Climate-driven convergent evolution on sky islands

Sarah Love (sjaynelove@gmail.com)
University of Tennessee at Knoxville https://orcid.org/0000-0001-8078-7373
Jennifer Schweitzer
University of Tennessee
Joseph Bailey
University of Tennessee

Article

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Abstract

Climate-induced evolution will determine population persistence in a changing world. However, finding natural systems in which to study these responses has been a barrier to estimating the impact of global change on a broad scale. Here we hypothesize that isolated sky islands (SI) and adjacent mountain chains (MC) can serve as natural laboratories for studying the impact of long-term climatic pressures on natural populations. We used greenhouse common garden trees to test whether populations on SI exposed to hot and dry climates since the Pleistocene have diverged from populations on MC, and if populations on SI have converged in their evolutionary responses. We show: (1) in the southwestern U.S., isolated SI are significantly hotter and drier than adjacent MC, (2) populations of *Populus angustifolia* from SI have diverged from MC in reproductive and productivity traits, (3) these traits (cloning and aboveground biomass, respectively) are significantly correlated, suggesting a genetic linkage between the traits, and (4) that the observed phenotypic change is driven both by natural selection and genetic drift. These results suggest that long-lived tree populations on distantly related SI have evolved in response to long-term selective pressures and genetic drift by converging on similar phenotypes and diverging from phenotypes on MC. These shifts are towards potentially beneficial phenotypes for population persistence in a changing world. These results also suggest that the SI-MC comparison is an ideal natural laboratory, as well as predictive framework, for studying responses to climate change across the globe.

Main

Identifying how dominant terrestrial species have adapted in response to climate change is fundamental to understanding which traits are most important for population persistence and subsequent ecosystem functioning under contemporary climate change. Recent studies have shown that atmospheric climate gradients shape the linkage and feedback between genetically based phenotypic variation and ecosystem functions at sub-continental scales across the western United States (Ware et al., 2019, 2021; Bayliss et al., 2020; Van Nuland et al., 2020). These include alterations to soil nutrient pools and bud break phenology driven by mean annual temperature and precipitation, elevation, and genetically based soil conditioning (Ware et al., 2019, 2021; Van Nuland et al., 2020), as well as genetic divergence at the provenance level in atmosphere-plant-ecosystem feedbacks (Bayliss et al., 2020). While these studies show how long-lived species may evolve along contemporary landscape-scale abiotic environmental gradients to affect ecosystem function, they do not directly address how *climate change* has driven evolutionary dynamics, or which traits have been important for population persistence and ecosystem functioning. Evolution can occur on rapid timescales and is critical to understand in a time of rapid environmental change (Grainger et al., 2021; Anstett *et al.*, 2021; Hendry 2017; Davis & Shaw, 2001). While multiple empirical approaches have been used to understand how species have and continue to *respond* to global *climate variation* (Davis & Shaw, 2001; Anderson *et al.*, 2015; Bokhorst *et al.*, 2011; Wooliver, Tittes, & Sheth, 2020), approaches that address how species have *evolved* in direct response to *climate change* are still not well developed.

Sky islands (SI) are globally distributed and have long been seen as laboratories for studying evolutionary ecology in response to geographic isolation and post-Pleistocene climate change (Heald, 1993; Warshall, 1995; McCormack, Huang & Knowles, 2009). On isolated SI, the distribution of many species can be explained by lowland extinctions after the Last Glacial Maximum (~ 18,000 years ago; Pielou, 1991; Wiens et al., 2019). Various studies suggest that before these lowland extinctions, populations on SI and connected mountain chains (MC) were larger, panmictic populations that maintained potential for gene flow (Pérez-Alquicira et al., 2010; Wiens et al., 2019). However, as historical climate change progressed and the habitat surrounding the isolated refugia of SI became less hospitable, some species either moved upslope or remained in place and many have become more isolated. With contemporary climatic changes and up-slope range contractions, the potential for gene flow between large, connected populations on MC and isolated populations on SI has further declined (Yanahan & Moore, 2019; Favé *et al.*, 2015; Hosner, Nyári, & Moyle, 2013). Previous work has shown that isolation of SI by environment and by distance has resulted in genetic divergence, shifts in species interactions, reduction in genetic variation, and rapid speciation on SI (DeChaine and Martin 2005; Baker, 2008; Robin et al., 2010; Manthey & Moyle, 2015; Vásquez et al., 2016; Mairal et al., 2017; Kidane, Steinbauer & Beierkuhnlein, 2019; Wiens et al., 2019; Williamson et al., 2019). These studies show the important roles of reduced gene flow, high environmental pressures, genetic drift, and historical population demographics that have shaped populations isolated atop SI since the Pleistocene (Knowles & Richards, 2005; Woolbright et al., 2014; Wiens et al., 2019). One limitation to these studies, however, is that most have examined only single SI or clusters of SI within a circumscribed geographic region. Thus, they lack the ability to compare adaptations on SI to evolutionary responses in more climatically buffered adjacent populations with greater habitat connectivity. Comparative studies of SI relative to adjacent, continuous MC can be used to examine trait evolution and evolutionary mechanisms important for population persistence and ecosystem function in response to past climate change. The sky island-mountain chain (SI-MC) comparison directly addresses climate-driven adaptive phenotypic change by using hot, dry, and isolated populations on SI to predict population responses at larger scales.

Across the intermountain western United States (U.S.), populations on SI have become increasingly fragmented and have been consistently exposed to the effects of a warming climate. Conversely, through historically maintained connectedness and climatically buffered habitat across population corridors (i.e., across watersheds in a chain of mountains), the potential for gene flow is hypothesized to have remained stable within populations on MC, and climatic selective pressures have remained relatively low (Woolbright et al., 2014). Here, we used the SI-MC comparison to determine if a long-lived tree species has adapted in response to climate change and isolation. Using clonal replicates of trees collected from SI and adjacent MC in riparian areas in the western U.S., we examined intra-specific, population-level divergence to test how reproductive and productivity traits respond to climate change and isolation. Under common garden conditions, we examined how long-term climatic variation in temperature and precipitation has altered cloning (i.e., asexual or vegetative reproduction) and aboveground biomass production (i.e., terrestrial productivity) in contemporary populations of the dominant foundation species *Populus angustifolia* (Evans *et al.*, 2013). We first examined the hypothesis that the climate on SI is

different than that of MC (Hypothesis 1). We found that SI have significantly hotter and drier climates than MC. Given this climatic variation and long-term isolation, we then tested the hypothesis that *P. angustifolia* populations on SI have phenotypically diverged from populations on MC. Specifically, based on previous research (see Ware et al., 2019), we hypothesized that with increased climatic pressures on SI (i.e., heat and drought), *P. angustifolia* populations grown in a common garden will have greater rates of cloning and aboveground biomass than populations on MC, and that these two traits are correlated across the entire study region (Hypothesis 2). Lastly, we used an ANCOVA approach that incorporated neutral genetic microsatellites to test the hypothesis that any observed phenotypic variation is due to climate-induced natural selection and not to genetic drift (Hypothesis 3).

Results

We found that trees from SI experienced different environmental conditions than trees collected from MC. Specifically, tree habitat from the sampled SI was 35% hotter (mean annual temperature; degrees Celsius) and 53% drier (mean annual precipitation; millimeters) than adjacent MC, suggesting stronger climatic selective pressure on *P. angustifolia* populations on SI (Fig. 1a & 1b; Wiens et al., 2019). We found no elevational differences in the population distributions across the SI-MC comparison (**Figure S1b**). This fact suggests that climates have shifted, but ranges have not shifted in response to climate. Therefore, any trait differences that are detected between these groups may be driven by natural selection. If ranges had shifted, the climatic profiles for each population would not vary as greatly as what we have observed, selective pressures would not be as profound, and the phenotypic trait differences would not as likely be due to natural selection.

Consistent with Hypothesis 2, and the strong environmental differences between SI and adjacent MC, we found that populations on SI, grown in a common garden, have converged in functional traits important to population persistence under stressful environmental conditions. Specifically, greenhouse cuttings from populations on SI had 34% higher rates of cloning (number of ramets) and 52% more aboveground biomass (g) than those from populations on MC (Fig. 2a & 2b; Table 1). One hypothesis for this pattern is that natural selection is acting on the fitness trait of aboveground biomass, and as cloning and aboveground biomass are positively correlated, high-aboveground biomass trees also produce more clones. Consistent with this hypothesis, we found a weak but significant positive correlation (R^2 = 0.022, p-value = 0.05016*) between cloning and aboveground biomass, suggesting a possible genetic linkage between the gene complexes responsible for the two traits (Fig. 2c).

Table 1

Reproductive and productivity traits (cloning and aboveground biomass, respectively) from sky island populations (SI) have diverged from mountain chain populations (MC).

	Fixed effects		**Chi-Square test**		**Analysis of Variance**		
Response:	Std. Error	Z-score	χ^2	$Pr(>\chi^2)$	SS	MS	F value
Cloning	0.2268	2.081	4.3305	0.03744 *	4.34	4.34	4.34
Biomass	0.1965	3.368	11.347	0.0007559 *	8.3097	8.3097	11.347

Data refer to summary statistics for generalized linear mixed-effects and linear mixed-effects models, respectively, where cloning and biomass were predicted by SI and MC as a fixed effect, with watershed/genotype as a nested random effect. Asterisks indicate quantitative trait differences that are significantly different from zero (*$p < 0.05$).

Lastly, in partial support of Hypothesis 3, we found that natural selection and genetic drift were responsible for patterns of genetic divergence for aboveground biomass and cloning, respectively. Based on previously established methods for determining the evolutionary roles of natural selection versus genetic drift, we used an ANCOVA approach with neutral genetic microsatellite data as a covariate in the phenotypic models described in Hypothesis 2 to account for population genetic structure (see Kooyers et al., 2015). Specifically, we performed a principal components analysis (PCA) on the z-scores of the microsatellite associated with individual genotypes and incorporated the first two principal component axes (PC1 and PC2) into the phenotypic models. Incorporating genetic markers in the trait models and observing the remaining significance attributed to mountain class (i.e., SI or MC) allows us to interpret any observed quantitative trait differences as a consequence of natural selection or genetic drift induced by mountain class. When microsatellite data were included in the phenotypic models (Table 1), we found that the differences in aboveground biomass due to mountain class remained significantly different even after accounting for neutral genetic variation (Table 2). This same framework showed that when neutral genetic variation is included in the cloning model, the variation attributed to mountain class is no longer significant (Table 2). Using Akaike Information Criterion (AIC), we found that both models performed best when the microsatellite data were included, suggesting that genetic drift is present in both populations, but does not significantly explain the phenotypic variation in aboveground biomass **(Table S1**). Overall, we conclude that natural selection from climate on SI is the driving evolutionary force behind variation in aboveground biomass, and genetic drift from isolation on SI is the driving force for variation in cloning.

Table 2
Convergent trait variation is driven by natural selection for aboveground biomass and genetic drift for cloning.

Factors	χ^2	Pr(>χ^2)
Response: Cloning		
Mtnclass	0.7226	0.3953
PC1	0.0908	0.7632
PC2	0.0257	0.8726
Response: Aboveground biomass		
Mtnclass	33.5313	7.013e-09 *
PC1	1.7287	0.1886
PC2	0.5824	0.4454

Data refer to summary statistics for mixed-effects models where cloning and aboveground biomass response variables were predicted by mountain class (“Mtnclass”: SI or MC) and microsatellite principal components axes (PC1 and PC2), with watershed/genotype as a nested random effect. Asterisks indicate differences that are significantly different from zero (*p < 0.05).

Discussion

Evolutionary responses to climate variation

Contemporary climate change is projected to increase temperatures and the prevalence of severe drought on a global scale, especially in the western U.S. (Dai, 2011; IPCC, 2021). Numerous studies suggest that plant species will likely respond to the stress caused by climate change by implementing shifts in their geographic ranges (Hughes, 2000; Walther *et al.*, 2002; Parmesan & Yohe, 2003; Parmesan, 2006; Chen et al., 2011). However, relying on bioclimatic envelopes to predict and model future species’ distributions in response to climate change has largely underestimated the adaptive capacities of long-lived species through phenotypic plasticity or rapid evolution (Fuller et al., 2010; Zhu et al., 2012). One phenotypically adaptive mechanism by which populations may persist under rapid climate change is through evolutionary shifts in cloning (i.e., asexual regeneration) and overall biomass production (i.e., productivity). Here we have shown that the SI of the intermountain West are significantly hotter and drier than adjacent MC, not due to variation in range elevation. Populations of *P. angustifolia* on SI overall have converged in responses to this climate variation and show significant increases in cloning frequency and aboveground biomass. Cloning as an asexual reproductive trait allows a genet to persist on the landscape, as well as “scavenge” for resources spatially that contribute to persistence and resilience in variable environments (Price & Marshall, 1999; Matsuo et al., 2014; Woolbright et al., 2014; Barrett 2015; Bittebiere, Benot & Mony, 2020). Adaptation toward greater vegetative growth (cloning) and productivity (aboveground biomass) in higher-stress populations of *P. angustifolia* on SI may indicate

that phenotypic trade-offs between reproduction and growth that are common in high-stress environments (King & Roughgarden, 1982; LaDeau & Clark, 2001; Qiu et al., 2021; Oddou-Muratorio et al., 2021) are not essential to the survival of this species (*sensu* Knops, Koenig, & Carmen, 2007). Across the SI-MC comparison, cloning and aboveground biomass were significantly correlated, suggesting genetic linkage between the two traits that may be influenced by environmental variation. Due to its value as a bioenergy crop, much research has gone into identifying genetic controls on aboveground biomass in the genus *Populus* (Bradshaw & Stettler, 1995; Rae et al., 2007; Rae et al., 2009; Allwright et al., 2016; Badmi et al., 2018). However, no research to our knowledge has attempted to tackle the genetic underpinnings of cloning as a reproductive strategy in *Populus*. Further research is needed to elucidate the genetic basis underlying the observed correlation between cloning and aboveground biomass. Finally, by examining the influence of neutral microsatellites on the observed phenotypic variation, we found that genetic drift is the primary mechanism driving differences in cloning between SI-MC populations. Conversely, natural selection, induced by strong selective climates like those seen on SI, is the primary evolutionary process shaping biomass responses in these populations (*sensu* Töpel et al., 2012; Reznik & Ghalambor, 2001; Levin, 2003; Jump & Peñuelas, 2005; Franks, Sim & Weis, 2007; Bertel et al., 2018).

Genes to Ecosystems

These results indicate that convergent evolution among SI can lead to predictable ecosystem-level consequences driven by climatic variation relative to MC, irrespective of the evolutionary mechanism at play. High aboveground biomass production and high rates of cloning in warmer and drier populations on SI represents carbon sequestration that influences overall ecosystem productivity (Nakamura et al., 2011; Robinson, Ryan, & Newman, 2012; Chen, 2017). Previous work detailing the evolutionary responses of species isolated atop individual or adjacent SI have shown the dramatic impact that isolation and climatic exposure on SI can have on a wide variety of taxa (DeChaine & Martin, 2005; Robin et al., 2010; Vásquez et al., 2016; Bertel et al., 2018; Knotek et al., 2020; Tusiime et al., 2020). We suggest that the study of SI may be made more robust by studying geographically adjacent MC comparatively to address evolution in direct response to climate change, as opposed to other approaches that infer evolution in response to climate variation. The climatic histories and demographic structure (i.e., low levels of gene flow and limited dispersal, and high selective pressures and potential for genetic drift) commonly found on SI, relative to MC, make SI-MC comparisons ideal for future climate change studies across the globe and will allow for direct tests of the role of climate change from genes to ecosystems.

Conclusions

These results show the empirical utility of the SI-MC comparison as a natural laboratory and predictive framework for studying evolution in response to climate change in natural populations. The SI-MC comparison should allow for a greater understanding of the genetically based phenotypes that promote population-level persistence in stressful environments and determine the ecological and evolutionary mechanisms (i.e., selection or drift) that drive ecosystems to persist globally (Zavaleta et al., 2009; Woolbright et al., 2014). The global breadth of the SI-MC comparison is ripe for studies that examine the

genetic basis by which populations persist or perish across a fragmented and vulnerable contemporary landscape. For example, SI and MC can be found across the entire natural range of *P. angustifolia* from northern Mexico to Alberta, Canada, and are present in all three genetic provenances identified for the species (Evans *et al.*, 2013). Moreover, SI and MC are found on almost every continent and similar comparisons are possible across many species of plants, animals and microorganisms, providing comparable opportunities to examine evolution in response to climate and isolation around the globe. Combined, using the SI-MC comparison across a large geographic scale to understand population- and community-level responses to climate change will allow for a better understanding and more robust predictions of climate-driven evolution, and subsequent ecosystem-level responses, across the globe.

Methods

Study species and site selection

Populus angustifolia James (Salicaceae) is a native high-elevation, foundation tree species dominant in riparian areas (900-3,500 m) across the intermountain western U.S. (Cooke & Rood, 2007). Throughout its natural range, *P. angustifolia* forms distinct genetic populations, largely driven by reduced gene flow resulting from variation in the intermountain landscape (Evans *et al.*, 2015). The fragmented contemporary range of *P. angustifolia* reflects historical bottlenecks driven by Pleistocene glacial cycles, as well as contemporary climate change (Pielou, 1991; Evans *et al.*, 2015). *Populus angustifolia* extends northwards from northern Mexico into southern Alberta, Canada; a geographic region also characterized by the presence of SI (Evans *et al.*, 2015; McCormack *et al.*, 2009). SI are isolated mountain habitats surrounded by desert lowlands or habitats outside of the range of species' thermal tolerance, serving as a barrier to species dispersal and migration (Knowles et al., 2020; McCormack *et al.*, 2009). Conversely, populations of trees from the continuous MC throughout the natural range of *P. angustifolia* are not dispersal-limited, as the riparian habitat between the high-elevation zones on MC is climatically suitable for within-population dispersal and migration. *Populus angustifolia* exhibits both asexual and sexual reproductive strategies, mostly by cloning and pollen/seed dispersal by wind and water (Braatne, Rood & Heilman, 1996; Schweitzer et al., 2002). Due to the obligate riparian nature of *P. angustifolia*, populations on SI used in this study constitute distinct populations, devoid of the potential for any significant gene flow (Evans *et al.*, 2013). Isolated SI across the landscape, paired with multiple adjacent MC, serve as natural replication for our study (Moore *et al.*, 2013). In May and June of 2012, 8 watershed populations (3 SI: Indian Creek "IC", UT; Lexington Creek "LEX", NV; Great Basin "GBS", NV. 5 MC: Dolores River "DOL", CO; Logan River "LOG", UT; Ogden Canyon "OGC", UT; San Miguel River "SMIG", CO; Weber River "WR", UT) of *P. angustifolia* were surveyed (**Figure S1a**). QGIS bioclimatic variables were determined for each sampling location (Ware et al., 2019; Hijmans et al., 2005). Georeferenced climate data for mean annual temperature and mean annual precipitation were collected for each tree from WorldClim (Fick & Hijmans, 2017). In the field, 826 genotypes (n = 505 genotypes from MC; n = 321 genotypes from SI) were georeferenced and sampled and 10 terminal branch cuttings (~ 20 cm long) were collected from each tree. The cuttings were initially established in general potting soil (equal parts vermiculite, perlite, and

peat) for 4 months (see Ware et al., 2019 for details). Surviving cuttings were replanted in 6.4 x 36 cm plastic pots and grown under identical ambient common garden conditions at the University of Tennessee Knoxville greenhouse.

Greenhouse common garden and quantitative trait measurements

To understand how *P. angustifolia* populations on SI have diverged in stress-adaptive functional traits (Hypothesis 2), cuttings were grown in the University of Tennessee Knoxville greenhouse. All plants in the common garden experienced similar environmental conditions: they were watered every 2–3 days under near-ambient seasonal temperature conditions. This setup allowed for the isolation of the genetic basis to trait variation. In 2012, aboveground biomass (g) was measured from the main stem of each replicated tree genotype using an allometric equation established by Van Nuland et al. (2017): *Aboveground biomass (g) = (stem volume (mm^3) x 0.41899) – 2.40137*. In 2016, cloning was quantified from all surviving greenhouse trees by counting the number of ramets present within each pot. Cloning was measured in 2016 as this was the year that cuttings began to produce ramets.

Molecular genetic analyses

We used microsatellite data to resolve the observed evolutionary patterns in quantitative traits, identify individual genotypes of each tree, and determine population genetic divergence in the study areas. In the field, leaves were collected from each genotype and were dried in a 70°C oven for 48 hours, finely ground using a SPEX SamplePrep 8000D Dual Mixer (SPEX SamplePrep, Metuchen, NJ, USA); genomic DNA (gDNA) was extracted using a Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) following manufacturer's protocols. To reduce PCR inhibition, the gDNA samples were diluted at 1:10 (1-part gDNA, 9 parts molecular-grade water). We used nine microsatellite markers from the following sources to determine multi-locus genotypes for each tree: one marker described by Tuskan *et al.*(2004), and eight described by Tuskan et al. (2006). PCR amplification and electrophoresis were executed using the techniques described by Ware et al. (2019). To control for clonal genotypes, we used the "multilocus matching" function in GenAlEx v.6.4 (Peakall & Smouse 2006, 2012) to identify samples with 100% microsatellite loci similarity.

Statistical analyses

To examine whether the climate on SI is hotter and drier than the climate on MC (Hypothesis 1), we employed linear mixed-effects models for mean annual temperature (MAT) and mean annual precipitation (MAP) response variables, separately, with mountain class (SI or MC) as the fixed effect and watershed population as a random effect ("lme4" package, R; Bates, Maechler, Bolker & Walker, 2014). To address the contribution of elevation to any observed population-level variation and to eliminate the alternative hypothesis that ranges have already shifted, we conducted a linear mixed-effects model with elevation as the response variable, mountain class (SI or MC) as the fixed effect, and watershed as a random effect. Subsequent ANOVAs were conducted for all models.

To address the hypothesis that isolated populations of SI have diverged in stress-adaptive functional traits, relative to MC (Hypothesis 2), we used a generalized linear mixed-effects model with a Poisson log link function for the cloning response variable, with mountain class (SI or MC) as a fixed effect, watershed/genotype as a nested random effect. This model was selected to account for the right-skewed cloning count data with a Poisson distribution. For the aboveground biomass response variable, we used a linear mixed-effects model with mountain class (SI or MC) as a fixed effect and watershed/genotype as a nested random effect. Aboveground biomass was log-transformed for normality. For both models in Hypothesis 2, a nested random effect of watershed/genotype was chosen to account for variation at each level (i.e., watershed, genotype). This procedure allowed for a more accurate assessment of any observed SI-MC effect and is a commonly used practice in classical studies in evolutionary ecology with nested design (Schielzeth & Nakagawa, 2013). Subsequent ANOVAs were performed on the cloning and aboveground biomass models to determine significance. A two-sided Pearson's product-moment trait correlation was used to test the hypothesized quantitative trait linkage between cloning and aboveground biomass across the SI-MC comparison.

To examine the mechanisms of evolution (i.e., natural selection or genetic drift) driving trait divergence (Hypothesis 3), we used an ANCOVA approach that incorporated neutral microsatellite data into the mixed-effects models employed for Hypothesis 2 (see Kooyers et al., 2015). To reduce dimensionality and account for loci collinearity, we first performed a principal component analysis (PCA) ordination on the z-scores of the microsatellite data. We then extracted the main principal components that explained the majority of the microsatellite variance (PC1 and PC2). The principal component axes were then added to the quantitative trait mixed-effects models described for Hypothesis 2. To test model fit for each trait, models employed in Hypothesis 2 without microsatellite PC axes were used as null models and were run in parallel with the alternate models that included microsatellite PC axes. Akaike Information Criterion (AIC) estimates were compared between each. For each trait, models containing PC1 and PC2 performed better than the null models without the PC axes, suggesting that genetic drift is present within both populations. For Hypothesis 3, if the observed variation in cloning and aboveground biomass across the SI-MC comparison seen in Hypothesis 2 remained significant after adding the microsatellite PC axes to the models, the evolutionary process driving the trait variation is consistent with adaptive trait differentiation (i.e., natural selection). Conversely, if the observed trait variation becomes insignificant after incorporating the microsatellite ordination axes, neutral and demographic processes (i.e., genetic drift) are more likely responsible for trait variation across the landscape (Kooyers et al., 2015; Price *et al.*, 2006). For all hypotheses, statistical significance ($p < 0.05$) was determined by Anova Type II Wald chi-square tests for each model ("Anova" function, "car" package, R; Fox et al., 2020).

Declarations

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Figures

Figure 1

The climate is different between sky islands (SI) and mountain chains (MC). A. Mean annual temperature (degrees Celsius) is 35% higher on SI than on MC. B. Mean annual precipitation (millimeters) is 53% lower on SI than MC. Asterisks denote a significant difference (*p < 0.05).

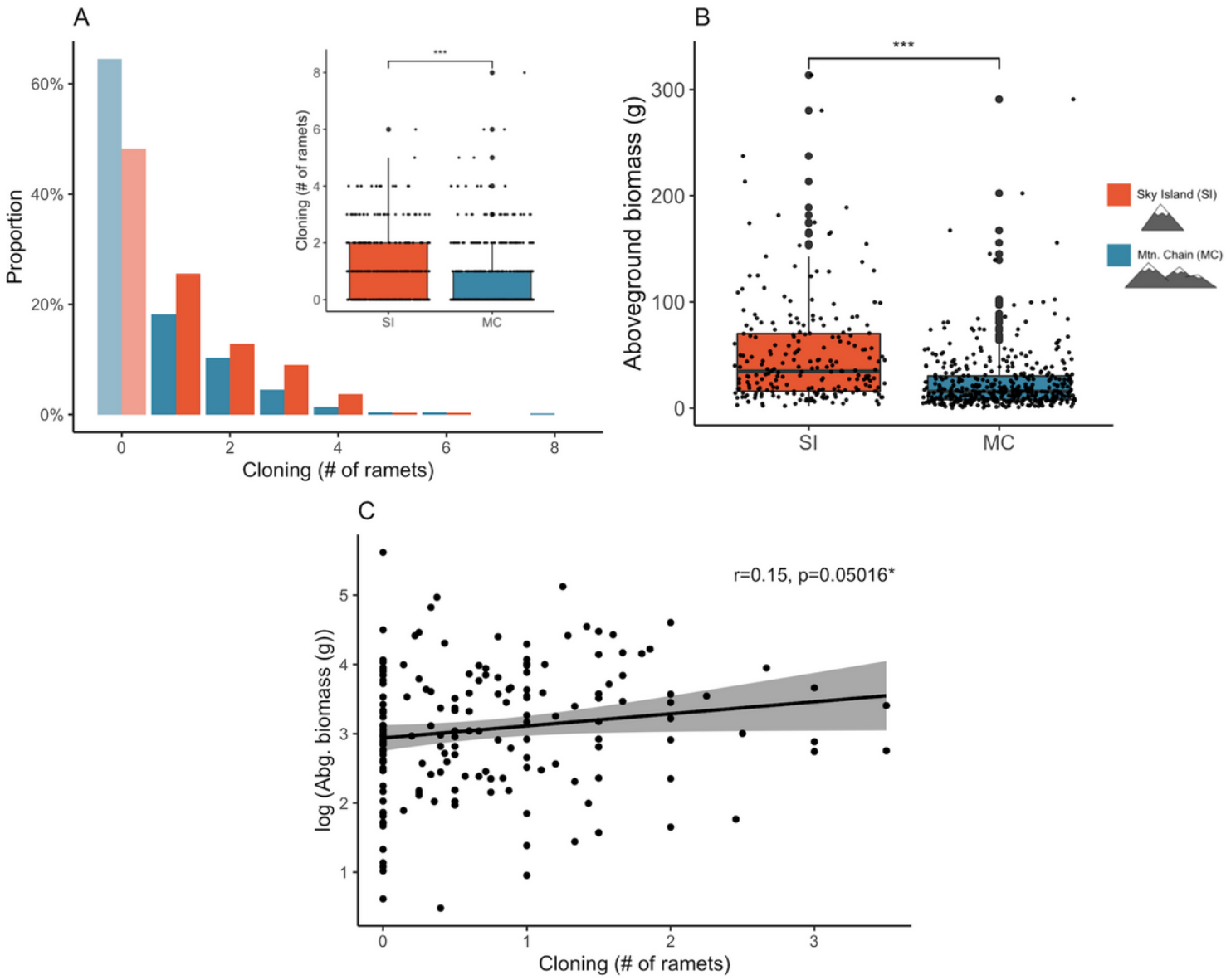

Figure 2

Functional traits of *Populus angustifolia* vary between sky islands (SI) and mountain chains (MC) when grown in a common environment, and are positively correlated. A. The mean number of clonal ramets and B. aboveground biomass are significantly higher on SI than MC. Panel A shows the normalized mass of cloning ramets (#) on SI (orange) and on MC (blue), separately. Each orange bar shows the proportion of ramets from trees from SI with a given cloning ramet number (x-axis), blue bars correspond to the same for MC; all bars in each color add up to 1. The inset is a box and whisker plot representative of these data. In each box and whisker plot, black circles denote genotype replicates (n = 505 genotypes on MC; n = 321 genotypes on SI). Asterisks denote a significant difference (*p < 0.05). C. Cloning (average number of clones per genetic replicate) and aboveground biomass (grams; log-transformed) are significantly, positively, correlated.

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