

1 Pre-adaptation to climate change through topography-driven evolution of  
2 traits and their plasticity

3 Running title: Drought-related trait plasticity evolution

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13 **ABSTRACT**

14 Climate change is expected to increase the level of drought stress experienced by many plant  
15 populations, yet the spatial distribution of changes in dryness remains highly uncertain. Species can,  
16 to some extent, adapt to climate uncertainty through evolving increased trait plasticity. Biodiversity  
17 conservation could capitalize on such natural variation in the ability of populations to cope with  
18 climate variability. Yet, disentangling evolution of trait means vs. trait plasticity is challenging, as it  
19 requires a sampling design with genetic replicates grown under distinct environmental conditions.  
20 Here, we applied different soil moisture treatments to clones of *Fragaria vesca* plants that were  
21 raised from seeds that were sampled in distinct mountainous topographical settings, to study  
22 adaptive trait and plasticity divergence in response to drought. We demonstrate that various fitness  
23 traits evolved along topographical gradients, including increased specific leaf area (SLA) with  
24 increasing slope, and increased growth plasticity with increasing altitude. Our results indicate that  
25 traits and their plasticity can evolve independently in response to distinct topographical stressors.  
26 We further show that trait heritability varies considerably among traits and topographical settings.  
27 Heritability of phenotypic plasticity tended to increase with altitude for all traits, with populations  
28 from high altitudes harboring more than twice the heritability for growth and SLA plasticity  
29 compared to populations from low altitudes. We conclude that (i) low altitudinal populations, which  
30 are expected to be **least** vulnerable to climate change, may only withstand limited increases in  
31 drought stress, while (ii) populations that evolved to thrive under more heterogeneous mountain  
32 conditions are pre-adapted to climate change through high plasticity and heritability. Highly  
33 heterogeneous landscapes may thus represent invaluable sources of quantitative genetic variation  
34 that could support conservation under climate change across the globe.

35

36 **Key-words:** clones, common garden, epigenetic variation, extreme climatic events, conservation,  
37 elevation, alpine ecosystems, landscape ecology

38 **1 INTRODUCTION**

39 Observed and projected changes in temperature and precipitation are destabilizing existing  
40 **interactions** between plant and animal communities and their abiotic context across the globe  
41 (Urban *et al.* 2015; Bertrand *et al.* 2016; Vázquez *et al.* 2017; Alexander *et al.* 2018). While drought  
42 stress levels will continue to change worldwide with high confidence, the regional and local spatial  
43 distribution and magnitude of these changes remains highly uncertain (IPCC 2014). This uncertainty  
44 complicates **biodiversity conservation anticipating climate change**, for example through assisted  
45 migration of genotypes pre-adapted to projected climate conditions. Yet, conservation strategies  
46 aiming to mitigate climate change impacts in the absence of accurate projections could capitalize on  
47 natural variation in the ability of populations to cope with climate variability and uncertainty.  
48 Populations that have evolved in highly heterogeneous and temporarily variable environments likely  
49 retain a higher fitness for a wide range of environmental conditions than populations that are  
50 adapted to more homogeneous and stable environments (Reed *et al.* 2010; Chevin & Lande 2011;  
51 **Chevin & Hoffmann 2017; Bonamour *et al.* 2019**).

52 Established variation in phenotypic traits that underlie drought tolerance can greatly increase the  
53 ability of populations to withstand unpredictable changes in soil moisture levels. While plastic  
54 phenotypic variation underlying drought tolerance may **support** fitness in environments **currently**  
55 featured by heterogeneous soil humidity levels, heritable phenotypic variation is required for  
56 **contemporary** evolution towards an increase in drought tolerance **and/or phenotypic plasticity for**  
57 drought tolerance (Anderson *et al.* 2011; Palacio-López *et al.* 2015; Hoffmann *et al.* 2017; Kingsolver  
58 & Buckley 2017). Genotypes that are capable of adjusting their phenotypes to changing soil moisture  
59 levels through plastic responses in particular represent a valuable yet understudied component of  
60 the evolutionary potential of natural populations in the context of climate change anticipated  
61 drought events (IPCC 2014; van Kleunen & Fischer 2005; Kingsolver & Buckley 2017; Bonamour *et al.*  
62 2019).

63 How particular environmental stressors drive evolution toward increased phenotypic plasticity in a  
64 population (i.e. increase the frequency of genotypes with enhanced levels of phenotypic plasticity) is  
65 a particularly challenging question, as answering it requires genetically identical replicates to be  
66 raised under distinct environmental settings (De Kort *et al.* 2016; Arnold *et al.* 2019). **Although quite**  
67 **some research relied on genetically similar individuals to study plasticity evolution approximately**  
68 **(e.g. Sultan 2001; Zhang *et al.* 2012; Scheepens *et al.* 2018), to our knowledge, only** one study has  
69 hitherto assessed how genetic variation in phenotypic plasticity varies across plant populations  
70 adapted to distinct climatic environments using genetically identical clones (Cooper *et al.* 2019).  
71 Transplantations of genotypic replicates from 16 populations of *Populus fremontii* to cool, warm and  
72 hot sites in the Southwestern United States revealed that genotypes originating from low latitude  
73 populations were significantly more plastic for bud set and bud flush as compared to genotypes  
74 originating from higher latitudes (Cooper *et al.* 2019). While this study provides rare evidence for  
75 natural variation in the magnitude of plastic responses to climate, the potential of natural  
76 populations to evolve this plasticity remains elusive. Elucidating the natural **occurrence and**  
77 **heritability** of trait plasticity evolution could thus shed renewed light on the ability of populations to  
78 cope with environmental changes. **Replication within genotypes across treatments** therefore  
79 represents a powerful experimental approach for (i) disentangling plastic (i.e. within-genotype) trait  
80 variation from genetic (i.e. among-genotype) trait variation, and (ii) studying heritable variation  
81 underlying trait plasticity.

82 Fine-scale environmental variation has frequently been associated with high levels of adaptive  
83 phenotypic variation. In particular, the highly heterogeneous landscapes from mountain ranges  
84 provide ample opportunity for adaptive radiation at fine spatial scales (Halbritter *et al.* 2018;  
85 Waterhouse *et al.* 2018). Moreover, high-altitudinal secondary valleys and cold air sinks typical of  
86 topographically complex landscapes designate topographical factors other than altitude as  
87 contributing determinants of temperature and soil moisture levels (Körner 2007; Günther *et al.*  
88 2016; O'Brien *et al.* 2017; Pfennigwerth *et al.* 2017). For natural populations, fine-scale phenotypic

89 variation coinciding with topographical variation could thus be key to cope with sudden  
90 environmental changes.

91 In the face of uncertain climate and soil moisture changes, the topographical complexity of  
92 mountainous landscapes may harbor an invaluable source of variation in drought resistance traits,  
93 including increased water use efficiency (e.g. through more and smaller stomata, Dittberner et al.  
94 2018), lower specific leaf area (SLA, Bansal et al. 2015) and advanced flowering (Wilczek *et al.* 2010).

95 In this regard, south-oriented slopes are assumed to be dominated by drought-resistant phenotypes,  
96 especially where strong inclinations facilitate water efflux. Populations inhabiting such challenging  
97 environments may thus evolve an enhanced ability to cope with drought stress through plastic or  
98 genetic phenotypic responses.

99 Here, we study topography-driven adaptive variation underlying drought stress-related traits and  
100 their plasticity, using genetic replicates of natural *Fragaria vesca* populations. A total of ca. 12  
101 genotypes × 11 mountainous populations × 3 soil moisture treatments × 4 clones was monitored in a  
102 common garden for growth, vegetative propagation, SLA, stomatal density and flowering rate. Traits  
103 and their plasticity (quantified as relative distance plasticity indices (RDPIs)) were subsequently  
104 modeled in relation to slope, aspect, topographical position index, absolute altitude, and relative  
105 altitude of the population sampling locations. Heritability was calculated for each trait and for each  
106 RDPI. This methodological strategy allowed us to tackle the following research questions: (i) how  
107 much adaptive variation do mountainous populations harbor for traits (and their plasticity) related  
108 to drought resistance? (ii) are topographical variables other than altitude strong determinants of  
109 adaptive trait variation, and (iii) does trait plasticity, measured as RDPI, have a strong heritable  
110 component? In parallel, we hypothesized that genotypes originating from low absolute altitude (~  
111 high temperatures), high relative altitude and high topographic position index (~ high water efflux)  
112 and steep, southerly oriented slopes (~ high water efflux and evapotranspiration) have evolved traits  
113 that allow to cope with drought stress.

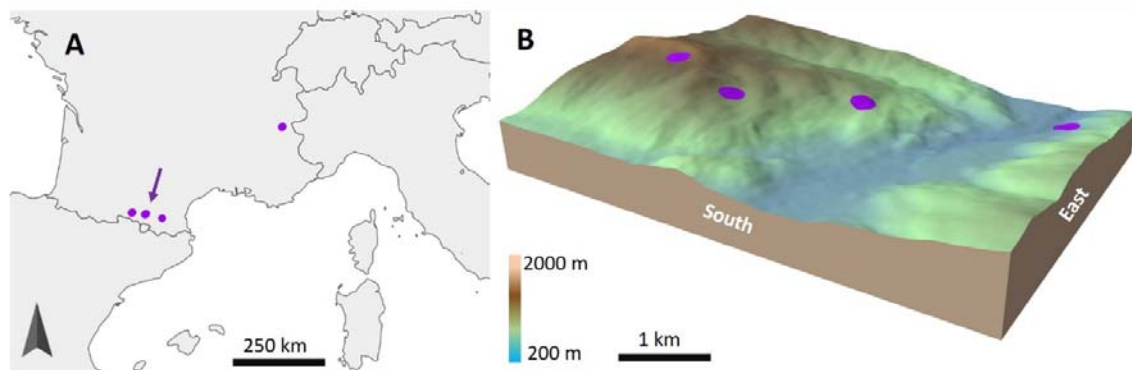
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## 115 2 METHODS

### 116 2.1 Study species and sampling

117 *Fragaria vesca* L., also known as the woodland strawberry, is a self-compatible, insect-pollinated  
118 perennial stoloniferous herb with a circumglobal temperate distribution. It is typically found along  
119 mixed and broadleaf forest edges, gaps and paths. Seeds from a total of eleven natural populations  
120 were sampled in the French Alps and Pyrenees, covering various topographical conditions. The  
121 populations were spatially clustered in four meta-populations (Fig. 1, Table 1), and distances  
122 between populations within a meta-population ranged from 550 m to 3700 m. **This spatial clustering**  
123 **can be assumed to result in substantially stronger genetic relatedness within than between meta-**  
124 **populations.** To minimize the risk of sampling the same genet twice, distance among samples was  
125 **maximized within locations.**

126



127 **Fig. 1.** Sampling locations (A) and topographical zoom on the sampling location with arrow (B). See Tables 1  
128 and S1 for topographical information on all populations.

129 **Table 1.** Sampling location features. NHeight (normalized height, 0-100%) reflects height relative to the  
130 nearest valley, and TPI (topographic position index, unitless) describes the curve in the landscape with TPI > 0  
131 near ridges and TPI < 0 near valleys (see 2.4 Topography). **Altitude is expressed as m a.s.l., and slope in**  
132 **percentages.** Grave is the Alpine meta-population; all other meta-populations are Pyrenean.

Pop	Meta-pop	Altitude	Aspect	Slope	NHeight	TPI
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<b>AT</b>	Sourroque	750	7.59	13.80	0.422	1.000
<b>BV</b>	Montoulieu	1261	143.53	13.68	0.825	2.875
<b>F</b>	Grave	1409	11.31	29.36	0.194	-0.625
<b>G</b>	Grave	1471	334.29	8.95	0.075	0.125
<b>LC</b>	Sourroque	489	58.50	6.06	0.211	-1.250
<b>ML</b>	Montoulieu	466	296.56	21.39	0.126	-2.500
<b>PN</b>	Axat	909	116.11	16.18	0.186	0.000
<b>SQ</b>	Sourroque	927	52.59	23.74	0.236	0.125
<b>SX</b>	Montoulieu	840	136.08	23.29	0.729	1.375
<b>TC</b>	Montoulieu	1021	80.94	25.54	0.535	6.000
<b>TP</b>	Sourroque	1184	42.99	8.62	0.985	1.500

133

## 134 **2.2 Clonal propagation and common garden**

135 After three weeks of cold treatment (°5C), seeds were germinated *in vitro* on sterile hormone free  
136 MS (Murashige & Skoog, 1962) medium. A total of five seedlings per mother plant were transplanted  
137 onto a meristem multiplication medium (MS supplemented with 0.5 mg/L BA (Benzyladenine)),  
138 which rendered ca. five **shoot** meristems per seedling. Each of these meristems was then transferred  
139 to new medium for a second multiplication cycle, aiming to obtain at least 15 clones **per seedling**  
140 **(i.e. per genotype)**. These clones were allowed to grow and set roots on a rooting medium (MS  
141 supplemented with 1 mg/L IBA (Indole butyric acid)) until transplantation into individual pots with  
142 0.45 L standard potting soil in February 2018. To maximize survival during acclimatization in  
143 greenhouse conditions, air humidity was kept at 90% for two weeks and then slowly decreased  
144 towards **standard** greenhouse conditions (ca. 70% humidity). Multiplication success, i.e. the average  
145 within genotype proportion of replicates surviving both the *in vitro* and acclimatization procedure,  
146 varied considerably among populations (from 31.6% in BV to 82.0% in G) (Table S2). We found a  
147 relatively strong correlation between multiplication success and vegetative propagation in the  
148 greenhouse (Pearson  $r = 0.59$ , see Fig S1), indicating a natural predisposition for clonal growth. No or  
149 weak correlations were found between multiplication success and other traits (Pearson  $r < 0.20$ , Fig  
150 S1), suggesting that the *in vitro* procedure did not considerably impact our results. A total of 1416

151 plants survived the acclimatization and were monitored throughout the experiment (population  
152 mean of 142, genotype mean of 12).

153 Pots were fully randomized across the greenhouse and across soil moisture treatments to minimize  
154 effects of micro-environmental variation on relations between drought treatments and phenotypic  
155 responses. Three soil moisture treatments (dry, moist, wet), which are assumed to capture at least  
156 part of the natural variation in soil moisture, were applied using a tube system (with 42 pots per  
157 tube) that allowed controlled watering (Fig S2). For a duration of two minutes, tubes were watered  
158 once or twice a week, depending on ambient temperatures (dry treatment), every (two) day(s)  
159 (moist treatment), and every four hours (wet treatment). Soil moisture was measured at two time  
160 points to quantify soil moisture differences between treatments, through measuring pot weights  
161 and through TDR (time-domain reflectometry) measurements. This indicated clear soil moisture  
162 differences among treatments (Fig S3). Starting in April 2018, the soil moisture treatments were  
163 applied during two cycles of six weeks, with a recuperation period of two weeks in between, in  
164 which all plants received equal amounts of water (cfr. moist treatment). During a third cycle of soil  
165 moisture treatments, far-red lamps (which mimic the light conditions typically experienced during  
166 flowering of *F. vesca* under tree cover) were installed to initiate flowering (Rantanen *et al.* 2014). We  
167 applied this treatment because, except for one population (“ML”), plants did not flower under  
168 regular greenhouse conditions.

### 169 **2.3 Phenotypic traits and their plasticity**

170 A total of five phenotypic traits were measured for all plants, namely dry above-ground biomass,  
171 specific leaf area for leaves without petiole (SLA for one leaf per plant, Pérez-Harguindeguy *et al.*  
172 2013), the total number of runners produced during the second soil moisture treatment (counted  
173 and cut each week), stomatal density, and relative flowering rate (RFR) (Table S3). Stomata were  
174 counted on nail polish leaf prints using a KEYENCE light microscope at 1000 x magnification. For  
175 three replicate counts per leaf print, median stomatal counts instead of averages were used to



176 minimize the impact of counting errors. **RFR** was calculated as  $(200 - t_{ff})/200$ , with  $t_{ff}$  = time of first  
177 flowering, expressed as days after initiation of the far-red treatment. Plants that did not flower after  
178 100 days of monitoring were given a 200 score to obtain an RFR of zero. Plants originating from the  
179 “ML” population flowered prior to far-red treatment and were therefore excluded from the RFR  
180 analyses. To remove redundant variation among the different traits, a principal component analysis  
181 (PCA) was performed on the vegetative traits (above-ground biomass, SLA, number of runners and  
182 stomatal density). This resulted in three principal components (PCs) retaining 91.1% of the original  
183 variation, with PC1 (hereafter **Growth**) mainly representing above-ground biomass and number of  
184 runners (positively correlated), PC2 (hereafter **Stomata**) correlating (positively) with stomatal  
185 densities, and PC3 (hereafter **SLA**) correlating (negatively) with SLA (Table S3). PC3 was inverted so  
186 that it increases with increasing SLA, thereby facilitating interpretation.

187 The relative distance plasticity index (RDPI), which quantifies phenotypic distances among  
188 individuals exposed to different environments, has been proposed as the most appropriate plasticity  
189 index among 17 different plasticity metrics (Valladares et al. 2006). We determined RDPI for each  
190 trait (PC) and for each genotype as the absolute trait difference between all pairs of clonal replicates  
191 grown under the **three** distinct drought treatments divided by the phenotypic sum of the respective  
192 pairs (Valladares *et al.* 2006). RDPI ranges from 0 (no plasticity) to 1 (maximal plasticity), and was  
193 calculated using the R package *Plasticity* (Ameztegui 2017). This rendered the additional trait  
194 variables **Growth plasticity**, **Stomatal plasticity**, **SLA plasticity** and **RFR plasticity** (Tables S4, S5).

## 195 **2.4 Topography**

196 A total of five topographical variables were extracted for each population using QGIS 2.18, namely  
197 aspect, slope (0-100%), elevation (m a.s.l.), normalized height (altitude relative to the nearest valley,  
198 0-100%) and topographic position index (TPI). Aspect was decomposed into two independent  
199 variables describing orientation relative to the South direction (aspect\_South) and orientation  
200 relative to the East direction (aspect\_East). TPI describes the curve in the landscape, with high TPI

201 (>0) near ridges (water efflux) and low TPI (<0) near valleys (water influx). Where no curve is present  
202 in the landscape (flat terrain and constant slopes), TPI reaches zero. The elevation map used for  
203 topographical extractions was at 30-meter resolution ([gdex.cr.usgs.gov](http://gdex.cr.usgs.gov)) and re-projected to a local  
204 coordinate reference system (Lambert Sud France). To obtain orthogonal topographical variables, a  
205 PCA was performed on all original topographical variables, and resulted in three PCs representing  
206 83.6% of the variation across the original topographical variables (Table S1). The first PC (hereafter  
207 **Relative height**) was mainly shaped by TPI, normalized height and aspect\_East, with low values  
208 representing populations in valleys with eastern aspect. The second PC (hereafter **Altitude**) was  
209 positively correlated with elevation and aspect\_South, and the third PC (hereafter **Slope**) was  
210 positively correlated with slope. Note that “Altitude” reflects maximum daily temperature ranges as  
211 the south-oriented, high altitude populations receive most sun and therefore heat up considerably  
212 over the day while temperatures are low during the night as compared to low altitude populations  
213 that are north-oriented.

## 214 **2.5 Modelling of traits and trait plasticity**

215 **Growth, Stomata** and **SLA** were modeled using linear mixed models as implemented in the R  
216 package *lme4*. We accounted for genetic differentiation through the random intercept factor  
217 “Genotype”:

218 Trait ~ Relative height + Altitude + Slope + Treatment + Relative height × Treatment + Altitude ×  
219 Treatment + Slope × Treatment + Meta-population + 1 | Genotype

220 **Growth RDPI**, **Stomatal RPD** and **SLA RDPI** across soil humidity treatments, are confined between 0  
221 and 1 and were therefore modelled as beta distributions with logit link. Using the R package  
222 *glmmTMB* (Brooks *et al.* 2017), a generalized mixed model with a beta response distribution was  
223 built as follows:

224 Trait plasticity ~ Relative height + Altitude + Slope + Meta-population + 1 | Genotype

225 Where required, response variables were transformed to improved model fits (see Table S6 for  
226 model residual plots). Pearson residuals, which are preferred when response variables follow a beta  
227 distribution (Espinheira *et al.* 2008), were used for evaluation of the generalized models (Table S7).  
228 Marginal (fixed model) and conditional (full model)  $R^2$  values were computed using the R package  
229 *sjstats* (Lüdtke 2019).

230 To improve the fit of **RFR** (excess of zeros) and **RFR plasticity** (excess of ones) models, genotype  
231 averages were computed and modeled using beta regression as implemented in R package *betareg*.  
232 This package also provides a pseudo- $R^2$  value as an overall effect size for beta regressions. To correct  
233 for increased Type I error due to multiple hypothesis testing, p-values were adjusted using the false  
234 discovery rate approach implemented in the R package “*qvalue*” (Storey *et al.* 2019).

## 235 **2.6 Heritability**

236 For each treatment, heritability in the broad sense ( $H^2$ ) was calculated as  $V_G/(V_G + V_E/r)$ , with  $V_G$   
237 representing the variance between genotypes,  $V_E$  the residual variance (i.e. random environmental  
238 variation among clones), and  $r$  the number of replicates (clones) (Lynch & Walsh 1998). Variance  
239 components and  $H^2$  confidence intervals were obtained using a random effects model with the delta  
240 method implemented in the pin function of R package *sommer* (Covarrubias-Pazarán 2018). Trait  $H^2$   
241 estimates at the population level were highly inaccurate (high SE), but were provided in Table S8 for  
242 completeness. We also calculated  $H^2$  at the population level for each of the plasticity traits, and  
243 plotted plasticity  $H^2$  against topographical PCs.

244

## 245 **3 RESULTS**

246 Drought significantly reduced growth, SLA and RFR compared to moist and wet treatments, while  
247 stomatal densities were significantly higher under drought stress than under moist and wet  
248 treatments (Figs. 2 and 3A, Table 2). The magnitude of these soil moisture treatment effects  
249 depended on the topographical features associated with the sampling locations of the populations,

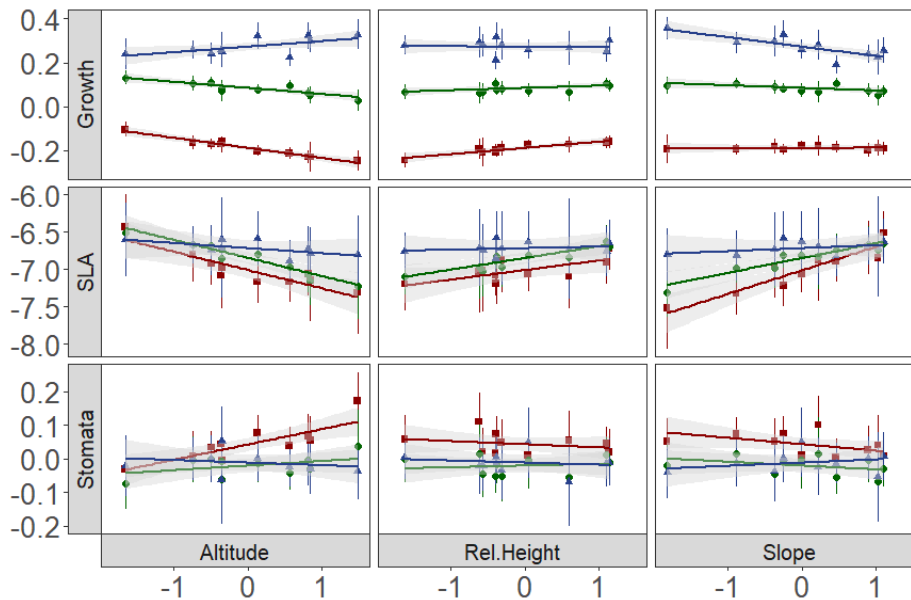
250 as demonstrated by significant interactions between treatment and at least one topographic  
 251 variable (Table 2). Specifically, populations from low altitude and from high relative height and steep  
 252 slopes experienced less growth reductions under drought stress than populations from high latitude,  
 253 low relative height and weak slopes. In addition, populations from steep slopes retained high SLA  
 254 under drought stress as opposed to populations from weak slopes (Fig. 2). The increase in stomatal  
 255 densities with drought stress was most pronounced for populations from high altitude (Fig. 2). RFR  
 256 did not vary significantly between populations from different topographical settings (Table 2).

257 **Table 2.** Significance of model terms, with \*, \*\* and \*\*\* representing FDR corrected p-values < 0.05, < 0.01  
 258 and < 0.001, respectively. R<sup>2</sup> represents R<sup>2</sup><sub>marginal</sub> for the mixed models, and pseudo-R<sup>2</sup> for the beta regressions.  
 259 See Tables S6 and S7 for detailed test statistics. NA = not applicable, NS = not significant (p>0.05).

	Treatment	Rel.Height <sup>(xTreatment)</sup>	Altitude <sup>(xTreatment)</sup>	Slope <sup>(xTreatment)</sup>	R <sup>2</sup>
Growth	***	*(*)	**(***)	NS <sup>(***)</sup>	0.66
Stomata	***	NS <sup>(NS)</sup>	*(*)	NS <sup>(NS)</sup>	0.06
SLA	***	NS <sup>(NS)</sup>	*(NS)	*(**)	0.08
RFR	**	NS <sup>(NS)</sup>	NS <sup>(NS)</sup>	NS <sup>(NS)</sup>	0.34
Growth plasticity	NA	*	**	NS	0.15
Stomatal plasticity	NA	NS	NS	NS	0.08
SLA plasticity	NA	NS	NS	NS	0.01
RFR plasticity	NA	NS	NS	NS	0.36

260

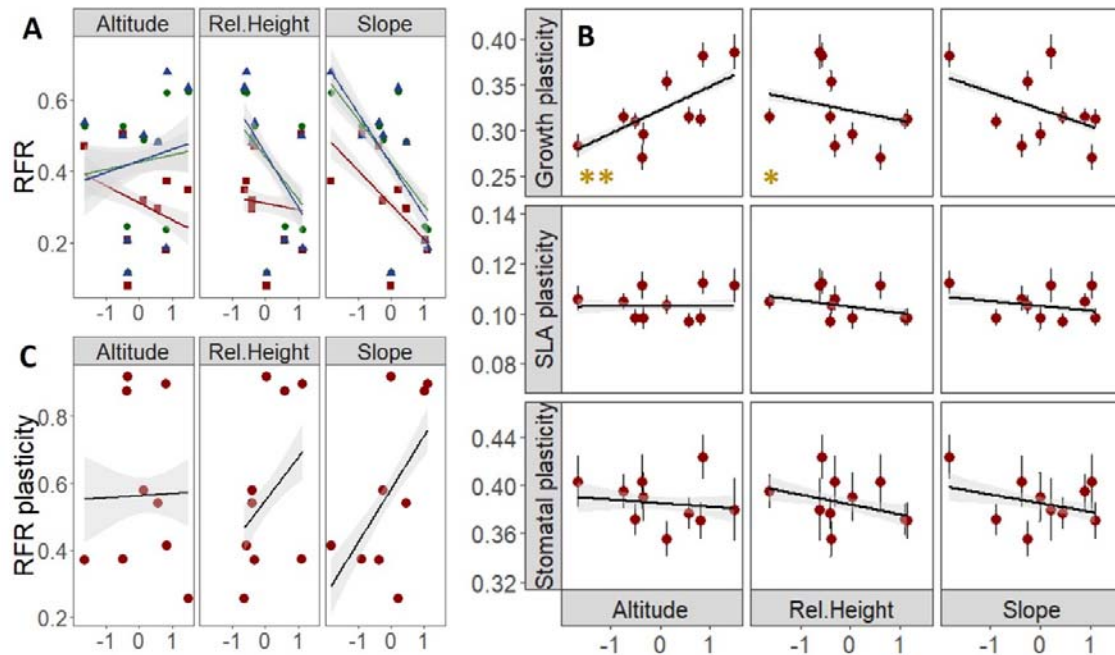
261 Altitude patterns systematically and markedly mirrored relative height and slope patterns across  
 262 traits and treatments (Figs. 2 and 3A), indicating that these topographical variables independently,  
 263 but predictably affect plant responses to soil moisture treatments. More specifically, whereas  
 264 growth under drought stress decreased with increasing latitude, it increased for populations situated  
 265 near mountain summits (Fig. 2). Similarly, drought stress decreased SLA for populations originating  
 266 from higher altitude, while it increased SLA for populations from relatively steep slopes.



267

268 **Fig. 2.** Relations between vegetative traits (principal components) and topographical variables (principal  
269 components) under three soil moisture treatments. Red squares, green dots and blue triangles represent dry,  
270 moist and wet soil moisture treatments, respectively. Plots present partial effects of the respective traits and  
271 their interaction with soil moisture treatment, extracted using the R package *remef* (Hohenstein 2013).

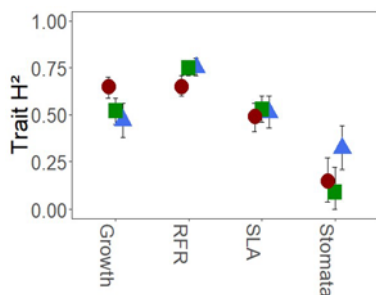
272 Trait plasticity was higher for RFR (between 0.2 and 1.0) than for all vegetative traits (between 0.1  
273 and 0.4), and could be explained by topography to a limited extent (Table 2, Fig. 3). Genotypes from  
274 low relative height and from high altitude in particular showed increased plasticity for growth (Fig.  
275 3B). Plasticity for SLA, stomata and RFR, on the other hand, did not differ significantly between  
276 topographical settings (Figs. 3B and 3C) (Table 2).



277

278 **Fig. 3.** Relations between traits and topographical variables (principal components). (A) RFR against  
 279 topography under three soil moisture levels, (B) vegetative trait plasticities against topography, and (C) RFR  
 280 plasticity against topography. Red squares, green dots and blue triangles represent dry, moist and wet soil  
 281 moisture treatments, respectively. Yellow asterisks represent the significance level for plasticity traits (\* and  
 282 \*\* are p-values <0.05 and <0.01, resp.)

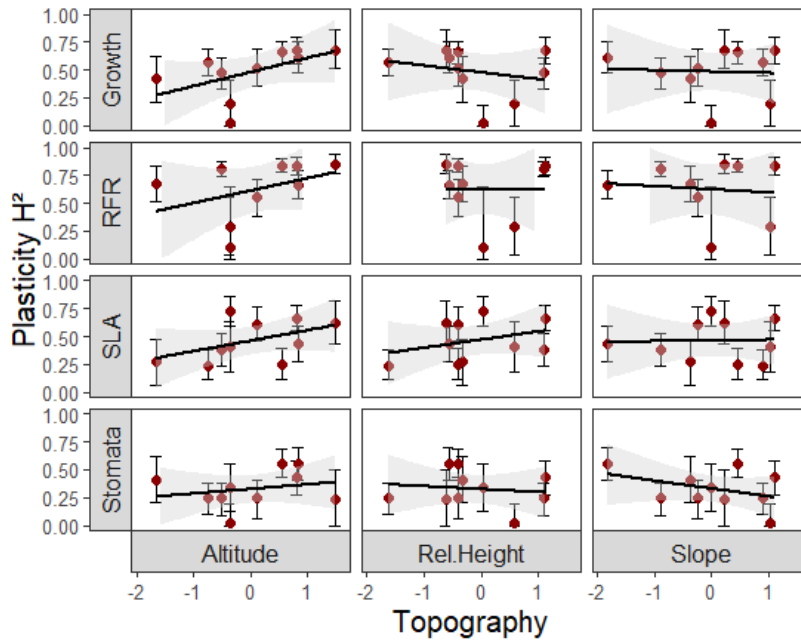
283 Trait  $H^2$  was highest for RFR and lowest for stomata (Table S8), indicating that RFR is under strong  
 284 genetic control while stomatal variation is predominantly environmental (Fig. 4). Trait  $H^2$  was  
 285 comparable among treatments (Fig. 4), suggesting that traits can evolve irrespective of soil moisture  
 286 levels and thus in response to increased drought stress.



287

288 **Fig. 4.** Trait heritability estimates across traits and soil moisture treatments. Red squares, green dots and blue  
 289 triangles represent dry, moist and wet soil moisture treatments, respectively.

290 Similar to trait  $H^2$ , plasticity  $H^2$  was on average highest for RFR ( $H^2=0.62$ ,  $SE=0.17$ ), followed by  
291 growth ( $H^2=0.48$ ,  $SE=0.16$ ), SLA ( $H^2=0.46$ ,  $SE=0.16$ ) and stomata ( $H^2=0.32$ ,  $SE=0.17$ ) (Table S8). For all  
292 traits, plasticity  $H^2$  tended to increase with increasing altitude (Fig. 5). Populations originating from  
293 higher altitudes may thus evolve increased trait plasticity when environmental conditions change. In  
294 addition,  $H^2$  of stomatal plasticity tended to decrease with increasing slope (Fig. 5).



295

296 Fig. 5. Plasticity heritability estimates across traits along topographical gradients.

297

#### 298 4 DISCUSSION

299 Adaptive trait variation could mitigate the impact of changing soil moisture levels on population  
300 dynamics and persistence, yet little is known about the potential of mountainous populations to  
301 withstand increased levels of drought stress. The temporal soil moisture heterogeneity presumably  
302 associated to various topographical settings could boost adaptive evolution towards (i) phenotypes  
303 that increase drought resistance, and (ii) phenotypic plasticity for traits underlying drought  
304 resistance. We used genetically identical replicates (clones) of *Fragaria vesca*, originating from  
305 distinct topographical settings, to study whether topography can drive adaptive trait variation that

306 allows population persistence under different levels of drought stress. Our results indicate that  
307 mountainous populations have a high potential to cope with drought stress, as they can evolve both  
308 through trait adaptation and through trait plasticity adaptation. However, low-altitudinal  
309 populations, which are expected to be most vulnerable to climate warming, have the lowest  
310 heritability and may thus only withstand modest increases in drought stress.

#### 311 **4.1 Genetic adaptive trait variation in mountainous populations**

312 Plants adapted to dry environments should be able to maintain growth under increased levels of  
313 drought to a larger extent than plants originating from moist environments. We correspondingly  
314 found less growth reduction under drought stress for plants originating from higher relative height  
315 (~ water efflux) and low absolute altitude (~ high minimal temperatures) than for plants originating  
316 from cool and moist locations (Fig. 2). To maintain growth under drought stress, plants adapted to  
317 dry environments should retain photosynthetic rates at relatively high levels and thus minimize  
318 reductions in SLA (e.g. Wellstein et al. 2017). Accordingly, the plants originating from dry  
319 environments (steep slopes and high relative height) had significantly higher SLA under increased  
320 drought than plants originating from moist environments (weak slopes and valleys). In addition,  
321 plant populations that evolved to thrive in dry conditions have frequently been found to escape  
322 summer drought through advanced flowering time (Franks 2011; Nguyen et al. 2016). Here, although  
323 flowering tended to advance with increasing relative height and slope (~ water efflux), these trends  
324 were deemed non-significant (Table 2, Fig. 3A).

325 Stomatal densities increased significantly under drought stress (Fig. 2). An increase in stomatal  
326 density can result in an improved water use efficiency through indirect effects of stomatal size, as  
327 size and density usually are inversely correlated (Dittberner *et al.* 2018). Small stomata not only have  
328 lower transpiration rates, they can also open and close at higher velocity than large stomata in  
329 response to local soil moisture changes (Drake *et al.* 2013; Raven 2014). Plants adapted to dry  
330 environments may strongly benefit from high densities of small and responsive stomata, allowing



331 quick re-initiation of photosynthesis when soil moisture levels temporarily increase. Here, plants  
332 from high absolute altitude in particular increased their stomatal density under drought (Fig. 2). High  
333 absolute altitude may correspond to decreased soil moisture levels where temperature inversions  
334 are frequent and/or where slopes are south-oriented. Our high-altitude populations were always  
335 south-oriented (Table S1), which may have driven evolution toward small stomata at high densities  
336 to deal with increased summer transpiration rates. Together, we could verify our hypothesis that  
337 genotypes originating from low altitude (~ high temperatures), high relative height and TPI (~ water  
338 efflux) and steep, southerly oriented slopes (~ water efflux and high evapotranspiration) have  
339 evolved traits that allow growth under drought stress. However, collinearity between altitude and  
340 aspect complicates disentangling the evolutionary roles of these two topographical in governing  
341 drought tolerance.

342 Although significant relations were observed between topography and phenotypic traits, much of  
343 the variation in SLA and stomatal density remained unexplained (Table 2, Table S6). Microclimatic  
344 variation not accounted for by the selected topographical variables (e.g. soil texture and shade) may  
345 have driven drought resistance evolution in addition to the selected topographical variables and thus  
346 explain some of the residual phenotypic variation. In addition, genetic drift likely contributes to the  
347 unexplained variation in phenotypic traits, as neutral processes are expected to shape at least part  
348 of the phenotypic variation observed in common gardens (McKay & Latta 2002; De Kort *et al.* 2013).  
349 We did not perform genetic marker analysis to exclude this neutral component ( $F_{ST}$ , Wright 1943),  
350 but instead assumed topographical variables as likely drivers of drought-related trait variation to be  
351 plausible cues for adaptive evolution.

#### 352 **4.2 Adaptive variation in trait plasticity of mountainous populations**

353 The use of genotypic replicates is key to studying adaptive divergence of phenotypic plasticity, which  
354 is predicted to drive evolutionary and demographic trajectories of natural populations. This study is  
355 among the first to quantify drought-related phenotypic plasticity as a trait that can vary between

356 genotypes within populations and thus evolve over contemporary time scales. Increased plasticity  
357 has been suggested to provide a fitness advantage where environments are heterogeneous but  
358 predictable (Reed *et al.* 2010; Chevin & Lande 2011; Chevin & Hoffmann 2017; Bonamour *et al.*  
359 2019). We found that within-genotype plastic responses to drought were common, but that only  
360 growth plasticity followed a pattern coinciding with topographical variation (Fig. 3B). Specifically,  
361 genotypes from low absolute altitude and from high relative height (near summits) were less plastic  
362 for growth. We suspect that increasing environmental heterogeneity with increasing absolute  
363 altitude, where south-facing populations experience fluctuating temperatures and soil moisture  
364 levels, may be an important driver for increased growth plasticity. In addition, environmental  
365 predictability may decrease away from valleys, thereby hampering evolution of phenotypic  
366 plasticity. Together, population inhabiting high altitudinal valleys seem to benefit most from high  
367 levels of growth plasticity.

368 The capacity of a genotype to adjust its gene expression in response to a change in environmental  
369 conditions is frequently referred to as epigenetic variation (Bossdorf *et al.* 2008; Thorson *et al.*  
370 2017). Here, we thus provide indirect evidence that populations can evolve their epigenetic  
371 machinery towards increased epigenetic flexibility in environments with heterogeneous soil  
372 moisture levels. Although habitat differences have been shown to result in epigenetic divergence  
373 between populations (e.g. Lele *et al.* 2018, Schmid *et al.* 2018), empirical evidence for evolution  
374 toward higher within-population epigenetic variation in heterogeneous environments is still lacking.

### 375 **4.3 Drought-related phenotypic plasticity has a substantial heritable component**

376 A large proportion of the phenotypic variation could be attributed to genetic rather than  
377 environmental variation, as indicated by the relatively high heritability estimates. This genetic  
378 variation can serve as a basis for natural selection to act upon, and thus reflects the potential of  
379 populations to evolve under changing environmental conditions (Mousseau & Roff 1987; Visscher *et al.*  
380 *et al.* 2008). A spatially varying balance between gene flow and natural selection pressures likely lies at

381 the basis of this accumulation of adaptive genetic variation. First, steep fine-scale environmental  
382 variation can cause strong and spatially variable selection pressures that, in combination with gene  
383 flow-facilitated spread of adaptive genetic variants, maintain adaptive genetic variation (Hämälä *et*  
384 *al.* 2018; Schmidt & Garroway 2018). Second, opposing selection pressures from several  
385 environmental factors affecting the same phenotypic traits (e.g. growth can be affected by soil  
386 moisture levels, other abiotic factors as well as the local biotic context) promote the maintenance  
387 adaptive genetic variation (Kruuk *et al.* 2002, 2008; Alberto *et al.* 2011).

388 With heritability estimates fluctuating around 0.5 (Figs. 4 and 5), phenotypic traits were found to be  
389 comparably heritable as their plasticity indices, indicating that traits as well as phenotypic plasticity  
390 are under genetic control and can evolve in response to changes in soil moisture levels. Growth,  
391 flowering rate, and their corresponding plasticity indices showed particularly high heritability (Figs. 4  
392 and 5), suggesting that these fitness traits have high potential to keep pace with local environmental  
393 changes (e.g. in pollinator phenology, drought levels and temperature). On the other hand, low-  
394 altitudinal populations harboured a considerably lower heritability than populations at high absolute  
395 altitude (Fig. 5). The higher environmental heterogeneity at high altitude may have facilitated the  
396 accumulation of heritable phenotypic variation, while the lack hereof at low altitude increases the  
397 vulnerability of low altitudinal populations to environmental change. We conclude that populations  
398 that evolved to thrive in heterogeneous conditions likely are pre-adapted to climate change. Such  
399 populations represent invaluable sources of quantitative genetic variation that could support  
400 conservation where climate projections are inconclusive.

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414

415 **DATA ARCHIVING**

416 All data required for the data analysis are available in the supporting files.

417

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