

# 1 **Plasticity in novel environments induces larger changes in genetic variance than adaptive divergence**

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15

## 16 Abstract

17 Genetic correlations between traits are expected to constrain the rate of adaptation by concentrating genetic  
 18 variation in certain phenotypic directions, which are unlikely to align with the direction of selection in novel  
 19 environments. However, if genotypes vary in their response to novel environments, then plasticity could  
 20 create changes in genetic variation that will determine whether genetic constraints to adaptation arise. We  
 21 tested this hypothesis by mating two species of closely related, but ecologically distinct, Sicilian daisies  
 22 (*Senecio*, Asteraceae) using a quantitative genetics breeding design. We planted seeds of both species across  
 23 an elevational gradient that included the native habitat of each species and two intermediate elevations, and  
 24 measured eight leaf morphology and physiology traits on established seedlings. We detected large significant  
 25 changes in genetic variance across elevation and between species. Elevational changes in genetic variance  
 26 within species were greater than differences between the two species. Furthermore, changes in genetic  
 27 variation across elevation aligned with phenotypic plasticity. These results suggest that to understand  
 28 adaptation to novel environments we need to consider how genetic variance changes in response to  
 29 environmental variation, and the effect of such changes on genetic constraints to adaptation and the evolution  
 30 of plasticity.

31 **Keywords:** adaptive divergence, additive genetic variance, covariance tensor, evolutionary rescue, genotype-  
 32 by-environment interactions, G-matrix, novel environments, phenotypic plasticity

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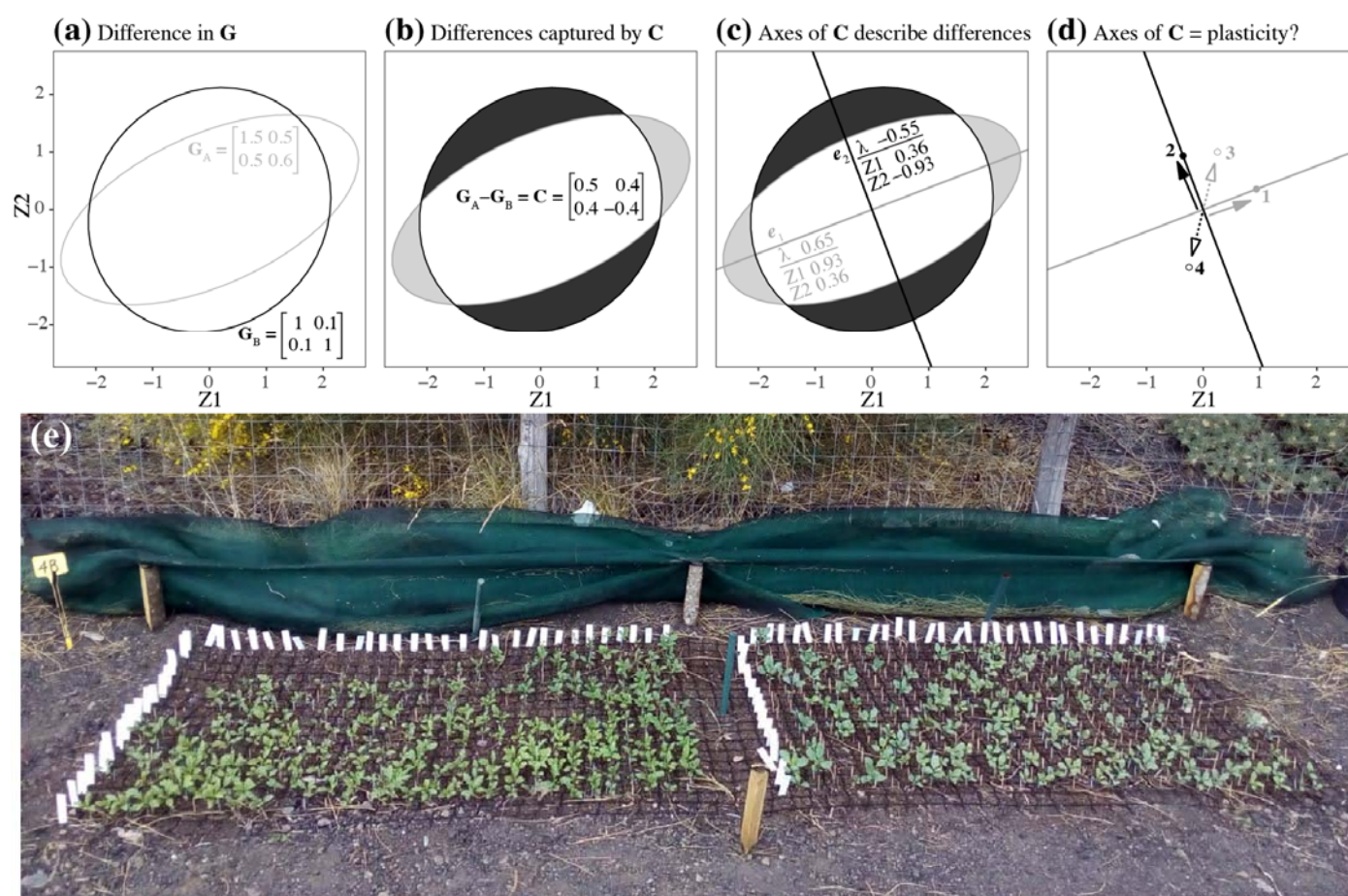
## 34 Introduction

35 Populations maintain resilience in response to novel environments if selection on existing genetic variation  
 36 (G) increases fitness over generations to create adaptation (termed 'evolutionary rescue'; Gomulkiewicz and  
 37 Holt 1995; Bell and Gonzalez 2009), or if the novel environment induces plastic changes for all genotypes  
 38 (E) that can maintain fitness (Via et al. 1995; Charmantier et al. 2008). In understanding population  
 39 responses to novel environments, studies often focus on the dichotomy of plasticity versus adaptation for  
 40 maintaining fitness and avoiding extinction. However, if genotypes vary in their sensitivity to the  
 41 environment, then genotype-by-environment interactions (G×E) underlying plasticity can change the amount  
 42 of genetic variation available to selection in novel environments (Wood and Brodie III 2015). Where  
 43 plasticity can no longer maintain fitness, the potential to persist in a novel environment will then be  
 44 determined by the extent to which G×E underlying plasticity changes genetic variation, and whether rapid  
 45 adaptation can ensue (Ghalambor et al. 2007).

46 The additive genetic variance-covariance matrix (**G**) describes the genetic architecture underlying  
 47 multivariate phenotypes (Lande 1979). Genetic correlations between traits are expected to concentrate  
 48 genetic variation in certain directions of the multivariate phenotype. If pleiotropy (or close linkage) underlies  
 49 genetic correlations, then any genetic changes in one trait will affect other traits similarly and **G** will be  
 50 stable, which will constrain adaptation when genetic variation lies in directions of the phenotype that differ to  
 51 selection (Lande 1980; Cheverud 1984; Arnold 1992; Arnold et al. 2008; Walsh and Blows 2009; Chenoweth  
 52 et al. 2010). However, if **G** changes in response to environmental variation, then G×E can determine the  
 53 availability of genetic variation in the direction of selection in novel environments, which will then determine  
 54 whether constraints to adaptation arise (Wood and Brodie III 2015), and therefore the potential for  
 55 evolutionary rescue.

56 Although **G** is expected to remain stable, at least in the short term (Zeng 1988), evidence suggests that **G** can  
 57 change during adaptive divergence (Doroszuk et al. 2008; Eroukhmanoff and Svensson 2011; McGlothlin et  
 58 al. 2018; Walter et al. 2018) and in response to environmental variation (Wood and Brodie III 2015;  
 59 Johansson et al. 2020). Evidence also suggests that plasticity in novel environments occurs along phenotypic  
 60 axes containing large amounts of genetic variation (Noble et al. 2019). However, we do not know whether, or  
 61 to what extent, shifts in **G** are associated with plasticity in novel environments. If plasticity creates changes  
 62 in **G**, then such changes in genetic variance can determine the potential for rapid adaptation to maintain  
 63 ecological resilience in novel environments. Therefore, by quantifying whether changes in **G** occur across  
 64 environments, and whether such changes align with plasticity, we can better understand how genetic  
 65 variation present in natural populations can respond to novel environments.

66 G-matrices can differ in the amount of variance in each trait, as well as in the genetic covariance between  
 67 traits. **Fig 1a-d** presents an example of how G-matrices for a hypothetical population could change across  
 68 two environments (A and B). Differences between two matrices can be captured by  $C = G_A - G_B$ , where  $C$  is  
 69 the matrix representing variance that is unique to each G-matrix (**Fig. 1b**). Eigenvectors of  $C$  then quantify  
 70 axes that describe the differences in genetic variance between the two original matrices (**Fig. 1c**). Using the  
 71 eigenvectors of  $C$  (i.e. the tensor of two matrices), we can test whether differences in  $G$  align with plastic  
 72 changes in mean phenotype across environments (**Fig. 1d**). Such an alignment would provide evidence that  
 73 genotype-by-environment interactions underlying plasticity can change  $G$ , and determine future evolutionary  
 74 responses to novel environments.



75  
 76 **Fig. 1 (a-d)** Conceptual diagram demonstrating, for two traits ( $Z1$  and  $Z2$ ), how differences in  $G$  for the same population exposed  
 77 to two environments (A and B) can be quantified with a two-matrix tensor, and then related to plasticity (change in mean  
 78 phenotype). **(a)** Hypothetical G-matrices are presented in the inset matrices, and visualised as two-dimensional ellipses ( $G_A$  in  
 79 gray, and  $G_B$  in black). The G-matrices for the two environments (inset tables) differ in shape due to different variances (along the  
 80 diagonal) and differences in covariances (off-diagonal). **(b)** Differences in  $G$  are represented by the gray shading for genetic  
 81 variance unique to environment A, and black shading for genetic variance unique to environment B. These differences in genetic  
 82 variance can be quantified using  $C = G_A - G_B$ , which has a positive difference in genetic variance in  $Z1$  (0.5) due to greater

83 genetic variance in Z1 for environment A. By contrast, Z2 has a negative genetic variance (-0.4) because environment B has  
 84 greater genetic variance in Z2. (c) Decomposing C identifies the two major axes (eigenvectors, which in this case are equivalent to  
 85 eigentensors), which are presented in the inset tables and represented by the black and gray lines. Each eigenvector describes  
 86 genetic variance that differs between the original matrices (eigenvalues represented by  $\lambda$ ), with the loadings of the traits describing  
 87 how each trait contributes to the differences in genetic variance described by each eigenvector. The first axis ( $e_1$ ) describes a  
 88 positive eigenvalue representing differences in genetic variance unique to environment A (gray shading along the gray line). The  
 89 second axis ( $e_2$ ) describes negative variance describing differences due to genetic variance unique to environment B (black shading  
 90 along the black line). (d) Changes in mean phenotype are represented by arrows and circles. If differences in genetic variance  
 91 underlie plasticity, we expect changes in mean phenotype along an axis representing genetic variance unique to either environment  
 92 A (point 1 and solid gray arrow), or environment B (point 2 and black arrow). However, if differences in genetic variance are not  
 93 associated with plastic responses to the two environments, then changes in mean phenotype would occur along an axis different to  
 94 changes in genetic variance (points 3 or 4, and dashed lines with unfilled arrows). (e) An example of a seedling block at 2,000m,  
 95 eight weeks after seeds were planted (*S. chrysanthemifolius* on left).

96

97 To test whether genotype-by-environment interactions create changes in genetic variance, we reciprocally  
 98 planted seeds of two ecologically contrasting, but closely related *Senecio* species across an elevational  
 99 gradient. *Senecio chrysanthemifolius* is a short-lived perennial with dissected leaves that occupies disturbed  
 100 habitats in the foothills of Mt. Etna (c.400-1,000 m.a.s.l [metres above sea level]), as well as across Sicily.  
 101 *Senecio aethnensis* is a perennial with entire glaucous leaves endemic to lava flows above 2,000m.a.s.l on  
 102 Mt. Etna, where individuals grow back each spring after being covered by snow in winter. The data we  
 103 analyse here are derived from an experiment where we mated among individuals within each species using a  
 104 quantitative genetics breeding design (Walter et al. 2021). We then reciprocally planted seeds (from each  
 105 family in the breeding design) of both species across an elevational gradient representing the home range of  
 106 each species, the edge of their range, and conditions outside their range (**Fig. 1e**). Previously we found  
 107 evidence for fitness trade-offs as differences in survival at elevational extremes, indicating specialisation of  
 108 each species to their native environment (Walter et al. 2021).

109 Here, we continue the analysis of the transplant experiment by including data on leaf morphology and  
 110 pigment traits, and testing whether genetic variance changes between species and across elevation.  
 111 Specifically, we test whether: 1) Seedlings show plasticity in novel environments that moves the phenotype  
 112 towards that of the native species, 2) Elevation or species differences are associated with larger changes in **G**,  
 113 and 3) Changes in **G** for each species aligned with the direction of plasticity as the elevational change in  
 114 mean phenotype.

115



# 116 **Methods and materials**

117 We only briefly describe the field experiment here, but refer the reader to the previous analysis where it is  
 118 presented in detail (Walter et al. 2021). We collected cuttings from naturally growing individuals, which we  
 119 propagated. We randomly assigned each individual as a sire (male) or dam (female) and mated each sire to  
 120 three dams (*S. aethnensis*  $n=36$  sires,  $n=35$  dams,  $n=94$  full-sibling families; *S. chrysanthemifolius*  $n=38$   
 121 sires,  $n=38$  dams,  $n=108$  full-sibling families).

122 We then planted 100 seeds from each family at four elevations on Mt. Etna that included the native habitats  
 123 of both species (500m and 2,000m) as well as two intermediate elevations (1,000m and 1,500m). We planted  
 124 25 seeds at each site, randomised into five experimental blocks (*S. aethnensis*  $n=432$  seeds/block,  $n=2,160$   
 125 seeds/site; *S. chrysanthemifolius*  $n=540$  seeds/block,  $n=2,700$  seeds/site; Total  $N=19,232$  seeds). To prepare  
 126 each experimental block, we cleared the ground of plant matter and debris, and then placed a plastic grid on  
 127 the ground with 4cm square cells. We attached each seed to the middle of a toothpick using non-drip super  
 128 glue and then pushed each toothpick into the soil so that the seed sat 1-2mm below the soil surface. To  
 129 replicate natural germination conditions, we suspended 90% shade-cloth 20cm above each plot and kept the  
 130 seeds moist until germination ceased (2-3 weeks). After this shade-cloth was removed and watering reduced.

131 When  $>80\%$  of plants had produced ten leaves at each transplant site, we collected the 5<sup>th</sup> and 6<sup>th</sup> leaves from  
 132 the base of the plant to quantify morphology and leaf pigment content. In total, we measured 6,454 plants  
 133 (500m  $n=2,369$ ; 1,000m  $n=1,929$ ; 1,500m  $n=1,030$ ; 2,000m  $n=1,126$ ), which included more than two  
 134 individuals for  $>90\%$  of the full-sibling families at each elevation (average number of individuals measured  
 135 per family: 500m  $=11.73 \pm 5.5$  [one standard deviation], 1,000m  $=9.55 \pm 3.7$ , 1,500m  $=5.10 \pm 2.8$ ,  
 136 2,000m  $=5.57 \pm 3.1$ ). This meant that all sires were measured at each site, and that mortality should not  
 137 influence the estimation of genetic variance. To quantify leaf pigment content, we used a Dualex instrument  
 138 (Force-A, France) to estimate the chlorophyll, flavonol and anthocyanin content of each leaf. To measure leaf  
 139 morphology, we scanned the leaves (Canoscan 9000F) and quantified morphology using the software Lamina  
 140 (Bylesjo et al. 2008), which produced leaf morphology traits that included leaf area, leaf complexity  
 141 ( $\frac{\text{leaf perimeter}^2}{\text{leaf area}}$ ), the width of leaf indents, and the number of leaf indents standardised by perimeter. We then  
 142 weighed the leaves of each plant and calculated specific leaf area ( $\text{SLA} = \frac{\text{leaf area}}{\text{leaf weight}}$ ). To analyse phenotype  
 143 data, we used R (v.3.6.1; R Core Team 2019 ) for all analyses. Prior to analysis, we standardised each trait by  
 144 their mean so that traits measured on different scales could be compared (Hansen and Houle 2008).

## 145 *1. Species differences in plasticity across elevation*

146 To quantify species differences in phenotypic plasticity across the elevational gradient, we used a

147 Multivariate Analysis of Variance (MANOVA), which tested for significant differences in mean multivariate  
 148 phenotype across elevation. We included all eight phenotypic traits as the multivariate response variable.  
 149 Elevation, species and their interaction were included as fixed effects. To visualise how the two species  
 150 differed across elevation we first constructed a D-matrix, the covariance matrix representing differences in  
 151 mean multivariate phenotype between species and across elevation (see glossary in **Table 1**). To construct **D**,  
 152 we extracted the Sums of Squares and Cross-Product (SSCP) matrices for each fixed effect ( $SSCP_S$  =  
 153 species;  $SSCP_E$  = elevation;  $SSCP_{S \times E}$  = species  $\times$  elevation) and the error term ( $SSCP_R$ ). We then estimated  
 154  $SSCP_H$  ( $SSCP_H = SSCP_S + SSCP_E + SSCP_{S \times E}$ ), which calculates the difference in mean across all elevations  
 155 for both species. We calculated Mean Square (MS) matrices by dividing the SSCP matrices by their  
 156 corresponding degrees of freedom ( $MS_H = \frac{SSCP_H}{7}$ ;  $MS_E = \frac{SSCP_E}{6,446}$ ). We then estimated **D** using

$$157 \quad \mathbf{D} = \frac{MS_H - MS_E}{nf}, \quad (1)$$

158 where  $nf$  represents the average number of individuals measured for each species at each elevation,  
 159 calculated from equation 9 in Martin et al. (2008). We used the eigenvectors of **D** to visualise differences in  
 160 multivariate phenotype across elevation for both species.

## 161 2. Quantifying species and elevational differences in genetic variance

162 Estimation of additive genetic variance: The additive genetic (co)variance matrix (**G**) represents the  
 163 multivariate genetic variance underlying morphological traits. To calculate **G** for each species at each  
 164 elevation, we used the package *MCMCglmm* (Hadfield 2010) and implemented the multivariate linear mixed  
 165 model

$$166 \quad y_{ijkl} = s_{i(j)} + d_{j(i)} + b_k + e_{l(ijk)}, \quad (2)$$

167 where  $s_{i(j)}$  represents the  $i$ th sire mated to the  $j$ th dam,  $d_{j(i)}$  the  $j$ th dam mated to the  $i$ th sire,  $b_k$  as the  
 168 variance among blocks within a transplant site and  $e_{l(ijk)}$  the residual error. The eight normally distributed  
 169 phenotypic traits were included as the multivariate response variable ( $y_{ijkl}$ ). We applied equation 2  
 170 separately to each species and transplant elevation, resulting in the estimation of eight G-matrices. For each  
 171 implementation, we extracted the sire variance component and multiplied it by four to calculate our observed  
 172 G-matrices (Lynch and Walsh 1998).

173 We implemented equation 2 using chains with a burn-in of 300,000 iterations, a thinning interval of 1,500  
 174 iterations and saving 2,000 iterations that provided the posterior distribution for all parameters estimated. We  
 175 confirmed model convergence by checking that the chains mixed sufficiently well and that autocorrelation

was lower than 0.05, and that our parameter-expanded prior was uninformative.

To test whether our experimental design captured biologically meaningful estimates of genetic variance, for each implementation of equation 2, we randomised offspring among sires and dams, and re-applied 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, which we used to estimate our randomised G-matrices representing the null distribution for our estimation of **G**. Observed estimates of genetic variance that exceed the null distribution provides strong evidence that our estimates of genetic variance are statistically significant.

**Table 1** Glossary of quantitative genetics terms

Term	Sym- bol	Definition
D-matrix	<b>D</b>	The variance-covariance matrix of mean phenotype. This captures how a group of traits differs in multivariate mean among levels of a covariate (e.g., elevation)
G-matrix	<b>G</b>	The additive genetic variance-covariance matrix underlying a set of traits. Genetic variances on the diagonal and genetic covariances among traits off the diagonal
$d_{\max}$		The first eigenvector of <b>D</b> , representing the axis along which the greatest differences in mean multivariate phenotype lie
$g_{\max}$		The first eigenvector of <b>G</b> , representing the axis that describes the direction containing the greatest amount of additive genetic variance
Sire variance		If a group of randomly selected sires are each mated to multiple dams in a breeding design, the variance among the sires represents 1/4 of the additive genetic variance after accounting for variance among dams and full-siblings
S-matrix	<b>S</b>	A symmetric matrix used for a tensor analysis. <b>S</b> describes the element-by-element differences among the original matrices
Eigntensor	<b>E</b>	Orthogonal axes describing differences among the original matrices. Eigntensors are constructed by scaling and arranging eigenvectors of <b>S</b>
Eigenvector ( $n$ ) of eigntensor ( $p$ )	$e_{p,n}$	The set of $n$ eigenvectors that describe the $p$ th eigntensor. Trait loadings describe how each trait contributes to differences among the original matrices that are captured by the eigenvector of an eigntensor.
Coordinates of an eigntensor		The correlation between the original matrices and each eigntensor. Quantifies which matrices contribute to the differences among all matrices that are captured by an eigntensor.



186

187 Quantifying differences in genetic variance: To quantify differences in **G**, we used a covariance tensor  
 188 approach (see glossary in **Table 1**). The strength of this approach is that, unlike other methods that focus on  
 189 pairwise comparisons, the covariance tensor can simultaneously compare multiple matrices. This simply  
 190 extends the two-matrix example (presented in **Fig. 1a-c**) to three or more matrices. The covariance tensor  
 191 quantifies differences among multiple matrices by first quantifying a matrix (the **S**-matrix) that captures the  
 192 raw differences among all matrices, and then identifying how each of the original traits and matrices  
 193 contribute to the differences captured by **S**. We only briefly describe the approach here, and refer readers to  
 194 more detailed descriptions in Basser and Pajevic (2007); Hine et al. (2009); Aguirre et al. (2014); Walter et  
 195 al. (2018), and a simplified description (**Fig. S4**). The covariance tensor is based on decomposition (i.e.  
 196 eigenanalysis, which is analogous to principal components) of symmetric matrices to construct a set of  
 197 orthogonal axes, known as eigentensors, which are used to identify and describe differences in the original  
 198 matrices being compared (e.g., elevation).

199 First, a symmetric matrix (**S**) is calculated, whose elements represent element-by-element variation among  
 200 the original matrices. Decomposing **S** identifies the orthogonal axes (eigenvectors) along which the original  
 201 matrices differ the most. Eigenvectors are scaled and rearranged to calculate the eigentensors, which are used  
 202 to identify how the original traits and matrices contributed to differences among all matrices. To identify  
 203 whether the observed eigentensors described significant differences in genetic variance, we constructed a null  
 204 distribution by randomising sire breeding values among treatments (here, elevations), and calculating a  
 205 randomised **G**-matrix for each MCMC iteration from the observed models. This calculates a null-distribution  
 206 based on the structure of the observed **G**-matrices (Aguirre et al. 2014). However, as suggested by Morrissey  
 207 et al. (2019), we also tested for significant eigentensors by randomising the sires among species and  
 208 elevations in the original dataset and re-implementing equation 2 on each randomisation. If the observed  
 209 eigentensors described greater differences in genetic variance than the eigentensors constructed from the null  
 210 distribution, then there is strong evidence for significant differences in our observed **G**.

211 To identify how each matrix (in our case, one elevation for a given species) contributes to differences among  
 212 all matrices (all elevations for a given species), the matrix coordinates of the eigentensors are calculated. The  
 213 coordinates are linear combination scores that are calculated between each eigentensor and the original  
 214 matrices, and can be interpreted similarly to a principal components analysis: larger scores indicate a greater  
 215 correlation between any given matrix and the differences among matrices described by that particular  
 216 eigentensor.

To identify how the original traits contribute to differences among matrices, each eigentensor is decomposed, and the eigenvectors interpreted in the same fashion as a principal components analysis. Traits with large loadings contribute to the differences described by the eigenvector of a particular eigentensor. Traits with loadings of different signs (positive and negative) describe traits that contribute to the differences in opposite ways. To identify how strongly each of the original matrices are associated with each eigenvector, we can use the matrix projection

$$V_{ijk} = \mathbf{e}_{ij}^T \mathbf{G}_k \mathbf{e}_{ij}, \quad (3)$$

where the  $V_{ijk}$  quantifies the amount of variance in the  $\mathbf{G}$ -matrix from the  $k$ th elevation that is described by the  $j$ th eigenvector from the  $i$ th eigentensor ( $\mathbf{e}_{ij}$ ). Greater values of  $V_{ijk}$  for any given matrix suggest that differences in that particular matrix underlie the differences in genetic variance captured by that eigenvector of the eigentensor.

We used the covariance tensor approach to make two comparisons. First, to identify whether elevation or adaptive divergence (i.e. differences between species) created larger differences in  $\mathbf{G}$ , we compared the  $\mathbf{G}$ -matrices of the two elevational extremes for both species. If adaptive divergence (i.e. exposure to different environments during the process of ecological speciation) created greater changes in  $\mathbf{G}$  than exposure to current environmental variation (i.e. to the elevational gradient), then differences between species would be greater than differences across elevation. Second, to identify the extent of elevational changes in  $\mathbf{G}$ , we quantified changes in  $\mathbf{G}$  across elevation for each species separately.

### *3. Testing whether elevational changes in genetic variance are associated with plasticity*

To test whether elevational changes in  $\mathbf{G}$  were associated with plasticity (change in mean phenotype), we compared the eigenvectors of eigentensors (capturing differences in  $\mathbf{G}$ ) with a  $\mathbf{D}$ -matrix representing multivariate change in phenotype across elevation. First, we conducted MANOVA as before, but for each species separately, and including experimental block (within elevation) as the error term, which tests whether elevational differences in mean multivariate phenotype are significantly greater than differences among blocks within elevation. We then used the output of the MANOVA to calculate a  $\mathbf{D}$ -matrix that captured the elevational change in mean phenotype for each species. Second, we used matrix projection (equation 3), to project the eigenvectors of eigentensors through the  $\mathbf{D}$ -matrix for each species separately. We predicted that if  $\mathbf{G} \times \mathbf{E}$  underlying plasticity can change the structure of  $\mathbf{G}$ , then eigenvectors (of eigentensors) that describe the largest differences in  $\mathbf{G}$  would also describe large changes in mean multivariate phenotype.

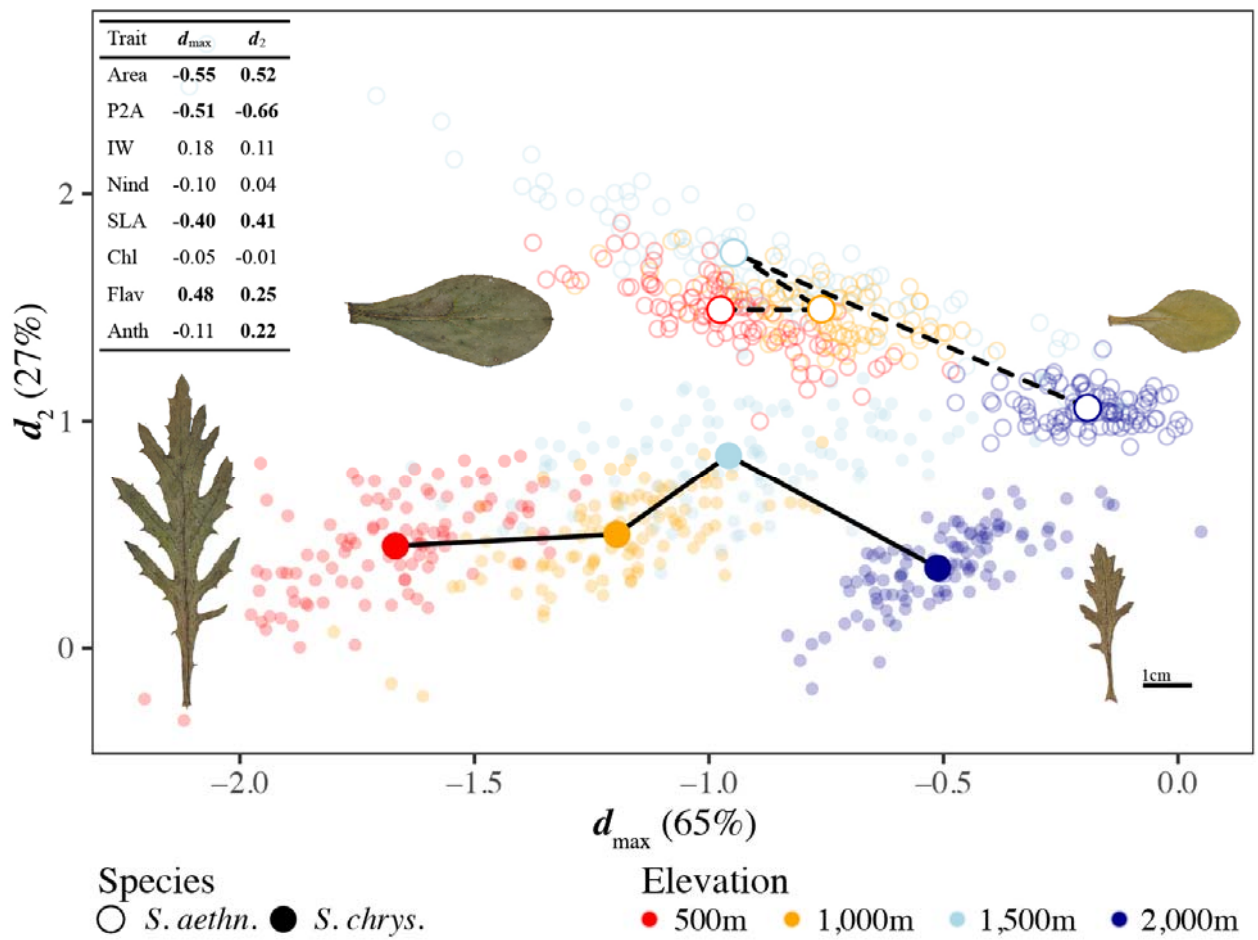
Estimating  $\mathbf{G} \times \mathbf{E}$  across elevation: We tested whether plasticity was associated with  $\mathbf{G} \times \mathbf{E}$  as a change in variance or as changes in rank of sire breeding values across elevation. We calculated the scores for the

first two eigenvectors of **D** (from equation 1) and used equation 2 to estimate the genetic variance at each elevation, and the genetic covariance among elevations. For each random component, we specified random slopes and intercepts for elevation. To specific the correct residual variance structure, we only estimated the residual variances at each elevation because two plants were not present at more than one elevation, preventing the estimation of residual covariance among elevations.

## Results

### *1. Species differed in their change in mean phenotype across elevation*

The MANOVA provided evidence that species (Wilks'  $\lambda = 0.21$ ,  $F_{1,6446} = 2940.56$ ,  $P < 0.0001$ ), elevation (Wilks'  $\lambda = 0.30$ ,  $F_{3,6446} = 401.12$ ,  $P < 0.0001$ ) and their interaction (Wilks'  $\lambda = 0.83$ ,  $F_{3,6446} = 50.62$ ,  $P < 0.0001$ ) all showed significant differences in mean multivariate phenotype. Changes in the univariate trait means are presented in **Fig. S2**. We used the MANOVA to estimate a D-matrix representing differences in mean multivariate phenotype between species and across elevation. We found that *S. chrysanthemifolius* shows a relatively gradual change in phenotype across elevation (**Fig. 2**). By contrast, *S. aethnensis* shows a sharper change in mean phenotype whereby the highest elevation (i.e., the native elevation) contrasts with all three lower elevations (**Fig. 2**).



**Fig. 2** Phenotypic plasticity creates large changes in mean multivariate phenotype. The first two axes of **D** together represent 92% of all change in mean phenotype, with the table inset displaying the trait loadings for each axis (loadings in bold contribute substantially to each axis). Large coloured circles represent the mean of each species at each transplant site, 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.

## 2. Genetic variance changed more across elevation than between species

We quantified G-matrices for each species and at each elevation (**Table S2**), and decomposed each matrix to identify the orthogonal axes (known as eigenvectors) that describe the distribution of genetic variance within each G-matrix (**Table 2**). The first four eigenvectors of **G** together described more than 80% of all genetic variance (**Table 2**), and were greater than expected under random sampling (**Fig. S2**), which suggests that our matrices captured biologically meaningful genetic variance underlying morphology. 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 fewer axes (than the number of traits) that contain higher proportions of the

281 total genetic variance. Differences in shape arise when matrices are more or less elliptical. Differences in  
 282 orientation arise when the linear combination of traits that are used to describe the major axes of genetic  
 283 variance differ between matrices.

284 Compared to the G-matrices estimated at the three lower elevations (500m-1,500m), we found that the G-  
 285 matrices of both species were smaller (i.e., contained less genetic variance) at the highest elevation (**Table 2**  
 286 and **Table S2**). *Senecio aethnensis* showed a similar shape across elevation, whereby three axes consistently  
 287 described >80% of the genetic variance at each elevation (**Table 2**). By contrast, G-matrices of *S.*  
 288 *chrysanthemifolius* were more elliptical at lower elevations (two axes described >70% of total genetic  
 289 variance), and much more spherical at the highest elevation (four axes described 80% of total genetic  
 290 variance). For both species the magnitude and sign (positive vs negative) of trait loadings changed across  
 291 elevation (**Table 2**), suggesting that different linear combinations of traits described axes of **G** at different  
 292 elevations.

293

**Table 2** The first four eigenvectors describing >80% of total genetic variation for each G-matrix estimated at each elevation for: (a) *S. aethnensis*, and (b) *S. chrysanthemifolius*. HPD represents the upper and lower 95% Highest Posterior Density intervals. ‘Proportion’ quantifies the proportion of total genetic variance that each eigenvector describes, and ‘Cumulative’ represents the cumulative proportion of genetic variance. Trait loadings in bold are greater than 0.2 to aid interpretation of the eigenvectors.

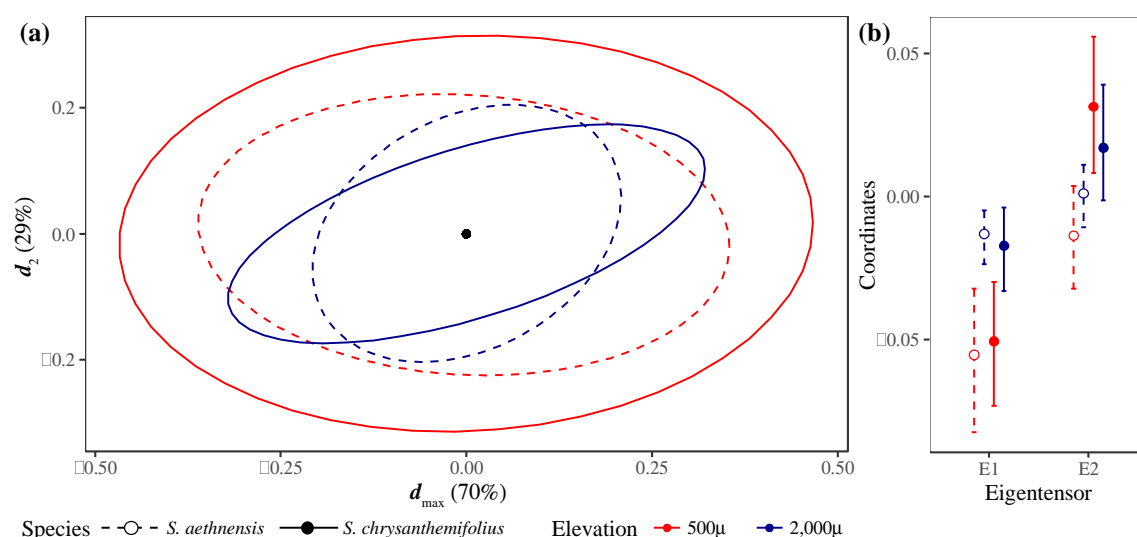
	500m				1,000m				1,500m				2,000m			
	$g_{\max}$	$g_2$	$g_3$	$g_4$	$g_{\max}$	$g_2$	$g_3$	$g_4$	$g_{\max}$	$g_2$	$g_3$	$g_4$	$g_{\max}$	$g_2$	$g_3$	$g_4$
<b>(a) <i>S. aethnensis</i></b>																
<b>Eigenvalues</b>	0.046	0.031	0.020	0.007	0.049	0.020	0.011	0.008	0.050	0.026	0.014	0.008	0.019	0.014	0.009	0.007
<b>HPDI<sub>lwr</sub></b>	0.020	0.011	0.008	0.001	0.022	0.008	0.003	0.001	0.019	0.002	0.005	0.001	0.002	0.004	0.003	0.001
<b>HPDI<sub>upp</sub></b>	0.076	0.053	0.034	0.017	0.080	0.034	0.022	0.018	0.084	0.064	0.025	0.021	0.038	0.026	0.017	0.020
<b>Proportion</b>	0.41	0.27	0.18	0.06	0.51	0.20	0.12	0.08	0.45	0.23	0.12	0.07	0.30	0.22	0.15	0.12
<b>Cumulative</b>	0.41	0.68	0.86	0.92	0.51	0.71	0.83	0.91	0.45	0.68	0.80	0.87	0.30	0.52	0.67	0.79
<b>Traits:</b>																
Area	0.19	<b>0.33</b>	0.04	-0.12	0.17	<b>0.45</b>	<b>0.67</b>	<b>-0.54</b>	<b>0.20</b>	<b>0.86</b>	<b>-0.44</b>	0.16	<b>0.21</b>	<b>0.29</b>	<b>0.26</b>	<b>0.87</b>
P2A	0.12	<b>-0.41</b>	<b>0.88</b>	0.07	<b>0.30</b>	<b>-0.83</b>	<b>0.44</b>	-0.08	<b>0.34</b>	<b>-0.52</b>	<b>-0.70</b>	<b>0.32</b>	<b>-0.47</b>	<b>-0.71</b>	<b>0.22</b>	<b>0.30</b>
Nind	<b>-0.51</b>	<b>-0.39</b>	-0.04	<b>-0.33</b>	<b>-0.26</b>	<b>-0.22</b>	<b>-0.29</b>	<b>-0.58</b>	<b>-0.59</b>	0.02	<b>-0.25</b>	-0.05	<b>-0.30</b>	0.01	-0.06	-0.10
IW	<b>0.47</b>	<b>0.28</b>	0.17	<b>0.27</b>	<b>0.24</b>	0.18	<b>0.25</b>	<b>0.55</b>	<b>0.64</b>	0.03	<b>0.33</b>	-0.01	0.17	-0.08	0.01	0.13
SLA	0.04	<b>-0.23</b>	<b>-0.23</b>	<b>0.63</b>	0.17	0.15	-0.16	<b>-0.20</b>	-0.03	-0.03	-0.16	<b>-0.53</b>	-0.07	<b>0.20</b>	<b>-0.76</b>	<b>0.20</b>
Chl	0.00	<b>0.34</b>	<b>0.20</b>	<b>-0.33</b>	-0.13	-0.08	0.06	0.11	0.10	0.00	<b>0.24</b>	0.13	<b>0.48</b>	-0.13	0.16	-0.14
Flav	<b>-0.69</b>	<b>0.50</b>	<b>0.29</b>	<b>0.43</b>	<b>-0.83</b>	-0.04	<b>0.42</b>	0.13	<b>-0.26</b>	0.02	<b>0.24</b>	<b>0.75</b>	0.08	<b>0.27</b>	<b>0.46</b>	<b>-0.27</b>
Anth	-0.03	<b>-0.27</b>	-0.08	<b>0.34</b>	0.15	0.06	0.06	-0.07	-0.05	-0.01	-0.04	0.06	<b>-0.61</b>	<b>0.53</b>	<b>0.23</b>	-0.01
<b>(b) <i>S. chrysanthemifolius</i></b>																
<b>Eigenvalues</b>	0.053	0.027	0.016	0.012	0.048	0.023	0.010	0.004	0.022	0.015	0.013	0.010	0.028	0.009	0.005	0.004
<b>HPDI<sub>lwr</sub></b>	0.024	0.012	0.007	0.004	0.020	0.011	0.001	0.001	0.005	0.001	0.002	0.001	0.003	0.000	0.000	0.000
<b>HPDI<sub>upp</sub></b>	0.087	0.044	0.028	0.022	0.083	0.039	0.023	0.008	0.041	0.035	0.028	0.025	0.058	0.022	0.013	0.013
<b>Proportion</b>	0.45	0.23	0.14	0.11	0.52	0.25	0.10	0.04	0.29	0.20	0.17	0.14	0.52	0.16	0.09	0.08
<b>Cumulative</b>	0.45	0.68	0.82	0.93	0.52	0.77	0.87	0.91	0.29	0.49	0.66	0.80	0.52	0.68	0.77	0.85
<b>Traits:</b>																
Area	<b>-0.21</b>	<b>0.74</b>	<b>-0.22</b>	<b>0.57</b>	<b>0.25</b>	-0.07	<b>0.92</b>	<b>0.21</b>	<b>0.43</b>	<b>0.53</b>	-0.05	<b>0.67</b>	-0.03	<b>-0.52</b>	<b>0.74</b>	<b>-0.38</b>
P2A	<b>-0.91</b>	<b>-0.35</b>	0.07	0.16	<b>-0.92</b>	0.02	<b>0.31</b>	<b>-0.23</b>	<b>-0.47</b>	<b>0.64</b>	<b>-0.52</b>	<b>-0.20</b>	<b>-0.97</b>	0.07	0.00	-0.05
Nind	0.10	<b>-0.30</b>	<b>-0.45</b>	0.11	0.02	<b>0.65</b>	0.04	<b>0.22</b>	<b>0.55</b>	0.11	0.01	<b>-0.38</b>	0.18	<b>0.58</b>	<b>0.38</b>	-0.03
IW	-0.05	<b>0.32</b>	<b>0.65</b>	-0.18	0.07	<b>-0.68</b>	-0.02	-0.07	<b>-0.41</b>	<b>-0.30</b>	-0.03	<b>0.35</b>	0.05	<b>-0.53</b>	<b>-0.34</b>	0.09
SLA	0.03	0.11	<b>-0.46</b>	-0.07	0.03	-0.01	-0.02	0.01	0.04	<b>0.20</b>	0.08	-0.15	-0.01	0.13	<b>0.23</b>	-0.03
Chl	0.01	0.07	0.01	-0.16	0.08	0.13	0.08	0.11	<b>0.32</b>	<b>-0.32</b>	<b>-0.73</b>	-0.17	0.09	-0.12	-0.17	<b>-0.27</b>
Flav	<b>0.34</b>	<b>-0.35</b>	<b>0.32</b>	<b>0.73</b>	<b>0.29</b>	<b>0.23</b>	0.14	<b>-0.91</b>	0.13	0.04	0.14	0.06	0.10	<b>-0.22</b>	0.07	0.09
Anth	-0.01	0.02	0.11	0.18	0.04	<b>-0.21</b>	0.15	-0.13	-0.07	<b>0.24</b>	<b>0.41</b>	<b>-0.43</b>	-0.05	-0.16	<b>0.32</b>	<b>0.87</b>

The first axis of **G**,  $g_{\max}$ , describes the greatest amount of genetic variance. It is expected that  $g_{\max}$  will remain stable due to pleiotropy preventing independent changes in different traits. However, for *S. aethnensis* we found that all elevations were nearly orthogonal to the home site (angle between  $g_{\max}$  at the home site



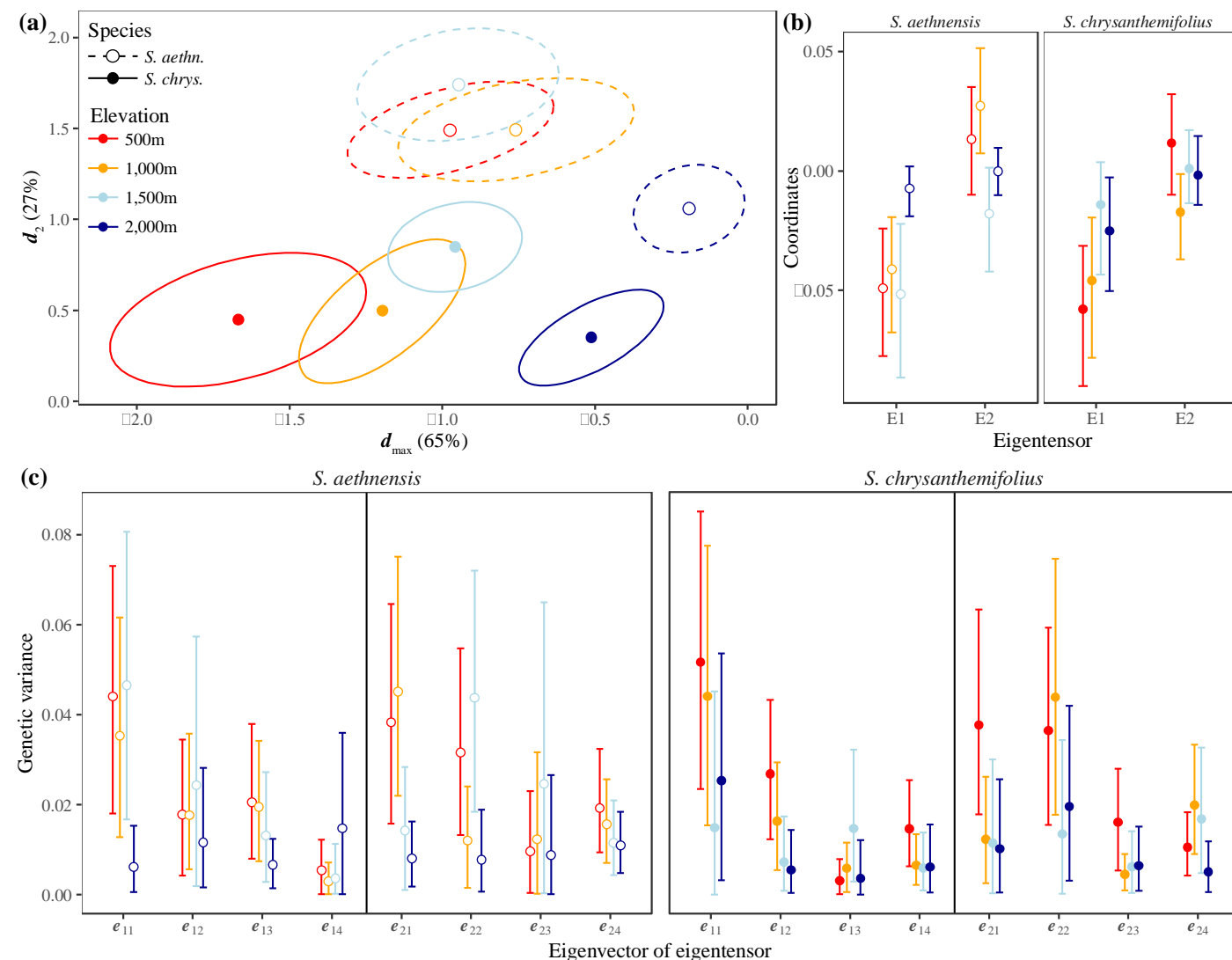
[2,000m] and  $g_{\max}$  at: 1,500m=76.2°; 1,000m=77.8°; 500m=79.7°). By comparison, for *S. chrysanthemifolius* the angle between the home site (500m) and the other elevations were much lower (1,000m=28.3°; 1,500m=62.2°; 2,000m=20.1°).

***G* changes more across elevation than between species:** To quantify differences in **G** we used a covariance tensor approach, which we applied to two separate analyses. To test whether species or elevation created larger changes in **G**, we applied a covariance tensor to the **G**-matrices of both species at the elevational extremes (both native elevations). Elevational differences in **G** appear to be substantial for both species (**Fig. 3a, Table2** and **Fig. S5**). Using the covariance tensor to quantify differences in genetic variance, we found that two (of three) eigentensors described greater differences in genetic variance compared to the null expectation (**Fig. S3a**). The coordinates capture how each matrix contributes to the differences described by an eigentensor. The first eigentensor, which captures 31.9% of all differences among **G**-matrices, describes large differences between extreme elevations, but not between species (**Fig. 3b**). By contrast, the second eigentensor captures 26.2% of all differences among **G**-matrices, and describes large differences between species, but not between elevations (**Fig. 3b**). Therefore, elevation created larger changes in **G** than adaptive divergence between the two species.



**Fig. 3** Differences in **G** are greater across elevational extremes than between species. **(a)** Visualising differences between species at the elevational extremes shows that the two species differ in their **G**-matrices, and that they respond to elevation differently. **(b)** The coordinates quantify how each matrix contributes to differences in genetic variance described by each eigentensor. Credible intervals represent the 95% HPD (Highest Posterior Density) intervals. The first eigentensor (describing 31.9% of the total difference in genetic variance) describes differences between the elevational extremes, but not differences between species. By contrast, the second eigentensor (describing 26.2% of the total difference in genetic variance) describes differences between species, but not between elevations. The summary of the tensor is located in **Table S3a**.

Second, we used the covariance tensor approach to quantify changes in  $\mathbf{G}$  across elevation for each species separately. Visualising the  $\mathbf{G}$ -matrices of the two species suggests large changes across elevation (**Fig. 4a**). We found that two eigentensors for *S. aethnensis*, and one eigentensor for *S. chrysanthemifolius* capture greater differences in genetic variance than expected under random sampling (**Fig. S3b-c**). For *S. aethnensis*, the coordinates of the first eigentensor reveal strong differences in  $\mathbf{G}$  between 2,000m and the lower elevations, while the second eigentensor quantifies differences between the two upper and lower elevations (**Fig. 4b**). Similarly, the first eigentensor captures differences between the upper and lower elevations for *S. chrysanthemifolius* (**Fig. 4b**). Projecting the eigenvectors of eigentensors through the original  $\mathbf{G}$ -matrices reveals how each original matrix (i.e. each elevation) contributes to the differences in genetic variance described by that particular eigenvector. We present only the first four eigenvectors from each eigentensor because these describe >80% of the differences captured by each eigentensor. Eigenvectors of eigentensors describe significant differences in genetic variance across elevation (**Fig. 4c**).

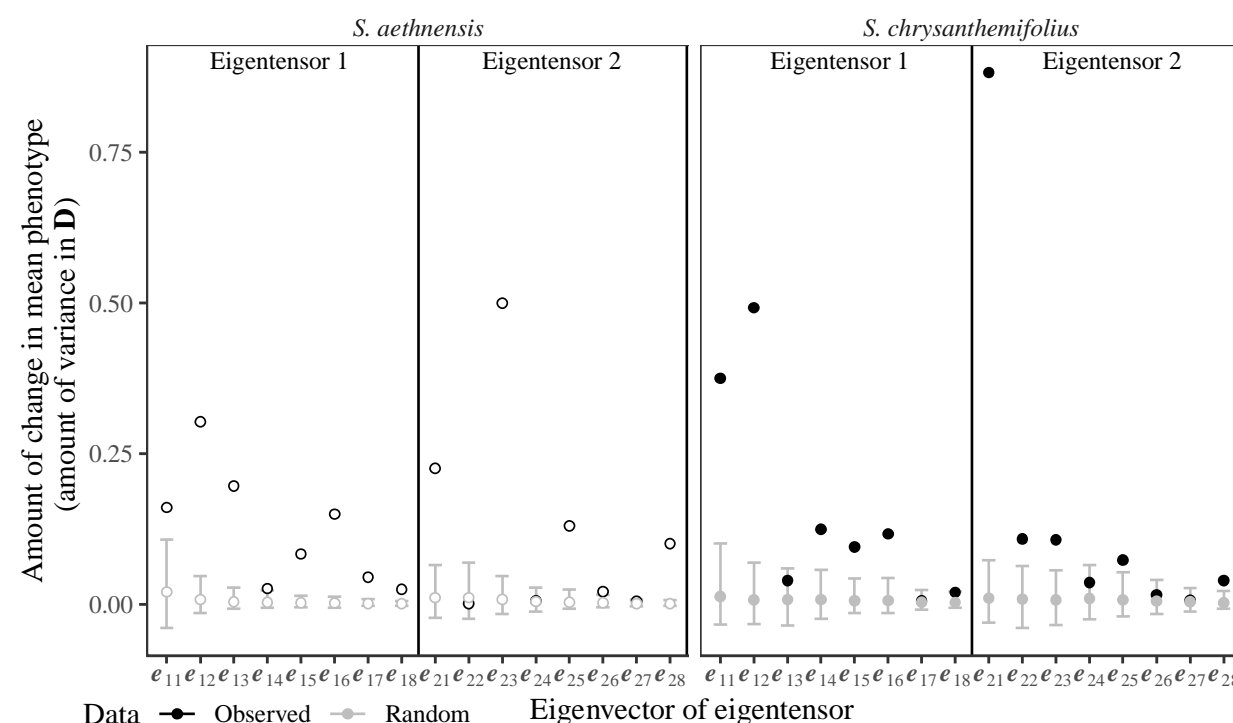


**Fig. 4** Elevation induces changes in  $\mathbf{G}$  for both species. (a) Visualising  $\mathbf{G}$ -matrices for both species at all elevations shows how

they change with the change in mean phenotype. **(b)** The coordinates show that, for both species, the first two eigentensors describe elevational differences in genetic variance. Credible intervals represent the 95% HPD intervals. **(c)** To identify how each elevation contributes to differences in **G** captured by the eigenvectors of eigentensors, we use matrix projection. **G**-matrices that describe more variance for a given eigenvector (of an eigentensor) contribute to the differences in elevation described by that particular eigenvector of the eigentensor. We only present the first four eigenvectors because they describe >80% of the difference in genetic variance captured by each eigentensor. The tensor summaries are located in **Table S3b-c**.

### 3. Changes in genetic variance are associated with changes in mean phenotype

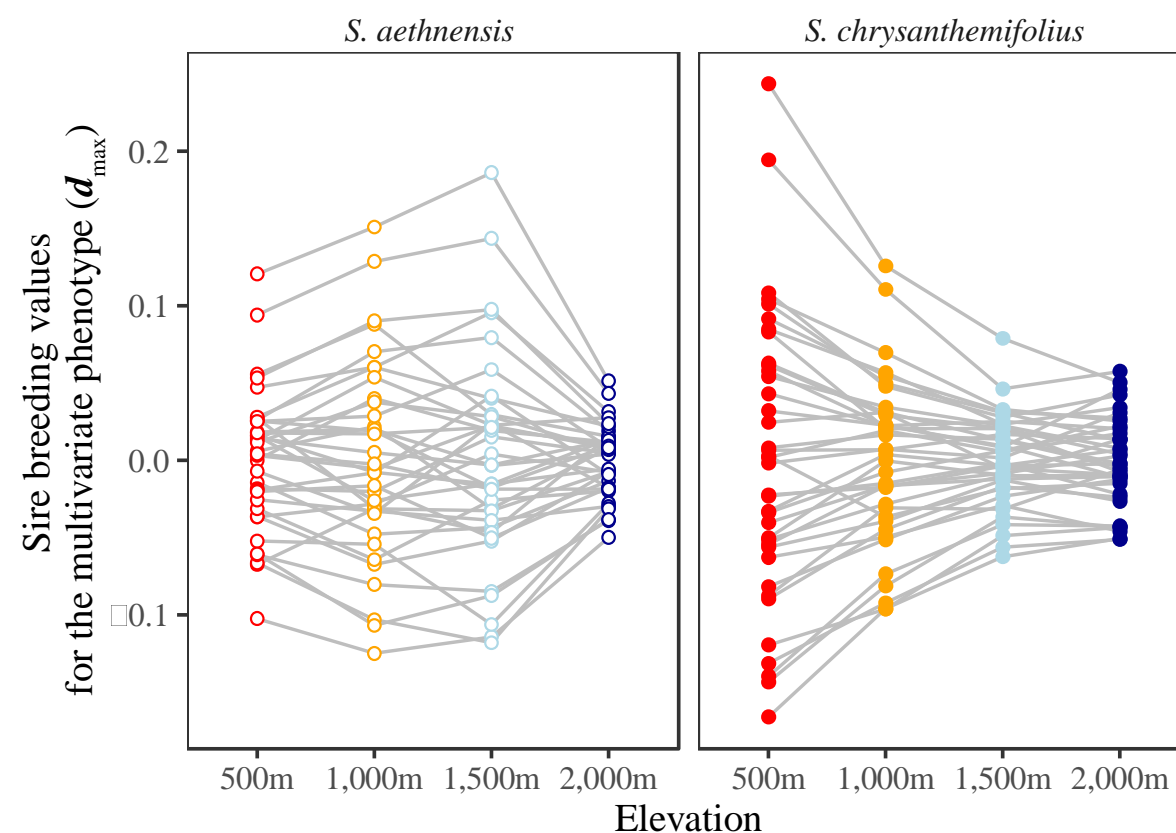
If **G**×**E** interactions that change **G** are associated with plasticity, we predicted that elevational differences in **G** would align with plastic changes in mean phenotype. To test this (for each species separately), we projected the eigenvectors of eigentensors (from **Fig. 4c**), which capture the greatest differences in **G**, through the **D**-matrix (representing elevational differences in mean multivariate phenotype). If changes in **G** were associated with plasticity, then eigenvectors of eigentensors that describe the greatest differences in **G** (i.e. the leading eigenvectors of each eigentensor) would also describe more variance in **D** than expected under random sampling. We found that for both species, our results supported our predictions, and that this was particularly strong for *S. chrysanthemifolius* (**Fig. 5**).



**Fig. 5** Eigenvectors of eigentensors that describe large differences in **G** also describe large changes in mean multivariate phenotype. The first two eigenvectors of each eigentensor capture >90% of the difference in genetic variance described by that eigentensor. We predicted that if the first two eigenvectors (that capture the greatest difference in genetic variance) describe large differences in mean multivariate phenotype, then changes in **G** align with plasticity. Projecting the eigenvectors of eigentensors

through the observed D-matrix (black circles) shows that the leading eigenvectors from each eigentensor describe greater differences in mean phenotype than expected under random sampling (gray circles and credible intervals representing 95% HPD intervals) and describe the greatest difference in mean phenotype. Therefore, we found evidence that changes in  $\mathbf{G}$  align with plastic changes in mean phenotype.

Changes in  $\mathbf{G}$  are associated with  $G \times E$  in plasticity: Estimating the G-matrix for the axis representing the largest change in mean phenotype ( $d_{\max}$ ), quantifies the genetic variance at each elevation and the genetic covariance between elevations. We found evidence of  $G \times E$  as large changes in genetic variance across elevation, with much smaller amounts of genetic variance at high elevation for both species (**Fig. 6; Table S4**). Genetic correlations between elevations are moderately strong and range from 0.42 to 0.72 (**Table S4**). Genetic correlations between elevations of less than one suggest that  $G \times E$  is also present as a change in sire rank across elevation (**Fig. 6**).



**Fig. 6** Sire breeding values for each species at each elevation show how sires change in genetic value relative to each other. Plasticity (as changes in mean phenotype captured by  $d_{\max}$  from **Fig. 2**) is associated with  $G \times E$  as a large change in genetic variance across elevation, as well as changes in sire rank across elevation (crossing of sires between elevations).

## 379 Discussion

380 We planted seeds from a breeding design of two closely related but ecologically distinct species across an  
 381 environmental (elevation) gradient that included each species' native environment and two intermediate  
 382 environments. We found that estimates of plasticity for eight leaf traits suggested that the phenotype of *S.*  
 383 *chrysanthemifolius* moved towards the phenotype of *S. aethnensis* at high elevations, while the phenotype of  
 384 *S. aethnensis* moved further away from the phenotype of *S. chrysanthemifolius* at lower elevations (**Fig. 2**).  
 385 This suggests that *S. chrysanthemifolius* shows a more appropriate phenotypic response to a novel  
 386 environment. Changes in genetic variance across elevation were both significant and stronger than  
 387 differences between species (**Fig. 3**), and were consistent across elevation for both species (**Fig. 4**).  
 388 Elevational differences in genetic variance aligned with plasticity as the change in mean phenotype (**Fig. 5**),  
 389 and were created by patterns of G×E as elevational changes in genetic variance and sire rank (**Fig. 6**).  
 390 Together, these results suggest that changes in genetic variance occur as a result of G×E underlying  
 391 phenotypic plasticity in novel environments, which will likely determine the potential for adaptation in novel  
 392 environments.

393 By analysing published studies, Wood and Brodie III (2015) found evidence that **G** is likely affected by the  
 394 environment as much as by evolution, but their results as to why **G** changed in response to the environment  
 395 were inconclusive. We help to resolve this by showing that novel environments not only create larger  
 396 changes in **G** than evolutionary history, but that such changes in **G** occur in the direction of plasticity as a  
 397 consequence of G×E interactions. Our findings not only support an alignment between plasticity and genetic  
 398 variation (Noble et al. 2019; Johansson et al. 2020), but suggest that to predict evolutionary responses to  
 399 environmental change, we need to better understand how genetic variation responds to environmental  
 400 variation. Therefore, future work needs to consider G×E to understand when and how constraints to  
 401 adaptation will prevent evolutionary rescue in novel environments, and to identify whether environment-  
 402 dependent genetic constraints could determine evolutionary trajectories.

403 Our results show that in order to better understand the potential for evolutionary rescue it will be necessary to  
 404 quantify the prevalence of G×E across a species' range and understand the potential for G×E to maintain  
 405 ecological resilience in novel environments. Evolutionary rescue will be possible if sufficient G×E in  
 406 plasticity is available, and selection on genetic variation in plasticity increases fitness in novel environments  
 407 (Chevin et al. 2010; Chevin and Hoffmann 2017), which can then lead to genetic assimilation of an initially  
 408 plastic response (Waddington 1953; Lande 2009). Although selection on plasticity should result in rapid  
 409 adaptation that facilitates evolutionary rescue (Charmantier et al. 2008; Wang and Althoff 2019; Walter et al.  
 410 2020), we still do not know whether environmental change will be too extreme or rapid to allow evolutionary

rescue. Furthermore, it is likely that in response to novel environments, not only will selection be for the appropriate phenotype (i.e. change in mean phenotype), it is likely that selection for new forms of plasticity that are appropriate to the novel environment (i.e. appropriate fluctuations around the new mean phenotype) will need to evolve. Given the unpredictable nature of novel environments however, selection for a new form of plasticity might be difficult (Leung et al. 2020).

The initial resilience of populations exposed to a novel environment will likely depend on how close plasticity is able to move the population towards a phenotypic optimum. Evidence suggests that plasticity in novel environments is more often maladaptive (Langerhans and DeWitt 2002; Palacio-López et al. 2015; Acasuso-Rivero et al. 2019), which means that populations will likely need to rely on rapid adaptation to maintain fitness and prevent extinction. However, there are two major obstacles for evolutionary rescue. Firstly, the adaptive potential for novel environments will be greatly diminished if genetic variance in the direction of selection is low (Walsh and Blows 2009), which can occur if G×E reduces genetic variance in novel environments. We found that the availability of genetic variance for evolutionary rescue will be species-specific. *Senecio aethnensis* showed an increase in genetic variance in the novel environment (500m), which contrasted with *S. chrysanthemifolius*, which showed a decrease in genetic variance at 2,000m (**Table 2**). These results therefore suggest that despite high elevation species having lowered plasticity compared to lower elevation species (Gugger et al. 2015; Schmid et al. 2017; de Villemereuil et al. 2018), selection on increased genetic variation in response to low-elevation (i.e. warmer) conditions could allow evolutionary rescue.

Secondly, the potential for rapid adaptation to a novel environment will be determined by the amount of genetic versus phenotypic variance underlying the multivariate phenotype. If plasticity common to all genotypes creates phenotypic variance that hides beneficial genetic variation from selection, then a demographic barrier to adaptation will arise because too few individuals will contribute to the following generation and the populations is more likely to go extinct (Chevin et al. 2013). In other words, if phenotypic variance is biased towards a direction in multivariate phenotype that is different to genetic variance, then it will make adaptation difficult because even if there is substantial genetic variation in the direction of selection, only a small fraction of the population would possess the beneficial alleles and adaptation will be difficult. Comparing genetic and phenotypic variance with the direction of selection using quantitative genetics in reciprocal transplant experiments can therefore identify whether evolutionary rescue in novel environments will be sufficiently rapid to avoid extinction. Such experiments can also be used to predict evolutionary trajectories during adaptation to novel environments by identifying whether evolutionary rescue favours adaptation towards the phenotype of species native to the novel environment, or whether adaptation



favours a different phenotypic optimum.

Although we show that  $G \times E$  can shift the  $G$ -matrix in response to novel environments, whether such shifts can help to promote evolutionary rescue requires estimates of selection and cross-generational selection experiments. A bottleneck event that occurs during the colonisation of (or exposure to) novel environments reduces population size, which can create instability in  $G$  (Arnold et al. 2008). Evolutionary rescue can only occur in small populations if adaptive alleles increase in frequency rapidly enough to allow adaptation before extinction occurs. Small population sizes can have important consequences for genetic variation by making  $G$  unstable (Jones et al. 2003). Rapid changes to the orientation and size of  $G$  can occur when rare alleles held at mutation-selection balance readily increase in frequency (Jones et al. 2003). If such alleles underlie  $G \times E$  interactions that have low benefit in the native environments, but increase fitness in novel environments (Walter et al. 2020), then the  $G \times E$  effects of new mutations (Roles et al. 2016) or rare/hidden variants (Schlichting 2008; Brennan et al. 2019) could facilitate evolutionary rescue. It is then likely that mutation will determine whether genetic constraints to rapid adaptation can be overcome for small populations. If pleiotropic mutations that provide beneficial genetic variation in the direction of selection arise readily, then the orientation of  $G$  can change rapidly for small populations, reducing the constraints to adaptation and making evolutionary rescue more likely (Arnold et al. 2008). Future studies should therefore determine the effect of mutation accumulation on  $G \times E$  and the response of  $G$  to novel environments.

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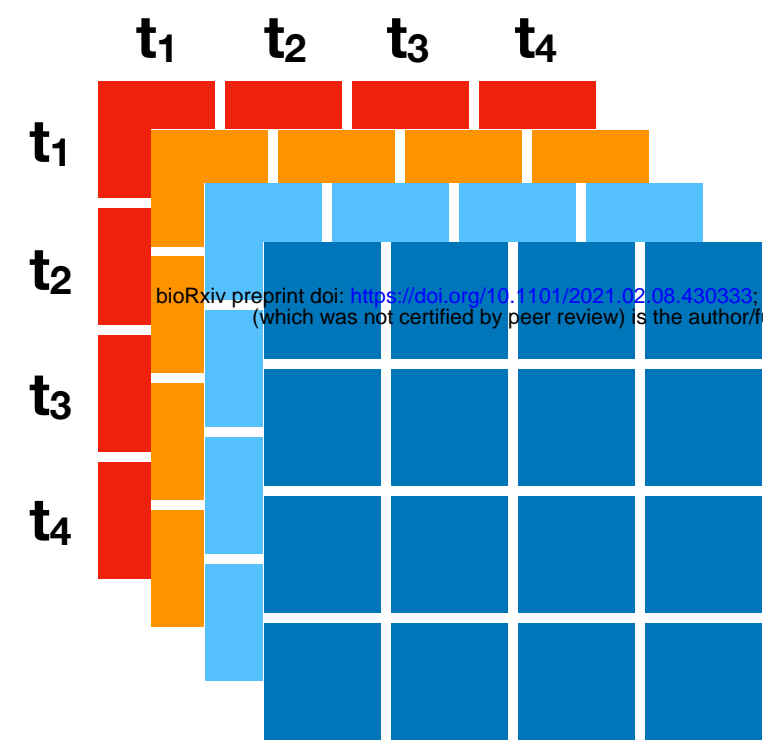
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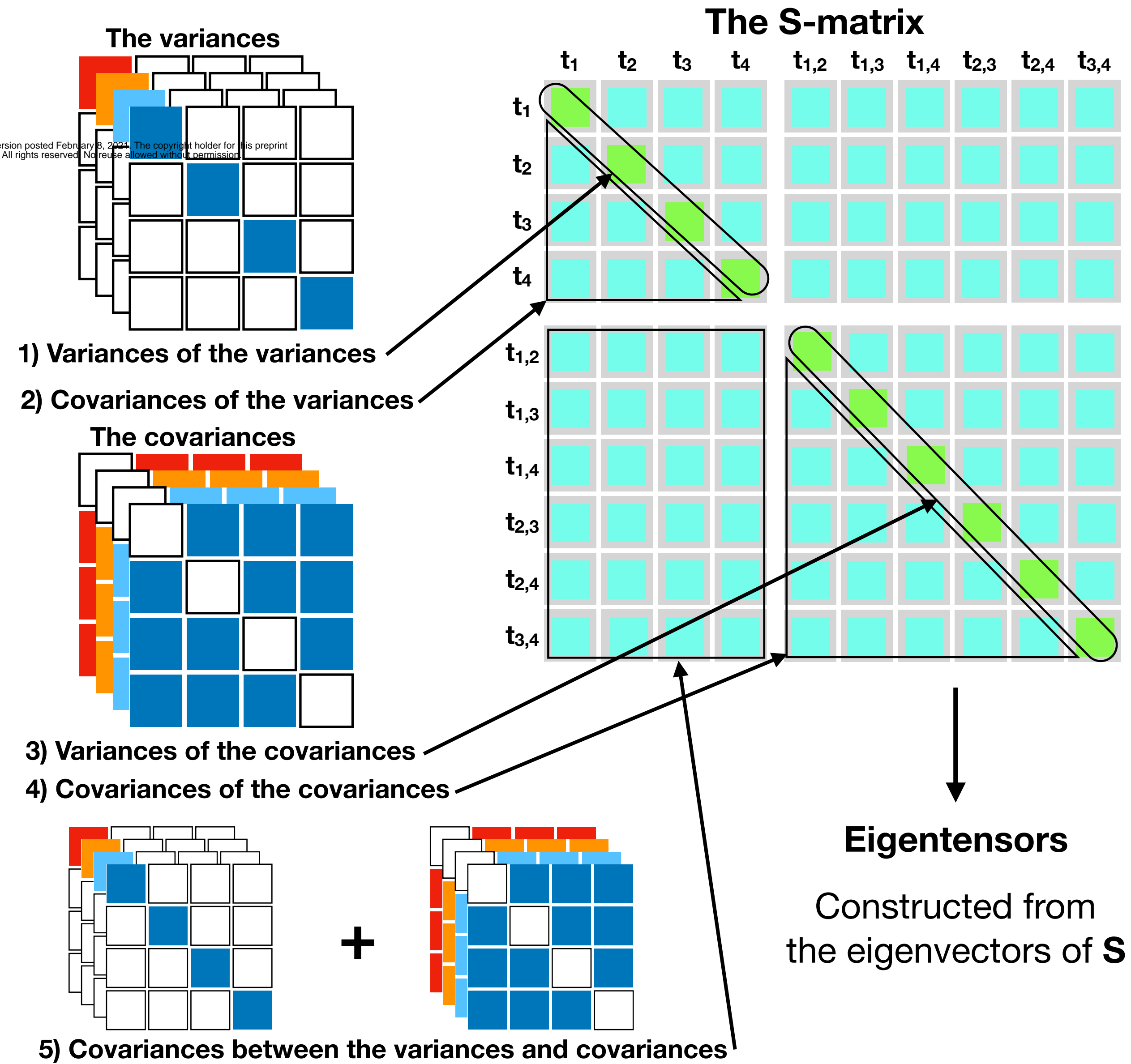


# Using a covariance tensor to quantify differences among multiple matrices

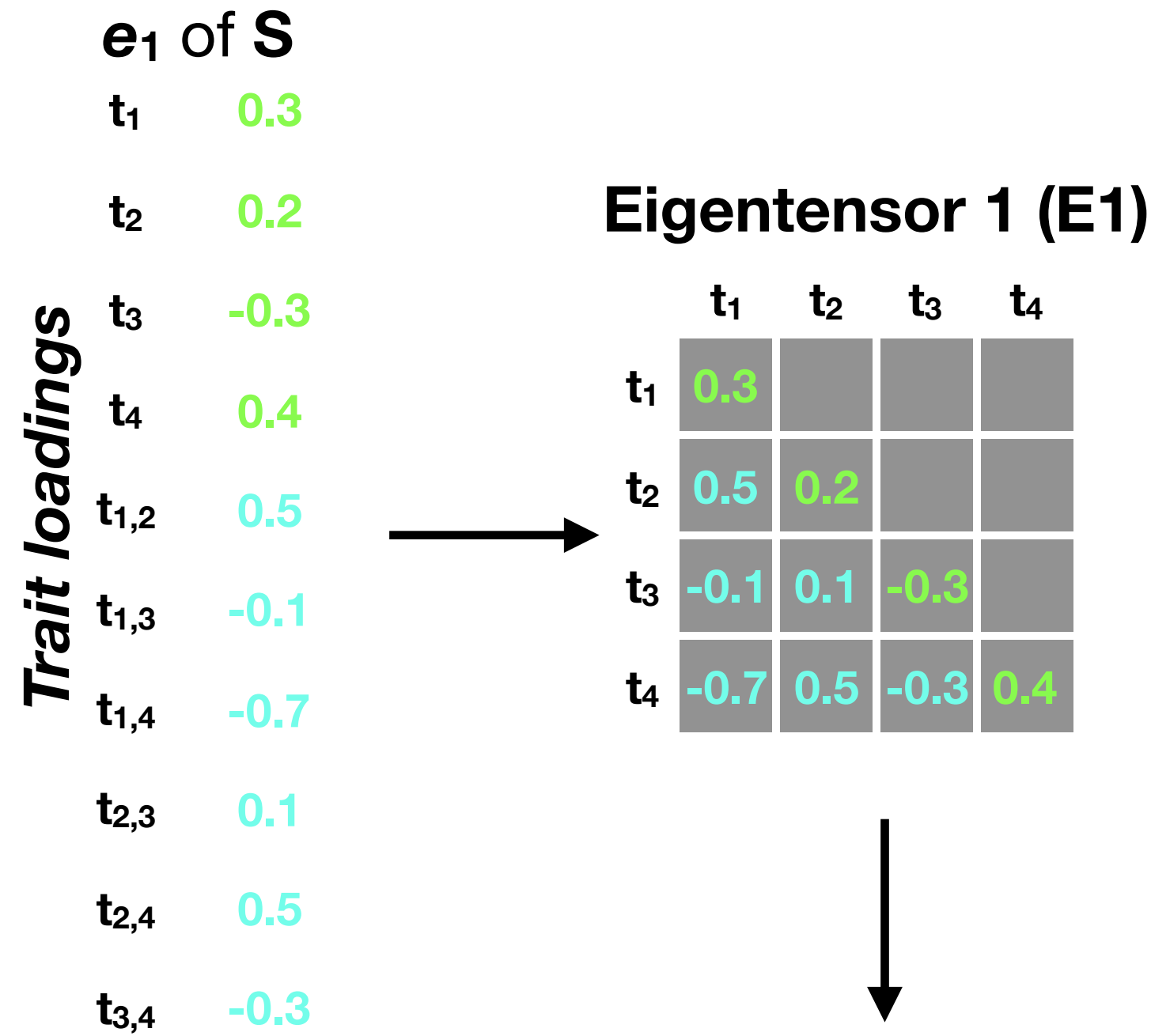
**Original matrices**  
4 traits, 4 matrices



**Step 1** Construct the **S-matrix**, the element-by-element differences among all matrices (i.e. the raw differences)



**Step 2** Construct eigentensors from the eigenvectors of **S**



**Step 3** Identify how the original traits and matrices contribute to the differences among all matrices described by **S**

