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# Hybrid introgression as a mechanism of rapid evolution and resilience to climate change in a riparian tree species



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Determining whether organisms can undergo adaptive evolution at a pace commensurate with contemporary climate change is critical to understanding and predicting the consequences of such change. Hybrid introgression is a mechanism of rapid evolution by which species may adapt to climatic shifts. Here, we examine variation in growth and survival in a long-term common garden experiment with a foundation tree species to determine if introgression is enhancing climate change resilience. Two naturally hybridizing tree species, low elevation *Populus fremontii* and high elevation *Populus angustifolia*, and hybrid and backcross genotypes were planted in a low elevation, warm common garden. We show that *P. angustifolia* and backcross trees are vulnerable to warming, and their survival is related to climate and transfer distance (proxies for climate change). Increased odds of survival are associated with genetic introgression, as indicated by RFLP genetic markers. Thus, for these long-lived foundation trees, hybrid introgression is associated with increased resistance to selection pressures in warmer, drier climates. These data highlight the importance of evolutionary patterns and processes in shaping ecosystem responses to climate change. If adaptive introgression through hybrid zones is common, hybrid-specific conservation policies and restoration should be reconsidered in the context of global change.

Identifying the ecological and evolutionary factors underlying ecosystem-level responses to climate change is an urgent research frontier. Climate change often acts as a selective force, shifting plant functional traits. The impacts of these shifts, especially when they occur in foundation species, extend to ecosystem function and associated communities (e.g., soil microbes, herbivores)<sup>1–8</sup>. Evolutionary changes in functional traits can occur on ecological timescales (i.e., rapid evolution)<sup>9–12</sup>. Investigations into the potential for rapid evolution and adaptation to warmer and drier conditions via interspecific gene flow (i.e., genetic introgression between hybridizing species) have demonstrated that for diverse taxa, ancient introgression has been an important mechanism of adaptation to historical climate change<sup>13–15</sup>. Evidence for the mitigation of some of the negative impacts of contemporary climate change via enriched genetic diversity and adaptive potential resulting from introgression has also been shown across levels of biological organization, from populations to ecosystems<sup>16–19</sup>. However, we lack data on genotype-level selection over multiple decades in concert with measurements of key organismal traits and information about the intraspecific genetic variation underlying phenotypic changes.

Variation in survival and growth under changing climates among foundation plant species represents a mechanism through which climate change-driven selection can affect ecosystem functions (e.g., carbon sequestration)<sup>6</sup>. This has been explored with naturally hybridizing foundation tree species in the *Populus* genus. Natural hybrid populations act as selective filters for introgressed genes from the low elevation, warm-adapted *Populus fremontii* moving into relatively high elevation, cool-adapted *Populus angustifolia* populations<sup>20</sup>. In a common garden, the presence of these introgressed *P. fremontii* genetic markers has been associated with unique arthropod communities and greater asexual reproduction<sup>21</sup>. Further, genotype-level variation has been demonstrated to influence ecosystem processes in this system. For example, genetically-based differences in herbivore susceptibility among these species and their hybrids influence aquatic leaf litter decomposition rates and carbon cycling<sup>16</sup> and ecosystem-level carbon budgets both in the field and in common gardens vary with tree cross type<sup>22,23</sup>. These studies demonstrate that hybrid introgression between foundation tree species can result in changes to key fitness traits and shape organismal and ecosystem-level responses to climate change.

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Here, we use a 31-year-old common garden experiment to understand the consequences of introgression for species' growth and survival in a climate change context to explore the potential ecosystem-level impacts of hybridization<sup>24</sup>. A change in climate was imposed on *Populus fremontii* and *Populus angustifolia* genotypes, as well as hybrid and backcross trees originating from natural populations along a temperature and elevational gradient, by planting them in a low-elevation, warm common garden. Over three decades, nearly half of all trees planted were lost to selective pressures (i.e., mortality in the climate change conditions in the warm, dry common garden) after reaching reproductive maturity. We hypothesized that introgression influences tree fitness and tested this by comparing measures of growth and selection over time. We tested three predictions: (1) In the common garden, *P. fremontii* trees will have higher survival and greater biomass accumulation (i.e., carbon) than *P. angustifolia* trees, and survival and growth for hybrid and backcross genotypes will fall between the parental species (i.e., will be additive), (2) among the more vulnerable *P. angustifolia* and backcross genotypes growing in warmer conditions, tree mortality will increase as a function of the magnitude of climate change (proxied by geographic and climatic transfer distance), and (3) among *P. angustifolia* and backcross trees the presence of introgressed genetic markers will be associated with increased survival and biomass accumulation. Testing these predictions is important because, if supported, they suggest a potentially common hybrid pathway by which a foundation tree species can adapt to a rapidly changing environment.

## Results

### Parental species and hybrid patterns of growth and survival

After 31 years in the common garden there were significant differences in survival among the four cross types that varied in their climatic origin (i.e., parental species *P. fremontii* and *P. angustifolia*, F<sub>1</sub> hybrids, and F<sub>1</sub> × *P. angustifolia* backcross hybrids). In the low elevation common garden approximately 90% of the low-elevation-adapted *P. fremontii* and 100% of F<sub>1</sub> hybrid cottonwood genotypes survived, while among the high-elevation-adapted backcross hybrid and *P. angustifolia* genotypes, only approximately 30% and 25% survived, respectively (Table S1, Fig. 1A). We also found significant differences in accumulated biomass among the four cross types, the patterns of which were consistent with previous studies (Table S2); F<sub>1</sub> hybrid genotypes had the highest biomass, followed by the *P. fremontii* genotypes. Mean biomass for the backcross and *P. angustifolia* genotypes was approximately 37% lower than for *P. fremontii* genotypes (Fig. 1B). Tukey post-hoc analyses showed that mean biomass for *P. angustifolia* and backcross genotypes were significantly different from *P. fremontii* genotypes, while F<sub>1</sub> genotypes were not significantly different from either parental species or the backcrosses.

### *Populus angustifolia* and backcross survival is related to climate change

Consistent with the second hypothesis that warming and drying would select against high elevation populations from cooler and wetter sites, survival among *P. angustifolia* and backcross trees varied significantly, with both geographic and climatic transfer distance, after 31 years in the common garden. *Populus angustifolia* and backcross trees from populations closer and more climatically similar to the common garden were more likely to survive than those originating from more distant and dissimilar populations (Fig. 2A; Table S3). Overall, we found that for each 1 °C increase in temperature, the odds of survival decreased by 7.5%, and that there was greater than 90% mortality among *P. angustifolia* and backcross trees when mean annual temperature (MAT) (MAT, here, a proxy for a number of temperature and precipitation variables, see Fig. S1) differed more than 4 °C between source populations and the common garden.

### Marker-trait associations among surviving backcross and *P. angustifolia* genotypes

While high elevation backcross hybrid and *P. angustifolia* genotypes had the highest mortality overall, survival among these cross types was associated

with the presence of a genetic marker introgressed from *P. fremontii*. We found that the presence of markers RFLP-755, RFLP-754, and RFLP-1286 were the most important predictors of survival for *P. angustifolia* and backcross genotypes, and that there was a significant difference in survival among individuals with RFLP-1286 compared to those without this marker (Table S4, single factor ANOVA results in Table S5). Overall, backcross hybrid and *P. angustifolia* trees that carried *P. fremontii* marker RFLP-1286 had approximately 75% greater survival after 31 years in the common garden relative to the trees that did not have the marker (Fig. 3). Further, all backcross individuals with this marker remained alive in 2022. This pattern had not yet emerged twelve years after planting; we found no significant difference in mortality based on marker presence for RFLP-1286 20 years ago, indicating that warming conditions over the last two decades have likely been important selective agents. We further investigated the effects of RFLP-1286 as a predictor for tree traits but found no significant effect (Table S6).

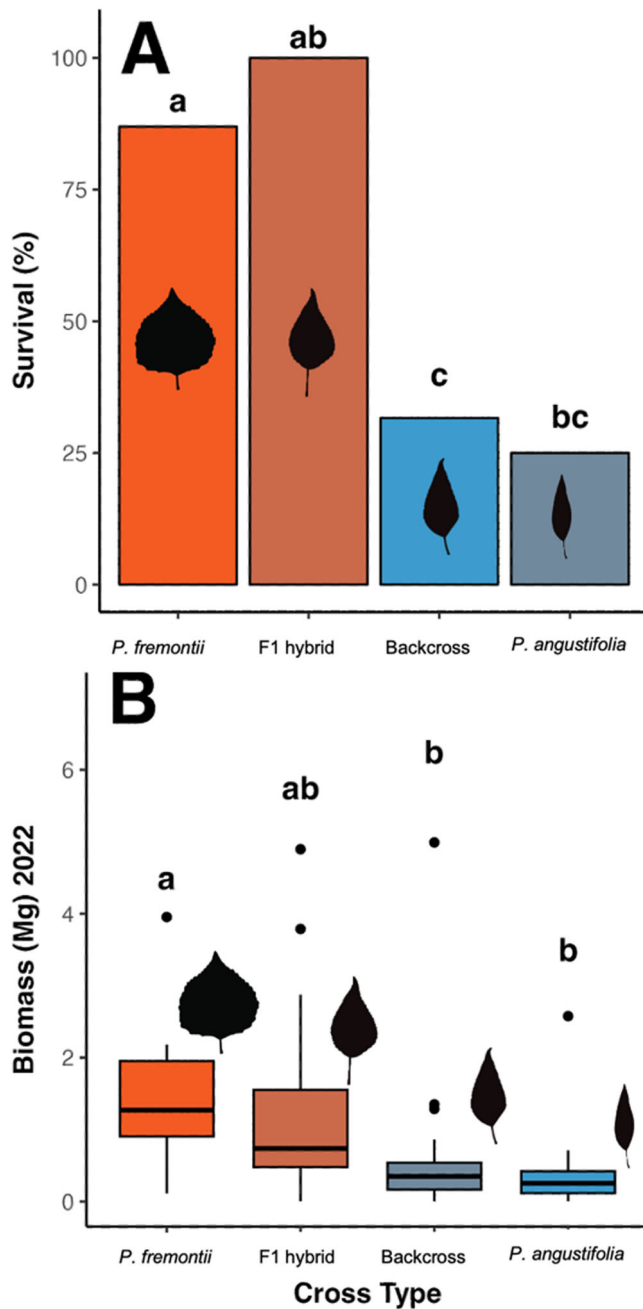
## Discussion

Long-term common garden experiments are rare, especially for large, long-lived species, but provide valuable insight into how interspecific interactions, such as hybridization and introgression, can influence ecosystem persistence (or change) under contemporary climate change. Our results shed light on the broad consequences of natural hybridization. We found strong differences in selection among two species and their hybrids, and that selection and growth differences were strongly associated with genetic factors. Specifically, the lower elevation *P. fremontii* had greater survival in the low elevation common garden than the higher elevation *P. angustifolia* (Fig. 1). F<sub>1</sub> hybrids were more similar in growth to *P. fremontii*, suggesting that *P. fremontii* traits in these hybrids are dominant over *P. angustifolia* traits. In another study examining *P. fremontii* and *P. angustifolia* survival after a record drought, Hultine et al.<sup>25</sup> found that both parental species suffered 3–4 times greater mortality in the wild relative to naturally occurring F<sub>1</sub> hybrids. This suggests that hybrid vigor, or heterosis, is also a potential outcome of hybridization in this system (also see refs. 23,24). Backcross trees had similar survival and biomass to *P. angustifolia* genotypes. F<sub>1</sub> hybrids did not significantly differ from either parental species in biomass or survival, suggesting that hybridization increases phenotypic variability in these individuals. In natural settings, these patterns are likely to have consequences for both individual species distributions and the composition and abundance of parental species and crosses in hybrid zones. Our results also show that the probability of survival decreased for trees as the distance and MAT between the common garden and source population increased. Thus, the deleterious impacts of climate change on *P. angustifolia* are likely to increase due to stronger selection pressures as the magnitude of climate change increases.

Although we saw high overall mortality among the *P. angustifolia* and backcross genotypes in the common garden, our study also provides evidence that hybrid introgression can be adaptive and has the potential to mitigate the negative impacts of climate change by enriching species' genetic variation. Greater genetic variation increases adaptive capacity, and therefore also increases the probability of persistence under climate change. Our results demonstrated this effect, as we show that a *P. fremontii* genetic marker is associated with greater survival among the surviving *P. angustifolia* and backcross trees. These findings provide evidence that introgression can be a means of surviving the strong selective forces of climate change. At larger scales, climate change effects are likely to alter the genetic and trait-based landscape of western riparian forests, and climate-driven shifts in genetic variation may result in altered ecosystem processes.

### Rapid evolution in response to climate change

Determining whether species can undergo adaptive evolution at a pace commensurate with contemporary climate change (i.e., rapid evolution) is a critical step in predicting the long- and short-term consequences of changes in abiotic conditions<sup>26–29</sup>. A growing body of evidence shows that rapid evolution over the course of a few years to decades is possible. For example, a resurrection study comparing *Brassica rapa* plants grown from four cohorts



**Fig. 1 | Cross types vary in survival and growth.** Survival (A) and growth (B) for two hybridizing species *Populus fremontii* and *P. angustifolia*, F1 hybrids and backcross genotypes differ after 31 years of growth in a common garden. Leaf icons correspond to cross types, letters correspond to significantly different groups identified with a Tukey HSD post hoc test.

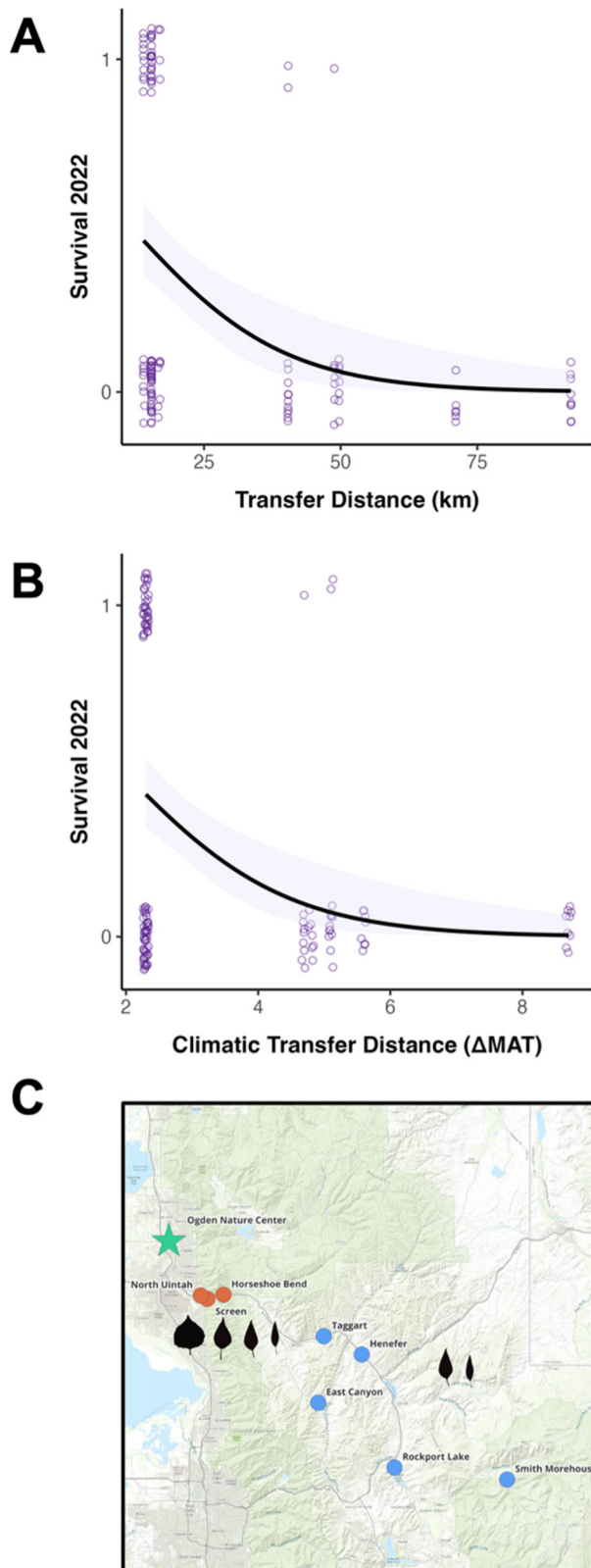
of seeds collected before and after major drought events found that evolutionary change had occurred within 18 generations<sup>30</sup>. Post-drought plants diverged in traits from pre-drought plants, overall showing both earlier phenology (here, flowering time), smaller stem diameters, and lower water use efficiency relative to pre-drought plants<sup>30</sup>. Interestingly, *B. rapa* flowering time shifts tracked oscillations between wet and dry periods. Seeds from the youngest cohort flowered earlier than the oldest cohort, but the intervening cohorts showed a shift back to later flowering time for seeds produced after a two-year wet period. After this intermediary wet period, the drought returned, and flowering time shifted back to the earlier timeframe. Rapid evolution has also recently been demonstrated for the marsh sedge *Schoenoplectus americanus*; Vahsen et al.<sup>6</sup> used a resurrection approach to

compare plants grown from seeds collected from an age-stratified seed bank. They found differences in the distribution and magnitude of belowground biomass and root:shoot ratios (~50–70% of which was explained by heritable variation) both between geographic provenances and between seed age cohorts. Importantly, these differences resulted in projected shifts in ecosystem-level processes related to the capacity of marsh resilience to sea level rise expected under climate change (i.e., soil surface accretion and carbon accumulation)<sup>6</sup>. Given this evidence demonstrating that rapid evolution in plants is possible, a current frontier in climate change adaptation studies is to determine the mechanisms by which the adaptive traits and underlying genetic variation associated with evolutionary change are obtained in populations.

### Hybridization as a mechanism for rapid evolution

Hybrid introgression is a mechanism for rapid adaptive evolution that has gained attention for its potential to increase organismal and ecosystem resilience to climate change<sup>17</sup>. It is particularly interesting to consider for plant species, among which hybridization is extremely common; about 25% of angiosperms can hybridize in natural settings, compared to ~10% of animal species<sup>31</sup>. Hybridization can have various consequences for parental species, ranging from the initiation of speciation (reduplication events associated with hybridization are thought to be a major speciation mechanism for vascular plants) to an overall loss of genetic diversity<sup>32,33</sup>, which can lead to extinction<sup>34</sup>. As early as the mid-20<sup>th</sup> century, researchers speculated that one of the most common and important outcomes of hybridization could be introgression, which is an intermediate to these extreme outcomes<sup>35</sup>. Alleles obtained through introgression can become fixed through neutral processes (i.e., genetic drift), but can also be acted upon by natural selection when they provide a fitness advantage. This process is designated as adaptive introgression, which is a specific case of hybrid introgression (the broader term includes the transmission of any allele, whether it be beneficial, neutral, or deleterious, between species). In fact, introgression is a well-established mechanism for adaptation to changing environmental conditions. Two examples of this are (1) among *Iris* species, in which the introgression of alleles from a flood tolerant species to a dry-adapted species resulted in increased flooding tolerance in the latter in a common garden setting<sup>36,37</sup>, and (2) in *Helianthus* species, in which introgression is associated with altered herbivory and reproductive output, as well as with increased drought tolerance<sup>38</sup>. Hybrid introgression has also been leveraged as a tool for increasing genomic diversity and tolerance to environmental stressors extensively in agricultural settings through introgression breeding or selective introgression. For example, in agricultural crops, introgression breeding has been used to increase disease resistance<sup>39</sup>; it is also frequently used in wheat crops to transfer beneficial alleles from wild to cultivated species (reviewed in ref. 40,41). Similarly, the potential use of introgression as a tool for restoration both via genetic engineering (e.g., to increase blight tolerance in the American Chestnut<sup>42</sup>) and through assisted gene flow has gained interest<sup>41</sup>. The frequency, magnitude, direction, and adaptive value of introgressed alleles in natural settings is not well understood, particularly at large spatial scales. However, as the evidence of its adaptive potential continues to grow, more researchers are identifying the genes and traits being transmitted by this mechanism.

Hybrid introgression has been explored in the *Populus* genus, and some progress has been made towards identifying both its fitness consequences and the specific genes transmitted by this mechanism. The gene regions associated with the RFLP markers we use here have not and cannot be identified<sup>43</sup>, nevertheless, previous studies provide some insight into the types of genes and traits that have moved between *Populus* species. For example, previous studies show evidence that hybrid introgression influences the range dynamics of *Populus* species. In a 2018 study, Suarez-Gonzales et al.<sup>44</sup> showed that introgression from the lower elevation species *P. trichocarpa* (black cottonwood) to the higher elevation species *P. balsamifera* (balsam poplar) facilitates northern range expansion. Further, they identified the nitrate transporter *AtNRT2* as a candidate gene for traits related to phenology (e.g., leaf out date) and biomass (height) that was



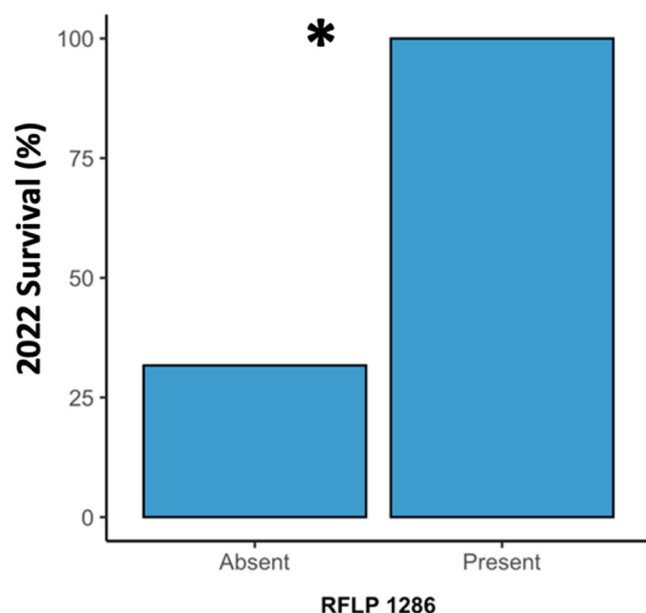
**Fig. 2 | *P. angustifolia* and backcross trees from populations climatically similar to the common garden have higher survival.** Mortality of *Populus angustifolia* and backcross trees (i.e., the percentage of trees from each population that died between 1991 and 2022) increased as both **A** geographic distance and **B** climatic distance (i.e., the absolute value of the difference in mean annual temperature (MAT) between the common garden and source population) increased. Individual data points have been jittered vertically to increase visibility. **C** A map of showing the location of the common garden (green star) and the populations from which the trees were collected. Orange dots indicate populations from which all four cross types were collected, while blue dots indicate populations from which only *P. angustifolia* and backcross trees were collected. Map in C was created using the Open Source Geospatial Foundation Project through QGIS Geographic Information System (2024).

observed as a transfer from the narrowleaf cottonwood to the balsam poplar. They identified introgression outliers (i.e., introgressed SNPs that were present more frequently than expected under a neutral demographic model and are thus likely under positive selection) and found that the gene regions flanking these outliers were related to photoperiodic regulation and the synthesis of cell wall components important for dormancy. Relative to the balsam poplar, the narrowleaf cottonwood is adapted to warmer climates with longer photoperiods in the growing season thus, it seems that the introgression of narrowleaf alleles related to photoperiodic regulation facilitates balsam poplar adaptation at its southern range margin. In this scenario, *Populus angustifolia* is analogous to *P. fremontii* in study system utilized here. Although we expect that many introgressed alleles will be neutral in their fitness effects, these examples demonstrate that adaptive introgression is likely common among *Populus* species and that it is likely possible to identify specific introgressed alleles associated with phenotypic traits that confer fitness advantages.

Previous work in this 31-year-old common garden also provides clues as to what kinds of genes and traits may be involved in adaptive introgression between *P. fremontii* and *P. angustifolia*. For example, marker RFLP-755 has been identified as being associated with increased cloning, which may be a particularly important as the Western US continues to undergo aridification<sup>21</sup>. Martinsen et al.<sup>20</sup> demonstrated the adaptive value of introgression by showing that hybrids act as a selective filter (i.e., introgressed fragments of different sizes move into *P. angustifolia* populations at different rates, and fragments which introgress further into the *P. angustifolia* zone are candidates for adaptive introgression). Our marker-trait associations support this idea, as individuals with RFLP-1286 had increased odds of survival relative to individuals without the marker. Although our study did not directly test the effects of introgression on range dynamics, we show that mortality increases with the magnitude of climate change based on transfer distance. Recent ecophysiological studies with *P. fremontii* have not yet attempted to tie functional traits to specific markers; however, they have identified traits that have clear ties to greater tolerance to increased temperatures associated with climate change that could potentially benefit *P. angustifolia* populations via adaptive introgression. In common garden studies, Blasini et al.<sup>46,47</sup> showed adaptive trait syndromes of phenology, wood density, xylem vessel size, and leaf economic spectrum traits of leaf area, stomatal densities, and stomatal conductance genetically varied among populations and allowed trees to tolerate temperatures exceeding 41 °C. Similarly, in a 2017 study Fischer et al.<sup>48</sup> found strong differentiation among *P. fremontii* genotypes in phenology and productivity in a southern Arizona common garden. In combination, these studies imply that local adaptation in *P. angustifolia* may be more likely to occur in warm edge, or low elevation populations that are more likely to overlap with *P. fremontii* populations.

Introgression between hybridizing species can alter allele frequencies in focal species' populations through positive selection on beneficial alleles or the integration of neutral alleles into the genome. These shifts can potentially impact larger scales of biological organization, depending on the trait these alleles are related to. Here, we show that climate-driven selection is favoring *P. angustifolia* backcrosses with genetic material obtained via introgression from *P. fremontii*. The strong selective pressures associated with climate

acquired through introgression. Nitrate regulation is an important component of physiological changes related to dormancy cycling<sup>45</sup>. Chhatre et al.<sup>15</sup> characterized introgression in a tri-species hybrid complex at the range margins of *P. angustifolia* (narrowleaf cottonwood), *P. balsamifera* (balsam poplar), and *P. trichocarpa* (black cottonwood) and found introgression occurring among all three species, although it was most commonly



**Fig. 3 | *P. angustifolia* individuals with *P. fremontii* marker RFLP 1286 are more likely to survive climate change.** In the common garden in 2022, survival among *P. angustifolia* and backcross genotypes was significantly different between individuals with marker 1286 present (100% surviving) and those with the marker absent (~30% surviving).

change (e.g., increased temperatures, altered precipitation regimes) will continue to act on these markers, likely making these more productive phenotypes dominant under global change. Under climate change, *P. fremontii* and  $F_1$  hybrid genotypes may become more dominant in regions where the range of the former overlaps with *P. angustifolia* due to greater heat tolerance. Such shifts may have implications for C sequestration, as we have shown *P. fremontii* trees are significantly more productive, and also cycle C faster due to enhanced tissue decomposition and belowground respiration<sup>22,23</sup>. Either of these scenarios, if occurring in natural populations, could represent examples of ecosystem evolution (i.e., a genetically-based change to an ecosystem process) occurring via genetic change to a foundation species but further investigation is required to characterize these impacts at large scales and across different climatic gradients.

### Hybridization and conservation policy

In the United States, the “hybrid policy” advocated by O’Brien and Mayr<sup>49</sup> is to “discourage hybridization between species” because hybridization may “disintegrate the genetic organization of the species in contact.” However, this view, especially with regard to plants, has long been challenged as there are a number of significant counter arguments (see refs. 50,51). In addition to hybrid zones being hot spots of biodiversity and critical habitat for unique species<sup>52</sup>, hybridization in plants has been documented as a common pathway in plant evolution and speciation<sup>32,33,53–56</sup>. In a 30-year review of the Endangered Species Act, Haig and Allendorf<sup>57</sup> note that hybrids are not included in the definition of species and thus not protected. Nevertheless, some hybrid species are assessed on a case-by-case basis. For example, specific hybrid palms and cliffroses are now officially protected and explicitly identified in recovery plans<sup>50</sup>. In the latter case, two species of cliffrose (*Purshia subintegra* and *P. stansburiana*) hybridize naturally, and although these hybrids are beyond the definition of a species, the U.S. Fish and Wildlife Services’ recovery plan recognizes that their hybrid swarms “illustrate the migratory and dynamic nature of evolving plant populations. Plants in the hybrid swarms are genetically and phenotypically variable, represent a piece of the evolutionary history of *Purshia*, and may provide the key to the future of the genus and species,” thus justifying the need for conservation of these specific hybrid populations<sup>58</sup>. The present study adds to this argument by presenting an example of adaptive introgression that

could be important for promoting evolutionary responses to global warming that are occurring naturally in native hybridizing species.

Long-term common garden experiments can provide a unique insight into the potential for natural hybridization to be utilized in conservation and management practices. It has been suggested that interspecific gene flow in the form of facilitated introgression is an underutilized conservation tool<sup>41</sup>. This tool has been explored as an option in the restoration of the American Chestnut (*Castanea dentata*), which has been driven to near extinction by the fungal pathogen *Cryphonectria parasitica*<sup>42</sup>. The intentional introgression of blight resistance genes from Asian chestnut species using genetic engineering is suggested as a potential avenue for reintroducing the American chestnut into natural settings. Our study shows that hybrid introgression has the potential to increase climate change resilience, and if the specific genes underlying resilience-related traits can be identified, intentional introgression could be more realistically considered for conserving this rare but important riparian forest ecosystem type.

While there has been interest in hybrid introgression as a form of gene flow and a mechanism for adaptive evolution, its prevalence in natural populations has long been unclear. Our results provide compelling evidence that hybrid introgression may be an under-recognized mechanism of rapid evolution and adaptation in response to climate change. Further, we show that hybridization, broadly, and introgression, specifically, can lead to shifts in traits, the consequences of which may extend beyond the species’ level to alter ecosystem function. Elucidating the extent, distribution, and fitness effects of introgression at larger spatial scales and identifying and characterizing the effects of candidate genes for these introgressed regions of the genome on populations, communities, and ecosystems will allow us to better understand and predict the fate of species under climate change.

Foundation species, like *P. angustifolia* and *P. fremontii*, have a disproportionately large impact on ecosystem function and modulate a number of ecosystem processes<sup>59</sup>. Because these species dominate the landscapes they inhabit and influence their associated communities through largely non-trophic and mutualistic effects (e.g., shaping microclimates, soil properties, providing habitat, etc.) changes in intraspecific genetic variation in traits related to those interactions and processes (i.e., shifts in the mean or variance of phenotypes within and/or among populations) can have cascading effects to influence higher levels of organization<sup>59–62</sup>. For example, it has been shown that declines in populations of a number of large foundation tree species, including eastern hemlocks (*Tsuga canadensis*) and whitebark pine (*Pinus albicaulis*), alters ecosystem function by decreasing net primary productivity<sup>60</sup>. Our results suggest that introgression and hybridization result in higher biomass phenotypes in populations of the dominant, foundation *Populus* species. *Populus angustifolia* co-occurs and hybridizes with *P. fremontii* in numerous populations across the Intermountain West; if introgression impacting biomass accumulation in this manner is widespread, it is likely to shift aspects of C cycling and soil properties (e.g., through leaf litter decomposition, moisture), and microclimates (through changes in canopy density associated with increased growth) across this geographic region. Characterizing how introgression and hybridization dynamics vary throughout the species’ range in future studies will give more insight into the potential ecosystem-level impacts of trait change driven by these processes.

## Methods

### Common garden

Many riparian forests across the western US are dominated by *Populus* species. High elevation localities are frequently occupied by *P. angustifolia* (narrowleaf cottonwoods), while *P. fremontii* (Fremont cottonwoods) occupy lower elevation sites. Hybridization is common where the distributions of these species overlap, and introgression has been shown to occur in a unidirectional pattern with *P. fremontii* genes moving into *P. angustifolia* populations, but not vice versa<sup>63,64</sup>. Four cross types were planted in the common garden we used for this study: “pure” *P. fremontii* and *P. angustifolia* trees,  $F_1$  hybrids, and backcross hybrids ( $F_1$  hybrid x *P. angustifolia* crosses). Genotypes were collected from multiple natural

populations along the Weber River, Utah, which has a 13 km hybrid zone<sup>20,63</sup>. *P. angustifolia* populations were collected at elevations between 1500–2350 m, hybrid and backcross hybrid populations between 1330–1500 m, and *P. fremontii* populations between 1280–1330 m. In 1990 and 1991, 199 clones, representing 63 naturally occurring genotypes of both parental species,  $F_1$ , and backcross hybrids, were randomly planted on 4 m centers in a common garden in Ogden, Utah, at the Ogden Nature Center (1300 m). Geographic transfer distance (the distance between the common garden and the population of origin) and climatic transfer distance (the absolute value of the difference between the MAT of the common garden and the population of origin) were calculated for each source population. The site receives approximately 459 mm of precipitation annually (MAP) and MAT is 10.3 °C. MAT among source populations ranges from 1.6 °C to 8 °C, while MAP ranges from 491 to 663 mm. The soil at the common garden is in the Entisol USDA Soil Taxonomic order and is composed of ~60% sand, ~30% silt, and ~10% clay<sup>22,65,66</sup>. The cross type status (i.e., *P. fremontii*, *P. angustifolia*,  $F_1$  hybrid, or backcross hybrid) for each tree was determined in earlier studies<sup>20,63</sup> based on the presence of *P. fremontii*-specific RFLP molecular markers (Fig. S2)<sup>67</sup>. Since RFLPs are codominant, it is possible to identify diagnostic species-specific markers (i.e., stable inter-specific polymorphisms), which can then be used to assign hybrid classes based on the frequency of these loci in individuals<sup>20</sup>. Keim et al. identified a set of 35 RFLP markers (originally developed by Bradshaw et al.<sup>67</sup>) that represented stable polymorphisms between *P. fremontii* and *P. angustifolia*. The genotypes used in this study were a randomly selected subset of the 550 individuals genotyped by Martinsen et al.<sup>20,23</sup>. Additionally, the parental species and their hybrids can be distinguished from one another based on morphological differences; *P. fremontii* has cordate leaves with wide-toothed margins, while *P. angustifolia* has lanceolate leaves with fine-toothed margins. Hybrids have an intermediate morphology, with ovate leaves with intermediately toothed margins (see leaf icons in Fig. 1). These morphological differences have been verified to reliably correspond with the cross types<sup>63</sup>. The combination of morphological differences and RFLP marker data thus provides a robust system for classifying trees by cross type.

In 2003, the 199 trees were surveyed for diameter at 1.4 m above the ground surface (diameter at breast height; hereafter DBH<sub>1.4</sub>) and survival<sup>23</sup>. In September 2022, nearly 20 years later, the remaining living trees in the common garden were resurveyed ( $n = 99$ ). DBH data from both surveys were used to estimate aboveground biomass carbon accumulation based on locally-developed allometric equations relating tree dry biomass to DBH for both species and hybrids between *P. angustifolia* and *P. fremontii* (where ~50% of biomass represents carbon)<sup>22,23</sup>.

## Statistics & reproducibility

**Species and cross type patterns of growth and survival.** To test our first prediction, that there would be differences in growth among the two species and their crosses, we used a linear mixed effects model (“lme4” and “car” in R<sup>68,69</sup>). Biomass was the response variable, cross type was the fixed effect, and genotype was included as a random effect. Biomass data were transformed to meet model assumptions. Ten trees were excluded from analyses due to missing data (source population and genotype,  $n = 185$ ). Similarly, to test whether there were differences in cross type survival, we used a generalized linear mixed effects model with a binomial distribution where mortality (live or dead) was included as the response variable, species cross type as the fixed effect, and genotype as a random effect (“lme4” and “car” packages in R<sup>68,69</sup>). P-values were adjusted using a sequential Bonferroni correction to account for multiple testing. For both models, we used a Tukey’s Honest Significant Difference test to identify differences among groups using the “multcomp” package in R<sup>70</sup>.

## *Populus angustifolia* and backcross survival is related to climate change

To test our second prediction, that mortality among *P. angustifolia* and backcross trees would be related to climatic differences between source

populations and the common garden, we used generalized linear models with geographic transfer distance (km) or climatic transfer distance (°C) as proxies for climate change for each source population that backcross and/or *P. angustifolia* trees were collected from as fixed effects and mortality (binary variable of live/dead) as the response variable and included genotype as a random effect<sup>68</sup>. P-values were adjusted using a sequential Bonferroni correction to account for multiple testing. These analyses utilized the same R packages described above. Transfer distance for *P. fremontii* and  $F_1$  hybrids was not analyzed as we expected them to be acclimated to the climatic conditions in the common garden due to their source populations being nearby. We extracted MAT for each source population from WorldClim<sup>71</sup>. Climatic transfer distance for each source population was calculated by taking the absolute value of the difference between the MAT for each population and the MAT for the common garden. MAT is correlated with numerous temperature and precipitation-related bioclimatic variables (Fig.S1) and, therefore, is employed here as a proxy for broad-scale climatic differences among source populations and the common garden. Additionally, previous ecological niche modelling studies have shown temperature and precipitation variables to be the primary environmental drivers of *P. angustifolia* occurrence patterns<sup>72</sup>. Both the geographic and climatic transfer distance response variables were transformed to meet model assumptions.

## Marker-trait associations among surviving backcross and *P. angustifolia* trees

To test our third prediction, that hybrid introgression would increase fitness in the common garden, we first identified associations between the RFLP markers and patterns of survival among backcross and *P. angustifolia* trees. To do this, we used a model selection approach based on Aikake’s information criterion to identify a subset of the RFLP markers to include in statistical analyses (“MASS” package in R<sup>73</sup>). Prior to model selection, we first took a subset of ten of the 35 *P. fremontii*-specific RFLP by removing highly correlated markers ( $r > 0.7$ ) so that final set of ten candidate markers could be used in analyses. We further investigated the impact of markers identified by this process on tree biomass using an LMM with markers of interest as fixed effects and genotypes as random effects. The p-values for all analyses were corrected using a sequential Bonferroni to account for multiple testing.

## Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

## Data availability

Data available in supplementary file.

## Code availability

Code available at <https://github.com/ashlynnhord/ONC>.

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## Author contributions

J.B. and D.F. collected data. J.B., D.F., and T.W. designed the experiment. A.H. performed data analyses. J.B., D.F., A.H., C.L., J.S., and T.W. wrote the manuscript.

## Competing interests

The authors declare no competing interests

## Additional information

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