Adaptive maternal effects in early life history traits help to maintain ecological resilience in novel environments for two contrasting *Senecio* species

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Abstract

Maternal effects can maintain fitness across environmental conditions regularly experienced by a population. However, the consequences of maternal effects for ecological resilience in novel environments remain poorly understood. We produced seeds by breeding field-collected individuals of two closely related, but ecologically distinct, species of Sicilian daisy (*Senecio,* Asteraceae) in a quantitative genetics design. Using siblings of the same families, we quantified seedling root morphology in the laboratory, and planted seeds at four elevations that included the native elevation of each species. Seedling survival of both species was high at their native elevations, but declined at the novel elevation furthest from their native range, demonstrating adaptive divergence. Seedling development rate changed with elevation similarly for both species, and aligned with selection for faster development at lower elevation and slower development at higher elevation. Selection on paternal (sire) variance in development rate was consistent across elevation, and opposed phenotypic selection in the novel elevations. By contrast, selection on maternal variance in development rate changed with elevation and aligned with the direction of selection at both the native and novel elevations, suggesting that maternal effects underlying plasticity in development rate can increase fitness in novel environments. Selection on root morphology was only strong for the high elevation species, and occurred in the direction of the native phenotype for maternal variance, but not for sire variance. Together, these results suggest that maternal variation in development time plasticity and root architecture can increase ecological resilience in novel habitats, despite theory suggesting that they may reduce fitness in such situations.

Keywords: additive genetic variance, early development, development rate, evolutionary rescue, fitness trade-offs, maternal effects, plasticity, root architecture, transgenerational plasticity

Introduction

Understanding how populations respond to fitness declines to avoid extinction will help predict ecological resilience in a rapidly changing world (Visser 2008). Where plasticity is adaptive, population fitness can be maintained across familiar environments by adjusting the phenotype to track the changing optima created by environmental variation (Charmantier et al. 2008). However, existing plastic responses are unlikely to be adaptive in environments that populations have never experienced before, leading to rapid declines in fitness outside the native range (Ghalambor et al. 2007; Valladares et al. 2007). In such situations however, selection on genetic variation can provide the potential for rapid adaptation that leads to the 'evolutionary rescue' of populations (Gomulkiewicz and Holt 1995; Bell and Gonzalez 2009). Understanding the adaptive potential of populations in novel environments is critical for identifying when and where populations and ecological communities can continue to persist in the new environments created by global change.

A key issue in understanding plasticity and its fitness effects in novel environments is identifying the consequences of transgenerational effects (Uller et al. 2013). Maternal effects contribute to plastic responses when mothers provide resources that help offspring cope with environmental variation (Donohue and Schmitt 1998; Galloway 2005; Räsänen and Kruuk 2007). Such adaptive maternal effects have been identified in a variety of taxa (e.g. Sultan 1996; Galloway and Etterson 2007; Galloway et al. 2009; Zhang et al. 2012; Dey et al. 2016), but such experiments are commonly conducted in the laboratory, or rarely test responses to novel environments that differ substantially from those experienced by either parent (but see Yeh and Price 2004). This means that the consequences of maternal effects for offspring fitness in novel environments are not well understood (Visser 2008; Uller et al. 2013; Moore et al. 2019). On one hand, maternal effects will reduce offspring fitness if they generate offspring phenotypes that are unsuitable in novel conditions. On the other hand, maternal effects, such as increased provisioning, could reduce offspring mortality, even if the offspring environments are substantially different to those experienced by the mother (Räsänen and Kruuk 2007).

Fitness declines in novel environments are often caused by selection early on in life history that results in high mortality (e.g. Kim and Donohue 2013; Postma and Agren 2016; Walter et al. 2018; Tysklind et al. 2020), which makes it difficult to identify the traits that are associated with fitness variation. This is because selection removes genotypes before their phenotypes can be measured (Hadfield 2008), and because traits important for early life history are often inherently difficult to measure (e.g. root morphology in small plants). However, it is adjustments in traits at early life history that are likely to be crucial for increasing fitness to maintain ecological resilience in novel environments (Nicotra et al. 2010; Kim and Donohue 2013). By using a quantitative genetics breeding design and connecting early life history phenotypes with survival in

natural conditions, we can test whether additive genetic variation (representing genetic variation in the absence of maternal effects) or maternal variation (representing maternal effects) underlying a given phenotype are associated with fitness in novel environments.

Germination success and development to seedling establishment are principal early life stages in plants. Variation in traits that increase the possibility of transitioning from earlier to later life history stages will therefore have important consequences for increasing plant performance in novel environments (Gray and Brady 2016). In particular, the speed at which plants develop early in life history is often environmentally dependent, can determine fitness later in life history (Burghardt et al. 2015; Walter et al. 2018), and will be likely to determine fitness in novel environments. Furthermore, variation in early root development can have major effects on resistance to stressful conditions (e.g., to drought or temperature extremes), and to different soil types and soil communities, all of which are likely to be critical for resilience in novel environments (Hodge 2004; Smith and De Smet 2012; Gray and Brady 2016). Identifying genetic variation and maternal effects in early development traits that increase survival or yield in novel environments can also identify the potential for improvements of crop traits through selective breeding.

To quantify how additive genetic and maternal variance for early life history traits can maintain resilience in novel environments, we generated seeds by breeding two Sicilian daisy species (*Senecio,* Asteraceae) in a quantitative genetics design and assayed offspring in field and laboratory experiments. *Senecio chrysanthemifolius* is native to disturbed habitats in lowland Sicily and occurs at 400-1,000m.a.s.l (metres above sea level) on Mount Etna. By contrast, *Senecio aethnensis* is endemic to high elevations on Mt. Etna (2,000m and above) where it occurs on lava flows that are covered by snow each winter. A previous reciprocal transplant of cuttings of both species showed that the two species have evolved different forms of plasticity associated with adaptive divergence to habitats at these contrasting elevations (Walter et al. 2020).

We used the seeds produced for each species in two separate experiments by assaying siblings from the same families. In the field, we reciprocally planted seeds across an elevation gradient that included the native habitat of each species, as well as two intermediate elevations. In the laboratory, we planted seeds *in vitro* and quantified the morphology of early root development for both species. We predicted that: (1) Each species would show reduced fitness in the other species' native habitat, providing evidence of adaptive divergence between these two species at the seedling stage. (2) If adaptive maternal effects can reduce fitness declines in novel environments, selection on the maternal component (i.e. maternal variation) of seedling development rate would align better with the development rate of the native species than selection on additive genetic variation (i.e. genetic variation in the absence of maternal effects). Furthermore, when planted at a novel elevation, maternal genotypes sampled from a more similar elevation would perform better

(e.g. lower elevation genotypes would perform better than higher elevation genotypes when planted at a novel low elevation). (3) In the novel environment, selection on the maternal component of early development root traits would align better (than selection on additive genetic variation) with the phenotype of the native species.

Methods and materials

Breeding design

To produce seeds of both species, we first sampled cuttings from c.80 individuals for each species, which we grew at the glasshouse under common garden conditions (see **Methods S1**). When plants produced buds, we covered branches with perforated bread bags to exclude pollinators while providing adequate airflow. We randomly assigned half of the plants 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. 1a**). To conduct controlled crosses, we removed flowers from the sires and rubbed their anthers on the flowers of the dams. Pollinated flowers were labelled and covered with bags made of fine mesh that prevented seed loss. 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.

Field experiment

In spring 2019 (9th May), we planted 25 seeds from each full-sibling family at each of four elevations (500m, 1,000m, 1,500m and 2,000m). These elevations include the home site of each species (2,000m for *S. aethnensis*; 500m for *S. chrysanthemifolius*) and two intermediate elevations (Walter et al. 2020). Five experimental blocks (with five seeds/family/block) were used to represent variation at each elevation (**Fig. 1b**). In total we planted 100 seeds for each family. However, due to a slow growth rate and small numbers of flowers produced by *S. aethnensis* in the glasshouse, we were unable to produce the same number of seeds for each family. This meant that four, eight and ten families were represented by 15, 20 and 25 seeds per elevation, respectively. We used a small drop of non-drip superglue to attach each seed to the middle of a tooth pick (Walter et al. 2016), which we randomised into 18×30 cell grids on polystyrene that replicated the blocks at the field sites (*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).

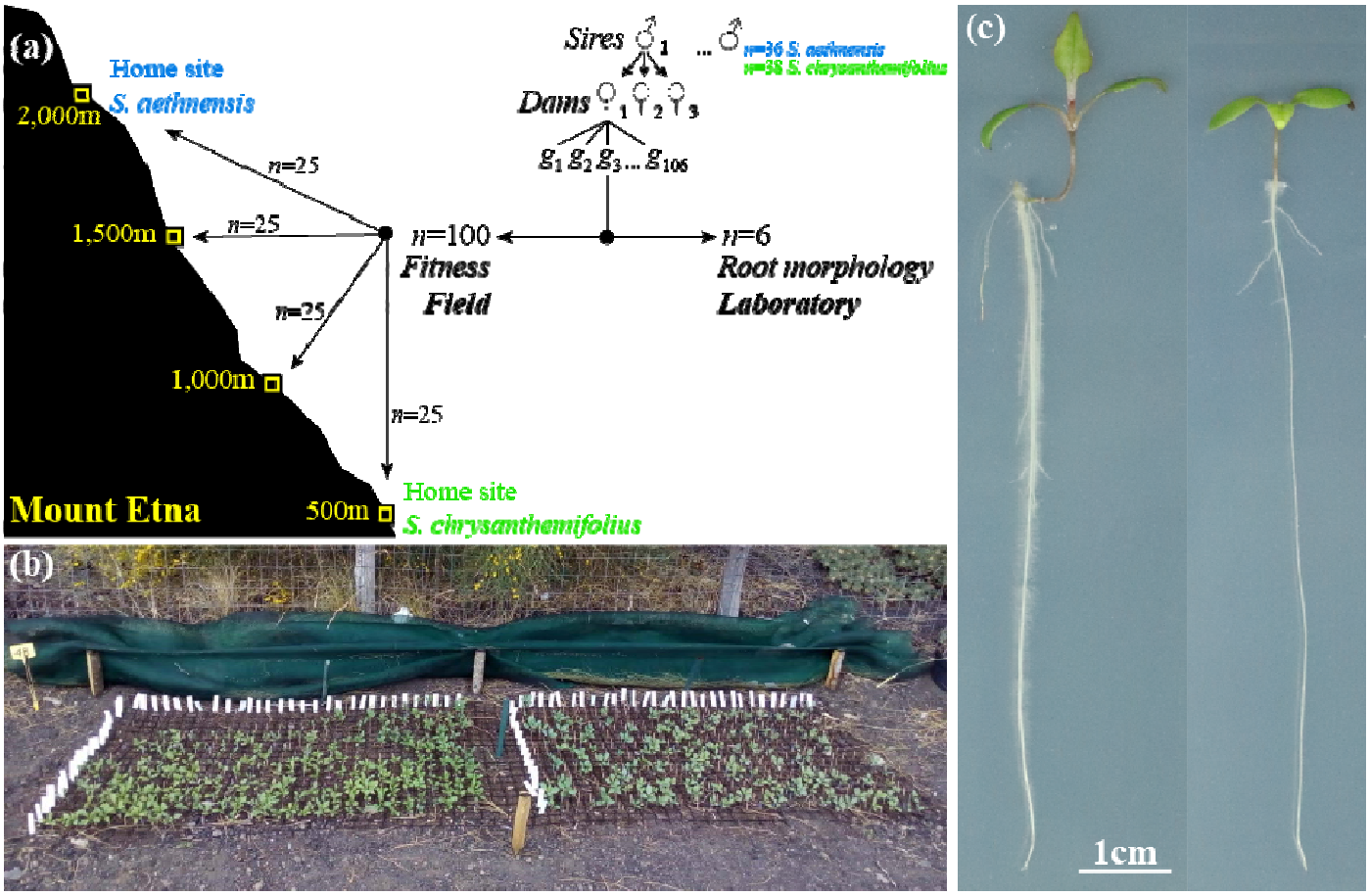

Fig. 1 (a) Schematic of the experimental design. In the glasshouse we used a full-sibling, half-sibling breeding design to mate among randomly sampled individuals from natural populations of each species. Offspring of the same families were planted as seeds at four elevations of Mount Etna and used to assay root morphology in the laboratory. **(b)** An example of a seedling block at 2,000m, eight weeks after the initial seed planting (*S. chrysanthemifolius* on left). **(c)** Pictures taken of example *S. aethnensis* (on left) and *S. chrysanthemifolius* (on right) individuals grown from seed in the lab, and used to quantify root morphology for each family planted in the field. Individuals represent the multivariate mean root morphology of each species. Compared to *S. chrysanthemifolius*, roots of *S. aethnensis* were generally larger, longer and with more branching.

To prepare the field sites, we placed a grid of plastic netting with 4cm square cells on flat soil cleared of plant matter and debris. To plant seeds, we pushed toothpicks into the soil until the seed sat 1mm under the soil surface. To replicate natural germination conditions in early spring, we suspended 90% shade-cloth 20cm above all blocks and kept plots moist until new germinations stabilised (three weeks). Watering was then reduced to once per week. After a further two weeks, we replaced the shade-cloth with a lighter 40% shade-cloth that replicated light shade that the plants are often found under. This maintained a strong environmental effect at each elevation without exposing plants to extreme environmental conditions.

We recorded binary measures of performance: seedling emergence, survival, whether plants reached seedling establishment (produced ten leaves) and whether plants reached maturity (produced a bud). To quantify seedling development rate, we calculated the number of days it took to reach seedling establishment. To record the temperature at all elevations for the duration of the experiment, we positioned three dataloggers (Tinytag, UK) at each site, adjacent to the experimental blocks. The dataloggers show a previously described pattern (Walter et al. 2020) of hot temperatures (>35°C) at low elevations in summer and cold temperatures (<0°C) at higher elevations in winter (**Fig. S2**). The experiment was completed in December 2019 when mortality stabilised at all sites (**Fig. S3**) and plants started growing into each other, which increased competition and would have likely biased further data collection.

Laboratory quantification of root traits for families grown in the field

To quantify root growth for seeds of the breeding design of each species, we grew six individuals from each full-sibling family on agar in sealed, sterile plates (*S. chrysanthemiolius* n=324 plates, N=647 seeds; *S. aethnensis* n=312 plates, N=624 seeds). Due to the large number of plates, and the availability of *S. aethnensis* seeds after *S. chrysanthemifolius*, and given comparisons within species were the main focus of this study, we conducted separate experiments for the two species, but used the same growth chamber and equipment. Methods were the same for both species, but after pilot experiments, slight adjustments were made to maximise germination for each species. These adjustments (detailed below) ensured germination and growth of the roots occurred on the agar plate and not in the petri dishes they were stratified in.

All seeds were sterilised using a 10% bleach solution, and transferred onto filter paper in Petri dishes with 3ml of water that were then sealed with Parafilm. Sterilised seeds were stratified by storing them in the dark at room temperature for seven days for *S. chrysanthemfolius* and three days for *S. aethnensis*. Two seeds were then sown onto agar (**Methods S1**) on each 12cm square plate at two points equidistant from the plate centre and edge. After sealing each plate with microporous tape, we randomised and stored them in a growth chamber set to 21°C and 50% humidity with a 15:9 hour light:dark cycle. Plates were kept inclined at 60° to promote downward root growth along the surface of the agar.

When 20% of plants reached the bottom of the plates (8 days for *S.chrysanthemfolius*; 9 days for *S.aethnensis*), we removed the plates from the growth chamber and photographed them using a Canon EOS 200D camera. The difference in growth period was based on a difference in growth rate between species observed in pilot studies (**Fig. 1c**). Plates were photographed and images processed using *Gia Roots* (Galkovskyi et al. 2012), which produced 14 root morphology traits for each plant. After exploring preliminary data, we found that three traits were sufficient to quantify root morphology: root network area, root network width and specific root length (total root system length divided by volume), where larger values

suggest long root systems with thin roots.

Statistical analyses

(1) Species differences in seedling performance across elevation: We used *R* (v.3.6.1; R Core Team 2019) for all analyses. To compare performance of the two species across elevation for several seedling performance traits (detailed below), we used the generalised linear mixed model:

$$y_{ijklm} = S_i + T_j + S_i \times T_j + g_{k(i)} + b_{l(j)} + e_{m(ijkl)}\,, \qquad (1)$$

where fixed effects included S_i represents the *i*th species, T_j the *j*th elevation and $S_i \times T_j$ the interaction between species and elevation. Differences among full-sibling families within species ($g_{k(i)}$) and differences among blocks within elevation ($b_{l(j)}$) were included as random effects. The residual error variance is represented by $e_{m(ijkl)}$. We applied equation 1 to several different estimates of field performance, which meant that we changed the response variable (y_{ijklm}) depending on which performance trait we were analysing. To quantify differences in survival, we included the total days survived and the censor date as the end of the experiment, and implemented a random-effects survival model using the package *coxme* (Therneau 2012). For the remaining performance traits (probability of emerging, reaching seedling establishment, reaching maturity and surviving to the end of summer) we applied equation 1 using the package *lme4* (Bates et al. 2015) with a binomial error distribution. For each implementation of equation 1, we tested the significance of the species×elevation component using a log-likelihood ratio test. Using the package *emmeans* (Lenth 2019), for each species we conducted post-hoc pairwise tests (with bonferroni correction for multiple testing) to test for significant differences in performance across elevation.

(2) Selection on seedling development rate: To estimate selection on development rate, we used the R package *MCMCglmm* (Hadfield 2010) to apply the generalized linear mixed model (see **Methods S1** for implementation details)

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

where s_i and d_j represents the sires and dams as random components. b_k represents the variance among blocks at each elevation, and $e_{l(ijk)}$ the residual variance. We used separate implementations of equation 2 to estimate selection on development rate at each elevation and for each species separately. We included development rate (poisson distributed) and survival (binomially distributed) as the bivariate response variable (y_{ijkl}). Extracting the sire and dam components provided the genetic (sire) and genetic plus maternal effects (dam) for the covariance between seedling development rate and survival.

To estimate phenotypic selection on development rate, we used *lme4* to implement an equation similar to equation 2, but with family as a random component replacing dam and sire. We included development rate as a fixed effect and kept survival as the univariate response variable. This estimates selection as the regression of development rate on survival. To test for a significant effect of development rate on survival we used a generalized linear mixed model, but to obtain coefficients of selection, we used a linear mixed model (Lande and Arnold 1983; Mitchell-Olds and Shaw 1987).

(3) Selection on root morphology: We combined the root morphology data from the laboratory experiment with our data on survival for the same sires and dams in the field to estimate selection on root architecture. To quantify selection, we estimated the covariance between root traits and field survival. This meant that we applied equation 2, but included all three root morphology traits and survival as the multivariate response variable. We included both b_k with p_m to account for variance among blocks in the field and among plates in the laboratory. We estimated the 4×4 matrix for both the sire and dam components, but for the residual variance we made sure not to estimate the residual covariance between root traits and fitness because only siblings from the same family could be measured in both experiments. The three covariances between survival and the root traits then represent the vector that quantifies the direction of selection on the multivariate phenotype.

To test whether selection on root architecture in novel environments favoured trait evolution in the direction of the native phenotype, we compared the direction of selection (vector standardised to unit length) with the vector representing differences in mean phenotype ($\Delta\bar{\boldsymbol{x}}$). To calculate $\Delta\bar{\boldsymbol{x}}$, we quantified changes in mean phenotype using

$$\Delta\bar{\boldsymbol{x}} = \bar{\boldsymbol{x}}_{S.\ aethnensis} - \bar{\boldsymbol{x}}_{S.chrysanthemifolius}, \qquad (3)$$

which estimates the vector representing differences in multivariate mean phenotype between species. We also tested whether differences in multivariate phenotype were significant using a multivariate analysis of variance (MANOVA). We predicted that if the angle between selection and the native phenotype was small and lower than the null expectation, then selection on a given species at a given elevation (away from its home range) was in the direction of the phenotype of the species native to that elevation.

Estimating a null distribution

To obtain a suitable null distribution for the observed estimates of genetic variance and selection, we randomised the pedigree within each species and for each experiment separately, and re-implemented equation 2 on the randomised data. To reduce computing time, we reduced the total number of iterations while keeping the thinning interval and burn-in period the same. The observed models showed that we

needed 100 MCMC iterations to obtain robust estimates of the mean for all parameters of interest from each model applied to the randomised data. We then compared the point estimates from our observed models with the distribution of the 1,000 models, each applied to randomised data. If our observed estimates exceed the randomised data, this suggests that our observed estimates capture significant genetic variance.

Results

(1) Survival differences reflect adaptive divergence between the two species

Seedling emergence was 30% greater for *S. chrysanthemifolius* than *S. aethnensis* across all elevations (**Fig. 2a**; species×elevation $\chi^2(3)=111.23$, $P<0.00001$), and only *S. aethnensis* showed significantly greater emergence at its home site than at novel elevations (**Fig. 2a**). Seedling establishment was similar across elevation for *S. aethnensis*, but was reduced at higher elevations for *S. chrysanthemifolius* (**Fig. 2b**; species×elevation $\chi^2(3)=387.55$, $P<0.00001$). At all elevations, the probability of reaching maturity was greater for *S. chrysanthemifolius* than *S. aethnensis*, and was lowest at the highest elevation for both species (**Fig. 2c**; species×elevation $\chi^2(3)=48.37$, $P<0.00001$). At the end of summer (September 2019), the survival of both species was greatest in their native habitats and lowest at the elevation furthest from their native site (**Fig. 2d**; species×elevation $\chi^2(3)=1678.30$, $P<0.00001$). Furthermore, for *S. chrysanthemifolius* survival declined gradually with increasing elevation (**Fig. 2e**; species×elevation $\chi^2(3)=1039.10$, $P<0.00001$). By contrast, *S. aethnensis* shows a relatively constant survival between 1,000m and 2,000m, but a sharp decline of >30% at 500m (**Fig. 2e**). Therefore, survival differences at elevational extremes provide evidence of ecological differentiation between these two species. Given the strongest decline in fitness was observed in the most novel elevation for each species (i.e. the native habitat of the other species), the following analyses focus on the elevational extremes.

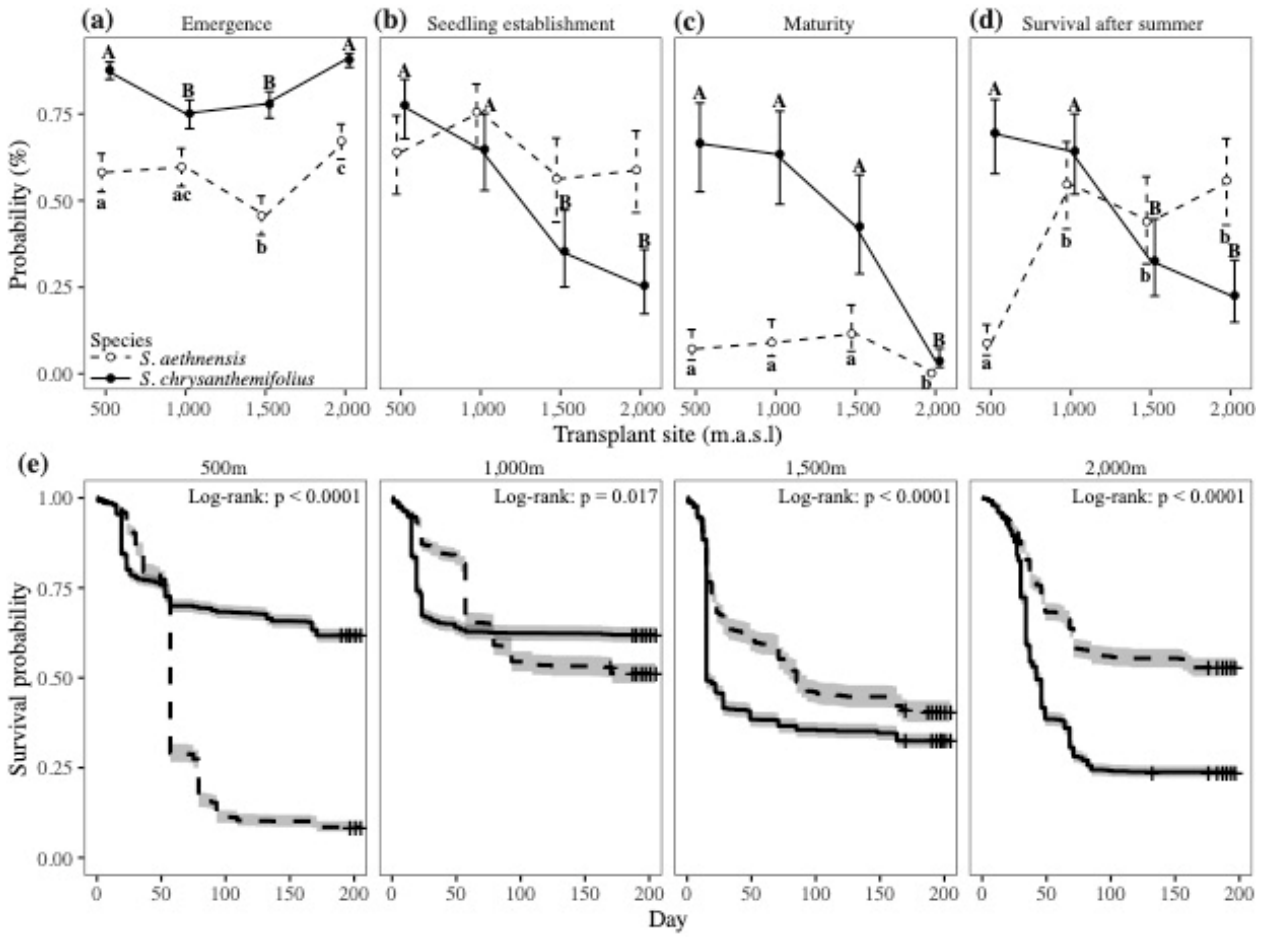

Fig. 2 Evidence for adaptive divergence whereby species perform better at their native elevation, and show reduced fitness at the novel elevation. Lowercase and uppercase letters denote significant differences (α=0.05 with bonferroni adjustment) across elevation for *S. aethnensis* and *S. chrysanthemifolius*, respectively. **(a)** Seedling emergence was greater for *S. chrysanthemifolius*, which was greater at the elevational extremes. Emergence was greater 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)** The probability of reaching maturity was lowest for both species at 2,000m, and *S. aethnensis* showed a much lower probability of reaching maturity across the elevational range. **(d)** 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. **(e)** Survival analysis across the duration of the experiment shows that the probability of survival was reduced as each species was planted further from their native elevations. For elevational extremes, each species performed better at its native elevation, and better than the foreign species. Gray shading represents 95% confidence intervals.

(2) Maternal effects on development rate can increase fitness in novel environments

Both species showed similar plasticity in seedling development rate, which was faster at lower elevations compared to higher elevations (**Fig. 3**). We tested whether selection on development rate for each species at the novel elevation matched the development rate of the native species. We found that phenotypic selection

was not significantly different for both species within a given elevation (development rate × species interaction at 500m: $\chi^2(1)=1.64$, P=0.20; at 2000m: $\chi^2(1)=0.0003$, P=0.99). However, both species showed significant changes in selection on development rate between elevations (**Fig. 4**; development rate × elevation interaction for *S. aethnensis* $\chi^2(1)=6.63$, P=0.01003*;* and *S. chrysanthemifolius:* $\chi^2(1)=13.09$, P=0.0003). Therefore, development rate and phenotypic selection on development rate was similar for both species at each elevation, and changed similarly across elevation. These findings also demonstrate that elevational changes in selection on development rate align with plasticity in development rate: slower development at 2,000m was associated with phenotypic selection for slower development, and faster development rate at 500m was associated with selection for faster development (**Fig. 4**).

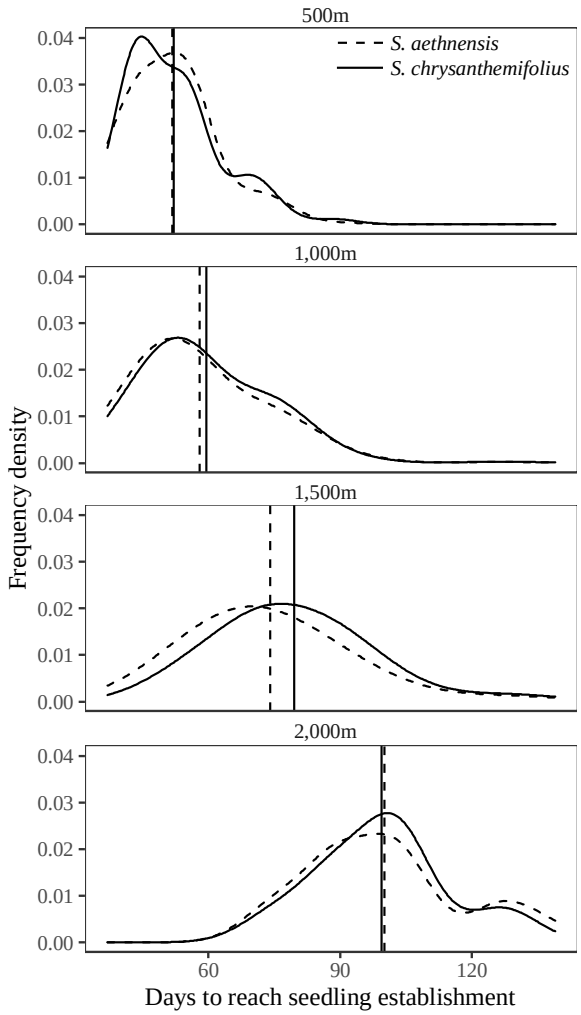

Fig. 3 Frequency distribution of seedling development rate for all plants that reached seedling establishment was similar for both species, and decreased at higher elevations. Vertical lines represent the mean at each elevation for each species.

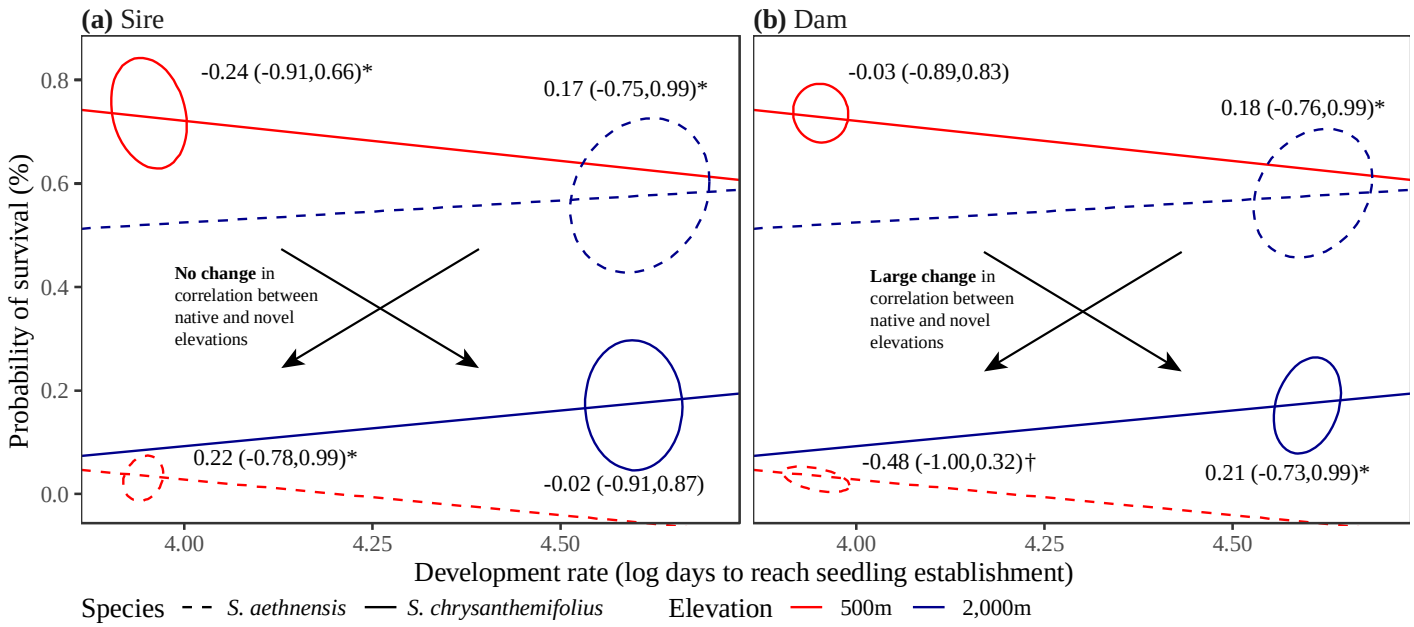

Fig. 4 Adaptive maternal effects are represented by changes in seedling development rate for the dam component (but not the sire component) at the novel elevation that align with selection and the optimum (development rate of the native species). For selection on **(a)** sire variance and **(b)** dam variance, dashed lines and ellipses represent *S. aethnensis*, and solid lines and ellipses represent *S. chrysanthemifolius*. Lines represent phenotypic selection on development rate at different elevations, calculated based on the regression of seedling development rate on survival. Both species show phenotypic selection for faster development at 500m (red lines), but slower development at 2,000m (blue lines). Ellipses present selection on sire and dam variance as the covariance between seedling development rate and survival, with numbers presenting their correlation (numbers in parentheses denoting the 95% HPD confidence intervals). Asterisks denote significant correlations at 63% HPD, and † at 85% HPD. In novel environments (*S. chrysanthemifolius* at 2,000m and *S. aethnensis* at 500m), genetic (sire) correlations are in the opposite direction of phenotypic selection (i.e. the optimal development rate). However, compared to the sire correlation, the dam correlation between development rate and fitness shows the opposite sign in novel environments, and aligns with phenotypic selection, indicating that maternal effects on plasticity in development rate increase the fitness of genotypes in novel environments.

Sire variance represents one quarter of the additive genetic variation, while dam variance represents one quarter of additive genetic variation plus environmental and genetic maternal effects (Lynch and Walsh 1998). Therefore, comparing sire and dam variance contrasts genetic variation (sire variance) with maternal effects (dam variance). To test whether selection on either sire or dam variance aligned with phenotypic selection on development rate, we quantified the sire and dam covariance between development rate and survival. We found that across elevations, the sign of the sire correlation between development and survival did not change for either species (**Fig. 4a**). *Senecio chrysanthemifolius* showed a significant negative correlation at 500m (-0.24), and a weaker but still negative correlation at 2,000m (-0.02), whereas *S. aethnensis* showed positive correlations at both 500m (0.22) and 2,000m (0.17). Therefore, sire correlations

between development rate and survival matched the direction of phenotypic selection only at the home sites of each species.

By contrast, we found significant changes across elevation for the dam correlation between development rate and survival (**Fig. 4a**). *Senecio chrysanthemifolius* showed a weak negative correlation at 500m (-0.03), which changed to a moderately strong and significant positive correlation at 2,000m (0.21). *Senecio aethnensis* showed positive dam correlation between development rate and survival at 2,000m (0.18), which changed to a strong negative correlation at 500m (-0.48), with the change in correlation across elevations significant at 77% HPD. Therefore, dam correlations between development rate and survival matched phenotypic selection at both native and novel elevations for both species, representing an adaptive maternal that adjusts development rate to match the development rate required in the novel environment.

If the elevation that the parental genotypes were sampled from determined their fitness in the novel environment, genotypes sampled from lower elevations within the native range would do better (than high elevation genotypes) when planted at the novel low elevation. However, although maternal genotypes of *S. aethnensis* from lower elevations performed slightly better than those sampled from higher elevations, the trend was not significant (**Fig. S4**).

Quantifying variation in root morphology

The two species differed significantly in their root architecture. Compared to *S. chrysanthemifolius* (trait means: root area=0.338±0.011 [1 SE]; width=0.542±0.023; specific root length [SRL]=1649.82±13.22), *S. aethnensis* (trait means: root area=0.628±0.023; width=0.750±0.025; SRL=1350.59±14.75) showed greater root depths and widths, but a lower specific root length. This meant that the difference in multivariate mean phenotype was significantly greater between species than among families within species (MANOVA Pillai's trace=0.53, $F_{1,195}$=73.30, $P<0.00001$). However, differences among species accounted for 10.3% of total variance, whereas differences among full-sibling families accounted for 39.5%, highlighting large differences in multivariate phenotype among families within species (**Fig. S5**).

Estimating sire and maternal covariance matrices within each species showed that root trait variances were similar for both the sire and dam components for *S. aethnensis*, whereas *S. chrysanthemifolius* showed very low sire variances but dam variances that were an order of magnitude greater (**Fig. 5**; **Table S2**). Sire variances for *S. chrysanthemifolius* are a magnitude lower than for *S. aethnensis*. By contrast, maternal variance in *S. aethnensis* is nearly an order of magnitude lower than *S. chrysanthemifolius* for root area and root width (**Fig. 5**; **Table S2**). All trait variances were greater than expected under random sampling for *S. aethnensis*, whereas only maternal variance in root area and width was greater than expected under random

sampling for *S. chrysanthemifolius* (**Fig. 5**).

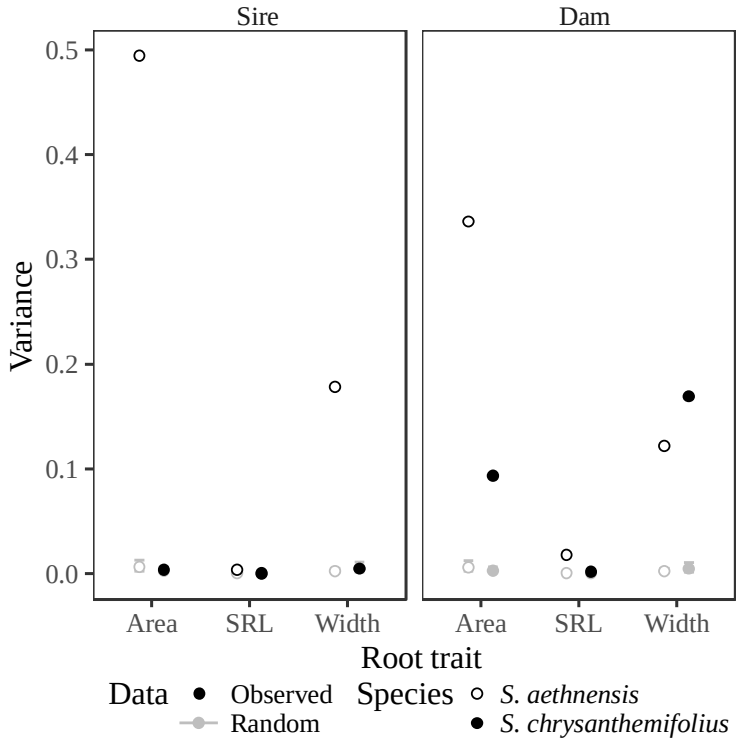

Fig. 5 Observed estimates of sire and dam variance were greater than expected under random sampling in all traits for *S. aethnensis*, except sire variance for specific root length (SRL). Observed trait variances only exceeded random sampling for dam variance in two traits (area and width) for *S. chrysanthemifolius*. Gray circles and credible intervals represent the mean and 95% HPD intervals for the variances estimated from the randomised data. Traits represent the area of the root network, Specific Root Length (SRL) as the total length of root network divided by root volume, and width of the root network.

(3) Estimating selection on root morphology

To quantify selection on root morphology, we calculated the covariance between the root traits measured for all families in the laboratory, and seedling survival of the same families measured in the field experiment. For *S. aethnensis*, our observed estimates of selection on root morphology were greater than for *S. chrysanthemifolius* and exceeded the null distribution at the novel elevations (**Fig. 6a**). Therefore, adaptation in root traits at novel elevations will be more likely in *S. aethnensis*, than in *S. chrysanthemifolius*.

To test whether selection at the novel elevation was in the direction of the phenotype of the native species, we estimated the angle between the vectors of selection and the vector representing species' differences in mean phenotype ($\Delta\bar{\boldsymbol{x}}$). If selection at the novel elevation was in the direction of the native species, then the angle between the vector of selection and $\Delta\bar{\boldsymbol{x}}$ would be low, and fall outside the null distribution. We found that for *S. aethnensis*, selection on dam variance at the novel elevation (500m) was in the direction of the native phenotype (*S. chrysanthemifolius*), but selection was in the opposite direction for sire variance

(**Fig. 6b-c**). By contrast, only selection on sire variance in root morphology for *S. chrysanthemifolius* was close to the direction of the native phenotype (*S. aethnensis*) at the novel elevation (2,000m; **Fig. 6b-c**). Therefore, maternal effects could increase ecological resilience at the novel 500m elevation for *S. aethnensis*, whereas selection on root morphology for *S. chrysanthemifolius* was overall weaker and unlikely to increase ecological resilience at the novel 2,000m elevation.

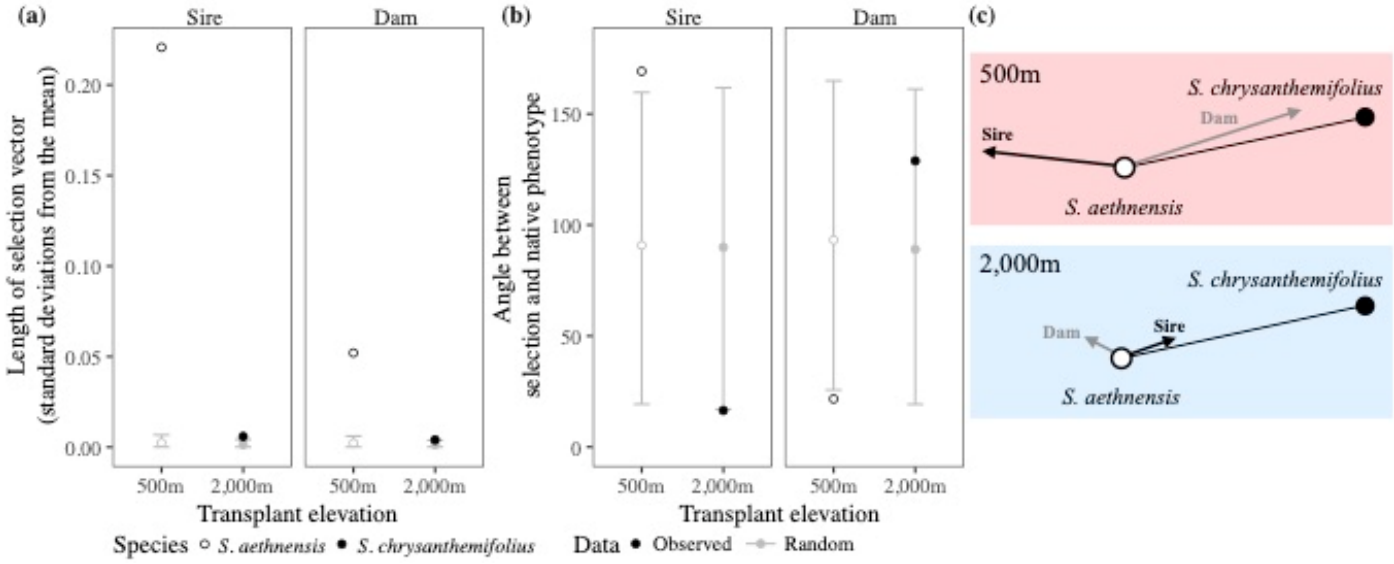

Fig. 6 Strength and direction of selection on root traits. **(a)** The length of the observed selection vectors (representing the direction of selection on root morphology) (black circles) are greater than expected under random sampling (gray circles and HPD credible intervals), but lower for *S. chrysanthemifolius* compared to *S. aethnensis*. **(b)** Gray credible intervals (90% HPD intervals) represent the angle between randomised selection vectors and the observed difference in mean phenotype between species ($\Delta\bar{x}$). If the observed angle (black circles) between selection and the phenotype of the native species is near zero and falls outside the random distribution, then selection occurred in a direction close to the difference in mean phenotype. Angles greater than 90° suggest that selection was in the direction opposite to the native phenotype. For *S. aethnensis*, selection on maternal variance at 500m was in the direction of the native phenotype (of *S. chrysanthemifolius*), but selection on sire variance was in the opposite direction. By contrast, selection on sire variance in *S. chrysanthemifolius* was in the direction of the native phenotype at 2,000 m (*S. aethnensis*). **(c)** Visual summary of **(b)**, where the large circles represent the multivariate mean phenotype of each species, and arrows represent the direction of selection on sire and dam variance.

Discussion

To our knowledge, no studies have combined field and laboratory experiments using siblings from the same families to test whether maternal effects can be adaptive, and increase resilience in novel environments. We produced seeds using a breeding design with two contrasting, but ecologically distinct species. In separate experiments, siblings of the same families were then assayed for root morphology in the laboratory, and planted across an elevational gradient representing conditions within and beyond their natural range. In

the field, we found evidence of adaptive divergence at early life history stages, where both species performed best in their native habitat, with reduced survival observed in the native habitat of the other species (**Fig. 2**). Seedling development rate changed across elevation similarly for both species (**Fig. 3**), and phenotypic selection on development rate was also similar for both species, favouring shorter development at low elevation and longer development at high elevations. However, selection on additive genetic variation (i.e. on the sire variance component) in development rate did not change across elevation, and aligned with phenotypic selection in the native, but not the novel environment (**Fig. 4**). This means that, additive genetic variance in plasticity for development rate at the novel elevation could not move the population closer to the optimum of the native species. By contrast, selection on the maternal component (i.e. on the dam variance) of development rate changed with elevation, and matched the direction of selection in both the native and novel environments (**Fig. 4**). Therefore, compared to additive genetic variation, maternal effects on development rate can better maintain ecological resilience in a novel environment by matching the local phenotypic optimum.

We detected substantial differences in root morphology between the two species, with *S. aethnensis* exhibiting greater root width and depth, but a lower specific root length (i.e. shorter, thicker roots). We also observed high levels of genetic and maternal variation in root morphology within species. By combining full-sibling estimates for root morphology (assayed in the laboratory) with field performance, we found evidence for significant selection on root morphology for *S. aethnensis*, and that selection on maternal variance in the novel environment (500m) was in the direction of the root phenotype of the native species at that elevation (**Fig. 6**). However, selection on the root morphology of *S. chrysanthemifolius* was much weaker and not consistently in the direction of the native phenotype at the novel elevation.

Overall, our results suggest that variation in maternal effects on progeny can reduce fitness declines at novel elevations. Maternal effects can therefore help maintain population mean fitness at ecological margins in this system, and promote subsequent evolutionary rescue in response to changing environments (Donelson et al. 2018).

Plasticity in development rate is associated with fitness declines at novel elevations

Similar to a previous study in *Senecio* (Walter et al. 2018), we found that fitness declines were associated with variation in development rate during early life history. Although plasticity in development rate meant that species developed at a similar rate to the native species when planted in the novel environment, large changes in development rate are likely to have downstream effects that cause the fitness reduction. For example, in non-stressful environments, reactive oxygen species act as chemical signals that mediate growth and development. However, in stressful environments, increased oxidation creates high toxicity of the same

compounds that reduce plant performance (Hossain et al. 2015; Farnese et al. 2016). Plasticity in development rate has been associated with increased stress that reduces performance due to oxidative damage (Janssens and Stoks 2017). Therefore, although selection might favour faster development of *S. aethnensis* at lower elevations, and slower development of *S. chrysanthemifolius* at higher elevations, temperature stress in novel environments could create oxidative damage (Morales and Munné-Bosch 2016). Developmental plasticity could then expose plants to stresses not present in their native range, reducing fitness substantially at early life history stages (DeWitt et al. 1998; Monaghan et al. 2009; Morales and Munné-Bosch 2016).

Adaptive maternal effects in development rate can reduce fitness declines in novel environments

Offspring that experience the same stressful environment as their mother can be more resilient (Latzel et al. 2014), suggesting that persistence in a novel environment across generations could rely on transgenerational maternal effects. Wadgymar et al. (2018) show extensive transgenerational plasticity for early life history traits in novel environments in *Boechera stricta*, but could not determine whether or not such plasticity was adaptive. In this study, we provide some of the first evidence for adaptive transgenerational maternal effects in novel environments. This is because, in contrast with sire variance (representing additive genetic variance), selection on maternal variance for development rate and root morphology was associated with greater fitness in novel environments, although these patterns were weaker for *S. chrysanthemifolius* compared to *S. aethnensis* (**Fig. 4**). Therefore, fitness declines in novel environments driven by selection on early life history are likely to be slowed by maternal genotypes that generate offspring phenotypes closer to the local phenotypic optimum. Further work needs to identify the limits of these maternal effects, and whether they are determined by the maternal genetic material (e.g. extra-nuclear DNA) or by the maternal environment (Galloway et al. 2009).

If maternal effects are largely environmental (i.e. non-genetic and determined by the environment that the mother developed in), there may be limits to the benefit of maternal effects in responding to novel environments. However, if adaptive maternal effects are genetic, then there is the potential for the maternal effects themselves to evolve and contribute to evolutionary rescue in novel environments (Wilson et al. 2005). A large proportion of heritability can be missed when maternal genetic variance is not taken into account (McAdam et al. 2002), suggesting that selection on maternal genetic effects can contribute substantially to evolutionary rescue. Furthermore, genetic variation in transgenerational plasticity can be high in some situations, which means that selection for appropriate maternal effects on offspring phenotypes could increase the probability of evolutionary rescue (Vu et al. 2015; Donelson et al. 2018), especially where, as in our study, the orientation of maternal effects aligns with the direction of selection in the novel environment.

Although we show evidence that adaptive maternal effects could help to maintain fitness in a novel environment, we acknowledge that with these data it is difficult to identify whether evolutionary rescue is possible before extinction occurs. The difficulty of measuring phenotypes before selection operates, combined with the difficulty of assaying important early life history traits and being unable to discern maternal genetic from environmental effects, mean that it is difficult to properly quantify the strength of selection on maternal variance, or whether the adaptive maternal effects are sufficiently large enough to help prevent extinction.

Selection on the maternal component of root traits can reduce fitness declines in novel environments

Root traits are critical traits for plant growth and survival, especially early in development. However, efforts to quantify selection on root traits in the field are rare (Murren et al. 2020). In this study, we show that selection on early root growth in a novel environment was in the direction of the phenotype of the native species, suggesting both that the species have diverged in their root morphology, and that ecological specialisation in root morphology is required to prevent fitness declines in novel environments. Although we did not measure plasticity in root architecture, root plasticity has been shown to be important for responding to water and nutrient heterogeneity (Hodge 2004; Zhu et al. 2010; Grossman and Rice 2012; Kumar et al. 2019; Kadam et al. 2020). Furthermore, Kadam et al. (2020) show that root plasticity has a moderate heritability, which suggests that selection for plasticity in root morphology could promote evolutionary rescue. Quantifying root plasticity across natural environments could reveal how plasticity in roots help to maintain fitness when novel environments are encountered.

Conclusion

Combining field and laboratory experiments, we show that selection on maternal variation in plasticity (for development rate) and root morphology increases fitness in novel environments, which could help to reduce fitness declines in novel environments. These patterns were especially strong for the high-elevation species, suggesting that the potential for evolutionary rescue in high-elevation communities may be greater than currently appreciated.

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