Estimating effective population size and mutation rate from sequence data using Metropolis-Hastings sampling

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ABSTRACT

We present a new way to make a maximum likelihood estimate of the parameter $4N_e\mu$ (effective population size times mutation rate per site, or Θ) based on a population sample of molecular sequences. We use a Metropolis-Hastings Markov chain Monte Carlo method to sample genealogies in proportion to the product of their likelihood with respect to the data and their prior probability with respect to a coalescent distribution. A specific value of Θ must be chosen in order to generate the coalescent distribution, but the resulting trees can be used to evaluate the likelihood at other values of Θ, generating a likelihood curve. This procedure concentrates sampling on those genealogies which contribute most of the likelihood, allowing estimation of meaningful likelihood curves based on relatively small samples. The method can potentially be extended to cases involving varying population size, recombination, and migration.

INTRODUCTION

The genealogy representing the relationship between a set of gene copies randomly chosen from a population can be thought of as a series of coalescences — points at which two lineages had a common ancestor (see Figure 1). The time intervals between one coalescence and the next are expected to have a distribution which depends on the effective population size $4N_e$ (in a diploid population; this paper will assume diploids, but the method is identical when applied to haploids, with $4N_e$ replaced by $2N_e$, and to mitochondria, with $4N_e$ replaced by $2N_f$). In the absence of an outside standard, molecular sequence data cannot give information on the actual durations of these intervals, but only on the amount of change that

occured during them. Therefore, instead of estimating $4N_e$ we must estimate its product with the neutral mutation rate μ. This paper discusses a new method for estimating the product $4N_e\mu$, also called Θ, using sequence data taken from a random sample of individuals from a population.

We wish to use the relationship between the intervals in the genealogy and Θ to make a maximum likelihood estimate of Θ from genealogies inferred from a population sample (for example, of nucleotide sequences). An earlier paper (FELSENSTEIN 1992b) approached this problem using bootstrapping. Since the true genealogy is generally unknown, we wish to base the estimate on a number of plausible genealogies, weighting each one according to its plausibility. FELSENSTEIN suggested bootstrap resampling the DNA data and using the genealogies reconstructed from each bootstrap sample to estimate Θ — arguing that this resampling procedure effectively chooses genealogies in proportion to their likelihood with respect to the data, which is equivalent (if a large number of samples are taken) to weighting the genealogies by their likelihood. For reasons which will be discussed below, we now believe this approach to be incorrect.

In the current paper we present a new method of sampling genealogies. The strategy is Metropolis-Hastings sampling: a repeated process of modifying a genealogy and accepting or rejecting it in proportion to the ratio of its probability to the probability of the previous genealogy, as described by METROPOLIS et al. (1953) and modified by HASTINGS (1970). We present the method as it applies to DNA or RNA sequence data, but it could readily be adapted to other types of information for which models of the change process are available, such as restriction site data. As presented, this method is appropriate for use in cases where recombination does not occur, such as mitochondrial DNA, but we hope in the future to extend it to cases involving recombination, migration, and varying population size.

We would like to compute the likelihood of the observed sequence data for a given value of Θ, $L(\Theta)$, in order to find the value of Θ which maximizes the likelihood of the data, and to assess how well supported this value is compared to others. For a given genealogy, $L(\Theta)$ is the product of the prior probability of the genealogy based on the coalescent distribution, $P(G|\Theta)$, and the probability of the sequence data given the genealogy, $P(D|G)$. This product should be summed over all possible genealogies to give the overall likelihood of the data set for a given value of Θ. The prior probability has been described by KINGMAN (1982a, b) and is straightforward to calculate. The probability of the sequence data for a given genealogy is also readily computable (FELSENSTEIN 1981). However, computation of the overall likelihood $L(\Theta) = \sum_G P(D|G)P(G|\Theta)$ demands a summation over a huge number of topologies, each with an infinite number of possible branch lengths.

Rather than sampling all genealogies, we could consider making a random sample; but in practice most genealogies are extremely implausible explanations of the sequence data and therefore contribute almost no information to the estimate. In order to get an accurate estimate, the random sample would have to be unmanageably large. Therefore, we use an importance sampling approach: we concentrate sampling on those genealogies which are plausible and therefore will contribute substantially to the estimate of Θ.

To use this approach, we need to choose a known distribution from which to sample. One approach would be to sample with respect to the coalescent prior $P(G|\Theta)$ — the prior probability of a genealogy at a given value of Θ, without regard to the data. This is easily done, but most of the genealogies drawn from $P(G|\Theta)$ do not contribute substantially to the likelihood because their topology is implausible for the given data, making this type of sampling very inefficient.

Another approach would be to sample genealogies from a probability density

proportional to the probability of the data given the genealogy, $P(D|G)$. One of us (Felsenstein 1992b) previously proposed to estimate Θ by bootstrapping: that is, repeatedly making new data sets by sampling with replacement from the original one, estimating the genealogy from each new data set, and treating each of the resulting genealogies as an independent sample from $P(D|G)$. Only limited simulation of this method was undertaken due to its slowness and to technical difficulties (when the true value of Θ is small, some bootstrap replicate data sets contain no variable sites, and such data sets disrupt the estimate). These simulations were not sufficient to establish whether or not this method (the bootstrap Monte Carlo method) is unbiased. We now know it to be biased, for the following reason.

The bootstrap resampling is attempting to sample points from a distribution proportional to $P(D|G)$. This is not a legitimate distribution to sample from: it has infinite area. Consider the case of only two sequences, and suppose that the data provide no information about the correct branch length back to their coalescence (for example, zero bases were sampled). In this case, the branch length could take any value from zero to infinity with equal probability, which means its expectation is infinitely large. If the data provide some information, but not enough to establish the branch length with perfect certainty, there will be an upwards bias in the estimate of Θ because the space of longer trees to sample is infinitely larger than the space of smaller trees, and longer trees lead to a higher estimate of Θ. The proposal by Felsenstein (1992b) to use Metropolis-Hastings sampling based on $P(D|G)$ in place of bootstrapping has proven, when implemented, to suffer from the same flaw, since it was sampling from the same illegitimate distribution.

The practical consequence of sampling from this illegitimate distribution is always an upward bias in the estimate of Θ. This has been verified empirically by Richard Hudson (pers. comm.) in simulations evaluating the initially proposed

form of the Metropolis-Hastings algorithm. HUDSON'S simulations showed this effect to be fairly severe with small data sets (200 bp from each of 20 individuals), with estimates two to three times higher than the true value (data not shown).

Therefore, the strategy which we have chosen is to sample with respect to the posterior probability of the genealogy, $P(D|G)P(G|\Theta)/P(D|\Theta)$, for a specific value of Θ which we will call Θ_0. (Although the denominator $P(D|\Theta)$ is unknown, we need only compute the ratio of the posterior probability for two genealogies, allowing this term to be cancelled.) To find the relative likelihood at other values of Θ we divide through by the importance sampling function:

$$\frac{L(\Theta)}{L(\Theta_0)} = \sum_G \left[\frac{P(D|G)P(G|\Theta)}{P(D|G)P(G|\Theta_0)} \right] \tag{1}$$

Use of the posterior probability as an importance function allows us to sample genealogies which will make a substantial contribution to the eventual value of the likelihood, and thus enables us to make a reasonable estimate of Θ by summing over a finite number of genealogies. It avoids the bias created by sampling proportional to $P(D|G)$, and practical experience suggests that it is much less computationally intensive than the bootstrap approach.

METHODS

Metropolis-Hastings Sampling: Our sampling strategy is to begin with an initial genealogy and make a small modification to it, choosing among a set of possible modifications according to their relative probabilities based on the distribution $P(G|\Theta_0)$. The probability of the data on the new genealogy ($P(D|G)$) is then calculated, and compared with the probability on the previous genealogy to decide whether or not the new genealogy should be accepted. If it is not, the old

genealogy is retained. Repeating this process creates a Markov chain of genealogies which, if run long enough, will travel among all genealogies in proportion to their posterior probabilities ($P(D|G)P(G|\Theta)/P(D\Theta_0)$ for the given Θ_0.

For the parameter $4N_e\mu$ we have chosen Θ rather than θ as in other studies because we are measuring μ in terms of mutations per site, not mutations per locus as in studies which use the infinite-sites model. Time is rescaled in terms of the mutation rate such that in 1 unit of time the expected number of mutations per site is 1 (this simplifies use of the coalescent approximation). We consider bifurcating, rooted, clocklike (ultrametric) genealogies. Throughout this discussion, "down" is towards the root. For ease of discussion, we will use the following convention: a node's "parent" is below it and its "children" are above it. (In actuality such a "child" represents a descendent of the "parent" a large number of generations later, at the time of the next coalescence event.)

Figure 1 shows the modification process: choosing a neighborhood (the region of the genealogy to be changed), rearranging the topology in that neighborhood, and choosing new branch lengths within the neighborhood. This is the fundamental operation of the algorithm, and if applied repeatedly can transform any genealogy into any other genealogy, thus allowing all possible genealogies to be searched. In practice, making larger rearrangements would probably make the sampling less efficient, because if a genealogy already has fairly high probability, a large rearrangement of it is liable to be much worse, and therefore be rejected. However, such techniques may prove useful in analyzing very large numbers of sequences, where the chance that the process will become trapped in a local maximum of the posterior probability distribution is greater.

To make a rearrangement, a node is chosen at random from among all nodes which have both parents and children (i.e. are neither tips nor the bottommost node

of the genealogy). This node will be referred to as the "target". The neighborhood of rearrangement consists of the target node, its children, parent, and parent's other child (see Figure 2A). A rearrangement makes changes of two kinds: it may reassort the three children among target and parent, and it modifies the branch lengths within the neighborhood. The new branch lengths must remain within the constraints imposed by the times of the three children and of the parent's ancestor; these times define the boundaries of the neighborhood. Conceptually, the portion of the genealogy involving these nodes is erased (see Figure 2B) and must now be redrawn. The lineages to be erased and redrawn will be referred to as "active" lineages, and the lineages existing at the same time but outside the neighborhood as "inactive" lineages.

To choose the times of the target and parent nodes, we draw from a conditional coalescent distribution with a given Θ, which we call Θ_0, conditioned on the number of inactive lineages. For each time interval, the probability of coalescence among the active lineages depends on the numbers of active and inactive lineages present in the genealogy during that interval. A random walk, weighted by these probabilities, is used to select a specific set of times. (This procedure is related to the Viterbi state-array algorithm (Viterbi 1967) and is explained in detail in the Appendices.) When the coalescence times have been determined, a topology compatible with them is chosen at random (incompatible topologies are those in which a child would be joined to a node whose branching time is above the child's time).

Once the new genealogy is generated, the probability of the sequence data on that genealogy is calculated under a standard model (Felsenstein 1981) much as is done in maximum likelihood phylogeny estimation. The Kimura 2-parameter model (Kimura 1980) of sequence evolution, modified to allow unequal base frequencies (J. Felsenstein unpublished, described by Kishino and Hasegawa 1989), is used

to assess the probability of generating the observed data for the given genealogy. A different model could be substituted in order to handle, for example, restriction site or amino acid data; the rest of the method would be unchanged.

The objective of this algorithm is to create a Markov chain whose states are genealogies, and whose stationary probabilities are equal to the posterior probability $P(D|G)P(G|\Theta)/P(D|\Theta)$ of each genealogy. HASTINGS (1970) shows that this can be done using the following relation, where G is the old genealogy and G' is the new:

$$r = \frac{P(D|G')}{P(D|G)} \frac{Q(G', G)}{Q(G, G')} \tag{2}$$

Q is the probability of generating the second genealogy starting from the first under the sampling strategy used. In the simple form of the Metropolis-Hastings algorithm presented here, the terms $Q(G', G)$ and $Q(G, G')$ are equal (they depend on the choice of target node and of final topology, both of which have equal probabilities in either direction) and therefore need not be calculated since their ratio is always 1. However, more complex versions of the algorithm, such as those dealing with recombination, will probably require calculation of the Q terms.

If r is greater than 1, the new genealogy is accepted, replacing the old. If it is less than 1, the new genealogy is accepted with probability r; otherwise the old one is retained.

Computing the likelihood curve for Θ: At intervals, genealogies created by this process can be sampled for use in constructing a likelihood curve for Θ. (The question of how often to sample will be touched on in the Discussion.) The genealogies were produced using importance sampling based on the known distribution $P(G|\Theta_0)$. Computation of their likelihood under other values of Θ must therefore take this importance sampling function into account:

$$L(\Theta) = \sum_G \frac{P(D|G)P(G|\Theta)}{P(D|G)P(G|\Theta_0)} \tag{3}$$

This equation can be reduced to a quickly calculatable form which depends only on the structure of the genealogies:

$$L(\Theta) = \sum_G \frac{P(G|\Theta)}{P(G|\Theta_0)} \tag{4}$$

To compute the term $P(G|\Theta)$ (the prior probability of the genealogy for the given Θ), consider the genealogy as a set of i time intervals, each with length t and number of lineages k; the total number of tips is n. The probability of the genealogy is a product over all intervals (KINGMAN 1982a, 1982b; FELSENSTEIN 1992b):

$$P(G|\Theta) = \left(\frac{2}{\Theta}\right)^{n-1} \exp\left[\sum_i \frac{-k(k-1)t_i}{\Theta}\right] \tag{5}$$

A likelihood curve can be constructed using equation 3 for various values of Θ. The maximum of this curve is a maximum likelihood estimate of Θ and can be found by standard methods. The curve is not guaranteed to have a single maximum, but in practice we have found that it generally does as long as the Markov chain has had sufficient time to approach equilibrium.

Combining multiple estimates: The closer the assumed value of Θ_0 is to the true value of Θ, the more efficient this strategy becomes. Therefore, it will often be useful to repeat the Markov chain sampling several times, using the estimate of Θ from each chain as the Θ_0 of the next. For maximum efficiency, the results of the earlier chains should not be discarded, but combined with the results of the final chain to produce an estimate of the overall likelihood curve, using an appropriate

weighting. The strategy we use is due to GEYER (1991) and treats the genealogies as having been sampled from a mixture distribution of their various values of Θ_0.

Suppose that m Markov chains have been run. For a given run j, n_j genealogies have been sampled, associated with a given value of Θ_0 which will be called Θ_j. The overall $L(\Theta_j)$ can be found by iterating the following relationship, where $\sum_G$ represents a summation over all of the sampled genealogies from all of the Markov chains:

$$L(\Theta_j) = \sum_G \frac{P(G|\Theta_j)}{\sum_{i=1}^{m} \left(n_i \frac{P(G|\Theta_i)}{L(\Theta_i)} \right)} \tag{6}$$

When Θ_j is the Θ_0 value at which one of the chains was run, this is a nonlinear set of equations in the $L(\Theta_j)$, which can be solved iteratively by calculating new values of the $L(\Theta_j)$ from the left hand side. Good starting values of the $L(\Theta_i)$ can be obtained using the genealogies from the final Markov chain. Likelihoods for other values of Θ_j can then be interpolated using the same set of equations.

The likelihood curves produced by this approach are not guaranteed to be unimodal, but in practice they usually are as long as enough iterations were done to approach equilibrium. We have found it best to run a series of very short chains whose results are not used in the combined estimate, in order for the genealogy and working value of Θ_0 to approach their final values. Then a small number of much longer chains can be used to make the final estimate.

RESULTS

Simulated data: We used computer simulation to explore the performance of this method. Trees were constructed randomly according to the coalescent

model, and DNA sequence data evolved according to the 2-parameter model of KIMURA (1980) using a transition/transversion ratio of 2.0. The UPGMA phylogeny reconstruction algorithm (as implemented in the PHYLIP program NEIGHBOR v3.5) was used to construct the starting tree to be used by the Metropolis-Hastings algorithm. We investigated several parameters which could influence the performance of the method: length of sequence, number of individuals sampled, and closeness of Θ_0 to the true Θ. The simulations presented are far from exhaustive, but can give a preliminary impression.

Table 1 shows results for samples of twenty individuals under three conditions: Θ_0 ten times too low, equal to the true Θ, and ten times too high. Results from the method of WATTERSON (1975) are provided for comparison. In general, the two methods perform about equally well. The Metropolis-Hastings method shows little or no bias towards Θ_0. This contrasts with runs in which only a single Markov chain was used, in which a substantial bias towards Θ_0 was seen (data not shown).

Table 2 shows similar results for samples of 100 individuals. Standard deviations for the Metropolis-Hastings method are a little lower than those for the method of Watterson.

Maximum likelihood methods in phylogenetics have typically been rather computer intensive. We timed our Metropolis-Hastings runs on a DECstation 5000/125 (a workstation of middling speed). A representative entry from Table 2 (105,500 steps total along the Markov chains) took 181.4 minutes. The majority of the runtime is consumed by likelihood calculations. When a change is made, only the likelihoods for the nodes in the neighborhood of rearrangement, and their ancestors down to the root of the tree, need to be re-evaluated. The mean number of such nodes increases slowly with number of sequences, and therefore runtime is not strongly dependent on number of sequences. For a given number of iterations,

runtime is expected to increase less than linearly with sequence length, since identical sites are collapsed together during likelihood calculation. However, more iterations will be needed to adequately search the space of plausible genealogies as the number of sequences increases.

When Metropolis-Hastings and related algorithms fail to perform well, it is generally because they become trapped in one part of their state space and fail to sample other parts. We have found it helpful to begin with a UPGMA genealogy rather than a random genealogy to avoid wasting time searching irrelevant parts of the genealogy space.

Mitochondrial DNA sequence data: WARD et al. (1991) examined 360 bp from the mitochrondial control region of 63 Amerindians of the Nuu-Chah-Nulth tribe. We analyzed both the full data set and two restricted data sets, purine-only and pyrimidine-only (there are no sites with both purines and pyrimidines in these data) in order to allow comparison with the results of TAVARÉ and GRIFFITHS (1993a). For the purine-only and pyrimidine-only data sets, base frequencies were set at 0.49 for bases appearing in the data set and 0.01 for bases not appearing; for the total data set they were calculated from the data. The transition/transversion ratio was set to 100.0. UPGMA was used to generate initial trees for each data set separately. The Θ estimate of WATTERSON (1975) based on the number of segregating sites was used as the initial value for Θ_0. We did ten short runs of 1500 steps (sampling every tenth genealogy from the final 500 steps) and two long runs of 12,000 steps (sampling every twentieth genealogy from the final 10,000 steps); the final estimate used only genealogies from the long runs.

For the full data the final estimate was 0.0396; the likelihood curve is shown in Figure 3. (Note that in this case $\Theta = 2N_f\mu$, where N_f is the number of females, since mtDNA is haploid and maternally inherited.) This is substantially higher

than the estimate of 0.0153 produced by the method of WATTERSON (1975) based on counting the number of segregating sites; this difference is expected, since some of the sites in this data set have clearly had multiple substitutions. Purine sites alone produced an estimate of 0.00466 (Watterson estimate 0.00667) and pyrimidine sites alone produced an estimate of 0.05237 (Watterson estimate 0.02217). Proportionally more of the pyrimidine sites are variable, suggesting that there may be a difference in mutation rate between the two classes. An appropriate extension of our method would be to assign purine and pyrimidine sites to different mutation rate categories.

DISCUSSION

Practical considerations: The Metropolis-Hastings sampler requires an initial value of Θ_0 and an initial genealogy. The results presented in Table 1 suggest that the initial value of Θ_0 is not critical as long as several Markov chains are run. However, the method is more efficient if Θ_0 is not too distant from Θ, and therefore we recommend using the method of WATTERSON (1975) or other quick estimators to select an initial value for Θ_0. The method is somewhat more succesful when it begins from a reasonable genealogy (data not shown).

We found the most successful search strategy to be running a fair number (5-10) of relatively short Markov chains to provide a good working estimate of Θ_0 and a good genealogy, and then 1-2 much longer chains to give the final estimate. Genealogies from the short chains should not be used in the final estimate, as such chains have not had time to approach equilibrium and can produce distortions in the likelihood curve.

Successive iterations in the Markov chain produce genealogies that are not independent. This is not a problem for likelihood estimation of Θ (except that

the number of genealogies sampled may sound more impressive than it actually is), but should be considered when using the sampled genealogies for other purposes. A sample of 100 successive genealogies is not an adequate replacement for 100 bootstrap samples, for example. It is not clear how many iterations are needed to make successive sampled genealogies approximately independent. Minimally, $n-2$ iterations are needed in order to transform any genealogy into any other (where n is the number of sequences). Practical experience suggests that on most data sets about 1/3 of the proposed modifications are accepted, so a minimal sampling increment for bootstrap use would be at least $3n$ steps along the chain.

Each individual step of the Metropolis-Hastings process is relatively quick, since it requires a likelihood evaluation of the genealogy rather than a likelihood maximization. However, more steps will be required as the number of individuals sampled increases, in order to make an adequate search of the region of plausible genealogies. We do not have an exact measure of the number of steps required.

Comparison with other approaches: It has been shown (FELSENSTEIN 1992a) that non-phylogenetic methods for estimating Θ do not make the most efficient possible use of the information present in the data. With small numbers of sequences (as in Table 1) the theoretical advantage of phylogeny-based methods is not visible, and the quick and simple method of WATTERSON (1975) is therefore preferable; but as the number of sequences increases (as in Table 2) phylogeny-based methods may begin to out-perform it.

A method based on a single genealogy has been proposed by FU (1993): he uses a UPGMA reconstruction of the genealogy, correcting the resulting estimate by a factor derived from simulations. For the WARD et al. (1991) Amerindian mtDNA data, FU's estimate of Θ was 13.32 per locus (0.037 per site), extremely close to our estimate of 0.0396. FU'S method is computationally simple, but may be difficult to

extend to cases such as migration, selection or recombination for which phylogeny reconstruction algorithms are not available.

Griffiths and Tavaré (1993a, b) have proposed a method which also sums across possible genealogies, but uses a random-sampling rather than a Metropolis-Hastings approach. For the infinite-sites model it is very fast (the set of possible genealogies is relatively small), but its performance under more complex models is not yet known. This method has been used to analyze the purine (Griffiths and Tavaré 1993a) and pyrimidine (Griffiths and Tavaré 1993b) sites of the Ward et al. (1991) data separately, omitting some sequences in order to make the data conform to the infinite-sites requirement. For the purine data their estimate of Θ was 1.19 (0.007 per site), slightly higher than our 0.005 per site; and for the pyrimidine data 3.61 (0.018 per site), considerably lower than our 0.052 per site. Further testing is needed to clarify the relationship between these methods.

Future directions: The basic method described here has several possible extensions. Since it uses a maximum likelihood genealogy evaluation, it can take advantage of any improvements which are developed in likelihood models, such as the work of Felsenstein and Churchill (in preparation) on using Hidden Markov Model methods to deal with mutation rates that vary from one site to another.

Other forms of data, such as protein sequences or restriction sites, can be analyzed as long as an appropriate likelihood method is available (for example the amino acid likelihood model of Kishino et al. 1990, or the restriction site likelihood models of Smouse and Li 1987, and Felsenstein 1992c); the rest of the algorithm will be unchanged.

A more complex model of genealogy structure is also possible. The genealogy space which the program searches could be extended to include genealogies involving population size changes, migration, recombination, or genetic rearrangement. This

would allow simultaneous estimation of the parameters controlling these processes. We are currently working on a version of the method which allows recombination and gene conversion. This will be very useful in analyzing nuclear DNA samples from sexual populations.

Finally, the collection of genealogies produced can be used to test other hypotheses; for example, it can be used in the same way as a bootstrap to measure the strength of support for a particular group or rooting by counting the number of sampled genealogies which show that group or rooting, as long as the interval between sampled genealogies is generous enough that they are reasonably independent.

Availability of software: The Metropolis-Hastings Monte Carlo algorithm described here is available from the authors as program COALESCE in the package LAMARC, which uses the same input/output formats as the PHYLIP package. The program is written in C and can be obtained by anonymous ftp from *evolution.genetics.washington.edu* in directory pub/lamarc.

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Appendix I: Calculating probabilities of coalescence

We use a modified Viterbi state-array approach (VITERBI 1967) to select coalescence times for the active lineages in the neighborhood of rearrangement. The strategy is to create a lattice showing the probability of each possible set of coalescences, and then select a path through this lattice in a manner proportional to the probability at each step. This has the effect of sampling randomly from the conditional coalescent distribution that is constrained by the limits of the neighborhood. (It differs from the standard Viterbi algorithm in that it chooses a random path, not the optimum path.) A legal set of coalescences is one in which all three active lineages have coalesced with each other by the time of the bottom of the neighborhood, and none have coalesced with any inactive lineages.

The genealogy is divided into a series of intervals with an interval boundary at each node. We can calculate the probability, within interval i, of no coalescence, one coalescence, or two coalescences among the active lineages. We will refer to these as $P_{j,j}^{(i)}$ (the probability that the number of active lineages is j at the top of the interval and j at the bottom), $P_{j,j-1}^{(i)}$, and $P_{j,j-2}^{(i)}$, respectively. Appendix II gives the full form of these probabilities.

At the top of the neighborhood there are two or three active lineages, depending on the genealogy structure. We work our way down the genealogy, calculating the cumulative probability of the presence of three, two or one active lineages ($S_3^{(i)}$, $S_2^{(i)}$, $S_1^{(i)}$ respectively for interval i) at the bottom of each interval. Figure 4 shows the structure of these probabilities. If only two lineages were active at the top of the neighborhood, the third is added at the interval in which it first becomes active. For example, the probability that there are two active lineages at the end of interval 4 is the sum of two components: the chance that interval 3 ended with two lineages and no coalescences occurred in interval 4 ($S_2^{(3)} \times P_{2,2}^{(4)}$), and the chance that interval

3 ended with three lineages and one coalescence occured among them in interval 4 ($S_3^{(3)} \times P_{3,2}^{(4)}$). (This example is shown by the bold arrows in Figure 4.)

The S_1 entry of the bottommost interval provides the total probability of an "allowed" series of events in this neighborhood (as opposed to the disallowed events of coalescence with an inactive lineage, or failure of the active lineages to coalesce with one another). Starting from this bottommost entry and working back upwards, we make a weighted random walk (choosing a specific set of coalescences) based on the cumulative probabilities in the state array and the transition probabilities among them. This is shown in Figure 5. For example, if the state in interval i has one active lineage, the state in the previous interval $(i-1)$ might have had one, two or three, corresponding to transition probabilities $P_{j,j}^{(i)}$, $P_{j,j-1}^{(i)}$ and $P_{j,j-2}^{(i)}$ respectively. The chance that j lineages in interval i came from j' lineages in interval $i-1$ (where $j' \leq j$) is:

$$\frac{S_{j'}^{(i-1)} P_{j,j'}^{(i)}}{S_j^{(i)}} \tag{7}$$

At each interval a random choice is made proportional to the transition probabilities. A complete series of such choices chooses a random path whose bottom end is in state 1, and thus defines a legal set of coalescences.

Once the interval in which coalescence occurs has been determined, the exact time of coalescence within that interval is needed. For cases in which two lineages coalesce during an interval, this can be solved explicitly by setting the integral of the density $P_{j,j-1}$ equal to a random fraction and then solving for the length x. For cases in which three lineages coalesce during the same interval a similar approach can be used, although an explicit solution is not available and iteration must be used to find the correct length x for the first coalescence. See Appendix II, equations 10 and 11, for the full form of these equations.

Appendix II: Transition probabilities

$P_{x,y}^{(i)}(t)$ gives the probability for a genealogy of n individuals that in time interval i (counting downwards from the tips of the genealogy), which is of length t, the number of active lineages will change from x to y.

These probabilities do not sum to one because of the possibility (disallowed in our procedure) that the active lineages could coalesce with inactive ones.

$P_{j,j}^{(i)}(t)$ is derived directly from the coalescent theory as the probability of no coalescence in interval i with duration t. $P_{j,j-1}^{(i)}(t)$ is then the probability of no coalescence from the start of the interval up to a time x, times the probability density of a coalescence at x, times the probability of no coalescence from x to the end of the interval. This is integrated over all possible values of x. $P_{j,j-2}^{(i)}(t)$ is constructed similarly by integrating over all possible values of the two coalescence times.

In these equations, $z = n - i + 1$, the number of inactive lineages during an interval.

$$P_{j,j}^{(i)}(t) = e^{-[j(j-1)+2jz]t/\Theta} \tag{8}$$

$$P_{j,j-1}^{(i)}(t) = \frac{j(j-1)}{2z+2(j-1)}\left[e^{-[2z(j-1)+(j-1)(j-2)]t/\Theta} - e^{-[2zj+j(j-1)]t/\Theta}\right] \tag{9}$$

$$\begin{aligned} &P_{j,j-2}^{(i)}(t) = \tfrac{j(j-1)^2(j-2)}{2z+2(j-2)} \\ &\left\{\left(\tfrac{1}{4z+4j-6}\right)\left[e^{-[2z(j-2)+(j-2)(j-3)]t/\Theta} - e^{-[2zj+j(j-1)]t/\Theta}\right]\right. \\ &\left.-\left(\tfrac{1}{2z+2(j-1)}\right)\left[e^{-[2z(j-1)+(j-1)(j-2)]t/\Theta} - e^{-[2zj+j(j-1)]t/\Theta}\right]\right\} \end{aligned} \tag{10}$$

In order to select a time within an interval where one coalescence occurs, we set (8) equal to a random fraction r, then solve for the length x:

$$x = \frac{-\Theta_0}{2(j-1)+2n} \ln\left[1 - r(1 - e^{-[2(j-1)+2z]t/\Theta})\right] \qquad (11)$$

In an interval where two coalescences occur, we find the time of the lower coalescence by setting (9) equal to a random fraction and solving for length, then use (10) to find the time of the upper one. We have not been able to find a non-iterative solution to this equation, but an approximate solution can be found by iteration:

$$x = \frac{-3e^{-2nt/\Theta}}{(n+1)(2n-3)(P^i_{j,j-2}(t))} \left[e^{-(4n+6)(x/\Theta)-1} - e^{-[2n+2]t/\Theta}\right]\left[e^{-[2n+4](x/\Theta)-1}\right]. \qquad (12)$$

Table 1: Estimates of Θ with 20 sampled individuals

A: Mean Θ estimate

Sites		200		500		1000
Θ_0	ML	WAT	ML	WAT	ML	WAT
0.001	0.01047	0.01001	0.00932	0.00896	0.00991	0.00958
0.01	0.00975	0.00944	0.01003	0.00998	0.00941	0.00958
0.1	0.00951	0.00948	0.00964	0.00962	0.01016	0.01010

B: Standard deviations

Sites		200		500		1000
Θ_0	ML	WAT	ML	WAT	ML	WAT
0.001	0.00549	0.00550	0.00364	0.00352	0.00307	0.00352
0.01	0.00484	0.00475	0.00338	0.00361	0.00293	0.00366
0.1	0.00460	0.00470	0.00325	0.00354	0.00315	0.00394

Means and standard deviations of estimated Θ from samples of 20 individuals with the true value of $\Theta = 0.01$. Five short Markov chains were run, each running for 1000 cycles without sampling and then 200 cycles sampling every 10th genealogy; then one longer Markov chain was run, running for 1000 cycles without sampling and then 5000 cycles sampling every 20th genealogy. Each entry is the mean or standard deviation of 100 replicates. The same data were used for the Watterson (WAT) and Maximum Likelihood (ML) estimations.

Table 2: Estimates of Θ with 100 sampled individuals

A: Mean Θ estimate

Θ_0	ML	WAT
0.001	0.01106	0.01078
0.01	0.01012	0.01002
0.1	0.01070	0.00985

B: Standard deviations

Θ_0	ML	WAT
0.001	0.00245	0.00289
0.01	0.00157	0.00257
0.1	0.00167	0.00239

Means and standard deviations of estimated Θ from samples of 100 individuals with the true value of $\Theta = 0.01$. Sequences were of length 1000 bp. Five short Markov chains were run, each running for 1000 cycles without sampling and then 500 cycles sampling every 10th genealogy; then one longer Markov chain was run, running for 2000 cycles without sampling and then 50,000 cycles sampling every 20th genealogy. Each entry is the mean of 20 replicates. The same data were used for the Watterson (WAT) and Maximum Likelihood (ML) estimations.

Figures

Figure 1. A coalescent genealogy

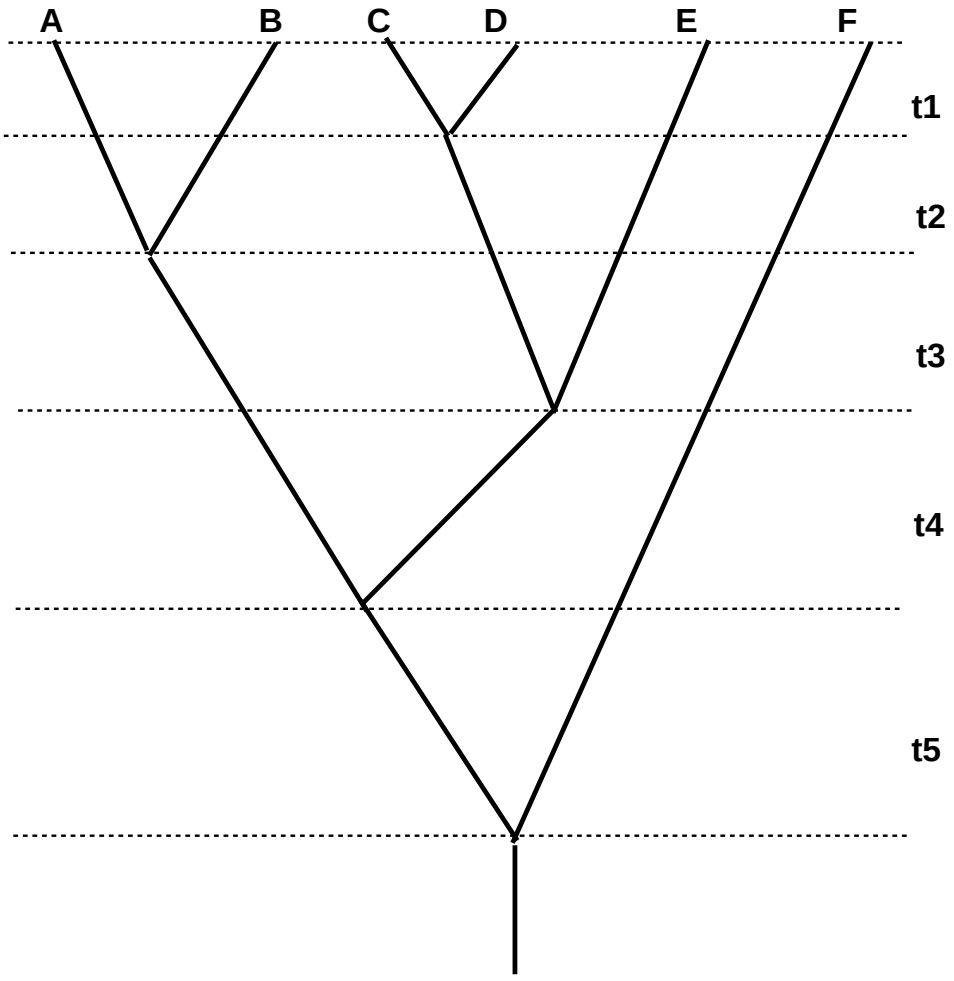
Figure 2. Steps in rearranging a genealogy

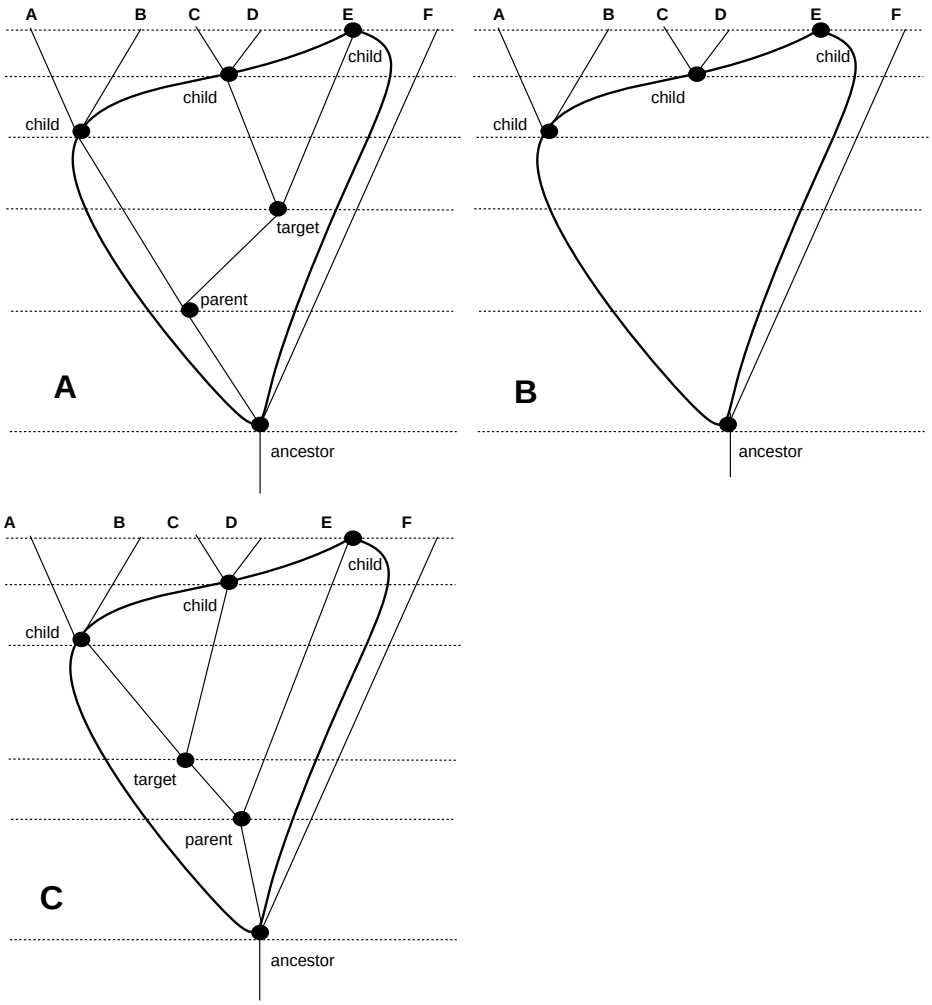
Dotted lines show active lineages, solid lines show inactive lineages. A: Selecting a neighborhood of rearrangement. B: Erasing the active lineages. C: Redrawing the active lineages.

Figure 3. Likelihood curve for the WARD et al. (1991) Nuu-Chah-Nulth mtDNA data

Figure 4. Viterbi state array. Labels on arrows are subscripts of the P terms. Bold arrows indicate example used in text.

Figure 5. One path through the state array. A tree structure corresponding to this path through the array is shown on the right. Only active lineages are indicated.


A
B
C
D
E
F
t1
t2
t3
t4
t5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C
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E
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child
child
child
target
parent
ancestor
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D
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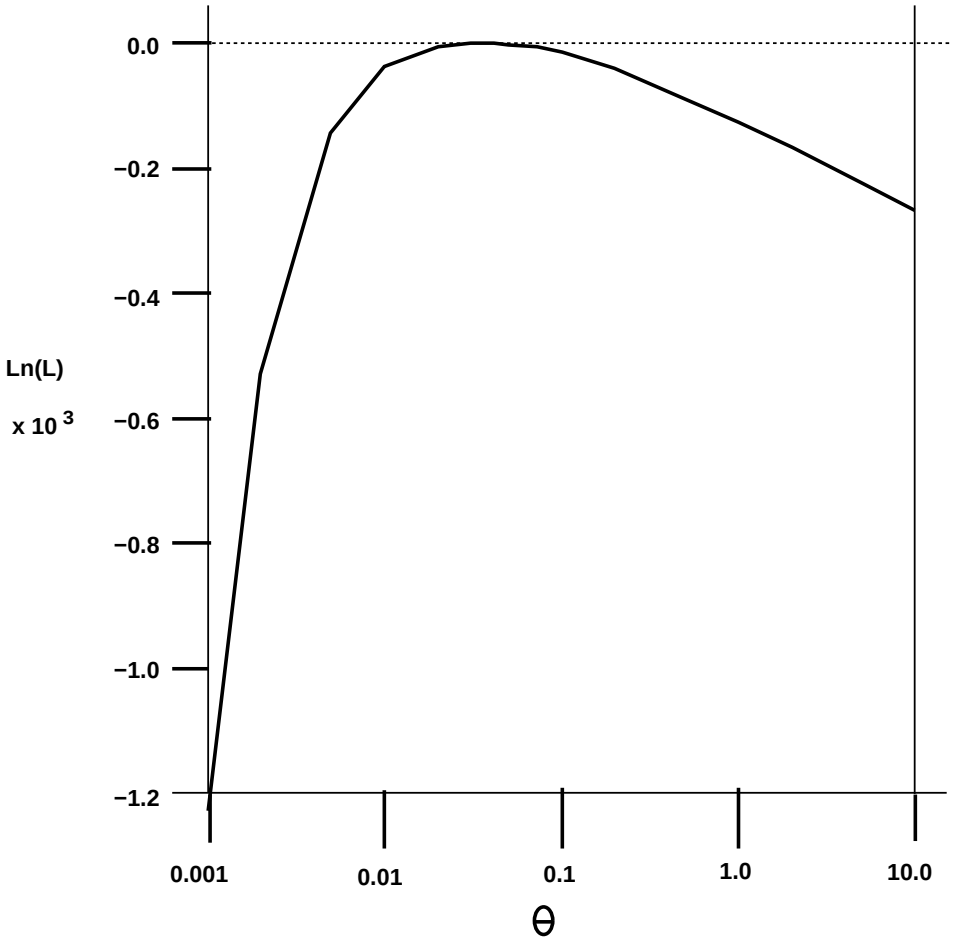
child
child
target
parent
ancestor
C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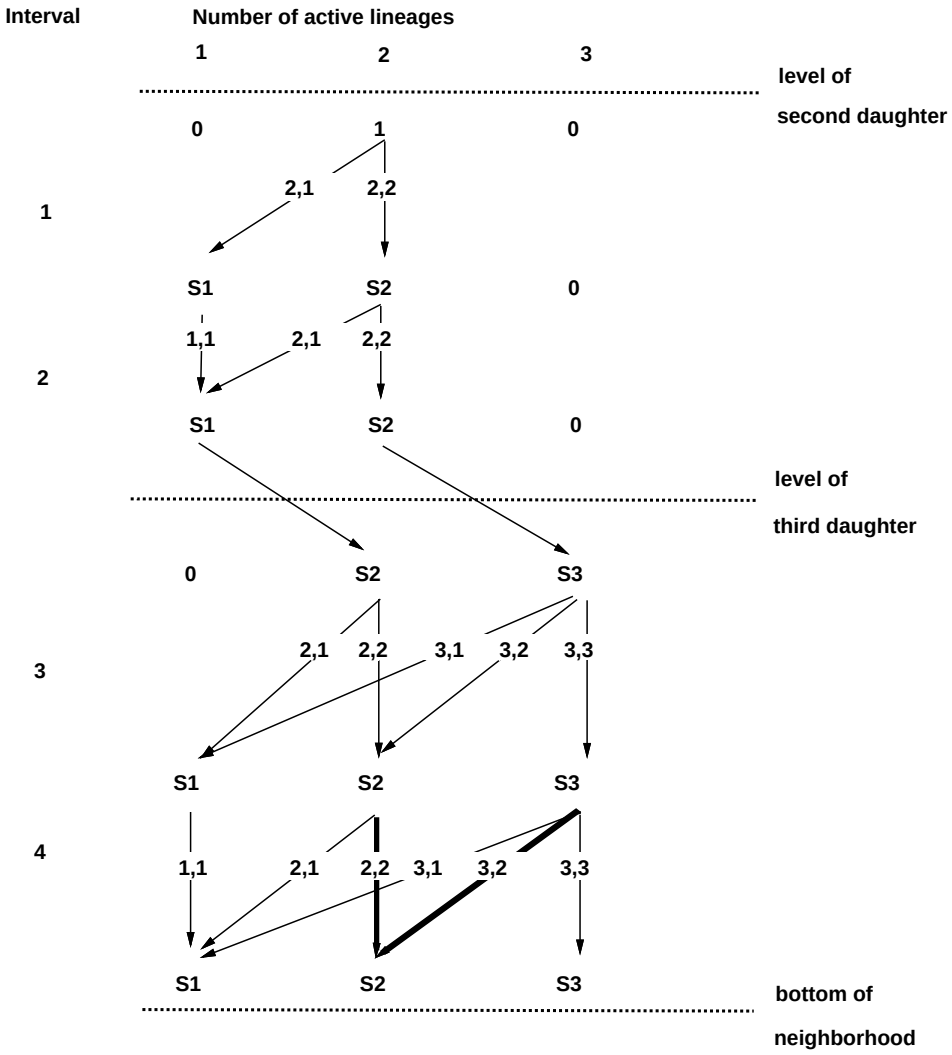
−0.4
Ln(L)
x 10 3
−0.6
−0.8
−1.0
−1.2
0.001
0.01
0.1
1.0
10.0
θ


Interval
Number of active lineages
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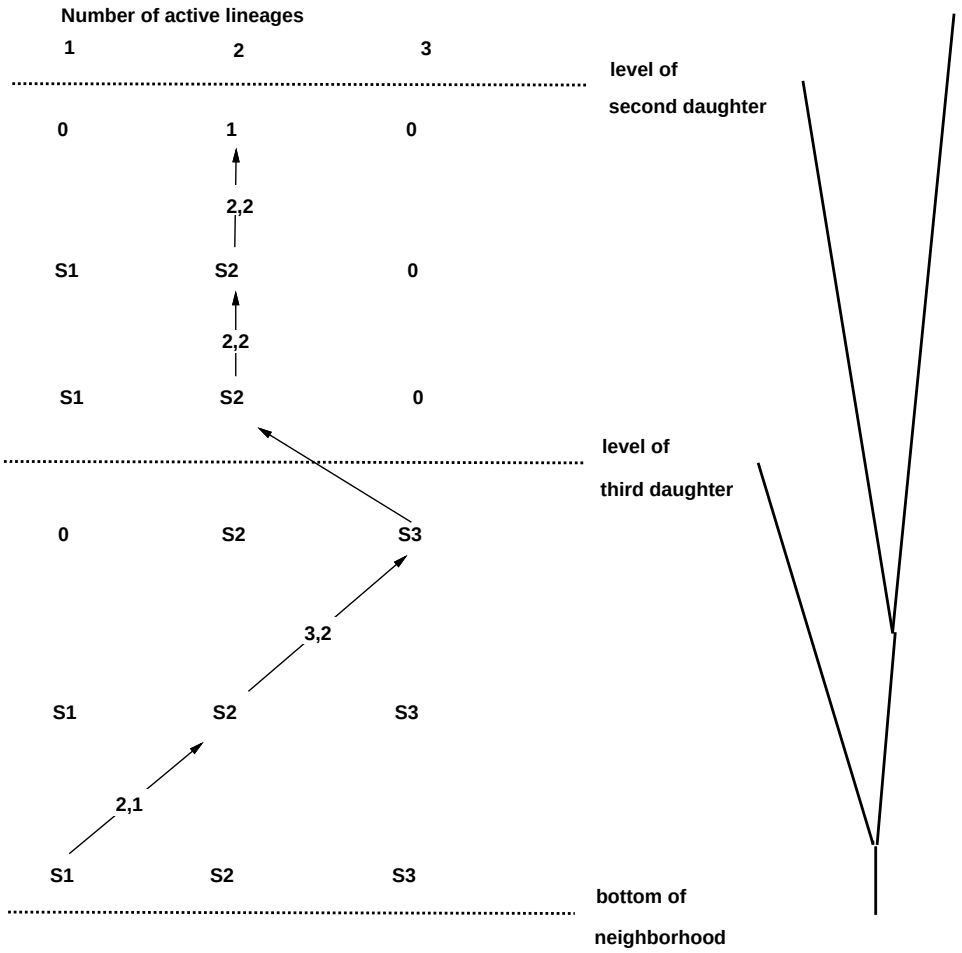
2
3
level of
second daughter
0
1
0
2,1
2,2
1
S1
S2
0
1,1
2,1
2,2
2
S1
S2
0
level of
third daughter
0
S2
S3
2,1
2,2
3,1
3,2
3,3
3
S1
S2
S3
4
1,1
2,1
2,2
3,1
3,2
3,3
S1
S2
S3
bottom of
neighborhood


Number of active lineages
1
2
3
level of
second daughter
0
1
0
2,2
S1
S2
0
2,2
S1
S2
0
level of
third daughter
0
S2
S3
3,2
S1
S2
S3
2,1
S1
S2
S3
bottom of
neighborhood