Heritable plant phenotypes track light and herbivory levels at fine spatial scales

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ABSTRACT

The biotic and the abiotic environment play a major role in shaping plant phenotypes and their geographic distributions. However, little is known about the extent to which plant phenotypes match local patterns of herbivory across fine-grained habitat mosaics, despite the strong effect of herbivory on plant fitness. Through a reciprocal transplant-common garden experiment with clonally propagated rhizomes, we tested for local phenotypic differentiation in bittercress (Brassicaceae: *Cardamine cordifolia*) plants collected across an ecotonal habitat mosaic. We found that bittercress in sunny meadows (high herbivory) and shaded understories (low herbivory) have diverged in heritable growth and herbivore resistance phenotypes. The expression of these differences was habitat dependent, mirroring patterns of adaptive divergence in phenotypic plasticity between plant populations in meadow and understory habitats at broader geographic scales, and showed no evidence for a constraint imposed by growth–defense tradeoffs. Most notably, plants derived from shade habitats exhibited a weaker shade-induced elongation response (i.e., shade avoidance syndrome, SAS) and reduced resistance to herbivory, relative to plants derived from sun habitats, when both were grown in shade common gardens. Greenhouse experiments revealed that divergent SAS phenotypes in shade conditions were expressed in offspring grown from seed as well. Finally, we observed partially non-overlapping flowering phenology between habitat-types in the field, which may be at least one factor that helps to reinforce habitat-specific phenotypic divergence. Altogether, our study illuminates how a native plant may cope with overlapping biotic and abiotic stressors across a fine-grained habitat mosaic.

INTRODUCTION

In species distributed across environmental gradients, individuals often exhibit phenotypes that track the local environment (Linhart and Grant 1996; Galloway 2005; Richardson et al. 2014). However, our knowledge regarding the conditions under which these patterns arise and persist has been disproportionately shaped by studies at course-grained rather than fine-grained spatial scales (Richardson et al. 2014). This bias has likely been shaped by the expectation that gene flow among interspersed habitat patches is generally a strong homogenizing force, preventing the establishment of habitat-associated phenotypic and genotypic variation at fine-grained spatial scales (Haldane 1930; Lenormand 2002). Although there is growing evidence that heritable phenotypes track habitat mosaics at fine-grained spatial

scales [i.e. *microgeographic* phenotypic divergence; (Richardson et al. 2014)] we have a limited understanding of the molecular, ecological, and evolutionary processes that facilitate and maintain this variation in nature.

Defoliation by insect herbivores exerts strong selection on plant phenotypes (Louda 1984; Prasad et al. 2012; Agrawal et al. 2012). In mustards (Brassicaceae), polymorphisms in genes that modify defensive chemicals underlie adaptation to local herbivore communities (Prasad et al. 2012; Zust et al. 2012), and the magnitude of geographic divergence at such loci is extreme compared to loci across the rest of the genome (Brachi et al. 2015). However, much of the strongest evidence for local adaptation to herbivory is from populations separated by large distances, on the scale of hundreds to thousands of kilometers (km). Although plants were the focus of pioneering research on variation in species distributions (Greig-Smith 1952) and phenotypic and genetic differentiation [reviewed in Linhart (1996)] across fine-grained habitat mosaics, relatively little is known about the relationship between anti-herbivore resistance traits and environmental herbivory pressure at microgeographic scales. A few studies have demonstrated that heritable divergence in herbivore resistance or defensive traits can correlate with variation in herbivore pressure at spatial scales ranging from a few km to only 500 m (Galen et al. 1991; Sork et al. 1993; Pellissier et al. 2014; Dostálek et al. 2016; Sato and Kudoh 2017).

The ability to mount strong defensive phenotypes in habitats where herbivores are abundant, either through phenotypic plasticity or through habitat-specific divergence in loci mediating resistance to herbivory, might be impeded by negative phenotypic correlations between defensive traits and those involved in response to other stressors. While the presence of multiple stressors can select for the coordinated expression of multiple inducible plant traits (Boege 2010), the response to selection may be limited by negative phenotypic correlations and insufficient genetic variance (Agrawal et al. 2010). For example, phenotypic investments in herbivore defense reduced stress tolerance in the mustard *Boechera stricta*, and this negative correlation itself may promote the patchy distribution of plants into habitats that do not impose a fitness cost arising from negative trait correlations (Siemens et al. 2009; Siemens and Haugen 2013; Alsdurf et al. 2013). Negative correlations between growth and defensive traits may impose a particularly strong constraint, and the question of how plants "solve" the dilemma of altering growth to compete for light *and* resist enemies in the face of growth-defense trade-offs has been the subject of intense study (Herms and Mattson 1992; Cipollini 2004; Züst and Agrawal 2017).

Herbaceous plant species abundant in both open habitats and deeply shaded forest understories, which often face distinct levels of both herbivory (Louda et al. 1987) and light competition (Dudley and Schmitt 1995), present an excellent opportunity for testing if both growth and anti-herbivore resistance phenotypes can diverge across microgeographic habitat mosaics, despite the potential tradeoffs between growth and defense. This is because decades of study have yielded clear expectations of which growth phenotypes are beneficial in high and low light habitats. In open and sunny habitats, plants express altered growth patterns in response to shade from herbaceous competitors. This change in plant growth traits and architecture, termed the shade avoidance syndrome (SAS), manifests as elongation of stems, petioles and hypocotyls, apical dominance, and early flowering (Keuskamp et al. 2010) induced by perception of light with a high far red (λ=700–800 nm):red (λ=600–700 nm) ratio or low blue light levels (λ=400–500 nm). Variation in SAS within species is an important example of locally adaptive plasticity (Van Kleunen and Fischer 2005; Valladares et al. 2007). Herbaceous plant species adapted to forest understories—where shading by forest canopies cannot be overcome—benefit from reduced SAS expression, while conspecifics adapted to open habitats benefit from their SAS expression in response to neighbor shade because neighbor shade can more easily be overcome (Dudley and Schmitt 1995; Schmitt et al. 1995; Dudley and Schmitt 1996; Donohue et al. 2000; Donohue et al. 2001; Bell and Galloway 2008). When light competition and herbivory are both encountered (e.g. in meadows), expression of SAS due to neighbor shade may attenuate herbivore resistance owing to an underlying trade-off between growth and defense (Uriarte et al. 2002; Cipollini 2004; Valladares et al. 2007). Whether herbivore resistance and SAS co-diverge at microgeographic scales, despite the commonly observed tradeoff between the two traits, remains largely unexplored for native plants.

Here, we quantified phenotypic divergence in growth and resistance to herbivory across a microgeographic habitat mosaic in bittercress (*Cardamine cordifolia* Gray; Brassicaceae), a forb native to montane regions of western North America. Bittercress grows in clumps across ecotonal patches with sharply contrasting selective regimes: high herbivory with high light competition from neighboring forbs in open meadow (sun) habitats, vs. low herbivory and shading from the evergreen tree canopy in nearby deeply shaded (shade) habitats with fewer herbaceous neighbors (Louda 1984; Collinge and Louda 1989; Louda and Rodman 1996; Alexandre et al. 2017). One of the primary herbivores of bittercress is a specialist leaf miner, *Scaptomyza nigrita* (Drosophilidae) (Collinge and Louda 1989; Gloss et al. 2014; Humphrey et al. 2014; Humphrey et al. 2016; Alexandre et al. 2017). When

given a choice in laboratory experiments, *S. nigrita* prefer to attack shade-derived compared to sun-derived bittercress, but abiotic factors over-ride this preference: *S. nigrita* adults strongly prefer brighter and warmer sunny habitats over darker and colder shade habitats, which largely restricts herbivory by *S. nigrita* to sunny habitats (Alexandre et al. 2017). For bittercress growing in the shade, one consequence of prolonged exposure to enemy-free space and decreased neighbor shade might be habitat-associated divergence in the investment in, and/or expression of, both SAS and inducible herbivore defenses. Thus, bittercress presents an opportunity to test for microgeographic divergence in growth and defensive phenotypes across habitats, in a context where such fine scale divergence might be constrained by growth–defense tradeoffs and homogenized by inter-habitat dispersal and gene flow.

Through reciprocal transplant–common garden experiments in the field and greenhouse, we tested if bittercress from deep shade habitats differed in growth phenotypes (including those reflecting SAS) compared to their sun-derived conspecifics. This design allowed us to measure the effect of phenotypic plasticity caused by growth environment (sunny vs. shaded), and whether source habitat impacts the average expression and/or the plasticity of expression of plant growth traits. We coupled these plant growth measurements with herbivore bioassays in which we measured bittercress resistance to *S. nigrita* in the common gardens. Finally, we determined whether flowering phenology differs between habitats in a manner that could facilitate phenotypic divergence by reducing gene flow among habitats.

Altogether, our study illuminates how a native plant may cope with overlapping biotic and abiotic stressors across distinct, fine-grained habitat types. We found that bittercress in sunny and shaded habitats have diverged in heritable growth and herbivore resistance traits. The expression of these differences was habitat dependent and in some cases counter to expected growth–defense tradeoffs. Finally, partially non-overlapping flowering phenology observed between habitat types may be at least one factor that helps to reinforce habitat-specific divergence.

MATERIALS AND METHODS

Common gardens in the field.

This study was conducted near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, USA, between 2011–2014. In 2011 at the RMBL, we conducted

a reciprocal transplant-common garden experiment to test if habitat of origin (sun vs. shade) impacted plant phenotypic responses to shading and realized resistance to herbivory. We chose nine sun and nine shade source sites from which to sample bittercress rhizomes for planting in common gardens that were either in meadows (sun) or under evergreen forest canopies (shade) (Fig. S1). At source and common garden sites, we recorded photosynthetically active radiation (PAR) using a light meter (Spectrum Technologies, Inc.), percent canopy cover using a densiometer, diameter at breast height (*dbh*) of the four largest trees within four meters, and latitude, longitude, and elevation (Garmin GPS) (Fig. S2, Tables S1). Source sites significantly differed in PAR, canopy cover, and *dbh* (ANOVAs, all $P<0.05$) but did not differ in elevation ($P>0.05$; Table S2).

From each source site, we collected rhizome tissue from 30 ramets and stored them at 4–10°C in low ambient light until planting. Using a randomized complete block design, 540 rhizomes—five from each of the 18 source sites (90 in total per garden)—were planted among six common gardens and spaced 16 cm apart to avoid shading from neighboring bittercress. Gardens were watered every 24–48 h with nearby water from a snow melt stream. Plants were shrouded using fine mesh cloth to prevent herbivory throughout the experiment.

After five weeks, we counted the number of leaves >10 mm and harvested and photographed the largest leaf. We measured petiole length and leaf area using ImageJ (Abràmoff et al. 2004) and leaf mass using a Sartorius CPA225D balance (Sartorius AG, Goettingen, Germany) following oven drying at 65°C for 2 d. Subsequently, we transplanted a single *S. nigrita* larva into the largest remaining leaf. Larvae were collected from mined leaves at Copper Creek, near the RMBL. After 72 h, we removed leaves and measured larval mass as a measure of larval performance (Whiteman et al. 2011; Whiteman et al. 2012), which is an index of realized resistance of the plants. Early instar larvae were excluded to approximately standardize larval developmental stage, but larvae were not weighed in order to minimize their manipulation. We therefore caution that the amount of variance within treatments is likely inflated by variation in initial larval mass. We compared larval mass using two-tailed two-sample *t*-tests of the larval mass distributions between sun and shade source plants in each of the garden types.

Greenhouse experiment.

We conducted a simulated shading experiment under greenhouse conditions using bittercress grown from seeds collected from sun and shade habitats. In August 2012, we collected seeds from bittercress growing in sun and shade habitats near

the source sites for the common gardens by tying bags to developing racemes (see Table S3 for collection sites). From the source sites we recorded % canopy cover using a densiometer and latitude, longitude, and elevation of each collection site as above. Seeds were transported to the University of Arizona and surface-sterilized with a solution of 50% bleach and 0.5% Triton-X-100 and stratified on moist filter paper in petri dishes for five weeks at 4°C. Seeds were germinated in the greenhouse in moist filter paper in petri dishes; seedlings were planted in soil in plastic pots as above and randomized to light filter environments to simulate sun or shade habitats. Evergreen forest canopy shade was simulated by growing 14 shade- and 23 sun-source plants under a blue plastic filter (Lee filter 115) that reduces the amount of red light available to plants (elevating the amount of FR:R light) (Runkle and Heins 2001). To simulate sun habitats, 19 shade- and 28 sun-source plants were grown under a control clear filter that does not alter the quality of light (Lee filter 130). When plants reached the 4-leaf stage and the 8-leaf stage, we measured vegetative traits as in the field common gardens. We did not have access to *S. nigrita* herbivores for the greenhouse experiment at to measure realized resistance in this trial.

We conducted all statistical analyses using R v.3.3 (R Core Team 2013). Source site characteristics were compared using ANOVA or linear mixed models (LMMs) using the *lme4* package (Bates et al. 2014). Hypothesis testing on fixed effects was conducted with conditional *F*-tests using denominator degrees of freedom (Kenward and Roger 1997) as implemented in R package *pbkrtest* (Halekoh and Højsgaard 2014). For the field common garden experiment, we modeled garden type, source habitat and interactions as fixed factors and garden number and source site number as random factors. To assess the role of garden and source types on plant phenotypes, we conducted principal component analysis (PCA) on total number of leaves, leaf area, petiole length, and specific leaf area (SLA, cm^2/g). We tested the relationship between garden and source types on PC1 + PC2 using linear discriminant function analysis (DFA) with R package *MASS* (Venables & Ripley 2002). To examine differences in petiole length among sun and shade-derived plants grown from seed in the greenhouse, we used R package *pbkrtest* as above to examine LMMs modeling petiole length at the 4- and 8- leaf stages as a function of environment type, source type, and their interaction as a fixed-effects with source site as a random effect. We separately analyzed petiole length at the 8-leaf stage in the FR:R light greenhouse treatment with source type as a fixed effect using the same approach as above. All field common garden data, as well as statistical analysis R scripts, are available on the Dryad digital repository (doi pending).

Field surveys of flowering phenology.

In 2015, we quantified floral abundance through time in sun and shade habitats in a sample of 240 bittercress plants spread across two sites (401 Trail and Copper Creek). At each site, we designated roughly equal numbers of plants in open sun, intermediate shade, and deep-shade and tracked plant-level flower abundance every 2–3 days for four weeks (between July 8 and August 5 of 2015). From these data, we designated a first, peak, and last flowering Julian day for each stem. In many cases, focal stems flowered before the observation window, and these stems were assigned a Julian date of 1 prior to the first observation day. Plants with no flowers or fruits by the end of the observation window were assigned a no-flowering status and were excluded from the phenology analysis. We also accounted for the presence of developing fruits on each stem, which allow us to designate plants with no *observed* flowers as having already completed flowering by the first observation date, thus distinguishing this class from those plants that had neither flowered nor fruited during the observation window. Many plants had just begun to flower by the end of the observation widow; the 'peak' and 'last' flowering events for these stems was assigned a Julian date of the last observation day +1. We compared the accumulation of each event across habitats and sites using non-parametric two-sample Kolmogorov–Smirnov tests, which calculates the difference between two empirical cumulative distributions; KS test *p*-values derived from the Kolmogorov distribution (by default) were compared to those obtained by permutation of the identity of the source habitat across data points (1000 replicates).

RESULTS

Effects source habitat and light environment on plant growth.

In the field, we measured plant growth traits in common gardens to investigate the strength of shade-induced growth phenotypes in bittercress genotypes derived from sun and shade habitats. After six weeks of growth in these common gardens, plants in shade gardens were strongly differentiated from those grown in sun gardens in a PCA using total number of leaves, leaf area, SLA, and petiole length (Fig. 1A). Using PC1 and PC2, which together comprised 80% of the variance, linear DFA correctly assigned 99.2% of plants to garden type, while 61.9% could be correctly assigned to source habitat type. Plants from both source habitats had similarly higher specific leaf area in shade gardens ($F_{1,4.24} = 46.7$, $P = 0.002$; Table 1, Fig. 1B), and plant from both source habitats regrew more leaves by 6 weeks in sun gardens than in shade gardens ($F_{1,4} = 18.16$, $P = 0.013$; Table 1, Fig. 1C.). Means and

297 standard errors of all growth traits for each source habitat in each garden are
298 compiled in Table S4.
299
300 In addition to these strong garden type effects, source habitat type affected various
301 individual plant growth phenotypes. Plants derived from shade habitats were
302 smaller than sun source plants in both garden types ($F_{1,15.68} = 15.81$, $P = 0.001$,
303 Table 1, Fig. 1B). Sun source plants had longer petiole length relative to their plant
304 size in both garden types ($F_{1,18.46} = 10.54$, $P = 0.0044$). But the increase in size-
305 specific petiole elongation for sun-derived plants from growth in the shade was far
306 stronger than that for shade-derived plants ($F_{1,265.65} = 13.47$, $P < 0.001$; Table 1, Fig.
307 1D). This result was recapitulated in the greenhouse, where sun bittercress grown
308 from seed exhibited significantly longer petioles at the eight-leaf stage compared to
309 shade source plants when both were grown under simulated neighbor shade (high
310 FR:R light; $z = 3.35$, $P = 0.0008$, Fig. S3). Leaf area exhibited no main source or
311 garden type effects but showed a strong interaction effect, with the changes in size-
312 specific leaf area being comparable in magnitude between garden types for plants
313 from both source habitats ($F_{1,264.34} = 9.5$, $P = 0.002$; Table 1, Fig. 1E).
314
315 *Effects of source habitat and light environment on realized herbivore resistance.*
316 In the field, we assayed realized plant resistance to *S. nigrita* larvae by measuring
317 larval mass as a proxy for herbivore performance in bittercress grown in the
318 common gardens. Despite high levels of variation in larval mass across both garden
319 types, shade-derived plants harbored larvae that had a higher average mass after
320 feeding for 24 hours than larvae randomized to any other condition. Specifically, in
321 shade gardens, larval mass was 21.3% higher when *S. nigrita* leaf-miners were
322 reared on plants from shade-derived compared to sun-derived plants (1.59 mg
323 vs.1.31 mg, two-tailed *t*-test, $P = 0.02$, Fig. 2). Within sun gardens, larval mass did
324 not differ between sun- or shade-derived plants (*t*-test, $P = 0.88$, Fig. 2).
325
326 *Effects of source habitat on flowering phenology in the field.*
327 Bittercress phenological progression and floral density curves differed both by
328 sampling site and by habitat type. At the 401 Trail site, plants in all habitats
329 exhibited very similar phenological progressions (K–S tests, all comparisons $P>0.05$;
330 Fig. 3A). In contrast, at the Copper Creek site, timing of first, peak, and last
331 flowering was significantly delayed for bittercress growing in intermediate shade
332 and deep shade compared to open sun habitats (sun vs. intermediate or deep shade
333 all $P<0.05$; Fig. 3A). The total density of flowering plants through time strongly
334 overlapped among habitats at the 401 Trail site, while shade habitats exhibited

their peak flower density after both the open sun and intermediate shade plants at Copper Creek (Fig. 3B).

DISCUSSION

Heritable divergence in plant growth and resistance traits across a fine-grained habitat mosaic

Two notable ecological features reliably distinguish sun and shade habitats for bittercress (Fig. S4), and thus could promote habitat-specific phenotypic divergence. First, herbivory by *S. nigrita*, a specialist leaf-miner that can heavily defoliate stands of bittercress, is primarily restricted to sun habitats (Collinge and Louda 1989; Louda and Rodman 1996; Alexandre et al. 2017). The enemy free space associated with shade habitats would be expected to favor lower resistance to herbivory in plants growing in shade (Agrawal et al. 2012). Second, the growth form of neighboring plants that filter incoming light, either forbs or canopy trees, differs among habitat types. Conspecific and heterospecific forbs grow at high densities in sun habitats of bittercress, and SAS is frequently observed to be adaptive in this context (Van Kleunen and Fischer 2005). In contrast, large evergreen trees filter the majority of light in shade habitats (Table S1). Canonical SAS phenotypes are typically an ineffective means to compete for light in forest understories, and their expression has been shown to reduce organismal fitness (Dudley and Schmitt 1995; Schmitt et al. 1995; Dudley and Schmitt 1996; Donohue et al. 2000; Donohue et al. 2001; Bell and Galloway 2008). We thus hypothesized that sun and shade plants should diverge in their expression of competitive growth (SAS) and resistance phenotypes.

Our reciprocal transplant/common garden assays revealed that heritable plant growth and resistance phenotypes were distinguishable between plants from the shade and the sun habitats. The directions of these differences were concordant with the hypothesis that light competition and herbivore prevalence drive phenotypic divergence among sun and shade habitats. Notably, some of the traits we measured were shaped by an interaction between light environment and parental habitat. In other words, although these phenotypes were plastic, differences in their expression across light environments (i.e., the degree of plasticity) were heritable.

Plant growth traits.

While light environment of the common gardens strongly influenced plant growth phenotypes, those phenotypes characteristic of adaptive plasticity to shade (i.e., SAS), most notably petiole elongation, differed markedly between plants from sun and shade habitat types (Fig.1). Rhizomes from sun source sites exhibited strong SAS when grown in shade gardens in the field (Fig. 1D), marked by increased petiole elongation relative to rhizomes from shade source sites grown under the same conditions. We observed this effect both in plants grown from seed derived from the same habitats in the greenhouse (Fig. S3), indicating that this phenotype is inherited by offspring through both clonal and sexual reproduction.

Resistance traits.

Herbivore resistance is a measure of how well herbivores can exploit a given host resource. We found that *S. nigrita* larvae feeding on shade-source plants gained more mass than larvae feeding on sun-source plants, but this pattern only emerged in shade common gardens (Fig. 2). This finding is consistent with the hypothesis that shade-derived plants invest less in defense when in their natal habitats, where investment in anti-herbivore defenses is likely costly in the absence of herbivory (Agrawal et al. 2012). More sensitive bioassays, as well as additional biomarkers of basal and inducible herbivore resistance, are required to identify the defense phenotypes responsible for the observed difference.

No evidence that a growth–defense tradeoff constrains habitat-associated phenotypic divergence

Negative phenotypic correlations between growth and defensive traits are widespread in plants (Herms and Mattson 1992; Cipollini 2004; Züst and Agrawal 2017). If these growth–defense tradeoffs are a consequence of resource allocation constraints, and selection on growth traits differs strongly between light habitats, tradeoffs may prevent local adaptation of herbivore resistance across the landscape. Observations from our common gardens suggest that any growth–defense tradeoffs that might exist in bittercress did not pose such a constraint. Plants from sun source sites were more resistant to *S. nigrita* than plants from shade source sites when both were grown in a common shade environment, even though plants from sun source sites concurrently expressed stronger SAS-related phenotypes under these conditions.

Our results are consistent with emerging evidence that a tradeoff between SAS and defense can be decoupled through mutation and natural selection (Züst and Agrawal 2017). Prolonged exposure to different regimes of herbivore pressure can

alter correlations between traits involved in competitive growth and resistance to herbivory in experimentally evolved plant populations, despite evidence that resource allocation constraints ultimately limit the extent to which both suites of traits can be co-expressed (Uesugi et al. 2017). Functional genetic studies of *Arabidopsis* mutants provide plausible mechanisms for the uncoupling of growth and defense at the molecular level (Moreno et al. 2009; Robson et al. 2010; Keuskamp et al. 2010; Kazan and Manners 2011; Keller et al. 2011; Cerrudo et al. 2012). Intriguingly, differences in the plastic response of bittercress to light habitat in our common gardens mirror those observed among *Arabidopsis* lines with mutations introduced to genes involved in light-responsive signaling (see Supplementary Discussion). Future studies could test if habitat-associated divergence in bittercress growth and defense phenotypes has evolved through genetic or epigenetic mutations to the well-characterized genes that mediate growth–defense tradeoffs in response to light environment in *Arabidopsis*.

We note, however, that our results do not provide unambiguous evidence for a decoupling of a growth–defense tradeoff in bittercress. Tradeoffs that arise through pleiotropy or resource allocation constraints can be masked when genotypes vary in their efficiency of resource acquisition or use (Agrawal et al. 2010; Züst and Agrawal 2017). Although rhizome size was standardized in our common garden experiment, differences in the quality or quantity of stored resources in rhizomes collected from different habitat sites, combined with habitat-dependent effects of this resource variation on growth and resistance phenotypes, might explain why plants from sun sources exhibited strong SAS without attenuated resistance to *S. nigrita*.

Potential mechanisms underlying microgeographic variation in bittercress

Future studies are needed to discern the mechanisms through which habitat-associated growth and resistance phenotypes are inherited in bittercress. Whether these traits are inherited through genetic or non-genetic mechanisms has implications for the ecological processes that generate the phenotype-habitat matching we observed across the landscape.

Transgenerational effects of parental environment on the phenotypes of clonally propagated offspring (Schwaegerle et al. 2000; Latzel and Klimešová 2010) and seed propagated offspring (Galloway 2005; Galloway and Etterson 2007) have been well documented in plants. These effects can shape offspring resistance against

herbivory (Agrawal 2001; Steets and Ashman 2010; Rasmann et al. 2012; González et al. 2017; Colicchio 2017), and epigenetic variants induced by (and affecting) resistance phenotypes can persist for multiple generations (Rasmann et al. 2012). Habitat matching of heritable phenotypes in bittercress could therefore be driven simply by parental experience, without requiring natural selection to filter locally unfit genotypes from sun and shade habitats.

If heritable variation in the expression of growth and defense phenotypes has a genetic basis, however, matching of these phenotypes to local habitats would require strong, spatially varying selection to overcome homogenizing effects of gene flow across fine-grained habitat mosaics (Lenormand 2002). There is strong evidence that this filtering process can occur in plants, sometimes at the scale of only a few meters (Waser and Price 1989; Schmitt and Gamble 1990; Antonovics 2006; Schemske and Bierzychudek 2007; Hendrick et al. 2016).

By reducing the frequency of inter-habitat mating events, differences in flowering time among bittercress in sun and shade habitats, coupled with natural selection filtering unfit genotypes in each habitat, could facilitate the buildup of locally adaptive genetic variants or maternal effects (Levin 2009). We quantified flowering time between sun and shade habitats at two sites, and found a marked difference in one of these sites, although the duration of flowering time still overlapped among habitats. The observed phenological differences between sun and shade sites are one of many potential mechanisms that may reinforce divergence between sun and shade habitats in bittercress.

Implications for the ecology and evolution of bittercress–*Scaptomyza* interactions

In a series of seminal field studies in the 1980s and 1990s, Louda and colleagues showed that higher herbivory on bittercress in open sun habitats, including by the abundant specialist herbivore *S. nigrita*, likely drives the distribution of bittercress toward more shaded habitats (Louda et al. 1987; Louda and Rodman 1996). These studies serve as a textbook example (Ricklefs and Miller 2000) of how natural enemies shape the distribution of their hosts across a heterogeneous landscape. However, the ultimate cause of higher herbivory in the sun remained unresolved.

Given that expression of SAS is frequently associated with reduced defenses against herbivory, and shade-derived bittercress exhibit reduced SAS relative to sun-derived bittercress in their source habitats, one possibility is that herbivory is

higher in the sun because bittercress plants in the sun are more palatable. The fact that shade source plants are less resistant than sun source plants to *S. nigrita* larval herbivory does not support this interpretation. Instead, our results further support the conclusion, emerging from other recent work (Alexandre et al. 2017), that a preference of *S. nigrita* for sun-exposed habitats is likely sufficient to explain higher herbivory on bittercress in the sun. Thus, this textbook example of how natural enemies shape the distribution of their hosts is ultimately driven by the effect of an extrinsic habitat characteristic (light) on the behavior of an herbivore, not by the herbivore's behavioral response to intrinsic differences in host plant quality across environments.

Conclusion

We found that bittercress offspring, whether produced clonally or from seed, exhibit growth and defense phenotypes that match their parental habitat across a fine-grained (i.e., microgeographic) habitat mosaic. If this process leads offspring to have higher viability or fecundity in their natal habitats, it could be an important mechanism that reduces rates of inter-habitat recruitment and gene flow, in turn facilitating the maintenance of genetic and epigenetic variation within populations (Felsenstein 1976; Hedrick 2006). The ecological, physiological, and evolutionary mechanisms underlying phenotype-habitat matching in bittercress—and their generality across plant systems—await future study.

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Fig. 1. Light environment and source habitat distinguish growth phenotypes of bittercress grown from rhizomes in common gardens. (**A**) Principle components analysis (PCA) of five growth phenotypes (see Methods) reveals distinct phenotypic clusters based on garden type. (**B–E**) Plant traits exhibited a garden type main effect (**B**, **C**), source habitat main effect (**C**, **D**), and interactions between garden type and source habitat (**D**, **E**). Error bars indicate ± standard error. 'ns' = $P>0.05$, *$0.05>P>0.01$, **$0.01>P>0.001$, ***$P<0.001$.

Fig. 2. Realized plant resistance to *Scaptomyza nigrita* larvae differed by source habitat in shade but not sun common gardens. *S. nigrita* larval mass was higher on average after feeding for 72 h on shade-derived plants than on sun-derived plants in shade common gardens, while larval mass was indistinguishable between plants from both sources in sun exposed common gardens. Larvae were initially of the same developmental instar but were not pre-weighted (see *Methods*). 'ns' = $P>0.05$, *$P<0.05$.

Fig. 3. Phenological progression and flowering curves indicate site-specific differences in phenology between open sun and shaded habitats. (A.) Phenological progression through first, peak, and last flowering depicted as empirical cumulative distributions indicate faster flowering for sun plants at Copper Creek but not 401 Trail site, as indicated by pairwise two-sample and two-tailed Kolmogorov–Smirnov (K–S) tests. (**B.**) Pooled average flower density through time shows delayed flowering peak in shade habitats at Copper Creek but not 401 Trail site.

FIGURES

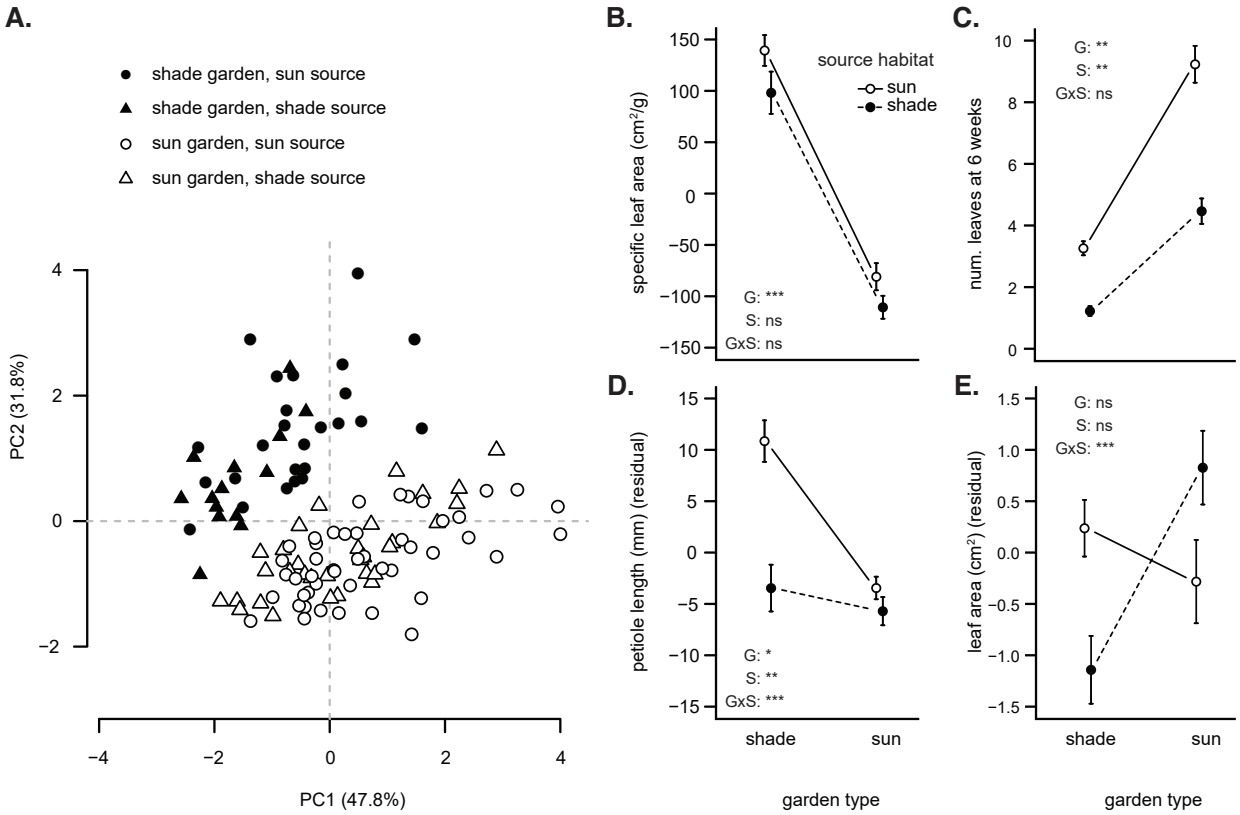

Fig. 1

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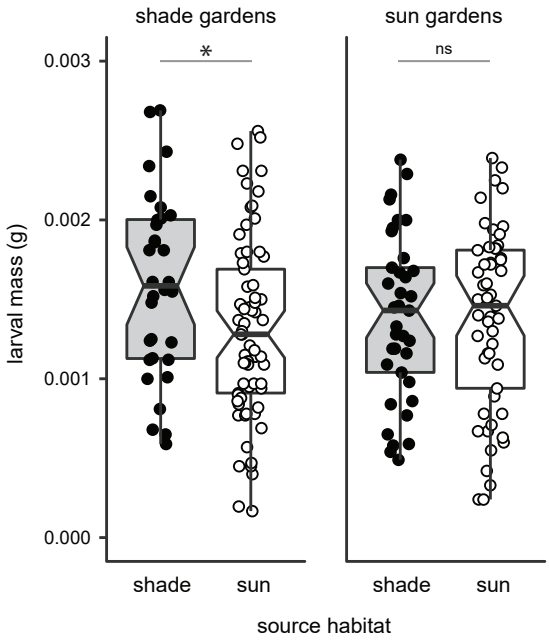

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845 **Fig. 2**

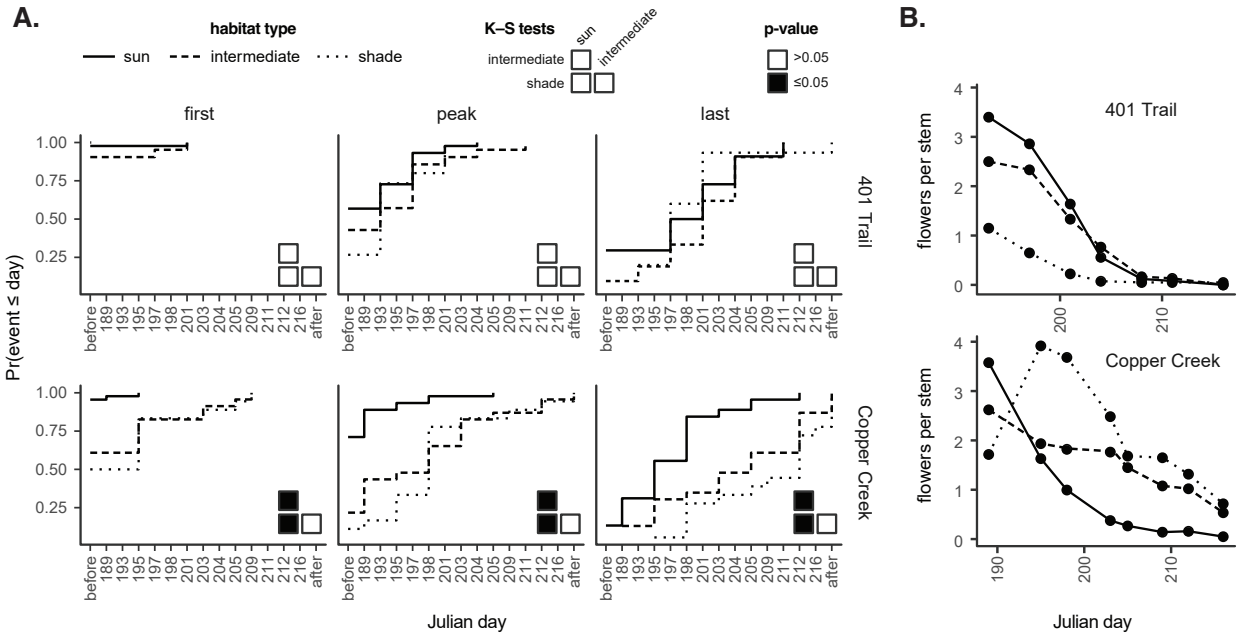

Fig. 3

TABLES

Table 1. Model results for plant growth traits in sun and shade common gardens.

		Response variables			
Predictor variables	Model statistic	Num. Leaves *	Leaf Area (cm2)†,[1]	Specific Leaf Area (cm^2/g)[1]	Petiole Length (mm)*,[1]
Garden Type	*MS*[a]	6.68	8.4	3.40E+05	5.923
	denDF††	4	4.7	4.24	4.22
	F	18.16	0.92	46.67	4.56
	P[b]	*0.013*	*0.383*	*0.002*	*0.096*
Source Type	*MS*[a]	5.977	0.31	3098	11.034
	denDF	15.68	17.35	16.84	18.45853
	F	15.81	0	0.42	10.54
	P	*0.001*	*0.853*	*0.527*	*0.004*
Garden Type X Source Type	*MS*	0.665	81.08	16921	13.236
	denDF	324.37	264.34	260.99	265.6573
	F	1.77	9.49	2.29	13.47
	P	*0.182*	*0.002*	*0.131*	*0.0003*
Covariate	*MS*	-	392.99	83500	42.23
	denDF	-	239.9	243.08	182.03
	F	-	38.57	4.51	32.26
	P	-	*0*	*0.0356*	*1.00E-07*
Genet X Garden Type	*Var*	0.0321	0.0167	17781	0.18922
	X2	5.207	0.735	5.3697	3.194
	df	3	3	3	3
	P	*0.157*	*0.865*	*0.147*	*0.363*

*- model results for square root transformed data

†- model results for *ln*-transformed data

†† - denominator degrees of freedom

a - MS from reduced model after testing for fixed and random interactions but retaining fixed interaction term if P<0.1

b - *F* and *P* values from Kendall-Rogers correction for denominator d.f. (see methods)

1 - modeled using leaf number as covariate

2 - modeled using SLA as covariate

ELECTRONIC SUPPLEMENTARY MATERIAL

Supplementary Figures.

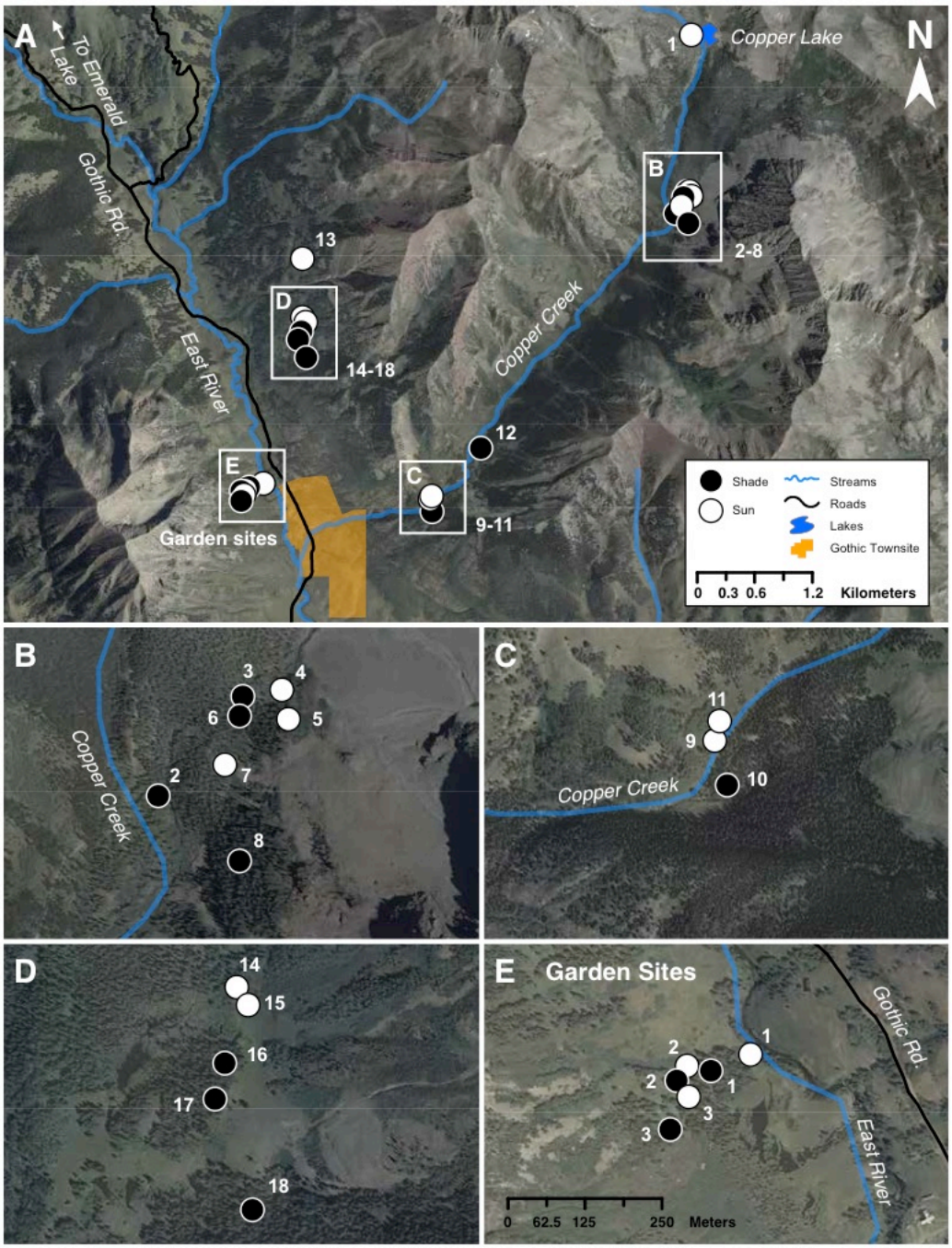

Fig. S1. Map of source and garden sites used in the field common garden study in the East River Valley and Copper Creek drainages, near the RMBL in Gothic, CO. **(A)**. Base map showing all sites within region (1:48,000). **(B–E)**. Maps showing detail of site locations (all same scale, 1:7,500). Scale bar in E applies to B–E.

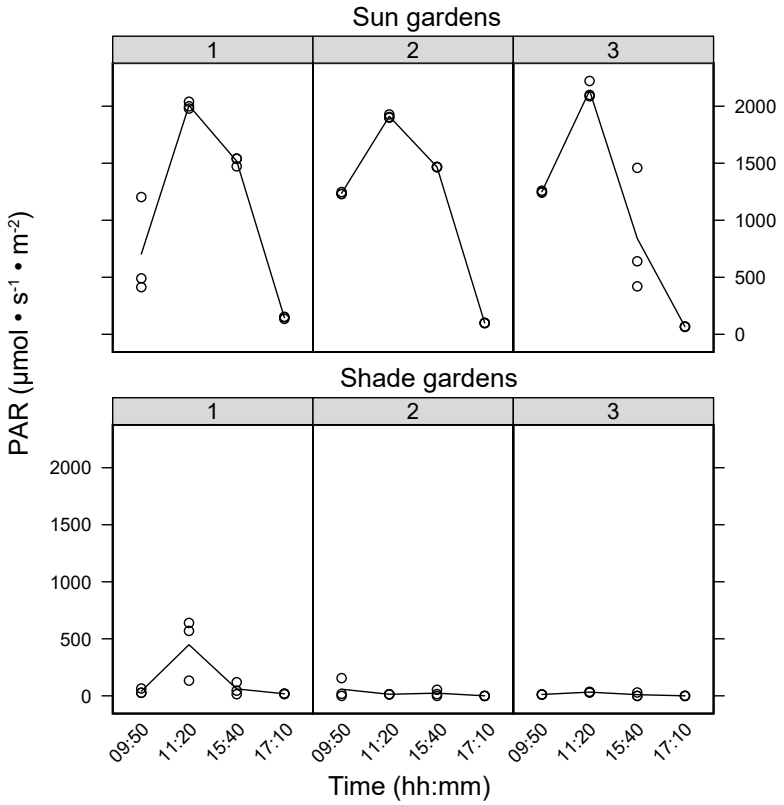

Fig. S2. PAR measurements for the six common garden sites showing daily natural variation in light abundance. Line depicts mean of three measurements per time point per garden.

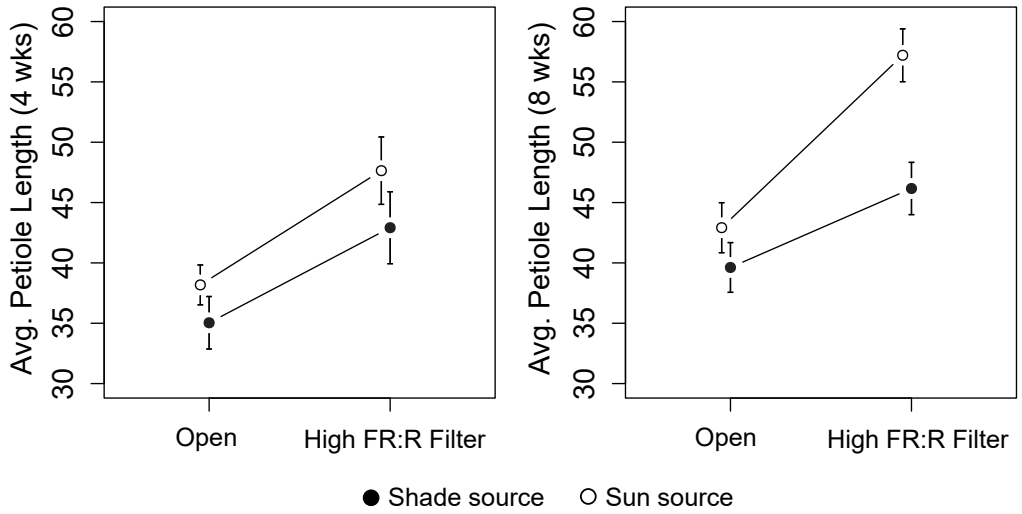

Fig. S3. Petiole length of shade and sun source bittercress re-grown from seed under neutral ("Open") or light filters ("High FR:R Filter") to simulate neighbor shading in the greenhouse. ± indicates standard error.

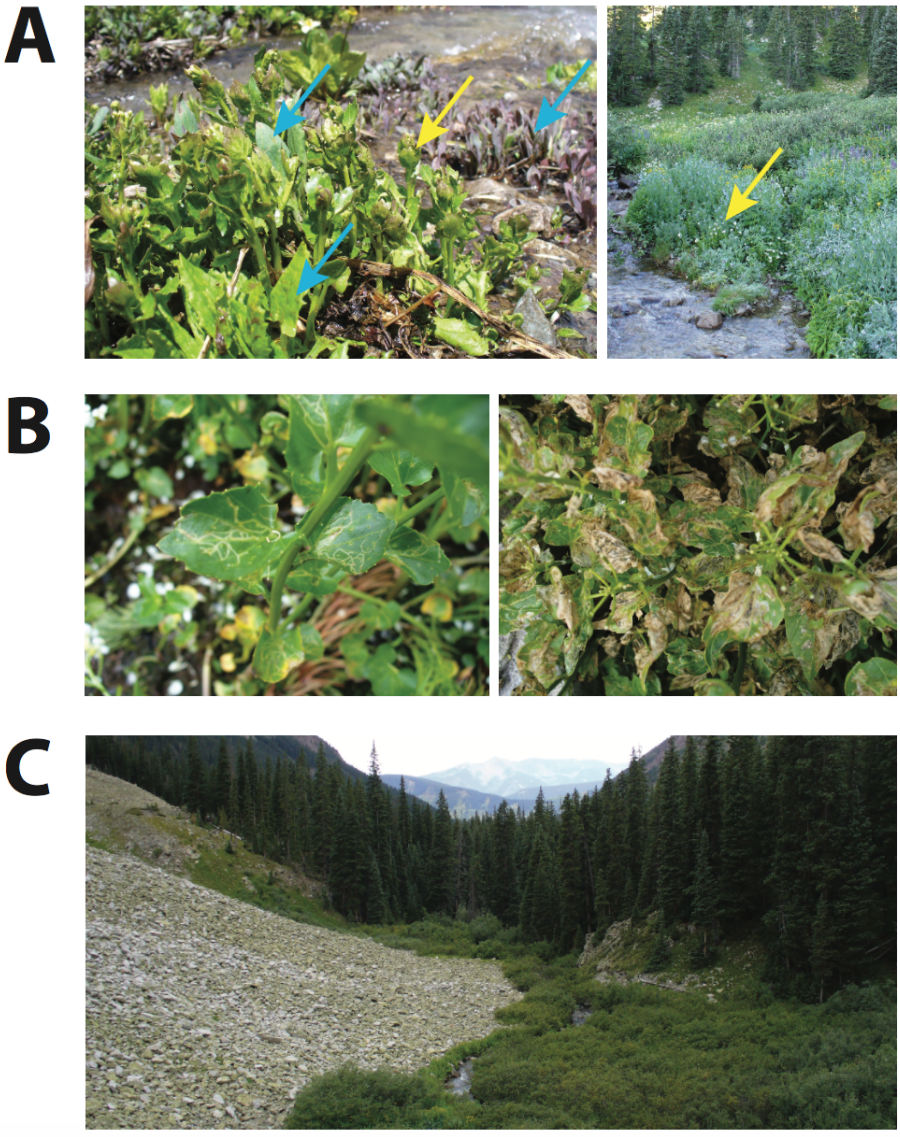

Fig. S4. Photographs illustrating characteristics of open sun habitats for bittercress near RMBL. (**A**) Bittecress densely interspersed among heterospecific forbs early (left) and later (right) in the growing season. Yellow arrows: bittercress (with white flowers in the image to the right); blue arrows: other forbs. (**B**) Leaf mines from *S. nigrita* early (left) and later (right) in the growing season. (**C**) An alpine stream providing a patch of sunny habitat for bittercress at the base of a talus slope, before flowing into a shaded evergreen forest.

Supplementary Tables.

Table S1. Attributes of sites used as sources for bittercress genets for common gardens.

Site #	Date Collected	Lat.	Long.	Elevation (m)	Soil Moisture	Light Env.	PAR (µmol•s•m-1)[1]	% Canopy Open	Average DBH (cm)
1	10-Jul-11	39.0050602776535	-106.943317166893	3461	Very Wet	Sun	1980	99.84	0
2	10-Jul-11	38.9885304491745	-106.945774321867	3218	Moist Loamy	Shade	300	6.76	10
3	11-Jul-11	38.9900162385746	-106.944210201638	3240	Moist Loamy	Shade	130	8.06	121.75
4	11-Jul-11	38.9900829890862	-106.943450029132	3253	Very Wet	Sun	2030	82.94	0
5	11-Jul-11	38.9896982273227	-106.9432894133	3261	Very Wet	Sun	2034	95.42	0
6	11-Jul-11	38.9897805369006	-106.94429611421	3244	Moist Loamy	Shade	130	4.16	83.25
7	11-Jul-11	38.9890385630266	-106.944483622637	3220	Very Wet	Sun	2134	99.84	0
8	11-Jul-11	38.9876108211294	-106.944190554921	3250	Moist Loamy	Shade	28	3.38	81.75
9	12-Jul-11	38.9607056556777	-106.973679741745	3023	Very Wet	Sun	2026	88.14	0
10	12-Jul-11	38.9600423351315	-106.973476684125	3013	Wet Loamy	Shade	35	5.72	72.25
11	12-Jul-11	38.9609507737634	-106.973571138078	3994	Very Wet	Sun	1933	86.84	0
12	12-Jul-11	38.9655594715881	-106.968516958823	3994	Very Wet	Shade	32	3.9	101
13	13-Jul-11	38.9828665210329	-106.989822099201	4058	Very Wet	Sun	1780	96.2	0
14	13-Jul-11	38.9774145794955	-106.98986568487	4082	Very Wet	Sun	1870	88.4	0
15	13-Jul-11	38.9771831850899	-106.989697600334	4057	Very Wet	Sun	1850	99.84	0
16	13-Jul-11	38.976356744414	-106.990078474138	4052	Very Wet	Shade	54	4.68	98
17	13-Jul-11	38.975757817399	-106.990327194402	4046	Moist Loamy	Shade	60	4.16	140.5
18	13-Jul-11	38.974195183581	-106.989498527449	4051	Very Wet	Shade	40	7.28	78.75

[1]Light meter was positioned above the center of each source collection plot, at times without cloud cover between 1:00 pm and 3:00 pm during the week of July 17, 2011.

Table S2. Environmental attributes of sun and shade source sites used in field common gardens.

	Sun Sites (N = 9)		Shade sites (N = 9)		ANOVA	
Attribute	μ	se	μ	se	F	P
PAR ($\mu mol \bullet s^{-1} \bullet m^{-2}$)	1959.7	37.0	89.0	29.4	1564	$<10^{-10}$
% Canopy Open	93.1	2.2	5.3	0.6	1527	$<10^{-10}$
DBH (cm)	0.00	0.00	87.5	12.2	51.77	$<10^{-10}$
Elevation (m)	3601	146	3568	150	0.025	0.875

Table S3. Source site attributes for plants from which seeds were collected and re-grown for use in greenhouse common garden experiment.

Site #[1]	Lat.	Long.	Elevation (m)	Light Env.	% Canopy Open
9	38.977203215	-106.992257330	3050	Shade	32.24
8	38.977172541	-106.992202530	3049	Shade	13.52
11	38.977208673	-106.991636863	3049	Shade	9.36
19	38.960075121	-106.973484675	3007	Shade	32.24
20	38.959999897	-106.973505522	3005	Shade	18.72
12	38.974598748	-106.990864137	3044	Sun	96.72
13	38.974504757	-106.990819289	3045	Sun	84.24
26	39.007275416	-107.040268306	3181	Sun	100

[1]Site numbers for plant from which seeds were collected differ from site numbers listed above in Table A1.

Table S4. Descriptive statistics for plant growth and herbivory phenotypes measured in common gardens.

	Shade gardens						Sun gardens					
	Shade source habitats			Sun source habitats			Shade source habitats			Sun source habitats		
Variable	N	μ	se[1]	N	μ	se	N	μ	se	N	μ	se
Num. leaves	103	1.36	0.17	108	3.68	0.23	113	4.84	0.44	114	9.98	0.61
Petiole length (mm)	41	32.22	2.27	76	47.04	2.06	62	31.94	1.5	90	35.59	1.18
Leaf area (cm^2)	41	3.18	0.33	76	5.04	0.3	62	6.82	0.46	90	7.03	0.44
Leaf mass (mg)	41	4.74	0.4	76	7.49	0.41	62	19.38	1.39	90	21.66	1.4
Specific leaf area (cm^2/g)	41	659.9	19.1	76	677.8	15.1	62	362.8	7.7	90	337.5	8.0
Larval mass (mg)	31	1.62	0.1	68	1.3	0.07	34	1.4	0.09	53	1.39	0.07

[1]Standard error

Supplementary Discussion.

Functional genetic studies of *Arabidopsis* mutants provide plausible, although speculative, mechanisms for the uncoupling of growth and defense at the molecular level in bittercress. Recall that bittercress from shade source habitats expressed relatively reduced SAS as well as reduced herbivore resistance when grown in shade gardens. This pattern was found in the SAS-deficient *A. thaliana* mutant *sav3-2,* which exhibits an attenuated defense phenotype but no SAS expression under high FR:R light (Moreno et al. 2009; Cerrudo et al. 2012). It is possible that bittercress in evergreen forest canopy understories do not *express* SAS but continue to express attenuated resistance to herbivores through a pleiotropic effect associated with detection of high FR:R light. In sun gardens, shade-derived bittercress resisted herbivores to the same degree as plants from sun source. Decoupling of shade detection from SAS expression would allow plants adapted to evergreen forest canopy shade to continue to resist herbivores in sun habitats while reducing the expression of SAS following shade detection under evergreen forest canopy shade, where its expression is unnecessary.

In contrast, plants from sun habitats could activate SAS upon detection of high FR:R light but may have decoupled the resistance attenuation that typically results from detection of neighbor shade (Robson et al. 2010; Kazan and Manners 2011). This could occur via modification of the physiological basis of shade detection. In *A. thaliana*, SAS is also induced by depletion of blue (B) light, which is detected by cryptochrome 1 (CRY1) (Keuskamp et al. 2010; Keller et al. 2011). Mutant *A. thaliana* with inactivated CRY1 constitutively express features of SAS but without attenuation of JA-dependent defenses. The same phenotype is produced in wild-type *A. thaliana* grown under B-depleted light (Cerrudo et al. 2012), suggesting that alternate light-sensing pathways may interact to determine the consequences of shade detection for plant defense against herbivores.

Mutations that reduce the ecological costs of negative correlations may be favored under particular combinations of selective agents, such as those faced by bittercress in sun habitats. Novel and habitat-specific genotypes may emerge that reveal novel insights into the genetic architecture of phenotypic plasticity. Extensive quantitative genetic variation has been found for SAS expression among *A. thaliana* accessions (Filiault and Maloof 2012), and testing for correlations between SAS and defense-related genetic variation coupled to shading and herbivory experiments may be one promising avenue of future research.

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