Title:

Plasticity of plant defense and its evolutionary implications in wild populations of *Boechera stricta*

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Abstract:

Phenotypic plasticity is thought to impact evolutionary trajectories by shifting trait values in a direction that is either favored by natural selection ("adaptive plasticity") or disfavored ("nonadaptive" plasticity). However, it is unclear how commonly each of these types of plasticity occurs in natural populations. To answer this question, we measured glucosinolate defensive chemistry and reproductive fitness in over 1,500 individuals of the wild perennial mustard *Boechera stricta*, planted in four common gardens across central Idaho, USA. Glucosinolate profiles—including total glucosinolate quantity as well as the relative abundances and overall diversity of different compounds—were strongly plastic both among gardens and along environmental gradients within gardens. The magnitude and direction of glucosinolate plasticity varied greatly among genotypes. We observed five cases of adaptive plasticity between gardens, in which glucosinolate profiles shifted in a direction that matched the direction of natural selection. In contrast, we found no evidence for nonadaptive glucosinolate plasticity between habitats. Evidence for within-habitat selection on glucosinolate reaction norm slopes (i.e., plasticity along a continuous environmental gradient) was inconclusive. Together, our results indicate that glucosinolate plasticity may improve the ability of *B. stricta* populations to persist after migration to new habitats.

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Introduction

The role of phenotypic plasticity in adaptive evolution has been a subject of great controversy and research interest for decades (Bradshaw 1965; Via and Lande 1985; Via *et al*. 1995; Pigliucci 2005; Ghalambor *et al*. 2015; Hendry 2015). It has long been recognized that both an organism's genotype and its environment shape its phenotype, which then determines its evolutionary fitness. Strictly speaking, phenotypic variation caused by environmental stimuli is not heritable and therefore cannot result in evolution through systematic changes in allele frequencies (Falconer and Mackay 1996). Nevertheless, plasticity is predicted to impact evolution by shifting phenotypes that are under natural selection (Bradshaw 1965). It remains unclear how commonly this occurs in natural populations, and whether the adaptive value of plasticity varies for different traits, environments, and spatial scales.

One way that plasticity could impact evolution is by accelerating or hindering adaptation to a novel environment—e.g., upon invasion of a new habitat or in response to a relatively sudden ecosystem shift, as might result from climate change (Donohue *et al*. 2001; Richards *et al*. 2006; Ghalambor *et al*. 2007; Anderson *et al*. 2012). Plasticity that moves a phenotype closer to the new phenotypic optimum is often called "adaptive" plasticity because it increases fitness relative to a non-plastic genotype (Figure 1g-i); however, whether this type of plasticity actually facilitates genetic adaptation is controversial. Strong adaptive plasticity could place an organism very near to the new adaptive peak, removing the selective force that would otherwise drive adaptation through allele frequency change and thereby inhibiting local adaptation. Alternatively, moderate adaptive plasticity may enable a population to survive in the new environment long enough for selection to increase the frequency of beneficial alleles, thus promoting local adaptation (Baldwin 1896; Price *et al*. 2003; Ghalambor *et al*. 2007). The opposite pattern—in which "nonadaptive" plasticity moves phenotypes farther from the new optimum—may either increase the risk of extinction or lead to rapid adaptive evolution by intensifying natural selection (Conover and Schultz 1995; Ghalambor *et al*. 2007; Ghalambor *et al*. 2015; Huang and Agrawal 2016). In this manuscript, we do not attempt to determine whether plasticity constrains or facilitates long-term genetic adaptation. Rather, our goal is to assess the relative frequency of adaptive versus nonadaptive plasticity in natural populations.

A related but distinct question is whether, and how, plasticity might evolve as an adaptation to environmental heterogeneity within a single habitat. Plasticity in response to fine-scale environmental variation is often imagined as a *reaction norm*, with the trait

value as some function of a continuous environmental predictor (Figure 2; Schmalhausen 1949). For traits that exhibit genotype-by-environment interactions, genetic variation exists for reaction norm shape. Natural selection acting on variation for plasticity can be detected using established quantitative genetic methods such as genotypic selection analysis on reaction norm coefficients (Figure 2g-h; Weis and Gorman 1990; Rausher 1992; Baythavong and Stanton 2010). Natural selection is predicted to favor increased plasticity if the spatial scale of changing selective pressures is similar to the organism's dispersal distance (Levins 1962; Gomulkiewicz and Kirkpatrick 1992; Baythavong 2011), if reliable environmental cues for the selection pressure are available (Levins 1963; Donohue *et al*. 2000; Schmitt *et al*. 2003; Reed *et al*. 2010), and if costs of plasticity are minimal (Auld *et al*. 2010).

Despite several excellent empirical studies (Dudley and Schmitt 1996; Schmitt *et al*. 1999; Donohue *et al*. 2000; Donohue *et al*. 2001; Sultan 2001; Baythavong 2011), more examples from natural populations are needed to test theoretical predictions about the fitness of both between-environment and within-environment plasticity (Hendry 2015). Data on the plasticity and evolution of physiological traits (as opposed to morphological or life-history traits) is particularly scarce (Palacio-López *et al*. 2015). Because variation in phytochemistry may affect not only the evolution of the plant but also entire communities and ecosystems (Wimp *et al*. 2007; Hopkins *et al*. 2009), phytochemical plasticity has been identified as a high priority research target (Hendry 2015). Here, we address these needs by studying plasticity and evolution of glucosinolate defensive chemistry in the wild perennial herb *Boechera stricta*, a close relative of Arabidopsis. Goals of this study were (1) to characterize genotype-by-environment

interactions underlying glucosinolate variation in *B. stricta*, (2) to assess whether glucosinolate plasticity alters relative fitness after transition to novel habitats, and (3) to test whether natural selection acts on glucosinolate reaction norms within habitats.

We measured glucosinolate profiles, size, and fecundity of 25 *B. stricta* genotypes replicated in 80 experimental blocks divided among four common gardens in diverse habitats (Figure 3). Because *Boechera* has limited dispersal (<0.5 m on average; Bloom *et al*. 2002), the environmental variation encompassed by the widely separated common gardens is much greater than what individual *B. stricta* populations normally encounter; thus, plasticity between field sites describes plasticity after a sudden environmental change or migration to a new habitat. To assess whether between-habitat glucosinolate plasticity exhibits an "adaptive" or "nonadaptive" pattern, we compared the direction of plasticity with the direction of selection in each site (Figure 1g-i). Then, we quantified within-habitat glucosinolate plasticity and assessed its relationship to fecundity in each habitat using genotypic selection analysis on reaction norm coefficients (Figure 2g-h). We found that substantial genotype-by-environment interactions underlie glucosinolate variation in *B. stricta*, and plasticity among sites tended to move trait values in an adaptive direction; however, we did not detect directional selection on glucosinolate plasticity within any habitat.

Methods

All statistical analyses were performed in R version 3.3.2 (R Core Team 2016) with heavy use of the packages ggplot2, lme4, lmerTest, dplyr, tidyr, and stringr (Wickham 2009; Bates *et al*. 2015; Kuznetsova 2015; Wickham and Francois 2015;

Wickham 2016a,b). Throughout, *P*-values were corrected for multiple comparisons using the sequential Bonferroni correction (Holm 1979). Additional details for all sections are available in Supplementary Methods, and our analytical approach is summarized in the Appendix. All data and R code will be made freely available in a Dryad repository upon article acceptance.

Study system

The short-lived perennial herb *Boechera stricta* (Graham) Al-Shehbaz is common in montane meadows and forests throughout its native range in western North America (Rushworth *et al*. 2011). Natural populations are strongly genetically differentiated (F_{ST}=0.56; Song et al. 2006) and have adapted to diverse habitats that vary in climate, water availability, elevation, soil composition, plant community diversity and density, and microbial community composition (Supplementary Figure 1; Anderson *et al*. 2013a,b; Wagner *et al*. 2016).

B. stricta produces a variety of glucosinolates, which are sulfur-rich, biologically active phytochemicals that protect against generalist insect herbivores and pathogens and may also affect nonpathogenic root-associated microbes (Agrawal 2000; Tierens *et al*. 2001; Brader *et al*. 2006; Halkier and Gershenzon 2006; Bednarek *et al*. 2009; Bressan *et al*. 2009; Hopkins *et al*. 2009; Schranz *et al*. 2009; Sanchez-Vallet *et al*. 2010). Glucosinolates are constitutively produced, although attack by natural enemies often induces additional production (Agrawal 1998, 2000; Brader *et al*. 2001; Agrawal *et al*. 2002; Textor and Gershenzon 2009; Abdel-Farid *et al*. 2010; Manzaneda *et al*. 2010). *B. stricta* produces four aliphatic glucosinolate compounds with differing biological activity (Figure 4a; Windsor *et al*. 2005; Schranz *et al*. 2009; Prasad *et al*. 2012). Total

glucosinolate concentration and relative abundances of these compounds vary extensively among individuals (Figure 4b-c).

Design and installation of field experiment

In October 2013, we planted 4,000 self-full siblings of 25 naturally inbred *Boechera stricta* genotypes (Supplementary Table 1) in fully randomized blocks (two replicates per genotype per block) in four common gardens in central Idaho (Figure 3). These field sites are all home to wild *B. stricta* populations, and are distinguished by many biotic and abiotic environmental characteristics (Supplementary Table 2; Supplementary Figure 1). Each genotype was derived from an accession from one wild *B. stricta* population (Figure 3a), which we propagated by self-fertilization in standard greenhouse conditions to minimize variation caused by maternal environmental effects. These genotypes represent the breadth of *B. stricta* genetic diversity, comprising 12 from the WEST subspecies and 13 from the EAST subspecies (Lee and Mitchell-Olds 2013). Because *B. stricta* primarily self-pollinates and is naturally inbred (F_{IS}=0.89; Song *et al.* 2006), self-full siblings are essentially genetically identical. Therefore, phenotypic differences between individuals of the same genotype describe that genotype's plastic response to environmental variation.

Measurement of plant performance in the field

During summer 2014, we returned to each site several times to measure survival, developmental stage, and height. At the end of the growing season, we measured fruit production for each surviving individual. Because *B. stricta* is predominantly self-pollinating (Song *et al.* 2006), fruit production reflects both male and female fecundity, and thus is a good estimate of reproductive fitness.

For phenotypic selection analyses (below), we used fecundity (in mm of fruit produced) as a measurement of reproductive fitness:

$$Fecundity = Number\ of\ siliques \times Length\ of\ average\ silique$$

For estimation of genotypic fitness, we also calculated the probability of survival for each genotype l: $P(survival) = \frac{number\ of\ surviving\ individuals\ of\ genotype\ l}{number\ of\ individuals\ of\ genotype\ l\ originally\ planted}$

We then calculated the total evolutionary fitness for each genotype as:

$$w = P(survival) \times mean(fecundity)$$

Measurement of glucosinolate profiles

Because insect attack can induce additional production of glucosinolates (Agrawal 1998), we measured glucosinolate profile as early as possible in the summer, before peak herbivory. On the earliest census date for each site, we collected ~20-30 mg of rosette leaf tissue from each surviving plant into tubes containing 70% methanol. Samples were shipped to Duke University, then fully randomized onto 96-well plates. Glucosinolates were extracted from the methanol leachates using established protocols (Supplementary Methods). We used high-performance liquid chromatography (HPLC) to measure the abundance of four aliphatic glucosinolates (Figure 4a-b) in each sample. Three of these compounds (1ME, 1MP, 2OH1ME) have branched-chain structures and biological activity that differs from that of the fourth, straight-chain compound (6MSOH; Figure 4a; Schranz *et al*. 2009; Prasad *et al*. 2012). We calculated absolute concentrations (μmol per mg dry weight) of each compound by comparing each peak to an internal standard and dividing by the dry weight of each leaf sample (Supplementary Methods).

From the absolute concentrations of all four compounds, we calculated three summary metrics for each sample's glucosinolate profile:

$$Total_{[GS]} = [2OH1ME] + [1ME] + [1MP] + [6MSOH]$$

$$BC_{ratio} = \frac{[2OH1ME] + [1ME] + [1MP]}{Total_{[GS]}}$$

$$BC_{diversity} = -\sum_{i=1}^{k} [BC_i] * \log([BC_i])$$

where k = the total number of branched-chain compounds present in the sample and $[BC_i]$ = the concentration of the ith branched-chain glucosinolate. Total [GS] describes the combined concentration of all aliphatic glucosinolates. BC-ratio describes the proportion of aliphatic glucosinolates that are derived from branched-chain amino acids, which is an ecologically and evolutionarily important trait in *B. stricta* (Schranz *et al*. 2009; Manzaneda *et al*. 2010; Prasad *et al*. 2012). Finally, BC-diversity describes the balance of the three types of branched-chain glucosinolates, taking low values when glucosinolate profiles are dominated by one compound and high values when multiple compounds are present in similar amounts (Figure 4b-c). We calculated BC-diversity using the Shannon diversity index in the **R** package **vegan** (Oksanen *et al*. 2013).

Partitioning variance in glucosinolate profiles

To assess plasticity of glucosinolate profiles among habitats, we used univariate REML mixed models and ANCOVA to partition variance in each of the three glucosinolate traits among genetic and environmental predictors. We modeled each trait as:

$$Trait = Genotype + Site + Genotype * Site + Block(Site) + Genotype * Block + Plant\ height + Developmental\ stage + Batch + error$$

where *Height, Developmental Stage,* and *Batch* were nuisance variables to control for (respectively) the "general vigor problem" of large plants having more resources to invest in defense (Agrawal 2011), ontogenetic changes in rosette glucosinolate profiles, and HPLC batch effects. One genotype was omitted from the analysis because no individuals of that genotype survived at one field site. Least-squares mean trait values (for *Site* and *Genotype* × *Site* fixed effects) resulting from this model were used to quantify between-habitat plasticity (see Appendix).

Testing for adaptive plasticity among habitats

The question of whether plasticity can aid survival in new environments hinges on whether the direction of plasticity matches the direction of selection (Figure 1g-i). Therefore, our approach was to determine (1) the direction of plasticity, if any, in each site (Figure 1g); (2) the direction of selection, if any, in each site (Figure 1h); and (3) whether the direction of plasticity matched the direction of selection more often than would be expected by random chance (Figure 1i). We evaluated plasticity and directional selection of three separate glucosinolate-related traits (Figure 4c) in each of four common gardens (Figure 3; Appendix).

First, to determine the direction of plasticity in each site, we calculated the plasticity deviation (ΔT_i) of each trait T in each site i using the formula:

$$\Delta T_i = T_i - \bar{T}$$

where T_i is the least-squares mean trait value for site i (calculated from the REML mixed model described above; depicted by the black points in Figure 1g) and $\bar{T}$ is the grand mean trait value from the entire experiment (depicted by the dotted line in Figure 1g). The sign of ΔT_i thus describes the direction of plasticity in site i.

Second, to determine the direction of selection (if any), we conducted twelve phenotypic selection analyses (3 glucosinolate traits × 4 sites). The within-site relative fecundity of all measured individuals was regressed onto standardized trait values, while controlling for microsite variation in habitat quality using a random intercept *Block* term:

$$Individual\ relative\ fecundity = Block + Trait_value + error$$

A significant *Trait_value* regression term indicated nonzero directional selection. Here, *Trait_value* indicates the product of an estimated regression coefficient x the observed trait value (see Supplementary Methods for details). This regression coefficient is the selection differential on one trait at one site (depicted as the slope of one line in Figure 1h). The signs of the significant regression coefficients from these models thus indicate the direction of selection on each trait at each site.

Next, to test whether selection differentials for each trait varied among sites (i.e., whether selection was spatially variable), we analyzed the relative fecundity and standardized phenotype data from all four sites together by fitting a mixed-effects ANCOVA model with an additional *Site* × *Trait* interaction term, which described heterogeneity of directional selection on the trait among habitats:

$$Individual\ relative\ fecundity = Site + Block(Site) + Trait + Site * Trait + error$$

Finally, using the outcomes of the above analyses (see Appendix), we applied two different methods to test the hypothesis that plasticity tends to move glucosinolate trait values in an adaptive direction. Whether plasticity of a trait is in an “adaptive” or “non-adaptive” direction in a given field site can only be evaluated if the trait both (1) exhibited significant plasticity at that site, and (2) was under significant directional

selection at that site (Figure 1g-i). Therefore, we considered only the cases in which we had detected both a significant plasticity deviation (ΔT_i) and a significant selection differential within a single site for any given trait. First, we conducted an exact binomial test to determine whether the sign of ΔT_i matches the sign of the selection differential more often than would be expected by random chance—i.e., to determine whether the direction of selection predicts the direction of plasticity (Figure 1i). Second, we performed a linear regression of ΔT_i on selection differentials (i.e., the linear coefficients of the phenotypic selection analyses) to test whether selection differentials in the four sites (both magnitude and direction) predict the magnitude and direction of plasticity.

Characterizing within-habitat plasticity using reaction norms

In this study, we focused on phenotypic plasticity induced by spatial environmental variation at a single time-point. Because each plant in this study only experienced a single spatial environment, plasticity of individual plants could not be measured. Instead, spatial plasticity of glucosinolate profiles is a property of a genotype, estimated by comparing the phenotypes of individuals that shared the same genotype but were growing in different experimental blocks. To infer whether natural selection was acting on fine-grained glucosinolate plasticity within *B. stricta* habitats, we (1) quantified block-scale plasticity for each genotype as a continuous function or *reaction norm*, and (2) used genotypic selection analysis to test whether reaction norm steepness—a measure of plasticity—predicted evolutionary fitness of each *B. stricta* genotype (see Appendix).

First, for each of 25 genotypes we fit one reaction norm to describe each of the three glucosinolate traits as a continuous linear function of an *environmental index* (EI, a numerical descriptor of microhabitat conditions within each experimental block)—for a

total of 75 reaction norms (3 traits × 25 genotypes). We assumed that most environmental factors causing glucosinolate plasticity are unknown, and so the relevant environmental characteristics are best "measured" using plant phenotype data. Therefore, we assigned the grand mean trait values observed in each block to be the environmental indices (Finlay and Wilkinson 1963). We then calculated a reaction norm for each genotype and each trait using linear regression of genotype-specific block mean trait values onto the EIs:

$$Genotype\ trait\ mean\ in\ block = Block\ environmental\ index + error$$

The linear regression coefficient estimated by each model is a reaction norm *slope*, which describes the magnitude and direction of plasticity for one genotype. We also calculated the *height* of each reaction norm by evaluating the linear function at the mean EI value; thus, reaction norm height describes the genotype's predicted trait value in an "average" block (Figure 2).

Second, to test whether reaction norm slopes were heterogeneous among genotypes, we analyzed genotype-specific block mean trait values and EIs from all 25 genotypes together by fitting an ANCOVA model with an additional *Genotype* × *EI* interaction term that described genetic variation for reaction norm slopes:

$$Genotype\ trait\ mean\ in\ block = Genotype + Block\ EI + Genotype * Block\ EI + error$$

If the interaction term was significant, we concluded that reaction norm slopes were heterogeneous among genotypes.

Testing for selection on reaction norm slope and reaction norm height

The slope and height of the reaction norms are measurable characteristics of plant genotypes, corresponding to plasticity and average trait values, respectively (Figure 2). We measured directional selection gradients on these reaction norm parameters at each site to determine whether glucosinolate plasticity in response to fine-grained environmental variation affects fitness within a habitat.

To test for directional selection on reaction norm slopes, we conducted genotypic selection analysis separately for each glucosinolate trait at each site. Genotypes' relative fitness within each site (genotypic survival rate × genotypic fecundity, divided by the mean fitness for all genotypes at that site) was regressed onto the genotypes' reaction norm height and slope, generating two partial regression coefficients that describe directional selection on average trait values and trait plasticity, respectively (Figure 2g-h; Supplementary Methods):

$$Genotype\ relative\ fitness = RN\ height + RN\ slope + error$$

Finally, to test whether directional selection on reaction norm components varied among habitats, we used relative fitness data from all sites to fit an additional ANCOVA model for each trait. This model included two additional interaction terms: *Site* × *Height* and *Site* × *Slope*, which tested for heterogeneous directional selection on reaction norm height and slope, respectively, among habitats:

$$Geno.rel.\ fit. = Site + RN\ height + RN\ slope + Site * RN\ height + Site * RN\ slope + error$$

Results

Genotype and environment synergistically controlled glucosinolate profiles

All three glucosinolate profile features—BC-ratio, Total [GS], and BC-diversity—differed among genotypes and among field sites (Figure 5a; Table 1). All three traits were also affected by developmental stage and plant size (Table 1; Supplementary Figure 2). Genotype differences in BC-ratio have been previously reported in *B. stricta* (Schranz *et al*. 2009; Manzaneda *et al*. 2010); our data show that Total [GS] and BC-diversity are also genetically controlled. Genotypes varied less in glucosinolate quantity than in glucosinolate quality, except for SAD12, which produced nearly twice the concentration of glucosinolates as the others. SAD12 is also notable as the only genotype in the experiment that originated in Colorado; the others are from Idaho or Montana. In this experiment the EAST subspecies harbored more genetic variation for BC-ratio than the WEST; however, genetic variability for the other traits was comparable between the subspecies (Figure 5a).

All three traits showed significant plasticity among sites. For Total [GS], the magnitude of the site effect was comparable to differences attributed to genotypes—in particular, plants growing at Mahogany Valley produced only 50% the quantity of glucosinolates as those growing at the other sites, on average (Figure 5a). In contrast, for BC-diversity and especially BC-ratio, the magnitude of among-site plasticity was minor compared to genotype differences. Additionally, BC-ratio and Total [GS] varied significantly among blocks within sites (6.3% and 11.2% of the total variance, respectively), indicating that meter-scale environmental heterogeneity affected expression of these traits (Supplementary Figure 3a; Table 1).

Finally, significant genotype-by-site interactions confirm that genotype and environment acted synergistically to shape glucosinolate profiles (Table 1). In general, EASTERN genotypes were more sensitive to environment than WESTERN genotypes, especially for BC-ratio and BC-diversity. However, the magnitude and direction of plasticity between sites varied even within subspecies (Figure 5b). In addition to these genotype × site interactions, genotype × block interactions accounted for 65.3% and 35.9% of the variance in BC-ratio and BC-diversity, respectively. This indicates strong genetic variation for plasticity of glucosinolate composition in response to environmental heterogeneity on the meter scale within habitats. Consistent with the observed subspecies difference in among-habitat plasticity (Figure 5b), EASTERN genotypes displayed greater within-habitat plasticity (Supplementary Figure 3b).

Evidence for frequent adaptive plasticity among sites

Direction of glucosinolate plasticity in each site

We used the experimental grand mean as a baseline to represent the expected value of each trait in the absence of plasticity among habitats. All three traits deviated significantly from this baseline in at least two sites (Figure 6a). Plasticity decreased BC-ratio and increased BC-diversity at both Jackass Meadow and Mahogany Valley; in contrast, at both the Alder Creek and Silver Creek sites, BC-diversity decreased significantly from the grand mean but BC-ratio exhibited no significant plastic response (Figure 6a). Plants growing at Alder Creek and Mahogany Valley produced relatively low quantities of glucosinolates. These plastic responses are averages across all genotypes; notably, individual genotypes varied strongly in their phenotypic deviations from the baseline (Supplementary Figure 4).

Directional selection on glucosinolate profiles in each habitat

We observed substantial genetic variation in survival rates, fecundity, and overall evolutionary fitness (Supplementary Figure 5). Phenotypic selection analysis revealed seven cases of directional selection on glucosinolate traits. High Total [GS] was associated with decreased fecundity in all four common gardens (Figure 6b; Supplementary Table 3); pooling the data from all sites and testing for a Site*Total [GS] interaction failed to reject the null hypothesis that these selection differentials were equivalent (Supplementary Table 4), suggesting that high glucosinolate concentrations are equally costly or disadvantageous in all four habitats. In contrast, directional selection on BC-diversity varied among sites (Supplementary Table 4). Specifically, high BC-diversity was associated with higher fecundity at Jackass Meadow but lower fecundity at Alder Creek and Silver Creek (Figure 6b); we detected no significant selection on BC-diversity at Mahogany Valley (Supplementary Table 3).

For thoroughness, we also calculated selection gradients to assess direct selection on each trait while controlling for indirect selection on the other glucosinolate traits (Supplementary Methods). The selection gradients generally agreed with the selection differentials, and also indicated that BC-ratio may be under negative selection at Mahogany Valley (Supplementary Table 5).

Plasticity generally moved glucosinolate profiles in an adaptive direction

We identified five cases in which a glucosinolate trait both exhibited a significant plastic response and experienced significant directional selection within a habitat. Total [GS] fit these criteria at Alder Creek and Mahogany Valley, while BC-diversity fit these criteria at all sites except Mahogany Valley (Figure 6). The direction of plasticity

matched the direction of selection in all five cases (**Figure 6**d), more frequently than expected by chance (exact binomial test, P=0.031).

Overall, selection differentials did not predict trait plasticity (single linear regression, R^2=0.31, P=0.19; **Figure 6**c). However, it is worth noting that selection on Total [GS] did not vary among sites; thus, there is less reason to expect plasticity among sites to track changing selection pressures. Considering only BC-diversity (i.e., the only trait under spatially variable selection), selection differentials strongly predicted plasticity (R^2=0.99, P=0.025).

Reaction norms were genetically variable but not under detectable selection

Reaction norm slopes of all three glucosinolate traits varied strongly among genotypes, indicating substantial genetic variation for glucosinolate plasticity in response to continuous environmental gradients (Figure 7; Supplementary Table 6). After correction for multiple testing, genotypic selection analysis failed to detect significant selection differentials on reaction norm slopes at any site (Supplementary Table 7), suggesting that glucosinolate plasticity in response to continuous environmental gradients was neither beneficial nor costly within any of these four habitats. However, a parallel analysis of selection gradients—measuring direct selection on the reaction norm slope of each trait, after accounting for indirect selection on the other traits (Supplementary Methods)—provided some evidence for directional selection on plasticity of all three traits at a single site, Silver Creek (Supplementary Table 8). At this site, flatter BC-ratio reaction norms were favored, whereas steeper reaction norms for Total [GS] and BC-diversity were associated with higher fitness (Supplementary Table 8).

Discussion

In *Boechera stricta*, plant genotype and environment synergistically affect the concentration and composition of glucosinolate profiles. All three glucosinolate traits measured—Total [GS], BC-ratio, and BC-diversity—were under both genetic and environmental control, with strong genotype-by-environment interactions. Genotype was the strongest determinant of BC-ratio and BC-diversity, whereas genotype and environment had similar effect sizes for Total [GS] (Figure 5a). We observed abundant genetic diversity for plasticity both among and within field sites (Figure 5b; Figure 7; Table 1). Particularly striking was the 65% of BC-ratio variation that was explained by genotype-by-block interactions (Supplementary Figure 3b; Table 1).

In general, EASTERN genotypes were more plastic than WESTERN genotypes, and there was additional genetic variation in reaction norm shape and inter-site plasticity within the EAST subspecies (Figure 5b; Figure 7). The lack of variation for glucosinolate plasticity among WESTERN genotypes is consistent with observed patterns of reduced molecular diversity relative to EASTERN genotypes (Baosheng Wang, personal communication), and might limit further evolution of reaction norms within the WEST but not the EAST subspecies.

Glucosinolate plasticity may aid colonization of new habitats

Because the distance between sites in this study is much greater than the dispersal distance of *Boechera* (Bloom *et al*. 2002), it is unlikely that the inter-site plasticity observed in this study evolved as an adaptation to heterogeneity among sites (Via and Lande 1985; Gomulkiewicz and Kirkpatrick 1992). Instead, the differences among sites are reasonable simulations of transitions to novel environments, or when streams carry

seeds to lower-elevation sites. Thus, we could assess whether this coarse-grained glucosinolate plasticity might impact the likelihood that a *B. stricta* population could survive a major environmental change or colonize a new habitat (Figure 1; Ghalambor *et al*. 2007).

The question of whether plasticity of a trait improves the relative fitness of a population upon encountering a new environment is only relevant to environments where traits are both plastic and under directional selection. In this experiment, we observed five such cases: plasticity and selection of Total [GS] in two sites, and of BC-diversity in three sites (Figure 6). Determining the direction and magnitude of plasticity in a given site requires the definition of a "baseline" against which the site-specific trait values can be compared; here we used the experiment-wide grand mean trait value as the baseline. We note that if the four field sites used in this experiment were somehow unrepresentative of the wider range of habitats occupied by wild *B. stricta* populations, then this baseline might not reflect the "true" average glucosinolate profile, and thus our estimations of plasticity might be incorrect. We have no reason to suspect this is the case, because we chose these sites to reflect the diversity of habitat types in the region (Supplementary Figure 1; Supplementary Table 2): Alder Creek and Silver Creek are riparian meadows—representative habitats of the WESTERN subspecies—while the other two sites are typical EASTERN subspecies habitats—dry, high-elevation meadows (Lee and Mitchell-Olds 2011, 2013). Nevertheless, repetition of this experiment in a wider range of *B. stricta* habitats would be necessary to rule out this possibility.

All five cases of simultaneous plasticity and directional selection showed a match between the direction of plasticity and the selection differential (Figure 6)—enough to

reject the null hypothesis of 50% probability of a match (exact binomial test, P=0.031). We noted that Total [GS] and BC-diversity were genetically correlated (correlation of least-squares genotype means, r=-0.57, P=0.023), which may impact the P-value of this test due to non-independence of the two data points from the same site, Alder Creek (Figure 6c-d). However, this result is strengthened by the observation that, in Mahogany Valley, the negative selection gradient on BC-ratio (Supplementary Table 5; Supplementary Methods) matches the negative plasticity deviation of BC-ratio (Figure 6a). Unlike BC-diversity, BC-ratio was not correlated with Total [GS] (r=-0.42, P=0.12). Furthermore, considering only BC-diversity, the strength and direction of selection strongly predicted the magnitude and direction of plasticity (Figure 6c; linear regression R^2=0.99; P=0.030).

The whole of the data suggest that glucosinolate plasticity often changes defensive chemistry to better match the local selection pressures, and therefore might aid *B. stricta* populations in colonizing new habitats (Ghalambor *et al*. 2007). Whether such adaptive plasticity promotes glucosinolate evolution in the long term, however, will be a more difficult question to answer (Ghalambor *et al*. 2007; Ghalambor *et al*. 2015; Huang and Agrawal 2016). Identifying the environmental cues perceived by the plants that induce changes in defensive chemistry, as well as the ecological causes of local selection pressures, should be a priority for future research on adaptive glucosinolate plasticity. Finally, it is worth noting that genotypes varied considerably in their plastic responses (Supplementary Figure 4). Although glucosinolate plasticity was adaptive on average, certain populations may lack the ability to exhibit adaptive plasticity or may even exhibit

maladaptive plasticity. Therefore, the contribution of glucosinolate plasticity to persistence after environmental change is not uniform across the species.

No strong evidence for selection on plasticity within habitats

In this experiment, we detected little evidence for selection on glucosinolate reaction norms, or plasticity in response to continuous environmental gradients. Because this study only included 25 genotypes, evidence for selection on reaction norms may become more clear as more genotypes are analyzed. Consistent with this, selection gradients on reaction norm height (i.e., mean trait values across all blocks) lacked statistical support but agreed qualitatively with the patterns detected using the phenotypic selection analysis (compare β values in Supplementary Table 3 with β_H values in Supplementary Table 7). Another possible reason for this negative result is that selection pressures on glucosinolate profiles may not vary on such a fine spatial scale, reducing the opportunity for adaptive plasticity within habitats (Via and Lande 1985; Gomulkiewicz and Kirkpatrick 1992). The lack of observed selection *against* plasticity suggests that glucosinolate plasticity does not carry a significant cost (Auld *et al*. 2010).

Finally, inter-annual variation is one potential cause of plasticity and variable selection that we did not address in this study. Other experiments have shown that herbivory pressure on *B. stricta* varies considerably over a span of a few years within a single site (Mitchell-Olds, unpublished); consecutive generations of a *B. stricta* lineage might therefore experience very different predatory environments, potentially causing temporally heterogeneous selection on glucosinolate profiles. The consistent negative selection on glucosinolate quantity in this study (Figure 6b; Supplementary Table 3) suggests that herbivory intensity may have been lower than usual, reducing the usefulness

of these expensive phytochemicals (Mauricio and Rausher 1997; Mauricio 1998). Temporal fluctuations in resource availability (e.g., due to limited or abundant rainfall) or pathogen pressure could also affect the relative costs and benefits of different properties of glucosinolate profiles. Because *B. stricta* is a perennial, even a single individual may need to adjust to several environments over its lifetime; in theory, plasticity could evolve as an adaptation to such fine-scale variability (Gomulkiewicz and Kirkpatrick 1992; Reed *et al*. 2010; Baythavong 2011). The relative importance of temporal environmental variation compared to spatial variation could be assessed by extending a similar experimental design over multiple growing seasons. Follow-up studies should prioritize temporal variation as a potential driver of adaptive plasticity in glucosinolate profiles and other important *B. stricta* traits.

Importantly, evidence of natural selection on reaction norms would not be sufficient to demonstrate that plasticity evolved as an adaptation. Reliable environmental cues for the different selection pressures are also required, preventing the evolution of plasticity as an adaptation to truly stochastic environmental fluctuations (Levins 1963; Donohue *et al*. 2000; Schmitt *et al*. 2003; Reed *et al*. 2010). In other words, if an organism's offspring reliably encounter multiple contrasting selection pressures, and if some perceptible environmental factor predicts how selection will act, plasticity (rather than habitat specialization) will evolve as a long-term adaptation to a heterogeneous environment (Donohue *et al*. 2000; Sultan and Spencer 2002). Even when these conditions are not met, however, plasticity can still improve short-term relative fitness—that is, it may sometimes be "adaptive" even if it did not evolve as an "adaptation" (Sultan 1987).

Future directions

The molecular basis of genotype-by-environment interactions is a key research goal for understanding the evolution of phenotypic plasticity and the robustness of genetic improvements in crop species (El-Soda *et al*. 2014). The ample genetic variation for plasticity of three glucosinolate traits provides an opportunity to explore this phenomenon at the molecular level in *B. stricta*, which offers resources such as near-isogenic lines varying in glucosinolate synthesis genes, a recently developed genome-wide association population, a fully sequenced genome, and genetic similarity to the model plant *Arabidopsis thaliana* (Rushworth *et al*. 2011; Prasad *et al*. 2012; Lee *et al*. 2017). The *Arabidopsis* glucosinolate biosynthetic pathway is well characterized (Halkier and Gershenzon 2006), although genes affecting plasticity are not necessarily part of this core pathway. Indeed, in *Arabidopsis*, patterns of flux through this pathway were robust to several biotic and abiotic environmental stimuli (Olson-Manning *et al*. 2015), suggesting that variation in environment-sensing genes or other upstream genes may be more important for glucosinolate plasticity, *per se*.

Finally, the exact ecological causes of glucosinolate plasticity in this experiment are still unclear and cannot be determined from this dataset; additional experiments with environmental manipulations will be necessary to identify the causal stimuli (Anderson *et al*. 2014). Many environmental features distinguish the field sites used in this study (Supplementary Figure 1). For this reason, we used plant phenotype to define the environmental index of each block, rather than guessing which environmental parameters were most relevant (Finlay and Wilkinson 1963). The disadvantage of this method is that the phenotype is a “black box” obscuring the actual ecological features driving trait

plasticity. The question of which ecological factors drive plasticity of glucosinolate profiles—and selection on them—will be an important step towards understanding the apparent link between natural selection and phenotypic plasticity in diverse habitats across a natural landscape.

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Author contributions

M.R.W. and T.M.-O. designed the experiment and statistical analyses. M.R.W. executed the experiment, analyzed the data, and wrote the manuscript with edits from T.M.-O.

Data accessibility

All data and R code will be archived in a Dryad repository upon article acceptance.

Appendix: Summary of Analyses

Plasticity **among** habitats				
	Question	***Data (per model)***	***Model***	***Notes***
[1]	What drives variation in glucosinolate traits?	Individual trait data • All 4 sites • 1 trait	Trait = Genotype + Site + Genotype*Site + Block + Genotype*Block + Height + Dev. Stage + HPLC batch	Site LS means used to calculate site-specific plasticity deviations
[2a]	At each site, is each trait under directional selection? (directly or via selection on correlated traits)	Individual trait & fecundity data, standardized • 1 sites • 1 trait	Relative fecundity = Trait + Block	Regression coefficient is the selection differential
[2b]	Does directional selection on each trait vary among sites? (directly or via selection on correlated traits)	Individual trait & fecundity data, standardized • All 4 sites • 1 trait	Relative fecundity = Site + Trait + Site*Trait + Block	
[2c]	At each site, is each trait under directional selection? (controlling for selection on correlated traits)	Individual trait & fecundity data, standardized • 1 site • All 3 traits	Relative fecundity = Trait_1 + Trait_2 + Trait_3 + Block	Partial regression coefficients are the selection gradients
[3]	Does direction of plasticity match direction of selection?	LS means from **[1]**, selection differentials from **[2a]**	Exact binomial test Linear regression: Plasticity deviation = selection differential	

Plasticity **within** habitats				
	Question	***Data (per model)***	***Model***	***Notes***
[4a]	How does each genotype's GS profile vary along env. gradients?	Genotype mean trait values in each block • 1 genotype • 1 trait • All 4 sites EI = grand mean trait value in each block	Trait = EI	Regression coefficient = RN slope
[4b]	Do genotypes differ in how their GS profiles vary along env. gradients?	Genotype mean trait values in each block • All 25 genotypes • 1 trait • All 4 sites	Trait = Genotype + EI + Genotype*EI	
[5a]	At each site, is RN slope under directional selection? (directly or via selection on correlated traits)	Genotype mean fitness Genotype RN slope & RN height from **[4a]** • All 25 genotypes • 1 trait • 1 site	Relative fitness = Height + Slope	Slope partial regression coefficient = Selection differential on plasticity
[5b]	Does directional selection on RN slopes (directly or via selection on correlated traits) vary among sites?	Genotype mean fitness Genotype RN slope & RN height from **[4a]** • All 25 genotypes • 1 trait • All 4 sites	Relative fitness = Site + Height + Slope + Site*Height + Site*Slope	
[5c]	At each site, is RN slope under direct directional selection? (controlling for selection on correlated traits)	Genotype mean fitness Genotype RN slope & RN height from **[4a]** • All 25 genotypes • 3 traits • 1 site	Relative fitness = Height_trait1 + Slope_trait1 + Height_trait2 + Slope_trait2 + Height_trait3 + Slope_trait3	Slope partial regression coefficients = Selection gradients on plasticity of each trait

Figures and Tables

Table 1: Statistics from REML mixed models of glucosinolate traits reveal strong genotype-by-site interactions. All effects are fixed except for Block, Genotype × Block, and HPLC batch, which were random-intercept terms. Significance of random effects was assessed using likelihood ratio tests. *N*=1,503 for Total [GS]. For BC-ratio and BC-diversity, *N*=1,486 because these traits could not be measured for individuals that produced no glucosinolates.

	BC-ratio	**Total [GS]**	**BC-diversity**
R^2	0.99	0.49	0.92
Genotype	$F_{23,1035}$=343.68 ***P*<3e^{-16}**	$F_{23,949}$=9.24 ***P* <3e^{-16}**	$F_{23,982}$=140.98 ***P* <3e^{-16}**
Site	$F_{3,109}$=21.28 ***P* =1.3e^{-10}**	$F_{3,99}$=14.07 ***P* =1.0e^{-07}**	$F_{3,1085}$=49.28 ***P* <3e^{-16}**
Genotype × Site	$F_{69,1035}$=3.93 ***P* <3e^{-16}**	$F_{69,899}$=3.02 ***P* =7.2e^{-14}**	$F_{69,972}$=12.91 ***P* <3e^{-16}**
Dev. Stage	$F_{2,785}$=4.31 ***P* =0.014**	$F_{2,1332}$=23.78 ***P* =2.1e^{-10}**	$F_{2,1226}$=6.49 ***P* =0.0031**
Height	$F_{1,1109}$=33.05 ***P* =2.3e^{-08}**	$F_{1,1344}$=46.86 ***P* =3.5e^{-11}**	$F_{1,1328}$=6.60 ***P* =0.010**
Block	χ^2_1=22.74 ***P* =3.7e^{-06}**	χ^2_1=49.23 ***P* =4.2e^{-14}**	χ^2_1=4.3e^{-11} *P*=1
Genotype × Block	χ^2_1=58.90 ***P* =5.0e^{-14}**	χ^2_1=0.03 *P*=0.86	χ^2_1=12.37 ***P* =0.00087**
HPLC batch	χ^2_1=4.09 ***P* =0.043**	χ^2_1=9.66 ***P* =0.006**	χ^2_1=8.33 ***P* =0.0078**

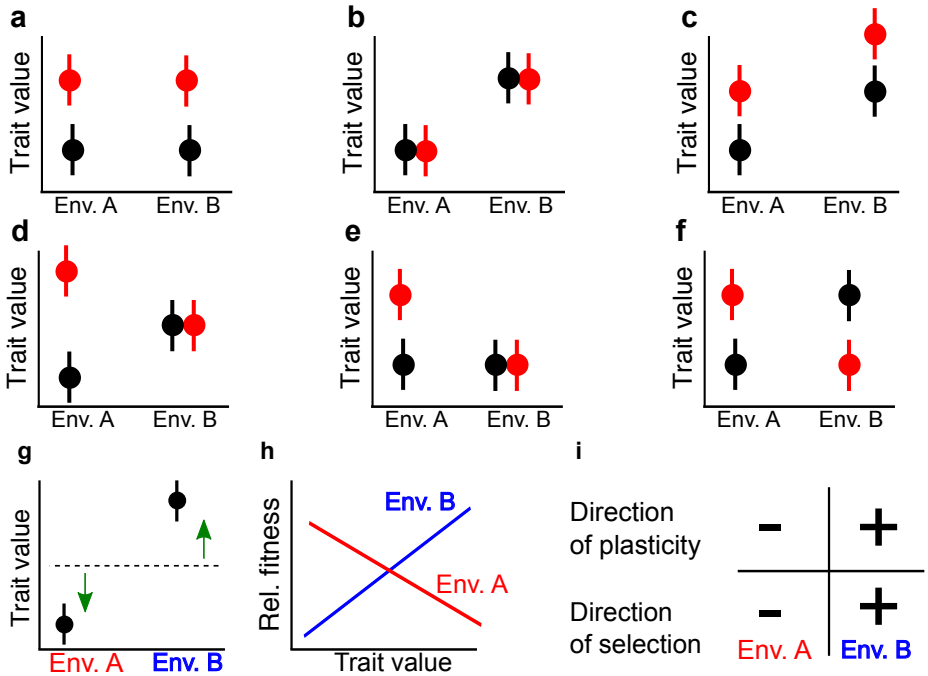


Figure 1: Schematic of genotype-by-environment interactions and adaptive plasticity between discrete environments. Points show the mean trait values of two genotypes ("red" and "black"). Panel **(a)** depicts a trait that is under pure genetic control with no plasticity. Panel **(b)** depicts a trait that is completely plastic and not genetically variable. Panel **(c)** shows both a genotype effect and plasticity, but no interaction between them. Panels **(d)-(f)** depict various examples of genotype-by-environment interactions. In **(d)**, a genetic difference is detectable only in one site; the genotypes have plasticity of equal magnitude but opposite sign so that the mean phenotype in each site is identical. In **(e)**, only one genotype is plastic. In **(f)**, the genotypes switch rank phenotype; averaged across sites, there is no genetic difference between them, and the average trait value within each site is the same. Note that panels **(a)-(f)** could represent six different traits measured simultaneously in one experiment: plasticity is a property of a particular trait, a particular genotype, and a particular environmental change. **(g)** The "plasticity deviation" in a given habitat (green arrows) can be calculated as the difference in mean trait value in that environment (black points) and the grand mean trait value across all environments (dotted line). For example, in hypothetical Environment A, plasticity causes trait values to be lower than average. **(h)** Directional selection is calculated as the linear regression of relative fitness on the trait value in a given habitat. For example, in hypothetical Environment A, lower trait values are associated with greater reproductive fitness compared to higher trait values. **(i)** Adaptive plasticity occurs when the direction of plasticity in a habitat (the sign of the plasticity deviation calculated in **[g]**) matches the direction of selection (the sign of the regression slope from **[h]**).

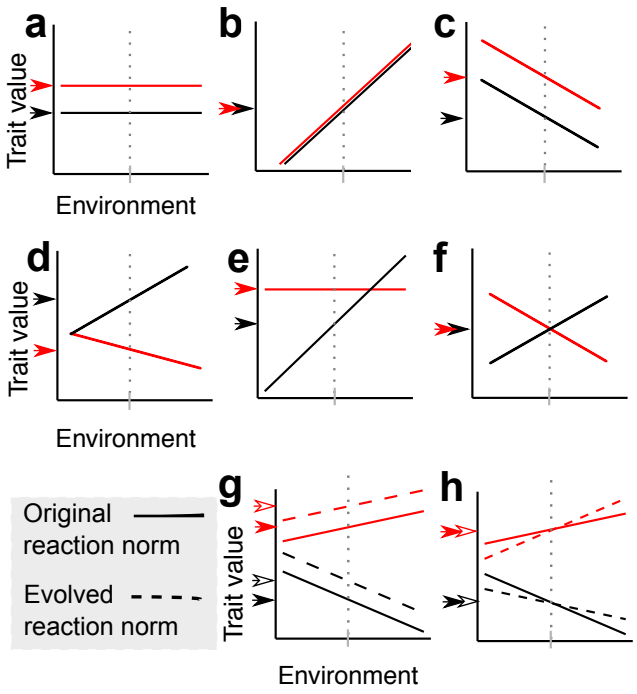


Figure 2: Genotype-environment interactions and selection on plasticity over continuous environmental gradients: Examples of reaction norms. Lines show the mean trait values of two genotypes ("red" and "black") across a range of some continuous environmental predictor. The arrows on the vertical axis indicate reaction norm *height*, or the trait value for each genotype evaluated at the average value of the environmental predictor (indicated by grey tick marks on horizontal axis and vertical dotted lines). Panel **(a)** shows a genetic difference with no plasticity—i.e., zero slope. Panel **(b)** shows plasticity with no genetic difference. Panel **(c)** shows both plasticity and a genotype effect, but no interaction between them, indicated by parallel reaction norms—the genotypes differ in reaction norm height, but not slope. Panels **(d)-(f)** all show possible genotype-by-environment interactions, or genetic variation for reaction norm shape. In panel **(d)** reaction norm slopes differ in sign but not magnitude; in **(e)** only one genotype is plastic. In panel **(f)** the genotypes are indistinguishable when averaged across all environments (e.g., if the environmental gradient was unobserved); the genotype difference is environment-dependent. When mean trait values and plasticity are genetically uncorrelated, they can evolve independently. Panel **(g)** illustrates changes in reaction norm height in response to directional selection for increased mean trait values. Panel **(h)** illustrates evolutionary change in reaction norm slope in response to directional selection for more positive linear reaction norm coefficients. Note that such selection may result in either increased or decreased overall plasticity (steeper or shallower slope), depending on the original shape of the reaction norm.

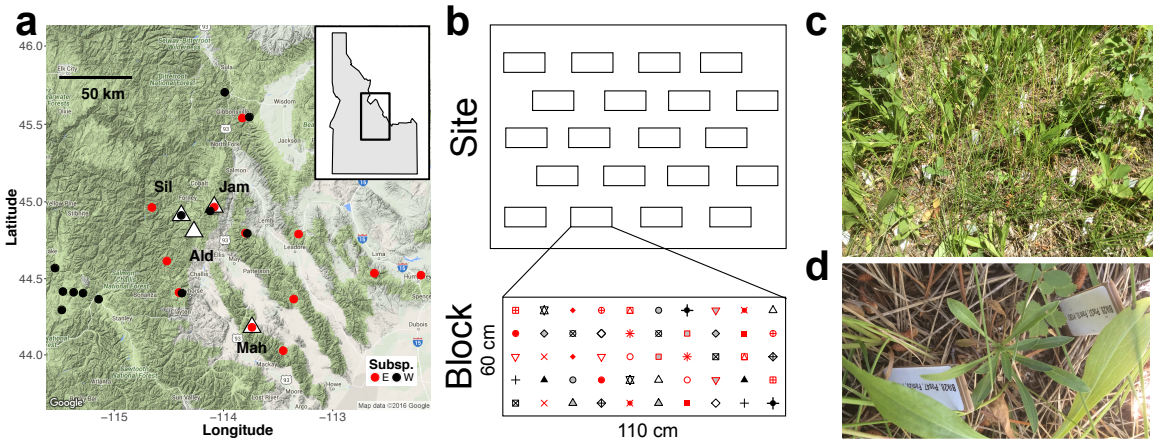


Figure 3: Field sites and experimental design. **(a)** Map of field sites and wild *B. stricta* populations used in common garden experiment. Common gardens are denoted with white triangles and labeled. Circles mark collection sites of the 25 genotypes included in the experiment. Not shown: one EASTERN genotype collected in Colorado. Map data: Google. **(b)** Each common garden contained 16 to 22 experimental blocks. Each randomized block contained two individuals of each of the 25 genotypes, planted in a 10-cm grid. Panel **(c)** shows a block placed within the natural vegetation; **(d)** shows one experimental rosette with its identifying tag.

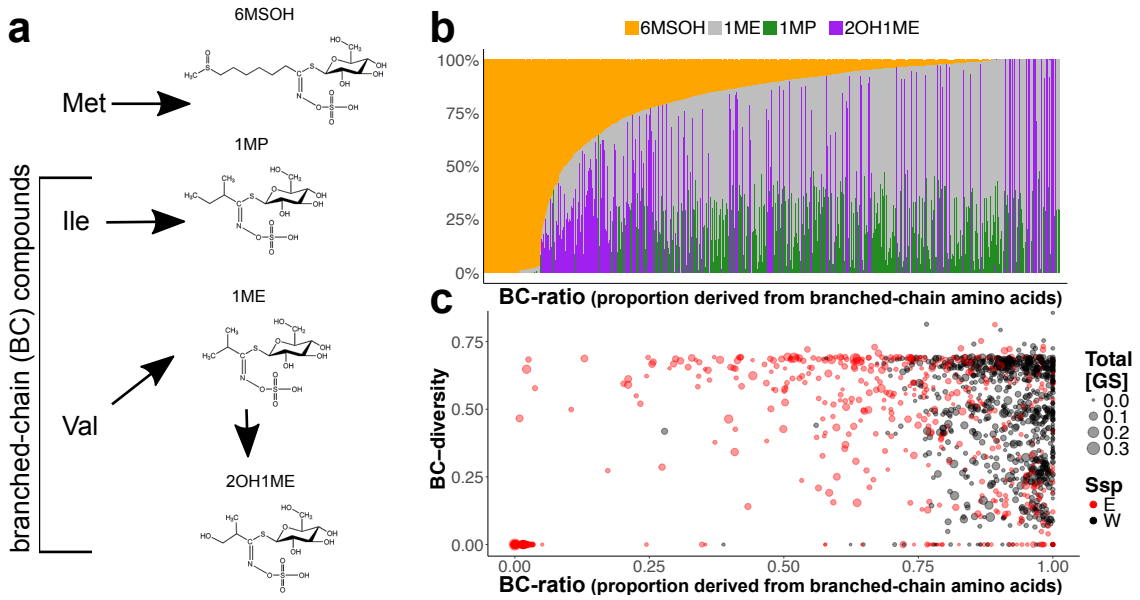


Figure 4: Glucosinolate variation in 1,505 field-grown *Boechera stricta* rosettes. **(a)** Chemical structures of the four primary glucosinolates in *B. stricta (*Kanehisa *et al*. 2002), with their amino acid precursors. 6MSOH is derived from methionine; the other compounds are all derived from branched-chain amino acids. **(b-c)** Rosette leaf glucosinolate profiles of 1,505 field-grown plants. In both panels, plants are sorted by increasing BC-ratio (i.e., decreasing 6MSOH). **(b)** The proportions of four aliphatic glucosinolates are shown for each measured plant. **(c)** Three summary metrics of each glucosinolate profile are shown for each individual. Three genotypes in this study lack branched-chain glucosinolate functionality and only produce 6MSOH; individuals of these genotypes are seen in the lower left-hand corner of panel **(c)**.

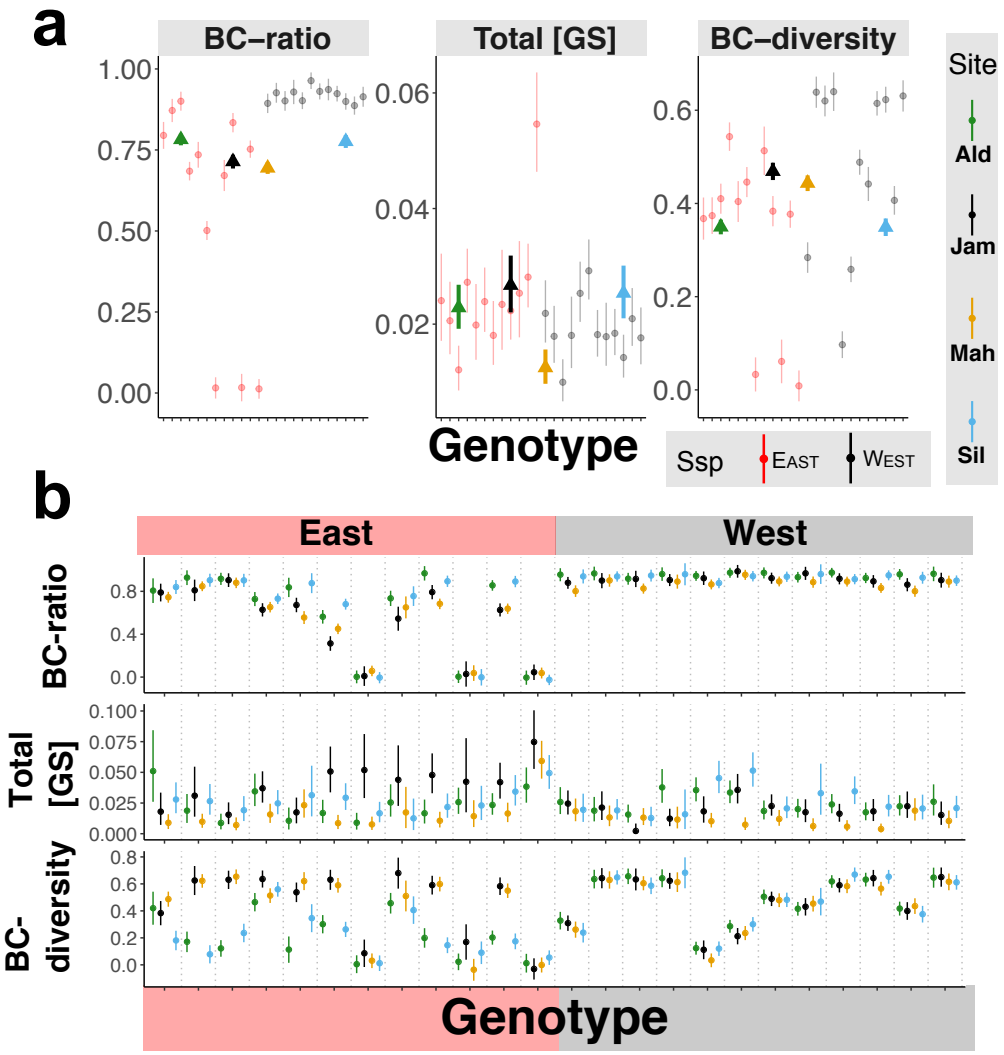


Figure 5: Genotype and environment influence glucosinolate profiles both independently and synergistically. **(a)** Both genotype and habitat influence glucosinolate profiles. Plotted are least-squares mean trait values for each Genotype (circles) and for each Site (triangles) from a REML mixed model that also controlled for developmental stage, plant size, genotype-by-site interactions, and block and batch effects (Table 1). Thus, circles show the mean trait value for each genotype (averaged across all sites); triangles show the mean trait value at each site (average for all genotypes). Note that the horizontal position of the triangles is meaningless—they were placed in order to not obscure the genotype means. Error bars are 95% confidence intervals. **(b)** Glucosinolate plasticity among sites is genetically variable. Genotypes are delimited by vertical dashed lines. The points are least-squares mean trait values for each genotype in each common garden, with 95% confidence intervals. BC-ratio and BC-diversity are unitless; units for Total [GS] are μmol/mg dry tissue.

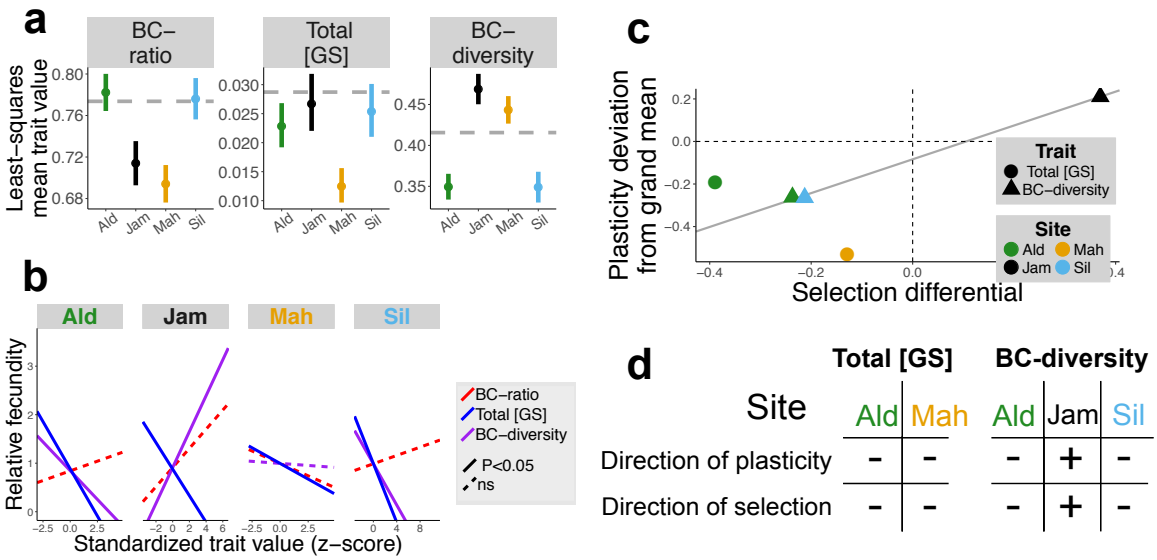


Figure 6: Evidence for adaptive glucosinolate plasticity among field sites. **(a)** Plasticity deviations from experimental grand mean for three glucosinolate traits in four common gardens (Sites). Least-squares mean trait values at each site are plotted with 95% confidence intervals, calculated from a linear mixed effects model that also controlled for plant genotype, block, height, developmental stage, genotype-by-site interactions, genotype-by-block interactions, and HPLC batch (Table 1). The grand mean trait value for the entire experiment is shown as a grey dashed line. The plasticity deviation, or the difference between the least-squares mean trait value at a site and the grand mean trait value (depicted with green arrows in Figure 1g)—was considered statistically distinguishable from zero if the grand mean did not fall within the 95% CI. These plasticity deviations represent the average for all genotypes; genotype-specific deviations are shown in Supplementary Figure 4. **(b)** Evidence for directional selection on glucosinolate traits at four field sites. Individuals' relative fecundity was regressed on standardized trait values separately for each glucosinolate trait at each site ($301<N<422$ for all sites). The slope of the resulting regression line (i.e., the selection differential), plotted here, reflects the strength and direction of selection (including indirect selection on correlated traits) as illustrated in Figure 1h. **(c)** Trait deviations from the experimental grand mean due to plasticity (from Panel **[a]**; shown here in units of 1 standard deviation) are positively correlated with selection differentials (slopes of the lines in Panel **[b]**); the best-fit single linear regression line for BC-diversity is shown in solid grey. **(d)** We observed five cases in which a trait was simultaneously plastic (i.e., deviated from the trait grand mean) and under selection within a given site. In all five cases, the direction of plasticity matched the direction of selection. This analysis is based on selection *differentials*; we observed one additional case where the direction of BC-ratio plasticity matched the selection *gradient* (direct selection on BC-ratio while controlling for indirect selection on the other glucosinolate traits; Supplementary Methods) at Mahogany Valley (Supplementary Table 5).

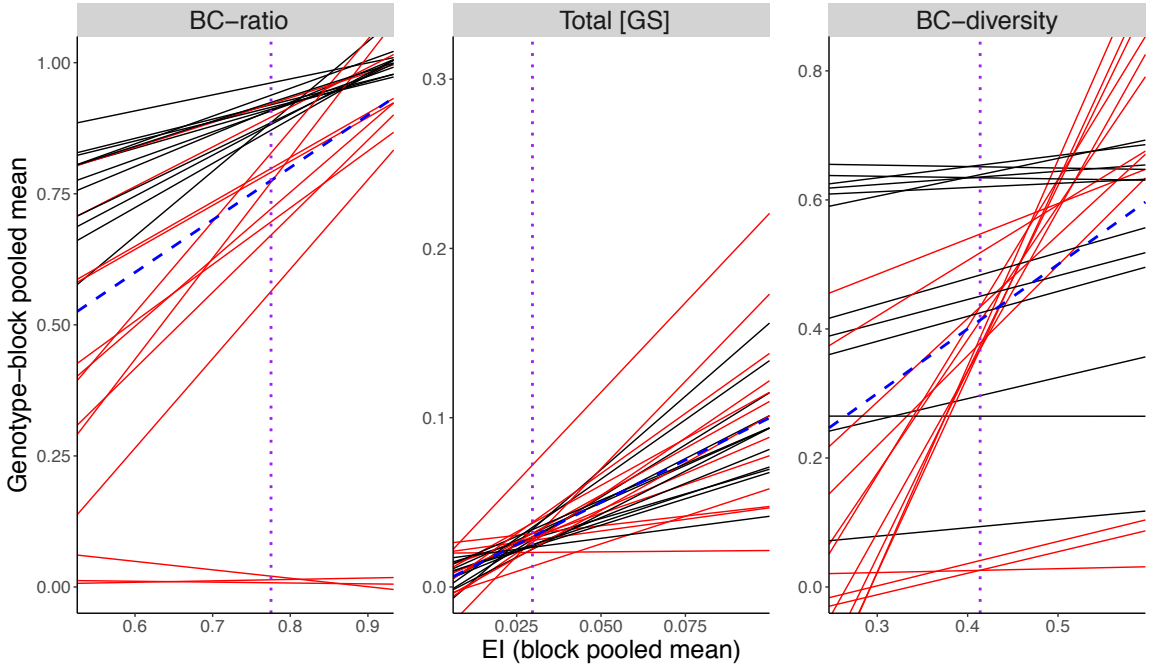


Figure 7: Genotype-specific glucosinolate reaction norms. The mean trait values of each genotype in each block were regressed onto the grand mean trait values for all genotypes in each block, or "environmental index". Each resulting regression line is a reaction norm for one genotype, plotted here in red or black for EASTERN or WESTERN genotypes, respectively. Genotypes with steeper reaction norm slopes exhibit more plasticity in response to continuous environmental gradients. The "average reaction norm" (equivalent to the line $y=1*x$, where the expressed trait value equals the block mean trait value) is shown as a blue dashed line. The purple vertical dotted line denotes the mean environmental index for each trait (i.e., an average environment), the value at which reaction norm height was evaluated.

Supplementary Information

Supplementary Table 1: List of *Boechera stricta* genotypes used in this experiment with the collection locations of the natural accessions from which they were descended.

Genotype	Abbreviation	Elevation (m)	Latitude (°N)	Longitude (°W)	Subspecies
Bayhorse Meadow	BHM	2465	44.406	114.381	WEST
Bayhorse Saddle	BHS	2650	44.410	114.408	EAST
Bannock	BNK	2493	44.791	113.313	EAST
Bearskin Creek	BSC	1965	44.416	115.470	WEST
Bear Valley Creek	BVC	1948	44.411	115.372	WEST
Bear Valley Meadow	BVM	2087	44.795	113.782	WEST
Deadwood	DDW	2028	44.296	115.480	WEST
Eagle Mountain	EAG	2210	45.541	113.827	EAST
East Creek Middle Fork	ECM	2647	44.536	112.618	EAST
Floodplain Forest	FPF	2138	44.797	113.798	EAST
Humphrey Crest	HUM	2156	44.523	112.192	EAST
Iron Flats	IRF	1880	44.941	114.121	WEST
Jackass Meadow	JAM	2691	44.967	114.085	EAST
Lost Trail Meadow	LTM	2462	45.705	113.989	WEST
Mahogany Camp	MAH	2526	44.182	113.739	EAST
Middle Fork Peak	MFP	2758	44.963	114.656	EAST
Mill Creek	MIL	2259	44.367	113.357	EAST
Pass Creek South	PCS	2319	44.027	113.453	EAST
Parker Meadow ‘A’	PMA	2681	44.616	114.518	EAST
Ruby Creek	RUB	2026	45.547	113.763	WEST
Silver Creek Upper	SIL	1843	44.912	114.387	WEST
Taylor River	SAD	2517	38.707	106.804	EAST
Thatcher	THA	2017	44.366	115.143	WEST
Van Horn	VAN	2014	44.406	115.286	WEST
Whiskey Creek	WHC	2061	44.570	115.542	WEST

Supplementary Table 2: List of natural Boechera stricta habitats featured in this experiment.

Site	Abbreviation	Elevation (m)	Latitude (°N)	Longitude (°W)
Silver Creek	Sil	1812	44.90	114.40
Mahogany Valley	Mah	2531	44.18	113.74
Jackass Meadow	Jam	2676	44.97	114.08
Alder Creek	Ald	2130	44.807	114.271

Supplementary Table 3: Phenotypic selection analysis of three glucosinolate traits at four field sites. Unlike selection gradients (Supplementary Table 5), selection differentials describe both direct and indirect selection on each trait. Significant F-tests indicate nonzero directional selection on a trait at a given site. $\boldsymbol{\beta}$ is the selection differential, or regression coefficient of relative fecundity onto standardized trait values after controlling for the random effect of experimental block. Denominator degrees of freedom for the mixed-model *F* test were estimated using the Satterthwaite approximation. *P*-values were calculated using permutation tests of trait values among individuals within experimental blocks and were corrected for multiple comparisons (each trait tested separately at 4 sites) using the sequential Bonferroni method.

Trait	Site	β	Num. DF	Den. DF	*F*	*P*
BC-ratio	Ald	0.080	1	394	0.74	0.76
	Jam	0.197	1	298	2.12	0.56
	Mah	-0.102	1	391	4.12	0.17
	Sil	0.043	1	340	0.57	0.76
Total [GS]	Ald	-0.389	1	409	15.63	**<0.001**
	Jam	-0.274	1	300	4.21	**0.049**
	Mah	-0.129	1	397	6.32	**0.008**
	Sil	-0.303	1	354	30.99	**<0.001**
BC-diversity	Ald	-0.237	1	393	6.60	**0.021**
	Jam	0.37	1	295	7.73	**0.024**
	Mah	-0.017	1	389	0.11	0.728
	Sil	-0.213	1	339	14.52	**<0.001**

Supplementary Table 4: Test for heterogeneity of directional selection on glucosinolate traits among habitats. Fixed effects are shown from three separate linear REML mixed models of relative fecundity on standardized trait values (*Z*-scores), with site-by-trait interaction terms, after controlling for the random effect of experimental block nested in site. $\boldsymbol{\beta}$ describes a test for nonzero mean directional selection across all sites; $\boldsymbol{\beta\ x\ Site}$ describes a test for heterogeneous selection differentials among sites. Denominator degrees of freedom for the mixed-model *F* test were estimated using the Satterthwaite approximation. *P*-values were calculated using permutation tests of trait values among individuals within experimental blocks.

	BC-ratio	**Total [GS]**	**BC-diversity**
$\boldsymbol{\beta}$ (average selection)	$F_{1,1429}$=1.87 *P*=0.17	$F_{1,1484}$=43.31 ***P*<0.001**	$F_{1,1418}$=0 *P*=0.99
Site	$F_{3,83}$=0.23 *P*=0.87	$F_{3,83}$=0.27 *P*=0.84	$F_{3,87}$=0.69 *P*=0.56
$\boldsymbol{\beta}$ **x Site** (heterogeneous selection)	$F_{3,1426}$=2.13 *P*=0.094	$F_{3,1476}$=2.26 *P*=0.079	$F_{3,1417}$=9.54 ***P*<0.001**

Supplementary Table 5: Phenotypic selection gradients on glucosinolate traits at each site. Unlike selection differentials (Supplementary Table 3), selection gradients describe direct selection on each trait after accounting for indirect selection on the others. Significant *F*-tests indicate nonzero directional selection gradients on a trait at a given site. $\boldsymbol{\beta}$ is the selection gradient, or partial regression coefficient of relative fecundity onto standardized trait values after controlling for the random effect of experimental block, and for regression onto the other two glucosinolate traits. Denominator degrees of freedom for the mixed-model *F*-test were estimated using the Satterthwaite approximation. *P*-values were calculated using permutation tests of trait values among individuals within experimental blocks and were corrected for multiple comparisons (each trait tested separately at 4 sites) using the sequential Bonferroni method.

Site	Trait	β	Num. DF	Den. DF	*F*	*P*
Ald	BC-ratio	0.17	1	393	2.63	0.33
	Total [GS]	-0.33	1	398	11.00	**<0.001**
	BC-diversity	-0.28	1	391	7.17	**0.018**
Jam	BC-ratio	0.02	1	294	0.01	0.93
	Total [GS]	-0.21	1	297	2.06	0.15
	BC-diversity	0.31	1	290	5.25	0.072
Mah	BC-ratio	-0.16	1	392	6.93	**0.016**
	Total [GS]	-0.16	1	396	8.89	**0.002**
	BC-diversity	0.04	1	388	0.59	0.44
Sil	BC-ratio	0.08	1	338	1.73	0.36
	Total [GS]	-0.28	1	345	24.62	**<0.001**
	BC-diversity	-0.23	1	337	15.19	**<0.001**

Supplementary Table 6: Test for heterogeneity of reaction norm slopes using three separate fixed-effect ANCOVA models. Mean trait values for each genotype in each block were regressed onto the block's environmental index (EI), calculated as the grand mean trait value for all genotypes in each block. The Genotype term tests for genetic variation in trait values averaged across all EI values. The EI term tests whether the regression coefficient of trait values on environmental index (i.e., the mean reaction norm slope across all genotypes) is statistically distinguishable from zero. The Genotype x EI term describes a test for heterogeneity of reaction norm slopes among genotypes, i.e., genetic variation for plasticity. *N*=1,299 for Total [GS] but *N*=1,283 for BC-ratio and BC-diversity, because these traits could not be measured in 16 genotype-block combinations that produced no glucosinolates.

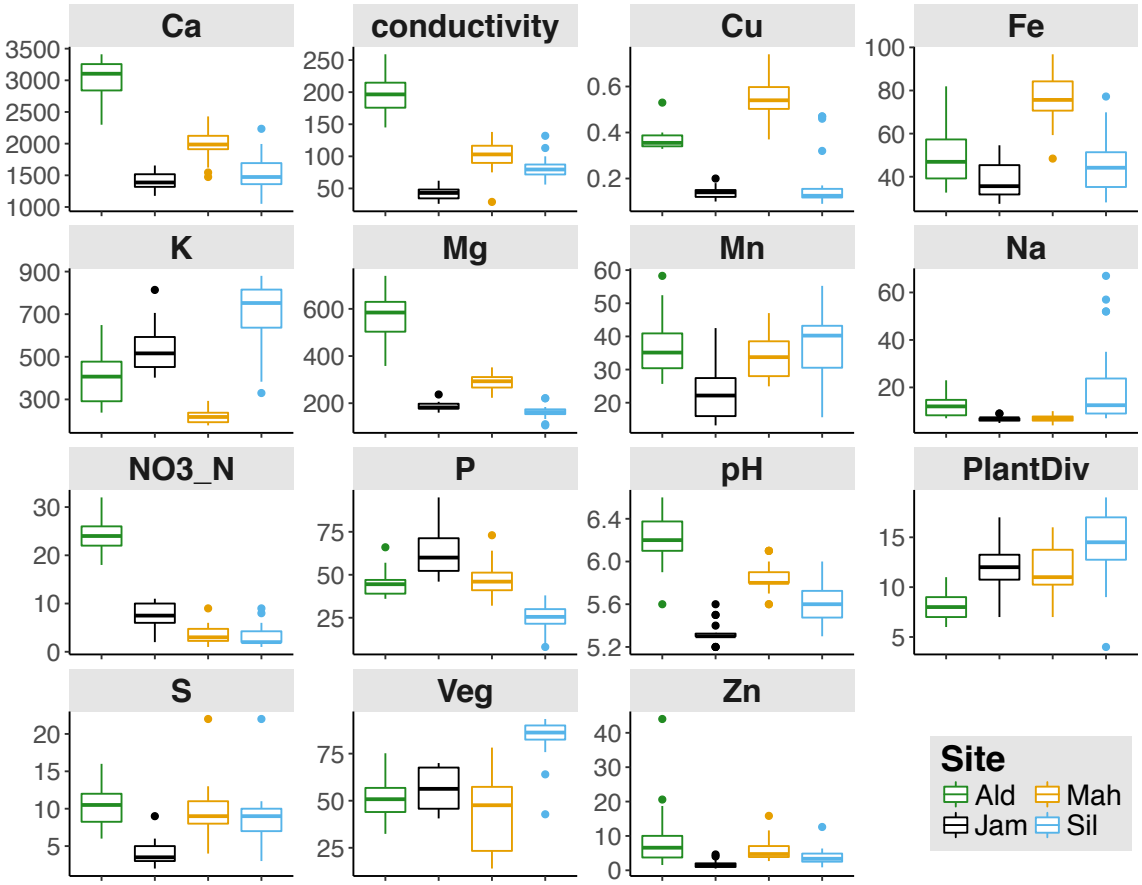
	BC-ratio	**Total [GS]**	**BC-diversity**
Genotype	$F_{24,1233}$=7.85 ***P*<3e^{-16}**	$F_{24,1249}$=1.90 ***P*=0.0056**	$F_{24,1233}$=30.37 ***P* <3e^{-16}**
EI	$F_{1,1233}$=33.63 ***P*=8.4e^{-9}**	$F_{1,1249}$=12.83 ***P* =0.00035**	$F_{1,1233}$=2.54 *P* =0.11
Genotype x EI	$F_{24,1233}$=7.19 ***P* <3e^{-16}**	$F_{24,1249}$=2.99 ***P* =1.9e^{-6}**	$F_{24,1233}$=25.49 ***P* <3e^{-16}**

Supplementary Table 7: Genotypic selection differentials on reaction norm coefficients in four field sites. Unlike selection gradients (Supplementary Table 8), selection differentials describe both direct and indirect selection on each trait. Relative fitness was regressed onto reaction norm height and reaction norm slope using a separate multiple linear regression for each glucosinolate trait in each site. Significant *F*-tests indicate nonzero directional selection on reaction norm parameters for a given trait at a given site. $\boldsymbol{\beta_H}$ is the partial regression coefficient of relative fitness onto reaction norm height; $\boldsymbol{\beta_m}$ describes directional selection on reaction norm slope. *P*-values were adjusted for multiple comparisons using the sequential Bonferroni correction.

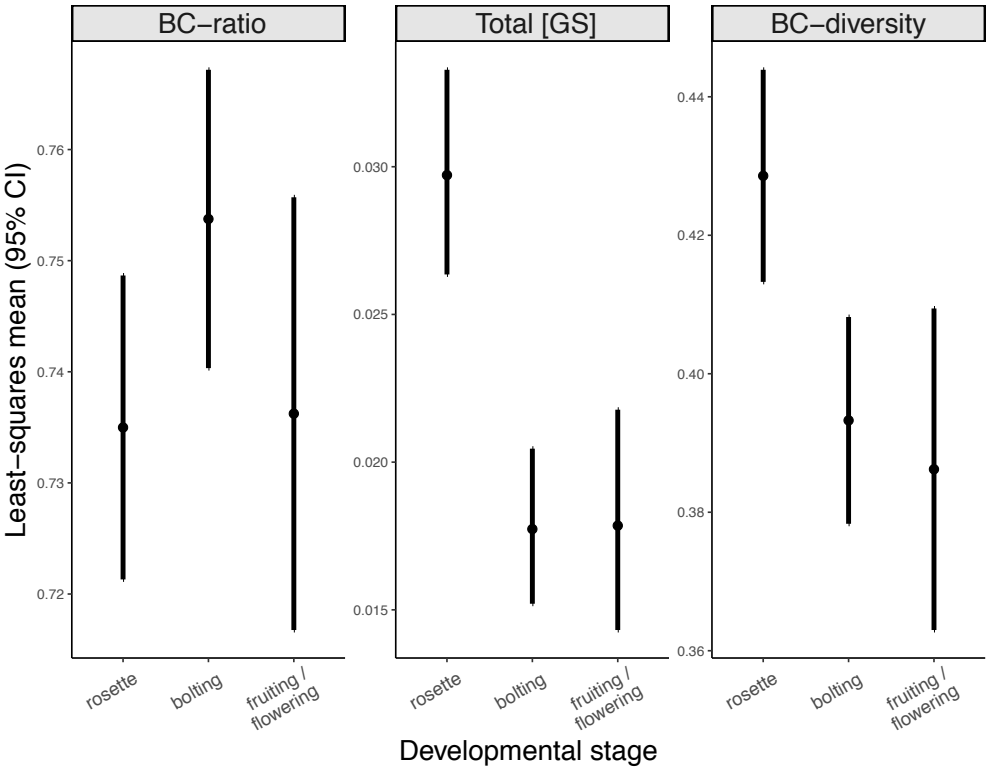
Trait	Site	Selection on reaction norm height			Selection on reaction norm slope		
		β_H	t	P	β_m	t	P
BC-ratio	Ald	0.14	0.32	0.75	-0.16	-0.70	0.84
	Jam	1.55	2.06	0.21	-0.56	-1.41	0.69
	Mah	0.58	1.48	0.46	-0.23	-1.10	0.84
	Sil	0.52	1.18	0.50	-0.21	-0.88	0.84
Total [GS]	Ald	-27.20	-2.27	0.13	0.56	2.39	0.10
	Jam	-33.03	-1.44	0.17	-0.52	-1.14	0.53
	Mah	-22.37	-1.81	0.17	0.10	0.42	0.68
	Sil	-29.30	-2.20	0.13	0.49	1.88	0.22
BC-diversity	Ald	-0.62	-1.07	0.89	0.04	0.41	1.00
	Jam	2.33	2.20	0.15	0.10	0.50	1.00
	Mah	-0.41	-0.73	0.95	0.12	1.14	0.80
	Sil	-0.29	-0.49	0.95	0.19	1.76	0.37

Supplementary Table 8: Selection gradients on reaction norm coefficients. Unlike selection differentials (Supplementary Table 7), selection gradients describe direct selection on each trait after accounting for indirect selection on the others. Relative fitness was regressed onto the heights and slopes of reaction norms for three glucosinolate traits, using a single multiple linear regression for each site. Significant *t*-tests indicate nonzero directional selection on reaction norm parameters for a given trait at a given site. $\boldsymbol{\beta_H}$ is the selection gradient, or partial regression coefficient of relative fitness onto reaction norm height; $\boldsymbol{\beta_m}$ is the selection gradient on plasticity measured as the reaction norm slope. *P*-values were adjusted for multiple comparisons (four per reaction norm coefficient) using the sequential Bonferroni correction.

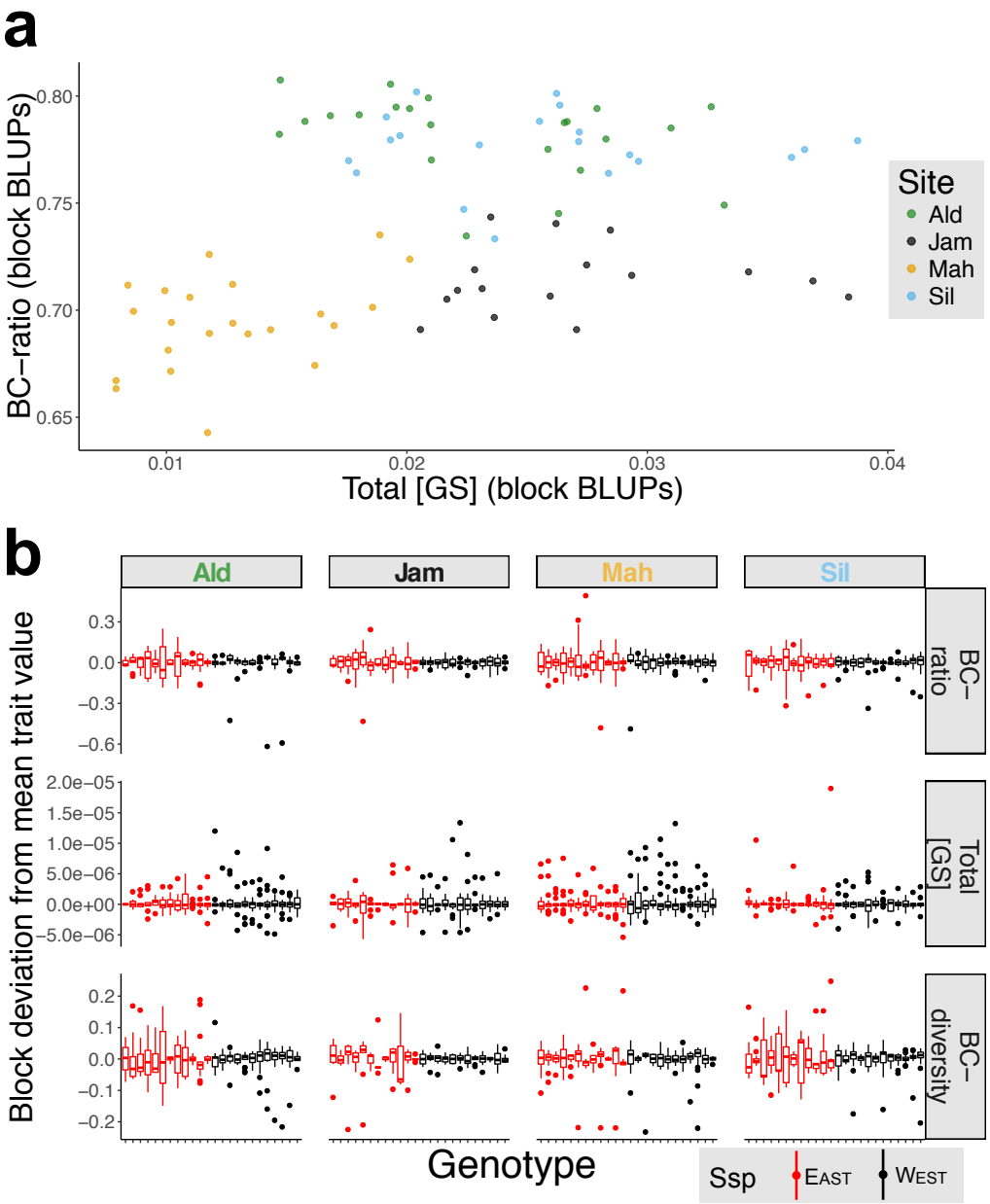
Site	Trait	Selection on reaction norm height			Selection on reaction norm slope		
		β_H	*t*	*P*	β_m	*t*	*P*
Ald	BC-ratio	0.46	0.88	0.78	-0.34	-1.19	0.35
	Total [GS]	-25.70	-1.89	0.30	0.58	2.19	0.13
	BC-diversity	-0.69	-0.82	0.85	0.09	0.62	0.74
Jam	BC-ratio	0.17	0.18	0.86	-0.97	-1.81	0.26
	Total [GS]	-20.70	-0.81	0.66	-0.09	-0.17	1.00
	BC-diversity	2.46	1.58	0.40	0.32	1.2	0.74
Mah	BC-ratio	1.07	2.3	0.14	-0.37	-1.42	0.35
	Total [GS]	-15.70	-1.27	0.66	0.06	0.25	1.00
	BC-diversity	-1.46	-1.92	0.28	0.15	1.13	0.74
Sil	BC-ratio	0.94	2.08	0.16	-0.82	-3.28	**0.02**
	Total [GS]	-14.20	-1.2	0.66	0.63	2.77	**0.05**
	BC-diversity	-0.13	-0.17	0.86	0.41	3.28	**0.02**



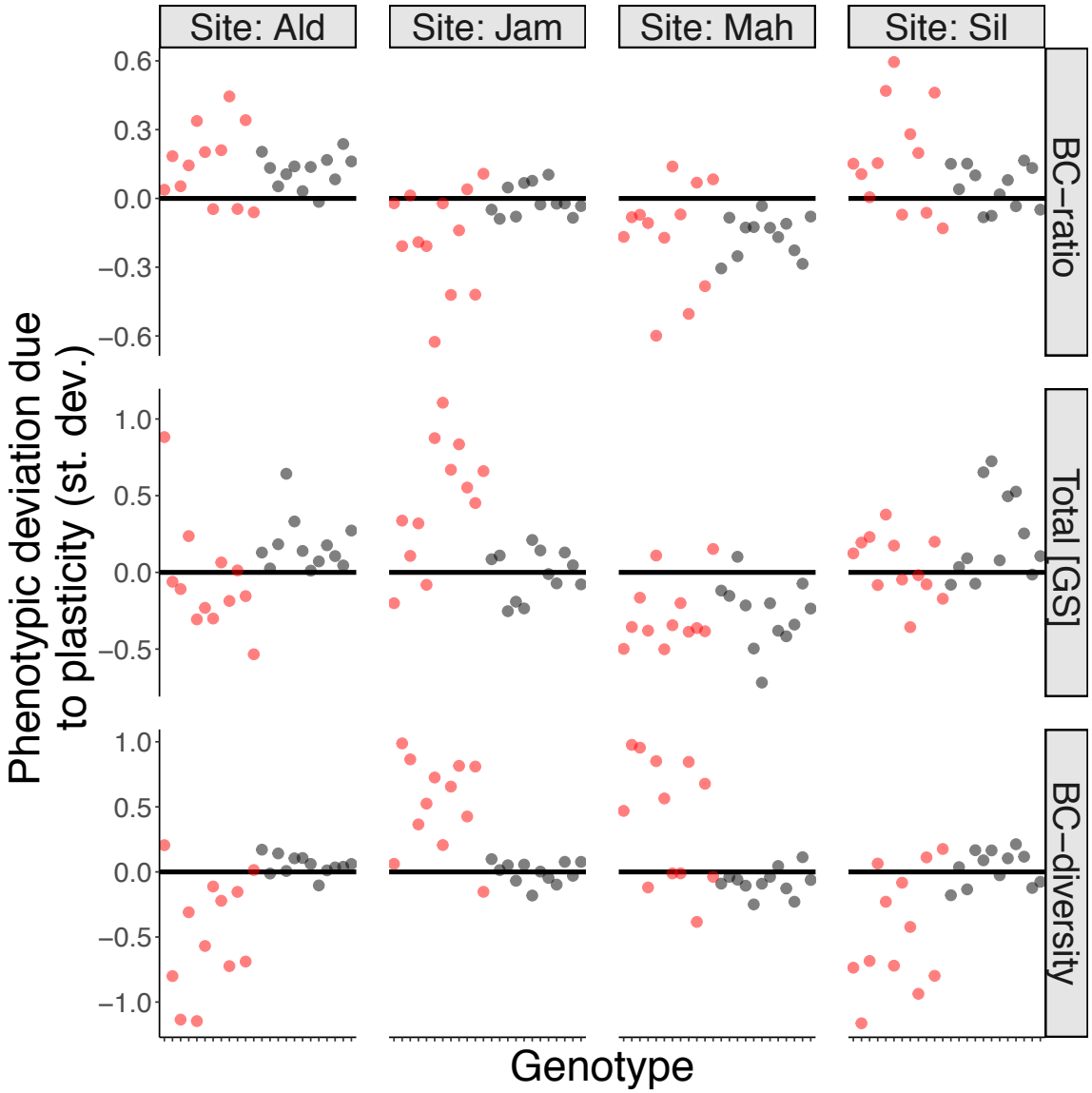
Supplementary Figure 1: Environmental characteristics of experimental blocks in four natural *Boechera stricta* field sites. Data were collected in summer 2014 from 16 to 22 half-square-meter plots spanning each common garden. "PlantDiv" = number of plant morphospecies present in each block (not counting *B. stricta*); "Veg" = percent vegetation cover of each block (estimated for each of 50 sub-blocks of area 10x10cm, then averaged). All other variables describe chemical content of soils (units: pH = none, conductivity = umho/cm, all others = ppm)



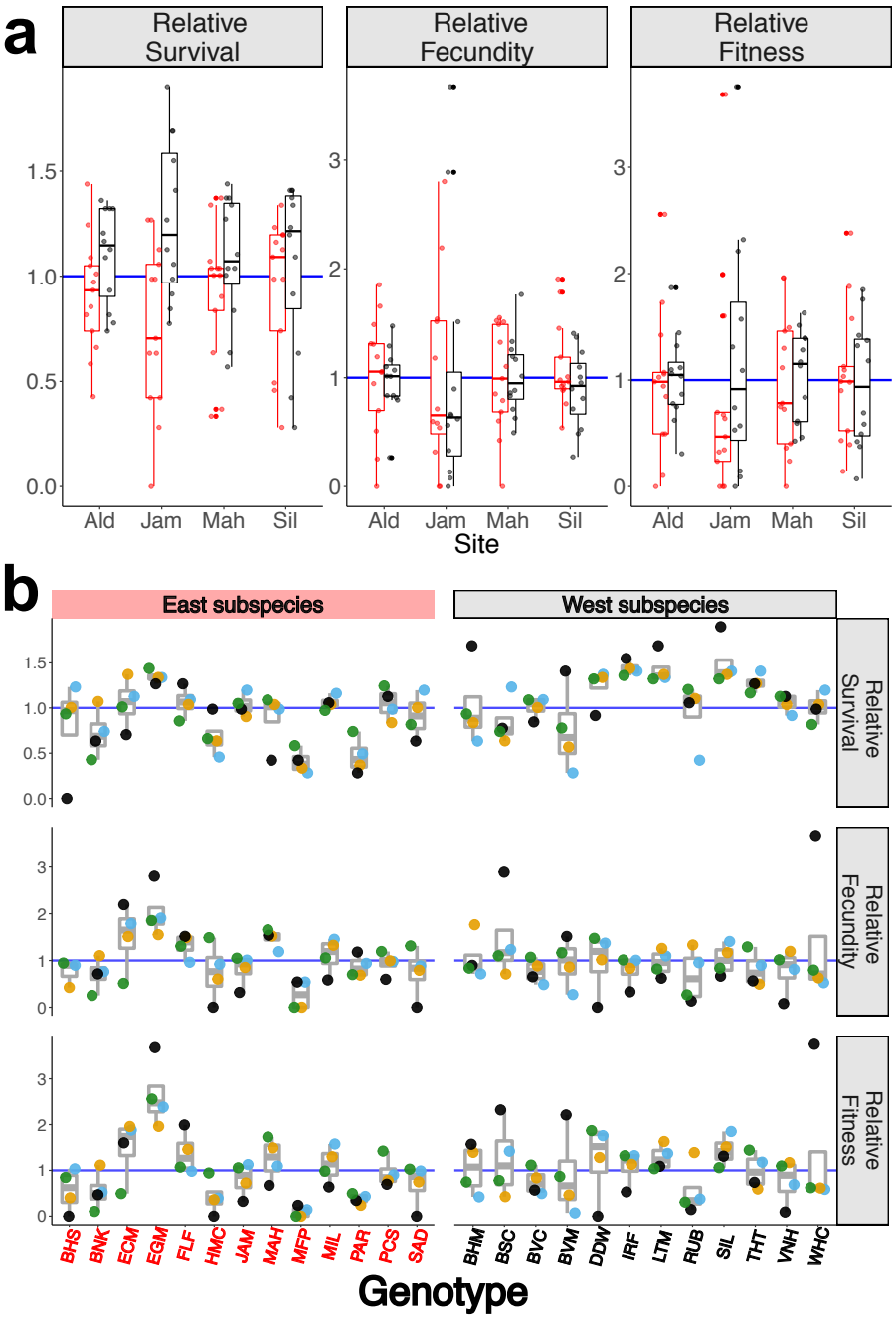
Supplementary Figure 2: Rosette glucosinolate profiles change as plants develop. All glucosinolate measurements in this study were from rosette leaves, regardless of the plant's overall developmental stage. Plotted are least-squares mean trait values with 95% confidence intervals, from a model that also controlled for genotype, site, plant size, and experimental block nested in site (Table 1).



Supplementary Figure 3: Within habitats, meter-scale environmental variation affects glucosinolate quality and quantity. Associated statistics are provided in Table 1. **(a)** Best linear unbiased predictions (BLUPs) of Total [GS] and BC-ratio are shown for each block nested within common gardens (Figure 3b,c). **(b)** Boxplots show distributions of Genotype × Block BLUPs (as deviations from site/genotype means) in each site. Deviations farther from zero indicate greater plasticity in response to block-scale heterogeneity. BC-ratio and BC-diversity are unitless; units for Total [GS] are µmol/mg dry tissue



Supplementary Figure 4: Genotype-specific plasticity deviations. Least-squares mean trait values for each genotype in each site are plotted in relation to the experiment-wide grand mean trait value (solid line), in units of standard deviations. Red points indicate EASTERN genotypes; black points indicate WESTERN genotypes. The plasticity deviations shown in Figure 6a are averages of all the genotypes shown here.



Supplementary Figure 5: Evolutionary fitness components in four natural habitats are shown for **(a)** the two *B. stricta* subspecies (red = EAST, black = WEST), and **(b)** the twenty-five *B. stricta* genotypes used in this study. "Fitness" was calculated for each genotype as the product of survival and fecundity (see Supplementary Methods). All fitness components are plotted relative to the mean value in each site (1, by definition, shown as a solid blue line).

Supplementary Methods

Field experiment

Plants were initially grown in 49-mL Cone-tainers (Stuewe and Sons Inc., Tangent, OR, USA) in the Duke University greenhouse, where photosynthetically active radiation was maintained between 600 and 2000 μmol sec^{-1} cm^{-2}, daytime and nighttime temperatures between 65-70°F and 55-60°F respectively, and relative humidity between 37-52%. We sowed seeds directly into moistened Metromix 200 potting soil (Sun Gro Horticulture Inc., Vancouver, BC, Canada) which filled the top quarter of each pot; the bottom 75% of each Cone-tainer contained Fafard 4P potting soil (Conrad Fafard Inc., Agawam, MA, USA). We randomized plants into blocks of 50, with two representatives of each genotype per block.

When plants were approximately 6 weeks old (late September, 2013) we flew them to central Idaho for transplant into common gardens. The original experimental design specified 16 blocks (N=16×50=800) in each of five field sites. We planted the first common garden (Jackass Meadow; "Jam") as planned; however, an early blizzard made the second garden (Parker Meadow) inaccessible. Therefore, we redistributed the blocks intended for Parker Meadow among the remaining field sites. The final sample sizes were N=800 at Jackass Meadow, N=1000 at Silver Creek ("Sil"), and N=1100 at Mahogany Valley ("Mah") and Alder Creek ("Ald").

We transferred plants in their soil plugs from their pots to pre-dibbled holes without disturbing the natural vegetation (Figure 3c-d). Each block comprised five rows of ten plants in a 10 cm grid, for a total block area of 0.5 m^2. Blocks were separated by ~0.5 m to several m, depending on space availability in each common garden and

position of obstacles such as trees and large rocks. To encourage establishment, we watered the blocks liberally just after transplant, but did not provide any additional water or other treatments for the remainder of the experiment. To deter winter browsing by mammalian herbivores, which caused extensive mortality in other experiments, we covered blocks with metal mesh cages (0.5 in^2 hardware cloth) over the winter.

Measurements of plant performance

We visited Alder Creek, Silver Creek, and Mahogany Valley within the first two weeks of June 2014, but the road to high-elevation Jackass Meadow was impassable due to snow until 28 June. Over-winter survival was 44.4% at Jackass Meadow; 71% at Silver Creek; 58.5% at Alder Creek; and 67.9% at Mahogany Valley. During these visits, we measured the height (from soil to the highest point of the plant) and developmental stage (either "rosette", "bolting", or "fruiting/flowering") of each survivor. We also collected rosette leaf tissue for glucosinolate analysis, although we did not collect samples from rosettes that were dried out or too small to provide adequate tissue (~20-30 mg fresh weight; see below for more detail).

At the end of the growing season, we counted the number of fruits (siliques) and estimated the length of an average-sized silique produced by each plant. Typically, most siliques were of very similar length and therefore measurement of an average fruit was straightforward; otherwise, we measured 2-3 representative siliques and calculated a mean fruit size from those measurements. Due to time limitation and the large sample size (N=1,164 reproducing plants with a mean of 5.1 siliques each), it was not feasible to measure the length of every silique.

We also collected a soil sample for each block, which comprised five pooled soil cores taken from the four corners and center of the block (soil corer diameter = 1 inch, length = 12 inches). Physiochemical properties of these samples were measured using standard protocols at the Texas A&M University AgriLife Extension Service Soil, Water, and Forage Testing Laboratory (http://soiltesting.tamu.edu/).

Measurement of glucosinolate profiles

During the first census at each site, we collected ~20-30 mg of rosette leaf tissue from each plant into tubes with 1.7 mL of 70% methanol. These collections were done as early as possible during the summer to minimize the influence of glucosinolate induction in response to insect attack; herbivory is relatively low until early July (Wagner and Mitchell-Olds, personal observation). The tubes were tightly capped and stored at ambient temperature for a minimum of one month, allowing glucosinolates to leach into the methanol, and shipped back to Duke University at the end of the summer.

Glucosinolate extraction and HPLC

Samples were fully randomized onto 96-well plates for glucosinolate extraction and HPLC analysis; each plate also contained two negative controls (a methanol blank and a sinigrin-only control). In each well we prepared a column of hydrated DEAE-Sephadex A-25 chloride (Sigma Aldrich, St. Louis, USA), equilibrated it for one hour in 20mM sodium acetate, and added 50 μL of 1 mM sinigrin (Sigma Aldrich, St. Louis, USA) as an internal standard. The leachate from each leaf sample was passed through these prepared columns and the bound glucosinolates were washed twice with 750 μL 70% methanol; twice with 750 μL diH_2O; once with 750 μL 20mM sodium acetate; and twice again with 750 μL diH_2O. The columns were incubated overnight at room

temperature in aqueous sulfatase. We then eluted desulfo-glucosinolates in two 75 μL fractions of 70% HPLC-grade methanol and two 75 μL fractions of HPLC-grade water.

We passed 50 μL of each sample through a Zorbax Eclipse XDB-C18 column (4.6 x 150 mm, 5-micron pore size; Agilent Technologies, Santa Clara, USA) on an Agilent 1100-series HPLC machine with a diode array detector. We separated individual desulfo-glucosinolates at 40°C on a gradient of water and acetonitrile (ACN): 6 minutes at 1.5% ACN, followed by a 2-minute increase to 2.5% ACN, a 7-minute increase to 5% ACN, a 2-minute increase to 18%, a 6-minute increase to 46%, a 1-minute increase to 92%, and finally a 5-minute decrease to 1.5%, for a total run time of 29 minutes per sample. We identified the separated compounds based on their retention times and UV absorption spectra at 229 nm (Windsor *et al*. 2005; Schranz *et al*. 2009; Prasad *et al*. 2012; Olson-Manning *et al*. 2013).

After removing the methanol leachate for desulfo-glucosinolate extractions, we air-dried and weighed each leaf sample. We then calculated the absolute concentration of each glucosinolate in each sample as:

$$[GS] = \frac{0.05\ \mu mol\ sinigrin}{Area_{sinigrin}} \times \frac{Area_{GS}}{Mass \times RRF_{GS}}$$

where $Area_{sinigrin}$ = the area under the chromatograph peak of the internal standard sinigrin, $Area_{GS}$ = the area under the chromatograph peak of the compound of interest, *Mass* = the dry mass in mg of the leaf sample from which the glucosinolates were extracted, and RRF_{GS} = the relative response factor of the glucosinolate of interest (Brown *et al*. 2003; Clarke 2010).

Glucosinolate profile summaries

The above protocols were optimized for quantification of aliphatic glucosinolates, and although indolic glucosinolates may also have been present in these samples, we did not measure them for this experiment. In *B. stricta*, the genetic control and eco-evolutionary significance of aliphatic glucosinolates are better understood than those of indolic glucosinolates (Schranz *et al*. 2009; Manzaneda *et al*. 2010; Prasad *et al*. 2012). Therefore, we focused on the four primary aliphatic compounds produced in leaves of this species: 6-methylsulfinylhexyl (6MSOH), 1-methylethyl (1ME), 2-hydroxy-1-methylethyl (2OH1ME), and 1-methylpropyl (1MP) (Figure 4a).

From the absolute glucosinolate concentrations (μmol per mg dry weight) of these four compounds, we calculated three summary descriptors:

$$Total_{[GS]} = [2OH1ME] + [1ME] + [1MP] + [6MSOH]$$

$$BC_{ratio} = \frac{[2OH1ME] + [1ME] + [1MP]}{Total_{[GS]}}$$

$$BC_{diversity} = -\sum_{i=1}^{k} [BC_i] * \log([BC_i])$$

where k = the total number of branched-chain compounds present in the sample and $[BC_i]$ = the concentration of the ith branched-chain glucosinolate. Total [GS] describes the combined concentration of all aliphatic glucosinolates. BC-ratio describes the proportion of aliphatic glucosinolates that are derived from branched-chain amino acids, which is an ecologically and evolutionarily important trait in *B. stricta* (Schranz *et al*. 2009; Manzaneda *et al*. 2010; Prasad *et al*. 2012). Finally, BC-diversity describes the balance of the three types of branched-chain glucosinolates, taking low values when glucosinolate profiles are dominated by one compound and high values when multiple compounds are

present in similar amounts (Figure 4b-c). We calculated BC-diversity using the Shannon diversity index implemented in the **R** package **vegan** (Oksanen *et al*. 2013).

Partitioning variance in glucosinolate profiles

We removed one genotype ('Bay Horse Saddle') from the dataset because no individuals of this genotype survived at one of the field sites ('Jam'), which prevented full analysis of variance (however, this genotype was not excluded from phenotypic selection analysis or reaction norm calculations—described below—because these procedures were not affected by empty cells in the genotype-site matrix). To assess plasticity of glucosinolate profiles among habitats, we used univariate REML linear mixed models to partition variance in each of the three glucosinolate traits among genetic and environmental predictors. We modeled each trait as:

$$Trait = Genotype + Site + Genotype * Site + Block(Site) + Genotype * Block + Plant\ height + Developmental\ stage + Batch + error$$

where *Plant height, Developmental stage,* and *Batch* were nuisance variables to control for (respectively) the "general vigor problem" of large plants having more resources to invest in defense (Agrawal 2011), ontogenetic changes in rosette glucosinolate profiles, and HPLC batch effects. *Block* (nested in Site), *Batch,* and $Genotype * Block$ were random-intercept terms; all other terms were fixed effects. We fit the above model a total of three times: once for each glucosinolate trait.

We considered *Genotype* to be a fixed effect because the genotypes used in this study were not sampled randomly from each subspecies: rather, they were chosen because they originated in the hybrid zone portion of the study region (Lee and Mitchell-Olds 2013). Furthermore, the genotype SAD12 is from Colorado (rather than

Montana/Idaho) and is somewhat genetically divergent from the other EASTERN genotypes in this study. We included SAD12 because it has been a focus of previous and ongoing work on *B. stricta*, has a sequenced genome, and is a parent of several useful near-isogenic lines and recombinant inbred lines. Therefore, although these resources were not utilized for this experiment, an improved understanding of the biology and ecology of the SAD12 genotype is of broader interest and has potential to generate future experiments on the genetic basis of plasticity in *B. stricta*.

To check whether our data satisfied the assumptions for ANOVA, we visually inspected the residuals of each model using Q-Q plots and by plotting them against fitted values. In the case of Total [GS], square-root transformation greatly improved homoscedasticity and reduced the influence of several outliers; therefore, we used square-root transformed Total [GS] as the response variable for the REML model of glucosinolate quantity. However, the resulting least-squares means were back-transformed and all subsequent analyses used Total [GS] measurements on the original scale. We adjusted *P*-values for the three separate tests using the sequential Bonferroni correction (Holm 1979). Statistical significance of random effects was assessed using likelihood ratio tests. We performed all linear mixed models using the **R** packages **lme4** and **lmerTest** (Bates *et al*. 2011; Kuznetsova *et al*. 2015). The resulting least-squares mean trait values for *Site* fixed effects were used to quantify between-habitat plasticity (below).

Testing for adaptive plasticity among habitats

The question of whether plasticity can aid survival in new environments hinges on whether the direction of plasticity matches the direction of selection (Figure 1g-i).

Therefore, to test for adaptive patterns of plasticity, we determined (1) the direction of plasticity, if any, in each environment (Figure 1g); (2) the direction of selection, if any, in each environment (Figure 1h); and (3) whether the direction of plasticity matched the direction of selection more often than would be expected by random chance (Figure 1i). We evaluated plasticity and directional selection of three separate glucosinolate-related traits (Figure 4c) in each of four common gardens (Figure 3).

Measuring the direction of plasticity in each site

We calculated the plasticity deviation (ΔT_i) of each trait T in each site i using the formula $\Delta T_i = T_i - \bar{T}$, where T_i is the least-squares mean trait value for site i (calculated from the REML linear mixed model described above; depicted by the black points in Figure 1g) and $\bar{T}$ is the grand mean trait value from the entire experiment (depicted by the dotted line in Figure 1g). We considered the plasticity deviation (ΔT_i, depicted by green arrows in Figure 1g) to be statistically distinguishable from zero if $\bar{T}$ did not fall within the 95% confidence interval of T_i. The sign of ΔT_i thus describes the direction of plasticity in site i.

Measuring the direction of selection in each site

The question of whether plasticity increases fitness in new environments rests on whether the plastic change in mean trait values (ΔT_i) is in the same direction as the change in mean trait values that would result from natural selection—i.e., directional selection (Figure 1g-i). Therefore, we focus on the results of directional (linear) selection analyses only. To account for both direct and indirect selection on glucosinolate traits (both of which could impact the adaptive value of trait plasticity), we calculated selection differentials of each trait in each site. To maximize power from this large dataset, we

used phenotypic selection analysis of fecundity and glucosinolate data from all individuals in the experiment (Lande 1979).

We conducted phenotypic selection analysis separately for each glucosinolate trait at each site using linear mixed effects models. Let z_{jk} equal the trait value of the kth individual in the jth block of a single site, centered and standardized as Z-scores such that mean and variance within each site equal 0 and 1, respectively. Letting F_{jk} equal the observed fecundity (proportional to mm of fruit produced) of the kth individual in the jth block, we then calculated the local relative fecundity of each individual as $f_{jk} = {}^{F_{jk}}/_{\bar{F}}$, where $\bar{F}$ is the mean observed fecundity at the individual's site, such that the mean of f_{jk} in each site equals 1. We then fit the linear mixed effects model:

$$Individual\ relative\ fecundity = Block + Trait\ value + error$$

$$f_{jk} = \mu + \alpha_j + \beta * z_{jk} + \varepsilon_{jk}$$

where μ is the mean relative fecundity; α_j is the random effect of the jth block; f_{jk} and z_{jk} are the relative fecundity and standardized trait value of the kth individual in the jth block; β is the selection differential or linear regression coefficient of relative fecundity onto standardized trait values; and ε_{jk} is the random error deviation. α_j was a random-intercept term included to control for covariance between fecundity and phenotype caused by block-scale environmental variation. We fit the above model a total of twelve times: once for each of the three traits at each of the four sites, generating twelve selection differentials (values of β).

Because model residuals often did not meet the assumptions of ANOVA, we used a permutation test to determine statistical significance. We compared the observed F

value to the distribution of F values generated by permuting standardized trait values among individuals within blocks and re-fitting the linear mixed model 1,000 times. For each trait, we adjusted P-values for the four separate tests using the sequential Bonferroni correction (Holm 1979). The regression coefficient β is the selection differential for one trait at one site (depicted as the slope of one line in Figure 1h). The sign of β thus describes the direction of selection.

To estimate selection differentials, we fit the above model separately for each site. However, to test whether these selection differentials varied among sites, we pooled the relative fecundity and phenotype data from all four sites and fit a mixed-effects ANCOVA model with an additional interaction term:

$$Individual\ relative\ fecundity = Site + Block(Site) + Trait + Site * Trait + error$$

$$f_{ijk} = \mu + S_i + \alpha_{ij} + \beta * z_{ijk} + (S * \beta)_i * z_{ijk} + \varepsilon_{ijk}$$

where μ is the experiment-wide mean relative fecundity; S_i is the main effect of the ith site; α_{ij} is the deviation caused by the random effect of the jth block nested in the ith site; β is the regression coefficient of f_{ijk} on z_{ijk} across all sites (i.e., the average selection differential); $z_{\square jk}$ is the trait value for the kth individual in the jth block in the ith site, centered and standardized as Z-scores so that the experiment-wide mean and variance equal 0 and 1, respectively; $(S * \beta)_i$ is the deviation attributed to the regression of f_{ijk} on z_{ijk} at the ith site (i.e., the interaction between site and selection differential); and ε_{ijk} is the random error deviation. A nonzero $(S * \beta)_i$ term (i.e., a significant site-by-trait interaction term) indicates heterogeneous directional selection among habitats. We fit the above model three times total, once for each glucosinolate trait.

We drew our main conclusions from the selection differentials calculated as described above, which reflects the combined influence of direct and indirect selection on each trait. However, for thoroughness we also calculated selection gradients in each site (i.e., selection on each trait after controlling for selection on the others, within one multiple linear regression):

$$Individual\ relative\ fecundity = Block + Total\ [GS] + BC_ratio + BC_diversity + error$$

$$f_{jk} = \mu + \alpha_j + \beta_1 * z_{1jk} + \beta_2 * z_{2jk} + \beta_3 * z_{3jk} + \varepsilon_{jk}$$

where μ_i is the mean relative fecundity; α_j is the random effect of the jth block; f_{jk} is the relative fecundity of the kth individual in the jth block; z_{1jk}, z_{2jk}, and z_{3jk} are the standardized values of three glucosinolate traits for the kth individual in the jth block; β_1, β_2, and β_3 are the selection gradients or partial regression coefficients of relative fecundity onto values of traits z_1, z_2, and z_3; and ε_{jk} is the residual error. We fit the above model four times total, once for each site.

In summary, we calculated the plasticity deviation for each trait at each site. Next, we estimated directional natural selection in each site using the linear regression of relative fecundity onto standardized trait values. We also tested whether selection on each trait was heterogeneous among sites. Finally, we tested whether the direction and magnitude of directional selection predict the direction and magnitude of plasticity in each site (see below).

Assessing the match between direction of plasticity and direction of selection

Whether plasticity of a trait is in an “adaptive” or “non-adaptive” direction in a given field site can only be evaluated if the trait both (1) exhibited significant plasticity at

that site, and (2) was under significant directional natural selection at that site (Figure 1g-i). These conditions were evaluated by the previously described analyses. We used two methods to test the hypothesis that plasticity tends to move trait values in an adaptive direction, considering all cases in which both a significant plasticity deviation (ΔT_i) and a significant selection differential (β_i) were detected within a single site i for any given trait. First, we conducted an exact binomial test to determine whether the sign of ΔT_i matches the sign of β_i more often than would be expected by random chance—i.e., to determine whether the direction of selection predicts the direction of plasticity (Figure 1i). Because we had observed that Total [GS] and BC-diversity were genetically correlated (potentially violating the assumption of independence for this test), we also removed the Total [GS] data points and then performed a linear regression of ΔT_i on β_i for BC-diversity only, to test whether selection differentials in the four sites (both magnitude and direction) are correlated with the magnitude and direction of plasticity.

Testing for adaptive plasticity within habitats

Separate from the question of whether plasticity increases fitness in new habitats (or "coarse-grained" environmental variation) is the question of whether plasticity in response to continuous environmental gradients (or "fine-grained" environmental variation) is adaptive within a single habitat. Theoretical work predicts that only the latter phenomenon can result in the evolution of plasticity as an adaptation to environmental heterogeneity (Via and Lande 1985).

In this study, we focused on phenotypic plasticity induced by spatial environmental variation at a single time-point. Because each plant in this study only experienced a single (spatial) environment, plasticity of individual plants cannot be

measured. Instead, spatial plasticity of glucosinolate profiles is a property of a genotype, estimated by comparing the trait values of individuals that shared the same genotype but grew in different experimental blocks or microhabitats.

To infer whether natural selection was acting on fine-grained glucosinolate plasticity within *B. stricta* habitats, we (1) quantified plasticity among blocks for each genotype as a continuous function or *reaction norm*, and (2) used genotypic selection analysis to test whether reaction norm steepness—a measure of plasticity—predicted evolutionary fitness of each *B. stricta* genotype.

Quantifying fine-grained plasticity by fitting reaction norms

We quantified fine-grained plasticity of glucosinolate profiles by expressing each glucosinolate trait as a genotype-specific continuous linear function of a block-scale *environmental index* (EI), or numerical descriptor of microhabitat conditions within each experimental block. Following Finlay and Wilkinson (1963), we used plant phenotype data to calculate EIs. This method assumes that the environmental factors that cause trait plasticity are unknown, and thus environmental characteristics are best "measured" using the mean trait values expressed in each environment. Therefore, for each glucosinolate trait T, we calculated the environmental index of each experimental block j as $E_j = \overline{t_j}$, where $\overline{t_j}$ is the grand mean of all trait values observed in the jth block.

Next, we fit a reaction norm for each trait T and each genotype l using linear regression:

$$Genotype\ trait\ mean\ in\ block = \square lock\ environmental\ index + error$$

$$g_{jl} = I_l + m_l * E_j + \varepsilon_{jl}$$

where g_{jl} is the mean trait value for one genotype-block combination (the lth genotype in the jth block); I_l is the regression intercept for the lth genotype; m_l is the reaction norm slope for the lth genotype (i.e., the linear regression coefficient of g_{jl} on E_j); E_j is the environmental index (i.e., the mean trait value) for the jth block as defined above; and ε_{jl} is the deviation attributed to random error. We fit the above model 75 times total: once for each of the three glucosinolate traits for each of the 25 genotypes.

We then calculated the "reaction norm height" H_l for each trait and each genotype by evaluating the genotype-specific reaction norm equation at the constant value $\bar{E}$, which is the mean of all environmental indexes: $H_l = I_l + m_l * \bar{E}$. Thus, the resulting reaction norm height H_l is the predicted trait value of the lth genotype in an "average" block or micro-environment (Figure 2).

As shown above, we estimated genotype-specific reaction norm parameters by fitting a separate linear regression for each genotype. To test whether reaction norm slopes were heterogeneous among genotypes, we pooled data from all genotypes and fit a fixed effects ANCOVA model:

$$Genotype\ trait\ mean\ in\ block = Genotype + Block\ EI + Genotype * Block\ EI + error$$

$$g_{jl} = \mu + G_l + m * E_j + (G * m)_l * E_j + \varepsilon_{jl}$$

where μ is the mean trait value; G_l is the main effect of the lth genotype; m is the regression coefficient of g_{jl} on E_j across all genotypes (i.e., the average reaction norm slope, predicted to be the line y=1*x by definition); $(G * m)_l$ is the deviation attributed to the regression coefficient of g_{jl} on E_j for the lth genotype (i.e., the genotype-specific reaction norm slope, or the interaction between genotype and block environmental

index); and ε_{jl} is the random error deviation. A nonzero $(G * m)_l$ (i.e., a significant genotype-by-environmental index interaction term) indicates genetic variation for reaction norm slopes. We assessed significance of this model using ANOVA with Type III sums of squares in the **R** package **car** (Fox and Weisberg 2011). We fit the above model a total of three times, once for each glucosinolate trait.

Testing for selection on reaction norm slope and reaction norm height

The slope and height of the reaction norms for each trait are themselves measurable characteristics of plant genotypes, corresponding to plasticity and average trait values, respectively. We measured directional selection gradients on these reaction norm parameters at each site to determine whether glucosinolate plasticity in response to fine-grained environmental variation affects fitness within a habitat.

First, we calculated the relative evolutionary fitness of each genotype within each habitat. Letting F_{kl} equal the fecundity of the kth surviving individual of the lth genotype in one common garden, and letting P_l equal the proportion of all planted individuals of the lth genotype to survive until summer 2014 at that common garden, site-specific genotypic fitness was calculated as $W_l = P_l \times \overline{F_l}$, where W_l is the absolute fitness of the lth genotype, and $\overline{F_l}$ is the mean fecundity of all individuals of the lth genotype at the same site. We then calculated the site-specific *relative* genotypic fitness as $w_l = {W_l}/{\overline{W_l}}$, where $\overline{W_l}$ is the mean absolute fitness of all genotypes in one site, such that the mean of w_l at any site equaled 1.

For each trait and each site, we then tested for directional selection on reaction norm components by fitting a multiple linear regression of relative fitness on reaction norm height and slope:

$$Genotype\ relative\ fitness = RN\ height + RN\ slope + error$$

$$w_l = I + \beta_H * H_l + \beta_{\square} * m_l + \varepsilon_l$$

where I is a constant intercept; H_l and m_l are the height and slope of the reaction norm of the lth genotype; β_H and β_m are the partial regression coefficients of relative fitness onto reaction norm height (H_l) and slope (m_l), respectively; and ε_{il} is the random error. A nonzero β_H or β_m term (i.e., a significant linear regression coefficient) constitutes evidence of directional natural selection on reaction norm height or reaction norm slope, respectively. We fit the above model twelve times total, once for each of the three glucosinolate traits for each of the four sites.

As described above, we estimated all β_H and β_m using separate linear regressions for each trait and each site. However, to test whether directional selection on reaction norm components varied among habitats, we pooled data from all four sites and fit an additional ANCOVA model for each trait:

$$Geno.rel.\ fit. = Site + RN\ height + RN\ slop\square + Site * RN\ height + Site * RN\ slope + error$$

$$w_{il} = \mu + S_i + \beta_H * H_l + \beta_m * m_l + (S * \beta_H)_i * H_l + (S * \beta_m)_i * m_l + \varepsilon_{il}$$

where w_{il} is the relative fitness of genotype l at site i; μ is the experiment-wide mean relative fitness; S_i is the deviation caused by the fixed effect of the ith site; β_H and β_m are the regression coefficients of w_{il} on H_l and m_l across all sites (i.e., the average selection gradients on reaction norm height and slope); $(S * \beta_H)_i$ and $(S * \beta_m)_i$ are the deviations attributed to the regression of w_{il} on H_l and m_l at the ith site (i.e., the site-specific selection gradients on reaction norm height and slope, in other words, the interaction

between site and each reaction norm parameter); and ε_{il} is the random error deviation for the lth genotype in the ith site. A nonzero $(S * \beta_H)_i$ or $(S * \beta_m)_i$ term (i.e., a significant site-by-height or site-by-slope interaction term) indicates heterogeneous directional selection on reaction norm height or slope, respectively, among habitats. We fit the above model three times total, once for each glucosinolate trait.

We drew our main conclusions about selection on reaction norms from the selection differentials calculated as described above, which account for both direct and indirect selection on each trait. However, for thoroughness we also calculated selection gradients on reaction norms for all traits in each site (i.e., selection on each trait's reaction norm coefficients after controlling for selection on the others', within a single multiple linear regression). We fit the multiple linear regression:

$$Geno.rel.fit. = RN\ height_{BC-ratio} + RN\ slope_{BC-ratio} + RN\ height_{Total\,[GS]} + RN\ slope_{Total\,[GS]} + RN\ height_{BC-div.} + RN\ slope_{BC-div.} + error$$

$$w_l = I + \beta_{H1} * H_{1l} + \beta_{m1} * m_{1l} + \beta_{H2} * H_{2l} + \beta_{m2} * m_{2l} + \beta_{H3} * H_{3l} + \beta_{m3} * m_{3l} + \varepsilon_l$$

where I is a constant intercept; H_{1l}, H_{2l}, and H_{3l} are the heights of the reaction norms of three glucosinolate traits for the lth genotype; m_{1l}, m_{2l}, and m_{3l} are the slopes of the reaction norms of three glucosinolate traits for the lth genotype; each β is the partial regression coefficient of relative fitness onto the height or slope of the reaction norm for one trait; and ε_l is the random error deviation. We fit the above model a total of four times, once for each site.

References

Abdel-Farid, I.B., Jahangir, M., Mustafa, N.R., van Dam, N.M., van den Hondel, C.A.M.J.J., Kim, H.K., *et al*. (2010). Glucosinolate profiling of *Brassica rapa* cultivars after infection by *Leptosphaeria maculans* and *Fusarium oxysporum*. *Biochem Syst Ecol*, 38, 612–620

Agrawal, A.A. (1998). Induced responses to herbivory and increased plant performance. *Science*, 279, 1201–1202

Agrawal, A.A. (2000). Benefits and costs of induced plant defense for *Lepidium virginicum* (Brassicaceae). *Ecology*, 81, 1804–1813

Agrawal, A.A. (2011). Current trends in the evolutionary ecology of plant defence: Plant defence theory. *Funct Ecol*, 25, 420–432

Agrawal, A.A., Conner, J.K., Johnson, M.T. & Wallsgrove, R. (2002). Ecological genetics of an induced plant defense against herbivores: additive genetic variance and costs of phenotypic plasticity. *Evolution*, 56, 2206–2213

Anderson, J.T., Inouye, D.W., McKinney, A.M., Colautti, R.I. & Mitchell-Olds, T. (2012). Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. *Proc Royal Soc B: Biol Sci*, 279, 3843–3852

Anderson, J.T., Lee, C.-R. & Mitchell-Olds, T. (2014a). Strong selection genome-wide enhances fitness trade-offs across environments and episodes of selection. *Evolution*, 68, 16–31

Anderson, J.T., Lee, C.-R., Rushworth, C.A., Colautti, R.I. & Mitchell-Olds, T. (2013). Genetic trade-offs and conditional neutrality contribute to local adaptation: Genetic basis of local adaptation. *Mol Ecol*, 22, 699–708

Anderson, J.T., Wagner, M.R., Rushworth, C.A., Prasad, K.V.S.K. & Mitchell-Olds, T. (2014b). The evolution of quantitative traits in complex environments. *Heredity*, 112, 4–12

Auld, J.R., Agrawal, A.A. & Relyea, R.A. (2010). Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proc Royal Soc B: Biol Sci*, 277, 503–511

Baldwin, J.M. (1896). A new factor in evolution. *Am Nat*, 30, 441–451

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J Stat Softw*, 67, 1–48

Baythavong, B.S. (2011). Linking the spatial scale of environmental variation and the evolution of phenotypic plasticity: Selection favors adaptive plasticity in fine-grained environments. *Am Nat*, 178, 75–87

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

Bednarek, P., Piślewska-Bednarek, M., Svatoš, A., Schneider, B., Doubskỳ, J., Mansurova, M., *et al*. (2009). A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. *Science*, 323, 101–106

Bloom, T., Baskin, J. & Baskin, C. (2002). Ecological life history of the facultative woodland biennial *Arabis laevigata* variety laevigata (Brassicaceae): seed dispersal. *J Torrey Bot Soc*, 129, 21–28

Brader, G., Mikkelsen, M.D., Halkier, B.A. & Tapio Palva, E. (2006). Altering glucosinolate profiles modulates disease resistance in plants. *Plant J*, 46, 758–767

Brader, G., Tas, E. & Palva, E.T. (2001). Jasmonate-dependent induction of indole glucosinolates in Arabidopsis by culture filtrates of the nonspecific pathogen *Erwinia carotovora*. *Plant Physiol*, 126, 849–860

Bradshaw, A.D. (1965). Evolutionary significance of phenotypic plasticity in plants. *Adv Genet*, 13, 115–155

Bressan, M., Roncato, M.A., Bellvert, F., Comte, G., el Zahar Haichar, F., Achouak, W., *et al*. (2009). Exogenous glucosinolate produced by *Arabidopsis thaliana* has an impact on microbes in the rhizosphere and plant roots. *ISME J*, 3, 1243–1257

Brown, P.D., Tokuhisa, J.G., Reichelt, M. & Gershenzon, J. (2003). Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*. *Phytochemistry*, 62, 471–481

Clarke, D.B. (2010). Glucosinolates, structures and analysis in food. *Analytical Methods*, 2, 310

Conover, D. & Schultz, E. (1995). Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends Ecol Evol*, 10, 248–252

Donohue, K., Messiqua, D., Pyle, E.H., Heschel, M.S. & Schmitt, J. (2000). Evidence of adaptive divergence in plasticity: Density-and site-dependent selection on shade-avoidance responses in *Impatiens capensis*. *Evolution*, 54, 1956–1968

Donohue, K., Pyle, E.H., Messiqua, D., Heschel, M.S. & Schmitt, J. (2001). Adaptive divergence in plasticity in natural populations of *Impatiens capensis* and its consequences for performance in novel habitats. *Evolution*, 55, 692–702

Dudley, S.. & Schmitt, J. (1996). Testing the adaptive plasticity hypothesis: Density-dependent selection on manipulated stem length in *Impatiens capensis*. *Am Nat*, 147, 445–465

El-Soda, M., Malosetti, M., Zwaan, B.J., Koornneef, M. & Aarts, M.G.M. (2014). Genotype × environment interaction QTL mapping in plants: lessons from Arabidopsis. *Trends Plant Sci*, 19, 390–398

Falconer, D. & Mackay, T. (1996). *Introduction to Quantitative Genetics*. 4th edn. Pearson Education Limited, Essex, England

Finlay, K. & Wilkinson, G. (1963). The analysis of adaptation in a plant-breeding programme. *Aust J Agric Res*, 14, 742–754

Fox, J. & Weisberg, S. (2011). *An R Companion to Applied Regression*. 2nd edn. Sage, Thousand Oaks, CA

Ghalambor, C.K., Hoke, K.L., Ruell, E.W., Fischer, E.K., Reznick, D.N. & Hughes, K.A. (2015). Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature*, 525, 372–375

Ghalambor, C.K., McKay, J.K., Carroll, S.P. & Reznick, D.N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct Ecol*, 21, 394–407

Gomulkiewicz, R. & Kirkpatrick, M. (1992). Quantitative genetics and the evolution of reaction norms. *Evolution*, 46, 390–411

Halkier, B.A. & Gershenzon, J. (2006). Biology and biochemistry of glucosinolates. *Plant Biol*, 57, 303–333

Hendry, A.P. (2015). Key questions on the role of phenotypic plasticity in eco-evolutionary dynamics. *J Heredity*, 2015, 1–17

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scand J Stat*, 6, 65–70

Hopkins, R.J., van Dam, N.M. & van Loon, J.J.A. (2009). Role of glucosinolates in insect-plant relationships and multitrophic interactions. *Annu Rev Entomol*, 54, 57–83

Huang, Y. & Agrawal, A.F. (2016). Experimental evolution of gene expression and plasticity in alternative selective regimes. *PLoS Genet*, 12, e1006336

Kanehisa, M., Goto, S., Kawashima, S. & Nakaya, A. (2002). The KEGG databases at GenomeNet. *Nucleic Acids Res*, 30, 42–46

Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. (2015). *lmerTest: Tests in Linear Mixed Effects Models. R package version 2.0-32. https://CRAN.R-project.org/package=lmerTest*

Lande, R. (1979). Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. *Evolution*, 33, 402–416

Lee, C.-R. & Mitchell-Olds, T. (2011). Quantifying effects of environmental and geographical factors on patterns of genetic differentiation: Environment & geography of genetic diversity. *Mol Ecol*, 20, 4631–4642

Lee, C.-R. & Mitchell-Olds, T. (2013). Complex trait divergence contributes to environmental niche differentiation in ecological speciation of *Boechera stricta*. *Mol Ecol*, 22, 2204–2217

Lee, C.-R., Wang, B., Mojica, J.P., Mandáková, T., Prasad, K.V.S.K., Goicoechea, J.L., *et al*. (2017). Young inversion with multiple linked QTLs under selection in a hybrid zone. *Nat Ecol Evol*, 1, 0119

Levins, R. (1962). Theory of fitness in a heterogeneous environment. I. The fitness set and adaptive function. *Am Nat*, 96, 361–373

Levins, R. (1963). Theory of fitness in a heterogeneous environment. II. Developmental flexibility and niche selection. *Am Nat*, 97, 75–90

Manzaneda, A.J., Prasad, K.V.S.K. & Mitchell-Olds, T. (2010). Variation and fitness costs for tolerance to different types of herbivore damage in *Boechera stricta* genotypes with contrasting glucosinolate structures. *New Phytol*, 188, 464–477

Mauricio, R. & Rausher, M.D. (1997). Experimental manipulation of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. *Evolution*, 51, 1435

Mauricio, R. (1998). Costs of resistance to natural enemies in field populations of the annual plant *Arabidopsis thaliana*. *Am Nat*, 151, 20–28

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., *et al*. (2016). *vegan: Community Ecology Package*. R package version 2.4-1. https://CRAN.R-project.org/package=vegan

Olson-Manning, C.F., Lee, C.-R., Rausher, M.D. & Mitchell-Olds, T. (2013). Evolution of flux control in the glucosinolate pathway in *Arabidopsis thaliana*. *Molec Biol Evol*, 30, 14–23

Palacio-López, K., Beckage, B., Scheiner, S. & Molofsky, J. (2015). The ubiquity of phenotypic plasticity in plants: A synthesis. *Ecol Evol*, 5, 3389–3400

Pigliucci, M. (2005). Evolution of phenotypic plasticity: Where are we going now? *Trends Ecol Evol*, 20, 481–486

Prasad, K.V.S.K., Song, B.-H., Olson-Manning, C., Anderson, J.T., Lee, C.-R., Schranz, M.E., *et al*. (2012). A gain-of-function polymorphism controlling complex traits and fitness in nature. *Science*, 337, 1081–1084

Price, T.D., Qvarnstrom, A. & Irwin, D.E. (2003). The role of phenotypic plasticity in driving genetic evolution. *Proc Royal Soc B: Biol Sci*, 270, 1433–1440

R Core Team. (2016). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria

Rausher, M. (1992). The measurement of selection on quantitative traits: Biases due to environmental covariances between traits and fitness. *Evolution*, 46, 616–626

Reed, T.E., Waples, R.S., Schindler, D.E., Hard, J.J. & Kinnison, M.T. (2010). Phenotypic plasticity and population viability: The importance of environmental predictability. *Proc Royal Soc B: Biol Sci*, 277, 3391–3400

Richards, C.L., Bossdorf, O., Muth, N.Z., Gurevitch, J. & Pigliucci, M. (2006). Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecol Lett*, 9, 981–993

Rushworth, C.A., Song, B.-H., Lee, C.-R. & Mitchell-Olds, T. (2011). *Boechera*, a model system for ecological genomics. *Molec Ecol*, 20, 4843–4857

Sanchez-Vallet, A., Ramos, B., Bednarek, P., López, G., Piślewska-Bednarek, M., Schulze-Lefert, P., *et al*. (2010). Tryptophan-derived secondary metabolites in *Arabidopsis thaliana* confer non-host resistance to necrotrophic *Plectosphaerella cucumerina* fungi. *Plant J*, 63, 115–127

Schmalhausen, I. (1949). *Factors of evolution: The theory of stabilizing selection*. Blakiston, Oxford, England

Schmitt, J., Dudley, S. & Pigliucci, M. (1999). Manipulative approaches to testing adaptive plasticity: Phytochrome-mediated shade-avoidance responses in plants. *Am Nat*, 154, S43–S54

Schmitt, J., Stinchcombe, J.R., Heschel, M.S. & Huber, H. (2003). The adaptive evolution of plasticity: Phytochrome-mediated shade avoidance responses. *Integr Comp Biol,* 43, 459–469

Schranz, M.E., Manzaneda, A.J., Windsor, A.J., Clauss, M.J. & Mitchell-Olds, T. (2009). Ecological genomics of *Boechera stricta*: identification of a QTL controlling the allocation of methionine-vs branched-chain amino acid-derived glucosinolates and levels of insect herbivory. *Heredity*, 102, 465–474

Song, B.H., Clauss, M.J., Pepper, A. & Mitchell-Olds, T. (2006). Geographic patterns of microsatellite variation in *Boechera stricta*, a close relative of Arabidopsis. *Mol Ecol*, 15, 357–369

Sultan, S.E. (1987). Evolutionary Implications of Phenotypic Plasticity in Plants. In: *Evolutionary Biology: Volume 21* (eds. Hecht, M.K., Wallace, B. & Prance, G.T.). Springer US, Boston, MA, pp. 127–178

Sultan, S.E. (2001). Phenotypic plasticity for fitness components in *Polygonum* species of contrasting ecological breadth. *Ecology*, 82, 328–343

Sultan, S.E. & Spencer, H.G. (2002). Metapopulation structure favors plasticity over local adaptation. *Am Nat*, 160, 271–283

Textor, S. & Gershenzon, J. (2009). Herbivore induction of the glucosinolate–myrosinase defense system: major trends, biochemical bases and ecological significance. *Phytochem Rev*, 8, 149–170

Tierens, K.F.-J., Thomma, B.P., Brouwer, M., Schmidt, J., Kistner, K., Porzel, A., *et al*. (2001). Study of the role of antimicrobial glucosinolate-derived isothiocyanates in resistance of Arabidopsis to microbial pathogens. *Plant Physiol*, 125, 1688–1699

Via, S., Gomulkiewicz, R., De Jong, G., Scheiner, S.M., Schlichting, C.D. & Van Tienderen, P.H. (1995). Adaptive phenotypic plasticity: consensus and controversy. *Trends Ecol Evol*, 10, 212–217

Via, S. & Lande, R. (1985). Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution*, 39, 505–522

Wagner, M.R., Lundberg, D.S., del Rio, T.G., Tringe, S.G., Dangl, J.L. & Mitchell-Olds, T. (2016). Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. *Nat Comm*, 7, 12151

Weis, A. & Gorman, W. (1990). Measuring selection on reaction norms: An exploration of the Eurosta-Solidago system. *Evolution*, 44, 820–831

Wickham, H. (2009). *ggplot2: Elegant Graphics for Data Analysis*. Springer, New York

Wickham, H. (2016). *tidyr: Easily Tidy Data with `spread()` and `gather()` Functions*. R.

Wickham, H. & Francois, R. (2015). *dplyr: A Grammar of Data Manipulation*

Wimp, G.M., Wooley, S., Bangert, R.K., Young, W.P., Martinsen, G.D., Keim, P., *et al*. (2007). Plant genetics predicts intra-annual variation in phytochemistry and arthropod community structure. *Molec Ecol*, 16, 5057–5069

Windsor, A.J., Reichelt, M., Figuth, A., Svatoš, A., Kroymann, J., Kliebenstein, D.J., *et al*. (2005). Geographic and evolutionary diversification of glucosinolates among near relatives of *Arabidopsis thaliana* (Brassicaceae). *Phytochemistry*, 66, 1321–1333