Adaptive plasticity in development rate and genetic variance in survival increase the potential for adapting to novel environments

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ABSTRACT

Plasticity in early development is expected to be crucial for helping natural populations adjust to environmental change. Where such plasticity fails to maintain fitness, genetic variance in plasticity or survival could help populations adapt to the novel conditions. To test this prediction, we used two closely related, but ecologically distinct, species of Sicilian daisy (*Senecio,* Asteraceae) adapted to high and low elevations on Mount Etna. Using a paternal half-sibling breeding design, we generated seeds of both species and planted them across an elevational gradient representing the native range of each species, and two intermediate elevations. We tracked mortality and measured the number of days it took seedlings to establish. We found that each species survived better at their native elevation, and better than the species from the other habitat. Genetic variance in relative survival was near zero within the native range, but increased in the novel environments for both species. Plasticity increased development time at higher elevations. At novel elevations, selection was in the direction of plasticity for the low-elevation species, but the opposite direction for the high-elevation species. Genetic variance for greater plasticity was associated with higher survival, but only for the low-elevation species. Together, these results suggest that while genetic variance in survival increased for both species, genetic variance for adaptive plasticity differs among species. Understanding adaptive potential in novel environments therefore needs to consider how species vary in their capacity and underlying genetic variation for adaptive plasticity.

Keywords: additive genetic variance, early development, evolutionary rescue, novel environments, phenotypic plasticity, selection gradient, survival

Introduction

Populations facing increasingly unfamiliar environments will need to shift their range or adapt if they are to persist. By adjusting the phenotype across environments, plasticity can help genotypes within the population to maintain fitness as environments vary (Via 1993; Via et al. 1995). In particular, plasticity during early development can be crucial for determining whether organisms reach maturity and are able to reproduce, which will be fundamental for maintaining fitness as environments change (West-Eberhard 2003; Smallegange 2022). Where plasticity can no longer maintain fitness, genotypes are expected to vary more in their sensitivity to the new environment, which can increase the potential for adaptation (Lande 2009; Chevin et al. 2010; Chevin and Lande 2011). Although genetic variation in plasticity at early life history stages could be important for both persistence and subsequent adaptation, the lack of field experiments that quantify genetic variance for early life history performance means that we lack the information to understand how the potential for population persistence in novel environments.

Understanding the adaptiveness and ecological limits of plasticity for natural populations remains an ongoing challenge. Plasticity has been shown to change as species become adapted to different environments (Murren et al. 2014; Murren et al. 2015; Walter et al. 2022), but plasticity is rarely linked to performance as environments become novel (but see Steinger et al. 2003; Ghalambor et al. 2015; Walter et al. 2020). Although plasticity in early development is widespread, it is often found have weak links with fitness and can be detrimental (i.e., nonadaptive) for persisting in a novel environment (Ghalambor et al. 2007; Ghalambor et al. 2015; Acasuso-Rivero et al. 2019). Understanding the limits to adaptive plasticity in early development is fundamental for quantifying the potential for species to shift their range or adapt to novel environments. (Angert and Schemske 2005; Angert et al. 2008; Walter et al. 2018; Hargreaves and Eckert 2019).

Where adaptive plasticity fails to help maintain fitness, selection on beneficial genetic variation in the novel environment will be crucial for determining the potential for adaptation to help persistence. Fisher's fundamental theorem of natural selection predicts adaptation as the amount of genetic variance in fitness, $V_A(W)$, relative to mean absolute fitness ($\overline{W}$). This captures genetic variance in relative fitness as $V_A(\omega) = \frac{V_A(W)}{\overline{W}}$, which predicts the potential to increase mean fitness in the next generation (Fisher 1930). Studies have found evidence for greater genetic variance in fitness in natural populations (Hendry et al. 2018; Bonnet et al. 2022), and in a novel environment (Walter et al. 2020). However, we lack data connecting relative fitness with variation in performance at early life history stages under natural conditions, especially as conditions at the edge, and then outside, the ecological range are encountered (Angert et al. 2020). This is because studies that quantify additive genetic variance in performance using field experiments across ecological gradients are rare. We therefore lack the data to understand the capacity for adaptation and plasticity to maintain fitness in

environments as they become less familiar.

To understand how genetic variance in survival changes as environments become less familiar, we generated seeds of two closely related but ecologically divergent species of Sicilian daisy (*Senecio*, Asteraceae), which we planted across an elevational gradient on Mt. Etna. *Senecio chrysanthemifolius* is a short-lived perennial with dissected leaves that occupies disturbed habitats (e.g. roadsides) at elevations under 1,000m.a.s.l. (metres above sea level) on Mt. Etna, as well as throughout Sicily. By contrast, *Senecio aethnensis* is a perennial with entire glaucous leaves endemic to lava flows above 2,000m.a.s.l. on Mt. Etna, where individuals grow back each spring after being covered by snow in winter. These two species show differences in plasticity, with *S. chrysanthemifolius* exhibiting a greater capacity for adaptive plasticity when faced with novel environments (Walter et al. 2021; Walter et al. 2022).

We used a paternal half-sibling breeding design to produce seeds for c.100 families for each species, which we reciprocally planted across an elevational gradient representing the native range of each species, the edge of their range, and conditions outside their range (**Fig. 1**). For seedlings that emerged, we tracked mortality, and measured the time it took them to establish. We used these data to test three hypotheses to understand how populations respond to novel environments. First, we test how adaptive divergence in early life history traits has occurred between the contrasting species on Mt Etna. Second, we estimate genetic variance in survival to test whether adaptive potential increases in novel environments. Third, we test whether plasticity in the time to reach development can predict fitness in novel environments, and whether genetic variance in development rate is associated with survival in novel environments.

Methods and materials

Breeding design and field experiment

To produce seeds of both species, we collected cuttings from c.80 individuals in natural populations of both species, and we propagated one cutting from each individual in the glasshouse. When plants produced buds, we covered branches with perforated bread bags to exclude pollinators while providing adequate airflow. For each species, we randomly assigned half of the individuals (which are hermaphroditic and self-incompatible) as females (dams), the other half as males (sires), and then mated three sires to three dams in a block design that produced c.100 full-sibling families for each species (**Fig. 2a**). We randomly mated sires to dams in 3×3 blocks (three sires mated to three dams) in a paternal half-sibling breeding design (Lynch and Walsh 1998) to produce 94 families of *S. aethnensis* (n=36 sires, n=35 dams) and 108 families of *S. chrysanthemifolius* (n=38 sires, n=38 dams). We performed crosses by removing flowerheads from sires and rubbing their

anthers on the flowerheads of the dams. Fertilised flowerheads were labelled and covered with mesh bags to catch the seeds once mature.

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

We planted 100 seeds from each family at four elevations on Mt. Etna that included the native habitats of both species (500m and 2,000m) and two intermediate elevations (1,000m and 1,500m) (**Fig. 2a**; Walter et al. 2022). Vagrant individuals of these species are often found between 1,000-1,500m, which means the intermediate elevations represent areas that could allow range expansions. We planted 25 seeds at each site randomised into five experimental blocks (*S. aethnensis* n=432 seeds/block, n=2,160 seeds/site; *S. chrysanthemifolius* n=540 seeds/block, n=2,700 seeds/site; Total N=19,232 seeds). However, *S. aethnensis* grew more slowly and produced less flowers in the glasshouse, which made it difficult to produce 100 seeds for each family, meaning that four, eight and ten families were represented by 15, 20 and 25 seeds per elevation, respectively.

To prepare each experimental block, we cleared the ground of plant matter and debris, and then placed a plastic grid (4cm square cells) on the ground. We attached each seed to the middle of a toothpick using non-drip super glue and then pushed each toothpick into the soil so that the seed sat 1-2mm below the soil surface (Walter et al. 2016). To replicate natural germination conditions, we suspended 90% shade-cloth 20cm above each plot and kept the seeds moist until germination ceased (2-3 weeks). After this initial period, we replaced the shade-cloth with a lighter 40% shade-cloth that replicated shade that naturally-growing plants are often found under. This maintained a strong environmental effect at each elevation without exposing plants to extreme environmental conditions. We recorded seedling emergence, survival and whether plants reached seedling establishment (produced ten leaves). The experiment was completed in January 2020 when mortality stabilised at all sites (**Fig. S2**) and plants started growing into each other, which increased competition and would have likely biased further data collection.

Quantifying survival and development across elevation for both species

To test how both species survived and developed across the elevation gradient, we applied the linear mixed model

131 $$y_{ijklm} = Sp_i \times E_j + b_k + f_l + e_{m(ijkl)}\,, \qquad (1)$$

132 where the fixed effects include $Sp_i \times E_j$ as the *i*th species planted at the *j*th elevation. Random effects include
133 b_k as the *k*th experimental block, f_l as the *l*th family within species, and $e_{l(ijk)}$ represents the residual
134 variation. We applied equation 1 separately for emergence, seedling establishment and survival to the end of
135 summer, with each variable included as a binary response variable (y_{ijkl}) using a logit link function with the
136 R package *lme4* (Bates et al. 2015). Second, to compare survival across time at each elevation, we used the R
137 package *coxme* (Therneau 2012) and included the censored survival at the end of the experiment as the
138 response variable. We used log-likelihood ratios to test for significant elevation×species interactions, and
139 then used the R package *emmeans* (Lenth 2019) to test for pairwise differences between species and across
140 elevations.

141 *Estimating genetic variance in survival*

142 To estimate genetic in survival for both species at each elevation, we used the R package *MCMCglmm*
143 (Hadfield 2010) to apply the generalised linear mixed model

144 $$y_{ijkl} = s_i + d_j + b_k + e_{l(ijk)}\,, \qquad (2)$$

145 where the response variable (y_{ijkl}) is survival as the presence/absence of plants after mortality stabilised at
146 the end of summer (September 12th; **Fig. S2**). We therefore used a probit link function to model survival as a
147 binary trait. Random effects include s_i as the *i*th sire, d_j the *j*th dam, b_k the variance among blocks within a
148 transplant elevation, and $e_{l(ijk)}$ the residual variation. After back-transforming parameters to the data scale
149 using *QGlmm* (de Villemereuil et al. 2016), we estimated genetic variance in relative fitness by dividing V_a
150 by the squared mean survival. To estimate genetic correlations between transplant elevations, we applied
151 equation 2 but we included transplant elevation as a fixed effect and specified unstructured covariance
152 matrices for each of the random effects, which estimates a 4×4 matrix with each elevation along the diagonal
153 and the off-diagonal containing the genetic covariances between elevations.

154 To apply equation 2, we used chains with a burn-in of 150,000 iterations, a thinning interval of 1,500
155 iterations and saving 2,000 iterations that provided the posterior distribution for all parameters estimated. We
156 confirmed model convergence by checking the chains mixed sufficiently well, that autocorrelation was lower
157 than 0.05, and that our parameter-expanded prior was uninformative (Hadfield 2010).

158 *Estimating selection on development time*

159 First, to understand how plasticity changed development time across elevation, we used equation 1 to
160 quantify differences in plasticity for both species at all elevations. Second, quantify how development time

161 changed with survival across all elevations, we calculated relative survival for each species by dividing by
162 mean survival (estimated across all elevations). We then used the linear regression

163 $$y_{ijkl} = Development \times Sp_i + b_j + f_k + e_{l(ijk)}\,, \qquad (3)$$

164 where y_{ijklm} is relative survival as the continuous response variable. Fixed effects include the interaction
165 between development time (across all elevations) and species ($Development \times Sp_i$). Random effects
166 include b_j as the *j*th block and f_k as the *k*th family within species, and $e_{l(ijk)}$ is the residual variation. To
167 analyse and interpret selection on the scale of development time, and not on the latent scale of a generalized
168 linear model, we included relative survival as a continuous response variable that assumes a gaussian
169 distribution. Although this violates the assumption of normality, this is the approach suggested and
170 commonly used (Lande and Arnold 1983; Mitchell-Olds and Shaw 1987; Hajduk et al. 2020).

171 To quantify selection on development time for both species reciprocally transplanted at the native habitats,
172 we analysed only the elevational extremes and included transplant elevation as an additional fixed effect
173 ($Development \times Elevation \times Sp_i$) in equation 3. A significant three-way interaction suggests that selection
174 on development rate differs between species and changes across elevation.

175 *Quantifying selection on plasticity in development time*
176 To identify whether genetic variation in plasticity is associated with survival at novel elevations, we
177 estimated plasticity in development time for each family using the coefficient of variation: variance in
178 development time across 500m and 2,000m divided by the mean development time. This yields plasticity as
179 the variance in development, but standardised for each family so that differences among families represent
180 genetic variance (in the broad-sense) in development time. Using generalised linear regression with a logit
181 link function, we then tested whether families with greater plasticity (across elevations) showed greater
182 survival at the novel elevation.

183

184 **Results**

185 *Evidence of adaptive divergence and greater genetic variance for survival in novel environments*
186 Seedling emergence was 75-85% for *S. chrysanthemifolius* across elevations, and 50-70% for *S. aethnensis*
187 (**Fig. 2a**). For *S. aethnensis,* c.60% of seedlings reached establishment at all elevations, which contrasted
188 with *S. chrysanthemifolius* where seedling establishment was 75% at its home site but gradually reduced to
189 25% at the 2,000m elevation (**Fig. 2b**). Mortality stabilised after summer (**Fig. S2**) at which point the two
190 species showed evidence of adaptive divergence to their respective habitats as: (1) greater survival

191 probability of both species at their home site compared to the habitat of the other species (**Fig. 2c**), supported
192 by a significant species×elevation interaction ($\chi^2(3)=1678.30$, $P<0.00001$); and (2) greater survival of the
193 native species compared to the species from the other habitat (**Fig. 2c**), meaning that *S. chrysanthemifolius*
194 survived better than *S. aethnensis* at low elevations (500m $Z=18.7$, $P<0.00001$; 1,000m $Z=3.2$, $P=0.0014$),
195 whereas *S. aethnensis* survived significantly better at higher elevations (1,500m $Z=8.4$, $P<0.00001$; 2,000m
196 $Z=14.9$, $P<0.00001$). Low survival of *S. aethnensis* at 500m and of *S. chrysanthemifolius* at 1,500m and
197 2,000m occurred as high mortality early in growth and development, within 50 days of emergences (**Fig. 3**).

198 *Genetic variance increases in novel environments*

199 For both species, genetic variance in relative survival was lowest (and near zero) at their home site and
200 elevations within their home range (**Fig. 4a**), but significantly greater at their novel elevations (**Fig. 4a**),
201 suggesting that genetic variance in relative seedling survival increases in more novel environments. Genetic
202 variance in absolute survival was greater at the novel elevation for *S. aethnensis,* and similarly greater for all
203 sites away from the home site for *S. chrysanthemifolius* (**Fig. 4b**).

204 *Selection on development time*

205 Development time changed significantly across elevation ($\chi^2(3)=291.81$, $P<0.00001$) with plants reaching
206 seedling establishment faster at lower elevations (**Fig. 5**). A significant interaction between development time
207 and elevation suggested that species responded differently to elevation ($\chi^2(3)=13.39$, $P=0.00387$). Seedlings
208 of *S. aethensis* tend to establish earlier than *S. chrysanthemifolius,* with greater differences in development
209 time observed at 1,500m (**Fig. 5**).

210 We predicted that if development time was associated with survival in novel environments, we would
211 observe selection for faster development at low elevations, and selection for slower development at high
212 elevations. We found a significant three-way interaction between development time, species and elevation
213 ($\chi^2(1)=50.22$, $P<0.00001$), suggesting that selection on development time changed across elevation and
214 differed between species (**Fig. 6**). At 500m, selection on the native species, *S. chrysanthemifolius*, favoured
215 faster development (slope for *S. chrysanthemifolius* = -0.0069±0.001 [1 SE]), whereas selection on *S.*
216 *aethnensis* favoured slower development (slope for *S. aethnensis* = 0.0098±0.002 [1 SE]). At 2,000m,
217 selection on the native species (*S. aethnensis*) was weak (slope = 0.00008±0.001), but strong and positive for
218 *S. chrysanthemifolius* (slope = 0.0035±0.001). Selection on development time in the novel environments was
219 therefore in the direction of plasticity for *S. chrysanthemifolius* at 2,000m (slower development at higher
220 elevations), but in the opposite direction of plasticity for *S. aethnensis* at 500m (**Fig. 6**).

221 *Selection on plasticity in development time*

222 Families of *S. chrysanthemifolius* that showed greater plasticity in development time, estimated as the

223 coefficient of variation across elevations, had significantly greater survival at novel elevations
224 (slope=0.14±0.02; $\chi^2(1)=34.02$, P<0.00001). At 500m, *S. aethnensis* showed the same trend, but it was not
225 significant (slope=0.017±0.02; $\chi^2(1)=0.52$, P=0.4708). These results suggest that genetic variation in
226 development time is associated with survival in a novel elevation for *S. chrysanthemifolius,* but not for *S.*
227 *aethnensis.*

228

229 **Discussion**

230 We find support for three hypotheses that are important for understanding how populations respond to
231 environmental gradients: First, adaptive plasticity maintains high survival in more familiar environments,
232 which is associated with low genetic variance in survival (**Figs. 3-4**). Second, where mean survival was
233 greatly reduced in novel environments, genetic variance in survival increased, which improves the adaptive
234 potential of the population (**Figs. 3-4**). Third, plasticity in the rate of development at early life history stages
235 can determine fitness in novel environments, with our results suggesting that genotypes that are better able to
236 cope with large environmental change will be those that can provide the potential to help populations persist
237 in novel environments (**Fig. 6**). These results provide further evidence that, compared to the high-elevation *S.*
238 *aethnensis, S. chrysanthemifolius* possesses a better capacity for adaptive plasticity, and genetic variation that
239 could be more beneficial for coping with environmental change.

240 *Plasticity in early life history traits*
241 Connecting plasticity in early development with performance as novel environments are encountered in the
242 field remains an ongoing challenge (Acasuso-Rivero et al. 2019). The extent to which plasticity is reversible
243 for particular traits is likely to determine the level of benefit that plasticity provides in novel environments
244 (Hoffmann and Bridle 2022). Walter et al. (2020) showed that lower phenotypic plasticity in
245 morphology/investment traits, which are irreversible once the plastic response has been decided, was
246 associated with greater fitness in a novel environment. By contrast, greater plasticity in gene expression
247 (which is likely to be more reversible) for genes associated with physiological responses to temperature and
248 light, was associated with greater fitness in the same novel environment. It is therefore likely that decisions
249 for irreversible traits, such as the rate of development and associated traits, early in life history will determine
250 the ability for genotypes to match large changes in the environment and maintain fitness in novel conditions.
251 Given that genetic variation in plasticity segregates in the population, the frequency of genotypes that are
252 pre-adapted to make adaptive decisions in novel conditions will be important to quantify in order to
253 understand adaptive potential and evolutionary rescue in response to environmental change (Hargreaves and
254 Eckert 2019).

Connecting genetic variance in plasticity with adaptive potential

In our results, greater plasticity in development time in the low elevation species increased survival at novel high elevations, suggesting that selection on genetic variation in development time plasticity could help populations to cope with environmental change. By contrast, the high elevation species only showed a weak association between plasticity and survival at low elevations. Genetic variation in plasticity that will help populations adapt to novel environments is therefore likely to depend on the evolutionary history of the species (Liu et al. 2022; Walter et al. 2022), with high elevation species likely to struggle to persist and then adapt to warmer conditions. However, the high elevation species was able to maintain greater survival across more of the elevational gradient than the low elevation species (**Fig. 3**). This suggests that even though genetic variance in plasticity might not be as adaptive, it is possible that the high elevation species has evolved to cope with greater environmental variation at early life history stages.

The potential for evolutionary rescue

We provide strong evidence that plasticity at early life history plays an important role in persisting in novel environments, and that genetic variation in plasticity could increase adaptive potential. However, we were unable to measure later life history stages or estimate demographic parameters that would allow us to predict the potential for evolutionary rescue. While genetic variance in survival can help to predict adaptive potential, if surviving plants do not produce many offspring, then evolutionary rescue will be unlikely to occur. Quantifying genetic variance in (relative and absolute) traits related to lifetime fitness is a challenge for all field experiments and requires tracking of plants from seed to maturity and fecundity (Sheth et al. 2018; Kulbaba et al. 2019). Such information will be vital for understanding adaptation at range margins, and the potential for evolutionary rescue in response to environmental change (Bridle and Hoffmann 2022).

Even if adaptive genetic variation is available, the likelihood of evolutionary rescue in a novel environment will be determined by whether the population can maintain a large enough population so as to remain viable as adaptation occurs (Chevin et al. 2013; Gonzalez et al. 2013; Polechová and Barton 2015; Bridle et al. 2019). Evolutionary rescue will not be possible if the population size in novel environments is reduced to the point where drift overcomes selection and prevents adaptation. Understand the potential for evolutionary rescue will require quantifying how genetic variation in early life history traits can not only allow persistence in the current generation, but whether genotypes that show adaptive plasticity can produce enough offspring to maintain population size and allow adaptation (Kulbaba et al. 2019).

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390 **Figures**

391

392 **Fig. 1** The experimental design. **(a)** For two Etnean *Senecio* species, we mated 36-38 sires each to 3 dams,
393 and produced 100 seeds from each mating (family). We planted 25 seeds from each family at each of four
394 elevations that represent the native site of each species and two intermediate elevations. **(b)** Photo of an
395 experimental block at 2,000m, 8 weeks after seeds were sowed (*S. chrysanthemifolius* on left).

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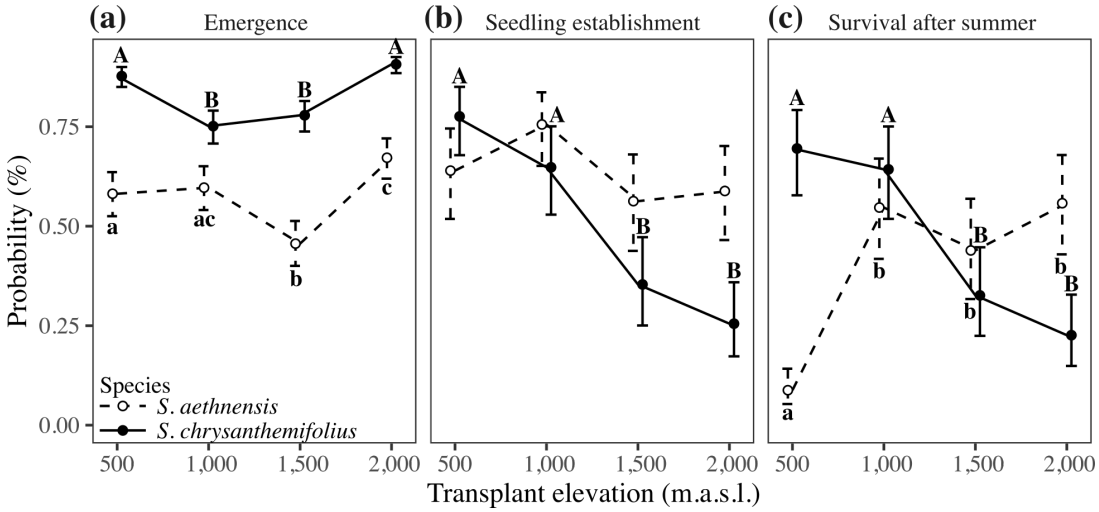

Fig. 2 Evidence for adaptive divergence in life history and survival for the two Etnean *Senecio* species. Lowercase and uppercase letters denote significant differences (α=0.05 with bonferroni adjustment) across elevation for *S. aethnensis* and *S. chrysanthemifolius*, respectively. **(a)** Seedling emergence was greater for *S. chrysanthemifolius,* which was greater at the elevational extremes. Emergence was greatest for *S. aethnensis* at its native elevation (2,000m). **(b)** The probability of reaching seedling establishment remained similar across elevation for *S. aethnensis*, but was reduced at higher elevations for *S. chrysanthemifolius*. **(c)** As evidence of adaptive divergence, both species survived to the end of summer (end of September) better at their home site than the foreign species, and better at their home site than at the novel elevation.

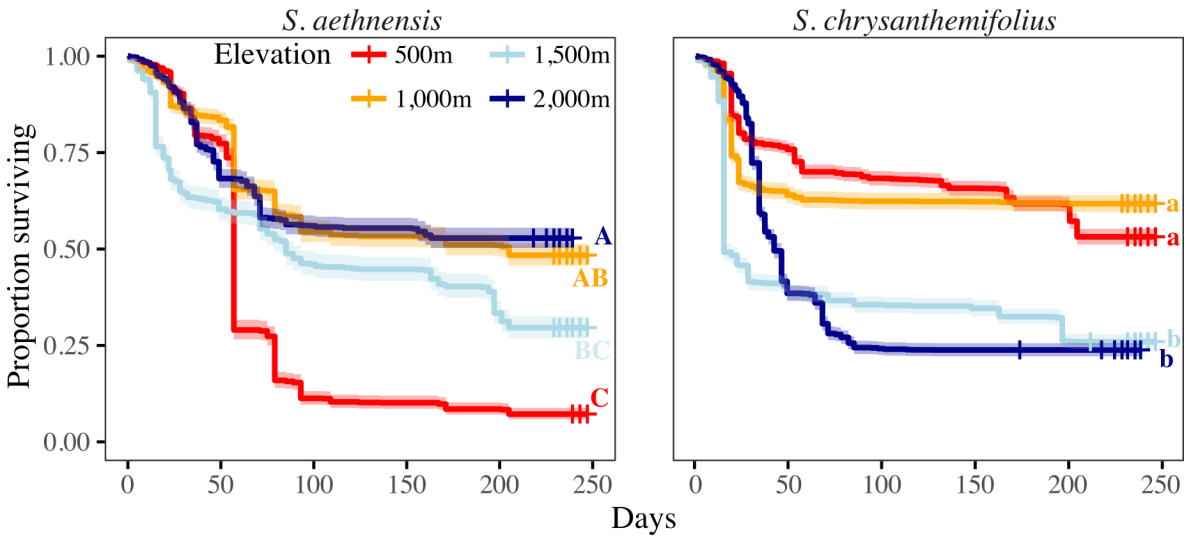

Fig. 3 Differences in survival for the two species reflect adaptive divergence to their natural habitats. Letters represent significant differences in survival. *Senecio aethnensis* survived best at its native 2,000m site, but suffered high mortality at 500m. While the 1,500m site showed intermediate survival, survival at the 1,000m site at the edge of the range was as high as the native site. By contrast, *S. chrysanthemifolius* shows high survival at the two lower elevations within the native range (500-1,000m), and lower survival at the two higher elevations.

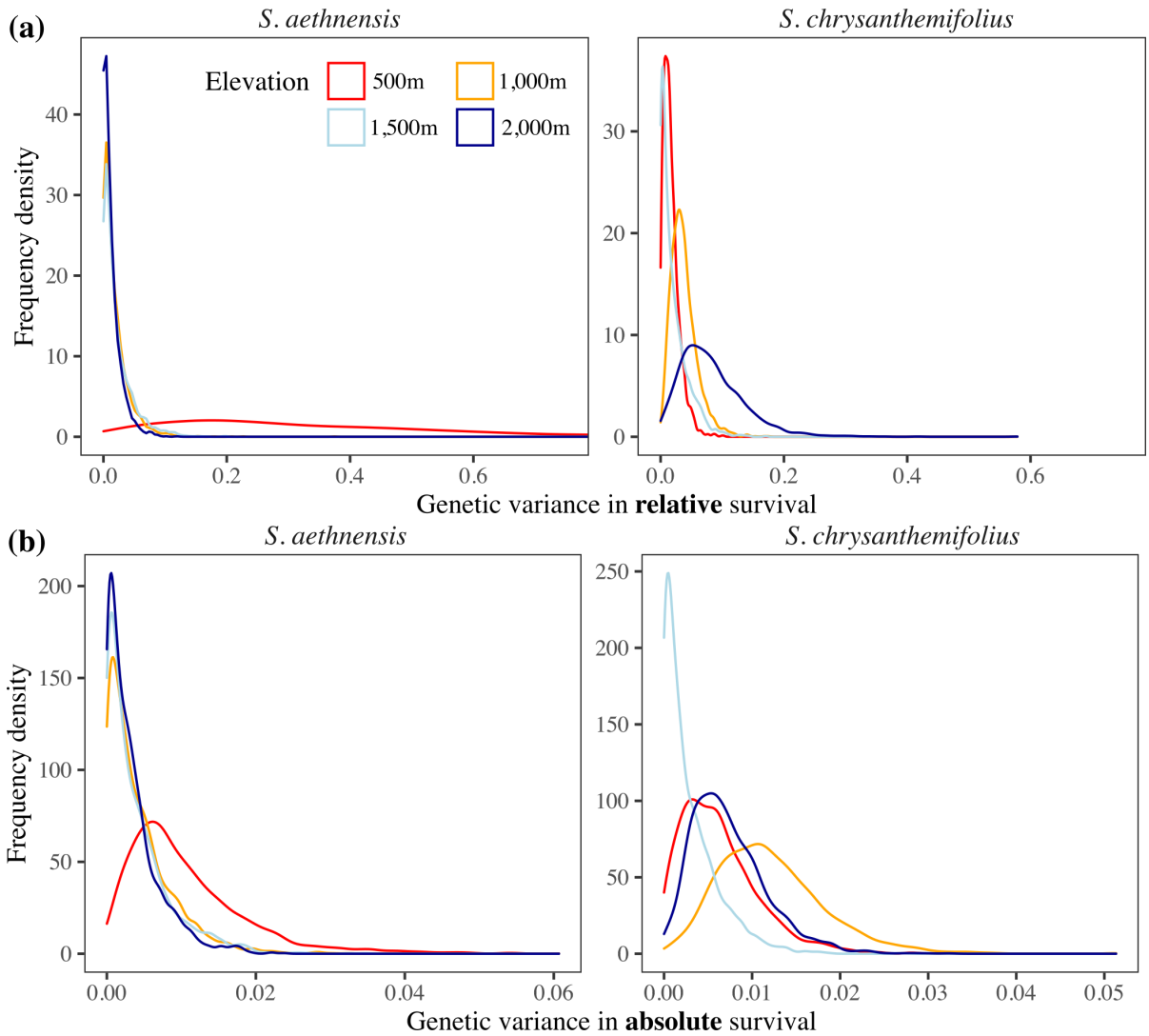

Fig. 4 Genetic variance in **(a)** relative and **(b)** absolute survival for both species at all elevations. **(a)** At their native sites, genetic variance in relative fitness was near zero, but significantly greater at the novel elevations, where their posterior distributions did not overlap at >85% Highest Posterior Density (HPD) interval. **(b)** Genetic variance in absolute fitness was also greater at the novel 500m elevation for *S. aethnensis* when compared to other elevations. For *S. chryanthemifolius,* however, genetic variance in absolute fitness was similarly high at all elevations, except 1,500m.

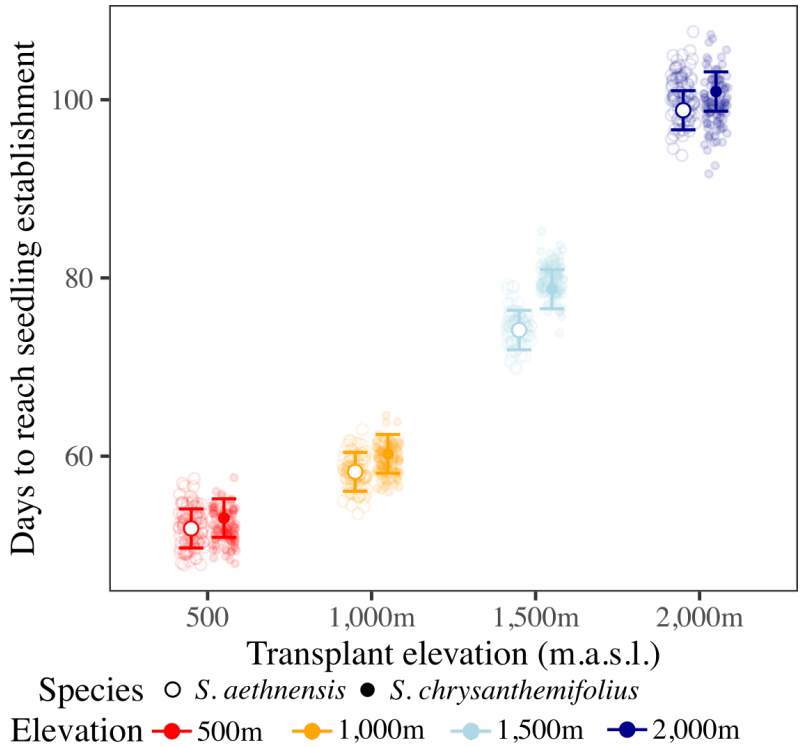

Fig. 5 Plasticity in development rate was similar for *S. aethnensis* (dashed lines) and *S. chrysanthemifolius* (solid lines) at each elevation. Development rate was much faster at lower elevations.

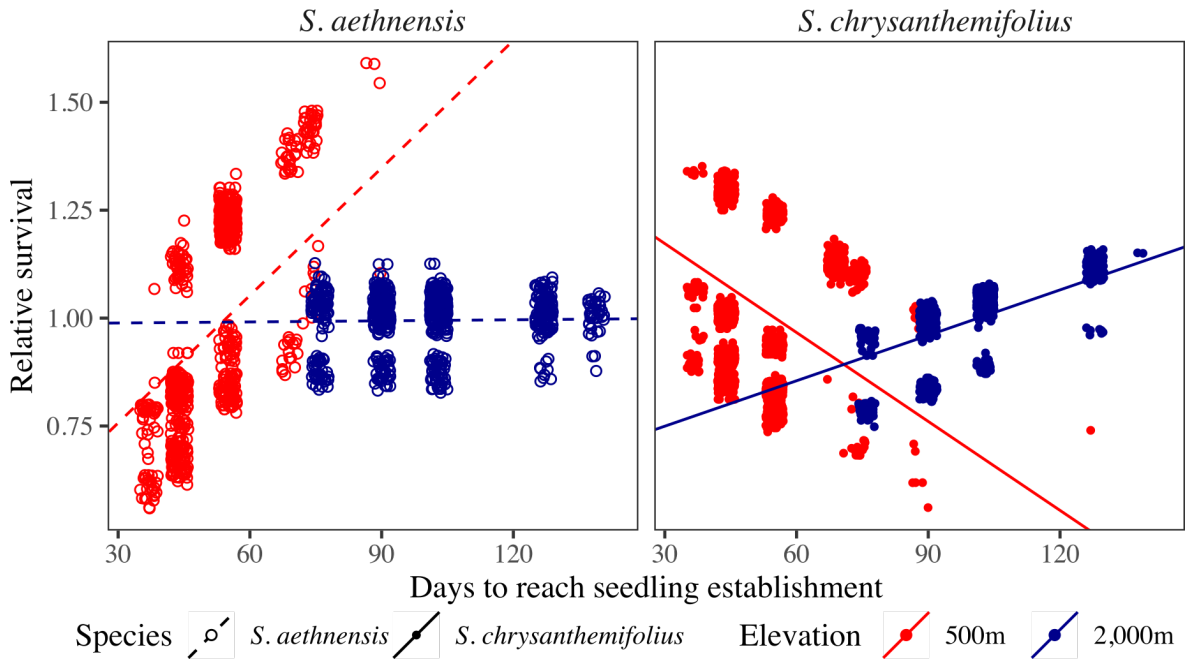

Fig. 6 Phenotypic selection on development rate at the home (native) sites of both species. Circles represent individual plants and lines represent the linear regression. Selection on development time changed across elevation for both species.