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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 a complete solution of the moments of this random variable can be obtained explicitly by recurrence, using a simple matrix shift method. This result leads to simple expressions for both the short and long time limits of the moments that can be computed directly from the substitution matrix used to model the neutral evolution. 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 needs 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 in general 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 exists 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 highlight 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

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

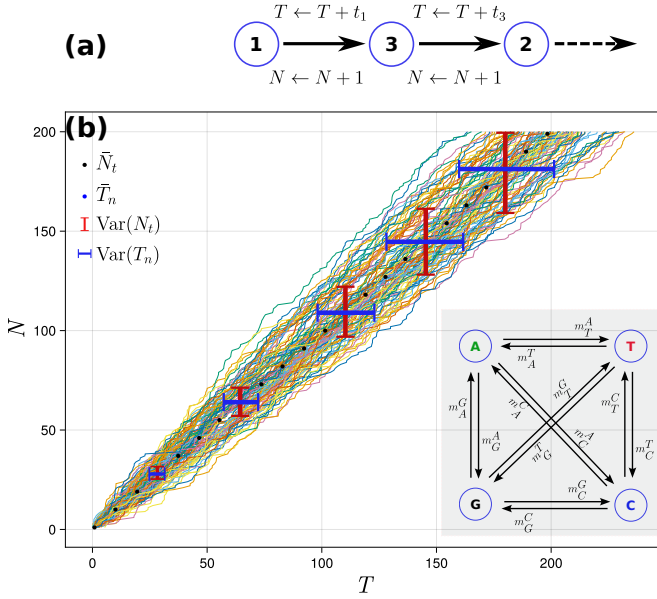


Figure 1. The random process of substitution number. Inset : a random process  $X$  can have  $K$  different values (here the four bases of DNA) with rates of transition  $m_{\beta}^{\alpha}$  between states. (a) Each time a transition occurs, the random variables  $(N, T)$  are incremented by  $(1, t_i)$  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).

of transition from one state  $\alpha$  into another  $\beta$  (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  $t_{\alpha}$ , an exponentially distributed time with a parameter depending on the state  $\alpha$  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 substitutions observed at time  $t$ . 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, and 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, a matrix shift, 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  $m_{\beta}^{\alpha}$  ( $\alpha \neq \beta$ ) between states while the diagonal matrix  $D = \text{diag}(m^{\alpha})$  contains the rate of leaving state  $\alpha$

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

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 invertible 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 manipulations of the matrix  $\tilde{Q}$ , its inverse  $\tilde{Q}^{-1}$  and the matrix  $\bar{M} = M - \bar{m}I$ .

In the long time limit the second (variance,  $\mu^2$ ), third ( $\mu^3$ ) and fourth ( $\mu^4$ ) 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  $\delta_2$  is found to be

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

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

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

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

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

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

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

Comparison to numerical simulations for  $\mu^3$  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  $\mu^3$  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. Variance of $T_n$ , time to reach $n$ substitution.

The matrix

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

contains the jump probabilities from state  $\alpha$  to  $\beta$ , knowing that a transition has occurred (see section IV). This matrix has one eigenvalue equal to unity, and its left and

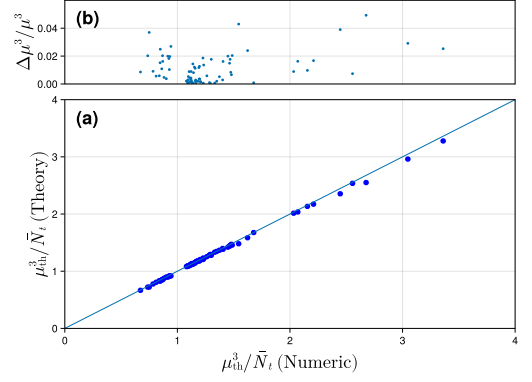


Figure 2. The third central moment  $\mu^3$  (relation 35) : comparison between numerical simulation and theory for four states system. 80 random  $4 \times 4$  substitution matrix  $M$  are generated and normalized to have  $\bar{m} = 1$ , so all numerical simulations take the same time. For each matrix,  $6 \times 10^7$  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  $\mu^3$  is plotted against its numerical value. Solid lines represents the diagonal. (b) The relative absolute difference between these two values for each matrix.

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  $\tilde{M}$ :

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

The long term variance of  $T_n$  is then given as

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

where the term in  $O(1/n)$  can be exactly evaluated (equation 57) 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 59 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  $\beta$  to a state  $\alpha$  is noted  $m_{\alpha}^{\beta}$ . The probability  $P_{\alpha}(t)$  of observing, at time  $t$ , the

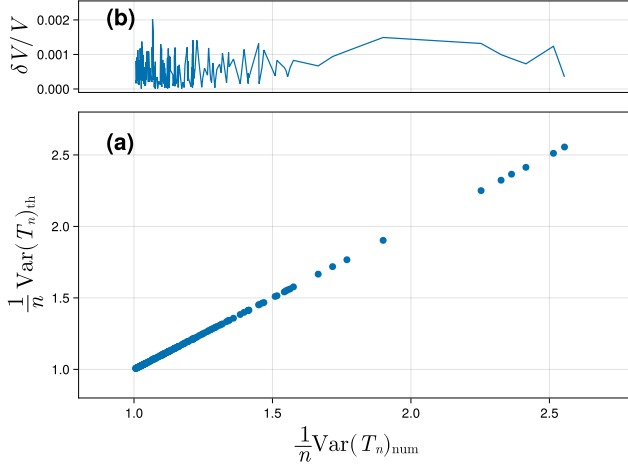


Figure 3. Comparison between theoretical and numerical limit of  $\text{Var}(T_n)/n$  for 200 random  $4 \times 4$  substitution matrices. Each matrix is normalized in order to have  $\bar{m} = 1$ . A standard Gillespie algorithm[19] is used to simulate  $10^6$  path for each matrix for a duration of  $n_{\text{max}} = 200$ . 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.

system in the state  $\alpha$  is given by the master equation

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

where  $m^\alpha = \sum_\beta m_\beta^\alpha$  is the rate of leaving the state  $\alpha$ . Collecting the components  $P_\alpha$  into the vector  $|P\rangle$  we can rewrite equation (15) in matricial notations as

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

where the elements of matrix  $M$  are given by the rates  $m_\alpha^\beta$  (with  $m_\alpha^\alpha = 0$ ) and  $D$  is the diagonal matrix of the elements  $m^\alpha$ . The matrix  $Q = M - D$ , called the substitution matrix (relation 2), 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  $\alpha$  after  $n$  substitutions is given by the Master equations

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

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

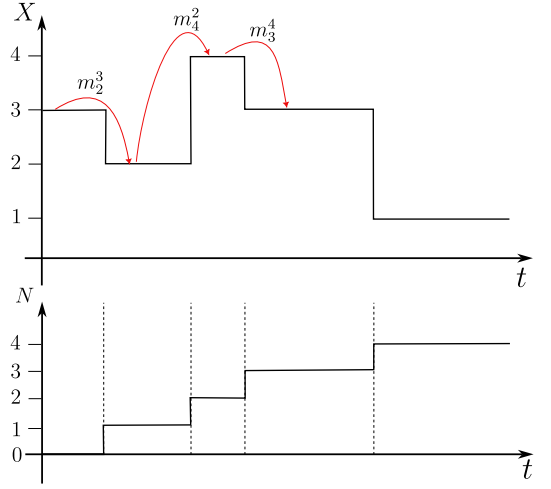


Figure 4. The stochastic process  $X$  as a function of time  $t$ . The probability density of jumping from state  $\alpha$  to state  $\beta$  is  $m_\beta^\alpha$  (equ. 1). Each time a transition occurs, the substitution number  $N_t$  is incremented by one unit.

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. :

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

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  $\alpha$  as

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

We group these moments into the vectorial moments

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

The true quantity of interest, 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

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

and therefore, 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 when 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  $|\pi\rangle$ . 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  $\lambda_i$  ( $< 0$ ) of  $Q$ , noting that  $\langle 1 | e_i \rangle = 0$ ,

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

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  $\mu^k$  from equation (27). This necessitates only to compute the 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  $\mu^k$ , compute the vectorial moment  $|\mu^k\rangle$  from the relation (26). This necessitates only the knowledge of  $|\mu^{k-j}\rangle$  and  $\mu^k$ .

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

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

where linear differential equations are transformed into algebraic ones. The long time limit  $t \rightarrow \infty$  can be obtained by the limit  $s \rightarrow 0$  in the Laplace domain. On the other hand, the short range limit  $t \rightarrow 0$  is obtained by the limit  $s \rightarrow \infty$  of the Laplace transform.

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

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

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. As  $|\mu^0\rangle = |\pi\rangle$ , from eq.(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, by a more intricate method, in [16] that has been shown to be exact by numerical simulations.

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

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

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

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

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

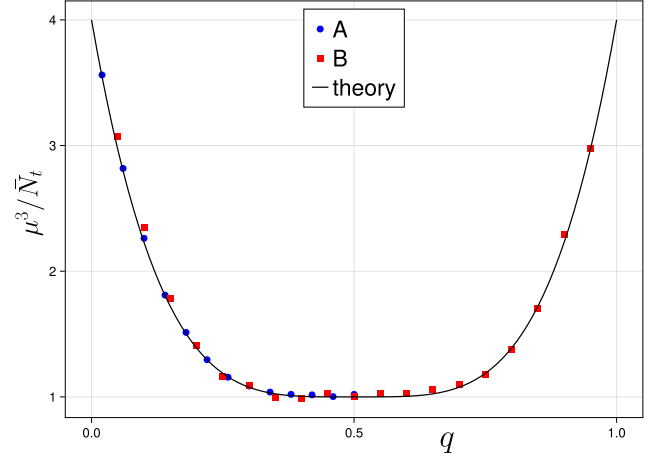


Figure 5. The third central moment  $\mu^3$  (relation 35): comparison between numerical simulation and theory for two states system. For each value of  $q$ , the  $2 \times 2$  matrix  $M$  (relation 36) is generated and is normalized to have  $\bar{m} = 1$ , so all numerical simulations take the same 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  $\mu^3$  at each time step and its limit value is computed from these paths. Solid line is the theoretical expression (38).

#### 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,

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

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

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  $4 \times 4$  substitution matrices.

#### E. Short term limit

The short term limit of the expression for the moments can also be simply retrieved by taking the  $s \rightarrow \infty$  limit of equations (31,34). Note that in this limit, the operator

$$U_s = (sI + \tilde{Q})^{-1} = \frac{1}{s} I - \frac{1}{s^2} \tilde{Q} + \dots$$

Therefore, expression (31) is developed into

$$\begin{aligned}\hat{\mu}^2 &= \frac{\bar{m}}{s^2} + \frac{2}{s^3} \langle 1 | \bar{M}^2 | \pi \rangle + O(1/s^4) \\ \mu^2 &= \bar{N} + \frac{\langle 1 | \bar{M}^2 | \pi \rangle}{\bar{m}^2} \bar{N}^2 + O(t^3)\end{aligned}\quad (39)$$

Note that the coefficient  $\nu_m = \langle 1 | \bar{M}^2 | \pi \rangle$  is a variance like expression of the leaving rates weighted by equilibrium probabilities :

$$\nu_m = \sum_{\alpha} (m^{\alpha})^2 \pi_{\alpha} - \left( \sum_{\alpha} m^{\alpha} \pi_{\alpha} \right)^2$$

By the same token, expression (34) is developed into

$$\begin{aligned}\hat{\mu}^3 &= \frac{\bar{m}}{s^2} + \frac{6}{s^3} \nu_m + O(1/s^4) \\ &= \bar{N} + 3 \frac{\nu_m}{\bar{m}^2} \bar{N}^2 + O(t^3)\end{aligned}\quad (40)$$

#### 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  $\alpha$ , knowing that  $n$  substitutions have taken place. Knowing that the system was in state  $\alpha$  and that a transition has occurred, the probability of finding the system in state  $\beta$  is

$$\tilde{m}_{\beta}^{\alpha} = m_{\beta}^{\alpha} / m^{\alpha}. \quad (41)$$

Collecting the coefficients  $P_{\alpha}$  into the vector  $|P(n)\rangle$ , and the coefficients  $\tilde{m}_{\beta}^{\alpha}$  into the matrix  $\tilde{M}$  (not to be confused with the matrix  $\bar{M}$  of preceding section), we can write the evolution of the system as

$$|P(n)\rangle = \tilde{M} |P(n-1)\rangle \quad (42)$$

By definition, the columns of the matrix

$$\tilde{M} = M D^{-1} \quad (43)$$

sum to 1 :

$$\langle 1 | \tilde{M} = \langle 1 | \quad (44)$$

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 :

$$\tilde{M} |\psi\rangle = |\psi\rangle ; \langle 1 | \psi\rangle = 1 \quad (45)$$

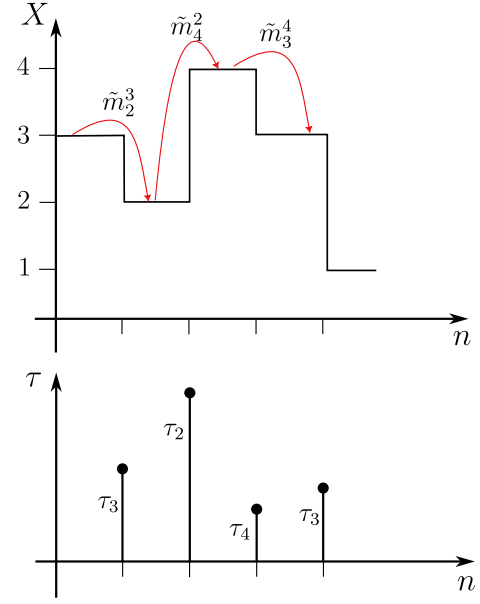


Figure 6. The stochastic process  $X$  as a function of the number of transitions  $n$ . Knowing that a transition has occurred, the probability of jumping from state  $\alpha$  to state  $\beta$  is  $\tilde{m}_{\beta}^{\alpha}$  (equ. 41). The residency time  $\tau_{\alpha}$  in the state  $\alpha$  is exponentially distributed with parameter  $m^{\alpha}$ . The total time  $t_n$  that the system takes to reach  $n$  transition is the sum of these residency times :  $t_n = \sum_{i=1}^n \tau_n$ .

$|\psi\rangle$  is the equilibrium probability of the  $X$  process, considered as a function of  $n$ . Without loss of generality, we suppose that initially, the system is in equilibrium

$$|P(0)\rangle = |\psi\rangle \quad (46)$$

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

$$D |\pi\rangle = \bar{m} |\psi\rangle \quad (47)$$

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

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

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  $\alpha$  is therefore

$$p_{\alpha}(t; n+1) = \int_{\tau=0}^t \sum_{\beta} p_{\beta}(t-\tau; n) \tilde{m}_{\alpha}^{\beta} f(\tau; \beta) d\tau. \quad (48)$$

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



Collecting the coefficients  $p_\alpha(t; n)$  into the vector  $|p(t; n)\rangle$ , equation (48) can be written as

$$|p(t; n+1)\rangle = \int_{\tau=0}^t M \exp(-D\tau) |p(t-\tau; n)\rangle d\tau \quad (49)$$

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 (49) transforms into a simple recurrence relation :

$$|\hat{p}(s; n+1)\rangle = \hat{K}(s) |\hat{p}(s; n)\rangle \quad (50)$$

where

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

is the Laplace transform of the kernel

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

in relation (49). Formally, the solution of the recurrence relation (50) is

$$|\hat{p}(s; n)\rangle = \left(\hat{K}(s)\right)^n |\psi\rangle \quad (51)$$

### B. Computing the variance of $T_n$

Various moments of the random variable  $T_n$  can be computed by the derivations of the expression (51) 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 (44,45)

$$\langle 1 | L_n(0) = \langle 1 | \left(\tilde{M}\right)^n = \langle 1 | \quad (52)$$

$$L_n(0) |\psi\rangle = \left(\tilde{M}\right)^n |\psi\rangle = |\psi\rangle \quad (53)$$

the mean of the random variable  $T_n$  can be computed by

$$\begin{aligned} \langle T_n \rangle &= \left\langle 1 \left| \frac{d}{ds} |\hat{p}(s; n)\rangle \right|_{s=0} \right\rangle \\ &= \langle 1 | L'_n(s=0) |\psi\rangle \end{aligned} \quad (54)$$

The matrix

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

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

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

Using the simplifications (44,52,53) and the relation (47), 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

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

and therefore (see appendix C)

$$\begin{aligned} \langle T_n^2 \rangle &= 2 \sum_{m=1}^{n-1} (n-m) \langle 1 | D^{-1} \tilde{M}^m D^{-1} |\psi\rangle \\ &+ 2n \langle 1 | D^{-2} |\psi\rangle \end{aligned} \quad (55)$$

As the matrix  $\tilde{M}$  has one eigenvalue equal to 1, we cannot explicitly compute the geometric series (55) as it is. However, we can use the shifted matrix method used in the preceding section and write

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

By the above definition, we have

$$\langle 1 | F = \langle 0 | \quad ; \quad F |\psi\rangle = |0\rangle \quad (56)$$

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

$$\begin{aligned} \frac{1}{n} \text{Var}(T_n) &= 2 \left\langle 1 | D^{-1} (I - F)^{-1} D^{-1} |\psi\rangle \right\rangle - \langle 1 | D^{-1} |\psi\rangle^2 \\ &- \frac{2}{n} \left\langle 1 | D^{-1} F (I - F)^{-2} D^{-1} |\psi\rangle \right\rangle \end{aligned} \quad (57)$$

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 stress that expression (55) involves only simple matricial operations: inversion, and left and right multiplication by row and column vectors.

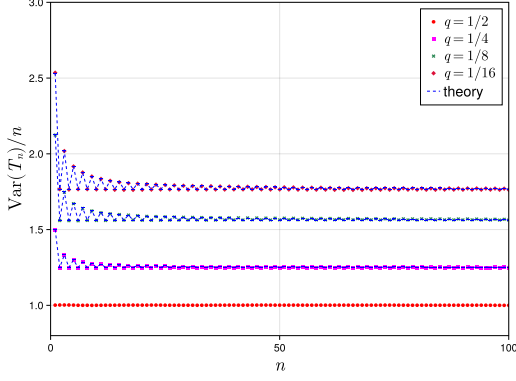


Figure 7. Comparison between theoretical and numerical values of  $\text{Var}(T_n)/n$  for a two states system (eq. 58) for four different values of  $q$ . Each matrix is normalized in order to have  $\bar{m} = 1$ . A standard Gillespie algorithm[19] is used to simulate  $10^6$  path for each matrix 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. 59).

### C. Applications.

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

$$\tilde{M} = \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix} ; |\psi\rangle = \frac{1}{2} \begin{pmatrix} 1 \\ 1 \end{pmatrix} \quad (58)$$

This is 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

$$\frac{\bar{m}^2}{n} \text{Var}(T_n) = 1 + (1 - 2q)^2 \left(1 + \frac{a_n}{n}\right) \quad (59)$$

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  $4 \times 4$  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

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

where the random variable  $\tau_i$  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  $\tau_i$  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  $\tau_i$  decreases the variance for  $T_n$ .

### V. DISCUSSION AND CONCLUSIONS.

The characterization of the random variable “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 simple matrix shift method, that allows for the exact computation of the moments of this random variable and its associated variable  $T_n$ : the results obey a moment closure scheme, *i.e.* 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.

Note that in molecular evolution, the main observable is the probability  $p_d(t)$  that two different sequences are different at a given site [20]:

$$p_d(t) = 1 - \text{tr}(e^{Qt}\Pi) \quad (60)$$

where  $\Pi$  is the diagonal matrix formed by the elements of the vector  $|\pi\rangle$ . One can estimate  $p_d(t)$  from the fraction of observed differences between two sequences  $\hat{p}$ . By eliminating time in relations (7,60), it is then possible to relate the estimators  $\hat{d}$  (of  $\langle n \rangle$ ) and  $\hat{p}$

$$\hat{d} = f(\hat{p}) \quad (61)$$

For sequences of length  $L$ ,  $\hat{p}$  is given by a binomial distribution  $B(L, p)$  and the variance of the distance estimator

$\hat{d}$  can be deduced from relation (61). This quantity however is very different from the *intrinsic* variance of the substitution number that we have computed here.

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

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

In order to simplify the computations involving index shift and without loss of generality, we extend the domain of  $N$  to negative integers :

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

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 components 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

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

where  $\bar{M} = M - \bar{m}I$ .

## Appendix B: Computation of the fourth moment of $N_t$

From the expression for the central moments  $\hat{\mu}^\ell$  ( $\ell = 0, \dots, 3$ ) and the vectorial moments  $|\hat{\mu}^j\rangle$  ( $j = 0, \dots, 2$ ), we can compute the third vectorial moment :

$$|\hat{\mu}^3\rangle = \frac{\bar{m}}{s + \bar{m}} \hat{\mu}^3 |\pi\rangle + U_s \bar{M} (3|\mu^2\rangle + 3|\mu^1\rangle + |\mu^0\rangle) + \bar{m} U_s (3|\mu^1\rangle + |\mu^0\rangle)$$

To compute the fourth moment, we only need to compute

$$\begin{aligned} \langle 1 | \bar{M} | \hat{\mu}^3 \rangle &= \frac{3\bar{m}A_s^1(\bar{m} + A_s^1)}{s^2(s + \bar{m})} + \frac{\bar{m}^2}{s(s + m)} \\ &+ \frac{1}{s}A_s^1 + \frac{6}{s}A_s^2 + \frac{6}{s}A_s^3 + \frac{3\bar{m}}{s}B_s^2 \end{aligned} \quad (B1)$$

where

$$\begin{aligned} A_s^k &= \langle 1 | \bar{M} (U_s \bar{M})^k | \pi \rangle \\ B_s^k &= \langle 1 | \bar{M} U_s^k \bar{M} | \pi \rangle \end{aligned}$$

The expression for the fourth moment deduced from (A8) is :

$$\begin{aligned} \hat{\mu}^4 &= \frac{4}{s} \langle 1 | \bar{M} | \hat{\mu}^3 \rangle + \frac{6}{s} \langle 1 | \bar{M} | \hat{\mu}^2 \rangle + \frac{4}{s} \langle 1 | \bar{M} | \hat{\mu}^1 \rangle \\ &+ \frac{1}{s} \langle 1 | \bar{M} | \hat{\mu}^0 \rangle + \frac{6\bar{m}}{s} \hat{\mu}^2 + \frac{4\bar{m}}{s} \hat{\mu}^1 + \frac{\bar{m}}{s} \hat{\mu}^0 \end{aligned} \quad (B2)$$

As we are mostly interested in the long time limit, we only need to collect the higher order terms in  $1/s^3$  in the above expression. Only two terms are of order  $1/s^3$  (the first term from the relation (B1) and the term from  $\hat{\mu}^2$ ) ; collecting these terms leads to

$$\hat{\mu}^4 = \frac{6\bar{m}^2}{s^3} \left( 1 + 4\frac{A_s^1}{\bar{m}} + 2\left(\frac{A_s^1}{\bar{m}}\right)^2 \right) \quad (B3)$$

As  $s \rightarrow 0$ ,  $A_s^1 \rightarrow \delta_2$ , where  $\delta_2$  was defined in relation (11). Taking the inverse Laplace transform, we obtain

$$\frac{\mu^4(t)}{(\bar{N}_t)^2} = 3 \left( 1 + 4\frac{\delta_2}{\bar{m}} + 2\left(\frac{\delta_2}{\bar{m}}\right)^2 \right) \quad (B4)$$

## Appendix C: deriving $\langle T_n^2 \rangle$

To compute  $\langle T^2(n) \rangle$  we have to compute explicitly

$$L_n''(s) = \sum_{m=0}^{n-1} L_m'(s) \hat{K}'(s) L_{n-m-1}(s) \quad (C1)$$

$$+ \sum_{m=0}^{n-1} L_m(s) \hat{K}''(s) L_{n-m-1}(s) \quad (C2)$$

$$+ \sum_{m=0}^{n-1} L_m(s) \hat{K}'(s) L_{n-m-1}'(s) \quad (C3)$$

where

$$\begin{aligned} K(s) &= M(D + sI)^{-1} \\ L_n(s) &= K(s)^n \end{aligned} \quad (C4)$$

$$L_n'(s) = \sum_{m=0}^{n-1} L_m(s) \hat{K}'(s) L_{n-m-1}(s) \quad (C5)$$

and its matrix element  $\langle 1 | L''(0) | \psi \rangle$ . Using the relation (C5), the first line (C1) of the above relation is :

$$J_1(s) = \sum_{m=1}^{n-1} \sum_{j=0}^{m-1} L_j(s) K'(s) L_{m-j-1}(s) K'(s) L_{n-m-1}(s)$$

Using the simplifications

$$\langle 1 | L_k(0) = \langle 1 | ; L_k(0) | \psi \rangle = | \psi \rangle$$

we obtain

$$\begin{aligned} \langle 1 | J_1(0) | \psi \rangle &= \sum_{m=1}^{n-1} \sum_{j=0}^{m-1} \langle 1 | D^{-1} \tilde{M}^{m-j} D^{-1} | \psi \rangle \\ &= \sum_{j=1}^{n-1} \sum_{m=j}^{n-1} \langle 1 | D^{-1} \tilde{M}^j D^{-1} | \psi \rangle \\ &= \sum_{j=1}^{n-1} (n-j) \langle 1 | D^{-1} \tilde{M}^j D^{-1} | \psi \rangle \end{aligned}$$

where the second line has been obtained by re-indexing and changing the order of summation.

## Appendix D: Numerical simulations.

Numerical simulations are performed by a classical Gillespie algorithm[19]. The algorithm is implemented using the Julia language[21]. The process is simulated as described in figure 1. To generate one path for a given substitution matrix  $M$ , we begin with a state  $\alpha_0$  drawn from the equilibrium state  $|\pi\rangle$  or  $|\psi\rangle$  depending on the problem. The time  $t_1$  to the next transition is drawn from an exponential distribution with parameter  $m^{\alpha_0}$  ; the next state  $\alpha_1$  is drawn at random using the  $\alpha_0$  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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