Estimation of site frequency spectra from low-coverage sequencing data using stochastic EM reduces overfitting, runtime, and memory usage

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Abstract

The site frequency spectrum (SFS) is an important summary statistic in population genetics used for inference on demographic history and selection. However, estimation of the SFS from called genotypes introduce bias when working with low-coverage sequencing data. Methods exist for addressing this issue, but suffer two problems. First, they have very high computational demands, to the point that it may not be possible to run estimation for genome-scale data. Second, existing methods are prone to overfitting, especially for multi-dimensional SFS estimation. In this article, we present a stochastic expectation-maximisation algorithm for inferring the SFS from NGS data that addresses these challenges. We show that this algorithm greatly reduces runtime and enables estimation with constant, trivial RAM usage. Further, the algorithm reduces overfitting and thereby improves downstream inference. An implementation is available at `github.com/malthesr/winsfs`.

1 Introduction

The site frequency spectrum (SFS) is the joint distribution of allele frequencies among one or more populations, and it serves as an important summary statistic in population genetics. For instance, the SFS is sufficient for computing nucleotide diversity [1], F_{ST} [2], and f-statistics [3]. Furthermore, the SFS may be used for inferring demographic history [4–6] and selection [7–9].

When working with high-quality data, it is usually straightforward to estimate the SFS from called genotypes. However, when genotype calls are uncertain, standard methods lead to significant bias in the estimated SFS [10], which propagates to downstream inference [11]. In particular, this situation arises when working with next-generation sequencing (NGS) data at low coverage and may be compounded by additional data-quality issues. Low-coverage NGS data is sometimes the only available option, for instance when working with ancient DNA [12–14]. Sequencing at low coverage is also

a popular choice to reduce sequencing costs, since most of the key population genetics analysis remain possible with such data [15].

To estimate the SFS from low-coverage data, several methods have been proposed which account for the genotype uncertainty in estimation of the SFS [10, 16]. These are based on finding the SFS that maximises the data likelihood using numeric optimisation. Two factors combine to create a computational challenge for such methods. First, in order to achieve an accurate estimate of the SFS, these methods usually require many iterations, each of which require a full pass over the input data. Second, unlike most genetics analyses, the SFS cannot be based on only the small subset of the variable sites, but must consider all sites. Taken together, this means that some summary of the full data must be held in RAM and iterated over many times. For genome-scale NGS data from more than a few dozen samples, or in more than one dimension, this is often not computationally feasible, as tens of hours of runtime and hundreds of gigabytes of RAM may be required. Current approaches for dealing with this issue restrict the analysis to fewer individuals and/or smaller regions of the genome [17], leading to less accurate results.

An additional problem with current methods is that they are prone to overfitting. In the multi-dimensional setting in particular, there is often very little information available for many of the entries in the frequency spectrum. Therefore, by considering the full data set, existing algorithms risk fitting noise, leading to estimates with poor generalisability.

In this paper, we present a novel version of the stochastic expectation-maximisation (EM) algorithm for estimation of the SFS from NGS data. In each pass through the data, this algorithm updates the SFS estimate multiple times in smaller blocks of sites. We show that for low-coverage whole-genome sequencing (WGS) data, this algorithm requires only a few full passes over the data. This considerably decreases running time, and means that it is possible to estimate the SFS using constant, negligible RAM usage by streaming data from disk. Moreover, by only considering smaller subsets of the data at a time, we show that this method reduces overfitting by smoothing the spectrum, which in turns leads to improved downstream inference.

2 Methods

Estimation of the SFS from low-coverage sequencing data requires pre-computing site allele frequency likelihoods for each site, and these are based on genotype likelihoods. We begin by briefly reviewing these concepts.

Genotype likelihoods Assume we have NGS data X sampled from K different populations (indexed by k), with N_k individuals in the kth population. Further, say that we have M diallelic sites (indexed by m), so that $G_{mkn} \in \{0, 1, 2\}$ is the genotype of a diploid individual n at site m in population k, coding genotypes as the number of derived alleles. In the same way, we use X_{mkn} to refer to the sequencing data at this location.

We define the genotype likelihood $p(X_{mkn} \mid G_{mkn})$ as the probability of

the data given a particular genotype. Genotype likelihoods form the basis of genotype calling and are calculated from aligned sequencing reads by various bioinformatic tools including `bcftools`/`samtools` [18, 19], `GATK` [20], and `ANGSD` [21], using slightly different models. For clarity, we outline the basic `GATK` model below, though the choice of model is not important for our purposes.

For D sequencing reads aligned to position m for individual n in population k, let b_d be the dth base. Assuming independence of base calls, we have

$$p(X_{mkn} \mid G_{mkn} = g) = \prod_{d=1}^{D} p(b_d \mid G_{mkn} = g)\,. \tag{1}$$

If we consider the genotype as two alleles $a_1, a_2 \in \{0, 1\}$ such that $G_{mkn} = a_1 + a_2$, then by random sampling of the parental alleles,

$$p(b_d \mid G_{mkn} = g) = \frac{1}{2} p(b_d \mid a_1) + \frac{1}{2} p(b_d \mid a_2)\,. \tag{2}$$

In turn, this probability is modelled by

$$p(b_d \mid a) = \begin{cases} \epsilon_d/3 & \text{if } b_d \neq a \\ 1 - \epsilon_d & \text{else} \end{cases}\,, \tag{3}$$

where ϵ_d is the sequencing error probability associated with the dth base.

Site allele frequency likelihoods Using genotype likelihoods, we can calculate site allele frequency (SAF) likelihoods. It is possible to think of the SAF likelihoods as the generalisation of genotype likelihoods from individuals to populations: instead of asking about the probability of the data for one individual given a genotype, we ask about the probability of the data for a population given the sum of their derived alleles.

More formally, define the sum of derived alleles for population k at site m,

$$Z_{mk} = \sum_{n=1}^{N_k} G_{mkn}\,, \tag{4}$$

with $Z_{mk} \in \{0, 1, \ldots, 2N_k\}$ each corresponding to possible sample frequencies $\{0, 1/2N_k, \ldots, 1\}$. Now define the SAF likelihood for a single population k,

$$p(X_{mk} \mid Z_{mk} = j_k) = \sum_{g \in \{0,1,2\}^{N_k}} p(g \mid Z_{mk} = j_k) \prod_{n=1}^{N_k} p(X_{mkn} \mid G_{mkn} = g_n)\,, \tag{5}$$

where X_{mk} is the data for all individuals sampled in population k at site m, $p(g \mid Z_{mk} = j_k)$ is the combinatorial probability of the genotype vector $g = (g_1, \ldots, g_{N_k})$ conditional on the sum of the genotypes being j_k, and $p(X_{mkn} \mid G_{mkn} = g_n)$ is a standard genotype likelihood. Using a dynamic programming algorithm, SAF likelihoods can be calculated from the genotype likelihoods of N individuals in $O(N^2)$ time per site [22], and a linear time approximation has also been given [23].

To extend this to the multi-dimensional SFS with K populations, let $\mathcal{J} = \times_{k=1}^{K}\{0, 1, \ldots, 2N_k\}$ be the set of possible derived allele count combinations across populations, let X_m be the data across all individuals in all populations at site m, and define $Z_m = (Z_{m1}, \ldots, Z_{mK}) \in \mathcal{J}$. Then

$$p(X_m \mid Z_m) = \prod_{k=1}^{K} p(X_{mk} \mid Z_{mk}) \,, \tag{6}$$

is the joint SAF likelihood for K populations.

Site frequency spectrum Using the definition of $\mathcal{J}$ above, we define the SFS as a parameter $\phi = \{\phi_j : j \in \mathcal{J}\}$ such that ϕ_j is the probability that $Z_m = j$. That is,

$$\phi_j = p(Z_m = j \mid \phi) \,, \tag{7}$$

for site m. That is, the SFS is the probability of a particular vector of derived allele sums at a site chosen at random.

When genotypes are available, the SFS can be estimated simply by counting observed allele count combinations. When genotypes cannot be called, the standard approach is maximum-likelihood estimation.

Assuming independence of sites, we write the likelihood function

$$\begin{aligned} p(X \mid \phi) &= \prod_{m=1}^{M} p(X_m \mid \phi) \\ &= \prod_{m=1}^{M} \sum_{j \in \mathcal{J}} p(X_m \mid \phi, Z_m = j) p(Z_m = j \mid \phi) \\ &= \prod_{m=1}^{M} \sum_{j \in \mathcal{J}} p(X_m \mid Z_m = j) \phi_j \,, \end{aligned} \tag{8}$$

where X_m refers to all sequencing data for site m. Note that the likelihood can be expressed solely in terms of joint SAF likelihoods.

The maximum likelihood estimate $\widehat{\phi} = \arg\max_{\phi} p(X \mid \phi)$ cannot be found analytically. Instead, $\widehat{\phi}$ is typically estimated using some iterative procedure such as BFGS [22] or an EM algorithm [16, 21]), of which the latter has become the standard choice. An overview of the this algorithm is given below. For details and proof, see supplementary text S1.

Standard EM algorithm Before optimization, we pre-compute the SAF likelihoods for all sites, populations, and possible sample frequencies. In addition, we make an arbitrary initial guess of the SFS $\widehat{\phi}^{(0)}$. The EM algorithm then alternates between an E-step, and an M-step.

The E-step consists of computing posterior probabilities of derived allele counts conditional on the current SFS estimate,

$$\begin{aligned} q_{mj}^{(t)} &= p(Z_m = j \mid X_m, \widehat{\phi}_j^{(t)}) \\ &= \frac{p(X_m \mid Z_m = j)\widehat{\phi}_j^{(t)}}{\sum_{j' \in \mathcal{J}} p(X_m \mid Z_m = j')\widehat{\phi}_{j'}^{(t)}} \,, \end{aligned} \tag{9}$$

for all sites $m \in \{1, \ldots, M\}$ and possibible derived allele counts $j \in \mathcal{J}$. Note that this conditional posterior depends only on the current SFS estimate and the (joint) SAF likelihoods.

Using the result of the E-step, the M-step updates the estimate by setting

$$\begin{aligned} \widehat{\phi}_j^{(t+1)} &= \frac{\sum_{m=1}^{M} q_{mj}^{(t)}}{\sum_{m=1}^{M} \sum_{j' \in \mathcal{J}} q_{mj'}^{(t)}} \\ &= \frac{1}{M} \sum_{m=1}^{M} q_{mj}^{(t)} \,, \end{aligned} \tag{10}$$

for all $j \in \mathcal{J}$.

The EM algorithm guarantees a monotonically increasing likelihood of successive values of $\widehat{\phi}^{(t)}$. The runtime of the algorithm is linear in the number of iterations required before convergence, with each iteration taking $O(M \prod_{k=1}^{K} N_k)$ time. In practice, the standard implementation is `realSFS` [22] from the software suite `ANGSD` [21] which uses a generic EM acceleration scheme [24]. The details of this acceleration will not be important in this context, so we omit the details.

Window EM algorithm As in standard EM, we pre-compute all SAF likelihoods and make an arbitrary initial guess $\widehat{\phi}^{(0)}$ of the SFS. In addition, we choose two hyperparameters B (the number of blocks) and W (the window size). Before starting optimization, all sites indices are shuffled into one of B blocks $\mathcal{B} = (\mathcal{B}_1, \ldots, \mathcal{B}_B)$ with $|\mathcal{B}_b| = \lfloor M/B \rfloor$ for $b < B$, and $|\mathcal{B}_B| = M \bmod B$. Blocks are non-overlapping and exhaustive, so that $\bigcup_{b=1}^{B} \mathcal{B}_b = \{1, \ldots, M\}$ and $\bigcap_{b=1}^{B} \mathcal{B}_b = \varnothing$.

After this initialisation, the window EM algorithm is defined as an iterative procedure that alternates between an E-step and an M-step, where the M-step in turn is split into an M1-step and an M2-step.

The E-step of the algorithm involves computing posteriors conditional on the current estimate of the SFS, much like standard EM. The difference is that we only process a single block of sites. Let $f(t) = (t-1) \bmod B + 1$, so that $f(1 + xB) = 1, f(2 + xB) = 2, \ldots$ for $x \geq 0$. Then, at time step t, we compute $q_{mj}^{(t)}$ for all $m \in \mathcal{B}_{f(t+1)}$ and all possible derived allele counts $j \in \mathcal{J}$ using eq. (9).

In the M1-step, the qs for the current block are used to give a block SFS estimate $\widehat{\psi}^{(t)}$. This is analogous to the standard M-step eq. (10), so that for each $j \in \mathcal{J}$

$$\widehat{\psi}_j^{(t+1)} = \frac{1}{|\mathcal{B}_{f(t+1)}|} \sum_{m \in \mathcal{B}_{f(t+1)}} q_{mj}^{(t)} \,, \tag{11}$$

These block estimates are then used in the M2-step to update the overall

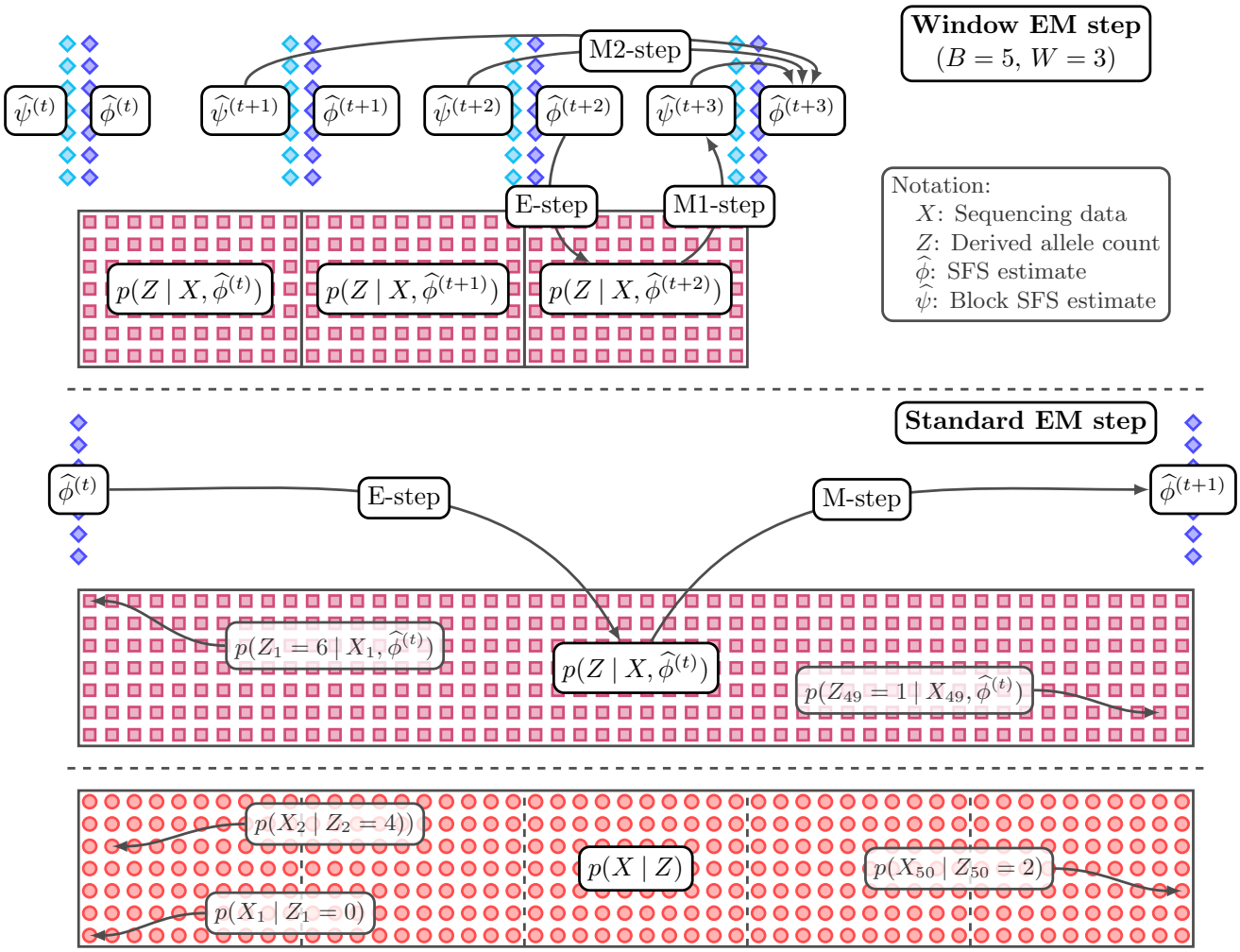

Figure 1: Schematic illustration of the standard and window EM algorithms for input consisting of a single population with $N = 3$ individuals and $M = 50$ sites. Sites are shown horizontally, derived allele frequencies are shown vertically. The pre-computed SAF likelihoods are illustrated at the bottom with blocks indicated by dashed lines. Standard EM computes the conditional posterior derived allele counts over all sites (E-step) and uses these to update the SFS estimate (M-step). Window EM computes the conditional posteriors for a small blocks of sites (E-step), computes a block SFS estimate after each block (M1-step), and updates the overall estimates as sliding window average (M2-step) of the W past block estimates. In this example, the sites have been split into $B = 5$ blocks with 10 sites each, and the sliding window covers $W = 3$ blocks.

Algorithm 1: Window EM algorithm

Input : (1) SAF likelihoods $p(X_{mk} \mid Z_{mk} = j_k)$ for sites $m \in \{1, \ldots, M\}$ and N_k individuals in each of populations $k \in \{1, \ldots, K\}$, with $j \in \mathcal{J} = \times_{k=1}^{K}\{0, 1, \ldots, 2N_k\}$. (2) Random, non-overlapping assignment of sites indices from 1 to M into B blocks $(\mathcal{B}_1, \ldots, \mathcal{B}_B)$. (3) Initial SFS estimate $\widehat{\phi}^{(0)}$.

Output : Estimate $\widehat{\phi}$ of the K-dimensional SFS.

Parameters : Number of blocks B, number of blocks per window W.

$t \leftarrow 0$

while not converged **do**

  $b_t \leftarrow t \bmod B + 1$ `// Block index`

  for $m \in \mathcal{B}_{b_t}$ **do**

    for $j \in \mathcal{J}$ **do**

      $q_{mj}^{(t)} \leftarrow \dfrac{p(X_m \mid Z_m = j)\widehat{\phi}_j^{(t)}}{\sum_{j' \in \mathcal{J}} p(X_m \mid Z_m = j')\widehat{\phi}_{j'}^{(t)}}$ `// E-step`

  $\mathcal{W}_t \leftarrow \{(t - w) \bmod B + 1 \mid w \in \{0, \ldots, \min(t, W - 1)\}\}$ `// Window indices`

  for $j \in \mathcal{J}$ **do**

    $\widehat{\psi}_j^{(t+1)} \leftarrow \dfrac{1}{|\mathcal{B}_{b_t}|} \sum_{m \in \mathcal{B}_{b_t}} q_{mj}^{(t)}$ `// M1-step`

    $\widehat{\phi}_j^{(t+1)} \leftarrow \dfrac{1}{\sum_{w \in \mathcal{W}_t} |\mathcal{B}_w|} \sum_{w \in \mathcal{W}_t} \widehat{\psi}_j^{(w)} |\mathcal{B}_w|$ `// M2-step`

  $t \leftarrow t + 1$

return $\widehat{\phi}^{(t)}$

SFS estimate for each $j \in \mathcal{J}$,

$$\begin{aligned}\widehat{\phi}_j^{(t+1)} &= \frac{1}{\sum_{w \in \mathcal{W}_t} |\mathcal{B}_w|} \sum_{w \in \mathcal{W}_t} \widehat{\psi}_j^{(w)} |\mathcal{B}_w| \\ &\stackrel{*}{=} \frac{1}{W} \sum_{w \in \mathcal{W}_t} \widehat{\psi}_j^{(w)} . \end{aligned} \tag{12}$$

where $\mathcal{W}_t = \{f(t + 1 - w) \mid w \in \{0, \ldots, \min(t, W - 1)\}\}$ is the window of the W latest block indices at time t. We use $\stackrel{*}{=}$ to express equality under the common special case when either $M/B = 0$ or $B \notin \mathcal{W}_t$, so that there are no issues with blocks of unequal sizes in the current window. In this case, the M2-step simplifies to the mean of the past W block estimates.

Pseudo-code for window EM is given in algorithm 1, and an illustration comparing window EM to standard EM is shown in figure 1.

In the below, we are interested in comparing standard EM and window EM. For clarity, we will use the term 'epoch' to refer to a full pass through the data for either algorithm. In the case of standard EM, an epoch is simply a single iteration; for window EM, an epoch corresponds to B iterations.

Convergence In the standard EM algorithm, the data log-likelihood (8) can typically be evaluated with little computational overhead during the

Population	Individuals	Sites	Median depth (range)	Contigs $\geq 100\,\text{kb}$	n_{50}	F_{ST}
Human						
YRI	10	$1.17 \cdot 10^9$	5.0 x (3.2 x–7.2 x)	52	$1.5 \cdot 10^8$	0.13
CEU	10	$1.17 \cdot 10^9$	6.2 x (2.9 x–8.0 x)			
Impala						
Masai Mara	12	$6.34 \cdot 10^8$	2.8 x (1.4 x–10.2 x)	7811	$3.4 \cdot 10^5$	0.24
Shangani	8	$6.34 \cdot 10^8$	2.9 x (2.6 x–16.8 x)			

Table 1: Overview of the input training data.

E-step. Therefore, a common convergence criterion is based on the difference between the log-likelihood values of successive epochs. That is, let

$$L_t = \frac{1}{M} \sum_{m=1}^{M} \log p(X \mid \widehat{\phi}^{(t)}) \,, \tag{13}$$

and convergence is reached when $L_{t+1} - L_t < \delta$, for some tolerance δ decided ahead of time.

For window EM, the same does not apply, since no full E-step is ever taken. However, the likelihood for each block can be calculated cheaply during each block E-step. Therefore, we define for epoch $e \in \{1, 2, \dots\}$,

$$L'_e = \sum_{b=1}^{B} \frac{1}{|\mathcal{B}_b|} \sum_{m \in \mathcal{B}_b} \log p(X_m \mid \widehat{\phi}^{(eB-b)}) \,, \tag{14}$$

that is, the sum of log-likelihoods of SFS estimates used over the past epoch, each evaluated in the block for which they were used in a block E-step, normalised by block size for convenience. We then propose the simple convergence criterion for window EM such that convergence is defined as $L'_{e+1} - L'_e < \delta$.

3 Results

To test the window EM algorithm, we implemented it in the `winsfs` program, available at `github.com/malthesr/winsfs`. We compare `winsfs` to `realSFS`, which implements the standard EM algorithm and serves as the current state of art. `realSFS` has already been validated on simulated data [21, 23]. Therefore, we compare directly against `realSFS`, and use split training and test data sets to evaluate any observed differences.

Evaluation data set We tested `winsfs` and `realSFS` on two WGS data sets of very different quality as described below. An overview is shown in table 1.

We first analyse 10 random individuals from each of the YRI (Yoruba Nigerian) and CEU (Europeans in Utah) populations from the 1000 Genomes Project [25]. This human data was sequenced to 3 x–8 x coverage and mapped to the high quality human refence genome. We created SAF files using `ANGSD`

[21] requiring minimum base and mapping quality 30 and polarising the spectrum using the chimpanzee as an outgroup. We then split this input data into test and training data, such that the first half of each autosome was assigned to the training set, and the second half to the test set. The resulting training data set contains $1.17 \cdot 10^9$ sites for both YRI and CEU, while the test data set contains $1.35 \cdot 10^9$ sites for both. Training set depth distributions for each individual are shown in supplementary figure 1.

We also analyse a data set of much lower quality from 12 and 8 individuals from two impala populations that we refer to as 'Maasai Mara' and 'Shangani', respectively, based on their sampling locations. These populations were sequenced to only 1 x–3 x with the addition of a single high-depth sample in each population (see supplementary figure 2). The data was mapped to a very fragmented assembly, and then we split the data into training and test sets just as for the human data. However, due to the low quality assembly we analysed only sites on contigs larger than 100 kb, and filtering sites based on depth outliers, excess heterozygosity, mappability, and repeat regions. We polarised using the impala reference itself. This process is meant to mirror a realistic workflow for working with low-quality data from a non-model organism. The impala input data ends up somewhat smaller than the human data set, with approximately $6.3 \cdot 10^8$ sites in both test and training data sets.

Broadly, the human data is meant to exemplify medium-quality data with coverage towards the lower end, but with no other significant issues. The impala data, on the other hand, represents low-quality data: not only is the coverage low and fewer sites are available, but the impala reference genome is poor quality with 7811 contigs greater than 100 kb and $n_{50} = 3.4 \cdot 10^{-5}$ (that is, 50 % of the assembly bases lie on contigs of this size or greater). This serves to introduce further noise in the mapping process, which amplifies the overall data uncertainty. Finally, the impala populations are more distinct, with $\mathrm{F_{ST}} \approx 0.24$ compared to 0.13 between the human populations. As we will see below, this creates additional challenges for estimation of the two-dimensional SFS.

Estimation Using the training data sets, we estimated the one-dimensional SFS for YRI and Maasai Mara, as well as the two-dimensional SFS for CEU/YRI and Shangani/Maasai Mara. We ran `winsfs` for 500 epochs using a fixed number of blocks $B = 500$ and window sizes $W \in \{100, 250, 500\}$. We will mostly focus on the setting with window size $W = 100$. For convenience, we introduce the notation $\texttt{winsfs}_{100}$ to refer to `winsfs` with hyperparameter settings $B = 500$, $W = 100$.

To compare, we ran `realSFS` using default settings, except allowing it to run for a maximum of 500 epochs rather than the default 100. We will still take the 100 epochs cut-off to mark convergence, if it has not occured by other criteria before then, but results past 100 will be shown in places.

In each case, we evaluated the full log-likelihood (eq. (8)) of the estimates after each epoch on both the training and test data sets. In addition, we computed various summary statistics from the estimates after each epoch.

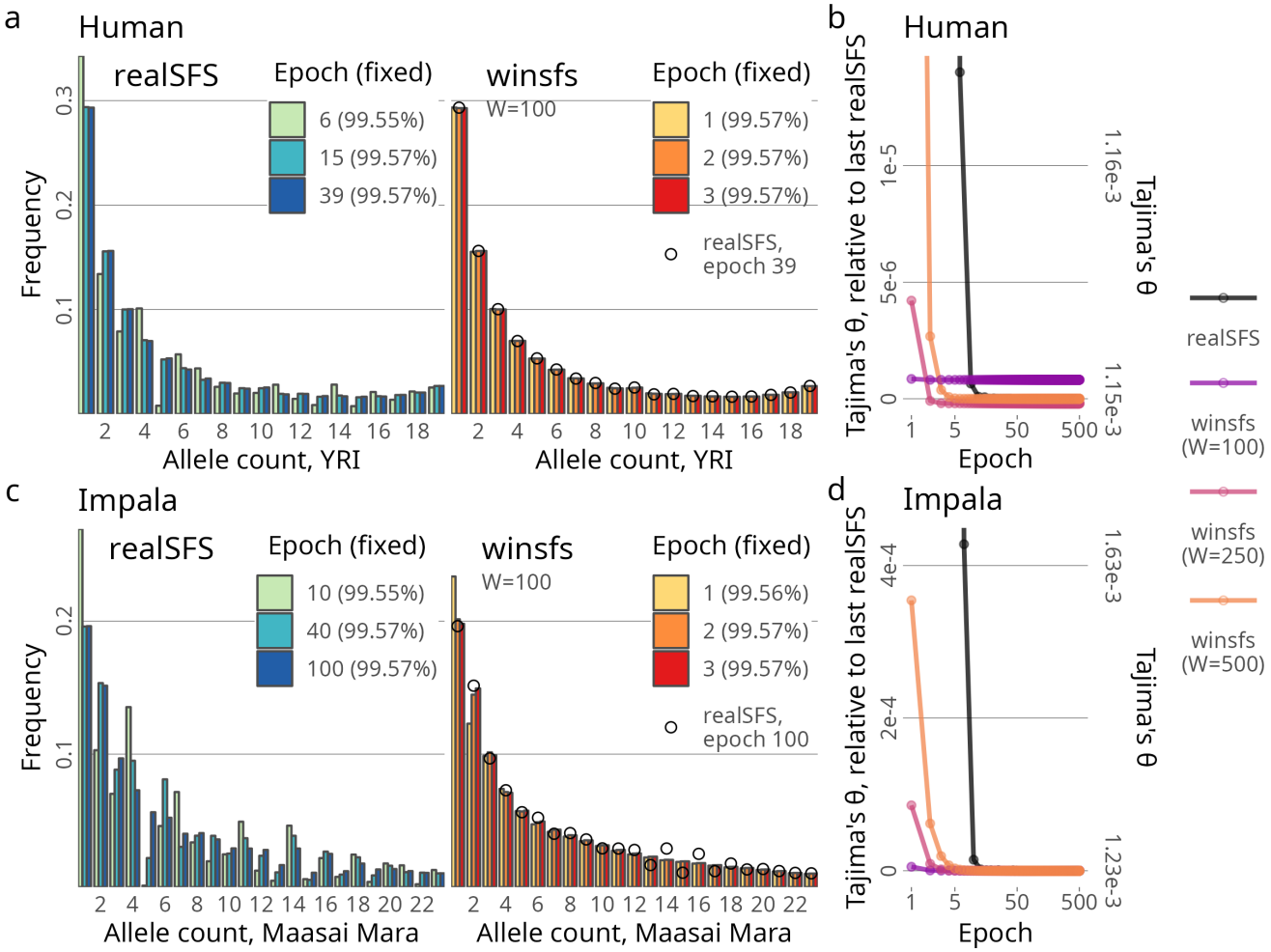

Figure 2: One-dimensional SFS estimation. **(a)**: YRI SFS estimates from `realSFS` and $\texttt{winsfs}_{100}$ after various epochs. Only variable sites are shown, proportion of fixed sites is shown in the legend. The final `realSFS` estimate is overlaid with dots on the `winsfs` plot for comparison. **(b)**: YRI Tajima's θ estimates calculated from `realSFS` and `winsfs` over epochs. **(c)**: Maasai Mara SFS estimates from `realSFS` and $\texttt{winsfs}_{100}$ after various epochs. Only variable sites are shown, proportion of fixed sites is shown in the legend. The final `realSFS` estimate is overlaid with dots on the `winsfs` plot for comparison. **(d)**: Maasai Mara Tajima's θ estimates calculated from `realSFS` and `winsfs` over epochs.

For details, see supplementary text S2.

One-dimensional SFS Main results for the one-dimensional estimates are shown in figure 2.

For the human YRI population, we find that a single epoch of $\texttt{winsfs}_{100}$ produces an estimate of the SFS that is visually indistinguishable from the converged estimate of `realSFS` at 39 epochs (figure 2a). Train and test set log-likelihoods (supplementary figure 3) confirm that the likelihood at this point is only very marginally lower for $\texttt{winsfs}_{100}$ than the last `realSFS`. By increasing the window size to 250 or 500, we get test log-likelihood values equal to or above those achieved by `realSFS`, and still within the first 5 epochs.

As an example of a summary statistic derived from the one-dimensional

SFS, figure 2b shows that $\texttt{winsfs}_{100}$ finds an estimate of Tajima's θ that is very near to the final `realSFS`, with a difference on the order of $1 \cdot 10^{-6}$. Increasing the window size removes this difference at the cost of a few more epochs.

In the case of Maasai Mara, `realSFS` runs for the 500 epochs, so we take epoch 100 to mark convergence. On this data, $\texttt{winsfs}_{100}$ requires two epochs to give a good estimate of the SFS, as shown in figure 2c. Some subtle differences relative to the `realSFS` results remain, however, especially at the middle frequencies: the `realSFS` estimate exhibits a 'wobble' such that even bins are consistently higher than odd bins. Such a pattern is not biologically plausible, and is smoothed out by `winsfs`.

Supplementary figure 4 shows train and test log-likelihood data for Maasai Mara, which again support the conclusions drawn from looking at the estimates themselves. In theory, we expect that the test log-likelihood should be adversely impacted by the `realSFS` 'wobble' pattern. In practice, however, with more than 99.5 % fixed sites, the fixed end of the spectrum dominate the likelihood to the extent that the effect is not visible. We return to this point below.

Finally, Figure 2d shows that Tajima's θ is likewise well-estimated by one or two epochs of $\texttt{winsfs}_{100}$ on the impala data.

Two-dimensional SFS Overall results for the joint spectra are seen in figure 3.

On the human data, $\texttt{winsfs}_{100}$ takes a single epoch for an estimate of the SFS that is near-identical to `realSFS` at convergence after 93 epochs. In fact, while the first $\texttt{winsfs}_{100}$ estimate has lower likelihood on the training data than the converged `realSFS` (figure 3b), it has a higher log-likelihood value on the test data (figure 3c) than any achieved by `realSFS`. This is likely caused by a faint 'checkerboard' pattern in the `realSFS` estimate due to overfitting, as we expect the spectrum to be smooth. Increasing the window size for `winsfs` increases both train and test log-likelihood scores further, and still in fewer epochs than `realSFS`.

In two dimensions, we compute both Hudson's F_{ST} (figure 3d) and the f_2-statistic (supplementary figure 5) from SFS estimates after all epochs, and we note similar patterns for these as we have seen before: one epoch of $\texttt{winsfs}_{100}$ gives an estimate of the summary statistic that is almost identical to the final `realSFS` estimate.

For the impalas, $\texttt{winsfs}_{100}$ requires two epochs for a good estimate of the spectrum, while `realSFS` again does not report convergence within the first 100. What is immediately striking about the impala results, however, is that the checkerboard pattern is very pronounced for `realSFS`, and again absent for `winsfs` (figure 3e). The problem for `realSFS` is likely exacerbated by two factors: first, the sequencing depth is lower, increasing the uncertainty; second, the relatively high divergence of the impala populations push most of the mass in the spectrum towards the edges. Together, this means that very little information is available for most of the estimated parameters. It appears that `realSFS` therefore ends up overfitting to the particularities of

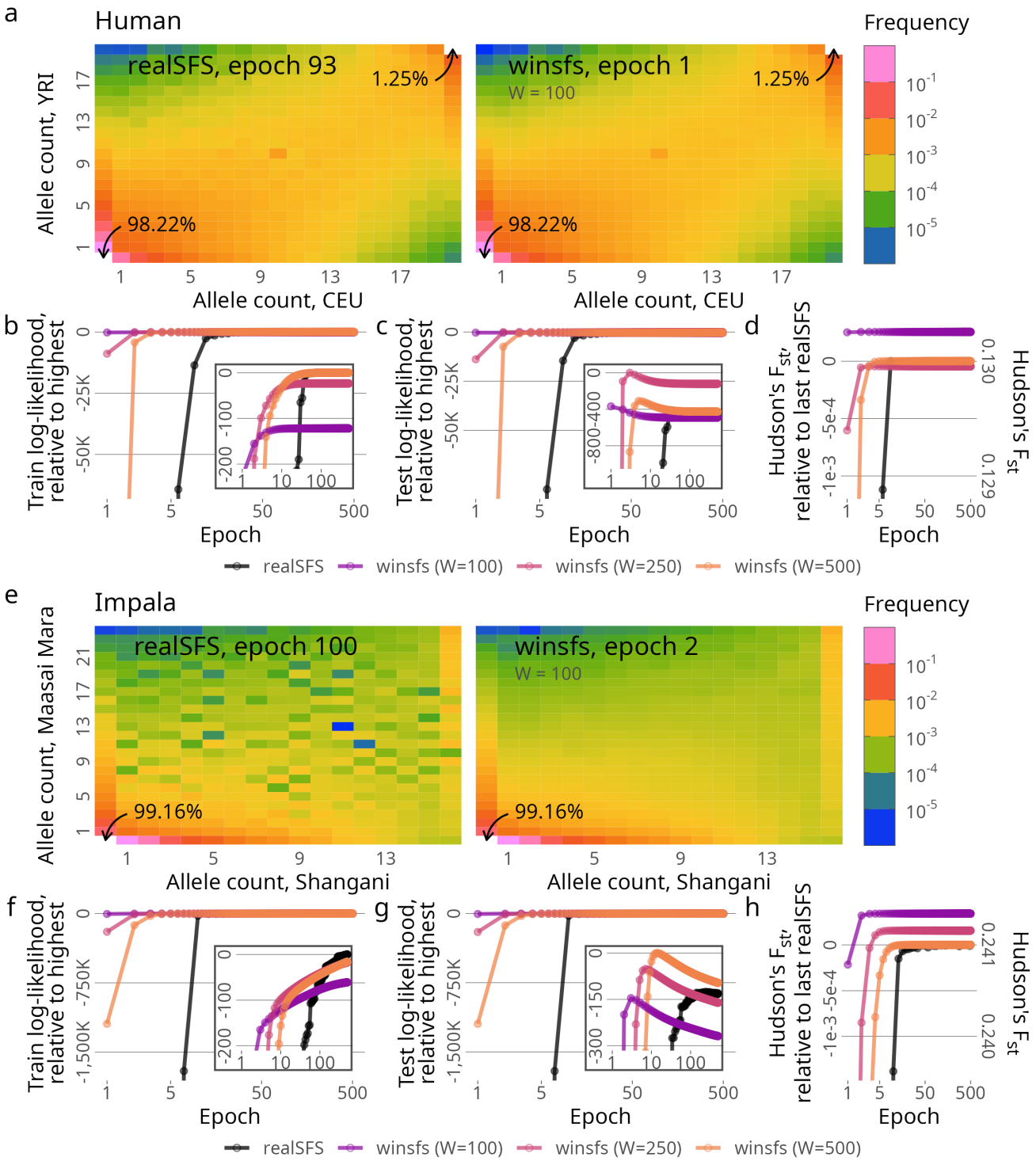

Figure 3: Two-dimensional SFS estimation. **(a)**: CEU/YRI SFS estimates from `realSFS` after 93 epochs (converged) and from $\texttt{winsfs}_{100}$ after a single epoch. Fixed sites not shown for scale, total proportion indicated by arrows. **(b)**, **(c)**: CEU/YRI SFS train and test log-likelihood over epochs for `realSFS` and `winsfs`. **(d)**: CEU/YRI Hudson's F_{ST} estimates calculated from `realSFS` and `winsfs` over epochs **(e)**: Shangani/Maasai Mara SFS estimates from `realSFS` after 100 epochs (converged) and from $\texttt{winsfs}_{100}$ after a single epoch. Fixed reference sites not shown for scale, proportions indicated by arrows. **(f)**, **(g)**: Shangani/Maasai Mara SFS train and test log-likelihood over epochs for `realSFS` and `winsfs`. **(h)**: Shangani/Maasai Mara Hudson's F_{ST} estimates calculated from `realSFS` and `winsfs` over epochs.

the training data at these bins.

This is also reflected in the difference between train and test log-likelihood (figures 3f and 3g), where `realSFS` does relatively better on the training set than the hold-out test set. All hyperparameter settings reaches test log-likelihoods equal to or greather than the final `realSFS` in less than 10 epochs, while they do not fare as well on the training data. Even so, the apparent difference in test log-likelihood between `winsfs` and `realSFS` is perhaps smaller than we would expect, given the marked checkerboarding. Again, we attribute this to the fact that the log-likelihood is dominated by all the mass lying in or around the zero-zero bin. We expect, therefore, that methods that rely on the 'interior' of the SFS should do better when using `winsfs`, compared to `realSFS`.

Before turning to test this prediction, we briefly note that F_{ST} (figure 3h) and the f_2-statistic (supplementary figure 5) are also adequately estimated for the impalas by $\texttt{winsfs}_{100}$ in one epoch.

Demographic inference All the SFS-derived summary statistics considered so far are heavily influenced by the fixed bins, or they are sums of alternating frequency bins. In either case, this serves to mask issues with irregular areas of the SFS in the lower-frequency bins. However, this will not be the case for downstream methods that rely on the shape of the spectrum in more detail.

To illustrate, we present a small case-study of trying to do demographic inference on the impala populations using the $\partial a \partial i$ [5] software with the estimated impala spectra shown in figure 3e, though folded due to the lack of an outgroup for proper polarisation. Briefly, based on an estimated SFS and a user-specified demographic model, $\partial a \partial i$ fits a model SFS based on the demographic parameters so as to maximise the likelihood of these parameters. Our approach was to fit a simple demographic model for the Shangani and Maasai Mara populations, and then gradually add parameters to the model as required based on the residuals of the input and model spectra. We take this to be representative of a typical workflow for demographic inference.

For each successive demographic model, we ran $\partial a \partial i$ on the folded spectra by performing 100 independent optimisation runs from random starting parameters, and checking for convergence by requiring the top three results to be within five likelihoods units of each other. If the optimisation had not converged, we did additional optimisation runs until either they converged or 500 independent runs where reached without likelihood convergence. In that case, we inspected the results for the top runs, to assess they were reliably reaching similar estimates and likelihoods. Results are shown in figure 4.

The first, basic model assumes that the populations have had constant populations sizes and a symmetric migration rate since diverging. The parameter estimates based on `realSFS` and `winsfs` are similar, though the `winsfs` model fit has significantly higher log-likelihood (figure 4a). However, when inspecting the residuals in figure 4b, the `realSFS` residuals suffer from a heavy checkerboard pattern, making it hard to distinguish noise from model misspecification. In contrast, the `winsfs` residuals clearly show areas of the

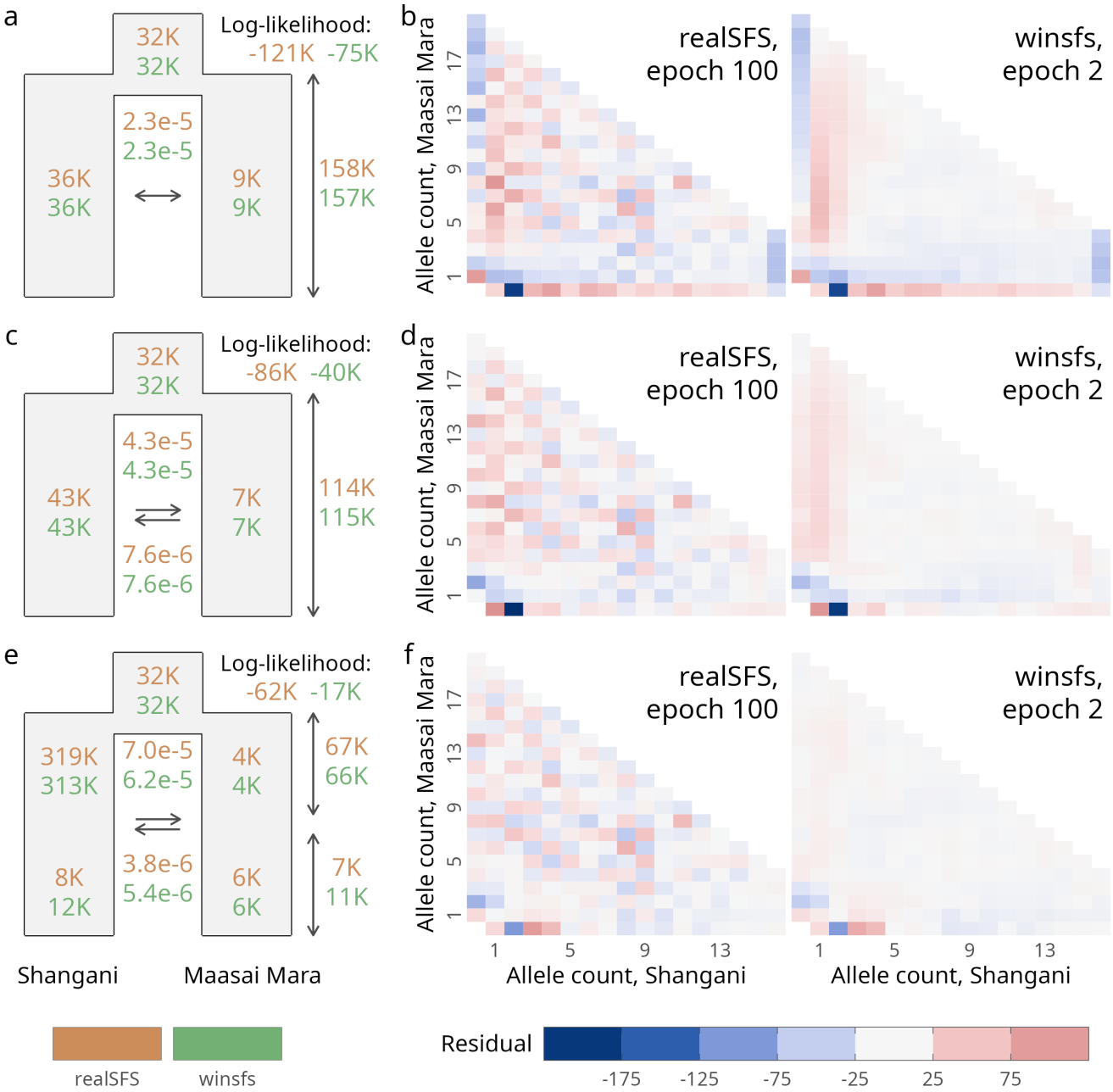

Figure 4: Demographic inference results. Each row corresponds to a demographic model fitted using $\partial a \partial i$. On the left, a schematic of the model is shown including parameter estimates using SFS estimates from `realSFS` after 100 epochs or from $\texttt{winsfs}_{100}$ after two epochs. Time is given in years, population sizes in number of individuals, and migration rates is per chromosome per generation. All parameters were scaled assuming a mutation rate of $1.41 \cdot 10^{-8}$ per site per generation and a generation time of 5.7 years. On the right, the residuals of the SFS fitted by $\partial a \partial i$. Note that $\partial a \partial i$ folds the input SFS, hence the residuals are likewise folded. The fixed category is omitted to avoid distorting the scale. **(a)**, **(b)**: Model with symmetric migration and constant population size. **(c)**, **(d)**: Model with asymmetric migration and constant population size. **(e)**, **(f)**: Model with asymmetric migration and a single, instantaneous population size change.

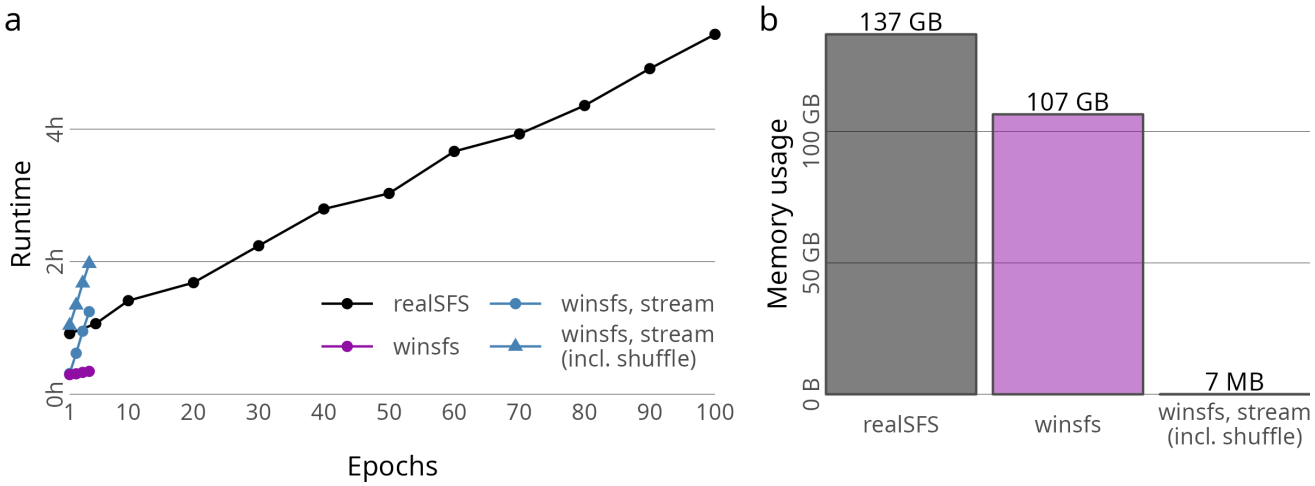

Figure 5: Computational resource usage of `winsfs` and `realSFS`. `winsfs` can be run while loading input data into RAM, or streaming through it on disk. In the latter case, data must be shuffled on disk before hand. **(a)**: Runtime required with 20 threads for various numbers of epochs. Results for `winsfs` are shown for in-memory usage and streaming mode. For streaming modes, times are given with and without the extra time taken to shuffle data on disk before running. **(b)**: Peak memory usage (maximum resident set size).

spectrum where the model poorly fits the data.

In particular, the residuals along the very edge of the spectrum suggest that a symmetric migration rate is not appropriate. Therefore, we fit a second model with asymmetric migration (figure 4c) Now $\partial a \partial i$ finds migration rates from Shangani to Maasai Mara an order of magnitude higher than *vice versa*. The results for `winsfs` (figure 4d) show improved residuals, while the `realSFS` residuals remain hard to interpret.

Finally, an area of positive residuals in the fixed and rare-variant end of the Shangani spectrum suggests that this population has recently undergone a significant bottleneck. Therefore, the third model allows for an instantaneous size change in each of the impala populations (figure 4e). At this point, the `winsfs` residuals (figure 4f) are negligible, suggesting that no more parameters should be added to the model. Once again, though, the `realSFS` residuals leave us uncertain whether further model extensions are required.

When looking at the final model fits, the $\partial a \partial i$ parameter estimates from `realSFS` and `winsfs` also start to differ slightly. In several instances, estimates disagree by about 50 %, and the log-likelihood remains much higher for `winsfs`, with a difference of 45 000 log-likelihood units to `realSFS`. We stress, however, that we would have likely never found the appropriate model without using `winsfs`, since the interpretation of the `realSFS` results is difficult. In relation to this point, we note that the final model results in considerably different estimates for parameters of biological interest, such as split times and recent population sizes, relative to the initial model.

Implementation The `winsfs` implementation is written in Rust as an efficient, parallelised program. In the main usage mode, pre-calculated SAF likelihoods are read into RAM, as in `realSFS`. However, it is also possible to

run `winsfs` while keeping the data on disk and streaming through sites. To do so, a preparation step is required in which SAF likelihoods are (jointly) shuffled into a new file. We confirmed that running in streaming mode produces equivalent result to the main `winsfs` mode (supplementary figure 7).

To assess the performance characteristics of `winsfs` with and without reading the data into RAM, we benchmarked `winsfs` in both modes as well as `realSFS` on the joint impala data. For each of the three modes, we ran estimation until convergence, as well as until various epochs before then, collecting benchmark results using Snakemake [26]. Both `realSFS` and `winsfs` were given 20 cores. Results are shown in figure 5. In terms of run-time, we find that running `winsfs` in RAM is significantly faster than `realSFS` (figure 5a). This is true in part because `winsfs` requires fewer epochs, but also since `winsfs` runs faster than `realSFS` epoch-by-epoch. As expected, when switching `winsfs` to streaming mode, run-time suffers as epochs increase. However, taking the number of epochs required for convergence into account, streaming `winsfs` remains largely competitive with `realSFS`, even when including the initial overhead to shuffle SAF likelihoods on disk.

Looking at memory consumption, streaming `winsfs` has a trivial peak memory usage of 7 MB, including the initial shuffle. In comparison, when reading data into RAM, `realSFS` and `winsfs` require 137 GB and 107 GB, respectively, even on the fairly small impala data set.

Finally, `winsfs` implements the convergence criterion based on differences in L'_e (eq. (14)) over successive epochs, with a default tolerance $\delta = 10^{-4}$. Using the default settings $B = 500$, $W = 100$, this leads to stopping after the third epoch on the human data sets, and after the fourth on the impala (supplementary figure 6). Stopping times increase appropriately with increasing window size as desired.

4 Discussion

We have presented the window EM algorithm for inferring the SFS from low-depth data, as well as the `winsfs` implementation of this algorithm. The window EM algorithm updates SFS estimates in smaller blocks of sites, and averages these block estimates in larger windows. We have argued that this approach has three related advantages relative to current methods. First, by updating more often, convergence happens one to two orders of magnitude faster. Due to the window averaging, this improvement in convergence times does not occur at the cost of stability. Second, due to the fast convergence, it is feasible to run the window EM algorithm out of memory. This brings the memory requirements of the algorithm from hundreds of gigabytes of RAM to virtually nothing. Third, by optimising over different subsets of the data in each iteration, the algorithm is prevented from overfitting to the input data. In practice, this means we get biologically more plausible spectra.

The window EM algorithm is *prima facie* quite similar in spirit to other versions of the stochastic EM algorithm [27–31]. They too work on smaller blocks, and seek some way of controlling stability in how the block estimate $\widehat{\psi}$ is incorporated in the overall estimate $\widehat{\phi}$. Typically, this involves an update

of the form $\gamma_t \widehat{\psi} + (1 - \gamma_t)\widehat{\phi}$ for some weight γ_t decaying as a function of iteration t. In initial experimentation, however, we found this unsuitable for the SFS domain. In the multi-dimensional setting in particular, we found no quickly converging scheme without unacceptable noise on the majority of bins with little available information in the data. We also found existing algorithms to be very sensitive to hyperparameter settings, making it difficult to choose settings to apply to various dimensionalities and data qualities. In contrast, the window EM algorithm works reliably for the SFS domain using the `winsfs` default settings of $B = 500$ and $W = 100$.

Within the SFS domain, prior work exists to improve estimation for low-depth sequencing data. For example, it has been proposed to 'band' SAF likelihoods to make estimation scale better in the number of sampled individuals [23, 32]. We note that such ideas are orthogonal to the work presented here, since it is concerned with the representation of the input data, and thereby indirectly modify all downstream optimisation methods. Others have focused on the implementation details of the EM algorithm, for instance using GPU acceleration [33]. Such efforts still have the typical high memory requirements, and do not address the overfitting displayed by the standard EM algorithm. Moreover, we find that the presented algorithmic improvements, combined with an efficient implementation, serve to make `winsfs` more than competitive with such efforts in terms of runtime. Indeed, with `winsfs` converging in-memory in less than an hour on genome-scale data, runtime is no longer a significant bottleneck for SFS estimation.

We emphasise, however, that the window EM algorithm and `winsfs` are unlikely to yield any meaningful benefits with sequencing data at above around 10 x–12 x coverage. With such data, the SFS should be estimated directly from genotype calls with appropriate filters. Nevertheless, efficient and robust methods remain important for low-coverage data. This is partly because low-coverage data may sometimes be the only option, for example when working with ancient DNA. Also, such methods allow intentionally sequencing at lower coverage, decreasing the financial cost.

Finally, to be clear, any improvements in the SFS estimates by `winsfs` are unlikely to be significant in the one-dimensional case, and for simple summary statistics like θ, $\mathrm{F_{ST}}$, or f-statistics. For such purposes, `winsfs` simply produces results similar to `realSFS`, although much faster. However, as the number of dimensions and samples increase, and as sequencing depth decreases, overfitting will start to influence the low-frequency bins of the spectrum. Where this information is used downstream, `winsfs` will lead to better and more interpretable results, and can potentially help solve commonly known biases in parameter estimates arising from model misspecification [34]. We have seen this in the $\partial a \partial i$ case study, but we believe the same would be true of other popular demographic inference frameworks including fastsimcoal [6, 35], moments [36], and momi [37]. It may also be significant for other methods for complex inference from the multidimensional spectrum, including inference of fitness effects using fit$\partial a \partial i$ [38, 39] or introgression using $\mathrm{D_{FS}}$ [40], though we have not explored these methods.

5 Acknowledgements

AA, CW, and MS are supported by the Independent Research Fund Denmark (grant numbers: 8021-00360B, 0135-00211B) and the University of Copenhagen through the Data+ initiative. GGE is supported by the Independent Research Fund Denmark (grant number: 8049-00098B).

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