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An Analysis of Potential Parallel Evolution between Two Spatial Scales of Balsam Poplar, Populus balsamifera

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Abstract

Local adaptation to climate provides strong evidence for the operation of natural selection. When climate gradients are shared, there is a potential for parallelism of local adaptation. By studying this potential parallelism, we gain insight into the balance between natural selection and gene flow operating within a given system. Here, I explore the idea of potential parallel evolution of local adaptation between a broad spatial scale and a fine spatial scale within *Populus balsamifera*. To do this, we grew trees from both spatial scales in a common garden and measured selected bud phenology traits (growing season length, bud set, bud flush, and leaf flush). These traits were first analyzed with linear mixed models to determine if there were significant clinal relationships between traits and source climate. Then the models of broad spatial scale individuals were used to predict trait values for the fine spatial scale individuals to determine if broad-scale relationships can accurately predict those of the fine scale. The results showed significant clinal relationships for the broad scale, but not for the fine scale. These broad-scale relationships successfully estimated the trait values of the fine scale but often left considerable variation unexplained. These results suggest that at the broad scale, there is a clinal relationship between phenology and source climate. The fine-scale individuals fall within this trend; however, they show no relationship of their own. Thus, selection is able to overcome the homogenizing effects of gene flow at broad spatial scales, but at fine spatial scales, the strength of gene flow cannot be overcome by selection. This study provides new insight into the evolutionary drivers operating at these spatial scales within *P. balsamifera*.

Introduction

Local adaptation describes the process by which members of a given species evolve to exhibit on average higher relative fitness in their local environment when compared to those members originating from a different environment. Thus, local adaptation specifically refers to this process observed among populations within a single, interconnected species across spatially dispersed populations (Kawecki & Ebert, 2004). Local adaptation is caused by spatially divergent selection, where different environments select for different genotypes with the highest fitness in that environment. When considering the different environments that a given species occupies, they are often distinguished through differences in climate. Climate is a primary agent of selection and is often looked to when studying local adaptation. Numerous studies across species have shown local adaptation in response to climate including in butterflies (Roy et al., 2015), invasive plants (van Boheemen, Atwater, & Hodgins, 2019), and even Siberian humans (Hallmark et al., 2019). However, other forces such as gene flow and genetic drift may also affect populations in ways that disrupt or prevent local adaptation.

Parallel evolution refers to the process wherein selection acts similarly across two or more groups of individuals, producing similar reactions to a given stressor. This process can be analyzed within the context of local adaptation. If local adaptation occurs in two distinct groups in a similar fashion as a result of similarities in the local environment, one could conclude the forces of selection are acting in a similar fashion producing parallel evolution of local adaptation. Cases of parallel evolution and local adaptation provide strong evidence for the role of repeatable natural selection in given systems. As a result of this, studies of parallel evolution often aim to elucidate the underlying evolutionary processes occurring within a given study species.

Investigating evidence for local adaptation and the potential for parallel evolution requires measuring genetic variation in phenotypes from samples originating across replicate environmental gradients and grown together using a common garden approach. The rationale behind this approach is that by growing individuals from different source populations in a single area, they are all experiencing the same environment, therefore any differences seen in phenotypic traits are in large part due to differences in genotype. One can then use quantitative genetics techniques to elucidate the genetic basis for complex traits (De Villemereuil, Gaggiotti, Mouterde, & Till-Bottraud, 2016). This study design is especially conducive to plants (Linhart & Grant, 1996) and therefore is often used when studying tree species. One of the most widely used and studied genera within this field is the genus *Populus*.

Populus trichocarpa and its sister species, *Populus balsamifera* are well-studied model organisms within the field of tree biology, therefore, there are lots of resources available for use relating to these study systems (Brunner, Busov, & Strauss, 2004), including the full sequence of the *P. trichocarpa* genome (Tuskan et al., 2006). *P. balsamifera* is an excellent study species due to its fast growth through propagation, its close relation to *P. trichocarpa* which makes use of genomic resources feasible, and because of its wide range (Eckenwalder, 1996). This wide range encompasses a great diversity of climates, making it an ideal candidate for local adaptation studies.

Previous work has been done on *P. trichocarpa* to investigate the impact of local adaptation. Through whole-genome sequencing of 544 unrelated individuals, one study identified 17.4 million SNPs across the genome and provided evidence of multiple selection pressures, climate being a major factor (Evans et al., 2014). Another study used a common garden approach to show strong evidence for phenotypic and genotypic variation being highly correlated to

geographical location and environmental differences (Mckown et al., 2014). Most recently, results have been put forth supporting those of the two previously mentioned studies as well as identifying altitude and latitude as important factors when considering divergent selection (Zhang, Suren, & Holliday, 2019). Together, all these studies present data supporting local adaptation in *P. trichocarpa*, with a strong influence of climatic factors.

Previous work on *P. balsamifera* has presented very similar conclusions to those observed for *P. trichocarpa*. One previous study used common garden approaches to test the hypothesis of local adaptation using Qst -Fst comparisons. The results supported local adaptation and suggested that selection is the primary mechanism for the variability in quantitative traits seen across the species range (Keller et al., 2011). Further research by Stephen Keller and colleagues used different methods to detect local adaptation, specifically concerning the flowering-time gene network of *P. balsamifera.* They first used a Bayesian approach to look for genetic markers showing Fst values greater than one would expect under neutrality. This led to a hierarchical analysis that identified Fst outliers. Finally, they tested for covariance between allele frequencies and environmental factors that are larger than those predicted using neutral genetic markers. Their analysis identified specific genes that were most strongly influenced by local adaptation, most notably GIGANTEA 5, the central circadian clock gene. Their research also illuminated the power of multiple approaches to gain more information on potential local adaptation (Keller, Levsen, Olson, & Tiffin, 2012). These works provide valuable information related to local adaptation in this species and identify potential hotspot genes to investigate further.

Previous research relating to *P. trichocarpa* has shown evidence of parallel adaptation among and between three transects, one latitudinal and two altitudinal. Up to 51.8% of outlier

loci were shared among two transects and 15% were shared across all three (Holliday, Zhou, Bawa, Zhang, & Oubida, 2016). This indicates relatively strong parallelism between two different geographic spaces along the same general scales. However, little is known about the potential parallel evolution of local adaptation across different spatial scales in *Populus*.

By comparing broad-scale samples to fine-scale samples, the evolutionary processes operating at these scales can be uncovered, more specifically, the balance between gene flow and selection. If parallel evolution is found between spatial scales, it indicates a similarity in this balance, favoring selection. If no parallel evolution is found, it indicates a difference in the balance, supporting the idea that gene flow is likely washing out the effects of selection at the fine spatial scale.

The current study sets out to explore this idea using the tree species, *Populus balsamifera* through a common garden approach. This tree is found across North America from Alaska to Maine, spanning across Canada and the northeastern portions of the United States (Fig. 1). Individuals from all across this range corresponding to two spatial scales were grown together in a common garden in Burlington, Vermont to test for parallel evolution of local adaptation.

Two spatial scales are compared during this study, a broad scale, and a fine scale. The broad scale consists of plants from populations scattered throughout the range, covering a Euclidean distance of approximately 5,500 km between the two furthest samples (Figure 1B). The fine scale consists of plants from populations within Vermont, New York, New Hampshire, and Maine covering a Euclidean distance of approximately 450 km between the two furthest samples (Figure 1A). Despite the vast difference in spatial scales, the two overlap along portions of common climatic gradients, and therefore phenotypic traits may be predicted to respond in similar ways. To explore this potential parallelism, I analyzed selected phenotypic traits to target

the following objectives: (a) Determine if there is evidence of local adaptation to climate in both the broad and fine spatial scales in a parallel fashion; and (b) whether the trait values of the broad-scale individuals could be used to predict those of the fine scale individuals. Based upon previous research, I hypothesize that the magnitude of selection in response to climate is strong enough to overcome the homogenizing effects of gene flow at both the broad and fine spatial scales. Therefore, I predict that these analyses will show evidence of local adaptation in the selected traits in both spatial scales such that they are considered parallel. This will be corroborated by the ability to use broad-scale trait values to predict those of fine scales. Conversely, if gene flow has overwhelmed selection, particularly at fine spatial scales, there will be no evidence of local adaptation to climate within fine scale individuals, although local adaptation may be more likely at broader spatial scales where gene flow is less expansive.

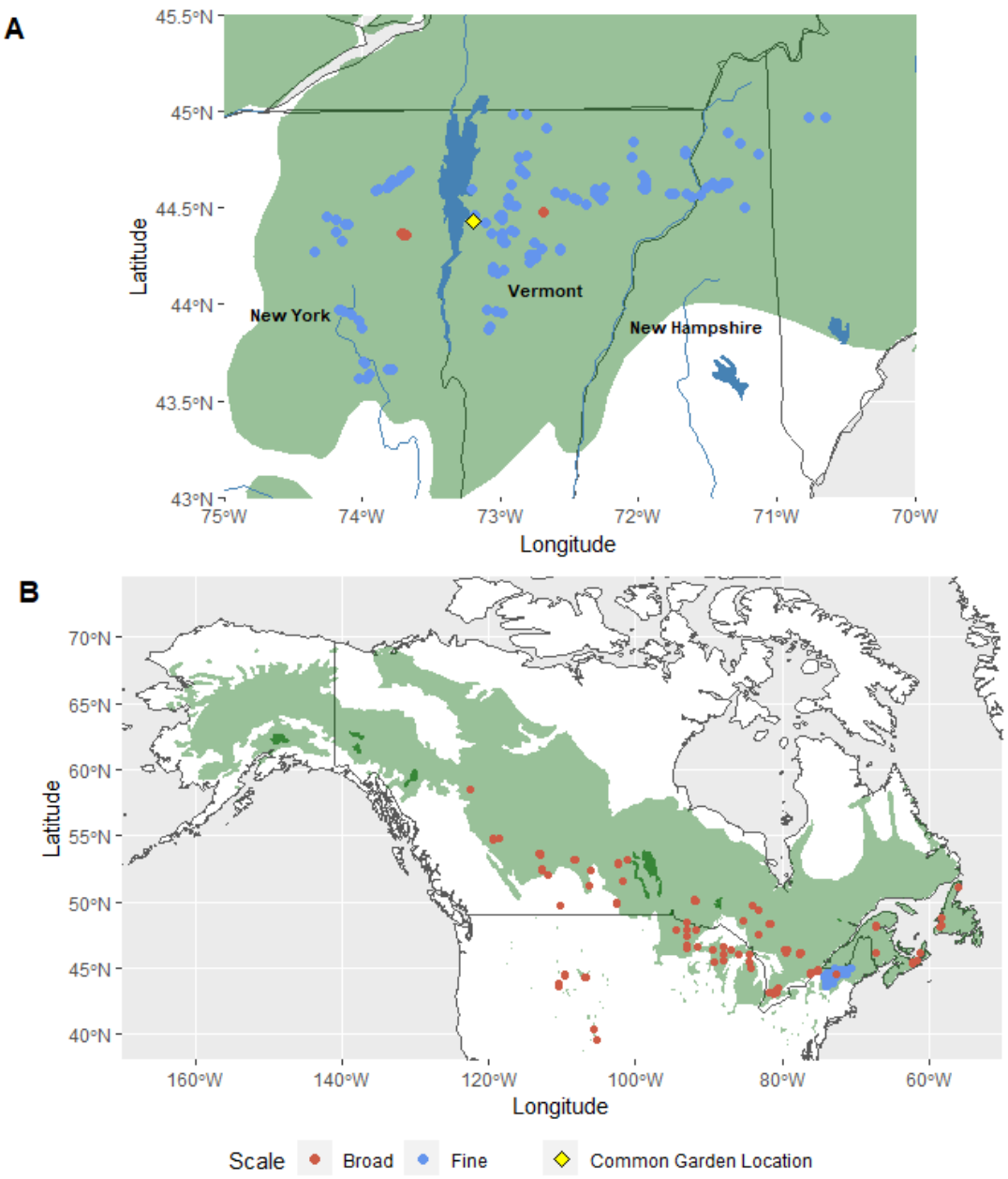


Figure 1: The source population locations of individuals planted in the common garden. (**A):** A map of the northeastern United States showing all fine-scale individuals, and the location of the common garden in Burlington, Vermont. (**B):** A map showing all broad and fine-scale samples as well as the range of *P. Balsamifera*, shown in green. Broad-scale samples are shown in orange, fine-scale samples are shown in blue, and the common garden site is shown in yellow.

Materials and Methods

The common garden located at the University of Vermont Horticulture Research and Education Center (Burlington, VT) was planted in the summer of 2019 using cuttings propagated from parent trees across the range. Each sample has associated metadata detailing the latitude and longitude of the parent tree, the population it belongs to, and the genotype name. The garden consists of 1,098 plants, which represent 3 replicates of 366 genotypes. Of these, 207 genotypes represent the broad scale (621 individuals), while 159 genotypes represent the fine scale (477 individuals) (Fig. 1). The garden is divided into 3 blocks, each with 1 replicate of the 366 genotypes. The order of the plants within each block was randomized. The plants were monitored closely, and dead individuals were replaced by a clone, as necessary, until the end of that growing season, to ensure the establishment of as many individuals as possible.

Data Collection:

This study selected bud phenology traits to analyze when exploring potential parallelism based on previous research indicating that these particular traits respond strongly to climate. When bud flush and bud set occur in trees is highly dependent on annual changes in temperature and determines the survival and growth of the tree (Hänninen & Kramer, 2007). Trees must flush bud early enough to take advantage of the growing season but cannot flush too early as cold spells can cause cold injury or death. Therefore, the timing of bud flush and bud set is highly dependent on annual temperature and climate, which has been seen in several species within *Populus* (Azad, 2012). Within *P. balsamifera*, previous work has shown strong responses to climate by genes responsible for flowering time, an important aspect of bud phenology (Keller et al., 2012). This work informed the selection of bud phenology traits in this study.

In the spring of 2020, bud flush data were collected for each plant. Bud flush was scored as the Julian day that the bud scales and leaf tips began to separate (Fig. S1). Data were collected three times per week starting when buds began flushing until all the individuals had flushed. It is important to note that data collection on bud flush began after some plants had already flushed. Therefore, the first day of data collection encompasses those plants that flushed before that date along with those that flushed on that date. Due to this, leaf flush data were also collected. Leaf flush was scored as the Julian day when the leaves of the apical bud fully emerged, and the petioles could be seen (Fig. S1). These data were collected three times per week until all individuals exhibited leaf flush.

In the fall of 2020, bud set data were collected for each plant. Bud set was scored as the Julian day when the apical bud fully closed. Data were collected three times per week starting when buds began to set until all individuals had set bud (Fig. S1).

Growing season length was calculated as the difference between the Julian date of bud set and the Julian date of bud flush. This additional trait was calculated because it includes two bud phenology traits, therefore it captures the variation seen in both. It also more completely captures the biological rationale for the timing of bud flush and bud set. As previously mentioned, this timing is important in allowing a tree to take advantage of its growing season adequately and safely, and climate has been seen to have an impact on this timing. Therefore, when grown in a common garden, variation in the length of the growing season is indicative of adaptation to source location conditions.

Analysis:

Climate PCAs

After the phenotypic data were collected, they were merged with the metadata from the initial collection and that of the garden plot. This gave a complete dataset with source latitude and longitude, and the location of the genotypes within the common garden. Historical climate data were obtained from the WorldClim 2 database at a spatial resolution of 30 seconds. This data represents the 30-year average from 1970 to 2000 (Fick and Hijmans, 2017). The latitude and longitude of each genotype source population were used to extract the value of 19 bioclimatic variables.

To narrow the focus of the analyses and determine which of the 19 variables contribute most strongly to the climate variation, three principal component analyses (PCAs) were conducted on the climate data for all individuals, only broad-scale individuals, and only fine-scale individuals. For each of these PCAs, the climate variable that loaded most strongly along principal component 1 and principal component 2 were used as proxies for the source climate in future models. It was important to conduct PCAs for the broad and fine spatial scales only as well as the global PCA to allow for the possibility that different bioclimatic variables are contributing most strongly in each spatial scale. The fine-scale individuals may not exhibit local adaptation to the bioclimatic variables that distinguish the broad-scale individuals, but instead, exhibit local adaptation to different bioclimatic variables. Therefore, the two variables that loaded strongest along the first two principal components in the global and broad only PCAs (because they are the same) and those two that loaded most strongly in the fine only PCA (because they were different) were used in the subsequent analyses as two sets of climatic variables.

These two sets of climatic variables were also used to subset the broad individuals to represent only those individuals that overlap in climatic space with the fine scale individuals. Hence, the maximum and minimum values for each variable in each set of the fine scale individuals were used to subset the broad scale individuals. This allows for a more direct comparison of trait values. This subset of broad individuals along with all the fine individuals make up the "Climate Subset" group in subsequent analyses.

Linear Mixed Models

The first analysis involved conducting linear mixed models to determine the association of different variables with phenotypic trait variations. Two sets of models were conducted using the "All Individuals" group and the "Climate Subset" group for each trait (bud flush, leaf flush, bud set, and growing season length). For the "All Individuals" group, three models were conducted, a global model, including individuals from both spatial scales, a broad scale only model, and a fine scale only model. For the "Climate Subset" group, two models were conducted, a global model including the climate subset broad scale individuals and all the fine scale individuals, and a broad scale only model, including only the climate subset broad scale individuals. An additional fine-scale only model is not necessary for this group, because this group also includes all the fine scale individuals and is, therefore, the same model as the fine-scale only model for the "All Individuals" group. These 5 models were run using both sets of climatic variables, for a total of 10 models per trait.

The models were defined as follows:

Global Model:

Trait ~ Climate Variable 1*Scale + Climate Variable 2*Scale + Block + Plant Number + (1|Genotype)

Broad and Fine only Models:

Trait ~ Climate Variable 1 + Climate Variable 2 + Block + Plant Number + (1|Genotype)

Note: Climate*Scale = Climate + Scale + Climate:Scale

Plant Number = The spatial position of each plant within its row

Each of these variables was tested for significance using ANOVA.

Under the hypothesis of parallel local adaptation to source climate, the results of this ANOVA analysis of linear mixed models would exhibit the following characteristics: All three models (global, broad only, and fine only) would show a significant association for those bioclimatic variables used; the global model would show no significant association of Scale, indicating that there are no intrinsic differences between spatial scales unrelated to climate; and the global model would show no significant association for the interaction between bioclimatic variables and Scale (bioX:Scale), indicating that the effect of climate in the form of the bioclimatic variable on a given phenotypic trait does not differ between the two spatial scales.

Prediction of Fine Scale Trait Values using Broad Scale Model

The second analysis approach involved using the linear mixed model for only the broad scale individuals to predict the trait values for the fine scale individuals. If the trait values for the fine scale individuals can be predicted successfully using the model produced using the broad scale individuals, it indicates the trait is responding to the same selection pressures in both spatial scales, therefore providing evidence for parallelism. The predictions and 95% confidence intervals were generated using the predictInterval function from the merTools R package (Knowles, 2020) as a result of 1,000 simulations. The number of observed values that fell outside

of the generated 95% confidence interval were counted. It is important to note that these results are not all out of 477 as some individuals did not have trait values associated with them (e.g., mortality). The final totals for each trait are as follows: Bud set: 470, bud flush: 475, leaf flush: 477, and growing season length: 469. To determine if these predicted trait values significantly matched those that were observed, a simple linear regression was conducted (observed trait values ~ predicted trait values), and associated adjusted R-squared and p-values were noted. These analyses were done for each set of climate variables and each grouping.

All analyses were run using R version 4.0.1 and RStudio version 1.3.959.

Best Linear Unbiased Predictors (BLUPs) vs. Climate Variables

To better visualize the relationship between the phenotypic trait values and the selected bioclimatic variables, the Best Linear Unbiased Predictors (BLUPs) of each trait for each genotype (3 replicates) were estimated and plotted against the four bioclimatic variables. The BLUPs estimate one value per genotype based on the 3 replicates, reducing the number of points plotted, making trends more visible.

Results

Climate PCAs:

After conducting the three climate PCAs including all individuals, only broad-scale individuals, and only fine-scale individuals, bioclimatic variables 3 and 17 (from here on referred to as bio3 and bio17) loaded most strongly in the PCAs for all individuals (Fig. 2), and only broad-scale individuals (Fig. 3). Bio3 is Isothermality ((mean of monthly max temperature – min temperature) / (max temperature of the warmest month – min temperature of the coldest month) x 100) and bio17 is the Precipitation of the Driest Quarter and therefore represent temperature variability and precipitation variables, respectively. This gives subsequent analyses representation of both types of climate variables. As seen in both of these PCAs, bio17 follows the same general direction and magnitude as many other bioclimatic variables, making it a good proxy for the climate in subsequent analyses.

For the fine-scale PCA, bioclimatic variables 10 and 12 (from here on referred to as bio10 and bio12) loaded most strongly (Fig 4). Bio10 is Mean Temperature of the Warmest Quarter and bio12 is Mean Annual Precipitation, therefore again there is a representation of both temperature and precipitation variables in subsequent analyses. Both bio10 and bio12 follow the same general direction and magnitude of several other climate variables, again making them good proxies for the climate in subsequent analyses.

Within the PCA including all individuals, one can see that the fine scale individuals occupy a climate space nested within that of the broad scale individuals. The climate subset performed using the two sets of climate variables (bio3 & bio17 and bio10 & bio12) captures those broad individuals that fall within the fine scale climate space (Fig. 2). For the bio3 & bio17

climate subset, 198 broad scale individuals were included, while the bio10 & bio12 climate subset included 150 broad scale individuals.

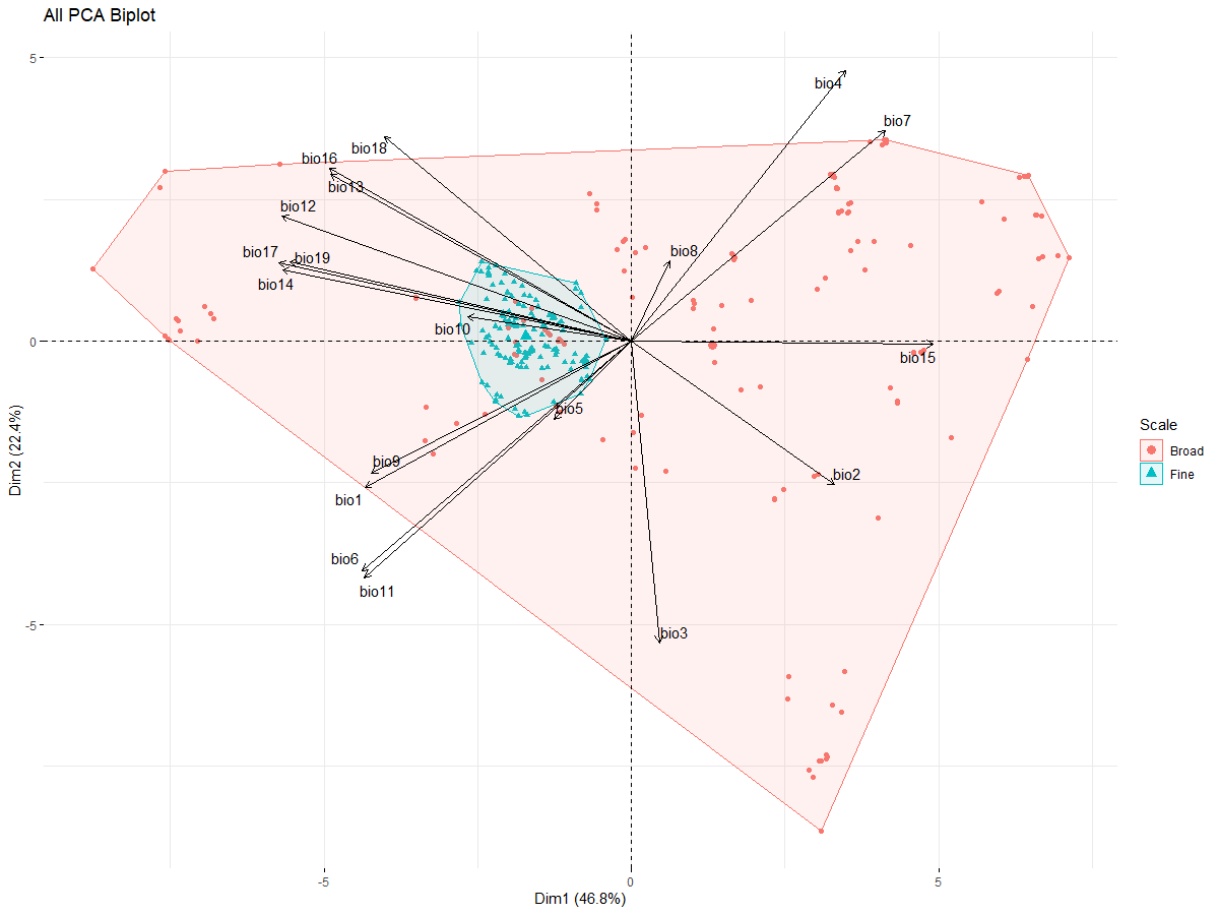


Figure 2: Principal Component Analysis Biplot of all individuals. Broad-scale individuals are shown with red points and fine-scale individuals are shown with blue points. Climate space occupied by the broad scale and fine-scale individuals are shown in red and blue, respectfully. The contribution of each bioclimatic variable is shown with black arrows.

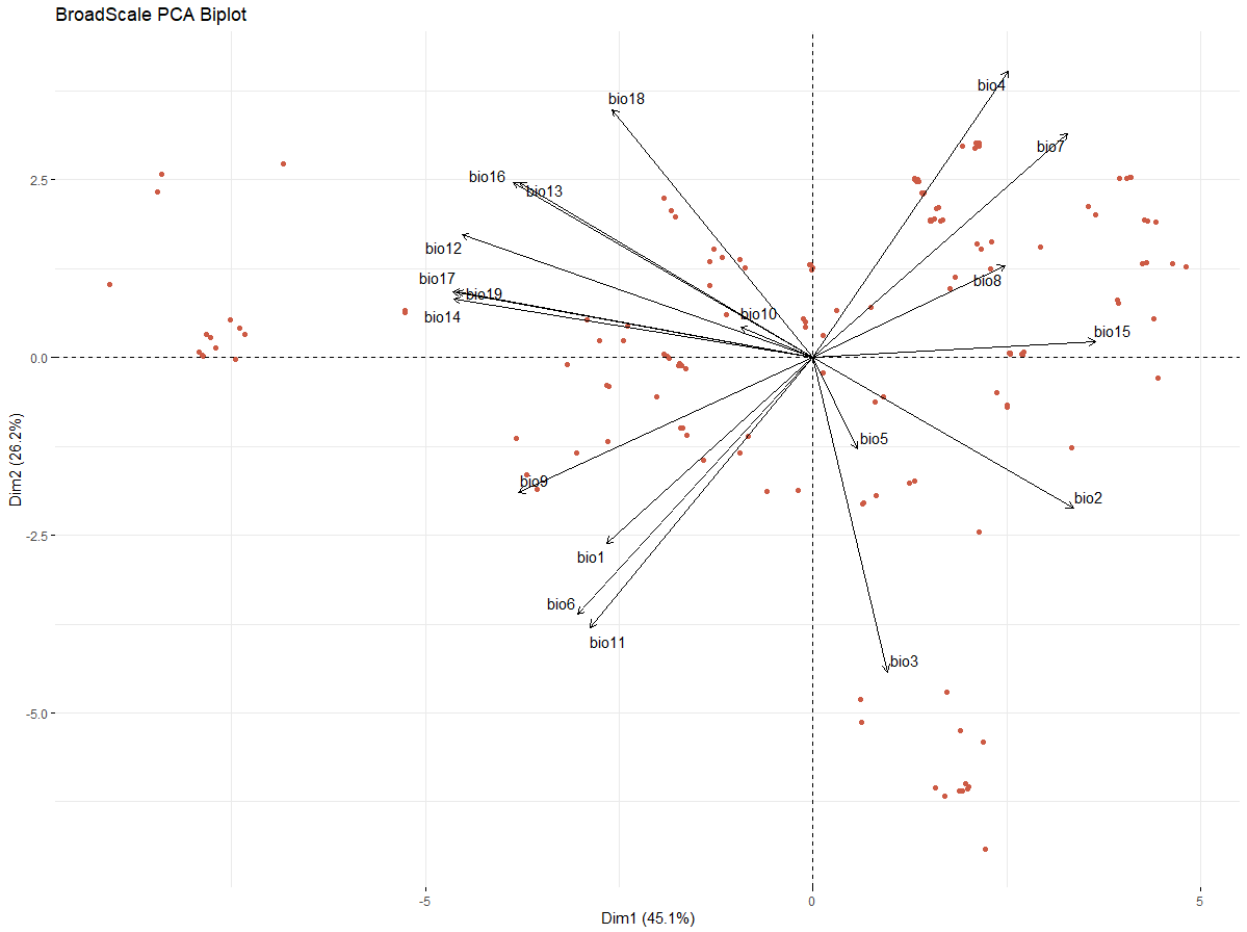


Figure 3: Principal Component Analysis Biplot of only broad-scale individuals. Broad-scale individuals are shown with red points. The contribution of bioclimatic variables is shown with black arrows. This contribution is very similar to that of the PCA including all individuals (Fig. 2).

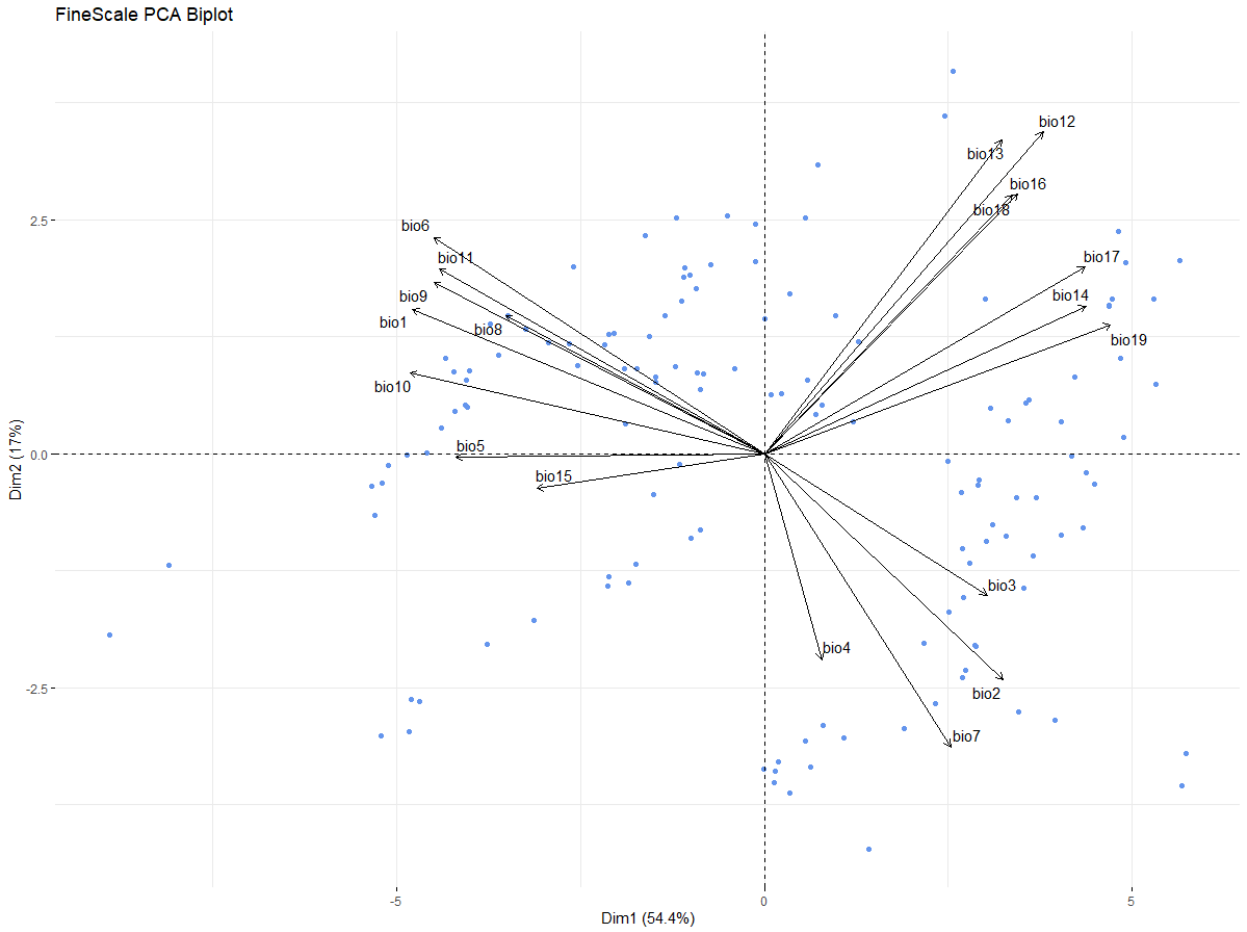


Figure 4: Principal Component Analysis Biplot of only fine-scale individuals. Fine-scale individuals are shown with blue points. The contribution of bioclimatic variables is shown with black arrows. This contribution is noticeably different than that of the PCAs for all individuals (Fig. 2) and only broad individuals (Fig. 3).

Growing Season Length

Linear Mixed Models

After conducting linear mixed models using both climate variable sets, the global and broad-scale only models including all individuals exhibited significant association for bio17 (Precipitation of the Driest Quarter), bio10 (Mean Temperature of the Warmest Quarter), and bio12 (Mean Annual Precipitation). The global and broad-scale only models including the climate subset individuals showed significance for bio17 and bio3 (Isothermality), but no significance for bio10 and bio12. The fine-scale only model, however, showed no significance for any bioclimatic variable. Additionally, those global models including all individuals showed no significant association for Scale or any of the interactions between bioclimatic variables and Scale (Table 1, Fig. 5).

Fine Scale Growing Season Length Predictions

After conducting prediction analysis using the broad-scale only models from each climate variable set and subset (all individuals vs. climate subset individuals), most observed trait values fell within the 95% confidence interval generated. The most observed values falling outside of the 95% confidence interval occurred with the bio3 & bio17 climate set model including all individuals, resulting in 83/469 values falling outside of the confidence interval (17.70%). When a linear regression was conducted to determine the association between the observed and predicted trait values, of the four, only the bio3 & bio17 climate set model including climate subset individuals did not exhibit a significant p-value. Therefore, most of the predictions showed a significant association between observed and predicted growing season length. However, each linear regression exhibited a very low adjusted R-squared value (Table 3, Fig. 6).

Figure 5: Growing Season Length vs. Bioclimatic Variables for Each Group. The best linear unbiased predictors (BLUPs) for growing season length are plotted against each bioclimatic variable (bio17, Precipitation of the Driest Quarter; bio3, Isothermality; bio10, Mean Temperature of the Warmest Quarter; and bio12, Mean Annual Precipitation). Plots A-D include all individuals, while plots E-H include only the climate subset individuals. The significant associations with bioclimatic variables for the broad-scale individuals, but not the fine-scale individuals are visualized. Broad-scale individuals are shown in red, and fine-scale individuals are shown in blue.

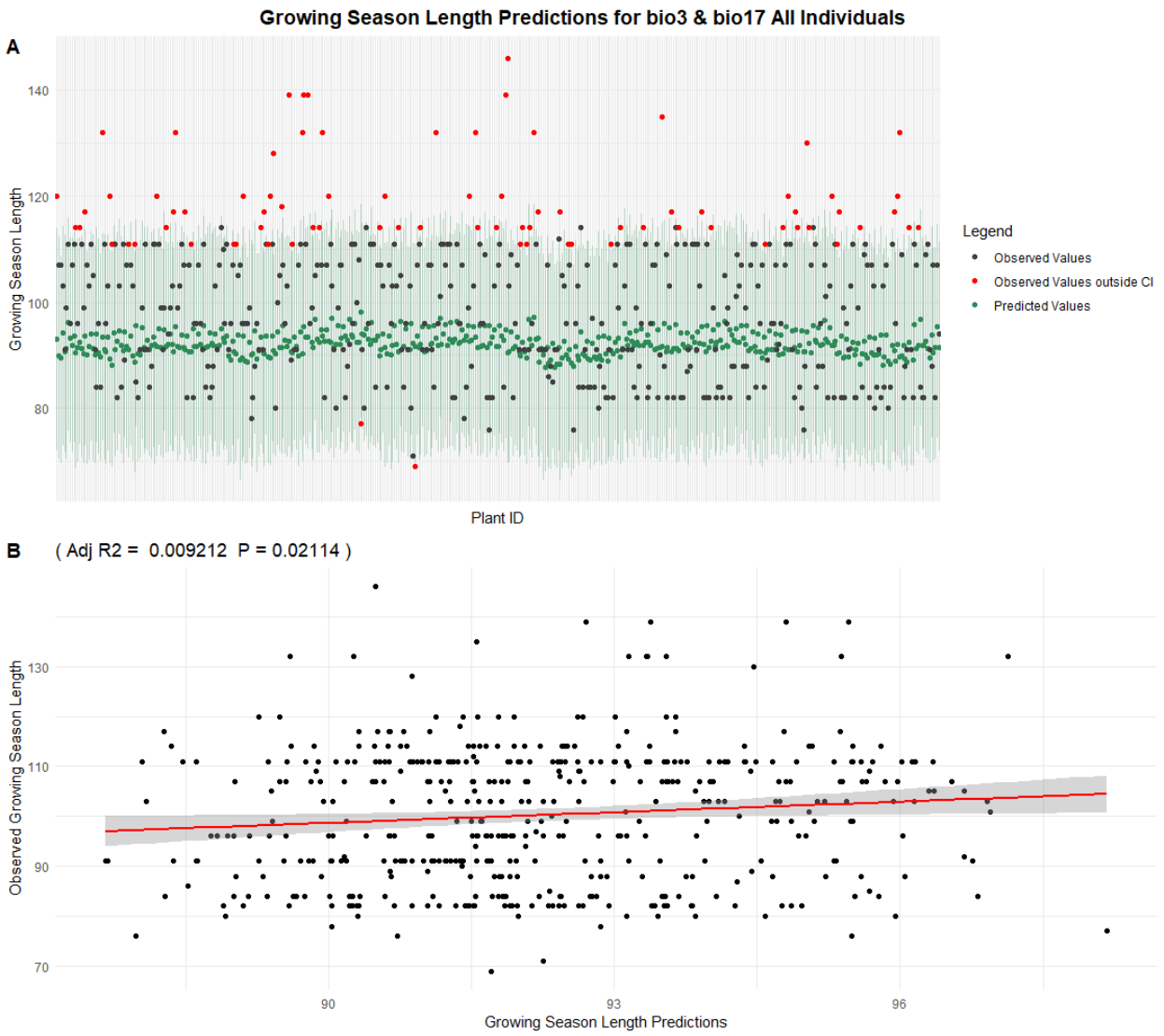


Figure 6: Visualization of the Prediction of Growing Season Length Values using Broad Scale Only Models. The results of the prediction of fine-scale growing season length values using the broad-scale only model including all individuals and using the bio3 & bio17 climate set. **(A):** Observed values of growing season length (black dots) plotted with the predicted values of growing season length (green dots) for each fine-scale individual. The 95% confidence interval for each prediction is shown with green bars and those observed values that fall outside of this confidence interval are shown with red dots. **(B):** Plot of observed growing season length against predicted growing season length values. The trend line is shown in red. The associated p-value and adjusted R-squared value are reported.

Phenology Traits - Bud Set, Bud Flush, and Leaf Flush:

Phenology traits generally showed evidence of clinal variation at the broad scale for both groups (All Individuals and Climate Subset), but not at the fine scale (Tables 1 and 2). Interestingly, the strength and significance of clinal associations often increased for those models that included climate subset individuals when compared to those that included all individuals.

Broad-scale estimates of clinal relationships were able to predict trait values at the fine scale but often left considerable variance unexplained. For bud set, no more than 10.21% of observed values fell outside of the 95% confidence interval of the prediction, and the relationships between predicted and observed values were significant. However, the adjusted R-squared value was very low, on the order of 2-3% (Table 3). For bud flush and leaf flush, each showed relatively low percentages of observed values falling outside of the generated 95% confidence interval; however, none of the regressions for observed vs. predicted trait values were significant (Table 3).

Table 1: Linear Mixed Model Results for Growing Season Length and Bud Set. The ANOVA output for each model conducted for growing season length and bud set showing the most important inputs when considering parallelism (each bioclimatic variable, Scale, and each interaction). The table is broken up by the climate set used (bio3 & bio17 as the first 5 rows, bio10 & bio12 as the second 5 rows), as well as by model, either global, broad only, or fine only (shown by different columns corresponding to either the 'All Individuals" or "Climate Subset" group for each trait). Those associations with significant p-values are noted with asterisks. The standard error is reported in parenthesis.

Trait		Growing Season Length					Bud Set				
Subset		All Individuals			Climate Subset		All Individuals			Climate Subset	
Model		Global	Broad	Fine	Global	Broad	Global	Broad	Fine	Global	Broad
bio3 & bio17 climate set models	bio3 (Isothermality)	0.288 (0.176)	0.285 (0.181)	-0.284 (0.839)	4.232** (1.464)	4.256** (1.583)	0.580*** (0.155)	0.578*** (0.148)	-0.277 (0.811)	2.171 (1.322)	2.183 (1.206)
	bio17 (Precipitation of the Driest Quarter)	0.054*** (0.011)	0.053*** (0.011)	0.033 (0.048)	0.213*** (0.052)	0.213*** (0.056)	0.054*** (0.010)	0.054*** (0.009)	0.040 (0.047)	0.145** (0.047)	0.145*** (0.042)
	Scale	28.164 (22.433)			161.999*** (45.339)		31.627 (19.781)			90.504* (40.949)	
	bio3:Scale	-0.572 (0.891)			-4.514** (1.702)		-0.856 (0.785)			-2.445 (1.537)	
	bio17:Scale	-0.020 (0.052)			-0.180* (0.072)		-0.014 (0.045)			-0.105 (0.065)	
bio10 & bio12 climate set models	bio10 (Mean Temperature of Warmest Quarter)	1.892*** (0.441)	1.911*** (0.448)	2.271 (1.222)	-0.629 (1.596)	-0.608 (1.792)	1.255** (0.402)	1.267** (0.391)	2.066 (1.179)	0.497 (1.506)	0.511 (1.600)
	bio12 (Mean Annual Precipitation)	0.010*** (0.003)	0.010*** (0.003)	0.023 (0.014)	0.030 (0.023)	0.030 (0.026)	0.008** (0.002)	0.008** (0.002)	0.023 (0.013)	0.035 (0.022)	0.035 (0.023)
	Scale	-14.133 (33.434)			-43.849 (51.385)		-25.456 (30.440)			-15.568 (48.522)	
	bio10:Scale	0.360 (1.329)			2.897 (2.042)		0.799 (1.210)			1.565 (1.927)	
	bio12:Scale	0.013 (0.014)			-0.007 (0.027)		0.015 (0.013)			-0.012 (0.026)	

Note: *p<0.05; **p<0.01; ***p<0.001

Table 2: Linear Mixed Model Results for Bud Flush and Leaf Flush. The ANOVA output for each model conducted for bud flush and leaf flush showing the most important inputs when considering parallelism (the bioclimatic variables, Scale, and each interaction). The table is broken up by the climate set used (bio3 & bio17 as the first 5 rows, bio10 & bio12 as the second 5 rows), as well as by model, either global, broad only, or fine only (shown by different columns corresponding to either the 'All Individuals" or "Climate Subset" group for each trait). Those associations with significant p-values are noted with asterisks. The standard error is reported in parenthesis.

Trait		Bud Flush					Leaf Flush				
Subset		All Individuals			Climate Subset		All Individuals			Climate Subset	
Model		Global	Broad	Fine	Global	Broad	Global	Broad	Fine	Global	Broad
bio3 & bio17 climate set models	bio3 (Isothermality)	0.293*** (0.061)	0.295*** (0.078)	-0.009 (0.121)	-2.158*** (0.386)	-2.163*** (0.645)	0.071 (0.043)	0.072 (0.049)	0.086 (0.157)	-1.516*** (0.319)	-1.527*** (0.423)
	bio17 (Precipitation of the Driest Quarter)	0.001 (0.004)	0.001 (0.005)	0.009 (0.007)	-0.072*** (0.014)	-0.072** (0.023)	-0.005 (0.003)	-0.004 (0.003)	-0.006 (0.009)	-0.063*** (0.011)	-0.063*** (0.015)
	Scale	3.760 (7.694)			-74.790*** (11.914)		-1.508 (5.368)			-54.219*** (9.847)	
	bio3:Scale	-0.305 (0.307)			2.148*** (0.448)		0.013 (0.214)			1.600*** (0.371)	
	bio17:Scale	0.008 (0.018)			0.081*** (0.019)		-0.002 (0.012)			0.057*** (0.016)	
bio10 & bio12 climate set models	bio10 (Mean Temperature of Warmest Quarter)	-0.670*** (0.155)	-0.677*** (0.199)	-0.086 (0.179)	1.192*** (0.297)	1.187* (0.461)	-0.307** (0.107)	-0.309* (0.124)	-0.154 (0.231)	0.785** (0.298)	0.781* (0.322)
	bio12 (Mean Annual Precipitation)	-0.002* (0.001)	-0.002 (0.001)	0.0004 (0.002)	0.005 (0.004)	0.005 (0.007)	-0.002* (0.001)	-0.002* (0.001)	-0.003 (0.003)	-0.005 (0.004)	-0.005 (0.005)
	Scale	-14.884 (11.798)			26.904** (9.551)		-1.666 (8.152)			15.838 (9.609)	
	bio10:Scale	0.595 (0.469)			-1.276*** (0.379)		0.157 (0.324)			-0.936* (0.382)	
	bio12:Scale	0.002 (0.005)			-0.005 (0.005)		-0.002 (0.004)			0.001 (0.005)	

Note: *p<0.05; **p<0.01; ***p<0.001

Table 3: Prediction of Fine Scale Trait Values Using Broad Scale Models. The table is divided by the climatic set used (bio3 & bio17 as the first 3 rows, bio10 & bio12 as the second 3 rows) and by group (shown by different columns for each trait). The number of observed values that fell outside of the generated 95% confidence interval is shown as a ratio of the total number of observations and shown as a percent. The p-value and adjusted R-squared values for the linear regressions conducted are reported for each model. Significant p-values are indicated with asterisks.

Trait		Growing Season Length		Bud Set		Bud Flush		Leaf Flush	
Subset		All Individuals	Climate Subset	All Individuals	Climate Subset	All Individuals	Climate Subset	All Individuals	Climate Subset
bio3 & bio17 climate set	# of observed values falling outside of 95% CI	83/469 (17.70%)	54/469 (11.51%)	48/470 (10.21%)	32/470 (6.81%)	7/475 (1.47%)	41/475 (8.63%)	59/477 (12.37%)	109/477 (22.85%)
	Adjusted R-squared	0.009212	8.72E-05	0.021704	0.0067175	0.0047379	4.05E-03	-0.0019561	-0.0021007
	p-value	0.02114*	0.30816	0.000793***	0.041662*	0.071779	0.087865	0.79042	0.96277
bio10 & bio12 climate set	# of observed values falling outside of 95% CI	58/469 (12.37%)	54/469 (11.51%)	45/470 (9.57%)	15/470 (3.19%)	8/475 (1.68%)	12/475 (2.53%)	57/477 (11.95%)	49/477 (10.27%)
	Adjusted R-squared	0.021193	0.008529	0.02922	0.023088	-0.000233	-0.0020448	-0.0019478	-0.00023746
	p-value	0.000916***	0.025439*	0.000115***	0.000555***	0.34612	0.85646	0.78483	0.34677

Note: *p<0.05; **p<0.01; ***p<0.001

Discussion

This study sought to determine if phenotypic traits of broad and fine-scale individuals responded to climate in a parallel fashion and if the broad-scale trait values could be used to predict those of the fine scale. After conducting both analyses, growing season length and bud set exhibited results most similar to those expected under the hypothesis of parallel local adaptation between spatial scales.

For growing season length, the linear mixed models including all individuals showed strong similarities to those results expected under parallelism. However, the fine-scale only models showed no significant association with any bioclimatic variables, indicating that these individuals are not locally adapting to source climate variation exhibited within this spatial scale. These results suggest that there is a clinal relationship between source climate and growing season length within the broad scale individuals, but no such relationship in the fine scale individuals. Therefore, parallelism is not supported. However, the lack of significance of Scale and both interactions (bioclimatic VariableX:Scale) indicates that the fine-scale individuals do fall within the clinal relationship between source climate and growing season length for the broad-scale individuals (Table 1). This conclusion is supported by the prediction analysis, which showed no more than 17.70% of observed values falling outside of the 95% confidence interval, and a significant association between observed and predicted trait values for the fine scale using all but one model, that being the bio3 & bio17 broad model including climate subset individuals. The successful prediction of fine scale growing season length using the broad-scale model supports the conclusion that the fine scale values fall within the clinal relationship of the broad scale but have no clinal relationship of their own. The low adjusted R-squared values for all prediction trends support this lack of clinal relationship in the fine scale, indicating that the broad

scale model is not able to accurately capture the variance within the fine scale individuals (Table 3). Growing season length is an important and telling trait in this analysis, as it includes both bud phenology traits (bud set and bud flush). In this way, it may be the best representation of phenotypic adaptive variation, of the traits tested in this study.

For bud set, both linear mixed models that included all individuals exhibited all the characteristics of what is expected under parallel adaptation aside from the significance of bioclimatic variables for the fine only model (Table 1). Despite the near-perfect similarity, the lack of significance of bioclimatic variables for the fine only model indicates that these individuals at the fine scale are not locally adapting to climate variation present within this spatial scale of sampling, therefore, parallelism is not supported. However, the lack of significance of Scale and both interactions indicates that the fine scale individuals are not significantly different in their response to source climate than the broad scale samples, therefore they may fit within the overall relationship of bud set and source climate. This is supported by the results of the predictions conducted using broad scale models to predict fine scale bud set values. For each of the four predictions, no more than 10.21% of observed values fell outside of the generated 95% confidence interval, and more importantly, each association between observed and predicted bud set values was significant (Table 3). It is important to note that despite the significant association, the adjusted R-squared values were very low, never exceeding a value of 0.029. This indicates that the fine scale individuals fit within the trend of the broad scale model, however, it does not accurately capture the variance within the fine scale individuals.

Although bud flush and leaf flush traits did not show strong similarities to those results expected under a parallel local adaptation hypothesis, all models for bud flush and the two

climate subset models for leaf flush showed associations with bioclimatic variables for the broad-scale only and global models (Table 2). This provides further evidence of a clinal relationship between source climate and the phenotypic trait within the broad scale.

When considering all traits, paying particular attention to growing season length, it can be concluded that there is a clinal relationship between source climate and phenology, and this relationship is seen most strongly in relation to the broad-scale individuals. The fine-scale individuals do fall within this clinal relationship, however, when parsed out from the broad-scale individuals, they do not show a significant association. This difference suggests a difference in the balance between selection in the form of local adaptation and gene flow across the spatial scales. In the broad-scale individuals, local adaptation of phenology occurs along the corresponding climatic gradient, indicating that selection is the stronger force in this balance. At the fine scale, however, the clinal relationship between source climate and phenology is not seen, indicating that gene flow is the stronger force in this balance. Selection in the form of local adaptation cannot overcome the homogenizing effects of gene flow at the fine spatial scale but is shown to do so in the broad spatial scale.

When making these conclusions it is important to consider the potential sources of error that may influence the results. Phenological traits such as the ones measured and analyzed in this study are difficult to quantify and score consistently, as these traits are more continuous than they are discrete. In this way, there is bound to be some deviation and variation in when these trait values are scored, which may influence the results of analyses.

This study gives insight into the trends in the clinal relationship between source climate and phenology and whether these trends show evidence of parallelism across spatial scales. By exploring this idea between a broad scale and a fine scale of *Populus balsamifera*, this study

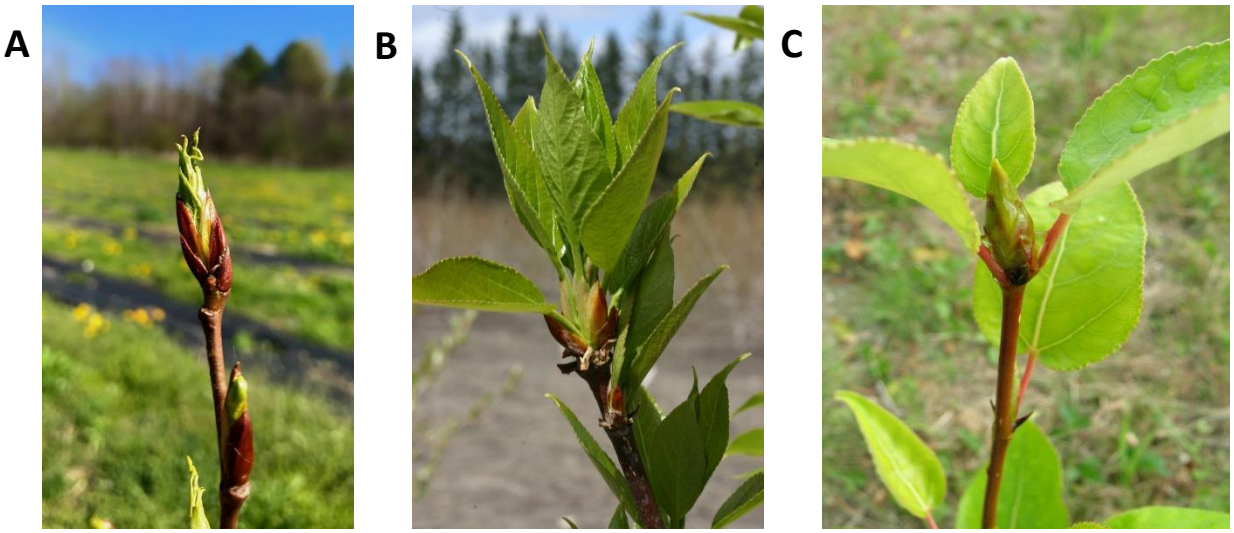
helps elucidate the balance between selection and gene flow operating at these scales. These analyses provide data and evidence which support a difference in this balance, indicating that selection cannot overcome the homogenizing effects of gene flow at fine scales, but is able to at broad scales. In this way, the results presented here help improve our understanding of the driving forces of evolution, a central question in the field of ecology and evolutionary biology.

Further Research and Conservation Implications

To corroborate the results seen in this study, a genetic analysis should be conducted on these samples to understand whether there is a significant overlap of adaptive loci between spatial scales and whether the SNPs, genes, and/or functional pathways being acted on are the same when comparing broad-scale and fine-scale individuals. These analyses will give a more complete understanding of potential parallelism occurring at these spatial scales. They will also enable a more direct comparison of results to previous studies that have seen significant overlap of loci across latitudinal gradients (Holliday, Zhou, Bawa, Zhang, & Oubida, 2016) as well as studies showing a significant number of SNPs relating to climate adaptation (Evans et al., 2014). More generally, studies similar to this should be repeated, using a fine spatial scale in a different part of the range of *P. balsamifera*, in order to determine if similar results are generated. Finally, studies of parallel local adaptation between spatial scales should be conducted in related species such as *P. trichocarpa,* as well as the hybrids that occur naturally between species of *Populus* (Hamzeh, Sawchyn, Périnet, & Dayanandan, 2007). This will determine if these conclusions relating to differences in the balance of selection and gene flow apply more generally to related tree species and hybrids.

These results and those of subsequent studies could be used to inform conservation decisions concerning transplantation and other forms of restoration of *Populus*. Specifically, this research and future studies like it can help to define the spatial scale at which selection is able to overcome gene flow and exhibit local adaptation, and therefore help to define the spatial scale at which conservation efforts should operate. Additionally, predictions similar to those done in these analyses can be done using bioclimatic variables projected for the future to predict future values of growing season length and phenology traits. These predictions can be used to inform transplantation efforts and other conservation decisions.

Supplementary Figures



Supplementary Figure 1: Photos showing the different stages of bud phenology. These represent the criteria on which each trait was scored. **(A)** Bud flush, showing bud scales and leaf tips separating. **(B)** Leaf flush, showing full emergence of leaves and the petioles visible. **(C)** Bud set, showing the apical bud closed.

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