

# Does plant apparency matter? Thirty years of data provide limited support but reveal clear patterns of the effects of plant chemistry on herbivores

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## Summary

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- According to the plant-apparency hypothesis, apparent plants allocate resources to quantitative defenses that negatively affect generalist and specialist herbivores, while unapparent plants invest more in qualitative defenses that negatively affect nonadapted generalists. Although this hypothesis has provided a useful framework for understanding the evolution of plant chemical defense, there are many inconsistencies surrounding associated predictions, and it has been heavily criticized and deemed obsolete.
- We used a hierarchical Bayesian meta-analysis model to test whether defenses from apparent and unapparent plants differ in their effects on herbivores. We collected a total of 225 effect sizes from 158 published papers in which the effects of plant chemistry on herbivore performance were reported.
- As predicted by the plant-apparency hypothesis, we found a prevalence of quantitative defenses in woody plants and qualitative defenses in herbaceous plants. However, the detrimental impacts of qualitative defenses were more effective against specialists than generalists, and the effects of chemical defenses did not significantly differ between specialists and generalists for woody or herbaceous plants.
- A striking pattern that emerged from our data was a pervasiveness of beneficial effects of secondary metabolites on herbivore performance, especially generalists. This pattern provides evidence that herbivores are evolving effective counteradaptations to putative plant defenses.

## Introduction

Plant secondary metabolites play a significant role in structuring interactions between plants and the network of organisms that comprise terrestrial communities (Ehrlich & Raven, 1964; Berenbaum, 1983; Roitberg & Isman, 1992; Barbosa *et al.*, 1991). In addition to conferring an array of physiological adaptations to plants, such as pigmentation (e.g. flavonoids and carotenoids), protection against UV (e.g. flavonoids), and structure (e.g. lignins), secondary metabolites play key ecological roles by defending plants from herbivores and pathogens (Fraenkel, 1953; Odum & Pinkerton, 1955; Ehrlich & Raven, 1964; Whittaker & Feeny, 1971), providing oviposition and feeding cues (Da Costa & Jones, 1971; Raybould & Moyes, 2001; Macel & Vrieling, 2003; Nieminen *et al.*, 2003), and attracting natural enemies of herbivores (Turlings *et al.*, 1990; Dicke & van Loon, 2000; Kessler & Baldwin, 2001). All plants invest resources in secondary metabolite production (Fraenkel, 1959; Dethier, 1954; Whittaker & Feeny, 1971) which can incur costs (reviewed in Huot *et al.*, 2014), but can also lead to increased plant fitness (Ehrlich & Raven, 1964; Cornell & Hawkins, 2003; Agrawal *et al.*, 2012; Moore *et al.*, 2014). The diversity of secondary

metabolites has resulted in a wealth of research examining the effects of these compounds on herbivores, generating numerous hypotheses focused on evolutionary and ecological patterns of plant defense (McKey 1979; Rhoades, 1979; Feeny, 1975, 1976; Rhoades & Cates, 1976; Bryant *et al.*, 1983; Coley *et al.*, 1985; Herms & Mattson, 1992). These hypotheses have been used to make predictions regarding plant life history traits that correspond to particular classes of antiherbivore defense and have also provided useful information for understanding how plants allocate resources between defensive and physiological functions.

Feeny (1975, 1976) and Rhoades & Cates (1976) formulated hypotheses explaining the evolution of plant defenses based on plant apparency. Apparent plants as defined by Feeny (1976) were plants that are 'bound to be found' or have 'susceptibility to discovery' by herbivores and were characterized as being mature plants that are dominant in late-successional communities. These plants were predicted to adaptively produce quantitative chemical defenses (i.e. high concentrations) as a consequence of the longevity of their leaf tissue. Quantitative compounds were hypothesized to defend plant tissues by reducing herbivore growth rate through decreased digestibility of consumed leaf tissue. As outlined in the original paper, these defenses evolved to

be effective against both specialist and generalist herbivores (in particular invertebrate folivores), although specialists were predicted to be less abundant on these plant types (Feeny, 1975). Rhoades & Cates (1976) elaborated that these secondary metabolites would be present in long-lived woody plants (highly apparent to herbivores) and mature tissue. They predicted that secondary metabolites fitting into this category would include phenolics and tannins; however, it is now known that many of these compounds are not actually defensive in function and do not always act as digestibility reducers in invertebrate herbivores (Bernays *et al.*, 1989; Barbehenn & Constabel, 2011).

Conversely, unapparent plants were defined as being 'hard to find' by their adapted herbivores and were characteristic of early successional communities (Feeny, 1975, 1976). As these plants were predicted to be fast-growing with high reproductive output, they would have a smaller resource pool for internal allocation of metabolites and would probably produce toxic (qualitative) secondary metabolites, which would typically be present at low concentrations in plant tissues. Rhoades & Cates (1976) posited that these metabolites would be present in ephemeral, herbaceous plants and young leaves. In addition, qualitative defenses were predicted to be most effective against nonadapted generalist herbivores as a consequence of their toxicity, while specialists would have evolved physiological or behavioral mechanisms for decreasing the toxicity of these defenses. These compounds were predicted to disrupt herbivore nervous system function, muscle action, and kidney and liver function (Rhoades & Cates, 1976). They predicted that secondary metabolites fitting into this category would include alkaloids, amines, and nonprotein amino acids (most nitrogen-containing compounds).

Although this hypothesis has provided an effective framework for developing experiments, many inconsistencies have been discovered, and many relevant criticisms have been levied against the hypothesis, including: it ignores the role of upper trophic levels, it has not yielded easily testable hypotheses, it is plagued by many unrealistic assumptions, and most plants include a complement of both qualitative and quantitative defenses, so that assigning chemical identities to plants or tissues is often an inaccurate generalization (Price *et al.*, 1980; Bernays & Graham, 1988; Brattsten & Ahmad, 1986; Fagerstrom *et al.*, 1987; Duffey & Stout, 1996). Another major problem with the plant-apparency framework is that it does not incorporate important aspects of chemically mediated interactions, such as synergy, dose dependence, and beneficial effects of secondary metabolites on adapted herbivores (e.g. Raubenheimer & Simpson, 2009; Richards *et al.*, 2010, 2015). For example, while cardenolides are toxic to most insects because they inhibit sodium/potassium (Na/K) ATPase, different concentrations and mixtures have different effects on specialist herbivores, based on their level of resistance as a result of adapted Na/K ATPase (Petschenka & Agrawal, 2015). Thus, high concentrations of putative qualitative defenses have the predicted detrimental effect on survivorship of herbivorous insects, but these compounds have highly beneficial effects on survivorship of *Danaus plexippus*, an adapted specialist, because sequestered cardenolides deter predators (Agrawal *et al.*, 2012). Consequently, this hypothesis of plant defense has been

dismissed as lacking generality and being ineffective in application (Bernays, 1981; Duffey & Stout, 1996; Agrawal *et al.*, 2006). Nevertheless, it is still prominent in the literature, and a modified plant-apparency hypothesis has not been proposed (Haukioja, 2003; Yamamura & Tsuji, 1995; Loehle, 1996; Silvertown & Dodd, 1996; Bustamante *et al.*, 2006). Although it is clear that many exceptions, discrepancies, and faults can be found in this hypothesis, a more quantitative approach is needed to objectively refute or revise it. We used meta-analysis to test the hypothesis that apparent and unapparent plant defenses produce differing effects on herbivore performance as outlined in the plant-apparency hypothesis, and to examine whether these predictions are useful for understanding the evolution of plant defenses.

A recent meta-analysis by Endara & Coley (2011) tested the predictions of the resource availability hypothesis and some of the predictions of the plant-apparency hypothesis. They found that herbivory varied significantly across species; this variation could be attributed to the resource environment of the species rather than species apparency, supporting the resource availability hypothesis. While this paper provided a strong test of the resource availability hypothesis, it did not fully address the plant-apparency hypothesis, as it did not include differences between specialist and generalist herbivores or a specific evaluation of the effects of secondary chemistry on the herbivores. We sought to more fully explore the plant-apparency hypothesis by testing for differences in the effect size of secondary metabolites from apparent and unapparent plants on specialist and generalist herbivores. Accordingly, the goal of this paper was to provide a strong quantitative synthesis with which to evaluate predictions of the plant-apparency hypothesis (Table 1). The hypothesis in its current form equates woody plants to apparent plants, and herbaceous plants to unapparent plants, (Fagerstrom *et al.*, 1987; Stamp, 2003; Bustamante *et al.*, 2006; Endara & Coley, 2011; Mas-sad *et al.*, 2011); thus, we used woody plants to encompass apparency and herbaceous plants to encompass unapparency.

A large body of literature demonstrating effects of various secondary metabolites on herbivores has been amassed since Feeny (1975) and Rhoades & Cates (1976) published their hypothesis of plant defense. To empirically test the validity of their predictions, we performed a meta-analysis on 30 yr of published effects of secondary metabolites on herbivores. We specifically focused on the effects of qualitative and quantitative defenses on specialist and generalist folivores feeding on apparent and unapparent plants. Defenses were categorized as either qualitative or quantitative based upon the description of the compound given in each paper (see the Materials and Methods section for more details). In addition, we investigated the mode of action of secondary metabolites on folivores, and whether apparent plant defenses have digestibility-reducing effects and unapparent plants act through toxic effects. Our results show that, while the plant-apparency hypothesis paved the way for studies and subsequent hypotheses on the evolution of plant defenses, it has in many ways lost its strength in explaining the occurrence and function of plant defenses in their current form.

**Table 1** Predictions made by the plant-apparency hypothesis comparing chemical defenses in apparent and unapparent plants, defenses against specialist and generalist herbivores, and the effects of apparent and unapparent plant defenses on herbivores

Comparison	Prediction	Statistical comparisons
Chemical defense in apparent (i.e. woody) vs unapparent (i.e. herbaceous) plants	Apparent (i.e. woody) plants will have a prevalence of quantitative defenses and unapparent (i.e. herbaceous) plants will have a prevalence of qualitative defenses	Traditional vote count from papers
Defense against specialist vs generalist herbivores	Apparent (i.e. woody) plant defenses will be effective against both generalist and specialist herbivores, while unapparent (i.e. herbaceous) plant defenses will be most effective against nonadapted generalists	Posterior distribution transformations: (1) negative impacts of compounds from woody plants on generalists vs impacts on specialists. (2) Negative impacts of compounds from herbaceous plants on generalists vs impacts on specialists
Effects of chemical defenses from apparent (i.e. woody) vs unapparent (i.e. herbaceous) plants	The effects of consuming compounds from apparent (i.e. woody) plants will be different from the effects of consuming compounds from unapparent (i.e. herbaceous) plants	Comparison of the effects of compounds from woody and herbaceous plants on herbivore (1) growth, (2) feeding, (3) weight, (4) survival, and (5) fecundity

## Materials and Methods

To quantitatively summarize 30 yr of chemical ecology, we utilized meta-analysis, which is a statistical synthesis of the results of many independent studies (Hedges *et al.*, 1999; Gurevitch & Hedges, 2001; Koricheva & Gurevitch, 2014). This technique combines the results of individual experiments into a single data set that allows for generalizable hypothesis tests. A standardized effect size for a specified set of parameters or manipulations is the statistic of interest in meta-analysis; here, we used the log response ratio to measure the effect size (Hedges *et al.*, 1999). The log response ratio is usually calculated as the ratio of the mean outcome of the experimental group ( $X_E$ ) to that of the control group ( $X_C$ ) ( $\log(X_E/X_C)$ ) (Hedges *et al.*, 1999), but this metric can be considerably biased when studies are included that have small sample sizes. To prevent sample size bias, corrected or modified log response ratios should be considered (Friedrich *et al.*, 2011; Lajeunesse, 2015), and thus we used a modified log response ratio equation that corrects bias of small sample size but converges on the traditional log ratio at larger sample sizes:  $\log_e(X_E/(N_E - X_E/N_E))/(X_C/(N_C - X_C/N_C))$ .

### Data collection

Our data search focused on studies in which the performance effects of secondary metabolites on herbivores were reported. In particular, we selected studies in which the herbivore's diet had been manipulated by altering (presence vs absence, and high vs low) secondary metabolite concentration or content and which reported the resulting effects on the herbivore's performance. Performance data relevant to the plant-apparency hypothesis included herbivore development time, feeding data, growth rates, and survivorship. In addition to these variables, we also collected data concerning fecundity and cellular interference. Also included were studies in which other variables were manipulated to change plant chemical concentration, such as CO<sub>2</sub>, temperature, plant age, or plant species, as long

as the resulting difference in concentration of secondary metabolites was reported. From each article, we recorded the mean treatment effect and control effect for the given performance variable being measured in the paper. As certain measurements indicate a detrimental effect when the mean is large (i.e. a larger mean for development time indicates a detrimental effect on the herbivore), we reversed the ratio for these measurements to reflect the true biological effect. Thus, negative effect sizes indicate detrimental effects of secondary metabolites on the herbivore's performance and positive effect sizes indicate a beneficial effect.

Articles were gleaned from all volumes of the *Journal of Chemical Ecology* (1975–2005), as this particular journal focuses on the interactions between animals and plant chemistry, and from an online search using the search engine ISI Web of Science (1975–2005). Each volume of the *Journal of Chemical Ecology* was examined by hand for appropriate articles. Keywords for the online search included: chem\* + defense, herbiv\*, plant + chem\*, secondary + chem\*, secondary + metab\*, qualitative defense\*, and quantitative defense\*. For inclusion in our database of articles, three criteria were required: the paper reported the mean, a measure of variation, and the sample size in either text, tables, or graphs; the system under study was terrestrial; the paper included the name or class and a quantification of the secondary metabolite(s) used in the study. In any study where repeated measurements had been taken over time, only the last measurement was used. From each paper, we obtained a maximum of three effect sizes, but no more than one effect size per individual experiment. Where more than one effect size was reported per experiment, the treatment and control means recorded were chosen randomly from among the available data, using a random number generator. To obtain numerical means and standard deviations from graphs, we used TECHDIG (2.0.0.1, 1998) to digitize the graphs. For our analysis, the treatment group was designated as the group in which the herbivore's diet was manipulated so that secondary metabolites were present or present in higher concentrations, and the control group was designated as the group where no

manipulation had occurred or the herbivore's diet contained lower concentrations of secondary metabolites.

From each paper that met our criteria, we recorded the mean, SD, and sample size, along with predictor variables of interest. If the standard error was reported, we converted it to the standard deviation. Predictor variables included classification as qualitative or quantitative defense, apparent (woody) or unapparent (herbaceous) plant, and specialist or generalist herbivore. A compound was classified as qualitative or quantitative based upon the classification given by the authors, or its chemical classification, or known effective concentration in plants (established from prior studies with the research system). In general, compounds such as phenolics, tannins, flavonoids and lignans that are composed of aromatic five-ring sugars were classified as quantitative defenses. Qualitative defenses included terpenes, saponins, cardenolides, iridoids, alkaloids, phenolic glycosides, glucosinolates and any other nonaromatic compounds.

Herbivore diet breadth was classified according to the designation given in each paper. The majority of papers used the classic definition of specialist and generalist: specialists use one or two host plants within the same family or genus, or feed within one or two families; generalists feed across many different plant families (Futuyma, 1991; Bernays & Chapman, 1994; Thompson, 1994).

The dependent variables in the sampled articles ranged from herbivore survivorship to inhibition of herbivore metabolism. The majority of the studies we used reported effects of secondary metabolites on herbivore growth, development time, fecundity, survivorship, or some measure of feeding efficiency. For the analysis, dependent variables were grouped into five categories based upon their effects on the herbivore (Table 2). Responses in the growth category included measurement of development time over the entire larval stage, one instar, or some other fixed amount of time, and relative growth rates ( $\text{mg d}^{-1}$ ). Responses in the weight category included final instar weight (mg), mean weight change (mg), mean weight gain (mg), adult weight gain (mg), and pupal weight (mg). Herbivore survivorship data typically consisted of the percentage of the total that survived. Feeding efficiency data included any of the main feeding indices: relative consumption rates (RCRs), efficiency of conversion of

digested food (ECD), efficiency of conversion of ingested food (ECI), approximate digestibility (AD), and relative consumption index (RCI). We also included other measurements of feeding efficiency such as mean per cent leaf area eaten (%), mean consumption (mg), per cent consumed (%), dry matter intake (mg), per cent damage (%), and other variants of these variables. Fecundity responses included the number of eggs oviposited, and pupal weights (mg), which can be used as a proxy for fecundity. Cellular effects variables included antioxidant capacity (mmol), metabolism of secondary metabolites (nmol), amount of secondary metabolites sequestered in tissue ( $\mu\text{mol}$ ), and enzyme activity ( $\text{unit mg per protein min}^{-1}$ ).

## Data analysis

We used a hierarchical Bayesian model to analyze our data. There has been a recent increase in studies utilizing a Bayesian approach to analyze meta-analysis data (Myers, 2001; Stewart, 2010; Mila & Ngugi, 2011; Verdu *et al.*, 2012; Ogle *et al.*, 2013, 2014; Dyer *et al.*, 2015) because the approach has several advantages compared with frequentist methods. As outlined by Mila & Ngugi (2011), Bayesian inference allows for parameter uncertainty and better incorporates data heterogeneity into the model. If prior information about the distribution of the data exists, then this information can be used in the prior probability. If several competing models exist, then Bayesian methods allow for full evaluation of the competing models. Finally, accepting null hypotheses in the Bayesian meta-analysis framework is more straightforward, as the posterior probability distributions are actual null hypothesis probabilities; thus, it allows one to report a probability that there are no differences between categories (within stated credibility intervals). All analyses were performed with SAS statistical software (v.9.4; SAS Institute, Cary, NC, USA), using the Markov chain Monte Carlo (MCMC) procedure. Noninformative priors were used with a normal distribution. MCMC runs were conducted for 50 000 generations with the first 40 000 generations discarded as a burn-in. Diagnostic plots of MCMC samples were examined in all cases to ensure adequate approximations of posterior distributions.

An initial examination of the raw data showed that there were balanced numbers of both beneficial and detrimental effects of plant chemistry on herbivore performance. For example, in some cases where the mean value for survival, herbivore mass, or a measure of feeding efficiency was greater in the experimental group (higher concentrations of secondary metabolites), we considered this a beneficial effect on the herbivore relative to the control group (none or lower concentrations of secondary metabolites). The mechanisms for beneficial vs detrimental effects are typically very different (e.g. milkweed cardenolide effects on different herbivores (Petschenka & Agrawal, 2015) or the effects of phytochemistry on specialists vs generalists (Dyer, 1995)). Instead of combining these into one effect size, we kept beneficial effects separate from detrimental effects for all analyses, but they were included in the same model, with all main effects and interactions nested within these different responses. Thus, for each comparison, there are two results to consider, one set of results for

**Table 2** Explanatory variables were grouped into six categories based upon their effects on herbivores. The right column lists the specific variables that were included in each category

Explanatory variable	Response variables taken from papers
Development	Growth rates, time to pupation, duration of instar, larval weight, weight change, and adult weight
Feeding	Feeding efficiency indices: relative consumption, conversion of ingested food, and conversion of digested food
Fecundity	Total number of eggs and pupal weights
Survivorship	Total number or per cent survival
Cellular interference	Antioxidant capacity, metabolism of secondary metabolites, amount sequestered, and enzyme activity

beneficial effect sizes and one for detrimental effect sizes. Corrected log response ratio values can be positive or negative for both of these types of response, but detrimental responses are on average more negative. This could create a problem when attempting to statistically compare the overall magnitude of the beneficial effect sizes (mostly positive values) with that of detrimental effect sizes (mostly negative values), however our posterior distributions reflected the overall magnitude of effect sizes (i.e. absolute values) – this allowed us to compare the overall magnitudes of beneficial vs detrimental effects. Thus, only positive effect sizes are possible in the posterior distributions reported here.

For specific comparisons relevant to plant-apparency hypotheses (Table 1), significant differences between log ratio estimates for different nested categories were assessed with simple transformations of the posterior distributions that were pairwise comparisons of sampled values for the last 10 000 MCMC steps (e.g. Fordyce *et al.*, 2011; Forister *et al.*, 2013). Using this approach, if the effect size for a particular set of categories (e.g. detrimental effects of qualitative defenses on specialists) is greater than the effect size for a comparable level of categories (e.g. detrimental effects of qualitative defenses on generalists) for > 95% of the 10000 MCMC iterations, then the two effect sizes are considered to be different (Fordyce *et al.*, 2011; Forister *et al.*, 2013). We report these as ‘posterior probabilities’ (PPs). We also report the mean posterior log ratio (mplog) for ease of comparison with other meta-analyses, as well as the posterior probability density (PPD), as this is a common Bayesian approach to demonstrating overlap in posterior distributions (e.g. Mila & Ngugi, 2011), and was more conservative than the transformation of the posterior distributions described above. We used the more conservative PPD approach for general unplanned comparisons between different categories examined in the meta-analysis.

## Results

### Summary statistics

Our search yielded 158 suitable papers (see Supporting Information Table S1 for the full data set with a bibliography) and 225 effect sizes spanning a 30-yr time period (1975–2005). We had 100 effect sizes for quantitative defenses and 108 effect sizes for qualitative defenses. Generalist herbivores were more commonly represented than specialist herbivores on woody plants (i.e. apparent plants; 67 vs 27 studies, respectively) (Fig. 1). For herbaceous plants (i.e. unapparent plants), generalist and specialist herbivores were nearly equally observed in the data set (59 vs 55 studies, respectively) (Fig. 1). Our data set was heavily biased toward invertebrate herbivores, with only 31 out of 225 effect sizes (14%) representing vertebrate herbivores. In woody plants, quantitative defenses were more common than qualitative defenses (68 vs 26 studies, respectively) (Fig. 1). By contrast, for herbaceous plants, qualitative defenses were more common than quantitative (82 vs 32 studies, respectively) (Fig. 1). Qualitative defense studies were dominated by the plant family Fabaceae, followed by Brassicaceae and Solanaceae (Fig. S1). Quantitative

defense studies were most represented by Salicaceae, followed closely by Fabaceae, Fagaceae, Lauraceae, and Solanaceae (Fig. S2). Overall, the number of plant families represented in the data set was rather low (33 families). Finally, 79% of papers were focused on the action of single compounds in an artificial diet, while the remaining 21% of the papers used leaf material only, or with compounds added to the surface.

### Beneficial vs detrimental impacts

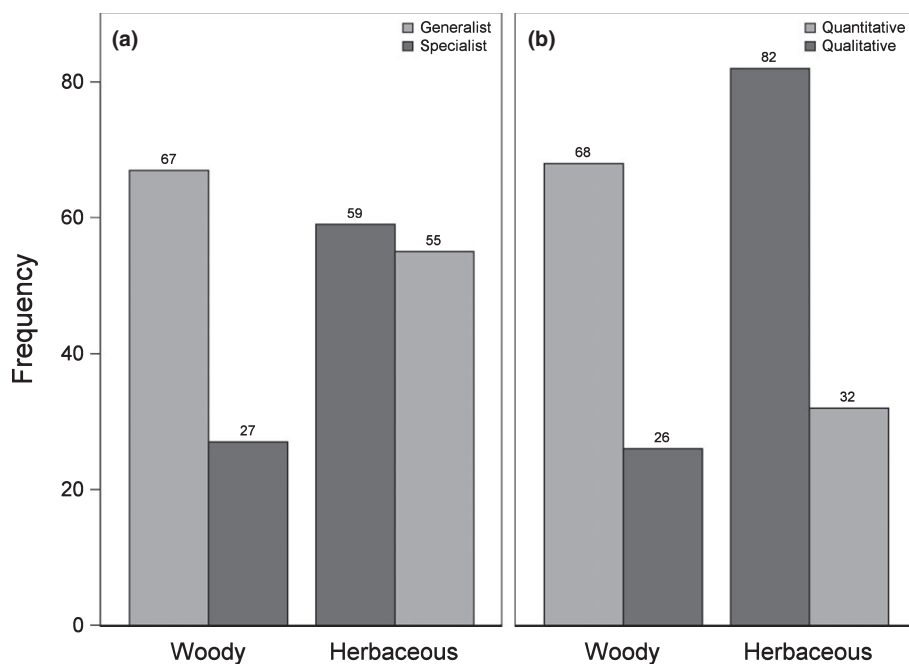
One major result from the meta-analysis was the clear distinction in the magnitude of effect sizes between detrimental and beneficial impacts on herbivore responses (mplog difference = 0.37; PP = 0.95; Fig. 2a); nevertheless, the beneficial impacts on herbivores were still large (mplog = 0.79; Fig. 3a). For qualitative defenses, there were large differences in detrimental impacts vs beneficial impacts (PP = 0.99; Figs 3c, S3), but for quantitative defenses this pattern was not as pronounced (PP = 0.91). The main category driving this difference was the detrimental impacts of qualitative defenses on specialist vs generalist herbivores (PP = 0.98; Figs 3d, S4).

### Chemical defenses in woody vs herbaceous plants

In the studies that we examined, woody (or ‘apparent’) plants were more likely to possess quantitative defenses (68%) rather than qualitative defenses (32%). The opposite was true for herbaceous (or ‘unapparent’) plants, where the majority of defenses were qualitative (75%) rather than quantitative (25%) (Fig. 1). Separating the effect sizes into beneficial and detrimental impacts showed that herbaceous plants had similar proportions of detrimental (52%) and beneficial (48%) impacts on herbivore performance; woody plants also had similar proportions of beneficial (51%) and detrimental (48%) impacts. The impact of defenses in woody plants (mplog = 1.2) was usually greater than that found in herbaceous plants (mplog = 0.9; Fig. 3e; posterior probability for difference = 0.94; Fig. 2c), and this difference was most pronounced for the detrimental impacts on herbivores (PP = 0.98; Fig. 2e) and less pronounced for the beneficial impacts on herbivores (PP = 0.91; Fig. 2g) (Fig. 3f). The highest mean posterior effect sizes were for the detrimental impacts of woody plant defenses on all herbivores (mplog = 1.53; Fig. 3f), and the detrimental impacts of qualitative defenses on specialist herbivores (mplog = 1.52; Fig. 3d).

### Specialist vs generalist herbivores feeding on woody and herbaceous plants

The effect sizes for defenses against specialists vs generalists were not significantly different (PP = 0.83; Fig. 2b), but impacts on specialists were typically higher than impacts on generalists (Fig. 3b). This effect on specialists was consistent whether these herbivores were feeding on compounds from woody or herbaceous plants, or whether the effects were detrimental or beneficial. There were no differences in the effects of woody plant defenses against specialists vs generalists (difference in



**Fig. 1** (a) Proportions of studies of different types of plants and antiherbivore defenses based on a quantitative literature review. For woody plants, there were more studies on generalist herbivores than specialist herbivores, while studies on herbaceous plants had an equal distribution of generalist and specialist herbivores. (b) In these studies, woody plants were more likely to have quantitative defenses than qualitative, and vice versa for herbaceous plants, which were more likely to have qualitative defenses than quantitative defenses. Numbers above bars represent the frequency of articles for each category.

mplog = 0.03; PP = 0.48; Fig. 2d) or herbaceous plant defenses against specialists vs generalists (difference in mplog = 0.24; PP = 0.73; Fig. 2f). The largest and most consistent difference was in the impact of qualitative defenses on specialists vs generalists (mplog difference = 0.41; PP = 0.99) and this was driven by greater detrimental impacts of qualitative defenses on specialists vs generalists (PP = 0.98; Figs 3d, 2h). By contrast, the impacts of quantitative defenses were only slightly greater on generalist (mplog = 1.24) vs specialist (mplog = 1.01) herbivores (PP = 0.76; Fig. 2i). For generalists feeding on compounds from woody plants, the mean detrimental effect size was larger (mplog = 1.50) than the mean beneficial effect size (mplog = 0.90), which is more than a 50% difference between the two means (PP = 0.91; Fig. 2j). For specialists feeding on compounds from woody plants, the mean posterior effect sizes for detrimental and beneficial impacts were similar (1.5 vs 1.3, respectively; PP = 0.57). Overall, the detrimental impacts of woody plant defenses were greater than those of herbaceous plant defenses (mplog difference = 0.63; PP = 0.98) and this pattern was consistent, but weaker for beneficial effects (mplog difference = 0.21; PP = 0.91).

#### Chemical defense and mode of action

**Growth data** There was no significant difference in the effect size for herbivore growth data between woody and herbaceous plants for both beneficial and detrimental analyses (beneficial effect sizes: woody plants, 95% PPD 0.434–1.092; herbaceous plants, 95% PPD 0.647–0.997; detrimental effect sizes: woody plants, 95% PPD 0.329–2.027; herbaceous plants, 95% PPD 0.022–4.392) (Fig. S5).

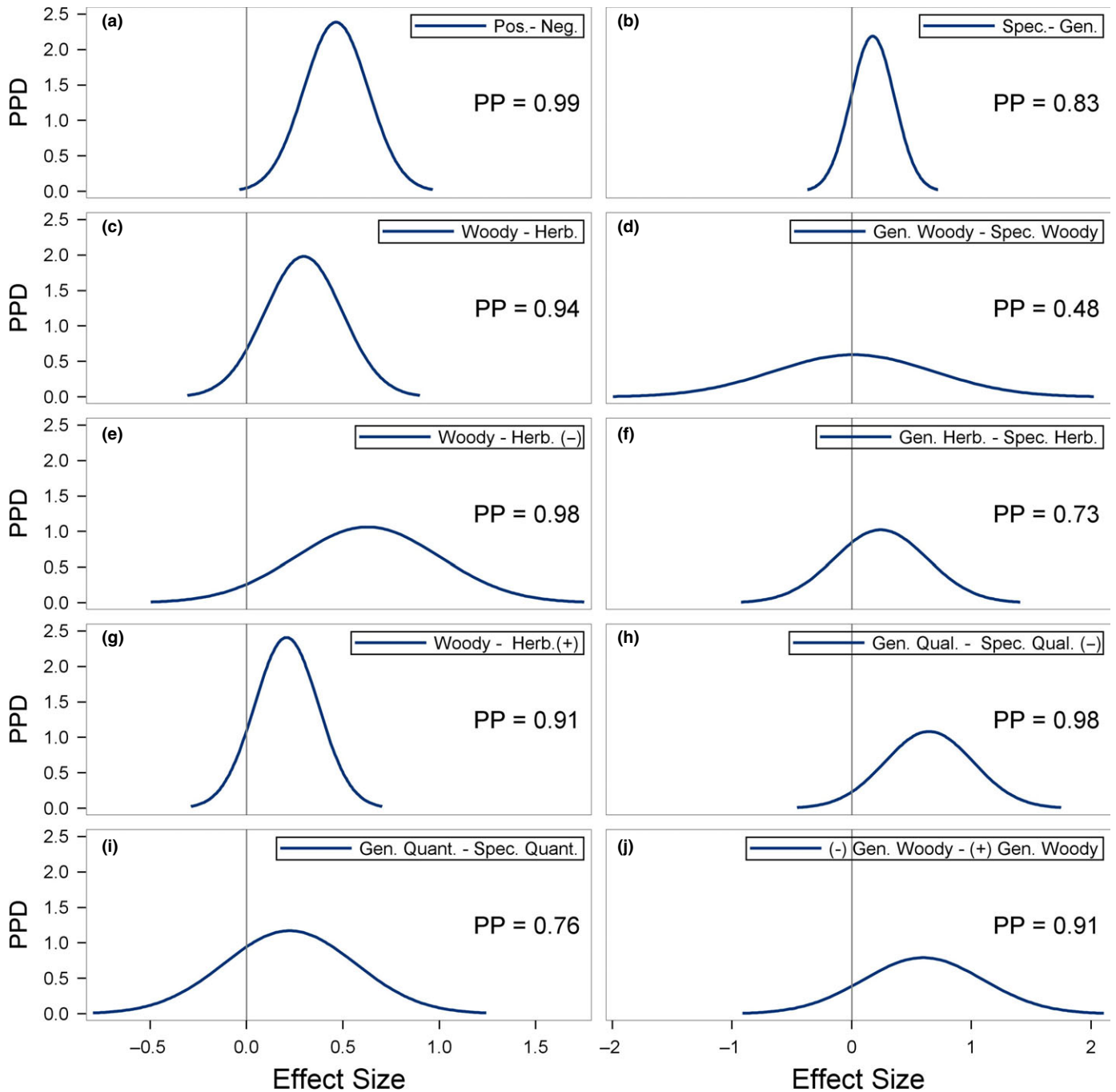
**Feeding data** There was no significant difference in the effect size for measures of herbivore feeding between woody and

herbaceous plants for both beneficial and detrimental analyses (beneficial effect sizes: woody plants, 95% PPD 1.100–2.114; herbaceous plants, 95% PPD 0.953–1.648; detrimental effect sizes: woody plants, 95% PPD 0.530–1.582; herbaceous plants, 95% PPD 0.270–0.855) (Fig. S6). Even though woody and herbaceous plants were not significantly different from each other, we found that beneficial effect sizes for both woody (mean = 1.307) and herbaceous (mean = 1.575) plants were much larger compared with detrimental effect sizes for herbaceous plants (mean = 0.548).

**Weight data** There was no significant difference in the effect size for herbivore weight data between herbivores on woody and herbaceous plants for both beneficial and detrimental analyses (beneficial effect sizes: woody plants, 95% PPD 0.243–1.669; herbaceous plants, 95% PPD 0.592–1.685; detrimental effect sizes: woody plants, 95% PPD 0.304–1.158; herbaceous plants, 95% PPD 0.631–1.326) (Fig. S7).

**Survival data** There was no significant difference in the effect size for herbivore survival between herbivores on woody and herbaceous plants for both beneficial and detrimental analyses (beneficial effect sizes: woody plants, 95% PPD 0.263–2.661; herbaceous plants, 95% PPD 0.180–2.547; detrimental effect sizes: woody plants, 95% PPD 0.472–2.597; herbaceous plants, 95% PPD 0.210–2.110) (Fig. S8).

**Fecundity data** There was no significant difference in the effect size for fecundity data between herbivores on woody and herbaceous plants for both beneficial and detrimental analyses (beneficial effect sizes: woody plants, 95% PPD 0.0495–9.503; herbaceous plants, 95% PPD 0.579–1.654; detrimental effect sizes: woody plants, 95% PPD 0.114–2.832; herbaceous plants, 95% PPD 0.100–1.669) (Fig. S9).



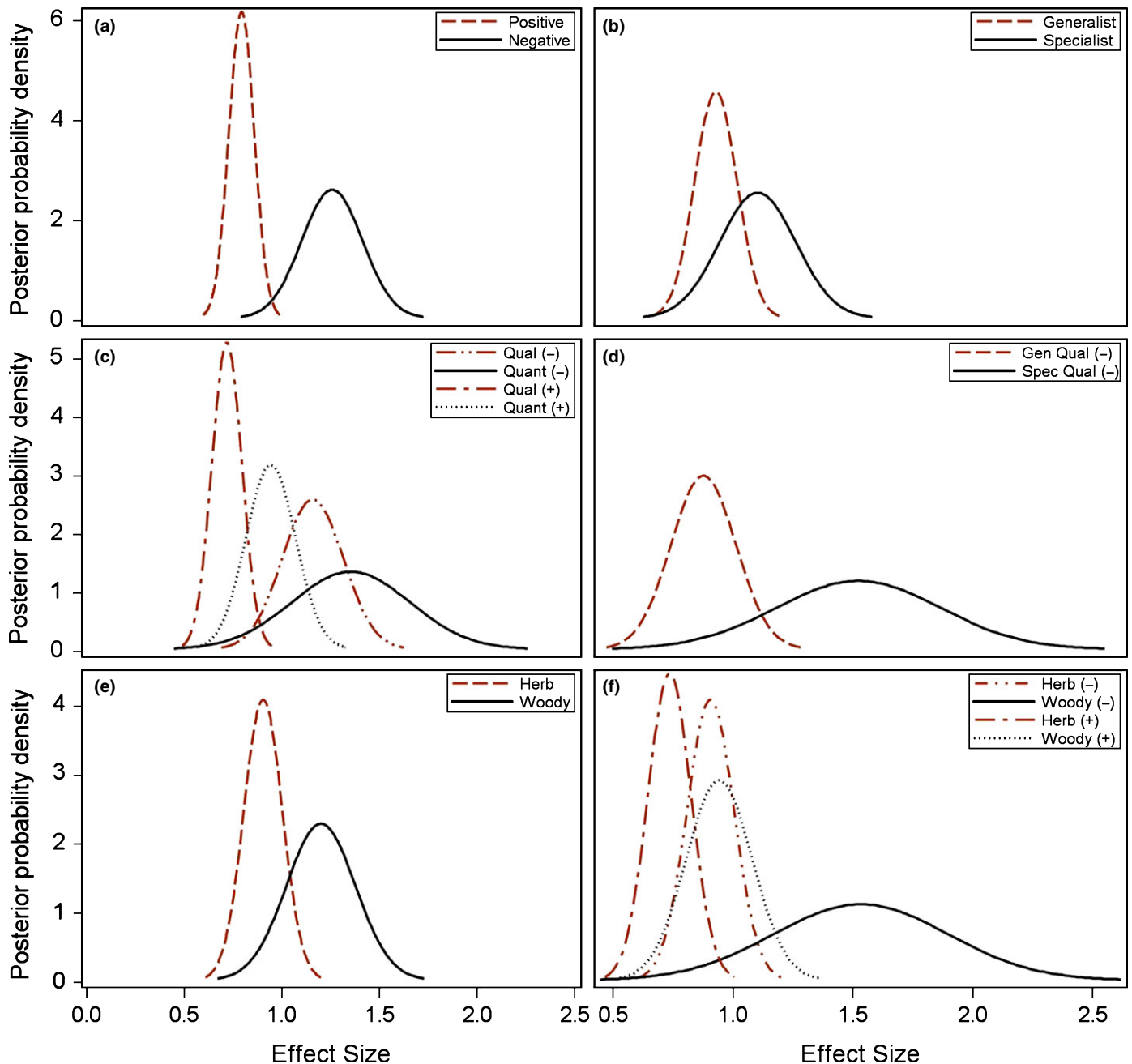
**Fig. 2** Bayesian meta-analysis results from transformations of posterior probability distributions (PPDs) for selected comparisons (a–j). The distributions are considered significantly different from zero if the effect size for a particular set of categories (e.g. negative effects of qualitative defenses on specialists) is greater than the effect size for a comparable level of categories (e.g. negative effects of qualitative defenses on generalists) for >95% of the 10 000 Markov chain Monte Carlo iterations. Gen., generalist; Herb., herbaceous; Neg., negative; Pos., positive; PP, posterior probability; Qual., qualitative; Quant., quantitative; Spec., specialist.

## Discussion

### Plant-apparency predictions

The plant-apparency hypothesis (Feeny, 1975, 1976) has been subject to many interpretations and has been modified from its form in the original papers by Feeny. While Feeny explicitly

focused on the successional stage of a plant community and age of plant and leaf tissue as predictors of a specific chemical profile, the hypothesis in its current form equates woody plants to apparent plants, and herbaceous plants to unapparent plants (Fagerstrom *et al.*, 1987; Stamp, 2003; Bustamante *et al.*, 2006; Endara & Coley, 2011; Massad *et al.*, 2011). Using woody and herbaceous plants as a proxy for plant successional stage broadens the



**Fig. 3** Bayesian meta-analysis results presented as posterior probability densities (PPDs) of corrected log response ratios. (a) PPD of effect sizes for negative and positive impacts of consuming plant secondary compounds for all herbivores. Effects sizes were larger for negative impacts, and significantly different from positive impacts (see Fig. 2a). (b) PPD for effects sizes on specialist and generalist herbivores feeding on diets with different plant secondary compounds. The effect sizes were not significantly different from each other (see Fig. 2b). (c) PPD for the effects of qualitative and quantitative plant defenses on herbivores. Negative qualitative and quantitative impacts were significantly larger than positive qualitative defenses. Overall, negative impacts had a larger effect size. (d) PPD for the negative impacts of qualitative defenses on generalist and specialist herbivores. The negative impacts of qualitative defenses were larger in specialists than generalists. (e) PPD for the effects of plant defenses from herbaceous and woody plants on all herbivores. Consuming chemical defenses from woody plants yielded a larger effect size than consuming chemical defenses from herbaceous plants. (f) PPD for the negative and positive effects of feeding on woody and herbaceous plants. The negative effects of feeding on woody plants were larger than the negative effects of feeding on herbaceous plants, and larger than the positive effects of feeding on both woody and herbaceous plants. Gen, generalist; Herb, herbaceous; Qual, qualitative; Quant, quantitative; Spec, specialist.

generality of the theory; however, it may also weaken the predictive power as the two categories are so broad. It must also be stated that the categories of apparent and unapparent plants are human constructs and plants are unlikely to be viewed in the

same way by small flying or crawling animals. The literature is replete with experimental results detailing the effects of plant secondary metabolites on herbivores, but it is difficult to assess the successional stage or age of the plant tissue used in a given study



(unless explicitly mentioned). With the assumption that woody plants are a good proxy for apparent plants and likewise herbaceous plants are a good representation of unapparent plants, our quantitative literature review provided evidence both supporting and undermining the predictions made by the plant-apparency hypothesis.

Feeny predicted that apparent plants would have quantitative defenses (e.g. tannins and phenylpropanoids) and unapparent plants would have qualitative defenses (e.g. glucosinolates, cardiac glycosides and alkaloids). While this prediction is difficult to test empirically, in our data set, 68% of woody plants (typically classified as ‘apparent’) contained quantitative defenses and 75% of herbaceous plants (typically classified as ‘unapparent’) contained qualitative defenses (Fig. 1). It is possible that investigators are biased toward studying quantitative defenses in woody plants because they have been well characterized, and the incidence and identity of qualitative defenses are less known for these types of plants. The low family-level richness (33 families; Figs S1, S2) of plants used in studies of the effects of secondary metabolites on herbivores indicates that investigators may be relying on a few, well-established study systems. The bias may be the same for herbaceous plants. Nonetheless, these simple summary statistics fit Feeny’s predictions for a predominance of quantitative defenses in woody plants and qualitative defenses in herbaceous plants.

### Secondary metabolites have beneficial and detrimental effects on herbivores

If woody plants are defended against both generalist and specialist herbivores, then we would expect a reasonably large detrimental effect size (i.e. significantly  $> 0$ ) for both these herbivore types, and that there would be no difference between them. Indeed, we found that the largest effect sizes were the detrimental impacts of woody plant defenses on all herbivores (Fig. 3f), and there was no difference in the effect size between specialist and generalist herbivores feeding on woody plants, supporting the plant-apparency hypothesis (Fig. 2d).

By contrast, if herbaceous plants are better defended against nonadapted generalists than specialists, then we would expect a significantly greater detrimental effect on generalist vs specialist herbivores. In fact, the detrimental impacts were not significantly different between generalists and specialists feeding on compounds from herbaceous plants, failing to support the plant-apparency hypothesis. This striking pattern that emerged in our data reveals that it is common for secondary metabolites to have both beneficial and detrimental impacts on herbivores, and in some cases beneficial impacts yield effect sizes that are as large as the effect sizes for detrimental impacts. For example, in some studies, secondary metabolites increased development time (Trumble *et al.*, 1991; Underwood *et al.*, 2002; Garcia *et al.*, 2003), while in other studies development time actually decreased (Zalucki & Malcolm, 1999; Cipollini & Redman, 1999; Li *et al.*, 2000). The same was true for measurements of herbivore mass, feeding, and survival. These seemingly contradictory effects demonstrate that in many cases herbivores have

adapted to the secondary metabolites in their diet, whether or not these compounds evolved as defenses against herbivores. Even more surprising was the fact that this was not limited to specialist herbivores. Generalist feeders experienced beneficial effects of similar magnitude from the secondary metabolites in their diet, despite the fact that most examples of chemical host plant adaptation in the literature focus on specialist herbivores (Dyer *et al.*, 2003; Mithofer & Boland, 2012; Forister *et al.*, 2012).

An alternative and likely interpretation of the prevalence of beneficial effects in the data set is a hormetic effect (Calabrese, 2005; Hayes, 2007; Raubenheimer & Simpson, 2009; Forbey & Hunter, 2012; Forbey *et al.*, 2013). This effect happens when secondary metabolites act in a dose-dependent fashion where low concentrations can have a beneficial effect on the herbivore, but become toxic at higher concentrations. Within this spectrum of effects lies a putative therapeutic window (Forbey & Hunter, 2012) where beneficial effects are observed, but there is a transition to detrimental effects at higher concentrations. In the data set here, it is possible that many of the beneficial effects are attributable to performance measurements gathered at low doses within the therapeutic window. To truly understand the effect of secondary metabolites on specialist and generalist herbivores, compounds should be tested using a dose-dependent method so that transitions between beneficial and detrimental can be captured.

### Interactive effects of chemical mixtures and natural enemies

The majority of studies in our data set focus on individual compounds, rather than natural mixtures in which those compounds are encountered in nature (Richards *et al.*, 2012). An increasing number of studies have demonstrated that individual compounds do not negatively affect herbivores (and in many cases enhance herbivore performance); rather they act additively or synergistically with other secondary metabolites (Dyer *et al.*, 2003; Richards *et al.*, 2010, 2012). Thus, it is possible that a large proportion of the studies summarized by our meta-analysis were erroneously testing specific compounds that only function in mixtures. Moreover, the unknown compounds in the plant may be the compounds that are active against herbivores, while the known compounds that are being tested are ineffective against the tested herbivore (but not necessary ineffective against other enemies), leading to erroneous conclusions about the defense of the plant.

Only three studies explicitly tested for a synergistic effect of multiple compounds present in the plant (although *c.* 50% of studies used plant-based diets). Studies testing for synergistic effects of plant secondary metabolites on herbivores or testing for chemical synergy in general are uncommon (Gertsch, 2011; Richards *et al.*, 2012). Some of the most outstanding examples of synergy are provided by the work of Berenbaum and colleagues with furanocoumarins from parsnip (*Pastinaca sativa*) (Berenbaum & Neal, 1985; Berenbaum *et al.*, 1991). Despite this early work showing the presence of synergistic effects of furanocoumarins on herbivores, only a handful of studies tested for

synergistic effects of plant chemistry on herbivores in other plant species (Calcagno *et al.*, 2002; Scott *et al.*, 2002; Dyer *et al.*, 2003; Richards *et al.*, 2010, 2012). If most secondary compounds act in a synergistic fashion, then the results reported here from 30 yr of data may not reflect the true effects that secondary metabolites have on herbivores.

It is also possible that many negative effects were underestimated, as we did not include third trophic level interactions. For example, Dyer *et al.* (2003) found that a mixture of amide compounds from the host plant *Piper cenocladum* had no direct effect on the performance of specialist geometrid caterpillars. However, the survival of the caterpillars was indirectly affected by the amide mixture as a result of increased parasitism when feeding on diets with elevated concentrations of amides (Richards *et al.*, 2010). None of the current plant defense hypotheses take into account third trophic level interactions. Given our knowledge of the importance of multitrophic interactions (Price *et al.*, 1980; Singer & Stireman, 2005; Gols, 2014), it is a logical progression to further include natural enemies in plant defense theory. One way to incorporate the third trophic level is by focusing on compounds that mediate interactions between primary and tertiary trophic levels. An obvious starting place is plant volatiles that attract natural enemies such as parasitoids to plants with herbivores (Turlings *et al.*, 1990; Gols, 2014). A simple hypothesis to test would be that plants that experience high amounts of damage caused by chemically adapted herbivores make use of volatiles to attract parasitoids for herbivore control. Studies could also focus on herbivore physiology such as the immune response which protects herbivores against the third trophic level. Secondary metabolites that handicap this response will benefit the plant by making herbivores more vulnerable to their natural enemies (Smilanich *et al.*, 2009), whereas chemicals that facilitate resistance to natural enemies (Singer *et al.*, 2009, de Roode *et al.*, 2013) provide another example of beneficial effects.

### Adaptations of generalist herbivores to secondary metabolites

What is novel from our quantitative summary of the effects of secondary metabolites from woody and herbaceous plants on herbivores is that generalist herbivores are just as likely as specialists to have beneficial responses to phytochemical defenses. While the idea that generalist herbivores adapt to host plant chemistry is not new, generalists are not as well represented in the literature as specialists (Mithofer & Boland, 2012; Ali & Agrawal, 2012). This may be because of the difficulty in finding a model system of generalists and their host plants to explore this question, as they feed on many chemically unique plants. One broad detoxification mechanism that generalists may be employing is the use of mixed function oxidases (Casida, 1970; Brattsten, 1979; reviewed by Price *et al.*, 2011). These oxidases act in a universal fashion to protect the herbivore from a suite of potentially toxic compounds, thus allowing generalists to effectively feed on a number of different plants with varying chemical identities. Furthermore, the prediction that woody (i.e. apparent) plants contain compounds that are not susceptible to adaptation by herbivores was

not supported in our data. Of the effect sizes for quantitative defenses in woody plants, 49% had a beneficial effect on herbivores, indicating that the defense did not evolve to deter herbivory, that it was not adequately tested (e.g. not examined in a dose-dependent fashion for hormesis, or in a natural mixture for synergy), that it may indirectly affect herbivores through the third trophic level, or that herbivores have adapted. The assumption that generalists are unable to breach chemical defense of herbaceous plants is not well supported (Bernays, 1981; Agrawal, 1998; Agrawal *et al.*, 1999; Agrawal & Kurashige, 2003; Singer *et al.*, 2009), and once again the data represented here show that generalists also have positive responses to secondary metabolites (Fig. S3).

### Defensive mechanisms

One component that has been missing from prior meta-analyses on plant defenses is an examination of mechanisms by which secondary metabolites affect herbivores (Koricheva, 2002; Endara & Coley, 2011; Massad *et al.*, 2011). The plant-apparency hypothesis predicts that woody plant defenses should reduce digestion and feeding, thus limiting growth of herbivores. By contrast, herbaceous plant defenses should act through toxicity effects given that they are present in low concentrations. We found that for all response variables measured there was no difference in the effect size between woody and herbaceous plants. In other words, herbaceous plant defenses were just as likely as woody plant defenses to affect growth, feeding, weight, survival, and fecundity of the herbivores, thus failing to support plant-apparency predictions and showing that categorizing the mode of action of a qualitative or quantitative defense is not informative or predictive (Hay & Fencial, 1988; Bernays *et al.*, 1989). In addition, assigning a mode of action based upon crude categories such as 'digestibility reducer' or 'toxin' is problematic because a compound that is cytotoxic as a result of disrupting specific enzymes could manifest downstream effects on digestion, thus blurring the demarcation between these two categories. Given the lack of detailed knowledge on the mode of action for many secondary metabolites, a worthy undertaking in the field of chemical ecology is to begin in-depth investigations of how defensive compounds are affecting different herbivores (e.g. Petschenka & Agrawal, 2015), or how herbivores have adapted.

Results from the feeding data showed that positive beneficial effect sizes in both woody and herbaceous plants were much stronger compared with the detrimental effect sizes in herbaceous plants. This finding suggests that some measures of feeding (amount consumed) are enhanced by plant defenses in some plants and herbivores. This may be interpreted as evidence for compensatory feeding where herbivores consume plant material in higher amounts to compensate for the low nutritional value of the diet (Slansky *et al.*, 1985; Suzuki-Ohno *et al.*, 2012; Flores *et al.*, 2014). If this is the case, herbivores are showing an adaptive response to nutritionally poor tissue by increasing their consumption. Another possible explanation for the beneficial effects in the data set is that many of the studies confounded the nutritional chemistry of the plants with defense chemistry such that

treatments with high concentrations of defensive chemistry also had higher nutritional quality. However, a recent study examining concurrent changes in nutritional and defensive chemistry found that higher levels of the latter led to poorer nutritional quality in plants (Landosky & Karowe, 2014). It is also worth noting that our data set is heavily biased towards invertebrate folivores, and vertebrate folivores may be responding differently as they may be more reliant on apparent plants as an energy source.

### Herbivore adaptation to putative defenses

One discovery from this meta-analysis, which was not addressed in the original plant-apparency hypothesis, is the proliferation of beneficial effects on both specialist and generalist herbivores. As mentioned earlier, it is expected that herbivores will evolve counteradaptations to plant defenses, and based on existing studies that ignore synergies, dose dependence, or other modes of mixture defense, a caricature of this pattern is that herbivores are 'ahead' in the 'chemical arms race', especially specialist herbivores. Updating this hypothesis to include the beneficial effects of chemistry on both specialist and generalist herbivores simply shows that herbivores are adapting to chemical defenses, which is an assumption made in the plant-apparency hypothesis. Our meta-analysis data support the recent papers by Endara & Coley (2011) and Massad *et al.* (2011) that used meta-analysis to investigate plant defense hypotheses. Both papers found partial support for the plant-apparency hypothesis in that woody plants were more likely to have quantitative defenses and herbaceous plants were more likely to have qualitative defenses, but the effect of these defenses did not differ between specialist and generalist herbivores. Thus, from an evolutionary perspective, it appears that specialist and generalist herbivores are not different in how they respond to plant chemical defenses; and, perhaps not surprisingly, what we observe and measure now is the legacy of herbivores adapting to these defenses.

Along similar lines, the plant-apparency hypothesis makes predictions about the chemical preference of specialist and generalist herbivores, assuming that only a single herbivore (or herbivore guild) is ecologically relevant. However, a more biologically sound scenario is that plant communities constantly experience pressure from both specialist and generalist herbivores. Thus, while plants may adapt to deter consumption by specialists, they are simultaneously under selection pressure from generalist feeders. Using specialist and generalist herbivores of the plant *Brassica nigra*, Lankau (2007) found that *B. nigra* increased concentrations of glucosinolates when the dominant herbivore was a generalist and, conversely, decreased chemical concentrations when the dominant herbivore was a specialist. When both herbivores were present, the concentrations stayed at an intermediate concentration. Castillo *et al.* (2014) found similar effects in wild populations of *Datura stramonium* across a geographical gradient in Mexico. Populations under attack by a specialist herbivore showed selection towards a reduction in secondary chemistry, while those populations where only the generalist herbivore was present showed increased selection of derived secondary metabolites. Clearly, plants are under selective pressure from both

specialist and generalist herbivores, regardless of their defensive chemistry, resulting in unique constitutive and inducible defense responses to these competing selective pressures (Firn & Jones, 2003; Agrawal & Heil, 2012). Herbivores may be selecting for multiple defense strategies in plants (Firn & Jones, 2003; Lankau, 2007; Carmona & Fornoni, 2013; Castillo *et al.*, 2014) such that chemical defense redundancy is widespread throughout plants to defend against the wide array of natural enemies that attack.

### Conclusions

Is plant apparency dead? Yes and no. It clearly remains useful to consider the apparency of woody vs herbaceous plants as one trait that can affect plant chemical defenses and herbivore communities. It is also useful to consider the effects of plant defenses against specialist vs generalist herbivores. However, most of the specific predictions of plant apparency are not useful and the focus of chemical ecology should continue to shift away from generalizations across broad categories such as apparent and unapparent plants and qualitative and quantitative defenses.

It is obvious that the evolution of plant defenses has been – and still is – influenced by herbivores (Becerra, 2015). A recent genomic study of plant metabolism by Chae *et al.* (2014) found that genes associated with secondary metabolism in angiosperms had significantly higher proliferation compared with genes associated with primary metabolism. This study supports the assertion that plant defenses have been under intense selection by herbivores since their first association 420 million yr ago (Labandeira, 2007). Of course evolutionary interactions among these trophic levels are ongoing, and our meta-analysis represents a snapshot of these dynamic interactions.

Future studies investigating plant defenses against herbivores must consider exploring the diversity of secondary metabolites in plants and whether naturally occurring combinations of compounds are more effective than single compounds alone (e.g. Richards *et al.*, 2015). Many of the studies in our data set ( $n=126$ ) were focused on the action of single compounds on herbivore performance and < 1% explicitly tested for a synergistic effect. With the diversity of compounds that are found in plants, the legacy of searching for effects of single compounds from plants at singular doses needs to be replaced with investigating the possibility of synergy between multiple compounds at a range of concentrations (Dyer *et al.*, 2003; Calabrese, 2005; Raubenheimer & Simpson, 2009; Richards *et al.*, 2010, 2012; Agrawal, 2011; Gertsch, 2011). Certainly this multidisciplinary approach is more laborious and requires a certain amount of knowledge of natural product chemistry to be executed successfully. Nonetheless, techniques in chemical ecology have improved vastly over the last decade, making it possible to explore plant chemistry to a greater depth than ever before (Dyer, 2011; Dyer *et al.*, 2014). In addition, collaborative research across disciplines (e.g. ecology and chemistry) is quickly becoming the rule rather than the exception and is better supported by funding agencies than in the past (Berenbaum, 2014). Using this approach, we can gain new insights into the

evolution of plant defenses that have previously gone unexplored.

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## Author contributions

A.M.S., R.M.F. and L.A.D. planned and designed the research. A.M.S. and R.M.F. performed the data collection. A.M.S. and L.A.D. performed the data analysis and wrote the manuscript.

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Relationship between plant species richness and the frequency at which the plant family was represented in the data set for qualitative defenses.

**Fig. S2** Relationship between plant species richness and the frequency at which the plant family was represented in the data set for quantitative defenses.

**Fig. S3** Three-dimensional plot showing the distribution of negative and positive effect sizes of qualitative and quantitative defenses.

**Fig. S4** Three-dimensional plot showing the distribution of negative and positive effects sizes for specialist and generalist herbivores feeding on plants with qualitative defenses.

**Fig. S5** Posterior probability distributions for growth data for herbivores feeding on woody and herbaceous plants.

**Fig. S6** Posterior probability distributions for feeding data for herbivores feeding on woody and herbaceous plants.

**Fig. S7** Posterior probability distributions for weight data for herbivores feeding on woody and herbaceous plants.

**Fig. S8** Posterior probability distributions for survival data for herbivores feeding on woody and herbaceous plants.

**Fig. S9** Posterior probability distributions for fecundity data for herbivores feeding on woody and herbaceous plants.

**Table S1** Meta-analysis data with the references for papers used in the analysis

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