Title: Number of HIV-1 founder variants is determined by the recency of the source partner infection

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One sentence summary: Multiple founder variant transmission of HIV-1 increased during early infection.

Abstract

During sexual transmission, the large genetic diversity of HIV-1 within an individual is frequently reduced to one founder variant that initiates infection. Understanding the drivers of this bottleneck is crucial to develop effective infection control strategies. Little is known about the importance of the source partner during this bottleneck. To test the hypothesis that the source partner affects the number of HIV founder variants, we developed a phylodynamic model calibrated using genetic and epidemiological data on all existing transmission pairs for whom the direction of transmission and the infection stage of the source partner are known. Our results suggest that acquiring infection from someone in the acute (early) stage of infection increases the risk of multiple founder variant transmission when compared with someone in the chronic (later) stage of infection. This study provides the first direct test of source partner characteristics to explain the low frequency of multiple founder strain infections.

Main Text

Sexual transmission of HIV-1 results in a viral diversity bottleneck due to physiological barriers as well as viral or cellular constraints that prevent most genetic variants within the source partner from establishing onward infection (*1–3*). Indeed, this diversity bottleneck results in around three quarters of new infections being founded by a single genetic variant (*4–9*). The extent of genetic diversity transmitted to a new partner is a crucial determinant in understanding the efficacy of putative vaccines and may shed light on the transmission of drug resistance to treatment naive individuals.

The factors leading to the diversity bottleneck during sexual transmission can be broadly categorized as those determined by the source partner—such as viral load and viral diversity available for transmission *(10)*, those determined by the recipient partner—such as target cell type and availability in the genital or rectal mucosa (e.g. (*3, 11, 12*)), and those connected with viral characteristics—such as glycosylation profiles and cell tropism (reviewed in (*13*)). While the impact of the recipient partner and the characteristics of transmitted founder variants have been widely discussed, little is known about how the source partner affects the viral diversity bottleneck. Modelling work suggests that infection stage of the source partner at the point of onward transmission may be a key driver in determining the number of transmitted variants (*14*). However, there is currently no empirical evidence to suggest how the infection stage of the source partner influences the viral diversity bottleneck. This gap has arisen because analyses are routinely conducted on individuals without information on the partner from whom they acquired infection. Phylogenetic analyses now offer a possible solution to this impasse.

Phylogenetic trees are representations of the ancestral relationships of organisms with the tips of the tree representing those that are sampled, the internal nodes representing their inferred common ancestors, and the branches as the evolutionary pathways between these actual and inferred individuals. When phylogenetic trees are constructed using sequence data from both partners in an HIV transmission pair, the relationship between the evolutionary histories of both sets of viral samples may reflect epidemiological relationships between the two individuals (*15-17*). Previous modelling studies suggest that the evolutionary histories of the viral populations in both partners can provide important information, such as the direction of transmission (*15*) and the number of transmitted founder variants (*18*). For this, each putative transmission pair can be classified into one of three 'topologies' that defines the evolutionary relationship between the viral populations of the two partners: monophyletic-monophyletic (*MM*, where the sequences from each partner form separate groups), paraphyletic-monophyletic (*PM*, where the sequences from one partner are embedded in the sequences from the second partner), or a combination of paraphyletic and polyphyletic (*PP*, where sequences from both partners are interspersed) (**Fig. 1A**). The number of monophyletic clusters in a PM (one) or PP (more than one) tree can be interpreted as the minimum number of transmitted founder variants. In practice, however, many factors may influence epidemiological interpretations from phylogenetic trees such as sampling times, sampling density of the viral populations and phylogenetic signal (*19, 20*).

Here we present a data-driven phylodynamic approach to overcome these empirical and methodological issues to evaluate the impact of the source partner's infection stage and route of exposure on the HIV diversity bottleneck (**Fig. 1B, C**). We first retrieved all available genetic and epidemiological information from published HIV sexual transmission pairs where the

direction of transmission is known, and kept for further analysis those pairs for whom transmission could be classified as having occurred in the source partner's acute stage (≤90 days after his/her infection) or chronic stage (later than 90 days after his/her infection). After further stratifying pairs into heterosexual (HET) and men-who-have-sex-with-men (MSM) risk groups, we found a significant difference in the timing of transmission between the two risk groups. Specifically, 10 of 36 MSM pairs were the result of acute stage transmission compared with 1 of 76 of HET pairs (**Fig. 2**).

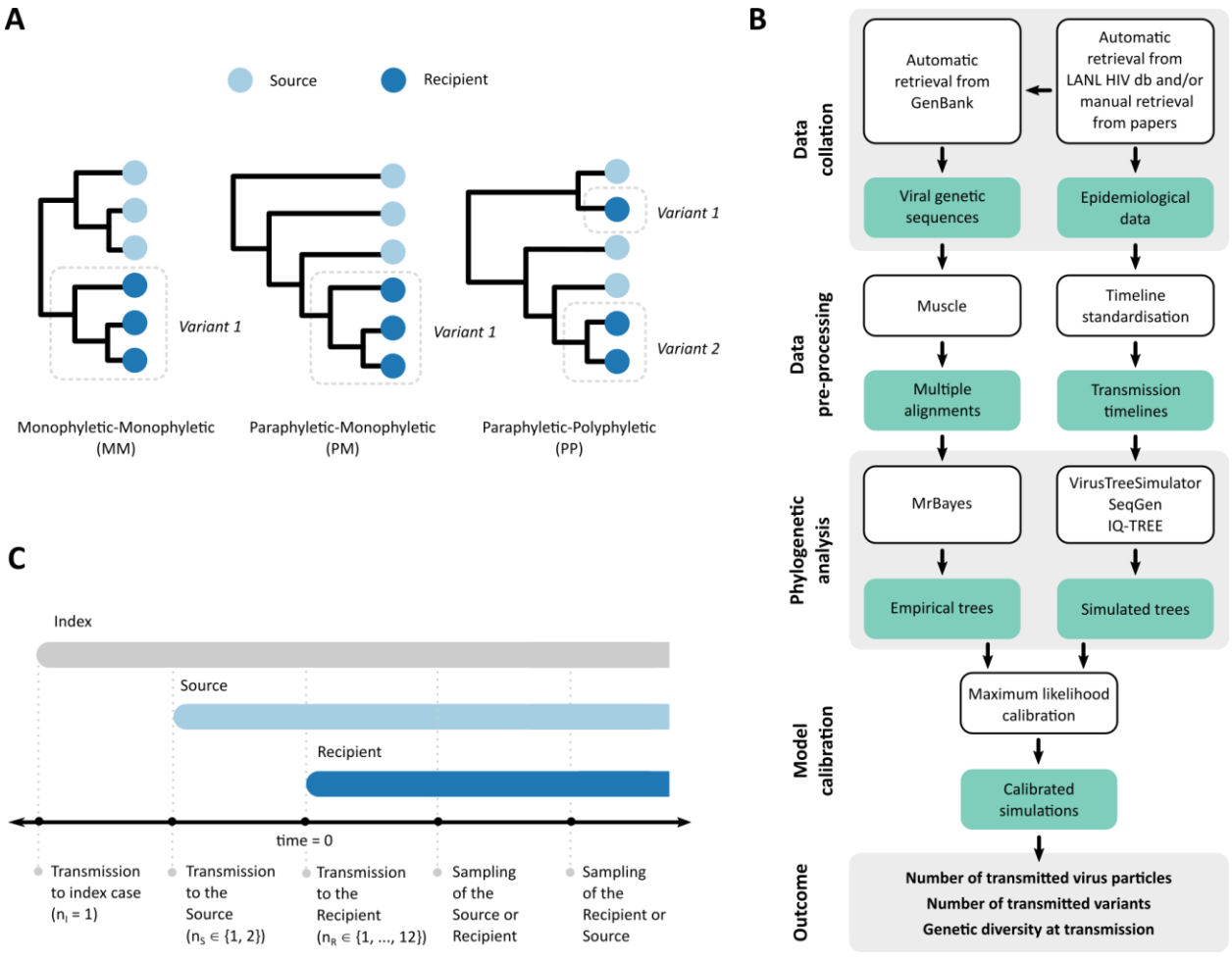


Fig. 1: Methods schematics. A) Phylogenetic tree topology class of known transmission pairs that have previously been used as a proxy for calculating the minimum number of founder variants transmitted to

the recipient: trees of class MM and PM both suggest a minimum of one founder variant while trees of class PP suggest a multiple founder variants, with the minimum number of founder variants being the number of recipient clades embedded in PP trees (here shown as two). B) Pipeline of phylodynamic analysis (LANLdb, Los Alamos National Laboratory HIV sequence database) where teal represents data or analysis output and white represents methods and analysis. An example of a standardised transmission timeline for a known source-recipient pair is provided in panel C. C) Schematic of the transmission pair model simulation that shows the transmission and sampling timelines. The simulated number of virus particles transmitted to the index case, and the source and recipient partners (n_I, n_S, n_R respectively) are shown on the transmission events timeline.

We then performed Bayesian phylogenetic tree reconstruction on the genetic sequences of the transmission pairs and classified the topology class of each tree in the posterior distribution as monophyletic-monophyletic (MM), paraphyletic-monophyletic (PM) or paraphyletic-polyphyletic (PP). The most likely topology class was PM (65% and 61% for HET and MSM, respectively), but with a higher number of PP trees in the MSM group (P=0.056, **Fig. 2**). This result has previously been reported as indicative of a higher number of founder variants for MSM (*18*). However, when we stratify the topology class by whether the source partner was in acute or chronic infection at the time of transmission, our results indicate that the infection stage of the source is the primary driver for any observed differences in topology class. Specifically, there is no difference between the HET and MSM groups in the PM/PP topology class ratio when transmission occurs in the chronic stage of infection (P=0.570). Note that only one HET transmission occurs during the acute stage, and the topology class for this pair is PP. These results remain qualitatively consistent when only data were analysed from the 66% of

transmission pairs for whom the posterior trees gave a certainty of over 95% for the most frequent topology class (**Fig. S3**). These results indicate that infection stage of the source partner, and not risk group *per se*, influences the diversity bottleneck at transmission.

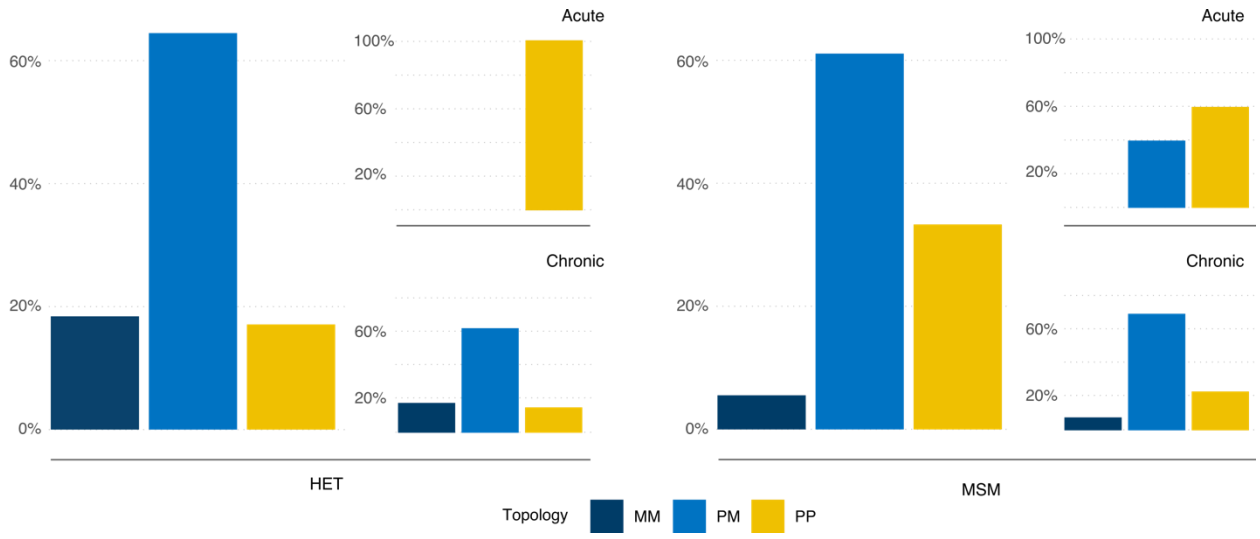


Fig. 2: Phylogenetic findings from the empirical transmission pairs. Fraction of phylogenetic tree topology class (MM: Monophyletic-Monophyletic, PM: Paraphyletic-Monophyletic and PP: Paraphyletic-Polyphyletic) where each tree topology class is classified as the most frequent topology class of each posterior distribution per transmission pair. Results are stratified by risk group: 76 heterosexual (HET) pairs and 36 men-who-have-sex-with-men (MSM) pairs) and infection stage of the source partner at transmission (11 acute pairs defined as <90d post infection and 101 chronic pairs defined as ≥90d post infection).

To test whether these empirical findings are indicative of a smaller diversity bottleneck in the chronic stage of HIV infection, we developed a phylodynamic framework in which we simulated the epidemiologic characteristics of each HET and MSM transmission pair, the timing of their sequence sampling, the transmission of virus particles, and the within-host genetic evolution in

both the source and recipient (**Fig. 1B)**. Specifically, using the epidemiological information from the transmission pairs, we simulated phylogenies under a coalescent model before generating genetic sequences from these simulations and performing Maximum Likelihood (ML) phylogenetic reconstruction on these simulated sequences. We classified each of these simulated trees as MM, PM or PP and determined the frequency of each topology class (*i.e.* the fraction of simulated trees that are classified as MM, PM and PP) for each simulated transmission pair across all the simulated sequences. However, as we could not directly observe the number of virus particles that are transmitted between source and recipient, we repeated the simulation of phylogenetic trees for each transmission pair under a range of plausible values of virus particles transmitted. By fitting the simulation output topology class distribution to the topology class distribution from the empirical phylogenetic trees using maximum likelihood inference, we then determined the most likely number of transmitted virus particles for each transmission pair and used this best fit model for further analysis. Note that two or more virus particles may have the same genetic sequence and would constitute a single founder variant (or haplotype), discussed later. Further, due to the analysis conditioning on extant lineages, we use the term 'founder variants' to describe those transmitted variants that found detectable viral lineages, thereby ignoring variants that are transmitted but the lineages of which become extinct.

Our fitting procedure selects a best fit model that clearly delineates between transmission pairs between whom one virus particle is transmitted (75% of pairs) and those between whom more than one virus particle is transmitted (25% of pairs, **Fig. 3A**). While there is a high degree of confidence in the result when one particle is transmitted, there is often uncertainty around the exact number when multiple particles are transmitted (**Fig. 3A**). Importantly, we found acute stage transmissions are more likely to lead to multiple particle infections compared with chronic

stage transmissions (73% vs. 20%, P = 0.0005). The topology class of the simulated phylogenetic trees is strongly influenced by the number of virus particles being transmitted (**Fig. 3B**). PM trees are more commonly found in the pairs that are better described by a model with a single transmitted virus particle (81%) whereas PP trees appeared more often when multiple particles are likely to have been transmitted (86%).

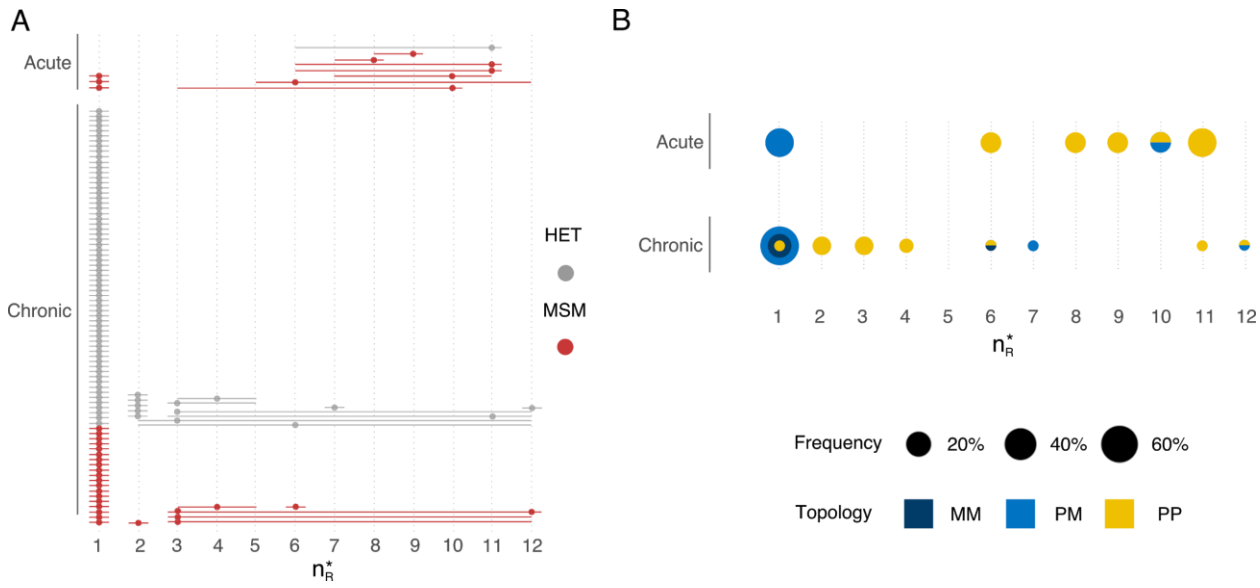


Fig. 3: **The estimated number of transmitted virus particles for the 112 transmission pairs.** The estimates of transmitted virus particles for each transmission pair were calculated by choosing the model simulation that generated a phylogenetic tree topology class distribution (that is, the number of MM, PM and PP trees constructed from the simulated genetic sequences) that best matched the topology class distribution from the phylogenetic trees constructed from the empirical genetic sequences. A) Maximum likelihood number of virus particles founding recipient infections, n_R^*, for each pair (stacked points) with 95% confidence intervals (lines) grouped by stage of infection (acute, 11 pairs or chronic, 101 pairs) and risk group (76 heterosexual pairs, HET and 36 men-who-have-sex-with-men pairs, MSM). B) Maximum

likelihood number of virus particles founding recipient infections coloured by topology class of the phylogenetic tree constructed from the simulated genetic sequences.

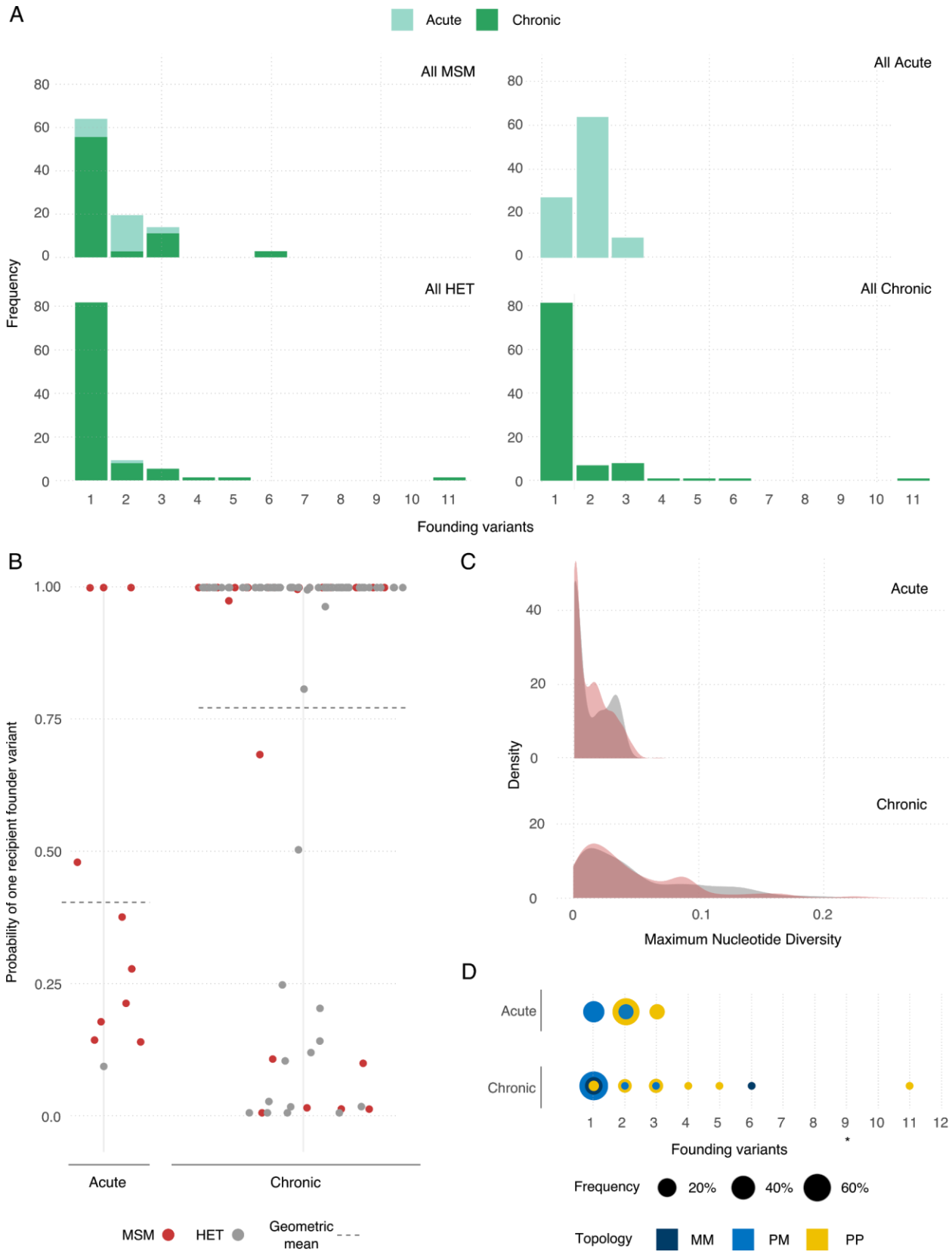
For each transmission pair, we then simulated the genetic sequences of the transmitted viral population under the best fit virus particle model and calculated the most likely number of founder variants for each transmission pair (*i.e.* the number of distinct haplotypes). The median number of founder variants transmitted across all pairs is 1 (range: 1-11, **Fig. 4A**). Using the full distribution of the number of transmitted founder variants for each pair, we also calculated the probability that a single founder variant was transmitted to the respective recipient. Our results suggest that across all pairs in both risk groups, the mean probability of observing one founder variant is 0.73. Stratifying by risk group, we find there is a higher probability that one founder variant founds HET infections than MSM infections (a geometric mean of 0.80 vs. 0.63, **Fig. 4B**). However, these risk group differences mostly disappear when we stratify the results by the infection stage of the source. Here, for example, when only chronic stage transmissions are considered, there is no difference in the probability of one founder variant between MSM transmissions and HET transmissions (means of 0.80 vs 0.71, P=0.398), and the pairwise diversity at transmission is similar between both groups (**Fig. 4C**). In contrast, when stratifying solely by infection stage of the source partner, we find that transmission during the acute stage has a much lower probability of one founder variant than during the chronic stage (means of 0.40 vs. 0.77) with a higher median number of founder variants transmitted, when only the most likely number of founder variants for each pair is considered (2 vs. 1, **Fig. 4A**). Nonetheless, if multiple founder variant transmission does occur, our results suggest that the number of founder variants

190 is higher during chronic stage transmission, consistent with a higher diversity measure during
191 this later stage of infection (**Fig. 4C**).

192 From these results, therefore, there is approximately double the chance of multiple founder
193 variant transmission during acute stage infection across both risk groups (relative risk = 0.52).
194 Assuming that transmission risk is weighted towards early transmission such that half of all
195 index case to source partner transmissions occur after 90 days of index case infection leads to
196 qualitatively similar results (Supplementary Materials). Similarly, calibrating the simulation
197 model to bootstrapped samples rather than Bayesian posterior distributions leads to similar
198 results (Supplementary Materials).
199
200 Our results suggest that there is an association between tree topology class and multiple founder
201 variant transmission, with 95% of MM and PM trees being due to one founder variant (**Fig. 4D**).
202 However, the number of embedded recipient clades is not always a proxy for the minimum
203 number of founder variants transmitted. For example, in chronic stage transmission, 11% of PP
204 topology class trees were due to single founder variant transmission (**Fig. 4D**). It is important to
205 stress that a PP topology class outcome may occur not only due to multiple genetically distinct
206 virus populations founding recipient infections but may also reflect a lack of phylogenetic signal
207 in the data; for instance, the sampled sequence lengths that gave rise to PP trees was on average
208 shorter than those for MM (P=0.096) and PM (P=0.004). Across both infection stages, we find
209 that if MM, PM or PP is assigned as the most likely tree topology class, then 92%, 96% and 15%
210 of transmissions are due to a single founder variant, respectively.
211

We have used a combination of empirical data and phylodynamic model simulation to evaluate the role of infection stage at transmission and route of transmission on the number of virus particles transmitted during sexual HIV exposure. This makes three important advances on previous work. First, it is the first empirically-based study that fits a model to data to understand the role of the source partner in multiple founder variant transmission. Second, while we use previously developed topology classification of phylogenetic trees to understand HIV transmission pairs, we extend this methodology by calibrating a phylodynamic model to empirical data. This new approach provides a means to validate the untested assumption that the number of embedded recipient partner lineages in a phylogenetic tree directly corresponds to the minimum number of founder variants transmitted. Third, our phylodynamic model explicitly incorporates virus particle number and the identity of genetic sequences. This advance produces results that contrast with previous work that has shown the number of founder variants has little impact on the topology class of the phylogenetic tree when only overall genetic diversity, rather than sequence identity, is tracked (*15*).

The relative importance of acute and chronic stages of HIV in determining both the number of virus particles and the number of founder variants transmitted is consistent with a recent modelling study (*14*). However, our study finds higher proportions of infections initiated by multiple founder variants overall during these two stages. This difference is likely due to the assumptions related to how the stages of infection are defined as well as the relative importance of transmission during late infection. Specifically, the previous modelling study finds that two thirds of multiple founder variant transmission occurs during the pre-AIDS stage of infection which is assumed to have both a high viral load and large haplotype diversity. If later stages of


A
Acute
Chronic
All MSM
All Acute
All HET
All Chronic
Frequency
Founding variants
B
Probability of one recipient founder variant
Acute
Chronic
MSM
HET
Geometric mean
C
Acute
Chronic
Density
Maximum Nucleotide Diversity
D
Acute
Chronic
Founding variants
Frequency
20%
40%
60%
Topology
MM
PM
PP

235

Fig. 4: **Phylogenetic findings from the calibrated simulations**. A) Frequency of number of transmitted founder variants for transmission pairs by either infection stage of source partner at transmission (left) or risk group (right). The number of multiple founder variants is calculated as the modal simulated value. B) Probability of one founder variant in the recipient for each pair stratified by infection stage of the source partner at transmission. C) Probability density distribution of maximum diversity (proportion of sites that differ) in the recipient partner across all simulations with more than one haplotype stratified by infection stage of the source at transmission. D) Number of founder variants coloured by topology class of the phylogenetic tree constructed from the best fit model of the simulated genetic sequences.

infection account for disproportionately less transmission then the previous model would predict higher proportions of multiple founder variant transmission in both the acute and chronic stages of infection, becoming more consistent with empirical estimates from our analysis. By contrast, our study is agnostic about the relative importance of early and late transmission and does not differentiate between chronic and a pre-AIDS stage of infection, which cannot easily be identified through analysis of empirical data.

Data from four of the MSM transmission pairs in this study have previously been used to estimate the number of variants founding infection using a combination approach of single genome amplification (SGA), direct amplicon sequencing and mathematical modelling (*7*). Our results broadly agree with this previous analysis, with both analyses suggesting two recipients were infected with one founder variant and one recipient was infected with multiple founder variants (our analysis suggests a mean of 2-3 founder variants and the previous analysis suggests 3 founder variants); there was disagreement with results from a fourth recipient, for whom a

single founder variant was 13% probable in this study (with a mode of 2 founder variants) but the most likely outcome in the previous analysis. Small differences likely arise because this study uses sequence data from both partners to evaluate the transmission of multiple founder variants to the recipient partner. These extra data can be used to parameterize a mathematical model that accounts for the evolutionary relationship between the virus samples from both partners, rather than relying solely on accumulating diversity. Specifically, neglecting the extent of genetic similarity between the source and recipient virus samples might misattribute borderline cases of diversity accumulation.

Our study finds a median of one founder variant and a maximum of 11, with little difference between HET and MSM risk groups. When only multiple founder variant transmissions are considered, our study finds a median of 2-3 founder variants. These values are consistent with a previous pooled analysis using results from four analyses that used the current gold-standard SGA combination approach as above (*9*).

At present, the genetic determinants of HIV-1 disease progression are not clear. However, it is important to note that even small differences between genotypes can have important clinical outcomes. For instance, single polymorphisms can affect replication capacity (*21*), or can lead to primary non-nucleoside reverse transcriptase inhibitor resistance with different amino acids changes at the same position conferring equivalent levels of resistance (*22*).

Previous studies have disagreed over the extent to which the elevated risk of transmission during the acute stage of infection (reviewed in (*23*)) is driven by increased viral load, elevated per particle transmission probability or other behavioural factors such as high rates of sex partner change or concurrent partnerships (*24-29*). Here, while we find strong evidence to support the

fact that acute stage transmissions are characterised by more virus particles and variants founding infection, this result alone cannot disentangle virus- and host-related drivers of elevated transmission. For example, the higher number of variants being transmitted during acute infection could arise if the number of transmissible variants declines as infection progresses or, because with more particles being transmitted, there are more opportunities for multiple variants to found infection (*14*,*30*) However, our study can shed light on the eight times elevated per-exposure risk of infection that has been found for MSM relative to HET transmission (*31*-*32*). In particular, the lack of difference in both the number of virus particles and the number of founder variants that establish infection after transmission from a chronically infected source in HET and MSM suggests that the observed heightened acquisition risk for MSM could in part be due to sampled MSM individuals being more likely to be in the acute stage at the time of transmission (*14*, *27*). Whether MSM partners are more likely to be sampled earlier in infection because of sampling procedures or because MSM are indeed more likely to transmit during early infection is unclear. While this observation raises the possibility that the role of sexual risk group in itself may have less of an impact on the transmission of multiple founder variant probability, from a pragmatic perspective, if more MSM infections are indeed caused by acute stage transmissions, the evolutionary and epidemiologic impact on public health will be the same irrespective of the mechanism.

There are two primary limitations to acknowledge. First, our model assumes a single transmission event between each source and recipient partner. Without detailed knowledge of the transmission pairs, we cannot distinguish between multiple infections each with a single founder variant and a single infection with multiple founder variants; if for some pairs, the former were

304 true then this might suggest an elevated transmission rate during the acute stage, as has been
305 observed previously (*28*, *29*). Second, our phylodynamic framework does not account for the
306 effect of selection and recombination. Specifically, selection, such as that for viruses which use
307 the CCR5 co-receptor (*33*), is thought to occur at the point of transmission , although the strength
308 may be dependent on the route of transmission (*34*).
309
310 Our study finds that the transmission of multiple HIV-1 founder variants is determined by
311 infection stage of the source partner, with transmission of more founder variants of HIV-1 in
312 acute compared with chronic infections. These findings stress that epidemiological or clinical
313 analysis of known transmission pairs should account for potential mediation by stage of
314 transmission when evaluating the effect of sexual risk group.
315
316 **Acknowledgements**

317 **Funding:** CJVA and KEA were funded by an ERC Starting Grant (award number 757688)
318 awarded to KEA. KAL was supported by The Wellcome Trust and The Royal Society grant no.
319 107652/Z/15/Z. MH was funded by The HIV Prevention Trials Network (grant number
320 H5R00701.CR00.01) and The Bill and Melinda Gates Foundation (grant number OPP1175094).
321 **Author contributions:** KEA conceived the study. CJVA, MH, KL, SH, KEA designed the
322 study. CJVA performed the experiments and analysed the data. CJVA, MH, KL, SH, KEA
323 interpreted the data. SGG created new software used in the study. KEA and CJVA drafted the
324 manuscript, with critical revisions from MH, RRR, KL, SH. All authors approved the final
325 version of the manuscript. **Competing interests:** The authors declare no competing interests.
326 **Data and materials availability:** All code and data are available at github.com/AtkinsGroup in

their respective repositories: data on the transmission pairs and sequence alignments (TransmissionPairs_Data), code for retrieval of transmission pair epidemiological data and metadata from Los Alamos National Laboratory HIV sequence database (TransmissionPairs_LANLRetrieval), code for sequence retrieval from GenBank (TransmissionPairs_GenBankRetrieval), code for phylodynamic analysis (TransmissionPairs_PhylodynamicAnalysis), and code for topological classification (TransmissionPairs_TreeTopologyAnalysis).

List of Supplementary Materials

Materials and Methods

Supplementary Text

Figs. S1 to S5

Data S1 to S4

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Reproducibility Checklist

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