

1 Bayesian inference of clonal expansions in a dated phylogeny

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20 ABSTRACT

21 Microbial population genetics models often assume that all lineages are constrained by the same
22 population size dynamics over time. However, many neutral and selective events can invalidate this
23 assumption, and can contribute to the clonal expansion of a specific lineage relative to the rest of
24 the population. Such differential phylodynamic properties between lineages result in asymmetries
25 and imbalances in phylogenetic trees that are sometimes described informally but which are difficult
26 to analyse formally. To this end, we developed a model of how clonal expansions occur and affect
27 the branching patterns of a phylogeny. We show how the parameters of this model can be inferred
28 from a given dated phylogeny using Bayesian statistics, which allows us to assess the probability
29 that one or more clonal expansion events occurred. For each putative clonal expansion event we
30 estimate their date of emergence and subsequent phylodynamic trajectories, including their long-term
31 evolutionary potential which is important to determine how much effort should be placed on specific
32 control measures. We demonstrate the usefulness of our methodology on simulated and real datasets.

33 INTRODUCTION

34 In a microbial population, a clonal expansion event happens when a single individual (or clone) acquires
35 an advantage relative to the rest of the population. This advantage could be selective, for example
36 a mutation conferring antimicrobial resistance (Blair et al. 2015; Holmes et al. 2016), or neutral, for
37 example a founder effect when the clone reaches a new population of susceptible hosts (Peter and
38 Slatkin 2015). Whatever the mechanism, clonal expansion causes a single lineage to grow suddenly,
39 leading to what were described as “epidemic clones” based on bacterial genotyping data (Maynard-
40 Smith et al. 1993; Smith et al. 2003; Feil et al. 2004; Fraser et al. 2005). Since the advent of whole
41 genome sequencing, clonal expansions have often been observed and described informally in pathogen
42 phylogenetic trees, when a branch suddenly seems to split into multiple branches (McVicker et al.
43 2014; Holden et al. 2013; Eldholm et al. 2015; Shapiro 2016; Stoesser et al. 2016; Ledda et al. 2017).

44 Phylodynamics can be used to infer past population size changes given pathogen genetic data (Ho
45 and Shapiro 2011; Volz et al. 2013). However, most phylodynamic methods assume that the same
46 population size function applies to the whole population, which is inappropriate if a clonal expansion
47 event affected only a subset of the sampled population. Differences between the branching observed in
48 a phylogeny and the branching expected in the absence of any population structure can be used to test
49 this assumption (Dearlove and Frost 2015; Volz et al. 2020). This principle provides a non-parametric
50 approach to the detection of hidden population structure, based on rejection of the null hypothesis of
51 an unstructured population. By contrast, here we develop and apply an explicit phylodynamic model
52 for how structure arises through one or more clonal expansion events.

53 We describe a phylogenetic model of clonal expansion which is an extension of the coalescent framework
54 (Kingman 1982; Donnelly and Tavaré 1995; Rosenberg and Nordborg 2002), and more specifically an
55 extension of the dated coalescent with heterochronous sampling and varying effective population size
56 (Griffiths and Tavaré 1994; Donnelly and Tavaré 1995; Drummond et al. 2002, 2003; Biek et al. 2015).

57 In brief, our population model consists of several subpopulations, including a “background” component
58 of constant size, plus an unknown number of additional components each of which corresponds to a
59 clonal expansion event, with an associated time of emergence, growth rate and maximum population
60 size (carrying capacity). We also describe how to perform Bayesian inference under this model, taking
61 as input a dated phylogeny, such that can be reconstructed using BEAST (Suchard et al. 2018),

62 BEAST2 (Bouckaert et al. 2019), treedater (Volz and Frost 2017), TreeTime (Sagulenko et al. 2018)
63 or BactDating (Didelot et al. 2018). In this inferential setting, our methodology allows us to detect
64 putative clonal expansions, assess their statistical significance and the specific parameters controlling
65 their growth. We performed inference on simulated datasets, where the correct clonal expansions that
66 took place are known, in order to benchmark the specificity and sensitivity of our methodology. We
67 also analysed several real datasets from recent studies on infectious diseases, and show that our new
68 method can reveal important features in pathogen evolutionary epidemiology that would otherwise be
69 difficult to analyse.

70 MATERIALS AND METHODS

71 Mathematical model description

72 We consider the ancestry of a sample of N individuals indexed by $i \in \{1, \dots, N\}$, with sampling times
73 denoted $\mathbf{t} = \{t_i\}_{i \in \{1, \dots, N\}}$. Here and elsewhere in this article, time is measured backward in time so
74 that for example if $t_1 < t_2$ then sample 1 is more recent than sample 2. The population is structured
75 into $M \geq 1$ subpopulations indexed by $j \in \{1, \dots, M\}$: the subpopulations $j \in \{1, \dots, M-1\}$ correspond
76 to $M-1$ “clonal expansion” subpopulations whereas the population $j = M$ is called the “background”
77 subpopulation. Each individual has the same probability θ_j of belonging to subpopulation j , with
78 $\boldsymbol{\theta} = \{\theta_1, \dots, \theta_M\}$ and $\sum_{j=1}^M \theta_j = 1$. This population structure therefore partitions the sampled
79 individuals $\{1, \dots, N\}$ into M mutually disjoint subsets $\mathbf{f} = \{f_1, \dots, f_{M-1}, f_M\}$ with $\bigcup_{i=1}^M f_i = \{1, \dots, N\}$.

80 The background subpopulation ($j = M$) is assumed to be ruled by the coalescent process with constant
81 population size N_M (Kingman 1982). Each of the other subpopulations ($j = 1, \dots, M-1$) on the other
82 hand is ruled by a coalescent model with its own varying population size function (Griffiths and Tavaré
83 1994). For each of these clonal expansion subpopulations we define a time of emergence t_j^{exp} , a carrying
84 capacity N_j and the time h_j it takes to reach half of the carrying capacity. Together these parameters
85 determine the size $\alpha_j(t)$ of the subpopulation j at time t as follows:

$$\alpha_j(t) = \begin{cases} \frac{N_j(t_j^{\text{exp}} - t)^2}{h_j^2 + (t_j^{\text{exp}} - t)^2} & \text{if } t \leq t_j^{\text{exp}} \\ 0 & \text{otherwise} \end{cases} \quad (1)$$

86 Note that this function has the property $\alpha_j(t_j^{\text{exp}}) = 0$ so that the population size reaches zero, when
 87 the expansion begins at t_j^{exp} . This forces the coalescent rate for a lineage to diverge to infinity as
 88 $t \rightarrow t_j^{\text{exp}}$. As such all lineages from the subpopulation are forced to coalesce before t_j^{exp} . From a
 89 modelling perspective this can be interpreted as the population being negligible at the time of the
 90 lineage diverging. Furthermore, $\alpha_j(t) \rightarrow N_j$ when $t \rightarrow -\infty$ in accordance with the definition of a
 91 carrying capacity being the size reached in the long term. Finally we note that $\alpha_j(t_j^{\text{exp}} - h_j) = N_j/2$,
 92 which means that h_j is indeed the time it takes to reach half of the carrying capacity. This function
 93 represents a qualitative approximation to the population dynamics of a clonal expansion.

94 To complete the definition of the joint ancestral process for all N individuals, we consider that each
 95 of the clonal expansions originated from either the background subpopulation or from one of the
 96 preexisting clonal expansions. Let d_j denote the origin of an expansion $j \in \{1, \dots, M - 1\}$. Therefore
 97 $d_j \in \{1, \dots, M\}$ with the condition that if $d_j < M$ then $t_j^{\text{exp}} < t_{d_j}^{\text{exp}}$ (if the origin is not the background
 98 subpopulation, it is another clonal expansion that much have emerged beforehand). Since each
 99 expansion starts with a negligible population size, this implies that the group of leaves sampled from
 100 a subpopulation is either monophyletic (if this subpopulation is not the origin of another one) or
 101 paraphyletic (otherwise) in the phylogeny of all N individuals.

Parameter description	Prior
Number of clonal expansions	$\pi(M - 1) = \text{poisson}(\phi)$
Subpopulation membership probabilities	$\pi(\boldsymbol{\theta} M) = \text{dirichlet}(\psi)$
Subpopulation membership	$\pi(\mathbf{f} \boldsymbol{\theta}) = \prod_{j=1}^M \theta_j^{ \mathbf{f}_j }$
Background population size	$\pi(N_M) = \text{lognorm}(\mu_{\text{anc}}, \sigma_{\text{anc}})$
Carrying capacities	$\pi(N_j N_M) = \text{lognorm}(N_M, \sigma_{\text{exp}})$
Times of clonal expansion emergence	$\pi(t_j^{\text{exp}} N_M) = \text{gamma}\left(\frac{\nu^2}{\kappa^2}, \frac{\kappa^2 N_M}{\nu}\right)$
Time to reach half of carrying capacity	$\pi(h_j N_M) = \text{exponential}(\lambda_r/N_M)$
Origin of each clonal expansion	$\pi(d_j t_{1..M}^{\text{exp}}) = \text{uniform}(\{i \in \{1, \dots, M\} : t_i^{\text{exp}} > t_j^{\text{exp}}\})$

Table 1: Summary of parameters and priors used for Bayesian inference

102 Table 1 summarises the parameters involved in this model, and lists the priors which were used to

103 perform Bayesian inference under this model. The background population size effectively acts as a
104 scale parameter on the entire process. First of all, we assume that the final effective population sizes
105 of the individual expansions are in the same order of magnitude as the background population size,
106 as defined by the prior probability $\pi(N_j | N_M)$. Furthermore, by affecting the expected time to most
107 recent ancestor of the phylogeny, the background population size strongly determines which clonal
108 expansions will be detectable and which will not. An expansion which occurred in the distant past,
109 or whose growth rate is slow is very likely to fully coalesce while its effective population size remains
110 near constant, making it undetectable. As such we condition both t_j^{exp} and h_j on N_M , leading to the
111 prior distributions $\pi(t_j^{\text{exp}}|N_M)$ and $\pi(h_j|N_M)$.

112 Bayesian inference

113 Performing inference under the clonal expansion model above for a given dated phylogeny \mathbf{g} requires
114 estimation of the value of all the underlying parameters of this model, including the unknown number
115 of subpopulations M . We consider the prior distributions summarised in Table 1. For convenience, let
116 $\boldsymbol{\alpha}$ denote the combination of the parameters N_M for the background population and $(N_j, t_j^{\text{exp}}, h_j, d_j)$
117 for each of the $j = 1, \dots, M - 1$ clonal expansions. The joint prior on $\boldsymbol{\alpha}$ is therefore:

$$\pi(\boldsymbol{\alpha}|M) = \pi(N_M) \prod_{j=1}^{M-1} \pi(N_j|N_M) \pi(t_j^{\text{exp}}|N_M) \pi(h_j|N_M) \pi(d_j|t_{1..M}^{\text{exp}}) \quad (2)$$

118 We can decompose the posterior probability of the model parameters given the dated phylogeny as
119 follows:

$$\begin{aligned} p(M, \mathbf{f}, \boldsymbol{\theta}, \boldsymbol{\alpha}|\mathbf{g}) &\propto p(\mathbf{g}|M, \mathbf{f}, \boldsymbol{\alpha}) \pi(M, \mathbf{f}, \boldsymbol{\theta}, \boldsymbol{\alpha}) \\ &= p(\mathbf{g}|M, \mathbf{f}, \boldsymbol{\alpha}) \pi(M - 1) \pi(\boldsymbol{\alpha}|M) \pi(\mathbf{f}|\boldsymbol{\theta}) \pi(\boldsymbol{\theta}|M) \end{aligned} \quad (3)$$

120 All other terms correspond to prior densities given in Table 1 and Equation 2, except for the first term
121 $p(\mathbf{g}|M, \mathbf{f}, \boldsymbol{\alpha})$ which is the likelihood of the dated phylogeny when all parameters are known, including
122 which leaves belong to which subpopulations, the population size function of each subpopulation, and
123 the origin of each clonal expansion subpopulation. In these conditions the likelihood is simply the

124 product of likelihoods of the coalescent process in each of the subpopulations. Let \mathbf{g}_j denote the part
 125 of the dated phylogeny that corresponds to the subpopulation j .

126 Knowledge of $(M, \mathbf{f}, \boldsymbol{\alpha})$ allows us to decompose exactly the genealogy \mathbf{g} into each of the \mathbf{g}_j components.
 127 Note in particular that a component \mathbf{g}_j contains all the leaves indexed in f_j plus a leaf dated at t_a^{exp}
 128 for each subpopulation a such that $d_j = a$, meaning that the origin of a is j . With these notations,
 129 the likelihood is therefore decomposed as:

$$p(\mathbf{g}|M, \mathbf{f}, \boldsymbol{\alpha}) = p(\mathbf{g}_M|N_M) \prod_{j=1}^{M-1} p(\mathbf{g}_j|N_j, t_j^{\text{exp}}, h_j) \quad (4)$$

130 The first term corresponds to the coalescent process in the background subpopulation, with constant
 131 population size $\alpha_M(t) = N_M$, and the remaining terms correspond to the coalescent process in the
 132 clonal expansion subpopulations, each with their own population size function $\alpha_j(t)$ as defined in
 133 Equation 1. These terms can be computed using standard coalescent theory (Griffiths and Tavare
 134 1994; Donnelly and Tavare 1995; Drummond et al. 2002). Briefly, if a population has size $\alpha(t)$ and
 135 $A(t)$ extent lineages at time t , then the probability of a dated phylogeny \mathbf{g} with $n - 1$ coalescent events
 136 at times c_1, \dots, c_{n-1} is given by:

$$p(\mathbf{g}|\alpha(t)) = \exp\left(-\int_{-\infty}^{\infty} \mathbb{1}[A(t) \geq 2] \binom{A(t)}{2} \frac{1}{\alpha(t)} dt\right) \prod_{i=1}^{n-1} \frac{1}{\alpha(c_i)} \quad (5)$$

137 Note the absence of the $\prod_{i=1}^{n-1} \binom{A(c_i)}{2}$ term as this is the likelihood of the entire genealogy, meaning
 138 both the branch lengths and the topology, so that this term from the probability of the waiting times
 139 cancel out with its reciprocal from the probability of the topology.

140 The computation in Equation 5 requires us to calculate the integral of the reciprocal of the population
 141 size function, for each interval of time in which $A(t)$ is constant and greater than one. This is
 142 straightforward for the background subpopulation, and for each clonal expansion subpopulation j
 143 with the population size function given in Equation 1 we can use the primitive function:

$$\int \frac{1}{\alpha_j(t)} dt = \frac{t}{N_j} + \frac{h_j^2}{N_j(t_j^{\text{exp}} - t)} \quad (6)$$

144 This completes the definition of the posterior probability in Equation 3. In order to sample from this
145 posterior distribution, we use a Reversible jump Markov Chain Monte-Carlo (Green 1995; Hastie and
146 Green 2012), since the dimensionality of the parameter space depends on the unknown parameter M .
147 The details of the updates used in this procedure are given in Supplementary Material. Unless otherwise
148 stated, during inference on all real and simulated datasets, we used the following hyperparameters:
149 $\theta = 1$, $\phi = 1$, $\mu_{\text{anc}} = 3$, $\sigma_{\text{anc}} = 3$, $\sigma_{\text{exp}} = 1$, $\nu = 1/2$, $\kappa = 1/2$, $\lambda = 5$.

150 **Simulation of testing data**

151 The process characterised above represents a standard Continuous Time Markov Chain (CTMC) and as
152 such can be simulated directly via Gillespie’s algorithm (Gillespie 1976). The waiting times are sampled
153 through inverse transform sampling with the inverse of the total process rate being approximated
154 numerically.

155 For the simulation of the genealogy in the first illustrative dataset presented, we used the following
156 hyperparameters: $\theta = 1$, $\phi = 2$, $\mu_{\text{anc}} = 4$, $\sigma_{\text{anc}} = 1/2$, $\sigma_{\text{exp}} = 1$, $\nu = 1/2$, $\kappa = 1/4$, $\lambda = 5$. For all other
157 simulated genealogies we used: $\theta = 1$, $\phi = 2$, $\mu_{\text{anc}} = 5$, $\sigma_{\text{anc}} = 1/2$, $\sigma_{\text{exp}} = 1/2$, $\nu = 1/3$, $\kappa = 1/4$,
158 $\lambda = 5$.

159 **Implementation**

160 We implemented the simulation and inference methods described in this paper into a new R package
161 entitled *CaveDive* which is available at <https://github.com/dhelekal/CaveDive>. The package uses
162 ape (Paradis and Schliep 2019) as a backend for handling phylogenies and ggtree (Yu et al. 2017) for
163 handling the visualisation of results. We also used the coda package (Plummer et al. 2006) to assess
164 the convergence and mixing properties of our MCMC algorithm, and found them to be satisfactory
165 with Gelman-Rubin statistics being less than 1.1 and the effective sample sizes in excess of 200 for all
166 parameters in the runs presented below. All runs were performed on a single core of Intel(R) Core(TM)
167 i7-3770 CPU with 8GB RAM.

168 RESULTS

169 Illustration of the clonal expansion model

170 In order to illustrate the concepts behind our clonal expansion model, we simulated from it the scenario
171 shown in Figure 1. In this example the population was made of $M = 4$ components: a background
172 subpopulation (pink) and three clonal expansions (blue, orange, green). Figure 1A shows the effective
173 population size of the four subpopulations as a function of time. The background subpopulation
174 remains of a constant size throughout, whereas each of the clonal expansions is characterised by a time
175 when the expansion started, a carrying capacity and a time to reach half of this carrying capacity.
176 The blue clonal expansion was the first one to have emerged, it has a large carrying capacity but this
177 potential is almost fully realised. The orange clonal expansion emerged next and very quickly reached
178 a relatively small carrying capacity. Finally, the green clonal expansion emerged and at the present
179 time it is still growing and far from having reached its capacity.

180 Figure 1B shows the corresponding dated phylogeny that was simulated in this example. Each point on
181 this dated phylogeny belongs to one of the subpopulations and is coloured accordingly as in Figure 1A.
182 A change of colour (highlighted by stars) therefore corresponds to the emergence of a clonal expansion.

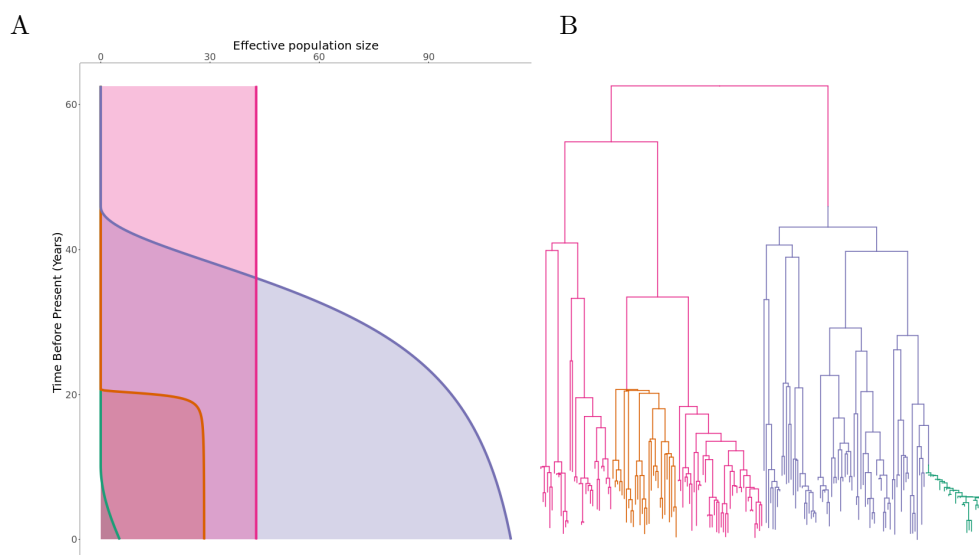


Figure 1: A realisation from the clonal expansion model. (A) Population size functions for each of the subpopulations. (B) Dated phylogeny coloured according to subpopulation as in part (A).

183 The blue and orange clonal expansions emerged out of the background subpopulation, whereas the
184 green expansion emerged out of the preexisting blue expansion, as can be seen from the transition
185 from blue to green.

186 For each of the four subpopulations, the population size function (Figure 1A) determines the branching
187 pattern in the corresponding part of the phylogeny (Figure 1B). For example, the background
188 subpopulation (pink) had a constant population size and the corresponding branches are therefore
189 consistent with expectation under the standard coalescent model. By contrast, the three clonal
190 expansions have been growing in size more or less suddenly resulting in star-like branchings soon after
191 their times of emergence. The orange and blue clonal expansions have almost reached their carrying
192 capacities so that recent branchings are similar to the expectation under a constant population size as
193 for the background subpopulation. The green clonal expansion on the other hand is still growing and
194 remains very small giving it a more linear structure.

195 **Application to a single simulated dataset**

196 We attempted to reconstruct the clonal expansion structure underlying the example shown in Figure 1.
197 In this inferential setting, the input data is therefore the dated phylogeny shown in Figure 1B, without
198 the colouring or location of stars that correspond to the emergence of clonal expansions. The aim is
199 to infer the correct number of clonal expansions (three in this case), their locations on the phylogeny
200 (stars in Figure 1B) as well as the demographic properties of each subpopulation (Figure 1A).

201 The priors used during the inference were the same as used for the simulation of this phylogeny.
202 The MCMC algorithm was run for 10^7 iterations with sampling every 1000 iterations, which took
203 approximately 3 hours. The results are shown in Figures 2 and S1. The correct number of three
204 clonal expansions was inferred with 67.5% of the posterior probability mass concentrated there, and
205 the majority of the remainder of the posterior probability mass shared between four and five clonal
206 expansions (Figure 2B). This suggests that although the phylogenetic data is informative about the
207 three correct expansions, it is not possible to rule out the existence of other expansions that would
208 have left little effect on the phylogeny, for example if they were very recent and if they would have
209 concerned only a small number of leaves. The correct position for the clonal expansions was inferred

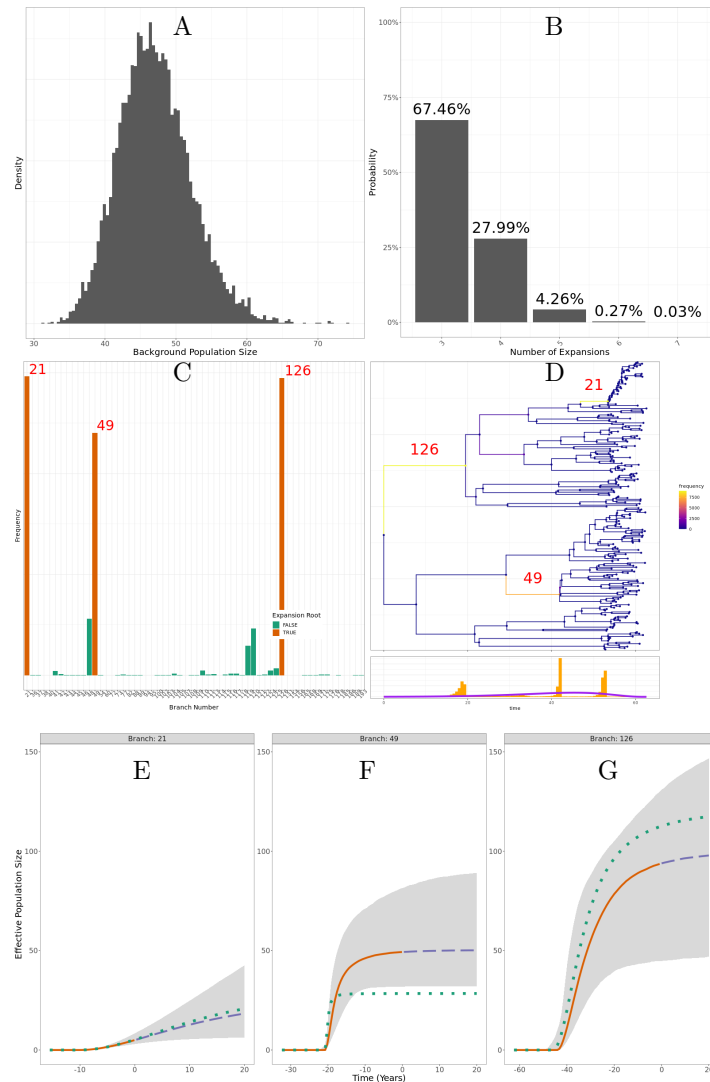


Figure 2: Application to the simulated dataset shown in Figure 1. (A) Posterior distribution of the background population size. (B) Posterior distribution of the number of clonal expansions. (C-D) Posterior probabilities of having a clonal expansions on different branches of the tree, with the indexes of three branches of interest shown. (E-G) Posterior reconstruction of the expansion population dynamics. 95% credible intervals in grey. Median in solid orange for past population dynamics and dashed blue for future prediction of the population dynamics. True population dynamics in dotted green.

210 with high probability, although it was not always possible to distinguish with certainty between the
211 correct branch or the ones directly above or below (Figure 2C-D). The demographic parameters of the
212 three clonal expansions (carrying capacity and time to reach half of it) were also correctly inferred,
213 resulting in posterior distributions for the effective population size of each expansion over time similar
214 to the ones used in the simulation (Figure 2E-G). The only exception concerned the carrying capacity
215 parameter of the orange expansion which was slightly overestimated (branch 49, cf Figure 2F), because
216 of the difficulty in correctly inferring such a sudden and self-limiting expansion.

217 **Application to multiple simulated datasets**

218 Firstly we performed inference based on 100 simulated dated phylogenies in which no clonal expansion
219 event occurred, so that the whole phylogeny is ruled by a single coalescent process with constant
220 population size. This allowed us to evaluate the false discovery rate of our methodology. For each
221 dataset in this test, the MCMC was run for 10^6 iterations with sampling every 100 iterations. We
222 found that in 98% of the replicates, the highest posterior probability was of having no clonal expansion,
223 corresponding to a 2% false positive rate. Such occasional false positive detection of clonal expansion
224 events is to be expected due to the fact that such events can leave little phylogenetic signature, and
225 therefore be difficult to rule out.

226 Secondly we performed inference based on 200 simulated dated phylogenies in which a single clonal
227 expansion event occurred, and the results are shown in Figure 3. In this benchmark, the MCMC was
228 run for 10^7 iterations with sampling every 1000 iterations. For nearly 74.5% of the simulated datasets
229 a single clonal expansion was found to be most likely (Figure 3A), as was indeed correct. In 15.5% of
230 the replicates no clonal expansion was found to be most likely, indicating a false negative case. This
231 result reflects the fact that some clonal expansion events are hard to infer if they left little phylogenetic
232 signature, for example if they occurred very recently, were sampled only a small number of times, or
233 occurred so long ago that almost all coalescent events occur before the period of rapid growth. Finally,
234 in 10% of the simulated datasets two clonal expansions were found to be most likely, representing a
235 relatively low rate of false positive detection, for the same reasons as in the previous simulations where
236 no clonal expansion had happened.

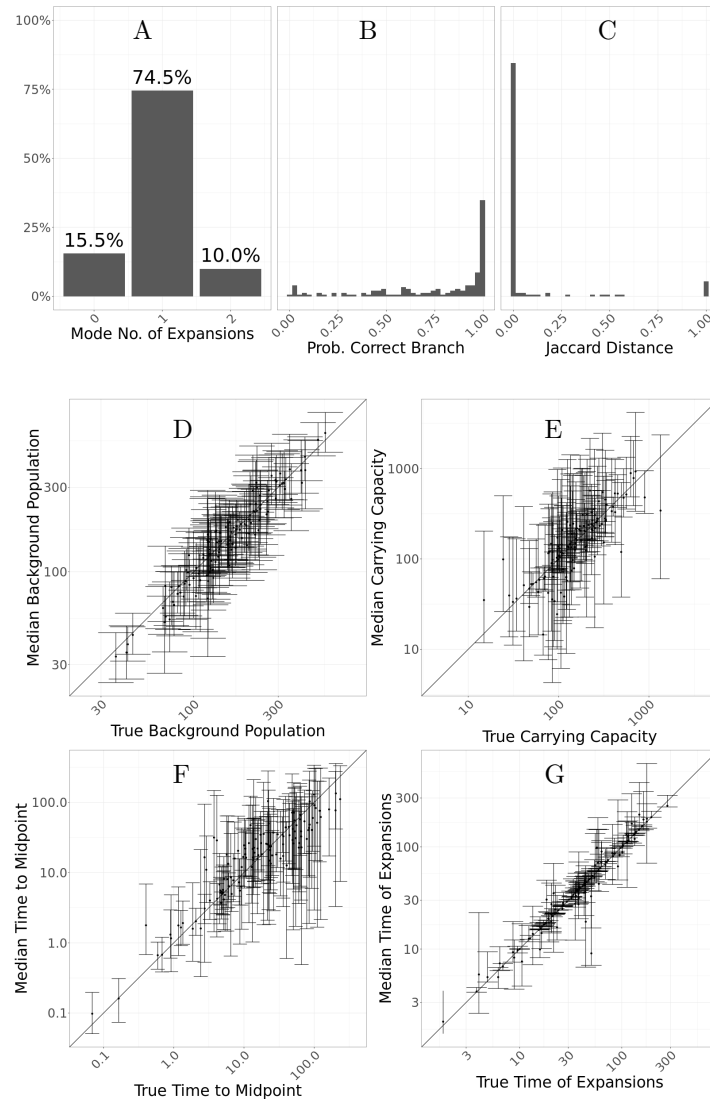


Figure 3: Application to 200 simulated trees containing one expansion. (A) Histogram of posterior modes for the number of expansions. (B) Histogram of probability to have a clonal expansion on the correct branch. (C) Histogram of Jaccard distances between the true expansion and the expansion corresponding to the mode branch. (D-G) Scatter plots showing posterior median and 95% credible interval for individual expansion parameters, with correct values on the x-axis and inferred values on the y-axis. Parts B-G only include simulations where the inferred mode of the number of expansions was one.

237 When a single clonal expansion was inferred, the probability of having this inferred event on the correct
238 branch was typically high (Figure 3B). However, when that was not the case, the clonal expansion was
239 almost always inferred on a very closely related branch, as can be seen when computing the Jaccard
240 distance between the correct and inferred expansion memberships (Figure 3C). The inferred effective
241 population size of the background population was highly consistent with the correct values (Figure
242 3D), and the same was true for the carrying capacity of the clonal expansion (Figure 3E). The time
243 taken to reach half of the carrying capacity was harder to infer, with little correlation between the
244 correct and inferred values (Figure 3F). The dating of the emergence of the clonal expansion was often
245 very precisely estimated (Figure 3G), although in some cases the credible interval on this parameter
246 was larger, which would be expected for example if the clonal expansion happened on a long branch.

247 Finally we performed inference based on 100 simulated dated phylogenies in which two or more clonal
248 expansion events occurred. We have simulated four sets of 25 phylogenies, with each set having
249 two, three, four, and five expansions respectively. The phylogenies were simulated using 60 tips plus
250 additional 40 per expansion. In this benchmark, the MCMC was run for 2×10^7 iterations with sampling
251 every 2000 iterations. The expected posterior (Figure 4A) marginals for the number of expansions show
252 a clear trend in probability mass being located on a greater number of putative clonal expansions as the
253 number of simulated expansions increases. We observe a slight tendency to underestimate the number

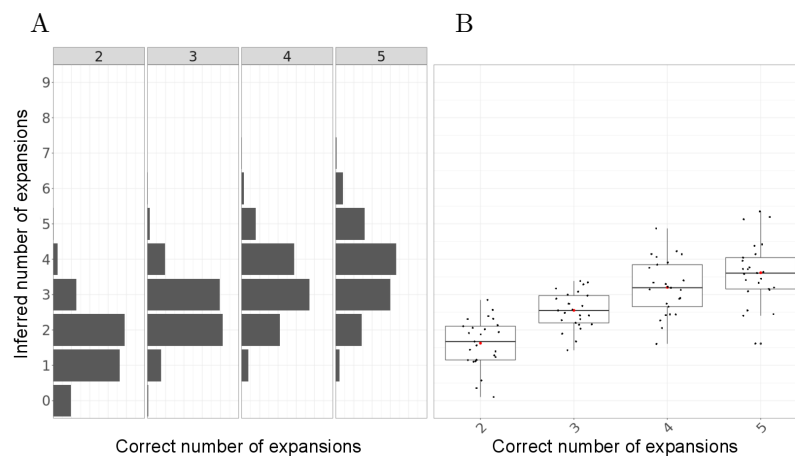


Figure 4: Application to 100 simulated datasets, with 25 per each scenario with 2, 3, 4 and 5 expansions. (A) Expected posterior distributions for the number of expansions for each scenario. (B) Box plots of the posterior mean number of expansions for each simulation by scenario.

254 of expansions relative to their true number. In terms of the posterior expectation of the number of
255 expansions (Figure 4B) we observe a clear increasing trend in terms of the medians, which initially
256 closely follow the true number of expansions in the case of two and three expansion phylogenies, and
257 underestimates the number of expansions for phylogenies with four and five expansions. This result
258 reflects our relatively conservative prior on the number of expansions, and the fact that they become
259 harder to detect as more and more occur on the same phylogeny, frequently with some expansions
260 originating from within another.

261 Application to *Streptococcus pneumoniae* dataset GPSC18

262 As the first real dataset to demonstrate our method, we used a global collection of genomes from the
263 Global Pneumococcal Sequence Cluster 18 (GPSC18) from a previously published study (Gladstone
264 et al. 2019). In this study, the authors described increased invasiveness in serotype 14 compared
265 to the background genotypes in the GPSC18 cluster. Indeed, serotype 14 is one of the leading
266 causes of invasive pneumococcal disease (Song et al. 2013), and its prevalence was reported to have
267 increased in recent years, despite its inclusion in pneumococcal conjugate vaccines (He et al. 2015).
268 This dataset consists of 228 genomes collected between 1991 and 2015, for which a dated phylogeny
269 has been previously published (Gladstone et al. 2020). Running our software for 10^8 iterations took
270 approximately 40 hours. The results are shown in Figures 5 and S2. The posterior inferred under
271 our model includes a single clonal expansion with very high certainty (Figure 5A), although other less
272 certain expansions can not be completely ruled out. The model therefore separates the genomes into
273 two categories, with about 80% of them belonging to the expansion and the remainder belonging to
274 the background population (Figure 5B). Notably, the expansion contains the vast majority of serotype
275 14 isolates, while containing only very few isolates corresponding to other serotypes (Figure 5C).
276 Conversely, the background population contained few isolates of serotype 14, with most of them being
277 of serotype 7C, 16F, 19A or 19F (Figure 5C). The inferred population size dynamics of clonal expansion
278 suggests that currently the expansion is of a slightly smaller size than the background population of
279 the GPSC18 cluster, but that it is still growing and might increase beyond the size of the background
280 population in the future (Figure 5D). This result is consistent with the fact that more genomes belonged
281 to the clonal expansion than to the background population: since serotype 14 is more associated with
282 disease, it would tend to be overrepresented in isolate collections (Didelot and Maiden 2010).

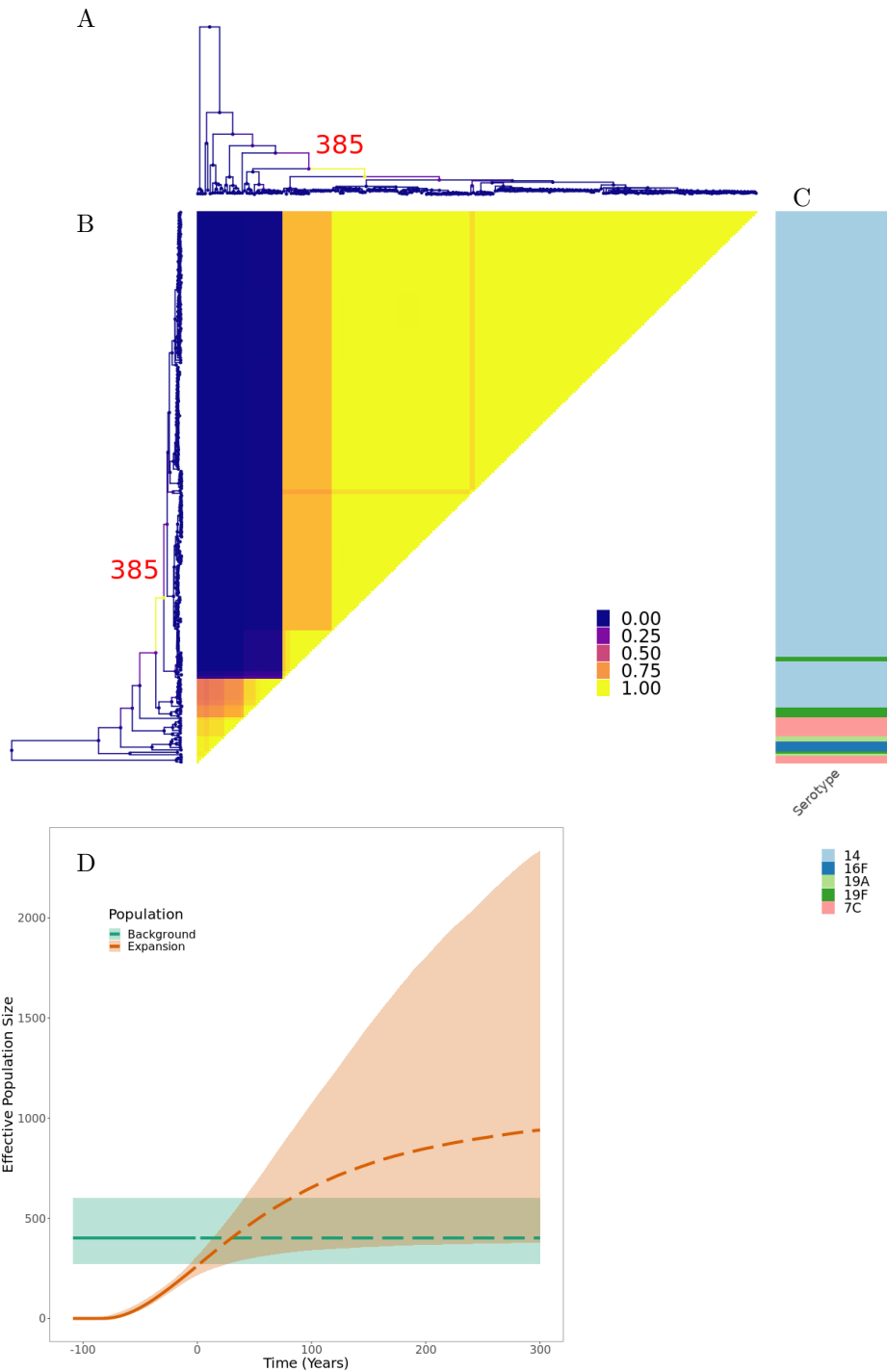


Figure 5: Application to GPSC18 *Streptococcus pneumoniae* phylogeny. (A) Dated phylogeny with branches colored according to the inferred probability of clonal expansion. (B) Pairwise matrix showing the posterior probabilities of any two samples belonging to the same subpopulation. (C) Color map showing serotype values. (D) Posterior summary of the inferred effective population size functions. The colored regions represent 95% credible interval and the lines represent median. Solid denotes past effective population size inference and dashed represents prediction of future effective population size.

283 **Application to methicillin-resistant *Staphylococcus aureus* dataset**

284 We reanalysed a previously published dataset of genomes of methicillin-resistant *Staphylococcus aureus*
285 (MRSA) from the USA300 lineage (Uhlemann et al. 2014). This lineage was first reported in the early
286 2000s but quickly spread throughout the United States to become a leading cause of community-
287 acquired skin infections (Challagundla et al. 2018). The dataset consists of 347 genomes isolated
288 between 2006 and 2011, for which we constructed a dated phylogeny using BactDating (Didelot et al.
289 2018) under the additive relaxed clock model (Didelot et al. 2021). The run time for our clonal
290 expansion analysis software was just under 54 hours for 10^8 iterations. The results are shown in
291 Figures 6 and S3. The posterior mean for the number of clonal expansions was 3.04, with 28%, 42%

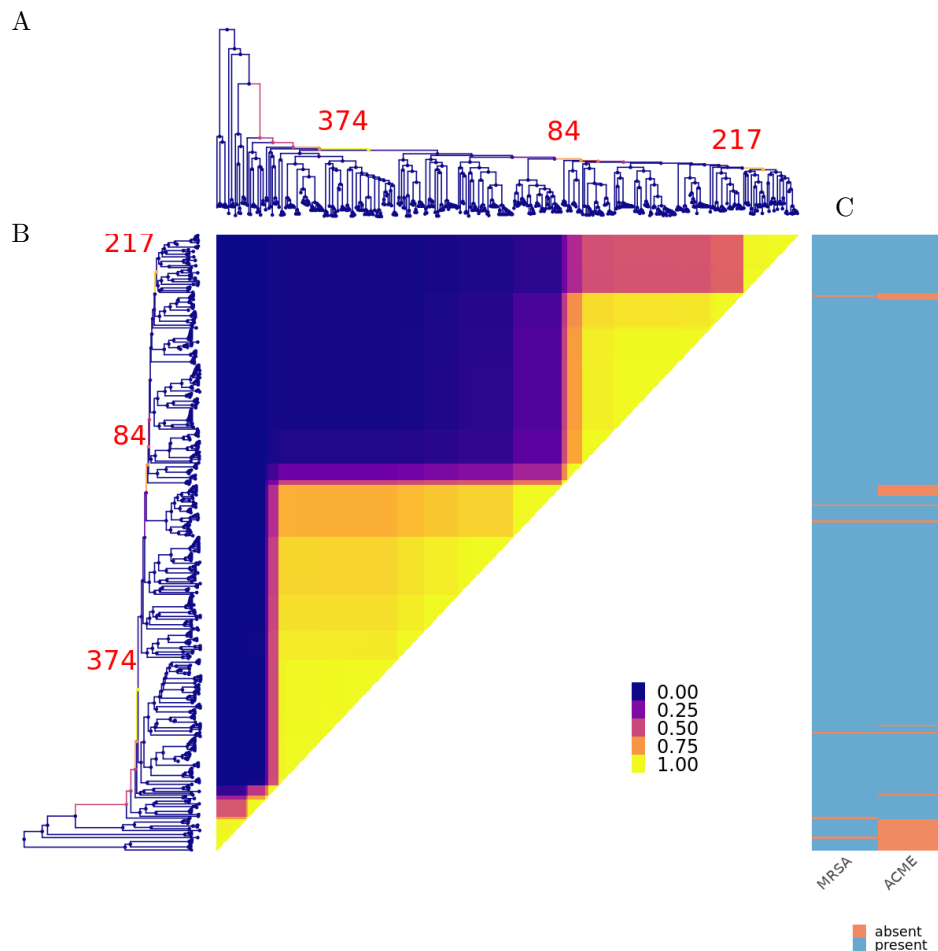


Figure 6: Application to Methicillin Resistant *Staphylococcus aureus* dataset. (A) Dated phylogeny with branches colored according to the inferred probability of clonal expansion. (B) Pairwise matrix showing the posterior probabilities of any two genomes belonging to the same subpopulation. (C) Color map showing the presence of phenotypes associated with virulence.

292 and 27% posterior probability assigned to having 2, 3 and 4 clonal expansions, respectively. The
293 most probable posterior population structure therefore consists of three expansions which are nested
294 into one another. The first expansion occurs at branch 374, which then gives rise to an expansion
295 associated with branch 84 and which finally gives rise to expansion starting from branch 217 (Figure
296 6). The first expansion on branch 374 is the most certain one, and also the most significant one since
297 it splits from the background population which is of a constant population size. This result therefore
298 suggests that it is not the whole of the USA300 MRSA lineage that expanded, but rather a large
299 subset of it which is associated almost perfectly with the presence of the arginine catabolic mobile
300 element (ACME) (Figure 6). ACME provides polyamine resistance as well as other functions (Joshi
301 et al. 2011). An association between ACME and the expansion within USA300 has been suggested
302 before (Uhlemann et al. 2014; Challagundla et al. 2018) but here for the first time we have detected it
303 using a well-suited model of clonal expansion. A previous phylodynamic analysis showed the temporal
304 association between the USA300 growth rate and the consumption of β -lactams assumed that the
305 whole population followed the same dynamic function (Volz and Didelot 2018). We show here that
306 this is not correct but this previous analysis remains approximately valid since the vast majority of
307 genomes are part of the ACME-associated clonal expansion. The other two putative expansions that
308 are nested within the first one do not seem associated with a clear genetic change that would provide a
309 selective advantages, but are more likely to correspond to founder effects occurring as USA300 spread
310 in different parts of the human population (Challagundla et al. 2018).

311 **Application to *Streptococcus pneumoniae* dataset GPSC9**

312 We also analysed a previously described global collection of genomes from the Global Pneumococcal
313 Sequence Cluster 9 (GPSC9) (Gladstone et al. 2020). This dataset consists of 277 genomes collected
314 between 1995 and 2016 for which a dated phylogeny has been previously published (Gladstone et al.
315 2020). The MCMC was run for 10^8 iterations and terminated within 51 hours. The results are shown
316 in Figures 7 and S4. The posterior mean for the number of expansions was approximately 3, with
317 56% of the posterior probability mass on this number. Approximately 25% of the probability mass
318 rests on a two expansion scenario, and the remainder is distributed between cases with four or more
319 expansions. The most certain clonal expansion occurred on branch 389 and corresponds to isolates
320 from all over the world, but are unique within GPSC9 in containing the ermB1 erythromycin resistance

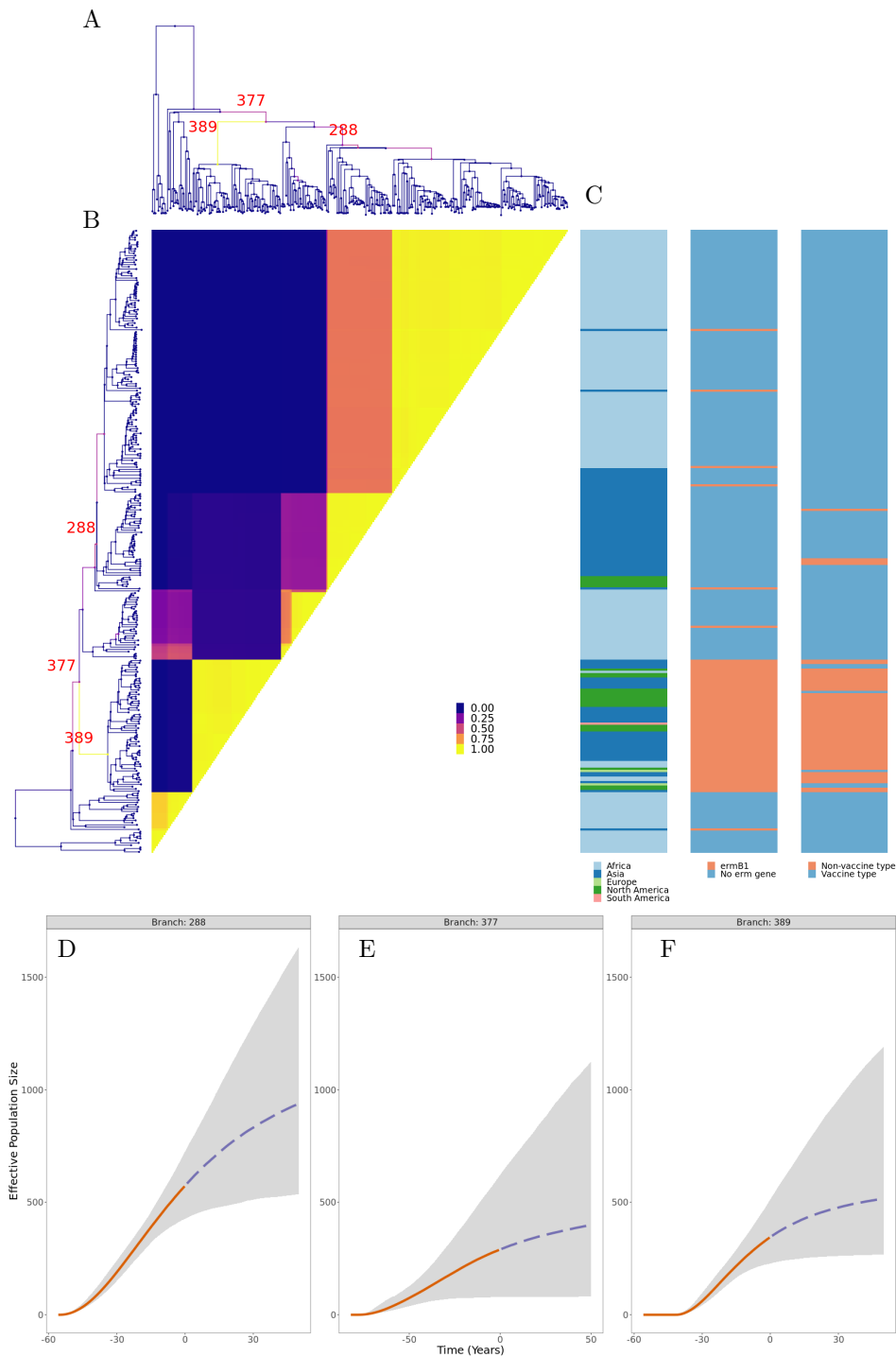


Figure 7: Application to GPSC9 *Streptococcus pneumoniae* phylogeny. (A) Dated phylogeny with branches colored according to the probability of clonal expansion. (B) Pairwise matrix showing the posterior probabilities of any two samples belonging to the same subpopulation. (C) Color map showing geographical sampling location, erm gene presence, and whether the serotype is covered by the vaccine. (D-F) Posterior summary of the inferred effective population size functions.

321 gene and being of a serotype not covered by the pneumococcal conjugate vaccines (Figure 7). This
322 clade therefore represents a clear example of vaccine escape by replacement of the capsular locus
323 (Mostowy et al. 2017), followed by worldwide spread. Other identified groups of genomes correspond
324 to locally successful clades as previously described (Gladstone et al. 2020). For example the expansion
325 on branch 288 corresponds to a clade that has successfully established itself throughout the African
326 continent as well as India, with around 50% posterior support to separate the Indian component
327 within this expansion. The background population corresponds to the first South African clade
328 previously identified (Gladstone et al. 2020). These results showcase once again how differences in
329 the phylodynamic trajectories of sublineages are not always caused by a selective advantage of the
330 pathogen, but often linked with the structure of the host population.

331 DISCUSSION

332 Detecting emerging microbial populations is a persistent and critical public health challenge.
333 However, robust solutions to this problem have been little explored. In this work, we describe a
334 novel, computationally tractable Bayesian approach to finding expanding populations within dated
335 phylogenies. Using simulated phylogenies, we estimated the false positive rate of the approach,
336 which was about 2% in the simulations performed. We also estimated the sensitivity of detection
337 of clonal expansions, which was of the order or 75%, with limited sensitivity attributable to the
338 limited phylogenetic signature left by expansions occurring in antiquity, very recently, or with limited
339 sampling. Importantly, in an analysis of real data from three separate microbial populations causing
340 high burdens of human disease, we identified clonal expansions associated with known virulent factors,
341 drug resistance loci, and absence from vaccine coverage, all biologically credible determinants of
342 clonal expansion. Thus, the application of the approach on both simulated and real world microbial
343 populations indicate the approach described may have wide application. To allow widespread use of
344 our new methodology, we provide an implementation in the form of a R package.

345 Our methodology has a number of limitations, inherent in the assumptions we have made in our
346 model. Firstly, we assume that the background population, before any clonal expansion occurred, has
347 a constant population size. This assumption would be invalidated for example if the whole population

348 under analysis has been expanding. However, in this case a clonal expansion event would be inferred
349 close to the root. Furthermore, the choice of a constant background population size is convenient from
350 a statistical point of view since it allows scaling of many parameters against the size of the background
351 population (see Table 1). Another choice we made concerns the form of the demographic function after
352 a clonal expansion occurs (Equation 1). Once again this is a choice of convenience, since this function
353 starts at zero when the expansion starts, plateaus at a well-defined carrying capacity value and its
354 reciprocal has an analytical primitive as needed (Equation 6). Our function approximates well the
355 logistic growth behaviour we seek to model and which arises for example in a susceptible-infectious-
356 susceptible SIS model (Allen 2008). Future work could seek to investigate other choices of functions,
357 but choosing another function with similar properties would probably not make much difference to
358 inference results. Our model also assumes that clonal expansions are the only type of phylodynamic
359 events to occur, disallowing for example the possibility for any population size reduction. This is partly
360 because the effect of reduction on phylogenies is less dramatic than sudden growth, so that such events
361 would be harder to detect, but also and mostly because our aim was to provide a method for clonal
362 expansion analysis rather. Further work should seek to expand on our method and develop a more
363 complete framework for the analysis of differential phylodynamic trajectories between lineages.

364 There are few previous methods to which our approach can be compared, as this is a first-in-class
365 principled approach to the key problem of detecting clonal expansions, whereas the vast majority
366 of existing phylodynamic methods assumes that all lineages follow the same demographic function
367 (Ho and Shapiro 2011). A recent study proposed a non-parametric test of this assumption which
368 can be used to split a phylogeny into separate components but which does not allow further analysis
369 of the phylodynamic properties of each component (Volz et al. 2020). Perhaps the closest existing
370 method is the recently proposed multi-type birth-death (MTBD) model (Barido-Sottani et al. 2020)
371 which is based on the birth-death model (Stadler 2010). In both cases the aim is to model the effect
372 of population heterogeneities in dated phylogenies. However, the model we present is based on a
373 coalescent process as opposed to a birth-death type process, and as such makes fewer assumptions
374 about sampling (Volz and Frost 2014). Furthermore the scenario being modelled is quite different, and
375 is underpinned by a completely different set of assumptions. Since our focus is specifically on clonal
376 expansions, an equivalent to birth-death changes only occurs when all members of a given clonal
377 expansion have coalesced, which is not the case with the MTBD model (Barido-Sottani et al. 2020).

378 Some comparison may also be drawn with genetic clustering based on fitting a Markov-modulated
379 Poisson process (MMPP) (McCloskey and Poon 2017), although this method focuses on detecting
380 small scale outbreaks, whereas we are interested in a phylodynamic behaviour on a significantly larger
381 scale. Furthermore, the assumptions are completely different: our model is phylodynamic and does
382 not represent an approximation of a transmission tree. Finally, our method is related with approaches
383 to detecting structure which are not based only on the phylogeny, but exploit integration with other
384 type of data (Baele et al. 2016), for example using the distribution of a phenotype (Ansari and Didelot
385 2016) or the geographical origin of the samples (Bloomquist et al. 2010).

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