Fixed effect variance and the estimation of repeatabilities and heritabilities:

Issues and solutions

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Abstract

Linear mixed effects models are frequently used for estimating quantitative genetic parameters, including the heritability, as well as the repeatability, of traits. Heritability acts as a filter that determines how efficiently phenotypic selection translates into evolutionary change, while repeatability informs us about the individual consistency of phenotypic traits. As quantities of biological interest, it is important that the denominator, the phenotypic variance in both cases, reflects the amount of phenotypic variance in the relevant ecological setting. The current practice of quantifying heritabilities and repeatabilities from mixed effects models frequently deprives their denominator of variance explained by fixed effects (often leading to upward-bias of heritabilities and repeatabilities) and it has been suggested to omit fixed effects when estimating heritabilities in particular. We advocate an alternative option of fitting models incorporating all relevant effects, while including the variance explained by fixed effects into the estimation of the phenotypic variance. The approach is easily implemented and allows optimising the estimation of phenotypic variance, for example by the exclusion of variance arising from experimental design effects while still including all biologically relevant sources of variation. We address the estimation and interpretation of heritabilities in situations in which potential covariates are themselves heritable traits of the organism. Furthermore, we discuss complications that arise in generalised and non-linear mixed models with fixed effects. In these cases, the variance parameters on the data scale depend on the location of the intercept and hence on the scaling of the fixed effects. Integration over the biologically relevant range of fixed effects offers a preferred solution in those situations.

Keywords: heritability, linear mixed modelling, fixed effects, quantitative genetics

Introduction

Additive genetic variance, phenotypic variance, and their ratio, the heritability of a trait, are key parameters in evolutionary quantitative genetics, because they allow the assessment of whether a phenotypic trait can evolve through natural and artificial selection (Falconer and Mackay, 1996; Lynch and Walsh, 1998). The heritability, h^2, of a trait corresponds to the fraction of the selection differential that can cause genetic change in the offspring generation. The heritability acts as a filter that determines how efficiently a population can respond to phenotypic selection. Therefore, heritability is relevant to assess the adaptive potential (e.g. in species threatened by global change (Hoffmann and Sgrò, 2011; Alberto et al., 2013)), as well as to investigate fundamental issues in evolutionary biology (Mousseau and Roff, 1987; Merilä and Sheldon, 2000; Kruuk et al., 2000; Hadfield et al., 2006). A related metric that is relevant in many biological contexts is the repeatability, R, that can be used. for

example, to describe the individual consistency in phenotypic traits such as behaviour. Repeatability can be used in a variety of context, but for the purpose of simplicity we here focus on individual consistency, which is arguably the most widespread application in evolutionary ecology.

Mathematically, the heritability (and repeatability) of a trait are defined as the ratio of its additive genetic variance V_A (between-individual variance V_I) to its total phenotypic variance V_P:

$$h^2 = \frac{V_\mathrm{A}}{V_\mathrm{P}}, \tag{1a}$$

$$R = \frac{V_\mathrm{I}}{V_\mathrm{P}}, \tag{1b}$$

As a measure of biological interest, heritability and repeatability should be estimated with the ecologically relevant phenotypic variance in the denominator, just as V_A and V_I should be estimated accounting for various confounding effects (Wilson et al., 2010) and in the relevant environment, since genotype-by-environment (or individual-by-environment) interactions are common (Falconer, 1952; Kawecki and Ebert, 2004; Stinchcombe, 2014). The phenotypic variance V_P could ideally be quantified by random sampling from the base population in a biologically relevant setting. But studies are often designed, for good reasons, primarily for estimating V_A and/or V_I without bias and with the highest possible precision. Optimal sampling for the estimation of these variances can sometimes generate conflicts between the precise estimation of the numerators and the denominators of Eq. 1a. To cope with these design choices, as well as to model experimentally and naturally arising confounding effects, quantitative genetic models have to be as thorough as possible in terms of covariates accounted for. This thoroughness inevitably leads to much complexity in the types and forms of effects included in the model, which in turns might render the computation of some parameters, especially V_P, more difficult than usually appreciated. As the two cases of heritability and repeatability have common issues regarding the correct estimation of V_P and the models to estimate heritability are generally slightly more complicated, we will focus on heritability throughout this article, but most arguments apply to repeatability estimation as well.

The most popular methods for estimating quantitative genetic parameters make use of the linear mixed models (LMM) framework (Kruuk, 2004; Wilson et al., 2010). In particular the so-called animal model (Thompson, 1976), a special case of a mixed effects model, is widely used in plant and animal breeding (Gianola and Rosa, 2015) and has been increasingly used in wild population studies over the past decade (Postma, 2014). One of the greatest advantages of mixed models is that they allow

accounting for various confounding effects (Kruuk, 2004; Wilson et al., 2010). A LMM fitted to explain a phenotype y can contain both fixed and random effects and is conventionally written as:

$$\mathbf{y} = \mu + \mathbf{Xb} + \mathbf{Z_a a} + \mathbf{Zu} + \mathbf{e}, \tag{2}$$

where $\mathbf{y}$ is the vector of phenotypes y, μ is the global intercept and $\mathbf{e}$ is a vector of residual errors. The $\mathbf{Xb}$ part stands for fixed effects (although not the intercept in the notation that we use here and in the following), whereas $\mathbf{Zu}$ refers to the random effects. Random effects, unlike fixed effects, are modelled as stemming from a normal distribution with a mean of zero and a variance to be estimated from the data. Because of the quantitative genetic context discussed here, we isolate the random effect $\mathbf{Z_a a}$ corresponding to the additive genetic value of the individuals from other random effect components. The matrices $\mathbf{X}$ and $\mathbf{Z}$ are referred to as the design and incidence matrices for the fixed and random effects, respectively. Especially, the $\mathbf{X}$ matrix contains the values of the co-factors included in the analysis. The vectors $\mathbf{b}$ and $\mathbf{u}$ contain the fitted fixed and potential random effect estimates, respectively.

When no fixed effects (apart from the intercept μ) are included in the analysis, the heritability is simply calculated as:

$$h^2 = \frac{V_\mathrm{A}}{V_\mathrm{A} + V_\mathrm{RE} + V_\mathrm{R}}, \tag{3}$$

where V_A stands for the variance in additive genetic values $\mathbf{a}$, V_RE for (the sum of) any relevant, i.e. accounting for natural sources of variation, additional random effect variance(s) and V_R for the residual variance. Since variance decomposition using LMM separates the phenotypic variance into additive components, Eq. 3 will generally give an unbiased estimate of Eq. 1a. Fixed effects, however, can be problematic for multiple reasons.

Substantial progress has been made in highlighting issues pertaining to fixed effects in quantitative genetic inferences (Wilson et al., 2010; Wolak et al., 2015), generating solutions for mixed model analysis in general (Nakagawa and Schielzeth, 2013), and in data-scale quantitative genetic inference using generalised mixed models (Nakagawa and Schielzeth, 2010; de Villemereuil et al., 2016). The purpose of this paper is to synthesise the ideas in these previous works so as to provide an accessible guidance to about what issues arise, and how to handle them, in a number of circumstances that are likely to occur in empirical evolutionary quantitative genetic studies.
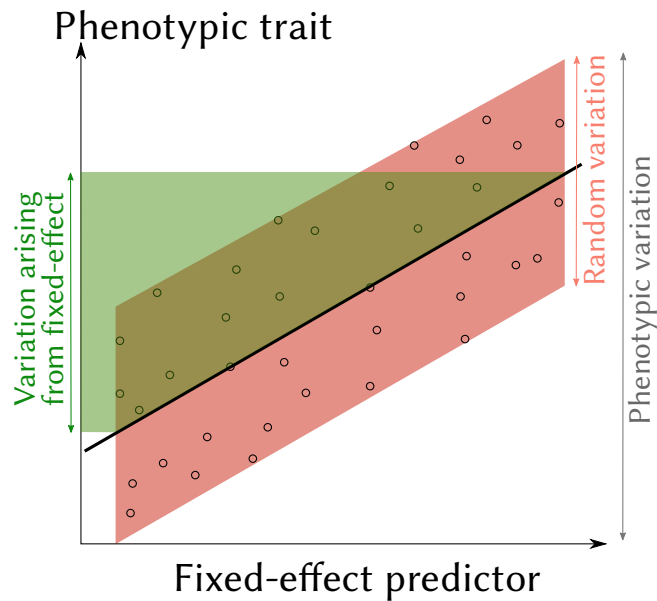
Figure 1: Schematic description of an analysis using a continuous fixed-effect predictor to model a phenotypic trait, possibly with random effects. The graph shows the relationship between the fixed-effect predictor and the phenotypic trait (individual data points in black circles, values predicted by the model as black thick line). The total phenotypic variation (black double-arrow on the right) is decomposed into the fraction explained by fixed-effect variation (i.e. the phenotypic variation "along" the predicted model, in green) on one hand, and random variation (i.e. variation from random effects and residual error arising "around" the predicted model, in red) on the other hand.

Phenotypic variance estimation in the presence of fixed effects

Fixed effects are often fitted with the intention to account for confounding effects and improve the goodness-of-fit of the models by accounting for complex patterns in the data. As illustrated in Fig. 1, the variance of the random effects, as well as the residual variance are estimated *around* the predicted values. Because of this, the sum of random variances (including additive genetic, random effects and residual variances) underestimates V_P, as it does not reflect the total phenotypic variance of the trait, but rather the variance after the fixed effects have been accounted for (i.e. related to the red part in Fig. 1).

As a consequence, fixed effects change the size of the phenotypic pie that is decomposed in different components, if the denominator is calculated as in Eq. 3. Wilson (2008) recommended particular care when fitting fixed effects in animal models and argued for a supplementary analysis without fixed effects. Note that the issues tackled here and by Wilson (2008) about reduction of the denominator variance when accounting for fixed effects also apply to the practice of two-step analyses by first fitting a linear model to account for confounding effects and then analysing the heritability of the residuals (Garland, 1988). The argument also applies to the estimation of repeatability.

Since it will typically not be possible to get a benchmark for V_P from an independent dataset, we need solutions that allow a reconstruction of V_P in the presence of fixed effects. A simple solution would be to replace the denominator $V_A + V_{RE} + V_R$ by the phenotypic variance in the original dataset V_{Po}, such that:

$$h^2 = \frac{V_A}{V_{Po}}. \tag{4}$$

V_{Po} will however be affected by various aspects of the experimental design and may not be representative of the phenotypic variance in the base population (even if biases may be small in some cases of well-balanced experimental designs).

A more proper solution is to account for the amount of variance that has been transferred from the random components to the fixed effects. The variance V_F arising from the fixed effect covariates will be the variance of the values predicted by the model (green part of Fig. 1) along all possible values for the fixed-effect predictor (x-axis in Fig. 1). If we note $\hat{y}$ the predicted value of the model according to a some specific predictor(s) value(s) and f_x the distribution of the fixed-effect predictor(s), the variance of fixed effects is thus the variance of $\hat{y}$ along the distribution f_x:

$$V_F = \int V_x(\hat{y}) \mathrm{d} f_x, \tag{5}$$

where V_x is the squared deviation from the mean (along the distribution f_x). In practice, however, a distribution f_x of the covariates will not be known, as such distributions are not part of the linear modelling assumptions. In the context of computing the coefficient of determination, Nakagawa and Schielzeth (2013) proposed constructing a fixed effect variance component as the variance of the linear predictor of the model $\hat{\mathbf{y}} = \mathbf{X}\hat{\mathbf{b}}$. In other words, $\hat{\mathbf{y}}$ are the data points projected on the black thick line in Fig. 1 and their corresponding variance V_F (i.e. related to the green part in the figure) can be computed as:

$$V_F = V(\hat{\mathbf{y}}) = V(\mathbf{X}\hat{\mathbf{b}}), \tag{6}$$

which is much simpler to compute than Eq. 5. When including this variance component in the heritability calculation, the denominator is no longer sensitive to the presence and number of fixed effects, because the variance transferred from random components to the fixed effects is now accounted for in the new component V_F (again, see Fig. 1 for a graphical illustration that V_P includes V_F):

$$h^2 = \frac{V_A}{V_A + V_F + V_{RE} + V_R}. \tag{7}$$

Table 1: Re-analysis of the unicorn dataset from Wilson (2008) in MCMCglmm (Hadfield, 2016), using models 1a, 1b and 1c from this reference (posterior mean of the estimates and 95% credible interval in bracket). We computed V_F and provide V_P and h^2 with or without accounting for this component. Discrepancies in values from h^2 compared to Wilson (2008) are due a typological error in this reference (A.J. Wilson, personal communication).

Fixed effect	V_F	V_A	V_R	V_P		h^2	
				No V_F	With V_F	No V_F	With V_F
None	0.00 [0.00-0.00]	0.34 [0.08-0.60]	3.14 [2.85-3.44]	3.49 [3.24-3.71]	3.49 [3.24-3.71]	0.098 [0.025-0.17]	0.098 [0.025-0.17]
Age + Sex	2.49 [2.36-2.62]	0.36 [0.27-0.47]	0.65 [0.58-0.73]	1.02 [0.94-1.08]	3.51 [3.35-3.65]	0.36 [0.28-0.45]	0.10 [0.08-0.13]
Age + Sex + Age:Sex	2.56 [2.42-2.68]	0.35 [0.26-0.46]	0.60 [0.52-0.67]	0.95 [0.88-1.03]	3.51 [3.36-3.66]	0.37 [0.28-0.46]	0.10 [0.072-0.13]

This is a straightforward calculation that can be applied for any analysis, and using most software, since it needs only the values of the co-factors (i.e. the design matrix $\mathbf{X}$) and the parameter estimates. The former is an aspect of the sampling and/or experimental design and the latter is part of the output of any statistical software. The computation of repeatability using V_F is straightforward by replacing V_A by V_I in Eq. 7, as in Eq. 1b.

It will be useful to provide estimates of this component in publications, in order to reflect how much variance was depleted because of the presence of fixed effects. The same kind of solution could be applied if the heritability was measured on the residuals of a regression (sometimes referred to as "corrected phenotypic values"): the variance of the regression model (V_F, following the exact same definition as in Eq. 6) could be computed and included in V_P, though a better practice anyway would be to run everything within a single LMM.

As an illustration, we re-analysed the unicorn example data from Wilson (2008) using the MCMCglmm R package (Hadfield, 2016). This analysis of unicorn horn length (Table 1) shows that accounting for the V_F component allow to recover the correct value for V_P and hence for h^2, whichever the structure of the fixed effect component. Hence, because this practice would answer the concerns raised by Wilson (2008), we encourage researchers to include fixed effects in their analyses. A decision not to fit influential fixed effects, despite their beneficial effect on the goodness-of-fit or for accounting for confounding effects, would likely harm model fit, parameter estimation and possibly the behaviour of the test statistics. Improving the fit of the model would most likely improve the precision of the estimates, which, for any particular dataset, would improve the precision of the heritability estimate (the point estimate would be more probably close to its true value and the confidence interval will be smaller). Furthermore, the inclusion of co-factors that account for non-genetic effects that are partly

confounded with the additive genetic component V_A (e.g. common environment effects) are likely to reduce upward bias in the heritability estimate and will tend to result in lower, but more accurate point estimates of heritabilities.

Table 2: Fixed effects literature survey. This literature survey does not claim completeness, but should be representative for heritability estimates in wild population using the animal model.

Référence	Nb. effects	Fixed effects	
		Natural variation	Experimental variation
Réale et al. (1999)	2	Sex, Years	—
Kruuk et al. (2000)	2	Age, Area	—
Milner et al. (2000)	8	Age, Parasite burden, Birth Year, Year of measurement, Birth type, Coat color, Horn type	Catch date
Kruuk et al. (2001)	1	—	Brood size manipulation
Merilä et al. (2001)	1	—	Brood size manipulation
Kruuk et al. (2002)	1	Age	—
MacColl and Hatchwell (2003)	6	Year, Sex, Helper, Hatch date, Area, Attempt	—
Sheldon et al. (2003)	1	Year	—
Hadfield et al. (2006)	5	Sex, Year, Hatch date	Carotenoid treatment, Immune treatment
Thériault et al. (2007)	4	Age, Year	Day of capture
Nilsson et al. (2009)	3	Age, Dyad	Brood size manipulation
Morales et al. (2010)	3	Eggmass, Age	Treatment
Charmantier et al. (2011)	2	Sex, Natal colony	—
Doligez et al. (2011)	2	Sex, Age	—
Lane et al. (2011)	2	Age-class, Year	—
Reid et al. (2011b)	1	Year	—
Reid et al. (2011a)	2	Year, Age-class	—
Evans and Sheldon (2012)	3	Sex, Age	Measurement day
Bérénos et al. (2014)	3	Sex, Litter size	Age at capture
Lane et al. (2015)	4	Age, Cone availability, Litter size, Year	—

Removing the influence of experimental design on V_P

In the context of estimating the phenotypic variance of a trait, fixed effects (as well as random effects) may be of two kinds. They can either reflect natural sources of variation that we are interested in, or variance arising from experimental and/or design effects. Since the latter category artificially inflates the variance in the data, we might wish to exclude this source of variance from the heritability calculation. For example, if we want to study the amplitude of insect songs in the field, we might want to improve our model fit by including effects accounting for natural sources of variation, such as the age of the individual (if the amplitude is age-dependent) and effects accounting for sampling

design, such as the distances between the animal and the microphone. Yet, in the computation of the phenotypic variance V_P, we might want to include the biological variance arising from age, but not the experimental variance arising from the distance.

We have categorised fixed effects in a cursory literature survey (Table 2) into sources of natural or experimental variation for illustration. Most of the fixed effects included in these analyses originate from natural variation (e.g. sex, year, age, area, litter size) and most likely should be included in V_P. Others are of experimental origin either being an experimental treatment or of design origin (e.g. due to variation in the time of measurement) and should probably be excluded from V_P. Of course, this separation between natural and experimental sources of variation can be quite difficult (e.g. year of sampling may represent error measurement or relevant natural variation depending on context). Furthermore, it can sometimes be interesting to also exclude natural sources of variation. For example, "age" or "sex" could be excluded from the denominator to get heritabilities conditional on those factors. This would allow performing evolutionary prediction for a particular age-class or sex.

In essence, what is required is to compute the phenotypic variance including the "natural" factors $\mathbf{x}_{\mathrm{nat}}$ conditional on the experimental factors $\mathbf{x}_{\mathrm{exp}}$:

$$V_{\mathrm{F}} = \int V_x(\hat{y}|\mathbf{x}_{\mathrm{exp}})\mathrm{d}f_{x_{\mathrm{nat}}|x_{\mathrm{exp}}}(\mathbf{x}_{\mathrm{nat}}). \tag{8}$$

To exclude the particular factor(s) in practice, the predictor(s) (i.e. the respective columns in the design matrix) and the related inferred parameters can simply be left out of a new linear predictor $\hat{\mathbf{y}}^{\star}$ in the calculation of V_{F} such that:

$$V_{\mathrm{F}} = V(\hat{\mathbf{y}}^{\star}). \tag{9}$$

This is equivalent to Eq. 8, in the sense that it is accounting for the variance due to the factors included in the computation of $\hat{y}^{\star}$ conditional on the effect of other factors that have been excluded. Note, however, that this computation is unfortunately not general and is based on the assumptions that the measured variance of the natural predictors is not caused by any of the experimental predictors. A more general solution relies on path analysis and the assumption of a causal pathway between variables (see Box 1).

A strong assumption in the equations above is that the sampled design matrix $\mathbf{X}$ for the cofactors is representative of their distribution f_x in the natural population. This might not be always the

case. For example, the insects that we are studying with respect to song amplitude might occur in distinct morphotypes (and these morphotypes differ in song amplitude) that are not equally common. For statistical reasons it may be useful to oversample rare morphs if we want to estimate the effect of morph on song amplitude. Such a sampling design will equalize morph frequencies in the sample and will thus tend to inflate V_{F} if calculated as $\mathbf{X}\hat{\mathbf{b}}$. Statistical requirements (balanced sampling) and biological realism (natural morph ratios) differ in this case and the calculation should account for this difference, especially we need to ensure the denominator actually reflect natural variation. A solution is to use Eq. 5 directly by assuming a distribution f_x for our morphotypes.

Although this might be feasible in this simple example, it will be more difficult as soon as many covariates have to be included, for which a joint distribution must be assumed. Two other solutions are possible. One possible solution would be to use Eq. 6, but replace the design matrix $\mathbf{X}$ by a modified design matrix $\mathbf{X}'$ that more closely matches the distribution of covariates to the natural population variation (e.g. sampled observations from the field) to compute the predicted values (noted $\hat{\mathbf{y}}'$). Note that, when constructing $\mathbf{X}'$, we should still take into account potential correlations between co-factors. For example if the rare morph is preferentially present in warm environments and temperature is included in the model, then $\mathbf{X}'$ should reflect that correlation. Additionally, the computation of $\mathbf{X}'$ will only represent a possible sampling of the true population, thus this solution will come with the drawback of sampling noise arising form this.

Another possible solution would be to use a slightly different (but exactly equivalent) approach compared to Eq. 6 and compute V_{F} as the product between the variance of the covariate in the natural population and their squared estimated effects. For a model with a simple continuous covariate $\mathbf{x}$ with an associated slope $\hat{b}_x$, this will be:

$$V_{\mathrm{F}} = \hat{b}_x^2 V(\mathbf{x}) \tag{10}$$

For a multivariate model, the variance-covariance $\mathbf{S}'$ of the covariates in the wild population is required (and can be easily computed from raw data) and one can compute V_{F} in the multivariate analogous of Eq. 10:

$$V_{\mathrm{F}} = \hat{\mathbf{b}}^{\mathsf{T}}\mathbf{S}'\hat{\mathbf{b}}, \tag{11}$$

where $^{\mathsf{T}}$ is the symbol for the transpose operation. The main advantage of this solution (again, exactly equivalent as the above approach) is that, compared to the resampling of a design matrix $\mathbf{X}'$, it does not introduce supplementary sampling noise (i.e. apart from the already existing sampling noise due to

data collection on the natural population and model estimates). All the issues and solutions discussed here equally apply to the computation of repeatability.

Fitting of genetic covariates and implicit assumptions about genetic covariances

A general consideration is whether fixed effects should cover only non-genetic sources of variation. Morphs in our example might be environmentally or genetically determined and it is usually advisable to model such discrete effects with potentially oligocausal control as fixed effects, no matter whether they are ultimately genetic or environmental in origin. With purely monogenic inheritance of morphs, morph phenotype is essentially a genetic marker for a (potential) quantitative trait locus (QTL) and thus represents the local heritability in linkage with the morph-determining locus (see e.g. Payne, 1918; Sax, 1923, for early QTL studies using Mendelian phenotypes as markers), while the polygenic contribution of the background is captured by V_A. Whether or not covariates cover genetic or non-genetic effects matters for the interpretation, since the estimate of V_A (and consequently h^2) might represent the total V_A or the background V_A other than the local heritability at the QTL.

Some potential covariates might also be (heritable) polygenic traits themselves. In many cases, relationships between a heritable focal trait and some other relevant heritable trait are best handled with multi-response models (see Hadfield, 2010; Wolak et al., 2015), wherein the potential covariate is treated as a response along with the focal trait. Such a model estimates the genetic (and non-genetic) variances for both traits along with genetic (and non-genetic) covariances among the traits treated as responses. This is not the case when the potential covariate is fitted as a fixed effect in the model: the fixed effect will explain the total influence of the covariate on the focal trait but not explicitly distinguish between (nor differentially estimate) different sources of covariances. There are situations where it does make sense to include polygenic traits as fixed covariates, particularly when studying questions where causal effects of traits on one another are relevant. Further discussions of such scenarios are presented in Gianola and Sorensen (2004) and Morrissey (2014, 2015).

To illustrate this, let us go back to Wilson (2008)'s unicorn dataset. It is a known fact that in unicorns, horn length varies according to the individual body mass (slope: $\beta = 0.403$ for a full model including age, sex and their interaction). It would thus seem relevant to add body mass as a covariate in our model of horn length. Doing so for our full model in Table 1 results in a

heritability (accounting for V_F) of $h^2_{\text{horn length|body mass}} = 0.066$ (Table S1 in Appendix). This is slightly lower than the estimate in Table 1, despite the fact that we accounted for the variance explained by the fixed effects. Yet, the inferred phenotypic variance ($V_{\text{P,horn length|body mass}} = 3.47$, Table S1) is comparable to the estimate in Table 1, what is now different is the estimate of the additive genetic variance ($V_{\text{A,horn length|body mass}} = 0.23$, Table S1, lower than $V_{\text{A,horn length}} = 0.35$ in Table 1). What is causing this lower additive genetic variance? Incidentally, we also know that body mass is a heritable trait ($V_{\text{A,body mass}} = 0.328$ and $h^2_{\text{body mass}} = 0.344$, Table S2). Moreover, a bivariate model shows that it is genetically correlated with horn length ($r_G = 0.63$, Table S3). Hence, by including body mass as a fixed effect, we have been "explaining away" some of the additive genetic variance of horn length, precisely because of this genetic correlation. A naive way to obtain a correct estimate of the additive genetic variance of horn length, without resorting to a bivariate model, would be to use the slope of the regression of horn length on body mass and compute the additive genetic variance as $V_{\text{A,horn length|body mass}} + \beta^2 V_{\text{A,body mass}} = 0.23 + 0.162 \times 0.328 = 0.283$, which doesn't quite restore $V_{\text{A,horn length}}$ to its original value. This is because this computation use the total slope of the relationship between horn length and body mass, hence assuming an effect of the phenotypic value of body mass on the genetic value of horn length, which is apparently not a good assumption here. Any more accurate recovery of V_A will almost always require to fit a bivariate model. Because of the issues highlighted here, we believe it is advisable, whenever possible, to fit multivariate models between heritable and putatively genetically correlated traits instead of using other traits as covariates.

More generally, whether the covariate variability is of genetic or environmental origin, we still make an implicit strong assumption about the structure of genetic variation of the response trait(s). In particular, we assume that the additive genetic effects (and especially their variance) are constant across the range of the covariate or categorical factor. In doing so, we assume that genotype-by-covariate interactions are negligible, or put differently, that there is a perfect genetic correlation e.g. between categories of the categorical covariate. This assumption is frequently violated. In the special case of sex, for example, it has been shown that fitting sex as a fixed effect in a LMM leads to (downward) biased estimates unless the cross-sex genetic correlation is perfect (Wolak et al., 2015).

Note that repeatability might also be impacted by the inclusion of biological covariates that are themselves repeatable and share some of the underlying mechanisms of this repeatability (e.g. genetics or environmental) with the response variable.

Non-linear models and non-Gaussian traits

The influence of fixed effects becomes more complex, and thus even more important to consider carefully, when the data are non-linear in the parameters of a model. Such non-linearity arises in non-linear mixed models (NLMMs), in generalised linear mixed models (GLMMs), through the non-linearity of their link functions, and when data are non-linearly transformed prior to analysis. In GLMMs, a distinction is critical between the latent scale and the data scale (Morrissey et al., 2014): on the former, we assume linearity, normality and perform most of the inferences, whereas the latter is a non-linear transformation from the latent scale (e.g. through the link function). Hence, the above framework could be used to compute heritabilities and repeatabilities on the linear, normally distributed, latent scale, but creates difficulties with methods transforming estimates from the latent scale to the data scale (see e.g. Table 1 and 2 in Nakagawa and Schielzeth, 2010).

Non-linearity causes dependence between fixed and random effects, with the direct consequences that quantitative genetic parameters can no longer be computed without accounting for the whole distribution of fixed effects. In other words, in a GLMM, variation associated with fixed effects does not only potentially contribute to the phenotypic variance, but the contribution to the phenotypic variance is not constant across the distribution of fixed effects (de Villemereuil et al., 2016). Hence V_A and V_P on the data scale become complex functions of all the parameters. This means that adding a V_F component to the computation of V_P will not work any longer, because the additivity of the fixed effects and random effects variance components is not valid in these models. De Villemereuil et al. (2016) showed that we need to integrate over the predicted values based on fixed effects to compute quantitative genetic parameters using a GLMM. The same logic applies when working with non-linear models or with data that was non-linearly transformed, unless we are specifically interested in the heritability of the transformed data.

Alastair Wilson collected data on the number of aggressive behaviours performed in a single day on all unicorn individuals for which he analysed the genetics of horn length in his 2008 paper. Here, we conduct analyses of these data to illustrate how, in addition to h^2, variance components such as V_A and V_P depend on the full distribution of fixed effects. Fig 2 show how, according to our Poisson GLMM model, the effect of the sex covariate impacts the observed number of aggressive behaviours: despite the two sexes having the same variance in their latent values and differing only in mean latent value (males having larger values, with little overlap), the counts of aggressive behaviours do not follow this patterns as clearly (variance in male values is larger with strong overlap of the distribution
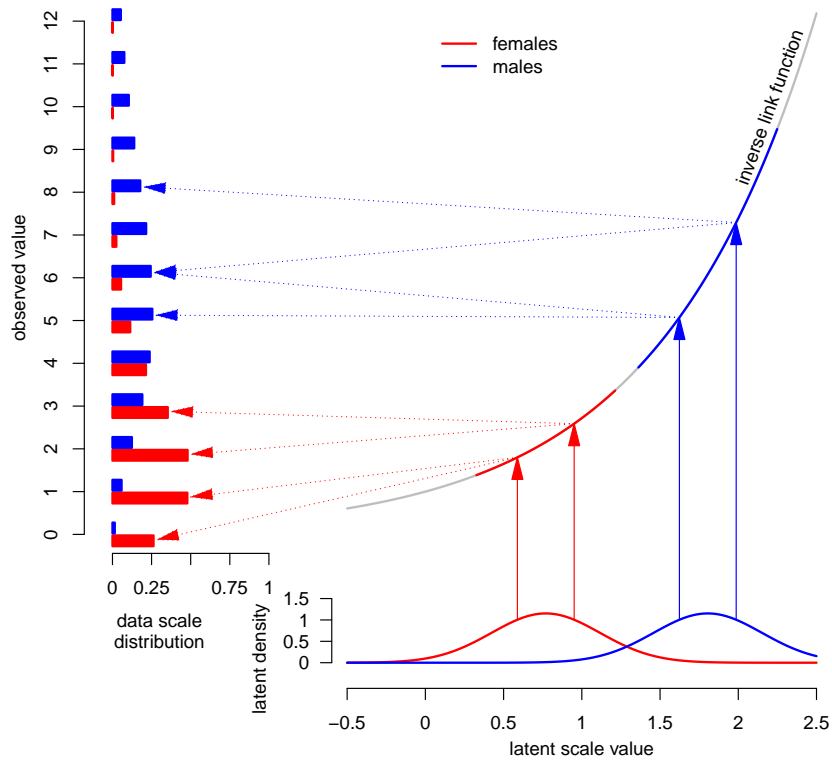
Figure 2: Illustration of the non-linearity of GLMMs using the unicorn aggressive behaviour trait. Plain arrows illustrate deterministic relationships and dotted arrows stochastic relationships. On the latent scale, males and females have the same variance in latent values, the males only differing from the female by a larger mean latent value. Because the inverse of the link function (here the exponential) is not linear, initially equally apart values for each sexes are more spread for males than for females (solid arrows). Because the variance depends on the mean for Poisson distributions, this effect is amplified for larger values, creating even more variances for males. The end result is that on the data scale, the two sexes no longer satisfy the assumptions on the latent scale: their variance are different (bigger for males), the shapes of their distributions now differ (females are more skewed toward). On the data scale, it is not possible to compute the variance arising from the fixed effect as simply the variance arising from differences in mean between the two sexes.

Latent scale	Intercept	V_F	V_R	V_A
Variances	0.0871 (-0.0533 - 0.254)	0.307 [0.273 - 0.342]	0.0973 [0.0686 - 0.13]	0.0317 [0.00766 - 0.056]
Observed scale	**Mean**	$V_{A,obs}$	$V_{P,obs}$	h^2_{obs}
(i) Using the intercept	1.17 (0.984 - 1.35)	0.0434 (0.0114 - 0.0813)	1.36 (1.14 - 1.61)	0.0318 (0.00825 - 0.0564)
(ii) Adding V_F	1.36 (1.16 - 1.55)	0.0589 (0.0154 - 0.11)	2.37 (1.95 - 2.79)	0.0247 (0.00722 - 0.0447)
(iii) Integrating over predictions	3.49 (3.37 - 3.62)	0.386 (0.113 - 0.709)	10.3 (9.3 - 11.4)	0.0377 (0.0119 - 0.0695)

Table 3: Results of fitting a model for number of observed aggressive behaviours using a Poisson distribution in MCMCglmm. First row: estimate of the intercept of the model and variance decomposition on the latent scale. Three last rows: estimates of population mean, additive genetic variance, phenotypic variance and heritability computed on the observed data scale using QGglmm *(i)* ignoring fixed effect and only providing the intercept; *(ii)* providing the intercept but including V_F in the latent total variance or *(iii)* using the whole latent predicted values distribution.

with females). This non-linearity of the effects can be linked to two subsequent phenomenons. To begin with, the exponential inverse link function assumed in the Poisson GLMMs is strongly non-linear: this results in the fact that two pairs (one for female, one for male, see red and blue arrows) of evenly spaced values on the latent scales are not evenly spaced after transformation through the inverse link-function. On top of this, the variance of a Poisson distribution is equal to the mean: this creates even more variance for large values (see dotted arrows and corresponding distributions of aggressive behaviours for males and females). The strong effect of this non-linearity on the variances of the phenotypic trait raises the question of how to best account for the variance "explained" by fixed effects.

We analysed the number of aggressive behaviours using the R package MCMCglmm and a Poisson family including sex, age and their interaction as fixed effects. The results of the analysis on the latent scale (direct output from MCMCglmm) are available in the first row of Table 3. We can see that the fixed effects account for a large part of the phenotypic variance (V_F is larger than V_A and V_R). Using the framework from de Villemereuil et al. (2016) implemented in the QGglmm R package, we then computed the quantitative genetic parameters on the observed data scale using three different approaches: *(i)* not accounting for fixed effects at all, *(ii)* accounting the variance of fixed effects in the total variance of the latent scale and *(iii)* using the whole distribution of predicted values to be integrated over rather a V_F component. As shown in the last three rows of Table 3, the three strategies drastically differ not only in the value for V_P, but also for all the other values, namely the population mean, the additive genetic variance and to a lesser extent, the heritability. Note that

accounting for the whole distribution of predicted values (last row) is the only approach that yields a population mean and phenotypic variance compatible with the sample estimates (number of aggressive behaviours, mean = 3.41 and variance = 9.77).

The analysis of unicorn aggression data illustrates that the approach of using a V_F component suggested here can only be applied directly to phenotypic traits with a normal error distribution and analysed using linear mixed models (or if the analysis is based on the latent scale of a GLMM). However, solutions to integrate over the distribution of predicted values do exist, such as assuming a distribution of the fixed-effect covariates (see Eq. 19 in de Villemereuil et al., 2016) or averaging over the predicted values according the fixed effects (i.e. averaging over $\hat{\mathbf{y}}$) within the integral computation (see Eq. 18 in de Villemereuil et al., 2016). This latter approach has been implemented in the QGglmm R package. This approach of course assumes that the distribution of fixed effects in the sample is representative for the base population of interest. Otherwise, the design matrices might need to be adjusted accordingly (e.g. by providing a construct such as $\hat{\mathbf{y}}^\star$ or $\hat{\mathbf{y}}'$ introduced above, or a mix of these constructs, as the predicted values to QGglmm).

On this subject, it must be noted that repeatability and heritability are computed differently for GLMMs as the narrow-sense heritability on the observed data scale of GLMMs is not an intra-class correlation coefficient any more (see the difference between Eqs. 9 and 16 in de Villemereuil et al., 2016).

Conclusion & Perspectives

Wilson (2008) identified an issue when fixed effects are included a quantitative genetic model: the inclusion of fixed effects in the model has an influence on the computation of the phenotypic variance. Based on recent work from several sources, we provide guidelines to overcome this and other related issues, in the hope this will facilitate the use and interpretation of quantitative genetic mixed models with fixed effects. We also discussed the complications arising from the diverse and complicated nature of covariates that can be fitted as fixed effects. We think that fixed effects are an opportunity to finely control confounding effects. Yet, when belonging to the phenotypic variance, they need to be included in the denominator of the heritability and repeatability. In order to do so, we here promote the practice of accounting for the "fixed-effect" variance component V_F (see Nakagawa and Schielzeth, 2013), which includes the variance of all or selected fixed effects to be added in the denominator of the heritability and repeatability calculation. We include an example of analysis using simulated

data (including how to implement Eqs. 9 and 11, see Supplementary Information) and the R package MCMCglmm (Hadfield, 2016) to show how these calculations can be implemented and how they can affect the output (h^2 estimates changing from 0.66 when not including V_F in the denominator to 0.15 when including it in our example). The code for the analysis of the unicorn examples is also provided.

This approach has several advantages. First, it overcomes Wilson (2008)'s legitimate reluctance of including fixed effects in the model. When including V_F in the denominator, there is no issue of "lost variance". Second, since we are now able to include fixed effects, we have gained a finer control on confounding effects on the additive variance. It also requires some careful consideration of which fixed effects represent experimental design effects and which are biologically relevant. Third, it provides us with the choice of whether or not to include effects in V_F, depending on whether or not we deem them part of the natural phenotypic variance of the studied population. Fourth, as argued above for the case of morphotypes in the context of song amplitude, the calculation of V_F can accommodate some discrepancies between the analysed data and the actual population in the distribution of covariates.

Overall we advocate for the inclusion of fixed effects in linear mixed models to estimate heritabilities and repeatabilities when *(i)* this improves the goodness-of-fit of the model and/or helps to account for confounding effects and *(ii)* a carefully computed V_F component is included in the calculation of the denominator. While this is generally also true for non-linear models and GLMMs, any model that involves non-linearity in the response to fixed effects will require particular attention and likely integration over their biologically relevant range in order to marginalize the influence of fixed effects.

Box 1: Using path analysis to obtain a partial V_F

Path analysis   Path analysis is a statistical analysis aiming at evaluating the *directed* influence of variables onto others. This directed relationship is referred to as *causality* (Wright, 1921). The direction of the relationship has a strong influence in our case, because it allows us to predict if the presence of one variable would inflate the variance of another.

Three examples   In the figure below are three different examples using a phenotypic variable of interest $\boldsymbol{P}$ influenced by a biological variable $\boldsymbol{B}$ and an experimental variable $\boldsymbol{E}$. The parameters b_{XY} stand for the coefficient of a model of the effect of X on Y (e.g. a slope). The parameters σ_X is the exogenous standard-deviation of the variable X, i.e. its standard-deviation due to influences outside of the causal pathway (e.g. stochasticity, unmeasured variables and measurement error). The parameters

σ_{XY} is the exogenous covariance between X and Y, i.e. a undirected covariance arising from common influences outside of the causal pathway or due to physical/logical constraints (e.g. size and volume are physically covarying).
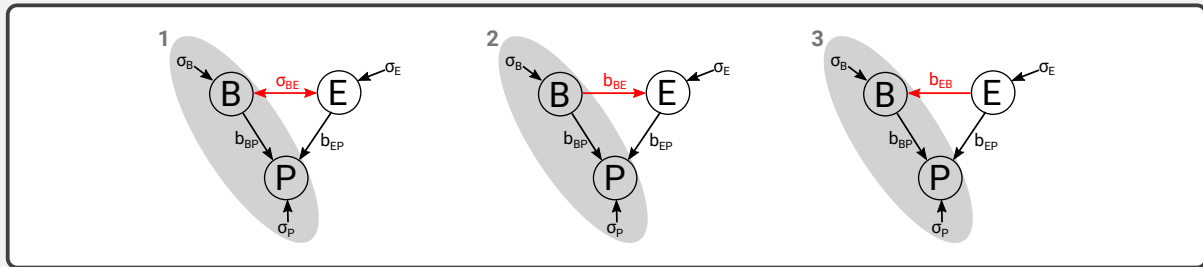
General principle   In all cases, we are only interested in computing the variance arising from the grey area of the pathway ($\boldsymbol{B}$ and $\boldsymbol{P}$), while excluding variance arising from $\boldsymbol{E}$. Excluding $\boldsymbol{E}$ from the graph means that we set its exogenous standard-deviation ($\sigma_{\boldsymbol{E}}$) and possible covariances (e.g. $\sigma_{\boldsymbol{BE}}$), as well as all the coefficients of its effect on any variable (e.g. $b_{\boldsymbol{EP}}$), to zero. Given that, the "fixed-effect variance" of $\boldsymbol{P}$ in this graph excluding $\boldsymbol{E}$ is simply the variance arising from the effect of $\boldsymbol{B}$:

$$V_{\mathrm{F}} = b_{\boldsymbol{BP}}^2 \sigma_{\boldsymbol{B}}^2$$

We will see that the difference between the three examples lies in the computation of $\sigma_{\boldsymbol{B}}$.

Example 1   In this example, the variables $\boldsymbol{B}$ and $\boldsymbol{E}$ share an undirected covariance σ_{XY}. In other words, we assume that a set of unmeasured variables have an effect on both $\boldsymbol{B}$ and $\boldsymbol{E}$, but not that a change in $\boldsymbol{E}$ will affect $\boldsymbol{B}$. In that case, the exogenous variance of $\boldsymbol{B}$ is merely its actual variance: $\sigma_{\boldsymbol{B}}^2 = V(\boldsymbol{B})$.

Example 2   In this example, the variable $\boldsymbol{B}$ has a direct effect on $\boldsymbol{E}$ (e.g. because an aspect of the species biology modulate the effect of the experimental treatment). In that case, changes of variance in $\boldsymbol{B}$ will affect the variance of $\boldsymbol{E}$, but this is not a problem for us since we want to exclude $\boldsymbol{E}$. Once again, the exogenous variance of $\boldsymbol{B}$ is merely its actual variance: $\sigma_{\boldsymbol{B}}^2 = V(\boldsymbol{B})$.

Example 3   In this example however, the variable $\boldsymbol{E}$ has a direct effect on the variable $\boldsymbol{B}$ (e.g. because the experimental treatment has an effect over different parts of the biological system). This means that, by experimentally introducing $\boldsymbol{E}$ into the biological system, we also experimentally increased the actual variance of $\boldsymbol{B}$. To compute the exogenous variance of $\boldsymbol{B}$, we need to remove this additional variance: $\sigma_{\boldsymbol{B}}^2 = V(\boldsymbol{B}|\boldsymbol{E})$. In other words, $\sigma_{\boldsymbol{B}}^2$ is here the residual variance of a model of the effect of $\boldsymbol{E}$ on $\boldsymbol{B}$ (e.g. the residual variance of the regression of $\boldsymbol{E}$ on $\boldsymbol{B}$).

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