# Environmental effects on genetic variance and the alignment with plasticity and selection

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**Abstract**

Adaptive plasticity allows populations to cope with environmental variation, but is expected to fail as conditions become unfamiliar. Under such novel conditions, populations will rely on rapid adaptation to increase fitness and avoid extinction. Adaptation will be most rapid when both plasticity and selection align with directions of the multivariate phenotype that contain abundant genetic variation. However, tests of this prediction from field experiments are rare. Here, we quantify how genetic variance in a multivariate phenotype changes across an elevational gradient, and test whether plasticity and selection align with genetic variation. We do so using two closely related, but ecologically distinct, species of Sicilian daisy (*Senecio,* Asteraceae) adapted to high and low elevations on Mount Etna. Using a paternal half-sibling breeding design, we generated seeds of both species, planted them across an elevational gradient spanning each species' native elevation, and then quantified mortality and leaf traits of emergent seedlings. We found that genetic variance in leaf traits changed more across elevation than between species. Plasticity across elevation aligned more closely with genetic variance estimated at their home sites compared to other elevations. For the high-elevation species at a novel low elevation, genetic variance changed in orientation, size and shape that aligned poorly with selection and the native phenotype. By contrast, genetic variance for the low-elevation species changed mainly in size at the novel high elevation, which aligned closely with the native phenotype. Our data suggest that changes in genetic variance are species-specific and only align with plasticity and selection under certain circumstances. While adaptive plasticity in some species could maintain genetic variance in the direction of selection, changes in genetic variance for other species will reduce the chance of rapid adaptation to novel environments.

**Keywords:** additive genetic variance, covariance tensor, elevation, environmental change, G-matrix, natural selection, novel environments, phenotypic plasticity, *Senecio*

## Introduction

Populations facing rapid environmental change must cope with increasingly novel environments if they are to persist. Plasticity can help populations to persist in different environments by changing their phenotype to track phenotypic optima and maintain fitness in the new conditions (Via et al. 1995; Charmantier et al. 2008). However, plasticity should only evolve to track environments that are regularly experienced by populations (Hermisson and Wagner 2004; Chevin et al. 2010; Ashander et al. 2016), and are unlikely to prevent fitness reductions in novel environments if phenotypic responses to the new conditions are inappropriate (Palacio-López et al. 2015; Acasuso-Rivero et al. 2019). When plasticity fails to maintain fitness, genotypes are likely to vary in the extent to which they suffer reduced fitness, which can increase the adaptive potential of populations and allow them to persist via rapid adaptation (Shaw and Shaw 2014; Walter et al. 2020b). Our understanding of the potential for adaptation in novel environments is limited because field experiments that combine simultaneous assays of plasticity, fitness and genetic variance along ecological gradients are very rare.

For adaptation to occur, selection must often operate on genetic variance in complex phenotypes (Blows 2007). The additive genetic variance-covariance matrix (**G**) summarises the genetic variation in multiple traits that define a multi-dimensional phenotype (Lande 1979; Walsh and Blows 2009). **G** represents variation among alleles at all loci that contribute to each trait, as well as genetic correlations between traits due to pleiotropy and linkage that concentrate genetic variation in certain directions of the phenotype (Steppan et al. 2002; Walsh and Blows 2009). Adaptation is expected to occur along the leading axis of **G** ($\boldsymbol{g}_{\max}$) because it contains the greatest amount of genetic variation (Schluter 1996; McGlothlin et al. 2018; Costa et al. 2020; Zu et al. 2020; Walter In press). Adaptation to novel environments will therefore be the most rapid if selection lies in the direction of $\boldsymbol{g}_{\max}$ (**Fig. 1a)**.

Some models also predict that $\boldsymbol{g}_{\max}$ will align with plasticity (Lind et al. 2015; Noble et al. 2019). Plasticity will be partially adaptive if it moves a population's mean phenotype towards its optimum in a new environment, even if the optimum is not reached. In such a scenario, plasticity maintains fitness to some extent, which could help populations to persist while adaptation occurs (Ghalambor et al. 2007). However, adaptation will be fastest if there is abundant genetic variation in the direction of the plastic response (i.e., $\boldsymbol{g}_{\max}$ aligns with plasticity) because genetic variation, plasticity, and selection will be aligned (**Fig. 1a**; Lande 2009; Levis and Pfennig 2016). While a recent synthesis found an alignment between genetic variance ($\boldsymbol{g}_{\max}$) and the direction of plasticity in novel environments, this finding was based on laboratory studies and was inconsistent among species, traits and environments (Noble et al. 2019). Given that plasticity is often non-adaptive (Acasuso-Rivero et al. 2019) and does not consistently align with genetic variation in laboratory

experiments (Noble et al. 2019), field experiments using natural populations are required to understand whether plastic responses to novel environments occur in directions of the phenotype with abundant genetic variation.

Genotypes often vary in their plastic responses to new environments, which create genotype-by-environment interactions (G×E) that can change the shape or orientation of **G**, and thereby influence adaptation (Sgrò and Hoffmann 2004; Wood and Brodie III 2015). Adaptation will be constrained if G×E causes genetic variation in the new environment to align poorly with the direction of selection, which could make population extinction likely (**Fig. 1b**; Chevin 2013). However, theory predicts that changes in **G** in new environments could align $\boldsymbol{g}_{\max}$ with selection and make rapid adaptation possible (**Fig. 1c**; Draghi and Whitlock 2012; Chevin 2013; Lind et al. 2015). To our knowledge, field experiments have not quantified plasticity, genetic variation and selection along environmental gradients as ecological margins are reached, and then exceeded. Consequently, we do not know whether changes in genetic variance across environments align with selection and thereby increase the adaptive potential of populations faced with novel conditions.

In this study, we planted seeds of two closely related, but ecologically contrasting, species of Sicilian daisy (*Senecio*, Asteraceae) across an elevational gradient on Mt. Etna (**Fig. 2a-b**). *Senecio chrysanthemifolius* is a short-lived perennial with dissected leaves that occupies disturbed habitats (e.g., roadsides and vineyards) at 500-1,000m.a.s.l. (metres above sea level) on Mt. Etna and throughout Sicily. By contrast, *Senecio aethnensis* is a perennial with entire glaucous leaves that is endemic to lava flows above 2,000m.a.s.l. on Mt. Etna, where individuals grow back each spring after being covered by snow in winter. Both species are obligate outcrossers that share generalist insect pollinators .

We used a paternal half-sibling breeding design to produce seeds for c.100 families per species and then reciprocally planted seeds across an elevational gradient representing the native range of each species, the edge of its range, and conditions outside its range. We tracked the mortality of all seedlings that emerged, and measured five ecologically-important leaf traits that are known to be plastic (Walter et al. 2022), correlate with fitness (Walter et al. 2020b), and are associated with adaptive divergence in other *Senecio* species (Richards et al. 2019; Walter et al. 2020a). Previous analyses of survival data from this experiment show that these two species are adapted to their contrasting habitats across the elevational gradient (**Fig. 2c**), and express more genetic variance in relative survival when planted in novel environments (**Fig. 2d**) (Walter et al. 2021). Here, we extend these results by analysing data for the five leaf traits to test how plasticity and genetic variance respond to elevation to determine the potential for adaptation at the edge, and then outside, their native range.

To understand how the two contrasting species respond to a natural environmental gradient, we first tested whether multivariate genetic variance (**G**) in leaf traits changed in size, shape and orientation across the elevational gradient for both species. We predicted that **G** would change the most at elevations further from their home (native) sites. We also predicted that for rapid adaptation to be possible, plasticity and selection at the novel elevation would align with $\boldsymbol{g}_{\max}$, the direction of greatest genetic variance (**Fig. 1a**).

## Methods and materials

*Breeding design and field experiment*

We briefly describe the field experiment, and refer readers to Walter et al. (2021) for more detail. We collected cuttings from c.80 individuals per species growing naturally at 2,200-2,600m.a.s.l for *S. aethnensis* and 526-790m.a.s.l for *S. chrysanthemifolius.* Locations of sampled individuals are presented in **Table S1** and **Fig. S1**. We then propagated one cutting per field individual in the glasshouse, which we randomly assigned as a sire (pollen donor) or dam (pollen receiver). We then randomly mated three sires to three dams in a block, and repeated this design for nine blocks (*S. aethnensis* $n$=36 sires, $n$=35 dams, $n$=94 full-sibling families; *S. chrysanthemifolius* $n$=38 sires, $n$=38 dams, $n$=108 full-sibling families). We then planted 100 seeds from each family on Mt. Etna at four elevations, including the native habitats ('*home sites*') of both species (500m and 2,000m) as well as two intermediate elevations (1,000m and 1,500m) that represent conditions within their native range, and the edge of their range. Vagrant individuals of both species are occasionally found at these intermediate elevations. Due to the difficulty of finding sites at the centre of each species' range (agricultural fields at 500m, and lava flows above 2,000m), we chose sites within 500m of local populations as close as possible to the correct elevation (Walter et al. 2022).

At each site, we planted 25 seeds randomised into five experimental blocks (*S. aethnensis* $n$=432 seeds/block, $n$=2,160 seeds/site; *S. chrysanthemifolius* $n$=540 seeds/block, $n$=2,700 seeds/site; Total N=19,232 seeds). To prepare each experimental block, we cleared the ground of plant matter and debris, and then placed a plastic grid on the ground with 4cm-square cells. We attached each seed to the middle of a toothpick using non-drip super glue and then pushed each toothpick into the soil so that the seed sat 1-2mm below the soil surface. To replicate natural germination conditions, we suspended 90% shade-cloth 20cm above each plot and kept the seeds moist until germination ceased (2-3 weeks). We then replaced the 90% shade-cloth with 40% shade-cloth to replicate shade that naturally-growing plants are often found under, which maintained a strong environmental effect at each elevation without exposing plants to extreme conditions. We recorded seedling emergence, survival and whether plants reached establishment (produced ten leaves). The experiment ended

in January 2020 when mortality stabilised at all sites (**Fig. S2**) and plants started growing into each other, which increased competition and would have likely biased further data collection.

*Quantifying leaf traits*

When more than 80% of plants had produced ten leaves at each transplant elevation, we collected the 5$^{th}$ and 6$^{th}$ leaves from the base of the plant to quantify morphology and leaf pigment content (N=6,454 plants). To quantify leaf content of flavonol pigments, we used a Dualex instrument (Force-A, France), measured in spectral reflectance. To measure leaf morphology, we scanned the leaves (Canoscan 9000F) and used *Lamina* (Bylesjo et al. 2008) to quantify leaf complexity ($\frac{\text{leaf perimeter}^2}{\text{leaf area}}$), width of leaf indents (mm) and number of leaf indents standardised by perimeter (indents per mm). We then weighed all leaves for each plant and calculated specific leaf area (SLA = $\frac{leaf\ area}{leaf\ weight}$ in mm$^2$/mg).

Prior to analyses, we mean standardised each trait to aid the comparison of traits measured on different scales (Hansen and Houle 2008) and to estimate the mean-standardised genetic variance (evolvability) of each trait, as appropriate for comparison with changes in mean phenotype (trait plasticity) across elevations. We used R (v.3.6.1; R Core Team 2021) for all analyses.

Due to slower growth at higher elevation, many plants died before they could be measured (**Fig. S2**), suggesting that mortality could influence our estimates of genetic variance. Nevertheless, we measured multiple offspring from all sires, and more than 97% of full-sibling families, meaning that estimates for all elevations are based on the entire pedigree and large sample sizes per family (*n*=482-1,683 individuals) (**Table S2**). In addition, more than two individuals were measured for more than 90% of families (average of 4.5-15.6 offspring measured per family), suggesting that we collected sufficient phenotypic data for most families in the pedigree to estimate genetic variance (**Table S2**).

*Quantifying plasticity as elevational changes in multivariate phenotype*

To quantify species differences in the plasticity of leaf traits across elevations, we used a multivariate analysis of variance (MANOVA) with the five leaf traits as the multivariate response variable and elevation, species, and their interaction as fixed effects. Block within elevation, and family within species, were used as error terms for species and elevation respectively. To visualise differences in plasticity, we estimated the **D** matrix of differences in mean multivariate phenotype between species and calculated scores for the first two axes of **D**. Methods for constructing **D** are presented in **Methods S1**.

*Estimating genetic variance in leaf traits*

To estimate genetic variation (**G**) in the five leaf traits for each species at each elevation, we used the package *MCMCglmm* (Hadfield 2010) to apply the linear mixed model

$$\boldsymbol{y_{ijkl}} = s_i + d_j + b_k + e_{l(ijk)}\,, \qquad (1)$$

where leaf traits are the multivariate response variable ($\boldsymbol{y_{ijkl}}$), $s_i$ is the *i*th sire, $d_j$ is the *j*th dam, $b_k$ is the *k*th block, and $e_{l(ijk)}$ is the residual variation. We applied equation 1 separately to the data for each species at each elevation (*n*=8). We used chains with burn-ins of 150,000 iterations and thinning intervals of 1,500 iterations, saving 2,000 thinned iterations (MCMC samples) as the posterior distributions for all parameters estimated. We confirmed model convergence by checking that chains mixed sufficiently well, that autocorrelations among samples were below 0.05, and that our parameter-expanded prior was uninformative (Hadfield 2010). For each model, we extracted the G-matrix as the sire (co)variance component, representing one-quarter of the additive genetic (co)variance in leaf traits (Lynch and Walsh 1998).

Since *MCMCglmm* constrains variance estimates to be positive, we tested the significance for our estimates of **G** by comparing them with suitable null distributions created by randomising offspring among sires and re-applying the model to randomised data. To maintain differences among blocks, we randomised offspring within each block separately. We conducted 1,000 randomisations for each observed **G** and concluded that observed estimates in **G** were significant if their posterior means exceeded those from null distributions.

*How does genetic variance change across elevation for contrasting species?*

To quantify differences in **G** matrices for leaf traits across elevations and species, we used an eigenanalysis to decompose each **G** into eigenvectors (linear combinations of the leaf traits) defining independent axes of genetic variance, and eigenvalues describing the amounts of genetic variance along those axes. For each matrix, we used the sum of eigenvalues (or trace) to quantify its size, the distribution of eigenvalues to quantify its shape (spherical if variance is similar along all axes, or elliptical if genetic correlations among traits condense variance into fewer axes), and eigenvectors to quantify orientation.

We then used a covariance tensor approach to formally compare **G** matrices within a single framework, unlike other approaches that are based on pairwise comparisons. Briefly, the covariance tensor is an eigenanalysis of the **S** matrix, which contains the variances and covariances of the individual elements across our eight original **G** matrices. Decomposing **S** therefore provides (after re-arrangement and scaling) a set of eigentensors that describe independent axes that describe differences in **G** across elevations or species. For more detail on the approach, see **Fig. S3** and supplementary code, and Hine et al. (2009); Aguirre et al. (2014); Walter et al. (2018).

To test the significance of observed eigentensors, we compared them to suitable null distributions created by re-applying equation 1 to data re-constructed from randomised breeding values that simulate changes in **G** due to random sampling only (see supplementary code; Morrissey et al. 2019; Walter In press). If observed

eigentensors described larger differences in **G** compared to null eigentensors, then we concluded that genetic variance for leaf traits changed significantly across elevations or species. To identify how much each original **G** matrix contributed to overall differences in genetic variance, we calculated the matrix coordinates, which are scores that describe correlations between eigentensors and the original matrices, akin to principal components scores. Larger scores indicate a greater correlation between any given matrix and the differences among matrices described by an eigentensor.

*Does plasticity occur in directions of the phenotype containing large amounts of genetic variance?*
To quantify the direction of multivariate plasticity in leaf traits between the home site and elevations away from the home site, we used

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

where for each species, $\Delta\bar{\boldsymbol{x}}_i$ is the vector representing plasticity as the difference in mean for each leaf trait between the home site ($\bar{\boldsymbol{x}}_{home\ site}$) and the *i*th elevation away from the home site ($\bar{\boldsymbol{x}}_i$).

To test whether plasticity in leaf traits across elevation occurred in directions of the phenotype containing large proportions of genetic variation in the same traits, we used the matrix projection

$$\mathrm{V}_{ij} = \frac{\Delta\bar{\boldsymbol{x}}_i{}^{\mathrm{T}}\mathbf{G}_{ij}\,\Delta\bar{\boldsymbol{x}}_i}{\lambda_{\boldsymbol{g}_{max\,ij}}}\ , \qquad (3)$$

where for each species, $\Delta\bar{\boldsymbol{x}}_i$ is the plasticity vector from equation 2, *T* is its transpose and $\mathbf{G}_{ij}$ is the *j*th MCMC sample of **G** at the *i*th elevation. The projection divided by $\lambda_{\boldsymbol{g}_{max\,ij}}$, which is the corresponding eigenvalue of $\boldsymbol{g}_{\max}$ that represents the maximum amount of genetic variance available in any direction. For each elevation away from the home site, equation 3 therefore yields the amount of genetic variation ($\mathrm{V}_{ij}$) in the direction of the plasticity vector, relative to the maximum available genetic variation in any direction (Noble et al. 2019).

*Does selection at elevations away from the home site align with genetic variance?*
To quantify selection acting on leaf traits through associations with survival at each elevation, we estimated phenotypic selection gradients (***β***). We did so for *S. aethnensis* only, given the substantial mortality of seedlings at 2,000m before leaves were sampled (**Fig. S2**) meant that we could not estimate selection for *S. chrysanthemifolius.* To estimate ***β***, we applied a multiple regression to the same data using the package *lme4* (Bates et al. 2015) and the linear mixed model

$$y = z_1 + z_2 + \cdots z_5 + e\ , \qquad (4)$$

223 where $y$ is relative survival to the end of summer, $z_n$ are leaf traits, and $e$ is the error variance. We extracted
224 the vector of trait coefficients (**$\beta$,** scaled to unit length), representing the direction of phenotypic selection on
225 leaf traits for *S. aethnensis* at elevations away from its home site (Lande and Arnold 1983).

226 To test whether genetic variance in leaf traits aligned with selection on leaf traits, we used equation 3 to
227 project selection vectors (**$\beta$**) through **G** matrices estimated at the home site and each of the elevations away
228 from the home site. We incorporated uncertainty in estimates of selection and **G** by projecting each of the
229 1,000 bootstrapped samples of **$\beta$**, through each MCMC sample of **G.**

230 To interpret coefficients of selection on the scale of the original traits (and not on the latent scale from a
231 model with a probit link function), we included relative survival as a continuous response variable that
232 assumes a gaussian distribution. Although this violates the assumption of normality, this is the approach
233 suggested and commonly used (Lande and Arnold 1983; Mitchell-Olds and Shaw 1987; Hajduk et al. 2020).

234 *Does genetic variance in leaf traits align with the native phenotype at the novel elevations?*
235 To test whether genetic variance in the novel environments align with a phenotypic optimum for that
236 environment, we focused on the home (native) sites of both species (500m and 2,000m) and quantified the
237 amount of genetic variance in leaf traits in the direction of the native phenotype (*S. chrysanthemifolius* at
238 2,000m and *S. aethnensis* at 500m). We estimated the direction of the native phenotype using equation 2 to
239 calculate the vector of differences in leaf phenotype between the two species at each elevation. We then used
240 equation 3 to quantify the amount of genetic variance in the direction of the vector representing the native
241 phenotype.

242

243 **Results**

244 *Species differences in leaf trait plasticity across elevation*
245 Leaf traits showed significant multivariate plasticity across elevation (elevation Wilks' $\lambda$=0.35, $F_{3,3011}$=257.6,
246 P<0.0001), and the two species showed evidence of differences in plastic responses to elevation (**Fig 3**,
247 species×elevation Wilks' $\lambda$=0.79, $F_{3,3011}$=49.8, P<0.0001). **Fig 3** shows that at 2,000m, plasticity in leaf traits
248 moved the mean phenotype of *S. chrysanthemifolius* towards the native phenotype expressed by *S.*
249 *aethnensis*. At 500m, however, plasticity moved the mean phenotype of *S. aethnensis* away from the native
250 phenotype expressed by *S. chrysanthemifolius*. Univariate trait means are presented in **Fig. S4**.

251 *2) Genetic variance changed more across elevation than between species*
252 For both species, genetic variance in leaf traits tended to be lower at 2,000m compared to 500-1,500m (**Fig.**

253 **S5**). Estimates of genetic variance in all traits (except SLA estimated for *S. chrysanthemifolius* at high
254 elevations) are greater than the null distribution (**Fig. S6**), suggesting that our estimates are statistically
255 significant. The shape and size of **G** was similar for both species at each elevation, and changed across
256 elevations similarly for both species (**Table 1**; the G-matrices are presented in **Table S3**). The first three
257 eigenvectors of **G** together described more than 80% of total genetic variance (**Table 1**) and described more
258 genetic variance than expected under the null distribution (**Fig. S7**), providing evidence that our estimates of
259 **G** are statistically significant. For most elevations, >50% of total genetic variance is concentrated along $\boldsymbol{g}_{max}$
260 (the primary axis of **G**), suggesting that **G** is elliptical (**Table 1**). At 2,000m, however, $\boldsymbol{g}_{max}$ describes 58% of
261 total genetic variance for *S. chrysanthemifolius*, which is greater than 37% for *S. aethnensis* at 2,000m, and
262 suggests that **G** is less elliptical for *S. aethnensis* at its home site.

263 For *S. aethnensis,* we found that a different combination of traits described $\boldsymbol{g}_{max}$ at its home site compared to
264 lower elevations (**Table 1**), suggesting that G changes in orientation across elevation for *S. aethnensis.* By
265 comparison, $\boldsymbol{g}_{max}$ was described by the same linear combination of traits for *S. chrysanthemifolius* at all
266 elevations (**Table 1**). Elevational differences in the orientation of **G** were only observed in the second and
267 third axes of **G** ($\boldsymbol{g}_2$ and $\boldsymbol{g}_3$) for *S. chrysanthemifolius*, which suggest differences between the two lower and
268 two higher elevations (**Table 1**). Overall, **G** was smaller at higher elevations, and changed in orientation and
269 shape more for *S. aethnensis* than *S. chrysanthemifolius*.

270 We found that three (of seven) eigentensors described greater differences in genetic variance compared to the
271 null expectation (**Fig. S8**), providing strong evidence that **G** estimated on leaf traits differs between species
272 and changes across elevation. The tensor summary is found in **Table S4**. The coordinates identify how each
273 matrix (i.e., elevation and species) contributes to the differences in genetic variance described by an
274 eigentensor. The first eigentensor (E1) captured 37.6% of all differences among G-matrices and described
275 large changes in genetic variance across elevations, but only small differences between species at each
276 elevation (**Fig. 4**). By contrast, the second eigentensor captures 28.1% of all differences among G-matrices,
277 and described large differences between species, but smaller difference between elevations compared to E1
278 (**Fig. 4**). The third eigentensor described smaller differences between the intermediate elevations. Elevation
279 therefore created larger changes in **G** than differences due to adaptive divergence between the two species.
280 For *S. aethnensis,* elevational changes in **G** occurred abruptly away from its 2,000m home site, which
281 contrasts to *S. chrysanthemifolius* that showed differences between the native range (500m and 1,000m) and
282 higher elevations (1,500m and 2,000m) (**Fig. 4**).

283 *Genetic variance in the direction of leaf trait plasticity is moderate*

284 We quantified the proportion of genetic variance in the direction of plasticity. For *S. aethnensis*, c.50% of the

maximum available genetic variance was in the direction of plasticity for all elevations, and only at 1,500m was plasticity observed to align better with genetic variance at the home site compared to elevations away from the home site (**Fig. 5a**). For *S. chrysanthemifolius*, however, plasticity was in a direction containing c.50-70% of the maximum genetic variance available, and at all elevations plasticity was associated with a better alignment with genetic variance estimated at the home site, compared to elevations away from the home site (**Fig. 5b**). Plasticity in both species therefore showed a closer alignment with genetic variance estimated at the home site compared with other elevations.

*Changes in genetic variance in leaf traits align poorly with phenotypic selection*

Within the range, phenotypic selection on leaf traits favoured larger specific leaf area ($\chi^2(1)=1.28$, P=0.00597) and flavonol content ($\chi^2(1)=2.00$, P=0.00062). At the range edge (1,000m), selection favoured greater leaf complexity ($\chi^2(1)=1.71$, P=0.00536) and SLA ($\chi^2(1)=3.00$, P=0.00023). Selection at the novel 500m elevation favoured more complex leaves ($\chi^2(1)=132.46$, P<0.0001) and greater flavonol content ($\chi^2(1)=41.50$, P=0.00103) (**Table S5**).

Across the elevational gradient, only a small proportion of genetic variance (<50%) relative to maximum available genetic variance was in the direction of phenotypic selection (***β***) (**Fig. 5c**). Furthermore, selection was in a direction that described more genetic variance at the home site compared to genetic variance estimated at the elevations away from the home (**Fig. 5c**). These results indicate that changes in genetic variance at elevations away from the home site did not align closely with selection.

*Genetic variance in leaf traits at novel elevations aligned with the native phenotype for one species*

For *S. aethnensis* at 500m, c.40% of the maximum available genetic variance was in the direction of the native phenotype of *S. chrysanthemifolius.* Furthermore, more genetic variance estimated at the home site (c.60%) was in the direction of the native phenotype at 500m, suggesting that changes in **G** at the novel 500m elevation created a poorer alignment with the native phenotype than if **G** were to remain the same across elevations (**Fig. 6**). By contrast, for *S. chrysanthemifolius* at 2,000m, a greater proportion (c.70%) of genetic variance was in the direction of the native phenotype of *S. aethnensis* (**Fig. 6**). These results suggest that although **G** showed smaller changes across elevation for *S. chrysanthemifolius* compared to *S. aethnensis*, more genetic variance in *S. chrysanthemifolius* aligned with the direction of the native phenotype at 2,000m. By contrast, *S. aethnensis* showed greater changes in **G** but that did not align with the phenotype of *S. chrysanthemifolius* at 500m.

## Discussion

316 Field studies combining estimates of selection, genetic variance and plasticity are rare, and to our knowledge,
317 have not included the effects of environmental variation at, and beyond, existing ecological margins. In an
318 extensive field transplant experiment, we planted seeds from a breeding design of two closely related but
319 ecologically distinct Sicilian *Senecio* species across an environmental gradient (elevation) and quantified
320 survival and five ecologically important leaf traits for the emergent seedlings. Both species showed
321 significant changes in multivariate phenotype across elevations (**Fig. 3**). Higher elevations reduced the
322 amount of multivariate genetic variance in both species (**Table 1**), and changed the orientation of **G** more for
323 *S. aethnensis* compared to *S. chrysanthemifolius* (**Fig. 4**). Plasticity, captured as the change in mean
324 phenotype between the home site and other elevations, occurred in a direction of the phenotype that did not
325 align closely with $\boldsymbol{g}_{\max}$ and only contained moderate amounts of genetic variance that tended to be greater for
326 genetic variance estimated at the home site (c.50% of the maximum available genetic variance) compared to
327 other elevations (c.10-40%) (**Fig. 5**). For *S. aethnensis*, changes in genetic variance at elevations away from
328 the home site aligned relatively poorly with phenotypic selection (**Fig. 5c**) and the native phenotype (at
329 500m; **Fig. 6**). By contrast, *S. chrysanthemifolius* showed a stronger alignment with the native phenotype at
330 the novel 2,000m elevation (**Fig. 6**).

331 Together, these results suggest that when exposed to a novel environment, the extent to which plasticity is
332 adaptive and the potential for adaptation will be species-specific. For *S. chrysanthemifolius* at the novel
333 2,000m elevation, changes in genetic variance were smaller and associated with plasticity and the direction of
334 the native phenotype at that elevation (reflecting the scenario in **Fig. 1a**). By contrast, for *S. aethnensis* at the
335 novel 500m elevation, larger changes in genetic variance were observed, but they were associated with a
336 poor alignment with plasticity, selection and the phenotypic optimum (reflecting the scenario in **Fig. 1b**).

337 Although we provide substantial evidence for changes in genetic variance, we could not measure all
338 individuals before selection, which means that estimates of genetic variance could be biased when viability
339 selection removes phenotypes before they can be measured (Hadfield 2008). However, this issue is
340 notoriously difficult to avoid in field experiments, and given that we measured individuals from almost all
341 families of *S. chrysanthemifolius*, and all families of *S. aethnensis* before strong selection, there should be
342 few genotypes missing data and our estimates of genetic variance should be robust.

343 *Low genetic variance in the direction of plasticity*

344 We only found moderate amounts of genetic variance in the direction of plasticity, which means our results
345 contrast with a meta-analysis that found plasticity in novel environments aligned with genetic variance
346 (Noble et al. 2019). However, they also found that the strength of the alignment varied across study species,
347 traits and environments, and that many of the studies used laboratory experiments with small numbers of

348 traits and limited environmental variables. The increased complexity and ecological breadth of our field
349 environment, including conditions beyond their natural range, could therefore reduce the alignment between
350 genetic variance and plasticity compared with estimates from laboratory experiments. It is also possible that
351 the alignment between plasticity and genetic variance varies greatly among studies because species (and
352 populations) vary in the amount of genetic variation in plasticity. During adaptation, if the environment is
353 predictable and selection on plasticity is strong, then genetic variation in the direction of plasticity is likely to
354 be eroded (Oostra et al. 2018; Hoffmann and Bridle 2022). If all genotypes of a given population show the
355 same plastic response, genetic variation in plasticity will be small (Debat and David 2001; Lande 2009; Shaw
356 et al. 2014), and plasticity of the population is likely to be in the direction of historical selection and not in
357 the direction of genetic variation.

358 *Environmental effects on genetic variance*

359 Wood and Brodie III (2015) found evidence that **G** is likely to be affected by the environment as much as by
360 evolution, although it remained unclear why **G** changed in response to the environment. We help to resolve
361 this by demonstrating that novel environments not only create larger changes in **G** than evolutionary history
362 (i.e., adaptive divergence between the sister *Senecio* species), but that such changes can be the result of
363 nonadaptive G×E (genotype-by-environment interactions) in response to stress when the high elevation
364 species experienced low elevation environments. By contrast, the low-elevation species showed little change
365 in orientation or shape, but changed mostly in size. Changes in **G** may often be maladaptive in novel
366 environments because plasticity has not evolved in the past to buffer those conditions. To better understand
367 the potential for rapid adaptation, it is therefore important to quantify how genotypes found across a species'
368 range vary in their response to environmental variation, and whether there are populations that contain
369 genetic variation that could help align **G** with selection in novel environments (Lande 2009; Chevin and
370 Lande 2011; Lind et al. 2015; Walter et al. 2020b; Bridle and Hoffmann 2022).

371 *Potential for persistence in novel environments*

372 The initial resilience of populations exposed to a novel environment is likely to depend on how close
373 plasticity moves the population towards a new phenotypic optimum (Ghalambor et al. 2007). Empirical
374 evidence suggests that plasticity is more often nonadaptive in novel environments (Langerhans and DeWitt
375 2002; Palacio-López et al. 2015; Acasuso-Rivero et al. 2019), which means that populations are likely to rely
376 on rapid adaptation to recover fitness and prevent extinction. Our results suggest that while plasticity in the
377 low-elevation species is adaptive to some extent, the high-elevation species showed stronger evidence of
378 nonadaptive plasticity, which is consistent with previous experiments with the *Senecio* on Mt Etna (Walter et
379 al. 2022), and high-elevation plants in general (Gugger et al. 2015; Nicotra et al. 2015; Schmid et al. 2017; de

Villemereuil et al. 2018). The potential for plasticity to maintain fitness as environments become more novel is therefore likely to depend on how plasticity has evolved for each species in the past (Liu et al. 2022).

Adaptation to novel elevations requires genetic variation in the direction of selection. Although we provided evidence that genetic variance in survival increased in the novel environments for both species (Walter et al. 2021), genetic variation aligned with the phenotypic optimum much more closely for the low-elevation species at 2,000m when compared to the high-elevation species at 500m. It is therefore likely that the high-elevation species will face genetic constraints that will make it difficult for rapid adaptation to occur at lower elevations, and by extension, warmer conditions created by climate change. By contrast, rapid adaptation to the high-elevation habitat should be more likely for the low-elevation species because genetic variation at 2,000m aligned more closely with plasticity and the native phenotype, which could be how a low-elevation species colonised and adapted to high elevations to give rise to *S. aethnensis*. Such variation, even between closely related species, in their responses to warmer versus cooler environments makes predicting adaptation to novel environments difficult. Although in this case it aligns with the growing evidence (at least for invertebrates) that limits to adaptation and plasticity at warmer margins are likely to be stronger than at cooler limits (Kellermann et al. 2009; Kellermann et al. 2012; van Heerwaarden et al. 2016).

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**Author contributions:** GW designed the study with AC, SH, SC and JB. GW, DT, ES, MM, GP and AC conducted the experiments, collected and curated the data. GW analysed the data and wrote the paper with help from KM, JC and input from all authors.

**Data availability:** Upon acceptance, all data and code will be deposited with Dryad.

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544

**Table 1** The first three eigenvectors of **G** that describe >80% of total genetic variance estimated at each elevation and for each species. 'Proportion' quantifies the proportion of total genetic variance that each eigenvector describes. HPD represent the 95% Highest Posterior Density (HPD) intervals. Trait loadings in bold are greater than 0.2 and suggest that they contribute to the genetic variance described by the eigenvector. Highlighted colours are linear combinations of traits that underlie elevational differences in $\boldsymbol{g}_{max}$ for *S. aethnensis*, and elevational differences in $\boldsymbol{g}_{2\text{-}3}$ for *S. chrysanthemifolius*.

| | **500m** | | | **1,000m** | | | **1,500m** | | | **2,000m** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\boldsymbol{g}_{max}$ | $\boldsymbol{g}_2$ | $\boldsymbol{g}_3$ | $\boldsymbol{g}_{max}$ | $\boldsymbol{g}_2$ | $\boldsymbol{g}_3$ | $\boldsymbol{g}_{max}$ | $\boldsymbol{g}_2$ | $\boldsymbol{g}_3$ | $\boldsymbol{g}_{max}$ | $\boldsymbol{g}_2$ | $\boldsymbol{g}_3$ |
| *S. aethnensis* | | | | | | | | | | | | |
| **Eigenvalues** | 0.051 | 0.023 | 0.016 | 0.047 | 0.017 | 0.011 | 0.051 | 0.016 | 0.008 | 0.015 | 0.010 | 0.009 |
| **HPD** | 0.029, 0.090 | 0.013, 0.038 | 0.004, 0.021 | 0.026, 0.082 | 0.009, 0.029 | 0.002, 0.015 | 0.025, 0.092 | 0.007, 0.031 | 0.000, 0.014 | 0.009, 0.032 | 0.005, 0.018 | 0.002, 0.010 |
| **Proportion** | 0.52 | 0.23 | 0.16 | 0.59 | 0.22 | 0.14 | 0.63 | 0.20 | 0.10 | 0.37 | 0.25 | 0.22 |
| **Traits:** | | | | | | | | | | | | |
| Complexity | -0.19 | **-0.93** | **0.28** | **-0.35** | **-0.89** | **0.23** | **-0.37** | **-0.92** | 0.14 | **-0.92** | 0.11 | -0.13 |
| Indent width | **-0.48** | 0.14 | **0.40** | **-0.27** | **0.27** | **0.54** | **-0.65** | **0.33** | 0.11 | 0.10 | -0.04 | **-0.52** |
| Number ind. | **0.52** | **-0.33** | **-0.45** | **0.29** | **-0.30** | **-0.60** | **0.61** | **-0.21** | -0.13 | **-0.28** | -0.05 | **0.69** |
| SLA | -0.03 | 0.03 | **-0.25** | -0.15 | 0.17 | **-0.33** | 0.02 | -0.06 | **-0.57** | 0.07 | **-0.80** | **0.26** |
| Flavonol | **0.67** | 0.09 | **0.70** | **0.84** | -0.14 | **0.42** | **0.27** | 0.04 | **0.79** | **0.25** | **0.58** | **0.41** |
| *S. chrysanthemifolius* | | | | | | | | | | | | |
| **Eigenvalues** | 0.053 | 0.019 | 0.013 | 0.043 | 0.023 | 0.004 | 0.016 | 0.008 | 0.004 | 0.024 | 0.009 | 0.004 |
| **HPD** | 0.026, 0.091 | 0.011, 0.032 | 0.004, 0.017 | 0.021, 0.078 | 0.01, 0.034 | 0.001, 0.007 | 0.005, 0.039 | 0.001, 0.015 | 0, 0.005 | 0.008, 0.058 | 0.001, 0.018 | 0, 0.006 |
| **Proportion** | 0.59 | 0.22 | 0.15 | 0.59 | 0.32 | 0.06 | 0.50 | 0.25 | 0.12 | 0.58 | 0.22 | 0.10 |
| **Traits:** | | | | | | | | | | | | |
| Complexity | **0.92** | 0.18 | **0.31** | **0.95** | 0.00 | **0.31** | -0.08 | **0.97** | 0.17 | **0.96** | 0.07 | **0.25** |
| Indent width | 0.04 | **-0.73** | 0.14 | -0.10 | **-0.71** | 0.14 | **0.69** | -0.06 | -0.03 | -0.08 | **0.57** | **-0.25** |
| Number ind. | -0.10 | **0.57** | -0.08 | 0.01 | **0.66** | -0.19 | **-0.69** | -0.16 | -0.15 | -0.15 | **-0.64** | **0.43** |
| SLA | -0.02 | **0.24** | **-0.39** | -0.03 | 0.00 | -0.05 | -0.17 | 0.05 | **-0.23** | 0.04 | -0.18 | -0.13 |
| Flavonol | **-0.36** | **0.21** | **0.85** | **-0.30** | **0.24** | **0.92** | -0.11 | -0.18 | **0.95** | **-0.23** | **0.47** | **0.82** |

## Figures

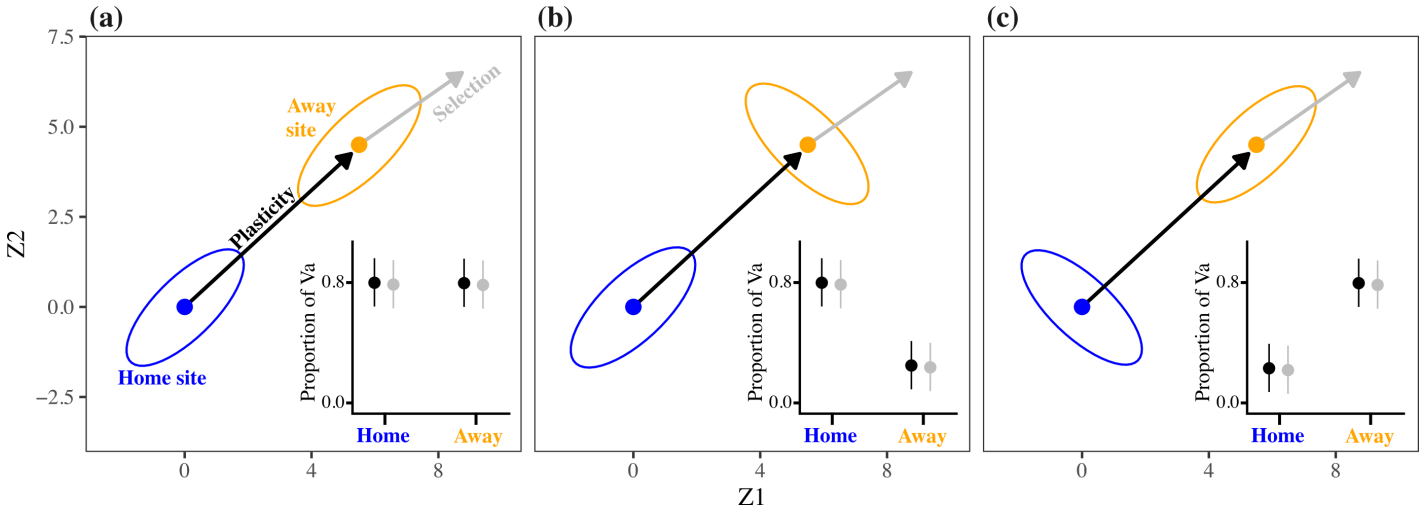



**Fig. 1** Hypothesised alignments of genetic variance, plasticity, and selection for two traits (Z1 and Z2) measured for a population measured at its home site (blue) and a site away from its native environment (orange). Ellipses plot the genetic variances of traits and solid circles within ellipses represent trait means. Large black arrows show changes in trait means across environments due to plasticity, and grey arrows show the directions of selection at the away site. Inset graphs present the proportion of genetic variation that align with the directions of plasticity and selection at the home and away environments. **(a)** Adaptation should be fastest if both plasticity and selection are in a direction of the phenotype containing a large amount of genetic variance in the traits (i.e., in a direction similar to $\boldsymbol{g}_{\max}$, the long axis of the ellipse). However, we know that genotype-by-environment (G×E) interactions (i.e. genetic variance in plasticity) are commonly found, which are represented here by changes in either the orientation or shape (not shown) of **G**. **(b)** Even if plasticity aligns with **G** from the home site, extinction will be likely if **G** changes away from the home site and becomes misaligned with the direction of selection. **(c)** If, however, **G** changes away from the home site, and such changes align **G** with selection and plasticity, then rapid adaptation would be possible.

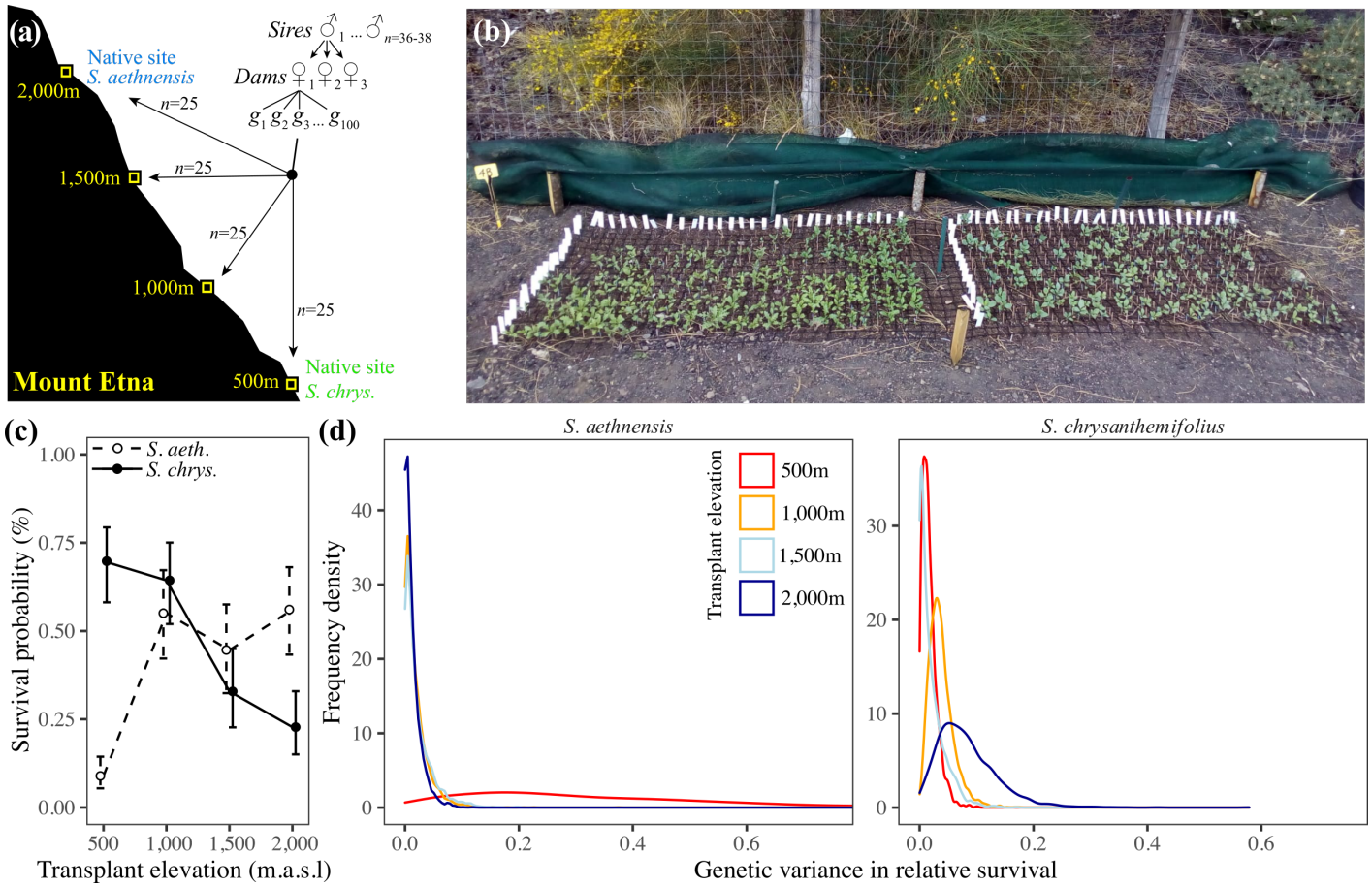



**Fig. 2** The experimental design. **(a)** For two Etnean *Senecio* species, we mated 36-38 sires each to 3 dams, and produced 100 seeds from each mating (family). We planted 25 seeds from each family at each of four elevations that represent the home (native) site of each species and two intermediate elevations. **(b)** Photo of an experimental block at 2,000m, 8 weeks after seeds were sowed (*S. chrysanthemifolius* on left). Panels **c-d** are analyses of the survival data (from this experiment) taken from Walter et al. (2021). **(c)** Mean survival probability after summer for both species across the elevation gradient (±1 SE). **(d)** Posterior distributions for estimates of genetic variance in relative survival, which was greater at the novel elevation for both species.

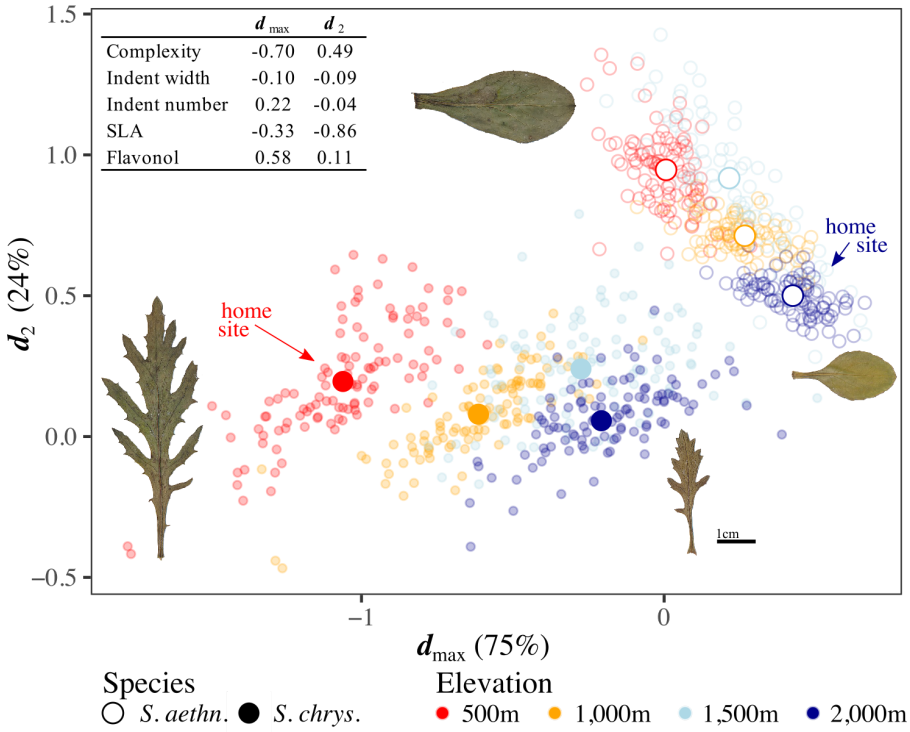
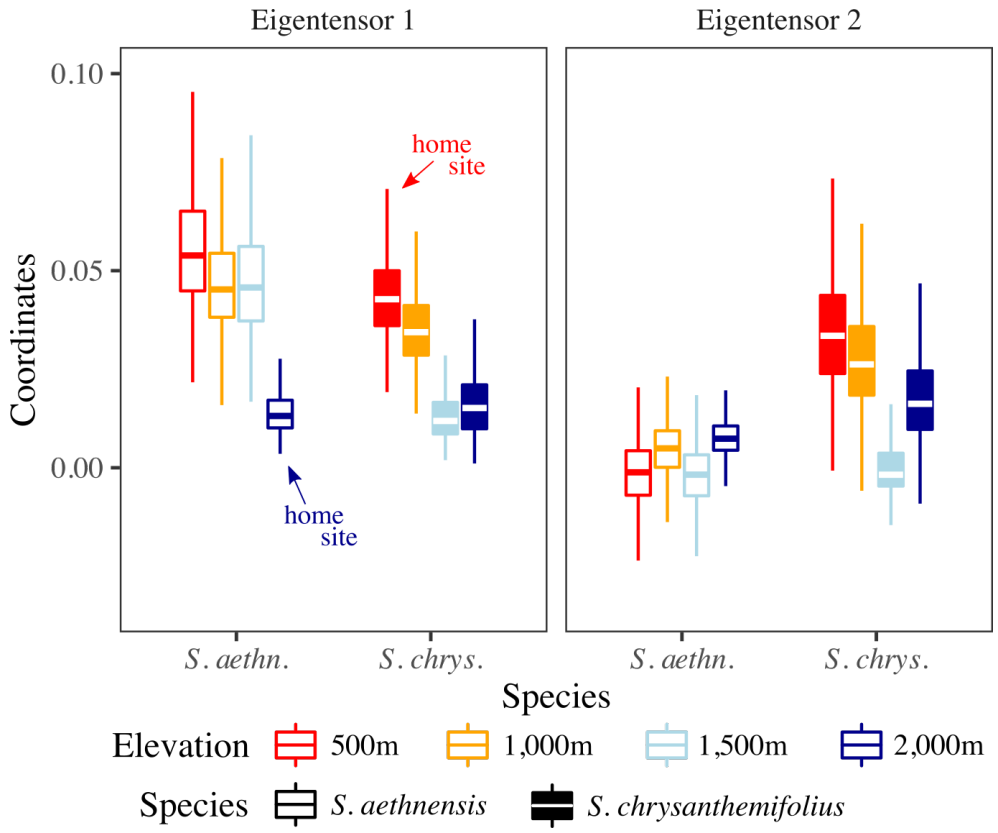



**Fig. 3 (a)** Multivariate phenotypic plasticity in leaf traits across elevation differs between species. The D-matrix represents plasticity as changes in mean multivariate phenotype across elevations for both species. The first two axes of **D** together describe 99% of all differences in mean phenotype. Trait loadings on each axis are in the table inset. Large, coloured circles represent the species means at different elevations, with the circles being larger than one standard error of the mean. Small circles represent means of the full-sibling families. Leaves represent a plant near the mean phenotype of each species at each elevational extreme.




586

587 **Fig. 4** Genetic variance differs more across elevation than between species. Eigentensors summarise
588 differences in **G** across elevation and between species, while the coordinates indicate how much each
589 original matrix contributes to the differences described by each eigentensor. Boxplots represent the posterior
590 distribution of the coordinates for **G** estimated for both species at all elevations. Eigentensor 1 describes large
591 elevational differences, but relatively small differences between species. Eigentensor 2, however, describes
592 larger differences between species, which is greater at the lower elevations.

593

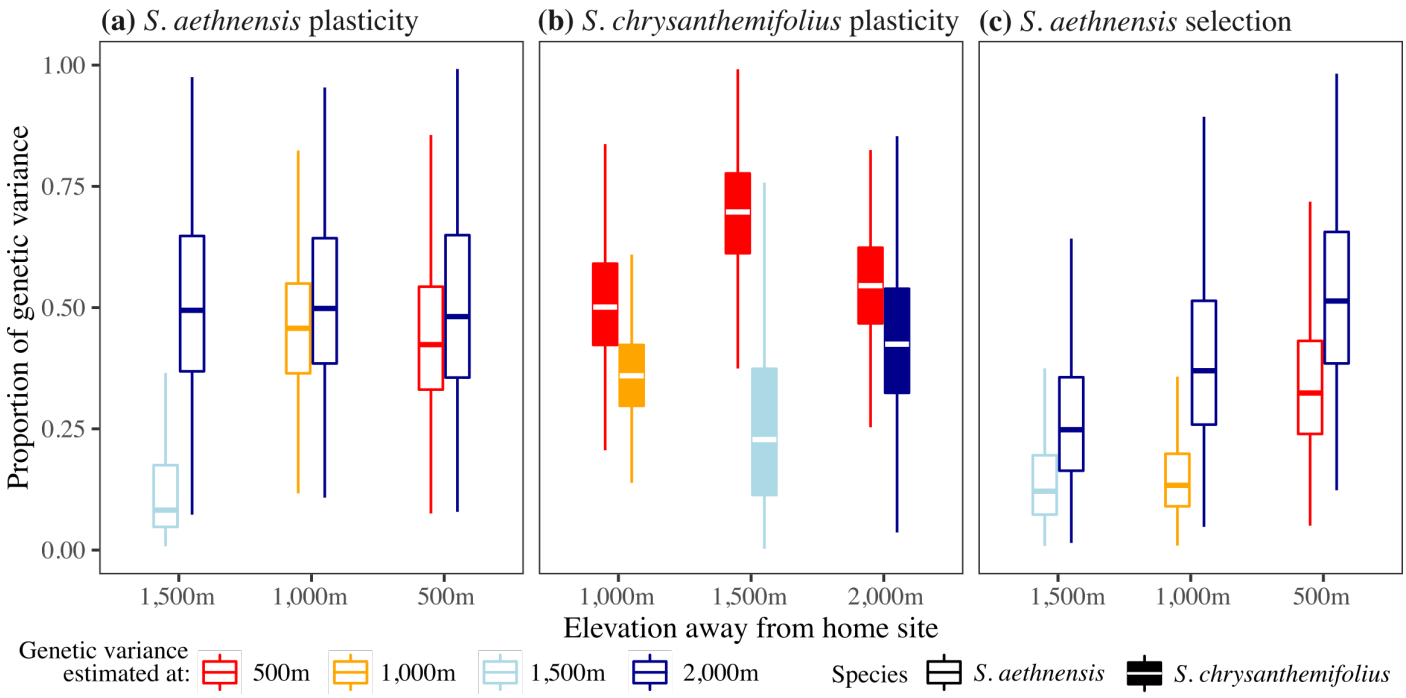



**Fig. 5** Boxplots representing the posterior distribution for the amount of genetic variance (as a proportion of $\boldsymbol{g}_{max}$, the maximum amount of genetic variation available) that lies in the direction of vectors that represent: **(a-b)** plasticity (change in mean phenotype) for both species, and **(c)** selection for *S. aethnensis.* The proportion of genetic variance is quantified for the home site, which is compared to each elevation away from the home site. Asterisks denote significant differences between genetic variance estimated at the home site versus away elevations (distributions do not overlap at >90% HPD). For both *S. aethnensis* **(a)** and *S. chrysanthemifolius* **(b)**, plasticity at each elevation away from the native site aligned more closely with genetic variance estimated at the home site compared to genetic variance estimated at lower elevations. **(c)** For *S. aethnensis,* selection aligned poorly with genetic variance, and aligned better with genetic variance estimated at the home site compared to sites away from the home site (lower elevations).

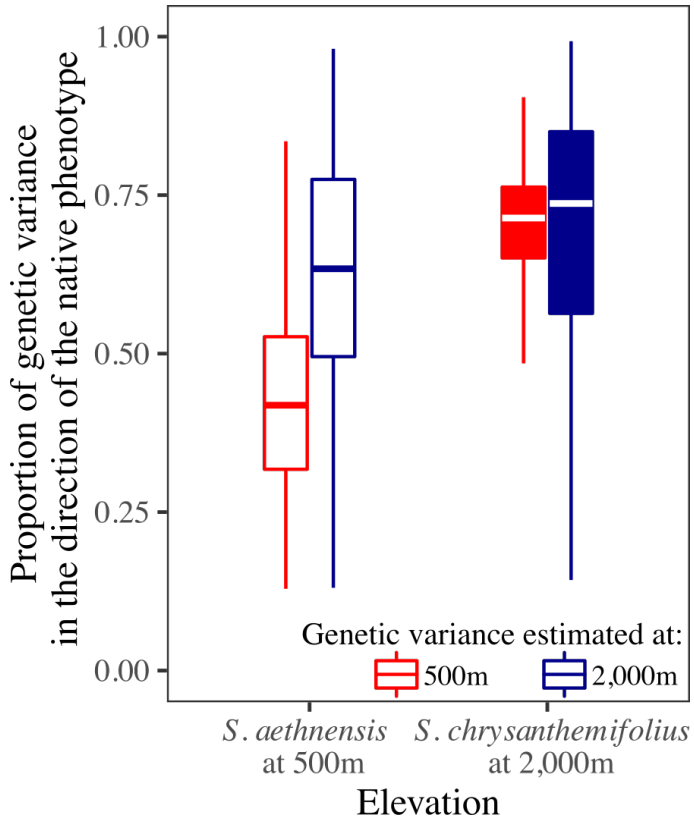



**Fig. 6** Boxplots representing the posterior distribution of the proportion of genetic variance in the direction of the native phenotype for the two species planted at both native habitats. For *S. chrysanthemifolius*, genetic variance in leaf traits align closely with the native phenotype at 2,000m. By contrast, genetic variation in *S. aethnensis* shows a weaker alignment with the native phenotype at 500m, with genetic variance estimated at 500m aligning more poorly with the native phenotype compared to genetic variance estimated at the home site (2,000m). At novel elevations, genetic variance in *S. chrysanthemifolius* therefore better aligned with the phenotypic optimum compared to *S. aethnensis*.