Genetic differences in plasticity across environmental scales determine fitness along an ecological gradient

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ABSTRACT

When populations suffer reduced fitness in novel environments, genotypes with less deleterious forms of plasticity could aid persistence by reducing the severity of fitness declines. However, we know little about how plasticity allows different genotypes track environmental variation to determine population persistence along ecological gradients, particularly as environments become novel. We transplanted numerous clones of 19 genotypes of a Sicilian daisy, *Senecio chrysanthemifolius*, at four elevations on Mt Etna. We estimated fitness at native and novel elevations, and quantified leaf plasticity both among and within elevations. Compared to genotypes with higher fitness within the native range, genotypes with higher fitness at the novel elevation showed lower variance in fitness, lower plasticity across elevations, but greater plasticity within elevations. Our results suggest there are genotypes hidden in a population whose plasticity better tracks novel environmental variation at fine and coarse scales, which will be crucial for population persistence under rapid environmental change.

Keywords: adaptive plasticity, adaptive potential, ecological gradient, elevation, environmental change, fitness, novel environments, phenotypic plasticity, population persistence, *Senecio*

INTRODUCTION

There is an urgent need to understand the capacity for natural populations to produce adaptive responses to ongoing global change (Martin *et al.* 2023; Urban *et al.* 2024). Adaptive phenotypic plasticity, defined as the ability of genotypes to express different but beneficial phenotypes as the environment changes, helps populations to maintain their fitness along spatial and temporal environmental gradients (de Jong 1995; Via *et al.* 1995; Sultan 2000; Charmantier *et al.* 2008). However, maintaining fitness via adaptive plasticity becomes difficult when novel environments are encountered, such as when new habitats are colonised, or when the environment changes rapidly and unpredictably. This is because adaptive plasticity should only evolve to buffer familiar variation in the environment, meaning that plastic responses shaped by current or historical environments should fail to prevent fitness declines under novel conditions (Bradshaw 1991; Ghalambor *et al.* 2007; Matesanz *et al.* 2010; Reed *et al.* 2010; Fierst 2011; Snell-Rood *et al.* 2018). Population persistence is then expected to be determined by the extent to which genotypes vary in their plastic response to novel environments, and how such differences in plasticity contribute to maintaining high enough mean fitness so as to avoid extinction (Chevin & Bridle 2025).

Genotypes often vary in plasticity within populations and therefore in their sensitivity to the environment (Bradshaw 1965; Schlichting 1986; Scheiner 1993). Such genetic variation in plasticity should aid population persistence in novel environments when genotypes with plasticity that is at least partially adaptive help to prevent more severe declines in fitness (Lande 1988; Lande & Shannon 1996; Bell 2013). Over generations, these genotypes can also help the population to adapt to the new conditions (Lande 2009; Chevin & Lande 2011; Kelly 2019; Chevin & Bridle 2025). These genotypes which are better at maintaining fitness in novel environments can have lower relative fitness within the native range, suggesting that genotypes important for persisting in novel environments could remain hidden within the native range (Angert *et al.* 2008; Walter *et al.* 2023). To accurately predict the response of natural populations to global change, we need to identify genotypes important for maintaining fitness in novel environments, understand how they better track novel changes in their environment, and identify the circumstances in which they could help population persistence in novel environments (Yeh & Price 2004; Morris 2014).

Genetic variance in plasticity is often reported (Saltz *et al.* 2018), and has been linked to performance in novel environments (Nussey *et al.* 2005; Peschel *et al.* 2020; Anderson *et al.* 2021; Walter *et al.* 2023) as well as adaptive responses to fine-scale environmental variation (Baythavong & Stanton 2010; Baythavong 2011). However, we still cannot predict how existing variation in plasticity will allow genotypes to track environmental variation across natural ecological gradients, especially as environments become novel. In particular, we need to know how genotypes that differ in plasticity make phenotypic adjustments to maintain fitness across fine versus coarse environmental variation within their native range, and then understand how these same genotypes vary in their ability to maintain fitness as environments become novel. Identifying and characterising genotypes that can help prevent more severe fitness declines in novel environments is challenging and requires field studies that connect measures of phenotype and fitness for naturally segregating genotypes transplanted across an ecological gradient that includes novel conditions (Chevin *et al.* 2013a). Such information identifies the ecological scale at which plasticity varies the phenotype, and the effect on fitness, to reveal how genetic variation in plasticity can support population persistence in novel environments (Valladares *et al.* 2007). This is an increasingly urgent question given the current rate of global change (Urban *et al.* 2024).

We focus on a Sicilian wildflower, *Senecio chrysanthemifolius* (Asteraceae), native to the foothills (c.400-1000m elevation) of Mt Etna, Sicily. This species is a self-incompatible, short-lived perennial that relies on generalist insect pollinators (e.g., hoverflies). A closely related sister species, *S. aethnensis,* occurs on old lava flows at high elevations on Mt Etna that are covered by snow each winter (Walter *et al.* 2020). Previous reciprocal transplant experiments with the two Etnean *Senecio* species have shown that they differ in leaf plasticity across elevation. In particular, *S. chrysanthemifolius* displayed leaf plasticity that was at least partly

adaptive when transplanted into a novel high-elevation habitat as plasticity moved its leaf phenotype towards that of the native *S. aethnensis* (Walter *et al.* 2022; Walter *et al.* 2024).

In this experiment, we quantified fitness and plasticity for 19 genotypes of *S. chrysanthemifolius* that showed contrasting fitness responses to higher elevations in a transplant experiment conducted in 2018. In the 2018 study, we connected genetic variance in plasticity with fitness at a novel 2000m elevation by transplanting cuttings (clones) of 314 genotypes across an elevation gradient on Mt Etna (Walter *et al.* 2023). Although mean fitness declined in the novel environment, we observed a threefold increase in additive genetic variance in fitness, which increased adaptive potential at the novel elevation compared to native elevations. This increased adaptive potential was associated with genetic differences in plasticity, where genotypes with higher plasticity showed lower fitness at the novel elevation. The contrasting fitness responses of these genotypes provide an exceptional opportunity to test how plasticity mediates fitness at different environmental scales along an ecological gradient.

From the 2018 experiment, we selected genotypes with the most contrasting fitness responses from 500 to 2000m elevation: 10 'Adaptive Potential' (AP) genotypes that displayed higher relative fitness in the novel environment, but lower fitness at the native site, and 9 'Home Range' (HR) genotypes that showed higher fitness at their native site, but lower relative fitness at the novel elevation (**Fig. 1a**). In 2020, we propagated 160 cuttings of each genotype and transplanted 40 cuttings/genotype to four elevations representing their home site (500m), a site within their native range (1000m), their range edge (1500m) and a novel elevation (2000m, outside the range and within the native habitat of *S. aethnensis*). We collected leaf phenotype and fitness data on mature plants. Transplanting clones at high replication across the entire elevational gradient allowed us to quantify plasticity as changes in phenotype across elevations for each genotype, as well as variation in phenotype among clones (within genotypes) within each elevation (i.e., plasticity as phenotypic variation in response to local environmental variation). We then tested how genotypic differences in plasticity determined fitness within and between elevations along the entire elevational gradient.

Given the fitness patterns observed in the 2018 study, we expected HR genotypes to show higher mean fitness than AP genotypes within the native range, whereas AP genotypes would perform better at the novel elevation (**Fig. 1b**). We then tested three hypotheses. *Hypothesis I* – Within the native range, adaptive plasticity would maintain high mean fitness, which would produce low variation in fitness similarly for both genotype classes. However, if AP genotypes consistently adjust their phenotype to better cope with novel environments, AP genotypes would show lower variance in fitness among genotypes and among clones (within genotypes) at the novel elevation when compared to HR genotypes (**Fig. 1c**). Conversely, we predicted that HR genotypes would show higher variance in fitness at 2000m if they produced a wider variety

117 of phenotypic responses resulting in lower mean fitness at the novel elevation. Given that changes to
118 phenotype should mediate variation in fitness, we then tested two hypotheses connecting plasticity to fitness.
119 For this, we calculated plasticity as changes in mean phenotype across elevation, and as the amount of
120 variation among clones (within genotypes) at each elevation (**Fig. 1d**). These metrics capture plasticity in
121 response to coarse changes in the environment across elevation, and to fine-scale environmental variation
122 within elevations.

123 *Hypothesis II* – At native elevations, we predicted that greater plasticity would be associated with higher
124 fitness because greater phenotypic adjustments would help all genotypes to successfully track changes in the
125 environment. By contrast, lower phenotypic plasticity would be favoured in novel environments because
126 greater phenotypic changes would be maladaptive **(Fig. 1e**; Snell-Rood *et al.* 2018**)**. *Hypothesis III* – If
127 plasticity in response to fine-scale environmental variation relies on predictable (familiar) environments,
128 plasticity as among-clone variation in phenotype (within genotypes) would only be strongly associated with
129 fitness at the novel elevation, but not within the native range. This is because all plasticity at native
130 elevations would help to maintain higher mean fitness and lower variance in fitness for all genotypes.
131 However, if plasticity could not predict environmental variation at the novel elevation, genotypes with
132 greater plasticity would show greater variance in fitness because varying the phenotype in response to
133 unfamiliar environments would increase variation in fitness (**Fig. 1f**).

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136 **METHOD AND MATERIALS**

137 *Field transplant*

138 In the Spring of 2020, we propagated 10 AP and 9 HR genotypes from the 2018 study by taking cuttings
139 from plants maintained in a greenhouse (Giarre, Italy). For each genotype, we propagated 10 clones in 14cm
140 diameter pots, randomised their location and let them grow into large plants (c.40cm high). We then removed
141 6-7 branches from each plant, which we cut into 4cm segments with 2-3 leaf nodes. We dipped the smaller
142 cuttings in rooting hormone (Germon Bew., Der.NAA 0.5%, L.Gobbi, Italy) and placed them in an 84-cell
143 tray containing an equal mix of compressed coconut coir and perlite. We kept trays on benches covered with
144 plastic for three weeks to maintain high humidity and promote root formation.

145 Rooted cuttings of each genotype were transplanted in early summer 2020 (29-30th June) to four elevations
146 on the south-eastern slope of Mt Etna: 500m, 1000m, 1500m and 2000m. The 500m site was in a garden
147 among fruit trees, the 1000m site in an abandoned vineyard among oak trees (*Quercus ilex*), the 1500m site

148 in an abandoned apple orchard, and the 2000m site on a lava flow from 1983 with pine trees present (Walter
149 *et al.* 2022; Walter *et al.* 2024). We transplanted 160 cuttings/genotype divided equally across four
150 elevations. At each elevation, we randomised the 40 cuttings/genotype into four spatially distinct
151 experimental blocks (*n*=190 plants/block; *n*=760 plants/elevation; N=3040 plants), within an area of
152 c.5000m^2. At each block, we cleared vegetation and debris, turned the soil 30cm deep, and then planted the
153 cuttings 30cm apart in a grid of 7×29 plants (**Fig. S1**). We irrigated the cuttings daily for three weeks so they
154 could establish, and then reduced irrigation to only the hottest days to prevent high mortality.

155 *Data collection*

156 We took data for each plant c.4 months after the initial transplant (13-23rd October). As our proxy for fitness,
157 we collected all flowerheads produced by each plant in paper bags, which we counted in the laboratory. This
158 trait is routinely used to estimate fitness in short-lived perennials (e.g. Pujol et al., 2014), including in our
159 previous experiment (Walter *et al.* 2023). To measure five ecologically important leaf traits, we sampled 3-4
160 fully expanded young leaves from each plant, which we weighed and then scanned to quantify morphology
161 using the program *Lamina* (Bylesjo *et al.* 2008). We used three leaf traits to represent leaf size, shape and
162 investment: leaf area (mm^2), perimeter (mm) and the number of indentations (count). To calculate the density
163 of leaf indents, we standardized the number of indentations by the perimeter. Using the weight of the leaves,
164 we estimated specific leaf area as $SLA = \frac{leaf\ area}{leaf\ weight}$, where greater values represent larger leaves per unit
165 mass. Using a Dualex instrument (Force-A, France), we measured chlorophyll and flavonol pigment content
166 (light absorbance units) for two mature leaves per plant. Flavonols are secondary metabolites produced under
167 stressful abiotic (e.g. temperature) and biotic (e.g. herbivore) conditions (Mierziak *et al.* 2014).

168 *Statistical analyses of fitness (Hypothesis I)*

169 All analyses were conducted in R version 4.3.2 (R Core Team 2024). To quantify mean fitness across
170 elevation (**Fig. 1b**), we used *MCMCglmm* (Hadfield 2010) to apply the generalised linear mixed model

171 $$y_{ijklm} = E_i \times R_j + g_k + b_l + e_{m(ijkl)}\ , \qquad (1)$$

172 where the interaction between genotype class (R_j; AP vs HR) and elevation (E_i) quantifies whether AP and
173 HR genotypes show different responses in fitness to elevation. g_k captures variation among the 19
174 genotypes. b_l represents the experimental blocks and $e_{m(ijkl)}$ the residuals. For each random effect, we
175 estimated variances at each elevation by specifying a 2×2 matrix. We included the number of flowers as the
176 poisson-distributed fitness response (y_{ijklm}). Equation 1 yielded the posterior distribution of mean fitness for
177 AP and HR genotypes at each elevation. We then used a second application of equation 1 on AP and HR
178 genotypes separately to calculate the among genotype variance in fitness at each elevation. This allowed us to

179 test whether among-genotype and among-clone (within genotypes, i.e., residual variation) variation were
180 different between AP and HR genotypes across elevations (*Hypothesis II*, **Fig. 1c**).

181 *Statistical analyses of phenotype and calculation of plasticity*

182 To test whether AP and HR genotypes showed differences in phenotype across elevation, we used *lme4*
183 (Bates *et al.* 2015) to apply equation 1, but included the five leaf traits as univariate response variables. We
184 used type-III ANOVA (Fox & Weisberg 2019) to test for significant $E_i \times R_j$ interactions, which indicate
185 genotypic differences in plasticity to elevation. We then used *emmeans* (Lenth 2019) to obtain estimated
186 marginal means for each genotype, which we used to calculate plasticity with

187 $$P_i = \frac{\bar{x}_i - \bar{x}_{home\ site}}{\bar{x}_{home\ site}}, \qquad (2)$$

188 where plasticity (P_i) for each genotype is the difference in mean between the home site and the *i*th elevation,
189 standardised by the home (500m) site mean (Valladares *et al.* 2006). This captures plasticity as the
190 elevational change in magnitude and direction (negative values reflect a trait decrease) of the phenotype
191 relative to the home site (Anderson *et al.* 2021).

192 To estimate plasticity within elevations, we calculated the coefficient of variation (CV) for each genotype
193 (and each leaf trait separately) using

194 $$CV_{ij} = \frac{\sigma_{ij}}{\bar{x}_{ij}}, \qquad (3)$$

195 where σ_{ij} and $\bar{x}_{ij}$ represent the standard deviation and mean, respectively, for the *i*th genotype transplanted at
196 the *j*th elevation. Equation 3 therefore captures plasticity as the among-clone (within-genotype) variance
197 including differences among blocks at each elevation (Hill & Mulder 2010). This is an appropriate use of CV
198 as we are comparing differences between AP and HR genotypes randomised into the same experimental
199 blocks, and because we do not estimate CV across elevations (Pélabon *et al.* 2020).

200 *Statistical analyses connecting plasticity with fitness*

201 In the following analyses, we pooled the HR and AP genotypes to use their combined variation in plasticity
202 and phenotype to test how associations with fitness change across elevations. First, to test phenotype-fitness
203 associations, we estimated phenotypic and genotypic selection. We divided each trait by its mean and tested
204 for elevational changes in selection using

205 $$y_{ij} = E_i \times T + e_{j(i)}, \qquad (4)$$

where E_i represents the *i*th elevation and T a leaf trait. We included relative fitness as the response variable (y_{ij}) and $e_{j(i)}$ represents the residuals. Significant $E_i \times T$ interactions provide evidence that associations between the trait and fitness changed across elevations. We used the same approach to estimate genotypic selection, but with the genotype means estimated at each elevation (Rausher 1992).

To test whether plasticity changed its association with fitness across elevation, we used equation 4 with plasticity as: (1) the change in trait mean across elevation, and (2) the amount of variation (CV) within elevations. A significant $E_i \times T$ would provide evidence that the association between plasticity and fitness changed across elevation for that leaf trait.

RESULTS

Genotypes differ in their reduction in mean fitness at 2000m

Consistent with *Hypothesis I*, changes in mean fitness across elevation were similar for AP and HR genotypes across experiments (2018 and 2020): HR genotypes showed higher fitness in the native range, whereas AP genotypes showed higher fitness at the novel 2000m elevation (**Fig. 2**; Walter *et al.* 2023). At 500m and 1000m, HR genotypes produced 144 and 74 more flowers on average than AP genotypes, respectively in this experiment. At 1500m, both genotypes showed similar fitness values, whereas AP genotypes produced 12 more flowers on average than HR genotypes at the novel 2000m elevation.

Hypothesis I: Variance in fitness changes across elevations differently for AP and HR genotypes

From low to high elevations, HR genotypes showed a significant 2-3-fold increase in among-genotype variance in fitness. By contrast, AP genotypes showed a significant 3-fold decrease in among-genotype variance in fitness as environments became novel (**Fig. 3a**). In addition, at 1500m and 2000m, HR genotypes showed c.5 times greater among-genotype variation in fitness than AP genotypes (**Fig. 3a**). We found the same patterns for the among-clone (within genotype) variance in fitness: HR genotypes showed an increase and AP genotypes a decrease, in among-clone variance at higher elevations (**Fig. 3b**). Therefore, consistent with *Hypothesis I*, HR genotypes showed an increase in variance in fitness within and among genotypes at higher elevations. At all elevations, variance in fitness was always greater for HR than AP genotypes. However, contrary to predictions, AP genotypes showed a reduction (rather than an increase) in variance in fitness at higher elevations.

Genotypic differences in plasticity across elevation

For both genotypes, all five leaf traits showed reductions in mean values at higher elevations, except for leaf indentation, which increased (**Fig. 4a**). Consistent with the 2018 study, flavonol content increased at the edge of the range, but then at 2000m returned to similar values as 500m. AP and HR genotypes showed significant differences in plasticity across elevations (i.e. significant genotype class×elevation interactions) for leaf area and chlorophyll content, and differences in mean phenotype across all elevations for specific leaf area and flavonol content (**Fig. 4a; Table S1**).

Quantifying plasticity as among-clone variance (within genotypes) within elevations (CV), AP and HR genotypes showed differences in plasticity for all traits. For leaf indents and flavonol content, AP genotypes showed greater plasticity than HR genotypes (greater CV) at each elevation (**Fig. 4b**). For leaf area, SLA and chlorophyll content, we found significant differences in the change in CV across elevation (genotype class×elevation interaction; **Fig.4b; Table S1**), suggesting that plasticity as among-clone variation changed across elevation differently for AP and HR genotypes.

Hypothesis II – Plasticity was associated with mean fitness within and across elevations

All five traits showed significant associations between phenotype and fitness, and, except for the number of leaf indents, higher values of each trait were favoured at all elevations (**Fig. S2**). Associations between traits and fitness changed significantly across elevation for leaf area, SLA and flavonol content, such that higher elevations favoured smaller leaves, lower flavonol content and greater SLA (**Fig. 5a; Table S2**). Genotypes with different trait values were therefore favoured in the native range compared to the novel elevation.

We predicted that greater plasticity would increase fitness within the native range, but reduce fitness at the novel elevation (**Fig. 1e**). We found support for *Hypothesis II*. Associations between plasticity and fitness changed significantly across elevation for three (of five) traits: leaf area, leaf indentation and chlorophyll content (**Fig. 5b; Table S3**). Only leaf indentation showed a significant elevation×plasticity interaction, which meant that plasticity as an increase in leaf indentation within the native range was favoured, whereas reduced indentation at higher elevations was favoured. For leaf area and chlorophyll content, associations between plasticity and fitness were positive at all elevations (**Fig. 5b**). However, because plasticity increased trait values within the range, but decreased them outside the range, different forms of plasticity were favoured at the 1000m native site compared to higher elevations. Specifically, at the 1000m native site, plasticity increased leaf area and chlorophyll content for all genotypes, and greater plasticity (i.e., larger increases in these traits) was favoured. By contrast, plasticity at 1500m and 2000m reduced leaf area and chlorophyll content for all genotypes, and lower plasticity (i.e., smaller reductions in these traits) was favoured (**Fig. 5b**). Consistent with the original study, higher fitness at 2000m was associated with smaller

267 changes in leaf area, indentation and chlorophyll content. However, unlike the original study, plasticity in
268 SLA and flavonol content did not show strong associations with fitness.

269 For each genotype, CV within an elevation represents plasticity as variation in phenotype in response to
270 environmental variation at that elevation. Plasticity within elevations was associated with mean fitness for
271 three traits: leaf indents, SLA and chlorophyll content, and this association changed across elevation for leaf
272 indents and chlorophyll (**Fig. 6a; Table S3**). However, our results contradicted *Hypothesis II:* greater
273 variance in phenotype (i.e., greater plasticity) was associated with lower fitness within the native range, but
274 greater fitness outside the native range (**Fig. 6a**). At the novel elevation, four traits showed positive
275 associations between plasticity and mean fitness, and only SLA showed the predicted negative trend (**Fig.**
276 **6a**). These results suggest that plasticity of different magnitudes were associated with mean fitness within
277 versus among elevations.

278 *Hypothesis III – Greater plasticity was associated with lower variance in fitness at the novel elevation*
279 We predicted that greater among-clone variation in phenotype (i.e., greater plasticity) within a genotype
280 would be associated with low variation in fitness within the range, but high variation in fitness at the novel
281 environment (**Fig. 1f**). Supporting *Hypothesis III*, four (of five) traits showed significant associations
282 between CV in phenotype and variance in fitness (**Fig. 6b**). As predicted, we found a weak positive
283 association between CV in phenotype and variance in fitness at elevations within the native range, suggesting
284 that plasticity maintained similarly high fitness for all clones and genotypes. Also as predicted, three traits
285 (leaf indentation, and chlorophyll and flavonol content) showed significant changes in the association
286 between CV in phenotype and variance in fitness across elevations (**Fig. 6b; Table S3**). However, contrary to
287 our predictions, these traits showed a strong negative association with variance in fitness at the novel
288 elevation, suggesting that greater plasticity reduced variance in fitness. Only SLA showed a positive
289 association between CV in phenotype and fitness at the novel elevation, as predicted (**Fig. 6b**).

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292 **DISCUSSION**

293 To test how genetic differences in plasticity influences fitness across an ecological gradient, we transplanted
294 large numbers of clones of 19 genotypes at four elevations on Mt Etna, Sicily. Genotypes were selected from
295 a previous experiment based on their contrasting fitness response to elevation (Walter *et al.* 2023): Adaptive
296 Potential (AP) genotypes showed lower fitness than the Home Range (HR) genotypes at the native 500m site,
297 but higher fitness than HR genotypes at the novel 2000m elevation, a result that was consistent in the current

experiment (**Fig. 2**). Supporting *Hypothesis I*, HR genotypes showed increased variation in fitness at higher elevations (among genotypes and among clones within genotypes), whereas AP genotypes showed a reduction in variation in fitness at higher elevations (**Fig. 3**). This suggests that AP genotypes cope better and show more consistent responses to the novel environment. By contrast, HR genotypes that do better within the native range, produce a variety of responses that result in lower mean fitness and higher variance in fitness in the novel environment.

We then tested how plasticity associated with fitness across elevations. Supporting *Hypothesis II*, genotypic differences in plasticity were associated with fitness, and this association changed across elevation. For plasticity across elevations, native elevations generally favoured higher plasticity, whereas the novel elevation favoured lower plasticity (**Fig. 5b**). By contrast, for plasticity within elevations, higher variance among clones (i.e., higher plasticity) at native elevations was associated with lower fitness, whereas higher variance at the novel environment was associated with higher fitness (**Fig. 6a**). Plasticity in response to finer-scale environmental variation therefore did not follow the same association with fitness as plasticity created by coarse changes in elevation. Consistent with *Hypothesis III*, plasticity within elevations captured by the among-clone (within genotype) phenotypic variation was weakly associated with variance in fitness, suggesting that phenotypic changes within the native range helps to maintain high mean fitness for all genotypes. However, contrary to *Hypothesis III*, higher plasticity (higher among-clone variation) was associated with lower variance in fitness at the novel elevation (**Fig. 6b**). Genotypes important for population persistence at a novel elevation therefore make smaller adjustments across elevations, but larger phenotypic adjustments within elevations.

Together, our results suggest that genotypes with different forms (magnitude and direction) of plasticity are favoured across an ecological gradient. These results also suggest that there is substantial genetic variation in environmental sensitivity within species that increases their potential to persist beyond their current range. Hidden genetic variation segregating in natural populations can therefore allow new forms of adaptive plasticity to evolve in marginal environments (Lande 2009), or help the population to maintain sufficient mean fitness to persist under short-term environmental change (Chevin & Hoffmann 2017). The frequency and genetic basis of such genotypes, as well as their absolute fitness in novel environments, will determine the potential for them to aid population persistence and adaptation to novel environments.

Genetic variation for population persistence under environmental change

Alleles that create AP genotypes could be maintained in the population through interannual or seasonal (within-year) variation. Environmental variation that causes fluctuating selection would then maintain genetic variation in plasticity that reduces vulnerability to environmental change (Gillespie & Turelli 1989;

330 Svardal *et al.* 2011; Wittmann *et al.* 2017). AP genotypes could represent 'generalist' genotypes that have
331 lower arithmetic fitness within their native range, but high geometric fitness across a broader range of spatial
332 or temporal environments (Gillespie 1974; Childs *et al.* 2010; Svardal *et al.* 2011; Tufto 2015; Draghi 2023).
333 This would suggest that AP genotypes are 'bet-hedging' genotypes that can buffer greater environmental
334 variation (Simons 2011). If so, then bet-hedging genotypes will be important for population persistence given
335 they reduce the fitness costs to the population as environments change rapidly and/or unpredictably
336 (Gillespie 1974; Childs *et al.* 2010; Bond *et al.* 2021; Draghi 2023). If so, identifying their molecular basis
337 and the gene networks that create such genotypes will be important for understanding adaptation and
338 persistence under global change. Alternatively, AP genotypes may have environmental sensitivities that are
339 only suitable for higher elevations, which would reduce their ability to help buffer novel low (and warmer)
340 elevations (Hargreaves & Eckert 2019). Testing whether AP genotypes increase relative fitness across a
341 broader range of novel environments, such as warmer conditions at lower elevations, will test whether they
342 represent bet-hedging genotypes that can assist population persistence in novel conditions more generally.

343 *Different forms of plasticity are favoured across an ecological gradient*
344 Consistent with other studies, we show that plasticity in different traits is likely to be important for
345 populations to maintain fitness across elevations (Anderson & Gezon 2015; Arnold *et al.* 2022). As
346 predicted, in three (of five) traits, a different direction and magnitude of plasticity was favoured as
347 environments became novel. Surprisingly, the traits most strongly associated with fitness in the original study
348 (SLA and flavonol content; Walter *et al.* 2023) were not associated with fitness in the current study. This
349 could be due to differences in climatic conditions between years. In 2018 the plants experienced a cooler
350 summer (lower temperatures than 2020) due to unusual summer storms (**Fig. S3**), which could have created
351 stronger selection on plasticity in SLA and flavonol content. Despite these among-year differences,
352 elevational patterns of mean fitness were consistent across experiments despite different traits responding,
353 suggesting consistent fitness responses of AP and HR genotypes to elevation.

354 *Contrasting patterns of plasticity in response to coarse versus local environmental variation*
355 AP genotypes showed lower plasticity as smaller elevational changes in trait means compared to HR
356 genotypes, suggesting that phenotypic stability helped them to maintain higher fitness in a novel
357 environment. Within elevations, however, AP genotypes typically showed greater among-clone phenotypic
358 variation than HR genotypes, which was associated with greater mean fitness and lower variation in fitness at
359 the novel elevation (**Fig. 6**). This is supported by our finding that AP genotypes showed greater changes in
360 gene expression across elevations than HR genotypes (Walter *et al.* 2023). AP genotypes are therefore
361 phenotypically robust across elevations, and are likely to be better than HR genotypes at minimising large
362 irreversible and potentially costly phenotypic changes across elevation (Hoffmann & Bridle 2022). Instead,

AP genotypes could maintain greater fitness than HR genotypes through larger changes in gene expression across elevations while making fine-scale phenotypic adjustments within an elevation. Such contrasting patterns of plasticity could be created by differences in their response to large climatic changes across elevation compared to variation in soil or biotic (e.g., soil biota) conditions within elevations (Paquette & Hargreaves 2021). Understanding the mechanisms that allow AP genotypes to better track novel environmental variation than HR genotypes can reveal how within-genotype variation could contribute to adaptive responses to novel environments (Hill & Mulder 2010; Prentice *et al.* 2020).

The genetic basis of increased adaptive potential in novel environments

Understanding the genetic architecture (e.g., dominance, number of loci) and population genetics (e.g., the frequency of alleles) in a population will improve predictions of the adaptive potential of populations in novel environments. However, adaptation to novel environments will only be possible if the effective population size remains large enough in the novel environment to avoid extinction (Chevin *et al.* 2013b; Gonzalez *et al.* 2013; Polechová & Barton 2015; Bridle *et al.* 2019). If AP genotypes are rare, then although they will be vital for producing adaptive responses to environmental change, they will need to help maintain a high enough population size to avoid extinction (Brennan *et al.* 2019). Simulations based on empirical estimates of ecological, life history and demographic parameters are therefore required to test whether selection for AP genotypes could increase to sufficient frequencies to allow adaptation to the novel environment (Bell & Gonzalez 2009; Connallon & Sgrò 2018; Bridle & Hoffmann 2022). In this study, however, we have shown that the hidden genetic variation in plasticity that could aid population persistence in novel environments involves genotypes that respond differently to coarse and fine-scale environmental variation, which differs from the adaptive plasticity that evolves within the native range.

Conclusions

In this study we show how hidden genetic variation in plasticity, which is likely to reflect bet-hedging genotypes, could aid population persistence in novel environments by better tracking coarse and fine variation in the environment to reduce the severity of fitness declines. We also show how different forms of plasticity are favoured across a natural ecological gradient from native to novel environments. Future experiments should focus on the genetic basis of AP genotypes to identify their frequency and demographic consequences in natural populations to predict adaptive responses to global change.

Statement of authorship: GW designed the study with JB, and input from AC, GE, JC, SC and SH. GE conducted the experiments, and collected and curated the data, with help from AC and DT. GW analysed the data and wrote the paper with help from GE and JB, and input from all authors.

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FIGURES

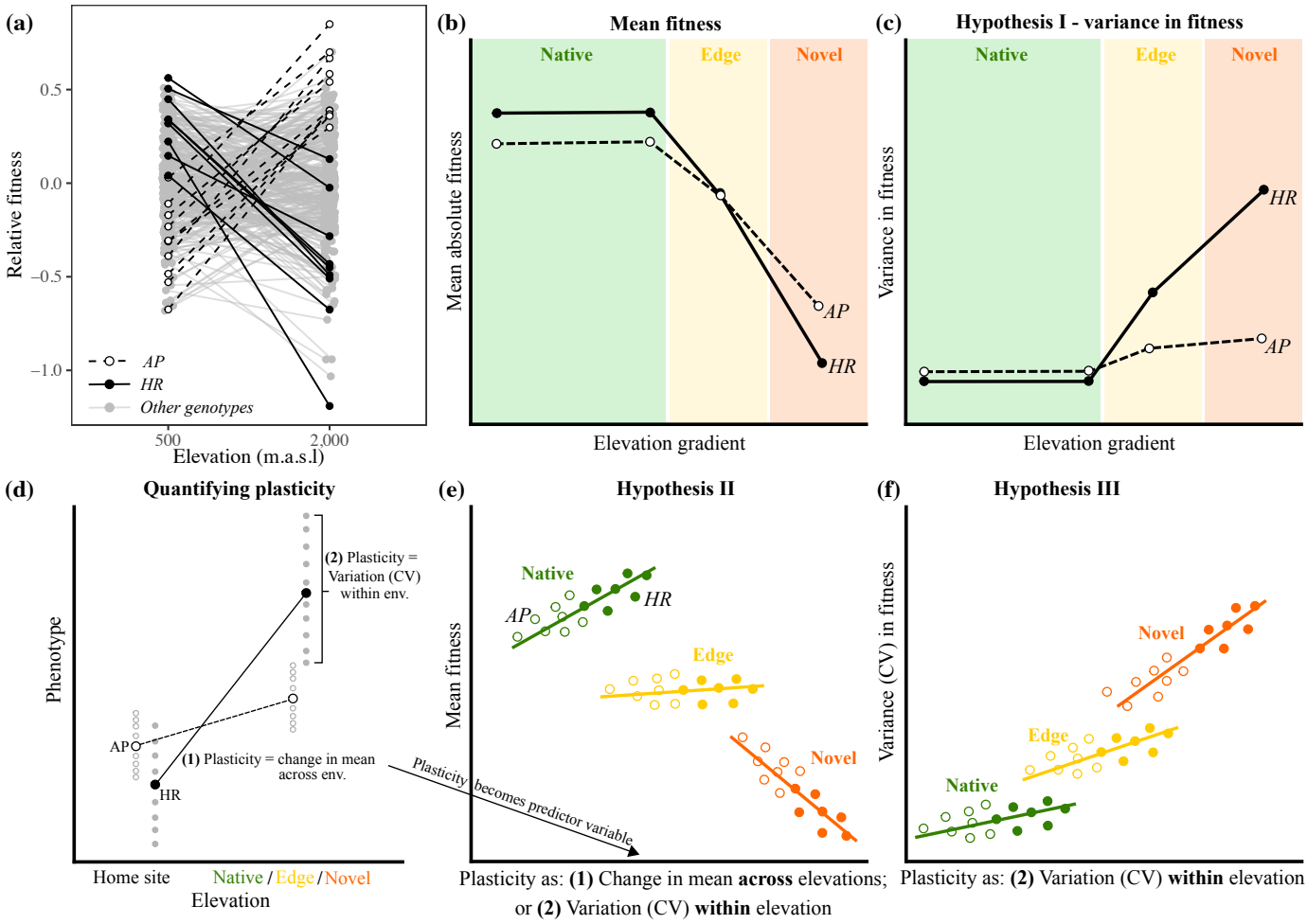

Fig. 1 Rationale for the choice of 'Adaptive Potential' and 'Home Range' genotypes from the results of the 2018 transplant experiment **(a)** and conceptual diagrams of the hypotheses **(c-f)**. **(a)** Genotypes were chosen from the 2018 experiment based on their change in relative fitness (independent of mean absolute fitness) from the home site (500m) to the novel elevation (2000m). Adaptive Potential (AP) genotypes showed greater relative fitness at the novel elevation, whereas Home Range (HR) genotypes showed greater relative fitness at the native elevation. This meant there was a negative genetic correlation in fitness of -0.11 between the native and novel elevation. In the current study, for each of the chosen 2018 genotypes, we transplanted c.40 cuttings at each of four elevations. We first expected to recover the same patterns of mean fitness as the original study, whereby HR genotypes would perform better than AP genotypes within the range, while AP genotypes would perform better in the novel environment. We then used this high level of replication per genotype at each elevation to test three hypotheses. **(c)** *Hypothesis I*: If AP genotypes have partially adaptive plasticity in the novel environment, they would show consistent fitness responses to the novel environment, which would result in low variance in fitness (among genotype and among-clone) in the novel environment compared to HR genotypes. **(d)** To connect fitness variation to plasticity, we first estimated two plasticity

metrics: (1) the change in mean phenotype across environments, and (2) the variation among clones within each environment. One AP genotype (open circles) and one HR genotype (closed circles) is depicted, with small grey circles representing clones within each environment, and large black circles representing the mean phenotype of that genotype. Reflecting the original study, we expected AP genotypes to show lower plasticity than HR genotypes. We then tested two hypotheses relating plasticity to fitness: **(e)** *Hypothesis II*: We predicted that the association between plasticity and fitness would change across the elevational gradient. Specifically, if greater phenotypic variation only helps to track changes within native environments, greater plasticity would be favoured within the native range, whereas lower plasticity would be favoured outside the native range. **(f)** *Hypothesis III:* If plasticity in response to fine-scale environmental variation determines fitness, we predicted that in native environments larger plasticity (variation in phenotype among clones within genotypes) would only weakly be associated with low variance in fitness because all genotypes would show similarly high fitness. By contrast, at the novel elevation, greater phenotypic changes would incur fitness costs and so greater plasticity (more variation among clones) would be associated with greater variance in fitness.

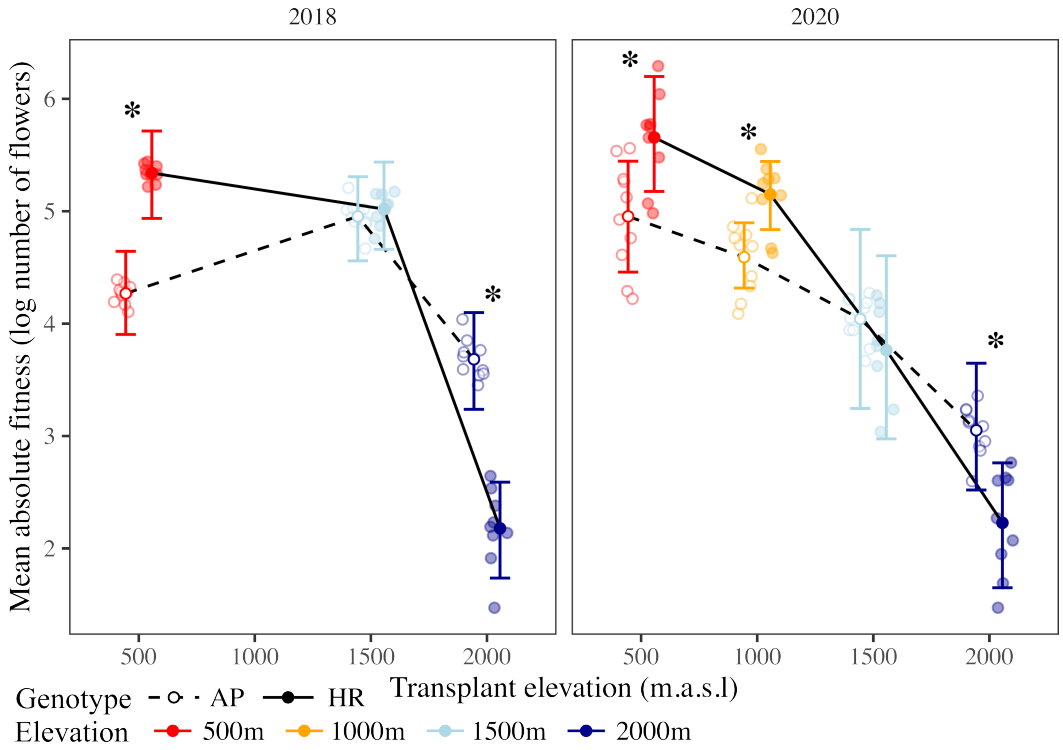

Fig. 2 Changes in mean fitness across elevation for the chosen genotypes in the original 2018 study and the current study (2020). Credible intervals represent the 90% Highest Posterior Density interval (HPD) of the mean. Asterisks denote significant differences in mean number of flowers between AP (Adaptive Potential: open circles and dashed lines) and HR (Home Range: closed circles and solid lines) genotypes at each elevation whereby their posterior distributions do not overlap at >90%. Small circles represent the mean for each genotype at each elevation.

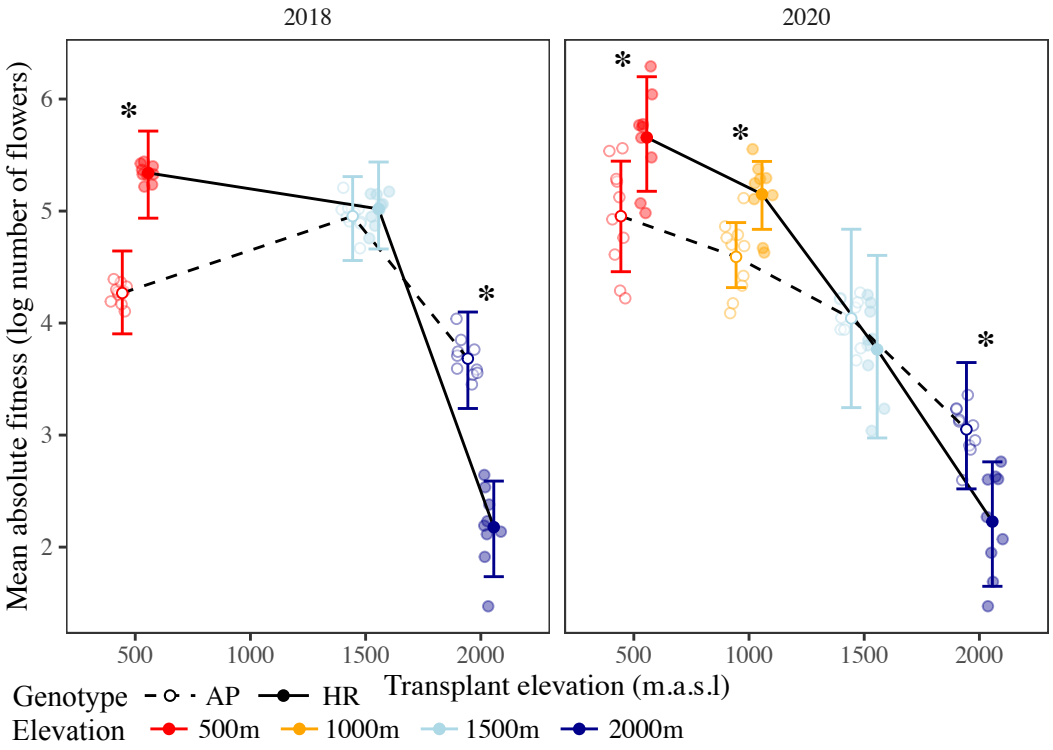

Fig. 3 *Hypothesis I:* Fitness variance among genotypes and among clones (within genotypes) changed across elevation differently for AP and HR genotypes, with the greatest difference in variation between the genotypes emerging at the novel elevation. Boxplots represent the posterior distribution of variance in fitness among: **(a)** genotypes, and **(b)** clones within genotype. Unfilled boxplots represent AP genotypes and filled boxplots HR genotypes. Asterisks denote significant differences where the posterior distributions do not overlap at >90%.

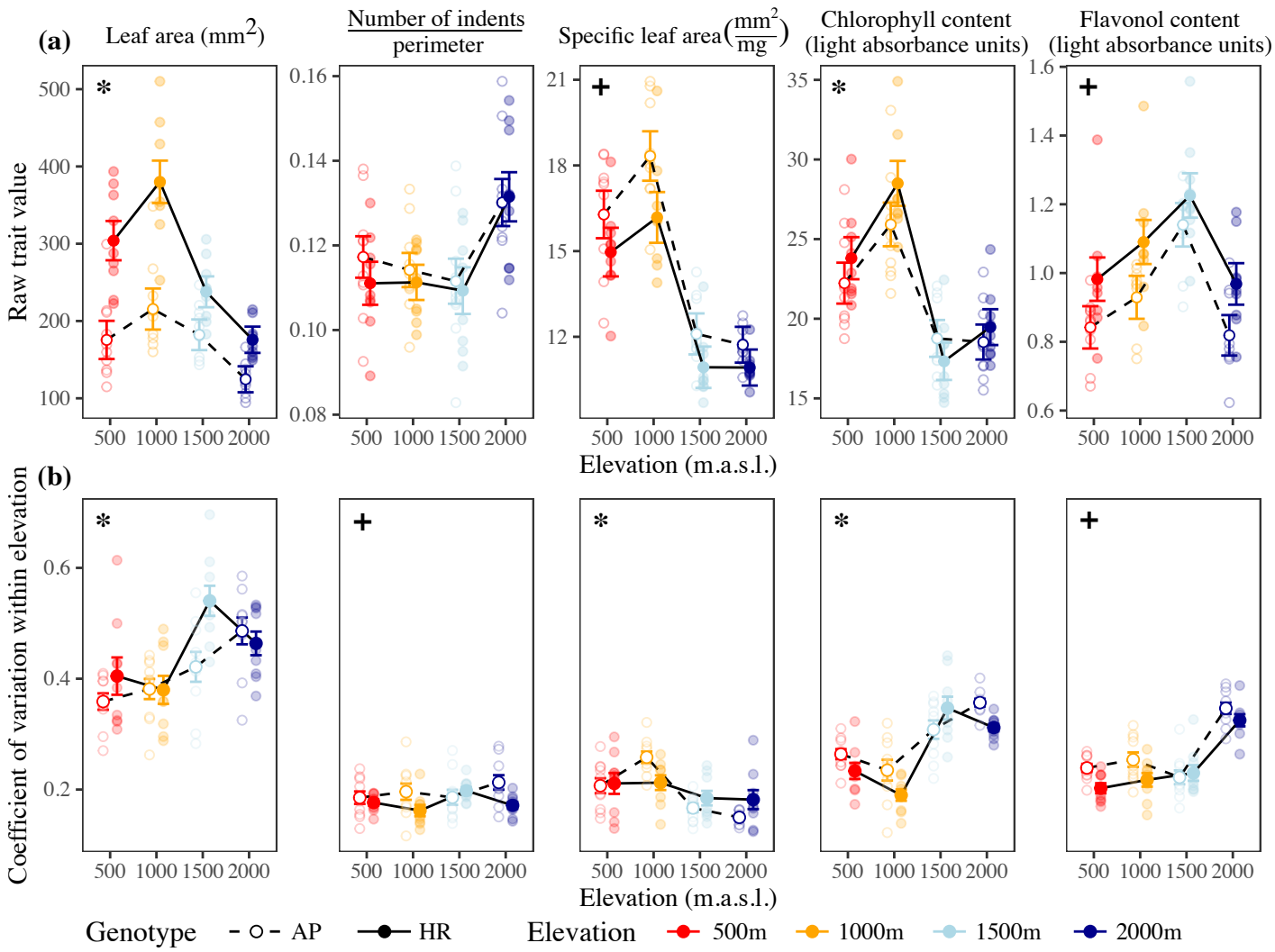

Fig. 4 Quantifying plasticity in leaf traits as **(a)** changes in trait means across elevation, and **(b)** and variance (CV) in phenotype within genotypes. AP genotypes are represented by open circles and dashed lines, and HR genotypes by closed circles and solid lines. Larger circles with credible intervals (±1 SE) represent the mean at each elevation, and small circles represent each genotype. Asterisks denote significant elevation×genotype (AP vs. HR) interaction, while plus (+) signs represent no significant interaction but significant differences between AP and HR genotypes. **(a)** For most traits, AP genotypes show smaller changes in phenotype across elevation compared to HR genotypes. **(b)** Most traits show an increase in variation among clones at higher elevations, with AP genotypes often showing greater variance compared to HR genotypes. Summary ANOVA tables are located in **Table S1**.

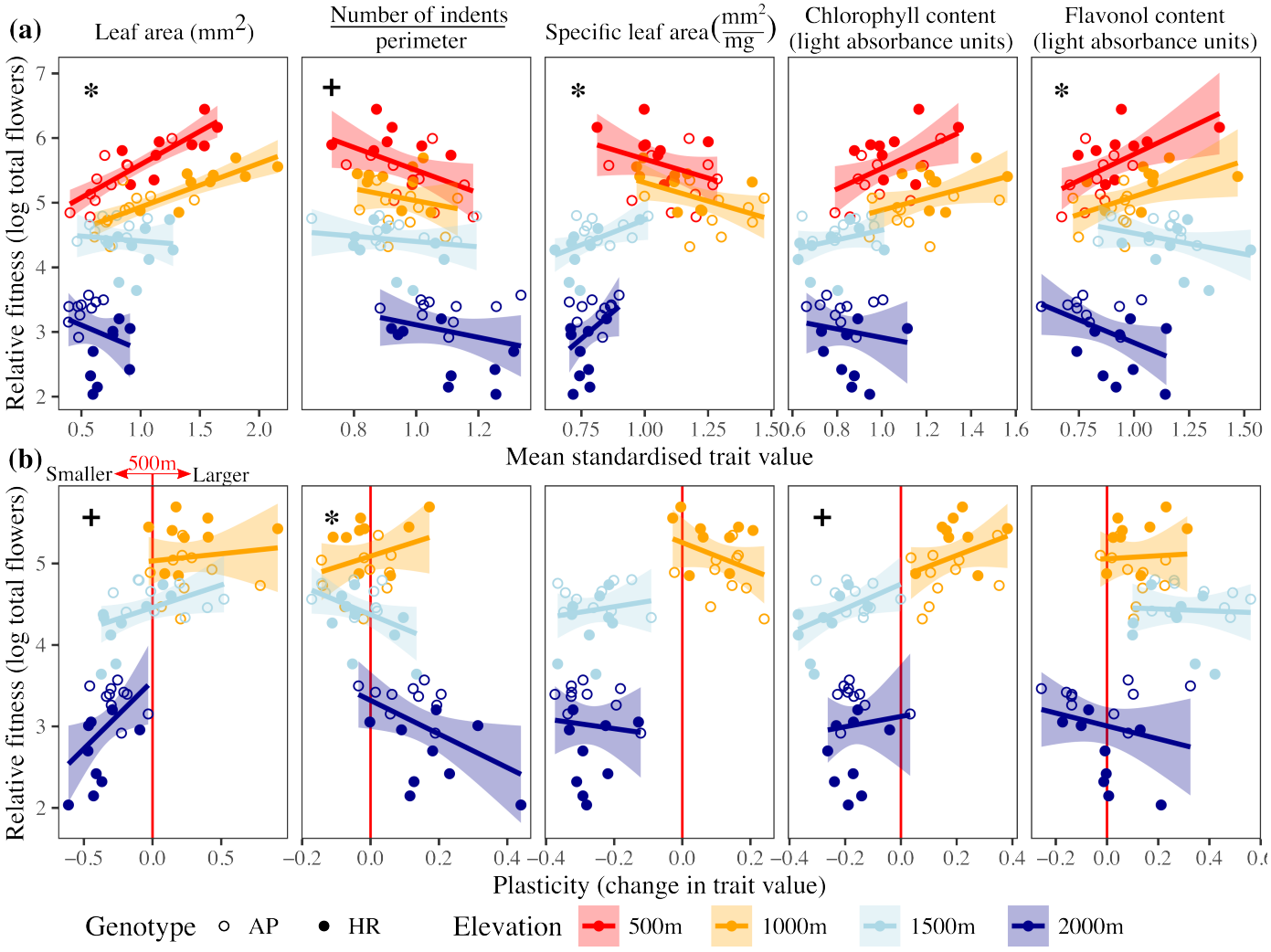

Fig. 5 Associations between **(a)** phenotype and **(b)** plasticity with fitness across elevation. Asterisks represent a significant interaction between the predictor variable (trait value or plasticity) and elevation. Addition (+) symbols represent significant regression slopes, but no significant interaction with elevation. AP and HR genotypes are represented by open and closed circles, respectively. **(a)** Genotypic values of each trait on fitness (phenotypic associations with fitness are located in **Fig. S2**). Three traits (leaf area, specific leaf area and flavonols) showed significant changes in selection on genotypes across elevation. **(b)** *Hypothesis II*: genotypic values of plasticity against fitness. Plasticity is represented as the change in mean phenotype from the home site (red vertical line) to each other elevation. Positive values represent plasticity as an increase in trait value, and negative values a decrease in trait value, from the home site. Leaf area and chlorophyll content show the same regression slopes across elevation, but have a different sign (positive versus negative), they show differences in plasticity because plasticity to 1000m is associated with an increase in each trait, whereas the change to 1500m and 2000m involves plasticity as a decrease in the same trait. Note that chlorophyll plasticity is only significantly associated with fitness for 1000m and 1500m, but not 2000m. Summary ANOVA tables are located in **Table S2-3**.

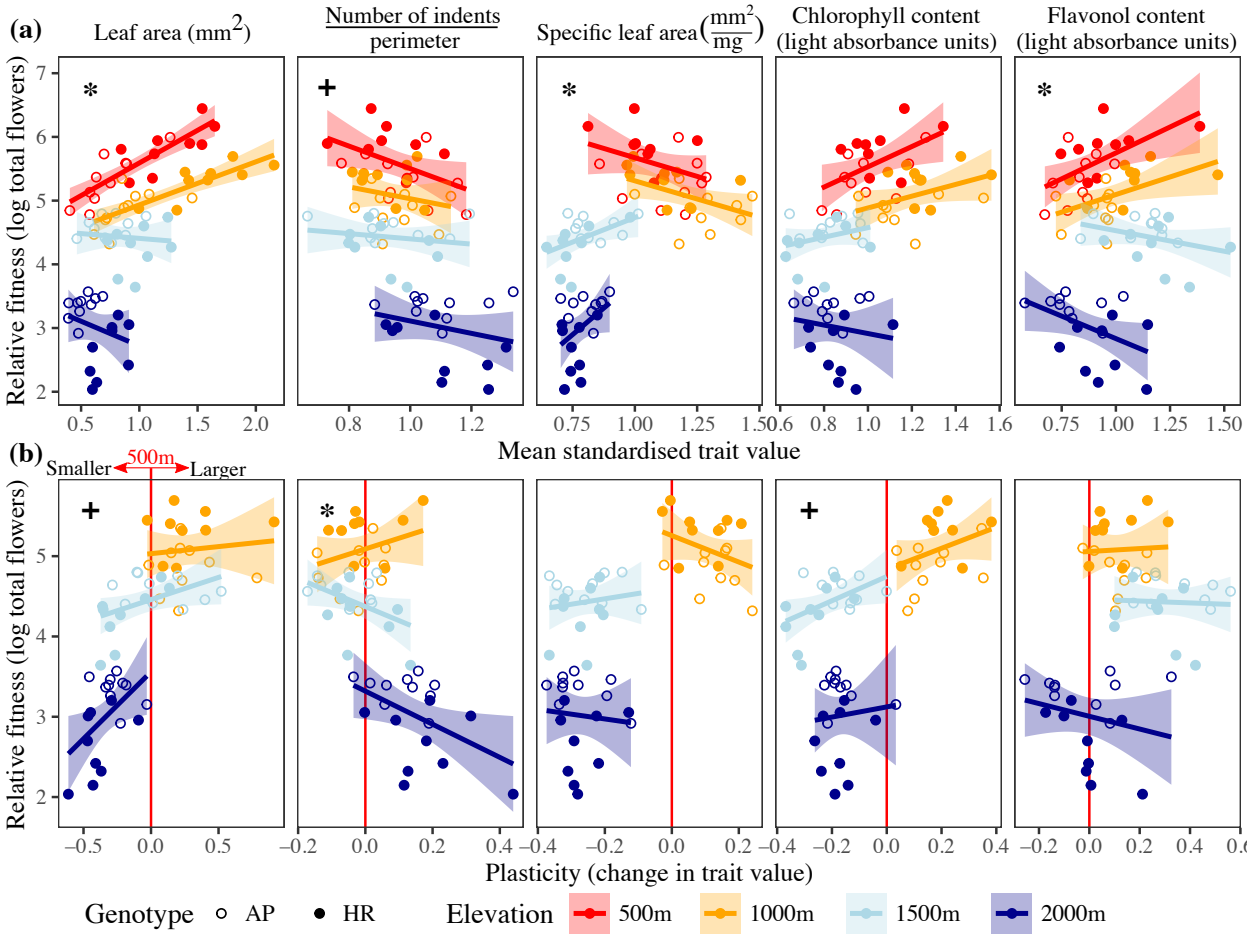

Fig. 6 Regression of plasticity as variance (CV) within each genotype at a given elevation against **(a)** mean absolute fitness (*Hypothesis II*), and **(b)** variance (CV) in fitness (*Hypothesis III*). Open circles represent AP genotypes, and closed circles HR genotypes. Shading represents one standard error of the regression. Asterisks denote significant interaction between plasticity and elevation, and plus (+) signs represent significant slopes but no significant interaction with elevation. Summary ANOVA tables are located in **Table S3**.