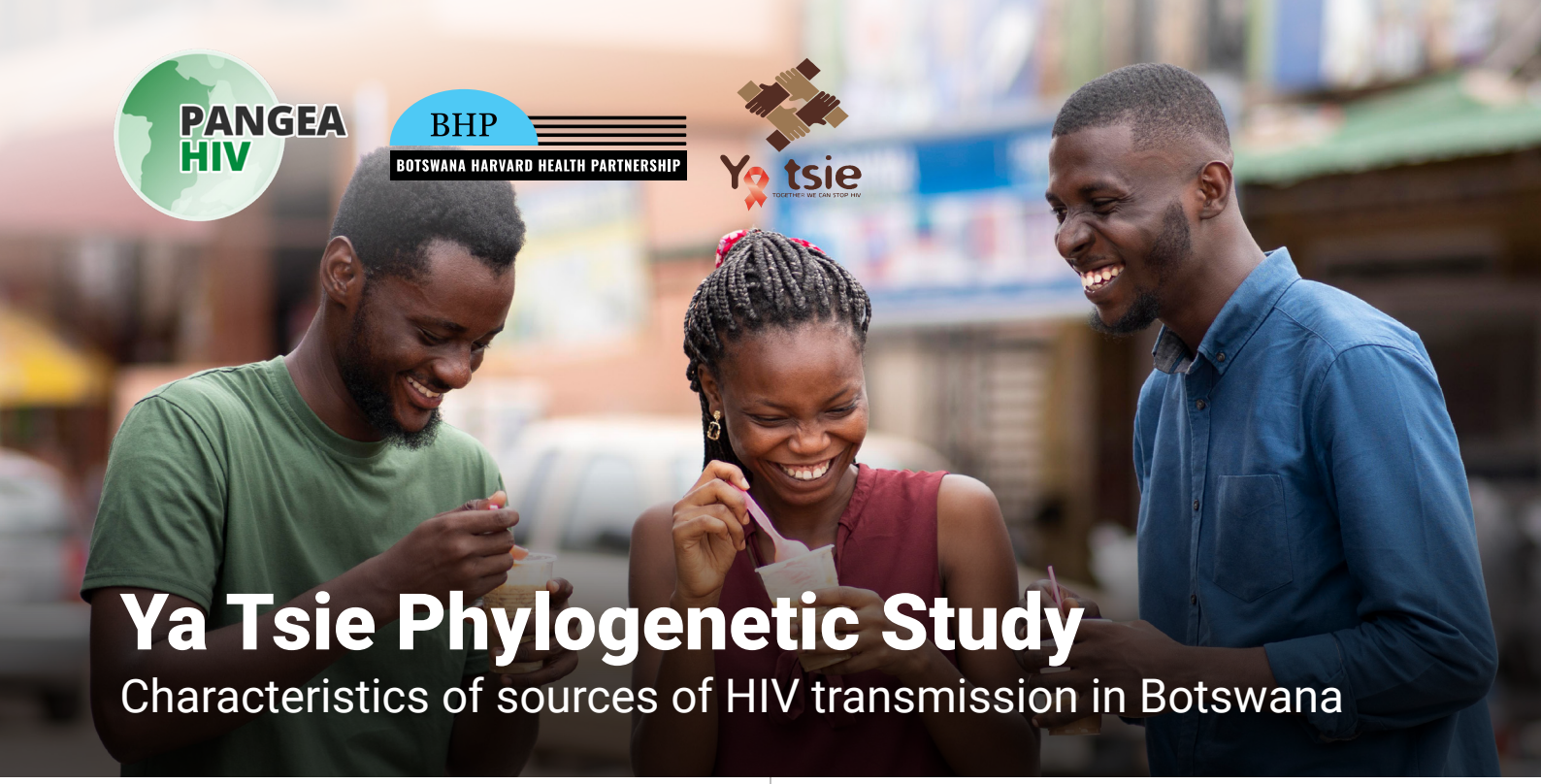


Ya Tsie Phylogenetic Study

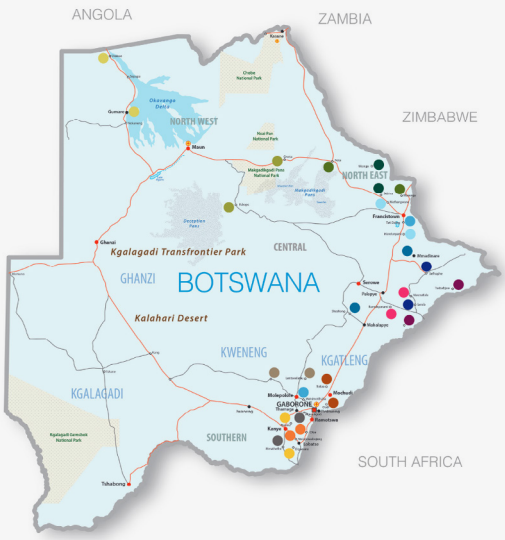
Characteristics of sources of HIV transmission in Botswana

About the Ya Tsie study

The Ya Tsie trial was conducted across 30 communities in Botswana from 2013 to 2018. The trial aimed to determine how much new infections could be reduced by providing HIV testing and antiretroviral therapy (ART) to everybody. These 30 communities were paired into control and intervention communities based on factors such as population size, age demographics, access to healthcare, and proximity to major urban centres. At the end of the study, HIV incidence was 31% lower in the intervention communities than in the standard-of-care communities. Both intervention and standard-of-care communities saw increases from baseline in population-levels of HIV-diagnosis, ART usage, viral suppression, and male circumcision, with greater increases observed in the intervention communities. To better understand the sources of new infections during the intervention period, a phylogenetic study was conducted in parallel with the trial.

During the trial period, the phylogenetic study gathered blood samples from over 5000 individuals aged 16 to 64 who were living with HIV. The genetic sequence of the HIV virus was determined from each of these samples. Our analysis focused on sequences from 3832 participants, representing approximately 14% of the estimated 27,000 individuals living with HIV within the trial communities during the trial period. The sequences were analysed phylogenetically to learn more about where transmissions were coming from within the population. 81% of the 3832 were proviral sequences obtained from individuals taking ART.

Figure 1
Map showing the 30 trial communities in the Ya Tsie trial matched into control and intervention pairs.



- Average community population 6,000;
- Total population ~180,800;
- Age -eligible (16-64) ~105,000

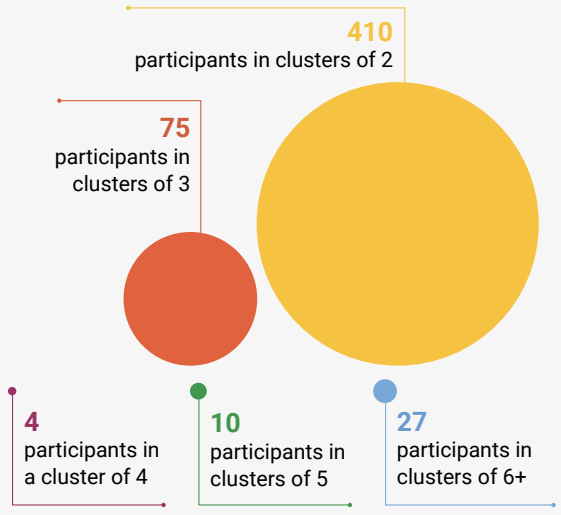
Ya Tsie
30 Villages in 15 pairs

- Ranaka Digawana
- Molapowabojang Otse
- Letlhakeng Lentswelatau
- Bokaa Oodi
- Mmathethe Mmankgodi
- Sefophe Lerala
- Ramokgonami Maunatlala
- Mmadinare Shoshong
- Metsimotlhaba Tati Siding
- Sebina Nkange
- Mandunyane Mathangwane
- Rakops Gweta
- Shakawe Gumare
- Tsetsebjwe Sefhare
- Nata Masunga

Most genetic clusters are small

In the first analysis, the sequences were grouped into genetic clusters. A cluster is a group of sequences which are genetically similar to each other. It can be assumed that individuals with very similar sequences are close to each other in the HIV transmission network. Large clusters are observed if a few individuals transmit HIV to many others within quick succession. Small clusters are consistent with an epidemic where people who transmit HIV only infect one or two people over longer periods of time. The study identified 236 clusters from the 3832 sequences. 97% of the clusters had either two or three individuals (Figure 2). This tendency was also observed in other countries in sub-Saharan Africa with predominantly heterosexual transmission. The small clusters in Botswana suggest a pattern of many individuals infecting only a few, as opposed to a scenario where a few people infect many, which is more common in the larger clusters typically seen in European and North American HIV epidemics. In those regions, the transmission often occurs within groups of men who have sex with men (MSM).

Figure 2
Most clusters consist of sequences from 2 or 3 individuals (yellow and orange circles) whereas few clusters consist of sequences from 4 or more individuals (purple, green and blue circles)



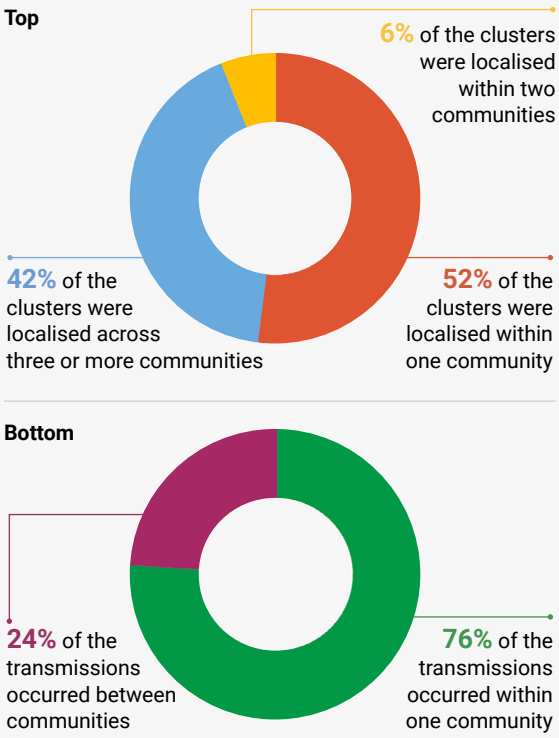
33% of all clusters had at least one sequence from a person that was still infectious at the time of sampling, but only 4% of clusters were entirely comprised of patients who were infectious at the time of sampling. In this context, identifying clusters to mitigate further infections would not have had a substantial impact as most members identified were already on ART.

Clusters and transmission events are geographically close

The study looked at geographical spread of both clusters and transmission pairs. A cluster analysis makes no assumptions about who infected whom in the cluster. More sophisticated phylogenetic analyses can use the rich information generated by next-generation sequencing to identify probable transmission pairs. These are pairs of sequences derived from one female and one male individual in which one likely infected the other. These analyses do not provide definitive answers for individual transmission pairs, but they work well for identifying the characteristics of sources of HIV transmission at population level, such as their typical age and sex.

Approximately 50% of all clusters contained sequences from one community only. Another 42% spanned two communities. Only 6% of clusters were localised across three or more communities (Figure 3 top), indicating that most transmissions happened locally. A similar geographical pattern was observed when looking at transmission pairs (Figure 3 bottom). 76% of transmissions occurred within one community and 24% between communities. The 24% is likely to be an underestimate because transmissions from outside the trial area could not be identified as cross-community transmissions (the source was not sampled).

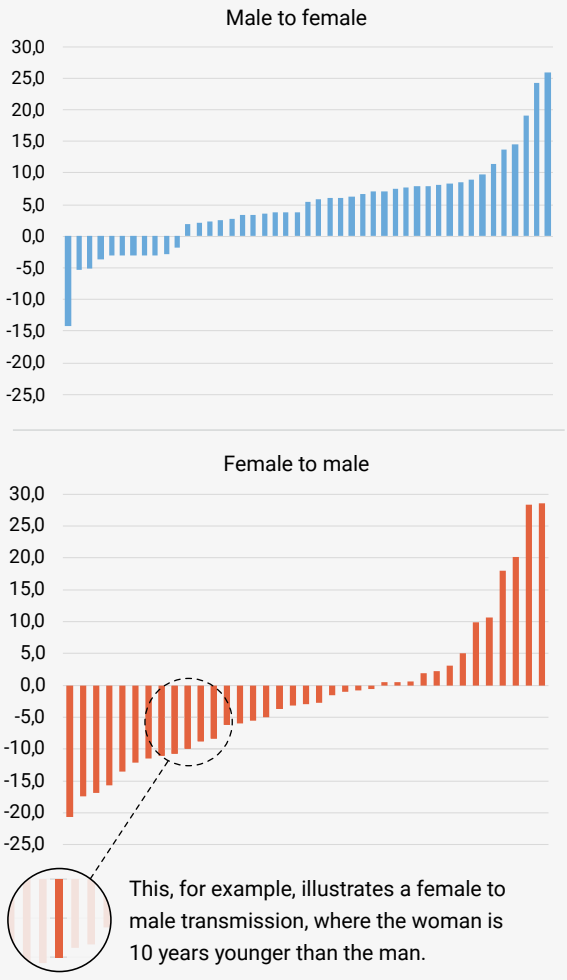
Figure 3
Clusters and transmission events are geographically close



In most transmission pairs, men are older than women

The study identified 82 likely transmission pairs among the study participants. Of these, 45 were male-to female transmission pairs and 37 were female-to-male transmission pairs (Figure 4). In most of these pairs, both male-to-female and female-to-male, the man was older than the women (76% and 65%, respectively). Adjusting for demographic differences between the study population and the whole population, men were on average 3.8 years older in male-to-female transmissions and 1.3 years older in female-to-male transmissions. More transmissions occurred from control to intervention communities.

Figure 4
Age gaps between partners vary widely (unadjusted numbers)



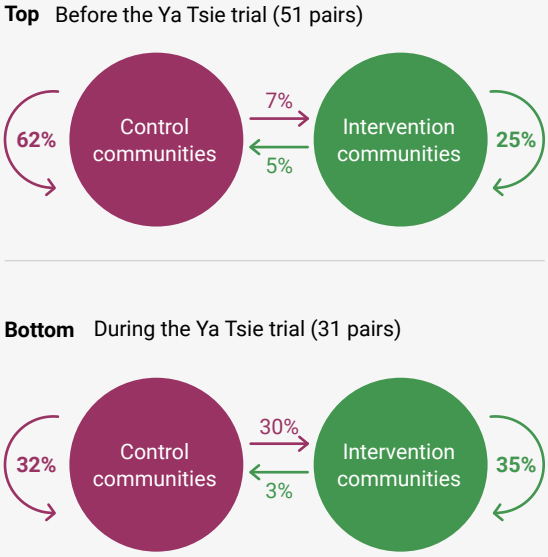
Overall, there was wide variation in the age gaps of the partners. Approximately 60% of transmissions happened between partners whose ages were five or less years apart and 40% of transmissions happened between partners whose ages were more than five years apart (Figure 4).

More transmissions occurred from control to intervention communities

Out of the 82 probable transmission pairs, for 51 the recipient was already living with HIV at the start of the trial, meaning that the transmission did not occur during the trial. 31 had a recipient who first tested positive during the trial period, so it is likely that these transmissions happened during the trial period and their pattern could have been influenced by the intervention. Figure 5 shows the proportions of transmission within and between the invention and the control communities before the trial (Figure 5 top) and during the trial period from 2013-2018 (Figure 5 bottom).

This analysis suggests that mobility in Botswana is high, and that the intervention likely had a larger impact on reducing HIV incidence than the trial results indicate: the trial results were diluted by infections coming from control communities and from areas outside the trial. However, given the small number of transmission pairs in this analysis, it is important to interpret these results with caution.

Figure 5
Transmission flows before and during the trial period



> *Widely distributed and easily accessible HIV testing, treatment and linkage to care remain crucial to fight HIV in the next decade.*

Conclusions

The Ya Tsie Phylogenetics study aimed to identify how and in which groups of the population the virus is spread. This information provides insights into the effectiveness of current approaches to reduce new infections, and informs the design of future HIV prevention strategies. The study's findings indicate that:

1. Small clusters of genetically similar HIV infections suggest an epidemic where many infect a few rather than a few infecting many.

2. 76% of transmissions occurred in the same community, but 24% occurred between communities, in line with high mobility in Botswana.

3. On average, men in male-to-female transmission pairs were 3.8 years older than their female partners, and 1.3 years older in female-to-male transmission pairs. 40% of pairs had an age gap of more than 5 years.

4. The high amount of sexual mixing between communities likely diluted the trial effect and made the reduction in HIV incidence look smaller than it would have been without an introduction of transmissions into the trial communities.

Overall, the study concluded that mobility patterns are fundamental to HIV transmission dynamics and to the impact of HIV control strategies. Transmission flows suggest that the intervention would have reduced HIV incidence even further if it had been rolled out country-wide.

PANGEA HIV

Phylogenetics And Networks for Generalised HIV Epidemics in Africa

PANGEA is a network of African, European and American researchers identifying individual and population-level factors that drive HIV transmission in Sub-Saharan Africa. For more information visit our website www.pangea-hiv.org

What must be done

1. Widely distributed and easily accessible HIV testing, treatment and linkage to care.

2. The implementation of additional programs providing pre-exposure prophylaxis (PreP) to younger women.

3. Establishing self-testing for men.

4. The establishing of a population-level genomic surveillance program to identify regions with high transmission rates for targeted interventions.

5. A reassessment of other UTT trials to establish how successful the intervention would have been if transmissions into the intervention communities had not diluted the trial results.

Adopting these measures has the potential to decrease new HIV infections and accelerate progress toward epidemic control.

The full phylogenetics study has been published as Magosi et al (2022), elife e72657. The trial itself has been published in Makhema et al (2019), NEJM 381:230-42 and Wirth et al (2020), Lancet HIV e422-e433.

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Partners

The Ya Tsie trial was carried out by the Botswana Harvard Health Partnership (BHP) (formerly the Botswana Harvard AIDS Institute Partnership) in collaboration with the Harvard T.H. Chan School of Public Health (HSPH), the U.S. Centers for Disease Control and Prevention (CDC), and the Botswana Ministry of Health.
The phylogenetics analysis was carried out by the Center for Communicable Disease Dynamics and the Department of Immunology and Infectious Diseases at HSPH, and by the BHP in collaboration with the PANGEA network. Sequencing was conducted by HSPH, BHP, and the PANGEA network.
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