The choice of the environmental covariate affects the power to detect variation in reaction norm slopes

Phillip Gienapp

Netherlands Institute of Ecology (NIOO-KNAW), Department of Animal Ecology, Wageningen, the Netherlands

Abstract

Many traits are phenotypically plastic, i.e. the same genotype expresses different phenotypes depending on the environment. Individuals can vary in this response to the environment and this individual variation (Individual-by-Environment interactions, IxE) can have important ecological or evolutionary consequences. Studies on individual variation in plasticity often fail to show IxE; this can indicate a genuine absence of IxE or simply a lack of power. There is, however, another factor that could potentially affect the power to detect IxE: the choice of the environmental variable included in the analysis. Identifying the genuine environmental driver of phenotypic plasticity will mostly be impossible and hence generally only a proxy for it can be included in the analysis. However, if this proxy is too weakly correlated with the real driver of plasticity, this will bias IxE downwards, could lead to spurious negative results and invalid conclusions. As the mean phenotype in a given environment captures all environmental effects on the phenotype, using it as 'environment' in the analysis should have better power to detect IxE. Using simulated data I here showed that proxies that are weakly correlated to the real driver of plasticity lead indeed to downward bias in IxE but that when using the environment-specific mean phenotypes this is not the case. Using environment-specific phenotypic means as a covariate has been commonly used in animal and plant breeding but rarely used outside these fields despite its potential usefulness as 'yardstick' to test whether IxE is present or absent in the phenotype of interest.

Introduction

Many traits are phenotypically plastic (Pigliucci, 2005), i.e. individuals or genotypes express different phenotypes in different environments. Phenotypic plasticity is generally described by a ‘reaction norm’ (Woltereck, 1909), which is defined by its intercept, i.e. the phenotype in the average environment, and its slope, i.e. the ‘sensitivity’ to the environment, in the case of a simple linear reaction norm. Genotypes and individuals can differ in their reaction norm slopes, in which case we talk about genotype-by-environment (GxE) and individual-by-environment interactions (IxE) (Nussey et al., 2007). Exploring individual variation in reaction norms (IxE), requires that an individual expresses the phenotype repeatedly; otherwise an individual-level reaction norm cannot be defined. Repeated expression of the phenotype is possible in so-called ‘labile’ traits, as, for example, many behavioural or physiological traits. Understanding individual and genetic variation in reaction norms is important because it can explain why different individuals respond differently to environmental ‘cues’ or whether reaction norm themselves could respond to selection as caused, for example, by climate change (Gienapp et al., 2014).

Individual and genetic variation in reaction norms is generally estimated with a specific form of the mixed model, the random regression or infinite dimensional model (Henderson, 1982, Kirkpatrick, 1989, Morrissey & Liefting, 2016). Instead of regarding phenotypes at discrete environmental states as separate traits and modelling variances and covariances, here the trait and the (co)variance in reaction norm slope and intercept is modelled as a continuous (linear) function of the environment, i.e. as an infinite-dimensional trait at an infinite number of discrete environmental states. Intercepts and slopes of individual reaction norms are modelled as random effects, i.e. random draws from a normal distribution with mean zero and (co)variances to be estimated.

Many studies explored individual and genetic variation in reaction norms, for example, in behaviour, phenology, i.e. the seasonal timing of life-cycle events, or physiology (e.g. Sih et al., 2004, Reed et al., 2009, Hau & Goymann, 2015, Stedman et al., 2017). While some studies found individual variation in reaction norms (e.g. Nussey et al., 2005, Brommer et al., 2008, Reed et al., 2009, Stedman et al., 2017), others did not (e.g. Reed et al., 2006, Charmantier et al., 2008). Negative results always raise the question whether it is a genuine absence, in this case of individual or genetic variation in reaction norms, or whether the study had enough power to detect this variation. Two simulation studies showed that the power to detect individual variation in reaction norms depends on both the number of observations per individual and the number of individuals sampled (Martin et al., 2011, van de Pol, 2012). However, another, so far overlooked, factor that could affect the power to detect individual variation in reaction norms is the choice of the environmental variable driving trait expression, i.e. against which covariate phenotypes are regressed in the analysis. Using a non-meaningful covariate will probably lead to a downward bias

in the population-level reaction norms and likely also to a downward bias in individual variation of reaction norms.

In experimental work it is obvious which variable to choose as covariate as this will obviously be the variable that was experimentally manipulated. In field work, however, a large number of variables could affect the trait and are potential covariates in an analysis of phenotypic plasticity. Biological knowledge will obviously help with the choice of this variable. For example, phenological traits, as, e.g., flowering, migration or breeding, generally depend on ambient temperatures (Sparks & Carey, 1995, Visser et al., 2009) and hence local temperatures would be an obvious choice as covariate. There are, however, many different ways to measure temperature as it can be averaged or summed over an almost infinite number of periods. This issue arises for virtually any trait in a natural population as there will always be more than one environmental variable to choose from. To complicate matters further traits can, of course, depend on more than one variable. For example, it has been shown that breeding time is affected by spring temperature and population density in tree swallows (*Tachycineta bicolor*) (Bourret et al., 2015) or spring temperature in interaction with day length in great tits (*Parus major*) (Gienapp et al., 2005). Consequently, it will very rarely be possible to include the real driver of plasticity as covariate in the analysis, simply because it is not known, and instead a proxy that correlates with the real causal driver(s) of plasticity has to be used.

If this proxy is, however, only weakly correlated to the real driver of plasticity, this will lead to an underestimation of population-level plasticity and likely also to an underestimation of individual variation in reaction norm slopes. Given sufficient sample size, any absence of individual variation in reaction norm slopes can hence indicate a true absence or that an unsuitable proxy, too weakly correlated with the real driver(s) of plasticity, was used. For example, Brommer *et al*. (2005) analysed IxE in breeding time in collared flycatchers (*Ficedula hypoleuca*) with three different environmental variables, temperature, rainfall, NAO. All three variables explained significant variation in the trait but significant IxE was found for only two of these variables. Similarly, no IxE was found for breeding time in the Wytham Wood great tit (*Parus major*) population when temperature sums from 1 March to 25 April were used as a covariate (Charmantier et al., 2008), while using the mean temperature from 15 February to 25 April as a covariate resulted in significant IxE (Husby et al., 2010).

The main problem here is that it will be virtually impossible to be sure that the chosen proxy is correlated closely enough with the true driver of plasticity to allow the detection of IxE or GxE. One potential solution to this is regressing individual phenotypes against environment-specific mean phenotypes. This, seemingly circular, method, called ‘joint-regression analysis’ or ‘Finlay-

Wilkinson regression', is commonly used in animal and plant breeding studies. It was first proposed by Yates and Cochran (1938) but only later made popular by Finlay and Wilkinson (Finlay & Wilkinson, 1963). It is widely used in animal and plant breeding to test reliably whether the (relative) merit of genotypes is constant across environments, i.e. whether GxE exists or not (James, 2009, Lynch & Walsh, 1998). Since the mean phenotype in each environment incorporates all plastic effects of environmental drivers, it would be correlated closely with the true, but unknown, driver of plasticity and would hence be a highly suitable description of the environment to explore individual variation in plasticity.

I here explored how the correlation of a proxy environmental variable with the real driver of plasticity can bias estimates of individual variation in plasticity and how the 'Finlay-Wilkinson regression' approach compares to using these various proxies. To do this, I regressed in a random regression model simulated phenotypes against environment-specific phenotypic means and against nine different environmental variables that are correlated with the real driver with an r of 0.9 to 0.1 but did not themselves have an effect on the phenotypes, i.e. are only proxies. In the simulated data, individual variation in reaction norms was present and the key point of interest was with which proxy it could be detected.

Methods

Individual phenotypes were simulated with an 'individual-based model'. Individual reaction norm (RN) intercepts and slopes were drawn from normal distributions with means of zero and variances of 5 and 0.1, respectively. Individual intercepts and slopes were assumed to be uncorrelated. Ten environmental variables (E1 to E10) were simulated for 20 years (i.e. one value per year for each variable across 20 years) by drawing values from a multivariate normal distribution with means of zero and the following covariance matrix:

$$\mathbf{E} = \begin{bmatrix} 1 & 0.9 & 0.8 & 0.7 & 0.6 & 0.5 & 0.4 & 0.3 & 0.2 & 0.1 \\ 0.9 & 1 & 0.7 & 0.6 & 0.5 & 0.4 & 0.3 & 0.2 & 0.1 & 0.01 \\ 0.8 & 0.7 & 1 & 0.5 & 0.4 & 0.3 & 0.2 & 0.1 & 0.01 & 0.001 \\ 0.7 & 0.6 & 0.5 & 1 & 0.3 & 0.2 & 0.1 & 0.01 & 0.001 & 0 \\ 0.6 & 0.5 & 0.4 & 0.3 & 1 & 0.1 & 0.01 & 0.001 & 0 & 0 \\ 0.5 & 0.4 & 0.3 & 0.2 & 0.1 & 1 & 0.001 & 0 & 0 & 0 \\ 0.4 & 0.3 & 0.2 & 0.1 & 0.01 & 0.001 & 1 & 0 & 0 & 0 \\ 0.3 & 0.2 & 0.1 & 0.01 & 0.001 & 0 & 0 & 1 & 0 & 0 \\ 0.2 & 0.1 & 0.01 & 0.001 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0.1 & 0.01 & 0.001 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

The correlations between E1 and the environments E2 to E10 were 0.9 to 0.1, see first column or first row of **E**. The correlations among E2 to E10 are not relevant for the simulation and could take any value but were set to the given values to make **E** approximately positive definite (see Appendix).

Phenotypes of 500 individuals were simulated by first determining how many observations each individual would have by drawing a random number from a Poisson distribution with a mean 3 and adding 1. This sample size was sufficient to detect IxE when using E1 as environmental variable, see Results. Adding 1 was done to ensure that each individual had at least two observations. A 'birth year' of each individual was then drawn randomly from the years but ensuring that the number of observations for the individual would fit within its 'life time'. Phenotypes were simulated as follows:

$$y_{i,j} = \mu + a_i + (b_{pop} + b_i)E1_j + \epsilon$$

with $y_{i,j}$ being the phenotype of individual *i* in year *j*, μ being the population-level RN intercept, a_i being the individual effect for RN intercept, b_{pop} being the population-level RN slope, b_i being the the individual effect for RN slope, $E1_j$ being the value of environmental variable E1 (i.e. the true causal driver of plasticity) in year *j*, and ϵ being a random error with mean zero and variance of 0.25.

To test whether additional but unidentified variables can bias results from an analysis with environment-specific means more than results from analyses with environmental variables, three such variables were modelled: age, habitat effects, and a systematic time trend, akin to a genetic change as response to a constant selection pressure. Age effects were -0.5, 0, 0.5, 0.5, 0.25, 0.0, -0.1, -0.2, -0.3, -0.4, -0.5, -0.6, -0.65, -0.7, -0.7, -0.75, -0.8, -0.8, -0.8, and -0.8 for ages of 1 to 20, roughly simulating an increase with an optimum at age 4 and slow senescence. Habitat effects were modelled by randomly distributing individuals over 5 different habitats with habitat-specific effects of -0.1, -0.05, 0.0, 0.05, and 0.1. The time trend was modelled by letting phenotypes increase by 0.01 per year.

Phenotypes were then regressed against all environments and environment-specific means (ESM) (all standardised before analysis) in separate analyses using a random regression model corresponding to the model above to estimate individual variation in RN intercepts and slopes.

Individual was fitted as a random effect and the environmental variable as a continuous fixed effect. The significance of variation in slopes was tested with a likelihood-ratio test that compared a model with individual variation in intercepts and slopes against a model with only variation in intercepts. Heterogeneous residual variation can lead to spurious individual variation in RN slopes but since all potential variation in phenotypic variation across a given environmental variable would be driven by variation in RN slopes, homogeneous residual variation across the environment was fitted. The difference in model likelihoods is approximately X^2-distributed with 1 df. For each model with a different environment (E1 to E10 and am) the estimated variances for intercept and slope, the significance for variation in slopes as well as the estimate for the population-level slope were stored. The simulation was repeated 1000 times. Simulation and analysis was done in R 3.1 (R Development Core Team, 2015). The code is provided as Supplementary Material.

Results

Unsurprisingly, using the environmental variable that was used to simulate the phenotypes (E1), i.e. the real driver of plasticity, as a covariate in the analysis recovered the input values for reaction norm (RN) slope and individual variation in intercept and slope of 0.5, 5.0, and 0.1, respectively. For the model without additional variables (age, habitat, year) the mean estimate for the population-level slope using E1 was 0.49, while the mean estimated individual variation in intercepts and slope was 5.02 and 0.099, respectively.

The estimates for population-level slope and individual variation in RN slopes clearly declined with a decreasing correlation of the used covariate with the 'real driver' (E1) (Fig. 1). As the estimated individual variation in RN slopes declined, the power, i.e. the proportion of replicates, in which IxE was statistically significant, decreased indicating that it would be highly unlikely to detect statistically significant individual variation in RN slopes if the covariate would be correlated with the real driver by 0.7 and less (Fig. 2). The estimates for individual variation in RN slopes from the model using environment-specific means as covariate were as close or closer to the estimates from the model using E1 than models using other environmental variables (E2 to E10) (Fig. 1). Other factors that affect the phenotype but are unaccounted for will alter the environment-specific mean phenotypes and can thereby potentially affect the results. Here, age, a second environmental variable, and a systematic time trend were tested, see Methods, but none of them led to any systematic bias in the probability to detect IxE and its estimates, see Supplementary Materials, Figs S1-4.

Discussion

As has been found previously (Brommer et al., 2005, Husby et al., 2010) and systematically explored here, the choice of the environmental variable that affects the phenotype in the reaction norm can affect the probability to detect individual variation in reaction norm slopes (IxE). The less related the included environmental variable is to the real driver of plasticity, the lower the probability to detect statistically significant IxE (Fig. 2). As it is in practice impossible to know how closely the used covariate is correlated to the real driver, it is not useful trying to develop any rules, even rules of thumb, for the power of such analyses. Instead, using environment-specific population means as covariate, i.e. using a so-called 'Finlay-Wilkinson' regression (Finlay & Wilkinson, 1963, Yates & Cochran, 1938), could provide an alternative means to explore IxE without the need to identify an environmental variable closely related to the real driver. While this approach does not give biological insight into the environmental variable underlying phenotypic plasticity and IxE in the analysed trait, it can reliably, given sufficient sample size, indicate the presence or absence of IxE.

If the presence of IxE has been shown using a 'Finlay-Wilkinson' regression, further analyses can aim to identify to meaningful biotic or abiotic environmental variables that drive IxE in the trait of interest. If IxE can be found using an environmental variable, it can then be concluded that this variable is reasonably closely correlated with the environmental variable that causally affects the trait. In this sense a 'Finlay-Wilkinson' regression can serve as a 'yardstick' against that models including other environmental variables can be compared.

Whether or not IxE, and also genetic variation in reaction norms slopes (GxE), is present or not in a population or species, is biologically relevant. For example, IxE can increase or decrease the amount of among-individual variation in novel environments, which affects the opportunity of selection and thereby potential evolutionary change, even in the absence of GxE. The absence of IxE can also be biologically interesting; for example, Reed et al. (2006) found no IxE in breeding time of common guillemots (*Uria aalge*) in response to the North Atlantic Oscillation and argued that this lack of IxE was a consequence of the benefits of coordinated breeding in colonial birds, such as the common guillemot. However, in such cases it would be desirable to be sure that this absence of IxE is not caused by an unsuitable choice of the environmental variable affecting the trait. In fact, when re-analysing this data using phenotypic environment-specific means, in this case annual mean egg-laying dates, as environmental variable, statistically significant IxE was found (T.E. Reed pers. comm.).

Trait expression is obviously not only affected by environmental variables but also by individual 'state' variables, such as age or physical condition and potentially other environmental variables, for example habitat in addition to temperature. Not accounting for such variables can bias environment-specific mean phenotypes, for example, if age structure in the population varies from year to year and this could potentially lead to specific biases in estimates from 'Finlay-Wilkinson' regressions. This potential bias may be especially problematic when there is a consistent time trend in phenotypes, as caused, for example, by a genetic selection response. Such potential biases would also affect the phenotypes, albeit not the covariate, of analyses based on environmental variables. The results here show that results from 'Finlay-Wilkinson' regressions are not biased compared to using environmental variables (see Appendix). It is, however, always preferable to include and test all variables potentially affecting the trait to obtain more accurate estimates of variations in reaction norms and also to gain a better understanding of which abiotic and biotic variables affect the trait.

An important assumption underlying the 'Finlay-Wilkinson' regression is that the response to the 'true' driver of plasticity is (approximately) linear as strongly non-linear reaction norms, for example, quadratic or sigmoidal, could lead to identical mean phenotypes in different environments, which would obviously interfere with reliably estimating IxE. It is also important that the environment-specific means are based on adequate sample sizes as it has been shown that unreliably estimated environment-specific means can bias estimates of GxE (Calus et al., 2004) and the same will apply for IxE, too.

Genetic variation in reaction norm slopes (GxE) is also relevant as environmental change likely leads to selection on both reaction norm intercept and slope (Gienapp et al., 2014). How the power to detect GxE depends on the choice of the covariate included in the analysis was not addressed here because of the specific issues with the power of quantitative genetic analyses. The sampling variation in heritability estimates, i.e. the power of quantitative genetic analyses, depends on the sample size but also on the variation of relatedness within the population (Visscher & Goddard, 2015). The variation in relatedness depends on a number of species- or population-specific ecological parameters, as, for example, dispersal or mating system. It would have been possible to simulate GxE but the obtained results would have been difficult to generalise. However, IxE is generally regarded as an 'upper limit' for GxE. Having found no IxE with sufficient sample size and using a 'Finlay-Wilkinson' regression it would hence be highly unlikely to find GxE in the same population. It should, however, be noted that this 'yardstick' cannot universally be applied as in short-lived species necessary sample sizes, in terms of repeat observations of individuals, may not ever be reached. Hence, IxE may not be well estimable but – given suitable relatedness information – quantitative genetic analyses could be possible and significant GxE could be found.

In animal and plant breeding the ‘Finlay-Wilkinson’ regression has long been used but very rarely outside this field (James, 2009). I argued here that it can be useful as a ‘yardstick’ in analyses exploring individual (and genetic) variation in reaction norm slopes as its results are unbiased by the correlation between it and the environmental variable causally affecting the trait. This can be especially relevant for studies not finding statistically significant IxE and could therefore give us a better understanding how prevalent (or not) individual-by-environment interactions really are.

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Figures

Figure 1

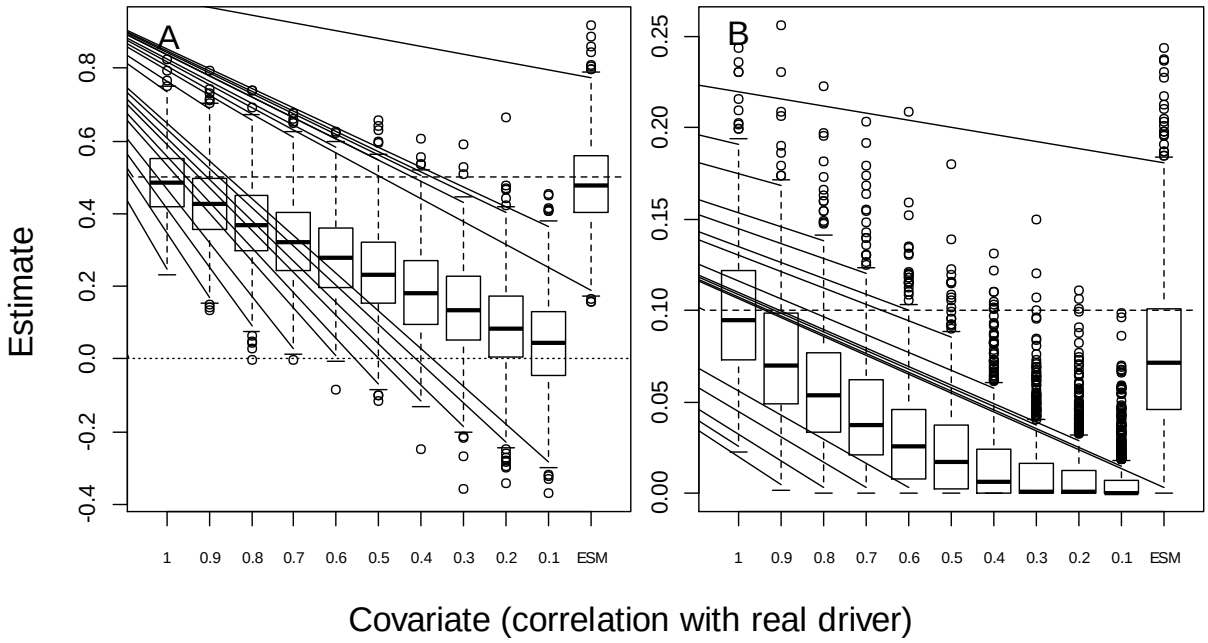

Fig. 1. Boxplot of estimates for the population-level reaction norm slope (A) and individual variation in reaction norm slopes (B) depending on the covariate included in the random regression model. The covariates included in the model are indicated by their correlation with the 'real driver' of plasticity (E1). ESM indicates environment-specific mean phenotypes as covariate. The dashed lines indicate the input values for the slope and variation in slopes. Bold line indicates median, box margins 1st and 3rd quartile and whiskers 1.5*inter-quartile range.

Figure 2

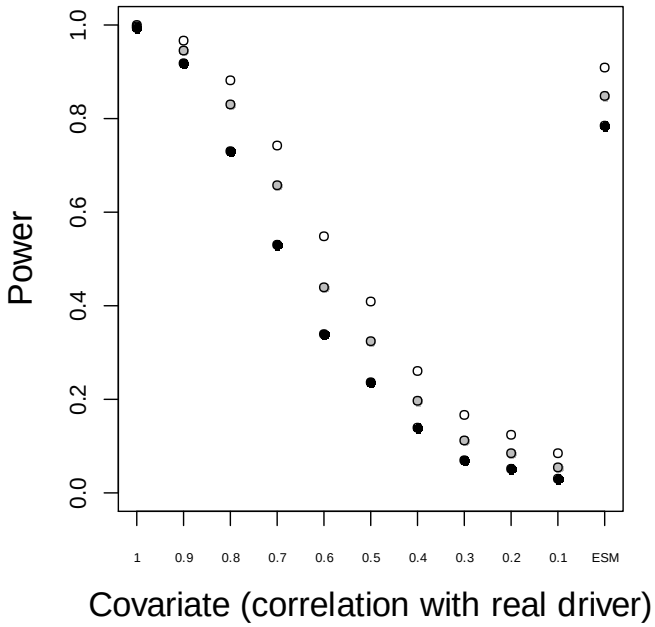

Fig. 2. The power to detect variation in reaction norms slopes (IxE) depends on the covariate included in the model. The proportion of replicates, in which IxE was statistically significant at a significance level of 0.05 (open symbols), of 0.01 (grey symbols), and 0.001 (black symbols), is plotted against the correlation of the covariate with the ‘real driver’ of plasticity (E1). ESM indicates environment-specific mean phenotypes as covariate.

Supplementary Figures

To test whether additional but unidentified variables can bias results from an analysis with environment-specific means more than results from analyses with environmental variables, three such variables were modelled: age, habitat effects, and a systematic time trend, akin to a genetic change as response to a constant selection pressure. As can be seen from the figures below neither age (Fig.S1), habitat effects (Fig.S2), nor a systematic time trend (Fig.S3) lead to bias in the estimates using environment-specific means as covariate. The power of analyses including these variables was also unaffected, see Fig.S4.

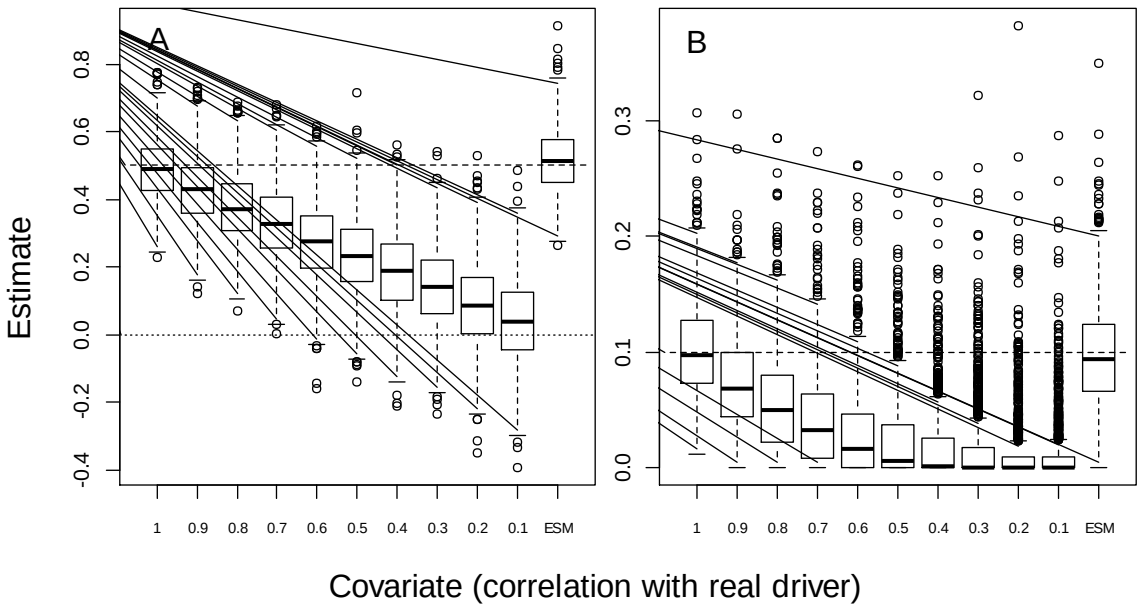

Fig. S1. Boxplot of estimates for the population-level reaction norm slope (A) and individual variation in reaction norm slopes (B) depending on the covariate included in the random regression model when the model included an age effect that was unaccounted for. The covariates included in the model are indicated by their correlation with the 'real driver' of plasticity (E1). ESM indicates environment-specific mean phenotypes as covariate. The dashed lines indicate the input values for the slope and variation in slopes. Bold line indicates median, box margins 1st and 3rd quartile and whiskers 1.5*inter-quartile range.

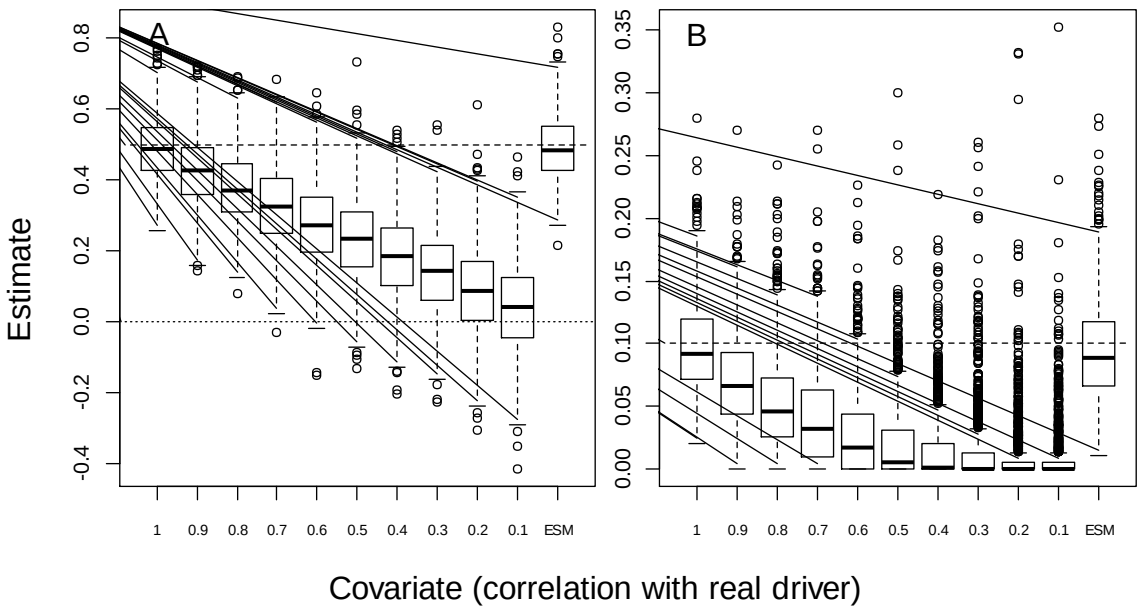

Fig. S2. Boxplot of estimates for the population-level reaction norm slope (A) and individual variation in reaction norm slopes (B) depending on the covariate included in the random regression model when the model included a habitat effect that was unaccounted for.

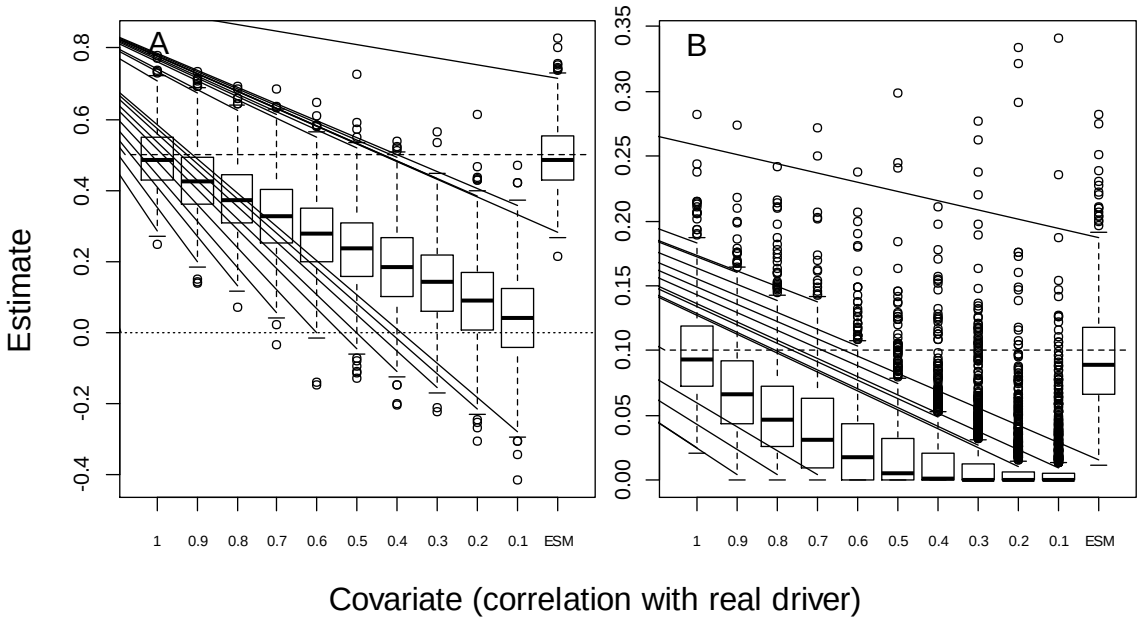

Fig. S3. Boxplot of estimates for the population-level reaction norm slope (A) and individual variation in reaction norm slopes (B) depending on the covariate included in the random regression model when the model included a habitat effect that was unaccounted for.

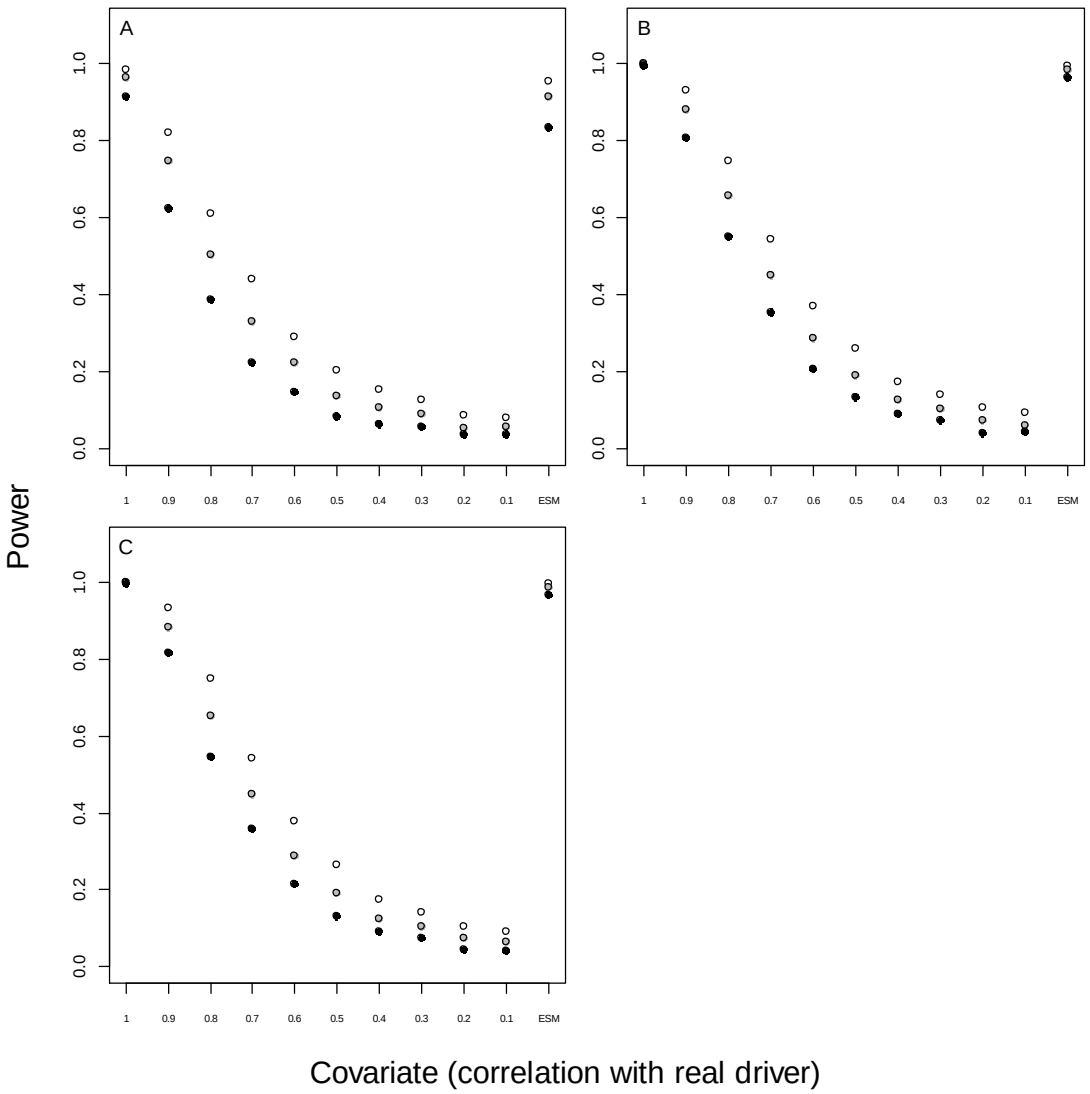

Fig. S4. The power to detect variation in reaction norms slopes (IxE) in models including age (A), habitat (B), and a systematic time trend (C) as un-accounted for variables depending on the covariate included in the model. The proportion of replicates, in which IxE was statistically significant at a significance level of 0.05 (open symbols), of 0.01 (grey symbols), and 0.001 (black symbols), is plotted against the correlation of the covariate with the 'real driver' of plasticity (E1). ESM indicates environment-specific mean phenotypes as covariate.