of sampling discontinuity within the north–north-western region of aspen's distribution (Frantz et al., 2009; Guillot et al., 2009), we did not detect any isolation-by-distance. Moreover, based on Jaramillo-Correa et al. (2009), we would expect to see some genetic separation in this broad geographic region.

High levels of standing genetic variation are important for populations of a species to persist and locally adapt over generations, because this variation includes polymorphism for resilience to future abiotic and biotic stressors and on which natural selection can act. For *P. tremuloides*, most variation was found within individuals, a general finding for outcrossing and highly heterozygous tree species along with geographic variation in allele frequencies (Porth & El-Kassaby, 2014). While for the NWNA and NENA clusters a highly similar

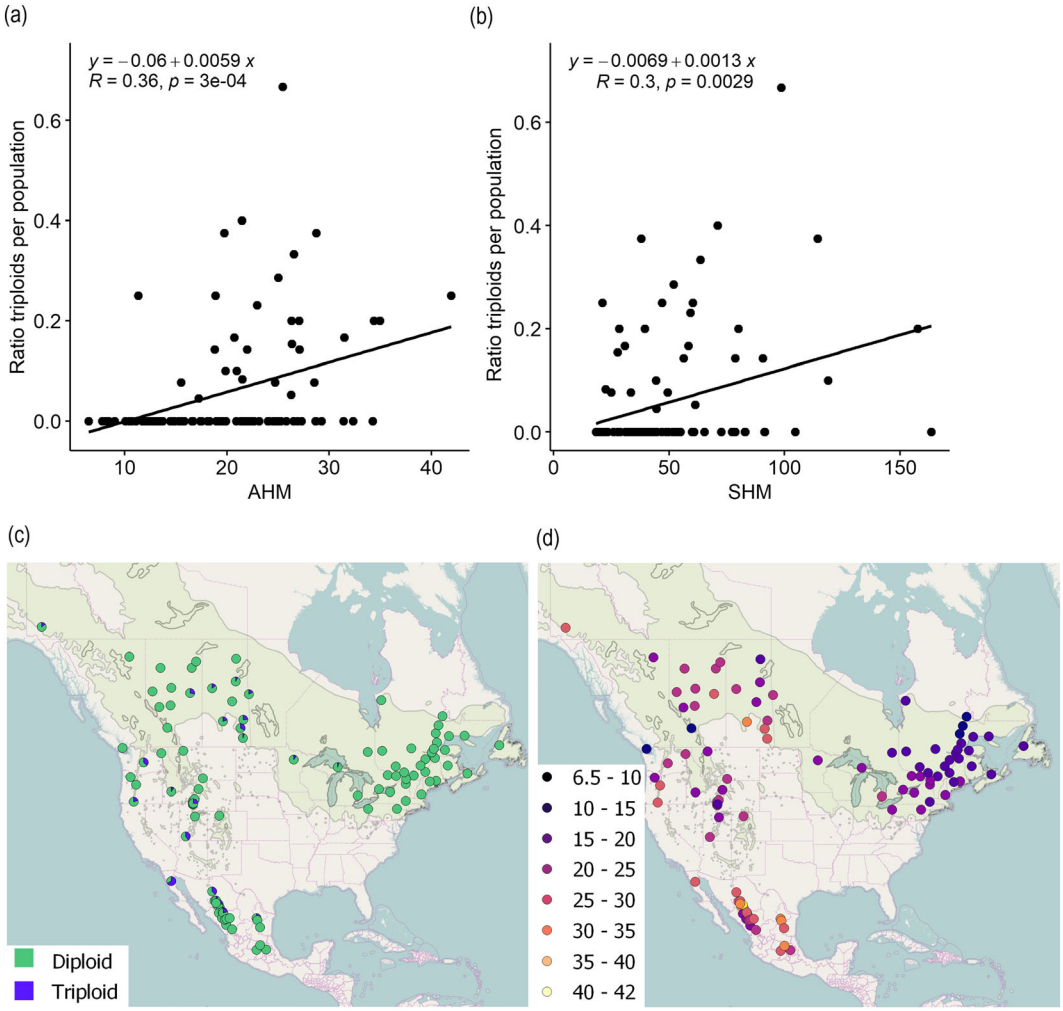

FIGURE 4 (a–d) Variables that drive environmental adaptation in *Populus tremuloides*. Annual heat–moisture index (AHM) and summer heat–moisture index (SHM) climatic variables are presented based on their highest correlation with ploidy occurrence out of all climatic variables from the ClimateNA database (Wang et al., 2016). (a) Correlation of the rate of triploid per population versus AHM and (b) correlation of the rate of triploid per population versus SHM. (c) Pie charts on map show distribution of triploids and diploids per population (only unique genotypes are shown). (d) Average AHM value per population mapped out for only unique genotypes. AHM is defined as (mean annual temperature + 10)/(mean annual precipitation/1000); SHM is defined as (mean warmest month temperature)/(May to September precipitation (mm)/1000)

TABLE 3 F_{ST} outliers in *Populus tremuloides* as obtained with Bayescan and false discovery rate (FDR) < 0.05

Set	Rel	Nb. of pops	Nb. of SNPs	Nb. of SNPs at FDR < 0.05	Gene space (%)	Blastx (%)
North-eastern NA	0.3	36	37,519	189	85.7	82.0
	0.85	36	39,862	198	85.4	80.3
North-western NA	0.3	19	37,519	7	100.0	85.7
	0.85	20	39,862	25	92.0	92.0
Western United States	0.3	9	37,519	74	86.5	89.2
	0.85	14	39,862	410	88.7	85.6
Mexico	0.3	19	37,519	110	86.4	80.0
	0.85	21	39,862	201	89.1	83.6

Note: Set: genetic clusters representing North-eastern North America (NA), North-western NA, Western United States and Mexico as depicted in Figure 1; Rel: relatedness threshold in population analysis; Nb. of pops: final number of populations included; Nb. of SNPs: final number of SNPs included in the analysis input; Nb. of SNPs at FDR < 0.05: number of reported F_{ST} outliers; Gene space(%): percentage of outlier SNPs within or close (<5 kb) to annotated genes; Blastx (%): percentage of outlier SNPs close to or in annotated genes with blastx hits (*E* value < 10e-5); in-depth results for outlier loci are provided in Datasets S2–S5, File S2.

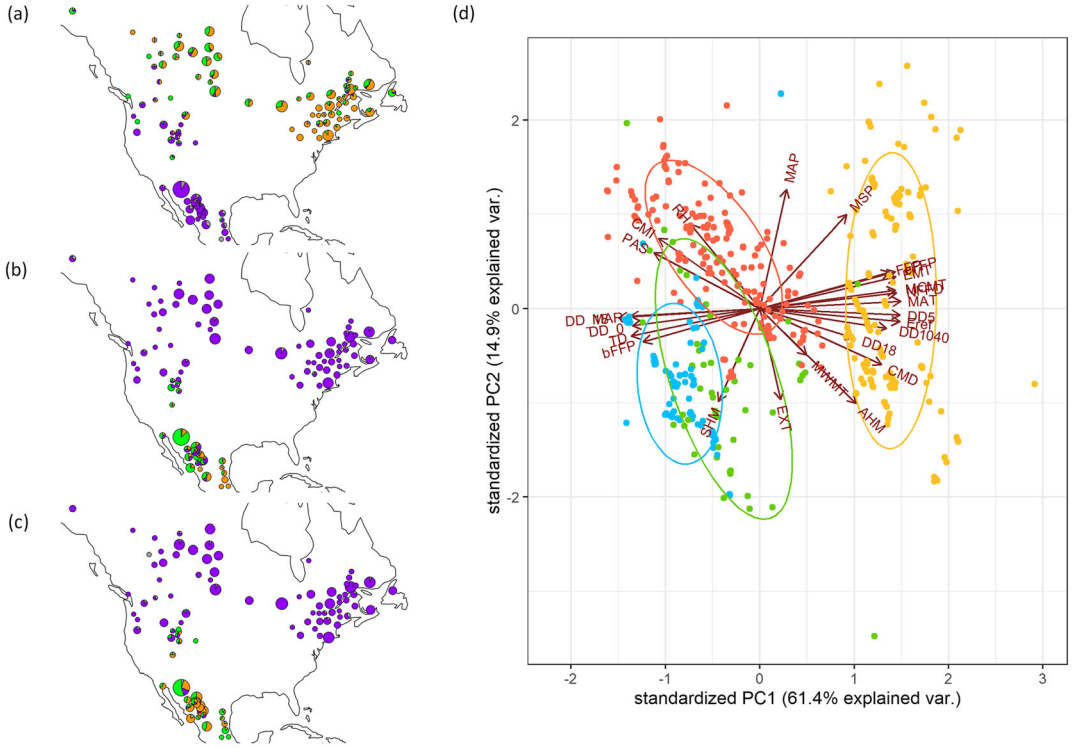

FIGURE 5 (a–d) Geographic and environmental context of adaptive genetic variation in *Populus tremuloides*. (a–c) Presentation of the allelic distribution across the sampling area for three F_{ST} outlier loci ([a] SNP = Potrs017598_19569, 5-prime-UTR variant; [b] SNP = Potrs000450_26480, synonymous variant; [c] SNP = Potrs001211_1443, downstream gene variant [<5 kb]) detected through Bayescan within three major genetic clusters; SNP Potrs017598_19569 was identified within the north-east North America (NENA) cluster, SNP Potrs000450_26480 within the western US (WUS) cluster and SNP Potrs001211_1443 within the Mexico (MX) cluster. Pie charts (a)–(c) indicate the allelic distribution per population, scaled to population size (min. 2, max. 45). Purple colour indicates homozygotes for the reference allele, orange colour indicates homozygotes for the alternative allele, light green colour indicates heterozygotes and grey colour indicates missed genotype calls; (d) principal component analysis (PCA) plot depicting major genetic and climatic variation in *P. tremuloides* (Figure 1). Individuals are colour coded according to their membership in any of the four major genetic clusters (red: NENA, blue: NWNA, green: WUS, yellow: MX). Ellipses represent the default 68% normal probability. Depicted climate variables encompass all available climatic variables from the ClimateNA database (see below for details). Based on these climatic variables, NENA, NWNA and WUS clusters are climatically most similar as their confidence intervals overlap. The MX cluster deviates most from the others for temperature variables (PC1, 61.4% explained variation). The MX and WUS clusters show more precipitation variation (PC2, 14.9% explained variation) compared with the remaining NENA and NWNA clusters. (a–c) SNP selection involved consecutive steps: First, F_{ST} outlier SNPs had to be located close (<5 kb) to genes with PFAMs that occurred at least five times over all four genetic clusters; second, genes with such PFAMs were further categorized based on literature research, GO terms and uniprot/TAIR databases; third, genes categorized within 'abiotic stress response for biological process' (n = 19) were prioritized; and finally, among those 19 SNPs, based on annotation and allelic distribution pattern, we display one representative F_{ST} outlier SNP for each of the most distinctive genetic clusters (i.e., NENA: *Potrs017598g19854* homologous to *AT2G47240 LACS1* gene, WUS: *Potrs000450g00618* homologous to *AT1G22640 MYB3* gene, MX: *Potrs001211g02063* homologous to *AT3G09010 Protein tyrosine and serine/threonine kinase* gene). Climate variables (d) encompass all available in ClimateNA (Wang et al., 2016), that is, mean annual temperature (°C) (MAT), mean warmest month temperature (°C) (MWMT), mean coldest month temperature (°C) (MCMT), temperature difference between MWMT and MCMT, or continentality (°C) (TD), mean annual precipitation (mm) (MAP), May to September precipitation (mm) (MSP), annual heat–moisture index (MAT + 10)/(MAP/1000) (AHM), summer heat–moisture index ((MWMT)/(MSP/1000)) (SHM), degree days below 0°C, chilling degree days (DD_0), degree days above 5°C, growing degree days (DD5), degree days below 18°C, heating degree days (DD_18), degree days above 18°C, cooling degree days (DD18), the number of frost-free days (NFFD), frost-free period (FFP), the day of the year on which FFP begins (bFFP), the day of the year on which FFP ends (eFFP), precipitation as snow (mm) (PAS), extreme minimum temperature over 30 years (EMT), extreme maximum temperature over 30 years (EXT), Hargreaves reference evaporation (mm) (Eref), Hargreaves climatic moisture deficit (mm) (CMD), mean annual relative humidity (%) (RH), Hogg's climate moisture index (mm) (CMI) and degree days above 10°C and below 40°C (DD1040)

diversity pattern was observed (H_{OBS}, H_S and F_{IS}), patterns for the WUS and MX were very distinct, which can be attributed to either common (as is the case for NWNA and NENA) or distinct (as is the case for all others) historical and environmental factors (see Ding et al., 2017; Jaramillo-Correa et al., 2009). Molecular evidence for glacial survival on ice-free mountain tops has already been reported for plants in the European Alps (Schönswetter et al., 2005). The presumably relict Mexican populations displayed high individual-based *F* and

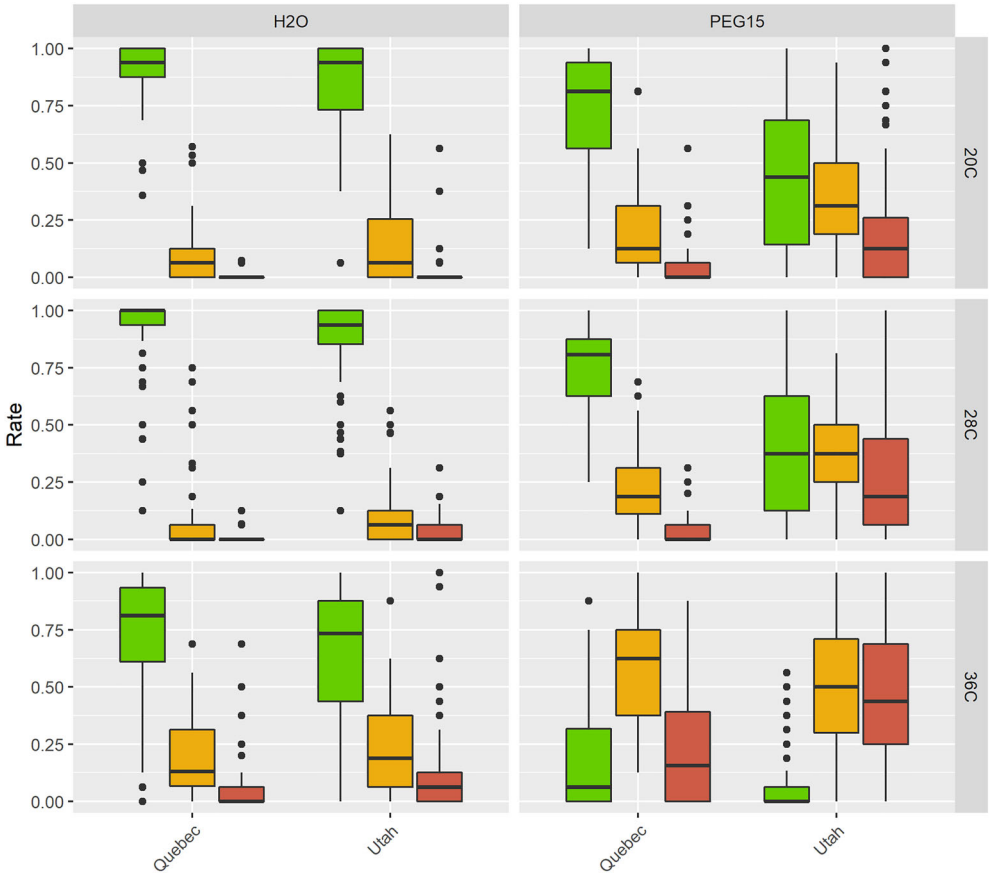

FIGURE 6 Boxplot of *Populus tremuloides* germination rates per region for the tested temperature and water regimes. Green indicates normal germination rate, orange indicates abnormal germination rate and red indicates absent germination. Overall, a significant reduction in normal germination rates was observed under 15% polyethylene glycol (PEG) stress and under 15% PEG stress in combination with 36°C compared with controls. Utah genotypes collectively exhibited a significantly reduced normal germination rate under 15% PEG stress (see Table S7 in File S2, Germ_region model)

low H_{OBS} because they occur in small, isolated high altitude stands (2200–3600 m a.s.l.) which rarely produced viable seeds (C. Wehenkel, personal observation). Hence, their between-population genetic variance was much higher compared with northern populations, yet their population-based diversity (especially inbreeding coefficient F_{IS}) was not different from the two northern clusters which could explain (1) why the Mexican populations have persisted and (2) maintained high levels of adaptive capacity.

P. tremuloides' ecosystems represent some of the most valuable vegetation types in many NA regions, and therefore, the species should be considered as keystone (Rogers & Gale, 2017). Its propagation strategy allows aspen as a pioneer species to come first by seed and then persist on the landscape through vegetative regeneration (i.e., root sprouting); the magnificent Pando (*lat*. I expand), a triploid aspen clone once made up of approximately 50,000 ramets covering an area of 43 hectares and located at Fishlake National Forest, Utah, USA (38.525, −111.750), is now declining (Rogers & Gale, 2017). Indeed, aspen clones covering large areas tend to be triploids and a general synergy between clonality and polyploidy in perennials as a selective advantage has been put forward (Mock et al., 2008, 2012). A recent study by Greer et al. (2018) suggested triploids may have a selective carbon uptake advantage over diploids, which would explain why triploid clones have higher shoot growth rates and are more vigorously propagating (Mock et al., 2008). However, triploids are also suggested to be more vulnerable to hydraulic stress (Greer et al., 2018). Long-standing interest in triploid aspen is not only based on their adaptive potential and higher growth rates but also certain favourable wood properties (higher specific gravity and longer fibres; Einspahr et al., 1963) for the forest industry.

The Salicaceae family is characterized by a considerable diversity in ploidy levels among its members. While in genus *Salix*, which is closely related to *Populus*, around 40% of the species are polyploid (Suda & Argus, 1968) with examples of extremely high ploidy (undecaploids in *Salix maccalliana* Rowlee), most pure *Populus* sect. *Tacamahaca* species are strictly diploid ($2n = 38$), while sect. *Populus* are facultative triploids (e.g., *P. tremuloides*, *P. tremula* and *P. alba*) (Rice et al., 2015; version 1.58). Outside this section, facultative polyploidy has only been reported in sect. *Aigeiros* (*P. nigra*) so far (Rice et al., 2015; version 1.58). However, information in this database stems from voucher samples; and while species range-wide assessments of ploidy levels are sparse, these are now vital to better understand species–environment interactions and forest resiliency to climate change (Greer et al., 2018).

There was no indication that all polyploids were sterile (we obtained viable seeds from western US triploid mother trees) or that germination or seedling growth was significantly impaired strictly based on mutation load. There is also evidence from *Populus tremula* that triploids are fertile and produce viable offspring (Johnsson, 1940). Contrary to current literature (e.g., Greer et al., 2018), we did find triploids at higher latitudes, especially within the NWNA cluster, and this

percentage was comparable with within aspen's most southern distribution in Mexico. Across aspen's entire range, we found a moderately positive correlation ($P < .01$) of triploid occurrence with dry and warm environments. An occurrence of polyploids could be beneficial because they may possess novel features for local adaptation, at environmental extremes or along latitudinal and elevation ranges (Parisod et al., 2010; Ramsey, 2011; Ramsey & Ramsey, 2014). With regard to sexual dimorphism in dioecious *Populus* (McKown et al., 2017), we noticed a prevalence of males, and male triploid aspen in particular, within our defined genetic clusters that harbour drought-prone environments (R. Goessen, unpubl.), where differences in resource allocation between sexes might explain a potential sex bias under stressful environmental conditions (Dorken & Van Drunen, 2018). Such imbalance, potentially aggravated through environmental stress, might indeed be a cause for concern with regard to aspen regeneration, especially in marginal populations such as those in Mexico. We put forward the hypothesis that it is dioecy of aspen and not polyploidy *per se* that might disadvantage aspen forest regeneration under future climate projections with frequent drought episodes and an increase in temperature. This needs further investigation in aspen, because sex locus markers have only become available recently (Pakull et al., 2015).

The strikingly lower number of F_{ST} outliers detected for the NWNA cluster in all cases agrees well with the climatic uniformity across this cluster. While statistical analyses did not expose an overrepresentation of certain PFAMs or GO terms between genetic clusters, environmental responses (biotic and abiotic stress) accounted overall for 41.5% of all categorized GO terms. These terms were most prevalent in WUS and MX clusters (49% and 39%, respectively), coinciding with higher variability in their respective environments and exposing patterns of important geographically adaptive genetic variation. Indeed, PFAMs with highest representations included *protein tyrosine and serine/threonine kinase* genes for the MX cluster and *myb-like DNA binding domain* genes for the WUS cluster, and the allele frequency patterns in such adaptive genes are clearly geographically differentiated. For the NENA cluster, we identified an aspen homologue to an *Arabidopsis LACS* gene involved in leaf cuticular wax and cutin biosynthesis, showing striking geographically differentiated genetic variation. *Lacs* mutants in *Arabidopsis* tend to have less wax and also cutin, rendering their cuticle more permeable, and these plants more prone to water loss through cuticular transpiration and highly susceptible to drought (Weng et al., 2010). Taken together, our study was able to match environmental with functional genetic variation across *P. tremuloides*' entire natural distribution, paving the way for future genomic applications. In addition to SNP variants, future work on larger structural genomic variations in aspen might also provide important diagnostics for local adaptation and stress responses, as already shown for the widely distributed balsam poplar (Prunier et al., 2019).

Our seed germination experiment further helped disentangle the effects of increased temperature from drought stress. Elevated temperatures alone had a minor effect compared with their combination with drought stress on germination rates and postgerminative growth. Indeed, on a range-wide basis, the NENA and WUS clusters, from where seeds for the experiment were sourced, are mostly differentiated by precipitation schemes (PCA, Figure 5d). While it is already known that aspen germination rate is extremely sensitive to drought (McDonough, 1979), seeds originating from seed sources locally adapted to drought (western United States) suppressed germination, potentially as a short-term adaptive seed survival strategy to avoid instant seedling mortality when water availability is suboptimal (Zeng et al., 2010). Another explanation for variable germination rates could possibly be maternal warming during seed development which would decrease germination success (Dewan et al., 2018).

Considering more episodes of drought and heat waves under climate change and shifting of climatic suitability of species towards higher elevations or northern regions, sexual reproduction in aspen might become even more difficult in the southern and western parts of this species' range where such episodes are already more prominent. Therefore, clonal reproduction in dry–system environments could become the major mechanism of persistence (Barrett, 2015; McDonough, 1979; Mock et al., 2012) for which epigenetic variation and accumulation of somatic mutations would be the source of important phenotypic variation (Barrett, 2015).

5 | CONCLUSION

With the separation into four main genetic clusters for *P. tremuloides*, and the uncovered genetic substructures within these, various hypotheses can now be tested around historical population movement (Jaramillo-Correa et al., 2009) and contemporary gene flow within and between the broader (bio)geographic regions on the North American continent as we defined them through population genomics, and further understanding these demographic histories, along with population structure and differentiation of populations will identify appropriate management units in aspen. Moreover, insights into the genetic differentiation between populations is also key to determine populations' potential candidacy for assisted gene flow, because the migration of local populations that are genetically too distant might cause outbreeding depression due to incompatibilities in local adaptation (Aitken & Whitlock, 2013). The information on the genetic makeup will also be particularly useful for developing a novel wood traceability tool in aspen for which the most informative genetic markers (species-specific, population-specific and adaptive variants) from our study will be extracted and made available to forest stakeholders involved in ecological restoration. Our study comprehensively provided information on ploidy and clonal diversity for aspen, with most triploids and aspen stands with highest numbers of clonemates identified within the western United States, Mexico and the Prairie provinces in Canada. The significant positive correlations of triploid occurrences in aspen stands with heat–moisture indices coinciding with a drier environment need to be further explored more exhaustively in common garden experiments. Under controlled conditions and drought stress, we

identified lower germination rates and postgerminative growth in aspen populations from Utah, which we attributed to a survival strategy related to imprinted differences in drought sensing based on annual precipitation at these sites or to potential maternal warming effects. Thus, follow-up experiments on the 'flexibility' of aspen's genome under environmental stress are needed to ascertain the importance of phenotypic plasticity versus natural selection for the species to persist. Finally, we identified several genetic variants under selection and influenced by temperature and precipitation variation across the four main genetic clusters. The adaptive diversity uncovered in our study can now be implemented in ecological niche modelling to improve the prediction of aspen distribution under varying climatic scenarios. This will also unravel potential genomic maladaptation of certain aspen populations to shifting climatic environments (Rellstab et al., 2021). This will pave the way for in-depth knowledge about local adaptation supporting predictions of aspen's response to climate change (Aguirre-Liguori et al., 2021; Savolainen et al., 2013).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

IMP, CW, NI, JB and RG planned and designed the research. CW, NI, MCGL, LTo, KM, RS, JHV and SLSR were involved in sample gathering and sharing. BB oversaw library preparation and sequencing procedures. RG performed experiments. LTi, MCGL, IG and LTo helped with conducting experiments (DNA extractions and germination experiment). JL performed SNP calling. RG analysed data. RG, IMP, NI, NP and JB interpreted the results and wrote the manuscript. IMP, NI, JB and CW obtained funding for this research.

DATA AVAILABILITY STATEMENT

Raw sequence data were deposited in the Sequence Read Archive with an embargo until 2023-03-01.

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