Chapter 3

Frequency estimation using prevalence data

3.1 Background

Considerable progress has been made in the fight against malaria in the past 15 years, manifest in a reduction in transmission in many regions [290, 27]. Nevertheless, there is a continued need for genetic surveillance of antimalarial resistance [286], and the changing landscape of transmission renders comparable measures of antimalarial resistance more important than ever. To assess spatiotemporal trends, we require estimates of the frequencies of the key determinants of parasite resistance [95, 96, 276], including alleles at individual SNPs and haplotypes and genotypes spanning multiple SNPs (for example, the quintuple mutant genotype associated with clinical resistance to SP [123]).

In areas of high endemicity people are often infected with multiple parasite clones [225, 258]. Multiclonal infections pose an analytical challenge, since standard analyses of blood sample surveys cannot resolve the clones within multiclonal infections, nor reconstruct the allelic sequences that comprise the haplotypes and genotypes of the constituent clones [276]. Instead, most blood sample surveys generate prevalence data, summaries at the level of the

blood samples [96].

Several statistical models have been designed to overcome the challenge of multiclonal infections and estimate allele, haplotype and genotype frequencies using prevalence data [39, 102, 224, 129, 95, 276, 125, 223]. They are described in detail in chapter 1. With the aim of harnessing the full potential of genetic markers for the surveillance of antimalarial resistance, we present a model that complements and builds upon the existing methods. Several differences set our model apart from existing methods of malaria haplotype and genotype frequency estimation using prevalence data [102, 224, 129, 95, 276, 125]. First, in contrast to all previously published methods, the model makes use of all available data, including those that are incomplete due to unsuccessful genotyping outcomes or study design (see, for example, [83]). Second, in contrast to the Bayesian method by Wigger *et al.* [276] and the model underpinning the freely available online software MalHaploFreq [95], our model is not reliant upon experimentally-derived estimates of sample-wise MOIs. Third, in contrast to most existing approaches [39, 102, 224, 95, 125, 223], it enables rapid analysis of data from three or more SNPs.

Akin to Wigger *et al.* [276], we construct our model within a Bayesian framework. Construction as such provides a straightforward yet comprehensive treatment of uncertainty [88]. We model each infection as an unobserved clonal conglomerate, while taking into account the sample-wise uncertainty in the population-level frequency estimates. Inference within a Bayesian framework also allows the incorporation of specialist knowledge [88], enabling the MOIs to be modelled as random variables whose prior distributions are centred about a reported average.

Also similar to Wigger *et al.* [276], we use a Markov chain Monte Carlo (MCMC) algorithm to sample from the posterior distribution of the haplotype frequencies conditional on the prevalence data. MCMC samplers are of great consequence because they allow sampling from distributions that do not belong to standard families of distributions [90]. Recursive sampling

within the MCMC scheme enables the sampler to efficiently average over the unobserved clonal conglomerates, allowing the analysis of prevalence data for more than three SNPs. Unlike methods designed to generate point estimates, the MCMC method generates a sample set that approximates the full posterior distribution. Posterior summaries, including mean estimates and credible intervals, are readily available given the MCMC sample.

The aim of this chapter is to provide full details of the model and its implementation. For exposition, we focus on results based on simulated data (which also feature in Additional file 2 of [248]). A full demonstration of the model's utility using data from the field can be found in [248] and in chapter 4. In the next section we introduce our notation. In section 3.2.2 we provide a hypothetical example to illustrate the challenge associated with multiclonal infections. We provide full details of the model and its implementation in sections 3.2.3 and 3.2.4, respectively, followed by details of the simulated data (section 3.2.5), convergence (section 3.2.6) and sensitivity analyses (section 3.2.7). Results can be found in section 3.3. The chapter ends with a discussion (section 3.4).

3.2 Methods

3.2.1 Notation

Suppose that I *P. falciparum* positive blood samples, each derived from an independent episode of malaria, are genotyped at J SNPs associated with antimalarial resistance. Due to the multiclonal nature of malaria, when the ith blood sample is genotyped at the jth SNP, the observed datum, y_{ij}, is a summary of all the alleles at the jth SNP belonging to all ≥ 1 clones within the sample. Let $y_{ij} = w$ denote the detection of wild type alleles only, $y_{ij} = m$ denote the detection of mutant type alleles only, $y_{ij} = h$ denote the detection of both wild and mutant type alleles (a *h*eteroallelic SNP) and $y_{ij} =?$ represent a missing genotyping outcome (due to assay failure, for example). For example, $\boldsymbol{y}_i = (h, ?, w)$ denotes the detection of both wild and mutant

I is the number of blood samples in the dataset (each from a distinct episode of malaria).
J is the number of SNPs genotyped.
$R \leq 2^J$ is the number of haplotypes compatible with the data.
$\boldsymbol{\pi} = (\pi_1, \cdots, \pi_R)$ is a vector of haplotype frequencies. $\boldsymbol{\pi} \in \mathbb{S}^R$, where $\mathbb{S}^R$ denotes the R dimensional simplex, and hence $\sum_{r=1}^{R} \pi_r = 1$.
$\boldsymbol{y} = (\boldsymbol{y}_1, \ldots, \boldsymbol{y}_I)^T$ is the collection of data for the $i = 1, \ldots, I$ blood samples, where $\boldsymbol{y}_i = (y_{i1}, \cdots, y_{iJ})$ is the vector of sample-wise genotyping outcomes for the ith blood sample and y_{ij} is the genotyping outcome of the ith blood sample at the jth SNP, where $y_{ij} \in \{w, m, h, ?\}\ \forall\ i = 1, \ldots I$ and $j = 1, \ldots J$.
m_{max} is the global maximum MOI set by the user.
$m_{i\text{min}}$ is the minimum MOI possible for the ith blood sample.
$\boldsymbol{a} = (\boldsymbol{a}_1, \cdots, \boldsymbol{a}_I)^T$ is the collection of unobserved haplotype counts for the $i = 1, \ldots, I$ blood samples, where $\boldsymbol{a}_i = (a_{i1}, \ldots, a_{iR})$ is the vector of haplotype counts for the ith blood sample, and a_{ir} denotes the number of clones in the ith blood sample characterised by the rth haplotype, where $a_{ir} \in \{0, \ldots, m_{\text{max}}\}\ \forall\ i = 1, \ldots, I,\ r = 1, \ldots R$.
$\boldsymbol{m} = (m_1, \ldots, m_I)$ is the collection of unobserved MOIs for $i = 1, \ldots, I$ blood samples, where the sample-wise MOI, $m_i \in \{1, \cdots, m_{max}\}\ \forall\ i = 1, \cdots, I$, is the total number of clones in the ith blood sample, $m_i = \sum_{r=1}^{R} a_{ir}$.
$\boldsymbol{H}$ is a $R \times J$ matrix summarising the allelic sequences of the R haplotypes over the J SNPs.
p_{ij} is the proportion of mutant type alleles at the jth SNP in the ith blood sample.
$\boldsymbol{\alpha} = (\alpha_1, \ldots, \alpha_R)$ is the hyperparameter of the Dirichlet prior on the haplotype frequencies, $\boldsymbol{\pi}$.
λ is the hyperparameter for the prior on the MOI, m_i.
ϕ is an additional hyperparameter for the prior on the MOI, m_i.

Table 3.1: Model notation.

type alleles at the first SNP in the ith blood sample, a missing genotype outcome at the second SNP and two or more wild type alleles at the third (since the first SNP is heteroallelic, the ith blood sample must comprise two or more clones). If $J > 1$ (as in the above example), the vector $\boldsymbol{y}_i = (y_{i1}, \ldots, y_{iJ})$ is a summary of the all the haplotypes or genotypes of the clones within the clonal conglomerate. Recall that the term haplotype applies if the SNPs are in the same gene, whereas the term genotype applies if the SNPs belong in multiple genes. Henceforth, haplotypes are referred to exclusively, noting that the same methods apply for genotypes. Let a_{ir} denote the unobserved haplotype count for the rth haplotype (the number of clones characterised by the rth haplotype) in the ith blood sample and $\boldsymbol{a}_i = (a_{i1}, \ldots, a_{iR})$ denote the vector of R haplotype counts for the ith blood sample, where R is the total number of haplotypes compatible across the dataset. Note that $\sum_{r=1}^{R} a_{ir} = m_i$ is the total number of clones in the ith blood sample, henceforth referred to as the MOI. Finally, let $\boldsymbol{\pi} = (\pi_1, \ldots, \pi_R)$ denote the vector of R haplotype frequencies (the proportions of parasite clones in the *P. falciparum* population characterised by haplotypes 1 to R). For reference, a full list of model notation can be found in table 3.1. To illustrate the structure of the data, let us consider the hypothetical example outlined below.

3.2.2 Running example

Suppose parasite DNA extracted from $I = 5$ blood samples is genotyped at $J = 3$ SNPs generating prevalence data (table 3.2) which can be represented by the 5×3 matrix $\boldsymbol{y}$,

Blood sample	SNP 1	SNP 2	SNP 3
1	w	w	w
2	w	m	m
3	h	h	h
4	w	h	?
5	w	h	h

Table 3.2: A hypothetical prevalence dataset based on five samples genotyped at three SNPs. For a given blood sample and SNP, w denotes the detection of wild type alleles only, m denotes the detection of mutant type alleles only, h denotes the detection of both wild and mutant type alleles and ? indicates the genotyping outcome is missing.

$$\boldsymbol{y} = \begin{array}{c} \\ i=1 \\ \vdots \\ \\ \\ i=5 \end{array} \begin{array}{c} \begin{array}{ccc} j=1 & \ldots & j=3 \end{array} \\ \begin{pmatrix} w & w & w \\ w & m & m \\ h & h & h \\ w & h & ? \\ w & h & h \end{pmatrix} \end{array}. \tag{3.1}$$

Since both wild and mutant type alleles are detected at all three SNPs (see equation (3.1)), $R = 2^3 = 8$ haplotypes are compatible with the observed data. The allele sequences of the R

haplotypes are stored in the rows of the $R \times J$ matrix $\boldsymbol{H}$,

$$\boldsymbol{H} = \begin{array}{c} \\ r=1 \\ \vdots \\ \\ \\ \\ \\ \\ r=8 \end{array} \begin{array}{c} \begin{array}{ccc} j=1 & \ldots & j=3 \end{array} \\ \begin{pmatrix} 0 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 1 & 1 & 0 \\ 1 & 0 & 1 \\ 0 & 1 & 1 \\ 1 & 1 & 1 \end{pmatrix} \end{array}, \tag{3.2}$$

where, arbitrarily, '0' denotes a wild type allele and '1' denotes a mutant type allele. Note that under the model, $R \leq 2^J$, because only haplotypes that are possible given the data are considered. Haplotypes that are impossible given the data are assigned zero frequency and do not feature in the $\boldsymbol{H}$ matrix. For example, if the hypothetical data are instead $\boldsymbol{y}_1 = (w, w, h)$ and $\boldsymbol{y}_2 = (m, w, m)$, the matrix of haplotypes would be,

$$\boldsymbol{H} = \begin{array}{c} \\ r=1 \\ \vdots \\ \\ r=4 \end{array} \begin{array}{c} \begin{array}{ccc} j=1 & \ldots & j=3 \end{array} \\ \begin{pmatrix} 0 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & 0 & 1 \\ 1 & 0 & 1 \end{pmatrix} \end{array}, \tag{3.3}$$

since the second SNP ($j = 2$) is homoallelic ($y_{12} = y_{22} = w$). Returning to the data in table 3.2 and equation (3.1), suppose the unobserved underlying haplotype count vectors for blood

samples $i = 1, \ldots, 5$ are

$$\boldsymbol{a} = \begin{array}{c} \\ i=1 \\ \vdots \\ \\ i=5 \end{array} \begin{array}{c} \begin{array}{cccccccc} 000_{r=1} & 100 & 010 & 001 & 110 & 101 & 011 & 111_{r=8} \end{array} \\ \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 4 & 0 \\ 0 & 1 & 1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 3 & 1 & 0 & 0 & 1 & 0 \\ 2 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \end{pmatrix} \end{array}, \tag{3.4}$$

where a_{ir} denotes the number of clones with the rth haplotype in the ith sample. For reference, the allele sequences of haplotypes $r = 1, \ldots, R$ are shown as column headings. For example, $\boldsymbol{a}_5 = (2,0,0,0,0,0,1,0)$ indicates that the 5th blood sample contains three clones, two with haplotype '000' and one with haplotype '011', such that when the first SNP is genotyped pure wild type alleles are detected and when the second and third SNPs are genotyped both wild and mutant type alleles are detected, giving rise to $\boldsymbol{y}_5 = (w,h,h)$, as seen in equation (3.1). Given $\boldsymbol{a}$, the empirical sample haplotype frequencies are directly calculable,

$$\begin{aligned} \boldsymbol{\pi} &= (\pi_1, \ldots, \pi_R) \equiv (\pi_{000}, \pi_{100}, \pi_{010}, \pi_{001}, \pi_{110}, \pi_{101}, \pi_{011}, \pi_{111}), \\ &= \frac{\sum_{i=1}^{I} \boldsymbol{a}_i}{\sum_{i=1}^{I} \sum_{r=1}^{R} a_{ir}}, \\ &= \frac{\sum_{i=1}^{I} \boldsymbol{a}_i}{\sum_{i=1}^{I} m_i}, \\ &= \left(\frac{3}{16}, \frac{1}{16}, \frac{4}{16}, \frac{2}{16}, \frac{0}{16}, \frac{0}{16}, \frac{6}{16}, \frac{0}{16} \right). \end{aligned}$$

In this hypothetical example, the vector of haplotype frequencies is trivial to estimate because the sample haplotype counts are known. In reality, the haplotype counts are not observed. To estimate the vector of haplotype frequencies, the following model is proposed.

3.2.3 The model

We propose the following model (figure 3.1) to estimate the vector of haplotype frequencies, $\boldsymbol{\pi}$, conditional on prevalence data. A number of simplifying assumptions are made in its construction:

1. blood samples are independently distributed;
2. clones are independently distributed (for example, the probability of being infected with two clones with allelic sequences '000' and '011' is $\pi_{000} \times \pi_{011}$);
3. perfect detection (for example, if a person is infected with ten clones, nine of which are characterised by '000' and one by '100', the mutant allele is detected);
4. alleles are error-free (for example '0' is correctly identified as '0' and not as '1').

The terms in which these assumptions are introduced are indicated below; their implications are discussed in section 3.4. Latent variables include the MOIs, m_i for $i = 1,\ldots,I$, and the haplotype count vectors, $\boldsymbol{a}_i$ for $i = 1,\ldots I$. The prevalence data for the ith blood sample, y_{ij} for $j = 1,\ldots,J$, are modelled directly upon the unobserved haplotype count vector, $\boldsymbol{a}_i$. Since the model is constructed within a Bayesian framework, we put prior distributions on $\boldsymbol{\pi}$, $\boldsymbol{a}_i$ and m_i for $i = 1,\ldots I$. The priors are specified according to the dependencies in figure 3.1. The joint posterior density is,

$$\begin{aligned}\rho\left(\boldsymbol{\pi},\boldsymbol{a},\boldsymbol{m} \mid \boldsymbol{y}\right) &= \frac{\rho\left(\boldsymbol{y}|\boldsymbol{a}\right)\rho\left(\boldsymbol{a}|\boldsymbol{m},\boldsymbol{\pi}\right)\rho\left(\boldsymbol{m}\right)\rho\left(\boldsymbol{\pi}\right)}{\rho\left(\boldsymbol{y}\right)} \\ &\propto \prod_{i=1}^{I}\left\{\prod_{j=1}^{J}\left\{\rho\left(y_{ij}|\boldsymbol{a}_i\right)\right\}\rho\left(\boldsymbol{a}_i|m_i,\boldsymbol{\pi}\right)\rho\left(m_i\right)\right\}\rho\left(\boldsymbol{\pi}\right), \qquad (3.5)\end{aligned}$$

where the product over $i = 1,\ldots,I$ results from the assumptions of independence between blood samples, and the product over $j = 1,\ldots,J$ results from an assumption of conditional independence between SNPs within the ith blood sample given the haplotype counts, $\boldsymbol{a}_i$. The

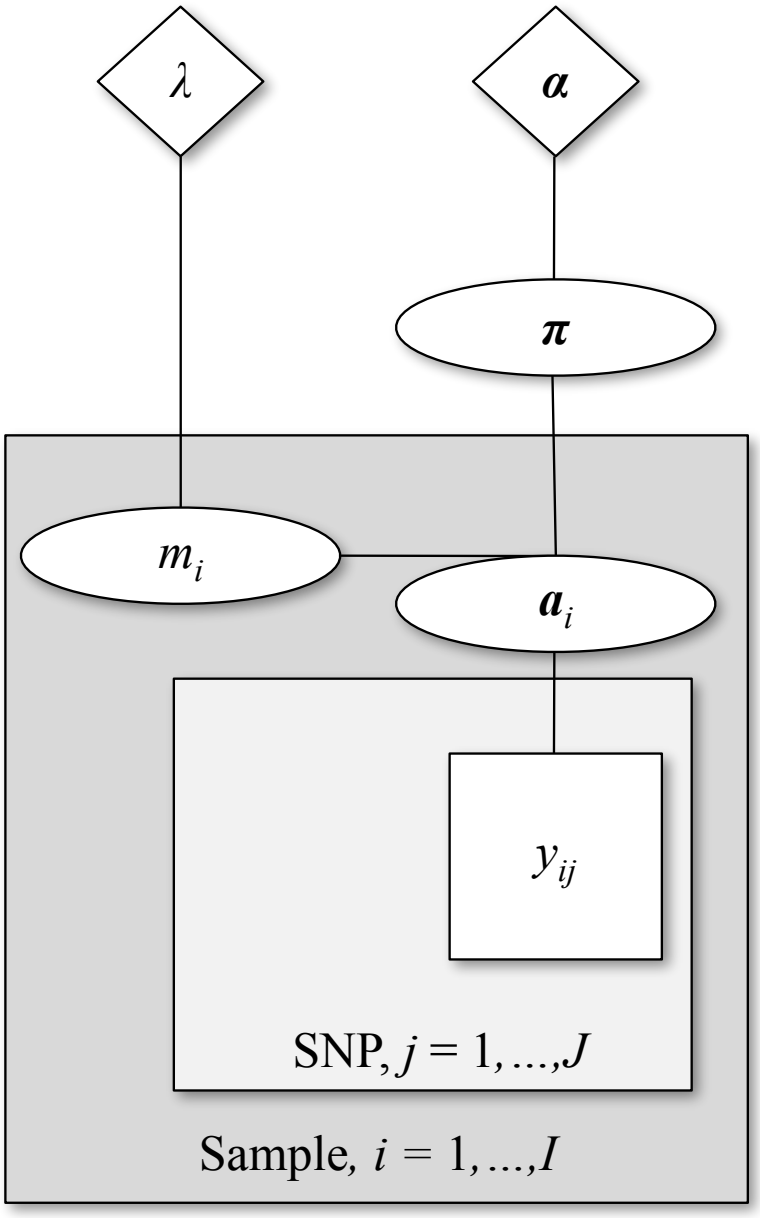


Figure 3.1: Haplotype frequency estimation model for prevalence data. The graph shows the model quantities and their conditional dependencies. The data y_{ij} for $I = 1, \ldots, I$ and $j = 1 \ldots, J$ are represented by a square. Ellipses denote unobserved variables: the vectors of haplotype counts, $\boldsymbol{a}_i$ for $I = 1, \ldots, I$; the MOI, m_i for $I = 1, \ldots, I$; and the haplotype frequency vector, $\boldsymbol{\pi}$. The diamonds represent the hyperparameters of the priors on m_i and $\boldsymbol{\pi}$ (λ and $\boldsymbol{\alpha}$, respectively). The density of the joint distribution is $\rho(\boldsymbol{\pi}, \boldsymbol{a}, \boldsymbol{m}, \boldsymbol{y}) = \rho(\boldsymbol{y} \mid \boldsymbol{a})\, \rho(\boldsymbol{a} \mid \boldsymbol{m}, \boldsymbol{\pi})\, \rho(\boldsymbol{m})\, \rho(\boldsymbol{\pi})$.

likelihood, $\rho\left(y_{ij}|\boldsymbol{a}_i\right)$, is specified as follows,

$$\rho\left(y_{ij}|\boldsymbol{a}_i\right) = \begin{cases} 1 \text{ if } y_{ij} = w \text{ and } p_{ij} = 0, \\ 1 \text{ if } y_{ij} = m \text{ and } p_{ij} = 1, \\ 1 \text{ if } y_{ij} = h \text{ and } 0 < p_{ij} < 1, \\ 1 \text{ if } y_{ij} \text{ is missing}, \\ 0 \text{ otherwise}, \end{cases} \tag{3.6}$$

where p_{ij} is the proportion of mutant type alleles at the jth SNP in the ith blood sample. That is, the number of haplotypes with a mutant type allele at the jth SNP, $\boldsymbol{a}_i \cdot \boldsymbol{h}_j$, where $\boldsymbol{h}_j$ is the jth column vector of the matrix $\boldsymbol{H}$, enlisting all the allele states (mutant '1' or wild type '0') of the R possible haplotypes at the jth SNP, normalised by the total number of haplotypes in the ith blood sample ($m_i = \sum_{r=1}^{R} a_{ir}$),

$$p_{ij} = \frac{\boldsymbol{a}_i \cdot \boldsymbol{h}_j}{\sum_{r=1}^{R} a_{ir}}. \tag{3.7}$$

Note that in equation (3.7) above, 100% detectability of the minority allele is assumed. For example, if the ith blood sample is infected with ten clones, nine with haplotype '000' and one with haplotype '100', $p_{i1} > 0$. That is, the clone with haplotype '100' is detected despite constituting only 10% of the total haplotype count for the ith blood sample. The correct identification of each allele is also assumed. For example, the alleles of both haplotypes at the second and third SNPs are identified correctly as '0' not '1', hence $p_{i\,2} = p_{i\,3} = 0$. As mentioned above, we discuss the implications of these assumptions in section 3.4.

The priors on $\boldsymbol{a}_i$ and $\boldsymbol{\pi}$ are specified as follows,

$$\rho\left(\boldsymbol{a}_i|m_i, \boldsymbol{\pi}\right) = \mathscr{M}\text{ultinomial}(\boldsymbol{a}_i \mid m_i, \boldsymbol{\pi}), \tag{3.8}$$

which implies independence between clones, and

$$\rho(\boldsymbol{\pi}) = \mathscr{D}\text{irichlet}(\boldsymbol{\pi} \mid \boldsymbol{\alpha}), \tag{3.9}$$

where $\boldsymbol{\alpha} = (\alpha_1, \ldots, \alpha_R)$ is the hyperparameter of the prior on $\boldsymbol{\pi}$. We set $\alpha_r = 1$ for $r = 1, \ldots, R$ with the effect that all R haplotypes are regarded *a priori* as biologically feasible and equally probable. Doing so provides an objective basis against which the validity of the results can be compared. Alternatively, one can incorporate prior knowledge about the viability of the different haplotypes by varying the elements of $\boldsymbol{\alpha}$. Instead of specifying one definitive prior for the MOI, we propose four options (equations (3.10) to (3.13)), with a view to selecting the one that provides the best fit to the data. All four are probability distributions (which assume independence between clones) on the set of integers $\{m_{i\min}, \ldots, m_{\max}\}$, where $m_{i\min} = 2$ if the ith blood sample is discernibly multiclonal (if for some $j \in \{1, \ldots, J\}$, $y_{ij} = h$) and 1 otherwise, while $m_{\max}$ is a global maximum set by the user and based on auxiliary data where available. All four follow classic distributions,

1. $$\rho(m_i) = \mathscr{U}\text{niform}(m_i \mid m_{i\min}, m_{\max}); \tag{3.10}$$

2. $$\begin{aligned}\rho(m_i) &= \mathscr{P}\text{oisson}_{\text{truncated}}(m_i \mid \lambda, m_{i\min}, m_{\max}),\\ &= \frac{\mathscr{P}\text{oisson}(m_i \mid \lambda)}{\left(\sum_{m_i=m_{i\min}}^{m_{\max}} \mathscr{P}\text{oisson}(m_i \mid \lambda)\right)};\end{aligned} \tag{3.11}$$

3. $$\begin{aligned}\rho(m_i) &= \mathscr{G}\text{eometric}_{\text{truncated}}(m_i \mid \lambda, m_{i\min}, m_{\max}),\\ &= \frac{\mathscr{G}\text{eomtric}(m_i \mid \lambda)}{\left(\sum_{m_i=m_{i\min}}^{m_{\max}} \mathscr{G}\text{eometric}(m_i \mid \lambda)\right)};\end{aligned} \tag{3.12}$$

4. $$\begin{aligned}\rho(m_i) &= \mathscr{N}\text{egative } \mathscr{B}\text{inomial}_{\text{truncated}}(m_i \mid \lambda, \phi, m_{i\min}, m_{\max}),\\ &= \frac{\mathscr{N}\text{egative } \mathscr{B}\text{inomial}(m_i \mid \lambda, \phi)}{\left(\sum_{m_i=m_{i\min}}^{m_{\max}} \mathscr{N}\text{egative } \mathscr{B}\text{inomial}(m_i \mid \lambda, \phi)\right)};\end{aligned} \tag{3.13}$$

where λ denotes the mean of the Poisson, geometric and negative binomial distributions and can be interpreted as an approximate *a priori* average MOI; and ϕ denotes the dispersion

parameter of the negative binomial distribution whose density is parameterised as follows,

$$\mathscr{N}\text{egative }\mathscr{B}\text{inomial}(m_i \mid \lambda, \phi) = \frac{\Gamma(m_i+\phi)}{\Gamma(\phi)\, m_i!}\left(\frac{\phi}{\lambda+\phi}\right)^{\phi}\left(1-\frac{\phi}{\lambda+\phi}\right)^{m_i} \tag{3.14}$$

The parameter λ is set by the user and based on auxiliary data where available. In this chapter, the Poisson prior is used for the sensitivity analyses based on simulated data. Upon analysing real data, the prior providing the best fit is selected using posterior predictive checks (see section A.2 for example).

3.2.4 The sampler

One cannot evaluate the posterior density given by equation (3.5) directly: it does not belong to a standard family of distributions and evaluating the normalising constant, $\rho(\boldsymbol{y})$, requires integrating over all possible $\boldsymbol{a}_i$, m_i and $\boldsymbol{\pi}$. Nevertheless, it is a distribution whose density can be evaluated pointwise up to a normalising constant. We therefore use a MCMC algorithm to sample from the distribution whose density is given by equation (3.5). More specifically, we use a Gibbs sampler. The Gibbs sampler works by breaking the problem down into blocks of variables which are iteratively sampled. For a given block, the variables within it are sampled from their full conditional distribution given the data and all variables outside the block at their current values. The variables under our model are $\boldsymbol{\pi}$, $\boldsymbol{a}_i$ and $m_i \;\; \forall\, i = 1,\ldots,I$; the target density is $\rho(\boldsymbol{\pi}, \boldsymbol{a}, \boldsymbol{m} \mid \boldsymbol{y})$ (equation (3.5)); and the blocks are

1. $\boldsymbol{a}_i$ and m_i given $\boldsymbol{\pi}$ and $\boldsymbol{y}$ for each $i = 1,\ldots,I$ independently,
2. $\boldsymbol{\pi}$ given $\boldsymbol{a}, \boldsymbol{m}$ and $\boldsymbol{y}$.

In other words, on each iteration of the sampler, for each $i = 1,\ldots,I$, we update the MOI and haplotype count vector, $\boldsymbol{a}_i$ and m_i, conditional on the current estimate of the haplotype frequency vector, $\boldsymbol{\pi}$, and the data, $\boldsymbol{y}$. Second, we update the haplotype frequency vector, $\boldsymbol{\pi}$,

given the collection of all the haplotype count vectors, $\boldsymbol{a}$, the collection of all the MOIs, $\boldsymbol{m}$, and the data, $\boldsymbol{y}$. The full conditional distribution of the variables in the first block, $\boldsymbol{a}_i$ and m_i, does not belong to a standard family of distributions, hence we use a Metropolis-Hastings step to update $\boldsymbol{a}_i$ and m_i. Due to conjugacy, the full conditional distribution of $\boldsymbol{\pi}$ is a Dirichlet distribution whose parameter vector is based on the haplotype count vectors. Hence we can Gibbs sample haplotype frequency vectors exactly. The mathematical details of the updates within both blocks are outlined in detail below. Starting at iteration $t = 0$, initial estimates, $\boldsymbol{\pi}^{(t)}$, $\boldsymbol{m}^{(t)}$ and $\boldsymbol{a}^{(t)}$, are either drawn from their respective priors (see section 3.2.3) or set equal to some specified values. For $t > 0$, the sampler proceeds as follows.

Update MOI and haplotype counts

The density of the joint conditional distribution of $\boldsymbol{a}_i$ and m_i is given by

$$\rho\left(\boldsymbol{a}_i, m_i \mid \boldsymbol{\pi}^{(t-1)}, \boldsymbol{y}\right) \propto \prod_{j=1}^{J} \left\{\rho\left(y_{ij} \mid \boldsymbol{a}_i\right)\right\} \rho\left(\boldsymbol{a}_i \mid m_i, \boldsymbol{\pi}^{(t-1)}\right) \rho\left(m_i\right) \;\forall\, i = 1, \ldots, I, \tag{3.15}$$

where $\rho\left(y_{ij} \mid \boldsymbol{a}_i\right)$ and $\rho\left(\boldsymbol{a}_i \mid m_i, \boldsymbol{\pi}^{(t-1)}\right)$ are given by equations (3.6) and (3.8), respectively, and $\rho\left(m_i\right)$ depends on the choice of MOI prior (equations (3.10) to (3.13)). Regardless of the MOI prior choice, the joint conditional distribution (equation (3.15)) does not belong to a standard family of distributions, hence cannot be sampled directly. Instead we use a Metropolis-Hastings update to sample from the joint conditional distribution whose density is given by equation (3.15). The Metropolis-Hastings step relies on the availability of a proposal distribution whose density can be evaluated. To ensure the MCMC algorithm only explores space compatible with the observed data, we use a proposal, q, conditioned upon the current vector of haplotype counts and the current MOI,

$$\left(\boldsymbol{a}_i^{\star}, m_i^{\star}\right) \sim q\left(\cdot \mid \boldsymbol{a}_i^{(t-1)}, m_i^{(t-1)}\right). \tag{3.16}$$

The joint proposal (equation (3.16)) is broken down into two stages, $m_i^\star \sim q_m\left(\cdot \mid \boldsymbol{a}_i^{(t-1)}, m_i^{(t-1)}\right)$ and $\boldsymbol{a}_i^\star \sim q_a\left(\cdot \mid m_i^\star, \boldsymbol{a}_i^{(t-1)}, m_i^{(t-1)}\right)$, described in detail below.

Propose a new MOI: The proposal q_m is implemented as follows. For, $i = 1, \ldots, I$, $m_i^\star$ is generated by either adding or subtracting a clone to the existing MOI,

$$m_i^\star \quad = \quad \begin{cases} m_i^{(t-1)} \pm 1 \text{ with probability } = 1/2 & \text{if } m_{\text{masked}\,i}^{(t-1)} > 0 \text{ and } m_i^{(t-1)} < m_{\max}, \\ m_i^{(t-1)} + 1 \text{ with probability } = 1 & \text{if } m_{\text{masked}\,i}^{(t-1)} = 0, \\ m_i^{(t-1)} - 1 \text{ with probability } = 1 & \text{if } m_i{}^{(t-1)} = m_{\max}, \end{cases} \tag{3.17}$$

where

$$m_{\text{masked}\,i}^{(t-1)} = \sum_{r=1}^{R} a_{\text{masked}\,ir}^{(t-1)} \text{ and} \tag{3.18}$$

$$\boldsymbol{a}_{\text{masked}\,i}^{(t-1)} = f(\boldsymbol{a}_i^{(t-1)} \mid \boldsymbol{y}_i). \tag{3.19}$$

The function, $f : \boldsymbol{a}_i^{(t-1)} \to \boldsymbol{a}_{\text{masked}}{}_i^{(t-1)}$ conditional on $\boldsymbol{y}_i$, ensures that the proposed MOI, $m_i^\star$, is compatible with $\boldsymbol{y}_i$. Essentially, $\boldsymbol{a}_{\text{masked}}{}_i^{(t-1)}$ is a template of $\boldsymbol{a}_i^{(t-1)}$, but with all counts whose removal would render $\boldsymbol{a}_i^{(t-1)}$ incompatible with $\boldsymbol{y}_i$ set equal to zero, thus 'masked', preventing their removal. The counts whose removal would render $\boldsymbol{a}_i^{(t-1)}$ incompatible with $\boldsymbol{y}_i$ include those that contribute either a solitary mutant allele or a solitary wild type allele. The function f is determined algorithmically as follows.

> First assign $\boldsymbol{a}_{\text{masked}}{}_i^{(t-1)} \leftarrow \boldsymbol{a}_i^{(t-1)}$. Second, if $\boldsymbol{a}_i^{(t-1)} \cdot \mathbf{h}_j = 1$, locate the solitary mutant count (r for which $a_{ir}^{(t-1)} \times h_{rj} = 1$) and, if $y_{ij} \neq ?$, set $a_{\text{masked}}{}_{ir}^{(t-1)} \leftarrow 0$ (see footnote[1]). Third, if $\boldsymbol{a}_i^{(t-1)} \cdot \mathbf{h}_j = \left(\sum_{r=1}^{R} a_{ir}^{(t-1)}\right) - 1$, locate the solitary wild type count (r for which $a_{ir}^{(t-1)} > 0$ and $a_{ir}^{(t-1)} \times h_{rj} = 0$) and, if $y_{ij} \neq ?$, set

[1] As an aside, in section 6.2.4 we encounter an application where $m_{i\min} = 2$ for all $i = 1, \ldots, I$. In this case, if $m_i^{(t-1)} = 2$, $a_{\text{masked}}{}_{ir}^{(t-1)} \leftarrow 0$ for all r corresponding to $a_{ir}^{(t-1)} > 0$.

$$a_{\text{masked}_{ir}}^{(t-1)} \leftarrow 0.$$

For example, if the hypothetical $\boldsymbol{a}$ given by equation (3.4) were our estimate at iteration $t-1$, $\boldsymbol{a}_{\text{masked}}^{(t-1)}$ would be given by

$$\boldsymbol{a}_{\text{masked}}^{(t-1)} = \begin{array}{c} \\ i=1 \\ \vdots \\ \\ \\ i=5 \end{array} \begin{array}{c} \begin{array}{cccccccc} 000_{r=1} & 100 & 010 & 001 & 110 & 101 & 011 & 111_{r=8} \end{array} \\ \begin{pmatrix} \mathbf{0} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 4 & 0 \\ 0 & \mathbf{0} & \mathbf{0} & \mathbf{0} & 0 & 0 & 0 & 0 \\ 0 & 0 & 3 & \mathbf{0} & 0 & 0 & 1 & 0 \\ 2 & 0 & 0 & 0 & 0 & 0 & \mathbf{0} & 0 \end{pmatrix} \end{array}, \tag{3.20}$$

where the elements that have been 'masked' are highlighted in bold, leading to,

$$\boldsymbol{m}_{\text{masked}}^{(t-1)} = \sum_{r=1}^{R} \boldsymbol{a}_{\text{masked}r}^{(t-1)} = \begin{array}{c} i=1 \\ \vdots \\ \\ \\ i=5 \end{array} \begin{pmatrix} 0 \\ 4 \\ 0 \\ 4 \\ 2 \end{pmatrix}. \tag{3.21}$$

For any $m_i^{\star}$, $m_i^{(t-1)}$ and $\boldsymbol{a}_i^{(t-1)}$, we can calculate $q_m\left(m_i^{\star} \mid m_i^{(t-1)}, \boldsymbol{a}_i^{(t-1)}\right)$, which is equal to $1/2$ or 1 as described by equation (3.17) and equation (3.22) below,

$$q_m\left(m_i^{\star} \mid m_i^{(t-1)}, \boldsymbol{a}_i^{(t-1)}\right) = \begin{cases} 1/2 & \text{if } m_{\text{masked}_i}^{(t-1)} > 0 \text{ and } m_i^{(t-1)} < m_{\max}, \\ 1 & \text{if } m_{\text{masked}_i}^{(t-1)} = 0, \\ 1 & \text{if } m_i{}^{(t-1)} = m_{\max}. \end{cases} \tag{3.22}$$

The probability density of the reverse step, $q_m\left(m_i^{(t-1)} \mid m_i^\star, \boldsymbol{a}_i^\star\right)$, is given by

$$q_m\left(m_i^{(t-1)} \mid m_i^\star, \boldsymbol{a}_i^\star\right) = \begin{cases} 1/2 & \text{if } m^\star_{\text{masked}\,i} > 0 \text{ and } m_i^\star < m_{\max}, \\ 1 & \text{if } m^\star_{\text{masked}\,i} = 0, \\ 1 & \text{if } m_i{}^\star = m_{\max}, \end{cases} \tag{3.23}$$

where $m^\star_{\text{masked}\,i}$ is derived from $\boldsymbol{a}_i^\star$ following equations (3.17) and (3.19). Note that the probability density of the forward step does not necessarily equal the probability of the reverse step, see for example figure 3.2, where $q_m\left(m_i^\star \mid m_i^{(t-1)}, \boldsymbol{a}_i^{(t-1)}\right) = 1$, but $q_m\left(m_i^{(t-1)} \mid m_i^\star, \boldsymbol{a}_i^\star\right) = 1/2$.

Propose a new haplotype count The proposal q_a is implemented as follows. For $i = 1, \ldots I$, the newly proposed haplotype count vector, $\boldsymbol{a}_i^\star$, is generated by either adding or subtracting a haplotype count vector representing a single clone, $\boldsymbol{a}_{\text{single clone}}$, to or from the current haplotype count vector, $\boldsymbol{a}_i^{(t-1)}$, conditional upon $m_i^\star$:

$$\boldsymbol{a}_i^\star = \begin{cases} \boldsymbol{a}_i^{(t-1)} - \boldsymbol{a}_{\text{single clone}}, \text{ where } \boldsymbol{a}_{\text{single clone}} \sim \mathscr{M}\text{ultinomial}\left(1, \boldsymbol{p}_{\text{sub}\,i}{}^{(t-1)}\right) \text{ if } m_i^\star = m_i^{(t-1)} - 1, \\ \boldsymbol{a}_i^{(t-1)} + \boldsymbol{a}_{\text{single clone}}, \text{ where } \boldsymbol{a}_{\text{single clone}} \sim \mathscr{M}\text{ultinomial}\left(1, \boldsymbol{p}_{\text{add}\,i}\right) \text{ if } m_i^\star = m_i^{(t-1)} + 1. \end{cases} \tag{3.24}$$

The probability vectors $\boldsymbol{p}_{\text{sub}\,i}{}^{(t-1)}$ and $\boldsymbol{p}_{\text{add}\,i}$ are calculated as follows,

$$\boldsymbol{p}_{\text{sub}\,i}{}^{(t-1)} = \frac{\boldsymbol{a}^{(t-1)}_{\text{masked}\,i}}{\sum_{r=1}^{R} a^{(t-1)}_{\text{masked}\,ir}} \text{ and } \boldsymbol{p}_{\text{add}\,i} = \frac{\boldsymbol{a}_{\text{compatible}_i}}{\sum_{r=1}^{R} a_{\text{compatible}_{ir}}}, \tag{3.25}$$

where $\boldsymbol{a}^{(t-1)}_{\text{masked}\,i}$ is given by equation (3.19) above. The vector $\boldsymbol{a}_{\text{compatible}_i}$ is the ith row of the look up matrix $\boldsymbol{a}_{\text{compatible}}$ in which the compatibilities of the $r = 1, \ldots, R$ haplotypes with $\boldsymbol{y}_i$ are

recorded. For example, for the hypothetical dataset (equation (3.1)),

$$\boldsymbol{a}_{\text{compatible}} = \begin{array}{r} \\ \boldsymbol{y}_1 = (w,w,w) \\ \boldsymbol{y}_2 = (w,m,m) \\ \boldsymbol{y}_3 = (h,h,h) \\ \boldsymbol{y}_4 = (w,h,?) \\ \boldsymbol{y}_5 = (w,h,h) \end{array} \begin{array}{c} \begin{array}{cccccccc} 000_{r=1} & 100 & 010 & 001 & 110 & 101 & 011 & 111_{r=8} \end{array} \\ \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 0 & 1 & 1 & 0 & 0 & 1 & 0 \\ 1 & 0 & 1 & 1 & 0 & 0 & 1 & 0 \end{pmatrix} \end{array} \tag{3.26}$$

where '1' denotes compatible and '0' denotes incompatible, and compatibility is defined as follows. If only wild or mutant type alleles are detected at the jth SNP of the ith sample ($y_{ij} = w$ or $y_{ij} = m$, respectively) only allele sequences with wild or mutant type alleles at the jth SNP are compatible, respectively; whereas if both wild and mutant type alleles are detected at the jth SNP of the ith sample, or if the datum is missing ($y_{ij} = h$ or $y_{ij} = ?$), allele sequences with both wild and mutant types alleles at the jth SNP are compatible. Akin to the dependence of $\boldsymbol{p}_{\text{sub}_i}^{(t-1)}$ upon $\boldsymbol{a}_{\text{masked}_i}^{(t-1)}$, the dependence of $\boldsymbol{p}_{\text{add}_i}$ upon $\boldsymbol{a}_{\text{compatible}_i}$ ensures the compatibility of $\boldsymbol{a}_i^\star$ with $\boldsymbol{y}_i$.

Note that given any $\boldsymbol{a}_i^\star$, $m_i^\star$, $\boldsymbol{a}_i^{(t-1)}$ and $m_i^{(t-1)}$, we can compute $q_a\left(\boldsymbol{a}_i^\star \mid m_i^\star, \boldsymbol{a}_i^{(t-1)}, m_i^{(t-1)}\right)$ following the multinomial distribution described above (equation (3.24)). That is to say,

$$q_a(\boldsymbol{a}_i^\star \mid m_i^\star, \boldsymbol{a}_i^{(t-1)}, m_i^{(t-1)}) = \begin{cases} p_{\text{add}_{ir}} \text{ if } m_i^\star = m_i^{(t-1)} + 1, \\ p_{\text{sub}_{ir}}^{(t-1)} \text{ if } m_i^\star = m_i^{(t-1)} - 1, \end{cases} \tag{3.27}$$

where the r specifies the rth element corresponding to $a_{\text{single clone}_r} = 1$ (the only element of $\boldsymbol{a}_{\text{single clone}}$ not equal to zero). The probability density of the reverse step, $q_a\left(\boldsymbol{a}_i^{(t-1)} \mid m_i^{(t-1)}, \boldsymbol{a}_i^\star, m_i^\star\right)$,

is also governed by equation (3.24), leading to

$$q_a(\boldsymbol{a}_i^{(t-1)} \mid m_i^{(t-1)}, \boldsymbol{a}_i^\star, m_i^\star) = \begin{cases} p_{\text{add}ir} \text{ if } m_i^{(t-1)} = m_i^\star + 1, \\ p^\star_{\text{sub}ir} \text{ if } m_i^{(t-1)} = m_i^\star - 1. \end{cases} \tag{3.28}$$

Acceptance probability: Having generated $m_i^\star$ and $\boldsymbol{a}_i^\star$, the newly proposed parameters are either rejected, in which case $\left(m_i^{(t)}, \boldsymbol{a}_i^{(t)}\right) \leftarrow \left(m_i^{(t-1)}, \boldsymbol{a}_i^{(t-1)}\right)$, or accepted with probability,

$$\mathbb{P}\left(\left(\boldsymbol{a}_i^{(t)}, m_i^{(t)}\right) \leftarrow (\boldsymbol{a}_i^\star, m_i^\star)\right) = \min\left\{1, \frac{\rho\left(\boldsymbol{a}_i^\star, m_i^\star \mid \boldsymbol{\pi}, \boldsymbol{y}_i\right)}{\rho\left(\boldsymbol{a}_i^{(t-1)}, m_i^{(t-1)} \mid \boldsymbol{\pi}, \boldsymbol{y}_i\right)} \frac{q\left(\boldsymbol{a}_i^{(t-1)}, m_i^{(t-1)} \mid \boldsymbol{a}_i^\star, m_i^\star\right)}{q\left(\boldsymbol{a}_i^\star, m_i^\star \mid \boldsymbol{a}_i^{(t-1)}, m_i^{(t-1)}\right)}\right\}, \tag{3.29}$$

where

$$\frac{\rho\left(\boldsymbol{a}_i^\star, m_i^\star \mid \boldsymbol{\pi}, \boldsymbol{y}_i\right)}{\rho\left(\boldsymbol{a}_i^{(t-1)}, m_i^{(t-1)} \mid \boldsymbol{\pi}, \boldsymbol{y}_i\right)} = \frac{\rho\left(\boldsymbol{a}_i^\star \mid m_i^\star, \boldsymbol{\pi}\right)}{\rho\left(\boldsymbol{a}_i^{(t-1)} \mid m_i^{(t-1)}, \boldsymbol{\pi}\right)} \frac{\rho\left(m_i^\star\right)}{\rho\left(m_i^{(t-1)}\right)}, \tag{3.30}$$

since $\prod_{j=1}^J \left\{\rho\left(y_{ij} \mid \boldsymbol{a}_i^\star\right)\right\} = \prod_{j=1}^J \left\{\rho\left(y_{ij} \mid \boldsymbol{a}_i^{(t-1)}\right)\right\} = 1$ by construction, and

$$\frac{q\left(\boldsymbol{a}_i^{(t-1)}, m_i^{(t-1)} \mid \boldsymbol{a}_i^\star, m_i^\star\right)}{q\left(\boldsymbol{a}_i^\star, m_i^\star \mid \boldsymbol{a}_i^{(t-1)}, m_i^{(t-1)}\right)} = \frac{q_m\left(m_i^{(t-1)} \mid \boldsymbol{a}_i^\star, m_i^\star\right)}{q_m\left(m_i^\star \mid \boldsymbol{a}_i^{(t-1)}, m_i^{(t-1)}\right)} \frac{q_a\left(\boldsymbol{a}_i^{(t-1)} \mid m_i^{(t-1)}, \boldsymbol{a}_i^\star, m_i^\star\right)}{q_a\left(\boldsymbol{a}_i^\star \mid m_i^\star, \boldsymbol{a}_i^{(t-1)}, m_i^{(t-1)}\right)}. \tag{3.31}$$

Each term on the right hand sides of equations (3.30) and (3.31) can be computed: $\rho\left(\boldsymbol{a}_i^\star \mid m_i^\star, \boldsymbol{\pi}\right)$ is a multinomial distribution (equation (3.8)), $\rho\left(m_i^\star\right)$ is one of the four prior distributions on the MOI (equations (3.10) to (3.13)), $q_m\left(m_i^{(t-1)} \mid \boldsymbol{a}_i^\star, m_i^\star\right)$ is equal to 1 or $1/2$ according to equation (3.23), $q_a\left(\boldsymbol{a}_i^{(t-1)} \mid m_i^{(t-1)}, \boldsymbol{a}_i^\star, m_i^\star\right)$ is equal to $p_{\text{add}ir}$ or $p^\star_{\text{sub}ir}$ according to equation (3.28), and likewise for the terms in the denominators (see, for example, figure 3.2). Note that although the notation does not make it explicit, the joint proposal (equation 3.16) is parameterised by $\boldsymbol{y}_i$, $m_{\max}$, $\boldsymbol{H}$ and $m_{i\min}$. These values do not feature in the notation, however, because they are fixed. Also note that there are no tuning parameters in the stage-wise proposals (equations

(3.17) and (3.24)), hence, under the Gibbs sampler described above, the acceptance rate of the update cannot be adjusted.

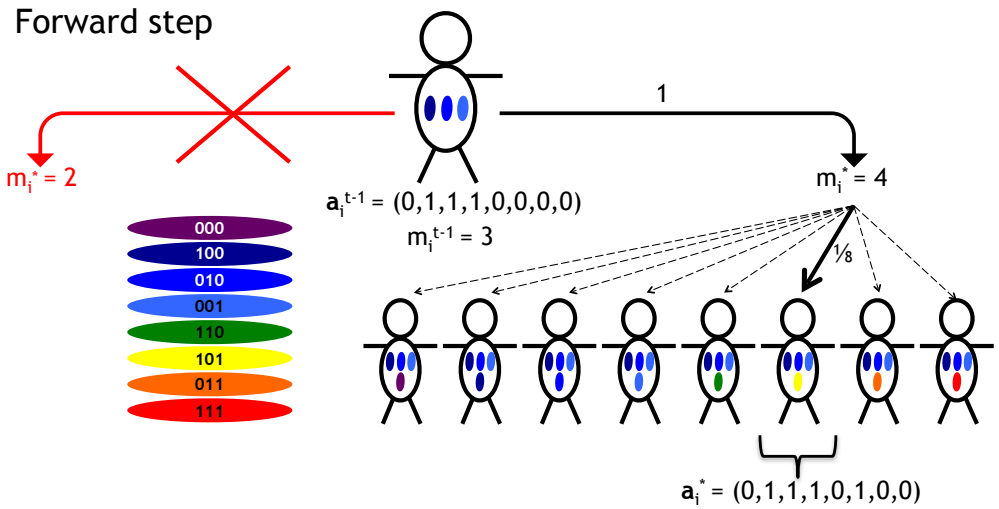
Update the vector of haplotype frequencies:

$\boldsymbol{\pi}$ is updated by sampling from its full conditional distribution with density,

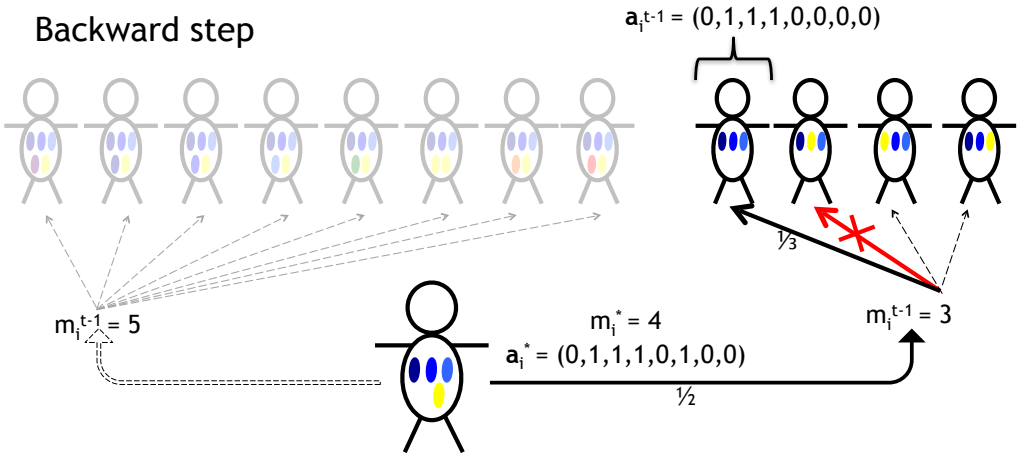
$$\begin{aligned}\rho(\boldsymbol{\pi} \mid \boldsymbol{a}^{(t)}, \boldsymbol{m}^{(t)}, \boldsymbol{y}) &\propto \prod_{i=1}^{\mathrm{I}} \left\{ \rho\left(\boldsymbol{a}_i^{(t)} \mid m_i^{(t)}, \boldsymbol{\pi}\right) \right\} \rho(\boldsymbol{\pi} \mid \boldsymbol{\alpha}), \\ &= \prod_{i=1}^{\mathrm{I}} \left\{ \mathscr{M}\text{ultinomial}\left(\boldsymbol{a}_i^{(t)} \mid m_i^{(t)}, \boldsymbol{\pi}\right) \right\} \mathscr{D}\text{irichlet}(\boldsymbol{\pi} \mid \boldsymbol{\alpha}), \\ &= \mathscr{D}\text{irichlet}\left(\boldsymbol{\pi} \mid \alpha_1 + \sum_{i=1}^{\mathrm{I}} a_{i1}^{(t)}, \ldots, \alpha_R + \sum_{i=1}^{\mathrm{I}} a_{iR}^{(t)}\right).\end{aligned}$$

Overview

The sampler is run for T iterations until convergence (see section 3.2.6). To spare computer memory, we sometimes set a thinning interval, meaning that only traces for each multiple of the thinning interval are retained. Following the general recommendation of Gelman *et al.* [88], we discard the first $t = 1, \ldots, T/2$ traces as burnin, leaving the MCMC sample, $\{\boldsymbol{\pi}^n, \boldsymbol{a}^n, \boldsymbol{m}^n\}_{n=1}^N$, which approximates the joint posterior (equation (3.5)), where N is the size of the MCMC sample post burnin and thinning. The sample $\{\boldsymbol{\pi}^n\}_{n=1}^N$, which approximates $\rho(\boldsymbol{\pi} \mid \boldsymbol{y})$, is obtained by discarding $\boldsymbol{a}^n$ and $\boldsymbol{m}^n$ from the joint sample, $\{\boldsymbol{\pi}^n, \boldsymbol{a}^n, \boldsymbol{m}^n\}_{n=1}^N$. Note that the inverse of the likelihood function (equation (3.6)) maps $\{0,1\}$ onto w, m or h conditional on $p_{ij} = (\boldsymbol{a}_i \cdot \boldsymbol{h}_j) / \sum_{r=1}^R a_{ir}$. By specifying an initial estimate of $\boldsymbol{a}_i^{(t)}$ at $t = 0$ for $i = 1, \ldots, I$, $p_{ij}^{(t)}$ is also specified for $j = 1, \ldots, J$, and thus each missing datum is assigned an initial estimate, $\hat{y}_{ij}^{(t)}$. Each time a new set of haplotypes is sampled for $t = 1, \ldots, T$, new imputed values for the missing data, $\hat{y}_{ij}^t$, are assigned. Imputation in this way assumes that the probability that a datum is missing does not depend on its value and that the missing mechanism is 'ignorable'. In other words, the parameters governing the missingness mechanism (for example, DNA quantity) are



(a) Forward step. At iteration $t-1$ of the sampler, an individual with sample data $\boldsymbol{y}_i = (h,h,h)$ is thought to be infected with $m_i^{(t-1)} = 3$ clones with haplotypes '100', '010' and '001', hence $\boldsymbol{a}_i^{(t-1)} = (0_{000}, 1_{100}, 1_{010}, 1_{001}, 0_{110}, 0_{101}, 0_{011}, 0_{111})$. The proposal involves the addition of a clone with probability one (subtraction is prohibited since $m_{\text{masked}i}^{(t-1)} = 0$, see $i=3$ equation (3.21)), hence $m_i^\star = 4$. All eight haplotypes are compatible with the observed data, hence $\boldsymbol{p}_{\text{add}\,i} = (1/8, \ldots, 1/8)$, again see $i=3$ equation (3.26)). We propose haplotype '101' (yellow ellipse), resulting in $\boldsymbol{a}_i^\star = (0_{000}, 1_{100}, 1_{010}, 1_{001}, 0_{110}, 1_{101}, 0_{011}, 0_{111},)$.



(b) Backward step. The reversal of the above proposal involves removal of the clone with haplotype '101' to recover $\boldsymbol{a}_i^{(t-1)}$. Following equations (3.18), (3.19) and (3.25), we have $\boldsymbol{a}_{\text{masked}i}^\star = (0_{000}, 1_{100}, \mathbf{0}_{010}, 1_{001}, 0_{110}, 1_{101}, 0_{011}, 0_{111})$, $m_{\text{masked}i}^\star = \sum_{r=1}^R a_{\text{masked}ir}^\star = 3$ and $\boldsymbol{p}_{\text{sub}i}^\star = \boldsymbol{a}_{\text{masked}i}^\star / \sum_{r=1}^R a_{\text{masked}ir}^\star = (0, 1/3, 0, 1/3, 0, 1/3, 0, 0)$. In other words, the probability of removing a clone is $1/2$ since addition is also possible (we could go down the lefthand branch resulting in $m_i^\star = 5$, since $m_{\text{masked}i}^\star > 0$ and $m_i^\star < m_{\max}$). We want to go down the righthand branch resulting in $m_i^{(t-1)} = 3$. Having chosen to remove a clone, the probability that we remove the clone with haplotype '101' (the yellow ellipse) is $1/3$, since we could also remove the clone with haplotype '100' (darkest blue ellipse) or the clone with haplotype '001' (lightest blue ellipse) without invalidating the compatibility of the ensuing vector of haplotype counts with the observed data.

Figure 3.2: A schematic of the proposal for the MOIs and haplotype counts. Malaria clones are represented by ellipses, colour-coded by haplotype (see stacked ellipse legend, subplot 3.2a). Branches with zero probability are depicted in red. They have zero probability because their outcomes violate compatibility with the observed data $\boldsymbol{y}_i = (h,h,h)$. Proposed branches are depicted by solid black lines. Alternative branches, that the proposal could have but did not take, are depicted by dashed lines. Each proposed branch is labeled by its probability, $\mathbb{P}(\text{proposed branch}) = 1/\text{number of available branches}$. The probabilities are equivalent to the terms in the proposal ratio, $q_m\left(m_i^{(t-1)} \mid \boldsymbol{a}_i^\star, m_i^\star\right) / q_m\left(m_i^\star \mid \boldsymbol{a}_i^{(t-1)}, m_i^{(t-1)}\right) \times q_a\left(\boldsymbol{a}_i^{(t-1)} \mid m_i^{(t-1)}, \boldsymbol{a}_i^\star, m_i^\star\right) / q_a\left(\boldsymbol{a}_i^\star \mid m_i^\star, \boldsymbol{a}_i^{(t-1)}, m_i^{(t-1)}\right) = 1/2 / 1 \times 1/3 / 1/8$.

not related to the parameters of interest (the frequencies) [132]. This assumption, does not hold if genotyping fails because of an unanticipated allele. Since sequencing is often used to identify *de novo* mutations before genotyping, the ignorable assumption is likely to hold. However, if the proposed model is used to analyse genotyping data in which failed assay attempts are likely due to unanticipated alleles, samples with missing data should be discarded.

3.2.5 Simulated data

Data are simulated to enable assessment of model performance. A haplotype frequency vector is drawn from a uniform Dirichlet distribution. A stated number of blood samples per dataset are then generated as follows. Unless otherwise stated, for each blood sample, a MOI is drawn from a non-zero conditioned Poisson distribution with $\lambda = 3$. For each blood sample, the haplotype count vector is drawn from a multinomial distribution with size equal to the MOI and probability vector equal to the vector of haplotype frequencies. The haplotype frequencies in the simulated dataset are calculated. Unless otherwise stated, for each blood sample, an observation is generated assuming 100% detectability using the inverse of the likelihood function (equation (3.6)).

3.2.6 Convergence

For every run of the sampler, log-posterior and frequency trace plots are visually inspected to monitor convergence. In addition to habitual visual inspection, a preliminary study to assess the number of iterations required for convergence is performed using 50 simulated datasets comprising one to five SNPs and 100 blood samples. For each dataset, the sampler is for 10,000, 20,000 and 50,000 iterations ($50 \times 3 = 150$ analyses in total). For each analysis, the within and between sequence variances of three parallel chains, initialised at different initial frequency vectors, are compared. Initial frequency vectors are generated by setting all but one of the initial frequencies (selected at random) to 0.02. The remaining frequency is fixed such that the

frequencies sum to unity. For datasets with only one SNP, one of the chains is initialised from a frequency vector equal to (0.5, 0.5). Comparison is based on the potential scale reduction factor (PSRF), a metric of convergence recommended by Gelman *et al.* [88]. The PSRF is an indicator of the factor by which the discrepancy in variation might be reduced if the current chains are continued for an infinite number of iterations. A value close to one supports the conjecture that the chain has converged. Gelman *et al.* advise running the chain long enough such that every PSRF < 1.1, with higher precision for final analyses. The PSRF values reported in this chapter are calculated for each haplotype frequency according to the equations on pages 303 and 304 of [88]. In total, 50,000 iterations are found to be sufficient, taking approximately five minutes to analyse a dataset comprising 100 blood samples and five SNPs.

3.2.7 Sensitivity analyses

Model performance is assessed using a series of simulated datasets, investigating the precision and accuracy of the frequency point estimates as a function of the data. For each dataset, frequency point estimates are defined by the medians of the MCMC sample. Their 95% credible intervals range from the 2.5th to the 97.5th percentiles of the MCMC sample. Accuracy is defined as the absolute error between the point estimate and the true frequency in the simulated sample, while precision is defined by the standard deviation of the marginal MCMC sample. Note that this is non-standard counterintuitive (in that lower values correspond to more accurate and precise estimates), and that both accuracy and precision decrease with the number of SNPs because the frequency mass is shared over a greater number of haplotypes. We also investigate the sensitivity of the frequency estimates to missing data, their initial values, the MOI prior and the assumption of perfect detectability. It is important to note that the tabulated results in the following section are averaged over the frequency estimates within each analysis, as well as across the analyses of ten different datasets for each combination of variables investigated. Doing so accounts for variation in the haplotype frequencies and datasets, but may also mask

haplotype specific effects. To see how average results translate into specific estimates, for each dataset we plot the frequency point estimates and their 95% credible intervals (see for example figure 3.3). Additional details of the specific sensitivity analyses are outlined below.

Precision and accuracy as a function of the data: In total, 150 simulated datasets varying in both width (one to five SNPs) and height (50, 100 and 1000 blood samples) are analysed and the average frequency and precision of the point estimates calculated as outlined above. For comparison, the datasets are also analysed using an approximate method: all blood samples with one or more heteroallelic SNPs are discarded, leaving a dataset with no discernibly multiclonal blood samples from which frequencies could be directly calculated using proportions. The frequencies of any unobserved sequences are set to zero to ensure accuracy is averaged over the same number of haplotypes as under the model.

The sensitivity of the frequency point estimates to missing data: From each of the 50 datasets used to assess convergence (section 3.2.6) data are erased from 0, 25, 50 and 75 of the blood samples selected at random. The number of genotyping outcomes erased per blood sample is selected at random, so too are the outcomes erased. Given each level of erosion, the datasets are analysed twice: first opting to impute missing data and second opting to discard blood samples with incomplete data.

The sensitivity of the frequency point estimates to their initial values: Each of the 50 datasets used to assess convergence (section 3.2.6) are reanalysed. For datasets with only one SNP, results generated post running three parallel chains with initial frequency vectors equal to (0.02, 0.98), (0.08, 0.98) and (0.5, 0.5) are compared. For datasets with two to five SNPs, results from five different chains are compared. The initial frequency vectors are selected at random from a set of frequency vectors containing a vector of uniform frequencies and all vectors generated by setting all but one of the frequencies to 0.02.

The sensitivity of the frequency point estimates to the MOI prior specification: Each of the 50 datasets used to assess convergence (section 3.2.6) are reanalysed another three times: first incorrectly assuming the distribution over the MOI is uniform; second, incorrectly assuming it is a truncated negative binomial (with $\lambda = 3$ and $\phi = 0.5$); and third, incorrectly assuming it is truncated geometric (with $\lambda = 3$). The same 50 datasets are further reanalysed twice, this time correctly assuming a Poisson prior, but with $\lambda = 1$, and then $\lambda = 5$, instead of $\lambda = 3$.

The sensitivity of frequency point estimates to the assumption that all clones are detected equally: For one to five SNPs, ten cohorts of 100 blood samples are generated as outlined above (section 3.2.5) but with parameter λ equal to one, three, five and seven. Observations are then calculated: first assuming 100% detectability; second, assuming 90% detectability (minority alleles that contributed less than 10% to a given SNP are ignored); and finally assuming 70% detectability (minority alleles that contributed less than 30% to a given SNP are ignored). All the datasets ($5 \times 10 \times 4 \times 3 = 600$ in total) are analysed assuming 100% detectability.

3.3 Results

Precision and accuracy as a function of the data: As one would hope from a valid model and functioning sampler, for a given number of SNPs, precision and accuracy increase with the number of samples in the dataset (table 3.3). Importantly, for any given dataset, the accuracies of the estimates generated under the statistical model are superior to those generated by discarding multiclonal samples (table 3.3).

The sensitivity of the frequency point estimates to missing data: Unsurprisingly, for a given number of SNPs, the impact of missing data on the mean accuracy and precision of

Number of SNPs	Number of blood samples	Statistical model		Approximate method
		Precision	Accuracy	Accuracy
1	50	0.034	0.015	0.075
	100	0.027	0.016	0.101
	1000	0.010	0.006	0.083
2	50	0.038	0.022	0.092
	100	0.034	0.022	0.058
	1000	0.011	0.007	0.046
3	50	0.039	0.035	0.074
	100	0.029	0.022	0.047
	1000	0.010	0.007	0.026
4	50	0.032	0.024	0.053
	100	0.024	0.017	0.040
	1000	0.008	0.007	0.014
5	50	0.021	0.017	0.040
	100	0.017	0.013	0.028
	1000	0.007	0.006	0.010

Table 3.3: Precision and accuracy as a function of the width (number of SNPs) and height (number of samples) of the simulated datasets. Lower values are indicative of higher accuracy and precision. Note that due to the way accuracy and precision are defined (see the introductory paragraph to section 3.2.7), neither accuracy nor precision is comparable across different numbers of SNPs.

Accuracy	Number of blood samples with incomplete data			
Number of SNPs	0	25	50	75
1	0.16	0.20 (0.20)	0.34 (0.33)	0.42 (0.43)
2	0.23	0.28 (0.28)	0.32 (0.33)	0.40 (0.42)
3	0.23	0.26 (0.26)	0.24 (0.28)	0.37 (0.46)
4	0.17	0.20 (0.20)	0.22 (0.25)	0.31 (0.30)
5	0.13	0.14 (0.15)	0.15 (0.16)	0.17 (0.19)

Table 3.4: The impact of incomplete data upon the mean accuracy of the frequency estimates. Lower values indicate higher accuracy. For those datasets with missing data, the mean accuracy obtained from analyses based on only the blood samples with complete data are included in parentheses.

Precision	Number of blood samples with incomplete data			
Number of SNPs	0	25	50	75
1	0.27	0.31 (0.31)	0.38 (0.38)	0.54 (0.54)
2	0.35	0.39 (0.40)	0.42 (0.45)	0.54 (0.64)
3	0.29	0.32 (0.33)	0.36 (0.39)	0.45 (0.53)
4	0.24	0.26 (0.27)	0.29 (0.31)	0.33 (0.38)
5	0.17	0.18 (0.18)	0.19 (0.20)	0.21 (0.23)

Table 3.5: The impact of incomplete data upon the mean precision of the frequency estimates. Lower values indicate higher precision. For datasets with missing data, the mean precision obtained from analyses based on only the blood samples with complete data are included in parentheses.

the frequency point estimates is unfavourable (compare column two with columns three, four and five, tables 3.4 and 3.5, respectively). However, in general, estimates are more accurate and precise upon imputation (compare the numbers within and outside the parenthesis). In summary, imputation enables use of all available data, whereas, for datasets with two or more SNPs, partial data are squandered when blood samples with incomplete data are discarded.

The sensitivity of the frequency point estimates to their initial values: Frequency point estimates are robust to their initial values. The mean difference between estimates obtained from chains initiated at different values is < 0.01, while the maximum is 0.02 (haplotype 00000, dataset B, figure 3.3).

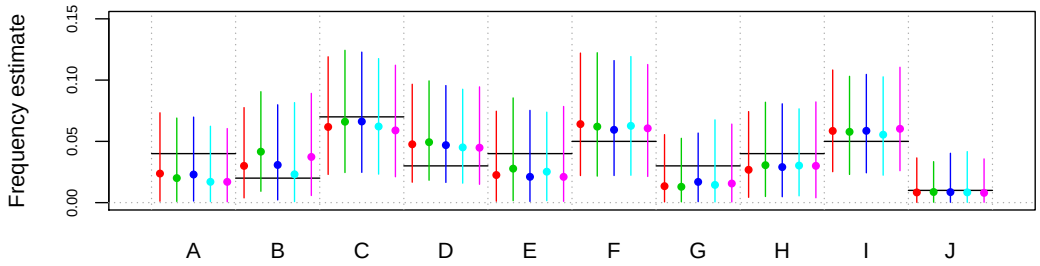


Figure 3.3: The sensitivity of a frequency point estimate to different initial values. The plot shows frequency point estimates (dots) with 95% credible intervals (vertical lines) for the haplotype with the allele sequence 00000, generated by five chains (five different colours) initiated at a different initial frequency vectors) for ten different datasets (A–J). For each dataset, the frequency of the haplotype with the allele sequence 00000 in the simulated sample is depicted by a black horizontal bar.

The sensitivity of the frequency point estimates to the MOI prior distribution: Unsurprisingly, the model with the correctly specified Poisson distribution over the MOI gives rise to the most accurate haplotype frequencies on average (table 3.6). At the level of the individual point estimates, the differences between estimates generated under the uniform, Poisson and negative binomial distributions are relatively small and the prior had little to no effect on precision (for example, see figure 3.4). Likewise, on average, the correct λ parameter specification gives rise to the most accurate frequency estimates (table 3.7). The detrimental effect of overestimating λ appears to be slightly less than that of underestimating it, but the range tested is small. In fact, it seems that no λ specification is preferable to misspecification (compare values in columns three and four of table 3.7 to values under the uniform prior in table 3.6). Overestimation has a spuriously favourable effect on precision, probably because overestimation augmented the number of clones per blood sample, thus leading to a greater number of haplotype assignments on which to base the haplotype frequencies. Sensitivity of the model to the parameter λ motivates the repeat analysis of field data, each time varying λ in order to establish the sensitivity of the results (for example, see section 4.2.3). Based on the accuracy when λ is unspecified compared with misspecified, if the *a priori* average MOI is unknown, a uniform prior is to be worth investigating.

Number of SNPs	MOI prior distribution			
	Uniform	Poisson	N. Binomial	Geometric
1	0.051	**0.016**	**0.016**	0.024
2	0.032	**0.023**	0.027	0.028
3	0.027	**0.022**	0.023	0.023
4	**0.017**	0.018	**0.017**	0.018
5	**0.013**	**0.013**	**0.013**	**0.013**

Table 3.6: The impact of MOI prior misspecification on the mean accuracy of the frequency estimates. All data are generated under a model with a MOI Poisson prior with $\lambda = 3$. Note that N. Binomial refers to a negative binomial distribution. Lower values (highlighted in bold) indicate higher accuracy.

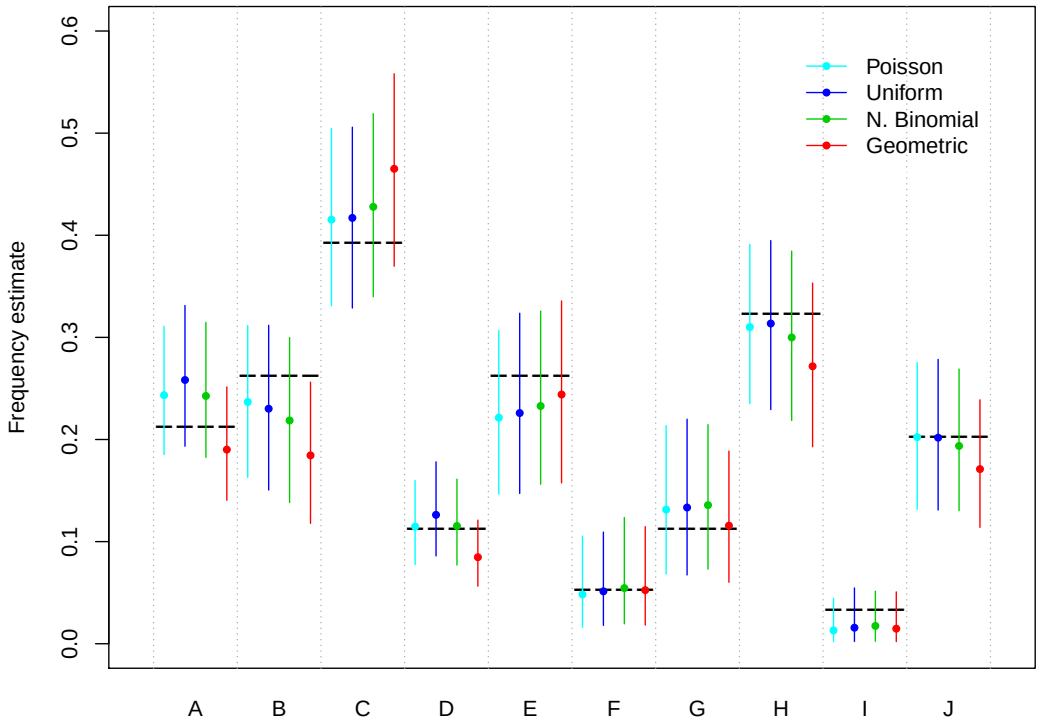


Figure 3.4: The impact of MOI prior misspecification on the frequency estimates of a single haplotype. The plot shows frequency point estimates (dots) and their 95% credible intervals (vertical lines) for the haplotype with allele sequence 11, colour-coded by the MOI prior distribution, across ten different datasets (A–J). The frequency in the simulated data set is denoted by the black horizontal bar. Note that N. Binomial refers to a negative binomial distribution. The data are simulated under the Poisson prior.

Number of SNPs	MOI prior parameter λ		
	Correct ($\lambda = 3$)	Underestimate ($\lambda = 1$)	Overestimate ($\lambda = 5$)
1	**0.016**	0.060	0.058
2	**0.023**	0.044	0.032
3	**0.023**	0.028	0.026
4	**0.017**	0.021	**0.017**
5	**0.013**	0.014	**0.013**

Table 3.7: The impact of the MOI prior parameter misspecification on the mean accuracy of the frequency estimates. Lower values indicate higher accuracy.

The sensitivity of frequency point estimates to the assumption that all clones are detected equally: Estimates are robust to suboptimal detectability when data are generated using MOI parameter, λ, of one or three (for example see figure 3.5). For data generated using $\lambda \geq 5$, estimates are robust to 90% detectability, but the accuracy decreases when the detectability drops to 70%, (figure 3.5). Unsurprisingly, the detrimental effect of suboptimal detectability has more impact upon datasets generated under a comparatively large MOI parameter, λ, since blood samples with a large number of clones are more likely to qualify as blood samples in which alleles might be in a minority. Suboptimal detectability appears to have a small spuriously positive effect on precision, seemingly due to the relative decline in the number of heteroallelic alleles. Suboptimal detectability primarily affects datasets comprised of three or fewer SNPs (table 3.8). In addition to the method used to define accuracy (see introductory paragraph to section 3.2.7), this may, in part, be due to the method used to generate the data, explained as follows. The data are generated using a frequency vector drawn from a uniform Dirichlet distribution. Since the number of possible haplotypes increases exponentially with the number of SNPs, haplotypes frequencies tend to be more uniform in datasets comprised of four or more SNPs. Since the likelihood that a single SNP is dominated by a single allele is smaller in a dataset comprising a large number of haplotypes over which mass is evenly distributed, suboptimal detectability primarily affects datasets comprised of three or fewer SNPs, especially

Number of SNPs	MOI parameter, λ	Limit of detection		
		100%	90%	70%
1	1	0.011	0.011	0.012
	3	0.015	0.015	0.051
	5	0.012	0.012	0.107
	7	0.033	0.029	0.160
2	1	0.017	0.017	0.017
	3	0.018	0.018	0.041
	5	0.028	0.028	0.081
	7	0.035	0.031	0.123
3	1	0.011	0.011	0.011
	3	0.019	0.019	0.025
	5	0.028	0.025	0.049
	7	0.039	0.04	0.065
4	1	0.010	0.010	0.010
	3	0.016	0.016	0.017
	5	0.023	0.023	0.028
	7	0.033	0.033	0.031
5	1	0.009	0.009	0.009
	3	0.013	0.014	0.014
	5	0.017	0.017	0.016
	7	0.019	0.019	0.018

Table 3.8: The impact of suboptimal detectability on the accuracy of the frequency estimates. For one to five SNPs, ten datasets are generated given detectability equal to 100%, 90% and 70%. The datasets are analysed assuming optimal detectability (100%). Lower values indicate higher accuracy.

when mass is unevenly distributed (for example, see the estimate for haplotype 010, cohort 26, $\lambda = 5$, figure 3.5).

3.4 Discussion

In this chapter, we present a statistical model designed to estimate population-level frequencies of *P. falciparum* allele and multi-SNP haplotype and genotype frequencies using prevalence data from malaria endemic regions where multiclonal infections are commonplace. Multiclonal

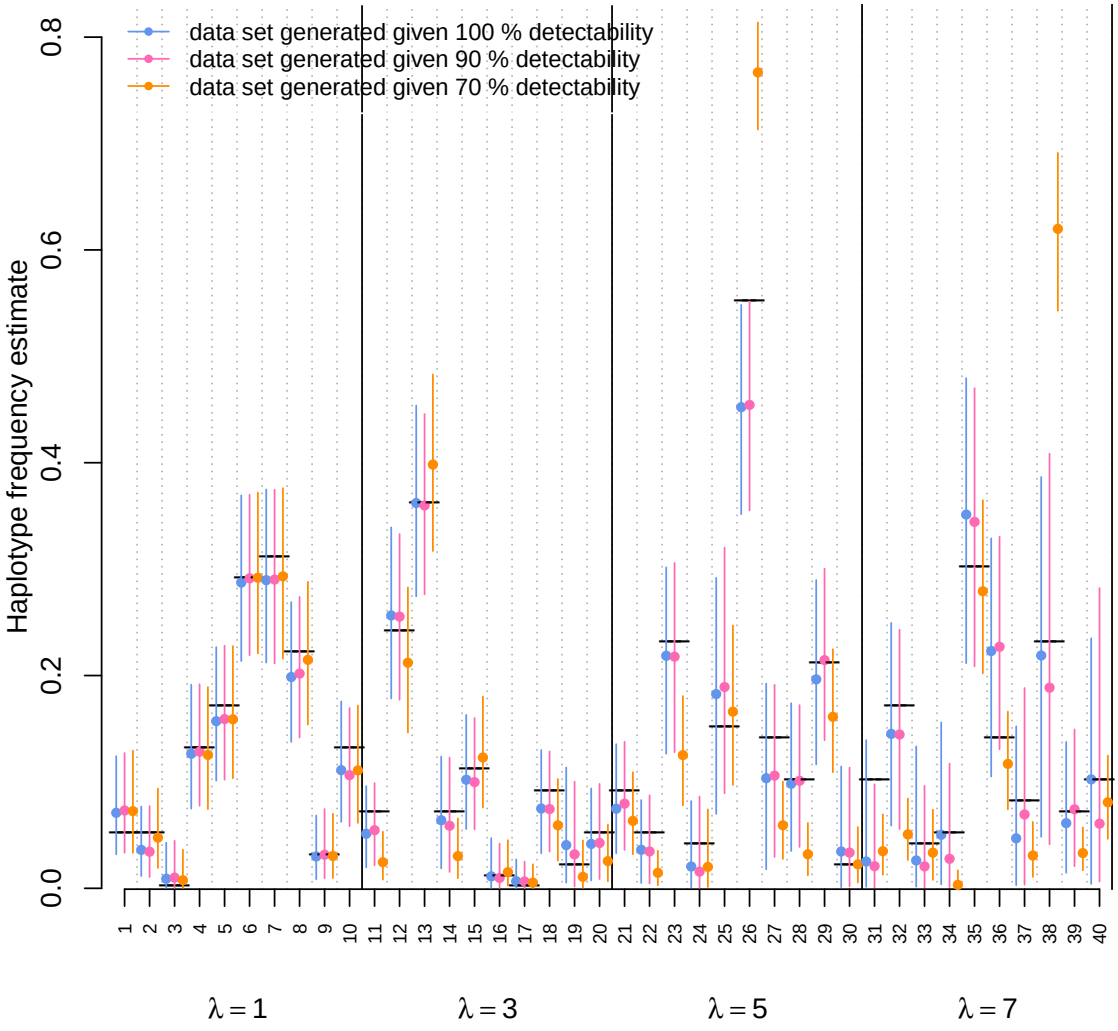


Figure 3.5: The impact of suboptimal detectability on the frequency estimates for a single haplotype with allele sequence 010. The plot shows haplotype frequency estimates (points) and their 95% credible intervals (vertical lines), given suboptimal detectability. Ten cohorts of one hundred infected blood samples are generated per specified MOI parameter, λ (40 cohorts in total: 1–10, $\lambda = 1$; 11–20, $\lambda = 3$; 21–30, $\lambda = 5$; 31–40, $\lambda = 7$). To enable comparison between results given different limits of detectability, three datasets are generated per cohort: one given 100% detectability (blue), another given 90% detectability (pink), and another given 70% detectability (orange). Each of the three datasets generated from a common cohort have the same frequencies in the simulated datasets (black horizontal bar), since the haplotypes in the infected blood samples remained the same despite changing the detectability. The datasets are all analysed assuming 100% detectability.

infections hamper the genetic surveillance of antimalarial resistance. The model is designed to overcome the problems associated with multiclonal infections. Its application generates comparable frequency estimates, allowing markers of resistance to be tracked in malaria endemic regions, yielding important information on the dynamics of resistance.

Importantly, the model does not require measurements of the sample-wise MOIs. Instead, it uses an *a priori* estimate of the average MOI (based on auxiliary data where available), an initial estimate of the vector of haplotype frequencies, and all available prevalence data to infer the haplotypes of the unobserved clones within individual blood samples. The initial frequency estimates are then revised based on clonal assignments. The implementation algorithm cycles over the aforementioned steps thousands of times until convergence. Application of the model reconstructs haplotypes within samples, provides a consistent method of frequency estimation, and avoids the loss of information that results from the usual adjustments made for multiclonal blood samples and unsuccessful genotyping outcomes.

To assess the impact of various model choices a suite of sensitivity analyses is performed using simulated data. The simulation study demonstrates that the frequencies estimated using the model are more accurate than estimates based on simply calculating proportions after discarding discernibly multiclonal blood samples. The model is robust to changes in the initial frequency estimates, but sensitive to deviations in the prior on the MOI. In light of these results, we recommend an investigation to find the MOI prior distribution that provides the best fit to the data (see section A.2 for an example), followed by repeat analyses of the data, each time varying the MOI prior parameter value within a reasonable range (such as the limits of its 95% confidence interval), to establish the sensitivity of the results to its value (see section 4.2.3 in the following chapter).

A number of simplifying assumptions are made in the construction of the model; they are listed below. As with any model, it is important to note that although the assumptions are likely to be violated in practice, the model may still be useful, as famously remarked upon by George

Box [31]. The assumptions include

1. blood samples are independently distributed;
2. clones are independently distributed (for example, the probability of being infected with two clones with allelic sequences '000' and '011' is $\pi_{000} \times \pi_{011}$);
3. perfect detection (for example, if a person is infected with ten clones, nine of which are characterised by '000' and one by '100', the mutant allele is detected);
4. alleles are error-free (for example '0' is correctly identified as '0' and not as '1').

The first two assumptions are common to all of the existing statistical methods of *P. falciparum* haplotype frequency estimation [102, 224, 129, 95, 276, 125], while more realistic assumptions regarding detectability and SNP miscalls are incorporated into alternative models ([95] and [276], respectively). We now discuss each assumption in turn.

Depending on the study design, the assumption of independence between samples is a valid one. For example, blood samples surveyed in a cross sectional study (such as [22]) should be independent. On the contrary, repeat sampling from the same child (for an example, see [51]) might lead to dependence. In chapter 4, we analyse the data from [51] under the assumption of independence. In chapter 5, we relax the assumption by adding an extension to our model.

The assumption that clones are independent depends on the manner in which multiclonal infections are acquired. An individual infected with clones obtained from multiple successive bites in a high transmission setting is likely to harbour independent clones, whereas the assumption is unlikely to hold for a person infected with multiple clones following a single inoculation from a mosquito harbouring a multiclonal infection [102]. Since both mechanisms are likely to occur, especially in high transmission settings, the assumption that clones are independently distributed is questionable. Reasons as to why the assumption might not harm inference are discussed in length by Hill and Babiker [102]. Perhaps the most compelling argument put forward by Hill and Babiker is the agreement between the experimentally-derived within-vector diversity (based on diploid oocysts from dissected mosquitoes collected in

the same village as the prevalence data), and the within-host diversity estimated under the assumption of independence. We agree with Hill and Babiker that the assumption is tenuous but pragmatic, noting (as do they) that there is not enough information in the data to support a model that distinguishes between inoculation with recombinant and independent clones [102]. In addition, we note that dependence between haplotypes will unlikely harm average estimates, since correlation typically leads to over-dispersed but unbiased realisations (see binomial example in [84]).

The validity of the assumption that all SNPs are correctly identified depends on the technology used to generate the data and differs for different SNPs. For example, concordances between calls based on microarray technology and calls based on RFLP analyses ranging from 63.9% (for *pdfhfr*-51) to 100% (for *pfmdr1*-86 and *pfdhps*-581) have been reported [145]. The model by Wigger *et al.*, includes an error probability term, which is fixed and equal for all SNPs [276] (see chapter 2 for a full description). Based on simulated data, Wigger *et al.* conclude that the error model is beneficial if the miscall rate exceeds 1–2%. Following Wigger *et al.*, it would be interesting to incorporate an error term into our model to account for miscalled SNPs. It is noted, however, that when analysing microarray data from the field (in which the probability of an error is thought to be 0.05 based on SNP-wise comparison with RFLP base calls [145]), the frequency estimates are statistically indifferent unless a large number of samples (> 500) are analysed [276]. If a large number of samples are analysed, omission of a fixed random error is likely to cause the model to overfit noise, hence underestimate dominant frequencies and overestimate rare frequencies [276]. A simple way to avoid overfitting noise without adding an error term, is to analyse the data twice, setting rare frequencies to zero in the second analysis [276].

The assumption that all clones in the blood sample are perfectly detected is almost certainly violated, especially when analysing data generated by PCR based methods [96]. Detection limits are thought to range between 80% and 99% [133, 113, 114, 58]. To assess the impact

of this assumption, simulated data are generated under imperfect detectability. Our model is robust to imperfect detection, providing the MOI prior parameter, λ, is less than or equal to three, or the limit of detectability is 90% or more.

Further to the problem of imperfect detection, is the fact that the blood sample itself might not contain a representative sample of the infection. This might occur because of low parasite numbers and/or because *P. falciparum* infections undergo complex cycles of sequestration [77]. Even when imperfect detection due to experimental procedures is taken into account (see [95], for example), what actually is estimated is the proportion of accessible parasite clones among the within-host parasite population. However, if the parameters of sequestration are independent to the frequencies of interest, which we assume they are, the inaccessible clones are ignorable and the estimates based on the accessible clones should be accurate; that is to say, collectively, the blood samples should equitably represent the host-infecting parasite population.

Following Hastings *et al.* [95, 96], we define frequency in terms of parasite clones (recall that, following convention, we use the word clone to denote a collection of genetically identical parasites). Alternatively, one could define frequency in terms of the proportion of parasites. In fact, one could see the former definition as an approximation of the latter, assuming clones represent populations of equal size. Unfortunately, there is not enough information in prevalence data to support a model that accommodates estimates in terms of biomass. Hence, all models that generate *P. falciparum* allele, haplotype and genotype frequencies based on prevalence data either define frequency in terms of parasite clones, or assume clones represent clones of equal size [39, 102, 224, 129, 95, 276, 125, 223].

As outlined in the introduction, several differences set our model apart from existing methods of *P. falciparum* allele, haplotype and genotype estimation using prevalence data. In contrast to preceding methods, the model presented here is able to analyse prevalence data for more than three SNPs, using all available data, including those that are incomplete due

to unsuccessful genotyping assays, without reliance upon experimentally-derived estimates of the sample-wise MOI, within a Bayesian framework, thus providing a readily extendable framework in which uncertainty is captured in a straightforward yet comprehensive manner. However, superior assumptions regarding detectability and experimental error are incorporated into alternative models [95, 276] (see above). It is especially important to take into account the suboptimal detectability of minority clones, addressed by Hastings *et al.* [95], when the experimentally-derived MOI estimates are regarded as fixed [96]. The latter is not the case in the current model (patient-level MOIs are treated as unobserved random variables), perhaps explaining why our model is comparatively robust to imperfect detectability.

In summary, genetic monitoring of *P. falciparum* plays an important role in the timely surveillance of antimalarial drug resistance. However, multiclonal infections present an analytic challenge, especially in areas of high transmission. We provide a full description of a model designed to overcome the challenge of multiclonal infections and estimate the frequencies of *P. falciparum* allele, multi-SNP haplotypes and genotypes. Its validity is demonstrated using a suite of sensitivity analyses, while its utility is demonstrated elsewhere using prevalence data for markers of resistance to SP [248]. Its applicability, however, extends beyond markers of SP resistance, as demonstrated in the following chapter. To the best of our knowledge, this is the first model that combines rapid analysis of three or more SNPs, using all available data without reliance upon measurements of the MOI in individual blood samples.

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