Estimation of Past Demographic Parameters From the Distribution of Pairwise Differences When the Mutation Rates Vary Among Sites: Application to Human Mitochondrial DNA

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ABSTRACT

Distributions of pairwise differences often called “mismatch distributions” have been extensively used to estimate the demographic parameters of past population expansions. However, these estimations relied on the assumption that all mutations occurring in the ancestry of a pair of genes lead to observable differences (the infinite-sites model). This mutation model may not be very realistic, especially in the case of the control region of mitochondrial DNA, where this methodology has been mostly applied. In this article, we show how to infer past demographic parameters by explicitly taking into account a finite-sites model with heterogeneity of mutation rates. We also propose an alternative way to derive confidence intervals around the estimated parameters, based on a bootstrap approach. By checking the validity of these confidence intervals by simulations, we find that only those associated with the timing of the expansion are approximately correctly estimated, while those around the population sizes are overly large. We also propose a test of the validity of the estimated demographic expansion scenario, whose proper behavior is verified by simulation. We illustrate our method with human mitochondrial DNA, where estimates of expansion times are found to be 10–20% larger when taking into account heterogeneity of mutation rates than under the infinite-sites model.

With the advent of the coalescent theory (Kingman 1982), people have become increasingly aware of the profound effect of demography on the amount of genetic variability maintained in a population (Hudson 1990; Donnelly and Tavaré 1997). Population expansions or contractions indeed leave recognizable signatures in the pattern of molecular diversity (reviewed in Harpending et al. 1998). For instance, sudden demographic expansions lead to star-shaped phylogenies and unimodal distributions of pairwise differences (Slatkin and Hudson 1991; Rogers and Harpending 1992), to a reduction of the number of segregating sites (Bertorelle and Slatkin 1995; Aris-Brosou and Excoffier 1996; Tajima 1996), to a lower amount of linkage disequilibrium between linked loci (Slatkin 1994), or to the occurrence of a large proportion of very low frequency mutations (Fu and Li 1993; Fu 1997). Note that population bottlenecks usually have other recognizable effects, often opposed to those of population expansions (e.g., Tajima 1993; Cornuet and Luikart 1996; Harpending et al. 1998).

When exposed to the evidence of a past demographic expansion, one might want to estimate the parameters of the expansion, such as the time at which it occurred and its magnitude, but the choice of parameters to be estimated depends on a particular scenario of population growth one might choose, such as exponential growth, logistic growth, or an instantaneous stepwise growth one might choose, such as exponential growth, logistic growth, or an instantaneous stepwise growth. The latter model has been favored in the literature due to its simplicity and because simulation studies have shown that it was a good approximation of rapid logistic growth (Rogers and Harpending 1992). Rogers and Harpending (1992) convincingly showed that under an infinite-sites model, the shape of the distribution of the number of observed differences between pairs of DNA sequences (often called the mismatch distribution) conveyed information on the timing and the amplitude of a stepwise expansion. They proposed a nonlinear least-squares approach (Rogers and Harpending 1992) or a method of moments (Rogers 1995) to find the theoretical mismatch distribution that would best fit the observations. Several authors noted that this approach could be inadequate (Lundstrom et al. 1992; Bertorelle and Slatkin 1995; Aris-Brosou and Excoffier 1996; Wakeley and Hey 1997) because the infinite-sites model was not realistic, especially in the case of the mitochondrial genome where an important heterogeneity of mutation rates had been observed (Lundstrom et al. 1992; Wakeley 1993). In fact, the presence of a few fast mutating sites can lead to unimodal mismatch distributions even for moderate
The mismatch distribution under the infinite-sites model: We assume that t generations ago, a population at equilibrium of size $N_0$ entered a demographic expansion phase to instantaneously reach a new size $N_1$ and that it remained at that size ever since. Under this demographic scenario described in Figure 1 and assuming that every new mutation occurs at a new site [the infinite-sites mutation model of Kimura (1969)], Li (1977) derived an expression for the probability of observing $i$ differences between two genes taken at random from this population, as

$$F_i(\theta_1, \theta_0, \tau) = F_i(\theta_1) + \exp\left(-\frac{\theta_1 + 1}{\theta_1}\right) \times \sum_{j=0}^{\infty} \frac{\tau^j}{j!} \left[ F_{i-j}(\theta_0) - F_{i-j}(\theta_1) \right], \quad (1)$$

where $\theta_0 = 2N_0u$, $\theta_1 = 2N_1u$, $\tau = 2ut$, and $u$ is the total mutation rate per generation per gene. Here, $F_i(\theta)$ is also the probability of observing $i$ differences between two genes in an equilibrium population as (Watterson 1975)

$$F_i(\theta) = \frac{\theta^i}{(\theta + 1)^{i+1}}. \quad (2)$$

Rogers and Harpending (1992) rederived Equation 1 and used it to describe the distribution of the number of pairwise differences between nonrecombining DNA sequences or RFLP haplotypes in a given sample, which they called the “mismatch distribution.” They proposed to estimate the demographic parameters $\theta_0$, $\theta_1$, as well as the expansion time $\tau$ directly from this mismatch distribution.

The mismatch distribution under a finite-sites model: Under the finite-sites model, $F^*_i(\theta_1, \theta_0, \tau)$ provides the distribution of the number of mutations having occurred during the evolution of a random pair of genes. Note that this number can be equal to or larger than the number of observed differences, depending on whether the same site has been hit several times by mutations or not. In this case, the expected mismatch probability distribution noted by $F^*_i(\theta_1, \theta_0, \tau)$ can be obtained by taking into account those cases where $j$ mutations ($j \geq i$) have led to exactly $i$ differences, as

$$F^*_i(\theta_1, \theta_0, \tau) = \sum_{j=i}^{\infty} F^*_j(\theta_1, \theta_0, \tau)H^m(i, j), \quad (3)$$

where $H^m(i, j)$ is the conditional probability of observing $i$ differences given $j$ mutations have occurred in the ancestry of two sequences of length $m$. We now describe how to obtain these conditional probabilities $H^m(i, j)$ starting with $m = 1$ and extending it to a sequence of arbitrary length.

One site case: We first solve the problem for one site ($m = 1$) assuming Kimura’s two-parameters model of mutation (Kimura 1980) with arbitrary relative tran-
tion (s) and transversion (v) rates subject to \( s + v = 1 \). Conditional on the number of mutations, we consider the mutation process as a random walk between the nucleotides A, C, G, and T. This Markov process has the single-step transition matrix

\[
\mathbf{M} = \begin{pmatrix}
A & C & G & T \\
0 & v/2 & s & v/2 \\
v/2 & 0 & v/2 & s \\
\frac{s}{2} & v/2 & 0 & v/2 \\
\frac{v}{2} & s & v/2 & 0
\end{pmatrix}
\]

where the elements of each row add up to one. The \( j \)th power of the matrix \( \mathbf{M} \) can be used to describe the impact of \( j \) mutations at that site. A closed-form expression for \( \mathbf{M}^j \) can be conveniently obtained by a diagonalization of \( \mathbf{M} \), as \( \mathbf{M} = \mathbf{VDV}^{-1} \), where \( \mathbf{D} = \text{diag} \{-s, -s, v + s, -v + s\} \) and \( \mathbf{V} \) is a matrix where the columns are the eigenvectors of \( \mathbf{M} \).

We thus obtain

\[
\mathbf{M}^j = \mathbf{VDV}^{-1} = \begin{pmatrix}
\alpha_i & \beta_i & \gamma_i & \beta_i \\
\beta_i & \alpha_i & \beta_i & \gamma_i \\
\gamma_i & \beta_i & \alpha_i & \beta_i \\
\beta_i & \gamma_i & \beta_i & \alpha_i
\end{pmatrix}
\]

where \( \alpha_i = \frac{1}{2}(1 + 2(-s) + (s - v)) \), \( \beta_i = \frac{1}{2}(1 - (s - v)) \), and \( \gamma_i = \frac{1}{2}(1 - (s - v)) \). The diagonal terms of \( \mathbf{M}^j \), here all equal to \( \alpha_i \), represent the probability of returning to the initial state after \( j \) mutations. It thus follows that

\[
H^j(0, j) := P(\text{no difference} | j \text{ mutations}) = \alpha_i,
\]

\[
H^j(1, j) := P(\text{one difference} | j \text{ mutations}) = 1 - \alpha_i.
\]

(4)

Multisite case, homogeneous mutation rates: Instead of deriving an explicit equation for \( H^n(i, j) \), when \( m > 1 \) we can compute these probabilities numerically using a recurrence equation, as shown below. Let us suppose that we have already derived the probability \( H^{n-i}(i, j) \) and that we want to study the case for an additional site and thus derive \( H^n(i, j) \). Suppose that \( i \) mutations have occurred at the \( m \)th site and that the \((j - 1)\) remaining mutations have occurred at the \((m - 1)\) other sites. The probability of observing overall \( i \) differences will depend upon whether we observe one or no difference at the \( m \)th site. With probability \( P_1 \), one difference will be observed at the \( m \)th site and \((i - 1)\) at the \((m - 1)\) other sites, and with probability \( P_2 \), all \( i \) differences will be observed among the \((m - 1)\) other sites. Therefore,

\[
P_1 = H^j(1, i)H^{n-i}(i - 1, j - l),
\]

\[
P_2 = H^j(0, i)H^{n-i}(i, j - l),
\]

(5)

where we admit that \( H^{n-i}(-1, j - l) = 0 \). Summing over all possible \( i \) values and multiplying by the probability that \( l \) mutations occur at the additional site, we finally have

\[
H^n(i, j) = \sum_{l=0}^{i} b(l, j, p)(P_1 + P_2)
\]

\[
= \sum_{l=0}^{i} b(l, j, p) H^{n-i}(i - 1, j - l) + H^j(0, i)H^{n-i}(i, j - l),
\]

(6)

where \( b(l, j, p) = (j-1)(j-1)!\) \( p(1-p)^{i-l} \) is the binomial probability with parameter \( p = 1/m \).

The mismatch distribution under a two-rates finite-sites model: Mutation rate heterogeneity arises when the mutation rates are not equal for all nucleotide sites. The simplest form of heterogeneity to be considered is a two-rates mutation model, where we make the distinction between fast and slow sites. As most mutations accumulate at a small number of fast sites, convergent or reverse mutations can become quite common. The consequence of this type of heterogeneity on the pattern of diversity has been studied in the case of the control region of human mitochondrial DNA (Wakeley 1993; Yang 1994, 1996; Bertorello and Slatkin 1995; Yang et al. 1995). Following Yang (1996), who inferred the number of segregating sites in a stationary population under the finite-sites model for two classes of mutation rates, we can modify Equation 3 by considering that \( m_f \) of the \( m \) sites are fast and that \( m_s \) are slow. In this case, we have to take into account all possible ways of partitioning the \( i \) differences among the slow and fast regions. If we assume that mutations occur independently, the probabilities of these partitions simply follow a binomial distribution. The expected mismatch probability distribution is thus given by

\[
F^{m=m_f} (\theta_f, \theta_o, \tau) = \sum_{k=1}^{m_f} F_{\tau}^{-} (\theta_f, \theta_o, \tau) \sum_{l=0}^{i} \sum_{k=0}^{l} b(k, j, p) \times H^{m_f}(l, k) H^{m_s}(i - l, j - k),
\]

(7)

where \( b(k, j, p) = (j-1)! \) is the same binomial probability function as in Equation 6 but with parameter \( p = m_f/m \) \((m_r + m_s) \) being the conditional probability that a mutation will hit one of the \( m_f \) fast mutating sites, and \( r \) is here the ratio of fast and slow mutation rates. Note that Equation 7 is the equivalent to Yang’s (1996) Equation 39, derived for the case of a stationary population. Yang used an infinite-alleles mutation model, stipulating that once a site is hit by one or more mutations, we observe one difference. Due to the high transition bias, this model also tends to overestimate the number of differences because it does not allow back mutations at non-segregating sites.

Multisite case, multisite mutation model: Suppose that we have a sequence of length \( m \) and that each nucleotide has a potentially different probability \( p_i \) \((i = 1 \ldots m) \) of being hit by a mutation, subject to the condition \( \sum_{i=1}^{m} p_i = 1 \). Under this multisite model, \( H^n(i, j) \), noted here \( H^{m}(i, j) \), can be also obtained by the recurrence
relation given in (6) except that the parameter $p$ is now equal to $p_t / \sum_{i=1}^n p_i$. Here $n$ is the index of the recursion step (1 ≤ $n$ ≤ m), and $p_t$ thus changes at every step of the recursion. It is important to note that, unlike the homogenous mutation rate case, the intermediate recurrence matrices are here meaningless, and only the last matrix obtained by this recurrence is correct for the required heterogeneity pattern. Under this m-rates mutation model, the expected mismatch distribution is not given in a more complex form than in the constant mutation model, the expected mismatch distribution $F_{\text{mR}} (\hat{\theta}_t, \hat{\theta}_0, \hat{\tau})$ is then obtained by setting $\hat{\tau} = \hat{\tau}$.

Note that the two-rates model is a special case of the present model and that it could be treated similarly. In that case, the double summation of Equation 7 could be condensed into the last term of Equation 8.

The mismatch distribution for gamma-distributed mutation rates: A gamma distribution of mutation rates can be seen as a special case of the m-rates mutation model. Such a distribution has been hypothesized for explaining the pattern of diversity in the control region of the mitochondrial genome (e.g., Kocher and Wilson 1991; Hasegawa et al. 1993; Wakeley 1993; Yang 1996). The density of the gamma distribution is given by

$$f(x) = \frac{1}{\Gamma(\alpha)} x^{\alpha-1} e^{-x},$$

where $\alpha$ is the shape parameter of the gamma distribution equal to $V(x)/\chi^2$, the inverse of the square of the coefficient of variation of mutation rates. We can discretize the gamma distribution over the m nucleotides as follows. We draw an arbitrarily large number of continuous gamma-distributed variates (say 1 million) with mean and variance equal to the shape parameter $\alpha$ (Ahrrens and Dieter 1974). The variates are then sorted and divided into m groups of equal size. The mean value of the ith group is then taken as a discretely distributed gamma variate $\tilde{p}_i$. The relative probability of being hit by a mutation $p_t$ is then obtained by setting $p_t = \tilde{p}_t / \sum_{i=1}^m \tilde{p}_i$. Those $\tilde{p}_i$'s can then be used directly in recursion Equation 6 to get the probabilities $H_{\text{mR}} (i, j)$ required in Equation 8.

In the present article, we used the values of the shape parameters $\alpha$ computed by S. Meyer (Meyer et al. 1999) on a human mtDNA control region sequence database (Handt et al. 1998) as $\alpha = 0.26$ for HV1 and $\alpha = 0.13$ for HV2.

Estimation of past demographic parameters using a least-squares approach: We estimated the demographic parameters $\theta_0$, $\theta_1$, and $\tau$ from the mismatch distribution, using a nonlinear least-squares approach. We use the Hooke and Jeeves algorithm (Hooke and Jeeves 1963) to find those parameters that minimize the sum of square deviations (SSD) between the observed mismatch distribution $F_{\text{exp}} (i)$ and its expectation $F_{\text{exp}}$ under a particular model. SSD is conventionally defined as

$$SSD = \sum_{i=0}^n (F_{\text{exp}} - F_{\text{obs}})^2. \quad (9)$$

Depending on which mutation model we consider, we replaced $F_{\text{exp}}$ by the quantities defined in Equations 3, 7, or 8. The Hooke-Jeeves algorithm starts from an arbitrary initial set of parameters and converges by an iterative process to a local minimum. This minimization procedure was mainly chosen for its robustness and its ability to converge under nontrivial conditions.

Bootstrap confidence intervals: We followed a parametric bootstrap approach to generate percentile confidence intervals around the estimated parameters $\hat{\theta}_0$, $\hat{\theta}_1$, and $\hat{\tau}$. For each of the $B$ simulated data sets, we applied our estimation procedure according to Equation 3, 7, or 8 to evaluate $B$ bootstrapped values $\hat{\theta}_0$, $\hat{\theta}_1$, and $\hat{\tau}$. For a given confidence level $\alpha$, the approximate limits of the confidence interval were obtained as the $1 - \alpha / 2$ and $1 + \alpha / 2$ percentile values (Efron and Tibshirani 1993, p. 168). It is important to emphasize that this form of parametric bootstrap assumes that the data are distributed according to the sudden expansion model.

Testing the validity of the sudden expansion model: We tested the hypothesis that the observed data fitted the sudden expansion model defined by the estimated parameters using the same parametric bootstrap approach as described above. We used here SSD defined in Equation 9 as a test statistic. We obtained its distribution under the hypothesis that the estimated parameters are the true ones by simulating B samples around the estimated parameters. As before, we reestimated each time new parameters $\hat{\theta}_0$, $\hat{\theta}_1$, and $\hat{\tau}$ and computed their associated sums of squares SSD_sim. The $P$ value of the test is therefore approximated by

$$P = \frac{\text{number of SSD_sim larger or equal to SSD_exp}}{B}.$$  

To check the accuracy of this procedure, we generated 1000 random data sets for the parameters $\theta_0 = 1$, $\theta_1 = 1000$, and $\tau = 3$ under a two-rates model with 270 slow sites and 30 fast sites mutating 20 times faster than the slow sites, which corresponds to the simulation conditions of the top of Table 1. The simulated distribution of the $P$ values for these parameters was almost uniform between 0 and 1 (data not shown), suggesting that the
SSD statistic provides a valid test of the sudden expansion model.

**RESULTS**

We show in Figure 2 the theoretical mismatch distributions and the demographic parameters estimated using different methods and mutation models for the two hypervariable segments of Mandenka mtDNA control region (Graven et al. 1995). Except for the curve based on Rogers’ method of moments (Rogers 1995), the shapes of the theoretical mismatch distributions are very similar to each other and provide no real indication of the validity of the underlying model. Note also that a much better fit is found for HV1 than for HV2. While the shape of the best-fitted mismatch does not seem to depend much on the mutation model, the values of the estimated parameters do change quite extensively, especially the expansion time \( \tau \), which shows larger values for finite-sites models than for the infinite-sites model. This is of course due to the fact that several mutations can accumulate at a given site in finite-sites models and that a longer evolutionary time is necessary to lead to the same number of observed differences. The magnitude of the expansion is also found to be smaller in finite-sites models, in agreement with previous simulation results (Aris-Brosou and Excoffier 1996).

In Figure 3, we show the expected mismatch distributions fitted for the Turkana sample (Watson et al. 1996) according to a finite-sites two-rates model (Figure 3a) and to a finite-sites gamma distribution model (Figure 3b). We also report the average mismatch distributions obtained from 5000 simulations performed according to the estimated parameters. Simulated and expected mismatch distributions are found to be in very good agreement, motivating the use of simulations to get empirical confidence intervals around the parameters.

To check if these confidence intervals have good coverage properties (i.e., the true parameters should be included in the confidence interval with a probability \( 1 - \alpha \)), we performed a series of simulations for a set of predefined parameters. For a given set of parameters \( \theta_0, \theta_1, \) and \( \tau \), we simulated 1000 data sets from which we estimated the parameters \( \hat{\theta}_0, \hat{\theta}_1, \) and \( \hat{\tau} \). For each set of estimated parameters, we simulated 1000 additional data sets from which new values \( \theta_0^*, \theta_1^*, \) and \( \tau^* \) were estimated. The distribution of these 1000 bootstrap values was used to evaluate the lower and upper limits of a \( 100(1 - \alpha) \% \) confidence interval around the \( \hat{\theta}_0, \hat{\theta}_1, \) and \( \hat{\tau} \) values as the \( \alpha/2 \) and \( 1 - \alpha/2 \) percentiles of the distribution, respectively. The results of these analyses are shown in Tables 1 and 2 for different types of mutation rate heterogeneity. It can be seen that the only parameter for which the bootstrap confidence interval has a good coverage is \( \tau \), as the proportion of the times the true value is outside the confidence interval is approximately equal to the significance level \( \alpha \). Note, however, that the confidence interval is not well centered, as the true values outside the confidence interval are always found on the left of the distribution. The bootstrap confidence intervals for \( \theta_0 \) and \( \theta_1 \) are much too broad (the true value of the parameter is found too often within the empirical confidence interval). In Figure 4, we plot the distributions of the two statistics \( x - \bar{x} \) and \( \bar{x} - x' \) for \( x = \tau \) and for \( x = \theta_0 \). To generate true
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**DISCUSSION**

In this study, we extend the model of Rogers and Harpending (1992) to estimate the parameters of a sudden stepwise demographic expansion by explicitly taking into account a possible heterogeneity of mutation rates. Contrary to previous claims (Rogers 1992; Rogers et al. 1996), we find that the estimated values of the parameters and their confidence intervals are quite sensitive to departure from the infinite-sites model. For instance, the estimated values of the expansion time (τ) shown in Figure 2 for the Mandenka population are found, respectively, 9 and 20% larger for HV1 and HV2 when using a model with gamma-distributed mutation rates than for the infinite-sites model. Even though our methodology appears computationally more intensive, it thus seems justified to take into account the known departures from the infinite-sites model to estimate the parameters of the stepwise demographic model. The present approach does not allow us to retrieve all the parameters of a demographic expansion with the same efficiency. As shown in Tables 1 and 2, the expansion time (τ) and the initial population size (θ0) are the only parameters that can be estimated without much bias and with reasonable precision, while the estimation of θ1 is clearly biased upward. The confidence intervals obtained from the parametric bootstrap approach are fairly estimated only for the expansion time τ, while those for the population sizes are clearly too large and thus overly conservative. This implies that the magnitude of the expansion cannot be precisely recovered by the present approach. This is understandable because once the expansion is sufficiently large, very few coalescent events (if any) will have occurred between the present time and the beginning of the expansion. As it is the accumulation of those coalescent events that can

![Image](https://via.placeholder.com/150)

Figure 3.—Theoretical and simulated mismatch distributions for Turkana's mtDNA HV1 (Watson et al. 1996). The simulated line was obtained as the mean mismatch distribution obtained after 5000 simulations according to the estimated parameters shown at the bottom of each graph. The estimated parameters were obtained for (a) two-rates model: of 370 sites, 34 were considered mutating 11 times faster than the others; and (b) finite sites (370 bp) m-rates model assuming a gamma distribution of mutation rates (shape parameter α = 0.26). The 95% confidence intervals of each estimated parameter are shown within brackets and are obtained from the simulations.
TABLE 1
Proportion of noninclusion of the exact parameter value: two mutation rates model

<table>
<thead>
<tr>
<th>Parameters of the two-rates mutation model</th>
<th>Demographic parameters</th>
<th>True values</th>
<th>Average estimated value</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>m₁  m₂  r</td>
<td></td>
<td></td>
<td></td>
<td>0.010  0.020  0.050  0.100</td>
</tr>
<tr>
<td>270 30 20</td>
<td>τ</td>
<td>3</td>
<td>3.726 (1.195)</td>
<td>0.008  0.024  0.095  0.203</td>
</tr>
<tr>
<td></td>
<td>θ₀</td>
<td>1</td>
<td>0.812 (1.137)</td>
<td>0      0      0.004  0.061</td>
</tr>
<tr>
<td></td>
<td>θ₀</td>
<td>1000</td>
<td>3777 (7638)</td>
<td>0      0      0.002  0.015</td>
</tr>
<tr>
<td>290 10 50</td>
<td>τ</td>
<td>3</td>
<td>3.854 (1.493)</td>
<td>0.010  0.023  0.089  0.189</td>
</tr>
<tr>
<td></td>
<td>θ₀</td>
<td>1</td>
<td>0.806 (1.051)</td>
<td>0      0      0.001  0.020</td>
</tr>
<tr>
<td></td>
<td>θ₀</td>
<td>1000</td>
<td>3246 (6664)</td>
<td>0      0      0.007  0.019</td>
</tr>
<tr>
<td>270 30 20</td>
<td>τ</td>
<td>9</td>
<td>9.253 (1.037)</td>
<td>0.003  0.005  0.015  0.057</td>
</tr>
<tr>
<td></td>
<td>θ₀</td>
<td>1</td>
<td>0.822 (1.019)</td>
<td>0      0      0      0</td>
</tr>
<tr>
<td></td>
<td>θ₀</td>
<td>1000</td>
<td>4183 (8444)</td>
<td>0      0      0      0</td>
</tr>
<tr>
<td>290 10 50</td>
<td>τ</td>
<td>9</td>
<td>9.286 (1.072)</td>
<td>0.004  0.009  0.023  0.056</td>
</tr>
<tr>
<td></td>
<td>θ₀</td>
<td>1</td>
<td>0.808 (1.019)</td>
<td>0      0      0      0</td>
</tr>
<tr>
<td></td>
<td>θ₀</td>
<td>1000</td>
<td>3986 (8917)</td>
<td>0      0      0      0.001</td>
</tr>
</tbody>
</table>

a  m₁ is the number of slow sites; m₂ is the number of fast sites; r is the ratio of mutation rates between the fast and the slow sites.

b Average value of the parameters estimated from 1000 coalescent simulations around the true values (standard deviations shown in parentheses).

Provide some information on the present population size, there will often be too few of them to get a reliable estimate of the present size, which will also tend to be overestimated. The present parametric bootstrap approach for defining the confidence intervals differs somewhat from that described in previous studies (Rogers 1995; Rogers et al. 1996). The previous approaches consisted of finding a set of values of the demographic parameter compatible with the observed data. The “compatibility” criterion was a statistic of goodness-of-fit (mean absolute error) between the observed and the theoretical mismatch distribution. A set of demographic parameters θ₀, θ₁, and τ was declared compatible if the goodness-of-fit statistic fell within a 95% confidence interval obtained by simulation. While this approach seems valid, it requires much heavier computations than ours if one wants to adequately explore the space of possible parameters, as a series of simulations needs to be carried out for each set of parameters. Moreover, the potential impact of the chosen goodness-of-fit statistic on the results and the reliability of the confidence intervals has not been addressed. The fact that the effective popula-

TABLE 2
Proportion of noninclusion of the exact parameter value: gamma distribution of mutation rates

<table>
<thead>
<tr>
<th>Parameters of the mutation model</th>
<th>Demographic parameters</th>
<th>True values</th>
<th>Average estimated value</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>m  α²</td>
<td></td>
<td></td>
<td></td>
<td>0.010  0.020  0.050  0.10</td>
</tr>
<tr>
<td>300 0.26</td>
<td>τ</td>
<td>3</td>
<td>3.514 (0.920)</td>
<td>0.014  0.028  0.076  0.183</td>
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<tr>
<td></td>
<td>θ₀</td>
<td>1</td>
<td>1.017 (1.227)</td>
<td>0      0      0.001  0.033</td>
</tr>
<tr>
<td></td>
<td>θ₀</td>
<td>1000</td>
<td>5,525 (10,095)</td>
<td>0      0      0.001  0.002</td>
</tr>
<tr>
<td>300 0.13</td>
<td>τ</td>
<td>3</td>
<td>3.457 (0.897)</td>
<td>0.007  0.019  0.069  0.146</td>
</tr>
<tr>
<td></td>
<td>θ₀</td>
<td>1</td>
<td>1.003 (1.265)</td>
<td>0      0      0.004  0.039</td>
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<tr>
<td></td>
<td>θ₀</td>
<td>1000</td>
<td>5,220 (9,873)</td>
<td>0      0      0      0</td>
</tr>
<tr>
<td>300 0.26</td>
<td>τ</td>
<td>9</td>
<td>9.210 (1.073)</td>
<td>0.013  0.024  0.081  0.152</td>
</tr>
<tr>
<td></td>
<td>θ₀</td>
<td>1</td>
<td>1.210 (1.111)</td>
<td>0      0      0      0</td>
</tr>
<tr>
<td></td>
<td>θ₀</td>
<td>1000</td>
<td>4538 (9,253)</td>
<td>0      0      0      0</td>
</tr>
<tr>
<td>300 0.13</td>
<td>τ</td>
<td>9</td>
<td>9.219 (1.146)</td>
<td>0.006  0.014  0.048  0.112</td>
</tr>
<tr>
<td></td>
<td>θ₀</td>
<td>1</td>
<td>1.195 (1.002)</td>
<td>0      0      0      0</td>
</tr>
<tr>
<td></td>
<td>θ₀</td>
<td>1000</td>
<td>4014 (8,300)</td>
<td>0      0      0      0</td>
</tr>
</tbody>
</table>

α Shape parameter of the gamma distribution.

b Average value of the parameters estimated from 1000 coalescent simulations around the true values (standard deviations shown in parentheses).
to small changes in the mismatch distribution (see also Rogers 1997). Conversely, small differences in the shape of the observed mismatch distribution will profoundly affect the values of the estimated parameters. As the estimation of the expansion time \( t \) depends essentially on the mean of the mismatch distribution (Rogers and Harpending 1992), while the other parameters \( \theta_0 \) and \( \theta_1 \) depend on higher moments of the distribution, those latter two parameters are more likely to be affected by the stochasticity of the genealogical process than \( t \). This is in keeping with our simulations, which show that the expansion time is usually quite well recovered from the mismatch distribution (Tables 1 and 2).

Even though we have refined the mutation model for mtDNA sequences, one can see that the theoretical mismatch distributions do not always perfectly fit with the observed distributions (Figures 2, 3, and 5). We can see two reasons explaining this discrepancy.

First, the single stepwise expansion model may be inadequate for some populations. Alternative population expansion models such as exponential growth or logistic growth could be more realistic than the stepwise growth used in this study (Polanski et al. 1998), but as long as the magnitude of the expansion is large and we start from a small population, they should lead to results very similar to those provided here (Rogers and Harpending 1992; Rogers 1997). It seems more likely that demographic scenarios very different from population growth may explain these discrepancies. Population contractions may indeed have occurred in some populations and could explain the rejection of the sudden expansion model. A population crash could have occurred in the Ngoebe population from Panama, as well as in other native Amerindian populations, where the hypothesis of sudden expansion is not supported, as in the Kuna from Panama (\( P = 0.05, \text{HV1} \)), the Huetar from Costa-Rica (\( P = 0.026, \text{HV2} \)), or the Mapuche from Argentina (\( P = 0.009, \text{HV2} \)). Additional evidence for a recent population contraction comes from the observation of large positive values for Tajima’s D-statistics (Tajima 1989) in those populations (results not shown), which are expected in the case of a recent population bottleneck (Tajima 1993). Note also that other factors like admixture events, population substructure, or inbreeding could all affect the shape of the mismatch distribution but to an extent that has not yet been quantified.

Second, the probabilities derived in Equations 1 and 2 and their derivatives apply to a pair of genes chosen at random from the population, while they are applied here to a random pair chosen from the sample. However, pairs drawn from the sample are not independent due to the shared portions of their genealogy. In populations having gone through a recent and large expansion, the internal branches are very short due to the star-like structure of the tree (Slatkin and Hudson 1991; Fu 1997), and a very few mutations will accumulate.

![Figure 4](image-url)

**Figure 4.**—Verification of the validity of the parametric bootstrap procedure. The distribution of the difference between the true value of the parameter (\( x \)) and its estimation from the simulation (\( \hat{x} \)) (1000 values) is compared to the distribution of the difference between the simulated (\( x \)) and bootstrapped parameter values (\( x^* \)) (1,000,000 values). The two statistics \( x - \hat{x} \) and \( x - x^* \) should be identically distributed for parametric bootstrap to work. (Top) \( x = \tau = 3, \theta_0 = 1, \theta_1 = 1000 \). (Bottom) \( x = \theta_0 = 1, \tau = 3, \theta_1 = 1000 \). Population sizes are not well recovered from the mismatch distribution would suggest that this previous approach may suffer from the same problems as the simple parametric bootstrap procedure and thus also lead to overly large confidence intervals.

A recent study has shown that time-dependent demographic models (including the present stepwise expansion model) were unstable with respect to the estimation of the demographic parameters describing the population sizes (Polanski et al. 1998), in the sense that large fluctuations in the demographic parameters lead only
Estimation of Past Demographic Parameters

Figure 5.—Empirical 95% confidence intervals for the mismatch distribution in four human populations analyzed for mtDNA control region. In all cases, a finite-sites mutation model was used, assuming gamma distribution of mutation rates (α = 0.26 for HV1 and α = 0.13 for HV2). For the Ngoebe sample (Kolman et al. 1995), the hypothesis of sudden expansion is rejected by the SSD test (P = 0.007). The SSD P values for the other samples are as follows: English HV1 (Piercy et al. 1995), 0.764; Mandenka HV2 (Graven et al. 1995), 0.0652; !Kung HV1 (Vigilant et al. 1991), 0.1368.

on those branches. In that case, the correlation between the number of pairwise differences (dij) will only be due to shared external branches (i.e., d12 and d13 will be correlated due to the shared lineage leading to sequence 1, but d12 and d13 should be almost independent), and our derivations should better hold at the sample level. On the other hand, for stationary populations or relatively small or remote expansions, some coalescent events will occur before and after the expansion. The internal branches will be longer and have a large associated variance. Those equations, while still being correct for a single pair of genes, will thus not allow us to get the sample distribution of pairwise differences as they do not take into account the covariance of pairwise differences. Therefore, the present method is not expected to recover the parameters of a demographic expansion efficiently unless the expansion has been very large.

Although mismatch distributions carry some information on the shape of the underlying gene genealogy and coalescent process, other aspects of molecular diversity are not explicitly taken into account by this approach. It has been shown that demographic parameters recovered from the mismatch distribution did not allow the correct prediction of the number of observed polymorphic sites (Bertorelle and Slatkin 1995) or of the distribution of mutation frequencies (Wakeley 1993, p. 614) for human mtDNA. This could either be due to departure from the infinite-sites mutation model or from the proposed simple demographic model. Even after having introduced a more realistic mutation model, we still find that the observed number of segregating sites is not always in agreement with the distribution obtained from simulations based on the estimated demographic parameters. For instance, considering the mismatch distributions shown in Figure 5, even if we get a perfect fit for the English HV1 sample, the estimated parameters lead on average to far fewer segregating sites than observed, although not significantly so (Sobs = 67; Smean = 60.6; SD(S) = 6.2; P = 0.872). Interestingly, the Mandenka HV2 sample presents a significant lack of segregating sites for HV2 as compared to the estimated expansion conditions (Sobs = 27; Smean = 38.0; SD(S) = 5.0; P = 0.014). This discrepancy could be explained by a very large heterogeneity of mutation rates in HV2 for this population, but it seems difficult to understand how and why the structural and functional constraints that are supposed to shape the heterogeneity of mutation rates (Wakeley 1993, p. 614) could differ between populations. Additional studies on that matter would nevertheless be needed to exclude this possibility.

To get absolute values for the demographic parameters inferred using the present approach, one should get an estimation of the substitution rate at the nucleotide level. The real value of mutation rate in humans has recently been the subject of an intense debate between those advocating the use of a phylogenetic mutation rate (~3 × 10⁻⁶ substitutions per site per generation of 20 yr) calibrated by the divergence between humans and chimpanzees (Jazin et al. 1998) and those studying
the mutation process directly on pedigrees giving numbers \( \sim 10 \) times larger \( (\sim 2.7 \times 10^3) \) substitutions per site per generation; Howell et al. 1996; Parsons et al. 1997; Parsons and Holland 1998). For the present methodology to be fully beneficial, it thus seems highly necessary to get reliable estimates of mutation rates. Otherwise, the importance of taking into account more realistic mutation models would seem rather futile.

Even if the present approach is an improvement over previous methods, it seems that the use of the mismatch distribution as a summary statistic may not exploit the full potential of molecular data and that maximum-likelihood methods that take into account phylogenetic relationships between DNA sequences (e.g., Griffiths and Tavaré 1994; Kuhner et al. 1995; Tavaré et al. 1997; Kuhner et al. 1998; Weiss and von Haeseler 1998) would be needed to get more reliable estimates of demographic parameters. However, considering the fact that these methods are extremely computer-intensive when heterogeneity of mutation rates is considered, the present approach may still be useful in most practical purposes.

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**LITERATURE CITED**


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