Fast and flexible linear mixed models for genome-wide genetics

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Abstract

Linear mixed effect models are powerful tools that account used to population structure in genome-wide association studies (GWASs) and estimate the genetic architecture of complex traits. However, fully-specified models are computationally demanding, and common simplifications often lead to reduced power or biased inference. We describe Grid-LMM (`https://github.com/deruncie/GridLMM`), a scalable algorithm for repeatedly fitting complex linear models that account for multiple sources of heterogeneity, such as additive and non-additive genetic variance, spatial heterogeneity, and genotype-environment interactions. Grid-LMM can compute approximate (yet highly accurate) frequentist test statistics or Bayesian posterior summaries at a genome-wide scale in a fraction of the time compared to existing general-purpose methods. We apply Grid-LMM to two types of quantitative genetic analyses. The first is focused on accounting for spatial variability and non-additive genetic covariance while scanning for QTL; and the second aims to identify gene expression traits affected by non-additive genetic variation. In both cases, modeling multiple sources of heterogeneity leads to novel discoveries.

Author summary

The goal of quantitative genetics is to characterize the relationship between genetic variation and variation in quantitative traits such as height, productivity, or disease susceptibility. A statistical method known as the linear mixed effect model has been critical to the development of quantitative genetics. First applied to animal breeding, this model now forms the basis of a wide-range of modern genomic analyses including genome-wide associations, polygenic modeling, and genomic prediction. The same model is also widely used in ecology, evolutionary genetics, social sciences, and many other fields. Mixed models are frequently multi-faceted, which is necessary for accurately modeling data that is generated from complex experimental designs. However, most genomic applications use only the simplest form of a linear mixed methods because the computational demands for model fitting can be too great. We develop a flexible approach for fitting linear mixed models to genome-wide scale data that greatly reduces their computational burden and provides flexibility for users to choose the best statistical paradigm for their data. We demonstrate improved accuracy for genetic association tests, increased power to discover causal genetic variants, and the ability to provide accurate summaries of model uncertainty using both simulated and real data examples.

Introduction

Population stratification, genetic relatedness, ascertainment, and other sources of heterogeneity lead to spurious signals and reduced power in genetic association studies [1–5]. When not properly taken into account, non-additive genetic effects and environmental variation can also bias estimates of heritability, polygenic adaptation, and genetic values in breeding programs [5–8]. Both issues are caused by departures from a key assumption underlying linear models: that observations are independent. Non-independent samples lead to a form of pseudo-replication, effectively reducing the true sample size. Linear mixed effect models (LMMs) are widely used to account for non-independent samples in quantitative genetics [9]. The flexibility and interpretability of LMMs make them a dominant statistical tool in much of biological research [9–18].

Random effect terms are used in LMMs to account for specific correlations among observations. Fitting an LMM requires estimating the importance of each random effect, called its variance component. General-purpose tools for this are too slow to be practical for genome-scale datasets with thousands of observations and millions of genetic markers [19]. This lack of scalability is caused primarily by two factors: (i) closed-form solutions of maximum-likelihood (ML or REML) or posterior estimates of the variance components are not available and numerical optimization routines require repeatedly evaluating the likelihood function many times, and (ii) each evaluation of the likelihood requires inverting the covariance matrix of random effects, an operation that scales cubically with the number of observations. Repeating this whole process millions of times quickly becomes infeasible.

To this end, several specialized approaches have been developed to improve the speed of LMMs, including the use of sparse matrix operations [20, 21] and spectral decomposition of the random effect covariance matrix [22–26]. These algorithms are particularly useful when the same random effect structure is used many times. For example, in genome-wide association studies (GWAS), each marker is tested with the same LMM. Similarly, in population-level transcriptomics, eQTLs or variance components are estimated for each of tens-of-thousands of genes expression traits. Fast and exact algorithms for fitting LMMs are limited to the case of only a single (full-rank) random effect term, besides the residuals [22–24]. Recently, approximate learning algorithms have been developed for the scalable extension to multiple random effects [28, 29], but few of these ensure guarantees in terms of estimation accuracy. One strategy applicable to studies with multiple random effects is to estimate the variance components only once in a model without additional marker effects and then test each marker either using a score test [30], which does not produce an effect size estimate, or with a conditional F-test assuming the variance component estimates are fixed [31–34]. Given the "known" variance components, closed-form solutions of all other parameters of an LMM can be found using a rotated version of the simple linear model. Unfortunately, both approximations suffer from reduced power when marker effects are large, do not permit posterior inference in a Bayesian analysis, and cannot be applied to parallel analyses of many traits (like gene expression). Table 1 summarizes these different methods, and their respective computational complexities, together with relevant references.

Grid-LMM takes a different approach to fitting LMMs: rather than directly optimizing the variance components separately for each test, we define a grid spanning all valid values of the variance components and fit simple linear models at each grid location. Each evaluation involves a single Cholesky decomposition of the random effect covariance matrix, which is then reused to calculate closed-form ML solutions or Bayesian posterior summaries (under a specific conjugate prior, see S1 Supplementary Methods) for all separate tests. After repeating these calculations across the whole grid, we select the highest ML (or REML) score for each marker to compute approximate

Table 1. Performance and limitations of reference models for linear mixed model GWAS. The time complexity of each algorithm is approximate, assuming a model with only a single marker effect and no other fixed effects. Here, l is used to index full-rank random effects; σ_l^2 are variance component parameters; n is the number of observations; p is number of markers to test; and t is the number of iterations needed for convergence. This value is expected to vary among methods, particularly for the iterations of grid search in Grid-LMM-fast. The rate limiting terms in common GWAS applications (where $p \gg n$) are in bold. "Method Type" describes the estimation of σ_l^2. "Exact" means effectively exact, up to machine precision and subject to possible convergence of algorithms to local maxima. "Null" means estimation of parameters under the null model with no marker effects. References list additional methods that are approximately equivalent to the given model classes.

Model	Max σ_l^2	Method Type	Approx. Time Complexity	References
GEMMA	1	exact	$\mathcal{O}(n^3 + \boldsymbol{pn^2} + ptn)$	[23, 24]
EMMAX	1	null	$\mathcal{O}(n^3 + tn + \boldsymbol{pn^2})$	[31, 32]
LDAK	>1	exact	$\mathcal{O}(\boldsymbol{ptn^3})$	[7, 21, 35, 36]
pylmm	>1	null	$\mathcal{O}(tn^3 + \boldsymbol{pn^2})$	[34, 37]
Grid-LMM	$\sim$ 1-8	grid	$\mathcal{O}(\boldsymbol{g}(n^3 + \boldsymbol{pn^2}))$	Present Work
Grid-LMM-fast	$\sim$ 1-8	grid-sampling	$\mathcal{O}(tn^3 + \boldsymbol{pn^2} + \sum_{i=2}^{t_2} \boldsymbol{g_i}(n^3 + \boldsymbol{p_i n^2}))$	Present Work

likelihood ratio and Wald tests [32, 38], or analogously derive posterior distributions and Bayes factors by summing appropriate statistics across the grid. The Grid-LMM approach relies on a re-parameterization of the typical LMM from individual variance components σ_l^2 to variance component proportions $h_l^2 = \sigma_l^2/\sigma^2$, where σ^2 without the subscript denotes the total sum of all variance components (including the residual). Since the variance components must be non-negative, their proportions are restricted to the unit interval $[0, 1]$ and sum to 1, forming a simplex. Therefore, a finite grid can span all valid parameter values. While the size of the grid increases rapidly with the number of random effects, for a small number of random effects ($\sim$1-8) and a moderate grid resolution, the size of the grid remains tiny relative to the number of models in a typical GWAS. As we show below, highly accurate test statistics are achieved even with a coarse grid in most reasonable situations, and further improvements to efficiency are possible by using heuristics to adaptively sample the grid or reduce the number of grid locations computed for the majority of tests. This strategy of conditioning on variance components over a grid and then combining solutions can be applied to many other tools in quantitative genetics including set tests for rare variants [39, 40], whole-genome regression models such as LASSO and elastic net [41, 42], and QTL mapping in controlled crosses [43].

In the following sections, we demonstrate the accuracy and advantages of the Grid-LMM approach in two genome-wide quantitative genetics contexts. The first is for GWAS where thousands-millions of markers are each tested for associations with a single phenotype, and the second is for gene expression where thousands of traits are each tested for non-additive genetic variance. In both cases, the same random effect structure is used for each model. While approximate, the test-statistics and posterior quantities calculated by Grid-LMM are accurate and improve power relative to other approximation methods — all while maintaining dramatically reduced computational burdens. Full derivations of the model and useful heuristics are described in detail in the Methods.

Results

As a first case-study, we used Grid-LMM to perform two types of genome-wide association studies (GWAS) that benefit from modeling multiple random effects: (1) the study of gene-environment interactions, and (2) detecting associations for phenotypes driven by non-additive genetic variation or spatial variation. In both cases, there are

multiple sources of covariation among observations that can inflate test statistics and bias estimates of heritability if not appropriately accounted for by using mixed models.

As an example of a GWAS with gene-environment interactions, we analyzed data on flowering times for *Arabidopsis thaliana* [44]. First, we benchmarked results from standard LMM methodologies to confirm that Grid-LMM association tests are accurate. We ran Grid-LMM on both a fine-grained grid with a small step size of 0.01 h^2-units, and a larger step size of 0.1 h^2-units, to test for associations between 216,130 single nucleotide polymorphisms (SNPs) and flowering times of 194 accessions measured at 10C (i.e. in a constant environment). We compared the Wald-test p-values computed by both Grid-LMM models to p-values computed using the exact method GEMMA [24], and the approximate method EMMAX [32]. Each method was applied as an LMM with only the additive relationship matrix as its single random effect. Grid-LMM p-values computed using the finer grid size (i.e. 0.01 h^2-units) were almost identical to those of GEMMA, and even p-values computed with the larger step size (i.e. 0.1 h^2-units) were more accurate than those resulting from EMMAX. There were particularly noticeable differences in performance for markers with larger scores, which were strongly underestimated by EMMAX since its approximations of h^2 are made strictly under the null model (Fig 1a). This pattern held true across all of the available 107 *Arabidopsis thaliana* phenotypes (see S1 Fig). However, despite these results, we do not advocate Grid-LMM in this setting; GEMMA (and similar methods) provides exact tests and is more computationally efficient. The real advantage of Grid-LMM is its ability to effectively model two (or more) random effects.

To demonstrate this advantage, we tested for gene-environment (G×E) interaction effects on flowering time. Here, we combined flowering time data from two conditions: constant 10C (as described above) and in the field. We limited our analysis to the 175 accessions that were grown under both conditions, yielding a total of $n = 350$ observations. When observations come from different environments, we might expect phenotypes to cluster for at least two reasons: (i) the sharing of alleles with constant effects across environments due to relatedness and population structure, commonly modeled with the additive genomic relationship matrix ($\mathbf{A}$) as a random effect, and (ii) the sharing of alleles with effects that differ among environments (or are specific to one environment). Previous work has shown that when testing for G×E effects with GWAS, modeling the second source of covariance using a second random effect can prevent spurious signals and increase power [34], which we replicated in this setting using simulations (S2 Fig and S3 Fig).

We therefore calculated G×E p-values for each genetic marker using Grid-LMM (grid step size = 0.01 h^2 units) on the full dataset using both the $\mathbf{A}$ and the G×E relationship matrices as two random effects. These were compared to p-values from an LMM which ignores the G×E covariance and only considers genetic similarity, and a model similar to `pylmm` [34] that considers both random effects but estimates variances components from the null model (this is referred to as null-LMM-G×E below). For each model, we included an additional random effect to account for the repetition of accessions which could induce additional covariance among observations. In this dataset, a simpler approach to testing for G×E was also available: test for associations between markers and the *plasticity* (i.e. the difference in flowering time between the field and 10C) of each accession, which requires only a single random effect and can be fit with GEMMA. This simple approach is only possible because every genotype was measured in both environments. Nonetheless, it is expected to produce identical tests to the full three-random-effect model and, therefore, serves as a viable benchmark for the Grid-LMM results. We used the plasticity GWAS approach as a baseline to compare the three models that use the raw data directly (Fig 1b). As expected, ignoring the G×E covariance leads to greatly inflated tests for the majority of markers. Grid-LMM

replicated GEMMA's plasticity p-values almost exactly when run with three random effects, while a portion of the null-LMM-G×E's tests were deflated, implying lower power. The full analysis using Grid-LMM with three random effects took 26 minutes. Fitting the same model for all 216,130 markers directly using an exact method like `LDAK` [7] would take approximately 6 hours (about 14× longer).

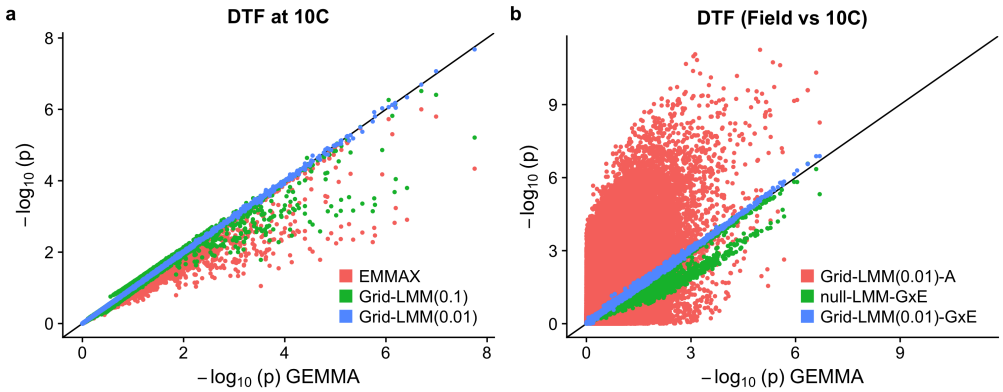

Fig 1. Comparisons of GWAS results for *Arabidopsis* flowering time. (**a**) Results for days-to-flower (DTF) at 10C. Compared are EMMAX and Grid-LMM with grid sizes 0.1 and 0.01, respectively. The exact method GEMMA is treated as a baseline. Each method was applied to the same LMM with only the additive relationship matrix as a random effect, and p-values were computed using the Wald test. (**b**) GWAS for the gene-environment (G×E) interactions effects on DTF between the constant 10C and field conditions. GEMMA results are for the plasticity of each accession (i.e. the difference $\mathrm{DTF}_{\mathrm{Field}}$ - $\mathrm{DTF}_{\mathrm{10C}}$), fit with a single random effect of the additive relationship matrix. The other three methods considered the full data with two observations per accession. Namely, Grid-LMM-A fit a "standard" GWAS with a grid step size 0.01, but with two random effects — the additive relationship matrix and an *iid* line effect. The other two models, null-LMM-G×E and Grid-LMM-G×E, fit three random effects — the additive relationship matrix, an *iid* line effect, and a G×E-additive relationship matrix representing the background covariation in gene-environment interactions among accessions.

Even when all individuals are measured in the same environment, the typical additive relationship matrix may not account for all sources of covariation. In particular, spatial variation and sharing of non-additive alleles may also induce covariance, lead to reduced power, and/or result in inflated tests in GWAS [45]. As an illustrative example, we tested for genetic association between 10,075 bi-allelic autosomal markers and body mass among 1,814 heterogenous stock mice from the Wellcome Trust Centre for Human Genetics [46]. We first computed additive and pairwise-epistatic relationship matrices, as well as a spatial-environmental covariance matrix based on the 523 different cages used in the experiment. Next, we compared p-values derived from an LMM considering only the additive relationship matrix (as in a typical GWAS) to those calculated by Grid-LMM using all three relationship matrices (Fig 2a-d). Using the three-random effect model, we identified associations on two chromosomes (numbers 4 and 11) that were not apparent when using just the additive relationship matrix as a random effect. Both loci have been previously identified as size-associated QTL (Table S1 Table) [47, 48]. The distribution of tests for markers in the remainder of the genome also showed less inflation with the three-random effect model (Fig 2b,d). The three-random effect model took 8.5 minutes to fit using Grid-LMM, while the full analysis on all 10,346 markers would have taken

approximately 10 hours with LDAK (more than 100× longer). Extrapolating to a consortium sized whole-genome analysis with 1 million markers would take ≈ 14 hours using Grid-LMM, as opposed to 40 days using LDAK. We see larger performance gains in the mouse dataset compared to the *Arabidopsis* dataset because of the larger sample size (n =1,814 vs. 350). LDAK (and other exact general-purpose REML methods) is dominated by matrix inversions which scale with n^3, while Grid-LMM is dominated by matrix-vector multiplications which scale with n^2 (again see Table 1). The performance advantage of Grid-LMM will increase even more for datasets with more individuals.

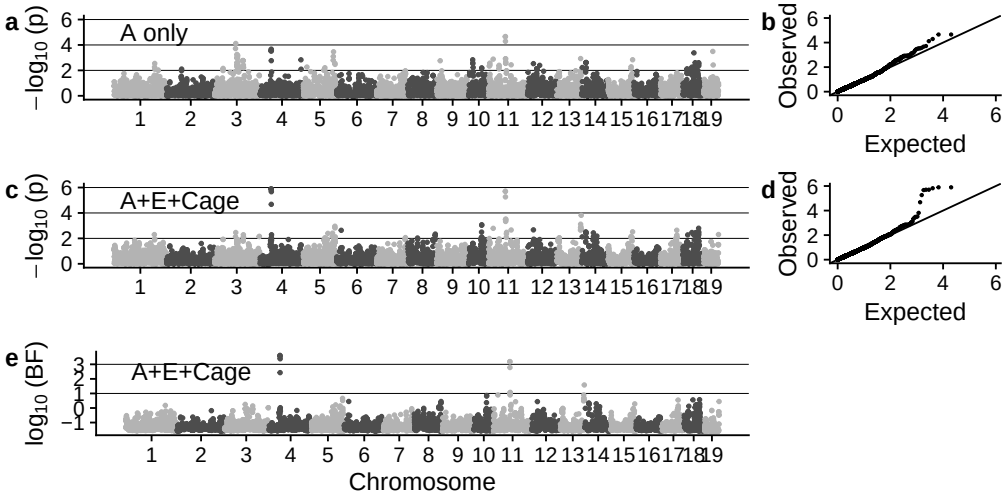

Fig 2. GWAS results for the body weight trait of the Wellcome Trust heterogenous stock mice. Grid-LMM was run using a grid size of 0.01 h^2-units for each model. (**a**-**b**) Wald test p-values from a model with only the additive relationship matrix (**A**) as a random effect, organized by chromosome or compared to a uniform distribution. (**c**-**d**) Wald test p-values from a model with three random effects: the additive, epistatic (**E**), and spatial (**Cage**) relationship matrices. (**e**) Approximate Bayes factors calculated from the three random effect model, assuming a standard normal $\mathrm{N}(0,1)$ prior for the marker effects and a uniform prior on the grid of variance component proportions.

To demonstrate the flexibility of Grid-LMM for GWAS, we also ran an analysis on the mice body weight trait using Bayesian inference and calculated Bayes Factors by comparing the models for each marker to the null model assuming no genetic effects. Here, we used a uniform prior over the grid of variance component proportions and assumed a standard normal prior for the marker effect sizes. In this setting, the Bayes Factors were highly correlated with the frequentist p-values — they also highlight the same associations on chromosomes 4 and 11 (Fig 2e). But in general, Bayes Factors provide additional flexibility for incorporating prior information on individual markers or even combining results among multiple studies [49, 50]. The full Bayesian analysis for Grid-LMM took just 7 minutes longer than the frequentist analysis, making it practical for genome-wide studies.

As a third case-study, we used Grid-LMM to estimate the additive and pairwise-epistatic variance components for each of 20,178 gene expression traits measured on 681 *Arabidopsis* accessions from the 1001 Genomes Project [51]. Using a grid-size of 0.05 h^2 units, we estimated the magnitude of each variance component by REML (Fig 3a). The whole analysis took ≈ 6 minutes. Finding REML solutions for the same two-random effect model, for each of the traits separately, using the exact method LDAK took ≈ 90 minutes. Grid-LMM variance component estimates replicated those of

LDAK accurately, but with less precision because of the coarse grid size (S4 Fig). Notably, for many genes, the proportion of variance in expression attributed to additive variance dropped considerably when the epistatic variance was also modeled (S5 Fig). Therefore including multiple random effects can have significant impacts on downstream conclusions for many traits.

Even with this relatively large sample size for a population-level gene expression dataset, considerable uncertainty remains in the estimated variance components. The point-estimates calculated by REML do not capture this uncertainty and estimating confidence intervals of variance components by REML is difficult. The full Grid-LMM approach can be used to calculate posterior distributions for each variance component with little additional cost — note that MCMC sampling is not needed because a reasonably-sized grid can span all valid values of each variance component proportion (see Methods). Using this approach, we identify 8,585 genes with a posterior probability of non-zero epistatic variance greater than 90%, and 28 genes with a posterior mean epistatic variance component greater than 80%. For two example genes, we show that the fitted posterior distributions are similar to those estimated by MCMC using `rstan` [52, 53] (see Fig 3b-c). The `rstan` analyses took ≈ 20 hours per gene to generate an effective sample size of $\approx 200 - 400$ for the variance component parameters. Therefore, posterior inference by MCMC for all 20,178 genes would take about 50 years of computational time.

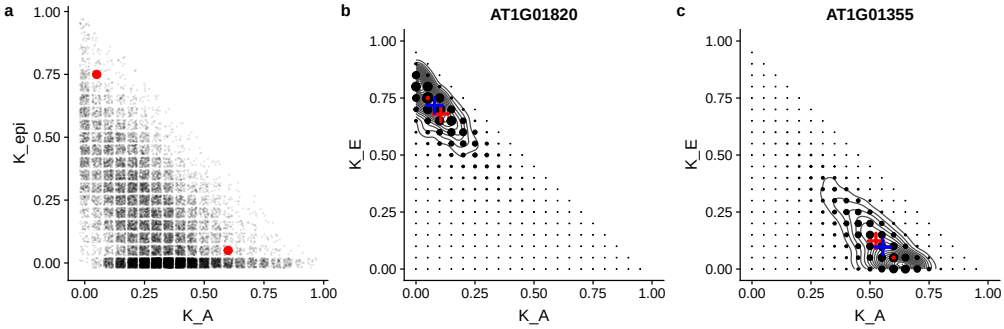

Fig 3. Variance component estimates for *Arabidopsis* gene exprssion traits. Grid-LMM was run using a grid size of 0.05 h^2-units for each of 20,178 genes. (**a**) REML estimates of the variance components proportions for the additive ($\mathbf{K}_A$) and pairwise-epistasis ($\mathbf{K}_E$) genetic variance. Estimates are jittered for clarity. (**b-c**) Posterior distributions of variance component proportions for two example genes, highlighted with red dots in (**a**). The area of each point is proportional to the posterior mass at that combination of the two variance components. A uniform prior over the grid was assumed, and the intercept was assigned a Gaussian prior with infinite variance. The grey contours show the posterior density as estimated by `rstan` using half-Student-t(3,0,10) priors on each standard deviation parameter. In each plot, the red dot shows the REML estimates. The red cross is the posterior mean as estimated by Grid-LMM. The blue cross shows the posterior mean as estimated by `rstan`.

Discussion

Grid-LMM addresses a central obstacle to the practical use of linear mixed models: the computational time needed to find optimal solutions for variance components. Our key observation is that for many common quantitative genetics analyses, optimizing variance component parameters to high precision is not necessary. From a Bayesian

perspective, the posterior distribution of variance components tends to be broad even with large sample sizes, and coarse approximations can be sufficient given the uncertainty in their true values. In GWAS applications, magnitudes of variance components are of less interest than the accuracy of test statistics for the (fixed) SNP effects, and we show that these are sufficiently accurate even with approximate variance component proportions. We believe that uncertainty in the estimation of random effect covariance matrices will have a larger effect on both types of statistics than the imprecision in the estimation of the variance components themselves.

The advantage to relaxing the need for perfect variance component solutions is a vast reduction in both computational time and algorithmic complexity. This reduces the time required for a typical GWAS sized dataset with two-or-more random effects from years to hours, and provides a framework for applying LMMs to even more powerful statistical tools [54–56]. We optimize variance component estimation with a grid search, the simplest type of optimization algorithms. At each grid vertex, after conditioning on (relative) variance component values, the LMM simplifies to a simple linear model; therefore, general purpose solutions to normal linear models are available. This means that the simple LMMs we explored in this paper can easily be generalized to more complex methods used in other GWAS-type applications that currently cannot easily be extended to experiments with heterogeneous samples (e.g. set-tests and multi-marker regressions).

We demonstrated Grid-LMM using three examples that are broadly representative of many experimental settings in quantitative genetics. The first was a study of gene-environment interactions, while the second and third focused on the partitioning of genetic variance among additive and non-additive components. Recent studies have shown how neglecting gene-environment covariance when estimating heritability [8] or testing genetic associations [34] can lead to biased estimates. There is little reason to expect these results to be limited to controlled environmental settings, as we have studied here. Previous work has found that incorporating spatial covariance through a Gaussian radial basis function improved estimates of trait heritability within a sample of individuals from Uganda [8]. Spatial variability also exists in agricultural field trials, and two-step approaches are frequently used where spatial variation is removed first and then genetic associations are tested on the residuals. This two-step procedure may be underpowered when true effect sizes are large. Similarly, epistatic and other non-linear genetic variation are known to be large for many traits [58–60] and accounting for this variation may improve our ability to detect both the main effects of markers (as we demonstrated above), as well as possibly interacting loci [17].

The slight differences in posterior means between the Grid-LMM and MCMC-based posterior estimates in Fig 3b-c are due to differences in the priors. MCMC-based LMM implementations classically use inverse-Gamma priors for variance components because of conjugacy [20]. However, others have recommended uniform or half-t-family priors for the standard-deviation parameters of hierarchical models [61], which are easily implemented in `Stan` [52]. We used a half-Student-t(3,0,10) distribution for each variance component in our `rstan` model to produce Fig 3b-c. This is easy to approximate in Grid-LMM; relative prior weights can simply be applied to each grid-vertex, resulting in much closer agreement of posterior summaries between the two methods (see S6 Fig). As we show in S7 Fig, supposedly "uniformative" versions of both the inverse-Gamma and half-Cauchy-type priors are actually highly informative for variance component *proportions*. In our experience, it is more natural to elicit priors on variance component proportions than variance components themselves, particularly when the phenotypes are on very different scales, because these can be interpreted as the relative importance of the various factors. This is an additional advantage of the LMM parameterization that we utilize in Grid-LMM.

The Grid-LMM approach does have limitations. First, the size of the grid spanning the variance components increases nearly exponentially with the number of random effects. Since each grid vertex requires a separate Cholesky decomposition of the observation-level covariance matrix, a grid search quickly becomes prohibitively expensive with more than ∼6-8 variance components. This is a general problem for mixed model algorithms, and it may be possible to adapt efficient derivative-based algorithms to the grid space. Our fast-heuristic search method converges on the correct answer in most cases we have examined; however, likelihood surfaces of linear mixed models are not always convex and this algorithm may converge onto a local maximum in such cases. We note that most general-purpose algorithms for LMMs with multiple random effects are also sensitive to this issue. Second, for REML or posterior inference of variance component proportions, Grid-LMM estimates are accurate but not precise; they are limited by the resolution of the grid. We show that this has little impact on hypothesis testing for fixed effects, for example in GWAS settings. However, boundaries of posterior intervals in particular may not be reliable. Nevertheless, summaries like posterior mean or (co)variance are highly accurate (Fig 3). Third, the Grid-LMM approach is limited to Gaussian linear mixed models. Generalized linear mixed model algorithms rely on iteratively re-weighting the observations, a function that changes the covariance matrix in a way that cannot be discretized. Finally, we have not explored LMMs with correlated random effects, although these are commonly used in quantitative genetics. Since correlation parameters are restricted to the interval $(-1, 1)$, discretizing correlations in parallel with the variance component proportions may be feasible and is an avenue that is worth future study.

Methods

Linear Mixed Models

We consider the following parameterization of the standard linear mixed model:

$$\begin{aligned} \mathbf{y} &= \mathbf{W}\boldsymbol{\alpha} + \mathbf{X}\boldsymbol{\beta} + \sum_{l=1}^{L} \mathbf{Z}_l \boldsymbol{u}_l + \mathbf{e}, \\ \boldsymbol{u}_l &\sim \mathrm{N}(\mathbf{0}, \sigma^2 h_l^2 \mathbf{K}_l), \\ \mathbf{e} &\sim \mathrm{N}(\mathbf{0}, \sigma^2 h_e^2 \mathbf{I}), \end{aligned} \tag{1}$$

where n is the number of observations, L is the number of random effect terms (not including the residuals), $\mathbf{y}$ is an $n \times 1$ vector of quantitative traits, and $\mathbf{W}$ and $\mathbf{X}$ are $n \times c$ and $n \times p$ design matrices for covariates and marker effects, respectively, with $\boldsymbol{\alpha}$ and $\boldsymbol{\beta}$ corresponding $c \times 1$ and $p \times 1$ vectors of coefficients. Similarly, $\mathbf{Z}_l$ are $n \times r_l$ design matrices with corresponding random effects $\boldsymbol{u}_l$, which are normally distributed around zero and have covariance matrices proportional to the known positive semi-definite $r_l \times r_l$ matrices $\mathbf{K}_l$. Lastly, $\mathbf{e}$ is a $n \times 1$ vector of uncorrelated normally distributed errors, and $\mathrm{N}(\bullet, \bullet)$ denotes the multivariate normal distribution. The common variance of all random effects are denoted by σ^2, and the vector $\mathbf{h}^2 = (h_1^2, \ldots, h_L^2, h_e^2)$ represents the proportion of variance attributed to each random effect term or the residual error. Elements of $\mathbf{h}^2$ are all non-negative and sum to one, forming an L-dimensional simplex.

In GWAS applications, we assume that $\mathbf{W}$ is constant across markers and the value of $\boldsymbol{\alpha}$ is not of central interest; meanwhile, $\mathbf{X}$ varies for each test and we aim to perform statistical inference on a subset of $\boldsymbol{\beta}$. In heritability estimation applications, we focus on inferring the vector $\mathbf{h}^2$. In both cases, the vectors $\boldsymbol{u}_l$ and $\mathbf{e}$ are nuisance parameters

and we can integrate them out resulting in the following equivalent model:

$$\begin{aligned} \mathbf{y} &\sim \mathrm{N}(\mathbf{W}\boldsymbol{\alpha} + \mathbf{X}\boldsymbol{\beta}, \sigma^2\mathbf{V}), \\ \mathbf{V} &= \sum_{l=1}^{L} h_l^2 \mathbf{Z}_l \mathbf{K}_l \mathbf{Z}_l^\intercal + h_e^2 \mathbf{I}. \end{aligned} \tag{2}$$

If the matrix $\mathbf{V}$ is full-rank (which is guaranteed if $h_e^2 > 0$), we can use the inverse of the Cholesky decomposition $\mathbf{V} = \mathbf{L}\mathbf{L}^\intercal$ to transform Equation 2 to the following:

$$\mathbf{y}^* \sim \mathrm{N}(\mathbf{W}^*\boldsymbol{\alpha} + \mathbf{X}^*\boldsymbol{\beta}, \sigma^2\mathbf{I}) \tag{3}$$

where $\mathbf{y}^* = \mathbf{L}^{-1}\mathbf{y}$, $\mathbf{W}^* = \mathbf{L}^{-1}\mathbf{W}$ and $\mathbf{X}^* = \mathbf{L}^{-1}\mathbf{X}$. Equation 3 is a simple linear model for $\mathbf{y}^*$, with the likelihood function:

$$\begin{aligned} &l_F(\mathbf{y}^*; \boldsymbol{\alpha}, \boldsymbol{\beta}, \sigma^2 \,|\, \mathbf{h}^2) = \\ &\qquad \frac{1}{2}\left[-n\log(2\pi\sigma^2) - \frac{1}{\sigma^2}(\mathbf{y}^* - \mathbf{W}^*\boldsymbol{\alpha} - \mathbf{X}^*\boldsymbol{\beta})^\intercal(\mathbf{y}^* - \mathbf{W}^*\boldsymbol{\alpha} - \mathbf{X}^*\boldsymbol{\beta}))\right], \end{aligned}$$

where efficient methods for inference of $[\boldsymbol{\alpha}, \boldsymbol{\beta}]$ and σ^2 are well known. We derive maximum-likelihood and restricted-maximum likelihood solutions for these parameters here, as well as posterior distributions under the conditional normal-inverse-gamma prior below. The log-likelihood and restricted-likelihood functions (respectively) for Equation 2 are:

$$l_F(\mathbf{y}; \boldsymbol{\alpha}, \boldsymbol{\beta}, \sigma^2, \mathbf{h}^2) = l_F(\mathbf{y}^*; \boldsymbol{\alpha}, \boldsymbol{\beta}, \sigma^2 \,|\, \mathbf{h}^2) - \log|\,\mathbf{L}| \tag{4}$$

and

$$\begin{aligned} &l_R(\mathbf{y}; \boldsymbol{\alpha}, \boldsymbol{\beta}, \sigma^2, \mathbf{h}^2) = \\ &\qquad l_F(\mathbf{y}; \boldsymbol{\alpha}, \boldsymbol{\beta}, \sigma^2, \mathbf{h}^2) + \frac{1}{2}\left[(c+p)\log(2\pi\sigma^2) + \log|\,\widetilde{\mathbf{X}}^\intercal\widetilde{\mathbf{X}}| - \log|\widetilde{\mathbf{X}}^{*\intercal}\widetilde{\mathbf{X}}^*|\right] \end{aligned} \tag{5}$$

which are simple (and computationally inexpensive) updates to the likelihood function of Equation 3. Here, we denote $|\bullet|$ as the matrix determinant and let $\widetilde{\mathbf{X}} = [\mathbf{W}; \mathbf{X}]$ and $\widetilde{\mathbf{X}}^* = [\mathbf{W}^*; \mathbf{X}^*]$, respectively. For ML and REML applications, we can calculate the profile likelihoods $l_F(\mathbf{y}; \mathbf{h}^2)$ and $l_R(\mathbf{y}; \mathbf{h}^2)$ as functions of the profile likelihood of the rotated data, which is formed by maximizing $l_F(\mathbf{y}^*; \boldsymbol{\alpha}, \boldsymbol{\beta}, \sigma^2 \,|\, \mathbf{h}^2)$ with respect to $\boldsymbol{\alpha}$, $\boldsymbol{\beta}$, and σ^2. Namely:

$$l_F(\mathbf{y}; \mathbf{h}^2) = \frac{n}{2}\left[\log\left(\frac{n}{2\pi}\right) - 1 - \log(\mathrm{RSS}_{\mathbf{y}^*})\right] - \log|\,\mathbf{L}| \tag{6}$$

where $\mathrm{RSS}_{\mathbf{y}^*} = \mathbf{y}^{*\intercal}[\mathbf{I} - \widetilde{\mathbf{P}}]\mathbf{y}^*$ is the residual sum of squares, and $\widetilde{\mathbf{P}} = \widetilde{\mathbf{X}}^*(\widetilde{\mathbf{X}}^{*\intercal}\widetilde{\mathbf{X}}^*)^{-1}\widetilde{\mathbf{X}}^{*\intercal}$ is a projection (hat) matrix.

Statistical inference by parallel grid search

We now outline the Grid-LMM approach for calculating approximate Wald-test statistics, and then show extensions for computing Bayesian posterior distributions of variance components and marker specific Bayes Factors.

A Wald test for the null hypothesis $\mathbf{M}\boldsymbol{\theta} = \mathbf{0}$ for $\boldsymbol{\theta} = [\boldsymbol{\alpha}^\intercal, \boldsymbol{\beta}^\intercal]^\intercal$ and an arbitrary $q \times (c+p)$ matrix $\mathbf{M}$ uses the general F-statistic:

$$F_{\mathrm{Wald}} = \frac{\widehat{\boldsymbol{\theta}}^\intercal\mathbf{M}^\intercal(\mathbf{M}\widetilde{\mathbf{X}}^\intercal\mathbf{V}^{-1}\widetilde{\mathbf{X}}\mathbf{M}^\intercal)\mathbf{M}\widehat{\boldsymbol{\theta}}}{q} \tag{7}$$

with q and $(n-c-p)$ degrees of freedom, where $\widehat{\boldsymbol{\theta}}$ is the estimate of $\boldsymbol{\theta}$ using the REML estimate of $\mathbf{V}$ [62].

To calculate genome-wide Wald statistics, we must estimate $\widehat{\mathbf{h}}^2$ for each of the p markers tested. There are no closed-form ML (or REML) solutions for $\mathbf{h}^2$; therefore, iterative algorithms are required. A naive approach involves repeatedly inverting $\mathbf{V}$, which scales cubically in the number of observations. Since the estimates for $\widehat{\mathbf{h}}^2$ differ for each test, the total computational complexity of in a GWAS setting is $\mathcal{O}(tpn^3)$ assuming an average of $\approx t$ inversions per run of the optimization algorithm (generally $\approx 3-100$, increasing with the number of random effect parameters). The fast algorithm used in GEMMA [24] and FaST-LMM [23] reduces this to $\mathcal{O}(n^3+pn^2+ptc^2n)$ by utilizing the eigenvalue decomposition of the similarity matrix $\mathbf{K}$. However, this only works for a single random effect. We are unaware of any exact algorithm with lower computational complexity for models with multiple random effects (and full-rank covariance matrices).

With Grid-LMM, rather than optimizing $\mathbf{h}^2$ separately for each marker, we instead define a grid of candidate values for $\mathbf{h}^2$ and calculate the restricted profile-likelihood $l_R(\mathbf{h}^2 \,|\, \mathbf{y}^*)$ at every grid vertex for each marker. At each grid vertex, we must invert $\mathbf{V}$ once, but we can re-use this calculation for every marker. This has a computational complexity of $\mathcal{O}(g(n^3+cn^2+pbn^2))$ for a grid with g vertices, assuming covariates $\mathbf{W}$ are constant across markers. For all analyses reported in the main text, we use a rectangular grid with a resolution of 0.1 or 0.01 h^2-units, with the restriction that all $h_l^2 \geq 0$ and $\sum_{l=1}^{L} h_l^2 < 1$. As described below, we either peg this grid to the origin (i.e. $h_l^2 = 0, \forall l$), or to the REML estimate $\widehat{\mathbf{h}}_0^2$ derived from the null model with no marker effects. This grid search generates a vector of g REML scores for each marker. We select the values of $\widehat{\mathbf{h}}^2$ that correspond to the highest score for each marker and use Equation 7 to calculate the approximate Wald statistics.

To calculate approximate likelihood ratio test statistics, we use the Grid-LMM approach to calculate the full profile-likelihoods for models under both the null and alternative hypothesis.

For Bayesian inference, rather than working with the profile likelihoods, we instead use a conditional normal-inverse-gamma prior $([\boldsymbol{\alpha}^\intercal, \boldsymbol{\beta}^\intercal]^\intercal, \sigma^2) \sim \mathrm{NIG}(\mathbf{0}, \boldsymbol{\Psi}, a_0, b_0)$, and then integrate over the prior to calculate the marginal likelihood of the data given $\mathbf{h}^2$. This results in the following:

$$p(\mathbf{y} \,|\, \mathbf{h}^2) = \frac{b_0^{a_0}}{(2\pi)^{n/2+(c+p)/2}|\mathbf{V}|^{1/2}|\boldsymbol{\Psi}|^{1/2}\Gamma(a)} \times \frac{(2\pi)^{(p+c)/2}|\boldsymbol{\Psi}^*|^{1/2}\Gamma(a^*)}{(b^*)^{a^*}}, \tag{8}$$

where $\Gamma(\bullet)$ is the gamma function, $\boldsymbol{\Psi}^* = (\boldsymbol{\Psi}^{-1} + \widetilde{\mathbf{X}}^\intercal \mathbf{V}^{-1}\widetilde{\mathbf{X}})^{-1}$, $a^* = a_0 + n/2$, and $b^* = b_0 + \mathrm{RSS}_{\mathbf{y}^*,\boldsymbol{\Psi}}/2$ with $\mathrm{RSS}_{\mathbf{y}^*,\boldsymbol{\Psi}}$ having the same form as $\mathrm{RSS}_{\mathbf{y}^*}$ in Equation 6 — except with $\widetilde{\mathbf{P}} = \widetilde{\mathbf{X}}^*\boldsymbol{\Psi}^*\widetilde{\mathbf{X}}^{*\intercal}\widetilde{\mathbf{X}}^*\boldsymbol{\Psi}^*\widetilde{\mathbf{X}}^{*\intercal}$. See S1 Supplementary Methods for more detail on the specific derivations. We calculate the marginal likelihood $p(\mathbf{y} \,|\, \mathbf{h}^2)$ at each vertex of the grid as described above. Assuming a discrete-valued prior $p(\mathbf{h}^2)$, we can then compute the posterior distribution of $\mathbf{h}^2$ as:

$$p(\mathbf{h}^2 \,|\, \mathbf{y}) = \frac{|\mathbf{V}_{\mathbf{h}^2}|^{-1/2}|\boldsymbol{\Psi}^*_{\mathbf{h}^2}|^{1/2}(b^*_{\mathbf{h}^2})^{-a^*}p(\mathbf{h}^2)}{\sum_{\mathbf{h}^2}|\mathbf{V}_{\mathbf{h}^2}|^{-1/2}|\boldsymbol{\Psi}^*_{\mathbf{h}^2}|^{1/2}(b^*_{\mathbf{h}^2})^{-a^*}p(\mathbf{h}^2)} \tag{9}$$

where, for clarity, parameters that are a function of $\mathbf{h}^2$ are denoted with a subscript. Continuous target densities $\pi(\mathbf{h}^2)$ can be approximated as $p(\mathbf{h}^2)$ by assigning each grid vertex a probability equal to the integral of $\pi(\mathbf{h}^2)$ over the L-dimensional rectangle centered at the corresponding value $\mathbf{h}^2$. We assume a uniform prior over our grid for all analyses presented in the main text. Bayes factors are computed by comparing models under the alternative and null hypotheses as the ratios in Equation 9. All analytical

calculations — including the summation in Equation 9 — can be performed on the log-scale to prevent numerical underflows. Terms common to both models drop out of the ratio; therefore, limiting improper priors on $\boldsymbol{\alpha}$ and σ^2 can be used, which results in scale-independence [49].

Accelerated grid searches

The full grid search described above is dramatically faster than the naive algorithm for mixed-model GWAS analyses, as long as the vertices of the grid is less than the number of genetic markers (i.e. $g < p$) and can easily be parallelized across multiple computers. However, g grows rapidly as the grid resolution and number of random effects increases:

$$g = \sum_{k=1}^{L} \binom{m}{k} \binom{L-1}{k-1}, \tag{10}$$

for a grid with m divisions per h_l^2 and, therefore, can still be slow. If we make two assumptions which are commonly true, we can develop heuristics for both the ML/REML and Bayesian algorithms that prevent the need to evaluate every grid vertex:

- The vast majority of markers have little association with the phenotype. This is a common hypothesis in GWAS settings [63–66], and for our purposes, we merely require that the percentage of variance explained individually by most markers is smaller than the difference between grid vertices $\approx 1/m$.
- The likelihood and/or posterior function is convex. This is not always true, since both the likelihood and posterior functions can have > 1 maximum. However, the conditions that cause these events are rare [67], and most exact LMM algorithms are also susceptible to converging to local maxima.

To search for the ML or REML solutions, we first find $\widehat{\mathbf{h}}_0^2$ under the null model with no marker effects. We calculate the profile (restricted)-likelihood scores for each test at $\widehat{\mathbf{h}}_0^2$, and then form a grid centered at this value by adding or subtracting $1/m$ to each h_l^2 in all combinations. For two random effects, this grid will be a ring around $\widehat{\mathbf{h}}_0^2$ with $g_1 \leq 8$ vertices (depending on if $\widehat{\mathbf{h}}_0^2$ is within $1/m$ of a boundary of the simplex). We calculate the scores for each test at each vertex of the grid and compare the maximum scores to the scores at $\widehat{\mathbf{h}}_0^2$. For every test, when no greater value is found, we choose $\widehat{\mathbf{h}}_0^2$ as the maximum and skip the corresponding marker in all future calculations. For the remaining p_2 markers, we select the set $\{\widehat{\mathbf{h}}_0^2, \mathbf{h}_1^2, \ldots, \mathbf{h}_j^2\}$ of grid vertices that maximized the scores for 1+ tests, and form a new grid of size g_2 around all of them, dropping vertices already tested. This procedure is repeated T times until the new grid no-longer increases scores for any test and we are confident that all (approximate) maximums have been found. This accelerated search has total complexity $\mathcal{O}(n^3 + pn^2 + \sum_{i=1}^{T} g_i(n^3 + p_i n^2))$.

Analogously, to accelerate evaluations of posterior distributions, we evaluate $p(\mathbf{y} \mid \mathbf{h}^2)$ over an initial grid of resolution $1/m_1$ with discrete prior $p_{m_1}(\mathbf{h}^2)$ and estimate the posterior distribution as in Equation 9. Summary statistics, such as the posterior mean and variance, will be more accurate if the posterior mass is distributed across multiple vertices of the grid. Therefore, we identify the set $\mathcal{H} = \{\mathbf{h}_l^2\}$ of vertices that together explain 99% of the posterior mass. If the size of this set is below a threshold (say $|\mathcal{H}| = 10$), we then form a new grid with double the resolution $m_2 = 2m_1$ and a new prior $p_{m_2}(\mathbf{h}^2)$. Vertices that overlap between the grids can be filled in directly. We then begin filling in the new grid by evaluating vertices within $1/m_2$ distance from any h_l^2 corresponding to the vertices in $\mathcal{H}$. After each iteration, we re-calculate the set $\mathcal{H}$, and

continue evaluating the neighboring vertices as long as $\mathcal{H}$ continues to grow. If the size of $\mathcal{H}$ remains smaller than the threshold after whole grid is evaluated, or no new vertices are added to $\mathcal{H}$ at the end of an iteration, we double the resolution again: $m_{i+1} = 2m_i$ and repeat the grid-filling steps again. We note that this procedure is only appropriate if the posterior is convex and, therefore, is limited to the case of uniform priors on $\mathbf{h}^2$.

To accelerate evaluations of GWAS Bayes Factors, we combine the two previously described algorithms. In particular, we define a grid centered on $\widehat{\mathbf{h}}_0^2$ with a resolution $1/m$ that is sufficiently fine such that we expect each posterior to be distributed across multiple vertices. We then calculate $p(\mathbf{y} \,|\, \mathbf{h}^2)$ for each test, starting from $\widehat{\mathbf{h}}_0^2$ and moving out in concentric rings on the grid. After each iteration, if the new ring contributes $< 0.01\%$ to the total posterior mass (among evaluated vertices) for that test, we assume the posterior is well characterized and stop evaluating $p(\mathbf{y} \,|\, \mathbf{h}^2)$ for that marker. As for the ML and REML solutions above, markers with little to no association with the phenotype will lead to posteriors of $\mathbf{h}^2$ that are concentrated close to $\widehat{\mathbf{h}}_0^2$; hence, only markers with large effects will shift $p(\mathbf{h}^2 \,|\, \mathbf{y})$ strongly to new regions of the grid.

Unless otherwise specified, we used accelerated grid searches for all Grid-LMM analyses presented here with grid step sizes of 0.01 h_l^2 units.

GWAS datasets

Genotype and phenotype data on 107 *Arabidopsis thaliana* traits and 216,130 genetic markers were downloaded from `https://github.com/Gregor-Mendel-Institute/atpolydb/wiki`. We follow practices suggested by the original authors of these data and log-transformed a subset of the phenotypes prior to analyses (except for traits that had values equal to zero) [44] . For the analysis of gene-environment interactions for flowering time, we (1) extracted the trait identifiers "7_FT10" (i.e. growth chamber at 10C) and "57_FT Field" (i.e. field setting), (2) selected data from the 175 accessions that were measured in both environments, and (3) concatenated the two datasets into a single vector of 350 observations. The two traits were individually standardized to have mean zero and standard deviation one prior to analysis. We used the `sommer` package in `R` to calculate an additive relationship matrix from all 216,130 markers [68], and then created a G×E kinship matrix as $\mathbf{DZKZ^\intercal D}$ where $\mathbf{K}$ is the 175×175 additive genomic relationship matrix, $\mathbf{Z}$ is a 350×175 incidence matrix linking observations to accessions, and $\mathbf{D}$ is a 350×350 diagonal matrix with elements equal to -1 or 1 corresponding to observations measured under "7_FT10" and "57_FT Field", respectively. Plasticities for each accession were calculated as the difference between "57_FT Field" and "7_FT10". We ran GEMMA (version 0.97) with the "-lmm1" option for Wald tests with the $\mathbf{K}$ matrix described above. We emulated EMMAX and `pylmm` functionality by estimating variance components using a null model with no marker effects, and either a single $\mathbf{K}$ matrix (for single-trait analyses) or 3 random effects (for G×E analysis). Wald test statistics were computed for each marker using our GridLMM `R` function with a grid consisting of only a single vertex. For G×E analyses, we fit a model with a main effect of the environment, a main effect for each marker of interest, and an interaction between the marker and the environment — and then calculated the Wald-F statistic only for the interaction effect.

The heterogeneous stock of mice data from the Wellcome Trust Centre for Human Genetics (`http://mtweb.cs.ucl.ac.uk/mus/www/mouse/index.shtml`) consists of 1,814 individuals from 85 families, all descending from eight inbred progenitor strains [46]. We used the marker and phenotype data provided in the `BGLR` `R` package [69] from which we extracted the "EndNormalBW" trait and information on the gender and cage of each mouse. Gender was used as a covariate in all analyses, and cage assignment was treated as a random effect in the three-random-effect models.

Additive and epistatic kinship matrices were calculated using `sommer`. Wald test statistics and Bayes Factors were calculated using the heuristic (accelerated) grid search of Grid-LMM. Bayes Factors were calculated assuming flat priors on the intercept and residual variance term, a standard normal prior $\mathrm{N}(0,1)$ on the marker effects — similar to the previously proposed D_2 prior [49] — as well as a uniform prior over the grid of variance component proportions.

Computational timings for GWAS analyses are reported for a MacBookPro14,3 with a 2.9Ghz Intel Core i7 processor and using only a single CPU core. Further speedups are possible by parallelizing the grid search. For accelerated grid searches, REML estimates of variance components under the null model (i.e. the starting points of the grid search) were calculated with `LDAK` and are included in the computational time estimates.

Gene expression dataset

Gene expression data on 24,175 genes from 728 *Arabidopsis* accessions was downloaded from `http://signal.salk.edu/1001.php` and subsetted to genes with average counts ≥ 10. A genomic relationship matrix ($\mathbf{K}_A$) for 1,135 accessions was downloaded from `http://1001genomes.org/data/GMI-MPI/releases/v3.1/SNP_matrix_imputed_hdf5/`. Both sets of data were subsetted to an overlapping set of 665 accessions. $\mathbf{K}_A$ was then centered by projecting out the intercept and normalized to have mean diagonal element equal to one. The pairwise-epistasis genomic relationship matrix $\mathbf{K}_E$ was calculated with element-wise multiplication as $\mathbf{K}_A \odot \mathbf{K}_A$, and then also normalized to have mean diagonal element equal to one. The gene expression matrix was normalized and variance-stabilized using the `varianceStabilizingTransformation` function of the `DEseq2` package [70]. Grid-LMM REML estimates were compared to exact REML estimates from the `LDAK` program with variance components constrained to be non-negative. Grid-LMM posterior distributions estimates were compared to those estimated using `Stan` [52] with the `rstan` `R` package [53]. To speed computation, we diagonalized the $\mathbf{K}_A$ covariance matrix by calculating the singular value decomposition $\mathbf{K}_A = \mathbf{U}\mathbf{S}\mathbf{U}^\intercal$, and pre-multiplying both sides of the LMM by $\mathbf{U}^\intercal$. We used a half-Student-t prior distribution with 3 degrees of freedom and a scale of 10 for the three standard deviation parameters. We ran four MCMC chains each of length 10,000, with the first 5,000 as warmup and a thinning rate of 20. Because of poor mixing, this resulted in an "$\mathrm{n}_{\mathrm{eff}}$" of approximately 200-400 per variance component parameter for each trait.

Power simulations

We compared the power of one and two-random effect models for detecting G×E markers based on the Arabidopsis flowering time data. We calculated additive genetic and G×E relationship matrices as described above using the actual genotypes. We then simulated phenotypes by summing together a G×E effect of a particular marker, (co)variance from the two random effects, and normally distributed error. For each combination of marker effect sizes ($\{0, 0.025, 0.5, 0.1, 0.15, 0.2\}$% of the total phenotypic variance), and random effect proportions ($h_l^2 \in \{0, 0.4, 0.8\}$ for each random effect), we ran 1000 simulations with different randomly selected markers from the Arabidopsis genotype data. We then fit five models to each dataset and calculated Wald-tests for marker G×E effects using each. The five methods consisted of three two-random effect approaches including: (1) an exact two-random effect model fit with the `sommer` package in `R` [68], (2) the approximate two-random effect model fit with Grid-LMM with a grid size of 0.1 h^2-units, and (3) the approximate two-random effect model `pylmm` that conditions on variance components estimated under the null model [34]. We also

consider two one-random effect models that could be fit with GEMMA: (4) a model that only included the additive genetic relationships and ignored the G×E covariance, and (5) a model that only included the G×E covariance and ignored the additve genetic relationships. We calculated statistical power for each GWAS method as the proportion of Wald test p-values exceeding the genome-wide significance level at the conventional Bonferroni corrected $P = 2 \times 10^{-7}$ (S2 Fig). For the simulations with a marker effect size of 0%, we compared the distribution of $-\log_{10}(p)$-values from each model to the expectation from a uniform distribution (S3 Fig). The results show that Grid-LMM maintains nearly identical power to the exact method in each simulation while maintaining an accurate null-distribution. The approximate method based on `pylmm` has uniformly lower power, but maintains an accurate null-distribution. The one-random effect methods show opposite results: when including only the additive relationship matrix, power for detecting G×E is uniformly high, but the null-distribution of $-\log_{10}(p)$-values is greatly inflated when G×E-variation is large. On the other hand, power for detecting G×E is low when only G×E covariance is modeled and yet the additive genetic-variance is large. Furthermore, the null-distribution of $-\log_{10}(p)$-values for this one random effect method is greatly deflated. These results all confirm our expectations that modeling covariance accurately, but not necessarily precisely, is important for accurate association mapping in GWAS.

Software Availability

Software for computing the Grid-LMM is carried out in R code, which is freely available at `https://github.com/deruncie/GridLMM`. Scripts for running the analyses reported in the manuscript are available at `https://github.com/deruncie/GridLMM_scripts`.

Author Contributions Statement

D.E.R. and L.C. conceived the experiment(s), D.E.R. conducted the experiment(s), D.E.R. and L.C. analyzed the results. Both authors wrote and revised the manuscript.

Competing Financial Interests

The authors have declared that no competing interests exist.

Supporting information

S1 Fig. Accuracy of the log-transformed p-values across the 107 Arabidopsis phenotypes [44]. GWASs were run for each phenotype using 216,130 markers and up to 199 accessions the single random effect of additive genetic relationships among lines. For each phenotype (represented as a single point in the plots), we compared the exact Wald-test -$\log_{10}(p)$ calculated by GEMMA to p-values calculated by the approximate methods EMMAX, and Grid-LMM using either the naive approach with a complete grid of size 0.1 h^2-units, or the fast heuristic algorithm Grid-LMM-fast with a fine grid size of 0.01-h^2 units. Grid-LMM p-values were always at least as accurate (as measured by root mean-squared-error, RMSE) as those calculated by EMMAX. Specifically, p-values calculated with a fine grid-size of 0.01 (using the fast algorithm) were nearly indistinguishable from those of GEMMA, except in the rare cases where the REML surface was not unimodal. This was generally restricted to a small subset of rare markers with small-moderate effect sizes, and

occurred for only a few traits. In these cases, the complete — but more coarse — grid search of Grid-LMM with step sizes of 0.1 was more accurate.

S2 Fig. Power analysis for simulated data. Bars show the genome-wide power for randomly selected SNPs in the Atwell genotype data under simulations with different proportions of additive (G) and gene-environment interaction (G×E) variation with different marker effect sizes. Each simulation included only a single marker with zero main effect and G×E effects scaled to a defined percentage of the phenotypic variation. The remaining phenotypic variation was simulated from a multivariate normal distribution constructed by appropriately weighting the additive relationship matrix, the G×E covariance matrix, and residual uncorrelated variation. Each simulation was run separately for 1000 randomly selected markers and Wald-test p-values were computed from five methods and compared to the Bonferroni corrected threshold $P = 2 \times 10^{-7}$ to determine significance. `exact-LMM-G+GxE` is an exact LMM algorithm fit with the `sommer` package [68]. The model included both random effects and the marker effect. At a genome-wide scale, it is very slow, with computational complexity $\mathcal{O}(ptn^3)$. `exact-LMM-G` and `exact-LMM-GxE` are exact LMM algorithms, similar to GEMMA, which included only one random effect and the marker effect. `null-LMM` is an approximation to the `pylmm` method that conditions on variance components estimated under a null model with no marker effect. It was run with both random effects. *Grid-LMM* was run with a grid size of 0.1 h^2-units and included both random effects and the marker effect.

S3 Fig. Null simulation results. QQ-plots of observed versus expected distributions of -$\log_{10}(p)$-values from simulations with no marker effect and residual phenotypic variation constructed with different proportions of additive (G) and gene-environment interaction (G×E) variation (as in S2 Fig).

S4 Fig. Comparison of REML estimates between Grid-LMM and LDAK for 20,843 *Arabidopsis* genes. (**a**) REML estimates for the additive genetic variance (variance component for $\mathbf{K}_A$). (**b**) REML estimates for the epistatic genetic variance (variance component for $\mathbf{K}_E$).

S5 Fig. Comparison of REML estimates for the additive genetic variance between models with or without an additional pairwise-epistasis random effect for 20,843 *Arabidopsis* genes. Both models were fit using Grid-LMM with a grid size of 0.05 h^2 units. Point positions are jittered for clarity.

S6 Fig. Posterior distributions of variance components for two genes under the half-Student-t(3,0,10) prior. Panels (**b**) and (**c**) of Fig 3 in the main text are repeated, except the half-Student-t(3,0,10) prior on the standard deviation of the variance components for $\mathbf{K}_A$ and $\mathbf{K}_E$ random effects was applied to each grid vertex. The prior was approximated by simulating 1×10^4 independent draws for σ_A, σ_E and σ_e, converting these to prior draws for h^2_A and h^2_E, and then measuring the proportion of draws closest to each grid vertex.

S7 Fig. Inverse-Gamma and half-Student-t priors are informative for variance component proportions. We compare the implied prior distributions on variance component proportions for three classes of priors in a two-random effect model (e.g. $\mathbf{K}_A$ and $\mathbf{K}_E$ as random effects plus uncorrelated random error). (**a**)-(**b**) Each standard deviation parameter was assigned a half-Student-t prior with 3 degrees of freedom and scale parameter of 10. (**c**)-(**d**) Each variance parameter was assigned an

inverse-Gamma prior with shape parameter 2 and scale parameter 1. (**e**)-(**g**) A uniform prior was applied to the 2-dimensional simplex of $[h^2_A, h^2_E, h^2_e]$. This is the default prior in GridLMM and equivalent to all analyses reported in the main text. (**a**)-(**c**)-(**e**) 2D-density plots for the two variance component proportions. Lighter blue denotes higher prior density. (**b**)-(**d**)-(**f**) Marginal densities for the $\mathbf{K}_A$ variance component proportion under each prior. The half-Student-t prior implies high probability that only one variance component is important. The inverse-Gamma prior implies high probability that all variance component proportions are non-zero.

S1 Table Markers associated with heterogenous stock mice body weight only when accounting for non-additive genetic covariance. Markers with -$\log_{10}(p) > 3$ on chromosomes 4 and 11 are shown. Position information for each marker is derived from [71]. Other studies that identified the same marker associations are listed. WTC: Wellcome Trust Center Heterogeneous Stock Mice. LG,SM Advanced Intercross: F_9 and F_{10} generation mice from of an intercross between LG/J and SM/J strains selected for differences in body size.

S1 Supplementary Methods Derivation of Bayesian posterior and Bayes Factors.

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