New Phytologist

Research

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

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

[1]School of Biological Sciences, University of Bristol, Bristol, BS8 1TQ, UK; [2]Department of Biology, University of Oxford, Oxford, OX1 3RB, UK; [3]Department of Biological, Geological and Environmental Sciences, University of Catania, Catania 95128, Italy; [4]Department of Biology, University of Naples Federico II, Naples 80126, Italy; [5]Department of Genetics, Evolution and Environment, University College London, London, WC1E 6BT, UK; [6]Present address: School of Biological Sciences, Monash University, Melbourne, Vic. 3800, Australia

Author for correspondence:
Greg M. Walter
Email: greg.walter@monash.edu

Received: *19 July 2022*
Accepted: *2 January 2023*

New Phytologist (2023)
doi: 10.1111/nph.18744

Key words: adaptive plasticity, additive genetic variance, differential gene expression, environmental change, evolutionary rescue, fitness, novel environments, population persistence.

Summary

- Rapid environmental change is forcing populations into environments where plasticity will no longer maintain fitness. When populations are exposed to novel environments, evolutionary theory predicts that genetic variation in fitness will increase and should be associated with genetic differences in plasticity. If true, then genetic variation in plasticity can increase adaptive potential in novel environments, and population persistence via evolutionary rescue is more likely.
- To test whether genetic variation in fitness increases in novel environments and is associated with plasticity, we transplanted 8149 clones of 314 genotypes of a Sicilian daisy (*Senecio chrysanthemifolius*) within and outside its native range, and quantified genetic variation in fitness, and plasticity in leaf traits and gene expression.
- Although mean fitness declined by 87% in the novel environment, genetic variance in fitness increased threefold and was correlated with plasticity in leaf traits. High fitness genotypes showed greater plasticity in gene expression, but lower plasticity in most leaf traits. Interestingly, genotypes with the highest fitness in the novel environment had the lowest fitness at the native site.
- These results suggest that standing genetic variation in plasticity could help populations to persist and adapt to novel environments, despite remaining hidden in native environments.

Introduction

Understanding how populations and ecological communities will respond to rapid environmental change remains a fundamental challenge (Parmesan, 2006; Shaw & Etterson, 2012; Bridle & Hoffmann, 2022). Populations respond to new environments either by genotypes adjusting their phenotypes to match changing conditions (adaptive plasticity) (Via *et al.*, 1995; Charmantier *et al.*, 2008), or by increases in the frequency of beneficial alleles that increase fitness 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 genetic variation in plasticity will be critical for persistence in novel environments (Lande, 2009; Chevin & Lande, 2011; Chevin & Hoffmann, 2017; Kelly, 2019).

Given that plasticity can only evolve to match the environmental variation previously encountered within the native range of a species, existing plasticity should only maintain fitness across a species' current or historical range (Ghalambor *et al.*, 2007; Chevin *et al.*, 2013). Such adaptive plasticity is expected to reduce genetic variation in fitness because plasticity common to all genotypes can successfully maintain fitness in familiar environments (Bradshaw, 1991). However, as adaptive plasticity becomes less effective in more novel environments and the absolute fitness of the population declines, genetic variation in fitness is expected to increase because genotypes will 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 increases the adaptive potential of the population that could allow 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 adaptive potential of the population when exposed to novel environments.

Populations that experience large reductions in mean absolute fitness ($\overline{W}$) in novel environments will face extinction if they cannot increase $\overline{W}$ sufficiently quickly to prevent population declines (Lynch & Lande, 1993; Lande & Shannon, 1996; Hendry *et al.*, 2018). Fisher's (1930) Fundamental Theorem of natural selection predicts adaptation for a population by quantifying genetic variance in relative fitness as $V_A(\omega) = \frac{V_A(W)}{\overline{W}}$, the ratio of

additive genetic variance in absolute fitness ($V_A(W)$) to mean fitness (Fisher, 1930; Sheth *et al.*, 2018; Walsh & Lynch, 2018; Bonnet *et al.*, 2019, 2022; Kulbaba *et al.*, 2019). If genetic variance in relative fitness increases in a novel environment, then the adaptive potential of the population will be improved because alleles that can rapidly increase mean fitness are already present (Shaw & Shaw, 2014; Shaw, 2019).

Although recent studies have found changes in additive genetic variation in fitness within a species' range (Sheth *et al.*, 2018; Kulbaba *et al.*, 2019; Peschel *et al.*, 2020), to our knowledge, increased genetic variance in relative fitness ($V_A(\omega)$) in novel environments has not been identified because studies typically quantify $V_A(\omega)$ in familiar environments (Hendry *et al.*, 2018) or focus on heritability (Hoffmann & Merilä, 1999; Charmantier & Garant, 2005), which does not directly quantify adaptive potential (Hansen *et al.*, 2011). Furthermore, studies that link fitness to plasticity in novel environments are even rarer (Steinger *et al.*, 2003; Wang & Althoff, 2019). We therefore have a remarkably limited understanding of whether genetic variation in relative fitness increases in novel environments, or the role of plasticity in explaining any changes in genetic variation in fitness. Without such information, the adaptive potential of populations as they become exposed to novel environments remains largely unknown (Shaw, 2019).

We focus on a Sicilian daisy, *Senecio chrysanthemifolius* (Asteraceae), which grows in disturbed habitats at low elevation (*c.* 400–1000 m above sea level (asl)) on Mount Etna and throughout lowland Sicily (Fig. 1a). This species occurs in small patches typically containing fewer than 100 individuals, with patches often separated by 1–2 km. Individuals are occasionally observed at elevations between 1000 and 1500 m (but never above 1500 m), indicating that these higher elevations represent the edge of the range for *S. chrysanthemifolius*. In natural populations, individuals of *S. chrysanthemifolius* typically live for < 2 yr and are obligate outcrossers that are pollinated by generalist insects (e.g. hoverflies), with wind-dispersed seeds that can often move hundreds of metres from their parent (Walter *et al.*, 2020a). Also found on Mt. Etna is a high-elevation sister species, *S. aethnensis*, which is endemic to lava flows above 2000 m, and is rarely found below 1500 m. Adaptive divergence between these two *Senecio* species is associated with contrasting leaf morphology and physiology, as well as differences in plasticity (Walter *et al.*, 2022a).

We collected cuttings from 72 naturally occurring *S. chrysanthemifolius* genotypes on the south-east slopes of Mt. Etna and propagated them in the glasshouse (Supporting Information Table S1; Fig. S1). We then conducted crosses among them in a paternal half-sibling breeding design (Lynch & Walsh, 1998) to produce 314 offspring genotypes, which represent matings that could easily be generated in the natural population (Fig. 1b). We then transplanted multiple cuttings (clones) of each offspring genotype at three elevations and quantified fitness, morphology, physiology and gene expression of each genotype in their native environment (500 m), the edge of their range (1500 m) and a novel environment (2000 m; Fig. 1b).

Using these data, we tested whether adaptive potential increases in novel environments with three predictions: (1) in more familiar environments (500 and 1500 m), we would observe high absolute mean fitness ($\overline{W}$) but low additive genetic variance in relative fitness because all genotypes successfully produce appropriate phenotypes within their native range and maintain high fitness. By contrast, at the novel elevation, absolute mean fitness should be reduced and associated with increased genetic variance in relative fitness that represents greater adaptive potential in the novel environment (Fig. 2a). (2) Significant genetic correlations between trait plasticity and fitness in the novel environment would provide evidence that genetic variation in plasticity is associated with greater fitness in the novel environment (Fig. 2b). (3) Given that changes in gene expression mediate plasticity, genotypes with greater fitness in a novel environment would show greater differential gene expression for more genes compared with genotypes with lower fitness, with such changes occurring in genes important for responding to the novel elevation (Fig. 2c).

Materials and Methods

Genotype sampling and crossing design

To quantify the adaptive potential of a local population, we sampled individuals from five sites at the centre of the species' range. To establish the parental generation, in June 2017, we collected cuttings from 72 individuals from five sites < 5 km apart on the foothills of Mt Etna between 526–790 m asl (Table S1; Fig. S1). The proximity of these sites and the fact that *S. chrysanthemifolius* (Poir.) is insect pollinated and its seeds wind-dispersed mean that gene flow is likely to routinely occur between them. Where possible, we sampled individuals that were at least 10 m apart to minimise chances of sampling close relatives. We removed all branches from mature plants that possessed vegetative material, which we cut into 4–5 cm segments at the glasshouse (Giarre, Italy), dipped them in a rooting plant growth regulator for softwood cuttings (Germon® Bew., Der. NAA 0.5%; L. Gobbi, Campo Ligure, Italy) and placed each cutting in one cell of an 84-cell tray containing a compressed mix of 1 : 1 perlite and coconut coir. For 3 wk, we kept cuttings in plastic tunnels to maintain humidity and encourage root growth. We then placed one randomly selected cutting per individual in a 30-cm-diameter pot with standard potting mix, which we watered regularly. To encourage growth, we suspended 25 W LED tubes (TSA Technology, Serravalle, Italy) 1 m above the bench. Once plants produced buds, we covered flowering branches with perforated bread bags 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 ($n = 36$) (Table S1). Because this species is self-incompatible, we could mate individuals by removing the flowers from sires and rubbing them on flowers of the dams. 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 family were germinated by cutting the top off each seed (< 1 mm) and placing them on moistened filter

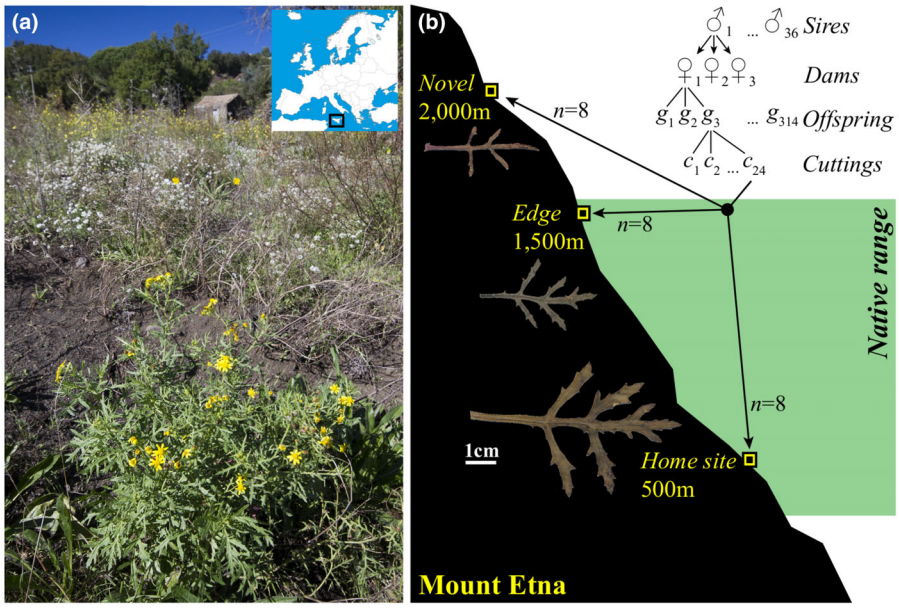

Fig. 1 Study system and experimental design. (a) *Senecio chrysanthemifolius* in its natural habitat. Inset map presents the location of the study system in Europe. (b) Schematic of Mount Etna showing the experimental design. We sampled individuals from five sites in the foothills of Mount Etna, which 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 multiple 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 representative genotype.

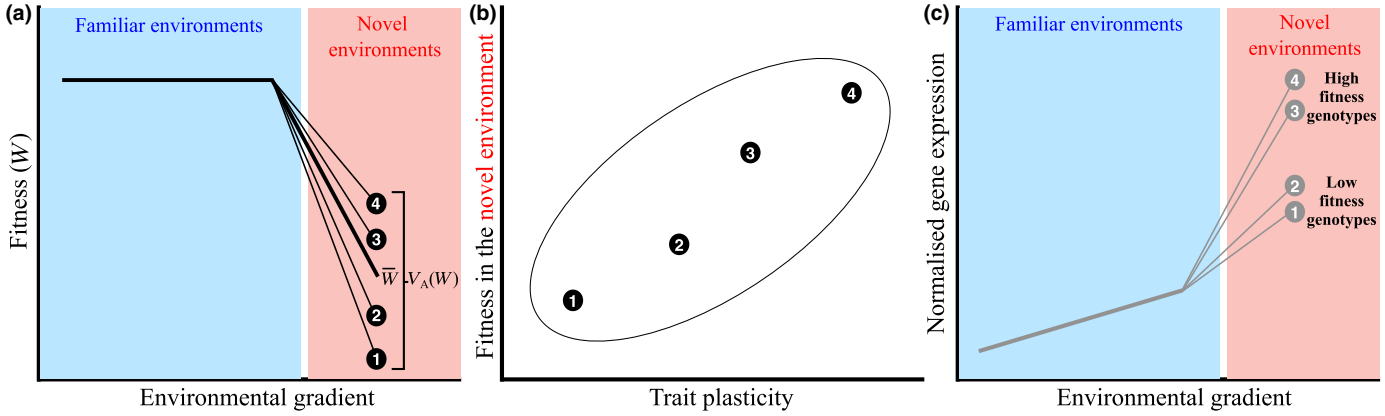

Fig. 2 (a) Conceptual diagram depicting the predicted change for fitness (*W*) in response to familiar (blue) and novel (red) environments. The thick line represents the change in mean fitness across environments, and the thin lines with circles represent changes in four different genotypes (represented by different numbers) within the population. If all genotypes can effectively buffer familiar environmental variation by generating appropriate phenotypes, we expect to observe high mean fitness within the range, which would be associated with low genetic variance in relative fitness (i.e. no difference among genotypes). However, in novel environments, we expect lower mean fitness associated with an increase in genetic variance in relative fitness because differences among genotypes emerge and increase adaptive potential. 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 observe moderately strong genetic correlations between trait plasticity and fitness, which is created by genotypes with higher fitness (3–4) showing greater trait plasticity in the novel environment than genotypes with lower fitness (1–2). (c) We also expect genotypes with low (1–2) and high (3–4) fitness to show differences in gene expression. Grey 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 high fitness genotypes show a stronger response by producing more gene product in the novel environment than low fitness genotypes. We use gene overexpression as an example, but either under or overexpression could be beneficial in a novel environment. Empirical support for these predictions would suggest that genotypes with particular plastic responses can help to increase fitness in novel environments and provide the potential for evolutionary rescue.

paper in a plastic Petri dish. Petri dishes were kept in the dark for 2 d, and then transferred to growth cabinets maintained at 22°C with a 12 h : 12 h, light : dark photoperiod. After 1 wk, we transferred seedlings to the glasshouse where three individuals from each family were grown in 14-cm-diameter pots containing standard potting mix (n = 312 individuals). When the main stem of each seedling reached *c.* 12 cm of growth, we cut the main stem 4 cm aboveground level to promote lateral branching and generate enough cuttings for the field transplant.

Field transplant of the cultivated offspring as cuttings

When most plants possessed branches that started producing buds, we removed all branches from each of the 312 individuals (hereafter, genotypes). Branches were cut into smaller segments each 4–5 cm long with 2–3 leaf nodes. For almost all genotypes, we were able to take 21 cuttings (transplant 1). To increase replication, we left the plants to regrow for 3 wk and then took a second round of cuttings (14 cuttings per genotype) from the same

genotypes (transplant 2). Due to the deaths of two genotypes in the glasshouse after taking the initial cuttings, we replaced these genotypes with siblings for the second round of cuttings, which led to two extra genotypes (n = 314 rather than 312). Cuttings were stored in high-humidity tunnels for 2 wk until they produced roots and were ready to transplant.

We transplanted 7–10 cuttings from each of the 314 genotypes at each of three transplant sites along an elevational gradient (n = *c.* 2700 cuttings per site; total n = 8149 cuttings) that represented a site within the species' native range at 545 m asl (hereafter, the 500 m homesite), the edge of its range at 1395 m asl (hereafter, the 1500 m site) and a novel elevation at 1942 m asl (hereafter, novel 2000 m site). The 500 m site was located on abandoned land near a local population, the 1500 m site in an apple and pear orchard where vagrant *S. chrysanthemifolius* individuals were found, and the novel 2000 m site on a lava flow from 1983 (Fig. S1). Not only are average temperatures lower at higher elevations, temperature extremes differ across elevations (Walter *et al.*, 2022a). Elevations under 1000 m experience high temperatures that regularly exceed 40°C during summer, and temperatures above 1000 m frequently drop below 0°C during winter. Soil is characterised as a silty sand at 500 and 1500 m, but changes to volcanic sand at 2000 m. Soil chemistry changes gradually across elevation, with lower nutrient content present at higher elevations (Walter *et al.*, 2022a).

To prepare each transplant site, the soil surface was cleared of plants and debris, and the soil turned (30 cm deep) immediately before transplanting. Due to the presence of snow at 2000 m following winter, we could only prepare the sites and transplant cuttings in May. We were, however, still able to transplant the cuttings during their natural growing period. At each site, we transplanted the cuttings into four large experimental blocks spacing each plant 30 cm apart. Transplanted cuttings were kept moist until they established, after which we reduced watering to minimal amounts that were sufficient to maintain survival while having a minimal effect on growth or flower production. Given that *S. chrysanthemifolius* grows on disturbed sites following rainfall, this experiment therefore replicates seminatural conditions for this species.

We took cuttings at two time points, which meant that we transplanted the cuttings in two temporal blocks. *Transplant 1, 23–25 May 2018*: at each transplant elevation, we randomised six cuttings of each of the 312 genotypes into two replicate experimental blocks (n = 936 cuttings per block; n = 1872 cuttings per transplant site; total n = 5634 cuttings). *Transplant 2, 10–11 July 2018*: we used all remaining cuttings to transplant two additional cuttings per genotype at each elevation into two additional blocks, as well as replace cuttings lost due to *c.* 10% mortality at 500 and 2000 m following a short spell of intense heat in late June (n = 321–525 cuttings per block; n = 718–927 cuttings per transplant site; total n = 2515 cuttings). The heat-related death was random among genotypes and was exacerbated by the hot black lava soil at 2000 m and the excessive heat at 500 m.

To account for environmental variation within each transplant elevation while accommodating the large number of cuttings in the available space, we split each block into 2–3 smaller sub-blocks (*c.* 35–50 m^2 each) placed next to each other (i.e. separated by < 3 m). Environmental conditions were relatively homogeneous across sub-blocks within each block, cuttings for each genotype were randomised across the block and we accounted for the sub-blocks statistically as described below. This field experimental design therefore estimates genetic variance for the breeding design while properly accounting for environmental variance among cuttings within genotype.

Estimating phenotype and fitness

Mortality of established 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. At the end of the growing season (ending September–October) after at least 3 months of growth, we counted all flower heads produced by each plant, which we used as an estimate of fitness for each clone. This trait is used routinely to assay fitness in short-lived perennials as total reproductive output for each plant in their first season (e.g. Pujol *et al.*, 2014). Given that plants of *S. chrysanthemifolius* rarely flower more than once and that the number of flowers was closely associated with the total seeds produced per plant (Methods S1), the total number of flowers represents a good proxy of fitness.

We measured leaf morphology and 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), from which we analysed four leaf traits: leaf area, leaf complexity $\left(\frac{\text{leaf perimeter}^2}{\text{leaf area}}\right)$, the number of leaf indents standardised by the perimeter and Specific Leaf Area $\left(\text{SLA} = \frac{\text{leaf area}}{\text{leaf weight}}\right)$. These leaf measurements represent leaf morphology and investment traits that show plastic responses to the abiotic environment in *S. chrysanthemifolius* (Walter *et al.*, 2022a,b), and in other plant systems, including sunflowers (Royer *et al.*, 2009). We also used a Dualex instrument (Force-A, Orsay, France) to measure the flavonol pigment content of the leaf. Flavonols 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). All data and code are provided in Dataset S1.

Quantifying genetic variance in fitness

To quantify genetic variance in fitness, we used MCMCGLMM (Hadfield, 2010) within R (v.3.6.1; R Core Team, 2021) to apply the generalised linear mixed model:

$$y_{ijklmnp} = T_i + s_{j(k)} + d_{k(j)} + g_{l(jk)} + b_{mn(i)} + e_{p(ijklmn)}, \quad \text{Eqn 1}$$

where the only fixed effect was transplant elevation (T_i). The random effects $s_{j(k)}$ represented the j^{th} sire, $d_{k(j)}$ the

k^{th} dam and $g_{l(jk)}$ the l^{th} individual (genotype) of the breeding design nested within dam and sire. We included fitness as a univariate, poisson-distributed response variable ($y_{ijklmnp}$), which quantifies genetic variance in relative fitness ($V_A(\omega)$) when estimates from a generalised linear model with a log-normal distribution are obtained on the latent scale (Bonnet *et al.*, 2019; Morrissey & Bonnet, 2019). To quantify genetic variance in relative fitness at each transplant elevation (and the genetic covariance among elevations), we estimated a 3 × 3 covariance matrix for the sire component by specifying random slopes and intercepts for transplant elevation.

To account for differences between transplant dates and among experimental blocks (within transplant date) at each elevation, we included the m^{th} experimental sub-block from the n^{th} transplant date as a random effect ($b_{mn(i)}$). Preliminary analyses showed that including separate random effects for transplant date and experimental block produced identical estimates of genetic variance. $e_{p(ijklmn)}$ represents the error variance. We included a substantial burn-in and thinning interval to allow model convergence, which we confirmed by checking that effective sample sizes exceeded 85% of the number of saved samples. We used weakly informative parameter-expanded priors for the random effects (Hadfield, 2010), and checked their sensitivity by changing the scale parameter while ensuring there was no effect on the posterior distribution.

Connecting genetic variance in plasticity with fitness

Identifying the ecological importance of leaf traits For all traits in the subsequent analyses, we calculated the average across all leaves sampled from each plant. To test whether variation in leaf traits was associated with fitness, we used multiple regression by applying generalised linear mixed models with 'LME4' (Bates *et al.*, 2015) for each elevation. We included all five traits as continuous predictors (each standardised by their global mean) and the number of flowers as a poisson-distributed response variable. Experimental block and genotype were included as random effects. We then used a multivariate analysis of variance to test whether plasticity significantly changed the multivariate phenotype across elevation by including all five traits as the multivariate response variable, transplant elevation as the categorical dependent variable, and the experimental blocks within transplant elevation as the error term.

Genetic correlations between leaf plasticity and fitness We calculated plasticity across elevation by standardizing all clones of each genotype at 1500 and 2000 m by dividing by the mean value of that genotype at the homesite (500 m). This standardization calculated the trait values for each of the 314 genotypes at the novel elevation, relative to their trait value at the homesite (see Fig. 4b,c, see later). 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 homesite. We estimated plasticity separately for each transplant date. To quantify the genetic correlation between phenotypic plasticity and fitness, we used Eqn 1, but only for the data collected at 2000 m and with all five leaf traits and fitness (number of flowers) as the multivariate response variable. This calculates the covariance between plasticity in each trait and fitness at 2000 m. We extracted the sire component and calculated the genetic correlations between each trait and fitness.

Gene expression analyses

To test whether gene expression variation across elevation was associated with fitness, we sampled RNA from 12 genotypes (of the 314) at all elevations. We were restricted to a subset of genotypes due to the high cost of sequencing and the need to sample all genotypes in the same environmental conditions. We chose two sets of genotypes: The six genotypes that showed the greatest fitness at 2000 m represent genotypes that increase the adaptive potential ('*AP*' genotypes) of the population at the novel elevation, and the six genotypes with the lowest fitness at 2000 m that represent genotypes more specialised to conditions within their native range ('*HR*' genotypes for '*Home Range*') (Fig. S2a). See Methods S2 for details on how genotypes were chosen.

At each transplant elevation, we sampled leaves from three randomly selected clones of each of the 12 chosen genotypes. Plants were sampled when they were flowering but were still growing vegetatively. From each plant, we sampled 3–4 young leaves (*c.* 15 mm long), which we immediately submerged in RNAlater and stored at 4°C for 24 h and then at −80°C before RNA extraction. To reduce environmental variation, we sampled 09:00–11:00 h on three consecutive days (19–21 November) under similar weather conditions.

We extracted RNA for the leaves of each plant and quantified gene expression at each elevation using 3′ RNAseq (QuantSeq). See Methods S2 for RNA extraction protocols and transcriptome assembly. Once assembled, we annotated the transcriptome using *Trinotate* and identified orthologous genes from our transcriptome in *Arabidopsis thaliana* using *OrthoFinder* (Emms & Kelly, 2019). 3′ reads were mapped to the reference transcriptome using SALMON v.1.1.0 (Patro *et al.*, 2017). 72.6–87.3% of reads were mapped. Transcript abundance estimates were imported into R using TXIMPORT (Soneson *et al.*, 2015), with estimates normalised according to library size but not by transcript length, as the 3′ sequencing method removes this bias. We visualised the broad patterns of variation in gene expression by normalizing transcript abundance using the variance stabilizing transformation function with DESEQ2 (Love *et al.*, 2014) followed by a principal components analysis (Fig. S3). We then calculated differential expression of transcripts using non-normalised transcript counts between transplant sites for each set of genotypes (*AP* and *HR*) separately using DESEQ2. Differentially expressed genes were defined as those showing both significant (adjusted P-value < 0.01) and strong ($\log_2$-fold change > 2 or < −2) changes in expression. We then tested for enrichment of gene ontology (GO) terms for differentially expressed genes using a Kolmogorov–Smirnoff test and Fisher's exact test with TOPGO (Alexa & Rahnenführer, 2019).

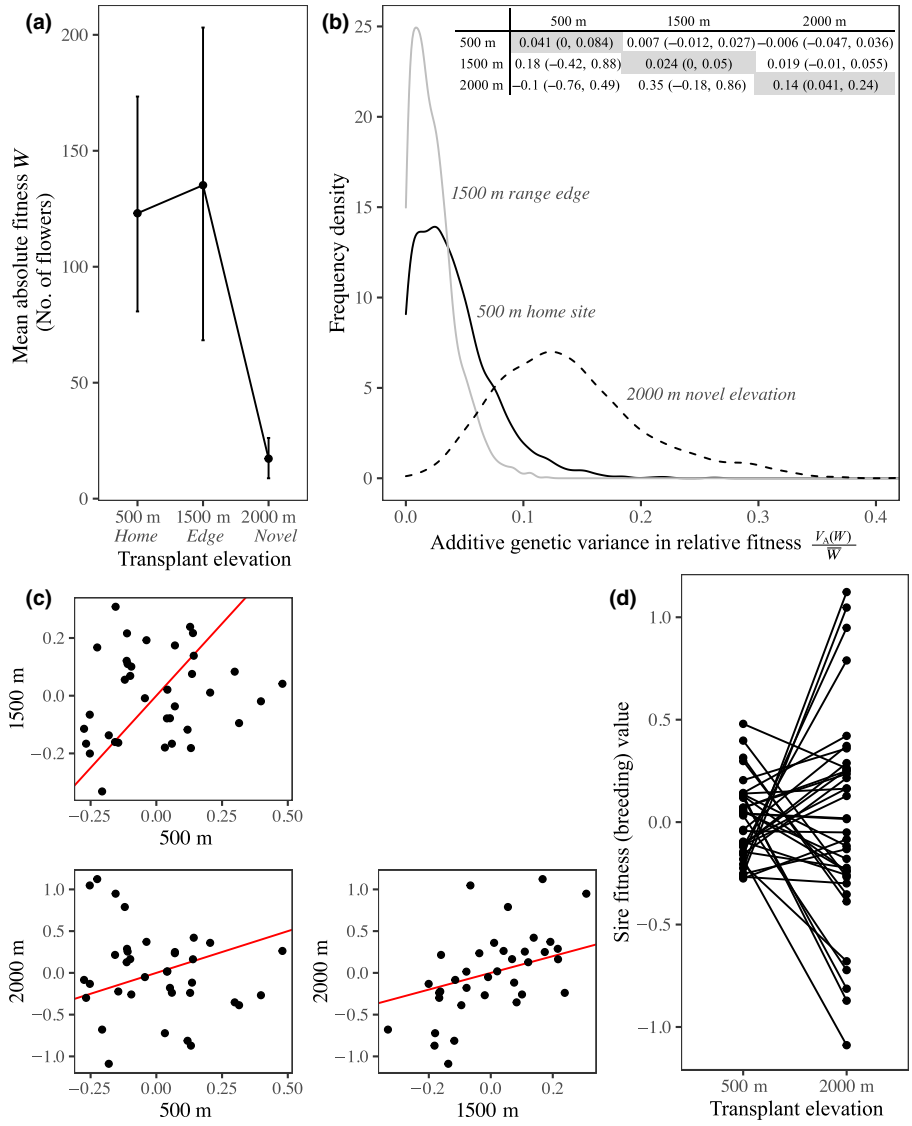

Fig. 3 Absolute mean fitness ($\overline{W}$) of *Senecio chrysanthemifolius* was 87% lower at the novel elevation, and this was associated with an increase in additive genetic variance in relative fitness ($\frac{V_A(W)}{\overline{W}}$). (a) Mean absolute fitness dropped outside the native range. Credible 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 relative fitness was significantly greater at the novel elevation (2000 m) where distributions did not overlap at 90% HPD with the edge of the range, and 80% with the homesite. Matrix inset presents the genetic variances along the diagonal (in grey), with the correlations below the diagonal and the genetic covariances above the diagonal. 90% HPD intervals are presented in parentheses. (c) By visualising the fitness values for the sires (i.e. their breeding values), we can see how the genotypes respond differently across elevations. Red lines represent the 1 : 1 relationship that would suggest the same relative fitness at any two transplant elevations. We observed positive genetic correlations between 500 and 1500 m, and between 1500 and 2000 m. A weak negative genetic correlation between 500 and 2000 m suggests that although most of the sires perform similarly well at both elevations, there are sires that change in relative fitness across elevations. (d) Sire breeding values for the home (500 m) and novel (2000 m) elevations relative to the population mean (represented as zero). The weak negative genetic correlation (−0.1) between the homesite and novel elevation suggests that genotypes that performed the best outside the range performed relatively poorly within the range, which is visualised by the high fitness genotypes at 2000 m having low fitness at 500 m.

Results

Reduced mean fitness but increased genetic variance in relative fitness in the novel environment

Consistent with our first prediction (Fig. 2a), absolute mean fitness (number of flowers) was high within the native range (500 and 1500 m) but decreased by 87% at 2000 m (Fig. 3a). Genetic variance in absolute fitness was also greater within the range (500 m = 732.1; 1500 m = 504.4), but an order of magnitude lower at the novel elevation (2000 m = 58.4; Table S2). At 2000 m, genotypes therefore have a lower fitness and vary less in absolute fitness. However, also consistent with our first prediction that adaptive potential increases in novel environments, genetic variance in relative fitness ($V_A(\omega)$) was near zero within the native range (500 and 1500 m), but three times greater at

2000 m, which predicted a 14% increase in mean fitness in the subsequent generation (Fig. 3b).

Genetic correlations were positive between 500 and 1500 m, and between 1500 and 2000 m, suggesting that sires showed similar relative performance between these elevations (Fig. 3c). However, genotypes with greater fitness at the novel elevation showed lower fitness at the 500 m homesite (Fig. 3c,d), as indicated by a weak and nonsignificant negative genetic correlation of −0.1 (−0.76, 0.49 90% Highest Posterior Density interval (HPD)) for fitness between the homesite and the novel elevation. While a positive correlation would suggest that genotypes that perform well at one site also perform well at the other site, a (nonsignificant) correlation near zero suggests that genotypes respond differently by showing changes in relative fitness across elevations. The near-zero correlation in our results shows that while many genotypes do not change in relative fitness across sites, the genotypes with the greatest fitness at 2000 m had lower fitness relative to other genotypes at the homesite (Fig. 3d). Genotypes that increase the adaptive potential of the population in novel environments could therefore have a selective disadvantage within their native range.

The 10 sires with the highest fitness in the novel environment were collected from three of the five sites (Table S3), suggesting that genetic variation from particular sites could be important for increasing the adaptive potential in novel environments. However, differences among sampling sites only accounted for a small proportion (0.5%) of the total variance in fitness compared with additive genetic variance (9.3%) (Methods S3). These results, combined with no evidence of local adaptation (Methods S3), suggest that the five sampling sites are essentially all part of the same population.

Genetic variance in fitness in the novel environment correlates closely with plasticity

Identifying the ecological importance of leaf traits We found that variance among genotypes was greater than among clones within genotype (Methods S4), suggesting that multiple clones reliably represent the response of each genotype to each elevation. We observed a significant association between all leaf traits and fitness at each elevation (Fig. 4a; Table S4), suggesting that these leaf traits are important for maintaining fitness as the environment varies. At all transplant elevations, selection was in a similar direction, except for flavonol content, where greater flavonol content is associated with greater fitness at 1500 and 2000 m, but with lower fitness at the 500 m homesite (Fig. 4a).

In support of other transplant experiments that measured the same traits (Walter *et al.*, 2022a,b), we found evidence of plasticity as large changes in leaf traits across elevation (Fig. 4b) associated with a large and significant change in mean multivariate phenotype with elevation (MANOVA $F_{2,23} = 44.521$, $P < 0.0001$). Plasticity reduced leaf area, leaf complexity and flavonol content similarly at the edge of the range (500–1500 m) and the novel elevation (500–2000 m). The number of indents and SLA increased from the homesite to the edge of the range, but then decreased at the novel elevation. Only leaf area showed a greater magnitude of plastic change in phenotype at 2000 m compared to 1500 m (Fig. 4b).

While selection at 2000 m favoured larger values of all traits, except the number of indents (Fig. 4a), plasticity created a reduction in trait values from the homesite to the novel 2000 m elevation (Fig. 4b), which suggests that fitness at 2000 m is likely to be greater for genotypes that change their phenotype less across elevation.

Genetic correlations between leaf plasticity and fitness We predicted that if genetic differences in plasticity underlie increased genetic variance in relative fitness at 2000 m, we would observe significant genetic correlations between trait plasticity and fitness (Fig. 2b). As predicted, we found strong and significant genetic correlations between plasticity and fitness at the novel elevation (> 90% of the posterior distribution did not overlap with zero) for four traits: leaf area (0.47; 0.01, 0.88 90% HPD), the number of indents (−0.51; −0.95, −0.07 HPD), SLA (0.61; 0.27, 0.94 HPD) and flavonol content (0.55; 0.13, 0.95 HPD) (Fig. 5a). The increase in genetic variance in fitness at the novel elevation was therefore genetically correlated with plasticity for four of five leaf traits, suggesting that genetic variation in plasticity increases the adaptive potential of a population exposed to a novel environment. Only plasticity in leaf complexity showed no association with fitness (−0.03; −0.54, 0.53 HPD) (Fig. 5a).

Genetic variation in fitness was associated with different patterns of plasticity that depended on the trait. For the number of indents, genotypes with higher fitness at 2000 m showed greater plasticity than low fitness genotypes, created by stronger reductions in trait values between 500 and 2000 m (Fig. 5b). For leaf area, SLA and flavonol content, genotypes with higher fitness at 2000 m showed lower plasticity across elevation than the genotypes with lower fitness (Fig. 5b). Reaction norms for the genotypes with the highest and lowest fitness at 2000 m shows how plasticity across elevation is associated with fitness at the novel elevation. Fig. 6(a) shows that when compared to the low fitness *home range* (*HR*) genotypes, high fitness *adaptive potential* (*AP*) genotypes show differences in plasticty across elevation and often arrive at different trait values at 2000 m. Differences in how the leaves change across elevation for *AP* and *HR* genotypes are visualised in Fig. 6b.

Plasticity in gene expression is associated with fitness in the novel environment

To test whether differences in fitness at the novel elevation were associated with differences in gene expression, we sampled and analysed RNA from clones (at all three elevations) of genotypes that showed high (*AP*) and low (*HR*) fitness at 2000 m. Overall, more genes were differentially expressed at 2000 m (i.e. showed higher or lower expression levels relative to the homesite) compared with the range edge, suggesting that plasticity under more novel conditions is created by broad transcriptional responses across the genome. *AP* genotypes with greater fitness at 2000 m showed significant changes (adj. $P < 0.01$) in more genes than the low fitness *HR* genotypes (1376 in *AP* vs 514 genes in *HR*)

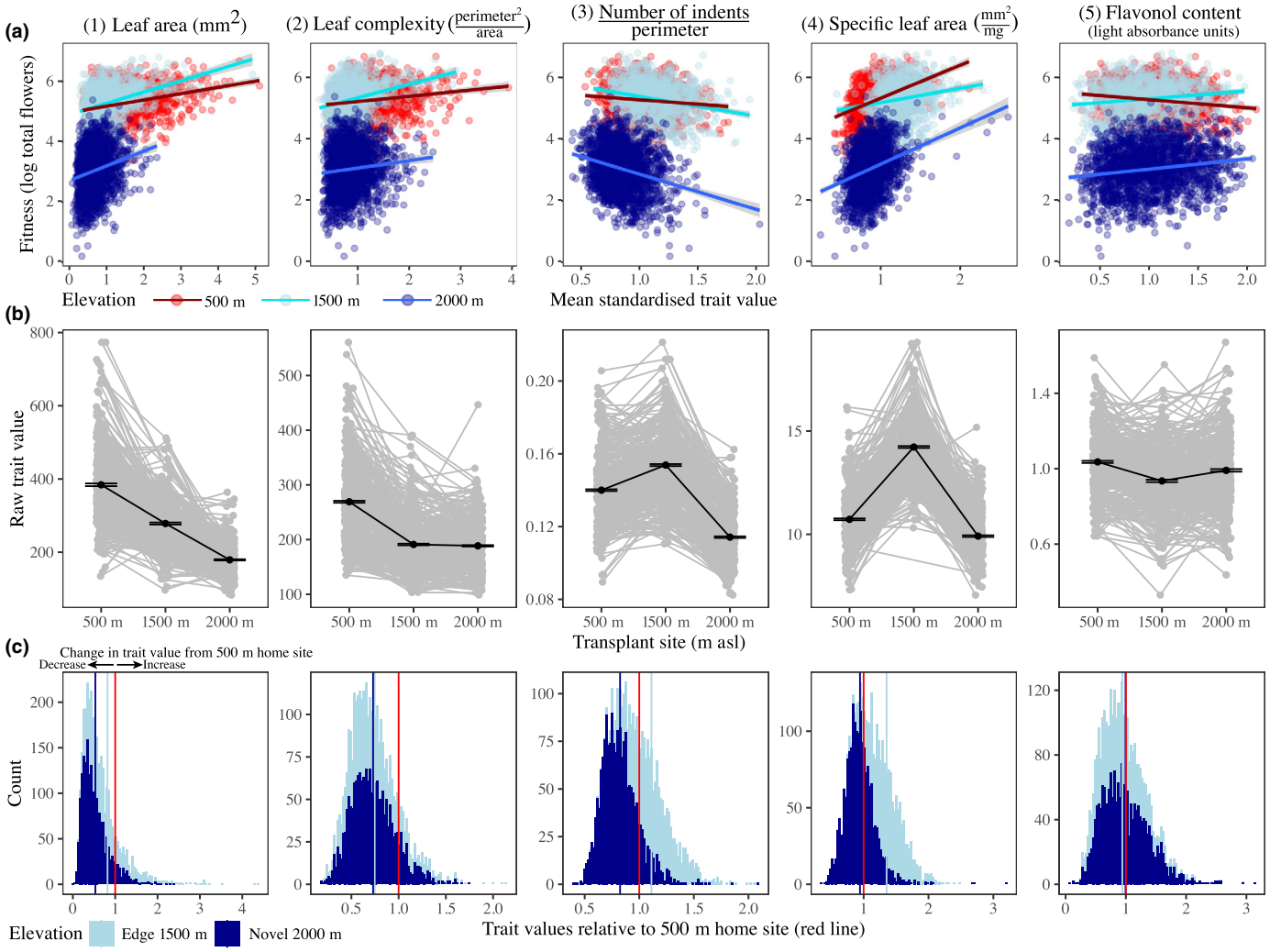

Fig. 4 Changes in phenotype and selection for *Senecio chrysanthemifolius* transplanted across elevations. (a) Phenotype-fitness associations at each elevation. Trait values are standardised by the global mean for each trait. Each point represents an individual plant and shows that selection is in a similar direction for all elevations, except for flavonol content. (b) 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 grey. Plasticity from the homesite (500 m) to the 1500 m is in the same direction as the 2000 m site for leaf area, complexity and flavonol content. For all traits, except specific leaf area, plasticity shows a stronger change in magnitude from 500 m to the novel 2000 m elevation, when compared to the 1500 m range edge. (c) Frequency distribution for plasticity in each trait, calculated as the degree to which each genotype changes trait values from the homesite (vertical red line) to the edge of the range (light blue) and to the novel elevation (dark blue). Vertical blue lines represent mean plasticity for all genotypes. Values of one represent no change from the homesite, whereas values above and below one represent plasticity as an increase and decrease in the trait value, respectively.

within the range (500–1500 m), suggesting that the *HR* genotypes adjusted fewer genes to maintain high fitness within their range (Fig. 7a). However, the number of genes that showed expression changes tripled outside the native range (500–2000 m) for both classes of genotypes (*AP* = 4876 genes vs *HR* = 4720) (Figs 7b, S4). The mean magnitude of expression change was also greater at the novel elevation (*AP* = 1.41, *HR* = 1.21) when compared to the edge of the range (*AP* = 1.19, *HR* = 1.04). Compared with the *HR* genotypes, *AP* genotypes showed strong overexpression in 10× more genes at the novel elevation, but underexpressed half as many genes (Fig. 7c). Therefore, as predicted (Fig. 2c), distinct patterns of gene expression between the home site and the novel elevation were associated with genotypic differences in fitness at the novel elevation.

Gene ontology We also predicted that greater fitness at 2000 m would be associated with changes in gene expression for ecologically important genes. *AP* and *HR* genotypes differed in the functional categories of genes that varied in expression at the novel elevation (Fig. 7d). *AP* genotypes showed differential expression in genes relating to biosynthesis, whereas *HR* genotypes differentially expressed genes related to photosystems. Comparing the enriched GO terms with those of a 2017 transplant experiment (Walter *et al.*, 2022a) that included the high elevation sister species (*S. aethnensis*), we found that four (of 17) GO terms were enriched both in *S. aethnensis* and the *AP* genotypes, compared with only one (of 20) GO term shared by *S. aethnensis* and the *HR* genotypes (Fig. 7d). Compared with the *HR*

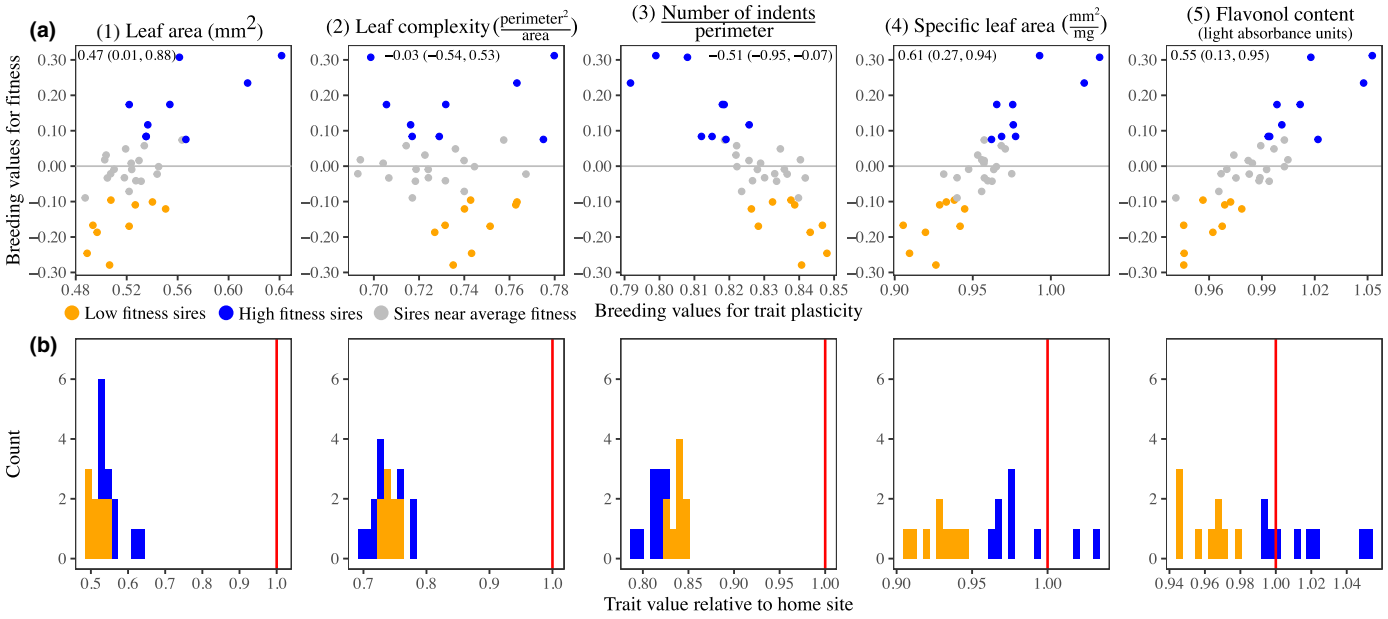

Fig. 5 Genetic variance in plasticity for *Senecio chrysanthemifolius* was significantly correlated with fitness at the novel elevation for all traits, except leaf complexity. (a) Visualization of the genetic correlations using the 36 sire genetic (i.e. breeding) values for plasticity (*x*-axis) vs fitness (*y*-axis). Sire genotypes with the greatest fitness (blue circles) at 2000 m show different levels of plasticity to genotypes with lowest fitness (orange circles). Grey circles represent genotypes that were closer to the average fitness of the population. Inset text presents the posterior mean (and 90% HPD intervals) of the genetic correlations between plasticity and fitness at 2000 m. (b) Histogram of the sire genetic values for trait plasticity that represent the magnitude of plastic change in trait means from the homesite (value of 1, represented by the red vertical line) to 2000 m. Compared to low fitness sires (orange), higher fitness sires (blue) at 2000 m showed differences in leaf plasticity as a smaller reduction in leaf area, specific leaf area and flavonol content, but a larger reduction in the number of indents.

genotypes, *AP* genotypes of *S. chrysanthemifolius* therefore showed a more similar gene expression response to the closely related *Senecio* species native to the 2000 m habitat.

Gene Ontology terms that were significantly enriched at 2000 m included light harvesting in photosystem I, cell wall organization, and responses to cold (Table S5). To illustrate the contrasting patterns of expression between *AP* 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. 7e), *AP* genotypes showed stronger underexpression than *HR* genotypes at the novel elevation. For genes involved in cold responses (Fig. 7c), *AP* genotypes showed a stronger response than *HR* genotypes, which included greater overexpression or underexpression depending on the gene.

We then tested whether differences in gene expression across elevations for *AP* and *HR* genotypes were associated with genes linked to leaf development and morphogenesis in *Arabidopsis* (Huala *et al.*, 2001). We found that 86 *Arabidopsis* orthologs were differentially expressed in either *AP* or *HR* genotypes between 500 and 2000 m (Table S6). These included two genes that are important for determining leaf shape and dissection in *Arabidopsis*: PINFORMED1 (PIN1) and ASYMMETRIC LEAVES1 (AS1) (Barkoulas *et al.*, 2008). In both cases, the orthologous genes in *S. chrysanthemifolius* showed significantly lower expression at 2000 m, with a greater decrease in expression shown by *AP* genotypes (Fig. S5), which supports the finding that greater reductions in leaf indentation is associated with higher fitness at 2000 m (Fig. 6).

Discussion

We provide empirical support for two fundamental hypotheses that are crucial for understanding how populations respond to novel environments: First, although adaptive plasticity was unable to maintain high fitness in the novel environment (Fig. 3a), as predicted by theory, genetic variance in relative fitness increased, which improves the adaptive potential of the population and could allow rapid adaptation to the novel environment (Fig. 3b). Second, increased genetic variance in relative fitness in the novel environment correlated closely with plasticity as elevational changes in leaf traits (Figs 5, 6), and in the expression of genes important for responding to the novel high-elevation habitat (Fig. 7), which suggests that genetic variation in plasticity increases the adaptive potential of populations exposed to novel environments.

Previous studies have shown high levels of genetic variance for fitness in natural populations (Hendry *et al.*, 2018; Sheth *et al.*, 2018; Kulbaba *et al.*, 2019), heritable variation in plasticity (Nussey *et al.*, 2005), adaptive plasticity in phenology (Charmantier *et al.*, 2008) and rapid evolutionary responses to range shifts (Buckley & Bridle, 2014). However, to our knowledge, this study provides the first experimental evidence that genetic variation in plasticity is associated with increased genetic variance in a trait closely associated with fitness in a novel environment, which increases the adaptive potential of the population in that environment. These results suggest that genetic variation important for rapid adaptation already segregates in the population, which makes evolutionary rescue more likely than if adaptation were to

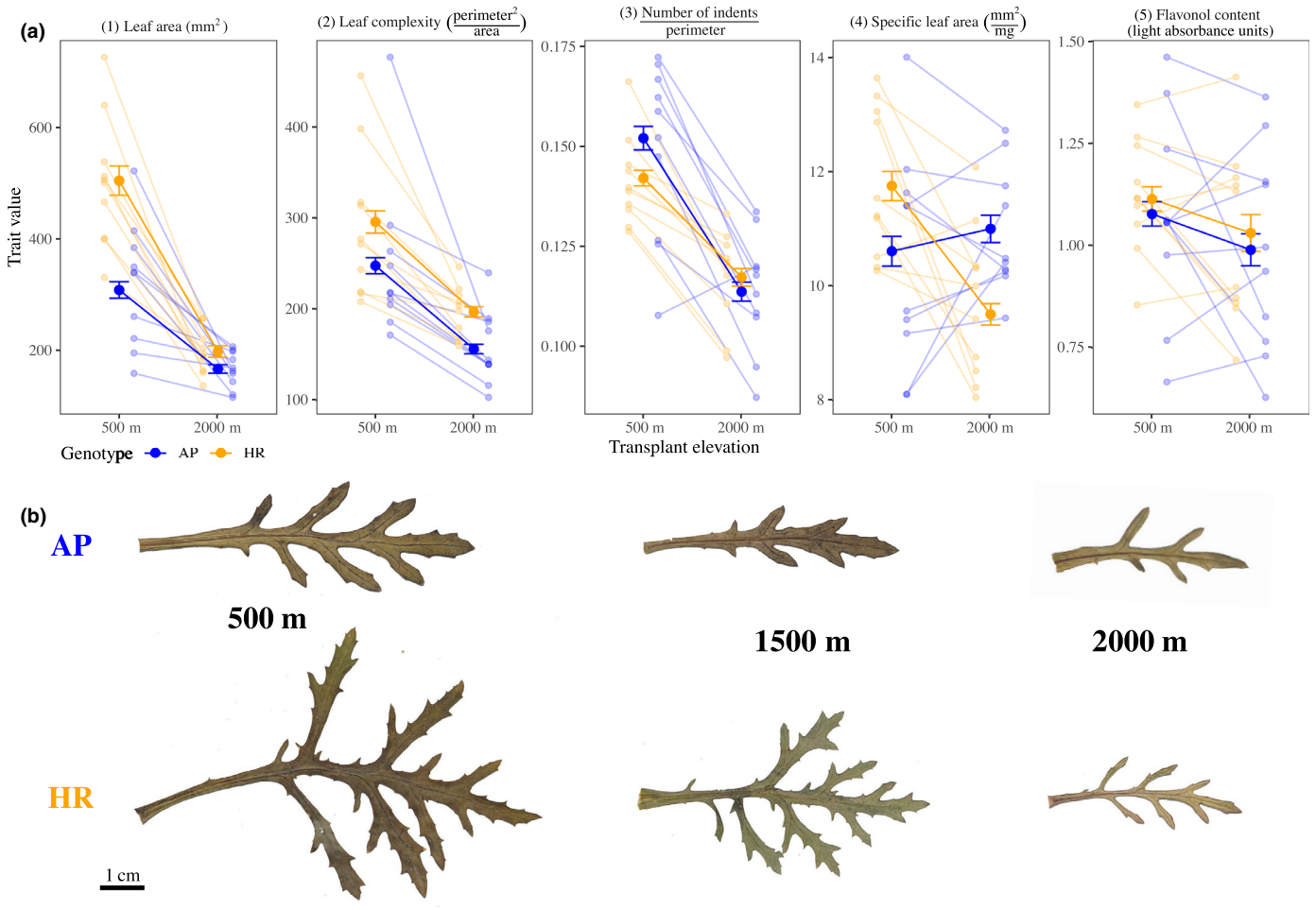

Fig. 6 Changes in leaf traits of *Senecio chrysanthemifolius* across elevation for genotypes selected for the gene expression analysis. *Adaptive potential* (*AP*) genotypes (blue) showed the higher fitness at 2000 m, compared to the low fitness *home range* (*HR*) genotypes (orange). (a) Large circles with error bars (±1 SE) represent the average for the 10 *AP* and *HR* genotypes (represented by small circles). *AP* and *HR* genotypes show different patterns of plasticity for leaf area, number of indents and specific leaf area. Note that plasticity in flavonol content is not different for *HR* vs *AP* genotypes, which is because although fitness shows a strong genetic correlation with plasticity, the phenotypic correlation is weak. (b) Images of leaves for a *AP* (upper row) and *HR* (lower row) genotype across elevation. While both genotypes show reduced leaf area and leaf complexity at higher elevations, *AP* genotypes show less of a reduction in leaf area and more of a reduction in the number of leaf indents across elevations, when compared to *HR* genotypes. These changes are associated with differences between *AP* and *HR* in gene expression across elevation for genes relating to leaf development and morphogenesis (see Gene expression results section).

rely on new mutation (Orr & Unckless, 2014). However, evolutionary rescue will only be likely if population size in the novel environment remains large enough to avoid extinction as adaptation occurs (Chevin *et al.*, 2013; Gonzalez *et al.*, 2013; Polechová & Barton, 2015; Bridle *et al.*, 2019), which is discussed in detail below.

Our results support empirical evidence that genetic variance in fitness depends on the environment in which it is quantified (Sheth *et al.*, 2018; Kulbaba *et al.*, 2019) and show that genotypes that could facilitate adaptation to novel environments may be under negative selection in native environments (Angert *et al.*, 2008). Genotypes with higher relative fitness in the novel 2000 m environment tended to show lower relative fitness in the homesite (Fig. 3c), suggesting that genotypes important for evolutionary rescue are likely to be rare within the native range where selection maintains them at low frequency (Brennan *et al.*, 2019). Our results also suggest that adaptive plasticity hides genetic variation important for evolutionary rescue by maintaining similarly high fitness for all genotypes within the native range. Studies that estimate genetic variation in relative fitness within the native range are therefore likely to underestimate the adaptive potential for novel environments.

The role of plasticity for increasing adaptive potential in novel environments

Genetic variance in relative fitness in the novel environment was correlated with plasticity in leaf traits and gene expression. Compared with low fitness genotypes at the novel elevation, high fitness genotypes showed greater plasticity in leaf indentation, but lower plasticity in leaf area, while trait values for specific leaf area and flavonol content were maintained from the homesite to the

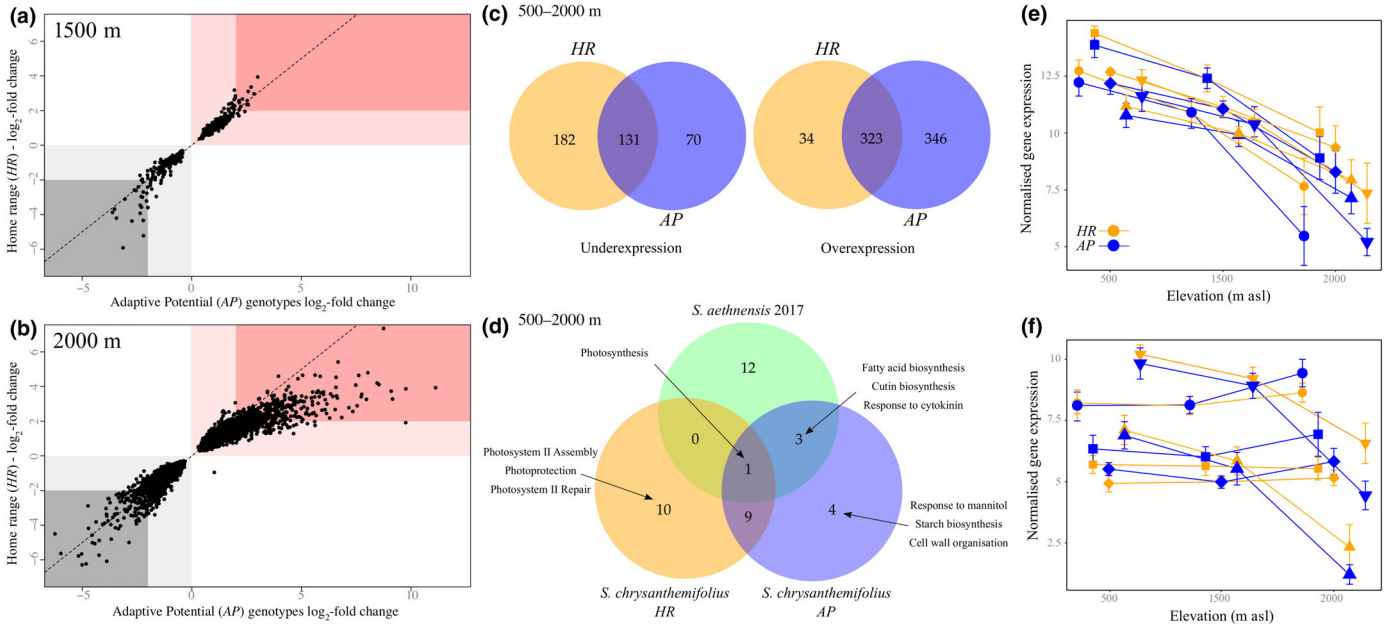

Fig. 7 High fitness *Adaptive Potential* (*AP*) and low fitness *Home Range* (*HR*) genotypes of *Senecio chrysanthemifolius* differ in gene expression at the novel elevation. (a, b) Comparing expression of the same genes (black circles), with deviations from the dotted line representing differences in expression between *AP* (*x*-axis) vs *HR* (*y*-axis) genotypes. Red and grey shading represents overexpressed and underexpressed genes, respectively. Darker shading denotes strong expression changes. (a) Within the native range (500 m vs 1500 m), more genes were differentially expressed in *AP* compared to *HR* genotypes. (b) Outside the native range (500 m vs 2000 m), more genes were differentially expressed, and more genes in the *AP* 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 *AP* and *HR* genotypes between the homesite (500 m) and outside the range (2000 m). *AP* genotypes overexpressed 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*., 2022a). Only the three most significant terms are shown. *AP* genotypes shared more GO terms with *S. aethnensis*, suggesting that high fitness at the high elevation 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 *AP* (blue) and *HR* (orange) genotypes. Each gene is represented by a different shape and credible intervals represent 95% confidence intervals. *AP* genotypes showed stronger underexpression at 2000 m. (f) Elevational changes in mean expression for five genes associated with responses to cold. *AP* genotypes show stronger under- and overexpression, but the reaction was gene dependent.

novel 2000 m elevation (Figs 5, 6). Similarly, our gene expression data suggest that the potential to adapt to novel environments is driven by genotypes with the most beneficial gene expression profiles (Wang & Althoff, 2019; Josephs, 2021). Compared with the low fitness *HR* genotypes, high fitness *AP* genotypes differentially expressed more genes at 2000 m with many genes exhibiting stronger overexpression (Fig. 7c). The changes observed in leaf traits across elevation were supported by changes in expression for genes that regulate leaf development and morphogenesis. Our results therefore suggest that population persistence in novel environments will depend on genotypes with plastic responses that are somewhat adaptive and able to prevent more drastic fitness declines (Lande, 2009; Chevin & Lande, 2011; Chevin & Hoffmann, 2017).

Walter *et al.* (2022a) showed that at the novel 2000 m elevation, plasticity moved the phenotype of *S. chrysanthemifolius* towards the native phenotype of its close relative, *S. aethnensis*. In the current study, high fitness genotypes of *S. chrysanthemifolius* displayed similar patterns of gene expression to *S. aethnensis* in genes associated with a response to elevation. These results reinforce our conclusion that *S. chrysanthemifolius* shows plasticity that is to some extent adaptive and should help persistence and then adaptation to the novel 2000 m environment. Given the abundance of moisture on Mt Etna in spring and autumn, the current range limit of *S. chrysanthemifolius* to lower elevations is likely to be created by snow and ice that is present over winter and that kills any seedlings before they establish at higher elevations. However, as climate change increases temperatures, *S. chrysanthemifolius* could expand and adapt to higher elevations as temperatures there become more conducive to growth, especially given that greater values of specific leaf area at 1500 m (and positive correlations with fitness at all elevations) suggest rapid plant growth could be possible at the range edge, and would be favoured at the novel elevation.

Given that genetic variation in fitness in *S. chrysanthemifolius* was associated with distinct patterns of plasticity, our findings contrast with evidence that: adaptation to novel environments involves nonadaptive plasticity in gene expression for Trinidadian guppies responding to predators (Ghalambor *et al.*, 2015); and that a lack of genetic variance in gene expression in native environments will prevent beneficial responses to novel environments in butterflies exposed to seasonal fluctuations (Oostra *et al.*, 2018). In our results, genotypes with greater fitness in the novel environment showed greater changes in gene expression but smaller changes in three of five leaf traits. Greater fitness in novel environments could therefore involve plasticity that is

adaptive when it minimises changes in traits that are irreversible once a plastic response occurs (e.g. leaf shape/size), while maximising changes in highly labile traits (e.g. gene expression associated with physiology). Such forms of plasticity are likely to be important for persisting in novel environments where genotypes have a lower capacity to anticipate the novel conditions (Velotta & Cheviron, 2018; Hoffmann & Bridle, 2022).

Greater adaptive potential does not guarantee evolutionary rescue

Despite the observed increase in adaptive potential in the novel environment, the likelihood of persistence will depend on the severity of the decline in mean fitness, which will determine whether the population size remains high enough to allow adaptation (Chevin *et al.*, 2013; Gonzalez *et al.*, 2013; Polechová & Barton, 2015; Bridle *et al.*, 2019). Evolutionary rescue will not be possible for populations that suffer fitness declines that reduce the effective population size so that selection cannot overcome drift, which will make extinction more likely than adaptation (Bridle & Vines, 2007; Chevin *et al.*, 2013; Carlson *et al.*, 2014). The decline in mean fitness and associated decline in genetic variance in absolute fitness in the novel environment suggests that the high fitness genotypes may not produce enough offspring to prevent extinction while adaptation occurs. Quantifying how environmental change affects genetic variance in absolute fitness and relative fitness is crucial to understanding the population dynamics in novel environments and the likelihood of evolutionary rescue (Chevin *et al.*, 2010; Shaw & Shaw, 2014). Empirical estimates of ecological and life history parameters within and beyond ecological margins are needed to identify when and where population declines are not so strong as to prevent evolutionary rescue (Connallon & Sgrò, 2018; Bridle & Hoffmann, 2022).

As climate change alters environments, the potential for populations to persist is likely to be determined by the number of genotypes with adaptive plasticity that can help them to persist and then facilitate adaptation to the new conditions. Genetic variation for adaptive plasticity is likely to be rare and unevenly distributed across a species' range (Stratton, 1994; Colautti & Barrett, 2013; Sheth & Angert, 2016; Walter *et al.*, 2020b), with local adaptation, mutation and drift determining which populations contain genetic variation that increase the potential for evolutionary rescue (Hargreaves & Eckert, 2019; Hoffmann & Bridle, 2022). Understanding how adaptive genetic variation is distributed across a species' range will determine the potential for evolutionary rescue, and will be useful for human-assisted conservation by identifying genotypes that can help to make threatened species more resilient (Sgrò *et al.*, 2011; Colautti *et al.*, 2012).

Acknowledgements

We are grateful to Leonie Moyle and three anonymous reviewers whose comments helped us to improve earlier versions of this work. We thank David Aguirre, Spencer Barrett, Roger Butlin, Robert Dugand, Ary Hoffmann and Carla Sgrò for advice and comments on previous versions. We are very grateful to those who helped with fieldwork: Alessandro Barbato, Octavia Brayley, Guy Burstein, Maria Castrogiovanni, Stefania Catara, Sarah du Plessis, Carmen Impelluso, Enrico la Spina, Mari Majorana, Jessica Menzies, Morgan Millen, Giuseppe Pepe and Daniel Ward. We are very grateful to Piante Faro for generously providing glasshouse resources, without which this study would not have been possible. This work was supported by NERC grants NE/P001793/1 and NE/P002145/1 awarded to JB and SH.

Competing interests

None declared.

Author contributions

GMW, SJH and JB designed the experiment with input from SC, JC and AC. GMW, AC and DT conducted the experiments. JC collected, extracted and processed RNA samples, and analysed the transcriptome data. GMW analysed the fitness and phenotype data. GMW wrote the manuscript with JC, JB and SJH, and input from all other authors.

ORCID

Jon Bridle https://orcid.org/0000-0002-5999-0307
James Clark https://orcid.org/0000-0003-2896-1631
Salvatore Cozzolino https://orcid.org/0000-0002-3176-8130
Antonia Cristaudo https://orcid.org/0000-0002-4607-9901
Greg M. Walter https://orcid.org/0000-0002-0883-3440

Data availability

Phenotype and fitness data with associated R code are included as Supporting Information. Raw RNA reads from sequencing experiments are located at the Sequence Reads Archive (SRA) under the project no. PRJNA603521.

References

Agashe D, Falk JJ, Bolnick DI. 2011. Effects of founding genetic variation on adaptation to a novel resource. *Evolution* **65**: 2481–2491.

Alexa A, Rahnenführer J. 2019. *Gene set enrichment analysis with* topGO. R package v.2.38.1. [WWW document] URL http://www.bioconductor.org/packages/release/bioc/html/topGO.html [accessed 1 April 2019].

Angert AL, Bradshaw HD Jr, Schemske DW. 2008. Using experimental evolution to investigate geographic range limits in monkeyflowers. *Evolution* **62**: 2660–2675.

Ashander J, Chevin LM, Baskett ML. 2016. Predicting evolutionary rescue via evolving plasticity in stochastic environments. *Proceedings of the Royal Society B: Biological Sciences* **283**: 20161690.

Barkoulas M, Hay A, Kougioumoutzi E, Tsiantis M. 2008. A developmental framework for dissected leaf formation in the *Arabidopsis* relative *Cardamine hirsuta*. *Nature Genetics* **40**: 1136–1141.

Bates D, Machler M, Bolker BM, Walker SC. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**: 1–48.

Bell G, Gonzalez A. 2009. Evolutionary rescue can prevent extinction following environmental change. *Ecology Letters* **12**: 942–948.

Bonnet T, Morrissey MB, de Villemereuil P, Alberts SC, Arcese P, Bailey LD, Boutin S, Brekke P, Brent LJN, Camenisch G *et al.* 2022. Genetic variance in

fitness indicates rapid contemporary adaptive evolution in wild animals. *Science* **376**: 1012–1016.

Bonnet T, Morrissey MB, Kruuk LEB. 2019. Estimation of genetic variance in fitness, and inference of adaptation, when fitness follows a log-normal distribution. *Journal of Heredity* **110**: 383–395.

Bradshaw AD. 1991. The Croonian Lecture, 1991 – genostasis and the limits to evolution. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **333**: 289–305.

Brennan RS, Garrett AD, Huber KE, Hargarten H, Pespeni MH. 2019. Rare genetic variation and balanced polymorphisms are important for survival in global change conditions. *Proceedings of the Royal Society B: Biological Sciences* **286**: 20190943.

Bridle J, Hoffmann A. 2022. Understanding the biology of species' ranges: when and how does evolution change the rules of ecological engagement? *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **377**: 20210027.

Bridle JR, Kawata M, Butlin RK. 2019. Local adaptation stops where ecological gradients steepen or are interrupted. *Evolutionary Applications* **12**: 1449–1462.

Bridle JR, Vines TH. 2007. Limits to evolution at range margins: when and why does adaptation fail? *Trends in Ecology & Evolution* **22**: 140–147.

Buckley J, Bridle JR. 2014. Loss of adaptive variation during evolutionary responses to climate change. *Ecology Letters* **17**: 1316–1325.

Bylesjo M, Segura V, Soolanayakanahally RY, Rae AM, Trygg J, Gustafsson P, Jansson S, Street NR. 2008. LAMINA: a tool for rapid quantification of leaf size and shape parameters. *BMC Plant Biology* **8**: 82.

Carlson SM, Cunningham CJ, Westley PA. 2014. Evolutionary rescue in a changing world. *Trends in Ecology & Evolution* **29**: 521–530.

Charmantier A, Garant D. 2005. Environmental quality and evolutionary potential: lessons from wild populations. *Proceedings of the Royal Society of London Series B: Biological Sciences* **272**: 1415–1425.

Charmantier A, McCleery RH, Cole LR, Perrins C, Kruuk LEB, Sheldon BC. 2008. Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science* **320**: 800–803.

Chevin LM, Gallet R, Gomulkiewicz R, Holt RD, Fellous S. 2013. Phenotypic plasticity in evolutionary rescue experiments. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **368**: 1–12.

Chevin LM, Hoffmann AA. 2017. Evolution of phenotypic plasticity in extreme environments. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **372**: 20160138.

Chevin LM, Lande R. 2011. Adaptation to marginal habitats by evolution of increased phenotypic plasticity. *Journal of Evolutionary Biology* **24**: 1462–1476.

Chevin LM, Lande R, Mace GM. 2010. Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biology* **8**: e1000357.

Colautti RI, Barrett SC. 2013. Rapid adaptation to climate facilitates range expansion of an invasive plant. *Science* **342**: 364–366.

Colautti RI, Lee CR, Mitchell-Olds T. 2012. Origin, fate, and architecture of ecologically relevant genetic variation. *Current Opinion in Plant Biology* **15**: 199–204.

Connallon T, Sgrò CM. 2018. In search of a general theory of species' range evolution. *PLoS Biology* **16**: e2006735.

Emms DM, Kelly S. 2019. OrthoFinder: phylogenetic orthology inference for comparative genomics. *Genome Biology* **20**: 238.

Fisher RA. 1930. *The genetical theory of natural selection*. Oxford, UK: Oxford University Press.

Ghalambor CK, Hoke KL, Ruell EW, Fischer EK, Reznick DN, Hughes KA. 2015. Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature* **525**: 372–375.

Ghalambor CK, McKay JK, Carroll SP, Reznick DN. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology* **21**: 394–407.

Gomulkiewicz R, Holt RD. 1995. When does evolution by natural selection prevent extinction? *Evolution* **49**: 201–207.

Gonzalez A, Ronce O, Ferriere R, Hochberg ME. 2013. Evolutionary rescue: an emerging focus at the intersection between ecology and evolution. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **368**: 20120404.

Hadfield JD. 2010. MCMC methods for multi-response generalized linear mixed models: The MCMCGLMM R package. *Journal of Statistical Software* **33**: 1–22.

Hansen TF, Pelabon C, Houle D. 2011. Heritability is not evolvability. *Evolutionary Biology* **38**: 258–277.

Hargreaves AL, Eckert CG. 2019. Local adaptation primes cold-edge populations for range expansion but not warming-induced range shifts. *Ecology Letters* **22**: 78–88.

Hendry AP, Schoen DJ, Wolak ME, Reid JM. 2018. The contemporary evolution of fitness. *Annual Review of Ecology, Evolution and Systematics* **49**: 457–476.

Hermisson J, Wagner GP. 2004. The population genetic theory of hidden variation and genetic robustness. *Genetics* **168**: 2271–2284.

Hoffmann AA, Bridle JR. 2022. The dangers of irreversibility in an age of increased uncertainty: revisiting plasticity in invertebrates. *Oikos* **2022**: 1–15.

Hoffmann AA, Merilä J. 1999. Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology & Evolution* **14**: 96–101.

Huala E, Dickerman AW, Garcia-Hernandez M, Weems D, Reiser L, LaFond F, Hanley D, Kiphart D, Zhuang M, Huang W *et al.* 2001. The *Arabidopsis* Information Resource (TAIR): a comprehensive database and web-based information retrieval, analysis, and visualization system for a model plant. *Nucleic Acids Research* **29**: 102–105.

Josephs EB. 2021. Adaptive and maladaptive expression plasticity underlying herbicide resistance in an agricultural weed. *Evolution Letters* **5**: 432–440.

Kelly M. 2019. Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **374**: 20180176.

Kulbaba MW, Sheth SN, Pain RE, Eckhart VM, Shaw RG. 2019. Additive genetic variance for lifetime fitness and the capacity for adaptation in an annual plant. *Evolution* **73**: 1746–1758.

Lande R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of Evolutionary Biology* **22**: 1435–1446.

Lande R, Shannon S. 1996. The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution* **50**: 434–437.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESEQ2. *Genome Biology* **15**: 550.

Lynch M, Lande R. 1993. Evolution and extinction in response to environmental change. In: *Biotic interactions and global change*. Sunderland, MA, USA: Sinauer Associates, 234–250.

Lynch M, Walsh B. 1998. *Genetics and analysis of quantitative traits*. Sunderland, MA, USA: Sinauer Associates.

Mierziak J, Kostyn K, Kulma A. 2014. Flavonoids as important molecules of plant interactions with the environment. *Molecules* **19**: 16240–16265.

Morrissey MB, Bonnet T. 2019. Analogues of the fundamental and secondary theorems of selection, assuming a log-normal distribution of expected fitness. *Journal of Heredity* **110**: 396–402.

Nussey DH, Postma E, Gienapp P, Visser ME. 2005. Selection on heritable phenotypic plasticity in a wild bird population. *Science* **310**: 304–306.

Oostra V, Saastamoinen M, Zwaan BJ, Wheat CW. 2018. Strong phenotypic plasticity limits potential for evolutionary responses to climate change. *Nature Communications* **9**: 1–11.

Orr HA, Unckless RL. 2014. The population genetics of evolutionary rescue. *PLoS Genetics* **10**: e1004551.

Parmesan C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics* **37**: 637–669.

Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. 2017. Salmon provides fast and bias-aware quantification of transcript expression. *Nature Methods* **14**: 417–419.

Peschel AR, Boehm EL, Shaw RG. 2020. Estimating the capacity of *Chamaecrista fasciculata* for adaptation to change in precipitation. *Evolution* **75**: 73–85.

Polechová J, Barton NH. 2015. Limits to adaptation along environmental gradients. *Proceedings of the National Academy of Sciences, USA* **112**: 6401–6406.

Pujol B, Marrot P, Pannell JR. 2014. A quantitative genetic signature of senescence in a short-lived perennial plant. *Current Biology* **24**: 744–747.

R Core Team. 2021. *R: a language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing.

Royer DL, Meyerson LA, Robertson KM, Adams JM. 2009. Phenotypic plasticity of leaf shape along a temperature gradient in *Acer rubrum*. *PLoS ONE* **4**: e7653.

Sgrò CM, Lowe AJ, Hoffmann AA. 2011. Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications* **4**: 326–337.

Shaw RG. 2019. From the past to the future: considering the value and limits of evolutionary prediction. *The American Naturalist* **193**: 1–10.

Shaw RG, Etterson JR. 2012. Rapid climate change and the rate of adaptation: insight from experimental quantitative genetics. *New Phytologist* **195**: 752–765.

Shaw RG, Shaw FH. 2014. Quantitative genetic study of the adaptive process. *Heredity* **112**: 13–20.

Sheth SN, Angert AL. 2016. Artificial selection reveals high genetic variation in phenology at the trailing edge of a species range. *The American Naturalist* **187**: 182–193.

Sheth SN, Kulbaba MW, Pain RE, Shaw RG. 2018. Expression of additive genetic variance for fitness in a population of partridge pea in two field sites. *Evolution* **72**: 2537–2545.

Soneson C, Love MI, Robinson MD. 2015. Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. *F1000Research* **4**: 1521.

Steinger T, Roy BA, Stanton ML. 2003. Evolution in stressful environments II: adaptive value and costs of plasticity in response to low light in *Sinapis arvensis*. *Journal of Evolutionary Biology* **16**: 313–323.

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

Velotta JP, Cheviron ZA. 2018. Remodeling ancestral phenotypic plasticity in local adaptation: a new framework to explore the role of genetic compensation in the evolution of homeostasis. *Integrative and Comparative Biology* **58**: 1098–1110.

Via S, Gomulkiewicz R, de Jong G, Scheiner SM, Schlichting CD, Van Tienderen PH. 1995. Adaptive phenotypic plasticity: consensus and controversy. *Trends in Ecology & Evolution* **10**: 212–217.

Walsh B, Lynch M. 2018. *Evolution and selection of quantitative traits*. Oxford, UK: Oxford University Press.

Walter GM, Abbott RJ, Brennan AC, Bridle JR, Chapman MA, Clark J, Filatov D, Nevado B, Ortiz-Barrientos D, Hiscock SJ. 2020a. *Senecio* as a model system for integrating studies of genotype, phenotype and fitness. *New Phytologist* **226**: 326–344.

Walter GM, Catara S, Bridle JR, Cristaudo A. 2020b. Population variation in early development can determine ecological resilience in response to environmental change. *New Phytologist* **226**: 1312–1324.

Walter GM, Clark J, Cristaudo A, Nevado B, Catara S, Paunov M, Velikova V, Filatov D, Cozzolino S, Hiscock SJ *et al.* 2022a. Adaptive divergence generates distinct plastic responses in two closely related *Senecio* species. *Evolution* **76**: 1229–1245.

Walter GM, Monro K, Terranova D, la Spina E, Majorana MG, Pepe G, Clark J, Cozzolino S, Cristaudo A, Hiscock SJ *et al.* 2022b. Environmental effects on genetic variance and the alignment with plasticity and selection. *BioRxiv*. doi: 10.1101/2021.02.08.430333.

Wang SP, Althoff DM. 2019. Phenotypic plasticity facilitates initial colonization of a novel environment. *Evolution* **73**: 303–316.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 Code and data for analysing phenotype and fitness.

Fig. S1 Map of experiment and sampling locations.

Fig. S2 Genotype choice for gene expression analysis.

Fig. S3 Visualizing variation in gene expression across genotypes, clones and elevation.

Fig. S4 Histogram of differentially expressed genes.

Fig. S5 Expression changes in the genes that function in *Arabidopsis* leaf morphology.

Methods S1 Comparing fitness as the number of flowers vs seed production.

Methods S2 RNA extraction, sequencing and transcriptome assembly.

Methods S3 Contribution of site variance to estimates of genetic variance.

Methods S4 Relating phenotypic traits to the elevational gradient.

Table S1 Sampling locations.

Table S2 Additive genetic (co)variance matrix for absolute fitness across elevation.

Table S3 Sire breeding values for fitness at 2000 m.

Table S4 Multiple regression to quantify phenotype-fitness associations.

Table S5 Significant gene ontology terms for differentially expressed genes.

Table S6 Differentially expressed genes for leaf development and shape in *Arabidopsis*.

Please note: Wiley is not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.