Inferring human population size and separation history from multiple genome sequences

Stephan Schiffels & Richard Durbin

The availability of complete human genome sequences from populations across the world has given rise to new population genetic inference methods that explicitly model ancestral relationships under recombination and mutation. So far, application of these methods to evolutionary history more recent than 20,000–30,000 years ago and to population separations has been limited. Here we present a new method that overcomes these shortcomings. The multiple sequentially Markovian coalescent (MSMC) analyzes the observed pattern of mutations in multiple individuals, focusing on the first coalescence between any two individuals. Results from applying MSMC to genome sequences from nine populations across the world suggest that the genetic separation of non-African ancestors from African Yoruban ancestors started long before 50,000 years ago and give information about human population history as recent as 2,000 years ago, including the bottleneck in the peopling of the Americas and separations within Africa, East Asia and Europe.

Human genome sequences are related to each other through common ancestors. Estimates of when these ancestors lived provide insight into ancestral population sizes and ancestral genetic separations as a function of time. In the case of non-recombining loci, such as the maternally inherited mitochondrial DNA or the paternally inherited Y chromosome, the time to common ancestors can be estimated from the total number of differences between sequences1–4. For autosomal sequences, which account for the vast majority of heritable sequence, the reconstruction of genealogical relationships is more complicated owing to ancestral recombination events that separate many different genealogical trees in different locations of the genome. Although inferring this pattern is more challenging, it provides, in principle, much more information about our past than non-recombining loci, as only a few samples yield many effectively independent genealogies, allowing the inference of a distribution of times to common ancestors with high resolution.

Reconstruction of the full underlying ancestral recombination graph is challenging because the space of possible graphs is extremely large. A substantial simplification was proposed by McVean and Cardin, who model the generating process of genealogical trees as Markovian along the sequence5,6. An application of this idea was presented by Li and Durbin in the pairwise sequential Markovian coalescent (PSMC) model7, a method that focuses on modeling the two genome sequences in one diploid individual. Because only two sequences are modeled, the coalescent event joining the sequences at the most recent common ancestor almost always occurred more than 20,000 years ago, meaning that PSMC can only infer population size estimates beyond 20,000 years ago. Also, with only two sequences, there is only limited scope for the analysis of population separations.

For more than two sequences, extending PSMC in the natural way by enumerating all possible trees with their branch lengths along the sequences would be very computationally costly, even under the Markovian model. A recent simplification was suggested by Song and colleagues8–10, which is based on approximating the conditional sampling process for adding an (n + 1)th sequence to the distribution of genealogies connecting n sequences. Here we propose an alternative approach that we call multiple sequential Markovian coalescent (MSMC), which overcomes the increase in complexity by introducing a different simplification. We characterize the relationship at a given location between multiple samples through a much reduced set of variables: (i) the time to the most recent common ancestor of any two sequences, that is, the first coalescence, along with the identities of the two sequences participating in the first coalescence (Fig. 1a), and (ii) the total length of all singleton branches in the tree, that is, branches that give rise to variants of minor allele count 1 if affected by a mutation. Given a demographic model, we can keep track of the likelihood distribution for these variables on the basis of the observed mutation data as we move along the sequences. As detailed in the Online Methods, we derive approximate transition and emission rates using the sequentially Markovian coalescent (SMC)5,6 framework. This approach allows efficient maximum-likelihood estimation of the free parameters, which include the piecewise constant population size as a function of time. If sequences are sampled from different subpopulations, we use additional free parameters for coalescence rates within and across population boundaries, which allow us to infer how subpopulations separated over time. We compare further the conditional sampling approach and our approach to determining first coalescence.

Because MSMC focuses on the first coalescence event for any pair of haplotypes, the inference limits are set by the distribution of first coalescence times (t), the mean of which scales inversely with the square
The model recovered the true hidden state with good local resolution. The typical length of segments of constant hidden state increased with sample size (Fig. 1b), whereas the typical time to the first coalescence decreased.

We then tried to infer back the simulation parameters from the simulated sequence data, applying MSMC to two different demographic scenarios (Fig. 2). First, we simulated a single population under a series of population growths and declines. MSMC recovered the resulting zigzag pattern in population size with good resolution (Fig. 2a), where two haplotypes (similar to in PSMC) yielded good estimates between 50,000 and 2 million years ago and eight haplotypes gave estimates as recent as 2,000 years ago, with a small bias toward smaller population sizes in the more distant past. We were able to test a reduced data set with reduced parameters using 16 haplotypes (Supplementary Fig. 2e), which suggested that the bias observed with 8 haplotypes in the distant past increased further with more haplotypes. We also tested other simulated histories with sharp changes in population size (Supplementary Fig. 2a,b). As expected from the experience with PSMC, very rapid changes in population size were smoothed out over an interval around the true time at which the change occurred.

Next, we simulated two population split scenarios where a single ancestral population split into two equally sized populations 10,000 or 100,000 years ago. For each population split scenario, we inferred effective coalescence rates across the two populations and within populations as a function of time (Online Methods). We provide a
Inference of population size from whole-genome sequences

We applied our model to the genomes from one, two and four individuals sampled from each of nine extended HapMap populations²⁰, YRI (Nigerian), M KK (Kenyan), LWK (Kenyan), CEU (Northern and Western European), TSI (Italian), GIH (North Indian), CHB (Chinese), JPT (Japanese) and MXL (Mexican admixed with European) (details in Supplementary Table 1). We statistically phased all genomes using a reference panel (Online Methods) and tested the impact of potential switch errors by comparing these sequences with family trio–phased sequences that were available for CEU and YRI populations (Online Methods and Supplementary Fig. 6).

The results from two individuals (four haplotypes) are shown in Figure 3a. In all cases, the inferred population history from four haplotypes matched the estimates from two haplotypes where their inference range overlapped (60,000–200,000 years ago; see thin lines for CEU and YRI in Fig. 3 and Supplementary Fig. 7a). We found that all non-African populations that we analyzed showed a remarkably similar history of population decline from 200,000 years ago until about 50,000 years ago, consistent with a single non-African ancestral population that underwent a bottleneck at the time of the exodus from Africa around 40,000–60,000 years ago²¹–²³. The separation of estimates for non-African and African ancestral population sizes began much earlier at 150,000–200,000 years ago, clearly preceding this bottleneck, as already observed using PSMC⁷. We quantify this separation further by directly estimating the relative cross coalescence rate over time. In contrast, we saw only a mild bottleneck in the African population histories, with an extended period of relatively constant population size more recent than 100,000 years ago. Between 30,000 and 10,000 years ago, we saw similar expansions in population size for the CEU, TSI, GIH and CHB populations. For the Mexican ancestors, we saw an extended period of low population size after the out-of-Africa bottleneck, with the lowest value around 15,000 years ago, which was particularly pronounced when we filtered out genomic regions of recent European ancestry due to admixture (dashed line in Fig. 3; Online Methods). This extended bottleneck is consistent with estimates of the time that the Native American ancestors crossed the Bering Strait and moved into the Americas²⁴–²⁶. We repeated all analyses based on four haplotypes on a replicate data set, using sequences for different individuals that were available for all populations except MXL. All results were well reproduced, and differences were only present in the most recent time intervals (Supplementary Fig. 8).

Analyzing eight haplotypes from each population except for the MXL and M KK populations (Online Methods), we could see recent changes in population size with higher resolution than with four haplotypes (Fig. 3b). Results from eight haplotypes were compatible to those from four haplotypes beyond 10,000 years ago,
with systematically slightly lower estimates of population sizes in the range of 10,000–30,000 years ago, as expected from Figure 2a (Supplementary Fig. 7b). We obtained new insights for periods more recent than 10,000 years ago, during the period of Neolithic expansion. Focusing first on Asian populations, we saw that the ancestors of the CHB population rapidly increased in number from 10,000 years ago to reach effective population sizes of over a million 2,000 years ago. The GH population ancestors also increased in number early, from around 10,000 years ago, with population growth occurring a little more slowly than with the CHB ancestors and numbers leveling off at around 4,000 years ago until recent times. The JPT ancestral population appeared to have split from the CHB ancestral population by 9,000 years ago and only slowly increased in size up until 3,000 years ago, since which time it also increased in size very rapidly. In Europe, Northern and Western European ancestors (CEU) experienced a relatively constant effective population size between 10,000 and 2,000 years ago, only rapidly increasing in number since 2,000 years ago; Southern European ancestors (TSI) had a consistently higher effective ancestral population size, appearing to show a more complex history of increase and decrease in number between 10,000 and 3,000 years ago and then increasing in number earlier than the CEU ancestors. As discussed previously, such a pattern of increase and decrease in population size can result from admixture with previously separated populations, consistent with multiple waves of peopling of Europe with a substantial genetic component from earlier waves in Southern Europe.

In Africa, the YRI (Yoruba) ancestral population expanded earliest, around 6,000 years ago, consistent with the introduction of agriculture and the Bantu expansion. This was followed by expansion of the LWK (Luhya) ancestral population, for which, before 6,000 years ago, there was a long ‘hump’ in ancestral population size extending back beyond 50,000 years ago, again possibly reflecting admixture within the last few thousand years between populations initially separated tens of thousands of years ago.

Divergence from and within African populations

MSMC lets us explicitly study the genetic separation between two populations as a function of time by modeling the relationship of multiple haplotypes, half of which are from one population and half of which are from the other. From analyzing four haplotypes for each pair of populations, we found that all relative cross coalescence rates between any non-African population and the Yoruba were very similar and exhibited a slow, gradual decline beginning earlier than 200,000 years ago and lasting until about 40,000 years ago (Fig. 4a). This similarity in rates gives additional information beyond estimates of population size and is consistent with all non-African populations diverging as a single population from the Yoruba ancestors.

To understand whether the gradual decline in relative cross coalescence rate between the YRI and non-African ancestors was due to the inability of MSMC to detect rapid changes (Fig. 2b) or due to true ongoing genetic exchange, we compared its results with simulated clean split scenarios at three different time points in the past (Fig. 4c). This comparison showed that no clean split could explain the inferred progressive decline in relative coalescence rate. In particular, the early beginning of the decline would be consistent with an initial formation of distinct populations before 150,000 years ago, whereas the late end of the decline would be consistent with a final split around 50,000 years ago. This comparison suggests a long period of partial divergence with ongoing genetic exchange between the Yoruban and non-African ancestors that began beyond 150,000 years ago, with population structure within Africa, and last for over 100,000 years.

The median point of this divergence was around 60,000–80,000 years ago, at which time there was still substantial genetic exchange, with half the coalescences between populations and half within. We also observed that the rate of genetic divergence was not uniform but could be roughly divided into two phases. First, up until about 100,000 years ago, the two populations separated more slowly, whereas after 100,000 years ago, the rate of genetic exchange decreased faster. We note that the fact that the relative cross coalescence rate did not reach 1, even around 200,000 years ago (Fig. 4c), might be owing to later admixture of archaic populations such as the Neanderthals into the CEU population after its split from the YRI ancestral population.

We also saw extended divergence patterns in eight-haplotype analysis between the ancestors of the three African populations (Fig. 4a), with the LWK and YRI ancestral populations being closest and the MKK ancestral population showing a very slow increase in relative cross coalescence rate going back in time with the YRI and LWK ancestral populations. These declines in rate were all more gradual than those shown in Figure 4b between out-of-Africa populations, suggesting that the separations of African populations were also not clean splits but gradual separations, perhaps reflecting complex ancestral structure with admixture. In addition, we saw a different separation history between CEU and MKK ancestral populations compared to the LWK and CEU ancestral populations, which in turn was very similar to our estimates of the YRI-CEU separation. Our results suggest that the Maasai ancestors were mixing extensively with non-African ancestors until about 80,000 years ago, much later than the separation of the YRI and non-African populations. This observation is consistent with a model in which the Maasai ancestors and non-African ancestors formed sister groups, which together separated from West African ancestors and continued to extensively mix until much closer to the time of the actual out-of-Africa migration. Nonzero estimates of relative cross coalescence rate between the MKK
and CEU ancestors after 50,000 years ago are probably confounded by more recent admixture from non-African populations back into East African populations, including the Maasai\textsuperscript{50,31}.

**Divergences outside Africa**

As expected, the oldest split among out-of-Africa populations was between European and East Asian (CHB and MXL) populations, most of which occurred between 20,000 and 40,000 years ago (Fig. 4b). Intriguingly, there might be a small component (10% or less) of this separation extending much further back toward 100,000 years ago, which is not compatible with a single out-of-Africa event around 50,000 years ago. Next oldest was a separation between Asian (CHB and GIH) and American (MXL) populations around 20,000 years ago. This was the most rapid separation we saw, compatible with a clean split. Passing over the GIH separations for now, we found in eight-haplotype analyses separations between the JPT and CHB ancestors around 8,000–9,000 years ago, which is compatible with the divergence in population size history described above, and between the CEU and TSI ancestors around 5,000–6,000 years ago, both also relatively sharp. Four-haplotype analyses of the same separations are shown in Supplementary Figure 9.

The pattern of divergence of the North Indian ancestors (GIH) from East Asian and European ancestors was more complex. We observed continued genetic exchange between the GIH ancestors and both of these groups until about 15,000 years ago, suggesting that, even though East Asians and Europeans separated earlier, there was contact between both of these populations and the GIH ancestors after this separation or, equivalently, that there was ancient admixture in the ancestry of North Indians. This deviation from a tree-like separation pattern was independently confirmed by D statistics from an ABBA-BABA test\textsuperscript{32} (Online Methods and Supplementary Table 2), which also indicated that the GIH population is genetically closer to the CEU population than to the CHB and MXL populations. This finding is consistent with the slower and later decline in relative coalescence rate between CEU and GIH populations compared to between CEU and CHB populations (Fig. 4b). These results suggest that the GIH ancestors remained in close contact with the CEU ancestors until about 10,000 years ago but received some historic admixture component from East Asian populations, part of which is old enough to have occurred before the split with the MXL ancestors.

**DISCUSSION**

We have presented here both a new method, MSMC, and new insight into the demographic history of human populations as they separated across the globe. We have shown that MSMC can give accurate information about the time dependence of demographic processes within and between populations from a small number of individual genome sequences. As with PSMC, it does this without requiring a simplified model with specific bottlenecks, hard population splits and fixed population sizes as are required by previous methods based on allele frequencies\textsuperscript{31–35} or more general summary statistics\textsuperscript{36–39}. However, MSMC extends PSMC by an order of magnitude to more recent times and also allows us to explicitly model the history of genetic separations between populations. Because MSMC measures the time to the first coalescence between all pairs of haplotypes, the analyzed time range decreases quadratically with the number of haplotypes. This should be compared with the more naive approach of combining data from PSMC run on different individuals, which would increase information at most linearly, as individuals’ histories are combined data from PSMC run on different individuals, which would increase information at most linearly, as individuals’ histories are not independent.

Although MSMC substantially advances the methodology from PSMC to multiple samples and much more recent times, we have also seen that its practical application appears to be limited to about 8 haploid sequences, both because of the approximations involved and because of computational complexity (see Supplementary Fig. 2e for estimates based on 16 haplotypes). It is intriguing, however, to imagine that larger numbers of samples, in principle, contain information about even more recent population history, potentially up until a few generations ago. The basic idea of looking at first coalescence events, as presented here, may lead to new developments that complement
MSMC in this direction, for example, by focusing on rare mutations such as doubletons in large samples\textsuperscript{40}. The alternative conditional sampling approach mentioned in the introduction\textsuperscript{9,10}, implemented in the software diCal, also allows for higher resolution at more recent times. However, when we applied diCal to our zigzag simulation of changes in population size, it did not appear to infer population history more recent than about 20,000 years ago (Supplementary Fig. 10). Also, there is currently no way to address the relationship between populations as characterized by MSMC through relative cross coalescence rate. Both of these points may improve in future developments of this method.

Applied to 34 whole-genome sequences from 9 populations, MSMC gives a picture of human demographic history within the last 200,000 years, beginning with the genetic separations of the Yoruban and non-African ancestors and extending well into the Neolithic (Fig. 4d). We find strong evidence that the Yoruban–non-African separation took place over a long time period of about 100,000 years, starting long before the known spatial dispersal into Eurasia around 50,000 years ago. Because we model directly an arbitrary history over time of the relative cross coalescence rate between populations, we can see more clearly a progressive separation than earlier analyses based on a single separation time with some subsequent migration\textsuperscript{17,33,41}. However, the Yoruban population does not represent all of Africa. We now see that the separation of the Maasai population from the out-of-Africa populations occurred within the last 100,000 years. The older part of the separation from the Yoruban ancestors might therefore be a consequence of ancient population structure within Africa, although the direct picture of relationships between African populations is complicated by more recent extensive exchange that we see between all three of the Yoruban, Maasai and Luhya populations within the last 100,000 years. This scenario still does not rule out a possible contribution from an intermediate modern human population that dispersed out of Africa into the Middle East or the Arabian peninsula but continued extensive genetic exchange with its African ancestors until about 50,000 years ago\textsuperscript{17,42,43}.

Our results are scaled to real times using a mutation rate of 1.25 × 10^{-8} mutations per nucleotide per generation, as proposed recently\textsuperscript{16} and supported by several direct studies of mutation\textsuperscript{14–16}. Using a value of 2.5 × 10^{-8}, as was common previously\textsuperscript{44,45}, would halve the times. This would bring the midpoint of the out-of-Africa separation to an uncomfortably recent 30,000–40,000 years ago, but, more discourtingly, it would bring the separation of Native American ancestors (MXL) from East Asian populations to 5,000–10,000 years ago, inconsistent with the paleontological record\textsuperscript{25,26}. We suggest that the establishment and spread of the Native American populations might provide a good time point for calibrating population genetic demographic models. We note that the extended period of divergence between African and non-African ancestors that we observe reconciles the timing of the most recent common ancestor of African and non-African mitochondrial DNA around 70,000 years ago\textsuperscript{1,13} with the lower autosomal nuclear mutation rate used here, which in simple-split models would suggest a separation around 90,000–130,000 years ago\textsuperscript{17,33,41,46}. Given that we observe extensive cross coalescence at nuclear loci around 60,000–80,000 years ago, sharing a common ancestor during that time for mitochondrial DNA, which acts as a single locus with reduced effective population size, is entirely likely.

**URLs.** D Programming language, http://www.dlang.org; \textcopyright; MSMC at GitHub, https://github.com/stschiff/msmc.

**METHODS**

Methods and any associated references are available in the online version of the paper.

**Note:** Any Supplementary Information and Source Data files are available in the online version of the paper.

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**AUTHOR CONTRIBUTIONS**

R.D. proposed the basic strategy and designed the overall study. S.S. developed the theory, implemented the algorithm and obtained results. S.S. and R.D. analyzed the results and wrote the manuscript.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

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ONLINE METHODS

Sequence data. Whole-genome sequences were generated by Complete Genomics13 and are freely available on their website (‘69 genomes’ public data set). Homozygous and heterozygous consensus calls are taken directly from the Complete Genomics tabular format file ‘masterVar’ in the ASM directory of each sample. All regions with a quality tag less than ‘VQHIGH’ were marked as missing data. Furthermore, sites for which more than 17 of the 35 overlapping 35-mers from the reference sequence could be mapped elsewhere with 0 or 1 missing data. Furthermore, sites for which more than 17 of the 35 overlapping each sample. All regions with a quality tag less than “VQHIGH” were marked as

Reference. In addition, we generated full chromosomal genomes from the 1000 Genomes Project reference panel48. In addition, we generated full chromosomal haplotype phases from available trio data for YRI and CEU to assess the impact of the accuracy of the population phasing method versus trio phasing. Current

Phasing. All segregating sites that were present in the 1000 Genomes Project integrated variant call set were phased with SHAPEIT2 software44 using the 1000 Genomes Project reference panel48. In addition, we generated full chromosomal haplotype phases from available trio data for YRI and CEU to assess the impact of the accuracy of the population phasing method versus trio phasing. Current

Unphased sites affect population size estimates and relative cross coalescence rate estimates differently (Supplementary Fig. 5). From a direct comparison with trio-phased data, we found that population size estimates were better derived allele (B). This left two configurations per setup named ABBA and BABA, respectively (Supplementary Table 2). For one two-haplotype setup, MSMC is very similar to PSW, with subtle differences due to the different underlying model SMC (ref. 6) versus SMC3. We therefore call our special case of two haplotypes PSW to distinguish it from PSMC.

Emission rate. For the emission rate, we assume that we are given local estimates of the singleton branch length of the tree, $T_S$ (Supplementary Note), which is treated as a ‘soft’ constraint; that is, we always consider max($T_S$, $M$) for $T_S$. For more than two haplotypes, an observation of alleles given some state $(t,i,j)$ could be classified into the following categories, each of which has its own emission probability $e(t;T_S)$, derived in the Supplementary Note.

(1) No mutation: $e(t;T_S) = 1 - \mu T_S$

(2) Singleton within the pair $[i,j]; e(t;T_S) = \mu t$

(3) Singleton outside the pair $[i,j]; e(t;T_S) = \mu t$

(4) Higher frequency variant: $e(t;T_S) = 1 - \mu T_S$

(5) Double mutation: $e(t;T_S) = 0$

(6) Missing data: $e(t;T_S) = 1$

If sites have multiple observations from ambiguous phasing results, we average the emission probability over those observations.

Parameter inference. To infer parameters with MSMC, we discretize time into segments, following the quantiles of the exponential distribution. The boundaries of the segments are

$$T_i = -\log \left( 1 - \frac{i}{n_T} \right) \sqrt{\frac{M}{2}}$$
where \( n_1 \) is the number of segments and \( M \) is the number of haplotypes, which determines the expected time to first coalescence. All times are given in units of \( 2N_0 \) generations, where \( 2N_0 \) is fixed from Watterson’s estimator. The number of segments used in the population size analysis was \( n_1 = 40 \) and in the estimation of relative cross coalescence rate was \( n_1 = 50 \). The denominator reflects the quadratically decreased time to first coalescence with increased sample size \( M \).

The limits on the inference are given by the second and the second-to-last boundaries, which correspond to the 2.5%- and 97.5%-quantile boundaries of the distribution of first coalescence times.

As in PSMC, we join segments to reduce the parameter search space. The segmentation we used was \( 10 \times 1 + 15 \times 2 \) for the population size inference, that is, the 30 rightmost segments were joined to pairs of 2. For relative cross coalescence rate estimates, we used a pattern of \( 8 \times 1 + 11 \times 2 \). For plots on a logarithmic time axis, we chose the left cutoff to be at \( T_i/4 \) and omitted the last time interval, as its value is shown also by the joined second-to-last time interval.

In each segment, the coalescence rate is kept constant. If all samples are from the same population, we have one parameter per segment, which is the scaled inverse population size \( \lambda_i \), where \( i \) enumerates the segments in time. If samples from two populations are analyzed, we have three parameters per segment: \( \lambda_i^1 \), \( \lambda_i^{12} \) and \( \lambda_i^2 \). Here \( \lambda_i^1 \) and \( \lambda_i^2 \) denote the coalescence rates within the two populations, and \( \lambda_i^{12} \) denotes the coalescence rate across populations. In principle, there are two further free parameters: the scaled recombination rate \( \rho \) and the scaled mutation rate \( \theta \). In practice, we fix \( \theta \) from Watterson’s estimator for \( \theta \). The recombination rate can be inferred relatively well from PSMC (Supplementary Fig. 1), and we fix it to that estimate for more than two haplotypes to reduce the parameter search space. We note that inference results on the coalescence rates are relatively independent of the recombination rate.

Maximum-likelihood estimates of all free parameters are generated iteratively by means of the Baum-Welch algorithm\(^{52,53} \) with a coarse-grained \( Q \) function and numerical maximizations using Powell’s direction set method. For the coarse-graining of the \( Q \) function, we use precomputed transition-emission matrices and evaluate the local variables only every 1,000 bp. This optimization makes it possible to analyze whole human genomes without the need to first bin the data into windows of 100 bp, as in ref. 7.

For only one population analyzed, an estimate of the scaled population size in time interval \( i \) is directly given as the inverse of the coalescence rate: \( N_i/2N_0 = 1/\lambda_i \). The scaling parameter \( N_0 \) is fixed from the scaled mutation rate: \( N_0 = \theta/(4\mu) \), where \( \mu = 1.25 \times 10^{-8} \) is the per-site, per-generation mutation rate. Relative cross coalescence rate estimates are obtained by dividing the cross-population coalescence rate by the average within-population coalescence rate: \( \gamma_i = 2\lambda_i^{12}/(\lambda_i^1 + \lambda_i^2) \). In the maximization step of the Baum-Welch algorithm, we constrained the optimization to \( \gamma \leq 1 \).

**Implementation.** We implemented the model and inference algorithm in the D programming language (see URLs). The source code, together with executables for common platforms, is freely available from GitHub (see URLs). We also provide additional information on the input file format and necessary preprocessing in the Supplementary Note.

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