Evaluating whole HIV-1 genome sequence for estimation of incidence and migration in a rural South African community

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Abstract

Background: South Africa has the largest number of people living with HIV (PLWHIV) in the world, with HIV prevalence and transmission patterns varying greatly between provinces. Transmission between regions is still poorly understood, but phylodynamics of HIV-1 evolution can reveal how many infections are attributable to contacts outside a given community. We analysed whole genome HIV-1 genetic sequences to estimate incidence and the proportion of transmissions between communities in Hlabisa, a rural South African community.

Methods: We separately analysed HIV-1 for gag, pol, and env genes sampled from 2,503 PLWHIV. We estimated time-scaled phylogenies by maximum likelihood under a molecular clock model. Phylodynamic models were fitted to time-scaled trees to estimate transmission rates, effective number of infections, incidence through time, and the proportion of infections imported to Hlabisa. We also partitioned time-scaled phylogenies with significantly different distributions of coalescent times.

Results: Phylodynamic analyses showed similar trends in epidemic growth rates between 1980 and 1990. Model-based estimates of incidence and effective number of infections were consistent across genes. Parameter estimates with gag were generally smaller than those estimated with pol and env. When estimating the proportions of new infections in Hlabisa from immigration or transmission from external sources, our posterior median estimates were 85% (95% credible interval (CI) = 78%–92%) for gag, 62% (CI = 40%–78%) for pol, and 77% (CI = 58%–90%) for env in 2015. Analysis of phylogenetic partitions by gene showed that most close global reference sequences
clustered within a single partition. This suggests local evolving epidemics or potential unmeasured heterogeneity in the population. **Conclusions:** We estimated consistent epidemic dynamic trends for *gag*, *pol* and *env* genes using phylodynamic models. There was a high probability that new infections were not attributable to endogenous transmission within Hlabisa, suggesting high inter-connectedness between communities in rural South Africa.

**Keywords**
HIV, phylodynamics

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Introduction

South Africa has the largest number of HIV-positive people in the world, with 7.5 million (95% CI = 6.9 million to 8.0 million) people living with HIV (PLWHIV) in 2019 (Satok & Boyer, 2019; UNAIDS, 2019). HIV prevalence varies between provinces with the lowest (8.3%) and highest (18.1%) prevalence observed in Northern Cape and KwaZulu-Natal provinces, respectively (Simbayi et al., 2019). HIV prevalence increased after 2012 corresponding to a period of increased uptake of antiretroviral therapy (ART) and reduced AIDS-related mortality (Simbayi et al., 2019).

The HIV epidemic in South Africa is dominated by HIV-1 subtype C (Hemelaar et al., 2011), which likely originated in Africa around 1960 (95% confidence interval (CI) = 1956 – 1964) (Wilkinson et al., 2015). Subtype C shows high genetic diversity in South Africa, as a result of multiple introductions from Zambia, Botswana, Malawi and Zimbabwe that occurred between 1985 and 2000, during a period of socio-political transformations in the country (Wilkinson et al., 2016). More recently, phylogenetic analyses of HIV-1 genetic sequences from South Africa and other African countries (Angola, Botswana, Democratic Republic of the Congo, Malawi, Mozambique, Swaziland, Tanzania, Zambia, and Zimbabwe) showed that as of 2014, 35% (95% CI = 20% – 60%) of new infections were attributable to external introductions into South Africa (Rasmussen et al., 2018). However, both studies (Rasmussen et al., 2018; Wilkinson et al., 2016) included only sequences from within Africa to analyse external introductions into South Africa.

In this study, we evaluated HIV-1 subtype C genetic sequences from individuals from Hlabisa, a rural South African community in KwaZulu-Natal province. We evaluated the potential of whole genome HIV-1 sequence data and phylogenetic analysis methods (Volz et al., 2013) to inform estimates of recent epidemic trends in Hlabisa.

The objectives and design of this analysis were aligned with the previous analysis by Rasmussen et al. (2018), which was based on data from a neighbouring community in KwaZulu-Natal. Specifically, our aim was to evaluate the potential of randomly sampled sequence data to estimate (1) the probability that a new infection arises from endogenous transmission within South Africa versus importation from outside of South Africa, and (2) trends in recent HIV incidence.

Methods

Ethics approval

Ethical approval for the cohorts we used in this study were obtained from the institutional review board (IRB) of the University of Cape Town, Research Ethics Committee (IRB doc number 213/400), University of Witwatersrand, HREC (IRB doc number 40206 and 060809), and University of KwaZulu-Natal Biomedical Research Ethics Committee (UKZN BREC) (numbers BFC104/11, BF052-010, and BF110/09). All participants provided written informed consent.

Sources of data

The data used in this study comprised HIV-1 genomic sequence data and metadata from 2,503 PLWHIV sampled in the Hlabisa sub-district of KwaZulu-Natal, South Africa. Samples came from five distinct cohorts, with most samples originating from the South African treatment as prevention trial (ZA-TasP) (Derache et al., 2019; Iwuji et al., 2018; Rossouw et al., 2017). Genetic sequencing data were generated by the PANGEA (Phylogenetics And Networks for Generalized Epidemics in Africa) consortium (Abelé-Dörner et al., 2019; Pillay et al., 2015). Metadata included the sample dates, age and gender of participants, as well as CD4 cell counts. CD4 counts were utilised to determine the stage of HIV infection for each participant; however, most CD4 data were not collected concurrently with samples used for HIV sequencing which reduced the utility of these data. From the 2,503 PLWHIV that we analysed, 48% had a CD4 cell count carried out within six months of sequence data, while 12% had a CD4 count carried out more than six months after genetic sequencing. For the remaining 40%, no CD4 count was available.

It is common for phylogenetic papers to report results based solely on the pol gene as pol sequences are abundant in sequence databases (Rasmussen et al., 2018; Wilkinson et al., 2019). We split genomic HIV-1 sequence data into gag, pol, and env genes using a subtype C reference sequence (GenBank accession number: KU319528) and the BLAST command line application tools version 2.5.0+. We chose to analyse the genomic data separated into genes 1) to reduce bias from recombination; 2) to have an appropriately calibrated substitution model and molecular clock model for each gene; and 3) to check whether phylogenetic analyses carried out using different genes yielded comparable results.

We used COMET (Struck et al., 2014) to determine the HIV-1 subtype of each genetic sequence. COMET classified less than 1% of sequences as non-subtype C and those were excluded from our analyses. Sequences with fewer than 800 nucleotides (nt) were also excluded from the analyses.

Because the global reservoir of HIV infections dwarfs the size of the sampled South African data, an imported lineage will have ancestral relationships that are much more strongly influenced by global HIV dynamics as opposed to local epidemiological dynamics. We accounted for this by identifying HIV sequences closely related to those within our collected South Africa data and including these sequences in our data set. We refer to these sequences as close global references (CGRs).

We were unable to select sequences from within South Africa outside of Hlabisa, as this information was not available in the sequence database. To select CGRs, we generated a global BLAST (McGinnis & Madden, 2004) database consisting of all available HIV-1 sequences where location of sampling was known and did not correspond to South Africa. This database consisted of all matching sequences in GenBank combined with the PANGEA-generated sequences.
not yet available in GenBank. Countries included in PANGEA are South Africa, Botswana, Zambia, Uganda, Kenya and Tanzania. Our CGR database included 786,571 sequences, around 9,000 of which were from other PANGEA sites. For each South African sequence, we used the BLAST tool and custom Python scripts (see Extended data [Nascimento, 2022]) to select the top three closest matches within the global database. Hypervariable regions of envelope (env) gene sequences were manually removed prior to using BLAST to prevent spurious matches for the env gene. We removed CGRs which were labelled as non-functional provirus, non-functional or truncated genes, and hypermutated sequences, and we verified the subtype classification for each of these CGR sequences using COMET. Any CGR which was not classified as subtype C was removed. Note that multiple South African sequences can share a closest match within the global set, and overlapping BLAST matches were deduplicated. After deduplication and curation, 30%, 17%, and 35% of sequences in the alignment were CGRs for the gag, pol, and env genes, respectively.

**Sequence alignment, drug resistance sites and recombination.**
We used Gene Cutter to generate, for each gene, a combined genetic sequence codon alignment consisting of the sampled South African sequences and the top three deduplicated BLAST matches from the global set. In all alignments, we also included two sequences (accessions AY371157 and K03454) from subtype D to be used as an outgroup in phylogenetic analyses (see section on molecular clock analysis). We further removed an average of 36% of columns of the alignment in which the majority of sequences was missing a nucleotide (Yang, 2014). The majority of these 36% of columns comprised nucleotide insertions observed in a single sequence and composed by unknown nucleotide (Ns).

Because drug resistance nucleotide sites are under strong selective pressure and may influence phylogenetic reconstruction, we next identified and masked drug resistance sites in the pol gene using the big.phylo R package version 1.0 (Ratmann, 2018) and HXB2 (accession K03455) reference sequence. The big.phylo R package uses the drug resistance mutation sites from the International Antiviral Society-USA (Wensing et al., 2015).

We examined the gag, pol and env alignments for evidence of within-sample recombination using RDP4 (Martin et al., 2015). We used the default setting and the option “auto mask for optimal recombination detection” to detect recombination within each alignment. Three, four and nine sequences were identified as potential recombinants in gag, pol and env alignments, respectively, and these were excluded from subsequent analysis.

After data preparation, maximum likelihood trees were estimated using alignments consisting of 2,455 sequences for the gag (of which 730 sequences were CGRs); 2,821 for the pol gene (of which 482 sequences were CGRs); and 2,422 for the env gene (of which 840 sequences were CGRs) including South African, CGRs and outgroup sequences.

**Phylogenetic analysis**
We estimated phylogenetic trees by maximum likelihood (ML) using RAxML-NG version 0.8.1 (Kozlov et al., 2019), the HKY + Γ (Hasegawa-Kishino-Yano (Hasegawa et al., 1985) plus gamma distribution (Yang, 1996)) DNA substitution model, and using two partitions for the data (1+2 and 3rd codon positions). This model choice was motivated by previous findings that partitioning by codon position is often superior for protein-coding sequences (Shapiro et al., 2006).

To estimate the ML tree, we replicated phylogeny inference 50 times using random starting conditions. From these 50 fits, 25 used parsimony-based randomized stepwise addition trees as starting trees, and 25 used random trees as starting trees to search for the best ML tree. Similarly, to calculate branch support for each tree, we ran 100 independent bootstrapped trees per alignment in parallel which were merged into a single file to calculate the bootstrap support for each branch of the best ML tree.

**Molecular clock analysis.** We estimated time-scaled phylogenetic trees using the ML trees using the uncorrelated lognormal relaxed clock model from the treedater R package version 1.0 (Volz & Frost, 2017) and dates of sampling where known, taking into account the alignment lengths of each gene. For CGRs, a range of dates was provided based on year of sampling where available. A total of 12, six and 44 samples for gag, pol and env, respectively, were missing date of collection, and for these we provided treedater with a very broad range of sample time limits (1980 – 2015). Molecular clock outliers were identified and excluded using the method from Benjamini and Hochberg at a 5% level (Benjamini & Hochberg, 1995), and treedater was re-run without outliers. Root position was based on a non-subtype C outgroup (accessions AY371157 and K03454). We also removed redundant CGR sequences from the trees. Redundant CGRs were sequences whose neighbour in the phylogenetic tree was also a CGR. Thus, the closest neighbour for a CGR was a sequence from South Africa.

After removing outliers and redundant CGRs, our final alignments used in phylodynamic analyses consisted of 1,962 sequences for the gag (of which 244 sequences were CGRs); 2,526 for the pol gene (of which 205 sequences were CGRs); and 1,766 for the env gene (of which 192 sequences were CGRs) including South African, CGRs and outgroup sequences.

We observed the highest number of sequences for the pol gene compared to gag and env genes. Because of that we down sampled the number of sequences in the pol alignment to those IDs observed in the env alignment. Our final alignment used in the phylodynamic analyses with phydynR (see section on phylodynamic analysis) for the down sampled pol gene consisted of 1,660 sequences (of which 194 were CGRs). Estimates of new infections and effective number of infections per year showed larger credible interval using the down sampled version of the pol alignment (Figure 1). Further reports were based on the complete data for the pol alignment.
Phylogenetic partitions. We used the treestructure method to partition time-scaled trees into clades with significantly different distributions of coalescent times (Volz et al., 2020). Phylogenetic partitions are correlated with metadata and provide insights into epidemiological structure, such as the presence or absence of association with geographic variables (Volz et al., 2020). We partitioned the South African data into smaller sets of two-three partitions with $n \in (400,1500)$ for each gene. For gag and pol, treestructure identified three partitions, while for env, treestructure identified two partitions.

Epidemiological models
We developed a mathematical model for epidemiological dynamics in Hlabisa, which was applied within a phylodynamic analysis framework to compute the likelihood of our genetic and clinical data. Our model was defined in terms of the number $I(t)$ of infected and infectious HIV individuals, and the number $Z(t)$ of infected individuals on ART as a function of time $t$. These variables described epidemic dynamics within the geographic region of focus, where sequence data were sampled. Additionally, we accounted for migration of HIV lineages between South Africa and the much larger global reservoir of HIV infections, denoted $X(t)$. We used a simple two-parameter exponential function to describe the size of the global reservoir, which grows at rate $\rho$.

The rate of new infections was controlled by the function $\beta(t)$ which specified the per-capita rate of transmissions from untreated individuals. The function $\beta(t)$ was specified by a linear interpolating spline with slope changes in 1980, 1990, 2000, 2005, 2010, and 2015. This model avoids making strong assumptions about how incidence scales with prevalence and does not require modelling the number of susceptible individuals through time. The function $\alpha(t)$ specified the per-capita rate of initiating antiretroviral therapy. The model did not account for treatment failure or loss to follow up. The function $\alpha(t)$ was given by a simple one-parameter linear function which increased from zero starting in 2005. The parameters $\mu$ and $\gamma$ denote per-capita rates of natural mortality and rates of disease-related mortality, respectively.

The dynamics of the number of infected individuals in each compartment is specified by the following system of ordinary differential equations where the time derivative of a variable $x$ with respect to time is $\dot{x}$:

$$
\dot{I}(t) = (\beta(t) - \mu - \gamma - \alpha(t))I(t) \\
\dot{Z}(t) = \alpha(t)I(t) - \mu Z(t) \\
\dot{X}(t) = \rho X(t)
$$

(1)

Phylo Dynamic analyses
We carried out fixed-tree phylodynamic analyses using dated phylogenies estimated by ML and treedater. This analysis was carried out with the BayesianTools R package version 0.1.6 (Hartig et al., 2018) which implements Bayesian Markov chain Monte Carlo (MCMC) methods and the phdynR R package version 0.2.0 (Volz, 2017) which implements the structured coalescent likelihood.

The structured coalescent model made use of the PL1 approximation (Volz & Siveroni, 2018) to the likelihood, which is slower to compute but more accurate. We estimated the parameters of our mathematical model (represented in Equations 1) using the differential-evolution MCMC zs sampler (MCMC-DEzs) (ter Braak & Vrugt, 2008) as implemented in the BayesianTools R package. We initially ran few MCMC-DEzs for 18,000 iterations, and we chose one run per analyses to provide initial conditions for subsequent longer runs. We then run final MCMC-DEzs ranging from 24,000 to 29,000 iterations.
using different initial conditions depending on the analyses (for gag, pol and env). These MCMC-DEzs were run in parallel using the computing resources of Imperial College London. We merged five independent runs in order to have two sets of runs to compare posterior distributions for each parameter and assess convergence of the chains. We also used the Gelman diagnostics from BayesianTools to check for convergence. For a list of the parameters we estimated and their corresponding priors see Table 1.

Non-parametric coalescent analyses
We carried out a non-parametric coalescent analysis in order to estimate effective population size through time. This was carried out on fixed time-scaled phylogenies reconstructed from gag, pol, and env genes separately to establish congruence of phylodynamic results based on different genes. Estimates were based on partitioned time-scaled trees per gene estimated using treeduct and treetreestructure as previously described. Prior to analysis, we pruned all CGR sequences from phylogenetic trees. These analyses were carried out with the skygrowth R package version 0.2.0 (Volz & Didelot, 2018) using MCMC and default parameter settings.

Results
Based on our epidemiological model, we were able to estimate the effective number of infections and the proportion of imports from outside Hlabisa as described below.

Epidemic dynamics
Coalescent analyses demonstrated rapid expansion of the epidemic during the 1980s and a smooth monotonic decrease of transmission rates into the 1990s (Figure 2 and Figure 3).

Table 1. List of priors used in the Bayesian MCMC analyses.

| Definition and symbol                                      | Prior               |
|------------------------------------------------------------|---------------------|
| Growth rate of global reservoir ($\rho$)                    | Lognormal(-3.352, 1)|
| Importation rate as imports per infected per unit time     | Lognormal(-2.9957, 1/4)|
| Initial number of global reservoir lineages ($X_0$)        | Lognormal(9.9035, 0.25)|
| Initial numbers of infected and infectious HIV individuals ($I_0$) | Exponential(1/10)|
| Changes in the transmission rate in intervals of 10 or 5 years (1980, 1990, 2000, 2005, 2010 and 2015) ($\beta$) | Lognormal(-1.792, 1)|
| Disease-related mortality rate ($\gamma$)                   | Fixed at 1/10.2$^a$ |
| Natural mortality rate ($\mu$)                              | Fixed at 1/(62.7-30)$^b$ |

$^a$ Based on Mangal (2017) who estimated the median survival time in Africa as 10.2 years when considering individuals 30 years old at seroconversion.

$^b$ We used a life expectancy of 62.7 years for females in 2016 (Life expectancy and Health Life expectancy Data by WHO region, WHO).

From 1980, estimates using the gag gene were lower than estimates using pol and env genes. The latter two were in agreement. From 1990, estimates using gag, pol and env genes were in agreement with overlapping credible intervals (Figure 3). We also observed that estimates using the pol gene were higher than those estimated using the gag or env genes after 1990 (Figure 3).

Non-parametric coalescent analysis to estimate the effective population size by gene and for each tree partition similarly showed agreement with the exception of gag partition 3 and env partition 1. These showed higher estimates and credible intervals than the other gene/partitions (Figure 4). We also observed a sharp decrease in effective population size after 2010. In general, analysis of the pol gene across the different partitions showed a smaller credible interval than the gag and env genes (Figure 4). This could be a consequence of the larger sample size for pol than for gag and env.

Migration and the role of the global HIV reservoir
Finally, we estimated the probability that a new infection was not attributable to endogenous transmission in Hlabisa. Posterior median estimates for the proportion of transmissions from outside of Hlabisa were 85% (95% CI = 78% – 92%) for gag, 62% (CI = 40% – 78%) for pol, and 77% (CI = 58% – 90%) for env gene in 2015.

We observed evidence that the process of migration and lineage importation was not evenly distributed through the population. The vast majority of lineage importation events were associated with a single partition within the HIV phylogeny. Figure 5 shows a down sampled time-scaled phylogeny for the gag gene and the distribution of CGRs in different partitions.
Figure 2. Transmission rates in the South Africa data set for gag, pol and env genes using phydynR. Boxplot showing the distribution of transmission rates per year.

Figure 3. Epidemic dynamics in the South Africa data set for gag, pol and env genes using phydynR. Left: New infections per year. Right: Effective number of infections per year. Posterior medians and 95% credible intervals are shown with solid line and shaded area, respectively.

detected using treestructure (see Methods). In the gag phylogeny, 230 of 244 CGRs clustered within a single partition. In the pol phylogeny, 199 of 205 CGRs clustered within a single partition. Finally, in the env phylogeny, 174 of 192 CGRs clustered within a single partition.

Discussion
Our data set enabled precise estimates of early epidemic growth rates, and model-based estimates were, in general, in agreement with non-parametric estimates for early growth rates (Figure 3 and Figure 4).
The Hlabisa HIV treatment and care programme initially had 1,800 PLWHIV enrolled and receiving ART in 2006, and was rapidly scaled up so that by the end of 2008, 7,576 PLWHIV were enrolled and receiving ART (Houlihan et al., 2011). Our models also showed a steady decline in transmission rates after 2005, coinciding with ART in Hlabisa (Figure 2).

Vandormael et al. (2019), using different epidemiological methods, reported an overall decline of 43% in incidence rate between 2012 and 2017 using data from Hlabisa. They observed that incidence declined after 2012 and 2014 for males and females, respectively, when approximately 35% of opposite-sex individuals were on ART. We simulated up to 2015 and we observed approximately 35% of individuals on ART in 2014. Between 2014 and 2015, we found a reduction in incidence of 52% for gag [from 20.54 (CI = 9.44 – 38.81) to 9.85 (CI = 4.29 – 18.81)], 56% for pol [from 83.13 (CI = 31.63 – 400.11) to 36.13 (CI = 13.09 to 169.79)] and 54% for env [from 30.75 (CI = 9.83 – 130.13) to 13.99 (CI = 3.74 – 60.99)].

Johnson et al. (2017), using a mathematical model for the South African HIV epidemic, estimated that incidence for KwaZulu-Natal peaked in 1997–1998. Our model-based analyses showed that for median values, the incidence peaked in 1988 using both gag and env genes, and in 1992 using the pol gene (Figure 3). Even though our analysis based on gag and env genes showed a median value much earlier than expected, the 95% credible interval peaked in 1992. Johnson et al. (2017) also showed that incidence estimates varied substantially depending on province. For example, incidence in Western Cape peaked in 2003–2004. In this context,
it is possible that for Hlabisa, incidence peaked slightly earlier than the rest of KwaZulu-Natal.

Model limitations
Our model-based phylodynamic analysis did not account for within-host evolution. This is a common but not universal assumption of population genetic modelling of infectious diseases which treats each infected host as a unit that corresponds to a single pathogen lineage. Bias due to neglecting within-host evolution is known to depend on sample proportion (Volz et al., 2017), and since these proportions were small in our cohort, is likely to be negligible. Our results may depend on variables which were not included in our epidemiological models, such as sex and age structure. It is likely that transmission rates vary between these categories. However, given the tenuousness of conclusions generated with the current models, it is also unlikely that the present data would support estimation of additional parameters for these categories. Finally, accurate estimation of global importation rates depends on inclusion of appropriate samples to represent diversity present in the global HIV reservoir. We have used all available sequences from GenBank and PANGEA for this purpose. However, inclusion of national-level databases could potentially identify additional lineages that are closely related to South African samples. Thus, the true rate of importation from the global reservoir may exceed the rates estimated here.

Migration and the role of the global HIV reservoir
We estimated the proportion of imports of HIV-1 lineages from outside the region sampled in South Africa. We summarised these estimates using a statistic that represents the probability that a new infection was endogenous, meaning that the new infection arose via transmission between individuals residing in South Africa. Exogenous transmission can arise from a variety of mechanisms (Rasmussen et al., 2018). For example, an individual may become infected when residing outside South Africa and then change residence to within South Africa. Alternatively, an individual from within South Africa may travel temporarily outside South Africa and become infected. Finally, an individual with primary residence outside South Africa may travel to South Africa and infect a local resident. We cannot distinguish between these three different mechanisms from the available data, as all three have very similar if not identical consequences for the HIV phylogeny.
Using gag, pol and env genes, we estimated a high probability of new infections in Hlabisa arising from immigration or transmission from external sources. The estimates from different genes had overlapping credible intervals.

Our estimated proportion of 62% (95% CI: 40% – 78%) of new infectious imported to Hlabisa using the pol gene was higher than the recent report of 35% (95% CI = 20% – 60%) in Rasmussen et al. (2018). Rasmussen et al. (2018) used a very similar model-based phylodynamic approach which also utilised pol sequence data from KwaZulu-Natal, but their catchment excluded the Hlabisa sub-district. We are not able to determine whether differences between our results and Rasmussen et al. (2018) were due to true differences between communities, patients sampled, or differences in inference methods, but both analyses support the presence of a very large proportion of transmissions occurring between rather than within communities.

In the phylogenetic tree, we observed a strong association between cladistic structure and placement of CGR sequences. The majority of CGRs were placed within a single partition. Similar arrangements were observed for HIV-1 subtype C in Ethiopia in which distinct clades composed by sequences from a single catchment (Amogne et al., 2016) grouped together. Both examples in Ethiopia and South Africa (involving a single catchment area) could potentially represent local evolving sub-epidemics. Another possibility is that these arrangements arose from unmeasured heterogeneity in the population, such as if a subset of the population, possibly a high-risk core group, has higher rates of migration outside of the sample catchment. In Hlabisa, males and females migrate to other areas in South Africa to work and males migrate further than females and return home less frequently. In addition, being a migrant and having lived in four or more places were found to be significant risk factors for HIV-1 infections (Lurie, 2006). Migrants had a 69% increase in acquiring HIV when compared to non-migrants due to increased prevalence of HIV risk behaviours (Dzomba et al., 2019). As an example of differences in behaviour: men who migrate to Carletonville, which is approximately 700 km away from Hlabisa, return home three to four times a year and the likelihood of infecting their partners is very low (Lurie, 2006). These behaviours suggest that there exists a subset within Hlabisa that is more likely to be infected by an external source. However, we were unable to test this hypothesis as we would need information on whether a patient was a migrant or not at the time of seroconversion.

Data availability

Underlying data

Genetic HIV-1 sequence data used in our analyses are available on request to the PANGEA consortium (https://www.pangea-hiv.org/). We are currently working on getting permission to release sequence into GenBank.

The R and Python scripts used in our analyses are available as a research compendium in GitHub (https://github.com/thednainus/pangeaZ).

Extended data

Analysis code available from: https://github.com/thednainus/pangeaZ

Archived analysis code as at time of publication: https://doi.org/10.5281/zenodo.6532287 (Nascimento, 2022)

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This study conducted in Hlabisa, Kwazulu-Natal, aimed to understand the transmission patterns of HIV-1 subtype C in the area, and to estimate various epidemiological parameters including transmission rates, viral effective population size, the effective number of infections over time and the proportion of infections originating from outside the community. The authors used a very thorough and up to date phylodynamics framework to analyse 2,503 local HIV-1 genetic sequences together with a large set of genetically close global references (CGRs). The results showed similar high trends in epidemic growth rates between 1980 and 1990, followed by a steep decline. Estimates using the pol gene tended to be higher than those estimated using the gag or env genes. Mean estimates of the proportion of new infections originating externally were rather high (between 62% and 85%, depending on the gene region) indicating a significant level of inter-connectedness between communities in rural South Africa.

Most of the objectives of this analysis were aligned with that of previous work done by Rasmussen et al. and/or Wilkinson et al., sometimes yielding different results. One key difference with previous analyses is the addition of non-African sequences to the dataset, expanding the scope of the migration analysis, and a comparison of estimates obtained from different HIV-1 genomic regions. The extent to which these additions generate new knowledge could be debated but the work is sufficiently novel and expertly conducted to be of interest to the HIV research community.

The phylogenetic and phylodynamic analyses are very well designed and conducted. The methodology is sound, appropriate and appreciably detailed in the manuscript. The authors have made appreciable efforts to make their data and scripts easily accessible, except for the sequence data which is only available on request. Note that I cannot comment on the epidemiological models, as modelling lies outside of my expertise.

The results seem robust and adequately interpreted. If anything, I find the reporting of the lineage importation analysis a bit too general. It is mostly circumscribed to estimates of the probability that a new infection was not attributable to endogenous transmission, but given that these
probabilities are rather large, it would have been useful to describe the close global references related to these exogenous transmissions a bit more. As it stands, we don't know much about where these CGRs come, whether particular sampling locations are more likely to be associated with import into Hlabisa etc.

The discussion is thorough and well documented, with insightful efforts to interpret differences with the previous work of Rasmussen et al. I do find the high proportion of imported new infections rather intriguing, but not concerning (concerning for the publication; it is of course concerning for the population at risk in Hlabisa...).

Minor comments:
1. The choice of some models (e.g. the HKY substitution model, the uncorrelated lognormal relaxed clock model) is not justified. Were model testing procedures used to determine goodness of fit? Are these choices based on previous work? This should be clarified.

2. It is said that the pol gene dataset was reduced to match the size of the gag and env ones. How was the down-sampling done?

3. The section on phylogenetic partitioning could be clearer. I understand the concept of partitions with different coalescent time distributions, but I am not sure I understand why partitioning is important here. Maybe the authors could expand a bit on why they partitioned the tree and what these partitions mean in epidemiological terms.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Viral genomics, molecular epidemiology, phylodynamics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.
This is an interesting study about the epidemiological dynamics of HIV-1 (subtype C) in a rural South African community. The process of data collection and curation is very thorough and the phylodynamic models are very neatly tailored to the problem of distinguishing importations from local transmission. My only major concern here is that there is not enough detail to follow what the phylodynamic model is doing. I accessed the github repositories, but again, there is not enough information there to understand it. Below I list specific points and suggestions before I would recommend this work for indexing:

**Major points:**
- Equations (1) model infected individuals that are infectious (I), in ART and thus not infectious (Z), and the size of the global reservoir (X). First, it would be useful to state clearly here that tips in the trees are labeled as belonging to either of these demes. Second, shouldn't there be a migration term here? Due to the way the data set is constructed, the global reservoir consists of singleton sequences, so I can't see how parameters related to X can be estimated without some sort of migration matrix. Moreover, distinguishing importations from local transmission would from a modelling standpoint would also require such a migration parameter.

- I am not clear on the rationale for removing so called 'redundant' non South African sequences. Is this just to make the data set more manageable, or would it have any impact on the model? In particular, wouldn't including such sequences inform about the coalescent rate from the 'reservoir' deme?

- I am aware that some of the authors have presented this in the past, but it would be very useful to include an expression, or at least a short section of text as to how the ODEs are integrated into the coalescent likelihood. As it reads presently, the epi modelling and phylodynamics are a bit disjointed.

**Minor comments:**
- On the less technical side, I am a bit concerned with the final sentences about migrants being more likely to acquire HIV in these communities. I can see that there is some empirical evidence from the references, but none of this was tested in this study because such metadata on seroconversion is not available. I would suggest making this section a bit less speculative.
Check that the abbreviation of 'confidence interval (CI)' actually occurs when it is first mentioned. I don't have page numbers, but this is somewhere at the start of the Introduction.

When discussing the role of importations and the global HIV reservoir, the probabilities are in percentages. Formally, probabilities should be between 0 and 1, to avoid confusion with other quantities (e.g. risk).

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Partly

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If applicable, is the statistical analysis and its interpretation appropriate?
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Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: infectious disease phylodynamics, phylogenetics, virus evolution

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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Summary

The authors study the HIV epidemic in a rural South-African community by applying phylogenetic and phylodynamic methods to a massive dataset comprising thousands of full genome sequences. They first estimated the temporal dynamics of incidence and prevalence and showed using the phydynR package that the former peaked around 1990. They further investigated these trends using a non-parametric coalescent approach. Finally, they use the phylogenetic structure to analyse migration patterns and show that the majority of the transmission events have their origin outside of the studied community. They analyse these results in the context of the South-African HIV epidemic and discuss potential limitations such as the absence of within-host evolution.

Review:

This study applies state-of-the-art inference software packages to a massive dataset to tackle a timely public-health question. My comment has to do with the interpretation of the results, which could perhaps be slightly more exhaustive (especially the comparison between gene regions and between methods). I think the model could also benefit from a few details.

1. Interpretation of the results

1.1 One of the added values of this study is that the authors perform their analyses on three different regions of the HIV genome. Although their results are qualitatively similar between these regions, there are some noticeable differences. In particular, with the phydynR approach, the epidemic appears to have a larger size when the analyses are performed on POL than when they are performed on GAG (with also a slightly slower epidemic). I think this trend should be discussed further. Is it due to the fact that there are more POL sequences?

1.2 Similarly, another asset of the approach is that the authors use two phylodynamics methods to infer past epidemic trends (phydynR and skygrowth). In the main text, they write that the two methods are consistent but I found this to be perhaps a bit hasty. Indeed, when comparing Figure 3B with Figure 4, there appear to be some differences. For example, for most partitions and genes, the prevalence peaks after 2000, whereas it is not the case in Figure 3B. Again, it would be interesting to discuss the potential origin of these divergences in the manuscript.

1.3 Intuitively, I would have expected the genomes resulting from introductions that are external to the community to cluster together. However, from the way the manuscript was written, I think my intuition is perhaps wrong. But I would appreciate it if the authors could clarify this point. Also regarding the proportion of transmission from outside the community, I was surprised that these appear to be the rule than the exception and I think it would be interesting to discuss this further (at least for a wide audience).

1.4 I found that the relatively early epidemic peak was not really discussed. The authors mention the test & treat policy in the Discussion (which might perhaps also deserve to be mentioned in the introduction) but the particular initiative cited here started in 2006, i.e. more than 10 years after the epidemic peak inferred here. I think that giving a few details
about the history of the measures implemented to control the HIV epidemic in Hlabisa in the Introduction could help better interpret the results obtained.

2. Methods

2.1 I found that some features of the model were unclear:

2.1.1 I was expecting to see a migration term in the equation for $\frac{dI}{dt}$ (otherwise, I wonder why to introduce this process at all in the model).

2.1.2 It was unclear to me why to mention $\frac{dZ}{dt}$ and $\frac{dX}{dt}$ since they do not affect the dynamics of $\frac{dI}{dt}$ (unless a term is missing).

2.1.3 About $\frac{dX}{dt}$, since this is the equation for exponential growth, wouldn't it be simpler to directly write that $X(t) = X_0 \exp(\rho t)$?

2.1.4 I found the notation $\alpha(t)$ potentially a bit misleading because, if I understand correctly, it corresponds to something like $a \tau$, where $a = 0$ before 2005.

2.1.5 From what I understood, the value of $\alpha(t)$ is fixed (it is not listed in Table 1). If so, the authors should explain how this value was set and, ideally, perform a sensitivity analysis.

2.1.6 Given all these unknowns about $\alpha(t)$, I am not sure I understand the rationale for its introduction because $\beta(t)$ will already be capturing the decrease in epidemic growth.

2.2 In the Discussion (page 8), the authors indicate that they performed “simulations”, which might explain some of the model features I found surprising in 2.1. If this is the case, the simulation results should be presented in the Results section and the methods could be clarified.

2.3 Wouldn’t it make sense to have the lineage importation parameter vary with time?

Minor comments

(I apologize for the lack of clarity in these comments but since Wellcome Open Research does not bother to add line numbers this is the best I can do)

- page 3: The formulation “We evaluated the potential of […] methods” seems inaccurate since this work is more about applying methods rather than comparing them or comparing their output to that obtained using other types of data.

- page 3: It was unclear to me whether the genomic data analysed in the context of this study was original (in which case the authors should specify it and it would clearly increase the impact of the study) or if they were re-analysing data that was already published in another context.
- page 4: The proportion of sequences with recombination events seems very low (16 in more than 2400 sequences). Is this me being wrong about the occurrence of these events (which is perhaps the most likely) or does this result from some of the filtering steps performed beforehand (e.g. subtype selection)?

- page 4: With such a massive dataset I would have naively expected a GTR model to fit better than an HKY model. Was there any specific reason for the model choice here?

- page 4: The authors do not seem to discuss the robustness of the phylogenetic signal in the data.

- page 6: It would perhaps be interesting to plot the prior distribution and the posterior for the migration rates.

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Yes

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Yes

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Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**

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**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** epidemiology, evolutionary biology, mathematical modelling

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