Making the Most of BLUPS : A Method for Estimating Nonlinear Selection on Behavioral Reaction Norms

A Preprint

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Abstract

Individuals' behavioral strategies are often well described by reaction norms, which are functions predicting repeatable patterns of phenotypic consistency, plasticity, and predictability across an environmental gradient. Reaction norms can be readily estimated using mixed-effects models and play a key role in current theories of adaptive individual variation. Unfortunately, however, it remains challenging to assess the directional effects of reaction norms on fitness-relevant outcomes, due to the high degree of uncertainty in random effect estimates of reaction norm parameters, also known as best linear unbiased predictors (BLUPs). Previous solutions to this problem avoid inferential bias by limiting analyses to the description of linear covariances and correlations among BLUPs and other measures. As such, empiricists currently lack a method for exploring and explaining reaction norms effects with non-linear structure, such as stabilizing, disruptive, or correlational selection, which are crucial for testing adaptive theory of individual variation. To address this issue, a solution is presented for unbiased estimation of nonlinear reaction norm effects on fitness or any other outcome of interest. This solution involves specifying BLUPs as both random and fixed effects in a single Bayesian multi-response model. By simultaneously accounting for uncertainty in BLUPs and their causal effects on other traits, the risks accompanying classical approaches can be effectively avoided. A novel method for visualizing multivariate selection with such models is also proposed. Simulations are then used to assess the power of these models under realistic empirical scenarios. Coding tutorials are provided to aid researchers in applying these models to their own datasets using R.

Keywords mixed-effects · multivariate · Bayesian · personality · plasticity · predictability

1 Introduction

A population will evolve by natural selection whenever heritable variation occurs in fitness-relevant phenotypes (Darwin 1859). Individual differences in behavior are, therefore, a fundamental ingredient of adaptive behavioral evolution. Across taxa, repeatable individual variation is observed not only in average behavior (Bell, Hankison, and Laskowski 2009), but also in the degree of behavioral responsiveness exhibited toward the environment (Dingemanse et al. 2010; Stamps 2016), as well as in the intra-individual variability of behavior across time (Biro and Adriaenssens 2013; Westneat, Wright, and Dingemanse 2015). These patterns of personality, plasticity, and predictability represent distinct but often integrated components of organisms' reaction norms (see **Figure 1**), which are functions expressing individual-specific behavioral strategies across environmental gradients (Dingemanse et al. 2010; McNamara and Leimar 2020). The evolution of such

function-valued traits is currently a central area of research within evolutionary ecology (Gomulkiewicz et al. 2018), which has led to a host of methodological innovations for estimating the RNs of labile phenotypes subject to measurement error (Dingemanse and Dochtermann 2013; Martin and Jaeggi 2021), as well as the development of a rich theoretical framework for explaining the adaptive processes maintaining individual variation in RNs within populations (Dall and Griffith 2014; Sih et al. 2015; Wolf and Weissing 2010).

For labile phenotypes such as behavior, hormones, and cognition, the magnitude of repeatable between-individual variation is generally modest in comparison to the total phenotypic variation observed across space and time (Bell, Hankison, and Laskowski 2009; Cauchoix et al. 2018; Fanson and Biro 2015). This is unsurprising, given that these traits are often the primary mechanisms by which organisms can flexibly respond to ephemeral and stochastic variation in their local environments, such as by up-regulating circulating testosterone in response to social challenges (Eisenegger, Haushofer, and Fehr 2011), or by temporarily inducing a fear state in response to odor cues of predation (Mathuru et al. 2012). As such, single measurements of these labile phenotypes are poor indicators of the underlying between-individual differences that are targeted by selection, and tend to instead reflect various sources of within-individual environmental heterogeneity (Brommer 2013; Dingemanse and Dochtermann 2013). Despite the unfortunate fact that many empirical studies still confound these distinct sources of trait (co)variation (Niemelä and Dingemase 2018; Royauté et al. 2018), the necessity of longitudinal data for studying RNs is increasingly appreciated and enforced within behavioral ecology (Dingemanse and Wright 2020). With the appropriate application of generalized mixed-effect models (GLMMs), these repeated measures data can then be used to estimate the unobserved but statistically identifiable RNs underlying raw behavioral measurements, thus effectively partitioning stochastic effects and measurement error from repeatable sources of individual variation (Dingemanse and Dochtermann 2013; Martin and Jaeggi 2021; Nakagawa and Schielzeth 2010; Nussey, Wilson, and Brommer 2007).

GLMMs are a powerful tool for not only estimating RNs from empirical data using random effects, but also for subsequently modeling the fixed effects of personality, plasticity, and predictability on fitness and the expression of other phenotypes. Nevertheless, although GLMMs are quite robust (Schielzeth et al. 2020), they can only give as much information about RNs and their effects as the model assumptions and empirical data provided to them. For labile phenotypes like behavior, this means that the predicted random effect values of RN parameters, also known as best linear unbiased predictors (BLUPs), are often estimated with non-trivial degrees of statistical uncertainty. The use of BLUP point estimates to predict outcomes in another response model will, therefore, artificially reduce uncertainty in the estimated effects of RNs and increase the risk of false positives (see Hadfield et al. 2010 for a detailed treatment, **Figure 1**). Previous solutions to this problem have provided effective antidotes to the anti-conservative inference encouraged by ignoring uncertainty in BLUPs (Houslay and Wilson 2017), but this comes at the cost of a reduced capacity to effectively model complex, directional effects of RNs on fitness-relevant outcomes and other phenotypes (see **Figure 2**). Therefore, the present study introduces a novel Bayesian multivariate modelling approach to facilitate unbiased estimation of RN effects of arbitrary complexity. The proposed solution is first motivated through a brief discussion of Houslay and Wilson (2017) 's approach to the misuse of BLUPs and its benefits and limitations, as well as introduction to Bayesian inference for unfamiliar readers. Novel Bayesian models are then introduced conceptually and demonstrated in multiple simulated empirical scenarios based on prior research. Code for estimating these models with the Stan statistical programming language (Carpenter et al. 2017) in the R statistical environment (R Core Team 2020) is also provided to aid researchers in unbiasedly modeling RN effects in their own datasets (see electronic supplementary material [**ESM**]).

2 The current solution

The basic challenge of modelling RN effects is to effectively account for the uncertainty in RN parameters (i.e. BLUPs) across all stages of analysis. Variation in phenotypes with low to moderate repeatability is, by definition, largely explained by factors other than between-individual differences. As a consequence, sampling designs with modest repeated measurements and uncontrolled environmental variation typically result in highly uncertain estimation of RNs. Failure to account for the uncertainty of RNs across subsequent stages of analysis artificially reduces uncertainty in the inferred effects of RNs, as uncertainty in individuals' trait values necessarily translates into uncertainty about the effects of these trait values. This is demonstrated in **Figure 1**, where it can be seen that the probability of false positives remains undesirably high for BLUP point estimates even when using conservative Bayesian priors. For this reason, Hadfield et al. (2010) discouraged all future use of BLUP point estimates in evolutionary ecology, so as to prevent the proliferation of misleading findings in the literature. Nevertheless, because the theoretical significance of RNs is not diminished by the difficulty of appropriately modeling their effects, many behavioral ecologists without clear alternative

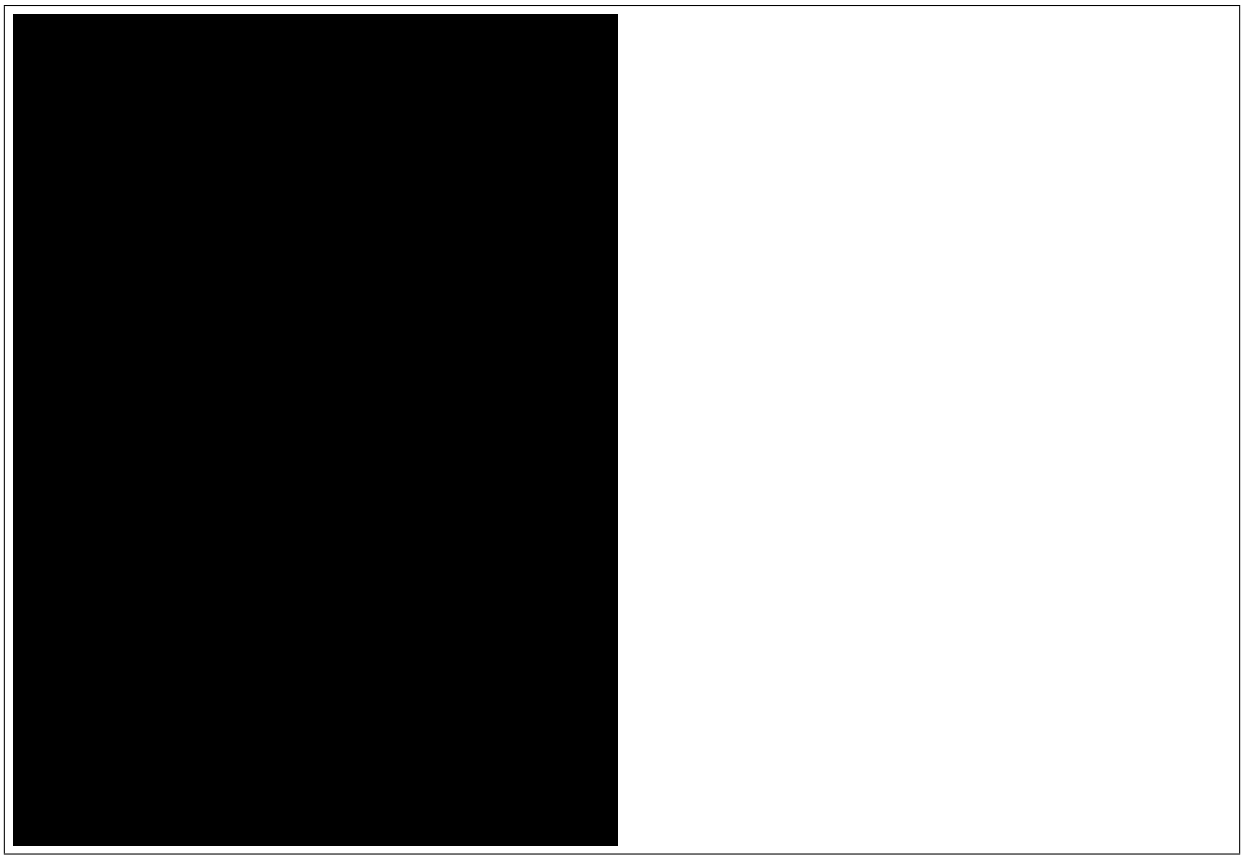

Figure 1: Sample figure caption.

solutions continued to misuse point estimates of BLUPs in their research. In response, Houslay and Wilson (2017) provided a detailed overview of appropriate strategies for tackling this challenge, emphasizing that multivariate GLMMs with correlated random effects can be used to effectively account for uncertainty in RNs across multiple response models. Despite these repeated cautionary notes, some researchers still continue to utilize BLUP point estimates (e.g. Dingemanse et al. 2020) or raw data (e.g. Brehm et al. 2019) for testing RN effects, even while acknowledging the work of Hadfield et al. (2010) and Houslay and Wilson (2017). This likely reflects the fact that the random effects models proposed by Houslay and Wilson (2017) do not readily extend to a variety of more complex RN effects that cannot be straightforwardly derived from random effect covariances and correlations (see **Figure 2**). This section briefly reviews the proposed solution of Houslay and Wilson (2017) for the misuse of BLUPs and discusses its benefits and limitations.

2.1 Multivariate GLMMs with covarying random effects

Houslay and Wilson (2017) note that many behavioral ecologists rely on GLMM software packages such as lme4 (Bates et al. 2014) that do not readily address multivariate, integrated phenotypes. As a consequence, researchers are often motivated to (i) estimate RNs from a univariate response model of the relevant behavior, and (ii) subsequently enter BLUP point estimates of these RNs as covariates in another response model. Fortunately, the risk engendered by this approach can be readily overcome by specifying a multivariate GLMM that simultaneously accounts for uncertainty in behavioral BLUPs and their associations with other responses. Houslay and Wilson (2017) demonstrate how this can be done with random effect correlations for phenotypic and quantitative genetic studies using both frequentist and Bayesian software. For simplicity, the present study focuses on the estimation of phenotypic GLMMs, although it should be noted that these models can be straightforwardly extended to quantitative genetic animal models (see **ESM**); further note that the present study relies on the flexibility of Bayesian modelling software for the novel solutions proposed below, and classical estimators are thus not considered further. Researchers unfamiliar with the general

benefits of fully Bayesian inference are encouraged to see McElreath (2020) for detailed discussion, as well as Gelman et al. (2020) for helpful tips on developing an effective Bayesian workflow for data analysis.

Consider a situation where we are interested to know whether personality, plasticity, and predictability are associated across phenotypes y and z. Plasticity is considered across some environmental gradient x, and we assume for simplicity that environmental exposures are randomized across individuals, so that there is no need to within-individual center the covariate (van de Pol and Wright 2009). A bivariate GLMM can be specified to estimate the associations among these RN parameters from repeated measures data. In particular, for observation i of individual j.

$$\begin{aligned} y_{ij} &\sim f\left(\eta_{ij}^{(y)}, \theta_j^{(y)}\right) \\ z_{ij} &\sim f\left(\eta_{ij}^{(z)}, \theta_j^{(z)}\right) \\ g^{(y)}\left(\eta_{ij}^{(y)}\right) &= \mu_j^{(y)} + \beta_j^{(y)} x_{ij} \\ g^{(z)}\left(\eta_{ij}^{(z)}\right) &= \mu_j^{(z)} + \beta_j^{(z)} x_{ij} \\ \left[\boldsymbol{\mu}^{(y)} \quad \boldsymbol{\beta}^{(y)} \quad \boldsymbol{\theta}^{(y)} \quad ... \quad \boldsymbol{\theta}^{(z)}\right]^{\mathrm{T}} &\sim \mathrm{MVNormal}\left(\left[\mu_0^{(y)} \quad \beta_1^{(y)} \quad \theta_0^{(y)} \quad ... \quad \theta_0^{(z)}\right]^{\mathrm{T}}, \mathbf{P}\right) \end{aligned} \tag{1.1}$$

Bold values are used to distinguish vectors and matrices from scalars, and superscripts (y) and (z) are used to distinguish parameters specific to the respective response models. The traits values are specified as being generated by some probability density function f with corresponding location $\boldsymbol{\eta}$ and dispersion $\boldsymbol{\theta}$ parameters, e.g. the means and standard deviations of a normal distribution or the means and shape parameters of gamma, negative binomial, and beta distributions. For GLMMs, these parameters are modelled on a latent linear scale using a link function g (e.g. an identity, log, logistic, or reciprocal transformation). We therefore refer to $g(\eta_{ij})$ as the linear predictor for observation i of individual j.

Typically, personality and plasticity are modelled through the linear predictor of the location parameters $\boldsymbol{\eta}$, capturing variation in expected behavior (i.e. predicted behavior averaged over dispersion). This is accomplished through the estimation of random intercept μ_j and random slope β_j for individual j, corresponding to the elevation and slope of the individual's behavioral RN. Predictability is instead modelled through the RN dispersion parameters $\boldsymbol{\theta}$, which are also random effects capturing individual-specific variability independent of the linear predictor. For simplicity, we ignore the possibility that individuals may also exhibit plasticity in their predictability as a function of the environment, although this could be readily estimated (along with other fixed and random effects) by introducing an additional linear predictor $g(\theta_{ij})$. For distributions without an explicit dispersion parameter, such as Poisson or binomial distributions, individual differences in predictability cannot be directly modelled in this way, but they can nonetheless be derived using the assumed mean-variance relations of these distributions (see **ESM** for example models handling both scenarios).

The individual-specific random effects are generated from a multivariate normal distribution centered on the population-average intercepts (μ_0), slopes (β_1), and dispersion parameters (θ_0) for each trait. The answer to our inquiry of interest–how do personality, plasticity, and predictability associate across traits y and z–is thus found in the covariance matrix $\mathbf{P}$ for the RN parameters.

$$\mathbf{P} = \begin{pmatrix} \mathrm{var}(\boldsymbol{\mu}^{(y)}) & \mathrm{cov}(\boldsymbol{\mu}^{(y)}, \boldsymbol{\beta}^{(y)}) & ... & \mathrm{cov}(\boldsymbol{\mu}^{(y)}, \boldsymbol{\theta}^{(z)}) \\ \mathrm{cov}(\boldsymbol{\beta}^{(y)}, \boldsymbol{\mu}^{(y)}) & \mathrm{var}(\boldsymbol{\beta}^{(y)}) & ... & \mathrm{cov}(\boldsymbol{\beta}^{(y)}, \boldsymbol{\theta}^{(z)}) \\ \vdots & \vdots & \ddots & \vdots \\ \mathrm{cov}(\boldsymbol{\theta}^{(z)}, \boldsymbol{\mu}^{(y)}) & \mathrm{cov}(\boldsymbol{\theta}^{(z)}, \boldsymbol{\beta}^{(y)}) & \cdots & \mathrm{var}(\boldsymbol{\theta}^{(z)}) \end{pmatrix} \tag{1.2}$$

In many cases, researchers will lack sufficient data to confidently estimate these parameters, and will instead consider simpler models such as models with only covarying personality components $\mathrm{cov}(\boldsymbol{\mu}^{(y)}, \boldsymbol{\mu}^{(z)})$. Houslay and Wilson (2017) also provide further considerations for modelling linear selection effects without repeated fitness measures. It is nevertheless important to recognize the full potential of such an analysis for capturing the structure of RNs and behavioral syndromes, which is further integrate into the novel models presented below.

2.2 Fully Bayesian inference

To estimate this model within a Bayesian framework, we simply need to specify prior distributions for all the population-level parameters, which are transformed within the model to derive the individual-level RN parameters.

$$\mu_0^{(y)}, \beta_1^{(y)}, ..., \sigma_0^{(z)}, \mathbf{P} \sim \boldsymbol{f}(\boldsymbol{\Phi}) \tag{1.3}$$

As above, $\boldsymbol{f}$ are probability density functions for each parameter and $\boldsymbol{\Phi}$ are the corresponding distributional parameters. Although it is common for methods papers to use and/or recommend using highly diffuse or flat priors (e.g. Houslay and Wilson 2017; Villemereuil et al. 2016), it is also well established within the statistics literature that weakly informative, regularizing priors–which pool hypotheses toward null values and provide low probability to extreme effect sizes–facilitate more robust inferences and should generally be preferred over flat priors whenever possible (Gelman and Tuerlinckx 2000; McElreath 2020; Lemoine 2019). This does not require that one has access to a relevant meta-analysis or is in a position to make strong a priori assumptions about the true effect size (cf. Ellison 2004). Rather, one can simply use general-purpose, conservative priors as a means of increasing the generalizability and robustness of their findings, even in a state of relative ignorance about the true effect size. See Lemoine (2019) for a more detailed discussion and recommendations for weakly regularizing priors in ecological research, which are described further in the **ESM**.

By specifying priors in the model, all parameters will be subsequently estimated as posterior distributions. For example, individual j's RN parameters on trait y will no longer be estimated with BLUP point estimates $\hat{\mu}_j^{(y)}$, $\hat{\beta}_j^{(y)}$, and $\hat{\theta}_j^{(y)}$, but will instead be estimated as probability distributions capturing all of the statistical uncertainty in the BLUPs

$$\Pr\left(\mu_j^{(y)} \mid \boldsymbol{x}, \boldsymbol{y}, \boldsymbol{z}, ..., \boldsymbol{\Phi}\right), \quad \Pr\left(\beta_j^{(y)} \mid \boldsymbol{x}, \boldsymbol{y}, \boldsymbol{z}, ..., \boldsymbol{\Phi}\right), \quad \Pr\left(\theta_j^{(y)} \mid \boldsymbol{x}, \boldsymbol{y}, \boldsymbol{z}, ..., \boldsymbol{\Phi}\right) \tag{2.1}$$

The answer to the question "What are the estimated BLUPs for individual j's RN parameters?" is now given as series of probability distributions, capturing all uncertainty in these values conditional on the observed dataset $(\boldsymbol{x}, \boldsymbol{y}, \boldsymbol{z})$ and other model parameters and priors $(...\Phi)$. Given that all uncertainty is captured in these distributions, the model provides nearly unlimited flexibility for direct forms of hypothesis testing. For example, to quantify our confidence that there is more repeatable variation in personality than predictability for trait y, we simply need to manipulate the relevant posteriors to calculate

$$\Pr\left(\text{var}(\boldsymbol{\mu}^{(y)}) > \text{var}(\boldsymbol{\theta}^{(y)}) \;\mid\; \boldsymbol{x}, \boldsymbol{y}, \boldsymbol{z}, ..., \boldsymbol{\Phi}\right) \tag{2.2}$$

When posterior distributions are estimated with Markov Chain Monte Carlo (MCMC), this value can be easily quantified by simply assessing this inequality across the relevant vectors of posterior samples and calculating the proportion of samples for which it is satisfied (see **SEM** for examples). Note that this is *not* an indirect null hypothesis test, which gives the probability of observing the data under the assumption that the null hypothesis is true. Instead, it is a direct test of a biologically substantive hypothesis given the observed data, the evaluation of which is generally the primary goal of scientific research. As such, intuitive interpretation can be made of this posterior probability, so that values closer to 1 indicate greater support for this directional hypothesis and values closer to 0 indicate stronger support for the opposite directional hypothesis. One could similarly perform a direct hypothesis test of a more robust null hypothesis than is typically considered, not merely quantifying the probability that the effect size is exactly zero, which is almost never true in reality (Amrhein, Trafimow, and Greenland 2019; Meehl 1978; Gelman and Carlin 2017), but rather the probability that the effect is of a biologically trivial magnitude (e.g. $< |0.1|$). For instance, considering the population-average regression coefficient for trait z

$$\Pr\left(-0.1 < \beta_1^{(z)} < 0.1 \;\mid\; \boldsymbol{x}, \boldsymbol{y}, \boldsymbol{z}, ..., \boldsymbol{\Phi}\right) \tag{2.3}$$

These Bayesian hypothesis tests help empiricists to avoid many common misinterpretations of classical tests, such as interpreting confidence intervals as reflecting the probable range of the true effect, interpreting P-values as providing the probability of the null hypothesis being true, or interpreting the rejection of a null hypothesis test as being indicative of the true ("alternative") hypothesis being correct (Greenland et al. 2016; McElreath 2020; McShane et al. 2019). Furthermore, as should be obvious from these examples, Bayesian posteriors can be easily manipulated to address a variety of questions which may not be easily specified

directly in a statistical model. This provides theoretically important benefits such as being able to perform hypothesis tests with random effects (e.g. comparisons of specific plot, group, or individual differences) or any other quantity that can be derived from the initial model parameters [e.g. comparisons of repeatabilities or R^2 values, or tests of the magnitude of assortment coefficients and social selection differentials; Martin and Jaeggi (2021)].

2.3 Benefits and limitations

As should be clear, the multivariate GLMMs proposed by Houslay and Wilson (2017) are an extremely valuable tool for behavioral ecologists interested in RNs, integrated phenotypes, and adaptive individual variation. When estimated within a Bayesian framework, these models provide a great deal of flexibility for addressing a variety of questions beyond simply quantifying random effect variances and covariances, although this on its own is quite an important task. As any student of multivariate statistics is well aware, trait covariance matrices such as **P** can be readily transformed to provide a veritable treasure chest of biological insights (Blows 2007), such as identifying trajectories of phenotypic conservation and divergence among closely related populations (Royauté, Hedrick, and Dochtermann 2020), discovering latent behavioral characters and networks causing covariance among multiple traits (Araya-Ajoy and Dingemanse 2014; Martin et al. 2019), and calculating selection differentials and genetic responses to selection (Stinchcombe, Simonsen, and Blows 2014). Thus, with the flexible hypothesis testing provided by Bayesian inference, Houslay and Wilson (2017) 's method can be used to accomplish many important empirical goals with relative ease.

Nonetheless, there are important cases where further information is desired that cannot be straightforwardly derived from random effect covariances alone, which places limitations on the flexibility of this method for exploring and explaining the causal effects of RNs on evolutionarily relevant outcomes. These considerations are not specific to the models proposed by Houslay and Wilson (2017), but are general limitations of all purely variance-partitioning models, which achieve accurate predictions and characterizations of (co)variance at the potential cost of reduced explanatory power and insight about the causal interactions generating (co)variances (Briley et al. 2019; Hadfield and Thomson 2017; Okasha and Otsuka 2020). This is why fixed effects remain important for testing evolutionary ecological theory, because we often want to directly parameterize specific functional relationships between traits, as well as to specify the direction of these effects. In other words, we often want to know whether trait y affects z in a specific, potentially non-linear manner, and perhaps in interaction with other traits or states, rather than merely asking whether trait y and z are linearly associated through any number of possible causal pathways in either direction.

In particular, testing adaptive theory of individual variation often requires evaluating nonlinear selection on behavioral RNs (**Figure 2**), which can be approximated using quadratic and interaction effects on RN parameters in a parametric fitness model (Lande and Arnold 1983). If the population RN is at an evolutionary equilibrium, soc that RN variation is non-adaptive within the population and results from processes such as mutation-selection balance or developmental noise (e.g. Bierbach, Laskowski, and Wolf 2017; Tooby and Cosmides 1990), then we should expect to find evidence of stabilizing selection around the population average RN parameters. This would be observed in a Lande-Arnold selection analysis on independent RN parameters as null or weak linear effects and negative quadratic effects (Stinchcombe et al. 2008). Alternatively, strong disruptive selection, potentially indicative of an ongoing behaviorally-mediated speciation process (Wolf and Weissing 2012), would be expected to surface as the opposite pattern–i.e. null or weak linear effects with positive quadratic effects. In other situations, such as when variation in RNs is maintained through spatially and/or temporally varying selection (e.g. Gurven et al. 2014; Le Cœur et al. 2015), interaction effects will also be expected between local ecological conditions (e.g. population density, resource abundance, climate) and individuals' RN parameters across multiple selection events. Similar considerations apply to social contexts as considered by evolutionary game theory, in which frequency-dependent fitness functions, such as cooperative strategies with diminishing returns or threshold payoffs as a function of partners' strategies (McNamara and Leimar 2020), will be observed through interactive selection effects (Araya-Ajoy, Westneat, and Wright 2020; Martin and Jaeggi 2021; Queller 2011). When considering behavioral syndromes, RNs may also be expected to evolve through correlational selection for specific parameter combinations, such as through female mate choice of males with high levels of both personality and predictability in aggressiveness among cichlids (*Pelvicachromis pulcher*, Scherer, Kuhnhardt, and Schuett 2018). When RN parameters are under correlational selection, trait interaction effects will be observed in a selection analysis, irrespective of the linear main effects of each trait (Blows 2003). Of course, these considerations also apply to a host of other RN effects on outcomes other than fitness, such as the exponential effects of personality in activity level and anxiety on seed removal and dispersal distance, respectively, among small mammals in the northeastern United States (Brehm et al. 2019). In all such cases, one could not detect these theoretically pertinent

relationships using random effect covariances alone, because covariance is by definition a measure of linear dependency and thus does not capture non-linear dependencies among traits.

It should be noted that one apparent but flawed solution to these challenges is to (i) first estimate BLUP posteriors in an initial Bayesian random effects model, and then to (ii) estimate a separate model with RN effects estimated by running the analysis repeatedly over every MCMC sample of the BLUP posteriors. While this is technically carrying the uncertainty in RNs forward, it nonetheless is expected to result in downwardly biased estimates of the RN effects, as Dingemanse and Wright (2020) observed in supplementary simulations. Although these authors do not provide an explanation for the observed bias, it can be attributed to a more general statistical phenomena known as attenuation bias, in which independent measurement error in a predictor variable causes downward bias in its association with an outcome measure (Adolph and Hardin 2007; Spearman 1904). This is caused by the fact that the BLUPs in the initial model are estimated independently of the RN effects on the outcome of interest, so that the estimated uncertainty in BLUPs is by design statistically independent of uncertainty in the RN effect estimated in the second stage of the analysis. This does not, however, make the use of BLUP point estimates any less dangerous, but is instead simply an artifact of not simultaneously accounting for both sources of uncertainty in the same model. It is important to remember that BLUPs are latent, statistical inferences, not directly measured trait values or mere averages of raw trait values, and as such are particularly sensitive to correct model specification (Hadfield et al. 2010; Postma 2006).

3 A novel solution

Given the limitations of relying solely on covarying random effects, behavioral ecologists stand to benefit from adding an additional modeling approach to their toolkit, one capable of directly estimating nonlinear RN effects of arbitrary complexity. Here I propose a novel solution that is a straightforward extension of Houslay and Wilson (2017) 's previous work: Bayesian multi-response GLMMs in which individuals' RNs are simultaneously treated as random effects on their observed behaviors as well as fixed effects on outcome measures of interest (e.g. survival and reproduction, habitat choice, performance in an experimental task, etc.). In this section, this basic modelling approach is formally introduced, along with various extensions of interest for specific empirical scenarios. A novel and straightforward method for visualizing the within-generation effects of multivariate selection is also proposed to compliment models considering selection on more than two RN parameters. Simulations are then used to explore the statistical properties of these models under realistic sampling regimes, providing a guidepost for researchers interested in applying these models to their own datasets. Finally, to aid in this effort, detailed R coding tutorials are provided in the **ESM**.

3.1 Multivariate GLMMs for nonlinear selection on RNs

Our goal in overcoming the limitations of previous approaches is to specify a multi-response GLMM for observation i of individual j on repeatedly measured behavior y, as well as for fitness-relevant outcome w predicted by individual variation in RN parameters of y. Given that researchers will often lack repeated measures of fitness or fitness-proxies (e.g. survival, clutch size, mate choice), the presented models assume that a single fitness measure is available per individual, although they can be straightforwardly extended for repeated measures by including additional random effects. It is also important to note that, to the best knowledge of the author, none of these models can be estimated with mainstream statistical software. This does not, however, reflect any fundamental issue with their parameterization or interpretation, but rather pragmatic limitations of the estimators employed and/or the software syntax. The Stan statistical programming language (Carpenter et al. 2017), which relies on cutting-edge and computationally efficient MCMC algorithms, provides exceptional flexibility for specifying and straightforwardly estimating such atypical GLMMs within a Bayesian framework.

3.1.1 Gaussian fitness measures

For simplicity, we begin by assuming that the fitness measure can be effectively modelled with a Gaussian distribution, which simplifies the estimation of selection gradients and differentials. As is appropriate for modelling relative fitness (Lande and Arnold 1983), w is mean-scaled so that $w_j = W_j/\bar{W}$, prior to entering the analysis. Our basic approach will be to specify the model of phenotype y as described in **Eq 1.1**. However, we need to change how the random effects are centered in the model, because we'd like for the individual RN trait values to be centered on zero in the fitness model. To accomplish this, we specify an alternative but equivalent non-centered parameterization, in which the scale of the population-average parameters is

separated out from the specification of the individual-specific parameters. This makes it so that the BLUPs are estimated are centered on zero and expressed as deviations from the population average. To accomplish this for the dispersion parameter, we also need to introduce an additional linear predictor $g^{(y)}\left(\theta_{ij}^{(y)}\right)$ to account for nonlinearity on the original scale due to adding parameters on the transformed linear scale.

$$
\begin{aligned}
y_{ij} &\sim f\left(\eta_{ij}^{(y)}, \theta_{ij}^{(y)}\right) \\
g_\eta\left(\eta_{ij}^{(y)}\right) &= \mu_0^{(y)} + \mu_j^{(y)} + \left(\beta_1 + \beta_j^{(y)}\right) x_{ij} \\
g_\theta\left(\theta_{ij}^{(y)}\right) &= \theta_0^{(y)} + \theta_j^{(y)} \\
\left[\boldsymbol{\mu}^{(y)} \quad \boldsymbol{\beta}^{(y)} \quad \boldsymbol{\theta}^{(y)}\right]^{\mathrm{T}} &\sim \mathrm{MVNormal}\left(\mathbf{0}, \mathbf{P}\right)
\end{aligned}
\tag{3.1}
$$

$$
\begin{aligned}
w_j &\sim \mathrm{Normal}\left(\mu_j, \sigma_j\right) \\
\mu_j &= \mu_0 + \beta_1\left(\mu_j^{(y)}\right) + \beta_2\left(\beta_j^{(y)}\right) + \beta_3\left(\theta_j^{(y)}\right) \\
&+\beta_4\left(\mu_j^{(y)}\mu_j^{(y)}\right) + \beta_5\left(\beta_j^{(y)}\beta_j^{(y)}\right) + \beta_6\left(\theta_j^{(y)}\theta_j^{(y)}\right) \\
&+\beta_7\left(\mu_j^{(y)}\beta_j^{(y)}\right) + \beta_8\left(\mu_j^{(y)}\theta_j^{(y)}\right) + \beta_9\left(\beta_j^{(y)}\theta_j^{(y)}\right)
\end{aligned}
$$

shorten to matrix notation...

3.1.2 Within-generation effects of selection

...

3.1.3 Non-Gaussian fitness measures

introduce ... approach of Morrissey ...

3.1.4 Visualizing multivariate selection

issues with canonical analysis, PCA, ...

propose simple p

Fig 3...

4 Simulation of model properties

Table 1...

5 Conclusion

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Of note is the command `\citet`, which produces citations appropriate for use in inline text. For example,

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5.1 Figures

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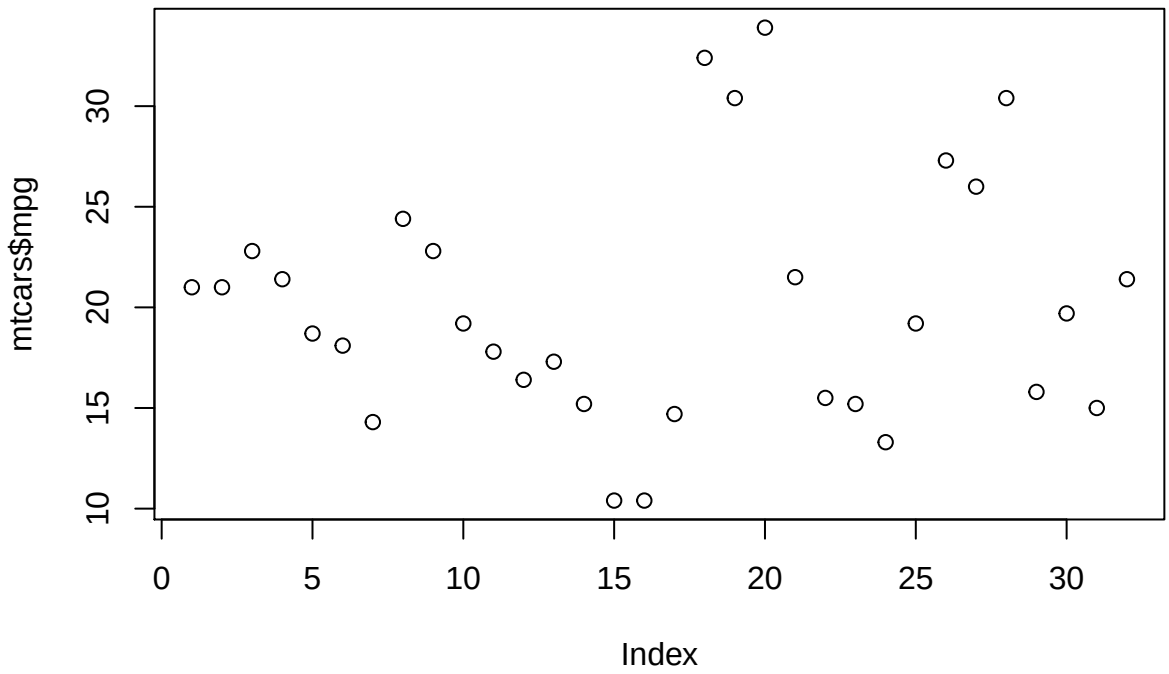
```
plot(mtcars$mpg)
```



5.2 Tables

Etiam euismod. Fusce facilisis lacinia dui. Suspendisse potenti. In mi erat, cursus id, nonummy sed, ullamcorper eget, sapien. Praesent pretium, magna in eleifend egestas, pede pede pretium lorem, quis consectetuer tortor sapien facilisis magna. Mauris quis magna varius nulla scelerisque imperdiet. Aliquam

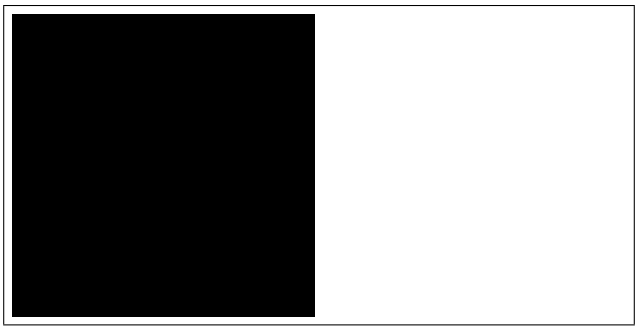


Figure 2: Sample figure caption.

Table 1: Sample table title

Part		
Name	Description	Size (μm)
Dendrite	Input terminal	$\sim$100
Axon	Output terminal	$\sim$10
Soma	Cell body	up to 10^6

non quam. Aliquam porttitor quam a lacus. Praesent vel arcu ut tortor cursus volutpat. In vitae pede quis diam bibendum placerat. Fusce elementum convallis neque. Sed dolor orci, scelerisque ac, dapibus nec, ultricies ut, mi. Duis nec dui quis leo sagittis commodo.

See awesome Table~1.

5.3 Lists

- Lorem ipsum dolor sit amet
- consectetur adipiscing elit.
- Aliquam dignissim blandit est, in dictum tortor gravida eget. In ac rutrum magna.

5.4 Leftovers

Previous To address this issue, empiricists have been encouraged to forgo the use of BLUP point estimates as directional predictors (i.e. fixed effect covariates), and to instead estimate random effect correlations (or covariances) among BLUPs and other relevant traits in a multi-response model [...]. This straightforward approach has undoubtedly been important for reducing the risk of false positives present in the literature, and is generally sufficient for empirical studies in which researchers are content to describe the (partial) correlations among traits. However, as with any purely variance-partitioning model, this approach trades off explanatory insight for descriptive accuracy [...]. As a result, empiricists currently lack a means of both avoiding bias due to the uncertainty in BLUPs, as well as effectively identifying the potentially complex directional effects of BLUPs on fitness and other phenotypes.

The present paper addresses this limitation by proposing an additional modelling approach for the empiricist's toolkit: Bayesian multi-response GLMMs with BLUPs specified as both random and fixed effects. This approach is first motivated through a brief discussion of previously proposed solutions and their limitations for identifying and explaining some important behavioral phenomena. I then explain how fully Bayesian multi-response models can be employed to avoid the misuse of BLUPs while also modelling BLUP effects of arbitrary complexity. This demonstrated through worked simulations of , with R code provided for estimating each

longitudinal datasets has GLMMs has increasingly become the norm within evolutionary ecology. Despite the many benefits of GLMMs, inferential bias has arisen in the literature from their application to causal questions regarding directional effects of RN parameters.

However, despite the centrality of RNs to behavioral evolution, it currently remains difficulty to unbiasedly investigate the causal effects of RNs on fitness and the phenotypic expression of other traits. Fundamentally, this is due to the high degree of , which is observed across a host of behaviors in both wild and captive contexts. ... While previous solutions have been provided for avoiding such anti-conservative inference, these approaches are limited in their capacity to address the complex, directional effects of RNs [see fig 2] .

Adolph, S. C., and J. S. Hardin. 2007. "Estimating Phenotypic Correlations: Correcting for Bias Due to Intraindividual Variability." *Functional Ecology* 21: 178–84.

Amrhein, V., D. Trafimow, and S. Greenland. 2019. "Inferential Statistics as Descriptive Statistics: There Is No Replication Crisis If We Don't Expect Replication." *The American Statistician* 73: 262–70.

Araya-Ajoy, Y. G., and N. J. Dingemanse. 2014. "Characterizing Behavioural 'Characters': An Evolutionary Framework." *Proceedings of the Royal Society B* 281: 20132645.

Araya-Ajoy, Y. G., D. F. Westneat, and J. Wright. 2020. "Pathways to Social Evolution and Their Evolutionary Feedbacks." *Evolution* 74: 1894–1907.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2014. "Fitting Linear Mixed-Effects Models Using Lme4." *arXiv Preprint* 1406.5823.

Bell, A. M., S. J. Hankison, and K. L. Laskowski. 2009. "The Repeatability of Behaviour: A Meta-Analysis." *Animal Behaviour* 77: 771–83.

Bierbach, D., K. L. Laskowski, and M. Wolf. 2017. "Behavioural Individuality in Clonal Fish Arises Despite Near-Identical Rearing Conditions." *Nature Communications* 8: 1–7.

Biro, P. A., and B. Adriaenssens. 2013. "Predictability as a Personality Trait: Consistent Differences in Intraindividual Behavioral Variation." *The American Naturalist* 182: 621–29.

Blows, M. W. 2003. "Measuring Nonlinear Selection." *The American Naturalist* 2003: 815–20.

———. 2007. "A Tale of Two Matrices: Multivariate Approaches in Evolutionary Biology." *Journal of Evolutionary Biology* 20: 1–8.

Brehm, A. M., A. Mortelliti, G. A. Maynard, and J. Zydlewski. 2019. "Land-use Change and the Ecological Consequences of Personality in Small Mammal." *Ecology Letters* 22: 1387–95.

Briley, D. A., J. Livengood, J. Derringer, E. M. Tucker-Drob, R. C. Fraley, and B. W. Roberts. 2019. "Interpreting Behavior Genetic Models: Seven Developmental Processes to Understand." *Behavioral Genetics* 49: 196–210.

Brommer, J. E. 2013. "On Between-Individual and Residual (Co) Variances in the Study of Animal Personality: Are You Willing to Take the 'Individual Gambit'?" *Behavioral Ecology and Sociobiology* 67: 1027–32.

Carpenter, B., A. Gelman, M. D. Hoffman, D. Lee, B. Goodrich, M. Betancourt, and... A. Riddell. 2017. "Stan: A Probabilistic Programming Language." *Journal of Statistical Software* 74. `https://www.jstatsoft.org/article/view/v076i01`.

Cauchoix, M., P. K. Y. Chow, J. O. Van Horik, C. M. Atance, E. J. Barbeau,...G. Barragan-Jason, and L. Cauchard. 2018. "The Repeatability of Cognitive Performance: A Meta-Analysis." *Philosophical Transactions of the Royal Society B* 373: 20170281.

Cœur, C. C., M. Thibault, B. Pisanu, S. Thibault, J. L. Chapuis, and E. Baudry. 2015. "Temporally Fluctuating Selection on a Personality Trait in a Wild Rodent Population." *Behavioral Ecology* 26: 1285–91.

Dall, S. R. X., and S. C. Griffith. 2014. "An Empiricist Guide to Animal Personality Variation in Ecology and Evolution." *Frontiers in Ecology and Evolution* 14: 3.

Darwin, C. 1859. *On the Origin of Species by Means of Natural Selection.* London, UK: J. Murray.

Dingemanse, N. J., and N. A. Dochtermann. 2013. "Quantifying Individual Variation in Behaviour: Mixed-effect Modelling Approaches." *Journal of Animal Ecology* 82: 39–54.

Dingemanse, N. J., A. J. Kazem, D. Réale, and J. Wright. 2010. "Behavioural Reaction Norms: Animal Personality Meets Individual Plasticity." *Trends in Ecology and Evolution* 25: 81–89.

Dingemanse, N. J., M. Moiron, Y. G. Araya-Ajoy, A. Mouchet, and R. N. Abbey-Lee. 2020. "Individual Variation in Age-dependent Reproduction: Fast Explorers Live Fast but Senesce Young?" *Journal of Animal Ecology* 89: 601–13.

Dingemanse, N. J., and J. Wright. 2020. "Criteria for Acceptable Studies of Animal Personality and Behavioural Syndromes." *Ethology* 126: 865–69.

Eisenegger, C., J. Haushofer, and E. Fehr. 2011. "The Role of Testosterone in Social Interaction." *Trends in Cognitive Sciences* 15: 263–71.

Ellison, A. M. 2004. "Bayesian Inference in Ecology." *Ecology Letters* 7: 509–20.

Fanson, K. V., and P. A. Biro. 2015. "Meta-Analytic Insights into Factors Influencing the Repeatability of Hormone Levels in Agricultural, Ecological, and Medical Fields." *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 316: R101–9.

Gelman, A., and J. Carlin. 2017. "Some Natural Solutions to the p-Value Communication Problem—and Why They Won't Work." *Journal of the American Statistician* 112: 899–901.

Gelman, A., and F. Tuerlinckx. 2000. "Type s Error Rates for Classical and Bayesian Single and Multiple Comparison Procedures." *Computational Statistics* 15: 373–90.

Gelman, A., A. Vehtari, D. Simpson, C. C. Margossian, B. Carpenter, Y. Yao, and... M. Modrák. 2020. "Bayesian Workflow." *arXiv Preprint* arXiv:2011.01808. `https://arxiv.org/abs/2011.01808`.

Gomulkiewicz, R., J. G. Kingsolver, P. A. Carter, and N. Heckman. 2018. "Variation and Evolution of Function-Valued Traits." *Annual Review of Ecology, Evolution, and Systematics* 49: 139–64.

Greenland, S., S. J. Senn, K. J. Rothman, J. B. Carlin, C. Poole, S. N. Goodman, and D. G. Altman. 2016. "Statistical Tests, p Values, Confidence Intervals, and Power: A Guide to Misinterpretations." *European Journal of Epidemiology* 31: 337–50.

Gurven, M., C. von Rueden, J. Stieglitz, H. Kaplan, and D. E. Rodriguez. 2014. "The Evolutionary Fitness of Personality Traits in a Small-Scale Subsistence Society." *Evolution and Human Behavior* 35: 17–25.

Hadfield, J. D., and C. E. Thomson. 2017. "Interpreting Selection When Individuals Interact." *Methods in Ecology and Evolution* 8: 688–99.

Hadfield, J. D., A. J. Wilson, D. Garant, and B. C. Sheldon. 2010. "The Misuse of BLUP in Ecology and Evolution." *The American Naturalist* 175: 116–25.

Houslay, T. M., and A. J. Wilson. 2017. "Avoiding the Misuse of BLUP in Behavioural Ecology." *Behavioral Ecology* 28: 948–52.

Lande, R., and S. J. Arnold. 1983. "The Measurement of Selection on Correlated Characters." *Evolution* 37: 1210–26.

Lemoine, N. P. 2019. "Moving Beyond Noninformative Priors: Why and How to Choose Weakly Informative Priors in Bayesian Analyses." *Oikos* 128. `https://onlinelibrary.wiley.com/doi/full/10.1111/oik.05985`.

Martin, J. S., and A. V. Jaeggi. 2021. "Social Animal Models for Quantifying Plasticity, Assortment, and Selection on Interacting Phenotypes." *Journal of Evolutionary Biology* XX: XX–.

Martin, J. S., J. J. Massen, V. Šlipogor, T. Bugnyar, A. V. Jaeggi, and S. E. Koski. 2019. "The EGA+ GNM Framework: An Integrative Approach to Modelling Behavioural Syndromes." *Methods in Ecology and Evolution* 10: 245–57.

Mathuru, A. S., C. Kibat, W. F. Cheong, G. Shui, M. R. Wenk, R. W. Friedrich, and S. Jesuthasan. 2012. "Chondroitin Fragments Are Odorants That Trigger Fear Behavior in Ffsh." *Current Biology* 22: 538–54.

McElreath, R. 2020. *Statistical Rethinking: A Bayesian Course with Examples in r and Stan.* 2nd ed. CRC Press. `https://xcelab.net/rm/statistical-rethinking/`.

McNamara, J. M., and O. Leimar. 2020. *Game Theory in Biology.* Oxford, UK: Oxford University Press.

McShane, B. B., D. Gal, A. Gelman, C. Robert, and J. L. Tackett. 2019. "Abandon Statistical Significance." *The American Naturalist* 73: 235–45.

Meehl, P. E. 1978. "Theoretical Risks and Tabular Asterisks: Sir Karl, Sir Ronald, and the Slow Progress of Soft Psychology." *Journal of Consulting and Clinical Psychology* 46: 806–34.

Nakagawa, S., and H. Schielzeth. 2010. "Repeatability for Gaussian and Non-gaussian Data: A Practical Guide for Biologists." *Biological Reviews* 85: 935–56.

Niemelä, P. T., and N. J. Dingemase. 2018. "Meta-Analysis Reveals Weak Associations Between Intrinsic State and Personality." *Proceedings of the Royal Society B* 285: 20172823.

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: 831–44.

Okasha, S., and J. Otsuka. 2020. "The Price Equation and the Causal Analysis of Evolutionary Change." *Philosophical Transactions of the Royal Society B* 375: 20190365.

Pol, M. van de, and J. Wright. 2009. "A Simple Method for Distinguishing Within- Versus Between-Subject Effects Using Mixed Models." *Animal Behaviour* 77: 753–58.

Postma, E. 2006. "Implications of the Difference Between True and Predicted Breeding Values for the Study of Natural Selection and Micro-evolution." *Journal of Evolutionary Biology* 19: 309–20.

Queller, D. C. 2011. "Expanded Social Fitness and Hamilton's Rule for Kin, Kith, and Kind." *Proceedings of the National Academy of Sciences USA* 108: 10792–99.

R Core Team. 2020. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. `https://www.R-project.org`.

Royauté, R., M. A. Berdal, C. R. Garrison, and N. A. Dochtermann. 2018. "A Meta-Analysis of the Pace-of-Life Syndrome Hypothesis." *Behavioral Ecology and Sociobiology* 72: 1–10.

Royauté, R., A. Hedrick, and N. A. Dochtermann. 2020. "Behavioural Syndromes Shape Evolutionary Trajectories via Conserved Genetic Architecture." *Proceedings of the Royal Society B* 287: 20200183.

Scherer, U., M. Kuhnhardt, and W. Schuett. 2018. "Predictability Is Attractive: Female Preference for Behaviourally Consistent Males but No Preference for the Level of Male Aggression in a Bi-Parental Cichlid." *PloS One* 13: e0195766.

Schielzeth, H., N. J. Dingemanse, S. Nakagawa, D. F. Westneat, H. Allegue, C. Teplitsky, and Y. G. Araya-Ajoy. 2020. "Robustness of Linear Mixed Effects Models to Violations of Distributional Assumptions." *Methods in Ecology and Evolution* 11: 1141–52.

Sih, A., K. J. Mathot, M. Moirón, P. O. Montiglio, M. Wolf, and N. J. Dingemanse. 2015. "Animal Personality and State–Behaviour Feedbacks: A Review and Guide for Empiricists." *Trends in Ecology and Evolution* 30: 50–60.

Spearman, C. 1904. "The Proof and Measurement of Association Between Two Things." *The American Journal of Psychology* 15: 72–101.

Stamps, J. A. 2016. "Individual Differences in Behavioural Plasticities." *Biological Reviews* 91: 534–67.

Stinchcombe, J. R., A. F. Agrawal, P. A. Hohenlohe, S. J. Arnold, and M. W. Blows. 2008. "Estimating Nonlinear Selection Gradients Using Quadratic Regression Coefficients: Double or Nothing?" *Evolution* 68. `https://onlinelibrary.wiley.com/doi/full/10.1111/evo.12321`.

Stinchcombe, J. R., A. K. Simonsen, and M. W. Blows. 2014. "Estimating Uncertainty in Multivariate Responses to Selection." *Evolution* 68. `https://onlinelibrary.wiley.com/doi/full/10.1111/evo.12321`.

Tooby, J., and L. Cosmides. 1990. "On the Universality of Human Nature and the Uniqueness of the Individual: The Role of Genetics and Adaptation." *Journal of Personality* 58: 17–67.

Villemereuil, P. de, H. Schielzeth, S. Nakagawa, and M. Morrissey. 2016. "General Methods for Evolutionary Quantitative Genetic Inference from Generalized Mixed Models." *Genetics* 204: 1281–94.

Westneat, D. F., J. Wright, and N. J. Dingemanse. 2015. "The Biology Hidden Inside Residual Within-individual Phenotypic Variation." *Biological Reviews* 90: 729–43.

Wolf, M., and F. J. Weissing. 2010. "An Explanatory Framework for Adaptive Personality Differences." *Philosophical Transactions of the Royal Society B* 365: 3959–68.

———. 2012. "Animal Personalities: Consequences for Ecology and Evolution." *Trends in Ecology & Evolution* 27: 452–61.