Review of *'Partitioning the phenotypic variance of reaction norms'.*

Jarrod Hadfield*

The authors have done a good job at responding to the previous comments on their manuscript. In particular, the further decomposition of V_{Gen} into V_G and $V_{G\times E}$ is, I think, a necessary addition to the manuscript if the focus is to be on phenotypic plasticity. However, a little more clarification on the exact meaning of V_G and $V_{G\times E}$ is required - for example, V_G can be negative which is inconsistent with the definition of a variance and may therefore worry the reader. I stand by my original point that the approach is about quantifying the contribution of plasticity to the phenotypic variance rather than quantifying the (genetic) variance in plasticity *per se*. I think the abstract and manuscript are now more clear on this point although this could still be improved. For example, I would not refer to h^2_I as the heritability of plasticity and I would certainly change the title to something like '*Quantifying the impact of phenotypic plasticity on the phenotypic and genetic variance.*' I would be happy to see this manuscript published and I think the changes I suggest are small and could me made without the need for further review.

Title and abstract

- Does the title clearly reflect the content of the article? No (see above).
- Does the abstract present the main findings of the study? Yes.

Introduction

- Are the research questions/hypotheses/predictions clearly presented? Yes.
- Does the introduction build on relevant research in the field? Yes (although reference to earlier work, as summarised in Lynch and Walsh (1998, Chapter 22), could have been made.)

Materials and methods

- Are the methods and analyses sufficiently detailed to allow replication by other researchers? Yes.

*I sign all reviews.

- Are the methods and statistical analyses appropriate and well described? Yes.

Results

- In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? Not Applicable.
- Are the results described and interpreted correctly? Yes (other than the‘ *heritability of plasticity*’).

Discussion

- Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? Yes.
- Are the conclusions adequately supported by the results (without overstating the implications of the findings)? Yes.

Comments

- L19: ‘*a base for an unifying*‘ should read ‘*a basis for a unifying*’.
- L32: ‘*requires for biologists*’ should read ‘*requires biologists*’.
- L34: ‘*to be comparable across context*’ should read ‘*to be comparable across contexts*’.
- L46: I forgot to mention the paper by Pélabon et al. (2020) in my previous review. In this paper, the authors discuss standardised approaches for quantifying plasticity (under a linear reaction norm) highlighting that a mean-standardised approach requires standardising by the mean of the trait *and* the environmental variable (if both are on a ratio or log-interval scale).
- L81: For a critique of Murren et al. (2014), see Pélabon et al. (2020) also.
- L83: ‘*More, even the notion*’ should read ‘*Moreover, even the notion*‘.
- L119: ‘*among environment*’ should read ‘*among environments*’.
- L125: ‘*with a number*’ should read ‘*with the number*’.
- Equation 2. I think this is OK, although the text for Equation 1 (and Equation 3) reads as if $\hat{z}$ is *conditional* on genotype, yet in Equation 2, genotypes are *marginalised*. This may confuse readers that are less familiar with the topic.
- L129: I think I would omit the mention of quantitative but discrete environments here, as it could throw the reader. After all, $\mathbf{G}_z$ can be computed for any specific values of z from any of the models discussed, although of course the character state and curve-parameter approaches may predict different $\mathbf{G}_z$.

- L160: '*variation surrounding such average*' should read '*variation surrounding such an average*'.

- L179-L212: These two paragraphs are very confusing. The partition $V_{Gen} = V_G + V_{G\times E}$ has been introduced previously, and so when it is stated that the genotypic variance V_{Gen} can be further decomposed in two steps, the reader is expecting a discussion of V_G versus $V_{G\times E}$. However, there is then an extensive discussion about dominance and additive effects. I would place the paragraph on L193-L212 first, but talk about V_G and $V_{G\times E}$ only. After this paragraph I would then (briefly) state that both V_G and $V_{G\times E}$ can be further decomposed into additive and non-additive components.

- L222 I think referring to h^2_I as the '*heritability of plasticity*' is misleading. Let's say a linear reaction norm was fitted to repeat-measure data. The total variance in slopes could be partitioned into a genetic variance and a permanent-environment variance, as is commonly done (Nussey et al. 2007). The heritability of plasticity, for me, would then be the genetic variance in slopes over the total variance in slopes.

- L247 This also requires no G by E covariance.

- L255: '*assumptions must valid*' should read '*assumptions must be valid*'.

- L262: Since $Var(\epsilon^2) = E[\epsilon^4] - E[\epsilon^2]E[\epsilon^2]$ and the variance and kurtosis are defined as $E[\epsilon^2]$ and $E[\epsilon^4]/Var(\epsilon)^2$, respectively, when ϵ is mean standardised, then $Var(\epsilon^2) = Kurt(\epsilon)Var(\epsilon)^2 - Var(\epsilon)^2 = Var(\epsilon)^2(Kurt(\epsilon) - 1)$. If ϵ is normal then $Kurt(\epsilon) = 3$ and so $Var(\epsilon^2) = 2Var(\epsilon)^2$. Not sure if this is worth mentioning but it does imply that π_{Cv} will be half of π_{SI} when the expected slopes/curvatures are equal.

- L266 & L295: '*linear on the parameters*' should read '*linear in the parameters*'.

- L289: Perhaps emphasise here that polynomials are linear in their parameters?

- L298-L401 As with the previous section, I think this is harder to follow than it needs to be. I would ignore the distinction between non-additive and additive components for now, and simply use the notation V_{Gen}, V_G and $V_{G\times E}$ and perhaps have a small section covering the distinction between non-additive and additive components that applies to all sections. For this reason, my following comments use the notation $V_{Gen}/V_G/V_{G\times E}$ rather than $V_{Add}/V_A/V_{A\times E}$.

- L300: After Equation 18, I think it would be good to show how V_{Gen} can be decomposed into V_{Gen} and $V_{G\times E}$ using the same notation (i.e. Equation 23 in the notation of Equation 18). I don't think it's obvious - see my next comment.

- L314: In the discrete case, I think the reader will have trouble understanding how V_G and $V_{G\times E}$, as defined in Equations 22 and 23, relate to the genetic correlations between traits in different discrete environments (probably the most common set-up by which people think about $G \times E$). I realise that this is covered later and in Appendix C, but I think an exact verbal statement of V_G is missing - it is the expected *covariance* in phenotype when genotypes are placed at random in two environments and the pair of environments are sampled with replacement and according to their frequency. Although I am happy with this interpretation, it does mean that V_G can be negative, which is inconsistent with the idea that V_G is a variance. To take a simple example, imagine the genetic variance, v, is the same in all k environments, and the genetic correlation, c, is identical between all pairs of environments. Then $\mathbf{G}_\theta = v(\mathbf{J}c + \mathbf{I}(1-c))$ where $\mathbf{J}$ and $\mathbf{I}$ are the unit and identity matrices respectively. Under this set up, $V_{Gen} = v$ and $V_G = (vk + vck(k-1))/k^2 = (v/k)(1 + c(k-1))$. If $c = 1$ then $V_G = V_{Gen} = v$ and $V_{G\times E} = 0$ and everyone would be comfortable with this fact. However, if c=-1 then $V_{Gen} = v$, $V_G = v(2-k)/k$ and $V_{G\times E} = v - v(2-k)/k$ which means that V_G can be negative when $k > 2$ and $V_{G\times E}$ can exceed v. I think this is OK (see Lynch and Walsh (Chapter 22 1998), and references therein ,where much of this is already covered) but some reassurance to the reader is required. If everyone was trained to think about quantitative genetics and mixed models in terms of covariances rather than variances I think life would be easier!

- L322: Is this really a '*marginal additive genetic variance*'? I would think Equation 21 is actually the marginal distribution: $V_{Gen} = \int \boldsymbol{\psi}_\epsilon^\top \mathbf{G}_\theta \boldsymbol{\psi}_\epsilon Pr(\boldsymbol{\psi}_\epsilon) d\boldsymbol{\psi}_\epsilon$.

- L325-L330. You could simply reference standard sum of squares theory (p355 Searle 2006):

$$E[\boldsymbol{\psi}_\epsilon^\top \mathbf{G}_\theta \boldsymbol{\psi}_\epsilon] = Tr(\mathbf{G}_\theta \mathbf{V}_{\boldsymbol{\psi}_\epsilon}) + E[\boldsymbol{\psi}_\epsilon^\top]\mathbf{G}E[\boldsymbol{\psi}_\epsilon]$$

  where $\mathbf{V}_{\boldsymbol{\psi}_\epsilon}$ is the (co)variance matrix of the $1,\ \epsilon,\ \epsilon^2 \ldots \epsilon^k$.

- L342 As stated previously, I think people would call V_b the (genetic) variance in plasticity not $V_b V(\epsilon)$.

  L364 $\gamma_i j$ should read γ_{ij}

  L386 I would omit the section on n_e as the reader is likely exhausted by this point.

  L408 & L699 It's not clear to me what is meant by a random-intercept model here.

  L410 Perhaps use the term random-parameter models rather than random-slope models?

  L413 '*Random effects are fitted to the parameters of this function (with the genotype as grouping factor), and any higher-order effects for a polynomial function.*' doesn't really make sense. Perhaps, '*Genotype-specific*

parameters, such as the intercept, slope, and any higher-order effects of a polynomial function, are treated as random'.

L435 Earlier, N_{Gen} is stated as 20 or 5 rather than 200 or 50.

L435 The sentence '*Residual noise was applied around each measure for each genotype with a residual variance* $V_{Res} = 0.25$' is redundant as it has been stated a few lines earlier.

L458 Shouldn't this be $h^2_I = 0.21$ rather than h^2_{RN}? Again, I would not refer to h^2_I as the heritability of plasticity.

L477 This phenomenon is well known (e.g. Hill and Thompson (1978)), and another reason to drop n_e from the manuscript. If it is retained, add the distribution of estimates of n_e to Figure 4 and change N_e to n_e in the legend.

L505 Presumably both $\hat{V}_{mod}$ and $\hat{V}_{Plas}$ are bias corrected? Personally I would use $\hat{V}_{Plas:CP}$ and $\hat{V}_{Plas:CS}$ to indicate the estimates of $\hat{V}_{Plas}$ under the curve-parameter and character state approaches.

L561 '*First focusing the*' should read '*First focusing on the*'.

L564 But isn't this partly due to the fact that the residual variance was set to be very small?

L564 '*their differ quite visibly* ' should read '*they differ quite visibly* '.

L577 '*is close to be maximised*' should read '*is close to being maximised*'.

L584 '*the low difference*' should read '*the small difference*'.

L683 I would write '*open the door to better commensurability and comparatibility across studies*' as '*opens the door for increasing comparatibility across studies*'. Not really sure what '*better commensurability*' means.

References

Hill, W. G., and R. Thompson. 1978. Probablilities of non-positive definite between-group or genetic covariance matrices. Biometrics 34:429–439.

Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer.

Murren, C. J., H. J. Maclean, S. E. Diamond, U. K. Steiner, M. A. Heskel, C. A. Handelsman, C. K. Ghalambor, J. R. Auld, H. S. Callahan, D. W. Pfennig, et al. 2014. Evolutionary change in continuous reaction norms. The American Naturalist 183:453–467.

Nussey, D. H., A. J. Wilson, and J. E. Brommer. 2007. The evolutionary ecology of individual phenotypic plasticity in wild populations. Journal of Evolutionary Biology 20:831–844.

Pélabon, C., C. H. Hilde, S. Einum, and M. Gamelon. 2020. On the use of the coefficient of variation to quantify and compare trait variation. Evolution letters 4:180–188.

Searle, S. R. 2006. Matrix Algebra Useful for Statistics. John Wiley & Sons, Hoboken NJ.