Regional genetic diversity in circadian period in Boechera stricta populations is high relative to the global range of diversity in Arabidopsis

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Abstract

Circadian clocks manifest adaptations to predictable 24-h fluctuations in the exogenous environment, but it has yet to be determined why the endogenous circadian period length in the wild varies genetically around the hypothesized optimum of 24 h. We quantified 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 elevational gradient spanning 2460-3300 m in the Rocky Mountains. Measuring over 3800 plants from 473 maternal families (7-20 per population), we found genetic variation that was of similar magnitude among vs. within populations, with population means varying between 21.9-24.9 h and maternal family means within populations varying by up to ˜6 h. After statistically factoring out spatial autocorrelation at the habitat extremes, we found that elevation explained a significant proportion of genetic variation in circadian period such that higher-elevation populations had shorter mean period lengths and less within-population variation. 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 provide evidence that spatially fine-grained environmental heterogeneity contributes to naturally occurring genetic diversity in circadian traits in wild populations.

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Introduction

Different species in a shared habitat may be exposed to divergent selective agents, but one selective force common to natural populations across the globe is the diel cycling of environmental conditions at an unvarying pace of 24 h as a result of the Earth´s rotation. The prevalence of such selection pressure is evidenced by the evolution of the circadian clock, an endogenous timekeeper, across the tree of life, which allows for varied biological functions from the expression of individual genes to physiology and behavior to be appropriately timed relative to anticipated environmental oscillations (e.g., Yerushalmi and 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 and Minis 1972); an arrhythmic *Synechococcus* bacterial strain was outcompeted by the wild type with a functional clock in cycling conditions (Woelfle et al. 2004), 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, the 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 and 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 and Minis 1972). Evidence for this hypothesis has been reported in studies on 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, Woelfle et al. 2004; Rubin et al. 2017), but results from resonance experiments are not always fully compatible with the classical theory (e.g., Graf et al. 2011, Horn et al. 2019, Woodley of Menie et al. 2019).

While experimental genotypes with induced mutations are useful in dissecting the explicit molecular components of varied biological functions, they are not representative of patterns of genetic diversity that have arisen in natural environments. In the wild, various evolutionary processes determine the partitioning of genetic variation in quantitative traits among *vs.* within populations (Mackay et al. 2009, Mitchell-Olds et al. 2007), and 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 and 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 migration is restricted (McRae and Beier 2007, Slatkin 1987, Wright 1943).

Intraspecific genetic variation that tracks spatial heterogeneity in the environment characterizes many plant traits related to fitness, including timing of growth and reproduction (e.g., Méndez-Vigo et al. 2011) which have overlapping molecular genetic underpinnings 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. 2017). Circadian traits are no different from other quantitative traits when it comes to substantial naturally occurring 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°, while Rees et al. (2021) restricted their sampling to Sweden and described a range of 4.4 h in delayed fluorescence among 191 genotypes sampled between 55° and 63° N. Since diel cycles are uniformly 24 h in length across the globe, variation in circadian period has predominantly been examined in relation to latitude that correlates closely with photoperiod and its variability over the course of a year (e.g., Hut et al. 2013). Yet, latitude alone explains only a minor proportion (less than 8 %) of the observed genetic variation in circadian period in *A . thaliana* (Michael et al. 2003, Rees et al. 2021), and thus the environmental agents that help maintain variation in the clock remain to a large extent unidentified (Salmela and Weinig 2019). It is noteworthy that animals also exhibit significant natural variability in circadian period which in 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.

Beside studies on diverse pools of *A . thaliana* accessions, plant studies sampling multiple genotypes per geographic location (i.e., populations) have demonstrated that genetic variation in the clock concurrently

segregates among and within populations (Greenham et al. 2017, Leinonen et al. 2020, Salmela et al. 2016) in a manner that is similar for instance to flowering time in natural populations of*A . thaliana* on the Iberian Peninsula (Méndez-Vigo et al. 2013). For instance, in an annual *Mimulus guttatus* population from southern Oregon, maternal seed families exhibited a genotypic range of almost 4 h in circadian period (Greenham et al. 2017). Further, in a population of *B . stricta* from southeastern Wyoming, circadian period varied by 3.5 h among maternal families sampled within a few hundred meters, 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 genetic variation in the circadian clock.

The contribution of environmental heterogeneity to the genetic diversity of circadian rhythms can be uncovered by sampling multiple populations across well-defined spatial gradients. Temperature conditions vary for instance with elevation such that growing seasons begin later and at longer day lengths at higher elevations, giving rise to genetic clines in quantitative traits even within relatively narrow spatial scales (e.g., Leinonen et al. 2020). Here, we sample plant populations intensively within a limited latitudinal but an 800-m elevational gradient in order 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 relative of *A . thaliana* with a wide North American distribution (Song et al. 2006). With the utilization of circadian assays of leaf movement across these populations and on over 3800 plants, we examine whether different evolutionary factors including stochastic and selective forces are associated with the spatial trends of variation in circadian period within a region that is small compared to previous studies on natural genotypes (e.g., Greenham et al. 2017, Michael et al. 2003, Rees et al. 2021) but that nonetheless comprises pronounced environmental heterogeneity. Based on a preceding study that found significant among- and within-population genetic diversity in circadian period in the same region but with only four populations and a smaller elevational gradient (Salmela et al. 2016), we hypothesize that **(1)** circadian period will exhibit genetic variation among and within the 30 populations,**(2)** that the range of variation among population 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, patterns of genetic variation among and within populations will correlate with elevation and associated local environmental variables.

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 elevational gradient (2460-3300 m above sea level) in three distinct mountain ranges (Fig. 1). Habitat included areas of sagebrush in the lower elevation populations 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) were 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 lineage.

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 to 20 family lines were selected for circadian analysis, and within each family, 18 replicates were included. We planted seeds into Redi-Earth potting mix (Sungro, Agawam, MA, USA) in 1-cm diameter pots and placed the pots into growth chambers (Percival Scientific, Perry, IN, USA) for germination using a 12-h/12-h light/dark cycle and 21 °C/18 °C day/night temperature cycles. After six days, seedlings were moved to 14-h/8-h light/dark cycle for entrainment; temperature conditions during entrainment simulated early May conditions in Laramie, WY

with hourly cycling for temperature from 4 °C to 17.5 °C (Table S1). After seven 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 prior to imaging. Images were taken every 15 min for five days to ascertain leaf positions, and stacked images of leaf movements were analyzed 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.

We estimated period values through BioDare2 (Zielinski et al. 2014). Values for 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 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 family line within the populations were removed. Mean values for each family line and subsequently each population were then determined, as well as the within-population range of values (the range from shortest to longest circadian period family within each population).

2.3 Environmental Variables

To identify environmental factors that could act as agents of selection on circadian period, we analyzed data for 19 bioclimatic variables in the WorldClim dataset (Fick and Hijmans 2017) for each of our population sites. For the analysis of environmental data, values for the climate variables collected at 30-s resolution (˜1 km^2) were utilized, including mean annual temperature and annual precipitation and measures of peak and low temperature and moisture 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. We also collected three soil samples; in the lab, we measured soil pH, soil electrical conductivity, soil moisture, bulk density, and soil texture (as percent of silt, sand, and clay composition).

2.4 Statistical Analysis

We tested for genetic differences among and within populations in circadian period and the range of circadian period. Because data distributions violated the assumptions of normality and heteroscedasticity, we also applied a non-parametric test, Welch's Heteroscedastic F test (Welch 1951) in R 3.6.3 with package "onewaytest" (Dag et al. 2018), to both the test of significant differences among populations as well as the test of significant differences among families within a population. The effect of "trial" was also tested, as 18 separate trials were conducted to screen the complete number of individual replicates. General linear mixed models were used to test for the statistical significance of different factors, with "population" as a fixed effect and "family within population" as a random effect. The linear mixed models were tested using the lme4 package (Bates et al. 2015) with the restricted maximum likelihood (REML) approach.

To determine if the *B. stricta* populations were structured by simple 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 neighborhoods. 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 center 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 population range (value from mean of shortest family to longest family). 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 3.6.3 with packages "sp", "spdep", "rgdal", "spgwr", and "spatstat" (Baddeley and Turner 2005, Bivand et al. 2013, Bivand et al. 2021, Bivand and Yu 2020, Pebsema et al. 2005, R Core Team 2020).

To test for associations between circadian values and environmental variables, we first used multivariate linear regression. We tested the response variables for the circadian period of population mean and within-population range. All 27 environmental predictors were included in the models. We first fit the complete model with all variables, and then used AIC modeling to determine the best fit model. Environmental variables, however, exhibited significant multicollinearity, and we therefore used principal component analysis to reduce the dimensionality of the data. Analyses were conducted in R 3.6.3 with packages "mctest", "GGally", and "corpcor" (Imdadullah et al. 2016, Schafer et al. 2017, Schloerke et al. 2021) for testing multicollinearity and "ggbiplot" (Vu 2011) for principal component analysis. Having reduced data dimensionality, we used partial least squares regression (PLS) and principal component regression (PCR), where the predictor variables included all climate and soil factors and the response variables were mean circadian period and the interpopulation range of circadian period. PCR first computes the principal components 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 (World and Eriksson 2001). Variables were standardized by dividing each by its standard deviation. 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 3.6.3 and packages "pls" and "caret" (Kuhn 2020, Mevik et al. 2020).

Results

3.1 Variation in Circadian Period among and within Populations

Families with at least 5 replicates were included in analysis of variance of circadian period, with the largest number of replicates within a single family being 18. Both ANOVA and the Welch's F-test found highly significant differences among and within populations in mean circadian period (Table 1). Among the 30 populations analyzed, mean circadian period varied between 21.91 to 24.92 h, or three hours (Fig. 2). Although most of the populations expressed a circadian period close to a 24 h, many populations had a period that is much shorter; 10 of the 30 populations had a mean period value that was shorter than 22.5 h, and only 2 such populations (LEW, LIB) exhibited spatial autocorrelation. Variation within the populations (among the families within each population) was similar (variance component for the family within population effect 0.687, or 21.3 % of total variation) compared to the variation among the 30 populations (variance component for the population effect 0.787, or 24.4 % of total variation), which defines the structure of the populations. Thus, though the range of family values within many of the populations was large, the populations were structured with slightly higher variation *among* the populations (Table 2).

Within populations, the difference between the family with the shortest *vs* . longest circadian period length (range) was 3 h on average. Two populations expressed exceptionally high ranges of >5 h (Happy Jack Trail, HJT, and Crow Creek, CRW, populations; Table 2). 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 >5 h range. The wide range of values for the HJT and CRW populations contrasted with another population, Sand Lake, which had a range of values among families of less than an hour (Fig. 3). Neither population mean value nor the number of replicates (both individuals and family lines) had a significant association with the within-population 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 families within some

populations (> 5 h) is greater than the mean 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 is 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 and the range of family values within each population. Clustering of populations was limited to two specific "hot spots," and these accounted for the autocorrelation. First, two populations located at highest elevation within the Snowy Range (Libby Flats (LIB) and Lewis Lake (LEW)) had similar values for circadian period. Second, the three easternmost populations, located in the Laramie Range of SE Wyoming (populations Crow Creek (CRW), Happy Jack Trail (HJT), and Middle Crow Creek (MCC)), had similar values for population mean and within-population range. Outside of these two clusters, values were randomly spaced across the populations. Further results are presented 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 strong predictor for both the mean circadian period among populations ($F = 7.01$; $p = 0.01$) as well as the range of circadian period values found within a population ($F = 14.45$; $p < 0.001$) (Fig. 4a and b). More specifically, shorter circadian periods with a more constrained range of values were observed at higher elevations; at lower elevations, a greater range of circadian period values were 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 range of family values within each population. Akaike Information Criterion, 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 800m difference in elevation; the lowest elevation population (North Brush Creek (NBC), 2460m) and the highest (Libby Flats (LIB), 3300m) were both located in the Medicine Bow Mountains. The most widely separated populations (Sandstone (SDS) vs Middle Crow Creek (MCC)) were found 150km apart. Over this spatial range, environmental variables estimated by the Worldclim models for the 30 populations were highly varied. Mean annual temperature varied by 4.5 degrees and annual precipitation varied by over 200mm (Table 3). Climate variables had a strong association with elevation, showing decreasing temperature and increasing moisture for sites at higher elevation. The tested soil samples were variable among the populations (Fig. 5). Higher elevation populations were more strongly associated with reduced pH, higher content of sand and silt within the soil, and increased soil moisture.

Given that spatial structure did not account for population differences in circadian traits, we were interested to test for associations between circadian parameters and not only elevation but also environmental variables. Many of the measured environmental variables correlated with elevation, and multicollinearity analysis demonstrated strong associations among the environment variables. Principal component analysis was used to reduce the dimensionality of the data (Table 4). Along the first axis in the PCA, populations from high elevation (>3000m) separated from those from low elevation (<2800m; Fig. 6). Populations between high and low elevation fell between them on the PCA but appeared to be more strongly associated with low elevation.

We used principal component and partial least squares regression models to reduce the dimensionality of the environmental variable. As the predictor variables were shown to have high multicollinearity, the PCR and PLS regression allow a stronger estimation of the variables at the population sites that may affect or impose selection on circadian period. By comparing the PC and PLS regressions to each other and to linear regression models, these analyses indicate the models that best explain the variation in period mean and within-population range. For population mean circadian period, the PCR model best explains the

variation (98.2% of the variation in predictors accounted for, 30.8% of the variation of the population mean) while reducing the data to three dimensions. For within-population range, the PLS model explains the data best (91.8% of the variation in predictor variables, 35.2% of the population range) by only using the first component. The most informative coefficients are the same for both models (PLS and PCR) for each of the response variables (population mean and within-population range). For population mean circadian period, annual precipitation, elevation, and annual range of temperature at the site were the strongest predictors (Table 5). Elevation was the single most important predictor in the model for within-population range, but total annual precipitation and annual temperature range were also strong contributors (Table 6).

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 genetic diversity was significant and of comparable magnitude among and within populations. Spatial proximity explained only a small proportion of among-population patterns of diversity, but elevation-related variation in the trait suggested environment-driven genetic differentiation in the region.

Circadian period manifests genetic diversity among and within natural plant populations

As hypothesized, we found that populations of *B. stricta* along an 800-m elevational gradient were genetically differentiated in circadian period of leaf movement, with population averages varying between 21.9 h and 24.9 h. Thus, the broad-ranging regional population sample in this study expanded the among-population range of variation by approximately 2 h from that previously described by Salmela et al. (2016) with a smaller elevational gradient. The only other study to estimate among-population genetic differentiation in circadian period found that in *Mimulus laciniatus* in the California Sierra Nevada, population averages 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 genetic variation documented among 150 *A. thaliana* accessions sampled across the Northern hemisphere (Michael et al. 2003), and for 68 % of the variation among 191 *A. thaliana* accessions sampled in 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 genetic diversity 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, i.e., on a scale of only a few hundred meters. Further, we observed that the magnitude of within-population genetic diversity was variable, with eight populations expressing a genetic range in period length that exceeded four hours. Variance components indicated that among-population genetic differences accounted for a slightly larger proportion of total variation in circadian period than did among-family differences within populations, but overall, the amounts of genetic diversity at these two levels were similar. This finding agrees with Song et al. (2006) who found that up to 40 % of molecular marker diversity segregated within and up to 47 % segregated among populations in *B. stricta* , but it differs from the study by Salmela et al. (2016) in which within-population genetic diversity in circadian period exceeded that found among populations. The difference most likely arises from a greater number of populations sampled in the current study. The 3.5-h within-population range in genotypic means found by Salmela et al. (2016) is in agreement with the current results. In *M. laciniatus* in the Sierra Nevada, among-population genetic differences explained ca. 12 % of total variation in circadian period while genetic variation within populations contributed only ca. 2 % (Leinonen et al. 2020). This interspecific difference may stem from the contrasting life histories of the two species: *M. laciniatus* is a small annual plant with a very rapid life and a limited distribution in the Sierra Nevada, whereas *B. stricta* is a short-lived perennial found across North America. In the widely distributed *M. guttatus* in western North America, within-population genetic variation in circadian period was large compared to the magnitude of among-population differentiation (Greenham et al. 2017).

In considering variation in biological rhythms, it is worth reviewing the observed range of natural genetic vari-

ation in circadian rhythms from the perspective of the classical circadian resonance theory, which 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 and 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 trait, 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 provide evidence that 24-h exogenous cycles do not select just for endogenous cycling patterns of a matching length. Considering that 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 association with another circadian trait like phase that is relevant in a naturally oscillating environment. Indeed, circadian period and phase are correlated 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, and experimental progeny from controlled crosses of *A. thaliana* and*Brassica rapa* accessions indicate that clock traits covary 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 relation to the steep spatial environmental gradients that characterize the study area in the Rocky Mountains.

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 averages or ranges of within-population genetic variation. On the whole, we found evidence for a moderate negative association between elevation and population averages of circadian period in our sample. This trend may be an outcome of populations sampled close to and above 3000 m exhibiting averages below 24 h while for instance at ca. 2700 m the full range of among-population genetic variation was evident. Similarly, the range of within-population genetic variation decreased towards higher elevations, which agrees with the results of Salmela et al. (2016) and which may result from more intense 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 (Fick and Hijmans 2017) 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 degC at 2500 m to ca. -1 degC at 3400 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 degC. On the other hand, average precipitation was estimated to increase towards higher elevations, while soil pH was lower at higher elevations. Although we found that elevation explained a larger proportion of genetic diversity in circadian period (22 %) than did latitude in *A. thaliana* (Michael et al. 2003, Rees et al. 2021), variation was still largely residual, suggesting that complex spatial gradients are behind the observed levels of diversity. Contrastingly, in *M. laciniatus* in the California Sierra Nevada, circadian period was not linearly related to elevation although the WorldClim data indicated a strong association between elevation and temperature conditions (Leinonen et al. 2020). Importantly, the WorldClim data provide model-based estimates for a given location with a 1 x 1 km grid, which may mask substantial micro-environmental heterogeneity in complex mountainous landscapes. When a species is found in highly divergent environments, it is possible that different environmental factors drive local adaptation even across adjacent populations. This has been noted for instance in *M. guttatus* in California, where coastal and inland populations seem to be influenced by divergent selective agents (Popovic and Lowry 2020). Such differentiation might give rise to large-scale spatial clines in quantitative traits with consequential levels of unexplained variation.

A population-level survey on reproductive phenology in this species has yet to be conducted, but in the study

by Anderson and Gezon (2014), which sampled one maternal family of *B. stricta* per location in Colorado at 2800-3700 m, higher-elevation genotypes tended to flower earlier in common garden. Consequently, selection on photoperiodic responses could be a contributing factor to the diversity of circadian period among populations. Beside the circadian clock, other traits will need to be investigated in the same genetic origins to understand adaptation in this species more comprehensively and to uncover the mechanisms that help maintain significant genetic diversity within populations. A limitation of the WorldClim data is that it does not provide information on the extent of potential among-year variation in temperature conditions in the region. However, weather station data from the Rocky Mountains reveal long-term annual variability for instance in spring temperatures and timing of snowmelt, which in turn are correlated with timing of flowering in nearby plant populations (Anderson et al. 2012). Genetic variation in the circadian clock might be sustained across short spatial distances within populations if selection on circadian period – or on a closely correlated trait - varied in strength or direction in response to such environmental fluctuations. Causes of very spatially localized genetic diversity in nature are often poorly understood (e.g., Delph and Kelly 2014), but in *A. thaliana* on the Iberian Peninsula, spatially and temporally replicated field experiments provide evidence that fluctuating natural selection could preserve genetic variation for instance in phenology within populations (Exposito-Alonso et al. 2018).

Conclusions

Our study on natural plant populations in the Rocky Mountains revealed significant genetic variation in circadian period on a relatively fine regional scope: populations separated by up to 100 km varied by up to 3 h, while maternal families within a population could vary by as much as 6 h – ranges of variation that are striking relative to the global range of natural variation reported among divergent accessions of the model system *A. thaliana* . Further, higher-elevation populations had shorter average circadian periods and narrower ranges of within-population variation than those sampled below 3000 m, suggesting that spatial environmental heterogeneity across elevations explains a proportion of the trait's diversity. 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 diversity could be linked to adaptation to climatic factors rather than diel cycles of varying lengths (Hozer et al. 2020, Mah et al. 2020, Salmela and 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, e.g., photoperiodic responses (Leinonen et al. 2020) and flowering time (Salmela et al. 2018).

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References

Abe MS, Matsumura K, Yoshii T, Miyatake T (2021) Amplitude of circadian rhythms becomes weaker in the north, but there is no cline in the period of rhythm in a beetle. PLoS ONE 16: e024511.

Anderson JT, Gezon ZJ (2015) Plasticity in functional traits in the context of climate change: a case study of the subalpine forb*Boechera stricta* (Brassicaceae). Global Change Biology 21: 1689-1703.

Anderson JT, Inouye D, Mckinney A, Colautti R, Mitchell-Olds T (2012) Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. Proceedings of the Royal Society B 279: 3843–3852.

Atamian HS, Creux NM, Brown EA, Garner AG, Blackman BK, Harmer SL (2016) Circadian regulation of sunflower heliotropism, floral orientation, and pollinator visits. Science 353: 587-590.

Baddeley A, Turner R (2005) spatstat: an R package for analyzing spatial point patterns. Journal of Statistical Software 12: 1-42.

Bates D, Machler M, Walker S (2015) Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67: 1-48.

Bivand R, Keitt T, Rowlingson B (2021). rgdal: bindings for the 'Geospatial' data abstraction library. R package version 1.5-19.

Bivand R, Yu D (2020) spgwr: geographically weighted regression. R package version 0.6-34.

Bivand RS, Pebesma E, Gomez-Rubio V (2013) Applied spatial data analysis with R, Second edition. Springer, NY.

Brachi B, Faure N, Horton M, Flahauw E, Vazquez A, Nordborg M, Bergelson J, Cuguen J, Roux F (2010) Linkage and association mapping of*Arabidopsis thaliana* flowering time in nature. PLoS Genetics 6: e1000940.

Dag O, Dolgun A, Konar NM (2018) onewaytests: an R package for one-way tests in independent group designs. The R Journal 10: 175–199.

Delph LF, Kelly JK (2014) On the importance of balancing selection in plants. New Phytologist 201: 45-56.

Dodd AN, Salathia N, Hall A, Kevei E, Toth R, Nagy F, Hibberd JM, Millar AJ, Webb AAR (2005) Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. Science 309: 630-633.

Edwards CE, Ewers BE, Williams DG, Xie Q, Lou P, Xu X, McClung CR, Weinig C (2011) The genetic architecture of ecophysiological and circadian traits in *Brassica rapa* . Genetics 189: 375–390.

Exposito-Alonso M, Brennan AC, Alonso-Blanco C, Pico FX (2018) Spatio-temporal variation in fitness responses to contrasting environments in *Arabidopsis thaliana* . Evolution 72: 1570-1586.

Fick SE, Hijmans RJ (2017) Worldclim 2: new 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37: 4302-4315.

Geary RC (1954) The contiguity ratio and statistical mapping. The Incorporated Statistician 5: 115–145.

Graf A, Schlereth A, Stitt M, Smith AM (2010) Circadian control of carbohydrate availability for growth in *Arabidopsis* plants at night. Proceedings of the National Academy of Sciences USA 107: 9458-9463.

Greenham K, Lou P, Puzey JR, Kumar G, Arnevik C, Farid H, Willis JH, McClung CR (2017) Geographic variation of plant circadian clock function in natural and agricultural settings. Journal of Biological Rhythms 32: 26-34.

Greenham K, Lou P, Remsen SE, Farid H, McClung CR (2015) TRiP: Tracking Rhythms in Plants, an automated leaf movement analysis program for circadian period estimation. Plant Methods 11: 33–44.

Horn M, Mitesser O, Hovestadt T, Yoshii T, Rieger D, Helfrich-Forster C (2019) The circadian clock improves fitness in the fruit fly,*Drosophila melanogaster* . Frontiers in Physiology 10: 1374.

Hozer C, Perret M, Pavard S, Pifferi F (2020) Survival is reduced when endogenous period deviates from 24 h in a non-human primate, supporting the circadian resonance theory. Scientific Reports 10: 18002.

Hut RA, Paolucci S, Dor R, Kyriacou CP, Daan S (2013) Latitudinal clines: an evolutionary view on biological rhythms. Proceedings of the Royal Society of London B 280: 20130433.

Imdadullah M, Aslam M, Altaf S (2016) mctest: an R package for detection of collinearity among regressors. The R Journal 8: 499-509.

Jolliffe IT (1982) A note on the use of principal components in regression. Journal of the Royal Statistical Society: Series C (Applied Statistics) 31: 300–303.

Kuhn M (2020) caret: classification and regression training. R package version 6.0-86.

Loveless MD, Hamrick JL (1984) Ecological determinants of genetic structure in plant populations. Annual Review of Ecology and Systematics 15: 65–95.

Mackay TFC, Stone EA, Ayroles JF (2009) The genetics of quantitative traits: challenges and prospects. Nature Reviews Genetics 10: 565–577.

Mah A, Ayoub N, Toporikova N, Jones TC, Moore D (2020) Locomotor activity patterns in three spider species suggest relaxed selection on endogenous circadian period and novel features of chronotype. Journal of Comparative Physiology A 206: 499–515.

McRae BH, Beier P (2007) Circuit theory predicts gene flow in plant and animal populations. Proceedings of the National Academy of Sciences USA 104: 19885–19890.

Mendez-Vigo B, Gomaa NH, Alonso-Blanco C, Pico FX (2013) Among- and within-population variation in flowering time of Iberian*Arabidopsis thaliana* estimated in field and glasshouse conditions. New Phytologist 197: 1332-1343.

Mendez-Vigo B, Pico FX, Ramiro M, Martinez-Zapater JM, Alonso-Blanco C (2011) Altitudinal and climatic adaptation is mediated by flowering traits and *FRI* , *FLC* , and *PHYC* genes in Arabidopsis. Plant Physiology 157:1942-1955.

Mevik B-H, Wehrens R, Liland KH (2020) pls: partial least squares and principal component regression. R package version 2.7-3.

Michael TP, Salome PA, Yu HJ, Spencer TR, Sharp EL, McPeek MA, Alonso JM, Ecker JR, McClung CR (2003) Enhanced fitness conferred by naturally occurring variation in the circadian clock. Science 302: 1049-1053.

Moran PAP (1950) Notes on continuous stochastic phenomena. Biometrika 37: 17–23.

Ouyang Y, Andersson CR, Kondo T, Golden SS, Johnson CH (1998) Resonating circadian clocks enhance fitness in cyanobacteria. Proceedings of the National Academy of Sciences USA 95: 8660-8664.

Palumbi SF (2003) Population genetics, demographic connectivity, and the design of marine reserves. Ecological Applications 13: 146–158.

Pittendrigh CS, Minis DH (1972) Circadian systems: longevity as a function of circadian resonance in *Drosophila melanogaster* . Proceedings of the National Academy of Sciences USA 69: 1537-1539.

Pivarciova L, Vaneckova H, Provaznik J, Wu BC, Pivarci M, Peckova O, Bazalova O, Cada S, Kment P, Kotwica-Rolinska J, Dolezel D (2016) Unexpected geographic variability of the free running period in the linden bug *Pyrrhocoris apterus* . Journal of Biological Rhythms 31: 568-576.

Popovic D, Lowry DB (2020) Contrasting environmental factors drive local adaptation at opposite ends of an environmental gradient in the yellow monkeyflower (*Mimulus guttatus*). American Journal of Botany 107: 298-307.

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rees H, Joynson R, Brown JKM, Hall A (2021) Naturally occurring circadian rhythm variation associated with clock gene loci in Swedish*Arabidopsis* accessions. Plant, Cell and Environment 44: 807– 820.

Rubin MJ, Brock MT, Davis AM, German ZM, Knapp M, Welch SM, Harmer SL, Maloof JN, Davis SJ, Weinig C (2017) Circadian rhythms vary over the growing season and correlate with fitness components. Molecular Ecology 20: 5528-5540.

Rubin MJ, Brock MT, Baker RL, Wilcox S, Anderson K, Davis SJ, Weinig C (2018) Circadian rhythms are associated with shoot architecture in natural settings. New Phytologist 219: 246-258.

Salmela MJ, Greenham K, Ping L, McClung CR, Ewers BE, Weinig C (2016) Variation in circadian rhythms is maintained among and within populations in *Boechera stricta* . Plant, Cell and Environment 39: 1293-1303.

Salmela MJ, McMinn RL, Guadagno CR, Ewers BE, Weinig C (2018) Circadian rhythms and reproductive phenology covary in a natural plant population. Journal of Biological Rhythms 33: 245-254.

Schafer J, Opgen-Rhein R, Zuber V, Ahdesmaki M, Silva APD, Strimmer K (2017) corpcor: efficient estimation of covariance and (partial) correlation. R package version 1.6.9.

Schloerke B, Cook D, Larmarange J, Briatte F, Marbach M, Thoen E, Elberg A, Crowley J (2021) GGally: extension to 'ggplot2'. R package version 2.1.0.

Siol M, Wright SI, Barrett SCH (2010) The population genomics of plant adaptation. New Phytologist 188: 313–332.

Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science 236: 787–792.

Song B, Clauss MJ, Pepper A, Mitchell-Olds T (2006) Geographic patterns of microsatellite variation in *Boechera stricta* , a close relative of *Arabidopsis.* Molecular Ecology 15: 357–369.

Vidigal DS, Marques ACSS, Willems LAJ, Buijs G, Mendez-Vigo B, Hilhorst HWM, Bentsink L, Pico FX, Alonso-Blanco C (2016) Altitudinal and climatic associations of seed dormancy and flowering traits evidence adaptation of annual life cycle timing in *Arabidopsis thaliana* . Plant, Cell and Environment 39: 1737–1748.

Vu VQ (2011) ggbiplot: A ggplot2 based biplot. R package version 0.55.

Woelfle MA, Ouyang Y, Phanvijhitsiri K, Johnson CH (2004) The adaptive value of circadian clocks: an experimental assessment in cyanobacteria. Current Biology 14: 1481-1486.

Woodley of Menie MA, Pawlik P, Webb MT, Bruce KD, Devlin PF (2019) Circadian leaf movements facilitate overtopping of neighbors. Progress in Biophysics and Molecular Biology 146: 104-111.

Wright S (1943) Isolation by distance. Genetics 28: 114–138.

Wright SI, Kalisz S, Slotte T (2013) Evolutionary consequences of self-fertilization in plants. Proceedings of the Royal Society B 280: 20130133.

Yarkhunova Y, Guadagno CR, Rubin MJ, Davis SJ, Ewers BE, Weinig C (2019) Circadian rhythms are associated with variation in photosystem II function and photoprotective mechanisms. Plant, Cell and Environment 41: 2518–2529.

Yerushalmi S, Green RM (2009) Evidence for the adaptive significance of circadian rhythms. Ecology Letters 12: 970-981.

Zielinski T, Moore AM, Troup E, Halliday KJ, Millar AJ (2014) Strengths and limitations of period estimation methods for circadian data. PLoS ONE 9: e96462.

Tables

Table 1. ANOVA results testing for differences among the populations and for within-population differences among family lines.

	d.f	MS	F-value	
Population	29	127.34	77.97	***
Family (population)	470	5.52	3.38	***
Trial	17	7.44	4.56	**

	d.f	MS	F-value	
Population (trial-corrected)	29	106.57	53.36	***
Population (Welch's ANOVA)	29	126.91	63.63	***

Table 2. Welch's ANOVA results per population test if family lines within each population were significantly different. Descriptions of population values, including mean value of circadian period, are included.

BAC, Barrett Creek; BBL, Barber Lake; BER, Bear Creek; BHM, Blackhall Mountain; BLR, Barber Lake Road; BRC, Bert Creek; CHP, Chimney Park; CRW, Crow Creek; DFC, Daminfour Creek; FOC, Foote Creek; GTC, Green Timber Creek; HJT, Happy Jack Trail; LBC, Little Brush Creek; LBL, Little Brooklyn Lake; LEW, Lewis Lake; LIB, Libby Flats; LKC, Lake Creek; MIC, Miner's Creek; MLT, Meadow Loop Trail; NBC, North Brush Creek; NFC, North French Creek; NMC, North Mullen Creek; SBC, South Brush Creek; SDL, Sand Lake; SDS, Sandstone; SLR, Slash Ridge; SWP, Sawmill Park; WBS, Webber Springs; WTO, Wildlife Turnout.

Population	**Replicates**	**Families**	**Elevation**	**F-stat**	**d.f.**	**p-value**	**Population mean**	**Population range**
BAC	59	10	2800	3.81	9	*	22.20	4.25
BBL	215	17	2625	8.82	16	***	22.04	3.12
BER	48	9	2980	1.21	8	ns	22.61	2.53
BHM	46	10	2880	10.22	9	***	22.31	4.04
BLR	83	10	2860	3.94	9	**	22.39	1.15
BRC	32	9	2680	1.65	8	ns	24.68	3.35
CHP	35	8	2570	4.84	7	**	24.53	3.75
CRW	164	20	2500	197.51	19	***	24.92	5.82
DFC	157	20	2790	2.14	19	*	22.62	1.89
FOC	185	19	2620	6.07	18	***	22.48	2.70
GTC	240	20	2670	5.23	19	***	23.05	3.00
HJT	140	20	2520	9.28	19	***	24.31	5.03
LBC	100	15	2690	4.27	14	***	21.94	4.54
LBL	165	20	3150	9.1	19	***	22.25	3.07
LEW	185	17	3270	4.33	16	***	22.79	2.52
LIB	199	17	3300	16.13	16	***	22.86	1.79
LKC	32	7	2770	26.12	6	**	22.62	2.80
MCC	88	17	2460	2.13	16	*	24.33	4.49
MIC	163	20	2750	1.86	19	*	22.13	3.67
MLT	68	10	2750	15.18	9	***	22.72	2.46
NBC	210	20	2460	5.71	19	***	23.62	3.57
NFC	64	14	3110	7.21	13	***	22.02	1.64
NMC	149	20	2670	3.47	19	**	22.78	4.24
SBC	231	20	2580	5.27	19	***	22.60	3.51
SDL	160	19	3070	2.55	18	**	23.12	0.65
SDS	165	18	2550	1.54	17	ns	23.60	3.48
SLR	34	8	2940	1.11	7	ns	23.30	3.84
SWP	217	20	2720	9.18	19	***	23.32	3.63
WBS	109	20	2830	3.3	19	**	24.40	4.62
WTO	105	19	3230	4.6	18	***	21.91	2.55

Table 3. Selected climate and soil variables from each of the 30 population sites.

Population Name	Mean Annual Temp. (@C)	Mean Annual Diurnal Range (@C)	Max. Temp. of Warm. Month (@C)	Mean Temp. of Cold. Quarter (@C)	Annual Precip. (mm)	Precip. of Wet. Month (mm)	Precip. Seasonality (mm)	Precip. of Warm. Quarter (mm)	Soil pH	Soil EC	% Sand	% C
BAC	1.7	13.8	21.9	-7.4	506	51	14	139	6.0	15.6	54.6	1
BBL	2.5	14.1	22.7	-6.6	464	49	19	137	6.4	10.8	64.3	1
BER	5.0	13.6	20.3	-8.4	565	56	14	155	5.8	4.6	63.2	1
BHM	1.4	14.4	21.8	-8.0	549	53	10	144	5.9	19.6	62.6	1
BLR	1.4	13.6	21.3	-7.4	507	53	17	145	6.1	6.0	63.3	1
BRC	2.1	14.6	22.6	-7.2	527	53	15	148	5.7	12.0	70.7	1
CHP	1.4	14.4	21.6	-7.9	546	55	15	154	6.0	17.6	68.6	1
CRW	3.0	14.0	23.0	-6.0	440	60	41	158	6.0	49.6	77.6	1
DFC	1.5	14.6	22.2	-8.0	542	52	9	140	6.1	18.3	76.6	1
FOC	3.0	13.8	23.4	-6.0	415	47	22	121	6.4	28.3	58.8	1
GTC	1.7	14.5	22.6	-7.9	509	50	9	130	6.4	30.0	79.0	1
HJT	3.5	14.2	23.8	-5.7	413	58	43	151	6.1	18.1	66.5	1
LBC	2.4	14.1	22.8	-6.8	472	49	15	130	6.0	13.3	65.8	1
LBL	-0.2	12.8	19.1	-8.8	584	60	15	161	6.4	39.3	62.5	1
LEW	-1.0	12.4	18.0	-9.5	620	63	14	167	5.3	44.0	53.8	1
LIB	-1.1	12.4	17.9	-10.0	628	64	14	169	5.2	43.6	60.8	1
LKC	1.4	14.4	21.6	-7.9	546	55	15	154	6.0	17.6	68.6	1
MCC	3.7	14.4	24.1	-5.4	415	59	44	153	6.0	42.6	78.6	9
MIC	1.7	14.3	22.4	-7.9	505	50	10	130	6.0	10.3	72.7	1
MLT	2.2	13.9	22.3	-6.9	470	50	19	138	6.2	33.3	60.2	1
NBC	3.4	14.3	24.2	-6.0	420	45	17	117	6.2	36.6	65.5	1
NFC	0.0	13.0	19.5	-8.7	573	58	14	156	5.6	23.3	58.6	1
NMC	2.2	14.3	22.6	-7.1	501	51	14	138	6.0	6.5	61.3	1
SBC	3.2	14.4	23.9	-6.3	438	46	17	122	5.8	18.6	55.6	1
SDL	0.4	12.9	19.8	-8.3	547	57	16	152	5.2	13.0	51.0	2
SDS	2.8	14.9	24.2	-7.4	441	43	10	113	6.8	36.0	54.2	1
SLR	0.9	13.4	20.7	-8.0	536	54	14	146	5.7	22.6	47.0	2
SWP	2.4	13.6	22.6	-6.6	445	49	19	128	6.3	9.7	58.2	1
WBS	1.3	14.0	21.8	-8.1	512	51	10	131	5.8	16.2	61.1	1
WTO	-0.6	12.7	18.6	-9.2	605	61	13	162	5.3	9.8	52.1	1

Table 4. Contributions to the PCA along the first three dimensions.

Environmental Variable	Dim 1	Dim 2	Dim 3
Mean Annual Temp.	8.175873	0.014046	0.259475
Mean Annual Diurnal Range	5.438736	2.738177	4.39212
Isothermality	3.659796	0.5577	4.968144
Temp. Seasonality	3.395135	8.05229	1.980139
Max Temp of Warmest Month	8.030107	0.459914	0.001444
Min Temp of Coldest Month	5.721602	1.741673	5.561818
Temp. Annual Range	0.204199	0.005347	0.630802
Mean Temp. of Wettest Quarter	3.971838	4.263307	0.470629
Mean Temp. of Driest Quarter	0.408852	4.40232	0.217807
Mean Temp. of Warmest Quarter	8.140053	0.074817	0.069247

Mean.Temp.of.Coldest.Quart	3.283379	0.011403	0.005012
Annual Precipitation	7.520978	0.071521	2.491851
Precip. Of Wettest Month	3.542352	12.31351	0.991667
Precip. Of Driest Month	6.224582	4.065052	2.522827
Precipitation Seasonality	1.965477	16.86486	0.746214
Precip. Of Wettest Quart	3.796683	11.59032	0.947383
Precip. Of Driest Quart	6.074684	4.518533	2.272777
Precip. Of Warmest Quart	3.782361	10.33083	1.372667
Precip. Of Coldest Quart	6.074684	4.518533	2.272777
Soil pH	4.076544	2.246973	0.221101
Soil Electrical Conductivity	0.000237	4.997221	0.016574
Percentage Soil Moisture	1.89885	0.131405	5.94154
Percentage Sand	1.755912	2.009057	23.00746
Percentage Silt	1.123645	2.868218	18.54711
Percentage Clay	1.733442	1.152973	20.09141

Table 5. Loadings for PCR model to explain mean population values

Loadings	Comp 1	Comp 2	Comp 3
Elevation	-0.963		0.249
Temp. annual range		-0.998	
Annual precipitation	-0.226		-0.786
Precip. of driest month			-0.101
Precip. Seasonality			0.141
Precip. of driest quarter			-0.360
Precip. of coldest quarter			-0.360

Table 6. Loadings for PLS model to explain range of within-population values

Loadings	Comp 1	Comp 2
Elevation	-0.964	
Temp. annual range		-1.193
Annual precipitation	-0.226	

Figure legends

Figure 1. Thirty populations of *Boechera stricta* are located in the Medicine Bow National Forest in south-eastern Wyoming

Figure 2. A comparison of the 30 populations for circadian values of the families within each population demonstrates a large range of the trait in many of the populations. In the figure, mean values are represented by a gray dot and populations are ordered by mean value of the population from shortest to longest circadian period.

Figure 3. The within-population variation of two populations (Sand Lake (a; SDL) and Crow Creek (b; CRW)) represent the two extremes of the range for family values. The CRW population has over 5 h range from the shortest to longest family, while the SDL population has just over 0.5 h range.

Figure 4. As a univariate explanation, elevation of the populations has a strong impact on the a) mean value of circadian period for the population and b) the range of family values within each population.

Figure 5. The relationship between elevation and the first 4 principal components, showing the strongest relationship along the first component.

Figure 6. Principal component analysis of the environmental variables across the 30 populations demonstrates a strong separation of the low and high elevation populations along the first component.

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