Caterpillars on a phytochemical landscape

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Summary

• Modern metabolomic approaches that generate more comprehensive phytochemical profiles than were previously available are providing new opportunities for understanding plant-animal interactions. Specifically, we can characterize the phytochemical landscape by asking how many individual compounds affect herbivores and how compounds covary among plants.

• Here we use the recent colonization of alfalfa (*Medicago sativa*) by the Melissa blue butterfly (*Lycaeides melissa*) to quantify primary and secondary plant metabolites and the performance of caterpillars as affected by both individual compounds and suites of covarying phytochemicals.

• We find that survival, development time and adult weight are all associated with variation in a large number of compounds, including biomolecules associated with plant cell function as well as putative anti-herbivore action. The dimensionality of the plant-insect interface is high, with clusters of covarying compounds in many cases encompassing divergent effects on different aspects of caterpillar performance.

• Individual compounds with the strongest associations tend to be secondary metabolites, including alkaloids, phenolic glycosides and saponins. The saponins are represented in our data by more than 25 individual compounds with beneficial and detrimental effects on caterpillars, which highlights the value of metabolomic data as opposed to approaches that rely on total concentrations within defensive classes.

Keywords: *Lycaeides melissa, Medicago sativa*, metabolomics, plant defense, specialization

Introduction

One of the conceptual pillars of trophic ecology is the idea that herbivores must overcome the barrier of plant secondary chemistry before extracting the nutrients necessary for growth and reproduction (Feeny *et al.*, 1992). The success of this idea is reflected in areas of research that include coevolution (Agrawal *et al.*, 2012), ecological specialization (Dyer, 1995), and nutrient flow in ecosystems (Olson, 1963). In most cases, progress has been made by chemical ecologists focusing on small subsets of the secondary metabolites produced by plants and consumed by herbivores. The focus on a few charismatic molecules or classes of compounds, such as furanocoumarins (Berenbaum, 1983) or cardiac glycosides (Zalucki *et al.*, 2001), was at least in part necessitated by early methods in natural products chemistry that were targeted and not easily optimized for the discovery of large suites of co-occurring primary and secondary metabolites (Maag *et al.*, 2015; Dyer *et al.*, 2018). As technological limitations have dissipated, the opportunity now exists for a deeper understanding of the challenges faced by herbivores, with the possibility of discovering, among other things, novel compounds and synergistic interactions among compounds (Prince & Pohnert, 2010; Richards *et al.*, 2010; Sardans *et al.*, 2011). More generally, an important task is to quantify the phytochemical dimensionality of the antagonistic interaction between plants and herbivores, with an eye towards understanding constraints on the evolution of both players (Fordyce & Nice, 2008; Macel *et al.*, 2010) and predicting the formation of new plant-herbivore interactions (Erbilgin, 2018). Here we use the example of a specialized herbivore and a novel host plant to investigate the phytochemical landscape from the perspective of developing caterpillars.

The Melissa blue butterfly, *Lycaeides melissa*, specializes on larval host plants in the pea family (Fabaceae), primarily in the genera *Astragalus* and *Lupinus*. Within the last 200 years, *L. melissa* has colonized introduced alfalfa, *Medicago sativa* (Fabaceae), at least twice and probably multiple times (Chaturvedi *et al.*, 2018), forming a heterogeneous patchwork of association throughout the range of the butterfly in western North America. *M. sativa* is a suboptimal host, relative to native hosts that have been examined, and populations of the butterfly that persist on *M. sativa* show evidence of loss of preference for native hosts (Forister *et al.*, 2012), reduced caterpillar performance on native hosts, and a slight increase in ability to develop on the suboptimal novel host (Gompert *et al.*, 2015). The genetic architecture of host

use in this system is known to be polygenic and characterized by loci with conditionally neutral (host-specific) effects (Gompert *et al.*, 2015). What is needed next is an understanding of how many independent or covarying phytochemical compounds have consequential effects on caterpillars eating the novel host. For example, will the trajectory of further local adaptation by *L. melissa* to *M. sativa* be a matter of evolving the ability to detoxify one or a large number of compounds? We would also like to know how key compounds covary among individual plants, which should help us understand the puzzle faced by ovipositing females encountering an array of co-occurring *M. sativa* chemotypes. Here we use a common garden approach (to minimize non-genetic phenotypic variation among plants) and caterpillars individually reared in a controlled environment to address these questions while describing the effects of metabolomic variation in *M. sativa* on *L. melissa*.

Materials and Methods

Plants and caterpillars

Plants used in this project were grown at the University of Nevada, Reno, Main Station experimental farm. The common garden was planted in 2016 with seeds collected the previous year from 45 plants (previously studied by Harrison et al. (Harrison *et al.*, 2018)) growing in a fallow field in north-western Nevada on the western edge of the Great Basin Desert. The focal butterfly, *L. melissa*, was present in the source field but has not yet colonized the university farm where experimental plants were grown. The 45 maternal plants each contributed 15 offspring to a randomized grid design in the common garden, irrigated with broadcast sprayers in 2016 and drip in 2017, without supplemental fertilization. Out of each maternal family, a single plant was randomly selected for use in the rearing experiment reported here as a way to capture as much genetic and phenotypic variation as possible.

On 17 and 18 July 2017, a total of 45 *L. melissa* females were collected from an alfalfa-associated population near Verdi, NV, and confined to oviposition arenas (500 mL plastic cups) in groups of three with host plant leaves and supplied with Gatorade on mesh lids. After three days, eggs were removed from leaves, pooled, and kept at room temperature until hatching, at which time caterpillars were placed individually in Petri dishes (100 x 25 mm) with leaves of a particular *M. sativa* individual (which became the only plant from which they were fed

113 throughout the experiment). Ten caterpillars were assigned to each of the 45 experimental *M.*
114 *sativa* plants (for a total of 450 independently-reared caterpillars) and kept in a growth chamber
115 set to 25 C and a 12 hour light / 12 hour dark cycle. From each caterpillar we recorded survival
116 to adult, date of eclosion (if successful) and adult weight to the nearest 0.01 mg on a Mettler
117 Toledo XP26 microbalance.
118
119 Phytochemistry and plant traits
120
121 Metabolomic variation among individual plants was characterized with liquid chromatography–
122 mass spectrometry (LC-MS, (Jorge *et al.*, 2016)) using leaves collected on a single day at the
123 start of the rearing experiment. Dried, ground leaves (10 mg) were extracted in 2 mL of 70%
124 aqueous ethanol and injected into an Agilent 1200 analytical high performance liquid
125 chromatograph paired with an Agilent 6230 Time-of-Flight mass spectrometer via an
126 electrospray ionization source. Resulting chromatograms were analyzed using MassHunter
127 Quantitative Analysis (v.B.06.00, Agilent, Santa Clara, CA); see electronic supporting
128 information for additional phytochemical protocols. Major classes of compounds were identified
129 using characteristic relative mass defects (Ekanayaka *et al.*, 2015). Leaf protein content was
130 quantified with three replicates (~2 mg each) per plant using the Bicinchoninic acid assay (Pierce
131 Biotechnology, Waltham, MA). Before grinding, five dried leaflets from each sample were
132 weighed to the nearest 0.1 mg, scanned, and area was measured using ImageJ (v.1.52a); specific
133 leaf area (SLA) was calculated as leaf area divided by dry mass. Finally, leaf toughness was
134 measured on fresh material in the common garden, at the start of the experiment (mid-July, when
135 leaves were also sampled for chemistry and protein) and at the end of the experiment (mid-
136 August), from three leaves per plant at each date, with a penetrometer (Chatillon 516 Series)
137 through the center of the middle leaflet, as in (Harrison *et al.*, 2018); the three leaves were
138 selected haphazardly, avoiding the oldest and youngest leaves. The six leaf toughness
139 measurements per plant were averaged for a single toughness measure used in analyses.
140
141 Analyses of plant traits and caterpillar performance
142 Overview
143

Our analytical strategy to understand the association between phytochemical variation and caterpillar performance followed two complementary paths, one focusing on dimension reduction and feature selection to produce relatively simple models, and the other on the estimation of effects of all individual compounds. For the first path, involving dimension reduction and feature selection, we utilized an approach developed for gene transcription studies that identifies groups or modules of correlated variables with hierarchical clustering (Langfelder & Horvath, 2008); after clustering, we reduced the number of independent variables by selecting among modules and other plant traits (specific leaf area, protein and leaf toughness) using lasso regression (Ogutu *et al.*, 2012). Selected modules (and other plant traits) were analyzed in Bayesian linear models that are useful in this context because they allowed us to quantify our confidence in the sign of effects (positive or negative) as continuous probabilities (as opposed to relying on arbitrary significance cutoffs). For the second analytical path, we utilized ridge regression (Ogutu *et al.*, 2012) to estimate effects for all compounds simultaneously, which allowed us to investigate the distribution of effects among compounds and classes of compounds. Both analytical paths incorporated cross-validation during the lasso and ridge regressions, and as a means of evaluating the predictive success of the Bayesian models. We also used randomization tests to compare the performance of modules and individual compounds with randomly-chosen suites of compounds.

Dimension reduction and feature selection

We chose an approach that reduces the number of independent variables while allowing us to learn something about the correlational structure of the data, specifically unsupervised hierarchical clustering as implemented in the blockwiseModules function of the WGCNA package (Langfelder & Horvath, 2008) in R (Team & R Development Core Team, 2016). Among the options in the pipeline, we used positive correlations among variables ("signed" network type), merge cut height at 0.25, and correlations raised to the power of five (which is where the scale free topology index reached a plateau). Through experimentation, we found that our results with LC-MS data were robust to variation in these choices, including the choice of signed or unsigned networks. After an initial round of clustering, we took a remaining 19 unassigned compounds and put them through a second round of clustering (although the majority

of consequential compounds were identified in the first round). One output of the WGCNA procedure is the first eigenvector from each cluster of compounds, which reduced our number of predictor variables by a factor of ten. The resulting eigenvectors plus protein, SLA (specific leaf area) and leaf toughness were then put through the feature reduction step of lasso regression (Ogutu *et al.*, 2012), a penalized regression that allows beta coefficients to be constrained to zero (thus excluding variables). We used the cv.glmnet function of the glmnet package (Friedman *et al.*, 2016) with cross-validation during error reduction set to leave out one plant (and associated caterpillars) at each iteration. The variables selected by the lasso were then put into a Bayesian linear model to estimate coefficients and associated credible intervals using JAGS (version 3.2.0) run in R with the rjags package (Plummer & others, 2003). Two Markov chains were run for 10,000 steps for each analysis (no burn in was required) and chain performance was assessed by plotting chain histories, and calculating the Gelman and Rubin convergence diagnostic and effective sample sizes (Gelman *et al.*, 1992; Brooks & Gelman, 1998). For all models, uninformative priors for the regression coefficients were modeled as a normal distribution with a mean of zero and variance of 100 (variance = 1/precision). We quantified our confidence in the sign of coefficients (positive or negative) as the fraction of the posterior samples that were less than zero (for coefficients with a median negative value) or greater than zero (for coefficients with a median positive value).

All analyses were done using the R statistical language (Team & R Development Core Team, 2016) on scaled (z-transformed) predictor variables, and both lasso and Bayesian models used binomial (for survival), Poisson (for development time) and Gaussian (for adult weight) errors. The latter two analyses (development time and adult weight) included sex as a factor. The analysis of development time also included adult weight as a covariate; while (reciprocally) the analysis of adult weight included development time as a predictor. These variables are negatively correlated (at -0.52), and they function as useful covariates of each other, allowing us to investigate the possibility of unique plant effects on weight gain and development time, which could not be discovered if, for example, these variables were combined into a single performance index.

The success of models developed with the dimension reduction and feature selection pipeline was judged in two ways. We used a cross-validation procedure in which we left out one plant (and associated caterpillars) in each iteration of the Bayesian model and then used the

estimated coefficients (for phytochemical variables and other plant traits) to predict the performance of the unobserved caterpillars. After 45 iterations (one for each plant), we calculated a simple correlation coefficient between the observed and predicted performance of caterpillars across plants. In addition, we repeatedly resampled the original LC-MS data to match the structure of the reduced set of predictor variables to ask to what extent randomly assembled modules could outperform the empirically-derived modules.

Individual compound effects

The second path of our two-part analytical strategy involved simultaneous estimation of the effects of all individual chemical compounds on caterpillar survival, development time and adult weight. For this approach, we again used penalized regression (in the glmnet package (Friedman *et al.*, 2016)), but this time with ridge regression (instead of lasso) which constrains beta coefficients to avoid variance inflation but does not eliminate variables. As with the analyses above, ridge regression was done using error structures appropriate to the specific response variables, and included additional covariates where possible (in models of development time and adult weight). The resulting coefficients associated with all individual compounds were examined as a second perspective on the modules examined in the first set of analyses, and were used to ask to what extent individual compound effects could be predicted by the degree to which they vary among individual plants as quantified with the simple coefficient of variation. To assess confidence in the results of ridge regressions, we used a bootstrap approach, repeatedly resampling the data and estimating coefficients 1000 times, noting the compounds whose bootstrap confidence intervals did or did not overlap zero (Delaney & Chatterjee, 1986). We also allowed for the discovery of interactions among compounds using penalized regression on all individual compounds and all pairwise interactions between compounds. For ease of interpretation, this final analysis of potential interactions used lasso (not ridge) regression, letting the coefficients for many of the individual compounds and pairwise interactions go to zero.

Results

Of the 450 caterpillars that started the experiment, 261 were reared to eclosion as adults (a mortality rate similar to previous work with this system (Gompert *et al.*, 2015)) on leaves from 45 individual alfalfa plants that were characterized for protein, leaf toughness, specific leaf area and 163 individual metabolomic features (see Fig. 1 for variation among plants in caterpillar performance and a subset of plant traits, and Supporting Information Table S1 for a list of compounds). Hierarchical clustering identified 14 subsets (or modules) of compounds with generally low correlations among modules and high correlations within modules (see Supporting Information Figs. S1 and S2 for correlations within and among modules, and Fig. S3 for module variation among plants). The correlational structure of the phytochemical data is illustrated as an adjacency network in Fig. 2, where it can be seen that some modules (e.g., modules 1 and 2) contain a great diversity of compound types, while other modules are made up of more narrow classes (e.g. modules 7 and 8 are mostly saponins, a class of defensive secondary metabolites (Levin, 1976)). From the 14 eigenvectors summarizing variation in the modules, as well as the other plant traits, lasso regression produced a reduced set of potential predictors which were then used in Bayesian multiple regression models that included between seven and nine independent variables (Table 1). The models had reasonably high performance in leave-one-out cross-validation (correlations between observed and predicted values were between 0.50 and 0.59, Table 1), and also in resampling analyses (Supporting Information Fig. S4), where a small fraction (never more than 4%) of randomly-generated models exceeded the variance explained of the models reported in Table 1.

Variation among plants in the suites of covarying compounds had large effects on caterpillar performance: for example, the beta coefficient of -2.33 (on the log-odds scale) associated with module 3 corresponds to a reduction in mean survival from 0.58 to 0.12 associated with a one unit change in variation associated with that phytochemical module (Table 1; note that in Table 1 and elsewhere negative coefficients for development time are associated with fewer days, and thus can be thought of as potentially beneficial effects, in contrast to negative coefficients for survival and weight that are detrimental to caterpillars). The phytochemical predictor variables are eigenvectors from clustering analysis, and thus are not entirely straightforward to interpret, especially when the clustering analysis was itself based on z-transformed data. It is useful to note that our LC-MS data consists of peak areas divided by the peak of an internal standard, and again divided by the dry weight of the sample (thus, in total,

referred to as "relative abundance per dry weight"). Variation in these numbers reflects variation in concentrations within compounds (among plants), but care should be used in comparing among compounds because of different ionization responses relative to the standard (thus the use of z-transformation for among-compound analyses). Nevertheless, the effects reported in Table 1 reflect real variation in suites of compounds, as can be seen in correlations between eigenvectors and individual compounds in Supporting Information Fig. S2, and in variation among plants in average z-scores in Fig. S3.

Modules included in multiple regression models frequently had common effects across response variables (e.g., the positive association of module 10 with both survival and adult weight), with the exceptions of module 11 that had a solitary effect on survival and module 6 with an effect only on development time (although the probability of the latter having a negative effect was only 0.75). Specific leaf area had a negative effect on survival and adult weight, and the coefficients for specific leaf area (-0.31 for survival and -0.42 for weight) were of smaller magnitude than most phytochemical effects.

Module-based analyses (as in Table 1) focused on feature reduction with lasso regression; as a complementary analytical approach, we used ridge regression on all of the compounds (which estimates effects of individual compounds without excluding variables as in lasso regression). Analyses of individual compounds by ridge regression (Fig. 3) were broadly consistent with the strongest module-specific effects, as can be seen, for example, with module 10 having positive effects on survival and adult weight in module analyses (Table 1) and in compound-specific analyses (Fig. 3). Similarly, the individual compounds in module 3 had negative compound-specific effects on survival (Fig. 3), and that module had the strongest negative effect on survival in the eigenvector-based analyses in Table 1. Not surprisingly, the larger modules (with a greater number of covarying compounds, including many primary metabolites) tended to have a more complex mix of positive and negative effects (for example, modules 1 and 2, Fig. 3). For ease of interpretation, the coefficients from compound-specific regressions of survival and development time (in Figs. 3 and 4) have been back-transformed to be on the scales of probability and days (respectively), and displayed as changes relative to intercepts. For example, a compound with a relatively large effect on survival in Fig. 3 could be associated with a one half percent (0.005) reduction in the probability of survival relative to average survival and while holding other compounds constant.

298 We also considered potential pairwise interactions among individual compounds, and
299 found few interactions that passed the filter of the penalized regression (Supporting Information
300 Table S2), at least relative to the large number of potential interactions. Saponins and alkaloids
301 tended to be overrepresented in the interactions that were detected, and phenolic glycosides were
302 involved in stronger negative interactions relative to other compounds (Fig. S5). We did not find
303 evidence that more or less variable compounds had differential effects on caterpillars, although
304 there was a trend towards both greater positive and greater negative effects being associated with
305 less variable compounds (Fig. S6). We saw some variation among classes of compounds in their
306 effects on caterpillars (Fig. 4). All classes included positive and negative effects, with saponins,
307 alkaloids and phenolic glycosides including some of the stronger negative effects of individual
308 compounds, while lipids and sterols tended towards positive associations with survival and
309 development (Fig. 4).
310
311 **Discussion**
312
313 The results reported here represent a dissection of the phytochemical landscape facing a
314 specialized insect herbivore attacking a novel host plant (Hunter, 2016). The phytochemical
315 landscape is both physical, referring to variation in compounds among individual plants in a
316 common garden, and hypothetical to the extent that effects of individual compounds on
317 caterpillars are estimated, although compounds are of course not encountered in isolation. Our
318 exploration of the phytochemical landscape facing *L. melissa* on *M. sativa* is necessarily a first
319 draft based on a snapshot in time, and the implications of this are discussed further (below) in the
320 context of within and among-population heterogeneity in the system. Nevertheless, models
321 including suites of covarying compounds and other plant traits had predictive success and
322 suggested different natural products affecting survival, development time and adult weight
323 (performance measured as adult weight is a proxy for fecundity (Forister *et al.*, 2009)). Previous
324 work with *M. sativa* and insect herbivores has focused on sapogenic glycosides (Levin, 1976),
325 and a simple outcome from our study could have been that one or a small number of saponins
326 have anti-herbivore properties that reduce fitness of our focal insect. Instead, we find large
327 numbers of compounds with potentially consequential effects on caterpillars (Fig. 3), and the

effects of those compounds were greater than the effects of leaf toughness and specific leaf area (Carmona *et al.*, 2011).

The precise identification of specific compounds is perhaps not as important as the result that prominent classes of secondary metabolites, such as saponins and peptides, include compounds with both positive and negative effects. Positive effects of these compounds are potentially associated with feeding stimulation, as has been observed (along with other positive effects) for other specialist herbivores and plant toxins (Seigler & Price, 1976; Smilanich *et al.*, 2016). Negative effects of saponins on insects potentially include disruption of hormone production (Chaieb, 2010), although exact modes of action on *L. melissa* will await further study. Although most of the individual compounds with strong effects appear to be secondary metabolites (including alkaloids and phenolic glycosides, as well as saponins and peptides), we also find both positive and negative effects associated with variation in certain primary metabolites (Fig. 4). For example, the larger detrimental effects of individual compounds on caterpillars include phospholipids (Fig. 2). These could be direct effects if a compound is suboptimal for development, or they could be associated with nutritional imbalance (Behmer, 2009), such that too much of one nutrient makes it difficult for caterpillars to consume a balanced diet. It has been suggested that the presentation of unbalanced nutrition can be a kind of anti-herbivore strategy (Berenbaum, 1995). Although this possibility has not been thoroughly investigated in many systems with full metabolomic profiling, the idea that nutritional imbalance could be as important as direct toxicity suggests that we might update theories of the evolution of plant defense that were built on differential investment into simple categories of plant growth versus defense (Stamp, 2003).

The finding that our specialist herbivore is affected by a wide range of metabolites, primary and secondary, that vary greatly even within a single host population has implications for our understanding of heterogeneity in the system and for the course of local adaptation of the herbivore to the novel host. *Lycaeides melissa* typically colonizes weedy or feral patches of *M. sativa* on roadsides or integrated into natural communities, and previous work has documented dramatic variation among individual alfalfa locations (often in close proximity) in the extent to which they can support caterpillar development (Harrison *et al.*, 2016). Previous phytochemical data with a lower resolution was less successful in explaining that variation (Harrison *et al.*, 2016), and the results reported here suggest that among patch variation could be explained by

359 future studies using metabolomic data as used here. The within-population complexity described
360 in the current study combined with previous evidence for dramatic among-population variation
361 in *M. sativa* suitability for the focal herbivore also raises the possibility that the novel host
362 presents a multi-faceted and potentially ever-shifting target from the perspective of evolving
363 butterfly populations. In particular, it is possible that *M. sativa* defense against a specialist
364 herbivore might be realized through different combinations (within and among populations) of
365 individually-acting compounds, thus making it unlikely for butterflies in any one population to
366 possess an effective suite of alleles that improve fitness on the novel host. In this context, it is
367 interesting to note that a molecular genetic dissection of caterpillar performance in this system
368 found a large number (potentially hundreds) of individual loci associated with performance on
369 *M. sativa*, yet evolution in populations associated with the novel host is primarily associated with
370 a loss (through genetic drift) of the ability to eat a native host and only slight improvement in the
371 ability to eat the novel host (Gompert *et al.*, 2015).

372 The correlational structure of the phytochemical variation that we observed has
373 implications for the evolution of plant defense and the accumulation of insect herbivores on *M.*
374 *sativa*. Specifically, correlations among modules should make it possible to hypothesize
375 directions of least resistance for defense evolution. Compounds in module 9 had a negative
376 effect on survival (Table 1), and module 9 negatively covaried with module 10 (Supporting
377 Information Fig. S1), which itself had a positive association with caterpillar survival. Thus an
378 increase in module 9 and an associated decrease in 10 would be beneficial for the plant, at least
379 with respect to herbivory by our focal herbivore. Of course, most plants do not have the luxury
380 of optimizing defense against a single herbivore, and it is easy to imagine that improvements in
381 defense against one enemy could lead to increased attraction to another, especially given the
382 diversity of effects even within major classes studied here, including saponins and phenolic
383 glycosides. Compounds in the latter class (phenolics) were found to have strong positive and
384 negative effects on assemblages of arthropods associated with the maternal plants from which
385 seeds were collected to start the common garden used in the present study (Harrison *et al.*, 2018).

386 The results reported here raise a number of avenues for future exploration, including the
387 apparent overrepresentation of both saponins and alkaloids in interactions with other compounds
388 (Fig. S5). Relative mass defect (RMD) is a useful tool for the categorization of compounds, but
389 it has limitations in complex mixtures; we are developing methods that use other data from high

resolution mass spectrometry to further refine categorization of *Medicago* metabolites. Also, in the present study, we have not attempted to separate constitutive and induced defenses (Jansen *et al.*, 2009) as the plants in the common garden were exposed to natural and continuous levels of herbivory. We also acknowledge that feeding under laboratory conditions is of course not natural, although we found in a previous study that genetic variants (in caterpillars) associated with success in laboratory feeding trials were at least partially predictive of genetic variation associated with alfalfa host use in the wild (Chaturvedi *et al.*, 2018). Thus it is clear that metabolomic data such as that analyzed here has the potential to both open up new avenues of conceptual development in plant-insect interactions and to link micro-evolutionary trajectories across hosts and herbivores.

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Author contributions

MLF: designed experiment, conducted analyses, wrote first draft. SY: conducted experiment and contributed to experimental design. CSP, CDD, BH: generated and interpreted phytochemistry and protein data. MLF, JGH, OS: developed and maintained common garden. JAF, ZHM, CCN, LAR: contributed to analyses and experimental design. CAB, JAF, ZG, CCN: contributed to experimental design. All authors: contributed to writing.

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515

516 **Figure legends** (also reproduced below individual figures)

517

518 **Fig. 1.** Variation among plants in caterpillar survival (a), development time (b) adult weight (c),
519 three individual compounds (d-e) and two external plant traits, specific leaf area (g) and leaf
520 toughness (h). The three example compounds shown here (out of the 163 assayed) were among
521 the top five most influential compounds for survival, development time and adult weight: cpd. 9
522 is an alkaloid with a negative association with survival, cpd. 94 (a peptide) has a negative
523 association with development time, and cpd. 160 is a phospholipid with a negative association
524 with adult weight. Individual plants in all panels are organized from left to right by decreasing
525 caterpillar survival in the top panel (a). Standard errors are shown for panels b, c, g and h. The
526 units for d-e are compound relative abundance per dry weight of sample; the units for specific
527 leaf area are cm^2/mg, and grams/newton for leaf toughness.

528

529 **Fig. 2.** Illustration of correlational structure among compounds: each node in the network is a
530 compound, and compounds are linked by a line if they are correlated among individual plants at
531 0.5 or above (links among compounds in modules 12-14 represent weaker correlations, greater
532 than 0.1; see main text for details). Two letter codes within nodes indicate compound classes, as
533 explained in the legend. Colors of nodes correspond to membership in modules as determined by
534 hierarchical cluster analysis; the color key to the 14 modules is shown in the lower left. Not shown are a
535 small number of compounds with weak connections to all other compounds, including two compounds
536 that were not included in any module (shown as module zero in Fig. 3).

537

538 **Fig. 3**. Effects of individual compounds on survival, development time and adult weight, as estimated by
539 ridge regression (using binomial, Poisson and Gaussian models, respectively). The strength of effect for
540 each compound is indicated by the horizontal extent of each bar, and compounds are grouped by modules
541 (m1, m2, etc.); the order of compounds along the vertical axis is arbitrary within modules and fixed across
542 columns. Orange colors indicate negative effects on survival, development and weight, while blue colors
543 are positive effects (note that negative effects for development time correspond to fewer days, or more
544 rapid development). The darker shades of orange and blue mark coefficients whose 95% confidence
545 intervals did not overlap zero in 1,000 bootstrap samples. Values for survival and development time have

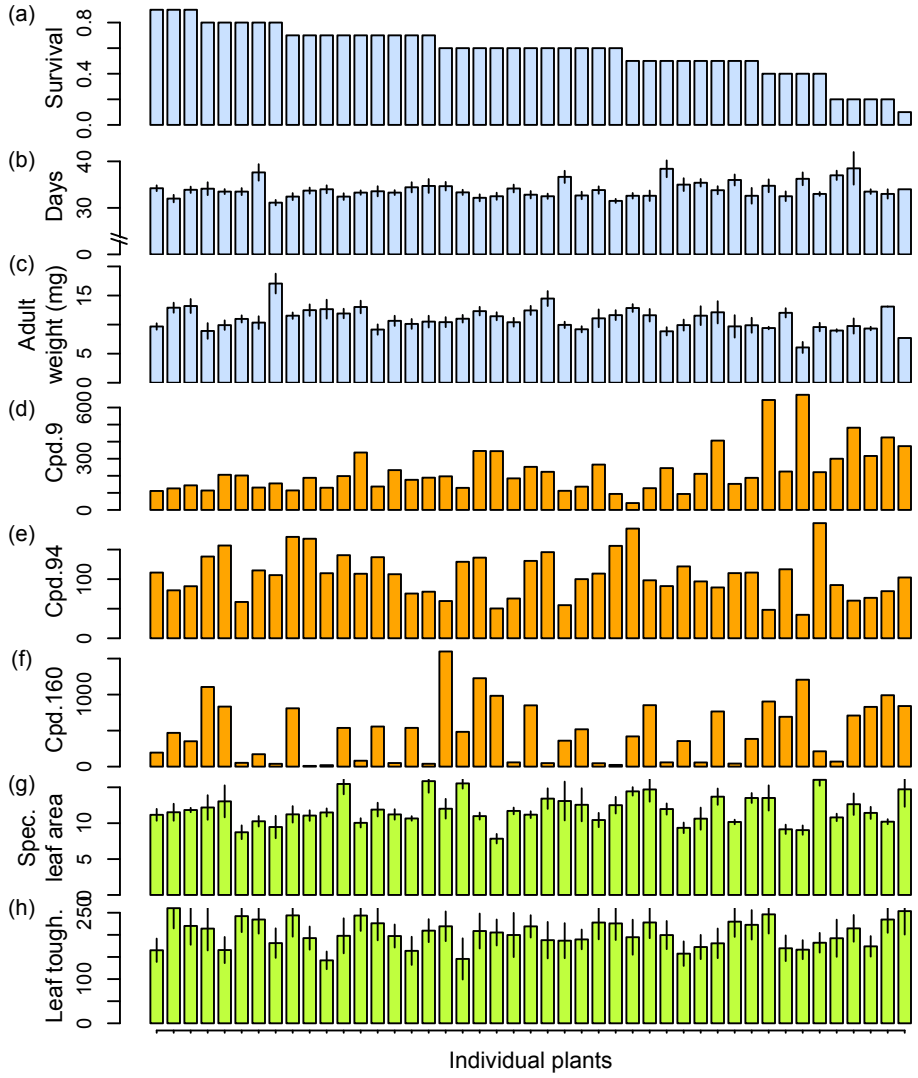
been back-transformed from units on the log-odds and log scales to units of probability and days to pupation, and are shown as changes from the mean or intercept values. For example, a negative (orange) survival coefficient of 0.005 means a one-half percent reduction in average probability of survival associated with variation in a particular compound. The fifteen compounds with the largest coefficients (by absolute value) and bootstrap intervals not overlapping zero are labelled by compound classes (see Fig. 2 for abbreviations) in each panel. Structural annotations are shown to the right for six compounds based on matches from the METLIN metabolomics database, as follows by compound number: 154 (unidentified sterol); 9 (unidentified alkaloid); 60 (soyasaponin A3); 40 (unidentified saponin); 46 (medicagenic acid 3-O-triglucoside); 45 (medinoside E). Those same compounds are identified in parentheses in the main panels next to the bars corresponding to their individual effects.

Fig. 4. Violin plots of compound-specific effects (coefficients from ridge regressions) summarized by chemical classes. Plots show median (black dot), interquartile range (box) and 95% confidence intervals (whiskers) surrounded by kernel density envelopes. Sample sizes for each category are shown above the top panel ("Other" includes 1 sugar, 2 pigments and 2 halogenated compounds). Categories are arranged from left to right based on the gradient of median positive to negative effects on survival. Coefficients for survival (a) and development time (b) have been back-transformed from the units of log-odds and log to probability and days to pupation, respectively, and shown as deviations from the mean or intercept value (as in Fig. 3). Note that negative effects for development time correspond to fewer days (more rapid development).

Table 1 Results from Bayesian regressions of module eigenvectors and covariates predicting caterpillar survival, development time and adult weight (as binomial, Poisson, and Gaussian regressions, respectively, with corresponding units in log-odds, log number of days, and milligrams).

	Survival coefficient (CI; prob.)	Development time coefficient (CI, prob.)	Weight coefficient (CI, prob.)
m2	0.37 (-1.87, 2.67; 0.63)	-0.07 (-0.26, 0.12; 0.78)	
m3	-2.33 (-3.94, -0.72; >0.99)		-2.33 (-4.87, 0.24; 0.96)
m6		-0.05 (-0.19, 0.09; 0.75)	
m9	-2.31 (-4.49, -0.15; 0.98)	0.064 (-0.11, 0.24, 0.78)	
m10	2.54 (0.81, 4.23; >0.99)		3.56 (1.22, 5.84; >0.99)
m11	2.01 (0.64, 3.42; >0.99)		
SLA	-0.31 (-0.55, -0.08; >0.99)		-0.42 (-0.78, -0.06; 0.99)
Protein	0.061 (-0.16, 0.28; 0.71)		0.08 (-0.26, 0.42; 0.68)
Tough.	0.036 (-0.17, 0.24; 0.36)	-0.002 (-0.02, 0.02; 0.57)	
Sex	/	0.06 (0.02, 0.10; >0.99)	1.10 (0.38, 1.83; >0.99)
mg	/	-0.03 (-0.05, -0.01; >0.99)	/
Days	/	/	1.10 (0.38, 1.83; >0.99)
Intercept	0.34 (0.14, 0.53; >0.99)	3.48 (3.45, 3.52; >0.99)	10.36 (9.81, 10.92; >0.99)
Validation	0.53	0.59	0.50

For each regression coefficient, numbers in parentheses are 95% credible intervals (the first two numbers) and the probability that the coefficient has the estimated sign (e.g., 0.63 for the m2 survival coefficient of 0.37 indicates a 63% probability that the m2 module has a positive effect on survival). Note that negative coefficients for development time indicate faster caterpillar development (fewer days) associated with variation in a particular compound. Modules (listed in the left column) are only shown if they were included in one of the three regressions following feature selection using lasso regression (see main text for additional details). Empty spaces in the Table appear if a particular module was selected through lasso regression for one or two analyses but not all three (m3, for example, was not selected by lasso regression for development time). Slash marks (/) indicate variables not considered for a particular analysis (e.g., sex, adult weight [mg] and development time [days] were not possible for the survival analysis because they are not observed on dead individuals). Values for "validation" shown in the last row are the correlation between observed and predicted values in cross-validation (Supporting Information Fig. S4).



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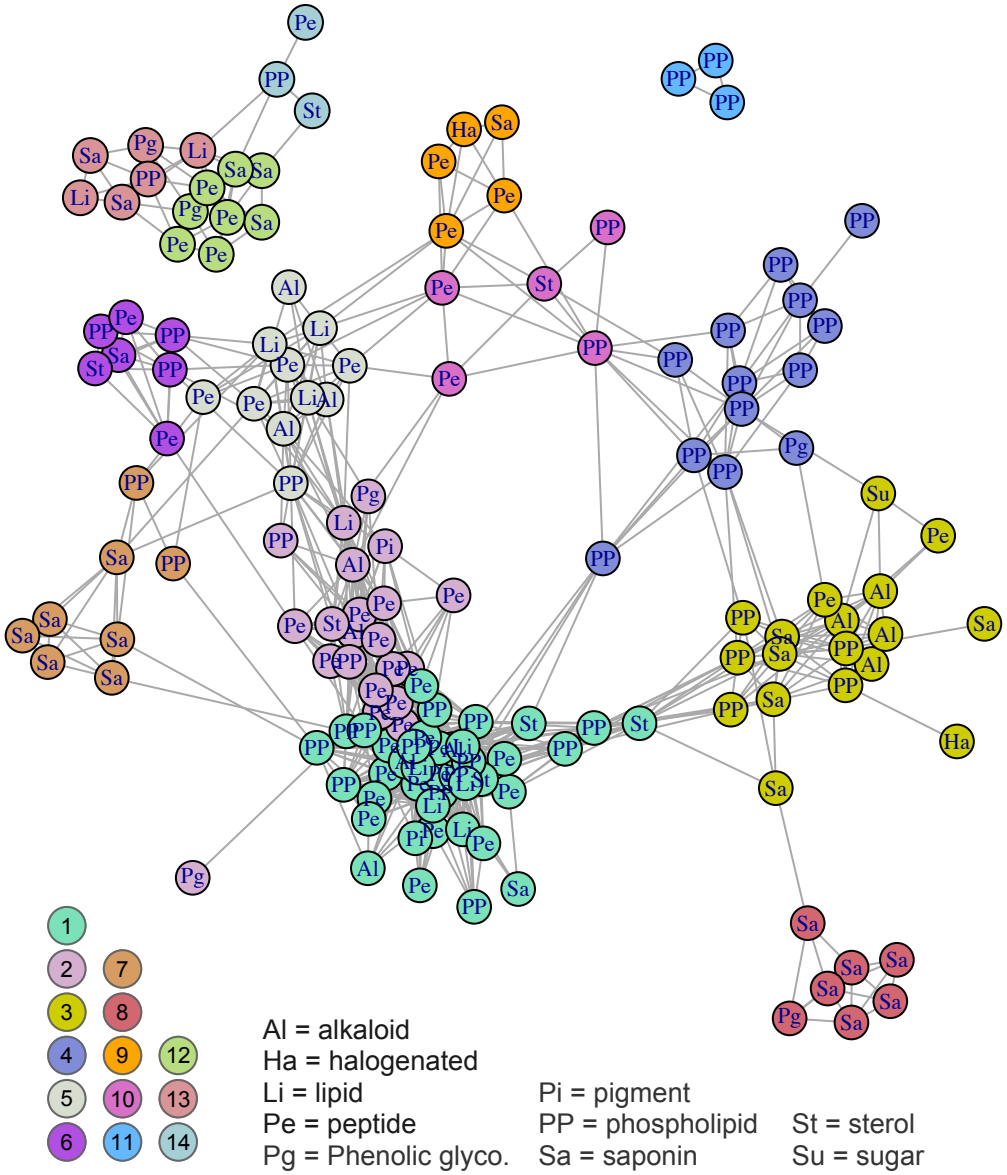

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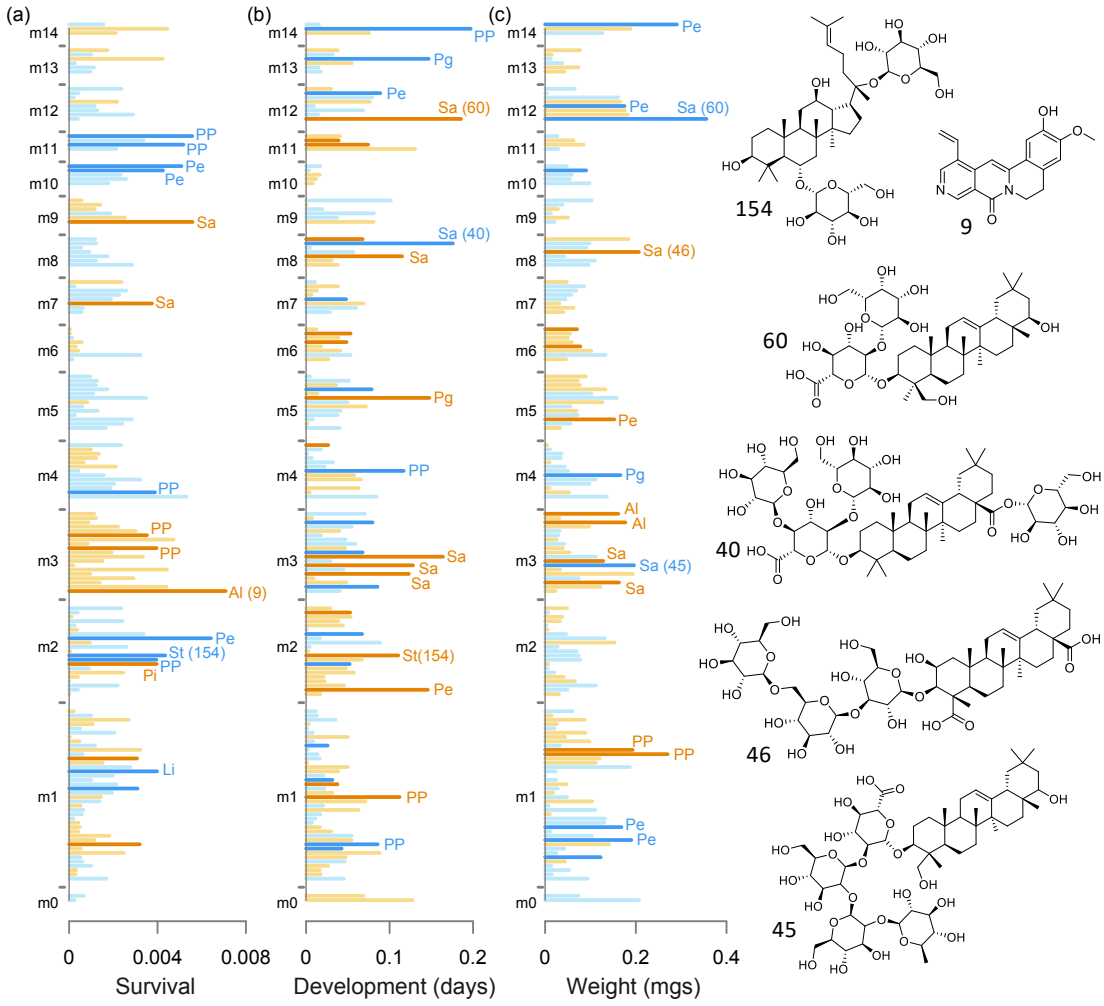

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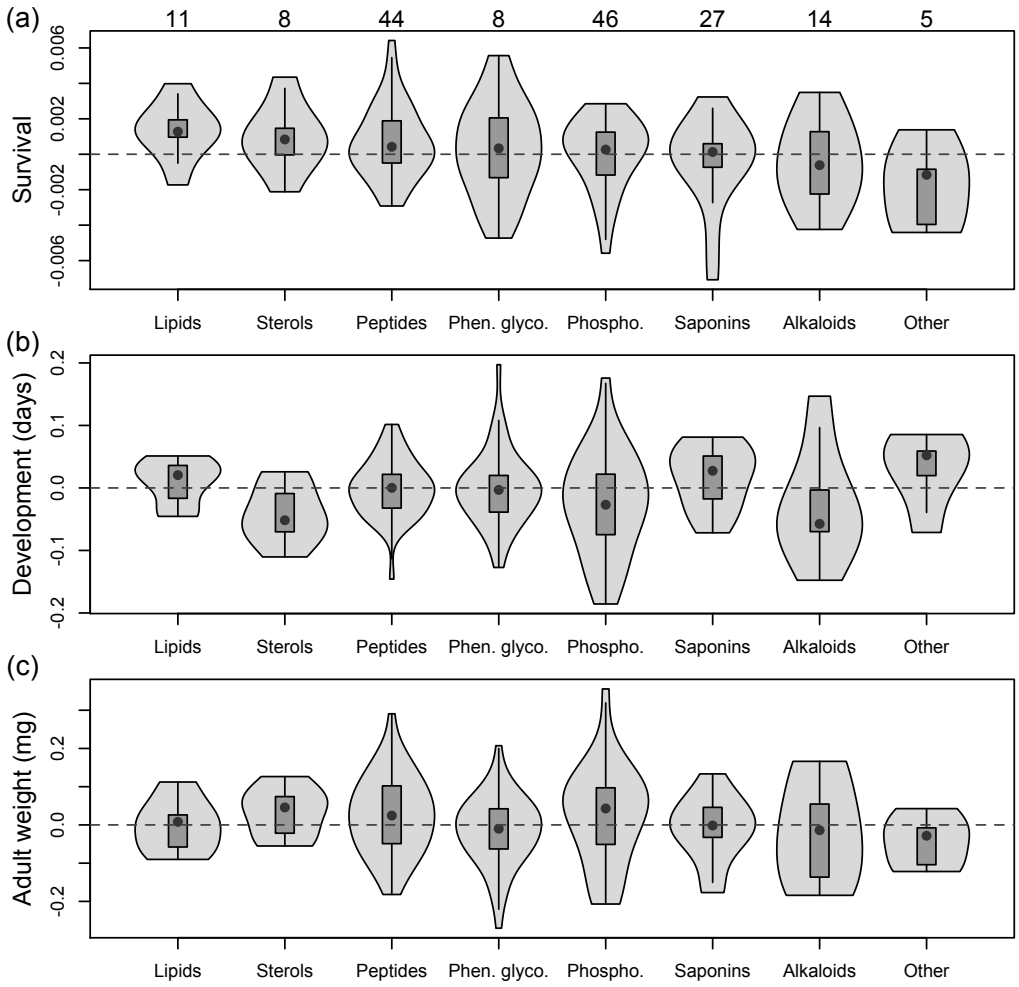

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