Conference on the Evolutionary Genetics of Infectious Disease

Unsupervised excursions into the deep evolutionary history of HIV-1 group M

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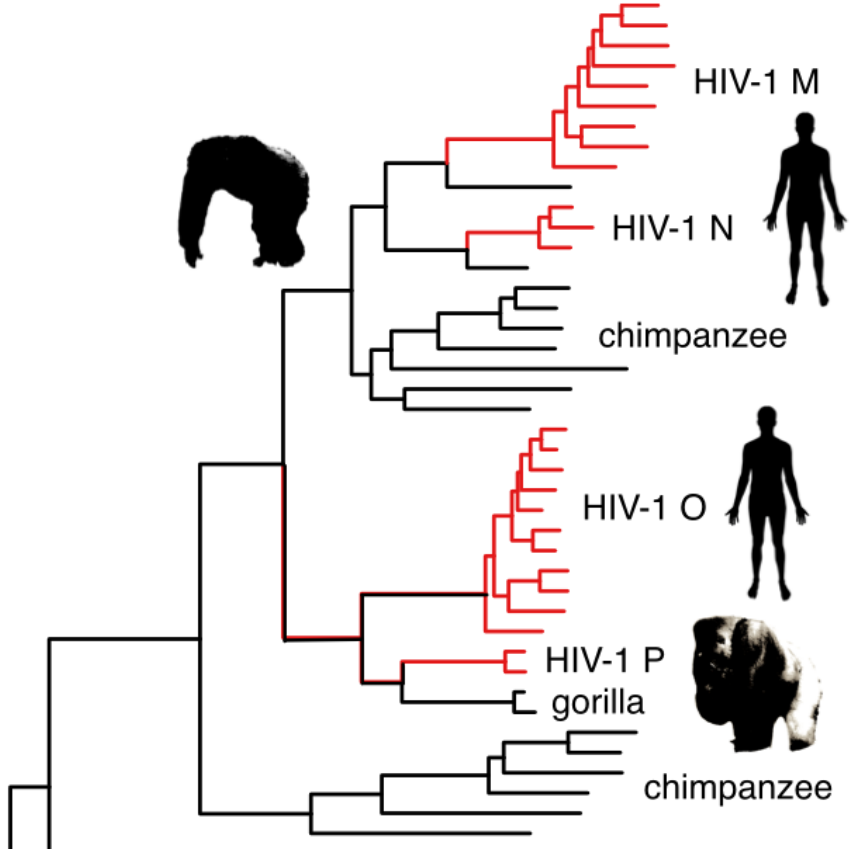
Phylogeny of HIV/SIV *gag*



Excerpt of figure from Joy *et al* (2015) Global Virology I, Springer. Generated by maximum likelihood.

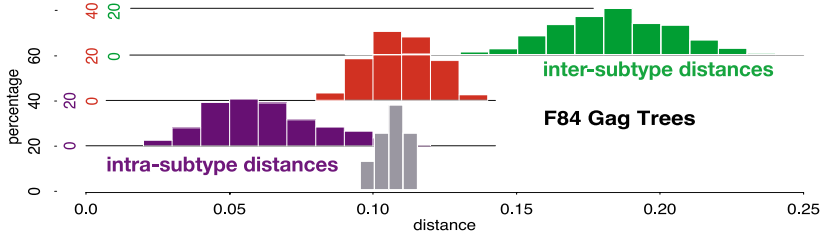
HIV-1 subtypes

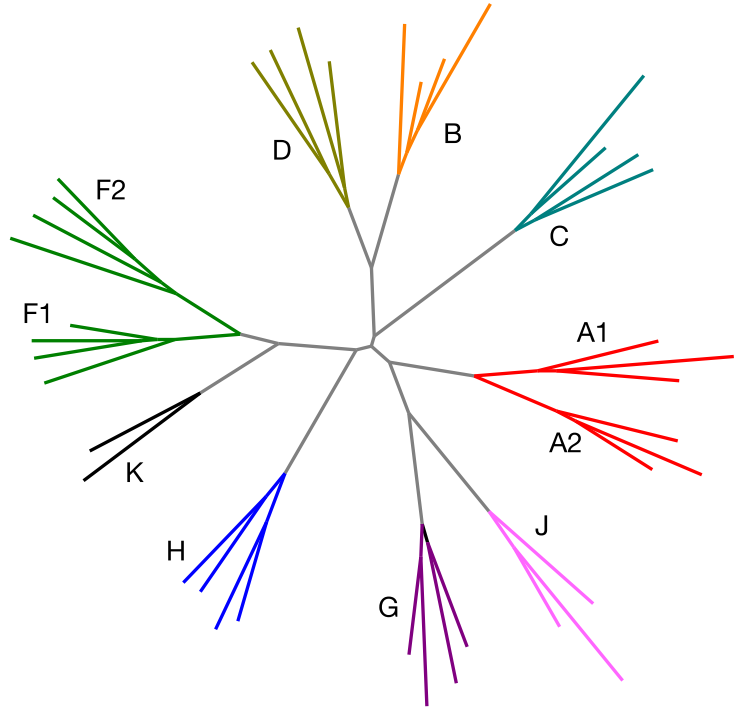
- Extraordinary diversity structured into subtypes.

HIV-1 Nomenclature Proposal

A Reference Guide to HIV-1 Classification

D.L. Robertson[1], J.P. Anderson[2], J.A. Bradac[3], J.K. Carr[4], B. Foley[5], R.K. Funkhouser[6], F. Gao[7], B.H. Hahn[7], C. Kuiken[5], G.H. Learn[2], T. Leitner[8], F. McCutchan[4], S. Osmanov[9], M. Peeters[10], D. Pieniazek[11], M.L. Kalish[11], M. Salminen[12], P. Sharp[13], S. Wolinsky[14], and B. Korber[5,6]





(*above*) ML tree from LANL curated (2010) HIV-1/M env subtype references, aligned with MAFFT (excluding indel-rich regions) and reconstructed with RAxML.

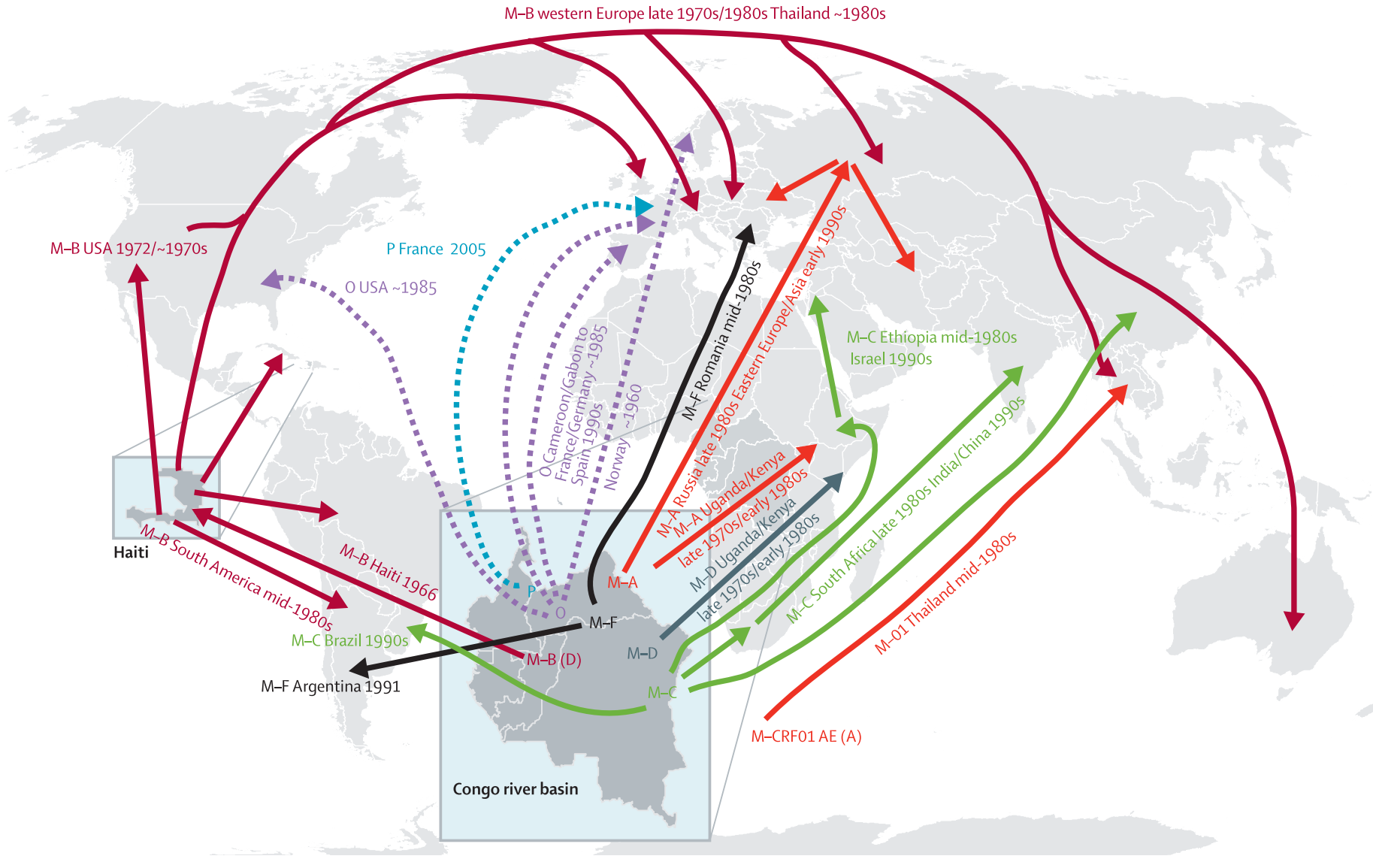
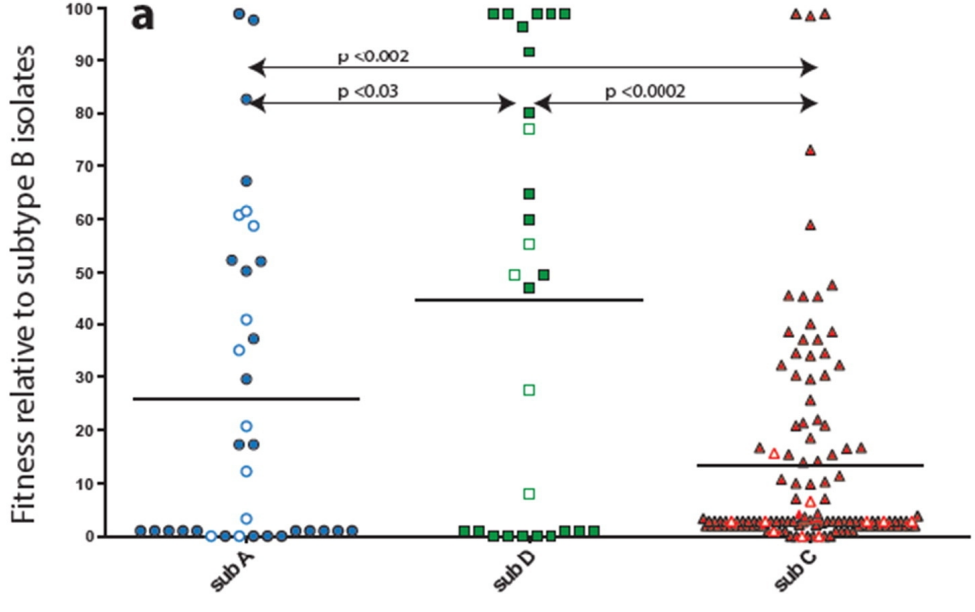


Figure from Tebit and Arts (2011) Lancet Inf Dis 11: 45-56.

Impact of HIV-1 subtypes

- Understanding the global epidemiology of HIV-1/M
- Variation in rates of progression to AIDS (subtypes C, D)



- Distinct mutational pathways to evolve drug resistance.

Figure from C Venner *et al.* (2016) EBioMedicine 13: 305-314.

Subtyping

- Compare a sequence to a reference database of "pure" subtype sequences
- Identify recombinant as a combination of two or more parent reference sequences.
- Sliding window methods (BOOTSCAN, REGA)
- Phylogenetic methods (SCUEAL)
- Linear classifiers (COMET)

Recombinant history of SIV

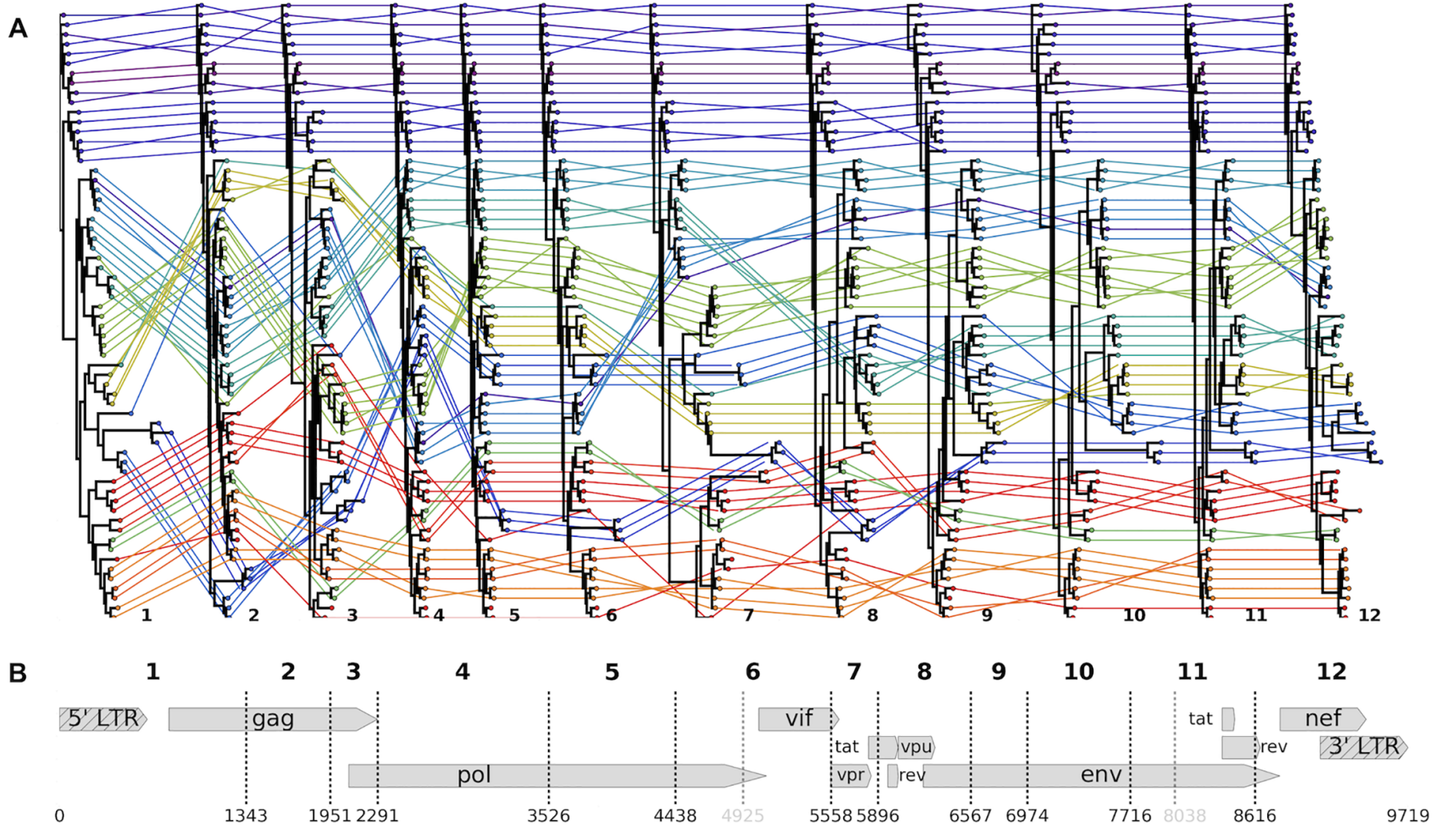


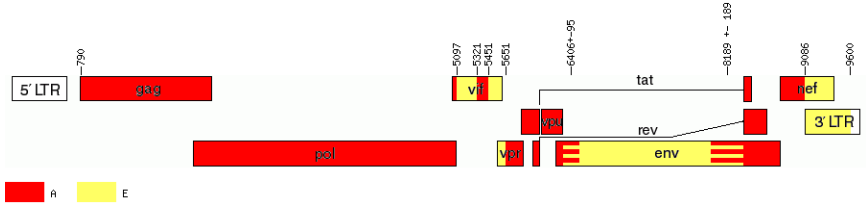
Figure from Bell and Bedford (2017) PLOS Pathog 13: e1006466.

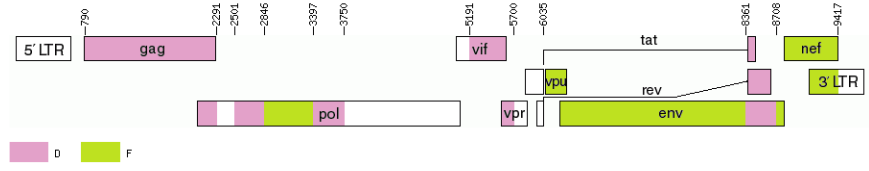
HIV-1 recombination

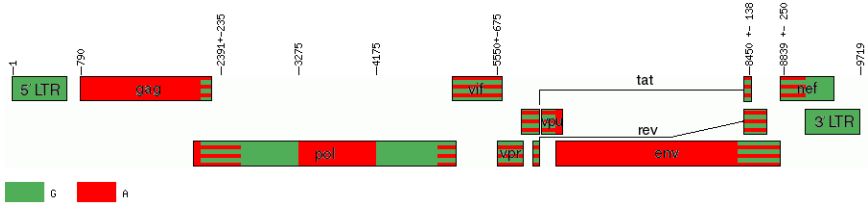
- Virus carries two copies of its genome.
- HIV reverse transcriptase switches templates during infection cycle.
- If a host cell is multiply infected, progeny virus can be recombinant.
- Effective recombination rate estimated at about 1%/genome/generation[1].
- Close to estimated mutation rate (0.2/genome/generation)[2].

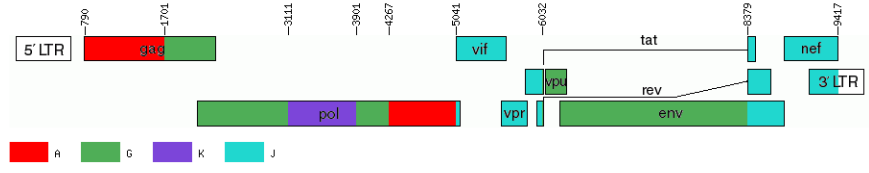
[1] RA Neher *et al.* (2011) PLOS Comput Biol 6(1); [2] F Zanini *et al* (2017) Virus Evol 3(1).

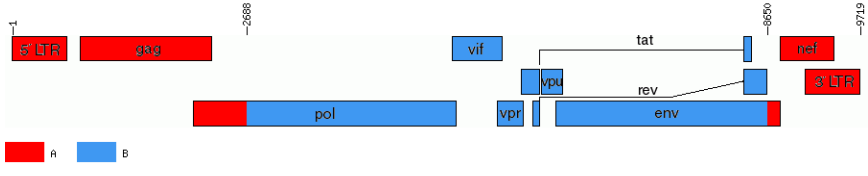
Circulating recombinant forms

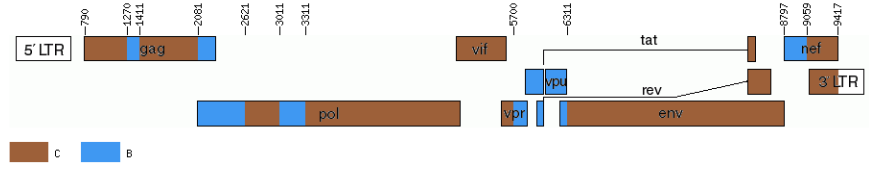


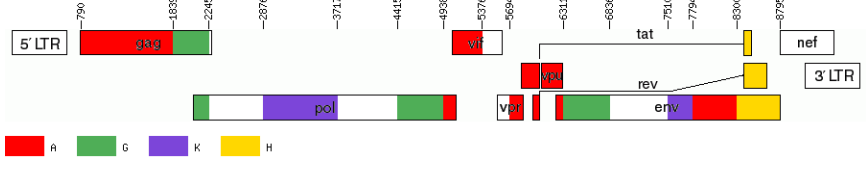


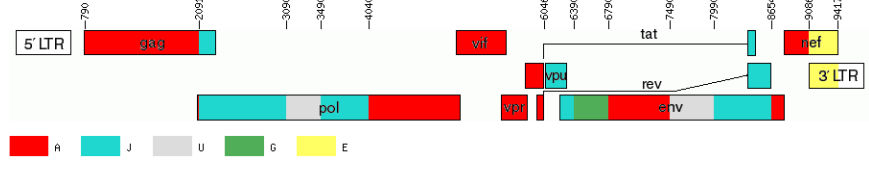






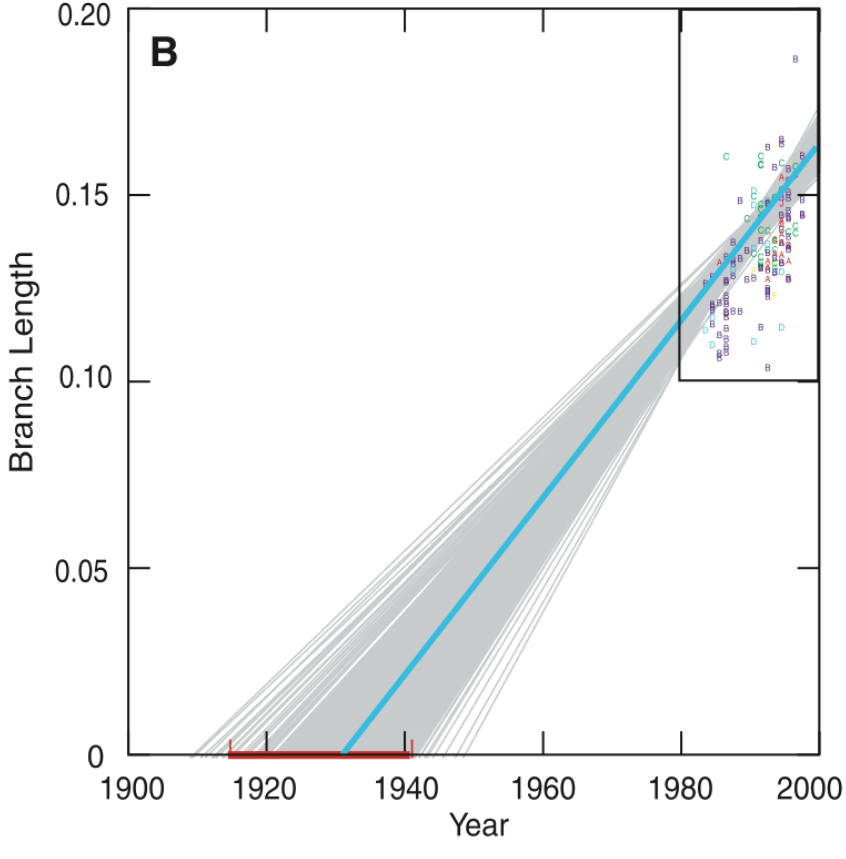






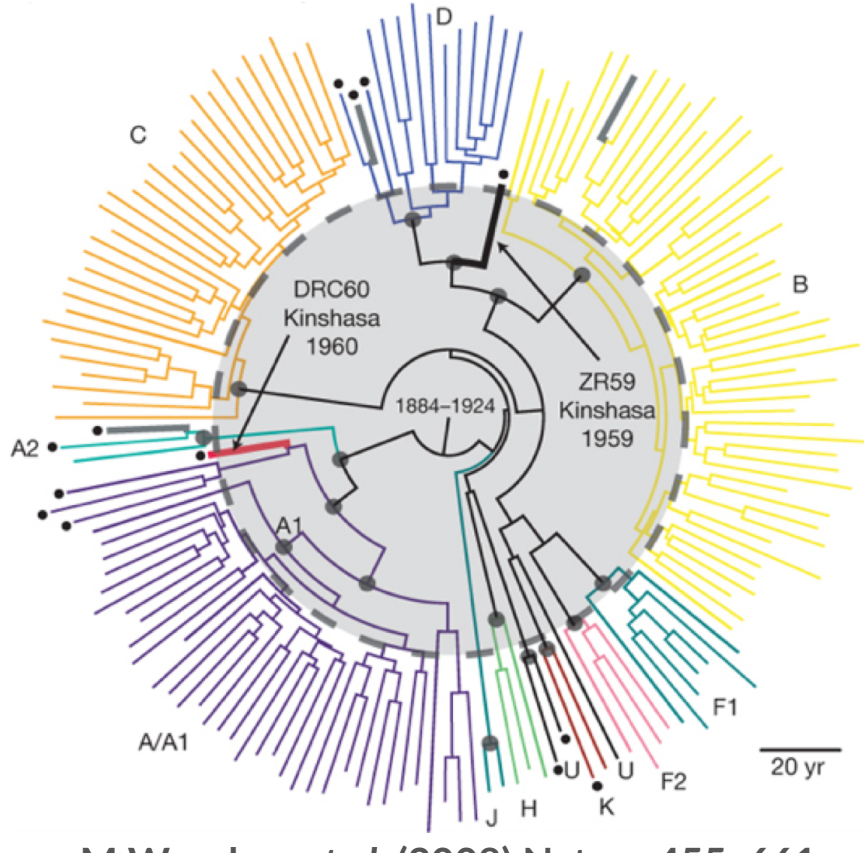
Dating the origin of HIV-1/M

Root-to-tip regression



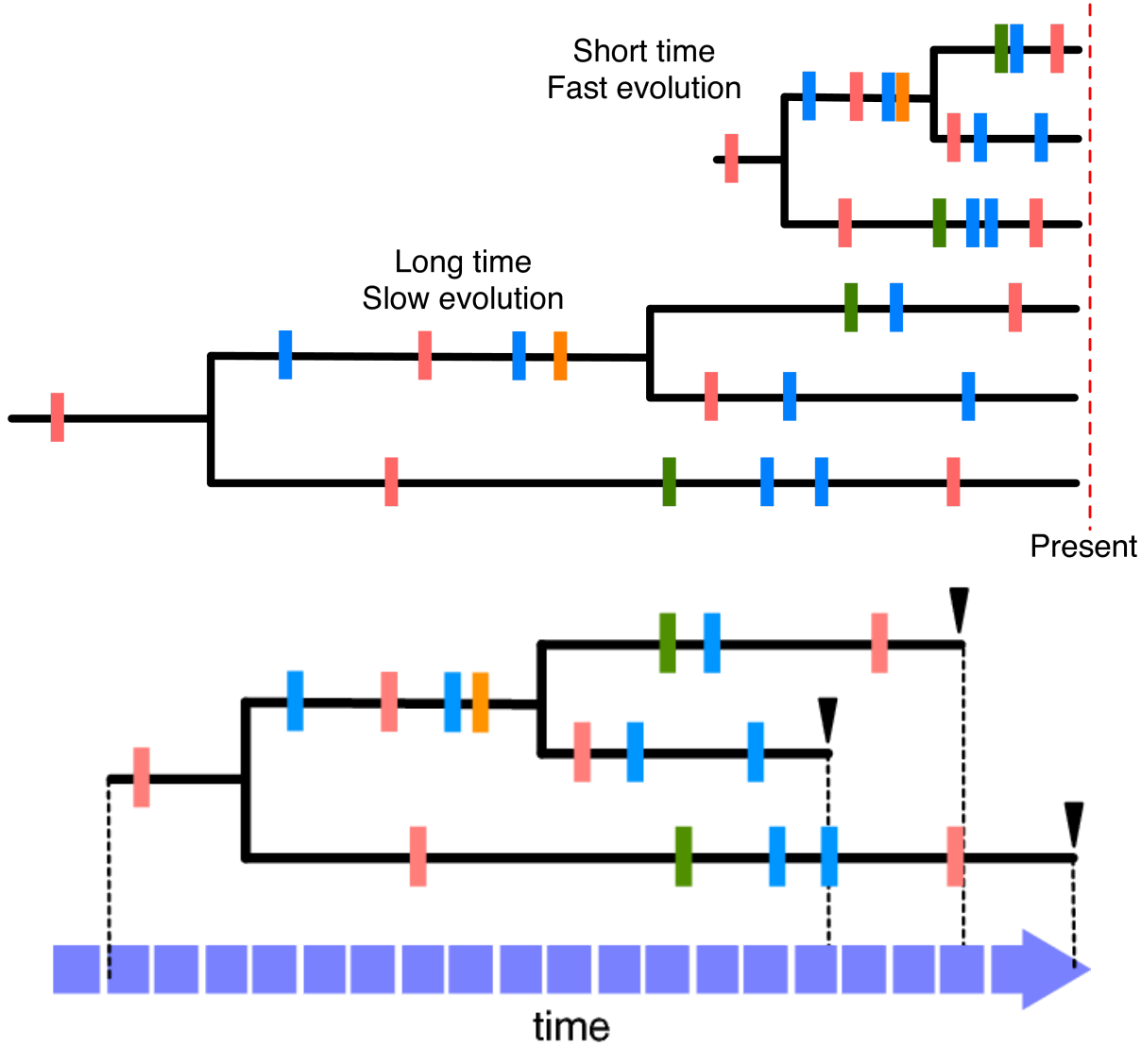
from B Korber *et al.* (2000) Science 288: 1789.

Bayesian sampling



from M Worobey *et al.* (2008) Nature 455: 661.

Molecular clock dating



More genomes

- The number of near full-length HIV-1 genomes in public databases is nearing 10,000
- A recent global consortium (PANGEA-HIV) is generating an *additional* 10,000 HIV-1 genomes sampled throughout Africa.
- An emerging consensus is that recombination is more widespread than we thought.

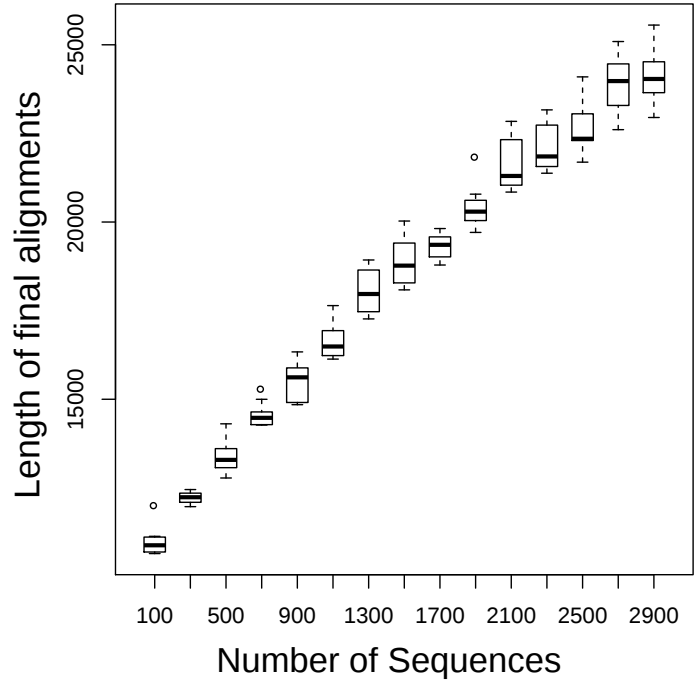
> *Given the inevitability of extensive recombination, it is highly unlikely that a single phylogeny can adequately represent the evolutionary history of HIV-1/M.*

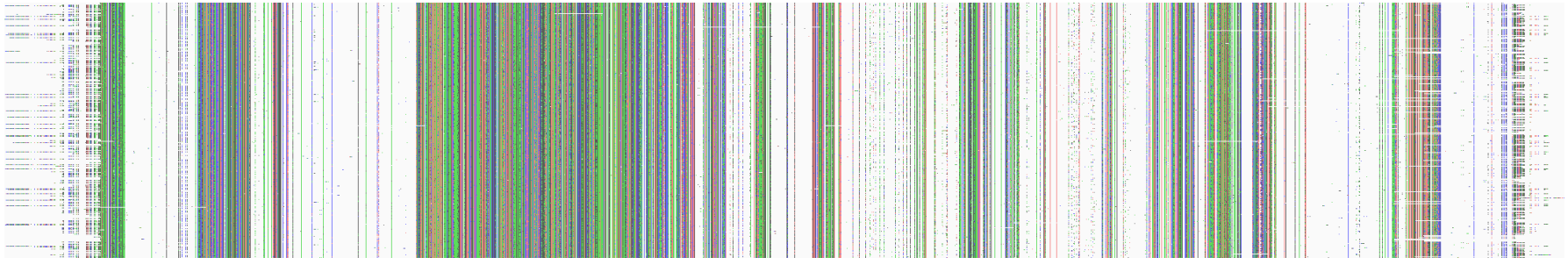
Data collection

- Queried Genbank for HIV-1 sequences of minimum length 8,000nt ($n = 7,816$)
- Manually reviewed all entries for *in vitro* clones, repeated samples, and non-group M variants.
- Reviewed associated literature for missing collection dates.
- Final total: $n = 3,900$ genome sequences.

Alignment

- An alignment is a hypothesis about the evolutionary homology of specific residues between two sequences.
- The genomic diversity of HIV-1/M causes the alignment to explode with regions of low homology.





Procrustean alignment

- Aggressive pairwise alignment of each sequence against a reference genome.
- Any insertions relative to the reference are *discarded*.
- We need the most representative reference possible!

Alignment-free clustering

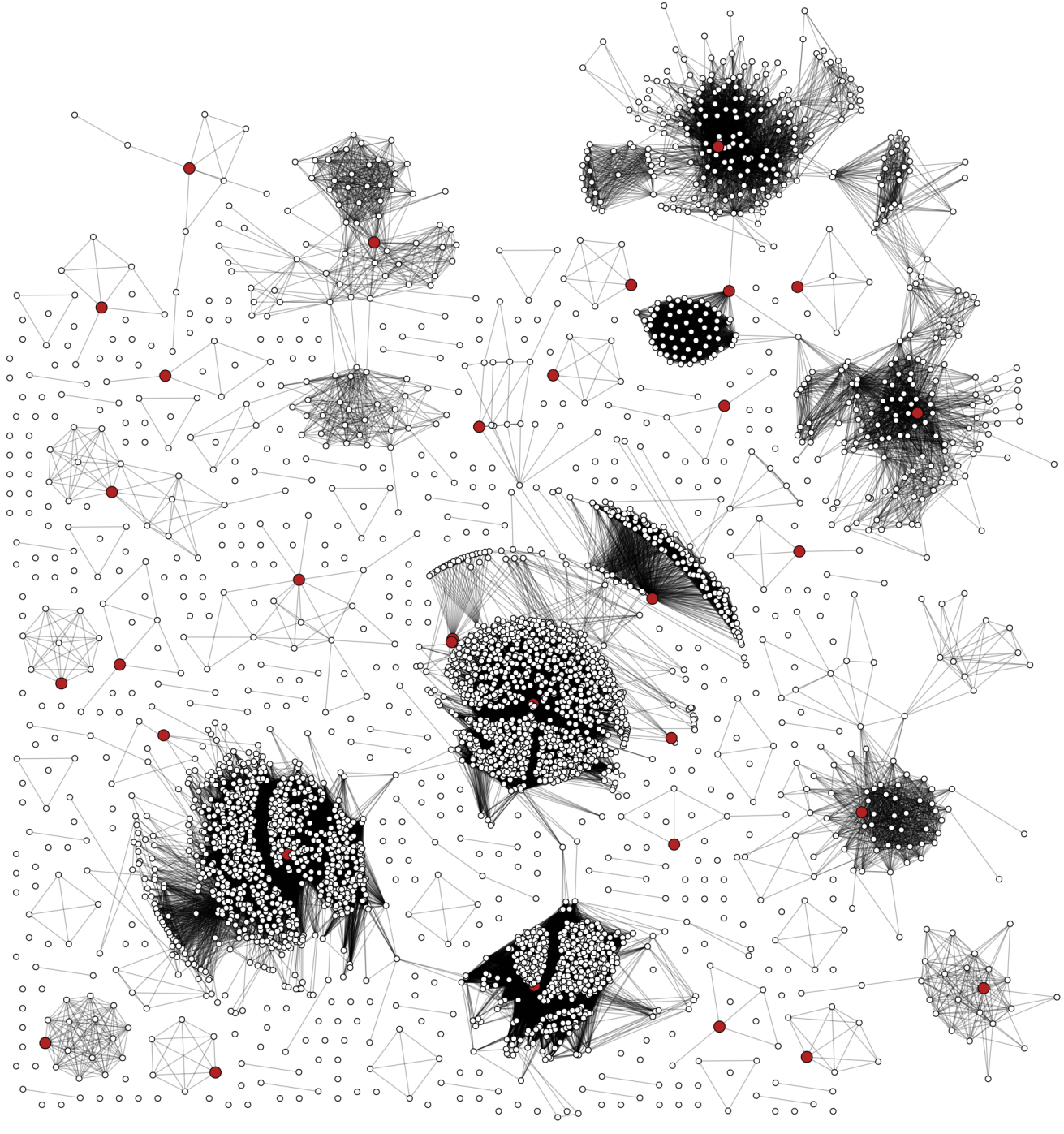
- Use a p-spectrum kernel distance to cluster genomes:

$$k(s,t) = \sum_{u \in \mathcal{A}^6} C(u,s)C(u,t)$$

where $\mathcal{A} = \{A, C, G, T\}$ and $C(x,y)$ counts occurrences of substring x in y.

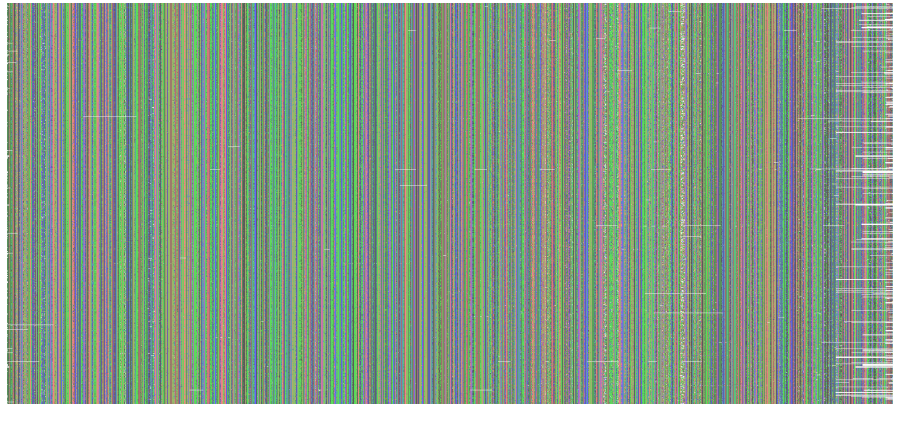
- We use the standard cosine normalization:

$$k'(s,t) = \frac{k(s,t)}{\sqrt{k(s,s)k(t,t)}}$$



Consensus sequence

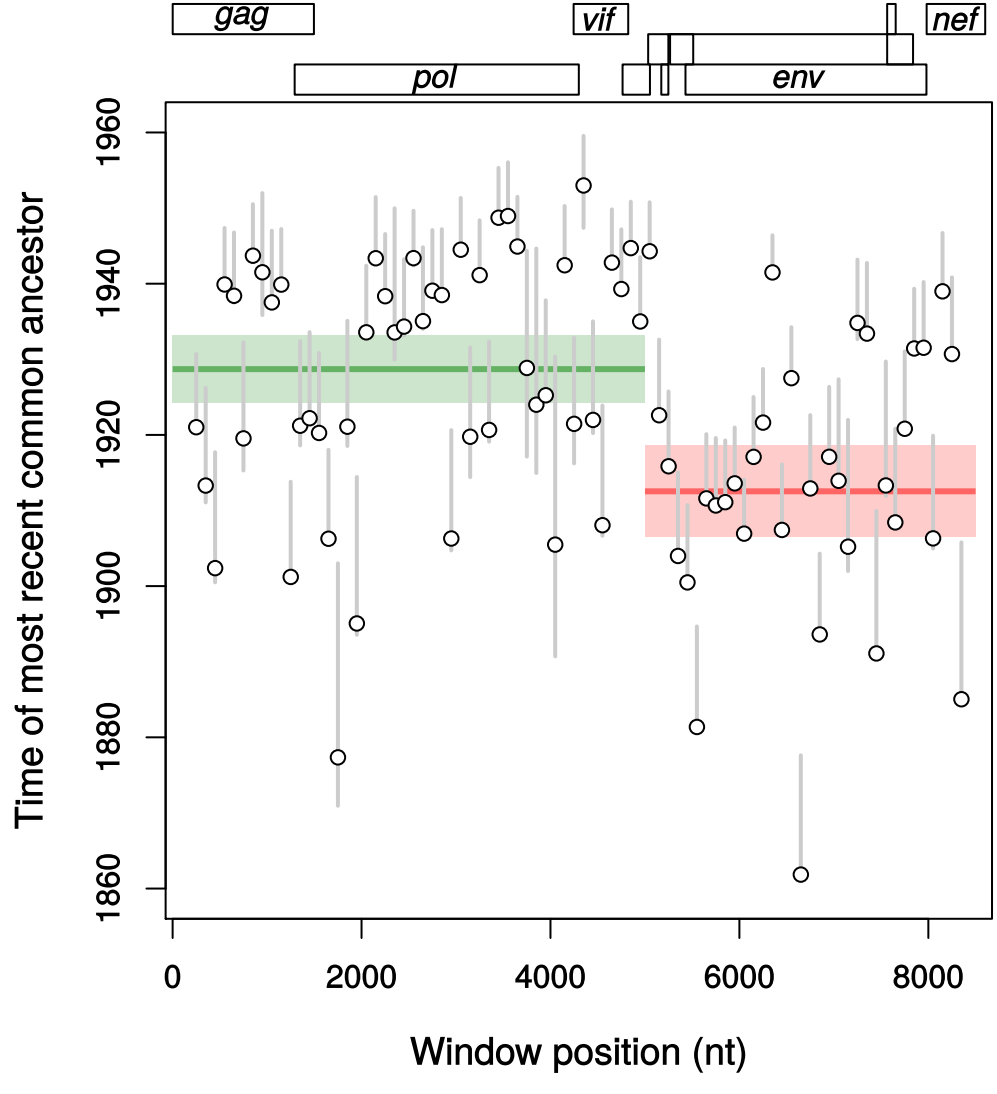
- Clustered genomes in this similarity graph with community detection algorithm (R *igraph* `cluster_leading_eigen`).
- Selected $n = 32$ centres with highest degree centrality.
- Multiple alignment of central genomes to make majority-rule consensus for Procrustean alignment.
- Final alignment 10,171nt in length.



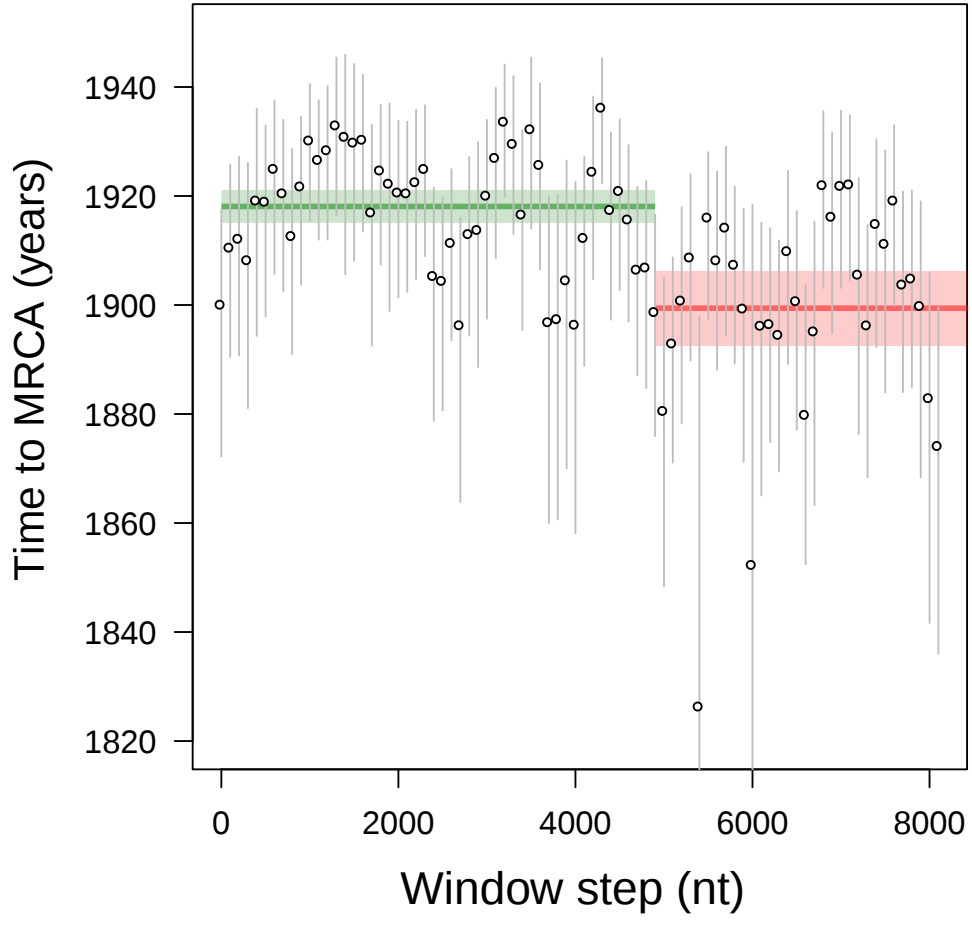
Sliding windows

- Extracted 81 windows of 500nt in steps of 100nt.
- For each window:
  - Reconstructed phylogeny by approximate ML (FastTree2).
  - Rooted phylogeny by root-to-tip regression (rtt)
  - Estimated time to the most recent common ancestor (TMRCA) under relaxed molecular clock (LSD).

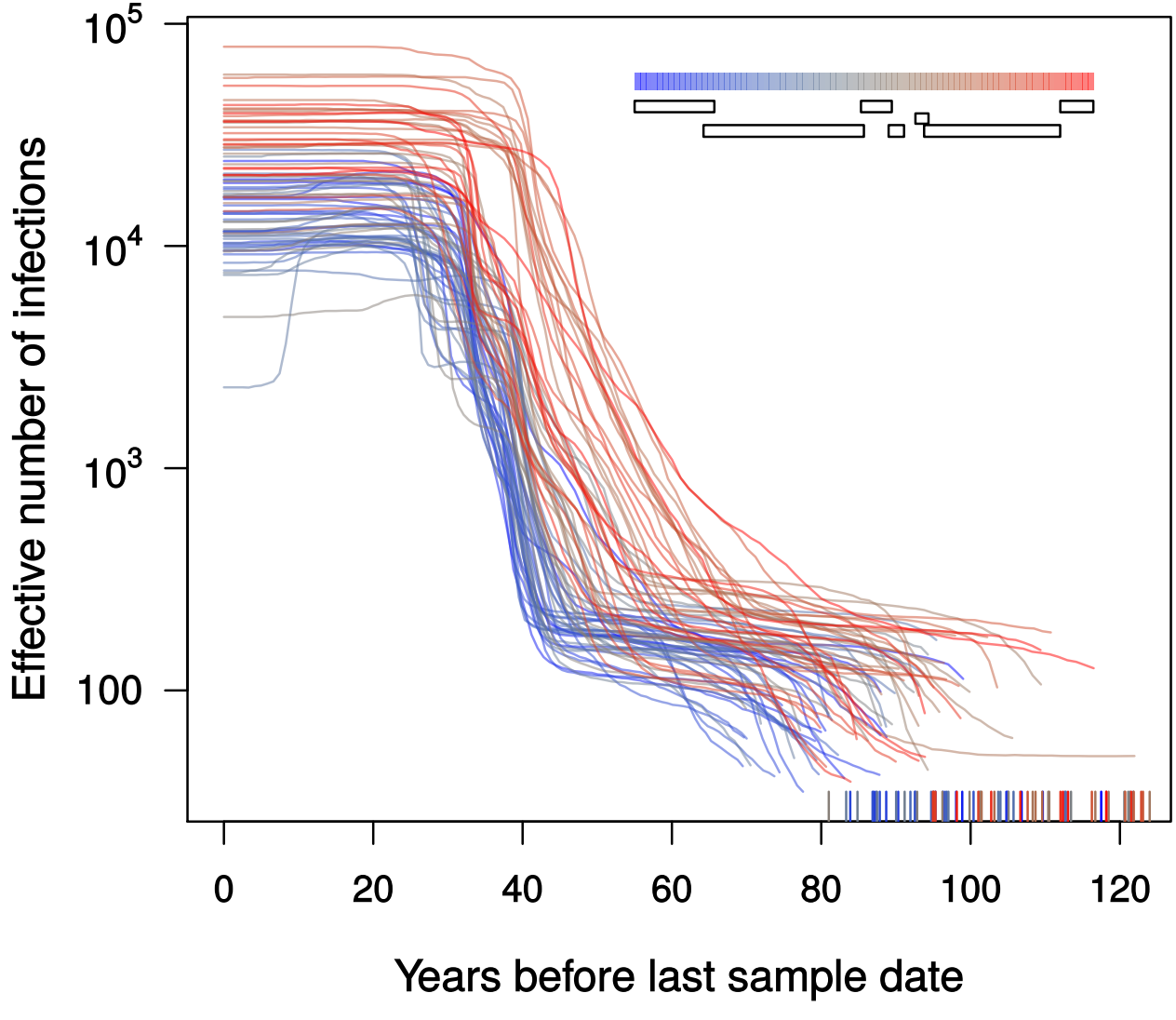
TMRCA are variable and autocorrelated along the HIV-1 genome



Similar results obtained using BEAST (data downsampled to ~300 tips).



Demographic "skyline" reconstructions from BEAST

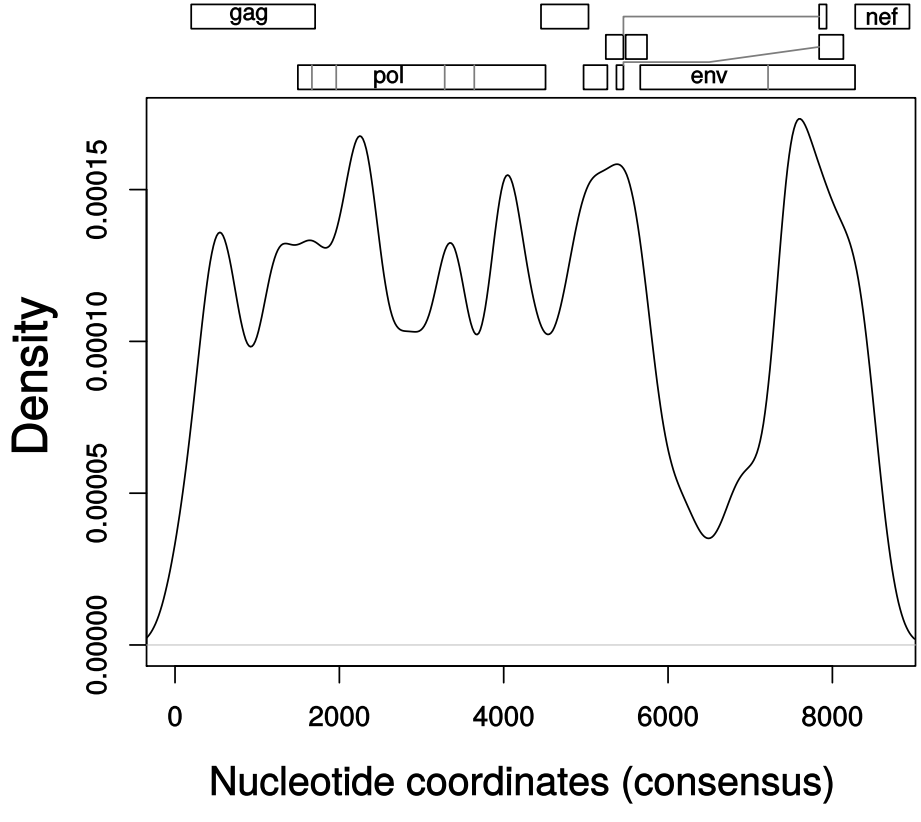


A deep recombination breakpoint?

- Why does HIV-1/M look older in *env*?
- Purifying selection may skew estimates of TMRCA.
  - No evidence of greater purifying selection in *gag* and *pol* versus *env*.
  - No evidence of saturation when comparing transitions to transversions.
- Examine signature of recombination.

Recombination analysis

- Implemented a conventional distance-based recombination detection method in Python.
- Putative breakpoints less frequent within *env*.



What does this mean?

- The deep history of HIV-1/M is recombinant.
- Is the concept of "pure" subtypes still useful?
- HIV-1 subtypes and sub-subtypes are being re-assessed.
- Selective introgression of HIV-1 *env*: first contact with the virus envelope?

A recombinant history

- How can we detect recombination if our reference points are themselves recombinant?
- Postulate 1: We cannot meaningfully describe recombinants as a mixture of "pure" subtypes on the time scale of HIV-1/M.
- Postulate 2: The recombinant history is too complex to reconstruct, *e.g.*, reticulate phylogenies.
- Postulate 3: There is enough residual homology to infer the evolutionary relationships of genome fragments.

Back to networks

- Let the homology of fragments be represented by a similarity graph.
- Graph clusters correspond to subtypes.
- As we move along the genome, fragments may shift from one cluster to another due to recombination.
- This process is analogous to a dynamic social network.

Stochastic blockmodeling

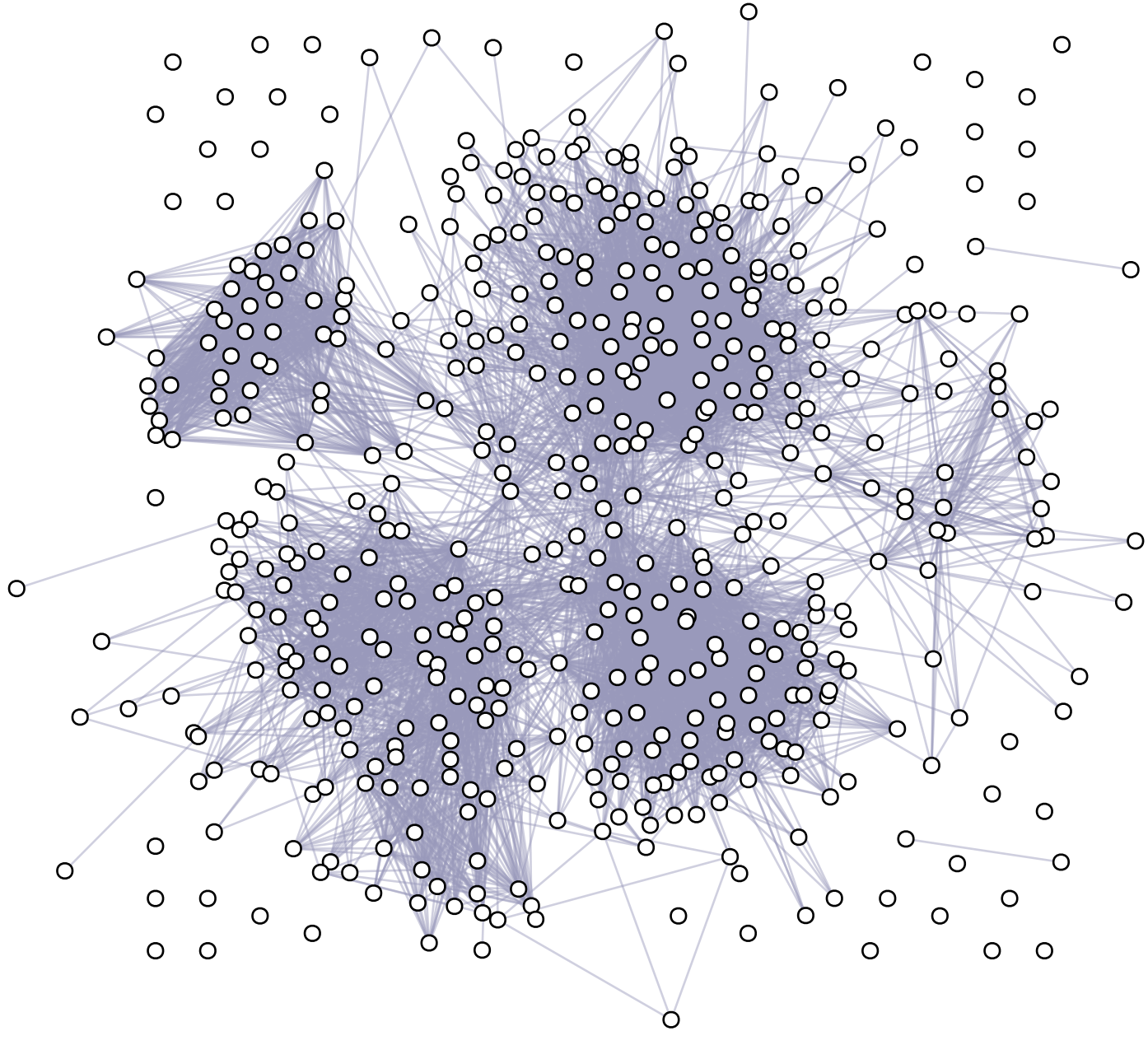
- Generative models for network communities.
- Each node is assigned to one of K communities (blocks) — block membership is a latent (unobservable) state of the node.
- Different edge probabilities within and between blocks.
- Transition of nodes among blocks over time is described by a Markov chain.

Data reduction

- SBM becomes computationally non-feasible with the complete data set.
- We reduced the $n = 3,900$ genome sequences to the most diverse $n = 500$.
- Progressive "pruning" of the shortest branches from the ML phylogeny.

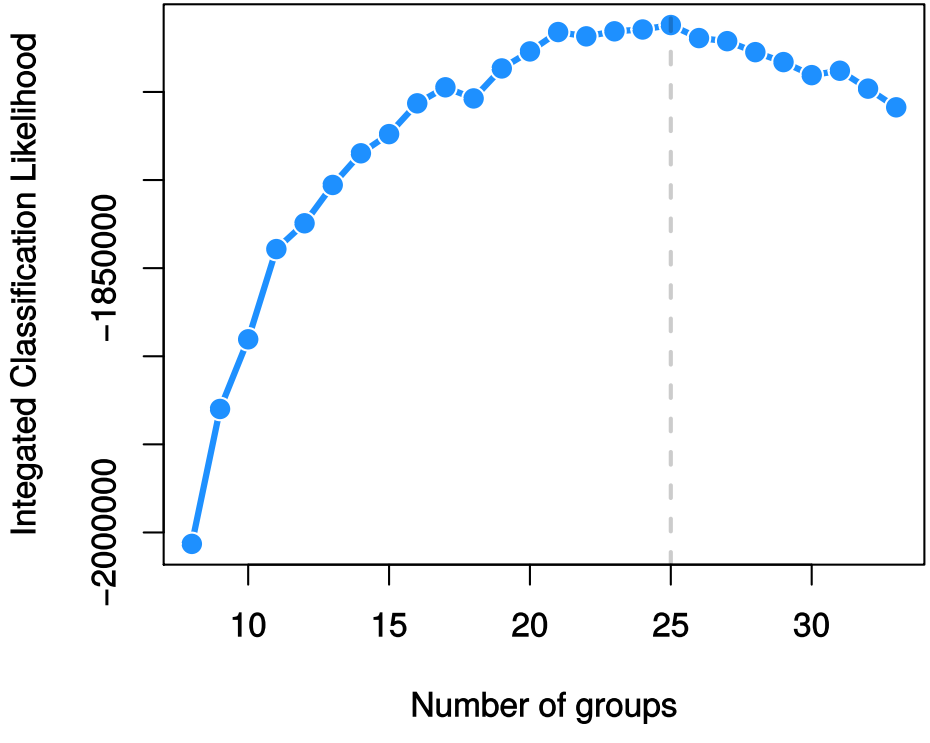
Constructing a graph

- We calculated the Tamura-Nei (1993; TN93) genetic distance for every pair of fragments in a window.
- Used the lower 10% quantile of the TN93 distribution as the graph-defining threshold for each window.
- Processed the series of graphs along the HIV-1 genome with dynamic stochastic block models (R package dynsbm; Matias and Miele).

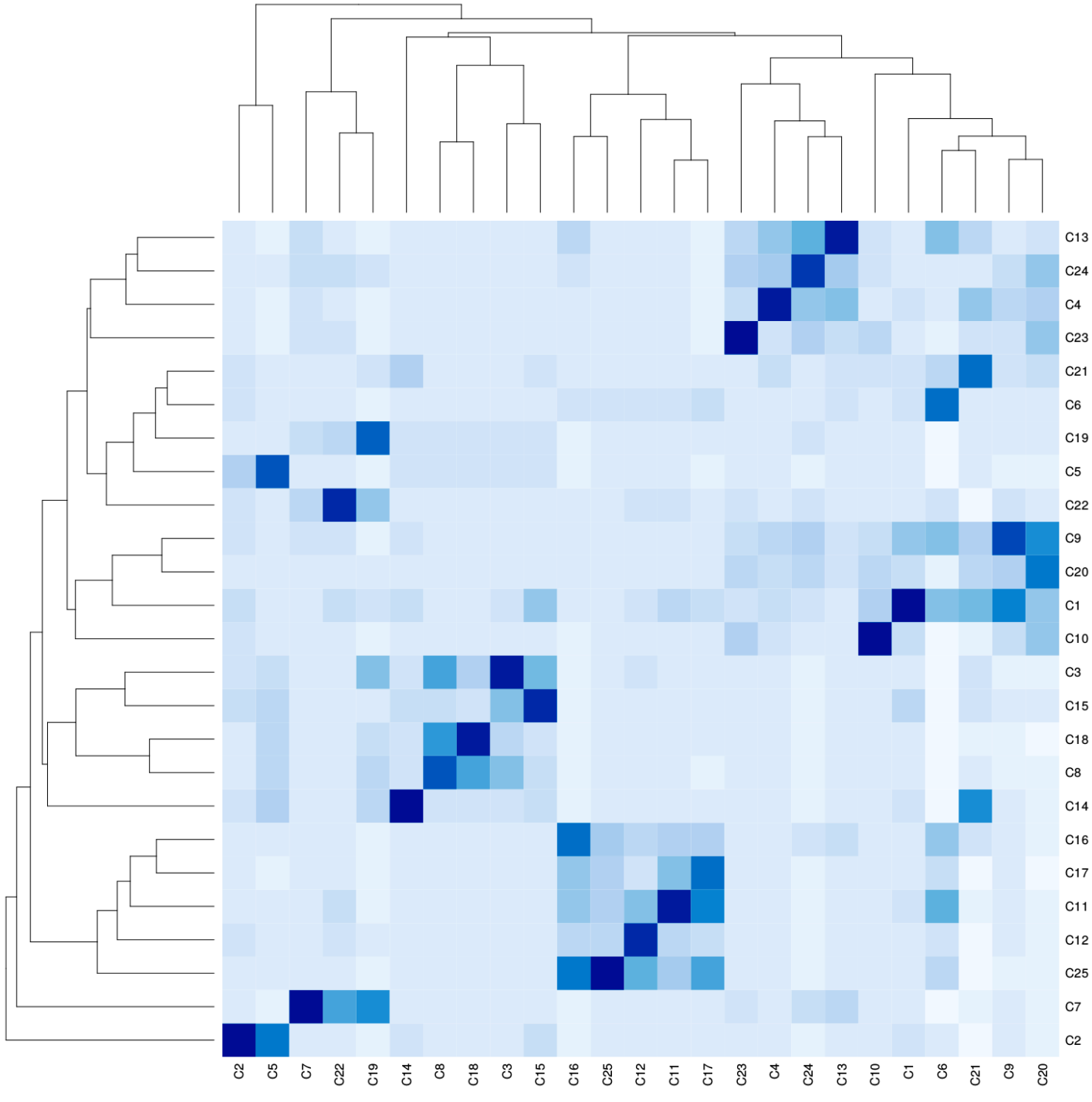


Optimal K=25

- Integrated classification likelihood (ICL) criterion avoids overestimation of the number of clusters by penalizing likelihood[1].



[1] C Biernacki *et al.* (1998) Assessing a Mixture Model for Clustering with the Integrated Classification Likelihood. INRIA.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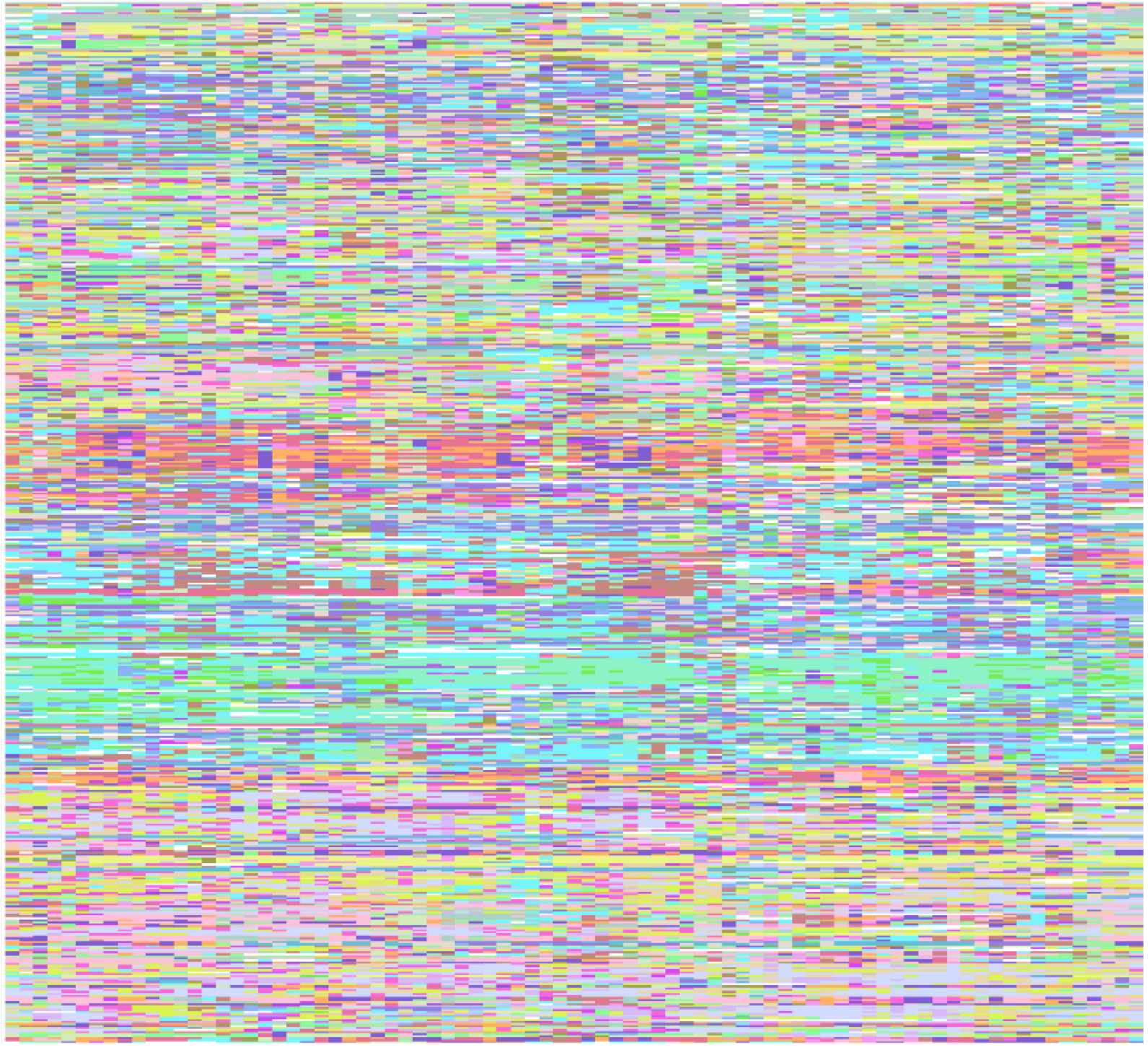
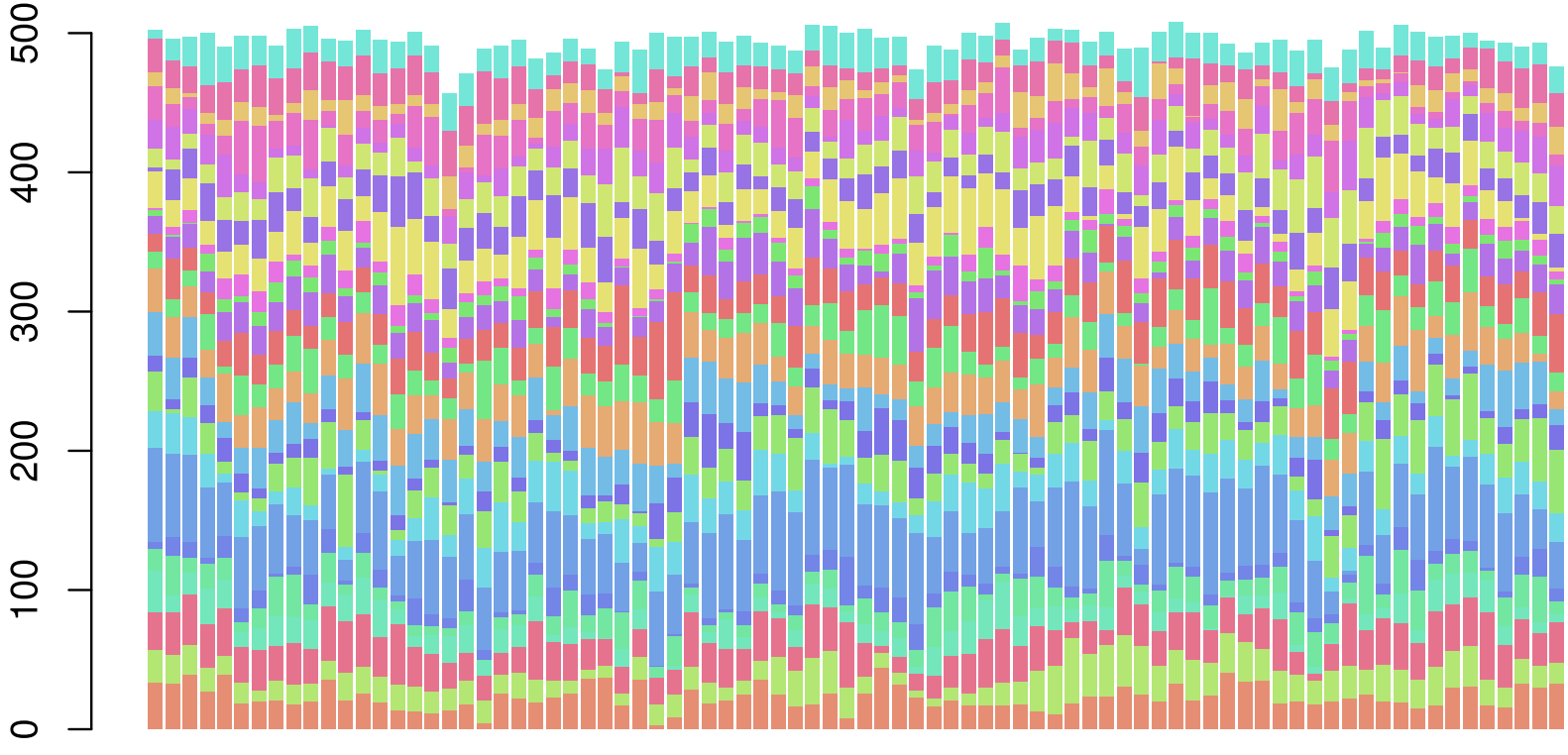
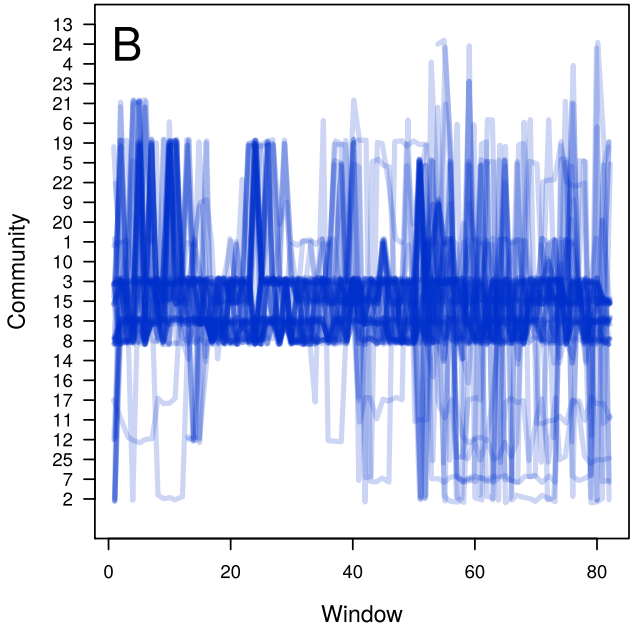
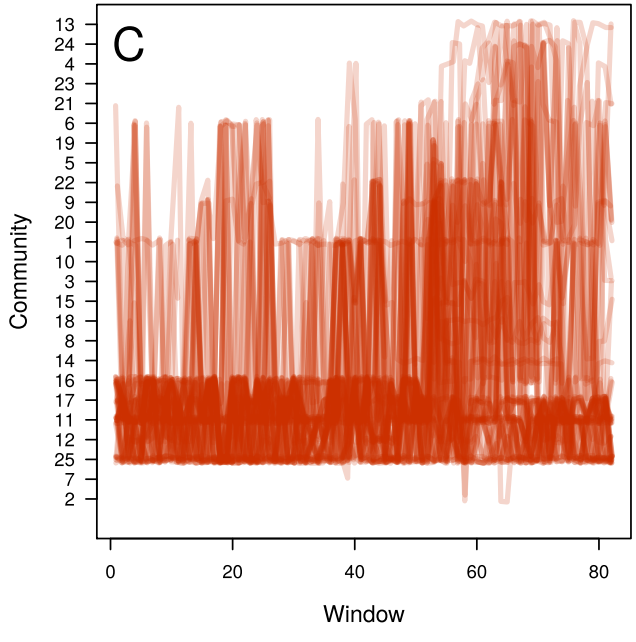


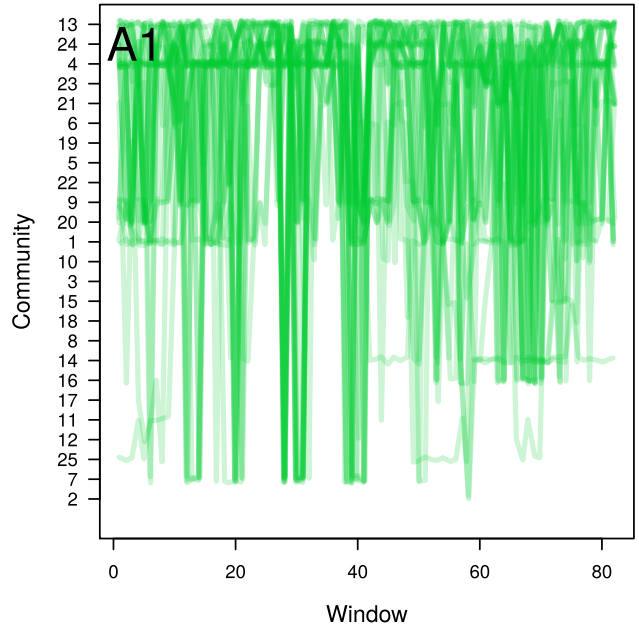
Communities are fairly stable



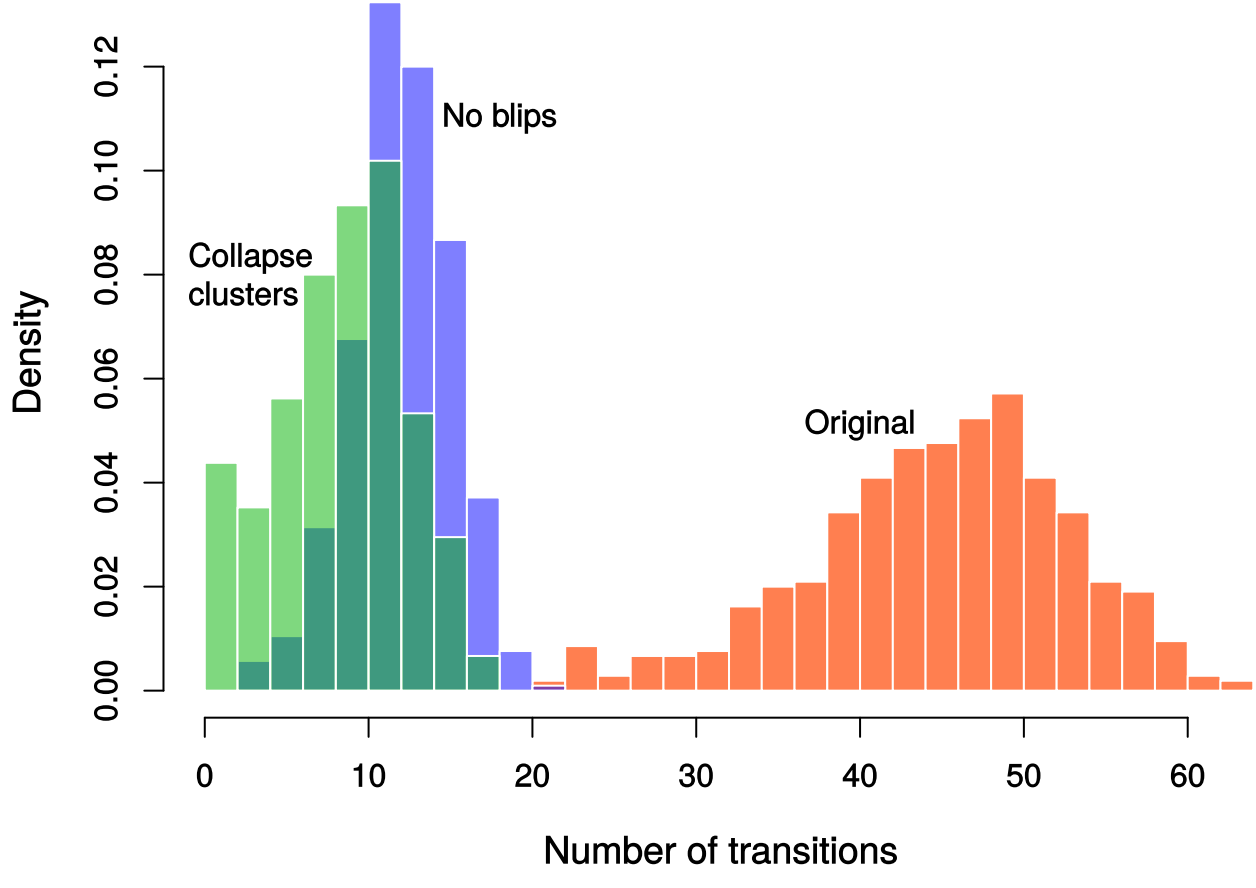
Communities correlate with major subtypes



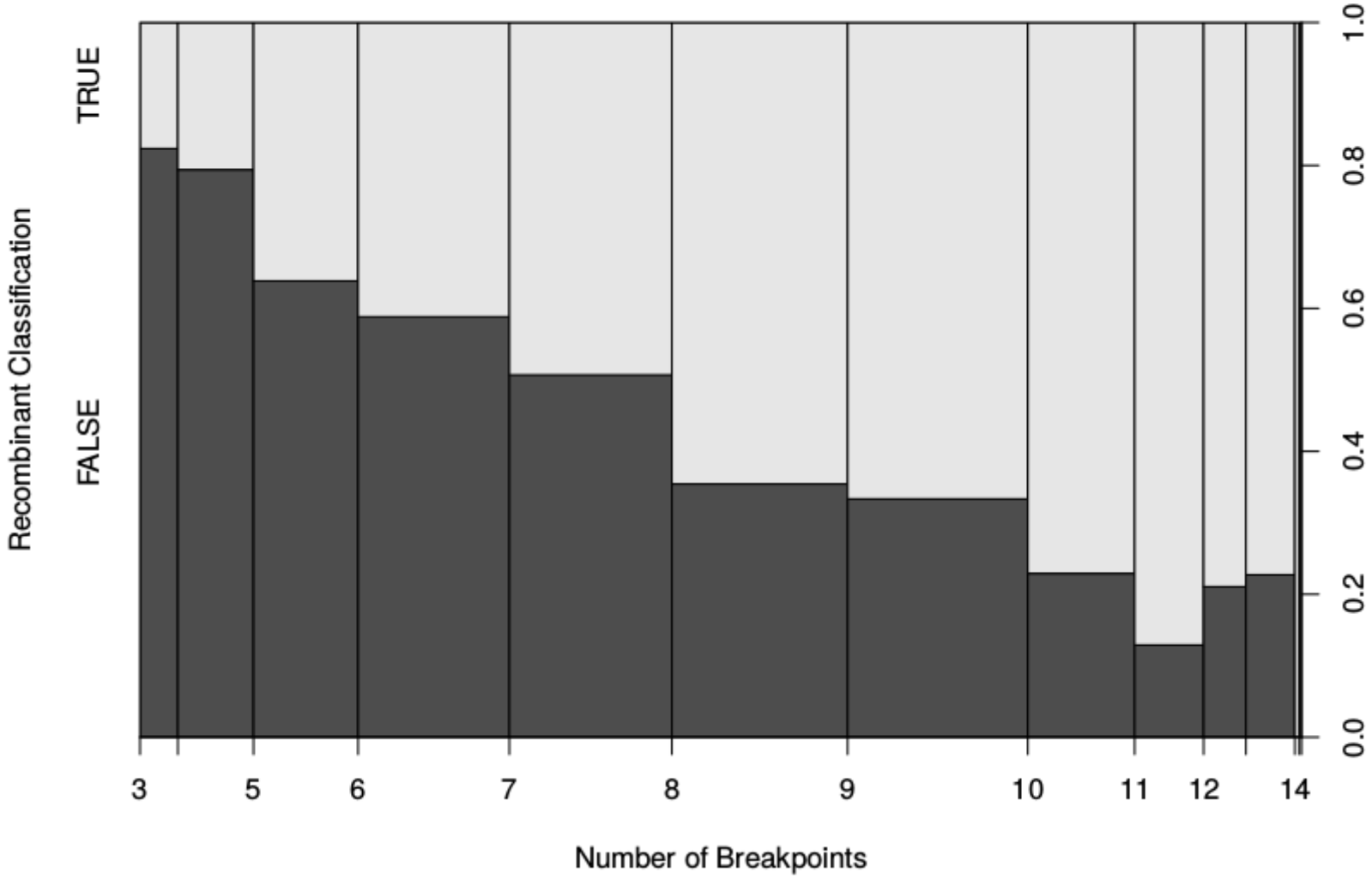




Number of putative breakpoints



Community transitions are consistent with "known" recombinants.



Plot excludes "blips" (transitions from A to B immediately followed by a revision from B to A in the next window).

A new nomenclature proposal?

- Unsupervised clustering of genomic variation may define new centres of diversity.
- Can we select new reference points for characterizing recent recombinants?
- Need simulation experiments to assess what network communities really mean.

Left to right: Dr. Mariano Avino, **Dr. Abayomi Olabode**, Garway Ng, Lisa-Monique Edward, John Palmer, Connor Chato, *Art and Fiona*, David Dick, Laura Muñoz-Baena. (Photo credit: Marin Poon.)

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