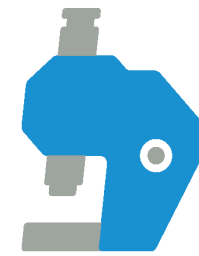


The NIH logo consists of the letters "NIH" in white, bold, sans-serif font, set against a dark blue background that is shaped like a right-pointing arrow.

National Institute of
Allergy and
Infectious Diseases



**Rakai Health
Sciences Program**

Improved Health Through Research

Rakai Couples Studies and Molecular Epidemiology Informing HIV Transmission and Pathogenesis

Oliver Laeyendecker MS, MBA, PhD

Epidemiologist, LIR, NIAID, NIH

Assistant Professor of Medicine, SOM, JHU

Assistant Professor of Epidemiology, JHSPH



**JOHNS HOPKINS
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SCHOOL OF PUBLIC HEALTH

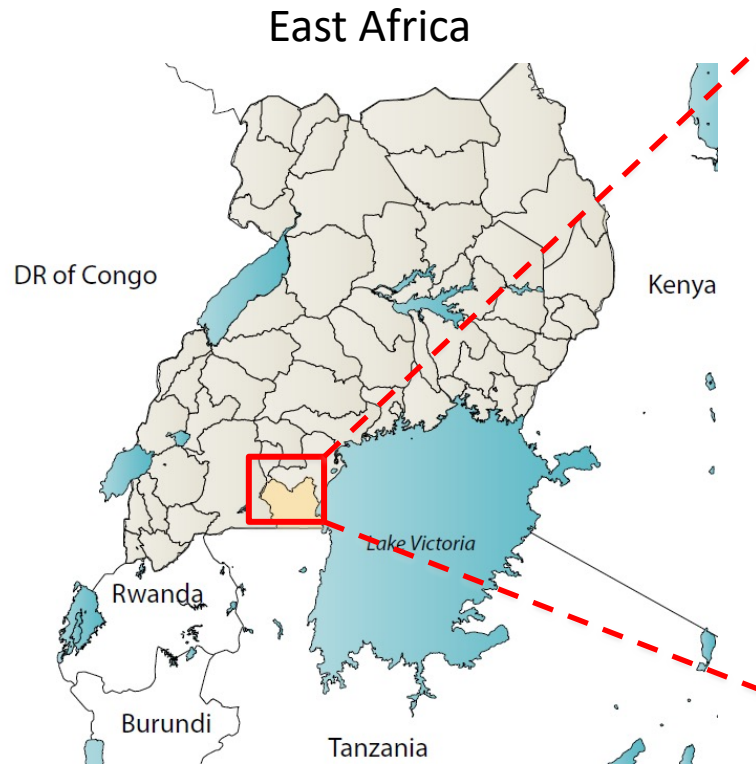
Objectives

- **Viral factors associated with transmission**
- **Linkage of transmission**
- **Viral factors associated with pathogenesis**
- **Population level changes in HIV subtype distribution**
- **Directionality of transmission and spatial studies**

Overall Purpose: To determine viral factors that influence transmission and the health of the host, identify potential targets for interventions
- vaccine and therapeutic designs
- direct and elucidate the outcome of interventions

Rakai, Uganda

- More than 700 agrarian, fishing and trading communities
- Population ~500,000
- Epicenter of HIV in East Africa
- HIV transmission endemic: prevalence ~15%; incidence ~0.6 per 100 person-years.



Rakai Community Cohort Study (RCCS)

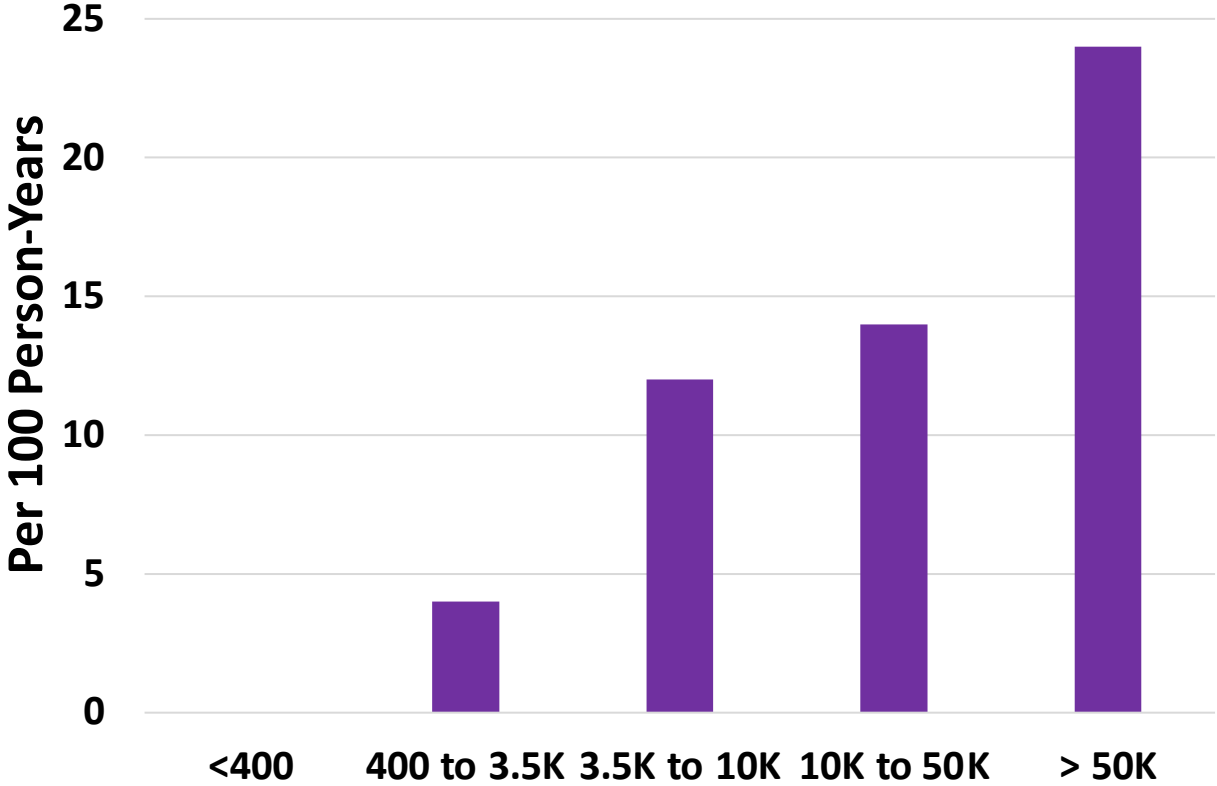
- Population-based HIV incidence cohort in 50 communities in Rakai District
 - Implemented by the Rakai Health Sciences Program
 - ~20,000 study participants in ~8000 households are surveyed at ~18 month intervals
- Ongoing since 1994 (19 survey rounds completed, 20th in progress)
 - ART introduced in 2004
 - Scale-up of male circumcision after 2006
- Study includes census and individual interviews.
 - Household census
 - Sociodemographic & behavioral survey
 - Detailed information on each sexual contact
 - Collection of biological specimens



**Impact of Viral Factors on
HIV Transmission and
How to Determine Linkage of
Transmission**

Viral Load and Duration of Infection on HIV Transmission

Viral load Set Point on sexual transmission

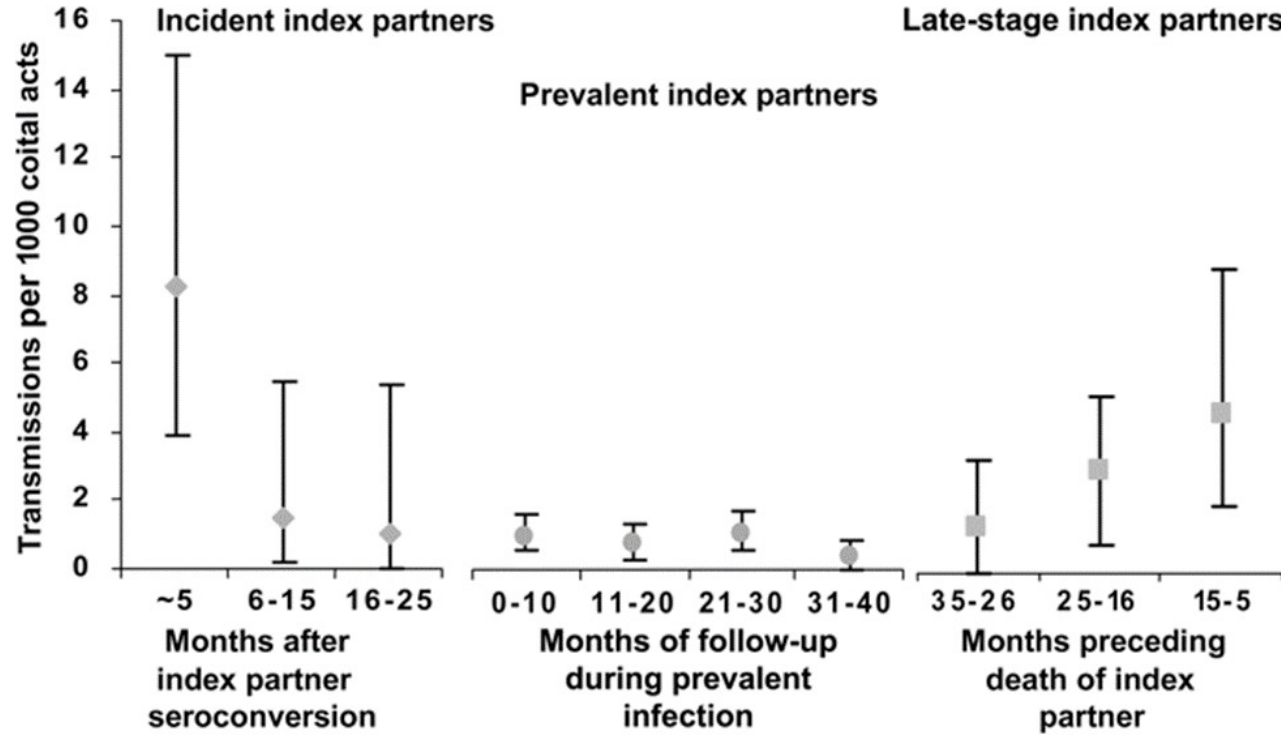


HIV Viral Load, RNA Copies / mL

Quinn et al. NEJM 2000



Transmission per coital act by stage of infection



Wawer... Gray JID 2005



Viral Linkage and Transmission among Monogamous Couples

Monogamous (N = 62)

Non - Monogamous (N=36)

<i>gag</i>	<i>gp41</i>	#	<i>gag</i>	<i>gp41</i>	#
Linked	Linked	36	Linked	Linked	9
Linked	No data	13	Linked	No data	3
No data	Linked	0	No data	Linked	0
Linked	Not linked	4	Linked	Not linked	6
Not linked	Linked	5	Not linked	Linked	2
Not linked	No data	2	Not linked	No data	8
No data	Not linked	2	No data	Not linked	0
Not Linked	Not linked	0	Not Linked	Not linked	8

Any linkage 93% (58/62)

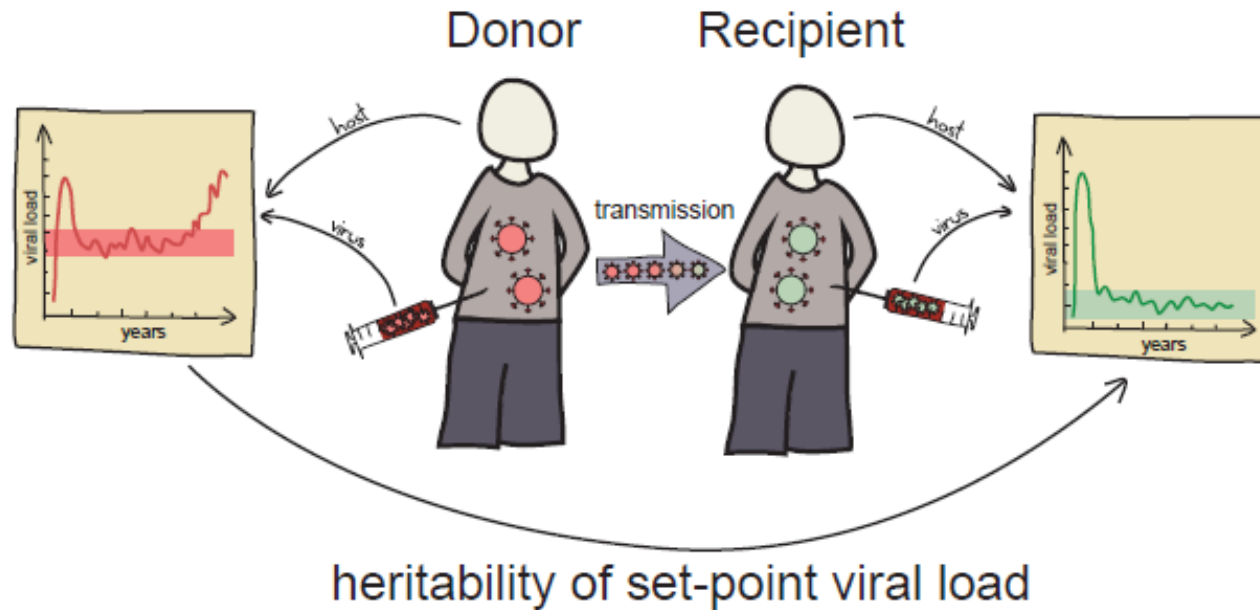
Any discordance 21% (13/62)

Any linkage 56% (20/36)

Any discordance 67% (24/36)

Viral Load is a Heritable Trait

Meta-analysis: $h^2 = 33\%$ (20 – 46%) *Fraser et al, Science 2014*

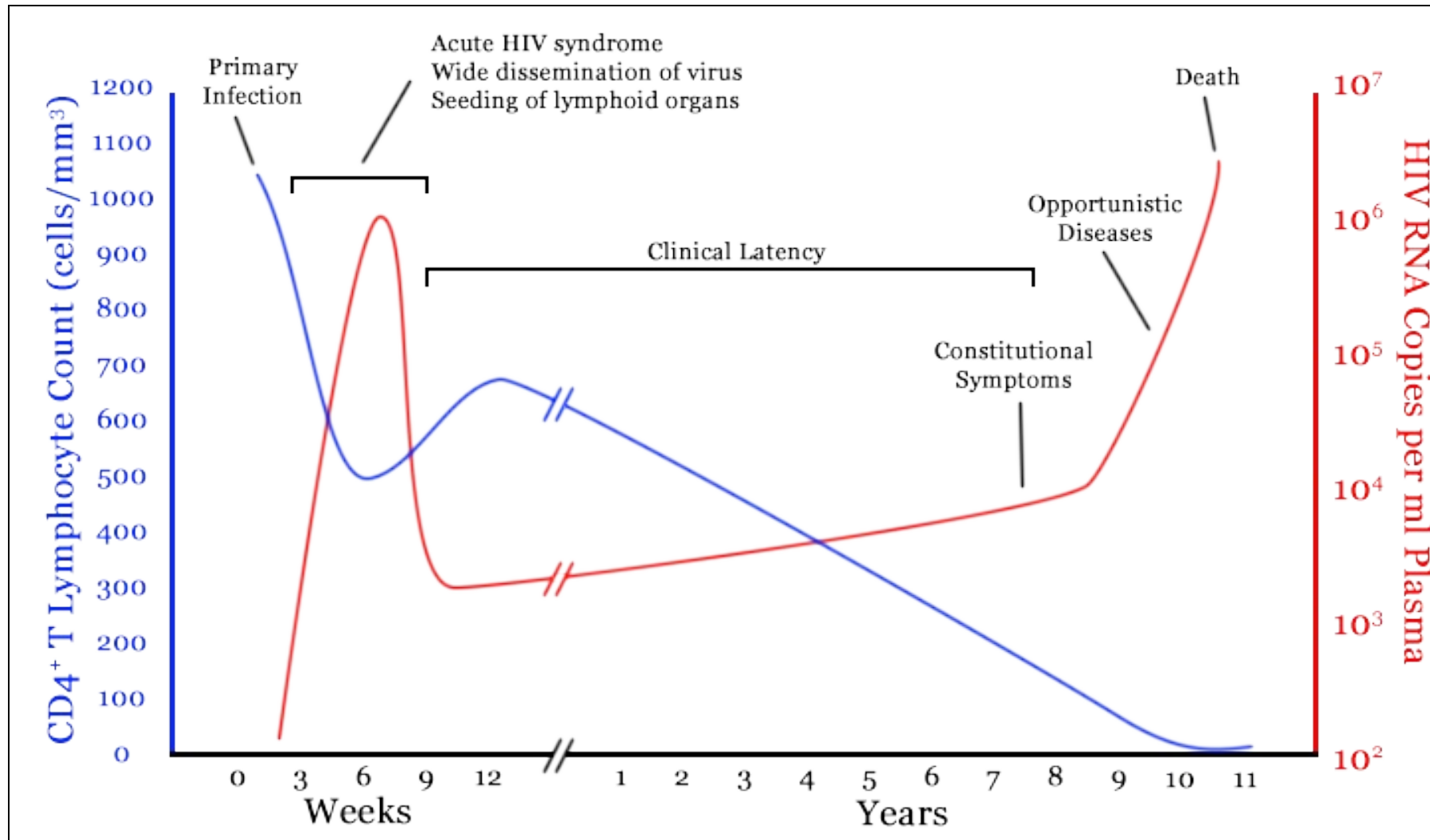


Hollingsworth...Fraser PLOS PATH 2010
Fraser et al. Science 2014

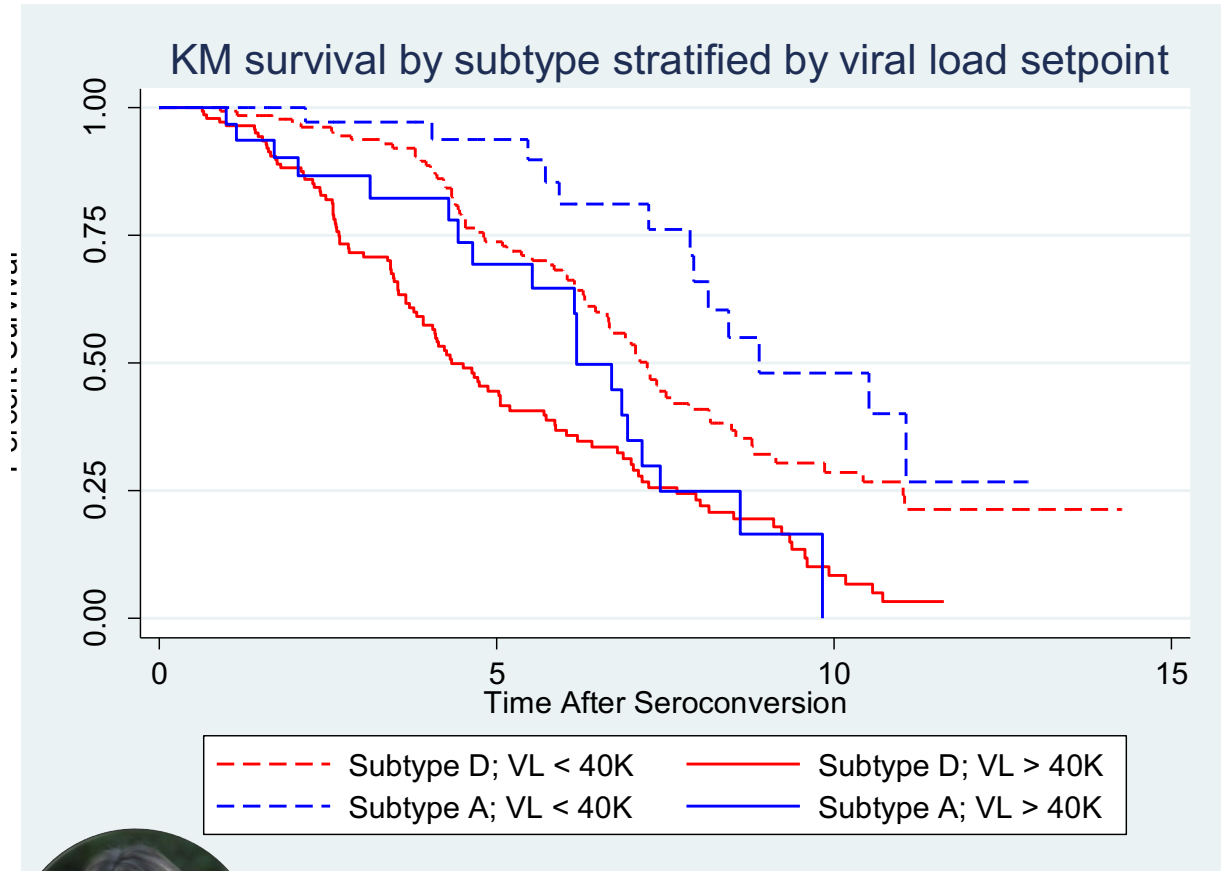
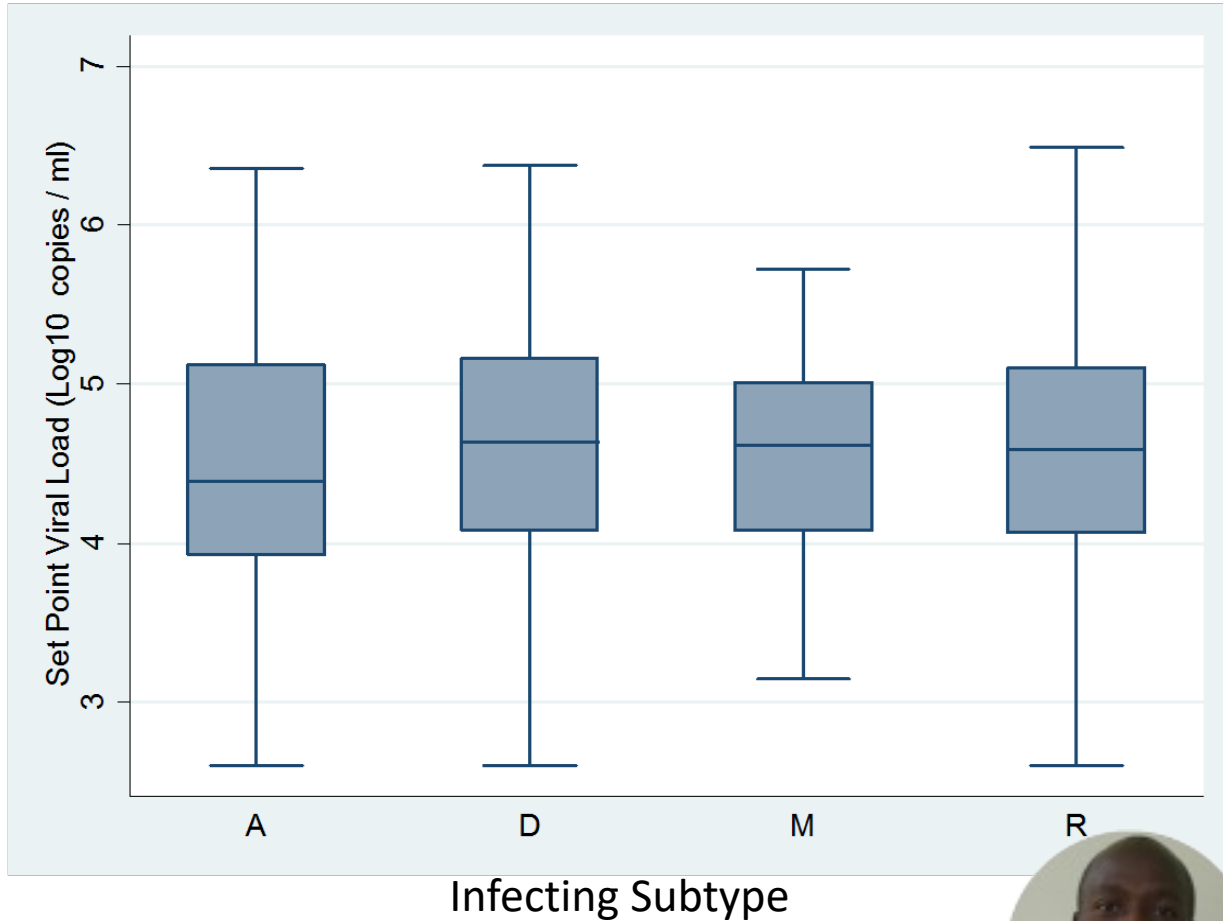
Summary – Qualitative Differences of the Virus that impact HIV Transmission and Pathogenesis

- Establishing linkage of transmission can be difficult
- Viral load is the primary predictor of HIV transmission
- Stage of infection is associated with differential risk of transmission
 - Some one who is acutely infected is much more likely to transmit than when they are chronically infected
- Viral load is a heritable trait
- Viral subtype is associated with differential transmission
 - Subtype **A** is more likely to transmit as subtype **D**

Natural History of HIV Infection



Viral Load Distribution by HIV Subtype and Its Impact on Disease Progression



Kiwanuka JID 2008

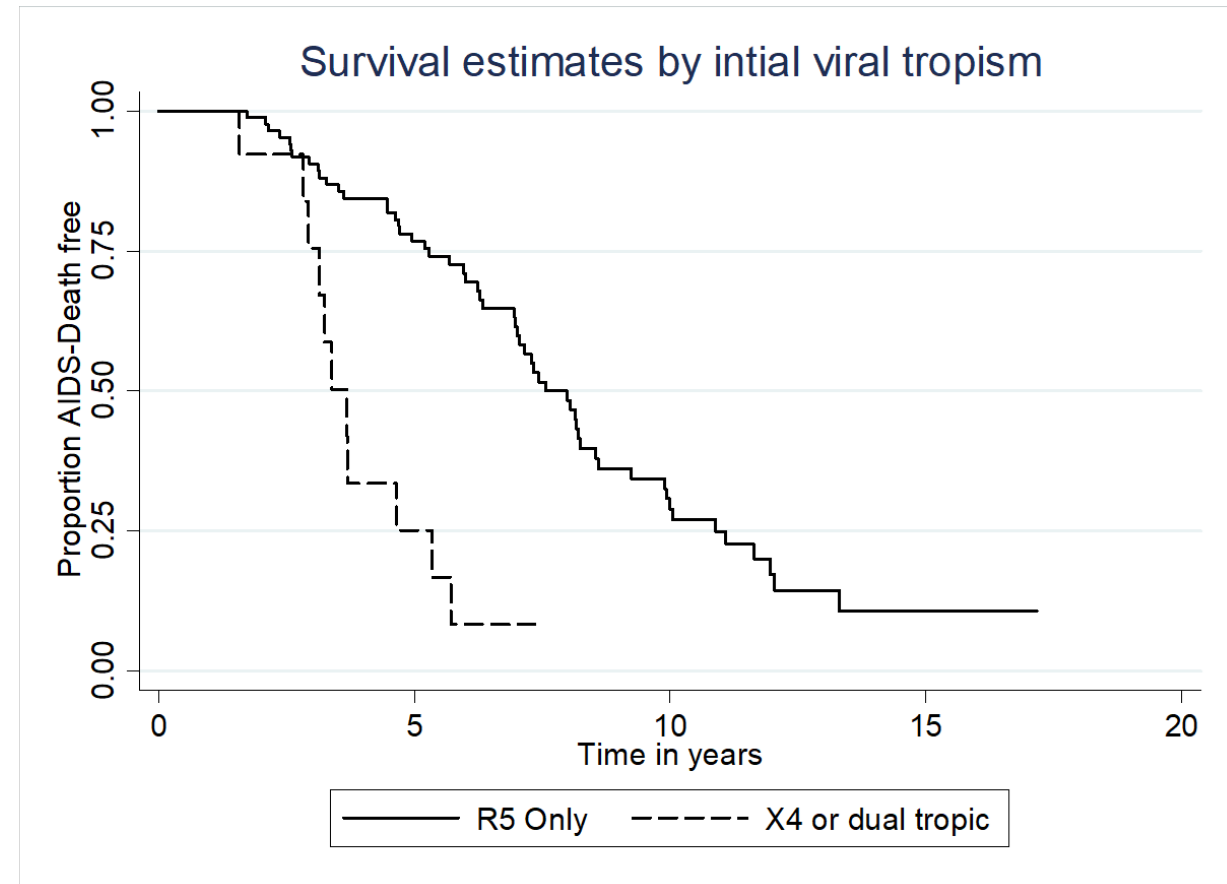
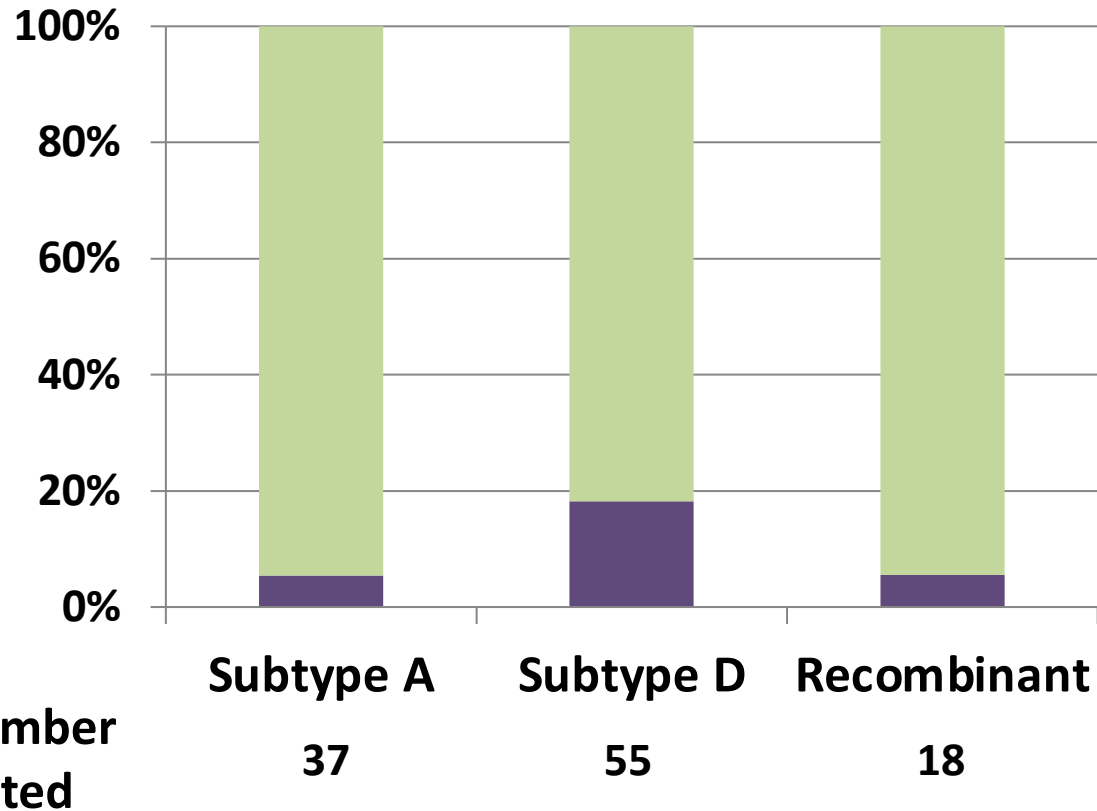


McPhee ARHR 2019

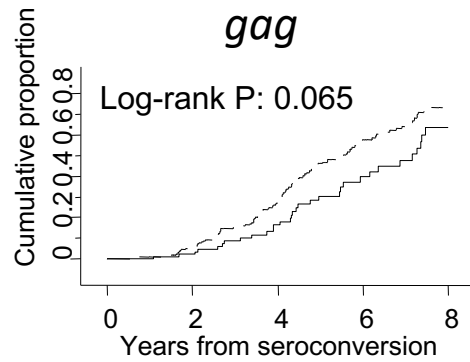
Viral Tropism Differences between HIV Subtypes

Co-receptor tropism in 1st year post seroconversion

■ X4/DM ■ R5

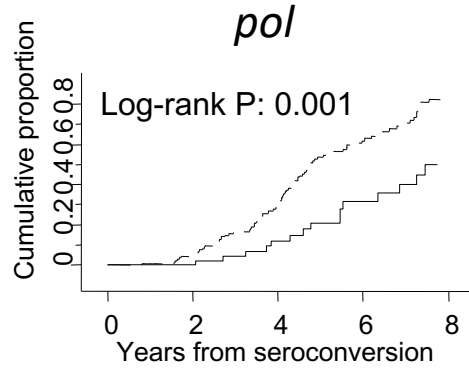


Subtype D and Disease Progression

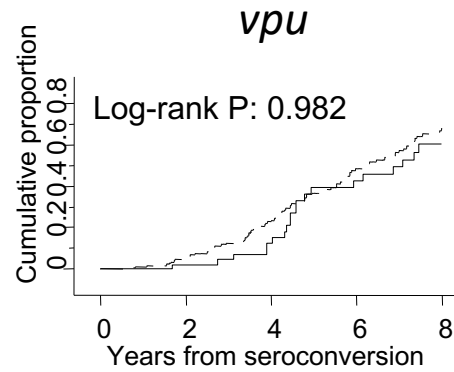


No. at risk, by subtype:

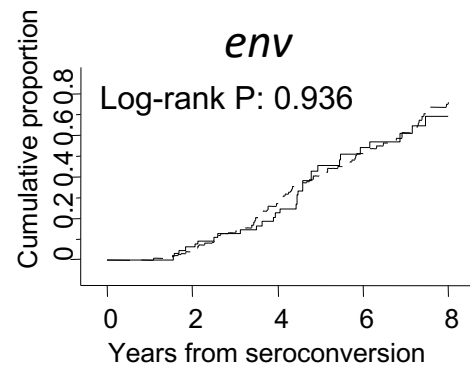
A	91	82	53	24	7
D	229	201	131	53	22



A	54	49	34	18	7
D	170	150	97	34	10

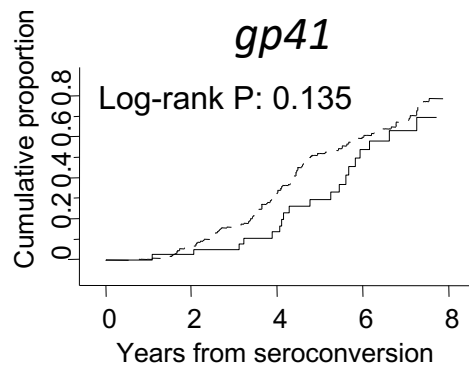


A	51	45	32	17	8
D	177	158	113	52	22



No. at risk, by subtype:

A	70	63	38	16	5
D	164	145	96	42	15

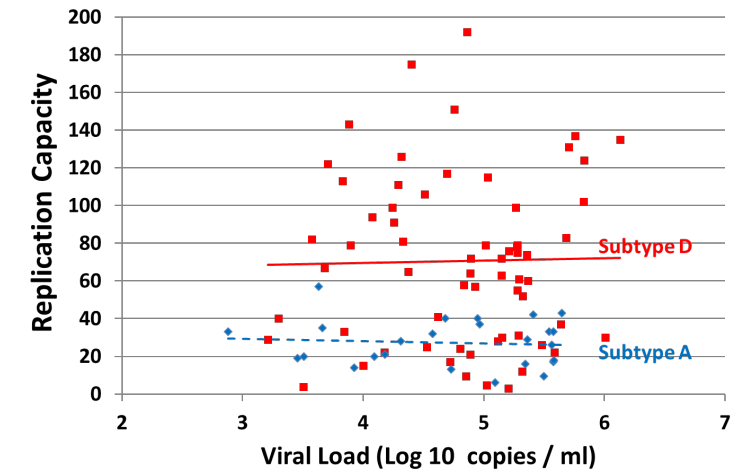
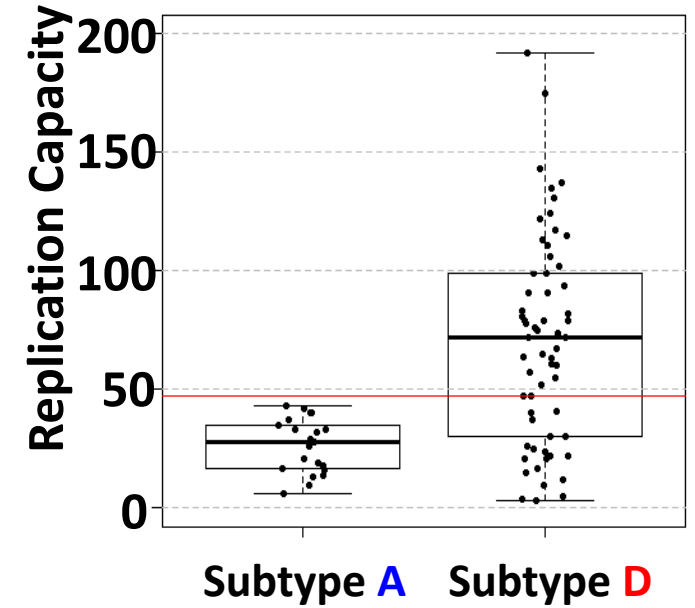


A	43	40	29	11	4
D	225	194	119	45	15

— Subtype A - - - Subtype D



Ng JID 2013

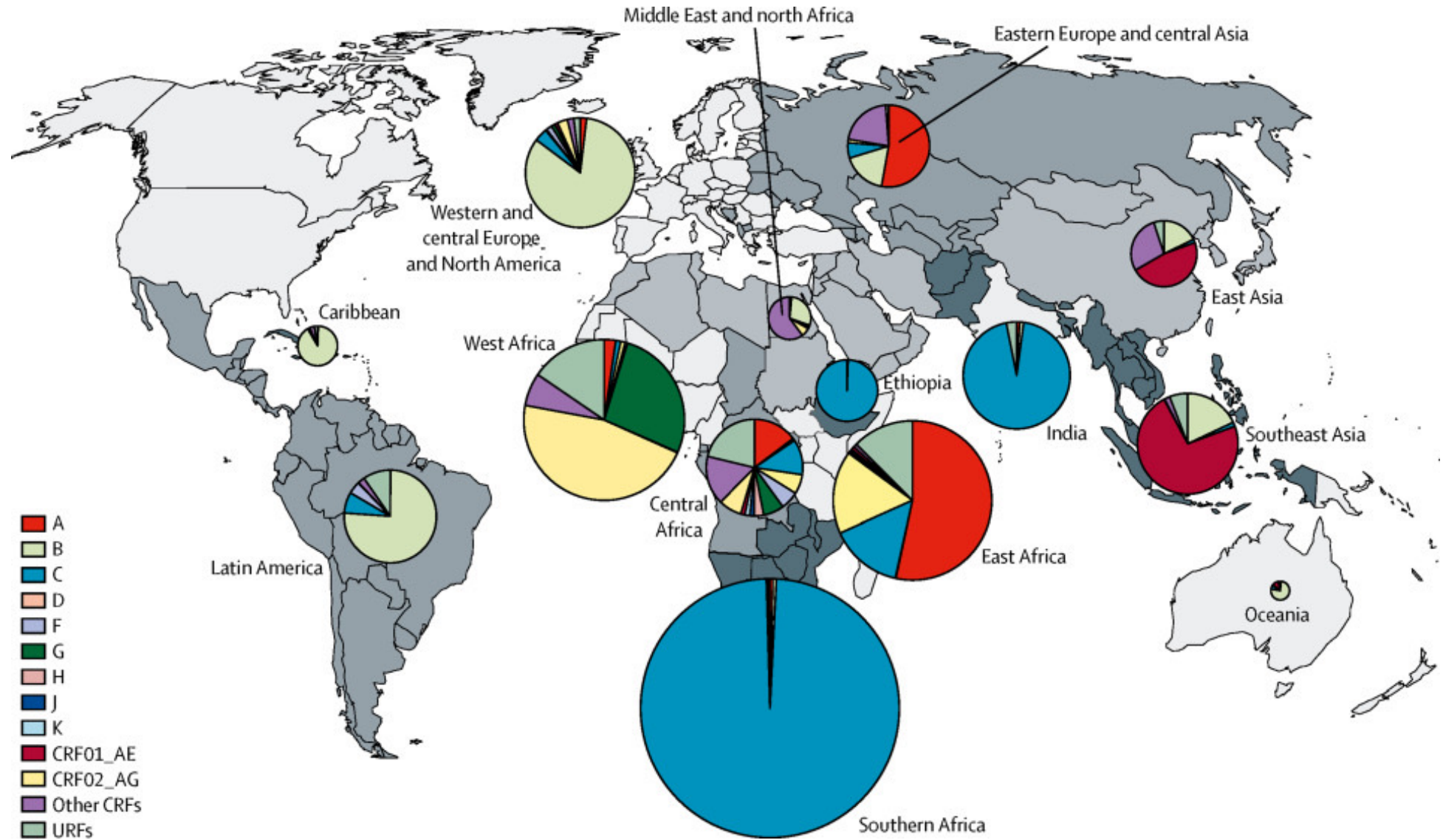


Summary – Qualitative Differences that impact HIV Pathogenesis

- Subtype **D** are more pathogenic than subtypes **A**
- Subtype **D** expresses X4 tropic virus more often than Subtype **A**
- X4 tropic Virus is associated with rapid disease progression
- Higher replication capacity in subtype **D** than **A**
- Replication capacity independent of viral load and is predicative of disease progression

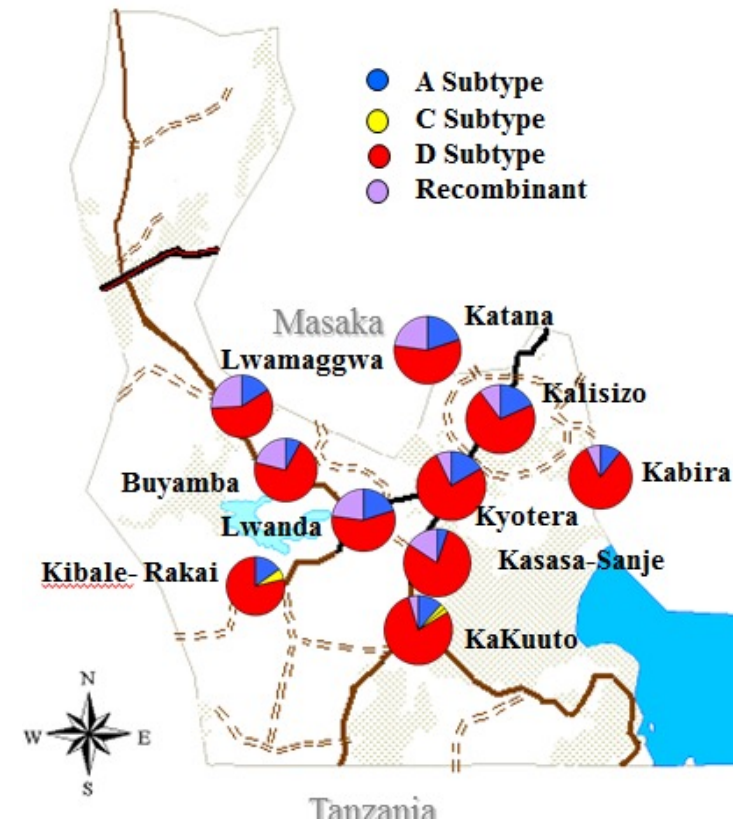
Population Level Changes in HIV Subtype Distributions and Viral Linkage

Global Distribution of HIV-1 Group M Subtypes



HIV-1 Subtype Distribution: 1994

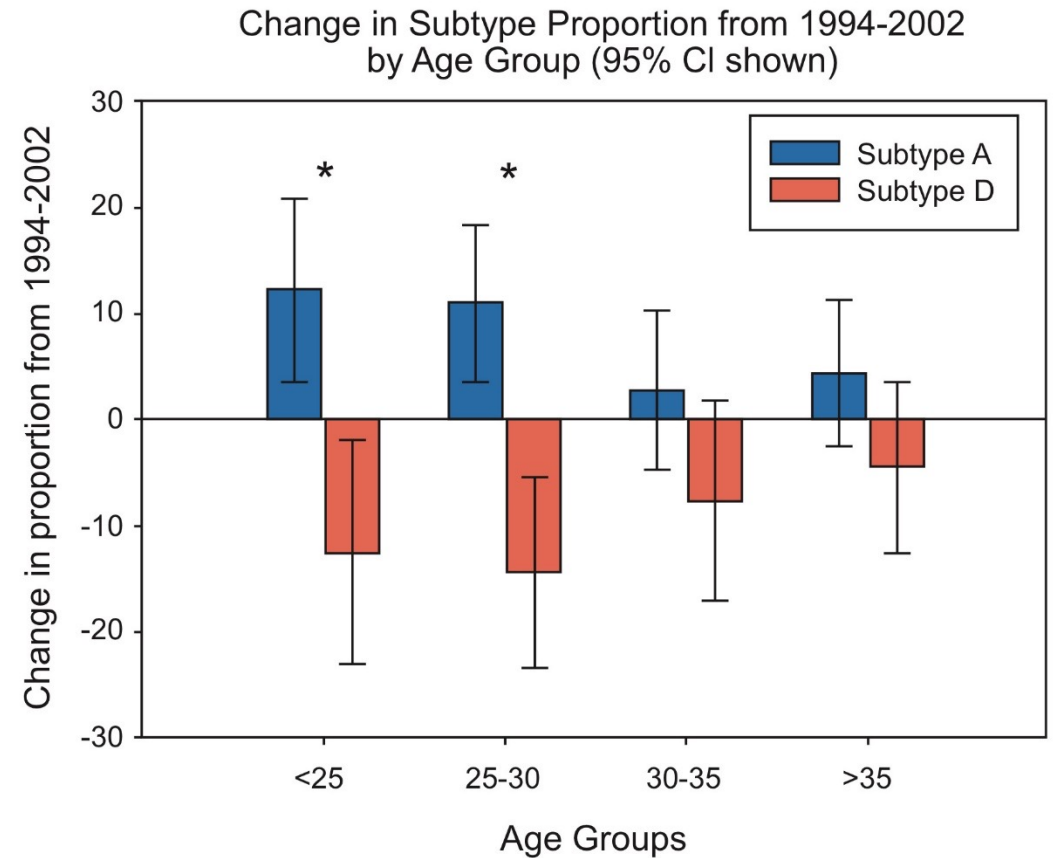
Community cluster	N	Subtype (N) (%)			
		A	D	C	R
North					
Kalisizo	140	22.1 (31)	66.4 (93)	0.7 (1)	10.7 (15)
Katana	106	23.6 (25)	60.4 (64)	0.9 (1)	15.1 (16)
Buyamba	72	9.7 (7)	77.8 (56)	1.4 (1)	11.1 (8)
Lwamaggwa	46	30.4 (14)	54.3 (25)	0	15.2 (7)
All northern region	364	21.2 (77)	65.4 (238)	0.8 (3)	12.6 (46)
Central					
Kyotera	78	15.4 (12)	75.6 (59)	0	9.0 (7)
Kabira	87	13.8 (12)	77.0 (67)	0	9.2 (8)
Lwanda	87	17.2 (15)	65.5 (57)	1.1 (1)	16.1 (14)
All central region	252	15.5 (39)	72.6 (183)	0.4 (1)	11.5 (29)
South					
Kibale-Rakai	42	9.5 (4)	88.1 (37)	2.4 (1)	0
Kakuuto	36	13.9 (5)	72.2 (26)	5.6 (2)	8.3 (3)
Kasasa-Sanje	79	5.1 (4)	74.7 (59)	0	20.3 (16)
All southern region	157	8.2 (13)	77.7 (122)	1.9 (3)	12.1 (19)
Total	773	16.7 (129)	70.2 (543)	0.9 (7)	12.2 (94)



Changes in HIV-1 Subtype Distribution: 1994 vs 2002

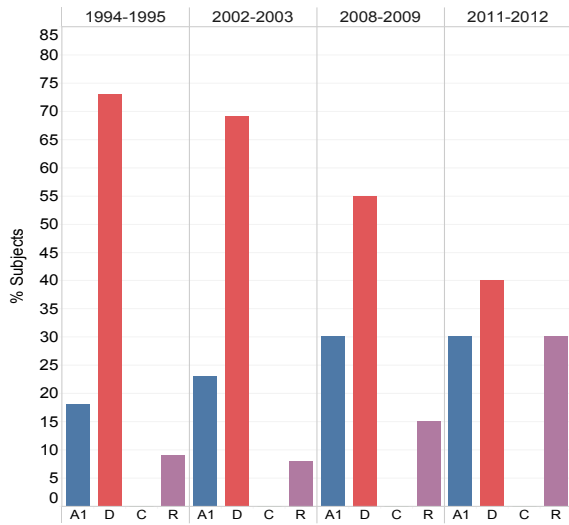
TABLE 1. COMPARISON OF OVERALL SUBTYPE DISTRIBUTION IN 1994 AND 2002

HIV-1 subtype	1994 n=773, (%)	2002 n=812, (%)	Change (%)	Chi-square p-value
A	129 (16.7)	189 (23.3)	6.6	0.005
C	7 (0.9)	7 (0.9)	0.0	
D	543 (70.2)	507 (62.4)	-7.8	
Recombinant	94 (12.2)	109 (13.4)	1.2	

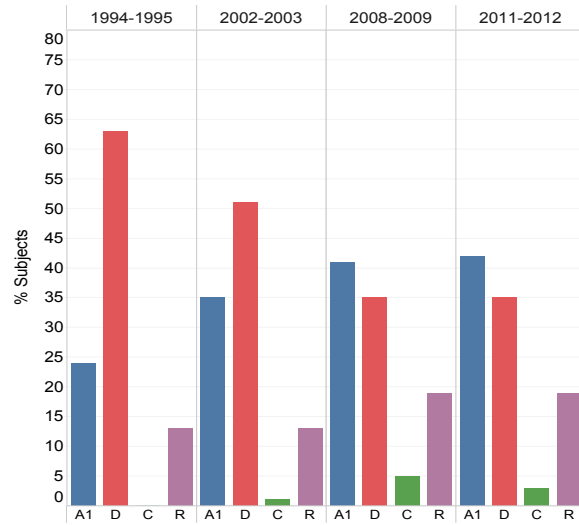


Change in Subtype Proportion by Region Over Time

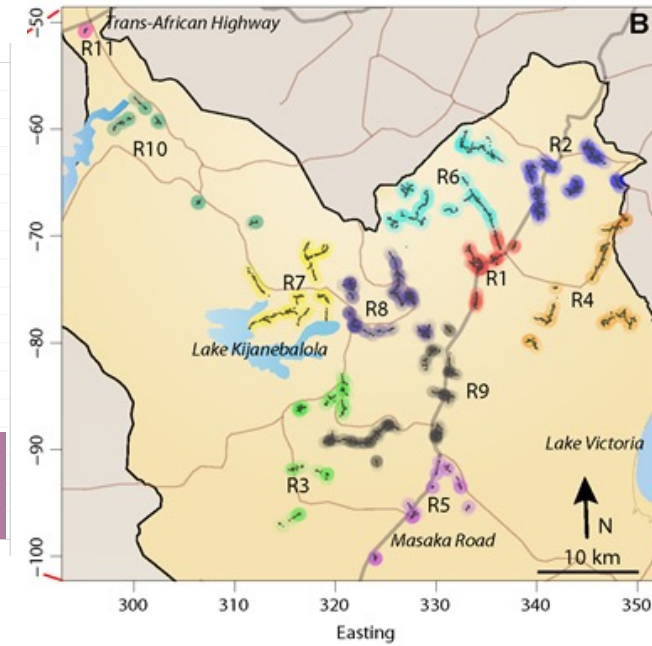
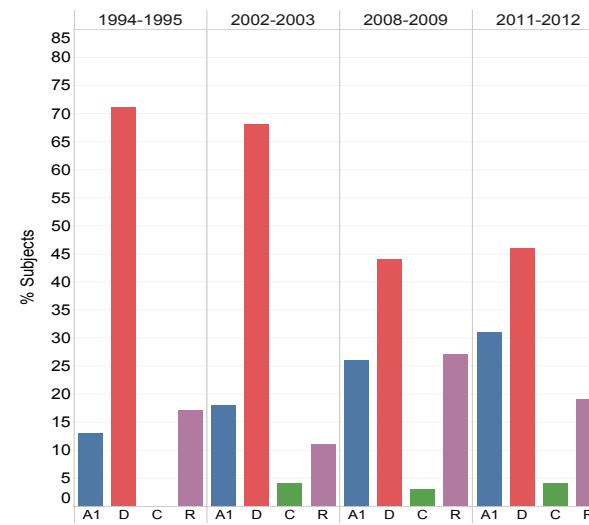
Region 1



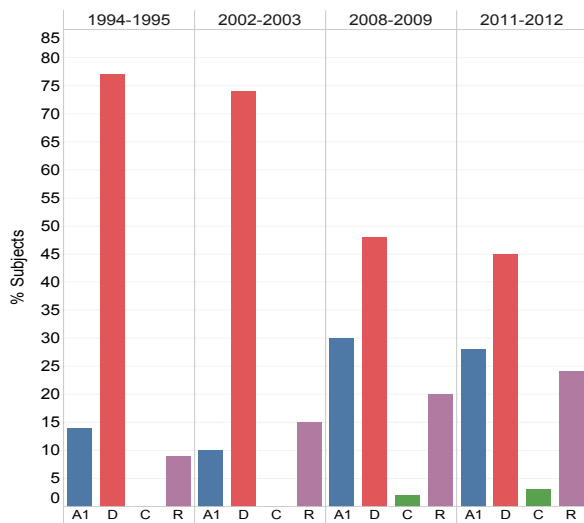
Region 2



Region 5



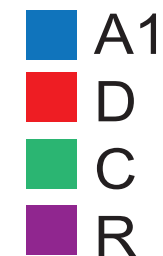
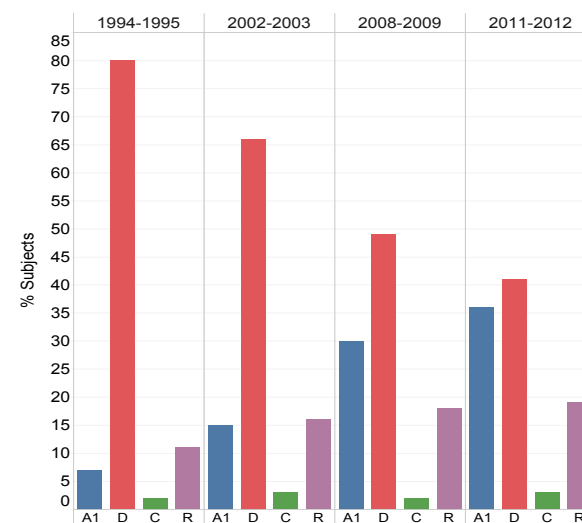
Region 4



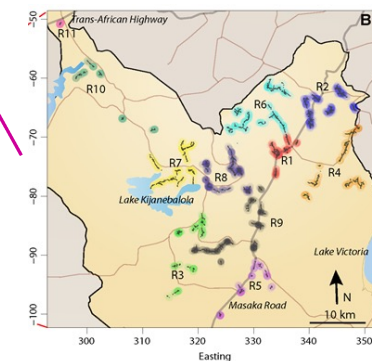
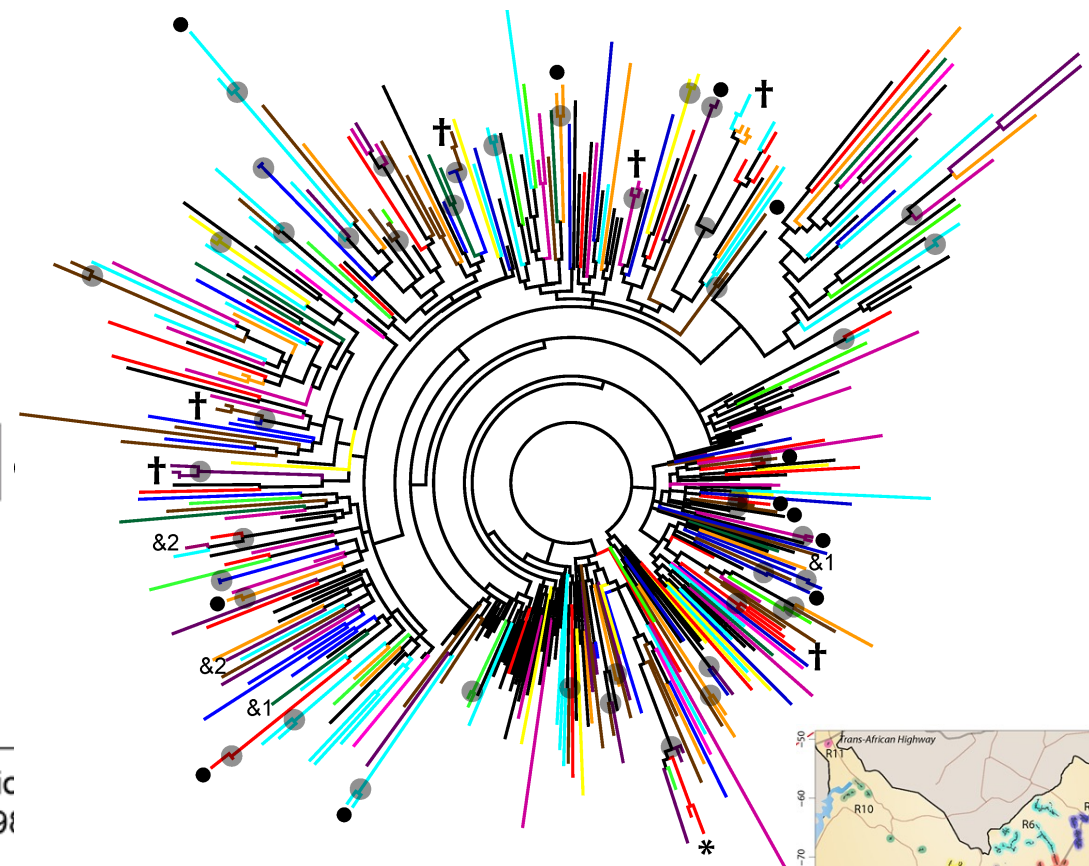
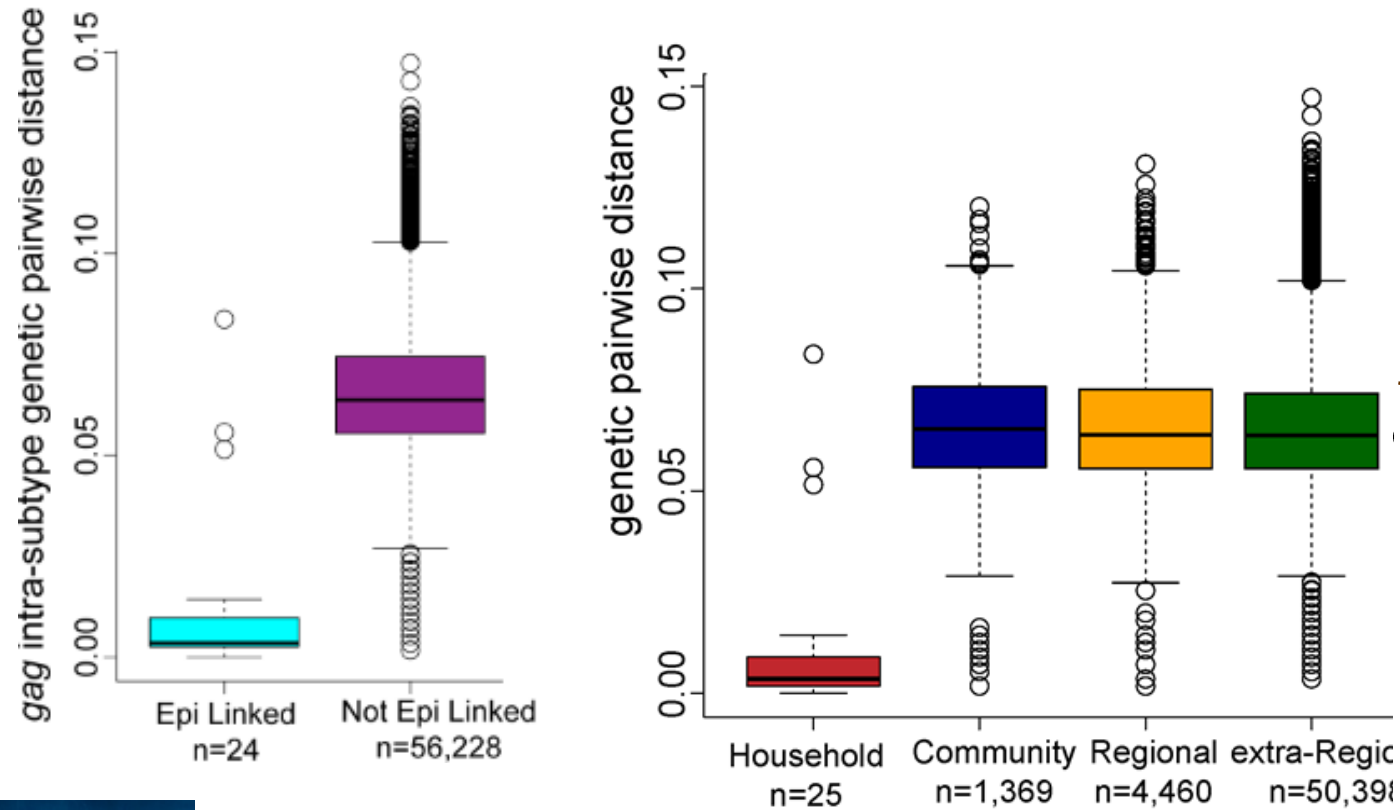
Region 8



Region 9



Geographic Distance and Viral Genetic Distance



Grabowski et al, Plos Med, 2014

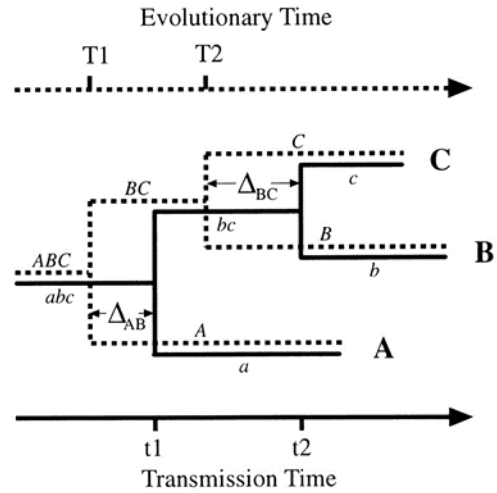
Summary – Population Level Changes in HIV Subtype Distributions and Infection Sources

- The frequency of subtype **D** decreasing, with recombinant and subtypes **A** increasing
- 39% of transmissions occurred in household sexual partnerships
 - Very high transmission rates (25% among incident cases)
- 40% were from known extra-household sexual partners
 - 62% of these were partners from outside the community.
- 20% unknown sources

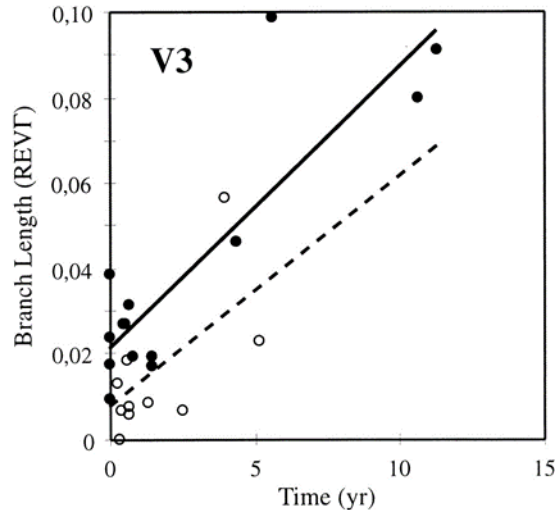
Directionality of Transmission

HIV Molecular Clock

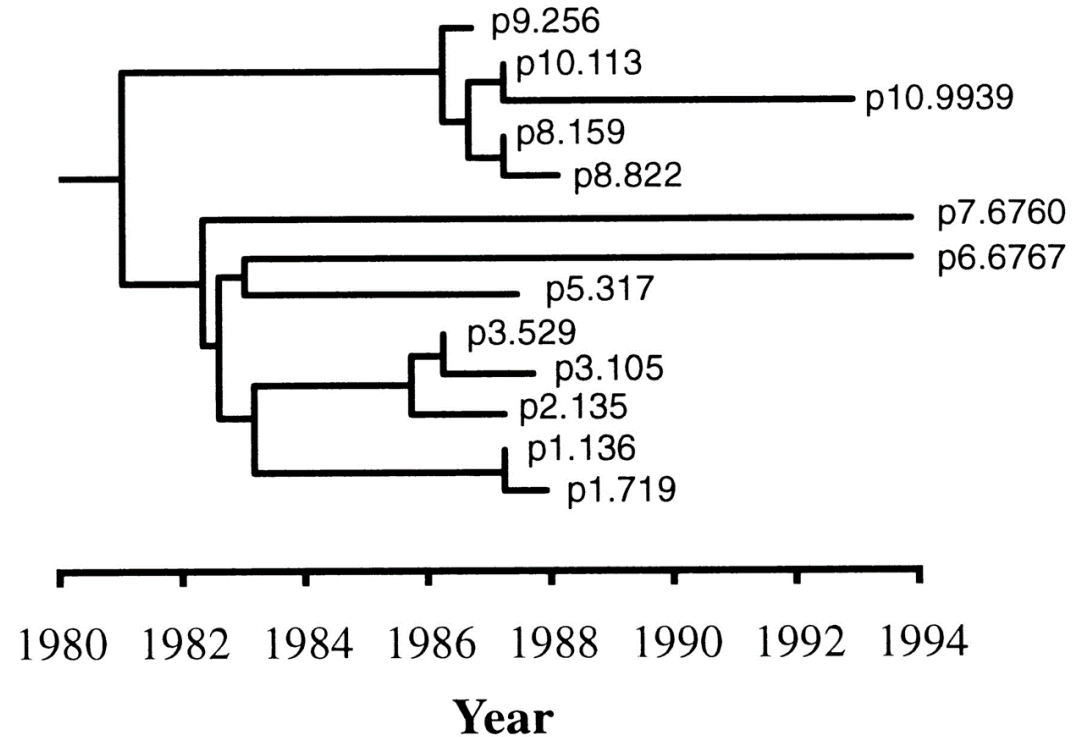
A



B



True Transmission History



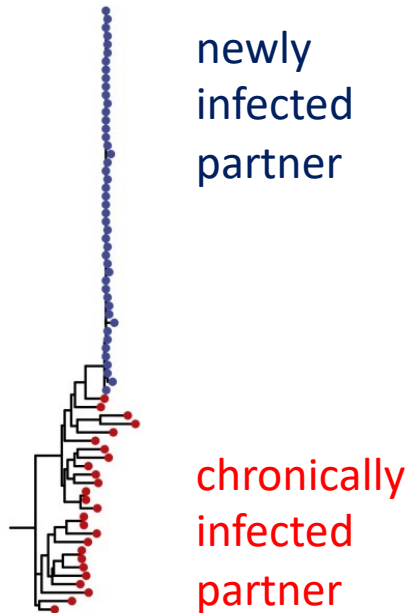
Distance in the tree is related to time



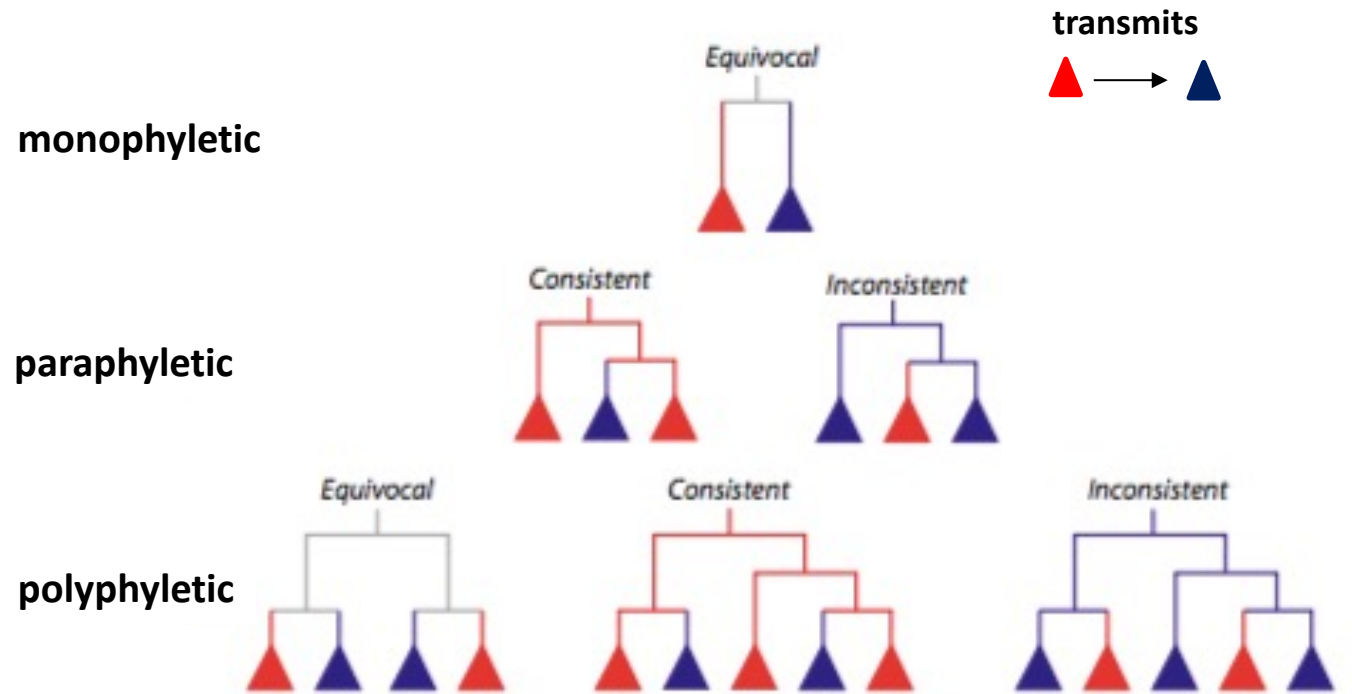
Leitner 1999 PNAS

Directionality of Infection

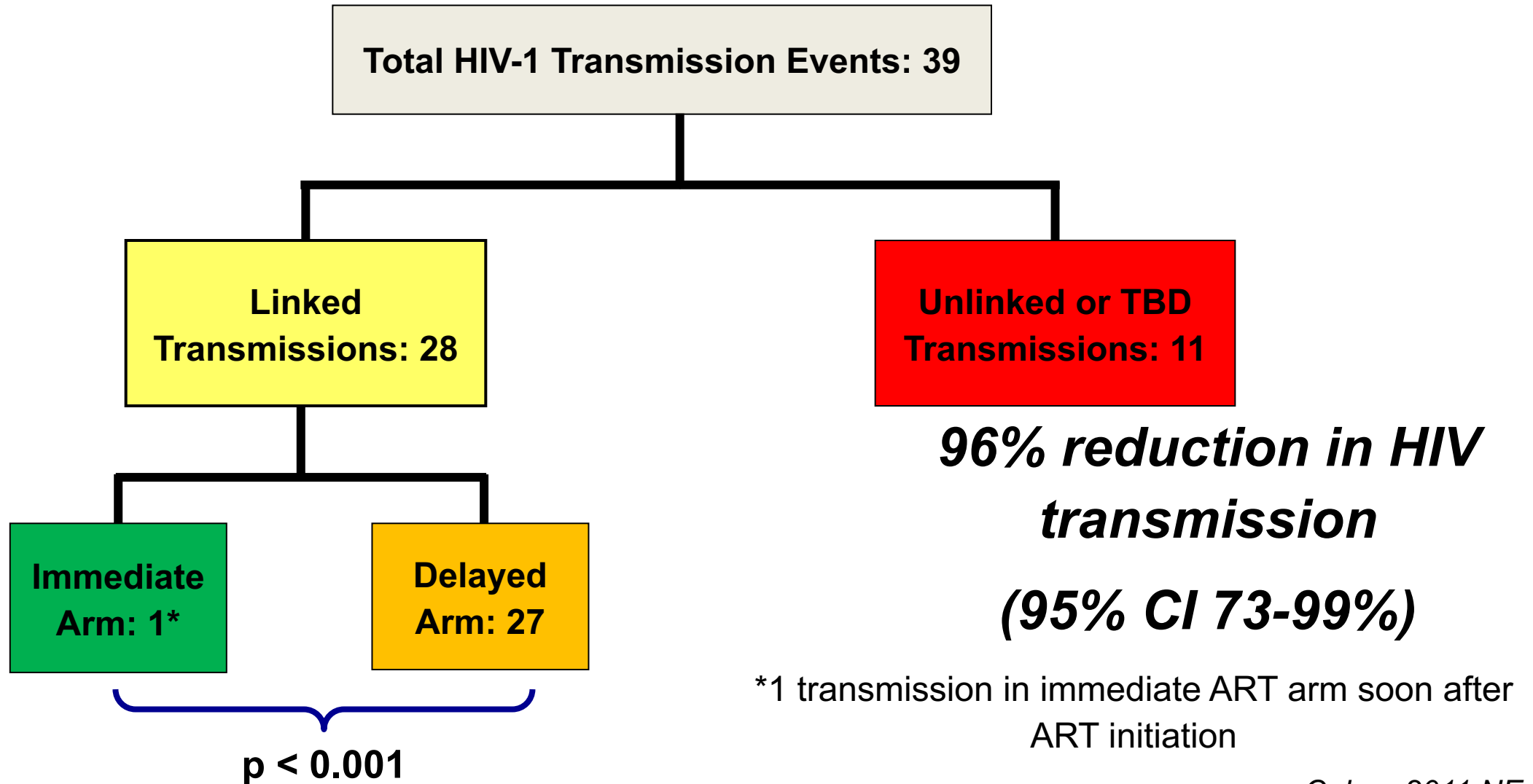
HIV exists as a quasispecies, or cloud of related viruses within an individual

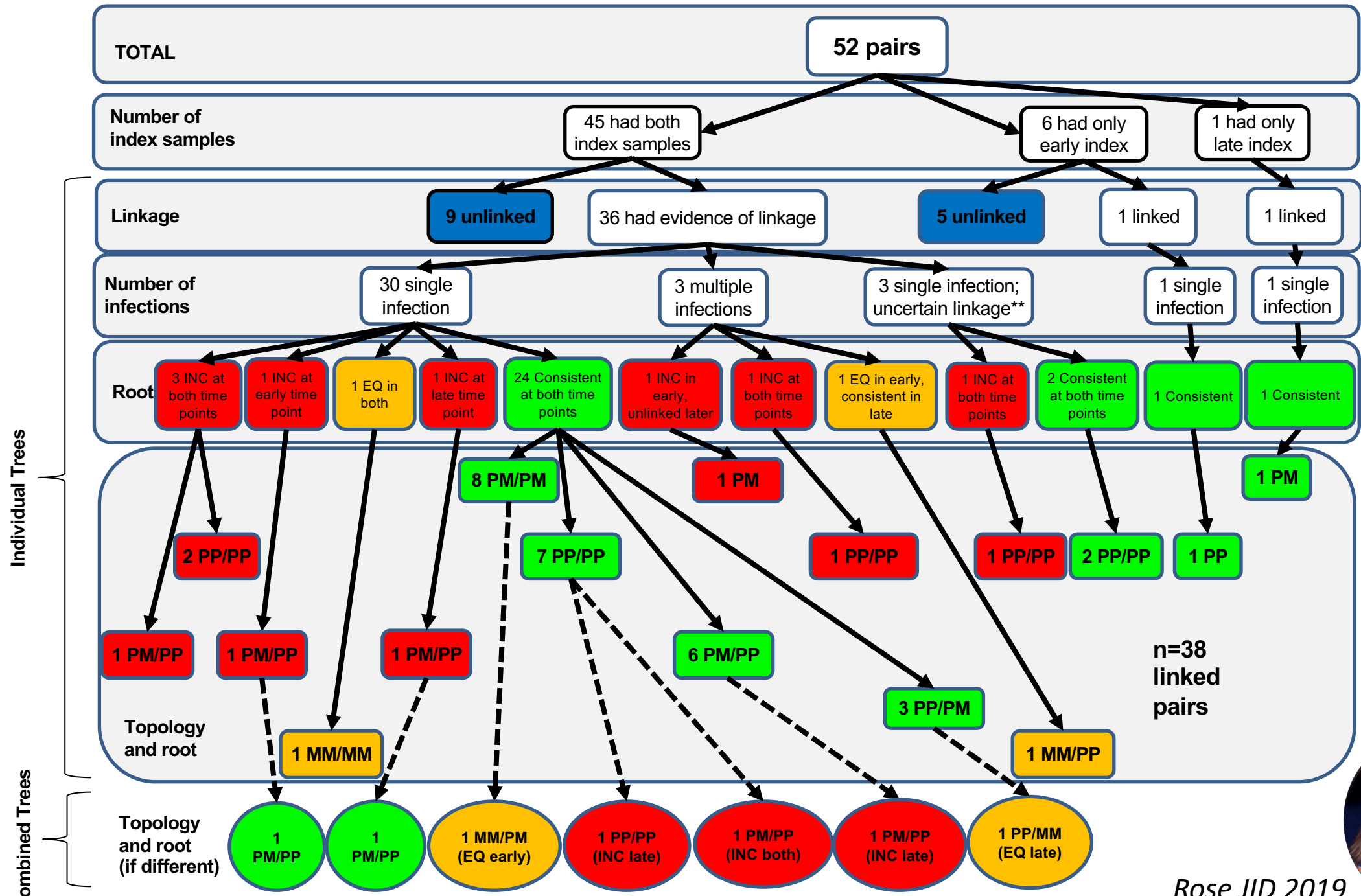


The structure of the tree can implicate the direction of the transmission

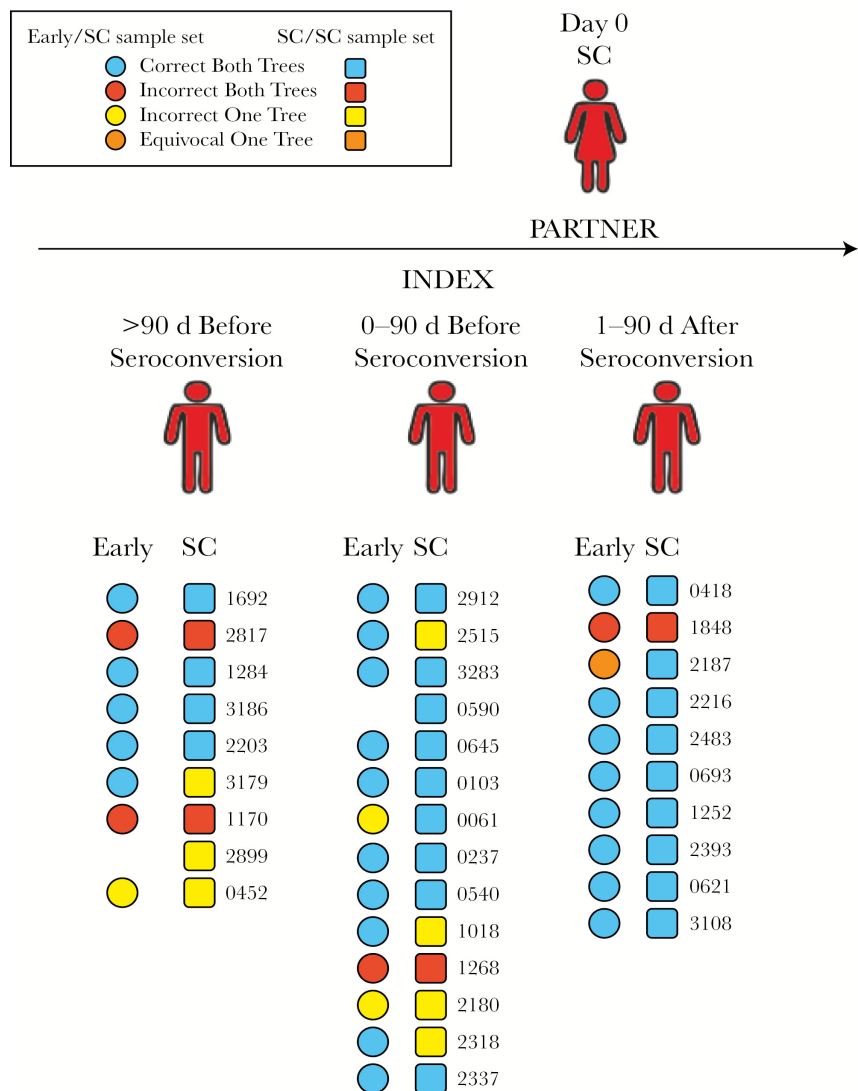


HPTN 052: Randomized Clinical Trial of Immediate vs. Delayed ART in Couples





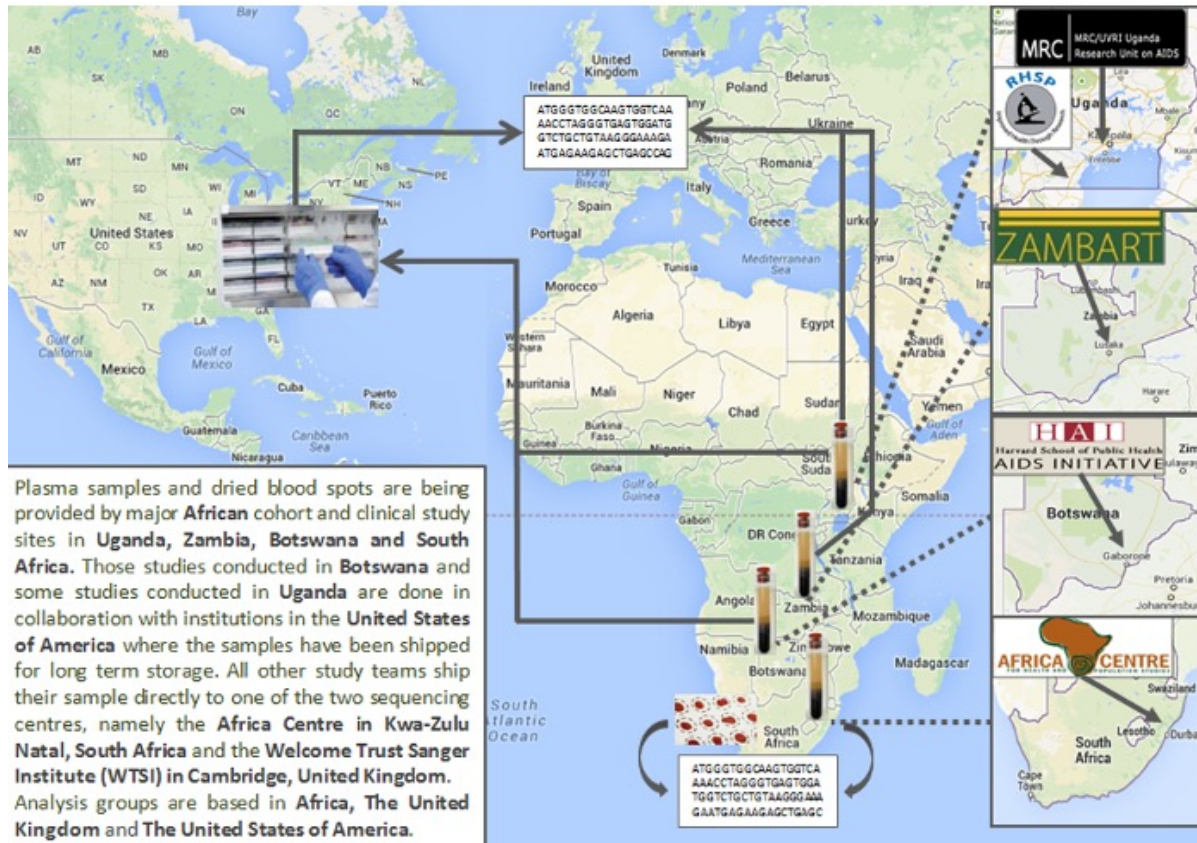
Directionality method validated using known transmission pairs from HPTN 052



- 33 pairs where the direction of transmission was known
 - Up to two time points sequenced from the index partner
- 454 pyrosequencing data of gp41
 - 450,336 sequences total
- predicted direction correctly 67%- 74%

Phylogenetics And Networks for Generalized HIV Epidemics in Africa (PANGEA- HIV)

- **Objective:** Generate 20,000 full-length HIV genomes to improve understanding of HIV transmission dynamics in Sub-Saharan Africa



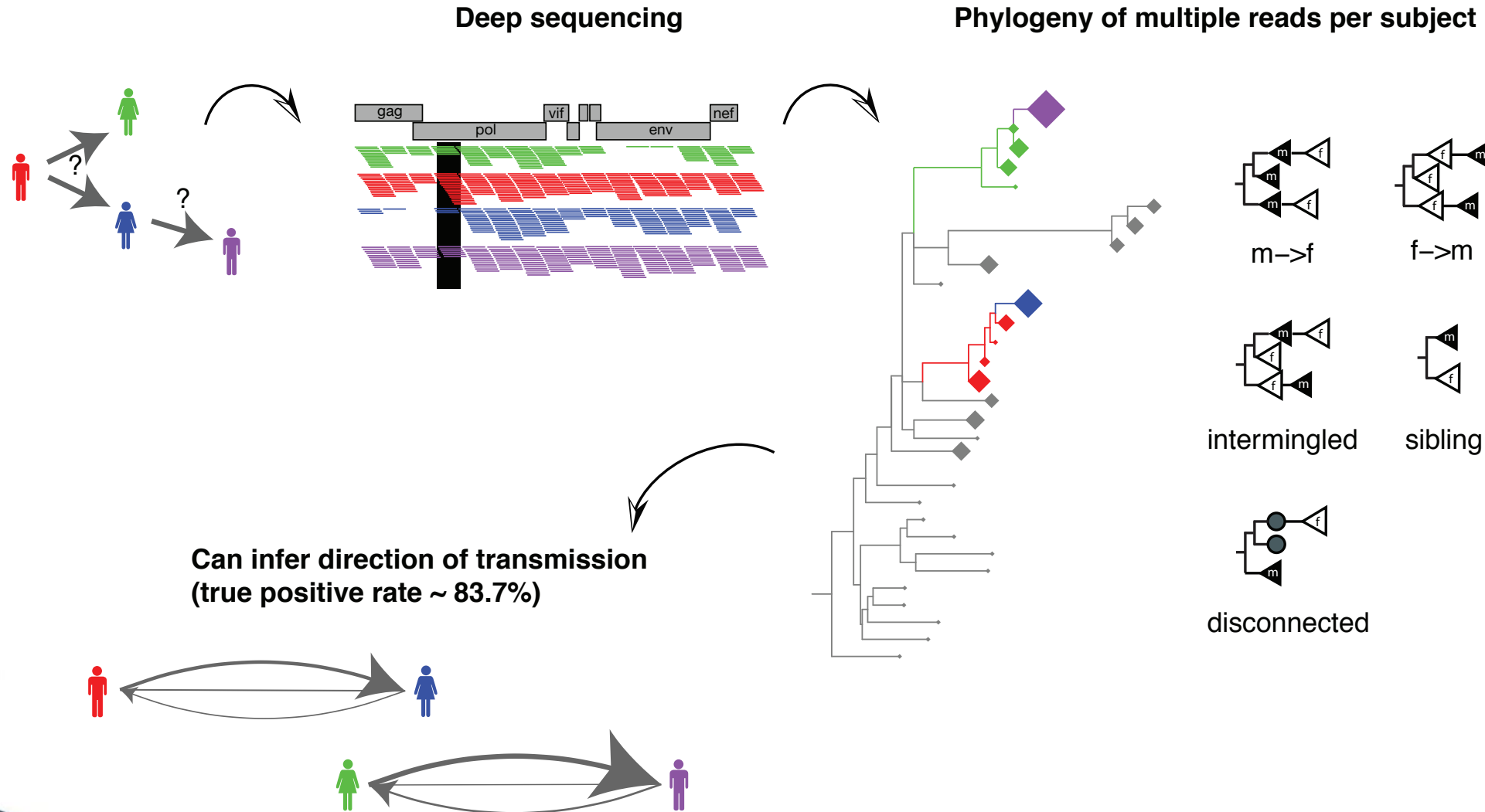
PANGEA-HIV: phylogenetics for generalised epidemics in Africa

Notable increases in coverage of antiretroviral treatment (ART) have been made in Africa in the past decade. More HIV-infected individuals are receiving treatment, and life-expectancy of infected individuals has increased.¹ However, the HIV epidemic continues and overall prevalence of HIV will increase.² The burden of HIV remains highest in sub-Saharan Africa, with

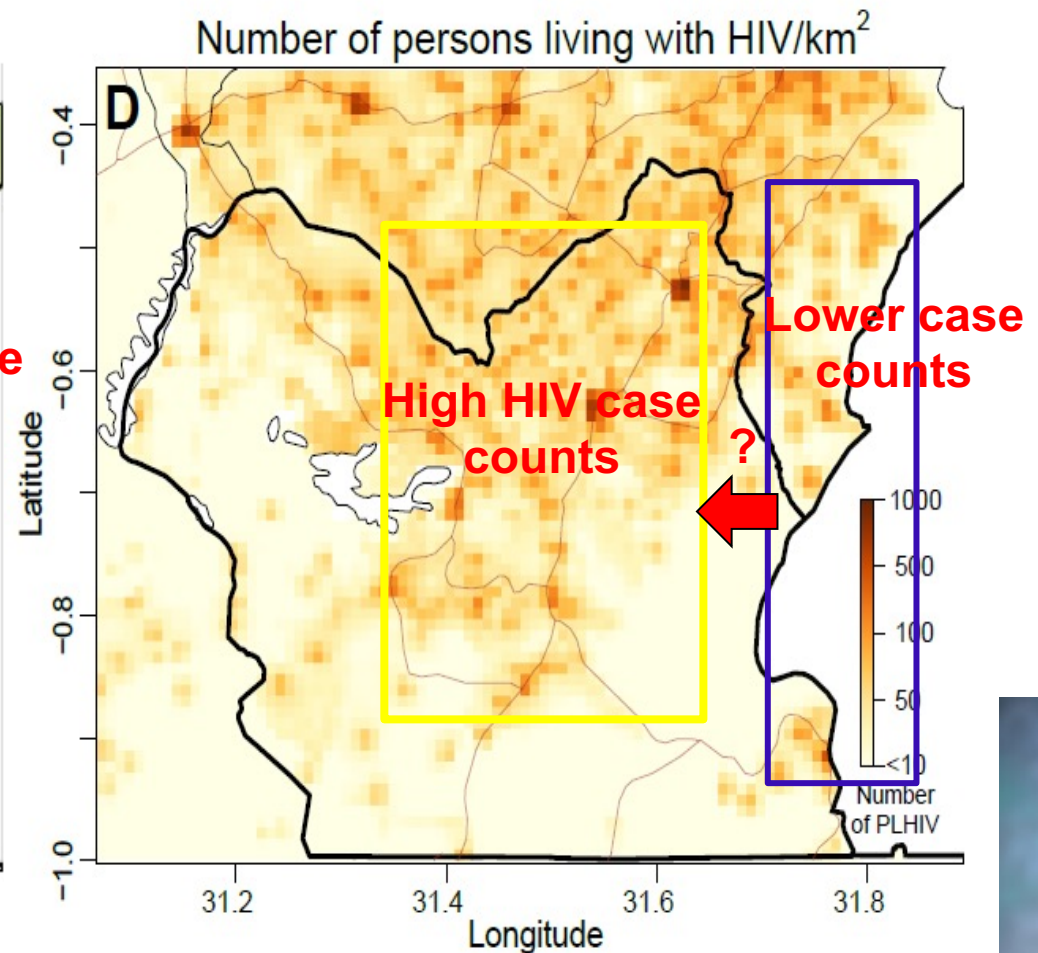
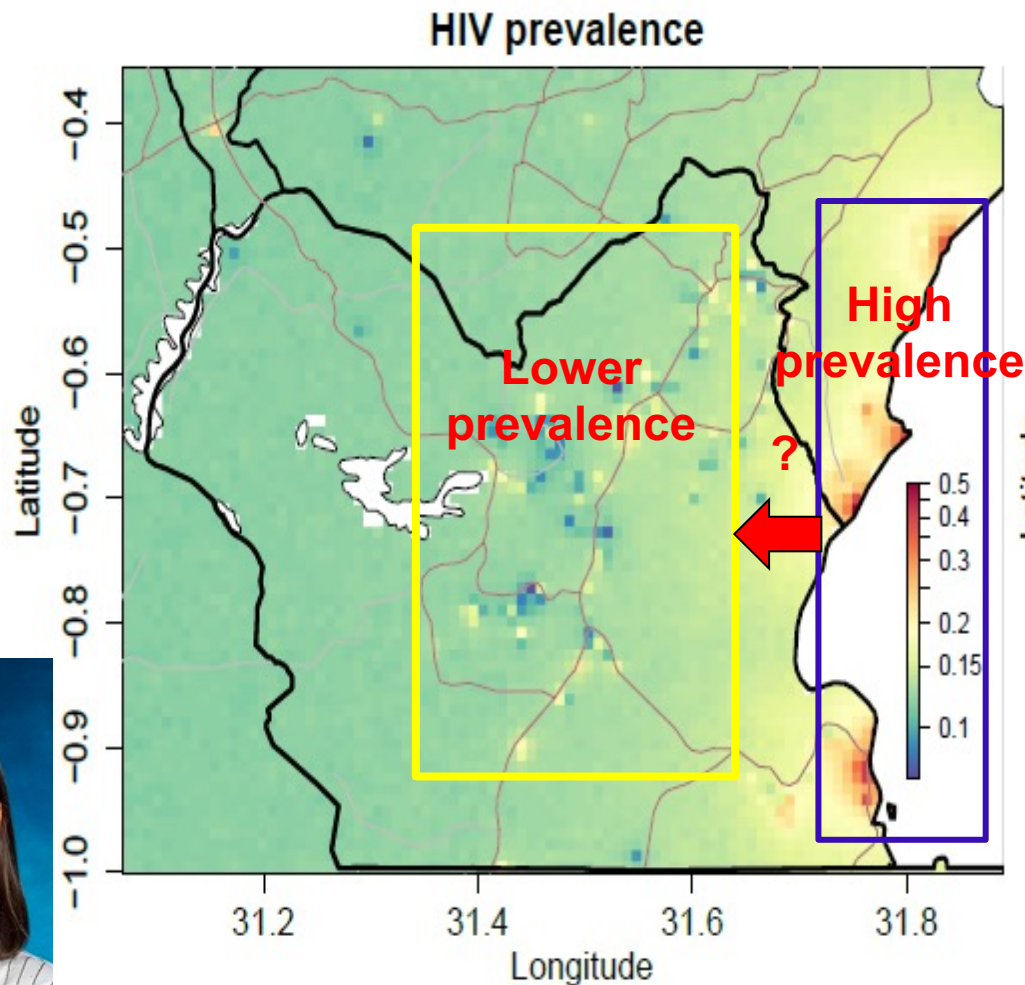
75% of all HIV infections and adult prevalence at 5%.³ The use of ART to reduce individual viral loads and viral transmission rates has emerged as a promising approach to further slow the epidemic.⁴ Yet, how to implement treatment as prevention (in combination with pre-exposure prophylaxis or behavioural change interventions) in the most effective and efficient ways



Deep sequence viral phylogenetics (phyloscanner)

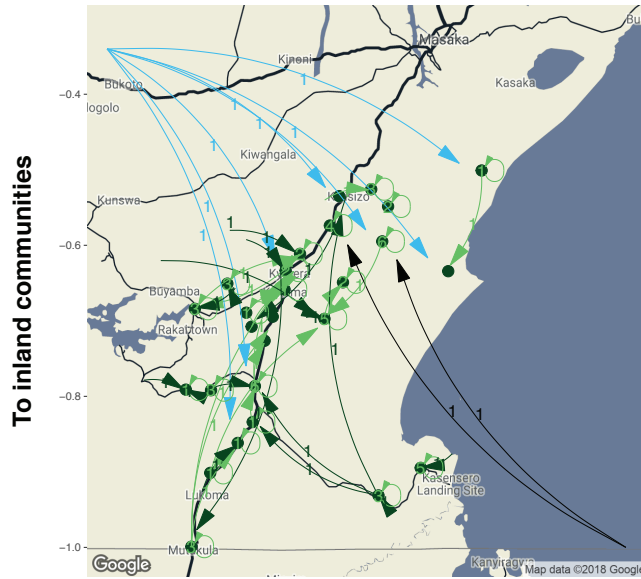


Are fishing communities a major source of HIV infection among neighboring inland populations in Rakai, Uganda?

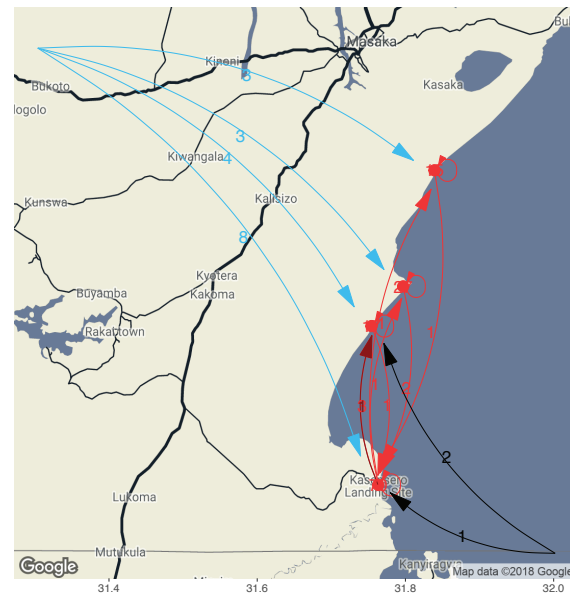


Reconstructed Transmission Flows

From inland communities



From fishing sites



reconstructed HIV-1 transmission events



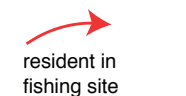
migrated from inland community



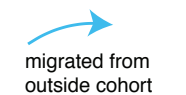
resident in inland community



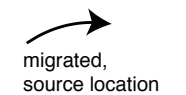
migrated from fishing site



resident in fishing site



migrated from outside cohort



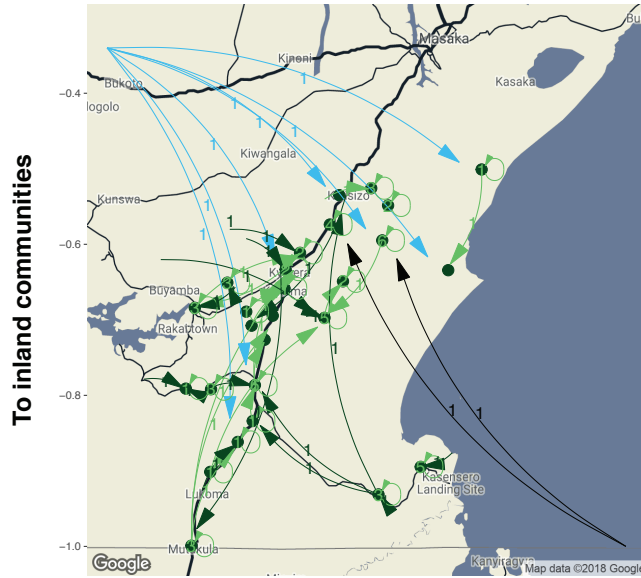
migrated, source location unknown

- **293 source-recipient pairs reconstructed**
- **232/293 (79%) within inland or within fishing**

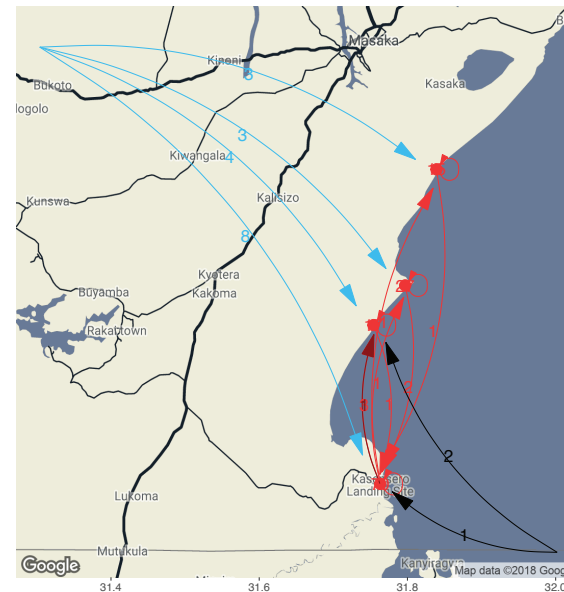
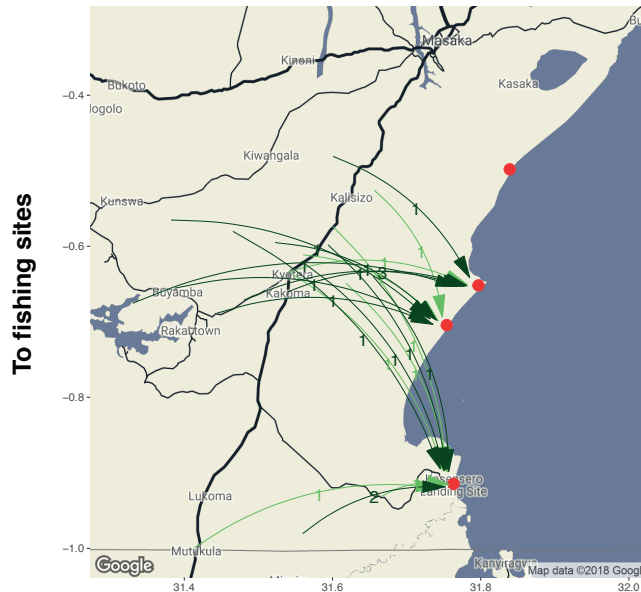
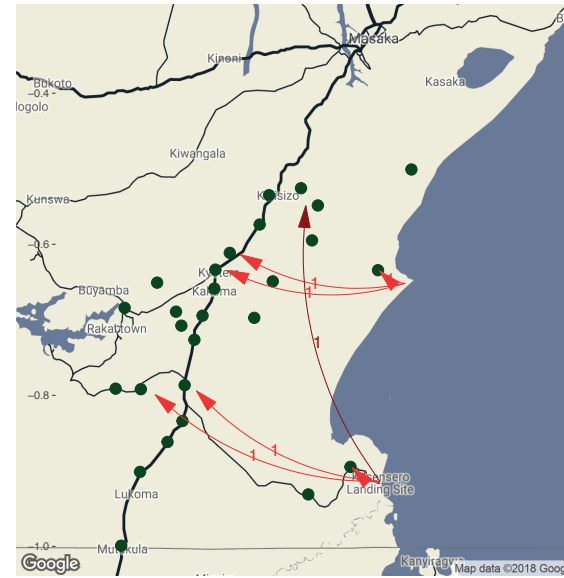


Reconstructed Transmission Flows

From inland communities



From fishing sites



reconstructed
HIV-1 transmission
events



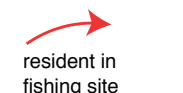
migrated from
inland community



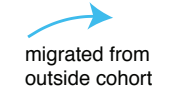
resident in
inland community



migrated from
fishing site



resident in
fishing site



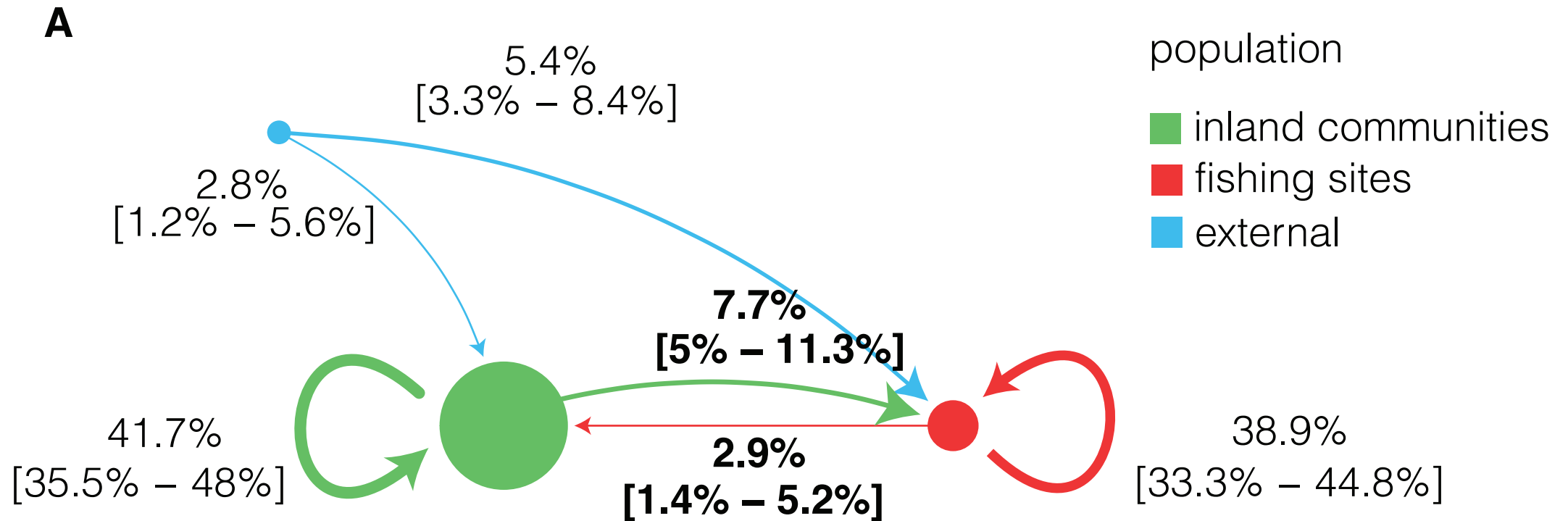
migrated from
outside cohort



migrated,
source location
unknown

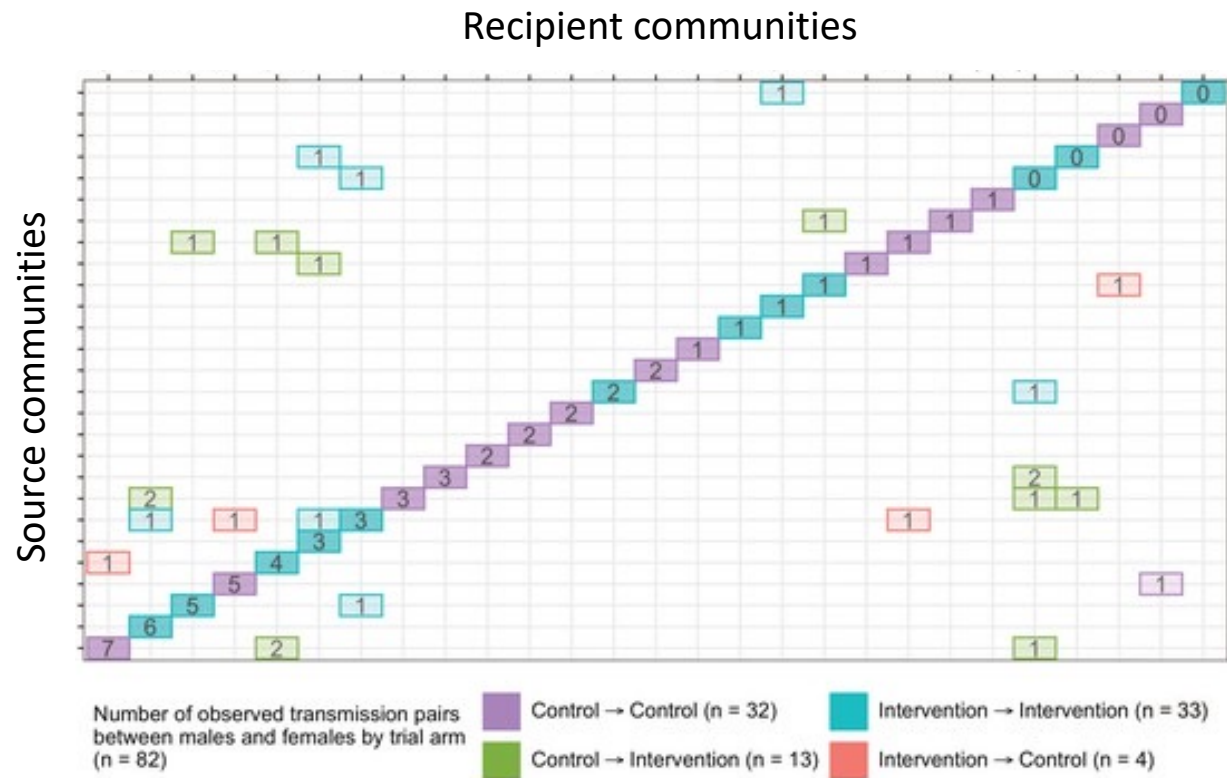
- 7/293 from fishing to inland
- 1/7 involved a source that had moved from fishing to inland
- 21/293 from inland to fishing
- 13/21 involved a source that had moved from inland to fishing

Fishing Sites are Viral Sinks Despite Being Hotspots



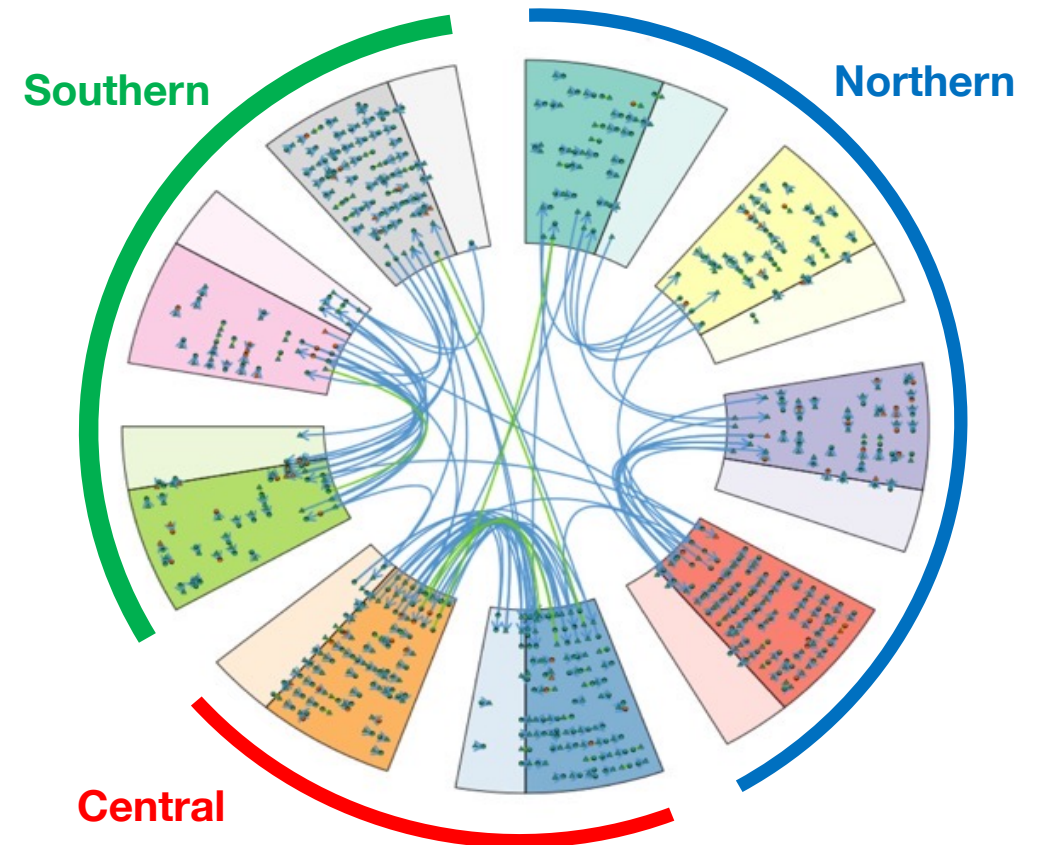
Similar analyses were carried out in Botswana and Zambia

Transmission between Ya Tsie communities in Botswana



Magosi et al, Elife 2022

Transmission between HPTN 071 communities in Zambia



Hall et al, in preparation

Conclusions - Directionality of Transmission and Spatial Studies

- **Direction of HIV transmission can be inferred from sequence data most of the time**
- **High HIV incidence/prevalence (hotspots) are not necessarily the main source of new infections in neighboring low risk areas**
- **Targeting of hotspots may not benefit to neighboring low risk areas**

Conclusions – Overall

- **Genetic features of HIV-1 impact its transmission and pathogenesis within a human host**
- **These viral genetic differences can be used to determine how the virus spreads within a population**
- **Understanding these molecular epidemiologic traits can be used for targeted intervention, both individually and at a population level**

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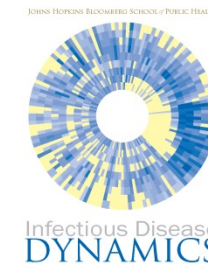
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Ronald Gray
Maria Wawer
Xiangrong Kong

- BioInfoExperts

Susanna Lamers
Rebecca Rose



BIOINFOEXPERTS



WRAIR

Walter Reed Army
Institute of Research
Soldier Health • World Health



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