Molecular Clock on a Neutral Network

Alpan Raval

Keck Graduate Institute of Applied Life Sciences,
535 Watson Drive, Claremont, California 91711, USA

School of Mathematical Sciences, Claremont Graduate University,
711 N. College Avenue, Claremont, California 91711, USA

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Abstract

The number of fixed mutations accumulated in an evolving population often displays a variance that is significantly larger than the mean (the overdispersed molecular clock). By examining a generic evolutionary process on a neutral network of high-fitness genotypes, we establish a formalism for computing all cumulants of the full probability distribution of accumulated mutations in terms of graph properties of the neutral network, and use the formalism to prove overdispersion of the molecular clock. We further show that significant overdispersion arises naturally in evolution when the neutral network is highly sparse, exhibits large global fluctuations in neutrality, and small local fluctuations in neutrality. The results are also relevant for elucidating the topological structure of a neutral network from empirical measurements of the substitution process.

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*Electronic address: araval@kgi.edu
**Introduction.** – The neutral theory of molecular evolution posits that most sequence substitutions at the nucleic acid or protein level are selectively neutral and do not appreciably alter the activity of the molecule in which they occur or the fitness of the host organism. It predicts that the number of substitutions accumulated in an evolving population of sequences in time $t$ follows a Poisson distribution with mean $\mu \nu t$, where $\mu$ is the mutation rate per sequence per generation and $\nu$ is the average fraction of neutral mutations (also called the neutrality). This prediction gives a simple explanation to the “molecular clock” – the idea that the number of accumulated fixed mutations in a population is proportional to the time elapsed – and implies that the variance in this number must equal its mean, leading to an index of dispersion (defined as the variance divided by the mean) of 1.

However, experimental studies often find that the index of dispersion is significantly larger than 1 (the overdispersed molecular clock). This finding can be reconciled with the neutral theory by assuming that the space of neutral sequences has fluctuating neutrality, causing the substitution process to be non-Poissonian, as verified by computer simulations that show significant overdispersion when the product $N \mu$ of the population size $N$ and the mutation rate $\mu$ is much smaller than 1.

There is limited theoretical understanding of the nature of the molecular clock. Cutler formally calculated the index of dispersion in terms of statistics of the mutation and fixation processes and argued that slow fluctuations in evolutionary parameters could lead to significant overdispersion in simple evolutionary models. Recent analytical results include a derivation of the index of dispersion for neutrally evolving protein populations constrained by a stability requirement. These results do not conclusively prove overdispersion of the molecular clock in a sufficiently general scenario, nor do they give an explicit characterization of the non-Poissonian nature of the full probability distribution of accumulated mutations.

A natural stage for fluctuating neutrality is presented by a neutral network of high- and equal-fitness genotypes in which two genotypes are linked by an edge if they differ by a single point mutation. The aim of this Letter is to theoretically clarify the non-Poissonian nature of the distribution of accumulated fixed mutations, to relate all cumulants of this distribution to graph invariants of the neutral network, to prove overdispersion of the molecular clock, and to identify features of the neutral network that could lead to significant overdispersion. We assume $N \mu \ll 1$, as is relevant for the majority of organisms in the plant and animal kingdom. For this limit to be valid, it is also
necessary that $\mu \ll 1$, as we assume below. We further assume that the neutral network is a connected graph; if it is not connected, the results below apply separately to populations evolving on each connected component of the neutral network graph.

*Substitution process when $N\mu \ll 1$. –* Consider a population of $N$ individuals evolving on a neutral network, represented by a graph $\mathcal{G}$ with $n$ nodes, $E$ edges, and adjacency matrix $\mathbf{G}$. The nodes of $\mathcal{G}$ represent high-fitness genotypes characterized by sequences of length $L$ over an alphabet of size $A$. Two nodes are connected by an edge if the corresponding genotypes differ by a single point mutation. The neutrality of a node $r$ in $\mathcal{G}$ is $d_r / (L(A - 1))$, where $d_r$ is the degree of $r$ in $\mathcal{G}$, and represents the fraction of point mutations of the genotype $r$ that are neutral. Following [17], we consider a discrete mutation-selection dynamics in which at each generation an individual suffers a point mutation with fixed probability $\mu$ that moves it to a neighboring genotype (which may or may not be of high fitness). $N$ individuals are then selected with replacement from the mutated population with probability proportional to their fitness, and the process is repeated. For $N\mu \ll 1$, the population at any point in time is converged on a single node of the neutral network [17]. At each generation it either stays at its current node or moves effectively as a single entity to a neighboring node. The probability $p_t(r)$ that the population is on node $r$ at time $t$ is governed by the equation

$$p_t = (\mathbf{I} - \tilde{\mu} \mathbf{D} + \tilde{\mu} \mathbf{G}) p_{t-1} = (\mathbf{I} - \tilde{\mu} \mathbf{L}) p_{t-1},$$

where $p_t(r)$ is the $r$th element of $\mathbf{p}_t$, $\mathbf{I}$ is the $n \times n$ identity matrix, $\tilde{\mu} \equiv \mu / (L(A - 1))$ is the reduced mutation rate, $\mathbf{D}$ is a diagonal matrix with node degrees on the main diagonal, and $\mathbf{L} \equiv \mathbf{D} - \mathbf{G}$ is the graph Laplacian of $\mathcal{G}$. The term $\mathbf{I} - \tilde{\mu} \mathbf{D}$ represents the probability that the population stays at its current node (either due to no mutation or a deleterious mutation that is culled by selection), and the term $\tilde{\mu} \mathbf{G}$ represents the probability that the population moves to a neighboring node.

$\mathbf{L}$ is a symmetric, positive semi-definite matrix and, if $\mathcal{G}$ is connected, has exactly one zero eigenvalue and all other eigenvalues positive [19, 20]. We denote eigenvalues of $\mathbf{L}$ by $\lambda_0 < \lambda_1 \leq \lambda_2 \leq \ldots \leq \lambda_{n-1}$, with $\lambda_0 = 0$. Further, because $\sum_j L_{ij} = \sum_i L_{ij} = 0$, the eigenvector of $\mathbf{L}$ corresponding to $\lambda_0$ is proportional to $\mathbf{1}$, the column vector with all entries equal to 1. The properly normalized limiting distribution over $\mathcal{G}$ is $\lim_{t \to \infty} \mathbf{p}_t = n^{-1} \mathbf{1}$, i.e., all nodes are occupied with equal probability [17].

Consider the joint distribution $p_t(r, m)$, representing the probability that the population
is on node $r$ at time $t$ and $m$ neutral substitutions have accumulated since time 0 (see [12] for a similar representation). The dynamics of the joint process is

$$p_t(m) = (I - \mu \mathbf{D}) p_{t-1}(m) + \mu \mathbf{G} p_{t-1}(m - 1),$$

where the $r$th element of $p_t(m)$ is $p_t(r, m)$. Assuming an equilibrated population at $t = 0$, the initial condition for Eq. (2) is $p_0(m) = \delta_{m,0} n^{-1}$.

To solve (2), it is convenient to define the vector moment generating function (mgf) $q_t(\theta) = \sum_{m=0}^{\infty} e^{m \theta} p_t(m)$. Noting that $q_0(\theta) = n^{-1} 1$, multiplying both sides of Eq. (2) by $e^{m \theta}$, summing over all $m$, and finally solving the resulting equation yields

$$q_t(\theta) = n^{-1} (I - \bar{\mu} L + \bar{\mu} (e^{\theta} - 1) G)^t 1.$$  (3)

The mgf $q_t(\theta)$ for the distribution of accumulated mutations is found by marginalizing over the vector mgf: $q_t(\theta) = \sum_r q_t(r, \theta) = 1^T q_t(\theta)$, where the superscript $T$ denotes the transpose operation. This yields

$$q_t(\theta) = n^{-1} 1^T (I - \bar{\mu} L + \bar{\mu} (e^{\theta} - 1) G)^t 1.$$  (4)

The probability $p_t(m)$ that $m$ mutations have accumulated in time $t$ may be recovered as the coefficient of $e^{m \theta}$ in the above mgf. Moments of $p_t(m)$ are obtained in the usual manner by taking multiple derivatives of Eq. (4) with respect to $\theta$. This procedure, however, becomes increasingly cumbersome for the calculation of higher moments, primarily because $L$ and $G$ do not, in general, commute. We therefore directly consider the late time and small $\bar{\mu}$ limit of the mgf $q_t(\theta)$ below.

**Late time behavior and cumulants.** – Consider a time scale long enough so that a sufficiently large number of mutations have accumulated in the population, i.e., $t \gg \bar{\mu}^{-1}$. It is then convenient to measure time in units of $\bar{\mu}^{-1}$, define a rescaled time variable $\tilde{t} = \bar{\mu} t$, and examine Eq. (1) in the limit $\bar{\mu} \ll 1$. Equation (1) may be rewritten as

$$q_t(\theta) = n^{-1} 1^T (I - \bar{\mu} L + \bar{\mu} (e^{\theta} - 1) G)^{\tilde{t}/\bar{\mu}} 1,$$  (5)

$$\simeq n^{-1} 1^T \exp \left[ \tilde{t} ((e^{\theta} - 1) G - L) \right] 1,$$  (6)

where we have used $\bar{\mu} \ll 1$ in making the approximation above. We now introduce the spectral expansion

$$(e^{\theta} - 1) G - L = \sum_{i=0}^{n-1} \lambda_i(\theta) u^{(i)}(\theta) u^{(i) T}(\theta),$$  (7)
where \( \{ \mathbf{u}^{(i)}(\theta) \} \) is an orthonormal basis of eigenvectors of \((e^\theta - 1)\mathbf{G} - \mathbf{L}\) with eigenvalues \(\lambda_i(\theta)\) ordered in decreasing order. Note that \(\lambda_i(0) = -\lambda_i\) (eigenvalues of \(-\mathbf{L}\)), and in particular, \(\lambda_0(0) = 0\) and \(\mathbf{u}^{(0)}(0) = n^{-1/2}\mathbf{1}\). For large \(\tilde{t}\), Eq. \(\text{(6)}\) is dominated by the leading term in Eq. \(\text{(7)}\), corresponding to the largest eigenvalue \(\lambda_0(\theta)\). Thus, in the late time limit, the cumulant generating function \(\ln q_t(\theta)\) and associated cumulants \(\{k_{(j)}\}\) are given by

\[
\ln q_t(\theta) \simeq \tilde{t} \lambda_0(\theta), \quad k_{(j)} \simeq \tilde{t} \frac{d^j}{d\theta^j} \lambda_0(\theta)|_{\theta=0}.
\]

(8)

Since \(\lambda_0(\theta)\) only depends on the topology of the neutral network graph, it follows that the ratio of any 2 cumulants depends only on the topology of the neutral network graph, and not on \(\tilde{\mu}\) and \(t\), at late times.

To obtain explicit formulae for the cumulants, we need to find \(\lambda_0(\theta)\) to any desired order in powers of \(\theta\). This is carried out in a recursive manner: expand \(\lambda_0(\theta)\) and \(\mathbf{u}^{(0)}(\theta)\) in power series in \(\theta\),

\[
\lambda_0(\theta) = \sum_{j=0}^{\infty} \lambda_0^{(j)} \theta^j, \quad \mathbf{u}^{(0)}(\theta) = \sum_{j=0}^{\infty} \mathbf{u}^{(0,j)} \theta^j,
\]

(9)

substitute these expansions in the eigenvalue equation

\[
[(e^\theta - 1)\mathbf{G} - \mathbf{L}] \mathbf{u}^{(0)}(\theta) = \lambda_0(\theta)\mathbf{u}^{(0)}(\theta),
\]

(10)

and compare the coefficients of equal powers of \(\theta\) on both sides of the above equation. Noting that \(\lambda_0^{(0)} = 0\), comparison of coefficients of \(\theta^0\) on both sides of Eq. \(\text{(10)}\) yields \(\mathbf{u}^{(0,0)} = n^{-1/2}\mathbf{1}\), and for \(j > 0\),

\[
\mathbf{L} \mathbf{u}^{(0,j)} = \frac{1}{\sqrt{n}} \left[ \frac{d}{j!} - \lambda_0^{(j)} \mathbf{1} \right] + \sum_{l=1}^{j-1} \left[ \frac{\mathbf{G}}{(j-l)!} - \lambda_0^{(j-l)} \mathbf{1} \right] \mathbf{u}^{(0,l)},
\]

(11)

where \(\mathbf{d}\) is a column vector containing node degrees (the main diagonal of \(\mathbf{D}\)), and it is understood that the sum on the right hand side vanishes for \(j = 1\). Multiplying both sides of Eq. \(\text{(11)}\) by \(\mathbf{1}^T\) and using \(\mathbf{1}^T\mathbf{L} = 0\), one obtains, for \(j > 0\),

\[
\lambda_0^{(j)} = \frac{\bar{d}}{j!} + \frac{1}{\sqrt{n}} \sum_{l=1}^{j-1} \left[ \frac{1}{(j-l)!} \mathbf{q}^T - \lambda_0^{(j-l)} \mathbf{1}^T \right] \mathbf{u}^{(0,l)},
\]

(12)

where \(\bar{d} = n^{-1} \sum_r d_r = 2n^{-1}E\) is the average degree of \(\mathcal{G}\). Equation \(\text{(12)}\) recursively expresses \(\lambda_0^{(j)}\) in terms of \(\lambda_0^{(k)}\) and \(\mathbf{u}^{(0,k)}\) for \(k < j\). To find \(\mathbf{u}^{(0,k)}\), one may consider inverting
in Eq. (11). However, \( L \), since it has a zero eigenvalue, has no inverse. We therefore introduce a pseudo-inverse of \( L \), denoted \( L^+ \), and defined by the spectral expansion

\[
L^+ = \sum_{i=1}^{n-1} \lambda_i^{-1} u^{(i)}(0) u^{(i)T}(0). \tag{13}
\]

Note that we have omitted the zero eigenvalue in carrying out the inversion. \( L^+ \) is a positive semi-definite symmetric matrix with \( 1^T L^+ = L^+ 1 = 0 \). Equation (11) may now be solved by writing \( u^{(0,j)} \) as \( L^+ \) multiplying the right hand side plus an arbitrary vector in the null space of \( L \). However, since \( L \) has only a single zero eigenvalue, this null space is 1-dimensional. Further, \( 1 \) lies in this null space; the null space is therefore spanned by \( 1 \), and the solution to Eq. (11) is

\[
\begin{align*}
 u^{(0,j)} &= \frac{L^+ d}{j! \sqrt{n}} + \sum_{l=1}^{j-1} \frac{1}{2 \sqrt{n}} \left( \sum_{l=1}^{j-1} u^{(0,j-l)} T u^{(0,l)} \right) 1,
\end{align*}
\tag{14}
\]

where the coefficient multiplying \( 1 \) above is found, after some algebra, by expanding the normalization condition \( u^{(0)}(\theta)^T u^{(0)}(\theta) = 1 \) in powers of \( \theta \).

Equations (12) and (14), together with the starting conditions \( \lambda_0^{(0)} = 0 \) and \( u^{(0,0)} = n^{-1/2} 1 \), are coupled nonlinear equations that allow one to recursively find \( \lambda_0^{(j)} \) (and therefore \( k^{(j)} \)) for all \( j \). For example, using these equations and noting that \( k^{(j)} = \tilde{t} j! \lambda_0^{(j)} \), the first three cumulants at late times are obtained as

\[
\begin{align*}
 k^{(1)} &= \tilde{t} d, \tag{15} \\
 k^{(2)} &= \tilde{t} \tilde{d} + \frac{2 \tilde{t}}{n} d^T L^+ d, \tag{16} \\
 k^{(3)} &= \tilde{t} \tilde{d} + \frac{6 \tilde{t}}{n} \left[ d^T L^+ d - 2 \tilde{d} d^T L^+ d + d^T L^+ G L^+ d \right].
\end{align*}
\]

Since a Poisson distribution with the same mean has all cumulants equal to \( \tilde{t} d \) (obtained from the first term in Eq. (12)), Eq. (12) shows systematic departures from Poissonian behavior at all cumulant orders in a manner that depends purely on the topology of the neutral network graph. Further, since for large \( \tilde{t} \), the Poisson distribution may be well approximated by a Normal distribution, the cumulants may be used to develop an Edgeworth expansion of \( p_t(m) \) around a Normal distribution to any desired accuracy. Fitting this distribution to an
empirically obtained \( p_t(m) \) distribution should then yield finer aspects of the topology of the neutral network than is accessible from mutational robustness studies alone \[17, 21\].

**Overdispersion of the molecular clock.**—Since the first cumulant is the mean and the second cumulant the variance, the index of dispersion \( R \) may be found as the ratio of \( k_{(2)} \) and \( k_{(1)} \) from Eqs. (15) and (16):

\[
R = 1 + \frac{2}{nd} \mathbf{d}^T \mathbf{L}^+ \mathbf{d}.
\]

Because \( \mathbf{d}^T \mathbf{L}^+ \mathbf{d} \) is a quadratic form associated with a positive semi-definite matrix \[22\], this shows that the molecular clock is generically overdispersed \((R \geq 1)\). \( R = 1 \) only if the neutral network graph is regular because for a regular graph (and only a regular graph), \( \mathbf{d} \propto \mathbf{1} \) lies in the null space of \( \mathbf{L} \) and \( \mathbf{L}^+ \). Using Eq. (6), it is in fact trivial to show that the substitution process is strictly Poissonian for regular neutral network graphs, since \( \mathbf{G} \) and \( \mathbf{L} \) commute for regular graphs. For all other graphs, \( \mathbf{d} \) will have a component orthogonal to the null space of \( \mathbf{L} \) and thus result in overdispersion. This is consistent with having “fluctuating neutrality”, i.e., unequal neutrality across the network, for overdispersion. To examine how the extent of overdispersion depends on neutrality fluctuations and other graph parameters, we now determine bounds on \( R \).

Using the spectral expansion (13), we obtain

\[
\mathbf{d}^T \mathbf{L}^+ \mathbf{d} = \sum_{i=1}^{n-1} \lambda_i^{-1} \left( \mathbf{d}^T \mathbf{u}^{(i)}(0) \right)^2,
\]

\[
\leq \lambda_1^{-1} \sum_{i=1}^{n-1} \left( \mathbf{d}^T \mathbf{u}^{(i)}(0) \right)^2 = n \lambda_1^{-1} \text{Var}(d),
\]

where we have used the fact that \( \lambda_1 \) is the second-smallest eigenvalue of \( \mathbf{L} \) and that \( \{\mathbf{u}^{(i)}(0)\} \) is an orthonormal basis of eigenvectors. \( \text{Var}(d) \) denotes the variance of the degree distribution of the graph. Noting that \( 2/(nd) = 1/E \), this results in an upper bound on the extent of overdispersion:

\[
R - 1 \leq \left( \frac{n}{E} \right) \lambda_1^{-1} \text{Var}(d).
\]

Thus the index of dispersion is bounded from above by an interesting combination of graph parameters: the sparseness (as measured by the ratio \( E/n \)), the fluctuations in neutrality (as measured by \( \text{Var}(d) \)), and \( \lambda_1^{-1} \), which has a number of interpretations. \( \lambda_1 \) is the algebraic connectivity of the graph \[23\] and measures its overall compactness and connectivity. Also,
λ_1^{-1} is the time scale (as measured in units of 1/μ) of relaxation of the distribution p_t (Eq. (11)) to its equilibrium value n^{-1}1. Therefore, for a fixed amount of neutrality fluctuation, R can be large if the neutral network is sparse (high n/E) and less well connected, or equivalently, if the network is sparse and the relaxation time scale is large. Since both of these conditions are expected to hold quite generally for large and sparse neutral networks, Eq. (20) is a weak upper bound. It is more interesting to examine the following lower bound on R. Returning to Eq. (18), and using the fact that f(λ_i) ≡ λ_i^{-1} is a convex function of λ_i for positive λ_i, we may apply Jensen’s inequality for convex functions:

\[ \sum_i a_i f(\lambda_i) \geq f \left( \sum_i a_i \lambda_i \right) \tag{21} \]

to Eq. (18) with the choice \( a_i = (\mathbf{d}^T \mathbf{u}(i)(0))^2 \). This yields a lower bound on R, namely

\[ R - 1 \geq \frac{n^2}{E} \sum_{i=1}^{n-1} \lambda_i \frac{\text{Var}(d)}{(\mathbf{d}^T \mathbf{u}(i)(0))^2} = 2 \left( \frac{n}{E} \right)^2 \frac{\text{Var}(d)}{\sum_{i,j} G_{ij} (d_i - d_j)^2}, \tag{22} \]

where we have used \( \sum_{i=1}^{n-1} \lambda_i (\mathbf{d}^T \mathbf{u}(i)(0))^2 = \mathbf{d}^T \mathbf{Ld} = (1/2) \sum_{i,j} G_{ij} (d_i - d_j)^2 \). Noting that the denominator measures the variation in the degree between neighboring nodes on the neutral network, we may define, analogous to Var(d), the local variation in neutrality \( \text{LVar}(d) \equiv (2E)^{-1} \sum_{i,j} G_{ij} (d_i - d_j)^2 \), where the normalizing factor of E appears because the sum is a sum over the edge set of the graph, and the factor of 2 prevents double counting of edges. We therefore get the lower bound

\[ R - 1 \geq \left( \frac{n}{E} \right)^2 \frac{\text{Var}(d)^2}{\text{LVar}(d)}. \tag{23} \]

Thus, although fluctuating neutrality is an essential component of overdispersion within the neutral evolution framework, the extent of overdispersion further increases if the graph is more sparse and has smaller local variation in neutrality, i.e., smaller fluctuations in neutrality across neighboring nodes (the latter requirement was first suggested in a different form by Cutler [11]). Significantly large overdispersion is then easily realized in, say, a sparse neutral network with large diameter in which large global fluctuation in neutrality (degree) occurs as a cumulative effect of small local fluctuations in neutrality.
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