

Investigating Viral Transmission Interpreting the Evidence

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Genetic Evidence for Virus Transmission

- Genetic diversity within virus populations
 - Existence of genetically diverse strains of HIV-1, HCV, HBV
 - May differ in genotype, strain, recombination pattern
- Viral strains infecting individuals after a recent transmission event
 - More similar to each than they are to viral strains infecting other individuals
 - Remain phylogenetically distinct over a variable period
- Identification of transmission clusters
 - Recent epidemic spread (eg. swine flu, West Nile virus)
 - Specific transmission events, risk sharing, iatrogenic routes
- Comparison of viral strains a key part of the investigation of transmission

Investigations of outbreaks

- HIV-1 in haemophiliacs
- HCV in anti-D recipients

Molecular epidemiology of an outbreak of infection with hepatitis C virus in recipients of anti-D immunoglobulin

J P Power, E Lawlor, F Davidson, E C Holmes, P L Yap, P Simmonds

Lancet 1995; **345**: 1211-13



Figure: Phylogenetic relations between sequences from the NS-5 region. Tree rooted by inclusion of a type 1a sequence (PT) as an outgroup

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Molecular epidemiology of an outbreak of infection with hepatitis C virus in recipients of anti-D immunoglobulin

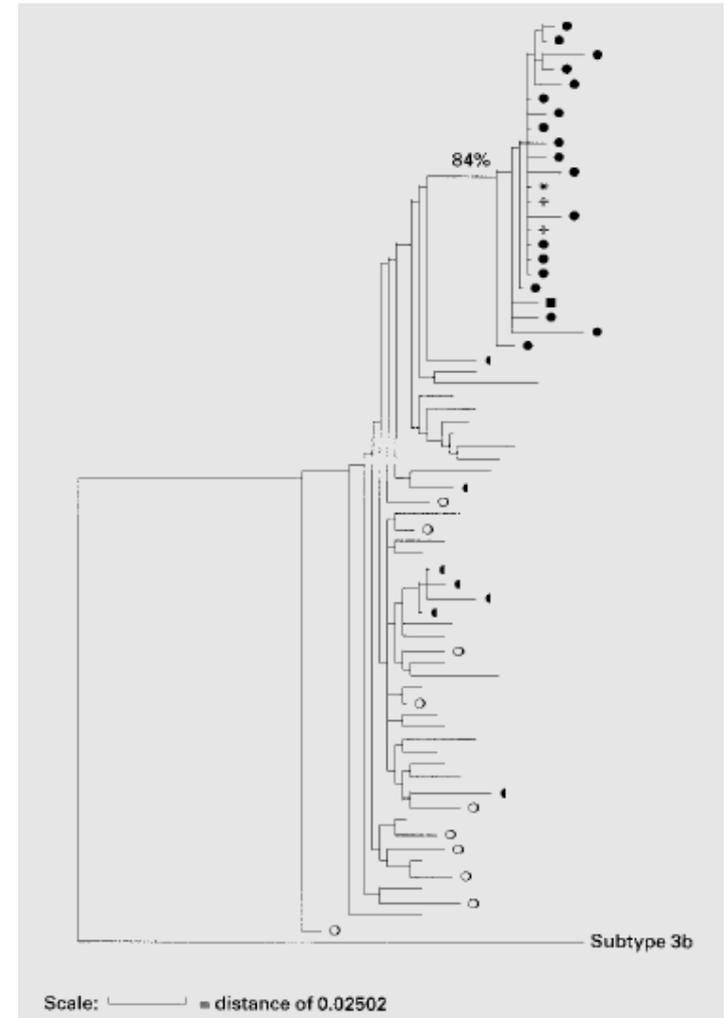
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A Second Outbreak of Hepatitis C Virus Infection from Anti-D Immunoglobulin in Ireland

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Jane McAllister^a Carol Lycett^c Fiona Davidson^c Steve Pathirana^a
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Investigations of outbreaks

- HIV-1 in haemophiliacs
- HCV in anti-D recipients
- PIV-3 in an outpatients department

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Epidemiology and clinical characteristics of parainfluenza virus 3 outbreak in a Haemato-oncology unit

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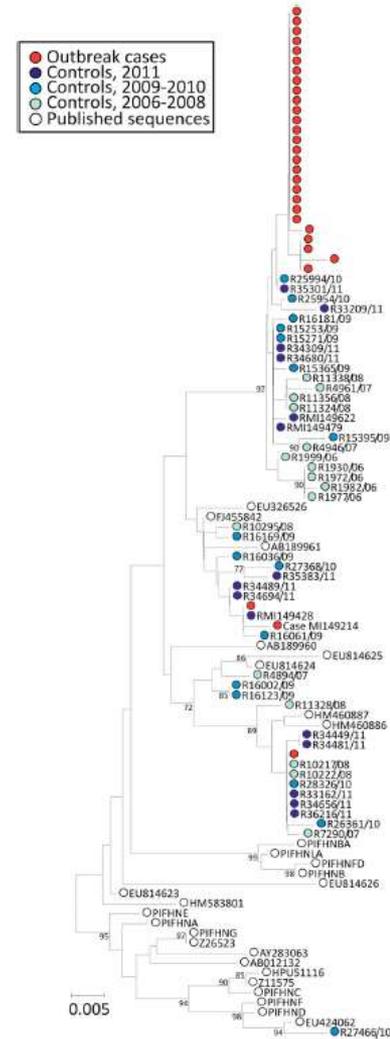


Figure 2 Sequence comparison of PIV3 sequences amplified and sequenced in the HN gene from suspected outbreak cases (red dots) with epidemiologically unrelated controls from Edinburgh (blue dot, labelled by collection year) and available published sequences from the target region (unfilled symbols; GenBank accession numbers). Bootstrap re-sampling was used to determine robustness of groupings; values $\geq 70\%$ shown.



Demonstration of transmission

■ Infection clusters

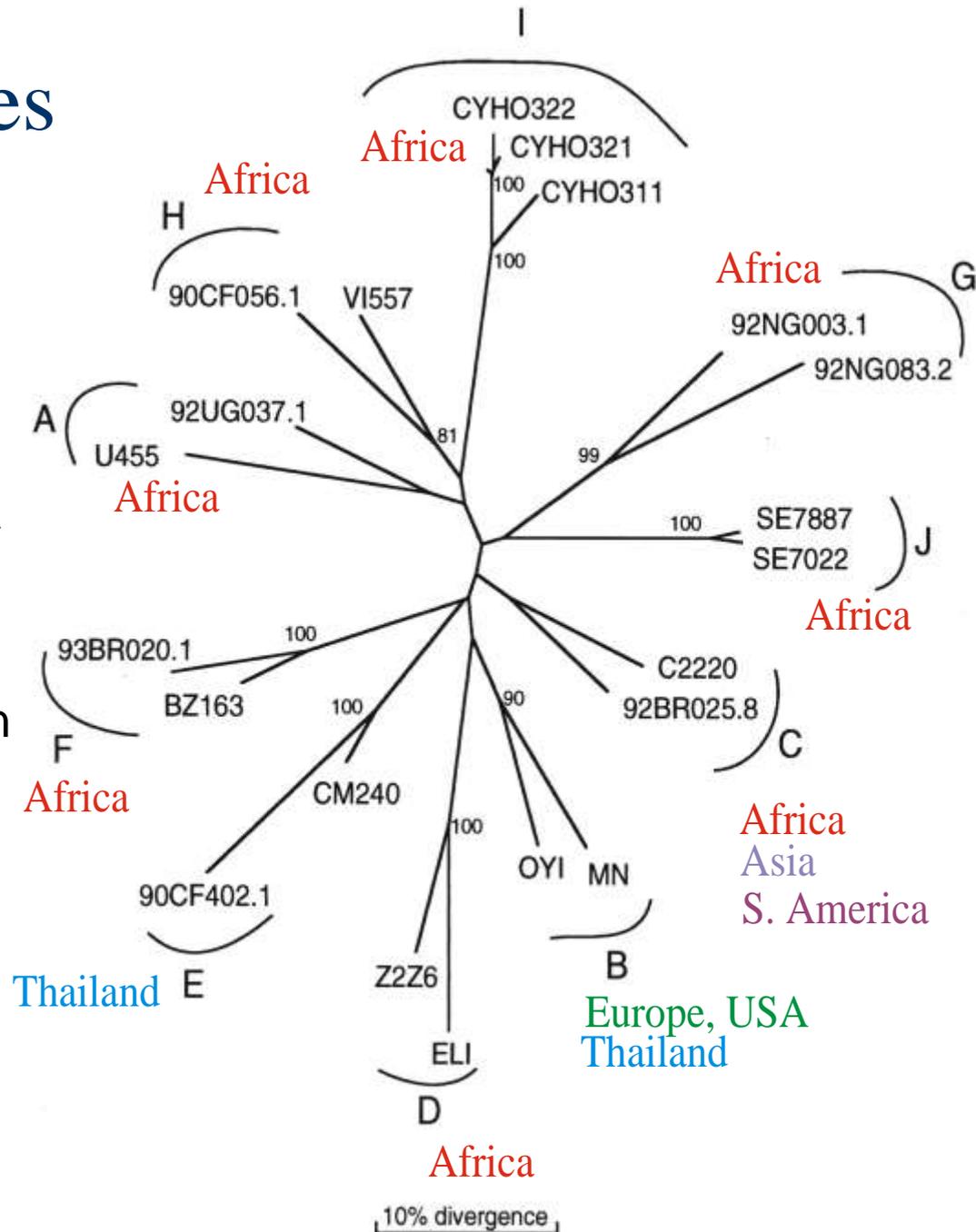
- Sequence relatedness between viruses infecting multiple individuals
- Contact histories point to a common source
- No other epidemiological factors to link them
- Transfusion history, contact tracing provides corroborating evidence for infection source

■ Individual transmission events

- HIV transmission, criminalised in most countries
- Important to perform robust analysis and recognise and explain limitations of evidence obtained
 - Finding of dissimilar strains **can rule out transmission**
 - Finding of closely related strains **cannot rule it in**
- Strain analysis cannot demonstrate direction of transmission

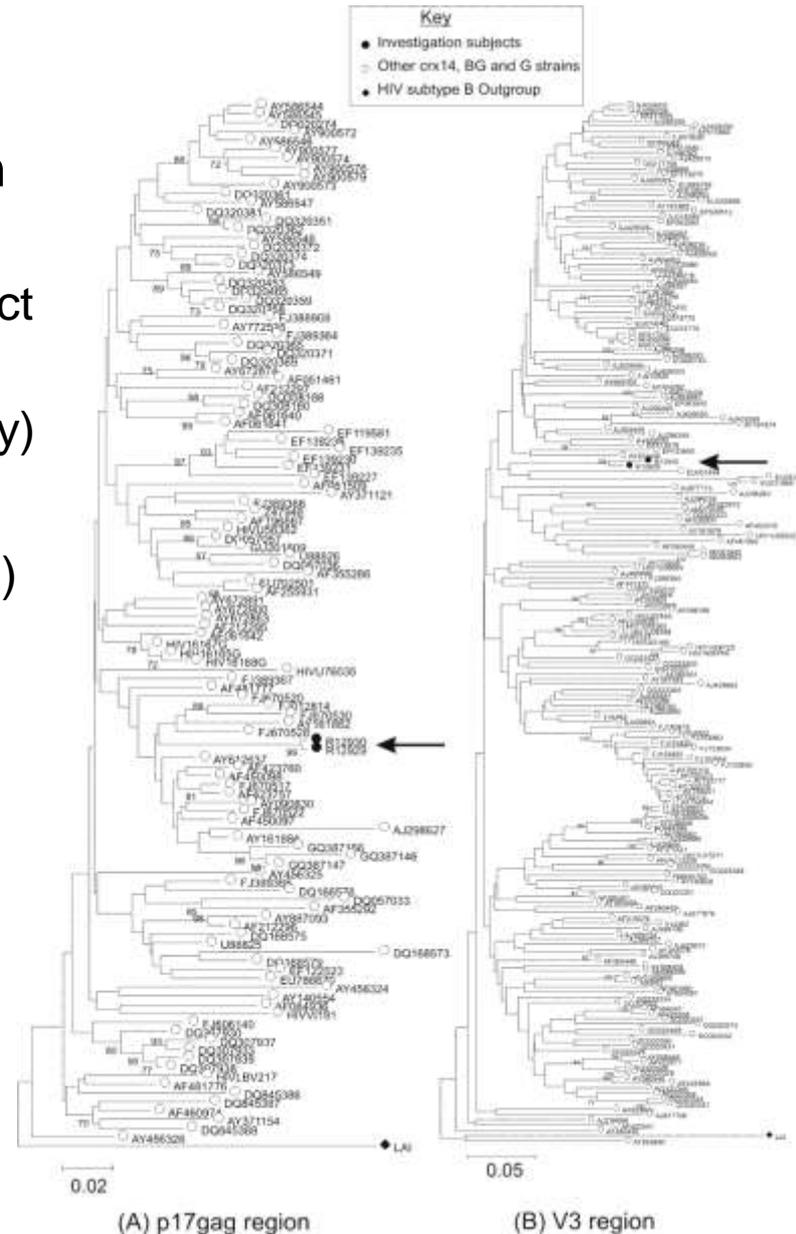
HIV-1 subtypes

- Group M isolates
 - > 12 subtypes
 - Differ by 25% from each other
- Most diversity in Africa
 - Almost all subtypes represented
- Relatively recent origin
 - 1920s-30s
 - Rapid sequence drift
- Recent spread characterized by appearance of mosaic genomes
 - Generated by recombination



A typical case

- CPS Request to test HIV strains in blood samples
 - Accusation of infection from contact with (non-disclosed) HIV infection
 - Escorted delivery (chain of custody)
- Laboratory procedures
 - Sample extraction (ideally PBMCs)
 - Sequencing two genome regions
 - Comparison with 500 closest matching sequencing on BLAST search of Genbank
- Construction of NJ or ML phylogenetic tree
 - Identification of sequence groups
 - Bootstrap support / other methods
- Repeat findings with second samples of blood

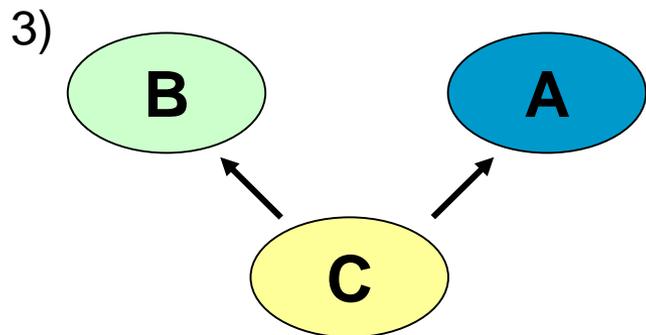
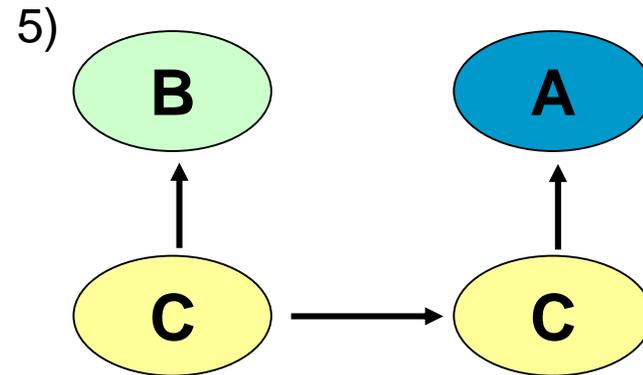
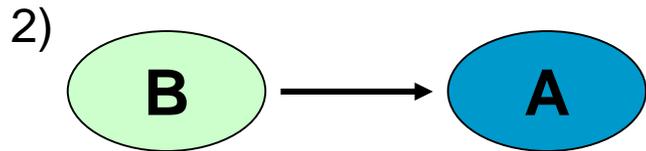
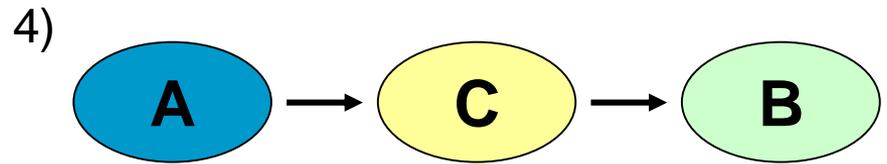
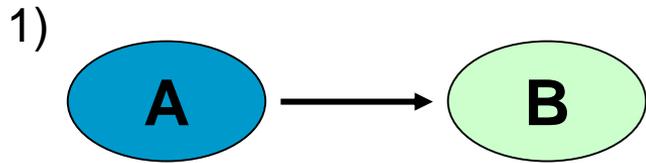


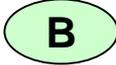


Interpreting genetic data in practice

- How related is “related”?
 - HIV changes rapidly over time
 - How long do HIV-1 strains stay measurably similar?
 - Diversity within individuals
 - Long-term infection – 10-15 years
 - One “unrepresentative” variant from a diverse population might transmit
- Could a third party be involved?
 - Infection of suspect (defendant) and contact (complainant) from a third party
 - Two or more intermediaries may lead to infection with similar strains

Transmission scenarios



-  **A** = Defendant
-  **B** = Complainant
-  **C** = Unexamined person(s) of the same transmission network

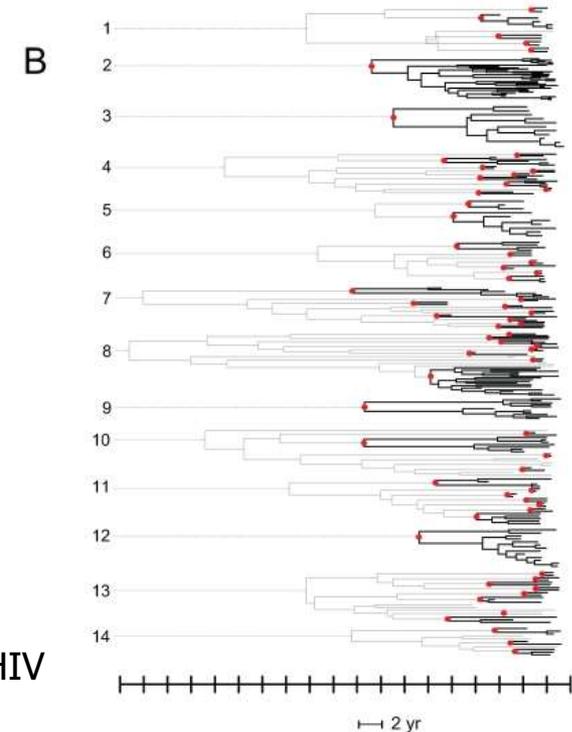
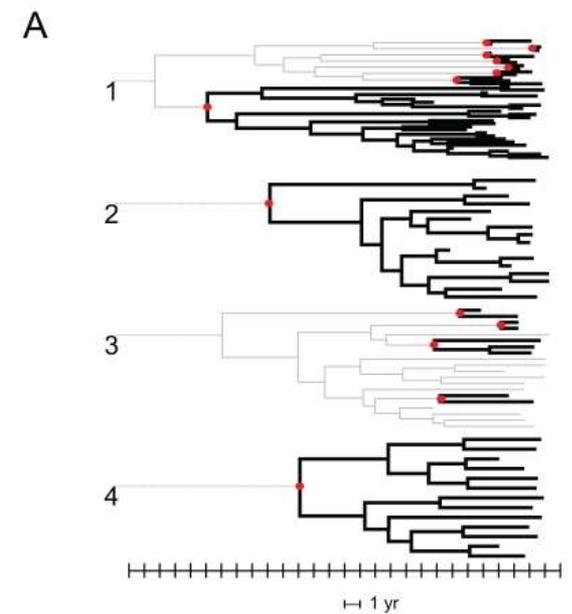


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- Could a third party be involved?
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 - Two or more intermediaries may lead to infection with similar strains
- How related are HIV-1 strains in a community?
 - Dynamic turnover of new strains within a risk group
 - New infections often occur with related strains

Patterns of HIV diversity

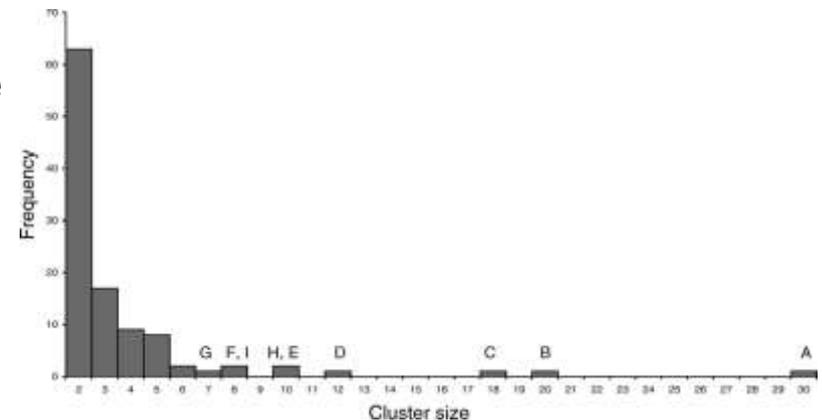
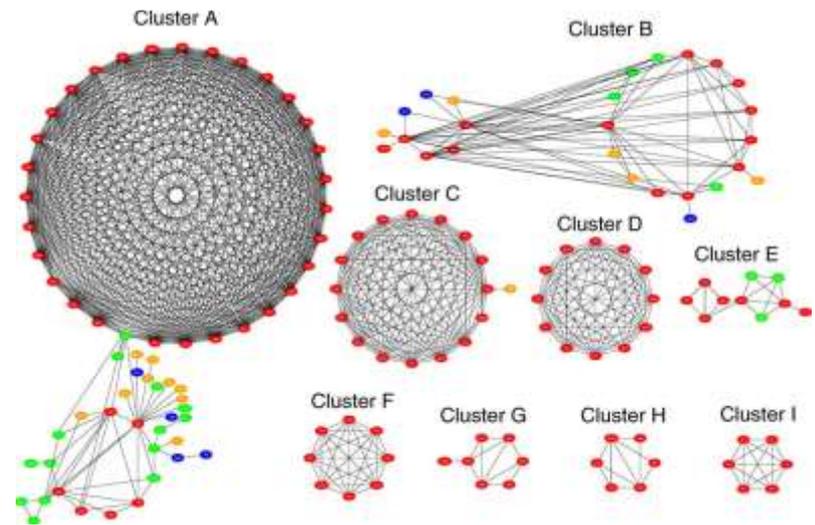
- Relatedness of HIV-1 strains infecting heterosexual risk group in the UK*
 - Non-subtype B
 - 2774 from 11071 pol sequences showed clustering
 - <4.5% sequence divergence



*Hughes *et al.*, Molecular Phylodynamics of the Heterosexual HIV Epidemic in the United Kingdom. PLoS Pathogens **5**: e1000590

Patterns of HIV diversity

- Relatedness of HIV-1 strains infecting heterosexual risk group in the UK
 - Non-subtype B
 - 2774 from 11071 pol sequences showed clustering
 - <4.5% sequence divergence
- Second investigation of subtype B in MSMs in London*
 - 402 / 2,126 showed <5% sequence divergence
 - 90 in clusters of 10 or more
- Relevance
 - Almost no individuals show divergence of <3%
 - Probability of showing even <4.5% divergence extremely low



*Lewis *et al.*, Episodic Sexual Transmission of HIV Revealed by Molecular Phylodynamics. PLoS Medicine **5**: e50



Investigating local outbreaks - Controls

- Investigation of pattern of HIV-1 sequence diversity to estimate likelihood of linkage
 - HIV-1 variants infecting same risk group
 - Same geographical area
 - Same time as alleged transmission
 - Potential to rule similarity arising from infection with a “local” strain
- Problems with sampling:
 - How many individuals need to be sampled locally to rule out a local network?
 - Availability of samples and access
 - Informed consent for retrospective testing needed
 - Potential for selective exclusion, danger of secondary prosecutions



What can be concluded from sequence analysis?

- Finding of unrelated HIV-1 strains in complainant and defendant
 - Most unlikely for transmission to have occurred
 - Sufficient to not pursue a prosecution irrespective of other evidence
- Finding of related HIV-1 strains
 - Consistent with transmission between the individuals
 - Cannot demonstrate directionality of transmission
 - Cannot rule infection through third party or from contact with a wider transmission network
- Other evidence needed for a successful prosecution
 - Dates of infection – defendant before complainant
 - Contact history of complainant – no credible alternative source of infection



Summary

- Strain comparisons can exclude transmission but cannot “prove” it without other evidence
- Finding related strains cannot:
 - Rule out other sources of infection
 - Cannot establish any likelihood of transmission
 - Statements such as “vastly improbable” seem intuitively correct but are misleading
 - Establish direction of transmission
- Demonstration of transmission requires:
 - Contact histories
 - Convincing exclusion of other sources of infection
 - Dates of infection to establish directionality
- Provided evidence for:
 - 55+ allegations of reckless HIV transmission
 - 5 outbreaks / iatrogenic transmission of HCV