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On the importance of accounting for intraspecific genomic relatedness in multi-species studies

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Running Head: Intraspecific genetic correlations

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Abstract

1. 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. Comparative approaches that account for the phylogenetic relationship of species—for which the benefits are demonstrated—are well developed. However, the importance of accounting for the intraspecific genetic structure, especially with the phylogenetic structure, is rarely addressed in the ecological literature.

2. We investigated whether it is beneficial to account for intraspecific genomic relatedness in multi-species studies. For this, we used a Phylogenetic Mixed Model to analyze simulated data and results from a budburst experiment where clippings of 10 tree and shrub species were subjected to different treatments in terms of temperature and photoperiod.

3. We found that accounting for intraspecific genomic relatedness yields more accurate and precise fixed effects as well as increased statistical power, but more so when the relative importance of the intraspecific to the phylogenetic genetic structure is greater. Analysis of the budburst 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.

4. Our results show that important statistical gains can be made by incorporating information on the intraspecific genomic relatedness of individuals in multi-species studies. This is relevant for investigations that are interested in intraspecific variation and that plan to include such observations in statistical tests.

Key-words: Phylogenetic mixed models (PMM), genetic structure, intraspecific variation, leaf phenology, climate change, phylogenetic generalized least squares (PGLS).

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Introduction

The reactions of different species to external stimuli are not independent. 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 the assumptions of most statistical tests, such as the independence of residuals in regression, and negatively impacts the results in terms of parameter estimates and p-values (e.g., Revell, 2010). This has been recognized for some time and a family of methods—comparative methods—have been developed to address this problem (Felsenstein, 1985; Grafen, 1989; Lynch, 1991; Ives et al., 2007; Felsenstein, 2008; Revell, 2010; Hadfield and Nakagawa, 2010).

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 increase studies of intraspecific trait variation (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). Similarly, 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; Garamszegi, 2014). Moreover, studies rarely 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.

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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 rapid development of sequencing techniques now allows precise estimation of genetic relatedness (Gienapp et al., 2017), at relatively affordable prices. This could allow a better understanding of how ecological responses are influenced by the genetic relationships among species and populations at once. 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 range shifts of northern versus southern populations' with warming (Anderson et al., 2009; Chen et al., 2011). Such results make clear that the 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. To achieve this, we use the phylogenetic mixed model (PMM) that allows multiple levels of genetic structure to be considered simultaneously (Lynch, 1991; Housworth et al., 2004; Hadfield and Nakagawa, 2010). Other approaches can be used to account for intraspecific correlations (reviewed in Stone et al., 2011; Garamszegi, 2014), but as we show below none currently provides as much flexibility as the PMM. We assess the importance of accounting for intraspecific genetic correlation structure using simulated data and provide a climate change-related empirical example where we investigate the importance of temperature and photoperiod on the timing of budburst of ten tree and shrub species.

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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 and 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

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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 is the heritability (h^2) parameter of quantitative geneticists (Housworth et al. 2006). In the present context, heritability is the quotient obtained by dividing the sum of 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 popular comparative method. A PGLS model that would include phylogenetic and intraspecific correlation structures could be denoted as:

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

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 PGLS residuals are completely structured by the genetic 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). We do not consider this PGLS model further here, but it is further developed and compared to the PMM using simulations in Appendix S1.

PMM simulations

We simulated data under the PMM model (equation 1) assuming $\mu = 0$ with various relative contributions of the phylogenetic and intraspecific variances, and tested how this affected the esti-

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mation of the fixed and random effects. The data simulations followed closely those of Revell (2010) and are described in Appendix S1. 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. In all cases, $\sigma_e^2 = 1$ and $\sigma_x^2 = 2$. 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, specifically $7 : 14$, $10 : 10$ and $14 : 7$.

Model fitting and performance

The PMM model was fitted using the `MCMCglmm` package in R (Hadfield, 2010). The phylogenetic structure was included in the model by giving the phylogeny to the pedigree argument. 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: 1) *null* (with no genetic structure, $\mathbf{y} = \mu + \beta\mathbf{x} + \mathbf{e}$); 2) *inter* (phylogenetic structure only, $\mathbf{y} = \mu + \beta\mathbf{x} + \mathbf{a} + \mathbf{e}$); 3) *intra* (intraspecific genetic structure only, $\mathbf{y} = \mu + \beta\mathbf{x} + \mathbf{b} + \mathbf{e}$); 4) *inter* + *intra* (phylogenetic and intraspecific genetic structures, $\mathbf{y} = \mu + \beta\mathbf{x} + \mathbf{a} + \mathbf{b} + \mathbf{e}$). 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 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 precision represents the standard deviation of the estimated slope in each MCMC run. We also report the number of simulations that gave a posterior probability > 0.95 for the slope to be greater than 0; this estimates the power of the model when $\beta > 0$ and the type I error when $\beta = 0$.

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Budburst experiment

Our simulations allowed us to examine the performance of PMM under a suite of conditions where accounting for intraspecific correlations may be critical to model performance; we also wanted to examine a case using empirical data for a common ecological study design that included both inter and intraspecific variation. Thus we analyzed a subset of a larger experiment, for which we additionally sampled material for genetic analysis. The experiment's objective was to determine the impact of temperature increases and longer photoperiods on the budburst timing for several tree and shrub species (full experiment described in Flynn and Wolkovich, in press). Clippings from 10 species (see Appendix S1) were collected from five individuals at two sites: Harvard Forest (MA, USA; 42.5 °N, 72.2 °W) and the *Station de biologie des Laurentides* (St. Hippolyte, 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 each clipping.

The interspecific phylogenetic structure used a published phylogenetic tree of 32,223 angiosperm species based on 7 genes (Zanne et al., 2014). To estimate the intraspecific genetic correlation structure, we generated thousands of genome-wide anonymous markers per species using a Genotyping-by-Sequencing approach (Elshire et al., 2011) from which we estimated genetic similarities using the `genpofad` method (Joly et al., 2015). Further details on our methods, including sequencing, read assembly and genotyping are provided in Appendix S1.

The data were analyzed in `MCMCglmm` with warming, photoperiod, and their interaction as fixed effects. For the random effects, we fitted the four models used in the simulations in terms of variance structure. We used the same priors as for the simulated data, but ran the chains for 100,000 generations after a burnin of 5000 generations, sampling every 20 generations. MCMC run convergence was assessed using the potential scale reduction factors (PRSF; converges to 1 with increasing convergence). We used the deviance information criterion (DIC) to select the best fitting

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model. Data and commented scripts to replicate the analyses are presented in Appendix S2.

Results

Simulations

The *null* model without genetic correlation structure always performed worst in terms of precision and accuracy, whereas the *intra* and *inter*+*intra* models 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 with increasing relative importance of the phylogenetic structure over the intraspecific structure. The models all performed better when the intraspecific structure was less important. To estimate the proportion of the total variance explained by genetic correlations (h^2), the *inter*+*intra* model was the most accurate (Fig. 1), 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 that 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 the phylogenetic effect and approached the performance of *intra* and *inter* + *intra* when the phylogenetic effect 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 impact on the results (Appendix S1; 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 to models that included the intraspecific structure, but mostly in terms of accuracy (Appendix S1; Figs. S3, S4). Finally, the advantage of taking into account intraspecific genetic correlations was also present

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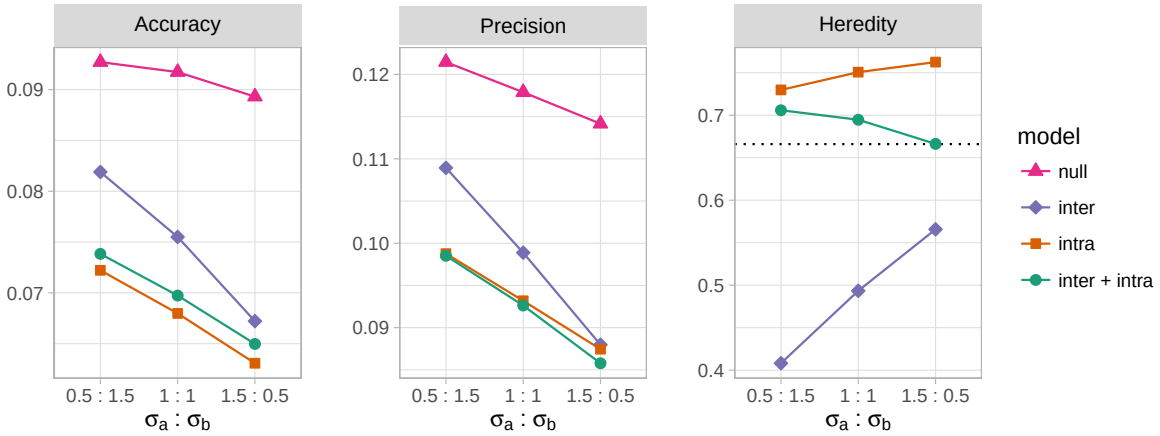

Figure 1: Results of the simulation study for the four variance structure models in terms of slope accuracy and precision, and for estimates of heredity (h^2) with 10 species and 10 individuals per species. Accuracy is the mean absolute distance between the estimated slope ($\hat{\beta}$) and the true 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.

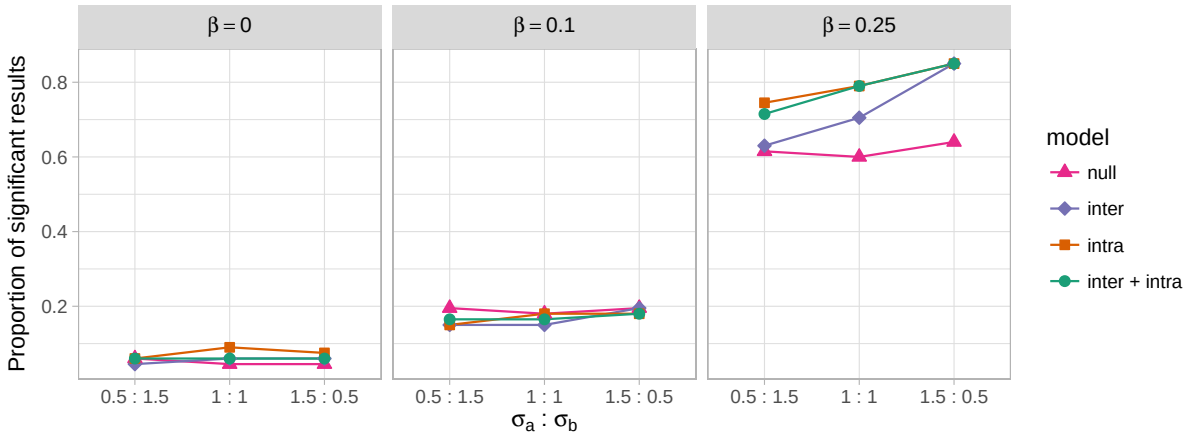

Figure 2: Proportion of the simulations that resulted in a significant regression slope ($\hat{\beta} > 0$) using a threshold of $\alpha = 0.05$. The results for $\beta = 0$ represent the type I error of the models whereas the results with $\beta \in \{0.1, 0.25\}$ represent the power of the models.

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when data were 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 (see Appendix S1 for detailed information on the genetic data). The mean locus-based population structure (Φ_{ST}) between sites was similar across species and ranged from 0.10 to 0.19, suggesting a moderate population structure. Similarly, phylogenetic trees built from the 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 (Appendix S1).

The MCMC runs showed good convergence (PRSF = 1 for fixed and random effects). The model that best fitted the data was $inter + intra$ according to the DIC (2097.3). The second best model was *intra* (2104.6), followed by *inter* (2134.5) and *null* (2426.0). Incorporating intraspecific structure thus resulted in an important improvement in fit (models *intra* and $inter + intra$), while not accounting for 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 warming and photoperiod was estimated with less variance when the intraspecific structure was accounted for, whereas the same interaction had higher variance (and the 95% posterior interval included 0) under the *null* model.

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

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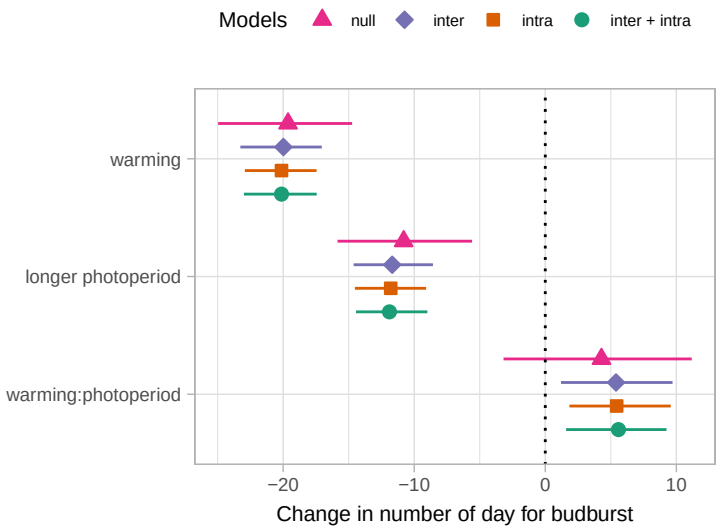

Figure 3: Fixed effects obtained in the phylogenetic mixed model for the four models tested. The symbols represent the means of the posterior distributions and the lines the 95% posterior intervals. The x-axis indicate the change in number of days for budburst, with negative number indicating that buds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

Table 1: Mean 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 [0.44,0.86]	
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]	0.042 [0.011, 0.95]	0.30 [0.12,0.51]	0.70 [0.49,0.88]	0.06 [0.013,0.17]

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.

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Discussion

Accounting for the intraspecific genetic correlation structure

An increasing number of studies mention the potential importance of accounting for intraspecific genetic structure in multi-species studies. 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 no phylogenetic effects (single-species analyses), when the relative importance of the intraspecific structure to the total variance was relatively small, and in the presence of weak population structure.

The models incorporating intraspecific genetic correlations performed well because they 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).

In addition to providing more accurate fixed effects, the finer partitioning of the total variance gives a better understanding of the study system by quantifying the proportion of the variance that is due to the intraspecific genetic structure. Notably, 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 that incorporates intraspecific structure also showed a marked improvement in performance compared to standard ordinary least squares (Appendix S1; 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

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and precision in simulations. This result may lead some researchers to consider including only the intraspecific structure, but we advise against it. First, the relative performance of the *intra* model decreased in the presence of stronger population structure and when the importance of the intraspecific correlation structure decreased relative to the phylogenetic variance (Figs. 1, Appendix S1; Fig. S1). Second, the $inter + intra$ model provided more precise estimates of the proportion of the total variance that is genetically structured in our simulations (Fig. 1) and more precise estimates of fixed effects, even where interspecific variation is large, as shown in our empirical example (Fig. 3). 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) offers more flexibility than other comparative methods (Hadfield and Nakagawa, 2010). 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, similar to how blocks are often treated in a randomized block design. 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 other methods, 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 due to intraspecific structure will simply be small.

Another advantage of the PMM is that it allows modeling several random effects simultaneously (Garamszegi, 2014). In our analyses, 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

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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 is particularly well suited for experimental studies that include several fixed effects. In such experiments, each species has several values for the response variable; at least one per fixed effect. Although such datasets can be analysed using most comparative methods, which often necessitate duplicating the terminal branches of the phylogeny to have—for each species—one tree tip that matches each observation for the response variable, the analysis is much more intuitive with PMM. For instance, the phylogenetic correlation structure can easily be included in the model by associating the species on the phylogeny to the factor representing the species in the dataset (see Appendix S2).

Modelling guidelines

The importance of accounting for intraspecific genomic relatedness will depend on the importance of the intraspecific genetic structure. Our results showed that the advantages gained from this approach are more important with greater population structure (obtained with smaller effective population sizes, restricted gene flow and longer divergence times) and when the intraspecific variance has a greater relative importance compared to the phylogenetic variance (provided that the phylogenetic structure is corrected for). 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 fitted the data.

The gain from modelling intraspecific correlation structure also depends on the genetic nature of the traits studied. Indeed, traits under strong genetic control could react in a way that more closely fit the genetic correlation structure. It has recently been reported that spring phenology is

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particularly plastic across populations (Aitken and Bemmels, 2016), and this might have contributed to the small estimated relative importance of the intraspecific genetic structure. 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.

Comparative methods are being increasingly used 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. Our results show that important gains can also be obtained by accounting for the intraspecific genetic structure.

Data and supplementary information

Additional tables and figures are available in Appendix S1 and commented R scripts in Appendix S2. Raw Genotyping-by-sequencing reads have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession number SRP126957. Further scripts, budburst data and processed sequence data will be deposited in a public archive.

Competing interests

We have no competing interests.

Author's contribution

SJ and EW conceived the work, DFBF and EW collected the data, SJ analysed the data, SJ and EW wrote the manuscript. All authors gave final approval for publication.

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