Accounting for intraspecific genetic correlations in ecological studies

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Abstract

Analyses in many fields of ecology are increasingly considering multiple species and multiple individuals per species. Premises of statistical tests are often violated with such datasets because of the non-independence of residuals due to phylogenetic relationships or intraspecific population structure. Ecologists are increasingly adopting comparative approaches that account for the phylogenetic relationship of species—for which the benefits are demonstrated—but the same cannot be said of the intraspecific population structure. Moreover, the importance of accounting for the intraspecific structure, solely or with the phylogenetic structure, is largely unaddressed. We show using simulations that accounting for intraspecific genetic correlations always yields more accurate and precise fixed effects and increased statistical power, but more so when the relative importance of the intraspecific to the phylogenetic structure is greater and with stronger intraspecific population structure. Analysis of a climate change experiment further showed that accounting for intraspecific and phylogenetic structures yields improved estimates of warming and photoperiod effects and their interactions in explaining the time to budburst for ten species sampled from two distinct populations. Based on their performance and flexibility, we argue that greater use of phylogenetic mixed models to account for genetic structure could advance research across many fields of ecology.

Key-words: Phylogenetic mixed models (PMM), comparative methods, population genetic structure, leaf phenology, climate change, MCMCglmm, phylogenetic generalized least squares (PGLS).

Introduction

The reactions of different species to external stimuli, either natural or experimental, are not independent. Indeed, because physiological responses have a genetic basis, closely related species are more likely to have similar responses to a specific treatment. This phylogenetic non-independence of species responses violates assumptions of most statistical tests, such as the independence of residuals in regression and negatively impact the results of statistical tests in terms of parameter estimates and p-values (e.g., Revell, 2010). This has been recognized for some time and a whole

family of methods—comparative methods—have been developed to address this problem (Felsenstein, 1985; Grafen, 1989; Lynch, 1991; Housworth et al., 2004; Ives et al., 2007; Felsenstein, 2008; Revell, 2010; Hadfield and Nakagawa, 2010; Kostikova et al., 2016), which are being increasingly used by ecologists.

Recently there has been growing awareness among ecologists of the need to also consider intraspecific variation within ecophylogenetic analyses. Within community ecology, there have been calls to greatly increase studies of intraspecific trait variation within community-level studies (Violle et al., 2012; Alofs, 2016) and to develop the necessary statistical models for such multilevel data (Funk et al., 2017; Read et al., 2016). Further, studies of climate change have repeatedly highlighted the need for models that incorporate variation in responses across both species and populations (Willis et al., 2008; Charmantier et al., 2008; Anderson et al., 2009; Chen et al., 2011). However, little attention has been given to the genetic correlation structure present below the species level within the field of comparative methods (but see Felsenstein, 2002; Stone et al., 2011; Read et al., 2016). Moreover, to our knowledge, no study has attempted to account for both phylogenetic and intraspecific genetic correlations simultaneously, even though the sampling structure in many ecological studies calls for such a design.

Presently, studies that account for phylogenetic correlation almost always ignore intraspecific genetic structure and as such assume that intraspecific samples are drawn from a single population. In contrast, many ecological studies explicitly sample individuals across important geographical ranges or from populations among which gene flow could be restricted, resulting in a potentially non-trivial correlation structure among samples. If this correlation is important, statistical tests that do not account for it are expected to be biased.

Until recently, the difficulty of obtaining genetic data to accurately estimate intraspecific genetic correlations provided sufficient justification for ignoring this source of variance in ecological studies. But the development of high-throughput sequencing techniques has changed the context and currently allows one to obtain thousands of markers for non-model organisms for a relatively affordable price.

This accessibility to genetic data could allow many areas of ecology to better understand how ecological responses are influenced by the genetic relationships between both species and populations. One area where this potential is particularly high is climate change research, where evidence of rapid ecological and evolutionary change is growing. Research has highlighted that species responses to climate change appear phylogenetically patterned, with species from certain clades and with particular traits appearing most vulnerable to local extinctions with warming (Willis et al., 2008). At the same time other work has highlighted discrepancies in species responses when studied over space (Charmantier et al., 2008), suggesting populations within species may show different responses to climate change. This is supported by population-level research that has found large differences in the responses of northern versus southern populations' range shifts with warming (Anderson et al., 2009; Chen et al., 2011). Such results make clear that best estimates of responses will need methods that consider variation at both the species and population levels, and the connections between different populations and different species, all at once.

The objectives of this report are to assess the importance of accounting for intraspecific genetic correlations in ecological studies, but also introduce introduce the phylogenetic mixed model (PMM) (Lynch, 1991; Housworth et al., 2004; Hadfield and Nakagawa, 2010) as a valuable statistical tool when genetic structure needs to be considered. Other approaches can be used to account for intraspecific correlations (reviewed in Stone et al., 2011), but currently none provides as much flexibility as the PMM. One advantage for ecologists is that it uses a familiar terminology, treating genetic correlations as random effects in a mixed model, similar to how blocks are often treated in a randomized block design. Another advantage is that more than one random effect can be used, which allows consideration of the phylogenetic and the intraspecific genetic structures simultaneously (Hadfield and Nakagawa, 2010). We assess the importance of accounting for intraspecific genetic correlation structure using simulated data to investigate a wide range of situations. We also provide a simple climate change-related empirical example where we investigate the importance of temperature, photoperiod and latitude on the leafout timing of ten tree and shrub species.

Methods

The phylogenetic mixed model

The phylogenetic mixed model has been described in detail elsewhere (Hadfield and Nakagawa, 2010; Villemereuil and Nakagawa, 2014), thus our description here is brief and focuses on the inclusion of phylogenetic and intraspecific correlations structures as random effects in the model, but also on the inclusion of fixed effects. In the following, we assume that phylogenetic and intraspecific correlations have been estimated independently, which allows the two structures to be included as separate effects and to quantify their relative importance. Here lowercase italic letters represent numbers, lowercase boldface letters vectors and uppercase boldface letters matrices. The phylogenetic mixed model (PMM) has the form:

$$\mathbf{y} = \mu + \beta\mathbf{x} + \mathbf{a} + \mathbf{b} + \mathbf{e}, \tag{1}$$

where $\mathbf{y}$ is the response variable, μ is the intercept, $\mathbf{x}$ is an explanatory variable, β the regression coefficient, $\mathbf{a}$ represents the effects due to the phylogenetic structure, $\mathbf{b}$ the effects due to the intraspecific structure, and $\mathbf{e}$ the residuals. $\mathbf{x}$ is a fixed effect (there could be more than one), whereas $\mathbf{a}$ and $\mathbf{b}$ are random effects. The random effects and residuals are assumed to follow normal distributions:

$$\mathbf{a} \sim \mathcal{N}(0, \sigma_a^2\mathbf{A})$$

$$\mathbf{b} \sim \mathcal{N}(0, \sigma_b^2\mathbf{B})$$

$$\mathbf{e} \sim \mathcal{N}(0, \sigma_e^2\mathbf{I}).$$

σ_a^2 is the phylogenetic variance, σ_b^2 is the intraspecific variance, and σ_e^2 is the residual variance. The matrices $\mathbf{A}$ and $\mathbf{B}$ represent the phylogenetic and the intraspecific correlation structures, respectively. The identity matrix $\mathbf{I}$ indicates that the residuals are independent and identically

distributed. Accordingly, the (co)variance structure ($\mathbf{V}$) of the model is $\mathbf{V} = \sigma_a^2\mathbf{A} + \sigma_b^2\mathbf{B} + \sigma_e^2\mathbf{I}$.

The PMM allows the estimation of the proportion of the total variance ($\sigma^2 = \sigma_a^2 + \sigma_b^2 + \sigma_e^2$) that is due to the genetic structure. This "heritability" (h^2) parameter is equivalent to the λ parameter often estimated in phylogenetic generalized least squares (PGLS) (Housworth et al. 2006). In the present context, heritability is the quotient obtained by dividing the phylogenetic and intraspecific variances by the total variance: $h^2 = (\sigma_a^2 + \sigma_b^2)/\sigma^2$. The remaining variance, $1 - h^2 = \sigma_e^2/\sigma^2$, is the non-genetic variance that could be due to the environment or other effects that impact the individuals in a way that is not defined by the genetic correlation structures. The PMM also allow the estimation of the relative contribution of the intraspecific correlation structure to the total genetic structure, which is $\sigma_b^2/(\sigma_a^2 + \sigma_b^2)$.

Phylogenetic generalized least squares

A brief mention of PGLS seems important as it is a commonly used phylogenetic comparative method. A PGLS model that would include phylogenetic and intraspecific correlation structure could be denoted as:

$$\mathbf{y} \sim \mathcal{N}(\mu + \beta\mathbf{x}, \delta\sigma_a^2\mathbf{A} + [1 - \delta]\sigma_b^2\mathbf{B}). \tag{2}$$

In other words, the residuals of the regression are normally distributed according to a correlation structure that is a combination of phylogenetic and intraspecific effects, with respective weights determined by the parameter δ. The main difference with the PMM is the absence of a residual term. In other words, the residuals need to be completely genetically structured by the correlation matrices provided. This assumption can be relaxed by rescaling the phylogenetic tree to give more or less weight to the terminal branches of the tree (Revell, 2010). Because of the flexibility provided by the PMM, that could also easily consider other random effects, we solely consider this approach here. However, this PGLS model (equation 2) is further developed and compared to the PMM using simulations in the supplementary materials.

PMM simulations

We simulated data under the PMM with various relative contributions of the phylogenetic and intraspecific variances and tested how this affected the estimation of the fixed and random effects. The data simulations followed closely those of Revell (2010). We simulated data using the PMM model outlined in equation (1) assuming $\mu = 0$. The fixed effects $\mathbf{x}$, assumed to be independent and identically distributed, were simulated as $\mathbf{x} = \sqrt{\sigma_x^2}\mathbf{u}$, where σ_x^2 is the variance of the trait and $\mathbf{u}$ is a vector of random values sampled from the normal distribution. The random effect $\mathbf{a}$ was simulated as $\mathbf{a} = \text{chol}(\sigma_a^2\mathbf{A})'\mathbf{v}$, where $\text{chol}(\sigma_a^2\mathbf{A})$ denotes the upper triangular Cholesky decomposite of $\mathbf{A}$ times the phylogenetic variance, and $\mathbf{v}$ is a vector of random normal deviates. Similarly, $\mathbf{b} = \text{chol}(\sigma_b^2\mathbf{B})'\mathbf{w}$, where $\mathbf{w}$ is a vector of random normal deviates. Finally, the residual errors, which are independent and identically distributed, were simulated as $\mathbf{e} = \sqrt{\sigma_e^2}\mathbf{z}$, where σ_e^2 is the error variance and $\mathbf{z}$ is a vector of random normal deviates. The response variable was obtained using $\mathbf{y} = \beta\mathbf{x} + \mathbf{p} + \mathbf{c} + \mathbf{e}$. The simulations were performed for different regression slopes $\beta \in \{0, 0.1, 0.25\}$ and different ratios of intraspecific and interspecific structure ($\sigma_a^2 : \sigma_b^2$) while keeping their sum to 2 (i.e., $0.5 : 1.5$, $1 : 1$, $1.5 : 0.5$). In all cases, $\sigma_e^2 = 1$ and $\sigma_x^2 = 2$.

The genetic correlation structures

The phylogenetic correlation structure was obtained by simulating a species tree with n_{sp} species with a pure birth model and extracting the correlation structure from the tree. The intraspecific correlation structure for n_{ind} individuals for each species corresponded to the mean correlation matrix obtained from 20 independent gene genealogies simulated within a population tree using the Coalescent with the `ms` software (Hudson, 2002). The population tree consisted of three populations without migration with speciations 0.3 and 0.8 coalescent units in the past, resulting in moderate population structure. The global intraspecific correlation structure consisted in a block diagonal matrix with the intraspecific correlation matrix of all species along the diagonal. The simulations were performed in `R` (R core team, 2015) and used functions from the packages `phytool` (Revell,

2012), `ape` (Paradis et al., 2004), `phyclust` (Chen, 2011) and `Matrix` (Bates and Maechler, 2016). Five hundred simulations were performed for each parameter combination. We simulated data with 98 or 100 individuals but different ratios of species vs. individuals per species ($n_{sp} : n_{ind}$), specifically $7 : 14$, $10 : 10$ and $14 : 7$.

Model fitting

The PMM model was fitted using the `MCMCglmm` package in R (Hadfield, 2010). The interspecific phylogenetic structure was included in the model using the 'pedigree' option. The intraspecific structure was incorporated using the single value decomposition of the genetic intraspecific correlation structure matrix, following Stone et al. (2011). We used the default priors for the fixed effects and diffuse inverse-Wishart priors for the random effects with $V = 1$ and $\nu = 0.002$. We fitted the following models, named according to their respective random effects:

$$null : y_i = \mu + \beta x_i + e_i$$

$$inter : y_i = \mu + \beta x_i + a_i + e_i$$

$$intra : y_i = \mu + \beta x_i + b_i + e_i$$

$$inter + intra : y_i = \mu + \beta x_i + a_i + b_i + e_i.$$

The MCMC chains were run for 2100 generations, removing the first 1000 as burnin and sampling the chain every 10 generations. These settings provided good convergence for all models and all simulation parameters. We report the accuracy and precision for the estimated slope, as well as the accuracy for the heredity (h^2). We also report the number of simulations that gave a posterior probability > 0.95 for the slope to be greater than 0; this represents the power of the model when $\beta > 0$ and the type I error when $\beta = 0$.

Budburst experiment

The experiment aimed at determining the impact of temperature increases, longer photoperiods, and latitude of origin on the budburst timing for several tree and shrub species. Clippings from 10 species were collected from five individuals at two sites: Harvard Forest (MA, USA; 42.5 °N, 72.2 °W) and St. Hyppolite (QC, Canada; 45.9 °N, 74.0 °W). Clippings were collected in January 2015 and kept cold until the start of the experiment. They were then subjected to different temperatures (15 °C or 20 °C) and photoperiods (8 or 12 hours of light per day) in growth chambers at the Arnold Arboretum. The number of days to budburst was recorded for all clippings. The data analyzed here is a subset of a larger experiment that studied more species (**see Flynn and Wolkovich**).

The interspecific phylogenetic structure used a published phylogenetic tree of 32,223 angiosperm species based on 7 genes (Zanne et al., 2014). The intraspecific genetic structure was estimated using the `genpofad` distance (Joly et al., 2015) from thousands of genome-wide anonymous markers per species obtained using a Genotyping-by-Sequencing approach (Elshire et al., 2011). Further details on our methods, including library construction, sequencing, read assembly and genotyping are provided in supplementary materials.

The data was analyzed in `MCMCglmm` with the following fixed effects: warming, photoperiod, latitude, as well as pairwise interaction between these. The experimental design was not set up to estimate the three-way interaction and, thus, it was not included in the model. For the random effects, we fitted the same four models as those used in the simulations in term of variance structure. We used the same priors as for the simulated data, but we ran the chains for 100,000 generations after a burnin of 5000 generations, sampling every 20 generations. MCMC run convergence was assessed visually, using the potential scale reduction factors (PRSF; converges to 1 with increasing convergence) and by assessing the estimated sampled sizes. We used the deviance information criterion (DIC) to select the best fitting model.

Results

Simulations

We compared the models based on their accuracy and precision with regards to the estimation of the fixed effects and heredity (h^2). Accuracy measures how close the estimated slope is to the true value and the precision represent the standard deviation of the estimated slope in each MCMC run. The *null* model without genetic correlation structure always performed worst in terms of precision and accuracy, whereas the *intra* and *inter* + *intra* models that included intraspecific correlations performed best (Fig. 1). The *inter* model with only phylogenetic structure did not perform as well as models *intra* and *inter* + *intra*, but its performance improved for increasing relative importance of the phylogenetic structure over the intraspecific structure. All models performed better when the intraspecific structure was less important. For the estimation of proportion of the total variance explained by genetic correlations (h^2), the *inter* + *intra* model was the most accurate (true value was 0.666), although it slightly overestimated the genetic contribution for greater contributions of intraspecific structure. The *inter* model underestimated the genetic structure of the data, but its estimates were greater than the strict interspecific variance included in the simulations. The *intra* model overestimated the total genetic structure of the data even though only the intraspecific structure was modelled.

All models had similar type I error rates (Fig. 2), except for the *intra* model was slightly higher, especially for increasing importance of the phylogenetic structure. The power of the models was similar for $\beta = 0.1$, whereas the models *intra* and *inter* + *intra* had the best power for $\beta = 0.25$. The power of model *inter* with $\beta = 0.25$ improved with increasing importance of phylogenetic effect to reach the performance of *intra* and *inter* + *intra* when the phylogenetic effects was three times as important as the intraspecific effect (i.e., when $\sigma_a^2{:}\sigma_b^2 = 1.5 : 0.5$).

Varying the amount of population structure had little effect on the results (See Supplementary Information; Figs. S1, S2). In contrast, increasing the ratio of the number of species to the number of individuals per species resulted in an improved relative performance of the *inter* model compared

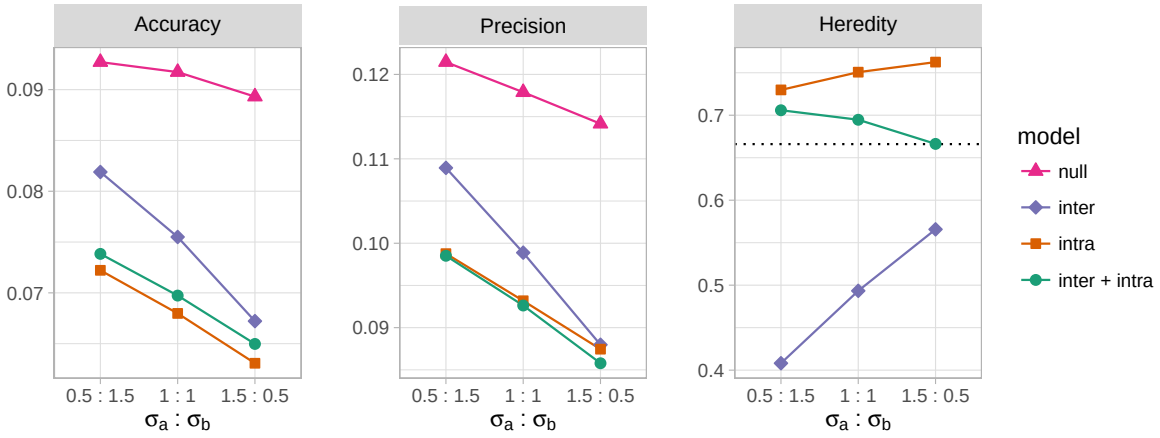

Figure 1: Results of the simulation study for the four variance structure models in terms of slope accuracy and precision, and for estimates of heredity (h^2) with 10 species and 10 individuals per species. Accuracy is the mean absolute distance between the estimated slope ($\hat{\beta}$) and the true slope (β), precision is the mean of the standard deviation of the posterior distribution of $\hat{\beta}$ for each simulation, and h^2 is proportion of the total variance explained by the genetic correlation structure (the dashed line indicates the true value). The x-axis indicates the ratio of phylogenetic (σ_a^2) to intraspecific (σ_b^2) variances used in the simulations. Only the results for $\beta = 0.25$ are shown as these results were not influenced by the slope.

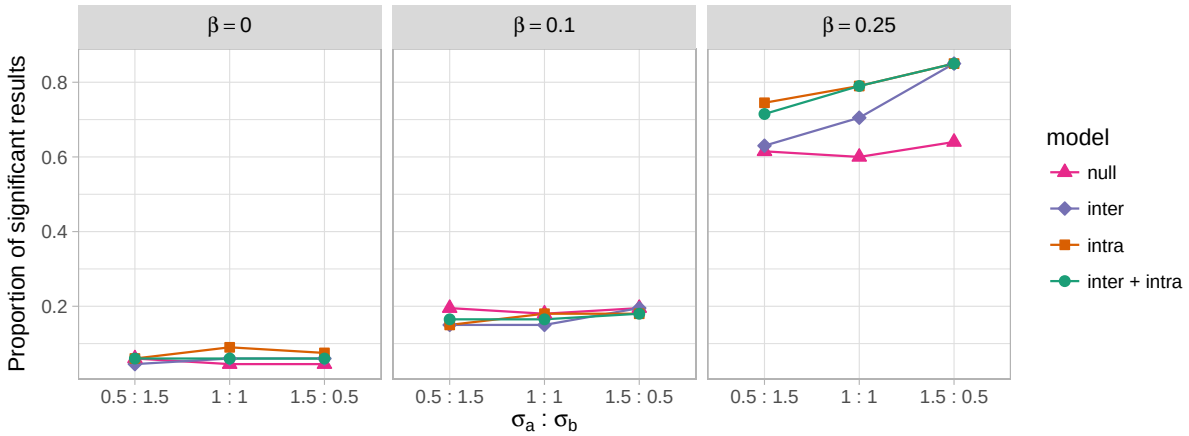

Figure 2: Proportion of the simulations that resulted in a significant regression slope ($\hat{\beta} > 0$) using a threshold of $\alpha = 0.05$. The results for $\beta = 0$ represent the type I error of the models whereas the results with $\beta \in \{0.1, 0.25\}$ represent the power of the models.

to models that included the intraspecific structure, but mostly in terms of accuracy (Figs. S3, S4). Yet, the *inter* model surpassed the *inter* + *intra* model only for accuracy when the ratio of the phylogenetic to the intraspecific variance was equal or greater than 1 ($\sigma_a \geq \sigma_b$). Finally, additional simulations also showed that the advantage of taking into account intraspecific correlations was also present when data was simulated for a single species, that is without phylogenetic effects (Figs. S5, S6).

Budburst data

The number of loci obtained per species ranged from 264 in *Prunus* to 2188 in *Vaccinium* and was broadly correlated with the genome size of species (detailed information on the genetic data is provided in the supplementary materials). Locus-based population structure (Φ_{ST}) between sites were similar across species and ranged from 0.10 to 0.19, suggesting moderate population structure. Similarly, phylogenetic trees built from the genome wide genetic distances showed that individuals from one site were generally more similar to individuals from the same site than to individuals from the other site (see Supplementary Material).

The MCMC runs showed good convergence (PRSF = 1 for fixed and random effects). The model that best fitted the data is *inter* + *intra* according to the DIC (2097.2). The second best model was *intra* (2102.5), followed by *inter* (2137.2) and *null* (2429.5). Incorporating intraspecific structure thus resulted in an important improvement in fit (models *intra* and *inter* + *intra*), while not accounting for any genetic correlation (*null*) clearly resulted in a poorer fit.

The wider posterior intervals obtained for the fixed effects with the *null* model illustrate the importance of taking into account the genetic structure present in the data (Fig. 3). The other models gave similar results, but there was a slight improvement in precision when the intraspecific genetic structure was included; notably, the interaction between the warming and photoperiod was more significant when the intraspecific structure was accounted for. Note that this interaction is not even significant (95% posterior interval includes 0) for the *null* model.

The random effects can be inspected to see how the total variance of the models was partitioned

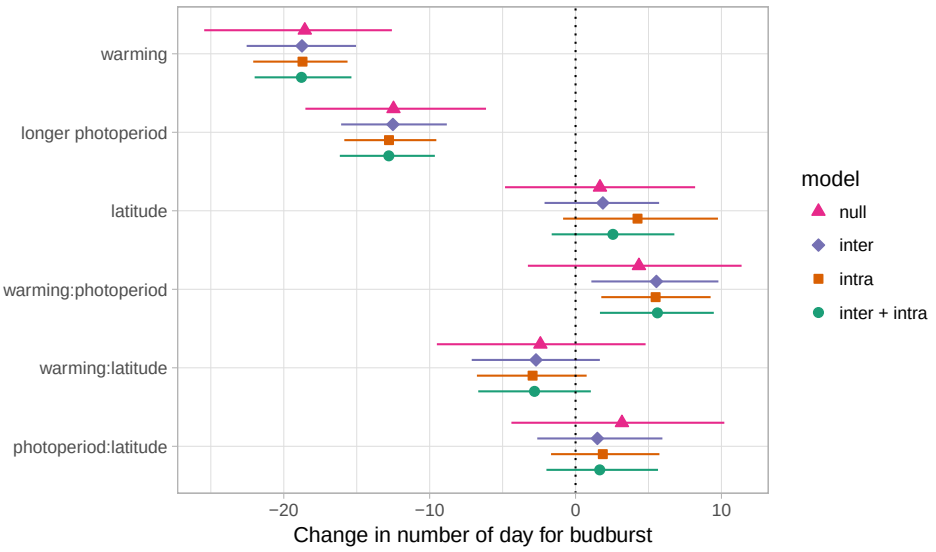

Figure 3: Fixed effects obtained in the phylogenetic mixed model for the four models tested. The symbols represent the means of the posterior distributions and the lines the 95% posterior intervals. The x-axis indicate the change in number of days for budburst, with negative number indicating that buds are opening earlier.

Table 1: Proportion of the total variance explained by the random effects of the models fitted to explain change in days for budburst, with their 95% posterior intervals (in brackets). The heredity (h^2) and the proportion of the total genetic structure due to the intraspecific correlation ($\sigma_b^2/(\sigma_a^2+\sigma_b^2)$) are given for models where they can be estimated.

Models	σ_a^2	σ_b^2	σ_e^2	h^2	$\sigma_b^2/(\sigma_a^2+\sigma_b^2)$
null	–	–	1 [1,1]	–	
inter	0.65 [0.44, 0.86]	–	0.35 [0.14,0.56]	0.65 [0.44,0.85]	
intra	–	0.33 [0.24, 0.43]	0.67 [0.57,0.76]	0.33 [0.24,0.43]	
inter + *intra*	0.65 [0.42, 0.87]	0.044 [0.011, 0.10]	0.30 [0.12,0.51]	0.70 [0.49,0.88]	0.068 [0.013,0.18]

(Table 1). The model where only the phylogenetic structure was incorporated (*inter*) explained 65% of the total variance, which is much more than when only the intraspecific variance was accounted for (*intra*; 33%). When both genetic sources of variance were estimated (model *inter* + *intra*), the h^2 was even greater (70 %) and the proportion explained by the phylogeny remained the same as for the *inter* model with only phylogenetic structure. Although the proportion of the total variance explained by the intraspecific variance is small in model *inter* + *intra* (4.4%), it is significant and helped to reduce the unexplained variance in the model (Table 1). The proportion of the total genetic variance due to the intraspecific structure in the *inter* + *intra* model was estimated to be 6.8%, which is modest but significantly greater than 0.

The results from the best model (*inter* + *intra*) suggest that the 5°C warming treatment had the strongest effect on budburst, followed by a longer (four hours) photoperiod (Fig. 3). The

interaction between these effects was positive and significant, suggesting that they are not additive in effect. Individuals coming from the northern site showed a slight delay in budburst, but this effect was not significant, as were the other interaction terms.

Discussion

Accounting for the intraspecific genetic correlation structure

An increasing number of ecological studies mention the potential importance accounting for intraspecific genetic structure in multi-species studies, yet no study has before directly investigated this question. Our results from simulations and empirical data showed multiple advantages of this approach. Perhaps most importantly, incorporating intraspecific correlation structure in statistical models led to a gain in accuracy and precision of the fixed effects, which are generally the parameters of interest in a study. Both the simulations and the empirical studies highlighted this result. The simulations further showed that this advantage persisted under various conditions, such as when there were not phylogenetic effects (single-species analyses), when the relative importance of the intraspecific structure to the total variance was relatively small and in presence of weak population structure.

The good performance of models incorporating intraspecific correlations in terms of fixed effects occurred because such models partition the total variance in the data—that would otherwise be classified mostly to the error term—to the genetic correlation structure(s). This is shown by the higher values of the variance due to genetic effects (heredity; h^2) in models that included intraspecific genetic correlations (Fig. 1; Table 1). But, in addition to providing more accurate fixed effects parameter estimates, the finer partitioning of the total variance gives a better understanding of the study system by quantifying the proportion of the variance in the model that is due to the genetic structure. Notedly, the improved performance observed when incorporating the intraspecific structure is not due to the specific modelling framework used in this study, namely the phylogenetic mixed model. Indeed, the simulations we performed under a PGLS model (see supplementary ma-

terial) that incorporates intraspecific structure also showed a marked improvement in performance compared to standard ordinary least squares (Figs. S7, S8).

One surprising result was the very good performance of the model that included only the intraspecific correlation structure (*intra*), which performed nearly as well as the model that accounted for both phylogenetic and the intraspecific genetic structures ($inter + intra$) in terms of accuracy and precision. These result may lead some researchers to consider including only the intraspecific structure, but we advise against it. First, the performance of the *intra* model decreased in the presence of stronger intraspecific population structure and when the the importance of the intraspecific correlation structure decreased relative to the phylogenetic variance, both in terms of parameter estimates and power (Figs. S1, S2). Second, the $intra + inter$ model provided more precise estimates of the proportion of the total variance that is genetically structured (Fig. 1), whereas the estimates of *intra* were upwardly biased. And last, as our fundamental biological understanding of ecological questions often stresses the multilevel nature of individuals within species, we argue it is important to include both structures in analyses.

The Phylogenetic Mixed Model

The phylogenetic mixed model (PMM), initially developed for quantitative genetics (Lynch, 1991; Housworth et al., 2004), offers much flexibility compared to other comparative methods (Hadfield and Nakagawa, 2010). As mentioned above, one advantage is that it uses a terminology familiar to most ecologists. The phylogenetic and the intraspecific genetic correlation structures are considered "random effects" in the model. That is, the model assumes that they add variance to the species response in a structured way that can be estimated and removed from the residual error, resulting in improved performance of the model. Further, because the residual variance is estimated by the model (in contrast to models which do not estimate residual variance, see Hadfield and Nakagawa, 2010), model performance is not affected if the intraspecific correlation structure has little effect on the data; in such cases the estimated variance will only be very small.

Another advantage of the PMM is that it allows for the possibility of modeling several random

effects simultaneously (Garamszegi, 2014). In our simulations and empirical example, the total variance of the model included a phylogenetic fraction, an intraspecific fraction, and a residual fraction. But it would be straight-forward to also add a random effect that could account for measurement error, given the appropriate study design. In contrast, the PGLS approach we introduced only considered a phylogenetic and an intraspecific variance with no residual error, which likely explains the increased Type I error compared to the PMM as residual errors were included in the simulations.

Finally, the PMM appears to be particularly well suited for experimental studies that include several fixed effects. In such experiments, each species will have several values for the response variable; at least one per fixed effect. Such a dataset is poorly compatible with some comparative methods for which one would need to multiply the terminal branches of the phylogeny to have—for each species—one tree tip that matches each observation for the response variable. This creates a phylogenetic tree where the tips have very small branch lengths, which can lead to a host of issues (Felsenstein, 2004). The PMM model avoids these unnecessary steps and instead allows to incorporate the phylogenetic correlations structure by associating the species on the phylogeny to the factor representing the species in the dataset (see R scripts in Supplementary Materials). In brief, we only see advantages for ecologists to use PMM when correcting for genetic correlations.

Modelling guidelines

The importance of accounting for intraspecific genetic correlations will depend on the amount of intraspecific structure and its importance relative to the phylogenetic structure. Our results showed that the advantages gained from this approach are more important with greater population structure and when the intraspecific structure variance has greater relative importance compared to the phylogenetic variance (provided you already correct for the phylogenetic structure). The relative importance of the intraspecific structure depends on how closely related the studied species are, whereas the amount of genetic structure will be affected by the (effective) population sizes as well as the divergence time and gene flow between populations (for each species). Small population

sizes and greater population isolation contribute to greater population structure. In our empirical example, the variance explained by the intraspecific correlation structure was small but significant. The modest effect might be due to the fact that the ten species sampled belonged to distinct angiosperm orders, representing important evolutionary distances that may strongly affect the reactions of individuals to the treatments compared to the intraspecific structure. However, the genetic data showed important population structure between the two sampled populations, which probably explains why the $inter + intra$ model clearly better fit the data.

The gain from modelling intraspecific correlation structure explicitly will also depend on how genetics control variation in the traits studied. Traits under strong genetic control will react more closely to the genome-wide correlation structure, and it will be more important to account for it in their analysis. Our empirical study was perhaps not the best to demonstrate the importance of genetic structure as it has recently been reported that spring phenology is particularly plastic across populations (Aitken and Bemmels, 2016). Therefore, it is possible that the study of other traits with a stronger genetic basis (e.g., timing of budset) could have resulted in larger improvements when accounting for the intraspecific correlation structure. Nevertheless, the results observed here are strong enough to show that there are clear advantages to incorporating intraspecific genetic correlation structures in ecological studies, even for plastic traits.

Comparative methods are being increasingly used in ecology to correct for the phylogenetic non-independence of species in statistical tests, in part because of the ease with which one can obtain a well resolved phylogeny, or the genetic data needed to infer the phylogeny of a given set of species. Our results also show that—in addition to accounting for phylogenetic structure—the intraspecific correlation structure should ideally be taken into account when incorporating multiple individuals per species, especially when studying closely related species or when sampling individuals from genetically distinct populations.

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