Environmentally induced development costs underlie fitness tradeoffs

GREG M. WALTER,[1,3] MELANIE J. WILKINSON,[1] J. DAVID AGUIRRE,[1,2] MARK W. BLOWS,[1] AND DANIEL ORTIZ-BARRIENTOS[1]

[1]*School of Biological Sciences, University of Queensland, St. Lucia, Queensland 4072 Australia*
[2]*Institute of Natural and Mathematical Sciences, Massey University, Auckland 0745 New Zealand*

Abstract. Local adaptation can lead to genotype-by-environment interactions, which can create fitness tradeoffs in alternative environments, and govern the distribution of biodiversity across geographic landscapes. Exploring the ecological circumstances that promote the evolution of fitness tradeoffs requires identifying how natural selection operates and during which ontogenetic stages natural selection is strongest. When organisms disperse to areas outside their natural range, tradeoffs might emerge when organisms struggle to reach key life history stages, or alternatively, die shortly after reaching life history stages if there are greater risks of mortality associated with costs to developing in novel environments. We used multiple populations from four ecotypes of an Australian native wildflower (*Senecio pinnatifolius*) in reciprocal transplants to explore how fitness tradeoffs arise across ontogeny. We then assessed whether the survival probability for plants from native and foreign populations was contingent on reaching key developmental stages. We found that fitness tradeoffs emerged as ontogeny progressed when native plants were more successful than foreign plants at reaching seedling establishment and maturity. Native and foreign plants that failed to reach seedling establishment died at the same rate, but plants from foreign populations died quicker than native plants after reaching seedling establishment, and died quicker regardless of whether they reached sexual maturity or not. Development rates were similar for native and foreign populations, but changed depending on the environment. Together, our results suggest that natural selection for environment-specific traits early in life history created tradeoffs between contrasting environments. Plants from foreign populations were either unable to develop to seedling establishment, or they suffered increased mortality as a consequence of reaching seedling establishment. The observation of tradeoffs together with environmentally dependent changes in development rate suggest that foreign environments induce organisms to develop at a rate different from their native habitat, incurring consequences for lifetime fitness and population divergence.

Key words: adaptation; development; divergence; heterogeneous landscape; natural selection; ontogeny; tradeoff.

INTRODUCTION

Tradeoffs arise when adaptation favours ecological specialization, which can reduce performance in alternative environments (Futuyma and Moreno 1988, Rice and Hostert 1993, Singer and McBride 2010, Poisot et al. 2011). Tradeoffs are expected to promote species diversification by preventing gene flow between populations adapted to contrasting environments (Nosil et al. 2005, Hereford 2009, Lenormand 2011). Theory predicts that tradeoffs arise when alleles adapted to one environment contribute negative fitness effects in foreign environments (Kawecki and Ebert 2004). However, the genes responsible for fitness tradeoffs remain largely undiscovered (e.g., Hall et al. 2010, Ågren et al. 2013, Anderson et al. 2013). Similarly, the ecological mechanisms underlying fitness tradeoffs remain elusive because natural environments vary in space and time, making it difficult to identify the traits responsible for fitness reductions in foreign environments (Mitchell-Olds et al. 2007, Anderson et al. 2011).

Populations inhabiting geographic regions with coarse environmental heterogeneity can experience divergent natural selection that promotes phenotypic divergence (Kawecki and Ebert 2004, Hereford 2009). Reciprocal transplants of populations across a heterogeneous landscape can be used to identify whether fitness tradeoffs have been created as a consequence of adaptive divergence (Blanquart et al. 2013). Populations that show higher relative performance when transplanted into their native environment than foreign environments provide evidence of fitness tradeoffs (Hereford 2009). Although evidence of fitness tradeoffs exists in many studies of local adaptation (reviewed in Hereford 2009), few have identified how natural selection creates fitness tradeoffs by reducing foreign population performance at key developmental stages (but see Angert and Schemske 2005, Peterson et al. 2016).

The intensity and mode of natural selection can change during organismal development, potentially determining when and how selection against foreign populations occurs (Arnold and Wade 1984, Aguirre et al. 2014). Lifetime fitness is defined by the individual contribution of offspring to the next generation, however the number of offspring depends on selection events occurring throughout the lifetime of an individual. At the population level, individuals transplanted into foreign environments can die (viability selection) or lack the ability to develop through life history, reducing fitness at later life history stages (Arnold and Wade 1984, Hadfield 2008, Shaw et al. 2008, Mojica and Kelly 2010, Postma and Agren 2016). If local adaptation to particular environments is associated with fitness tradeoffs, and we can assess the strength of selection during early and late stages of organismal development, we can explore how

fitness tradeoffs emerge during ontogeny (Kingsolver et al. 2001, Geber and Griffen 2003).

During early development, ecophysiological traits determine how plants uptake and utilize resources to promote growth and development in any particular environment (Ackerly et al. 2000). Local adaptation of environment-specific ecophysiological traits may be responsible for fitness tradeoffs when foreign populations possess a mismatch between their ecophysiological traits and the transplant environment (Kawecki and Ebert 2004). Identifying when natural selection reduces the fitness of foreign populations by reducing survival or impairing development can reveal how adaptation in one environment creates fitness tradeoffs with other environments. Organisms transplanted into foreign environments may suffer reduced fitness via several mechanisms. Individuals may not grow and reproduce if they are unable to acquire sufficient resources to reach important life history stages (Angert et al. 2008, Reich 2014, Friedman and Rubin 2015), they may allocate resources to any one life history stage at a cost to performance at later life history stages (Stearns 1989, Reznick 1992, Anderson et al. 2014), or they might develop at a rate that compromises resource acquisition in an unfamiliar environment (Ricklefs and Wikelski 2002, Metcalfe and Monaghan 2003, Lee et al. 2013). By quantifying development rate and exploring whether native and foreign populations experience the same rate of mortality after crossing developmental transitions we can investigate how natural selection reduces fitness in foreign environments to create fitness tradeoffs.

We analyzed data from a reciprocal transplant experiment that used three replicate populations of four ecotypes from a widespread, and diverse native Australian wildflower species complex (*Senecio pinnatifolius*) transplanted into the four environments in which the ecotypes naturally occur. In this study, we focussed on varieties distributed across central eastern Australia that occur on sand dunes exposed to high solar irradiance and temperatures (Dune ecotype; *S. pinnatifolius* var. *pinnatifolius*), rocky headlands exposed to high wind and salt spray (Headland ecotype; *S. pinnatifolius* var. *maritimus)*, dry sclerophyll woodlands exposed to ephemeral water availability and intermediate shading (Woodland ecotype; *S. pinnatifolius* var. *dissectifolius*) and moist subtropical rainforest (on elevated tablelands) exposed to high shading and numerous insect herbivores (Tableland ecotype; *S. pinnatifolius* var. *serratus*) (Radford et al. 2004, Thompson 2005, Roda et al. 2013*a*, Walter et al. 2016). Populations occupying these contrasting environments are considered ecotypes as they display strong phenotype-environment correlations and genetically based differences in morphology (Radford et al. 2004, Thompson 2005). Ecotypes share insect pollinators and are obligate outcrossers. The Woodland ecotype has an annual life history strategy, while the Tableland ecotype is a short-lived perennial. Life history strategies in the coastal environments range between annual and short-lived perennial, depending on whether individuals are found in more favorable microenvironments. For example, plants in the dune environment possess large root structures from several years of growth, but mostly when found amongst native grasses protecting them from solar irradiance and desiccation (Ali 1968; Walter, *personal observation*). Seeds are dispersed by wind and have the

potential to travel large distances. Previous reciprocal transplants found local adaptation between contrasting dune and headland coastal habitats (Melo et al. 2014, Richards et al. 2016, Walter et al. 2016). A previous study showed that the genetic variance underlying morphological traits has diverged between contrasting environments, providing evidence that natural selection has also shaped genetic correlations during adaptive radiation (Walter et al. 2018). Nonetheless, despite adaptation to contrasting environments, ecotypes show little genetic divergence, suggesting ecotypic divergence has occurred recently (Roda et al. 2013*a,b*, Melo-Hurtado 2014).

In the current study we used transplant experiments with three replicate populations of each of the four ecotypes to identify whether tradeoffs emerged at three key life history stages: emergence, seedling establishment and maturity. We then assessed whether the transition to key life history stages influenced mortality risk to identify how fitness tradeoffs emerge between ecotypes adapted to contrasting environments. Finally, we recorded the time taken to reach each life history stage to explore whether tradeoffs were associated with differences in development rates. A previous analysis of field performance using the same data found strong evidence for adaptation to each environment, but weaker adaptation between populations within environments. Population performance correlated negatively with environmental distance from the transplant site, suggesting that differences in the environment are responsible for patterns of local adaptation (Walter et al. 2016).

MATERIALS AND METHODS

Sample collection and creation of seed resources

We sampled seeds from three natural populations of each ecotype. Dune and Headland ecotypes occur as parapatric population pairs along the coastline, with pairs separated by 50–100 km and ecotypes within each parapatric pair separated by 3–12 km for the locations we sampled. We sampled sites for the Woodland and Tableland ecotypes from patches of habitat separated by 60–400 km and 70–210 km, respectively (see Fig. 4 in Walter et al. 2016). At each location we collected seeds from 30 mature plants separated by at least 10 meters to reduce the risk of sampling closely related individuals (Appendix S1: Table S1). One representative seed from each plant sampled in the field was grown in The University of Queensland glasshouses to reduce environment-specific maternal effects (Bischoff and Muller-Scharer 2010); these plants were used to produce seeds for the transplant experiment. Germination was induced in the laboratory by scarifying seeds and placing them on moist filter paper in a petri dish. Petri dishes were kept in the dark for 2 d before being transferred to a controlled temperature room where light was maintained at a 12 h:12 h day-night cycle at 25°C. After a week, plants were transferred to the glasshouse where they were transplanted individually into 140 mm round pots containing soil (70% pine bark: 30% coco peat) with 5 kg/m³ slow release osmocote fertilizer and 830 g/m³ Suscon Maxi insecticide. When plants commenced flowering we conducted controlled crosses by rubbing two flower heads from different individuals of the same

population together repeatedly over several days, allowing all flowers within each inflorescence to donate and accept pollen. Crossing tags were used to track fertilized flower heads and seeds were collected in seed envelopes once they emerged. We produced seeds for 11–30 full-sibling families for each population (Appendix S1: Table S1) that we used in a reciprocal transplant experiment in the four natural environments.

Reciprocal transplant experiment measuring field fitness

To investigate population performance in the field, we conducted a reciprocal transplant experiment in the four environments including a coastal sand dune (S 28°47′1.23″, E 153°35′38.56″), a rocky headland (S 28°48′47.22″, E 153°36′19.15″) a dry sclerophyll woodland (S 28°20′19.82″, E 152°2′23.75″) and a warm-temperate rainforest (S 28°21′15.55″, E 152°23′48.44″). We located the woodland transplant site after all seeds were produced in the glasshouse, and as such were unable to include the local population at this site. Therefore, we relied on seeds from other locations to represent the performance of the Woodland ecotype in its native environment. All seeds were transplanted into the four environments on the 18 and 19th of March 2014 (i.e., the start of Autumn in Australia).

Each transplant site contained seeds from the 12 parental populations (7,670 seeds total) randomized into 6 blocks of 320 seeds each. We included one seed from each full-sibling family in each environmental block (environment $n = 6$, total $N = 24$ per family), however due to problems in the glasshouse some populations produced fewer full-sibling families and consequently we used several families multiple times (see Appendix S1: Table S1). To prepare the seeds we used non-drip superglue to glue each individual seed to a toothpick and stored them in boxes in a pre-prepared randomized grid that replicated the grid we set up in the field. In the field, toothpicks with seeds were planted individually into the centre of a 25 mm × 25 mm grid cell so that the seed sat 1–2 mm below the soil surface. Shade cloth (50%) was suspended 150 mm above the plots, which were kept moist for 3 weeks. Field observations have shown that native populations can germinate year-round whenever there is sufficient rain and accordingly, we used supplemental shade and water to replicate natural germination conditions and ensure all populations across the four ecotypes grew at the same time. During the first 3 weeks of the transplant emergence and mortality were recorded every second day. Following the initial period we recorded emergence, mortality, whether plants reached seedling establishment (produced 10 leaves) and whether they reached maturity (produced a bud) in weeks 4, 5, 7 and 9, then monthly until 20 months. We also recorded the day when each plant reached germination, seedling establishment and maturity to calculate development rates to each life history stage. Shade cloth was replaced after 30 d with very light bird netting once germination ceased, which was then removed after 100 d. The dune environment was treated slightly differently because high temperatures and sun exposure makes it a very harsh environment. The natural plants in the dune habitat grow in amongst native grass (*Spinifex* sp.), which provides some shelter. However, we needed a systematic approach for planting seeds on toothpicks using grids. To replicate natural conditions we planted seeds alongside the native *Spinifex* and retained the shade cloth for an additional 70 d and the bird netting until the end of the experiment. More details of the field experiment can be found in Walter et al. (2016).

Population performance in the reciprocal transplant experiment

The reciprocal transplant data was collected as binary responses indicating the success or failure to reach each of the three life history stages (emergence, seedling establishment and maturity) and development rate was recorded as the number of days taken to reach each of these life history stages. With 12 populations from four ecotypes, we were interested in quantifying natural selection by comparing population performance in their native environment and the three foreign environments. Therefore, in the linear model outlined below population was considered a fixed effect with 12 levels. Using the data collected at each life history stage, we applied the same statistical analysis to estimate population performance and development rate in each transplant environment. We used the R package "MCMCglmm" (Hadfield 2010) to implement the generalized linear mixed model,

$$y_{ijklm} = E_i + P_j + EP_{ij} + F_{k(j)} + B_{l(i)} + e_{m(ijkl)}, \quad (1)$$

where transplant environment (E_i), population (P_j) and their interaction (EP_{ij}) were included as fixed effects. Family nested within population ($F_{k(j)}$) and environmental block ($B_{l(i)}$) within transplant environment were fitted as random effects and $e_{m(ijkl)}$ was the residual error. To quantify the effect of natural selection on population performance at each life history stage, we first applied the model in Eq. 1 to the three binary life history traits (emergence, seedling establishment and maturity), which we included as a multivariate response variable (y_{ijklm}) (see Data S1). The model described in Eq. 1 estimated the probability of reaching each life history stage, conditional on performance at the previous life history stage. Second, to identify whether development rate was environmentally dependent and whether native populations developed at a different rate to foreign populations we implemented Eq. 1 with development rate as a univariate, Gaussian response variable. To do so, we used three separate applications of Eq. 1 using the number of days to reach emergence, the number of days to transition between emergence and seedling establishment and, the number of days to transition between seedling establishment and maturity.

All models were run for 2,100,000 Markov Chain Monte Carlo (MCMC) sampling iterations with a burn-in period of 100,000 MCMC iterations and a thinning interval of 2,000 MCMC iterations. We checked that the effective sample size for each parameter exceeded 85% of the total number of iterations saved, and autocorrelation was <0.05 between the thinned samples of each parameter. We used a Cauchy prior distribution for the random effects with a location parameter of zero and a scale parameter equal to the variance of the raw data or $p \times (1-p)$ for the binary data, where p is the distribution mean. To examine the sensitivity of the parameter

values to our prior specifications we repeated the analyses while adjusting the scale parameter of the Cauchy distribution to excessively large and small values. Comparing model outputs showed that altering the prior made minimal difference to the parameter estimates, suggesting our prior did not strongly influence the parameter values. To assess whether fitness tradeoffs and patterns of local adaptation emerged throughout ontogeny we extracted 1,000 MCMC iterations from each model, which provided the posterior distribution of field performance for each population, in each transplant environment. Calculating the 95% Highest Posterior Density (HPD) intervals for the posterior distribution of each parameter captured the uncertainty in estimating field performance. Comparing the posterior distribution for population performance in native and foreign environments then quantified whether natural selection produced tradeoffs between contrasting habitats.

Due to the small number of plants that reached maturity, models including maturity as a binary response variable performed poorly. Consequently, we increased the number of sampling iterations to 10,100,000 with a burn-in of 100,000 iterations and a thinning interval of 10,000 iterations. Unfortunately, models still struggled to obtain the desired autocorrelation or number of effective samples for each parameter. On closer examination of the model output, there were 11 parameters with effective sample sizes of <85% of the total iterations saved. Inspection of the raw data showed all 11 parameters were estimated on populations with less than four plants that reached maturity, meaning a near zero probability of plants from the population reaching maturity in that particular environment. Therefore, the poor estimation of these parameters was due to the difficulty in obtaining a precise estimate as the probability of reaching maturity approached zero. However, posterior predictive checks showed that the mean of the raw data aligned closely with the mean of the posterior distribution, suggesting that the observed and posterior distributions aligned closely and all models estimated the parameters well (Appendix S1: Fig. S1). We took this as evidence that the model performed sufficiently well in estimating performance of each population in each environment, and the model output could be used for the subsequent analyses.

Survival consequences for developing in a foreign environment

We then explored whether the probability of survival for native and foreign plants changed depending on whether they reached, or failed to reach, key life history stages. To do so, we conducted survival analyses in each environment for native ($n = 3$ populations) vs. foreign plants ($n = 9$ populations) that reached seedling establishment vs. those that did not. We then repeated the analyses for native and foreign plants that reached maturity vs. those that failed. We conducted the survival analyses using mixed effects Cox models implemented with the R package "coxme" (Therneau 2012). For seedling establishment we used a subset of the data that only contained the plants that reached germination, and for maturity we used only the plants that reached seedling establishment. In each dataset we created a new variable that identified the native and foreign plants in each environment, as well as the plants that reached seedling establishment (for

the seedling establishment dataset) and maturity (for the maturity dataset), and those that did not. As such, the new variable contained four levels, "Fail-Foreign" and "Fail-Native" for the foreign and native plants that failed to reach the life history stage and "Pass-Foreign" and "Pass-Native" for the foreign and native plants that reached the life history stage.

For all models, transplant environments were analyzed separately, the variable representing native/foreign plants was analyzed as a fixed effect, with population and environmental block included as random effects. To compare mortality rate with the timing of life history stages, we graphed the survival curves and included the interquartile range representing the time at which plants reached a particular life history stage. We could then visualize whether plants died at a faster rate before or after reaching each life history stage. The proportional hazard assumption was often violated in the survival analyses, suggesting that (as predicted) the survival hazard changes across life history. However, if the lines of the survival curves (and cumulative hazard function) representing different groups (e.g., populations) remain parallel and do not intersect, then hazard estimates taken from a Cox model can still be used to quantify differences in mortality risk between groups. This is because the hazard estimate represents the average effect (over time) for a given level of the covariate and differences between groups will be captured by differences in average hazard over time. Therefore, although the hazard ratio changes over time, it only changes in magnitude depending on the levels of the covariate. We found that the survival curves and cumulative hazard function for the four levels of the contrast variable were parallel and rarely intersected (Appendix S1: Fig. S2). Survival curves only intersected for one comparison each for the dune and headland environments, which we interpreted with caution (Appendix S1: Fig. S2).

RESULTS

Emergence of fitness tradeoffs during ontogeny

To quantify how natural selection creates fitness tradeoffs we estimated population performance at each life history stage (conditional on performance at the previous stage) using the generalized linear mixed effects model in Eq. 1. We identified the life history stage with the strongest pattern of local adaptation by contrasting the fitness of local, native and foreign populations across transplant environments. Local referred to populations transplanted into their local environment, native included populations from the native ecotype but from a different location, and foreign referred to populations from a foreign ecotype (see Walter et al. 2016). Where HPD intervals do not overlap there is evidence of a significant difference in performance. We found that local populations performed consistently better than native populations, suggesting local adaptation within ecotypes. Furthermore, a significant difference in performance between native and foreign ecotypes suggested natural selection reduced the performance of foreign populations, and these effects were especially strong at seedling establishment (Fig. 1).
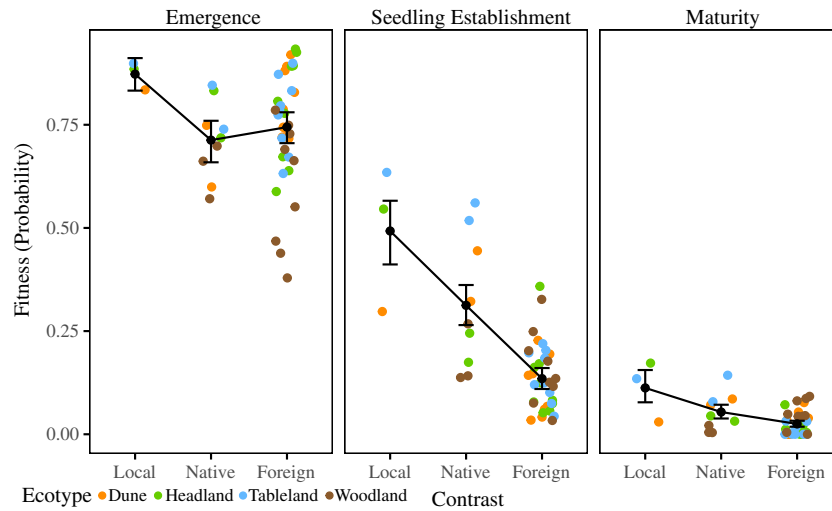
Fig. 1. The Local-Native-Foreign contrast for transitions between life history stages. The greatest difference in field performance between local/native populations and foreign populations was for the probability of reaching seedling establishment. Error bars are 95% Highest Posterior Density (HPD) intervals.

To identify whether natural selection produced fitness tradeoffs, we estimated the relative performance of all populations in each environment, for each life history stage. As such, we calculated relative performance as the difference in performance between each population, and the mean of the transplant environment. Plotting relative performance in foreign environments (x-axis) against relative performance in the native environment (y-axis) tested whether natural selection created fitness tradeoffs (Fig. 5 in Hereford 2009). We expected that if tradeoffs were present, natural selection would have reduced relative fitness in foreign environments and increased relative fitness in native environments. As such, we expected populations to occur in the upper left quadrant where they display high relative fitness in their native environment, but low relative fitness in foreign environments with HPD intervals that do not overlap zero. The

lower left and upper right quadrants signify relative population performance that is worse, or better, in both environments, respectively. The bottom right-hand quadrant would suggest an inverse tradeoff where populations performed better in foreign environments, than native environments.

We found that tradeoffs in field performance emerged as ontogeny progressed (Fig. 2). At emergence populations exhibited no fitness tradeoffs, occurring in both the upper right quadrant where performance was higher in both foreign and native environments, and the lower left quadrant where performance was lower in both environments. As development proceeded, population performance shifted into the upper left quadrant of Fig. 2 for both seedling establishment and maturity, suggesting that natural selection created tradeoffs in fitness as life history progressed. For seedling establishment and maturity, six and three
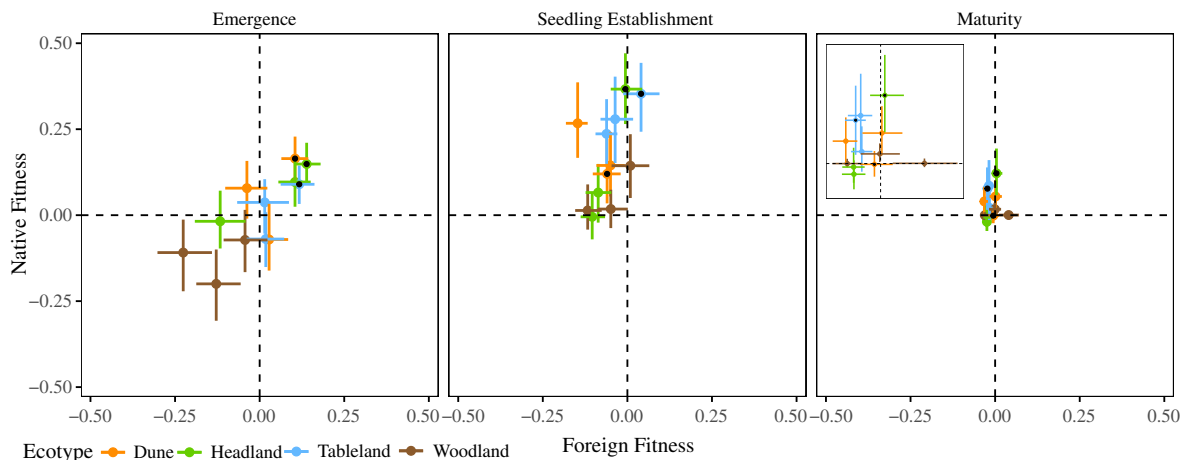

Fig. 2. Relative fitness of a population in foreign environments (x-axis) vs. relative fitness in its native environment (y-axis). Each panel is a different life history stage with black dots indicating the native populations transplanted into their local environment. Insert within the maturity panel shows an expanded view of the data. As life history stages progressed, populations moved from the area signifying no tradeoff (upper right and lower left section of the quadrant) into the upper left quadrant, indicating a fitness tradeoff. Error bars are 95% HPD intervals.

populations did not overlap zero for both axes at 90% HPD, respectively. Therefore, tradeoffs in performance were strongest at seedling establishment where more populations occurred in the tradeoff region of the graph. Given ecotypes can be annual or short-lived perennial, it is possible that differences in development rate reflect differences in the life-history strategy common to each ecotype. However, at the completion of the experiment, alive but immature plants were mostly from the native ecotypes, except in the woodland environment (Appendix S1: Table S2a). Therefore, the patterns of adaptation and fitness tradeoffs we present should be conservative given that more native plants could have reached maturity if the experiment was run for longer.

Survival consequences of developing in a foreign environment

We then assessed whether there were increased mortality risks associated with transitioning to important life history stages, which included seedling establishment (growth to ten leaves) and maturity (producing a bud). More plants from native than foreign ecotypes both survived at the end of the transplant (Appendix S1: Table S2a) and transitioned to seedling establishment (Appendix S1: Table S2b) and maturity (Appendix S1: Table S2c). Therefore, more foreign plants than native plants died without reaching important life history stages.

To identify whether natural selection created higher mortality risks for foreign plants depending on whether they transitioned between key life history stages, we conducted survival analyses on native and foreign plants that reached, and failed to reach, seedling establishment and maturity. Figure 3 is conceptual and depicts predictions for the survival of native and foreign plants as time progresses, with cartoons depicting different life history stages. We predicted that if tradeoffs were created when natural selection reduced fitness at specific developmental stages, then the survival of foreign plants would be greatly reduced after they reached seedling establishment and maturity (Fig. 3a). We also predicted that if natural selection increased the risk of mortality when plants fail to reach key life history stages then native

and foreign plants would show similar survival trajectories (Fig. 3b).

Generally, we found that native and foreign plants that reached seedling establishment survived better than plants that failed to reach seedling establishment, suggesting that plants cannot survive for long periods (i.e., to a time with good growing conditions) without developing to seedling establishment (red vs. blue lines; Fig. 4). Native and foreign plants that failed to reach seedling establishment both died rapidly, with similar survival trajectories that suggest a strong developmental cost in increased mortality for all plants that failed to reach seedling establishment (blue lines; Fig. 4). However, for plants that reached seedling establishment, foreign plants died significantly faster than native plants (red lines; Fig. 4), suggesting foreign plants suffered increased mortality after reaching seedling establishment. Appendix S1: Table S3a contains the Tukey post-hoc comparison (with Bonferroni correction) for each survival model, highlighting significant differences in survival between plants that reached seedling establishment and those that did not.

In contrast to the effect of reaching seedling establishment on longevity, we found that native plants survived much longer than foreign plants regardless of whether they reached maturity (Fig. 5). Therefore, selection against foreign plants was similarly strong for those that reached maturity, and those that failed to do so. However, foreign plants died rapidly after reaching maturity in the dune and headland environments, suggesting a cost of development once maturity has been reached in the coastal environments. Appendix S1: Table S3b contains the Tukey post-hoc comparison (with Bonferroni correction) of each survival model, highlighting significant differences in survival between native and foreign plants (except in the woodland), but no significant differences between plants that reached and did not reach maturity (except in the tableland environment).

Overall, survival curves differed between environments. In the woodland environment foreign plants survived better than native plants at seedling establishment (Fig. 4), which can be due to the native populations requiring specific growth conditions at certain times of the year. For the
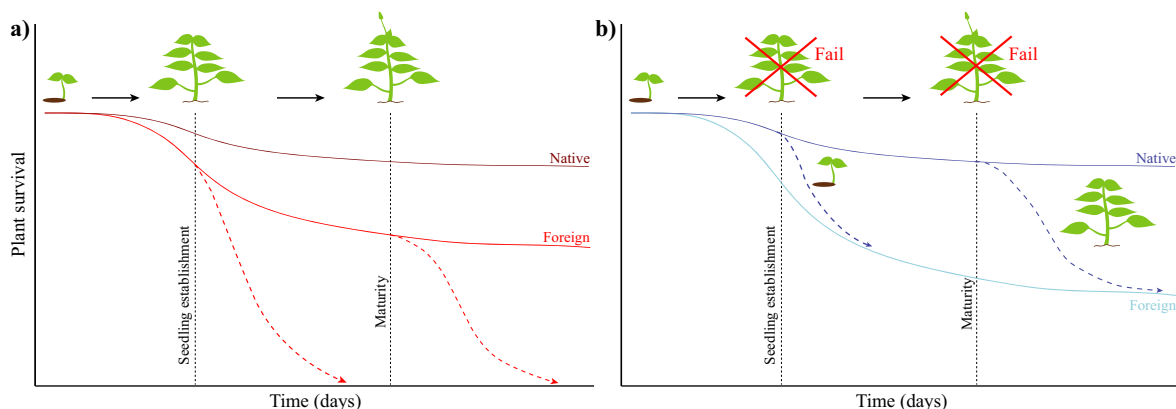

FIG. 3. Conceptual figure depicting the predictions for when development may induce higher mortality risks, with curves representing survival curves and dashed lines the predicted drop in survival. Cartoon plants along the top depict the timing of transitions between life history stages. (a) If costs of development emerged for foreign plants that reached seedling establishment and maturity, then foreign plants would die rapidly after reaching each life history stage (dashed lines). (b) However, if higher mortality risks are incurred when plants fail to reach life history stages, native plants would die similarly to foreign plants (dashed lines). Red crosses depict plants that fail to transition to the next life history stage, with the cartoon plants underneath depicting the life history stage they remained at.
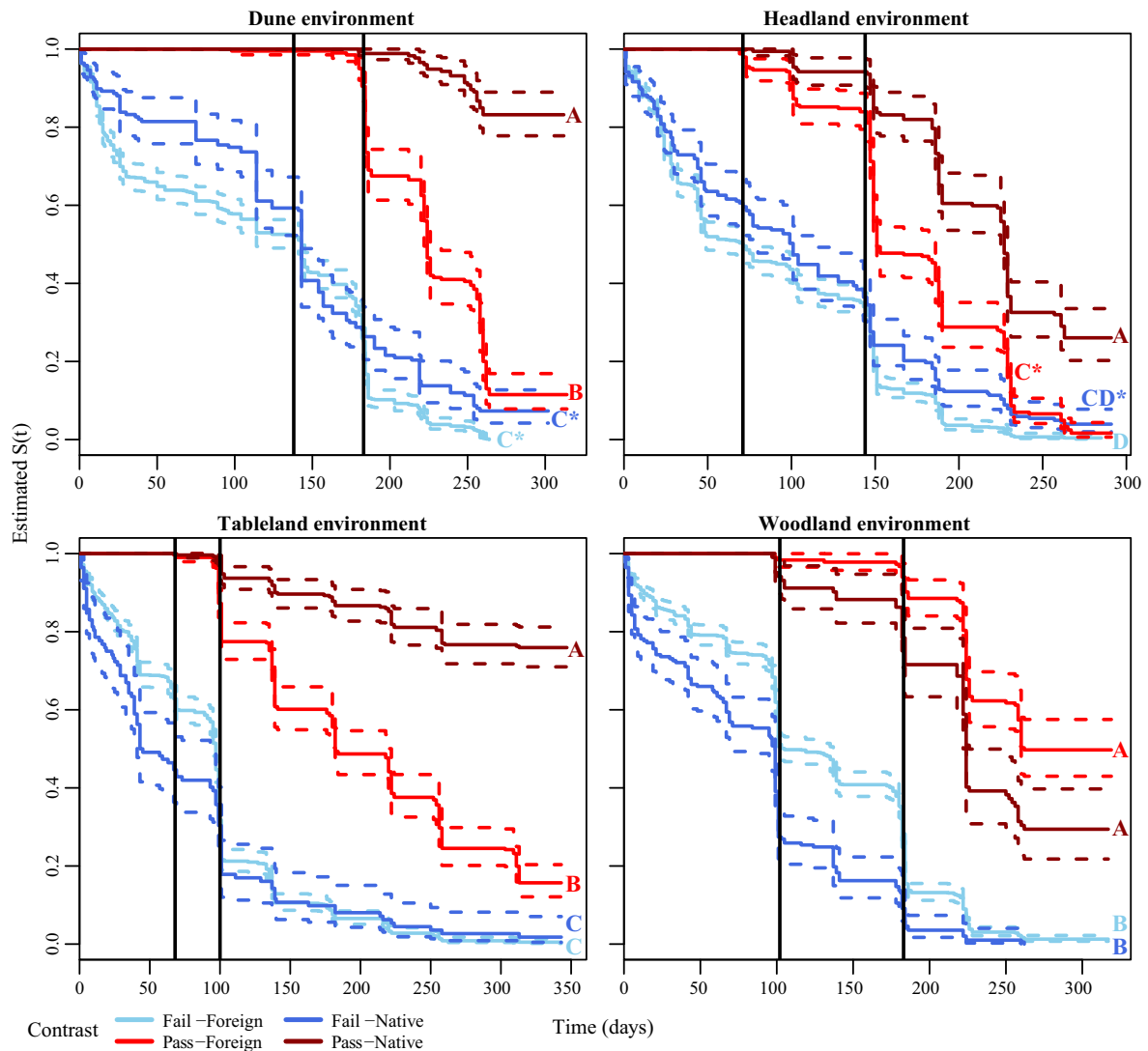
Fig. 4. Survival analysis for native and foreign plants that reached (=Pass) and failed (=Fail) to reach seedling establishment. Dashed lines are 95% confidence intervals and letters denote Tukey's post-hoc groups. Asterisks denote comparisons that violated proportional hazards with intersecting survival curves. Native and foreign plants that failed to reach seedling establishment died at a similar rate. Vertical lines represent the interquartile range for the time period that the transplant population reached seedling establishment. Foreign plants that reached seedling establishment died rapidly after reaching the life history stage.

tableland environment, most mortality occurred immediately after seedling establishment but was gradual for the plants that reached maturity, suggesting selection was strongest on foreign plants at seedling establishment and reduced after they reached maturity (Figs. 3, 4). For both coastal environments, survival of the foreign populations decreased rapidly after plants reached both seedling establishment and maturity (Figs. 3, 4).

Development rate

To identify whether differences in development rate explained patterns of fitness tradeoffs (Figs. 1, 2) and differences in survival (Figs. 4, 5) we tested whether populations showed different rates of development in different transplant environments. We found that populations responded similarly to transplant environments, with the exception for the

time to emergence in the dune environment, seedling establishment in the woodland environment and maturity in the tableland environment (Fig. 6). If natural selection favored adaptation of a specific developmental rate, we expected that native populations would develop differently to foreign populations. However, we found that native and foreign populations showed no clear differences in development rates in each transplant environment (Appendix S1: Fig. S3), suggesting the environment determined development rate.

Discussion

In studying the ecological basis of fitness tradeoffs between ecotypes of *S. pinnatifolius* adapted to contrasting environments, we found tradeoffs in field fitness emerged as development progressed and natural selection
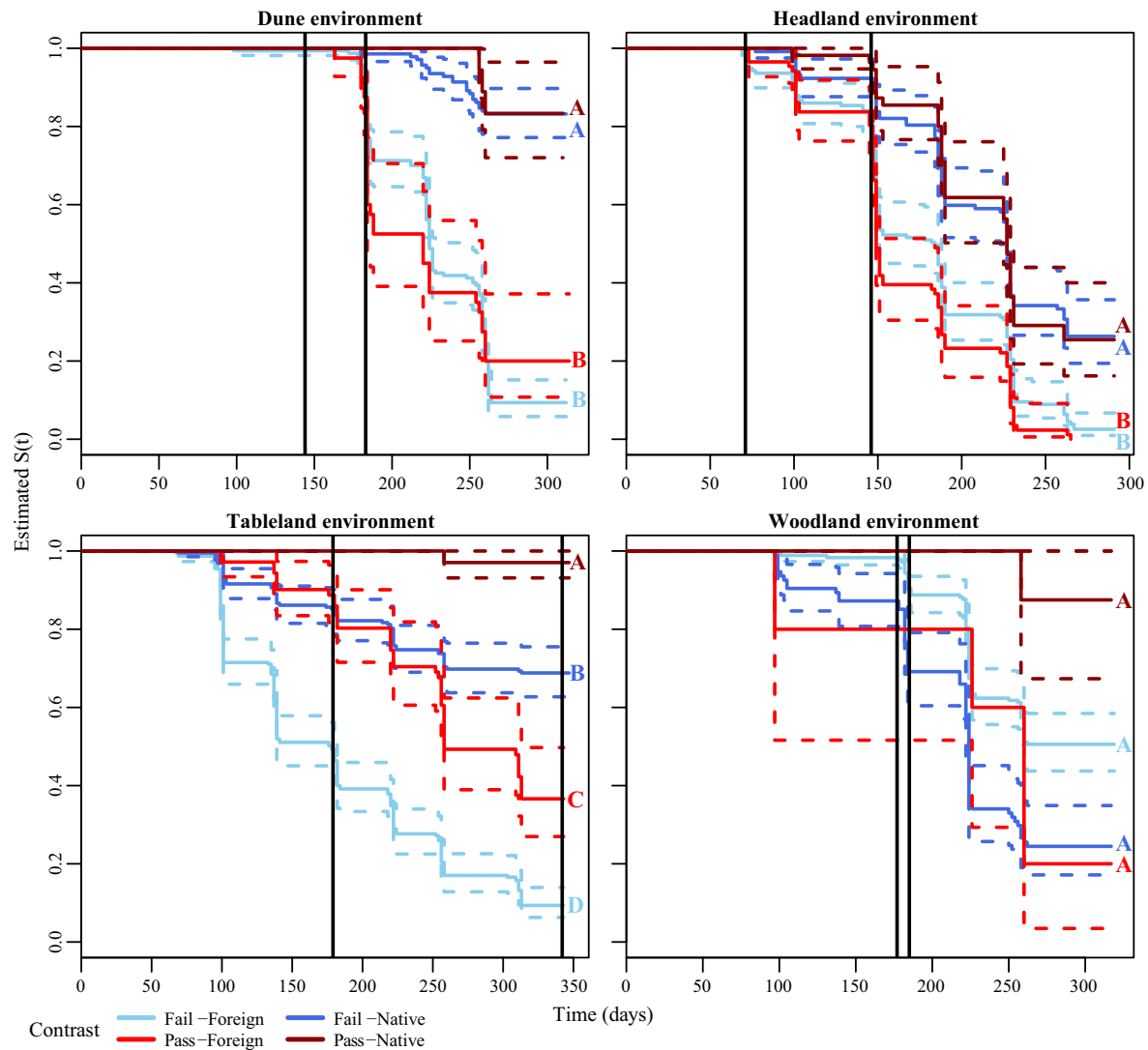
FIG. 5. Survival analysis for native and foreign plants that reached seedling establishment and either reached (=Pass) or failed to reach (=Fail) maturity. Dashed lines are 95% confidence intervals and letters denote Tukey's post-hoc groups. Native plants survived similarly well, often regardless of whether they reached maturity or not. Vertical lines show the interquartile range for the time period that the transplant population reached maturity. Foreign plants died rapidly in the coastal environments shortly after reaching maturity.

reduced foreign population performance. These tradeoffs created patterns of local adaptation that were strongest at seedling establishment. The patterns of fitness tradeoffs were partly explained by high mortality of foreign populations after they established as seedlings, while both native and foreign plants died rapidly if they failed to reach seedling establishment. Tradeoffs were unrelated to how fast individuals transitioned between life history stages, even though developmental rate was specific to each environment. Therefore, natural selection on specific developmental stages may be responsible for adaptive divergence between contrasting environments, resulting in fitness tradeoffs that maintain ecological divergence during diversification.

Understanding how tradeoffs in fitness arise can provide insights into the developmental, morphological or physiological traits that underlie natural selection and patterns of local adaptation. If natural selection creates patterns of

tradeoffs at a given life history stage, then environment specific traits associated with that life history stage could be identified. For example, if the fitness of foreign populations declines early in life history, then physiological traits associated with early development may underlie patterns of local adaptation (Donohue 2014). However, if tradeoffs only emerge after maturity, then natural selection only reduces foreign population fitness once phenotypic traits associated with reproduction are expressed. Inducing germination allowed us to study how fitness changed as plants developed at the same time, during the same environmental conditions. Nevertheless, it is possible we missed fitness tradeoffs at emergence where the decision to germinate may be an environment-specific trait important in these contrasting environments (Vandelook et al. 2008). Our data showed that natural selection was strongest on the traits that facilitated the transition from germination to seedling establishment, suggesting that tradeoffs were
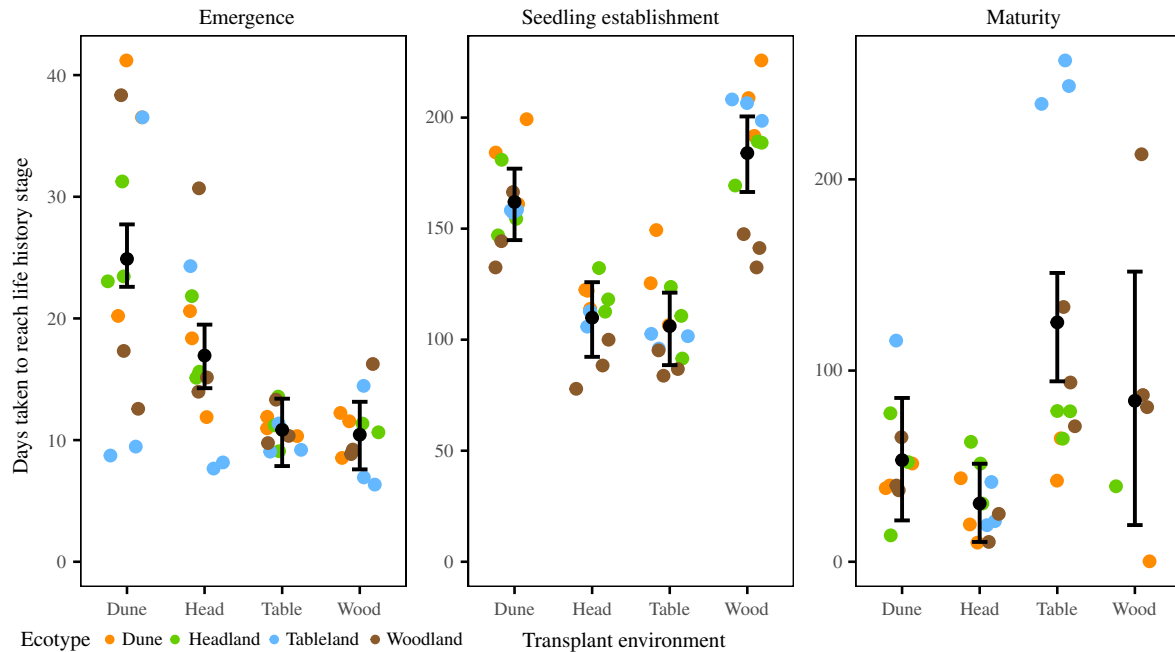
FIG. 6. Days taken to reach each life history stage for populations planted in each environment. Overall means and 95% HPD intervals show that the environment determined development rate, and populations often responded similarly to each transplant environment.

created by selection on environment-specific traits early in life history.

Foreign populations either failed to reach key life history stages, or died shortly after reaching them. In the coastal and tableland environments, reductions in survival followed seedling establishment. However, large reductions in survival for foreign populations only occurred after maturity in the coastal environments, suggesting strong selection after plants reached both seedling establishment and maturity in these environments. The woodland displayed no similar patterns to the remaining ecotypes, likely due to this ecotype being a strict annual that may rely on environment-specific germination and growth cues not captured by our experiment (also discussed in Walter et al. 2016).

It is possible for plants to survive without developing, waiting for suitable environmental conditions for growth. However, we found that both foreign and native plants suffered enhanced mortality if they failed to reach seedling establishment, suggesting this is a key life history stage that must be reached within a given period of time to ensure survival. Therefore, selection was strongest at seedling establishment and it is likely that ecophysiological traits mediating development underlie the evolution of tradeoffs between contrasting environments. Studies have shown that traits such as water use efficiency, nitrogen accumulation or traits that allow stress tolerance/avoidance may promote survival and development to enhance reproductive output (reviewed in Ackerly et al. 2000). At seedling establishment, morphological traits (e.g., number of branches, leaf morphology) are still developing and may not yet be seen by natural selection. Following seedling establishment, foreign plants died faster than native plants regardless of whether maturity was attained, meaning that environment-specific morphological or physiological traits unrelated to

development were important for growth and survival after reaching seedling establishment.

Together, our results suggest that adaptation of traits linked to early development (seedling establishment) may promote successful growth in native environments at a cost to performance in alternative environments, creating fitness tradeoffs. We see two non-mutually exclusive explanations. First, plants transplanted outside their native environment may possess mismatched traits, producing a stress response and altering resource allocation during development. Stressed plants allocate resources to development to attempt to reach reproductive stages without accumulating sufficient resources to grow and survive (Galloway 1995, Simpson and Dean 2002, Baythavong and Stanton 2010). Shifting resource allocation to development allows plants to reach maturity, but reduces the resources available during the reproductive life history stage (Stearns 1989, Sultan 2000). Second, we found that development rate was environmentally dependent, suggesting the environment may elicit a stress response by changing the interaction between physiological traits and plant development (Sultan 2000). Therefore, plasticity in development rate may allow adjustments to different environments to increase survival or growth early in life history, but with consequences for fitness at later life history stages. Consequently, selection on growth and development would produce fitness tradeoffs when plants fail to adjust to a different environment, or when the traits themselves are maladapted outside their native environment (Angert et al. 2008).

Identifying how natural selection changes across development and creates fitness tradeoffs has implications for understanding the evolution of extrinsic reproductive isolation. This is important because ecotypes of *S. pinnatifolius* can disperse over long distances and tradeoffs in

performance between environments can contribute to reproductive isolation, maintaining divergence (Hereford 2009, Lenormand 2011). For instance, if selection is strong during early development, then adaptive divergence in phenotypic traits expressed at early developmental stages may cause barriers to gene flow. We showed that this is likely in *S. pinnatifolius*, where natural selection was strongest on traits expressed during early development. Consequently, natural selection could only operate on phenotypic traits expressed later in development if they were either physically linked to adaptive traits earlier in development (via pleiotropy or linkage disequilibrium), or if they promote increased fitness at later life history stages (Donohue 2014). Overall, our data suggests that traits associated with fitness at early life history stages likely create a large proportion of the total extrinsic reproductive isolation between contrasting habitats, with traits expressed later in development (e.g., number of branches) maybe playing a lesser role in directly creating extrinsic reproductive isolation.

Conclusions

Our results suggest that the early stages of adaptive divergence may be created by divergent natural selection on traits important at early life history stages. We suggest that expression of traits early in development may be determined by the environment in which organisms grow, leading to developmental trajectories that are optimal for local, but not migrant individuals. Our results suggest that developmental plasticity could play an important role in the early stages of ecotypic differentiation by creating developmental mismatches between populations across contrasting environments. Consequently, the costs of developmental plasticity might contribute to origins of extrinsic reproductive isolation between ecotypes (Chevin and Lande 2015).

Literature Cited

Ackerly, D. D., et al. 2000. The evolution of plant ecophysiological traits: Recent advances and future directions. BioScience 50:979–995.

Ågren, J., C. G. Oakley, J. K. McKay, J. T. Lovell, and D. W. Schemske. 2013. Genetic mapping of adaptation reveals fitness tradeoffs in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences of the United States of America 110:21077–21082.

Aguirre, J. D., M. W. Blows, and D. J. Marshall. 2014. The genetic covariance between life cycle stages separated by metamorphosis. Proceedings of the Royal Society B-Biological Sciences 281:20141091.

Ali, S. I. 1968. *Senecio lautus* Complex in Australia. 4. The biology of the complex. Phyton-Annales Rei Botanicae 13:53–62.

Anderson, J. T., J. H. Willis, and T. Mitchell-Olds. 2011. Evolutionary genetics of plant adaptation. Trends in Genetics 27:258–266.

Anderson, J. T., C. R. Lee, C. A. Rushworth, R. I. Colautti, and T. Mitchell-Olds. 2013. Genetic tradeoffs and conditional neutrality contribute to local adaptation. Molecular Ecology 22:699–708.

Anderson, J. T., C. R. Lee, and T. Mitchell-Olds. 2014. Strong selection genome-wide enhances fitness tradeoffs across environments and episodes of selection. Evolution 68:16–31.

Angert, A. L., and D. W. Schemske. 2005. The evolution of species' distributions: reciprocal transplants across the elevation ranges of *Mimulus cardinalis* and *M. lewisii*. Evolution 59:1671–1684.

Angert, A. L., H. D. Bradshaw, and D. W. Schemske. 2008. Using experimental evolution to investigate geographic range limits in monkeyflowers. Evolution 62:2660–2675.

Arnold, S. J., and M. J. Wade. 1984. On the measurement of natural and sexual selection: applications. Evolution 38:720–734.

Baythavong, B. S., and M. L. Stanton. 2010. Characterizing selection on phenotypic plasticity in response to natural environmental heterogeneity. Evolution 64:2904–2920.

Bischoff, A., and H. Muller-Scharer. 2010. Testing population differentiation in plant species - how important are environmental maternal effects. Oikos 119:445–454.

Blanquart, F., O. Kaltz, S. L. Nuismer, and S. Gandon. 2013. A practical guide to measuring local adaptation. Ecology Letters 16:1195–1205.

Chevin, L. M., and R. Lande. 2015. Evolution of environmental cues for phenotypic plasticity. Evolution 69:2767–2775.

Donohue, K. 2014. Why ontogeny matters during adaptation: developmental niche construction and pleitorpy across the life cycle in *Arabidopsis thaliana*. Evolution 68:32–47.

Friedman, J., and M. J. Rubin. 2015. All in good time: understanding annual and perennial strategies in plants. American Journal of Botany 102:497–499.

Futuyma, D. J., and G. Moreno. 1988. The evolution of ecological specialization. Annual Review of Ecology Evolution and Systematics 19:207–233.

Galloway, L. F. 1995. Response to natural environmental heterogeneity: maternal effects and selection on life-history characters and plasticities in *Mimulus guttatus*. Evolution 49:1095–1107.

Geber, M. A., and L. R. Griffen. 2003. Inheritance and natural selection on functional traits. International Journal of Plant Sciences 164:S21–S42.

Hadfield, J. D. 2008. Estimating evolutionary parameters when viability selection is operating. Proceedings of the Royal Society of London Series B-Biological Sciences 275:723–734.

Hadfield, J. D. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. Journal of Statistical Software 33:1–22.

Hall, M. C., D. B. Lowry, and J. H. Willis. 2010. Is local adaptation in *Mimulus guttatus* caused by tradeoffs at individual loci? Molecular Ecology 19:2739–2753.

Hereford, J. 2009. A quantitative survey of local adaptation and fitness tradeoffs. American Naturalist 173:579–588.

Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. Ecology Letters 7:1225–1241.

Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang, P. Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. American Naturalist 157:245–261.

Lee, W. S., P. Monaghan, and N. B. Metcalfe. 2013. Experimental demonstration of the growth rate - lifespan tradeoff. Proceedings of the Royal Society B-Biological Sciences 280:20122370.

Lenormand, T. 2011. From local adaptation to speciation: specialization and reinforcement. International Journal of Ecology 2012:11.

Melo, M. C., A. Grealy, B. Brittain, G. M. Walter, and D. Ortiz-Barrientos. 2014. Strong extrinsic reproductive isolation between parapatric populations of an Australian groundsel. New Phytologist 203:323–334.

Melo-Hurtado, M. C. 2014. Ecological speciation in *Senecio lautus*. University of Queensland.

Metcalfe, N. B., and P. Monaghan. 2003. Growth versus lifespan: perspectives from evolutionary ecology. Experimental Gerontology 38:935–940.

Mitchell-Olds, T., J. H. Willis, and D. B. Goldstein. 2007. Which evolutionary processes influence natural genetic variation for phenotypic traits? Nature Reviews Genetics 8:845–856.

Mojica, J. P., and J. K. Kelly. 2010. Viability selection prior to trait expression is an essential component of natural selection. Proceedings of the Royal Society B-Biological Sciences 277:2945–2950.

Nosil, P., T. H. Vines, and D. J. Funk. 2005. Reproductive isolation caused by natural selection against immigrants from divergent habitats. Evolution 59:705–719.

Peterson, M. L., K. M. Kay, and A. L. Angert. 2016. The scale of local adaptation in *Mimulus guttatus*: comparing life history races, ecotypes, and populations. New Phytologist 211:345–356.

Poisot, T., J. D. Bever, A. Nemri, P. H. Thrall, and M. E. Hochberg. 2011. A conceptual framework for the evolution of ecological specialisation. Ecology Letters 14:841–851.

Postma, F. M., and J. Agren. 2016. Early life stages contribute strongly to local adaptation in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences of the United States of America 113:7590–7595.

Radford, I. J., R. D. Cousens, and P. W. Michael. 2004. Morphological and genetic variation in the *Senecio pinnatifolius* complex: Are variants worthy of taxonomic recognition? Australian Systematic Botany 17:29–48.

Reich, P. B. 2014. The world-wide 'fast–slow' plant economics spectrum: a traits manifesto. Ecology 102:275–301.

Reznick, D. 1992. Measuring the costs of reproduction. Trends in Ecology and Evolution 7:42–45.

Rice, W. R., and E. E. Hostert. 1993. Laboratory experiments on speciation: What have we learned in 40 years? Evolution 47:1637–1653.

Richards, T. J., G. M. Walter, K. McGuigan, and D. Ortiz-Barrientos. 2016. Divergent natural selection drives the evolution of reproductive isolation in an Australian wildflower. Evolution 70:1993–2003.

Ricklefs, R. E., and M. Wikelski. 2002. The physiology/life-history nexus. Trends in Ecology and Evolution 17:462–468.

Roda, F., L. Ambrose, G. M. Walter, H. L. L. Liu, A. Schaul, A. Lowe, P. B. Pelser, P. Prentis, L. H. Rieseberg, and D. Ortiz-Barrientos. 2013a. Genomic evidence for the parallel evolution of coastal forms in the *Senecio lautus* complex. Molecular Ecology 22:2941–2952.

Roda, F., et al. 2013b. Convergence and divergence during the adaptation to similar environments by an Australian groundsel. Evolution 67:2515–2529.

Shaw, R. G., C. J. Geyer, S. Wagenius, H. H. Hangelbroek, and J. R. Etterson. 2008. Unifying life-history analyses for inference of fitness and population growth. American Naturalist 172:E35–E47.

Simpson, G. G., and C. Dean. 2002. Flowering - *Arabidopsis*, the rosetta stone of flowering time? Science 296:285–289.

Singer, M. C., and C. S. McBride. 2010. Multitrait, host-associated divergence among sets of butterfly populations: implications for reproductive isolation and ecological speciation. Evolution 64:921–933.

Stearns, S. C. 1989. Tradeoffs in life-history evolution. Functional Ecology 3:259–268.

Sultan, S. E. 2000. Phenotypic plasticity for plant development, function and life history. Trends in Plant Science 5:537–542.

Therneau, T. 2012. Mixed Effects Cox Models. Mayo Clinic, Mayo Foundation for Medical Education and Research.

Thompson, I. R. 2005. Taxonomic studies of Australian *Senecio* (Asteraceae): 5. The *S. pinnatifolius/S. lautus* complex. Muelleria 21:23–76.

Vandelook, F., D. V. de Moer, and J. A. Van Assche. 2008. Environmental signals for seed germination reflect habitat adaptations in four temperate Caryophyllaceae. Functional Ecology 22:470–478.

Walter, G. M., M. J. Wilkinson, M. E. James, T. J. Richards, J. D. Aguirre, and D. Ortiz-Barrientos. 2016. Diversification across a heterogeneous landscape. Evolution 70:1979–1992.

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