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A general method for the estimation of moments of substitution number in molecular evolution

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The random variable “substitution number” N_t , *i.e.* the number of mutations that have accumulated in a sequence under neutral evolution during a time t , is a cornerstone of the field of molecular evolution. We show here that both the short and long time limits of its moments can be computed from the substitution matrix used to model the neutral evolution by elementary means. The method developed here is also used to compute the moments of the complementary variable, T_n , the time it takes to accumulate n mutations. The method we develop here necessitates only elementary operations on the substitution matrix and does not involve spectral decomposition.

I. INTRODUCTION.

Substitution models are the cornerstone of the field of molecular evolution and describe the Markovian stochastic process of genetic variation through fixed mutations. One of their most important application is to evaluate the length of the branches in a phylogeny tree, and hence the time of divergence from an ancestor, through the estimation of the number of substitutions N that has occurred between two sequences. A substitution model describes the rate of mutation between symbols of an alphabet, that can describe for example base pairs in DNA (4 symbols), codons (61 symbols, excluding the STOP), amino acids (20 symbols), or more complicated alphabets taking into account various intricacy of the evolutionary process under consideration. Since their inception by Jukes and Cantor[1] and Kimura and Ohta [2, 3], many substitution models have been proposed, documented in textbooks [4, 5] and incorporated into phylogenetic software such as IQ-Tree[6]. A review of these models and advances in this field can be found in [7].

Once a substitution model Q has been chosen, it can be used to characterize the random variable N_t of the number of substitutions that have occurred during a time t . It is straightforward to deduce the *mean* number of substitutions $\langle N_t \rangle$ from the substitution matrix Q . However, the mean is only the first step to characterize a random variable and one need its higher moments (variance, skewness, kurtosis, ...) to set the confidence limits on this estimation. The task of evaluating the higher moments has proved extremely difficult and for example has led to the much discussed controversy of the “overdispersion” of the molecular clock[8–11].

Zheng[12] was the first to use Markov chains to investigate the variance of substitution number as a solution of a set of differential equations. His investigation was further developed by Bloom, Raval and Wilke[13] who gave the general solution in terms of the spectral decomposition of the substitution matrix; this solution was extended by Raval[14] for a specific class of matrices used for random walk on neutral graphs. Minin and Suchard[15] used the same spectral method to derive an analytical form for the generating function of a binary process. Note that the substitution matrix is singular and asymmetric,

its eigenvalues are in general complex and the spectral decomposition cannot be obtained exactly. We recently published[16] a simple, exact result for the variance of the substitution number and showed, as a consequence, that for the large class of Generalized time reversible matrices, the dispersion index, *i.e.* the variance to mean ratio is always larger than one. Although the result for the variance was simple, the method to obtain it was intricate and could not be generalized to compute the higher moments of the substitution number.

In the present article, we show that there exist a simple transformation of the substitution matrix that decomposes the problem into a series of simple equations that can be solved by elementary matrix algebra and allows for the computation of moments of arbitrary order. The same method allows us also to compute directly the moments of the complementary random variable T_n , the time it takes to reach n substitutions. This provides for a more direct approach to estimate the divergence *time* from an ancestor.

This article is organized as follows. In the next section, we display the main results of this article and their comparison to numerical simulations of the stochastic process. Section III then details the methods used to compute the moments of N_t . Section IV address the complementary method of computing the variance of T_N . Section V is devoted to discussions and conclusions. The appendices contain details of some of the computations.

II. MAIN RESULTS

A. Definitions and notations.

The problem of substitution number N can be formulated as follows. A discrete finite Markov process X has K possible values and is described by substitutions rates

$$m_{\beta}^{\alpha} \quad \alpha, \beta \in \{1 \dots K\} \quad (1)$$

of transition from one state α into another β (Fig.1, inset). Each time a transition occurs, the random variable N is incremented by one unit, while the random variable time T is incremented by t_{α} , an exponentially distributed

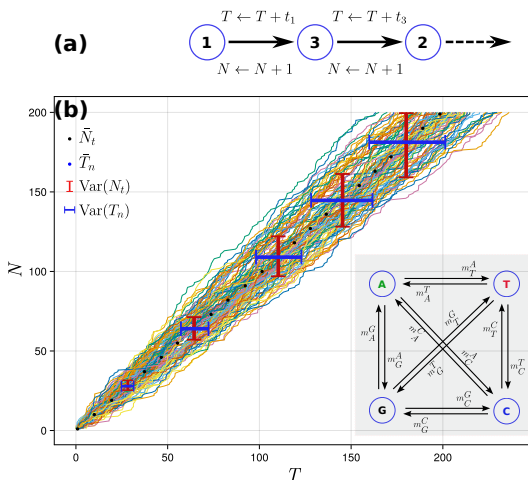


Figure 1. The random process of substitution number. Inset : a random process X can have K different values (here the four bases of DNA) with rates of transition m_{β}^{α} between states. (a) Each time a transition occurs, the random variables (N, T) are incremented by $(1, t_i)$ where t_i is an exponential time depending on the state that is left. (b) A realization of this process can be represented by a random path in the (N, T) space (solid lines for 200 such random paths, simulated by a Gillespie algorithm). Given a time t , one could then study the probability $P(n; t)$ of observing n substitutions at time t and the moments of the random variable N_t (vertical red slices). On the other hand, given a substitution number n , one could study the probability density $p(t; n)$ of the time it takes to reach n substitutions, and the moments of the random variable T_n (horizontal, blue slices).

time with a parameter depending on the state α that the system leaves (Fig 1a). We can represent each realization of this process by a random path (N, T) (Fig 1b). The problem of the substitution number is to evaluate the moments (variance and higher order moments) of N_t , the number of substitution observed at a given time. A complementary and valuable information is the moments of the random variable T_n , the time it takes to reach n substitution numbers. To our knowledge, this last problem has not been addressed in the literature. The substitution number is used to evaluate the length of the branches in a phylogeny tree[5]. Under the assumption of molecular clock[9, 17], the time of divergence can be estimated from the substitution number. Its intrinsic uncertainty could be in principle deduced from the moments of the substitution number. Computing the moments, in particular the variance, of T_n is a more direct approach to the problem of divergence time.

The main result of this article is that a simple transformation allows one to compute the quantities described above, both for N_t and T_n .

First, let us recall the basic definitions used in the field of molecular evolution. The substitution matrix

$$Q = M - D \quad (2)$$

contains all the information about the rates : The matrix

M contains the transition rates m_{β}^{α} ($\alpha \neq \beta$) between states while the diagonal matrix $D = \text{diag}(m^{\alpha})$ contains the rate of leaving state α

$$m^{\alpha} = \sum_{\beta=1}^K m_{\beta}^{\alpha} \quad (3)$$

The matrix Q is not symmetric and we must make a clear distinction between column (right) and row (left) vectors (*i.e.* linear forms). The Dirac notations[18] are extremely useful to manage this distinction and will be used through this article. A column vector is noted $|u\rangle$, a row vector $\langle u|$, left (right) multiplication of a matrix by a row (column) vector are $\langle u| R$ and $R |u\rangle$; $\langle u|v\rangle$ is a scalar while $|u\rangle\langle v|$ is a matrix.

By definition, each column of Q sums to zero, therefore it has a zero eigenvalue :

$$\langle 1| Q = \langle 0| \quad (4)$$

where $\langle 1| = (1, 1, \dots, 1)$ is the left (row) eigenvector. The right (column) eigenvector associated to the zero eigenvalue is denoted $|\pi\rangle$:

$$Q |\pi\rangle = |0\rangle \quad (5)$$

and is called the equilibrium probability of the states.

The average rate \bar{m} , *i.e.* the weighted average of the leaving rates is defined as :

$$\bar{m} = \sum_{\alpha=1}^K m^{\alpha} \pi_{\alpha} = \langle 1| D |\pi\rangle = \langle 1| M |\pi\rangle \quad (6)$$

The first moment (average) of N_t and T_n are straightforward to compute :

$$\bar{N}_t = \bar{m} t \quad (7)$$

$$\bar{T}_n = \frac{1}{\bar{m}} n \quad (8)$$

B. Moments of Substitution number N_t

As we discussed above, the average quantities are not sufficient to evaluate a random variable and one must possess the higher central moments such as variance, third and fourth moments. An exact estimation of the higher moments had proved extremely difficult. The source of this difficulty resides precisely in the existence of the zero eigenvalues of Q (see section III). We show here that this difficulty can be circumvented by making a simple transformation of the substitution matrix : using the matrix $|\pi\rangle\langle 1|$ whose columns are all equal and formed of the eigenvector $|\pi\rangle$ and defining the reversible matrix

$$\tilde{Q} = \bar{m} |\pi\rangle\langle 1| - Q \quad (9)$$

one can obtain the higher moments of N_t in terms of simple manipulation the matrix \tilde{Q} , its inverse \tilde{Q}^{-1} and the matrix $\tilde{M} = M - \bar{m}I$.

In the long time limit the second (variance, μ^2), third (μ^3) and fourth (μ^4) central moments of N_t are as follows. For the variance, we obtain

$$\frac{\mu_2}{\bar{N}_t} = 1 + 2\frac{\delta_2}{\bar{m}} + O(1/t) \quad (10)$$

The quantity $2\delta_2/\bar{m}$ is called ‘‘the overdispersion’’ of the molecular clock and δ_2 is found to be

$$\delta_2 = \langle 1 | \tilde{M} \tilde{Q}^{-1} \tilde{M} | \pi \rangle \quad (11)$$

Expression (10) is equivalent to the expression published in our earlier work[16].

For the third central-moment, the result is found to be

$$\frac{\mu_3}{\bar{N}_t} = 1 + \frac{6\delta_3}{\bar{m}} + O(1/t) \quad (12)$$

where

$$\delta_3 = \langle 1 | \tilde{M} \tilde{Q}^{-1} \tilde{M} (\tilde{Q}^{-1} \tilde{M} + I) | \pi \rangle \quad (13)$$

Note that for a Poisson process, $\mu_3/\bar{N} = 1$.

For the fourth central-moment, the result is found to be

$$\frac{\mu_4}{(\bar{N}_t)^2} = 3 + 4\frac{\delta_2}{\bar{m}} \left(1 + \frac{\delta_2}{\bar{m}} \right) + O(1/t) \quad (14)$$

For a Poisson process, $\mu_4/\bar{N}^2 = 3 + O(1/\bar{N})$.

Comparison to numerical simulations for μ^3 is given in figure 2 and detailed in section III. For a two state system with only one free parameter (beyond time scaling), a simple formula for μ^3 can be derived (equation 38 and figure 7).

Similar expression for the short time limit can also be found by the same method (see section III).

C. Time T_n to reach n substitution.

The matrix

$$\tilde{M} = MD^{-1}$$

contains the jump probabilities from state α to β , knowing that a transition has occurred (see section IV). This matrix has one eigenvalue equal to unity, and its left and right eigenvectors are noted $\langle 1 |$ and $|\psi\rangle$, where $\langle 1 | = (1, \dots, 1)$:

$$\langle 1 | \tilde{M} = \langle 1 | ; \tilde{M} |\psi\rangle = |\psi\rangle$$

As in the preceding discussion, we can decompose \tilde{M} :

$$\tilde{M} = |\psi\rangle \langle 1 | + F$$

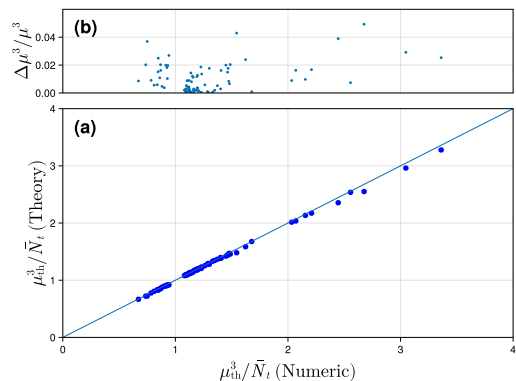


Figure 2. The third central moment μ^3 (relation 35) : comparison between numerical simulation and theory for four states system. 80 random 4×4 substitution matrix M are generated and normalized to have $\bar{m} = 1$, so all numerical simulations take the same time. For each matrix, 6×10^7 paths are generated for a total time of $T = 1000$ using a standard Gillespie algorithm[19] ; each simulation for a given matrix takes approximately 5 hours. (a) For each matrix, the theoretical value of μ^3 is plotted against its numerical value. Solid lines represents the diagonal. (b) The relative absolute difference between these two values for each matrix.

The long term variance of T_n is then given as

$$\frac{1}{n} \text{Var}(T_n) = 2 \left\langle 1 | D^{-1} (I - F)^{-1} D^{-1} | \psi \right\rangle - \left\langle 1 | D^{-1} | \psi \right\rangle^2 + O(1/n)$$

where the term in $O(1/n)$ can be exactly evaluated (equation 55) and the residual is of the form F^n that converges exponentially to zero. Figures 3 shows the comparison of this result to numerical simulations. For a two state system with only one free parameter, a simple formula for $\text{Var}(T_n)$ can be derived (equation 57 and figure 7).

The next sections are devoted to the derivation of the above results.

III. COMPUTING MOMENTS OF N_t

A. Background.

Consider a random process X that can take K values. The rate of transition from a state β to a state α is noted m_{α}^{β} . The probability $P_{\alpha}(t)$ of observing, at time t , the system in the state α is given by the master equation

$$\frac{d}{dt} P_{\alpha}(t) = \sum_{\beta=1}^K m_{\alpha}^{\beta} P_{\beta}(t) - \sum_{\beta=1}^K m_{\beta}^{\alpha} P_{\alpha}(t) \quad (15)$$

Collecting the components P_{α} into the vector $|P\rangle$ and noting $m^{\alpha} = \sum_{\beta} m_{\beta}^{\alpha}$ the rate of leaving the state α , we can rewrite equation (15) in matricial notations as

$$\frac{d}{dt} |P\rangle = M |P\rangle - D |P\rangle \quad (16)$$

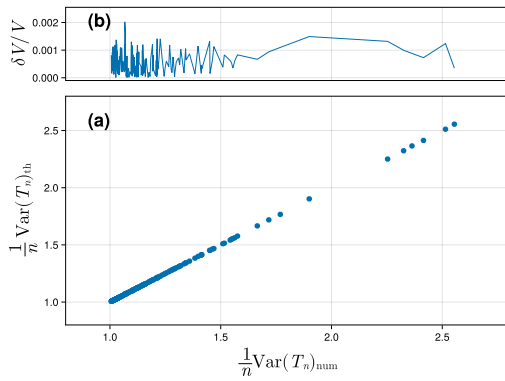


Figure 3. Comparison between theoretical and numerical limit of $\text{Var}(T_n)/n$ for 200 random 4×4 substitution matrices. Each matrix is normalized in order to have $\bar{m} = 1$. A standard Gillespie algorithm[19] is used to simulate 10^6 path for each matrix for a duration of $n_{max} = 200$. The Variance at each index n is computed using these paths. The numerical limiting value is extracted from the last 100 indexes. (a) for each random matrix, its theoretical variance is plotted against its numerical value. (b) The relative absolute difference between these two values for each matrix. The relative difference is inversely proportional to the number of paths used for the numerical simulation.

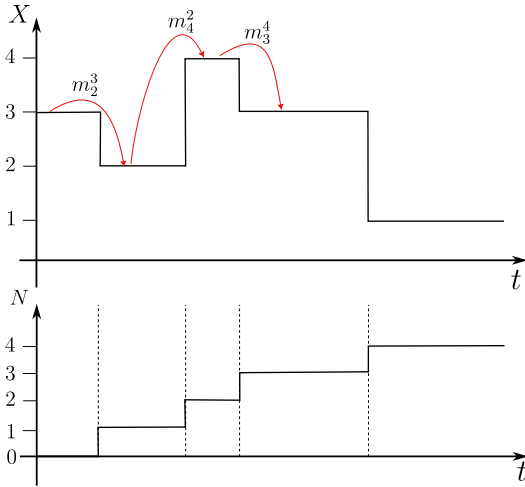


Figure 4. The stochastic process X as a function of time t . The probability density of jumping from state α to state β is m_{β}^{α} (equ. 1). Each time a transition occurs, the substitution number N_t is incremented by one unit.

where the elements of matrix M are given by the rates m_{α}^{β} (with $m_{\alpha}^{\alpha} = 0$) and D is the diagonal matrix of the elements m^{α} . The matrix $Q = M - D$, called the substitution matrix (relation2), has one zero eigenvalue with the associated left and right eigenvectors $\langle 1|$ and $|\pi\rangle$ (relations 4-5).

We can now set a counter N called the substitution number (Figure 4) : each time a transition occurs, N is incremented by one unit. The *joined* probability $P_{\alpha}^n(t)$ of observing at time t the system in state α after n sub-

stitutions is given by the Master equations

$$\frac{d}{dt} P_{\alpha}^0(t) = - \sum_{\beta=1}^K m_{\beta}^{\alpha} P_{\alpha}^0(t) \quad (17)$$

$$\frac{d}{dt} P_{\alpha}^n(t) = \sum_{\beta=1}^K m_{\alpha}^{\beta} P_{\beta}^{n-1}(t) - \sum_{\beta=1}^K m_{\beta}^{\alpha} P_{\alpha}^n(t) \quad n \neq 0 \quad (18)$$

or, in matricial notations introduced above :

$$\frac{d}{dt} |P^n\rangle = M |P^{n-1}\rangle - D |P^n\rangle \quad (19)$$

where, without loss of generality, we suppose that the system at $t = 0$ is in equilibrium. Moreover, in order to simplify the computations we extend the domain of N to negative integers :

$$|P^0(t=0)\rangle = |\pi\rangle ; |P^n(t)\rangle = |0\rangle \quad n < 0$$

From the expression of $|P^n\rangle$ the average of the random variable N_t is computed by

$$\bar{N} = \langle N_t \rangle = \sum_{n=0}^{\infty} \langle 1|n P^n(t)\rangle$$

and is trivial (see appendix A) to show that :

$$\bar{N} = \bar{m}t \quad (20)$$

The focus of this article is the higher moments of N_t . Let us define the *central* moment of order k for the state α as

$$\mu_{\alpha}^k(t) = \sum_n (n - \bar{N})^k P_{\alpha}^n(t)$$

We can group these moments into the *vectorial* moments

$$|\mu^k(t)\rangle = (\mu_1^k(t), \dots, \mu_K^k(t))^T$$

The (scalar) central moment for the substitution number is therefore

$$\mu^k = \sum_{\alpha=1}^K \mu_{\alpha}^k(t) = \langle 1| \mu^k(t) \rangle \quad (21)$$

B. Deriving the moments equation.

From the recurrence equation (19) we can directly derive a set of hierarchical ordinary differential equations (see appendix A) for the central moments :

$$\begin{aligned} \frac{d}{dt} |\mu^k\rangle &= Q |\mu^k\rangle + k \bar{M} |\mu^{k-1}\rangle \\ &+ M \sum_{\ell=2}^k \binom{k}{\ell} |\mu^{k-\ell}\rangle \end{aligned} \quad (22)$$

where

$$\bar{M} = M - \bar{m}I \quad (23)$$

Let us recall that, by definition,

$$\langle 1|\bar{M}|\pi\rangle = 0$$

Time derivative of a moment of order k depends only on the moments of equal and lower order. The zero eigenvalue of matrix Q causes the moments to grow as a power of time. This zero eigenvalue however prevents us to solve the equations in terms of convolutions of $\exp(-Qt)$ with some functions, as the matrix Q^{-1} does not exist. One could theoretically use the spectral decomposition of Q to solve equation (22); Q being asymmetric in general, some of the eigenvalues are complex and this is not a practical solution, specially where the number of states $K > 2$. Surprisingly, this difficulty can be circumvented by a simple matrix shift :

$$\tilde{Q} = \bar{m}|\pi\rangle\langle 1| - Q \quad (24)$$

The matrix $|\pi\rangle\langle 1|$ is just a matrix whose columns are the repetition of the eigenvector $|\pi\rangle$. Consider the set of and left right normalized eigenvectors of Q : $\langle e^i|$ and $|e^i\rangle$ ($i = 1, \dots, K$) where by definition, $\langle e^i|e_j\rangle = \delta_j^i$. The matrix \tilde{Q} has the same eigenvectors as the matrix Q . The zero eigenvalue is shifted to \bar{m} :

$$\tilde{Q}|\pi\rangle = \bar{m}|\pi\rangle \quad (25)$$

while the sign of other eigenvalues is reversed : for $|e_i\rangle$ associated to the eigenvalue λ_i (< 0)

$$\tilde{Q}|e_i\rangle = -\lambda_i|e_i\rangle$$

The matrix \tilde{Q} is invertible, as all its eigenvalues have their real part greater than 0. Using relation (24), equation (22) transforms into :

$$\begin{aligned} \frac{d}{dt}|\mu^k\rangle &= -\tilde{Q}|\mu^k\rangle + \bar{m}\mu^k|\pi\rangle + k\bar{M}|\mu^{k-1}\rangle \\ &+ M\sum_{\ell=2}^k \binom{k}{\ell} |\mu^{k-\ell}\rangle \end{aligned} \quad (26)$$

while the main quantity of interest, the *scalar* moment $\mu^k = \langle 1|\mu^k\rangle$, is obtained from (22) by projecting the equation on the $\langle 1|$ linear form:

$$\frac{d}{dt}\mu^k = k\langle 1|\bar{M}|\mu^{k-1}\rangle + \sum_{\ell=2}^k \binom{k}{\ell} \langle 1|M|\mu^{k-\ell}\rangle \quad (27)$$

The set of equations (26,27) allows us now to obtain, by the usual techniques of ordinary differential equations, all the moments. The algorithm is as follows :

- Knowing the vectorial moment up to $|\mu^{k-1}\rangle$, compute the scalar moment μ^k from equation (27). This necessitates only to compute the known quantities $\langle 1|M|\mu^{k-\ell}\rangle$, *i.e.* left and right multiplication of the Matrix M by known vectors.

- Knowing the scalar moment μ^k , compute the vectorial moment $|\mu^k\rangle$ from the relation (26). This necessitates only the knowledge of $|\mu^{k-j}\rangle$ and μ^k .

By definition, $|\mu^0\rangle = |\pi\rangle$, so the recurrence in the above process can be processed step by step. This two step resolution allows for the isolation of the effect of the zero eigenvalue from the other ones, as detailed below.

C. Solving the moments equations.

Equations (26,27) are best studied in the Laplace domain

$$|\hat{\mu}^k(s)\rangle = \int_0^\infty |\mu^k(t)\rangle dt$$

where differential equations are transformed into algebraic ones. We are mostly interested in the long time limit $t \rightarrow \infty$, which corresponds in the Laplace domain to the limit $s \rightarrow 0$: we need only develop algebraic equations in s and keep the leading order. The short range limit is also easily obtained by development for $s \rightarrow \infty$. Using the basic rules of Laplace transforms, equations (26,27) are transformed into

$$\hat{\mu}^k = \frac{k}{s} \langle 1|\bar{M}|\hat{\mu}^{k-1}\rangle + \frac{1}{s} \sum_{\ell=2}^k \binom{k}{\ell} \langle 1|M|\hat{\mu}^{k-\ell}\rangle \quad (28)$$

and

$$\begin{aligned} |\hat{\mu}^k\rangle &= \frac{\bar{m}}{s + \bar{m}} \hat{\mu}^k |\pi\rangle + kU_s \bar{M} |\hat{\mu}^{k-1}\rangle \\ &+ U_s M \sum_{\ell=2}^k \binom{k}{\ell} |\hat{\mu}^{k-\ell}\rangle \end{aligned} \quad (29)$$

where we have used the shorthand notation

$$U_s = (sI + \tilde{Q})^{-1}$$

As $(sI + \tilde{Q})|\pi\rangle = (s + \bar{m})|\pi\rangle$, the simplification

$$U_s|\pi\rangle = \frac{1}{s + \bar{m}}|\pi\rangle$$

has been used to derive expression (29). Moreover, development of U_s in power of s is straightforward

$$U_s = \tilde{Q}^{-1} - s\tilde{Q}^{-2} + \dots$$

and is used below to obtain the long time limit of the moments.

Let us now apply the resolution algorithm to solve for the first moments of N_t . The resolution process, as we described above, uses the successive steps

$$\hat{\mu}^1 \Rightarrow |\hat{\mu}^1\rangle \Rightarrow \hat{\mu}^2 \Rightarrow |\hat{\mu}^2\rangle \Rightarrow \hat{\mu}^3 \dots$$

We already know that $\hat{\mu}^1 = 0$ by definition. Therefore, from (29), we obtain

$$|\hat{\mu}^1\rangle = \frac{1}{s} U_s \bar{M} |\pi\rangle \quad (30)$$

From the above expression, we compute the variance of N_t and its long time behavior:

$$\hat{\mu}^2 = \frac{2}{s^2} \langle 1 | \bar{M} U_s \bar{M} | \pi \rangle + \frac{\bar{m}}{s^2} \quad (31)$$

$$= \frac{2}{s^2} \langle 1 | \bar{M} \tilde{Q}^{-1} \bar{M} | \pi \rangle + \frac{\bar{m}}{s^2} + O(1/s) \quad (32)$$

The dispersion index R , *i.e.* the variance to mean ratio, converges to

$$R = 1 + 2 \frac{\delta_2}{\bar{m}} \quad (33)$$

where

$$\delta_2 = \langle 1 | \bar{M} \tilde{Q}^{-1} \bar{M} | \pi \rangle$$

The above expression is equivalent to the expression obtained in [16] and has been shown to be exact by numerical simulations.

Continuing the resolution, from expression (31), we compute $|\hat{\mu}^2\rangle$:

$$|\hat{\mu}^2\rangle = \frac{\bar{m}}{s^2(s + \bar{m})} (\langle 1 | \bar{M} U_s \bar{M} | \pi \rangle + \bar{m}) |\pi\rangle + \frac{2}{s} (U_s \bar{M})^2 |\pi\rangle + \frac{1}{s} U_s M |\pi\rangle$$

The expression for $\hat{\mu}^3$ is trivially obtained if we recall that $\langle 1 | \bar{M} | \pi \rangle = 0$:

$$\hat{\mu}^3 = \frac{6}{s^2} \langle 1 | \bar{M} (U_s \bar{M})^2 | \pi \rangle + \frac{6}{s^2} \langle 1 | \bar{M} U_s \bar{M} | \pi \rangle + \frac{\bar{m}}{s^2} \quad (34)$$

In the long time limit ($s \rightarrow 0$), keeping the leading terms in the above expression, we have

$$\frac{\mu^3}{\bar{N}_t} = 1 + 6 \langle 1 | \bar{M} \tilde{Q}^{-1} \bar{M} (\tilde{Q}^{-1} \bar{M} + I) | \pi \rangle + O(1/t) \quad (35)$$

The explicit computation of μ^4 (relation A) is given in the appendix A.

D. Applications.

As an example, consider a two states system. Noting the two transition rates mq and $m(1-q)$, we have

$$M = m \begin{pmatrix} 0 & 1-q \\ q & 0 \end{pmatrix} ; |\pi\rangle = \begin{pmatrix} 1-q \\ q \end{pmatrix} \quad (36)$$

and $\bar{m} = 2mq(1-q)$. All the matricial computations can be performed symbolically and we have, for large times,

$$\mu^2/\bar{N}_t = 1 + (1-2q)^2 \quad (37)$$

$$\mu^3/\bar{N}_t = 1 + 3(1-2q)^4 \quad (38)$$

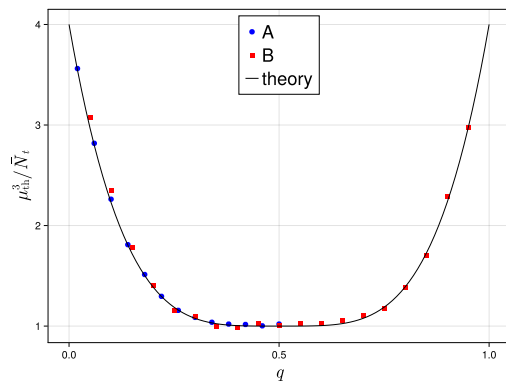


Figure 5. The third central moment μ^3 (relation 35) : comparison between numerical simulation and theory for two states system. For each value of q , the 2×2 matrix M (relation 36) is generated and is normalized to have $\bar{m} = 1$, so all numerical simulations take the same time. For each matrix, 10^7 (A series, blue circles) or 10^6 (B series, red squares) paths are generated for a total time of $T = 1000$ using a standard Gillespie algorithm[19] ; each simulation for a given matrix takes approximately 50 minutes. The value of μ^3 at each time step and its limit value is computed from these paths. Solid line is the theoretical expression (38).

figure 5 shows the excellent agreement between the above theoretical results and numerical simulations of the stochastic process.

For higher dimensional matrices, the symbolic expressions are too cumbersome to compute symbolically. Figure 2 shows the comparison of the numerical simulations to the theory for random 4×4 substitution matrices.

IV. DERIVING THE VARIANCE EQUATION FOR T_n

A. Evolution of the probability density of T_n .

Consider again the stochastic process X , but this time as a function of the substitution number and not time (Fig; 6). We note $P_\alpha(n)$ the probability of observing the system in state α , knowing that n substitutions have taken place. Knowing that the system was in state α and that a transition has occurred, the probability of finding the system in state β is

$$\tilde{m}_\beta^\alpha = m_\beta^\alpha / m^\alpha. \quad (39)$$

Collecting the coefficients P_α into the vector $|P(n)\rangle$, and the coefficients \tilde{m}_β^α into the matrix \tilde{M} , we can write the evolution of the system as

$$|P(n)\rangle = \tilde{M} |P(n-1)\rangle \quad (40)$$

By definition, the columns of the matrix

$$\tilde{M} = MD^{-1} \quad (41)$$

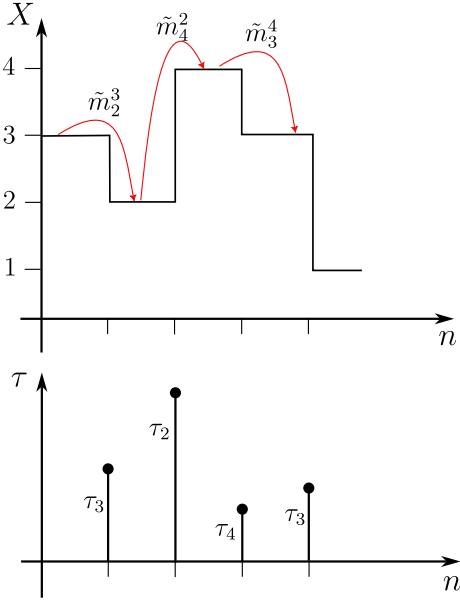


Figure 6. The stochastic process X as a function of the number of transitions n . Knowing that a transition has occurred, the probability of jumping from state α to state β is $\tilde{m}_{\beta}^{\alpha}$ (equ. 39). The residency time τ_{α} in the state α is exponentially distributed with parameter m^{α} . The total time t that the system takes to reach n transition is the sum of these residency times.

sum to 1 :

$$\langle 1 | \tilde{M} = \langle 1 | \quad (42)$$

therefore $\langle 1 |$ is the left (row) eigenvector of \tilde{M} associated to the eigenvalue 1. We denote by $|\psi\rangle$ the right (column) normalized eigenvector of \tilde{M} associated to the same eigenvalue 1 :

$$\tilde{M} |\psi\rangle = |\psi\rangle ; \langle 1 | \psi\rangle = 1 \quad (43)$$

$|\psi\rangle$ is the equilibrium probability of the X process, considered as a function of n . In the limit of large substitution numbers, ψ_{α} is the relative number of times the system is in state α . Without loss of generality, we suppose that initially, the system is in equilibrium

$$|P(0)\rangle = |\psi\rangle \quad (44)$$

The vector $|\psi\rangle$ is related to the zero eigenvector of the matrix Q (relation 2) through the relation

$$D |\pi\rangle = \tilde{m} |\psi\rangle \quad (45)$$

The probability density $f(\tau; \alpha)$ that the system resides a time τ in the state α is exponentially distributed with parameter m^{α} :

$$f(\tau; \alpha) = m^{\alpha} e^{-m^{\alpha} \tau}$$

We can now derive the expression for the random variable T_n . The probability density $p_{\alpha}(t; n)$ of the total time

t to reach n substitutions and to be in state α is therefore

$$p_{\alpha}(t; n+1) = \int_{\tau=0}^t \sum_{\beta} p_{\beta}(t-\tau; n) \tilde{m}_{\alpha}^{\beta} f(\tau; \beta) d\tau \quad (46)$$

in plain words, the probability density that the total time at $n+1$ is t and the system in state α equals the probability density that (i) at n the total time was $t-\tau$ and (ii) the system was in β and made a transition $\beta \rightarrow \alpha$ and (iii) the system resided a time τ in state β , summed over all β and τ .

Collecting the coefficients $p_{\alpha}(t; n)$ into the vector $|p(t; n)\rangle$, equation (46) can be written as

$$|p(t; n+1)\rangle = \int_{\tau=0}^t M \exp(-D\tau) |p(t-\tau; n)\rangle d\tau \quad (47)$$

where we have used the relation $\tilde{M}D = M$. The above expression is a convolution product, therefore using the Laplace transform

$$|\hat{p}(s; n)\rangle = \int_{t=0}^{\infty} e^{-st} |p(t; n)\rangle dt$$

equation (47) transforms into a simple recurrence relation :

$$|\hat{p}(s; n+1)\rangle = \hat{K}(s) |\hat{p}(s; n)\rangle \quad (48)$$

where

$$\hat{K}(s) = M(D + sI)^{-1}$$

is the Laplace transform of the kernel

$$K(\tau) = M \exp(-D\tau)$$

Formally, the solution of the recurrence relation (48) is

$$|\hat{p}(s; n)\rangle = \left(\hat{K}(s) \right)^n |\psi\rangle \quad (49)$$

B. Computing the variance of T_n

Various moments of the random variable T_n can be computed by the derivations of the expression (49) as a function of s for $s=0$. Let us define

$$L_n(s) = \left(\hat{K}(s) \right)^n$$

and note that, from equations (42,43)

$$\langle 1 | L_n(0) = \langle 1 | \left(\tilde{M} \right)^n = \langle 1 | \quad (50)$$

$$L_n(0) |\psi\rangle = \left(\tilde{M} \right)^n |\psi\rangle = |\psi\rangle \quad (51)$$

the mean of the random variable T_n can be computed by

$$\begin{aligned} \langle T_n \rangle &= \left\langle 1 \left| \frac{d}{ds} \left[\hat{p}(s; n) \right] \right|_{s=0} \right\rangle \\ &= \langle 1 | L'_n(s=0) | \psi \rangle \end{aligned} \quad (52)$$

The matrix

$$\hat{K}'(s) = M(D + sI)^{-2}$$

and $\hat{K}(s)$ don't commute in general. Therefore, the derivative of the matrix the matrix $L_n(s)$ has to be computed explicitly :

$$L'_n(s) = \sum_{m=0}^n L_m(s) \hat{K}'(s) L_{n-m-1}(s)$$

Using the simplifications (42,50,51) and the relation (45), it is straightforward to compute the average

$$\langle T_n \rangle = n \langle 1 | D^{-1} | \psi \rangle = \frac{n}{\bar{m}}$$

where \bar{m} is the average leaving rate defined in equation (6).

For the second moment,

$$\langle T_n^2 \rangle = \left\langle 1 \left| \frac{d^2}{ds^2} |\hat{p}(s; n)\rangle \right|_{s=0} \right\rangle$$

We note that

$$\begin{aligned} L''_n(s) &= \sum_{m=0}^n L'_m(s) \hat{K}'(s) L_{n-m-1}(s) + \\ &+ \sum_{m=0}^n L_m(s) \hat{K}''(s) L_{n-m-1}(s) \\ &+ \sum_{m=0}^n L_m(s) \hat{K}'(s) L'_{n-m-1}(s) \end{aligned}$$

and therefore (see appendix)

$$\begin{aligned} \langle T_n^2 \rangle &= 2 \sum_{m=1}^{n-1} (n-m) \langle 1 | D^{-1} \tilde{M}^m D^{-1} | \psi \rangle \\ &+ 2n \langle 1 | D^{-2} | \psi \rangle \end{aligned} \quad (53)$$

As the matrix \tilde{M} has one eigenvalue equal to 1, we cannot explicitly compute the geometric series (53) as it is. However, we can use the method used in the preceding section and write

$$\tilde{M} = |\psi\rangle \langle 1| + F$$

By the above definition, we have

$$\langle 1 | F = \langle 0 | \quad ; \quad F | \psi \rangle = |0\rangle \quad (54)$$

and therefore

$$\tilde{M}^m = |\psi\rangle \langle 1| + F^m$$

The Matrix $(F - I)$ is therefore invertible and

$$\sum_{m=1}^{n-1} (n-m) F^m = F(I - F)^{-2} [I - F^n - n(I - F)]$$

All the eigenvalues of F are $\in [-1, 1[$ and in the limit of large n , $F^n \rightarrow 0$ exponentially. Note that the special case when F has a -1 eigenvalue does not change the result (see for example, the two states system below). Finally, grouping all terms, it is found that

$$\begin{aligned} \frac{1}{n} \text{Var}(T_n) &= 2 \langle 1 | D^{-1} (I - F)^{-1} D^{-1} | \psi \rangle - \langle 1 | D^{-1} | \psi \rangle^2 \\ &- \frac{2}{n} \langle 1 | D^{-1} F (I - F)^{-2} D^{-1} | \psi \rangle \end{aligned} \quad (55)$$

where $\text{Var}(T_n) = \langle T_n^2 \rangle - \langle T_n \rangle^2$. We observe that $\text{Var}(T_n)/n$ converges as $1/n$ toward its limit. We insist that expression (53) involves only simple matricial operations: inversion, and left and right multiplication by row and column vectors.

C. Applications.

As a first example, consider the two state system (equation 36). From the definition of the matrix M , it is trivial to show that

$$\tilde{M} = \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix} ; \quad |\psi\rangle = \frac{1}{2} \begin{pmatrix} 1 \\ 1 \end{pmatrix} \quad (56)$$

This a particular case where \tilde{M} possesses a -1 eigenvalue with the left and right associated eigenvector $\langle i | = (1, -1)$ and $|\psi_1\rangle = (1/2)(1, -1)^T$. The matrix \tilde{M} can therefore be written as

$$\begin{aligned} \tilde{M} &= |\psi\rangle \langle 1| - |\psi_1\rangle \langle i| \\ \tilde{M}^m &= |\psi\rangle \langle 1| + (-1)^m |\psi_1\rangle \langle i| \end{aligned}$$

For this simple system, we don't have to use the large n approximation and the result is found to be

$$\frac{\bar{m}^2}{n} \text{Var}(T_n) = 1 + (1 - 2q)^2 \left(1 + \frac{a_n}{n} \right) \quad (57)$$

where

$$a_n = \frac{1}{2} (1 + (-1)^n)$$

figure 7 shows the excellent agreement between the above expressions and numerical simulations of the stochastic process.

For higher dimensional matrices, the symbolic expressions are too cumbersome to compute explicitly. Figure 3 shows the comparison of the numerical simulations to the theory for random 4×4 substitution matrices, and the excellent agreement between them.

A final remark is in order. We saw in section III that the variance to mean ratio for the random variable N_t is always superior to 1 and is overdispersed compared to a Poisson process. For the random variable T_n , the situation is reversed. We can write

$$T_n = \sum_{i=1}^n \tau_i$$

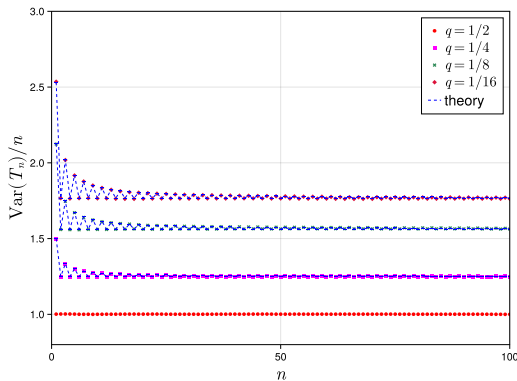


Figure 7. Comparison between theoretical and numerical values of $\text{Var}(T_n)/n$ for a two states system (eq. 56) for four different values of q . Each matrix is normalized in order to have $\bar{m} = 1$. A standard Gillespie algorithm[19] is used to simulate 10^6 paths for each matrix for a duration of $n_{max} = 100$. The Variance at each index n is computed using these paths. For each random matrix, its numerical variance (symbols) is compared to the theoretical prediction of eq. 57).

where the random variable τ_i is the time the system takes to make one transition. These variables are exponentially distributed and in particular,

$$\text{Var}(\tau_i) = 2 \langle 1|D^{-2}|\psi\rangle - \langle 1|D^{-1}|\psi\rangle^2$$

If τ_i were independent, we would have

$$\text{Var}(T_n) = n\text{Var}(\tau)$$

Our numerical simulations show that, although $\text{Var}(T_n)$ is linear in n ,

$$\text{Var}(T_n) < n\text{Var}(\tau)$$

i.e. correlations between τ_i *decreases* the variance for T_n .

V. DISCUSSION AND CONCLUSIONS.

The substitution number N_t is a problem that is stated simply, but its solution has proved, until now, to be extremely difficult. In this article, we have developed a method, based on a simple shift of the substitution matrix, that allows for the simple solution for the moments of this random variable and its associated variable T_n . Once the basic principle is derived, the solution is obtained by a simple moment closure: results from step k , which are obtained by simple matrix algebra, are used to obtain results for step $k+1$. Even though we have restricted the computation to the first four moments, a formal algebra software can be programmed in principle to derive the result for arbitrary moments. The above method is very general and can be used for any stochastic process that has a unique equilibrium probability.

Appendix A: Deriving the moments equation for N_t

Consider the system of equations

$$\frac{d}{dt} |P^n\rangle = M |P^{n-1}\rangle - D |P^n\rangle \quad (\text{A1})$$

with the initial condition

$$|P^0(t=0)\rangle = |\pi\rangle$$

First, let us note that

$$\sum_n |P^n(t)\rangle = |\pi\rangle \quad (\text{A2})$$

as the left hand side is the probability of finding the system in a given state, *whatever* the number of substitution.

We define the moment-vector of order k as

$$|N^k\rangle = \sum_n n^k |P^n\rangle$$

The moments of the random variable N are obtained simply by summing over all the component of the corresponding vector-moment:

$$\langle N^k\rangle = \sum_{\alpha=1}^K (|N^k\rangle)_{\alpha} = \langle 1|N^k\rangle \quad (\text{A3})$$

Consider the *mean*-vector

$$|N\rangle = \sum_n n |P^n(t)\rangle$$

Applying $\sum_n n$ to both sides of equations (A1) we obtain

$$\frac{d}{dt} |N\rangle = Q |N\rangle + M |\pi\rangle \quad (\text{A4})$$

where $Q = M - D$ and we have used the change of index $n \rightarrow n+1$ to simplify the expression

$$\begin{aligned} M \sum_n n |P^{n-1}\rangle &= M \sum_n (n+1) |P^n\rangle \\ &= M |N\rangle + M |\pi\rangle \end{aligned}$$

Summing over the component of $|N\rangle$ by applying the left form $\langle 1|$ to expression (A4), we have

$$\frac{d}{dt} \langle N(t)\rangle = \frac{d}{dt} \langle 1|N(t)\rangle = \langle 1|M|\pi\rangle = \bar{m} \quad (\text{A5})$$

As $\langle 1|M = \langle 1|D$, the coefficient

$$\bar{m} = \sum_{\alpha=1}^K m^{\alpha} \pi_{\alpha}$$

is simply the weighted average of the leaving rates. Integrating equation (A5), we have the linear growth law of the average:

$$\bar{N}(t) = \langle N(t)\rangle = \bar{m}t$$

We apply the same method to obtain higher orders moments. We define the *central* moment-vector of order k as

$$|\mu^k(t)\rangle = |(N - \bar{N}(t))^k\rangle = \sum_n (n - \bar{m}t)^k |P^n(t)\rangle \quad (\text{A6})$$

Deriving the above expression as a function of time, we have

$$\frac{d}{dt} |\mu^k(t)\rangle = -\bar{m}k |\mu^{k-1}(t)\rangle + \sum_n (n - \bar{m}t)^k \frac{d}{dt} |P^n(t)\rangle$$

We can now use equation (A1) to develop the last term of the above expression. To compute the term

$$\sum_n (n - \bar{m}t)^k |P^{n-1}\rangle$$

we make the usual change of index $n \rightarrow n + 1$ and use the binomial expression

$$(n - \bar{m}t + 1)^k = \sum_{\ell=0}^k \binom{k}{\ell} (n - \bar{m}t)^{k-\ell}$$

Grouping all the terms and recalling that $Q = M - D$, we obtain

$$\begin{aligned} \frac{d}{dt} |\mu^k\rangle &= Q |\mu^k\rangle + k(M - \bar{m}I) |\mu^{k-1}\rangle \\ &+ M \sum_{\ell=2}^k \binom{k}{\ell} |\mu^{k-\ell}\rangle \end{aligned} \quad (\text{A7})$$

The above expression is an *exact* moment closure : the differential equation of moments-vector of order k depends only in moments-vector of order $\leq k$. Projecting on the $\langle 1|$ form and recalling that $\langle 1|Q = \langle 0|$, we get finally the expression for the centered moments

$$\begin{aligned} \frac{d}{dt} \langle \mu^k \rangle &= k (\langle 1|(M - \bar{m}I)|\mu^{k-1}\rangle) \\ &+ \sum_{\ell=2}^k \binom{k}{\ell} \langle 1|M|\mu^{k-\ell}\rangle \end{aligned} \quad (\text{A8})$$

Appendix B: Numerical simulations.

Numerical simulations are performed by a classical Gillespie algorithm[19]. The algorithm is implemented using the Julia language[20]. The process is simulated as described in figure 1. To generate one path for a given substitution matrix M , we begin with a state α_0 drawn from the equilibrium state $|\pi\rangle$ or $|\psi\rangle$ depending on the problem. The time t_1 to the the next transition is drawn from an exponential distribution with parameter m^{α_0} ; the next state α_1 is drawn at random using the α_0 column of the matrix \tilde{M} of transition probabilities. A loop until the final index n is used to continue the process and the path is recorded as the vector (t_1, \dots, t_n) .

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