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Review

Tansley insights

How to analyse plant phenotypic plasticity in response to a changing climate

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Summary

Plant biology is experiencing a renewed interest in the mechanistic underpinnings and evolution of phenotypic plasticity that calls for a re-evaluation of how we analyse phenotypic responses to a rapidly changing climate. We suggest that dissecting plant plasticity in response to increasing temperature needs an approach that can represent plasticity over multiple environments, and considers both population-level responses and the variation between genotypes in their response. Here, we outline how a random regression mixed model framework can be applied to plastic traits that show linear or nonlinear responses to temperature. Random regressions provide a powerful and efficient means of characterising plasticity and its variation. Although they have been used widely in other fields, they have only recently been implemented in plant evolutionary ecology. We outline their structure and provide an example tutorial of their implementation.

I. Introduction

In a rapidly changing climate, plants face challenges from the environment distinct to those experienced by their ancestors in the recent evolutionary past. The capacity of a single genotype to generate alternative phenotypes based on shifts in environment – or phenotypic plasticity (Bradshaw, 1965, 2006) – is a potential mechanism by which plants can respond quickly to changes in their environment (Nicotra *et al.*, 2010). In the face of a warming world, research interest in phenotypic plasticity and its genetic basis has accelerated dramatically, as researchers endeavour to understand its potential role for population persistence and adaptation (Chevin & Hoffmann, 2017). However, a precursor to addressing such questions is the need to characterise the nature of the phenotypic responses to environmental variation, namely the shape of reaction norms.

Reaction norms provide information on the direction and magnitude of phenotypic change elicited in response to environmental variation (Box 1), and can be compared across genotypes, populations or species. Here we focus on plastic responses to temperature – one of the most important aspects of our changing climate for plants – but the arguments we present are equally applicable to other biotic and abiotic environmental drivers (e.g. herbivory, light, water, nutrients, CO_2 and predation). Reaction norms in plants are frequently analysed by comparing trait expression between two temperatures or by assuming a linear response to temperature. However, this assumption – whilst statistically convenient – may obscure key properties of the plants' responses to changing environments, and could ultimately impede a comprehensive biological understanding of plasticity (Chevin & Lande, 2011). Furthermore, reaction norms can have markedly dissimilar shapes for different traits across the same environmental

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Box 1 Glossary

The terms used to describe phenotypic plasticity are numerous and frequently confused or confusing. For clarity, we therefore set out here the definitions we are using. *Phenotype* refers to traits of an organism resulting from both genetic and environmental influences. We use the term *genotype* in a broad, population-genetic sense, to refer to the complete genome of a genetic unit (e.g. an *individual*, *clone*, *family* or *line*). *Phenotypic plasticity* is then the ability of a single genotype to express different phenotypes under different environmental conditions; note that this may include a maladaptive response to a change in environment (Nicotra *et al.*, 2010; Gianoli & Valladares, 2012). *Acclimation* is a facultative and reversible form of plasticity, which alters short-term physiological processes and phenotype in response to environmental variation (Beaman *et al.*, 2016; Sándor *et al.*, 2018). A *reaction norm* describes the shape or specific form of the phenotypic response to the environment of an individual or genotype (Scheiner, 1993; Via *et al.*, 1995). *Genotype-by-environment interactions* ($G \times E$) occur when genotypes differ in the slope of their reaction norms (Nussey *et al.*, 2007; Josephs, 2018). The term $G \times E$ is sometimes used synonymously with plasticity, where differences among genotypes rather than plasticity are of specific interest (e.g. crop models: Heslot *et al.*, 2014; plant breeding: Elias *et al.*, 2016). *Population-level* plasticity refers to the average across the population of *individual-level* reaction norms of individual or genotype.

range within a species (e.g. Vitasse *et al.*, 2010) and among different species with the same trait and environmental range (e.g. Schou *et al.*, 2017). Characterising the shape of reaction norms as nonlinear or continuous functions of the environment will thus be more realistic and informative than assuming simple linear reaction norms (Stinchcombe *et al.*, 2012). However, doing so introduces complexity that can be challenging to quantify (Valladares *et al.*, 2006).

Whether reaction norms are simple or more complex, analyses of plasticity in response to a changing environment can address two key questions: (1) What is the average response across all individuals (genotypes, lines, clones or families could equally be interchanged with individuals) in a population? (2) How much do different individuals within a population vary in their plasticity? Question (1) considers a single 'population-level' reaction norm for the average response of individuals to a range of conditions (e.g. a temperature gradient), whereas question (2) considers variation in individual reaction norm shapes driven by among-individual differences within a population.

We outline here how a random regression mixed model (RRMM) approach offers a statistically efficient and appropriate way to describe both the population-level response and the variation among individuals in that response, using nonlinear functions if required (Nussey *et al.*, 2007; Morrissey & Liefting, 2016). RRMMs have been widely used in the animal breeding (Schaeffer, 2004) and animal evolutionary ecology literature (Nussey *et al.*, 2007; Stinchcombe *et al.*, 2012), and, very recently, to represent changes with age in tree or plant breeding studies (e.g. Pujol *et al.*, 2014; Sun *et al.*, 2017; Campbell *et al.*, 2018; Marchal *et al.*, 2019). However, despite previous recommendations (Pujol & Galaud, 2013), to our knowledge, RRMMs have not yet been widely implemented in many disciplines of plant biology to analyse plasticity. Our aim here is to provide a brief overview of the random regression approach and some tools to apply it, in the hope of encouraging use of the technique.

II. The many shapes of phenotypic plasticity

Plant phenotypic traits exhibit a variety of plastic response shapes to changes in temperature. Reaction norms of most phenotypic traits, if sampled across a wide enough range of temperatures, are typically curved (nonlinear). For example, a shallow parabolic curve typically describes the optimal temperature for carbon gain, which is highest at a range of intermediate growth temperatures, declining either side of this range (Fig. 1a; e.g. Gunderson *et al.*, 2010). Traits such as seed germination rate might exhibit a more peaked but still nonlinear response to temperature (Fig. 1b), where germination is optimal within an intermediate temperature

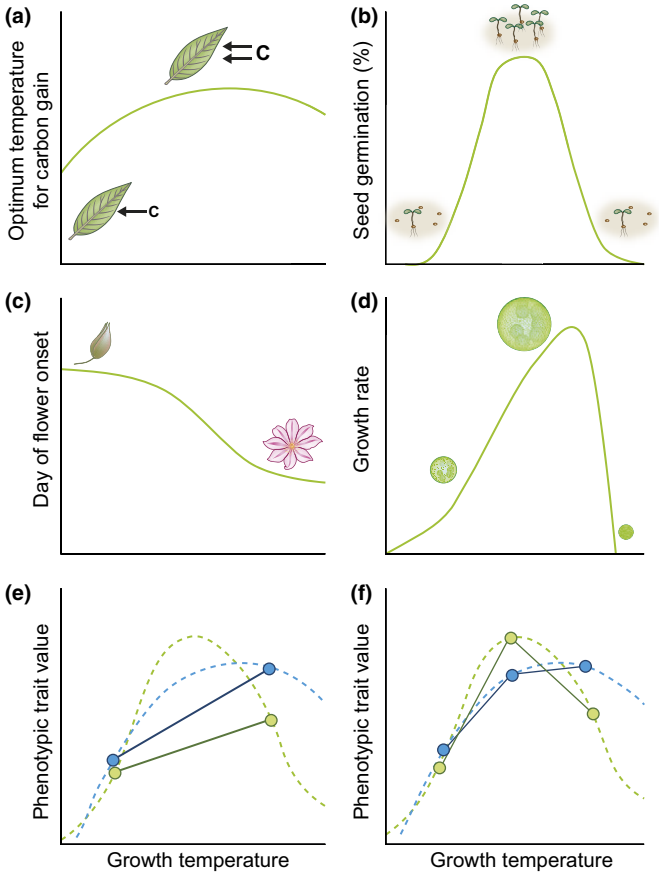

Fig. 1 Typical nonlinear reaction norm examples demonstrating the variety of shapes of plasticity in response to growth temperature: (a) shallow parabolic reaction norm shape of optimal temperature for carbon gain; (b) peaked response of seed germination percentage; (c) sigmoidal response of day of flowering onset; and (d) threshold response of growth rate of an algal colony in response to temperature. (e) Describing reaction norms with only two points (solid lines and points) may miss fundamental and biologically meaningful aspects of the underlying reaction norm shape (dashed line). (f) Adding just one more point (a third environmental level) captures far more of the underlying reaction norm shape.

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range and decreases sharply either side of this optimum (e.g. Cochrane *et al.*, 2014). Although often modelled linearly, phenological measures such as date of flowering onset may sometimes be better described with a sigmoidal response to temperature (independent of photoperiod) that incorporates temperature extremes (Fig. 1c), and which would otherwise be missed with a narrower temperature range (Jochner *et al.*, 2016). Finally, although not a plant example, growth rates of phytoplankton show a strong threshold relationship with temperature (Fig. 1d), where growth rate drastically declines beyond a critical temperature (Hinners *et al.*, 2017).

Characterising plasticity from only a small subset of discrete environments may obscure aspects of plasticity that would only be apparent from consideration of a wider range of environmental conditions (Schlichting, 2008). Standard experiments typically assess plastic responses by comparing phenotypes in a single 'low' vs 'high' temperature, often focusing on historically realistic values in the current environment (e.g. Frei *et al.*, 2014; Nicotra *et al.*, 2015). However, when reaction norms are nonlinear across a wider range of biologically relevant temperatures, choosing two arbitrary points may result in a highly misleading representation of plasticity. For example, if the underlying reaction norm is nonlinear, then fitting a linear reaction norm between two points will severely underestimate or overestimate the phenotypic response at intermediate or extreme temperatures (Fig. 1e). A solution to this oversimplified reaction norm problem is to map the phenotypic trait response across multiple temperatures (at least three and ideally as many as can be managed logistically) to characterise the whole reaction norm shape (Fig. 1f). This reaction norm approach can also be applied to 'natural' sources of temperature variation such as years of a study or elevation of sites, for studies of wild populations, by assigning an arbitrary index value (e.g. 1–2–3) to the environmental treatment levels.

With an appropriate statistical framework, both population- and individual-level plasticity can then be assessed. Population-level plasticity in response to a changing climate has broad implications for crop production, tree breeding, evolutionary ecology and ecophysiology. Coupled with some measure of fitness, assessing variation in plasticity between individuals or genotypes within a population potentially allows us to investigate whether differences in plasticity are associated with differences in fitness, and hence whether plasticity is under selection. Furthermore, we can ask whether that variation in plasticity has a heritable genetic basis (additive genetic variance underlying genotype-by-environment interactions: G × E), and so could potentially evolve or respond to artificial selection. RRMMs provide an efficient means of characterising population-level and individual variation in both linear or nonlinear reaction norms within a population (Morrissey & Liefting, 2016). From the output of these models, measures of plastic responses can then be extracted to compare across individuals, genotypes, populations or even species.

III. Random regression mixed model framework

Myriad approaches to quantifying plasticity are available in the literature. These include derivation of quantitative indices or metrics of plasticity (Valladares *et al.*, 2006; Sadras *et al.*, 2009; Murren *et al.*, 2014) and decomposition of reaction norm shape changes (Izem & Kingsolver, 2005). However, many approaches share a commonality of assessing variation in reaction norms over multiple steps, by first extracting individual- or genotype-level indices and then analysing these. This 'statistics-on-statistics' may have pitfalls of interpretation (Morrissey & Liefting, 2016). In contrast, random regression (or random slopes) mixed model (RRMMs) analyses fit individual-level reaction norms and thus assess their variation in a single step. Typical implementations of RRMMs also have the flexibility to model virtually any shape of linear or nonlinear response to a changing environment.

RRMMs, like other forms of mixed models, are composed of two predictor components: 'fixed' effects (the variable(s) of interest because of their direct effects on the response variable) and 'random' effects (the variable(s) that contribute variance among values of the response; Zuur *et al.*, 2009). These models are incredibly versatile and vary in complexity, and hence we outline a series of increasingly complex scenarios here, with model formulae described in Box 2 and Supporting Information Notes S1, and a worked example with data and R code in Notes S2–S4. Consider the following example: we are interested in determining the effect of temperature on seed germination, and as such, we measure reaction norms of a set of genotypes (e.g. full-sibling families). The primary objective is to determine the overall population-level average effect of temperature on seed germination, and hence temperature is a fixed effect function (which may be nonlinear). However, we might also be interested in quantifying how much variation there is among genotypes around the average population-level function, and hence genotype is fitted as a random effect (random intercepts), so that the model quantifies the overall variance among genotypes in their mean germination rates. To quantify variation around the average responses in the shape of reaction norms of the individual genotypes, a random regression term (or terms) can then be added to the model, to represent the reaction norm for each genotype, such that variance in the terms describing these individual reaction norms can then be estimated. We give a brief outline of the respective statistical models in Box 2.

To illustrate the RRMM, we present four hypothetical population-level (fixed) reaction norm shapes of phenotypic trait values in response to changing temperature, along with different random effects (Fig. 2). The functions describing the four population-level (i.e. average) reaction norm shapes are: linear (Fig. 2a–c), quadratic polynomial (Fig. 2d–f), sigmoidal (Fig. 2g–i) and a cubic spline estimated from a generalised additive model (GAM; Fig. 2j–l). The GAM is a highly flexible model for complex reaction norm shapes (e.g. Hinners *et al.*, 2017). Each population-level reaction norm shape is modelled along with three different scenarios of variation in the individual reaction norms, which are represented by different functions of each of the random effects separately (the three rows of Fig. 2). In the final column, we have visually isolated each random effect by presenting the relative difference between each individual reaction norm and the population-level average response (Fig. 2m–o).

The simplest mixed model is a linear fixed effects and random intercepts-only model (Fig. 2a; Box 2, Eqn 1). The phenotypic trait

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Box 2 Random regression mixed models

We outline here three mixed models to complement Fig. 2. A basic mixed model is:

$$y_{ij} = \alpha + \beta x_j + a_i + e_{ij} \quad \text{Eqn 1}$$

where y_{ij} is the phenotypic trait value of individual i on occasion j; α and β are fixed effects of the overall intercept (the population mean phenotype if x is mean-centred) and slope regression coefficients, respectively; x_j is the environment (e.g. temperature) experienced on occasion j (mean-centred for convenience); and the random effects in the model are a_i, the random intercept coefficient for individual i (representing the difference in means between individuals), and e_{ij}, the residual for individual i on occasion j. Mean-centring of the environmental variable x means that intercepts α and a_i reflect average values, for the population and individual respectively.

A 'random regression mixed model' (RRMM) includes an additional component b_i for the random slope of individual i in response to environment x:

$$y_{ij} = \alpha + \beta x_j + a_i + b_i x_j + e_{ij} \quad \text{Eqn 2}$$

The fixed components of the model, the intercept (α) and slope (β), are as above and describe the population mean reaction norm. The right-hand side of the equation contains the random regression component, and fits individual-level intercepts (a) and slopes (b). It is important to note that these are relative to the population-level fixed effects. Hence, if individuals vary in their average trait value but not in their reaction norm shapes, a model of the form of Eqn 1 would suffice.

RRMMs can incorporate nonlinear functions in either or both the fixed and the random effects, for example:

$$y_{ij} = \alpha + \beta x_j + \gamma x_j^2 + a_i + b_i x_j + c_i x_j^2 + e_{ij} \quad \text{Eqn 3}$$

where the fixed effect component γx_j^2 describes a quadratic curve for the population-level response and the additional random effect component $c_i x_j^2$ reflects the difference between individuals in the nonlinear component of their response to the environment, relative to the population mean. However, a quadratic random coefficient can make covariance matrices difficult to interpret, and it is worth noting that variation among individual reaction norms relative to the population mean might be adequately described by a random regression in which the random component is linear, even when the overall population response is nonlinear (Morrissey & Liefting, 2016). The choice of which form of model to use can be guided by Akaike's information criterion (AIC) or likelihood-ratio tests of the improvement (or not) on adding further complexity. We direct the reader to Supporting Information Notes S1 for further information on software resources available, and a few cautionary points on fitting the RRMMs we have outlined, and Notes S2 for a worked example of fitting and comparing RRMMS. In addition, Zuur *et al.* (2009) is an excellent resource for further reading on mixed models.

is modelled as a population-level (fixed component) linear function of temperature, where genotypes differ only in their mean (intercept) deviation from the population mean, but not in the slope of their reaction norms (Fig. 2m). However, when the slopes (as well as intercepts) of the individual genotype reaction norms vary, fitting a linear random regression term (Fig. 2b,n; Box 2, Eqn 2) will give a better representation of plastic responses. When individual reaction norms also vary in their nonlinear component (e.g. curvature), random regressions can also be further extended to incorporate nonlinear deviations from the population average (Fig. 2c,o; Box 2, Eqn 3).

The principle of increasing complexity of the fixed effect (population-level) component of a mixed model can then be extended to other basis functions (Fig. 2d–l; Notes S1). For example, higher-degree polynomial and sigmoidal functions might be appropriate for estimating the population-level average response to temperature (see Section II; Fig. 1). Furthermore, as reaction norm complexity increases, a basis function that allows for more variation in the reaction norm shape, such as a GAM, may be worth investigating (Fig. 2j; Notes S1).

Increasing complexity can also be added to the random effect component of the model. However, importantly, irrespective of the complexity of the fixed (population-level) component of the model, it may be sufficient to model variation among genotypes with just a linear term if the random slope component (relative to the mean) does not meaningfully deviate from linearity (e.g. Fig. 2n). For example, phenotypic trait values might exhibit greater variation among genotypes as temperature increases toward extremes (e.g. Kronholm *et al.*, 2016), such that genotypes expressing similar trait values at lower temperatures express much greater variation in trait values at higher temperatures (Fig. 2b,e,h, k). This is a valuable property of RRMMs: a complex nonlinear function can be used to describe the fixed component of the model, whilst a simpler (e.g. linear) function may be sufficient for describing variation in the random regression component (Morrissey & Liefting, 2016). In all cases, models of different levels of complexity in both the fixed and the random effect functions can be evaluated statistically (Box 2).

Analysing plasticity data in a single mixed model analysis avoids the drawbacks of multistep approaches, whilst being flexible for unbalanced designs and different reaction norm functions, and having vast potential for extension. For example, RRMMs can incorporate additional experimental covariates as either fixed (e.g. number of leaves) or random (e.g. replicate observations) effects. Models will typically return estimates of the covariance between genotype-specific intercepts and slopes, which will then give an indication of whether genotypes with higher trait values are typically those associated with more (or less) plasticity (Nussey *et al.*, 2007). If the trait in question is a component of fitness that may vary across the environment, this covariance can even be interpreted as a test of costs or benefits of plasticity in the fitness component. A useful extension, which allows an analysis of selection on plasticity, is to use a bivariate model combining a random regression of the focal trait with a basic model of measures of individual fitness: here, the covariance between trait slopes and individual fitness represents selection on plasticity (Arnold *et al.*, in

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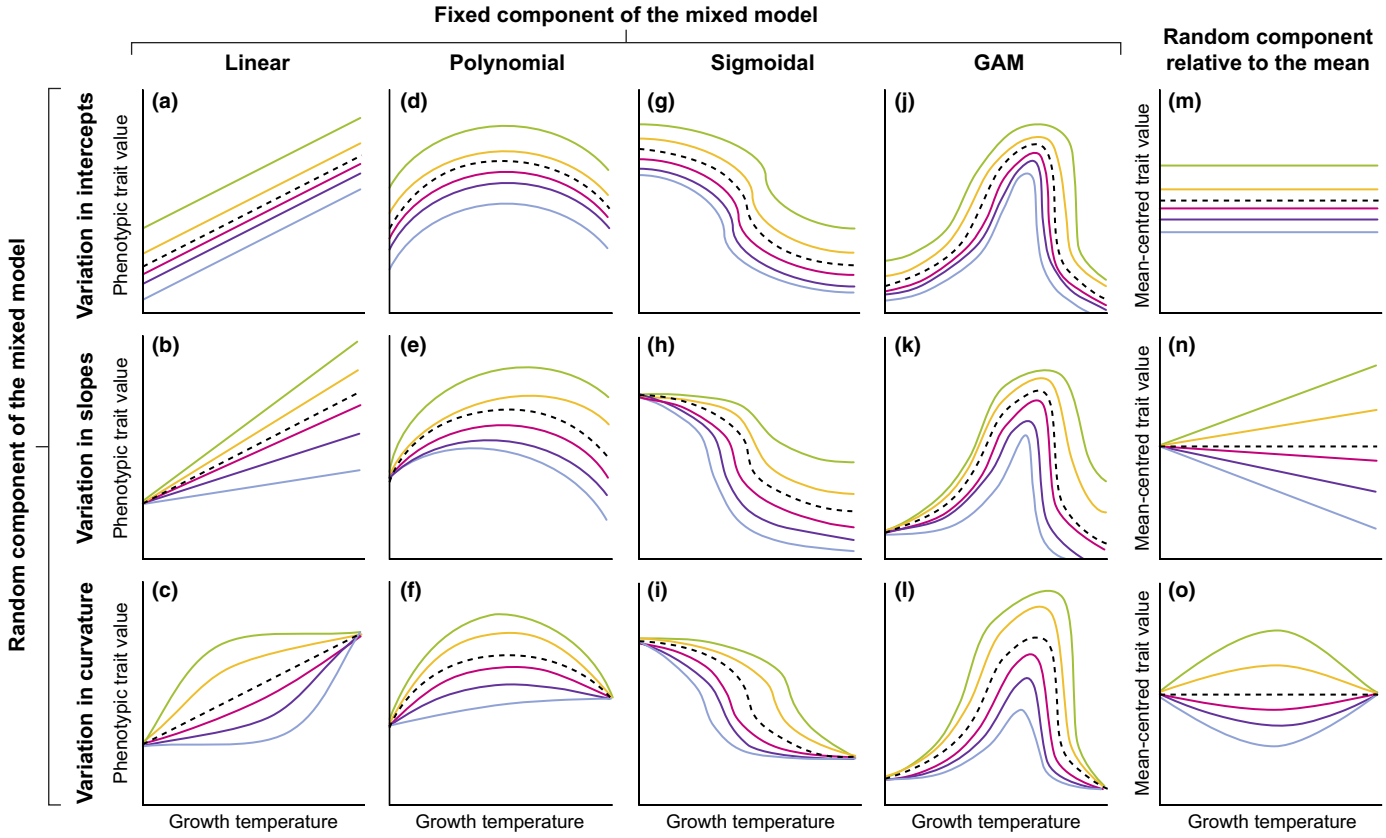

Fig. 2 Graphical representation of both the overall population average and individual reaction norms of a phenotypic trait value in response to changing temperature when analysed with mixed models of varying complexity. The core mixed model contains a fixed component that describes the population average reaction norm (black dashed line) and a random component that describes variation in the individual reaction norms (coloured solid lines). Each of the first four columns represents an increasingly complex fixed component of the mixed model: (a–c) linear, (d–f) quadratic polynomial, (g–i) sigmoidal and (j–l) generalised additive model (GAM) that describe the population average reaction norm. The final column (m–o) represents the individual reaction norm slopes (the random component) relative to the mean-centred phenotypic trait value of the population in response to temperature. Each of the three rows represents increasingly complex scenarios of variance in the random component of the mixed model, independently: variation in intercepts (a, d, g, j), variation in slopes (b, e, h, k), and variation in curvature (a quadratic term; c, f, i, l) among the individual reaction norms. See Box 2 for model formulae, the main text for additional descriptions and Supporting Information Notes S1–S4 for basis functions, software and a worked example with R code for implementing these models.

press; for empirical examples see Hayward *et al.*, 2014; Boulton *et al.*, 2018). Furthermore, where plasticity in multiple traits has been measured, a multivariate mixed model can also be used to analyse plastic responses in several traits, and will return estimates of covariances between the different traits in both intercepts and slopes (e.g. Husby *et al.*, 2010).

Plasticity may be compared amongst different populations of the same species (e.g. Husby *et al.*, 2010) or amongst different species (e.g. Gardner *et al.*, 2016). With data on related individuals (e.g. families or lines), relatedness information can be incorporated in a mixed model, for example including pedigree information in an 'animal model' (which, despite the name, is equally applicable to plants), to estimate levels of additive genetic variance in the relevant traits (Kruuk, 2004; Wilson *et al.*, 2010). Extension of the animal model to include an interaction between the additive genetic effect and the environmental variable, a random regression animal model, then allows a test for G × E interactions (Nussey *et al.*, 2007). With multispecies analyses, phylogenetic information can be included (Hadfield & Nakagawa, 2010), and models can then be extended to test for a phylogenetic signal in plasticity. This approach is exactly analogous to estimation of G × E within a population, but with species' phylogeny replacing the population pedigree (e.g. Gardner *et al.*, 2016).

In addition to investigating whether there is overall variation in reaction norms, researchers may also want to identify which genotypes show the most or least plastic response. From the RRMM output, the random regression slope (or higher order terms if nonlinear random functions are being considered) coefficient for each genotype can be extracted: these are known as best linear unbiased predictors, or BLUPs, and can be used to rank which genotypes are more or less plastic. Such a ranking approach might be desired for breeding crops that are tolerant to projected changes in climate ('climate-ready crops'), for designing restoration and revegetation strategies, or might feed into investigations of the evolution and adaptive nature of plasticity in wild species. However, use of BLUPs for anything beyond ranking should be done in conjunction with appropriate treatment of their associated uncertainty, for example within a Bayesian Markov chain Monte Carlo framework (see Hadfield *et al.*, 2010; Houslay & Wilson, 2017). Finally, there are cautionary points to consider when using

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an RRMM approach, including whether statistical power is sufficient to estimate variance among individual reaction norms confidently, and whether the mathematical functions necessarily match biological function. We expand on these points in Notes S1.

IV. Conclusions

To date, plant biologists have seldom used RRMMs for measuring variation in reaction norms in studies of phenotypic plasticity. We suggest here that a RRMM approach offers an efficient, powerful, statistically appropriate and broadly applicable way both of analysing nonlinear population-level plasticity and of characterising variation in reaction norm shapes. It also has huge potential for extension, for example to include multivariate responses, multiple environmental variables, and quantitative genetic or comparative analyses. There are prospective benefits not only for our fundamental understanding of the genetic basis and evolution of plant phenotypic plasticity in a changing climate, but also application to breeding climate-ready crops and management of natural systems.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Notes S1 Common basis functions for nonlinear reaction norms and software resources for fitting random regression mixed models.

Notes S2 Worked example tutorial to apply increasingly complex models and model selection procedures to an example plant phenotypic plasticity dataset in R.

Notes S3 Dataset of flowering phenology plasticity used in the worked example tutorial.

Notes S4 R code for the worked example tutorial.

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