

# Hyperaccumulators and Herbivores—A Bayesian Meta-Analysis of Feeding Choice Trials

Peter A. Vesik · Suzie M. Reichman

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**Abstract** The leading hypothesis for the evolution of the metal hyperaccumulation trait in plants is as a defense against herbivores. A central piece of evidence expected for this hypothesis is that plants benefit from herbivores being deterred from eating high metal tissues. While many studies have investigated whether or not herbivores are deterred by high metal feeds, there has been no quantitative synthesis of these studies. We performed a Bayesian meta-analysis of 31 feeding choice trials from ten published studies, where invertebrates were offered diets of plant tissue from hyperaccumulating species with high and low metal concentrations. Results of individual trials ranged from distinct preference to distinct aversion for high metal diets. The overall mean effect was for herbivore aversion to high metal diets, whether we used fixed or random effects. However, random effect models were better supported than fixed effect models, indicating there was much real variation between trials. This variation could be attributed partly to each of herbivores, plants, studies, and metals, with herbivores being the greatest source of variation. On average, high metal diets deterred insects but not gastropods, which is supported by other research of metal tolerance and sequestration by gastropods. This suggests that the evolution of hyperaccumulation may have differing selective pressures depending upon the suite of herbivores the plants are naturally exposed to. Future studies should give greater consideration to the selection of herbivores and plants tested.

**Keywords** Elemental plant defense · Heavy metal · Literature synthesis · Metal uptake · Nickel · Selenium · Zinc

## Introduction

Metal hyperaccumulation, i.e., the ability to accumulate and tolerate large concentrations of metal<sup>1</sup> in aboveground tissues, has been identified in over 415 species of plants across a range of genera, families, and orders worldwide (Baker et al. 2000). Threshold concentrations for the metal hyperaccumulation trait are orders of magnitude higher than the concentrations found in “normal” plant species, e.g., the thresholds for classification as a zinc, nickel, or selenium hyperaccumulator are 10,000, 1,000, or 100  $\mu\text{g g}^{-1}$  metal in the shoots (dry weight), respectively (Baker et al. 2000; Reeves and Baker 2000). Interest in metal hyperaccumulating plants is high within both the research and management communities because of the potential use of hyperaccumulating species in phytoremediation of metal-contaminated sites and the need to conserve the unique and often endangered metalliferous ecosystems in which hyperaccumulating species are found (Whiting et al. 2004).

Boyd and Martens (1992) evaluated early research on hypotheses for the evolution of hyperaccumulator plants and concluded that the herbivory/pathogen-defense hypothesis was the most plausible. Their review stimulated much research that explored the defensive role of hyperaccumulated metals against terrestrial invertebrate herbivores. Boyd’s review of 34 studies of the defense hypothesis

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P. A. Vesik (✉)  
School of Botany, The University of Melbourne,  
Parkville 3010 Victoria, Australia  
e-mail: pvesk@unimelb.edu.au

S. M. Reichman  
School of Chemistry, The University of Melbourne,  
Parkville 3010 Victoria, Australia

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<sup>1</sup> Metal hyperaccumulating plants generally are considered to include those that hyperaccumulate metals, such as zinc or copper, and/or metalloids, such as selenium or boron. Throughout this paper, the term metal is used to include both metals and metalloids.

(Boyd 2007) has clarified the categories of evidence needed for demonstration of a selective benefit of hyperaccumulation. There is considerable, but not total, support for the herbivory-defense hypothesis among the hyperaccumulator research-community (e.g., Boyd et al. 2002; Jhee et al. 1999; Pollard 2000; Pollard and Baker 1997). One form of evidence for the herbivory-defense hypothesis is herbivore deterrence in feeding choice trials (Boyd 2007). A number of researchers have investigated the potential of hyperaccumulated metals to protect plants from herbivory via feeding-choice experiments where invertebrate herbivores were offered a choice of leaf tissue (high or low metal concentration) from hyperaccumulator plants, and the feeding preferences were analyzed. Results have varied widely among these, ranging from aversion (e.g., Boyd et al. 2002; Hanson et al. 2004; Martens and Boyd 2002) to preference for a high metal diet (e.g., Boyd and Martens 1999; Hanson et al. 2003; Noret et al. 2005). Findings from choice trials were tabulated, reporting the number that showed a defensive effect (Boyd 2007). This methodology, called vote counting, is limited in its utility for quantitative synthesis (Hedges and Olkin 1980; Bushman 1994). Specifically, Boyd (2007) did not examine the magnitude of the effect and variation therein. By building on Boyd's review (2007), we report here a Bayesian meta-analysis of the published data from feeding-choice trials. Our objective was to investigate quantitatively the following questions: (1) Is there a deterrent effect of high metal concentrations in the leaf tissue of hyperaccumulator plants against herbivory by terrestrial invertebrates? (2) If so, how large is the effect? (3) How does the effect vary among trials? (4) Can the variation be explained?

## Methods and Materials

Meta-analysis is a process of quantitative synthesis of results of multiple studies of the same process. By combining results of multiple studies, greater statistical power to examine hypotheses is attained. Moreover, the generality of a model can be examined and effects of covariates determined. We used a Bayesian statistical framework for our analysis for two main reasons. First, we were interested in estimating effect sizes or parameters and confidence in them, rather than the statistical significance of a test of a null hypothesis, as what occurs with classical or frequentist statistical approaches (McCarthy 2007). Second, in many cases, random effect models are more appropriate than fixed effects models (Raudenbush 1994). This is because we expect variation of parameters for biological and logistic reasons, i.e., a range of effect sizes, due to differences in biological interactions that are beyond sampling variation. The technical aspects of

performing frequentist random effects meta-analyses are complex, and procedures are neither straightforward nor accepted universally (Raudenbush 1994). By contrast, random effect models, and models with a mixture of fixed and random effects, can easily be constructed and evaluated with Bayesian inference by using Markov Chain Monte Carlo sampling methods (Clark 2005; Link et al. 2002). For an introduction to Bayesian modeling, we suggest Link et al. (2002) and McCarthy (2007).

Our aim here was to compile quantitatively and analyze a data set of invertebrate binary choice feeding trials. We obtained the data set from peer-reviewed publications that reported feeding choice trials of invertebrate herbivores offered control and high metal concentration diets of metal hyperaccumulating plants. We located the publications through personal bibliographies, searches of the online database, Thomson ISI Web of Science (latest search April 2008), and the tabulation provided by a recent review (Boyd 2007). Ten papers were found that fit our criteria for analysis, namely, that means, sample sizes, and precisions (standard errors) for consumption of plant tissue were accessible in the paper. Multiple trials often were reported in a single publication, either as repeat runs or by using different herbivores or plant population combinations. Thus, our data set comprised 31 individual trials (Table 1).

To account for differences in the way trials were conducted (e.g., age and size of herbivores, trial duration) and how response variables were measured (e.g., area vs. mass consumed, absolute vs. relative consumption), we transformed the response data to reflect the proportional change in consumption between control and high metal diets. Because the minimum consumption of a herbivore is zero and because most individuals ate small amounts with a few individuals eating large amounts (i.e., a right skewed distribution), we modeled the data with log-normal distributions. In addition, the skewed data led to standard errors that increased in magnitude with the mean. Hence, we used means and standard errors as presented in the original papers to calculate the parameters for log-normal distributions by using the following formulae (McCarthy 2007):

$$c = \sigma^2 / \mu^2 + 1, \quad (1)$$

$$a = \ln(\mu) - 0.5 \ln(c), \quad (2)$$

$$t = 1 / \ln(c), \quad (3),$$

where  $\mu$  is the reported mean, and  $\sigma$  the reported standard error of the mean,  $c$  is a scaling parameter for the conversion,  $a$  is the mean of the log-normal, and  $t$  is the

**Table 1** Summary of 31 binary choice feeding trials of invertebrate herbivores offered high and low metal concentration diets from metal hyperaccumulator plants

Source	Plant	Herbivore	$E$	$\tau$	Metal	Trial no.
(Boyd et al. 2002)	<i>Senecio coronatus</i>	<i>Helix aspersa</i> (G)	-0.14	4.00	Ni	22
(Boyd et al. 2002)	<i>Senecio coronatus</i>	<i>Helix aspersa</i> (G)	2.04	1.30	Ni	8
(Boyd et al. 2002)	<i>Senecio coronatus</i>	<i>Helix aspersa</i> (G)	2.41	2.20	Ni	7
(Hanson et al. 2003)	<i>Brassica juncea</i>	<i>Mesodon ferrissi</i> (G)	-2.49	1.89	Se	31
(Hanson et al. 2003)	<i>Brassica juncea</i>	<i>Mesodon ferrissi</i> (G)	-1.15	10.88	Se	27
(Hanson et al. 2003)	<i>Brassica juncea</i>	<i>Mesodon ferrissi</i> (G)	-0.48	46.15	Se	25
(Hanson et al. 2003)	<i>Brassica juncea</i>	<i>Pieris rapae</i> (I)	3.01	1.47	Se	4
(Jhee et al. 2005)	<i>Streptanthus polygaloides</i>	<i>Melanoplus femurrubrum</i> (I)	1.86	6.02	Ni	9
(Jhee et al. 1999)	<i>Thlaspi caerulescens</i>	<i>Pieris napi oleracea</i> (I)	0.27	34.18	Zn	19
(Jhee et al. 1999)	<i>Thlaspi caerulescens</i>	<i>Pieris napi oleracea</i> (I)	1.01	43.73	Zn	13
(Martens and Boyd 1994)	<i>Streptanthus polygaloides</i>	<i>Pieris rapae</i> (I)	0.50	14.65	Ni	18
(Noret et al. 2005)	<i>Thlaspi caerulescens</i>	<i>Helix aspersa</i> (G)	-0.38	3.08	Zn	24
(Noret et al. 2005)	<i>Thlaspi caerulescens</i>	<i>Helix aspersa</i> (G)	1.29	3.22	Zn	11
(Noret et al. 2005)	<i>Thlaspi caerulescens</i>	<i>Helix aspersa</i> (G)	-2.14	4.33	Zn	30
(Noret et al. 2005)	<i>Thlaspi caerulescens</i>	<i>Helix aspersa</i> (G)	-0.91	8.32	Zn	26
(Noret et al. 2005)	<i>Thlaspi caerulescens</i>	<i>Helix aspersa</i> (G)	0.91	15.79	Zn	14
(Noret et al. 2005)	<i>Thlaspi caerulescens</i>	<i>Helix aspersa</i> (G)	-1.33	3.26	Zn	28
(Noret et al. 2005)	<i>Thlaspi caerulescens</i>	<i>Helix aspersa</i> (G)	-1.76	4.64	Zn	29
(Noret et al. 2005)	<i>Thlaspi caerulescens</i>	<i>Helix aspersa</i> (G)	-0.02	15.57	Zn	20
(Noret et al. 2005)	<i>Thlaspi caerulescens</i>	<i>Helix aspersa</i> (G)	0.80	11.24	Zn	15
(Noret et al. 2005)	<i>Thlaspi caerulescens</i>	<i>Helix aspersa</i> (G)	-0.13	5.42	Zn	21
(Noret et al. 2005)	<i>Thlaspi caerulescens</i>	<i>Helix aspersa</i> (G)	0.56	6.05	Zn	17
(Pollard and Baker 1997)	<i>Thlaspi caerulescens</i>	<i>Deroceras caruanae</i> (G)	1.57	15.42	Zn	10
(Pollard and Baker 1997)	<i>Thlaspi caerulescens</i>	<i>Pieris brassicae</i> (I)	4.39	1.4	Zn	3
(Pollard and Baker 1997)	<i>Thlaspi caerulescens</i>	<i>Schistocerca gregaria</i> (I)	1.28	9.81	Zn	12
(Behmer et al. 2005)	<i>Thlaspi caerulescens</i>	<i>Schistocerca gregaria</i> (I)	0.73	20.95	Zn	16
(Freeman et al. 2006)	<i>Stanleya pinnata</i>	<i>Pieris rapae</i> (I)	6.79	0.22	Se	1
(Freeman et al. 2006)	<i>Stanleya pinnata</i>	<i>Plutella xylostella</i> Stanleyi (I)	-0.19	5.17	Se	23
(Freeman et al. 2006)	<i>Stanleya pinnata</i>	<i>Plutella xylostella</i> G88 (I)	5.35	0.31	Se	2
(Freeman et al. 2007)	<i>Stanleya pinnata</i>	<i>Orthoptera</i> spp. (I)	2.88	4.56	Se	5
(Freeman et al. 2007)	<i>Stanleya pinnata</i>	<i>Orthoptera</i> spp. (I)	2.50	7.12	Se	6

$E$  is the effect size and is positive where control diets were preferred to high metal diet.  $\tau$  is the precision of the estimated effect size and is the inverse of the variance. See text for details. Trial refers to the trial number in Fig. 1.  $G$  after the herbivore indicates gastropod,  $I$  indicates insect

precision (inverse of the variance) of the log-normal. Where data were only presented as graphs, we first scanned the figures and then used the program Datathief (<http://www.datathief.org/>) to recover mean and standard error values. The effect size ( $E$ ) of the metal on consumption was then calculated as:

$$E = a_{\text{control}} - a_{\text{metal}} \quad (4).$$

The precision of the effect size was calculated as (assuming independence, so that the variances are additive):

$$\tau = \frac{1}{\left(\frac{1}{t_{\text{metal}}} + \frac{1}{t_{\text{control}}}\right)} \quad (5).$$

In this way, we obtained the basic dataset where the response variable was the effect size,  $E$ , and associated precision,  $\tau$ , for each binary choice trial (Table 1). Note that these effect sizes are not *differences* but *differentials* that reflect how many times more of one diet is consumed than another. Because the effect is on a natural log scale, values for  $E$  of 0.5, 1, 2, 3 correspond to eating 1.6, 2.7, 7.4, and 20 times as much of the control diet over the high metal diet; negative values of  $E$  imply preference for the high metal diet.  $\tau$  is the inverse of the variance and, thus, can be converted to a standard error by taking the square root of the inverse. Large values of  $\tau$  equal small standard errors; e.g.,  $\tau=0.5$ , 1, 5, and 10 yield standard errors of 1.4, 1, 0.45, and 0.32, respectively.

The differentials obtained above were modeled with simple linear models by using Bayesian statistics. Models were fitted by using the Bayesian statistical modeling software WINBUGS 1.4.1 (Lunn et al. 2000), available as freeware from <http://www.mrcbsu.cam.ac.uk/bugs/>. We began by modeling the differential as a fixed coefficient, such that observed effect sizes were distributed around the grand mean with only random error variance. Next, we modeled the differential as a random coefficient, such that observed effect sizes were random draws from a population of effect sizes for which we inferred the mean and variance. This allowed all trials to have their own effect size, which is drawn from some unknown distribution that we seek to model. Third, we sought to examine the effect of introducing information about covariates as random effects into the models with the differential being a fixed effect or a random effect. Four covariates were included: (1) the publication; (2) the plant species (one of *Brassica juncea*, *Senecio coronatus*, *Stanleya pinnata*, *Streptanthus polygaloides*, *Thlaspi caerulescens*); (3) the herbivore species (see Table 1); and (4) the metal studied (nickel, selenium, or zinc). Finally, we fitted a model with an herbivore type as fixed effect to examine the difference between gastropods and insects.

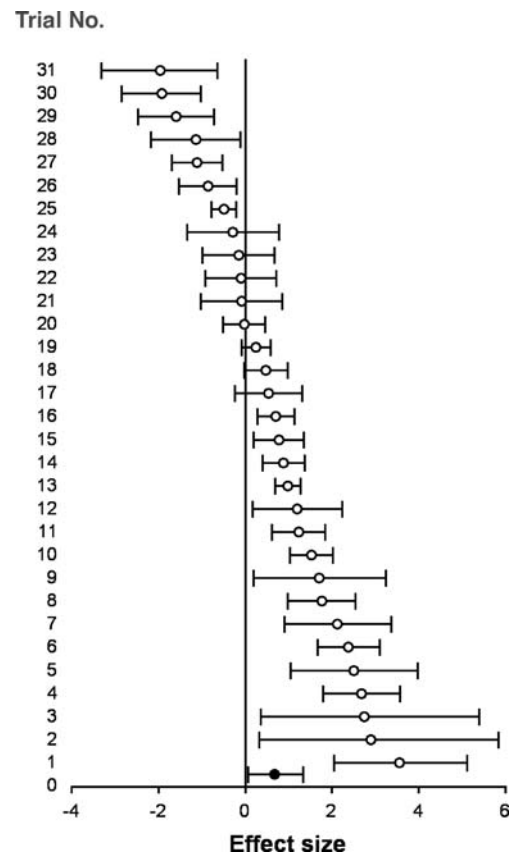
Although Bayesian inference has the potential to incorporate prior information as expert belief or ancillary data, we chose not to use this, and rather used minimally informative prior distributions for the parameters of interest. All fixed effect coefficients were modeled as being drawn from normal distributions with mean 0.0 and SD 1,000. Random effect coefficients were modeled as normally distributed with mean 0.0 and SDs drawn from a uniform distribution over the interval (0, 100). Three Markov chain Monte Carlo (MCMC) chains with different initial values (-1, 0, 1) were run simultaneously for each model. In all cases, burn-in phases were >1,000 iterations. Standard Bayesian MCMC diagnostics were used to assess convergence, including plots of each of the chain traces, chain autocorrelations, and the Gelman–Rubin statistic (Spiegelhalter et al. 1995; Zuur et al. 2002). Bayesian 95% credible intervals were calculated from the posterior probability density of monitored parameters, in each case by using >50,000 samples. When minimally informative priors are used, as used here, credible intervals are practically equivalent to frequentist confidence intervals calculated with maximum likelihood estimation. The estimates for the effect sizes ( $E$ ) (and credible intervals) were then converted back to an arithmetic scale by exponentiating, i.e., differential consumption =  $e^E$ .

For model selection, the series of models was compared by using the deviance information criterion (DIC) (Spiegelhalter et al. 2002), calculated with WINBUGS. In a given dataset, the model with the lowest DIC is the model

that would be most likely to produce a dataset of the same structure as that observed. That is, it is the model that best balances model fit, as measured by the deviance, and model complexity, as measured by the estimated number of parameters in the model. The percentage of deviance explained by a fitted model (similar to  $R^2$ ) was calculated as the total deviance minus the deviance for the fitted model, divided by the total deviance, using the deviances at the posterior mean of the parameters ( $\hat{D}$ ).

## Results

The effect sizes ( $E$ ) modeled for individual trials ranged widely from strong aversion to strong preference (Fig. 1). For the null model, which posits that all measured effect



**Fig. 1** Effect sizes (with 95% Bayesian credible intervals) of 31 binary choice trials of invertebrate herbivores offered control and high metal concentration plant tissue from a random effects model. Effect size is a natural log scale; positive values indicate aversion to the high metal, and negative ones indicate preference. Zero indicates no preference, equal amounts of control and high metal diets are chosen. Individual effect sizes of trials (open circles) range widely from strong preference to strong aversion to the metal (see Table 1 for identification of the trial the numbers refer to). The credible interval around the mean effect size (filled circle, at bottom of graph) reflects uncertainty about the location of the mean. The distribution of 95% of population effect sizes expected is about twice as wide

sizes should be equal and only vary through sampling error, the mean effect size (and 95% credible interval) was 0.43 (0.32, 0.54). This implies that, on average, invertebrates avoided the high metal plant leaves and ate a mean of 1.54 (1.38–1.72) times as much control diet as high metal diet. However, compared by DIC, the fixed effect model was the poorest of those examined (Table 2). The random effects model had a mean effect size of 0.71 (0.10, 1.36; Fig. 1) corresponding to herbivores eating 2.03 (1.10, 3.89) times as much control diet as high metal diet. The SD of the estimated mean effect was 0.32, but that among trials was fivefold greater, 1.62 (1.19, 2.29), which underscores the relevance of trial-to-trial variation. In other words, the mean effect is larger, but less certain when we account for real variation among trials.

Introduction of covariates improved fixed effect models beyond the null model (see model 11 in Table 2). This is because, for a given level of a covariate, e.g., for the herbivore *Pieris rapae*, the observed effect sizes from different trials were far from equal. In comparison, once the random effect of the trials was included, the addition or removal of other covariates had relatively little influence on model selection (Table 2). Models that included herbivore or plant were better supported than those that included metal or study. There was little evidence for an effect of

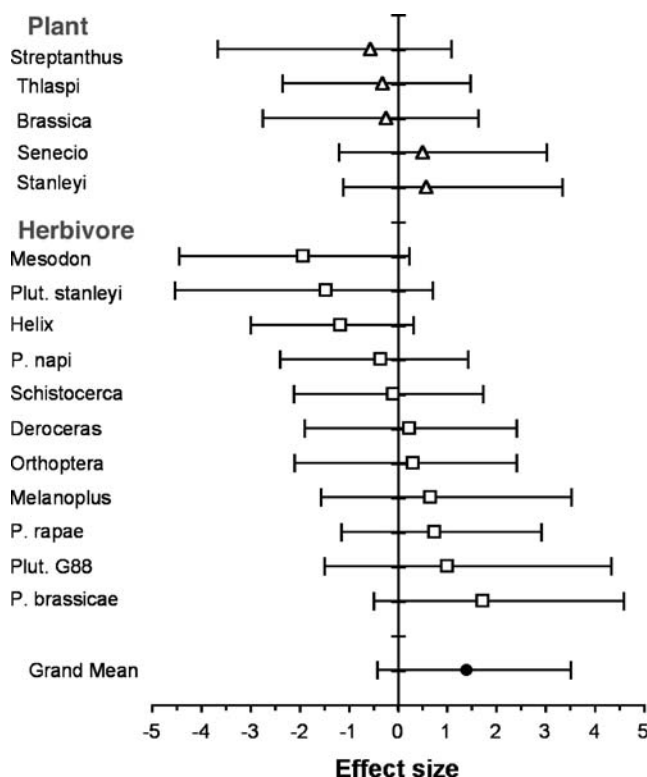
metal type. The effects for the individual metals all had SDs at least three times as large as the means.

The best-supported model had random effects of herbivore, plant, and trial (Model 1 in Table 2). According to this model, the mean effect size was 1.41 (−0.40, 3.53), corresponding to 4.1 (0.67, 34.1) times as much control food as high metal food being consumed (Fig. 2). Hence, the uncertainty increased over the model of trial-to-trial variation only, and we cannot be certain that, on average, high metals deter herbivores. Examining the SD of the various random effects revealed important variation among herbivores [ $SD_h=1.63$  (0.52, 3.52)], and among plants [ $SD_p=1.16$ , (0.10, 4.63)], and variation between trials was reduced [ $SD_t=1.06$  (0.70, 1.69)] relative to the model with a random trial effect only. The uncertainty about variation among plants was in part because so few hyperaccumulators have been studied. On average, *Stanleya pinnata* and

**Table 2** Comparison of models of the effect of high metal concentrations in plant tissue on invertebrate consumption

Model	DIC	$\hat{D}$	Explained deviance (%)
1 Trial, herbivore, plant	66.0	11.0	96.9
2 Trial, herbivore, study	66.6	11.7	96.7
3 Trial, herbivore, metal	66.8	12.0	96.6
4 Trial, herbivore	67.7	13.2	96.3
5 Trial, plant	68.0	11.8	96.7
6 Trial, study, plant	68.2	13.4	96.2
7 Trial, metal	68.6	12.3	96.5
8 Trial, study	68.8	13.4	96.2
9 Trial	69.0	13.0	96.3
10 Herbivore, plant, study	143.5	115.8	67.4
11 Null model, fixed mean effect only	356.9	354.9	0

DIC is deviance information criterion,  $\hat{D}$  is the deviance at the posterior mean of the parameters, Explained deviance reports how much residual deviance after the fitting of a null model (no. 11) is explained by a particular target model. Models with low DIC values are more parsimonious than those with high ones. Models with DIC values that differ by less than about 2 are of equivalent predictive value. All models that included a random effect of trial were more parsimonious than those that did not. Only one such model that lacked an effect of trial is included here for comparison, model 10



**Fig. 2** Plot of the mean (and 95% Bayesian credible intervals) for the effect of plants (triangles) and herbivores (squares) on differential consumption of control and high metal concentration plant tissue in feeding choice trials. The Grand Mean (filled circle) effect is at the bottom of the graph. Zero indicates no preference, equal amounts of control and high metal diets are chosen. The effects of the plants and herbivores are centered on zero and would be added to the Grand Mean. Effect size is a natural log scale; positive values indicate aversion to the high metal, and negative ones indicate preference. Credible intervals here reflect the uncertainty around mean effect sizes. The distribution of 95% population effect sizes expected is about twice as wide

*Senecio coronatus* tended to evoke greater herbivore deterrence (Fig. 2).

Of the herbivores, the snails, *Mesodon ferrissi* and *Helix aspersa*, were less likely to be deterred by high metal concentrations, thus suggesting a difference in tolerance between gastropods and insects (Fig. 2). Further, the demonstrably Se-tolerant moth, *Plutella xylostella* Stanleyi (Freeman et al. 2006), also was less likely to be deterred by high metal (Se) than other herbivores.

Probing deeper into the herbivore effect, we fitted a model with a fixed effect for herbivore (insect vs. gastropod) as well as random effects for plant and trial (Table 3). This revealed that gastropods were unlikely to be deterred from eating high metal plants; the credible interval around the mean effect for the gastropods included zero, and the mean effect was close to zero (Table 3). By contrast, high metals were likely to deter insects. The contrast between the effects of the herbivores (insects minus gastropods) was 1.84 (0.67, 3.08), which means that the deterrent effect of high metals was on average 6.3 times (1.95, 21.7) greater for insects than for gastropods. Note that the Se-tolerant *Plutella* was included in the insects, yet this did not influence the average deterrence of insects greatly.

## Discussion

The meta-analyses revealed three major findings. (1) On average, high metal concentrations in hyperaccumulators deterred herbivory by terrestrial invertebrates. (2) The variation was so great, however, that individual trials revealed results that ranged from distinct metal aversion to distinct metal preference. Some of this variation could be explained by the identity of herbivores, plants, metals, and studies. (3) Herbivores were the greatest source of variation in deterrence, with the suggestion that hyperaccumulation deterred insects but not gastropods.

This work supports an interpretation that metal hyperaccumulation by plants leads to herbivore deterrence, which is a key piece of evidence expected for antiherbivory defense being a reason for the evolution of hyperaccumulation (Boyd 2007). The variability between the effect sizes of individual trials was too great to be considered the result of simple “sampling error”. Rather, there is a wide distribution of true effect sizes. It is expected that future studies will report results that range from distinct metal aversion to distinct metal preference, and that some of the range is because there are situations where the true preference is for a high metal diet, rather than just being a result of stochastic sampling variation.

The study identity explained some of the variation in feeding choice. There are a number of possible interpretations of this result. A skeptical interpretation is that experimenters have strong influence on results. A more positive and probable interpretation is that although the herbivore, plant, and metal all influence the effect of high metal on feeding choice, these factors are more similar within studies than between studies. For instance, while three studies compared different herbivores, none compared different plant species. Indeed, one study (Noret et al. 2005) contributed 11 trials, all with the same herbivore and plant species combination. That publication points to a possible “file drawer effect,” whereby single or underpowered trials that fail to detect herbivore deterrence are either not submitted for publication or are rejected because they do not match with (unspoken) expectations (Moller and Jennions 2001). The study of Noret et al. (2005) contributed a disproportionate share of trials that indicated preference for high metal diets; perhaps so many trials were needed to convince authors, editors, and reviewers that the findings were not “mistakes.” Ecotypic variation within plants and herbivores studied in multiple studies also may contribute to the variation.

Because the distribution of herbivores and plants across studies was limited, uneven, and confounded, the power of

**Table 3** Parameter estimates for a model with a fixed effect of insects versus gastropods and random effects for plants and trials

The fixed effect for the herbivore type was modeled such that gastropods were the reference class. This model had a DIC value of 66.6, and  $\bar{D} = 11.9$ . Insects–gastropods is the contrast between the two classes of herbivore

	Mean	SD	2.5% CI	Median	97.5% CI
Insects	1.89	0.72	0.51	1.86	3.42
Insects–Gastropods	1.85	0.61	0.67	1.84	3.08
Gastropods	0.03	0.75	–1.34	0.01	1.53
<i>Brassica</i>	–0.19	0.67	–1.68	–0.11	1.05
<i>Thlaspi</i>	–0.30	0.82	–2.26	–0.14	1.12
<i>Streptanthus</i>	–0.41	0.79	–2.28	–0.25	0.86
<i>Stanleyi</i>	0.53	0.86	–0.79	0.35	2.58
<i>Senecio</i>	0.29	0.77	–1.12	0.17	2.01
SD <sub>p</sub>	0.97	1.02	0.04	0.72	3.43
SD <sub>t</sub>	1.34	0.24	0.94	1.32	1.89

the analysis to estimate effects of herbivore or plant or metal was limited. Estimates of mean effect sizes for plants and herbivores varied over threefold and 30-fold, respectively, when back-transformed (Fig. 2), but the credible intervals for predictions were broad. The most striking result was that high metal diets did not deter snail herbivory. Our random effect analysis recovered the effect of the evolution of Se-tolerance in a taxon of *Plutella xylostella* (Freeman et al. 2006; compare the two *Plutella* effects in Fig. 2). Yet, high metals deterred the two snail taxa even less than the Se-tolerant *P. xylostella stanleyi*. Indeed, in a study that we could not include in our analysis because we could not extract the relevant statistics, *Helix aspersa* did not distinguish between low and high metal diets of *Arabidopsis halleri* (Huitson and Macnair 2003), thus supporting our results for snails. We note that snails, particularly the species most frequent in our dataset, *Helix aspersa*, have been shown to be tolerant of, and to accumulate, high levels of metals (Coughtrey and Martin 1976, 1977). Snails also regulate free metal concentrations through binding to metallothioneins and calcium phosphate granules (Berger and Dallinger 1989; Howard et al. 1981; Marigomez et al. 2002). If the two snail species, *Mesodon ferrissi* and *Helix aspersa*, and the Se-tolerant *Plutella xylostella Stanleyi* were omitted from the analysis (e.g., in Fig. 2), the effect would be to increase the grand mean deterrent effect and reduce its uncertainty due to these herbivores being the least deterred by metal-rich diets.

Our fixed effect contrast of the gastropods and insects showed that while insects were deterred from eating high metal plants, gastropods were not. This is an important result for better understanding the evolution of hyperaccumulation as a defense. Caution is required because, strictly, the fixed effect analysis cannot be used as a basis for inference about other gastropods and insects, unlike the random effects analysis. The fixed effect also ignores herbivore species variation within those classes of insects and gastropods. This clearly is incorrect; compare the two snails *Helix aspersa* and *Mesodon ferrissi* with the slug *Deroceras caruanae* (Fig. 2). However, visual interpretation of the parameter estimates from the random effect model in Fig. 2 supports the finding that, on average, hyperaccumulation deters insects but not gastropods. Whether this is a general result requires targeted research. Our analyses suggest the evolution of hyperaccumulation may have differing selective pressures that depend upon the suite of herbivores the plants are naturally exposed to. Where gastropods are a large component of potential herbivores, selection for hyperaccumulation as a defense may be diminished.

Explanation for the wide variability in the results may lie in the choice of widespread, generalist herbivores for most herbivory-defense trials on hyperaccumulating plants,

including those analyzed here. Herbivores endemic to, or at least occurring in, areas inhabited by hyperaccumulator species may be better able to identify metals in plant tissues and avoid them. Further, with sufficiently long exposure to hyperaccumulators, one might expect the evolution of deterrence and/or detoxification mechanisms in some of these endemic herbivores (e.g., Freeman et al. 2006; Wall and Boyd 2006). We suggest that future researchers pay more attention to the choice of plant–herbivore pairings. Studies that cover multiple plant–herbivore combinations reduce the confounding of studies, plants, and herbivores. Further research also could use the results of this work as priors to be updated with new data using the Bayesian statistical framework (McCarthy and Masters 2007).

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