Fixed or random? On the reliability of mixed-effects models for a small number of levels in grouping variables

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Abstract

1. Biological data are often intrinsically hierarchical. Due to their ability to account for such dependencies, mixed-effects models have become a common analysis technique in ecology and evolution. While many questions around their theoretical foundations and practical applications are solved, one fundamental question is still highly debated: When having a low number of levels should we model a grouping variable as a random or fixed effect? In such situation, the variance of the random effect is presumably underestimated, but whether this affects the statistical properties of the ecological effects (fixed effects) is unclear.
2. Here, we analyzed the consequences of including a grouping variable as fixed or random effect and possible other modeling options (too simple or too complex models) for data with small number of levels in the grouping variable (2 - 8). For all models, we calculated type I error rates, power and coverage. Moreover, we show how these statistical properties change with the study design.
3. We found that mixed-effects models already correctly estimate the variance of a random effect with only two groups. Moreover, model choice does not influence the statistical properties of the ecological effect when there is no random slope in the data-generating process. However, if an ecological effect differs among groups, using a random slope and intercept model, and switching to a fixed-effect model only in case of a singular fit, avoids overconfidence in the results. Additionally, power and type I error are strongly influenced by the number of and difference between groups.
4. We conclude that inferring the correct random effect structure is of high importance to get correct statistical properties. When in doubt, we recommend starting with the simpler model and using model diagnostics to identify missing components. When

having identified the correct structure, we encourage to start with a mixed-effects model independent of the number of groups and switch to a fixed-effect model only in case of a singular fit. With these recommendations, we allow for more informative choices about study design and data analysis and thus make ecological inference with mixed-effects models more robust for small number of groups.

Introduction

Many biological data have a hierarchical grouping structure that introduces dependencies among observations (McMahon & Diez 2007; Bolker *et al.* 2009; Zuur *et al.* 2009; Harrison *et al.* 2018). When conducting statistical analyses, models have to reflect these dependencies in their assumptions for correct statistical properties (Arnqvist 2020), a task for which linear and generalized mixed-effects models (LMMs or GLMMs) were designed (Laird & Ware 1982; Chen & Dunson 2003). Mixed-effects models have also replaced ANOVAs as the common tool for variance analysis (Wainwright *et al.* 2007; Boisgontier & Cheval 2016) because they allow simultaneous analysis of variance at different hierarchical levels (Krueger & Tian 2004; Boisgontier & Cheval 2016), their better handling of unbalanced study designs (Swallow & Monahan 1984; Lindstrom & Bates 1988; Pinheiro & Bates 1995; Littell 2002) , and their better statistical properties for missing data (Baayen *et al.* 2008). Therefore, mixed-effects models have become one of the most popular methods in ecology and evolution (Bolker *et al.* 2009) and other fields (e.g. psychology, see Meteyard & Davies 2020).

The ability of mixed-effects models to adapt to different data structures (i.e. their flexibility to handle many different hierarchical structures, see Box 1), which makes them in the first place so powerful (Wainwright *et al.* 2007), raises also discussions about their proper application (Nakagawa & Schielzeth 2013). These issues include data-related problems such as overdispersion (Harrison 2014, 2015), robustness to wrong distributional assumptions (Schielzeth *et al.* 2020), and their correct evaluation (Nakagawa & Schielzeth 2013). Despite these rather technical challenges, there are also application-oriented issues (Harrison *et al.* 2017; Meteyard & Davies 2020) such as the question whether to use the

most complex random effect structure (Barr *et al.* 2013; but see Matuschek *et al.* 2017) or in which situations we should model a grouping variable as random or fixed effect (Harrison *et al.* 2018).

A priori, modeling a grouping variable as fixed or random effect are equally well suited for multilevel analysis (Townsend *et al.* 2013) and strict rules don't exist because the best strategy generally depends on the goal of the analysis (Gelman & Hill 2007, see Box 2). There are, however, technical differences between these two options (Millar & Anderson 2004). The most prominent difference is that modeling a grouping variable as a random effect implicitly makes the assumption that the individual levels of the grouping variable are realizations of a common distribution, usually a normal distribution, for which the standard deviation is unknown and needs to be estimated (e.g. DerSimonian & Laird 1986). This assumption shrinks the estimates of each random effect level to the mean of the underlying distribution. In contrast, modeling a grouping variable as a fixed effect makes no distributional assumptions about the individual level estimates. Thus, compared to modeling the grouping variable as a fixed effect, the random effect model uses fewer degrees of freedom (e.g. Schelldorfer *et al.* 2011), which leads to higher power to detect significant effects on the remaining fixed effects (p-values are inversely proportional to degrees of freedom) at the cost of a small bias towards zero on the random effect estimates (e.g. Johnson et al. 2014).

Based on these properties, random-effect modeling seems preferable over fixed-effect modeling if the estimates of the grouping variable are not of particular interest, or if the modeler is willing to accept the normal distribution assumption on those estimates. It is, however, not clear if these advantages remain when the number of groups is small (cf. also

Harrison *et al.* 2018), because this might cause a wrong estimate of the random effects' standard deviation (Harrison, 2018), which then could influence other estimates of the mixed-effects model (e.g. the fixed effects, Hox *et al.* 2017).

The ecological literature suggests, as a rule of thumb, to only fit a grouping variable as random effect if it has a minimum of five, sometimes eight, groups to properly estimate the standard deviation of the random effect (Bolker 2015; Harrison 2015; Harrison *et al.* 2018). With four or fewer levels in the grouping variable, fitting it as a fixed effect is recommended as the preferred alternative (Gelman & Hill 2007; Bolker *et al.* 2009; Bolker 2015). In other disciplines other thresholds are proposed, e.g. 10-20 in psychology (McNeish & Stapleton, 2016) or 30-50 in sociology (Maas & Hox, 2005). To our knowledge, however, none of these values were based on a systematic analysis of how the choice of the grouping variable affects the statistical estimates (i.e. type I error rate, power, and coverage) of other effects of interest in the model (i.e. the slope effect of some explanatory variable, henceforth called the ecological effect).

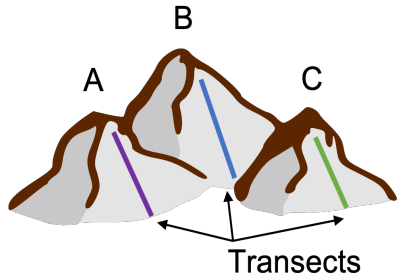
Here, we simulated a hypothetical unbalanced study design on reproductive success and height of a plant on a temperature gradient (altitudinal gradient among different mountains (levels), see Box 1) with two in complexity increasing data-generating processes to compare statistical properties with a varying number of groups (two to eight mountains). To represent the challenge of an analyst to correctly specify the model structure and the consequences if not, we additionally tested miss-specified models (too complex or too simple versions of the fixed and mixed-effects models). To investigate the consequences of these modeling choices on the ecological effect with different number of levels in the grouping variable, we compared: type I error rates (how often a non-existing temperature effect would be

interpreted as existing), statistical power (the rate at which a truly existing temperature effect would be interpreted as existing), and statistical coverage (i.e. how often the true effect of temperature falls into the 95% confidence interval of the model). Based on our results, we give practical recommendations on when to include grouping variables as random effects and in which situations it might be more beneficial to include them as fixed effects.

Box 1: Example of an ecological study design with hierarchical/grouping variables

Sampling design. We want to understand the effect of temperature on the reproductive success of a plant that grows in mountains. We hypothesize that: (H1) increasing temperature (lower altitude) increases the probability of flowering (reproduction), and (H2) the height of flowering plants. To do so, we establish altitudinal transects in many mountains (populations) and collect information from a certain number of plants.

Sampling design

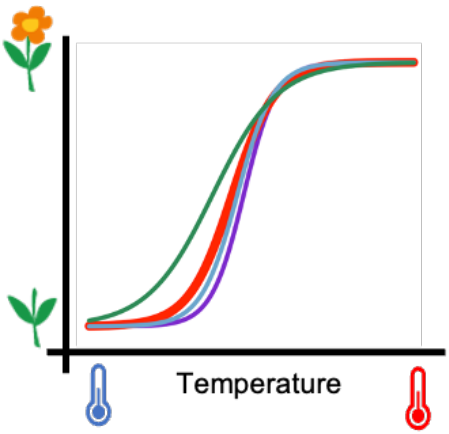


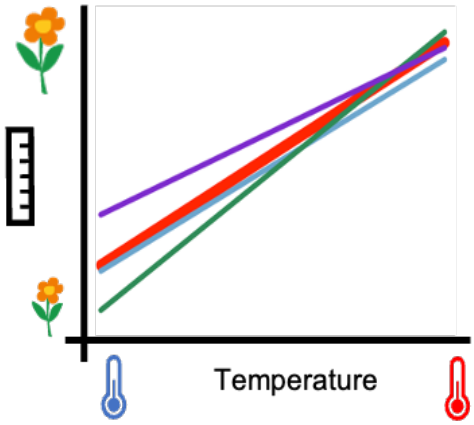
Problem. The transects are not in the same geographical alignment, the type of soil varies in each mountain, and the plants are genetically very distinct among populations. All these factors introduce differences among populations that are not exactly of our interest (given our hypotheses), but statistically, plants of the same mountain are non-independent observations. The mountains can be considered as grouping, blocking or control variables.

Modeling options. We may use a mixed-effects model with a random intercept and slope (Box 2) for mountain to account for the differences among populations (colored lines in H1 and H2), while still modeling the relationship of interest as fixed effects (red lines). An alternative may be to use a simple fixed-effects model, i.e., to include mountain as a categorical predictor (Box 2).

Hypothesis 1 - The reproductive success (flowering or not) of plants increases with temperature.

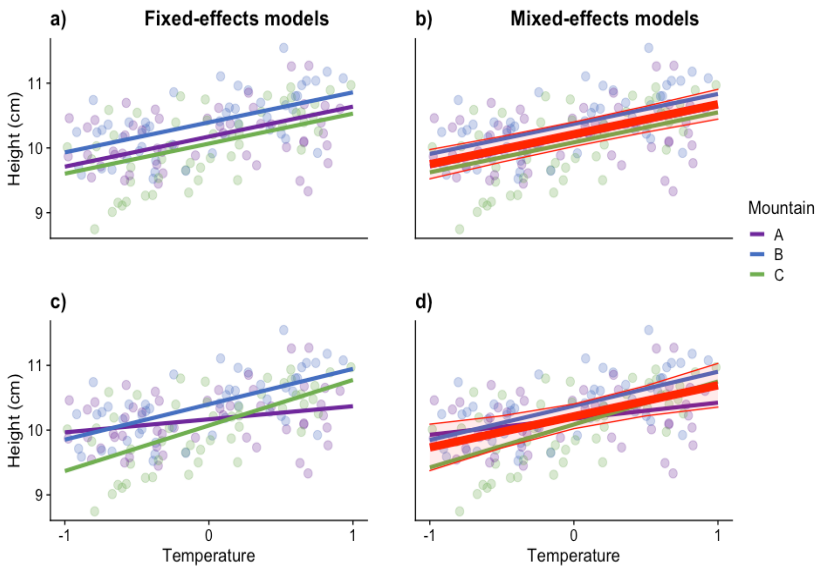
Hypothesis 2 - The height of flowering plants increases with temperature.





Box 2: Differences between modeling a grouping variable as random or fixed effect

Fixed or random effects. The question of whether to include a hierarchical/grouping variable as a random or fixed effect in the analysis depends on several factors. Fixed effects are usually used when the analysts are interested in the individual level estimates of a grouping variable (Bolker *et al.* 2009) and these are independent, mutually exclusive, and completely observed (e.g. control and treatment in experiments, male and female when analyzing differences between sex) (e.g. Hedges & Vevea 1998; Gunasekara *et al.* 2014). Random effects, in comparison, are modeling choices when the variance between the different levels is of interest (Bolker *et al.* 2009) and not the exact estimates of the different levels (e.g. DerSimonian & Laird 1986). Additionally, random effects can be used when not every realization of the underlying mechanism can be observed (e.g. species across a number of observational sites in different geographic areas) but the analysts want to control for its influence (i.e. pseudo-replication, see Arnqvist 2020).



F
intercept (a,b) and random intercept and slope (c,d) for each mountain in the example from H2 Box 1. Dots represent the data points, lines the individual estimates for each mountain. The red line and shaded red intervals are the mean and its estimated standard deviation for mixed-effects models.

Technical differences between random and fixed effects. When specifying a grouping variable as fixed effect, the model estimates the effect of one reference level and, for the default contrast method (see Schielzeth 2010), differences between the reference level and possible linear combinations of other levels (Fig. B1a,c). Fixed-effect models don't estimate mean effect over groups, but it can be calculated using uncertainty propagation (see Supporting Information S2). Mixed-effects models, nevertheless, estimate the grand mean effects and their standard deviation additionally to each individual level effects (Fig. B1b, d). Grouping variables may have not only different intercepts (Fig B1 a,b), but also different slopes (Fig B1 c, d - the temperature "ecological" effect). In fixed-effects models, this is done by introducing an interaction between the ecological effect and the grouping variable, and in mixed-effects models by estimating random slopes for each group. The choice of modeling different slopes for each group is related to the complexity of the data and may have impact on modeling structure and inference.

Models in R. Here are the formula R syntax[1] of fixed and mixed-effects models fitted in Figure B1

Scenario A random intercept only

a) Height ~ Temperature + Mountain (fixed-effect model)
b) Height ~ Temperature + (1| Mountain) (mixed-effects model)

Scenario B random intercept and slope

c) Height ~ 0 + Mountain + Temperature:Mountain (fixed-effect model[2])
d) Height ~ Temperature + (1|Mountain) + (0 + Temperature| Mountain) (mixed-effects model[3])

1 From lm() or glm() base R functions, lme4 (Bates et al. 2015) and glmmTMB (Brooks et al. 2017) packages.
2 This formula parametrization estimates a separate intercept and slope for each group independently. The most common parametrization is *Mountain* + *Temperature* + *Mountain:Temperature*, which is the same as *Mountain*Temperature*, that uses one group as a reference intercept and slope and the other parameters estimate differences to the reference group. For purposes of prediction as in Fig B1 c, these parametrizations don't change anything.
3 Uncorrelated random intercept and slope. For correlated models, the formula for the random terms is (1+Temperature|Mountain) or just (Temperature|Mountain).

123

Methods

Simulated example

To compare random- and fixed-effect modeling of a grouping variable with small number of groups, we simulated data based on our hypothetical example from Box 1. We hypothesized first that a higher temperature improves the reproductive success (either yes or no) of a plant species (H1), and second, that a higher temperature also increases the average height of the reproductive plants (H2). To test these hypotheses, we simulated a data collection in a randomly chosen number of mountains from a varying number of plants for each mountain (H1: expected range between 40-360 plants per mountain, H2: expected range between 10-90 plants per mountain) while keeping the overall number of plants constant (H1: on average 200 plants per mountain, H2: on average 50 plants per mountain) along altitudinal transects. We varied the number of mountains (groups) from two to eight and simulated 5,000 datasets for each case. We investigated performance for models with binomial (GLMM and GLM, H1) and normal distribution (LMM and LM, H2). The different choices of observations per group was made to meet the higher data request of binomial models compared to linear models. The varying number of plants per mountain reflects an unbalanced study design – a common scenario in ecology and evolution (Shielzeth et al. 2020).

Scenarios of data complexity and model fitting

Table 1: Data-generating and tested models for each scenario of data simulation: Scenario A random intercept for each mountain and B random intercept and slope for each mountain. For the fixed-effects models, we used R syntax for model formula in *lm()* function and for the mixed-effects models we used syntax from *lmer()* or *glmmTMB()* functions from *lme4* and *glmmTMB* packages. Response variables are reproductive success (RS) for the binomial distribution models (H1, Box

1) and height of flowering plants for the normal distribution models (H2, Box 1). T is the temperature effect on both response variables.

		Scenario A Random intercept only		**Scenario B** Random intercept and slope	**Description**
Data-generating model	(1)	Height/RS ~ T + (1|mountain)	(6)	Height/RS~ T + (1|mountain) + (0+T|mountain)	Effect of intercept (and slope in B) vary across mountains
Tested models					
Fixed-effect models	(2)	Height/RS ~ T	(7)	Height/RS ~ T	Temperature only main effect – too simple model
	(3)	Height/RS ~ T + mountain	(8)	Height/RS ~ 0 + mountain + T:mountain	Main effects of temperature and mountain (and interaction in B) – correctly specified models
Mixed-effects models			(9)	Height/RS ~ T + (1|mountain)	Temperature and mountain both vary – too simple models
	(4)	Height/RS ~ T + (1|mountain)	(10)	Height/RS ~ T + (1|mountain) + (0+T|mountain)	Effect of intercept (and uncorrelated slope temperature in B) vary across mountains – correctly specified models
	(5)	Height/RS ~ T + (1|mountain) + (0+T|mountain)	(11)	Height/RS ~ T + (T|mountain)	Effect of intercept and slope temperature (correlated effects in B) across mountains – too complex models

Scenario A - random intercepts per mountain

In scenario A, we assumed mountains differ in their intercepts (mean height/reproductive success, Table 1, Eq. 1). For this scenario we tested two different mixed-effects model structures: a correctly specified model which corresponds to the data generating model (Table 1, Eq. 4) and a too complex model (Table 1, Eq. 5) with an additional random slope for each mountain. Since in real data analysis the true underlying data generating process is unknown, it is useful to understand if a too complex model can correctly estimate a zero (or nearly zero) random slope and, thus, approximate the true model structure (Table1, Eq. 1).

As fixed-effect alternatives, we tested the correctly specified model with mountain as fixed intercept together with temperature as slope (Table1, Eq. 3), and a too simple model omitting mountain at all (Table 1, Eq. 2). This last model corresponds to a mixed-effects model that estimates the random intercept of mountain as zero.

Scenario B - random intercepts and random slopes per mountain

In scenario B, we assumed a random intercept and a random slope (without correlation among them) for each mountain as data-generating process (Table 1, Eq. 6). We tested three different mixed-effects model structures: a correctly specified model corresponding to the data generating model (Table 1, Eq. 10), a too complex model with a correlation term for the random intercept and random slope (Table 1, Eq. 11), and a too simple model with only a random intercept for each mountain (Table 1, Eq. 9). We used the too simple model to test the effect of not accounting for important contributions to the data-generating process.

As fixed-effect alternatives, we tested the correctly specified model with the main effects of temperature and mountain, and the interaction term (Table 1, Eq. 8), and the too simple model dropping mountain as predictor (Table 1, Eq. 7). We tested the last model since mixed-effects models, with standard deviation estimates of zero for both random effects, correspond to fixed-effects models omitting the grouping variable.

Model fitting

We fitted mixed-effects models to our simulated data with two of the most commonly used packages in R: *lme4* (Bates *et al.* 2015) and *glmmTMB* (Brooks *et al.* 2017). We present here *lme4* results because it reports optimization issues as singular fit, i.e., when some of

179 the parameters of the variance-covariance Cholesky decomposition used in the optimization
180 are exactly zero. Results with glmmTMB can be found in Supporting Information S1.

181 For LMMs we used the restricted maximum likelihood estimator (REML). For GLMMs we
182 used the maximum likelihood estimator (MLE) since REML is not supported in *lme4* for
183 GLMMs (for a comparison of REML and MLE see Supporting Information S1). All results
184 presented in scenario A and B are for the datasets without singular fit convergence problems
185 (see section *Standard deviation of random effects and singular fits*).

186 **Statistical properties and simulation setup**

187 We compared the modeling options for both data generating scenarios based on important
188 statistical properties of the temperature effect (ecological effect) in our simulated data: type
189 I error rate, statistical power, and coverage. Type I error rate is the probability to identify a
190 temperature effect as statistically significant although the effect is zero. Statistical power is
191 the probability to detect the temperature effect as significant if the effect is truly greater than
192 zero. Coverage is the probability that the true temperature effect falls into the estimated 95%
193 confidence interval. For a correctly calibrated statistical test, the type I error is expected to
194 be equal to the alpha-level (in our case 5%), the coverage to be equal to the confidence
195 level (in our case 95%), and the higher the statistical power the better the test.

196 To investigate the behavior of type I error, we simulated data with no slope effect, i.e., the
197 effect of temperature on plant reproduction (average reproductive success of 0.5) and on
198 height (average 10 cm) is zero. To additionally investigate statistical power and coverage,
199 we simulated an example with a weak effect, i.e., an effect that is barely statistically
200 significant. In our example, a weak effect corresponds to an average increase in size per

unit step of the standardized temperature (linear scale) of 0.4 cm (Hypothesis 2) and 0.4 gain in reproductive success (Hypothesis 1) at scale of the linear predictors.

For scenario A and scenario B, the individual effects for each mountain were drawn from a normal distribution with standard deviation of 0.1 around the average effects: 10.0 cm average height (intercept), and 0.4 cm average increase in size or 0.4 (logit link scale) gain in reproductive success with temperature (slope).

Standard deviation of random effects and singular fits

To understand how the number of levels affected the estimation of the standard deviation of the random effect in mixed-effects models, we recorded and compared standard deviation estimates for random intercepts and slopes from the correctly specified mixed-effects model in scenario B (Table 1, Eq. 10). We also compared optimization routines (REML and MLE) in terms of estimating zero standard deviations (singular fits, see below) for both LMMs and GLMMs (see supporting information S1).

Technically, singular fits occur when at least one of the variances (diagonal elements) in the Cholesky decomposition of the variance-covariance matrix are exactly zero or correlations between different random effects are estimated as -1 or 1. As singular fits represent models that have problems in the optimization process, we have classified our simulated datasets into the ones that showed singular fit warnings or not for mixed-effects models in *lme4* package for the correctly specified model in scenario B (Table 1, Eq. 10). We calculated the above-mentioned statistical properties separately for the two groups of data: singular and non-singular fit.

222 **Quantifying the influences of study design on power and type I error**

223 The statistical properties of the ecological effect may depend not only on the number of
224 groups (mountains) but also on the standard deviation of the random effect, the overall
225 number of observations and the balance of observations among groups. To further quantify
226 their impacts on the statistical properties of the ecological effect, we additionally ran 300
227 iterations (each with 1,000 non-singular model fits) with the data generating model from
228 scenario B for hypothesis 2 (height of plants, uncorrelated random slope and random
229 intercept). Thereby, we sampled uniformly the number of mountains from 2 to 20, the
230 standard deviations of the random effects from 0.1 to 2, the overall number of observations
231 from 10*number of mountains to 500*number of mountains. Additionally, to create different
232 degrees of unbalance in data, we sampled for each mountain the average share of total
233 observations from 0.1 to 0.9. Thereby, we ensured that there are at least 3 observations per
234 mountain. We used the difference between the largest and the lowest proportion as proxy
235 for the degree of unbalance between the groups.

236 We fitted the correctly specified LMMs and LMs (Table 1, Eq 8), with the same structure as
237 the data generating model (Table 1, Eq 10), and calculated type I error rate and statistical
238 power of the temperature effect. Based on if the simulations had more than 10 groups, we
239 split the results to better distinguish the effects of different study design parameters as the
240 pattern changed (see Supporting Information S1.5). We then fitted a linear regression with
241 the statistical property (power and type I error rate) as response and standard deviation,
242 number of groups, number of observations per group and the unbalance proxy and their
243 two-way interactions as predictors. All predictors were standardized to allow for a correct
244 comparison of effect sizes across variables.

Results

Scenario A - random intercepts per mountain

When the data-generating model had a random intercept, irrespectively of the number of groups (mountains), all models except for the too complex model (random intercept and slope) showed a type I error rate of 5% and coverage around 95% (Fig. 1), as long as we made sure that they converged (no singular fit). Power increased (Fig. 1A) with the number of mountains for LMMs from 0% (2 mountains) to 100% (5 to 8 mountains), and for GLMMs (Fig. 1B) from 40% (2 mountains) to 95% (8 mountains). Note that the model omitting the grouping variable mountain presented similar properties as the other models. However, when increasing the standard deviation of the random intercept in the simulation, this model showed much lower power (Fig. S8).

For the too complex model for LMMs, we found on average a lower type I error rate of around 1-2% (Fig. 1A), lower statistical power to detect the temperature effect for a small number of mountains, and higher coverage (of around 98-99%) than the other models (Fig. 1A). Binomial datasets with small number of observations per mountain (25, 50, 100) presented similar results regarding type I error and coverage but as expected, very low power among models (Figure S).

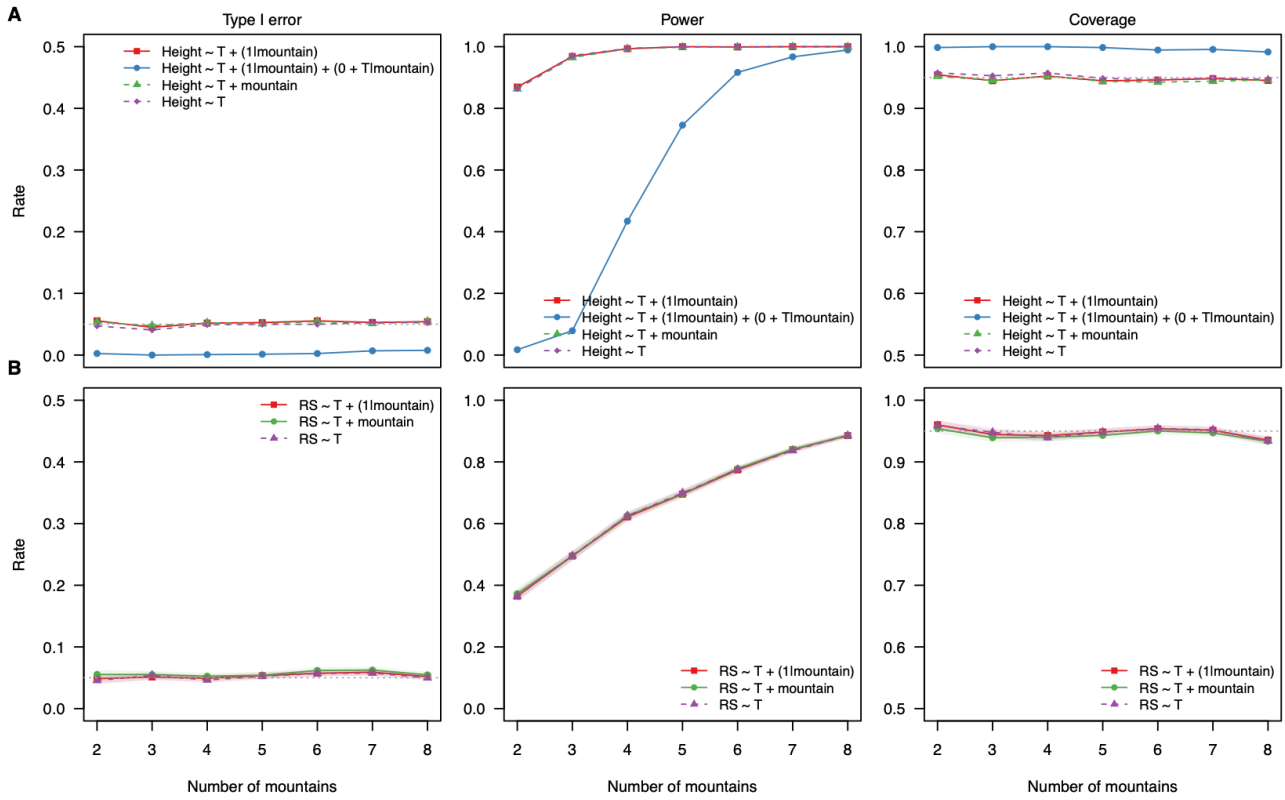


Figure 1: Type I error rates, power, and coverage for linear fixed and mixed effect models (A) and generalized linear fixed and mixed-effects models (B), fitted to simulated data with 2-8 mountains (random intercept for each mountain - Scenario A) and 50 observations per mountain. For each scenario, 5,000 simulations and models were tested. These results are only for datasets in which mixed-effects models converged without presenting singular fit problems.

Scenario B - random intercepts and slopes per mountain

In scenario B, where mountains differed in their average height/reproductive success (intercept) and their response to increasing temperature (slope of the ecological effect), the differences among models were greater (Fig. 2). We found that type I error rate of the correctly specified mixed-effects model (Table 1, Eq. 10) slightly increased, while coverage decreased slowly with the number of groups towards the respective nominal values (0.05 and 0.95, respectively). The too complex model with correlated random intercept and random slope (Table 1, Eq. 11) presented similar properties, but with slightly increased type

I error and decreased power (Fig. 2). For the correctly specified fixed-effect model, type I error (≈ 2%) and coverage (≈ 98%) stayed constant with the number of groups (Fig. 2). For the correctly specified fixed- and mixed-effects models, power increased with the number of mountains, but the mixed-effects model showed overall higher power than the fixed-effects model irrespective of the number of mountains. For both normal and binomial models, the too simple model omitting the grouping variable resulted in a higher type I error rate (> 0.10), too low coverage (<0.90), but higher power than the other models (Fig. 2).

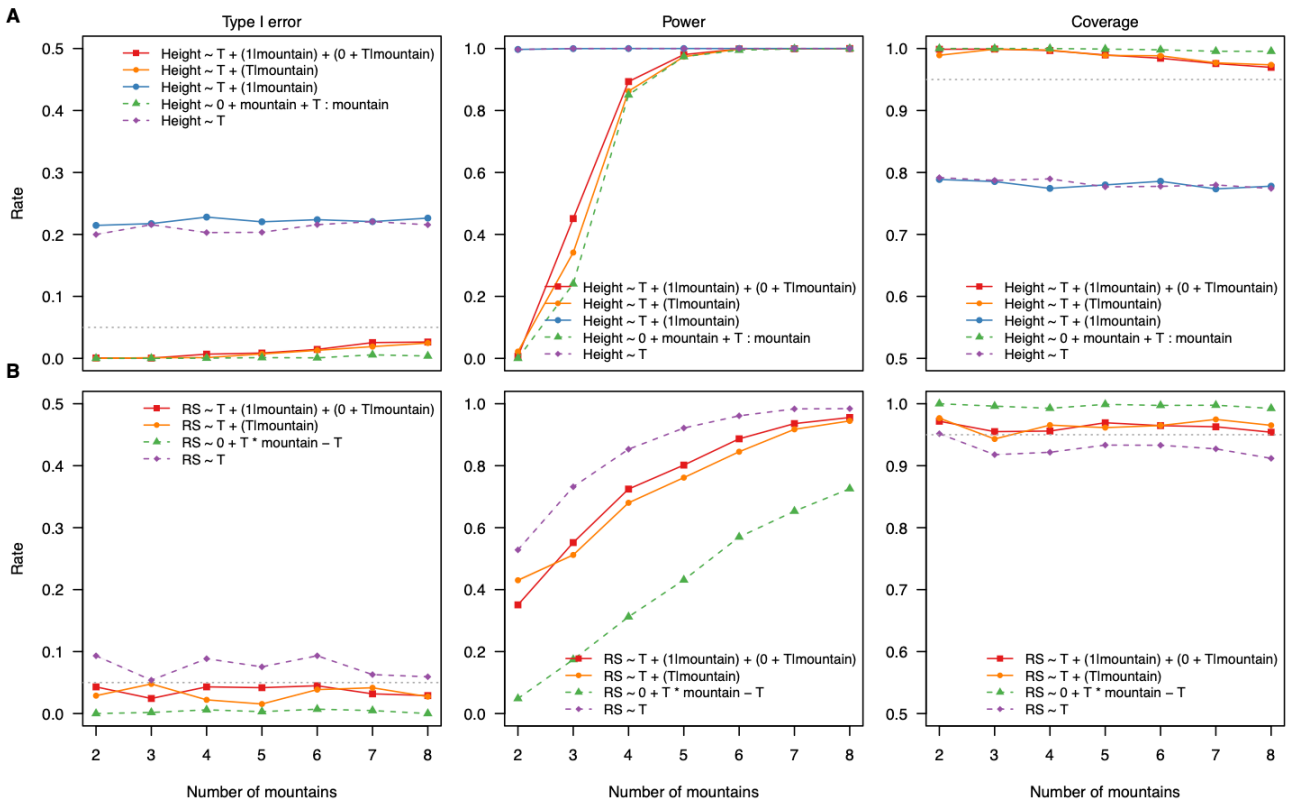


Figure 2: Type I error rates, power, and coverage for linear (mixed effect) models (A) and generalized linear (mixed-effects) models (B), fitted to simulated data with 2-8 mountains for scenario B (random intercept and random slope for each mountain range). For each scenario, 5,000 simulations and models were tested. These results are only for datasets in which mixed-effects models converged without presenting singular fit problems.

288 **Standard deviation of random effects and singular fits**

289 We found for LMMs (singular and non-singular fits combined) in Scenario B (random
290 intercept and slope) that the standard deviations of the random effects of the correctly
291 specified model (Table 1, Eq. 10) showed bimodal distributions with one peak around the
292 correct value (0.1) and one peak around zero (Fig. 3A, B). The peak around zero decreased
293 in height, i.e. less models estimated a standard deviation of zero with an increasing number
294 of mountains (Fig. 3A, B, see also Table S1). When looking at models without singular fits
295 (Fig. 3 C and D), the distribution of standard deviations is unimodal around the correct value.
296 Results for *glmmTMB* were consistent with *lme4* when using a threshold of 10^{-3} to classify
297 a standard deviation as zero (singular fit) (Fig. S2).

298 By comparing the fitting algorithms, we found that using MLE led to more standard deviation
299 estimates of zero, i.e. singular fit convergence problems, (Fig. S3, S4) than REML.
300 Additionally, using MLE, the distribution of standard deviation estimates of the non-singular
301 fits are not at the correct value (Fig. S3, S4), but the bias decreases with increasing number
302 of groups. For both optimization routines, increasing the number of levels reduced the
303 number of singular fits (Table S1).

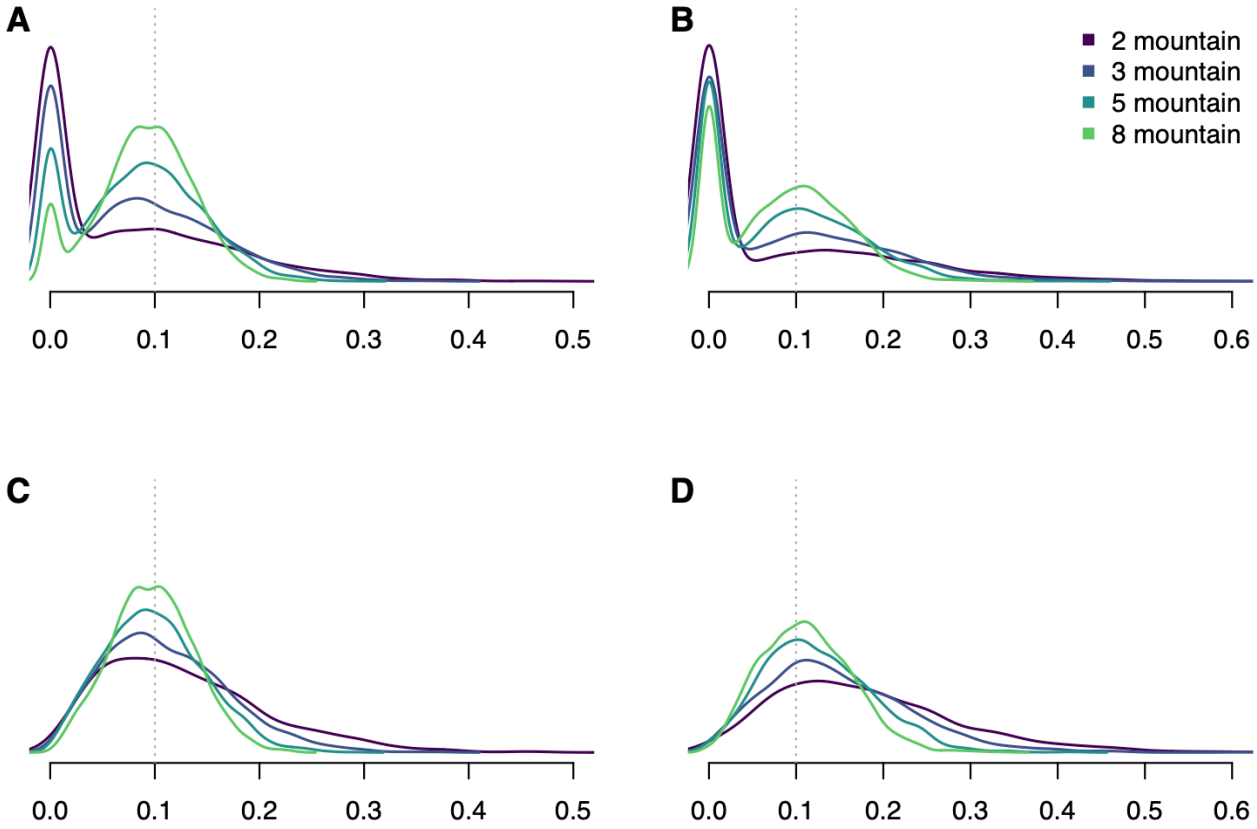


Figure 3: Standard deviation estimates of the random intercepts (A, C) and random slopes (B, D) for linear mixed-effects models (LMM, Table 1. eq. 10) in Scenario B, fitted with lme4 using REML to simulated data with 2-8 mountains. Figures A and B show the results for all models (with and without singular fits) and figures C and D show the results for the models without singular fits. For each scenario, 5.000 simulations and models were tested. The grey lines represent the true standard deviation used in the simulation (0.1).

We found that singular fits had a high influence on type I error and power (Fig. 4) of mixed-effects models. For a singular fit, the type I error rate of the correctly specified mixed-effects model was constant around 10% (similar to the model omitting the grouping variable), while with non-singular fits it was 1% for two groups and increased towards the correct value of 5% with increasing number of groups (Fig. 4A). In comparison, the fixed effect model had similar type I error rates for data that presented singular and non-singular fits in mixed-

effects models, both increasing towards the nominal value with increasing number of groups (Fig. 4C).

We also found differences in power for the models between singular and non-singular fits (Fig. 4B, D). For the correct LMM and correct LM, power increased with the number of groups, but the increase for the LM was stronger. The power of the mixed-effects model with correct structure is higher for singular than non-singular fits especially for a low number of mountains (Fig. 4B). For the fixed-effects model, the difference is less strong, however the model has still higher power with singular fit than for non-singular fits (Fig. 4D).

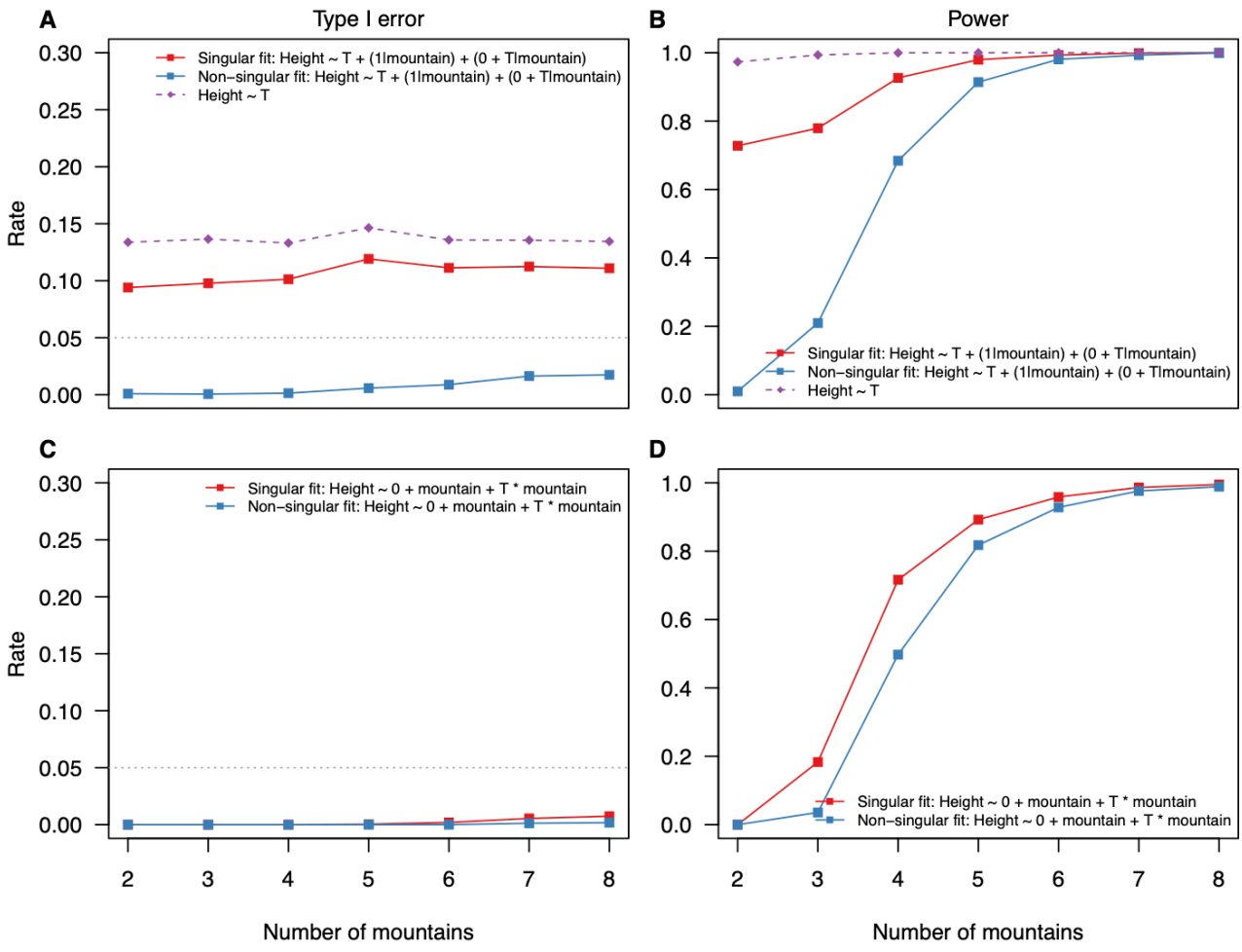


Figure 4: Type I error rate and power of the correctly specified linear fixed and mixed-effects models in scenario B separated by datasets that presented singular fit (red lines) and non-singular fit (blue lines) when fitted with lme4 package. Figure A and B are results for the linear mixed-effects models, and C and D for the linear fixed-effect models. For comparisons, we show also results for the fixed-effect model that omits the grouping variable (mountain).

Quantifying the influences of study design on power and type I error

With less than ten mountains (Fig. 5A, B), we found that the correctly specified LM started with a higher deviation to the expected type I error rate (0.05) for the ecological effect (Fig. 5A, average value) than the correctly specified LMM. The LM was also more strongly affected by the standard deviation of the random effects, the number of observations and the number of mountains, i.e., increasing standard deviation/number of observations/number of mountains decreased the deviation to the nominal type I error rate (Fig 5A). Additionally, for both models we found a tradeoff between the unbalance in the data and the number of mountains (interaction terms *unbalanced:mountain* in Fig 5A). When increasing the unbalance of observations between mountains, the number of mountains must also increase to obtain similar type I error rates. For power, we found that both LMs and LMMs have similar values, and both are similarly affected by the study design factors (Fig. 5B). Increasing the standard deviation decreased power, while increasing the number of mountains increased power (Fig. 5B). Notably, we also found a strong negative interaction between the standard deviation and the number of mountains.

For more than 10 levels, the LM had a higher average deviation of the observed to the expected type I error rate but was stronger positively affected by the standard deviation (in the sense that the deviation was decreasing), but negatively affected by unbalance between

levels, however with high standard errors on this effect estimate (Fig. 5C). Power was equal for both models. Overall, the influence of the study design factors became less strong with more than 10 levels (Fig 5D).

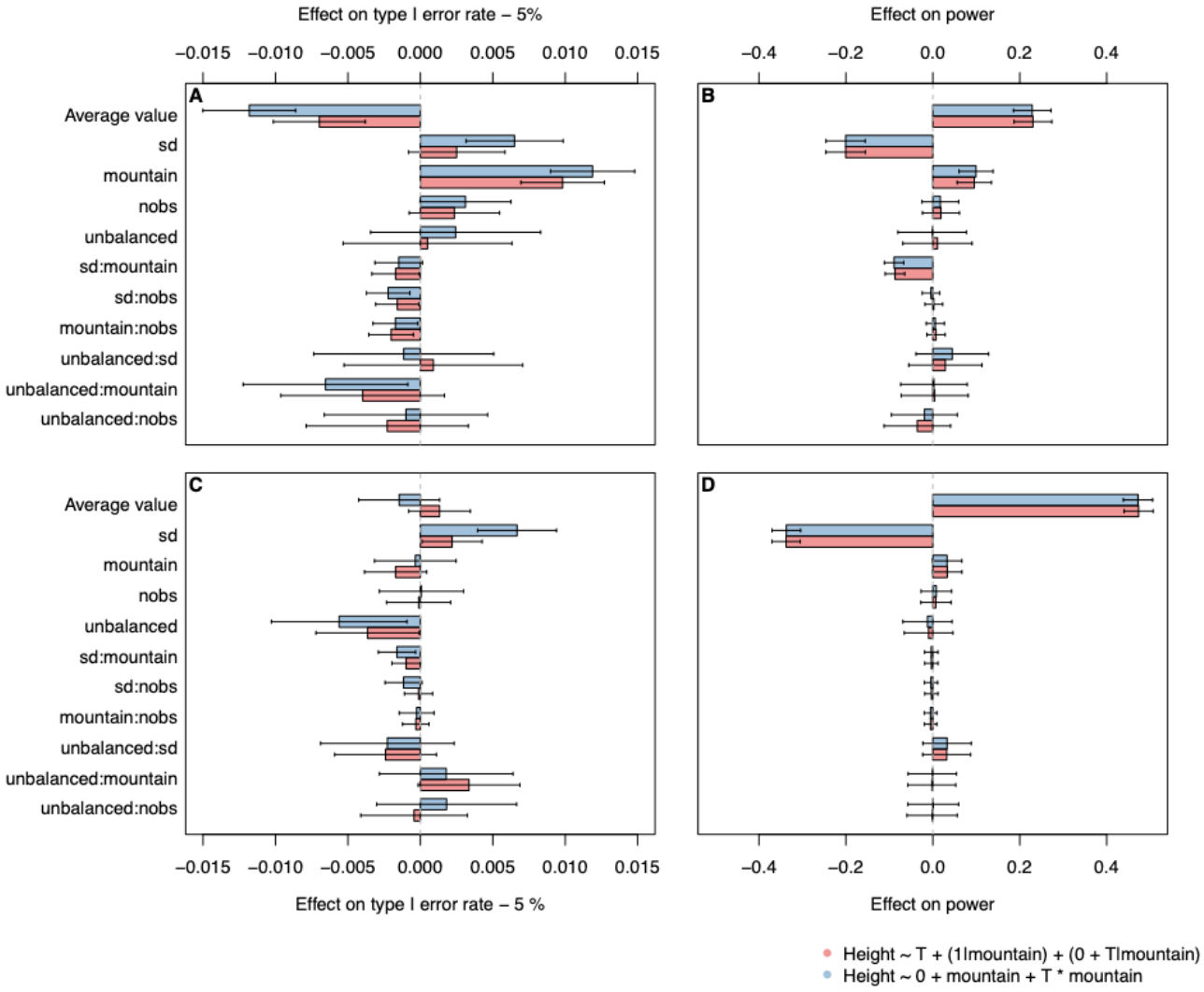


Figure 5: Comparing the influence of study design factors on the type I error rate (A, C) and power (B, D) for less than 10 levels (A, B) and more than 10 levels (C, D) of linear mixed- (red bars) and fixed-effects models (blue bars). We found that the standard deviation of the random effects (*sd*) and the number of groups (*mountain*) are the most important values to get correct statistical properties and improve the chance to find a significant effect. For this analysis, we used the plant height example (Hypothesis 2, Box 1) for Scenario B (random intercept and random slope). *Average value* is the intercept,

nobs is the overall number of observations and *unbalanced* is the degree of unbalance of observation between groups. Interactions between study design factors are represented by the colon symbol (:).

Discussion

Ecological data collections or experiments often produce data with grouping structures and mixed-effects models can account for these dependencies. When such data, however, has a small number of groups, analysts are faced with a decision: should they model the grouping variable as fixed or random effect? How does this decision influence the ecological effect of interest? Here, we showed with simulations that mixed-effects models with small number of levels in the grouping variable are more robust than previously assumed (Fig. 3) and that the decision between fixed and random effect matters most when the data-generating processes also included a varying slope for each group (Fig. 2).

When groups (mountains) differed only in their intercept (scenario A), almost all models independent of the number of groups presented the same statistical properties for the ecological effect (temperature) (Fig. 1, see also Gomes 2021). The only exception, the too complex model, had too low type I errors (close to zero, Fig. 1) and lower power. We speculate this is caused by the lower degrees of freedom and that the model was unable to correctly estimate the random slopes to zero. Notably, for scenario A, the too simple model omitting the grouping variable presented correct statistical properties (Fig. 1). However, power decreased strongly with increasing differences between groups (Fig. S8, Fig. 5), which confirms the importance of including grouping variables to correctly partition the variance among the different predictors (Gelman 2005; Gelman & Hill 2007; Bell *et al.* 2019).

When the intercept and slope varied per group (Scenario B) and the model fitted correctly, the model choice influenced the statistical properties more strongly. The mixed-effects

model had a better coverage, type I error, and a slightly higher power than the fixed-effects model, especially for a larger number of mountains (Fig. 2). A higher power may be linked to the distributional assumption of the random effects (see Gelman & Hill 2007): with increasing numbers of mountains this assumption reduces the “effective” number of parameters needed to be estimated for the variations between mountains. In summary, mixed-effects models are less conservative but have higher power than fixed-effects models for low number of groups especially for generalized linear (mixed) models.

Too complex mixed-effects models presented in both scenarios slightly lower type I error and power compared to the correctly parameterized mixed-effects model (Fig. 1, 2). This trade-off between type I error and power is in line with Matuschek *et al.* (2017) for different model complexities. Overall, the more complex (higher parametrized) models are more conservative but have less power than the simplified models.

In scenario B, too simple models exhibited inflated type I errors (in line Schielzeth & Forstmeier 2009; Barr *et al.* 2013; Bell *et al.* 2019), too low coverage but very high power (Fig. 2). We speculate that additional variance coming from the difference between levels in the grouping variable, which is not accounted, is attributed to the ecological effect and this leads to overconfident estimates.

Standard deviation of random effects and singular fits

The standard deviation estimates of the random effects of a correctly fitted model (no-singular fit) were around the correct value, showing that mixed-effects models were able to correctly estimate the random effect variance for a small number of groups (McNeish 2017), although with a higher uncertainty (Fig. 3B). However, singular fit problems were also higher

405 for small number of groups, creating a high peak in zero in the distribution of the standard
406 deviations estimates (Fig. 3, Table S1). A singular fit in our simulations corresponded to a
407 zero-variance estimate as we excluded the other possibility (we set the correlations between
408 random slope and intercept to zero, see Methods). Here, the correctly specified mixed-
409 effects model had similar statistical properties as a fixed-effect model dropping the grouping
410 variable (Fig. 4). This behavior becomes clearer when we think about the actual
411 consequences of a variance estimate of zero: no difference between the groups and hence
412 no influence of the grouping variable in the model (as a fixed-effect model without the
413 grouping variable). However, the models still differed in their number of parameters (and
414 degrees of freedom) which might explain the slight differences in statistical properties (Fig.
415 4).When switching to fixed-effects models for singular fits in the random effect, the type I
416 error rate and power were similar to the random effect model with non-singular fits (Fig. 4),
417 suggesting that in case of a singular fit, the fixed-effects model with the correct structure is
418 favorable over the mixed-effects model because of its more conservative estimates.

419 **Connection to study design**

420 Earlier studies reported mixed recommendations about important study design factors.
421 While some studies stressed only the importance of the number of observations for each
422 level (Martin *et al.* 2011; Pol 2012), we found, in accordance with Aarts *et al. (*2014), that
423 the number of groups and the standard deviation between groups have higher influence on
424 type I error rates and power.

425 The influence of the standard deviation on the statistical properties is mixed. On the one
426 hand, increasing the standard deviation had a positive effect on the type I error for both

models but the fixed-effect model was more strongly affected (Fig. 5). The different assumptions about the distribution of the groups might explain this different behavior: the mixed-effects model assumes the groups to be normally distributed and estimates flexibly the standard deviation of the groups, whereas the fixed-effects model makes implicitly the assumption of an infinite standard deviation. On the other hand, increasing the standard deviation decreased the power of both models because more variance is explained by the different groups, making the slope effect estimate more uncertain.

To avoid the tradeoff between the influence of increasing standard deviation on type I error rate and power, we encourage to design a study with at least 10 levels because in this situation the effects of the study design on the statistical properties were small (Fig. 5). However, we are aware that financial or time resources are limited, so we believe that sampling similar grouping variables (i.e. mountains with a small standard deviation) is more beneficial as this leads to too conservative type I errors, but higher power. In our scenarios, the influence of the unbalanced number of observations between groups was small (Fig. 5) suggesting that unbalanced designs are not the main concern as long as mixed-effects models are used for analysis, given their robustness to unbalanced data (Swallow & Monahan 1984; Pinheiro & Bates 1995; Schielzeth et al. 2020). Moreover, the impact of study design on type I error and power stresses the importance of pre-experiments and power analyses (e.g. Johnson *et al.* 2015; Green & MacLeod 2016; Brysbaert & Stevens 2018) to maximize the meaningfulness and efficiency of a study.

447 **Practical suggestion**

448 Coming back to our original question, we found no harsh threshold for the minimum number
449 of levels in a grouping variable necessary to model it as a random effect (Fig. 2), but rather
450 found that a singular fit in the mixed-effects model indicates switching to the fixed-effects
451 model is beneficial (more conservative type I error rates, Fig. 2). Acknowledging that most
452 singular fits occur with a low number of levels (Table S1), this might also explain the rule of
453 thumb.

454 We therefore recommend starting with the mixed-effects model, regardless of the number
455 of groups, and switching to a fixed-effects model only in case of a singular fit (more
456 conservative type I error rate). For a random intercept and slope in the data-generating
457 process, we recommend starting with correlated random slope and intercept (following Barr
458 et al. 2013). If this model converges, the resulting estimates have approximately the correct
459 nominal type I error. When obtaining a singular fit, one should first try uncorrelated random
460 effects (following Matuschek *et al.* 2017) and only in case of a recurring singular fit switch to
461 a fixed-effects model. These recommendations are summarized in Fig. 6.

462 Previous recommendations assumed that we know whether only the intercept, or both slope
463 and intercept differ across groups. In practical data analysis, however, we are never totally
464 sure about the underlying data-generating process. Here, specifying a too complex model
465 (including a random slope if there is none) leads to too low type I error rates and less power,
466 while a too simple model (not including a random slope if there is one) has inflated type I
467 error rates and higher power. Thus, to prevent being overly confident, it is essential to
468 include random slopes if they are present (Schielzeth & Forstmeier 2009). But because of

the power issue, we doubt that always starting with the maximally complex random effect structure (as suggested in Bell *et al.* 2019) is generally a good advice. We therefore need a decision criterion for deciding on the complexity of the random effect structure.

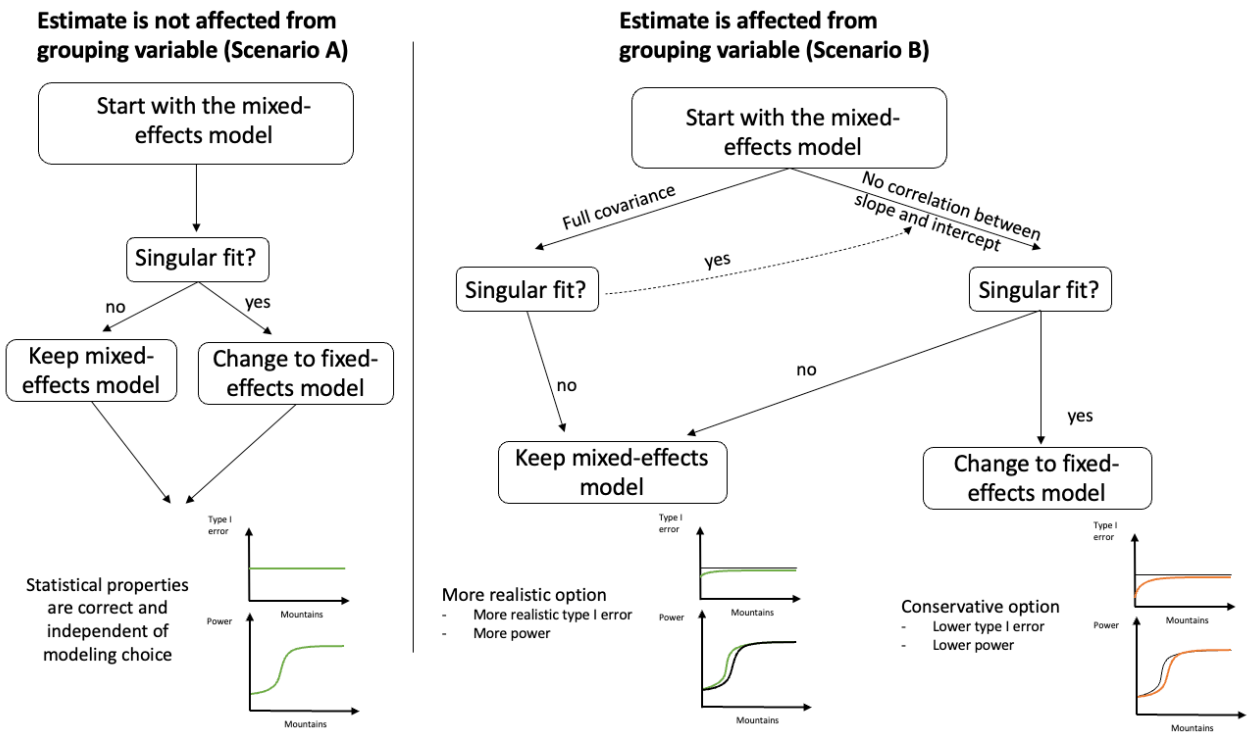


Figure 6: Consequences of mixed-effects models with less than 5 levels in a random effect. Two extreme events can be observed for the variance estimate of the random effect. On the one hand (left side), the variance estimate can be close to zero, which can result (if the random effect is not changed to a fixed effect) in a higher probability of a type I error for the associated fixed effects. On the other hand (right side), the variance estimate can be overestimated, which can result in a higher probability of a type II error for the associated fixed effects.

Unfortunately, model selection on random effects is relatively complicated, because the exact degrees of freedom, that are used by a random effect, are unclear (Kuznetsova *et al.* 2017), only approximations exist and for low number of levels a Kenward-Rogers correction is preferred (McNeish 2017). Although this prevents a naïve use of AIC or likelihood ratio tests (LRT), other methods such as simulated (restricted) LRTs (Wiencierz *et al.* 2011) can

be used to decide if adding a random slope is justified. Thus, we recommend starting with the simpler structure (typically random intercepts) and then use residual checks (e.g. Hartig 2019) or appropriate model selection criteria (e.g. Matuschek *et al.* 2017) to decide if a random slope should be added.

Another, somewhat perpendicular option is to modify the assumption that the individual groups stem from a normal distribution, which is mainly due to mathematical convenience (Beck & Katz 2007). This assumption corresponds to a L2 (RIDGE) regularization on the random effects (regularization is common over all statistical fields and techniques, e.g. in Bayesian statistics regularization is done via shrinkage priors), which purposefully biases parameter estimates towards zero but it is unable to produce sparse estimates (Zou & Hastie 2005). This means the random effect estimates in the too complex model will typically not be shrunk to exactly zero.

From regularization theory we know that a L1 (LASSO) regularization, which corresponds to a Laplace distribution (Park & Casella 2008; Hans 2009; Tibshirani 2011), produces more sparse estimators. Because mixed-effects models are relatively robust to distributional assumptions (Bell *et al.* 2019; Schielzeth *et al.* 2020), switching distributions to LASSO will make random effect estimates more sparse while maintaining most of their statistical advantages. Therefore, using mixed-effects models with L1 or a combination of L1 and L2 regularization (elastic net, see Zou & Hastie 2005) could potentially allow starting with the maximal complex random effect structure (see also McNeish & Bauer 2020), as the stronger shrinkage towards zero from the L1 structure ameliorates the loss of power observed here and thus should be investigated in further studies.

Conclusion

In conclusion, we showed that, contrary to common opinions, mixed-effects models correctly estimate the variance components of the random effects for small numbers of groups. We also found that the statistical properties of the ecological effect are robust against the model choice when the grouping variable does not share variance with the ecological effect in the data-generating process. When in doubt about the data-generating process, we encourage starting with a simplified model (random intercept only) because of power-issues with too complex models, and then consulting model diagnostics and simulated LRTs to check for evidence of random slope effects. When finding evidence for random slopes in these tests, we recommend starting with the mixed-effects model and switching only to a fixed-effects model in case of a singular fit problem. These recommendations ensure conservative type I error rates and avoid overconfidence. Moreover, we demonstrated that the statistical properties of both choices, the fixed-effects and the mixed-effects model, depends on the study design, particularly on the number of groups and the magnitude of difference between groups further supporting an informative decision on the study design itself. With this work, we provide a practical guideline, which helps ecologists in the study design, the data analysis and thus making ecological inference more informative and robust.

Data availability statement:

No empirical data was used in this study. Code to run and analyze the experiments can be found at https://github.com/JohannesOberpriller/RandomEffect_Groups.

Author Statement

MP, JO and MSL designed the study. MP and JO ran the experiments, analyzed the results and wrote a first draft. All authors contributed equally to revising the manuscript and interpreting and discussing results.

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