

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

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Running Head: Intraspecific genetic correlations

1 Abstract

2 1. Analyses in many fields of ecology are increasingly considering multiple species and multiple
3 individuals per species. Premises of statistical tests are often violated with such datasets be-
4 cause of the non-independence of residuals due to phylogenetic relationships or intraspecific
5 population structure. Comparative approaches that account for the phylogenetic relationship
6 of species—for which the benefits are demonstrated—are well developed. However, the im-
7 portance of accounting for the intraspecific genetic structure, especially with the phylogenetic
8 structure, is rarely addressed in the ecological literature.

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

13 3. We found that accounting for intraspecific genomic relatedness yields more accurate and
14 precise fixed effects as well as increased statistical power, but more so when the relative
15 importance of the intraspecific to the phylogenetic genetic structure is greater. Analysis of
16 the budburst experiment further showed that accounting for intraspecific and phylogenetic
17 structures yields improved estimates of warming and photoperiod effects and their interactions
18 in explaining the time to budburst.

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

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

25 Introduction

26 The reactions of different species to external stimuli are not independent. Because physiological
27 responses have a genetic basis, closely related species are more likely to have similar responses to a
28 specific treatment. This phylogenetic non-independence of species responses violates the assump-
29 tions of most statistical tests, such as the independence of residuals in regression, and negatively
30 impacts the results in terms of parameter estimates and p-values (e.g., Revell, 2010). This has been
31 recognized for some time and a family of methods—comparative methods—have been developed to
32 address this problem (Felsenstein, 1985; Grafen, 1989; Lynch, 1991; Ives et al., 2007; Felsenstein,
33 2008; Revell, 2010; Hadfield and Nakagawa, 2010).

34 Recently there has been growing awareness among ecologists of the need to also consider in-
35 traspecific variation within ecophylogenetic analyses. Within community ecology, there have been
36 calls to increase studies of intraspecific trait variation (Violle et al., 2012; Alofs, 2016) and to de-
37 velop the necessary statistical models for such multilevel data (Funk et al., 2017; Read et al., 2016).
38 Similarly, studies of climate change have repeatedly highlighted the need for models that incorpo-
39 rate variation in responses across both species and populations (Willis et al., 2008; Charmantier
40 et al., 2008; Anderson et al., 2009; Chen et al., 2011). However, little attention has been given to the
41 genetic correlation structure present below the species level within the field of comparative meth-
42 ods (but see Felsenstein, 2002; Stone et al., 2011; Read et al., 2016; Garamszegi, 2014). Moreover,
43 studies rarely account for both phylogenetic and intraspecific genetic correlations simultaneously,
44 even though the sampling structure in many ecological studies calls for such a design.

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

51 Until recently, the difficulty of obtaining genetic data to accurately estimate intraspecific genetic
52 correlations provided sufficient justification for ignoring this source of variance in ecological stud-
53 ies. But the rapid development of sequencing techniques now allows precise estimation of genetic
54 relatedness (Gienapp et al., 2017), at relatively affordable prices. This could allow a better under-
55 standing of how ecological responses are influenced by the genetic relationships among species and
56 populations at once. One area where this potential is particularly high is climate change research,
57 where evidence of rapid ecological and evolutionary change is growing. Research has highlighted
58 that species responses to climate change appear phylogenetically patterned, with species from cer-
59 tain clades and with particular traits appearing most vulnerable to local extinctions with warming
60 (Willis et al., 2008). At the same time other work has highlighted discrepancies in species re-
61 sponses when studied over space (Charmantier et al., 2008), suggesting populations within species
62 may show different responses to climate change. This is supported by population-level research
63 that has found large differences in the range shifts of northern versus southern populations' with
64 warming (Anderson et al., 2009; Chen et al., 2011). Such results make clear that the best estimates
65 of responses will need methods that consider variation at both the species and population levels,
66 and the connections between different populations and different species, all at once.

67 The objectives of this report are to assess the importance of accounting for intraspecific genetic
68 correlations in ecological studies. To achieve this, we use the phylogenetic mixed model (PMM)
69 that allows multiple levels of genetic structure to be considered simultaneously (Lynch, 1991; Hous-
70 worth et al., 2004; Hadfield and Nakagawa, 2010). Other approaches can be used to account for
71 intraspecific correlations (reviewed in Stone et al., 2011; Garamszegi, 2014), but as we show below
72 none currently provides as much flexibility as the PMM. We assess the importance of accounting
73 for intraspecific genetic correlation structure using simulated data and provide a climate change-
74 related empirical example where we investigate the importance of temperature and photoperiod on
75 the timing of budburst of ten tree and shrub species.

76 **Methods**

77 **The phylogenetic mixed model**

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

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

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

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

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

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

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

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

95 The PMM allows the estimation of the proportion of the total variance ($\sigma^2 = \sigma_a^2 + \sigma_b^2 + \sigma_e^2$) that
96 is due to the genetic structure; this is the heritability (h^2) parameter of quantitative geneticists
97 (Housworth et al. 2006). In the present context, heritability is the quotient obtained by dividing
98 the sum of the phylogenetic and intraspecific variances by the total variance: $h^2 = (\sigma_a^2 + \sigma_b^2)/\sigma^2$.
99 The remaining variance, $1 - h^2 = \sigma_e^2/\sigma^2$, is the non-genetic variance that could be due to the
100 environment or other effects that impact the individuals in a way that is not defined by the genetic
101 correlation structures. The PMM also allow the estimation of the relative contribution of the
102 intraspecific correlation structure to the total genetic structure, which is $\sigma_b^2/(\sigma_a^2 + \sigma_b^2)$.

103 **Phylogenetic generalized least squares**

104 A brief mention of PGLS seems important as it is a popular comparative method. A PGLS model
105 that would include phylogenetic and intraspecific correlation structures could be denoted as:

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

106 In other words, the residuals of the regression are normally distributed according to a correlation
107 structure that is a combination of phylogenetic and intraspecific effects, with respective weights
108 determined by the parameter δ . The main difference with the PMM is the absence of a residual
109 term: in PGLS residuals are completely structured by the genetic correlation matrices provided.
110 This assumption can be relaxed by rescaling the phylogenetic tree to give more or less weight to
111 the terminal branches of the tree (Revell, 2010). We do not consider this PGLS model further here,
112 but it is further developed and compared to the PMM using simulations in Appendix S1.

113 **PMM simulations**

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

116 mation of the fixed and random effects. The data simulations followed closely those of Revell (2010)
117 and are described in Appendix S1. The simulations were performed for different regression slopes
118 $\beta \in \{0, 0.1, 0.25\}$ and different ratios of intraspecific and interspecific structure ($\sigma_a^2 : \sigma_b^2$) while
119 keeping their sum to 2. In all cases, $\sigma_e^2 = 1$ and $\sigma_x^2 = 2$. Five hundred simulations were performed
120 for each parameter combination. We simulated data with 98 or 100 individuals but different ratios
121 of species vs. individuals per species, specifically 7 : 14, 10 : 10 and 14 : 7.

122 **Model fitting and performance**

123 The PMM model was fitted using the `MCMCglmm` package in R (Hadfield, 2010). The phyloge-
124 netic structure was included in the model by giving the phylogeny to the pedigree argument. The
125 intraspecific structure was incorporated using the single value decomposition of the genetic intraspe-
126 cific correlation structure matrix, following Stone et al. (2011). We used the default priors for the
127 fixed effects and diffuse inverse-Wishart priors for the random effects with $V = 1$ and $\nu = 0.002$.
128 We fitted the following models, named according to their respective random effects: 1) *null* (with
129 no genetic structure, $\mathbf{y} = \mu + \beta\mathbf{x} + \mathbf{e}$); 2) *inter* (phylogenetic structure only, $\mathbf{y} = \mu + \beta\mathbf{x} + \mathbf{a} + \mathbf{e}$);
130 3) *intra* (intraspecific genetic structure only, $\mathbf{y} = \mu + \beta\mathbf{x} + \mathbf{b} + \mathbf{e}$); 4) *inter + intra* (phylogenetic
131 and intraspecific genetic structures, $\mathbf{y} = \mu + \beta\mathbf{x} + \mathbf{a} + \mathbf{b} + \mathbf{e}$). The MCMC chains were run for 2100
132 generations, removing the first 1000 as burnin and sampling the chain every 10 generations. These
133 settings provided good convergence for all models and all simulation parameters.

134 We compared the models based on their accuracy and precision with regards to the estimation
135 of the fixed effects and heredity (h^2). Accuracy measures how close the estimated slope is to the
136 true value and precision represents the standard deviation of the estimated slope in each MCMC
137 run. We also report the number of simulations that gave a posterior probability > 0.95 for the
138 slope to be greater than 0; this estimates the power of the model when $\beta > 0$ and the type I error
139 when $\beta = 0$.

140 **Budburst experiment**

141 Our simulations allowed us to examine the performance of PMM under a suite of conditions where
142 accounting for intraspecific correlations may be critical to model performance; we also wanted to
143 examine a case using empirical data for a common ecological study design that included both
144 inter and intraspecific variation. Thus we analyzed a subset of a larger experiment, for which we
145 additionally sampled material for genetic analysis. The experiment's objective was to determine
146 the impact of temperature increases and longer photoperiods on the budburst timing for several
147 tree and shrub species (full experiment described in Flynn and Wolkovich, in press). Clippings from
148 10 species (see Appendix S1) were collected from five individuals at two sites: Harvard Forest (MA,
149 USA; 42.5 °N, 72.2 °W) and the *Station de biologie des Laurentides* (St. Hippolyte, QC, Canada;
150 45.9 °N, 74.0 °W). Clippings were collected in January 2015 and kept cold until the start of the
151 experiment. They were then subjected to different temperatures (15 °C or 20 °C) and photoperiods
152 (8 or 12 hours of light per day) in growth chambers at the Arnold Arboretum. The number of days
153 to budburst was recorded for each clipping.

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

160 The data were analyzed in `MCMCglmm` with warming, photoperiod, and their interaction as fixed
161 effects. For the random effects, we fitted the four models used in the simulations in terms of
162 variance structure. We used the same priors as for the simulated data, but ran the chains for
163 100,000 generations after a burnin of 5000 generations, sampling every 20 generations. MCMC run
164 convergence was assessed using the potential scale reduction factors (PSRF; converges to 1 with
165 increasing convergence). We used the deviance information criterion (DIC) to select the best fitting

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

167 Results

168 Simulations

169 The *null* model without genetic correlation structure always performed worst in terms of precision
170 and accuracy, whereas the *intra* and *inter+intra* models performed best (Fig. 1). The *inter* model
171 with only phylogenetic structure did not perform as well as models *intra* and *inter+intra*, but its
172 performance improved with increasing relative importance of the phylogenetic structure over the
173 intraspecific structure. The models all performed better when the intraspecific structure was less
174 important. To estimate the proportion of the total variance explained by genetic correlations (h^2),
175 the *inter+intra* model was the most accurate (Fig. 1), although it slightly overestimated the genetic
176 contribution for greater contributions of intraspecific structure. The *inter* model underestimated
177 the genetic structure of the data, but its estimates were greater than the strict interspecific variance
178 included in the simulations. The *intra* model overestimated the total genetic structure of the data
179 even though only the intraspecific structure was modelled.

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

186 Varying the amount of population structure had little impact on the results (Appendix S1; Figs.
187 S1, S2). In contrast, increasing the ratio of the number of species to the number of individuals
188 per species resulted in an improved relative performance of the *inter* model compared to models
189 that included the intraspecific structure, but mostly in terms of accuracy (Appendix S1; Figs. S3,
190 S4). Finally, the advantage of taking into account intraspecific genetic correlations was also present

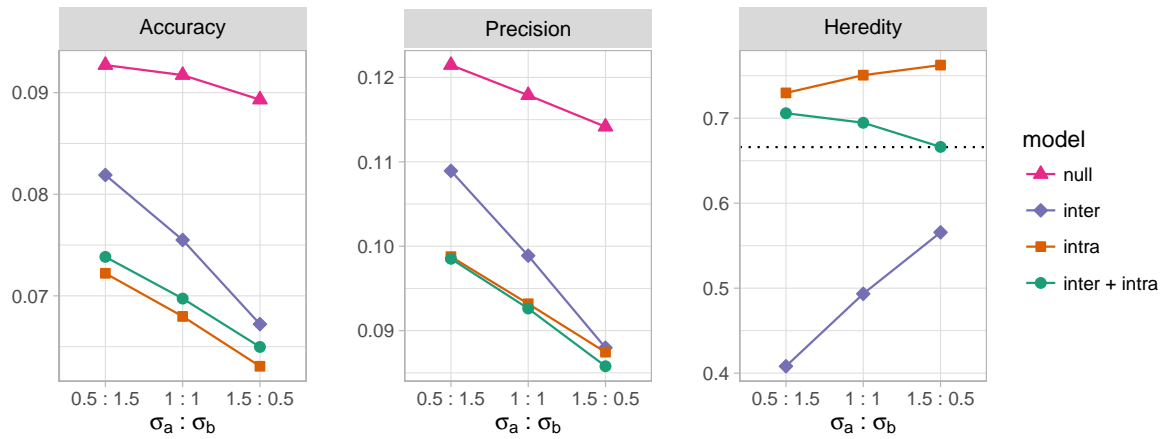


Figure 1: Results of the simulation study for the four variance structure models in terms of slope accuracy and precision, and for estimates of heredity (h^2) with 10 species and 10 individuals per species. Accuracy is the mean absolute distance between the estimated slope ($\hat{\beta}$) and the true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

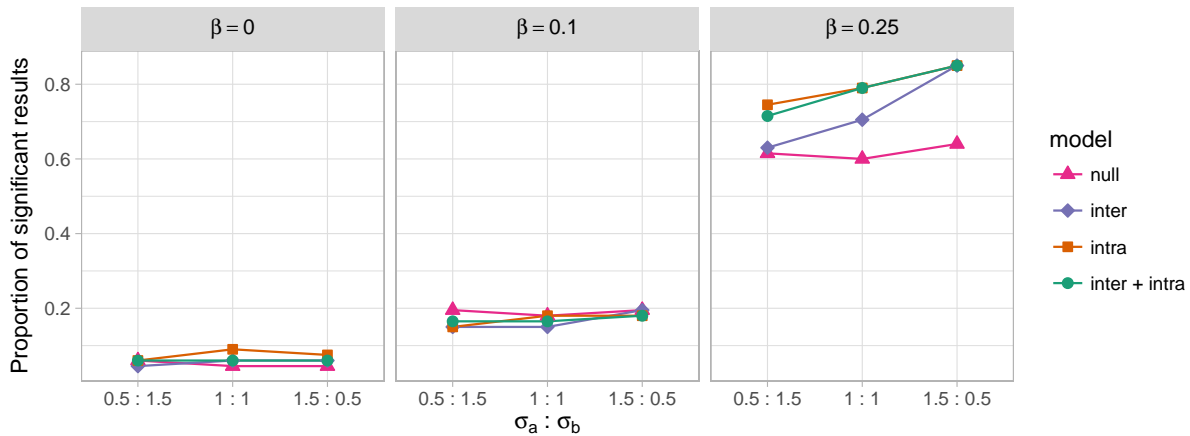


Figure 2: Proportion of the simulations that resulted in a significant regression slope ($\hat{\beta} > 0$) using a threshold of $\alpha = 0.05$. The results for $\beta = 0$ represent the type I error of the models whereas the results with $\beta \in \{0.1, 0.25\}$ represent the power of the models.

191 when data were simulated for a single species, that is without phylogenetic effects (Figs. S5, S6).

192 **Budburst data**

193 The number of loci obtained per species ranged from 264 in *Prunus* to 2188 in *Vaccinium* and
194 was broadly correlated with the genome size of species (see Appendix S1 for detailed information
195 on the genetic data). The mean locus-based population structure (Φ_{ST}) between sites was similar
196 across species and ranged from 0.10 to 0.19, suggesting a moderate population structure. Similarly,
197 phylogenetic trees built from the genetic distances showed that individuals from one site were
198 generally more similar to individuals from the same site than to individuals from the other site
199 (Appendix S1).

200 The MCMC runs showed good convergence (PRSF = 1 for fixed and random effects). The
201 model that best fitted the data was *inter + intra* according to the DIC (2097.3). The second best
202 model was *intra* (2104.6), followed by *inter* (2134.5) and *null* (2426.0). Incorporating intraspecific
203 structure thus resulted in an important improvement in fit (models *intra* and *inter + intra*), while
204 not accounting for genetic correlation (*null*) clearly resulted in a poorer fit.

205 The wider posterior intervals obtained for the fixed effects with the *null* model illustrate the
206 importance of taking into account the genetic structure present in the data (Fig. 3). The other
207 models gave similar results, but there was a slight improvement in precision when the intraspecific
208 genetic structure was included. Notably, the interaction between warming and photoperiod was
209 estimated with less variance when the intraspecific structure was accounted for, whereas the same
210 interaction had higher variance (and the 95% posterior interval included 0) under the *null* model.

211 The random effects can be inspected to see how the total variance of the models was partitioned
212 (Table 1). The model where only the phylogenetic structure was incorporated (*inter*) explained 65%
213 of the total variance, which is much more than when only the intraspecific variance was accounted
214 for (*intra*; 33%). When both genetic sources of variance were estimated (model *inter + intra*), the
215 h^2 was even greater (70 %) and the proportion explained by the phylogeny remained the same as
216 for the *inter* model with only phylogenetic structure. Although the proportion of the total variance

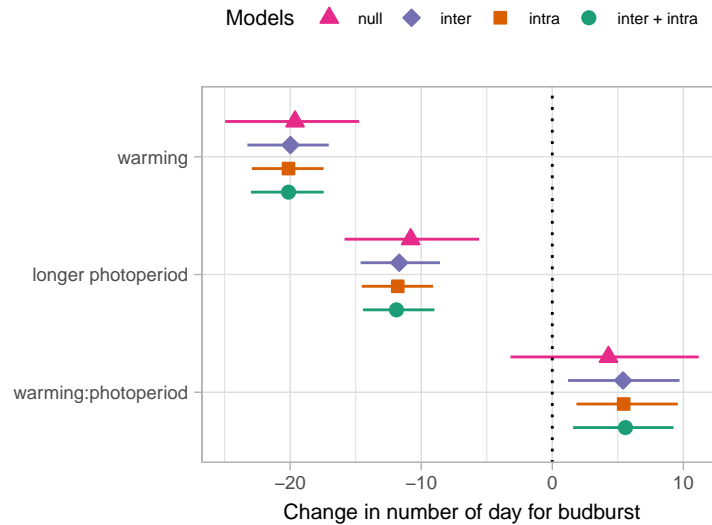


Figure 3: Fixed effects obtained in the phylogenetic mixed model for the four models tested. The symbols represent the means of the posterior distributions and the lines the 95% posterior intervals. The x-axis indicate the change in number of days for budburst, with negative number indicating that buds 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	$\sigma_b^2/(\sigma_a^2 + \sigma_b^2)$
<i>null</i>	–	–	1 [1,1]	–	–
<i>inter</i>	0.65 [0.44, 0.86]	–	0.35 [0.14,0.56]	0.65 [0.44,0.86]	–
<i>intra</i>	–	0.33 [0.24, 0.43]	0.67 [0.57,0.76]	0.33 [0.24,0.43]	–
<i>inter + intra</i>	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]

217 explained by the intraspecific variance is small in model *inter + intra* (4.4%), it is significant and
 218 helped to reduce the unexplained variance in the model (Table 1). The proportion of the total
 219 genetic variance due to the intraspecific structure in the *inter + intra* model was estimated to be
 220 6.8%, which is modest but significantly greater than 0.

221 The results from the best model (*inter + intra*) suggest that the 5°C warming treatment had
 222 the strongest effect on budburst, followed by a longer (four hours) photoperiod (Fig. 3). The
 223 interaction between these effects was positive and significant, suggesting that they are not additive.

224 Discussion

225 Accounting for the intraspecific genetic correlation structure

226 An increasing number of studies mention the potential importance of accounting for intraspecific
227 genetic structure in multi-species studies. Our results from simulations and empirical data showed
228 multiple advantages of this approach. Perhaps most importantly, incorporating intraspecific cor-
229 relation structure in statistical models led to a gain in accuracy and precision of the fixed effects,
230 which are generally the parameters of interest in a study. Both the simulations and the empirical
231 studies highlighted this result. The simulations further showed that this advantage persisted under
232 various conditions, such as when there were no phylogenetic effects (single-species analyses), when
233 the relative importance of the intraspecific structure to the total variance was relatively small, and
234 in the presence of weak population structure.

235 The models incorporating intraspecific genetic correlations performed well because they parti-
236 tion the total variance in the data—that would otherwise be classified mostly to the error term—to
237 the genetic correlation structure(s). This is shown by the higher values of the variance due to ge-
238 netic effects (heredity; h^2) in models that included intraspecific genetic correlations (Fig. 1; Table
239 1).

240 In addition to providing more accurate fixed effects, the finer partitioning of the total variance
241 gives a better understanding of the study system by quantifying the proportion of the variance that
242 is due to the intraspecific genetic structure. Notably, the improved performance observed when
243 incorporating the intraspecific structure is not due to the specific modelling framework used in this
244 study, namely the phylogenetic mixed model. Indeed, the simulations we performed under a PGLS
245 model that incorporates intraspecific structure also showed a marked improvement in performance
246 compared to standard ordinary least squares (Appendix S1; Figs. S7, S8).

247 One surprising result was the very good performance of the model that included only the in-
248 traspecific correlation structure (*intra*), which performed nearly as well as the model that accounted
249 for both phylogenetic and the intraspecific genetic structures (*inter + intra*) in terms of accuracy

250 and precision in simulations. This result may lead some researchers to consider including only the
251 intraspecific structure, but we advise against it. First, the relative performance of the *intra* model
252 decreased in the presence of stronger population structure and when the importance of the intraspe-
253 cific correlation structure decreased relative to the phylogenetic variance (Figs. 1, Appendix S1;
254 Fig. S1). Second, the *inter + intra* model provided more precise estimates of the proportion of the
255 total variance that is genetically structured in our simulations (Fig. 1) and more precise estimates
256 of fixed effects, even where interspecific variation is large, as shown in our empirical example (Fig.
257 3). And last, as our fundamental biological understanding of ecological questions often stresses the
258 multilevel nature of individuals within species, we argue it is important to include both structures
259 in analyses.

260 **The Phylogenetic Mixed Model**

261 The phylogenetic mixed model (PMM) offers more flexibility than other comparative methods
262 (Hadfield and Nakagawa, 2010). One advantage is that it uses a terminology familiar to most
263 ecologists. The phylogenetic and the intraspecific genetic correlation structures are considered
264 “random effects” in the model, similar to how blocks are often treated in a randomized block
265 design. That is, the model assumes that they add variance to the species response in a structured
266 way that can be estimated and removed from the residual error, resulting in improved performance
267 of the model. Further, because the residual variance is estimated by the model (in contrast to other
268 methods, see Hadfield and Nakagawa, 2010), model performance is not affected if the intraspecific
269 correlation structure has little effect on the data; in such cases the estimated variance due to
270 intraspecific structure will simply be small.

271 Another advantage of the PMM is that it allows modeling several random effects simultaneously
272 (Garamszegi, 2014). In our analyses, the total variance of the model included a phylogenetic
273 fraction, an intraspecific fraction, and a residual fraction. But it would be straight-forward to also
274 add a random effect that could account for measurement error, given the appropriate study design.
275 In contrast, the PGLS approach we introduced only considered a phylogenetic and an intraspecific

276 variance with no residual error, which likely explains the increased Type I error compared to the
277 PMM as residual errors were included in the simulations.

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

286 **Modelling guidelines**

287 The importance of accounting for intraspecific genomic relatedness will depend on the importance
288 of the intraspecific genetic structure. Our results showed that the advantages gained from this
289 approach are more important with greater population structure (obtained with smaller effective
290 population sizes, restricted gene flow and longer divergence times) and when the intraspecific vari-
291 ance has a greater relative importance compared to the phylogenetic variance (provided that the
292 phylogenetic structure is corrected for). In our empirical example, the variance explained by the
293 intraspecific correlation structure was small but significant. The modest effect might be due to
294 the fact that the ten species sampled belonged to distinct angiosperm orders, representing impor-
295 tant evolutionary distances that may strongly affect the reactions of individuals to the treatments
296 compared to the intraspecific structure. However, the genetic data showed important population
297 structure between the two sampled populations, which probably explains why the *inter + intra*
298 model clearly better fitted the data.

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

302 particularly plastic across populations (Aitken and Bemmels, 2016), and this might have contributed
303 to the small estimated relative importance of the intraspecific genetic structure. It is possible that
304 the study of other traits with a stronger genetic basis (e.g., timing of budset) could have resulted
305 in larger improvements when accounting for the intraspecific correlation structure.

306 Comparative methods are being increasingly used to correct for the phylogenetic non-independence
307 of species in statistical tests, in part because of the ease with which one can obtain a well resolved
308 phylogeny. Our results show that important gains can also be obtained by accounting for the
309 intraspecific genetic structure.

310 **Data and supplementary information**

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

315 **Competing interests**

316 We have no competing interests.

317 **Author's contribution**

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

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