Hidden genetic variation in plasticity increases the potential to adapt to novel environments

Greg M. Walter[1,2*], *James Clark*[1,3], *Delia Terranova*[4], *Salvatore Cozzolino*[5], *Antonia Cristaudo*[4], *Simon J. Hiscock*[3] *and Jon R. Bridle*[1,6]

[1]University of Bristol, School of Biological Sciences, Bristol BS8 1TQ, UK

[2]Current address: Monash University, School of Biological Sciences, Melbourne 3800, Australia

[3]University of Oxford, Department of Plant Sciences, Oxford, OX1 3RB, UK

[4]University of Catania, Department of Biological, Geological and Environmental Sciences, Catania 95128, Italy

[5]University of Naples Federico II, Department of Biology, Naples 80126, Italy

[6]Current address: University College London, Department of Genetics, Evolution and Environment, London WC1E 6BT, UK

* Corresponding Author: Greg M. Walter

Email: greg.walter@monash.edu

Abstract

Adaptive plasticity increases population persistence, but can slow adaptation to changing environments by hiding the effects of different alleles on fitness. However, if plastic responses are no longer adaptive in novel environments, then differences among alleles can emerge and increase genetic variation in fitness that allows rapid adaptation. We tested this hypothesis by transplanting cuttings and seeds of a Sicilian daisy within and outside its native range, and quantifying variation in morphology, physiology, gene expression and fitness. We show that genetic variance in plasticity increases the potential for rapid adaptation to novel environments. Genetic variation in fitness was low across native environments where plasticity effectively tracked familiar environments. In the novel environment however, genetic variation in fitness increased threefold, and correlated with genetic variation in plasticity. Furthermore, genetic variation that can increase fitness in the novel environment had the lowest fitness at the native site, suggesting that adaptation to novel environments relies on genetic variation in plasticity that is selected against in native environments.

Introduction

Understanding how populations can respond to novel environments is vital for predicting the resilience of ecological communities to environmental change (Parmesan 2006; Shaw and Etterson 2012). Populations persist in novel environments either by genotypes adjusting their phenotype to match the new conditions (adaptive plasticity) (Via et al. 1995; Charmantier et al. 2008), and/or by increases in the frequency of alleles that increase fitness in novel environments, causing adaptation (termed 'evolutionary rescue') (Gomulkiewicz and Holt 1995; Bell and Gonzalez 2009). However, if adaptation to novel environments relies on alleles that increase fitness via beneficial plastic responses, then selection on plasticity can promote evolutionary rescue (Lande 2009; Chevin and Lande 2011).

Given that plasticity can only evolve to buffer environmental variation encountered within its native range, existing plasticity should only maintain fitness in previously experienced conditions (Chevin et al. 2013), which reduces genetic variation in fitness in familiar environments (Bradshaw 1991). However, the buffering effect of adaptive plasticity should become less effective in novel environments, exposing genetic variation to selection because genotypes will vary more in their phenotype and fitness depending on their (previously untested) sensitivity to the new conditions (Hermisson and Wagner 2004; Lande 2009; Chevin et al. 2010; Chevin and Lande 2011; Ashander et al. 2016). This exposure of genetic variation in novel environments could allow rapid evolutionary rescue in novel conditions (Nussey et al. 2005; Lande 2009). If so, estimates of genetic variation in fitness within a species' current geographical range are likely to underestimate the potential for evolutionary rescue in novel environments, and therefore the resilience of populations and ecological communities to environmental change.

54 The amount of additive genetic variance in relative fitness (i.e. the amount of genetic variation
55 relative to mean fitness) predicts the increase in mean fitness in the subsequent generation
56 (Fisher 1930; Bonnet et al. 2019), and therefore the potential for evolutionary rescue (Shaw
57 2018). Although genetic variation in fitness is known to vary depending on the environment
58 (Sheth et al. 2018), changes in the amount of genetic variation in fitness is rarely characterized in
59 novel environments, and has not been linked to genetic variation in plasticity in natural
60 environments.

61 In this study, we transplanted multiple clones of 314 genotypes (produced by a quantitative
62 genetics breeding design) across three environments to test: (1) In familiar environments,
63 adaptive plasticity maintains high mean fitness but low genetic variance in fitness because all
64 genotypes can track the environment. However, in a novel environment, the ability for plasticity
65 to allow genotypes to track their environment will be limited, which will reduce mean fitness,
66 but increase genetic variation in fitness that can facilitate evolutionary rescue. (2) Genetic
67 variation in fitness in the novel environment will be correlated with genetic variation in plasticity
68 for morphology, physiology and gene expression.

69 We use a short-lived perennial Sicilian daisy, *Senecio chrysanthemifolius* (Asteraceae), that
70 grows in disturbed habitats at low altitudes (c.400-1,000 m.a.s.l [metres above sea level]) on
71 Mount Etna, an active volcano, and throughout lowland Sicily (**Fig. 1A**). We mated naturally-
72 occurring *S. chrysanthemifolius* genotypes in a quantitative genetic full-sibling, half-sibling
73 breeding design (Lynch and Walsh 1998) to produce 314 offspring genotypes (**Fig. 1B**). We then
74 used multiple clones of each genotype to estimate the fitness, morphology, physiology and gene
75 expression of each genotype at three elevations, both within and outside the species' native range

(**Fig. 1B**). Our breeding design allowed us to calculate the among-sire variance, which represents the additive genetic variance for all phenotypic and fitness measurements recorded.

Results

(1) Reduced mean fitness but increased genetic variance in fitness in the novel environment

Mortality of cuttings was low, even at the novel elevation (12% across all elevations), allowing us to assay fitness for 6-8 cuttings per genotype at each transplant site. In September-October, after 5 months of growth, we estimated fitness by counting all flower heads produced by each plant. This trait correlates closely with total seeds produced per plant (*see Supplementary Material* and **Fig. S1**), and has been used routinely to assay fitness in other short-lived perennials (e.g. Pujol et al. 2014). Fitness (number of flowers) was consistently high within the native range (500m and 1,500m), but decreased by 87% at 2,000m (**Fig. 1C**). Additive genetic variance in relative fitness was near zero within the native range (500m and 1,500m), but more than three times greater at 2,000m where it predicts a 13.8% increase in mean fitness in the subsequent generation (**Fig. 1D**; **Table S2**). Low genetic variance in relative fitness within the native range suggests that adaptive plasticity allows all genotypes to track phenotypic optima and maintain high mean fitness in familiar environments. By contrast, the plastic responses of genotypes at the novel elevation fail (to differing extents) to maintain high mean fitness, creating high levels of genetic variation in relative fitness, and increasing the potential for evolutionary rescue in unfamiliar environments (Shaw 2018). Furthermore, seven of the nine sires with the highest fitness in the novel environment were collected from two sites (**Table S3**), suggesting that

genetic variation present at certain sites within the native range might be key to promoting evolutionary rescue.

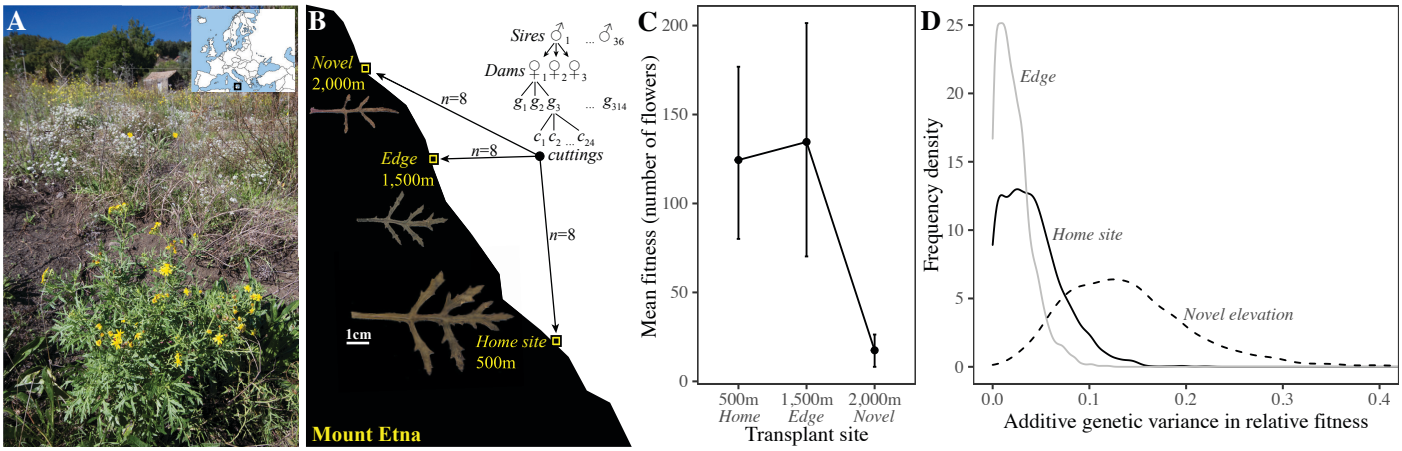


Fig. 1. The experimental design (**A-B**) and the reduction in mean fitness, but increase in genetic variance in fitness at the novel elevation (**C-D**). (**A**) *Senecio chrysanthemifolius* in its natural habitat. Inset map presents the location of the study system in relation to Europe. **(B)** Schematic of Mount Etna showing the experimental design. We sampled individuals from five sites in the foothills of Mount Etna, which we crossed in the glasshouse and transplanted cuttings of their offspring at three elevations. We mated 36 sires each to three dams; we grew three offspring per cross in the glasshouse from which we sampled cuttings that were transplanted at the three elevations. Inset leaves show the average change in leaf morphology with elevation observed for a single genotype. (**C**) Mean fitness dropped outside the native range. Credible intervals represent 95% Highest Posterior Density (HPD) intervals of the mean. (**D**) Posterior distributions for the estimates of additive genetic variance in fitness. Genetic variance in fitness was significantly greater at the novel elevation (2,000m).

(2) Fitness in the novel environment correlates with genetic variance in plasticity

Quantifying phenotypes across elevation: We measured leaf morphology and leaf pigment content by sampling 3-4 young, but fully expanded leaves from each plant. We scanned the leaves and quantified morphology using the morphometric software '*Lamina*' (Bylesjo et al. 2008), which produced five leaf morphology traits: leaf area, leaf complexity ($\frac{leaf\ perimeter^2}{leaf\ area}$), the number of leaf indents standardized by the perimeter, the width of leaf indents and Specific

Leaf Area ($SLA = \frac{leaf\ area}{leaf\ weight}$). These leaf measurements represent leaf morphology and investment traits that show plastic responses to the abiotic environment in *S. chrysanthemifolius* (Walter et al. 2020b), and in other plant systems, including sunflowers (Royer et al. 2009). We also used a Dualex instrument (Force-A) to measure the chlorophyll, flavonol and anthocyanin pigment content of the leaf. Flavonols and anthocyanins are secondary metabolites that combat oxidative stress created by stressful abiotic (e.g. light and temperature) and biotic (e.g. herbivore) conditions (Mierziak et al. 2014). Within each transplant site, we observed a significant association between phenotype and fitness (**Table S5** and **Fig. S2**), and a significant change in multivariate phenotype across elevation (MANOVA $F_{2,23} = 35.647$, $P<0.0001$; *see Supplementary Material*), suggesting that these traits are ecologically important and show significant plasticity across elevation (**Fig. 2A**).

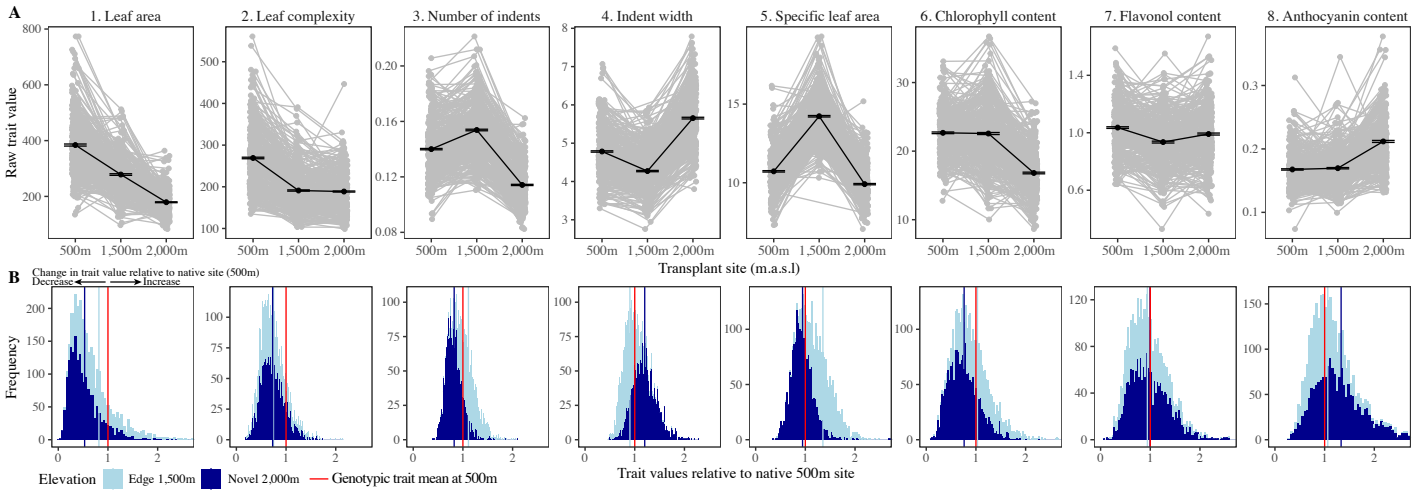


Fig. 2. When compared to the home site (500m), the magnitude of plasticity was greater at the novel elevation (2,000m) compared to the edge of the range (1,500m). (**A**) The change in raw trait values across elevation. Black lines and circles represent the overall mean (±1 SE), with all the genotypes from the breeding design in gray. (**B**) Frequency distribution for plasticity in each trait, which was calculated as the degree to which each genotype changed trait values from the home site (vertical red line) to the edge of the range (light blue) and to the novel elevation (dark blue). Values of one represent no change from the home site. Values above and below one represent plasticity as an increase and decrease in the trait value, respectively.

140 *Identifying genetic variance in plasticity:* To estimate genetic variation in plasticity, we first
141 quantified plasticity as the phenotypic change from the home site to the edge of the range (500-
142 1,500m), and separately, to the novel elevation (500m-2,000m). We calculated plasticity
143 separately for each of the 314 genotypes by dividing the trait values of each genotype at 1,500m
144 and 2,000m by the mean trait value of that genotype at the home site. This standardization
145 calculates the trait values at 1,500m and 2,000m relative to the home site for each genotype.
146 Values of one reflect no change in phenotype between sites, while values above and below one
147 respectively reflect increases and decreases in trait values from the home site (**Fig. 2B**). We
148 estimated plasticity for all traits and then used multivariate linear mixed effects models
149 (including fitness and plasticity in all eight traits) to partition the total variance into sire variance,
150 representing additive genetic variance in plasticity for each trait. We tested whether our estimates
151 of genetic variance in plasticity were statistically significant by comparing our observed
152 estimates to a null distribution (*see Methods*).

153 Our observed estimates of genetic variance in plasticity to the edge of the range (500 to 1,500m)
154 were within the null distribution for four traits, while four traits showed greater plasticity than
155 the null expectation (**Fig. 3**). By contrast, our observed estimates exceeded the null distribution
156 for all eight traits at 2,000m (**Fig. 3**), suggesting more genetic variance in plasticity at the novel
157 elevation. Genetic variation in plasticity was created by genotype-specific responses to the novel
158 environment (**Fig. S3**). Such genetic variance in plasticity could promote evolutionary rescue if
159 correlated with fitness variation at the novel elevation.

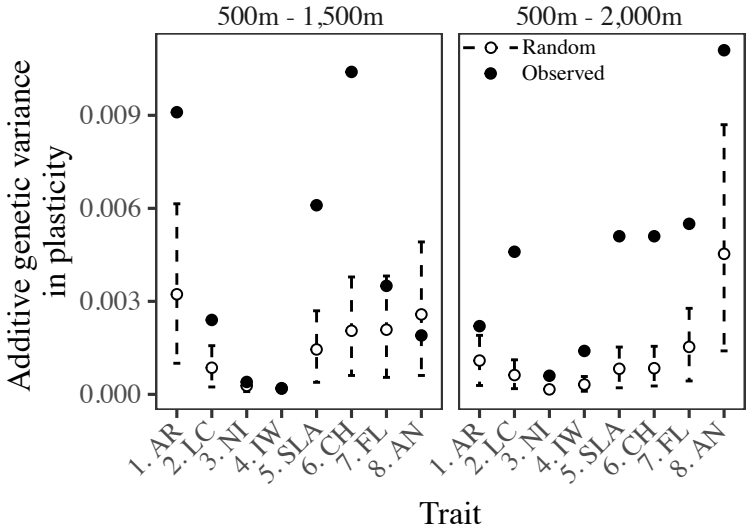


Fig. 3. Greater additive genetic variance in plasticity is observed at the novel elevation than at the range edge. Credible intervals represent 95% HPD for the null distribution. Where the observed estimate exceeds the random distribution indicates statistically significant estimates of genetic variance in plasticity. Significant genetic variance in plasticity was observed for all traits at the novel elevation, compared to only four traits at the range edge. Traits: AR=leaf area, LC=leaf complexity, NI=number of indents, IW=indent width, SLA=specific leaf area, CH=chlorophyll content, FL=flavonol content and AN=anthocyanin content.

Genetic correlations between leaf plasticity and fitness: We detected significant genetic correlations (>70% of the posterior distribution did not overlap with zero) between the magnitude of trait plasticity and fitness at the novel elevation for four traits: leaf complexity (-0.25), number of indents (-0.31), SLA (0.4) and flavonol content (0.39) (**Fig. 4A**). Therefore, genetic variance in plasticity for four leaf traits was correlated with genetic variation in fitness, and so could cause evolutionary rescue in novel environments. Furthermore, depending on the trait, genetic variation in fitness was associated both with increases and decreases in the magnitude of plastic responses. For leaf complexity and the number of indents, *ER* genotypes show greater plasticity than *HR* genotypes, which was created by stronger reductions in trait values between 500m and 2,000m (**Fig. 4B)**. By contrast, for SLA and flavonol content, *ER* genotypes show less plasticity than the *HR* genotypes (**Fig. 4B**).

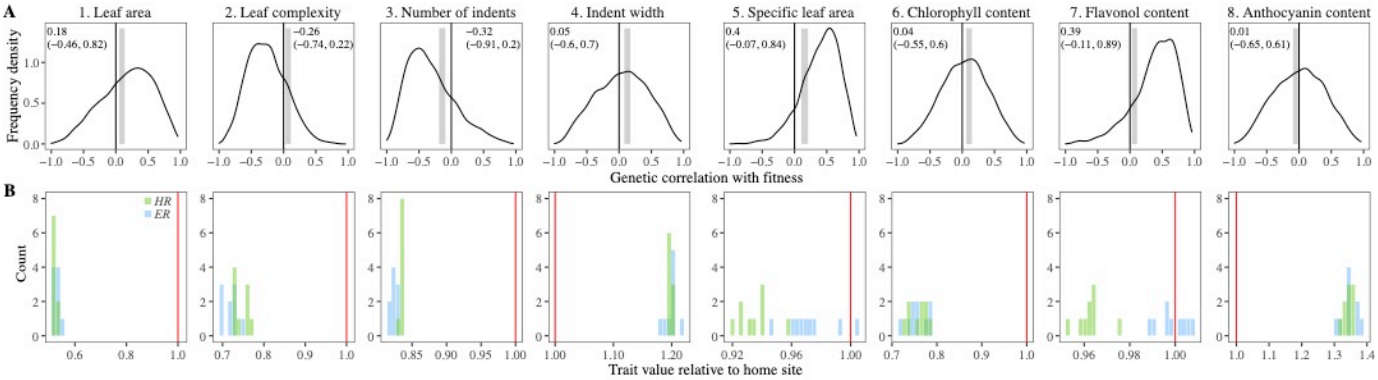


Fig. 4. Genetic variance in plasticity was significantly correlated with fitness at the novel elevation. (**A**) Genetic correlations between plasticity and fitness were moderately strong for leaf complexity, the number of indents, specific leaf area and flavonol content. Numbers inset represent genetic correlations with the 95% HPD interval in parentheses. Gray shading represents the 95% HPD intervals for phenotypic correlations with fitness. (**B**) Histogram for the sire genetic values (that underlie the genetic correlations from **A**), which represent the magnitude of plastic change in trait means from the home site (red vertical line) to 2,000m. Shown are the 25% of sires with the highest (*ER* genotypes in blue) and lowest (*HR* genotypes in green) fitness at 2,000m. Greater fitness at 2,000m is associated with plasticity as a greater reduction in leaf complexity and number of indents, but with a lesser change in SLA and flavonol content.

Variation in gene expression: Overall, a larger number of genes were differentially expressed with increasing distance from the home site. To the edge of the range (500m-1,500m), the *ER* genotypes showed significant changes (adj. $P<0.01$) in more genes than the *HR* genotypes (1,376 in *ER* vs 514 genes in *HR*), suggesting that the *HR* genotypes adjusted fewer genes to maintain fitness within their range (**Fig. 5A**). However, the number of genes that showed expression changes tripled outside the native range (500m-2,000m) for both classes of genotypes (*ER*=4,876 genes vs *HR*=4,720) with the *ER* genotypes again showing a greater magnitude (1.2-fold mean difference) of differential expression per gene than *HR* genotypes (**Fig. 5B**). For both classes of genotypes, the mean magnitude of expression change was greater at the novel elevation (*ER*=1.41, *HR*=1.21) when compared to the edge of the range (*ER*=1.19, *HR*=1.04). Compared to the *HR* genotypes, *ER* genotypes showed twice as many underexpressed genes at the novel

203 elevation, but half as many overexpressed genes (**Fig. 5C**). Distinct patterns of gene expression
204 were therefore associated with genotypic differences in fitness at the novel elevation.

205 *Gene ontology:* To identify the function of differentially expressed genes, we tested for
206 functional enrichment of Gene Ontology (GO) terms. *ER* and *HR* genotypes differed in the
207 functional categories of genes that varied in expression at the novel elevation (**Fig. 5D**). *ER*
208 genotypes showed differential expression of genes relating to biosynthesis, while differentially
209 expressed genes in the *HR* genotypes were related to photosystems. Comparing the enriched GO
210 terms to those of a 2017 transplant experiment (Walter et al. 2020b) that included the high
211 elevation sister species (*S. aethnensis*), we found that four (of 17) GO terms were enriched in
212 *S.aethnensis* as well as in the *ER* genotypes, compared to only one (of 20) GO term shared by *S.*
213 *aethnensis* and the *HR* genotypes (**Fig. 5D**). Therefore, genotypes of *S. chrysanthemifolius* that
214 showed the highest fitness at the novel elevation also showed a more similar gene expression
215 response to the species native to that elevation.

216 GO terms that were significantly enriched at 2,000m included light harvesting in photosystem I,
217 cell wall organization, and responses to cold (**Table S8**). To illustrate the contrasting patterns of
218 expression, we selected the five most differentially expressed genes from each GO term and
219 compared the reaction of the *ER* and *HR* genotypes across elevation. For genes involved in
220 photosystem I (**Fig. 5E**), *ER* genotypes showed stronger patterns of underexpression than *HR*
221 genotypes at the novel elevation. For genes involved in cold responses **(Fig. 5F)**, *ER* genotypes
222 showed a stronger response than *HR* genotypes, which included greater overexpression or
223 underexpression depending on the gene.

224

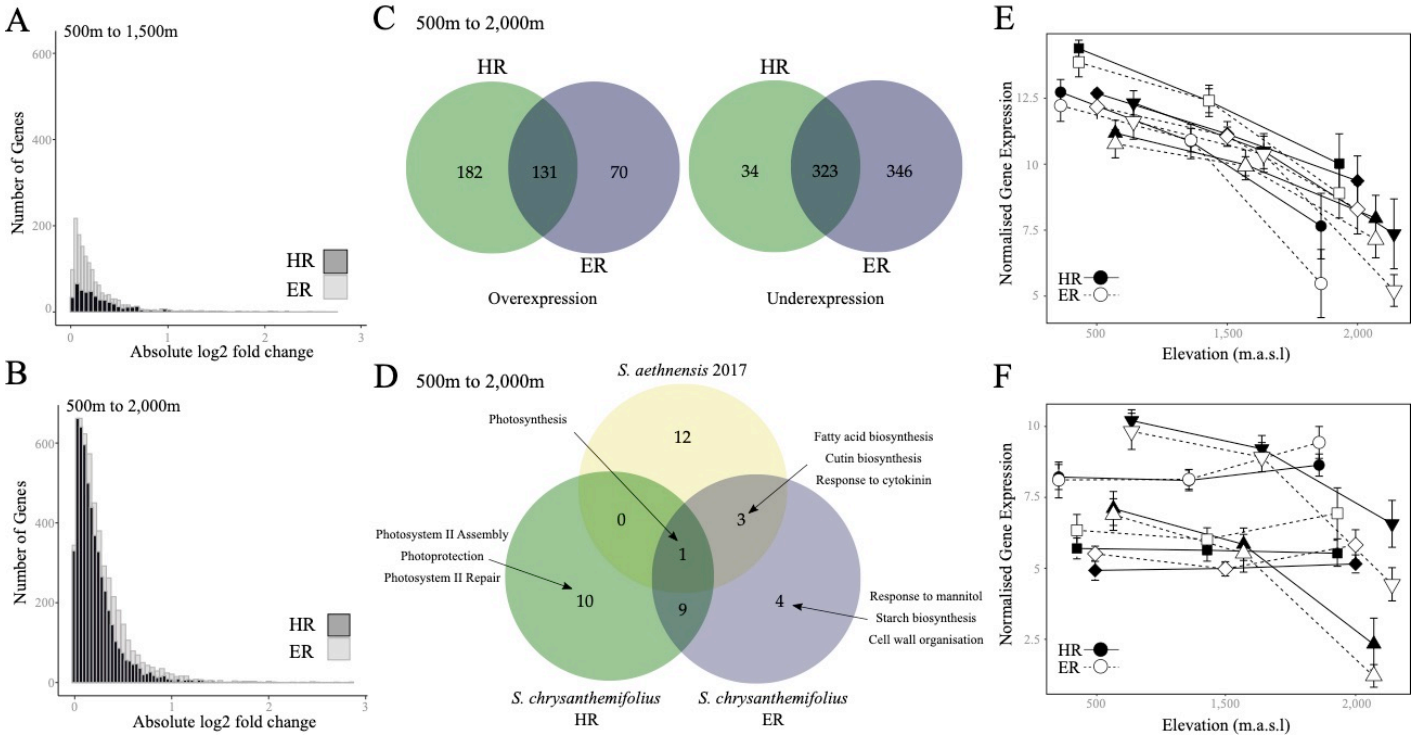


Fig. 5. *ER* and *HR* genotypes differ in gene expression at the novel elevation. **(A)** Within the native range, more genes were differentially expressed in the *ER* genotypes. (**B**) Outside the native range, more genes were differentially expressed, and more genes in the *ER* genotypes showed a greater magnitude of differential gene expression. **(C)** Numbers of differentially expressed genes (adjusted $P<0.01$ and log-fold change <2 or >-2 for over- and underexpression) for the *ER* and *HR* genotypes between the home site (500m) and outside the range (2,000m). *ER* genotypes underexpressed more genes than *HR* genotypes. **(D)** Enriched Gene Ontology (GO) terms for differentially expressed genes for both genotypes and for a high elevation sister species (*S. aethnensis*) transplanted along the same elevation gradient in 2017 (Walter et al. 2020b). Only the three most significant terms are shown. *ER* genotypes shared more GO terms with *S. aethnensis,* suggesting that high fitness is associated with similar genetic pathways to the native species. **(E)** Comparing elevational changes in mean expression for five genes (associated with light harvesting) for the *ER* (unfilled shapes, dashed lines) and *HR* (filled shapes, solid lines) genotypes. Each gene is represented by a different shape and credible intervals represent 95% confidence intervals. *ER* genotypes showed stronger patterns of underexpression at 2,000m. **(F)** Elevational changes in mean expression for five genes associated with responses to cold. *ER* genotypes show stronger under- and overexpression, but the pattern was gene-dependent.

Selection at the native environment hides genetic variation that could promote evolutionary rescue in novel environments

Surprisingly, genetic variance in fitness at the novel environment was uncorrelated with fitness at the native environment (**Table S2**; genetic correlation of -0.1 [-0.79,0.6 HPD]). **Fig 6A** shows that the sires with the highest fitness at the novel elevation had the lowest fitness at the home site and *vice versa,* suggesting that genetic variance in relative fitness not only increases in novel environments, but that a genetic trade-off exists between the native and novel elevations. Therefore, although evolutionary rescue may be facilitated by genetic variation that is present in natural populations, these genotypes are selected against within the native range and only have positive effects on fitness in novel environments.

Comparing fitness of seeds vs. cuttings: The 314 genotypes used in this study were generated by crossing among adult genotypes sampled from the field. They were then grown from seed in the greenhouse and transplanted into the field as cuttings. This means they did not experience selection in the field at early life history stages. To test whether genetic variation that increases fitness in novel environments is selected against at the home site during early life history, we transplanted seeds from all 104 full-sibling families at the same elevations in spring (April 2019).

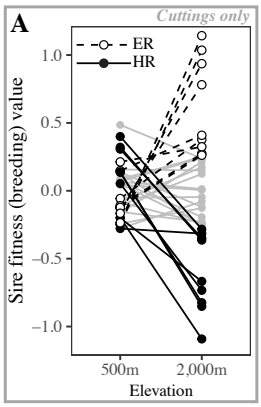
We transplanted 25 seeds of each full-sibling family at each transplant site (n=2,600 seeds/site; total N=8,000), and measured the number of seedlings that emerged and tracked their survival to autumn (October, 2019). We then estimated the genetic covariance in performance between seeds and cuttings, and between the home site and the novel elevation (*see Supplementary Material* for the analysis between 500 to 1,500m). Using a multivariate generalized mixed

267 model, we calculated a 4×4 covariance matrix with the diagonal representing genetic variance in
268 relative fitness as seeds and cuttings, and the off diagonal representing the genetic covariance
269 (and correlation) between seeds and cuttings both within and between elevations (**Fig. 6B**).

270 If genetic variance that can increase fitness at novel elevations is selected against at the home
271 site, sires that performed well as cuttings at the novel elevation (2,000m) should perform
272 similarly well as seedlings at 2,000m. However, the same sires that performed best at 2,000m
273 should show lower performances at the home site (500m). **Fig. 6B-D** presents seedling
274 emergence as the fitness component for early life history, but results were similar for survival
275 (**Fig. S8**). As predicted, performance as seedlings and cuttings at 2,000m were positively
276 correlated (0.19; -0.55,0.88 HPD; **Fig. 6B**), suggesting genetic variation in relative fitness at the
277 novel elevation was common to both life history stages. However, consistent with the lower
278 performance of *ER* genotypes as seedlings at the home site, seedling fitness at a given elevation
279 (500m or 2,000m) showed weak negative genetic correlations with fitness as cuttings at the other
280 elevation (-0.15 [-0.85,0.6 HPD] and -0.04 [-0.67,0.6 HPD]; **Fig. 6B**). The fittest sires at the
281 novel elevation therefore had the lowest fitness at the native site, while the sires with the lowest
282 fitness at the novel elevation showed the highest fitness at the native site (**Fig. 6C**).

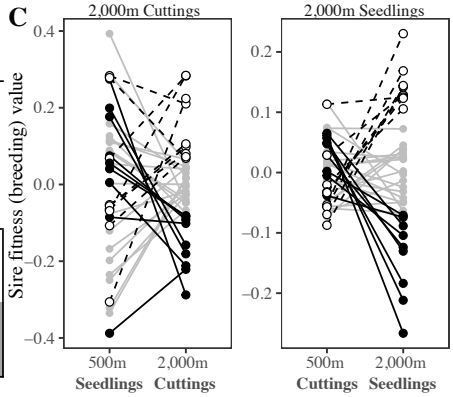
283 To confirm that selection on seedlings at the home site reduces genetic variation suitable for
284 evolutionary rescue at the novel elevation, we used matrix decomposition to describe the matrix
285 in **Fig. 6B**. This method, analogous to principal components analysis, identifies axes that
286 describe genetic variation in fitness that is shared between elevations and life history stages. The
287 first axis represents genetic variance in seedling emergence common to both elevations (**Fig.
288 6D**), largely because genetic variance in seedling performance was much greater (**Fig. 6B**).
289 However, the second axis, representing 25% of total genetic variance in fitness, describes genetic

variation in fitness that differed between the native and novel elevations because the 500m and 2,000m sites possess loadings with high values that have different signs (**Fig. 6D**). Genetic variation that increased fitness at the novel elevation showed lower fitness at the native elevation, suggesting that selection on seedlings within the native range reduces the viability of genotypes that would allow evolutionary rescue in novel environments.



B

	500m **Seedlings**	500m **Cuttings**	2,000m **Seedlings**	2,000m **Cuttings**
500m **Seedlings**	0.553 (0,1.241)	-0.01 (-0.127, 0.098)	0.126 (-0.167, 0.529)	-0.015 (-0.200, 0.195)
500m **Cuttings**	-0.07 (-0.84,0.6)	0.047 (0,0.114)	-0.017 (-0.118, 0.068)	-0.01 (-0.066, 0.038)
2,000m **Seedlings**	0.3 (-0.43,0.95)	-0.15 (-0.85,0.6)	0.299 (0,0.955)	0.044 (-0.083, 0.242)
2,000m **Cuttings**	-0.04 (-0.67,0.6)	-0.13 (-0.76,0.47)	0.19 (-0.55,0.88)	0.147 (0.022, 0.289)



D

Eigenvector	e_1	e_2
Eigenvalue	0.6	0.27
HPD interval	(0.00, 1.29)	(0.02, 0.69)
Prop. of variance	0.58	0.25
Traits		
500m **Seeds**	**0.92**	**-0.36**
500m **Cuttings**	-0.03	-0.07
2,000m **Seeds**	**0.38**	**0.85**
2,000m **Cuttings**	0.02	**0.37**

Fig. 6. Genetic variation that could promote evolutionary rescue shows lower performance at the home site. **(A)** Sire fitness values as cuttings at the home site and novel elevation show that the *ER* genotypes had lower fitness than the *HR* genotypes at the home site. **(B)** The 4×4 covariance matrix representing genetic variation for fitness as seedlings and cuttings at the home site and novel elevation. Gray shading represents genetic variance in fitness along the diagonal, with the lower diagonal presenting genetic correlations, and the upper diagonal the genetic covariances. (**C**) Sires that performed well at the novel elevation (as both seedlings and cuttings) showed lower fitness than the *HR* genotypes at the native elevation as both seedlings (left panel) and cuttings (right panel). (**D**) Axes of genetic variation in fitness describing the matrix in (**B**). Numbers in bold emphasize trait loadings greater than 0.2 to aid interpretation. The second axis describes genetic variation underlying differences in fitness between the home site and the novel elevation.

Discussion

This study provides evidence that adaptive plasticity is restricted to native environments, and fails to sustain high mean fitness in a novel environment, exposing genetic variation in fitness that could promote evolutionary rescue by allowing rapid changes in allele frequencies.

313 Increased genetic variation in fitness of *S. chrysanthemifolius* at the novel elevation was
314 correlated with genetic variation for greater plasticity that reduced the trait values of leaf
315 complexity and leaf indentation, but lower plasticity that maintained the trait values of specific
316 leaf area and flavonol content across elevation. Our data also suggest that genetic variation that
317 would increase fitness in novel environments showed lower fitness in the native environment.
318 Therefore, genetic variation important for evolutionary rescue is likely to be maintained at low
319 frequency by selection within the native range (Brennan et al. 2019). Studies estimating genetic
320 variation in fitness *in situ* are therefore likely to underestimate the potential for evolutionary
321 rescue because the effects of alleles important for evolutionary rescue only emerge in novel
322 environments (Sheth et al. 2018; Kulbaba et al. 2019).

323 Gene expression responses are determined through complex genetic and epigenetic regulation,
324 which are likely to determine plasticity in phenotype, and how environmental sensitivity can
325 evolve and promote resilience in novel environments (Oostra et al. 2018). Interestingly, the gene
326 expression data revealed that, compared to the low fitness genotypes at 2,000m, genotypes with
327 high fitness differentially expressed more genes, which was associated with greater
328 underexpression. Furthermore, differential expression in the high fitness genotypes occurred in
329 functional categories associated with plasticity in the closely related high elevation (2,000m+)
330 sister species. This suggests that adaptation to novel environments requires genotypes that create
331 the most beneficial gene expression profiles (Wang and Althoff 2019), which contrasts with
332 previous evidence that adaptation to novel environments involves non-adaptive plasticity in gene
333 expression (Ghalambor et al. 2015), or that a lack of genetic variance in gene expression will
334 prevent beneficial responses to novel environments (Oostra et al. 2018).

335 Overall, our results provide strong evidence that evolutionary rescue depends on genetic
336 variation that increases fitness by selecting for the most beneficial plastic responses in novel
337 environments (Lande 2009; Chevin and Lande 2011; Chevin and Hoffmann 2017), created by a
338 fine-tuning of gene-products and trait values in novel environments. Our results also support
339 studies that show that genetic variance in fitness depends on the environment in which it is
340 quantified (Sheth et al. 2018; Kulbaba et al. 2019). Genetic variation for sensitivity to the
341 environment is likely to be unevenly distributed across a species' range (Stratton 1994; Colautti
342 and Barrett 2013; Sheth and Angert 2016; Walter et al. 2020a), with local adaptation determining
343 which populations provide the potential to promote evolutionary rescue (Hargreaves and Eckert
344 2019). To quantify rates of adaptation to novel environmental regimes, research should therefore
345 assess genetic variation in environmental sensitivity for multiple populations exposed to a range
346 of novel environments. It is also important to determine how and when the expression of genetic
347 variation likely to cause evolutionary rescue is masked by existing environmental conditions.

348

349 **Methods**

350 ***Genotype sampling and crossing design***: To establish the parental generation, in June 2017 we
351 collected cuttings from 72 individuals located at five geographically separate sites on the
352 foothills of Mt. Etna (Table S1; also see Fig. 1 in Walter et al. 2020b). Where possible, we
353 sampled individuals that were at least ten meters apart from each other to minimize chances of
354 sampling close relatives. We removed whole branches from mature plants, which we cut into 4-
355 5cm segments at the glasshouse (Giarre, Italy), dipped them in a rooting plant growth regulator
356 for softwood cuttings (Germon® Bew., Der. NAA 0.5%, L. Gobbi, Italy) and placed each cutting
357 in one cell of an 84-cell tray containing a compressed mix of 1:1 perlite and coconut coir. For

three weeks, we kept cuttings on benches covered by plastic to maintain humidity and encourage root growth. For each individual sampled in the field, we placed one cutting in a 30cm diameter pot with standard potting mix, which we watered regularly and left to grow. To encourage growth, we suspended 25W LED tubes (TSA Technology, Italy) 1m above the bench. Once plants produced buds, we covered flowering branches with perforated bread bags in order to prevent pollinators from entering while allowing airflow. We randomly designated each individual as a dam or sire and grouped them into 12 blocks, each containing three sires (n = 36) and three dams (n = 36), see **Table S1**. Within each block we mated all sires to all dams to produce nine full-sibling families per block (n = 104 total full-sibling families, with four crosses failing to produce seeds).

Six seeds from each full-sibling family were germinated by cutting the top off each seed (<1mm) and placing them on moistened filter paper in a plastic Petri dish. Petri dishes were kept in the dark for two days, and then transferred to growth cabinets maintained at 22°C with a 12h:12h light:dark photoperiod. After one-week we transferred seedlings to the glasshouse where one individual was transplanted into one cell of an 84-cell tray containing a mix of 1:1 perlite and coconut coir. After growing 3-4 true leaves, we transferred seedlings to 14cm diameter pots containing standard potting mix. We transferred three seedlings (in three separate pots) for each full-sibling family, which we arranged randomly in the greenhouse. When the main stem of each seedling reached c.12cm of growth, we cut the main stem c.4cm above ground level to promote lateral branching and generate enough cuttings to replicate each genotype in the field transplant. This meant that each of the 312 individuals grew 4-5 side branches, each long enough to take 5-6 cuttings.

380 ***Field transplant of the breeding design as cuttings:*** When branches started producing buds, we
381 took cuttings from all 312 individuals (hereafter, genotypes), cutting each branch from each plant
382 into smaller segments each 4-5cm long with 2-3 leaf nodes. For almost all genotypes we were
383 able to take 21 cuttings (transplant 1). Each cutting was treated as described above and left for
384 two weeks to produce roots. To produce a second round of cuttings, all plants were left to regrow
385 for three weeks, at which point we repeated the process to generate another 14 cuttings per plant
386 (transplant 2). Due to the deaths of two genotypes in the glasshouse after taking the initial
387 cuttings, we replaced these genotypes with siblings for the second round of cuttings, which led to
388 two extra genotypes (n=314 rather than 312). Cuttings were collected as above and stored in
389 high-humidity tunnels for two weeks until they produced roots and were ready to transplant.

390 *Transplant 1, May 23-25 2018:* To prepare the cuttings, for each transplant site we randomized
391 six cuttings per genotype into 24 84-cell trays. We split the 24 trays into two sets of 12, each
392 containing three cuttings for each genotype, which provide two replicate environmental blocks at
393 each site, each containing three cuttings for each of the 312 genotypes (transplant 1: n = 936
394 cuttings per block; n = 1,872 cuttings per transplant site; total N = 5,634 cuttings).

395 *Transplant 2, 10-11 July*: We used all remaining cuttings available to transplant two additional
396 cuttings/genotype at each field site in two additional blocks, as well as transplanting extra
397 cuttings to replace cuttings lost due to c.10% mortality at 500m and 2,000m due to a short spell
398 of intense heat in late June (transplant 2: n = 321-525 cuttings per block; n = 718-927 cuttings
399 per transplant site; total N = 2,515 cuttings). In total, we transplanted 8,149 cuttings for the 314
400 genotypes.

401 In total, we transplanted 7-10 cuttings from each of the 314 genotypes at each of three transplant
402 sites along an elevational gradient (n=c.2,700 cuttings/site; total N=8,149 cuttings) that
403 represented typical conditions within the species' native range (500m.a.s.l), the edge of its range
404 (1,500m.a.s.l), and a novel elevation (2,000m.a.s.l). The 500m site was located on abandoned
405 land, the 1,500m site in an apple and pear orchard, and the 2,000m site on a lava flow from
406 c.1980. Elevations under 1,000m are exposed to high temperatures that regularly exceed 40°C
407 during summer, and above 1,000m the temperature regularly drops below 0°C during winter.
408 Soil at lower elevations soil is characterized as a sandy loam, but changes to volcanic sand above
409 1,500m (Walter et al. 2020b).

410 To prepare experimental plots, the soil surface was cleared of plants and the soil turned (30cm
411 deep) immediately prior to transplanting the cuttings. We then transplanted cuttings into large
412 grids of different sizes, spacing each plant 30cm apart. Transplanted cuttings were maintained
413 moist until they established, after which we reduced watering to minimal amounts at regular
414 intervals, sufficient to maintain survival for all genotypes while having a minimal effect on
415 growth or flower production.

416 ***Bayesian implementation of statistical tests:*** We used Bayesian models to estimate genetic
417 variance in fitness and phenotypic plasticity across elevation. For each equation, we included a
418 substantial burn-in and thinning interval to allow model convergence. We checked
419 autocorrelation by confirming that the effective sample sizes exceeded 85% of the number of
420 samples that were saved. We used uninformative parameter-expanded priors, which we checked
421 by changing the scale parameter and ensuring that there was no effect on the posterior
422 distribution. From each implementation, we extracted 2,000 samples, which provided the
423 posterior distribution for each parameter estimated.

424 ***Quantifying genetic variance in fitness:*** To quantify genetic variance in fitness, we used the R
425 (v3.6.1; R Core Team 2019) package '*MCMCglmm*' (Hadfield 2010) to implement the
426 generalized linear mixed effects model

427 $$y_{ijklmn} = T_i + s_{j(k)} + d_{k(j)} + g_{l(jk)} + b_{m(i)} + e_{n(ijklm)}\,, \qquad (1)$$

428 where the only fixed effect represented transplant site (T_i). The random effects $s_{j(k)}$ represented
429 the *j*th sire, $d_{k(j)}$ the *k*th dam and $g_{l(jk)}$ the *l*th individual of the breeding design nested within
430 dam and sire. Additional random effects included block within transplant site ($b_{m(i)}$), which
431 removed variance due to differences between the two transplants and between the experimental
432 blocks. $e_{n(ijklm)}$ represented the error variance. For the $s_{j(k)}$, $d_{k(j)}$ and $g_{l(jk)}$ components we
433 were interested in estimating the variance among genotypes within each transplant site, as well
434 as the covariance among sites. To do this, we estimated random intercepts for each transplant
435 site, and random slopes between transplant sites, resulting in a 3×3 covariance matrix for each
436 random effect. We implemented equation 1 while estimating residual variance at each transplant
437 site, but not the covariance among sites because plants could not be at both sites at once. Fitness
438 was included as a univariate, poisson-distributed response variable (y_{ijklmn}), which meant that
439 for each random component our estimates of variance represented variance in relative fitness
440 (Bonnet et al. 2019). We implemented equation 1 with 1 million iterations, a burn-in of 100,000
441 iterations and thinning interval of 500 iterations. The additive genetic (co)variance matrix for
442 fitness is presented in **Table S2**.

443 ***Quantifying genetic variance in plasticity****:* For the subsequent analyses, for all traits we
444 calculated the average across all leaves sampled from each plant. We calculated plastic changes
445 in phenotype across elevation by standardizing all clones of each genotype (at 1,500m and

446 2,000m) by the mean value of that genotype at the home site (500m). This standardization
447 calculated the trait values for each genotype at the edge of the range, and outside the range,
448 relative to their trait value at the home site (see **Fig. 2**). We estimated plasticity separately for
449 each transplant date to account for differences between transplants in the linear mixed model
450 below. To quantify genetic variance in plasticity, and the genetic correlation between phenotypic
451 plasticity and fitness, we used a multivariate linear mixed effects model

452 $$y_{ijklm} = s_{i(j)} + d_{j(i)} + g_{k(ij)} + b_l + e_{m(ijkl)}\,, \qquad (2)$$

453 where the random effects $s_{i(j)}$ represented the *i*th sire, $d_{j(i)}$ the *j*th dam and $g_{k(ij)}$ the *k*th
454 individual of the breeding design nested within dam and sire. Additional random effects included
455 block within transplant site (b_l), which removed variance due to differences between the two
456 transplants and between the experimental blocks. The error variance is represented by $e_{m(ijkl)}$.
457 We applied equation 2 to plasticity in all eight phenotypic traits as a multivariate response
458 variable (y_{ijklm}). Fitness was included as the ninth trait as a poisson-distributed response
459 variable. We applied equation 2 separately for the edge of the range (1,500m) and outside the
460 range (2,000m). We implemented equation 2 with 2 million iterations, a burn-in of 200,000
461 iterations and thinning interval of 1,000 iterations.

462 Extracting the sire component from the output of equation 3 and multiplying it by four (Lynch
463 and Walsh 1998) quantified the 9×9 additive genetic variance-covariance matrix for plasticity,
464 separately for the 1,500m and 2,000m elevations. In **Table S6** we present the estimates for
465 genetic variance in plasticity for each trait, and each comparison. To quantify the genetic
466 correlation between plasticity and fitness we calculated the correlation matrix and extracted
467 genetic correlations between trait plasticity and fitness, which are presented in **Fig. 4**.

468 In a Bayesian framework, estimates of genetic variance in plasticity are constrained to be
469 positive, making comparisons to zero uninformative. To test whether we detected biologically
470 meaningful observed estimates of genetic variance in plasticity, we created a null distribution by
471 randomizing the parentage of all individuals and re-implementing the same equation. To prevent
472 the fixed effect of the two transplants and the experimental blocks having undue influence on the
473 randomizations, we randomized the pedigree within each experimental block. We randomized
474 and re-implemented equation 2 1,000 times to create a suitable null distribution. To reduce
475 computing time, we reduced the total number of iterations while retaining the same burn-in and
476 thinning interval to ensure the same mixing properties of the chain (Walter et al. 2018). The
477 convergence of our observed models showed that 100 iterations were required to obtain a stable
478 estimate of the mean for each variance parameter. We calculated the mean of each model
479 implemented on the randomized data, which gave us the null distribution of genetic variance in
480 plasticity expected under random sampling. If our observed estimates of genetic variance
481 exceeded our null distribution, this is evidence for significant genetic variance in plasticity.

482 ***Gene expression analyses:*** To prioritize genotypes for gene expression analyses, we quantified
483 changes in fitness between the home site (500m) and the novel elevation (2,000m). We chose six
484 genotypes that showed the greatest increase in relative fitness from the home site to outside the
485 range (*see Supplementary Material*), representing genotypes that could promote evolutionary
486 rescue in the novel environment (hereafter, '*ER*' genotypes). We also identified six genotypes
487 with the greatest decrease in relative fitness outside their range, suggesting a higher level of
488 specialization to their native range (hereafter, '*HR*' genotypes).

489 For each of the chosen genotypes, we sampled leaves from three clones chosen at random at each
490 transplant site. From each plant, we sampled 3-4 young leaves (c.15mm long), which we

491 immediately submerged in RNAlater and stored at 4°C for 24 hours and then at -80°C before
492 RNA extraction. To control for variation due to weather and diurnal effects, sampling was
493 constrained to three consecutive days between 9am and 11am under similar weather conditions.
494 We extracted RNA for the leaves of each plant and quantified gene expression at each elevation
495 using 3' RNAseq (QuantSeq). See *Supplementary Material* for RNA extraction protocols and the
496 reference transcriptome assembly.

497 ***Detecting Differentially Expressed Genes:*** 3' reads were mapped to the reference transcriptome
498 using Salmon v1.1.0 (Patro et al. 2017). On average 79.15% of reads were mapped (72.6-87.3%).
499 Transcript abundance estimates were imported into R using the 'txImport' package (Soneson et
500 al. 2015), with estimates normalized according to library size but not by transcript length, as the
501 3' sequencing method removes this bias. We visualized the broad patterns of variation in gene
502 expression among samples by first normalizing the transcript abundance using the variance
503 stabilizing transformation function in DESeq2 and then subjecting the transcript abundance
504 matrix to a principal components analysis **(Fig. S5**). Differential expression of transcripts was
505 then calculated using non-normalized transcript counts between transplant sites for each set of
506 genotypes (*ER* and *HR*) separately using DESeq2 (Love et al. 2014). Differentially expressed
507 genes were defined as those showing both significant (adjusted p-value < 0.01) and strong (log2-
508 fold change > 2 or < -2) changes in expression between treatments.

509 We tested for enrichment of gene ontology (GO) terms among differentially expressed genes
510 between each transplant site for both sets of genotypes (**Table S8**). Enrichment tests were
511 performed using both a Kolmogorov-Smirnoff test with the 'weight' algorithm and Fisher's
512 exact test in the topGO package (Alexa and Rahnenführer 2019).

513 ***Estimating seedling performance across elevation*:** To test the performance of the same sires at
514 early life history stages, we planted siblings from all full-sibling families as seeds at the same
515 sites as the cuttings. At each transplant site, we transplanted 25 seeds from each family
516 randomized into five replicate blocks at each transplant site (family n = 104, n = 520 seeds per
517 block, n = 2,600 seeds per transplant site, total N = 8,000 seeds). We used a small drop of non-
518 drip superglue to attach each seed to the middle of a toothpick, which we randomized into five
519 environmental blocks at each site (Walter et al. 2016). We then placed each toothpick into a
520 randomized grid (replicating the experimental grids in the field) made of 3cm thick polystyrene
521 glued to plywood (**Fig. S6A**). To construct the experimental blocks in the field, we cleared the
522 ground and attached 4x4cm grid fishing net to the ground using 20cm steel u-shaped pegs.

523 Seeds in natural populations germinate in autumn and spring with available water. To replicate
524 natural germination conditions, we initiated the transplant in spring (7-8 May 2019), suspended
525 80% shade-cloth 20cm above each block and kept the seeds moist for three weeks, after which
526 we reduced watering. During this period it rained often, suggesting the experiment matched
527 natural germination timing. Shortly after reducing water, and once seedling emergence
528 stabilized, we replaced the shade-cloth with a lighter shade-cloth (40%) that represented light
529 shading common in natural populations (5 weeks at 500m; 7 weeks for 1,500m; 8 weeks for
530 2,000m). We removed all shade-cloth once seedlings reached their first two true leaves (after 9
531 weeks at 500m, and after 10 weeks for 1,500m and 2,000m). The differences in timing were
532 necessary because the lower temperatures at higher elevations slowed the rate of emergence and
533 growth. To make conditions as similar as possible for the four sites, and as natural as possible,
534 we removed shade-cloth and ceased watering at the same average developmental point at each
535 site. We collected data weekly by recording whether seedlings emerged and then tracking their

survival. **Fig. S6B** presents a photo of a seedling block at 2,000m, two months after the initial transplant.

Seedling emergence was high (500m=86.8%; 1,5000m=76.5%; 2,000m=90.0%). Compared to the home site, mortality after emergence was higher at the edge of the range and outside the range (**Fig. S7**). For the analyses of genetic variance below, we used seedling emergence and survival as measures of seedling performance. For survival, we used the data collected at the end of summer (24 September, 139 days from commencement of the experiment), at which point mortality stabilized and 67.8% of the plants that emerged still remained across all transplant sites (**Fig. S7**).

Our *a priori* hypothesis focused on describing the genetic correlation between seedlings and cuttings across the elevational extremes (i.e. between the home site and the novel elevation). Consequently, we focus on the 500m and 2,000m elevations, but we also provide the analogous analysis with the intermediate (1,500m) elevation in the *Supplementary Material*. To connect fitness for the same sires measured as seedlings and cuttings, we re-implemented equation 1 with the combined seedling and cutting data. To account for the difference among blocks in the seed experiment we included an additional random term ($r_{p(i)}$) representing the *p*th experimental seed block within the *i*th transplant site. To estimate the genetic covariance between fitness as seeds and as cuttings, we implemented equation 1 with seed and cutting fitness included as a bivariate response variable. Both random intercepts and random slopes were estimated for each transplant site and life history stage, resulting in a 4×4 genetic covariance matrix ($\mathbf{G}_{\mathrm{m}}$) representing genetic variance in fitness as both cuttings and seeds within each transplant site, as well as the genetic covariance among transplant sites for seedlings and cuttings. We implemented equation 1 for

558 emergence and separately for survival. In the main text we present seedling emergence, but
559 results for seedling survival were consistent (**Fig. S8**).

560 To identify genetic variation common to both seed and cuttings experiments, and common
561 among transplant sites, we decomposed $\mathbf{G}_m$, which quantified the orthogonal axes of genetic
562 variation underlying total genetic variation in fitness. We predicted that if genetic variance
563 underlying evolutionary rescue was removed at early life history stages, we would observe an
564 axis of genetic variance with trait loadings opposite for the home site and the site outside the
565 home range. To identify whether our axes of genetic variation described biologically meaningful
566 genetic variance in fitness, we created a suitable null distribution for which to compare our
567 observed matrix decomposition. We constructed our random distribution using the same method
568 of randomizing the pedigree information and re-implementing the same model. To prevent
569 random and fixed effects having undue influence on the randomizations, we randomized the
570 pedigree within each experimental block (separately for seedlings and cuttings).

571 We found that all axes describing genetic variance in fitness represented greater genetic variance
572 in fitness than expected under random sampling (**Fig. S9**). This means that the second
573 eigenvector that described differences in fitness between the home site and the site outside the
574 range also described greater genetic variance than expected under random sampling.

575

576 **References**

577 Alexa, A., and J. Rahnenführer. 2019. Gene set enrichment analysis with topGO. R package
578 version 2.38.1.

579 Ashander, J., L. M. Chevin, and M. L. Baskett. 2016. Predicting evolutionary rescue via evolving
580 plasticity in stochastic environments. Proceedings of the Royal Society B-Biological
581 Sciences 283.
582 Bell, G., and A. Gonzalez. 2009. Evolutionary rescue can prevent extinction following
583 environmental change. Ecology Letters 12:942-948.
584 Bonnet, T., M. B. Morrissey, and L. E. B. Kruuk. 2019. Estimation of genetic variance in fitness,
585 and inference of adaptation, when fitness follows a log-normal distribution. Journal of
586 Heredity 110:383-395.
587 Bradshaw, A. D. 1991. The Croonian Lecture, 1991 - Genostasis and the Limits to Evolution.
588 Philosophical Transactions of the Royal Society B-Biological Sciences 333:289-305.
589 Brennan, R. S., A. D. Garrett, K. E. Huber, H. Hargarten, and M. H. Pespeni. 2019. Rare genetic
590 variation and balanced polymorphisms are important for survival in global change
591 conditions. Proceedings of the Royal Society B-Biological Sciences 286:20190943.
592 Bylesjo, M., V. Segura, R. Y. Soolanayakanahally, A. M. Rae, J. Trygg, P. Gustafsson, S.
593 Jansson et al. 2008. LAMINA: a tool for rapid quantification of leaf size and shape
594 parameters. BMC Plant Biology 8.
595 Charmantier, A., R. H. McCleery, L. R. Cole, C. Perrins, L. E. B. Kruuk, and B. C. Sheldon.
596 2008. Adaptive phenotypic plasticity in response to climate change in a wild bird
597 population. Science 320:800-803.
598 Chevin, L. M., R. Gallet, R. Gomulkiewicz, R. D. Holt, and S. Fellous. 2013. Phenotypic
599 plasticity in evolutionary rescue experiments. Philosophical Transactions of the Royal
600 Society of London Series B-Biological Sciences 368:1-12.

601 Chevin, L. M., and A. A. Hoffmann. 2017. Evolution of phenotypic plasticity in extreme
602 environments. Philosophical Transactions of the Royal Society of London Series B-
603 Biological Sciences 372:20160138.
604 Chevin, L. M., and R. Lande. 2011. Adaptation to marginal habitats by evolution of increased
605 phenotypic plasticity. Journal of Evolutionary Biology 24:1462–1476.
606 Chevin, L. M., R. Lande, and G. M. Mace. 2010. Adaptation, plasticity, and extinction in a
607 changing environment: towards a predictive theory. PLoS Biology 8:e1000357.
608 Colautti, R. I., and S. C. Barrett. 2013. Rapid adaptation to climate facilitates range expansion of
609 an invasive plant. Science 342:364-366.
610 Fisher, R. A. 1930, The genetical theory of natural selection. Oxford, Oxford University Press.
611 Ghalambor, C. K., K. L. Hoke, E. W. Ruell, E. K. Fischer, D. N. Reznick, and K. A. Hughes.
612 2015. Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in
613 nature. Nature 525:372-375.
614 Gomulkiewicz, R., and R. D. Holt. 1995. When does evolution by natural selection prevent
615 extinction? Evolution 49:201-207.
616 Hadfield, J. D. 2010. MCMC Methods for multi-response generalized linear mixed models: The
617 MCMCglmm R package. Journal of Statistical Software 33:1-22.
618 Hargreaves, A. L., and C. G. Eckert. 2019. Local adaptation primes cold-edge populations for
619 range expansion but not warming-induced range shifts. Ecology Letters 22:78-88.
620 Hermisson, J., and G. P. Wagner. 2004. The population genetic theory of hidden variation and
621 genetic robustness. Genetics 168:2271-2284.

622 Kulbaba, M. W., S. N. Sheth, R. E. Pain, V. M. Eckhart, and R. G. Shaw. 2019. Additive genetic
623 variance for lifetime fitness and the capacity for adaptation in an annual plant. Evolution
624 73:1746-1758.
625 Lande, R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic
626 plasticity and genetic assimilation. Journal of Evolutionary Biology 22:1435-1446.
627 Love, M. I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion
628 for RNA-seq data with DESeq2. Genome biology 15:550.
629 Lynch, M., and B. Walsh. 1998, Genetics and analysis of quantitative traits. Sunderland, Sinauer
630 Associates, Inc.
631 Mierziak, J., K. Kostyn, and A. Kulma. 2014. Flavonoids as important molecules of plant
632 interactions with the environment. Molecules 19:16240-16265.
633 Nussey, D. H., E. Postma, P. Gienapp, and M. E. Visser. 2005. Selection on heritable phenotypic
634 plasticity in a wild bird population. Science 310:304-306.
635 Oostra, V., M. Saastamoinen, B. J. Zwaan, and C. W. Wheat. 2018. Strong phenotypic plasticity
636 limits potential for evolutionary responses to climate change. Nature Communications
637 9:1-11.
638 Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annual
639 Review of Ecology, Evolution, and Systematics 37:637-669.
640 Patro, R., G. Duggal, M. I. Love, R. A. Irizarry, and C. Kingsford. 2017. Salmon provides fast
641 and bias-aware quantification of transcript expression. Nature methods 14:417.
642 Pujol, B., P. Marrot, and J. R. Pannell. 2014. A quantitative genetic signature of senescence in a
643 short-lived perennial plant. Current Biology 24:744-747.

R Core Team. 2019 R: A language and environment for statistical computing, version 3.6.1. R Foundation for Statistical Computing, Vienna, Austria.

Royer, D. L., L. A. Meyerson, K. M. Robertson, and J. M. Adams. 2009. Phenotypic plasticity of leaf shape along a temperature gradient in *Acer rubrum*. PLoS One 4:e7653.

Shaw, R. G. 2018. From the Past to the Future: Considering the Value and Limits of Evolutionary Prediction. The American Naturalist 193:1-10.

Shaw, R. G., and J. R. Etterson. 2012. Rapid climate change and the rate of adaptation: insight from experimental quantitative genetics. New Phytologist 195:752-765.

Sheth, S. N., and A. L. Angert. 2016. Artificial Selection Reveals High Genetic Variation in Phenology at the Trailing Edge of a Species Range. The American Naturalist 187:182-193.

Sheth, S. N., M. W. Kulbaba, R. E. Pain, and R. G. Shaw. 2018. Expression of additive genetic variance for fitness in a population of partridge pea in two field sites. Evolution 72:2537-2545.

Soneson, C., M. I. Love, and M. D. Robinson. 2015. Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. F1000Research 4.

Stratton, D. A. 1994. Genotype-by-environment interactions for fitness of *Erigeron annuus* show fine-scale selective heterogeneity. Evolution 48:1607-1618.

Via, S., R. Gomulkiewicz, G. De Jong, S. M. Scheiner, C. D. Schlichting, and P. H. Van Tienderen. 1995. Adaptive phenotypic plasticity: consensus and controversy. Trends in Ecology & Evolution 10:212-217.

Walter, G. M., J. D. Aguirre, M. W. Blows, and D. Ortiz-Barrientos. 2018. Evolution of genetic variance during adaptive radiation. The American Naturalist 191:E108–E128.

667 Walter, G. M., S. Catara, J. R. Bridle, and A. Cristaudo. 2020a. Population variation in early
668 development can determine ecological resilience in response to environmental change.
669 New Phytologist 226:1312-1324.
670 Walter, G. M., J. Clark, A. Cristaudo, B. Nevado, S. Catara, M. Paunov, V. Velikova et al.
671 2020b. Adaptive divergence generates distinct plastic responses in two closely related
672 Senecio species. bioRxiv.
673 Walter, G. M., M. J. Wilkinson, M. E. James, T. J. Richards, J. D. Aguirre, and D. Ortiz-
674 Barrientos. 2016. Diversification across a heterogeneous landscape. Evolution 70:1979-
675 1992.
676 Wang, S. P., and D. M. Althoff. 2019. Phenotypic plasticity facilitates initial colonization of a
677 novel environment. Evolution 73:303-316.

678

679 **Acknowledgements:** We thank J.D. Aguirre, S. Barrett, R. Dugand and A. Hoffmann for
680 comments on previous versions. We are very grateful to those who helped with fieldwork: A.
681 Barbato, O. Brayley, G. Burstein, M. Castrogiovanni, S. du Plessis, C. Impelluso, E. la Spina,
682 M.G. Majorana, J. Menzies, M. Millen, G. Pepe and D. Ward, and to Piante Faro for providing
683 glasshouse resources. This work was carried out using the computational facilities of the
684 Advanced Computing Research Centre, University of Bristol. **Funding:** This work was
685 supported by joint NERC grants NE/P001793/1 and NE/P002145/1 awarded to JB and SH.
686 **Author contributions:** GW, SH and JB designed the experiment with input from SC and AC.
687 GW, AC and DT conducted the experiments. JC collected, extracted and processed RNA
688 samples, and analyzed the transcriptome data. GW analyzed the data, and wrote the manuscript
689 with help from JC, JB and SH, and input from all other authors.