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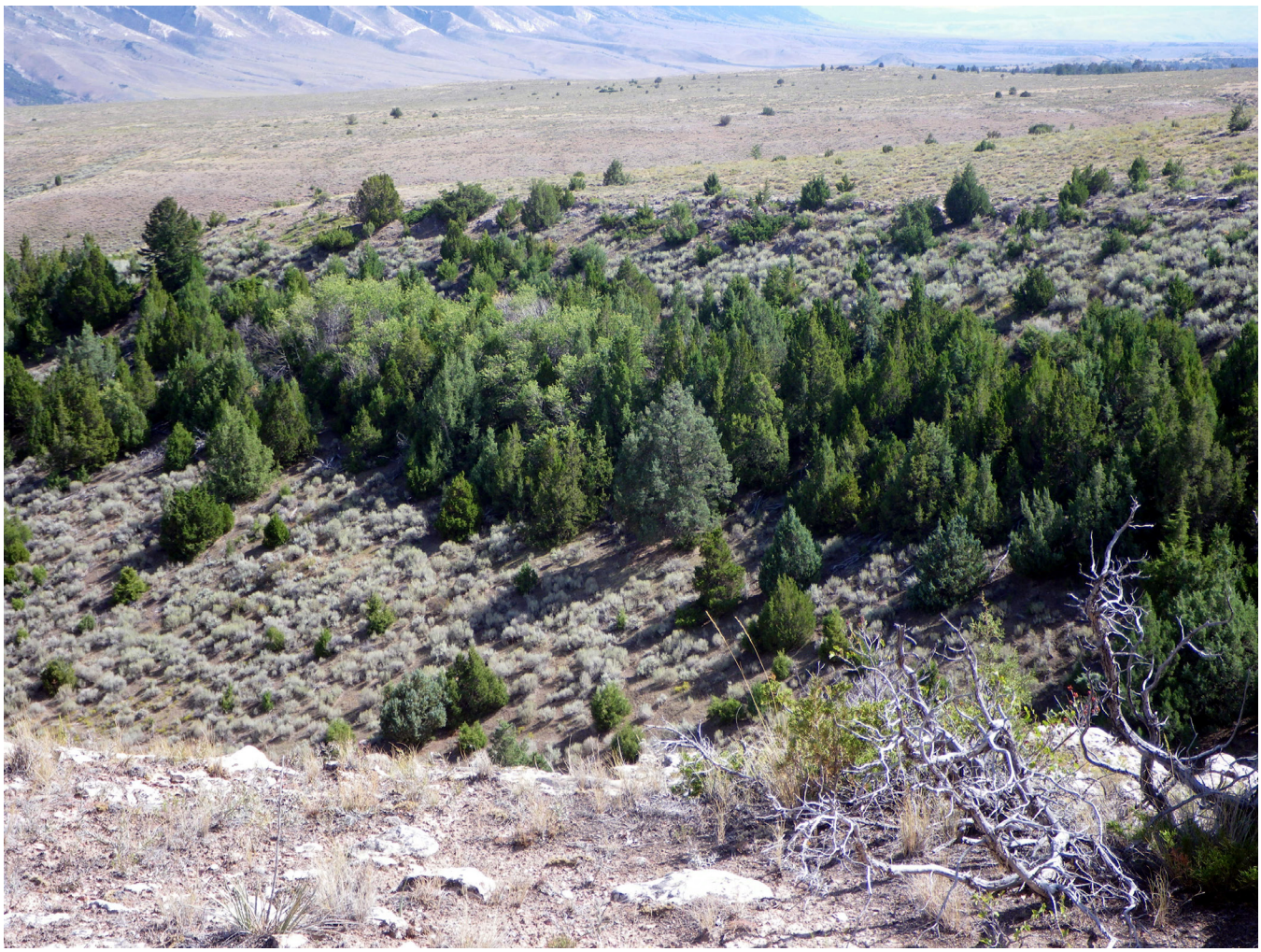
Genetic characterization and comparison of three disjunct *Populus tremuloides* Michx. (Salicaceae) stands across a latitudinal gradient

Natural Resource Report NPS/NRSS/IMD/NRR—2020/2073



ON THE COVER

View to the southeast showing the disjunct aspen stand on Dryhead Creek at Bighorn Canyon National Recreation Area (BICA). Note the surrounding Pinyon-Juniper and sagebrush communities.

Photo by: Joe Stevens, CNHP

Genetic characterization and comparison of three disjunct *Populus tremuloides* Michx. (Salicaceae) stands across a latitudinal gradient

Natural Resource Report NPS/NRSS/IMD/NRR—2020/2073

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Abstract

Populus tremuloides has the largest species range of any deciduous tree in North America and is ecologically and economically important. Since the melting of Cordilleran and Laurentide ice sheets, the range of *P. tremuloides* has expanded northward. With the northward expansion, many areas in western North America that would have been suitable during the early Holocene no longer support *P. tremuloides*. However, microrefugia still harbor small *P. tremuloides* stands in ecologically anomalous and spatially disjunct sites across western North America. Theoretical and empirical studies suggest that individuals in such sites may harbor locally adapted genes, having been under strong selection pressure for long periods of time given the more arid environment and presumed relictual origins. Microsatellite markers and a continent-wide dataset for *P. tremuloides* were employed to compare disjunct stands in three different sites across a latitudinal gradient to assess genetic diversity and possible relictual origins. All disjunct stands were comprised of a single diploid genotype. Each unique diploid genotype contained alleles that were rare and distantly geographically dispersed in the range-wide dataset. This finding suggests that these stands are relictual, i.e. that they are remnants from a time when now broadly separated populations were connected through geneflow. From these findings further detailed study of these and similar stands are needed, as well as in-situ and ex-situ conservation to preserve the genetic resources present in these disjunct stands.

Introduction

The quaking aspen or aspen (Salicaceae: *Populus tremuloides* Michx.) is an iconic North American tree species ranging from northern Canada to south-central Mexico and from California to Newfoundland, making it the most widespread deciduous tree species in North America (Little 1971; Eckenwalder 1996) (Figure 1). Aspen stands provide important ecosystem functions such as harboring a high diversity of mammal, bird, plant, and insect life (e.g. Debyle and Winokur 1985; Rumble et al. 2001; Simonson et al. 2001; Griffis-Kyle and Beier 2003), a high capacity for storing and releasing snow pack (LaMalfa and Ryel 2008), and acting as fire breaks during wildfires (Fechner and Barrows 1976). Aspen are also economically important as a source of timber, pulp, and wood fiber, as well as for their aesthetic value as an ornamental tree (Debyle and Winokur 1985).

The current extensive range of aspen is largely the result of recent range expansion since the last glacial maximum (LGM), as much of the current northern extent of the range was covered by the Cordilleran and Laurentide ice sheets until about 10,000 ybp (Figure 1). Phylogeographic work has shown that recent expansion of aspen is evident in the genetic structuring of the range into northern and south-western genetic clusters (Callahan et al. 2013). This division between the groups generally occurs along the LGM. The northern cluster is characterized by having limited sub-structuring both within populations and individuals, as well as high rates of inferred geneflow, and almost no effect of isolation by distance (Latutrie et al. 2016). By contrast, the south-western genetic cluster is sub-structured, with lower relative diversity within populations but high allelic diversity regionally (Callahan et al. 2013). These genetic patterns suggest that the south-western genetic cluster is a set of stable relictual populations moving between low and high elevations during climate fluctuations, while the northern genetic cluster has expanded into areas of northern North America previously occupied by continental glaciers. Niche modeling (Ding et al. 2017) and genotyping of relictual populations along the Niobrara River (Deacon et al. 2017) suggest that the north central Great Plains east to the central Appalachians may have been refugial areas for aspen during the last ice age. While in the south-west portion of the aspen range lower elevation valleys below glaciated mountains likely possessed suitable aspen habitat during the last ice age (Mitton and Grant 1996; Ding et al. 2017).

In addition to the differences in population genetic structure between the north and south-western genetic clusters within the aspen species range, geographic differences in ploidy have also been described (Mock et al. 2012). In the western mountainous portion of the aspen range triploid genotypes were found, in some cases, to constitute over half of the samples examined for some sites (Colorado and Utah), while in the northern and eastern portions of the range very few if any triploids were found (Mock et al. 2012). While polyploidy is common in plants the persistence of non-diploidized polyploids over longer evolutionary time scales is rare (Thompson and Lumaret 1992; Arrigo and Barker 2012; Van de Peer et al. 2017). Polyploidy is thought to provide competitive advantages over diploids in the early stages of colonizing newly available habitats or expanding range limits as polyploids have been shown in some instances to be more drought tolerant (Li et al. 1996; Ravi et al. 2013), have increased growth rates (Maceira et al. 1993; Liao et al. 2016), and increased cold hardiness (Sugiyama 1998; Ozherelieva and Sedov 2017). It has been posited that the south-western distribution of triploidy in aspen may be due to the hotter more arid climate in this area

and the possible increased drought tolerance of triploids over diploids (Mock et al. 2012). However recent physiological comparisons between diploid and triploid aspens indicated that triploid aspen may be less drought tolerant than diploids because of higher transpiration rates (Greer et al. 2017). Finding recurrent triploid-genotype stands that have been exposed to long term aridity without the presence of diploids would support the idea that triploid aspen are more drought tolerant.

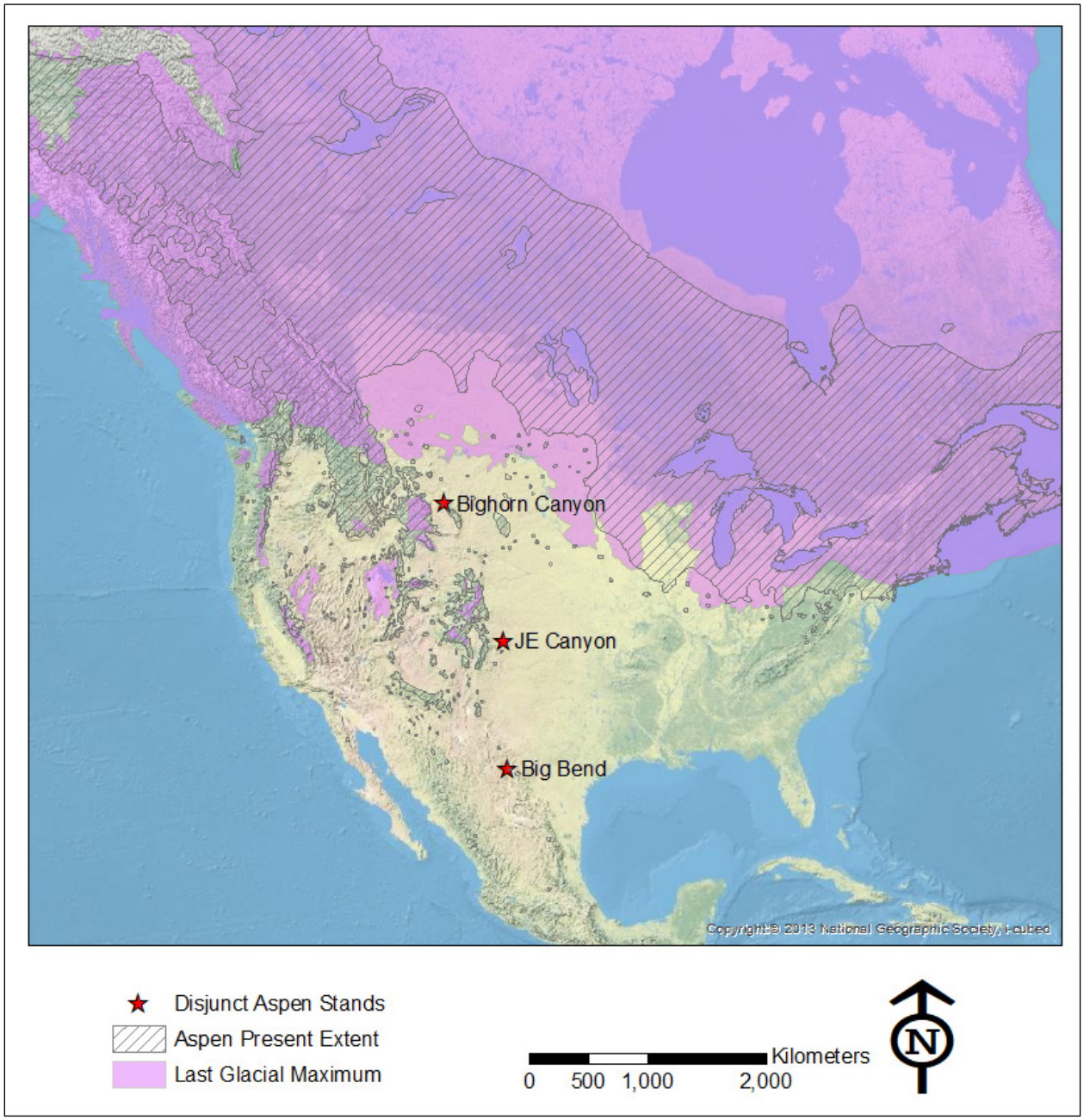

Figure 1. Present extent of Populus tremuloides (Little 1971) relative to the last glacial maximum (LGM; Erhlers et al. 2011). Disjunct aspen stands were sampled in Bighorn Canyon National Recreation Area, Montana, JE Canyon Ranch, Colorado, and Big Bend National Park, Texas.

Across the range of aspen, numerous small disjunct stands have been noted to occur distant from any larger core stands of aspen (Mitton and Grant 1996). The sites in which these stands occur are considered anomalous as they are often in areas thought to be too arid for aspen survival (Stevens et al. 2017). The origin of these stands is either recent long-distance dispersal by seed (Debyle and Winokur 1985) from adjacent stands or the remnants of once larger aspen forests that existed during the last glaciation (Ding et al. 2017). In either case these disjunct stands of aspen are common throughout the south-western genetic cluster range and persist due to the presence of favorable microclimatic conditions and/or locally adapted genes. Strong selection on locally adapted genes for drought and heat tolerance are expected to be present in these disjunct stands because of the arid environments in which they are found. In two *Populus* species, *P. balsamifera* and *P. tremula,* clear genetic evidence has been found for strong selection on different adaptive traits at the trailing and leading edges of these species ranges (Wang et al. 2018; Keller et al. 2018). Specifically, Keller et al. (2018) suggested that the adaptive genes at the trailing edge for the range of *P. balsamifera* were under the greatest threat of extinction given the current climate trend and general shift of the range to the north. As such, these trailing edge genotypes are strong candidates for germplasm preservation. Similar contractions are projected for the southern portion of the aspen range (Rehfeldt et al. 2009) with evidence of this contraction already being recorded in some areas (Bretfield et al. 2016).

Numerous National Park units have been established throughout the range of aspen, and because of the non-random placement of these parks in areas with unique landscape attributes, they have inadvertently preserved numerous disjunct aspen stands. However, the main focus of aspen research in US National Park units has been on larger scale ecological questions such as stand persistence and recruitment in the absence of ungulate control by predators (e.g. Ripple and Larsen 2000; Beschta and Ripple 2009). The integration of population genetic and genomic studies with larger ecologic questions would be an important addition to understanding these trophic interactions in greater depth. More importantly, an assessment of the diversity and uniqueness of aspen genotypes found in US National Park units will help to guide future conservation efforts including in situ and ex situ germplasm preservation, and will enhance visitor interpretation and education (e.g. Fancy et al. 2009). The preservation of locally adapted and genetically diverse germplasm will be essential in proposed assisted migration programs (Gray et al. 2011) and an invaluable resource in developing disease and drought resistant lines for various purposes (e.g. Benetka et al. 2012).

Herein we apply genetic approaches to characterize four disjunct stands occurring in ecologically anomalous sites across a latitudinal gradient. These sites are regarded as anomalous because they deviate from the surrounding arid scrublands by having a greater abundance of subsurface water and thus vegetation. Two stands occur in southern Montana at Bighorn Canyon National Recreation Area (BICA; NPS abbreviation), one stand on the private JE Canyon Ranch held by the Nature Conservancy in southeastern Colorado, and one stand in Big Bend National Park (BIBE; NPS abbreviation) in southern Texas (Figure 1). Given the results of Callahan et al. (2013) and the theoretical work predicting the genetic expectations of species range shifts (Hampe and Petit 2005; Mock et al. 2012; Woolbright et al. 2014; Kiedrzynski et al. 2017) we aim to test the following

hypotheses: 1) the southern disjunct stands should be older than the northern (as measured by coalescent times to the inferred genetic cluster of origin; Sun et al. 2009), 2) genetic divergence between disjunct stands and inferred genetic clusters of origin should increase with increase in latitudinal separation, and 3) Triploidy should be common among disjunct stands occupying arid environments if triploids are more drought and heat resistant than diploids. If hypotheses one and two are supported by the data, then the disjunct stands will be inferred to be relictual, and not from a recent dispersal. Numerous tree species have shown similar patterns of genetic divergence between core (high geneflow) and marginal (low geneflow) populations (e.g. Peakall et al. 2003; Wei et al. 2016). Given the limitations associated with using single genotype stands for population-based inferences, complimentary analyses were conducted to find common patterns for making inferences regarding the origin of disjunct stands. In addition to testing these theoretical expectations, the resulting data will be useful in helping to prioritize conservation efforts and guiding future studies examining the functional genomics of aspen throughout the species range (*sensu* Keller et al. 2018).

Materials and Methods

Sampling

Four disjunct aspen stands in three sites, Bighorn Canyon National Recreation Area (BICA), JE Canyon Ranch (JE Canyon), and Big Bend National Park (BIBE) were identified for study based on previous fieldwork in which they were noted as being abnormal for persisting outside the habitat expected for aspen. The disjunct stands range in distance from 15 to 450 km from the nearest adjacent large stands that could be characterized as typical montane aspen stands. In addition to collecting specimens from disjunct stands, collections from adjacent large montane aspen stands (at BICA and JE Canyon) were made to improve local sampling density. Adjacent sampling for the BICA stands consisted of collections from the Pryor Mountains in Montana to the east and the Bighorn Mountains to the southeast in Wyoming, and for the JE Canyon stand in Colorado the adjacent Spanish peaks to the west were sampled. The three sites (BICA, BIBE, and JE Canyon Ranch) were chosen based on their distribution across a broad latitudinal gradient.

All collections from BICA, JE Canyon Ranch and their respective adjacent montane stands consisted of two specimens from each tree to be deposited in the Colorado State University herbarium (LR Tembrock collection numbers 17110 – 17209), a sample of three or four leaves preserved in silica gel for DNA extractions, geographic coordinates, estimated tree height, a dbh (diameter at breast height) measurement to roughly assess age class of trees, and notes regarding the tree sampled, associated species, and slope/aspect (see Appendix A).

Bighorn Canyon National Recreation Area Site

On the 29th of August 2017, we sampled tissue from trees in each of the two known *P. tremuloides* stands in BICA (Figure 2). The BICA Dryhead and BICA Bench stand, separated by 12 kilometers, were each sampled by dispersing collections across the stands to maximize the chance of detecting multiple clones if present.

The BICA Dryhead stand from which 11 trees were sampled is in a branch canyon of Dryhead Creek (latitude 45.20906, longitude -108.16962) near where the main creek flows into the Bighorn River (hereafter referred to as the Dryhead stand). The approximately 1.5 ha Dryhead stand (Figure 3A, B) occurs at the edge of an arid plateau (Figure 4) on a north-facing slope where groundwater is perched close to the surface and emanates in one or two small springs (see Stevens et al. 2017 for a detailed description of the stand). The nearest large montane aspen stands to the Dryhead stand are located 15 kilometers to the west in the Pryor Mountains and approximately 40 kilometers to the southeast in the northern Bighorn Mountains. From a 50 square kilometer area in the Pryor Mountains 29 aspen specimens were collected and from a 600 square kilometer area within the northern Bighorn Mountains 28 aspen specimens were collected. These geographically adjacent collections were made to improve inferences for the origin of BICA aspen. The individual trees at the Dryhead stand are mostly small (<0.5m dbh) with a few larger specimens (>1.4m dbh) in protected areas and some newly emergent suckers (~0.5 m in height) in open sites. The main tree species that co-occur in and around the Dryhead stand are *Juniperus scopulorum* (Rocky Mountain juniper) and *Pinus flexilis* (limber pine). Other common species on the site include *Mahonia repens* (creeping Oregon grape),

Artemisia spp. (sagebrush)*, Rhus trilobata* (skunkbush)*, Symphoricarpos* spp (snowberry)*., Ribes* spp. (currant), and *Amelanchier alnifolia* (western serviceberry).

The BICA Bench aspen stand is near the northwest corner of the park on a natural bench (hereafter referred to as the Bench stand) on the eastern slope of the Pryor Mountains (latitude 45.13148, longitude -108.28117) (Figure 5). A total of six individuals were collected from this approximately 1.6 ha stand. The nearest large montane aspen stands to the Bench stand are located six kilometers to the northwest in the Pryor Mountains and approximately 40 kilometers to the southeast in the northern Bighorn Mountains. Likely due to the geology at the Bench stand, springs have provided suitable conditions for its survival in an otherwise semiarid habitat. The individuals in the stand are a mix of relatively large (0.9m dbh) and presumably older trees with furrowed bark to moderately sized trees (0.5m dnh) to young newly emergent suckers (0.25m in height). The condition and large size of the trees in the Bench stand suggest that the habitat in this area are more favorable for aspen survival and clearly less arid than the Dryhead stand. The main plant species co-occurring in and around the Bench stand were *Acer glabrum* (Rocky Mountain maple), *Cirsium* spp. (thistle), *Cornus sericea* (western dogwood), *Juniperus scopulorum*, *Mahonia repens*, *Physocarpus* spp. (ninebark), *Pseudotsuga menziesii* (Douglas fir), and *Symphoricarpos albus* (common snowberry).

JE Canyon Ranch Site

The JE Canyon Ranch site, held by the Nature Conservancy, is operated as a cattle ranch and managed for the preservation of its unique ecological attributes. A single disjunct stand was sampled at this site (hereafter referred to as the JE Canyon stand) (Figure 6). The JE Canyon stand is associated with a seep along the north facing wall of a branch canyon of the larger Chacuaco Canyon (latitude 37.35664, longitude -103.697748) (Figure 7). The nearest large montane aspen stands to the JE Canyon stand are located 120 kilometers to the west in the Spanish Peaks from which 15 specimens were collected from a 300 square kilometer area. The 0.4 ha JE Canyon stand (Figure 3C, D) is made up of about 100 trees. The surrounding landscape, like that of the Dryhead stand, is ecologically anomalous to the normal conditions in which aspen are generally found. Associated species include *Pinus ponderosa* (Ponderosa pine), *Quercus gambelii* (Gambel oak), *Juniperus scopulorum*, *Gutierrezia sarothrae* (broom snakeweed), *Cercocarpus montanus* (mountain mahogany), *Rhus trilobata*, and *Vitis riparia* (riverbank grape).

Big Bend National Park site

Collections from Big Bend NP in September 2005 were made during a separate study (Mock, personal communication). A single disjunct stand, hereafter referred to as the Big Bend stand, was sampled within this site. This stand is centrally located within Big Bend NP, approximately 240 meters southwest of the summit of Mt Emory (latitude 29.243837, longitude -103.307006) (Figs. 8, 9). Several other disjunct aspen stands are thought to exist near the Big Bend stand in sky island mountain ranges like the Maderas del Carmen in Mexico, but the nearest large montane stands to Big Bend occur in the Sacramento Mountains of New Mexico, 450 kilometers to the northwest. We were unable to find previously collected aspen specimens, DNA, or genotypes from the Sacramento Mountains, so samples taken for Callahan et al. (2013) from a 50 square kilometer area in the Black Range of New Mexico, 580 kilometers NW from the Big Bend site and 180 kilometers west of the

Sacramento Mountains, were used for comparison. Unlike the Dryhead and JE Canyon stands, which are in protected north facing sites, the Big Bend stand is in an open west facing talus slope with little nearby vegetation (Fig. 3E, F). The 20 or so trees in the stand are broadly spaced and found across an area of about 0.3 hectares. Common plant species adjacent to the Big Bend stand includes *Arbutus xalapensis* (Texas madrone), *Pericome caudata* (mountain tail-leaf), *Pinus cembroides* (Mexican pinyon), and *Quercus* ssp. (oaks).

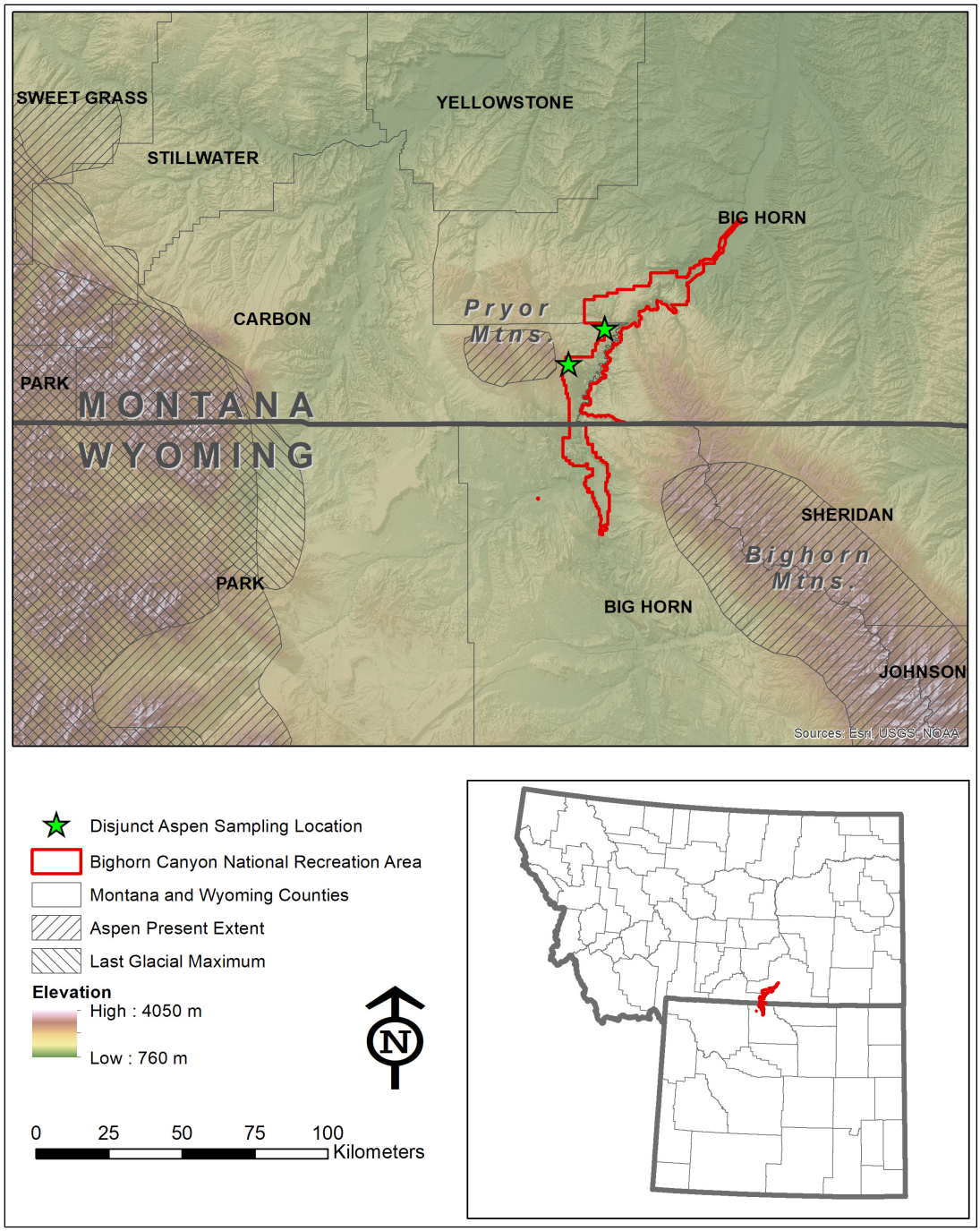

Figure 2. Disjunct aspen sampling locations in Big Horn Canyon National Recreation Area relative to the present extent of aspen in northcentral Wyoming and southcentral Montana.

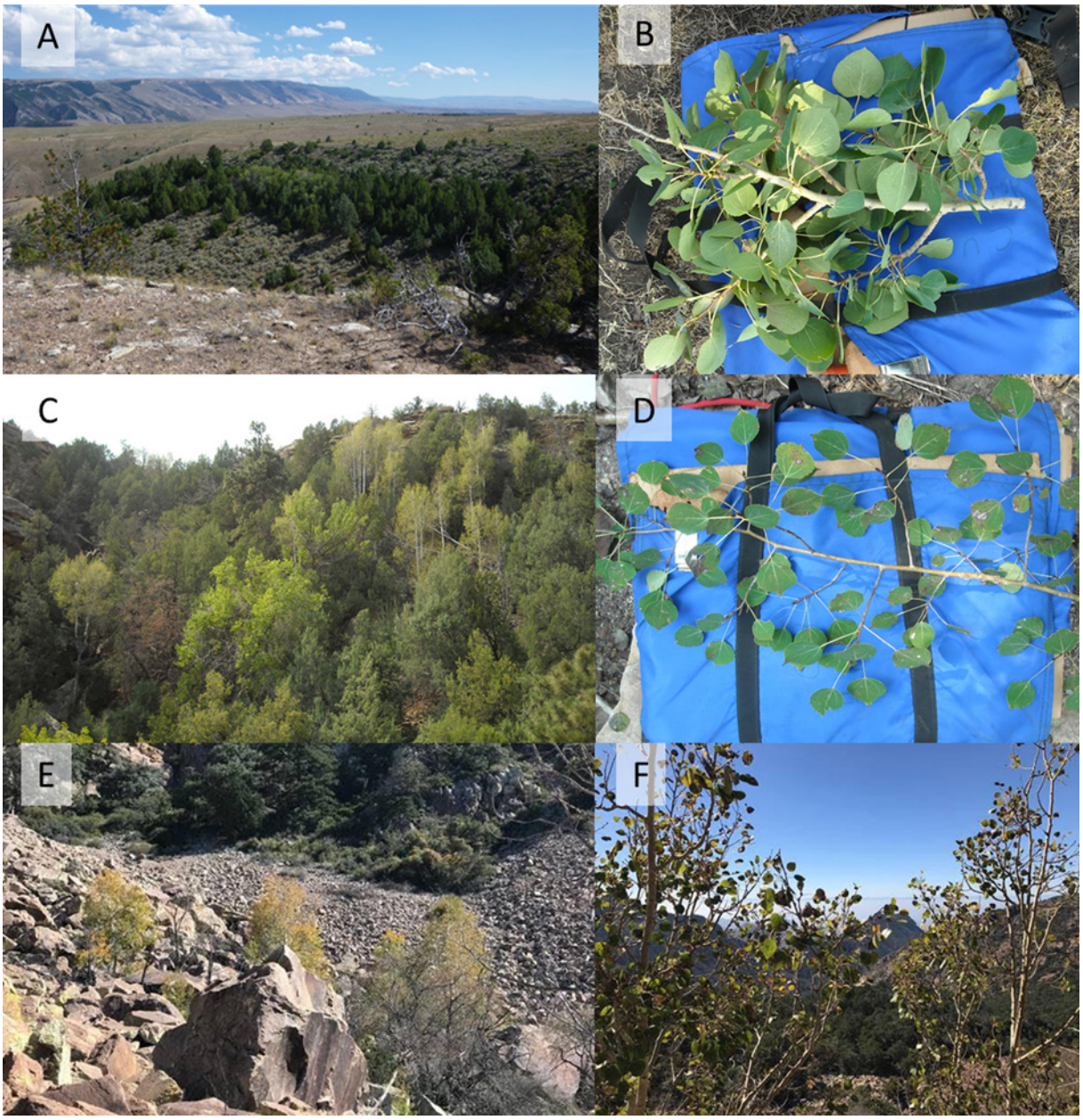

Figure 3. Three disjunct stands and samples from (A) BICA Dryhead stand, (B) sample 17114 made from BICA Dryhead stand, (C) JE Canyon Ranch stand, (D) sample 17202 made from JE canyon stand, (E) Big Bend stand looking east towards Mt Emory, and (F) Big Bend stand detail of leaves and stems.

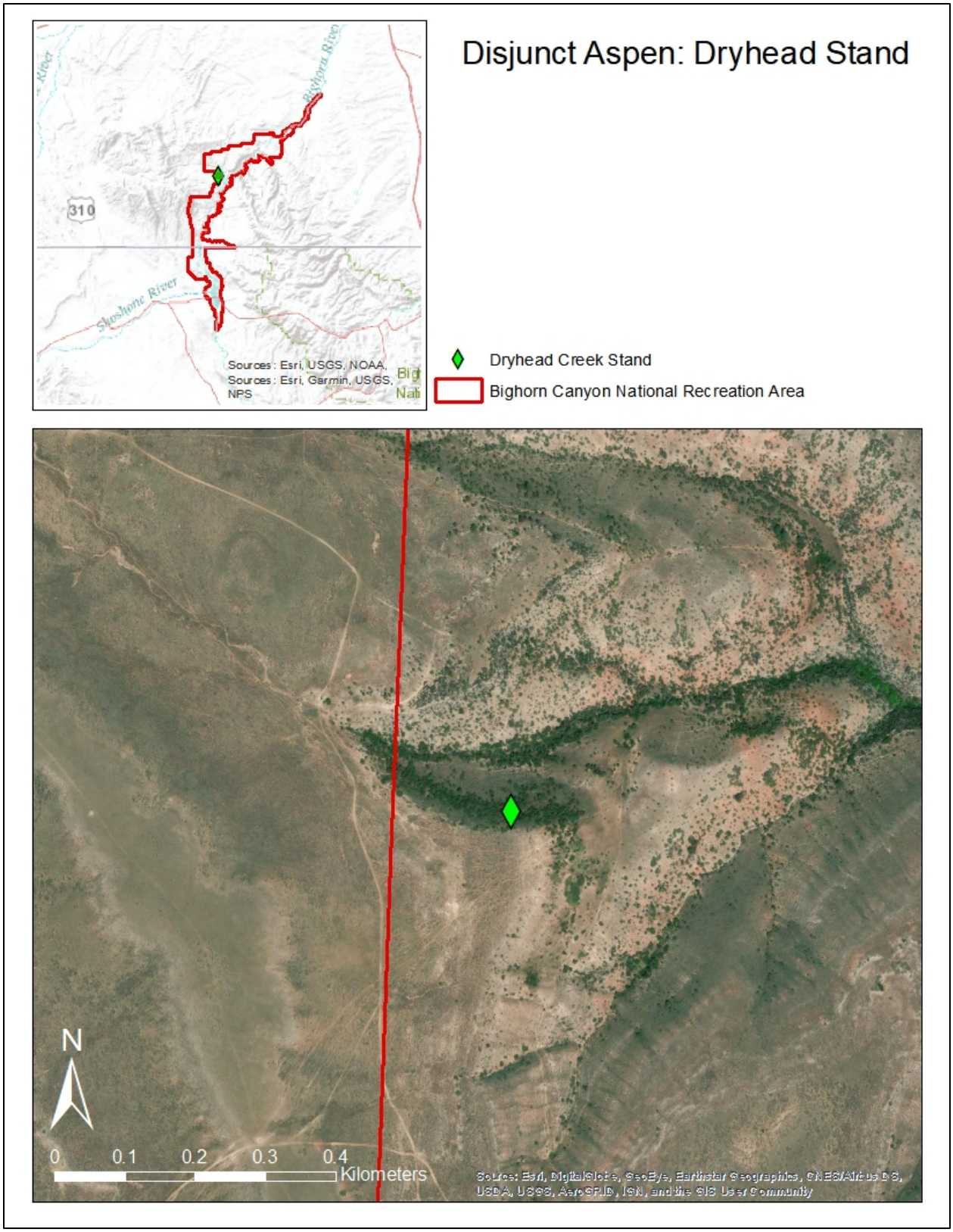

Figure 4. Dryhead Creek stand where sampling of disjunct aspen occurred in Bighorn Canyon National Recreation Area, Montana (note arid setting).

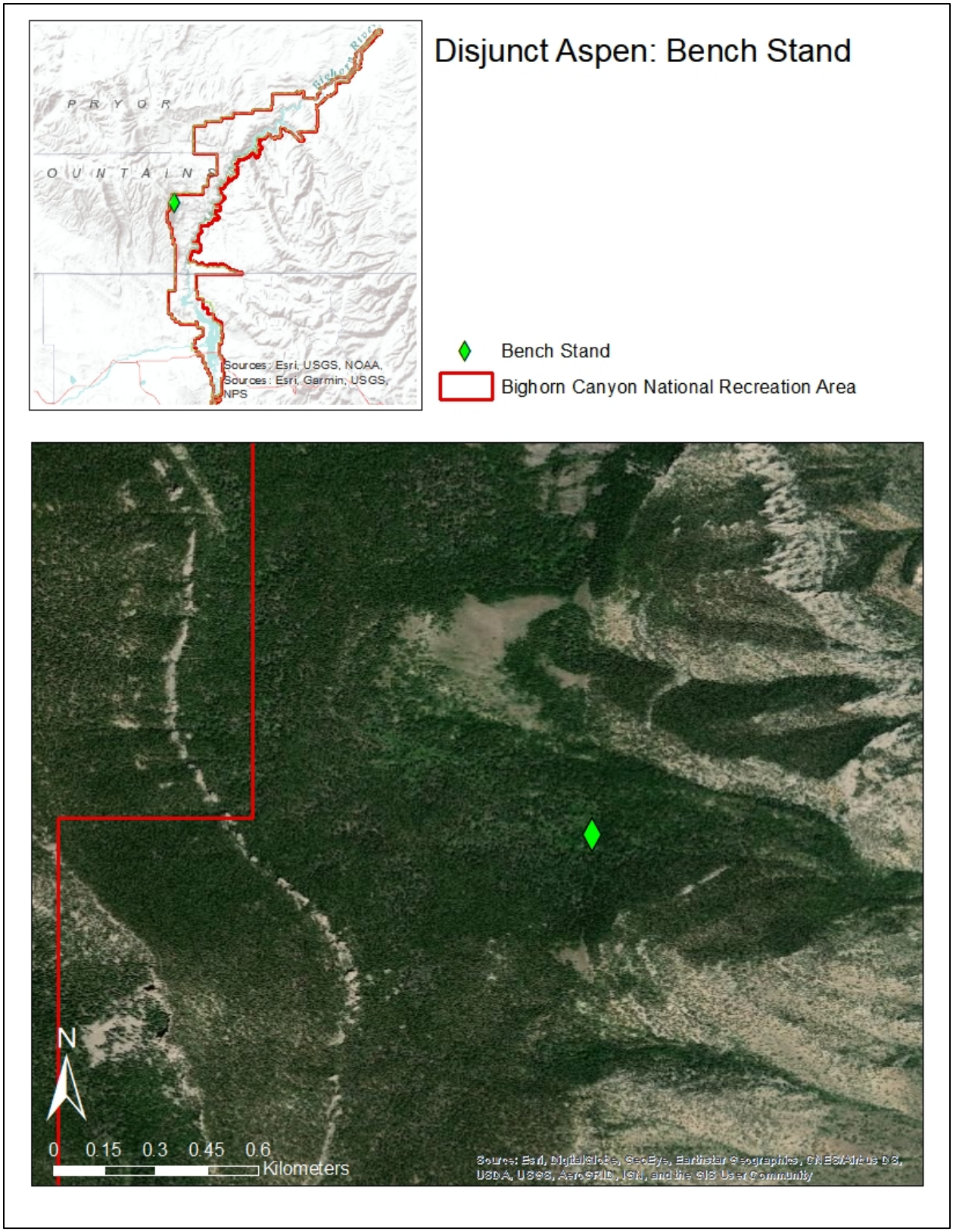

Figure 5. Bench stand where sampling of disjunct aspen occurred in Bighorn Canyon National Recreation Area, Montana.

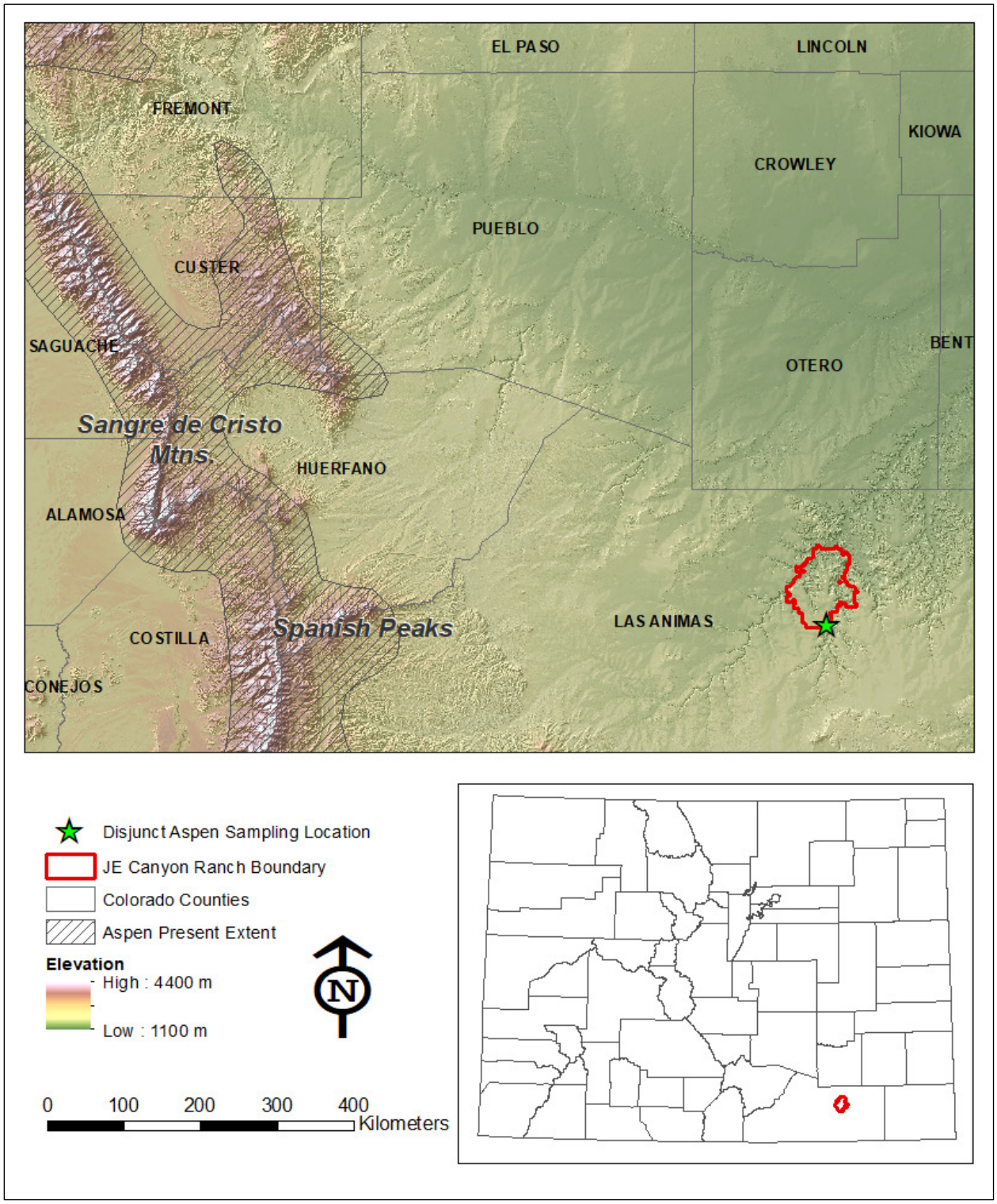

Figure 6. Disjunct aspen sampling location in JE Canyon Ranch relative to the present extent of aspen in southeast Colorado. Last glacial maximum extent not displayed because it does not occur within bounds of the map – montane glaciers were present in high elevation mountain valleys through the early Holocene.

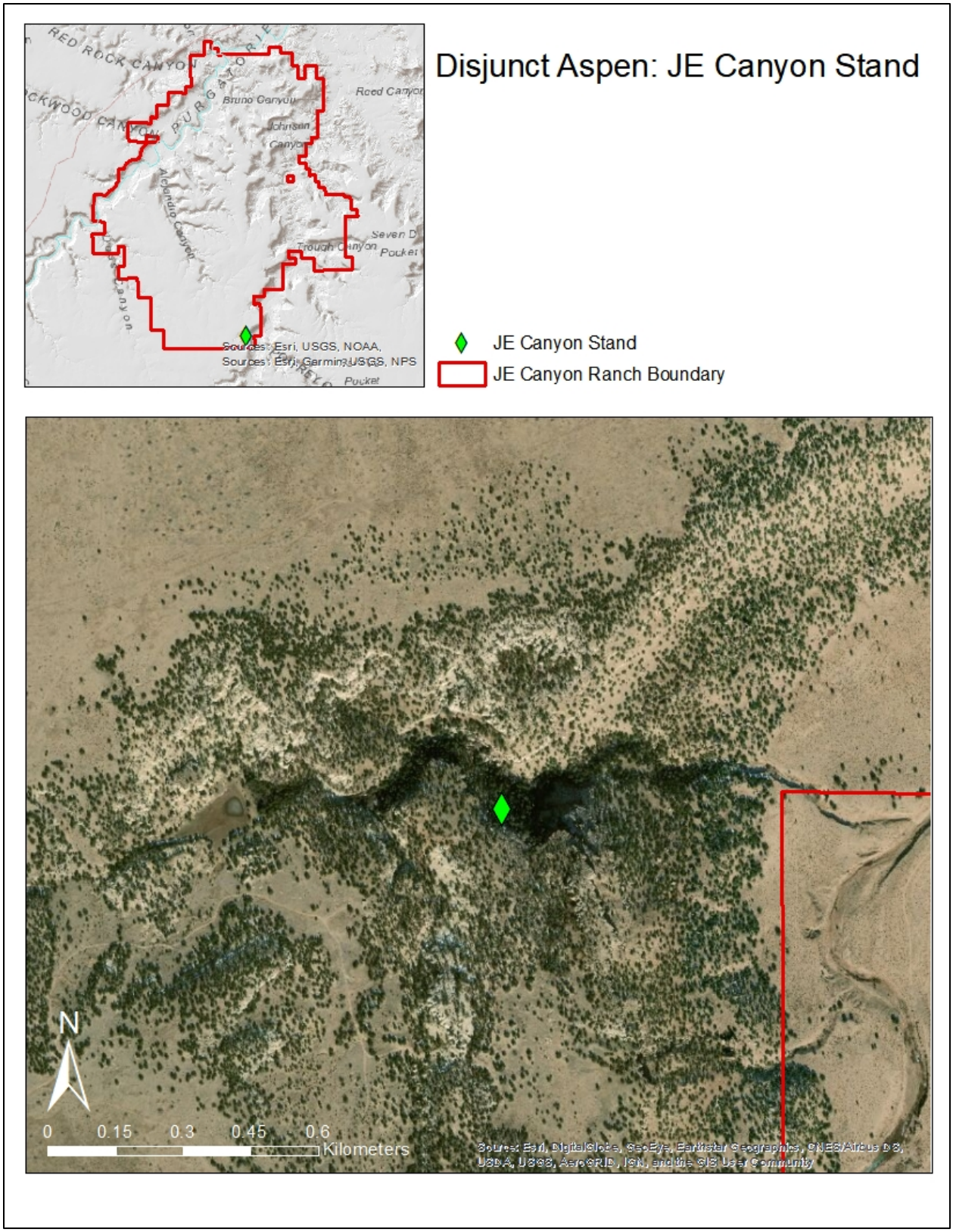

Figure 7. JE Canyon stand where sampling of disjunct aspen occurred on JE Canyon Ranch, Colorado (note arid setting).

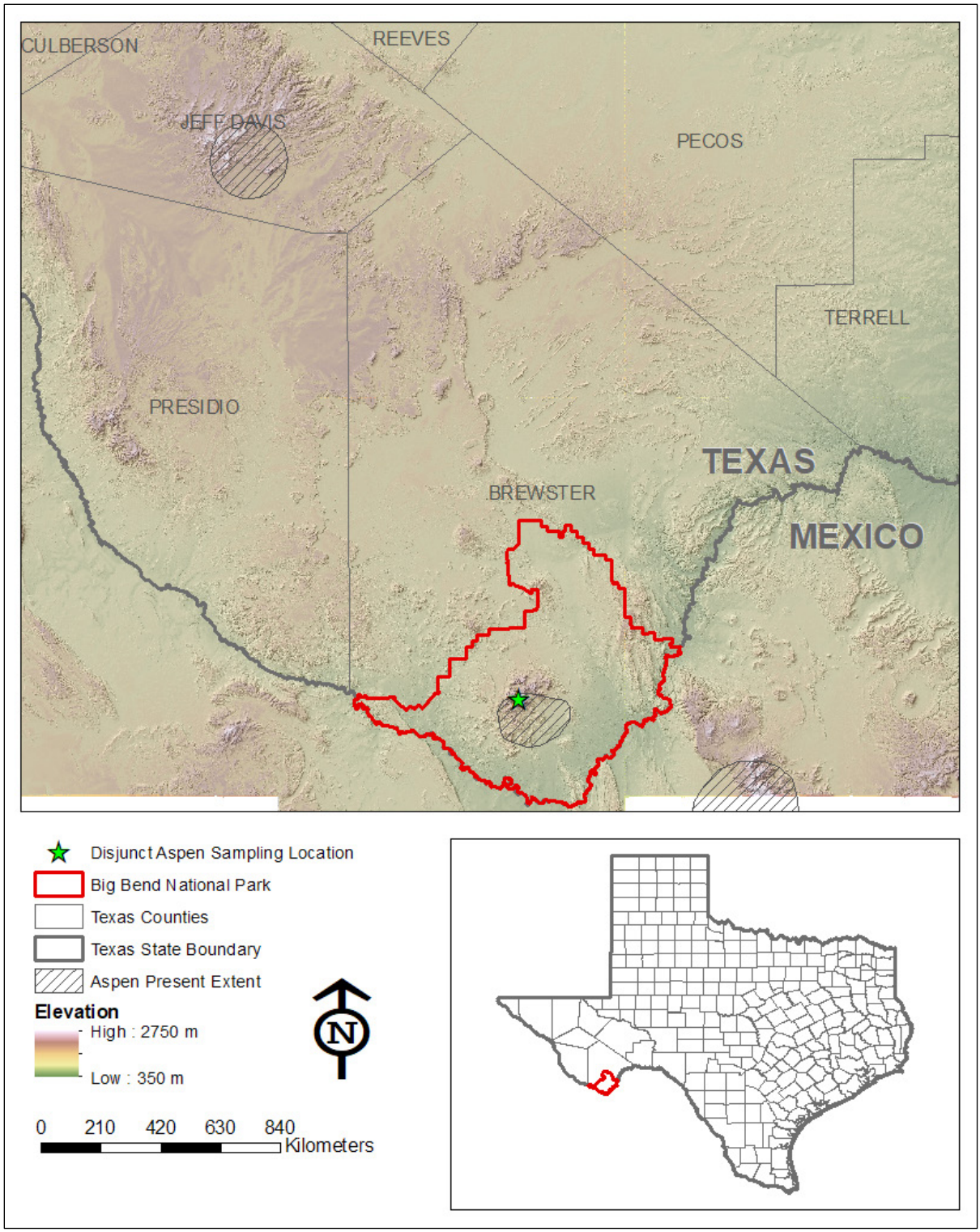

Figure 8. Disjunct aspen sampling location in Big Bend National Park relative to the present extent of aspen in southwest Texas and northern Mexico. Last glacial maximum extent not displayed because it does not occur within the bounds of the map. Present aspen extent shown in Big Bend is based on the disjunct aspen stand described in this study.

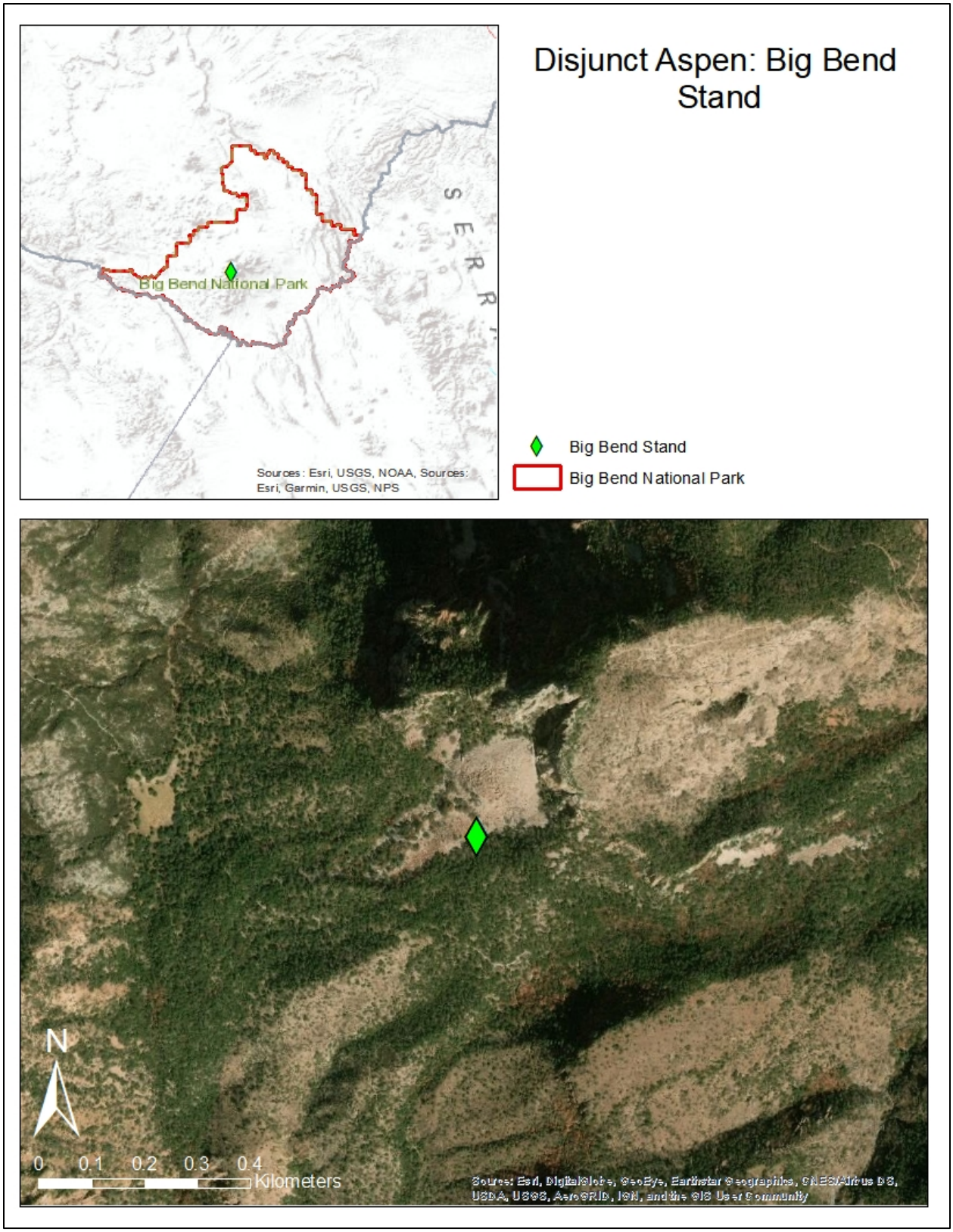

Figure 9. Big Bend stand where sampling of disjunct aspen occurred in Big Bend National Park, Texas (note arid setting).

DNA Extraction, PCR, and Genotyping

Leaf samples collected during 2017 from disjunct stands in BICA and JE Canyon as well as from adjacent large montane stands in the Pryor, Bighorn, and Spanish Peaks mountain ranges were processed for DNA extraction, PCR amplification, and fragment analyses. All other samples used in this study (see genetic data assembly section below) were processed previously for different aspen genetics projects (Callahan et al. 2012; Mock et al. 2013) and combined with the data collected in 2017. For all data collected in 2017, (Appendix A; LT collection numbers 17110-17209) genomic DNA was extracted from leaf material using a Qiagen (Venlo, Netherlands) DNeasy plant kit following the manufacturer's instructions except for the final elution, where volume was reduced from 100 µL to 50 µL. Prior to extraction, leaf tissue was pulverized in a ball mill (Biospec Products, Bartlesville, Oklahoma, USA) using silica-zircon beads. The DNA extractions (n=100) were diluted to approximately 5.5 ng/µL. Diluted samples were then amplified at 12 microsatellite loci, including seven described in Callahan et al. (2013; W14, W15, W17, W20, P86, P10, and P71. See Table 1). Microsatellite loci were amplified in 10µl reactions containing 2x MyTaq HS Master Mix (BioLine), 0.5 mM $MgCl_2$, 0.3 µM forward and reverse primers, and approximately 15 ng DNA. Thermocycling conditions were 95°C for 3 min. followed by 35 cycles of 95°C for 30 seconds, a locus-specific annealing temperature (50°C, 54°C, 57°C, or 60°C) for 40 seconds, and 72°C for 50 seconds, followed by a final extension of 72°C for 10 minutes. All loci were amplified separately with the exception of W20/P14 and P86/P71, then pooled prior to fragment analysis into 4 multiplex groups (Table 2).

Extracted DNA from 14 samples described by Callahan et al. (2013) were run alongside the newly acquired samples. These 14 samples were chosen to represent the range of allele sizes observed in Callahan et al. (2013), and 5 of these samples were used as a ladder in genotyping PCRs. Alleles were scored using GeneMarker v2.6.7 (SoftGenetics, LLC.) and the allele sizes adjusted and binned to match allele sizes from Callahan et al. (2013), based on the replicated samples from that study.

Table 1. Microsatellite loci, primer sequences, and attached 5'-modified fluorophores (see Appendix A for microsatellite lengths by locus and specimen).

Locus	Primer ID	5' Fluorophore	Primer Sequence, orientation is 5' - 3'
W14	WPMS-14-L	6FAM	CAGCCGCAGCCACTGAGAAATC
	WPMS-14-R	–	GCCTGCTGAGAAGACTGCCTTGAC
W15	WPMS-15-L	6FAM	CAACAAACCATCAATGAAGAAGAC
	WPMS-15-R	–	AGAGGGTG TTGGGGGTGACTA
W17	WPMS-17-L	6FAM	ACATCCGCCAATGCTTCGGTGTTT
	WPMS-17-R	–	GTGACGGTGGTGGCGGATTTTCTT
W20	WPMS-20-L	–	GTGCGCACATCTATGACTATCG
	WPMS-20-R	VIC	ATCTTGTAATTCTCCGGGCATCT

Table 1 (continued). Microsatellite loci, primer sequences, and attached 5'-modified fluorophores (see Appendix A for microsatellite lengths by locus and specimen).

Locus	Primer ID	5' Fluorophore	Primer Sequence, orientation is 5' - 3'
P86	PMGC-486-L	VIC	AGAAGTTGTTGAACCCGATGGG
	PMGC-486-R	–	GCTACAAACTTTGTTGTACCC
P10	PMGC-510-L	VIC	AGTCCTGGTCCTGGATTGG
	PMGC-510-R		CTACATTAATTTCCCTGTCATC
P71	PMGC-2571-L	6FAM	TCTCGCAGATTCATGTAACCC
	PMGC-2571-R	–	GACTGTATGTTGACCATGCCC
P33	PMGC-433L	–	GCAGCATTGTAGAATAATAAAAG
	PMGC-433Rt	NED	AAGGGGTCTATTATCCACG
P76	PMGC-576-L	–	GCTGTCTAACATGCCATTGC
	PMGC-576-R	NED	AATTTACATTTCTTTATCATCACC
P14	PTR14-F	NED	TCCGTTTTTGCATCTCAAGAATCAC
	PTR14-R	–	ATACTCGCTTTATAACACCATTGTC
W16	WPMS-16-L	NED	CTCGTACTATTTCCGATGATGACC
	WPMS-16-R	–	AGATTATTAGGTGGGCCAAGGACT
LG65	LG7165-L	–	CTGTCTTTGATTCGATCCAT
	LG7165-R	PET	AGAGTCTTTCGTTCGTGAAA

Table 2. Multiplexing groups and pre-fragment analysis pooling.

Multiplex Group	Individually Amplified Loci	Loci Amplified Together
1	W14, W16	P10, LG65
2	W15, P76	_
3	W17	W20, P14
4	P33	P86, P71

Genetic Data Assembly

In order infer the origin and test hypotheses for disjunct and adjacent genotypes collected for this study, several datasets from across the range of aspen were assembled into a single combined matrix for analyses (see Table 3 in Results section). The majority of genotypes (784) were from Callahan et al. 2013, 37 genotypes from Mock et al. 2013, as well as one genotype from Big Bend National Park and five genotypes from Merrimack County, New Hampshire provided by Karen Mock constituted the previously generated dataset used in this study. These previously generated datasets genotyped at the same seven loci were combined with 46 unique diploid genotypes generated as part of this study and employed to provide a range-wide context for inferences regarding origin and divergence of the disjunct and adjacent genotypes.

For this project 100 specimens were genotyped from disjunct stands in BICA and adjacent stands in the Pryor and Bighorn Mountains of Montana and Wyoming respectively as well as a disjunct stand at JE Canyon and the adjacent stands in the Spanish peaks of Colorado. Before combining the newly generated data in this study with the 827 genotypes from previous studies, 33 repeat/clonal genotypes, 18 triploid individuals, and five *P. angustifolia* or *P. deltoides* individuals were removed from the newly generated data. These additional *Populus* species were sampled to test for possible recent interspecific gene flow as they were found near the Dryhead and JE Canyon stands. No evidence of interspecific gene flow was found between these genotypes. All genetic analyses for this study was conducted using the combined dataset of 873 individual diploid genotypes across seven loci (W14, W15, W17, W20, P86, P10, and P71) unless otherwise indicated.

Potential triploids were identified from the 100 newly generated genotypes by having three peaks at one or more locus (Appendix A). For triploid detection five additional loci (LG65, P14. P33, and P76, and W16) were employed to search for triple peaks. These five loci were not used with the combined dataset analyses as they were not part of the previously generated datasets. Triploids had already been removed from the previously generated datasets. The putative triploids found in this study were recorded and discussed in reference to clonal diversity and stand genetic diversity but were excluded from population genetic analyses that require diploid genotypes. Identification of triploids was necessary for testing hypothesis three wherein if triploids are more heat and drought resistant they should be overrepresented at the arid disjunct sites.

Because aspen grow as large clonal organisms it is common to have repeat genotypes between individual collections (i.e. ramet) from a stand (i.e. genet). However clonal reproduction is imperfect and genotypic differences can sometimes be found between ramet genotypes (O'Connell and Ritland 2004; Ally et al. 2008; Mock et al. 2008). Fortunately, signatures of somatic mutation are, in most cases, easily differentiated from sexual recombination as the number of allelic differences between sexual recombinants are many and the number of allelic differences between somatic mutants are few when compared across an entire dataset. Population genetic analyses that rely on allele frequencies for inference can be biased if repeat and/or somatic mutant genotypes remain in a dataset (Putman and Carbone 2014). As such, repeat and somatic mutants were removed from the 100 genotypes generated for this study and had already been removed from the previously generated datasets. Somatic mutants were identified as individuals that differed at only one or two loci. The cutoff of two loci was determined by comparison of all newly generated genotypes to determine what the lowest number of allelic mismatches was and if the mismatch count was discontinuous from the lowest level to the highest level (i.e. a gap). The gap between the lowest number of mismatches and the next highest mismatch was set one mismatch above the lowest number. After the removal of somatic mutants from the newly generated dataset was complete the newly generated dataset was compared to the previously generated dataset using the gap detection approach. This method is like that used in Mock et al. (2008) for somatic mutant detection in aspen. Metrics of genotypic diversity (number of alleles per locus, Shannon diversity index, heterozygosity and fixation index) and gap analyses were calculated in GenAlex 6.5 (Peakall and Smouse 2006, 2012) for the newly generated and combined datasets.

In addition to gap analysis PI and PIsibs (probability of identity of individuals and probability of identity of siblings) were calculated for the combined dataset to determine the power of the seven-locus marker panel to distinguish multilocus genets. The average PI value was 5.3E-09 and the average PIsibs value was 1.15E-03. Given these values for PI and PIsibs a high level of confidence was given to the determination of repeat genotypes as arising from clonality and not the result of duplicative genotypes arising by chance from sexual recombination.

Genetic Analyses

In order to assess the newly collected samples with the previously generated dataset from across the range of aspen we employed distance-based and model-based methods for resolving population structure using the combined dataset of 873 genotypes. For the distance-based approach, PCoA was used based on a pairwise distance matrix of covariance as implemented in GenAlex 6.5 (Peakall and Smouse 2006, 2012). The distance-based approach was used to complement the HWE-model-based approaches (STRUCTURE, Geneland, and DIYABC) such that similar genotypes produced from somatic mutation that can be found in long-lived clonal stands would not bias estimates of population structure (e.g. Tembrock et al. 2017). The model-based approach for population structure inference was carried out in STRUCTURE 2.3.4 (Pritchard et al. 2000; Falush et al. 2003, 2007; Hubisz et al. 2009) on the complete set of 873 genotypes. Run parameters were the same as those used in Callahan et al. (2013) with exception of increasing the number of iterations from 10 to 40 at each level of K to more thoroughly assess the stability of assignments at each level of K (Kopelman et al. 2015). The ΔK method as implemented in Structure Harvester 0.6.94 (Earl and vonHoldt 2012) was used to select the best-supported value of K across all iterations (Evanno et al. 2005). CLUMPAK 1.1 (Kopelman et al. 2015) was used to address the problems of multi-modality and label switching that can occur when analyzing STRUCTURE results. Sub-structure was assessed by separately running groups of individuals from a given partition determined from the run with the complete set of 873 genotypes (Rosenberg et al. 2001). The run parameters for the subdivided groups were identical to those used for the complete dataset. A sub-grouping was considered unsupported if no individuals with a PP $\geq$ 0.95 were found among the clusters under the best supported partitioning as determined by the ΔK method. These genetic clusters were used as reference sets for the assignment of BICA, JE Canyon Ranch, and Big Bend genotypes to a given genetic cluster of origin. Assignments were made hierarchically to genetic clusters resolved in the STRUCTURE analyses. Individual assignments for genotypes from the disjunct stands were determined based on the STRUCTURE genetic clusters as reference sets using the Bayesian assignment method of Baudouin and Lebrun (2001) as implemented in GeneClass 2.0 (Piry et al. 2004). Individual assignment of the disjunct genotypes was not made to collection sites as this provides misleading spatial resolution and low statistical support.

Population structure was also assessed on the complete dataset of 873 genotypes using the spatial model in Geneland 4.0.0 (Guillot et al. 2005a; Guillot et al. 2005b; Guillot et al. 2008; Guillot 2008; Guillot and Santos 2010; Guedj and Guillot 2011), wherein the geographic location and allele frequencies are priors that inform the model. This approach allows for the identification of areas of more- or less-frequent gene flow between genetic clusters and possible boundaries to gene flow between genetic clusters. The spatial-genetic approach was employed to identify the boundary

between the northern and south-western genetic clusters and infer the origin and/or past dispersal of disjunct genotypes. Additionally, because multiple levels of *K* are tested between Geneland runs it provides an alternative test to the Δ*K* method employed from the STRUCTURE analyses. For each of 100 independent runs, 100,000 iterations were conducted with every 1000th iteration saved to memory, optimal cluster searches were set to allow a *K* from 1 to 10, uncertainty on coordinates was set to 50 to allow for gene flow across the entire sampling area and for multiple genotypes at the same geographic location, post processing burn-in was set to 200. The run with the highest average posterior probability (PP) was used for interpretation. Robust assessment of population structure is needed for proper assignment of individual genotypes to a genetic cluster of origin, for genetic based comparisons, and for alternative model testing regarding origin and age of disjunct genotypes in the coalescent analyses.

Coalescent times of the disjunct stands to the northern and south-western genetic clusters was inferred using DIYABC 2.0 (Cornuet et al. 2014). The northern and south-western genetic clusters used in the coalescent analyses were those used for assignments (i.e. all individuals that are in the northern or south-western cluster with a PP ≥ 0.95). Three separate simulations were run: one for each disjunct genotype (BICA Dryhead, JE Canyon, and Big Bend) with two scenarios per simulation; one scenario in which the disjunct clone diverged more recently from the northern genetic cluster; and one in which it diverged more recently from the south-western genetic cluster. Each scenario was run for 500,000 iterations. Uniform priors were chosen for model parameters, and coalescent time two was set to be greater than or equal to coalescent time 1, such that a realistic range of coalescent times could be assessed without model conflict. Coalescent analyses were employed to estimate the number of generations between disjunct genotypes and the genetic cluster of origin as well as infer the number of generations between the two genetic clusters of origin. By testing alternative models for the origin of each disjunct stand the confidence of assignments can be assessed in a coalescent framework. Model comparison also provides a useful tool for phylogeographic interpretation (Thomé and Carstens 2016). Using the simple model comparisons outlined here if model support is conflicting or split between scenarios an alternative origin such as parentage from the north and south-western clusters can be inferred.

Results

Genetic Structuring and Assignment of Disjunct and Adjacent Stands

The addition of 46 unique genotypes from the BICA disjunct stands and adjacent Pryor and Bighorn Mountains in Wyoming and Montana, and disjunct stands from JE Canyon and adjacent stands in the Spanish Peaks in southern Colorado made during this study, to 827 genotypes from previous studies resolved a similar north south-western genetic structure as found in Callahan et al. 2013. However, some degree of separation can be seen in the northern genetic group between east and west across the range of *K* (Figure 10). This east/west split with the northern genetic cluster does not resolve in the substructure analyses and further confirms the assertion of Callahan et al. (2013) that the northern population structure is the result of recent expansion following the last glacial maximum. The sub-structure analyses did support the split of the south-western genetic cluster into a western and southwest cluster like that of Callahan et al. (2013). Interestingly, Pryor and Bighorn Mountain genotypes assigned to both northern and south-western genetic clusters (Figure 10), with the proportion of membership split nearly evenly between north and south-western genetic clusters for the Pryor Mountain site (Table 3). In addition to The Pryor Mountain collection site, the Flathead National Forest site in Montana, and the BICA site have the most evenly split proportion of membership of any site sampled in North America (Table 3).

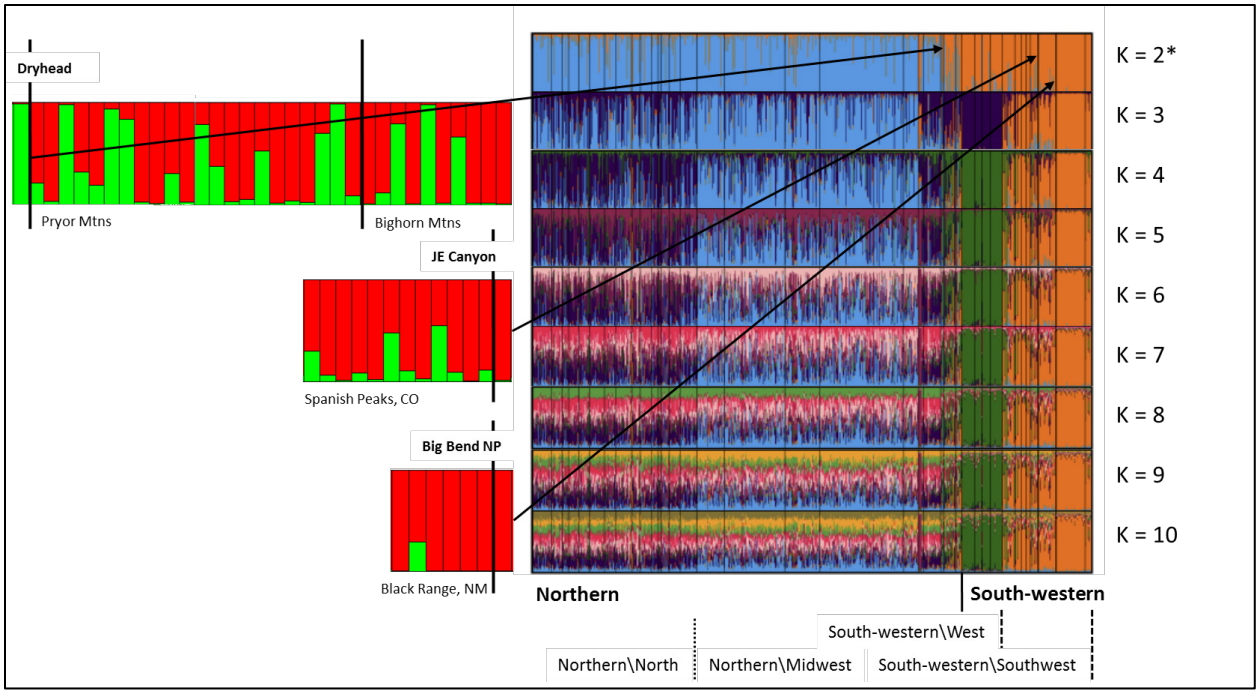

Figure 10. P. tremuloides samples across North America, indicating the stability of the south-western genetic cluster across all levels of K tested. Samples arranged north to south. *indicates the best supported partition using the Delta K method. No well-supported partitions were resolved within the northern genetic cluster, however subtle differences were maintained in assignments across K indicating some divergence between a midwestern and northern subcluster. The southwestern cluster is divided into two well-supported partitions having a western (mainly in California, Idaho, Montana, Nevada, and Oregon) distribution, and a southwestern (mainly Arizona, Colorado, Nevada, New Mexico, and Utah) distribution. These well-supported partitions are in green (south-western\west) and orange (south-western\southwest) at K =4 and above, and orange and purple at K =3. Location of disjunct genotypes and adjacent genotypes in the barplot indicated by arrows. Inset shows assignments at K =2 taken from run including all individuals. Recolored inset plots with northern cluster in green and south-western in red.

Table 3. Proportion of membership of each collection site at *K*=2, number of unique genotypes from each collection site, proportion of genotypes from each collection site containing alleles that occur 1 – 5 times in the entire dataset (values in parentheses are average across entire dataset), proportion of genotypes from each collection site containing alleles that occur 6 – 10 times in the entire dataset (values in parentheses are average across entire dataset), and the number of private alleles found from each collection site. Note the nearly split membership proportions of the Flathead, Bighorn Canyon, and Pryor Mtn sites. Collection sites sorted generally from north to south.

Collection Sites	**Northern**	**South-western**	**Number of unique diploid genotypes**	**Genotypes with rare allele 1 - 5 (0.12)**	**Genotypes with rare allele 6 - 10 (0.14)**	**Private alleles**
Coldfoot, Alaska (AKCF) [B]	0.926	0.074	29	0.21	0.07	2
Kenai, Alaska (AKK) [B]	0.906	0.094	17	0.18	0.12	0
Hinton, Alberta, Canada (HIN) [B]	0.888	0.112	10	0.1	0.1	0
La Loche, Saskatchewan, Canada (LALO) [B]	0.903	0.097	16	0.06	0	0
Flin Flan, Manitoba, Canada (FLFL) [B]	0.846	0.154	17	0.12	0.18	1
St. Felicien, Quebec, Canada (SFQ) [B]	0.931	0.069	23	0.13	0.04	0
Baie Comeau, Quebec, Canada (BCQ) [B]	0.853	0.147	18	0.17	0.06	0
Haure St. Pierre, Quebec, Canada (HSPQ) [B]	0.873	0.127	25	0.16	0	1
Deer Lake, Newfoundland, Canada (DLN) [B]	0.831	0.169	13	0.08	0.08	0
Wentworth, Nova Scotia, Canada (WNC) [B]	0.89	0.11	18	0.22	0.17	0
University of Maine (UME) [B]	0.937	0.063	10	0.2	0.1	0
Merrimack County, New Hampshire [E]	0.951	0.049	5	0.4	0.4	0
Catskill Mountains, New York (CMNY) [B]	0.84	0.16	12	0.17	0.17	0
Itasco County, Minnesota (MN) [B]	0.892	0.108	18	0.16	0.18	0
Ontonagon County, Michigan; Ottawa NF (MI) [B]	0.963	0.037	26	0.04	0.08	0
St Louis County, Minnesota (MNSL) [B]	0.922	0.078	43	0.16	0.19	0
Lake County, Minnesota (MNL) [B]	0.929	0.071	94	0.07	0.1	1
Iron County, Wisconsin (WII) [B]	0.883	0.117	55	0.18	0.16	4
Wisconsin/Michigan Border (WIMI, 103) [B] and Oakland County, Michigan (WIO, 28) [B]	0.942	0.058	131	0.13	0.12	2
Emmet County, Michigan (NMI) [B]	0.94	0.06	23	0.09	0	1
Flathead National Forest, Montana (*MTF*) [C]	0.36	0.64	8	0.63	0	4
Yellowstone NP, Montana (POTR) [B]	0.848	0.152	27	0	0.07	0
Bighorn Canyon, NRA, Montana [A, D]	0.557	0.443	4	0.5	0	0
Pryor Mountains, Montana [A, D]	0.336	0.664	16	0.11	0	2
Northern Bighorn Mountains, Wyoming [D]	0.271	0.729	12	0.1	0	0
Boise National Forest, Idaho (BNF) [B]	0.062	0.938	20	0	0.1	0

[A] Sites from which disjunct genotypes were sampled (also highlighted in green).

[B] Collection sites used in Callahan et al. 2013

[C] Sites with the diploid samples from Mock et al. 2012

[D] Sites sampled for this study.

[E] Sites where genotypes were provided by Karen Mock of Utah State University

Table 3 (continued). Proportion of membership of each collection site at *K*=2, number of unique genotypes from each collection site, proportion of genotypes from each collection site containing alleles that occur 1 – 5 times in the entire dataset (values in parentheses are average across entire dataset), proportion of genotypes from each collection site containing alleles that occur 6 – 10 times in the entire dataset (values in parentheses are average across entire dataset), and the number of private alleles found from each collection site. Note the nearly split membership proportions of the Flathead, Bighorn Canyon, and Pryor Mtn sites. Collection sites sorted generally from north to south.

Collection Sites	**Northern**	**South-western**	**Number of unique diploid genotypes**	**Genotypes with rare allele 1 - 5 (0.12)**	**Genotypes with rare allele 6 - 10 (0.14)**	**Private alleles**
Northern Bighorn Mountains, Wyoming D	0.271	0.729	12	0.1	0	0
Boise National Forest, Idaho (BNF) B	0.062	0.938	20	0	0.1	0
Wenatchee NF, Kittitas County, Washington (WWA) [B]	0.137	0.863	12	0.17	0.25	1
Klamath Falls, Oregon (KFO) [B]	0.08	0.92	13	0	0	0
South Lake Tahoe, California and Nevada (CANV) [B]	0.108	0.892	18	0	0	0
Swan Flats, Utah (USF) [B]	0.099	0.901	20	0.05	0.1	0
Humboldt NF, Nevada (NVW) [B]	0.017	0.983	10	0	0.1	0
Garfield County, Utah (*GUT*) [C]	0.021	0.979	8	0	0.13	0
Gunnison County, Colorado (*COG*) [C]	0.017	0.983	6	0	0	0
Spanish Peaks, San Isabel NF, Colorado [D]	0.154	0.846	13	0.08	0	1
JE Canyon Ranch, Branson, Colorado [A, D]	0.115	0.885	1	0	1	0
Sangre (NMT, 12) [B] and Black Ranges (*NM*, 15) [C], New Mexico	0.076	0.924	27	0.04	0.15	0
Big Bend NP, Texas [A, E]	0.009	0.991	1	0	1	0
Coconino NF, Arizona (AZ) [B]	0.016	0.984	45	0.11	0.09	1
Hidalgo and Queretaro States, Mexico (MXQ) [B]	0.012	0.988	9	0	0	0

[A] Sites from which disjunct genotypes were sampled (also highlighted in green).

[B] Collection sites used in Callahan et al. 2013

[C] Sites with the diploid samples from Mock et al. 2012

[D] Sites sampled for this study.

[E] Sites where genotypes were provided by Karen Mock of Utah State University

Four diploid genotypes were identified from the BICA site, one (11 samples taken) occurred at the Dryhead stand and three (one genotype sampled three times, one genotype sampled twice and a third sampled once) from the Bench stand (Appendix A). The Dryhead genotype assigned with high confidence to the northern genetic cluster using both methods (Table 4). The three genotypes in the Bench stand each assigned to one of the three reference genetic clusters (north, south-western\southwest, and south-western\west) making it much more diverse compared to the other disjunct stands (and stands in general throughout the range of aspen), as all other disjunct stands studied herein were made up of a single genotype. Given the more mesic conditions, higher elevation, proximity to extant montane stands, and habitat type of the Bench stand compared to the

Dryhead stand, it would seem that the Bench stand was more recently connected to larger stands. The high genotypic diversity of the Bench stand may persist due to the reduced selection pressures associated with the more mesic setting. Given that the BICA Bench stand is more reflective of larger montane stands in regard to genotypic diversity and environmental setting it was excluded from some of the analyses regarding coalescence but included in all other analyses.

Table 4. Probability of assignment of disjunct genotypes to supported genetic clusters. Assignments using the method described in Baudouin and Lebrun (2001). Reference sets based on well-supported genetic clusters (Figure 10) made up of groups of individuals with 0.95 PP or higher membership as calculated in STRUCTURE. Assignments were conducted in two rounds: first to north or south-western clusters and second to southwest or west clusters if the initial assignment was to the south-western genetic cluster; PP of assignment refers to the final assignment value. The original BBNP_2_CC1 name was retained such that it can be cross referenced with other publications or databases, see appendix A.

Individual	Origin	Assignment (genetic cluster)	PP of Assignment
BBNP_2_CC1	Big Bend NP	south-western\southwest	99.997
17202	JE Canyon Ranch	south-western\southwest	99.84
17114	Bighorn Canyon NRA (Dryhead)	north	100
17130	Bighorn Canyon NRA (Bench)	south-western\southwest	98.959
17128	Bighorn Canyon NRA (Bench)	south-western\west	99.683
17127	Bighorn Canyon NRA (Bench)	north	99.984

The JE Canyon stand in southeastern Colorado was, like the Dryhead stand, made up of a single diploid genotype. The genotype of this stand assigned with high confidence to the south-western genetic cluster and then to the southwest genetic subcluster (Table 4, Figure 10). The Big Bend stand was also a single diploid genotype and assigned to the same genetic sub-cluster as the single genotype in the JE Canyon stand.

The distance-based PCoA analysis largely corroborated the findings from the model-based STRUCTURE analyses (Figure 11). The Dryhead genotype, two (represented by collections 17130 and 17128) of the three Bench stand genotypes, and the JE Canyon genotype cluster between the northern and south-western\west clusters. The Big Bend stand genotype clearly resolves with genotypes from the south-western\southwest group and sample 17127 from the Bench stand clearly clusters with the northern genotypes. The PCoA graph indicates a clear separation between the north and south-western\southwest clusters but a less pronounced separation between the north and south-western\west groups, suggesting less divergence between these genetic clusters.

The addition of geography to the genetic model further reinforced the findings of Callahan et al. (2013) in that two genetic clusters in a north/south-western arrangement was the best-supported partition across multiple runs. Two important patterns are resolved in the Geneland analysis 1) the separation between the north and south-western genetic clusters is generally abrupt suggesting some barrier to geneflow or perhaps recent secondary contact between the groups, as suggested by

Callahan et al. (2013) and 2) the Bighorn Mountain, Pryor Mountain (including BICA), and nearby sites are resolved on a more moderate (compared to the genetic cluster boundary in the rest of the range) sloping cline between the north and south-western genetic clusters (Figure 12).

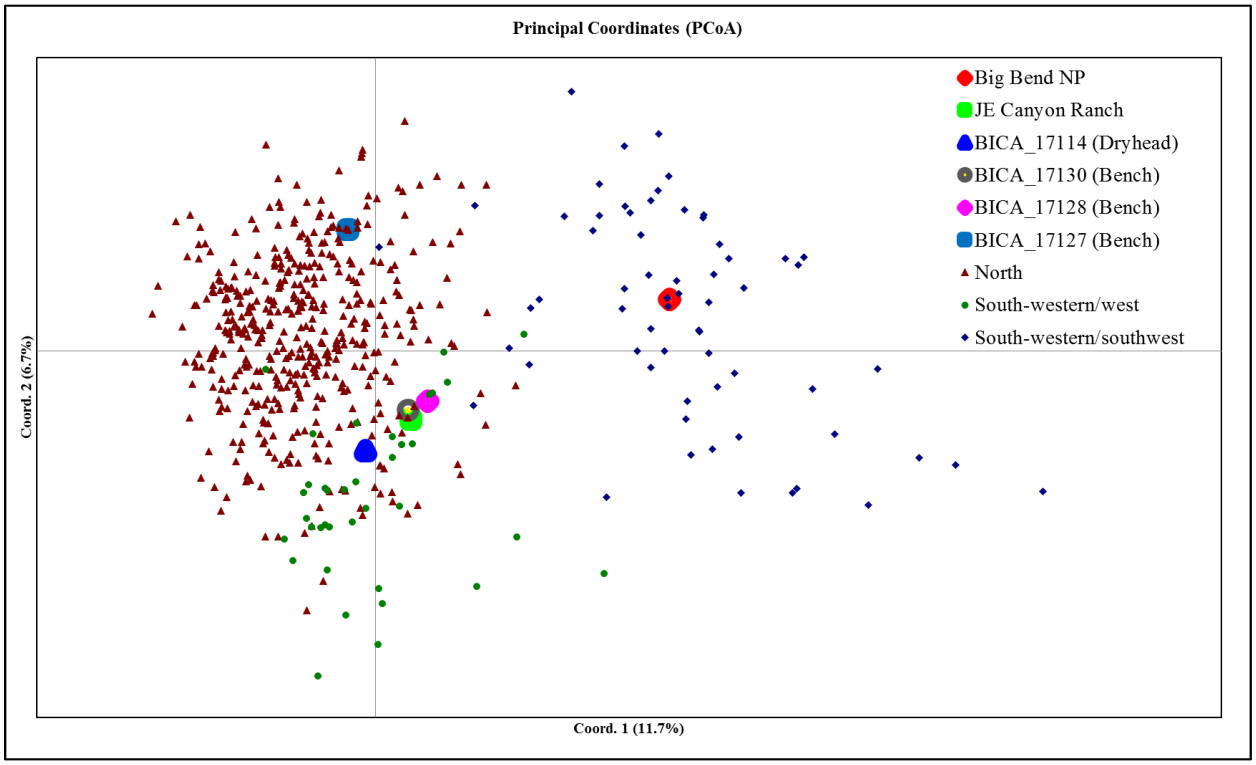

Figure 11. The genetic distance of disjunct P. tremuloides stands from three genetic clusters (north, south-western\west, and south-western\southwest as defined for the assignment analyses using the 0.95 PP cutoff) as represented by PCoA. Numbers for BICA genotypes refer to LT collection numbers.

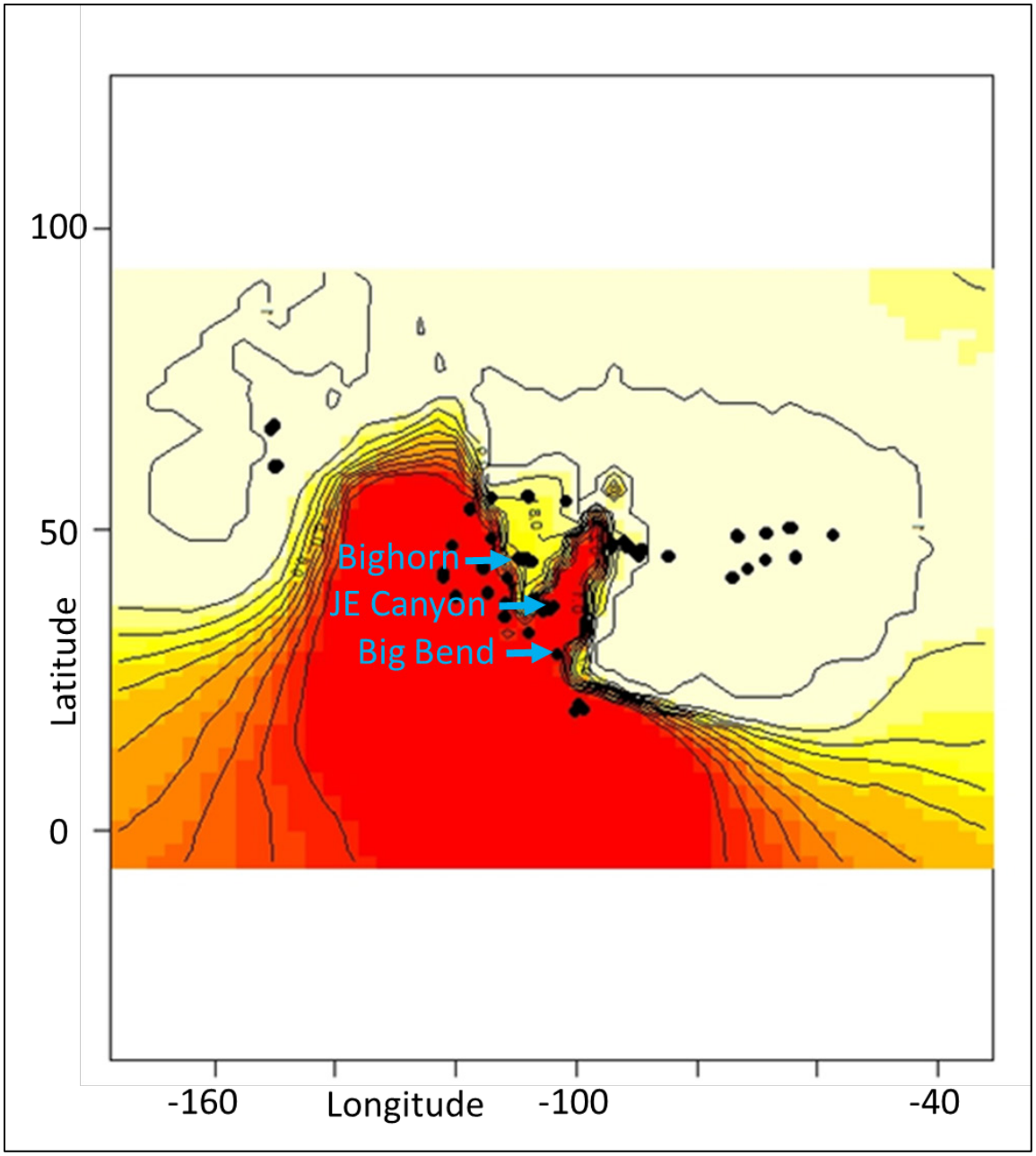

Figure 12. Distribution of all collection sites (Table 3) on best-supported cluster landscape of two partitions when geographic location is included as a model prior; red = south-western cluster, white = northern cluster. Black dots denote geographic locations of rangewide collection sites. Contour lines in 0.1 intervals depict Posterior Probability of assignment. Note the "steepness" between clusters and the arrangement of collection sites on these slopes. Disjunct stands identified by blue arrows.

Genetic and Genotypic Diversity

Measures of genetic diversity including number of alleles per locus, Shannon diversity index, heterozygosity and fixation index for the disjunct and the adjacent montane stands were calculated to compare disjunct stands to adjacent stands (Table 5). The Dryhead stand is identical to the West Harsten stand in the nearby Pryor Mountains with regard to proportion of heterozygosity, effective

number of alleles and Shannon diversity index (Table 5). All but one stand (Teeple Spring) examined in the Pryor Mountains was found to have one or more repeated/clonal genotypes. Surprisingly, no triploid genotypes were found in the Pryor Mountains while 40% and 20% of the sampled genotypes in the Bighorn and Spanish Peaks respectively were triploid. No genotypes were observed more than once in the Spanish Peaks of Colorado, which is likely a result of the dispersed sampling and difficulty in determining stand boundaries among the mixed forest types found in the area. The Spanish Peak and JE Canyon genotypes were found to have a high proportion of heterozygosity compared to genotypes in the Pryor, Bighorn, and Black Range Mtns. Sampling from stands in the Guadalupe and Sacramento Mountains of southern New Mexico needs to be conducted so that these stands can be analyzed along with the range wide sample set and compared to the Big Bend stand.

Lastly, the characterization of alleles based on abundance and in what geographic areas they are found can provide useful direct insights into the genetic diversity and/or uniqueness of a population or individual (e.g. Xue et al. 2018). The three disjunct stands possess rare alleles (as compared to allele frequencies at each locus across all samples from across the range) at a high rate compared to other collection sites in the range and in nearby stands (Table 3). Rare and private alleles are more frequently found in genotypes of the northern genetic cluster potentially because drift is less efficient at purging rare alleles in forests with large effective population sizes. Additionally, more sampling throughout the south-western portion of the range might provide better resolution regarding rare and private alleles. The genotype of the JE Canyon stand possesses allele 158 at locus W17 (Appendix A), which is found across the entire continental sampling range (873 genotypes) at a rate of 0.51% (Appendix B). Three Arizona genotypes and four northern genotypes contain allele 158 (Appendix 3). The Big Bend genotype possesses allele 227 at locus W14 (Appendix A), which is found across the entire continental sampling range at a rate of 0.46% (Appendix B). One Arizona and Utah genotype contain allele 227 and five northern genotypes contain allele 227 (Appendix C). The Dryhead genotype possesses allele 143 at locus W14 (Appendix A), which is found across the entire continental sampling range at a rate of 0.11% (Appendix B). Within the entire continental sample set, only one other diploid genotype from Nova Scotia possesses this allele (Appendix C).

Table 5. Comparison of genetic metrics by stand for disjunct stands and closest sampled areas with extensive aspen stands. Area of stand estimated from aerial imagery and ground-based observations. Na average alleles per locus, Ne is effective number of alleles; I Shannon diversity index; Ho observed heterozygosity; He expected heterozygosity; uHe unbiased expected heterozygosity; and F is fixation index Population based metrics for single genotype stands should be treated with caution given the inherently low number of samples; see total row for more accurate values. Total number of genotypes in an area may exceed the total from stands as it may include samples taken from singleton trees. #Triploids were documented using flow cytometry (Mock et al. 2012) in this area but not in any of the genotypes used for this study.

Geographic origin	Stand	Area of stand (ha)	Number of clonal genotypes	Number of genotypes	Number of triploid genotypes	Na	Ne	I	Ho	He	uHe	F
Bighorn Canyon, NRA, MT	Dryhead	1.5	1	1	0	1.714	1.714	0.495	0.714	0.357	0.714	-1.000
Bighorn Canyon, NRA, MT	Bench	1.6	2	3	0	3.286	2.759	1.082	0.667	0.627	0.752	-0.069
Pryor Mtn, MT	Crooked Creek Headwaters	2.3	1	3(1 somatic)	0	2.571	2.110	0.724	0.571	0.421	0.505	-0.334
Pryor Mtn, MT	Dryhead Vista Bench	13	1	3	0	3.714	3.220	1.193	0.762	0.659	0.790	-0.098
Pryor Mtn, MT	West Harsten Flats	0.5	1	1	0	1.714	1.714	0.495	0.714	0.357	0.714	-1.000
Pryor Mtn, MT	East Harsten Flats	3.1	1	3	0	3.857	3.286	1.218	0.667	0.659	0.790	-0.028
Pryor Mtn, MT	Teeple Spring	5.4	0	5	0	4.571	3.552	1.347	0.800	0.697	0.775	-0.173
Pryor Mtn, MT	FR2073 Jct	0.1	1	1	0	1.143	1.143	0.099	0.143	0.071	0.143	-1.000
Pryor Mtn, MT	Sage Creek Side Drainage	1.9	1	1	0	1.714	1.714	0.495	0.714	0.357	0.714	-1.000
Pryor Mtn, MT	**Total (including single ind.)**	**NA**	**9**	**25**	**0**	**8.857**	**4.974**	**1.739**	**0.669**	**0.753**	**0.769**	**0.110**
Bighorn Mtn, WY	Hwy 14 Hairpin	2.1	0	2	0	2.571	2.267	0.854	0.643	0.536	0.714	-0.181
Bighorn Mtn, WY	Hwy 14 Truck Ramp*	1.6	1	2	1	1.429	1.429	0.297	0.429	0.214	0.429	-1.000
Bighorn Mtn, WY	Bucking Mule TH	1	0	3	1	2.857	2.714	0.941	0.643	0.554	0.738	-0.111
Bighorn Mtn, WY	Galloway Cabin	0.6	1	1	1	NA	NA	NA	NA	NA	NA	NA
Bighorn Mtn, WY	Wheddon Spring*	0.5	0	2	1	1.571	1.571	0.396	0.571	0.286	0.571	-1.000
Bighorn Mtn, WY	Tongue River Exclosure	2.3	1	1	1	NA	NA	NA	NA	NA	NA	NA
Bighorn Mtn, WY	Burgess Jct	2.9	0	3	0	3.286	2.671	1.020	0.667	0.579	0.695	-0.120
Bighorn Mtn, WY	Steamboat Point*	1.7	0	3	1	3.143	3.000	1.040	0.714	0.589	0.786	-0.200
Bighorn Mtn, WY	Antelope Butte	17.5	1	2	2	NA	NA	NA	NA	NA	NA	NA
Bighorn Mtn, WY	**Total (including single ind.)**	**NA**	**4**	**20**	**8**	**7.429**	**5.027**	**1.719**	**0.619**	**0.778**	**0.812**	**0.212**
Spanish Peaks, CO	Wahatoya	0.5	0	2	0	3.286	3.048	1.139	0.714	0.661	0.881	-0.067

* Stands that are comprised of both diploid and triploid genotypes, allelic diversity values from these stands are calculated from the diploid genotypes.

Table 5 (continued). Comparison of genetic metrics by stand for disjunct stands and closest sampled areas with extensive aspen stands. Area of stand estimated from aerial imagery and ground-based observations. Na average alleles per locus, Ne is effective number of alleles; I Shannon diversity index; Ho observed heterozygosity; He expected heterozygosity; uHe unbiased expected heterozygosity; and F is fixation index. Population based metrics for single genotype stands should be treated with caution given the inherently low number of samples; see total row for more accurate values. Total number of genotypes in an area may exceed the total from stands as it may include samples taken from singleton trees. #Triploids were documented using flow cytometry (Mock et al. 2012) in this area but not in any of the genotypes used for this study.

Geographic origin	**Stand**	**Area of stand (ha)**	**Number of clonal genotypes**	**Number of genotypes**	**Number of triploid genotypes**	**Na**	**Ne**	**I**	**Ho**	**He**	**uHe**	**F**
Spanish Peaks, CO	Blue Lake	~10.0	0	2	0	2.857	2.552	0.953	0.786	0.571	0.762	-0.371
JE Canyon Ranch, CO	JE Canyon	0.4	1	1	0	2.000	2.000	0.693	1.000	0.500	1.000	-1.000
Spanish Peaks, CO	**Total (including single ind.)**	**NA**	**1**	**16**	**3**	**7.857**	**4.753**	**1.673**	**0.607**	**0.744**	**0.774**	**0.105**
Big Bend, NP, TX	BigBend	0.3	1	1	0	1.429	1.429	0.297	0.429	0.214	0.429	-1.000
Black Range, NM	**Total (including single ind.)**	**NA**	**0**	**6**	**0#**	**4.286**	**3.114**	**1.21**	**0.671**	**0.635**	**0.695**	**-0.061**

* Stands that are comprised of both diploid and triploid genotypes, allelic diversity values from these stands are calculated from the diploid genotypes.

Coalescence Times for Disjunct Stands

Coalescent analyses were used to roughly infer the age of, and moreover the reproductive periodicity between disjunct stands and genetic clusters (north and south-western) from which they are inferred to have originated as well as between the north and south-western clusters. Two scenarios were run for each disjunct stand to test the assumptions of each scenario and provide further support to the above assignments. For all three disjunct genotypes the coalescent analyses clearly supported the assumed origins – the BICA Dryhead stand had an origin from the northern genetic cluster and the JE Canyon Ranch and Big Bend stands originated from the south-western genetic cluster (Figure 13). Comparison across the three model tests also indicates that support between models deviates with latitude. Such a pattern is expected to be the result of the Big Bend genotype being more divergent from the northern cluster than the JE Canyon genotype is from the northern cluster. This result matches the expectation for an isolation by distance pattern and the inferred relictual status of the south-western genetic cluster genotypes. Oddly, the coalescent times for the Big Bend genotype to the south-western genetic cluster were the most recent of the three. However, such a discrepancy may arise by applying the coalescent to aspen, a species that rarely reproduces and produces viable seedlings in marginal conditions (Kay 1993), which would result in more recent times to coalescence. Additionally, sampling a single genotype for time to coalescence one may also bias the estimates.

Despite the challenges of dating aspen clones with the coalescent, it should provide some insights into how often reproduction and seedling establishment occurs in different parts of the species range. The coalescent times for the disjunct stands to the most closely related genetic clusters was 151, 209, and 60 generations for the Dryhead, JE Canyon Ranch, and Big Bend NP stands, respectively (Table 6). These are modal values from the coalescent analyses which is the value that was output most often across 500,000 iterations. The average and median values are larger in all cases, but the modal values were used for interpretation as this method of results selection mirrors other Bayesian method interpretations (Kopelman et al. 2015).

Table 6. Coalescent times for each disjunct stand to its parent genetic cluster as supported from direct model comparison of two alternative scenarios. Time to coalescence 1 for the Dryhead stand is to the northern genetic cluster and time to coalescence 2 is to the south-western genetic cluster; times to coalescence for JE Canyon and Big Bend are to the south-western and then to the northern genetic cluster. Best supported modal values presented.

Relict Clones	**Time to coalescence 1 in generations (mode)**	**range**	**Time to coalescence 2 in generations (mode)**	**range**
Bighorn Canyon NRA, MT (Dryhead)	151 northern	36-1670	967 south-western	399-7140
JE Canyon Ranch, CO	209 south-western	52-2400	1240 northern	495-7490
Big Bend NP, TX	60 south- western	22-837	1430 northern	546-8070

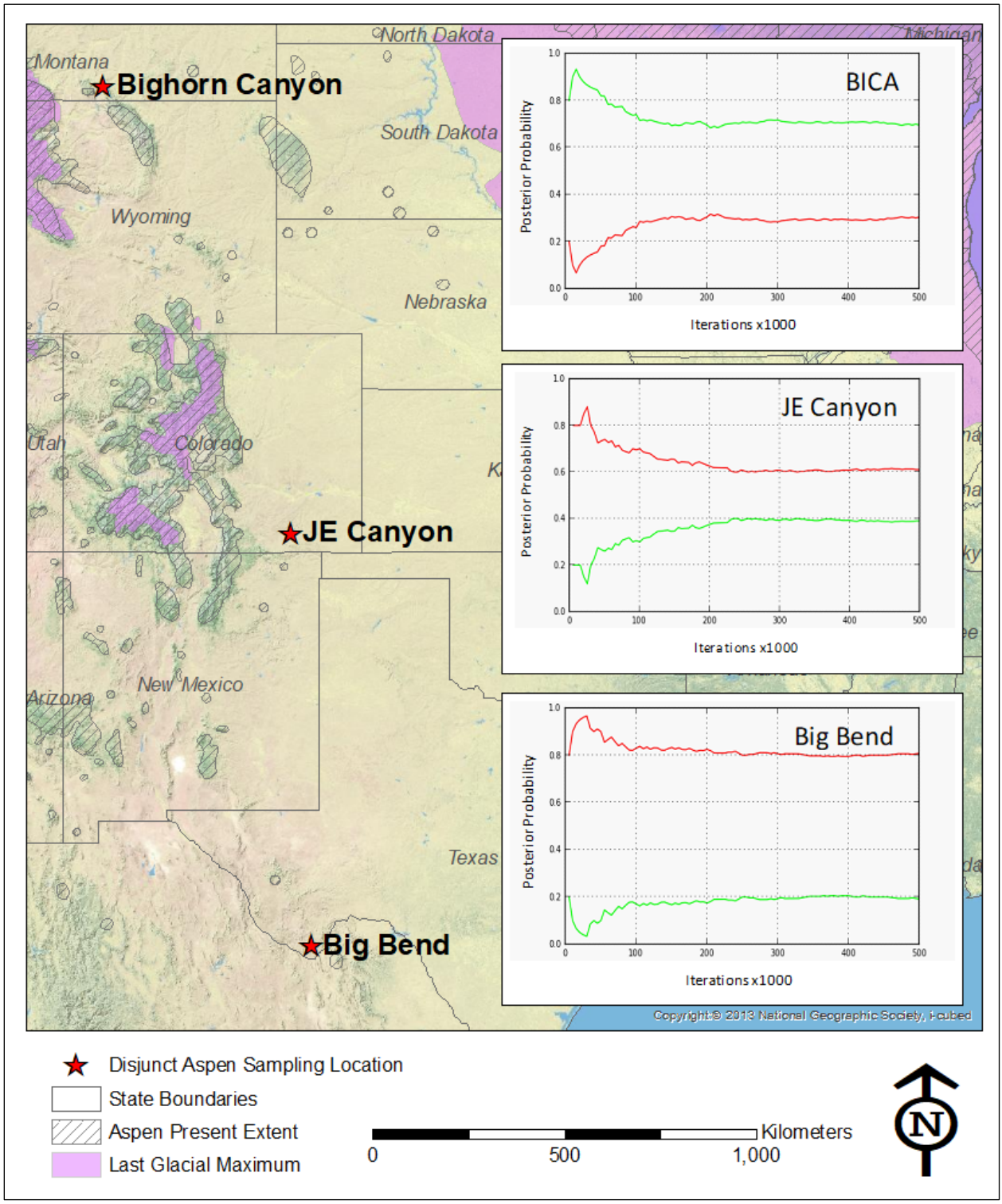

Figure 13. Alternative model testing for each disjunct genotype. The red lines represent scenarios in which the disjunct genotype originated from the south-western genetic cluster and the green lines in which they originated from a northern genetic cluster.

Discussion

Origin of Disjunct Aspen Clones

For all three disjunct stands examined (BICA Dryhead, JE Canyon, and Big Bend) the genotypes were consistent with an origin from a genetic cluster that is geographically adjacent but inconsistent with a recent long-distance seed dispersal origin from the closest montane stands sampled (especially when considering coalescent times – see below). For the Dryhead stand the Pryor and/or Bighorn Mountain stands could have been connected or founded from a larger forest that occupied the Bighorn river valley during the last glacial period. While the Dryhead stand is similar to the other stands in JE Canyon Ranch and Big Bend in being in an ecologically anomalous setting, different selection pressures (such as day length, e.g. Wang et al. 2018) may have resulted in a northern genotype persisting in the arid Dryhead stand. From other studies in *Populus* (e.g. Wang et al. 2018; Keller et al. 2018) and molecular ecology principles (Callahan et al. 2013), one would expect genes for tolerating aridity to be associated with south-western genotypes. However, given the presence of both northern and south-western genotypes in the Pryor Mountains, this area may represent a midpoint in the conditions selecting for northern and south-western genotypes and/or simply a geographic midpoint that has suitable habitat during climatic oscillations and harbors genotypes from both contracting and expanding range fluctuations (Abellán and Svenning 2014). While the Pryor and Bighorn stands possess both northern and south-western genotypes, the lack of triploid genotypes in the Pryor Mountains is more similar to stand dynamics found in the Northeast and Midwest. Comparative functional genomic studies focused on the Pryor Mountains and range margin genotypes are needed to elucidate what processes maintain the north south-western division in some areas and not others, as well as what factors select for high rates of triploidy.

No evidence for triploidy was found for any genotypes in either fragment analysis or morphological examination of individuals in the three disjunct stands studied herein. Triploidy in aspen has been thought to be associated with tolerance to higher temperatures and drought stress given the higher abundance of triploids in the south-western portion of the aspen range (Mock et al. 2012). However, triploids are often associated with higher elevation locations (Mock et al. 2012) that are generally cooler and wetter and, in many cases, would have been glaciated or unsuitable during the last glacial period (Ding et al. 2017). Furthermore, physiological studies comparing triploid and diploid stands indicate that triploids have a higher transpiration rate and thus require a greater water supply than diploids, potentially making them more sensitive to drought and heat stress (Greer et al. 2017). Thus triploids may have been at a competitive advantage during the shift from lower elevation occupied during glaciation to higher elevation during glacial retreat. The shift from broad lowland valleys to more narrow mountainous valleys and slopes with newly opened niches from glacial retreat is expected to have increased competition between species wherein neopolyploids could have had a selective advantage over diploids (e.g. Sugiyama 1998; Sutherland and Galloway 2018). While polyploidy may present a selective advantage during colonization it could in turn be disadvantageous at the trailing edge of a species range where environmental selection is expected to intensify (Kiedrzynski et al. 2017). Our findings of disjunct stands in arid settings containing only diploid genotypes supports such a model of aspen range shift in the mountainous south-western portion of the range. Despite the lack of triploids in any of the disjunct stands examined for this study, triploid

stands remain important candidates for conservation and future study as they possess unique gene combinations/interactions, physiological attributes, wood characteristics, and ecological interactions more likely to be lost as triploids have higher rates of sterility than diploids (Bradshaw and Stettler 1993).

The allelic combination of the Dryhead genotype (Appendix A) is unique across the range of aspen, in particular the presence of allele 243 at locus W14 is worth noting. The pattern of shared rare alleles from northeastern North America to the Pryor\Bighorn area is also found with specimen 17144 from the Pryor mountains containing allele 138 (monomorphic) at locus P510 with only one other genotype in the Catskill mountains of New York. This further suggests a relictual connection when these or parental genotypes may have been connected through geneflow in the past. Allele 243 at locus W14 was also found in a triploid individual (17181; Appendix D) collected as part of this study in the Bighorn Mountains. From the examination of the morphology and the genotype of 17181 it may be a cross between *P. tremuloides* and *P. balsamifera*. However, Eckenwalder (1996) asserts that claims of this particular cross have been misidentified as *P. ×jackii*. Alternatively, the triploid may be a cross between *P. tremuloides* and *P. trichocarpa*, which is also considered improbable (Eckenwalder 1996). The Bighorn Mountains have been identified as a hybrid zone among *P. angustifolia*, *P. balsamifera*, and *P. trichocarpa* (Chhatre et al. 2018) and as such, 17181 may represent a highly admixed genotype. No matter the case, *P. tremuloides* parentage seems possible as shared alleles were found in 17181 to other 'typical' diploid *P. tremuloides* including those in the same stand (Table 5; Steamboat Point), and all loci but one primed and produced readable amplicons from PCR. Thus, the presence of allele 243 in the Dryhead genotype may have been the result of an ancient hybridization event or is a relict allele shared with *P. tremuloides*, *P. angustifolia, P. balsamifera* and/or *P. trichocarpa*. As such, a relict or introgressed assemblage of alleles may explain how the Dryhead genotype persists in such arid conditions as well as the atypical morphology described for this genotype (Figure 3B; Stevens et al. 2017). Introgression appears to be an important part of the adaptive history of *Populus* in general (Chhatre et al. 2018) and likely *P. tremuloides* as well (Deacon et al. 2017). The detailed study of the Dryhead and similar genotypes using broad scale genomic techniques will be important in understanding this pattern of adaptive hybridization among *P. tremuloides*.

The genotypes at JE Canyon and Big Bend, like the Dryhead genotype, also possess rare alleles. The occurrence of rare alleles shared between extant north and south-western genetic clusters in all disjunct genotypes compared herein may represent historic gene flow (based on expectations from Slatkin 1985) when the species range was contiguous in the areas where the disjuncts are currently present. The northern genetic cluster aspen forests today, with high rates of gene flow, large effective population sizes and a high proportion of private alleles may reflect what was found in southeast Colorado 20,000 ybp. These patterns among disjunct aspen support some of the expectations for trailing edge populations (Kiedrzyński et al. 2017). The JE Canyon and Big Bend stands comport to the microrefugia as presented by Hannah et al. (2014) given the small size of these populations, there location in the species range, and the occupancy of isolated microclimates relative to the surrounding landscape. Furthermore, with the current understanding of species range fluctuations in aspen (*sensu* Elliott and Baker 2004; Ding et al 2017) the disjunct relict stands surviving in microrefugia like those

in this study might harbor more ancient clones than the massive montane clones like 'Pando' which are often touted as some of the oldest stands based on size as a proxy for age (Barnes 1975; Ally et al, 2008). Given these findings, aspen in general and disjunct stands in particular are well suited for studying the evolution of species ranges and genomic adaptation across a wide breadth of ecological, demographic, and genetic conditions. As such, NPS units widely distributed across the US having documented anomalous aspen stands within their boundaries are uniquely situated for the study of the genetics, evolution, and conservation of disjunct relict aspen stands. In conjunction with genetic studies the ecological importance of aspen stands requires in-depth research to understand their role in supporting biodiversity.

Age of Aspen Disjunct Stands: the Coalescent and its Relation to the "Window of Opportunity" in Historic Seedling Establishment

The question of how disjunct aspen stands established in microrefugia is not fully understood. However, based on hindcasting of aspen niche models (Ding et al. 2107) and current observations of new aspen stand establishment and expansion after disturbance via seed (i.e. window of opportunity; Kay 1993; Elliot and Baker 2004), it seems probable that the now disjunct stands were either part of or closer to larger aspen stands during times of more favorable conditions at lower elevations in the last ~20,000 years. Additionally, the long-distance dispersal capacity of aspen seeds may have been important in the establishment of disjunct stands like those at Big Bend, where little past or present habitat (Ding et al. 2017) is located between present large montane stands and Big Bend National Park.

Coalescent analyses as implemented in DIYABC and most other methods outputs the coalescent time in generations, which for annual plant species can be roughly equated to a mean tropical year. Aspen, are known to reproduce, disperse seeds, germinate, and grow to reproductive age dispersing viable seed (one generation) very infrequently. Thus coalescent times in generations are likely to be greatly decoupled from mean tropical years in aspen. Nonetheless, coalescent analyses in concert with other information sources, past climate reconstructions, or known earth history events can offer some insight into the largely unknown past reproductive periodicity of aspen.

In the Pryor Mountains, evidence from radiocarbon dated woodrat middens (Lyford et al. 2002) and analyses of niche models (Ding et al. 2017) suggests that the Dryhead stand and surrounding area would likely have had the proper conditions for aspen establishment between 15,000 and 10,000 years ago. The coalescent analyses estimates that approximately 150 reproductive cycles separate the Dryhead genotype from the extant genotypes making up the northern genetic cluster, thus supporting the findings for rare sexual reproduction between aspen individuals (e.g., Kay 1993). However, as the climate became drier and warmer through time the rate of establishment most likely decreased. Thus the periodicity of reproduction and establishment was probably more frequent 10,000 years ago than it is today.

The JE Canyon stand might have been part of an older and larger aspen forest based on nearby sediment cores in the northern Sangre de Christo Mountains (Armour et al. 2002), geological analyses of nearby volcanic eruptions (Sayre and Ort 2011), and hindcasted niche modeling (Ding et al. 2017). From the Ding et al. (2017) model, the area in southeast Colorado where the JE Canyon

stand currently occurs was part of a large area in southeast Colorado and northeast New Mexico suitable for aspen 21,000 years ago. By 14,000 years ago the suitable habitat had contracted westward to the Spanish Peaks area where large stands exist today. Analyses of glacial sediment in the nearby Sangre de Christo Mountains confirm that the late Pleistocene in southeast Colorado was cooler and wetter (Armour et al. 2002). Volcanic activity in the Raton-Clayton volcanic field (part of which is included in Capulin Volcano National Monument) ceased around 56,000 years ago (Sayre and Ort 2011) and could have set the older bounds as well as exceptional conditions (bare mineral soil) for establishment of aspen in the Purgatoire River Basin of southeast Colorado. As with the coalescent analyses of the Pryor Mountain area, few (209) reproductive cycles separate the JE Canyon stand from the south-western genetic cluster.

The pathway by which aspen established in Big Bend NP is less clear with the niche models of Ding et al. (2017) not showing any large scale suitable habitat in Big Bend or surrounding areas in the last 21,000 years or previous to that in their multiple glaciation scenario (however the Sacramento mountains of NM are inferred to be refugia through multiple glaciations). Analyses of packrat middens from Big Bend do indicate that climates were cooler and wetter 10,000 years ago (Tweet et al. 2012). The expansion-contraction model for aspen in the southwest, wherein aspen expand to lower elevations during cooler wetter periods and contract to higher elevations during hotter drier periods (Callahan et al. 2013), does not seem as applicable to the Big Bend stand based on its geographical setting. For both the Dryhead and JE Canyon stands, large extant populations occur relatively close by and paleontological evidence and niche modelling reasonably indicate that these now separate populations were likely in closer genetic and geographic proximity at the beginning of the Holocene. Not so for the Big Bend stand, indicating it may have established via a long-distance dispersal event or be part of a much more ancient range expansion. The results from the coalescent analyses appear to support a recent dispersal, as the time to coalescence between the Big Bend genotype and the south-western genetic cluster are the most recent among the disjunct stands surveyed when theory suggests they should be the oldest (Kiedrzyński et al. 2017). Alternatively, the Big Bend and south-western stands might be quite old but generational turnover is less frequent because of the extreme conditions in which these stands persist (Jelinski and Cheliak 1992). The low proportion of heterozygosity in the Big Bend genotypes might also point to historical inbreeding as expected from distant island populations cut off from gene flow (Vásquez et al. 2016). Interestingly, the estimated periodicity of reproduction for the Dryhead and JE Canyon genotypes was similar, suggesting a possibly shared attribute such as ecological disturbance after glacial melting (Kay 1993) and/or masting reproductive cycles (Kelly 1994). No matter the situation, the presence of aspen in Big Bend NP is both geographically and ecologically anomalous and requires further study in order to understand its migration pathway and adaptive traits.

While the coalescent values reported herein should be interpreted with caution given the broad range of values output from the Bayesian simulations and the decoupling of mean tropical years from sexual reproduction in aspen, it is a useful exercise in attempting to clarify the age of disjunct aspen stands. While other approaches using somatic mutation rate have been employed to date aspen clones they also inferred a broad range for clone ages (160 – 9652 years old; Ally et al. 2008). Using somatic mutation to infer age can also be problematic in small stands with few ramets such as the

disjunct stands studied herein. Clearly, alternative approaches such as comparative dendrochemistry (e.g., Poussart et al. 2006), pollen viability assays (Ally et al. 2010), and genome wide assays for somatic mutations are needed to more accurately estimate the ages of aspen clones throughout the species range and provide insights into the often-debated maximum age for relictual clones (Eckenwalder 1996). Additionally, combining methods that focus on different spatial and temporal scales, such as coalescence for historical establishment estimates and somatic mutation rates for time since establishment, might provide improved resolution for clone ages at a larger scale.

Genetics of Isolated Aspen Stands and Considerations for Conservation

An isolated stand made up of a single genotype is inherently reduced in genetic diversity. To recover from this state of reduced genetic diversity would require 1) selfing and subsequent recombination (Roze and Lenormand 2005), 2) long distance dispersal of seeds and/or pollen to these stands (Whiteley et al. 2015), or 3) somatic mutation (Mock et al. 2008), all of which would be extremely rare in these disjunct aspen stands. Furthermore, somatic mutations and selfing are unlikely to result in a genetic rescue that will provide sufficient adaptive capacity against rapidly changing environmental conditions. Over the next 100 years aspen habitat is predicted to decline by 94% (Rehfeldt et al. 2009), with marginal populations like those studied herein expected to be extinct as the microclimates and adapted genes in these disjunct genotypes will be insufficient to buffer against the rapidly warming climate (Hannah et al. 2014). Aspen is by no means a rare species threatened with extinction in the short term; however, the resource of rare genes and gene complexes adapted to the extreme conditions found at range margins are at risk of loss. Ironically these adapted gene complexes for survival in the most marginal conditions would be those most sought after in assisted migration efforts.

Based on the presence of rare alleles and theoretical expectations, the stands found at Dryhead, JE Canyon Ranch and Big Bend NP are prime candidates for conservation and further inquiry regarding drought, and heat adapted genes. The Dryhead genotype and aspens across the Pryor Mountain area require further study to more fully understand what genetic resources are present in these stands and what historical and environmental conditions were responsible for maintaining such high genotypic diversity (north and south-western genotypes) in this area. The Pryor Mountains are known to have high rates of plant endemism and plant biodiversity (e.g. Lesica 1994; McCarthy 1996), but they also appear to be an area in which high genotypic diversity for common species like aspen are conserved. As such, the Pryor Mountain aspens in general and the Dryhead and Bench stands specifically should be studied in greater detail to elucidate the overall genetic resources of Pryor Mountain aspens and the phylogeographic importance of this area. With conservation funds limited, areas of high genotypic diversity like the Pryor Mountains and stable-edge microrefugia with potentially locally adapted gene complexes like the JE Canyon Ranch and Big Bend NP should also be prioritized for conservation and study.

While rapid changes in climate are among the most often discussed threats to aspen, numerous other factors such as pest insects, fungal pathogens, overgrazing, and land use change (e.g. DeByle and Winokur 1985; Rogers and McAvoy 2018), alone or in concert with a warming climate, threaten aspen stands generally and small disjunct stands in arid settings in particular. The US National Parks

have conservation as a central tenant of their establishment and possess numerous aspen stands making them a well-suited agency for conservation of aspen genetic resources. However, other agencies like the US Forest Service, US Fish and Wildlife Service, Bureau of Land Management, and US Geological Survey all have expertise in biological conservation and would be excellent partners in providing services for the conservation of aspen as well. Lastly, private agencies and landholders have been and continue to be an important part of aspen conservation and should be integrated into future conservation strategies. Specifically, in-situ and ex-situ conservation measures are needed for short- and long-term conservation of disjunct and relict aspen genotypes respectively (Volis and Blecher 2010). Preservation and propagation of the disjunct genotypes discussed here and in other studies could be directly employed for assisted migration efforts in wild forest lands and/or large-scale afforestation projects (Gray et al. 2011; Bastin et al. 2019) as well as integrated into the development of new cultivars for restoration and timber production on marginal lands (Benetka et al. 2012).

Conclusions

Since the description and characterization of continent wide patterns of genetic structuring and diversity in aspen (Mock et al. 2012; Callahan et al. 2013), a need for finer scale genetic studies has been apparent in an effort to understand how genetic structuring at the population, stand, and individual level contributes to genetic structure and range dynamics at the continental scale. This and other studies such as Deacon et al. (2017) are efforts towards elucidating fine scale genetic patterns in glacial refugia, which are helping to resolve larger scale questions regarding the formation and maintenance of continental scale patterns. As such, several findings are noted: 1) the disjunct stands studied herein follow an expected pattern of divergence from south to north, suggesting relictual origins associated with geographically adjacent stands during cooler climatic conditions; 2) south-western disjuncts are progressively older with decreasing latitude in regard to inferred coalescent times to northern genetic clusters but young when compared to south-western genetic clusters; 3) all disjunct genotypes possessed rare alleles not found in adjacent stands, suggesting a relictual origin when now distant populations were connected through geneflow; and 4) no triploid genotypes were found among the disjunct stands suggesting that triploidy is not selected for in drought resistance and heat tolerance but could be at a competitive advantage during colonization after recession of montane glaciers. These results require further study employing broader genomic sampling and more detailed ecological and physiological studies to elucidate how these disjunct stands have established and persisted in microrefugia. Given the threats to these disjunct stands, conservation efforts should be enacted to preserve the unique genetic, ecological, and physiological attributes contained in these stands.

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Appendix A. Collected Aspen Specimen Data

See Table A1 for collected aspen specimen data and Table A2 for specimen molecular data.

Table A1. Physical description, locality, site information, and associated plant species of collected aspen specimens.

LT Collection No	Collectors	Family	Genus	specific epithet	authority	specimen description	main associated species	site/stand description	State	County	date collected	DBH (meters)	height (estimate; meters)	elevation (meters)
17_110	Luke R Tembrock, Bill Picket	Salicaceae	*Populus*	*angustifolia*	E. James	large tree growing along stream, sticky cataphylls, furrowed bark	*Rhus trilobata, Juniperus scopulorum, Artemisia* spp, *Achillea millefolium, Gutierrezia sarothrae*	Bighorn Canyon NRA, headwaters of side drainage to Dry Head Creek a western side drainage to the Bighorn River, sandstone and limestone geology, area heavily disturbed from wild horses, numerous flowing springs and seeps in the area.	Montana	Carbon	29-Aug-17	0.62	10	1376
17_111	Luke R Tembrock, Bill Picket	Salicaceae	*Populus*	*angustifolia*	E. James	large tree growing along stream, sticky cataphylls, furrowed bark	*Rhus trilobata, Juniperus scopulorum, Artemisia* spp, *Achillea millefolium, Gutierrezia sarothrae*	Bighorn Canyon NRA, headwaters of side drainage to Dry Head Creek a western side drainage to the Bighorn River, sandstone and limestone geology, area heavily disturbed from wild horses, numerous flowing springs and seeps in the area.	Montana	Carbon	29-Aug-17	0.13	10	1392
17_112	Luke R Tembrock, Bill Picket	Salicaceae	*Populus*	*angustifolia*	E. James	large tree growing along stream, sticky cataphylls, furrowed bark	*Rhus trilobata, Juniperus scopulorum, Artemisia* spp, *Achillea millefolium, Gutierrezia sarothrae*	Bighorn Canyon NRA, headwaters of side drainage to Dry Head Creek a western side drainage to the Bighorn River, sandstone and limestone geology, area heavily disturbed from wild horses, numerous flowing springs and seeps in the area.	Montana	Carbon	29-Aug-17	1.25	15	1365
17_113	Luke R Tembrock, Bill Picket	Salicaceae	*Populus*	*angustifolia*	E. James	large tree growing along stream, sticky cataphylls, furrowed bark	*Rhus trilobata, Juniperus scopulorum, Artemisia* spp, *Achillea millefolium, Gutierrezia sarothrae*	Bighorn Canyon NRA, headwaters of side drainage to Dry Head Creek a western side drainage to the Bighorn River, sandstone and limestone geology, area heavily disturbed from wild horses, numerous flowing springs and seeps in the area.	Montana	Carbon	29-Aug-17	1.17	15	1357
17_114*	Luke R Tembrock, Bill Picket	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, evidence of browsing, thick leaves, die back in upper part of crown, suckering heavily browsed	*Rhus trilobata, Juniperus scopulorum, Artemisia* spp, *Achillea millefolium, Gutierrezia sarothrae*	Bighorn Canyon NRA, headwaters of side drainage to Dry Head Creek a western side drainage to the Bighorn River, sandstone and limestone geology, area heavily disturbed from wild horses, numerous flowing springs and seeps in the area.	Montana	Carbon	29-Aug-17	1.4	12	1406
17_115*	Luke R Tembrock, Bill Picket	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, evidence of browsing, thick leaves, die back in upper part of crown, suckering heavily browsed	*Rhus trilobata, Juniperus scopulorum, Artemisia* spp, *Achillea millefolium, Gutierrezia sarothrae*	Bighorn Canyon NRA, headwaters of side drainage to Dry Head Creek a western side drainage to the Bighorn River, sandstone and limestone geology, area heavily disturbed from wild horses, numerous flowing springs and seeps in the area.	Montana	Carbon	29-Aug-17	0.14	7	1408
17_116*	Luke R Tembrock, Bill Picket	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, evidence of browsing, thick leaves, die back in upper part of crown, suckering heavily browsed	*Rhus trilobata, Juniperus scopulorum, Artemisia* spp, *Achillea millefolium, Gutierrezia sarothrae*	Bighorn Canyon NRA, headwaters of side drainage to Dry Head Creek a western side drainage to the Bighorn River, sandstone and limestone geology, area heavily disturbed from wild horses, numerous flowing springs and seeps in the area.	Montana	Carbon	29-Aug-17	0.26	7	1412
17_117*	Luke R Tembrock, Bill Picket	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, evidence of browsing, thick leaves, die back in upper part of crown, suckering heavily browsed	*Rhus trilobata, Juniperus scopulorum, Artemisia spp, Achillea millefolium, Gutierrezia sarothrae*	Bighorn Canyon NRA, headwaters of side drainage to Dry Head Creek a western side drainage to the Bighorn River, sandstone and limestone geology, area heavily disturbed from wild horses, numerous flowing springs and seeps in the area.	Montana	Carbon	29-Aug-17	0.41	8	1416

* Samples from disjunct stands.

Table A1 (continued). Physical description, locality, site information, and associated plant species of collected aspen specimens.

LT Collection No	Collectors	Family	Genus	specific epithet	authority	specimen description	main associated species	site/stand description	State	County	date collected	DBH (meters)	height (estimate; meters)	elevation (meters)
17_118*	Luke R Tembrock, Bill Picket	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, evidence of browsing, thick leaves, die back in upper part of crown, suckering heavily browsed	*Rhus trilobata, Juniperus scopulorum, Artemisia spp, Achillea millefolium, Gutierrezia sarothrae*	Bighorn Canyon NRA, headwaters of side drainage to Dry Head Creek a western side drainage to the Bighorn River, sandstone and limestone geology, area heavily disturbed from wild horses, numerous flowing springs and seeps in the area.	Montana	Carbon	29-Aug-17	0.52	8	1422
17_119*	Luke R Tembrock, Bill Picket	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, evidence of browsing, thick leaves, die back in upper part of crown, suckering heavily browsed	*Rhus trilobata, Juniperus scopulorum, Artemisia spp, Achillea millefolium, Gutierrezia sarothrae*	Bighorn Canyon NRA, headwaters of side drainage to Dry Head Creek a western side drainage to the Bighorn River, sandstone and limestone geology, area heavily disturbed from wild horses, numerous flowing springs and seeps in the area.	Montana	Carbon	29-Aug-17	0.47	7	1422
17_120*	Luke R Tembrock, Bill Picket	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, evidence of browsing, thick leaves, die back in upper part of crown, suckering heavily browsed	*Rhus trilobata, Juniperus scopulorum, Artemisia* spp, *Achillea millefolium, Gutierrezia sarothrae*	Bighorn Canyon NRA, headwaters of side drainage to Dry Head Creek a western side drainage to the Bighorn River, sandstone and limestone geology, area heavily disturbed from wild horses, numerous flowing springs and seeps in the area.	Montana	Carbon	29-Aug-17	0.45	8	1419
17_121*	Luke R Tembrock, Bill Picket	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, evidence of browsing, thick leaves, die back in upper part of crown, suckering heavily browsed	*Rhus trilobata, Juniperus scopulorum, Artemisia* spp, *Achillea millefolium, Gutierrezia sarothrae*	Bighorn Canyon NRA, headwaters of side drainage to Dry Head Creek a western side drainage to the Bighorn River, sandstone and limestone geology, area heavily disturbed from wild horses, numerous flowing springs and seeps in the area.	Montana	Carbon	29-Aug-17	0.41	7	1401
17_122*	Luke R Tembrock, Bill Picket	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, evidence of browsing, thick leaves, die back in upper part of crown, suckering heavily browsed	*Rhus trilobata, Juniperus scopulorum, Artemisia* spp, *Achillea millefolium, Gutierrezia sarothrae*	Bighorn Canyon NRA, headwaters of side drainage to Dry Head Creek a western side drainage to the Bighorn River, sandstone and limestone geology, area heavily disturbed from wild horses, numerous flowing springs and seeps in the area.	Montana	Carbon	29-Aug-17	0.51	7	1407
17_123*	Luke R Tembrock, Bill Picket	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, evidence of browsing, thick leaves, die back in upper part of crown, suckering heavily browsed	*Rhus trilobata, Juniperus scopulorum, Artemisia* spp, *Achillea millefolium, Gutierrezia sarothrae*	Bighorn Canyon NRA, headwaters of side drainage to Dry Head Creek a western side drainage to the Bighorn River, sandstone and limestone geology, area heavily disturbed from wild horses, numerous flowing springs and seeps in the area.	Montana	Carbon	29-Aug-17	0.36	7	1408
17_124*	Luke R Tembrock, Bill Picket	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, evidence of browsing, thick leaves, die back in upper part of crown, suckering heavily browsed	*Rhus trilobata, Juniperus scopulorum, Artemisia* spp, *Achillea millefolium, Gutierrezia sarothrae*	Bighorn Canyon NRA, headwaters of side drainage to Dry Head Creek a western side drainage to the Bighorn River, sandstone and limestone geology, area heavily disturbed from wild horses, numerous flowing springs and seeps in the area.	Montana	Carbon	29-Aug-17	0.59	10	1420
17_125*	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, little evidence of browsing, trunk white, unforrowed, leaves nearly round	*Mahonia repens, Symphoricarpus albus, Juniperus scopulorum, Cirsium* sp, *Cornus sericea, Acer glabrum, Physocarpus* sp, *Pseudotsuga menziesii*	Bighorn Canyon NRA, 15 acre grove situtated on a bench between limestone escarpments on the eastern slope of the Pryor mtns, numerous flowing springs and seeps, area wetter than surrounding slopes, grove very healthy with trees of multiple ages classes.	Montana	Carbon	29-Aug-17	0.5	10	1866
17_126*	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, little evidence of browsing, trunk white, unforrowed, leaves nearly round	*Mahonia repens, Symphoricarpus albus, Juniperus scopulorum, Cirsium* sp, *Cornus sericea, Acer glabrum, Physocarpus* sp, *Pseudotsuga menziesii*	Bighorn Canyon NRA, 15 acre grove situtated on a bench between limestone escarpments on the eastern slope of the Pryor mtns, numerous flowing springs and seeps, area wetter than surrounding slopes, grove very healthy with trees of multiple ages classes.	Montana	Carbon	29-Aug-17	0.75	15	1868

* Samples from disjunct stands.

Table A1 (continued). Physical description, locality, site information, and associated plant species of collected aspen specimens.

LT Collection No	Collectors	Family	Genus	specific epithet	authority	specimen description	main associated species	site/stand description	State	County	date collected	DBH (meters)	height (estimate; meters)	elevation (meters)
17_127*	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, little evidence of browsing, trunk white, unforrowed, leaves nearly round	*Mahonia repens, Symphoricarpus albus, Juniperus scopulorum, Cirsium* sp*, Cornus sericea, Acer glabrum, Physocarpus* sp*, Pseudotsuga menziesii*	Bighorn Canyon NRA, 15 acre grove situtated on a bench between limestone escarpments on the eastern slope of the Pryor mtns, numerous flowing springs and seeps, area wetter than surrounding slopes, grove very healthy with trees of multiple ages classes.	Montana	Carbon	29-Aug-17	0.33	10	1876
17_128*	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, little evidence of browsing, trunk white, unforrowed, leaves nearly round	*Mahonia repens, Symphoricarpus albus, Juniperus scopulorum, Cirsium sp, Cornus sericea, Acer glabrum, Physocarpus sp, Pseudotsuga menziesii*	Bighorn Canyon NRA, 15 acre grove situtated on a bench between limestone escarpments on the eastern slope of the Pryor mtns, numerous flowing springs and seeps, area wetter than surrounding slopes, grove very healthy with trees of multiple ages classes.	Montana	Carbon	29-Aug-17	0.89	12	1862
17_129*	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, little evidence of browsing, trunk white, unforrowed, leaves nearly round	*Mahonia repens, Symphoricarpus albus, Juniperus scopulorum, Cirsium* sp*, Cornus sericea, Acer glabrum, Physocarpus* sp*, Pseudotsuga menziesii*	Bighorn Canyon NRA, 15 acre grove situtated on a bench between limestone escarpments on the eastern slope of the Pryor mtns, numerous flowing springs and seeps, area wetter than surrounding slopes, grove very healthy with trees of multiple ages classes.	Montana	Carbon	29-Aug-17	0.55	12	1864
17_130*	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	young tree, little evidence of browsing, trunk white, unforrowed, leaves nearly round	*Mahonia repens, Symphoricarpus albus, Juniperus scopulorum, Cirsium* sp*, Cornus sericea, Acer glabrum, Physocarpus* sp*, Pseudotsuga menziesii*	Bighorn Canyon NRA, 15 acre grove situtated on a bench between limestone escarpments on the eastern slope of the Pryor mtns, numerous flowing springs and seeps, area wetter than surrounding slopes, grove very healthy with trees of multiple ages classes.	Montana	Carbon	29-Aug-17	0.22	3	1867
17_131	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp*, Shepherdia canadensis, Juniperus communis, Pseudotsuga menziesii, Potentilla fruticosa*	Custer National Forest, ~20 acre grove 140 meters west of FR 2849 in saddle between Big Pryor Mtn and East Pryor Mtn, grove associated with flowing springs amidst sagebrush park lands	Montana	Carbon	30-Aug-17	0.35	8	2157
17_132	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp*, Shepherdia canadensis, Juniperus communis, Pseudotsuga menziesii, Potentilla fruticosa*	Custer National Forest, ~20 acre grove 140 meters west of FR 2849 in saddle between Big Pryor Mtn and East Pryor Mtn, grove associated with flowing springs amidst sagebrush park lands	Montana	Carbon	30-Aug-17	0.31	5	2138
17_133	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp*, Shepherdia canadensis, Juniperus communis, Pseudotsuga menziesii, Potentilla fruticosa*	Custer National Forest, ~20 acre grove 140 meters west of FR 2849 in saddle between Big Pryor Mtn and East Pryor Mtn, grove associated with flowing springs amidst sagebrush park lands	Montana	Carbon	30-Aug-17	0.305	10	2145
17_134	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp*, Shepherdia canadensis, Juniperus communis, Pseudotsuga menziesii, Potentilla fruticosa*	Custer National Forest, ~20 acre grove 140 meters west of FR 2849 in saddle between Big Pryor Mtn and East Pryor Mtn, grove associated with flowing springs amidst sagebrush park lands	Montana	Carbon	30-Aug-17	0.32	12	2154
17_135	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Salix* spp*, Abies lasiocarpa, Physocarpus* sp*, Iris missouriensis, Potentilla fruticosa*	Custer National Forest, large grove spread over several miles 850 meters ENE from Dry Head Vista on East Pryor Mtn, grove situtated on a bench between limestone escarpments, numerous flowing springs and seeps, area wetter than surrounding slopes, grove very healthy with trees of multiple ages classes.	Montana	Carbon	30-Aug-17	0.68	8	2201

* Samples from disjunct stands.

Table A1 (continued). Physical description, locality, site information, and associated plant species of collected aspen specimens.

LT Collection No	Collectors	Family	Genus	specific epithet	authority	specimen description	main associated species	site/stand description	State	County	date collected	DBH (meters)	height (estimate; meters)	elevation (meters)
17_136	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Salix* spp, *Abies lasiocarpa, Physocarpus* sp, *Iris missouriensis, Potentilla fruticosa*	Custer National Forest, large grove spread over several miles 850 meters ENE from Dry Head Vista on East Pryor Mtn, grove situtated on a bench between limestone escarpments, numerous flowing springs and seeps, area wetter than surrounding slopes, grove very healthy with trees of multiple ages classes.	Montana	Carbon	30-Aug-17	0.255	6	2176
17_137	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Salix* spp, *Abies lasiocarpa, Physocarpus* sp, *Iris missouriensis, Potentilla fruticosa*	Custer National Forest, large grove spread over several miles 850 meters ENE from Dry Head Vista on East Pryor Mtn, grove situtated on a bench between limestone escarpments, numerous flowing springs and seeps, area wetter than surrounding slopes, grove very healthy with trees of multiple ages classes.	Montana	Carbon	30-Aug-17	0.35	7	2135
17_138	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Salix* spp, *Abies lasiocarpa, Physocarpus* sp, *Iris missouriensis, Potentilla fruticosa*	Custer National Forest, large grove spread over several miles 850 meters ENE from Dry Head Vista on East Pryor Mtn, grove situtated on a bench between limestone escarpments, numerous flowing springs and seeps, area wetter than surrounding slopes, grove very healthy with trees of multiple ages classes.	Montana	Carbon	30-Aug-17	0.5	8	2066
17_139	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Juniperus communis, Symphoricarpus albus, Abies lasiocarpa, Picea engelmannii, Rosa woodsii, Prunus virginiana, Artemesia* spp, *Geranium* sp	Custer National Forest, located on both sides of FR 2097, associated with springs and seeps at south end of Harsten Flats, North and East facing slopes, trees generally healthy.	Montana	Carbon	31-Aug-17	0.57	7	2072
17_140	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Juniperus communis, Symphoricarpus albus, Abies lasiocarpa, Picea engelmannii, Rosa woodsii, Prunus virginiana, Artemesia* spp, *Geranium* sp	Custer National Forest, located on both sides of FR 2097, associated with springs and seeps at south end of Harsten Flats, North and East facing slopes, trees generally healthy.	Montana	Carbon	31-Aug-17	0.305	6	2083
17_141	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Juniperus communis, Symphoricarpus albus, Abies lasiocarpa, Picea engelmannii, Rosa woodsii, Prunus virginiana, Artemesia* spp, *Geranium* sp	Custer National Forest, located on both sides of FR 2097, associated with springs and seeps at south end of Harsten Flats, North and East facing slopes, trees generally healthy.	Montana	Carbon	31-Aug-17	0.28	5	2070
17_142	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Juniperus communis, Symphoricarpus albus, Abies lasiocarpa, Picea engelmannii, Rosa woodsii, Prunus virginiana, Artemesia* spp, *Geranium* sp	Custer National Forest, located on both sides of FR 2097, associated with springs and seeps at south end of Harsten Flats, North and East facing slopes, trees generally healthy.	Montana	Carbon	31-Aug-17	0.205	3	2070
17_143	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Juniperus communis, Symphoricarpus albus, Abies lasiocarpa, Picea engelmannii, Rosa woodsii, Prunus virginiana, Artemesia* spp, *Geranium* sp	Custer National Forest, located on both sides of FR 2097, associated with springs and seeps at south end of Harsten Flats, North and East facing slopes, trees generally healthy.	Montana	Carbon	31-Aug-17	0.21	3	2072

* Samples from disjunct stands.

Table A1 (continued). Physical description, locality, site information, and associated plant species of collected aspen specimens.

LT Collection No	Collectors	Family	Genus	specific epithet	authority	specimen description	main associated species	site/stand description	State	County	date collected	DBH (meters)	height (estimate; meters)	elevation (meters)
17_144	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Juniperus communis, Symphoricarpus albus, Abies lasiocarpa, Picea engelmannii, Rosa woodsii, Prunus virginiana, Artemesia* spp, *Geranium* sp	Custer National Forest, located on both sides of FR 2097, associated with springs and seeps at south end of Harsten Flats, North and East facing slopes, trees generally healthy.	Montana	Carbon	31-Aug-17	0.24	3	2072
17_145	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Juniperus communis, Symphoricarpus albus, Abies lasiocarpa, Picea engelmannii, Rosa woodsii, Prunus virginiana, Artemesia* spp, *Geranium* sp	Custer National Forest, located on both sides of FR 2097, associated with springs and seeps at south end of Harsten Flats, North and East facing slopes, trees generally healthy.	Montana	Carbon	31-Aug-17	0.28	6	2073
17_146	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Juniperus communis, Symphoricarpus albus, Abies lasiocarpa, Picea engelmannii, Rosa woodsii, Prunus virginiana, Artemesia* spp, *Geranium* sp	Custer National Forest, located on both sides of FR 2097, associated with springs and seeps at south end of Harsten Flats, North and East facing slopes, trees generally healthy.	Montana	Carbon	31-Aug-17	0.55	7	2093
17_147	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Rubus parviflorus, Salix spp, Juniperus communis, Symphoricarpus albus, Abies lasiocarpa, Picea engelmannii, Rosa woodsii, Prunus virginiana, Artemisia* spp, *Geranium* sp	Custer National Forest, located at headwaters of Sage Creek south of Roberts Bench, associated with springs (Teeples spring)and seeps in NW facing drainage, trees generally healthy.	Montana	Carbon	31-Aug-17	0.45	8	2150
17_148	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Rubus parviflorus, Salix* spp, *Juniperus communis, Symphoricarpus albus, Abies lasiocarpa, Picea engelmannii, Rosa woodsii, Prunus virginiana, Artemisia* spp, *Geranium* sp	Custer National Forest, located at headwaters of Sage Creek south of Roberts Bench, associated with springs (Teeples spring)and seeps in NW facing drainage, trees generally healthy.	Montana	Carbon	31-Aug-17	0.46	5	2109
17_149	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Rubus parviflorus, Salix* spp, *Juniperus communis, Symphoricarpus albus, Abies lasiocarpa, Picea engelmannii, Rosa woodsii, Prunus virginiana, Artemisia* spp, *Geranium* sp	Custer National Forest, located at headwaters of Sage Creek south of Roberts Bench, associated with springs (Teeples spring)and seeps in NW facing drainage, trees generally healthy.	Montana	Carbon	31-Aug-17	0.37	6	2075
17_150	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Rubus parviflorus, Salix* spp, *Juniperus communis, Symphoricarpus albus, Abies lasiocarpa, Picea engelmannii, Rosa woodsii, Prunus virginiana, Artemisia* spp, *Geranium* sp	Custer National Forest, located at headwaters of Sage Creek south of Roberts Bench, associated with springs (Teeples spring)and seeps in NW facing drainage, trees generally healthy.	Montana	Carbon	31-Aug-17	0.275	3	2057

* Samples from disjunct stands.

Table A1 (continued). Physical description, locality, site information, and associated plant species of collected aspen specimens.

LT Collection No	Collectors	Family	Genus	specific epithet	authority	specimen description	main associated species	site/stand description	State	County	date collected	DBH (meters)	height (estimate; meters)	elevation (meters)
17_151	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Rubus parviflorus, Salix* spp, *Juniperus communis, Symphoricarpus albus, Abies lasiocarpa, Picea engelmannii, Rosa woodsii, Prunus virginiana, Artemisia* spp, *Geranium* sp	Custer National Forest, located at headwaters of Sage Creek south of Roberts Bench, associated with springs (Teeples spring)and seeps in NW facing drainage, trees generally healthy.	Montana	Carbon	31-Aug-17	0.32	4	2098
17_152	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	young tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp, *Achillea millefolium, Phleum pratense, Rosa woodsii, Iris missouriensis*	Custer National Forest, very small grove of young trees located at edge of park at the intersection of FR 2097 and 2073.	Montana	Carbon	31-Aug-17	0.31	5	2164
17_153	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	young tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp, *Achillea millefolium, Phleum pratense, Rosa woodsii, Iris missouriensis*	Custer National Forest, very small grove of young trees located at edge of park at the intersection of FR 2097 and 2073.	Montana	Carbon	31-Aug-17	0.345	6	2166
17_154	Luke R Tembrock	Salicaceae	*Populus*	*angustifolia*	E. James	young tree, trunk forrowed, leaves nearly elliptical, cataphylls sticky	*Salix* sp, *Abies lasiocarpa, Picea engelmannii*	Custer National Forest, small cluster of trees growing from borrow ditch along FR 23085	Montana	Carbon	31-Aug-17	n	n	2124
17_155	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides x angustifolia*		young tree, trunk white, unforrowed, leaves sub-elliptical, cataphylls somewhat sticky	*Salix* sp, *Abies lasiocarpa, Picea engelmannii*	Custer National Forest, small cluster of trees growing from borrow ditch along FR 23085	Montana	Carbon	31-Aug-17	0.455	7	2130
17_156	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Salix* sp, *Abies lasiocarpa, Picea engelmannii, Artemisia* spp., *Rubus parviflorus, Amelanchier alnifolia, Acer glabrum*	Custer National Forest, grove growing along tributary of Sage Creek, North of FR 23085	Montana	Carbon	31-Aug-17	0.365	7	2037
17_157	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Salix* sp, *Abies lasiocarpa, Picea engelmannii, Artemisia spp., Rubus parviflorus, Amelanchier alnifolia, Acer glabrum*	Custer National Forest, grove growing along tributary of Sage Creek, North of FR 23085	Montana	Carbon	31-Aug-17	0.27	4	2005
17_158	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	young tree, trunk white, unforrowed, leaves sub-elliptical, cataphylls somewhat sticky	*Salix* sp, *Abies lasiocarpa, Picea engelmannii*	Custer National Forest, small cluster of trees growing at the base of the road fill on FR 23085	Montana	Carbon	31-Aug-17	0.67	10	1993
17_159	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Salix* sp, *Abies lasiocarpa, Picea engelmannii*	Custer National Forest, small cluster of trees growing at the base of the road fill on FR 23085	Montana	Carbon	31-Aug-17	0.57	8	1863
17_160	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	very large mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp, *Alnus* sp, *Pinus* sp, *Abies lasiocarpa, Ribes* sp, *Iris missouriensis*	Bighorn National Forest, eastern drainage of the Bighorn mtns, aspen trees surrounding marshy meadow between two levels of Alt 14	Wyoming	Bighorn	31-Aug-17	1.02	10	2130
17_161	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	young tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp, *Alnus* sp, *Pinus* sp, *Abies lasiocarpa, Ribes* sp, *Iris missouriensis*	Bighorn National Forest, eastern drainage of the Bighorn mtns, aspen trees surrounding marshy meadow between two levels of Alt 14	Wyoming	Bighorn	31-Aug-17	0.145	3	2163

* Samples from disjunct stands.

Table A1 (continued). Physical description, locality, site information, and associated plant species of collected aspen specimens.

LT Collection No	Collectors	Family	Genus	specific epithet	authority	specimen description	main associated species	site/stand description	State	County	date collected	DBH (meters)	height (estimate; meters)	elevation (meters)
17_162	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp, *Juniperus communis, Lupinus* sp, *Abies lasiocarpa, Picea engelmannii*	Bighorn National Forest, ~20 acre grove on east facing slope of Big Willow Creek, 200 meters ENE from the Galloway cabin on FR 159	Wyoming	Sheridan	1-Sep-17	0.655	7	2559
17_163	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp, *Juniperus communis, Lupinus* sp, *Abies lasiocarpa, Picea engelmannii*	Bighorn National Forest, ~20 acre grove on east facing slope of Big Willow Creek, 200 meters ENE from the Galloway cabin on FR 160	Wyoming	Sheridan	1-Sep-17	1.02	8	2574
17_164	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp, *Juniperus communis, Lupinus* sp, *Abies lasiocarpa, Picea engelmannii*	Bighorn National Forest, ~20 acre grove on east facing slope of Big Willow Creek, 200 meters ENE from the Galloway cabin on FR 161	Wyoming	Sheridan	1-Sep-17	1.07	8	2604
17_165	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	very large mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp, *Lupinus* sp, *Achillea millefolium, Picea engelmannii, Pinus contorta*	Bighorn National Forest, clones scattered on South facing slope of Little Tepee Creek, 340 meters south of Bucking Mule Falls TH, in open rocky sagebrush parklands	Wyoming	Bighorn	1-Sep-17	1.67	12	2512
17_166	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp, *Lupinus* sp, *Achillea millefolium, Picea engelmannii, Pinus contorta*	Bighorn National Forest, clones scattered on South facing slope of Little Tepee Creek, 340 meters south of Bucking Mule Falls TH, in open rocky sagebrush parklands	Wyoming	Bighorn	1-Sep-17	0.89	9	2507
17_167	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	young tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp, *Lupinus* sp, *Achillea millefolium, Picea engelmannii, Pinus contorta*	Bighorn National Forest, clones scattered on South facing slope of Little Tepee Creek, 340 meters south of Bucking Mule Falls TH, in open rocky sagebrush parklands	Wyoming	Bighorn	1-Sep-17	0.57	7	2476
17_168	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp., *Achillea millefolium, Balsamorhiza sagittata*	Bighorn National Forest, clones scattered on South and West facing slopes above Alt 14, in open rocky sagebrush parklands associated with springs and seeps	Wyoming	Bighorn	1-Sep-17	0.36	4	2487
17_169	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp., *Achillea millefolium, Balsamorhiza sagittata*	Bighorn National Forest, clones scattered on South and West facing slopes above Alt 14, in open rocky sagebrush parklands associated with springs and seeps	Wyoming	Bighorn	1-Sep-17	0.545	4	2506
17_170	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp., *Achillea millefolium, Balsamorhiza sagittata*	Bighorn National Forest, clones scattered on South and West facing slopes above Alt 14, in open rocky sagebrush parklands associated with springs and seeps	Wyoming	Bighorn	1-Sep-17	0.34	4	2507
17_171	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp., *Achillea millefolium, Balsamorhiza sagittata*	Bighorn National Forest, clones scattered on South and West facing slopes above Alt 14, in open rocky sagebrush parklands associated with springs and seeps	Wyoming	Bighorn	1-Sep-17	0.59	5	2485
17_172	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	young tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp., *Picea engelmannii, Pinus contorta, Juniperus communis*	Bighorn National Forest, clones on Southwest facing slope of North Tongue River, 520 meters west of Wheddon spring and 200 north of Alt 14, in open rocky sagebrush parklands associated with springs and seeps	Wyoming	Sheridan	1-Sep-17	0.315	4	2659
17_173	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp., *Picea engelmannii, Pinus contorta, Juniperus communis*	Bighorn National Forest, clones on Southwest facing slope of North Tongue River, 520 meters west of Wheddon spring and 200 north of Alt 14, in open rocky sagebrush parklands associated with springs and seeps	Wyoming	Sheridan	1-Sep-17	0.44	4	2645

* Samples from disjunct stands.

Table A1 (continued). Physical description, locality, site information, and associated plant species of collected aspen specimens.

LT Collection No	Collectors	Family	Genus	specific epithet	authority	specimen description	main associated species	site/stand description	State	County	date collected	DBH (meters)	height (estimate; meters)	elevation (meters)
17_174	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	very large, mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp., *Achillea millefolium, Lupinus* sp, *Frasera speciosa, Picea engelmannii, Pinus contorta*	Bighorn National Forest, large clones on Southeast facing slopes of the North Tongue River, 340 meters North of the angler access parking lot on Alt 14, in open rocky sagebrush parklands, two experimental clearcut and enclosure sites are contained in this grove	Wyoming	Sheridan	1-Sep-17	1.275	15	2521
17_175	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	very large, mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Artemisia* spp., *Achillea millefolium, Lupinus* sp, *Frasera speciosa, Picea engelmannii, Pinus contorta*	Bighorn National Forest, large clones on Southeast facing slopes of the North Tongue River, 340 meters North of the angler access parking lot on Alt 14, in open rocky sagebrush parklands, two experimental clearcut and enclosure sites are contained in this grove	Wyoming	Sheridan	1-Sep-17	1.085	15	2530
17_176	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, evidence of browsing, trunk white, unforrowed, leaves nearly round	*Picea engelmannii, Pinus contorta, Potentilla fruticosa, Salix* sp, *Bromus* spp, *Lupinus* sp	Bighorn National Forest, large clones on both sides of Alt 14, 850 meters Northeast of Burgess Junction, in open marshy parklands, one experimental clearcut and enclosure site is contained in this grove	Wyoming	Sheridan	1-Sep-17	0.805	10	2443
17_177	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, evidence of browsing, trunk white, unforrowed, leaves nearly round	*Picea engelmannii, Pinus contorta, Potentilla fruticosa, Salix* sp, *Bromus* spp, *Lupinus* sp	Bighorn National Forest, large clones on both sides of Alt 14, 850 meters Northeast of Burgess Junction, in open marshy parklands, one experimental clearcut and enclosure site is contained in this grove	Wyoming	Sheridan	1-Sep-17	0.895	9	2447
17_178	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, evidence of browsing, trunk white, unforrowed, leaves nearly round	*Picea engelmannii, Pinus contorta, Potentilla fruticosa, Salix* sp, *Bromus* spp, *Lupinus* sp	Bighorn National Forest, large clones on both sides of Alt 14, 850 meters Northeast of Burgess Junction, in open marshy parklands, one experimental clearcut and enclosure site is contained in this grove	Wyoming	Sheridan	1-Sep-17	0.825	9	2452
17_179	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, little evidence of browsing, trunk white, unforrowed, leaves nearly round	*Pinus contorta* dominated forests	Bighorn National Forest, lone tree at outside edge of road cut/fill 700 meters up from hairpin corner on Alt 14, Eastern side of Bighorn Mtns.	Wyoming	Sheridan	3-Sep-17	0.435	3	1783
17_180	Luke R Tembrock	Salicaceae	*Populus*	*angustifolia*	E. James	mature tree, trunk forrowed, leaves nearly elliptical	*Pinus contorta* dominated forests	Bighorn National Forest, small group of trees in drainge below road fill west of Alt 14 in Crystal Spring Draw drainage, Eastern side of Bighorn Mtns.	Wyoming	Sheridan	3-Sep-17	0.59	10	1872
17_181	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides x balsamifera*		mature tree, trunk forrowed, leaves lanceolate to elliptical, petioles mostly cylindrical, abaxial leaf surface nearly white	*Picea engelmannii, Ribes* sp, *Poaceae* spp	Wyoming State land, clones scattered along headwater area of Turkey Creek 1000 meters SW from Steamboat Point near Hwy 14	Wyoming	Sheridan	3-Sep-17	1	3	2237
17_182	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, little evidence of browsing, trunk white, unforrowed, leaves nearly round	*Picea engelmannii, Ribes* sp, *Poaceae* spp	Wyoming State land, clones scattered along headwater area of Turkey Creek 1000 meters SW from Steamboat Point near Hwy 14	Wyoming	Sheridan	3-Sep-17	1.01	9	2237
17_183	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, little evidence of browsing, trunk white, unforrowed, leaves nearly round	*Picea engelmannii, Ribes* sp, *Poaceae* spp	Wyoming State land, clones scattered along headwater area of Turkey Creek 1000 meters SW from Steamboat Point near Hwy 14	Wyoming	Sheridan	3-Sep-17	0.73	9	2216
17_184	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Picea engelmannii, Poaceae spp, Artemisia* spp., *Achillea millefolium, Juniperus communis*	Bighorn National Forest, numerous large clones associated with headwater springs and seeps of Granite Creek, 1300 NW from Antelope Butte Ski Area off Hwy 14	Wyoming	Bighorn	3-Sep-17	0.955	10	2634

* Samples from disjunct stands.

Table A1 (continued). Physical description, locality, site information, and associated plant species of collected aspen specimens.

LT Collection No	Collectors	Family	Genus	specific epithet	authority	specimen description	main associated species	site/stand description	State	County	date collected	DBH (meters)	height (estimate; meters)	elevation (meters)
17_185	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Picea engelmannii, Poaceae spp, Artemisia* spp., *Achillea millefolium, Juniperus communis*	Bighorn National Forest, numerous large clones associated with headwater springs and seeps of Granite Creek, 1300 NW from Antelope Butte Ski Area off Hwy 14	Wyoming	Bighorn	3-Sep-17	0.44	5	2656
17_186	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Picea engelmannii, Poaceae spp, Artemisia* spp., *Achillea millefolium, Juniperus communis*	Bighorn National Forest, numerous large clones associated with headwater springs and seeps of Granite Creek, 1300 NW from Antelope Butte Ski Area off Hwy 14	Wyoming	Bighorn	3-Sep-17	0.36	3	2640
17_187	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Picea engelmannii, Poaceae* spp, *Artemisia* spp., *Achillea millefolium, Juniperus communis*	Bighorn National Forest, numerous large clones associated with headwater springs and seeps of Granite Creek, 1300 NW from Antelope Butte Ski Area off Hwy 14	Wyoming	Bighorn	3-Sep-17	0.91	9	2632
17_188	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	young tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Picea engelmannii, Pinus ponderosa, Quercus gambelii, Lonicera involucrata, Bromus* sp.	San Isabel National Forest, several scattered clones near the intersection of CR 360 and FR 442	Colorado	Huerfano	4-Sep-17	0.16	3	2569
17_189	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	young tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Picea engelmannii, Pinus ponderosa, Quercus gambelii, Lonicera involucrata, Bromus* sp.	San Isabel National Forest, several scattered clones along Wahatoya Creek riparian zone, 250 meteres upstream from the southern edge of Camp Wahatoya	Colorado	Huerfano	4-Sep-17	0.25	3	2644
17_190	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Picea engelmannii, Pinus ponderosa, Quercus gambelii, Lonicera involucrata, Bromus* sp.	San Isabel National Forest, several scattered clones near the intersection of CR 360 and FR 442	Colorado	Huerfano	4-Sep-17	0.54	6	2567
17_191	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Pinus ponderosa, Quercus gambelii, Juniperus communis*	San Isabel National Forest, vast aspen forests throughout the area on the East slopes of the Sangre de Cristo mtns, collection made at Spring Creek TH, 50 meters west of the Cuchara water filtration plant	Colorado	Huerfano	4-Sep-17	0.65	8	2622
17_192	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Pinus ponderosa, Quercus gambelii, Juniperus communis*	San Isabel National Forest, vast aspen forests throughout the area on the East slopes of the Sangre de Cristo mtns, collection made 10 meters west from the edge of Co Hwy 12	Colorado	Huerfano	4-Sep-17	0.82	10	2783
17_193	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	very large mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Pinus ponderosa, Quercus gambelii, Juniperus communis, Picea engelmannii*	San Isabel National Forest, vast aspen forests throughout the area on the East slopes of the Sangre de Cristo mtns, collection made on first harpin corner on FR 422 below Blue Lakes	Colorado	Huerfano	4-Sep-17	1.36	12	3145
17_194	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Pinus ponderosa, Quercus gambelii, Juniperus communis, Picea engelmannii*	San Isabel National Forest, vast aspen forests throughout the area on the East slopes of the Sangre de Cristo mtns, collection made on east side of FR 422 1 km down road from Bear Lake	Colorado	Huerfano	4-Sep-17	n	n	3228
17_195	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, little evidence of browsing, trunk white, unforrowed, leaves large and nearly lanceolate, young stems reddish, cataphylls somewhat sticky	*Pinus ponderosa, Quercus gambelii, Juniperus communis, Picea engelmannii*	San Isabel National Forest, vast aspen forests throughout the area on the East slopes of the Sangre de Cristo mtns, collection made at pinic area adjacent to confluence of Texas and Cucharas Creeks off FR 422	Colorado	Huerfano	4-Sep-17	0.79	8	2878

* Samples from disjunct stands.

Table A1 (continued). Physical description, locality, site information, and associated plant species of collected aspen specimens.

LT Collection No	Collectors	Family	Genus	specific epithet	authority	specimen description	main associated species	site/stand description	State	County	date collected	DBH (meters)	height (estimate; meters)	elevation (meters)
17_196	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, little evidence of browsing, trunk white, unforrowed, leaves nearly round	*Pinus ponderosa, Quercus gambelii, Juniperus communis, Picea engelmannii*	San Isabel National Forest, vast aspen forests throughout the area on the East slopes of the Sangre de Cristo mtns, collection made 600 meters north of Cuchara pass at parking area east of CO Hwy 12	Colorado	Huerfano	4-Sep-17	0.785	8	3015
17_197	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Pinus ponderosa, Quercus gambelii, Juniperus communis, Picea engelmannii*	North Lake State Willife Area, vast aspen forests throughout the area on the East slopes of the Sangre de Cristo mtns, collection made on Northwest corner of North Lake 8 meters west of CO Hwy 12	Colorado	Las Animas	4-Sep-17	0.36	4	2629
17_198*	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Pinus ponderosa, Quercus gambelii, Juniperus scopulorum, Gutierrezia sarothrae, Cercocarpus montanus, Rhus trilobata, Vitis riparia*	JE Canyon Ranch, isolated aspen population in side canyon of Chacuaco Canyon 2 km east of CR 50 and 300 meters ENE from rock homestead, population located just beneath Dakota sandstone rimrock and associated with springs and seeps, desert scrub habitat dominant in area	Colorado	Las Animas	5-Sep-17	0.5	9	1619
17_199*	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Pinus ponderosa, Quercus gambelii, Juniperus scopulorum, Gutierrezia sarothrae, Cercocarpus montanus, Rhus trilobata, Vitis riparia*	JE Canyon Ranch, isolated aspen population in side canyon of Chacuaco Canyon 2 km east of CR 50 and 300 meters ENE from rock homestead, population located just beneath Dakota sandstone rimrock and associated with springs and seeps, desert scrub habitat dominant in area	Colorado	Las Animas	5-Sep-17	0.34	8	1618
17_200*	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Pinus ponderosa, Quercus gambelii, Juniperus scopulorum, Gutierrezia sarothrae, Cercocarpus montanus, Rhus trilobata, Vitis riparia*	JE Canyon Ranch, isolated aspen population in side canyon of Chacuaco Canyon 2 km east of CR 50 and 300 meters ENE from rock homestead, population located just beneath Dakota sandstone rimrock and associated with springs and seeps, desert scrub habitat dominant in area	Colorado	Las Animas	5-Sep-17	0.865	10	1611
17_201*	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Pinus ponderosa, Quercus gambelii, Juniperus scopulorum, Gutierrezia sarothrae, Cercocarpus montanus, Rhus trilobata, Vitis riparia*	JE Canyon Ranch, isolated aspen population in side canyon of Chacuaco Canyon 2 km east of CR 50 and 300 meters ENE from rock homestead, population located just beneath Dakota sandstone rimrock and associated with springs and seeps, desert scrub habitat dominant in area	Colorado	Las Animas	5-Sep-17	0.65	10	1615
17_202*	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	young tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Pinus ponderosa, Quercus gambelii, Juniperus scopulorum, Gutierrezia sarothrae, Cercocarpus montanus, Rhus trilobata, Vitis riparia*	JE Canyon Ranch, isolated aspen population in side canyon of Chacuaco Canyon 2 km east of CR 50 and 300 meters ENE from rock homestead, population located just beneath Dakota sandstone rimrock and associated with springs and seeps, desert scrub habitat dominant in area	Colorado	Las Animas	5-Sep-17	0.15	4	1578
17_203	Luke R Tembrock	Salicaceae	*Populus*	*deltoides*	Marshall	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Pinus ponderosa, Quercus gambelii, Juniperus scopulorum, Gutierrezia sarothrae, Cercocarpus montanus, Rhus trilobata, Vitis riparia*	JE Canyon Ranch, isolated aspen population in side canyon of Chacuaco Canyon 2 km east of CR 50 and 300 meters ENE from rock homestead, population located just beneath Dakota sandstone rimrock and associated with springs and seeps, desert scrub habitat dominant in area	Colorado	Las Animas	5-Sep-17	0.49	8	1567
17_204*	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Pinus ponderosa, Quercus gambelii, Juniperus scopulorum, Gutierrezia sarothrae, Cercocarpus montanus, Rhus trilobata, Vitis riparia*	JE Canyon Ranch, isolated aspen population in side canyon of Chacuaco Canyon 2 km east of CR 50 and 300 meters ENE from rock homestead, population located just beneath Dakota sandstone rimrock and associated with springs and seeps, desert scrub habitat dominant in area	Colorado	Las Animas	5-Sep-17	0.645	8	1594
17_205	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Pinus ponderosa, Quercus gambelii, Juniperus communis*	CO Hwy 12 ROW, vast aspen forests throughout the area on the East slopes of the Sangre de Cristo mtns, collection made in borrow ditch west of CO Hwy 12, ~5.5 km north of Stonewall Gap	Colorado	Las Animas	6-Sep-17	1.05	8	2616

* Samples from disjunct stands.

Table A1 (continued). Physical description, locality, site information, and associated plant species of collected aspen specimens.

LT Collection No	Collectors	Family	Genus	specific epithet	authority	specimen description	main associated species	site/stand description	State	County	date collected	DBH (meters)	height (estimate; meters)	elevation (meters)
17_206	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Pinus ponderosa, Quercus gambelii, Juniperus communis*	San Isabel National Forest, vast aspen forests throughout the area on the East slopes of the Sangre de Cristo mtns, forests heavily impact by SAD with large areas of die back in this part of the Purgatoire drainage, collection made at north end of Purgatoire campground on west side of parking lot for TH of trail 1309	Colorado	Las Animas	6-Sep-17	0.595	8	2988
17_207	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Pinus ponderosa, Quercus gambelii, Juniperus communis*	North Lake State Willife Area, vast aspen forests throughout the area on the East slopes of the Sangre de Cristo mtns, collection made 22 meters south of FR 34 along the Purgatoire River	Colorado	Las Animas	6-Sep-17	0.44	5	2745
17_208	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Picea engelmannii, Pinus contorta, Vaccinium* sp.	FR 46 ROW, 1 km east of Cordova Pass at edge of road fill	Colorado	Las Animas	6-Sep-17	0.74	8	3379
17_209	Luke R Tembrock	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree, some evidence of browsing, trunk white, unforrowed, leaves nearly round	*Pinus ponderosa, Quercus gambelii, Salix* spp.	FR 46 ROW at confluence of Apishapa River and Herlick Creek in riparian corridor, single tree growing above borrow ditch north of road	Colorado	Las Animas	6-Sep-17	0.82	8	2649
BBNP_2*	Raymond Skiles	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree	*Arbutus xalapensis, Pericome caudata, Pinus cembroides, Quercus* ssp.	West side of Mt. Emory growing at base of talus slope	Texas	Brewster	2016	–	–	2138
BBNP_3*	Raymond Skiles	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree	*Arbutus xalapensis, Pericome caudata, Pinus cembroides, Quercus* ssp.	West side of Mt. Emory growing at base of talus slope	Texas	Brewster	2016	–	–	2149
BBNP_5*	Raymond Skiles	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree	*Arbutus xalapensis, Pericome caudata, Pinus cembroides, Quercus* ssp.	West side of Mt. Emory growing at base of talus slope	Texas	Brewster	2016	–	–	2160
BBNP_6*	Raymond Skiles	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree	*Arbutus xalapensis, Pericome caudata, Pinus cembroides, Quercus* ssp.	West side of Mt. Emory growing at base of talus slope	Texas	Brewster	2016	–	–	2186
BBNP_9*	Raymond Skiles	Salicaceae	*Populus*	*tremuloides*	Michx.	mature tree	*Arbutus xalapensis, Pericome caudata, Pinus cembroides, Quercus* ssp.	West side of Mt. Emory growing at base of talus slope	Texas	Brewster	2016	–	–	2189

* Samples from disjunct stands.

Table A2. Molecular data of collected aspen specimens.

LT Collection No	latitude	longitude	date extracted	ng/ul	260/280	LG65[A]		P 10		P 14[A]		P 33[A]		P 71		P 76[A]		P86		W 14		W 15		W 16		W 17		W 20	
17_110	45.21009	-108.16977	21-Nov-17	21.379	1.829	384	384	158	162	192	195	199	203	88	88	149	161	–	–	204	204	196	196	–	–	140	140	228	228
17_111	45.20973	-108.17122	21-Nov-17	28.009	1.952	370	384	158	162	192	192	201	203	88	88	–	–	–	–	204	204	199	199	–	–	140	149	228	228
17_112	45.21041	-108.16859	14-Nov-17	22.207	1.9	384	384	158	162	192	195	199	203	88	88	147	147	–	–	204	204	196	196	–	–	140	140	228	228
17_113	45.21063	-108.16742	14-Nov-17	30.792	1.962	370	384	158	162	192	192	201	203	88	88	–	–	–	–	204	204	199	199	–	–	140	149	228	228
17_114[B]	45.20906	-108.16962	14-Nov-17	21.221	1.884	349	349	148	160	195	195	187	191	88	88	157	165	177	221	213	243[C]	199	199	191	191	140	143	212	230
17_115[B]	45.2091	-108.17	14-Nov-17	31.469	1.915	349	349	148	160	195	195	187	191	88	88	157	165	177	221	213	243[C]	199	199	191	191	140	143	212	230
17_116[B]	45.20907	-108.17054	14-Nov-17	11.184	2.238	349	349	148	160	195	195	187	191	88	88	157	165	177	221	213	243[C]	199	199	191	191	140	143	212	230
17_117[B]	45.20908	-108.17084	14-Nov-17	13.936	2	349	349	148	160	195	195	187	191	88	88	157	165	177	221	213	243[C]	199	199	191	191	140	143	212	230
17_118[B]	45.209	-108.17103	14-Nov-17	25.879	2.008	349	349	148	160	195	195	187	191	88	88	157	165	177	221	213	243[C]	199	199	191	191	140	143	212	230
17_119[B]	45.20901	-108.17116	14-Nov-17	20.789	1.893	349	349	148	160	195	195	187	189	88	88	157	165	177	221	213	243[C]	199	199	191	191	140	143	212	230
17_120[B]	45.20913	-108.17158	14-Nov-17	43.645	1.922	349	349	148	160	195	195	187	189	88	88	157	165	177	221	213	243[C]	199	199	191	191	140	143	212	230
17_121[B]	45.20967	-108.17215	14-Nov-17	40.847	1.934	349	349	148	160	195	195	187	191	88	88	157	165	–	–	213	243[C]	199	199	191	191	140	143	212	230
17_122[B]	45.20954	-108.17221	31-Oct-17	21.211	1.91	349	349	148	160	195	195	187	191	88	88	157	165	177	221	213	243[C]	199	199	191	191	140	143	212	230
17_123[B]	45.20966	-108.17258	31-Oct-17	19.799	1.98	349	349	148	160	195	195	187	191	88	88	157	165	177	221	213	243[C]	199	199	191	191	140	143	212	230
17_124[B]	45.20985	-108.17321	14-Nov-17	30.054	1.949	349	349	148	160	195	195	187	191	88	88	157	165	177	221	213	243[C]	199	199	191	191	140	143	212	230
17_125[B]	45.13148	-108.28117	14-Nov-17	43.195	1.951	–	–	156	164	157	180	193	193	98	102	157	165	191	193	–	–	196	196	191	191	143	149	212	228
17_126[B]	45.131535	-108.281257	14-Nov-17	71.819	1.899	358	358	156	164	157	180	193	193	98	102	157	165	191	193	210	215	196	196	185	185	143	149	212	228
17_127[B]	45.131409	-108.281794	14-Nov-17	54.882	1.905	358	358	156	164	157	180	193	193	98	102	157	165	191	193	210	215	196	196	185	185	143	149	212	228
17_128[B]	45.131879	-108.281991	14-Nov-17	60.099	1.912	361	361	166	166	195	195	189	197	82	96	169	169	179	179	210	213	193	193	179	179	143	149	212	224
17_129[B]	45.132009	-108.281917	14-Nov-17	87.588	1.925	361	361	166	166	195	195	189	197	82	96	169	169	179	179	210	213	193	193	179	179	143	149	212	224
17_130[B]	45.13239	-108.281896	14-Nov-17	66.334	1.922	358	367	164	170	195	195	187	189	98	98	163	163	193	193	213	215	193	199	179	197	143	146	212	212
17_131	45.176818	-108.439736	14-Nov-17	64.133	1.931	358	358	148	158	195	195	187	187	96	98	157	169	193	193	221	221	196	196	191	191	134	143	218	218
17_132	45.176984	-108.440813	14-Nov-17	54.26	1.943	358	358	148	158	195	195	187	187	96	98	157	169	193	193	221	221	196	196	191	191	134	143	218	218
17_133	45.175932	-108.441184	14-Nov-17	66.377	1.939	349	349	160	166	186	195	189	195	86	102	151	151	179	193	213	221	196	199	185	191	143	143	218	218
17_134	45.176434	-108.440276	14-Nov-17	40.862	1.918	358	358	148	158	195	195	187	187	96	98	157	169	193	201	221	221	196	196	191	191	134	143	218	218
17_135	45.189056	-108.348705	14-Nov-17	56.51	1.901	349	349	148	152	157	195	179	189	90	90	151	151	183	193	207	213	193	196	185	185	143	143	218	228
17_136	45.18876	-108.347313	14-Nov-17	74.483	1.921	358	361	150	156	195	195	187	189	88	98	142	142	183	221	210	221	184	190	191	191	140	140	212	212
17_137	45.18811	-108.345474	14-Nov-17	97.496	1.922	379	379	150	166	195	195	189	189	90	94	142	151	183	221	207	213	184	187	185	185	143	143	212	228
17_138	45.187827	-108.342964	15-Nov-17	74.885	1.911	379	379	150	166	195	195	189	189	90	94	142	151	183	221	207	213	184	187	185	185	143	143	212	228
17_139	45.194546	-108.425779	15-Nov-17	64.618	1.91	352	352	164	170	195	195	181	189	86	90	159	159	179	179	210	213	190	190	179	191	134	143	212	218
17_140	45.195132	-108.425676	15-Nov-17	64.372	1.929	352	352	164	170	195	195	181	189	86	90	159	159	179	179	210	213	190	190	179	191	134	143	212	218
17_141	45.194741	-108.425364	15-Nov-17	63.018	1.933	352	352	164	170	195	195	181	191	86	90	–	–	179	179	210	213	190	190	179	191	134	143	212	218
17_142	45.192988	-108.422345	15-Nov-17	74.161	1.874	352	352	154	164	195	195	179	185	90	96	151	151	179	193	210	213	187	193	185	185	143	143	224	228
17_143	45.19236	-108.423478	15-Nov-17	71.415	1.919	352	352	154	164	195	195	179	185	90	96	151	151	179	193	210	213	187	193	185	185	143	143	224	228
17_144	45.191717	-108.424303	15-Nov-17	75.545	1.964	352	355	138	138	186	195	191	197	94	102	142	151	193	195	213	213	184	184	179	185	152	171	212	212

[A] Loci used with other loci for triploid determination (also in red).

[B] Samples from disjunct stands (also highlighted in green).

[C] Rare alleles in stands from disjunct stands (also highlighted in green).

Table A2 (continued). Molecular data of collected aspen specimens.

LT Collection No	latitude	longitude	date extracted	ng/ul	260/ 280	LG65[A]		P 10		P 14[A]		P 33[A]		P 71		P 76[A]		P86		W 14		W 15		W 16		W 17		W 20	
17_145	45.191535	-108.425101	15-Nov-17	49.85	1.926	358	358	166	170	161	195	179	193	100	102	151	161	177	193	210	213	181	193	185	191	134	134	212	212
17_146	45.190827	-108.427927	15-Nov-17	86.713	1.931	352	352	142	164	195	195	187	187	98	100	157	157	193	233	210	221	187	190	185	191	143	143	212	218
17_147	45.187137	-108.43372	15-Nov-17	87.589	1.947	361	361	150	156	195	195	193	193	90	98	149	149	193	221	210	213	181	190	185	191	134	143	212	216
17_148	45.186938	-108.435112	15-Nov-17	65.329	1.908	361	361	158	166	195	195	179	187	102	102	157	157	179	193	210	232	193	196	179	179	140	143	212	216
17_149	45.187371	-108.437158	15-Nov-17	87.91	1.892	379	379	158	170	195	195	193	193	82	100	151	167	179	193	207	221	190	196	185	191	134	143	212	212
17_150	45.18749	-108.438261	15-Nov-17	70.119	1.93	361	361	150	160	195	200	187	197	98	98	157	165	179	193	210	213	202	202	185	191	140	143	212	218
17_151	45.188741	-108.437377	31-Nov-17	7	1.94	361	361	166	166	186	195	185	197	94	98	157	157	193	221	207	213	190	190	191	191	143	143	216	228
17_152	45.181662	-108.448723	31-Nov-17	8.1	2.28	349	349	150	150	161	195	187	189	86	98	151	151	193	193	213	213	190	190	173	185	143	143	212	212
17_153	45.181272	-108.448678	31-Nov-17	41.1	1.94	349	349	150	150	161	195	187	189	86	98	151	165	193	193	213	213	190	190	173	185	143	143	212	212
17_154	45.179635	-108.465538	31-Nov-17	5.5	2.24	–	–	158	162	183	192	201	201	86	88	153	153	–	–	–	–	196	199	–	–	140	140	228	228
17_155	45.178446	-108.463745	31-Nov-17	10.9	1.98	349	349	148	150	195	195	187	187	88	88	151	180	179	221	210	232	196	196	185	185	143	149	212	212
17_156	45.189853	-108.474712	31-Nov-17	5.5	2.01	–	–	166	170	195	195	179	193	98	102	157	157	193	221	213	221	193	196	185	191	140	140	212	212
17_157	45.191803	-108.473696	31-Nov-17	6.3	1.9	361	361	166	170	195	195	179	193	98	102	157	157	193	221	213	221	193	196	185	191	140	140	212	212
17_158	45.193576	-108.483266	31-Nov-17	6.3	1.96	352	352	152	152	195	195	187	197	110	116	163	169	179	193	224	232	196	196	185	185	140	143	212	212
17_159	45.202503	-108.514795	31-Nov-17	5.1	2.18	367	367	150	152	157	198	187	197	84	90	157	163	193	193	210	213	187	187	173	191	143	143	212	212
17_160	44.795634	-107.960122	31-Nov-17	5.9	1.94	370	370	152	152	195	195	187	195	90	90	169	169	185	185	195	221	181	193	185	185	140	146	218	222
17_161	44.795211	-107.957793	31-Nov-17	7.6	2.1	355	355	150	168	157	195	187	187	90	100	149	169	193	193	213	221	181	184	185	191	134	140	218	218
17_162	44.730669	-107.559533	20-Nov-17	88.437	1.903	352	355	160	160	157	161	183	187	96	98	151	151	179	189	207	213	181	193	170	173	143	143	212	218
17_163	44.730531	-107.558992	20-Nov-17	50.792	1.914	352	355	160	160	157	161	183	187	96	98	151	151	179	189	207	213	181	193	170	173	143	143	212	218
17_164	44.730758	-107.557826	20-Nov-17	58.859	1.909	352	355	160	160	157	161	183	187	96	98	151	151	179	189	207	213	181	193	170	173	143	143	212	218
17_165	44.881191	-107.907224	20-Nov-17	44.456	1.935	355	355	150	160	195	195	187	187	90	96	157	163	183	183	213	221	184	184	173	185	134	143	212	216
17_166	44.880512	-107.908639	20-Nov-17	41.865	1.914	355	355	156	160	195	195	187	187	90	98	169	169	221	221	210	213	187	193	173	185	137	143	216	216
17_167	44.879602	-107.909792	20-Nov-17	41.313	1.9	–	–	152	170	195	195	187	187	96	98	163	163	193	193	213	232	199	199	191	203	140	146	216	216
17_168	44.791861	-107.919229	20-Nov-17	50.869	1.936	355	355	156	162	161	189	183	187	86	98	142	149	179	193	207	213	196	196	191	197	134	134	212	212
17_169	44.793051	-107.91996	20-Nov-17	47.398	1.874	355	355	156	162	161	189	183	187	86	98	142	149	179	193	207	213	196	196	191	197	134	134	212	212
17_170	44.793528	-107.920756	20-Nov-17	60.644	1.82	355	355	156	162	161	189	183	187	86	98	142	149	179	193	207	213	196	196	191	197	134	134	212	212
17_171	44.792728	-107.922202	20-Nov-17	51.902	1.868	364	364	150	166	157	189	183	187	86	96	149	149	193	193	213	213	187	196	167	185	143	143	216	216
17_172	44.74666	-107.68447	20-Nov-17	64.685	1.832	361	361	160	168	195	195	187	189	94	98	163	163	193	193	213	213	184	184	173	185	143	146	212	218
17_173	44.745458	-107.68389	20-Nov-17	49.436	1.888	361	364	158	170	195	195	187	189	94	98	151	167	179	183	207	213	184	196	173	179	143	143	216	218
17_174	44.759066	-107.624236	20-Nov-17	62.71	1.869	352	352	144	158	195	195	187	189	90	98	151	163	185	193	213	213	190	190	179	185	134	149	212	224
17_175	44.761452	-107.622589	20-Nov-17	54.079	1.872	352	352	144	158	195	195	187	189	90	98	151	163	185	193	213	213	190	190	179	185	134	149	212	224
17_176	44.776527	-107.511555	20-Nov-17	49.824	1.826	355	361	152	166	195	195	183	187	102	104	155	163	189	195	210	221	187	196	179	185	143	143	212	212
17_177	44.775086	-107.510358	20-Nov-17	36.108	1.792	355	355	166	166	195	195	191	203	88	96	149	161	183	183	210	218	184	193	179	185	143	143	212	216
17_178	44.774373	-107.507222	20-Nov-17	40.896	1.892	352	355	160	166	195	195	187	191	88	98	149	163	183	221	210	210	193	196	185	185	134	134	212	216
17_179	44.823531	-107.334226	20-Nov-17	55.915	1.797	352	352	140	140	195	195	179	189	102	102	161	163	179	181	210	210	190	196	179	185	143	146	212	212

[A] Loci used with other loci for triploid determination (also in red).

[B] Samples from disjunct stands (also highlighted in green).

[C] Rare alleles in stands from disjunct stands (also highlighted in green).

Table A2 (continued). Molecular data of collected aspen specimens.

LT Collection No	latitude	longitude	date extracted	ng/ul	260/280	LG65[A]		P 10		P 14[A]		P 33[A]		P 71		P 76[A]		P86		W 14		W 15		W 16		W 17		W 20	
17_180	44.81452	-107.333506	20-Nov-17	28.221	1.942	370	370	158	158	186	192	205	215	88	96	159	159	–	–	235	235	196	202	–	–	146	146	224	228
17_181	44.798962	-107.366623	20-Nov-17	33.476	1.945	367	367	158	172	157	186	193	203	106	106	161	173	185	185	243	249	202	202	–	–	146	146	222	234
17_182	44.79894	-107.366666	20-Nov-17	50.345	1.923	355	355	150	158	195	195	183	199	90	92	163	163	185	193	204	213	196	196	191	191	140	149	212	212
17_183	44.801919	-107.36724	20-Nov-17	45.286	1.856	367	367	160	160	195	195	193	195	88	108	157	165	181	183	210	213	184	187	185	185	134	143	212	212
17_184	44.620621	-107.526944	20-Nov-17	61.084	1.925	352	361	160	160	195	195	187	193	88	100	163	184	183	193	207	213	193	196	185	197	134	149	204	212
17_185	44.621962	-107.525499	20-Nov-17	66.19	1.936	352	361	160	160	195	195	187	193	88	100	163	184	183	193	207	213	193	196	185	197	134	149	204	212
17_186	44.622562	-107.523369	21-Nov-17	14.24	1.959	352	361	160	160	195	195	187	193	88	100	163	184	183	193	207	213	193	196	185	197	134	149	204	212
17_187	44.624458	-107.520133	21-Nov-17	17.829	1.989	355	361	144	152	157	195	189	189	98	114	155	157	183	193	213	218	184	193	–	–	140	143	212	216
17_188	37.421939	-104.977658	21-Nov-17	28.863	1.94	355	355	154	156	154	195	185	195	88	90	163	165	193	193	210	232	193	196	179	185	143	143	212	216
17_189	37.411973	-104.963692	21-Nov-17	42.998	1.929	352	352	156	166	157	195	189	201	90	104	151	163	193	193	204	213	193	193	179	191	143	143	218	218
17_190	37.422068	-104.977074	21-Nov-17	15.027	1.925	352	352	152	160	195	195	183	183	96	102	151	163	195	197	213	213	184	184	179	191	140	149	212	228
17_191	37.371973	-105.105982	21-Nov-17	21.424	1.938	367	367	162	164	195	195	189	197	90	94	169	178	183	197	213	224	187	193	179	185	143	143	216	216
17_192	37.34031	-105.095909	21-Nov-17	40.792	1.856	361	364	158	162	157	195	187	201	86	98	151	163	193	193	210	210	184	184	179	185	140	143	216	216
17_193	37.319102	-105.134164	21-Nov-17	22.322	1.956	346	346	162	170	157	195	187	187	90	96	151	151	195	199	210	213	184	196	179	191	143	152	216	216
17_194	37.317223	-105.141159	21-Nov-17	18.182	1.916	352	361	160	160	195	195	185	187	90	114	163	163	193	195	224	232	193	196	179	185	143	143	216	230
17_195	37.329613	-105.106486	21-Nov-17	25.238	1.977	346	349	156	160	195	195	187	189	86	88	163	163	179	193	213	213	184	193	191	191	143	143	212	216
17_196	37.321205	-105.078716	21-Nov-17	16.802	2	349	349	150	166	195	195	187	189	86	90	157	182	193	209	213	213	196	196	179	191	140	143	212	216
17_197	37.248857	-105.045969	21-Nov-17	52.45	1.945	346	346	152	156	165	195	183	187	84	86	149	163	197	197	201	210	184	196	179	179	140	143	212	212
17_198[B]	37.35664	-103.697748	21-Nov-17	11.313	1.841	361	361	158	166	195	195	183	189	88	98	163	163	195	197	213	218	184	196	191	191	149	158	212	230
17_199[B]	37.357071	-103.698424	21-Nov-17	19.715	1.642	361	361	158	166	195	195	183	189	88	98	163	163	195	197	213	218	184	196	191	191	149	158	212	230
17_200[B]	37.357114	-103.698249	21-Nov-17	23.941	1.96	361	361	158	166	195	195	183	189	88	98	163	163	195	197	213	218	184	196	191	191	149	158	212	230
17_201[B]	37.35679	-103.697849	21-Nov-17	20.473	2.124	361	361	158	166	195	195	183	189	88	98	163	163	195	197	213	218	184	196	191	191	149	158	212	230
17_202[B]	37.357449	-103.697805	21-Nov-17	23.203	1.926	361	361	158	166	195	195	183	189	88	98	163	163	195	197	213	218	184	196	191	191	149	158	212	230
17_203	37.357698	-103.697807	31-Nov-17	9.6	2.06	–	–	158	158	192	192	211	213	–	–	–	–	–	–	237	237	196	196	–	–	152	152	216	222
17_204[B]	37.357401	-103.698208	21-Nov-17	17.358	1.966	361	361	158	166	195	195	183	189	88	98	163	163	195	197	213	218	184	196	191	191	149	158	212	230
17_205	37.195975	-105.045749	21-Nov-17	22.189	1.991	364	364	160	160	195	195	187	197	98	98	163	163	179	193	213	213	184	184	185	191	143	143	212	216
17_206	37.254845	-105.109752	21-Nov-17	18.443	1.909	364	364	154	158	195	195	183	189	90	94	151	163	195	221	213	213	196	196	185	185	143	143	204	212
17_207	37.239358	-105.073636	21-Nov-17	18	1.937	364	364	156	158	195	195	187	189	90	98	151	163	179	209	207	213	184	196	173	185	143	171	216	216
17_208	37.343665	-105.015855	21-Nov-17	18.119	1.887	352	352	150	150	157	157	183	183	98	100	163	163	193	197	213	224	193	199	179	209	143	143	212	212
17_209	37.327088	-104.9499	21-Nov-17	31.231	1.938	361	361	166	170	195	195	187	189	84	86	151	163	187	191	213	224	184	196	179	191	126	146	212	230
BBNP_2	29.2436117	-103.30766	–	–	–	–	–	144	162	–	–	–	–	100	100	–	–	181	193	213	227[C]	184	184	–	–	143	143	216	216
BBNP_3	29.243686	-103.307293	–	–	–	–	–	144	162	–	–	–	–	100	100	–	–	181	193	213	227[C]	184	184	–	–	143	143	216	216
BBNP_5	29.2438183	-103.307001	–	–	–	–	–	144	162	–	–	–	–	100	100	–	–	181	193	213	227[C]	184	184	–	–	143	143	216	216
BBNP_6	29.244213	-103.306836	–	–	–	–	–	144	162	–	–	–	–	100	100	–	–	181	193	213	227[C]	184	184	–	–	143	143	216	216
BBNP_9	29.244615	-103.307063	–	–	–	–	–	144	162	–	–	–	–	100	100	–	–	181	193	213	227[C]	184	184	–	–	143	143	216	216

[A] Loci used with other loci for triploid determination (also in red).

[B] Samples from disjunct stands (also highlighted in green).

[C] Rare alleles in stands from disjunct stands (also highlighted in green).

Appendix B. Distributions of Allele Frequencies

Figures B1 and B2 show distributions of allele frequencies. Tables B1 and B2 show the genotypes for disjunct stands at the respective loci.

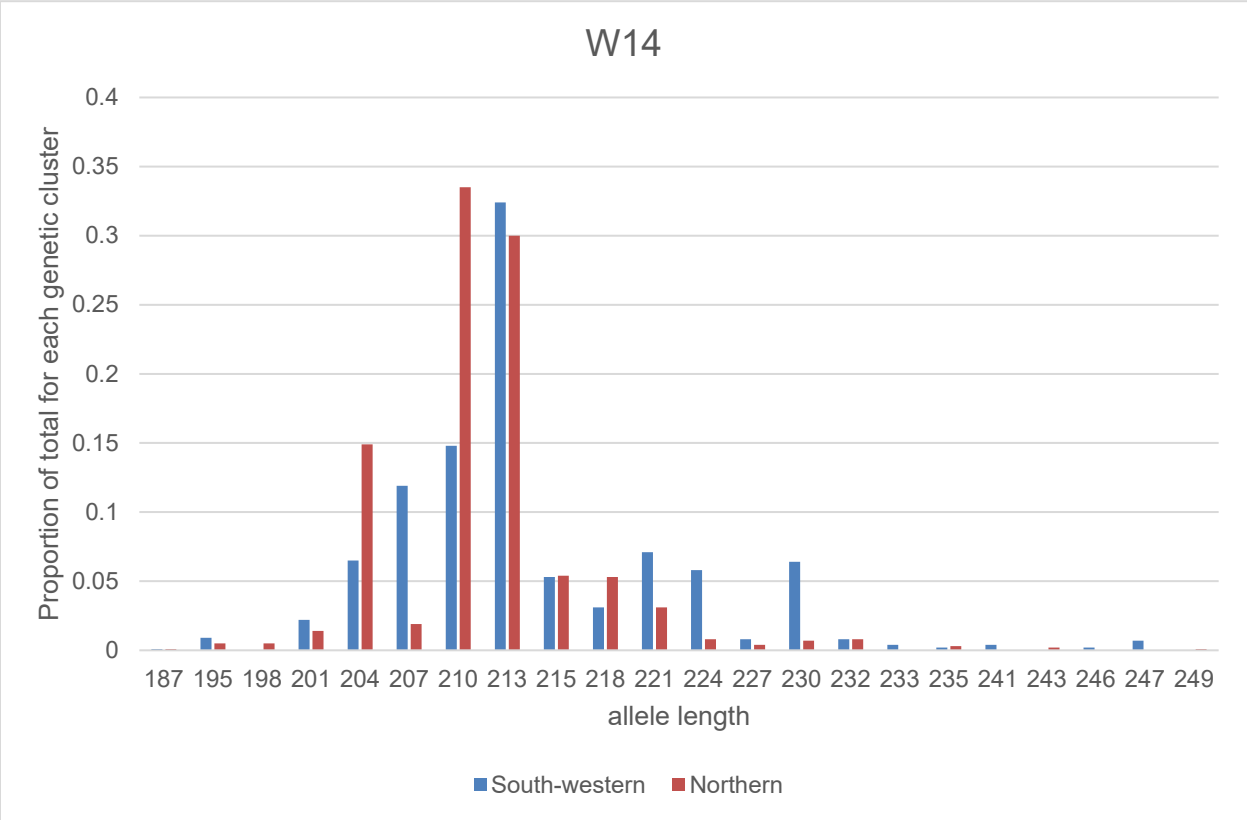

Figure B1. Distribution of allele frequencies for each genetic cluster at locus W14 for the entire combined dataset of 873 genotypes.

Table B1. Genotypes for disjunct stands at locus W14.

Stand	W14_1	W14_2
BICA Dryhead	213	243
JE Canyon	213	218
Big Bend	213	227

* Allele that occurs at less than 0.6% across the entire dataset for that locus (also highlighted in green).

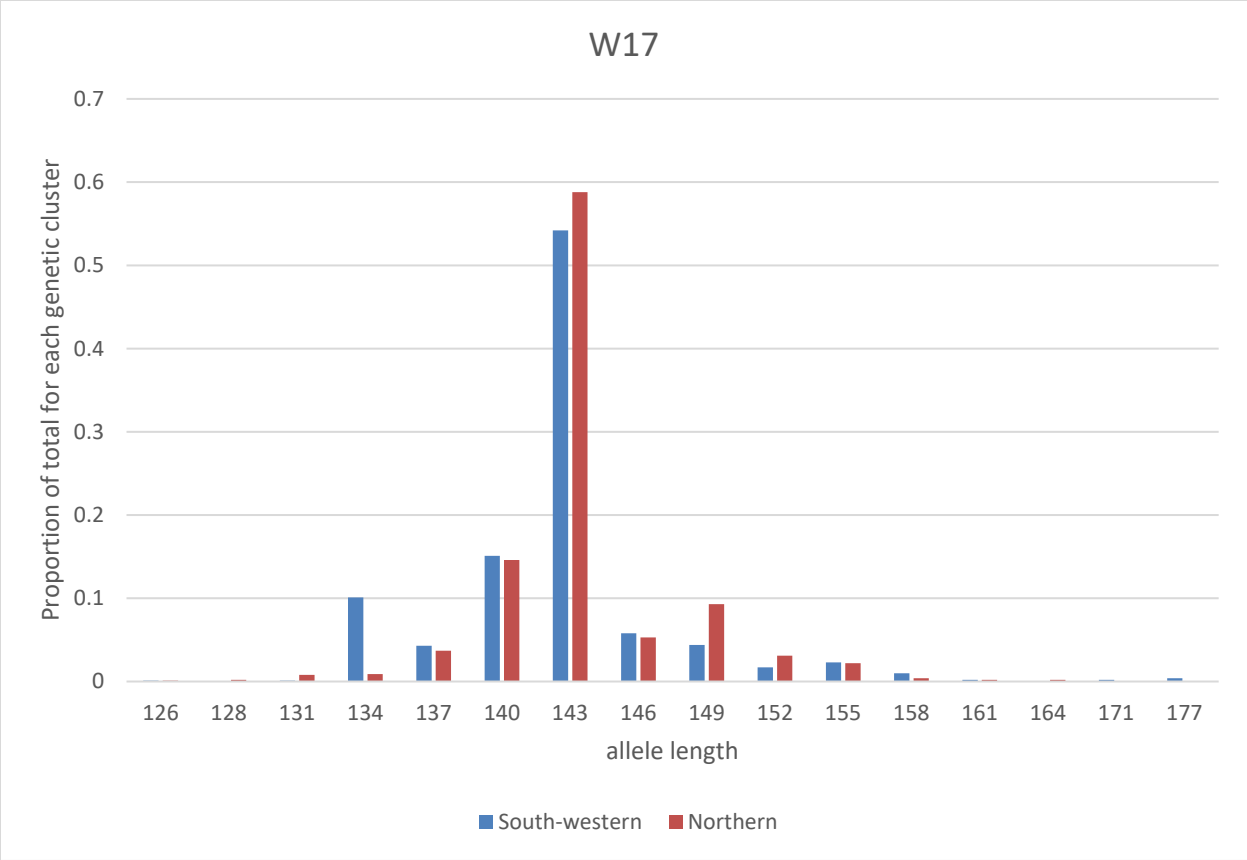

Figure B2. Distribution of allele frequencies for each genetic cluster at locus W17 for the entire combined dataset of 873 genotypes. Genotypes for disjunct stands at the respective loci included after graph.

Table B2. Genotypes for disjunct stands at locus W17.

Stand	**W17_1**	**W17_2**
BICA Dryhead	140	143
JE Canyon	149	158
Big Bend	143	143

* Allele that occurs at less than 0.6% across the entire dataset for that locus (also highlighted in green).

Appendix C. Geographic Distribution of Rare Alleles

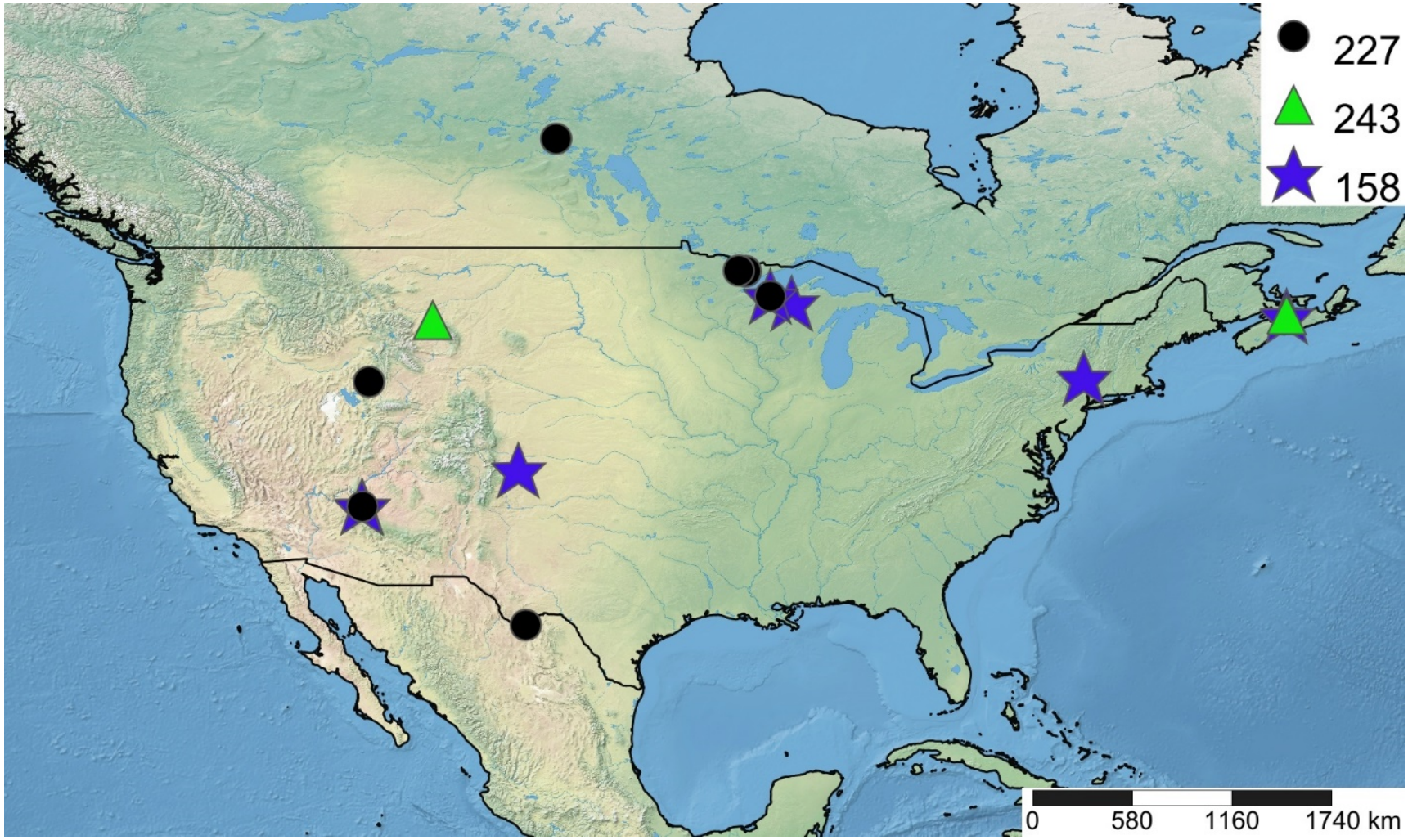

Figure C1. Geographic distribution of rare alleles found in each of three disjunct genotypes.

Appendix D. A Possible Triploid Hybrid

Figure D1. Sample 17181 possible triploid hybrid of *P.tremuloides* with other *Populus* species.

Figure D2. Sample 17181 possible triploid hybrid of *P.tremuloides* with other *Populus* species.

The Department of the Interior protects and manages the nation's natural resources and cultural heritage; provides scientific and other information about those resources; and honors its special responsibilities to American Indians, Alaska Natives, and affiliated Island Communities.

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