

HIV-phyloTSI: IAVI workshop

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Intrahost diversity and recency of infections

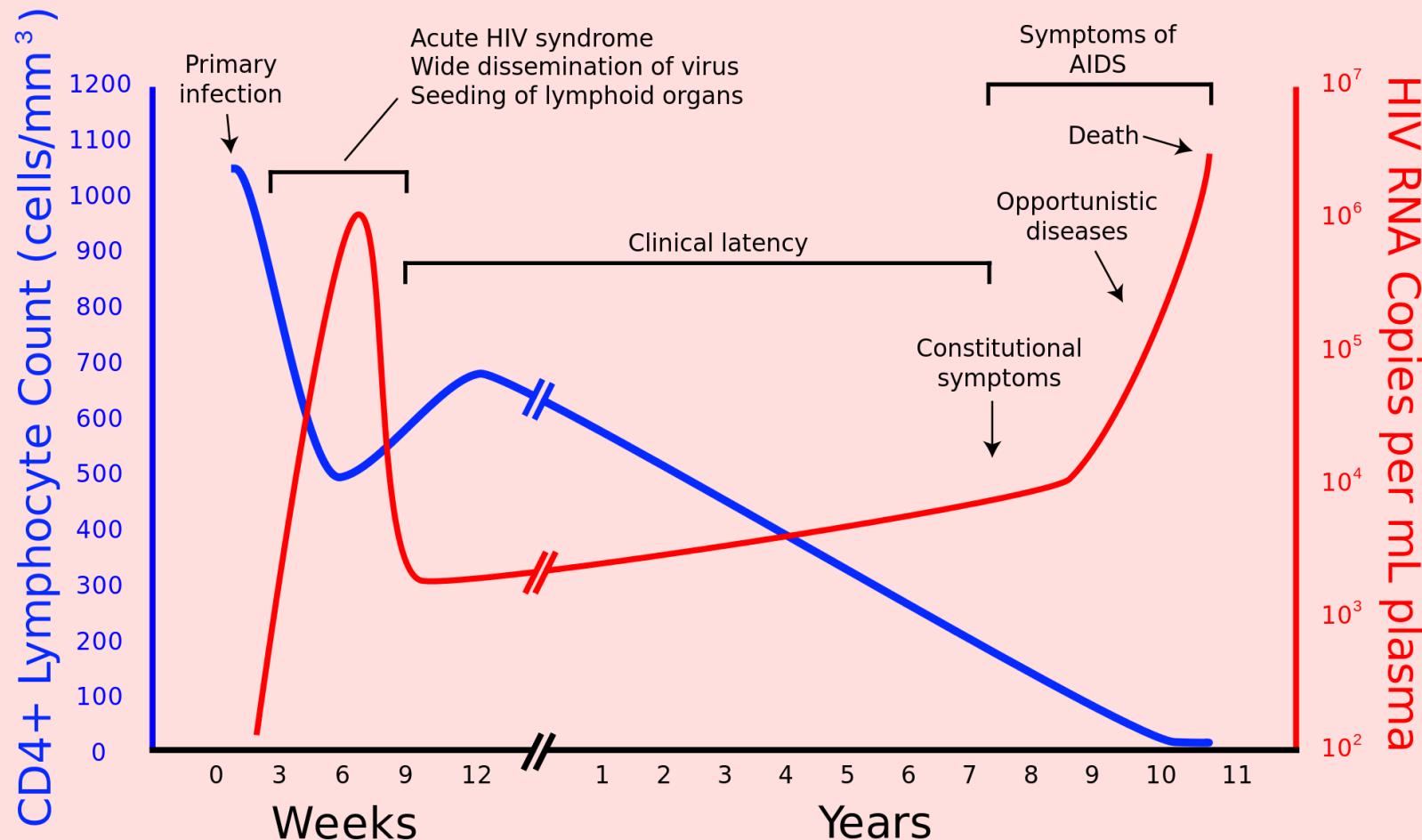
why do we care about recency?

- incidence estimation
- identifying sub-populations suffering from recent infections are happening.
- determining generation intervals.

but getting data is hard:

- longitudinal cohorts as gold standard
- alternatives which are discussed in this section

What is recency? Natural history of HIV

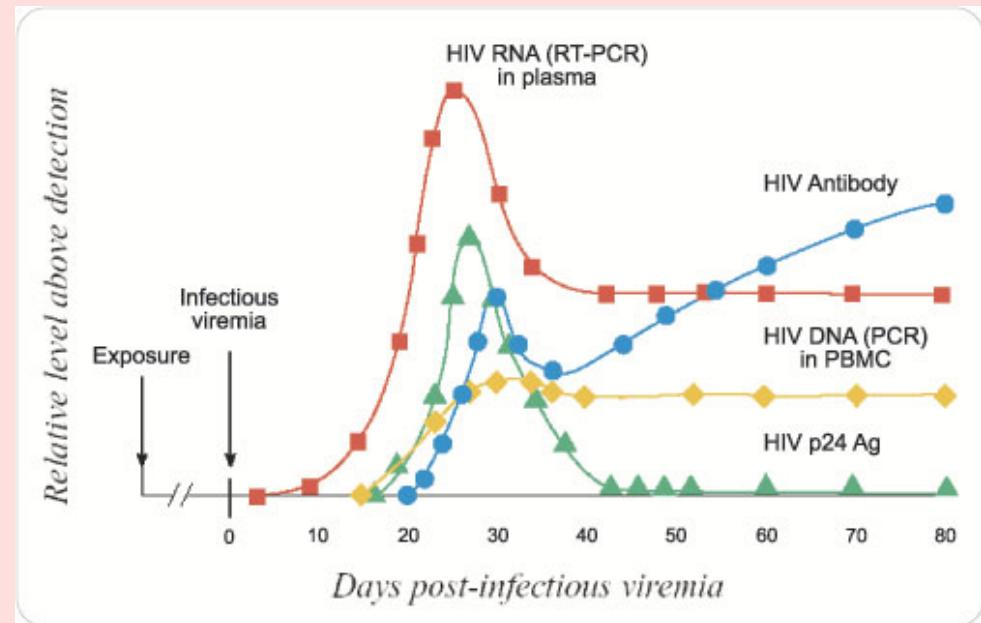


dynamics define infection stages: (CDC: 2014) ; (Fiebig et al. 2003)

Lab assays to identify infection stages

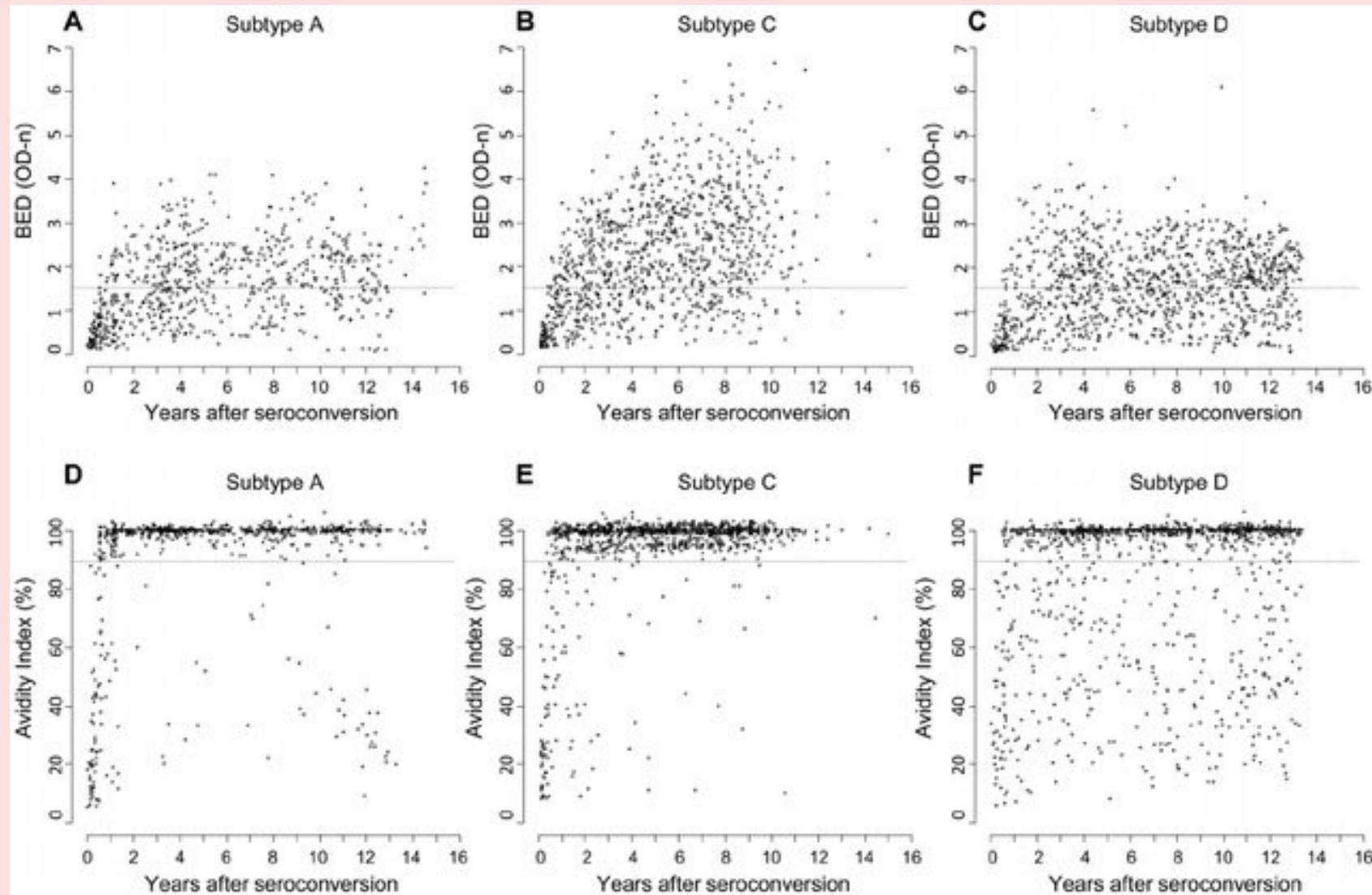
Based on the quality and type of immune response.

- **Less sensitive Enzyme Immunoassays**, antigen test (1998)
- **Lag-Avidity** (2012)
- **Bed-CEIA** (2011)

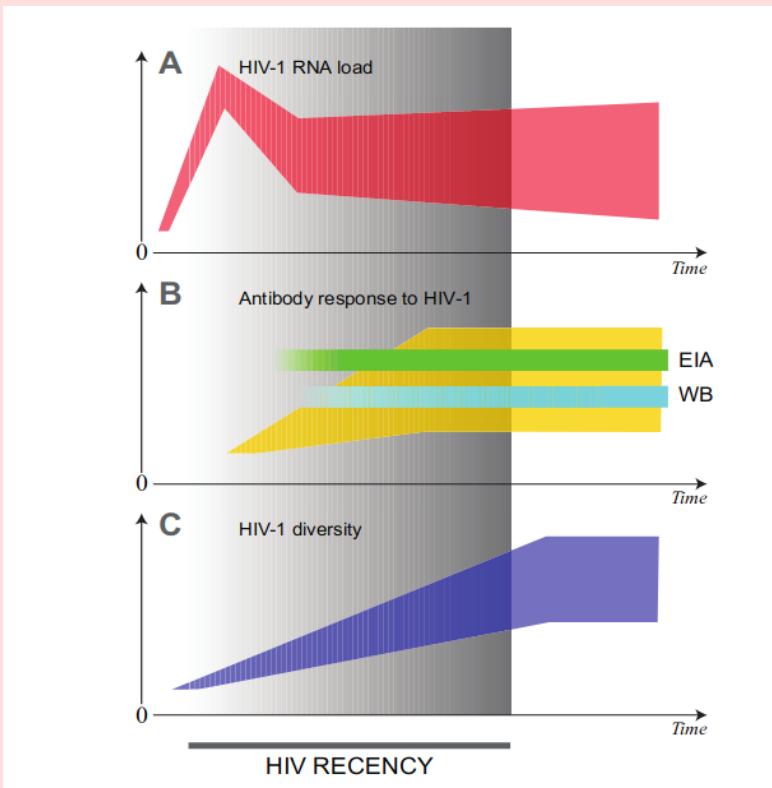


This is great, but specificity and sensitivity are hard to control:

- individual level variation, confounding subtypes, ART status...



An alternative unit of time: genetic diversity



- HIV infection is a multi-faceted phenomenon: immune response is just one facet.
- HIV-1 diversity grows as a function on time since infection.
- NGS is more informative than Sanger ([Carlisle et al. \(2019\)](#))

Case study: recency in Botswana

Ragonnet-Cronin et al. (2022) developed recency classifier

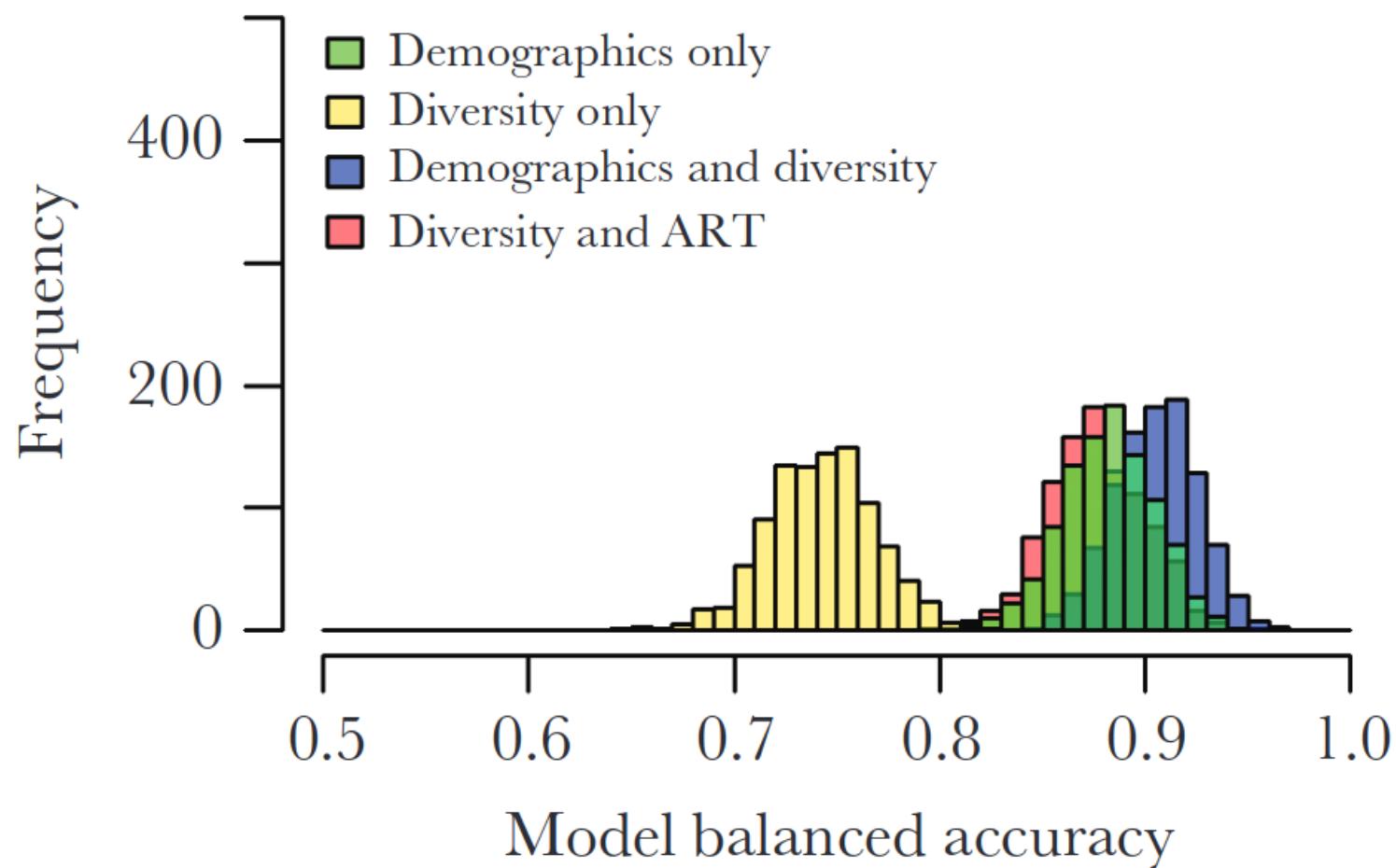
- **end goal:** characterizing undiagnosed cases in Botswana (Bhebhe et al. (2022))
- **data:** demographic data, some testing data and individual level NGS
- **how:** train ML model on known recent and known chronic infections, and use it to classify unknown.

comments on model:

1. still required cohort study, and *not directly generalisable* to other contexts.
2. *simple measures of diversity*: entropy and mean pairwise distance
3. these alone are *not enough* to provide good classification

Case study: recency in Botswana

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HIV-phylo TSI

Golubchik et al. (2022) tackles weaknesses of previous approach:

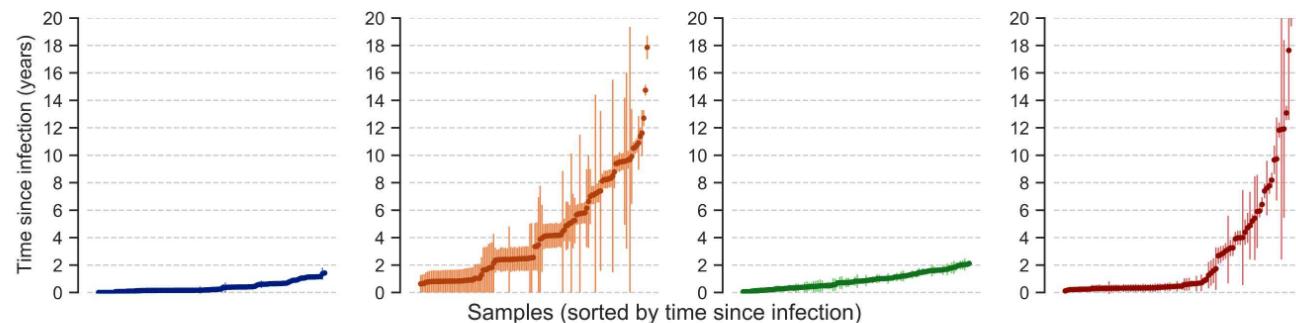
1. **Pre-trained**: no need for expensive training data.
2. Captures more complex **measures of divergence**.
3. Comparatively **low false recency rate** using exclusively NGS data.

HIV-phylo TSI: training data

sequences from PANGEA and BEEHIVE with known infection range.

	UW Partners in Prevention (UWP)	Rakai CCS (RAK)	BEEHIVE (BEE)	MRC Uganda (MRC)
Participants	108	132	104	94
Samples	150	132	104	94
Age, median (range)	33.3 (19.0 - 55.1)	32.0 (17.0 - 50.0)	32.9 (20.4 - 62.8)	29.0 (17.0 - 75.4)
Proportion female	0.43	0.49	0.03	0.45
Years since infection, mean (range)	0.4 (0.0 - 1.4)	2.9 (0.6 - 17.9)	0.8 (0.1 - 2.1)	0.5 (0.1 - 21.0)
Predominant subtypes	A1, D, C	D, A1, A1D	B	D, A1, B
log10 VL, mean (range)	4.9 (3.4 - 6.8)	4.8 (3.2 - 6.0)	4.6 (3.1 - 5.6)	4.4 (2.9 - 5.2)
CD4 count, mean (range)	n/a	407 (15 - 1626)	501 (140 - 1062)	639 (197 - 1597)
Proportion of infections: <=12 months	0.83	0.23	0.59	0.63
<=18 months	1.0	0.27	0.79	0.65

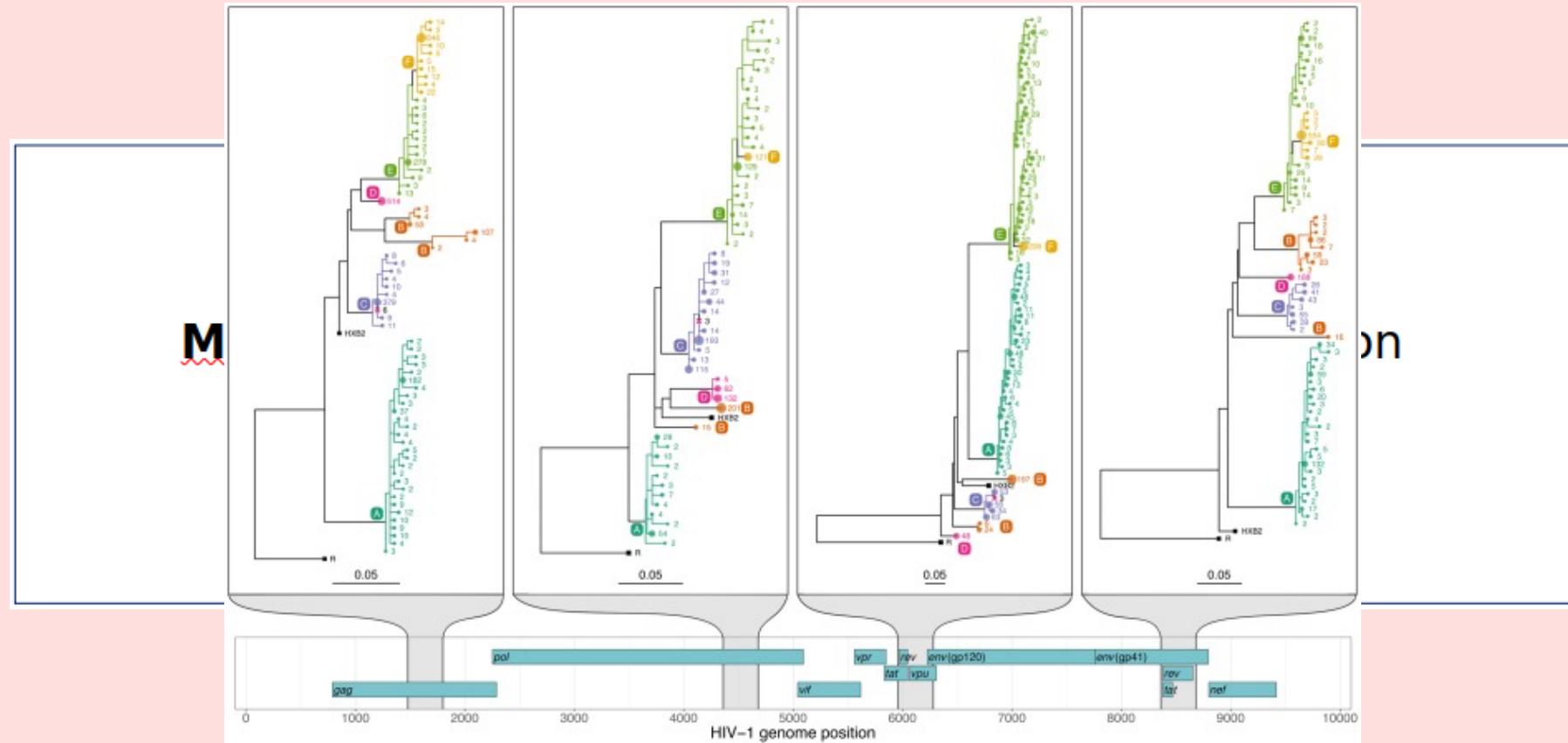
Duration of infection
(seroconversion interval)



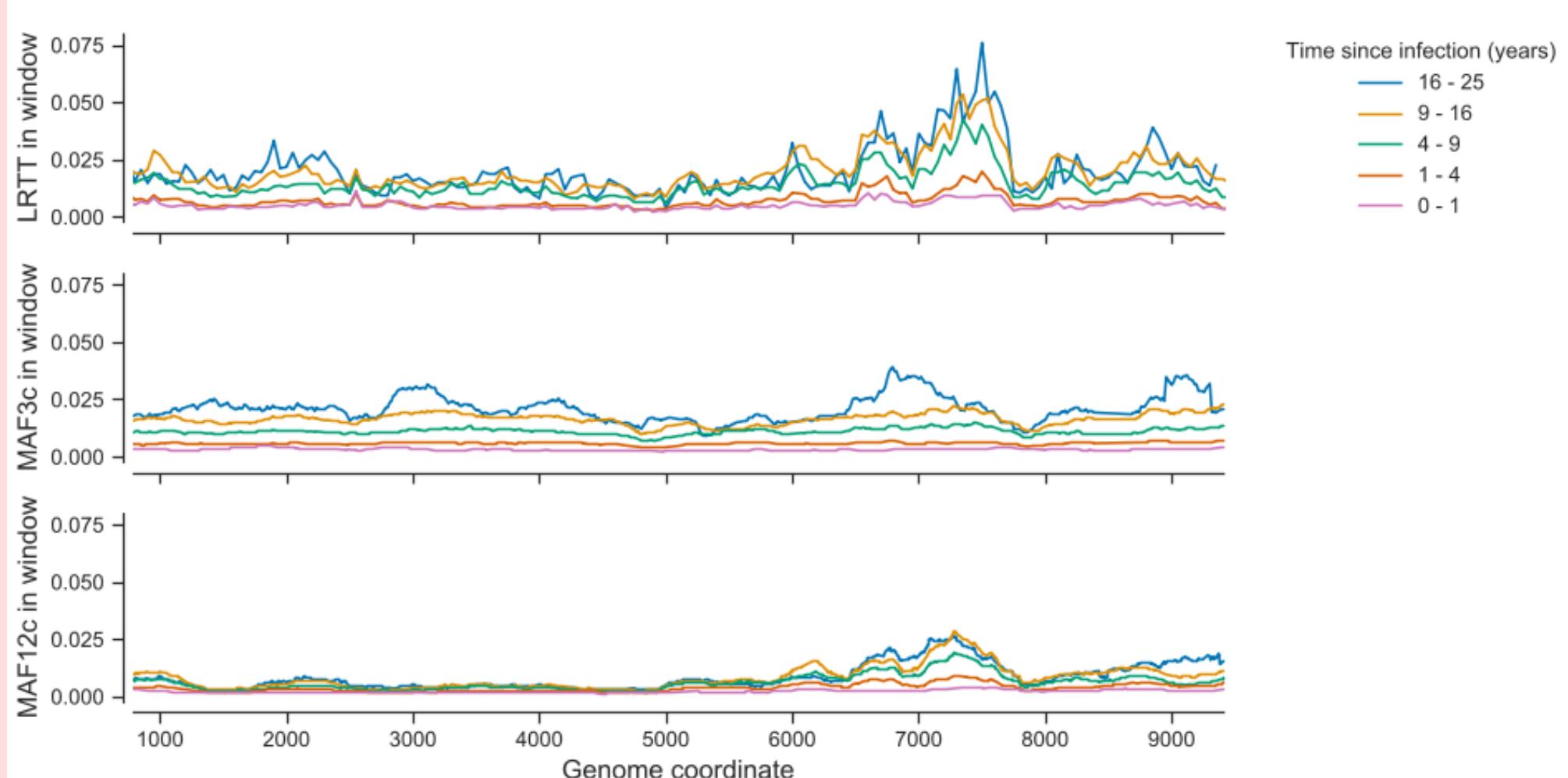
HIV-phylo TSI: intrahost diversity

choice of predictors based on viral diversification following infection.

1. "Accumulated" mutations on codons.
2. "Depth" of host-specific subtree in phylogeny. (LRTT)



But why these measures of intrahost diversity?



HIV-phylo TSI model

different signal in different part of the genome. How to combine predictors?

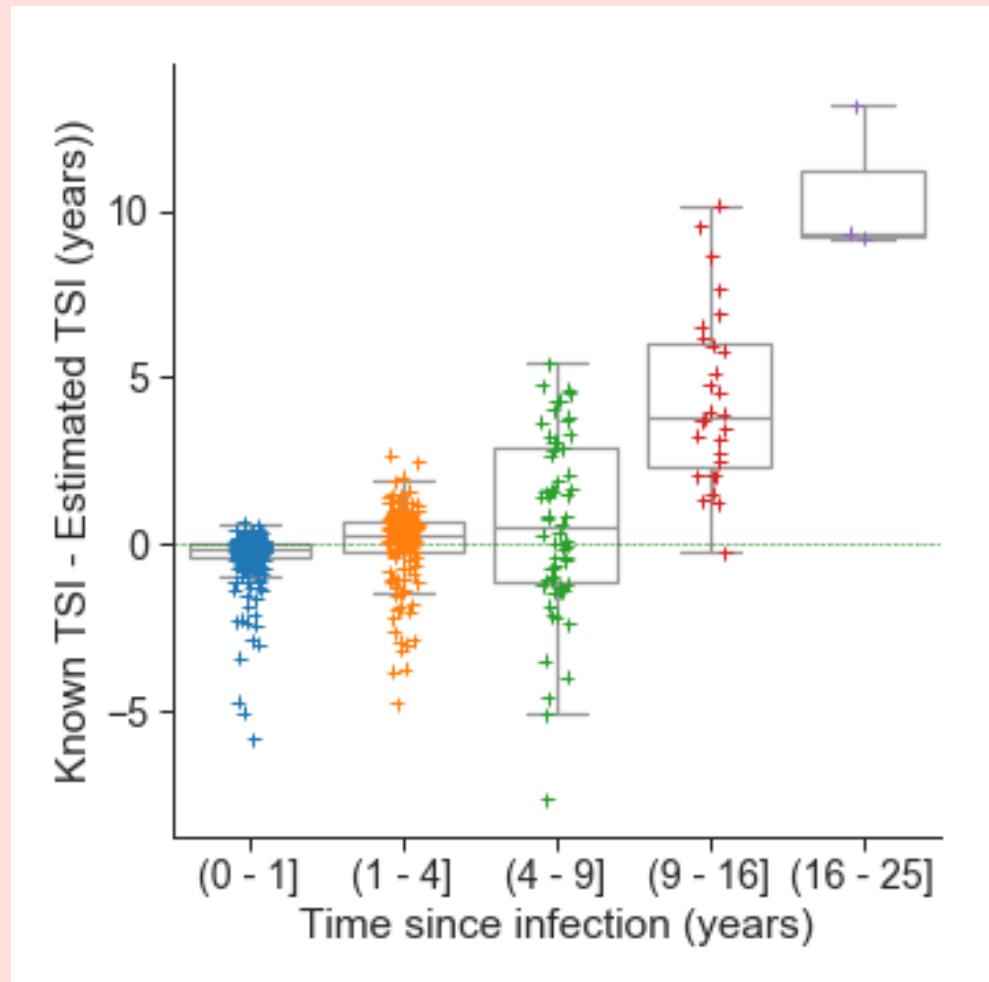
- take average over gene
- let the model pick up the informative covariates.
- Best performing set of features chosen through LOOCV
- Regressor performs well as a classifier too, but FRR (~10%)

HIV-phylo TSI performances: bias

- bias is low in infections < 9 years.
- range of individual-level errors can be quite wide.

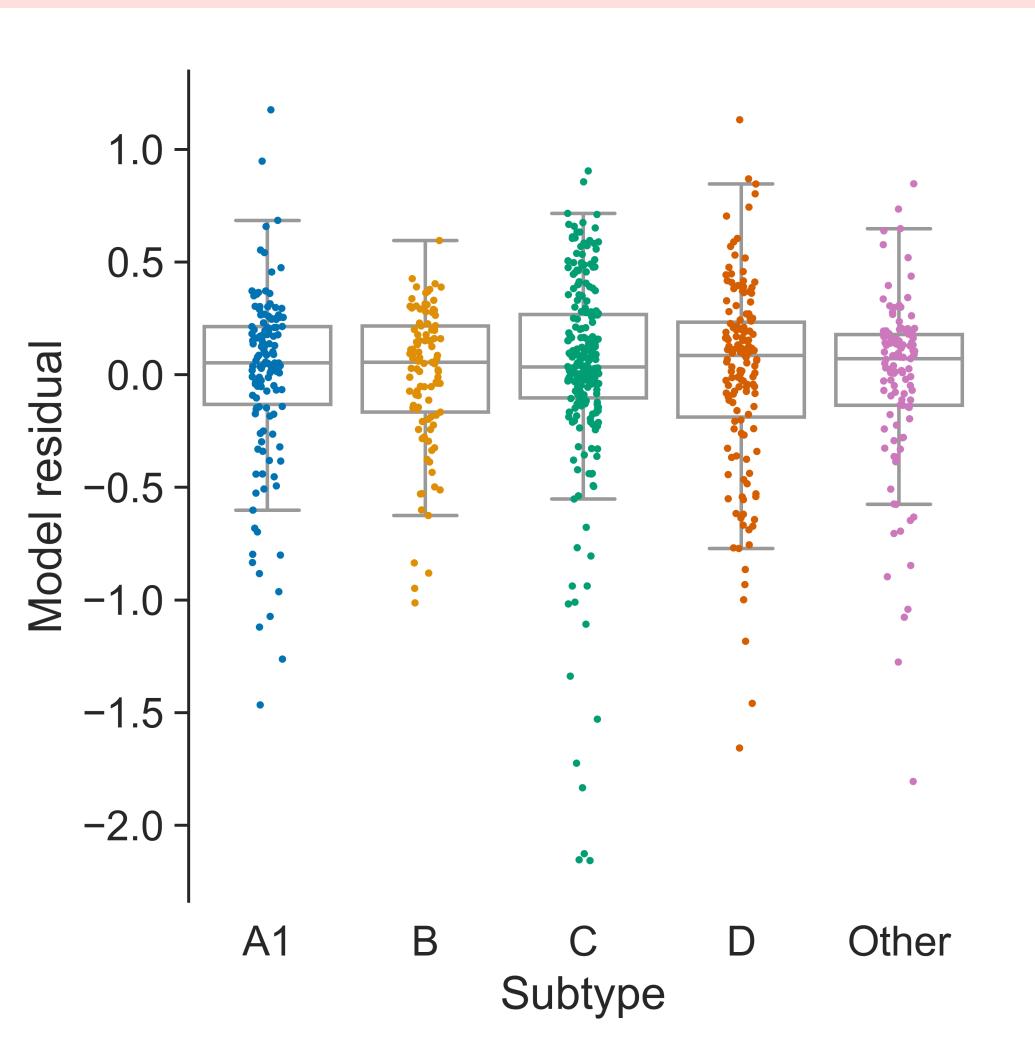
For applications:

- estimates are not precise at the individuals level BUT
- there is signal in medians taken among subpopulation.



HIV-phyloTSI performances: robustness to subtype

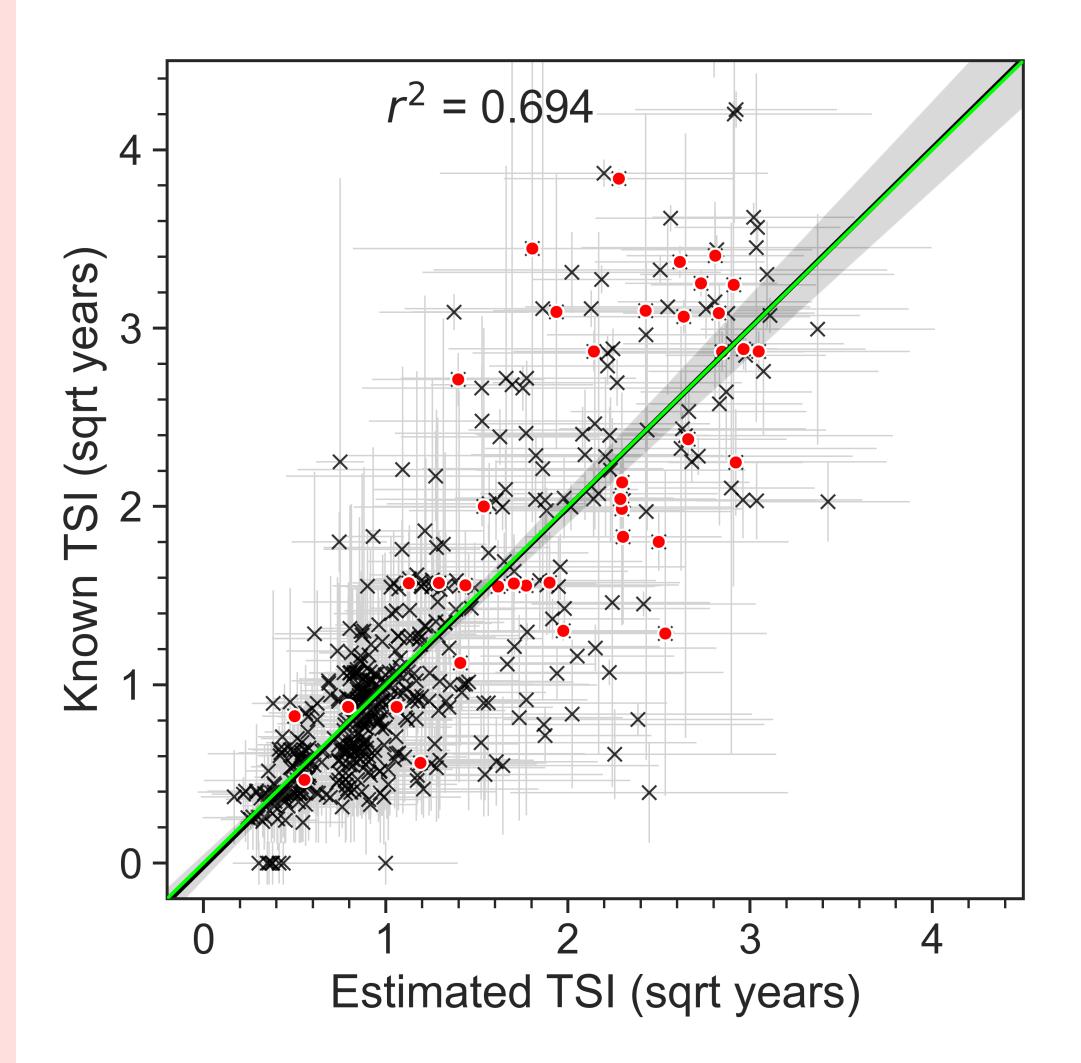
model bias was close to 0 for all subtypes included in the dataset.



HIV-phyloTSI performances: robustness to ART (?)

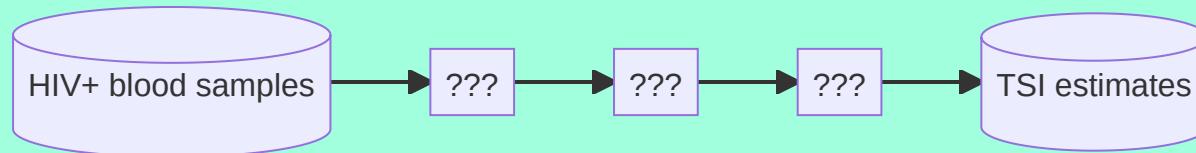
Some individuals in the training data reported prior ART use.

- followed pattern
- BUT: small sample size
- AND: no predictions for suppressed individuals.



HIV-PhyloTSI bioinformatics pipeline

Demistifying the process



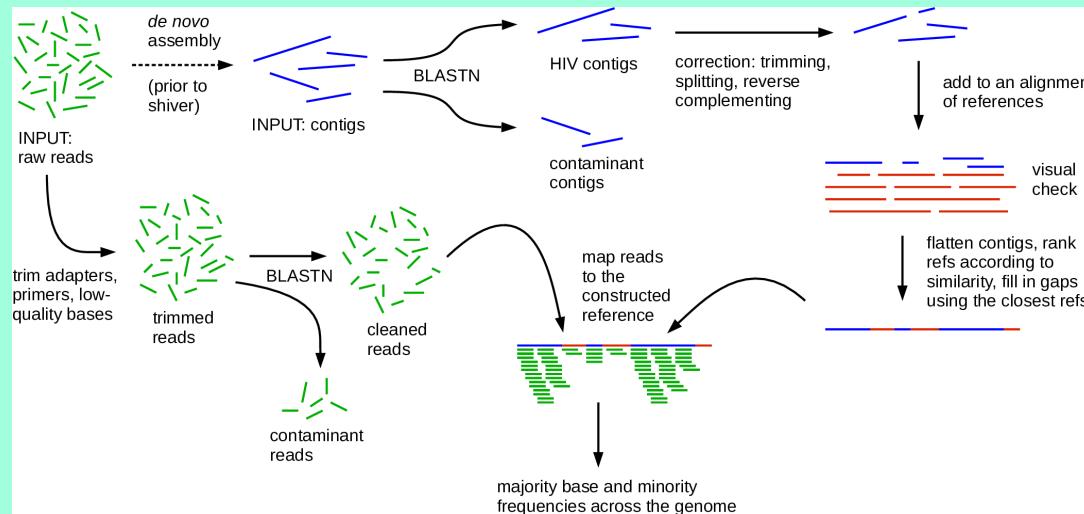
- In this section, want to **describe** processes and **data manipulation** carried out by the pipeline.
- **Not** the actual **implementation** of the pipeline.

Main steps:

- Next Generation Sequencing (NGS)
- Constructing phylogenies **MANY!**
- Analysing phylogenies

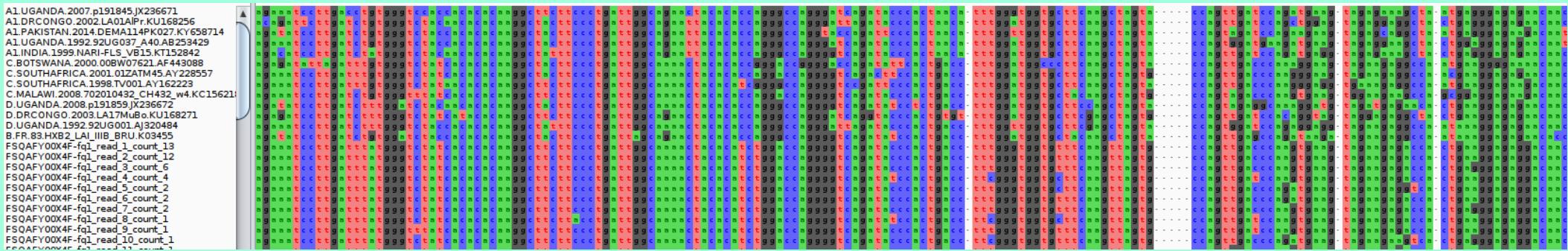
Next Generation Sequencing

- simultaneous sequencing of multiple viral particles to capture within-host diversity
- PANGEA protocols: I ([2012](#)) and II ([2018](#))
- TSI pipeline starts with the outputs produced by [shiver \(2018\)](#): * .bam, * .ref .fasta



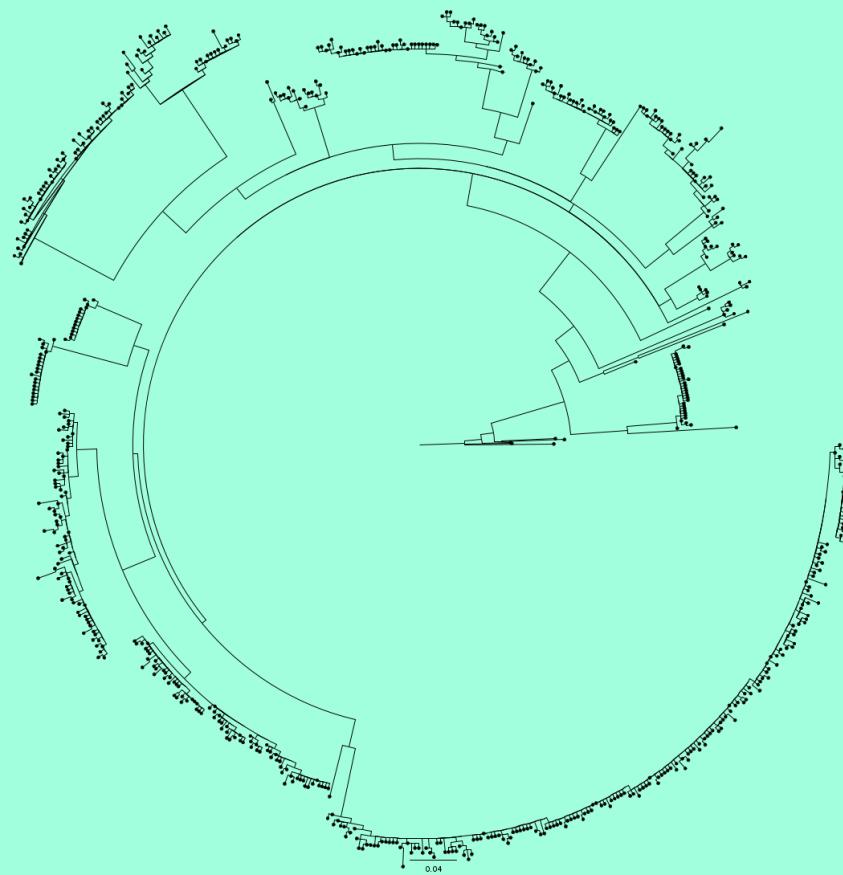
Grouping & multiple sequence alignment

- Many sequences, many hosts, many genome windows -> **group by host!**
- aligning to compare “apples to apples”: MAFFT (Katoh 2002)



Inferring phylogenies

- Phylogenies are made through IQTREE by group and window through IQTREE (Nguyen et al. 2015)



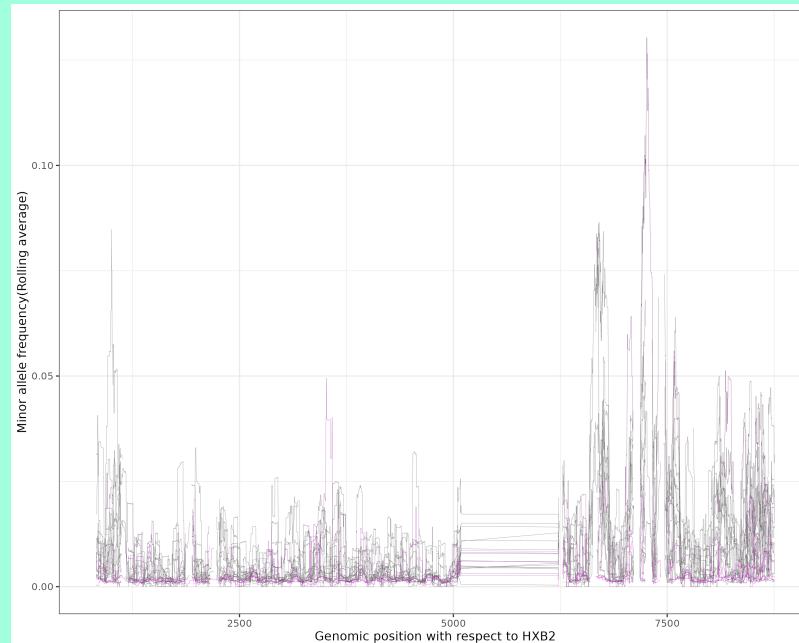
Code and slides

Analysing phylogenies

- `phyloscanner` (TOCITE) summarizes each tree through summary statistics:
- `patStats.csv`: contains LRTT, number of tips, etc...

Minor Allele Frequencies

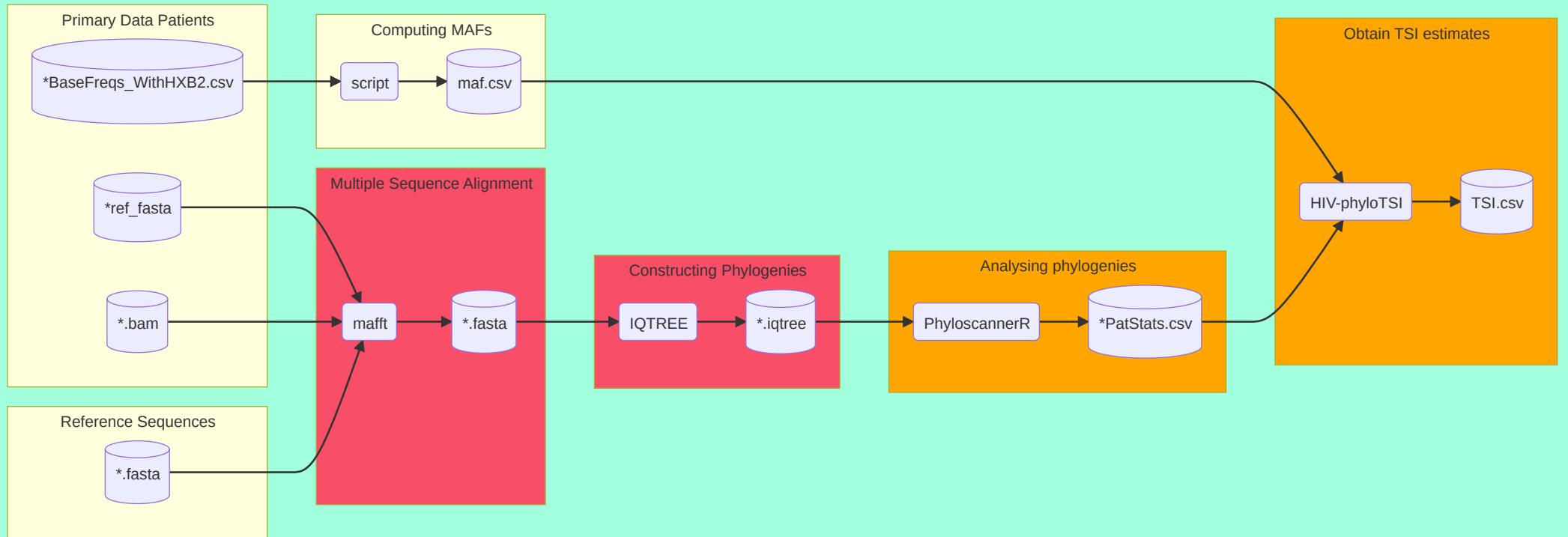
- $\text{MAF} = (1 - \text{proportion of majority bases}) / \text{depth}$
- Evaluated at first 2 codons positions (**MAF12c**) and/or third codon position (**MAF3c**)



HIV-phyloTSI

- described in Golubchik et al. (2022), code
- Focus of the practical session,
- takes as input features of phylogenies and MAFs.
- returns a csv file with TSI estimates and some uncertainty range.

Summary



Steps run for each group and window are shown in red, while those running by group in orange

But what is HIV-phyloTSI doing?

- **ML algorithm**, in particular Random Forest.

TODOOOOOOOOOOOOOOOOOOOOOOOOOOOOOooooo

HIV-PhyloTSI obtaining TSI estimates

Plan

- divide in group of 4, each group with a machine running Linux or MacOS
- introduction of sample dataset
- installing dependencies
- running hiv-phyloTSI
- if there is time, visualising outputs in R.

Preliminary Tools

There are two tools that will make our lives easier:

- **Terminal access** allows to run programs and give instructions to computer. <> ([Ctrl+Alt+T](#) on Linux, [Terminal](#) on MacOS, see [WSL for Windows](#)).
- **Conda** allows to download the exact requirements needed for HIV-phyloTSI. Installation instructions can be found [here](#)

Installing dependencies

The ‘ingredients’ to run the analyses are: the **ML algorithm**; the **data** and the **code dependencies**. The below code chunk allows you to download everything that is needed for the analyses.

```
1 # change directory to where you want to install HIV-phyloTSI repo
2 cd $HOME/git          # this is where I install git packages
3 # cd $HOME && mkdir git && cd git
4
5 # clone directories necessary to run the analysis
6 # BDIs code and workshop materials
7 git clone git@github.com:BDI-pathogens/HIV-phyloTSI.git
8 git clone git@github.com:abriz97/HIV-phyloTSI-workshops.git
9 # store paths to 2 above directories
10 DIR_WORKSHOP="$(pwd)/HIV-phyloTSI-workshops"
11 DIR_PROGRAM= "$(pwd)/HIV-phyloTSI"
12
13 # install python dependencies for HIV-phyloTSI and load the environment
14 conda env create -f HIV-phyloTSI-workshops/hivphylotsi.yml
15 conda activate hivphylotsi
```



Note

When interacting with a terminal, learning the power of the `$` operator is key. The operator allows to evaluate variables (eg. `$HOME`) or to evaluate commands surrounded by brackets (eg. `$(pwd)`).

Running the algorithm

Once all the ingredients are there, we can start cooking. It is relatively simple to run the analyses, even though we need to be precise in the way we specify the paths to the input data.

```
1 # Run HIV-phyloTSI on input data.  
2 python $DIR_PROGRAM/HIV-phyloTSI.py \  
3   -d $DIR_PROGRAM/Model \  
4   -p $DIR_WORKSHOP/HIV-phyloTSI-workshops/input/ptyr1_patStats.csv \  
5   -m $DIR_WORKSHOP/HIV-phyloTSI-workshops/input/phsc_input_samples_maf.csv \  
6   -o $DIR_WORKSHOP/HIV-phyloTSI-workshops/output/ptyr1_tsi_workshop.csv  
7  
8 # print header of output to make sure it exists:  
9 head $DIR_WORKSHOP/HIV-phyloTSI-workshops/output/ptyr1_tsi_workshop.csv
```

Note

The first 2 lines point to `$DIR_PROGRAM` because they refer to the code we want to use. On the other hand, the bottom 3 lines refer to the input data and output paths, and this is why they point to `$DIR_WORKSHOP`.

Visualising results

I provide some **R functions and scripts** to visualise results, which **can be found** in the github **repository**:

- script: \$DIR_WORKSHOP/src/workshop_analyses.R
- functions: \$DIR_WORKSHOP/src/R/workshop_R_helpers.R

Again, we can use conda to **install the necessary packages**:

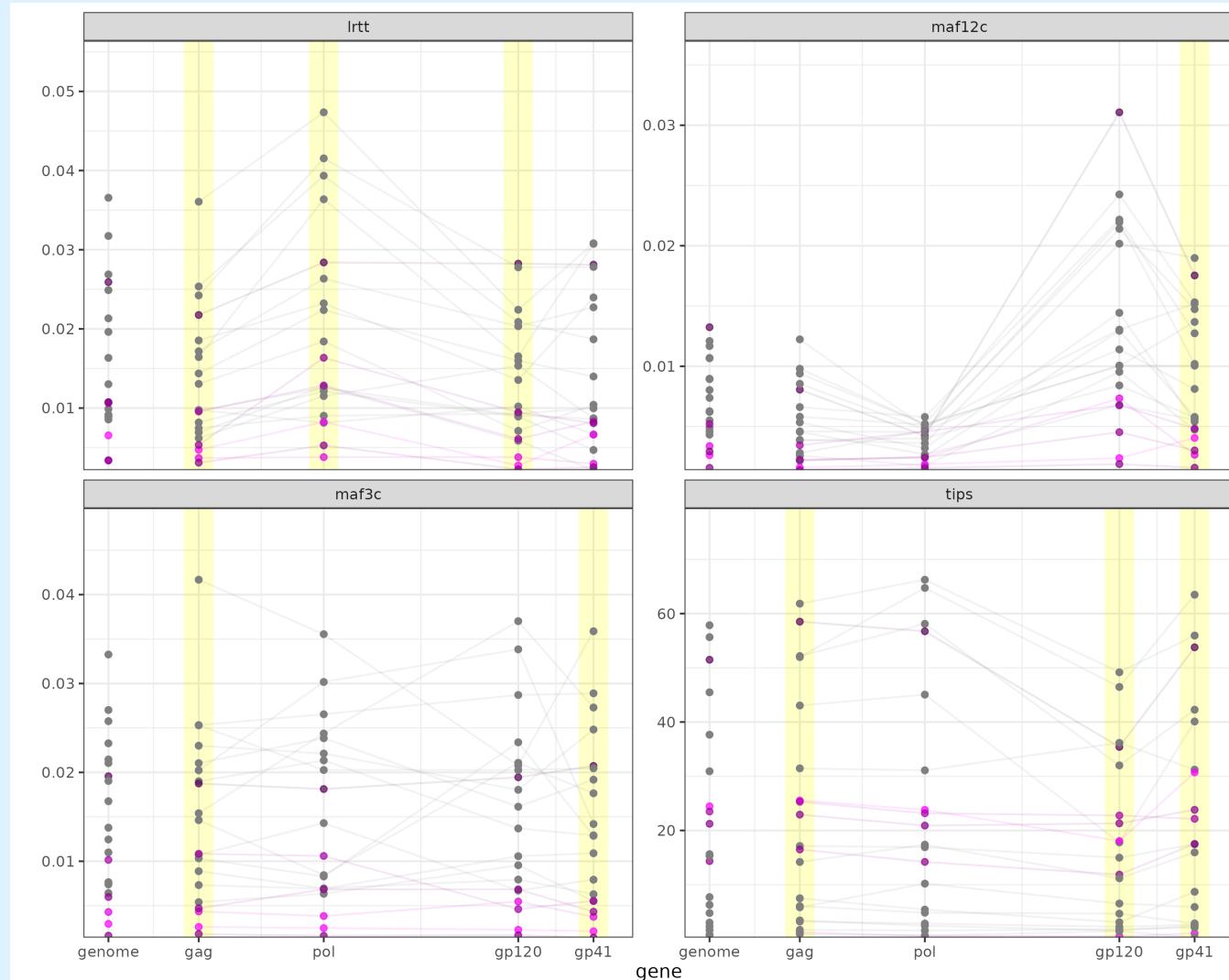
```
1 # install few R dependencies for visualisation and load the environment
2 cd $DIR_WORKSHOP
3 conda env create -f workshopR.yml
4 conda activate workshopR
```

I will be showing snippets of the above code together with the plots they produce.

You can reproduce the steps by opening up the script in RStudio.

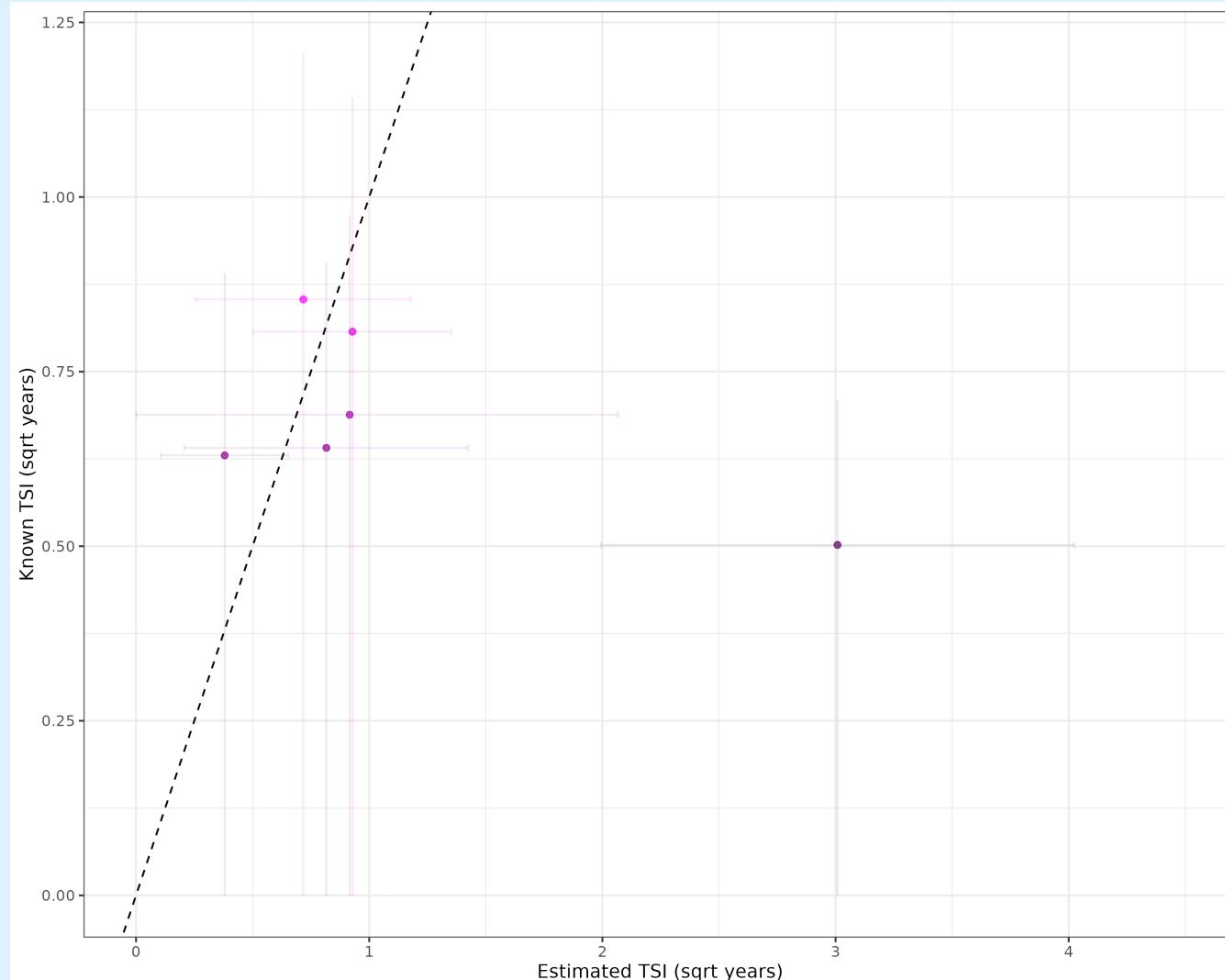
Predictors used

```
1 dtsi <- fread(file.path(git_root, "data/ptyr1_tsi.csv"))
2 plot_predictors_from_tsi_output(dtsi, exclude="dual")
```



Evaluation on seroconverters

```
1 dall <- merge(dtsi, ddates)
2 plot_cross_interval_tsisero(dall, sqroot=TRUE)
```

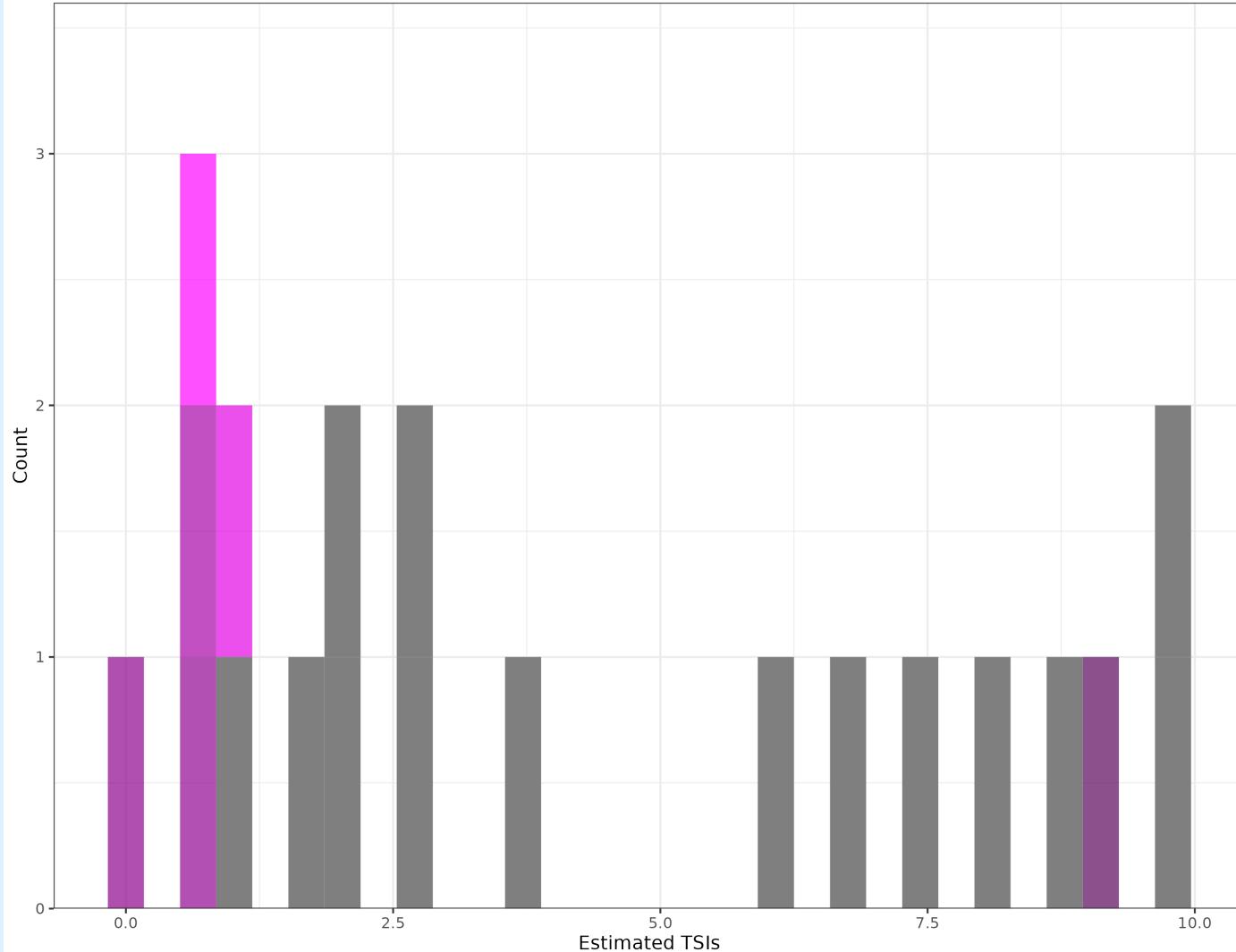


Histogram of estimates TSIs

```
1 p_hist <- plot_histogram_tsi(dtsi)
```



Shortest predictions are from seroconverters

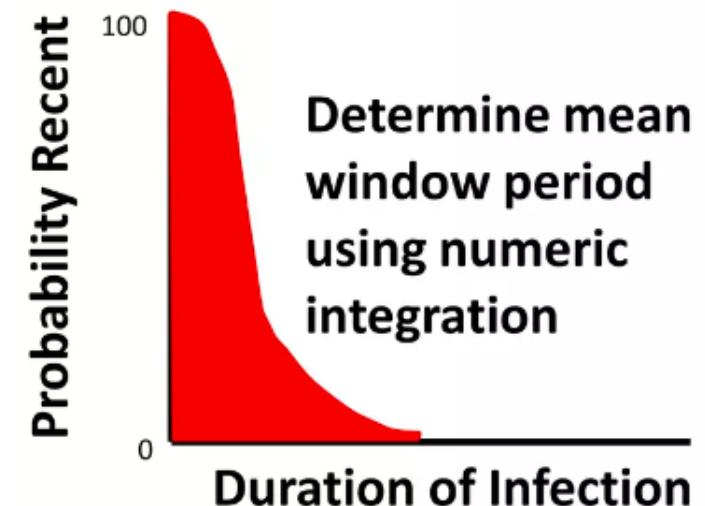


Possible follow-up analyses

Prevalence of recent infections

Following Freeman and Hutchison (1980) and Brookmeyer and Quinn (1995), if incidence is constant, it can be estimated as:

$$I = \frac{\text{# recent}}{\text{# diagnoses}} \times MDRI$$



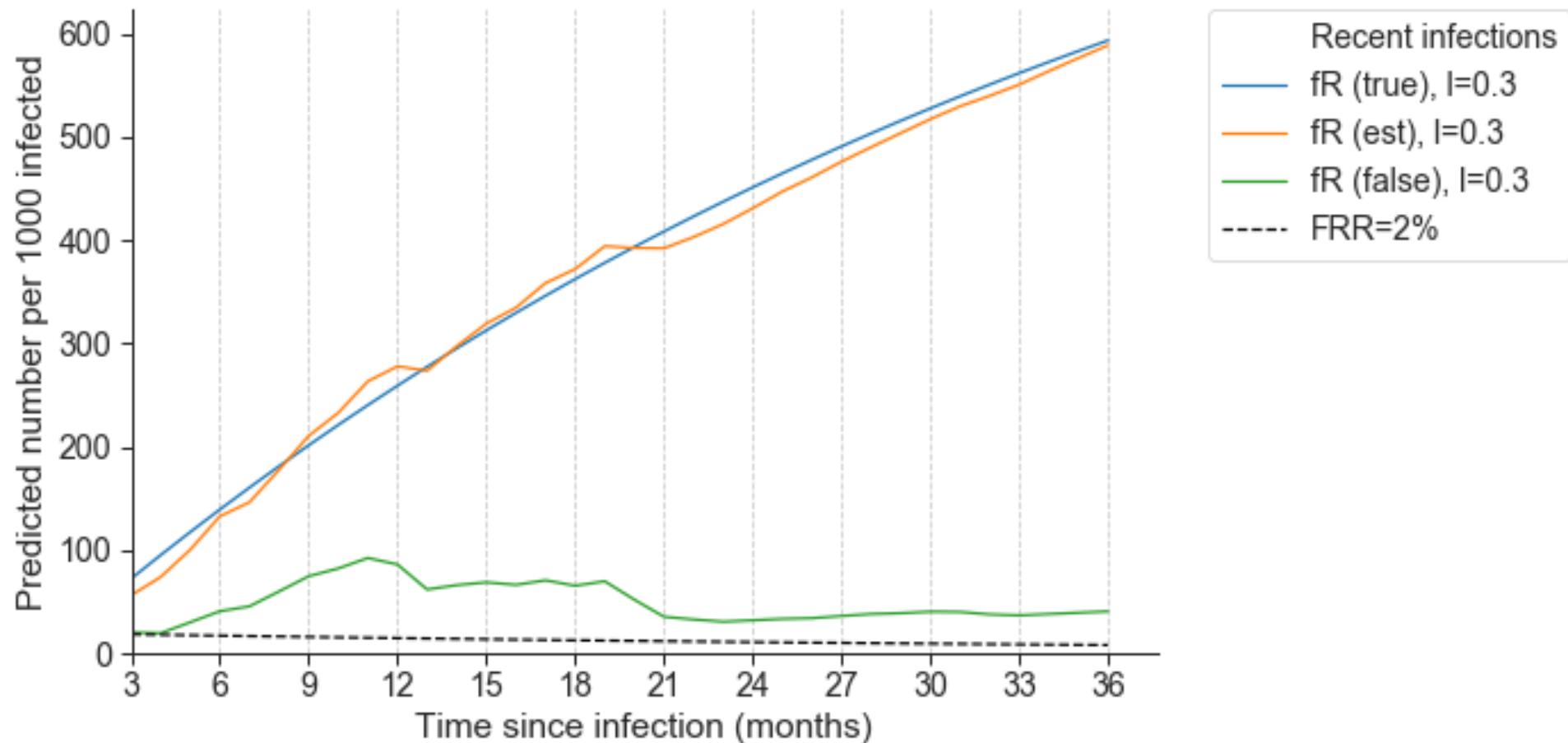
where $(MDRI)$ = mean duration of recency of infection.

- $(MDRI = P[\text{ever being classified as recent}] \times E[TSI | \text{classified as recent}])$
- **not a constant**: viral suppression, underlying population.

Here we **focus on the numerator**.

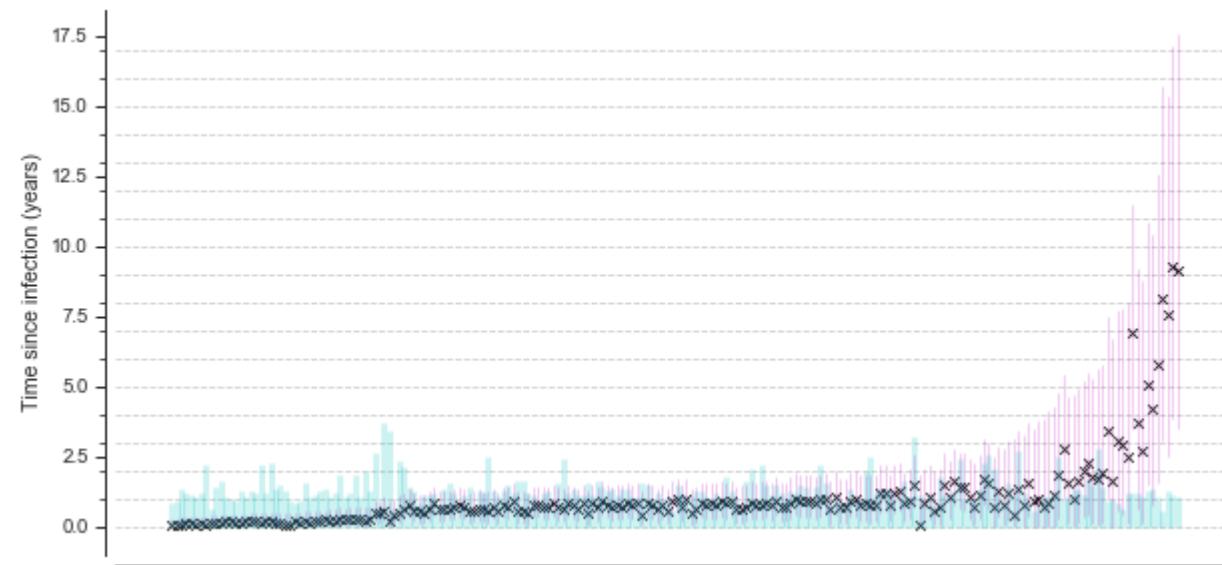
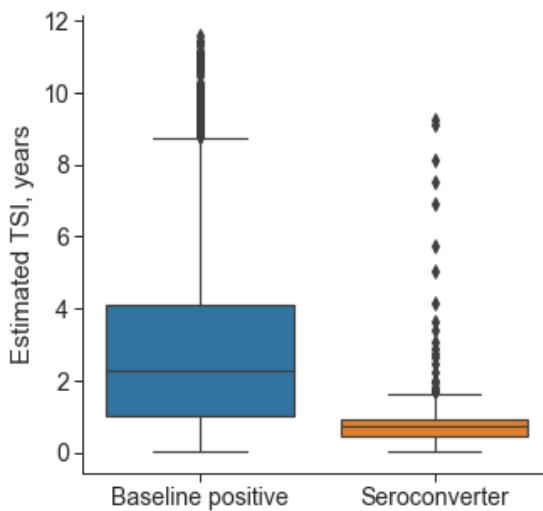
Simulation study:

In simulated settings, the prevalence of recent infections is well estimated, despite at least 2% of recent classifications are wrong.



Application in Zambia

When directly applied to real-world data, HIV-phyloTSI generally produces smaller TSI for known recent infections as compared to people with unknown first positive date:



=> Can be used to compare median TSIs among population subgroups.

Suggestions on TSI comparison among groups

Comparing simple summary statistics can be misleading:

- need to make sure statistic is *reliable*
- need to *choose* summary statistic: **median**

For reliability:

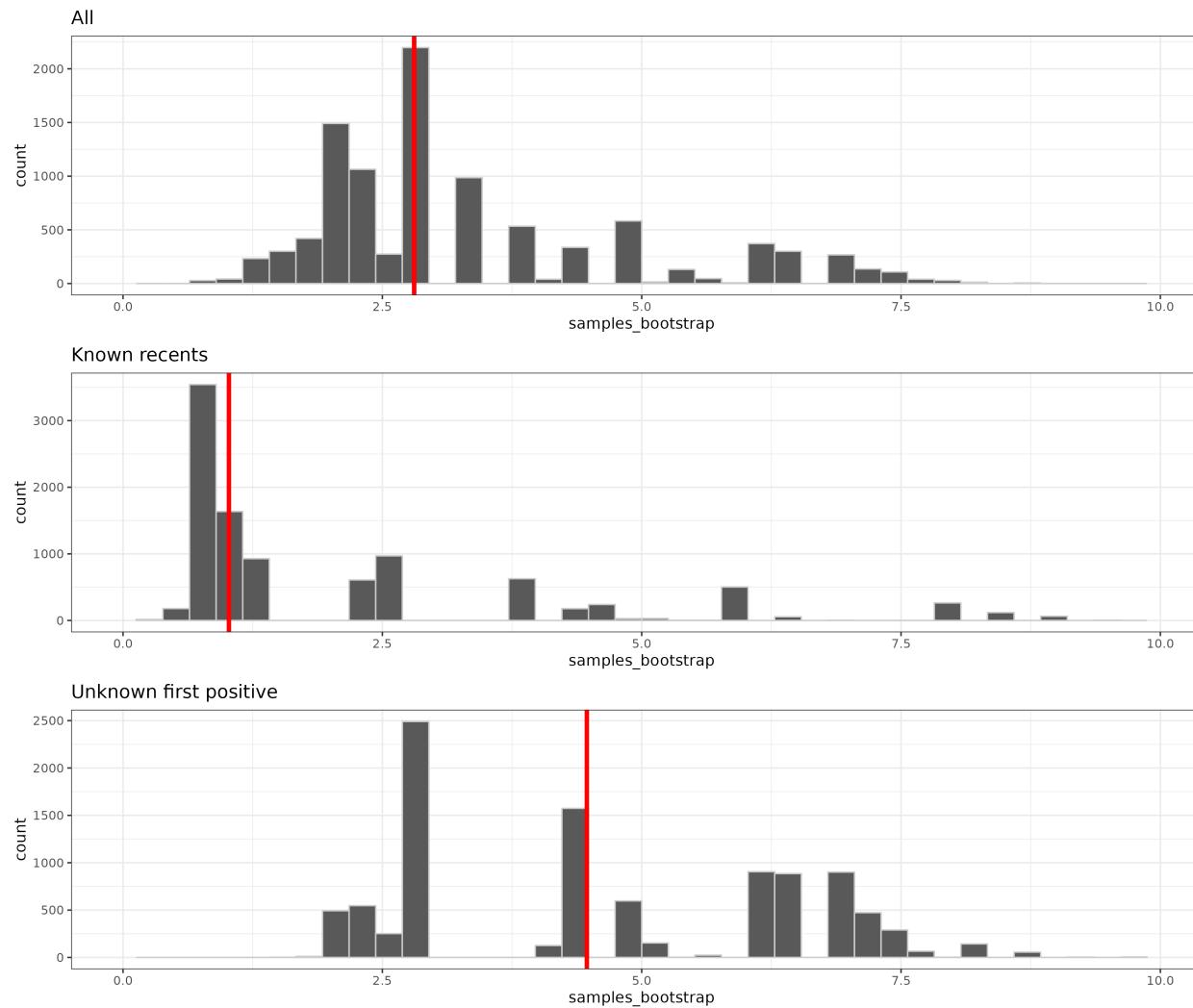
1. make sure groups are large enough (*minimum of 40 per group*)
2. Perform **bootstrap** to estimate uncertainty around

Suggestions on TSI comparison: bootstrap

Bootstrapping: statistical technique which *recycles* analysis data to estimate uncertainty around an estimator (e.g. median).

1. **Synthetic data** sets of the same size are obtained by sampling with replacement from the data.
2. The **estimator is computed** on each synthetic data set.
3. The distribution of the estimators obtained on the syntetic data sets is summarised through 95% quantiles.

Results on sample dataset:



How NOT to perform grouping:

Groups should be made based on covariates different than HIV-phyloTSI inputs or outputs.

Do NOT:

1. Group by estimate of HIV-phyloTSI
2. Group by estimated date of infection
3. Again: chose groups of sizes < 40

Other analyses on population level outputs:

Estimation of generation time distribution

Enriching source-recipient pairs by providing time since infection.

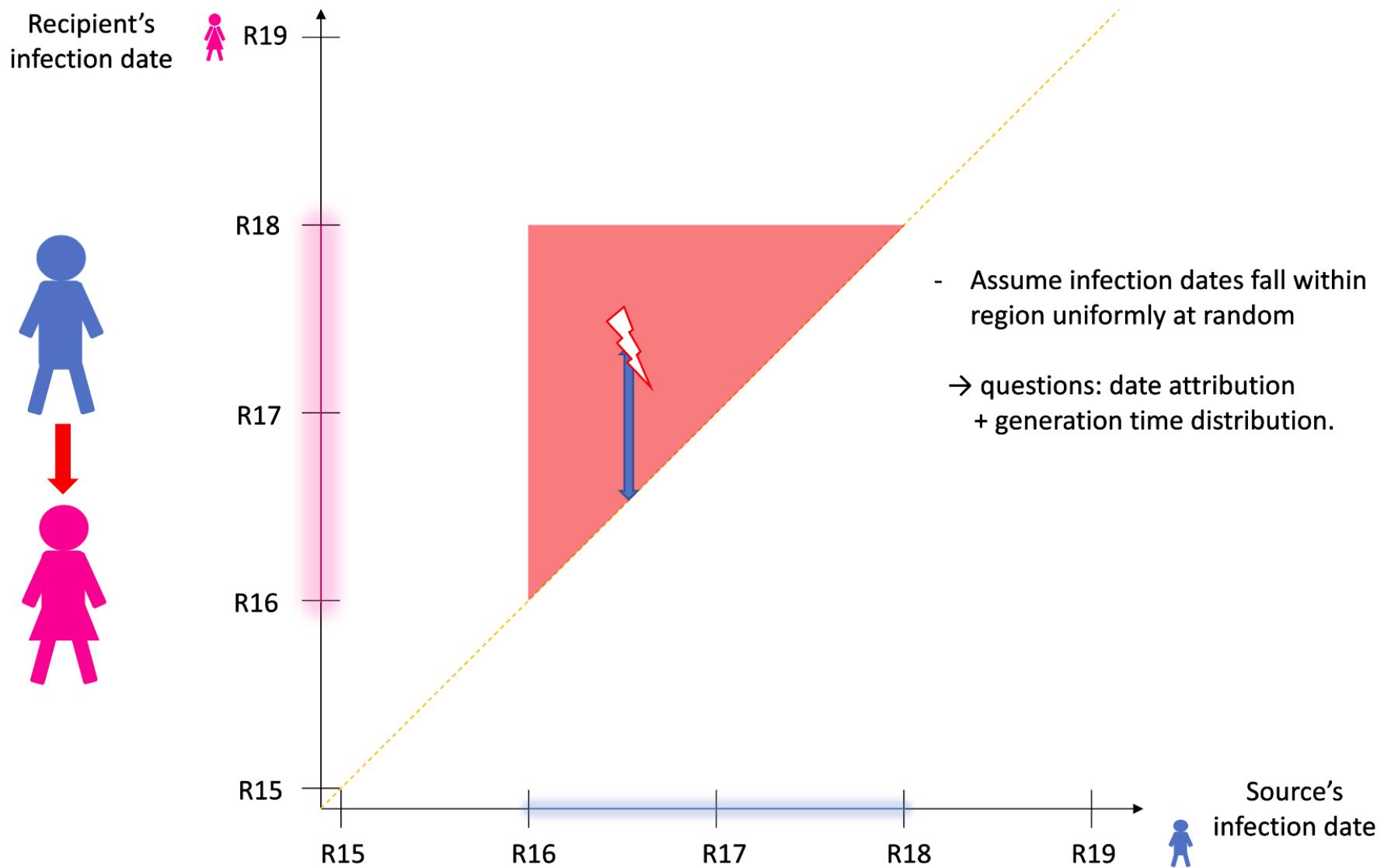
- Zambia ([Hall et al. 2021](#))
- Rakai: ([Monod et al. 2023](#))

 **Important**

Dating infection events in Rakai

- Infection pairs data from Rakai Community Cohort Study
- Transmission pairs detected with phyloscanner Ratmann et al. (2020)
- Question: how did transmission pattern change over time?
- Need to date infections

Dating infection events in Rakai



Summing up

- HIV-phyloTSI is a novel algorithm to estimate infection dates.
- alternative to serological assays which allows more control on definition of recency (Robust to subtype and ART usage)
- preliminary analyses and simulation studies demonstrate good performances at population level
- but may be inaccurate at the individual level: very large prediction intervals.
- Can help us explore answers to unanswered questions

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[Code and slides](#)