

# Presence of substitute diets alters plant resistance to specialist and generalist herbivores: a meta-analysis

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**Abstract.** How herbivores respond to resource heterogeneity is important for predicting plant resistance to herbivores. Experimental studies thus far have revealed that herbivore responses differ depending on whether herbivores are offered single or multiple plant types, but the reports have rarely been combined. Here, we conducted a meta-analysis of 47 publications on choice and no-choice experiments to reveal how the presence/absence of multiple plants (i.e., choice/no-choice conditions) alters the extent of behavioral avoidance by herbivores. The herbivore diet breadth and response traits (feeding, growth, or oviposition) explained a significantly large amount of heterogeneity in the herbivore response under choice and no-choice conditions. In contrast, a small amount of heterogeneity was explained by the herbivore types (vertebrates, exophagous, or endophagous invertebrates), plant resistance traits (chemical or nonchemical), plant life form, and relatedness of plant pairs (intraspecific or interspecific) as well as interactions between the herbivore and plant characteristics. Compared with the no-choice conditions, specialist herbivores further avoided suboptimal plants under choice conditions. Generalist herbivores more evenly utilized optimal and suboptimal plants under choice conditions. The avoidance of suboptimal plants under choice conditions was the most prominent in oviposition response. Thus, our meta-analysis found that herbivore characteristics rather than plant traits were more responsible for driving behavioral avoidance by herbivores to a particular plant. The contrasting response between specialist and generalist herbivores to plant heterogeneity may be more ubiquitous than previously thought.

**Key words:** antibiosis; antixenosis; choice experiment; generalist; meta-analysis; plant–herbivore interaction; specialist.

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## INTRODUCTION

Plant–herbivore interactions are a major component of terrestrial ecosystem, where plants defend themselves, while herbivores seek to overcome the defense. As plants and herbivores have been studied intensively, a number of examples are available for modern ecologists. Thus, recent authors have characterized general patterns of plant–herbivore interaction by means of meta-analysis (Barton and Koricheva 2010, Gripenberg et al. 2010, Carmona et al.

2011). For instance, meta-analyses of plant defense uncovered which plant traits can predict antiherbivore resistance (Carmona et al. 2011) and when plants express these resistance traits (Barton and Koricheva 2010). At the same time, herbivore host selection is a major subject of meta-analyses in relation to their diet specialization (Lefcheck et al. 2013) and adaptation to host plants (Gripenberg et al. 2010).

To cope with attacks by herbivores, plants have developed various resistance traits, such as mechanical structures and toxic secondary

metabolites (Schoonhoven et al. 2005). The plant resistance to herbivores has often been evaluated by mitigation of plant damage (Simms and Rausher 1987) or by decreases in preference or performance of herbivores (Karban and Baldwin 1997). In a plant–herbivore system, there are two major mechanisms of plant resistance: One is plant defense that reduces size, fecundity, and survival rate of herbivores (*sensu* antibiosis: Painter 1951), while the other is defense that evokes behavioral avoidance of herbivores (antixenosis: Kogan and Ortman 1978). Thus far, practical studies comparing multiple plants were conducted using two distinct settings, that is, no-choice and choice experiments. The no-choice setting comprises a set of subexperiments in which herbivore responses to a single plant are examined, whereas in the choice setting, preference among multiple plants is examined. The former and the latter are often conducted with an aim of evaluating antibiosis and antixenosis, respectively (Spencer 1996, Casteel et al. 2006, Sato et al. 2014). However, there are few meta-analyses that compare herbivore responses between choice and no-choice experiments.

Because antixenosis is largely involved in behavioral avoidance by herbivores (Schoonhoven et al. 2005), it may depend on how herbivores respond to an array of their host plants. For example, specialist herbivores often concentrate on a few specific cues to locate host plants (Bernays and Minkenberg 1997) and thus can detect their host from a series of plants faster than generalists (Bernays and Funk 1999). Conversely, it has been hypothesized that a mixed use of multiple plants improves performance of generalist herbivores by diluting toxins (Freeland and Janzen 1974) or by balancing nutrient intake (Behmer et al. 2001). Several studies have illustrated the diet mixing behavior of generalist mammals (Freeland and Saladin 1989, Nersesian et al. 2012) and insect herbivores (Behmer et al. 2001, Singer et al. 2002), indicating that the defensive effects of a particular compound depend on the foraging context for herbivores (Behmer et al. 2005, Bergvall et al. 2006, Nersesian et al. 2012). Recently, Lefcheck et al. (2013) have conducted a meta-analysis to examine the effects of diet mixing behavior on consumer fitness. This meta-analysis suggests that the diet mixing behavior is likely driven by ecological contexts rather than physiological

benefits for herbivores, yet their effects in the context of plant resistance remain unexplored.

We should note here that the results for related taxa are not statistically independent, because of their shared evolutionary history (Harvey and Pagel 1991, Adams 2008, Lajeunesse 2009). For example, related plants often share a similar composition of secondary metabolite, such as the glucosinolates of Brassicaceae and relatives (Spencer 1996, Borgen et al. 2012). In addition, plant phylogeny may be correlated with other life-history traits. As the Brassicaceae consists of herbaceous species, a plant life form may also be related to a certain lineage of the plant family. Therefore, the concept of an evolutionary comparative method should be taken into consideration to understand the phylogenetic constraints on the general pattern of plant–herbivore interactions.

In the current study, we performed a meta-analysis of 144 measures from 47 published studies that experimentally compared herbivore responses under choice and no-choice conditions. In this meta-analysis, we assumed that herbivore response under no-choice conditions reflected net physiological effects of a given plant type on herbivore growth or fecundity (i.e., antibiosis), while choice conditions represented behavioral avoidance of herbivores (antixenosis) in addition to the antibiotic effects (Fig. 1). By focusing on the differential herbivore responses between the two choice conditions, we intended to evaluate the relative importance of antibiosis and antixenosis in the presence of substitute diets for herbivores (Fig. 1). Primarily, the following question was addressed: What kinds of characteristics of herbivores and plants are most responsible for the antibiotic–antixenotic difference in herbivore responses? In this question, we tested three contrasts of herbivore characteristics (specialist or generalist; vertebrates, exophagous invertebrates, or endophagous invertebrates; feeding, growth, or oviposition) and three contrasts of plant characteristics (chemical, nonchemical, or both; tree or herb; intraspecific or interspecific plant variation). Additionally, we asked the following two questions. Are there any interactive effects between the herbivore and plant characteristics on the herbivore response? Are these responses affected by the design of the choice experiment, that is, the number of plant types offered to herbivores simultaneously?

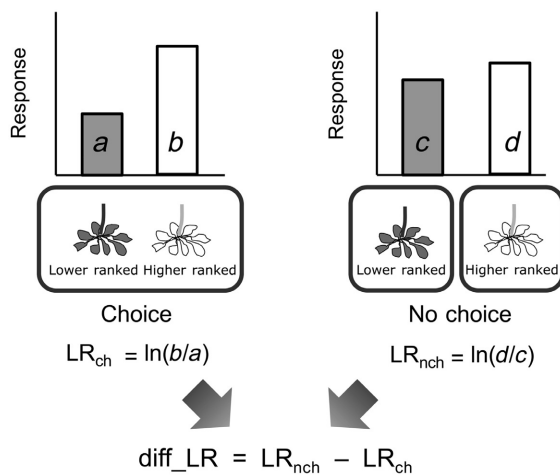


Fig. 1. A schematic diagram for the estimation of the log response ratio under choice conditions ( $LR_{ch}$ ) or no-choice conditions ( $LR_{nch}$ ). Parameters  $a$  and  $b$  indicate herbivore response to lower-ranked (dark) and higher-ranked (light) plants under choice conditions, respectively, while  $c$  and  $d$  represent those under no-choice conditions. The negative or positive sign of the difference of LRs ( $diff\_LR$ ) indicates a decreased or increased response to lower-ranked plants, respectively, under the presence of higher-ranked plants. The higher-ranked plant was defined as a plant type that had the highest value of response under a no-choice condition, whereas other plant types in comparison with the higher-ranked plant were defined as lower-ranked plants.

## MATERIALS AND METHODS

### Compilation of the database

We searched the online database of the ISI Web of Science with the keyword “choice\*” AND a keyword indicating herbivore (“herbivor\*” OR “phytophag\*”) to identify candidate literature. We further searched with the keywords, “preference” AND “performance,” instead of “choice\*” to find candidates from preference–performance studies. Of >2000 abstracts indexed till the end of 2012, we screened for empirical studies on plant–herbivore interactions. Furthermore, we selected data sets of 47 publications from the prescreened literature based on the criteria detailed below. First, experiments were conducted under enclosed conditions that focused on plant–herbivore interactions and excluded the other trophic interactions with antagonists (e.g., predators,

parasitoids, or pathogens) and mutualists (pollinators or mycorrhizal fungi). The enclosed conditions included the laboratory experiment or field experiment protected from any accesses by external organisms. Second, herbivore response was evaluated by the variables of their feeding, oviposition preference, and growth of herbivores (collectively hereafter termed “herbivore response”). These criteria were consistent with Simms and Rausher’s (1987) definition that plant resistance indicates 1, which represents the ratio of leaf area loss, and Karban and Baldwin’s (1997) definition that plant resistance traits denote plant traits altering preference or performance of herbivores, which has, in addition, been adopted by a meta-analysis of plant resistance (Carmona et al. 2011). Thirdly, we chose studies of both choice and no-choice conditions that were designed for common herbivores of the same stage in a single publication, and where the same variables were measured between the two conditions. Most of preference–performance studies were excluded as they measured different variables between the choice and no-choice conditions. The fourth criterion was that studies were chosen where focal traits of plants were either quantitatively presented or explicitly mentioned within a publication. Because a few publications did not explicitly mention focal plant traits, we searched further for publications reporting trait information to confirm their assumptions regarding putative resistance traits. We described such additional information as “Notes” in the metadata (Data S1). Lastly, studies were chosen where studied plant pairs belonged to a common species or genus. This criterion is intended to minimize the differences in other life-history traits and evolutionary history between plant pairs by focusing on closely related plants. We excluded studies using synthetic foods, unless they explicitly assumed a certain plant taxon (Dearing and Cork 1999, Nersesian et al. 2012 for *Eucalyptus* spp.: “Notes” in Data S1). Livestock/grazer studies were excluded due to the last criterion. As several publications performed the choice and no-choice experiments using multiple states of herbivores (e.g., herbivore sexes, developmental stage, and preconditioning with different hosts: see also “Notes” in Data S1), we used average values of the different conditions. Mean and standard errors of the herbivore responses as

well as the sample size of experiments were extracted from each publication. Data presented in figures were obtained using the ImageJ software (Abramoff et al. 2004). The standard errors were not available for some publications due to pooled reports or no presentation (11 measures from five publications: Data S1). These data were excluded in our statistical analysis that required the variance information but listed in the metadata (Data S1). In total, the current study compiled 144 measures from 47 publications, encompassing studies of 12 orders, 19 plant families, 32 genera, and 51 herbivore species. The metadata and the entire list of papers reviewed are available in supplementary materials (Data S1 and Appendix S1).

#### Explanatory variables

Our meta-analysis focused on the following seven factors as explanatory variables: herbivore diet breadth (specialist or generalist), herbivore type (vertebrates, exophagous invertebrates, or endophagous invertebrates), herbivore response traits (feeding, growth, or oviposition), plant resistance traits (chemical, nonchemical, or both), plant life form (tree or herb), taxonomic relatedness of plant pairs (intraspecific or interspecific), and the design of choice experiments (pairwise or simultaneous choice on multiple plant types).

For herbivore data, we listed the diet breadth, herbivore type, and response traits. The diet breadth was categorized as either specialist (e.g., oligophagous beetles, butterflies, and leaf miners) or generalist (mammals, grasshoppers, and snails) for the herbivores that fed on plants belonging to single or multiple plant families, respectively. As to the herbivore type, we distinguished between herbivorous vertebrates (which include possums and beavers), exophagous invertebrates (which include beetles, caterpillars, grasshoppers, and snails) and endophagous invertebrates (which include aphids, leaf miners, gallmakers, and herbivorous mites). We also distinguished three types of herbivore response traits (feeding, preference, or performance).

For plant data, we distinguished between the resistance trait type, life form, and taxonomic relatedness. The resistance trait type was classified as chemical, nonchemical, or both. The chemical type, for example, included secondary metabolites (e.g., glucosinolates and their

enzyme, myrosinase), metal concentrations (zinc and nickel), and nutritional (carbon and nitrogen contents) chemicals. The nonchemical type included physical traits (e.g., spine, trichomes, and leaf thickness) and studies that focused on leaf position without specifying any traits. Combinations of both chemical and nonchemical traits were identified as “both chemical and physical.” Based on specific traits measured in the study, we classified studies on leaf position (Hoffman and Rao 2011) and induced response (Agrawal 2000) as chemical, nonchemical, or both. The plant life form was classified into a tree or herb. The plant taxonomic relatedness was determined by whether multiple plant types offered to herbivores were intraspecific or interspecific pairs.

#### Estimation of effect size

The log response ratio (LR) was utilized as an effect size that estimates the changes in a certain variable (an indicator variable of herbivore response in the current study) between treatment and control groups (Hedges et al. 1999). We applied LRs to convert herbivore responses to lower-ranked plants relative to higher-ranked plants under choice ( $LR_{ch}$ ) and no-choice conditions ( $LR_{nch}$ ; Fig. 1). With the assumption that herbivore response under no-choice conditions reflects net effects of a given plant type without being confounded by the other plant types, we first defined the higher-ranked plant as a plant type that had the highest value of herbivore response under a no-choice condition (Fig. 1). On the other hand, other plant types in comparison with the higher-ranked plant were defined as lower-ranked plants (Fig. 1). We calculated the log response ratio for the choice condition as  $LR_{ch} = \ln(b/a)$  and for the no-choice condition as  $LR_{nch} = \ln(d/c)$ . The parameters  $a$  and  $b$  denote the mean value of herbivore response to lower-ranked and higher-ranked plants, respectively, under choice conditions. The parameters  $c$  and  $d$  denote the mean value of herbivore response to lower-ranked and higher-ranked plants under no-choice conditions, respectively. The sampling variance was calculated as follows:

$$\sigma_{ch}^2 = [(SD_b)^2 / (N_b \times b^2)] + [(SD_a)^2 / (N_a \times a^2)];$$

$$\sigma_{nch}^2 = [(SD_d)^2 / (N_d \times d^2)] + [(SD_c)^2 / (N_c \times c^2)];$$



where  $SD_x$  and  $N_x$  indicate standard deviation and sample size of each variable, respectively. When zero values occurred in the logarithm, the lowest value observed within an experiment was added to all observations for that experiment following the procedure of Viola et al. (2010). We used the `escalc` function of the `metafor` package (Viechtbauer 2010) in R software version 3.2.1 (R Core Team 2015) to estimate LRs (implemented as log-transformed ratio of means). To visualize the difference in herbivore responses, we compared the LR between the choice and no-choice conditions as  $\text{diff\_LR} = \text{LR}_{\text{no-choice}} - \text{LR}_{\text{choice}}$ , where  $\text{diff\_LR}$  indicates the degree to which the presence of higher-ranked plants (i.e., the choice condition) increased (positive sign) or decreased (negative sign) the herbivore response to lower-ranked plants as compared to the response to lower-ranked plants in the solitary condition (no-choice condition; Fig. 1).

According to Lajeunesse (2015), we conducted a bias diagnosis and correction for our LR measures because the sample sizes were relatively small ( $n < 10$ ) for many experiments compiled in this study (Data S1). On the basis of  $< 3$  values of the Geary's rule (see Eq. 13 in Lajeunesse 2015), 22% of our LR measures were considered potentially biased; therefore, the mean and variance of all the LR measures were corrected with the delta method (Eqs. 8 and 9 in Lajeunesse 2015). Another specific problem encountered in the current study was that pairwise comparisons for all plant types within a single multiple-choice experiment (i.e., simultaneous choice among more than two plant types) led us to exponentially increase the samplings done on a single experiment; therefore, to avoid this exaggerated sampling, for the multiple-choice design we calculated LRs for combinations of the highest ranked plants with the other plant types.

### Statistical analysis

We conducted a random-effect and mixed-effect meta-analysis to assess the sampling heterogeneity and to test factors moderating herbivore response, respectively. In the random-effect model, we used the `rma` function (in the `metafor` package) to calculate summary statistics of heterogeneity (total heterogeneity  $Q_T$ ; percentage of among-study heterogeneity relative to that of the total heterogeneity  $I^2$ ). We further used the `rma.mv` function to perform a

multilevel random-effect meta-analysis. In this analysis, the publication ID was included as a random factor to deal with nonindependent samplings from a single publication and to consider variation in experimental designs among publications (hereafter referred to as experimental set level analysis). We then used the `rma.mv` function to perform a multilevel mixed-effect meta-analysis that examined the fixed effects of herbivore diet breadth (specialist or generalist), herbivore type (vertebrates, exophagous invertebrates, or endophagous invertebrates), response traits (feeding, growth, or oviposition), plant resistance traits (chemical, nonchemical, or both), plant life form (tree or herb), plant relatedness (within or between species), and choice design (multiple- or pairwise choice experiments) on LRs. The following three interactions were incorporated as additional fixed factors: (1) The herbivore diet breadth  $\times$  plant resistance traits interaction was examined to test whether the response to a specific kind of plant trait depended on the herbivore diet breadth; (2) the herbivore diet breadth  $\times$  relatedness of plant pairs was considered to inspect a potential confounding effect between plant relatedness and the herbivore diet breadth; and (3) the herbivore type  $\times$  plant resistance traits interaction was also included to test a differential response of three herbivore types to plant traits. The publication ID was included as a random factor in the mixed-effect model. The significance of each fixed factor was analyzed using Cochran's  $Q$  tests. We first analyzed the interaction terms, and then we examined the main effects using the models excluding the interactions. Resampling tests were not performed in this meta-analysis, because nonindependent samplings were dealt with as a random factor in multilevel models. The  $\text{diff\_LR}$ s were calculated from the raw data and compared with model prediction by the mixed-effect meta-analysis including all the fixed and random factors.

In the same way as described above, we also analyzed multilevel mixed-effect models that incorporated the plant family as an additional random factor to consider phylogenetic influences of host plants (referred hereafter to as plant family level analysis). This additional model corresponded to a nested ANOVA approach to partition the total variance into within taxon and between taxon (Harvey and Pagel 1991).

Although there are limits to this approach as a phylogenetic comparative method, alternative approaches, such as phylogenetic autocorrelations (Adams 2008) or phylogenetically independent contrasts (Lajeunesse 2009), were difficult to apply to our metadata, which included multiple measures from a single taxonomic group (specifically due to a rank deficient of a genetic distance matrix). The random factor of the publication ID was nested below that of the plant family, because data from a single plant family were composed of multiple publications. The variance component of publication ID and plant families were compared in the random-effect multilevel meta-analyses, and then fixed factors were tested using the mixed-effect multilevel meta-analyses.

Additionally, we inspected publication biases by analyzing asymmetry of funnel plots. We used the sample size as a measure statistic because it was available for all data (i.e., 144 measures: Data S1). Kendall's rank correlation was calculated to examine nonparametric asymmetry between the sample size and effect size in the random-effect meta-analyses. We also used the trimfill function to perform a trim-and-fill method that estimated the number of missing studies due to the suppression of the most extreme results on one side of the funnel plot (Duval 2005). The R script of this study is available in supplementary materials (Data S1).

## RESULTS

### *Random-effect meta-analysis: source of heterogeneity*

The random-effect meta-analysis showed a significant amount of total heterogeneity in our

metadata ( $Q_T = 4422$  and  $2622$ ,  $df = 132$  and  $132$ ,  $P < 0.0001$  and  $0.0001$  for  $LR_{nch}$  and  $LR_{ch}$ , respectively; Table 1). The large percentage of total heterogeneity was attributable to differences between studies (as indicated by  $I^2$ ; Table 1). The variable degree of LRs can be observed in forest plots that present a mean effect size for each measure (Appendix S2: Fig. S1). Our multilevel meta-analysis divided the variance component between the levels of experimental set (i.e., publication ID) and plant families (Table 1). The variance among plant families was similar with those among publications under the both no-choice and choice conditions (indicated by  $\sigma^2$  and  $\tau^2$ ; Table 1).

### *Mixed-effect meta-analysis: factors moderating herbivore response*

The herbivore diet breadth and response traits explained a significant amount of heterogeneity in their response across the experimental conditions (under choice or no-choice conditions:  $LR_{nch}$  and  $LR_{ch}$ ) and the two levels of analyses (experimental set level and plant family level: Table 2). The moderate amount of heterogeneity was explained by plant resistance traits and plant relatedness (intraspecific or interspecific), but this amount was marginally significant in some cases (Table 2). The explained amount of heterogeneity was not significant for the other factors encompassing herbivore types, plant life form, and choice experiment design (Table 2). There was a moderate amount of heterogeneity attributable to interactions between the plant resistance traits and either of the diet breadth or types of herbivores: The significant amount of heterogeneity was explained by an interaction term between the

Table 1. Summary statistics from the random-effect meta-analysis for herbivore responses under no-choice and choice conditions.

Condition	$Q_T$	$I^2$ (%)	Mean	Random factors				
				95% CI		~1 Publication ID		
				Lower	Upper	$\sigma^2$	Inner $\tau^2$	Outer $\rho$
No-choice	4422	98.3	0.74	0.51	0.97	0.213	0.191	0.336
Choice	2622	98.2	0.86	0.62	1.11	0.184	0.188	0.638

Notes: Shown are the total heterogeneity ( $Q_T$ ), percentage of heterogeneity attributable to study differences ( $I^2$ ), mean of the log response ratio (mean) with its upper and lower limits of 95% confidence interval (95% CI), and variance components of random factors. The mean and variance components are those estimated in multilevel meta-analytic models.  $\sigma^2$ , the amount of heterogeneity in the level of the random factor;  $\tau^2$ , the amount of heterogeneity in the level of the inner factor;  $\rho$ , correlation between the levels of the inner and outer factors.

Table 2. The amount of heterogeneity ( $Q$ ) explained by the herbivore diet breadth, herbivore type, herbivore response traits, plant resistance traits, life form, taxonomic relatedness, and design of choice experiment for the herbivore response under no-choice ( $LR_{nch}$ ) and choice ( $LR_{ch}$ ) conditions.

Fixed factors	df	(A) Experimental set level				(B) Plant family level			
		$LR_{nch}$		$LR_{ch}$		$LR_{nch}$		$LR_{ch}$	
		$Q$	$P$	$Q$	$P$	$Q$	$P$	$Q$	$P$
Herbivore diet breadth	1	<b>94.57</b>	<b>&lt;10<sup>-22</sup></b>	<b>29.40</b>	<b>&lt;10<sup>-7</sup></b>	<b>94.34</b>	<b>&lt;10<sup>-21</sup></b>	<b>26.03</b>	<b>&lt;10<sup>-6</sup></b>
Herbivore type	2	1.87	0.39	<b>6.67</b>	<b>&lt;0.05</b>	1.81	0.41	4.65	0.10
Herbivore response traits	2	<b>35.68</b>	<b>&lt;10<sup>-7</sup></b>	<b>69.18</b>	<b>&lt;10<sup>-15</sup></b>	<b>35.68</b>	<b>&lt;10<sup>-7</sup></b>	<b>71.04</b>	<b>&lt;10<sup>-15</sup></b>
Plant resistance traits	2	5.24	0.07	<b>6.83</b>	<b>&lt;0.05</b>	5.23	0.07	5.59	0.06
Plant life form	1	0.01	0.94	0.08	0.78	0.00	0.95	0.02	0.88
Plant relatedness	1	<b>6.54</b>	<b>&lt;0.05</b>	2.55	0.11	<b>6.60</b>	<b>&lt;0.05</b>	<b>7.55</b>	<b>&lt;0.01</b>
Choice design	1	0.15	0.70	0.21	0.65	0.12	0.73	1.99	0.16
Diet × Resistance	2	4.81	0.09	2.89	0.24	4.78	0.09	2.20	0.33
Diet × Relatedness	1	0.85	0.36	0.47	0.49	0.88	0.35	0.16	0.69
Herb. type × Resistance	2	<b>12.84</b>	<b>&lt;0.01</b>	1.75	0.42	<b>12.83</b>	<b>&lt;0.01</b>	2.04	0.36
Residuals	118	<b>2198</b>	<b>&lt;0.001</b>	<b>1827</b>	<b>&lt;0.001</b>	<b>2198</b>	<b>&lt;0.001</b>	<b>1827</b>	<b>&lt;0.001</b>

Notes: The experimental set level analysis (A) included the publication ID as a random factor. The plant family level analysis (B) nested a random factor of the publication ID below that of the plant family. Bold values indicate <5% significances with Cochran's  $Q$  tests. Degree of freedom (df) is listed for each fixed factor.

herbivore type and plant resistance traits under no-choice conditions (Table 2). Residual heterogeneity remained significant even after incorporating these fixed factors (Table 2).

The difference of LRs (diff\_LR) indicates the extent to which the presence of higher-ranked plants increases (positive sign) or decreases (negative sign) the herbivore response to lower-ranked plants (Fig. 1). The overall mean of diff\_LR displayed a slightly negative deviation from zero (Fig. 2). As for the two factors significantly responsible for the herbivore response detected above (the herbivore diet breadth and response traits: Table 2), some groups showed contrasting trends within a category. We identified positive and negative deviations of diff\_LR from the overall mean for generalist and specialist herbivores, respectively (Fig. 2), indicating a weakened and strengthened avoidance by generalist and specialist herbivores to lower-ranked plants under choice conditions, respectively. The oviposition response had a negative diff\_LR compared with feeding and growth (Fig. 2). The herbivores tended to show a more negative diff\_LR to nonchemical traits than to chemical traits. The interspecific plant pairs had wider confidence intervals of diff\_LR compared with intraspecific plant pairs (Fig. 2).

Based on the certain amount of heterogeneity explained by some interactions between herbivore categories and plant resistance traits

(Table 2), we plotted each group of the herbivore diet breadth or types separately against the plant resistance traits (Fig. 3). The specialist and generalist herbivores exhibited contrasting trends in their response to chemical plant traits (negative and positive deviation of diff\_LR from the overall mean), whereas this contrasting pattern was not observed for the other trait types (Fig. 3). Exophagous and endophagous invertebrates were predicted to possess different tendencies in their responses between chemical and nonchemical traits, but the sample size was small for the nonchemical cases to test the prediction (Fig. 3). The vertebrate examples were available only for the chemical traits.

#### Funnel plot asymmetry: test of potential publication bias

No significant asymmetry and missing studies were detected for the no-choice condition (Kendall's rank correlation,  $\tau = 0.06$ ,  $Z = 1.05$ ,  $P = 0.29$ ; Appendix S2: Fig. S2) and choice condition ( $\tau = -0.05$ ,  $Z = -0.83$ ,  $P = 0.40$ ; Appendix S2: Fig. S2), indicating that little publication bias existed in the present metadata.

## DISCUSSION

Our meta-analysis characterized several tendencies in the herbivore responses to a set of

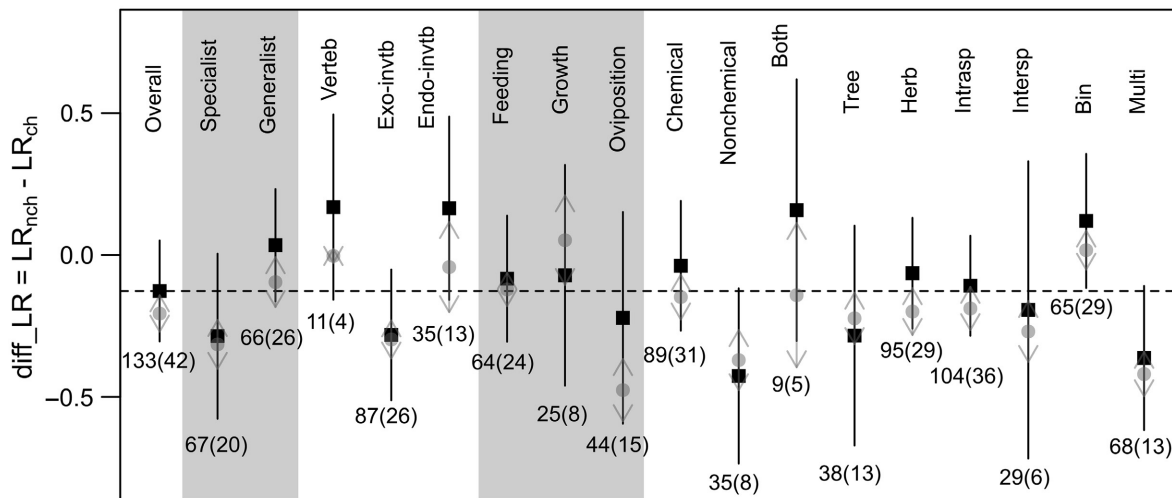


Fig. 2. Difference of the log response ratio ( $\text{diff\_LR} = \text{LR}_{\text{ch}} - \text{LR}_{\text{nch}}$ ) between choice ( $\text{LR}_{\text{ch}}$ ) and no-choice ( $\text{LR}_{\text{nch}}$ ) conditions (means and 95% confidence intervals indicated by black squares and bars, respectively) plotted against seven factors: (1) the herbivore diet breadth (specialist or generalist); (2) herbivore type (vertebrates, exophagous invertebrates, or endophagous invertebrates); (3) herbivore response traits (feeding, growth, or oviposition); (4) plant resistance traits (chemical, nonchemical, or both); (5) plant life form (tree or herb); (6) taxonomic relatedness (intraspecific or interspecific); and (7) design of choice experiments (pairwise or multiple choice). The number of measures is listed beside each plot, while the number of publications is described within parentheses. Gray dots and arrows indicate model prediction (means and 95% confidence intervals) by the mixed-effect meta-analysis including all the fixed and random factors. Shaded area highlights categories that explain the significant amount of heterogeneity under both the choice and no-choice conditions (Table 2). Note that studies without standard errors are not included in this plot.

multiple plants under choice and no-choice conditions. The two characteristics of herbivores, diet breadth and response traits, contributed remarkably to the heterogeneity in the herbivore response under both the choice and no-choice conditions. As shown by the contrast values of  $\text{diff\_LR}$ , antixenotic effects of lower-ranked plants against specialist herbivores increased under choice conditions, whereas antixenotic effects of lower-ranked plants against generalist herbivores increased under choice conditions. Additionally, there was weak evidence for interactions between herbivore and plant characteristics, although the stronger avoidance by specialist herbivores seemed prominently related to chemical plant traits. The experimental contexts of pairwise or multiple choice also unlikely explained the herbivore response under choice and no-choice conditions. These results indicate that, with less regard to the interactive roles and choice context, herbivore characteristics rather than plant traits are more important for evoking

behavioral avoidance by herbivores to a particular plant type.

Evidence for the contrasting response of specialist and generalist insects to plant chemicals can be found in the publications reviewed here: Ballhorn et al. (2010) illustrated that cyanogenic chemicals of a lima bean less likely protected plant leaves when provided as a part of the diet to a generalist locust, whereas these chemicals conferred stronger defensive effects against a specialist beetle through behavioral avoidance. Our meta-analysis estimated opposite signs and a greater degree of  $\text{diff\_LR}$ s from two paired cases of Ballhorn et al. (2010). Furthermore, mixed foraging on various diets is known to allow generalist mammals to be released from their constraints of poor detoxification ability (Freeland and Janzen 1974, Freeland and Saladin 1989) or to balance their nutrient intake (Nersesian et al. 2012). In our meta-analysis, several publications tested the diet mixing hypothesis in generalist mammals (Dearing and Cork 1999, McArthur



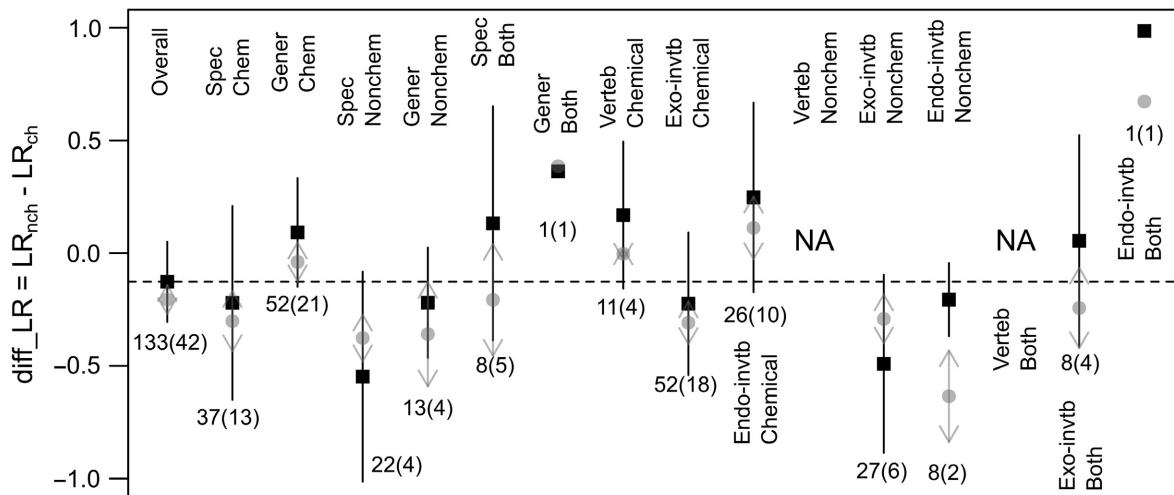


Fig. 3. Difference of the log response ratio ( $\text{diff\_LR} = \text{LR}_{\text{ch}} - \text{LR}_{\text{nch}}$ ) between choice ( $\text{LR}_{\text{ch}}$ ) and no-choice ( $\text{LR}_{\text{nch}}$ ) conditions (mean and 95% confidence intervals: indicated by black square and bars, respectively) plotted against 15 combinations between the herbivore diet breadth (specialist or generalist) or herbivore type (vertebrates, exophagous invertebrates, or endophagous invertebrates) and plant resistance traits (chemical, nonchemical, or both). The number of measures is listed beside each plot. The number of publications is described within parentheses. Gray dots and arrows indicate model prediction (mean and 95% confidence intervals) by the mixed-effect meta-analysis including all the fixed and random factors. Note that studies without standard errors are not included in this plot. NA means no data available.

et al. 2010, Nersesian et al. 2012). Indeed, we estimated the positive  $\text{diff\_LR}$ s from publications demonstrating the diet mixing (Nersesian et al. 2012), but could not obtain such positive signs from studies partially supporting the diet mixing hypothesis (Dearing and Cork 1999). Therefore, it is plausible that diet mixing by generalists could weaken antibiotic effects of defensive chemicals under the choice condition.

As Bernays and Funk (1999) proposed, specialist herbivores respond rapidly to host plant cues owing to their relaxed constraint on processing information from multiple hosts. This “neural constraint hypothesis” would expect stronger antixenotic effects of a particular plant trait against specialist herbivores under choice conditions. In this study, we observed a negative deviation in the difference of LRs for specialist herbivores. Specifically, negative values of  $\text{diff\_LR}$  were estimated from Spencer (1996) who illustrated antixenotic effects of various chemicals of a Brassica cultivar on oviposition preference of the crucifer-feeding diamondback moth *Plutella xylostella* (except for the sinigrin, which induces the oviposition preference only in the

presence of alkanes). Thus, combined with the generalist responses we discussed above, our results may agree with the notion of the neural constraint hypothesis.

There are a series of literatures that are relevant to be discussed here, although they were not included in our meta-analysis due to mismatches with the criteria of selecting literatures. For example, a number of studies on host choice experiments for herbivorous insects have aimed to address why the oviposition preference is not always linked to the larval performance in insect herbivores (e.g., Wiklund 1975, Gripenberg et al. 2010, Liu et al. 2012). In particular, the previous meta-analysis of the preference and performance studies documented that oligophagous herbivorous insects showed a stronger preference–performance linkage than that of polyphagous herbivores (Gripenberg et al. 2010). Here, we revealed a stronger avoidance in oviposition preference to lower-ranked plants than that in performance. In our metadata, the oviposition by specialist herbivores displayed a negative  $\text{diff\_LR}$  (mean =  $-0.37$ ), but that of generalist herbivores was slightly positive (mean =  $0.06$ ). Hence, the

heterogeneity among herbivore response traits may be ascribed to the stronger linkage between the oviposition preference and host suitability in specialist herbivores.

Although it is still unknown how important our results are in the field, there would be remarkable notions on intra- or interspecific plant variation that drives their interaction with herbivores. In our metadata, for example, intraspecific variation is often considered in the context of phenotypic plasticity and induced response (Agrawal 2000, McArthur et al. 2010, Hoffman and Rao 2011). Contrarily, interspecific variation is a major viewpoint of apparent competition and intercropping between plant species (reviewed by Barbosa et al. 2009). In this context, our study found a marginally significant amount of heterogeneity explained by the plant relatedness, where interspecific plant pairs exhibited wider intervals of effect size than intraspecific ones did. If the results reflect the two processes discussed so far, our meta-analysis may have some implications on the predictability of anti-herbivore resistance conferred by the two sources of plant variation.

Because many species of endophagous herbivores (such as leaf miners) are known as specialists (Cornell 1989, Gaston et al. 1992), it is possible that herbivore feeding habits have shown a similar trend with the diet breadth. However, the endophagous invertebrate category displayed a different tendency from the specialist herbivores. In addition, the specialist vs. generalist patterns turned out are not a simple reflection of the difference between generalist mammals and specialist insects, because the amount of heterogeneity attributable to the diet breadth remained significant even if the vertebrate–invertebrate difference was included as a covariate. Although the small amount of heterogeneity was attributable to the different herbivore types, we should notice a potential correlation between the diet breadth and the other characteristics of herbivores.

Our results should be interpreted carefully considering the limited sample size and the presence of phylogenetic bias. The current metadata suffered from sample size biases when comparing the chemical and nonchemical groups or the different herbivore types. This bias might have prevented us from detecting interactive effects between herbivore and plant traits. Another

caveat is the restricted consideration for the phylogenetic influence in the plant family level analysis. This has occurred because the nested ANOVA approach did not reflect all the phylogenetic information (Harvey and Pagel 1991). Additionally, it is difficult to apply this nested method to the herbivore category due to the unrealistic assumption that phylogenetic distance is treated as equal among vertebrate and invertebrate taxa. Further examples and detailed phylogeny are required for evaluating combinations between the herbivore behavioral response and plant defense traits in coevolutionary contexts.

In conclusion, our meta-analysis revealed the contrasting response between specialist and generalist herbivores to a mixed array of their diet plants, highlighting one of the patterns of specialist vs. generalist prevalent in plant–herbivore systems. While a recent meta-analysis has shown that fitness gain for generalist consumers by diet mixing are relatively limited (Lefcheck et al. 2013), diet breadth may still have some impacts on herbivore behaviors, at least under enclosed conditions. Additionally, specialist–generalist differences have also been reported for herbivore responses to vegetation diversity in the field (e.g., Castagneyrol et al. 2014). Given that plants often serve as a heterogeneous resource for herbivores, the contrasting response between specialists and generalists may account for heterogeneity of plant damage and herbivore abundance at population or community levels.

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