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

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Abstract

Adaptive plasticity helps populations to cope with environmental variation but is expected fail as conditions become more novel. Plasticity can, however, move the phenotype towards the optimum and maintain population persistence while adaptation occurs. Adaptation will then be the most rapid when plasticity and selection lie in a direction of the multivariate phenotype that contains a large amount of genetic variation. However, evidence from field experiments that quantify fitness, selection and genetic variance is rare. Here, we tested whether plasticity and selection lie in the direction of the phenotype containing a large proportion of genetic variation as ecological limits are reached, and then exceeded. We used 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 and planted them across an elevational gradient that represented the native range of each species, and two intermediate elevations. We tracked mortality of emergent seedlings and measured five leaf traits. We found that each species survived better at their native elevation and although mortality was higher at more novel elevations, genetic variance in relative survival was greater. Genetic variance in leaf traits changed more across elevations than between species. At elevations away from the native site, genetic variance was associated with selection in the high-elevation species, and with the direction of plasticity and the local phenotypic optimum in the low-elevation species. Our data therefore suggest that when adaptive plasticity fails to maintain fitness, adaptive potential increases, which is associated with changes in genetic variance that align with selection in high-elevation species, but with plasticity and the phenotypic optimum in the low-elevation species. The potential for adaptation to novel environments is therefore likely to be species-specific.

Keywords: additive genetic variance, evolutionary rescue, G-matrix, natural selection, novel environments, phenotypic plasticity, phenotypic variance, selection differential, selection gradient

Introduction

Under conditions of rapid environmental change, populations need to cope with increasingly novel environments if they are to persist. If the sensitivity of genotypes to the environment mean that they change their phenotype and match the changing phenotypic optima created by environmental change, then phenotypic plasticity can help the population to maintain high fitness (Via et al. 1995; Charmantier et al. 2008). However, plasticity should only evolve to track environmental conditions that are regularly experienced by a population (Hermisson and Wagner 2004; Chevin et al. 2010; Ashander et al. 2016), making it difficult for plasticity to buffer novel conditions because phenotypic responses are unlikely to be appropriate for the new conditions (Palacio-López et al. 2015; Acasuso-Rivero et al. 2019). When plasticity fails to maintain high fitness, genotypes are expected to vary in the extent to which they suffer reduced fitness, which can increase the adaptive potential of the population (Walter et al. 2020b), and could allow populations to persist via rapid adaptation (termed 'evolutionary rescue'; Gomulkiewicz and Holt 1995; Bell and Gonzalez 2009). Our ability to predict the potential for adaptation (and therefore, evolutionary rescue) in more stressful environments is limited because field experiments that combine simultaneous assays of plasticity, fitness and genetic variance along ecological gradients are rare.

The additive genetic variance-covariance matrix (**G**) summarises the genetic variation that underlies multi-dimensional phenotypes (Lande 1979). **G** represents variation among alleles that determines how much genetic variation exists in each trait, and how genetically correlated multiple phenotypic traits are to each other (Walsh and Blows 2009). Pleiotropy, linkage disequilibria and physical linkage create strong genetic correlations that concentrate genetic variation in certain directions of the phenotype (Steppan et al. 2002). Evolution is expected to occur most easily and rapidly along the leading axis of **G** ($\boldsymbol{g}_{\max}$) because it contains the greatest amount of genetic variation (Schluter 1996). Although adaptation does tend to occur in the direction of $\boldsymbol{g}_{\max}$ (McGlothlin et al. 2018; Costa et al. 2020), we know that genetic correlations can also change rapidly with the environment, which could determine whether populations can adapt to novel environments (Sgrò and Hoffmann 2004; Wood and Brodie III 2015). Genotypes often vary in their response to new environments, which creates genotype-by-environment interactions (G×E) that are expected to change the shape or orientation of **G** (Wood and Brodie III 2015). In new environments, G×E will be maladaptive if changes in **G** result in low genetic variance in the direction of selection. However, theory suggests that changes in **G** in a new environment are predicted to align $\boldsymbol{g}_{\max}$ with selection, which would make rapid adaptation and therefore, evolutionary rescue, more likely (Draghi and Whitlock 2012; Chevin 2013; Lind et al. 2015).

Under some models, plasticity is also expected to align with $\boldsymbol{g}_{\max}$ (Noble et al. 2019). Plasticity is partially adaptive if it moves the phenotype of the population towards the phenotypic optimum in a novel environment, even if it fails to reach the phenotypic optimum. In such a scenario, plasticity can maintain fitness to some extent, which could help populations to persistence while adaptation occurs (Ghalambor et al. 2007). Rapid adaptation will be possible if there is abundant genetic variation in the direction of the plastic response (i.e., plasticity aligns with $\boldsymbol{g}_{\max}$) because both genetic variation and plasticity align with the direction of the optimum phenotype in the new environment (Lande 2009; Levis and Pfennig 2016). While genetic variance ($\boldsymbol{g}_{\max}$) has been shown to align with the direction of plasticity in novel environments, this finding was limited to studies from the laboratory and was not consistent between species, traits and environments studied (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 with natural populations are required to understand whether plastic responses to novel environments occur in the direction of the phenotype that contains a large amount of genetic variation.

The potential for a population to adapt to a new environment will be determined by how much genetic variation lies in the direction of selection under the new conditions. Adaptation will be most rapid if genetic variation remains stable across environments and both selection and plasticity lie in the direction of $\boldsymbol{g}_{\max}$ (**Fig. 1a**) (Levis and Pfennig 2016; Noble et al. 2019). Given that genotype-by-environment (G×E) interactions are common and can change the shape or orientation of **G** (Wood and Brodie III 2015), genetic constraints are likely to slow adaptation when genetic variation in the new environment lies in a direction different to selection, which could make extinction more likely than evolutionary rescue (**Fig. 1b**) (Chevin 2013). If, however, **G** changes in response to the new environment and genetic variation aligns with selection, then rapid adaptation could still be possible (**Fig. 1c**) (Draghi and Whitlock 2012). The lack of field experiments that quantify **G** and selection for environmental gradients as ecological margins are reached, and then exceeded, mean that (to our knowledge) there are no empirical tests of whether genetic variance changes across environments, and whether such changes align genetic variation with selection that increases the adaptive potential of populations faced with novel conditions.

We generated seeds of two closely related but ecologically divergent species of Sicilian daisy (*Senecio*, Asteraceae), which we planted across an elevational gradient on Mt. Etna. *Senecio chrysanthemifolius* is a short-lived perennial with dissected leaves that occupies disturbed habitats (e.g. roadsides) at elevations under 1,000m on Mt. Etna, as well as throughout Sicily. By contrast, *Senecio aethnensis* is a perennial with entire glaucous leaves 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. We used a paternal half-sibling breeding design to

produce seeds for c.100 families for each species, which we reciprocally planted across an elevational gradient representing the native range of each species, the edge of their range, and conditions outside their range (**Fig. 2**). We tracked mortality for seedlings that emerged, and measured five ecologically-important leaf traits that show plasticity (Walter et al. 2022), correlate with fitness (Walter et al. 2020b), and are associated with adaptive divergence in other *Senecio* species (Walter et al. 2018; Richards et al. 2019; Walter et al. 2020a). In previous transplant experiments (Walter et al. 2022), plasticity in *S. chrysanthemifolius* at the novel 2,000m elevation was in the direction of the native phenotype of *S. aethnensis*. By contrast, at 500m, the phenotype of *S. aethnensis* moved further from the native phenotype of *S. chrysanthemifolius.* These results suggest that *S. chrysanthemifolius* shows more evidence of adaptive plasticity compared to *S. aethnensis*, and that the Etnean *Senecio* provide an ideal system to test how plasticity and genetic variance in ecologically-important leaf traits change with fitness across an ecological gradient.

We tested four hypotheses to understand how the two contrasting species respond to a natural environmental gradient that included conditions at the edge and outside their native ranges: (1) Where adaptive plasticity fails to maintain high fitness, we would observe greater genetic variance in fitness that increases adaptive potential in novel environments. (2) Across the elevational gradient, multivariate genetic variance (**G**) would change in size, but not orientation, and the greatest change would be observed in more novel environments. If evolutionary rescue is likely (**Fig. 1a**), then (3) plasticity (elevational changes in mean phenotype for the population), and (4) selection would occur in a direction of the phenotype containing a large amount of genetic variance. However, given that G×E could change the orientation of genetic variance (Wood and Brodie III 2015), we might expect elevational changes in **G** to occur that would either make evolutionary rescue less likely in novel environments if there is low genetic variation in the direction of selection in the new environment is (**Fig. 1b**), or could provide some potential for evolutionary rescue if changes in **G** increase the amount of genetic variation in the direction of selection in the novel environment (**Fig. 1c**).

Methods and materials

Breeding design and field experiment

To produce seeds of both species, we collected cuttings from c.80 individuals in natural populations of both species, and we propagated one cutting from each individual in the glasshouse. When plants produced buds, we covered branches with perforated bread bags to exclude pollinators while providing adequate airflow. For each species, we randomly assigned half of the individuals (which are hermaphroditic and self-incompatible) as females (dams), the other half as males (sires), and then mated three sires to three dams in a block design that produced c.100 full-sibling families for each species (**Fig. 2a**). We randomly mated sires to dams in

3×3 blocks (three sires mated to three dams) in a paternal half-sibling breeding design (Lynch and Walsh 1998) to produce 94 families of *S. aethnensis* (*n*=36 sires, *n*=35 dams) and 108 families of *S. chrysanthemifolius* (*n*=38 sires, *n*=38 dams). We performed crosses by removing flowerheads from sires and rubbing their anthers on the flowerheads of the dams. Fertilised flowerheads were labelled and covered with mesh bags to catch the seeds once mature.

Due to limitations in glasshouse space, we performed crosses on both species separately. We sampled individuals of *S. chrysanthemifolius* in June-July 2017 from five independent locations at the base of Mt. Etna (**Table S1** and **Fig. S1**), and between January and February (2018) we crossed 38 sires with 38 dams to produce 108 families. For *S. aethnensis*, we sampled individuals from a range of elevations on both the North and South of Mt. Etna in October 2018 (**Table S1** and **Fig. S1**), and between January and March (2019) we crossed 36 sires to 35 dams to produce 94 families.

We planted 100 seeds from each family at four elevations on Mt. Etna that included the native habitats of both species (500m and 2,000m) and two intermediate elevations (1,000m and 1,500m) (**Fig. 2a**; Walter et al. 2022). We planted 25 seeds at each site 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). However, *S. aethnensis* grew more slowly and produced less flowers in the glasshouse, which made it difficult to produce 100 seeds for each family, meaning that four, eight and ten families were represented by 15, 20 and 25 seeds per elevation, respectively. To prepare each experimental block, we cleared the ground of plant matter and debris, and then placed a plastic grid (4cm square cells) on the ground. 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 (Walter et al. 2016). 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). After this initial period, we replaced the shade-cloth with a lighter 40% shade-cloth that replicated shade that naturally-growing plants are often found under. This maintained a strong environmental effect at each elevation without exposing plants to extreme environmental conditions. We recorded seedling emergence, survival and whether plants reached seedling establishment (produced ten leaves). The experiment was completed 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 5th and 6th leaves from the base of the plant to quantify morphology and leaf pigment content (N=6,454 plants).

Unfortunately, due to slower growth at higher elevations many plants died before reaching 10 leaves (**Fig. S2**), which meant that mortality could influence the estimation of genetic variance. Despite this, multiple offspring from all sires and more than 97% of full-sibling families were measured, meaning that our estimates of genetic variance are based on measurements collected from the entire pedigree with large sample sizes within each family (n=482-1,683 individuals) at each elevation (**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**). Mortality should only be an issue in the estimation of genetic variance for *S. chrysanthemifolius* at higher elevations (2,000m) because only 20-30% of seedlings that grew were measured and measurements were taken after mortality. Estimates of genetic variance for *S. aethnensis* at all elevations should be robust because we were able to measure a large number of plants (>45%) before high mortality.

To quantify leaf flavonol content, we used a Dualex instrument (Force-A, France), measured in spectral reflectance. To measure leaf morphology, we scanned the leaves (Canoscan 9000F) and quantified morphology using *Lamina* (Bylesjo et al. 2008), which produced leaf morphology traits that included leaf complexity ($\frac{\text{leaf perimeter}^2}{\text{leaf area}}$), the width of leaf indents (mm) and the number of leaf indents standardised by perimeter (indents per mm). We then weighed the leaves of each plant and calculated specific leaf area (SLA $= \frac{leaf\ area}{leaf\ weight}$ in $mm^2/\mu g$). We used R (v.3.6.1; R Core Team 2019) for all analyses.

Prior to analysis we standardised each trait by their mean so that traits measured on different scales could be compared (Hansen and Houle 2008). We chose this method of standardisation because it captures evolvability as the amount of genetic variance relative to the mean phenotype, which is the most appropriate standardisation for comparing changes in mean phenotype across elevations (i.e. plasticity) with genetic variance.

Quantifying differences in multivariate phenotype

To quantify species differences in phenotypic plasticity across the elevational gradient, we used a Multivariate Analysis of Variance (MANOVA), which tested for significant differences in mean multivariate phenotype across elevation. We included all five phenotypic traits as the multivariate response variable. Elevation, species and their interaction were included as fixed effects. Block within elevation and family within species was used as the error term to test whether differences among species and elevation were greater than among blocks and families. To visualise differences in phenotype, we estimated **D**, the matrix representing differences in mean multivariate phenotype, and calculated the scores for the first two axes of **D**. Methods for constructing **D** are presented in **Methods S1**.

Estimating genetic variance in leaf traits

We estimated genetic for each species at each elevation with the package *MCMCglmm* (Hadfield 2010) by applying the linear mixed model

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

where s_i represents the *i*th sire, d_j the *j*th dam, b_k the variance among blocks within a transplant elevation and $e_{l(ijk)}$ the residual variation. The five normally distributed leaf traits were included as the multivariate response variable ($\boldsymbol{y_{ijkl}}$). To calculate **G** for both species at each elevation (*n*=8), we applied equation 1 separately to the data collected for each species at each elevation. We then extracted the sire component, which represents one-quarter of the additive genetic variance in the response variable (Lynch and Walsh 1998). We used chains with a burn-in of 150,000 iterations, a thinning interval of 1,500 iterations and saving 2,000 iterations that provided the posterior distribution for all parameters estimated. We confirmed model convergence by checking the chains mixed sufficiently well, that autocorrelation was lower than 0.05, and that our parameter-expanded prior was uninformative (Hadfield 2010).

MCMCglmm estimates of genetic variance are constrained to be positive, which means that tests for significant levels of genetic variance need to compare the observed estimates with a suitable null distribution created by randomising offspring among sires and dams, and re-applying the model to the randomised data. To maintain differences among the experimental blocks, we randomised the parentage of offspring within each block separately. We conducted 1,000 randomisations for each observed G-matrix. Where the posterior mean of our observed estimates of genetic variance exceeds the null distribution there is strong evidence of statistically significant genetic variance.

(1) Survival reflects adaptive divergence

To test whether these two species display patterns of adaptive divergence, we used survival models implemented within the R package *coxme* (Therneau 2012). We included the censored survival at the end of the experiment and the interaction between transplant elevation and species as a fixed effect. Experimental block and family were included as random effects. We tested for a significant elevation×species interaction using a log-likelihood ratio test. We used the package *emmeans* (Lenth 2019) to test for pairwise differences between species and elevations.

To test whether genetic variance in survival changed across elevation for each species, we applied equation 1 using *MCMCglmm* and included survival as the presence/absence of plants after mortality stabilised at the end of summer (September 12th). We included survival as a binomial trait with a probit link. After back-

transforming parameters to the data scale using *QGlmm* (de Villemereuil et al. 2016), we estimated genetic variance in relative fitness by dividing V_a by the squared mean survival.

(2) Quantifying differences in genetic variance

G-matrices can differ in size (the total amount of genetic variance), shape or orientation. If all traits are genetically independent, all axes of a G-matrix will describe a similar amount of genetic variance and the matrix will be spherical. However, the shape of a G-matrix becomes more elliptical when genetic correlations among traits condense genetic variance into a smaller number of independent axes than the number of traits, that contain higher proportions of the total genetic variance. The orientation of **G** changes when different linear combinations of traits describe the major axes of different matrices. We decomposed the G-matrix at each elevation and used the eigenvalues to identify differences in shape, and the eigenvectors to identify differences in orientation.

To quantify differences in **G** between species and across elevations, we used a covariance tensor approach. The strength of this approach is that, unlike other methods that focus on pairwise comparisons, the covariance tensor can compare multiple matrices within a single framework. We only briefly describe the approach here, but more detailed descriptions are found in Hine et al. (2009); Aguirre et al. (2014); Walter et al. (2018), and a simplified description in **Fig. S3**. The covariance tensor is analogous to principal components analysis, but that uses a (co)variance matrix representing differences in variance. The tensor approach is therefore based on decomposition (i.e. eigenanalysis, analogous to principal components) of a matrix (**S**) that contains the raw element-by-element differences among the original matrices being compared. **S** is constructed by calculating the variances and covariances among all elements of the original matrices. The diagonal of **S** contains the *among-matrix variances* of both the trait variances and trait covariances. The off-diagonal of **S** then contains the *among-matrix covariances* of the trait variances and covariances. **S** therefore captures how each element of **G** varies among the original matrices (here, across elevations or between species). Decomposing **S** calculates the eigenvectors that describe the differences among the original matrices, which are then re-arranged and scaled to construct the eigentensors, the orthogonal axes that describe the differences in genetic variance contained in **S**.

The covariance tensor is used to quantify whether differences in **G** are statistically significant, and to identify which of the original matrices (here, species and elevations) underlie the differences in genetic variance that are captured by the covariance tensor.

To identify whether the observed eigentensors capture significant differences in genetic variance, we compared the observed differences among **G** to a suitable null distribution, created by re-applying equation 1 to data containing no expected differences in **G** (see supplementary code; Morrissey et al. 2019).

Breeding values are sampled from the observed matrices and randomly assigned to different elevations and species. Next, the phenotype is re-constructed around the randomised breeding values. Re-applying equation 1 then quantifies differences in genetic variance that are solely due random sampling (Morrissey et al. 2019). If the observed eigentensors described greater differences in genetic variance than the eigentensors constructed from the null distribution, then there is strong evidence for significant differences in observed **G** among elevations and between species.

To identify how each matrix contributes to differences among all matrices, we calculated the matrix coordinates of the eigentensors. The coordinates are scores that represent the correlation between each eigentensor (describing differences in genetic variance) and each of the original matrices. The coordinates can be interpreted similarly to a principal components analysis: larger scores indicate a greater correlation between any given matrix and the differences among matrices described by that particular eigentensor.

We applied the covariances tensor analysis to test: (1) Whether elevation or species differences created larger differences in genetic variance by comparing **G** for both species transplanted in the native habitats of both species (i.e. the elevational extremes). (2) Whether elevation created strong differences in **G** across elevation for each species separately.

(3) Does population-level plasticity occur in a direction of the phenotype containing large amounts of genetic variance?

To quantify the direction of multivariate plasticity between the home site of each species and each of the elevations away from the home site, we estimated the change in trait mean from the native elevation to each of the other elevations using

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

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

To test whether the direction of plasticity, represented by changes in mean phenotype between elevations, describes a large proportion of genetic variation, we used the matrix projection (on each species separately):

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

where $\Delta\bar{\boldsymbol{x}}_i$ represents the vector of plasticity calculated between the native site and the *i*th elevation away from the home site. *T* represents the transpose of the vector, and *Tr* represents the trace, i.e. the total variance in **G**, such that equation 3 yields the proportion of genetic variation described by the vector, $\Delta\bar{\boldsymbol{x}}_i$. The vector

of plasticity is projected through the *j*th MCMC sample of the genetic (co)variance matrix estimated at the *i*th elevation ($\mathbf{G}_{ij}$) away from the home site to identify what proportion of genetic variance (V_{ij}) lies in the direction of the plasticity vector. To compare whether genetic variation at the native site or at the *i*th elevation described more genetic variation, we also projected the plasticity vector through **G** of the home site.

(4) Does selection at elevations away from the native site align with genetic variance?

Given that we were unable to sample *S. chrysanthemifolius* leaves before substantial mortality of seedlings occurred at 2,000m (**Fig. S2**), we were not able to estimate selection on *S. chrysanthemifolius.* We therefore only estimate selection on leaf traits for *S. aethnensis* by calculating the genetic selection differential ($\boldsymbol{s}_g = cov_a(z, w)$) and the phenotypic selection gradient ($\boldsymbol{\beta}$) at each elevation away from the native 2,000m site. Because we wanted to interpret the coefficients of selection on the scale of the original traits (and not on the liability scale from a model with a probit error distribution), we included relative survival as a continuous response variable that assumes a gaussian distribution. Although this violates the assumption of normality, this is the approach suggested and commonly used (Lande and Arnold 1983; Mitchell-Olds and Shaw 1987; Hajduk et al. 2020). In the supplementary material we present estimates of selection on the latent scale.

To estimate $\boldsymbol{s}_g$, we used *MCMCglmm* to apply equation 1 to the data collected from each elevation, but we also included relative survival as a sixth response variable. We then extracted the additive genetic covariance between each trait and fitness, which is a vector (standardised to unit length) that represents the direction of selection on genetic variation (Price 1970; Queller 2017; Hajduk et al. 2020).

To estimate the phenotypic selection gradient ($\boldsymbol{\beta}$), we applied a multiple regression on the same data at each transplant elevation using the R package *lme4* (Bates et al. 2015) with the linear mixed model,

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

where z_n represents each leaf trait as a fixed effect and e represents the error variance. We included relative survival to the end of summer as the response variable (y) and extracted the trait coefficients to estimate the vector $\boldsymbol{\beta}$ (normalized to unit length) that captures the direction of phenotypic selection.

To test whether genetic variance aligned with selection, we used equation 3 to project both vectors that describe the direction of selection ($\boldsymbol{s}_g$ and $\boldsymbol{\beta}$) through **G** estimated at the home site and each elevation away from the home site. We chose this approach because we were interested in testing whether genetic variance aligned with the direction of selection without concern for the amount of genetic variance in survival that would determine the potential for adaptation to each elevation. To incorporate uncertainty in the estimates of

selection and in **G**, through each MCMC sample of **G** we projected each MCMC sample of $\boldsymbol{s}_g$, and each of 1,000 bootstrapped samples of $\boldsymbol{\beta}$.

Results

(1) Evidence of adaptive divergence and greater genetic variance for survival in novel environments

Seedling emergence was 75-85% for *S. chrysanthemifolius* across elevations, and 50-70% for *S. aethnensis* (**Fig. S4**). For *S. aethnensis,* c.60% of seedlings reached establishment at all elevations. By contrast, 75% *S. chrysanthemifolius* seedlings established at its native 500m site, fewer seedlings reached establishment at the edge of the range (35%) and the novel 2,000m elevation (25%) (**Fig. S4**). As evidence of adaptive divergence, each species survived better at their native elevation compared to elevations away from their native site (**Fig. 3a**). Furthermore, at the elevational extremes (i.e. the native habitats of each species), the native species performed better than the foreign species, which is supported by a significant species×elevation interaction ($\chi^2(3)=1678.30$, $P<0.00001$). At low elevations, *S. chrysanthemifolius* survived better than *S. aethnensis* (500m $Z=18.7$, $P<0.00001$; 1,000m $Z=3.2$, $P=0.0014$), whereas *S. aethnensis* survived significantly better at higher elevations (1,500m $Z=8.4$, $P<0.00001$; 2,000m $Z=14.9$, $P<0.00001$). For both species, genetic variance in survival was lowest (and near zero) at their native site, and greatest at their novel elevations (**Fig. 3b**), suggesting that genetic variance in seedling survival increases in more novel environments.

Changes in multivariate phenotype across elevation

The two species show significant differences in multivariate phenotype (**Fig. 4**; Wilks' $\lambda=0.17$, $F_{1,3011}=2918.4$, $P<0.0001$). As evidence of plasticity, multivariate mean phenotype changed significantly across elevation (**Fig. 4**; Wilks' $\lambda=0.35$, $F_{3,3011}=257.6$, $P<0.0001$). A significant species×elevation interaction (Wilks' $\lambda=0.79$, $F_{3,3011}=49.8$, $P<0.0001$) suggests that leaf plasticity across elevation differed between the two species. Plasticity in seedling morphology moved the phenotype of *S. chrysanthemifolius* towards the native phenotype of *S. aethnensis* at 2,000m, but the phenotype of *S. aethnensis* away from the native phenotype of *S. chrysanthemifolius* at 500m (**Fig. 4**). Univariate trait means are presented in **Fig. S5**.

2) Genetic variance changed more across elevation than between species

We quantified G-matrices for each species and at each elevation (**Table S3**). Compared to *S. aethnensis* and *S. chrysanthemifolius* at lower elevations (500m-1,500m), high mortality of *S. chrysanthemifolius* at the 2,000m novel elevation occurred before seedling establishment and meant that we could not measure leaves for as many individuals (see methods). Although we measured multiple individuals per family for *S.*

chrysanthemifolius at 2,000m, the reduced replication meant that estimates of genetic variance for all traits were bounded by zero. However, we believe that estimates of genetic variance for this species at 2,000m are still robust because the posterior distributions of genetic variance are normally-distributed for all but one trait (SLA; **Fig. S6**), and also because our observed estimates of genetic variance for all traits (except SLA) describe greater genetic variance than the null distribution (**Fig. S7**), suggesting that our observed estimates of genetic variance are statistically significant. Estimates of genetic variance for *S. chrysanthemifolius* at 2,000m should nevertheless be interpreted with caution because measurements were obtained after high mortality. By using the entire posterior distribution in all subsequent analyses, we can however, take into account the high uncertainty in the estimation of genetic variance for *S. chrysanthemifolius*.

We found that the shape and size of **G** was similar for both species at each elevation, and changed across elevations similarly for both species (**Table 1a**). We decomposed **G** at each elevation to identify the orthogonal axes (eigenvectors) that describe **G**. The first three eigenvectors of **G** together described more than 80% of total genetic variance (**Table 1b**) and described more genetic variance than expected under random sampling (**Fig. S8**), suggesting that our observed estimates of **G** describe more genetic variance than expected under a null distribution. For both species, genetic variance in leaf traits is lower at 2,000m compared to 500-1,500m (**Figs. S3-5**). For most elevations, >50% of total genetic variance is concentrated along $\boldsymbol{g}_{\max}$ (the primary axis of **G**), suggesting that **G** is elliptical (**Table 1b**). At 2,000m, however, $\boldsymbol{g}_{\max}$ describes 58% of total genetic variance for *S. chrysanthemifolius*, which is greater than 37% for *S. aethnensis* at 2,000m, and suggests that **G** is less elliptical for *S. aethnensis* at its native elevation.

The magnitude and sign (positive vs negative) of trait loadings quantifies how genetic variance in each of the original traits contribute to describing axes of **G** (**Table 1b**). Where different linear combinations of traits describe $\boldsymbol{g}_{\max}$ (the direction in **G** that contains the greatest amount of genetic variance) for different species or at different elevations suggests that **G** differs in orientation. For *S. aethnensis*, we found that a different combination of traits described $\boldsymbol{g}_{\max}$ at the native 2,000m site compared to the other lower elevations (**Table 1b**), which means that all elevations are nearly orthogonal to the native site (angle between $\boldsymbol{g}_{\max}$ at the native site [2,000m] and $\boldsymbol{g}_{\max}$ at: 1,500m=80.1°; 1,000m=65.8°; 500m=81.3°). By comparison, $\boldsymbol{g}_{\max}$ was described by the same linear combination of traits for *S. chrysanthemifolius* at all elevations (**Table 1b**), which is supported by small angles between $\boldsymbol{g}_{\max}$ of the native site compared to higher elevations (1,000m=11.8°; 1,500m=85.9°; 2,000m=11.8°). Elevational differences in the orientation of **G** are only observed for *S. chrysanthemifolius* in the second and third axes of **G** ($\boldsymbol{g}_2$ and $\boldsymbol{g}_3$), which suggest differences between the two lower and two higher elevations (**Table 1b**). Furthermore, the orientation of **G** was similar for both species at 2,000m (25.8°), but more different at 500m (60.2°). Overall, **G** was smaller at higher elevations, and changed

in orientation and shape more for *S. aethnensis* than *S. chrysanthemifolius*.

To quantify differences in **G** we used a covariance tensor approach. We tested whether species or elevation created larger changes in **G** by applying the tensor to the G-matrices of both species at the elevational extremes (i.e. at both native elevations). We found that two (of three) eigentensors described greater differences in genetic variance compared to the null expectation (**Fig. S9**), providing strong evidence for significant differences in **G**. Tensor summary tables are found in **Table S4**. The coordinates identify how each matrix contributes to the differences described by an eigentensor. The first eigentensor, which captures 54.3% of all differences among G-matrices, describes large differences between extreme elevations, but little difference between species (**Fig. 5a**). By contrast, the second eigentensor captures 39.1% of all differences among G-matrices, and describes large differences between species at 500m, but little difference between elevations (**Fig. 5a**). Elevation therefore created larger changes in **G** than differences due to adaptive divergence between the two species.

Applying the covariance tensor to each species separately, we tested for changes in genetic variance across elevation. For *S. aethnensis*, genetic variance changed abruptly away from its native 2,000m site (**Fig. 5b**), which contrasts to *S. chrysanthemifolius* that showed differences between the native range (500m and 1,000m) and higher elevations (1,500m and 2,000m) (**Fig. 5b**). Given that genetic correlations among elevations are greater than 0.6 for all traits (**Table S5**), changes in **G** are likely to have been created by changes in variance (**Table 1**) rather than from changes in the relative values (i.e., crossing of reaction norm).

(3) Genetic variance in the direction of plasticity is moderate

To test whether plasticity (change in mean phenotype across elevations) occurred in the direction of the phenotype containing large amounts of genetic variance, we projected vectors of plasticity through **G** estimated at each elevation. For *S. aethnensis*, only c.25% of total genetic variance was in the direction of plasticity for all elevations, and only at 1,500m is plasticity observed to align better with genetic variance at the native site compared to the alternative elevation (**Fig. 6a**). For *S. chrysanthemifolius*, however, plasticity occurred in a direction that contained slightly more genetic variance (c.25-45%), and that is associated with a better alignment with the native site at all elevations, but is only statistically significant at the edge of the range (1,500m) (**Fig. 6b**). Confirming that genetic variance in the direction of plasticity is lower for *S. aethnensis* compared to *S. chrysanthemifolius*, the angle between plasticity and $\boldsymbol{g}_{\max}$ of the native site was greater for *S. aethnensis* at 1,500m=75.8°, 1,000m=70.8° and 500m=89.8° than for *S. chrysanthemifolius* at 1,000m=51.3°, 1,500m=37.2° and 2,000m=46.1°.

(4) Changes in genetic variance align with genetic but not phenotypic selection

For *S. aethnensis* planted at each elevation away from the native 2,000m site, we calculated selection on

leaf traits as genetic selection differentials ($\boldsymbol{s}_g$) and phenotypic selection gradients ($\boldsymbol{\beta}$). Within the range, phenotypic selection 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) (**Fig S10** and **Table S6**). We detected no significant estimates of selection on leaf indent widths or the number of indents.

The direction of genetic ($\boldsymbol{s}_g$) selection described a large proportion (55-65%) of genetic variance within the range (1,500m) and at the edge (1,000m) compared to genetic variance estimated at the native elevation (<25%) (**Fig. 6a**). A greater proportion of genetic variance at the novel elevation (c.37%) also aligned with selection compared to the native site (25%), but the difference is not statistically significant. Reflecting a close alignment between genetic variance and selection, the direction of $\boldsymbol{g}_{max}$ is very similar to genetic selection ($\boldsymbol{s}_g$) for *S. aethnensis*, except at the novel elevation: Angle between selection and $\boldsymbol{g}_{max}$ measured at 1,500m=20.8°, 1,000m=16.4° and 500m=65.0°.

In contrast with genetic selection, a much smaller proportion of genetic variance (<25%) aligns with the direction of phenotypic selection ($\boldsymbol{\beta}$) (**Fig. 6a**). Furthermore, selection is in a direction that describes more genetic variance at the native site than at the elevations away from the native site, although this difference is not statistically significant (**Fig. 6a**). However, a significantly greater proportion of genetic variance lies in the direction of phenotypic selection at the novel 500m elevation (c.20%) compared to elevations within the range and at the range edge (c.10%) (**Fig. 6a**).

At the novel elevations, the phenotype of the native species represents a possible phenotypic optimum. We found that for *S. aethnensis* at 500m, only c.25% of total genetic variance is in the direction of the native phenotype of *S. chrysanthemifolius* (**Fig. 6c**). By contrast, at 2,000m, a much greater proportion (c.50%) of genetic variance in *S. chrysanthemifolius* is in the direction of the native phenotype of *S. aethnensis* (**Fig. 6c**). These results suggest that although **G** estimated for *S. chrysanthemifolius* does not change across elevation as much as *S. aethnensis*, $\boldsymbol{g}_{max}$ aligns with the direction of the phenotypic optimum at 2,000m. By contrast, *S.* aethnensis shows greater changes in **G** but that do not align with the phenotype of *S. chrysanthemifolius* at 500m.

Discussion

Field studies combining estimates of selection, genetic variance and plasticity are rare, and to our knowledge, have not included the effects of novel environmental variation in determining likely future evolutionary trajectories. We planted seeds from a breeding design of two closely related but ecologically distinct Sicilian *Senecio* species across an environmental (elevation) gradient that included their native habitats, elevations at the edge of their native ranges, and a novel elevation (the native habitat of the other species). We quantified five ecologically-important leaf traits, and survival.

We find support for four hypotheses that are important for understanding how populations respond to environmental gradients: First, adaptive plasticity can maintain high survival across elevations up to the range edge, which is associated with low genetic variance in survival. However, where survival is greatly reduced in novel environments, genetic variance in survival increases, which could allow adaptation to the novel environments (**Fig. 3b**). Second, the environment altered the distribution of multivariate genetic variance (**Fig. 5**), which reduced genetic variance at higher elevations for both species. However, *S. aethnensis* showed larger changes in orientation when compared to *S. chrysanthemifolius*. Third, plasticity of the population occurred in a direction of the phenotype that only contained moderate amounts of genetic variance (25-45% of total genetic variance), which was greater for *S. chrysanthemifolius* when compared to *S. aethnensis* (**Fig. 6a-b**). Furthermore, genetic variance estimated at the native site was more closely associated with plasticity than genetic variance estimated at elevations away from the native site, which suggests that plasticity occurs in the direction of genetic variation. Fourth, changes in genetic variance for *S. aethnensis* at elevations away from the native site aligned with genetic, but not phenotypic selection (**Fig. 6a**). Furthermore, in novel environments, genetic variance was in the direction of the native phenotype (the local optimum) for *S. chrysanthemifolius* but not for *S. aethnensis.*

Together, these results suggest that whether plasticity is adaptive or non-adaptive in novel environments, and the potential for adaptation to novel environments will be species-specific. For *S. chrysanthemifolius* at the novel 2,000m elevation, changes in genetic variance were smaller and associated with plasticity and the direction of the phenotypic optimum at that elevation (**Fig. 7a**). By contrast, for *S. aethnensis* at the novel 500m elevation, larger changes in genetic variance were associated with an alignment with genetic selection, but not with the direction of the phenotypic optimum or plasticity (**Fig. 7b**). Both species, however, displayed greater genetic variance in fitness at novel elevations, suggesting a greater potential for adaptation in novel environments.

Although we provide substantial evidence for changes in genetic variance, we acknowledge that we were unable to measure all individuals before selection, and that estimates of genetic variance and selection can be biased when viability selection removes phenotypes before they can be measured (Hadfield 2008). However,

this issue is notoriously difficult to avoid in field experiments, and given that we measured individuals from almost all families of *S. chrysanthemifolius*, and all families of *S. aethnensis* before selection, there should be few genotypes missing data and our estimates of genetic variance and selection should be robust.

Low genetic variance in the direction of plasticity

Given that we only found moderate amounts of genetic variance in the direction of plasticity, our results contrast with a meta-analysis that found that plasticity in novel environments aligned with genetic variance (Noble et al. 2019). However, they also found that the strength of the alignment varied across study species, traits and environments, making broad conclusions difficult. In addition, many of the studies were conducted under laboratory conditions with small numbers of traits and environmental variables, and the increased complexity of the field environment could reduce any alignment between genetic variance and plasticity in our data. It is possible that variation among studies in the alignment between plasticity and genetic variance could be determined by the level of canalisation in the plastic response, which occurs when all genotypes of a given species show the same plastic response and there is little genetic variation in plasticity (Debat and David 2001). During adaptation, if the environment is predictable and selection on plasticity is strong, then genetic variation in the direction of plasticity is likely to be reduced, leading to canalization (Oostra et al. 2018). We need to better understand the genetic basis of plasticity under natural conditions to identify when and where we would expect genetic variance to align with plasticity, and to allow rapid evolutionary responses in novel environments.

Environmental effects on genetic variance

Wood and Brodie III (2015) found evidence that **G** is likely to be affected by the environment as much as by evolution, however it remained unclear why **G** changed in response to the environment. We help to resolve this by showing that novel environments not only create larger changes in **G** than evolutionary history, but that such changes can also align **G** with the response to selection. To better understand the potential for evolutionary rescue, we should therefore focus on quantifying how genotypes from across a species' range vary in their response to environmental variation, which create genotype-by-environment interactions (G×E) that change **G**, and whether such changes align with selection that can lead to rapid adaptation. Evolutionary rescue will be possible if sufficient G×E underlying plasticity is available, and selection on genetic variation in plasticity increases fitness in novel environments (Lande 2009; Chevin and Lande 2011; Walter et al. 2020b) that can then lead to genetic assimilation of an initially plastic response (Waddington 1953; Lande 2009). However, we still do not know whether environmental change will be too extreme or rapid to allow evolutionary rescue.

Potential for persistence in novel environments

The initial resilience of populations exposed to a novel environment is likely to depend on how close plasticity moves the population towards a phenotypic optimum. Evidence suggests that plasticity is more often nonadaptive in novel environments (Langerhans and DeWitt 2002; Palacio-López et al. 2015; Acasuso-Rivero et al. 2019), which means that populations will probably need to rely on rapid adaptation to maintain fitness and prevent extinction. Our results suggest that while plasticity in the low-elevation species is (to some extent) adaptive, the high-elevation species showed stronger evidence of nonadaptive plasticity, which is consistent with other studies of high-elevation plants (Gugger 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.

Adaptation to the novel elevations requires genetic variation in the direction of selection. While we provided evidence that genetic variance in survival increased in the novel environments for both species, 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 to adaptation that will make it difficult for rapid adaptation to occur and allow evolutionary rescue. By contrast, rapid adaptation to the high-elevation habitat is much more likely for the low-elevation species. Given that *S. chrysanthemifolius* is suspected to be similar to the common ancestor of both species, it is likely that the large proportion of genetic variation in the direction of the phenotypic optimum at 2,000m promoted adaptation to the high-elevation habitat that led to adaptive divergence and the emergence of the high elevation species.

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Tables

Table 1 Summaries of **G** estimated for each species at each elevation. **(a)** Estimates of multivariate evolvability: n_D = effective number of dimensions, $\boldsymbol{e}_{max}$ = maximum evolvability, and Tr = total genetic variance. 95% Highest Posterior Density (HPD) intervals are presented in parentheses. Both species show similar values for all three metrics at each elevation, and change similarly across elevations with smaller genetic variances and evolvabilities at higher elevations. **(b)** The first three eigenvectors of **G** that describe >80% of total genetic variance estimated at each elevation and for each species. HPD = 95% HPD intervals. 'Proportion' quantifies the proportion of total genetic variance that each eigenvector describes. Trait loadings in bold are greater than 0.2 to aid interpretation of the eigenvectors. Highlighted in yellow and blue are linear combinations of traits that underlie elevational differences in $\boldsymbol{g}_{max}$ for *S. aethnensis*, and elevational differences in $\boldsymbol{g}_2$ and $\boldsymbol{g}_3$ for *S. chrysanthemifolius.*

	500m			**1,000m**			**1,500m**			**2,000m**		
(a)	n_D	$\boldsymbol{e}_{max}$	Tr	n_D	$\boldsymbol{e}_{max}$	Tr	n_D	$\boldsymbol{e}_{max}$	Tr	n_D	$\boldsymbol{e}_{max}$	Tr
S. aethnensis	1.81 (1.29, 2.36)	0.23 (0.17, 0.30)	0.10 (0.06, 0.14)	1.64 (1.26, 2.15)	0.22 (0.16, 0.29)	0.08 (0.05, 0.12)	1.49 (1.13, 1.98)	0.23 (0.16, 0.31)	0.08 (0.04, 0.13)	2.03 (1.49, 2.68)	0.14 (0.10, 0.18)	0.04 (0.02, 0.06)
S. chrysanth.	1.65 (1.27, 2.12)	0.23 (0.17, 0.31)	0.09 (0.05, 0.13)	1.59 (1.22, 1.99)	0.21 (0.14, 0.28)	0.07 (0.04, 0.11)	1.49 (1.05, 2.05)	0.14 (0.08, 0.20)	0.03 (0.01, 0.06)	1.51 (1.09, 2.07)	0.17 (0.09, 0.25)	0.04 (0.01, 0.08)
(b)	$\boldsymbol{g}_{max}$	$\boldsymbol{g}_2$	$\boldsymbol{g}_3$	$\boldsymbol{g}_{max}$	$\boldsymbol{g}_2$	$\boldsymbol{g}_3$	$\boldsymbol{g}_{max}$	$\boldsymbol{g}_2$	$\boldsymbol{g}_3$	$\boldsymbol{g}_{max}$	$\boldsymbol{g}_2$	$\boldsymbol{g}_3$
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 lower	0.023	0.010	0.004	0.024	0.008	0.002	0.023	0.007	0.000	0.006	0.003	0.001
HPD upper	0.083	0.036	0.031	0.080	0.028	0.022	0.087	0.029	0.022	0.025	0.019	0.021
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 lower	0.023	0.010	0.005	0.015	0.010	0.001	0.004	0.000	0.000	0.000	0.001	0.001
HPD upper	0.087	0.032	0.024	0.075	0.039	0.009	0.029	0.027	0.010	0.051	0.021	0.011
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

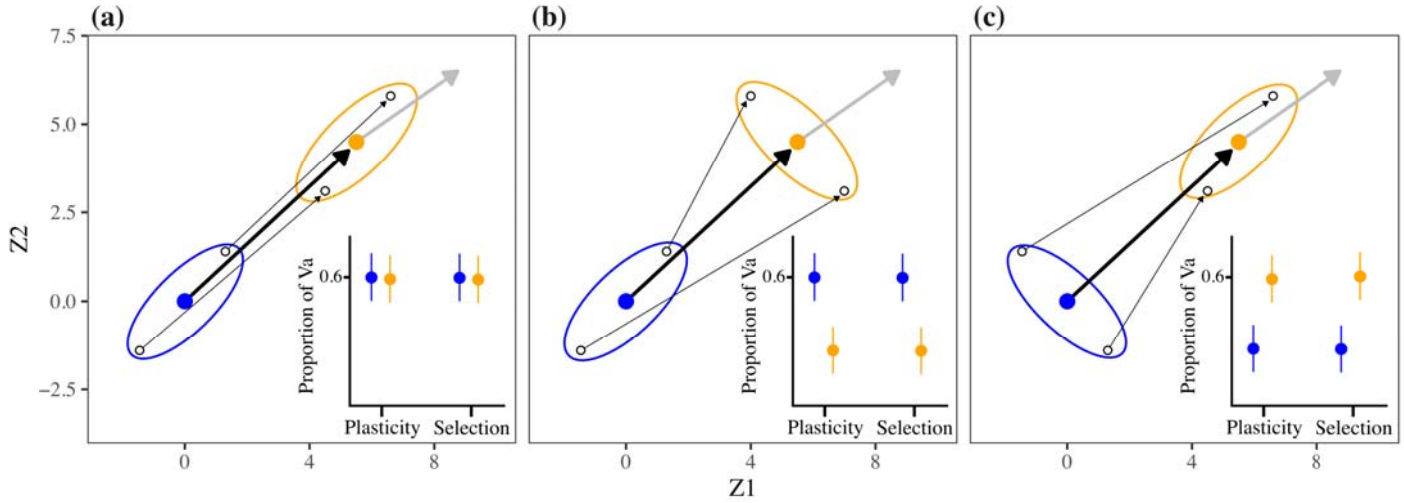

Fig. 1 Conceptual diagram depicting the trait means (large circles) and genetic (co)variances (ellipses) for two traits (Z1 and Z2) measured for a population in its native environment (blue) and a novel environment (orange). Large black arrows represent change in the population mean due to plasticity, and the grey arrow represents the direction of selection in the novel environment. Unfilled circles and small arrows represent the change in two hypothetical genotypes. Inset graphs quantify the proportion of genetic variation that the vectors of plasticity and selection describe in the native and novel environments. **(a)** We expect adaptation to be most rapid if both plasticity and selection are in the direction of the phenotype containing a large amount of genetic variance, i.e. in a direction similar to the long axis of the ellipse of **G**. In this scenario, both plasticity and selection describe similar amounts of genetic variation and genotypes show the same plasticity. **(b)** However, we know that genotype-by-environment (G×E) interactions (i.e. genetic variance in plasticity) are commonly found, which are represented here by different responses of the two genotypes to the novel environment. G×E is likely to change either the orientation or shape (not shown) of **G** (Wood and Brodie III 2015), in which case genetic variance could align with plasticity, but only in the native environment. Such changes in **G** could be maladaptive in a novel environment if **G** becomes misaligned with the direction of selection. In this scenario, G×E is not beneficial because both plasticity and selection align with **G** in the native environment, but not in the novel environment. **(c)** G×E could also create changes in **G** that align with selection and plasticity in the novel environment, which would increase the adaptive potential of the population in the novel environment because there would be more genetic variation available for selection to promote adaptation.

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 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).

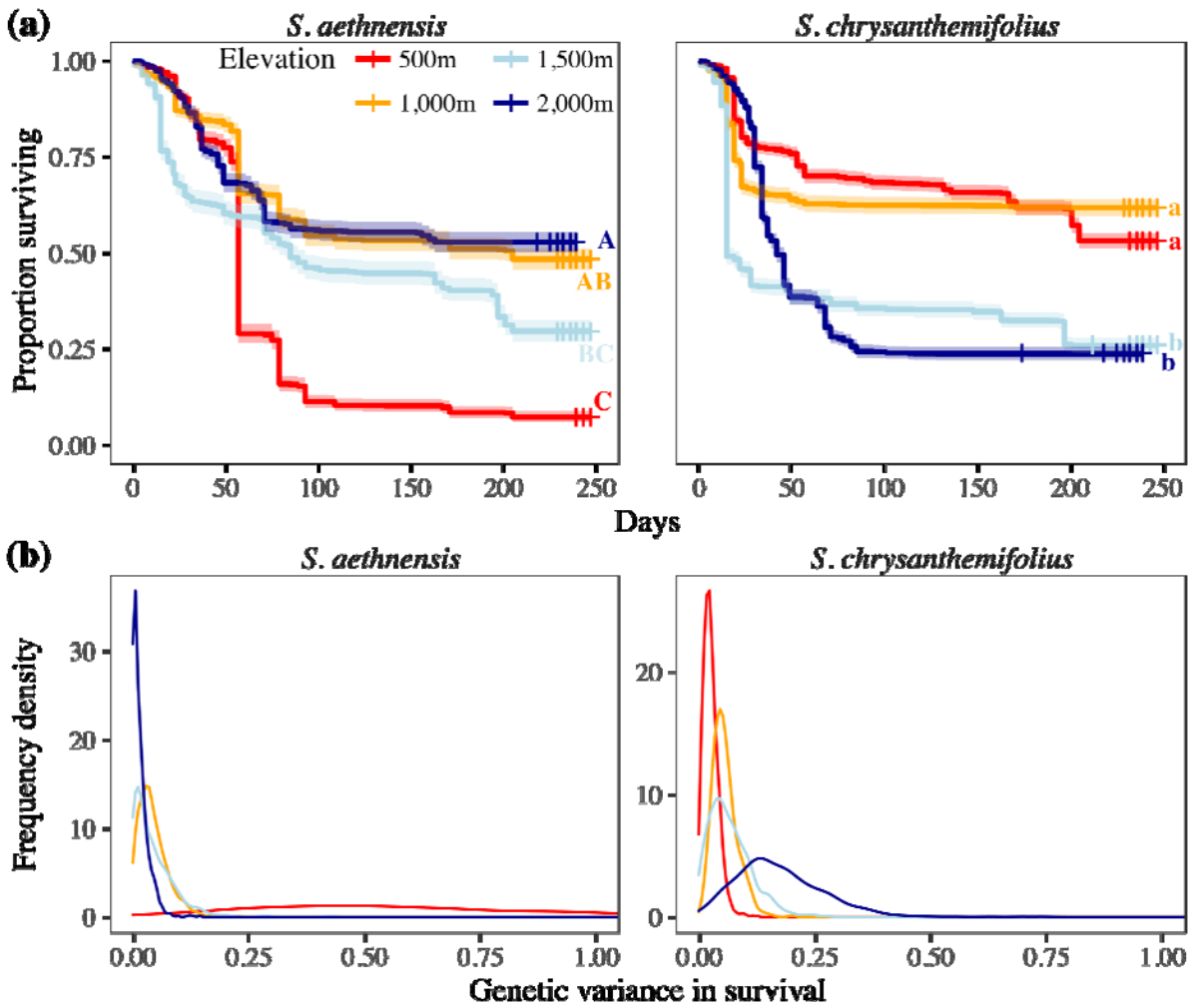

Fig. 3 (a) Differences in survival for the two species reflect adaptive divergence to their natural habitats. Letters represent significant differences in survival. *Senecio aethnensis* survived best at its native 2,000m site, but suffered high mortality at 500m. While the 1,500m site showed intermediate survival, survival at the 1,000m site at the edge of the range was as high as the native site. By contrast, *S. chrysanthemifolius* shows high survival at the two lower elevations within the native range (500-1,000m), and lower survival at the two higher elevations. **(b)** For both species, additive genetic variance in survival was near zero at their native sites, and significantly greater at the novel elevations, where their posterior distributions did not overlap at >85% Highest Posterior Density (HPD) interval. The 1,000m and 1,500m sites showed an intermediate level of genetic variance in survival in both species.

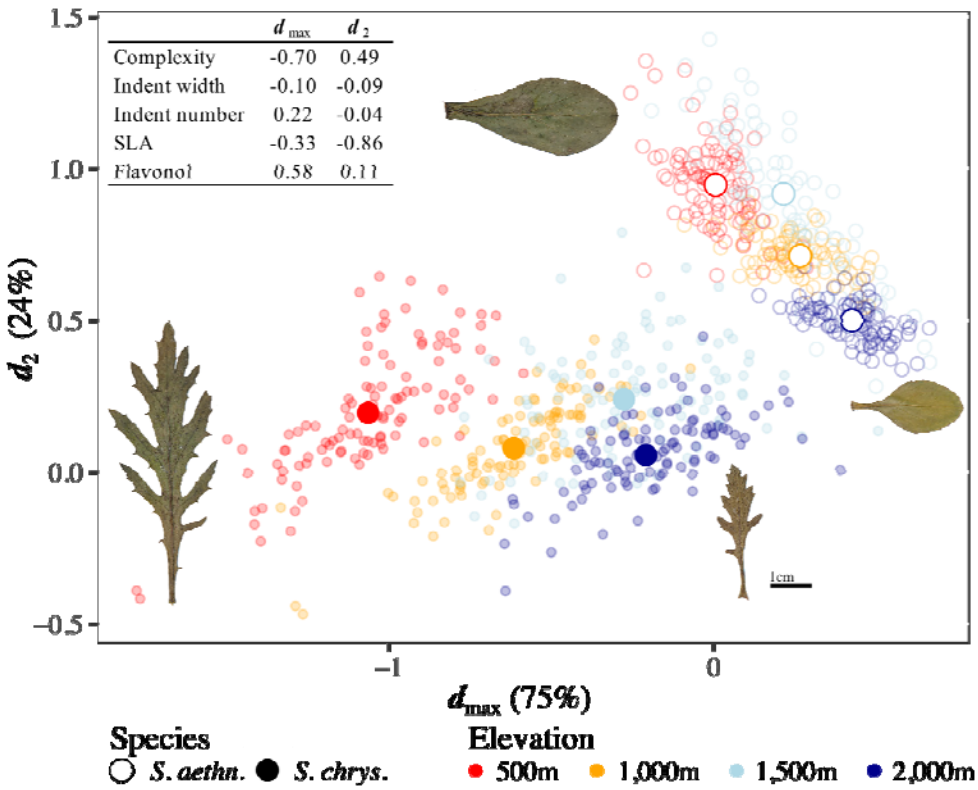

Fig. 4 (a) Evidence of phenotypic plasticity as large changes in mean multivariate phenotype across elevation. The first two axes of **D** together represent 99% of all change in mean phenotype, with the table inset displaying the trait loadings for each axis. Large, coloured circles represent the mean of each species at each transplant elevation, with the size of the circle exceeding one standard error. Small circles represent the mean for each full-sibling family. Inset leaves represent a plant near the mean phenotype of each species for the elevational extremes.

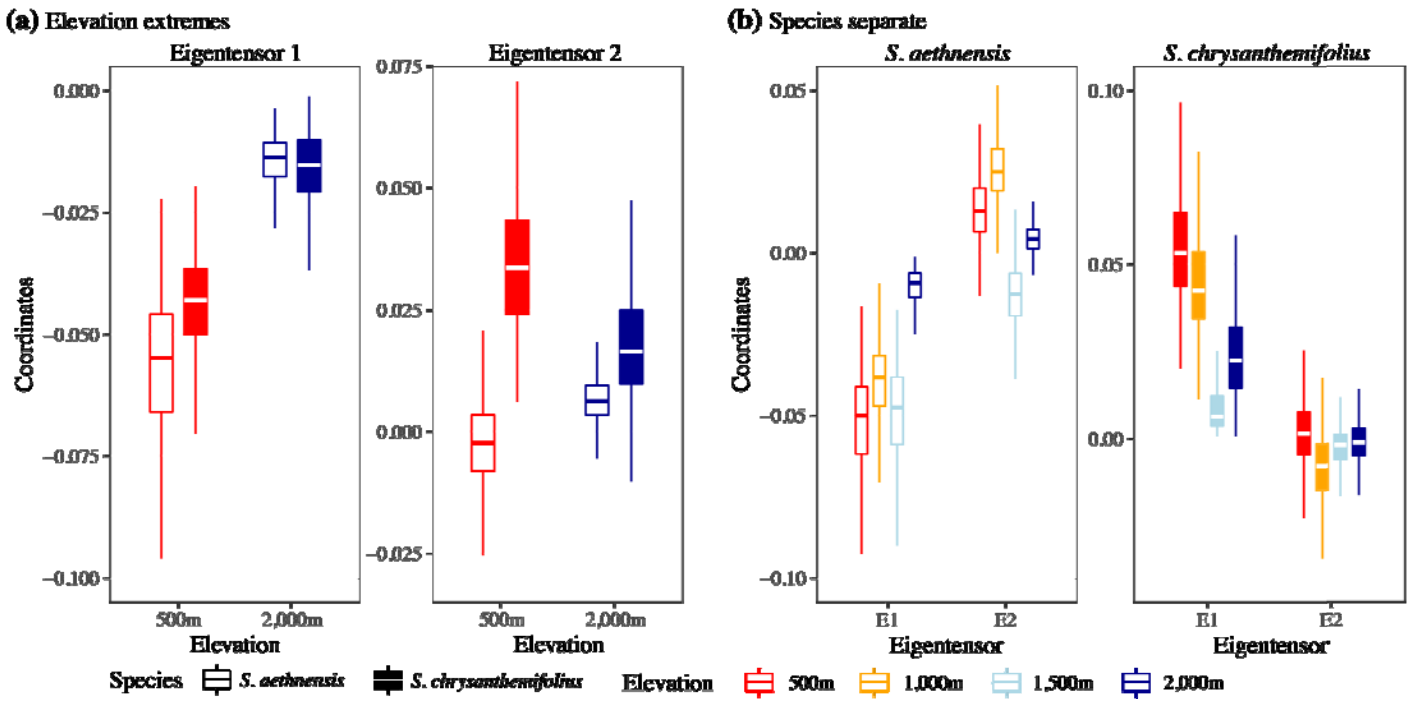

Fig. 5 Elevation induces larger changes in **G** than differences between species. **(a)** Boxplots represent the posterior distribution of the coordinates for **G** estimated for both species at the elevational extremes (i.e. reciprocally planted at the native habitats of both species). The coordinates quantify how each matrix contributes to differences in genetic variance described by each eigentensor. Differences in **G** due to elevation (red vs blue) are greater than differences between species (filled vs unfilled). This is because the first eigentensor, which describes the largest difference in genetic variance, describes differences across elevation but not between species. The second eigentensor describes a smaller difference in genetic variance, which is associated with larger differences between the two species than across elevations. **(b)** Elevational changes in **G** within each species. Differences among elevations are stronger for *S. aethnensis* than for *S. chrysanthemifolius.* For *S. aethnensis*, **G** changes consistently for all elevations away from the native site, whereas *S. chrysanthemifolius* shows differences between the two lower and two higher elevations.

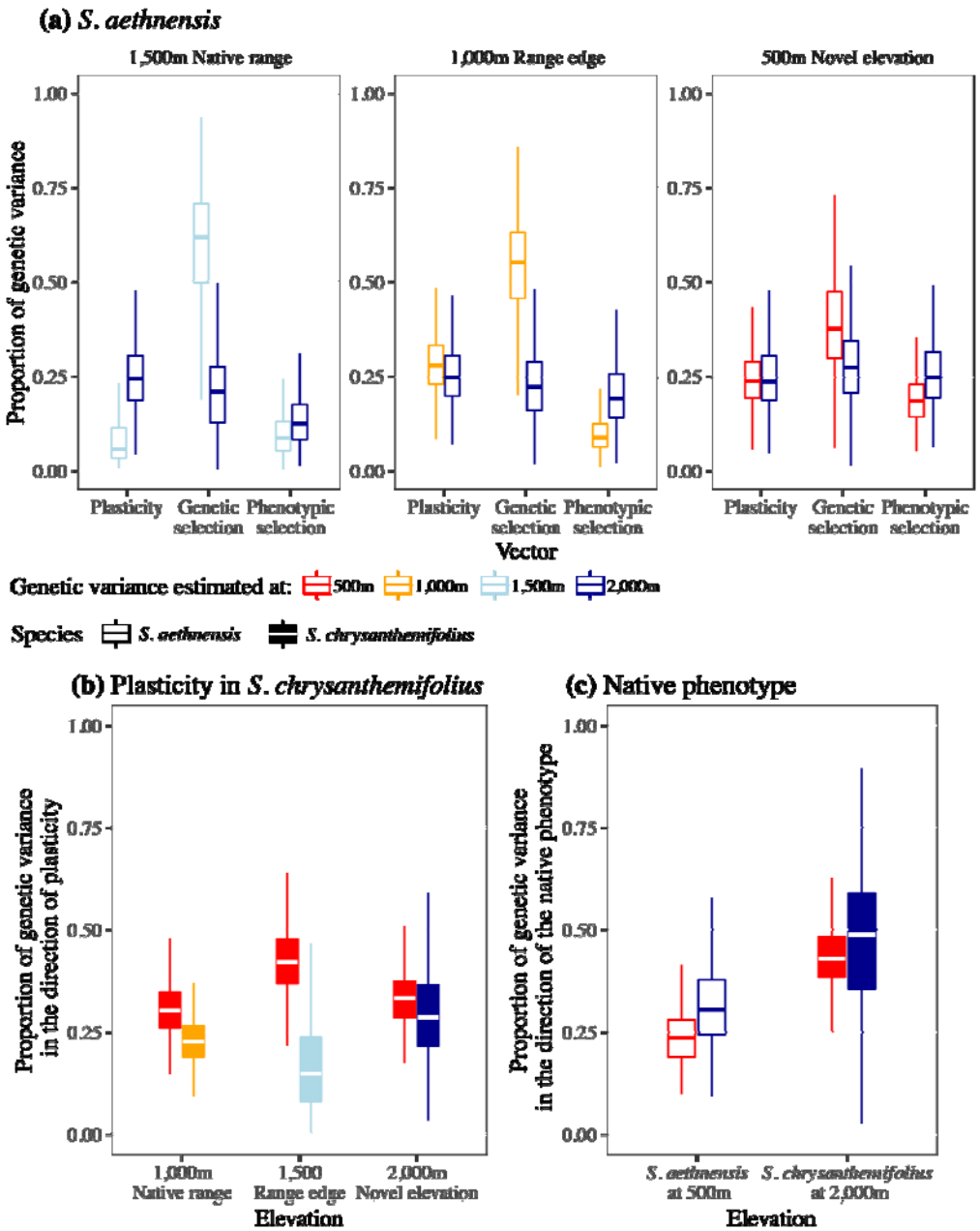

Fig. 6 Boxplots representing the proportion of genetic variance that lies in the direction of vectors that represent plasticity (change in mean phenotype), selection and the native phenotype (optimum phenotype). The proportion of genetic variance is quantified for the native site, which is compared to each elevation away from the native site with asterisks denoting significant differences (distributions do not overlap at >90% HPD). **(a)** Plasticity and selection for *S. aethnensis* at each elevation away from the native site. Plasticity: Only c.25% of genetic variance is in the direction of plasticity, suggesting that plasticity does not occur in directions of the phenotype that contain large amounts of genetic variance. Plasticity only aligns better with genetic variance at the native site for *S. aethnensis* within the range (1,500m). Genetic selection: Compared to genetic variance estimated at the native site (c.25%), a larger proportion (c.65%) of genetic variance quantified at elevations away from the native site lies in the direction of genetic selection. Phenotype

selection: When compared to genetic selection, a smaller proportion (<25%) of genetic variance lies in the direction of the phenotypic selection gradient. Phenotypic selection describes a greater proportion of genetic variance at the native site compared to elevations away from the native site. **(b)** The proportion of genetic variance in the direction of plasticity for *S. chrysanthemifolius*. At all elevations, there is more genetic variance at the native elevation compared to each of the other elevations, but differences are only significant at the range edge (1,500m). A greater proportion of genetic variance at the native site lies in the direction of plasticity (30-45%), when compared with *S. aethnensis* (c.25%). **(c)** For each species at the novel elevation (i.e., the native elevation for the other species), the phenotype of the native species represents an optimum phenotype. More genetic variance lies in the direction of the native phenotype for *S. chrysanthemifolius* at 2,000m (c.45-50%) when compared to *S. aethnensis* at 500m (c.25%). Genetic variation in *S. chrysanthemifolius* is therefore better aligned with the phenotypic optimum compared to *S. aethnensis*.

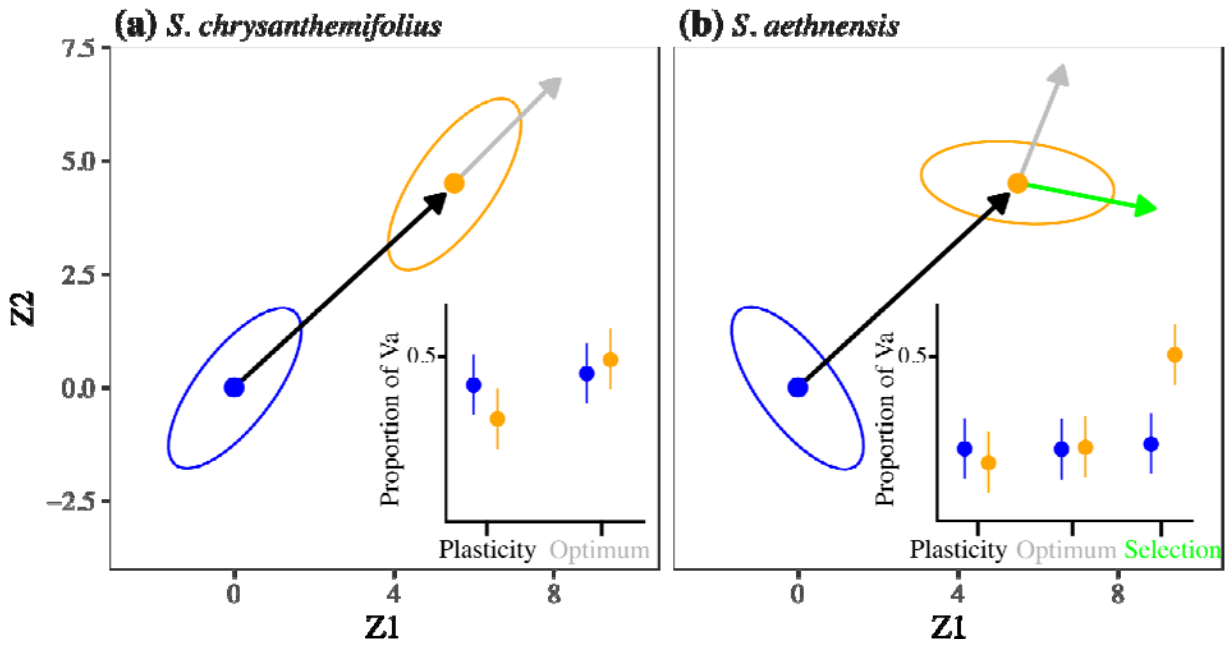

Fig. 7 Conceptual summary of the results for **(a)** *S. chrysanthemifolius* and **(b)** *S. aethnensis* for two traits (Z1 and Z2) that are genetically correlated. Changes in mean (filled circles and black arrows) represent plasticity, while changes in the ellipse represent changes in genetic variance. Blue represents each species at its native elevation, and orange at elevations away from their native elevation. Grey arrows represent the direction of the optimum, while the green arrow represents the direction of genetic selection. **(a)** *Senecio chrysanthemifolius* showed smaller changes in genetic variance, and the direction of plasticity and the native phenotype contained relatively high amount of genetic variance. **(b)** For *S. aethnensis*, genetic variance changed in orientation, with the direction of the optimum (native phenotype and phenotypic selection) and plasticity containing small proportions of genetic variance. However, changes in genetic variance aligned with genetic selection at the sites away from the native elevation.