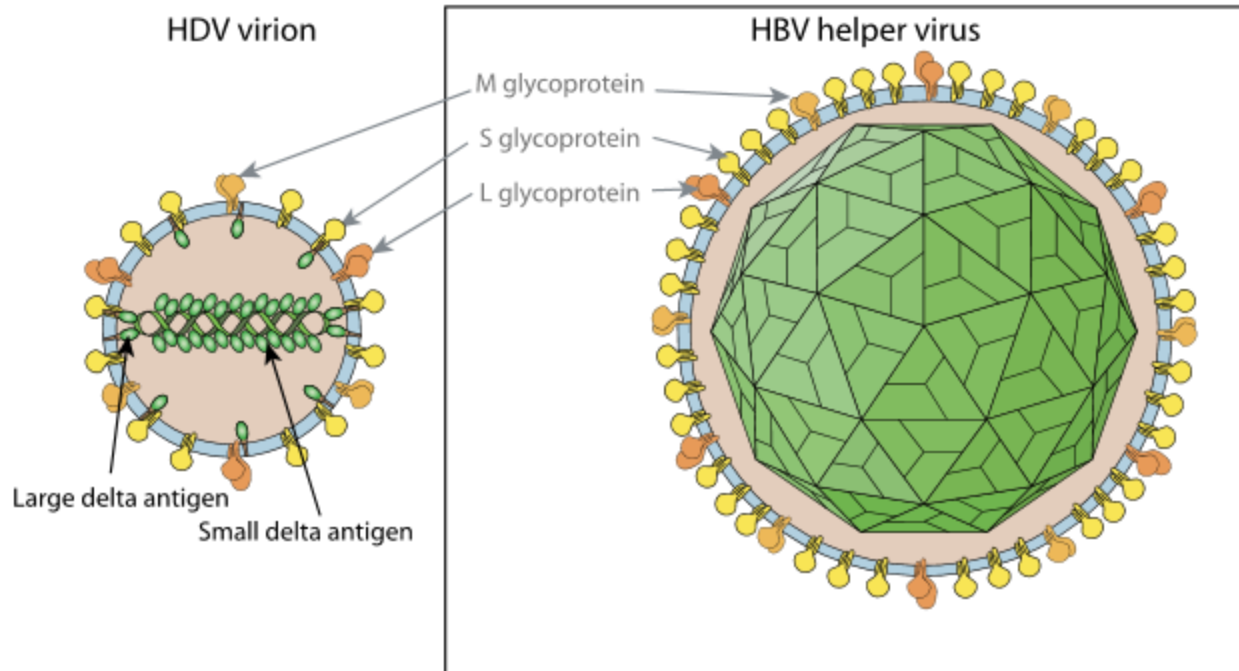


Intro to Project 2: Recombination events in Hepatitis D virus

301520 VO+UE Virus Evolution and Ecology (2025W)

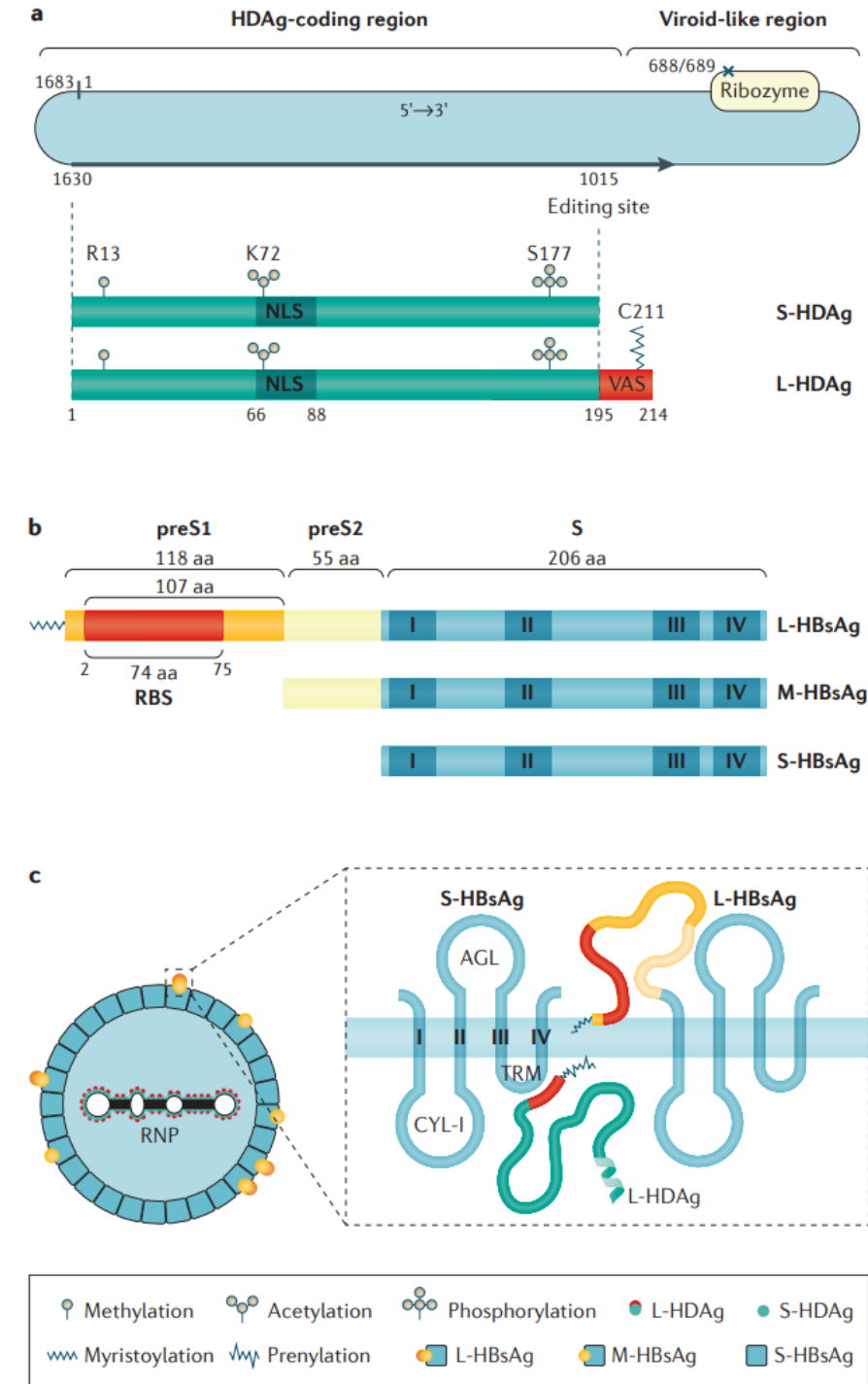
Hepatitis D (HDV)

- Genus: *Deltavirus*
 - 35-37 nm (smallest human infecting virus)
 - circular -ss RNA genome, ~1.7 kb in length
 - Enveloped virus
- Satellite virus of Hepatitis B (HBV)
 - relies on HBV for viral packaging, infectivity, transmission, and inhibition of host immunity
 - But not for replication of own genome
- Cell tropism: hepatocytes (liver cells)



HDV genome and genetic diversity

- Encodes a single protein: delta antigen (HDAg), with 2 isoforms:
 - small (S-HDAg), ~195 amino acids, 24 kDa
 - large (L-HDAg), ~214 amino acids, 27 kDa

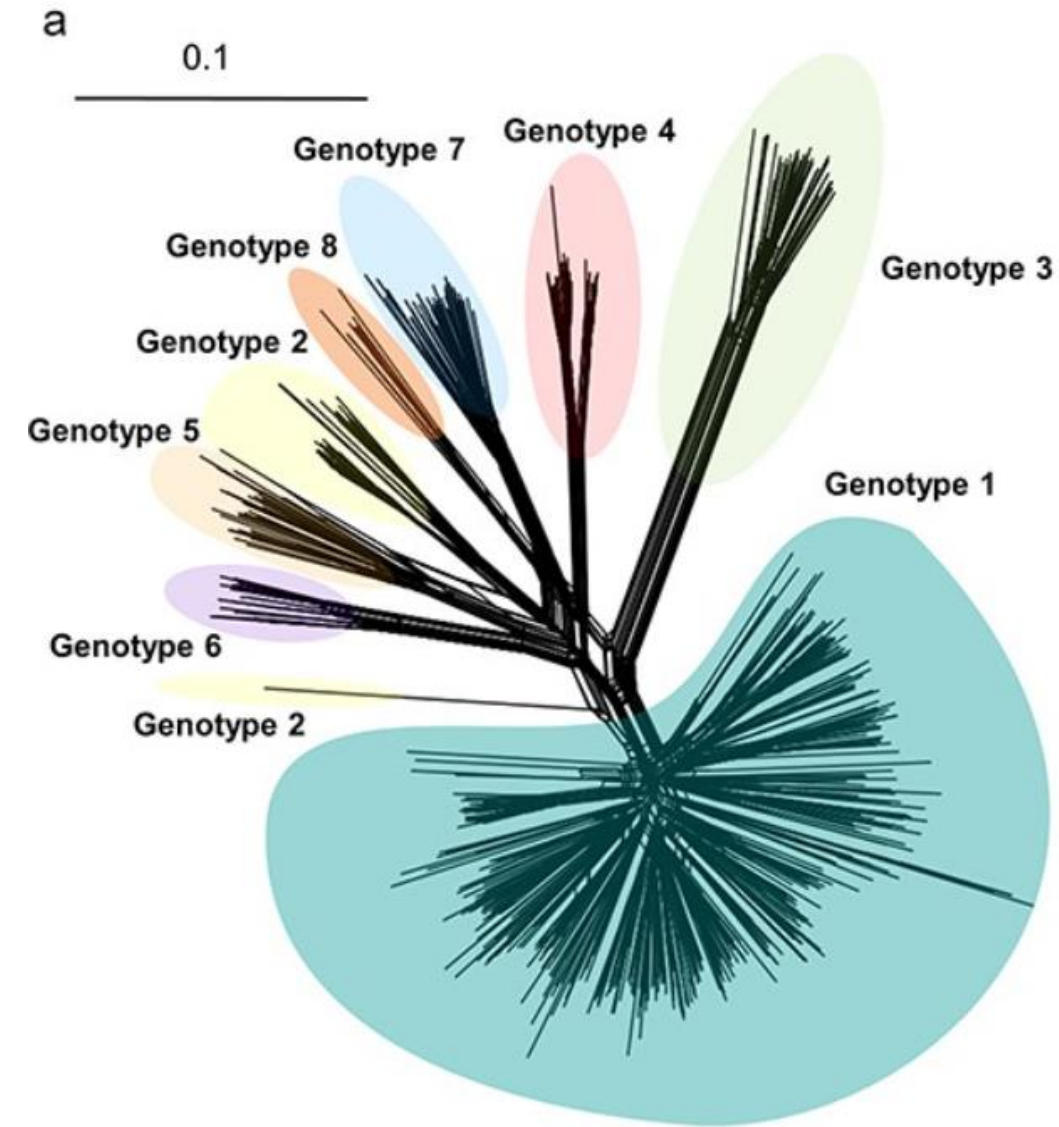


Lempp, Ni & Urban 2016.
Nature Reviews

B Külekci

HDV genome and genetic diversity

- Encodes a single protein: **delta antigen (HDAg)**, with 2 isoforms:
 - small (S-HDAg), ~195 amino acids, 24 kDa
 - large (L-HDAg), ~214 amino acids, 27 kDa
- **8 genotypes** are circulating worldwide, with 81% to 89% sequence homology, with multiple subtypes
- clusters exhibiting an inter-genotypic difference $\geq 10\%$ are classified as genotypes



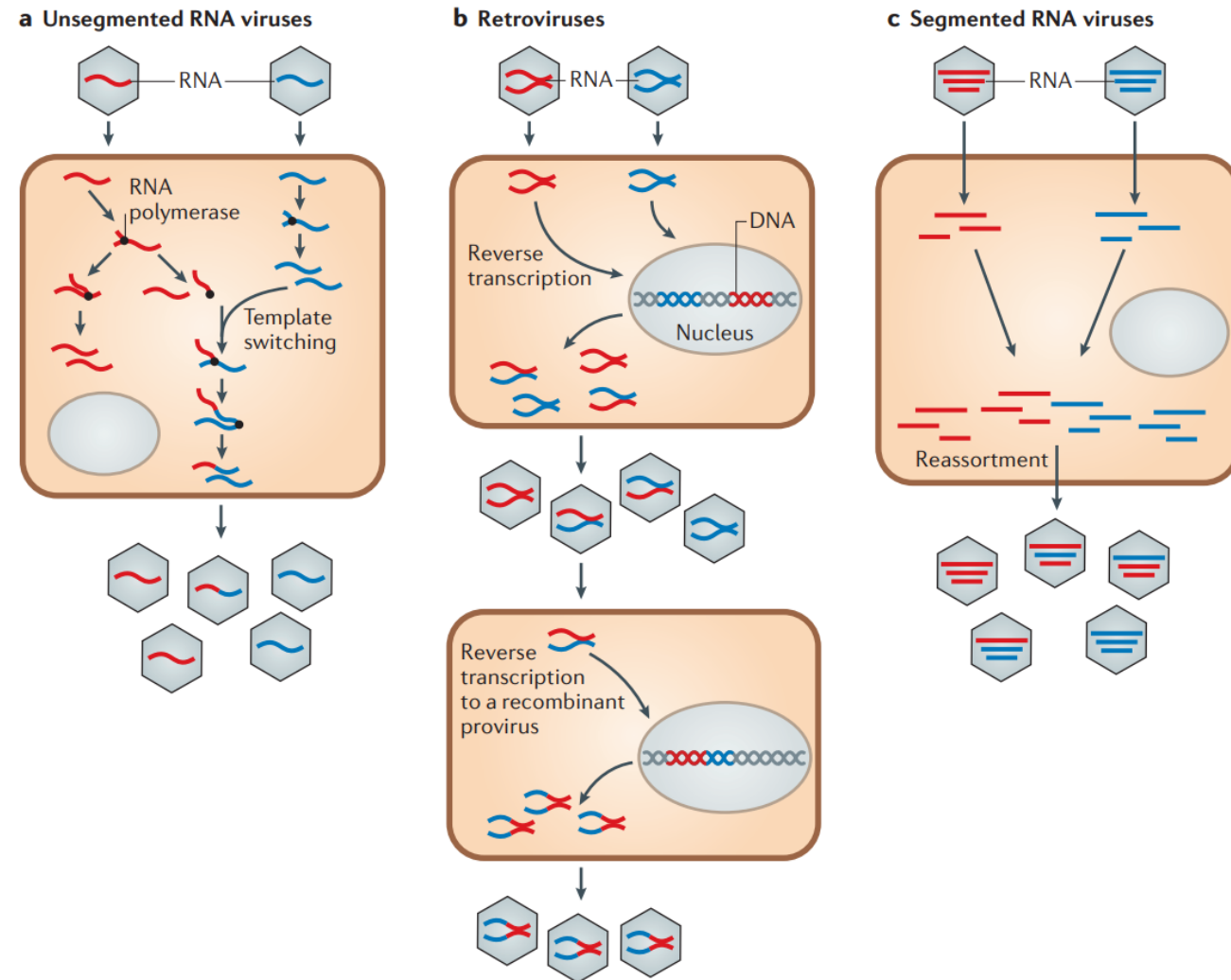
Chowdhury 2025. Virus Evolution

Virus recombination

- Recombination as an evolutionary mechanism to increase host range
- Requires co-infection of host cell by genetically distinct strains
- template switching vs. reassortment
- -ss RNA viruses recombine at low rates due to their genomic organisation e.g.
 - quick association of viral RNA with ribonucleoprotein subunits --> template switching is less likely
 - lack of substrates for template switching (single copy)

HDV specifics:

- Chronic infection --> more chances to acquire mixed infections
- Might contribute to immune evasion (together with T-cell-induced selection pressure)



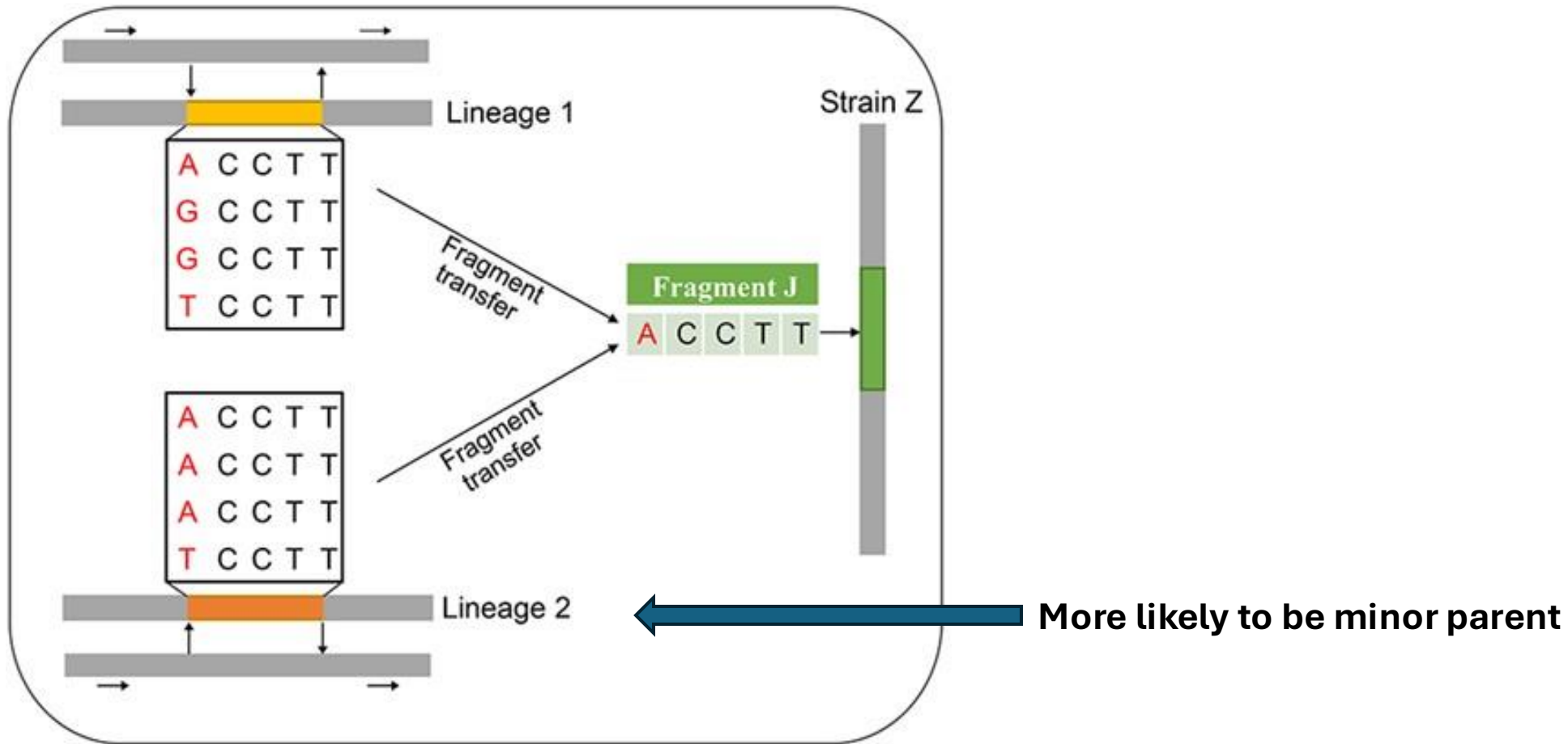
Simon-Loriere and Holmes 2011. Nat Rev Microbiol

Identifying recombination

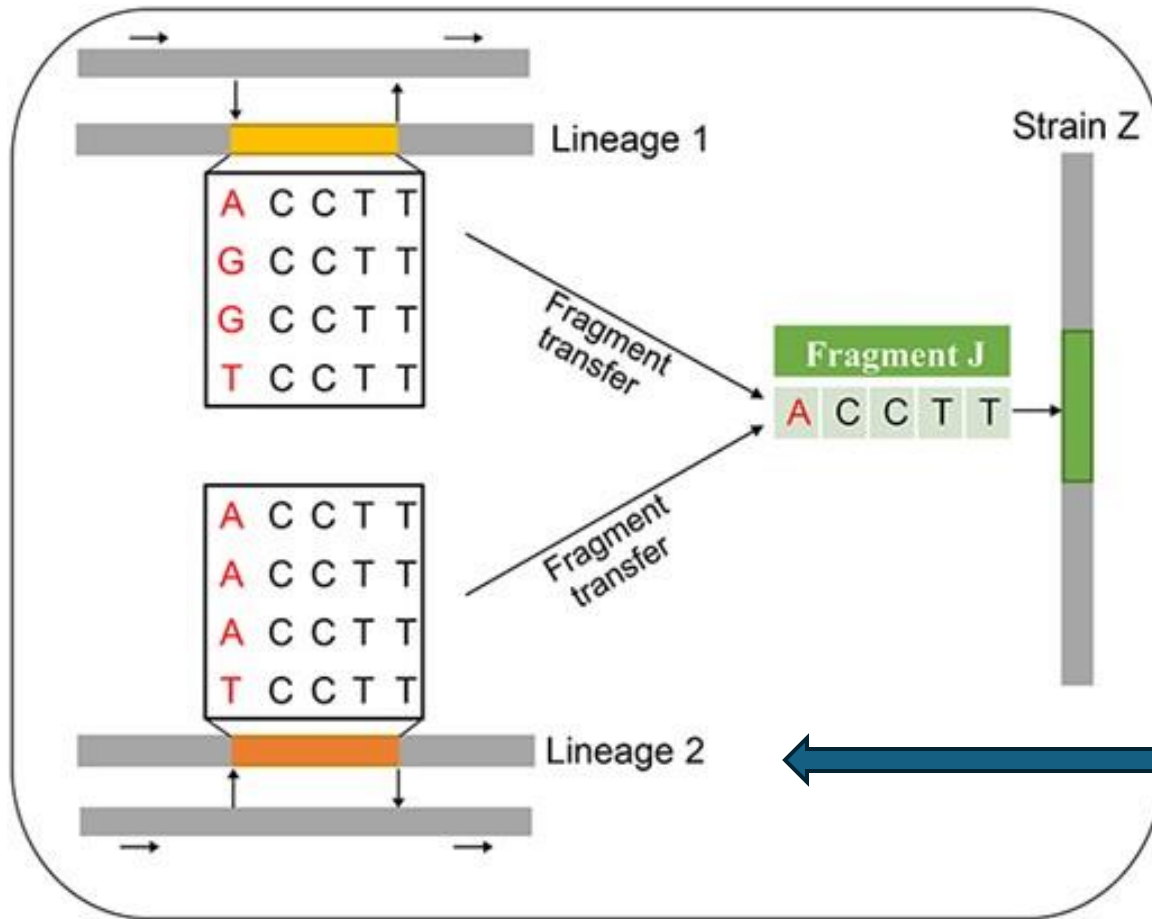
- Challenging in low-divergence viruses due to uncertainty: recombination or *in-situ* mutation (or sequencing error)?
 - "low-divergence viruses" i.e. high genomic similarity between lineages
- Tool we will use: **VirusRecom** (<https://doi.org/10.1093/bib/bbac513>)
- Method: "evaluates the likelihood of recombination by quantifying recombination contribution using **weighted information content (WIC)**" + statistical testing

Recombination = transmission of information

Whose contribution is larger?

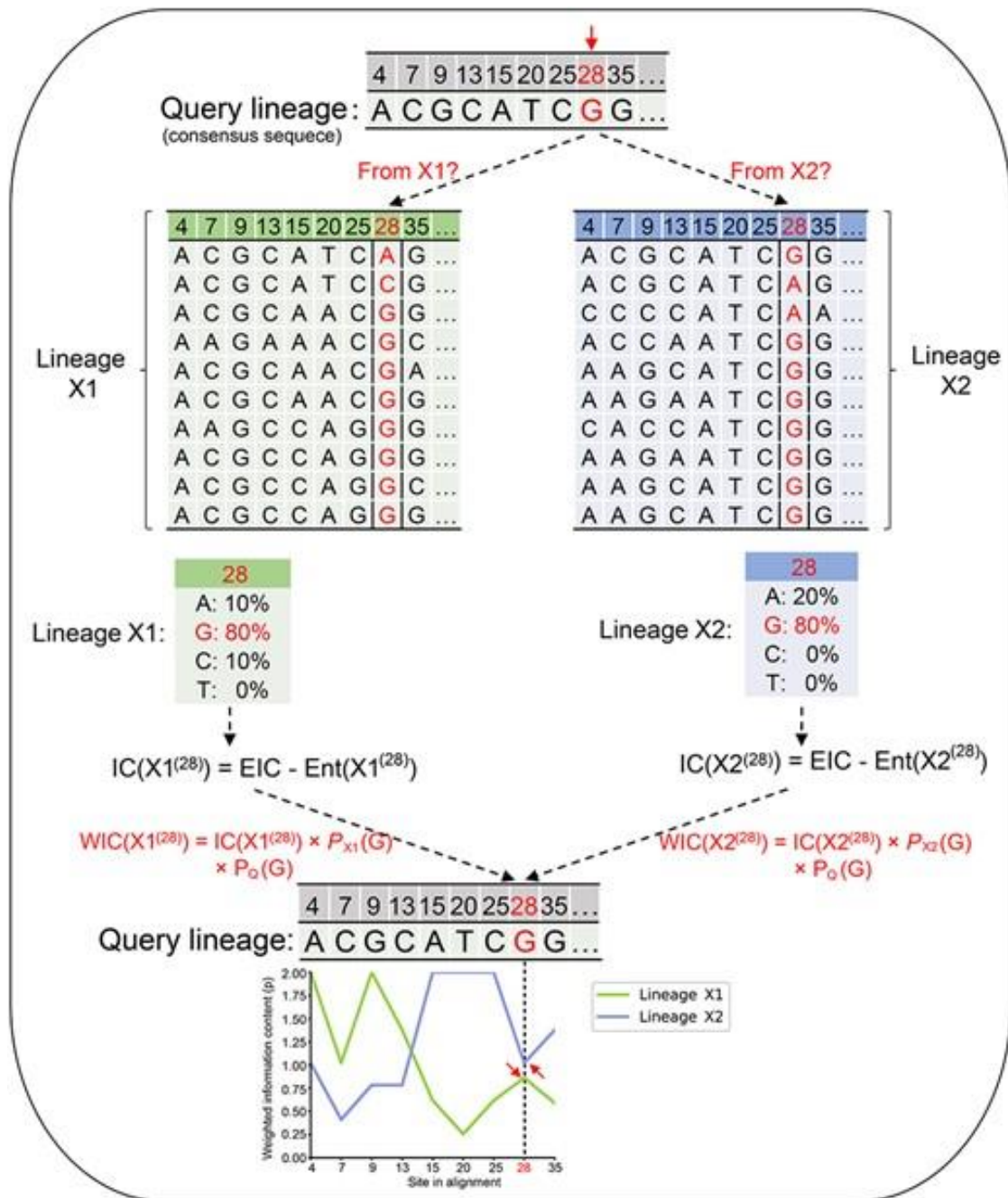


Whose contribution is larger?



More likely to be minor parent contributing "A"
"Is this nucleotide likely due to recombination or mutation?"

--> quantification of contribution by calculating WIC

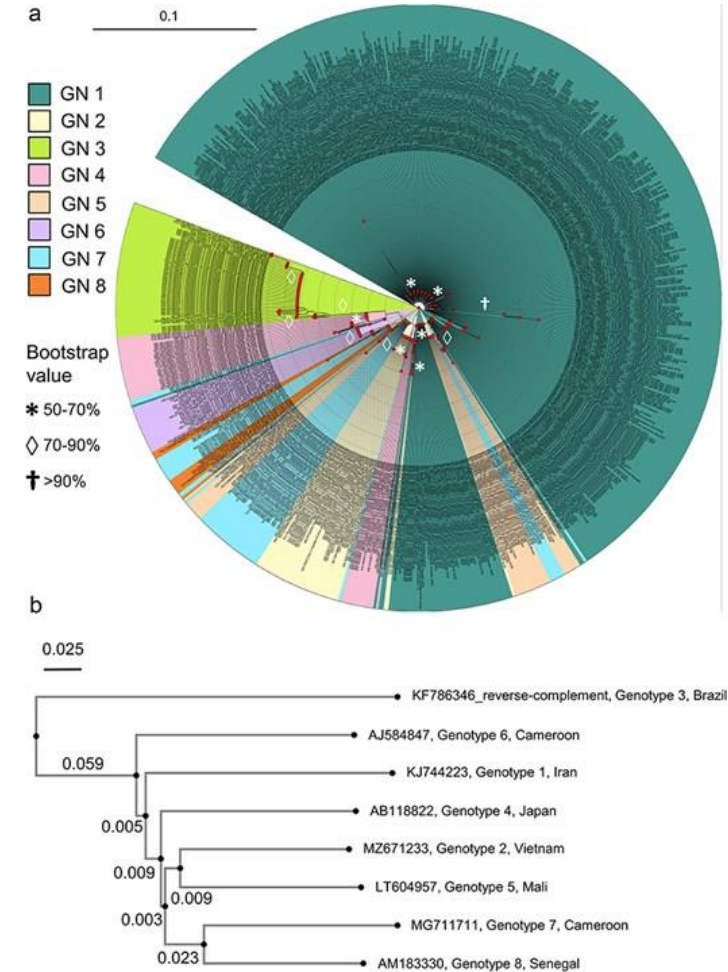


Steps

1. Multiple sequence alignment (including query)
2. Check for polymorphic sites
3. Calculation of WIC in all sites from each genotype contributing to query
4. Splitting into regions/"sliding windows"
5. Determine major parent: The genotype with the maximum cumulative length of recombination regions is regarded as the major parent.
6. Significance testing for WIC contribution on the recombinant region from the major parent and other parents

Outline of Project II

- Your lab has recently sequenced a new Hepatitis D Virus (HDV) sample. To understand its evolutionary origin, you aim to:
 - Identify the most probable parent genotype of your HDV sequence.
 - Investigate whether your sequence shows potential recombination signals with other HDV genotypes.
- **Tools & Data:**
 - **VirusRecom** for recombination analysis.
 - **HDVdb** for publicly available HDV sequences.
 - The most recent reference sequences for all eight HDV genotypes (Chowdhury, 2025).



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<https://doi.org/10.1093/bib/bbac513>