

EVOLUTIONARY ECOLOGY OF PLANT DEFENCES

Plant traits that predict resistance to herbivores

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Summary

1. Although secondary metabolites are recognized as fundamental to the defence of plants against insect and mammalian herbivores, their relative importance compared to other potential defensive plant traits (e.g. physical resistance, gross morphology, life-history, primary chemistry and physiology) are not well understood.

2. We conducted a meta-analysis to answer the question: What types of genetically variable plant traits most strongly predict resistance against herbivores? We performed a comprehensive literature search and obtained 499 separate measurements of the strength of covariation (measured as genetic correlations) between plant traits and herbivore susceptibility – these were extracted from 72 studies involving 19 plant families.

3. Surprisingly, we found no overall association between the concentrations of secondary metabolites and herbivore susceptibility – plant traits other than secondary metabolites most strongly predicted herbivore susceptibility. Specifically, genetic variation in life-history traits (e.g. flowering time, growth rate) consistently exhibited the strongest genetic correlations with susceptibility. Genetic variation in gross morphological traits (e.g. no. branches, plant size) and physical resistance traits (e.g. latex, trichomes) were also frequently correlated with variation in herbivore susceptibility, but these relationships depended on attributes of the herbivores (e.g. feeding guild) and plants (e.g. longevity).

4. These results call into question the conventional wisdom that secondary metabolites are the most important anti-herbivore defence of plants. We propose the hypothesis that herbivores select most strongly on genetic variation in life-history, morphological and physical resistance traits, but the greater pleiotropic effects of genes controlling these traits impose strong constraints on their evolution. Meanwhile, secondary metabolites could have evolved to be important defensive mechanisms not because they have the largest effect on herbivores, but because the constraints on their evolution are the weakest.

Key-words: antibiosis, co-evolution, genetic covariance, plant defence, plant resistance, plant-insect, secondary chemistry

Introduction

‘The examples cited of [herbivorous] insects ... clearly demonstrate the function of secondary substances in these plants as means of repelling or attracting insects.’

p. 1470 Fraenkel 1959 *Science*

‘The observed patterns clearly point to the critical importance of plant biochemistry in governing the [co-evolutionary] relationships between the two groups.... Of secondary, but still possibly major importance, are mechanical plant defenses...’

p. 605 Ehrlich & Raven 1964 *Evolution*

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Interactions between plants and herbivores are among the most dominant species interactions in nature. Plants form the basal resource of virtually all food webs, and herbivores consume 10–15% of the plant biomass produced annually in both natural and managed ecosystems (Cyr & Pace 1993).

This herbivory can have cascading ecological and ecosystem-level effects (Crowley 1983; Bardgett, Wardle & Yeates 1998; Wimp & Whitham 2001; Stark, Julkunen-Tiitto & Kumpula 2007). These ecological interactions have fueled ongoing co-evolution between plants and herbivores, whereby plants have evolved an arsenal of defences that reduce the amount and impact of herbivory, and herbivores have evolved countermeasures to thwart these defences (Ehrlich & Raven 1964; Futuyma & Slatkin 1983; Karban & Baldwin 1997; Karban & Agrawal 2002).

As the opening quotations imply, the earliest appreciation for the role of plant traits in providing resistance against herbivores came with the recognition that secondary metabolites (e.g. terpenoids, glucosinolates, tannins) and physical plant traits (e.g. latex, trichomes) influence the feeding patterns of arthropod herbivores (Dethier 1941; Fraenkel 1959; Krieger, Feeny & Wilkinson 1971). More recent research has found, for instance, that higher concentrations of glucosinolates and greater densities of trichomes in *Arabidopsis thaliana* reduced herbivory by two flea beetle species (Mauricio 1998). These traits can also evolve as adaptive defences since there exists heritable variation for glucosinolate and trichome levels, and herbivores selected for an increase in these levels (Mauricio & Rausher 1997). Similar patterns associated with the functional role and evolution of plant secondary chemistry have been observed in several plant-herbivore systems in a micro-evolutionary (within-species) context (Fordyce & Malcolm 2000; Kessler, Halitschke & Baldwin 2004; Agrawal 2005; Despres, David & Gallet 2007; Mitra *et al.* 2008; Johnson *et al.* 2009a). These patterns are not universal, however, as increased concentrations of secondary compounds can confer susceptibility to specialist and generalist herbivores (Mithen, Raybould & Giamoustaris 1995; Agrawal, Gorski & Tallamy 1999; Lankau 2007; Bidart-Bouzat & Kliebenstein 2008).

Macroevolutionary (between-species) patterns associated with the evolution of chemical and physical plant traits, and the co-evolution of their herbivores, also provide some of the strongest support for the hypothesis that these traits are adaptive defences (Ehrlich & Raven 1964; Berenbaum 1990; Agrawal 2007). For example, the innovation of laticifers and cardiac glycosides in *Asclepias* provided novel physical and chemical defences effective against most herbivores, which contributed to the rapid diversification of the clade (Farrell, Dussourd & Mitter 1991; Farrell & Mitter 1998). Recent evidence shows that the initial evolution for increased latex and cardenolides was followed by a decline in the concentrations of these traits (Agrawal & Fishbein 2008), probably because of counter adaptations and diversification in specialist herbivores (Holzinger & Wink 1996; Farrell & Mitter 1998). Based on these types of data (also see Becerra 1997, 2003), it seems likely that chemical and physical plant traits play important ecological and evolutionary roles in defence as proposed by early pioneers in the field (Dethier 1941; Fraenkel 1959; Ehrlich & Raven 1964). Nevertheless, the role of these traits might not be as straightforward as originally believed.

Many empirical studies have found that plant traits without obvious associations with resistance can influence the prefer-

ence and performance of herbivores. For example, variation within and between plant species for physiological traits such as water content and nitrogen concentration are correlated with the performance of many herbivore species (Scriber & Feeny 1979; Mattson 1980; White 1984; Agrawal 2004; Johnson 2008). Phenological traits can also have large effects on herbivory, as in *Helianthus annuus* where late flowering individuals experience reduced damage by weevils and moths (Pilson 2000). Studies like these have shown that a large diversity of traits, including primary and secondary chemistry, physiological, morphological and life-history traits, all relate to a plant's resistance to herbivores. As such, it is increasingly recognized that a plant's defence may depend on the effects of these traits acting in concert (Coley, Bryant & Chapin 1985; Kursar & Coley 2003; Agrawal & Fishbein 2006; Agrawal 2007).

Despite these advances, we lack an understanding of the traits that are most strongly associated with resistance against herbivores, and the relative importance of different types of traits involved in defence (Karbon & Baldwin 1997; Stamp 2003). This information is needed to fully understand the ecology and evolution of plant defence, and is necessary to broaden current applications of plant-defence theory to agricultural systems. In this quantitative review, we attempt to fill this gap by answering the following questions: At a general level, does variation in secondary chemistry (SM) correlate with resistance more strongly than all non-secondary metabolite plant traits (NSM)? On a narrower but related level, what plant traits (secondary chemistry, morphology, life-history, primary chemistry and physiology) are the best predictors of resistance to herbivores? Are certain functional groups of herbivores, such as chewers and piercing-sucking feeders, influenced more strongly by particular types of plant traits? And finally, does variation in plant form (e.g. herbaceous versus woody) moderate effects of certain classes of plant traits on herbivores? To answer these questions at the microevolutionary scale, we collated data from published studies that estimated genetic correlations between genetic variation in plant traits and herbivore susceptibility using an ecological genetics design (Table S1), and then synthesized these data with meta-analysis. The paucity of data at the macroevolutionary scale (Table S2) prevented a robust meta-analysis, but we also provide a preliminary quantitative review of the trends found among existing datasets at this scale.

Materials and methods

DATA MINING, STUDY SELECTION CRITERIA AND EFFECT SIZE ESTIMATION

We compiled and synthesized a comprehensive dataset of published studies testing pairwise correlations between trait values and herbivore susceptibility. A study was included in our dataset if it estimated variation in at least one plant trait and one measure of herbivore susceptibility. Relevant studies were identified by querying Thomson Scientific's Web of Science online database (ISI; <http://apps.isiknowledge.com>) using the following keyword searches: 'resistance', 'mean famil*', 'plant resistance', 'genetic var*', 'plant damage', 'genetic correlatio*', 'antibiosis', 'antixenosis', 'herbivor*',

'plant defens*', 'plant defence*', 'plant-insect', 'insect damage', 'herbivor* damage' and 'plant trait'. We also examined studies cited in the papers identified above as well as those reported in a previous meta-analysis (Leimu & Koricheva 2006).

We then narrowed our selection criteria to studies only reporting genetic correlations between plant traits and susceptibility to herbivores – these studies have an advantage over research testing phenotypic correlations because their results can be directly interpreted in an evolutionary context (Lande & Arnold 1983; Rausher 1992). These studies typically used the means of families (e.g. full-sib, paternal half-sibs), genotypes, isogenic lines, clones, accessions, and cultivars as their unit of replication. Plant susceptibility to herbivores was estimated according to the amount of damage, the preference and performance of individual herbivores. Insects can damage plants in many different ways and so we utilized herbivore damage data that included any measure of the quantity of tissue removed by herbivores, including: the number or % of damaged leaves, severity of aphid damage, biomass removed, leafminer sting/leaf area (see Supporting Information Table 1 for a complete list of response variables). Gall and miner density were also included as 'damage' because the presence of such herbivores implies foliar damage. We defined herbivore preference as any measure of herbivore abundance or density in which herbivores were allowed to naturally colonize or choose between plants. Herbivore performance included the growth rate of individual herbivores or populations, herbivore mass, survival, number of hatched larvae and insect maturation time. When resistance (1–relative damage), antibiosis (a reduction in herbivore performance) or antixenosis (a reduction in herbivore preference) were reported, the sign was inverted to reflect plant susceptibility. Since we found no difference in effect sizes among these three classes of herbivore susceptibility (i.e. damage, preference and performance; between-group heterogeneity test using a traditional fixed-effects meta-analysis; $Q_b = 2.96$, d.f. = 2, $P = 0.23$), we combined these data and do not distinguish among them when reporting results. In

general, a positive effect size describes that on average, an increase in the value of a plant trait is associated with an increase in susceptibility to herbivores.

Genetic correlations were quantified as Pearson product-moment correlation coefficients (r). When Spearman correlations (ρ) were reported, we transformed these data with $r = 2 \sin(\pi\rho/6)$ when the sample size of the study was $N < 90$, and we did not transform the data when $N > 90$ since $r \approx \rho$ at this level of replication (Lajeunesse in press). If the coefficient of determination R^2 was reported we took the square root to estimate r . When authors reported a range of R^2 values we used the highest value as per Leimu & Koricheva (2006). We also did not include studies reporting partial coefficients from multiple regression analyses. Very few studies reported results using multiple regressions, and although they contain information valuable for our review, it was too difficult to extract r from partial correlations given that they are dependent on which traits were included in the final model. Only a fully reported model would be useful to extract these data. However, when possible, raw pairwise r was recovered by contacting the corresponding authors of the publication. Finally, all r correlations were transformed into Z-score effect sizes prior to analyses (following Rosenthal 1991).

Our final dataset included $K = 499$ genetic correlations from 66 studies published between 1983–2010 (Table S1, Appendix S1). These correlations were reported for 40 species (including plant varieties and hybrids) from 19 plant families. These published studies also included 65 herbivore species from 33 families and 15 orders.

TRADITIONAL AND PHYLOGENETICALLY-INDEPENDENT META-ANALYSIS

The Z-transformed correlation coefficients were pooled using both traditional and phylogenetically-independent meta-analysis

Table 1. Phylogenetically-independent meta-analysis of genetic correlations between plant traits and susceptibility of plants to herbivory. Pooled effect sizes (\bar{Z}_i^p) and review sample sizes (K^p in brackets) are reported for two major subgroups: (a) contrast between secondary (SM) and non-secondary metabolites traits (NSM), and (b) contrast among traits described as physical, gross morphology, life–history, and primary chemistry and physiology. Significant non-zero genetic correlations (i.e. effect sizes) are in bold and are based on 95% confidence intervals, and Q_b^p test evaluating between-group differences are reported in Table S5

	NSM trait subgroups					
	SM	NSM	Physical	Gross morphological	Life-history	Primary chemistry and physiology
Feeding guild						
Browsers	0.159 (2)	0.108 (3)	0.004 (2)	0.273 (2)	–0.237 (1)*	0.064 (2)
Chewers	–0.035 (15)	–0.107 (25)	–0.136 (14)	–0.145 (19)	–0.240 (10)	–0.162 (5)
Endophytes	0.096 (3)	0.086 (7)	–0.362 (1)*	0.278 (5)	0.049 (3)	0.001 (2)
Piercing/sucking	0.132 (4)	0.131 (18)	0.133 (6)	0.230 (9)	–0.062 (5)	–0.026 (2)
Herbivore specificity						
Specialist	–0.041 (13)	–0.171 (28)	–0.190 (13)	–0.011 (17)	–0.417 (10)	0.005 (5)
Generalist	0.035 (13)	–0.072 (28)	–0.107 (11)	–0.076 (14)	–0.078 (10)	–0.084 (5)
Plant longevity						
Annual	–0.141 (5)	–0.207 (17)	–0.130 (5)	–0.168 (13)	–0.441 (9)	–0.266 (1)*
Biennial	0.413 (2)	–0.039 (1)*	0.030 (1)*	0.101 (1)*	–0.065 (1)*	–0.309 (1)*
Perennial	0.044 (13)	0.162 (24)	0.019 (13)	0.223 (12)	0.164 (6)	0.058 (7)
Plant life–form						
Herbs	0.011 (13)	–0.086 (29)	–0.069 (14)	–0.034 (20)	–0.244 (14)	–0.116 (5)
Woody plants	0.070 (6)	0.208 (10)	–0.110 (5)	0.338 (5)	0.274 (2)	0.068 (4)

*These effect sizes should be interpreted with caution because they were derived from single species and thus are the statistical equivalent of a pooled effect derived from a traditional fixed-effects meta-analysis.
NSM, non-secondary metabolite, SM, Secondary metabolites.

(see Hedges & Olkin 1985; Lajeunesse 2009). All traditional meta-analyses (regression weighted by the within-study variances) were performed using METAWIN (version 2.1; Rosenberg, Adams & Gurevitch 2000). In these analyses, the unit of replication was individual *Z*-scores for specific correlations among plant traits, but for many studies multiple *Z*-scores were available for multiple categories of plant traits. These multiple effect sizes extracted from single studies were treated as independent because we were interested in testing for potential moderator effects among multiple plant trait categories. Finally, our results from phylogenetically-independent and traditional meta-analyses were similar, and so we focus our interpretation and description of results on the phylogenetically-independent methods. All results from traditional analyses are provided online (Tables S3 and S4).

To control for the evolutionary history of plants, we applied Lajeunesse's (2009) method to integrating phylogenetic history into meta-analysis using a weighted GLS approach. First, a phylogenetic hypothesis of the 42 plant species was assembled from a modified megatree of all major plant groups based on APG III. (2009) using PHYLOMATIC (Webb & Donoghue 2004). The internal branch-length (BL) distances of this phylogeny (i.e. temporal ordering of nodes) were based on the estimated divergence times of major plant lineages compiled by Hedges, Dudley & Kumar (2006) and Hedges & Kumar (2009). Species lacking phylogenetic information were placed as polytomies at the root of their family or genus should multiple species have the same genus/family (see Fig. S1). We then converted this ultrametric tree into a phylogenetic correlation matrix (*P*) that has the standardized shared BL distance of each species in off-diagonals and ones in the main diagonal (Grafen 1989; Rohlf 2001). These correlations are then used to modify the weighted regression scheme of meta-analysis (see Lajeunesse 2009; Lajeunesse, Jennions & Rosenberg in press). For each moderator variable (i.e. for each categorical factor or predictor that could explain structure in the variation of effect sizes), a subset tree was used to estimate *P* for each category – this *P* contains only the relevant species for which effect size data was available and conserves all the BL information found in Fig. S1. Finally, for many studies, there were multiple correlations available for each moderator category for a given species. To resolve this issue, we pooled these multiple effect sizes prior to our phylogenetically-independent meta-analysis. This pooling resulted in one representative (pooled) effect size and variance for each species within a given moderator category, and also resulted in smaller sample sizes for each moderator category given that the unit of the review was now individual species (K^P = number of species). These multiple effect sizes for each species were pooled using a traditional meta-analysis with a fixed-effects model (following Lajeunesse, Jennions & Rosenberg in press).

For both the traditional and phylogenetically-independent meta-analysis, the statistical significance of pooled correlations (\bar{Z}_+) between plant traits and herbivore susceptibility was assessed using the bias-corrected 95% bootstrap confidence intervals (CI) generated from 4999 iterations. Pooled effects sizes were considered statistically significant if CI did not overlap zero (Adams, Gurevitch & Rosenberg 1997). We tested the explanatory power of moderator effects (details below) using the between-group Chi-squared test Q_B (Hedges & Olkin 1985). A significant Q_B (Q_B^P for phylogenetically-independent test) indicates that the moderator grouping is a significant explanatory variable for heterogeneity among effect sizes. Finally, a random-effects model was assumed for all analyses (following Gurevitch & Hedges 1999).

MODERATOR EFFECTS AND HYPOTHESIS TESTS

To identify which category of plant traits best predicted the correlations to herbivore susceptibility, we pooled effect sizes into moderator subgroups of plant traits among five major functional categories: (i) secondary metabolites (SM); (ii) physical traits; (iii) gross morphology; (iv) life-history; and (v) primary chemistry and physiology. SM included any compound not directly involved in the primary function of a plant's physiology, growth or resource acquisition, which included the activity of enzymes directly involved in secondary metabolite production (e.g. myrosinase). Physical traits were non-chemical leaf and stem characteristics that could prevent insect herbivores from damaging a plant (e.g. trichome density, leaf toughness). Gross morphological traits included large physical structures (e.g. biomass, height, number of branches) and the size of plants. Life-history included traits that described the rate of growth, phenology and investment in reproductive structures. Finally, primary chemistry and physiological traits included concentrations of elements or plant processes directly involved in growth and resource acquisition. We were also interested in whether the strength of correlations between plant traits and herbivore susceptibility could depend on aspects related to the biology of either the plants or herbivores. Each insect species was categorized according to: feeding guild (browsers, chewers, endophytes like miners, gallers and seed predators, piercing/sucking), herbivore type (vertebrate vs. invertebrate), herbivore specificity (specialist: one or two plant families; generalist: three or more plant families). We also categorized plant species according to their longevity (annual, biennial, perennial) and life-form (herbs or woody plant).

To test the relative importance of secondary metabolites as resistance traits, we initially contrasted secondary metabolites (SM) vs. non-secondary metabolite (NSM) plant attributes as factors that could explain variation in susceptibility to herbivores. We then tested whether this result was conditional on: feeding guild, host specificity, plant longevity and plant life-form. To more precisely identify the types of traits that best predict herbivore susceptibility, we performed the same analyses but contrasted SM, physical traits, gross morphological traits, life-history traits, and primary chemistry and physiology traits.

Finally, we examined for publication bias using funnel plots (Hunter & Schmidt 2004; Fig. S2). In the absence of publication bias, it is expected that the variation around the overall mean effect would have the shape of a symmetric funnel – where the variation in effect sizes decrease with increasing within-study sample sizes and that the effect size is independent of sample size (Palmer 1999). We tested for a bias against nonsignificant (null) results by visually inspecting funnel plots for a characteristic gap in the lower inner area of the funnel, which would suggest that nonsignificant results were missing (Hunter & Schmidt 2004). Finally, we tested for the independence of effect sizes and their sample sizes using a Spearman's rank correlation, and assessed their overall distribution using a weighted histogram and a normal quantile plot (Fig. S3).

Results

BIAS AND VARIATION AMONG CORRELATIONS

We found no evidence for publication bias in our data (Fig. S2). Effect sizes were symmetrically and normally distributed (Figs S2 and S3), and the correlation between sample size and *Z*-transformed effect sizes was negligible (Spearman correlation; $R_s = 0.04$, d.f. = 498, $P = 0.381$).

Importantly, there was significant variation among effect sizes (phylogenetically-independent within-study heterogeneity test assuming a fixed-effects model: $Q_T^P = 1353.0$, d.f. = 41, $P < 0.0001$). This is evidence that variation among the effect sizes was not due to sampling error and it provides justification for: exploring which types of plant traits provide the strongest predictors of herbivore susceptibility; whether genetic correlations varied as a function of herbivore and plant attributes; and for assuming a random-effects model for pooling correlations (see Gurevitch & Hedges 1999).

WHAT PLANT TRAITS PREDICT RESISTANCE TO HERBIVORES?

Overall, genetic correlations between SM and herbivore susceptibility were not significantly stronger than correlations between NSM and herbivore susceptibility (phylogenetically-independent between-group test: $Q_B^P = 0.07$, d.f. = 1, $P = 0.786$; Table S3). When NSM were further subdivided, a significant difference was detected among plant trait categories ($Q_B^P = 10.77$, d.f. = 3, $P = 0.013$; Table S3), but genetic variation in life-history traits was the only consistently significant predictor of herbivore susceptibility ($\bar{Z}_+^P = -0.22$, 95% CI: -0.37 to -0.06 , $K^P = 16$). Correlations involving SM and other traits were weaker and did not differ from zero.

ARE CERTAIN FUNCTIONAL GROUPS OF HERBIVORES INFLUENCED MORE STRONGLY BY PARTICULAR TYPES OF PLANT TRAITS?

Genetic correlations between plant traits and herbivore susceptibility depended on the feeding guild of the focal herbivore ($Q_B^P = 15.58$, d.f. = 3, $P = 0.001$; Fig. 1). On average,

genetic variation in SM was not related to the performance of herbivores from any feeding guild, while NSM had relatively strong correlations with chewing insects, mammalian browsers and endophytes (Table 1). Specifically, gross morphological and life-history traits negatively correlated with chewing insect performance (Table 1). Life-history traits also negatively correlated with damage by mammalian browsers while gross morphology was positively related to damage. The strongest negative genetic correlations across all feeding guilds were those observed between physical plant traits and susceptibility to endophytic herbivores (Table 1). Trait classes did not significantly vary in their ability to predict damage by piercing-sucking herbivores (Table 1).

The effects of plant traits on herbivore susceptibility did not vary between specialist and generalist feeding herbivores ($Q_B^P = 0.07$, d.f. = 1, $P = 0.785$). However, in the case of specialist herbivores, while genetic variation in SM was not consistently correlated with susceptibility, correlations with NSM were significantly negative and nonzero (Fig. 2). Among NSM traits, physical and life-history traits were both negatively correlated with susceptibility, while there was no clear association with other traits. No plant trait consistently correlated with susceptibility to generalist herbivores (Table 1).

DOES VARIATION IN PLANT ATTRIBUTES LEAD TO DIFFERENTIAL EFFECTS OF CERTAIN CLASSES OF PLANT TRAITS ON HERBIVORES?

The association between plant traits and herbivore susceptibility greatly depended on whether a plant species was annual, biennial or perennial (i.e. plant longevity) ($Q_B^P = 11.12$, d.f. = 2, $P = 0.004$). Within annuals, only genetic

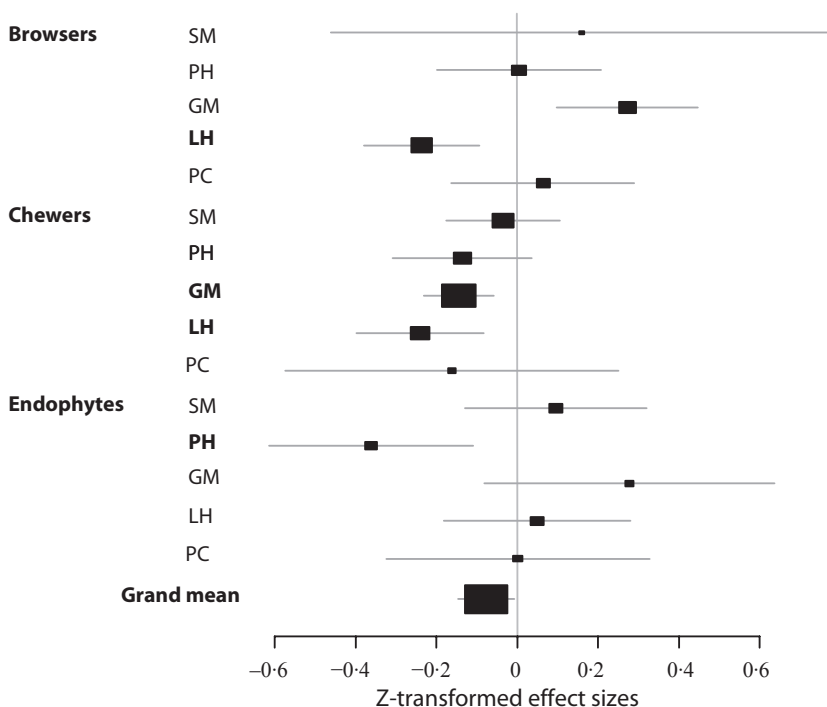


Fig. 1. The difference among the strength of the correlation between susceptibility to herbivory and plant traits among feeding guilds. Trait categories are abbreviated as follows: Secondary metabolites (SM), physical leaf and stem traits (PH), gross morphological (GM), life-history (LH) primary chemistry and physiology (PC). A positive effect size describes that on average, an increase in the value of a plant trait is associated with an increase in susceptibility to herbivores. The effect sizes for piercing-sucking insects did not significantly differ from zero and so they are not shown (see Table 1). The size of the square around each mean effect size is proportional to the weight of this mean effect in the overall meta-analysis; that is, it indicates which trait category contributed the most to the grand mean (Lewis & Clarke 2001). Bold abbreviations indicate non-zero mean effects.

Fig. 2. The differences in effect sizes of genetic correlations between plant traits and plant susceptibility for specialist herbivores. Effect sizes for generalist herbivores not shown because they did not significantly deviate from 0 (see Table 1). The abbreviations and interpretation of results are the same as Fig. 1.

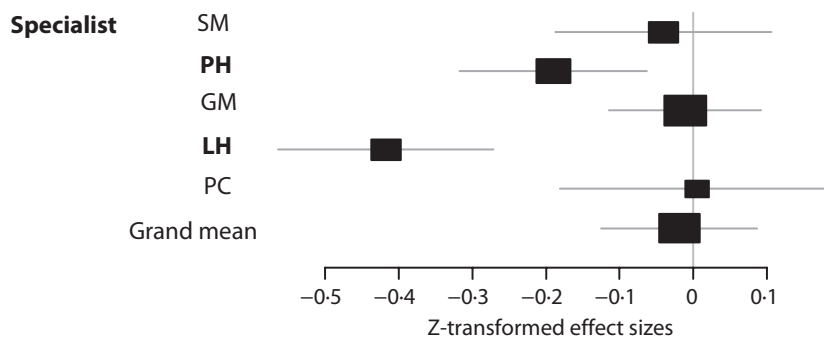
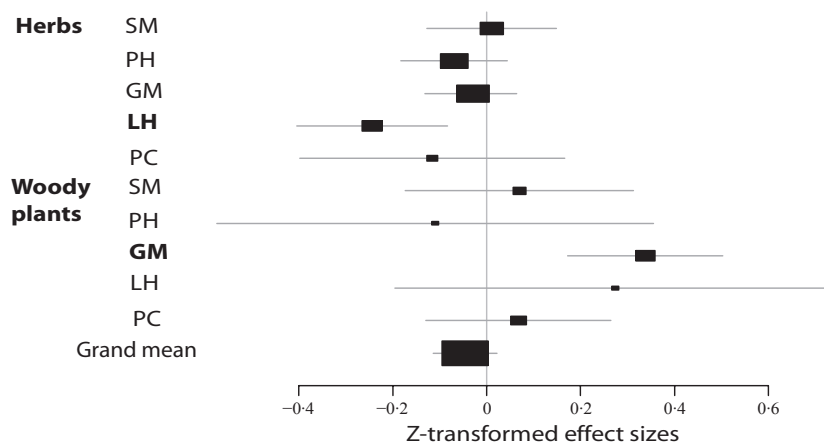


Fig. 3. Comparison of the strength of correlation between plant traits and plant susceptibility among herbaceous and woody plants. The abbreviations and interpretation of pooled effects are the same as in Fig. 1.



variation in NSM was significantly related to herbivore susceptibility, where gross morphology, life-history and primary chemistry exhibited the highest negative correlations (Table 1). In biennials, SM significantly correlated with susceptibility, but on average these correlations were positive. For perennial plants, gross morphology and life-history traits exhibited the highest correlations but all confidence intervals overlapped with zero (Table 1).

Finally, plant life-form (herbaceous vs. woody plants) also explained differences among genetic correlations between plant traits and susceptibility to herbivores ($Q_B^P = 4.81$, d.f. = 1, $P = 0.028$). Life-history traits were significantly negatively correlated with susceptibility in herbaceous plants while gross morphology positively correlated with susceptibility in woody plants (Table 1; Fig. 3). SM provided a non-significant and weak predictor of herbivore susceptibility across life-forms (Table 1; Fig. 3).

RELATIONSHIP BETWEEN PLANT TRAITS AND HERBIVORE SUSCEPTIBILITY AT A MACROEVOLUTIONARY SCALE

To understand whether our results observed at the microevolutionary scale could also be replicated at a macroevolutionary scale, we performed a second traditional meta-analysis where correlations between plant traits and herbivore susceptibility were estimated at the interspecific levels instead of the intraspecific level. Few datasets are available at this scale (6 studies which used 6 plant species and 90 effect sizes; Table S2) and so we interpret them with caution.

Similar to our results at the microevolutionary scale, variation in NSM were negatively related to susceptibility ($\bar{Z}_+ = -0.33$, 95% CI = -0.49 to -0.17 , $K = 78$), whereas variation in SM did not differ from zero ($\bar{Z}_+ = -0.02$, 95% CI = -0.24 to 0.23 , $K = 12$). Within NSM, physical traits were negatively related to susceptibility ($\bar{Z}_+ = -0.44$, 95% CI = -0.60 to -0.23 , $K = 42$), while other traits showed no overall relationship. Unfortunately life-history traits could not be evaluated due to a very small review sample size. Despite the deterrent effect of physical traits, there was no overall difference among the mean pooled effects ($Q_B = 0.31$, d.f. = 3, $P = 0.959$).

Discussion

The most striking result of our meta-analysis is that plant secondary metabolites did not significantly predict resistance to mammalian and insect herbivores. By contrast, genetic variation in life-history, gross morphology, physical leaf and stem traits, and primary chemistry and physiology were most strongly related to herbivore susceptibility. This result is surprising because it is widely believed that the primary function of secondary metabolites is to defend plants against herbivores and pathogens. Our findings call into question a paradigm that has pervaded the thinking and direction of research on the evolution of plant defences for five decades (Fraenkel 1959; Ehrlich & Raven 1964; Rosenthal & Janzen 1979; Fritz & Simms 1992; Karban & Baldwin 1997; Stamp 2003; Berenbaum & Zangerl 2008). We argue below that secondary metabolites are still important in the evolution of defence, however, perhaps not for the reasons commonly invoked.

Our results further suggest that the recently advocated 'syndromes' approach to the study of plant defence evolution might be the key to understanding why the world is green (Kursar & Coley 2003; Agrawal 2007).

WHY DO SECONDARY METABOLITES NOT PREDICT SUSCEPTIBILITY TO HERBIVORES?

Although it has long been recognized that many types of traits are involved in defence against herbivores, it is widely believed that secondary metabolites play the dominant role in the ecology and evolution of plant defence (Rosenthal & Janzen 1979; Bennett & Wallsgrrove 1994; Theis & Lerdau 2003; Berenbaum & Zangerl 2008; Orians & Ward 2010). Why then does our review of ecological genetics experiments, which estimate the strength of genetic correlations between secondary chemistry and herbivore susceptibility, not support this view? We offer three potential explanations.

Firstly, individual plants contain a vast array of secondary compounds and specific secondary metabolites that might have evolved to defend plants against a specific herbivore or specific groups of herbivores (Bennett & Wallsgrrove 1994; Harborne & Williams 2000; Theis & Lerdau 2003; Macel, Van Dam & Keurentjes 2010; J.P. Salminen, unpublished data). Although some classes of chemicals have been portrayed as having general effects on herbivores (e.g. condensed tannins, see Feeny 1976), other chemicals appear to be effective against specific subsets of natural enemies (Linhart & Thompson 1999; Macel *et al.* 2005; Leiss *et al.* 2009; J.P. Salminen, unpublished data), or are most effective in combination with a specific mixture of other secondary metabolites. Moreover, specialist insect herbivores often evolve counter-adaptations to overcome or even benefit from specialized chemical defences (Karban & Agrawal 2002; Despres, David & Gallet 2007). Thus, correlations between herbivore susceptibility and genetic variation in the amount of a specific chemical, or an entire class of chemicals (e.g. cardenolides), can range from negative to positive, and may therefore be expected to show little or no relationship with the level of secondary metabolites.

Secondly, co-evolution and non-adaptive evolutionary processes may cause most secondary metabolites to have no biological function (Jones & Firn 1991). The classic view of co-evolution predicts that plant and insect populations are locked into a co-evolutionary arms-race, where novel defensive chemicals experience strong positive selection because they allow plants to escape herbivory (Ehrlich & Raven 1964). Herbivore populations may subsequently evolve counter-adaptations to overcome these novel defences, which in turn, would again selectively favor novel plant defence chemistry. This co-evolutionary process is thought to have generated the wide diversity of chemical defences observed in many plant clades (Berenbaum & Feeny 1981; Becerra 1997; Farrell & Mitter 1998; Wink 2003; Agrawal & Fishbein 2008), as well as variation in the levels of specific chemical compounds (Zangerl & Berenbaum 2005; Agrawal *et al.* 2009). If such an arms-race renders specific defensive compounds ineffective

against herbivores, then this would lead to the gradual accumulation of chemical diversity – provided the cost of maintaining non-functional metabolites is not too high. If this accumulation process is common, then most secondary metabolites are perhaps relics of past co-evolutionary interactions.

Gene duplications – including chromosome doubling, tandem duplication, and RNA-mediated movement of genes to other parts of the genome – are also likely to be an important mechanism for adaptive and non-adaptive evolution of chemical diversity in plants. Gene duplication can lead to the adaptive evolution of new secondary metabolic functions (Rausher 2006; Des Marais & Rausher 2008), or the non-adaptive divergence of two gene copies (Innan & Kondrashov 2010). Even when one duplicated gene diverges neutrally from a functional gene copy, it might still result in the production of enzymes that catalyze biosynthetic reactions that produce non-functional secondary metabolites. If we accept the argument that most plants do contain an abundance of biological inactive secondary metabolites (Jones & Firn 1991), then detecting those chemicals involved in defence will be akin to finding a 'needle in a haystack'. In which case, we will require the use of newly developed genomic and metabolomic technologies that are not well suited to conventional ecological genetics approaches (Barakat *et al.* 2009; Macel, Van Dam & Keurentjes 2010).

The third explanation for our findings is that traits other than secondary metabolites have larger effects on the preference and performance of herbivores. The relatively strong and consistent genetic correlations involving life-history variation, gross morphology and physical plant traits indicate that this is at least a partial explanation (Table 1). Since increased herbivory must on average result in negative fitness effects, our results imply that herbivores select these traits more strongly than on secondary metabolites. For example, a field experiment that measured genetic variation and selection on all of the classes of traits reviewed here found that the strength of directional selection was 2.6–4.5 stronger on life-history variation (plant longevity) and gross morphology (plant biomass) than on specific secondary metabolites, although the relative contribution of herbivory to the measured selection gradients was unclear (Johnson *et al.* 2009a). Therefore, in systems where herbivores are a potent agent of natural selection, phenology, growth rate, the thickness and hairiness of leaves, and the size and architecture of plants, may evolve as adaptive defences against herbivory. This argument is not a new one. The importance of these traits have been recognized and studied over several decades and they have been explicitly incorporated into the most influential theories of plant defence evolution (Feeny 1976; Coley 1980; Coley, Bryant & Chapin 1985; Herms & Mattson 1992). Nevertheless, the recognition of traits such as phenology and gross morphology as defences runs against the existing paradigm that the ecology and evolution of secondary metabolites represent the *most important* plant defence against herbivores.

The three explanations offered above are not mutually exclusive and all are likely to have contributed to our results.

Indeed, secondary metabolites can be relatively specialized in their functions and are not restricted to a role in defence; many secondary metabolites have no apparent biological function; and, variation in secondary chemistry within natural populations often has less of an effect on herbivores than variation in non-chemical traits. Does this mean less focus should be paid to the defensive role of plant secondary chemistry? Not necessarily, but perhaps a more balanced and pluralistic approach is needed.

DO SECONDARY METABOLITES PLAY A ROLE IN PLANT DEFENCE?

Secondary metabolites do play a role in plant defence. However, the findings of our review lead us to conclude that their role in anti-herbivore defence is more complex than often appreciated. We propose that life-history, morphology and physical leaf and stem traits typically have larger effects on the preference and performance of herbivores than secondary metabolites. However, these traits, critical to a plant's fitness, are controlled by many genes from multiple primary and secondary biosynthetic and physiological pathways, and are subject to selection by many biotic and abiotic factors. Therefore it is likely that there is strong stabilizing selection that maintains an optimal level within populations for these traits and the genes controlling them. Herbivores likely impose selection on life-history and plant morphology, but they are one selective agent among many, and the ability for plant populations to respond might be limited. In other words, the evolutionary constraints acting on non-secondary metabolic traits are strong and natural selection by herbivores may result in little evolutionary response.

We propose the hypothesis that secondary chemistry is important in plant defence, not because of large effects that specific chemicals have on herbivores, but because the evolutionary constraints acting on these traits are relatively weak compared to those acting on other traits. The production of secondary metabolites does involve many genes within branching pathways, and there are often many pleiotropic effects of mutations in these genes (e.g. Rausher 2006). Nevertheless, the pleiotropic effects of genes involved in the biosynthesis of secondary metabolites are likely smaller than traits associated with life-history or morphological variation. Even very weak selection can lead to large adaptive phenotypic changes in the levels and diversity of defensive chemicals over long periods of time. If secondary chemistry is important in defence not because of its large effects but because of weak selective constraints, then we predict that the importance of secondary metabolites in defence would be more evident when comparing the effects of secondary metabolites on herbivores among plants species that reflect macroevolutionary timescales. Although recent studies support this prediction (Agrawal *et al.* 2009; Johnson, Smith & Rausher 2009b), our review of existing macroevolutionary datasets does not, but given the scarcity of data across a small number of systems, we believe the prediction remains to be rigorously tested.

Conclusions

We propose that in a microevolutionary context, plant secondary chemistry has had a secondary role in defending plants against herbivores – second to life-history, morphology and physical resistance traits. We further argue that plant secondary metabolites may still evolve to be potent defences against herbivory over macroevolutionary timescales, not because of their large effects on herbivores but because of the relatively weak selective constraints acting on these traits. Our findings support recent calls for a reevaluation and pluralistic approach to the study of plant defence evolution, which considers the role of traditional and non-traditional resistance traits, as well as correlations between them, on herbivores (Kursar & Coley 2003; Agrawal 2007).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1. The hypothesized phylogenetic relationships of the 41 plant species from 20 different families used in our phylogenetically-independent meta-analysis.

Figure S2. From the traditional meta-analysis: Funnel plot of Z-transformed effect sizes against their within-study sample size ($K = 498$). White dots denote secondary metabolites (SM) and black dots,

non-secondary metabolites (NSM) including physical, gross morphological, life-history and primary chemistry and physiology traits.

Figure S3. From the traditional meta-analysis: (a) weighted frequency histogram of Z-transformed correlations between plant traits and herbivore susceptibility. White bars denote secondary metabolites (SM) and black bars, non-secondary metabolites (NSM) including physical, gross morphological, life-history and primary chemistry and physiology traits. (b) Normal quantile plot testing normal distribution.

Table S1. Database for meta-analysis at microevolutionary level.

Table S2. Database for meta-analysis at macroevolutionary level.

Table S3. Differences among plant trait categories in the strength of pooled genetic correlations (\bar{Z}_+) between plant traits and susceptibility of plants to herbivory. The number of pooled effects are K , and the Q_B test evaluates differences on the strength of the correlation among plant traits. All analyses designated with ^P are phylogenetically-independent.

Table S4. Traditional meta-analysis of correlations between traits and susceptibility of plants to herbivory. Pooled effect sizes and sample sizes (in brackets) are reported for two major subgroups: (a) contrast between secondary (SM) and non-secondary metabolite traits (NSM), and (b) contrast among traits described as physical (PH), gross morphology (GM), life-history (LH), and primary chemistry and physiology (PC). Q_b tests differences in the strength of the correlation among plant traits.

Table S5. The phylogenetically-independent between-group heterogeneity test (Q_b^p) for differences among categories of plant traits potentially related with plant susceptibility to herbivory (see pooled effects for each group in Table 1). The between-group heterogeneity test for differences among feeding guild, herbivore specificity, plant longevity and plant life-form can be found in the text.

Appendix S1. References cited on the meta-analyses databases

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