Estimation of the covariance structure from SNP allele frequencies

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Abstract: We propose two new statistics, \( \hat{V} \) and \( \hat{S} \), to disentangle the population history of related populations from SNP frequency data. If the populations are related by a tree, we show by theoretical means as well as by simulation that the new statistics are able to identify the root of a tree correctly, in contrast to standard statistics, such as the observed matrix of \( F_2 \)-statistics (distances between pairs of populations). The statistic \( \hat{V} \) is obtained by averaging over all SNPs (similar to standard statistics). Its expectation is the true covariance matrix of the observed population SNP frequencies, offset by a matrix with identical entries. In contrast, the statistic \( \hat{S} \) is put in a Bayesian context and is obtained by averaging over pairs of SNPs, such that each SNP is only used once. It thus makes use of the joint distribution of pairs of SNPs. In addition, we provide a number of novel mathematical results about old and new statistics, and their mutual relationship.

Keywords: admixture model; \( F_2 \)-statistics; SNP evolution.

1 Introduction

A common situation in population genetics is ancestral disentanglement of related populations (Korunen and Goldberg 2021; Leppala et al. 2017; Lipson 2020; Patterson et al. 2012; Pickrell and Pritchard 2012). Imagine we observe genetic data in the form of allele frequencies from \( n \) SNPs and \( m \) related populations, and assume the population history is described by an unknown admixture graph. This graph is estimated from the data under the assumption of neutral evolution. The estimation typically takes place in two steps. First the covariance matrix of the SNP allele frequencies is estimated from the data, which in turn is used to determine the admixture graph. This covariance matrix is at the core of much inference on population history. In this article we are interested in efficient estimation of the covariance matrix.

To put some notation, assume we observe data vectors, \( X^1, \ldots, X^n \) (one for each SNP), where \( X^k = (X^k_1, \ldots, X^k_m) \) is an \( m \)-dimensional real-valued vector with common expectations \( \mathbb{E}(X^k_i) = \mu_k, i = 1, \ldots, m \), and \( m \times m \) covariance matrix \( \Sigma^k, k = 1, \ldots, n \). Here, \( X^k_i \) is the frequency of a particular allele (say, the reference allele) of the \( k \)th SNP in population \( j \). While it is standard to assume an underlying admixture graph or tree (Lipson 2020; Patterson et al. 2012), we will not impose this here. However, we do assume the populations share a common ancestor (‘root’) at some point in the past, represented by the mean value \( \mu_k \).

The objective is to estimate

\[
\Sigma = \frac{1}{n} \sum_{k=1}^{n} \Sigma^k = \frac{1}{n} \sum_{k=1}^{n} \mathbb{E} \left[ (X^k - \mu_k e) (X^k - \mu_k e)^\top \right],
\]
the average covariance matrix over all sites. The mean values \( \mu_k \), \( k = 1, \ldots, n \), are nuisance parameters of little interest. In the absence of any knowledge about \( \mu_k \), Pickrell and Pritchard (2012) suggests a surrogate statistic \( \hat{W} \) for a related covariance matrix \( W \), obtained from \( \Sigma \) by replacing \( \mu_k \) in Eq. (1) with the average allele frequency. If the population history is a tree, one cannot infer the placement of the root from \( \hat{W} \). Consequently, to rectify this, one might choose manually one population as an outgroup and use this to place the root (Pickrell and Pritchard 2012). The same situation appears for another surrogate statistic, the observed distance matrix \( \hat{D} \), that is an estimator of the pairwise \( F_2 \)-distance matrix \( D \) (for formal definitions, see Section 2) (Patterson et al. 2012).

In the present paper, we are concerned with two things. The first is to make available some results on the statistics \( \hat{W} \) and \( \hat{D} \), and their mutual relationship. The second is to propose two new statistics, \( \tilde{V} \) and \( \hat{S} \), that both can be used to recover the placement of the root, without using an outgroup. Whereas, \( \tilde{V} \) is similar in spirit to \( \hat{W} \) and \( \hat{D} \) in the sense of averaging over all SNPs, \( \hat{S} \) is based on pairwise comparison of SNPs, and is put in a Bayesian context. This statistic might open for new ways to explore the data.

The results are stated generally and do not rely on any specific distributional assumptions on the SNP allele frequencies. In particular, the \( \lambda_k \)'s do not need to be frequencies at all, but could be arbitrary random variables with mean and variance. Hence, the proposed theory and methodology might have wider applications in population genetics and genomics, as well as outside these fields.

**Notation** If \( A \) is a matrix, then \( A' \) denotes the transposed matrix. Vectors are assumed to be column vectors. If \( v \) is a vector, then \( v' \) is a row vector. Let \( I \) be the \( m \times m \) identity matrix, \( E \) the symmetric \( m \times m \) square matrix with all entries equal to one, and \( e = (1, \ldots, 1)' \) the vector in \( \mathbb{R}^m \) with all entries one. Furthermore, let \( e_i \) be the \( i \)th unit vector, \( i = 1, \ldots, m \). So, \( (e_i)_i = 1 \) and \( (e_i)_j = 0 \) for \( j \neq i \).

For an \( m \times m \) matrix \( A \), the Frobenius norm of \( A \) is \( \|A\|_F = \sqrt{\sum_{a=1}^m \sum_{b=1}^m A_{ab}^2} \). For a linear operator \( \mathcal{X} : \mathbb{R}^m \to \mathbb{R}^m \), the image is \( \text{im}(\mathcal{X}) = \{ \mathcal{X}(A) : A \in \mathbb{R}^m \} \), and the operator norm is

\[
\|\mathcal{X}\|_{\text{op}} = \sup_{\|A\| = 1} \|\mathcal{X}(A)\|_F.
\]

If \( \mathcal{X} \) is an orthogonal projection then the operator norm is one.

## 2 Estimation of the covariance matrix

The theory to be developed holds for general random vectors, \( X_1, \ldots, X_m \) with values in \( \mathbb{R}^m \), \( m \geq 2 \). However, we put the theory in the context of population genetics as this is the application area we have in mind. Thus, we think of \( X_k = (X^1_k, \ldots, X^m_k) \), \( k = 1, \ldots, n \), as vectors of observed allele frequencies, either population or sample based.

Recall the covariance matrix in Eq. (1),

\[
\Sigma = \frac{1}{n} \sum_{k=1}^m X_k X_k' - \frac{1}{n} \sum_{k=1}^m X_k X_k' = \frac{1}{n} \sum_{k=1}^n \left( X_k - \mu_k e \right) \left( X_k - \mu_k e \right)'.
\]

In the case the means \( \mu_k, k = 1, \ldots, n \), are known, then a natural unbiased estimator of \( \Sigma = (\Sigma_{ij})_{i,j=1,\ldots,m} \), is

\[
\hat{\Sigma}_{ij} = \frac{1}{n} \sum_{k=1}^n \left( X^i_k - \mu_k \right) \left( X^j_k - \mu_k \right), \quad i, j = 1, \ldots, m.
\] (2)

However, in the absence of such knowledge, we cannot estimate \( \Sigma \) from the data without further assumptions. This has led to the proposal of alternative approaches, for example by substitution of \( \hat{\mu} \) with an estimated mean (Pickrell and Pritchard 2012). A natural unbiased estimator of \( \mu_k \) is the moment estimator

\[
\hat{\mu}_k = \frac{1}{m} \sum_{i=1}^m X^i_k.
\]

Plugging this into Eq. (2), yields the statistic \( \hat{W} \) given by

\[
\hat{W}_{ij} = \frac{1}{n} \sum_{k=1}^n \left( X^i_k - \hat{\mu}_k \right) \left( X^j_k - \hat{\mu}_k \right), \quad \text{where} \quad \hat{\mu}_k = \frac{1}{m} \sum_{i=1}^m X^i_k.
\]
is the empirical mean (Pickrell and Pritchard 2012). This is not an estimator of $\Sigma$ per se, but it still contains information about the data generating process. In Pickrell and Pritchard (2012), $\hat{W}$ is used as a surrogate for $\hat{\Sigma}$.

Obviously, $\hat{W}$ is a symmetric matrix and

$$
\hat{W}_{ij} = \frac{1}{n} \sum_{k=1}^{n} \left( X_i^k - \mu_k \right) \left( X_j^k - \mu_k \right) = \hat{\Sigma}_{ij} - \frac{1}{m} \sum_{a=1}^{m} \hat{\Sigma}_{ia} - \frac{1}{m} \sum_{b=1}^{m} \hat{\Sigma}_{jb} + \frac{1}{m^2} \sum_{a=1}^{m} \sum_{b=1}^{m} \hat{\Sigma}_{ab}
$$

(Pickrell and Pritchard 2012, Eq. (23)).

Patterson et al. (2012) suggest a different statistic to capture the evolutionary distances between the populations. For populations $i$ and $j$, and SNP $k$, the distance between the populations (at SNP $k$) is defined as the variance of $X_i^k - X_j^k$, which is known as an $F_2$ statistic. Let $D$ be the matrix with $(i, j)$ entry $D_{ij} = \frac{1}{n} \sum_{k=1}^{n} \text{var} \left( X_i^k - X_j^k \right)$. An obvious estimator of $D$ is defined by

$$
\hat{D}_{ij} = \frac{1}{n} \sum_{k=1}^{n} \left( X_i^k - X_j^k \right)^2.
$$

Also, $D$ and $\hat{D}$ are symmetric matrices, and

$$
\hat{D}_{ij} = \frac{1}{n} \sum_{k=1}^{n} \left( X_i^k - \mu_k + \mu_k - X_j^k \right)^2 = \hat{\Sigma}_{ii} + \hat{\Sigma}_{jj} - 2\hat{\Sigma}_{ij}.
$$

Thus, both $\hat{D}$ and $\hat{W}$ are linear transformations of $\hat{\Sigma}$. Furthermore, they are related to each other by an isomorphism (see Theorem 2), and hence carry the same information. To formalise this, we need some further notation.

Let $S_m$ be the vector space of symmetric $m \times m$-matrices with dimension $m(m+1)/2$, and define linear operators $\mathcal{D}, \mathcal{W}: S_m \rightarrow S_m$ by

$$
\mathcal{W}(A)_{ij} = A_{ij} - \frac{1}{m} \sum_{a=1}^{m} A_{ia} - \frac{1}{m} \sum_{a=1}^{m} A_{ja} + \frac{1}{m^2} \sum_{a=1}^{m} \sum_{b=1}^{m} A_{ab},
\mathcal{D}(A)_{ij} = A_{ij} + A_{ji} - 2A_{ij}.
$$

Obviously, $\hat{D} = \mathcal{D}(\hat{\Sigma})$ and $\hat{W} = \mathcal{W}(\hat{\Sigma})$.

**Lemma 1.** The linear operator $\mathcal{W}$ has the representation

$$
\mathcal{W}(A) = \left( I - \frac{1}{m} E \right) A \left( I - \frac{1}{m} E \right).
$$

Consequently, if $A$ is positive definite, then $\mathcal{W}(A)$ is positive semi-definite. In particular, $\mathcal{W}(\Sigma)$ is positive semi-definite.

**Proof.** The first part follows by straightforward evaluation. For the second part, let $x \in \mathbb{R}^m$. We have

$$
x^t \mathcal{W}(A) x = x^t \left( I - \frac{1}{m} E \right) A \left( I - \frac{1}{m} E \right) x = \left( \left( I - \frac{1}{m} E \right) x \right)^t A \left( I - \frac{1}{m} E \right) x \geq 0,
$$

as $A$ is positive semi-definite by assumption. The final part follows by noting that $\Sigma$ is positive definite, since it is a covariance matrix. □
Theorem 2. We have
\[ \mathcal{D} = \mathcal{D} \circ \mathcal{W}, \quad \mathcal{W} = -\frac{1}{2} \mathcal{W} \circ \mathcal{D}, \quad \mathcal{D} \circ \mathcal{D} = -2\mathcal{D} \quad \text{and} \quad \mathcal{W} \circ \mathcal{W} = \mathcal{W}. \]

The operator \( \mathcal{W} \) is an orthogonal projection (hence has operator norm one), while \(-\frac{1}{2} \mathcal{D}\) is a non-orthogonal projection with operator norm
\[ \sqrt{m - 1} \leq \| -\frac{1}{2} \mathcal{D} \|_{\text{op}} \leq 1 + \sqrt{m - 1}. \]

The operators \( \mathcal{D} \) and \( \mathcal{W} \) have the same \( m \)-dimensional kernel, given by
\[ K = \{ ev^t + ve^t : v \in \mathbb{R}^m \}. \]

The restrictions
\[ \mathcal{W} : \text{im}(\mathcal{D}) \to \text{im}(\mathcal{W}), \quad -\frac{1}{2} \mathcal{D} : \text{im}(\mathcal{W}) \to \text{im}(\mathcal{D}) \]
are each other's inverse. The images of \( \mathcal{W} \) and \( \mathcal{D} \) have dimension \( \frac{1}{2} m(m - 1) \).

The proof of Theorem 2 is deferred to Section 7.1. Based on simulation, we conjecture the operator norm of \(-\frac{1}{2} \mathcal{D}\) to be \( \sqrt{m} \), but we are unable to prove it.

Let \( W := \mathbb{E}(\hat{W}) \) and \( D := \mathbb{E}(\hat{D}) \).

Theorem 3. It holds that \( W = \mathcal{W}(\Sigma) \), and \( D = \mathcal{D}(\Sigma) \).

Proof. By linearity of the expectation and the definition of \( \mathcal{W} \) and \( \mathcal{D} \). \( \square \)

The interpretation of the results are well understood in the case the populations are related by a tree, see Figure 1. In this case, it is standard to associate independent random variables to the edges and the root of the tree, such that
\[ X_i^k = C_r^k + \sum_{e \in \Gamma_{ri}} C_e^k, \quad k = 1, \ldots, n, \quad i = 1, \ldots, m, \quad (4) \]
where the sum is over all edges \( e \) on the unique path \( \Gamma_{ri} \) from the root \( r \) to population \( i \), \( C_r^k \) is the random variable associated the root, and \( C_e^k \) the random variable associated the edge \( e \). This model naturally arises from the normal approximation model,
\[ X_i \sim \text{Normal} \left( x_0, \frac{t}{N_e} x_0(1 - x_0) \right) \]
(Nicholson et al. 2002), where \( x_0 \) is the SNP frequency at time \( 0 \), \( N_e \) is the effective population size, and \( X_i \) is the SNP frequency \( t \) generations later. The change in frequency might be found by summing independent increments over different time epochs, leading to the model in Eq. (4).

Consider the case of \( m = 2 \), and let \( \Sigma \) be given as
\[ \Sigma = \begin{pmatrix} \sigma_1 + \tau & \tau \\ \tau & \sigma_2 + \tau \end{pmatrix}, \quad (5) \]
where \( \sigma_1, \sigma_2, \tau \geq 0 \), corresponding to the graph in Figure 1(a). Typically, in an evolutionary context, \( \tau \) might be taken to be zero (the variance of the root variable), as data from the two populations will not contain any information about the evolution of the two populations prior to their most recent common ancestor. However, one might alternatively think of \( \tau \) as the variance of the SNP means \( \mu_k, k = 1, \ldots, n \) (to be explored in Section 5).

It follows from Eq. (5) and \( D = \mathcal{D}(\Sigma) \) that
\[ \mathcal{D}(\Sigma) = \begin{pmatrix} 0 & \sigma_1 + \sigma_2 \\ \sigma_1 + \sigma_2 & 0 \end{pmatrix}. \]
Figure 1: A tree with two (a) and three (b) leaves. In (c), the length \( \tau \) of the root tip is extended by \( x \) and slid by \( y \) to the right. We refer to the full edge labelled tree as a rooted tree with a root tip; the tree without the root tip but with the placement of the root as a rooted tree without the root tip; and the tree without the root tip and the placement of the root as an unrooted tree. In the latter case, the branches labeled \( \sigma_{11} \) and \( \sigma_{1} \) in (b) are replaced by a single branch of length \( \sigma_{11} + \sigma_{12} \).

From which only the sum \( \sigma_{1} + \sigma_{2} \) might be recovered. Hence, neither the length of the “root tip” (\( \tau \)) nor the placement of the root can be recovered. The elements of the kernel might be seen as operations on the tree, while preserving \( \mathcal{D}(\Sigma) \). This is perhaps best illustrated for \( m = 3 \), in which case we take the tree of Figure 1(b) as starting point, with

\[
\Sigma = \begin{pmatrix}
\sigma_{11} & 0 & 0 \\
0 & \sigma_{12} + \sigma_{2} & \sigma_{12} \\
0 & \sigma_{12} & \sigma_{12} + \sigma_{3}
\end{pmatrix} + \tau E.
\]

Adding the kernel element

\[
e \begin{pmatrix} y & 0 & 0 \end{pmatrix} + \begin{pmatrix} y \end{pmatrix} e' + (x - y)E = xE + \begin{pmatrix} y & 0 & 0 \end{pmatrix} \]

\[
\begin{pmatrix} 0 & -y & -y \\
-0 & -y & -y \\
0 & -y & -y
\end{pmatrix}
\]

to \( \Sigma \) corresponds to extending the outgoing edge from the root with \( x \) and sliding the root by \( y \) to the right on the edge, see Figure 1(b) and (c). Choosing \( y = \sigma_{12} \) yields a trifurcated star-shaped tree. Now, using other similar kernel elements, the star-shaped tree might be turned into many other trees while preserving the distance matrix

\[
\mathcal{D}(\Sigma) = \begin{pmatrix}
0 & \sigma_{1} + \sigma_{2} & \sigma_{1} + \sigma_{3} \\
\sigma_{1} + \sigma_{2} & 0 & \sigma_{2} + \sigma_{3} \\
\sigma_{1} + \sigma_{3} & \sigma_{2} + \sigma_{3} & 0
\end{pmatrix},
\]

where \( \sigma_{11} + \sigma_{12} = \sigma_{1} \).

The same applies for higher \( m > 3 \) by iteratively applying kernel matrices to move ancestral nodes of the tree while preserving \( \mathcal{D}(\Sigma) \).
3 A new statistic

We suggest a third symmetric statistic, that carries more information about $\Sigma$ than the other two statistics. It is defined by

$$\hat{V} = \frac{1}{n} \sum_{k=1}^{n} X^k(X^k)' - \hat{\mu}_k^2E.$$ 

The second term is a correction term that makes the expectation of $\hat{V}$ independent of the mean $\mu_k$. Also, it ensures the sum of all entries of $\hat{V}$ is zero. In contrast to the other two statistics, $\hat{V}$ is not linear in $\Sigma$. However, if we define $Y = \frac{1}{n} \sum_{k=1}^{n} X^k(X^k)'$, then $\hat{W} = \mathcal{Y}(Y), \hat{D} = \mathcal{D}(Y)$ and $\hat{V} = \mathcal{V}(Y)$, where $V$ is defined in Eq. (6).

By inspection, it holds that $\mathcal{Y} (\hat{V}) = \hat{W}$ and $\mathcal{D} (\hat{V}) = \hat{D}$. Let $\mathcal{V} : S_m \to S_m$ be defined as

$$\mathcal{V}(A) = A - \frac{1}{m^2} EAE.$$ 

The following holds.

Theorem 4. The operator $\mathcal{V}$ is an orthogonal projection, $\mathcal{V} = \mathcal{V} \circ \mathcal{V}$ with kernel $\{\lambda E : \lambda \in \mathbb{R}\}$. In particular, $\mathcal{V}$ has operator norm one. Furthermore, $V := \mathcal{E}(V) = \mathcal{V}(\Sigma)$.

Proof. Let $V = \mathcal{V}(A)$. Note that $e'Ve = 0$. Hence, $\mathcal{V}(V) = V$, which implies $\mathcal{V} \circ \mathcal{V} = \mathcal{V}$. It follows from Eq. (6), that $\mathcal{V}(E) = 0$, but also that $A$ differs from $\mathcal{V}(A)$ by a constant times $E$. Hence $\ker(\mathcal{V}) = \{\lambda E : \lambda \in \mathbb{R}\}$. Note that an $m \times m$-matrix $F$ is orthogonal to $E$ if and only if $\sum_{i=1}^{m} \sum_{j=1}^{m} F_{ij} = 0$. We have

$$\sum_{i=1}^{m} \sum_{j=1}^{m} \mathcal{V}(A)_{ij} = \sum_{i=1}^{m} \sum_{j=1}^{m} A_{ij} - \sum_{i=1}^{m} \sum_{j=1}^{m} A_{ij} = 0.$$

Hence, $\mathcal{V}$ is an orthogonal projection. In particular, $\mathcal{V}$ has operator norm one, for $m \geq 2$. Define $\hat{V}^k = X^k(X^k)' - \hat{\mu}_k^2E$, such that $\hat{V} = \frac{1}{n} \sum_{k=1}^{n} \hat{V}^k$. Note that

$$\mathbb{E} (\hat{\mu}_k^2) = \frac{1}{m^2} \sum_{a=1}^{m} \sum_{b=1}^{m} \mathbb{E} (X^a X^b)^k \quad \text{and} \quad \mathbb{E} (X^k(X^k)') = \Sigma^k + \mu_k^2E.$$

Then, $\mathbb{E} (\hat{\mu}_k^2) = \mu_k^2 + e' \Sigma^k e / (m^2)$, and

$$\mathbb{E} (\hat{V}) = \frac{1}{n} \sum_{k=1}^{n} \Sigma^k - \frac{1}{m^2} (e' \Sigma^k e)E = \Sigma - \frac{1}{m^2} E \Sigma E,$$

and the proof is complete. \(\square\)

In the case of a tree, the placement of the root is identifiable from $V = \mathcal{V}(\Sigma)$, but not the length of the root tip. For $m = 2$, we find

$$\mathcal{V}(\Sigma) = \frac{1}{4} \begin{pmatrix} 3\sigma_1 - \sigma_2 & -\sigma_1 - \sigma_2 \\ -\sigma_1 - \sigma_2 & 3\sigma_2 - \sigma_1 \end{pmatrix}.$$ 

from which $\sigma_1, \sigma_2$ might be recovered. The general statement for arbitrary $m$ is here:

Theorem 5. The unrooted tree is identifiable from $D$ (or $W$). The rooted tree without the root tip is identifiable from $V$. The rooted tree with the root tip is identifiable from $\Sigma$.

Proof. The first statement is well known in literature (Semple and Steel 2003, theorem 7.1.8, page 148). By Theorem 2, it also holds true for $W$. 

Let $\mathcal{T}_1 = (\mathcal{V}_1, \xi_1)$ and $\mathcal{T}_2 = (\mathcal{V}_2, \xi_2)$ be two rooted trees with vertex sets $\mathcal{V}_1, \mathcal{V}_2$, respectively, and edge sets $\xi_1, \xi_2$, respectively, and common $m \times m$ covariance matrix $\Sigma$.

Define the $(m + 1) \times (m + 1)$ matrix $A$ as follows

$$
\begin{pmatrix}
\Sigma_{11} & \Sigma_{12} & \cdots & \Sigma_{1m} \\
\Sigma_{21} & \Sigma_{22} & \cdots & \Sigma_{2m} \\
\vdots & \vdots & \ddots & \vdots \\
\Sigma_{m1} & \Sigma_{m2} & \cdots & \Sigma_{mm}
\end{pmatrix}.
$$

The element $A_{ij}$ is the distance between the leaves $i$ and $j$ of $\mathcal{T}_1$ (or $\mathcal{T}_2$) for $i, j \leq m$, and $A_{i,m+1} = A_{m+1,i} = \Sigma_{ii}$ is the distance from the root to the leaf $i$. If we consider the root as another ‘leaf’, then there is an isomorphism $\varphi: \mathcal{T}_1 \rightarrow \mathcal{T}_2$, such that $x - y$ is an edge in $\mathcal{V}_1$ if and only if $\varphi(x) - \varphi(y)$ is an edge in $\mathcal{T}_2$ (indifferent of the direction). Moreover, the length of $x - y$ is equal to the length of $\varphi(x) - \varphi(y)$, and $\varphi$ maps the $i$th leaf of $\mathcal{T}_1$ to the $i$th leaf of $\mathcal{T}_2$, and the root of $\mathcal{T}_1$ to the root of $\mathcal{T}_2$. It follows that $\mathcal{T}_1$ and $\mathcal{T}_2$ are isomorphic as directed labelled trees.

Let $\mathcal{T}_1$ and $\mathcal{T}_2$ be two trees with the same matrix $V$. Let $\Sigma_1$ and $\Sigma_2$ be their corresponding covariance matrices. Then, $\Sigma_1 - \Sigma_2 = \lambda E$ for some $\lambda$. Without loss of generality, $\lambda \geq 0$. If we make the root tip of $\mathcal{T}_2$ $\lambda$ longer, resulting in a tree $\mathcal{T}_2'$, with corresponding covariance matrix $\Sigma_2'$, then $\Sigma_1 - \Sigma_2' = 0$. It follows that $\mathcal{T}_1$ and $\mathcal{T}_2'$ are isomorphic. Consequently, it follows that $\mathcal{T}_1$ and $\mathcal{T}_2$ are equal except for the length of the root tip.

The following theorem relates $\mathcal{V}$ with $\mathcal{D}$ and $\mathcal{W}$, analogous to Theorem 2. Simple examples show that $\mathcal{V} \circ \mathcal{D} \neq \mathcal{D}$.

**Theorem 6.** It holds that $\mathcal{V} \circ \mathcal{W} = \mathcal{W} \circ \mathcal{V} = \mathcal{W}$ and $\mathcal{D} \circ \mathcal{V} = \mathcal{D}$.

**Proof.** Let $A \in \mathbb{S}_m$ and let $V = \mathcal{V}(A)$. Note that $A$ and $V$ differ only by a constant times $E$. As $E$ is in the kernel of $\mathcal{W}$ and $\mathcal{D}$, we have $\mathcal{W}(V) = \mathcal{W}(A)$ and $\mathcal{D}(V) = \mathcal{D}(A)$. This proves $\mathcal{D} \circ \mathcal{V} = \mathcal{D}$ and $\mathcal{W} \circ \mathcal{V} = \mathcal{W}$.

Note that $\mathcal{W}(A)e = e(I - E/m)A(I - E/m)e = 0$. It follows that $\mathcal{V}(\mathcal{W}(A)) = \mathcal{W}(A)$. Hence, $\mathcal{V} \circ \mathcal{W} = \mathcal{W}$. \hfill $\square$

We end by showing consistency of the statistic $\hat{V}$, assuming (almost) independence between sites, large $n$ and not too large $m$.

**Theorem 7.** Assume $X^1, \ldots, X^n$ are random vectors in $\mathbb{R}^m$ with mean $\mu_k e$ and $m \times m$ covariance matrix $\Sigma_k$, and that there is an integer $t \geq 1$, such that $X^k$ and $X^\ell$ are independent whenever $|k - \ell| \geq t$. Moreover, if there exists a constant $C > 0$, such that the forth moments of $X^k_i$, $k = 1, \ldots, n$, $i = 1, \ldots, m$, are smaller than $C$, then

$$
\mathbb{E}(\|\hat{V} - V\|_F) \leq 4\sqrt{\frac{m^2 t}{n}},
$$

$$
\mathbb{E}(\|\hat{D} - D\|_F) \leq 16\sqrt{\frac{m^2 t}{n} C},\quad \mathbb{E}(\|\hat{W} - W\|_F) \leq 16\sqrt{\frac{m^2 t}{n} C},
$$

for any $m, n, t$.

We defer the proof of Theorem 7 to Section 7.2. If $X^1, \ldots, X^n$, are frequencies, then the boundedness assumption is naturally met. The bound provides means to establish convergence in Frobenious norm as $n, m$ become large, and highlights the individual importance of $m, n, t$, respectively.
4 Least square estimation

In TreeMix (Pickrell and Pritchard 2012), the basic observation is \( \hat{W} \) from which parameters are estimated, for example, assuming the populations are related by a tree. One might alternatively take \( \hat{V} \) to be the basic observation. We pose the question whether the parameter estimates obtained from \( \hat{W} \) and \( \hat{V} \), respectively, are compatible?

A natural estimation procedure is Least Square (LS) estimation, which we will consider here. (We note that TreeMix in principle uses weighted LS estimation, where the weights are empirically obtained.) Let \( L \) be a linear subspace of \( \mathcal{V}(S_m) \) and \( H: V \in L \subseteq \mathcal{V}(S_m) \) a linear hypothesis about \( V \). We define the LS estimator of \( V \) under \( H \) by

\[
\hat{V}_L = \text{argmin}_{A \in L} \| \hat{V} - A \|_F.
\]

Similarly, one might estimate \( W \) from \( \hat{W} \) under the corresponding linear hypothesis \( H' : W \in \mathcal{W}(L) \subseteq \mathcal{W}(S_m) \),

\[
\hat{W}_L = \text{argmin}_{A \in \mathcal{W}(L)} \| \hat{W} - A \|_F.
\]

In either case, the LS estimator is the projection of the observation \( \hat{V} \) (respectively, \( \hat{W} \)) onto the linear space \( L \) (respectively, \( \mathcal{W}(L) \)).

**Theorem 8.** Let \( L \subseteq \mathcal{V}(S_m) \) be a linear subspace. If \( \mathcal{W}(L) \subseteq L \), then \( \hat{W}_L = \mathcal{W}(\hat{V}_L) \). Additionally, \( \hat{W}_L = \text{argmin}_{A \in L} \| \hat{W} - A \|_F \).

**Proof.** As \( \mathcal{W} \) is an orthogonal projection, we have for \( A \in L \),

\[
\| \hat{V} - A \|_F^2 = \| \mathcal{W}(\hat{V} - A) \|_F^2 + \| (I - \mathcal{W})(\hat{V} - A) \|_F^2.
\]

Further using that \( \mathcal{W}(\hat{V}) = \hat{W} \). As \( \mathcal{W}(L) \subseteq L \) by assumption, hence also \( (I - \mathcal{W})(L) \subseteq L \), and \( \mathcal{W}(L) \oplus (I - \mathcal{W})(L) = L \) by orthogonality of \( \mathcal{W} \). Hence, the minimum can be found as \( \hat{V}_L = \hat{V}_1 + \hat{V}_2 \), where

\[
\hat{V}_1 = \text{argmin}_{A \in \mathcal{W}(L)} \| \hat{W} - A \|_F, \quad \hat{V}_2 = \text{argmin}_{A \in (I - \mathcal{W})(L)} \| \hat{V} - \hat{W} - A \|_F.
\]

This implies \( \hat{V}_1 = \hat{W}_L \) by definition and \( \hat{W}_L = \mathcal{W}(\hat{V}_L) \).

For the last statement, \( \min_{A \in \mathcal{W}(L)} \| \hat{W} - A \|_F \geq \min_{A \in L} \| \hat{W} - A \|_F \), as \( \mathcal{W}(L) \subseteq L \). Using orthogonality of \( \mathcal{W} \), we have for \( A \in L \),

\[
\| \hat{W} - A \|_F^2 = \| \hat{W} - \mathcal{W}(A) \|_F^2 + \| \mathcal{W}(A) - A \|_F^2.
\]

Further, for \( A \in L \), \( \mathcal{W}(\mathcal{W}(A)) = \mathcal{W}(A) \). Hence, \( \| \hat{W} - \mathcal{W}(\mathcal{W}(A)) \|_F = \| \hat{W} - \mathcal{W}(A) \|_F \) and \( \| \mathcal{W}(\mathcal{W}(A)) - \mathcal{W}(A) \|_F = 0 \). It follows that \( \| \hat{W} - \mathcal{W}(A) \|_F \leq \| \hat{W} - A \|_F \) for \( A \in L \). Therefore, \( \min_{A \in \mathcal{W}(L)} \| \hat{W} - A \|_F \leq \min_{A \in L} \| \hat{W} - A \|_F \), and consequently, \( \min_{A \in \mathcal{W}(L)} \| \hat{W} - A \|_F = \min_{A \in L} \| \hat{W} - A \|_F \).

Note that \( \mathcal{W}(L) \subseteq L \) if and only if \( (I - \mathcal{W})(L) \subseteq L \). Hence, provided \( \mathcal{W}(L) \subseteq L \) holds, it follows from Theorem 8 by symmetry that the LS estimator \( \hat{U}_L \) under the linear hypothesis \( H'' : U \in (I - \mathcal{W})(L) \subseteq S_m \),

\[
\hat{U}_L = \text{argmin}_{A \in (I - \mathcal{W})(L)} \| \hat{W} - A \|_F
\]

fulfills \( \hat{U}_L = (1 - \mathcal{W})(\hat{V}_L) \). It leads to a reverse statement to that of Theorem 8.

**Theorem 9.** Let \( B \) be the support of the random variable \( \hat{V} = \hat{V}(X_1, \ldots, X_m) \) and assume \( \text{span}(B) = \mathcal{V}(S_m) \). Furthermore, let \( L \subseteq \mathcal{V}(S_m) \) be a linear subspace. If \( \hat{W}_L = \mathcal{W}(\hat{V}_L) \) and \( \hat{U}_L = (1 - \mathcal{W})(\hat{V}_L) \) hold for all \( \hat{V} \in B \), then \( \mathcal{W}(L) \subseteq L \).
Proof. We proceed by contradiction. By the remark above, we might assume that \( \mathcal{W}(L) \not\subseteq L \) or \((1 - \mathcal{W})(L) \not\subseteq L \), and show that it leads to a contradiction. Choose an arbitrary point \( \hat{V} \in B \) such that \( \hat{V} = \hat{V}_1 + \hat{V}_2 \in \mathcal{W}(L) \oplus (I - \mathcal{W})(L) \) \( \setminus L \), where \( \hat{V}_1 \in \mathcal{W}(L) \) and \( \hat{V}_2 \in (1 - \mathcal{W})(L) \). Such a point exists due to the span condition.

The LS estimate \( \hat{V}_L \) fulfills \( \hat{V}_L \neq \hat{V} \), as \( \hat{V} \not\in L \), while the LS estimates \( \hat{W}_L \) and \( \hat{U}_L \) clearly fulfill \( \hat{W}_L = \hat{V}_1 \) and \( \hat{U}_L = \hat{V}_2 \), respectively, as \( \hat{V}_1 \in \mathcal{W}(L) \) and \( \hat{V}_2 \in (1 - \mathcal{W})(L) \) by assumption. Since \( \hat{V}_L \neq \hat{V} \), then either \( \mathcal{W}(\hat{V}_L) \neq \hat{V}_1 \) or \((I - \mathcal{W})(\hat{V}_L) \neq \hat{V}_2 \), contradicting the conditions of the theorem. The proof is completed. \( \square \)

Example 10. Two populations related by a tree as in Figure 1(a) corresponds to the linear hypothesis, \( H: V \in L \), given by

\[
V \in L = \left\{ \frac{1}{4} \begin{pmatrix} 3 \sigma_1 - \sigma_2 & -\sigma_1 - \sigma_2 \\ -\sigma_1 - \sigma_2 & 3 \sigma_2 - \sigma_1 \end{pmatrix} \bigg| \sigma_1, \sigma_2 \in \mathbb{R} \right\}
\]

\[
= \left\{ \frac{1}{4} \begin{pmatrix} \sigma + 2 \delta & -\sigma \\ -\sigma & \sigma - 2 \delta \end{pmatrix} \bigg| \sigma, \delta \in \mathbb{R} \right\},
\]

where \( \sigma = \sigma_1 + \sigma_2 \) and \( \delta = \sigma_1 - \sigma_2 \), see Eq. (7). The projection of \( L \) by \( \mathcal{W} \) fulfills

\[
\mathcal{W}(L) = \left\{ \frac{1}{4} \begin{pmatrix} \sigma & -\sigma \\ -\sigma & \sigma \end{pmatrix} \bigg| \sigma \in \mathbb{R} \right\} \subseteq L,
\]

Hence, Theorem 8 applies.

However, assuming the branch lengths are related by \( \sigma_1 = 2 \sigma_2 \), then \( \sigma = 3 \sigma_2 \) and \( \delta = \sigma_2 \), and \( L \) reduces to a one-dimensional linear subspace,

\[
\bar{L} = \left\{ \frac{1}{4} \begin{pmatrix} 5 \sigma_2 & -3 \sigma_2 \\ -3 \sigma_2 & \sigma_2 \end{pmatrix} \bigg| \sigma_2 \in \mathbb{R} \right\},
\]

while \( \mathcal{W}(\bar{L}) = \mathcal{W}(L) \). Clearly,

\[
\mathcal{W}(\bar{L}) \cap \bar{L} = \left\{ \begin{pmatrix} 0 \\ 0 \end{pmatrix} \right\} \neq \mathcal{W}(\bar{L}),
\]

and the conclusion of Theorem 8 does not hold. This case might be seen as an instance of Figure 2.

Figure 2: Imagine \( \hat{V} \in \mathbb{R}^2 \), and that \( \mathcal{W} \) and \( I - \mathcal{W} \) are the projections onto the two coordinate axes, respectively. Furthermore, assume the hypothesis \( H: V \in L \) corresponds to the red line. The corresponding hypothesis for \( W \) is \( H': W \in \mathcal{W}(L) = \mathbb{R} \times \{0\} \). The LS estimate of \( V \) under \( H \) is \( \hat{V}_L \) (top red point), while the LS estimate of \( W \) under \( H' \) is \( \hat{W}_L = \hat{W} \) itself. However, this LS estimate is different from the projection of \( \hat{V}_L \) onto \( \mathcal{W}(L) \) (bottom red point). Compared to the conditions of the theorem, \( \mathcal{W}(L) \not\subseteq L \).
5 Combining information across SNPs

By combining information across SNPs, one might derive more informative about the data generating process and also derive other useful statistics. In this case, it is necessary to require some regularity across sites for reasons of comparison. We propose one such statistic, which is closely related to $\Sigma$ in the previous section, by

$$\hat{S} = \frac{1}{2} \left[ \frac{n}{2} \right] \sum_{k=1}^{[n/2]} (X^{2k} - X^{2k-1})(X^{2k} - X^{2k-1})^t,$$

assuming the number of SNPs is even (if it is odd, one might discard one SNP). Assuming the true allele frequencies are draws from a common distribution then the average allele frequency cancels out in the difference $X^{2k} - X^{2k-1}$. Thus, we are left with an expression for the variance alone, see below.

As $\hat{S}$ makes use of information from pairs of variables, it is natural to impose some regularity conditions on the parameters $(\mu_k, \Sigma^k)$, $k = 1, \ldots, n$, of the model. Perhaps the simplest approach is to embed the model into a Bayesian framework (as is often used for simulation purposes (Escalona et al. 2016)). Specifically, we assume $(\mu_k, \Sigma^k)$, $i = 1, \ldots, n$, are draws (at this point not necessarily independent) from a common distribution $F$, and the random vector $X^k$ subsequently is a draw from a distribution $G$, characterised by $(\mu_k, \Sigma^k)$,

$$\begin{align*}
(\mu_k, \Sigma^k) & \sim F \\
X^k | \mu_k, \Sigma^k & \sim G(\cdot | \mu_k, \Sigma^k).
\end{align*} \tag{8}$$

Here, we assume $F$ is a distribution concentrated on $\mathbb{R} \times P_m$, where $P_m \subseteq S_m$ is the space of real symmetric positive definite matrices with mean $(\mu_0, \Sigma_0)$, and the marginal distribution of $\mu_k$ has variance $\tau$.

Then, $X^k$ has mean,

$$E(X^k) = E(\{E(\{X^k | \mu_k, \Sigma^k\})\}) = E(\mu_k e) = \mu_0 e,$$

and covariance

$$\text{cov}(X^k) = E\left[(X^k - \mu_0 e)(X^k - \mu_0 e)^t\right] = E\left[E\left[(X^k - \mu_k e + \mu_k e - \mu_0 e)(X^k - \mu_k e + \mu_k e - \mu_0 e)^t | \mu_k, \Sigma^k\right]\right]$$

$$= E(\Sigma^k) + E((\mu_k - \mu_0)^2 E) = \Sigma_0 + \tau E,$$

where $\tau = E((\mu_k - \mu_0)^2)$. Set $\Sigma_k = \Sigma_0 + \tau E$. Since $\Sigma^k$ is assumed to be positive definite, then so is $\Sigma_0$, and hence also $\Sigma_k$. The latter follows directly from $x^t(\Sigma_0 + \tau E)x = x^t\Sigma_0 x + \tau (\sum_{i=1}^m x_i)^2 \geq 0$ (with equality if and only of $x = 0$).

Assuming $X^{2k-1}$ and $X^{2k}$ are independent, then

$$E[(X^{2k} - X^{2k-1})(X^{2k} - X^{2k-1})^t] = E[(X^{2k} - \mu_0 e + \mu_0 e - X^{2k-1})(X^{2k} - \mu_0 e + \mu_0 e - X^{2k-1})^t] = 2\Sigma_k,$$

hence $E(\hat{S}) = \Sigma_k$.

To connect to the model of Section 2, we might think of $\Sigma$ as $\Sigma_k = \Sigma_0 + \tau E$, and $\tau$ as the variance of the means across sites.

In the context of population genetics, the assumption that $X^{2k-1}$ and $X^{2k}$ are independent, is quite mild. We only ask for a pairing of the variables, $X^1, \ldots, X^n$, such that the two variables of each pair are independent, not that pairs of variables themselves are independent. One could, for example take one member of the pair from one chromosome and the other from another chromosome, assuming there are sufficient number of SNPs for such pairing. A precise condition is given here.

**Lemma 11.** Assume that each SNP with a corresponding random variable is associated to one of $C$ chromosomes, such that random variables associated to SNPs on different chromosomes are independent of each other. Let
$n_i$ be the number of SNPs associated to chromosome $i$, $i = 1, \ldots, C$. Furthermore, assume the chromosomes are ordered such that $n_1 \geq n_2 \geq \ldots \geq n_C$. If $n_1 + \cdots + n_C$ is an even number and $\sum_{i=2}^{C} n_i \geq n_1$, then the SNPs can be ordered in pairs, such that the corresponding random variables of each pair are independent.

A proof can be found in Hakemi (1962). A multi-graph (a graph potentially with multiple edges between two nodes) is constructed with $C$ nodes, representing chromosomes. Each edge between two nodes represents a pair of variables. Then there is a simple automated method for ordering the pairs: the $n_C$ variables on chromosome $C$ are linked to $n_C$ variables on chromosome 1. Then, there are $n_2, \ldots, n_{C-1}$ and $n'_1 = n_1 - n_C$ variables left on $C-1$ chromosomes. These are reordered from large to small and the pairing reiterated Hakemi (1962).

The proof of the next statement can be found in Section 7.3.

**Theorem 12.** Assume $X^1, \ldots, X^n$ are random vectors in $\mathbb{R}^m$ defined by Eq. (8), and that there exists an integer $t \geq 1$, such that the pairs $(X^{k-1}, X^{k})$ and $(X^{k-2}, X^{k})$ are independent whenever $|k - \ell| \geq t$, and that $X^{k-1}$ and $X^{k}$ are independent for $k = 1, \ldots, |n/2|$. Moreover, if there exists a constant $C > 0$, such that the forth moments of $X_i^k$, $k = 1, \ldots, n$, $i = 1, \ldots, m$, are smaller than $C$, then

$$
\mathbb{E}[\|\hat{S} - \Sigma\|_2^2] \leq 4 \sqrt{\frac{m^2 t}{n^2}} C,
$$

for all $n, m$.

This estimator has as the additional benefit that it accurately estimates the variance of $X^k$, while $\hat{V}$ only estimates it up to a constant.

### 5.1 Sampling bias

In the previous section, we did not make any specific assumptions about the random vectors $X^1, \ldots, X^n$, though it would be natural to think of them as population allele frequencies. However, typically, we do not have access to population allele frequencies, but only sample allele frequencies.

To make this specific, let $X^1, \ldots, X^n$ denote population allele frequencies and $X^{s,k} = \left( X_{i}^{s,k}, \ldots, X_{m}^{s,k} \right)$, $k = 1, \ldots, n$, be the corresponding sample allele frequencies. We will assume the sample allele counts are binomial, that is, $X_{i}^{s,k} = Z_{i}^{k}/(2N_{ik})$, where $Z_{i}^{k} \sim \text{Bi} (2N_{ik}, \pi_{ik})$, and $N_{ik}$ denotes the sample size at site $k$ in population $i$. By allowing $N_{ik}$ to vary over $k$, we allow for missing data across loci.

Define

$$
Y = \frac{1}{n} \sum_{k=1}^{n} X^k(Y)^t, \quad Y^s = \frac{1}{n} \sum_{k=1}^{n} X^{s,k}(Y)^t.
$$

Then, the three statistics $\hat{W}, \hat{D}$ and $\hat{V}$ are linear maps of $Y$, namely, $\hat{W} = \mathcal{W}(Y), \hat{D} = \mathcal{D}(Y)$ and $\hat{V} = \mathcal{V}(Y)$ (the proof is left to the reader). Conditioned on $X^k$, the variable $X^{s,k} - X^k$ has zero mean, such that

$$
\mathbb{E}[X^{s,k}(X^{s,k})^t] = \mathbb{E}[(X^{s,k} - X^k)(X^{s,k} - X^k)^t] + \mathbb{E}[X^k(Y)^t],
$$

by adding and subtracting $X^k$.

Also conditioned on $X^k$, the sample variables $X_{a}^{s,k}$ and $X_{b}^{s,k}$ are independent for $a \neq b$. Hence,

$$
\mathbb{E} \left[ (X_{a}^{s,k} - X_{a}^k) (X_{b}^{s,k} - X_{b}^k) \right] = 0 \text{ for } a \neq b. \text{ Furthermore,}
$$

$$
\mathbb{E} \left[ (X_{a}^{s,k} - X_{a}^k)^2 \right] = \frac{X_{a}^k (1 - X_{a}^k)}{2N_{ik}}.
$$
Thus, the bias correction of $Y_n^r$ is the diagonal matrix

$$\text{bias}(Y_n^r) = \frac{1}{n} \sum_{k=1}^{n} \text{diag} \left( \frac{X_n^r \left( 1 - X_n^r \right)}{8N_n^2 (n_{ik} - 1)}, \ldots, \frac{X_n^r \left( 1 - X_n^r \right)}{8N_n^2 (n_{mk} - 1)} \right)$$

(Pickrell and Pritchard 2012, text S1, supplementary material).

By the linearity of the mean (and hence the bias) the bias of $\hat{D}$, $\hat{W}$ and $\hat{V}$ are $\mathcal{O}(\text{bias}(Y))$, $\mathcal{O}(\text{bias}(Y))$, and $\mathcal{O}(\text{bias}(Y))$, respectively.

Similarly, the bias correction for $\hat{S}$ is

$$\text{bias}(\hat{S}) = \frac{1}{2 \lfloor n/2 \rfloor} \sum_{k=1}^{[n/2]} \text{diag} \left( \frac{X_1^{s,2k-1} \left( 1 - X_1^{s,2k-1} \right)}{8N_1^{2,s,2k-1} (N_{1,2k-1} - 1)}, \ldots, \frac{X_m^{s,2k-1} \left( 1 - X_m^{s,2k-1} \right)}{8N_m^{2,m,2k-1} (N_{m,2k-1} - 1)} \right).$$

### 6 Simulation results

Here we present simulation results and analyses of real data that show one may identify the position of the root in a genealogical tree from both $\hat{V}$ and $\hat{S}$ directly. This is in contrast to TreeMix that relies on an outgroup to place the root onto the tree.

For each of the scenarios below, we compute $\hat{W}$, $\hat{V}$, and $\hat{S}$, as well as run TreeMix by specifying an outgroup. To estimate the placement of the root from $\hat{V}$ and $\hat{S}$, respectively, we simply search for the partition of the $m$ populations into two groups that minimizes the average covariance between populations in different groups. The rationale for this is that the covariance $\text{Cov} \left( X_i^k, X_j^k \right)$, the $(i,j)$th entry of $\Sigma^k$, is smallest among the covariances when population $i$ and $j$ descend from opposite branches emanating from the root. The same holds for the $(i,j)$th entry of $V$ and $\Sigma_i$ (the expectation of $\hat{S}$).

#### 6.1 Two simulation scenarios

We adopt a test scenario used in Pickrell and Pritchard (2012) and originally proposed in DeGiorgio et al. (2009) to study human evolution. We consider 20 populations related by a tree as shown in Figure 3. At each split in the tree, the ‘outbranching’ ancestral population goes through a bottleneck, but population sizes are otherwise constant. We simulated two scenarios using the same commands as in Pickrell and Pritchard (2012, p4 of the supplementary information), a short branch and a long branch scenario. Specifically, we assume

- 200 Mb long genome distributed into 400 independent regions, each 500 kb long,
- 20 individuals sampled from each of the 20 populations,
- Time and parameters are scaled by the effective population size, see Hudson (1983, 2002) for details, using an effective population size of $N_e = 10,000$, and a per base per generation mutation/recombination rate of $10^{-8}$. This yields a population scaled mutation rate of $\theta = 200$, and population scaled recombination rate of $\rho = 200$ for each region,
- Splits happen at equidistant times, the $i$th population splits out from the $(i-1)$th population at time $T(21 - i), i = 2, \ldots, 20$, in the past. In the short branch scenario $T = 0.00275$; in the long branch scenario $T = 0.1375$ (50 times longer than in the short branch scenario),
- Immediately after the $i$ population has split from the $(i-1)$th population, its population size is reduced to 2.5% of its original size. The bottleneck lasts for $B$ time units before regaining its original size. In the
Figure 3: Schematic drawing of the simulation set-up. Sequential splitting at equidistant times. After each split, the one of the populations undergoes a severe bottleneck. The placement of the root can be identified from the two groups of populations descending from the two branching emanating from the root; here population 1 and populations 2–20.

short branch scenario $B = 0.00005$; in the long branch scenario $B = 0.0025$ (50 times longer than in the short branch scenario).

The simulation results in 1,225,747 SNPs in the short branch scenario, and 6,530,862 SNPs in the long branch scenario. Since we simulate a large number of SNPs, we do not bias correct.

We compute the covariance $\Sigma$ assuming the normal approximation and a fixed root frequency $x^0_k$ for SNP $k = 1, \ldots, n$, see Eq. (4). Then, the entries become

$$
\Sigma_{ij}^k = (i - 1) \left( T - B + \frac{B}{0.025} \right) x^k_0 \left( 1 - x^k_0 \right), \quad \text{for} \quad 1 \leq i < j \leq m
$$

$$
\Sigma_{ii}^k = \left[ (i - 1) \left( T - B + \frac{B}{0.025} \right) + (21 - (i + 1)T \right] x^k_0 \left( 1 - x^k_0 \right), \quad \text{for} \quad i = 1, \ldots, m.
$$

The variance $\Sigma_{ii}^k$ increases with increasing $i$. The covariance $\Sigma_{ij}^k$ is independent of $j > i$, and increases with increasing $i$. The difference between $\Sigma$ and $V$ is a constant matrix; hence the same conclusions hold for $V$.

Using population 1 as an outgroup, Treemix constructs the tree topology exactly as modeled. However, if there is not an outgroup specified or a wrong outgroup is used, then Treemix cannot return the correct tree topology. With our statistics $\hat{V}$ and $\hat{S}$, we correctly identify the split into one group consisting of population 1 and another group consisting of the remaining populations, both in the short as well as the long branch scenario, see Figures 4 and 5.

6.2 Data from the 1000 Genomes Project

We selected data from six populations from the 1000 Genomes Project (see https://www.internationalgenome.org/data-portal/data-collection/30x-grch38) that are supposedly not admixed: YRI (Yoruba in Ibadan, Nigeria; 108 individuals), LWK (Luhya in Webuye, Kenya; 99 individuals), CEU (Northern and Western European; 99 individuals), FIN (Finnish; 99 individuals), CHB (Han Chinese; 103 individuals), CDX (Dai Chinese; 93 individuals). The number of SNPs is 4,391,887; all SNPs with MAF > 5%. Since the data set contains a large number of SNPs, we do not bias correct.

Using YRI as an outgroup, TreeMix produces the tree in Figure 6. In contrast, using either $\hat{V}$ or $\hat{S}$, we identify the root to separate the clades (YRI, LWK) and (CEU, FIN, CHB, CDX), see Figure 7. Placing the root between the two clades would produce a more balanced, molecular clock-like tree.
Figure 4: Short branch scenario. Color-coding runs from dark red (small values) to dark blue (large values). The diagonal elements $\hat{V}_{ii}(\hat{S}_{ii})$ increases from population 1 to 20 due to an increasing number of bottlenecks (zero for population 1; 19 for population 20). Also, the off-diagonal elements $\hat{V}_{ij}(\hat{S}_{ij})$ are roughly constant for $j > i$. Both observations are in accordance with theoretical expectations, Eq. (9).

Figure 5: Long branch scenario. Color-coding runs from dark red (small values) to dark blue (large values).

7 Proofs

7.1 Proof of Theorem 2

Let $A$ be a symmetric $m \times m$ matrix. Let $W = \mathcal{W}(A)$. So
Figure 6: TreeMix tree of the six 1000 Genomes Project populations, forcing YRI to be the outgroup.

Figure 7: Color-coding runs from dark red (small values) to dark blue (large values). Population 1: YRI, 2: CHB, 3: CDX, 4: LWK, 5: CEU, 6: FIN. For both statistics, the entries for pairs of populations in the two different clades, (YRI, LWK) and (CEU, FIN, CHB, CDX), are smaller than any other entry.

\[
\mathcal{D}(W)_{ij} = W_{ii} + W_{jj} - 2W_{ij} = A_{ij} - \frac{2}{m} \sum_{k=1}^{m} A_{ik} + \frac{1}{m^2} \sum_{k=1}^{m} \sum_{i=1}^{m} A_{ik} + A_{jj} - \frac{2}{m} \sum_{k=1}^{m} A_{jk} + \frac{1}{m^2} \sum_{k=1}^{m} \sum_{j'=1}^{m} A_{jk'}
\]

\[
- 2 \left( A_{ij} - \frac{1}{m} \sum_{k=1}^{m} A_{ik} - \frac{1}{m} \sum_{k'=1}^{m} A_{jk} + \frac{1}{m^2} \sum_{k=1}^{m} \sum_{j'=1}^{m} A_{jk'} \right) = A_{ii} + A_{jj} - 2A_{ij} = \mathcal{D}(A)_{ij}.
\]

It follows that \( \mathcal{D} = \mathcal{D} \circ W' \).
Let $D = \mathcal{D}(A)$. Then

$$-\frac{1}{2} \mathcal{W}(D)_{ij} = -\frac{1}{2} D_{ij} + \frac{1}{2m} \sum_{k=1}^{m} D_{ik} + \frac{1}{2m} \sum_{k=1}^{m} D_{jk} - \frac{1}{2m^2} \sum_{k=1}^{m} \sum_{\ell=1}^{m} D_{k\ell}$$

$$= -\frac{1}{2} (A_{ii} + A_{jj} - 2A_{ij}) + \frac{1}{2m} \sum_{k=1}^{m} (A_{ik} + A_{kj} - 2A_{ik})$$

$$+ \frac{1}{2m^2} \sum_{k=1}^{m} \sum_{\ell=1}^{m} (A_{ik} + A_{\ell k} - 2A_{ik})$$

$$= A_{ij} + \frac{1}{2m} \sum_{k=1}^{m} (A_{ik} - 2A_{ik}) + \frac{1}{2m^2} \sum_{k=1}^{m} \sum_{\ell=1}^{m} (A_{ik} + A_{\ell k} - 2A_{ik})$$

$$= A_{ij} - \frac{1}{m} \sum_{k=1}^{m} A_{ik} - \frac{1}{m} \sum_{k=1}^{m} A_{jk} + \frac{1}{m^2} \sum_{k=1}^{m} \sum_{\ell=1}^{m} A_{k\ell} = \mathcal{W}(A)_{ij}.$$
That is, \( F = EF / m + FE / m \). Note that

\[
(EF)_{ij} = \sum_{k=1}^{m} F_{kj}
\]

does not depend on \( i \). So there is a vector \( x \in \mathbb{R}^m \), so that

\[
EF = \begin{pmatrix} x' \\ \vdots \\ x' \end{pmatrix}.
\]

And we have \( FE = (EF)' = (x \ldots x) \).

\[
F = \frac{1}{m} \begin{pmatrix} x' \\ \vdots \\ x' \end{pmatrix} + \frac{1}{m} (x \ldots x) = ex' / m + xe' / m.
\]

It follows that \( \ker(\mathcal{W}) = \{ ev' + ve': v \in \mathbb{R}^m \} \). The kernel has dimension \( m \). Since \( \dim(\mathcal{S}_m) = m(m + 1) / 2 \), it follows by the rank-nullity theorem that \( \dim(\im(\mathcal{W})) = m(m - 1) / 2 \).

It follows from \( (-\frac{1}{2} \mathcal{D}) \circ (-\frac{1}{2} \mathcal{D}) = -\frac{1}{2} \mathcal{D} \) and \( \mathcal{W} \circ \mathcal{W} = \mathcal{W} \) that \( -\frac{1}{2} \mathcal{D} \) and \( \mathcal{W} \) are projections.

Next we demonstrate that \( \mathcal{W} \) is an orthogonal projection by showing that the image space of \( \mathcal{W} \) is orthogonal to the kernel of \( \mathcal{W} \). Let \( B \) be a symmetric \( m \times m \)-matrix. Then \( B \) is orthogonal to the kernel if and only if for all \( v \in \mathbb{R}^m \),

\[
0 = \langle B, ev' + ve' \rangle = \sum_{i=1}^{m} \sum_{j=1}^{m} B_{ij}(v_i + v_j) = \sum_{i=1}^{m} v_i \sum_{j=1}^{m} B_{ij} + \sum_{j=1}^{m} v_j \sum_{i=1}^{m} B_{ij} = 2 \sum_{j=1}^{m} v_j \sum_{i=1}^{m} B_{ij}.
\]

Note that \( \{ e_i e' + e'e_i: 1 \leq i \leq m \} \) is a basis for the kernel (where \( e_i \) is the \( i \)-th unit vector), and

\[
\langle B, e_i e' + e'e_i \rangle = 2 \sum_{j=1}^{m} B_{ij}, \quad i = 1, \ldots, m.
\]

Thus \( B \) is orthogonal to \( \ker(\mathcal{W}) \) if and only if all rows of \( B \) sum to zero.

Denote \( W = \mathcal{W}(A) \). Note that

\[
\sum_{j=1}^{m} W_{ij} = \sum_{j=1}^{m} A_{ij} - \sum_{k=1}^{m} A_{ik} - \frac{1}{m} \sum_{j=1}^{m} \sum_{k=1}^{m} A_{jk} + \frac{1}{m} \sum_{k=1}^{m} \sum_{\ell=1}^{m} A_{k\ell} = 0,
\]

for all \( i \in \{1, \ldots, m\} \). It follows that \( \mathcal{W} \) is an orthogonal projection. Consequently, the operator norm is one.

From the fact that \( -\frac{1}{2} \mathcal{D} \) and \( \mathcal{W} \) have the same kernel, and \( \im(\mathcal{W}) \neq \im(\mathcal{D}) \) (elements of \( \im(\mathcal{D}) \) has zero diagonal), it follows from unicity of orthogonal projections that \( -\frac{1}{2} \mathcal{D} \) cannot be an orthogonal projection.

Finally, we derive bounds on the operator norm of \( -\frac{1}{2} \mathcal{D} \). Consider the normalized identity matrix \( \Delta = \frac{1}{\sqrt{m}} I \) with \( \| \Delta \|_F = 1 \). Note that \( \Delta' = \mathcal{D}(\Delta) \) has \( \Delta'_{ii} = 0 \) and \( \Delta'_{ij} = \frac{2}{\sqrt{m}} \) for \( i \neq j \). Hence, \( \| \Delta' \|_F = 2 \sqrt{m - 1} \leq \| \mathcal{D} \|_{\text{op}} \) gives the lower bound. For the upper bound, we write a symmetric matrix as \( A = B + \Lambda \), where \( B \) has all diagonal elements zero, \( \Lambda \) is a diagonal matrix, and \( \| A \|_F \leq 1 \). Then

\[
-\frac{1}{2} \mathcal{D}(A) = -\frac{1}{2} \mathcal{D}(B + \Lambda) = B + \Lambda',
\]

where \( \mathcal{D}(\Lambda) = \Lambda' \) is the matrix with entries \( \Lambda'_{ij} = -\frac{1}{2}(\Lambda_{ii} + \Lambda_{jj}), i \neq j \), and zero otherwise. Then,
\[ ||\Lambda' ||_F^2 = \frac{1}{2} \sum_{i<j} (\Lambda_{ii} + \Lambda_{jj})^2 = \frac{1}{2} \sum_{i<j} \Lambda_{ii}^2 + \Lambda_{jj}^2 + 2\Lambda_{ii}\Lambda_{jj} = \frac{1}{2}(m-1) \sum_{i=1}^{m} \Lambda_{ii}^2 + \frac{1}{2} \sum_{i=1}^{m} \sum_{j=1}^{m} \Lambda_{ii}\Lambda_{jj} - \frac{1}{2} \sum_{i=1}^{m} \Lambda_{ii}^2 \]

\[ = \frac{1}{2}(m-2) \sum_{i=1}^{m} \Lambda_{ii}^2 + \frac{1}{2} \left( \sum_{i=1}^{m} \Lambda_{ii} \right)^2 \leq \frac{1}{2}(m-2) + \frac{1}{2} \left( \frac{m}{\sqrt{m}} \right)^2 = m - 1, \]

assuming \( ||A||^2 \geq 1 \), hence \( ||\Lambda' ||_F^2 \leq ||A||_F^2 \leq 1 \) and \( \sum_{i=1}^{m} \Lambda_{ii} \leq \frac{m}{\sqrt{m}} \). Consequently,

\[ || - \frac{1}{2} \mathcal{D}(A)||_F \leq ||B||_F + ||\Lambda'||_F \leq ||A||_F + ||\Lambda'||_F \leq 1 + \sqrt{m - 1}. \]

### 7.2 Proof of Theorem 7

Define \( \hat{V}^k = X^k(X^k)' - \hat{\mu}^2F \) with entries \( \hat{V}_{ab}^k, a, b = 1, \ldots, m \), then \( \hat{V} = \frac{1}{n} \sum_{k=1}^{n} \hat{V}^k \).

Trivially for \( x_1, x_2, x_3, x_4 \in \mathbb{R} \), \( |x_1x_2x_3x_4| \leq x_1^2 + x_2^2 + x_3^2 + x_4^2 \). So we have for

\[ \hat{V}_{ab}^k = X_{a}^kX_{b}^k - \frac{1}{m^2} \sum_{c=1}^{m} \sum_{d=1}^{m} X_{c}^kX_{d}^k \]

that

\[ \mathbb{E}\left( \left( \hat{V}_{ab}^k \right)^2 \right) \leq \mathbb{E}(X_{a}^k)^2(X_{b}^k)^2 + \frac{1}{m^4} \left( \sum_{c=1}^{m} \sum_{d=1}^{m} X_{c}^kX_{d}^k \right)^2 + \frac{2}{m^2} \mathbb{E}\left( X_{a}^kX_{b}^k \sum_{c=1}^{m} \sum_{d=1}^{m} X_{c}^kX_{d}^k \right) \]

\[ \leq 4C + 4C + 8C = 16C, \]

where it is assumed that all moments of \( X_{a}^k \) up to order four are bounded uniformly in \( k = 1, \ldots, n \) and \( a = 1, \ldots, m \), by some number \( C > 0 \). Hence,

\[ \var\left( \hat{V}_{ab}^k \right) \leq \mathbb{E}\left( \left( \hat{V}_{ab}^k \right)^2 \right) \leq 16C. \]

As \( \hat{V}^k, \hat{V}^{k+1}, \ldots, \hat{V}^{k+[\frac{n-k}{t}]/t} \) are independent, for \( k = 1, \ldots, t \), we have

\[ \var\left( \frac{1}{n} \left( \hat{V}_{ab}^k + \hat{V}_{[k+1]ab} + \cdots + \hat{V}_{[k+[\frac{n-k}{t}]/t]ab} \right) \right) \leq \frac{[n - k]/t}{n^2} 16C \leq \frac{16C}{nt}. \]

Applying Corollary 15 gives

\[ \var(\hat{V}_{ab}) \leq \frac{16t^2C}{nt} = \frac{16tC}{n}. \]

It follows that

\[ \mathbb{E}\left[ \left\| \hat{V} - V \right\|_F^2 \right] = \sum_{a=1}^{m} \sum_{b=1}^{m} \var(\hat{V}_{ab}) \leq \frac{16m^2tC}{n}. \]

Consequently, by Jensen’s inequality the claim follows: \( \mathbb{E}(||\hat{V} - V||_F^2) \leq \mathbb{E}\left( \left\| \hat{V} - V \right\|_F^2 \right) \).

It follows from Theorem 6 in combination with the definition of \( \mathcal{D} \), that \( \hat{D}_{ij} = \hat{V}_{ii} + \hat{V}_{jj} - 2\hat{V}_{ij} \). Similarly, using Corollary 15 again and the definition of \( \mathcal{D} \), gives \( \var(\hat{D}_{ij}) \leq \frac{256C}{n} \). Using \( \mathbb{E}(\hat{D}) = D \), the claim for \( \mathbb{E}(\|\hat{D} - D\|_F) \) follows similarly to that for \( \hat{V} \).

Finally, from Theorem 6,

\[ \hat{W}_{ij} = \hat{V}_{ij} - \frac{1}{m} \sum_{a=1}^{m} \hat{V}_{ia} - \frac{1}{m} \sum_{a=1}^{m} \hat{V}_{ja} + \frac{1}{m} \sum_{a=1}^{m} \sum_{b=1}^{m} \hat{V}_{ab}. \]
Applying Corollary 15 gives
\[
\text{var}(\hat{W}_{ij}) \leq 4 \left( \text{var}(\hat{V}_{ij}) + \text{var}\left(\frac{1}{m} \sum_{a=1}^{m} \hat{V}_{ja}\right) + \text{var}\left(\frac{1}{m} \sum_{a=1}^{m} \hat{V}_{ja}\right) + \text{var}\left(\frac{1}{m^2} \sum_{a=1}^{m} \sum_{b=1}^{m} \hat{V}_{ab}\right) \right) \leq \frac{256t}{n} C.
\]

Again, in a similar way to that of \(\hat{V}\), the claim for \(\mathbb{E}(\|\hat{W} - W\|)\) follows.

### 7.3 Proof of Theorem 12

Define \(\hat{S}_k = (X^{2k} - X^{2k-1})(X^{2k} - X^{2k-1})^t\). Then we have
\[
\text{var}\left(\hat{S}_{ab}^k\right) \leq \mathbb{E}\left[\left(\hat{X}_a^{2k} - \hat{X}_a^{2k-1}\right)^2 \left(\hat{X}_b^{2k} - \hat{X}_b^{2k-1}\right)^2\right]
\]
\[
= \mathbb{E}\left[\left(\hat{X}_a^{2k} \hat{X}_b^{2k} - \hat{X}_a^{2k} \hat{X}_b^{2k-1} - \hat{X}_a^{2k-1} \hat{X}_b^{2k} + \hat{X}_a^{2k-1} \hat{X}_b^{2k-1}\right)^2\right].
\]

The latter can be written as a sum of 16 elements \(\mathbb{E}\left[\hat{X}_c^{2k} \hat{X}_d^{2k} \hat{X}_e^{2k} \hat{X}_f^{2k}\right]\), where \(c, d, e, f \in \{2k - 1, 2k\}\). Applying the Jensen’s inequality and then Hölder’s inequality twice gives
\[
\mathbb{E}\left[\left|\hat{X}_c^{2k} \hat{X}_d^{2k} \hat{X}_e^{2k} \hat{X}_f^{2k}\right|\right] \leq \sqrt{\mathbb{E}\left[\hat{X}_c^{2k}\right]^4 \mathbb{E}\left[\hat{X}_d^{2k}\right]^4 \mathbb{E}\left[\hat{X}_e^{2k}\right]^4 \mathbb{E}\left[\hat{X}_f^{2k}\right]^4} \leq \sqrt{C^4} = C.
\]

It follows that \(\text{var}(\hat{S}_{ab}^k) \leq 16C\).

So \(\hat{S} = \frac{1}{[n/2]} \sum_{k=1}^{[n/2]} \hat{S}_k\). As \(\hat{S}_1, \hat{S}_2, \ldots, \hat{S}_{[n/2]}\) are independent, for \(k = 1, \ldots, t\), we have
\[
\text{var}\left(\frac{1}{[n/2]} \left(\hat{S}_{ab}^k + \hat{S}_{ab}^{k+t} + \cdots + \hat{S}_{ab}^{[([n/2]-k)/t]}\right)\right)
\]
\[
\leq \frac{([([n/2]-k)/t]}{[n/2]^2} 16C \leq \frac{16C}{[n/2]^t}.
\]

Applying Corollary 15 gives
\[
\text{var}(\hat{S}_{ab}) = \text{var}\left(\frac{1}{[n/2]} \left(\hat{S}_{ab}^k + \hat{S}_{ab}^{k+t} + \cdots + \hat{S}_{ab}^{[([n/2]-k)/t]}\right)\right)
\]
\[
\leq \frac{t}{[n/2]} \text{var}\left(\frac{1}{[n/2]} \left(\hat{S}_{ab}^k + \hat{S}_{ab}^{k+t} + \cdots + \hat{S}_{ab}^{[([n/2]-k)/t]}\right)\right) \leq t \frac{16C}{[n/2]^t} = \frac{16C}{[n/2]^t}.
\]

It follows that
\[
\mathbb{E}\left[\left\|\hat{S} - \Sigma\right\|^2\right] = \sum_{a=1}^{m} \sum_{b=1}^{m} \text{var}(\Sigma_{ab}) \leq \frac{16m^2t}{[n/2]} C,
\]
and by Jensen’s inequality that
\[
\mathbb{E}\left[\left\|\hat{S} - \Sigma\right\|^2\right] \leq \sqrt{\mathbb{E}\left[\left\|\hat{S} - \Sigma\right\|^4\right]} \leq 4 \sqrt{\frac{m^2t}{[n/2]} C}.
\]
8 Auxiliary results

Lemma 13. Let $x, y \in \mathbb{R}$. Then $|xy| \leq \frac{1}{2}(x^2 + y^2)$.

Proof. It follows from $0 \leq (x - y)^2 = x^2 + y^2 - 2xy$ and $0 \leq (x + y)^2 = x^2 + y^2 + 2xy$ that $|2xy| \leq x^2 + y^2$.

Lemma 14. Let $x_1, \ldots, x_m \in \mathbb{R}$. Then $\left(\sum_{i=1}^{m} x_i\right)^2 \leq m \sum_{i=1}^{m} x_i^2$.

Proof. From Lemma 13, $\left(\sum_{i=1}^{m} x_i\right)^2 = \sum_{i=1}^{m} \sum_{j=1}^{m} x_i x_j \leq \frac{1}{2} \sum_{i=1}^{m} \sum_{j=1}^{m} \left(x_i^2 + x_j^2\right) = m \sum_{i=1}^{m} x_i^2$.

Corollary 15. Let $X_1, \ldots, X_m$ be random variables. Then, $\text{Var} \left(\sum_{k=1}^{m} X_k\right) \leq m \sum_{k=1}^{m} \text{Var}(X_k)$.

Proof. Take $x_k = X_k - \mathbb{E}[X_k]$ and expectation in Lemma 14.

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