

The effect of population kinship estimation bias in heritability estimation and genetic association

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Abstract

Population kinship matrices are estimated for a variety of applications, including estimation of heritability and to control for population structure in genetic association studies. Recent work found that the most common family of kinship estimators can be severely biased. In this work, we investigate the effect of this kinship bias on the two downstream applications of heritability estimation and genetic association. We present a novel trait simulation strategy that accurately parametrizes heritability, even when utilizing real genotypes. Using these simulations, we find that heritability estimation becomes biased when using such biased kinship matrices. Remarkably however, this kinship bias does not affect genetic associations based on either Principal Components Analysis (PCA) or Linear Mixed-effects Models (LMM). Lastly, we explain our empirical observations using theory. In particular, the exact form of the bias of the standard kinship estimator is such that it is compensated for by fitting the intercept in both PCA and LMM approaches, which model population structure via covariates, suggesting that only downstream applications with this precise arrangement will be robust to this kinship bias.

1 Introduction

Kinship is utilized in principal components analyses and linear-mixed effects models to correct for structure in Genome-Wide Association Studies (GWAS) (Xie et al., 1998; Yu et al., 2006; Aulchenko et al., 2007; Price et al., 2006; Astle and Balding, 2009; Kang et al., 2008; Kang et al., 2010; Zhou and Stephens, 2012; Loh et al., 2015; Sul et al., 2018) and to estimate genome-wide heritability (Yang et al., 2010; Yang et al., 2011; Speed et al., 2012). The most commonly-used kinship estimator (Price et al., 2006; Astle and Balding, 2009; Rakovski and Stram, 2009; Thornton and McPeck, 2010; Yang et al., 2010; Yang et al., 2011; Zhou and Stephens, 2012; Speed et al., 2012; Speed and Balding, 2015; Loh et al., 2015; Wang et al., 2017; Sul et al., 2018) was recently determined to have a complex bias (Ochoa and Storey, 2016b; Weir and Goudet, 2017).

Heritability has long been estimated from the trait correlation between close relatives, particularly identical and fraternal twins, or siblings (Falconer and Mackay, 1996). These initial estimation approaches were based on a regression model (Falconer and Mackay, 1996). A different model was needed to achieve accurate estimates when estimating on more distant relatives, such as the variance component model of the SOLAR approach (Almasy and Blangero, 1998), which is based on estimating kinship from pedigrees. This idea was extended from pedigrees to populations with the GCTA approach (Yang et al., 2010; Yang et al., 2011), which has spawned much research (Visscher et al., 2010; Speed et al., 2012; Krishna Kumar et al., 2016; Yang et al., 2016; Jiang et al., 2016). However, we previously found that the most common family of kinship estimators employed by these approaches for populations can be severely biased (Ochoa and Storey, 2016b). Speed et al. (2012) focused on some biases related to other assumptions of the multivariate normal heritability model, but biases in kinship estimation were not known or included in these evaluations.

There are alternative approaches for estimating heritability, such as LD Score Regression (Bulik-Sullivan et al., 2015) and SumHer (Speed and Balding, 2019), which we do not consider in this proposal since they are not based on kinship matrices.

2 Methods

2.1 Genetic model

Suppose there are m biallelic loci and n diploid individuals. The genotype $x_{ij} \in \{0, 1, 2\}$ at a locus i of individual j is encoded as the number of reference alleles, for a preselected but otherwise arbitrary reference allele per locus. These genotypes can be treated as random variables structured according to relatedness. If φ_{jk} is the kinship coefficient of two individuals j and k , and p_i is the ancestral allele frequency at locus i , then under the kinship model (Ochoa and Storey, 2016a; Ochoa and Storey, 2016b) the expectation and covariance are given by

$$\mathbb{E}[\mathbf{X}] = 2\mathbf{p}\mathbf{1}_n^T, \quad \text{Cov}(\mathbf{x}_i) = 4p_i(1 - p_i)\mathbf{\Phi},$$

where \mathbf{x}_i is the length- n column vector of genotypes at locus i , $\mathbf{X} = (\mathbf{x}_i^\top)$ is the complete $m \times n$ genotype matrix, $\Phi = (\varphi_{jk})$ is the $n \times n$ kinship matrix, $\mathbf{p} = (p_i)$ is a length- m column vector of ancestral allele frequencies, $\mathbf{1}_n = (1)$ is a length- n column vector where every element is 1, and the \top superscript denotes matrix transposition. Both kinship (Φ) and ancestral allele frequencies (\mathbf{p}) are parameters that depend on the choice of ancestral population, for which the Most Recent Common Ancestor (MRCA) population is the most sensible choice (Ochoa and Storey, 2016a; Ochoa and Storey, 2016b). In this work, to simplify notation, we omit cumbersome notation that marks this dependence of parameters on the choice of ancestral population, not do we explicitly condition on the ancestral population when calculating expectations and covariances as done in previous work, although it is done implicitly. This and later notation is summarized in Table 1.

The length- n quantitative trait vector \mathbf{y} for all individuals is assumed to follow a linear polygenic model,

$$\mathbf{y} = \mathbf{1}_n \alpha + \mathbf{X}^\top \beta + \epsilon, \quad (1)$$

where α is the intercept coefficient, $\beta = (\beta_i)$ is a length- m column vector of effect size coefficients for each locus i (which may be zero), and ϵ is a length- n column vector of non-genetic effects. To analyze the covariance structure of the trait, we shall treat α and β are fixed parameters, while \mathbf{X} and ϵ are random. The non-genetic effects are assumed to be independent with variance $(1 - h^2)\sigma^2$

Table 1: **Mathematical notation.**

Variable	Dimensions	Description
m	1	Number of loci
n	1	Number of individuals
i	1	Locus (variant) index
j, k	1	Individual indexes
μ	1	Trait mean
σ^2	1	Trait variance scale
h^2	1	(Narrow-sense) Heritability
$\mathbf{X} = (x_{ij})$	$m \times n$	Genotype matrix
$\mathbf{x}_i = (x_{ij})$	$n \times 1$	Genotype vector at locus i
\mathbf{y}	$n \times 1$	Trait vector
α	1	Intercept
$\beta = (\beta_i)$	$m \times 1$	Effect size coefficients
ϵ	$n \times 1$	Non-genetic random effect
$\mathbf{p} = (p_i)$	$m \times 1$	Ancestral allele frequencies
$\Phi = (\varphi_{jk})$	$n \times n$	Kinship matrix
$\mathbf{1}_n$	$n \times 1$	Vector of ones
\mathbf{I}_n	$n \times n$	Identity matrix

given by the total trait variance scale σ^2 and the narrow-sense heritability h^2 :

$$\mathbf{E}[\epsilon] = \mathbf{0}_n, \quad \text{Cov}(\epsilon) = (1 - h^2)\sigma^2\mathbf{I}_n,$$

where $\mathbf{0}_n$ is a length- n column vector of zeroes. The expectation of the trait is therefore

$$\mathbf{E}[\mathbf{y}] = \alpha\mathbf{1}_n + \mathbf{E}[\mathbf{X}^\top]\beta + \mathbf{E}[\epsilon] = \mu\mathbf{1}_n, \quad \text{where} \quad \mu = \alpha + 2\mathbf{p}^\top\beta.$$

The covariance matrix of the trait is

$$\text{Cov}(\mathbf{y}) = \left(\sum_{i=1}^m \text{Cov}(\mathbf{x}_i)\beta_i^2 \right) + \text{Cov}(\epsilon) = \mathbf{\Phi} \left(\sum_{i=1}^m 4p_i(1 - p_i)\beta_i^2 \right) + (1 - h^2)\sigma^2\mathbf{I}_n.$$

Therefore, we can write the covariance in terms of the heritability and the overall variance scale, in a formulation that matches previous work (Yang et al., 2010; Yang et al., 2011; Speed et al., 2012):

$$\text{Cov}(\mathbf{y}) = \sigma^2 (h^2 2\mathbf{\Phi} + (1 - h^2)\mathbf{I}_n), \quad \text{where} \quad \sigma^2 h^2 = \sum_{i=1}^m 2p_i(1 - p_i)\beta_i^2.$$

This last equation suggests a clear definition for the single-locus i contribution to the heritability, given by

$$h_i^2 = 2p_i(1 - p_i)\beta_i^2\sigma^{-2}, \quad (2)$$

which is such that the total heritability is a sum of these single-locus heritabilities: $h^2 = \sum_{i=1}^m h_i^2$. Since the earlier expectations and covariances are conditioned on the choice of ancestral population, and given in terms of parameters that depend on it (p_i and $\mathbf{\Phi}$), then the parameters μ, σ^2, h^2 are all also dependent on the choice of ancestral population.

The parametrization of our model is equivalent to setting separate absolute scales to the genetic and environment variance components, as $\sigma_G^2 = h^2\sigma^2$ and $\sigma_E^2 = (1 - h^2)\sigma^2$, respectively, which results in $\sigma_G^2 + \sigma_E^2 = \sigma^2$ and $\sigma_G^2/(\sigma_G^2 + \sigma_E^2) = h^2$, as desired.

The factor of two in front of $\mathbf{\Phi}$ is traditionally there so that for an unstructured population $2\mathbf{\Phi} = \mathbf{I}_n$, in which case the trait covariance simplifies to $\text{Cov}(\mathbf{y}) = \sigma^2\mathbf{I}_n$ for any value of h^2 . More broadly, the variance of the trait for any outbred individual is σ^2 under this parametrization. In many previous presentations this factor of 2 does not appear there explicitly, but instead the kinship matrix is defined as $2\mathbf{\Phi}$ (Yang et al., 2011; Speed et al., 2012).

2.2 Multivariate Normal estimation model

Here we focus on heritability estimation from the Multivariate Normal (MVN) variance component model, namely (Speed et al., 2012; Yang et al., 2011)

$$\mathbf{y} \sim \text{MVN}(\mu\mathbf{1}, \sigma^2 (2h^2\mathbf{\Phi} + (1 - h^2)\mathbf{I}_n)). \quad (3)$$

Note that, by construction, the above trait vector has the same mean and covariance matrix of the genetic trait model in Eq. (1). However, the trait in Eq. (3) is not drawn from genotypes anymore,

but solely from the kinship matrix; for this reason we refer to this as a *MVN* trait, in contrast to the *genetic* trait model in Eq. (1).

This MVN trait model (from Eq. (3)) is justified as arising from the genetic trait model (from Eq. (1)) in the limiting case of infinite causal locus effects, each with equal per-locus variance (heritability) according to Eq. (2) (equal $p_i(1 - p_i)\beta_i^2$ for all i), the condition under which the genetic trait approaches the MVN distribution as a consequence of the multivariate central limit theorem. However, convergence may be slower in practice since both effect sizes (β_i) and ancestral allele frequencies (p_i) vary for realistic causal loci, so the product $p_i(1 - p_i)\beta_i^2$ is not expected to be the same at all causal loci i . Another way of stating the difference is that Eq. (1) and Eq. (3) agree in the first two moments, but disagree in higher moments.

2.3 Kinship estimation

2.3.1 Standard kinship estimator

The “standard” kinship estimator is the most common estimator employed across various applications for population structure (Astle and Balding, 2009; Speed and Balding, 2015; Wang et al., 2017), including heritability estimation (Speed et al., 2012; Speed and Balding, 2015; Speed et al., 2017) and genetic association tests based on PCA (Price et al., 2006), LMMs (Astle and Balding, 2009; Zhou and Stephens, 2012; Loh et al., 2015; Sul et al., 2018) and other models (Rakovski and Stram, 2009; Thornton and McPeck, 2010). The popular heritability estimation approach GCTA (Yang et al., 2010; Yang et al., 2011) employs a variant of this estimator detailed in the next paragraph. The standard estimator, and its almost sure limit as the number of loci m and number of individuals n go to infinity (Ochoa and Storey, 2016b), are given by

$$\hat{\varphi}_{jk}^{\text{std}} = \frac{1}{m} \sum_{i=1}^m \frac{(x_{ij} - 2\hat{p}_i)(x_{ik} - 2\hat{p}_i)}{4\hat{p}_i(1 - \hat{p}_i)} \xrightarrow[n, m \rightarrow \infty]{\text{a.s.}} \frac{\varphi_{jk} - \bar{\varphi}_j - \bar{\varphi}_k + \bar{\varphi}}{1 - \bar{\varphi}}, \quad (4)$$

where $\hat{\varphi}_{jk}^{\text{std}}$ is the estimated kinship of individuals j and k , $\hat{p}_i = \frac{1}{2n} \sum_{j=1}^n x_{ij}$ is the standard ancestral allele frequency estimator, $\bar{\varphi}_j = \frac{1}{n} \sum_{k=1}^n \varphi_{jk}$ is the mean kinship of individual j with all others, and $\bar{\varphi} = \frac{1}{n^2} \sum_{j=1}^n \sum_{k=1}^n \varphi_{jk}$ is the overall mean kinship. This is a complex bias that varies for every pair of individuals, and which is on average a downward bias. (Note that the mean $\hat{\varphi}_{jk}^{\text{std}}$ is exactly zero, regardless of the true value of the mean kinship.)

Variants of this approach that weigh loci according to linkage disequilibrium (Speed et al., 2017; Wang et al., 2017) do not alter the bias calculated above, since the same bias is present in each individual locus (Ochoa and Storey, 2016b). Our previous work also considered a more general form where the ancestral allele frequency estimator $\hat{p}_i = \frac{1}{2} \sum_{j=1}^n w_j x_{ij}$ is calculated with weights w_j per individual j (such that $\sum_{j=1}^n w_j = 1$), and found that these weights alter the values of the bias terms $\bar{\varphi}_j$ and $\bar{\varphi}$ to be weighted averages, but no choice of weights eliminates these biases (Ochoa and Storey, 2016b). Such weighted \hat{p}_i estimates encompass the best unbiased linear

estimator (Aistle and Balding, 2009; Thornton and McPeck, 2010), with weights corresponding to $\mathbf{w} = (\mathbf{1}^\top \mathbf{\Phi}^{-1} \mathbf{1})^{-1} \mathbf{1}^\top \mathbf{\Phi}^{-1}$.

2.3.2 GCTA kinship estimator

The GCTA software (Yang et al., 2011) estimate what they refer to as a Genetic Relatedness Matrix (GRM), which is evidently twice a kinship matrix estimate due to the similarity to Eq. (4) and due to the historical connection between kinship and heritability in Eq. (3) and in previous work (Falconer and Mackay, 1996). In fact, the GCTA kinship estimates for two different individuals is identical to Eq. (4) (after taking into account the factor of 2 in Eq. (3)):

$$\hat{\varphi}_{jk}^{\text{GCTA}} = \hat{\varphi}_{jk}^{\text{std}} \quad \text{for} \quad j \neq k.$$

The GCTA kinship estimator differs from the standard estimator only for $j = k$ (Yang et al., 2011), where the estimator and its limit are instead given by (Ochoa and Storey, 2016b):

$$\hat{\varphi}_{jj}^{\text{GCTA}} = \frac{1}{2} + \frac{1}{m} \sum_{i=1}^m \frac{x_{ij}^2 - (1 + 2\hat{p}_i) x_{ij} + 2\hat{p}_i^2}{4\hat{p}_i(1 - \hat{p}_i)} \xrightarrow[n, m \rightarrow \infty]{\text{a.s.}} \frac{\varphi_{jj} - \bar{\varphi}_j}{1 - \bar{\varphi}}. \quad (5)$$

2.3.3 Popkin kinship estimator

The popkin (population kinship) estimator is given by (Ochoa and Storey, 2016b)

$$\begin{aligned} A_{jk} &= \frac{1}{m} \sum_{i=1}^m (x_{ij} - 1)(x_{ik} - 1) - 1, \\ \hat{A}_{\min} &= \min_{u \neq v} \frac{1}{|S_u||S_v|} \sum_{j \in S_u} \sum_{k \in S_v} A_{jk}, \\ \hat{\varphi}_{jk}^{\text{new}} &= 1 - \frac{A_{jk}}{\hat{A}_{\min}}, \end{aligned} \quad (6)$$

where S_u are subpopulations that partition individuals. This estimator is accurate ($\hat{\varphi}_{jk}^{\text{new}} \xrightarrow[m \rightarrow \infty]{\text{a.s.}} \varphi_{jk}$) under the assumption that \hat{A}_{\min} is calculated over individual pairs whose true kinship is zero. In other words, the two subpopulations S_u and S_v with the minimum mean A_{jk} value should have its true mean kinship value φ_{jk} be zero.

2.4 Heritability and genetic association software

TODO: state versions, download links, etc.

Outline:

- SOLAR-Eclipse (herit only) (Almasy and Blangero, 1998).
- GCTA (both heritability and genetic association) (Yang et al., 2011).
- PCA: used plink2 (genetic association only) (Chang et al., 2015).

2.5 Simulations

2.5.1 Trait simulation algorithm

Suppose the genotype matrix \mathbf{X} is available, and we have fixed values for the number of causal loci m_1 , the trait mean, variance scale, and heritability (μ, σ^2, h^2) . The goal is to choose the intercept α and draw random effect sizes β that result in the desired trait parameters. First we randomly select m_1 loci to be causal, and subset the genotype matrix \mathbf{X} and ancestral allele frequency vector \mathbf{p} so that from this point on they contain only those causal loci (they now have dimensions $m_1 \times n$ and length m_1 , respectively).

Below we divide the algorithm into two steps: (1) scaling the effect sizes, and (2) centering the trait. Each step forks into two cases: whether the true ancestral allele frequencies \mathbf{p} are known or not (the latter requires a known kinship matrix Φ).

Scaling effect sizes. The initial effect sizes β_i are drawn independently from a standard normal distribution:

$$\beta_i \sim N(0, 1).$$

First we consider the simpler case of known ancestral allele frequencies $\mathbf{p} = (p_i)$. The initial genetic variance scale is

$$\sigma_0^2 = \sum_{i=1}^{m_1} 2p_i(1 - p_i)\beta_i^2.$$

We obtain the desired variance by dividing each β_i by σ_0 (which results in a variance of 1) and then multiply by $h\sigma$ (which results in the desired variance of $h^2\sigma^2$). Combining both steps, the update is

$$\beta \leftarrow \beta \frac{h\sigma}{\sigma_0}.$$

Now we consider the case of unknown ancestral allele frequencies but known kinship matrix. First, sample estimates $\hat{\mathbf{p}} = (\hat{p}_i)$ of the ancestral allele frequencies are constructed from the genotype data as

$$\hat{p}_i = \frac{1}{2n} \mathbf{1}_n^\top \mathbf{x}_i.$$

Although this estimator is unbiased ($E[\hat{\mathbf{p}}] = \mathbf{p}$), the resulting variance estimates of interest $\hat{p}_i(1 - \hat{p}_i)$ are downwardly biased (Ochoa and Storey, 2016b):

$$E[\hat{p}_i(1 - \hat{p}_i)] = p_i(1 - p_i)(1 - \bar{\varphi}),$$

where $\bar{\varphi} = \frac{1}{n^2} \mathbf{1}_n^\top \Phi \mathbf{1}_n$ is the mean kinship coefficient in the data. Therefore the initial genetic variance scale, estimated as

$$\hat{\sigma}_0^2 = \sum_{i=1}^{m_1} 2\hat{p}_i(1 - \hat{p}_i)\beta_i^2,$$

has an expectation of

$$E[\hat{\sigma}_0^2] = \sigma_0^2(1 - \bar{\varphi}).$$

Therefore, assuming that this additional factor $(1 - \bar{\varphi})$ is known, the update

$$\beta \leftarrow \beta \frac{h\sigma\sqrt{1 - \bar{\varphi}}}{\hat{\sigma}_0}$$

results in the desired variance.

Centering the trait. Here we consider the problem of selecting the intercept coefficient α that, together with the previous effect size coefficient vector β , result in the desired trait mean μ .

When ancestral allele frequencies are known, the trait can be centered precisely. Given our model, we obtain the desired overall trait mean μ by choosing the intercept coefficient to be

$$\alpha = \mu - 2\mathbf{p}^\top \beta.$$

When ancestral allele frequencies are unknown, the solution is to choose the intercept coefficient

$$\alpha = \mu - 2\hat{p}\mathbf{1}_{m_1}^\top \beta, \quad \hat{p} = \frac{1}{m_1} \mathbf{1}_{m_1}^\top \hat{\mathbf{p}} = \frac{1}{2m_1n} \mathbf{1}_{m_1}^\top \mathbf{X}^\top \mathbf{1}_n = \frac{1}{2} \bar{X},$$

where $\mathbf{1}_{m_1}$ is a length- m_1 column vector of ones. Note that this overall mean allele frequency \hat{p} is computed among causal loci only. This works very well in practice since β is drawn randomly, so it is uncorrelated to \mathbf{p} and therefore

$$\frac{1}{m_1} \mathbf{p}^\top \beta = \frac{1}{m_1} \sum_{i=1}^{m_1} p_i \beta_i \approx \left(\frac{1}{m_1} \sum_{i=1}^{m_1} p_i \right) \left(\frac{1}{m_1} \sum_{i=1}^{m_1} \beta_i \right) = \frac{1}{m_1} \bar{p} \mathbf{1}_{m_1}^\top \beta$$

is a good approximation.

Now we discuss why the more obvious naive approach, which would be to center the trait using estimated ancestral allele frequencies as $\alpha = \mu - 2\hat{\mathbf{p}}^\top \beta$, does not work. This approach is equivalent to centering genotypes at each locus as

$$\mathbf{y} = \alpha \mathbf{1}_n + \sum_{i=1}^{m_1} (\mathbf{x}_i - 2\hat{p}_i \mathbf{1}_n) \beta_i + \epsilon.$$

However, this operation introduces a distortion in the covariance of the genotypes (Ochoa and Storey, 2016b):

$$\text{Cov}(\mathbf{x}_i - 2\hat{p}_i \mathbf{1}_n) = p_i(1 - p_i) (\Phi + \bar{\varphi} \mathbf{1}_n \mathbf{1}_n^\top - \varphi \mathbf{1}_n^\top - \mathbf{1}_n \varphi^\top),$$

where $\bar{\varphi}$ is the overall mean kinship, as before, and $\varphi = \frac{1}{n} \Phi \mathbf{1}_n$ is a length- n column vector of per-row mean kinship values. These undesirable distortions propagate to the trait, which we confirmed in simulations (not shown). Note that the intercept version we chose instead does not induce this genotype centering, which prevents the undesirable distortions in the trait covariance.

2.5.2 Admixture simulation for genotype matrices

TODO: describe the BNPSD simulation.

3 Results

3.1 Empirical demonstration of biases in heritability estimation

TODO: add results using SOLAR and GCTA, varying the input kinship matrices (estimates and limits from methods).

GCTA results:

Fig. 1 shows the distribution of heritability estimated by GCTA with different input kinship matrices. The performance of the default kinship matrix of GCTA is surprisingly different from the other kinship matrices. Using popkin and std lim kinship matrices perform most similar to the one using true kinship matrix.

3.2 Empirical demonstration of robustness to kinship bias in PCA and LMM genetic association studies

To quantify the effect of the various kinship matrix estimates, and their limiting biases, we calculated effect size coefficients and p-values for all variants of the PCA and LMM methods, and calculated

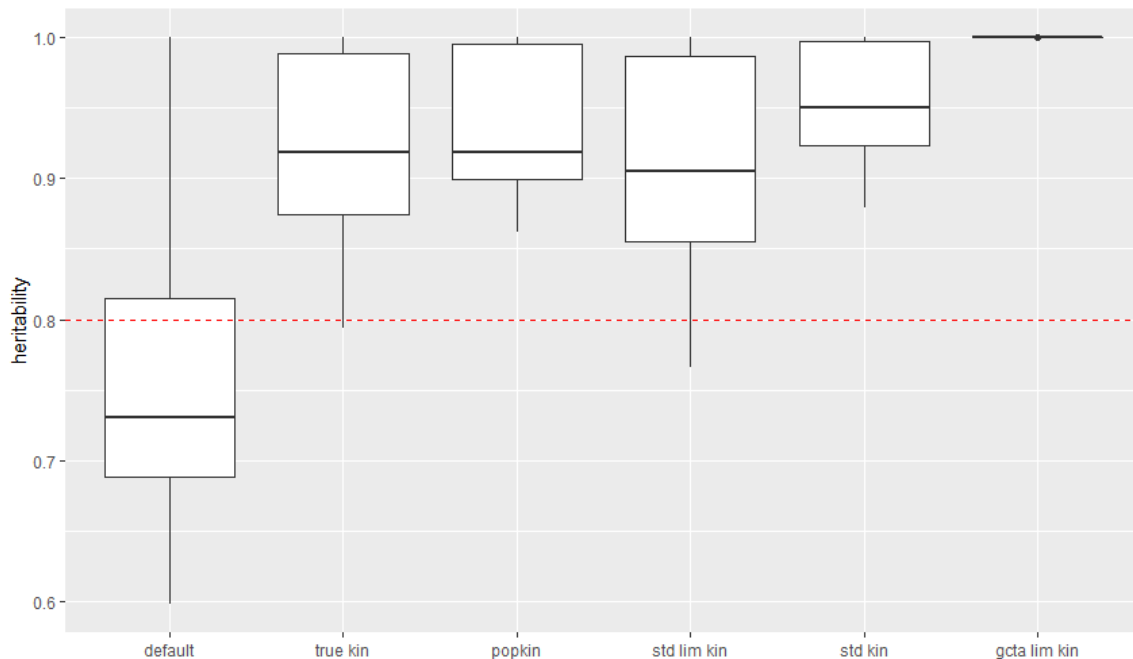


Figure 1: **Heritability estimation by GCTA with different kinship matrices.** $n = 2000$ individuals for 10 replicates, others are default settings ($m = 100000$ loci, $k = 3$ admixture ancestral subpopulations, $F_{ST} = 0.3$ for admixed individuals, bias coefficient $s = 0.5$, no family structure, and the trait had a heritability of $h^2 = 0.8$ and $m_1 = 100$ causal loci).

correlation coefficients of these vectors across methods. To further differentiate methods, we reduced the number of loci in the simulation to $m = 10,000$, which results in greater noise in all kinship matrix estimates (compared to a typical association study, which often contains millions of loci), which allows us to better distinguish that effect from the effect of bias (which remains in the limit of infinite loci).

We found that all the methods yield highly correlated values (for p-values in Fig. 2, effect sizes $\hat{\beta}$ in Fig. 3). However, while all PCA variants cluster together, LMM statistics do not cluster with each other. Instead, LMMs using the limiting kinship values cluster strongly with PCA, while LMMs that use estimated kinship matrices formed a separate cluster. This suggests that PCA is more robust to estimation noise than the LMM approach. Nevertheless, these effects are expected to be very small in real datasets, which typically have greater numbers of loci (recall we artificially reduced this number here to create greater differences between methods).

3.3 Theoretical justification of empirical observations

Here, to eliminate random estimation noise from the analysis (which our empirical evaluations suggest play a minor role), we shall focus on the limiting bias of the standard kinship estimator. Therefore, our theoretical results only consider the true kinship matrix Φ and the limit of the standard kinship estimator from Eq. (4), which can be restated in terms of matrix operations as

$$\hat{\Phi}^{\text{std-lim}} = \frac{1}{1 - \bar{\varphi}} (\Phi + \bar{\varphi} \mathbf{1}_n \mathbf{1}_n^\top - \varphi \mathbf{1}_n^\top - \mathbf{1}_n \varphi^\top).$$

The two kinship matrices are related more succinctly using the centering matrix \mathbf{C} :

$$\hat{\Phi}^{\text{std-lim}} = \frac{1}{1 - \bar{\varphi}} \mathbf{C} \Phi \mathbf{C}, \quad \mathbf{C} = \mathbf{I}_n - \frac{1}{n} \mathbf{1}_n \mathbf{1}_n^\top.$$

The centering matrix has been well studied, and we review its properties here. For any length- n vector \mathbf{v} we have

$$\mathbf{C} \mathbf{v} = \mathbf{v} - \mathbf{1}_n \bar{v},$$

where $\bar{v} = \frac{1}{n} \mathbf{1}_n^\top \mathbf{v}$ is the mean value of the elements of \mathbf{v} . Therefore, $\mathbf{v} = \mathbf{1}_n$ gets transformed to the zero vector, so it is an eigenvector with an eigenvalue of zero:

$$\mathbf{C} \mathbf{1}_n = \mathbf{0}_n.$$

Moreover, any vector \mathbf{v} orthogonal to $\mathbf{1}_n$ has a zero mean element ($\bar{v} = 0$) by hypothesis and it is not altered by \mathbf{C} ($\mathbf{C} \mathbf{v} = \mathbf{v}$). Therefore, the nullspace of \mathbf{C} is spanned by $\mathbf{1}_n$.

This centering matrix provides the key insight as to why PCA and LMM approaches are robust to this specific kinship bias, namely that by fitting the intercept term $\alpha \mathbf{1}_n$ together with the eigenvectors (for PCA) or random effects (in the same rowspace as the eigenvectors; for LMM) of $\hat{\Phi}^{\text{std-lim}}$, they complete the rowspace of $\hat{\Phi}^{\text{std-lim}}$ to equal the rowspace of the true kinship matrix Φ plus the intercept. First we show the following lemma.

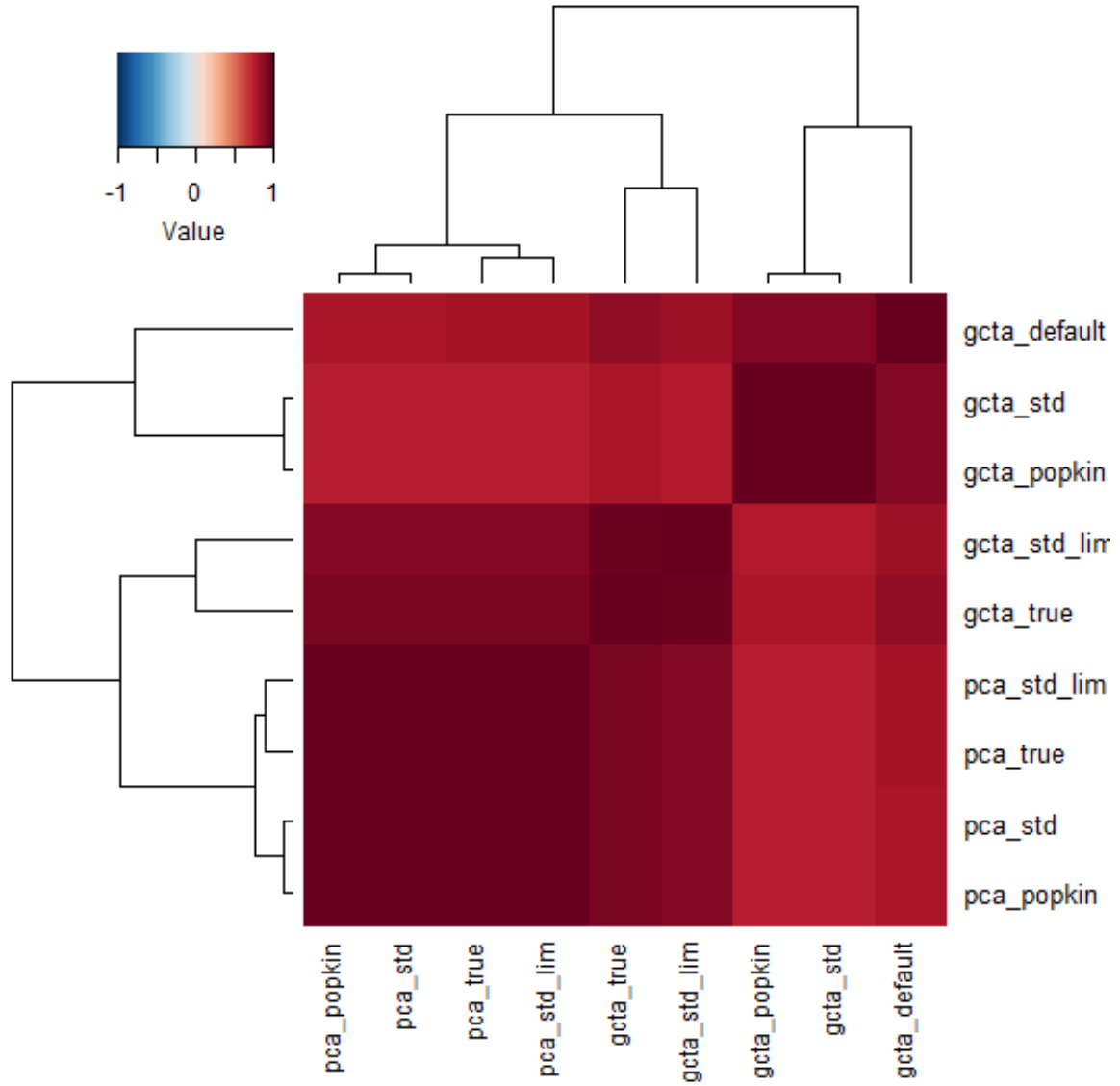


Figure 2: Heatmap and dendrogram for p-values of GCTA and PCA with different kinship matrices ($m = 10,000$).

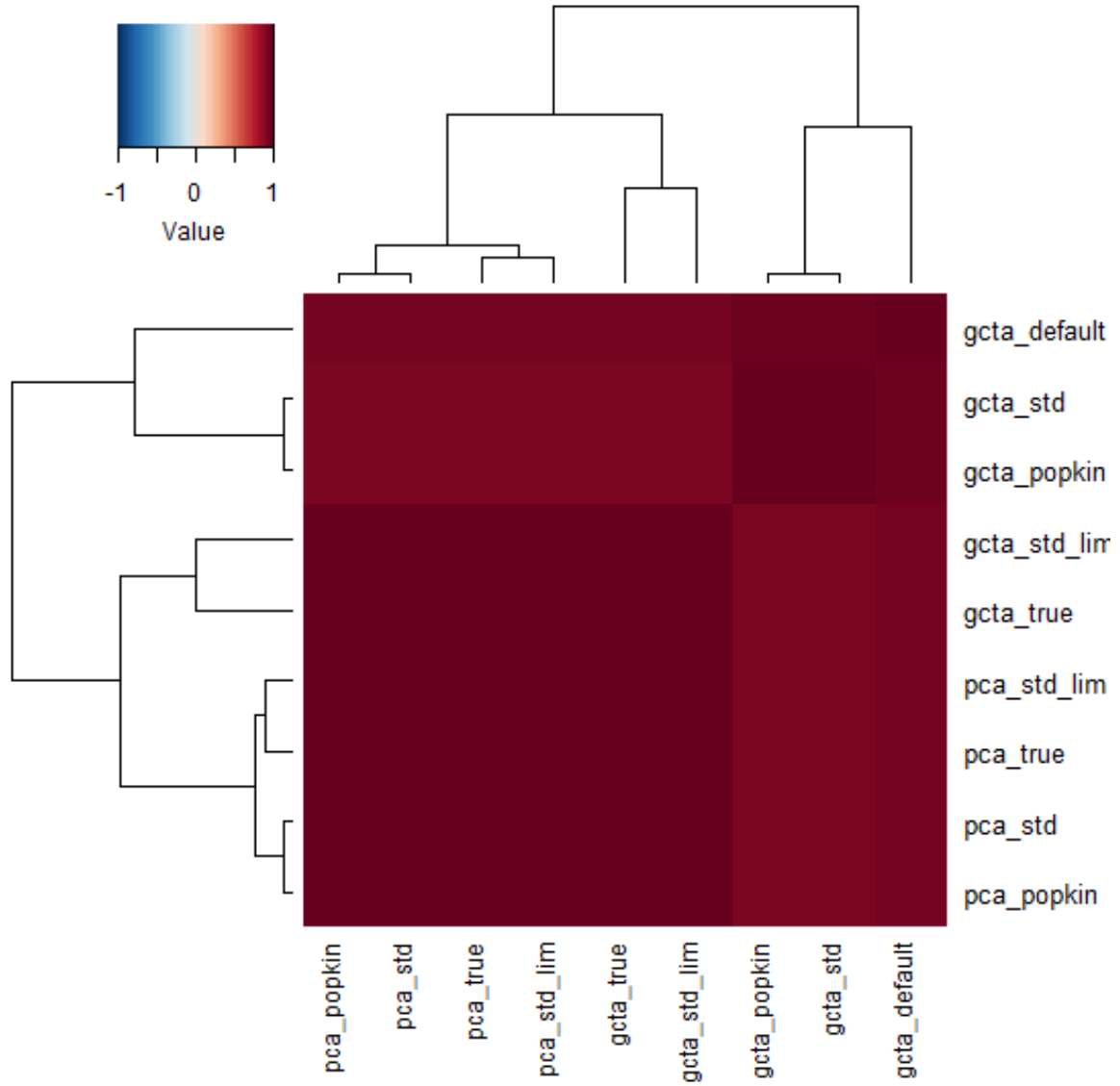


Figure 3: Heatmap and dendrogram for $\hat{\beta}$ of GCTA and PCA with different kinship matrices ($m = 10,000$).

Lemma. $\mathbf{1}_n$ is in the nullspace of $\hat{\Phi}^{\text{std-lim}}$ but not of Φ .

Proof. The vector $\mathbf{1}_n$ is not in the nullspace of any true kinship matrix Φ , since $\Phi\mathbf{1}_n \neq \mathbf{0}_n$, which follows since all kinship values are non-negative and the diagonal of the kinship matrix is strictly positive (it has a minimum value of $\frac{1}{2}$). However, $\mathbf{1}_n$ is in the nullspace of $\hat{\Phi}^{\text{std-lim}}$ since $\mathbf{C}\mathbf{1}_n = \mathbf{0}_n$:

$$\hat{\Phi}^{\text{std-lim}}\mathbf{1}_n = \frac{1}{1 - \bar{\varphi}}\mathbf{C}\Phi\mathbf{C}\mathbf{1}_n = \mathbf{0}_n.$$

□

Now we may prove the desired theorem.

Theorem. The rowspace of Φ and $\mathbf{1}_n$ equals rowspace of $\hat{\Phi}^{\text{std-lim}}$ and $\mathbf{1}_n$.

Proof. Since $\mathbf{1}_n$ is in both rowspaces, it suffices to consider vectors \mathbf{v} orthogonal to $\mathbf{1}_n$, which satisfy $\mathbf{C}\mathbf{v} = \mathbf{v}$. We shall prove below that any such vector is in the nullspace of Φ if and only if it is in the nullspace of $\hat{\Phi}^{\text{std-lim}}$. Then, since the nullspace of Φ and $\mathbf{1}_n$ is the same as the nullspace of $\hat{\Phi}^{\text{std-lim}}$ and $\mathbf{1}_n$, it follows from the fundamental theorem of linear algebra that their rowspaces are also the same.

If \mathbf{v} is in the nullspace of Φ , then $\Phi\mathbf{v} = \mathbf{0}_n$. It follows that

$$\hat{\Phi}^{\text{std-lim}}\mathbf{v} = \frac{1}{1 - \bar{\varphi}}\mathbf{C}\Phi\mathbf{C}\mathbf{v} = \frac{1}{1 - \bar{\varphi}}\mathbf{C}\Phi\mathbf{v} = \mathbf{0}_n,$$

so \mathbf{v} is also in the nullspace of $\hat{\Phi}^{\text{std-lim}}$.

Conversely, if \mathbf{v} is in the nullspace of $\hat{\Phi}^{\text{std-lim}}$, then $\hat{\Phi}^{\text{std-lim}}\mathbf{v} = \mathbf{0}_n$, which implies that $\mathbf{C}\Phi\mathbf{C}\mathbf{v} = \mathbf{C}\Phi\mathbf{v} = \mathbf{0}_n$. Left multiplying by \mathbf{v}^\top results in $\mathbf{v}^\top\mathbf{C}\Phi\mathbf{v} = \mathbf{v}^\top\Phi\mathbf{v} = 0$, which implies that \mathbf{v} is also in the nullspace of the positive-semidefinite matrix Φ , as desired. If Φ were positive definite, then no such $\mathbf{v} \neq \mathbf{0}_n$ would exist (Φ would have the trivial nullspace $\{\mathbf{0}_n\}$). □

3.3.1 Theoretical justification for PCA genetic association

In PCA-based genetic association, the desired result follows from the previous theorem, as follows. Here the goal is to fit the trait \mathbf{y} using a model similar to our main model in Eq. (1), namely

$$\mathbf{y} = \mathbf{1}_n\alpha + \mathbf{x}_i\beta_i + \mathbf{U}_r\gamma_r + \epsilon, \tag{7}$$

where instead of including the whole genotype matrix \mathbf{X} as we did in Eq. (1), here the genotype vector \mathbf{x}_i at a single locus i is fit, and the effect of the rest of the genome is approximated using the top r eigenvectors of the kinship matrix, contained in the $n \times r$ matrix \mathbf{U}_r and its length- r vector of coefficients γ_r . At each locus i the coefficients α , β_i , and γ_r are fit to minimize the squared error between the observed trait and the model, and the residuals and possibly the degrees of freedom are used to evaluate the significance of the fit for the genotype under the null hypothesis that $\beta_i = 0$.

Here the kinship matrix in question is not the full kinship matrix Φ , but its r -dimensional approximation $\Phi_r = \mathbf{U}_r \mathbf{\Lambda}_r \mathbf{U}_r^\top$, where $\mathbf{\Lambda}_r$ is an $r \times r$ diagonal matrix of the top r eigenvalues. One key requirement in need of verification is that $\mathbf{1}_n$ is not in the nullspace of Φ_r , which certainly holds for reasonable approximations as $\mathbf{1}_n^\top \Phi_r \mathbf{1}_n$ estimates the mean kinship of the data, which is non-zero except for completely unstructured populations. The other key assumption,

$$\hat{\Phi}_r^{\text{std-lim}} (1 - \bar{\varphi}) = (\mathbf{C} \Phi \mathbf{C})_r \approx \mathbf{C} \Phi_r \mathbf{C},$$

is only approximately true. In other words, centering the kinship matrix first and then approximating to the top r dimensions is not exactly equal to first approximating to r dimensions and then centering, although in simulations we found it to be a very good approximation (especially in the absence of family structure, which is where use of PCA is most appropriate anyway [TODO: cite Yao, other papers]). Therefore, in this case the rowspace of Φ_r and $\mathbf{1}_n$ only approximately equals the rowspace of $\hat{\Phi}_r^{\text{std-lim}}$ and $\mathbf{1}_n$.

The rowspace of \mathbf{U}_r is the same as the rowspace of the r -dimensional kinship matrix it is derived from, so either Φ_r or $\hat{\Phi}_r^{\text{std-lim}}$. Thus, when fitting the model, the space spanned by $\mathbf{1}_n \alpha + \mathbf{U}_r \gamma_r$ is approximately the same whether the eigenvectors \mathbf{U}_r are derived from the true kinship matrix Φ or the limit of the biased estimator $\hat{\Phi}^{\text{std-lim}}$, and this is so because the intercept vector $\mathbf{1}_n$ is present in the model. That implies that, while the coefficients α and γ_r may change when fit based on Φ or $\hat{\Phi}^{\text{std-lim}}$, the sum of the term $\mathbf{1}_n \alpha + \mathbf{U}_r \gamma_r$ will be approximately the same, as it is being chosen to minimize the least square error and the subspace is approximately the same, so the value of this solution must be approximately the same. Thus, the fit of the focal coefficient β_i is approximately the same either way, and so is its evaluation of significance (the sum of square errors is also approximately the same). Therefore, the genetic association test is approximately unchanged when the eigenvectors of either Φ or $\hat{\Phi}^{\text{std-lim}}$ are included as covariates.

3.3.2 Theoretical justification for LMM genetic association

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4 Discussion

The biased kinship matrix may be more desirable in PCA, from a numerical standpoint, as the resulting eigenvectors are not only orthogonal to each other but also to the intercept (whereas the eigenvectors of the true kinship matrix are not orthogonal to the intercept; see our Lemma).

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