Hidden genetic variation in plasticity provides the potential for rapid adaptation to novel environments

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ABSTRACT

If genetic variation in fitness emerges in novel environments, then rapid adaptation can overcome fitness declines and allow population persistence. Although plasticity is expected to underlie such genetic variance in fitness, this has not been tested empirically. We transplanted cuttings of a Sicilian daisy (*Senecio,* Asteraceae) within and outside its native range, and quantified genetic variation in fitness, which we connect to plasticity in leaf traits and gene expression. Compared to native environments, mean fitness in the novel environment declined by 87%, but was associated with a threefold increase in genetic variation. Fitness in the novel environment was genetically correlated with plasticity: genotypes with greater fitness showed higher plasticity in leaf form, lower plasticity in leaf physiology and more gene underexpression. Surprisingly, genotypes with greater fitness in the novel environment had the lowest fitness at the native site. Therefore, genetic variance in plasticity increases the potential for rapid adaptation to novel environments, but adaptive plasticity and stabilising selection in native (non-stressful) environments hides such genetic variation in natural populations.

INTRODUCTION

Understanding how adaptation and phenotypic plasticity contribute to population resilience in novel environments is vital for predicting the response of natural populations to environmental change (Parmesan 2006; Shaw & Etterson 2012). Populations persist in novel environments either by genotypes adjusting their phenotype to match the changed conditions (adaptive plasticity) (Via *et al.* 1995; Charmantier *et al.* 2008), or by changes in the frequency of alleles that increase fitness in novel environments and promote adaptation (termed 'evolutionary rescue') (Gomulkiewicz & Holt 1995; Bell & Gonzalez 2009). However, if evolutionary rescue relies on alleles that increase fitness via beneficial plastic responses, then selection on plasticity will be important for adaptation to novel environments (Lande 2009; Chevin & Lande 2011; Chevin & Hoffmann 2017; Kelly 2019).

Given that plasticity should only evolve to buffer environmental variation encountered within the native range of a species, existing plasticity should only maintain fitness in previously experienced conditions (Chevin *et al.* 2013). Such adaptive plasticity should reduce genetic variation in fitness because all genotypes successfully track familiar environments (Bradshaw 1991). As adaptive plasticity becomes less effective in more novel environments, genetic variation in fitness is expected to increase as genotypes vary more in their (previously untested) sensitivity to the new conditions (Hermisson & Wagner 2004; Lande 2009; Chevin *et al.* 2010; Chevin & Lande 2011; Ashander *et al.* 2016). Such an exposure of genetic variation in novel environments could allow rapid evolutionary rescue (Nussey *et al.* 2005; Lande 2009; Agashe *et al.* 2011), and would suggest that estimates of genetic variation in fitness within a species' current geographical range will underestimate the potential for evolutionary rescue in novel environments.

Populations are likely to experience reduced mean absolute fitness ($\overline{W}$) in novel environments, which will lead to extinction if they cannot increase $\overline{W}$ sufficiently quickly to recover. The potential to increase mean fitness in the subsequent generation is determined by the ratio of additive genetic variance in fitness $V_A(W)$ to mean fitness ($\frac{V_A(W)}{\overline{W}}$) (Fisher 1930; Bonnet *et al.* 2019; Kulbaba *et al.* 2019), which therefore determines the potential for evolutionary rescue (Shaw 2018; Sheth *et al.* 2018). Increased genetic variance improves the chance of adapting to a new environment because alleles that are beneficial in the novel environment can rapidly increase mean fitness. Evidence for increased additive genetic variation in fitness under novel conditions is scarce, rarely linked to plasticity and mostly derived from laboratory rather than field experiments. We therefore have a remarkably limited understanding of whether genetic variation in fitness increases in novel environments, or the role of plasticity in creating any increases in genetic variation in fitness. Without such information, the patterns and rates of evolutionary rescue that we can expect in novel environments remain largely unknown (Shaw 2018).

We focus on a Sicilian daisy, *Senecio chrysanthemifolius* (Asteraceae), that grows in disturbed habitats at low elevation (c.400-1,000m.a.s.l [metres above sea level]) on Mount Etna (an active volcano), and throughout lowland Sicily (**Fig. 2A**). This species occurs in small patches typically containing fewer than 100 individuals, and patches are often separated by 1-2km. Individuals are occasionally observed at elevations between 1,000m and 1,500m, suggesting that these higher elevations represent the edge of the range for *S. chrysanthemifolius*. In natural populations, individuals of *S. chrysanthemifolius* typically live for less than two years and are obligate outcrossers that are pollinated by generalist insects (e.g. hoverflies). Also found on Mt. Etna is a high-elevation sister species, *S. aethnensis*, that is endemic to lava flows above 2,000m.a.s.l.

83 Adaptive divergence between these two *Senecio* species is strongly associated with contrasting
84 leaf morphology and distinct patterns of plasticity, suggesting that leaf traits are under divergent
85 selection (Walter *et al.* 2020b; Walter *et al.* 2021a; Walter *et al.* 2021b).

86 We collected cuttings from 72 naturally-occurring *S. chrysanthemifolius* genotypes at five sites
87 on the south-east slopes of Mt. Etna (**Table S1**, **Fig. S1**). Sites were located <5km apart, which
88 means that gene flow could routinely occur between them. Following propagation in the
89 greenhouse, we conducted crosses among the genotypes in a paternal half-sibling breeding
90 design (Lynch & Walsh 1998) to produce 314 offspring genotypes (**Fig. 2B**). We then
91 transplanted multiple cuttings (clones) of each genotype at three elevations and quantified
92 fitness, morphology, physiology and gene expression of each genotype in their native
93 environment (500m.a.s.l), the edge of their range (1,500m) and a novel environment (2,000m;
94 **Fig. 2B**).

95 Using these data, we tested the potential for adaptation to a novel environment in the field with
96 three predictions: (1) In more familiar environments (500m and 1,500m), we would observe high
97 absolute mean fitness ($\overline{W}$) but low additive genetic variance in fitness ($\frac{V_A(W)}{\overline{W}}$) because all
98 genotypes can successfully track the environment. By contrast, at the novel elevation, absolute
99 mean fitness would be reduced and be associated with increased genetic variance in fitness that
100 could allow evolutionary rescue to the novel environment (**Fig. 1A**). (2) Strong genetic
101 correlations between trait plasticity and fitness in the novel environment would provide evidence
102 that particular forms of plasticity increase fitness in the novel environment (**Fig. 1B**). (3) Given
103 that changes in gene expression mediate plasticity, genotypes with greater fitness would show

104 distinct patterns of gene expression compared to genotypes with lower fitness, and help to
105 identify the genomic basis of plasticity (**Fig. 1C**).

106

107 **METHODS**

108 ***Genotype sampling and crossing design***

109 To establish the parental generation, in June 2017 we collected cuttings from 72 individuals
110 located at five geographically separate sites on the foothills of Mt. Etna (**Table S1** and **Fig. S1**).
111 Where possible, we sampled individuals that were at least ten meters apart from each other to
112 minimize chances of sampling close relatives. We removed whole branches from mature plants,
113 which we cut into 4-5cm segments at the glasshouse (Giarre, Italy), dipped them in a rooting
114 plant growth regulator for softwood cuttings (Germon® Bew., Der. NAA 0.5%, L. Gobbi, Italy)
115 and placed each cutting in one cell of an 84-cell tray containing a compressed mix of 1:1 perlite
116 and coconut coir. For three weeks, we kept cuttings in plastic tunnels to maintain humidity and
117 encourage root growth. We then placed one cutting per individual in a 30cm diameter pot with
118 standard potting mix, which we watered regularly. To encourage growth, we suspended 25W
119 LED tubes (TSA Technology, Italy) 1m above the bench. Once plants produced buds, we
120 covered flowering branches with perforated bread bags to prevent pollinators from entering
121 while allowing airflow. We randomly designated each individual as a dam or sire and grouped
122 them into 12 blocks, each containing three sires (n=36) and three dams (n=36), see **Table S1**.
123 Within each block we mated all sires to all dams to produce nine full-sibling families per block
124 (n = 104 total full-sibling families, with four crosses failing to produce seeds).

125 Six seeds from each family were germinated by cutting the top off each seed (<1mm) and
126 placing them on moistened filter paper in a plastic Petri dish. Petri dishes were kept in the dark
127 for two days, and then transferred to growth cabinets maintained at 22°C with a 12h:12h
128 light:dark photoperiod. After one-week we transferred seedlings to the glasshouse where three
129 individuals from each family were grown in 14cm diameter pots containing standard potting mix
130 (*n*=312 individuals). When the main stem of each seedling reached c.12cm of growth, we cut the
131 main stem 4cm above ground level to promote lateral branching and generate enough cuttings for
132 the field transplant.

133 ***Field transplant of the breeding design as cuttings***

134 When branches started producing buds, we took cuttings from all 312 individuals (hereafter,
135 genotypes). Branch were cut into smaller segments each 4-5cm long with 2-3 leaf nodes. For
136 almost all genotypes we were able to take 21 cuttings (transplant 1). Each cutting was treated as
137 described above and left for two weeks to produce roots. To produce a second round of cuttings,
138 all plants were left to regrow for three weeks, at which point we repeated the process to generate
139 another 14 cuttings per plant (transplant 2). Due to the deaths of two genotypes in the glasshouse
140 after taking the initial cuttings, we replaced these genotypes with siblings for the second round of
141 cuttings, which led to two extra genotypes (*n*=314 rather than 312). Cuttings were stored in high-
142 humidity tunnels for two weeks until they produced roots and were ready to transplant.

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

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

154 In total, we transplanted 7-10 cuttings from each of the 314 genotypes at each of three transplant
155 sites along an elevational gradient (n=c.2,700 cuttings/site; total N=8,149 cuttings) that
156 represented typical conditions within the species' native range (500m.a.s.l), the edge of its range
157 (1,500m.a.s.l), and a novel elevation (2,000m.a.s.l). The 500m site was located on abandoned
158 land, the 1,500m site in an apple and pear orchard, and the 2,000m site on a lava flow from
159 c.1980. Elevations under 1,000m are exposed to high temperatures that regularly exceed 40°C
160 during summer, and above 1,000m the temperature regularly drops below 0°C during winter
161 (Walter *et al.* 2020b).

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

168 ***Data collection***

169 Mortality of cuttings transplanted into the field was low, even at the novel elevation (12% across

170 all elevations), allowing us to assay fitness for 6-8 cuttings per genotype at each transplant site.
171 In September-October, after 5 months of growth, we estimated fitness by counting all flower
172 heads produced by each plant. This trait has been used routinely to assay fitness in short-lived
173 perennials (e.g. ref Pujol *et al.* 2014) and was closely associated with the total seeds produced
174 per plant in a previous transplant experiment (see **Methods S1**).

175 We measured leaf morphology and leaf pigment content by sampling 3-4 young, but fully
176 expanded leaves from each plant. We scanned the leaves and quantified morphology using the
177 morphometric software '*Lamina*' (Bylesjo *et al.* 2008), from which we analyzed four leaf traits:
178 leaf area, leaf complexity ($\frac{leaf\ perimeter^2}{leaf\ area}$), the number of leaf indents standardized by the
179 perimeter and Specific Leaf Area ($SLA = \frac{leaf\ area}{leaf\ weight}$). These leaf measurements represent leaf
180 morphology and investment traits that show plastic responses to the abiotic environment in *S.*
181 *chrysanthemifolius* (Walter *et al.* 2020b; Walter *et al.* 2021b), and in other plant systems,
182 including sunflowers (Royer *et al.* 2009). We also used a Dualex instrument (Force-A, France) to
183 measure the flavonol pigment content of the leaf. Flavonols are secondary metabolites that
184 combat oxidative stress created by stressful abiotic (e.g. light and temperature) and biotic (e.g.
185 herbivore) conditions (Mierziak *et al.* 2014).

186 ***Bayesian implementation of statistical tests***

187 We used Bayesian models to estimate genetic variance in fitness, and to estimate genetic
188 correlations between plasticity and fitness at 2,000m. We included a substantial burn-in and
189 thinning interval to allow model convergence. We confirmed that effective sample sizes
190 exceeded 85% of the number of samples that were saved. We used uninformative parameter-
191 expanded priors, which we checked by changing the scale parameter and ensuring that there was

192 no effect on the posterior distribution. We applied each equation using 2 million iterations, a
193 burn-in of 200,000 iterations and thinning interval of 1,000 iterations.

194 ***Quantifying genetic variance in fitness***

195 To quantify genetic variance in fitness, we used *MCMCglmm* (Hadfield 2010) within R (v3.6.1;
196 R Core Team 2019) to implement the generalized linear mixed effects model

197 $$y_{ijklmn} = T_i + s_{j(k)} + d_{k(j)} + g_{l(jk)} + b_{m(i)} + e_{n(ijklm)}\,, \qquad (1)$$

198 where the only fixed effect was transplant elevation (T_i). The random effects $s_{j(k)}$ represented
199 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
200 dam and sire. For the sire, dam and genotype components, we estimated both random slopes and
201 intercepts for transplant elevation, which calculates a 3×3 covariance matrix containing the
202 variance among genotypes within each transplant elevation, and the genotypic covariance among
203 transplant sites. Additional random effects included block within transplant site ($b_{m(i)}$), which
204 removed variance due to differences between the two transplants and between the experimental
205 blocks. $e_{n(ijklm)}$ represented the error variance. Fitness was included as a univariate, poisson-
206 distributed response variable (y_{ijklmn}), which meant that our estimates of variance represent
207 variance in relative fitness (Bonnet *et al.* 2019).

208 ***Quantifying genetic variance in plasticity***

209 In the subsequent analyses, for all traits we calculated the average across all leaves sampled from
210 each plant. We calculated plastic changes in phenotype across elevation by standardizing all
211 clones of each genotype at 2,000m by the mean value of that genotype at the home site (500m).
212 This standardization calculated the trait values for each genotype at the novel elevation, relative
213 to their trait value at the home site (see **Fig. 4**). We estimated plasticity separately for each

214 transplant date. To quantify the genetic correlation between phenotypic plasticity and fitness, we
215 used equation 1, but only for the data collected at 2,000m and with all five leaf traits and fitness
216 (number of flowers, a poisson-distributed trait) as the multivariate response variable (y_{ijklm}).
217 This calculates the covariance between plasticity in each trait and fitness at 2,000m. We
218 extracted the sire component (representing additive genetic variance) and calculated the genetic
219 correlations between each trait and fitness.

220 ***Gene expression analyses***

221 To compare gene expression variation across elevation, we chose two sets of genotypes from the
222 crossing design that showed differences in fitness at the novel elevation (2,000m). The genotypes
223 that showed the greatest fitness outside the home range represent genotypes that could promote
224 evolutionary rescue ('*ER*' genotypes) and the genotypes with the lowest fitness outside the range
225 represent genotypes more specialized to conditions with their native range ('*HR*' genotypes for
226 'Home Range'). Because our collection of young leaves for RNA samples had to be completed
227 before the winter snow arrived, we were restricted to choosing genotypes before the flowers of
228 all cuttings were counted. After flowers were counted for two clones of each genotype at each
229 transplant site, we estimated genetic variation for fitness as the among-genotype variance (i.e.
230 among the individuals in the crossing design) at each transplant site, and the covariance between
231 transplants sites (see equation 1). We then chose the 15 genotypes with the highest (*ER*
232 genotypes) and the 15 genotypes with the lowest (*HR* genotypes) relative fitness at 2,000m.

233 For the *ER* and *HR* genotypes chosen for gene expression analyses, we sampled leaves from
234 three clones of each genotype chosen at random at each transplant site. Plants were sampled
235 when they were flowering, but were still growing vegetatively. From each plant, we sampled 3-4
236 young leaves (c.15mm long), which we immediately submerged in RNAlater and stored at 4°C

237 for 24 hours and then at -80°C before RNA extraction. To control for variation due to weather
238 and diurnal effects, sampling was constrained to three consecutive days (19-21 November)
239 between 9am and 11am under similar weather conditions.

240 After fitness was quantified for all cuttings, we repeated the analysis for choosing the genotypes
241 (this time with all the fitness data included), and from the sampled genotypes we chose the six
242 *ER* genotypes and six *HR* genotypes for gene expression analysis that maintained the strongest
243 fitness differences at 2,000m (**Fig. S2A**). Importantly, the chosen genotypes also represented
244 changes in additive genetic variance in relative fitness, whereby sires of the chosen genotypes
245 exhibited similar fitness responses to elevation (**Fig. S2B**).

246 We extracted RNA for the leaves of each plant and quantified gene expression at each elevation
247 using 3' RNAseq (QuantSeq). See **Methods S2** for RNA extraction protocols and the reference
248 transcriptome assembly. 3' reads were mapped to the reference transcriptome using Salmon
249 v1.1.0 (Patro *et al.* 2017). 72.6-87.3% of reads were mapped. Transcript abundance estimates
250 were imported into R using *txImport* (Soneson *et al.* 2015), with estimates normalized according
251 to library size but not by transcript length, as the 3' sequencing method removes this bias. We
252 visualized the broad patterns of variation in gene expression by first normalizing transcript
253 abundance using the variance stabilizing transformation function with *DESeq2* (Love *et al.* 2014)
254 followed by a principal components analysis **(Fig. S3**). We then calculated differential
255 expression of transcripts using non-normalized transcript counts between transplant sites for each
256 set of genotypes (*ER* and *HR*) separately using *DESeq2*. Differentially expressed genes were
257 defined as those showing both significant (adjusted p-value <0.01) and strong (log2-fold change
258 >2 or <-2) changes in expression. We then tested for enrichment of gene ontology (GO) terms

259 for differentially expressed genes using both a Kolmogorov-Smirnoff test and Fisher's exact test
260 using *topGO* (Alexa & Rahnenführer 2019).

261

262 **RESULTS**

263 ***(1) Reduced mean fitness but increased genetic variance in fitness in the novel environment***
264 Consistent with our first prediction (**Fig. 1A**), absolute mean fitness (number of flowers) was
265 consistently high within the native range (500m and 1,500m), but decreased by 87% at 2,000m
266 (**Fig. 3A**). Also consistent with our first prediction, additive genetic variance in relative fitness
267 ($\frac{V_A(W)}{\overline{W}}$) was near zero within the native range (500m and 1,500m), but more than three times
268 greater at 2,000m, which predicted a 13.8% increase in mean fitness in the subsequent generation
269 (**Fig. 3B**; **Table S2**). Near zero genetic variance in fitness within the native range (500m and
270 1,500m) suggests that all genotypes could track the phenotypic optima to maintain high mean
271 fitness in familiar environments. By contrast, genotypes at the novel elevation fail (to differing
272 extents) to maintain high fitness, increasing genetic variation in fitness, and therefore improving
273 the potential for evolutionary rescue in the unfamiliar environment (Shaw 2018).

274 We also found that genotypes with greater fitness at the novel elevation showed lower fitness at
275 the native site (**Fig. 3C**), as indicated by a weak negative genetic correlation of -0.1 (-0.79, 0.60
276 95% Highest Posterior Density interval [HPD]) for fitness between the native site and the novel
277 elevation. This suggests that genotypes that could promote adaptation to the novel elevation (i.e.
278 those with the greatest fitness at 2,000m) have lower fitness relative to other genotypes within
279 the native range. Seven of the nine sires with the highest fitness in the novel environment were

collected from two out of five sites (**Table S3**), suggesting that genetic variation from particular sites will be important for evolutionary rescue.

(2) Genetic variance in fitness in the novel environment correlates closely with plasticity

Identifying the ecological importance of leaf traits: We used multiple regression to estimate phenotypic selection gradients based on partial regression coefficients of all traits on fitness. We observed a significant association between all leaf traits and fitness at each elevation (**Table S4, Fig. S4**, **Methods S3**), suggesting that these leaf traits are associated with maintaining fitness at each elevation. In support of other transplant experiments that measured the same leaf traits (Walter *et al.* 2020b; Walter *et al.* 2021b), we found evidence of plasticity as large changes in leaf traits across elevation (**Fig. 4B**) associated with a large and significant change in multivariate phenotype with elevation (MANOVA $F_{2,23} = 44.521$, $P<0.0001$; **Methods S3**). These results provide strong evidence that these traits are ecologically important and show high levels of plasticity across elevations.

Genetic correlations between leaf plasticity and fitness: If plasticity can promote evolutionary rescue in novel environments, we should observe strong genetic correlations between trait plasticity and fitness in the novel environment (see **Fig. 1B**; Lande 2009; Chevin & Lande 2011). We quantified plasticity separately for each of the 314 genotypes by dividing the trait values of each genotype at 1,500m and 2,000m by the mean trait value of that genotype at the home site. This standardization calculates the trait values at 1,500m and 2,000m relative to the home site for each genotype. Values of 1 reflect no change in phenotype between sites, while values above and below 1 respectively reflect increases and decreases in trait values from the home site (**Fig. 4B**).

302 We found that plasticity reduced leaf area, leaf complexity and flavonol content similarly at the
303 edge of the range (500-1,500m) and the novel elevation (500-2,000m). The number of indents
304 and SLA showed a contrasting response to elevation whereby trait values increased to the edge
305 of the range, but then decreased at the novel elevation. Only leaf area showed a greater
306 magnitude of plastic change in phenotype at 2,000m compared to 1,500m (**Fig. 4B**).

307 We estimated the genetic correlation between plasticity for all traits and fitness at the novel
308 2,000m elevation. We found strong and significant genetic correlations between plasticity and
309 fitness at the novel elevation (>90% of the posterior distribution did not overlap zero) for two
310 traits: SLA (0.51; 0.09, 0.94 90 % HPD) and flavonol content (0.47; 0.02, 0.97 HPD) (**Fig. 5A**).
311 This means that the increase in genetic variance in fitness at the novel elevation was genetically
312 correlated with plasticity for these two leaf traits, and so could promote evolutionary rescue at
313 the novel elevation. We also detected moderately strong genetic correlations between plasticity
314 and fitness at 2,000m for the remaining traits: leaf area (0.38; -0.18, 0.97 HPD), leaf complexity
315 (-0.24; -0.86, 0.30 HPD) and the number of indents (-0.38; -0.93, 0.23 HPD) (**Fig. 5A**).
316 However, the uncertainty for these traits overlapped zero more substantially and leaf area and
317 number of indents were only marginally significant (no overlap with zero at 80% HPD).

318 Depending on the trait, genetic variation in fitness was associated with different patterns of
319 plasticity, which were represented by increases or decreases in the magnitude of plastic
320 responses. For leaf complexity and the number of indents, genotypes with high fitness at 2,000m
321 show greater plasticity than low fitness genotypes, created by stronger reductions in trait values
322 between 500m and 2,000m (**Fig. 5B-C)**. By contrast, for leaf area, SLA and flavonol content,
323 genotypes with high fitness at 2,000m show less plasticity than the low fitness genotypes (**Fig.**
324 **5B-C**).

325 ***(3) Plasticity in gene expression is associated with fitness in the novel environment***

326 Overall, a larger number of genes were differentially expressed with increasing distance from the
327 home site, suggesting that plasticity under novel conditions invokes broad transcriptional
328 responses involving loci across the genome. *ER* genotypes (high fitness 'evolutionary rescue'
329 genotypes) showed significant changes (adj. $P<0.01$) in more genes than the low fitness *HR*
330 genotypes (1,376 in *ER* vs 514 genes in *HR*) within the range (500m-1,500m), suggesting that
331 the *HR* genotypes adjusted fewer genes to maintain fitness within their range (**Fig. 6A**).
332 However, the number of genes that showed expression changes tripled outside the native range
333 (500m-2,000m) for both classes of genotypes (*ER*=4,876 genes vs *HR*=4,720) with the *ER*
334 genotypes again showing a greater magnitude (1.2-fold mean difference) of differential
335 expression per gene than *HR* genotypes (**Fig. 6B**). For both classes of genotypes, the mean
336 magnitude of expression change was greater at the novel elevation (*ER*=1.41, *HR*=1.21) when
337 compared to the edge of the range (*ER*=1.19, *HR*=1.04). Compared to the *HR* genotypes, *ER*
338 genotypes showed twice as many underexpressed genes at the novel elevation, but half as many
339 overexpressed genes (**Fig. 6C**). Therefore, as predicted (**Fig. 1C**), distinct patterns of gene
340 expression between novel and native environments were associated with genotypic differences in
341 fitness at the novel elevation.

342 *Gene ontology: ER* and *HR* genotypes differed in the functional categories of genes that varied in
343 expression at the novel elevation (**Fig. 6D**). *ER* genotypes showed differential expression of
344 genes relating to biosynthesis, while differentially expressed genes in the *HR* genotypes were
345 related to photosystems. Comparing the enriched GO terms to those of a 2017 transplant
346 experiment (Walter *et al.* 2020b) that included the high elevation sister species (*S. aethnensis*),
347 we found that four (of 17) GO terms were enriched in *S.aethnensis* as well as in the *ER*

genotypes, compared to only one (of 20) GO term shared by *S. aethnensis* and the *HR* genotypes (**Fig. 6D**). Therefore, the high fitness genotypes of *S. chrysanthemifolius* also showed a more similar gene expression response to the closely-related *Senecio* species native to that elevation.

GO terms that were significantly enriched at 2,000m included light harvesting in photosystem I, cell wall organization, and responses to cold (**Table S5**). To illustrate the contrasting patterns of expression between *ER* and *HR* genotypes, we selected the five most differentially expressed genes from each GO term and compared the reaction of these genotypes across elevation. For genes involved in photosystem I (**Fig. 6E**), *ER* genotypes showed significantly stronger patterns of underexpression than *HR* genotypes at the novel elevation. For genes involved in cold responses **(Fig. 6F)**, *ER* genotypes showed a stronger response than *HR* genotypes, which included greater overexpression or underexpression depending on the gene.

DISCUSSION

We provide strong evidence that hidden genetic variation segregating in natural populations increases the potential to adapt to novel environments. Previous studies have shown heritable responses in plasticity (Nussey *et al.* 2005), adaptive plasticity in phenology (Charmantier *et al.* 2008) and important range shifts (Parmesan 2006) that allow populations to track environmental change, however, to our knowledge, this study provides the first experimental evidence under natural conditions showing how adaptation to novel environments can occur via selection on different forms of plasticity.

Our results reveal that when the limits of adaptive plasticity are reached in novel environments, populations suffer reduced mean fitness (**Fig. 3A**). At the same time however, genetic variation

370 in fitness increases (**Fig. 3B**), some of which could promote evolutionary rescue by allowing
371 rapid increases in the frequency of beneficial alleles. Interestingly, the genotypes that could
372 increase mean fitness in novel environments showed lower fitness in the native environment
373 (**Fig. 3C**). Genotypes important for evolutionary rescue may therefore be selected against within
374 the native range, meaning that genetic variation important for evolutionary rescue at ecological
375 margins may only exist at low frequency throughout much of a species' range (Brennan *et al.*
376 2019). Studies estimating genetic variation in fitness *in situ* are therefore likely to underestimate
377 the potential for evolutionary rescue because the fitness effects of alleles (and their importance
378 for evolutionary rescue) are only seen in novel environments (Sheth *et al.* 2018; Kulbaba *et al.*
379 2019). Genotypes with greater fitness at the novel elevation showed greater plasticity that
380 reduced the trait values of leaf complexity and leaf indentation, but lower plasticity that
381 maintained the trait values of leaf area, specific leaf area and flavonol content from the home site
382 to the novel elevation (**Fig. 5**). These results support the theory that selection for particular forms
383 of plasticity can promote evolutionary rescue (Lande 2009; Chevin & Hoffmann 2017).

384 Gene expression responses are determined through genetic and epigenetic regulation, which are
385 likely to determine plasticity in phenotype, and how such plasticity will evolve to increase
386 population persistence in novel environments (Oostra *et al.* 2018). Compared to the edge of the
387 range, the novel elevation induced stronger gene expression changes at similar loci as well as
388 inducing differential gene expression at more loci across the genome (**Fig. 6A-B**). In contrast
389 with the low fitness *HR* genotypes at 2,000m, genotypes with high fitness (*ER*) differentially
390 expressed more genes, which was associated with many genes exhibiting stronger
391 underexpression (**Fig. 6C-F**). Furthermore, differential expression in the high fitness genotypes
392 occurred in functional categories associated with plasticity in the closely related high elevation

393 (2,000m+) sister species. These results suggest that adaptation to novel environments requires
394 genotypes that create the most beneficial gene expression profiles (Wang & Althoff 2019;
395 Josephs 2021). This finding contrasts with evidence that adaptation to novel environments
396 involves non-adaptive plasticity in gene expression (Ghalambor *et al.* 2015), or that a lack of
397 genetic variance in gene expression will prevent beneficial responses to novel environments
398 (Oostra *et al.* 2018).

399 Our results suggest that genetic variation segregating in natural populations can promote
400 adaptation to novel environments. However, the likelihood of evolutionary rescue will depend
401 heavily on demographic parameters of the population, as well as the genetic load that is
402 associated with any increased genetic variation in fitness (Chevin *et al.* 2013; Gonzalez *et al.*
403 2013; Polechová & Barton 2015). In particular, if a population experiences a particularly severe
404 decline in mean fitness (and therefore decline in density), evolutionary rescue will become less
405 likely even if beneficial genetic variation is available to selection. This is because the number of
406 individuals in the population will likely fall to the point where extinction is more likely than
407 adaptation (Bridle & Vines 2007; Chevin *et al.* 2013; Carlson *et al.* 2014). Future work needs to
408 identify the ecological and life history parameters within which population declines are not so
409 strong as to prevent evolutionary rescue, even though (as we show here) a population's adaptive
410 potential increases in novel environments.

411 Overall, our results provide strong evidence that evolutionary rescue depends on genetic
412 variation that increases fitness by selecting for the most beneficial plastic responses in novel
413 environments (Lande 2009; Chevin & Lande 2011; Chevin & Hoffmann 2017), created by a
414 fine-tuning of gene-products and trait values in novel environments. Our results also support
415 studies that show that genetic variance in fitness depends on the environment in which it is

quantified (Sheth *et al.* 2018; Kulbaba *et al.* 2019), and shows for the first time that genotypes suitable for adaptation at range margins may be under negative selection in native environments. Genetic variation for sensitivity to the environment is likely to be unevenly distributed across a species' range (Stratton 1994; Colautti & Barrett 2013; Sheth & Angert 2016; Walter *et al.* 2020a), with local adaptation also likely to determine which populations contain the genetic variation that can increase the potential for evolutionary rescue (Hargreaves & Eckert 2019). To quantify rates of adaptation to novel environmental regimes, research should therefore estimate genetic variation in environmental sensitivity for multiple populations exposed to a range of novel environments. It is also important to determine how and when the expression of genetic variation likely to cause evolutionary rescue is masked by existing environmental conditions.

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materials availability: Upon acceptance, data will be deposited with the Environmental Information Data Centre (UK) and RNA sequences will be deposited with the Short Reads Archive.

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613

FIGURES

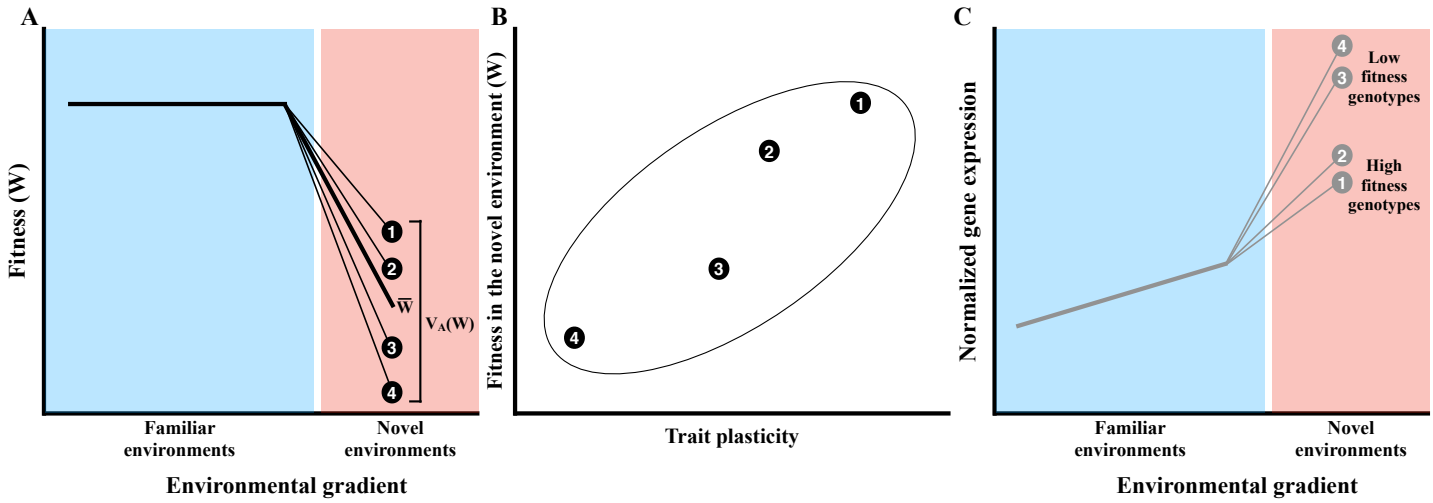

Fig. 1 (A) Conceptual diagram depicting the predicted change for fitness (W) in response to familiar (blue) and novel (red) environments. The thick line represents the change in mean fitness across environments, and the thin lines with circles represent changes in four different genotypes (represented by different letters) within the population. If genotypes can effectively track their native environment, we would expect low genetic variance for fitness within the native range to be associated with high mean fitness. This is shown as a high mean fitness in the familiar environments, associated with no differences among genotypes. However, in novel environments, we expect a reduction in mean fitness associated with an increase in genetic variance, where differences among genotypes emerge and provide the potential for evolutionary rescue. If genetic differences in plasticity underlie the increase in genetic variation in fitness in the novel environment, we can make two predictions: **(B)** We would expect plasticity to be genetically correlated with genetic variation in fitness. Compared to the genotypes with lower fitness (3-4), the genotypes with greater fitness (1-2) at the novel environment are also associated with different levels of plasticity in a given trait. This creates a moderately strong genetic

correlation between trait plasticity and fitness. **(C)** We would also expect that high (1-2) and low (3-4) fitness genotypes to show differences in their patterns of gene expression. Gray lines represent the gene expression profiles for a single gene (as an example) where gene products are similar for high and low fitness genotypes within the native range, but produce different levels of the gene product in the novel environment. Empirical support for these predictions would provide strong evidence that genotypes with particular plastic responses help to maintain fitness in novel environments and increase the potential for evolutionary rescue.

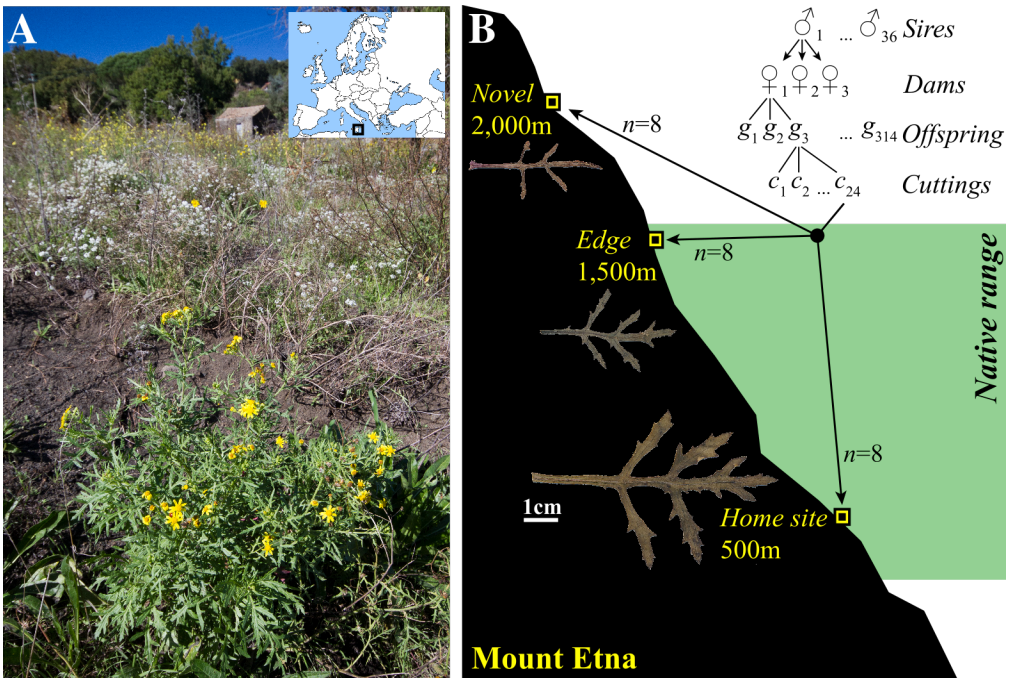

Fig. 2. The system and experimental design. (**A**) *Senecio chrysanthemifolius* in its natural habitat. Inset map presents the location of the study system in relation to Europe. **(B)** Schematic of Mount Etna showing the experimental design. We sampled individuals from five sites in the foothills of Mount Etna, which we crossed in the glasshouse and transplanted cuttings of their offspring at three elevations. We mated 36 sires to 36 dams in 12 blocks of 3×3 (presented in the figure as a nested design for simplicity). Each sire was therefore mated to three dams; we grew three offspring per cross in the glasshouse from which we sampled cuttings that were transplanted at the three elevations. Green shading denotes the native range of *S. chrysanthemifolius* on Mt. Etna and inset leaves show the average change in leaf morphology with elevation observed for a single genotype.

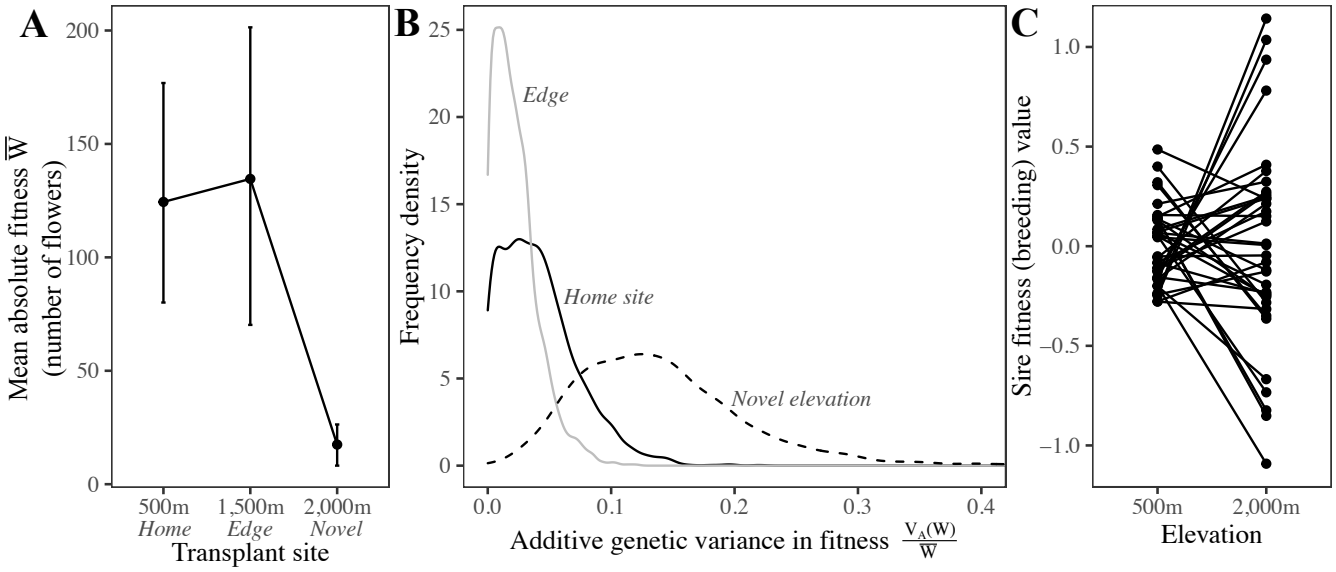

Fig. 3. Absolute mean fitness ($\overline{W}$) was lower at the novel elevation, and this was associated with an increase in additive genetic variance in fitness ($\frac{V_A(W)}{\overline{W}}$). (**A**) Mean absolute fitness dropped outside the native range. Credible intervals represent 95% Highest Posterior Density (HPD) intervals of the mean. (**B**) Posterior distributions for the estimates of additive genetic variance in fitness. Genetic variance in fitness was significantly greater at the novel elevation (2,000m) where distributions did not overlap at 95% HPD. (**C**) A negative genetic correlation in fitness between the native site (500m) and novel elevation (2,000m) of -0.1 (-0.79, 0.60 HPD; see **Table S2**) suggests that genotypes that perform best outside the range perform relatively poorly within the range. This is visualized using the breeding values of the sires, which show that the reaction norms of the two genotypes cross, creating a genetic trade-off in fitness between the native site and the novel elevation.

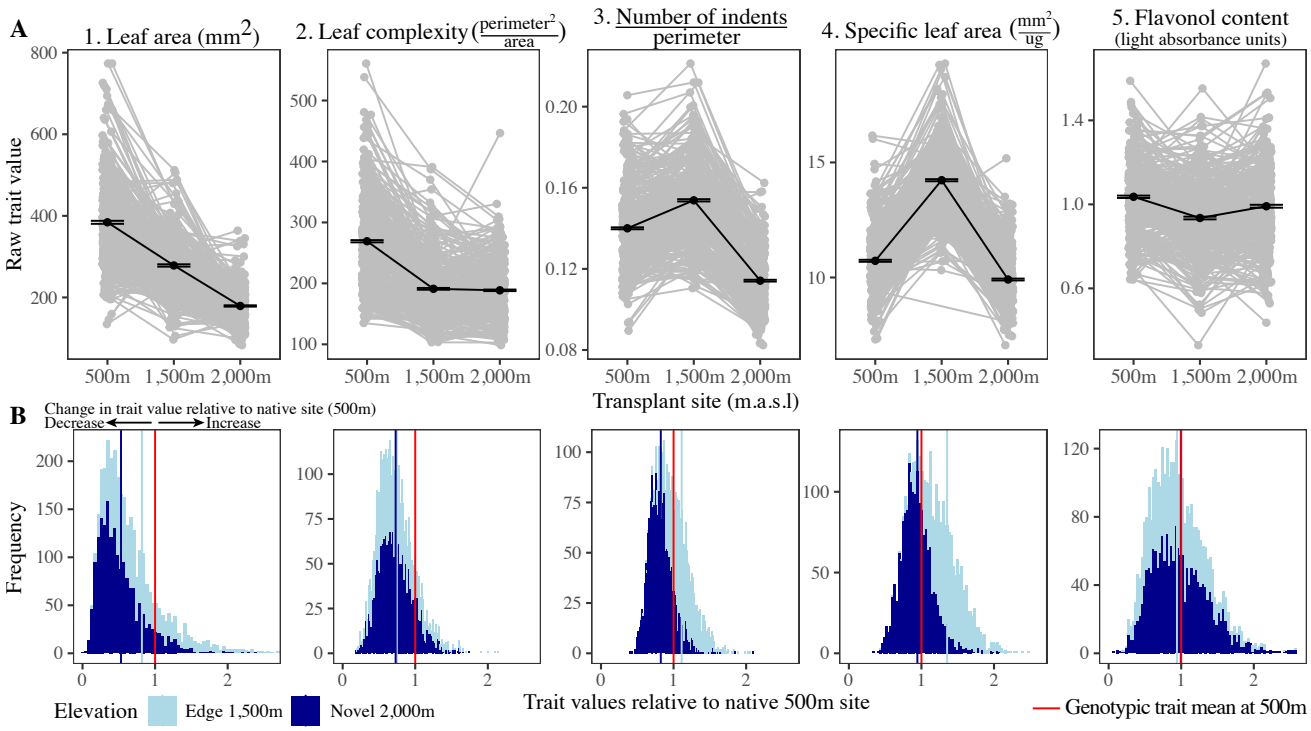

Fig. 4. When compared to the home site (500m), the magnitude of plasticity was greater at the novel elevation (2,000m) compared to the edge of the range (1,500m). (**A**) The change in raw trait values across elevation. Black lines and circles represent the overall mean (±1 SE), with all the genotypes 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.

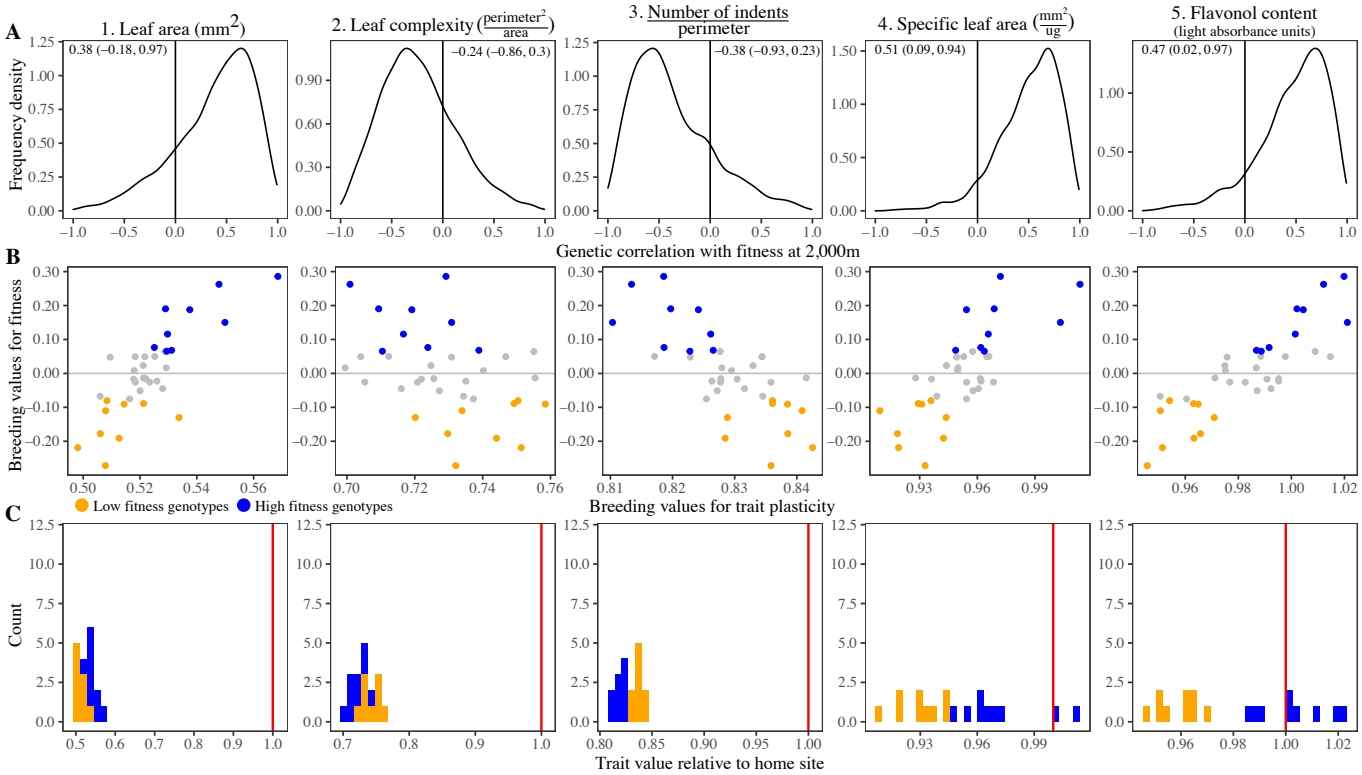

Fig. 5. Genetic variance in plasticity was significantly correlated with fitness at the novel elevation. (**A**) Genetic correlations between plasticity and fitness at 2,000m (with the posterior mean and 90% HPD intervals inset). Genetic correlations with fitness were strong and significant for specific leaf area and flavonols, but weaker for leaf area, leaf complexity and the number of indents. (**B**) Visualization of the genetic correlations (from **A**) using the sire genetic (i.e. breeding) values for plasticity (x-axis) versus fitness (y-axis). Genotypes with the greatest fitness (blue circles) show different levels of plasticity to genotypes with lowest fitness (orange circles). (**B**) Histogram for the sire genetic values for trait plasticity that represent the magnitude of plastic change in trait means from the home site (value of 1, represented by the red vertical line) to 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 leaf area, SLA and flavonol content.

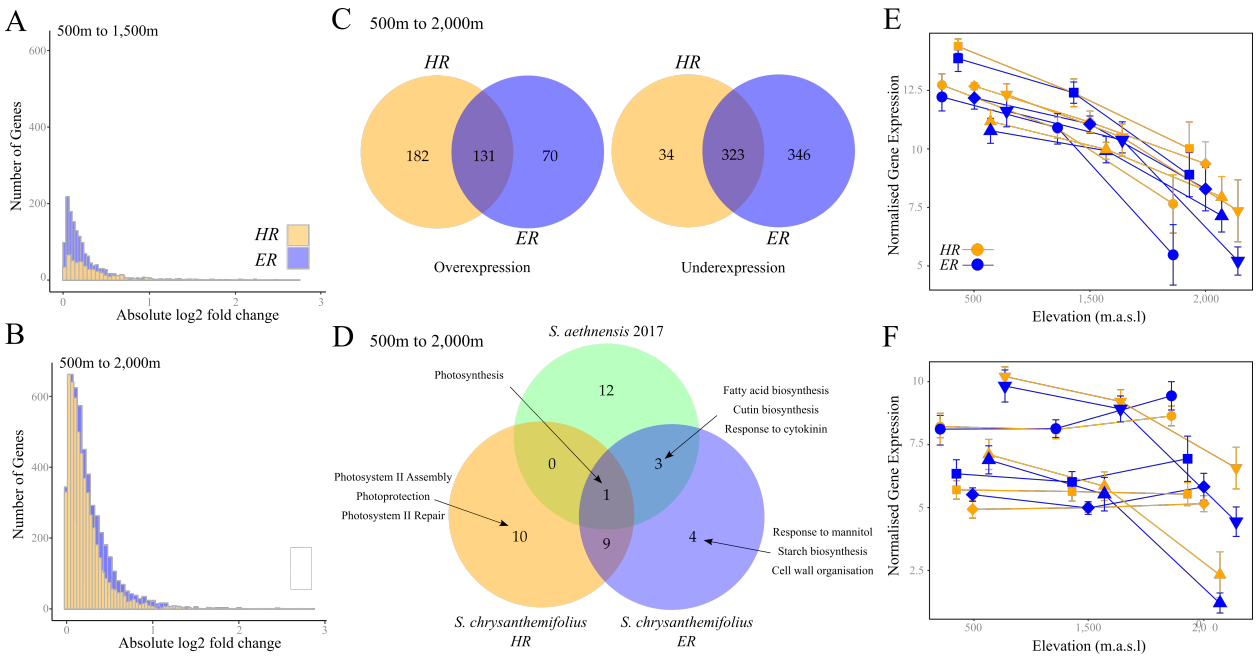

Fig. 6. *ER* and *HR* genotypes differ in gene expression at the novel elevation. **(A)** Within the native range, more genes were differentially expressed in the *ER* genotypes. (**B**) Outside the native range, more genes were differentially expressed, and more genes in the *ER* genotypes showed a 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%

701 confidence intervals. *ER* genotypes showed stronger patterns of underexpression at 2,000m. **(F)**
702 Elevational changes in mean expression for five genes associated with responses to cold. *ER*
703 genotypes show stronger under- and overexpression, but the pattern was gene-dependent.