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Naturally segregating genetic variation in circadian period exhibits a regional elevational and climatic cline

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Abstract

Circadian clocks confer adaptation to predictable 24-h fluctuations in the exogenous environment, but it has yet to be determined what ecological factors maintain natural genetic variation in endogenous circadian period outside of the hypothesized optimum of 24 h. We estimated quantitative genetic variation in circadian period in leaf movement in 30 natural populations of the *Arabidopsis* relative *Boechera stricta* sampled within only 1° of latitude but across an elevation gradient spanning 2460–3300 m in the Rocky Mountains. Measuring ~3800 plants from 473 maternal families (7–20 per population), we found that genetic variation was of similar magnitude among versus within populations, with population means varying between 21.9 and 24.9 h and maternal family means within populations varying by up to ~6 h. After statistically accounting for spatial autocorrelation at a habitat extreme, we found that elevation explained a significant proportion of genetic variation in the circadian period, such that higher-elevation populations had shorter mean period lengths and reduced intrapopulation ranges. Environmental data indicate that these spatial trends could be related to steep regional climatic gradients in temperature, precipitation, and their intra-annual variability. Our findings suggest that spatially fine-grained environmental heterogeneity contributes to naturally occurring genetic variation in circadian traits in wild populations.

KEYWORDS
biological rhythms, circadian clock, environmental gradient, genetic differentiation, local adaptation, spatial heterogeneity

1 | INTRODUCTION

Different species in a shared habitat may be exposed to divergent selective agents, but one selective agent common to natural populations across the globe is the diel cycling of environmental conditions that arises due to the Earth´s rotation. The importance of this selective agent is evidenced by the evolution across the tree of life of the circadian clock, an endogenous timekeeper, which allows for varied biological functions from the expression of individual genes to physiology and behaviour to be appropriately timed relative to anticipated environmental oscillations (e.g., Yerushalmi & Green, 2009). Experimental disruptions of the clock by loss-of-function mutations or by manipulations of the exogenous surroundings often reveal deleterious consequences on fitness components. For example, *Drosophila melanogaster* had lower fitness in unnatural day lengths than under 24-h cycles (Pittendrigh & Minis, 1972), *Arabidopsis* mutant genotypes with altered clock function had lower fitness than wild-type genotypes in field settings (Rubin et al., 2017), and in the common sunflower (*Helianthus annuus*) an interruption of natural solar tracking reduced biomass and the number of pollinator visits (Atamian et al., 2016).

Circadian traits are measured in constant conditions where the clock can 'free-run' and where endogenous cycles often deviate from

24-h rhythms maintained under naturally cycling settings. The free-running length of one full cycle, circadian period, is a key trait that is expected to be closely related to fitness. The circadian resonance theory postulates that peak fitness is achieved when the length of the endogenous circadian period matches that of the exogenous environmental cycle (Pittendrigh & Minis, 1972). Consequently, 24-h circadian periods would be expected to be advantageous in natural environments, whereas deviations from 24 h in either direction would improve fitness in unnatural diel cycles shorter or longer than 24 h. These tradeoffs might arise from physiological costs associated with constantly re-entraining the clock to an environmental cycle that is out of sync with the endogenous circadian rhythm (Pittendrigh & Minis, 1972). Support for this hypothesis is provided by studies in experimental mutant genotypes of the plant model *Arabidopsis thaliana* and cyanobacteria that express marked variation in circadian period (Dodd et al., 2005; Ouyang et al., 1998; Rubin et al., 2017, 2018; Woelfle et al., 2004), but results from resonance experiments are not always fully compatible with the classical theory (e.g., Graf et al., 2010; Horn et al., 2019; Woodley of Menie et al., 2019; Yamamoto & Tabata, 2022).

While experimental genotypes with induced single-gene mutations are useful in dissecting the molecular components of varied biological functions, they are not representative of patterns of genetic variation in quantitative traits that have arisen and segregate in natural environments. In the wild, various evolutionary processes determine the partitioning of quantitative genetic variation among versus within populations (Mackay et al., 2009; Mitchell-Olds et al., 2007). Populations may become genetically differentiated from each other as a consequence of stochastic events such as random genetic drift (Palumbi, 2003; Slatkin, 1987) or systematic spatially varying natural selection (Loveless & Hamrick, 1984; Siol et al., 2010). Populations may also exhibit genetic isolation by distance, in which physically proximate populations are more similar than distant ones, if populations originate by chance from a limited number of genetically related founders or if migration is restricted (McRae & Beier, 2007; Slatkin, 1987; Wright, 1943). Regardless of the underlying evolutionary forces, natural plant populations, including those of *A. thaliana*, consistently express significant among- and within-population genetic variation in diverse, continuously varying quantitative traits when examined in a common-garden environment (e.g., Linhart & Grant, 1996; Méndez-Vigo et al., 2013; Stenøien et al., 2005). This trait variation reflects the collective effect of numerous simultaneously segregating genes rather than the effect of a single mutation (Hill, 2010).

Patterns of intraspecific quantitative genetic variation correlate with spatial heterogeneity in the environment in many plant traits related to fitness including timing of growth and reproduction (e.g., Méndez-Vigo et al., 2011), and these traits have molecular genetic underpinnings that overlap with the circadian clock (Brachi et al., 2010). For example, flowering time is highly variable among natural genotypes of *A. thaliana* sampled across the Iberian Peninsula, with lower-elevation origins generally flowering earlier in common garden (Vidigal et al., 2016). Like most other quantitative traits, circadian traits express substantial naturally occurring quantitative genetic variation. Michael et al. (2003) documented a range of 6.5 h in circadian period in leaf movement among 150 *A. thaliana* genotypes sampled across the Northern Hemisphere and a latitudinal gradient spanning ~50°, whereas Rees et al. (2021) identified a range of 4.4 h in delayed fluorescence among 191 genotypes sampled in Sweden alone between 55° and 63°N. Because diel cycles are uniformly 24 h in length across the globe, genetic variation in circadian period has been examined predominantly in relation to latitude and with regard to correlated changes in photoperiod and its variability over the course of a year (e.g., Hut et al., 2013). Yet, latitude alone explains only a minor proportion (<8%) of the observed genetic variation in circadian period among accessions of *A. thaliana* (Michael et al., 2003; Rees et al., 2021), and thus the environmental agents that help maintain natural variation in the clock remain to a large extent unidentified (Salmela & Weinig, 2019). It is noteworthy that animals also exhibit significant genetic variation in circadian period. For instance, locomotor activity of insects varies by up to 8 h among mostly European populations of *Pyrrhocoris apterus* (Pivarciova et al., 2016) and in *Tribolium castaneum* in Japan (Abe et al., 2021), with no evidence for marked linear latitudinal trends.

Aside from studies sampling a single genotype to represent a population, plant studies sampling multiple genotypes per geographic location have demonstrated that genetic variation in the clock segregates not only among but also within populations (Greenham et al., 2017; Leinonen et al., 2020; Salmela et al., 2016), in a pattern reminiscent of segregation observed for flowering time in populations of *A. thaliana* on the Iberian Peninsula (Méndez-Vigo et al., 2013). For example, in an annual *Mimulus guttatus* population from southern Oregon, maternal families exhibited a genotypic range of almost 4 h in circadian period (Greenham et al., 2017). Further, in a population of the *Arabidopsis* relative *Boechera stricta* from southeastern Wyoming, circadian period varied by 3.5 h among maternal families sampled within a few hundred metres, with evidence for a positive correlation between period length and first-year growth (Salmela et al., 2016). Together, these patterns point to the role of fine-grained regional environmental heterogeneity in shaping the distribution of natural genetic variation in the circadian clock.

The potential contribution of environmental heterogeneity to genetic variation of circadian rhythms can be estimated by sampling multiple populations across well-defined spatial gradients. For instance, temperature conditions vary with elevation such that growing seasons begin later and at longer day lengths at higher elevations, giving rise to genetic clines in quantitative traits like phenology even within relatively narrow spatial scales (e.g., Leinonen et al., 2020). Here, we sample plant populations intensively within a limited latitude but over an 800-m elevation gradient to determine how quantitative genetic variation in circadian period is partitioned among and within Rocky Mountain populations of *B. stricta*, a short-lived and predominantly self-fertilizing perennial with a wide North American distribution (Song et al., 2006). With the utilization of circadian assays of leaf movement across these populations on a large sample (~3800 plants), we examine whether different

evolutionary factors including stochastic and selective forces are associated with the spatial patterns of genetic variation in circadian period. The sampled geographic region is small compared to previous studies on natural genotypes (e.g., Greenham et al., 2017; Michael et al., 2003; Rees et al., 2021), but nonetheless includes significant heterogeneity in environmental factors like temperature and precipitation. Following on the results of another study sampling fewer populations of *B. stricta* (Salmela et al., 2016), we hypothesize that (1) circadian period will exhibit genetic differentiation among and within the 30 populations, (2) that the range of variation among populations will be larger than in Salmela et al. (2016) due to a greater range of environments sampled, and that (3) if variation in the clock contributes to adaptation to environmental heterogeneity, then patterns of genetic variation among and within populations will correlate with elevation and associated local environmental variables.

2 | MATERIALS AND METHODS

2.1 | Study populations

For the current project, local populations of *B. stricta* were identified in the Medicine Bow National Forest near the University of Wyoming. Locations for plant populations were first estimated from records from the Rocky Mountain Herbarium to determine appropriate habitat types within the region. The 30 populations utilized for this project were found along an elevation gradient (2460–3300 m above sea level) in three distinct mountain ranges (Figure 1). Habitat included areas of sagebrush at lower elevation and open meadows within montane forests at higher elevation. Within each population, a representative sample of individuals (at least 30 individuals along an approximately 50-m transect) was identified. We collected seeds when the fruits on each plant had begun to dehisce; on each plant, most fruits were removed and seeds were subsequently sorted. We pooled the seeds from each individual plant, and these were considered a single maternal family. Maternal effects in wild-collected seed can contribute to genotypic variation for some traits in some species (Bischoff & Müller-Schärer, 2010), although the magnitude of potential maternal effects on circadian clock parameters remains unknown. In general, significant differences among populations and among maternal families within populations for a phenotypic trait such as circadian period measured under common-garden settings reveal quantitative genetic variation in the trait (Supporting Information: Figure S1) emerging from multiple genes and their interactions with the environment (Rees et al., 2021; Rubin et al., 2019).

2.2 | Leaf movement assays

To determine circadian period in natural populations of *B. stricta*, we adapted the protocol from Tracking Rhythms in Plants (TRiP, Greenham et al., 2015). For each of the 30 populations, 10–20 maternal families were selected for circadian analysis, and within each maternal family, 18 replicates were included. We planted seeds into Redi-Earth potting mix (Sungro) in 1-cm diameter pots and placed the pots into growth chambers (Percival Scientific) for germination using a 12-h/12-h light/dark cycle and 21°C/18°C day/night temperature cycles. After 6 days, seedlings were moved to 14-h/10-h light/dark cycle for entrainment; temperature conditions during entrainment simulated early May conditions in Laramie, WY with daily cycling for temperature from 8°C to 17.5°C (Supporting

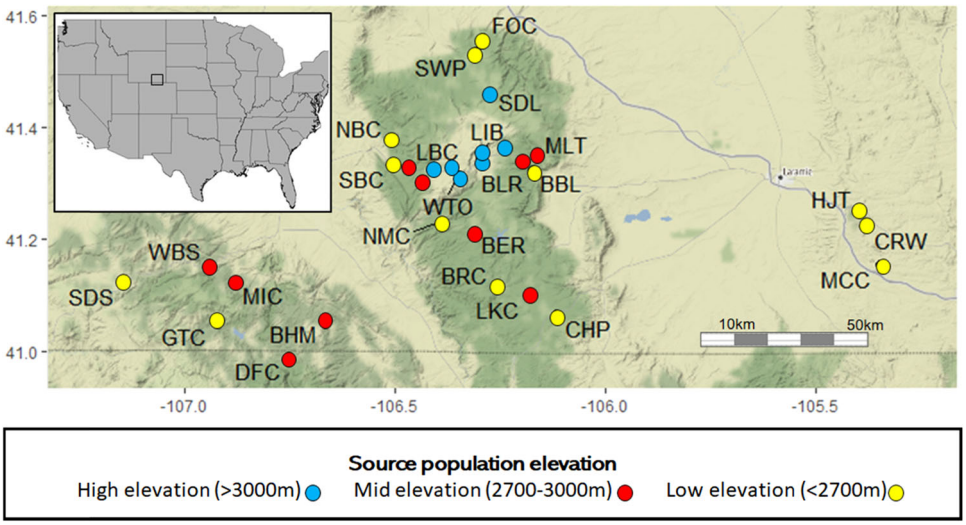

FIGURE 1 Locations of 30 populations of *Boechera stricta* in the Medicine Bow National Forest in southeastern Wyoming and northern Colorado that were sampled for the determination of natural quantitative genetic variation in circadian period in leaf movement. Sites are grouped into three categories: high elevation (>3000 m), mid-elevation (2700–3000 m), and low elevation (<2700 m). Each population is represented by 7–20 maternal families, allowing for the partitioning of genetic variation in circadian period segregating among populations and among maternal families within populations.

Information: Table S1). After 7 days in the entrainment conditions, seedlings were transferred to imaging stands and placed in constant light and temperature (17.5°C) conditions for 24 h before imaging. Images were taken every 15 min for 5 days to ascertain leaf positions, and stacked images of leaf movements were analysed in MatLab (The MathWorks, Inc.) through the TRiP pipeline to determine circadian period (i.e., the duration of one endogenous cycle). These germination and entrainment windows were selected to allow sufficient time for plant growth and for cotyledons to be imaged, but before the development of the first true leaves, which can obscure cotyledon imaging. Due to the large number of maternal families (100 s), the large number of replicates (1000 s), and the complexity of imaging, it was not possible to fully randomize replicates of all maternal families and all populations across trials. To account for the possible environmental variation among growth chambers, 6 of the 18 replicates for each maternal family were imaged in separate compartments (three compartments for each maternal family), and trial chamber was noted.

We estimated period values through BioDare2 (Zielinski et al., 2014). Values for circadian period were determined by two algorithmic methods: MFourFit (a curve-fitting method) and MESA (maximum entropy spectral analysis; an autoregressive model based on stochastic modelling). Settings for both analyses included removing estimates of circadian period lower than 18 h and higher than 30 h. Traces from experimental replicates that displayed no rhythmicity of pattern were discarded. For each individual replicate measurement, we compared the difference between the two analyses and values that differed by more than 10% (~2 h) were removed. Statistical outliers for each maternal family within the populations were removed through the R package 'EnvStats' (v2.4.0; Millard, 2013). After the quality control steps, 3823 replicates remained that represented 5–18 individuals within each of 473 maternal families (Supporting Information: Table S2). Mean values for each maternal family and subsequently each population were then determined as well as the intrapopulation range of values (as the difference between the shortest to longest maternal family mean within each population).

2.3 | Environmental variables

To identify environmental factors that could act as agents of selection on circadian period, we analysed data for 19 bioclimatic variables in the WorldClim data set (Hijmans et al., 2005; downloaded in R 4.1.2 by package 'raster'—v3.5-2; Hijmans, 2021) for each of our population sites. For the analysis of environmental data, values for the climate variables collected at 30-s resolution (~1 km × 1 km grids) were utilized, including mean annual temperature and annual precipitation and measures of peak and low temperature and precipitation through the year. As soil variables were not found in any online database for the areas of the experimental *B. stricta* populations, we collected soil samples and analyzed soil features from each of the 30 population sites. At each site, along a 10-m transect, we measured soil temperature and moisture approximately 2 weeks after snowmelt at each site. We also collected three soil samples; in the lab, we measured soil pH, soil electrical conductivity, soil moisture, and soil texture (as percent of silt, sand, and clay composition).

2.4 | Statistical analysis

To determine whether there was significant genetic variation in circadian period among and within populations (Supporting Information: Figure S1), we tested for the main effects of population (fixed factor) and maternal family nested within population (random factor) using analysis of variance (ANOVA) in IBM SPSS Statistics v. 28. Maternal families with at least five replicates were included in ANOVA of circadian period, with the largest number of replicates within a single maternal family being 18. Because data distributions violated the assumptions of normality and homoscedasticity, we also applied a nonparametric test, Welch's Heteroscedastic *F* test (Welch 1951) in R 4.1.2 with package 'onewaytests' (v2.6; Dag et al., 2018; R Core Team, 2020), to examine whether the deviations from model assumptions affected our results. Within each population, one-way ANOVA was used to test for variation among maternal families using base R 4.1.2. The effect of trial was also tested, as 18 separate imaging trials were required to screen all individual replicates. Due to the large number of replicates and complexities of plant imaging, we could not use a fully randomized design of replicates of all populations in all trials; however, multiple replicates of all populations were present in multiple trials. Therefore, we estimated residual variation in circadian period after accounting for the effect of trial, and then re-tested the effect of population using the residuals. To compare the magnitudes of variation in circadian period due to population and maternal family within population, we estimated variance components for both factors using the restricted maximum likelihood approach and the lme4 package (v1.1-27.1; Bates et al., 2015). For this descriptive purpose, both factors were considered random.

To determine if the *B. stricta* populations were structured by spatial proximity and to evaluate global spatial autocorrelation for circadian period, we estimated two spatial statistics, Moran's *I* and Geary's *C* (Geary, 1954; Moran, 1950). Moran's *I* is a standard for spatial data and is widely utilized to provide an overall statistic for large-scale analysis of spatial patterns. Geary's *C* is better used to determine differences between pairs of observations and can be more sensitive to smaller neighbourhoods. Within the data, some pairs of populations were closer than others, indicating that Geary's *C* was more appropriate. To initially evaluate global spatial autocorrelation, we used Moran's *I*, which considers the directionality of spatial association among populations. With Moran's *I*, values centre around 0, with a negative statistic indicating clustering of dissimilar values and a positive statistic suggesting the clustering of similar values; '0' would indicate randomness and no autocorrelation. For these analyses, we used the population mean and the intrapopulation

range of circadian period. Spatial regression was used to determine if the values for spatial autocorrelation affect the overall distribution of the populations within the environmental variables. To test for spatial dependence in the regression, spatial error (spatial correlation between error terms) and spatial lag (using a variable to account for autocorrelation) models with elevation, mean annual temperature, annual precipitation, and soil texture as predictor variables were used. We tested for the significance of the spatial autocorrelation and used Akaike Information Criterion (AIC) to identify the best-fit linear models, specifically if models with or without the spatial estimates were better. Analyses were conducted in R 4.1.2 with packages 'sp' (v1.4-5; Bivand et al., 2013; Pebesma & Bivand, 2005), 'spdep' (v1.1-12; Bivand et al., 2013), 'rgdal' (v1.5-27; Bivand et al., 2021), 'spgwr' (v0.6-34; Bivand & Yu, 2020), and 'spatstat' (v2.2-0; Baddeley & Turner, 2005; R Core Team, 2020).

As an important aspect of our analysis, we wanted to test for associations between circadian values and a range of environmental variables. We used principal component analysis (PCA) to reduce the dimensionality of the environmental data with 25 climate and soil variables. Analyses were conducted in R 4.1.2 with packages 'mctest' (v1.3.1; Imdadullah et al., 2016), 'GGally' (v2.1.2; Schloerke et al., 2021), and 'corpcor' (v1.6.10; Schafer et al., 2017) for testing multicollinearity and 'ggbiplot' (v0.55; Vu, 2011) for PCA. Having reduced data dimensionality, we used partial least-squares (PLS) regression and principal component regression (PCR), where the predictor variables included all climate and soil factors—we did not include elevation in these models, as it is a determinant variable for the other environmental predictors. We used both mean circadian period and the intrapopulation range of circadian period as the response variables in separate models. PCR first computes the PCs of the predictors, and then uses these components as predictors in a regression against the response variable (Jolliffe, 1982). PLS regression is a similar analysis to PCR but works in a supervised framework for the predictors as they are combined into the components (Wold et al., 2001). Predictor variables were standardized by dividing each by its SD and a decomposition step removes uninformative predictor variables. The strongest model was used based on cross-validation both within each model and as a comparison of the PLS and PCR models. After the optimal model was determined, we calculated the contribution of each coefficient. For this analysis, we used R 4.1.2 and packages 'pls' (v2.8-0; Mevik et al., 2020) and 'caret' (v6.0-90; Kuhn, 2020).

3 | RESULTS

3.1 | Variation in circadian period among and within populations

Both nested ANOVA and the Welch's *F*-test found highly significant differences in circadian period among populations, and in ANOVA the effect of maternal family within population was significant (Table 1). The effect of population remained significant after statistically factoring out the effect of trial, suggesting limited micro-environmental differences among trials over time or among growth chambers (Table 1). Among the 30 populations analyzed, mean circadian period varied between 21.91 and 24.92 h, or 3 h (Figure 2). Although most of the populations expressed a mean circadian period close to 24 h, many populations had a mean period that was shorter; 10 of the 30 populations had a mean period value that was shorter than 22.5 h.

The effect of maternal family was significant within most individual populations (Supporting Information: Table S2), and the difference between the maternal family with the shortest versus longest mean circadian period length (intrapopulation range) was 3 h on average. In the complete data set the variance component for the effect of maternal family within population was 0.687, or 21.3% of total variation, while the variance component for the population effect was 0.787, or 24.4% of total variation. This indicates that overall, the magnitude of genetic variation was very similar among *versus* within populations. Residual (i.e., within-maternal family) variation explained the remaining 54.3% of total variation (Supporting Information: Fig. S1). Two populations expressed exceptionally high intrapopulation ranges of >5 h (Happy Jack Trail, HJT, and Crow Creek, CRW; Supporting Information: Table S2). HJT and CRW exhibited spatial autocorrelation, such that one cannot exclude the possibility of a founder event and that HJT and CRW are one genetic population; nevertheless, under this hypothesis, at least one population exhibits a >5 h range. The wide intrapopulation range of variation in circadian period for the HJT and CRW populations contrasted with another population, Sand Lake, SDL, which had a range of values among maternal families of less than an hour (Figure 3). Neither population mean value nor the number of replicates (both

TABLE 1 A summary of three statistical tests on natural genetic variation in circadian period in leaf movement in populations of *B. stricta*.

	d.f.	MS	*F*-value	Significance
Population	29	78.88	12.47	****
Maternal family (population)	443	7.35	4.19	****
Residuals	3350	1.75		
	Welch's *F*-test			
Population	29	126.91	63.63	***
Trial	18	172.36	77.7	**
Population (trial-corrected by residuals)	29	83.44	34.70	***

Note: Results from a nested analysis of variance (ANOVA) are shown on top. The second test, Welch´s Heteroscedastic F, allows deviations from normality and homoscedasticity. Eighteen separate trials were needed to screen all replicates for circadian period, and the trial was included as a factor together with population in the final test.

**$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

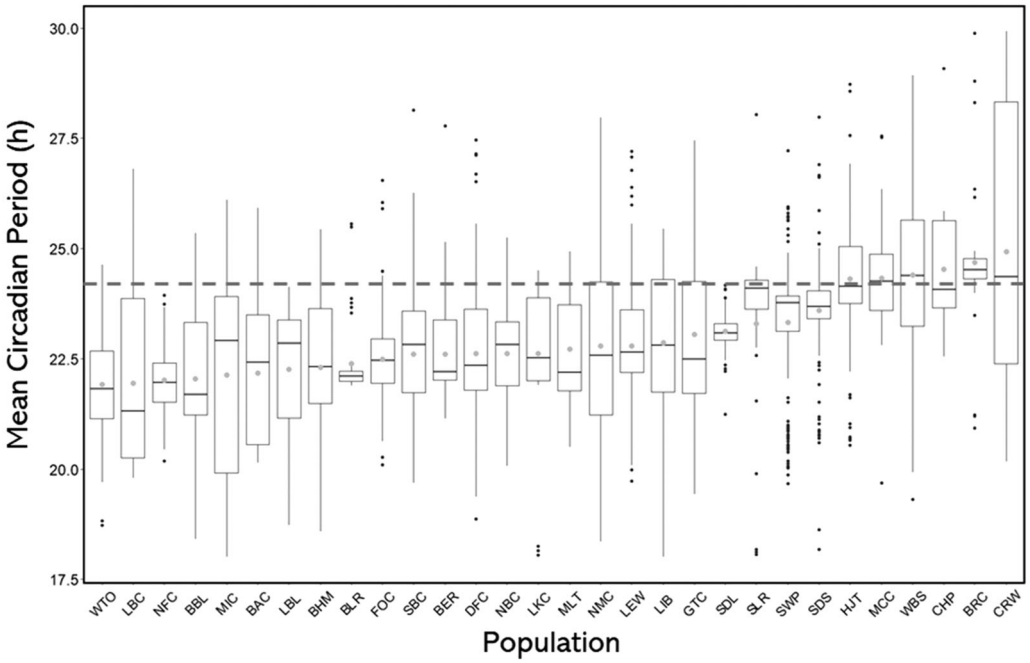

FIGURE 2 A comparison of the range of variation in circadian period in leaf movement among and within *B. stricta* populations from the central Rocky Mountains. Mean values are represented by a grey dot and populations are ordered by population mean value from the shortest to the longest circadian period. Dashed line represents 24 h to indicate populations that fall above or below natural daylength. All 3823 replicates are represented, fitting into 473 families that come from the 30 populations.

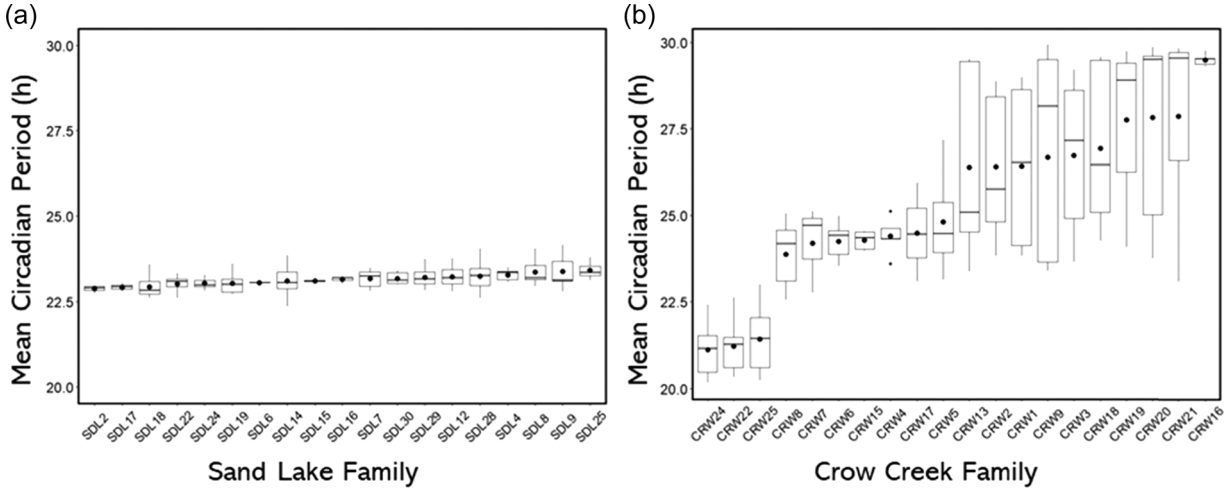

FIGURE 3 The two extremes of intrapopulation range in circadian period in leaf movement in *B. stricta*: (a) the Sand Lake population located at 3070 m (least variation; a 0.65-h range among 19 maternal families) and (b) the Crow Creek population located at 2500 m (most variation; a 5.82-h range among 20 maternal families). Maternal family means are indicated by a black dot.

individuals and maternal families) had a significant association with the intrapopulation range, indicating the results for circadian range within populations are not a consequence of slight differences in replicate number and potential sampling bias. In sum, the range of variation among maternal families within some populations (>5 h) is greater than the difference among populations (3 h).

3.2 | Spatial analysis

The appearance of among-population genetic variation can be influenced by isolation by distance, in which geographically proximate populations are more closely related. Isolation by distance can also affect the appearance of genotypic correlations because data from different populations are not independent.

We therefore tested first for evidence of spatial autocorrelation. Spatial autocorrelation was significant among the populations both for the mean values of the circadian period (Moran's $I = 0.24$, $p = 0.04$; Supporting Information: Fig. S2) and the intrapopulation range (Moran's $I = 0.29$, $p = 0.02$; Supporting Information: Fig. S3). Clustering of populations was limited to one specific 'hot spot', and this accounted for the autocorrelation. The three easternmost populations, located in the Laramie Range of southeastern Wyoming (CRW, HJT and Middle Crow Creek, MCC), had similar values for population mean and the intrapopulation range. Outside of this single cluster, values were randomly spaced across the populations. The results of the Geary's *C* test, which is better suited for local populations, found no significant autocorrelation among populations. Further results are presented both as observed and after adjusting for the occurrence of spatial autocorrelation.

3.3 | Variation in local environments and association with circadian period

Univariate linear regression revealed elevation as a significant predictor for both the mean circadian period among populations ($F = 7.01$; $p = 0.01$) as well as the intrapopulation range ($F = 14.45$; $p < 0.001$) (Figure 4a,b). More specifically, shorter mean circadian periods with a more constrained intrapopulation range were observed at higher elevations; at lower elevations, a greater intrapopulation range was found. For both the spatial error and spatial lag multivariate regression models, the spatial independent variables were not significant for either of the two response variables, mean circadian period for the population or the intrapopulation range. AIC, used to select the best-fit model, further indicated that linear models excluding a spatial component had greater explanatory power than those with spatial variables. The latter two results indicate that spatial autocorrelation did not account for the association between elevation and either circadian period or circadian range.

Populations were separated by an 800-m difference in elevation; the lowest elevation population (North Brush Creek [NBC], 2460 m) and the highest (LIB, 3300 m) were both located in the Medicine Bow Mountains. The most widely separated populations (Sandstone, SDS, vs MCC) were found 150 km apart. Over this spatial range, environmental variables estimated by the Worldclim models for the 30 population sites were highly varied. Mean annual temperature varied by 4.5°C and annual precipitation varied by over 200 mm (Supporting Information: Table S3). Many of the measured environmental variables correlated with elevation, and multicollinearity analysis demonstrated strong associations among the environment variables. PCA identified four PCs among the 25 climate and soil variables, with the main component, PC1, correlating negatively with temperature and positively with precipitation (Supporting Information: Table S4). Further, PC1 varied along elevation, showing decreasing temperature and increasing precipitation at sites located at higher elevation, while elevational trends were not as striking for the other PCs (Figure 5 and Supporting Information: Fig. S4). Soil pH and soil moisture were the only soil variables that had a strong association with PC1, with lower pH and higher moisture at higher elevation.

Given that spatial structure did not account for population differences in circadian period, we were interested to test for associations between circadian parameters and not only elevation but also environmental variables. We used PLS and PCR models to reduce the dimensionality of the environmental variables. As the predictor variables were shown to have high multicollinearity, the PCR and PLS models allow a stronger estimation of the variables at the population sites that may affect or impose selection on circadian period. By comparing the PCR and PLS models to each other and to linear regression models, these analyses indicate the models that best explain the variation in mean period and the intrapopulation range (Table 2). For population mean circadian period, the PLS model that best explains the response variable reduced the data to two components and accounted for 29.7% of the variation in this trait (Supporting Information: Figure 5a and 5b). For the intrapopulation range, the PLS model that best explains the data included only one

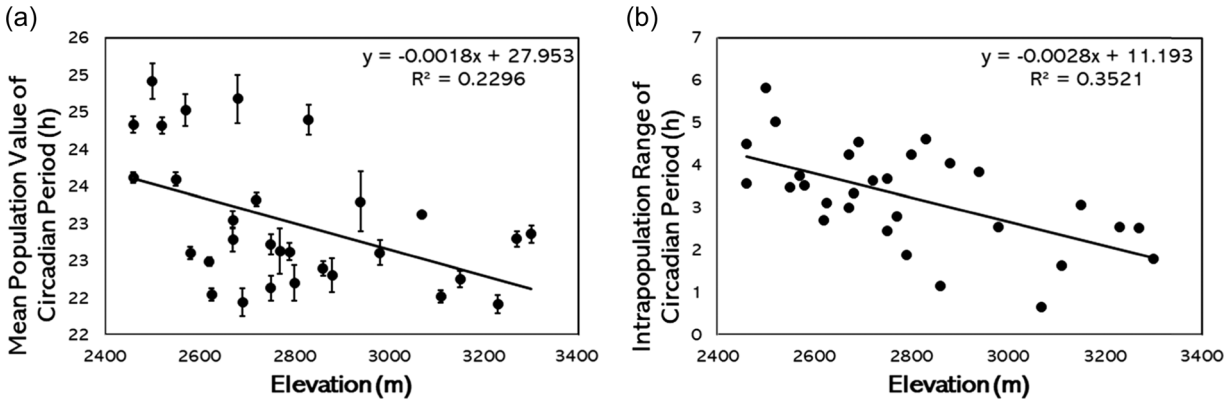

FIGURE 4 Associations between elevation and (a) mean circadian period (±standard error) and (b) intrapopulation range of circadian period in 30 populations of *B. stricta* sampled in the central Rocky Mountains.

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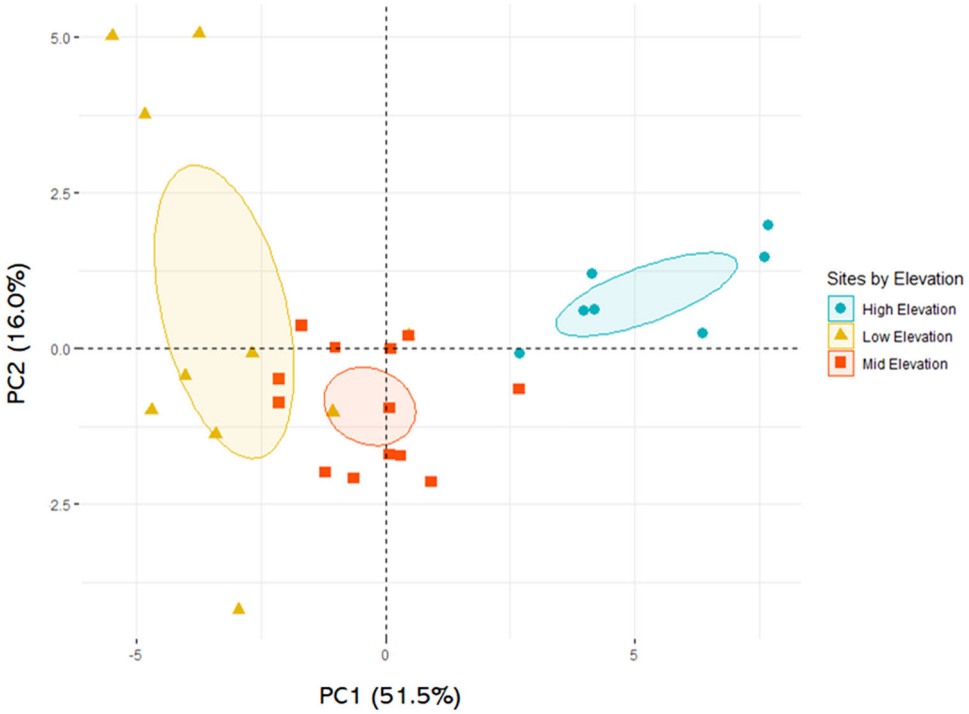

FIGURE 5 Environmental variation among the home sites of Rocky Mountain *B. stricta* populations visualized with two principal components (PC), PC1 and PC2. Loadings of 25 climate and soil variables on the PCs are shown in Supporting Information: Table S4. In brief, PC1 correlates negatively with temperature and soil pH and positively with precipitation, while PC2 is best associated with precipitation seasonality. Sites are grouped into three categories: high elevation (>3000 m), mid-elevation (2700–3000 m), and low elevation (<2700 m). Ellipses represent 95% confidence intervals for each category.

component and accounted for 28.39% of the population range (Supporting Information: Figure 5c). The most informative coefficients are the same for both models. For both response variables, annual precipitation and precipitation during the coldest and driest portion of the year accounted for the majority of the predictor variables (Table 2) and mean annual temperature and the minimum temperature in the winter were also highly informative. Higher precipitation and lower mean temperatures were associated with higher elevation populations, which corresponds with populations with shorter circadian periods and lower intrapopulation range found at high elevations.

4 | DISCUSSION

We quantified natural genetic variation in circadian period among and within 30 populations of *B. stricta* sampled across a heterogeneous landscape in the central Rocky Mountains. With multiple maternal families representing each population, we found that quantitative genetic variation was significant and of comparable magnitude among and within populations. Spatial proximity explained only a small proportion of among-population patterns of variation in mean circadian period and its intrapopulation range, but elevation-related variation in the trait suggested environment-driven genetic differentiation in circadian rhythms in the region.

4.1 | Circadian period manifests genetic variation among and within natural plant populations

As hypothesized, we found that populations of *B. stricta* sampled along an 800-m elevational gradient expressed significant differences in circadian period in a common-garden environment. Along with accompanying differentiation among maternal families within populations, this result is indicative of quantitative genetic variation in circadian period controlled by several simultaneously varying genes whose effects may be environment-dependent (e.g., Brachi et al., 2010; Hill, 2010; Mackay et al., 2009; Rees et al., 2021). Population means varied between 21.9 and 24.9 h, and the broad-ranging regional population sample in this study expanded the among-population range of variation in circadian period by approximately 2 h from that previously described by Salmela et al. (2016) with a slightly smaller elevational gradient spanning approximately 600 m. The only other study to estimate among-population genetic differentiation in circadian period across elevations found that in *Mimulus laciniatus* in the California Sierra Nevada, population means varied by 1.6 h within ca. 100 km and along an elevational range of 1000–2600 m (Leinonen et al., 2020). The current 3-h range among population means accounts for approximately 46% of the genotypic range documented among 150 *A. thaliana* accessions sampled across the Northern hemisphere (Michael et al., 2003), and for 68% of the corresponding range among 191 *A. thaliana* accessions sampled

TABLE 2 Weights of individual environmental variables on one or two components that explain genetic variation in circadian period in leaf movement among or within populations of *B. stricta* and that were identified by partial least-squares (PLS) regression.

	Component 1	Component 2
Weights—PLS model for population mean		
Mean annual temperature	0.180	
Min. temperature of coldest month	0.134	−0.466
Annual precipitation	−0.891	0.350
Precipitation of wettest month		0.172
Precipitation seasonality		0.251
Precipitation of wettest quarter	−0.122	0.489
Precipitation of driest quarter	−0.325	−0.213
Precipitation of warmest quarter	−0.124	0.442
Precipitation of coldest quarter	−0.325	−0.213
Weights—PLS model for intrapopulation range		
Mean annual temperature	0.173	
Min. temperature of coldest month	0.146	
Annual precipitation	−0.852	
Precipitation of wettest quarter	−0.129	
Precipitation of driest quarter	−0.301	
Precipitation of warmest quarter	−0.129	
Precipitation of coldest quarter	−0.301	

within Sweden (Rees et al., 2021). These contrasts are notable because we restricted our sampling to a considerably narrower geographic range.

Although a high degree of self-fertilization in species like *B. stricta* is often expected to erode various forms of genetic variation on a fine spatial scale (e.g., Wright et al., 2013), a significant effect of maternal family nested within population showed that genetic variation in circadian period was present locally, that is, on a scale of only a few hundred metres. Further, the observed magnitude of intrapopulation genetic variation was comparable to that observed by Salmela et al. (2016). Variance components estimated for the experimental factors indicated that among- and intrapopulation genetic differences were of similar magnitude. This finding of quantitative genetic variation occurring at different hierarchical levels agrees with Song et al. (2006). Their research found that up to 40% of variation in 21 polymorphic microsatellite loci segregated within and up to 47% segregated among populations in *B. stricta*. This conclusion differs somewhat from the study by Salmela et al. (2016) in which intrapopulation quantitative genetic variation in circadian period exceeded that found among populations; this difference most likely arises from a greater number of populations sampled in the current study. Studies in other species document varying degrees of intra- relative to among-population variation (Greenham et al., 2017; Leinonen et al., 2020), and it remains to be seen if these patterns generalize to life-history or other species-specific attributes.

In considering variation in biological rhythms, it is worth reviewing the observed range of natural genetic variation in circadian rhythms from the perspective of the classical circadian resonance theory. This theory postulates a fitness advantage to endogenous circadian periods whose length matches that of the exogenous environmental cycle (Dodd et al., 2005; Ouyang et al., 1998; Pittendrigh & Minis, 1972; Woelfle et al., 2004). We detected a wide range of genetic variation in circadian period in natural populations that have experienced only 24-h diel cycles in their native habitats. This raises the possibility of weak daylength-imposed natural selection on the circadian clock, which in recent animal studies has been proposed to explain the considerable variation detected in period length of locomotor activity within and among three spider species and in mouse lemurs (Hozer et al., 2020; Mah et al. 2019). Indeed, these parallel observations across plant and animal species suggest that 24-h exogenous cycles do not select just for endogenous cycling patterns of a matching length. Considering that genetic variation in circadian period is not manifested under 24-h environmental cycles, it is possible that the trait is influenced by selection indirectly via its correlation with another circadian trait-like phase that is relevant in a naturally oscillating environment and that is correlated with circadian period in some (Rees et al., 2021; Rubin et al., 2017) but not all datasets (Michael et al., 2003) in *A. thaliana*. Genetic variation in the clock could be affected by selection on genetically related phenological traits like flowering time: Salmela et al. (2018) found that maternal families from a single population of *B. stricta* that had longer circadian periods tended to flower earlier and at a larger size after vernalization. Experimental progeny from controlled crosses of *A. thaliana* and *Brassica rapa* accessions indicate that clock traits covary also with shoot growth patterns (Rubin et al., 2018) and photosynthetic traits (Edwards et al., 2011; Yarkhunova et al., 2019). To identify environmental factors that may select for natural genetic variation in circadian period in *B. stricta*, it is necessary to investigate among-population differences in biological rhythms in relation to the steep spatial environmental gradients that characterize the study area in the Rocky Mountains.

4.2 | Genetic variation in circadian period follows an elevational gradient in the Rocky Mountains

We detected limited spatial autocorrelation in our data, indicating that in rare cases spatially adjacent populations exhibited similar population means or intrapopulation ranges of genetic variation in circadian period. On the whole, we found evidence for a moderate negative association between elevation and population means of circadian period in our sample. This trend may be an outcome of populations sampled close to and above 3000 m exhibiting means below 24 h while for instance at ca. 2700 m the full range of

among-population genetic variation was evident. Similarly, the range of intrapopulation genetic variation decreased towards higher elevations, which agrees with the results of Salmela et al. (2016) and which may result from more intense natural selection in marginal habitats with long winters and limited growing seasons and resources.

We estimated home site conditions of the populations using the WorldClim data (Hijmans et al., 2005) and found evidence for pronounced temperature and precipitation gradients along the 800-m elevational range, with estimates of average annual temperature varying from ca. +4°C at 2500 m to ca. −1°C at 3300 m. This pattern denotes that growing seasons begin at different timepoints and photoperiods depending on the elevation: by June, the two highest-elevation sites may have yet to reach a monthly mean of +5°C. On the other hand, average precipitation was estimated to increase towards higher elevations, while soil pH was lower at higher elevations. Elevation-dependent variation in multiple environmental factors related to temperature and precipitation complicates the identification of explicit selective agents acting on the circadian clock in the region. It is possible that pronounced differences, for instance, in overall temperature conditions across the elevational gradient affect optimal daily timing of various biological processes in a manner that generates spatially varying selection on the clock directly. Alternatively, differences in growing season length and attendant selection on phenology could exert indirect selection on the circadian clock due to genetic correlations between flowering time and the clock. When a species is found in highly divergent environments, it is possible that different environmental factors drive local adaptation even across adjacent populations, as is the case with nearby populations of other species (Popovic & Lowry, 2020). Such differentiation in selective pressures might give rise to large-scale spatial clines in quantitative traits with consequential levels of unexplained genetic variation.

4.3 | Conclusions

Our study on natural plant populations in the Rocky Mountains revealed significant genetic variation in circadian rhythms on a relatively fine regional scope: populations sampled close to 41°N and separated by 150 km at most in distance varied by up to 3 h in circadian period, while maternal families within a population could vary by as much as 6 h—ranges of among- and intrapopulation variation that are striking relative to the global range of natural genetic variation reported among divergent accessions of the model system *A. thaliana*. Further, higher-elevation populations had shorter mean circadian periods and narrower intrapopulation ranges of variation than those sampled below 3000 m, suggesting that spatial environmental heterogeneity across elevations explains a proportion of the trait´s variation. Overall, the observed magnitude of natural genetic variation in circadian period within the sampled area supports the idea that the trait might not be under constant selective pressure in the wild and that its variation could be linked to adaptation to climatic factors rather than diel cycles of varying lengths (Hozer et al., 2020; Mah et al., 2020; Salmela & Weinig, 2019). To determine what role the clock plays in local adaptation in variable environments, it would be important to simultaneously assess other fitness-related traits with common genetic bases, for example, photoperiodic responses (Leinonen et al., 2020) and flowering time (Salmela et al., 2018). With a wider selection of quantitative traits, ecological strategies across environmental gradients can be described as trait syndromes, that is, sets of divergent traits that are correlated with each other (e.g., Takou et al., 2019).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support these findings of this study are available in Dryad at doi:10.5061/dryad. kh189327x.

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SUPPORTING INFORMATION

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