Dear PCI editors,

Please find attached the revision of our manuscript previously entitled “Partitioning the phenotypic variance of reaction norms”. We revised the manuscript according to the remaining comments from reviewer 1. We changed the title of the manuscript for “Partitioning the phenotypic and genetic variance of reaction norms”. Furthermore, we reorganised how the partitioning was introduced, as suggested by the reviewer, to make the distinction between additive and non-additive variances appear slightly later in the manuscript. We now also mention that π_{Sl} and π_{Cv} are not equal when slope and curvature are equal. We also now clarify that our framework (and the Reacnorm package) can be applied in a context of repeated measurements, where e.g. the heritability of the parameters θ is not 1 (Appendix C5). Finally, we explain why our variance decomposition cannot yield negative variances, contrary to what the reviewer suggested.

We hope that these modifications fully address the remaining issues raised by reviewers in the manuscript, and that you will consider this version suitable for a recommendation for PCI Evolutionary Biology.

Sincerely,
Pierre de Villemereuil and Luis-Miguel Chevin

Reviewer 1: Jarrod Hadfield

The authors have done a good job at responding to the previous comments on their manuscript. In particular, the further decomposition of VGen into VG and VG×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 VG and VG×E is required - for example, VG 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_I^2 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.

We thank Dr Hadfield for his in-depth evaluation of our revised manuscript. We respond to these points in more detail below, notably regarding the naming of h_I^2. Regarding the title, we wish to retain the genericity of our original one (‘Partitioning the phenotypic variance of reaction norms’), because our approach is not limited to ‘Quantifying the impact of phenotypic plasticity on the phenotypic and genetic variance’; indeed, one of our main interests is in further decomposing the plasticity components into the π, φ, and γ parameters to estimate contributions from different parameters of reaction norm shape. However to emphasize genetics a bit more, we now entitle the ms: ‘Partitioning the phenotypic and genetic variances of reaction norms’.

• L19: ‘a base for an unifying‘ should read ‘a basis for a unifying’.

Corrected, thank you.

• L32: ‘requires for biologists’ should read ‘requires biologists’.

Corrected, thank you.

• L34: ‘to be comparable across context’ should read ‘to be comparable across contexts’.

Corrected, thank you.

• 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).
We have now added a reference to this paper when mentioning our choice for variance-standardisation (l. 218-220): "From this, it is possible to derive unitless quantities of interest, for instance by standardising by the phenotypic variance, which is more widely applicable and appropriate than mean-standardisation in the context of reaction norms (Pelabon et al., 2020)."

• L81: For a critique of Murren et al. (2014), see Pélabon et al. (2020) also.
We now cite this reference when mentioning critiques of Murren et al. (2014).

• L83: 'More, even the notion' should read 'Moreover, even the notion'.
Corrected, thank you.

• L119: 'among environment' should read 'among environments'.
Corrected, thank you.

• L125: 'with a number ' should read 'with the number '.
Corrected, thank you.

• 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.
Our entire framework relies on marginalising over the genotypes. While Equation 3 seems to be conditional, it must be understood together with Equation 4, which marginalises genotypes in the same fashion as Equation 2.

• L129: I think I would omit the mention of quantitative but discrete environments here, as it could throw the reader. After all, Gz 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 Gz.
We have removed the reference to this scenario for simplicity.

• L160: 'variation surrounding such average' should read 'variation surrounding such an average'.
Corrected, thank you.

• L179-L212: These two paragraphs are very confusing. The partition VGen = VG + VG×E has been introduced previously, and so when it is stated that the genotypic variance VGen can be further decomposed in two steps, the reader is expecting a discussion of VG versus VG×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 VG and VG×E only. After this paragraph I would then (briefly) state that both VG and VG×E can be further decomposed into additive and non-additive components.
We modified the structure of this paragraph (l.310-312), which now starts with the $V_{Gen} = V_G + V_{G\times E}$ decomposition, and only after tackles the subject of additive v. non-additive variances. We would like to stress that the application of this variance decomposition at the level of the additive genetic

variances is the reason why we have such a unifying framework, because we can express everything in terms of reaction norm gradients, which simplifies notations and analyses.

• L222 I think referring to h2 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.
We understand this criticism: indeed when one parameter of the reaction norm can summarize plasticity (eg slope for a linear reaction norm), then "heritability of plasticity" should be used to describe the heritability of this parameter. In contrast, h^2_I in eq. 9 rather corresponds to the joint contribution of genetic variances in all reaction norm parameters to overall phenotypic variation. To address this, we have made two changes to the ms.
First, we now call h^2_I the "heritability from plasticity" and V_{AxE} the "additive genetic variance arising from plasticity", to make it clear that it is the component of heritability across environments that stems from plasticity being genetically variable.
Second, a subtle point is that, for the sake of simplicity, we had defined reaction norms as exclusive properties of genotypes (thus h^2 of θ is 1 by definition). However, traits with repeated measurements (such as breeding time across reproductive seasons) allow reaction norms to be estimated for individuals ("individual plasticity" sensu Nussey 2007). Our framework can easily account for such scenarios, although this makes it necessary to slightly change the way V_{Plas} and T^2_{RN} are computed, the rest remaining unchanged. In fact, our Reacnorm package was already able to account for such cases. To keep things simple, we have retained the assumption that the genotype fully determines the reaction norm in most of the ms, but we *(i)* now explicitly state and discuss this assumption (l.139-144 and l.237-239), *(ii)* include a new section in the Appendix explaining the slight changes necessary to account for further random effects affecting variation in θ (l.1082-1104), *(iii)* mention in the Appendix that Reacnorm can already account for such cases.

• L247 This also requires no G by E covariance.
Yes, this is now specified below Equation 6.

• L255: 'assumptions must valid ' should read 'assumptions must be valid '.
Corrected, thank you.

• 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.
Thank you for this comment, we have now added below Eq. (14) that $Var(\epsilon^2) = 2Var(\epsilon)^2$ for a normal distribution, as we agree this may be of interests to our readers. This is now mentioned in the manuscript.

• L266 & L295: 'linear on the parameters' should read 'linear in the parameters'.
Corrected, thank you.

• L289: Perhaps emphasise here that polynomials are linear in their parameters?
Yes, this was indeed a cryptic premise. This is now fully stated.

• 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 VGen , VG and VG×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 VGen /VG /VG×E rather than VAdd /VA/VA×E
We have reworded sections introducing $V_{Gen}/V_G/V_{G\times E}$ to always introduce such decomposition before $V_{Add}/V_A/V_{A\times E}$. However, as explained above, we would like to retain the focus on the additive genetic variance. First, because this is ultimately the variance component that matters the most for predicting (short-term) evolution. Second, because it simplifies and unifies the computation of all variance components, using the "reaction norm gradients" that we define in Eq. 19.

• L300: After Equation 18, I think it would be good to show how VGen can be decomposed into VGen and VG×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.
We have added a mentioned to this decomposition below Eq. 18.

• L314: In the discrete case, I think the reader will have trouble under standing how VG and VG×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 × E). I realise that this is covered later and in Appendix C, but I think an exact verbal statement of VG 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 VG can be negative, which is inconsistent with the idea that VG 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 $G\theta = v(Jc + I(1 - c))$ where J and I are the unit and identity matrices respectively. Under this set up, $VGen = v$ and $VG = (vk + vck(k - 1))/k2 = (v/k)(1 + c(k - 1))$. If c = 1 then VG = VGen = v and VG×E = 0 and everyone would be comfortable with this fact. However, if c=-1 then VGen = v, $VG = v(2 - k)/k$ and $VG\times E = v - v(2 - k)/k$ which means that VG can be negative when k > 2 and VG×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!
While we agree we the numerical computation presented here, we disagree that VG can be negative. We provide attached a proof that VG is always non-negative, which is due to the positive semi-definiteness of variance-covariance matrices (i.e. G in this case). The example with correlations set to c = -1 for all pairs of traits result in non-positive definite matrices for k > 2, and are thus not possible values for G. (Note that because Jarrod Hadfield won't get to see our ms again, we have sent him this this document, and he agreed with our point).

• L322: Is this really a 'marginal additive genetic variance' ? I would think Equation 21 is actually the marginal distribution: $VGen = \int \psi_\epsilon^T G_\theta \psi_\epsilon \Pr(\psi_\epsilon) d\psi_\epsilon$.
It is the variance of the breeding values *after* averaging across environments. So, it's marginal in that sense (see the green dots in Figure 1). To avoid this misunderstanding, we now call this variance (and its corresponding heritability) "environment-blind additive genetic variance", as it is the variance one would get should variation in the focal environment be completely ignored.

• L325-L330. You could simply reference standard sum of squares theory (p355 Searle 2006):
$E[\psi_\epsilon^T G_\theta \psi_\epsilon] = T\, r(G_\theta V_{\psi\epsilon}) + E[\psi_\epsilon]^T G_\theta [\psi_\epsilon]$

where $V_{\psi\epsilon}$ is the (co)variance matrix of the 1, ϵ, ϵ2 . . . ϵk.
This would be shorter, but we wanted to express the reasoning leading the separation of these terms in our case, and why they are linked to V_{Add}, V_A and V_{AxE} so we chose not to use that existing mathematical shortcut.

• L342 As stated previously, I think people would call Vb the (genetic) variance in plasticity not VbV (ϵ).
We changed the wording throughout the manuscript.

• L364 γi j should read γij
Corrected, thank you.

• L386 I would omit the section on ne as the reader is likely exhausted by this point.
We drastically reduced the length of this section and now directly mention that n_e is an interesting theoretical value, but in practice suffer from estimation issues.

• L408 & L699 It's not clear to me what is meant by a random-intercept model here.
The idea was that the environment-specific averages ("intercepts", by opposition to the slopes in a random-slope model) were treated as random, but this was, strictly speaking, an abuse of the word 'intercept'. We thus changed for 'random-parameter model' as suggested below.

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

• 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'.
Yes, this reads better, thank you.

• L435 Earlier, NGen is stated as 20 or 5 rather than 200 or 50.
Yes, this is because the sampling is with repeat in the discrete case (described earlier) and without repeat in the continuous case (described here). We have now made this more explicit.

• L435 The sentence 'Residual noise was applied around each measure for each genotype with a residual variance VRes = 0.25' is redundant as it has been stated a few lines earlier.
Yes, this was because the sampling mechanism is not exactly comparable across the discrete and continuous case, resulting in slightly different wording. Nonetheless, we've now grouped both sentences into one, to avoid this redundancy (l.434-435).

• L458 Shouldn't this be h2 = 0.21 rather than h2 ? Again, I would not refer to h2 as the heritability of plasticity.
Yes, thank you.

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

We now directly mention (l.390-401) the ultimate source on the matter (Lawley, 1956) when we introduce n_e, to explain it is useful in theory, but not in practice, and removed further mentions of n_e from the manuscript. We also removed mention to n_e in Figure 4.

• 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.
Yes, they are the bias-corrected estimators. Since we know that $\hat{V}$Plas:CP is a very crude and incorrect estimator for $\hat{V}$Plas, we prefer to keep the notations $\hat{V}$mod and $\hat{V}$Plas in this particular section to reflect this. In other sections, there is either only one $\hat{V}$Plas, or $\hat{V}$Plas from both approaches are equal.

• L561 'First focusing the' should read 'First focusing on the'.
Corrected, thank you.

• L564 But isn't this partly due to the fact that the residual variance was set to be very small?
Yes, it could be. This is now stated.

• L564 'their differ quite visibly ' should read 'they differ quite visibly '.
Corrected, thank you.

• L577 'is close to be maximised ' should read 'is close to being maximised '.
Corrected, thank you.

• L584 'the low difference' should read 'the small difference'.
Corrected, thank you.

• 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.
We changed the wording of this sentence.

Proof that V_G is positive

Pierre de Villemereuil

1 Context

The question is that, if G is a variance-covariance matrix, and we define :

- V_{Gen} as the average of the diagonal elements of G (total variance across components of G)
- V_G as the average of all elements of G (variance of the average across components of G)
- $V_{G\times E}$ as the difference $V_{\text{Gen}} - V_G$.

Is it true that V_G checks the properties of a variance, i.e. at least that it is non-negative?

If K is the dimension of G, then we can write the average of all its elements as:

$$V_G = \frac{1}{K^2}\left(\sum_k V_k + 2\sum_{k<l} C_{kl}\right) \tag{1}$$

where V_k is the kth variance on the diagonal and C_{kl} is the covariance between the kth and lth components of G. Because covariances can be negative, it is not evident from this expression whether V_G can be negative or not.

2 A counter-example with negative correlation

It is proposed that in a situation where G is composed of equal variances $V_k = v$ on the diagonal, and equal covariances such that the resulting correlation is $c = -1$, the value of V_G above would be negative. This is correct.

The problem with this counter-example is that it constructs a matrix G that is not a variance-covariance matrix. Indeed, variance-covariance matrices must be positive semidefinite, i.e. all of its eigenvalues must be non-negative. Yet, here G = vM where M contains 1 on the diagonal, and -1 elsewhere. The spectrum of M is composed of 2 with multiplicity $K - 1$ and a Kth eigenvalue $\lambda_K = 2 - K$, which is negative for $K > 2$.

As a result, V_G is negative in that example (for $K > 2$), but G is not a proper variance-covariance matrix because it is not positive semi-definite. So the negativity in V_G is likely to be the result of G being ill-defined here.

3 A proof that V_G is positive

A definition for the positive semi-definiteness of a matrix A is that for all real vector $\boldsymbol{x}$ (of proper dimension), we have $\boldsymbol{x}^T A\boldsymbol{x} \geq 0$. Let $\boldsymbol{u}$ be a unit vector (all element of $\boldsymbol{u}$ are 1), then the sum S of all elements in A can be written as $S = \boldsymbol{u}^T A\boldsymbol{u}$. By the property above, we thus have $S \geq 0$ if A is positive semi-definite.

Since V_G is the average of all elements of G, if we define $S = \boldsymbol{u}^T G\boldsymbol{u}$, we can write $V_G = \frac{S}{K^2}$. Since $S \geq 0$ (because G is positive semi-definite, as a variance-covariance matrix), we thus have $V_G \geq 0$.

We can even say more: if $V_G = 0$, then G is positive semi-definite (since it is a variance-covariance matrix), but not positive definite (i.e. one of its eigenvalue is 0).