

Growth of riparian cottonwoods: heterosis in some intersectional *Populus* hybrids and clonal expansion of females

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Abstract

Key message A field study revealed that some native intersectional hybrids of riparian poplars (*Populus* species) demonstrated faster growth than the parental species, and this was increased for female genets due to their greater clonal expansion.

Abstract To investigate the diversity and adaptation of cottonwoods, riparian poplars, we observed all trees on an island in southern Alberta, Canada over a span of two decades. This woodland included the section *Tacamahaca* balsam poplar, *Populus balsamifera* (B), and narrowleaf cottonwood, *P. angustifolia* (A); and the section *Aigeiros* prairie cottonwood, *P. deltoides* (D), along with their natural interspecific hybrids. There were differences in sizes and growth across the taxa, and basal area increments revealed that some of the intersectional hybrids, A×D (*P.×acuminata*) and B×D (*P.×jackii*), displayed the greatest growth, followed by the sequence: B~D>A×B (intra-sectional hybrids) >A. This revealed variation across the taxa and suggests that sufficient genetic distance is required for heterosis, although only some intersectional hybrids displayed hybrid vigor. Within some taxa, female trunks were generally larger and females produced more clonal ramets, providing ‘clonal amplification’ that resulted

in larger overall genet sizes, especially for the intersectional hybrids. Thus, these females outperformed males in this environmentally favorable environment caused by an artificially stabilized water table. In addition, members of a large clone displayed synchronous mortality, indicating that physiological interconnections persist over decades. These results reveal interacting genetic and environmental influences on the growth, clonality, and mortality of these *Populus* species and their native hybrids in a natural riparian woodland. The differing characteristics among taxa and the sexes would influence their adaptation and their suitability for reclamation or other plantings and for contributions to hybrid poplar breeding programs.

Keywords Basal area increment · Hybrid vigor · LiDAR · Oldman River · *Populus angustifolia* · *P. balsamifera* · *P. deltoides*

Introduction

Poplar biodiversity

Southwestern Alberta, Canada, provides a focus for *Populus* biodiversity, with four native cottonwoods, or riparian (streamside) poplar species overlapping in distribution and interbreeding (Cooke and Rood 2007; Floate et al. 2016). The black cottonwood, *P. trichocarpa* Torr. & Gray (syn. *P. balsamifera* subsp. *trichocarpa*), extends from Pacific drainages to the west and is very closely related to the balsam poplar, and *P. balsamifera* L. (‘B’, Fig. 1) extends from the north. Due to their close similarity, these two taxa are combined. In addition, in the section *Tacamahaca*, the narrowleaf cottonwood, *P. angustifolia* James (‘A’), reaches its northern limit in this area. The section *Aigeiros* prairie

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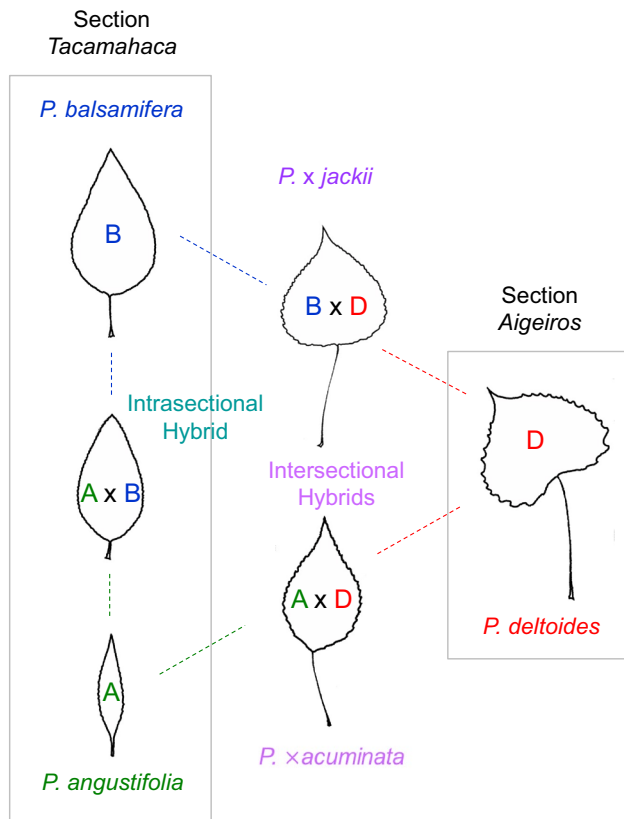


Fig. 1 Leaf shapes and designations of riparian cottonwoods of southern Alberta, Canada, modified from Gom and Rood (1999b)

cottonwood, *P. deltoides* W. Bartram ex Marshall ('D'), is at its western limit. There are consequently three cottonwood types with distinctive leaf shapes (Fig. 1, Gom and Rood 1999b), and confirmation by phytochemical profiles (Greenaway et al. 1991) and molecular markers (Khasa et al. 2003; Hamzeh and Dayanandan 2004; Floate et al. 2016).

This interbreeding of the three taxa produces a dissectional, trispecific hybrid swarm, with intrasectional hybrids involving B×A, and the more distant intersectional hybrids, *P. x acuminata* Rydb., A×D, and *P. x jackii* Sarg., B×D (Eckenwalder 1984; Floate 2004). These designations recognize *P. deltoides* as typically the maternal parent of the initial hybrid, and there are subsequent limitations in backcrossing (Keim et al. 1989; Hamzeh et al. 2007; Thompson et al. 2010). Of these regional hybrid combinations, *P. trichocarpa* has been artificially crossed with *P. deltoides* to produce intersectional 'D×T' hybrid poplars that are extensively cultivated (Ceulemans et al. 1992; Dillen et al. 2010). This system provides an ideal opportunity to investigate ecophysiological adaptation across the cottonwood species and their interspecific hybrids (Rood et al. 2003). This should reveal patterns across these *Populus* taxa that would apply to other regions across North America,

where poplar hybridization is common in sympatric zones (Whitham et al. 2006, 2008).

Commencing in 1994, we characterized all of the cottonwood trunks in an island grove along the Oldman River in Lethbridge, Alberta (Kalischuk et al. 1997; Gom and Rood 1999a, b). This assessed the species, hybrids, and clonal groupings, and allows for comparisons of growth vigor and other characteristics within and across the taxa. The island hydrology is favorable, since a weir extends across the river and stabilizes the water level, thus compensating for the upstream water withdrawal for irrigation. We undertook the present study to determine if the relative rankings for growth vigor of the mature trees of these taxa would match rankings of seedlings or trees in adjacent native riparian sites, or clonal saplings in greenhouse or nursery studies (Table 1). The prior comparisons suggest the sequence: A/ B×D (intersectional hybrids) > B > or ~D > A×B (intrasectional hybrids) > A.

Since poplars are dioecious, we also investigated differentiation across the sexes, expecting a slight influence but possibly increased growth of females, reflecting the favorable environment due to the stabilized groundwater level (Freeman et al. 1976; Grant and Mitton 1979; Xu et al. 2008; Hultine et al. 2016). We also investigated patterns of clonality, where the emergence of multiple trunks from a common root system increases the biomass production of particular genets (or genotypes). We expected differentiation across the taxa, with greater clonality of the section *Tacamahaca* trees. We also considered possible differentiation of clonality across the sexes and possible interactions of taxa and sex, which have rarely been investigated.

Materials and methods

Study site

We investigated all of the cottonwood trees in the upriver zone of an island in the Oldman River at Lethbridge, Alberta, Canada (49°41'N; 112°51'W; Fig. 2). The site had been a natural island in the early 20th century (Fig. 2), and by mid-century, the island had become connected to the floodplain, probably due to natural channel migration and infilling. Around 1956, the Coalbanks Canal was excavated in association with the construction of the concrete weir across the Oldman River and across the canal to the municipal water intake (Fig. 2). The weir elevated the water surface about 1.6 m and impounds streamflow to create a 1.1 km long upstream reservoir with a stabilized water level.

Our island woodland study commenced in 1994, with numbered metal tags nailed to all 391 trees with trunk diameters exceeding 10 cm at 1.4 m height. Trunk

Table 1 Growth rate rankings of riparian cottonwood species and hybrids that occur naturally in southern Alberta and adjacent regions, in field or controlled-environment studies, including *Populus angus-*

tifolia (A), *P. balsamifera* (B¹), *P. deltoides* (D), *P. trichocarpa* (T¹), and intrasectional (A×B) and intersectional (A/B×D) hybrids

System	Finding	Inheritance ² (I)	Source
Nursery	Artificial D×T > T > D	Heterosis and variation	Ceulemans et al. 1992; Scarascia-Mugnozza et al. 1997
Nursery 3 year saplings	Artificial B×D > D ~ B ~ A×B > A	Dominance and heterosis	Campbell et al. 1993
Nursery	Artificial D×T > T ~ D	Heterosis	Ridge et al. 1986
Greenhouse clonal saplings	B > D > A	Interspecific variation	Kranjcec et al. 1998
Native seedlings	D > B ~ A×B	Variation and dominance	Rood et al. 1998
Native seedlings	D > A/B×D ~ B > A×B ~ A	Dominance	Kalischuk et al. 2001
Greenhouse 1. Seedlings	1. B > D	Interspecific variation	Amlin and Rood 2002
Greenhouse 2. Clonal saplings	2. B > A		
Native stands	A/B×D > or ~ B > A×B > A	Dominance and additive I	Willms et al. 2006
Nursery	Artificial D×T > T ~ D	Heterosis	Dillen et al. 2010
Natural stands and nursery	<i>P. fremontii</i> (F ³) > A×F > A	Additive I	Lojewski et al. 2009
Greenhouse	B×D > A	Variation	Nielsen et al. 2010
Natural stand	A/B×D > D ~ B ~ A×B > A	Heterosis and additive I	This study

¹*P. trichocarpa* is very closely related to *P. balsamifera*

²For Inheritance: Additive—hybrid is intermediate between parents; Dominance—hybrid equals a parent; Heterosis—hybrid exceeds both parents

³*P. fremontii* is very closely related to *P. deltoides*

Fig. 2 Aerial photograph of the study site along the Oldman River in 2012 with an inset image from 1926



diameters were measured at the tags, and the apparent health of each tree was assessed based on the extent of branch and crown die-back. The phenotype of each tree was assessed over the following 3 years, with species or hybrid assignments were based on foliar morphology, and this was combined with assessments of sex, foliar and floral phenology, and gall abundance, for complete clonal determinations (Kalischuk et al. 1997; Gom and Rood 1999a, b).

We observed the grove frequently from 1994 to 2016, noting seasonal phenology and tree losses due to beaver cutting especially along the river bank, some further natural mortality, and clearing of some trees for weir and canal maintenance. In spring 2008, the sex, phenology, and health were again assessed for all trunks, and in the spring of 2009, trunk diameters were remeasured. Final measurements of the 227 remaining trees were undertaken during dormancy from December 2014 to March 2015, and the leafless condition facilitated cm resolution localizations with a Topcon (Livermore, CA, USA) HiPer SR Global Navigation Satellite System with a nearby static base station.

The analyses revealed a deficiency of remaining narrowleaf cottonwoods (*P. angustifolia*, A) on the island, and consequently, in September 2015, we measured diameters and extracted increment cores from adjacent trees situated in a band that extended southward (upstream) from the natural island grove across the artificial canal (Fig. 2). Ring measurements of those cores permitted additional determinations of the 1994 and 2009 diameters of additional narrowleaf cottonwoods.

Topography and canopy analyses with LiDAR

For topographic analysis, we surveyed the island with a Teledyne Optech Aquarius combined topo/bathymetric airborne LiDAR (Light Detection and Ranging) system during dry, clear-sky daytime conditions in mid-August 2015 at an approximate sample density of 1 point/m². These 3D data points simultaneously sampled both the ground surface and the woodland vegetation. Following the application of a ground-classification routine (Axelsson 1999; Hopkinson et al. 2006), the resultant terrain (or bare earth) points were gridded using a Triangulated Irregular Network (TIN) algorithm in the Surfer software package (Golden Software, Boulder, CO) to produce a 1 m resolution digital elevation model (DEM; Fig. 3). The study area was isolated, and water surface elevations surrounding the island were manually digitized at distances of ~5 m. These channel water surface elevations were used to interpolate a planar TIN surface that was assumed to approximate a flat water-table surface under the island. By subtracting the positions of this surface from the DEM, depth to groundwater (Z_{gw}) values was determined for each tree.

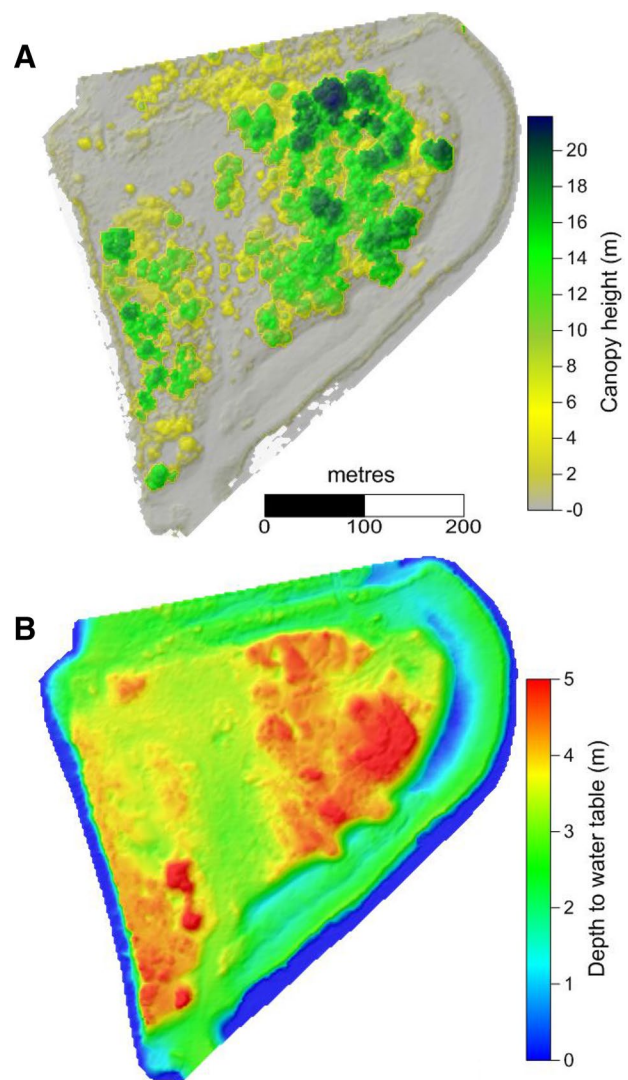


Fig. 3 LiDAR analysis of the study island along the Oldman River displaying **a** canopy height and **b** depth to groundwater (Z_{gw})

The LiDAR analysis also detected the riparian vegetation, and from the uppermost rebounds, the woodland canopy was also plotted (Fig. 3). We attempted to determine heights of individual trees, but this analysis was confounded, since some trunks were angled or forked (bifurcated), and crown die-back was substantial, complicating the trunk and canopy structures.

Quantitative analyses

We have previously described the species and hybrid distributions, and clonal associations (Gom and Rood 1999a, b). We had reported the 1994 trunk circumferences (Fig. 6 in Gom and Rood 1999a), and the current study emphasized growth increases over the two-decade interval from 1994 to

2014, and considered other measurements and observations from 1994 to 2016.

Riparian cottonwoods display an inherent growth pattern in radial increments (RI) and in basal area increments (BAI) (Willms et al. 2006; Berg et al. 2007). Following maturation at about 20 years, RI progressively decline in association with increasing circumference. Conversely, BAI are relatively constant and are thus more suitable for comparisons across mature trees of differing ages. Consequently, from the 1994, 2009, and 2014 diameters, we determined radii ($r=D/2$) and calculated trunk basal areas ($\pi \times r^2$). We then calculated the average yearly BAI as: $[(BA_{2014} - BA_{1994})/20]$ with consideration of the intermediate 2009 diameters and basal areas to ensure correct correspondences. In addition to growth values per trunk, we also assessed the values per genet, by accumulating the values across the clonal ramets.

Analyses with SPSS 19 (IBM, Armonk, NY) included descriptive statistics, t tests for paired comparisons, and univariate linear model Analyses of Variance (ANOVAs) commonly followed by Tukey's HSD and Duncan post-hoc tests for group comparisons to investigate prospective effects of taxa or clones. We applied two different taxon groupings, with five categories A, A×B (intraspecific hybrids), B, A/B×D (interspecific hybrids, with A×D and B×D were combined due to overlapping leaf shapes) and D, for the analyses of all trunks, including the clonal ramets. For analyses based on genets, due to the reduced numbers of A and B genotypes, we combined these two *Tacamahaca* species to provide four categories: A/B (A or B), A×B, A/B×D, and D, with clonal means providing single values for each genet. We also undertook Analyses of Covariance (ANCOVAs), with the 1994 diameters as the covariate to consider possible influence from variation across the initial tree sizes.

We also explored spatial patterns of trunk growth rate for all trees (including ramets) to identify correspondences between increased or decreased rates and position on the island. A Moran's I spatial autocorrelation analysis was performed in ArcGIS (ESRI, Redlands, CA) at different spatial scales and followed by a hot spot analysis (heat mapping) using the Getis-Ord G_i^* test to identify clusters of fast- or slow-growing trees. The results were mapped and interpreted for potential environmental influences, such as proximity to water or following beaver cutting (Gom and Rood 1999a).

Results

Cottonwood growth

From the 391 trees observed in 1994, 262 and 227 trunks remained in 2009 and 2014, respectively. There was 96%

correspondence across the 2014 and 1994 diameters and the linear regression provided a slope of 1.033 (Fig. 4). This near-unity slope suggests that analyses of diameter or radial increment could have been suitable for this woodland grove. The regression intercept reflected an upward offset that represented the typical diameter growth over the two-decade interval, with an average radial increase of 2.50 mm/year or an annual diameter increment of 0.5 cm. We undertook analyses of diameters and BAI, and the interpretations were very consistent. As planned with the study design, we emphasize BAI in the subsequent presentations. This avoids the apparently slight influence from the inherent growth pattern, and increases comparability with other regional cottonwood growth studies (Willms et al. 2006; Berg et al. 2007).

The LiDAR analysis provided the digital elevation model (DEM), which demonstrated that the island surface was relatively flat and generally 3 to 5 m above the water surfaces of the adjacent reservoir and of the Coalbanks Canal (Fig. 3). On the island, there were two woodland groves that were separated by a slightly lower band with a roadway for weir access (Fig. 2). The grove closer to the river was smaller, and the woodland was sparser and with a lower canopy (Fig. 3). The larger, denser, and taller grove was closer to the canal (Fig. 3). With the stabilized reservoir and canal water surfaces, the alluvial groundwater table would also have been stabilized and this would have reduced the influence of elevation, which is more relevant for woodlands along a natural river zone that displays seasonal changes in water level, or stage (Rood et al. 2013). Supporting this expectation, there was no association between the tree size or growth, and the depth to groundwater (Z_{gw} , Fig. 3; for example,

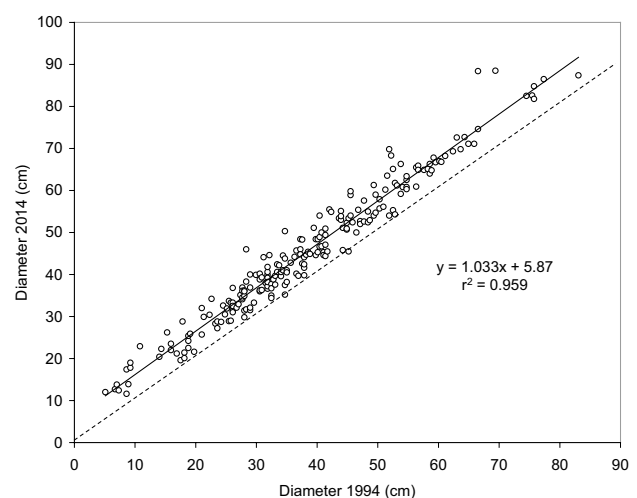


Fig. 4 Diameters of individual cottonwood trees in 2014 versus 1994. The best-fit line is plotted, and the dashed line provides the 1:1 line with unit slope and origin intercept

$BAI = 0.115(Z_{gw}) + 0.935$, $r = 0.040$, not significant). The apparent slope was even positive, suggesting increasing growth with higher surfaces, contrary to expectation (Rood et al. 2013). This could partly reflect accretion within the groves due to the trapping of suspended sediments by the woodland shrubs and small trees during flood events.

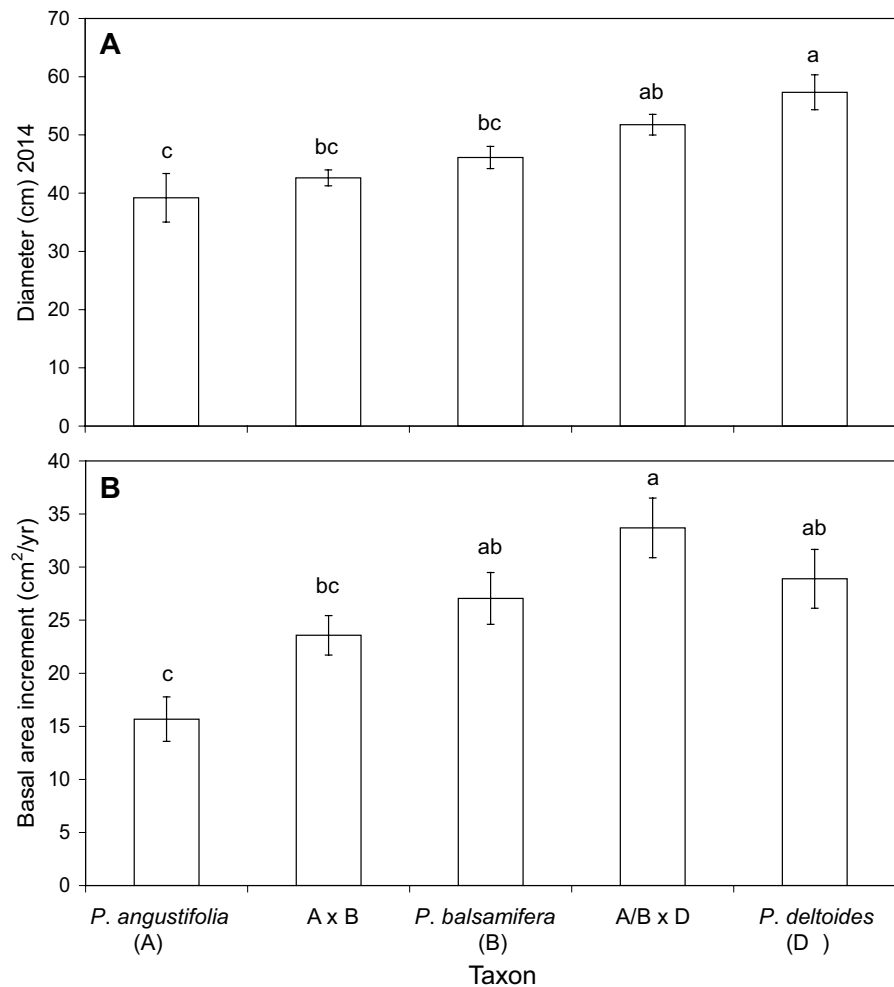
The 2014 diameters provided the total tree sizes and reflect the growth over the full life of each tree, including the growth prior to and after initial study in 1994. There were patterns across the 2014 sizes (Fig. 5A), with an ANOVA revealing the highly significant influence of taxa ($F_{4,190} = 8.035$, $p < 0.001$). The *P. deltoides* (D) trees were largest, with apparently descending sizes for the intersectional hybrids (A/B×D), *P. balsamifera* (B), the *Tacamahaca* intrasectional hybrids (A×B), and finally *P. angustifolia* (A).

Of these studied trees, 24 had trunk diameters smaller than 20 cm in 1994 (Fig. 4). These smaller trees were excluded from the subsequent BAI analyses to ensure that trees were in the mature growth phase. Of the 195

larger trees, 30 had been judged as unhealthy in 2009, partly due to beaver girdling. These consistently displayed lower trunk diameter growth and BAI than the apparently healthy trees (BAI, healthy: 28.76 ± 1.38 versus unhealthy: 13.91 ± 1.64 cm²/year; $t = 4.472$, $df = 193$, $p = 0.030$), and included all cottonwood taxa and with generally similar proportions to the overall grove (1A, 8A×B, 7B, 8D, and 6A/B×D). Since these trees were identified as unhealthy at the onset of the growth interval, they were excluded from subsequent analyses. We also undertook statistical analyses including the smaller trees and the unhealthy trees, and the patterns across the taxa were quite consistent but the variation was increased.

The subsequent BAI analyses investigated this refined data set with 171 trees, including clonal ramets and the supplemental *P. angustifolia* from across the canal. There was substantial variation across the taxa (ANOVA: $F_{4,166} = 4.05$, $p = 0.004$). The intersectional hybrids (A/B×D) grew the fastest, followed by *P. deltoides* and *P. balsamifera*, and then the intrasectional *Tacamahaca* hybrids, while *P. angustifolia* grew more slowly (Fig. 5B).

Fig. 5 **A** Trunk diameters in 2014 and average annual basal area increases from 1994 to 2014 of riparian cottonwoods, with representation by species or hybrid type. Different letters indicate significant difference ($p < 0.05$). **B** Clonal ramets are included, resulting in 7, 54, 35, 72, and 27 trunks (left to right). Different letters indicate significant difference ($p < 0.05$)



To remove duplication from the clonal ramets, we calculated trunk growth averages for each clone to enable genet analyses. Since there were limited numbers of genets for *P. angustifolia* and *P. balsamifera*, we combined these into a single, section *Tacamahaca* group for this analysis. This revealed a significant taxon effect ($F_{3,64} = 4.29, p = 0.008$), with more vigorous growth of the collective intersectional hybrids, while the trees from the two parental sections *Tacamahaca* and *Aigeiros* (*P. deltoides*) displayed generally similar growth increments over the two-decade interval (Fig. 6).

The various clones clearly displayed differences in growth rates ($F_{57,107} = 5.382, p < 0.001$), and we were especially interested in the comparison of intrasectional hybrids within section *Tacamahaca* (B×A) versus the intersectional

hybrids (A/B×D). These provided the two hybrids groups, with lower genetic distance for the intrasectional hybrids versus greater genetic distance for the intersectional hybrids, and both hybrid types were well represented in the island grove. Ranked ordering of the genets of each hybrid type are shown in Fig. 7. There were slow-growing genets of each type, but the distribution is clearly different with the intersectional hybrids averaging 41% greater BAI over the two-decade interval (33.7 ± 2.8 versus 23.9 ± 1.9 cm²/year; $t = -2.79, df = 42, p = 0.008$; equal variance not assumed). The distribution of the intersectional hybrids was broader (Std. Dev.: intrasectional 10.9 versus intersectional 25.6), indicating greater diversity of growth rates. Thus, more intersectional hybrids grew rapidly, and some grew much faster than any of the intrasectional hybrids.

Fig. 6 Average trunk basal area increments from 1994 to 2014 for cottonwood genets (for each genet, the clonal ramets are averaged), for the combined section *Tacamahaca* species (A and B, $n = 12$), for the intrasectional (A×B, 17) and intersectional hybrids (A×D and B×D combined, 29), and for the section *Aigeiros*, *P. deltoides* (10). Different letters indicate significant difference ($p < 0.05$)

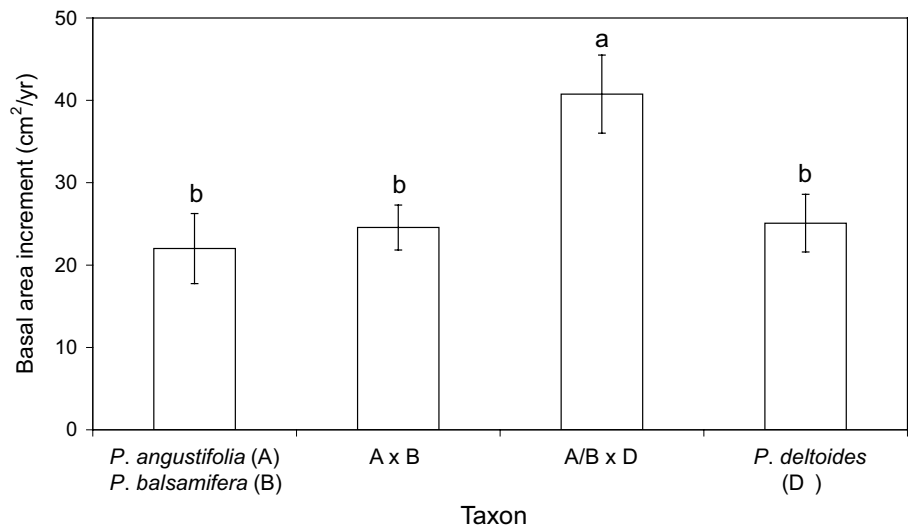
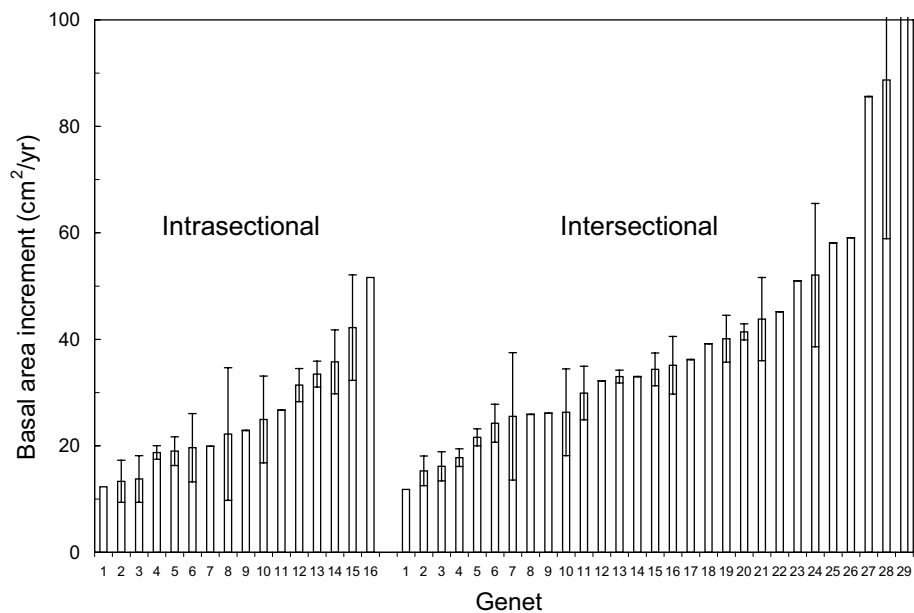


Fig. 7 Averaged trunk basal area increments for genets of intrasectional hybrids [*P. angustifolia* (A) × *Populus balsamifera* (B)] (within section *Tacamahaca*, left), and for clones of intersectional hybrids of A or B × *P. deltoides* (right). Genets within each type are ordered by increasing BAI, and for clonal trees, the means ± SE are plotted



Sex and clonality

Trunk growth rates within the taxonomic types were similar across the two sexes. However, females were apparently slightly larger at the commencement of the study in 1994 (Fig. 8A) and the increased size of some females persisted through the measurements in 2009 and 2015 (not shown). Some females had more clonal ramets in 1995 (Fig. 8B; Gom and Rood 1999a), and this pattern persisted. As revealed by the product of clonal number \times ramet size, some females produced larger genet total trunk area (Fig. 8C). This was apparently displayed for the different taxa, with the greatest difference among the intersectional hybrids (Fig. 8C), and this demonstrated heterosis for genet trunk growth and trunk biomass for the females.

We anticipated that clonality might increase trunk competition, but we did not observe reduced growth rates with increasing clonal size (for intersectional hybrids: $n=43$; $r=0.172$, not significant). Conversely, we found that ramet spacing increased with increasing clonality (for intersectional hybrids: spacing (m) = $0.674 \times$ ramet number + 0.255 ; $n=25$, $r^2=0.473$, $p<0.01$). Thus, trunks within larger clones were apparently more dispersed, and this would reduce intraclonal competition.

Clonal mortality

We had recognized the clonal groups especially by synchronous bud flushing, while autumn senescence was more variable within the clones (Gom and Rood 1999b). This suggested that the various ramets experienced different degrees of environmental stress and may thus have been physiologically independent. However, in contrast to this conclusion, we observed synchronous mortality of the largest clone with a drought interval in 2015 (supplemental figure), and this could reflect continuing physiological coordination. Following this observation, we investigated clonal mortality in other riparian groves, and in the summer of 2016, we observed a similarly synchronous mortality of a substantial clone of *P. balsamifera* along Pincher Creek (49°32'49"N; 113°47'38"W). This contrasted with the apparent health of a spatially intermixed *P. angustifolia* clone. We have also observed synchronous mortality of clonal trees in shelterbelts and farmstead plantings, and these have included juvenile saplings from clonal suckers as well as the larger trees from the original plantings (for example, 49°41'55"N; 112°54'16"W). These confirm the occurrence of synchronous mortality within cottonwood clones and suggest that physiological interdependence persists through decades.

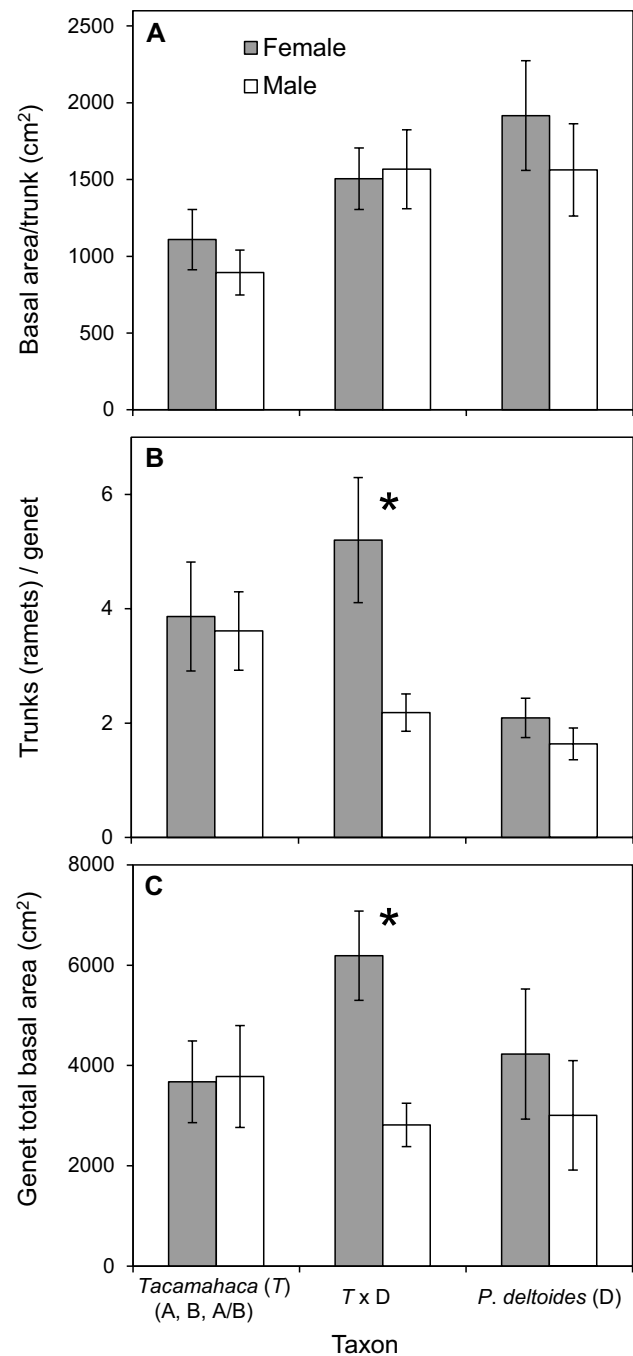


Fig. 8 Average (\pm SE) trunk basal areas (**A**, top), numbers of clonal trunks per genet (**B**, middle), and the product of those to provide the average total basal area per genet, for the different groupings by *Populus* section (section *Tacamahaca* species and intrasectional hybrids, intersectional hybrids, and *P. deltoides* of section *Aigeiros*). *Significant difference between sexes ($p<0.05$)

Spatial patterning

In addition to the genetic differentiation across the taxa, we expected some environmental influence and the spatial

analysis revealed some localized differentiation in growth rates. Trees in a patch towards the centre of the island displayed slower growth, while a patch to the northeast, closer to the canal, displayed faster growth (Fig. 9). Trunks in both of these zones contained various poplar taxa, including substantial D and A/BxD, the two fast-growing types, although the fast-growth patch contained the largest trees on the island (Fig. 3a), and these were intersectional hybrids. The fast-growth patch was near the perimeter of the grove, and this would reduce competition. Trunk density was also lower here, being about one-half of that in the slow-growth patch (~144 versus 320 trees/ha; based on 25×50 m quadrats), supporting an influence from competition.

Discussion

Growth across the cottonwood taxa

Our first prediction anticipated that there would be differing growth rates across the species and hybrids, with

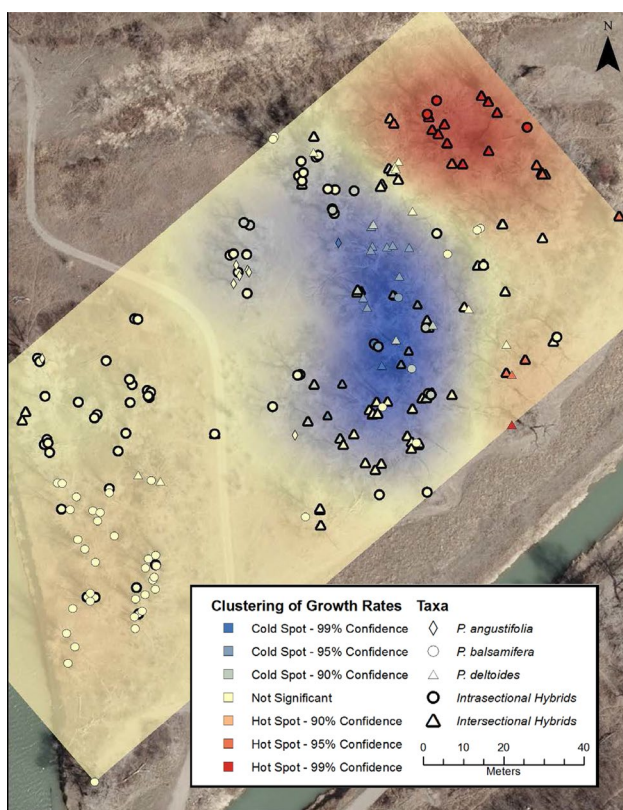


Fig. 9 Section of the study island (Fig. 2) with cottonwood tree positions plotted and a heat map to represent zones of low (blue) or high (red) trunk growth from 1994 to 2014. The plotted trees in the upper right represent the periphery of the grove, and the canal is just outside of the field of view. (Color figure online)

sequential ranking that would be consistent with that from prior field and controlled-environment studies (Table 1). Supporting this, we did observe significant differences in the sizes and growth rates across the taxa, and the relative growth rates in this study were generally as predicted: $A/B \times D > D \sim B > A \times B > A$. The slower growth of the narrowleaf cottonwood, *P. angustifolia*, is well established in this region as this willow-like poplar (Rood et al. 2010) is typically smaller and grew less rapidly in field and controlled-environment conditions (Table 1). The other section *Tacamahaca* species, balsam poplar, *P. balsamifera*, is typically larger and faster growing in natural and artificial environments. The present study confirms this pattern and also supports an intermediate size and growth rate for the intrasectional hybrids of *P. angustifolia* × *P. balsamifera*. This suggests additive inheritance, whereby the hybrid is intermediate in phenotype (Fig. 10); rather than dominance, where the hybrids would have been similar to the larger *P. balsamifera* (Rood et al. 1988).

Similar to prior studies (Table 1), the relative performance of *P. deltoides* versus *P. balsamifera* was less consistent. These two species overlap in occurrence and hybridize in a very long band along the southern Canadian border, extending from the study region eastward through the prairies (Ronald 1973). Despite this extensive

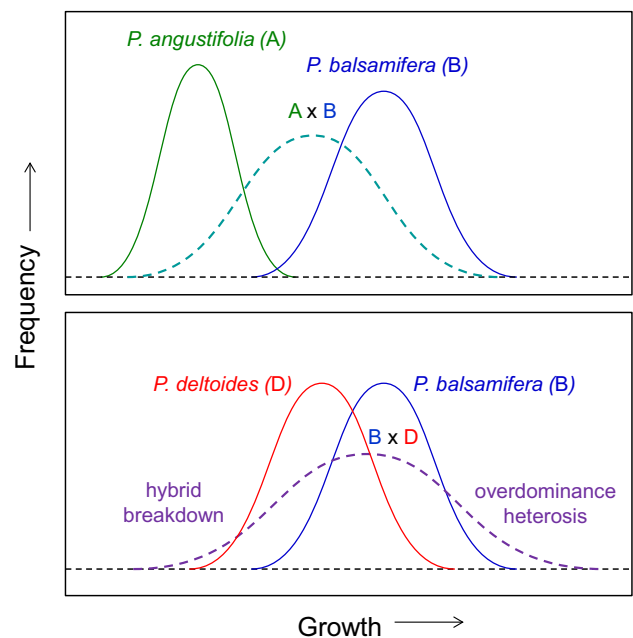


Fig. 10 Schematic diagram representing the possible distribution of cottonwood growth rates with groupings by species or hybrid type. Normal distributions are plotted for each but especially for the intersectional hybrid, a skewed distribution might occur and the mean placement for that type is also less certain. The relative positioning of B versus D is also less certain, as this has varied across prior studies

occurrence, there has been limited prior comparison of growth patterns of *P. deltoides* and *P. balsamifera* in zones of overlap.

Heterosis in natural intersectional hybrids

An important finding from our study was the increased growth rates of some of the natural intersectional *P. ×jackii* (B×D) or *P. ×acuminata* (A×D) hybrids in southern Alberta. This indicates heterosis, or hybrid vigor, for trunk growth in some hybrid cottonwoods. We had previously investigated the prospect of heterosis in natural interspecific hybrids but that prior comparison largely involved intrasectional *Tacamahaca* hybrids (Campbell et al. 1993) that would have had less genetic differentiation. Our present study supports the view that sufficient genetic distance is required for heterosis (Campbell et al. 1993), and consequently, some intersectional hybrids, but not intrasectional hybrids, would out-perform both parental species.

There was substantial variation in the growth rates across the different intersectional hybrids and we further conclude that rather than intersectional hybrids being typically faster growing, only some particular intersectional hybrids display overdominance. There is the subsequent prospect that the population of intersectional hybrids would display a broader phenotypic range of growth rates, and probably other physiological characteristics (Bate et al. 1988; Dillen et al. 2010). We consequently propose a system, as indicated in Fig. 10. Each *Populus* species displays a distribution of responses, as is typical for most biological characteristics, and these distributions in growth rate or other performance characteristics would vary across the taxa. The narrowleaf cottonwood is consistently slow growing, while the balsam poplars and prairie cottonwoods display faster growth. With the combination of traits from these two parents, along with the genetic diversity provided by gamete segregation and combination, the intersectional hybrids would be expected to display a broader range of physiological performance.

From this model, we would predict that there would be some very vigorous intersectional hybrids, but also that there would be some hybrids with slower growth. This prediction is consistent with the finding of Lojewski et al. (2009) for intersectional hybrids of *P. angustifolia* × *P. fremontii*, with the latter parent being very closely related to *P. deltoides*. In that study, segregating seedlings were monitored and there was greater mortality for the intersectional hybrids than for the parental species. This could represent ‘hybrid break-down’ from inferior genetic combinations, and this would provide the opposing outcome from hybrid vigor, broadening the physiological distribution (Fig. 10). We might thus conclude that rather than intersectional hybrids typically displaying hybrid vigor,

the more likely situation is that intersectional hybrids display a wider range of variation and associated with this breadth, and some will display hybrid vigor, particularly in some environments (Orians et al. 1999).

This study also indicates that there is substantial variation across the different genets *within* the species or hybrid types. The magnitude of this clonal variation was greater than we had anticipated, and while some clones displayed strong consistency in growth rate, other clones displayed variances that approached the variance across the taxa. We have cultivated native regional species and hybrid clones in a nursery and observed strong consistency in their characteristics, including growth patterns (Campbell et al. 1993). Conversely, there was greater variation in a study of *P. angustifolia* clones that originated from across the full latitudinal range (Evans et al. 2015; Kaluthota et al. 2015). We thus conclude that the genotype defines the upper limit for growth rate but that there are multiple environmental factors in riparian zones that can reduce the growth of individual trees.

Spatial patterns

To investigate prospective environmental factors, we considered spatial patterns across the island. Despite the presumably stabilized alluvial water table, the spatial autocorrelation analysis revealed two zones with lower versus faster growth, respectively. The slower growth zone was closer to the center of the island and thus further from the river or canal, while the zone of faster growth was closer to the canal. While the increased growth may have partly reflected improved access to moisture, the fast-growing zone was at the edge of the grove, where light exposure would have been increased and competition decreased. An influence from competition was consistent with the doubled trunk density in the slow growth zone, and the thinner density in the fast-growth zone would reflect the increased beaver cutting that was observed around the perimeter of the grove (Gom et al. 1999a). There was another zone with dense trunks in the southwestern zone, but in that patch, the balsam poplars were smaller in largely members of a single large clone (Gom et al. 1999a), with the adjacent zones being treeless, reducing competition.

Sex and clonality

Following from prior studies (reviewed in Hultine et al. 2016), we expected subtle difference across the sexes, but slightly more vigorous performance of the females on this island, reflecting the favorable water status due to the stabilized groundwater level. We found that some females

tended to be larger but the differences were not statistically significant. More substantial differentiation was observed for the extent of clonality (Fig. 8). Females had more trunks per genet, and this resulted in a sex ratio that approached 2:1, with many more females (Gom 1999a). Due to their increased clonality, females of the intersectional hybrids had total genet trunk areas that were about double that of the males (Fig. 8C), and these patterns represent ‘clonal amplification’, whereby clonal expansion increases eco-physiological differentiation, resulting in substantial difference between females and males. This pattern supports the conclusion that for dioecious riparian plants, females will especially thrive with favorable environmental conditions relative to water status (Freeman et al. 1976; Comtois et al. 1986; Dawson and Ehleringer 1993; Ward et al. 2002; Hultine et al. 2007, 2016; Nielsen et al. 2010; Juvany and Munné-Bosch 2015). The increased overall growth and size of female genets were probably due to their increased clonality and this has also been observed for upland aspens, *P. grandidentata* and *P. tremuloides* (reviewed in Sakai and Sharik 1988). The increased clonality provides superior performance of females with favorable environmental conditions and is consistent with the concept of strategic positioning, whereby females thrive in conditions that would be suitable for seedling recruitment (Nielsen et al. 2010).

Conclusions

This study involved two decades of observations and measurements of all of the riparian cottonwood trees in an island grove comprised of a complex swarm of poplar species and hybrids. Uniquely, all of the clonal groups had been previously identified within this grove. We observed environmental influences within zones of the island that supported more rapid or slower growth, probably influenced by tree density and competition. Our investigation of trunk growth rates revealed differences across the clones and across taxa, with rankings that were fairly consistent with prior reports from field or controlled-environment studies, producing the declining growth rate sequence: intersectional hybrids of *P. balsamifera* (B) or *P. angustifolia* (A) × *P. deltoides* (D) (A/B×D) > B ~ D > intersectional hybrids of A×B > A. This sequence indicated heterosis, or hybrid vigor, for some naturally occurring intersectional hybrids. However, while some intersectional hybrids displayed very vigorous growth, others display hybrid breakdown and poor performance. Vigorous intersectional hybrids could display rapid colonization and early growth within native woodlands and could also provide fast-growing clones for reclamation plantings or as prospective parents for breeding of fast-growing artificial poplar hybrids.

We also observed differences in the extent of clonality across the taxa and also across the sexes. Some females, and particularly those of the intersectional hybrids, produced more clonal trunks (ramets), and thus, some female genets were larger. This supports the interpretation that females will thrive under environmentally favorable conditions, and for this island, the stabilization of the adjacent water level with the reservoir and canal would have produced a similarly stabilized groundwater table that would have provided a favorable environment in which some females thrived. This study thus revealed that genetic and environmental factors, as well as interactions between these major factors, influenced the growth and clonality of the riparian cottonwoods with a very biodiverse natural woodland.

Author contribution statement LAG and SBR recognized the study opportunity, and LAG undertook the initial field study and analyses. DWP contributed to the subsequent field measurements and led the quantitative analyses across the taxa, DM and JSM undertook the final field measurements and spatial analyses, and CH undertook the LiDAR analyses. SBR supervised the project and led the manuscript development, with contribution from all co-authors.

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