

1 **Title:**

2 Environmental contributions to the evolution of trait differences in *Geum triflorum*: implications
3 for restoration

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15 **Short title:**

16 Environment predicts trait differences in *Geum triflorum*

17 **ABSTRACT**

18 ***Premise of the Study***

19 Understanding how environment influences the distribution of trait variation across a species'
20 range has important implications for seed transfer during restoration. Heritable genetic
21 differences associated with environment could impact fitness when transferred into new
22 environments. Here, we test the degree to which the environment shapes the evolution and
23 distribution of genetic effects for traits important to adaptation.

24 ***Methods***

25 In a common garden experiment, we quantified trait differentiation for populations of *Geum*
26 *triflorum* sourced from three distinct ecoregions and evaluated the ability of climate to predict
27 trait variation. Populations were sourced from alvar ecoregions which experience predictable
28 extremes in seasonal water availability and the prairie ecoregion which exhibits unpredictable
29 changes in water availability.

30 ***Key Results***

31 Plants sourced from alvar ecoregions exhibited smaller but more numerous stomata and greater
32 intrinsic water use efficiency relative to prairie plant populations supporting the evolution of
33 ecotypic differences. Estimates of standing genetic variance and heritable genetic variation for
34 quantitative traits suggest alvar populations have greater adaptive potential. However, reduced
35 evolvability suggest all populations of *G. triflorum* may have limited capacity to evolve in
36 response to environmental change.

37 ***Conclusions***

38 These results point towards the importance of understanding the role of environment in shaping
39 the distribution and evolution of genetic differences across seed populations and how these data
40 may inform recommendations for seed transfer across novel environments and our expectations
41 of populations' adaptive potential.

42

43 **Key Words**

44 Heritability, evolvability, ecotypic variation, water-use efficiency, grassland restoration,
45 common garden, stomata, alvar, prairie

46

47 INTRODUCTION

48 Understanding how the environment influences trait variation is essential, particularly
49 within the context of restoration (Wang et al., 2010). The evolution of ecotypic differences for
50 vegetative, physiological, or reproductive life history traits can lead to differential success
51 following seed transfer across environments during restoration (McKay et al., 2005; Anderson et
52 al., 2016; Braasch et al., 2021; VanWallendael, Lowry & Hamilton, 2022). In addition, the
53 history of selection may influence the distribution of genetic variance of traits important to
54 adaptation. Variance in the heritability or evolvability of traits is expected to impact the success
55 of ecotypes when planted in novel restored environments (Broadhurst et al., 2008; Crowe &
56 Parker, 2008; Havens et al., 2015). Thus, quantifying how environment contributes to the
57 evolution of trait differences and the distribution of genetic variance provides important insight
58 into contemporary adaptation and future adaptive capacity (Broadhurst et al., 2008; Bucharova et
59 al., 2019; Hamilton et al., 2020; Kulbaba et al., 2021). This is particularly important to
60 restoration, which aims to establish populations resilient to change.

61 Trait differences arise through a combination of deterministic and stochastic processes
62 (Kawecki and Ebert, 2004; Crow et al 2018; Galliard et al., 2018). For example, climatic
63 gradients have contributed to ecotypic differentiation among grass species for morphological
64 (Aspinwall et al., 2013; Olsen et al., 2013), phenological (Lowry et al., 2019), physiological
65 (Aspinwall et al., 2013; Maricle et al., 2017), and fitness traits (McMilan, 1959; Galliard et al.,
66 2018). To establish seed transfer recommendations during restoration, teasing apart the
67 contributions of environment to the evolution of trait differences may be useful to predicting
68 populations' response to new environments. In this study, we focus on the evolution of
69 physiological traits, which may evolve in response to varying water availability (Dudley et al.,

70 1996; Picotte et al., 2007; Dittberner et al., 2019). Range wide variation in *Arabidopsis thaliana*
71 for stomatal characteristics suggests that climatic factors have led to the evolution of changes in
72 stomatal size and density (Dittberner et al., 2019). With smaller, but more numerous stomata,
73 plants have a greater ability to respond rapidly to changing water availability associated with
74 increased temperatures (Drake et al., 2013; Dittberner et al., 2019). Thus, variation in
75 physiological traits may correspond with the evolution of ecotypes associated with environments
76 across a species' range.

77 The history of selection, particularly the degree to which environmental heterogeneity has
78 been predictable or unpredictable across a species' range, may impact the distribution of genetic
79 variation underlying traits and consequently their capacity to adapt. Here, we define
80 environmental predictability as repeatable seasonal cues associated with a given climate variable
81 (Reed et al., 2010). Theory suggests that where populations have experienced predictable
82 environmental cues, heritable genetic variance for phenotypic traits will increase as the total
83 phenotypic variance is reduced (Fig. 1; Levins, 1963; Reed et al., 2010; Baythavong, 2011;
84 Kulbaba et al., 2021). In such a scenario, heritable trait differences among ecotypes may lead to
85 increased risk of maladaptation when seed is transferred to new environments (Reed et al., 2010;
86 March-Salas et al., 2019). In contrast, populations sourced from unpredictable environments are
87 expected to exhibit greater plasticity and reduced trait heritability (Fig 1; Chevin et al., 2010;
88 Reed et al., 2010; Ghalambor et al., 2007; Baythavong, 2011; March-Salas et al., 2019). If
89 adaptive, plasticity enables plants to modify their phenotype in response to the changed
90 environment to maintain fitness (Reed et al., 2010; Baythavong, 2011; Becklin et al., 2016;
91 March-Salas et al., 2019). If plasticity is non-adaptive it may come with a fitness cost (Gilbert et
92 al., 2019). Evolvability, which is the expected change in a trait per generation for a given

93 selection coefficient (Hansen and Houle, 2008; Hansen et al., 2011), quantifies how rapidly
94 adaptation is predicted in a continuously shifting environment (Shaw and Etterson, 2001;
95 Kulbaba et al., 2021). Therefore, quantifying trait heritability and evolvability for seeds sourced
96 from predictable and unpredictable environments provides complimentary metrics to predict
97 populations' capacity to respond to changing selective pressures. These metrics can be used to
98 guide seed transfer recommendations and aid in determining both the initial risk of transfer
99 across environments and the likelihood populations will adapt once established.

100 *Geum triflorum* Pursch., is an early season perennial forb associated with remnant prairie
101 habitat across much of the Great Plains of North America (Hamilton and Eckert 2007, Yoko et
102 al., 2020). The Great Plains are critically imperiled due to habitat loss associated with land
103 conversion, fragmentation, and urban expansion and thus are important habitats for restoration
104 efforts (Hoekstra et al., 2005; Gascoigne et al., 2011; Comer et al., 2018; Wimberly et al., 2018;
105 Bengtsson et al., 2019). Populations of *G. triflorum* also persist as isolated 'islands' across alvar
106 habitats scattered throughout the Great Lakes and into Manitoba, Canada (Hamilton and Eckert
107 2007; Yoko et al., 2020). Alvars are habitats characterized by a thin layer of soil over limestone
108 bedrock that harbor a unique assemblage of plants largely disjunct from the core of their
109 distribution (Hamilton and Eckert 2007). Alvars experience extreme, but predictable annual
110 fluctuation in water availability from flooding in the spring to early summer desiccation (Catling
111 and Brownell 1995; Hamilton et al., 2002; Yoko et al., 2020). In contrast, while prairies
112 experience flooding and drought, compared to the predictable interannual extremes of the alvar
113 ecoregion, the onset of these events is less predictable. In addition, the deep, organically rich soil
114 characterizing prairie ecoregions provides a buffer to extreme water fluctuations (Anderson,
115 2006). Thus, we suggest the alvar ecoregion reflects a 'predictable' history of selection, whereas

116 the prairie ecoregion reflects an 'unpredictable' history of selection in response to changing
117 water availability. These ecoregions provide an ideal system to evaluate the role predictability of
118 the environment may play in influencing the amount and distribution of genetic variance for
119 phenotypic traits. Physiological traits, including stomatal size and density along with water-use
120 efficiency (WUE) are expected to vary between prairie and alvar ecoregions. Given the
121 importance of stomatal traits and WUE to plant persistence, examining how environment of
122 origin has influenced variation in these traits will inform seed transfer recommendations.

123 Using a common garden experiment of maternal seed families for *G. triflorum* sourced
124 from both prairie and alvar ecoregions, we evaluated the role source environment has had on the
125 distribution of physiological trait variation linked to plant water use. We quantified ecoregional
126 differentiation for each trait and tested for correlations between functional traits and climate of
127 origin for all sampled populations. Lastly, we quantified standing genetic variance for stomatal
128 traits, including estimates of heritability and evolvability. Specifically, we ask 1) do
129 physiological traits exhibit ecoregional differences, 2) is there a relationship between
130 physiological trait variation and source climate, and 3) does the history of selection associated
131 with seed source environment structure the distribution of additive genetic variance and the
132 heritability or evolvability of physiological traits? We predict alvar ecoregions will exhibit
133 smaller, but more numerous stomata in addition to greater WUE relative to prairie populations.
134 We also expect populations from the alvar ecoregions will have greater heritability for
135 quantitative traits relative to prairie populations due to the history of selection associated with
136 predictable changes in water availability. An understanding of how differentiation in
137 physiological traits evolve and the role selection may play in shaping the distribution of heritable

138 trait variation will be valuable for predicting the response of seeds to restored environments and
139 estimating their longer-term evolutionary potential.

140

141 **MATERIALS AND METHODS**

142 **Field sampling** – In the spring of 2015, seed from 19 populations of *Geum triflorum* Pursch.
143 (Rosaceae) spanning much of its distribution were collected, including 11 populations from the
144 Great Lake alvar ecoregion (GLA), two from the Manitoba alvar ecoregion (MBA), and six from
145 the midwestern prairie ecoregion (PRA, Fig. 2). Forty individual seed heads, each representing a
146 maternal family, were harvested approximately every two meters along a 100 m transect within
147 each population (as in Hamilton and Eckert 2007). In addition to field collections, three seed
148 populations with known provenance were provided by commercial growers from within the
149 prairie ecoregion and incorporated. Two populations were provided by commercial growers (SD-
150 PMG, MN-PMG), and one from the United States Department of Agriculture collected near
151 Pullman, WA (WA-BLK). In total, twenty-two populations were sampled across much of the
152 species' distribution for inclusion in the common garden experiment (Fig. 2).

153 **Common garden experiment** – On November 7th, 2015, seeds were planted in a greenhouse at
154 North Dakota State University in Fargo, ND. Using a half-sib design, 12 seeds from each of ten
155 maternal seed families from each population were planted across 12 randomized complete
156 blocks. In addition, two individual seeds from each commercial collection were planted in each
157 block, for a total of 24 seeds per commercial seed population. In May 2016, surviving seedlings
158 (58% of the total, Yoko et al., 2020) were transferred to a permanent field common garden
159 location at Minnesota State University at Moorhead's Ecoregional Science Center (46.86913N, -

160 96.4522W) in Moorhead, MN. The randomized-complete block design was maintained, and a
161 full description of the establishment and maintenance of the common garden experiment can be
162 found in Yoko (2020).

163 *Measurement of physiological traits*

164 *Stomatal Traits* – In July 2016, using mature plants from the field common garden experiment,
165 both abaxial (lower) and adaxial (upper) leaf surfaces were assessed for stomatal trait variation.
166 Using a thin layer of Newskin "liquid bandage," two randomly selected leaves per individual
167 were sampled to quantify lower and upper leaf surface stomatal trait variation (N=650, 417 GLA,
168 91 MBA, 142 PRA). 'Liquid bandage' leaf impressions were mounted onto slides and
169 photographed using a Zeiss Stereo Discovery (V8) digital microscope (Carl Zeiss Microscopy,
170 LLC, Thornwood, NY, USA) with a Canon Rebel T3 E0S 1100D digital camera (Canon Virginia
171 Inc., Newport News, VA, USA). Photographs were standardized to a 0.32 x 0.42 mm grid and
172 were subsequently analyzed for stomatal trait variation using ImageJ software (v1.52a, National
173 Institutes of Health, USA). Stomatal traits evaluated included guard cell length (GCL, μm),
174 which is a proxy for stomatal size, stomatal density (SD, mm^2), which represents the number of
175 stomata per unit leaf area, and area of the leaf occupied by stomata, stomatal area index (SAI,
176 mm^2). Measurements are reported independently for both abaxial (ab) and adaxial (ad) leaf
177 surfaces. Individual stomatal size measurements represent an average of three guard cells per
178 individual. Stomatal density was calculated by dividing the total number of stomata per slide by
179 the area of the grid. The stomatal area index is reported as the product of average guard cell
180 length and stomatal density (Bertel et al., 2017).

181 *Water-use efficiency* -- In May 2018, leaf samples were harvested to estimate intrinsic water-use
182 efficiency (WUE). Foliar carbon isotopes were analyzed because they provide an ability to assess
183 water-use efficiency over the lifetime of a leaf (Farquhar et al., 1989). We sampled leaves from
184 approximately five individuals per population (53 GLA, 9 MBA and 31 PRA). Leaf tissue was
185 oven-dried at 55 °C over 24 hours and then homogenized into a fine powder using a TissueLyser
186 II (Qiagen, Hilden, Germany). Between 4-5 mg of homogenized leaf tissue were weighed and
187 placed into a tin capsule (Costech, Valencia, CA, USA) for ¹³C isotope analysis using a
188 continuous-flow isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at UC Davis Stable
189 Isotope Facility (Davis, CA, USA). The reported $\delta^{13}\text{C}$ values are expressed relative to the Vienna
190 Pee Dee Belemnite.

191 *Statistical analysis*

192 *Assessing genetic differences across ecoregions* – To test for differentiation in stomatal traits and
193 WUE associated with ecoregion of origin, we used an analysis of variance (ANOVA). All traits
194 were first assessed for normality and homogeneity of variance. Following the ANOVA, a
195 posthoc Tukey honest significance test was performed to identify significant pairwise differences
196 between ecoregions. All statistical tests were performed in R (R Core Team, 2018).

197 *The role of climate to population trait variation* – To evaluate the relationship between climate
198 of origin and its contribution to physiological trait variation, we extracted annual climatic
199 variables, representing the most recent 30-year averages spanning 1980-2010, from ClimateNA
200 v.5.50 using source population latitude, longitude, and elevation (Wang et al., 2016). Climate
201 variables were highly correlated; therefore, we performed a principal component analysis (PCA)
202 to reduce multicollinearity across traits using R (R Core Team, 2018). PC1 explained 47% of the

203 climatic variation across populations, and PC2 explained 30%. Given the first two PC axes
204 explained over 77% of the variation across traits, subsequent analyses reflect only PC1 and PC2.
205 (Supporting Table 1).

206 To test whether differences in stomatal traits and WUE could be explained by climatic
207 variation summarized as PC1 and PC2, we fit linear regressions using the `lm` function in R (R
208 Core Team, 2018). We tested three models using PC1 and PC2 as predictor variables. Two
209 models included each PC as a single predictor, and the third model included both factors as
210 additive predictors. The best-fitting model was identified as that with the lowest Akaike
211 Information Criterion (AIC, Supporting Table 1).

212 *Ecoregion-specific genetic variance for stomatal traits* –Additive genetic variance (V_A), broad-
213 sense heritability (H^2), narrow-sense heritability (h^2), and evolvability (CV_A) were estimated for
214 all stomatal traits. As only a subset of individuals were assessed for WUE, it was not possible to
215 estimate genetic variance components for this trait. Phenotypic variance attributable to
216 ecoregion, population, family, and block were evaluated using a linear mixed-effect model and
217 the `lmer_alt` function within the `afex` package in R (Singmann et al., 2021):

$$y_{ijkl} = \mu + b_i + p_j + f_{i,k} + b_i \times f_{i,k} + e_{ijk}$$

218 where b_i is the phenotypic variance attributable to the random effect of the i th block, p_j is the
219 random effect of the j th population, $f_{i,k}$ is the random effect of the k th family within the i th
220 population, $b_i \times f_{i,k}$ is the interaction effect of block x family, and e_{ijk} is the random error. We
221 calculated the additive genetic variance (V_A) as:

$$222 \quad V_A = 2.5 \times f_{i,k}$$

223 Where $f_{i,k}$ is the random effect of the k th family within the i th population. Traditionally,
224 narrow-sense heritabilities use a coefficient of relationship ($p=1/4$) to account for a half-sib
225 design like that used in our experiment. However, we modified our model based on Ahrens et al.,
226 (2020) to account for the potential mixed mating system of *G. triflorum* adopting a coefficient of
227 relationship of $p=1/2.5$ to generate conservative values.

228 Broad-sense heritability (H^2) was calculated as follows using variance components from the
229 mixed model:

$$230 \quad H^2 = \frac{p_j + f_{i,k} + b_i \times f_{i,k}}{b_i + p_j + f_{i,k} + b_i \times f_{i,k} + e_{ijk}}$$

231 The variance components associated with the k th family within the i th population and the block \times
232 family ($b_i \times f_{i,k}$) interaction effect was subsequently used to estimate narrow-sense heritability
233 and the evolvability. Narrow-sense heritability (h^2) for leaf surface traits was estimated using the
234 following equation:

$$h^2 = \frac{2.5 \times \sigma_{f_{i,k}}^2}{\sigma_{f_{i,k}}^2 + \sigma_{f_{i,k} \times block}^2 + \sigma_{error}^2}$$

235 where h^2 is the narrow-sense heritability, $\sigma_{f_{i,k}}^2$ is the family within population component of
236 variance, $\sigma_{f_{i,k} \times block}^2$ is the variance in the interaction effect of block and family ($b_i \times f_{i,k}$), and
237 σ_{error}^2 is the error component (e_{ijk}) representing individual variance. Standard errors for h^2
238 were calculated by dividing the pooled standard deviations of the model by the trait sample size.

239 In addition to broad and narrow-sense heritability estimates, we included estimates of
240 evolvability to standardize comparisons of additive genetic variance as a means for comparison

241 across traits and ecoregions (Hansen and Houle 2008; Hansen et al., 2011; Cava et al., 2019). We
242 calculated evolvability as follows:

$$CV_A = \frac{\sqrt{2.5 \times \sigma_{f_{i,k}}^2}}{\chi}$$

243 where $\sigma_{f_{i,k}}^2$ is the family component of variance, and χ is the mean of the trait under evaluation.

244 RESULTS

245 *Physiological trait differentiation*

246 Using an ANOVA, we tested for differences in stomatal traits and WUE for populations
247 of *G. triflorum* sourced from distinct ecoregions. Significant trait differentiation was observed
248 between ecoregions for all traits (Table 1). Populations from both GLA and MBA ecoregions
249 exhibited reduced guard cell length for abaxial (GLA=27.1 $\mu\text{m} \pm 0.1 \mu\text{m}$; MBA=26.5 $\mu\text{m} \pm 0.2$
250 μm) and adaxial (GLA=26.5 $\mu\text{m} \pm 0.1 \mu\text{m}$; MBA=26.1 $\mu\text{m} \pm 0.2 \mu\text{m}$) leaf surfaces relative to
251 PRA values (abaxial 28.2 $\mu\text{m} \pm 0.2 \mu\text{m}$ and adaxial, 28.1 $\mu\text{m} \pm 0.2 \mu\text{m}$, Fig 3A-B). While
252 populations sourced from both GLA and MBA did not differ significantly in abaxial stomatal
253 density, both had greater overall stomatal density (260.8 $\text{mm}^2 \pm 3.7 \text{mm}^2$; 246.3 $\text{mm}^2 \pm 7.5 \text{mm}^2$
254 respectively) relative to populations sourced from the PRA ecoregion (PRA=206.6 $\text{mm}^2 \pm 5.8$
255 mm^2 , Fig3C). Interestingly, all three ecoregions differed significantly from each other for adaxial
256 stomatal density, with MBA exhibiting the greatest (MBA=193.3 $\text{mm}^2 \pm 5.3 \text{mm}^2$), followed by
257 GLA (GLA=176.6 $\text{mm}^2 \pm 2.6 \text{mm}^2$), and PRA (PRA=141.32 $\text{mm}^2 \pm 4.1 \text{mm}^2$, Fig3D). For
258 stomatal area index (SAI) significant ecoregional differences were observed across both leaf
259 surfaces (Fig3E-F). Populations sourced from the GLA ecoregion had, on average, the largest

260 abaxial stomatal area index (GLA=5.28 mm² ± 0.1 mm²), followed by MBA (MBA=4.87 mm² ±
261 0.1 mm²), with PRA exhibiting the lowest (PRA=4.30 mm² ±0.1 mm²). For adaxial stomatal area
262 index, MBA exhibited the greatest SAI (MBA=5 mm²± 0.1 mm²), followed by GLA (GLA=4.7
263 mm²±0.1 mm²), while PRA consistently exhibited reduced SAI for both leaf surfaces relative to
264 alvar ecoregions (PRA=3.93 mm²±0.1 mm²). Plants sourced from GLA exhibited higher water
265 use efficiency (GLA=-29.4 ± 0.1) relative to MBA (-29.7 ±0.2), although there was no
266 significant difference between the two alvar ecoregions. In contrast, plants sourced from the
267 PRA ecoregion exhibited significantly reduced water use efficiency relative to both GLA and
268 MBA (-30.1 ±0.1) (Fig4).

269 *Physiological trait variation associated with climate of origin*

270 Using a PCA based on 30-year climate averages we assessed the distribution of climate
271 variation for populations sourced from prairie and alvar ecoregions (Table S1). The first two
272 axes of the PCA explained over 77% of the variation, with PC1 explaining 47% and PC2
273 explaining 30%. The climatic variables that exhibited the highest loadings on PC1 were all
274 associated with temperature and photoperiod, including mean annual temperature (MAT),
275 growing degree days above 18°C (DD_18), and variables related to the frost-free period (eFFP
276 and FFP). Climate variables with the highest loadings on PC2 were related to water availability,
277 including climate-moisture deficit (CMD) and annual heat moisture index (AHM) which indicate
278 the amount of water available for plant uptake. A linear regression with combinations of PC1 and
279 PC2 was used to predict stomatal trait and WUE variation for plants grown in the common
280 garden. The model that best predicted physiological trait variation was assessed by comparing
281 AIC scores, with the chosen model exhibiting the lowest AIC value. In nearly all cases PC2 was
282 the best predictor for stomatal traits and WUE (Table S2), indicating water availability likely

283 explains variance in physiological traits for *G. triflorum*. Populations sourced from regions of the
284 species' range that experience increased climate moisture deficit and higher annual heat moisture
285 indices were associated with increased abaxial and adaxial guard cell length ($r^2=0.39$, $P<0.01$;
286 $r^2=0.39$, $P<0.01$ respectively, Fig5 A-B). In contrast, greater climate moisture deficit and higher
287 annual heat moisture associated with the PC2 axis predicted reduced stomatal density ($r^2=0.40$,
288 $P<0.01$; $r^2=0.37$, $P<0.01$ respectively, Fig5 C-D). This indicates that populations from
289 ecoregions with reduced water availability on average produced fewer, but larger, stomata.
290 Similarly, both abaxial and adaxial stomatal area indices decreased with larger values of PC2
291 ($r^2=0.41$, $P<0.01$; $r^2=0.33$, $P<0.01$, respectively, Fig5 E-F). Finally, WUE decreased across the
292 PC2 axis ($r^2=0.37$, $P<0.01$, Fig. 6), indicating the control over water-use decreases under
293 increased CMD and AHM for *G. triflorum* populations.

294 ***Distribution of additive genetic variance for stomatal traits***

295 Additive genetic variance (V_A) provides an estimate of the amount of genetic variation
296 available for selection to act upon (Falconer and Mackay, 1996). Ecoregion-specific estimates
297 for additive genetic variance (V_A) of stomatal traits were quantified using the half-sibling design
298 (Table 2). In the common garden, Great Lake alvar individuals exhibited the greatest V_A for
299 adaxial and abaxial guard cell length (0.60, 0.50), stomatal density (425, 353) and for abaxial
300 stomatal density (0.10). These results suggest that for *G. triflorum*, the majority of standing
301 genetic variation for stomatal traits is associated with the Great Lake alvar ecoregion.

302 ***Heritability of stomatal traits***

303 We estimated both broad-sense and narrow-sense heritabilities for traits across eco-
304 regions to understand how history of selection may influence the distribution of phenotypic trait

305 variance. Broad-sense heritability (H^2) accounts for all genetic components of total phenotypic
306 variance and was calculated for each ecoregion. Estimates of stomatal trait H^2 ranged from 0.09-
307 0.9 (Table 2). H^2 was comparable for GLA and MBA individuals for abaxial and adaxial GCL
308 (GLA= 0.9±0.28, MBA= 0.8±0.2; GLA= 0.9±0.32, MBA= 0.8±0.21, respectively), and PRA
309 exhibited the lowest H^2 for abaxial and adaxial GCL (0.09±0.05; 0.12±0.1, respectively). Across
310 all three ecoregions, large variances were observed around H^2 estimates for abaxial and adaxial
311 stomatal density traits limiting our ability for comparison of stomatal density H^2 across
312 ecoregions. Finally, abaxial and adaxial stomatal area index showed similar trends to H^2
313 estimates for GCL, where individuals sourced from the alvar ecoregions had comparable H^2
314 estimates (GLA= 0.86±0.18, MBA= 0.9±0.12; GLA= 0.85±0.16, MBA= 0.84±0.15,
315 respectively) and were greater than individuals sourced from the PRA ecoregion (0.34±0.09,
316 0.33±0.12). These results suggest physiological trait differences associated with ecoregions are
317 likely attributable to genetic effects.

318 While broad sense heritabilities includes total genetic variance and provide an
319 understanding of total genetic effects contributing to a trait phenotype, narrow-sense
320 heritabilities (h^2) account for the proportion of genetic variance attributed to additive effects,
321 providing an understanding of potential response to selection. There was substantial variability in
322 narrow sense heritabilities estimated for physiological traits across ecoregions (Table 2). On
323 average, individuals from the GLA ecoregion exhibited greater h^2 for all traits, excluding
324 stomatal area index, which was greatest for individuals sourced from the PRA ecoregion (Table
325 2). Narrow-sense heritability was similar between GLA (0.43±0.10) and MBA (0.39±0.20) for
326 abaxial guard cell length and h^2 for individuals sourced from the GLA ecoregion were relatively
327 consistent across leaf surfaces (adaxial GLA=0.39±0.20). However, for individuals sourced

328 from the MBA ecoregion there was no heritability observed for adaxial guard cell length, likely
329 reflecting a lack of maternal families for this ecoregion. For abaxial guard cell length, GLA
330 exhibited the highest h^2 (0.43 ± 0.10), followed by MBA (0.39 ± 0.20) and PRA, which exhibited
331 the lowest degree of h^2 (0.22 ± 0.15). Additionally, GLA exhibited the greatest h^2 for adaxial
332 guard cell length traits (0.39 ± 0.10), followed by PRA (0.21 ± 0.15). Across all three ecoregions,
333 large variances were observed for heritabilities for abaxial and adaxial stomatal density,
334 hindering our ability to make ecoregion comparisons for stomatal density. For abaxial stomatal
335 area index, PRA exhibited the greatest h^2 (0.17 ± 0.06), followed by GLA (0.1 ± 0.04), however, no
336 h^2 was observed for MBA individuals as seen with adaxial guard cell length. Lastly, MBA had
337 the greatest h^2 for adaxial stomatal area index (0.52 ± 0.15), followed by PRA (0.44 ± 0.10), and
338 GLA exhibited the lowest (0.41 ± 0.06). These values suggest a proportion of the ecotypic
339 differences observed between regions are likely attributable to additive genetic effects with some
340 variance across ecoregions.

341 *Evolvability of stomatal traits*

342 To determine whether the adaptive capacity of stomatal traits differs across ecoregions
343 we calculated evolvability. While alvar and prairie ecoregions exhibited eco-region differences
344 in V_A , evolvability did not vary by ecoregion (Table 2). This suggests, that while the amount of
345 additive genetic variance is greater for alvar ecoregions, the per-generation change expected due
346 to any given selection coefficient is similar across ecoregions. Although evolvability did not vary
347 by ecoregion it did vary across traits (Table 2). Abaxial and adaxial guard cell length had the
348 lowest evolvabilities (0-0.04), and adaxial stomatal area index exhibited the greatest (0.15-0.18),
349 suggesting that the per generation change will be greater for stomatal area index traits than for

350 guard cell length. Overall, evolvabilities for all traits were low ranging from 0- 0.18 indicating
351 that the expected per generation change in these traits is likely limited (Table 2).

352 **DISCUSSION**

353 Selection associated with environment can influence the distribution of genetic variance
354 underlying traits across a species' range. Here, by examining populations of *G. triflorum* sourced
355 from distinct ecoregions with contrasting predictability in water availability, we observed
356 substantial differentiation in physiological traits that could impact recommendations for seed
357 transfer across environments. Climate factors associated with varying water availability strongly
358 predicted physiological trait variation across ecoregions, indicating that environment has likely
359 contributed to the evolution of trait differences. Populations sourced from the alvar ecoregion
360 exhibited increased stomatal density but reduced stomatal size and greater water use efficiency
361 relative to prairie populations when grown in a common environment. This suggests that plants
362 sourced from alvar ecoregions may have evolved increased control over water use. In addition,
363 additive genetic variance for physiological traits was greater for populations sourced from the
364 environmentally predictable alvar ecoregions relative to those sourced from the prairie
365 ecoregion. Heritability estimates suggest the alvar populations exhibit increased genetic control
366 over the phenotypic expression of physiological traits. However, estimates of evolvability
367 suggest that exposure to varying selection coefficients may lead to limited change in traits over
368 generations across ecoregions. Thus, our results suggest that while the environment contributes
369 to the evolution of genetic differences across ecoregions and the distribution of genetic variance
370 in traits important to adaptation, the adaptive capacity overall of *G. triflorum* may be limited
371 range wide. Combined, the evolution of genetic differences may lead to environment-trait

372 mismatches following movement of seed across ecoregions and populations may have limited
373 capacity to buffer the fitness consequences of mismatches via plasticity.

374 *Physiological trait differentiation associated with seed source environment*

375 Physiological traits often exhibit differentiation associated with environment of origin
376 (Dudley, 1996; Didiano et al., 2016; Dittberner et al., 2019; Galliard et al., 2018; Ramirez-
377 Valiente et al., 2018). Here we observed that when grown in a common environment,
378 populations sourced from the alvar ecoregion exhibited, on average, smaller and more numerous
379 stomata relative to populations sourced from prairie ecoregions. In addition, alvar populations
380 exhibited greater intrinsic WUE relative to prairie populations suggesting that physiological
381 traits are differentiated across ecoregions. Alvar environments exhibit annual cycles of extreme
382 variation in water availability; from flooding in the spring to early summer desiccation (Catling
383 and Brownell, 1995, Yoko et al., 2020;). Thus, variation across ecoregions may reflect the
384 evolution of physiological traits required to maintain fitness under seasonal extremes in water
385 availability. Many, but small stomata may enable plants to respond rapidly to varying extremes
386 within the alvar ecoregion (Drake et al., 2013). Previous studies have shown the evolution of
387 traits in response to water stress (Anderson et al., 2011; Wadgymar et al., 2016) or have directly
388 linked physiological trait variation and water-availability to source environment (Dudley, 1996;
389 Didiano et al., 2016; Dittberner et al., 2019; Galliard et al., 2018; Ramirez-Valiente et al., 2018).
390 Our results demonstrate that the environment of seed source may contribute to the evolution of
391 phenotypic differences in physiological traits, which could impact fitness if seed is transferred to
392 a new environment during restoration.

393

394 Stochastic evolutionary processes may also contribute to trait differentiation observed for
395 populations of *G. triflorum*. Previous studies suggest that alvar populations were likely founded
396 from an expansion of the prairie ecoregion during the warming Hypsithermal but have
397 subsequently become isolated following the consequent cooling period (Hamilton and Eckert,
398 2007). Thus, genetic differences may have accumulated across alvar populations due to
399 stochastic processes associated with isolation and reduced connectivity relative to prairie
400 populations (Lande 1992, Young et al., 1996). If lack of gene flow or drift following isolation
401 were the primary mechanisms contributing to differentiation, we would expect geographically
402 proximal MBA populations to be more similar to PRA populations, where there is a common
403 history and high probability of gene flow between ecoregions that would limit the evolution of
404 trait differences. However, our results suggest that Great Lake and Manitoba alvar populations
405 are more similar to each other, suggesting that selection associated with environment has likely
406 driven the evolution of physiological trait differences among ecoregions.

407 *Climate of origin predicts physiological trait variation*

408 Using climate associated with population origin we performed a PCA to identify those
409 climate variables that structure population variation across the range of *G. triflorum*. We found
410 ecoregions were differentiated primarily by temperature (PC1) and water availability (PC2).
411 While PC1 explained the most variation between ecoregions, it did not predict physiological trait
412 variation. However, we did observe a relationship between PC2 and physiological trait variation.
413 Using the PC2 axis, we observed greater annual climate moisture deficit (CMD) and annual heat
414 moisture index (AHM) were associated with fewer, but larger stomata and lower WUE
415 characteristic of the prairie ecoregion. Previous studies found similar patterns where drier
416 conditions led to reduced stomatal control impacting plant water use (Didiano et al., 2016; Guo

417 et al., 2017). Yoko (2020) suggested that stomatal traits and WUE in *G. triflorum* were likely
418 under strong divergent selection due to ecoregional differences in water availability.
419 Interestingly, other traits examined by Yoko (2020) may be related to climatic variables along
420 the PC1 axis. For example, Yoko (2020) found that prairie populations invest more energy
421 towards resource allocation than alvar populations, which may be related to variables observed
422 along the PC1 axis.

423

424 ***Distribution of genetic variance across ecoregions***

425 The amount of additive genetic variance in fitness-related traits is proportional to the
426 amount of genetic variance available for selection to act upon (Kulbaba et al., 2019). Here, we
427 found that individuals sourced from the alvar ecoregions, which exhibit predictable seasonal
428 extremes in water availability, exhibited the greatest amount of additive genetic variance for
429 stomatal traits (Table 2). Temporally varying, but predictable environments like those featured in
430 the alvar ecoregion likely favor the maintenance of additive genetic variance (Levins, 1963;
431 Baythavong, 2011; Kulbaba et al., 2021). As such, estimates of V_A for prairie populations, which
432 experience unpredictable changes in water availability, may reflect selection for plasticity
433 (Baythavong, 2011; Kulbaba et al., 2021). Increased estimates of V_A from the alvar ecoregion for
434 stomatal traits also suggest that these populations may harbor greater capacity to respond to
435 selection (Kulbaba et al., 2019). However, it is important to note the variance in V_A estimates
436 across ecoregions may reflect variance in the number of families included for each regional
437 estimate of trait variance in our common garden experiment (GLA=60; MBA=12; PRA=18).
438 Fewer maternal families evaluated in the MBA and PRA region may lead to conservative
439 estimates of V_A .

440

441 *Heritability of stomatal traits*

442 Quantifying heritability provides insight into the degree to which trait variation is largely
443 mediated by genetic or environmental effects. We predicted that heritable trait variation would
444 be greater within alvar environments as they experience environmental heterogeneity that is
445 predictable. Interestingly, broad-sense heritabilities for stomatal traits ranged from 0.09-0.9 and
446 narrow-sense heritability estimates ranged from 0.1-0.5 and were similar across ecoregions
447 (Table 2). This suggests that while the genetic effects for some traits is substantial, there is also a
448 substantial proportion of variance attributable to environmental variance. Indeed, unpredictable,
449 heterogeneous environments may select for the maintenance of plasticity to ensure plant
450 resilience to change (Chevin et al., 2010; Reed et al., 2010; Ghalambor et al., 2007; Baythavong,
451 2011; March-Salas et al., 2019). As maternal effects are strongest in first generation seedlings
452 and the first year of growth (Donohue, 2009), it is possible that heritability estimates in our study
453 would decrease in a second generation. However, the perennial life-history and time to produce
454 seed did not facilitate the inclusion of a second generation. To limit the potential effect of the
455 maternal environment we evaluated trait variation following at least six months of establishment
456 in the field as previous studies have indicated the impact of the maternal environment may
457 diminish over time (Donohue, 2009). However, given these caveats, our estimates of both broad
458 and narrow-sense heritability likely represent upper bounds (Falconer and Mackay, 1996).

459

460 Understanding the heritability of traits remains important in the context of restoration,
461 where the degree to which traits are mediated by genetic or environmental effects can be used to
462 inform seed transfer guidelines (Broadhurst et al., 2008; Espeland et al., 2017; Bucharova et al.,

463 2017). Here, we observed that individuals sourced from the alvar ecoregion exhibited greater
464 broad-sense heritability relative to individuals from the prairie ecoregion. This may suggest that
465 ecotypic differences that may have arisen in one environment may impact expression of
466 phenotypes in novel restored environments. Where there is a difference between seed source and
467 transferred environment an increased probability of environmental mismatch may reduce fitness
468 in the restored environment (Reed et al., 2010; March-Salas et al., 2019). Despite this, narrow-
469 sense heritability estimates indicate that populations may be able to produce a plastic response to
470 the environment, potentially mitigating negative effects associated with seed transfer and climate
471 change (Arntx and Delph, 2001).

472 *Evolvability of stomatal traits*

473 Populations require sufficient genetic variation for selection to act upon for adaptation to
474 occur (Shaw & Etterson, 2001, Jump & Peñuelas, 2005; Cotto et al., 2017). We estimated
475 evolvability for stomatal traits based on the standardization of additive genetic variance and
476 noted that all estimates were close to zero with little to no differences across ecoregions (Table
477 2). This suggests that populations used in this experiment may have limited capacity to respond
478 to selection. This is a concern in the context of restoration, which will require seed transferred to
479 a new environment to adapt. In *G. triflorum*, limited evolvability in traits associated with water
480 use could lead to adaptational lags when seed is transferred across environments. Reduced
481 evolvability may leave populations more susceptible to demographic declines (Shaw & Etterson,
482 2001, Jump & Peñuelas, 2005; Cotto et al., 2017). As restorations are multi-species, we advocate
483 for studies that quantify the distribution of genetic variation and evolvability for traits important
484 to adaptation. In this way we may predict long-term evolutionary potential of seed populations
485 used in restoration. Finally, as the work presented here was conducted in a common garden in the

486 prairie region, we urge caution when extrapolating our results into other systems and recognize
487 that a reciprocal transplant experiment allows for the evaluation of additive genetic variance in
488 alvar environments and quantification of plasticity.

489 **CONCLUSIONS**

490 Identifying how the environment influences the evolution of ecotypes is important to
491 development of seed transfer guidelines. For *G. triflorum* populations, we observed ecoregional
492 differentiation for physiological traits and variation in the distribution of genetic variation. This
493 suggests different seed source populations may exhibit varying evolutionary trajectories that
494 could impact seed transfer decisions. Thus, minimizing environmental differences when
495 transferring seed across environments may be necessary where genetic differences exist among
496 seed sources. However, sourcing local seed may not be enough to create restored populations
497 capable of withstanding climate change (Broadhurst et al., 2008; Bucharova et al., 2018;
498 Espeland et al., 2017). By evaluating heritable genetic variation for traits important to adaptation,
499 it may be possible to quantify the effect selecting seed for restoration beyond local sources will
500 have to long-term adaptive potential.

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510

511 **DATA AVAILABILITY STATEMENT**

512 All data and scripts associated with this manuscript are available on GitHub

513 (<https://github.com/KateLVolk/AJB-common-garden-physiology>).

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- 685
- 686

Table 1. Summary of ANOVA for stomatal traits and WUE comparing differences associated with ecoregion of origin for *Geum triflorum* seedlings planted in a common environment. Bold values are significant $P < 0.001$

Trait	df	SS	F	P
GCLab	2.00	1.5E+02	19.15	<0.001
GCLad	2.00	2.7E+02	29.83	<0.001
SDab	2.00	2.7E+05	29.12	<0.001
SDad	2.00	1.6E+05	35.29	<0.001
SAIab	2.00	8.7E+01	27.59	<0.001
SAIad	2.00	6.7E+01	24.75	<0.001
WUE	2.00	9.1E+00	12.12	<0.001

Note: GCLab, abaxial guard cell length; GCLad, adaxial guard cell length; SDab, abaxial stomatal density; SDad, adaxial stomatal density; SAIab, stomatal area index; WUE, water-use efficiency

687

688

Table 2. Ecoregional-specific broad-sense heritabilities (H^2), additive genetic variance (V_A), narrow-sense heritabilities (h^2) and evolvability (CV_A)

Trait	PRA				MBA				GLA	
	H^2	V_A	h^2	CV_A	H^2	V_A	h^2	CV_A	H^2	V_A
GCLab	0.09±0.05	0.42	0.22±0.15	0.04	0.8±0.2	0.51	0.39±0.20	0.04	0.9±0.28	0.60
GCLad	0.12±0.1	0.42	0.21± 0.15	0.04	0.8±0.21	0.00	0.0±0.0	0.00	0.9±0.32	0.50
SDab	0.32±4.35	168	0.13±2.93	0.10	0.9±9.1	239.00	0.13±4.19	0.10	0.86±10.12	425.00
SDad	0.4±4.17	240	0.37±3.49	0.17	0.9±5.8	252.00	0.27±4.35	0.13	0.85±7.1	353.00
SAIab	0.34±0.09	0.07	0.17±0.06	0.10	0.9±0.12	0.00	0	0.00	0.86±0.18	0.10
SAIad	0.33±0.12	0.2	0.44±0.10	0.18	0.84±9.15	0.30	0.52±0.15	0.17	0.85±0.16	0.19

Note: GCLab, abaxial guard cell length; GCLad, adaxial guard cell length; SDab, abaxial stomatal density; SDad, adaxial stomatal density; SAIab, stomatal area index; WUE, water-use efficiency; PRA, prairie; MBA, Monte Alvar.

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691 **Fig 1.** Predictive scenarios for how the distribution of heritable genetic variation could be
692 influenced by environmental predictability. Under greater environmental predictability, the
693 distribution of heritable genetic variation should increase (blue line) while decreasing variation
694 attributed to plasticity (red line).

695 **Fig 2.** Collection sites of *G. triflorum* populations. Green points represent Great Lake Alvar
696 (GLA) populations, blue points represent Manitoba Alvar populations (MBA), and yellow points
697 represent Prairie populations (PRA). The red star shape indicates the common garden location.

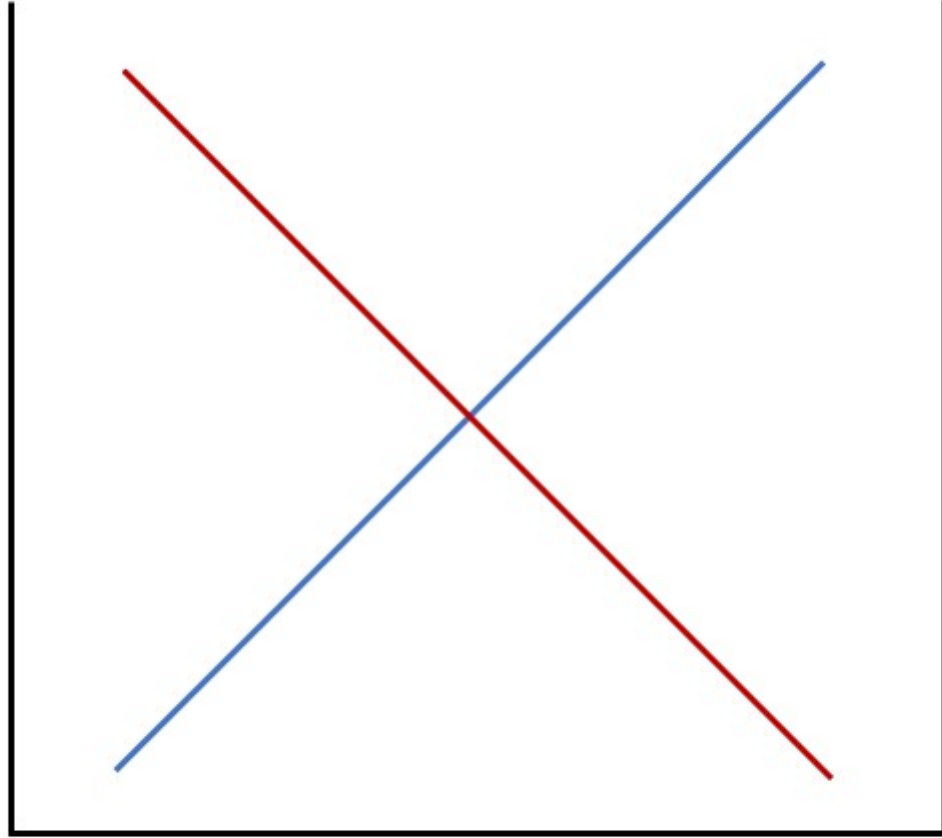
698 **Fig 3.** Box plots indicate regional differences in physiological traits associated with water-use,
699 including abaxial guard cell length (A), adaxial guard cell length (B), abaxial stomatal density
700 (C), adaxial stomatal density (D), abaxial stomatal area index (E), and adaxial stomatal area
701 index (F). The horizontal line in the box plot indicates the median, and white diamonds indicate
702 the mean. Boxplots with the same letter are not significantly different based on Tukey's
703 comparison of means ($\alpha = 0.05$).

704 **Fig 4.** Box plot indicates regional differences in water-use efficiency (WUE). The horizontal line
705 in the box plot indicates the median, and white diamonds indicate the mean. Boxplots with the
706 same letter are not significantly different based on Tukey's comparison of means ($\alpha = 0.05$).

707 **Fig 5.** Relationships between stomatal traits and principal component 2 (PC2), including abaxial
708 guard cell length (A), adaxial guard cell length (B), abaxial stomatal density (C), adaxial
709 stomatal density (D), abaxial stomatal area index (E), and adaxial stomatal area index (F). Data
710 points represent population-level averages for each region. Lines depict the shape of the
711 association between PC2 and trait values surrounded by a 95% confidence shading. The
712 significance of each relationship is indicated in the top right corner of each graph ($p < 0.01$).

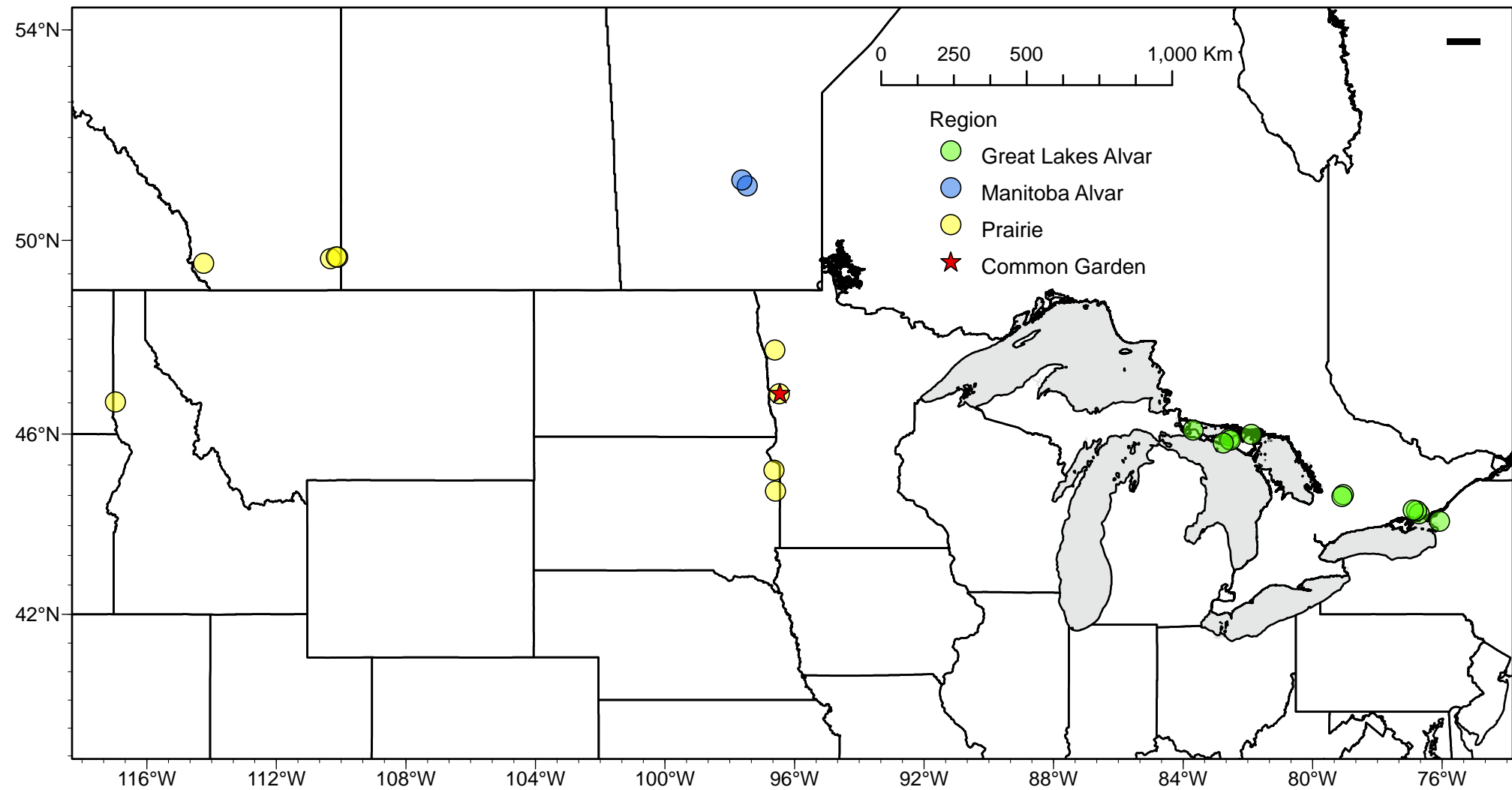
713 **Fig 6.** Relationships between water-use efficiency (WUE) and principal component 2 (PC2).
714 Data points represent population-level averages for each region. Lines depict the shape of the
715 association between PC2 and trait values surrounded by a 95% confidence shading. The
716 significance of the relationship is indicated in the top right corner of each graph ($p < 0.01$).
717

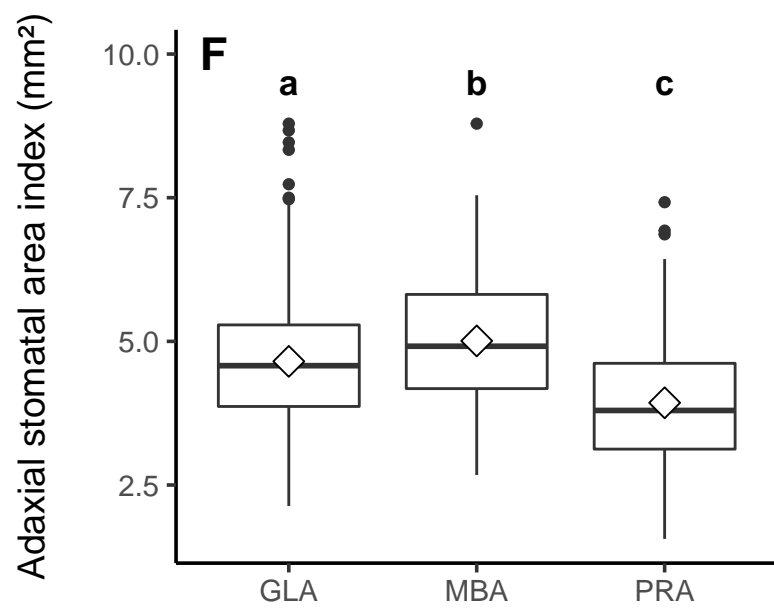
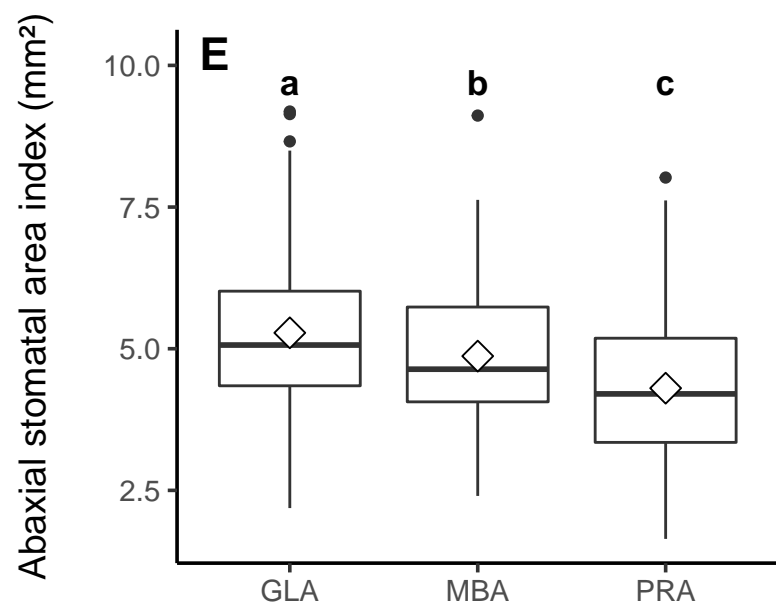
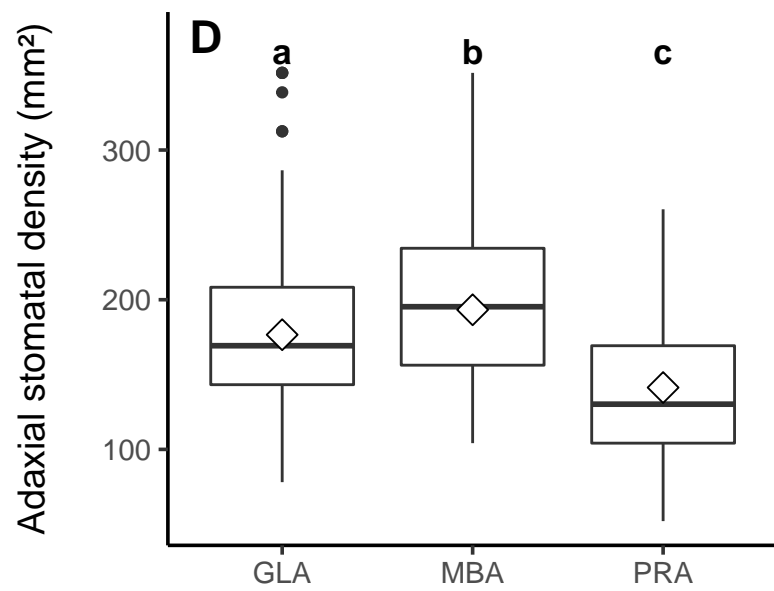
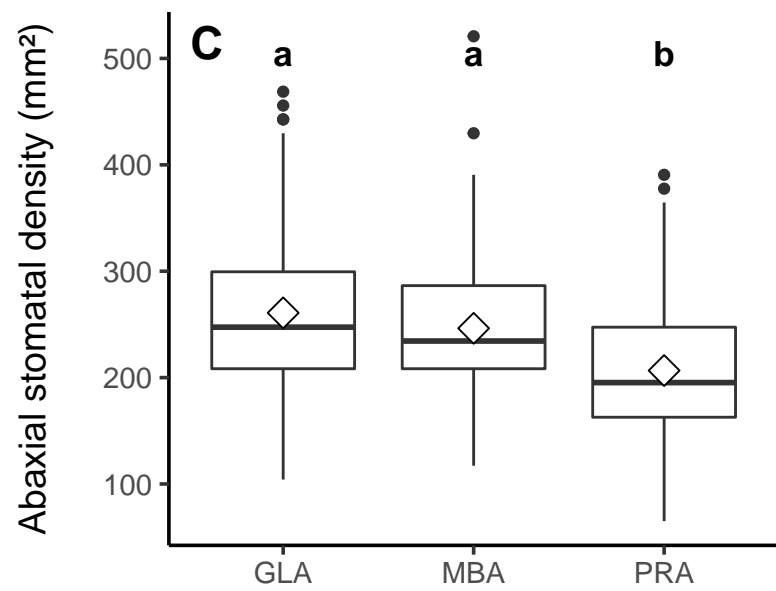
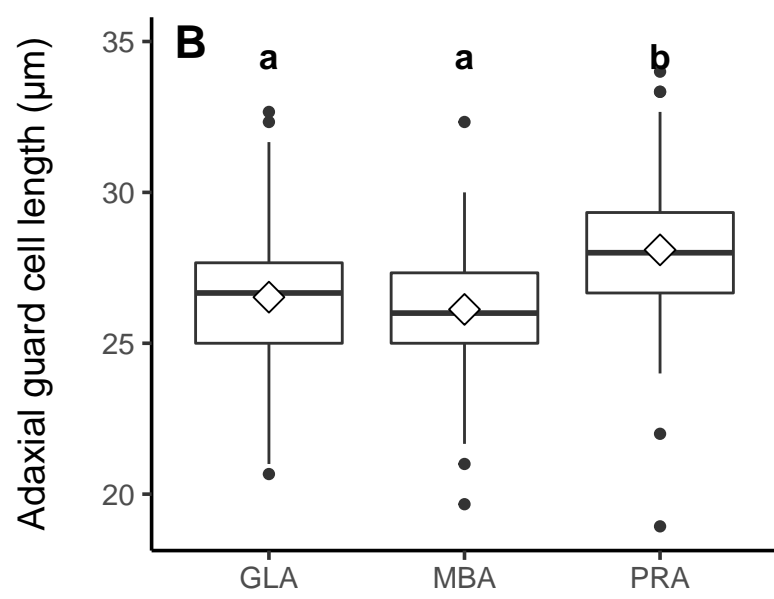
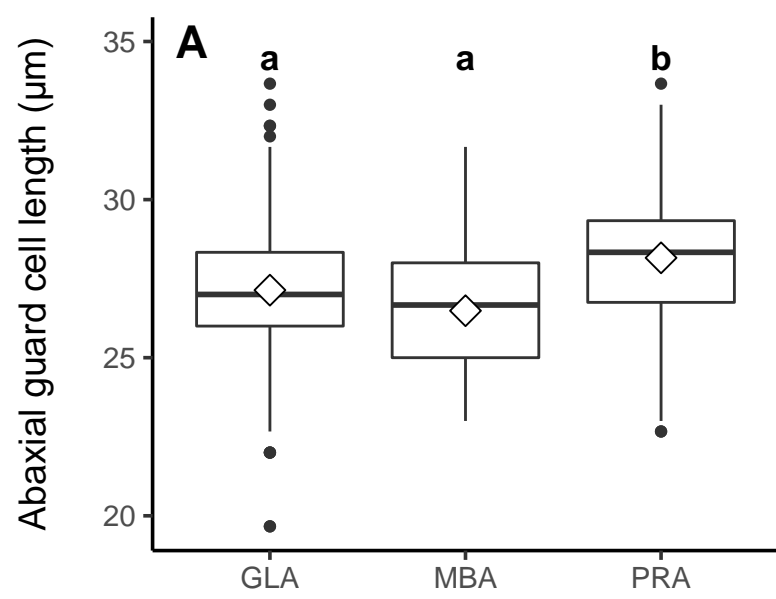
Heritable genetic variation



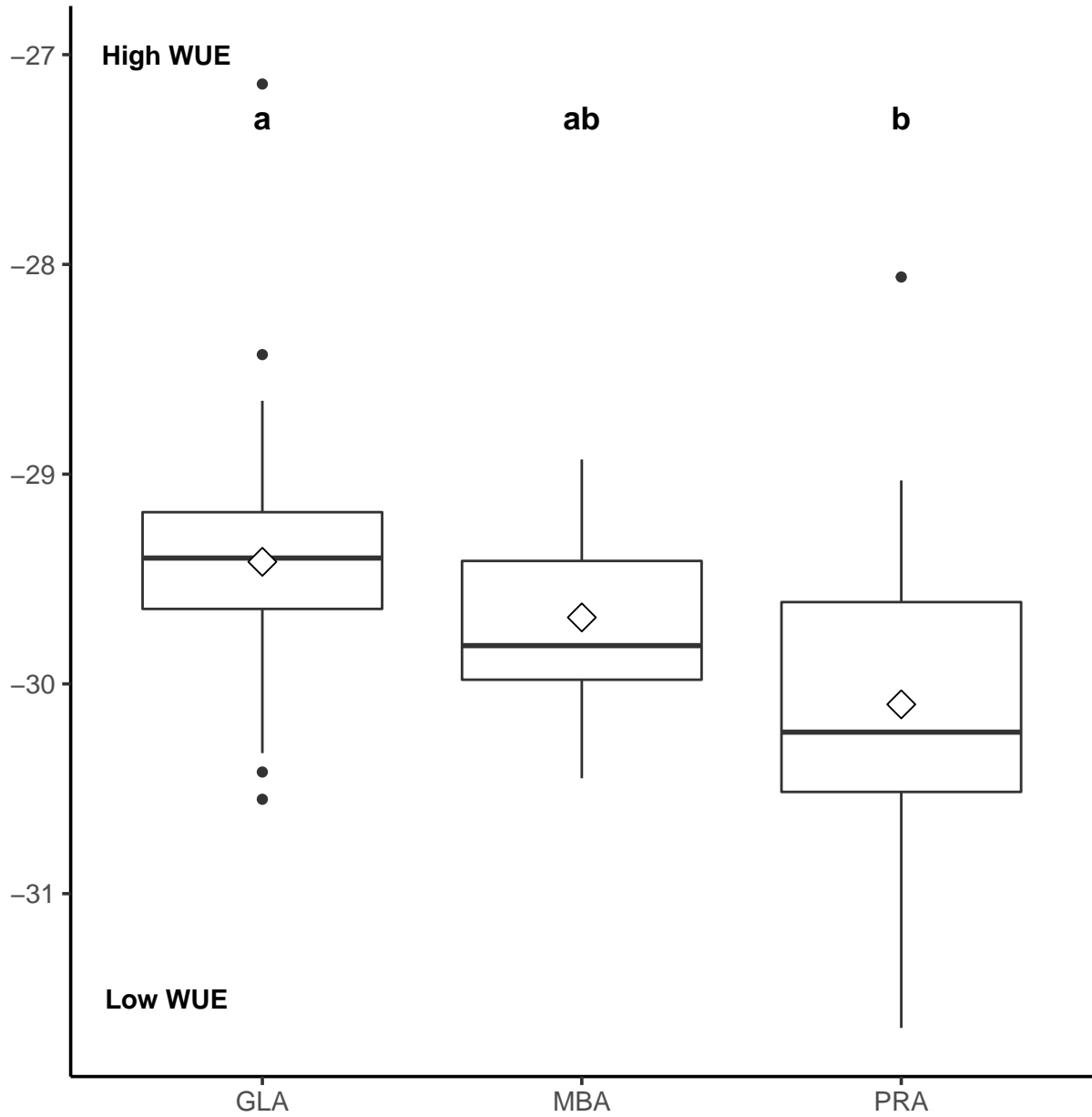
Predictability

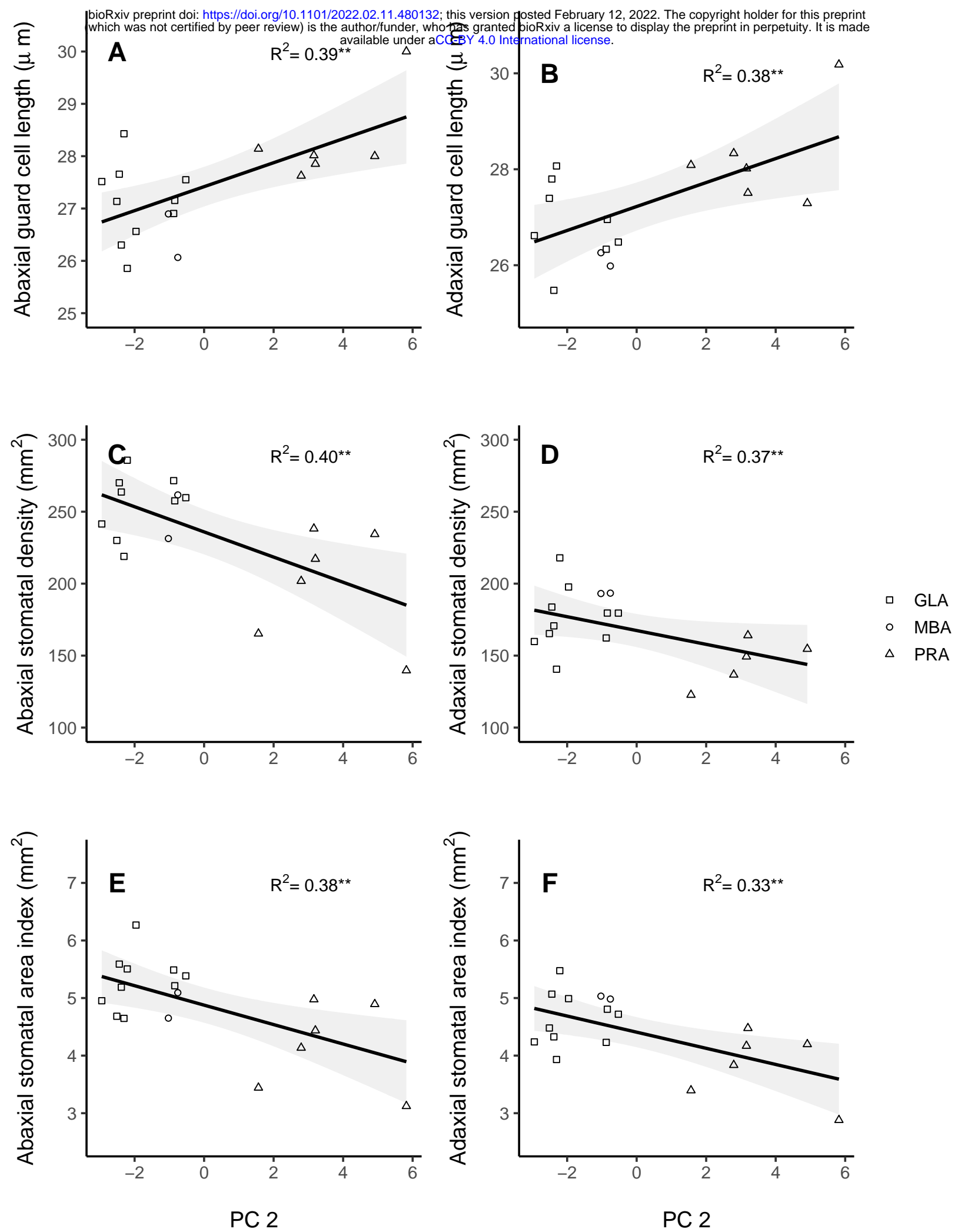
Plasticity





Carbon isotope composition





Carbon isotope composition

