# 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 that better adjust their phenotype to cope with environmental change can aid persistence by reducing the severity of fitness declines. However, we know little about how plastic changes in phenotype allow different genotypes to track environmental variation across 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 assessed fitness at native and novel elevations and quantified leaf plasticity among and within elevations. Genotypes with higher fitness at novel elevations showed lower variance in fitness, lower plasticity across elevations, but higher plasticity within elevations compared to those with higher fitness in the native range. Our results suggest that there are genotypes hidden in a population whose plasticity better tracks novel environmental variation at multiple 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 beneficial phenotypes as the environment changes, allows populations to maintain fitness as the environment varies (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 environmental change is rapid and unpredictable. 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 result in fitness (and population) 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 should then be determined by the extent to which genotypes vary in their plastic response to novel environments, and how such differences in plasticity can maintain high enough mean fitness to avoid extinction (Chevin & Bridle 2025).

Within populations, genotypes often vary in plasticity and therefore in their sensitivity to the environment (Bradshaw 1965; Schlichting 1986; Scheiner 1993). Predicting population persistence under global change then requires identifying when and how genetic differences in plasticity can help populations maintain fitness as environments become novel (Yeh & Price 2004; Morris 2014). However, the need to assay hundreds of genotypes means that field studies quantifying genetic variation in plasticity are rare, and limited to a single model species and a few select environments (Matesanz *et al.* 2010; Gianoli & Valladares 2012; Merilä & Hendry 2014; Peschel *et al.* 2020). Consequently, field experiments typically lack the capacity to determine how genetic variation in plasticity determines fitness along environmental gradients. We therefore do not know how genotypes that differ in plasticity make phenotypic adjustments to maintain fitness within native environments, or how the same plasticity determines fitness as environments become novel. This gap in knowledge remains a significant barrier to understanding the adaptive potential of populations facing rapid global change (Chevin *et al.* 2010; Hendry 2016; Snell-Rood *et al.* 2018; Fox *et al.* 2019).

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 fitness declines (Lande 1988; Lande & Shannon 1996; Bell 2013). These genotypes that are better at maintaining fitness in novel environments can have lower relative fitness in native environments and remain hidden within the native range (Hermisson & Wagner 2004; Angert *et al.* 2008; Brennan *et al.* 2019; Walter *et al.* 2023). Such hidden genetic variation is a critical yet poorly understood source of adaptive potential for populations facing rapid environmental change. By selecting genotypes that differ in their ability to cope with novel environments, and then replicating these genotypes across native and novel environments, it is possible to test how genetic differences in plasticity determine fitness (Chevin *et al.* 2013). Comparing genotypes with higher relative fitness within the native range (HR, 'Home Range' genotypes) to genotypes with higher relative fitness in novel environments (AP, 'Adaptive Potential' genotypes) provides a powerful framework that harnesses the hidden genetic potential present in populations (**Fig. 1a**; Hermisson & Wagner 2004; Angert *et al.* 2008; Brennan *et al.* 2019; Walter *et al.* 2023). Transplanting numerous clones of HR and AP genotypes across an environmental gradient can test how genetic differences in fitness arise due to differences in plasticity in response to native and novel environmental variation at different scales along the gradient. This approach identifies the ecological scale at which plasticity varies the phenotype, and the effect on fitness, to reveal how genetic variation in plasticity could help populations to track environmental variation (e.g., during environmental change or range shifts) or support population persistence as environments become marginal (Valladares *et al.* 2007; Valladares *et al.* 2014; Donelson *et al.* 2019; Zettlemoyer 2023; Lewin *et al.* 2024).

Genotypes with contrasting fitness responses to novel environments can be used to test three hypotheses to understand how variation in plasticity determines fitness across native and novel environments. ***Hypothesis***

85 ***I:*** Adaptive plasticity reduces variance in fitness because all genotypes make the same adjustments to
86 maintain fitness (Richards *et al.* 2006; Ghalambor *et al.* 2007). Beneficial plasticity in AP genotypes will
87 then mean they consistently adjust their phenotype to better cope with novel environments, and so will show
88 lower variance in fitness at novel environments compared to HR genotypes (**Fig. 1b**). Conversely, HR
89 genotypes should show higher variance in fitness in novel environments if they produce a wider variety of
90 phenotypes that reduce mean fitness. ***Hypotheses II-III*** compare plasticity in response to fine-scale variation
91 within environments, with coarse changes across environments, to test how environmental scale influences
92 plasticity to determine fitness in native and novel environments (**Fig. 1c**). ***Hypothesis II:*** While selection in
93 fine-scale heterogeneous environments favours the evolution of adaptive plasticity (Baythavong & Stanton
94 2010; Baythavong 2011), we do not know if plasticity with the same magnitude or direction is favoured
95 across native and novel environments, or whether this changes with environmental scale. Greater plasticity
96 should be favoured at fine and coarse scales in native environments because all phenotypic adjustments will
97 maintain high mean fitness. By contrast, novel environments should favour reduced plasticity at both scales
98 because large phenotypic changes become maladaptive (**Fig. 1d**; DeWitt *et al.* 1998; Murren *et al.* 2015;
99 Snell-Rood *et al.* 2018). ***Hypothesis III:*** All plastic responses to fine-scale variation should maintain high
100 fitness in native environments, but if the same plasticity becomes maladaptive in novel environments, then
101 any variation in phenotype will affect fitness and higher plasticity should increase variance in fitness (**Fig.**
102 **1e**).

103 *Senecio* (Asteraceae) wildflower species that inhabit Mt Etna (Sicily) are a powerful system to test how
104 plasticity is linked to adaptive capacity across elevations under semi-natural field conditions (Walter *et al.*
105 2020). We focus on *S. chrysanthemifolius* that is native to c.400-1500m elevation on Mt Etna. This species is
106 a self-incompatible, short-lived perennial that relies on generalist insect pollinators (e.g., hoverflies). A
107 closely related sister species, *S. aethnensis,* occurs on old lava flows at high elevations that are covered by
108 snow each winter. Previous reciprocal transplant experiments have shown that the two *Senecio* species are
109 adapted to their contrasting habitats, which has created differences in plasticity and genetic variance
110 underlying leaf traits (Walter *et al.* 2022a; Walter *et al.* 2024).

111 Here we present a large field experiment conducted in 2020, which significantly extends our 2018 field
112 experiment that transplanted cuttings of a breeding design of *S. chrysanthemifolius* on Mt Etna. We showed
113 greater adaptive potential at a novel 2000m elevation was created by a three-fold increase in genetic variance
114 in fitness, which was associated with genetic variance in plasticity (Walter *et al.* 2023). From the 2018 study,
115 we selected 19 genotypes that showed contrasting fitness responses across elevations (**Box 1**). In 2020, we
116 transplanted numerous clones (n=40 per elevation) of the 19 genotypes at each of four elevations and
117 quantified fitness and phenotype to test the three hypotheses outlined above (**Fig. 1**). Transplanting clones at

high replication across the entire elevational gradient provided two benefits. First, it allowed us to test whether different forms of plasticity (magnitude and direction) are favoured within the native range compared to novel elevations, which builds on the original study that focused on plasticity at the novel elevation. Second, we could test how plasticity determines fitness at different ecological scales, both across elevations that represent large-scale environmental changes, as well as within elevations where plasticity varies the phenotype in response to fine-scale environmental variation.

## MATERIAL AND METHODS

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. 2a**). In 2020, we propagated 160 cuttings of each genotype and transplanted them at four elevations representing two sites within their native range (500m and 1000m), their range edge (1500m) and a novel elevation (2000m). We measured leaf traits and fitness on mature plants.

*Field transplant*

In Spring (2020), we propagated the 19 genotypes from the 2018 study by taking cuttings from plants maintained in a greenhouse (Giarre, Italy). For each genotype, we propagated 10 clones in 14cm diameter pots, randomised their location and let them grow into large plants (c.40cm high). We then removed 6-7 branches from each plant, which we cut into 4cm segments with 2-3 leaf nodes. We dipped the smaller cuttings in rooting hormone (Germon Bew., Der.NAA 0.5%, L.Gobbi, Italy) and placed them in an 84-cell tray containing an equal mix of compressed coconut coir and perlite. We kept trays on benches covered with plastic for three weeks to maintain high humidity and promote root formation.

Rooted cuttings of each genotype were transplanted in early summer (29-30$^{th}$ June) to four elevations on the south-eastern slope of Mt Etna: 500m, 1000m, 1500m and 2000m. The 500m site was in a garden among fruit trees, the 1000m site in an abandoned vineyard, the 1500m site in an abandoned apple orchard, and the 2000m site on a lava flow from 1983 with pine trees present (Walter *et al.* 2022a; Walter *et al.* 2024). Higher elevations experience consistently colder temperatures (**Fig. S1**), and soil across the elevation gradient changes from silty sand at elevations 500-1500m to volcanic soil at 2000m (Walter *et al.* 2022a). We transplanted 160 cuttings/genotype divided equally across elevations. At each elevation, we randomised the

40 cuttings/genotype into four spatially distinct experimental blocks (*n*=190 plants/block; *n*=760 plants/elevation; N=3040 plants), within an area of c.5000m$^2$. At each block, we cleared vegetation and debris, turned the soil 30cm deep, and then planted the cuttings 30cm apart in a grid of 7×29 plants (**Fig. S2**). We irrigated the cuttings daily for three weeks so they could establish, and then reduced irrigation to only the hottest days to prevent high mortality.

*Data collection*

We took data for each plant c.4 months after the initial transplant (13-23$^{rd}$ October). As our proxy for fitness, we collected all flowerheads produced by each plant in paper bags, which we counted in the laboratory. This trait is routinely used to estimate fitness in short-lived perennials (e.g., Gross *et al.* 2004; Pujol *et al.* 2014), including in our previous experiment that showed a close association with seed production (Walter *et al.* 2023). To measure five ecologically important leaf traits, we sampled 3-4 fully expanded young leaves from each plant, which we weighed and then scanned to quantify morphology using the program *Lamina* (Bylesjo *et al.* 2008). We used three leaf traits to represent leaf size, shape and investment: leaf area (mm$^2$), perimeter (mm) and the number of indentations (count). To calculate the density of leaf indents, we standardized the number of indentations by the perimeter. Using the weight of the leaves, we estimated specific leaf area as $SLA = \frac{leaf\ area}{leaf\ weight}$, where greater values represent larger leaves per unit mass. Using a Dualex instrument (Force-A, France), we measured chlorophyll and flavonol pigment content (light absorbance units) for two mature leaves per plant. Flavonols are secondary metabolites produced under stressful abiotic (e.g. temperature) and biotic (e.g. herbivore) conditions (Mierziak *et al.* 2014). We chose leaf morphology and investment traits because they show strong plasticity in response to elevation, and are associated with reproductive fitness in *Senecio* (Brennan *et al.* 2009; Walter *et al.* 2023; Walter *et al.* 2024), and other plants (Dudley 1996; Ackerly *et al.* 2000; Van Kleunen & Fischer 2005; Gianoli & Saldaña 2013; Damián *et al.* 2020).

*Hypothesis I: Statistical analyses of fitness*

All analyses were conducted in R (v.4.3.2; R Core Team 2024). To quantify mean fitness across elevation, we used *MCMCglmm* (Hadfield 2010) to apply

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

where the interaction between fixed effects of genotype class ($R_j$; AP vs HR) and elevation ($E_i$) quantifies whether AP and HR genotypes show different fitness responses to elevation. Random effects include $g_k$ that captures variation among the 19 genotypes, and $b_l$ variation among experimental blocks. $e_{m(ijkl)}$ are the residuals. For each random effect, we estimated variances at each elevation. We included the number of

flowers as the poisson-distributed fitness response ($y_{ijklm}$). Equation 1 yielded the posterior distribution of mean fitness for AP and HR genotypes at each elevation. We then used a second application of equation 1 on AP and HR genotypes separately to test whether among-genotype and among-clone (within genotypes, i.e., residual variation) variation were different between AP and HR genotypes across elevations (*Hypothesis I*, **Fig. 1b**).

*Statistical analyses of phenotype and calculation of plasticity*

To test whether AP and HR genotypes showed differences in phenotype across elevation, we used *glmmTMB* (Brooks *et al.* 2017) to apply equation 1, but included the five leaf traits as univariate response variables. We used type-III ANOVA (Fox & Weisberg 2019) to test for significant $E_i \times R_j$ interactions, which indicate genotypic differences in plasticity to elevation. We then used *emmeans* (Lenth 2019) to obtain estimated marginal means for each genotype, which we used to calculate plasticity with

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

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

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

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

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

*Hypotheses II-III: Connecting plasticity with fitness*

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

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

where $E_i$ represents the *i*th elevation and $T$ a leaf trait. We included fitness as the response variable ($y_{ij}$) and $e_{j(i)}$ are 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 using genotype means 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

*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 (1) greater variance in fitness (within and among genotypes) than AP genotypes at novel elevations, and (2) an increase in genetic variance in fitness at higher elevations. Contrary to predictions, AP genotypes showed a reduction (rather than a slight 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**).

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

245 *Hypothesis II – Different forms of plasticity were favoured across versus within elevations*
246 All five traits showed significant associations between phenotype and fitness, and, except for the number of
247 leaf indents, higher values of each trait were favoured at all elevations (**Fig. S3**). Associations between traits
248 and fitness changed significantly across elevation for leaf area, SLA and flavonol content, such that higher
249 elevations favoured smaller leaves, lower flavonol content and greater SLA (**Fig. 5a; Table S2**). Genotypes
250 with different trait values were therefore favoured in the native range compared to the novel elevation.

251 We predicted that greater plasticity would increase fitness within the native range, but reduce fitness at the
252 novel elevation (**Fig. 1d**). We found support for *Hypothesis II*. Associations between plasticity and fitness
253 changed significantly across elevation for three (of five) traits: leaf area, leaf indentation and chlorophyll
254 content (**Fig. 5b; Table S3**). Only leaf indentation showed a significant elevation×plasticity interaction,
255 which meant that plasticity as an increase in leaf indentation within the native range was favoured, whereas
256 reduced indentation at higher elevations was favoured. For leaf area and chlorophyll content, associations
257 between plasticity and fitness were positive at all elevations (**Fig. 5b**). However, because plasticity increased
258 trait values within the range, but decreased them outside the range, different forms of plasticity were
259 favoured at the 1000m native site compared to higher elevations. Specifically, at the 1000m native site,
260 plasticity increased leaf area and chlorophyll content for all genotypes, and greater plasticity (i.e., larger
261 increases in these traits) was favoured. By contrast, plasticity at 1500m and 2000m reduced leaf area and
262 chlorophyll content for all genotypes, and lower plasticity (i.e., smaller reductions in these traits) was
263 favoured (**Fig. 5b**). Consistent with the original study, higher fitness at 2000m was associated with smaller
264 changes in leaf area, indentation and chlorophyll content. However, unlike the original study, plasticity in
265 SLA and flavonol content did not show strong associations with fitness.

266 For each genotype, CV within an elevation represents plasticity as variation in phenotype in response to
267 environmental variation within that elevation. Plasticity within elevations was associated with mean fitness
268 for three traits: leaf indents, SLA and chlorophyll content, and this association changed across elevation for
269 leaf indents and chlorophyll (**Fig. 6a; Table S3**). However, our results contradicted *Hypothesis II:* greater
270 variance in phenotype (i.e., greater plasticity) was associated with lower fitness within the native range, but

271 greater fitness outside the native range (**Fig. 6a**). At the novel elevation, four traits showed positive
272 associations between plasticity and mean fitness, and only SLA showed the predicted negative trend (**Fig.**
273 **6a**). These results suggest that plasticity of different magnitudes were associated with mean fitness within
274 versus among elevations.

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

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288

289 **DISCUSSION**

290 To test how genetic differences in plasticity influence fitness across an ecological gradient, we transplanted
291 large numbers of clones of 19 genotypes at four elevations on Mt Etna, Sicily. Genotypes were selected from
292 a previous experiment based on their contrasting fitness response to elevation (Walter *et al.* 2023): Home
293 Range (HR) genotypes showed higher relative fitness at native elevations, but lower relative fitness than
294 Adaptive Potential (AP) genotypes at the novel 2000m elevation (**Box 1**). Supporting *Hypothesis I*, HR
295 genotypes showed increased variation in fitness at higher elevations, whereas AP genotypes showed a
296 decrease (**Fig. 3**). AP genotypes therefore coped better and showed more consistent responses to the novel
297 environment. By contrast, HR genotypes produced a variety of responses that resulted in lower mean fitness
298 and higher variance in fitness in the novel environment.

299 Supporting *Hypothesis II*, genotypic differences in plasticity were associated with fitness. Across elevations,
300 higher plasticity was favoured within the native range, whereas lower plasticity was favoured at the novel
301 elevation (**Fig. 5b**). However, plasticity as variation within elevations showed a contrasting result: within

native elevations lower plasticity was often favoured, whereas within the novel elevation higher plasticity was favoured (**Fig. 6a**). Consistent with *Hypothesis III*, plasticity within native elevations was weakly associated with variance in fitness, suggesting that phenotypic changes in native environments maintained high mean fitness. However, contrary to *Hypothesis III*, higher plasticity was often associated with lower variance in fitness at the novel elevation (**Fig. 6b**).

Our results provide two important insights for understanding how genetic variation in plasticity contributes to population persistence across environments. First, we provide some of the first evidence that genotypes with different forms (magnitude and direction) of plasticity are favoured within their native range compared to novel environments. Second, we show that plasticity in response to fine-scale environmental variation did not follow the same association with fitness as plasticity created by coarse changes in elevation. Specifically, we show that genotypes important for population persistence at novel elevations make smaller phenotypic adjustments across elevations, but larger adjustments within elevations.

*Implications for predicting population persistence under global change*

Our results provide some of the strongest evidence that where populations face novel environments, adaptive plasticity fails to maintain high mean fitness, and genetic variation in plasticity will be important for population persistence. By focussing on genotypes with contrasting fitness responses to elevation, the current study is the first to show that selection on plasticity changes across elevation, and that genotypes present in populations can track native and novel environments differently, which also depends on the spatial scale of environmental variation. Our in-depth comparison of AP and HR genotypes moves beyond demonstrating genetic variance in plasticity to reveal how hidden genetic variation in plasticity in natural populations could allow new forms of adaptive plasticity to evolve (Lande 2009; Usui *et al.* 2023), help populations to maintain fitness and persist as environments change (Chevin & Hoffmann 2017), or help them shift their geographical range in response to global change (Valladares *et al.* 2007; Valladares *et al.* 2014; Donelson *et al.* 2019; Zettlemoyer 2023; Lewin *et al.* 2024). The frequency and genetic basis of such genotypes, as well as their absolute fitness in novel environments, will determine their potential to aid population persistence under environmental change.

To predict the resilience of ecological communities, future experiments should expand the framework to multiple species and assay genotypes from across a metapopulation in conditions predicted under climate change. This would test whether AP genotypes are broadly common across populations and species, or whether they are only present in species with large populations that experience greater environmental heterogeneity, which would favour the generation and maintenance of AP genotypes. Encouragingly, a field experiment with both Etnean *Senecio* species showed increased genetic variance in seedling survival at both

334 novel (high and low) elevations (Walter *et al.* 2022b), suggesting that AP genotypes are present (1) at early
335 life history stages, and (2) in species from contrasting environments that experienced different novel
336 environments. Including a variety of novel environments would test whether AP genotypes can aid
337 persistence across a range of environments, rather than only high elevations. Also testing for AP genotypes in
338 species showing range stasis versus expansion or shifts (or species with different sized distributions) would
339 confirm whether AP genotypes emerge as a broader phenomenon across different ecological contexts for
340 coping with environmental change. Identifying AP genotypes is challenging but would benefit conservation
341 efforts to increase resilience in species from vulnerable ecosystems.

342 *Genetic variation for population persistence under environmental change*

343 AP genotypes could be maintained in populations through interannual or seasonal variation. Environmental
344 variation that causes fluctuating selection would then maintain genetic variation in plasticity that reduces
345 vulnerability to environmental change (Gillespie & Turelli 1989; Svardal *et al.* 2011; Wittmann *et al.* 2017).
346 AP genotypes could represent 'generalist' genotypes that have lower arithmetic fitness within their native
347 range, but high geometric fitness across a broader range of spatial or temporal environments (Gillespie 1974;
348 Childs *et al.* 2010; Svardal *et al.* 2011; Tufto 2015; Draghi 2023). This would suggest that AP genotypes are
349 'bet-hedging' genotypes that can buffer greater environmental variation (Simons 2011). Bet-hedging
350 genotypes would then be important for population persistence given they reduce the fitness costs to the
351 population as environments change rapidly and/or unpredictably (Gillespie 1974; Childs *et al.* 2010; Bond *et*
352 *al.* 2021; Draghi 2023). Compared to HR genotypes, clones of AP genotypes showed more beneficial
353 plasticity in response to fine-scale variation at the novel elevation, which supports the idea that clonal (i.e.,
354 environmental) responses are under genetic control and contributes to the genetic basis of adaptive plasticity
355 (Prentice *et al.* 2020). Identifying the molecular mechanisms that determine how AP genotypes track novel
356 variation at fine and coarse scales will help to more accurately predict population persistence under global
357 change.

358 *Contrasting patterns of plasticity in response to coarse versus fine-scale environmental variation*

359 AP genotypes showed lower plasticity as smaller phenotypic changes across elevation compared to HR
360 genotypes, suggesting that phenotypic stability or robustness helps maintain higher fitness in novel
361 environments. AP genotypes therefore seemed better than HR genotypes at minimising large irreversible and
362 costly phenotypic changes across elevations (Hoffmann & Bridle 2022). However, AP genotypes typically
363 showed higher plasticity than HR genotypes within elevations, which was associated with higher mean
364 fitness and lower variation in fitness at the novel elevation (**Fig. 6**). Previously, AP genotypes showed larger
365 changes in gene expression across elevations compared to HR genotypes (Walter *et al.* 2023). AP genotypes
366 could therefore maintain greater fitness than HR genotypes at the novel elevation through larger changes in

gene expression across elevations and more fine-scale phenotypic adjustments within elevation. Fine-scale adjustments could be less important within the native range where large phenotypic responses to reliable environmental cues maintain fitness, whereas the same plasticity becomes costly in response to unreliable cues in novel environments that favour phenotypic stability and a fine-tuning of phenotype and gene expression. Such contrasting patterns of plasticity could be created by differences in the response to large climatic changes across elevation compared to variation in edaphic or biotic (e.g., soil biota) conditions within elevations (Paquette & Hargreaves 2021).

*Idiosyncrasies that could determine the generality of AP genotypes*

Here we provide a framework for identifying genotypes important for population persistence in novel environments. While we used flower output as our fitness metric, which may not reflect variation in growth or survival, future experiments should test whether AP genotypes emerge using other performance measures, such as accumulated biomass, which could be valuable to conservation efforts seeking to increase resilience in long-lived species. It will then be important to understand whether AP genotypes are broadly beneficial across many novel environments or whether their fitness benefits are specific to high elevations. Testing AP and HR genotypes across a broader range of novel environments, such as warmer conditions at lower elevations, will identify whether they represent bet-hedging genotypes that can assist population persistence in novel conditions more generally. While fine-scale edaphic or biotic effects could create phenotypic and fitness variation within elevations, large temperature differences could underlie the large phenotypic changes across elevations. Further work is therefore necessary to separate temperature from other environmental cues to identify whether, and at what temporal and spatial scales, AP genotypes could aid population persistence to global change.

*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 that reduces the severity of fitness declines. We also demonstrate that different forms of plasticity are favoured across a natural ecological gradient from native to novel environments. To predict the potential for AP genotypes to aid population persistence under global change, future experiments should focus on their molecular basis, their frequency within and across species, the generality of their fitness benefits across novel environments, and the demographic consequences of their spread.

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**Statement of authorship:** GW designed the study with input from JB, 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.

**Data availability:** All data and code are available at https://osf.io/yjk5g/.

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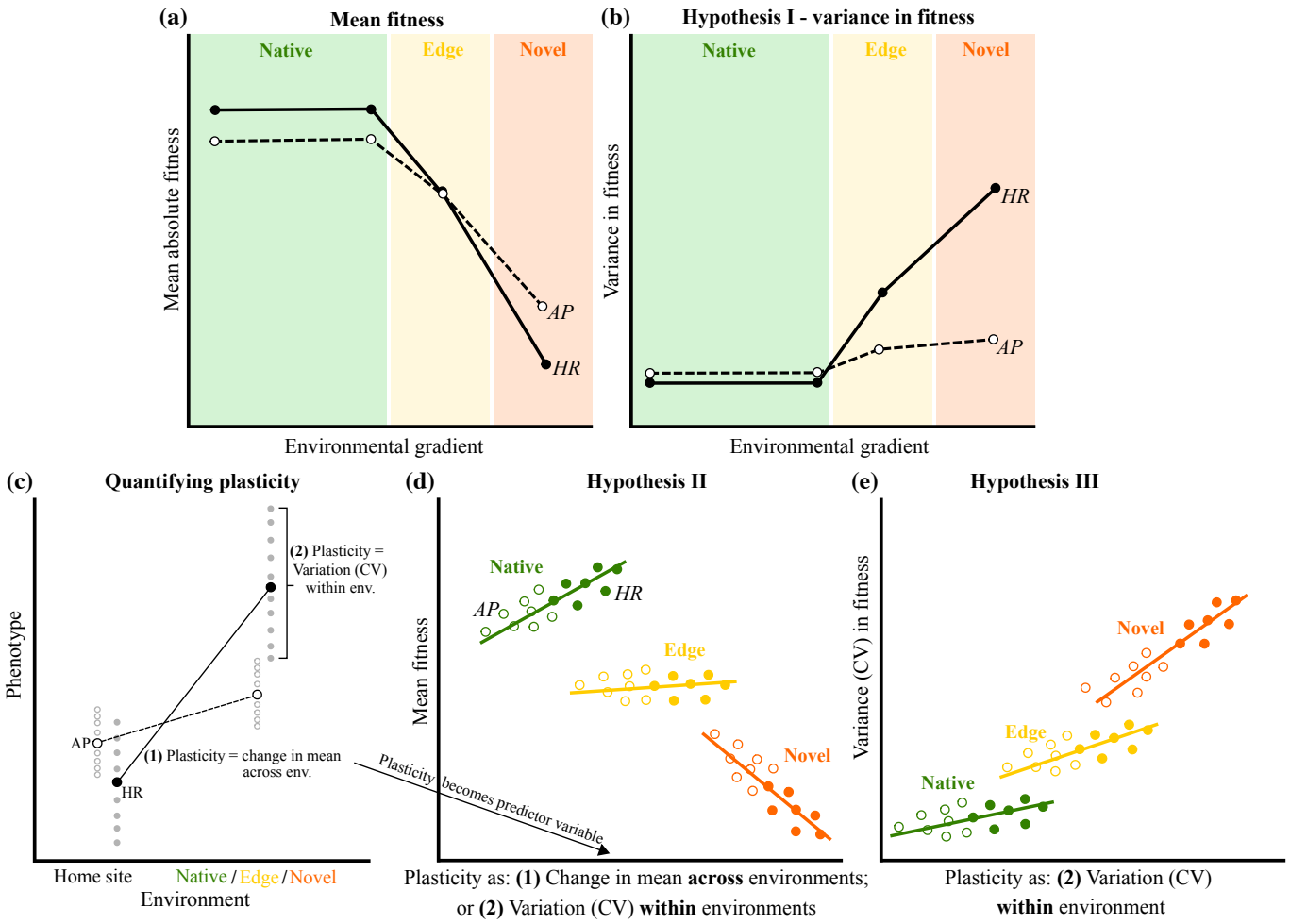
675 **FIGURES & BOXES**

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677 **Fig. 1** Conceptual framework for testing how genetic variation in plasticity maintains fitness across
678 environmental scales as environments become novel. **(a)** Selection of genotypes that include HR ('Home
679 Range'; closed circles and solid lines) genotypes that show greater relative fitness in native environments,
680 compared to AP ('Adaptive Potential', open circles and dashed lines) genotypes that show greater relative
681 fitness in novel environments. **(b)** ***Hypothesis I*****:** If AP genotypes have partially adaptive plasticity in the
682 novel environment, they would show consistently higher fitness in responses to the novel environment,
683 which would result in lower variance in fitness in the novel environment compared to HR genotypes. **(c)** To
684 connect fitness variation to phenotypic plasticity, we first estimated two plasticity metrics: (1) the change in
685 mean phenotype across environments, and (2) the variation among clones in response to small-scale variation
686 within each environment. One AP genotype (open circles) and one HR genotype (closed circles) is depicted,
687 with small grey circles representing clones within each environment, and large black circles representing the
688 mean of that genotype. Reflecting the original study, we expected AP genotypes to show lower plasticity
689 than HR genotypes. We then tested two hypotheses relating plasticity to fitness: **(d)** ***Hypothesis II*****:** We
690 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. **(e)** ***Hypothesis III:*** If plasticity in response to fine-scale environmental variation determines fitness, we predicted that in native environments larger plasticity would only be weakly associated with variance in fitness because all genotypes would show similarly high mean fitness. By contrast, at the novel elevation, greater phenotypic changes would incur fitness costs and so greater plasticity would be associated with greater variance in fitness.

**- Box 1 -**

In the 2018 study, we connected genetic variance in plasticity with fitness at a novel 2000m elevation by transplanting cuttings (clones) of 314 genotypes 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 a native elevation. This increased adaptive potential was associated with genetic differences in plasticity. 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 chose genotypes 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 (**Fig. 2a**). In the current study, for each of the chosen 2018 genotypes, we transplanted c.40 cuttings at each of four elevations. We recovered the same patterns of mean fitness as the original study: HR genotypes performed better than AP genotypes at native elevations, and AP genotypes performed better at the novel environment (**Fig. 2b**). Our findings were therefore consistent across years, and including an additional 1000m elevation showed that AP genotypes had consistently lower fitness in the native range.

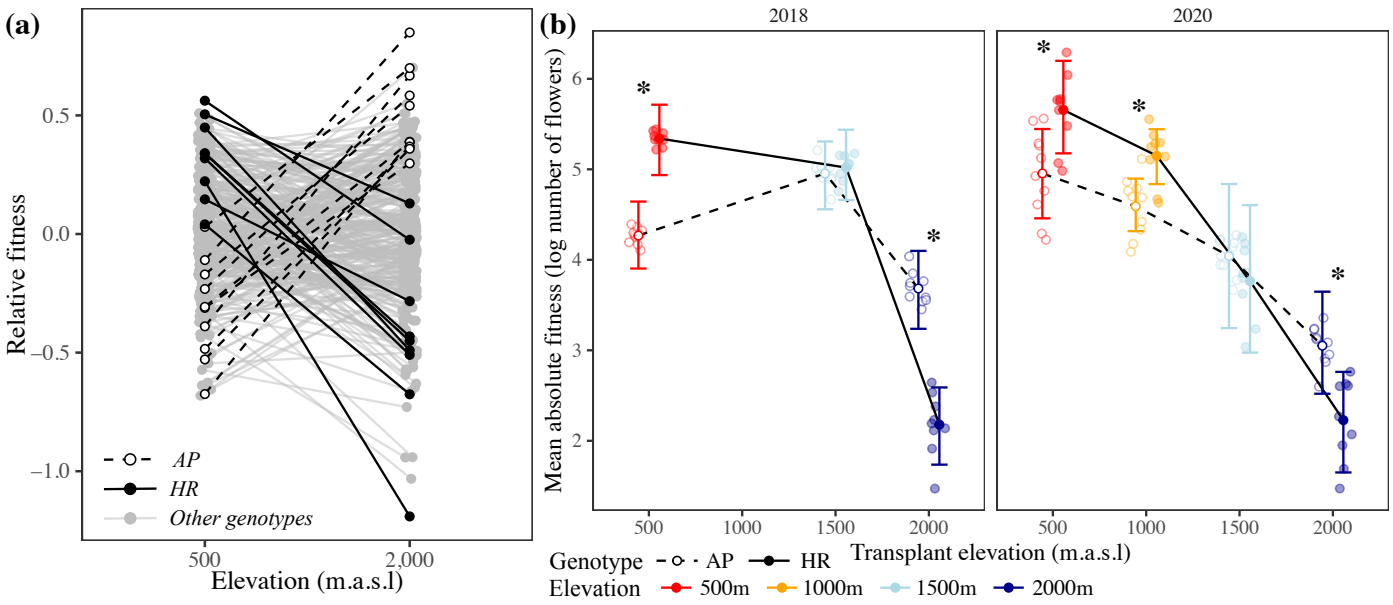


**Fig. 2 (a)** Genotypes were chosen from the 2018 experiment based on their change in relative fitness from their home site to the novel 2000m elevation. **(b)** Changes in mean fitness across elevation for the chosen genotypes in the original 2018 study and the current study (2020). Credible intervals 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:

721 closed circles and solid lines) genotypes at each elevation whereby their posterior distributions do not overlap
722 at >90%. Small circles represent the mean for each genotype at each elevation.

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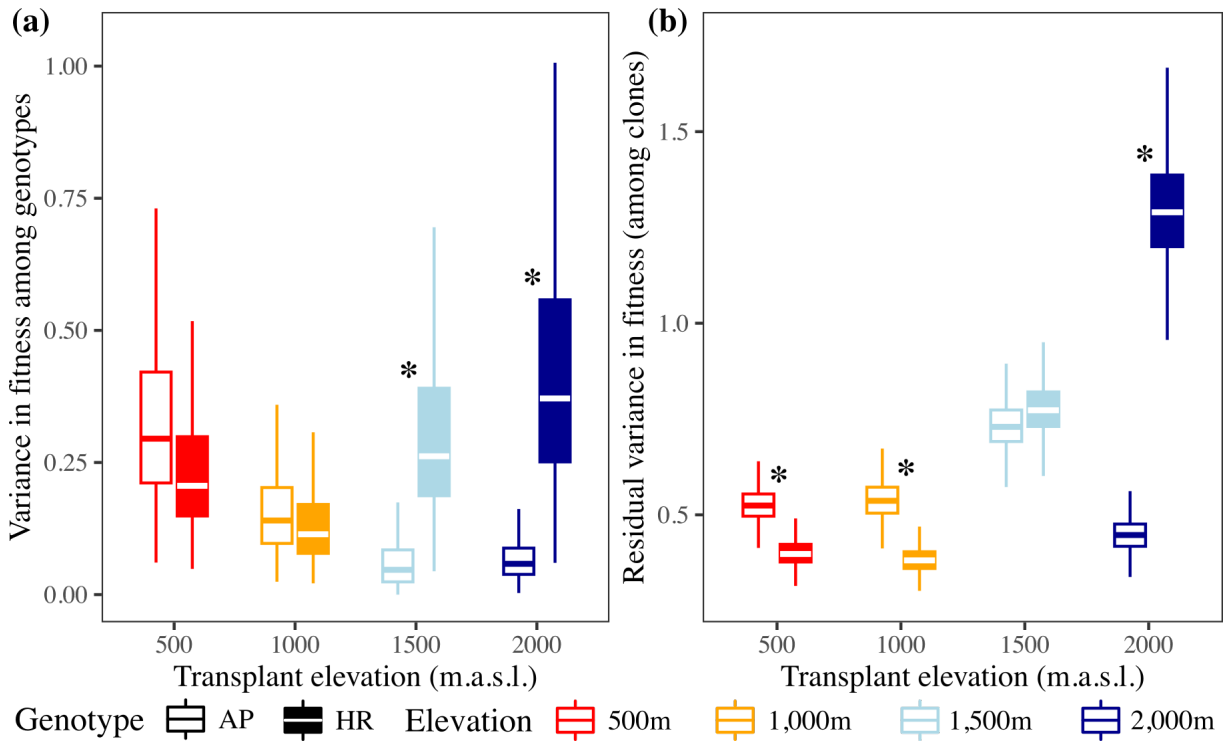



**Fig. 3** *Hypothesis I:* Fitness variance among genotypes and among clones (within genotypes) changed across elevation differently for AP and HR genotypes, with the greatest difference in variation between the genotypes emerging at the novel elevation. Boxplots represent the posterior distribution 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%.

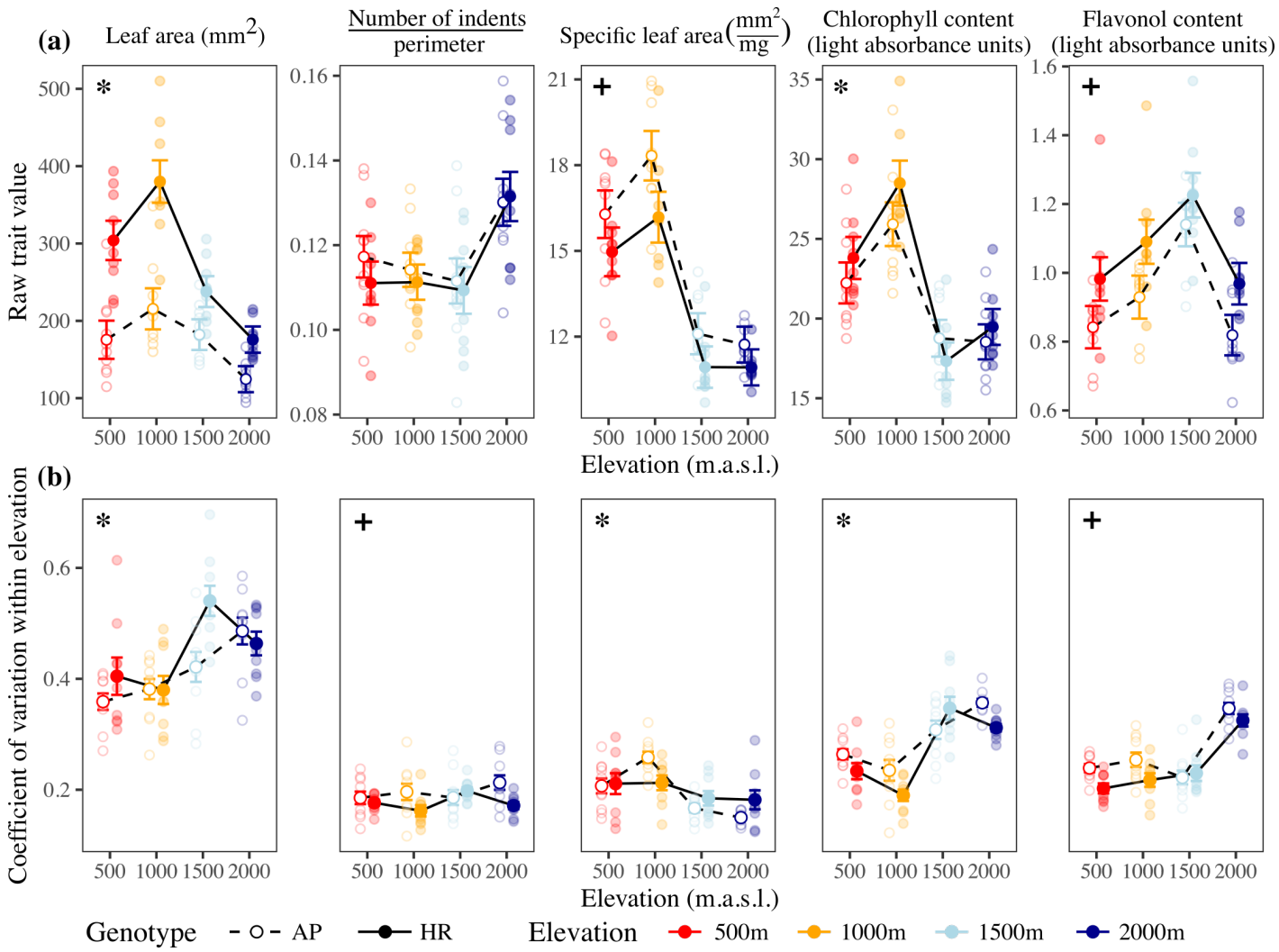



**Fig. 4** Quantifying plasticity in leaf traits as **(a)** changes in trait means across elevation, and **(b)** and variance (CV) in phenotype within genotypes. AP genotypes are represented by open circles and dashed lines, and HR genotypes by closed circles and solid lines. Larger circles with credible 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**.

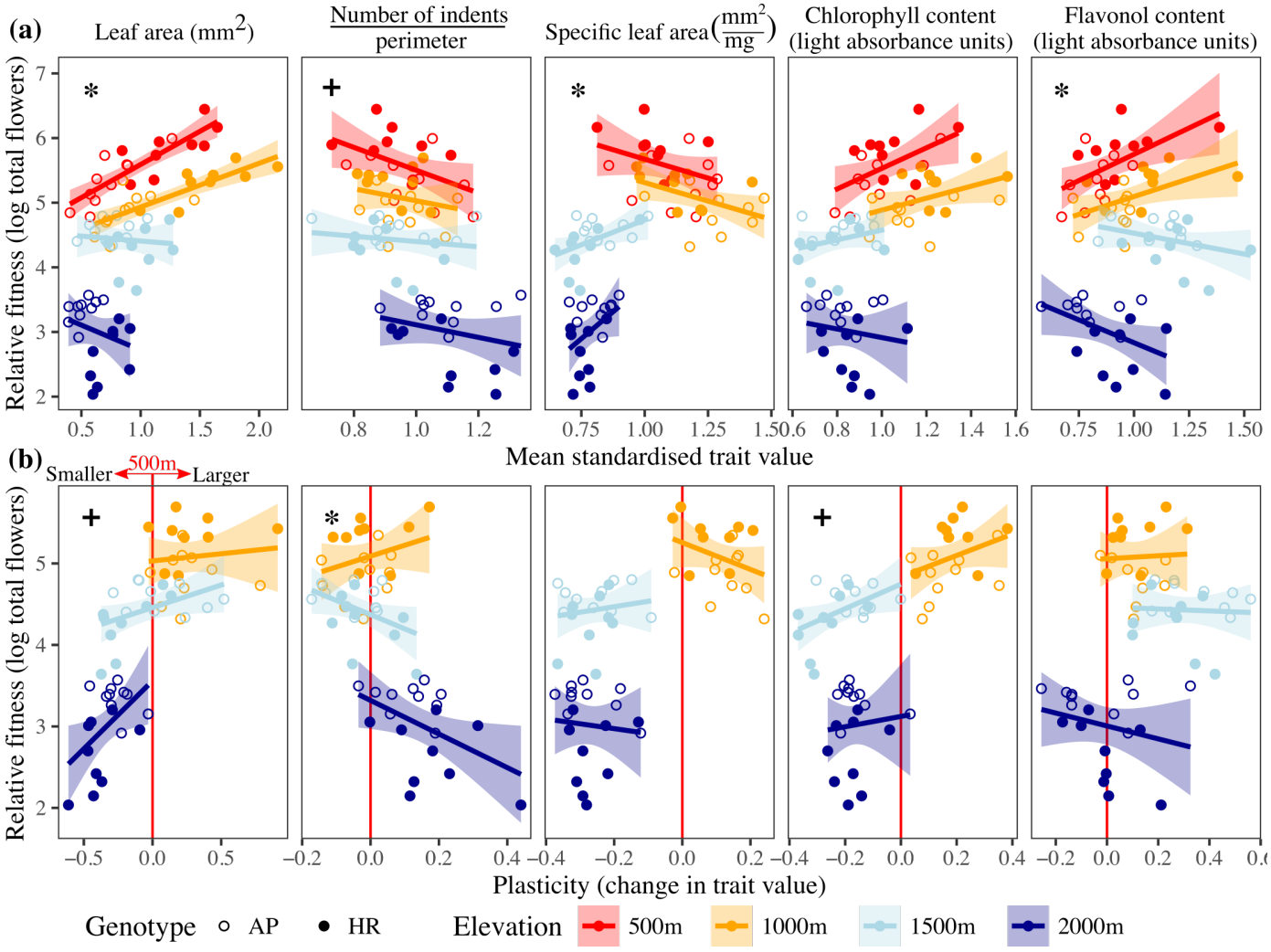
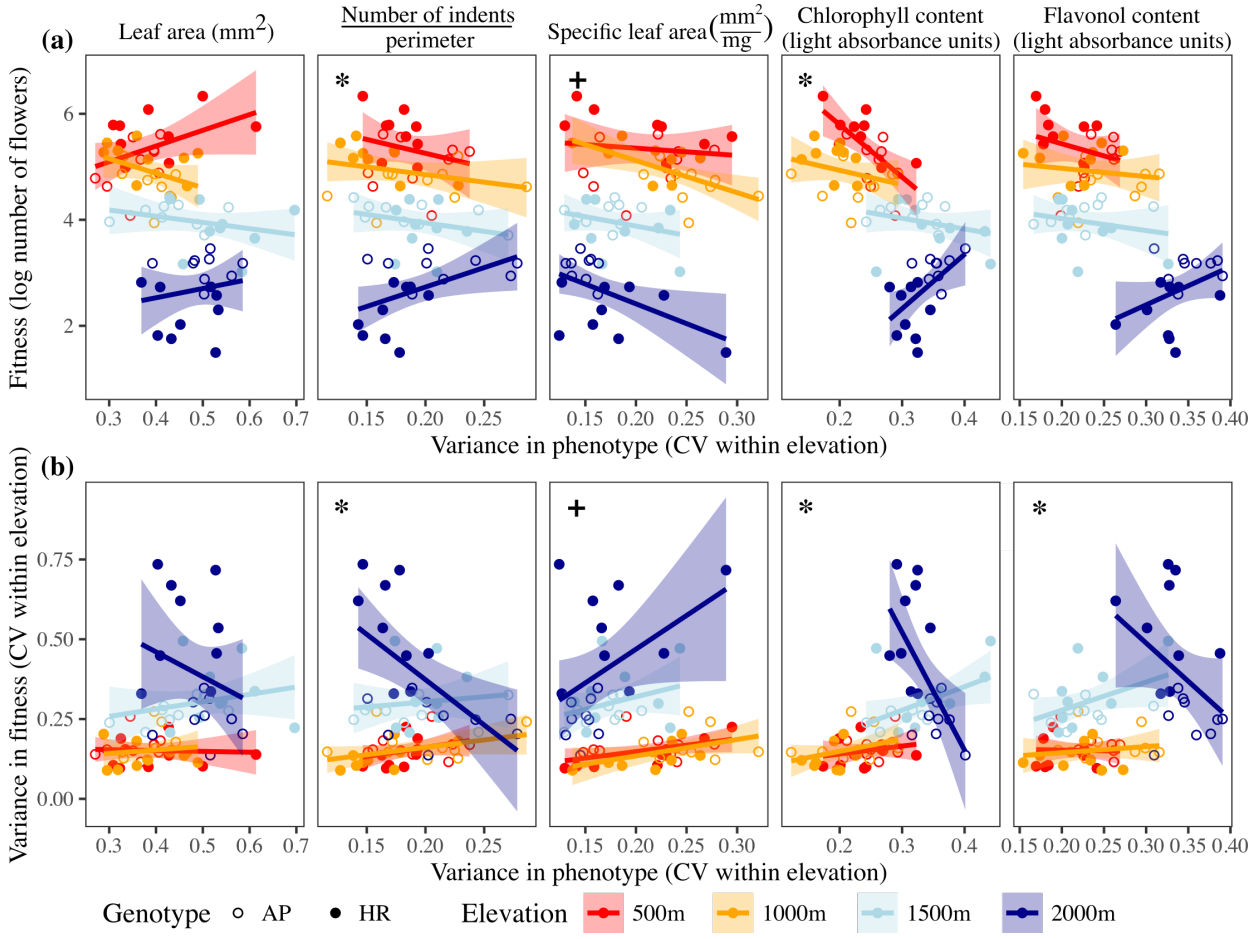



**Fig. 5** Associations between **(a)** phenotype and **(b)** plasticity with fitness across elevation. Asterisks represent a significant interaction between the predictor variable (trait value or plasticity) and elevation. Addition (+) symbols represent significant regression slopes, but no significant 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. S3**). 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**.

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