Comparison of REML methods for the study of phenome-wide genetic variation

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October 2022

Abstract

It is now well documented that genetic covariance between functionally related traits leads to an uneven distribution of genetic variation across multivariate trait combinations, and possibly a large part of phenotype-space that is inaccessible to evolution. How the size of this nearly-null genetic space translates to the broader phenome level is unknown. High dimensional phenotype data to address these questions are now within reach, however, incorporating these data into genetic analyses remains a challenge. Multi-trait genetic analyses, of more than a handful of traits, are slow and often fail to converge when fit with REML. This makes it challenging to estimate the genetic covariance (\(G\)) underlying thousands of traits, let alone study its properties. We present a previously proposed REML algorithm that is feasible for high dimensional genetic studies in the specific setting of a balanced nested half-sib design, common of quantitative genetics. We show that it substantially outperforms other common approaches when the number of traits is large, and we use it to investigate the bias in estimated eigenvalues of \(G\) and the size of the nearly-null genetic subspace. We show that the high-dimensional biases observed are qualitatively similar to those substantiated by asymptotic approximation in a simpler setting of a sample covariance matrix based on i.i.d. vector observation, and that interpreting the estimated size of the nearly-null genetic subspace requires considerable caution in high-dimensional studies of genetic variation. Our results provide the foundation for future research characterizing the asymptotic approximation of estimated genetic eigenvalues, and a statistical null distribution for phenome-wide studies of genetic variation.

1 Introduction

To answer the most compelling questions in evolutionary biology we must uncover the causal connection between genotypes, phenotypes, and the environment (selection). Unlike the finite genomes that underlie them, organisms are comprised of essentially infinite phenotypes that may genetically vary and covary. While not all of those phenotypes will be meaningful for fitness, there are at least many thousands to consider, if we are to begin to understand the genotype-phenotype map. Efforts to increase the scope and throughput of phenotyping have been cited as an urgent priority in evolutionary biology since at least 2010 [Houle, 2010, Houle et al., 2010]. High-throughput phenomic technologies such as such as RNA sequencing [Schrag et al., 2018], drone based plant imaging [Furbank and
Tester, 2011], wearable sensors [Haleem et al., 2021] Sharma et al., 2021 Neethirajan, 2017, metabolomics for physiological measurements [Jin et al., 2020] Freimer and Sabatti, 2003, and computer vision for morphometrics [Lüörg et al., 2021] are now within reach. However, incorporating these high-dimensional phenotype data into genetic analyses remains a challenge.

Multivariate studies of genetic variation focusing on relatively small sets of traits \( p \ll 20 \) (small \( p \)) have transformed our understanding of how genetic variation is distributed across phenotypes, and how this affects evolutionary outcomes. While almost all individual traits studied have been shown to have genetic variation [Lynch et al., 1998], and responses to artificial selection are often rapid and of large magnitude [Hill and Kirkpatrick, 2010], multivariate studies of the genetic variance-covariance matrix (\( G \)) show that this genetic variation is distributed unevenly across multivariate phenotypes [Kirkpatrick, 2009] Sztepanacz and Houle, 2019]. The concentration of genetic variance onto fewer multivariate trait combinations than the number of phenotypes measured is biologically caused by the pleiotropic effects of alleles on multiple traits which leads to their genetic covariance [Lande, 1980]. Multivariate trait combinations with high genetic variation form a genetic subspace where traits are predicted to have high evolvability. Evolution is predicted to occur more quickly along these multivariate trait combinations than in any individual trait [Agrawal and Stinchcombe, 2009], contributing to divergence among populations (eg. [McGlathlin et al., 2022]) Schluter, 1996], species (eg. Innocenti and Chenoweth, 2013) Bégin and Roff, 2004), and sexes (eg. Gosden and Chenoweth, 2014). The set of orthogonal multivariate trait combinations with low genetic variation, form a complementary subspace which is termed the nearly-null genetic subspace [Gomulkiewicz and Houle, 2009] Gaydos et al., 2013. This subspace putatively represents important evolutionary constraints in natural populations, where phenotypic evolution is expected to be constrained [Gomulkiewicz and Houle, 2009], occur slowly [Kirkpatrick, 2009] or stochastically [Hine et al., 2014].

Estimating the size of the nearly-null genetic subspace is an avenue to quantify both genetic constraints that may lead to evolutionary limits, and the extent of pleiotropy underlying organisms, which determines their genetic dimensionality. Past studies have typically found that genetic variance is restricted to less than half of the phenotype space with most multivariate trait combinations having no detectable genetic variation [Blows and McGuigan, 2015]. One notable exception to this pattern is Drosophila wing shape, which as been shown to have genetic variation in all multivariate wing shape traits (ie. a full rank \( G \)) [Mezey and Houle, 2005] Sztepanacz and Blows, 2015] Sztepanacz and Houle, 2019 Houle and Meyer, 2015]. These multivariate studies have typically dealt with small sets of functionally related traits such as wing [Mezey and Houle, 2005], skeletal Garcia et al., 2014 or phyto [Walsh and Lynch, 2018] morphology, or traits that have a strong physiological Caruso et al., 2005] or biochemical relationships Sztepanacz and Rundle, 2012. Therefore, genetic covariance among these traits may be relatively high compared to the range of traits that are possible to study with phenomics. Whether inferences for the size of the nearly null subspaces found in these small \( p \) studies can be extrapolated to phenomes is an open biological question.

Confounded with the biological phenomena that nearly-null genetic subspaces represent, are the statistical phenomena that arise from their estimation. Even in low \( p \) studies, estimating \( G \) and its eigenvalues is a challenge. The standard approach is to fit a multivariate mixed model in REML to estimate \( G \) as an unstructured covariance matrix. These models commonly fail to converge with only a small number of traits, and run-times are long because of the quadratic growth \( \sim p^2/2 \) in the number of parameters to estimate with the
number of traits. As shown in the one-way MANOVA context, small $p$ models that do converge, often produce estimated matrices that are not positive definite [Hill and Thompson, 1978] and that have overdispersed eigenvalues [Hayes and Hill, 1981], properties which are exacerbated in the two-way hierarchical model, such as the nested half-sib design common in quantitative genetic studies. In particular, [Hayes and Hill, 1981] show that the magnitude of overdispersion of the genetic eigenvalues is determined by $p/n$ with smaller sample sizes or more traits leading to larger dispersion. The overdispersion of estimated eigenvalues is a pattern remarkably similar to that we interpret biologically as genetic subspaces of high evolvability and those that are nearly null. In the phenomic context, where $p/n \sim 1$ or possibly even greater than 1, it could even obscure any biological signal. Disentangling how much of the dispersion of eigenvalues of $G$ is due to biological covariance versus statistical estimation is therefore important, if we want to predict evolutionary constraints.

In simpler settings, recent work has provided a quantitative description of the bias and error related to the dispersion of estimated eigenvalues when $p$ is of order comparable to $n$, summarized for example in [Johnstone and Paul, 2018]. The bulk eigenvalue distribution of sample (i.i.d.) covariance matrices, such as those describing among-individual covariance matrices such as phenotypic covariance matrices $P$, or genomic relatedness matrices (GRMs), is known to follow the Marchenko Pastur distribution [Marˇ cenko and Pastur, 1967, Yao et al., 2015]. For a large class of mixed models, including full-sib and half-sib designs, and for the particular case of MANOVA estimators, overdispersion of sample eigenvalues in the bulk eigenvalue distribution is described by a generalization of the Marchenko-Pastur distribution [Fan and Johnstone, 2019].

The individual eigenvalues of sample covariance matrices also follow particular distributions. The leading eigenvalue conforms to the Tracy-Widom distribution, both for sample (i.i.d.) covariance matrices [Johnstone, 2001] and for MANOVA estimates from mixed models [Fan and Johnstone, 2022]. The trailing (smallest) eigenvalue converges to a reflected Tracy-Widom distribution [Fan and Johnstone, 2022]. Again for MANOVA estimates in mixed models, the leading estimated eigenvalues and eigenvectors can be influenced by an alignment between directions of sufficiently elevated variance in the different covariance components [Fan et al., 2018]. For example, an alignment between the major axis of genetic variance $g_{max}$ and environmental variation could lead to an estimated $g_{max}$ that is biased toward the major axis of environmental variation and with an eigenvalue that is much larger than it should be. These quantitative descriptions provide appropriate null distributions for MANOVA eigenvalues, and enable bias-correction for estimated eigenvalues in these contexts.

Simple among line (one-way) genetic simulation studies estimating $G$ for $p=5$ using REML, combined with an empirical centering and scaling approach [Saccenti et al., 2011], suggest that REML eigenvalues may have similar properties. The bulk distribution of eigenvalues appears to follow the Marchenko Pastur distribution [Blows and McGuigan, 2015], and the leading eigenvalues of estimated $G$ are consistent with the Tracy Widom distribution [Sztepanacz and Blows, 2017]. Notably, however, the leading eigenvalues of $G$ estimated using factor analysis or MCMCglmm do not follow the Tracy Widom distribution [Sztepanacz and Blows, 2017], showing that the method used to estimate $G$ influences the sampling distribution of its eigenvalues. These studies laid the groundwork for determining the properties of REML eigenvalues in the one-way design, however, they are limited to small $p$ and $n \gg p$. A major hurdle in extrapolating these studies to the phenomic-scale, is the difficulty in employing REML for high $p$.

Alternative approaches to REML have been developed in recent years and employed to
estimate $G$ and its eigenvalues for high $p$ phenomic studies. The Bayesian sparse factor approach of Runcie and Mukherjee [2013] enables the estimation of genetic covariance for thousands of traits by placing a prior on the latent factors underlying $G$ and $E$ that few traits contribute to them (ie. they are sparse). Studies using this method have qualitatively found the same uneven distribution of standing genetic variation [Garcia et al., 2014], mutational variation [Hine et al., 2018], and a large nearly-null genetic subspace, as seen in small $p$ studies. While biologically motivated, the assumption that sparse factors underlie both $G$ and $E$ may upwardly bias estimates of the nearly null subspace. MegaLMM [Runcie et al., 2021] uses strong Bayesian priors on the sparsity and number of important latent factors underlying $G$ and $E$ to fit linear mixed models for $> 20,000$ traits and relatively small $n$. The primary goal of this approach, however, is for genomic prediction, not estimation. Consequently, the properties that make the method feasible for including phenome-level data, and which are beneficial for genomic prediction, may have undesirable properties with respect to estimation. Blows et al. [2015] circumvented convergence problems in REML by constructing large $G$ from a set of overlapping principal sub-matrices, estimated for a small numbers of traits using REML. Since the constructed matrix is not guaranteed to be positive definite, a subsequent bending [Hayes and Hill, 1981, Meyer, 2019] of $G$ was required. This approach enabled a coarse description of the distribution of genetic variation across $G$, finding that the vast majority of genetic variation was found in few dimensions. However, a quantitative description of the size of the nearly null subspace was not possible. While each of these approaches have value in certain circumstances, they all make some undesirable assumptions that lead to challenges in interpreting the size of the nearly null space and performing statistical hypothesis testing.

In this paper we demonstrate that REML is feasible for high $p$ phenomics in the specific setting of a balanced nested half-sib design, without having to make any additional assumptions about the structure of $G$ or perform any bending, and we begin to study the properties of estimated eigenvalues from these high $p$ models. In particular, we perform simulation study investigating the spectral characteristics of the REML estimate of $G$ for a family of balanced half-sib breeding designs in the moderate-$p$ ($p \approx 50$) setting.

As algorithms that are usually used to fit mixed models often fail to converge even for $p$ as small as 5, we use an algorithm designed specifically for fitting balanced nested designs introduced in Calvin and Dykstra [1991] that will allow us to find REML estimates for larger values of $p$.

In section 3, we compare this Calvin-Dijkstra algorithm with various other procedures used to compute or approximate the REML estimate, showing that it does at least as well as its common alternatives. We then demonstrate that the algorithm is a practical method for REML estimation for the balanced designs even for $p \approx 50$, in which regard it substantially outperforms the alternatives presented.

Sections 4 and 5 investigate the illustrate the biases inherent in using functions of eigenvalues of the REML estimate $\hat{G}$ to estimate the corresponding functions of the eigenvalues of $G$. We show that, as $p$ gets large, the large eigenvalues of $\hat{G}$ have a substantial upward bias when used to estimate the large eigenvalues of $G$. We also investigate the bias in estimators of the nearly-null dimension of $G$ based on counting small eigenvalues of $\hat{G}$. These biases are more difficult to describe, so we demonstrate the behavior of such estimators on a family of qualitatively different between-sire and between-dam covariance matrices. Section 6 has some summary conclusions and discussion.
2 Description of methods compared

Our simulations use a balanced half-sib (or nested two-way) design

\[ Y_{ijk} = \mu + \alpha_i + \beta_{ij} + \epsilon_{ijk} \]  

(1)

Here \( \alpha_i, \beta_{ij} \) and \( \epsilon_{ijk} \) are the random effects due to sire, dam and individual respectively, and \( \mu \) is a fixed intercept.

Each \( Y_{ijk} \) is a vector of \( p \) traits; there are \( I, J \) and \( K \) possible values of the indices \( i, j \) and \( k \). Each random effect vector is assumed to follow independent normal distributions

\[ \alpha_i \sim \mathcal{N}(0, \Sigma_A), \quad \beta_{ij} \sim \mathcal{N}(0, \Sigma_B), \quad \epsilon_{ijk} \sim \mathcal{N}(0, \Sigma_E). \]  

(2)

We are particularly interested in estimation of the genetic covariance matrix \( G = \Sigma_A \) and its eigenvalues, but will also look at the concomitant estimates of \( \Sigma_B \) and \( \Sigma_E \). The estimates we will compare are

- **MANOVA**, which are easy to compute but do not necessarily produce a positive-definite estimate for \( \Sigma_A \),
- **REML-lme**, fitted using a generic mixed-effects model solver (we use \texttt{R} function \texttt{lme()}), which should converge to the true REML estimates but are prohibitively slow to compute for high (or even moderate) numbers of traits,
- **pseudo-REML**, as described by Amemiya [1985]. These are easy to compute, and in the full-sub (one-way) design are exactly the REML estimates. In the half-sib case, they are only approximations.
- **stepwise REML**, as described by Calvin and Dykstra [1991]. This is an iterative algorithm that converges to the true REML estimates. Although we know of no results describing the rate of convergence, in practice it is very fast, allowing for easy REML estimation even for 100 \( \times \) 100 matrices.
- **pairwise REML**, in which the estimates of the individual entries \( \Sigma_{lm} \) are computed using a bivariate analysis with traits \( l \) and \( m \). This estimate is sometimes proposed as a computational fall-back when the dimensionality of \( \Sigma \) is too high for REML to converge; it need not be non-negative definite, as will be seen.

3 Initial examples

3.1 A small \( p \) example with zero eigenvalues

As a warm-up, we first illustrate the methods in a setting with a small number of traits, \( p = 4 \). Specifically, we take

\[ p = 4, \quad I = 100, \quad J = 3, \quad K = 5, \]  

(3)

and diagonal structures for the variance component matrices:

\[ \Sigma_A = \sigma_A^2 \text{diag}(1, 1, 0, 0), \quad \sigma_A^2 = 25 \]

\[ \Sigma_B = \sigma_B^2 \text{diag}(1, 0, 0, 1), \quad \sigma_B^2 = 9, \]  

(4)

\[ \Sigma_E = \sigma_E^2 \text{Id}_4, \quad \sigma_E^2 = 1. \]

\(^1\)indeed, this was the largest number of traits for which \texttt{lme()} runs relatively quickly.
This information is not used when fitting – all procedures are run with no assumptions on the covariances. The vector $\mu = (1, 2, 3, 4)$, but its value is immaterial to all methods used here.

All simulations were done in R; the software and technical descriptions of code used to produce the results of this paper are available in an R package hosted at [https://github.com/damian-t-p/REMLSimulationPaper](https://github.com/damian-t-p/REMLSimulationPaper).

Table 1 shows the results of one sample realization, about which the following remarks may be made.

- For MANOVA, as expected, the zero eigenvalues of $\Sigma_A$ lead to some negative estimated eigenvalues. The REML criterion is higher than that of the actual REML estimate found by stepwise REML. This is because MANOVA in this balanced setting in fact maximizes the likelihood over a larger set, i.e. without enforcing the non-negativity constraints.

- the Calvin-Dykstra (CD) algorithm for stepwise-REML exhibited linear convergence in the REML criterion (not shown here). The CD convergence criterion uses

$$d^2(\tilde{\Sigma}^l, \tilde{\Sigma}^{l-1}) := \sum_{k \in \{A,B,E\}} n_k \|\tilde{\Sigma}^l_k - \tilde{\Sigma}^{l-1}_k\|^2,$$

where $\tilde{\Sigma}^l = (\tilde{\Sigma}^l_A, \tilde{\Sigma}^l_B, \tilde{\Sigma}^l_E)$ is the vector of covariance estimates after the $l$th iteration. We stopped when $d(\tilde{\Sigma}^l, \tilde{\Sigma}^{l-1}) < 10^{-6}$.

- The pseudo REML estimates are close, but not identical, to the stepwise REML ones, and the REML criterion score is lower.

- REML-lme is slow and often fails to converge: we record the results at termination, even before convergence, and for this reason the REML criterion score is less than that of stepwise REML, which does converge to the maximum.

| Component | Method       | $\lambda_1$ | $\lambda_2$ | $\lambda_3$ | $\lambda_4$ | REML-Crit. |
|-----------|--------------|-------------|-------------|-------------|-------------|-------------|
| $\Sigma_A$ | MANOVA       | 26.24       | 24.62       | -0.01       | -0.13       | -7039       |
|           | REML-LME     | 26.27       | 24.46       | 0.34        | 4.3e-3      | -7083       |
|           | Stepwise REML | 26.24       | 24.61       | 0           | 0           | -7040       |
|           | Pseudo REML  | 26.24       | 24.62       | 0           | 0           | -7054       |
| $\Sigma_B$ | MANOVA       | 9.35        | 8.72        | 0.015       | -2.5e-3     |             |
|           | REML-LME     | 9.17        | 8.47        | 0.025       | 0.015       |             |
|           | Stepwise REML| 9.31        | 8.65        | 7.8e-3      | 0           |             |
|           | Pseudo REML  | 9.35        | 8.74        | 0.015       | 0           |             |
| $\Sigma_E$ | MANOVA       | 1.10        | 1.00        | 0.93        | 0.93        |             |
|           | REML-LME     | 1.09        | 1.01        | 0.96        | 0.92        |             |
|           | Stepwise REML| 1.10        | 1.00        | 0.93        | 0.93        |             |
|           | Pseudo REML  | 1.10        | 1.00        | 0.93        | 0.93        |             |

Table 1: Sample eigenvalues $\lambda_1 \geq \lambda_2 \geq \lambda_3 \geq \lambda_4$ for one sample realization drawn from (1)–(2) with parameters as in (3)–(4).
3.2 An example with $p = 50$

An important advantage of stepwise REML is that it is can easily be run for relatively large numbers of traits. In this section, we use $p = 50$, but $p$ can be in the thousands before the stepwise REML estimate takes more than a second to compute.

The parameters are as in (3)–(4), but with some modifications:

$$p = 50, \quad \Sigma_A = \sigma_A^2 \text{diag}(|Z_i|), \quad \Sigma_B = \sigma_B^2 \text{diag}(|Z_i'|),$$

(5)

where $Z_i, Z_i'$ are all independent standard normal deviates.

Figure 1 compares the eigenvalues of $\hat{\Sigma}_A$ (in blue) next to the true eigenvalues of $\Sigma_A$ (in red). Now that $p = 50$ is of the same order as $I = 100$, the sample eigenvalues are much more spread out than those of $\Sigma_A$ – we see that the REML estimate zeroes out many eigenvalues while overestimating a few others.

![Figure 1: Histograms of eigenvalues of $\Sigma_A$ and its REML estimate](image)

3.3 Pairwise REML with $p = 50$

We investigate the strategy of computing pairwise REML estimates for each pair of traits. We create a new estimate $\hat{\Sigma}_{A,P}$ such that the diagonal entries $[\hat{\Sigma}_{A,P}]_{ii}$ are computed by doing a single-trait analysis on trait $i$ and the off-diagonal entries $[\hat{\Sigma}_{A,P}]_{ij}$ are computed by doing a 2-trait analysis on the traits $(i,j)$.

The setting is the same as the previous subsection, especially (5). In fact, we used the stepwise-REML estimates for the 2-trait analyses, since there are 1250 entries of $\hat{\Sigma}_{A,P}$ to fill in, and even in the 2-dimensional case, fitting a generic mixed model is too slow for this. However, we can still compare the resulting $\hat{\Sigma}_{A,P}$ with the REML estimate $\hat{\Sigma}_{A,R}$ to see how the eigenvalues produced by the pairwise procedure differ from those done by computing the whole stepwise REML estimate at once.
Let $\lambda_1, R > \ldots > \lambda_p, R$ be the eigenvalues of $\hat{\Sigma}_{A,R}$ and $\lambda_1, P > \ldots > \lambda_p, P$ be the eigenvalues of $\hat{\Sigma}_{A,P}$. Figure 2 plots the differences $\lambda_{i,R} - \lambda_{i,P}$ against $\lambda_{i,R}$. We may observe that

- the pairwise REML estimate $\hat{\Sigma}_{A,P}$ is not positive definite: some 15 of its estimated eigenvalues $\lambda_{i,P}$ lie between 0 and $-0.75$,
- the pairwise REML eigenvalues are all smaller than the actual REML ones: $\lambda_{i,P} \leq \lambda_{i,R}$ for all $i$,
- when the pairwise REML eigenvalues are positive, the differences are not large: an empirical summary (in this case) might be that typically,

\[ 0 \leq \lambda_{i,R} - \lambda_{i,P} \leq 0.05 \lambda_{i,R} \]

### 4 Repeated sampling

The Calvin-Dykstra algorithm is fast enough that it is possible to study the properties of REML estimates of eigenvalues through simulation.

#### 4.1 Bias in top eigenvalues $\lambda_{1,R}, \ldots, \lambda_{5,R}$

This example confirms the upward bias in the largest eigenvalues of the REML estimates and confirms that the bias increases with the number of traits $p$.

Again we use the half-sib design (1)–(2) with $I = 100, J = 3, K = 5$ and

\[ \Sigma_A = I, \quad \Sigma_B = 4I, \quad \Sigma_E = I. \]  

(6)
The number of traits $p$ grows from 10 to 100 in increments of 10. Over $R = 50$ replications, the averages of the top 5 eigenvalues of $\hat{\Sigma}_{A,R}$, $\hat{\Sigma}_{B,R}$ and $\hat{\Sigma}_{E,R}$ are plotted against $p$. Also shown (thick red lines) are the corresponding population eigenvalues (1, 4 and 1 respectively).

Figure 3: The largest 5 eigenvalues of the REML estimates of $\Sigma_A$, $\Sigma_B$ and $\Sigma_E$ plotted against $p$. Each point is the mean of 50 replicates. The largest population eigenvalue of each matrix is depicted with a horizontal red line.

Some remarks on the results in Figure 3:

- the means are biased upwards, as expected, but the biases are much more substantial for $\Sigma_A$ (especially) and $\Sigma_B$ than for $\Sigma_E$. Also notable is the rapid increase in the biases with $p$ in each case,

- The standard deviations of the 50 replicates were computed for each eigenvalue (results not shown here). These were markedly larger for $\hat{\Sigma}_{A,R}$ and $\hat{\Sigma}_{B,R}$ (between 0.2 and 0.35) than for $\hat{\Sigma}_{E,R}$ (between 0.02 and 0.03). Although obscured by sample fluctuation, it appears — as might be expected — that the SDs are ordered, decreasing as we move down from the top eigenvalue to the 5th. There is no clear dependence on $p$ in the SDs.

- In this setting the REML estimates coincide with the MANOVA estimators. This is because all of the population eigenvalues of $\Sigma_A$, $\Sigma_B$ and $\Sigma_E$ in [6] are well separated from 0, and the MANOVA estimates were in all cases positive definite.

4.2 Differences between the largest REML and MANOVA eigenvalues

In contrast to the previous example, when nearly null genetic subspaces are present we expect the REML estimates to differ significantly from MANOVA as the smallest MANOVA
eigenvalues will be negative. Here we examine the difference between the largest eigenvalues \( \lambda_i(\hat{\Sigma}_{k,R}) \) and \( \lambda_i(\hat{\Sigma}_{k,M}) \) for REML and MANOVA for \( k \in \{A, B, E\} \).

We set half of the diagonal entries of \( \Sigma_A \) and \( \Sigma_B \) to zero at random, leaving \( \Sigma_E \) and all other parameters unchanged from the previous subsection. Figure 4 shows the mean differences (averaged over 50 replications) of \( \lambda_i(\hat{\Sigma}_{k,R}) - \lambda_i(\hat{\Sigma}_{k,M}) \) as a function of \( p = 10, 20, 30, \ldots, 100 \). We observe that

- the mean differences again increase in absolute value, nearly linearly, as \( p \) increases,
- for \( \Sigma_A \) the top REML eigenvalues are larger than MANOVA, while for \( \Sigma_B \) and \( \Sigma_E \) they are smaller,
- the magnitude of the (negative) difference is much larger for \( \Sigma_B \),
- the SDs of the differences over the 50 replications (not shown) are approximately 10% of the mean differences, and in particular are generally increasing with \( p \).

These variations in sign and magnitude of the differences between the top REML and MANOVA eigenvalues call for further study. At the least they indicate a dependence on the level of nesting of the respective variance components.

![Figure 4: Differences between the REML and MANOVA estimates of the largest 5 eigenvalues of \( \Sigma_A, \Sigma_B \) and \( \Sigma_E \) plotted against \( p \). Each point is the mean of 50 replicates.](image)

5 Nearly Null Subspaces

5.1 Counting zero eigenvalues in REML estimator \( \hat{\Sigma}_{A,R} \)

In this section we simulate several half-sib designs in which \( \Sigma_A \) has null spaces of various dimensions \( d \). We might expect the number of zero (or small) eigenvalues of the corre-
sponding REML estimates \( \hat{\Sigma}_{A,R} \) to track the null space dimension \( d \). We will see that this is broadly true, but that again significant biases are present.

**Specification of \( \Sigma_A, \Sigma_B, \Sigma_E \) – generalities.** In general, the principal axes of variation (i.e. eigenvectors) of the covariance matrices \( \Sigma_A, \Sigma_B, \Sigma_E \) need not be aligned, and so to use only diagonal matrices in our investigations would entail a loss of generality.

We will therefore allow for examples of non-alignment of the axes of \( \Sigma_A \) and \( \Sigma_B \), but for simplicity we keep the error covariance scalar: \( \Sigma_E = I \). Because the model (1)–(2) is Gaussian, the REML and MANOVA estimates are unchanged by rotation (an orthogonal transformation) of the data. This means that we can take \( \Sigma_A \) to be diagonal (and \( \Sigma_E = I \) is unchanged), but that \( \Sigma_B \) need not be.

Specifically suppose that \( \Sigma_A \) has eigendecomposition \( \Sigma_A = P \Lambda P^T \) for suitable orthogonal and diagonal matrices \( P \) and \( \Lambda \) respectively. Then the REML estimates are the same for the model with parameters \( (\Sigma_A, \Sigma_B, \Sigma_E = I) \) as for the model with parameters \( (\Lambda, P^T BP, I) \).

In summary, the following are important to note:

- We can only assume a diagonal structure for one of \( \Sigma_A \) or \( \Sigma_B \), but not both,
- If we are interested in estimating something about \( \Sigma_A \) other than the eigenvalues, we cannot assume a diagonal structure for \( \Sigma_A \),
- If we do not assume that \( \Sigma_E \) is a scalar matrix, then it cannot be assumed to be diagonal either.

**Settings for \( \Sigma_A \):** Thus, we will consider the following specifications for \( \Sigma_A \):

\[
\Sigma_A = \begin{cases} 
    c_A \text{ diag}(1_{p-d}, 0_d) & \text{Identity} \\
    c_A \text{ diag}((X_i)_1^{p-d}, 0_d) & X_i \text{ iid } \chi_5^2 & \text{Chi-squared} \\
    c_A \text{ diag}(|X_i + 5|)_1^{p-d}, 0_d) & X_i \text{ iid } \text{Cauchy}(0) & \text{Heavy-tail}
\end{cases}
\]

(7)

with the choices

\[ p = 50, \quad d = 0, 5, 10, \ldots, 50, \quad c_A = 0.5, 1, 2. \]

A typical draw from each of these cases (with \( d = 10 \) zeros) might be summarized qualitatively as follows:

- Identity: 40 non-zero eigenvalues all equal to 1,
- Chi-squared: 40 nonzero eigenvalues spread unevenly over the range \((0, 15)\),
- Heavy-tailed: 39 nonzero eigenvalues spread unevenly over \((0, 30)\) with an outlier (e.g. at 750).

**Settings for \( \Sigma_B \):** We consider one scalar and three non-diagonal cases:

\[
\Sigma_B = \begin{cases} 
    I & \text{‘Identity’} \\
    W_q(q, I)/q & \text{‘Wishart’} \\
    P \text{ diag}((X_i)_1^q)P' X_i \text{ iid } \chi_5^2, & P \text{ random orthogonal} \text{‘High-rank’} \\
    0.811' + 0.2I & \text{‘High-corr’}
\end{cases}
\]

(8)
In a little more detail:

- **Identity**: sets all 50 eigenvalues equal to 1,

- **Wishart**: Let $X$ be a $p \times p$ matrix of iid $N(0,1)$ random variables. Then we form $B = XX^T/p$, which is the sample covariance of $X$. Since the population covariance of the columns of $X$ is $I$, $B$ is not too far from $I$, while still having uniformly distributed eigenvectors. The eigenvalues of $B$ are unevenly spread between 0 and 4.

- **High-rank**: Let $P$ be a matrix of orthonormal vectors drawn uniformly. Let $D$ be diagonal matrix with iid $\chi^2$ entries on the diagonal. We then take $B = PDP^T$. This matrix has eigenvectors independent from those of $A$, but the eigenvalues are more spread out than those in the Wishart case.

- **High-corr**: $B$ is a square matrix with 1s on the diagonal and 0.8s in every other entry. This can be represented as $B = 0.811^T + 0.2I$, where $1$ is a vector of ones. This means that the eigenvalues of $B$ are 0.8$p = 40$ with multiplicity 1 and 0.2 with multiplicity $p - 1 = 49$.

**Remaining parameter settings**: In all cases, the error covariance is taken to be the identity $\Sigma_E = I$. We use the following set parameter values:

- **Number of traits**, $p = 50$,

- **Number of sires, dams per sire, and individuals per dam**: $I = 100, J = 3, K = 5$,

- **Number of repetitions**, $n = 10$.

Moreover, the following variables:

- **Dimensions of null space** $d = 0, 5, 10, \ldots, 50$,

- **Additional sire covariance scaling** $c_A = 0.5, 1, 2$.

**Results**: For each combination of $\Sigma_A$ and $\Sigma_B$ structures and the null space dimension and scaling variables $d$ and $p$ described above, we compute the eigenvalues of the REML estimate $\hat{\Sigma}_{A,R}$ of $\Sigma_A$.

Let $\hat{d}$ be the number of eigenvalues of $\hat{\Sigma}_{A,R}$ that are exactly zero. Figure 5 plots $\hat{d}$ (vertical axis) against $d$, the ‘true’ null space dimension. The solid line and ribbon show the mean and interquartile range computed from the $n = 10$ replicates. As a reference, the line $\hat{d} = d$ is shown on each plot with black dashes.

In all cases, the estimated nearly-null dimension tends to be moderated: when $d$ is large, $\hat{d}$ is an underestimate, and vice-versa when $d$ is small. Moreover, it seems that the structure of $B$ that is the main factor governing the shape of the $\hat{d}$ curve.

We can perform similar analyses with different estimators of $d$. Consider, for example

$$\hat{d}(\delta) = \#\{i : \lambda_i \leq \delta\}.$$ 

Figure 6 has the same features as fig. 5 but instead displays the number of “small” eigenvalues of $A$, defined here as the number of eigenvalues with a value below 1, i.e. $\hat{d}(1)$.

\footnote{and are approximately a sample from a Marcenko-Pastur quarter circle law with parameter $\gamma = 1$}
Figure 5: Number of zero eigenvalues of the REML estimate of $\Sigma_A$ plotted against the nearly-null dimension of $\Sigma_A$. The mean and inter-quartile range of 10 replicates are shown.
This always exceeds the number of eigenvalues of $A$ that are exactly zero, and so, as we
might expect, it improves the under-estimates at the expense of the over-estimates.

This estimator does particularly well when $B$ has well-separated eigenvalues - that is in
the high-correlation and identity cases and when the eigenvalues of $A$ are larger (chi-squared
and heavy-tailed cases).

5.2 Bias in $\lambda_{1,R}$

Again we allow the dimension $d$ of the null space of $\Sigma_A$ to vary, but now turn attention to
biases in the REML estimate of the largest eigenvalue.

The previous simulation setting is used, except that the following structures are used
for $\Sigma_A$:

$$
\Sigma_A = \begin{cases} 
  c_A \text{diag}(1_{p-d}, 0_d) & \text{Identity} \\
  c_A \text{diag}((X_i)_1^{p-d}, 0_d) & X_i \overset{iid}{\sim} \chi^2_{5/5} \text{ Chi-squared}(*) \\
  c_A \text{diag}(5, 1_{p-d-1}, 0_d) & \text{Spiked.}
\end{cases}
$$

(*): the variates $X_i$ are held the same across replications to reduce variation.

We focus on the top eigenvalue of the MANOVA and REML estimates of the sire covari-
ance $\Sigma_A$, namely $\lambda_{1,M}$ and $\lambda_{1,R}$ respectively across the settings, with $R = 10$ replications.
Figure 7 shows the behavior of the relative differences $(\lambda_{1,M} - \lambda_{1,R})/\lambda_{1,R}$.

- Empirically, it always happens that $\lambda_{1,R} \geq \lambda_{1,M}$,
- The size of the relative differences $(\lambda_{1,M} - \lambda_{1,R})/\lambda_{1,R}$ depends strongly on $\Sigma_B$, being
  much larger for $\Sigma_B = \text{‘highcorr’}$ (which has a single large spike eigenvalue) than for
  the other cases, where the difference is smaller, but not insignificant,
- the relative differences also tend to increase in magnitude with the null space dimen-
  sion $d$.
- the relative differences also tend to vary inversely with the sire variance scaling $c_A$.

We can also investigate the bias in $\lambda_{1,R}$ by plotting the relative difference $(\lambda_{1,R} - \lambda_1)/\lambda_1$
where $\lambda_1$ is the largest eigenvalue of the true underlying covariance matrix $\Sigma_A$, Figure 8.

We may make the following remarks about the upward bias of the REML estimate
relative to the truth: $(\lambda_{1,R} - \lambda_1)/\lambda_1$

- it depends on the structure, rank and magnitude of both $\Sigma_A$ and $\Sigma_B$
- it is particularly large when the nonzero eigenvalues of $\Sigma_A$ are all the same (‘identity’),
  though in this case the eigenvalues of $\Sigma_A$ are smaller than for ‘chisquared’ and ‘spiked’,
- it is smallest in the high correlation case $\Sigma_B$. 

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Figure 6: Number of eigenvalues of the REML estimate of $\Sigma_A$ smaller than 1 plotted against the nearly-null dimension of $\Sigma_A$. The mean and inter-quartile range of 10 replicates are shown.
Figure 7: Relative difference between the largest eigenvalue of the MANOVA and REML estimate of $\Sigma_A$ for various choices of $\Sigma_A$ and $\Sigma_B$. $\Sigma_E = I$ throughout. The mean and inter-quartile range of 10 replicates are shown.
Figure 8: Relative bias in the largest eigenvalue of the REML estimate of $\Sigma_A$ for various choices of $\Sigma_A$ and $\Sigma_B$. $\Sigma_E = I$ throughout. The mean and inter-quartile range of 10 replicates are shown.
6 Discussion

We have shown that the Calvin-Dykstra convex duality algorithm renders REML feasible for high-$p$ phenomics in the very specific setting of balanced half-sib designs.

While the balanced observation assumption in this paper is unrealistic in practice, it does allow initial demonstration of statistical estimation phenomena for large $p$ that will likely carry over to unbalanced settings and more complex designs. We have seen examples of two such phenomena in the simulations conducted here.

First there are significant biases in estimating the dimension $d$ of a null space in the genetic covariance by simply counting the number of zero REML eigenvalues: it is typically biased high when $d/p$ is small and biased low when $d/p$ is large. Second, there are significant biases of overestimation in the REML estimates of the largest eigenvalues of genetic covariance matrices.

Both these high dimensional biases are not unexpected if one recalls similar phenomena (eigenvalue spreading and biases) that have been observed and substantiated by asymptotic approximation in two simpler settings: estimation of a single $p$-dimensional covariance matrix based on i.i.d. vector observations (reviewed in Johnstone and Paul [2018]), and MANOVA estimates in the present high dimensional variance component models Fan and Johnstone [2019, 2022, Fan et al., 2018]. It is a natural topic for future research to look for greater understanding of REML in high-$p$ settings, for example via asymptotic approximations.

In summary, the simulations in this work, even with their limitation to specific settings in special balanced designs, already indicate that considerable caution will be needed in interpreting REML estimates in phenome-wide studies of genetic variation.

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Calvin [1993] proposed an extension of the Calvin-Dykstra algorithm to unbalanced half-sib designs, using the EM algorithm to impute missing observations. In current work we are implementing this algorithm in order to apply it to some publicly available datasets that are beyond current capabilities of more generic REML solvers.
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