

Cytotype differences in radial increment provide novel insight into aspen reproductive ecology and stand dynamics

R. Justin DeRose, Karen E. Mock, and James N. Long

Abstract: High rates of triploidy have recently been described in quaking aspen (*Populus tremuloides* Michx.) of the Intermountain West, raising questions about the contributions of triploidy to stand persistence and dynamics. In this study, we investigated cytotypic differences between diploid and triploid aspen clones using dendrochronological techniques. We used tree-ring data collected from stems within an aspen stand near Fish Lake, Utah, to test for differences in stem age, population structure, growth, and response to climate. This stand contains the well-known Pando clone, which is purported to be the largest organism documented on earth. Our results show that triploid aspen stems grew more rapidly than diploids, and that this difference was most pronounced early in stand development. Growth response to climate varied little between triploids and diploids, where wide rings were associated with cool, moist years, and narrow rings were associated with above-average growing season temperatures. Stand development processes and inherent genetic differences are mechanisms possibly controlling the observed differences in aspen ring width between triploids and diploids. Regardless of the mechanism, the results have specific management implications. Conventional regeneration methods involving coppicing and the associated intermediate treatments will promote asexually reproducing triploids, leading to static or reduced genetic diversity. Enhanced genetic diversity will be favored by management actions that explicitly account for (i) the potential existence of multiple cytotypes within a stand and (ii) the observed differences in growth rates between diploid and triploid individuals.

Key words: dendroecology, genetic diversity, ploidy, *Populus tremuloides*, silviculture.

Résumé : Des taux élevés de triploïdie ont récemment été rapportés chez le peuplier faux-tremble (*Populus tremuloides* Michx.) dans la région intramontagneuse de l'Ouest. Cela soulève des questions au sujet de la contribution de la triploïdie à la dynamique et la persistance des peuplements. Dans cette étude, nous avons examiné les différences de cytotypic entre des clones diploïdes et triploïdes à l'aide de techniques dendrochronologiques. Nous avons utilisé des données dendrométriques collectées sur des tiges dans un peuplement de peuplier faux-tremble près de Fish Lake, dans l'Utah, pour déterminer s'il y a des différences dans l'âge de la tige, la structure de la population, la croissance et la réaction au climat. Ce peuplement contient le clone « Pando » considéré comme le plus vaste organisme documenté sur terre. Nos résultats montrent que la tige des peupliers triploïdes croît plus vite que celle des peupliers diploïdes et que cette différence est plus prononcée tôt dans le développement du peuplement. La réaction en croissance au climat variait peu entre les triploïdes et les diploïdes; les cernes larges étaient associés aux années fraîches et humides et les cernes étroits à des températures au-dessus de la moyenne pendant la saison de croissance. Les processus de développement du peuplement et les différences génétiques inhérentes sont probablement les mécanismes responsables des différences dans la largeur des cernes observées entre triploïdes et diploïdes. Peu importe le mécanisme, les résultats ont des implications spécifiques en aménagement. Les méthodes conventionnelles de régénération impliquant le recépage et les traitements intermédiaires associés vont favoriser les triploïdes qui se reproduisent asexuellement et la diversité génétique cessera d'évoluer ou diminuera. L'augmentation de la diversité génétique sera favorisée par des pratiques d'aménagement qui tiennent explicitement compte : (i) de la possibilité qu'il y ait de multiples cytotypes dans un peuplement et (ii) des différences de taux de croissance observées entre individus diploïdes et triploïdes. [Traduit par la Rédaction]

Mots-clés : dendroécologie, diversité génétique, ploïdie, *Populus tremuloides*, silviculture.

Introduction

The Pando quaking aspen (*Populus tremuloides* Michx.) clone in central Utah, USA, is putatively the largest living organism ever described (Mitton and Grant 1996; Kemperman and Barnes 1976). In the absence of detailed morphological or genetic data, a large homogeneous aspen stand such as the one containing Pando would traditionally have been characterized as an even-aged, single-genotype stand. However, an emerging body of literature is challenging earlier conceptions of aspen ecology and establishing that quaking aspen stand and clonal dynamics are much more complicated than once thought (reviewed in Long and Mock

(2012)). This new insight includes, for example, the documentation of substantial within-stand variability in size structures (Rogers et al. 2009), age structures (Rogers et al. 2009; Binkley 2008), genotypes (DeWoody et al. 2008; Mock et al. 2008), clone size (DeWoody et al. 2008), and ploidy, or cytotypes. The occurrence of triploid aspen has long been documented (Every and Wiens 1971; Einspahr et al. 1963; Van Buijtenen et al. 1957), but until recently, the high frequency of triploids and their contributions to stand and landscape diversity has been underappreciated (Mock et al. 2012). Using the stand containing Pando as an example, we investigate the role of triploidy in aspen regeneration ecology and stand dynamics and illustrate how an improved un-

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R.J. DeRose. Forest Inventory and Analysis, USDA Forest Service, Rocky Mountain Research Station, 507 25th Street, Ogden, UT 84401, USA.

K.E. Mock and J.N. Long. Department of Wildland Resources and Ecology Center, Utah State University, 5230 Old Main Hill, Logan, UT 84322, USA.

Corresponding author: R. Justin DeRose (e-mail: rjderose@fs.fed.us).

derstanding of triploidy fundamentally affects interpretations of aspen ecology and management.

Populus spp., including aspen, are generally diploid, although triploid forms have been known to arise through spontaneous production of unreduced gametes within a species or through hybridization (Ramsey and Schemske 1998; Harlan and DeWet 1975). Because triploids generally have greatly reduced fertility, they are primarily limited to vegetative reproduction, whereas diploids may undergo sexual and (or) asexual reproduction. Triploids are expected to differ from diploids with respect to both physiology and structure, based on findings in other plant species. It has been observed in aspen that large stems can be triploid (Einspahr et al. 1963) and that large clones are often triploid (Mock et al. 2008, 2012). Although vegetative sprouting is the dominant regeneration mechanism in the western North American aspen clones (DeByle and Winokur 1985), diploid aspen is capable of naturally reproducing sexually (via seed), as has been documented following fires (Fairweather et al. 2014; Kay 1997; Turner and Romme 1994). It has also been known for decades that aspen can be reliably propagated from seed (Campbell 1984). Because seedling reproduction is thought to be relatively uncommon and episodic in western landscapes, the management of aspen has focused primarily on vegetative reproduction.

Aspen stands often exhibit abundant reproduction, rapid growth, and early self-thinning following disturbance, resulting in a simple, even-aged population structure. Based on these homogeneous stand conditions, foresters have traditionally assumed that each stem in the stand was genetically similar and the result of vegetative reproduction (DeByle and Winokur 1985). However, using contemporary genetic tools, western aspen stands have been shown to contain many different clones, often including both diploid and triploid genotypes (Mock et al. 2008). Given the different possible regeneration mechanisms, it is possible that variation between aspen cytotypes could result in different regeneration strategies, growth rates, and population structures. That some aspen stems growing on the same site are potentially of recent seed origin, exhibit different growth rates, or were not the result of a stand-replacing disturbance represents a departure from conventional views of aspen ecology. If evidence for these differences were found, it would precipitate a fundamental shift in our approach to aspen management from the current simple-structure paradigm to one that accepts a more complex population structure. Recent findings of high clonal diversity and high rates of triploidy in western landscapes point to a need for further exploration into the possible effects of cytotype differences on aspen stand dynamics.

We paired dendrochronological methods with genetic data to investigate potential differences between diploid and triploid aspen stems across multiple clones within the “Pando stand” (Pando is the most common genotype previously identified in the stand) near Fish Lake, Utah, USA. Our study goals were to compare diploid and triploid stems with respect to the following: (i) regeneration ecology by examining stem ages, (ii) population structure by assessing relative and absolute densities and stem sizes, (iii) stem growth rates by examining chronologies of annual ring widths, and (iv) the influence of climate on radial growth. We tested the null hypotheses that diploid and triploid stems would be similar with respect to age, population structure, growth rates, and growth responses to climate.

Study area

The Pando stand is situated on the Fish Lake Plateau, within the Fish Lake graben, in the Fishlake National Forest, central Utah (38°31'N, 111°45'W). Elevation within the stand varies little, ranging from 2700 m to 2790 m. The high-elevation Fish Lake Plateau is situated near the western edge of the Colorado Plateau and is characterized by dominant Pacific-driven winter precipitation,

primarily in the form of snow, and a minor component of summer precipitation delivered by the North American monsoon system (Mock 1996). Mean annual precipitation varied from 422 mm to 1230 mm over the period 1895–2007. Mean monthly growing season (May–September) temperature varied from 15.3 to 20.3 °C over the period 1895–2007 (Western Regional Climate Centre (WRCC), www.wrcc.dri.edu/). The Pando clone, which comprises 75% of the Pando stand by area, has been described as the largest organism on the earth because it covers ~43 ha (Kemperman and Barnes 1976) and has been estimated to weigh over 6 million kg (Grant et al. 1992). It has also been suggested that the clone must be from several thousand years old to perhaps 1 million years old (Mitton and Grant 1996) to have achieved its current size (Kemperman and Barnes 1976). The Pando site was not glaciated during the last glacial maximum ~21.1 ka (Marchetti et al. 2011). Well-drained soils were relatively uniform across the site, and the entire stand supports an understory of common juniper (*Juniperus communis* L.).

Methods

A previous study designed to explore genetic diversity in Pando and adjacent stands employed a 50 m² sampling grid from which leaf or stem tissue was collected to determine genotype using a panel of microsatellite loci and cytotype and both flow cytometry (based on nuclear staining density) and allelic variation at microsatellite loci (three alleles at one or more loci) (DeWoody et al. 2008; Mock et al. 2008). The presence of large clones in the Pando stand and the 50 m² grid sampling design provided a high level of replication for determination of clonal genotype and cytotype.

On the same grid used by Mock et al. (2008), we randomly relocated 40 previously tagged ramets, which resulted in a total of 37 usable sample trees, $n = 16$ diploid stems (representing 13 genotypes) and $n = 21$ triploid stems (representing four genotypes). Three trees were too rotten for tree-ring analysis. Our sample was designed with ramets as the unit of replication within cytotype groups. Although the absolute number of genotypes represented differed among cytotypes, our sample was broadly representative of the landscape in terms of the overall proportion of stems of each cytotype and their spatial representation. Further replication within genotypes in the Pando stand was not possible for diploids or triploids due to the relatively high number of genotypes represented by only one or two stems on the Mock et al. (2008) sampling grid; therefore, all analyses focused on cytotype-specific differences. All sampled stems were revisited during the spring to determine the gender of each sample tree. By observing catkins, we determined that virtually all of the triploid stems were male (two stems were dead), and all but two diploid stems were male. We carefully scrutinized ring-width variability for the two female diploid stems and determined that they were indistinguishable from the other diploids. This suggests that gender differences were not an issue in this study.

One increment core was extracted from breast height (1.3 m) for each sample tree and was taken as carefully as possible so as to intersect the center of the tree. The Pando stand occurs on relatively gentle slopes, and the aspen in the stand exhibited strong circuit uniformity so that one core per stem was adequate to characterize annual variation in ring-width increment. A sampling point was designated 1 m north of each sample tree, where a variable radius plot (3 m²·ha⁻¹ basal area factor) was established. For each tree determined to be in the plot, with the exception of the sampling tree to avoid nonindependence, the diameter at breast height (DBH) was measured and the species and status (live or dead) were noted.

Chronology development

Increment cores were mounted in grooved boards with the transverse section accentuated. Mounted samples were sanded with progressively finer sand paper until ring-width boundaries

Table 1. Mean (standard deviation; SD), minimum values, and maximum values for diploid and triploid stem attributes (diameter and age) and plot attributes (trees per hectare and basal area) within the Pando stand.

	Diploid			Triploid		
	Mean (SD)	Minimum	Maximum	Mean (SD)	Minimum	Maximum
Stem attributes						
Diameter (cm)	17.7 (4.35)	12.3	26.8	26.1 (6.08)	12.9	37.5
Age (years)	106 (26)	64	151	111 (19)	82	140
Plot attributes						
Trees per hectare	2222 (1485)	125	5065	1442 (1111)	76	4869
Basal area (m ²)	20.1 (9.2)	6	39	23.4 (10.2)	3	42

Note: A Wilcoxon's rank-sum test was run for each attribute and the values are as follows: diameter, $W = 44$ and $p < 0.001$; age, $W = 135$ and $p < 0.426$; trees per hectare, $W = 210$ and $p < 0.111$; basal area, $W = 122.5$ and $p < 0.229$.

were clearly visible under a microscope. Tree-ring series were initially crossdated using the marker year approach (Yamaguchi 1991). The data were then digitized with a microscope and stage to 1 μm accuracy, relying heavily on the “shadow technique” (DeRose and Gardner 2010) to identify annual ring boundaries. Crossdating was verified at the $P < 0.01$ significance level using a 50-year window, overlapped by 25 years, in the program COFECHA (Holmes 1983). Independent chronologies were developed for the cores from triploid and diploid trees and were checked against unpublished regional chronologies (R.J. DeRose, unpublished data). We used negative exponential curves to remove the geometric growth pattern before they were averaged into a standardized tree-ring index. We used a standard index that does not include the pooled variance (variance shared by each tree-ring series). For increment cores that did not intersect the pith ($n = 16$), estimates of the number of additional rings to the center were made using a geometric model (Duncan 1989) and counts of less than five rings were considered appropriate for analysis. We interpreted age as age at breast height.

Climate data

Climate data for the Fish Lake Plateau were downloaded from the WRCC, central Utah Climate Division, for the period 1895–2006. Reconstructed monthly Palmer drought severity indexes (PDSI) (www.cgd.ucar.edu/cas/catalog/climind/pdsi.html) covered the period 1888–2004. We selected mean monthly precipitation, maximum monthly temperature, and monthly PDSI as possible limiting climatic factors for our diploid and triploid tree-ring chronologies.

Data analysis

To test whether stem age at breast height and stem diameters differed by cytotype, we compared sample means between diploids and triploids. Similarly, to test for differences by cytotype in competition and stand dynamics that potentially influence the sampled trees, basal area and trees per hectare of the plot surrounding the sample trees were compared using the Mann–Whitney U test. To test for differences in growth between diploids and triploids year by year, we compared raw ring widths using the Mann–Whitney U test. For each genotype, we also assessed possible variability in growth for each cytotype by aligning individual series by their date of origin (assumed to be breast height in this study), taking into account the estimated number of rings to the pith. Using the Mann–Whitney U test, growth differences by cytotype were tested for (i) the first 50 years, (ii) the first 100 years, and (iii) all years of growth. Calendar-year ring widths were converted to basal area increments to further test for differences in growth over the entire growth period (1875–2005) using ANOVA and to assess the effect of differential growth on the mean cumulative basal area increment.

The cytotype-specific responses to climate variables were assessed using the standard chronology in a bootstrapped correlation function analysis in the package bootRes in R (Zang and

Biondi 2013; Biondi and Waikul 2004). Bootstrapped significance was calculated at the 0.05 level. We also tested for possible temporal instability in the response of ring width to climate using a moving window analysis. Three monthly climate parameters, total precipitation, maximum temperature, and the PSDI, were assessed for their relationship to diploid and triploid chronologies during the current year and previous year. Differences between cytotypes were considered significant when bootstrapped 95% confidence intervals did not overlap, and then only if the month-to-month trend in sign was also different. All analyses were conducted in the R statistical computing environment (R Core Team 2012).

Results

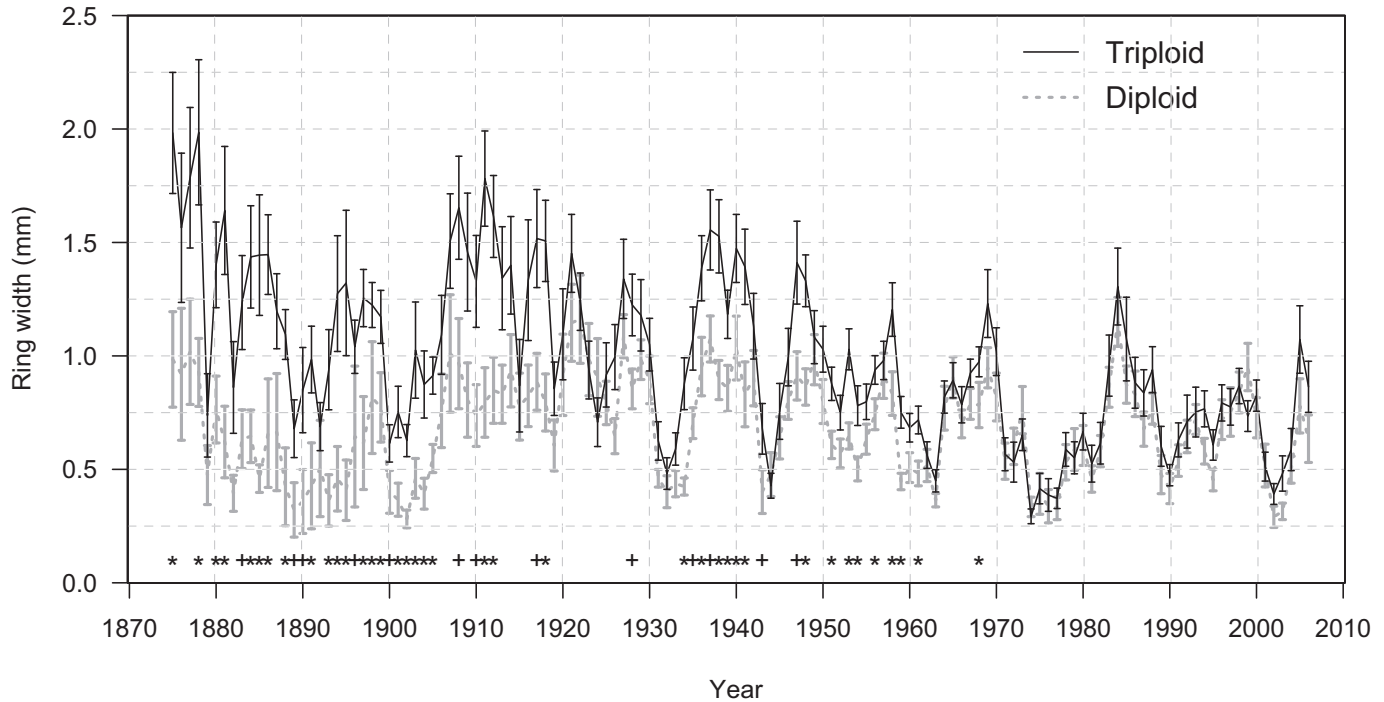
Mean stem breast-height ages were just over 100 years and were not significantly different between diploids and triploids ($P = 0.468$; Table 1). This result was not likely to be influenced by estimating the number of rings to the center for increment cores that did not reach the pith ($n = 16$ cores), where the range of the estimated number of missing rings to the pith was 1–4 (mean = 2), which represented 0.5%–5% of total tree age.

Metrics of population structure indicated that the plots around diploid and triploid stems were in similar stages of stand development. Relative density, measured as basal area, was not significantly different between plots surrounding diploid and triploid stems ($P = 0.257$; Table 1). Similarly, absolute density (stems·ha⁻¹) in plots around diploid stems was not significantly different than in plots around triploid stems ($P = 0.128$; Table 1). There was, however, a large difference in mean stem diameter, with diploid stems significantly smaller than triploid stems ($P < 0.001$; Table 1).

Diploids typically had substantially lower calendar-year growth than triploids (Fig. 1), which likely varied partially as a result of the variation in putative establishment dates (breast-height ages). Specifically, in 20 of the first 26 (77%) calendar years (1875–1900), mean ring width was significantly lower for diploids than for triploids ($P < 0.1$; Fig. 1). Frequency distributions for diploid and triploid ring widths over the first 50, 100, and all years combined were highly significant ($P < 0.001$) and portrayed the diminishment of larger radial growth by triploid stems over time (Fig. 2). Basal area increment varied considerable for both cytotypes, but an overall pattern of higher increment for triploids was clear (Fig. 3a) and highly significant over the entire study period ($P < 0.001$). Consequently, because the diploid stems were much smaller overall, their mean cumulative basal area by the year 2005 was less than half that of the triploids (Fig. 3b).

Increment cores crossdated well for both diploids and triploids and exhibited relatively strong interseries correlation coefficients (0.55–0.58) and moderate ring-width sensitivity (0.37), and COFECHA indicated no crossdating problems (Table 2). Chronology statistics were strikingly similar between diploid and triploid stems, with the exception of mean ring width, which was significantly different (Table 2). Strong crossdating verification

Fig. 1. Mean ring width (mm; ± 1 standard error) for diploid and triploid stems from 1875 to 2005 showing that triploid individuals grew more rapidly early in stand development. Mann-Whitney U tests for year-by-year differences in mean ring width are denoted by an asterisk (*) for significant differences at $P < 0.05$ and by a plus sign (+) for significant differences at $P < 0.1$. Triploid data are represented by the darker, solid line, and diploid data are represented by the lighter, broken line.



statistics indicated that one core per tree was adequate for describing individual-tree growth variability, and that all of the sampled aspen from this site exhibited a similar response, on average, to climatic conditions.

Diploid and triploid chronologies exhibited strikingly similar responses to total monthly precipitation, monthly drought, and maximum monthly temperature, and no statistically significant differences were found between cytotypes (Figs. 4a–4c). All responses to climate for both cytotypes were found to be temporally consistent over the period 1895–2007 (data not shown). Significant positive associations between ring width and previous cool season precipitation (December) and current growing season precipitation (June, July) were found for both diploids and triploids (Fig. 4a). Diploids had a significant positive response to the previous growing season October and cool season January precipitation. The triploid response was not significant but had the same sign (positive). A strong pattern between positive PDSI (non-drought years) and the tree-ring indices was found for cool season drought through the end of the current growing season for both cytotypes (Fig. 4b). During July, diploids and triploids both had significant negative relationships between temperature and annual tree-ring increment. In addition, diploids showed a significant negative response to growing season April and June temperatures.

Discussion

This study quantifies growth-rate differences between aspen ramets from two fundamentally different cytotypes (i.e., diploids and triploids) occurring on the same site. We failed to reject our first hypothesis that diploid and triploid stems were similar in age. We also failed to reject our second hypothesis that plots surrounding diploid and triploid stems were similar with respect to population structure. However, we have strong evidence for a large difference in mean stem diameter (greater for triploid stems than for diploid stems). The hypothesis that diploids and triploids

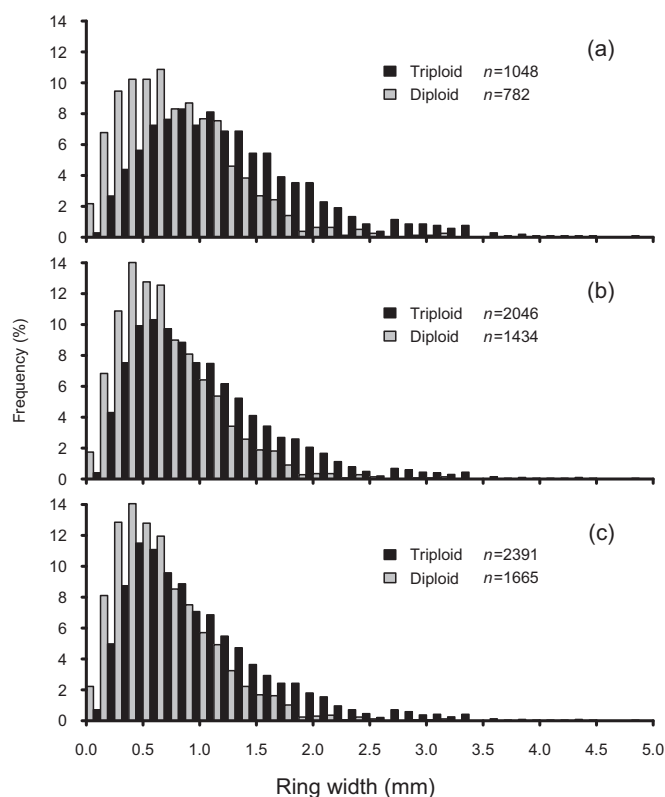
had similar growth rates can be rejected based on both calendar-year and time-since-origin tests. Specifically, growth rates differed profoundly early in stand development (greater in triploids). Finally, we failed to reject the hypotheses that diploids and triploids had similar growth responses to climate for precipitation, temperature, and drought.

Our findings indicate that diploid and triploid stems in the study area were generally similar with respect to age, population structure, and growth responses to climate but had important differences with respect to growth rates. Specifically, triploids exhibit substantially faster growth than diploids early in stand development, resulting in a significant size advantage. The cytotype-specific growth differences might provide an ecological explanation for recent observations showing differences between diploids and triploids with respect to their frequency and clone size on the landscape (Mock et al. 2008). For example, although there were many more diploid genets, their mean clone size and combined total area was substantially less than for triploid genets (Mock et al. 2008). Considering cytotype-specific growth differences in the context of the variation in disturbance frequency and severity of aspen stands yields intriguing hypotheses regarding the mechanism behind the observed range-wide variation in triploidy proportion (Mock et al. 2012).

Pando stand development

It is unlikely that during the establishment of the Pando stand in the late 1800s, initial stand density was lower for the triploids than for the diploids; therefore, differences in initial density are an unlikely explanation for the growth differences observed. Although the origin of ramets in the Pando stand is unclear, on average, diploid and triploid stems were of similar age, which suggests a common establishment event or period. Therefore, although some of the ramets might be of seedling origin, the vast majority were likely from suckers and, therefore, would have

Fig. 2. Frequency distribution by cytotype of ring widths for: (a) the first 50 years of growth, (b) the first 100 years of growth, and (c) all ring widths. Mann–Whitney U tests indicated highly significant ($P < 0.001$) cytotype differences between the first 50 years, the first 100 years, and all ring widths. n = number of ring widths by cytotype in the distribution. Frequency values sum to 100% by cytotype.



exhibited substantial initial stem density. This claim is indirectly supported by a regeneration harvest undertaken in the 1990s squarely in the center of the Pando stand, which resulted in greater than 10 000 stems·ha⁻¹ (Henningson 2012). The stems regenerated during the 1990s have been positively identified as belonging to the same genotype as the triploid Pando stems measured in this study (Mock et al. 2008).

The sampled diploid and triploid stems were growing on the same site, established during the same time period (with similar stem age and likely the same stand-initiating disturbance), and are currently at full site occupancy (similar basal areas in the surrounding plot). Therefore, the large difference in diameter between cytotypes is attributable to the large discrepancy in ring-width increment, which primarily occurred early in the stand development (i.e., the first ~25 years; Fig. 1), but was also accentuated again from ~1935 to 1960 (Fig. 1 and Fig. 3a), and secondarily from the cumulative basal area increment over the life of the trees (Fig. 3b). Therefore, although the differences in ring width later in stand development were generally diminished, on average, cumulative basal area increment of the triploids was more than double that of diploids. Interestingly, a similar pattern was found between live and dead aspen stems in Arizona, where live trees exhibited a higher lifetime growth than trees that had recently died (Ireland et al. 2014). Paired with our observation that diploids grew slower overall but particularly early in stand development, it is highly likely that identifying genetic information such as cytotype differences in large data sets such as reported by Ireland et al. (2014) could help determine possible predisposing factors (e.g., ploidy) for the recent widespread mortality in western aspen currently attributed to climate (Worrall et al. 2013).

Our observations of the Pando stand are consistent with previous knowledge of aspen stand development in the absence of information about relative proportion of ploidy. Postdisturbance aspen reproduction through suckering is associated with exceedingly high stem densities. In addition, aspen has very low shade tolerance, and aggressive self-thinning is initiated within a few years of stand initiation. With information about intrastand differences in ploidy, our results indicate that triploids have an early size advantage. In the course of stand development (i.e., in the first few decades following stand reinitiation), this means that relative proportions of cytotypes could shift dramatically in favor of triploids, and a loss of genetic diversity is possible if infertile triploids increasingly dominate the landscape.

Response to climate

Although other studies of aspen ring-width sensitivity to climatic factors have documented temperature responses similar to those on this study, none has controlled for cytotype (Hanna and Kulakowski 2012; Leonelli et al. 2008). Indeed, in northern Colorado, Hanna and Kulakowski (2012) noted that the growth of most aspen had a negative relationship to seasonal temperature and that aspen stems within a single stand had differential responses to various climatic factors (seasonal temperature and annual PDSI). It is likely that cytotype differences not accounted for by Hanna and Kulakowski (2012) may help to explain such within-stand differences. That we observed a negative relationship to temperatures over a considerable portion of the growing season for the diploids (from April to July, with May being nearly significant) but not the triploids was noteworthy given that they occupy the same site and experience virtually the same climate. These months represent a key growth period for aspen, suggesting that slightly reduced ring width in response to warmer temperatures could result in a cumulative reduction in stem diameter over time. Furthermore, if a particular cytotype is more likely to have reduced growth during drought or high temperature periods (Galvez et al. 2011), that might result in carbon being redirected to reserves earlier in the growing season, potentially resulting in a reduction in diameter growth.

A positive relationship between previous and current year precipitation and aspen growth has been noted in previous work (Leonelli et al. 2008). We suspect that the positive relationship with precipitation found in our study likely mirrors the strong relationship that we observed with the drought record (Fig. 4). Hogg et al. (2005, 2008) found that interior Canadian aspen growth and mortality were controlled largely by drought. Similarly, Leonelli et al. (2008) found that the previous year's drought limited aspen growth. In contrast, we found the current year's moist periods to be significantly positively associated with growth, and this was evident for both diploids and triploids. It is likely, in this droughty region of the Interior West, that the PDSI characterizes the moisture and temperature regimes responsible for driving long-term, year-to-year variation in aspen ring width for both diploid and triploid stems.

Inherent genotypic differences

Aspen frequently show pronounced phenotypic and chemical differences among genets (Stevens et al. 2012; Kanaga et al. 2008; Osier and Lindroth 2004). Collectively, the triploid stems in the current study belonged to four genotypes (clones), whereas the diploids stems belonged to 13 genotypes. Furthermore, the triploid clones examined in this study included the three largest clones in the stand (in terms of area). Although representative of the local landscape (i.e., Pando), it is possible that the triploid clones were not broadly representative of triploids in aspen, and observed cytotype differences might be due to extreme values for these particular triploid clones. To distinguish variability among genotypes within cytotypes requires replication within multiple genotypes, which was not available in the Pando stand based on

Fig. 3. (a) Mean basal area increment (mm^2 ; ± 1 standard error), and (b) comparison of cumulative basal area increment (cm^2), on average, for the diploid and triploid chronologies from 1875 to 2005. Lifetime basal area increment was significantly different between cytotypes (ANOVA; $F = 122.3$, $P < 0.001$).

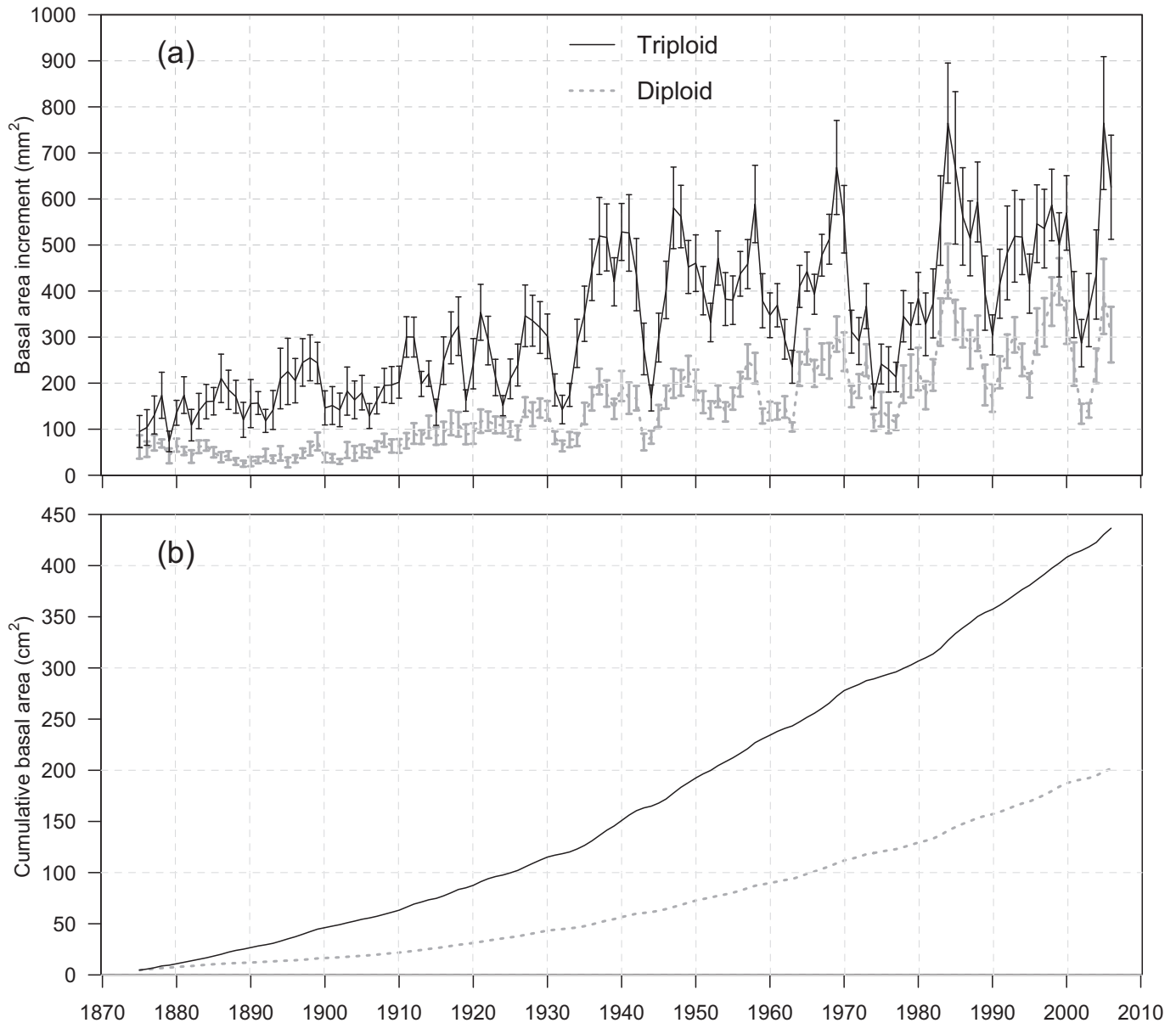


Table 2. Statistics for diploid and triploid ring-width chronologies.

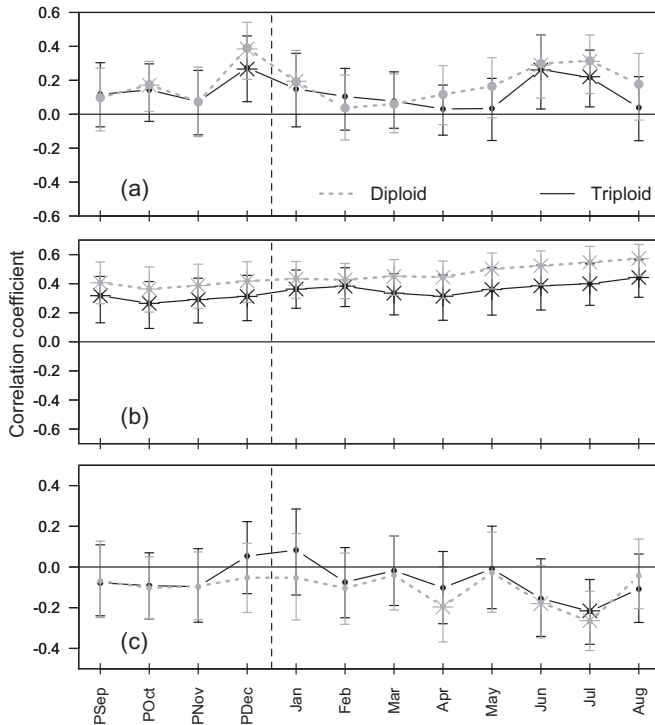
	Diploid	Triploid
Number series (trees)	16	21
Interseries correlation	0.548	0.578
Average mean sensitivity	0.370	0.367
Mean length of series	107	113
Mean ring width (mm)	0.69	1.44
Average first-order autocorrelation	0.593	0.638
Segment problems	0	0

Note: The statistics are from the program COFECHA (Holmes 1983), which was run using a 50-year segment length with a 25-year overlap. All results were significant at the 0.01 level.

the Mock et al. (2008) sampling grid. Furthermore, although the clones may be (or were in the past) physiologically connected, these connections can be among genotypes (Jelínková et al. 2009), and these connections do not necessarily imply that resources are being shared (Jelínková et al. 2012). Regardless, we were careful to sample both the triploids and diploids in the Pando stand proportional to their representation on the landscape, and given the large observed differences in mean ring-width increment and its variation among cytotypes (Figs. 1 and 2), it is unlikely that any genotype effect has limited control when compared with cytotype. In this regard, a broader study involving more triploid clones and taking into account clonal size differences between diploid and triploids could be informative.

It is also possible that other genotypic differences among genets not measured in this study could help further elucidate the differences found between diploid and triploid stem growth. Previous work has shown that aspen genets can show pronounced phenotypic differences (Kanaga et al. 2008). Gender, for example, is a

Fig. 4. Bootstrapped correlation function analysis results for the diploid and triploid chronologies and (a) monthly total precipitation, (b) monthly Palmer Drought Severity Index, and (c) monthly maximum temperature for the period 1895–2006. Asterisks superimposed on correlation values indicate months with significant correlations at the 0.05 level. Months beginning with “P” indicate correlation analysis between previous year climate and current year chronologies. No significant differences in climate response were found between cytotypes.



possible confounding factor because physiological differences are expected between males and females (Grant and Mitton 1979). However, nearly all of the clones sampled in our study were males, and we carefully scrutinized ring-width variability of the two female clones to make sure that gender was not responsible for the observed differences between diploids and triploids.

Novel insights on aspen ecology

Rapid growth early in stand development appears to give the triploids in the Pando stand a substantial competitive advantage over the diploids. This presumes that early height growth is reflected in the diameter increment data, which is reasonable considering the shade intolerance of aspen. During the stem-exclusion (self-thinning) phase of stand development, which would occur very early for aspen as a result of high initial densities, competition would favor triploids. Via the process of self-thinning, the triploids will outcompete the diploids, which succumb disproportionately to density-dependent mortality or are relegated to the periphery of the stand (as observed in the Pando stand by Mock et al. (2008)). Over the course of many “natural rotations” of high-severity fire, this would yield an advantage to the triploids in terms of relative proportion of ramets and the area that they occupy. Indeed, Mock et al. (2008) noted the disproportionate size (i.e., area) of triploid clones relative to their genotype representation when compared with diploids in two separate landscapes. It is likely that the relative cytotype-specific clone size differences could be due to the triploid growth advantage early in stand development, further mediated by the frequency at which stand-replacing disturbances occur for a given stand. This also

suggests that the relative sizes of diploid and triploid clones in a landscape could be a measure of time since disturbance.

In a given landscape and presuming only suckering regeneration, the shorter the “natural rotation”, the faster the shift toward triploid dominance will be. For example, there are dramatic regional differences in the proportion of cytotypes in the Interior West versus boreal populations, which may be a result of the lack of ice sheets at southerly latitudes during the last glacial maximum (Mock et al. 2012). Similarly, differences in regional fire frequencies may result in great disparity in the mean length of natural rotations. In other words, during the last few thousand years, boreal aspen will have experienced fewer stand reinitiation events compared with the Interior West (although boreal fire frequency is likely increasing, e.g., Kasischke and Turetsky (2006)). Because the creation of new triploid clones is expected to be rare (e.g., ~1% per century), this suggests that the triploid boreal aspen have not had sufficient geologic time to exert their dominance. Similarly, the inverse relationship between disturbance frequency and severity also helps to frame an ecological explanation for the continued presence of diploids in a given stand over long time scales. Extremely high severity disturbances, which occur infrequently, result in the conditions thought to be necessary for the establishment of seedlings. For example, particularly severe fires that damage or kill existing root systems and expose mineral soil result in large openings and limited competition from either the overstory or conspecific suckers.

Aspen management

This study quantifies and explains the ecological ramifications of growth-rate differences between aspen ramets from two cytotypes. Given that triploids have a strong stem growth advantage, particularly early in stand development, the conventional regeneration approach (i.e., simple coppice, in which all of the mature stems are clearfelled to promote suckering), which might initially be neutral with respect to cytotype, is likely to favor triploid clones over time. Over multiple rotations, the early growth advantage of triploid stems will likely result in a progressive increase in dominance of the stand by triploids at the expense of diploids. Over time, a loss of genetic diversity on the landscape is likely under this scenario. Furthermore, because of this early growth difference, the potential to favor triploid genotypes during midrotation activities (e.g., weeding, thinning, etc.), although not commonly practiced, is real. Based on our results, the substantially smaller diploids would be removed, and triploids favored, particularly, when midrotation activities (i.e., thinning and perhaps ungulate browsing) have a size preference. This type of intervention may unintentionally result in the reduction of genetic diversity and a possible minimization of sexual reproduction via loss of the diploid genotypes. This may be a particular concern given that genetic diversity is necessary for species adaptation, in particular to rapidly changing climates.

Ideally, a pragmatic conservative approach to in situ management should focus on the long-term maintenance of aspen genetic diversity and cytotype diversity (i.e., both diploids and triploids) on the larger landscape. For example, high-severity fire is likely a necessary process in many aspen communities to maintain genetic diversity, along with protection of seedlings (Fairweather et al. 2014). Over the long term, the management of low-severity fires may not be sufficient to maintain diversity as there would be limited opportunities for the establishment of diploid aspen, and it could also lead to the loss of diploid genotypes and increasing proportions of triploid genotypes. Alternative aspen management strategies to preserve genetic diversity might include coppicing with reserves to promote potential seeding from diploids. In particular, when direct assessment of ploidy is not possible, leaving aspen across the diameter distribution would maximize the possibility of maintaining diploid trees. Additionally, intermediate treatments could be modified to main-

tain all size classes of aspen to increase the chances that diploid genotypes are maintained.

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