Received: 1 October 2024 | Accepted: 14 January 2025

DOI: 10.1111/1365-2435.70004

Functional Ecology

PERSPECTIVE

Causes and Consequences of Phenotypic Plasticity

Quantifying phenotypic plasticity: A call for consistency

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Funding information
Spanish Ministry of Science and Innovation, Grant/Award Number: PID2021-126456NB

Handling Editor: Isabel Smallegange

Abstract

1. The interest of evolutionary, functional and applied ecologists in the study of phenotypic plasticity has grown considerably in recent decades. From being considered irrelevant in the mid-20th century, phenotypic plasticity is now considered ubiquitous and essential for organisms to adapt to changing environments and to meet the challenges posed by anthropogenic global change.
2. Consequently, an increasing number of studies are investigating phenotypic plasticity in many systems and ecological scenarios. This has led to the development of multiple and disparate methodological approaches.
3. In this article, we propose a methodological framework that considers phenotypic plasticity as a trait property detected by constructing genotype-based reaction norms that can be modelled using mixed-effect models.
4. We do not claim that this methodology is the only existing alternative for studying plasticity, but we believe it is a standard and consistent approach that allows for a rigorous assessment of the magnitude and between-genotype variation in plasticity.

KEYWORDS
G×E interaction, mixed models, phenotypic plasticity, polyphenic traits, reaction norms, within-individual plasticity, within-module plasticity

1 | INTRODUCTION

Phenotypic plasticity permeates life on Earth. There is virtually no species, from short-lived unicellular microorganisms to massive multicellular species hundreds of years old, which is not capable of expressing plasticity in some of its traits in response to environmental changes of various kinds. Evolutionary biologists have long recognised this and investigated plasticity for over a century. It is not surprising, therefore, that in one of the foundational textbooks of the Modern Synthesis, Dobshansky (1937) stated that "what is inherited in a living being is not this or that morphological character, but a definite norm of reaction to environmental stimuli".

This universal feature of organisms can become a curse for researchers, as it makes it extremely difficult to achieve conceptual and methodological unification. This discrepancy started very early on, with the very definition of phenotypic plasticity (Sultan, 2021). Although in basic terms there is universal agreement that phenotypic plasticity is the ability of a given genotype to express different phenotypes in different environments, the emphasis given to different aspects of this process varies. While some approaches to the study of plasticity focus on the active and, many times, adaptive response of organisms to environmental changes, others emphasise the role of the environment in passively inducing phenotypic, sometimes maladaptive changes (Schilichting & Pigliucci, 1998; West-Eberhard, 2003).

Furthermore, although it is clear from the definition that plasticity is a property of individual traits or characters (Bradshaw, 1965; Via, 1987), it is often considered an attribute of an organism or even a population or species. This misconception makes it very difficult to quantify plasticity when, for example, some traits may have plastic responses to environmental changes, while other traits may have canalised responses. Similarly, the definition of plasticity tells us that this property is closely associated with specific changes in the environment. Thus, for a given trait, different environmental variables may trigger a plastic response while other environmental variables have no effect on the expression of the trait. The situation becomes more complex when we notice that for a given trait and a given environmental variable, the same genotype may have a plastic response to some values of the environmental variable but a canalised response to other values (Sultan, 2021). Genotypes cannot then be characterised as fully plastic or canalised (Sultan, 2021). All these considerations imply that the expression of plasticity is specific to genotype, trait, type of environmental variable and the range of values of that environmental variable.

An important consequence of this disparity of approaches to defining and studying phenotypic plasticity is the existence of an equally diverse collection of methods for determining the occurrence and quantifying the magnitude of phenotypic plasticity. Here, we describe the main ways plasticity has been empirically quantified. We are aware that our study is not exhaustive because our aim is not to provide a complete review of the ways in which plasticity has been quantified. Our proposal is intentionally based on the idea that phenotypic plasticity is a property of phenotypic traits that is detected by constructing genotype-based reaction norms (Schilichting & Pigliucci, 1998). Thus, the methods we propose are conceptually and analytically connected and form a coherent corpus firmly based on mixed models (Pinheiro & Bates, 2000), a family of statistical models particularly suited to study reaction norms. These models are versatile and robust and, in recent years, have demonstrated their ability to detect subtle aspects of how phenotypic plasticity is expressed in complex contexts. We start by describing the simplest, but no less important, methodological aspects associated with the quantification of plasticity and continue adding layers of complexity that allow us to study this phenomenon in more realistic situations.

2 | FUNDAMENTAL CONSIDERATIONS WHEN STUDYING PLASTICITY

2.1 | Genotype-implicit versus genotype-explicit estimates of plasticity

A first issue to consider when studying plasticity relates to how to include genotypic values in the assessment of plasticity. This is important because when individuals from different environments are not from the same genotypes, no formal genetic estimates can be obtained (Via, 1987). That is, studying traits from individuals occupying two habitats or environments, or from individuals growing together from different habitats or sites can just tell us the average change in trait values (Tian et al., 2024), and only if there is no sampling error in obtaining the individuals to be subjected to the experimental environments (Figure 1a). However, it can tell us nothing about the between-genotype variation in plasticity and gives us no information about the G×E interaction (Sultan, 2021). These studies might be good starting points for future examination of plasticity, but researchers must refrain from interpreting their present results as evidence for plasticity. Even when carried out accurately, a design not including genetic information does not provide information on how phenotypic plasticity may evolve, whether it is adaptive, or how organisms express plastic responses to cope with environmental changes. Thus, experimental or observational studies exploring plasticity must take into account a good proxy of genotype (e.g. clones, recombinant inbred lines, lines, full- or half-siblings, relatives). This statement may seem naïve, considering that the accepted definition of plasticity states that plasticity only occurs when the same genotype expresses different phenotypes in different environments. Unfortunately, it is not uncommon to still find studies that assess plasticity without controlling for genotypes (see Appendix S1, where we show that only 48% of 194 studies published between 2022 and 2024 considered some proxy of the genotypic value to estimate phenotypic plasticity).

2.2 | Environment-implicit versus environment-explicit estimates of plasticity

Plasticity can be calculated by comparing the expression of the trait in different environmental qualitative categories (high or low nutrient level, presence or absence of predators, sun or shade, etc.). Because the exact values of the environments are not quantified, we will refer to the plasticity obtained through this first approach as *environment-implicit estimates of plasticity*. There are several types of environment-implicit estimates depending on whether what it is compared across environments is the difference in trait means, the ratio of trait means or the trait variation (Cheplick, 1995; Poorter & Nagel, 2000; Schlichting, 1986; Schlichting & Levin, 1984; Valladares et al., 2006).

A second approach focuses on considering any phenotypic trait as a reaction norm, a function relating the expression of a trait (z) with the variation in one or several environmental variables (x) (Schmalhausen, 1949; Woltereck, 1909),

$$z = f(x). \quad (1)$$

This alternative approach, in contrast, requires a quantitative estimation of the environmental variables putatively triggering plasticity. Consequently, we will call those estimates obtained through this approach *environment-explicit estimates*. Plasticity is calculated as the slope of this function between the expression of a trait and the change in an environmental variable (Arnold et al., 2019; Morrisey & Liefting, 2016). The simplest scenario is to compare the expression of a trait in two discrete environments. In this case, the average slope of

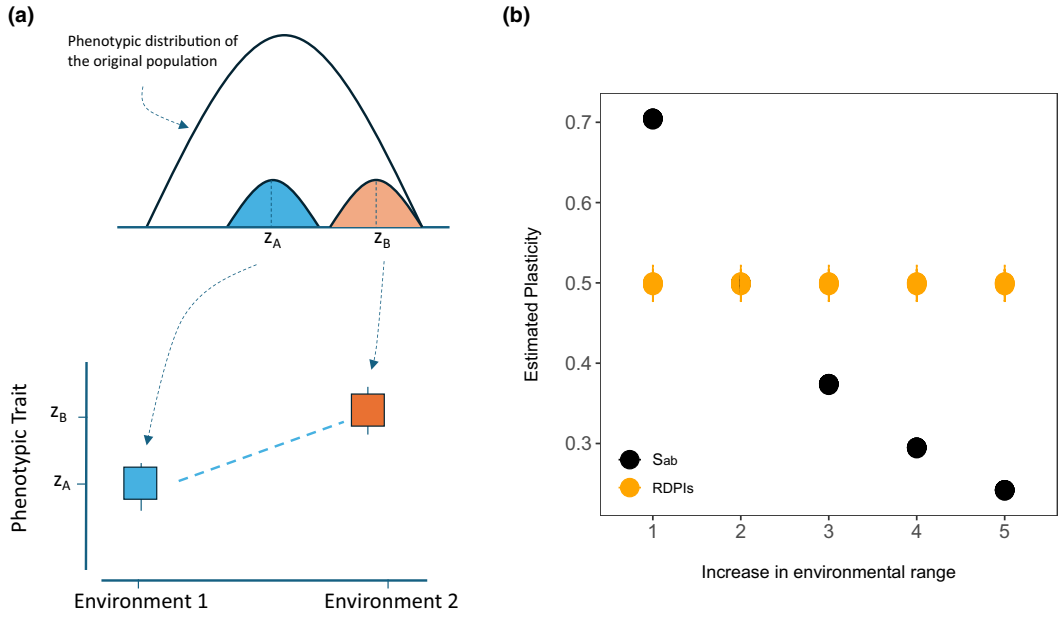

FIGURE 1 (a) A simple illustration showing a potential caveat arising when the experiments to estimate plasticity do not consider genotypes. If no control for genotype occurs and sampling is not large enough, we could randomly assign individuals/genotypes with different phenotypic values to different environments, wrongly concluding the occurrence of plasticity. (b) A simple simulation study showing how the intensity of phenotypic plasticity between two environments changes as a function of the differences between these environments in the value of the environmental variable if plasticity is calculated as an explicit estimate of the environment (the slope of the reaction norm; S_{ab}), but remains constant when calculated as an implicit estimate in the environment (the Relative Distance Plasticity Index; RDPIs $= |(z_i - z_j)|/(z_i + z_j)$, where z_i and z_j are the value of the phenotypic trait z in environments x_i and x_j, respectively; Valladares et al., 2006). Simulations were done by performing 50 runs for each of eight scenarios, varying in number of genotypes per population (10, 20, 40, and 80), individuals per genotype (15 or 30) and assuming that trait values change twice between environments.

the reaction norm is the most commonly used environment-explicit plasticity estimate. It is calculated as the difference in mean phenotype between two environments divided by the difference between the two environments themselves (Morrisey & Liefting, 2016),

$$S_{ab} = \frac{z_a - z_b}{x_a - x_b}. \quad (2)$$

An important property of S_{ab} is that it is an unbiased estimator of the average slope of a reaction norm between points x_a and x_b, weighting all values in the environment equally.

By omitting any quantitative variation in environmental variables, environment-implicit estimates yield the same values regardless of the severity of environmental change (Figure 1b).

A simple simulation study indicates that the bias of environment-explicit estimates, calculated as the deviation from expected plasticity (Araya-Ajoy et al., 2015), tends to be smaller than the bias of environment-implicit indices to changes in the number of genotypes, replicates per genotype and plasticity intensity (see Appendix S2 details). For all these reasons, one might think that environment-explicit estimates are the approach used today to characterise plasticity. However, it is still not uncommon to find studies that assess plasticity without quantifying the environment (only 65% of the 194 studies included in our review did some quantification of the environment, and only 17% of them used this quantitative information to estimate reaction norms; Appendix S1).

It is worth mentioning that the classification based on implicit and explicit considerations of the environment is not equivalent to the classical debate on the two approaches to modelling plasticity, the character state approach versus the polynomial approach (De Jong, 1995; Van Tienderen, 1991; Van Tienderen & Koelewijn, 1994; Via et al., 1995; Via & Lande, 1985). Thus, while the debate arose mainly from the idea of considering the environment as discrete versus continuous, our classification is motivated by the convenience of quantifying the values of the environment, being this continuous or discrete. In fact, as noted from Equation (2), the environment-explicit estimate can be applied not only to continuous environments but also to discrete environments (De Jong, 1995; Gavrilets & Scheiner, 1993a; Van Tienderen, 1991).

2.3 | Discrete versus continuous variation of the environment

Although many studies on plasticity have considered only two values of a given environmental variable, there are many situations in which the environmental variables vary continuously, meaning that they can take on more than two values. There are several important consequences of considering more than two environments. First, experiments designed to estimate plasticity will require more than two environmental treatments, making them more complex and requiring larger sample sizes

(Scheiner & Gurevitch, 2001). Second, plasticity will be more difficult to assess using environment-implicit estimates of plasticity because there are several potential points to compare phenotypic values, and it is not always possible to decide which ones to use (De Jong, 1995). In continuous environments, environment-explicit reaction norms describe plasticity much more accurately than environment-implicit plasticity indices (Gomulkiewicz & Stinchcombe, 2022; Kingsolver et al., 2015; Stinchcombe et al., 2012). Third, the relationship between phenotypic traits and environmental variables will often be nonlinear when there are more than two environments (Gomulkiewicz & Stinchcombe, 2022).

In this situation, standard multivariate models can be used to model reaction norms (Gomulkiewicz et al., 2018). Although other statistical models could be used, polynomial regressions are good statistical tools to characterise these linear and nonlinear reaction norms (Morrisey & Liefting, 2016; Rocha & Klaczko, 2014). First- (linear), second- (quadratic) or higher-order polynomial regressions can be fitted to describe monotonic, optimal, or more complex reaction norms by expanding the function to *n*th order as

$$z_k = a + b_1 x_k + \varepsilon_k, \tag{3a}$$

$$z_k = a + b_1 x_k + b_2 x_k^2 + \varepsilon_k, \tag{3b}$$

$$z_k = a + b_1 x_k + b_2 x_k^2 + \cdots + b_n x_k^n + \varepsilon_k = a + \sum_{n=1}^{N} b_n x_k^n + \varepsilon_k, \tag{3c}$$

where z_k is the value of a given phenotypic trait in the environment k and x_k is the value of a environmental variable in that environment. Two considerations should be borne in mind. First, the residual error term ε_k is drawn from a normal distribution with mean zero and variance σ^2_k. Second, estimates of reaction norms are influenced by how the environmental variables are measured and scaled (Araya-Ajoy et al., 2015; Morrisey & Liefting, 2016). For this reason, when relating trait expression with an environmental variable to build a reaction norm, it is convenient to mean-centring the environmental covariate (Enders & Tofighi, 2007). By mean-centring the environmental variable, the regression analyses indicate how the environment influences not only the magnitude of the plasticity (the slope of the models) but also the mean-value of the trait (the intercept of the models) (Dingemanse & Dochtermann, 2013; Morrisey & Liefting, 2016; Nussey et al., 2007).

3 | QUANTIFYING GENOTYPIC DIFFERENCES IN PHENOTYPIC PLASTICITY

Different genotypes often respond to environmental variation in different ways, a phenomenon reflecting the amount of genetic variation existing for plasticity. This genotype-by-environment (G×E) interaction is vital because it is a necessary, albeit not sufficient, condition for plasticity to evolve (Gavrilets & Scheiner, 1993b; Via & Lande, 1985). Consequently, studies on the evolutionary ecology of plasticity must consider the existence of genotypic differences in reaction norms.

An appropriate experimental design to accurately evaluate between-genotype differences in reaction norms requires replication within genotype and environment. That is, here, the experimental units are not the genotypes within environment but the individuals within genotypes×environment. This is possible when plasticity is studied experimentally in species where replicates of each genotype can be obtained. Although this may seem difficult, many species meet these conditions, including those reproducing asexually by budding, vegetative propagation, asexual spore formation, fragmentation or agamogenesis (parthenogenesis and apomixis), as well as those species reproducing sexually by autogamy. Likewise, genotype replicates can be also obtained by using the widely known breeding designs employed since long time ago in quantitative genetics to obtain isogenic lines. When obtaining replicates per genotype turns out difficult or impossible due to the characteristic of the studied species or the study conditions, a possibility is to assess between-genotype differences in reaction norms using a pedigree-derived matrix of relatedness across individuals (Kruuk, 2004; Lynch & Walsh, 1998).

The polynomial regressions described in Equation (3) cannot model G×E interactions. Hence, statistical methods have been proposed to estimate the magnitude of this interaction. One analytical tool that is widely used because it avoids many of the problems of other multistep inference models is the random slope regression mixed model (RRMM), a type of hierarchical model in which data are structured in groups and (regression) coefficients can vary by group. RRMM fits genotype-level reaction norms and thus assesses their variation in a single step (Arnold et al., 2019; Morrisey & Liefting, 2016). The model, in its most basic form, is

$$z_{ik} = a + \sum_{n=1}^{N} b_n x_k^n + \alpha_i + \sum_{n=1}^{N} \beta_{ni} x_k^n + \varepsilon_{ik}, \tag{4}$$

where z_{ik} is the trait value of genotype i in environment k, the fixed coefficient a is the overall intercept (that corresponds to the population mean phenotype if the environmental variable is mean-centred), and the fixed slope coefficients b_n are the overall slope regression coefficients from order 1 to N in response to changes in the environmental variable x. RRMMs include a random intercept coefficient for each genotype i (that corresponds to the mean phenotype of each genotype if the environmental variable is mean-centred), and the random coefficient β_{ni} (that corresponds to the slope regression coefficients from order 1 to N of each genotype i in response to changes in the environment). Finally, ε_{ik} is the residual variation of genotype i in each environment k, that like before is drawn from a normal distribution ~ $N(0, \sigma^2_{ik})$. Consequently, the magnitude and significance of the population-level plasticity can be found by estimating the coefficient of the fixed effect terms, whereas the random effect term indicates how much variation there is among genotypes around the population-level average (random intercepts). Finally, the RRMMs quantify the variation around the average responses in the slopes of the individual reaction norms using the random polynomial terms. Although we have described here a general polynomial function, first-order RRMMs ($N=1$) are the most widely used because increasing the order of the function does not improve the interpretation

of the individual reaction norms whereas it hinders the interpretation of the covariance matrices (Arnold et al., 2019; Morrisey & Liefting, 2016). Detailed technical information on how to construct and interpret RRMMs is provided in Morrisey and Liefting (2016) and Arnold et al. (2019).

4 | QUANTIFYING PHENOTYPIC PLASTICITY BELOW INDIVIDUAL LEVEL

4.1 | Within-individual phenotypic flexibility in unitary organisms

We have seen how plasticity can be measured when different individuals of the same genotype develop in different environments and express phenotypic traits in a permanent and non-reversible manner. However, many life-history, phenological, physiological and behavioural traits are labile because they are expressed multiple times during an individual's lifespan and its expression changes quickly depending on environmental variables (Brommer, 2013; Inouye et al., 2019). Thus, labile traits may alter their expression when a given individual faces different environments. This type of phenotypic plasticity is called phenotypic flexibility (Piersma & van Gils, 2011) or labile plasticity (Reid & Acker, 2022). In this scenario, phenotypic variance due to plasticity is partitioned into a between-genotype, between-individual component and a within-individual component. Within-individual variation is traditionally considered the lowest level at which phenotypic variance can be assessed (Lynch & Walsh, 1998). When studying intraindividual variation in traits, it is necessary to ensure that the changes are not merely a consequence of the ontogenetic process, in which case they should not be considered plastic changes. This is important because many ontogenetic changes can give rise to patterns of intraindividual phenotypic variation similar to those of plasticity (Diggle, 2002). For example, individual metameres vary in size, shape and other traits during ontogeny in almost all plant species, a phenomenon termed heteroblasty (Zotz et al., 2011).

The calculation of within-individual plasticity requires some methodological and analytical considerations. First, in contrast to those cases where plasticity emerges when different individuals of the same genotype face different environments (Figure 2a), phenotypic plasticity is expressed here at two levels, at the genotype level and at the individual level ('I × E' sensu Nussey et al., 2007) nested within genotypes

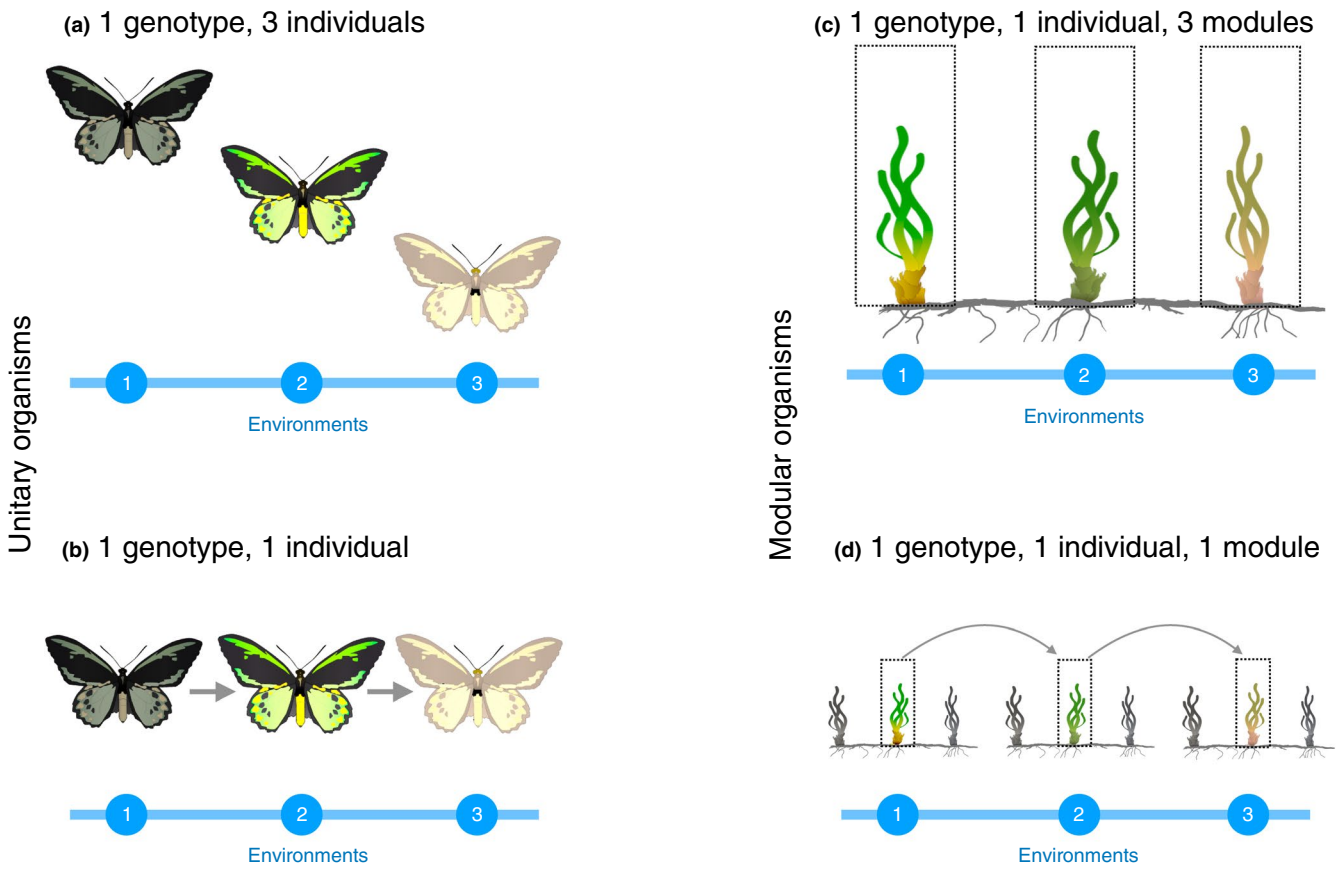

FIGURE 2 (a) Traditionally, plasticity is assessed using different individuals of the same genotype facing different environments. This type of plasticity is common for developmentally plastic traits. The finest level of analysis of plasticity here is the genotype. (b) Plasticity can be expressed when the same individuals face different environments and change the expression of a labile trait (within-individual plasticity). (c) In modular organisms, within-individual plasticity can emerge when different modules confront different environments. In this case, within-individual plasticity can occur not only for labile traits but also for developmentally plastic traits. (d) Plasticity can also occur in modular organisms when the same module faces different environments. Within-module plasticity will affect labile traits. Icons modified from vecta.io.

(Figure 2b) (Brommer, 2013). Therefore, to obtain reliable information on plasticity at the genotype and individual levels, it is necessary to know to which genotype each individual belongs. As seems in the previous section, this is possible when plasticity is studied experimentally in species where individual replicates can be obtained. It is also necessary to study plasticity in a trait expressed several times in the same individual. Environmentally sensitive sequential variations in trait expression are frequent in behavioural, phenological and physiological traits. Consequently, there is a long tradition of studies of within-individual plasticity in behavioural ecology (Dingemanse et al., 2010; Dingemanse & Dochtermann, 2013) and physiological ecology (Froy et al., 2019; Guindre-Parker, 2020; Guindre-Parker et al., 2019).

Likewise, models used to calculate within-individual plasticity should consider this hierarchical structure (Schielzeth & Forstmeier, 2009; Schielzeth & Nakagawa, 2013; Van de Pol & Wright, 2009). In addition, the sequential measurements taken to the same individual are not statistically independent (Diaz-Uriarte, 2002; Schielzeth & Forstmeier, 2009). Consequently, models to assess plasticity should also consider this serial correlation (Saarinen, 2004; Schielzeth & Forstmeier, 2009). In this respect, studies assessing within-individual plasticity are methodologically analogous to the longitudinal crossover studies developed in pharmaceutical and medical studies (Andersen & Millen, 2013; Detry & Ma, 2016; Putt & Chinchilli, 1999). The RRMM model in Equation (4) can be extended to include the within-individual (auto)correlation structure to calculate plasticity at the genotype and individual levels. One possibility is to use multilevel mixed effects models for repeated measures with autocorrelated errors (MMRM):

$$z_{ijk} = a + \sum_{n=1}^{N} b_n x_k^n + \alpha_i + \sum_{n=1}^{N} \beta_{ni} x_k^n + \alpha_{j(i)} + \sum_{n=1}^{N} \beta_{nj(i)} x_k^n + \varepsilon_{j(i)k}, \quad (5)$$

where z_{ijk} is the trait value of individual j of genotype i in environment k, a and b are the fixed coefficients indicating the overall intercept and environmentally dependent slope, α_i and β_i are the random coefficients describing the intercept and the change in slope with environment of each genotype i, and $\alpha_{j(i)}$ and $\beta_{j(i)}$ are the random coefficients describing the intercept and change in slope of each individual j nested within each genotype i. Within-subject autocorrelation can be controlled by modelling a correlation structure between residual errors $\varepsilon_{j(i)k}$ (Galecki & Burzykowski, 2013). A widely used error covariance structure is the first-order autoregressive structure, which assumes that the correlation between two within-individual measurements decreases as the difference in environment increases (Funatogawa & Funatogawa, 2019; Galecki & Burzykowski, 2013; Saarinen, 2004; Zuur et al., 2009). But researchers can use the ones that best fit their data.

Reliable information on the genetic relatedness of individuals may not be available when studying plasticity in nature, which greatly hampers the use of MMRM models. In those cases, the presence of I×E does not necessarily mean that plasticity also occurs at the genetic level. In fact, classical quantitative genetics assumes that the expression of phenotypic traits at the individual level is determined by the combination of additive genetic effects and permanent environmental effects that may encompass any non-additive genetic effects as well as maternal or individual-specific environmental effects (Lynch & Walsh, 1998; Meyer & Kirkpatrick, 2005). The effect of the genotype and the individual on trait variation can be elucidated in these circumstances by using a random regression animal model (RRAM):

$$z_{ik} = a + b_1 x_k + (\alpha_{bi} + \alpha_{ei}) + (\beta_{b1i} + \beta_{e1i}) x_k + \varepsilon_{ik}, \quad (6)$$

where z_{ik} is the trait value of individual i in environment k, α_{bi} and α_{ei} are the genetic and non-genetic components (the 'breeding value' and 'permanent environment effect' of classical quantitative genetic models, respectively) of the intercept of the reaction norm of each individual i, and the random coefficient β_{b1} and β_{be1} are genetic and non-genetic effects on the reaction norm slope of each individual i in response to changes in the environment. The genetic components of variance can be calculated in natural populations, when there is no experimental control of genetic relatedness, by using an 'animal model', a linear mixed model that incorporates a pedigree-derived matrix of relatedness across individuals (Kruuk, 2004; Lynch & Walsh, 1998). We refer the reader to Nussey et al. (2007) and Brommer (2013) for more details on how to proceed with these models.

4.2 | Within-individual developmental plasticity in modular organisms

The theory of phenotypic plasticity is largely based on unitary organisms, those organisms with a closed developmental programme that begins with a unicellular sexually produced zygote and produces a single functional unit (sometimes called 'genets') (Buss, 1983; Tuomi & Vuorisalo, 1989). However, most plants, algae and fungi, and many animals and bacteria have a modular structure in which the individual develops by an asexual repetition of physically interrelated subunits called modules (Andrews, 1998; Harper, 1977; Hiebert et al., 2020; Tuomi & Vuorisalo, 1989).

Modular organisms can express within-individual plasticity when different modules are confronted with different biotic or abiotic environments (Figure 2c). Nevertheless, unlike unitary organisms, within-individual plasticity in modular organisms can involve not only labile but also fixed traits, meaning that it can manifest in these organisms not only as phenotypic flexibility but also as developmental plasticity (Gómez et al., 2020). Although variation in trait expression is frequent in modular organisms, the consequences of modularity for the expression of plasticity have been seldom studied (De Kroon et al., 2005; Diggle, 2002; Herrera, 2009). Equation (5) and its associated experimental design can be used to explore the occurrence of within-individual developmental plasticity in modular organisms (Appendix S3).

4.3 | Within-modular plasticity

Modular organisms, in addition, can express plasticity at a lower organisational level. Plasticity can emerge at the subindividual level if modules of the same individual face different levels of the environmental variable (Figure 2d; Appendix S3). This occurs, for example, when leaves of a

tree, polyps of a coral colony, zooids of a bryozoan colony or hyphae of a fungus experience distinctive changes in an environmental variable or in the intensity of damage caused by pathogens, herbivores or grazers. This is the case, for example, of plants in which herbivores only attack some modules and the induced defences are expressed locally and disappear when the herbivore stops eating in that module (Volf et al., 2022). In this scenario, a single module is confronting several environments. This means that, whereas in unitary organisms the individual is the lowest level of organisation at which plasticity can arise, plasticity can arise at the module level in modular organisms. Within-modular plasticity is likely to be more evident in organisms with clonal modularity (each module can perform all the essential functions required for independent life), sometimes called 'ramets' (Tuomi & Vuorisalo, 1989), than in those with organismic modularity (each module includes some but not all the basic elements of the organism and cannot thereby live autonomously; Tuomi & Vuorisalo, 1989). Likewise, within-module plasticity could also be influenced by the presence of intraorganismal genetic heterogeneity originated from somatic mutations, gene conversion or genome duplications (Pineda-Krch & Lehtila, 2004).

Although we presume that within-modular plasticity is common in many modular organisms, very little has been studied so far. Here, we propose a way to study and quantify within-module plasticity, in the hope of encouraging its study in natural systems. Because plasticity emerges when a genotype expresses different phenotypes in different environments, assessing within-modular plasticity requires modules to confront different environments (Figure 2d). The experimental design necessary to do this requires quantifying how trait expression in a particular module of several individuals of some genotypes varies among environments. This can be performed if the environment can be changed easily for single modules, as the leaves-herbivorous insects system studied by Volf et al. (2022). Analytically, Equation (5) can be extended to include an additionally nested level describing trait variation within modules:

$$z_{m(ij)k} = a + \sum_{n=1}^{N} b_n x_k^n + \alpha_i + \sum_{n=1}^{N} \beta_{ni} x_k^n + \alpha_{j(i)} + \sum_{n=1}^{N} \beta_{nj(i)} x_k^n + \alpha_{m(ij)} + \sum_{n=1}^{N} \beta_{nm(ij)} x_k^n + \varepsilon_{m(ij)k}, \quad (7)$$

where $\alpha_{m(ij)}$ and $\beta_{nm(ij)}$ are the random coefficients describing the intercept and change in slope of each module m within each individual j of the genotype i. The remaining parameters are as in Equation (5). Although exploring intramodular plasticity may seem excessive, we believe that many modular organisms use this mechanism to cope with rapidly and heterogeneously changing environments. We therefore suspect that it is worth exploring systematically.

5 | QUANTIFYING PHENOTYPIC PLASTICITY OF POLYPHENIC TRAITS

Some plastic traits, instead of varying continuously, show alternative discrete values in different environments. This type of plastic traits is known as polyphenism (Mayr, 1963), threshold trait (Roff, 1996), or conditional strategy (Hazel et al., 1990). Examples of polyphenic traits are the castes of social insects, the solitary and gregarious phases of migratory locusts, the winged and wingless forms of aphids, the alternative larval coloration of some Lepidoptera species, the seasonal polyphenism of some butterflies, or the predator-induced phenotypes of water fleas (Nijhout, 2003). Despite being one of the first types of plasticity to be formally recognised (Woltereck, 1909), the development of methods to quantitatively estimate the G×E interaction in polyphenic traits has lagged far behind those used to study continuous plasticity (but see Carter et al., 2017; Dennis et al., 2011). We believe that this is partly because researchers' interest has focused primarily on detecting the environmental values that trigger developmental shifts and partly because polyphenic reaction norms are very nonlinear and cannot be easily studied with linear mixed models.

Two mechanisms cause the emergence of polyphenic traits: continuous reaction norms only partially expressed in discontinuous environments or developmental switches of threshold traits (Nijhout, 2003). The first type of polyphenism can be studied using one of the previous methods if a broader range of environmental values can be reproduced experimentally. This approach has already been followed when studying floral polyphenism (Gómez et al., 2020), seasonal polyphenism in butterflies (Wijngaarden & Brakefield, 2001) or locust phase polyphenism (Foquet et al., 2021). On the contrary, threshold traits show discrete, alternative phenotypic states due to the presence of an underlying latent quantitative trait, termed liability, which triggers the phenotypic switching when crossing an environmental threshold (Reid & Acker, 2022; Roff, 1996). Although the liability-scale reaction norm may be linear (Reid & Acker, 2022), organismic-level reaction norms of threshold traits are, in most cases, highly sigmoidal (Nijhout, 2003; Sakamoto & Innan, 2024; Suzuki & Nijhout, 2006). The expression of polyphenic traits z_k can thus be described by the function:

$$z_k = \frac{L}{1 + e^{-b(x_k - x_0)}}, \quad (8a)$$

where the asymptote of the curve (L) is the value of the phenotypic trait when it is expressed, the steepness of the curve (b) is the slope of the curve in the environmental region where the trait switches, and the midpoint of the function (x_0) is the environmental value at which the phenotype switches (maximum, reactivity and sensitivity, respectively, sensu Carter et al., 2017). Genotypes may vary in each of these parameters (Figure 3), and this variation is what defines the G×E interaction in polyphenic traits. A nonlinear mixed-effects model (NLMEM) following the Lindstrom and Bates (1990) formulation can be used to describe the nonlinear relationship of the expression of the trait z_{ik} of each genotype i in each environment k as

$$z_{ik} = \frac{L + L_i}{1 + e^{-(b+b_i)(x_k - (x_0 + x_{0i}))}} + \varepsilon_{ik}, \quad (8b)$$

where the three parameters can be modelled as random effects with not only mean overall effect on the phenotypic trait (L, b, x_0) but have also genotypic deviation (L_i, b_i, x_{0i}). This model allows any of the three

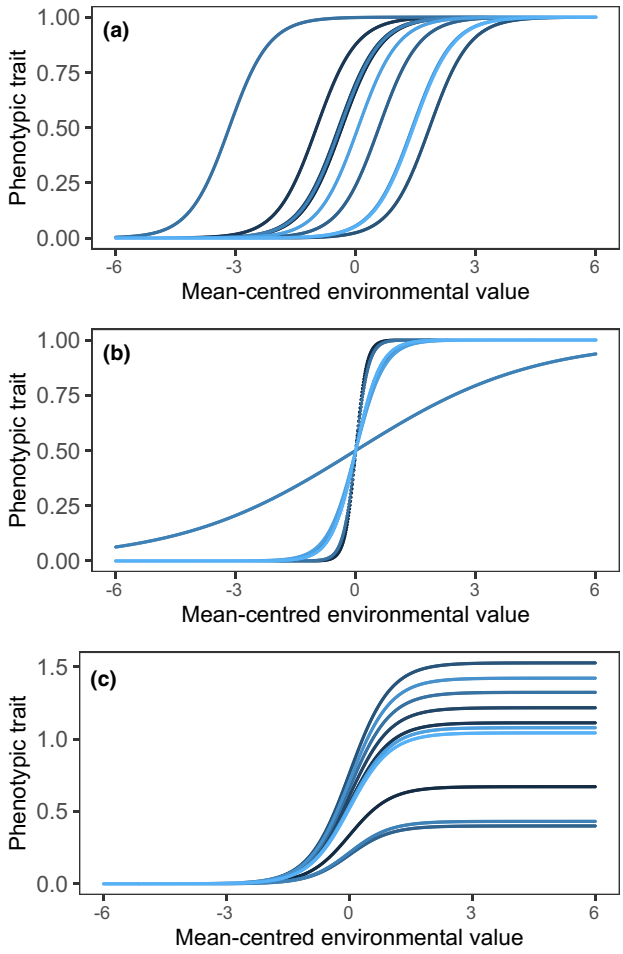

FIGURE 3 Between genotypes differences in (a) midpoint, (b) steepness and (c) asymptote of the polyphenic reaction norms.

parameters to vary between genotypes independently or in combination, and to test specific hypotheses about expression in polyphenic traits in individual systems.

6 | QUANTIFYING MULTIDIMENSIONAL PHENOTYPIC PLASTICITY

Plasticity rarely occurs in nature as a response to variation in one single environmental variable. Instead, most plastic traits respond to the simultaneous variation of several environmental factors. This phenomenon is called multidimensional phenotypic plasticity (MDPP; Morel-Journel et al., 2020; Westneat et al., 2019). These complex scenarios make plasticity more challenging to assess, requiring more elaborate experimental designs and larger sample sizes. Moreover, sophisticated analytical tools are also required. Researchers have used different approaches to consider the combined effect of two or more environmental variables. A robust way could be to expand previous equations to incorporate additional environmental variables (Hudak & Dybdahl, 2023; Westneat et al., 2019). We can expand the first-order Equation (4) ($N=1$) to include the effect of p environmental variables as

$$z_{ik_p} = a + \sum_{p=1}^{P} b_p x_{pk_p} + \alpha_i + \sum_{p=1}^{P} \beta_{pi} x_{pk_p} + \varepsilon_{ik_p}, \tag{9a}$$

where k_p are the different values that each p environmental variable can take, and z_{ik_p} is the trait value of genotype i in each k value of each p environmental variable. Equation (9a) considers that the effects of several environmental variables are only additive. But their effects can be also interactive, with either pairwise or higher-order interactions. For two environmental variables x_1 and x_2 ($p=2$) and a pairwise interaction effects, the model is

$$\begin{aligned} z_{ik_1k_2} = a + b_1 x_{1k_1} + b_2 x_{2k_2} + \left(b_{12} x_{1k_1} x_{2k_2}\right) \\ + \alpha_i + \beta_{1i} x_{1k_1} + \beta_{2i} x_{2k_2} + \left(\beta_{12i} x_{1k_1} x_{2k_2}\right) + \varepsilon_{ik_1k_2}. \end{aligned} \tag{9b}$$

Although in some situations are important, higher-order interactions will make the experiments impractical because the sample size required will be too large, the analytical models needed to solve the experiment will be too complex, and our ability to interpret them will be too limited.

7 | CONCLUSIONS

Our understanding of the relevance of phenotypic plasticity to the ecology and evolution of organisms will improve if studies on this phenomenon are carried out within a coherent conceptual and methodological framework. We summarise here the most important points:

1. The quantification of phenotypic plasticity requires the use of good proxies of genotype.
2. Reaction norms at the genotype level capture the essence of plasticity. Thus, we encourage the use of reaction norms over other approaches to assess phenotypic plasticity.
3. We recommend, whenever possible, quantifying the environmental variables used to develop the reaction norms.
4. Different genotypes often respond to environmental variation in different ways, a phenomenon reflecting the amount of genetic variation existing for plasticity. We recommend considering this G×E interaction when possible. Random slope regression mixed models (RRMM) are well-suited for this task.
5. Many traits are expressed multiple times during an individual's lifespan, and their expression changes depending on environmental variation. Plasticity of labile traits requires assessing the trait expression in various environments for several individuals of a given genotype. Models used to calculate within-individual plasticity should consider this hierarchical structure and the statistical non-independence of measurements taken sequentially in the same individuals. Autoregressive multilevel mixed effects models for repeated measures (MMRM) can be used in this situation.
6. Some organisms can express plasticity at a lower organisational level: the module. Because plasticity emerges when a genotype expresses different phenotypes in different environments,

assessing within-modular plasticity requires modules to confront different environments. Two-level nested MMRM can be useful to quantify within-modular plasticity.

7. Polyphenic traits, instead of varying continuously, show alternative discrete values in different environments. The expression of polyphenic traits is, in most cases, highly sigmoidal. Nonlinear mixed-effects models (NLMEM) can be used to explore the magnitude of plasticity as well as the G×E interaction in polyphenic traits.
8. Most plastic traits respond to the simultaneous variation of several environmental variables, a phenomenon termed multidimensional phenotypic plasticity (MDPP). Any of the previously proposed mixed models can be accommodated to include more than one fixed factor and can be thus used to quantify MDPP.

We are aware that our proposal will be difficult to apply in some systems, or that different researchers will have different analytical and/or experimental preferences or views. Even so, we hope that our proposal will at least serve as a stimulus to try to establish a common framework of study that will allow us to advance more deeply into the role that such a fundamental phenomenon as phenotypic plasticity plays in the ecology and evolution of organisms.

AUTHOR CONTRIBUTIONS

José María Gómez, Adela González Megías, Francisco Perfectti and Cristina Armas conceived the idea. José María Gómez decided the methodology. José María Gómez did the modelling. José María Gómez led the writing of the manuscript. Adela González Megías, Francisco Perfectti and Cristina Armas reviewed the literature. All authors analysed the data. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

We are grateful to Luis Navarro, Eduardo Narbona and Rubén Torices for their insightful comments and profound reflections on the nature of phenotypic plasticity and to Raquel Sánchez, Angel Caravantes, Gema Batanero, Isabel Sánchez Almazo and María José Jorquera for technical support. This research is supported by grants from the Spanish Ministry of Science and Innovation (PID2021-126456NB, including EU FEDER funds). This is a contribution to the Research Unit Modelling Nature, funded by the Consejería de Economía, Conocimiento, Empresas y Universidad (QUALIFICA 00011) and to Evoflor (UGR), Unidad Asociada I+D+I al CSIC.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

No empirical data have been used in this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1: Dataset of studies quantifying phenotypic plasticity between 2022 and 2024.

Appendix S2: Bias of environment-implicit and -explicit estimates of plasticity.

Appendix S3: Experimental design to study within-individual phenotypic plasticity.

How to cite this article: Gómez, J. M., Perfectti, F., González-Megías, A., & Armas, C. (2025). Quantifying phenotypic plasticity: A call for consistency. *Functional Ecology*, *00*, 1–11. https://doi.org/10.1111/1365-2435.70004