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Relaxation of herbivore-mediated selection drives the evolution of genetic covariances between plant competitive and defense traits

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Insect herbivores are important mediators of selection on traits that impact plant defense against herbivory and competitive ability. Although recent experiments demonstrate a central role for herbivory in driving rapid evolution of defense and competition-mediating traits, whether and how herbivory shapes heritable variation in these traits remains poorly understood. Here, we evaluate the structure and evolutionary stability of the G matrix for plant metabolites that are involved in defense and allelopathy in the tall goldenrod, *Solidago altissima*. We show that G has evolutionarily diverged between experimentally replicated populations that evolved in the presence versus the absence of ambient herbivory, providing direct evidence for the evolution of G by natural selection. Specifically, evolution in an herbivore-free habitat altered the orientation of G, revealing a negative genetic covariation between defense- and competition-related metabolites that is typically masked in herbivore-exposed populations. Our results may be explained by predictions of classical quantitative genetic theory, as well as the theory of acquisition-allocation trade-offs. The study provides compelling evidence that herbivory drives the evolution of plant genetic architecture.

KEY WORDS: **Allelopathy, acquisition-allocation theory, G-matrix, trade-off, plant-herbivore interaction, plant secondary metabolites.**

Herbivory-mediated selection is central to the evolution of plant traits that impact growth, competitive ability, and defense (Rausher and Simms 1989; Mauricio and Rausher 1997; Strauss et al. 2002; Carmona et al. 2011; Agrawal et al. 2012). Competing demands for resources invariably lead to trade-offs in investment between different traits, such as defense against herbivores versus competitive ability against other plant species (Coley et al. 1985; Herms and Mattson 1992). Nevertheless, empirical signals of such trade-offs (i.e., negative genetic covariance) are rare, and may often be obscured by other forms of variation (Van Noordwijk and de Jong 1986). The optimal balance of investment in defense versus competition should ultimately depend on ecological conditions, including the local density and diversity of herbivores and competitor plant communities, which are likely to vary over time and across species' ranges. Such environmental variability can potentially drive the rapid evolution of plant traits on ecological time-scales (Agrawal et al. 2012; Bode and Kessler 2012; Züst et al. 2012; Uesugi and Kessler 2013), and contribute to divergence in key traits that differentiate native from invasive plant populations (Blossey and Notzold 1995).

A population's capacity to respond to natural selection (i.e., its "evolvability") ultimately depends on patterns of genetic variance and covariance among selected traits (as summarized by the **G** matrix). A rich literature on plant-herbivore coevolution documents significant levels of genetic variance and covariance for plant defense traits, including resistance and tolerance against

herbivory (Simms and Rausher 1987; Fineblum and Rausher 1995; Juenger and Bergelson 2000; Pilson 2000; Weinig et al. 2003; Franks 2008), and morphological and chemical defenses (Mauricio and Rausher 1997; Shonle and Bergelson 2000; Andrew et al. 2007; Johnson et al. 2009). Moreover, by measuring natural selection on these traits in environments with and without herbivory, several studies demonstrate that insect herbivores can act as agents of selection on plant resistance traits (Simms and Rausher 1987; Mauricio and Rausher 1997; Shonle and Bergelson 2000; Johnson et al. 2009). Herbivores are therefore likely to drive the evolution of plant defenses and other correlated traits.

Many studies have estimated the **G** matrix for a set of traits within single populations, and such studies facilitate predictions about the short-term evolutionary responsiveness of these populations to natural selection. However, single estimates of **G** may reveal little about each population's capacity for evolutionary change across many generations, as the predictability of long-term evolutionary trends depends on the stability of **G** (Lande 1979; Schluter 1996). Changes in allele frequencies and associations between alleles (i.e., linkage disequilibrium) can ultimately reshape quantitative genetic variation, and alter the multivariate structure of **G** (Phillips and McGuigan 2006; Wood and Brodie 2015). For example, selection may alter the shape and orientation of **G** by eroding genetic variance along phenotypic dimensions that experience strong stabilizing selection (Bulmer 1980; Turelli 1988; Shaw et al. 1995; Blows and Walsh 2009). Genetic drift may systematically reduce the overall size of **G** by stochastically altering its individual elements (Roff 2000; Jones et al. 2003). The evolutionary stability of **G** under directional selection may also be context-dependent (Jones et al. 2004; Arnold et al. 2008), with some forms of selection expected to stabilize **G** over time (e.g., when the peak of an adaptive landscape moves in parallel to $\boldsymbol{g}_{\max}$, the dimension of **G** for which there is maximum genetic variance) and others expected to destabilize it (e.g., when peak movement is orthogonal to $\boldsymbol{g}_{\max}$; Jones et al. 2004).

Variation in herbivory may destabilize **G** by altering the sign of genetic covariance between defense and competitive traits. Theoretically, a shift in the sign of correlational selection can reshape genetic covariance from positive to negative (Arnold et al. 2008). In the presence of herbivory, plants must both defend and compete, leading to positive correlational selection in favor of both traits. Relaxation of herbivory may generate negative correlational selection—selecting against energetically costly defense traits (Herms and Mattson 1992) and favoring increased competitive ability. Life-history theory provides a complementary perspective on the evolution of genetic covariances between defense and competitive traits (Arnold 1992). These traits may negatively covary because they directly trade off against one another—investment of limited resources into one trait necessarily reduces the allocation of resources to others (Coley et al. 1985; Herms and Mattson 1992; Campos et al. 2016). Nevertheless, negative genetic covariation between competing functions may often be obscured by genetic variation in resource acquisition (Van Noordwijk and de Jong 1986; Agrawal 2011; Metcalf 2016). Theory suggests that empirical signals of allocation trade-offs should therefore depend on population-specific patterns of variation in acquisition and allocation (van Noordwijk and de Jong 1986). As we demonstrate in the discussion, relaxation of herbivory and selection for decreased allocation to defense, can expose a negative genetic covariance between defense and competitive ability that might otherwise be masked in populations where herbivores are abundant.

Despite strong theoretical motivation for assessing the evolutionary stability of **G**, there are currently few clear-cut empirical examples of its evolutionary response to natural selection (Arnold et al. 2008). Several studies have documented parallel divergence of **G** in populations exposed to similar habitats (Cano et al. 2004; Calsbeek et al. 2011; Eroukhmanoff and Svensson 2011; Franks et al. 2012; Wood and Brodie 2015), which suggests that environment-dependent selection drives predictable divergence in **G**. For example, Franks et al. (2012) found that **G** matrices for plant defensive metabolites differed between native and invasive populations of *Melaleuca quinquenervia*, suggesting that **G** has evolved in herbivore-free environments. However, these studies typically lack key information about the evolutionary histories of their focal populations, limiting their ability to identify the selective agents responsible for divergence (McGuigan et al. 2005; Doroszuk et al. 2008). Controlled selection experiments can directly test for effects of selection on **G** matrix evolution, but such studies are rare, and mostly limited to the experimental evolution of lab-adapted model species (Shaw et al. 1995; Blows and Higgie 2003; Hine et al. 2011; Careau et al. 2015). These studies provide valuable insights into the evolution of **G**, yet their simplified laboratory settings exclude biotic and abiotic factors that are important selective agents within natural contexts. To our knowledge, only one study to date has evaluated the evolution of **G** in response to selection within the field (Doroszuk et al. 2008), and none have examined the effect of herbivory on the evolution of **G** in plants. Additional, field-based evolution experiments are therefore needed to evaluate the role of selection in **G** matrix evolution within ecologically relevant contexts.

Here, we use a recently developed Bayesian analysis approach (Hine et al. 2009; Aguirre et al. 2014) to evaluate the evolutionary stability of the **G** matrix for plant chemical traits that mediate herbivore defense and interspecific competition via allelopathy in the tall goldenrod (*Solidago altissima*; Asteraceae). We previously showed—in a 12-year field manipulation of replicated experimental *S. altissima* populations—that populations where insect herbivores were excluded (hereafter, "no-herbivore" populations) evolved increased allelopathic compound production in their roots (Kobayashi et al. 1980; Johnson et al. 2010; Uesugi

and Kessler 2013), and decreased production of defense-related compounds in their roots and leaves (Uesugi and Kessler 2016). No-herbivore populations also evolved increased competitive ability against heterospecific competitors, relative to control populations that evolved under herbivore-exposed conditions ("herbivore" populations; Uesugi and Kessler 2013). Parallel patterns of metabolite divergence have also occurred between native and invasive populations of *S. altissima*, with invasive populations that have escaped from their ancestral herbivores for ~100 years evolving high-competition/low-defense metabolite profiles (Uesugi and Kessler 2016). This set of observations implies that competition and defense may covary genetically in goldenrod, with greater allocation of resources to one function diverting resources away from the other.

We estimated **G** matrices for secondary metabolites involved in defense and competition in replicated *S. altissima* populations that each experimentally evolved in herbivore or no-herbivore habitats. We show that herbivore-mediated natural selection has led to divergence in **G** between the populations, and that selection has reshaped genetic covariances between metabolites that function in defense and competition. Specifically, defense and competition-mediating traits evolved to positively covary in the presence of herbivores, and to negatively covary in their absence. Our results provide new evidence for the important role of herbivory as an evolutionary driver of complex plant phenotypes.

Material and Methods

STUDY SYSTEM

The tall goldenrod, *S. altissima* L. (Asteraceae), is a dominant perennial forb of old-field plant communities native to eastern North America (Werner et al. 1980). The species can be diploid, tetraploid, or hexaplod across its range, but only hexaploid individuals have been reported from the north east where this study took place (Halverson et al. 2008). In this region, *S. altissima* is attacked by a diverse group of herbivores, many of which are specialists on *Solidago* species (Root and Cappuccino 1992).

Solidago altissima has annual cycles of sexual reproduction by outcrossed seeds and asexual reproduction via the die-off and new growth of aboveground biomass from previous year's rhizomes. Old fields are initially colonised by seeds, but subsequent population growth depends largely on asexual reproduction (Cain 1990; Meyer and Schmid 1999). Thus, the changes that we characterize in experimentally evolved populations are likely due largely to genotype sorting.

Between 1995 and 2008, a selection experiment was conducted in an old field dominated by *S. altissima* at Whipple Farm, Tompkins Co., NY (for details, see Uesugi and Kessler 2013). Twelve plots (each 5×5 m^2) were established within the field in a 4×3 configuration, each separated by a 1-m buffer zone. Plots were spread evenly across the field and assigned randomly to "herbivore" and "no-herbivore" habitats, ensuring that adjacent plots were not clustered in the same treatment, and minimizing initial differences in plant genotype composition between habitats. Insect herbivores were removed from no-herbivore plots by biannual spraying with the insecticide fenvalerate (ORTHO Group, Marysville, OH). Herbivore plots were exposed to ambient levels of herbivory. Hence, our field manipulation of herbivory on *S. altissima* was spatially replicated, allowing us to separate effects of herbivore-mediated selection on plant metabolite divergence from genetic drift; Conner 2016).

In 2008, we randomly sampled 16 *S. altissima* genotypes from each plot to establish a clone library at Cornell University, Ithaca, NY. Genotypes were propagated in a common greenhouse environment from rhizome cuttings, with three cycles of annual growth—prior to trait measurement—to minimize shared environmental ("maternal") effects. An analysis of 16 microsatellite loci (following methods described in Sakata et al. 2013) in randomly selected individuals from this library detected similar numbers of genotypes in the herbivore and no-herbivore populations (29 of 30 individuals sampled from the herbivore populations were genetically unique, as were all 26 individuals sampled from the no-herbivore populations; Shiojiri and Uesugi, unpubl. data). Thus, any differences in the quantitative genetic variation of plant metabolites observed between herbivore and no-herbivore populations are unlikely to reflect differences in genotypic richness between them.

EXPERIMENTAL DESIGN AND DATA COLLECTION

In 2010, we clonally propagated 29 genotypes from the no-herbivore populations (3–8 per plot) and 30 genotypes from the herbivore populations (2–7 per plot). We then planted six clonal replicates per genotype into pots, assigning one replicate per genotype to each of two contemporary herbivory environments (with and without damage from *Trirhabda virgata,* a major specialist beetle in the study area; Root and Cappuccino 1992) and three competitive environments (no competition, intraspecific competition, and interspecific competition from *Poa Pratensis*, a common grass competitor; Carson and Root 2000) in a factorial design. We randomly arranged all plants in a common environment (a rooftop growing space at Cornell University) in early spring, and collected leaf and root tissues for analyses of plant secondary metabolites in early summer.

We measured leaf and root secondary metabolites that are associated with anti-herbivore defense (phenolics, flavonoids, and diterpene acids; Hull-Sanders et al. 2007; Uesugi et al. 2013) and competitive ability via allelopathy (polyacetylenes; Kobayashi et al. 1980; Johnson et al. 2010; Uesugi and Kessler 2013), using HPLC and following methods described in Uesugi and Kessler (2016). Metabolite concentrations were calculated for five focal

metabolite classes: leaf phenolics ("*Lph*") and flavonoids ("*Lfl*"), and root phenolics ("*Rph*"), polyacetylenes ("*Rpa*"), and diterpene acids ("*Rdt*").

ESTIMATION OF G MATRICES FOR PLANT SECONDARY METABOLITES

To estimate genetic variance/covariance (**G**) matrices for plant metabolite traits in herbivore and no-herbivore populations, and to explore the divergence of **G** matrices in response to 12 years of herbivore removal, we used a two-step approach combining restricted maximum likelihood and Bayesian analyses. Because our analyses are based on clonal replicates, they estimate broad-sense genetic parameters that include additive and nonadditive genetic effects plus shared environmental effects (Lynch and Walsh 1998). We minimized environmental effects by propagating each genotype for three cycles in a common environment prior to metabolite measurement. Nevertheless, we emphasise that our **G** matrices estimate the upper limits of genetic effects. Data for each of the five focal metabolite classes were standardized to a mean of 0 and variance of 1 to place them on similar scale for analysis.

First, we fitted a multivariate linear mixed model to the five metabolite traits using *ASReml-R* (Butler et al. 2009), and used likelihood-based model reduction to identify the simplest model that best fit the data. Specifically, the final model (fitted by population) in matrix form was

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{ZG} + \varepsilon$$

where **y** was the vector of observations on all clonal replicates. **X** was the design matrix relating clonal replicates to **β**, a vector representing the fixed effects of trait (a dummy variable ensuring that all parameters were estimated separately by metabolite), contemporary competition and herbivory treatments, and their interactions. **Z** was the design matrix relating the random effects of genotype (**G**) to clonal replicates (Lynch and Walsh 1998). **G** itself was an unstructured covariance matrix containing the variances of (and covariances between) the traits, partitioned from the equivalent residual matrix (**ε**). Elements of **G** were tested for significance using standard likelihood-ratio tests based on a χ^2 distribution. Fixed effects and their interactions were tested using standard Wald tests (reported in Table S1).

We also tested whether metabolites varied among plots within each habitat (the level at which herbivory manipulation was replicated in the initial selection experiment) by including them as another random effect in our model. In both habitats, plot-level variances were negligible (precluding covariance estimation) and contributed little to model fit ($\chi^2 = 6.00$, *d.f.* $= 10$, $P = 0.81$). Hence, phenotypically at least, the metabolites of plants from different plots responded similarly to the herbivory manipulation (we had too few genotypes per plot to formally quantify divergence in genetic (co)variance among plots—but within each selection treatment—that occurred through genetic drift). We therefore removed plot terms from our final model to limit overfitting. Doing so did not affect subsequent tests.

Once the appropriate model was identified, we refitted it in a Bayesian framework using the *MCMCglmm* package of *R* (R Core Team 2014) to sample the marginal posterior distribution of each **G** matrix (Hadfield 2010). We used weakly informative inverse-Wishart priors with the scale parameter defined as a diagonal matrix containing values of one-half of the phenotypic variance, and distribution parameters set to 0.001 for the degrees of freedom (Hadfield 2010; note that using parameter-expanded priors gave similar results). Posterior distributions were estimated from 10,100,000 MCMC iterations sampled every 1000 iterations following an initial burn-in of 100,000 iterations. To check model convergence, we inspected plots of traces and posterior distributions, and calculated autocorrelations between samples (all were below the recommended level of 0.1, yielding an effective sample size close to 10,000 for each parameter). Note that likelihood and Bayesian analyses yielded similar **G** matrices (Table 1 and Table S2, respectively).

ESTIMATION G MATRIX DIVERGENCE USING A GENETIC COVARIANCE TENSOR

To test whether **G** matrices diverged in response to herbivore removal, we used a fourth-order genetic covariance tensor to identify the combinations of metabolites that differ most between matrices. Tensors can be used to compare any number of **G** matrices, as explained by Hine et al. (2009) and Aguirre et al. (2014; see Sztepanacz and Rundle 2012; Careau et al. 2015 for its applications). For our two **G** matrices, the comparison simply involves an eigenanalysis of their pairwise differences (the matrix **E**: Sztepanacz and Rundle 2012). Hence, the eigenvectors ($\boldsymbol{e_1}$, $\boldsymbol{e_2}$, etc.) of **E** describe the combinations of metabolites in which our matrices differ the most; their corresponding eigenvalues describe the amount of variation in each combination. We applied the tensor analysis to the 10,000 MCMC samples obtained above using the *R* routine provided by Aguirre et al. (2014), which compared observed divergence of our **G** matrices to a null model that assumed divergence was driven by random sampling of variation. The null **G** matrices for each of the 10,000 MCMC samples were constructed by first estimating the genetic value of each individual based on genotype structure of each population, and then randomly assigning individuals to herbivore and no-herbivore populations. Thus, excess divergence in observed **G** matrices relative to randomized **G** indicates a significant effect of the selection treatment on **G** matrix differentiation. Finally, we projected the leading eigenvectors of **E** onto observed **G**

Table 1. Genetic variance-covariance (G) matrices for plant secondary metabolites estimated in (a) the herbivore habitat and (2) the no-herbivore habitat.

(a) Herbivore habitat					
	Lph	*Lfl*	*Rph*	*Rpa*	*Rdt*
Lph	**0.26 ± 0.10**				
Lfl	**0.17 ± 0.10**	**0.43 ± 0.14**			
Rph	**0.20 ± 0.09**	0.10 ± 0.09	**0.30 ± 0.11**		
Rpa	0.16 ± 0.11	0.05 ± 0.12	0.16 ± 0.11	**0.68 ± 0.20**	
Rdt	0.15 ± 0.09	**0.23 ± 0.11**	0.12 ± 0.10	0.19 ± 0.13	**0.50 ± 0.16**
(b) No-herbivore habitat					
	Lph	*Lfl*	*Rph*	*Rpa*	*Rdt*
Lph	**0.16 ± 0.08**				
Lfl	0.04 ± 0.09	**0.58 ± 0.17**			
Rph	0.03 ± 0.06	−0.07 ± 0.09	**0.19 ± 0.09**		
Rpa	**−0.18 ± 0.09**	0.17 ± 0.13	0.02 ± 0.09	**0.66 ± 0.19**	
Rdt	**0.20 ± 0.09**	−0.11 ± 0.12	0.13 ± 0.09	**−0.31 ± 0.14**	**0.54 ± 0.16**

Values are REML-estimated variance components (± SE), with bold indicating $P < 0.05$.

matrices to estimate the relative amounts of genetic variance in the directions of most divergence.

To further characterize evolution in **G**, we explored the overall geometry of each matrix by calculating its total size (its trace, or sum of its diagonal elements), and using an eigenanalysis to identify its leading dimension of variance ($\boldsymbol{g}_{\max}$), and its effective number of dimensions (the sum of the eigenvalues each divided by the largest eigenvalue). This number will be 1 if all genetic variance lies in a single dimension, or five if all metabolite classes have similar variance and do not covary genetically (Kirkpatrick 2009). We compared metrics between **G** matrices based on posterior means and the overlap of 95% HPD intervals calculated from the 10,000 MCMC samples.

Results

All five metabolite traits varied significantly among *S. altissima* genotypes from herbivore and no-herbivore populations (Table 1). Significant genetic covariances were detected for some pairs of traits in both populations, but differed in strength and sign. The herbivore population (Table 1A) exhibited positive covariances between leaf flavonoids and phenolics (*Lfl* and *Lph*), between leaf and root phenolics (*Lph* and *Rph*), and between leaf flavonoids and root diterpenes (*Lfl* and *Rdt*). In contrast, the no-herbivore population (Table 1B) exhibited negative covariances between root polyacetylenes (*Rpa*) and root diterpenes (*Rdt*), and between *Rpa* and leaf phenolics (*Lph*), but positive covariance between *Rdt* and *Lph*.

The tensor analysis revealed that the divergence in **G** between herbivore and no-herbivore populations is greater than expected by chance alone. Despite considerable uncertainty in our observed **E** (approximated by pairwise differences between matrices in Table 1), its 95% HPD interval did not overlap with **E** from randomized data (Fig. 1A), indicating significant effect of herbivore removal on **G**. The leading eigenvector of **E** ($\boldsymbol{e}_1$), accounting for 41% of all changes in **G**, contrasts *Rpa* and *Lfl* with *Rph*, *Lph*, and *Rdt* (Fig. 1B). This suggests the evolution of negative covariance between these metabolites, in line with the contrasting patterns seen for pairwise covariances in Table 1. The second eigenvector of **E** ($\boldsymbol{e}_2$), accounting for 37% of all changes in **G**, describes positive associations among all metabolites (Fig. 1B). Projection of these eigenvectors onto our **G** matrices revealed that genetic variance in $\boldsymbol{e}_1$ has increased significantly in response to herbivore removal, whereas genetic variance in $\boldsymbol{e}_2$ has decreased in response to herbivore removal but not significantly so (Fig. 1C).

Herbivore and no-herbivore populations did not differ in their total amount of genetic variance (i.e., the trace of **G**), which was 2.59 (95% HPD: 1.59–3.75) for the former and 2.47 (95% HPD: 1.49–3.54) for the latter. They did not differ in the proportion of genetic variance in the direction of $\mathbf{g}_{\max}$, which was 0.60 (95% HPD: 0.30–0.96) for the herbivore population and 0.57 (95% HPD: 0.30–0.95) for the no-herbivore population. Nor did they differ in the effective number of dimensions in **G**, which was 1.81 (95% HPD: 1.35–2.34) for the herbivore population and 1.72 (95% HPD: 1.28–2.19) for the no-herbivore one. Overall, these tests imply that **G** matrices have not diverged in size or dimensionality: both matrices describe similar amounts of genetic variance in metabolite traits, and a similar proportion of variance (roughly half) is explained by the first

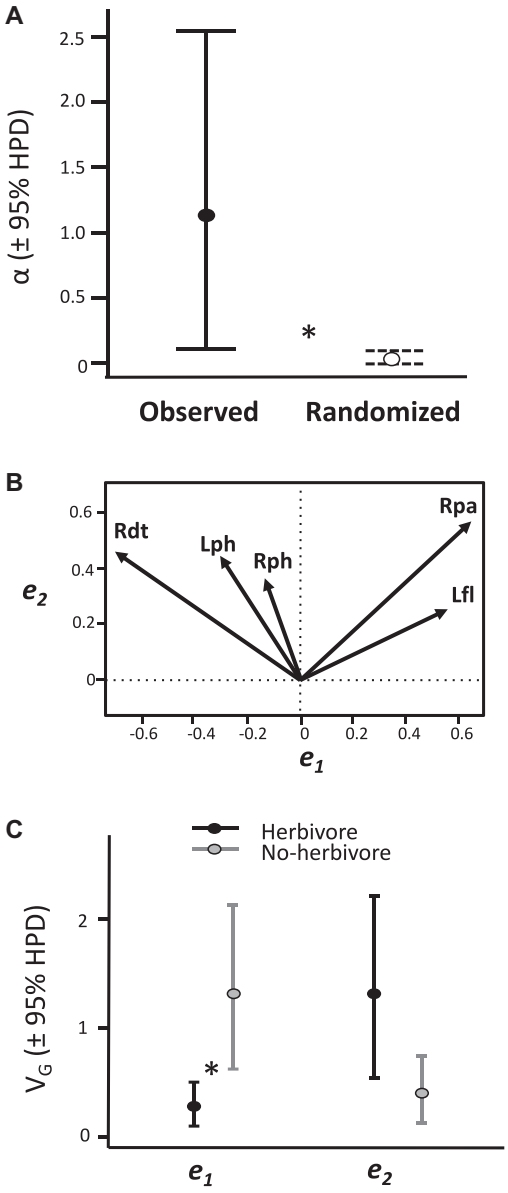

Figure 1. Results of the genetic covariance tensor analysis, which compares the G matrix of populations from herbivore and no-herbivore habitats. (A) The observed level of divergence between G matrices of herbivore and no-herbivore populations (variance captured by the eigentensor, α) is contrasted against the randomized level expected by chance alone. The 95% HPD intervals of observed and randomized estimates do not overlap, indicating significant divergence between G matrices. (B) Combinations of plant secondary metabolites described by the leading eigenvectors (e_1 and e_2) of the eigentensor E. The first axis (e_1), explaining 41% of variance in E, describes negative covariation among focal traits and suggests that G has diverged in the level of trade-offs among compound classes. The second axis (e_2), explaining 37% of variance in E, describes positive associations among metabolite traits. (C) Relative magnitudes of genetic variance (V_G) expressed by herbivore (black) and no-herbivore populations (gray) in the direction of e_1 and e_2. Asterisks indicate no overlap between 95% HPD intervals.

eigenvector in both matrices. Hence, the matrices have diverged primarily in orientation, through changes in their off-diagonal elements.

Discussion

The long-term evolutionary trajectories of populations exposed to novel environments critically depend upon the evolutionary stability—or lack thereof—of the **G** matrix among traits that affect fitness. Although replicated experimental evolution studies can isolate the effects of selection on the evolution of the **G** matrix, and control for potentially confounding effects of mutation and genetic drift, such experiments are rarely carried out in natural habitats (Doroszuk et al. 2008). By applying a cutting-edge analytical approach to an ecologically relevant field manipulative experiment on *S. altissima*, we demonstrated that 12 years of herbivore removal resulted in evolutionary divergence of the **G** matrix for secondary metabolites that mediate plant defense and competitive ability. Divergence in **G** exceeds expectations based solely on random sampling, and the excess is attributable to herbivore-mediated selection.

The major axis of divergence in **G** between herbivore and no-herbivore populations (eigenvector e_1) corresponds to a shift in covariance between allelopathic compounds that mediate interspecific competition and metabolites that associate with plant defense against herbivory. The most pronounced shift in covariance structure involved *Rpa*, which includes a known allelopathic polyacetylene, dehydromatricaria ester (Kobayashi et al. 1980; Johnson et al. 2010; Uesugi and Kessler 2013). In the no-herbivore habitat, *Rpa* exhibited a significant negative covariance with both *Rdt* (diterpene acids) and *Lph* (leaf phenolics); the latter two compound classes influence herbivore defense (Hull-Sanders et al. 2007; Uesugi et al. 2013). In both cases, the covariance shifted from (marginally) positive in herbivore-exposed populations to negative in no-herbivore populations (see Table 1). This pattern suggests that evolutionary divergence between herbivore and no-herbivore populations—owing to the removal of herbivory within the latter—exposed a negative genetic covariance between secondary metabolites that mediate plant competitive ability and anti-herbivore defense.

Our results are consistent with previous work that demonstrated significant heritable variation for plant defense and competitive traits (Mauricio and Rausher 1997; Shonle and Bergelson 2000; Andrew et al. 2007; Johnson et al. 2009). Yet despite theoretical expectations that defense and competitive ability should trade off (Coley et al. 1985; Herms and Mattson 1992; Campos et al 2016), negative genetic covariation between these traits is rarely found (Mitchell-Olds 1986; Simms and Rausher 1987; Rausher and Simms 1989; Mauricio and Rausher 1997; Weinig et al. 2003; Andrew et al. 2007; Johnson et al. 2009;

Agrawal 2011). Demonstrations of negative covariation between defense and competitive traits are, to our knowledge, limited to our study and that of Franks (2008), who found that stem elongation rate (a proxy for competitive ability) and herbivore resistance negatively covaried genetically in native and invasive populations of *Melaleuca quinquenervia*.

Two scenarios of selection may contribute to the divergent patterns of genetic covariance between competition and defense traits in herbivore versus no-herbivore populations (i.e., positive and negative genetic covariances, respectively). First, the change in sign of the genetic covariance should evolve if correlational selection is positive in the presence of herbivores and negative under no-herbivore conditions. The change in correlational selection shifts the axis of phenotypic variation where stabilizing selection is weak, leading to orthogonal axes of high genetic variance (Arnold et al. 2008).

Second, models of resource allocation trade-offs can account for the evolution of a negative genetic covariance following herbivore removal. Consider an example based on van Noordwijk and de Jong's (1986) influential model of resource acquisition and allocation between a pair of traits. In a population where individuals genetically vary for resource acquisition ability, and resource allocation to competition versus defense, the genetic covariance between competition (T_1) and defense (T_2) will be:

$$\mathrm{cov}(T_1,T_2) = \bar{A}^2\bar{B}\,(1-\bar{B})\left[\frac{\sigma_A^2}{\bar{A}^2} - \frac{\sigma_A^2}{\bar{A}^2}\,\frac{\sigma_B^2}{\bar{B}(1-\bar{B})} - \frac{\sigma_B^2}{\bar{B}(1-\bar{B})}\right], \quad (1)$$

(see van Noordwijk and de Jong 1986), where $\bar{A}$ and ${\sigma_A}^2$ represent the population mean and genetic variance for acquisition ability (respect*i*vely), $\bar{B}$ is the mean allocation to competition (the remainder is allocated to defense), and ${\sigma_B}^2$ is the genetic variance for allocation. The traits positively covary when $\sigma_A^2/\bar{A}^2 >> \sigma_B^2/\bar{B}(1-\bar{B})$. This condition is likely to arise in populations evolving in the presence of herbivores, where selection favors equal allocation to competition and defense, and there is strong stabilizing selection on optimal allocation—conditions that minimize $\sigma_B^2/\bar{B}(1-\bar{B})$ (Fig. 2, panels A and B). In herbivore-free environments, directional selection should favor increased allocation to competition, leading to two consequences: (1) an upward shift in $\bar{B}$ (and consequent decrease in $\bar{B}(1-\bar{B})$); and (2) a transient inflation of σ_B^2 as alleles that increase B reach intermediate frequencies on their way to fixation. Both factors should (at least transiently) inflate $\sigma_B^2/\bar{B}(1-\bar{B})$, producing a negative covariance between competition and defense (Fig. 2, panels A and C). Thus, selection for increased resource allocation to competition (allelopathy) in herbivore-free environments can alter the **G** matrix for secondary metabolite compounds and reveal a

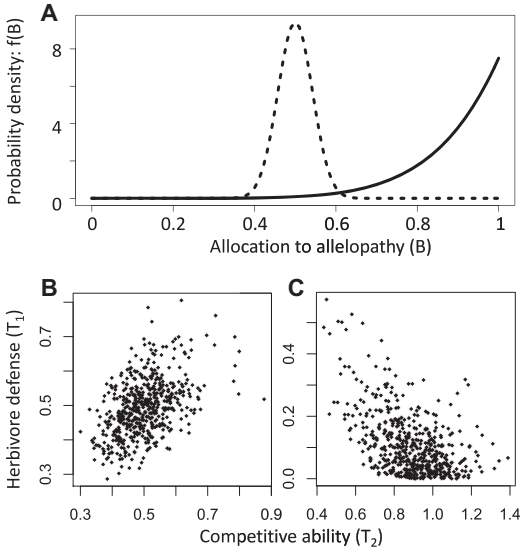

Figure 2. Evolutionary divergence in allocation can potentially restructure the genetic correlation between competition and defense traits. Panels A–C illustrate the conceptual model described in the Discussion (see eq. (1) and surrounding text). The two traits, T_1 and T_2, are expressed as a function of an individual's resource acquisition level (denoted *A*) and the proportion of resources allocated to competition versus defense (denoted *B*). Trait expression follows the functions: $T_1 = AB$ and $T_2 = A(1-B)$. As in van Noordwijk and de Jong (1986), we assume that *A* and *B* are genetically independent; cov(*A*, *B*) = 0. In the scenario illustrated here, the distribution of B diverges between herbivory and no-herbivory populations; the distribution of *A* is assumed to be the same in both populations (i.e., *A* is a gamma distributed random variable with shape and scale parameters $k = 50$ and $\theta = 0.02$, where $\bar{A} = k\theta$ and $\sigma_A^2 = k\theta^2$). Scenarios in which *A* also evolves are plausible, and will modify the change in *B* that is required to reveal a negative covariance between the traits. (A) In the presence of herbivores, selection favors allocation to both competition and defense. Individuals from herbivore populations allocate a relatively equal proportion of resources to competition and defense, so that *B* follows a symmetrical distribution with mean of $\bar{B} = 0.5$ (dotted curve; $B \sim$ beta($\alpha = 70$, $\beta = 70$)). In a novel environment without herbivores, selection favors increased investment in competition. Removal of herbivory relaxes selection for defense traits and drives the evolution of increased allocation to competition (solid curve; $B \sim$ beta($\alpha = 7.5$, $\beta = 1$) is a hypothetical state of the population after generations of experimental evolution). Panel (B) shows simulated trait values for 500 individuals from an herbivore population (using the *B* distribution outlined in dotted curve, from panel (A), where there is a positive genetic correlation between competition and defense traits (correlation coefficient of $r = 0.45$). Panel (C) shows simulated trait values for 500 individuals from a no-herbivore population (using the *B* distribution outlined in solid curve, from panel (A), where there is now a negative genetic correlation between competition and defense traits ($r = -0.51$). As panels B and C show, the evolution of allocation causes a transition from positive covariance between competition and defense, to negative covariance.

previously hidden trade-off between competitive ability and herbivore defense.

The altered pattern of genetic covariance between herbivore and no-herbivore populations, unaccompanied by changes in the trace or dimensionality of **G**, further argues that selection under herbivore removal has shifted the orientation of **G** while preserving other aspects of its geometry. Other studies of divergence in **G** under selection present conflicting results, with some reporting shifts in matrix orientation (e.g., Donohue et al. 2000; Cano et al. 2004; Doroszuk et al. 2008) and others reporting proportional changes (Phillips et al. 2001; Blows and Higgie 2003; Eroukhmanoff and Svensson 2011; Careau et al. 2015). This disparity in results indicates that the effect of selection on **G** matrices is context-dependent, and may depend on the alignment of directional selection relative to the orientation of **G** (Jones et al. 2004; Arnold et al. 2008). For example, **G** should be evolutionarily stable when the optimum of an adaptive landscape moves in parallel with the genetic line of least resistance (i.e., $\boldsymbol{g}_{\max}$), whereas **G** is destabilized when the optimum shifts orthogonally to $g_{\max}$ (Jones et al. 2004). Our results are consistent with the latter scenario, although the ancestral $g_{\max}$ for this system is unknown.

In conclusion, we found that herbivore removal induced evolution of the genetic covariances among secondary metabolites of *S. altissima*, including emergence of a negative covariance between competition- and defense-related compounds. Genetic variance in the trait combination that summarizes these changes (e_1) also increased in response to relaxed herbivory, suggesting that populations released from herbivory may continue to evolve greater competitive ability at the expense of lower herbivore defense, and they may do so rapidly. This effect of **G**-matrix evolution may facilitate rapid evolution of increased allelopathy in invasive ranges of *S. altissima* (Blossey and Notzold 1995; Uesugi and Kessler 2016). Future studies will examine whether escape from herbivory in invasive *S. altissima* populations has resulted in patterns of **G** evolution in parallel to those observed in this field experimental study.

AUTHOR CONTRIBUTIONS

A.U. and A.K. collected data, A.U. and K.M. designed and analyzed the data; A.U., T.C., and K.M. contributed to data analysis and interpretation; A.U., T.C., A.K., and K.M. wrote the article.

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DATA ARCHIVING

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LITERATURE CITED

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

Agrawal, A. A., A. P. Hastings, M. T. J. Johnson, J. L. Maron, and J.-P. Salminen. 2012. Insect herbivores drive real-time ecological and evolutionary change in plant populations. Science 338:113–116.

Aguirre, J. D., E. Hine, K. McGuigan, and M. W. Blows. 2014. Comparing G: multivariate analysis of genetic variation in multiple populations. Heredity 112:21–29.

Andrew, R. L., I. R. Wallis, C. E. Harwood, M. Henson, and W. J. Foley. 2007. Heritable variation in the foliar secondary metabolite sideroxylonal in Eucalyptus confers cross-resistance to herbivores. Oecologia 153:891–901.

Arnold, S. J. 1992. Constraints on phenotypic evolution. Am. Nat. 140:S85–S107.

Arnold, S. J., R. Burger, P. A. Hohenlohe, B. C. Ajie, and A. G. Jones. 2008. Understanding the evolution and stability of the G-matrix. Evolution 62:2451–2461.

Blossey, B., and R. Notzold. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. J. Ecol. 83:887–889.

Blows, M., and B. Walsh. 2009. Spherical cows grazing in flatland: constraints to selection and adaptation. Pp. 83–101 *in* Adaptation and fitness in animal populations. van der Werf, J. H., Graser, H., Frankham, R., and Gondro, C. (eds). Springer, Netherlands.

Blows, M. W., and M. Higgie. 2003. Genetic constraints on the evolution of mate recognition under natural selection. Am. Nat. 161:240–253.

Bode, R. F., and A. Kessler. 2012. Herbivore pressure on goldenrod (*Solidago altissima* L., Asteraceae): its effects on herbivore resistance and vegetative reproduction. J. Ecol. 100:795–801.

Bulmer, M. G. 1980. The mathematical theory of quantitative genetics. Clarendon Press, UK.

Butler, D., B. Cullis, A. Gilmour, and B. Gogel. 2009. ASReml-R reference manual. The State of Queensland, Department of Primary Industries and Fisheries, Brisbane.

Cain, M. L. 1990. Patterns of *Solidago altissima* ramet growth and mortality—the role of below-ground ramet connections. Oecologia 82:201–209.

Calsbeek, B., S. Lavergne, M. Patel, and J. Molofsky. 2011. Comparing the genetic architecture and potential response to selection of invasive and native populations of reed canary grass. Evol. Appl. 4: 726–735.

Campos, M. L., Y. Yoshida, I. T. Major, D. de Oliviera Ferreira, S. M. Weraduwage, J. E. Froehlich, B. F. Johnson, D. M. Kramer, G. Jander, T. D. Sharkey, et al. 2016. Rewiring of jasmonate and phytochrome B signalling uncouples plant growth-defense tradeoffs. Nat. Comm. 7: 12570

Cano, J. M., A. Laurila, J. Palo, and J. Merilä. 2004. Population differentiation in G matrix structure due to natural selection in *Rana temporaria*. Evolution 58:2013–2020.

Careau, V., M. E. Wolak, P. A. Carter, and T. Garland. 2015. Evolution of the additive genetic variance–covariance matrix under continuous directional selection on a complex behavioural phenotype. Proc. R. Soc. B 282: 20151119.

Carmona, D., M. J. Lajeunesse, and M. T. J. Johnson. 2011. Plant traits that predict resistance to herbivores. Funct. Ecol. 25:358–367.

Carson, W. P., and R. B. Root. 2000. Herbivory and plant species coexistence: community regulation by an outbreaking phytophagous insect. Ecol. Monogr. 70:73–99.

Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant antiherbivore defense. Science 230:895–899.

Donohue, K., E. H. Pyle, D. Messiqua, M. S. Heschel, and J. Schmitt. 2000. Density dependence and population differentiation of genetic architecture in *Impatiens capensis* in natural environments. Evolution 54:1969–1981.

Doroszuk, A., M. W. Wojewodzic, G. Gort, and J. E. Kammenga. 2008. Rapid divergence of genetic variance—covariance matrix within a natural population. Am. Nat. 171:291–304.

Eroukhmanoff, F., and E. I. Svensson. 2011. Evolution and stability of the G-matrix during the colonization of a novel environment. J. Evol. Biol. 24:1363–1373.

Fineblum, W. L., and M. D. Rausher. 1995. Tradeoff between resistance and tolerance to herbivore damage in a morning glory. Nature 377:517–520.

Franks, F. J. 2008. Selection on herbivory resistance and growth rate in an invasive plant. Am. Nat. 171:678–691.

Franks, S. J., G. S. Wheeler, and C. Goodnight. 2012. Genetic variation and evolution of secondary compounds in native and introduced populations of the invasive plant *Melaleuca quinquenervia*. Evolution 66:1398–1412.

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

Halverson, K., S. B. Heard, J. D. Nason, and J. O. Stireman. 2008. Origins, distribution, and local co-occurrence of polyploid cytotypes in *Solidago altissima* (Asteraceae). Am. J. Bot. 95:50–58.

Herms, D. A., and W. J. Mattson. 1992. The dilemma of plants-to grow or defend. Q Rev. Biol. 67:478–478.

Hine, E., S. F. Chenoweth, H. D. Rundle, and M. W. Blows. 2009. Characterizing the evolution of genetic variance using genetic covariance tensors. Philos. Trans. R Soc. Lond. B Biol. Sci. 364:1567–1578.

Hine, E., K. McGuigan, and M. W. Blows. 2011. Natural selection stops the evolution of male attractiveness. Proc. Natl. Acad. Sci. USA 108:3659–3664.

Hull-Sanders, H. M., R. Clare, R. H. Johnson, and G. A. Meyer. 2007. Evaluation of the evolution of increased competitive ability (EICA) hypothesis: loss of defense against generalist but not specialist herbivores. J. Chem. Ecol. 33:781–799.

Johnson, M. T. J., A. A. Agrawal, J. L. Maron, and J. P. Salminen. 2009. Heritability, covariation and natural selection on 24 traits of common evening primrose (*Oenothera biennis*) from a field experiment. J. Evol. Biol. 22:1295–1307.

Johnson, R. H., R. Halitschke, and A. Kessler. 2010. Simultaneous analysis of tissue- and genotype-specific variation in *Solidago altissima* (Asteraceae) rhizome terpenoids, and the polyacetylene dehydromatricaria ester. Chemoecology 20:255–264.

Jones, A. G., S. J. Arnold, and R. Bürger. 2003. Stability of the g-matrix in a population experiencing pleiotropic mutation, stabilizing selection, and genetic drift. Evolution 57:1747–1760.

Jones, A. G., S. J. Arnold, R. Burger, and J. Fry. 2004. Evolution and stability of the G-matrix on a landscape with a moving optimum. Evolution 58:1639–1654.

Juenger, T., and J. Bergelson. 2000. The evolution of compensation to herbivory in scarlet gilia, Ipomopsis aggregata: herbivore—imposed natural selection and the quantitative genetics of tolerance. Evolution 54:764–777.

Kirkpatrick, M. 2009. Patterns of quantitative genetic variation in multiple dimensions. Genetica 136:271–284.

Kobayashi, A., S. Morimoto, Y. Shibata, K. Yamashita, and M. Numata. 1980. C-10-polyacetylenes as allelopathic substances in dominants in early stages of secondary succession. J. Chem. Ecol. 6:119–131.

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

Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer Sunderland, MA.

Mauricio, R., and M. D. Rausher. 1997. Experimental manipulation of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. Evolution 51:1435–1444.

McGuigan, K., S. F. Chenoweth, and M. W. Blows. 2005. Phenotypic divergence along lines of genetic variance. Am. Nat. 165:32–43.

Metcalf, C. J. E. 2016. Invisible trade-offs: Van Noordwijk and de Jong and life-history evolution. Am. Nat. 187:iii–v.

Meyer, A. H., and B. Schmid. 1999. Seed dynamics and seedling establishment in the invading perennial *Solidago altissima* under different experimental treatments. J. Ecol. 87:28–41.

Mitchell-Olds, T. 1986. Quantitative genetics of survival and growth in *Impatiens capensis*. Evolution 40:107–116.

Phillips, P., and K. McGuigan. 2006. Evolution of genetic variance-covariance structure. Evolutionary genetics: concepts and case studies. Oxford Univ. Press, Oxford, England pp. 310–325.

Phillips, P. C., M. C. Whitlock, and K. Fowler. 2001. Inbreeding changes the shape of the genetic covariance matrix in *Drosophila melanogaster*. Genetics 158:1137–1145.

Pilson, D. 2000. Herbivory and natural selection on flowering phenology in wild sunflower, *Helianthus annuus*. Oecologia 122:72–82.

Rausher, M. D., and E. L. Simms. 1989. The evolution of resistance to herbivory in *Ipomoea purpurea*. I. Attempts to detect selection. Evolution 43:563–572.

Roff, D. 2000. The evolution of the G matrix: selection or drift? Heredity 84:135–142.

Root, R. B., and N. Cappuccino. 1992. Patterns in population-change and the organization of the insect community associated with goldenrod. Ecol. Monogr. 62:393–420.

Sakata, Y., S. Kaneko, A. Hayano, M. Inoue-Murayama, T. Ohgushi, and Y. Isagi. 2013. Isolation and characterization of microsatellite loci in the invasive herb *Solidago altissima* (asteraceae). Appl. Plant Sci. 1:1200313.

Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. Evolution 50:1766–1774.

Shaw, F. H., R. G. Shaw, G. S. Wilkinson, and M. Turelli. 1995. Changes in genetic variances and covariances: G whiz! Evolution 49:1260–1267.

Shonle, I., and J. Bergelson. 2000. Evolutionary ecology of the tropane alkaloids of *Datura stramonium* L. (Solanaceae). Evolution 54:778–788.

Simms, E. L., and M. D. Rausher. 1987. Costs and benefits of plant resistance to herbivory. Am. Nat. 130:570–581.

Strauss, S. Y., J. A. Rudgers, J. A. Lau, and R. E. Irwin. 2002. Direct and ecological costs of resistance to herbivory. Trends Ecol. Evol. 17:278–285.

Sztepanacz, J. L., and H. D. Rundle. 2012. Reduced genetic variance among high fitness individuals: inferring stabilizing selection on male sexual displays in *Drosophila serrata*. Evolution 66:3101–3110.

Turelli, M. 1988. Phenotypic evolution, constant covariances, and the maintenance of additive variance. Evolution 42:1342–1347.

Uesugi, A., and A. Kessler. 2013. Herbivore exclusion drives the evolution of plant competitiveness via increased allelopathy. New Phytol. 198:916–924.

———. 2016. Herbivore release drives parallel patterns of evolutionary divergence in invasive plant phenotypes. J. Ecol. 104:876–886.

Uesugi, A., E. H. Poelman, and A. Kessler. 2013. A test of genotypic variation in specificity of herbivore-induced responses in *Solidago altissima* L. (Asteraceae). Oecologia 173:1387–1396.

Uesugi, A., Connallon, T., Kessler, A., and Monro, K. 2017. Data from: Relaxation of herbivore-mediated selection drives the evolution of genetic

covariances between plant competitive and defense traits. Dryad Digital Repository. https://doi.org/10.5061/dryad.1j4f2.

Van Noordwijk, A. J., and G. de Jong. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. Am. Nat. 128:137–142.

Weinig, C., J. R. Stinchcombe, and J. Schmitt. 2003. Evolutionary genetics of resistance and tolerance to natural herbivory in *Arabidopsis thaliana*. Evolution 57:1270–1280.

Werner, P. A., I. K. Bradbury, and R. S. Gross. 1980. The biology of Canadian weeds. 45. *Solidago canadensis* L. Can. J. Plant Sci. 60:1393–1409.

Wood, C. W., and E. D. Brodie. 2015. Environmental effects on the structure of the G-matrix. Evolution 69:2927–2940.

Züst, T., C. Heichinger, U. Grossniklaus, R. Harrington, D. J. Kliebenstein, and L. A. Turnbull. 2012. Natural enemies drive geographic variation in plant defenses. Science 338:116–119.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Tests of fixed effects in the linear mixed models fitted to the five metabolite traits by population (see details in main text). Note that trait was effectively a dummy variable, ensuring that all parameters were estimated separately by metabolite, and that data for each metabolite were scaled to a mean of 0 and variance of 1 before analysis.

Table S2. Genetic variance-covariance (**G**) matrices for plant secondary metabolites estimated in a Bayesian framework; a) the herbivore habitat and 2) the no-herbivore habitat. Values are posterior means, with 95% HPD intervals in parentheses beneath.