An empirical approach to demographic inference

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Abstract

Inference with population genetic data usually treats the population pedigree as a nuisance parameter, the unobserved product of a past history of random mating. However, the history of genetic relationships in a given population is a fixed, unobserved object, and so an alternative approach is to treat this network of relationships as a complex object we wish to learn about, by observing how genomes have been noisily passed down through it. This paper explores this point of view, showing how to translate questions about population genetic data into calculations with a Poisson process of mutations on all ancestral genomes. This method is applied to give a robust interpretation to the $f_4$ statistic used to identify admixture, and to design a new statistic that measures covariances in mean times to most recent common ancestor between two pairs of sequences. The method more generally interprets population genetic statistics in terms of sums of specific functions over ancestral genomes, thereby providing concrete, broadly interpretable interpretations for these statistics. This provides a method for describing demographic history without simplified demographic models. More generally, it brings into focus the population pedigree, which is averaged over in model-based demographic inference.

Introduction

For most of the history of the field, geneticists made great progress with data that comprise a tiny fraction of all that is in the genome. Even the hundreds of thousands of markers on modern genotyping chips make certain inferences impossible due to ascertainment of these markers. Today, whole-genome sequence is being used to make striking new discoveries about health and human history [Haak et al., 2015]; such data will soon be commonplace as well in nonmodel organisms. The scale and unbiased nature of these data opens up new opportunities, but will also require new methods, as many assumptions made previously may no longer be necessary.

To do this, it is helpful to begin with the process that generated these data. The rules by which the majority of genetic material is inherited by diploid, sexually reproducing organisms are simple: each new individual carries two distinct copies of the genome – one from mom, one from dad – and each of these copies is formed by recombining randomly chosen parts of the parent’s two copies so as to make a whole. The inherited copies may be modified by mutation, which provides the raw material not only for evolution, but also the means for us to learn about those genomes’ histories.

The network describing parent-offspring relationships between past and present members of a population is known as the population pedigree; adding an encoding of the genetic relationships – which parts of each genome was inherited from which copy of the parental genomes – produces the ancestral recombination graph [ARG, described by Griffiths and Marjoram, 1997]. Supposing that any particular position in one of those copies of the genome was inherited from a single one of the two ancestral copies – for the most part true – then at each point on the genome these genetic relationships form a tree (known as the gene tree) that is embedded within the larger pedigree. Just as the population pedigree can be thought of as constraining the collection of gene trees, so the ancestral recombination graph constrains the patterns of concordance and discordance of genetic variants created by mutation within each population.

Inference with population genetic data usually works by specifying a population model – e.g., a randomly mating population of fixed size $N$ – that determines a probability distribution on the population pedigree,
then assuming that mutation and recombination occurs independently within this [Ewens, 2004]. This paper
deals with the intermediate layer – if we take the population pedigree, or even the ancestral recombination
graph, as fixed, then what can we learn about it? What are the statistics of population genetics telling us
about it? This is partly a matter of philosophy: should the population pedigree that has actually occurred
be treated as an instantiation of a random process, with the goal of inferring parameters of that process?
Or, is it better to treat the population pedigree as a nonrandom but complex and partially observed object
that we wish to estimate summary statistics of? The former view may be most appropriate if we have one or
several well-defined demographic models we believe are close to the truth, but the latter is useful for making
robust inferences in the absence of any realistic demographic model, as well as for identifying whether we
have power to distinguish such models.

The approach of this paper is simple: Assuming that mutations occur independently of demography and
recombination (and are hence neutral), these can be treated as an inhomogeneous Poisson process on the set
of all ancestral genomes. Many population genetics statistics can be written as integrals of specific functions
against this Poisson process. By treating the ancestral recombination graph as fixed, the means and variances
of these statistics can be found in terms of integrals of the same functions against the mutation rate, and can
therefore be interpreted as descriptive statistics of the unknown, but real, ancestral recombination graph.
Stochastic models of population genetics generally include demography, recombination, and mutation: this
paper takes the outcomes of the first two as fixed, dealing only with the randomness of mutation. More
information is obtainable by also treating recombination as random, but it is useful to begin with only
mutation, especially as most commonly used population genetics statistics do not model recombination (e.g.,
the site frequency spectrum). The addition of recombination will be described in a companion paper.

The remainder of this paper is structured as follows. After a review of some of the literature, two main
results are presented in non-technical terms, given for their inherent interest as well as examples of the
general method of calculation that is presented in the subsequent section. Next come proofs of the main
results, and some more material, followed by some more discussion of the results and future work.

Background

Perhaps the main point of this paper is to see what can be learned about the population pedigree, or
more properly the ARG, by taking an empirical point of view, i.e. treating it as a fixed, but unknown
object. Slatkin [1991] interprets probabilities of identity by descent and $F_{ST}$ in terms of genealogies; the
discussion is framed in terms of randomly mating populations, but much of the discussion is applicable
without thinking of the genealogies as random. McVean [2002, 2009] continued in this vein, interpreting two
common population genetics statistics, linkage disequilibrium and the principal components decomposition,
in terms of the realized genealogy of the population. Wakeley et al. [2012] begins from a similar philosophical
point, that the pedigree should be treated as a fixed parameter, and goes on to show that data generated
under a fixed pedigree differ very little from those generated under a standard coalescent model (which does
not incorporate correlations due to a fixed pedigree).

Formal methods describing expectations of gene transmission in a known pedigree have long been used
by plant and animal breeders. In particular, Wright [1922]’s method of “path coefficients” treated the
pedigree was known and averaged over recombination and segregation. These methods are related to a
parallel issue arising in genetic association studies [reviewed by Speed and Balding, 2014] where the pedigree
is a confounding factor, and alternative approaches can be seen as estimating the actual degree of genetic
relatedness or as averaging over it.

The empirical point of view for genealogies is much more natural in the context of phylogenetics, where
between most groups of species, all loci have nearly the same genealogical relationship, and the goal is
to directly infer this common tree. For instance, Gillespie and Langley [1979] pointed out that variation
in coalescence time contributed to variance in between-species divergence, and Edwards and Beerli [2000]
further partition the variance in divergence into components induced by the substitution process and by
the demographic process. Pluzhnikov and Donnelly [1996] also described, in a model of random mating, the
variance of Watterson’s and Tajima’s estimates of $\theta$ expected along the genome as a function of recombination
rate. Similarly, studies of nonrecombining loci often treat the demography as an unknown parameter. For
instance, Tang et al. [2002] took the frequentist approach to estimating time to most recent common ancestor (TMRCA) for modern human Y-chromosomes. Hudson [2007] continued this work, finding the variance of Thomson et al. [2000]’s TMRCA estimator (which is based on the density of segregating derived mutations).

Early examples of using whole-genome data to estimate an empirical quantity of the population pedigree were the inferences of gene flow between diverged species by Kulathinal et al. [2009] (between Drosophila species), and by Green et al. [2010] (Neanderthal to modern humans). A wider family of similar statistics were subsequently developed in the human population genetics literature [e.g., Durand et al., 2011, Patterson et al., 2012], and have formed the basis for many new insights into human history and evolution. However, their behavior under complex, non-deme-based models of population structure is thus far unclear. Understanding these in a more general context was a major impetus for the present work.

Main results

The purpose of this paper is to outline a general method of calculation, the utility of which is best communicated with examples. First, a few assumptions. I assume that at each site there are no more than two variants seen (the proportion of triallelic sites within populations is extremely small, but see Jenkins and Song [2011]); and that at each site a reference allele has been chosen; “allele frequencies” are frequencies of variants seen (the proportion of triallelic sites within populations is extremely small, but see Jenkins and Song [2011]); and that at each site a reference allele has been chosen; “allele frequencies” are frequencies of the other, alternate allele. However, all statistics developed here are invariant under relabeling of alleles.

I also make the standing assumption of low per base pair mutation rate – precisely, the infinite sites model of mutation [Watterson, 1975], under which each new mutation occurs at a distinct site, and is hence observable. This assumption is quite common in population genetics, where the typical time separating samples is small enough to make this assumption a good one (but I return to this below).

As a first example, consider the \( f_4 \) statistic [Patterson et al., 2012]: if \( X, Y, U, \) and \( V \) are four sets of sampled genomes, and \( p_X(\ell) \) is the allele frequency among the samples in \( X \) at site \( \ell \), etcetera, then the \( f_4 \) statistic of these samples is

\[
f_4(X, Y; U, V) = \frac{1}{G} \sum_{\ell=1}^{G} (p_X(\ell) - p_Y(\ell))(p_U(\ell) - p_V(\ell)) \tag{1}
\]

where the sum is over some set of \( G \) sites. Notice that \( f_4(X, Y; U, V) = -f_4(Y, X; U, V) \). This implies that if the relationship of \( X \) and \( Y \) to \( U \) and \( V \) is symmetric, then the expected value of the statistic is zero. In particular, it is zero under a tree-based model of populations, in which \( X \) and \( Y \) diverged from a common ancestral population more recently than their common ancestors with \( U \) or \( V \), and there was no subsequent gene flow from \( U \) or \( V \). Green et al. [2010] and Reich et al. [2010] used these statistics to provide evidence for gene flow from archaic hominids into modern humans. For example, taking \( X \) to be a sample of subsaharan Africans, \( Y \) a sample of western Europeans, \( U \) a Neanderthal, and \( V \) a chimpanzee, a significantly negative value implies that European allele frequencies are more correlated with Neanderthal than expected under a model of no admixture; and given archaeological evidence, this supports interbreeding between Neanderthals and modern humans after leaving Africa. However, it is not at first clear how the statistic should perform under more realistic population histories (e.g. asymmetric gene flow between subsaharan Africa and Mediterreanean populations). The results below provide a model-free interpretation:

**Theorem 1** (\( f_4 \) statistic). Let \( X, Y, U, \) and \( V \) be collections of \( n_X, n_Y, n_U, \) and \( n_V \) chromosomes, respectively, that have been genotyped at \( G \) sites. (I) For each position \( \ell \) in the genome and each ancestral individual’s chromosome, \( a \), let \( F_X(a, \ell) \) denote the proportion of the samples in \( X \) that have inherited from ancestor \( a \) at position \( \ell \), and similarly for \( F_Y, F_U, \) and \( F_V \). Then

\[
\mathbb{E}[f_4(X, Y; U, V)] = \frac{1}{G} \sum_{\ell=1}^{G} \sum_{a} \mu(\ell)(F_X(a, \ell) - F_Y(a, \ell))(F_U(a, \ell) - F_V(a, \ell)), \tag{2}
\]

where \( \mu(\ell) \) is the per-generation mutation rate at site \( \ell \), and the sum over \( a \) is the sum over all ancestral chromosomes. (II) Equivalently, for any set of samples \( (x, y, u, v) \), let \( S_{(x,u);(y,v)}(\ell) \) denote the length of the
branch between the most recent common ancestor of \(x\) and \(u\) and the most recent common ancestor of \(y\) and \(v\) (which may be zero). Then

\[
\mathbb{E}[f_4(X,Y;U,V)] = \frac{1}{n_Xn_Yn_Un_V} \sum_{x,y,u,v} \frac{1}{G} \sum_{\ell=1}^G \mu(\ell)(S_{(x,u):(y,v)}(\ell) - S_{(x,v):(y,u)}(\ell)),
\]

where the sum is over all choices of \(x \in X\), \(y \in Y\), \(u \in U\), and \(v \in V\). Furthermore,

\[
\text{var}[f_4(X,Y;U,V)] \leq \frac{1}{n_Xn_Yn_Un_V} \sum_{x,y,u,v} \frac{1}{G^2} \sum_{\ell=1}^G \mu(\ell)(S_{(x,u):(y,v)}(\ell) + S_{(x,v):(y,u)}(\ell)).
\]
consider relationships on only a single chromosome, and let \( A \) denote the collection of all copies of this chromosome in any individual alive at any point back to some arbitrarily distant time. The population pedigree, denoted \( P \), is the directed acyclic graph with vertex set \( A \) that has an edge \( a \rightarrow b \) if \( a \) is a parent of \( b \), so that each vertex in \( A \) has in-degree equal to two. (Although this is a different object than the pedigree relating the diploid individuals, rather than their constituent chromosomes, the two are simple transformations of each other.) Under the standing assumption of the infinite sites model, no two mutations fall in the same location, and so locations on the chromosome are indexed by the continuous interval \( G = [0, G] \), where \( G \) is the total length of the chromosome, in nucleotides. At each site in \( G \) all ancestral chromosomes are related by a tree, embedded in the population pedigree, and this collection of trees is equivalent to the ARG.

Mutation Under the infinite sites model, the mutation process is Poisson: concretely, the sites at which ancestral chromosome \( a \in A \) differs from their parent (i.e., has a mutation) are distributed as the points of an inhomogeneous Poisson process on \( G \). This Poisson process of mutations has intensity measure given by the function \( \mu \), where \( \mu(\ell) \) is the mutation rate at \( \ell \). To study the inheritance of mutations through the pedigree, these are next collected into a Poisson process on all ancestral chromosomes: formally, \( M \) is the point measure on \( A \times G \) formed by putting one point mass at each mutation. Equivalently, \( M \) counts mutations: for any region of the chromosome \([i, j] \subset G \) and any set of ancestral chromosomes \( A \subset A \), the value \( M(A \times [i, j]) \) is the total number of mutations that occurred in the region \([i, j] \) on any of the chromosomes in \( A \).

Under the assumption that mutation occurs independently of the pedigree, the expected number of mutations occurring in any collection of pieces of ancestral chromosomes is simply the sum of \( \mu(\ell) \) across these pieces. Formally, \( M \)'s mean measure has density \( \mu \) with respect to \( n \otimes d\ell \), where \( n \) is counting measure on \( A \) and \( d\ell \) is Lebesgue measure on the chromosome. Below, I will abuse notation slightly and write \( d\mu \) for this measure, so that for any (measurable) subset of ancestral genomes \( S \subset A \times G \),

\[
\mathbb{E}[M(S)] = \int_S d\mu(a, \ell). \tag{8}
\]

The key simplifying assumption of the infinite sites model is that every mutation is observable: if two ancestral chromosomes \( a \) and \( b \) have both inherited a region of genome \([i, j] \) from their most recent common ancestor on this region, then given the genomes of \( a \) and \( b \) on this segment, we also know the number of mutations that have occurred in this segment in any of the ancestors going back to the common ancestor.

Additive statistics The formulation of mutations as a point measure on ancestral genomes is designed to make a certain set of statistics easy to formulate and analyze, namely, those that can be formed using integrals of test functions against the mutation process. Recall that since \( M \) is a point measure on the set of all ancestral genomes, \( A \times G \), with unit mass at each ancestral mutation, then if \( \chi \) is a function on \( A \times G \), the integral of \( \chi \) against \( M \) is the sum of the values of \( \chi \) at the locations of all mutations: defining \( \{m_{a,k} : 1 \leq k \leq n_a\} \) to be the locations of the mutations that occurred on ancestral chromosome \( a \),

\[
\int \chi(a, \ell)dM(a, \ell) = \sum_{a \in A} \sum_{k=1}^{n_a} \chi(a, m_{a,k}). \tag{9}
\]

It is easier to write this as simply \( \int \chi dM \). Here is a simple example (for which all this notation is unnecessary):

Example 1 (Number of mutations). Given a sample of chromosomes \( A \subset A \) let \( \zeta_A(a, \ell) = 1 \) if \( a \in A \) and \( \zeta_A(a, \ell) = 0 \) otherwise. Then \( \int \zeta_A dM \) counts the number of mutations appearing de novo in the sampled chromosomes.

This is a simple prototype for what we would like to do more generally. But, notice that \( \int \zeta_A dM \) is unobservable given only the genomes in \( A \) – to identify new mutations, we need the parental genomes as well. Statistics of this form work by first, based on the samples, constructing a function \( \chi \) on ancestral genomes, and then adding up the value that \( \chi \) takes at the location of each mutation on any ancestral
Figure 1: A cartoon of values taken by the path between (left) two sampled chromosomes \( x \) and \( y \), and (right) two groups of samples, \( X = \{x_1, x_2\} \), and \( Y = \{y_1, y_2, y_3\} \). The path between two single chromosomes is, marginally at each locus, the indicator of the path from each back to their common ancestor; but between larger groups the values it takes depends on the local topology of the tree relating the samples.

genome. To be observable, such statistics will need to satisfy certain conditions: in particular, \( \chi \) must be zero for any piece of ancestral genome not inherited by any sampled genome, or inherited by all of them.

This idea of observability under the infinite sites model is encoded by the following set of special functions:

**Definition 1 (Paths).** (I) For any two chromosomes \( x \) and \( y \) in \( \mathcal{A} \), the path from \( x \) to \( y \) is the function

\[
[x \leftrightarrow y](a, \ell) = \begin{cases} 
1 & \text{if } x \text{ or } y, \text{ but not both, inherits from } a \text{ at } \ell \\
0 & \text{otherwise.}
\end{cases}
\]  

(II) For any two collections of chromosomes \( X \) and \( Y \), having \( |X| \) and \( |Y| \) elements each, the path from \( X \) to \( Y \) is the function

\[
[X \leftrightarrow Y](a, \ell) = \frac{1}{C(X, Y)} \sum_{x,y} [x \leftrightarrow y](a, \ell),
\]

where the sum is over distinct pairs \( x \in X \) and \( y \in Y \) with \( x \neq y \), and \( C(X, Y) \) is the number of such pairs. These are depicted in figure 1.

In other words, the path \([x \leftrightarrow y]\) is the indicator function of the pieces of ancestral chromosomes that are inherited by \( x \) or \( y \), but not both. At each locus \( \ell \), the function \([x \leftrightarrow y](\cdot, \ell)\) on \( \mathcal{A} \) is the indicator function of the tree connecting \( x \) and \( y \) at \( \ell \) (but notably excluding the root). \([X \leftrightarrow Y]\) is the average of these indicators, which gives the proportion of paths between pairs of samples from the two groups that pass through each ancestor in the pedigree.

**Example 2 (Sequence divergence).** The sequence divergence between two chromosomes \( x \) and \( y \), denoted \( \pi(x,y) \), is the mean density of distinguishing mutations; it is

\[
\pi(x,y) = \frac{1}{G} \int [x \leftrightarrow y]dM.
\]
For a group of samples \( X \) we define the average pairwise divergence similarly:

\[
\pi(X) = \frac{1}{|X|(|X|-1)} \sum_{x_1 \neq x_2 \in X} \pi(x_1, x_2)
\]

\[
= \frac{1}{G} \int (|X \leftrightarrow X|) \, dM.
\]  

Tajima [1983] proposed \( \pi(X) \) as an estimator for \( \theta = 4N_e \mu \), under the model of a large, randomly mating population of diploids with constant mutation rate \( \mu \) and effective population size \( N_e \). Indeed, \( \mathbb{E}[\pi(X)] = \theta \), but the variance of \( \pi(X) \) for a nonrecombining locus does not go to zero as the number of samples grows, and so this is regarded as a poor estimator of \( \theta \). However, it is clear that the variance does go to zero if calculated with the genealogy fixed, i.e., thought of as an estimator of the mean time to most recent common ancestor (multiplied by twice the mutation rate). Denoting by \( \overline{\pi t} \) the empirical mean mutation-rate-scaled time to most recent common ancestor, the variance can be partitioned (as in Edwards and Beerli [2000]):

\[
\text{var}[\pi(X)] = \mathbb{E}[\text{var}[\pi(X) \mid \overline{\pi t}]] + \text{var}[\mathbb{E}[\pi(X) \mid \overline{\pi t}]].
\]

The first term here goes to zero as the size of the sample increases, but the second, due to randomness in the demographic process, does not. The question of whether \( \pi(X) \) is a good estimator, and for what, becomes a question of whether we want to think of it as estimating a summary statistic of actual relationships or a parameter in a certain stochastic model of demography.

**Observable statistics** For application to data, it only makes sense to consider statistics that can be obtained by comparing the genomes of a given set of chromosomes. As discussed above, under the infinite sites model this means that the genome sequence of a pair of samples \( x \) and \( y \) on subset of the genome \( L \) is determined by the number of mutations inherited by \( x \) or \( y \) but not both on \( L \), or \( \sum_{a \in A} \sum_{\ell \in L} [x \leftrightarrow y](a, \ell) \).

If \( 1_L(a, \ell) = 1 \) for \( \ell \in L \) and is zero otherwise, this is \( M(1_L(x \leftrightarrow y)) \), so the set of observable statistics, given a collection of samples \( A \), is generated by the set of linear combinations of functions of this form, i.e., products of indicators of a segment of genome with a path between two samples. It is clear that we cannot learn about parts of the pedigree from which no samples have inherited, or about anything occurring longer ago than the most recent common ancestor of the samples at each site, but it is not clear how to formulate more generally what information about the ARG is or is not obtainable from finite samples of chromosomes.

This notion of observability is made formal by the following definition:

**Definition 2** (Observable statistics). A function \( \chi(a, \ell) \) on \( A \times G \) is observable given a collection of chromosomes \( A \) if it is in the algebra generated by functions of the form \( 1_{[u, v]}(a, \ell) \), where \( [u, v] \) is an interval of the chromosome, and \( x \) and \( y \) are samples in \( A \).

There are further, unavoidable, limitations to what it is possible to learn from the data. For instance, the sequence divergence \( \pi(x, y) \) between \( x \) and \( y \) is an estimate of the mean of the empirical distribution of times to most recent common ancestors (TMRCA) between \( x \) and \( y \), multiplied by the mutation rate. Theorem 2 gives an estimator of the variance of the mean TMRCA in windows. However, it is not possible to estimate the variance of the distribution of single-site TMRCA values without somehow modeling dependencies between sites induced by recombination. An easy way to see this is if each site has at most one mutation, then without using inter-site dependencies, the model is equivalent to one where the probability of a segregating mutation is constant, equal to the mean. To make further progress, it is necessary (and reasonable) to make further assumptions, that are beyond the scope of this paper.

**Moments**

Calculations with statistics in this formalism can be made with the help of the following general formula for integrals of test functions against a Poisson processes:
Lemma 1. [Generating function] For any test function \( \phi : \mathcal{A} \times \mathcal{G} \to \mathbb{R} \) for which \( \int \phi d\mu \) is absolutely convergent, the statistic \( \int \phi dM \) is well-defined, and

\[
\mathbb{E} \left[ \exp \left( \int \phi dM \right) \right] = \exp \left( \int \left( e^\phi - 1 \right) d\mu \right). \tag{15}
\]

Proof. This is Campbell’s Theorem; see e.g. Kingman [1993]. Recall that \( \int \phi dM \) is the sum of the value that \( \phi \) takes over all mutations occurring in \( M \); “well-defined” means that this sum is absolutely convergent even if there are an infinite number of mutations.

We immediately get, by differentiating this formula, the moments:

Lemma 2 (Mean and covariance). Let \( \phi \) and \( \psi \) be test functions on \( \mathcal{A} \times \mathcal{G} \) for which \( \int \phi d\mu \), \( \int \psi d\mu \), and \( \int \phi \psi d\mu \) are all absolutely convergent. Then

\[
\mathbb{E} \left[ \int \phi dM \right] = \int \phi d\mu \tag{16}
\]

\[
\text{cov} \left[ \int \phi dM, \int \psi dM \right] = \int \phi \psi d\mu. \tag{17}
\]

Proof. These are a standard calculations, but we carry out the second for covariance for concreteness. Let \( F(a,b) = \exp \left( \int (a\phi + b\psi) dM \right) \). Using (15),

\[
\frac{\partial}{\partial a} \frac{\partial}{\partial b} \log \mathbb{E} [F(a,b)] \bigg|_{a=b=0} = \int \phi \psi d\mu. \tag{18}
\]

On the other hand, exchanging the expectation and the derivatives,

\[
\frac{\partial}{\partial a} \frac{\partial}{\partial b} \log \mathbb{E} [F(a,b)] \bigg|_{a=b=0} = \frac{\mathbb{E} \left[ \frac{\partial}{\partial a} \frac{\partial}{\partial b} F(a,b) \right] - \mathbb{E} \left[ \frac{\partial}{\partial a} F(a,b) \right] \mathbb{E} \left[ \frac{\partial}{\partial b} F(a,b) \right]}{\mathbb{E} [F(a,b)^2]} \bigg|_{a=b=0} \tag{19}
\]

\[
= \mathbb{E} \left[ \int \phi \psi dM \right] - \mathbb{E} \left[ \int \phi dM \right] \mathbb{E} \left[ \int \psi dM \right]. \tag{20}
\]

Expressions for more general moments, or statistics whose expected values match desired quantities, can be found using the following general formula:

Lemma 3. [General Moments] Let \( S \) be the set of upper triangular \( n \times n \) matrices with entries in \( \{0,1\} \) and whose columns sum to 1 (i.e. ways of partitioning the set \( \{1,2,\ldots,n\} \) into sets), and for \( \sigma \in S \) let \( |\sigma| \) denote the number of nonzero rows of \( \sigma \) (i.e. the number of sets in the partition). Then for any collection of test functions \( \phi_1, \ldots, \phi_n \) on \( \mathcal{A} \times \mathcal{G} \) for which the following integrals against \( \mu \) are absolutely convergent,

\[
\mathbb{E} \left[ \prod_{i=1}^n \int \phi_i dM \right] = \sum_{\sigma \in S} \prod_i \int \left( \prod_j \phi_j^{\sigma_{ij}} \right) d\mu \tag{21}
\]

and

\[
\int \prod_{i=1}^n \phi_i d\mu = \sum_{\sigma \in S} (-1)^{1+|\sigma|} \mathbb{E} \left[ \prod_i \int \left( \prod_j \phi_j^{\sigma_{ij}} \right) dM \right]. \tag{22}
\]

Note: methods for efficient computation of mixed moments using orthogonal polynomials are presented in a general framework by Peccati and Taqqu [2011].

Proof. By induction, by differentiating either the generating function (for the first formula) or the log of the generating function (for the second) of \( \sum_i \alpha_i \phi_i \).
Application of the method

We can now use the formalism developed above to easily prove the theorems given in the Introduction.

Proof of Theorem 1. Write $G_{x\ell}$ for the genotype of haplotype $x$ at position $\ell$, coded as ‘0’ for the reference allele and ‘1’ for the alternate allele. First note that

$$f_4(X, Y; U, V) = \frac{1}{G} \sum_{\ell=1}^{G} \frac{1}{n_X n_Y n_U n_V} \sum_{x,y,u,v} (G_{x\ell} - G_y\ell)(G_{u\ell} - G_v\ell),$$

(23)
i.e., $f_4$ is the average product of differences between pairs of haplotypes chosen from $(X, Y)$ and from $(U, V)$, averaging over site in the genome and choices of pairs $(x, y)$ and $(u, v)$. For a given quadruple $(x, y, u, v)$, sites will add to this sum if there is a mutation shared by $x$ and $u$ that $y$ and $v$ do not carry, or vice versa; and sites will subtract from the sum if there is a mutation that is shared by $x$ and $v$ that $y$ and $u$ do not carry, or vice versa. The theorem for $f_4(x, y, u, v)$ then follows easily by writing the statistic as the difference of two Poisson random variables, divided by $G$. To carry this through using the notation here, note that the function $[x \leftrightarrow v][y \leftrightarrow u] - [x \leftrightarrow u][y \leftrightarrow v]$ takes the value +1 on the central branch of trees with unrooted topology $((x, u), (y, v))$, −1 on the central branch of trees with unrooted topology $((x, v), (y, u))$, and is zero on the remaining topology $((x, y), (u, v))$; therefore,

$$f_4(X, Y; U, V) = \frac{1}{G} M \left( [(X \leftrightarrow V)[Y \leftrightarrow U] - [X \leftrightarrow U][Y \leftrightarrow V]) \right).$$

(24)
The expected value of $f_4$ is therefore just the integral of $[(X \leftrightarrow V)[Y \leftrightarrow U] - [X \leftrightarrow U][Y \leftrightarrow V]$ against $\mu$; using linearity this can be rewritten in various ways to give the two interpretations given in the theorem. Since

$$\mu(1[\ell] x \leftrightarrow v][y \leftrightarrow u]) = \mu(\ell) \left( S(x,y):\{u,v\}(\ell) + S(x,u):\{y,v\}(\ell) \right),$$

(25)
expression (3) follows immediately.

For the second interpretation, define $\star$ to be all ancestral genomes alive at some point in the remote past (longer ago than the maximum time to most recent common ancestor across the genome), and note that $[x \leftrightarrow u] = |\star| [\star \leftrightarrow x][\star \leftrightarrow u]$. (The factor of $|\star|$ is only here to cancel the denominator in the definition of the path function.) Therefore, $[x \leftrightarrow v][y \leftrightarrow u] - [x \leftrightarrow u][y \leftrightarrow v] = |\star|^[\star \leftrightarrow x][\star \leftrightarrow u] - |\star|^[\star \leftrightarrow y][\star \leftrightarrow u]$. Since $\mu(\star)[\star \leftrightarrow x](a, \ell) = 1$ if $a$ is more recent than $\star$ and $x$ has inherited from $a$ at $\ell$, averaging over choices of $x$ gives the function $F_X$ defined in the theorem: $\star|\mu(\star \leftrightarrow X)(a, \ell) = \mu(\ell)F_X(a, \ell)$, so that equation (4) follows. Note that in this interpretation, each term in the product is not summable on its own (e.g., $\sum_a F_X(a, \ell) = \infty$), but cancellation and a reasonable assumption about finiteness of recent common ancestors makes differences like $F_X - F_Y$ summable.

Finally, consider the variance. By theorem 2,

$$\text{var}[f_4(X, Y; U, V)] = \frac{1}{G^2}\mu \left( [(X \leftrightarrow V)[Y \leftrightarrow U] - [X \leftrightarrow U][Y \leftrightarrow V])^2 \right).$$

(26)

By Jensen’s inequality,

$$[(X \leftrightarrow V)[Y \leftrightarrow U] - [X \leftrightarrow U][Y \leftrightarrow V])^2 \leq \frac{1}{n_X n_Y n_U n_V} \sum_{x,y,u,v} ([x \leftrightarrow v][y \leftrightarrow u] - [x \leftrightarrow u][y \leftrightarrow v])^2.$$ 

(27)

Since the summand is equal to $(S(x,u):\{y,v\}(\ell) - S(x,v):\{y,u\}(\ell))$, summed over $\ell$ this gives equation (4).



heterozygosities as an estimator of at least a similar quantity. This would be arguably more natural from the population genetics point of view, as mean heterozygosities are an easily observed statistic. The following is therefore a useful contrast to theorem 2.

First, some more notation. Let \((x_1, x_2)\) and \((y_1, y_2)\) be two pairs of sampled chromosomes, and for each position \(\ell\) in the genome, recall that \(t_x(\ell)\) is the total number of ancestors in the tree leading from \(x_1\) and \(x_2\) back to their common ancestor at \(\ell\) (not including the common ancestor), and likewise for \(t_y(\ell)\). We will also need \(t_{x\cap y}(\ell)\), the number of ancestors in the intersection of these two trees. Just as \(\bar{\mu}_x\) was defined above to be the mean time to common ancestor, scaled by mutation rate; so also \(\mu t_{x\cap y}(\ell)\).

**Theorem 3** (Empirical covariance of heterozygosities). Let \(\theta_x(\ell) = 1\) if the genotypes of \(x_1\) and \(x_2\) differ at \(\ell\), and \(\theta_x(\ell) = 0\) otherwise, and let \(\bar{\theta}_x = \frac{1}{G} \sum_{\ell=1}^G \theta_x(\ell)\) be the mean heterozygosity of \((x_1, x_2)\); and likewise for \(\theta_y(\ell)\) and \(\bar{\theta}_y\). Then

\[
\begin{align*}
\mathbb{E} \left[ \frac{G-1}{G^2} \sum_{\ell=1}^G \theta_x(\ell)\theta_y(\ell) - \bar{\theta}_x \bar{\theta}_y \right] &= \bar{\mu} t_{x\cap y} - \bar{\mu}_x \bar{\mu}_y \\
\text{and} \quad \text{var} \left[ \frac{G-1}{G^2} \sum_{\ell=1}^G \theta_x(\ell)\theta_y(\ell) - \bar{\theta}_x \bar{\theta}_y \right] &= \frac{1}{G} t_{x\cap y} + O(1/G^3).
\end{align*}
\]

Note that since \(\mu t_x \ll 1\), the value of (28) is positive.

**Proof of Theorem 3.** Under the infinite-sites model, \(\theta_x(\ell)\) and \(\theta_y(\ell)\) are both nonzero only if there is a mutation at \(\ell\) that falls on both the path from \(x_1\) to \(x_2\) and the path from \(y_1\) to \(y_2\), and so

\[
\sum_{\ell} \theta_x(\ell)\theta_y(\ell) = \int [x_1 \leftrightarrow x_2](a, \ell)[y_1 \leftrightarrow y_2](a, \ell) dM(a, \ell).
\]

To reduce the amount of ink below, for the meantime let \(\phi_x = [x_1 \leftrightarrow x_2]\), \(\phi_y = [y_1 \leftrightarrow y_2]\), and for a test function \(\psi\) write \(M(\psi) = \int \psi dM\) and \(\mu(\psi) = \int \psi d\mu\). In this shorthand, the statistic is

\[
\frac{G-1}{G^2} \sum_{\ell=1}^G \theta_x(\ell)\theta_y(\ell) - \bar{\theta}_x \bar{\theta}_y = \frac{G-1}{G^2} M(\phi_x\phi_y) - \frac{1}{G^2} M(\phi_x)M(\phi_y).
\]

Using Lemma 3, \(\mathbb{E}[M(\phi_x\phi_y)] = \mu(\phi_x\phi_y)\) and \(\mathbb{E}[M(\phi_x)M(\phi_y)] = \mu(\phi_x\phi_y) + \mu(\phi_x)\mu(\phi_y)\), which combine to give

\[
\mathbb{E} \left[ \frac{G-1}{G^2} M(\phi_x\phi_y) - \frac{1}{G^2} M(\phi_x)M(\phi_y) \right] = \frac{1}{G} \mu(\phi_x\phi_y) - \frac{1}{G^2} \mu(\phi_x)\mu(\phi_y),
\]

which is equation (28).

Again using Lemma 3, and the fact that \(\phi_x(\alpha, \ell)^2 = \phi_x(\alpha, \ell)\), \(\text{var}[M(\phi_x\phi_y)] = \mu(\phi_x\phi_y)\) and

\[
\text{cov}[M(\phi_x\phi_y), M(\phi_x)M(\phi_y)] = \mu(\phi_x\phi_y) (1 + \mu(\phi_x) + \mu(\phi_y))
\]

and

\[
\text{var}[M(\phi_x)M(\phi_y)] = \mu(\phi_x\phi_y) (1 + 2(\mu(\phi_x) + \mu(\phi_y)) + 2\mu(\phi_x\phi_y) + 4\mu(\phi_x)\mu(\phi_y))
\]

so finally, equation (29) is \(1/G^4\) multiplied by

\[
(G-1)^2 \text{var}[M(\phi_x\phi_y)] - 2(G-1) \text{cov}[M(\phi_x\phi_y), M(\phi_x)M(\phi_y)] + \text{var}[M(\phi_x)M(\phi_y)]
\]

which, after some algebra, is

\[
\mu(\phi_x\phi_y) \left\{ (G-1)^2 - 2(G-1)(1 + \mu(\phi_x) + \mu(\phi_y)) + 1 + 2(\mu(\phi_x) + \mu(\phi_y)) + 2\mu(\phi_x\phi_y) + 4\mu(\phi_x)\mu(\phi_y) \right\}.
\]

□
We now turn to the proof of Theorem 2, which is similar to the previous proof, but somewhat more involved.

**Proof of Theorem 2.** Here, the goal is to design a statistic that estimates the covariance in mean coalescent times (where time is always scaled by mutation rate). Therefore, if we define

\[ \psi_{k,x}(a, \ell) = \begin{cases} \frac{1}{|W_k|} [x_1, x_2](a, \ell) & \text{if } \ell \in W_k \\ 0 & \text{otherwise} \end{cases} \]  

(37)

then \( \mu(\psi_{k,x}) = \overline{\mu}_{k,x} \) gives the mean coalescent time on window \( W_k \). We next need something whose expectation is \( \mu(\psi_{k,x}) \mu(\psi_{k,y}) \). By Lemma 3, this is

\[ \mathbb{E}[M(\psi_{k,x})M(\psi_{k,y})] - M(\psi_{k,x}\psi_{k,y}) = \overline{\mu}(\psi_{k,x}) \mu(\psi_{k,y}). \]  

(38)

Similarly, if we define \( \overline{\psi}_x(a, \ell) = \frac{1}{n} \sum_{k=1}^{n} \psi_{k,x}(a, \ell) \), so that \( \mu(\overline{\psi}_x) = \overline{\mu}_x \), we need something whose expectation is \( \mu(\overline{\psi}_x) \mu(\overline{\psi}_y) \), which by the same lemma is

\[ \mathbb{E}[M(\overline{\psi}_x)M(\overline{\psi}_y)] - M(\overline{\psi}_x\overline{\psi}_y) = \mu(\overline{\psi}_x) \mu(\overline{\psi}_y). \]  

(39)

These two combine to get

\[ C_{x,y} = \frac{1}{n} \sum_{k=1}^{n} \{M(\psi_{k,x})M(\psi_{k,y}) - M(\psi_{k,x}\psi_{k,y})\} - \{M(\overline{\psi}_x)M(\overline{\psi}_y) - M(\overline{\psi}_x\overline{\psi}_y)\}. \]  

(40)

Now note that by linearity of \( M \),

\[ M(\overline{\psi}_x)M(\overline{\psi}_y) - M(\overline{\psi}_x\overline{\psi}_y) = \frac{1}{n^2} \sum_{j=1}^{n} \sum_{k=1}^{n} (M(\psi_{j,x})M(\psi_{k,y}) - M(\psi_{j,x}\psi_{k,y})). \]  

(41)

Defining \( Z_{jk} = M(\psi_{j,x})M(\psi_{k,y}) - M(\psi_{j,x}\psi_{k,y}) \), then

\[ C_{x,y} = \frac{1}{n} \sum_{k=1}^{n} Z_{kk} - \frac{1}{n^2} \sum_{j=1}^{n} \sum_{k=1}^{n} Z_{jk}, \]  

(42)

which, since \( M(\psi_{k,x}) = N_k(x)/|W_k| \) and \( M(\psi_{k,x}\psi_{k,y}) = N_k(x,y)/|W_k|^2 \), is equation (5).

The substantially lengthier calculation of the variance is postponed until the Appendix.

\[ \square \]

**The infinite sites assumption**

Some of the simplicity above (and more generally in population genetics) relies on the assumption that only one mutation can occur at each site, which generally results in expressions that are correct up to a factor proportional to the fraction of sites at which more than one mutation has occurred. To illustrate this, suppose that more than one mutation can occur per site; in other words, the Poisson process of mutations happens on a discrete, not continuous, set.

Assume for the moment that all mutation rates are equal: \( \mu_{\ell} = \mu \). Fix a pairs of sampled chromosomes \( (x_1, x_2) \), and define \( \xi_n(\ell) \) is defined to be 1 if \( n \) mutations have occurred at site \( \ell \) between \( x_1 \) and \( x_2 \), and let \( \bar{\xi}_n = (1/G) \sum_{\ell=1}^{G} \xi_n(\ell) \). The expected value of \( \bar{\xi}_n \) under this model is

\[ \frac{1}{G} \sum_{\ell=1}^{G} e^{-\mu_{x}(\ell)} \left( \mu_{x}(\ell) \right)^n / n!, \]  

(43)

and so an estimate of \( n^{th} \) moment of the empirical distribution of \( t_x(\ell) \) could be \( n! \bar{\xi}_n^{\frac{n}{G}} \). However, since this is essentially estimating the density of sites at which there were \( n \) mutations, to have any accuracy requires a reasonable number of such sites, and distinguishing these from sequencing error. In practice, this is highly problematic, and is confounded by mutation rate heterogeneity.
Discussion

In this paper I have explored the empirical approach to population genetics, by treating the (empirical, realized) ancestral recombination graph as a complex, unobserved, object we wish to learn about, rather than as an intermediate layer that is averaged over in the course of inferring parameters of interest in higher-level stochastic models (e.g., coalescent models). This approach certainly does not replace coalescent theory, but seems useful in that it can provide more concretely interpretable results to non-specialists (e.g., “numbers of common ancestors” rather than “coalescent rates”), and intuition about what statistics have the best power to distinguish between alternative population models. This way of thinking is certainly not new, but data that give us power to infer quantities directly at this level of abstraction is.

Additionally, I have described a new formalism, that can simplify calculations related to population genetics statistics by writing these as integrals of functions against a Poisson random measure, which models the locations of mutations on the genomes of all possible ancestors.

This point of view, and this formalism, leads to two general sorts of interpretations for a given statistic: first, in terms of the distribution of trees along which the samples are related at each locus; and second, in terms of weighted sums over ancestral chromosomes. This duality is easy to see considering simple examples such as pairwise divergences, and is less obvious for more complex statistics such as $f_4$.

$f_4$ and family Theorem 1 gives an interpretation of the $f_4$ statistic as a sum of products of differences in ancestry, over all ancestral genomes. Patterson et al. [2012] interprets this and related statistics in terms of shared drift along branches of an admixture graph (in which each edge is a randomly mating population). Since genetic drift is determined by the sharing of common ancestors, Theorem 1 can be seen as a more precise statement of the same observation. Since the main point of this paper is to lay out the general framework, I have not undertaken the task of recasting the entire family of related statistics (e.g., ABBA-BABA), but the general way forward can be seen by analogy to $f_4$.

The unknown network of ancestors The problem at hand, to infer aspects of the unknown pedigree through which genetic material has been noisily inherited, has some parallels to the problem of active network tomography [reviewed in Lawrence et al., 2006]: given information on losses and delays of “probe” packets sent between a subset of peripheral nodes in a network, infer the topology of the network and certain characteristics of its internal nodes. Results in this field on identifiability [e.g., Singhal and Michailidis, 2007, Gopalan and Ramasubramanian, 2012] would be very interesting in this context, although genomic data are substantially noisier, more sparsely collected, and more numerous. Others have already made use of the parallels to phylogenetics, in the other direction [Ni and Tatikonda, 2011].

Recombination A glaring omission in this paper is any treatment of linkage between sites: for instance, when calculating variances, each site is treated as independent; surely this cannot be right, as genealogies at nearby sites are highly correlated? But, we begin by assuming that the mutation process at each site is independent given the genealogies (which seems for the most part reasonable), and take the entire empirical ancestral recombination graph as fixed, which includes the locations of ancestral recombination breakpoints. If the aim is to estimate descriptive statistics of the ARG then this is the correct point of view; but an intermediate position would be to treat only the population pedigree as given, and to additionally model randomness in recombination. This could allow for recovery of additional information left behind by recombination, and was the point of view taken in Ralph and Coop [2013]. This will be the subject of a companion paper.

Mutation Above, we have allowed for heterogeneity in mutation rate, as this is known to be substantial [e.g., Misawa and Kikuno, 2009]. When applying these methods, it seems necessary to choose regions of the genome with comparable large-scale mutation rates, and then within these, to average over enough sites that small-scale heterogeneity will not hopelessly confound comparisons. Methods such as described in Lipson et al. [2015], may be useful in disentangling mutation rate heterogeneity from heterogeneity in demographic
histories along the genome. Furthermore, this paper only models diallelic markers, and completely ignores both sequence context and backmutation. This is common in population genetics methods, since the infinite sites assumption should be a good one at the typical levels of divergence encountered within species, especially if mutation rate heterogeneity is accounted for. Reconciliation of more realistic models of mutation with the Poisson process method described here would be difficult.

Selection

An important assumption behind this method is that mutations are independent of the ARG. This is not true for alleles under selection, but neither does this approach entirely assume neutrality: since the ARG is taken as given, if we knew which sites were under selection and excluded these from the analyses, then the assumptions would be satisfied. In other words, linked, selected sites are not an issue, as they act by distorting local genealogies. On the other hand, if a large fraction of segregating polymorphisms are actively under selection, this violates the model (but it is unclear what to replace it with).

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The variance of $C_{x,y}$

Continuation of proof of Theorem 2. First note that (42) implies that

$$C_{x,y} = \frac{1}{n} \sum_{j=1}^{n} \sum_{k=1}^{n} z_{jk} \left( \delta_{jk} - \frac{1}{n} \right),$$

(44)
where \( \delta_{jk} = 1 \) if \( j = k \) and is zero otherwise. To find the variance of \( C_{x,y} \), we need to compute covariances of the \( Z_{jk} \) terms. To do this, it is most efficient to record the general case. The following lemma follows directly from Lemma 3, which uses upper-case roman characters for test functions to make the result more visually intuitive and easier to apply.

**Lemma 4.** If \( A, B, C, \) and \( D \) are test functions as in Lemma 3, then

\[
\text{cov}[M(A)M(B), M(C)] = \mu(ABC) + \mu(A)\mu(BC) + \mu(B)\mu(AC)
\]

and

\[
\text{cov}[M(A)M(B), M(C)M(D)] = \mu(ABCD) + \mu(A)\mu(BCD) + \mu(B)\mu(ACD) + \mu(C)\mu(ABD)
\]

\[
+ \mu(D)\mu(ABC) + \mu(AC)\mu(BD) + \mu(AD)\mu(BC)
\]

\[
+ \mu(A)\mu(C)\mu(BD) + \mu(A)\mu(D)\mu(BC)
\]

\[
+ \mu(AC)\mu(B)\mu(D) + \mu(AD)\mu(B)\mu(C)
\]  \hspace{1cm} (46)

If \( j \neq k \), then \( \psi_{j,x} \) and \( \psi_{k,x} \) are supported on disjoint parts of the ancestral genomes, and so \( \psi_{j,x}\psi_{k,y} = 0 \). For the same reason, the covariance of \( Z_{jk} \) and \( Z_{lm} \) is zero unless at least one of \( j \) and \( k \) match at least one of \( l \) and \( m \). We are further helped by the fact that \( \psi_{j,x}^n = \psi_{j,x}/W_j^{n-1} \).

First, we apply Lemma 4 with \( A = C = \psi_{j,x} \) and \( B = D = \psi_{k,y} \) and \( j \neq k \) (so, note that \( AB = AD = CB = CD = 0 \)).

\[
\text{var}[Z_{jk}] = \frac{\mu(\psi_{j,x})\mu(\psi_{k,y})}{|W_j||W_k|} \left( 1 + |W_j|\mu(\psi_{j,x}) + |W_k|\mu(\psi_{k,y}) \right)
\]  \hspace{1cm} (47)

Similarly, if \( A = \psi_{j,x}, B = \psi_{k,y}, C = \psi_{k,x}, \) and \( D = \psi_{j,y}, \) and \( j \neq k \) (so, note that \( AB = AC = BD = CD = 0 \)).

\[
\text{cov}[Z_{jk}, Z_{kj}] = \mu(\psi_{j,x}\psi_{j,y})\mu(\psi_{k,y}\psi_{k,x}) + \mu(\psi_{j,x})\mu(\psi_{j,y}\psi_{k,y}\psi_{k,x}) + \mu(\psi_{j,x}\psi_{j,y})\mu(\psi_{k,y}\mu(\psi_{k,x})
\]  \hspace{1cm} (48)

Now, with \( A = C = \psi_{j,x}, B = \psi_{k,y}, \) and \( C = \psi_{l,y}, \) and \( j, k \) and \( l \) all distinct,

\[
\text{cov}[Z_{jk}, Z_{jl}] = \frac{1}{W_j}\mu(\psi_{j,x})\mu(\psi_{k,y})\mu(\psi_{l,y})
\]  \hspace{1cm} (49)

and similarly,

\[
\text{cov}[Z_{kj}, Z_{jl}] = \mu(\psi_{j,x}\psi_{j,y})\mu(\psi_{k,x})\mu(\psi_{l,y}).
\]  \hspace{1cm} (50)

Now, with \( A = C = \psi_{j,x}, B = \psi_{j,y}, \) and \( D = \psi_{k,y}, \)

\[
\text{cov}[M(\psi_{j,x})M(\psi_{j,y}), M(\psi_{j,x})M(\psi_{k,y})] = \mu(\psi_{k,y})\left\{ \mu(\psi_{j,x}\psi_{j,y})\left( \frac{1}{W_j} + \mu(\psi_{j,x}) \right) + \frac{1}{W_j}\mu(\psi_{j,x})\mu(\psi_{j,y}) \right\}
\]  \hspace{1cm} (51)

and with \( A = \psi_{j,x}, B = \psi_{k,y}, \) and \( C = \psi_{j,x}\psi_{j,y}, \)

\[
\text{cov}[M(\psi_{j,x})M(\psi_{k,y}), M(\psi_{j,x}\psi_{j,y})] = \frac{1}{W_j}\mu(\psi_{k,y})\mu(\psi_{j,x}\psi_{j,y}).
\]  \hspace{1cm} (52)

These combine to give

\[
\text{cov}[Z_{jj}, Z_{jk}] = \mu(\psi_{k,y})\left\{ \mu(\psi_{j,x}\psi_{j,y})\mu(\psi_{j,x}) + \frac{1}{W_j}\mu(\psi_{j,x})\mu(\psi_{j,y}) \right\}
\]  \hspace{1cm} (53)
Finally, taking \( A = C = \psi_{j,x} \) and \( B = D = \psi_{j,y} \), we get

\[
\begin{align*}
\text{var}[M(\psi_{j,x})M(\psi_{j,y})] &= \frac{1}{W_j} \mu(\psi_{j,x} \psi_{j,y}) + \frac{2}{W_j} \mu(\psi_{j,x}) (\mu(\psi_{j,x}) + \mu(\psi_{j,y})) \\
&\quad + \frac{1}{W_j} \mu(\psi_{j,x}) \mu(\psi_{j,y}) + \mu(\psi_{j,x} \psi_{j,y})^2 + \frac{1}{W_j} \mu(\psi_{j,x})^2 \mu(\psi_{j,y}) \\
&\quad + \frac{1}{W_j} \mu(\psi_{j,x})^2 \mu(\psi_{j,y})^2 + 2 \mu(\psi_{j,x}) \mu(\psi_{j,y}) \mu(\psi_{j,y} \psi_{j,x}) 
\end{align*}
\]

\( (54) \)

and

\[
\begin{align*}
\text{cov}[M(\psi_{j,x})M(\psi_{j,y}), M(\psi_{j,x} \psi_{j,y})] &= \frac{1}{W_j^2} \mu(\psi_{j,x} \psi_{j,y}) + \frac{1}{W_j} \mu(\psi_{j,x} \psi_{j,y}) (\mu(\psi_{j,x}) + \mu(\psi_{j,y})) \\
&\quad + \frac{1}{W_j} \mu(\psi_{j,x}) \mu(\psi_{j,y})^2 + 2 \mu(\psi_{j,x}) \mu(\psi_{j,y}) \mu(\psi_{j,y} \psi_{j,x}) 
\end{align*}
\]

\( (55) \)

and

\[
\begin{align*}
\text{var}[M(\psi_{j,x} \psi_{j,y})] &= \frac{1}{W_j^2} \mu(\psi_{j,x} \psi_{j,y}) \\
&\quad + \frac{1}{W_j} \mu(\psi_{j,x} \psi_{j,y}) (\mu(\psi_{j,x}) + \mu(\psi_{j,y})) + 2 \mu(\psi_{j,x}) \mu(\psi_{j,y}) \mu(\psi_{j,y} \psi_{j,x}) 
\end{align*}
\]

\( (56) \)

so

\[
\begin{align*}
\text{var}[Z_{jj}] &= \text{var}[M(\psi_{j,x})M(\psi_{j,y})] - 2 \text{cov}[M(\psi_{j,x})M(\psi_{j,y}), M(\psi_{j,x} \psi_{j,y})] + \text{var}[M(\psi_{j,x} \psi_{j,y})] \\
&= \mu(\psi_{j,x}) \mu(\psi_{j,y}) \left( \frac{1}{W_j^2} + \frac{1}{W_j} (\mu(\psi_{j,x}) + \mu(\psi_{j,y})) + 2 \mu(\psi_{j,x} \psi_{j,y}) \right) + \mu(\psi_{j,y} \psi_{j,x})^2 
\end{align*}
\]

\( (57) \)

\( (58) \)
We can put these together to obtain that

\[
\begin{align*}
\var[C_{x,y}] &= \frac{1}{n^2} \sum_{1 \leq j,k,\ell,m \leq n} \cov[Z_{jk}, Z_{\ell m}] \left( \delta_{jk} - \frac{1}{n} \right) \left( \delta_{\ell m} - \frac{1}{n} \right) \\
&= \frac{(n-1)^2}{n^4} \sum_{j} \var[Z_{jj}] - \frac{2(n-1)}{n^4} \sum_{j \neq k} \cov[Z_{jj}, Z_{jk} + Z_{kj}] + \frac{1}{n^4} \sum_{j \neq k} \var[Z_{jk}] \\
&\quad + \frac{1}{n^4} \sum_{j \neq k} \cov[Z_{jk}, Z_{kj}] + \frac{1}{n^4} \sum_{j \neq k \neq l \neq j} \cov[Z_{jk}, Z_{jl}] \\
&\quad + \frac{1}{n^4} \sum_{j \neq k \neq l \neq j} \cov[Z_{jk}, Z_{lj}] + \frac{2}{n^4} \sum_{j \neq k \neq l \neq j} \cov[Z_{kj}, Z_{lj}] \\
&= \frac{(n-1)^2}{n^4} \sum_{j} \left( \mu(\psi_{j,x}) \mu(\psi_{j,y}) \left( \frac{1}{W_j} \mu(\psi_{j,x}) + \frac{1}{W_j} \mu(\psi_{j,y}) \right) \right) \\
&\quad - \frac{2(n-1)}{n^4} \sum_{j \neq k} \left( \mu(\psi_{k,x}) \left( \mu(\psi_{j,x}) \mu(\psi_{j,y}) + \frac{1}{W_j} \mu(\psi_{j,x}) \mu(\psi_{j,y}) \right) \right) \\
&\quad + \mu(\psi_{k,x}) \left( \mu(\psi_{j,y}) \mu(\psi_{j,y}) + \frac{1}{W_j} \mu(\psi_{j,y}) \mu(\psi_{j,y}) \right) \\
&\quad + \frac{2}{n^4} \sum_{j \neq k} \left( \frac{1}{W_j} \mu(\psi_{j,x}) \mu(\psi_{k,y}) \left( \frac{1}{W_j} + \mu(\psi_{j,x}) + \mu(\psi_{k,y}) \right) \right) \\
&\quad + \frac{1}{n^4} \sum_{j \neq k} \left( \mu(\psi_{j,x}) \mu(\psi_{k,y}) \psi_{k,x} + \mu(\psi_{j,x}) \mu(\psi_{k,y}) \psi_{k,x} + \mu(\psi_{j,x}) \mu(\psi_{k,y}) \mu(\psi_{k,x}) \right) \\
&\quad + \frac{1}{n^4} \sum_{j \neq k \neq l \neq j} \frac{1}{W_j} \mu(\psi_{j,x}) \mu(\psi_{k,y}) \mu(\psi_{l,y}) \\
&\quad + \frac{1}{n^4} \sum_{j \neq k \neq l \neq j} \frac{1}{W_j} \mu(\psi_{j,x}) \mu(\psi_{k,y}) \mu(\psi_{l,x}) \\
&\quad + \frac{2}{n^4} \sum_{j \neq k \neq l \neq j} \mu(\psi_{j,x}) \mu(\psi_{k,y}) \mu(\psi_{l,y}).
\end{align*}
\]

The above is dominated by terms of the form \((\mu^3/nW)^3\), where \(\mu^3\) is an average of \(\mu(\psi)\) terms. To put a crude bound on this, assume that \(\mu(\psi_{j,x})\) and \(\mu(\psi_{j,y})\) are bounded by \(\epsilon\) for every \(j\), resulting in \(\var[C_{x,y}] \leq \frac{n^3}{nW}\), as in the theorem.