**Running title: Climate explains population divergence in plasticity**

# Climate explains population divergence in drought-induced plasticity of functional traits and gene expression in a South African *Protea*

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## Abstract

Long term environmental variation often drives local adaptation and leads to trait differentiation across populations. Additionally, when traits change in an environment-dependent way through phenotypic plasticity, the genetic variation underlying plasticity will also be under selection. These processes could create a landscape of differentiation across populations in traits and their plasticity. Here, we studied drought responses in seedlings of a shrub species from the Cape Floristic Region, the common sugarbush (*Protea repens*). We measured morphological and physiological traits, and sequenced whole transcriptomes in 8 populations that represent both the climatic and the geographic distribution of this species. We found that there is substantial variation in how populations respond to drought, but we also observed common patterns such as reduced leaf size and leaf thickness, and upregulation of stress- and down-regulation of growth-related gene groups. Both high environmental heterogeneity and milder source site climates were associated with higher plasticity in various traits and co-expression gene networks. Associations between traits, trait plasticity, gene networks and the source site climate suggests that temperature may play a bigger role in shaping these patterns when compared to precipitation, in line with recent changes in the region due to climate change. We also found that traits respond to climatic variation in an environment dependent manner: some associations between traits and climate were apparent only under certain growing conditions. Together, our results uncover common responses of *P. repens* populations to drought, and climatic drivers of population differentiation in functional traits, gene expression and their plasticity.

Keywords: phenotypic plasticity, climatic variation, transcriptomics, *Protea*, drought, climate change

## INTRODUCTION

Populations can persist in changing environmental conditions through three mechanisms: by tracking their climatic niche in space through migration or dispersal, by shifting their niche through adaptation, and/or by matching traits to the new environment through phenotypic plasticity (Chevin, Lande, and Mace 2010). For plants, plasticity can be the fastest response to environmental change, since it involves rapid adjustments of morphology, physiology and gene expression within a single generation. Like mean trait values, the form and magnitude of trait plasticity can be under selection and thus plasticity can also vary among populations along environmental gradients (Geber and Griffen 2003; Wennersten and Forsman 2012; Lázaro-Nogal et al. 2016, 2015; Wadgymar, Mactavish, and Anderson 2018). Previous studies have demonstrated that such population differences in plasticity might or might not have climatic drivers (Pratt and Mooney 2013; Lázaro-Nogal et al. 2015; Pohlman, Nicotra, and Murray 2005; Cochrane et al. 2015).

While plasticity can have strong consequences on functional traits (e.g. (Etterson 2004), it remains less clear how often plasticity confers environment-specific fitness benefits (Forsman 2015; Acasuso-Rivero et al. 2019). Plasticity has been directly shown to be adaptive: for example, Dudley and Schmitt (1996) showed that plastic stem elongation was beneficial at high but not low plant densities. Other studies have characterized patterns of plasticity that suggest adaptive plasticity (Sultan 2003; Nicotra et al. 2015; Baythavong 2011; Lázaro-Nogal et al. 2016; Cavender-Bares and Ramírez-Valiente 2017). Resurrection studies using historical and modern *Daphnia magna* populations showed evolution of plasticity for heat tolerance and fish predation

(Cuenca Cambronero et al. 2018; Stoks et al. 2016). In light of these studies and theoretical predictions (Grime 1994; Bradshaw and Hardwick 1989; Alpert and Simms 2002; Schlichting and Pigliucci 1995; West-Eberhard 2003; Ghalambor et al. 2015), we expect that selection should promote local adaptation in plasticity under two main circumstances. First, plants from environments with strong and relatively predictable forms of spatial or temporal heterogeneity should be more plastic than those from homogeneous environments (Ackerly et al. 2000; Van Kleunen and Fischer 2005; Baythavong and Stanton 2010; Scheiner 2013; Lázaro-Nogal et al. 2015; Bonamour et al. 2019; Chevin and Hoffmann 2017; Reed et al. 2010); but see Palacio-López et al. (2015). Second, plants from harsh climates are expected to be less plastic than those from milder climates (Vitasse et al. 2013; Gugger et al. 2015), because the fitness costs of mismatch between the phenotype and the environment should be greater in resource-poor conditions (Alpert and Simms 2002). Elevation contrasts provide one example of this latter pattern: for an alpine herb, *Wahlenbergia ceracea,* inhabiting high montane and alpine elevations, plants from colder, higher-elevation environments tend to have both lower and less plastic growth rates than plants from warmer sites (Nicotra et al. 2015).

The form and magnitude of functional plasticity which organisms express can be expected to influence the vulnerability of populations to environmental change. This study focuses on a widespread plant species from the Cape Floristic Region (CFR) of South Africa, a biodiversity hotspot (Cowling 2009), that is predicted to be strongly affected by climate change. Our study species, *Protea repens* (common sugar bush), is the most widespread species in this plant genus with 112 species, ~85% of which are endemic (Sauquet et al. 2009; Rebelo 2001). Climate models project warming up

85 to 3 °C by 2070 (Wilson, Latimer, and Silander 2015), and precipitation projections
86 being more variable, most models project drying in the super-diverse western
87 mountains (Wilson, Latimer, and Silander 2015; Hewitson and Crane 2006). Most
88 Mediterranean-climate regions, including California and Southern Australia as well as
89 the CFR, have recently experienced unprecedented droughts, which caused large
90 mortality events in trees (Young et al. 2017; Allen et al. 2010) and shrubs (Valliere et
91 al. 2017). Thus, understanding the patterns and effects of drought plasticity in
92 functional traits is a vital issue for plant vulnerability in these semi-arid regions.

93 Plasticity in key plant traits, including stomatal density and conductance, growth
94 rate, and leaf morphology, has been observed to vary among populations in long-lived
95 woody plants (e.g. (Aubin et al. 2016) as well as herbaceous annuals (Jacobs and
96 Latimer 2012). Likewise, along the environmental gradients *Protea* species inhabit, we
97 have found evidence for local adaptation and environment-dependent benefits in
98 several traits *(Carlson, Adams, and Holsinger 2016; Carlson, Holsinger, and Prunier*
99 *2011; Latimer et al. 2009; Mitchell, Carlson, and Holsinger 2018; Prunier, Holsinger,*
100 *and Carlson 2012)*. Specifically, we found evidence for positive selection on stomatal
101 density in populations from hotter and drier sites, with links to higher stomatal
102 conductance in *P. repens (Carlson, Adams, and Holsinger 2016)*. Additionally, in the
103 monophyletic group of white proteas, *P. subvestita* shows correlations between root
104 length and rainfall seasonality. Biomass also decreases with increasing elevation in *P.*
105 *mundii*, *P. punctata* and *P. lacticolor*, but shows the opposite trend in *P. mundii*
106 (Prunier, Holsinger, and Carlson 2012). On a broader phylogenetic scale, we also
107 found associations between temperature and plant size related traits such as leaf area
108 and plant height (Mitchell, Carlson, and Holsinger 2018). There is also evidence for

109 differential developmental plasticity and drought plasticity in leaf traits of several other
110 *Protea* species (*Protea sect. Exsertae*: (Carlson and Holsinger 2012; Heschel et al.
111 2014).

112 Previously, we also showed that gene expression in populations grown in a
113 common garden show patterns of differentiation corresponding to climatic variables
114 such as elevation (a good proxy for temperature in CFR), minimum winter temperature
115 and mean annual precipitation in *P. repens* (Akman et al. 2016). These gene networks
116 also show correlations with key traits such as height, leaf area, stomatal density/size,
117 providing insights into the genes involved in climate driven differentiation.
118 Measurements of plasticity in gene expression, in which thousands of "phenotypes"
119 can be measured simultaneously, are also valuable for investigating the causes and
120 consequences of plasticity. Gene expression patterns can change within seconds of
121 a stimulus, long before larger biochemical shifts and morphological changes become
122 apparent. Gene expression patterns can also be linked to morphological and
123 physiological adjustments, potentially providing insights into the functional genetic
124 basis of adaptation and/or plasticity (Whitehead 2012; Akman et al. 2016) We note
125 that such links do not pinpoint the genetic locus associated with the phenotypic
126 variation, as loci influencing expression may lie in regulatory regions or in others genes
127 whose expression is upstream of the focal gene. Previous studies have revealed
128 patterns of adaptive plasticity in gene expression in fish (Dayan, Crawford, and
129 Oleksiak 2015; Morris et al. 2014; Schneider et al. 2014), plants (Lasky et al. 2014;
130 Colicchio et al. 2015) and corals (Barshis et al. 2013; Granados-Cifuentes et al. 2013).
131 These studies demonstrate that gene expression profiling can usefully complement

132 standard functional trait measurements to help understand the functional genetic basis
133 and physiological significance of plasticity and how it varies across populations.

134 With this study, we aim to answer two questions: 1) How do populations of *P.*
135 *repens* respond to drought, both in terms of functional traits and gene expression,
136 together with their plasticity, and how do these responses vary across populations?
137 and 2) Do the climatic conditions of the source sites covary with the mean values and
138 plasticity of functional traits and gene expression levels? To do so, we imposed a
139 severe, experimental drought on seedlings in a dry down experiment, and measured
140 functional traits and gene expression patterns. Then, we tested for population
141 differentiation in trait means and drought-response plasticity in functional traits and
142 gene expression patterns, and evaluated associations between patterns of population
143 differentiation and their potential climatic drivers.

## 144 MATERIALS AND METHODS

### 145 Species description

146 *Protea repens* L. is a common evergreen shrub endemic to South Africa, with
147 particularly high abundance in semi-arid fynbos of the CFR (Rebelo 2001). This
148 species has narrow, sclerophyllous leaves and reaches 2-4 m in height. All viable and
149 nonviable fruits remain within a woody, serotinous infructescence until the adult plant
150 is killed by fire, which occurs at ~10-20 year intervals (with variation among geographic
151 regions (Van Wilgen, Forsyth, and De Klerk 2010). Fruits disperse by tumbling, and
152 seeds germinate during the next rainy season (May-Aug for much of the CFR). Due to
153 the Mediterranean rainfall patterns in the western CFR, some western sites receive
154 far more wintertime precipitation than do eastern population sites.

155 **Field sampling and climate data**

156 In 2011, we collected seeds from 8 wild populations of *P. repens* spanning its
157 geographic distribution in the CFR (**Figure 1A** and **Table S1**). The populations
158 encompass a range of precipitation levels and degrees of seasonal concentration --
159 some sites have strong winter rainfall seasonality, while others experience both winter
160 precipitation and some summer monsoonal rain (Table S1). The elevation range was
161 deliberately wide, from ~150m to ~1500m. The reason why the lower elevation,
162 warmer sites are "milder" is that they have little or no frost in winter, while being only
163 moderately dry. High-elevation sites, in contrast, receive snow in winter and have high
164 winds and shorter growing seasons. There are hot, dry, low-elevation sites in the
165 interior of the Cape region that are not mild, but these tend to be too dry to support our
166 study species, and were not included in our sampling. From 40-50 plants per
167 population (mean n= 45 plants), we collected 1-2 mature seed heads from the previous
168 year's growth. Plants were sampled along transects through the population centre,
169 with at least 1 m between each sampled plant for small populations (<100 plants), and
170 5 m for larger populations. Seed heads were allowed to air-dry until achenes were
171 released. GPS coordinates of each site near the population center were used to
172 extract elevation and six climate metrics for rainfall and temperature from GIS layers.
173 A principal component analysis performed on these data confirmed that the sample
174 sites cover most of the range of variation evenly in major environmental factors
175 (**Figure 1B**).

176 The climate data layers were from the South African Atlas of Agrohydrology and
177 Climatology (Roland Edgar Schulze 1997) with higher resolution for 4 and 5 below,
178 and (R. E. Schulze 2007) for the rest, and were based on 50 year (collected between

179 1950-1999 when data was available from field stations) climate averages, extremes,
180 or variances. The climate layers used were (1) mean annual rainfall, (2) average daily
181 maximum temperature for summer (January), (3) average daily minimum temperature
182 for winter (July), (4) precipitation concentration (an seasonality indicator we refer to as
183 "rainfall seasonality"), measured as Markham's concentration index (Markham 1970),
184 which ranges from 0 to 100, with a value of 0 indicating perfectly even precipitation
185 across all months, and 100 indicating that all precipitation falls in a single month (5)
186 inter-annual rainfall variability, measured as the coefficient of variation in mean annual
187 rainfall across years, and (6) extreme temperature range within a year, measured as
188 the difference between summer maximum and winter minimum temperatures. Our
189 seventh variable was elevation, which was extracted separately from ASTER GDEM
190 (Daac 2011). Elevation is correlated strongly with winter minimum temperature, and
191 we interpret it primarily as an indicator of temperature regime, although some other
192 factors such as precipitation and wind speed are more weakly associated with
193 elevation in the region (**Table S2**). Most of these layers have been used in our earlier
194 work on *P. repens*, except for (5) and (6), which were added to complement rainfall
195 seasonality as additional measures of environmental heterogeneity. Soil analyses for
196 these source population locations were not available, but we standardized the general
197 soil type: populations occurred (as is typical for this species) on low-nutrient, coarse,
198 sandy soil derived primarily from quartzite sandstone parent material.

199 Only two pairs of climate variables were strongly correlated with each other:
200 winter minimum temperature with elevation (Pearson's R= -0.91) and summer
201 maximum temperature with annual temperature range (R=0.70; **Table S2**). Rainfall
202 seasonality in our dataset is positively associated with wintertime rainfall (total for Dec-

Feb, Pearson's R=0.80) and negatively associated with summertime rainfall (total for June-August, Pearson's R=-0.90). The 12-year gap between the last collection year of the climate variables and seed collection date might hinder some of the associations we detect in later sections. However, the age of the plants (9-23 years based on veld age through burn history when available) from which seeds were collected will potentially eliminate this gap. We predict that selection should have already acted on the maternal plants and pollen sources, and their adaptations, if any, should be reflected in the germplasm we performed our experiments on.

**Experimental setup and dry-down treatments**

In July 2014, we sowed seeds from 8 *Protea repens* populations on low nutrient mix (50% bark, 50% sand) in trays of 100 individual plugs 2x2 cm in size with a complete random design. Trays were placed in a long-day growth chamber (8 hours in dark at 8℃, 16 hours in light at 20℃). The trays were randomized every 3 days with each watering in the growth chambers. After 1.5 months, we transplanted germinated seedlings into small, deep pots (5 cm diameter x 17 cm deep) in trays of 50 each, in the same low nutrient mix. After transplanting, seedlings were rested for two weeks indoors under growth lights before being transferred to a single greenhouse bench at Nicholls State University Farm, Thibodaux, LA.

Two weeks post-greenhouse transfer and two weeks prior to the start of the dry-down experiment, we selected a total of 316 out of 419 seedlings (19-20 seedlings/population x 8 populations x 2 treatments) for uniform size for the experiment. We then reorganized the 2.5 month-old seedlings into trays containing 20-25 seedlings each, in a complete random design. In the experiment, seedlings were

226 assigned to one of two treatments: control, in which we applied regular watering
227 (~every 3 days, equal amounts of water was given to each plant) and drought, in which
228 we stopped watering entirely for 12 days. Because we had a variable number of
229 seedlings per maternal plant, we could not perfectly balance maternal lines across
230 populations and treatments. To the extent possible, however, we included at least one
231 seedling per maternal family per treatment; for 82% of the 83 maternal families, we
232 were able to include at least one seedling per maternal family in each treatment, and
233 for 51% of families, we included multiple seedlings per treatment (Table S3). The
234 drought experiment began in late October 2014, when greenhouse conditions were
235 warm but relatively dry, and thus most similar to summer conditions in the native range.

236 **Trait measurements and gene expression sampling**

237 At 6 days and 12 days into the drought experiment, plants were measured for
238 functional traits and leaves were sampled for transcriptomics. Our sampling design is
239 depicted in **Figure S1**. Functional traits that were measured at both timepoints
240 included plant height (cm), stomatal conductance, leaf area, leaf length:width ratio
241 (LWR), specific leaf area (fresh leaf area/dry leaf mass; SLA), stomatal density
242 (number per $mm^2$), stomatal pore length (mm) and stem pigmentation. Stem
243 pigmentation, measured as stem hue, was derived using Endler's segment
244 classification (Maia *et al.* 2013), based on the average of two reflectance spectra
245 readings (400-700 nm) taken at mid-stem (JAZ, Ocean Optics). On the segment
246 classification scale for hue, higher values represent redder stems that contain higher
247 levels of anthocyanin. Stomatal conductance was measured using a steady-state leaf
248 porometer (SC-1, Decagon) on one leaf per plant, with an additional adjacent leaf
249 measured simultaneously if the chamber was not filled by a single leaf due to small

250 leaf size.

251 All other leaf measurements were taken from the 2nd or 3rd youngest
252 expanding leaf from the stem apex. Immediately following conductance measurement,
253 the leaf was removed to measure leaf area, width, length and stomatal traits. Fresh
254 leaves were digitally scanned shortly after harvest at 400 dpi on a flatbed scanner.
255 After scanning, acrylic peels were taken from the adaxial leaf surface and placed on
256 cellophane, which were later viewed under a light microscope at 40x for stomatal
257 measurements (see Carlson *et al.* 2016). Although height and pigmentation were
258 measured on all plants on both timepoints (mean sample size per population=39), all
259 other traits were measured on only a subset of plants on each of these days, typically
260 in association with the transcriptomic data (10-20 individuals per population for each
261 day). Two additional variables were calculated by subtracting day 6 from day 12 values
262 on the same plant: pigment accumulation (day 12 hue - day 6 hue) and growth (day
263 12 height - day 6 height). A principle component analysis was performed on the traits
264 measured separately for day 6 and day 12. Details of sampling for gene expression
265 and carbohydrate analysis are included in Supplementary Materials and Methods.
266 Briefly, leaves were sampled at both time points for whole transcriptome sequencing,
267 and at day 12, the whole plants were freeze dried and extracted with 70% methanol,
268 and carbohydrates were measured with anthrone method (as described in (Akman et
269 al. 2012)).

270

271 **Gene expression analyses and bioinformatics**

272 See Supplementary Materials and Methods for transcriptome library preparation and
273 sequencing details. Raw reads were trimmed and demultiplexed with trimmomatic
274 (Bolger, Lohse, and Usadel 2014) and fastx toolkit (Hannon, n.d.). By using bowtie
275 (Langmead and Salzberg 2012), trimmed reads were mapped to a previously
276 published de-novo transcriptome of *P. repens* (Akman et al. 2016a) assembled by
277 using sequences of individuals that also include all of our focal populations. Before
278 reads were mapped to the transcriptome, a further clustering of the contigs were done
279 using cd-hit (Fu et al. 2012). Read counts, extracted using eXpress (Pachter 2011),
280 averaged 6.02 million reads per sample (ranged between 2.46-21.80 million). Contigs
281 with <1 counts per million (cpm) were excluded from the analyses. Differential gene
282 expression analyses were performed using edgeR. For each time point, differentially
283 expressed genes per population were revealed using pairwise comparisons using
284 negative binomial models. The common set of differentially expressed genes was
285 obtained by extracting the overlap of differentially expressed genes for each
286 population at day 12 (**Table S5**). We then used linear models in edgeR, including
287 sequencing lane as a fixed factor, to detect contigs that showed variation between
288 treatments and among populations (population x treatment). Multi-dimensional scaling
289 (MDS) analysis was performed for each time point to visualize general patterns of
290 gene expression for all the samples using the top 500 genes that explain the most
291 variation (**Figure 2A-B and Figure S6**).

292 Co-expression gene networks were computed using WGCNA (Langfelder and
293 Horvath 2008) with consensus clustering option for the 2 timepoints together.
294 Normalized and cpm-filtered read counts were transformed with “voom” in limma
295 package. A soft-threshold of 6 was used for clustering and 14 co-expressed gene

296 networks were constructed. The number of genes within each gene network varied
297 between 49 (GN13) to 7682 (GN1). The gene network “grey” (called GN0 in this study)
298 as constructed by WGCNA for genes that do not fit any gene network has 3124 genes.
299 A representative “eigen gene” value, which is the first principal component axis for all
300 genes within the network, was used for further analyses of climate and trait
301 associations. By using gene orthology of our genes with Arabidopsis (Akman et al.
302 2016a), we also tested for Gene Ontology (GO) enrichment for the 13 networks. This
303 analysis was performed in Gorilla (Eden et al. 2009), using the network genes as target
304 set and the whole gene set as the background. Please note that, when we refer to a
305 statistical test for a given gene network (GN), an association or a linear mixed model
306 test, we are referring to the eigengene value of that gene network. However, when we
307 mention an associated function, this is based on all the genes on that network and the
308 corresponding GO analyses. Contigs within each module, GO enrichments and the
309 genes with the highest module membership for a single network are provided in **Table**
310 **S4**.

311 **Statistical analyses**

312 *How do populations respond to drought and how do these responses vary?*

313 We tested whether drought significantly affected traits in *Protea repens* using linear
314 mixed models. We used a total of 23 plant metrics in our analyses, including 8 traits
315 on day 6, and 15 traits on day 12. We analyzed each trait on each day separately,
316 because preliminary analyses on combining both days revealed significant timepoint
317 effects for almost every response variable. Each model included the fixed effects of
318 treatment, population, and the population by treatment interaction (G x E) and random

319 effects of maternal line nested within population. In these models, the treatment-by-
320 population interaction term indicates whether there was differential plasticity among
321 populations, or in other words, that plants from different populations responded
322 differently to the treatment (G x E effect). These analyses were performed using the
323 lme4 package (Bates, Maechler, and Bolker 2012) in R 3.1 (Team 2014). To obtain p-
324 values for each fixed effect, we successively dropped one predictor from the full model
325 and used a chi-square test to compare AIC scores between models. Given the large
326 number of statistical tests that this entailed, we also adjusted p-values according to
327 estimated false discovery rates (FDR; R package "fdrtool", local method).

328 Gene expression analyses followed the same general approach as for traits.
329 As response variables, these analyses focused on eigengenes in the 14 gene
330 networks (GN) that were produced through the gene clustering analysis. Models for
331 gene networks included fixed effects of treatment, population, and their interaction,
332 random effects of maternal line nested within population, and a random effect of
333 sequencing lane. We also tested for genes that are differentially expressed within a
334 population. Commonly regulated genes for all populations are given in **Table S5**. We
335 also tested for genes differentially expressed among populations for both timepoints
336 and days separately.

337 *Do the climatic conditions of the source sites covary with traits, gene expression and*
338 *their plasticity?*

339 To test whether characteristics of the home climate predicted population
340 variation in traits and gene expression, we used mixed effect models. In these models,
341 traits and gene networks (GN) were response variables, the climate variables were

342 fixed effect terms, and maternal line nested in population was a random effect variable.
343 For testing the significance of the fixed factors, we calculated p-values based on chi-
344 square tests as explained above. FDR corrections were applied to p-values in two
345 groups: traits and gene networks. Day 6 and Day 12 FDR corrections were also
346 applied separately. For variables with significant climate and treatment interaction,
347 each treatment was also analyzed separately to estimate slopes and test their
348 significance within treatments.

349 We used another set of linear mixed models to test whether there were
350 significant associations between traits and gene expression patterns. We performed
351 these analyses with lme4 in R as above, with each pairwise comparison between a
352 trait and a gene network in its own model. Response variables were one of 8 (at day
353 6) or 15 (at day 12) traits and predictor variables were one of 14 eigen gene values for
354 a gene network. Random effects were sequencing lane, population and maternal line
355 nested within population. Correlations were tested separately for the two timepoints,
356 and p-values were calculated as explained previously. An FDR correction was applied
357 separately to the two time points (Table S6).

358 To characterize plasticity for individual traits and to quantify the direction and
359 magnitude of plasticity, we used maternal line plasticity indices. For each maternal
360 family, we calculated plasticity as the family mean trait value in the wet treatment
361 minus the family mean trait value in the drought treatment, divided by the overall family
362 mean trait value (for families with only one plant per treatment, we used the trait value
363 of that plant rather than a family mean). Note that this plasticity metric can take on
364 both positive and negative values: values close to 0 indicate low plasticity (little

365 difference between treatments), while large negative and large positive values both
366 indicate high plasticity.

367 We used mixed effect models to determine whether characteristics of the home
368 climate predicted cross-population variation in plasticity of traits and gene expression.
369 In our analyses, plasticity in traits and gene networks were response variables (these
370 are the above mentioned family means), the 7 climate variables were fixed effect
371 variables and population was included as a random effect. Each climate variable was
372 tested against each response variable in a separate model, with day 6 and day 12
373 measurements analyzed separately (lmer library). In order to do this, we used a subset
374 of gene network and trait pairs that already showed a climate x treatment effect (before
375 FDR-correction;**Table S7&8**). These included 5 and 11 tests for gene networks, and
376 2 and 15 for traits for day 6 and day 12, respectively.

## 377 RESULTS

### 378 How do responses to drought vary among populations?

379 *Drought responses are stronger in gene expression compared to traits*

380 Six days into the drought experiment, only three of the 8 traits differed significantly
381 between treatments: unwatered plants had shorter stems, lower stomatal conductance
382 and more variable SLA (**Table 1**). At the later stage, treatment effects became
383 pronounced for more traits measured (12 of 15): unwatered plants had lower stomatal
384 conductance, decreased height, slower growth in height (from day 6 to day 12), higher
385 SLA, smaller leaf area, denser stomata, smaller stomatal pore size, increasing
386 pigment accumulation, lower carbohydrates aboveground (but not belowground). In

387 the earlier stages of the stress, morphological and physiological traits did not show
388 responses as pronounced as those observed in gene expression patterns, potentially
389 due to time required for morphological changes to happen (**Figure 2**). In contrast to
390 most of the traits, the treatment effect was significant for over half of the gene networks
391 already at day 6 into the treatment. This trend of more pronounced gene expression
392 response was visible both in co-expression gene networks (**Table 3**, **Figure S2**) and
393 Multi-dimensional Scaling (MDS) analyses (**Figure 2A-B**), which indicates a lag
394 between the rapid adjustments in gene expression and trait onset. However, we
395 should also note that the MDS for gene expression is based on the top 500 genes that
396 explain the most variation, and we might not have measured morpho-physiological
397 traits that might also respond as quickly. Notably, population V (Baviaanskloof, a high-
398 elevation eastern site) did not show a treatment effect in traits at the later stage,
399 although the effect was quite pronounced in gene expression (**Figure 2B&D** and **3A**).

400 At day 6 into the treatment, unwatered plants had higher eigengene values for
401 gene networks GN 2, 4, 5, 6, and 7 and lower values for gene networks GN 1, 9 and
402 11, relative to watered plants (**Table 3**, **Figure S3**). These modules show gene
403 enrichment in GO categories for ATPase activity (GN2), oxidoreductase activity (GN4),
404 cytoskeleton organization (GN5), photorespiration (GN6), response to high light
405 intensity and temperature (GN7; note that p-value<0.0005, fdr-corrected p-value is not
406 significant), cell wall organization (GN1) and pyridine-containing compound metabolic
407 process (GN9). As the stress advanced after 12 days into the drought treatment,
408 watered and unwatered plants differed significantly for most traits and gene networks.
409 Significant treatment responses were detected for 10 of 14 gene networks and 12 of
410 15 traits. (**Tables 1-3**, **Figures S3-5**). In addition to the gene networks with treatment

411 effects at day 6, GN3 (tropism and carbohydrate metabolism related, and shows
412 associations with leaf area, height and aboveground soluble carbohydrates) and GN13
413 (homeostasis related) also had evident treatments effects at day 12, with GN3 having
414 lower values and GN13 higher values under drought.

415 *Drought up-regulates stress related genes and down-regulates growth related genes*

416 The number of genes differentially regulated between earlier and later stages of the
417 stress and among populations differed significantly (**Figure 3A&B**). For example,
418 population G (Garcia's Pass) does not have any genes differentially expressed at day
419 6, indicating either a lower level of stress or lack of accurate perception and timely
420 signal transduction of the environmental cues to gene expression phenotypes. In
421 contrast, population B (Bredasdorp) had several hundred differentially expressed
422 genes by day 6. All populations had at least several hundred differentially expressed
423 genes by day 12. Two populations (C-Cederberg and S-Swartberg) showed few
424 differentially expressed genes initially, but over 1500 differentially expressed genes by
425 day 12 (**Figure 3A**). Although the number of genes regulated upon drought stress was
426 highly variable, we found 110 and 63 genes to be consistently up- or down- regulated,
427 respectively, among populations (**Table S5**). The up-regulated genes include heat
428 shock proteins, auxin signaling and ABA catabolism genes, genes related to drought
429 stress and photosynthesis, and a leaf amylase; whereas down-regulated genes
430 included ARF genes (involved in cell elongation and division), and salt stress-, fatty
431 acid and amino acid biosynthesis-related genes.

432 *Population differentiation in stress response becomes more evident as the stress*
433 *advances*

434 Variation among populations is evident in most of the traits, 11 out of 15 traits were
435 significantly different between the populations at day 6, and all traits except for root
436 length showed a population effect at day 12 (**Table 1&2**). We also observed G x E
437 effects (variation in population responses to drought) to a limited extent on day 6, and
438 to a greater extent on day 12 (**Table 1**). At day 6, only SLA and gene network 13
439 (enriched for homeostasis GO term) showed variation in responses to drought across
440 populations (**Table 1&3**). SLA was lower under drought for western populations (R, G,
441 C and F), but it was higher under drought in the remaining 4 populations (V, A, B, S;
442 **Table 1**, **Figure S3**). At day 12, populations showed variation in their response to
443 drought for SLA, stomatal size, pigment accumulation, stomatal conductance, stem
444 growth rate, starch biomass aboveground, sugar biomass above and belowground. In
445 addition to GN13 at day 6, GN7 expression (linked to high light intensity and high
446 temperature GO term) also showed a G x E effect at day 12 (**Table 3**).

447 For each treatment, we also tested for genes differentially expressed among
448 populations (testing for population effect) and timepoints separately. This analysis
449 reveals which genes are differentially expressed among populations, indicating
450 population differentiation, within a treatment and a timepoint. With these analyses, we
451 find 851 and 668 genes to be differentially regulated among populations for the control
452 treatment, and 717 and 786 for drought treatment at day 6 and 12, respectively (FDR-
453 corrected p-value<0.01). We also observed some overlap between these groups
454 (**Figure 3B**); 45 of the genes being differentially expressed among populations in both
455 of the treatments for both days. Interestingly, the majority of genes differentially
456 expressed in a treatment did not overlap between the two timepoints and this holds
457 true for both of the treatments (e.g. purple and pink in **Figure 3B**). Likewise, the genes

458 differentially expressed in the different treatments did not show overlap within a single
459 timepoint (e.g. purple and yellow in **Figure 3B**). These results show that together with
460 a strong treatment effect, there is a pronounced timepoint effect in our experiment for
461 gene expression (note that the plants were all harvested at the same time of the day
462 but 6 days apart).

463 *Most gene networks are associated with physio-morphological traits*

464 Testing for associations between gene networks and traits, we showed that 12 of the
465 14 gene networks show correlations with at least one trait (Table S6). For example,
466 GN 4 (oxidoreductase activity), 5 (cytoskeleton organization), 7 (response to high light
467 intensity and temperature), 9 (pyridine-containing compound metabolic process) and
468 13 (homeostasis) all show correlations with plant height at day 6. At day 12, these
469 same gene networks and an additional GN3 (tropism and carbohydrate metabolism-
470 related) are correlated with plant height. GN13 also shows correlation with leaf area
471 and stomatal conductance at day 6, and additionally SLA, stomatal size, aboveground
472 starch and soluble carbohydrates at day 12. GN4 and 5 show correlations with SLA
473 and leaf area at day 6, and above- and belowground soluble carbohydrates
474 additionally at day 12. Similarly, GN6 (photorespiration) is associated with leaf area,
475 stomatal conductance, aboveground starch and soluble carbohydrates and
476 belowground soluble carbohydrates at day 12. Additionally, GN7 shows correlations
477 with SLA at day 6, and GN12 (no GO enrichments) shows correlations with leaf area
478 at day 12. GN9 shows correlations with leaf area and SLA at both time points, and
479 with conductance at day 6 and above ground soluble carbohydrates and starch and
480 below ground soluble carbohydrates at day 12.

481

482 **Do the climatic conditions covary with traits, gene expression and their**
483 **plasticity?**

484 *Gene networks and functional traits correlate with temperature*

485 In order to assess climatically driven trait and gene expression variation among
486 populations, we tested for correlations between source-site climate variables and co-
487 expression gene networks and functional traits. We found several gene networks that
488 covary with climatic variables (**Figure 4**; boxed combinations). These include GN 3, 4,
489 5, 9 and 13 at day 6, and GN3, 4, 5, 6, 7, 10, 12 and 13 at day 12. Most of these gene
490 networks showed strong correlations with elevation and the closely related variables
491 winter minimum temperature and summer maximum temperature. We also observed
492 correlations between mean annual precipitation and GN6, 10 and 13, stomatal
493 conductance and starch content in shoots at day 12. Stem growth showed correlations
494 with elevation, summer maximum and winter minimum temperature. Aboveground
495 starch content co-varied with elevation, mean annual precipitation and intra-annual
496 precipitation variation. Similarly, aboveground soluble carbohydrates showed
497 associations with elevation.

498 We tested for climate by treatment interaction which revealed if associations
499 between traits and the climate are significant for only one of the treatments. We found
500 that gene expression patterns for GN7 at day 12 show a positive correlation with
501 elevation (and minimum winter temperature) only for plants under drought conditions.
502 Contrastingly, the correlations between GN12 and elevation, and GN13 and mean
503 annual precipitation are significant only for watered plants. Similarly, for stem growth
504 and aboveground starch, the correlations with elevation are significant only for watered

505 plants, and stomatal conductance with mean annual precipitation only under watered
506 conditions.

507 *Plasticity differentiation is associated with climate*

508 We tested if plasticity in traits and gene expression reflects signatures of climatic
509 effects. At day 6, plasticity in GN5 (related to cytoskeleton organization) is the only
510 gene network that show a correlation with a climatic variable: low elevation populations
511 showed higher plasticity in gene expression patterns of this gene network (**Table 4**).
512 We also observe higher plasticity in stomatal density in wetter sites. At day 12, we
513 found that plasticity in GN10 (related to translation) and GN13 (linked to homeostasis)
514 covaries with mean annual precipitation. For GN10, populations from both the driest
515 and the wettest sites show higher plasticity, and populations from wetter sites show
516 higher plasticity for GN13 (**Table 4**, **Figure 5D**). Additionally, plasticity in stem growth
517 correlates with both elevation and summer maximum temperature. High plasticity in
518 growth is observed in populations from low elevation and warmer sites (**Figure 5A**).
519 Similar to GN13, plasticity in leaf area shows correlations with mean annual
520 precipitation with higher plasticity in populations from wetter sites (**Figure 5B**).
521 Pigment accumulation shows correlations with temperature heterogeneity
522 (temperature range within years) (**Figure 5C**) and maximum temperature. However,
523 pigment-related associations are driven by a single population in Cederberg, the most
524 northern population we studied. We should also note that this trend is driven by the
525 direction of plasticity in this population for pigment content, which is opposite to what
526 we observe in all the other populations:, plants from the Cederberg population have
527 higher pigment under drought and lower pigments when watered (Figure S3).

528 **DISCUSSION**

529 Local adaptation can lead to trait divergence among populations along environmental
530 gradients. Plasticity in traits, as any other phenotypic character, can also be under
531 selection, creating matching patterns between plasticity differentiation and the source
532 site climatic variation among populations. In this study, we show that functional traits
533 and gene expression patterns, and their plasticity are associated with source-site
534 climate in *Protea repens*, suggesting local adaptation in both trait means and their
535 plasticity.

536

537 *Drought imposes a complex stress regulating many gene expression networks*

538 Drought has been a strong driver of plant evolution in the Cape Floristic Region and
539 other Mediterranean climate zones (Allsopp, Colville, and Verboom 2014). When
540 water availability is limited, as it typically is for several months during the summer in
541 most of the region, plants experience a suite of complex stresses (West, Dawson, and
542 February 2012; Ramachandra Reddy, Chaitanya, and Vivekanandan 2004) reflected
543 in regulation of various pathways including ABA-dependent pathways, carbohydrate
544 metabolism, ROS scavenging and photosynthesis under drought (Heschel et al. 2014;
545 Miller et al. 2010). Accordingly, we observe differential gene expression in 10 out of
546 13 gene networks under drought conditions. For example, the genes with the highest
547 gene network membership in GN5 (related to cytoskeleton organization) are
548 downregulated under drought in all the populations, and this gene network also shows
549 correlations with height, leaf area and aboveground carbohydrate content.
550 Cytoskeleton organization related pathways were also shown to be highly hampered

551 during drought stress in Arabidopsis (Bhaskara et al. 2017), linking this process to
552 reduced growth. A general pattern similar to that of GN5 is seen in other gene networks
553 important for growth (such as GN1 related to cell wall organization and other
554 carbohydrate metabolism related gene networks). This "shut-off" of growth also
555 explains the shorter stems and smaller carbohydrate content aboveground under 12
556 days of drought for all populations. Additionally, the genes that were consistently up-
557 regulated among all the populations were genes involved in stress responses.
558 Together these results indicate a trade-off in which resources are channeled from
559 growth to stress acclimation. A similar trade-off has been observed across many plant
560 species for herbivory (Kempel et al. 2011; Züst, Rasmann, and Agrawal 2015), cold
561 stress (Loehle 1998) and drought (Haugen et al. 2008). We also detected reduced
562 stomatal conductance in all populations in the drought treatment. This indicates a
563 potential drop in photosynthesis, which might further contribute to the energy deficit
564 hampering growth. The only gene network, GN6 (enriched for photorespiration related
565 genes) that shows a correlation with conductance at the later stage of the stress is
566 also correlated with leaf area and carbohydrate content. Interestingly, we also find a
567 correlation between this gene network and mean annual precipitation suggesting local
568 adaptation to water availability via regulation of carbohydrate accumulation.

569 *Associations with the climate are context dependent*

570 When there are fitness consequences of a trait that is induced conditionally in
571 response to environmental stressors, with no fitness costs/benefits when those
572 stressors are absent, genetic variation that controls trait plasticity will be under
573 selection only under the inducing stressful environment (Van Dyken and Wade 2010;
574 Ledón-Rettig et al. 2014). Strikingly, we observed climatic correlations in gene

575 expression and traits that only exist in either one of the treatments (**Figure 4**). Gene
576 network 7, for example, shows a correlation with elevation and winter minimum
577 temperature only when the plants are under drought stress. Gene ontology
578 enrichments in this module point to regulation by high light and temperature stress. A
579 similar gene network in our previous study in a common garden with more mature
580 plants (18 months old) was also enriched for GO terms related to high light intensity
581 and temperature, and this module showed correlations with stem diameter and
582 stomatal density, but surprisingly not with any climate variables (Akman et al. 2016b).
583 GN7 in this study also shows significant correlations with height at both timepoints and
584 SLA in the earlier timepoint (**Table S6**). The top members of this gene network with
585 Arabidopsis homology are down-regulated in populations coming from higher
586 elevation, one of these genes is 4CL3 (AT1G65060) involved in last step of
587 phenylpropanoid pathway - the upstream pathway anthocyanins are derived from. We
588 also found that plants from warmer sites (higher maximum summer temperature) have
589 higher pigment accumulation (p-value =0.029, fdr-corrected value = 0.106). Previous
590 studies showed a negative effect of heat stress on plant growth (Olszyk et al. 1998;
591 Hasanuzzaman et al. 2013; Bita and Gerats 2013), and anthocyanin accumulation as
592 a protective mechanism against heat, cold, oxidative stress and UV radiation (Hatier
593 and Gould 2009; Steyn et al. 2009; Zeng et al. 2010; Wang et al. 2013). The higher
594 amount of anthocyanins accumulated in populations originating from warmer sites in
595 this study might enable a better protection against stressors such as reactive oxygen
596 species, and thus lead to taller stature. In a parallel finding, under drought conditions,
597 differentially expressed genes among populations were enriched for only oxidative

598 stress related genes, suggesting selection on expression of these genes along the
599 climate gradient.

600 Contrastingly, GN13 (homeostasis related; the best representative gene
601 showing homology with an Arabidopsis heat shock protein- AT5G59720) shows a
602 correlation with mean annual precipitation only in watered conditions. Heat shock
603 proteins are shown to be involved in responses to stresses such as heat, cold, UV
604 radiation, salt and drought (Chen, Feder, and Kang 2018). Under control conditions,
605 plants from wetter source sites express genes in this network at lower levels compared
606 to populations from drier sites, and show a bigger up-regulation under drought,
607 eventually reaching similar levels of expression for all populations. This gene network
608 also shows correlations with leaf area, SLA, stomatal pore size and aboveground
609 carbohydrate content. In plants from drier environments, higher constitutive
610 expression of these genes might be crucial for a prompt response to drought via
611 regulation of stomatal pore size, leaf size and thickness. However, plants from
612 populations that rarely experience drought might not benefit from constitutive
613 expression of these genes, creating a mismatch between costs and benefits.

614 *Minimum temperature and elevation show widespread associations with trends in*
615 *gene expression*

616 Previously, we showed that functional traits and gene expression patterns as
617 measured in a common garden with older plants (1.5 year old plants vs 2 months-old
618 plants in this study) correlated with source site climate (Akman *et al*., 2016). Although
619 using fewer populations in this study, we observed similar patterns in those
620 correlations. For example, in both studies we observe associations between gene

621 expression networks and minimum winter temperature, elevation and mean annual
622 precipitation (note that minimum winter temperature and elevation are highly
623 correlated: Pearson's R = -0.91, **Table S2)**. Taken together, these variables have
624 several associations with gene network variation, as we also found in the previous
625 study. Although *Protea* populations throughout the CFR experience drought at least
626 occasionally, the drought metrics used in this study are not as strong predictors of trait
627 variation as temperature/elevation is under the tested conditions (drought and control
628 in a greenhouse and a common garden). Since gene expression networks include all
629 major groupings of coexpressed genes, they reflect an unbiased pattern in plant
630 responses, and in this case, this pattern indicates fairly widespread variation in gene
631 expression across temperature/elevation gradients.

632 Why was temperature/elevation associated with population differences in gene
633 expression more often than precipitation? We can't say conclusively, but note that
634 populations in the CFR experience drought periodically, and although some of the
635 populations we studied experienced a decrease in precipitation over the past 115
636 years, this effect is not as strong as the increase in temperature over the same period
637 (**Figure** 6, (Kruger and Shongwe 2004; Mason and Jury 1997). Both historically and
638 during recent warming, temperature regimes potentially impose a more continuous
639 selection force than precipitation patterns. Precipitation has been more variable
640 historically and recent climate change trends are variable across the region, and this
641 variability might make it harder to detect patterns of selection in response to
642 precipitation changes across climate gradients even if measurements are performed
643 under drought conditions as in our experiments. These results also line up with a
644 broader trend: a meta-analysis of 21 plant traits from 25354 species concluded that

645 temperature is a stronger driver of plant trait evolution compared to precipitation
646 (Moles et al. 2014).

647 *Carbohydrate reserves play a role in survival*

648 Surprisingly, we found a positive correlation between survival under drought and
649 precipitation; plants from wetter sites survived longer under drought. Populations from
650 wetter sites also showed higher stomatal conductance and shoot starch content,
651 indicating higher photosynthetic rates. The fact that populations from wetter sites
652 survived better under drought is counterintuitive. However, in a sudden drought onset,
653 like the one imposed in this experiment, carbohydrate reserves might be a decisive
654 factor in survival, especially in earlier stages of development, and thus if plants from
655 wetter sites have built up larger reserves, they may survive longer (O'Brien et al. 2014).

656 *Both climatic heterogeneity and favorable environmental conditions promote plasticity*
657 *evolution*

658 Here, we also tested if plasticity is favored and thus selected for in heterogeneous
659 and/or milder climatic conditions. A heterogeneous environment in theory should
660 promote plasticity evolution as this can allow for a better match between the phenotype
661 and the changing environment, potentially surpassing cost of plasticity. Similarly, in
662 mild environments, plasticity can arise through a relaxed selection in which a mismatch
663 between the phenotype and the climate does not impose strong fitness costs. We
664 found evidence for both of these patterns for the functional traits and the gene
665 expression patterns. However, in this study, we have more evidence for growth
666 plasticity evolving under milder conditions than heterogeneous environments, i.e.
667 higher plasticity for growth and GN5 (cytoskeleton organization) in populations from

668 lower elevation/warmer sites (representing milder climates). Although heat stress can
669 be detrimental, in the CFR cold stress may impose a stronger selective pressure for
670 growth (Wilson, Latimer, and Silander 2015). In fact, in a study of 56 Proteaceae
671 species looking at climate and plant trait correlations, Mitchell et al, showed the most
672 strongly supported evolutionary correlations to be between winter minimum
673 temperature (next to mean annual temperature and elevation) and plant height
674 (Mitchell, Carlson, and Holsinger 2018). Authors also note that this negative effect of
675 elevation on plant height might be coupled potentially with shallower soils in the high
676 elevation sites (Campbell and Werger 1988). These combined effects might create
677 harsher environments which increase the cost of mismatch between phenotype and
678 the environment, thus decreasing fitness of more plastic individuals. We also find
679 higher plasticity in wetter sites for GN13 (homeostasis related) and leaf area, and
680 again these sites represent milder climates in this drought-prone region. The only
681 correlation we observe for heterogeneity is also related to temperature: plasticity in
682 pigment accumulation increases with increasing heterogeneity. In environments
683 where temperature change across seasons is high, levels of anthocyanins might
684 effectively protect plants against abiotic stressors, thus promoting selection on higher
685 plasticity.

686 To conclude, our results suggest that both phenotypic traits and gene expression, and
687 their plasticity can be under selection along a climate gradient. However it is also
688 important to note that without studying fitness consequences of the climate, our results
689 do not prove that this variation is adaptive. We also showed that detecting associations
690 between these traits and the environmental variables can be a challenging task as
691 these associations are environment-dependent, and thus can be detected only when

692 they are measured under certain conditions. Additionally, we found that trait variation
693 can be explained better with temperature related-variables rather than precipitation
694 related variables we chose. This could arise from recent temperature increase
695 associated with climate change that can potentially impose a stronger and more
696 continuous selection pressure compared to the more variable changes in precipitation.
697 This study also provides insights into the drivers of plasticity evolution: we found more
698 evidence for higher plasticity in mild environments compared heterogeneous
699 environments. Although our results demonstrate population differentiation in traits and
700 their plasticity and their links to climate, we should also note that we found only 24/350
701 trait/gene expression and climate associations to be significant together with 9/33 for
702 plasticity. This relatively low number could either be due to the fact that the climate
703 variables we chose cannot fully represent the source site environments, that our
704 experimental design does not capture the genetic variation fully, or that drift has
705 affected population differentiation. In this regard, future work with long-term transplant
706 experiments in several common gardens and collection of genomics/transcriptomics
707 data would enable heritability and Fst/Qst calculations, and coupling these with fitness
708 measurements would conclusively uncover patterns of local adaptation and the
709 underlying genetic/gene expression mechanisms in this vulnerable species in the
710 CFR.

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## REFERENCES

Acasuso-Rivero, Cristina, Courtney J. Murren, Carl D. Schlichting, and Ulrich K. Steiner. 2019. "Adaptive Phenotypic Plasticity for Life-History and Less Fitness-Related Traits." *Proceedings. Biological Sciences / The Royal Society* 286 (1904): 20190653.

Ackerly, David D., Susan A. Dudley, Sonia E. Sultan, Johanna Schmitt, James S. Coleman, C. Randall Linder, Darren R. Sandquist, et al. 2000. "The Evolution of Plant Ecophysiological Traits: Recent Advances and Future Directions: New Research Addresses Natural Selection, Genetic Constraints, and the Adaptive Evolution of Plant Ecophysiological Traits." *Bioscience* 50 (11): 979–95.

Akman, Melis, Amit V. Bhikharie, Elizabeth H. McLean, Alex Boonman, Eric J. W. Visser, M. Eric Schranz, and Peter H. van Tienderen. 2012. "Wait or Escape? Contrasting Submergence Tolerance Strategies of Rorippa Amphibia, Rorippa Sylvestris and Their Hybrid." *Annals of Botany* 109 (7): 1263–76.

Akman, Melis, Jane E. Carlson, Kent E. Holsinger, and Andrew M. Latimer. 2016. "Transcriptome Sequencing Reveals Population Differentiation in Gene Expression Linked to Functional Traits and Environmental Gradients in the South African Shrub Protea Repens." *The New Phytologist* 210 (1): 295–309.

Allen, Craig D., Alison K. Macalady, Haroun Chenchouni, Dominique Bachelet, Nate McDowell, Michel Vennetier, Thomas Kitzberger, et al. 2010. "A Global Overview of Drought and Heat-Induced Tree Mortality Reveals Emerging Climate Change Risks for Forests." *Forest Ecology and Management* 259 (4): 660–84.

Allsopp, Nicky, Jonathan F. Colville, and G. Anthony Verboom. 2014. *Fynbos: Ecology, Evolution, and Conservation of a Megadiverse Region*. Oxford University Press.

Alpert, Peter, and Ellen L. Simms. 2002. "The Relative Advantages of Plasticity and Fixity in Different Environments: When Is It Good for a Plant to Adjust?" *Evolutionary Ecology* 16 (3): 285–97.

Aubin, I., A. D. Munson, F. Cardou, P. J. Burton, N. Isabel, J. H. Pedlar, A. Paquette, et al. 2016. "Traits to Stay, Traits to Move: A Review of Functional Traits to Assess Sensitivity and Adaptive Capacity of Temperate and Boreal Trees to Climate Change." *Environmental Review* 24 (2): 164–86.

Barshis, Daniel J., Jason T. Ladner, Thomas A. Oliver, François O. Seneca, Nikki

Traylor-Knowles, and Stephen R. Palumbi. 2013. “Genomic Basis for Coral Resilience to Climate Change.” *Proceedings of the National Academy of Sciences* 110 (4): 1387–92.

Bates, Douglas, Martin Maechler, and Ben Bolker. 2012. “lme4: Linear Mixed-Effects Models Using S4 Classes.”

Baythavong, Brooke S. 2011. “Linking the Spatial Scale of Environmental Variation and the Evolution of Phenotypic Plasticity: Selection Favors Adaptive Plasticity in Fine-Grained Environments.” *The American Naturalist* 178 (1): 75–87.

Baythavong, Brooke S., and Maureen L. Stanton. 2010. “Characterizing Selection on Phenotypic Plasticity in Response to Natural Environmental Heterogeneity.” *Evolution; International Journal of Organic Evolution* 64 (10): 2904–20.

Bhaskara, Govinal Badiger, Tuan-Nan Wen, Thao Thi Nguyen, and Paul E. Verslues. 2017. “Protein Phosphatase 2Cs and Microtubule-Associated Stress Protein 1 Control Microtubule Stability, Plant Growth, and Drought Response.” *The Plant Cell* 29 (1): 169–91.

Bita, Craita E., and Tom Gerats. 2013. “Plant Tolerance to High Temperature in a Changing Environment: Scientific Fundamentals and Production of Heat Stress-Tolerant Crops.” *Frontiers in Plant Science* 4 (July): 273.

Bolger, Anthony M., Marc Lohse, and Bjoern Usadel. 2014. “Trimmomatic: A Flexible Trimmer for Illumina Sequence Data.” *Bioinformatics* , April. https://doi.org/10.1093/bioinformatics/btu170.

Bonamour, Suzanne, Luis-Miguel Chevin, Anne Charmantier, and Céline Teplitsky. 2019. “Phenotypic Plasticity in Response to Climate Change: The Importance of Cue Variation.” *Philosophical Transactions of the Royal Society B: Biological Sciences*. https://doi.org/10.1098/rstb.2018.0178.

Bradshaw, A. D., and K. Hardwick. 1989. “Evolution and Stress—genotypic and Phenotypic Components.” *Biological Journal of the Linnean Society. Linnean Society of London* 37 (1-2): 137–55.

Campbell, B. M., and M. J. A. Werger. 1988. “Plant Form in the Mountains of the Cape, South Africa.” *The Journal of Ecology* 76 (3): 637–53.

Carlson, Jane E., Christopher A. Adams, and Kent E. Holsinger. 2016. “Intraspecific Variation in Stomatal Traits, Leaf Traits and Physiology Reflects Adaptation along Aridity Gradients in a South African Shrub.” *Annals of Botany* 117 (1): 195–207.

Carlson, Jane E., and Kent E. Holsinger. 2012. “Developmental Plasticity in <italic>Protea</italic> as an Evolutionary Response to Environmental Clines in the Cape Floristic Region.” *PloS One* 7 (12): e52035.

Carlson, Jane E., Kent E. Holsinger, and Rachel Prunier. 2011. “Plant Responses to Climate in the Cape Floristic Region of South Africa: Evidence for Adaptive Differentiation in the Proteaceae.” *Evolution; International Journal of Organic Evolution* 65 (1): 108–24.

Cavender-Bares, Jeannine, and José Alberto Ramírez-Valiente. 2017. “Physiological Evidence from Common Garden Experiments for Local Adaptation and Adaptive Plasticity to Climate in American Live Oaks (Quercus Section Virentes): Implications for Conservation under Global Change.” In *Oaks Physiological Ecology. Exploring the Functional Diversity of Genus Quercus L.*, 107–35. Springer.

Chen, Bing, Martin E. Feder, and Le Kang. 2018. “Evolution of Heat-Shock Protein Expression Underlying Adaptive Responses to Environmental Stress.” *Molecular*

*Ecology*. https://onlinelibrary.wiley.com/doi/abs/10.1111/mec.14769.
Chevin, Luis-Miguel, and Ary A. Hoffmann. 2017. "Evolution of Phenotypic Plasticity in Extreme Environments." *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 372 (1723). https://doi.org/10.1098/rstb.2016.0138.
Chevin, Luis-Miguel, Russell Lande, and Georgina M. Mace. 2010. "Adaptation, Plasticity, and Extinction in a Changing Environment: Towards a Predictive Theory." *PLoS Biology* 8 (4): e1000357.
Cochrane, Anne, Gemma L. Hoyle, Colin J. Yates, Jeff Wood, and Adrienne B. Nicotra. 2015. "The Phenotypic Response of Co-Occurring Banksia Species to Warming and Drying." *Plant Ecology* 216 (1): 27–39.
Colicchio, Jack M., Patrick J. Monnahan, John K. Kelly, and Lena C. Hileman. 2015. "Gene Expression Plasticity Resulting from Parental Leaf Damage in Mimulus Guttatus." *The New Phytologist* 205 (2): 894–906.
Cowling, R. M. 2009. "From Winter Wet to Winter Dry: Fynbos Diversity and Evolution along the West–east Gradient." *South African Journal of Botany: Official Journal of the South African Association of Botanists = Suid-Afrikaanse Tydskrif Vir Plantkunde: Amptelike Tydskrif van Die Suid-Afrikaanse Genootskap van Plantkundiges* 2 (75): 386–87.
Cuenca Cambronero, Maria, Jordan Beasley, Stephen Kissane, and Luisa Orsini. 2018. "Evolution of Thermal Tolerance in Multifarious Environments." *Molecular Ecology* 27 (22): 4529–41.
Daac, L. P. 2011. "ASTER L1B." *Sioux Falls, South Dakota: USGS/Earth Resources Observation and Science (EROS) Center*.
Dayan, David I., Douglas L. Crawford, and Marjorie F. Oleksiak. 2015. "Phenotypic Plasticity in Gene Expression Contributes to Divergence of Locally Adapted Populations of Fundulus Heteroclitus." *Molecular Ecology* 24 (13): 3345–59.
Dudley, Susan A., and Johanna Schmitt. 1996. "Testing the Adaptive Plasticity Hypothesis: Density-Dependent Selection on Manipulated Stem Length in Impatiens Capensis." *The American Naturalist* 147 (3): 445–65.
Eden, Eran, Roy Navon, Israel Steinfeld, Doron Lipson, and Zohar Yakhini. 2009. "GOrilla: A Tool for Discovery and Visualization of Enriched GO Terms in Ranked Gene Lists." *BMC Bioinformatics* 10 (1): 48.
Etterson, Julie R. 2004. "Evolutionary Potential of Chamaecrista Fasciculata in Relation to Climate Change. I. Clinal Patterns of Selection along an Environmental Gradient in the Great Plains." *Evolution; International Journal of Organic Evolution* 58 (7): 1446–58.
Forsman, A. 2015. "Rethinking Phenotypic Plasticity and Its Consequences for Individuals, Populations and Species." *Heredity* 115 (4): 276–84.
Fu, Limin, Beifang Niu, Zhengwei Zhu, Sitao Wu, and Weizhong Li. 2012. "CD-HIT: Accelerated for Clustering the next-Generation Sequencing Data." *Bioinformatics* 28 (23): 3150–52.
Geber, Monica A., and Lauren R. Griffen. 2003. "Inheritance and Natural Selection on Functional Traits." *International Journal of Plant Sciences* 164 (S3): S21–42.
Ghalambor, Cameron K., Kim L. Hoke, Emily W. Ruell, Eva K. Fischer, David N. Reznick, and Kimberly A. Hughes. 2015. "Non-Adaptive Plasticity Potentiates Rapid Adaptive Evolution of Gene Expression in Nature." *Nature* 525 (7569): 372–75.

850 Granados-Cifuentes, Camila, Anthony J. Bellantuono, Tyrone Ridgway, Ove Hoegh-
851 Guldberg, and Mauricio Rodriguez-Lanetty. 2013. "High Natural Gene Expression
852 Variation in the Reef-Building Coral Acropora Millepora: Potential for Acclimative
853 and Adaptive Plasticity." *BMC Genomics* 14 (1): 1–12.
854 Grime, J. P. 1994. "The Role of Plasticity in Exploiting Environmental Heterogeneity."
855 *Exploitation of Environmental Heterogeneity by Plants: Ecophysiological*
856 *Processes above-and Belowground* 1: 19.
857 Gugger, Simona, Halil Kesselring, Jürg Stöcklin, and Elena Hamann. 2015. "Lower
858 Plasticity Exhibited by High- versus Mid-Elevation Species in Their Phenological
859 Responses to Manipulated Temperature and Drought." *Annals of Botany* 116 (6):
860 953–62.
861 Hannon, G. J. n.d. "Fastx-Toolkit. 2010." *GNU Affero General Public License* 706.
862 Hasanuzzaman, Mirza, Kamrun Nahar, Md Mahabub Alam, Rajib Roychowdhury, and
863 Masayuki Fujita. 2013. "Physiological, Biochemical, and Molecular Mechanisms
864 of Heat Stress Tolerance in Plants." *International Journal of Molecular Sciences*
865 14 (5): 9643–84.
866 Hatier, Jean-Hugues B., and Kevin S. Gould. 2009. "Anthocyanin Function in
867 Vegetative Organs." In *Anthocyanins: Biosynthesis, Functions, and Applications*,
868 edited by Chris Winefield, Kevin Davies, and Kevin Gould, 1–19. New York, NY:
869 Springer New York.
870 Haugen, Riston, Lexi Steffes, Joy Wolf, Paul Brown, Steven Matzner, and David H.
871 Siemens. 2008. "Evolution of Drought Tolerance and Defense: Dependence of
872 Tradeoffs on Mechanism, Environment and Defense Switching." *Oikos* 117 (2):
873 231–44.
874 Heschel, M. Shane, Ann Evankow, Katherine Bibee Wolfson, Jane E. Carlson, and
875 Kent E. Holsinger. 2014. "Drought Response Diversification in African Protea
876 Species." *International Journal of Plant Sciences* 175 (4): 442–49.
877 Hewitson, B. C., and R. G. Crane. 2006. "Consensus between GCM Climate Change
878 Projections with Empirical Downscaling: Precipitation Downscaling over South
879 Africa." *International Journal of Climatology* 26 (10): 1315–37.
880 Jacobs, B. S., and A. M. Latimer. 2012. "Analyzing Reaction Norm Variation in the
881 Field vs. Greenhouse: Comparing Studies of Plasticity and Its Adaptive Value in
882 Two Species of Erodium." *Perspectives in Plant Ecology, Evolution and*
883 *Systematics* 14 (5): 325–34.
884 Kempel, Anne, Martin Schädler, Thomas Chrobock, Markus Fischer, and Mark van
885 Kleunen. 2011. "Tradeoffs Associated with Constitutive and Induced Plant
886 Resistance against Herbivory." *Proceedings of the National Academy of Sciences*
887 *of the United States of America* 108 (14): 5685–89.
888 Kruger, Andries C., and Stephen Shongwe. 2004. "Temperature Trends in South
889 Africa: 1960--2003." *International Journal of Climatology* 24 (15): 1929–45.
890 Langfelder, Peter, and Steve Horvath. 2008. "WGCNA: An R Package for Weighted
891 Correlation Network Analysis." *BMC Bioinformatics* 9 (1): 559.
892 Langmead, Ben, and Steven L. Salzberg. 2012. "Fast Gapped-Read Alignment with
893 Bowtie 2." *Nature Methods* 9 (4): 357–59.
894 Lasky, Jesse R., David L. Des Marais, David B. Lowry, Inna Povolotskaya, John K.
895 McKay, James H. Richards, Timothy H. Keitt, and Thomas E. Juenger. 2014.
896 "Natural Variation in Abiotic Stress Responsive Gene Expression and Local
897 Adaptation to Climate in Arabidopsis Thaliana." *Molecular Biology and Evolution*

31 (9): 2283–96.
Latimer, Andrewm, J. A. Silander Jr., A. G. Rebelo, and G. F. Midgley. 2009. "Experimental Biogeography: The Role of Environmental Gradients in High Geographic Diversity in Cape Proteaceae." *Oecologia* 160 (1): 151–62.
Lázaro-Nogal, Ana, Silvia Matesanz, Alice Godoy, Fernanda Pérez-Trautman, Ernesto Gianoli, and Fernando Valladares. 2015. "Environmental Heterogeneity Leads to Higher Plasticity in Dry-Edge Populations of a Semi-Arid Chilean Shrub: Insights into Climate Change Responses." *The Journal of Ecology* 103 (2): 338–50.
Lázaro-Nogal, Ana, Silvia Matesanz, Lea Hallik, Alisa Krasnova, Anna Traveset, and Fernando Valladares. 2016. "Population Differentiation in a Mediterranean Relict Shrub: The Potential Role of Local Adaptation for Coping with Climate Change." *Oecologia* 180 (4): 1075–90.
Ledón-Rettig, Cris C., David W. Pfennig, Amanda J. Chunco, and Ian Dworkin. 2014. "Cryptic Genetic Variation in Natural Populations: A Predictive Framework." *Integrative and Comparative Biology* 54 (5): 783–93.
Loehle, Craig. 1998. "Height Growth Rate Tradeoffs Determine Northern and Southern Range Limits for Trees." *Journal of Biogeography* 25 (4): 735–42.
Markham, Charles G. 1970. "SEASONALITY OF PRECIPITATION IN THE UNITED STATES." *Annals of the Association of American Geographers. Association of American Geographers* 60 (3): 593–97.
Mason, S. J., and M. R. Jury. 1997. "Climatic Variability and Change over Southern Africa: A Reflection on Underlying Processes." *Progress in Physical Geography: Earth and Environment* 21 (1): 23–50.
Miller, Gad, Nobuhiro Suzuki, Sultan Ciftci-Yilmaz, and Ron Mittler. 2010. "Reactive Oxygen Species Homeostasis and Signalling during Drought and Salinity Stresses." *Plant, Cell & Environment* 33 (4): 453–67.
Mitchell, Nora, Jane E. Carlson, and Kent E. Holsinger. 2018. "Correlated Evolution between Climate and Suites of Traits along a Fast-Slow Continuum in the Radiation of Protea." *Ecology and Evolution* 8 (3): 1853–66.
Moles, Angela T., Sarah E. Perkins, Shawn W. Laffan, Habacuc Flores-Moreno, Monica Awasthy, Marianne L. Tindall, Lawren Sack, et al. 2014. "Which Is a Better Predictor of Plant Traits: Temperature or Precipitation?" Edited by Aveliina Helm. *Journal of Vegetation Science: Official Organ of the International Association for Vegetation Science* 25 (5): 1167–80.
Morris, Matthew R. J., Romain Richard, Erica H. Leder, Rowan D. H. Barrett, Nadia Aubin-Horth, and Sean M. Rogers. 2014. "Gene Expression Plasticity Evolves in Response to Colonization of Freshwater Lakes in Threespine Stickleback." *Molecular Ecology* 23 (13): 3226–40.
Nicotra, Adrienne B., Deborah L. Segal, Gemma L. Hoyle, Aaron W. Schrey, Koen J. F. Verhoeven, and Christina L. Richards. 2015. "Adaptive Plasticity and Epigenetic Variation in Response to Warming in an Alpine Plant." *Ecology and Evolution* 5 (3): 634–47.
O'Brien, Michael J., Sebastian Leuzinger, Christopher D. Philipson, John Tay, and Andy Hector. 2014. "Drought Survival of Tropical Tree Seedlings Enhanced by Non-Structural Carbohydrate Levels." *Nature Climate Change* 4 (June): 710.
Olszyk, D., Claudia Wise, Erica VanEss, and David Tingey. 1998. "Elevated Temperature but Not Elevated CO2 Affects Long-Term Patterns of Stem Diameter

and Height of Douglas-Fir Seedlings." *Canadian Journal of Forest Research. Journal Canadien de La Recherche Forestiere* 28 (7): 1046–54.
Pachter, Lior. 2011. "Models for Transcript Quantification from RNA-Seq." *arXiv [q-bio.GN]*. arXiv. http://arxiv.org/abs/1104.3889.
Palacio-López, Kattia, Brian Beckage, Samuel Scheiner, and Jane Molofsky. 2015. "The Ubiquity of Phenotypic Plasticity in Plants: A Synthesis." *Ecology and Evolution* 5 (16): 3389–3400.
Pohlman, Catherine L., Adrienne B. Nicotra, and Brad R. Murray. 2005. "Geographic Range Size, Seedling Ecophysiology and Phenotypic Plasticity in Australian Acacia Species." *Journal of Biogeography* 32 (2): 341–51.
Pratt, Jessica D., and Kailen A. Mooney. 2013. "Clinal Adaptation and Adaptive Plasticity in Artemisia Californica: Implications for the Response of a Foundation Species to Predicted Climate Change." *Global Change Biology* 19 (8): 2454–66.
Prunier, R., K. E. Holsinger, and J. E. Carlson. 2012. "The Effect of Historical Legacy on Adaptation: Do Closely Related Species Respond to the Environment in the Same Way?" *Journal of Evolutionary Biology* 25 (8): 1636–49.
Ramachandra Reddy, Attipalli, Kolluru Viswanatha Chaitanya, and Munusamy Vivekanandan. 2004. "Drought-Induced Responses of Photosynthesis and Antioxidant Metabolism in Higher Plants." *Journal of Plant Physiology* 161 (11): 1189–1202.
Rebelo, A. 2001. "Saso Proteas: A Field Guide to the Proteas of Southern Africa." http://kbd.kew.org/kbd/detailedresult.do?id=352313.
Reed, Thomas E., Robin S. Waples, Daniel E. Schindler, Jeffrey J. Hard, and Michael T. Kinnison. 2010. "Phenotypic Plasticity and Population Viability: The Importance of Environmental Predictability." *Proceedings. Biological Sciences / The Royal Society* 277 (1699): 3391–3400.
Sauquet, Hervé, Peter H. Weston, Cajsa Lisa Anderson, Nigel P. Barker, David J. Cantrill, Austin R. Mast, and Vincent Savolainen. 2009. "Contrasted Patterns of Hyperdiversification in Mediterranean Hotspots." *Proceedings of the National Academy of Sciences* 106 (1): 221–25.
Scheiner, Samuel M. 2013. "The Genetics of Phenotypic Plasticity. XII. Temporal and Spatial Heterogeneity." *Ecology and Evolution* 3 (13): 4596–4609.
Schlichting, Carl D., and Massimo Pigliucci. 1995. "Gene Regulation, Quantitative Genetics and the Evolution of Reaction Norms." *Evolutionary Ecology* 9 (2): 154–68.
Schneider, Ralf F., Yuanhao Li, Axel Meyer, and Helen M. Gunter. 2014. "Regulatory Gene Networks That Shape the Development of Adaptive Phenotypic Plasticity in a Cichlid Fish." *Molecular Ecology* 23 (18): 4511–26.
Schulze, R. E. 2007. "South African Atlas of Climatology and Agrohydrology: WRC Report 1489/1/06." Water Resource Commission.
Schulze, Roland Edgar. 1997. "South African Atlas of Agrohydrology and-Climatology." 1868452719. Pretoria, South Africa: Water Research Commission.
Steyn, Willem J., Stephanie J. E. Wand, Gerard Jacobs, Richard C. Rosecrance, and Stephanie C. Roberts. 2009. "Evidence for a Photoprotective Function of Low-Temperature-Induced Anthocyanin Accumulation in Apple and Pear Peel." *Physiologia Plantarum* 136 (4): 461–72.
Stoks, Robby, Lynn Govaert, Kevin Pauwels, Bastiaan Jansen, and Luc De Meester. 2016. "Resurrecting Complexity: The Interplay of Plasticity and Rapid Evolution

in the Multiple Trait Response to Strong Changes in Predation Pressure in the Water Flea Daphnia Magna." *Ecology Letters* 19 (2): 180–90.

Sultan, Sonia E. 2003. "Phenotypic Plasticity in Plants: A Case Study in Ecological Development." *Evolution & Development* 5 (1): 25–33.

Team, R. Core. 2014. "R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2014."

Valliere, Justin M., Irina C. Irvine, Louis Santiago, and Edith B. Allen. 2017. "High N, Dry: Experimental Nitrogen Deposition Exacerbates Native Shrub Loss and Nonnative Plant Invasion during Extreme Drought." *Global Change Biology* 23 (10): 4333–45.

Van Dyken, J. David, and Michael J. Wade. 2010. "The Genetic Signature of Conditional Expression." *Genetics* 184 (2): 557–70.

Van Kleunen, Mark, and Markus Fischer. 2005. "Constraints on the Evolution of Adaptive Phenotypic Plasticity in Plants." *The New Phytologist* 166 (1): 49–60.

Van Wilgen, B. W., G. G. Forsyth, and H. De Klerk. 2010. "Fire Management in Mediterranean-climate Shrublands: A Case Study from the Cape Fynbos, South Africa." *Journal of Applied*. https://agupubs.onlinelibrary.wiley.com/doi/full/10.1111/j.1365-2664.2010.01800.x.

Vitasse, Yann, Günter Hoch, Christophe F. Randin, Armando Lenz, Chris Kollas, J. F. Scheepens, and Christian Körner. 2013. "Elevational Adaptation and Plasticity in Seedling Phenology of Temperate Deciduous Tree Species." *Oecologia* 171 (3): 663–78.

Wadgymar, Susana M., Rachel M. Mactavish, and Jill T. Anderson. 2018. "Transgenerational and Within-Generation Plasticity in Response to Climate Change: Insights from a Manipulative Field Experiment across an Elevational Gradient." *The American Naturalist* 192 (6): 698–714.

Wang, Hongxia, Weijuan Fan, Hong Li, Jun Yang, Jirong Huang, and Peng Zhang. 2013. "Functional Characterization of Dihydroflavonol-4-Reductase in Anthocyanin Biosynthesis of Purple Sweet Potato Underlies the Direct Evidence of Anthocyanins Function against Abiotic Stresses." *PloS One* 8 (11): e78484.

Wennersten, Lena, and Anders Forsman. 2012. "Population-Level Consequences of Polymorphism, Plasticity and Randomized Phenotype Switching: A Review of Predictions." *Biological Reviews of the Cambridge Philosophical Society* 87 (3): 756–67.

West, A. G., T. E. Dawson, and E. C. February. 2012. "Diverse Functional Responses to Drought in a Mediterranean-type Shrubland in South Africa." *New*. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1469-8137.2012.04170.x.

West-Eberhard, Mary Jane. 2003. *Developmental Plasticity and Evolution*. Oxford University Press.

Whitehead, Andrew. 2012. "Comparative Genomics in Ecological Physiology: Toward a More Nuanced Understanding of Acclimation and Adaptation." *The Journal of Experimental Biology* 215 (6): 884–91.

Wilson, Adam M., Andrew M. Latimer, and John A. Silander. 2015. "Climatic Controls on Ecosystem Resilience: Postfire Regeneration in the Cape Floristic Region of South Africa." *Proceedings of the National Academy of Sciences* 112 (29): 9058–63.

Young, Derek J. N., Jens T. Stevens, J. Mason Earles, Jeffrey Moore, Adam Ellis, Amy

L. Jirka, and Andrew M. Latimer. 2017. “Long-Term Climate and Competition Explain Forest Mortality Patterns under Extreme Drought.” *Ecology Letters* 20 (1): 78–86.

Zeng, Xue-Qin, Wah Soon Chow, Li-Juan Su, Xin-Xiang Peng, and Chang-Lian Peng. 2010. “Protective Effect of Supplemental Anthocyanins on Arabidopsis Leaves under High Light.” *Physiologia Plantarum* 138 (2): 215–25.

Züst, Tobias, Sergio Rasmann, and Anurag A. Agrawal. 2015. “Growth--Defense Tradeoffs for Two Major Anti-Herbivore Traits of the Common Milkweed Asclepias Syriaca.” *Oikos* 124 (10): 1404–15.

## Data Accessibility

All sequencing data will be made available at NCBI SRA. Bioinformatics and R scripts will be made available upon acceptance of the manuscript on Akman’s github. Climate data, phenotypic measurement and gene expression counts will be made available in Dryad.

## Author contributions

Akman, Carlson and Latimer designed the research. Akman and Carlson performed the dry down experiments. Akman prepared sequencing libraries and performed gene expression analyses. Akman, Carlson and Latimer performed climate association analyses and wrote the manuscript.

## FIGURES

Fig 1. Map of CFR and locations of sampling sites (A); Principal component analysis for the climatic variables used in this study

Fig 2. Multi-dimensional scaling (MDS) plots for gene expression patterns at day 6 (A) and day 12 (B); principal component analysis of measured traits at day 6 (C) and day 12 (D) into the drought treatment.

Fig 3. Number of differentially expressed genes for the 8 populations for after 6 and 12 days of the drought treatment (A). Note that population A is not sampled at day 12, and population G does not have any differentially expressed genes between the treatments at day 6. Number of differentially expressed genes among populations under control and drought conditions for day 6 and 12 separately (B). For example, the intersecting ellipse between Day6 control and DAY6 drought indicates number of genes that are differentially expressed among populations, regardless of the treatment.

Fig 4. Heatmap of standardized regression coefficients for climate variables as predictors and traits and gene networks (GN) as response variables for measurements (A) on day 6 or (B) day 12. For variables with significant interaction effects, each treatment was analyzed separately to estimate slopes and test their significance within treatments. Solid boxes indicate a significant association and the box is divided in cases where there is a significant treatment and climate effect, indicating that the climate association was significant only in one of the treatments. The upper half of the box represents the watered treatment and the lower half represents the dry treatment. Stars indicate significant correlations for either of the two treatments.

Fig 5. Response curves for the studied 8 populations under well-watered and drought conditions and the associations between plasticity and climate variables for growth (A), leaf area (B) and gene network 13 (C).

Fig 6. Mean annual precipitation (A) and mean annual temperature (B) averaged across the 8 population sites between 1901-2016.

## TABLES

Table 1. Results of linear mixed models testing trait differences of *Protea repens* seedlings at 6 and 12 days into the dry down experiment. The model compares values between watered and unwatered treatments as well as among 8 wild-sourced populations and the population by treatment interaction. Significant values are bolded.

Table 2. Results of linear mixed models testing for differences in growth and carbohydrate storage between watered and unwatered *Protea repens* seedlings and among 8 populations at the end of the dry-down experiment (day 12). Significant values are bolded.

Table 3. Results of linear mixed models comparing eigengene values of the gene networks (GN0-GN13) at 6 and 12 days into the dry down experiment for watered and unwatered plants of *P. repens*. Significant values are bolded.

Table 4. Significant effects from mixed models testing associations between climate variables and plasticity measures, for traits and eigengenes of gene networks (GN) on day 6 and day 12.

**SUPPLEMENTARY INFORMATION**

Table S1. Population source site climatic information

Table S2. Correlations between climatic variables

Table S3. Maternal lines used in the experiment, with sample sizes for each maternal line in the two treatments (drought and watered)

Table S4. WGCNA GO categories and highest gene network membership genes

Table S5. Up and down-regulated genes for all the populations under drought

Table S6. Gene network and trait correlations

Table S7. Results of linear mixed effect models for climate, treatment and climate x treatment effects for traits.

Table S8. Results of linear mixed effect models for climate, treatment and climate x treatment effects for gene networks.

Figure S1. Sampling scheme

Figure S2. WGCNA sample clustering

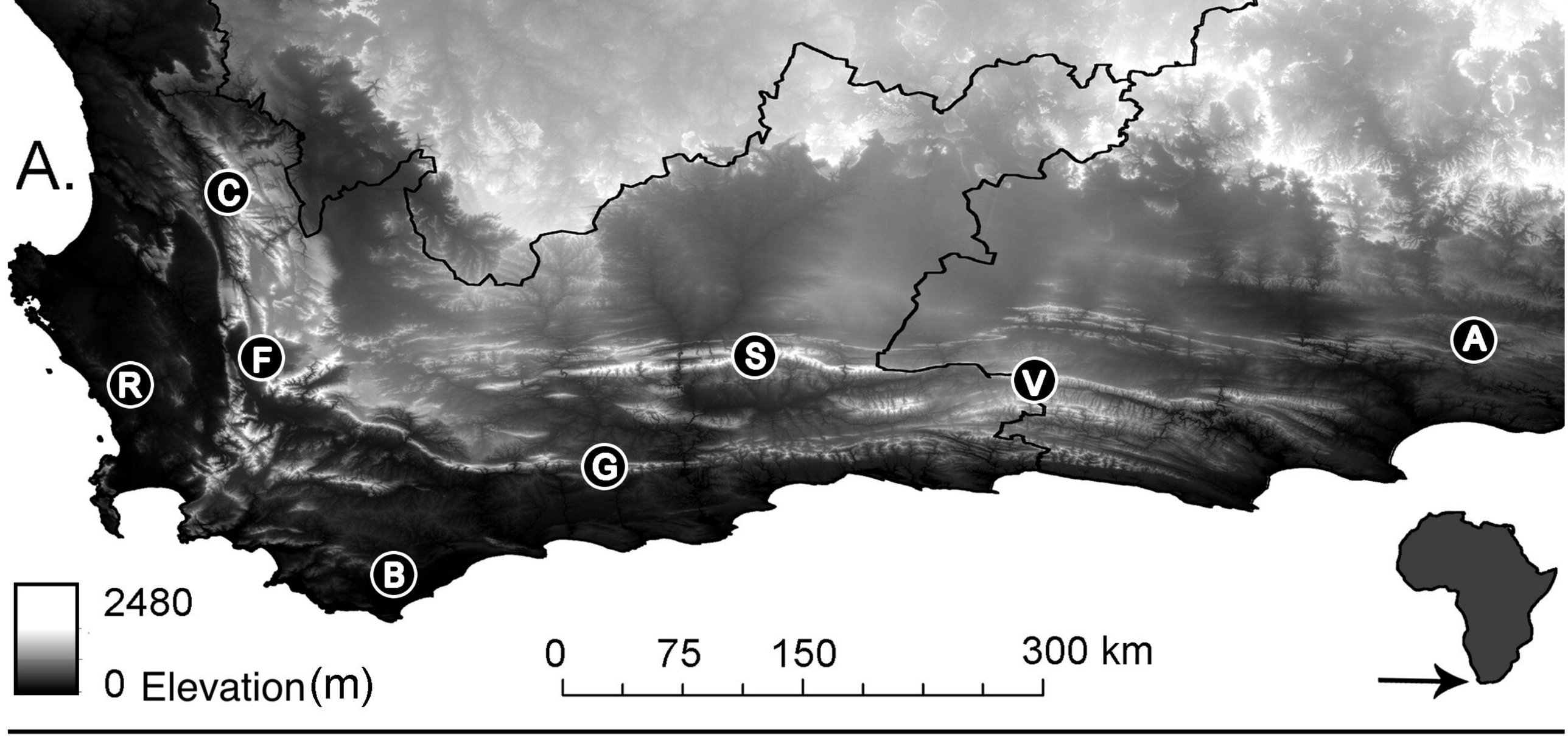
Figure S3. Functional traits response curves

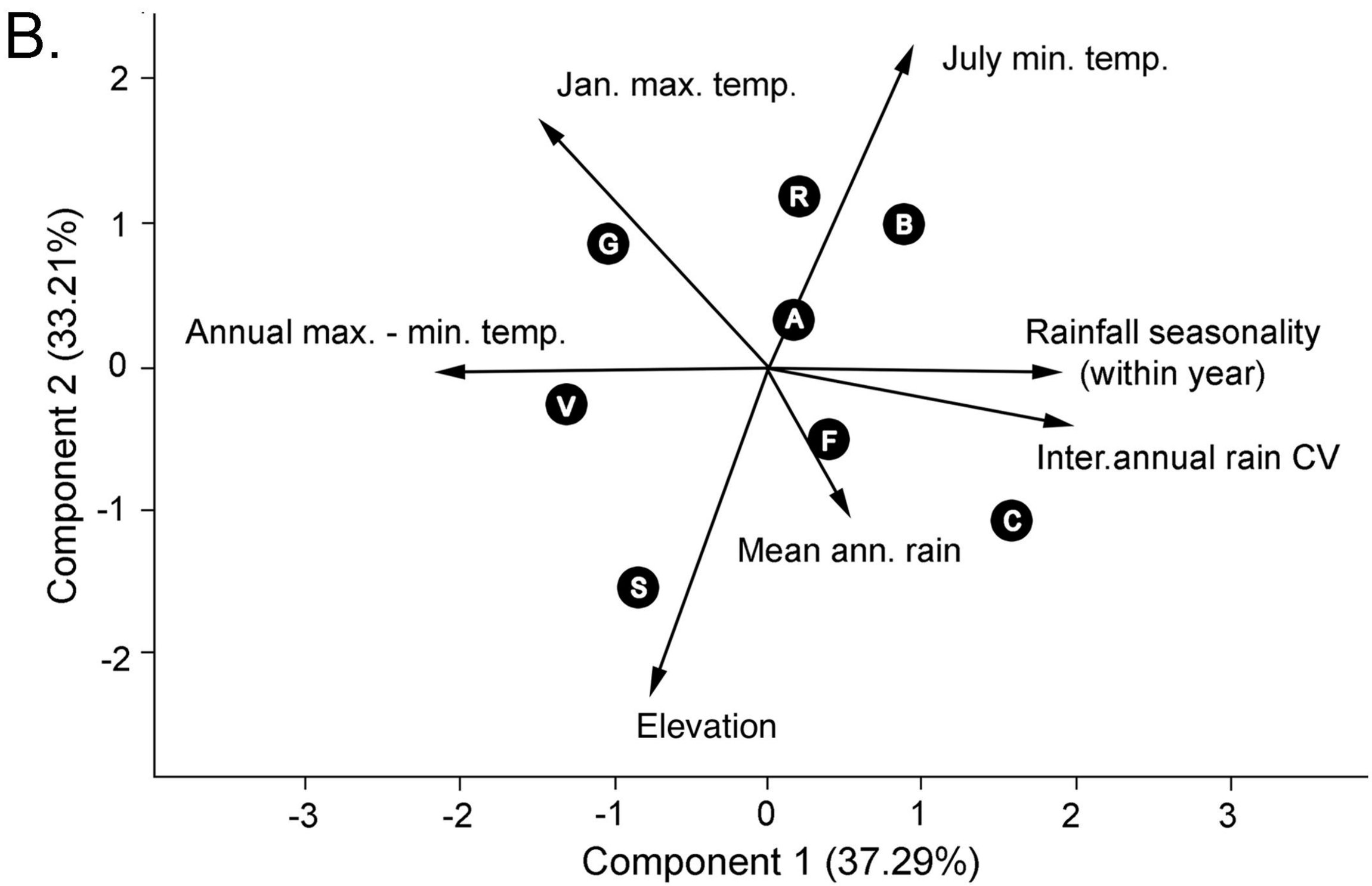
Figure S4. Gene network response curves

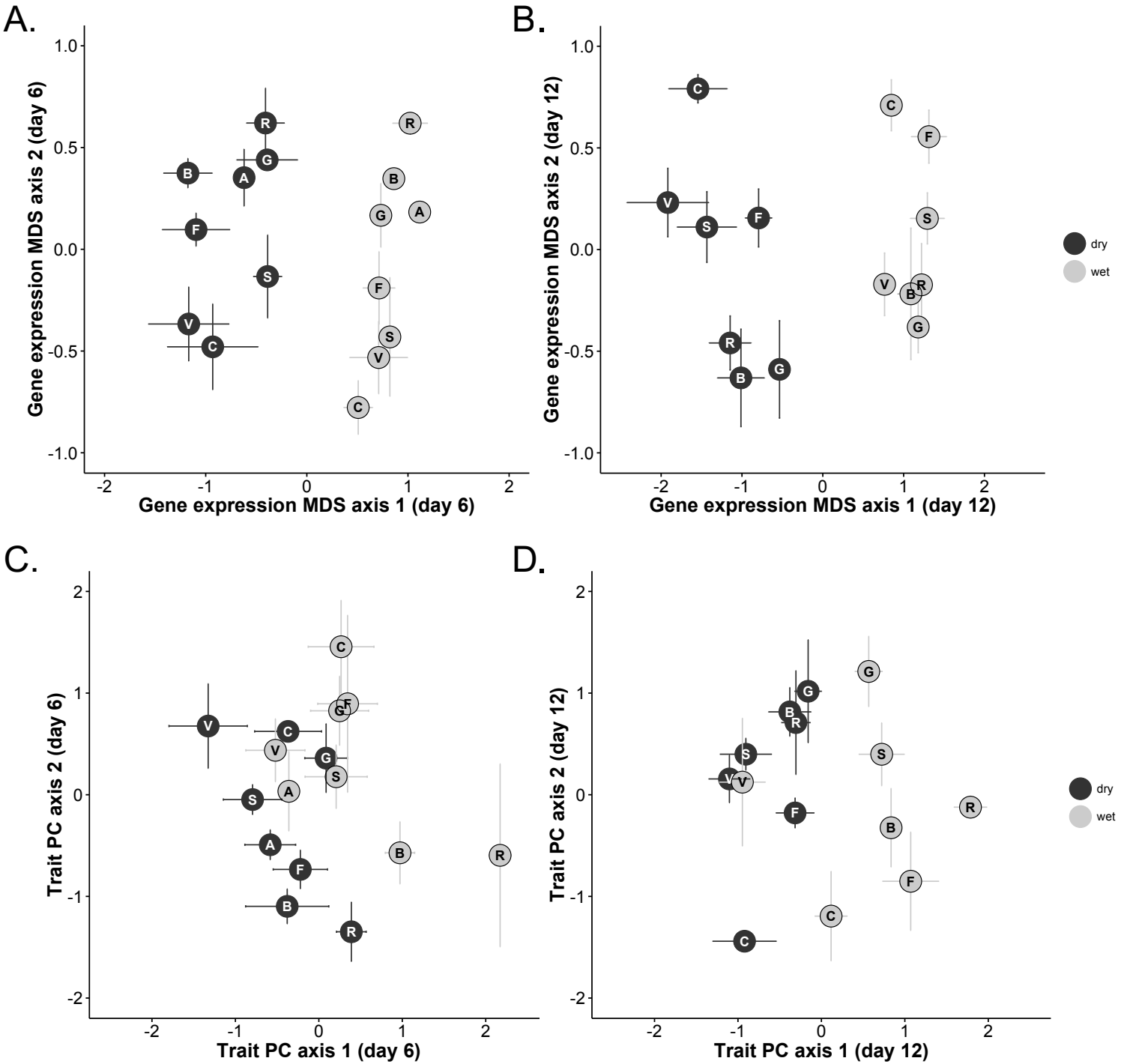
Figure S5. Performance traits response curves

Figure S6. MDS plot with all samples for day 6 and day 12

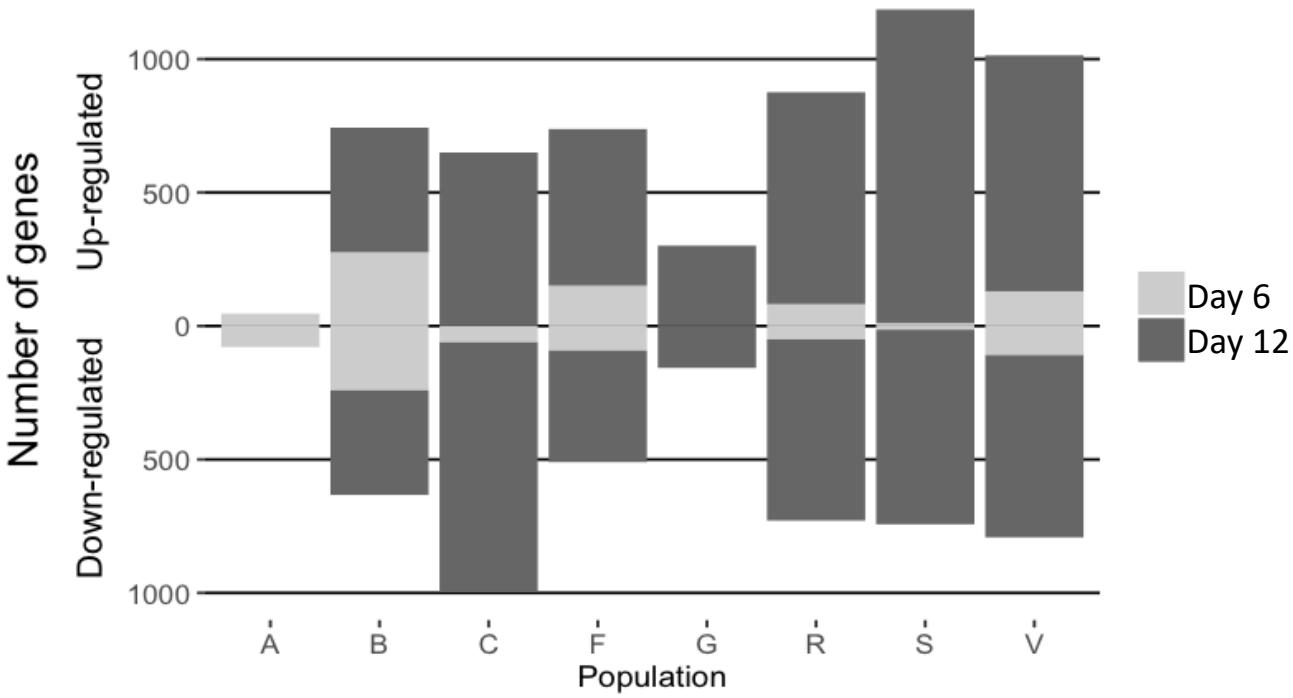
Supplementary Materials and Methods


A.
C
F
R
S
V
A
G
B
2480
0 Elevation (m)
0
75
150
300 km

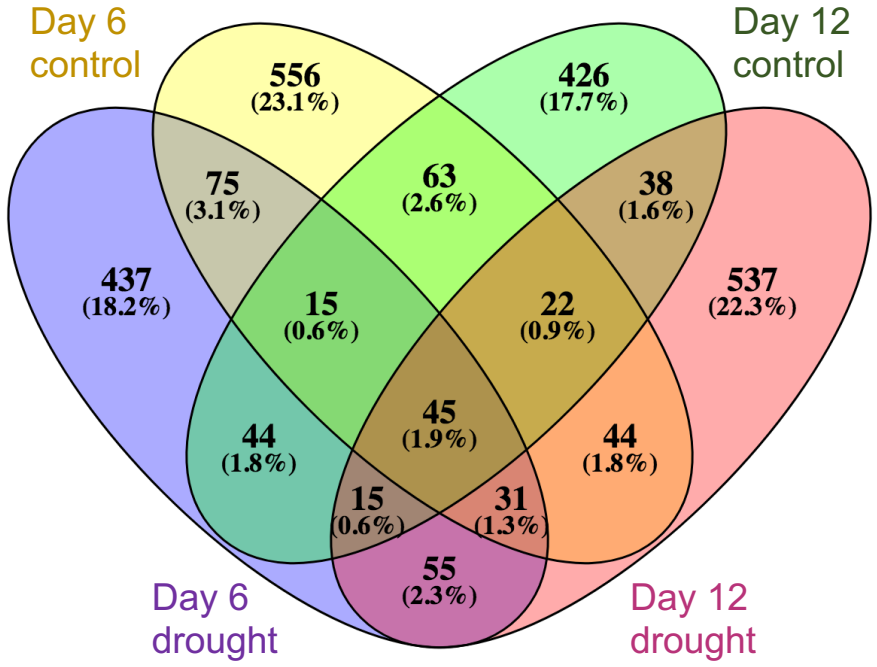

B.
Jan. max. temp.
July min. temp.
Annual max. - min. temp.
Rainfall seasonality
(within year)
Inter.annual rain CV
Mean ann. rain
Elevation
R
B
G
A
V
F
C
S
Component 2 (33.21%)
Component 1 (37.29%)
2
1
0
-1
-2
-3
-2
-1
0
1
2
3

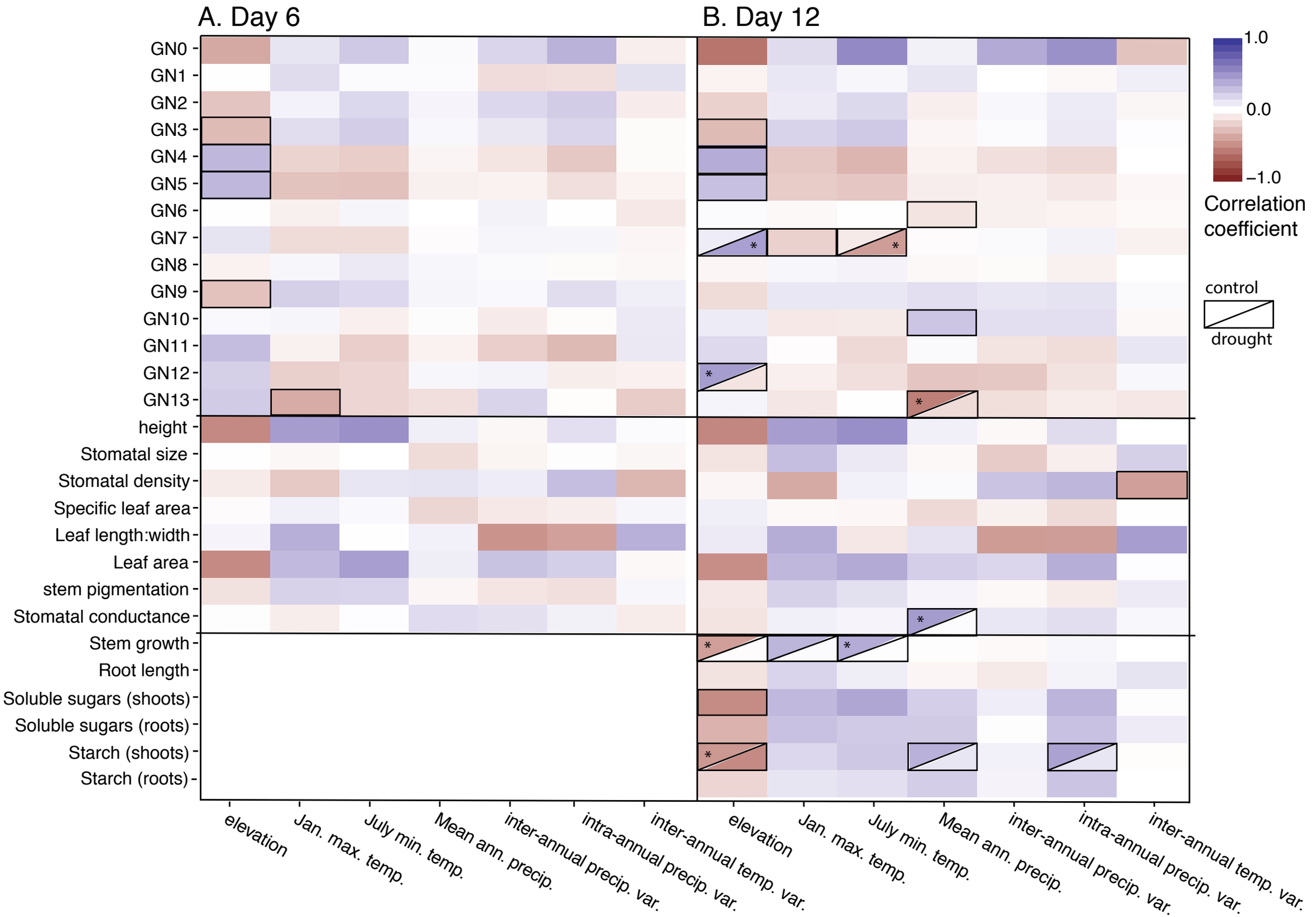

A.
B.
C.
D.
Gene expression MDS axis 2 (day 6)
Gene expression MDS axis 1 (day 6)
Gene expression MDS axis 2 (day 12)
Gene expression MDS axis 1 (day 12)
Trait PC axis 2 (day 6)
Trait PC axis 1 (day 6)
Trait PC axis 2 (day 12)
Trait PC axis 1 (day 12)
dry
wet

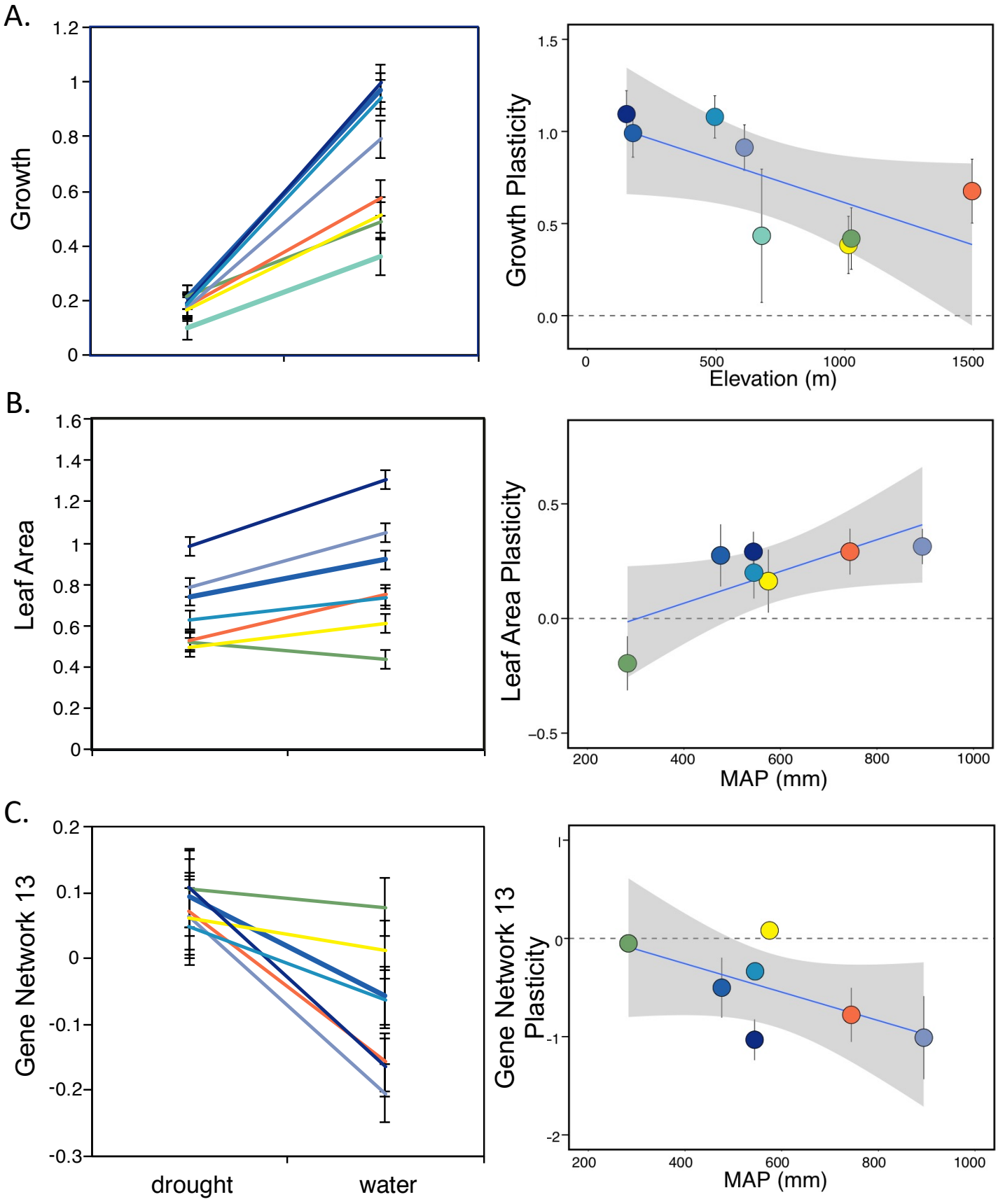
A.

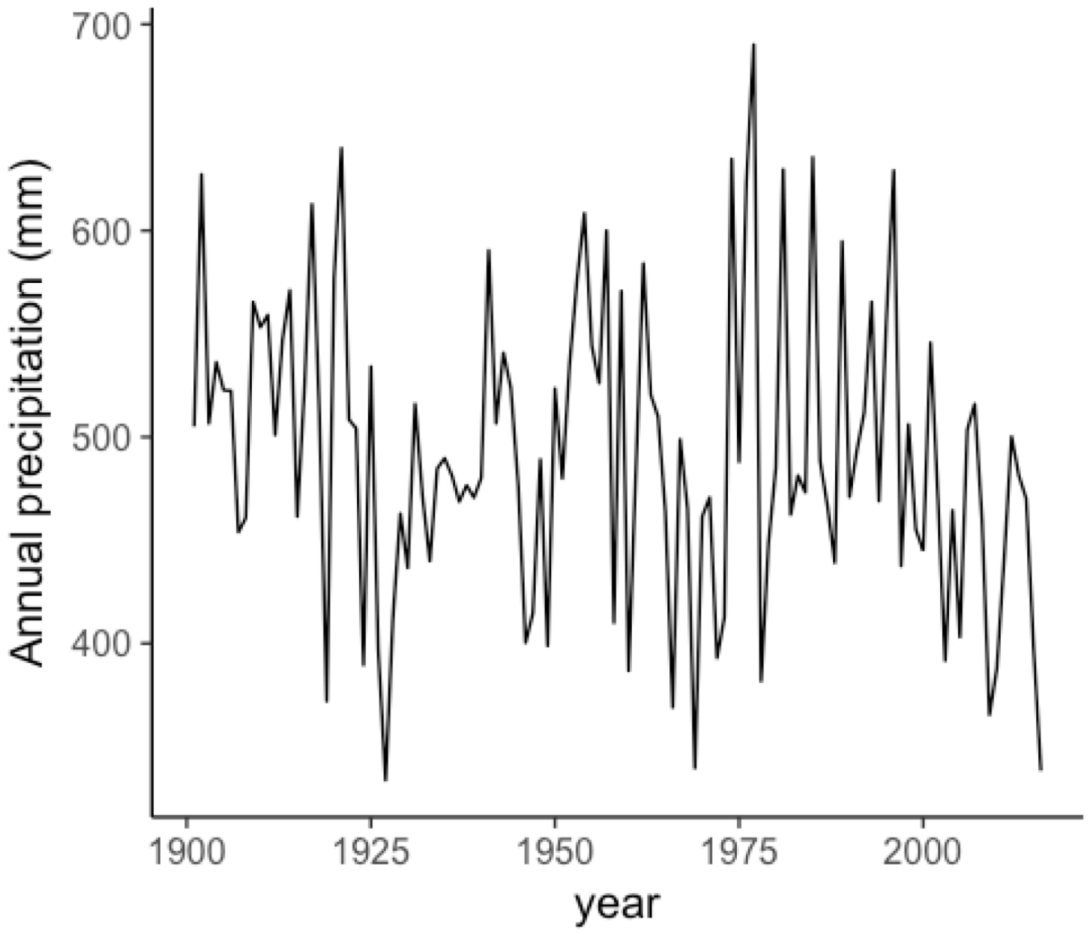

Number of genes
Up-regulated
Down-regulated
1000
500
0
500
1000
A
B
C
F
G
R
S
V
Population
Day 6
Day 12

B.


Day 6 control
Day 12 control
Day 6 drought
Day 12 drought
556 (23.1%)
426 (17.7%)
437 (18.2%)
537 (22.3%)
75 (3.1%)
63 (2.6%)
38 (1.6%)
15 (0.6%)
22 (0.9%)
45 (1.9%)
44 (1.8%)
44 (1.8%)
15 (0.6%)
31 (1.3%)
55 (2.3%)


A. Day 6
B. Day 12
GN0
GN1
GN2
GN3
GN4
GN5
GN6
GN7
GN8
GN9
GN10
GN11
GN12
GN13
height
Stomatal size
Stomatal density
Specific leaf area
Leaf length:width
Leaf area
stem pigmentation
Stomatal conductance
Stem growth
Root length
Soluble sugars (shoots)
Soluble sugars (roots)
Starch (shoots)
Starch (roots)
elevation
Jan. max. temp.
July min. temp.
Mean ann. precip.
inter-annual precip. var.
intra-annual precip. var.
inter-annual temp. var.
1.0
0.0
−1.0
Correlation coefficient
control
drought


A.
Growth
1.2
1
0.8
0.6
0.4
0.2
0
Growth Plasticity
1.5
1.0
0.5
0.0
0
500
1000
1500
Elevation (m)
B.
Leaf Area
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Leaf Area Plasticity
0.5
0.0
−0.5
200
400
600
800
1000
MAP (mm)
C.
Gene Network 13
0.2
0.1
0
-0.1
-0.2
-0.3
drought
water
Gene Network 13 Plasticity
0
-1
-2
200
400
600
800
1000
MAP (mm)


Annual precipitation (mm)
700
600
500
400
1900
1925
1950
1975
2000
year

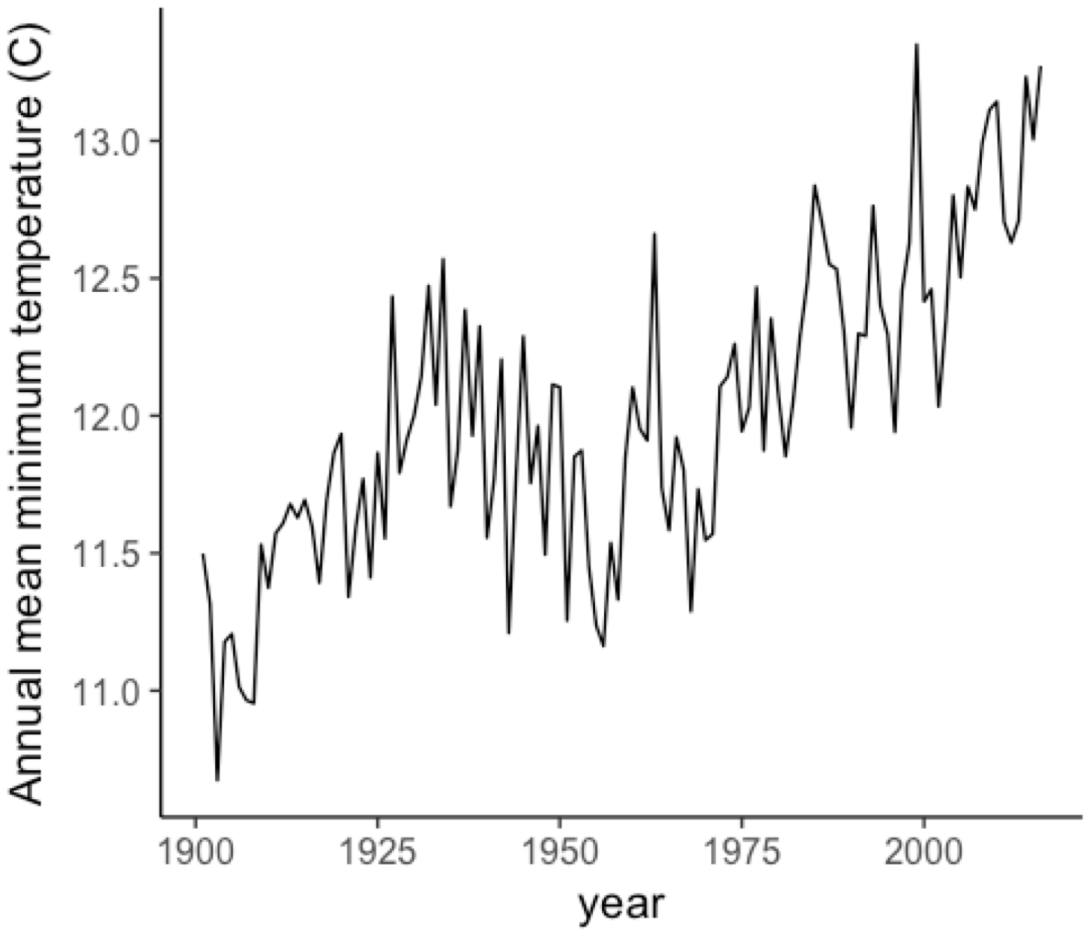

Annual mean minimum temperature (C)
13.0
12.5
12.0
11.5
11.0
1900
1925
1950
1975
2000
year

**Table 1**. Results of linear mixed models testing functional trait and performance differences of *Protea repens* seedlings at 6 and 12 days into the dry down experiment. The model compares values between watered and unwatered treatments as well as among 8 wild-sourced populations and the population by treatment interaction. Significant values are bolded.

| Response variable | Fixed effects | *day 6* | | | | *day 12* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | df | Chi-sq | P-value | q-val | df | Chi-sq | p-value | q-value |
| Leaf area ($cm^2$) | Population | 7 | 49.91 | **<0.0001** | **<0.0001** | 6 | 69.16 | **<0.0001** | **<0.0001** |
| | Treatment | 1 | 0.17 | 0.678 | 0.881 | 1 | 23.39 | **<0.0001** | **<0.0001** |
| | Pop. × Trt. | 7 | 8.61 | 0.282 | 0.423 | 6 | 13.3 | 0.039 | 0.050 |
| SLA ($cm^2$/g) Log transform | Population | 7 | 5.15 | 0.642 | 0.881 | **6** | **15.35** | **0.018** | **0.027** |
| | Treatment | **1** | **12.13** | **0.0005** | **0.002** | 1 | 73.14 | **<0.0001** | **<0.0001** |
| | Pop. × Trt. | **7** | **19.23** | **0.008** | **0.027** | **6** | **16.71** | **0.010** | **0.017** |
| LWR | Population | 7 | 67.48 | **<0.0001** | **<0.0001** | 6 | 78.55 | **<0.0001** | **<0.0001** |
| | Treatment | 1 | 0.06 | 0.814 | 0.881 | 1 | 1.86 | 0.173 | 0.210 |
| | Pop. × Trt. | 7 | 3.47 | 0.839 | 0.881 | 6 | 1.52 | 0.958 | 0.958 |
| Stomatal density | Population | **7** | **17.46** | **0.015** | **0.040** | **6** | **29.73** | **<0.0001** | **0.0001** |
| | Treatment | 1 | 3.07 | 0.080 | 0.192 | **1** | **12.95** | **0.0003** | **0.0007** |
| | Pop. × Trt. | 7 | 9.56 | 0.215 | 0.369 | 6 | 3.59 | 0.732 | 0.765 |
| Stomatal pore size (um) | Population | 7 | 3.87 | 0.795 | 0.881 | **6** | **21.92** | **0.001** | **0.002** |
| | Treatment | 1 | 0.04 | 0.844 | 0.881 | **1** | **27.68** | **<0.0001** | **<0.0001** |
| | Pop. × Trt. | 7 | 3.05 | 0.881 | 0.881 | **6** | **14.27** | **0.027** | **0.038** |
| Stem pigmentation | Population | 7 | 34.13 | **<0.0001** | **<0.0001** | 7 | 38.51 | **<0.0001** | **<0.0001** |
| | Treatment | 1 | 1.85 | 0.174 | 0.336 | 1 | 0.42 | 0.515 | 0.579 |
| | Pop. × Trt. | 7 | 10.12 | 0.182 | 0.336 | 7 | 5.71 | 0.574 | 0.630 |
| Stem height (cm) | Population | **7** | **105.34** | **<0.0001** | **<0.0001** | 7 | 121.24 | **<0.0001** | **<0.0001** |
| | Treatment | **1** | **5.9** | **0.015** | **0.040** | 1 | 49.4 | **<0.0001** | **<0.0001** |
| | Pop. × Trt. | 7 | 4.1 | 0.768 | 0.881 | 7 | 6.86 | 0.443 | 0.511 |
| Stomatal conductance | Population | 7 | 0 | 0.254 | 0.406 | **6** | **17.52** | **0.008** | **0.014** |
| | Treatment | **1** | **0** | **<0.0001** | **<0.0001** | **1** | **104.7** | **<0.0001** | **<0.0001** |
| | Pop. × Trt. | 7 | 0 | 0.148 | 0.323 | **6** | **23.43** | **0.0007** | **0.001** |

**Table 2**. Results of linear mixed models testing for differences in growth, carbohydrate storage, root length and pigment accumulation between watered and unwatered *Protea repens* seedlings and among 8 populations at the end of the dry-down experiment (day 12). Significant values are bolded.

| Response variable | Fixed effects | df | Chi-sq | p-val | q-val |
|---|---|---|---|---|---|
| Soluble sugars mass (g) belowground | Population | **7** | **55.57** | **<0.0001** | **<0.0001** |
| | Treatment | **1** | **6.33** | **0.012** | **0.019** |
| | Pop. × Trt. | **7** | **17.52** | **0.014** | **0.022** |
| Soluble sugars mass (g) aboveground | Population | **7** | **80.08** | **<0.0001** | **<0.0001** |
| | Treatment | **1** | **25.69** | **<0.0001** | **<0.0001** |
| | Pop. × Trt. | **7** | **24.27** | **0.001** | **0.002** |
| Starch mass (g) belowground | Population | **7** | **21.06** | **0.004** | **0.008** |
| | Treatment | 1 | 0.10 | 0.748 | 0.765 |
| | Pop. × Trt. | 7 | 4.57 | 0.712 | 0.763 |
| Starch mass (g) aboveground | Population | **7** | **57.40** | **<0.0001** | **<0.0001** |
| | Treatment | **1** | **60.10** | **<0.0001** | **<0.0001** |
| | Pop. × Trt. | **7** | **100.76** | **<0.0001** | **<0.0001** |
| Growth rate (height on Day 12-6) | Population | **7** | **34.85** | **<0.0001** | **<0.0001** |
| | Treatment | **1** | **194.34** | **<0.0001** | **<0.0001** |
| | Pop. × Trt. | **7** | **26.33** | **0.0004** | **0.0009** |
| Root length (mm) | Population | 6 | 9.36 | 0.154 | 0.192 |
| | Treatment | **1** | **6.66** | **0.010** | **0.017** |
| | Pop. × Trt. | 6 | 6.3 | 0.391 | 0.463 |
| Stem pigment accumulation | Population | **7** | **15.47** | **0.031** | **0.041** |
| | Treatment | **1** | **5.08** | **0.024** | **0.035** |
| | Pop. × Trt. | **7** | **15.58** | **0.029** | **0.040** |

**Table 3**. Results of linear mixed models comparing eigengene values (GN0-GN13) at 6 and 12 days into the dry down experiment for watered and unwatered plants of *P. repens*. Significant values are bolded.

| | Fixed effects | *Fixed effects at 6 days* | | | | *Fixed effects at 12 days* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | df | Chi-sq | p-value | q-val | ddf | Chi-sq | p-value | q-val |
| GN0 | Population | **7** | **24.89** | **0.0008** | **0.0042** | **6** | **80.38** | **<0.0001** | **<0.0001** |
| | Treatment | 1 | 2.05 | 0.152 | 0.281 | 1 | 0.77 | 0.380 | 0.479 |
| | Pop. × Trt. | 7 | 5.72 | 0.573 | 0.655 | 6 | 8.51 | 0.203 | 0.294 |
| GN1 | Population | **7** | **18.59** | **0.010** | **0.042** | **6** | **16.49** | **0.011** | **0.026** |
| | Treatment | **1** | **91.40** | **<0.0001** | **<0.0001** | **1** | **123.77** | **<0.0001** | **<0.0001** |
| | Pop. × Trt. | 7 | 11.30 | 0.126 | 0.252 | 6 | 6.71 | 0.348 | 0.457 |
| GN2 | Population | 7 | 8.91 | 0.259 | 0.363 | 6 | 13.87 | 0.031 | 0.0566 |
| | Treatment | **1** | **13.01** | **0.0003** | **0.0018** | **1** | **50.11** | **<0.0001** | **<0.0001** |
| | Pop. × Trt. | 7 | 9.65 | 0.210 | 0.351 | 6 | 3.22 | 0.781 | 0.841 |
| GN3 | Population | 7 | 8.56 | 0.286 | 0.387 | **6** | **18.70** | **0.005** | **0.013** |
| | Treatment | 1 | 0.07 | 0.796 | 0.836 | **1** | **9.88** | **0.00167** | **0.005** |
| | Pop. × Trt. | 7 | 9.30 | 0.232 | 0.351 | 6 | 1.47 | 0.962 | 0.962 |
| GN4 | Population | 7 | 16.16 | 0.024 | 0.078 | **6** | **36.29** | **<0.0001** | **<0.0001** |
| | Treatment | **1** | **7.52** | **0.006** | **0.028** | **1** | **75.50** | **<0.0001** | **<0.0001** |
| | Pop. × Trt. | 7 | 6.04 | 0.536 | 0.643 | 6 | 3.80 | 0.704 | 0.799 |
| GN5 | Population | 7 | 12.91 | 0.074 | 0.177 | **6** | **26.58** | **0.0002** | **0.0007** |
| | Treatment | **1** | **13.37** | **0.0003** | **0.0018** | **1** | **68.66** | **<0.0001** | **<0.0001** |
| | Pop. × Trt. | 7 | 9.32 | 0.231 | 0.351 | 6 | 2.90 | 0.821 | 0.841 |
| GN6 | Population | 7 | 12.84 | 0.076 | 0.177 | 6 | 11.76 | 0.067 | 0.113 |
| | Treatment | **1** | **56.14** | **<0.0001** | **<0.0001** | **1** | **107.13** | **<0.0001** | **<0.0001** |
| | Pop. × Trt. | 7 | 9.00 | 0.253 | 0.363 | 6 | 7.72 | 0.260 | 0.352 |
| GN7 | Population | 7 | 15.34 | 0.032 | 0.096 | **6** | **19.99** | **0.003** | **0.008** |
| | Treatment | **1** | **67.46** | **<0.0001** | **<0.0001** | **1** | **86.20** | **<0.0001** | **<0.0001** |
| | Pop. × Trt. | 7 | 10.67 | 0.154 | 0.281 | **6** | **14.79** | **0.022** | **0.049** |
| GN8 | Population | 7 | 15.11 | 0.035 | 0.098 | 6 | 3.69 | 0.719 | 0.799 |
| | Treatment | 1 | 0.65 | 0.420 | 0.540 | 1 | 1.49 | 0.222 | 0.311 |
| | Pop. × Trt. | 7 | 9.96 | 0.191 | 0.334 | 6 | 4.10 | 0.663 | 0.796 |
| GN9 | Population | 7 | 12.56 | 0.084 | 0.186 | **6** | **23.63** | **0.0006** | **0.0019** |
| | Treatment | **1** | **26.83** | **<0.0001** | **<0.0001** | **1** | **102.74** | **<0.0001** | **<0.0001** |
| | Pop. × Trt. | 7 | 6.21 | 0.515 | 0.636 | 6 | 13.97 | 0.030 | 0.057 |
| GN10 | Population | 7 | 1.76 | 0.972 | 0.972 | 6 | 10.58 | 0.102 | 0.165 |
| | Treatment | 1 | 0.04 | 0.840 | 0.860 | 1 | 0.13 | 0.723 | 0.799 |
| | Pop. × Trt. | 7 | 5.46 | 0.604 | 0.655 | 6 | 14.12 | 0.028 | 0.056 |
| GN11 | Population | 7 | 13.34 | 0.064 | 0.168 | 6 | 12.34 | 0.055 | 0.096 |
| | Treatment | **1** | **31.91** | **<0.0001** | **<0.0001** | **1** | **64.04** | **<0.0001** | **<0.0001** |
| | Pop. × Trt. | 7 | 9.27 | 0.234 | 0.351 | 6 | 6.33 | 0.388 | 0.479 |
| GN12 | Population | 7 | 7.05 | 0.424 | 0.540 | 6 | 9.38 | 0.154 | 0.231 |
| | Treatment | 1 | 2.75 | 0.097 | 0.204 | 1 | 0.06 | 0.808 | 0.841 |
| | Pop. × Trt. | 7 | 5.53 | 0.596 | 0.655 | 6 | 9.41 | 0.152 | 0.231 |
| GN13 | Population | 7 | 16.84 | 0.018 | 0.063 | 6 | 14.14 | 0.028 | 0.056 |
| | Treatment | 1 | 0.26 | 0.608 | 0.655 | **1** | **30.16** | **<0.0001** | **<0.0001** |
| | Pop. × Trt. | **7** | **17.85** | **0.013** | **0.0496** | **6** | **16.57** | **0.011** | **0.026** |

**Table 4.** Significant effects from mixed models testing associations between climate variables and plasticity measures, for traits and eigengenes on day 6 and day 12.

| Day | Plasticity measure | Climate variable | p-value | Plasticity high in pops. from: |
|---|---|---|---|---|
| Day 6 | GN5 | Elevation | 0.015 | lower elevation |
| Day 6 | Stomatal density | Mean annual precip. | 0.008 | wetter sites |
| Day 12 | GN10 | Mean annual precip. | 0.035 | wettest and driest sites |
| Day 12 | GN13 | Mean annual precip. | 0.021 | wetter sites |
| Day 12 | Growth | Elevation | 0.014 | lower elevation |
| Day 12 | Growth | Summer max. temp. | 0.016 | warmer sites |
| Day 12 | Leaf area | Mean annual precip. | 0.019 | wetter sites |
| Day 12 | Pigment accumulation | Temperature range | 0.039 | higher heterogeneity |
| Day 12 | Pigment accumulation | Summer max. temp. | 0.041 | coolest and warmest sites |