The expected neutral frequency spectrum of two linked sites

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Abstract

We present an exact, closed expression for the expected neutral Site Frequency Spectrum for two neutral sites, 2-SFS, without recombination. This spectrum is the immediate extension of the well known single site \(\theta/f\) neutral SFS. Similar formulae are also provided for the case of the expected SFS of sites that are linked to a focal neutral mutation of known frequency. Formulae for finite samples are obtained by coalescent methods and remarkably simple expressions are derived for the SFS of a large population, which are also solutions of the multi-allelic Kolmogorov equations. We also present the single-locus triallelic spectrum. Beyond their fundamental interest, these results can be used to improve neutrality tests, composite likelihood and Poisson random field methods.
1 Introduction

According to the “neutral theory of molecular evolution” ([Kimura] 1983), polymorphisms segregating in the populations are best described by a mutation-drift equilibrium, that is often referred to as the standard neutral model. One of the major features that characterizes nucleotide polymorphisms is the Site Frequency Spectrum (SFS), that is the distribution of the mutation frequencies at each site. The SFS can be computed either for the whole (large) population, assuming that the frequency $f$ is a continuous value in $[0, 1]$ or for a sample of $n$ individuals, for which the frequency is a discrete variable $f = k/n$, where $k \in [0, n]$. Typically sites with alleles at frequency 0 or 1 are not included in the SFS, as they are summarized instead in the divergence between the population and the outgroup.

Using coalescent theory, [Fu] (1995) derived the means and covariance matrix for each bin of the sample SFS, by averaging tree realizations across the whole tree space. For a single realization of the coalescent tree, results are different and depend on the realization; for example, mutations of high frequencies can be present only for highly unbalanced genealogies ([Ledda et al.] 2015). The SFS was also studied in scenarios including selection ([Kim and Stephan] 2002; [Fay and Wu] 2000) or demography ([Griffiths and Tavaré] 1994; [Zivković and Wiehe] 2008), or population structure ([Alcala et al.] 2016).

Besides its general interest, the SFS has been used to devise goodness-of-fit statistical tests to estimate the relevance of the standard neutral model for an observed dataset. SFS-based neutrality tests ([Tajima] 1989a; [Fu and Li] 1993) contrast estimations of the nucleotide variability from different bins of the sample SFS ([Tajima] 1993; [Achaz] 2009). Interestingly, it has been shown that, once the SFS under an alternative scenario (e.g. selection, demography or structure) is known, the optimal test to reject the standard neutral model is based on the difference between the standard neutral SFS and the alternative scenario SFS ([Ferretti et al.] 2010). All these tests assume complete linkage among variants in their null model. Assuming independence between the sites, the observed SFS can also be used
to estimate model parameters. An interesting recent approach is the estimation of piece-wise constant demography from genomewide SFS (e.g., Liu and Fu (2015)). More sophisticated methods based on the expected SFS, such as Poisson Random Field (Sawyer and Hartl, 1992; Bustamante et al., 2001, 2002) and Composite Likelihood approaches (e.g., Kim and Stephan, 2002; Li and Stephan, 2005; Kim and Nielsen, 2004; Nielsen et al., 2005), have also played an important role in the detection of events of selection across regions of the genome. However, the assumption of linkage equilibrium is often violated in genetic data. In fact, while the average spectrum is insensitive to recombination, the presence of linked variants affects the distribution of summary statistics, therefore the spread (and possibly the mean) of the estimated parameters (Hudson et al., 1990; Thornton, 2005). For this reason, simulations of the evolution of linked sequences are required for an accurate estimation of the statistical support for different models (Gutenkunst et al., 2009).

The joint SFS for multiple sites has been the subject of longstanding investigations. The simplest spectrum for multiple sites is the “two-locus frequency spectrum” (Hudson, 2001), which we will name hereafter the “two-Sites Frequency Spectrum” or 2-SFS. Assuming independence between the sites (i.e., free recombination), it simply reduces to the random association between two single-sites spectra (1-SFS). For intermediate recombination, a recursion solvable for small sample size has been provided (Golding, 1984; Ethier and Griffiths, 1990) as well as a numerical solution relying on simulations (Hudson, 2001). Without recombination, finding an analytical expression for the spectrum has proven to be difficult.

There is a close relation between the $m$-SFS (the joint SFS of $m$ sites) and the multi-allelic spectrum of a single locus (hereafter a locus is a sequence with one or more sites). In a large sequence with a low mutation rate per site, under the so-called infinite-sites model, sites are assumed to have at most two alleles as new mutations occur exclusively at non-polymorphic sites. At the locus scale, the multiple alleles are the haplotypes that are specific com-
binations of the alleles carried at each site. Therefore, at least conceptually, the SFS for \( m \) biallelic sites at low mutation rate is closely related to the spectrum of \( m + 1 \) alleles in a multi-allelic locus. Indeed, it is possible to retrieve the latter from the former by considering the \( m + 1 \) alleles that result from the \( m \) polymorphic sites. However, the \( m \)-SFS contains extra-information on the different linkage between the sites that is not available in the multi-allelic locus spectrum.

For an infinite population, the multi-alleles locus spectrum is the solution of a multiallelic diffusion equation \[\text{Ewens} \ 2012\]. Some approaches proposed polynomial expansions to solve the diffusion equations for the SFS of an infinite population. A final result for the polynomial expansion of the 2-SFS has been recently found for two sites without recombination and with general selection coefficients \[\text{Xie} \ 2011\]. However, the reported solution is an infinite series that is in sharp contrast with the simple solution existing for a single neutral site: \( E[\xi(f)] = \theta/f \). Furthermore, no closed form was provided for the sample 2-SFS.

Using a coalescent framework, the probability and size of two nested mutations were expressed by \[\text{Hobolth and Wiuf} \ 2009\] as sums of binomial coefficients. Their formulae can be rewritten as an expected SFS in terms of a finite series. However their conditioning on exactly two nested mutations skews the spectrum and simulations show that even under this condition their result is valid only for \( L\theta \ll 1 \). Interesting recent results on a tri-allelic locus \[\text{Jenkins and Song} \ 2011; \text{Jenkins et al.} \ 2014\] used an approach similar to the one presented here, but did not lead to the full 2-SFS. More recently, \[\text{Sargsyan} \ 2015\] broadened the result of \[\text{Hobolth and Wiuf} \ 2009\] by conditioning on any two mutations (nested or not) and extending it to populations of variable size. Moreover, he clarified the notion and classification of the 2-SFS.

In this work, we present a simple closed-form solution for the expectation of the 2-SFS without recombination. We report the solution both for the discrete sample 2-SFS and the continuous population 2-SFS. The solution for
a finite sample was obtained in a coalescent framework (Fu, 1995; Ferretti et al., 2012) and its extension to the limit of infinite sample sizes yields the continuous spectrum. We also derive the expected 1-SFS of sites that are completely linked to a focal mutation of known frequency. Finally, we translate our results on the 2-SFS into closed expressions for a locus with three alleles.

1.1 Model definitions and notations

We consider a population of size $N$ of haploid individuals without recombination. All subsequent results can be applied to diploids, provided that $2N$ is used instead of $N$, and to other cases by substituting the appropriate effective population size. We denote by $\mu$ the mutation rate per site and by $\theta = 2N\mu$ the population-scaled mutation rate per site. We work in the infinite-sites approximation, that is valid in the limit of small mutation rate $\theta \ll 1$. More properly, our results are derived in the limit $\theta \to 0$ with fixed non-zero $\theta L$, where $L$ is the length of the sequence. We use mutation as a synonym for derived allele.

Connection between sample and population SFS

We denote $\xi(f)$ the density of mutations at frequency $f$ in the whole population and $\xi_k$ the number of sites having mutations at frequency $k/n$ in a sample of size $n$. Importantly, in both cases $f$ or $k$ refer to frequency of the mutation, i.e. the derived allele, and thus $\xi$ corresponds to the unfolded SFS. The two spectra (sample and population) are related. Assuming that a mutation has frequency $f$ in the population, the probability of having $k$ mutant alleles in a sample of size $n$ is simply given by the Binomial $\binom{n}{k} f^k (1 - f)^{n-k}$.

As the expected density of mutations at frequency $f$ in the population is given by $E[\xi(f)]$, one can easily derive the sample frequency from the population frequency using the following sampling formula:

$$E[\xi_k] = \int_{\frac{1}{N}}^1 \binom{n}{k} f^k (1 - f)^{n-k} E[\xi(f)] \, df$$

(1)
assuming that \( n \ll N \).

Conversely, the population SFS can be derived from the sample SFS using the limit of large sample size \( n \to \infty \). For a sample of \( n \) individuals, the interval between the frequency bins is \( 1/n \) and therefore the density of mutations at the continuous frequency \( f = k/n \) can be approximated by \( E \left[ \xi \left( \frac{k}{n} \right) \right] \approx \frac{E[\xi_k]}{1/n} = nE[\xi_k] \). The expected population spectrum can then be constructed from the limit:

\[
E[\xi(f)] = \lim_{n \to \infty} nE[\xi_{[nf]}] \tag{2}
\]

for frequencies not too close to \( \frac{1}{N} \) or \( 1 - \frac{1}{N} \).

For a sample of size \( n \), the expected neutral spectrum for constant population size is \( E[\xi_k] = \theta L/k \) and consequently, we have \( E[\xi(f)] = \theta L/f \). These results are exact for the Kingman coalescent and the diffusion equations respectively, and they are approximately valid for neutral models for frequencies \( f \gg \frac{1}{N} \).

For frequencies of order \( \frac{1}{N} \), model-dependent corrections are expected and equation (2) is not valid anymore.

**Conditional 1-SFS and joint 2-SFS**

In the following, we will use two related but different kinds of spectra.

The first one is the joint 2-SFS of two bi-allelic sites. It is denoted \( \xi(f_1, f_2) \) for the population and \( \xi_{k,l} \) for the sample. It is defined as the density of pairs of sites with mutation frequencies at \( f_1 \) and \( f_2 \) for the population (resp. \( k/n \) and \( l/n \) for the sample). This is a natural generalization of the classical SFS for a single site. The expected spectrum \( E[\xi(f_1, f_2)] \) has two equivalent interpretations in the small \( \theta \) limit: (a) for a locus, it is the expected number of pairs of sites that harbor mutations with frequencies \( f_1 \) and \( f_2 \); (b) for two randomly chosen linked polymorphic sites, it is the probability that they contain mutations with frequencies \( f_1 \) and \( f_2 \).

The second is a conditional 1-SFS, a frequency spectrum of sites that are

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\( ^1 \)More formally, eq. (2) can be obtained from eq. (1) under the assumptions that \( \frac{1}{N} \ll f \ll 1 - \frac{1}{N} \) and that the population SFS is smooth over a range of frequencies \( \Delta f \sim \frac{1}{N} \).
linked to a focal mutation of frequency $f_0$. It is denoted $\xi(f|f_0)$ for the population and $\xi_{kl}$ for the sample. Again, this spectrum represents both (a) the expected density of single-site mutations of frequency $f$ in a locus linked to a focal neutral mutation of frequency $f_0$ and (b) the probability density that a randomly chosen site (linked at the focal site) hosts a mutation at frequency $f$.

Note that despite the similarity in notation, the two spectra $\xi(f, f_0)$ and $\xi(f|f_0)$ are different. The difference is basically the same as the one between the joint probability $p(f, f_0)$ that two sites $x$ and $x_0$ have mutations of frequency $f$ and $f_0$ respectively, and the conditional probability $p(f|f_0)$ that a mutation at site $x$ has frequency $f$ given that there is a mutation of frequency $f_0$ at a focal linked site $x_0$. Furthermore, the joint spectrum $\xi(f, f_0)$ refers to pairs of sites – i.e. it is a 2-SFS – while the spectrum of linked sites $\xi(f|f_0)$ is a single-site SFS.

The relation between both types of spectra can be understood from the relation between the probabilities. The expected spectrum $E[\xi(f)]$ is given by the probability to find a mutation of frequency $f$ at a specific site, multiplied by the length of the sequence: $E[\xi(f)] = p(f) L$. Note that, as explained above, when $L = 1$ (when a locus with a single site is considered), $E[\xi(f)]$ becomes a proper probability. Assuming the presence of a mutation of frequency $f_0$ at a focal site, we have $E[\xi(f|f_0)] = p(f|f_0)(L-1)$. For pairs of sites, the expected number of mutations at frequencies $(f, f_0)$ is $E[\xi(f, f_0)] = p(f, f_0)L(L-1)$ when $f \neq f_0$ or $p(f_0, f_0)L(L-1)/2$ when $f = f_0$. The additional factor $\frac{1}{2}$ accounts for the symmetrical case of equal frequencies $f = f_0$. The equality $p(f, f_0) = p(f|f_0)p(f_0)$ applied to sample and population spectra, results in the following relations:

$$E[\xi(f, f_0)] = \begin{cases} E[\xi(f|f_0)] \cdot E[\xi(f_0)] & \text{for } f \neq f_0 \\ \frac{1}{2} \cdot E[\xi(f|f)] \cdot E[\xi(f)] & \text{for } f = f_0 \end{cases} \quad (3)$$
\[ E[\xi_{k,l}] = \begin{cases} E[\xi_{k,l}] \cdot E[\xi_l] & \text{for } k \neq l \\ \frac{1}{2} \cdot E[\xi_{k,l}] \cdot E[\xi_l] & \text{for } k = l \end{cases} \] (4)

Note that by definition, the 2-SFS includes only pairs of sites that are both polymorphic. The probability that a pair of sites contains a single polymorphism of frequency \( k/n \) depends only on the 1-SFS and it is approximately equal to \( 2E[\xi_k] \) for \( \theta \ll 1 \). Consequently, on a sequence of size \( L \) hosting \( S \) polymorphic sites, the number of pairs of sites for which only one of the two is polymorphic of frequency \( k/n \) is \( E[(L-S)\xi_k] = L \cdot E[\xi_k] - E[S\xi_k] \approx L \cdot E[\xi_k] \) for small \( \theta \).

2 Results

2.1 Decomposition of the 2-SFS

We follow [Sargsyan (2015)] and divide the 2-SFS \( \xi(f_1, f_2) \) without recombination into two different components: one nested component \( \xi^N(f_1, f_2) \) for cases where there are individuals carrying the two mutations (one is “nested” in the other), and a disjoint component \( \xi^D(f_1, f_2) \) that includes disjoint mutations only present in different individuals. The overall spectrum is given by:

\[ \xi(f_1, f_2) = \xi^N(f_1, f_2) + \xi^D(f_1, f_2) \] (5)

\[ \xi_{k,l} = \xi^N_{k,l} + \xi^D_{k,l} \] (6)

It is noteworthy to mention that that the overall spectrum cannot fully describe the genetic state of the two sites, while the two components \( \xi^N(f_1, f_2) \), \( \xi^D(f_1, f_2) \) give a complete description up to permutations of all the haplotypes, similarly to the usual SFS for one site. As an example, given an outgroup sequence \( \ldots \text{TGCATTAC} \ldots \), the two inequivalent sets of sequences
are identical from the point of view of the overall spectrum (in both sets there is just a pair of mutations with allele count 1 and 2 respectively, therefore the only nonzero contribution is $\xi_{1,2} = \xi_{2,1} = 1$) but they are distinguished by the two components (the only nonzero contributions are $\xi_{1,2}^N = \xi_{2,1}^N = 1$ for the first set and $\xi_{1,2}^D = \xi_{2,1}^D = 1$ for the second). For this reason, we these two components constitute the core of the two-locus SFS or 2-SFS.

Without recombination, the conditional 1-SFS $\xi(f|f_0)$ can be also decomposed further into different subspectra. They are illustrated in Figure 1:

- $\xi^{(i)}(f|f_0)$: inner mutations, where the mutation is carried only by a subset of individuals with the focal mutation;
- $\xi^{(co)}(f|f_0)$: co-occurring mutations, where both mutations are systematically carried by the same individuals;
- $\xi^{(e)}(f|f_0)$: enclosing mutations, where only a subset of individuals with the mutation also carry the focal one;
- $\xi^{(cm)}(f|f_0)$: complementary mutations, where each individual has only one of the two mutations;
- $\xi^{(o)}(f|f_0)$: outer mutations, where the mutation is carried by a subset of the individuals without the focal one.

Importantly, without recombination, enclosing and complementary mutations cannot be present together in the same sequence.

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2We subdivide the “strictly nested” mutations of [Sargsyan 2015] into inner and enclosing mutations while we refer to his “identical” mutations as co-occurring.
Given the rules of conditional probabilities \( p(f, f_0) = p(f|f_0)p(f_0) \) and the interpretations above, the relations between the two sets of population sub-spectra are:

\[
E[\xi^N(f, f_0)] = \left( E[\xi^{(i)}(f|f_0)] + E[\xi^{(co)}(f|f_0)] + E[\xi^{(e)}(f|f_0)] \right) \cdot \frac{E[\xi(f_0)]}{1 + \delta_{f,f_0}} \tag{7}
\]

\[
E[\xi^D(f, f_0)] = \left( E[\xi^{(cm)}(f|f_0)] + E[\xi^{(o)}(f|f_0)] \right) \cdot \frac{E[\xi(f_0)]}{1 + \delta_{f,f_0}} \tag{8}
\]

Similarly, for sample spectra, we have

\[
E[\xi^N_{k,l}] = \left( E[\xi^{(i)}_{k|l}] + E[\xi^{(co)}_{k|l}] + E[\xi^{(e)}_{k|l}] \right) \cdot \frac{E[\xi_{l}]}{1 + \delta_{k,l}} \tag{9}
\]

\[
E[\xi^D_{k,l}] = \left( E[\xi^{(cm)}_{k|l}] + E[\xi^{(o)}_{k|l}] \right) \cdot \frac{E[\xi_{l}]}{1 + \delta_{k,l}} \tag{10}
\]

We denote the Kronecker delta by \( \delta_{x,y} \) which value is 1 if \( x = y \) and 0 otherwise. Note that \( x \) and \( y \) can be either discrete or continuous variables.

### 2.2 The unfolded joint and conditional SFS

In this section, we report the conditional and joint spectra both for the sample and the population. The derivations and proofs of all equations in this section are given in the Methods and in the Supplementary Material. A comparison of the formulae with simulations is provided in the Supplementary Material. The folded version of the 2-SFS is provided in Appendix for completeness.
2.2.1 The sample joint 2-SFS

Using equations 9 and 10 one can derive the two components of the 2-loci spectrum as:

\[
E[\xi_{N,k,l}] = \begin{cases} 
\theta^2 L^2 \frac{\beta_n(k) - \beta_n(k+1)}{2} & \text{for } k < l \\
\theta^2 L^2 \frac{\beta_n(k)}{2} & \text{for } k = l \\
\theta^2 L^2 \frac{\beta_n(l) - \beta_n(l+1)}{2} & \text{for } k > l 
\end{cases}
\]

\[
E[\xi_{D,k,l}] = \begin{cases} 
\theta^2 L^2 \left( \frac{1}{k!} - \frac{\beta_n(k) - \beta_n(k+1) + \beta_n(l) - \beta_n(l+1)}{2} \right) \frac{2 - \delta_{k,l}}{k} & \text{for } k + l < n \\
\theta^2 L^2 \left( \frac{a_n - a_k}{n-k} + \frac{a_n - a_l}{n-l} - \frac{\beta_n(k) + \beta_n(l)}{2} \right) \frac{2 - \delta_{k,l}}{k} & \text{for } k + l = n \\
0 & \text{for } k + l > n 
\end{cases}
\]

with

\[a_n = \sum_{i=1}^{n-1} \frac{1}{i}, \quad \beta_n(i) = \frac{2n}{(n-i+1)(n-i)} \left( a_{n+1} - a_i \right) - \frac{2}{n-i}\]

As shown by equation 6, the full spectrum is simply the sum of the two above equations.

2.2.2 The population joint 2-SFS

Similarly, the 2-SFS for the whole population is given by the sum of the two following equations:

\[
\begin{align*}
E[\xi^{N}(f, f_0)] &= \theta^2 L^2 \cdot \left[ \frac{1}{(1 - \min(f, f_0))^2} \left( 1 + \frac{1}{\min(f, f_0)} + \frac{2 \ln(\min(f, f_0))}{1 - \min(f, f_0)} \right) - \frac{\ln(f_0)}{1 - f_0} - \ln(f) \right] \\
E[\xi^{D}(f, f_0)] &= \theta^2 L^2 \cdot \left[ \frac{1}{f f_0} - \frac{1}{(1 - f)^2} \left( 1 + \frac{1}{f} + \frac{2 \ln(f)}{1 - f} \right) - \frac{1}{(1 - f_0)^2} \left( 1 + \frac{1}{f_0} + \frac{2 \ln(f_0)}{1 - f_0} \right) \right] \\
&\quad + \delta(f - 1 + f_0) \left( \frac{1 - f_0}{f_0^2} \log(1 - f_0) + \frac{f_0}{(1 - f_0)^2} \log(f_0) + \frac{1}{f_0(1 - f_0)} \right)
\end{align*}
\]

Note that the related formula (14) in the paper by Ferretti et al. (2012) has a sign error. It should be identical to the second equation in (11) apart for a multiplicative factor.
with \( E[\xi^D(f, f_0)] = 0 \) for \( f + f_0 > 1 \).

Here, we denote \( \delta(f - f_0) \) the density of the Dirac distribution concentrated in \( f_0 \): \( \delta(f - f_0) = 0 \) for \( f \neq f_0 \), normalized such as \( \int \delta(f - f_0) df = 1 \).

### 2.2.3 The sample conditional 1-SFS

The conditional 1-SFS for sites that are linked to a focal mutation of count \( l \) is simply the sum of all its components, given by the following equations:

\[
E[\xi^{(i)}_{k|l}] = \theta L \cdot l \frac{\beta_n(k) - \beta_n(k + 1)}{2} \quad \text{for} \quad k < l
\]

\[
E[\xi^{(co)}_{k|l}] = \theta L \cdot l \beta_n(k) \delta_{kl}
\]

\[
E[\xi^{(e)}_{k|l}] = \theta L \cdot l \frac{\beta_n(l) - \beta_n(l + 1)}{2} \quad \text{for} \quad k > l
\]

\[
E[\xi^{(cm)}_{k|l}] = \theta L \cdot l \left( \frac{a_n - a_k}{n - k} + \frac{a_n - a_l}{n - l} - \frac{\beta_n(k) + \beta_n(l)}{2} \right) \delta_{k,n-l}
\]

\[
E[\xi^{(o)}_{k|l}] = \theta L \cdot \left( \frac{1}{k} - l \frac{\beta_n(k) - \beta_n(k + 1) + \beta_n(l) - \beta_n(l + 1)}{2} \right) \quad \text{for} \quad k + l < n
\]

and 0 otherwise.

### 2.2.4 The population conditional 1-SFS

For the whole population, this becomes:

\[
E[\xi^{(i)}(f|f_0)] = \theta L \cdot \frac{f_0}{(1 - f)^2} \left( 1 + \frac{1}{f} + \frac{2 \ln(f)}{1 - f} \right), \quad f < f_0
\]

\[
E[\xi^{(co)}(f|f_0)] = \theta L \cdot \delta(f - f_0) \frac{2f_0}{1 - f_0} \left( \frac{\ln(f_0)}{1 - f_0} - 1 \right)
\]

\[
E[\xi^{(e)}(f|f_0)] = \theta L \cdot \frac{f_0}{(1 - f_0)^2} \left( 1 + \frac{1}{f_0} + \frac{2 \ln(f_0)}{1 - f_0} \right), \quad f > f_0
\]

\[
E[\xi^{(cm)}(f|f_0)] = \theta L \cdot \delta(f - 1 + f_0) \left[ \frac{1}{f_0} \log(1 - f_0) + \left( \frac{f_0}{1 - f_0} \right)^2 \log(f_0) + \frac{1}{1 - f_0} \right]
\]

\[
E[\xi^{(o)}(f|f_0)] = \theta L \cdot \left[ \frac{1}{f} - \frac{f_0}{(1 - f)^2} \left( 1 + \frac{1}{f} + \frac{2 \ln(f)}{1 - f} \right) \right. \left. - \frac{f_0}{(1 - f_0)^2} \left( 1 + \frac{1}{f_0} + \frac{2 \ln(f_0)}{1 - f_0} \right) \right], \quad f < 1 - f_0
\]
2.3 Shape of the SFS

We report the full joint 2-SFS as well as each the nested and disjoint component (Figure 2). Nested mutations have preferentially a rare mutation in either site – so that the mutation at low frequency is easily nested into the other – or are co-occurring mutations –corresponding to mutation found in the same branch. Disjoint mutations are dominated by cases where both mutations are rare – mostly disjoint – or by complementary mutations. The large contribution of co-occurring (nested component) and complementary mutations (disjoint component) is a direct consequence of the two long branches that coalesce at the root node of a Kingman tree.

The conditional 1-SFS of linked sites and the relative contributions of each component to each frequency are shown in Figure 3. Co-occurring and complementary mutations also account for a considerable fraction of the spectrum, especially when the focal mutation \( f_0 \) is at high frequency. The rest of the spectrum is biased towards mutations which frequency is lower than the focal one. Inner mutations are important only when the frequency of the focal mutation is not too low. Enclosing mutations are typically negligible and their abundance is uniform as it was also noticed by Hobolth and Wiuf (2009).

Finally, in Figure 4 we show the impact of having a focal mutation of a known frequency on two estimators of \( \theta \). The Watterson (1975) estimator, \( \hat{\theta}_S \), depends on the total number of polymorphic sites, which increases with the frequency of the focal mutation, while Tajima (1983) estimator, \( \hat{\theta}_\pi \), is more sensitive to mutations of intermediate frequency. Therefore the comparison between the two illustrates how the spectrum is skewed towards common or rare mutations. As Tajima’s \( D \) (Tajima, 1989b) is proportional to the difference \( \hat{\theta}_\pi - \hat{\theta}_S \), positive values for this test statistic suggest an excess of common mutations while negative values point to an excess of rare mutations. Figure 4 shows that the spectrum has a slight excess of rare mutations at low frequencies of the focal mutation, an excess of common mutations for intermediate frequencies, while it is apparently dominated again by rare
mutations if the focal mutation is at high frequencies.

2.4 2-SFS for ordered pairs of sites

The expected spectrum described in the previous section applies to unordered pairs of sites. As an example, consider a sequence containing just two SNPs at positions 37 and 42, with nested mutations of frequency 0.3 and 0.1 respectively. The nonzero components of the spectrum would be 

\[ \xi(0.1, 0.3) = \xi(0.3, 0.1) = 1, \]

irrespective of which of the two SNPs has frequency 0.1.

However, it can be useful to rewrite our results in terms of the spectrum \( \xi_{\text{ordered}} \) for ordered pairs of sites. Sites can be ordered by their position along the sequence, or by any other criterion. In the previous example, the components of the ordered spectrum would be \( \xi_{\text{ordered}}(0.3, 0.1) = 1 \) but \( \xi_{\text{ordered}}(0.1, 0.3) = 0 \).

The relation between the 2-SFS and the ordered 2-SFS is the following. For different frequencies \( k \neq l \), the 2-SFS of unordered pairs is symmetric, so \( \xi_{k,l} = \xi_{l,k} \) are actually the same object. However, for the ordered 2-SFS, they are different. Their sum correspond to the total number of unordered pairs:

\[ \xi_{k,l} = \xi_{k,l}^{\text{ordered}} + \xi_{l,k}^{\text{ordered}} \]  

and since the expected values do not depend on the order,

\[ E[\xi_{k,l}^{\text{ordered}}] = E[\xi_{l,k}^{\text{ordered}}] = E[\xi_{k,l}]/2 \]  

On the other hand, the order does not matter for pairs of identical mutations, i.e. \( \xi_{k,k} = \xi_{k,k}^{\text{ordered}} \) and therefore

\[ E[\xi_{k,k}^{\text{ordered}}] = E[\xi_{k,k}] \]  

These relations can be extended to the population spectrum in a straightforward way. Note that this factor 2 between both cases relates to the same factor in equations 3 and 4. In fact, \( E[\xi_{k,l}^{\text{ordered}}] = E[\xi_{k,l}] \cdot E[\xi_{l}] \).
2.5 Triallelic spectrum

As discussed before, the evolutionary dynamics of two non-recombining SNPs is the same as the one of a triallelic locus, where the three alleles are represented by the possible haplotypes of the sequence containing the SNPs. Therefore we can extract the frequency spectrum of neutral mutations in a triallelic non-recombining locus from our results.

Triallelic loci can represent many possible types of variants in genomes. They can be triallelic SNPs in any set of nucleotide sequences - these sites are rare compared to biallelic SNPs, but they exist. Or they could be Copy Number Variants, or microsatellites with variable number of repeats.

The unfolded tri-allelic spectrum for two derived alleles of frequency \( f_1, f_2 \) generated with rescaled mutation rates per locus \( \theta_1^{loc}, \theta_2^{loc} \) is

\[
\text{E}[\xi_{3al}^3(f_1, f_2)] = \theta_1 \theta_2 \left( \text{E}[\xi^D(f_1, f_2)] + \text{E}[\xi^N(f_2 - f_1, f_1)] + \text{E}[\xi^N(f_1 - f_2, f_2)] \right),
\]

where the expectations are given by equation (12) with \( \theta = 1 \).

Similarly, the sample triallelic spectrum for derived alleles of count \( k, l \) is

\[
\text{E}[\xi_{3al}^3_{k,l}] = \theta_1 \theta_2 \left( \text{E}[\xi^D_{k,l}] + \text{E}[\xi^N_{k-l,l}] + \text{E}[\xi^N_{l-k,k}] \right),
\]

where the expectations are given by equation (11) with \( \theta = 1 \).

The spectrum presented here is the unnormalised spectrum, i.e. the expected number of triallelic segregating sites with given frequencies. Note that the normalised spectrum \( \text{E}[\xi_{3al}^3_{k,l}] / \sum_{k',l'} \text{E}[\xi_{3al}^3_{k',l'}] \) – i.e. the probability that a triallelic segregating site contains alleles of a given frequency – was obtained by different methods in JENKINS and SONG (2011).

3 Methods

3.1 Two mutations

As discussed also by SARGSYAN (2015), it is easy to see that our mutation classes cover all possible relations of two mutations in a non-recombining co-
alescent. The two bi-allelic sites were created by two independent mutations: an old mutation followed by a young one. They both occurred in a single individual and then rose in frequency throughout the action of genetic drift.

The young mutation could have occurred in an individual that also carried the old mutation, leading to what we have name the “nested” case. Conversely, if the young mutation has occurred in an individual who did not have the old mutation, it leads to the “disjoint” case. As recombination is forbidden here, the complete linkage prevents any further mixing between these two cases and the derived allele that corresponds to the young mutation will remain fully linked to the background allele it occurred in.

In the nested case, the young mutation can be fixed in sequences carrying the old one (that is co-occurring case) or not. In the latter case, the young mutation can be the focal one (enclosing case) or the other one (inner case). In the disjoint case, the young mutation can get fixed among the individuals lacking the old mutation (complementary case) or not (outer case). Therefore, without recombination, these 5 types are the only possible cases.

Because these are the only possible classes of mutations without recombination, there are constrains on the frequency spectrum for linked sites. For example, the presence of an enclosing mutation of count \( k \) is incompatible with complementary mutations or outer mutations of count greater than \( n - k \); this can be shown by considering the enclosing mutation as focal one, and noticing that the other mutations would not fall in any of the previous classes.

3.2 The sample joint 2-SFS

To obtain the sample spectrum for pairs of mutations, we notice that this spectrum can be defined in terms of the expected value of crossproducts of the usual SFS. In detail, we have

\[
E[\xi_{k,l}] = E[\xi_k \xi_l], \text{ if } k \neq l
\]  

(20)
and

\[ E[\xi_{k,k}] = E[\xi_k(\xi_k - 1)]/2. \]  

These expected values have been derived by \(\text{[Fu] (1995)}\) by coalescent methods. However his results do not distinguish the different contributions from nested and disjoint mutations to the spectrum.

Tracking the origin of each term in the derivation, it is easy to show that equations (24) and (28) of \(\text{[Fu] (1995)}\) contribute to nested pairs of mutations, while equations (25), (29) and (30) contribute to disjoint pairs of mutations. All these terms combine linearly and do not interfere, therefore we can decompose the resulting \(E[\xi_k \xi_l]\) into contributions coming from equations (24),(28) and (25),(29) and (30) of \(\text{[Fu] (1995)}\). This can be obtain directly by Fu’s expression for the covariance matrix \(\sigma_{kl}\), since \(E[\xi_k \xi_l] = \delta_{k,l}E[\xi_k] + E[\xi_k]E[\xi_l] + \theta L^2 \sigma_{kl}\) and \(E[\xi_k] = \theta L/k\).

A detailed review of the calculations of \(\text{[Fu] (1995)}\), tracking the parts that lead to our mutation classes, is provided in the Supplementary Material.

### 3.3 The sample conditional 1-SFS

The spectrum for sites linked to a focal mutation of count \(l\) (equation \([13]\)) can be obtained from the previous spectrum \([11]\). The first step is simply to condition on the frequency \(l/n\) of the focal mutation, i.e. dividing the 2-SFS \(E[\xi_{k,l}]\) by \(E[\xi_l]^{1+\delta_{k,l}}/2\) following equations \([9]\) and \([10]\). In fact, \(E[\xi_{k,l}] = (L-1)P[c(x) = k|c(y) = l] = L(L-1)P[c(x) = k, c(y) = l] / LP[c(y) = l] = \frac{2}{1+\delta_{k,l}}E[\xi_{k,l}] / E[\xi_l]\) where \(c(x)\) is the derived allele count at site \(x\).

The second step is to break further the two contributions of the resulting conditional spectrum into the different components. Inner, co-occurring and enclosing mutations are derived from the nested contribution and are distinguished by site frequencies only: inner ones correspond to \(k < l\), co-occurring ones to \(k = l\) and enclosing ones to \(k > l\). Similarly, from the disjoint contribution, mutations belonging to the outer component can be obtained by selecting the frequency range \(k + l < n\) while complementary ones correspond to \(k + l = n\).
3.4 Population spectra

In the limit of large samples, the frequency spectra converge to the continuous SFS for infinite populations. However, the limit $n \to \infty$ should be taken with care. The easiest derivation proceeds as follows: since the conditional 1-SFS (eq [14]) is a single-locus spectrum, its population components can be obtained from the corresponding ones for finite samples (eq. [13]) by direct application of the equation (2). Then the population 2-SFS (eq [12]) can be reconstructed from equations (7) and (8), by multiplying by the neutral spectrum $E[\xi(f_0)] = \theta L/f_0$ and by $\frac{1}{1+\delta f,f_0}$ and joining the nested and disjoint contributions. The only tricky passage of the derivation is the functional limit of the Kronecker delta as a Dirac function: $n\delta_{\lfloor nf \rfloor,\lfloor nf_0 \rfloor} \to \delta(f-f_0)$ for $n \to \infty$.

3.5 Triallelic spectrum

The mutation process for two non-recombining loci - resulting in the generation of three alleles - resembles the mutation process for a single triallelic locus once the different mutation rates are taken into account. The rescaled mutation rates for the two mutations are $(\theta_1^{loc}, \theta_2^{loc})$ instead of $(2\theta, \theta)$ for the two-site case (the first mutation can appear in either of the loci, hence the factor of 2). Moreover, we consider a single locus instead of $L(L-1)/2 \sim L^2/2$ pairs of sites. The overall factor is therefore $\theta_1^{loc}\theta_2^{loc}/\theta^2 L^2$. Both nested and disjoint components contribute to the triallelic spectrum.

4 Discussion

In this article, we have provided the first exact closed formulae for the joint 2-SFS as well as for the conditional 1-SFS, both for sample and population. Using the basic results from [FU 1995], we were able to derive the formulae for sample spectra which we used then to derive the population spectra by letting $n \to \infty$. Importantly, our results only hold when there is no
recombination, and are averaged across the tree space.

The analytical expressions provided in this paper are consistent with the intuition on the evolution of linked mutations. Consider a new mutation increasing in frequency by neutral drift and reaching low/intermediate frequency. We would expect to find a large number of outer linked mutations and a low number of inner mutations, since at the birth time of the mutation all other mutations were outer. For similar reasons, enclosing mutations would be less abundant than outer mutations. The spectrum of inner mutations should be more skewed towards rare alleles than the neutral one, since inner mutations evolve inside an expanding subpopulation. On the other hand, the spectrum of outer mutations should resemble the neutral one. These expectations are confirmed by our results.

Note that for sequences linked to a mutation close to fixation, co-occurring and complementary mutations dominate and produce a strong “haplotype structure” due to the residual ancestral haplotype.

Interestingly, conditioning on the presence of a mutation of frequency $f$ impacts the length and balance of the tree, as apparent from Figure 4. This can be understood as follows. Rare mutations are common in any possible tree, therefore they just increase slightly the tree length. Instead, mutations of intermediate frequency tend to occur mostly in the upper branches of the tree, therefore the presence of such mutations implies higher, more balanced trees. The effect is even stronger for high frequency mutations, which reside only in the uppermost branches, implying high unbalanced trees.

There are several potential applications of these results. Direct applications include the improvement of inference techniques based on the SFS, like composite likelihood (e.g., Kim and Stephan 2002; Li and Stephan 2005; Kim and Nielsen 2004; Nielsen et al. 2005) and Poisson Random Field methods Sawyer and Hartl (1992). These methods use analytical expressions for the SFS for a single site together with approximations of independence between different sites. For non-recombining sequences, they could be improved by assuming independence between different pairs of sites, while
taking pairwise dependence between sites into account through the two-locus SFS developed here.

The spectrum could also be useful for new neutrality tests based on linkage between mutations. Our results lead to a better understanding of the linkage disequilibrium (LD) structure among neutral loci, therefore they can be immediately applied to LD-related statistics, for example to compute the average LD across non-recombining neutral loci. Furthermore, they can be used to build neutrality tests optimised to detect positive or balancing selection through its effect on the frequency spectrum of linked sites.

The classical correspondence between the Kingman model in the large $n$ limit and the diffusion approximation suggests that the 2-SFS spectrum presented here is a solution of the diffusion equations for three alleles (Ewens 2012). As in the case of the standard 1-SFS, the remarkably simple form of the solutions could be due to the simplicity of the diffusion equations. In fact, it is easy to check that the inner and enclosing components are solutions of the corresponding diffusion equation:

$$\frac{\partial \xi}{\partial t} = \frac{1}{2N_e} \left( \frac{\partial^2}{\partial f^2} \left[ f(1-f)\xi \right] + 2 \frac{\partial^2}{\partial f \partial f_0} \left[ f(1-f_0)\xi \right] + \frac{\partial^2}{\partial f^2} \left[ f_0(1-f_0)\xi \right] \right)$$

while the disjoint part is a solution of the equation:

$$\frac{\partial \xi}{\partial t} = \frac{1}{2N_e} \left( \frac{\partial^2}{\partial f^2} \left[ f(1-f)\xi \right] - 2 \frac{\partial^2}{\partial f \partial f_0} \left[ f f_0\xi \right] + \frac{\partial^2}{\partial f^2} \left[ f_0(1-f_0)\xi \right] \right)$$

Our work suggests that the whole solution (12) or equivalently (14) is actually the solution of the appropriate full set of equations for the system (including conditions and equations at the boundaries). A direct proof of this result could lead to interesting developments towards new solutions for selective equations as well.

The SFS presented here is the simplest two-locus spectrum for neutral, non-recombining mutations in a population of constant size. These results could be extended to variable population size using the approach of Živković and Wiehe (2008); Jenkins and Song (2011) and to mutations in rapidly adapting populations using the Λ-coalescent approximation and the results
of Birkner et al. (2013). However, the most interesting extensions would be to consider (a) non-neutral mutations and (b) recombination.

Adding selection to the two-locus SFS would significantly enhance its potential for most of the applications discussed above. The SFS for pairs of selected mutations has been obtained by Xie (2011) as a polynomial expansion. However, the computation is still cumbersome, while flexible numerical alternative could be soon available. Given the simplicity of the expression for the single-locus SFS $\xi(f) = \frac{\theta(1 - e^{-2Ne_s(1-f)})/f}{(1-f)(1-e^{-2Ne_s})} \left[\text{Wright}\right]$ 1938; Sawyer and Hartl 1992), we expect that closed expressions could be found for pairs of mutations with different selective coefficients. This would be a promising development for future investigations.

On the other hand, finding the exact two-locus SFS with recombination appears to be a difficult problem. Recombination is intrinsically related to the two-locus SFS via the same definition of linkage disequilibrium. For this reason, many approximations and partial results have been developed since Hudson (2001), like expansions in the limit of strong recombination (Jenkins and Song 2012). The SFS of linked loci presented in this paper could be indirectly useful as a starting point for more precise investigations of the effect of recombination events. For example, it could be used as a starting point for perturbation expansions at low recombination rates.

Since recombination events follow the same Poisson process as mutation events, although with a different rate, the spectrum $\xi_{k,l}$ could also be reinterpreted (up to a constant) as the probability that a recombination event reached frequency $k$ in a sequence linked to a specific mutation of frequency $l$, i.e. it is equivalent to the spectrum of mutation-recombination events.

Obtaining the full two-locus spectrum with selection and recombination could open new avenues for model inference and analysis of genomic data.
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A  The folded spectra

When no reliable outgroup sequence is available, one cannot assess if the allele is derived or ancestral. In that case, alleles can only be classified as minor (less frequent) and major (most frequent). The distribution of minor allele frequencies, known as the folded SFS, will be noted \( \eta(f^*) \), where \( f^* \) denotes the minor allele frequency that ranges from 0 to 0.5. Importantly, the folded SFS can be retrieved from the full SFS by simply summing alleles at complementary frequencies:

\[
\eta(f^*) = \frac{\xi(f^*) + \xi(1 - f^*)}{1 + \delta_{f^*,(1-f^*)}} 
\]

As a consequence, the single site SFS under the standard neutral model then become \( E[\eta(f^*)] = \theta/[f^*(1 - f^*)(1 + \delta_{f^*,(1-f^*)})] \) and \( E[\eta_k] = \theta n/[k^*(n - k^*)(1 + \delta_{k^*,n-k^*})] \), where \( k^* \) denotes the count of the minor allele.

Following the same idea, we define a conditional folded 1-SFS and a joint folded 2-SFS using the minor allele frequencies. Minor alleles can also be classified as “nested” or “disjoint” depending on the presence or absence of individuals enclosing both minor alleles. As for the unfolded case, this classification gives a complete description of the linkage between pairs of mutations. However, in contrast to the unfolded case, the classification has no strict evolutionary meaning. For example, “disjoint” minor alleles do not necessarily correspond to pairs of alleles born in different backgrounds. Moreover, alleles of frequency \( f^* = 0.5 \) (or allele count \( k^* = n/2 \)) suffer from an ambiguity in the choice of the minor allele and therefore should be treated separately. Note also that with the exception of alleles with frequency 0.5, folded spectra do not contain complementary alleles, since the frequency of one of the two complementary alleles will exceed 0.5.

Pairs of mutations with \( f, f_0 \) both larger or smaller than 0.5 will be classified identically (as nested or disjoint) in the folded case. However, pairs of mutations with \( f < 0.5 \) and \( f_0 > 0.5 \) (or vice-versa) will swap their classification.
As a consequence, the two components of the 2-SFS are:

\[
E[\eta^N(f^*, f_0^*)] = E[\xi^N(f^*, f_0^*)] + E[\xi^N(1 - f^*, 1 - f_0^*)] + E[\xi^D(f^*, 1 - f_0^*)] \\
E[\eta^D(f^*, f_0^*)] = E[\xi^D(f^*, f_0^*)] + E[\xi^N(f^*, 1 - f_0^*)] + E[\xi^N(1 - f^*, f_0^*)]
\] (25)

To obtain the conditional 1-SFS, we proceed similarly to the unfolded case. First we separate the 2-SFS above into components based on frequency. The inner component corresponds to frequencies \(f^* < f_0^*\) of the nested part, while the cooccurring and enclosing components correspond to \(f^* = f_0^*\) and \(f^* > f_0^*\) respectively. The outer component corresponds to the disjoint part, since there cannot be any complementary component. Then we divide each component by the expected 1-SFS \(E[\eta(f_0^*)]\) to obtain

\[
E[\eta^{(i)}(f^*|f_0^*)] = \frac{f_0^*(1 - f_0^*)}{\theta} E[\eta^N(f^*, f_0^*)] \quad \text{for } f^* < f_0^* \\
E[\eta^{(co)}(f^*|f_0^*)] = 2 \cdot \frac{f_0^*(1 - f_0^*)}{\theta} E[\eta^N(f^*, f_0^*)] \quad \text{for } f^* = f_0^* \\
E[\eta^{(e)}(f^*|f_0^*)] = \frac{f_0^*(1 - f_0^*)}{\theta} E[\eta^N(f^*, f_0^*)] \quad \text{for } f^* > f_0^* \\
E[\eta^{(co)}(f^*|f_0^*)] = 0 \\
E[\eta^{(o)}(f^*|f_0^*)] = (1 + \delta f^*, f_0^*) \cdot \frac{f_0^*(1 - f_0^*)}{\theta} E[\eta^D(f^*, f_0^*)]
\] (26)

While the classification of the pairs with frequencies \(f^* = 0.5\) and/or \(f_0^* = 0.5\) is ambiguous, these pairs are usually irrelevant for the population spectrum. The sample spectra are similar. For \(n\) even, there are ambiguous pairs with \(k\) or \(l = n/2\) that can be easily retrieved from the equations (11), (13) and treated separately. Considering only \(k, l < n/2\), the sample 2-SFS is:

\[
E[\eta^{N}_{k^*, l^*}] = E[\xi^{N}_{k^*, l^*}] + E[\xi^{N}_{n-k^*, n-l^*}] + E[\xi^{D}_{k^*, n-l^*}] + E[\xi^{D}_{n-k^*, l^*}] \\
E[\eta^{D}_{k^*, l^*}] = E[\xi^{D}_{k^*, l^*}] + E[\xi^{N}_{k^*, n-l^*}] + E[\xi^{N}_{n-k^*, l^*}] + E[\xi^{D}_{n-k^*, l^*}]
\] (27)
and the conditional 1-SFS is:

\[
\begin{align*}
E[\eta_{k^*|l^*}^{(i)}] &= \frac{l^*(n - l^*)}{\theta_n} E[\eta_{k^*|l^*}^N] \quad \text{for } k^* < l^* \\
E[\eta_{k^*|l^*}^{(co)}] &= 2 \cdot \frac{l^*(n - l^*)}{\theta_n} E[\eta_{k^*|l^*}^N] \quad \text{for } k^* = l^* \\
E[\eta_{k^*|l^*}^{(e)}] &= \frac{l^*(n - l^*)}{\theta_n} E[\eta_{k^*|l^*}^N] \quad \text{for } k^* > l^* \\
E[\eta_{k^*|l^*}^{(em)}] &= 0 \\
E[\eta_{k^*|l^*}^{(o)}] &= (1 + \delta_{k^*,l^*}) \cdot \frac{l^*(n - l^*)}{\theta_n} E[\eta_{k^*|l^*}^D] 
\end{align*}
\]
Figure 1: Two examples of non-recombining loci and their corresponding genealogical trees. This figure illustrates the classification of all possible types of mutations with respect to the focal mutation (in red) and their occurrence on the sequence tree.
Figure 2: Plots of nested and disjoint contributions to the two-locus frequency spectrum for \( \theta L = 1, n = 20 \). Note the different scales of the two plots.

Figure 3: Barplot of the spectrum of linked sites for \( \theta L = 1, n = 20 \), each column colored according to the different contributions. The focal mutation has frequency 5/20=0.25 (left), 10/20=0.5 (middle) and 15/20=0.75 (right) respectively.
Figure 4: Mean values of the Watterson estimator ($\hat{\theta}_S$) and Tajima estimator ($\hat{\theta}_π$) of $\theta$ conditioned on the presence of a linked mutation, for $\theta = 1$, $n = 20$. In the inset, approximate mean value of Tajima’s $D$ (computed substituting $S$ with its mean value in the denominator).
Supplementary Material

A.1 Simulations

In this section we present a numerical result as an example to check the consistency of our results. In Figure S1 the analytical sample spectrum is compared with those obtained by coalescent simulations. We parsed the output of *ms* [Hudson 2002] to count the number of mutations conditional on a focal mutation of given frequency. The good agreement between the spectra supports our equations.

The source code (C++) for computing analytical as well as simulated spectra can be found in the package *coatli* developed by one of the authors and available on [http://sourceforge.net/projects/coatli/](http://sourceforge.net/projects/coatli/).

Figure S1: Frequency spectrum of nested mutations in linked sites for n=20, \( L\theta = 1 \) and a focal mutation of frequency \( k = 10 \), compared with coalescent simulations (averages for different numbers of samples).
A.2 Fu 1995 reloaded

The 1995 paper by [Fu (1995)] derived the second moments of the Kingman coalescent [Kingman (1982)], more precisely the covariance of mutations of size $i$ and $j$: $\text{Cov}[\xi_i, \xi_j]$. Unfortunately the very tight presentation and some typos may make it hard to follow the transformations. Here we lay out some essential parts in greater detail and show how they lead to our expressions for different mutations classes.

Figure S2: A coalescent tree describing the genealogy of a non-recombining locus for a sample of size $n = 4$. The topology of the tree is defined by the relationship between the lines, e.g. line $\xi_{43}$ is a descendant of lines $\xi_{33}$ and $\xi_{22}$, but not of any other line. A mutation happening “on” line $\xi_{33}$ is of size 2, since it has two descendant lines (and hence leaves) at state $n = 4$, i.e. two individuals of the sample carry it. All lines of the same state have the same length, reflecting the same mutation probability. Hence the amount of mutations of size 1 (“singletons”) occurring on $\xi_{31}$ and $\xi_{32}$ is correlated with the amount of mutations of size 2 arising on $\xi_{33}$. Averaging over different topologies leads to more complicated correlations.

As a starting point for the combinatorics let us note that the descendance of lines in the coalescent can be described by a Polya urn process, and the
two expressions given beneath are special cases of a general formula (c.f. e.g. Griffiths and Tavaré (2003)). We introduce the following notation: let $p_{k \rightarrow n}(t \rightarrow i)$ denote the probability that $t$ lines at state $k$ have $i$ descendents at state $n$. This probability is

$$p_{k \rightarrow n}(t \rightarrow i) = \frac{(i-1) \binom{n-i-1}{t-1}}{\binom{n-1}{k-t-1}}$$

and the probability that $t$ and $u$ lines at state $k$ have respectively $i$ and $j$ descendents at state $n$ is

$$p_{k \rightarrow n}(t \rightarrow i, u \rightarrow j) = \frac{(i-1) \binom{j-1}{u-1} \binom{n-i-j-1}{k-t-u-1}}{\binom{n-1}{k-1}}.$$ 

In order to avoid case distinctions it is helpful to abuse for a while the notation by defining $\binom{-1}{1} = 1$ and $\binom{n}{k} = 0$ for any other combination of $n < 0$ or $k < 0$. This makes it possible to subsume in the above and following formulas “boundary cases” such as $k$ lines of state $k$ yielding the $n$ lines of state $n$ (with probability 1). Later on these special cases will be considered separately and the final expressions don’t contain any negative values.

The probability that a line at state $k$ is of size $i$ is referred to as $p(k, i)$. The probability that two lines at state $k$ are of size $i$ and $j$ is referred to as $p(k, i; k', j)$. The probability that a line at state $k$ and another at state $k' > k$ are of size $i$ respective $j$ is split up with respect to the latter line being a descendant of the former line or not: $p(k, i; k', j) = p_a(k, i; k', j) + p_b(k, i; k', j)$. 
The two formulas above suffice to derive these probabilities:

\[ p(k, i) = p_{k \rightarrow n}(1 \rightarrow i) = \frac{(n-i-1)}{(k-1)} \]

\[ p(k, i; k, j) = p_{k \rightarrow n}(1 \rightarrow i, 1 \rightarrow j) = \frac{(n-i-j-1)}{(k-3)} \]

\[ p_a(k, i; k', j) = \sum_{t=1}^{k'-1} \frac{k'-t}{k'} p_{k \rightarrow k'}(1 \rightarrow t) \frac{t}{k'} p_{k' \rightarrow n}(1 \rightarrow j, t-1 \rightarrow i-j) \]

\[ = \sum_{t=1}^{k'-1} \frac{(k'-t-1)}{(k-1)} \frac{t}{k'} \left( \frac{(n-i-1)}{k'-t-1} \right) \]

\[ p_b(k, i; k', j) = \sum_{t=1}^{k'-1} \frac{k'-t}{k'} p_{k \rightarrow k'}(1 \rightarrow t) \frac{k'-t}{k'} p_{k' \rightarrow n}(1 \rightarrow j, t \rightarrow i) \]

\[ = \sum_{t=1}^{k'-1} \frac{(k'-t-1)}{(k-1)} \frac{k'-t}{k'} \left( \frac{(n-i-j-1)}{k'-t-1} \right) \]

In the latter two formulas, the summation index \( t \) stands for the number of descendants, that the line from state \( k \) may have at state \( k' \).

Now we consider the “mutational” correlation between the lines. Other than in our main article, \( \theta \) denotes here the locus mutation rate (not the site mutation rate), i.e. includes the locus length \( L \).

Let \( X \) be a random variable. It can be easily shown that, if \( X \) is exponentially distributed (\( X \sim \text{Exp}(\lambda) \)), then the first two moments of \( X \) are \( E[X] = \frac{1}{\lambda} \) and \( E[X^2] = \frac{2}{\lambda^2} \). If \( X \) is Poisson-distributed (\( X \sim \text{Pois}(\mu) \)), then \( E[X] = \mu \) and \( E[X^2] = \mu + \mu^2 \). By definition of the coalescent the \( \xi_{kl} \) are distributed like \( \xi_{kl} \sim \text{Pois}(\theta T_k) \) with \( T_k \sim \text{Exp}(\frac{2}{k(k-1)}) \). \( \xi_{kl} \) and \( \xi_{kl'} \) are independent if \( k \neq k' \) while \( \xi_{kl} \) and \( \xi_{kl'} \) are independent conditional on \( T_k \) for \( l \neq l' \). We have thus

\[ T_k \sim \text{Exp}(\lambda_k) \] with \( \lambda_k = \frac{k(k-1)}{2} \) and \( \xi_{kl} \sim \text{Pois}(\mu) \) with \( \mu = \frac{\theta}{2} T_k \)
\[
E[\xi_{kl}] = E[E[\xi_{kl}|T_k]] = E[\frac{\theta}{2} T_k] = \frac{1}{k(k-1)} \theta
\]

\[
E[\xi_{kl}^2] = E[E[\xi_{kl}^2|T_k]]
= E[\frac{\theta}{2} T_k + (\frac{\theta}{2} T_k)^2]
= \frac{\theta}{2} E[T_k] + \frac{\theta^2}{4} E[T_k^2]
= \frac{2}{k(k-1)} \frac{\theta}{2} + 2 \frac{2^2}{k^2(k-1)^2} \frac{\theta^2}{4}
= \frac{1}{k(k-1)} \theta + \frac{2}{k^2(k-1)^2} \theta^2
\]

\[
E[\xi_{kl} \xi_{kl'}] = E[E[\xi_{kl} \xi_{kl'}|T_k]]
= E[E[\xi_{kl}|T_k]E[\xi_{kl'}|T_k]]
= E[(\frac{\theta}{2} T_k)^2]
= \frac{2}{k^2(k-1)^2} \theta^2
\]

\[
E[\xi_{kl} \xi_{k'l}] = E[\xi_{kl}] E[\xi_{k'l}]
= \frac{1}{k(k-1)k'(k'-1)} \theta^2
\]

For a particular topology, the number of mutations of size \(i\) can be parcelled onto lines as

\[
\xi_i = \sum_{k=2}^{n} \sum_{l=1}^{k} \epsilon_{kl}(i) \xi_{kl}
\]

with the “indicator-variable” \(\epsilon_{kl}(i) = 1\) if line \(\xi_{kl}\) has \(i\) descendent leaves and
0 otherwise. We take the expectation over all topologies and branch lengths:

$$E[\xi_i \xi_j] = E\left[\left(\sum_{k=2}^n \sum_{l=1}^k \epsilon_{kl}(i) \xi_{kl}\right)\left(\sum_{k'=2}^n \sum_{l'=1}^{k'} \epsilon_{k'l'}(j) \xi_{k'l'}\right)\right]$$

$$= \sum_{k=2}^n \sum_{k'=2}^n \sum_{l=1}^k \sum_{l'=1}^{k'} E[\epsilon_{kl}(i) \epsilon_{k'l'}(j)] E[\xi_{kl} \xi_{k'l'}]$$

$$= \sum_{k=2}^n \sum_{l=1}^k E[\epsilon_{kl}(i) \xi_{kl}] + \sum_{k=2}^n \sum_{l=1}^{k-1} \sum_{l'=l+1}^n E[\epsilon_{kl}(i) \epsilon_{k'l'}(j)] E[\xi_{kl} \xi_{k'l'}] + \sum_{k=2}^n \sum_{k'=2}^n \sum_{l=1}^k \sum_{l'=1}^{k'} E[\epsilon_{kl}(i) \epsilon_{k'l'}(j)] E[\xi_{kl} \xi_{k'l'}]$$

$$= \delta_{i=j} \sum_{k=2}^n kp(k, i) E[\xi_{kl}^2] + \sum_{k=2}^n k(k-1)p(k, i; k, j) E[\xi_{kl} \xi_{k'l}] + \sum_{k=2}^n \sum_{k'=k+1}^{n-1} kk'p(k, i; k', j) + p(k, j; k', i)) E[\xi_{kl} \xi_{k'l'}]$$

If we define for $k < k'$

$$s^1(i) = \sum_{k=2}^n kp(k, i) \frac{1}{k(k-1)}$$

$$s^2(i) = \sum_{k=2}^n kp(k, i) \frac{2}{k^2(k-1)^2}$$

$$s(i, j) = \sum_{k=2}^n k(k-1)p(k, i; k, j) \frac{2}{k^2(k-1)^2}$$

$$s_a(i, j) = \sum_{k=2}^{n-1} \sum_{k'=k+1}^{n} kk'p_a(k, i; k', j) \frac{1}{k(k-1)k'(k'-1)}$$

$$s_b(i, j) = \sum_{k=2}^{n-1} \sum_{k'=k+1}^{n} kk'p_b(k, i; k', j) \frac{1}{k(k-1)k'(k'-1)}$$

then

$$E[\xi_i \xi_j] = \delta_{i=j} s^1(i) \theta +$$

$$\left(\delta_{i=j} s^2(i) + s(i, j) + s_a(i, j) + s_a(j, i) + s_b(i, j) + s_b(j, i)\right) \theta^2 .$$
The different relations between lines correspond to our subdivision of the conditional frequency spectrum. In particular, we have

\[ E[\xi_{ij}] = \delta_{i<j} \theta^2 j \ s_a(j, i) \]
\[ E[\xi_{ij}^{(co)}] = \delta_{i=j} \theta^2 j \ (s^2(i) + 2s_a(i, i)) \]
\[ E[\xi_{ij}^{(c)}] = \delta_{i>j} \theta^2 j \ s_a(i, j) \]
\[ E[\xi_{ij}^{(cm)}] = \delta_{i+j=n} \theta^2 j \ (s(i, j) + s_b(i, j) + s_b(j, i)) \]
\[ E[\xi_{ij}^{(o)}] = \delta_{i+j<n} \theta^2 j \ (s(i, j) + s_b(i, j) + s_b(j, i)) \]

The following derivations simplify these expressions until we finally yield the equations [13].

The simplification makes use of two known formulas for binomial coefficients:

\[ \sum_{m=0}^{n} \binom{m}{k} = \binom{n+1}{k+1} \] \hspace{1cm} (B1)

\[ \sum_{j=0}^{k} \binom{m}{j} \binom{n-m}{k-j} = \binom{n}{k} \] \hspace{1cm} (B2)

The second equation is called the “Chu-Vandermonde identity”. In the first equation, the summation can start as well at \( m = k \) since \( \binom{m}{k} = 0 \) for \( m < k \).

Furthermore we need three helping equations from [Fu (1995)]:

The straight-forward computable equation 14

\[ \frac{n-i-1}{k-2} = \frac{n-i-2}{k-1} \frac{k-1}{i} \]

and the technically more demanding equations 34

\[ 2 \sum_{k=2}^{n} \frac{n-k}{i-1} \frac{1}{k} = \beta_n(i) \]

and 36

\[ \sum_{k=3}^{n} \frac{n-k-2}{k-3} \frac{1}{k(k-1)} = \frac{\beta_n(i) - \beta_n(i+1)}{2} \].
A useful variation of equation (34), needed repeatedly, can be derived using his equation (33) (not replicated here):

\[
\frac{1}{\binom{n-1}{i}} \sum_{k=2}^{n} \frac{\binom{n-k}{i-1}}{k-1} = \frac{1}{\binom{n-1}{i}} \sum_{k=1}^{n-1} \frac{\binom{n-1-k}{i-1}}{k}
\]

\[
= \binom{n}{i} \frac{1}{i^2} \binom{n-1}{i-1} (a_n - a_i)
\]

\[
= \frac{a_n - a_i}{n - i}.
\]  

(34a)

Now we have to account for the “boundary cases” in the probability expressions \(p()\). As defined above, a binomial coefficient \(\binom{a}{b}\) with \(a = -1\) is non-zero only for \(b = -1\), which translates to additional constraints on the state \(k\) and the number of descendants that the line from this state can have at state \(k'\):

For example, if in the expression

\[
p(k, i; k, j) = \binom{n-i-j-1}{k-3} \binom{n-1}{k-1}
\]

we have \(i + j = n\), then the descendants of two lines encompass the whole sample. However this is only possible for the two lines of state \(k = 2\). The same reasoning applied on \(p_a(k, i; k', j)\) for \(i = j\) leads to the condition, that the summation is only over one element, namely \(t = 1\). Finally, if \(i + j = n\) in the expression for \(p_b(k, i; k', j)\), then \(k = 2\) and \(t = k' - 1\).
\[ s^1(i) = \sum_{k=2}^{n} kp(k, i) \frac{1}{k(k-1)} \]
\[ = \sum_{k=2}^{n} k \left( \frac{n-k}{i} \right)(k-1) \frac{1}{(n-i)^i} k(k-1) \]
\[ = \frac{1}{i} \sum_{k=0}^{n-2} \left( \frac{k}{i} \right) \left( \frac{n-k}{i} \right) \]
\[ B_1 = \frac{1}{i} \]

\[ s^2(i) = \sum_{k=2}^{n} kp(k, i) \frac{k}{k^2(k-1)^2} \]
\[ = \sum_{k=2}^{n} k \left( \frac{n-k}{i} \right)(k-1) \frac{2}{(n-i)^i} k(k-1)^2 \]
\[ = \sum_{k=2}^{n} \left( \frac{n-k}{i} \right) \frac{k}{k(k-1)} \]
\[ = 2 \sum_{k=2}^{n} \left( \frac{n-k}{i} \right) \left( \frac{1}{k} - \frac{1}{k-1} \right) \]
\[ B^1_{ii} = \frac{2a_n - a_i}{n-i} - \beta_n(i) \]

\[ s(i, j) = \sum_{k=2}^{n} k(k-1)p(k, i; k, j) \frac{2}{k^2(k-1)^2} \]
\[ = \sum_{k=2}^{n} \left( \frac{n-i-j}{k-3} \right) \frac{2}{k(k-1)} \]
\[ = \delta_{i+j<n} \sum_{k=3}^{n} \left( \frac{n-i-j-2}{k-3} \right) \frac{2}{k(k-1)} + \delta_{i+j=n} \frac{1}{n-1} \]
\[ = \delta_{i+j<n} \left( \beta_n(i + j - 1) - \beta_n(i + j) \right) + \delta_{i+j=n} \frac{1}{n-1} \]
Case $i > j$ ($\Rightarrow t \geq 2$)

$$s_a(i, j) = \sum_{k'=3}^{n} \sum_{k=2}^{k'-1} kk'p_a(k, i; k', j) \frac{1}{k(k-1)k'(k'-1)}$$

$$= \sum_{k'=3}^{n} \sum_{k=2}^{k'-1} \sum_{t=2}^{k'-1} \frac{(k'-1)_{t-1}}{k-1} t \frac{(i-j-1)(n-i-1)}{(k'-t-1)(k'-1)} \frac{1}{(k-1)(k'-1)}$$

$$= 14 \sum_{k'=3}^{n} \sum_{k=2}^{k'-1} \sum_{t=2}^{k'-1} \frac{(i-j-1)(n-i-1)}{(k'-1)} \frac{1}{k'(k'-1)}$$

$$= \sum_{k'=3}^{n} \sum_{t=2}^{k'-3} \frac{(i-j-1)(n-j-2-(i-j-1))}{(k'-3-t)} \frac{1}{(k'-1)} \frac{1}{k'(k'-1)}$$

$$= 36 \frac{\beta_n(j) - \beta_n(j+1)}{2}$$

Case $i = j$ ($\Rightarrow t = 1$)

$$s_a(i, j) = \sum_{k'=3}^{n} \sum_{k=2}^{k'-1} kk'p_a(k, i; k', j) \frac{1}{k(k-1)k'(k'-1)}$$

$$= \sum_{k'=3}^{n} \sum_{k=2}^{k'-1} \frac{(k'-2)(k'-1)}{k-1} \frac{1}{k'(k'-1)} \frac{(n-i-1)}{(k'-2)} \frac{1}{(k-1)(k'-1)}$$

$$= \sum_{k'=3}^{n} \sum_{k=2}^{k'-1} \frac{k-1}{k'-1} \frac{1}{k'(k'-1)} \frac{(n-i-1)}{(k'-2)} \frac{1}{(k-1)(k'-1)}$$

$$= \sum_{k'=3}^{n} \frac{(n-i-1)}{(k'-1)} \frac{k'-2}{k(k'-1)^2}$$

$$= 14 \sum_{k'=3}^{n} \frac{(n-i-1)}{(k'-1)} \frac{k'-2}{i k'(k'-1)}$$

$$= \sum_{k'=2}^{n} \frac{(n-i-1)}{i} \left( \frac{2}{k'} - \frac{1}{k'-1} \right)$$
34 \beta_n(t) - \frac{a_n - a_i}{n - t}

Case i + j < n (\Rightarrow t \leq k' - 2)

\begin{align*}
s_b(i, j) &= \sum_{k'=3}^{n} \sum_{k=2}^{k'-1} kk'p_b(k, i; k', j) \frac{1}{k(k-1)k'(k'-1)} \\
&= \sum_{k'=3}^{n} \sum_{k=2}^{k'-1} \sum_{t=1}^{(k'-2)} \frac{(k'-t-1)}{(k'-1)} \frac{k' - t (i-j)}{(k'-1)} \frac{(n-i-j-1)}{(k'-2)} \frac{1}{k-1} \\
&= \sum_{k'=3}^{n} \sum_{k=2}^{k'-1} \sum_{t=1}^{(k'-2)} \frac{1}{tk'k'(k'-1)} \frac{k' - t (i-j)}{(k'-1)} \frac{(n-i-j-1)}{(k'-2)} \frac{1}{k-1} \\
&= \sum_{k'=3}^{n} \sum_{k=2}^{k'-1} \left( \sum_{t=2}^{(k'-2)} \frac{1}{tk'k'(k'-1)} \frac{k' - t (i-j)}{(k'-1)} \frac{(n-i-j-1)}{(k'-2)} \frac{1}{k-1} \right) \\
&= \sum_{k'=3}^{n} \sum_{k=2}^{k'-1} \frac{1}{tk'k'(k'-1)} \frac{k' - t (i-j)}{(k'-1)} \frac{(n-i-j-1)}{(k'-2)} \frac{1}{k-1} \\
&\quad - \sum_{k'=3}^{n} \sum_{t=1}^{(k'-2)} \frac{1}{tk'(k'-1)} \frac{(i-j)}{(k'-2)} \frac{(n-i-j-1)}{(k'-3)} \\
&\quad + \sum_{k'=3}^{n} \frac{1}{k'-1} \frac{(i-j)}{(k'-2)} \frac{(n-i-j-1)}{(k'-3)} \\
&= \sum_{k'=3}^{n} \frac{1}{tk'(k'-1)} \frac{(i-j)}{(k'-2)} \frac{(n-i-j-1)}{(k'-3)} \\
&\quad - \sum_{k'=3}^{n} \frac{1}{tk'(k'-1)} \frac{(i-j)}{(k'-2)} \frac{(n-i-j-1)}{(k'-3)} \\
&= \frac{1}{i} \sum_{k'=3}^{n} \frac{1}{k'-1} \frac{(i-j)}{(k'-2)} \frac{(n-i-j-1)}{(k'-3)} \\
&= \frac{1}{i} \sum_{k'=3}^{n} \left( \sum_{t=0}^{(k'-2)} \frac{1}{k'-1} \frac{(i-j)}{(k'-2-t)} \frac{(n-i-j-1)}{(k'-3)} - \frac{1}{k'-1} \frac{(i-j)}{(k'-2-t)} \right)
\end{align*}
\[-\sum_{k'=3}^{n} \sum_{t=0}^{k'-3} \frac{1}{k'(k'-1)} \left( \frac{1}{k'-1} \right)^{t} \left( \frac{n-j-2-(i-1)}{k'-3-t} \right) - \sum_{k'=3}^{n} \frac{1}{k'(k'-1)} \left( \frac{n-i-j-1}{k'-3} \right) \]

\[\frac{1}{i} \sum_{k'=3}^{n} \left( \frac{1}{k'-1} \left( \frac{n-j-1}{k'-2} \right) - \frac{1}{k'-1} \left( \frac{n-i-j-1}{k'-2} \right) \right) \]

\[= \frac{1}{i} \sum_{k'=3}^{n} \left( \frac{n-k'}{n-j-1} \right) \frac{1}{j} - \frac{1}{i} \frac{1}{i+j} \]

\[= \frac{1}{2} (\beta_n(j) - \beta_n(j + 1) + \beta_n(i + j - 1) - \beta_n(i + j)) \]

Case \(i + j = n\) (\(\Rightarrow k = 2\) and \(t = k' - 1\))

\[s_b(i, j) = \sum_{k'=3}^{n} k' p_b(2, i; k', j) \frac{1}{k'(k'-1)} \]

\[= \sum_{k'=3}^{n} \frac{1}{k'-1} \left( \frac{1}{k'-1} \right) \frac{1}{k'-1} \]

\[= \sum_{k'=3}^{n} \frac{1}{(k'-1)} \frac{1}{k'\left(k'-1\right)^2} \]

\[= \sum_{k'=3}^{n} \frac{1}{(n-j-1)} j k'\left(k'-1\right) \]

\[= \sum_{k'=2}^{n} \frac{1}{(n-j-1)} j k'\left(k'-1\right) - \frac{1}{(n-j-1)} \frac{1}{2j} \]

\[= \sum_{k'=2}^{n} \frac{1}{(n-j-1)} j \left( \frac{1}{k'-1} - \frac{1}{k'} \right) - \frac{1}{2(n-1)} \]

\[\frac{a_n - a_j}{n - j} - \frac{1}{2} \beta_n(j) - \frac{1}{2(n-1)} \]

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