Rethinking Ecology
RESPONSES

A comment on "Variability in plant nutrients reduces insect herbivore performance"

Apostolos-Manuel Koussoroplis[1], Toni Klauschies[2], Sylvain Pincebourde[3], David Giron[3], Alexander Wacker[2]

1 *Université Clermont Auvergne, Aubière, France* **2** *Universitäet Potsdam, Potsdam, Germany* **3** *CNRS, Tours, France*

Corresponding author: *Apostolos-Manuel Koussoroplis* (a-manuel.koussoroplis@uca.fr)

Abstract

In their recent contribution, Wetzel et al. [Wetzel et al. (2016) Variability in plant nutrients reduces insect herbivore performance. Nature 539: 425-427] predict that variance in the plant nutrient level reduces herbivore performance via the nonlinear averaging effect (named *Jensen's effect* by the authors) while variance in the defense level does not. We argue that the study likely underestimates the potential of plant defenses' variance to cause Jensen's effects for two reasons. First, this conclusion is based on the finding that the average Jensen's effect of various defense traits on various herbivores is zero which does not imply that the Jensen's effect of specific defense traits on specific herbivores is null, just that the effects balance each other globally. Second, the study neglects the nonlinearity effects that may arise from the synergy between nutritive and defense traits or between co-occurring defenses on herbivore performance. Covariance between interacting plant defense traits, or between plant nutritive and defense traits, can affect performance differently than would nutritive or single plant defense variance alone. Overlooking the interactive effects of plant traits and the traits' covariance could impair the assessment of the true role of plant trait variability on herbivore populations in natural settings.

Plant trait variance can have important consequences for herbivore populations in natural systems via nonlinear averaging effects of herbivore performance (Stockhoff 1993; Ruel and Ayres 1999). In their recent contribution, Wetzel et al. (Wetzel et al. 2016) predict that variance in plant nutrient level reduces herbivore performance via nonlinear averaging effects while variance in defense level does not.

Using an analogy of the Hedge's *d* metric (Hedges and Olkin 1985), Wetzel et al. (Wetzel et al. 2016), calculate the average of the normalized Jensen's effect (Ruel and Ayres 1999) which is based on the difference between the average performance in the absence and the presence of plant trait variance, *i.e.* $F(\overline{x}) - \overline{F(x)}$. They inferred this metric from a large number of curves fitted to published datasets relating insect herbivore performance to different levels of various defense and nutrition traits. They found that the average Jensen's effect size is significantly negative for plant nutritive traits but is nearly zero for plant defense traits. The latter motivated the authors to conclude that the relationships between insect herbivore performance and plant defense levels are linear *on average* and that consequently plant defense variance should not affect herbivore performance via nonlinear averaging contrary to the overall negative effects of variance in plant nutritive traits.

We argue that this study may have underestimated the potential of plant defense trait variance to generate Jensen's effects. The conclusion is based on the *average* shape of the relationship between herbivore performance and plant defenses, *i.e.* $F(\overline{x}) - \overline{F(x)}$. This approach seems to be valid when considering a group of random datasets with no true nonlinearities (Fig. 1A). Although many of the individual data sets will show some nonlinearity due to sampling error, the mean nonlinearity will be close to zero, as positive and negative curvatures would on average balance each other. In this scenario, the Jensen's effect size is accurately predicted to be close to zero (Fig. 1C). However, when considering a collection of strongly concave-up and concave-down relationships and thus truly non-linear coherences (Fig. 1B), estimating the average effect size of Jensen's inequality based on the raw differences leads to a kind of *fallacy of the average*. The resulting positive and negative Jensen's effects cancel each other and the average effect is zero despite the relationships being strongly nonlinear (Fig. 1D). A more reliable estimate of the average Jensen's effect size in such cases could be obtained by the average of the absolute differences, *i.e.* $|(F(\overline{x}) - \overline{F(x)})|$ (Fig. 1C, D). Hence, a linear shape on average does not necessarily imply that the Jensen's effect is generally close to zero but only that the average of the observed Jensen's effects is zero. Nevertheless, using the mean absolute value is not entirely unbiased and a mean absolute value that differs from zero might also result from sampling error (see Fig. 1C, purple points). It is thus very important to develop statistical tools to distinguish between variation in Jensen's Inequality effects that are based on sampling error versus actual differences in the curvature of the functional relationships.

The choice of the most appropriate way to report and analyze such data depends on prior physiological knowledge. Growth limitation by macro- or micronutrients involves similar physiological mechanisms: Anabolic rate limitation when nutrient supply is limiting (Kooijman 2010) and extra metabolic costs when they are in excess (Boersma and Elser 2006; Simpson and Raubenheimer 2012). We can thus safely assume a common Gaussian-like or parabolic shape of the growth reaction norm to
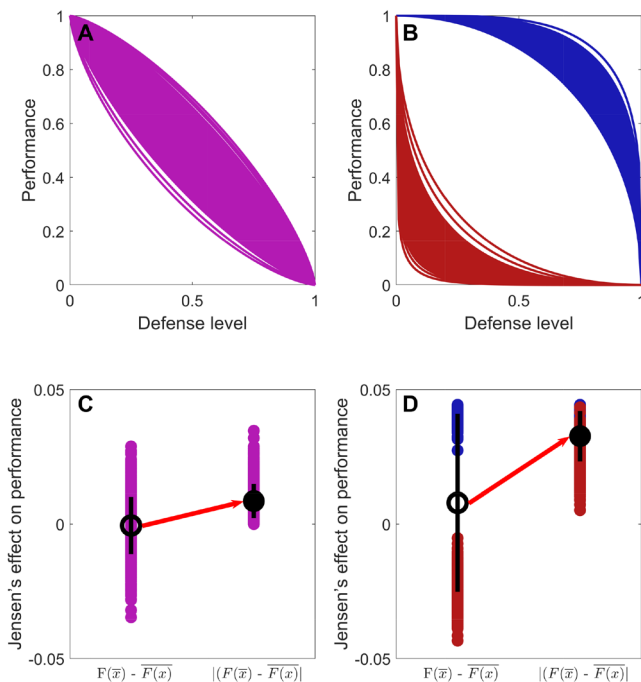
Figure 1. An illustration of the "fallacy of the averaged shape". Mixtures of randomly generated quasi-linear (**A**) or strongly nonlinear concave-up (blue) and concave-down (red) (**B**) relationships between herbivore performance and plant defense level. **C, D** Mean (open and closed black dots) and standard deviation (black bars) of the effect size of Jensen's Inequality based on the individual values (colored dotes) of the corresponding relationships shown in **A** and **B**.

limiting plant nutrient levels (Raubenheimer et al. 2005; Elser et al. 2016; Sperfeld et al. 2017). This seems to be confirmed by Wetzel et al. (2016) since the vast majority of the reaction norms extracted from their literature data base are concave-down (see fig. 3D in Wetzel et al. 2016). This homogeneity of reaction norms justifies the use of $F(\bar{x}) - \overline{F(x)}$ for nutrient variance. In the case of defense variance however, the use of $F(\bar{x}) - \overline{F(x)}$ is not appropriate. Wetzel et al. (2016) report that the empirical observations about the relationships between defensive traits and performance do not show a single clear pattern but rather include linear, concave-up or concave-down relationships. This diversity of relationships seems logical given the immense diversity of plant defense strategies and related underlying physiological mechanisms, which range from deterrence to various post-ingestive toxic effects. In agreement with that, the large number of significant nonlinear relationships shown by Wetzel et al. (Wetzel et al. 2016) in their figures 3C and 3D point towards all type of relationships being possible rather than to a single underlying average linear relationship. Given this diversity, the choice to consider defense effects on performance as a single process that can be averaged is questionable. We think that it would be more appropriate to report the number of instances with linear, concave-up and concave-down relationships and to consider effect sizes for significant concave-up and concave-down curves separately.

Another important aspect that is not considered in the study is the possibility for interactive effects (i.e. synergy or antagonism) between traits which can also generate nonlinear averaging effects on insect performance (Simpson and Raubenheimer 2001; Tao et al. 2014; Hunter 2016a). Such relationships can occur between nutritive and defense traits (Simpson and Raubenheimer 2001; Tao et al. 2014) and between co-occurring defensive traits (Agrawal and Fishbein 2006; Whitehead and Bowers 2014) (Fig. 2). The performance of the herbivore can be related nonlinearly to the combination of two factors despite the relationship between the performance and these two factors taken separately is linear. This is the case when different combinations of the factors act non-additively on the herbivore's performance (synergistic or antagonistic effects, Fig. 2B). Provided that the two factors vary concomitantly (Hunter 2016b) and in a correlated manner (Agrawal and Fishbein 2006; Agrawal et al. 2012; Tao and Hunter 2012), the emerging nonlinearity generates nonlinear averaging effects that are proportional to the covariance of the two factors (Koussoroplis et al. 2017) (Fig. 2, see Methods). Mathematically, covariance is defined as the product of the standard deviations of the two factors with their Pearson coefficient of correlation, i.e. $cov_{x,y} = \sigma_x \sigma_y \varrho x,y)$. Hence, keeping everything else constant, any modification of plant defensive trait variance changes the value of covariance between defensive and nutritive traits, which in turn can affect performance. In addition, the variance of plant defensive traits can affect herbivorous insect performance via nonlinear averaging effects even when the relationship between performance and plant defense levels is linear (Fig. 2A). For the same reasons, the variance in two interacting defensive traits (Whitehead and Bowers 2014) can affect herbivore performance via nonlinear averaging effects even if herbivore performance relates to each individual trait linearly.

In conclusion, we consider Wetzel et al. (Wetzel et al. 2016) as a major contribution highlighting the role of plant trait variance in regulating herbivore populations and as a general call for quantifying the nonlinearity of biological relationships (Ruel and Ayres 1999; Denny 2017; Koussoroplis et al. 2017). However, future meta-analyses like the one of Wetzel et al. (Wetzel et al. 2016) need to adjust data analysis based on prior physiological knowledge to achieve accurate conclusions. While we agree with their prediction that nutritive trait variance generally has a negative effect on herbivore performance (because most reaction norms to nutrients are concave-down) we argue that it is unlikely that variance in single plant traits is sufficient to accurately predict the negative effects of plant (trait) diversity on herbivore performance. Most importantly, we think that it is essential to consider plant traits in interaction rather than alone. Because of covariance effects, variance in plant defense traits that yield linear responses can nevertheless alter nutrient variance effects and consequently the performance of herbivores (Fig. 2A). Like most biological and ecological processes in nature (Darling and Côté 2008), herbivore performance is influenced by multiple interacting factors (Simpson and Raubenheimer 2001; Tao et al. 2014; Sperfeld et al. 2016). By failing to consider these interactions, we might overlook an important array of nonlinear averaging effects and thus impair our ability to understand the true role of plant diversity on herbivore populations and their potential use in pest-control strategies.
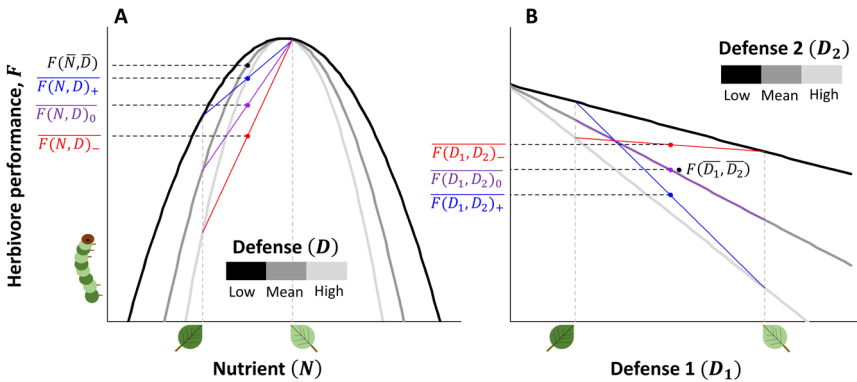
Figure 2. An illustration of the covariance effects. Plant nutrient-defense (**A**) or defense-defense (**B**) covariance effects on herbivore performance in the cases of a hypothetical antagonistic effect between the plant nutrient and chemical defense concentrations and two synergistically acting defense traits, respectively. When herbivores feed on a plant population with both nutritive and defense variance (the two types of plants on the x-axis), the Jensen's effect on performance, $F(\overline{x}) - \overline{F(x)}$, depends on whether the covariance between the traits is positive (blue), negative (red) or null (purple). See methods for procedure.

Methods

Estimating the average effect size of Jensen's Inequality

In the presence of truly concave-up and concave-down relationships (such as in Fig. 1B), the average effect size of Jensen's Inequality might be strongly underestimated when based on raw deviations, i.e. naive differences or log-response ratios between the functional mean and the functional value at the mean, because positive and negative effect sizes may cancel each other. In this case, a robust estimate has to account for potential changes in the sign of the effect of Jensen's Inequality due to different curvatures, i.e. a mixture of concave-up or concave-down shapes. Hence, the average effect size of Jensen's Inequality might be more reliably estimated from the absolute deviations (note that the use of absolute means does not allow to estimate the direction of the effect size).

To test this approach, we compared two different estimates of the average effect size of Jensen's Inequality based on a linear versus strongly non-linear relationship between herbivore performance and plant defense level, by including both a set of concave-up and concave-down curvatures.

We used a general monotonic function to describe the impact of a plant defense trait x on the performance, *i.e.* maximum growth rate F of an herbivore:

$$F(x) = \left(1 - x^n\right)^{\frac{1}{n}} \tag{1}$$

The parameter n determines the shape of the herbivores' functional response to plant defense level. The functional relationship is concave-up for $n > 1$ and concave-down for $n < 1$. In contrast, the functional relationship will be approximately linear

for n≈1. To compare the two different estimates of an average effect size of Jensen's Inequality under two different conditions we first generated a set of weakly non-linear (~linear) functions, by drawing 1000 values of n from a log-normal distribution with μ equal to zero and σ equal to 0.1. Hence, in this case, randomly drawn values of *n* will be often very similar to 1. Afterwards, we generated a set of strongly non-linear concave-up and concave-down functions, by drawing 500 values of n from a log-normal distribution with μ equal to one and σ equal to 0.1 (concave-up) and 500 values of n from a log-normal distribution with μ equal to minus one and σ equal to 0.1. In this case, randomly drawn values of *n* will often diverge strongly from 1.

We then calculated the average of the herbivore's performance in the absence $F(\bar{x})$ and in the presence $\overline{F(x)}$ of variation in the plant defense level. For the latter, we assumed the trait distribution to follow a beta distribution with mean equal to 0.5 and variance equal to 0.05. In line with the function defined above, the upper and lower limits of the plant defense trait were set to 1 and 0. Then, we calculated the average of the raw differences, *i.e.* $\overline{F(x) - \overline{F(x)}}$ and of the absolute differences, *i.e.* $|(F(\bar{x}) - \overline{F(x)})|$. The corresponding results are shown in Figure 1.

Covariance effects

We illustrate the non-linear averaging effects on performance caused by the covariance of two interacting factors through two examples. In the first example, we consider a hypothetical interaction between a plant limiting nutrient and chemical defense concentrations (i.e. a secondary metabolite). We assume a typical response to nutrients that follows Bertrand's rule (Raubenheimer et al. 2005) and we model it using a quadratic function. For simplicity, we assume that the relationship between performance and chemical defense concentration is linear. The herbivore performance is modeled as:

$$F(N,D) = ND^2 + bN + c \qquad (2)$$

where N and D are nutrient and chemical defense concentrations, respectively. b and c are arbitrarily chosen constants. In this model, the toxicity of the defense increases proportionally to the deviation of the plant nutrient concentration from the optimum, a commonly observed pattern (Simpson and Raubenheimer 2001; Tao and Hunter 2012; Hunter 2016a). Under certain assumptions (see (Koussoroplis et al. 2017)), the Jensen's effect can be approximated by

$$\overline{F(N,D)} - F(\bar{N},\bar{D}) = \frac{1}{2}F_N''(\bar{N},\bar{D})var(N) + \frac{1}{2}F_D''(\bar{N},\bar{D})var(D) + F_{ND}''(\bar{N},\bar{D})covar(N,D) \quad (3)$$

where $F_i''(\bar{N},\bar{D})$ are the partial or cross-partial second order derivatives of F which quantify the various non-linearities of the herbivore's reaction norms to the two factors. Note that the term $F_{ND}''(\bar{N},\bar{D})$ quantifies the non-additivity of the interaction between the two factors. When the two factors act in synergy, then $F_{ND}''(\bar{N},\bar{D}) > 0$, whereas $F_{ND}''(\bar{N},\bar{D}) < 0$ when the two factors act antagonistically on performance. In

the case of an additive effect of the two factors $F''_{ND}(\bar{N},\bar{D}) = 0$ and the covariance effect vanishes. In our example, the herbivore response to defense is linear, $F''_D = 0$ and equation (3) simplifies to

$$\overline{F(N,D)} - F(\bar{N},\bar{D}) = \frac{1}{2}F''_N(\bar{N},\bar{D})var(N) + F''_{ND}(\bar{N},\bar{D})covar(N,D) \qquad (4)$$

In the second example, we consider a case of two co-occurring chemical defenses. Their interactive effect on herbivore performance is modeled as

$$F(D_1,D_2) = D_1 D_2 + c \qquad (5)$$

where D_i are the concentrations in the two defensive chemicals in plant tissues and c an arbitrarily chosen constant. The Jensen's effect can be approximated using eq. 3. However, we assume that the herbivore performance relates linearly to each chemical alone, i.e. $F''_{D1} = 0$ and $F''_{D2} = 0$. So the equation collapses to:

$$\overline{F(D_1,D_2)} - F(\overline{D_1},\overline{D_2}) = F''_{D_1 D_2}(\overline{D_1},\overline{D_2})covar(D_1,D_2) \qquad (6)$$

The equations 4 and 6 demonstrate that even when the relationship between herbivore performance and plant defense traits is linear, defense variance can affect herbivore performance. This situation is realized when (1) defense covaries with another limiting plant nutritional or defensive trait and (2) the two covarying traits interactively affect herbivore performance. A simple graphical illustration of this phenomenon is provided in Figure 2.

Aknowledgements

Author contribution

AMK and TK conceived and wrote the manuscript. TK performed the analyses for Fig.1. AMK conceived the model in Fig. 2. All other authors contributed to revisions of the MS.

Authors	Contribution	ACI
AMK	0.45	3.273
TK	0.40	2.667
SP	0.05	0.211
DG	0.05	0.211
AW	0.05	0.211

References

Agrawal AA, Fishbein M (2006) Plant defense syndromes. Ecology 87: S132–S166. https://doi.org/10.1890/0012-9658(2006)87[132:pds]2.0.co;2

Agrawal AA, Petschenka G, Bingham RA, Weber MG, Rasmann S (2012) Toxic cardenolides: chemical ecology and coevolution of specialized plant-herbivore interactions: Tansley review. New Phytologist 194: 28–45. https://doi.org/10.1111/j.1469-8137.2011.04049.x

Boersma M, Elser JJ (2006) Too much of a good thing: On stoichiometrically balanced diets and maximal growth. Ecology 87: 1325–1330. https://doi.org/10.1890/0012-9658(2006)87[1325:tmoagt]2.0.co;2

Darling ES, Côté IM (2008) Quantifying the evidence for ecological synergies. Ecology Letters 11: 1278–1286. https://doi.org/10.1111/j.1461-0248.2008.01243.x

Denny M (2017) The fallacy of the average: on the ubiquity, utility and continuing novelty of Jensen's inequality. The Journal of Experimental Biology 220: 139–146. https://doi.org/10.1242/jeb.140368

Elser J, Kyle M, Learned J, McCrackin M, Peace A, Steger L (2016) Life on the stoichiometric knife-edge: effects of high and low food C:P ratio on growth, feeding, and respiration in three Daphnia species. Inland Waters 6: 136–146. https://doi.org/10.5268/iw-6.2.908

Hedges LV, Olkin I (1985) Statistical Methods for Meta-Analysis. Academic Press, 369 pp. https://doi.org/10.1016/C2009-0-03396-0

Hunter MD (2016a) Effects of primary producer chemistry on trophic interactions. In: The phytochemical landscape, Monographs in population biology. Princeton University Press, Princeton, 39–137.

Hunter MD (2016b) The variable chemistry of primary production. In: The phytochemical landscape, Monographs in population biology. Princeton University Press, Princeton, 24–38.

Kooijman B (2010) Dynamic energy budget theory for metabolic organisation (3th edn). University Press, Cambridge.

Koussoroplis A-M, Pincebourde S, Wacker A (2017) Understanding and predicting physiological performance of organisms in fluctuating and multifactorial environments. Ecological Monographs 87: 178–197. https://doi.org/10.1002/ecm.1247

Raubenheimer D, Lee K, Simpson S (2005) Does Bertrand's rule apply to macronutrients? Proceedings of the Royal Society B: Biological Sciences 272: 2429–2434. https://doi.org/10.1098/rspb.2005.3271

Ruel JJ, Ayres MP (1999) Jensen's inequality predicts effects of environmental variation. Trends in Ecology & Evolution 14, 361–366. https://doi.org/10.1016/s0169-5347(99)01664-x

Simpson SJ, Raubenheimer D (2001) The geometric analysis of nutrient allelochemical interactions: A case study using locusts. Ecology 82: 422–439. https://doi.org/10.1890/0012-9658(2001)082[0422:tgaona]2.0.co;2

Simpson SJ, Raubenheimer D (2012) The nature of nutrition a unifying framework from animal adaptation to human obesity. Princeton University Press, Princeton. https://doi.org/10.1515/9781400842803

Sperfeld E, Raubenheimer D, Wacker A (2016) Bridging factorial and gradient concepts of resource co-limitation: towards a general framework applied to consumers. Ecology Letters 19: 201–215. https://doi.org/10.1111/ele.12554

Sperfeld E, Wagner ND, Halvorson HM, Malishev M, Raubenheimer D (2017) Bridging Ecological Stoichiometry and Nutritional Geometry with homeostasis concepts and integrative models of organism nutrition. Functional Ecology 31: 286–296. https://doi.org/10.1111/1365-2435.12707

Stockhoff BA (1993) Diet heterogeneity: implications for growth of a generalist herbivore, the gypsy moth. Ecology 74: 1939–1949. https://doi.org/10.2307/1940837

Tao L, Berns AR, Hunter MD (2014) Why does a good thing become too much? Interactions between foliar nutrients and toxins determine performance of an insect herbivore. Functional Ecology 28: 190–196. https://doi.org/10.1111/1365-2435.12163

Tao L, Hunter MD (2012) Does anthropogenic nitrogen deposition induce phosphorus limitation in herbivorous insects? Global Change Biology 18: 1843–1853. https://doi.org/10.1111/j.1365-2486.2012.02645.x

Wetzel WC, Kharouba HM, Robinson M, Holyoak M, Karban R (2016) Variability in plant nutrients reduces insect herbivore performance. Nature 539: 425–427. https://doi.org/10.1038/nature20140

Whitehead SR, Bowers MD (2014) Chemical ecology of fruit defence: synergistic and antagonistic interactions among amides from Piper. Functional Ecology 28: 1094–1106. https://doi.org/10.1111/1365-2435.12250