The Swarm: Causes and consequences of HIV quasispecies diversity

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The Swarm

Within an infected person, HIV exists as a genetically diverse population of competing **quasispecies**

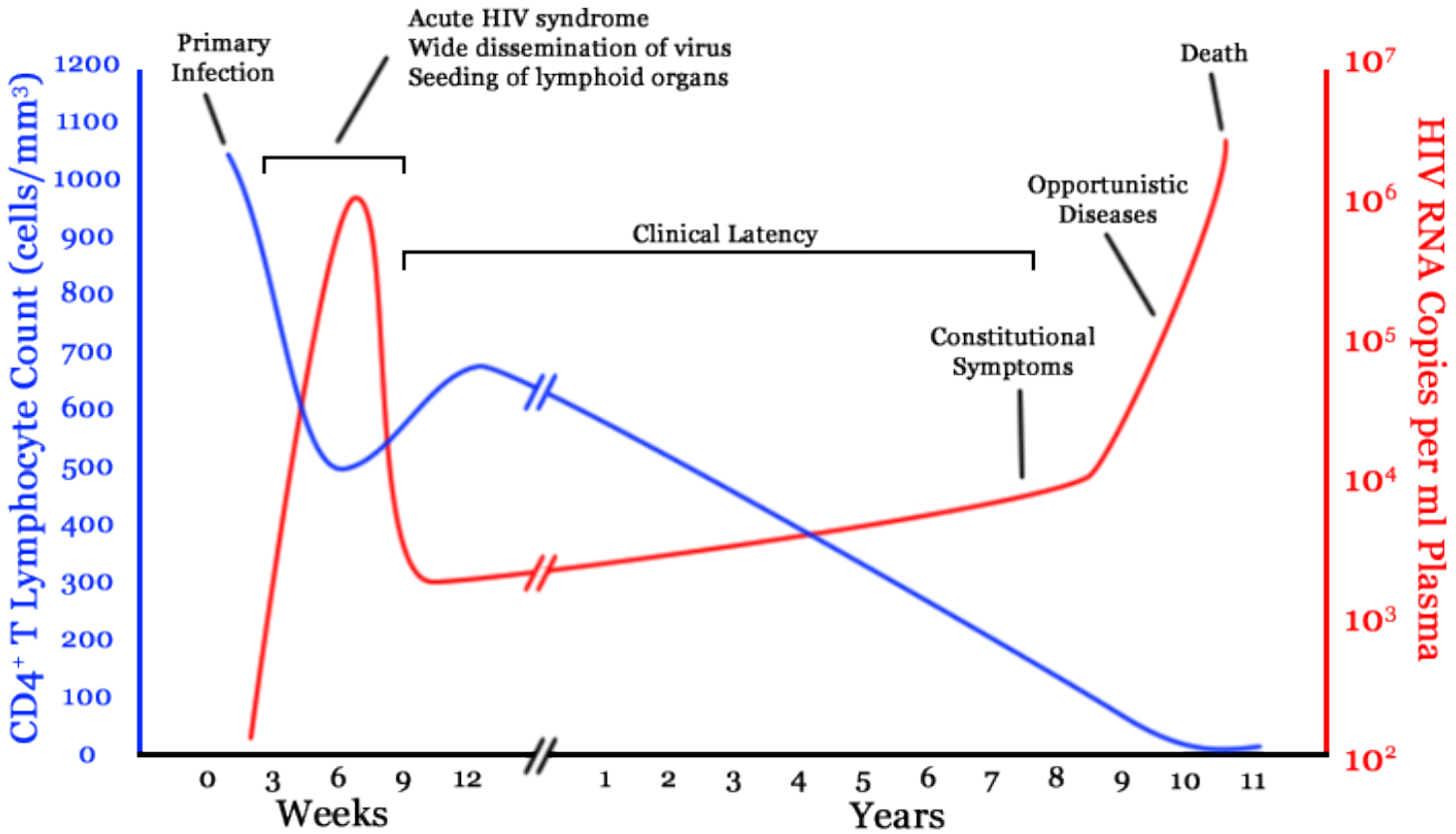
Quasispecies

Questions:

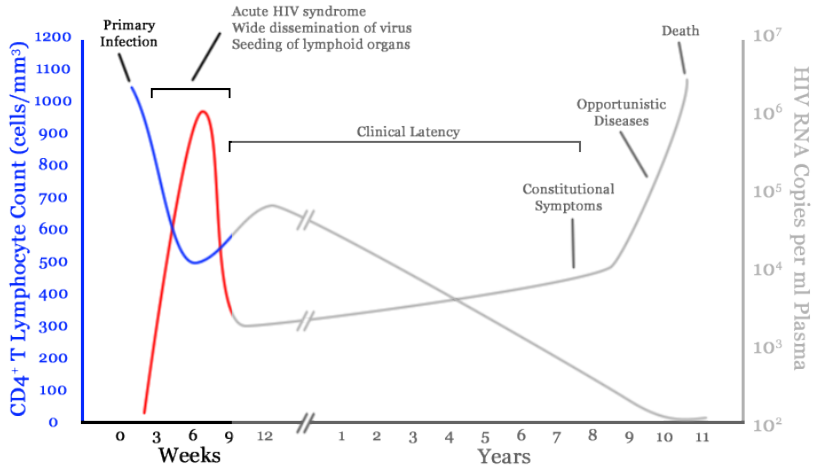
- How do we characterize HIV sequence diversity in an infected patient?
- What is the balance point between ability to resist selective pressure and overall viral function?
- How is quasispecies diversity maintained in the face of this pressure?
- What are the consequences of viral diversity?

1. HIV Infection, briefly
2. Sequencing Techniques
3. Viral Fitness
4. Inducing Diversity
5. Maintaining Diversity
6. Impact of Diversity
7. Summary

Natural Course of Infection



Stage 1: Acute/Early Infection



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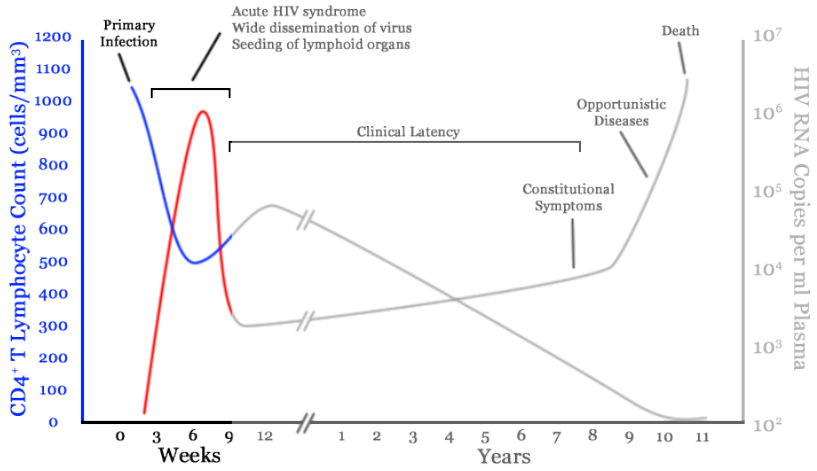

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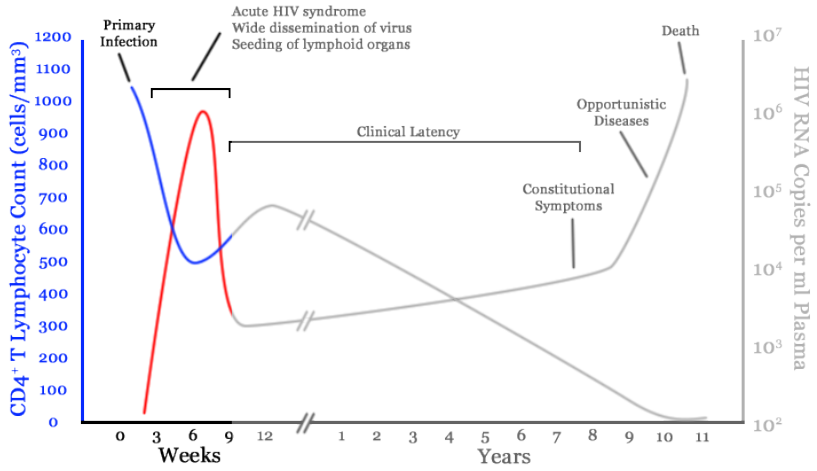

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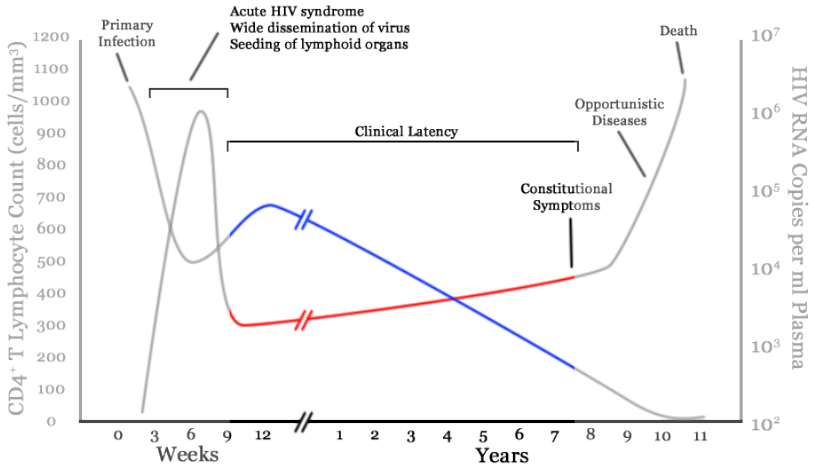
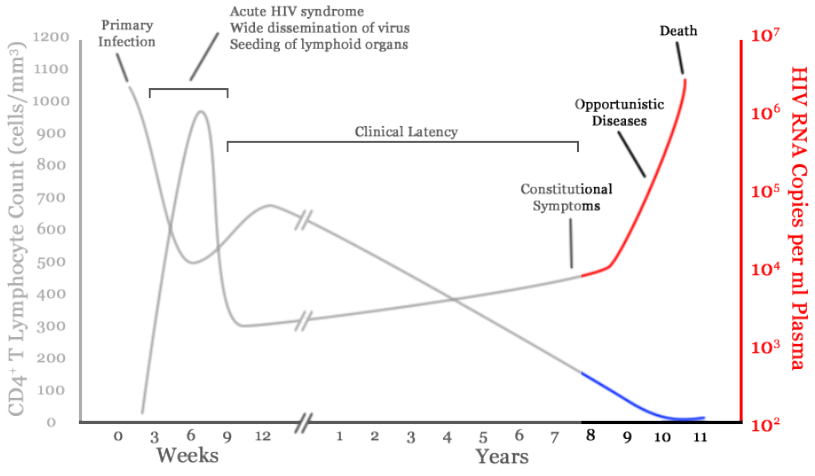
- Very high viral loads, CD4+ (T "helper" cell) levels drop dramatically
- Flu-like symptoms
- Vigorous CD8+ (T "killer" cell) response, killing many HIV-infected cells. CD4+ levels rebound, but do not reach original levels.

Stage 2: Immune Control



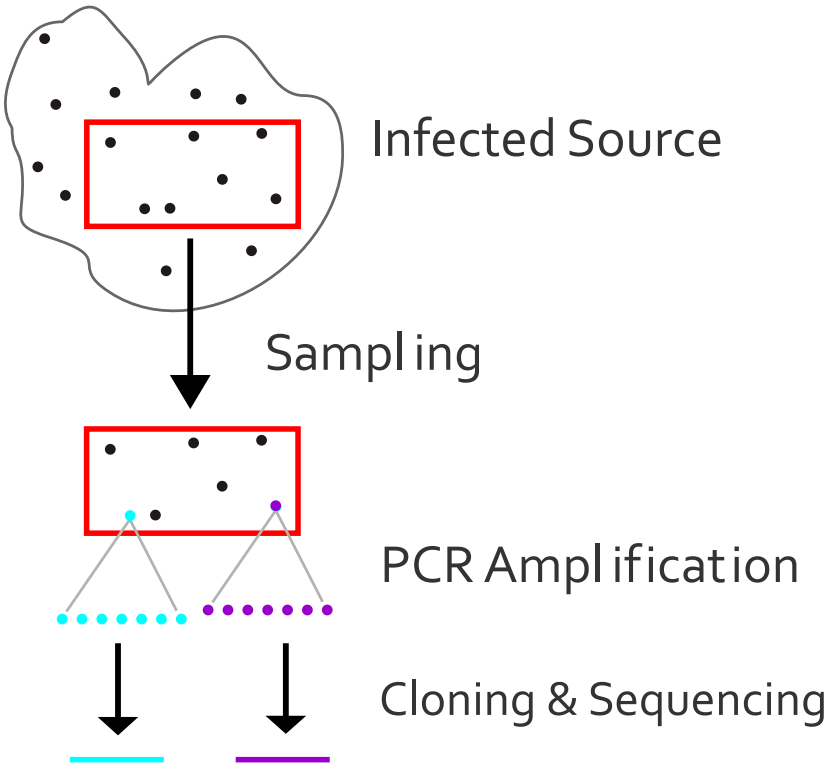
- Virus remains active in lymphoid tissue, mutations accumulate
- Reservoirs persist in other parts of the body (i.e. gut)
- Gradual decrease in CD4+ levels over a two-week to 20-year period

Stage 3: Immune Failure



- CD4+ count drops to level where cell-mediated immunity is no longer effective
- Viral load rises sharply, progression to AIDS

Basic Sequencing



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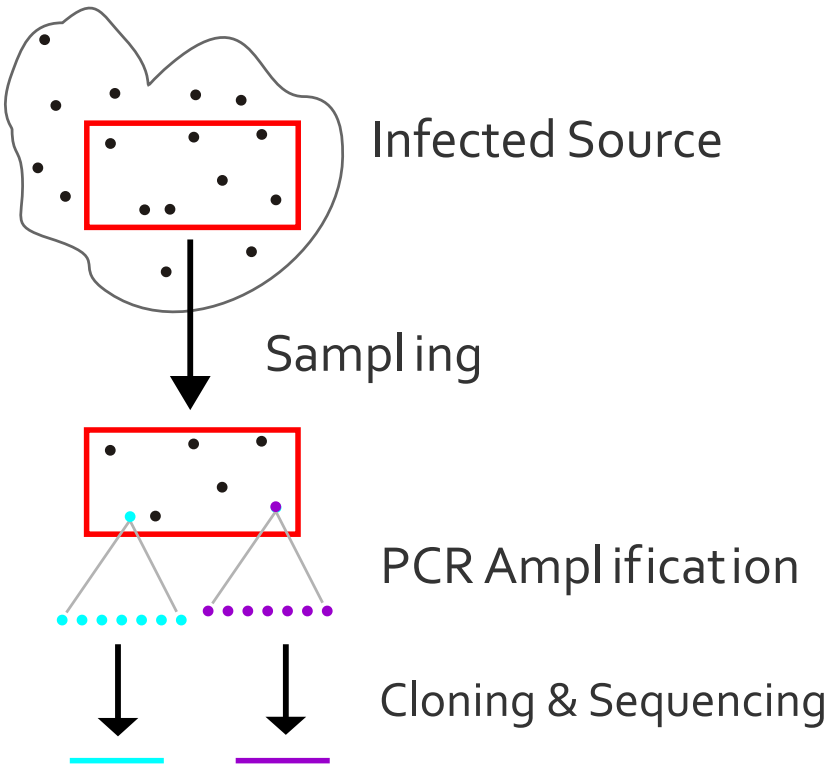

Issues

- Viral sequences may differ across tissues

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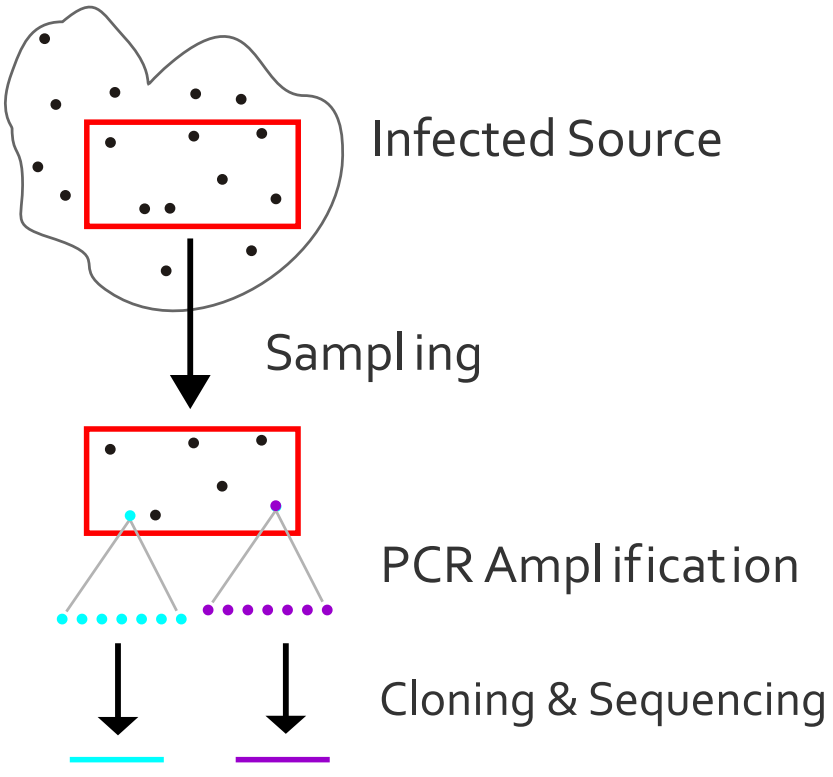
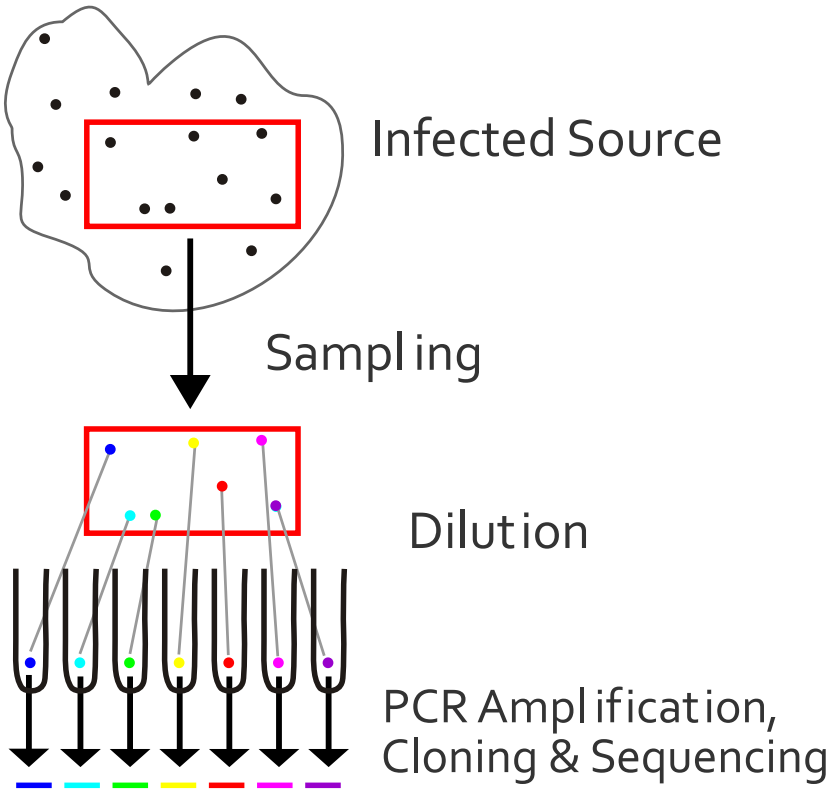


Issues

- Viral sequences may differ across tissues
- May miss rare sequences, misrepresent population diversity
- If small number of genomes in original sample (ie. very low viral load), may amplify same template sequence more than once[a]

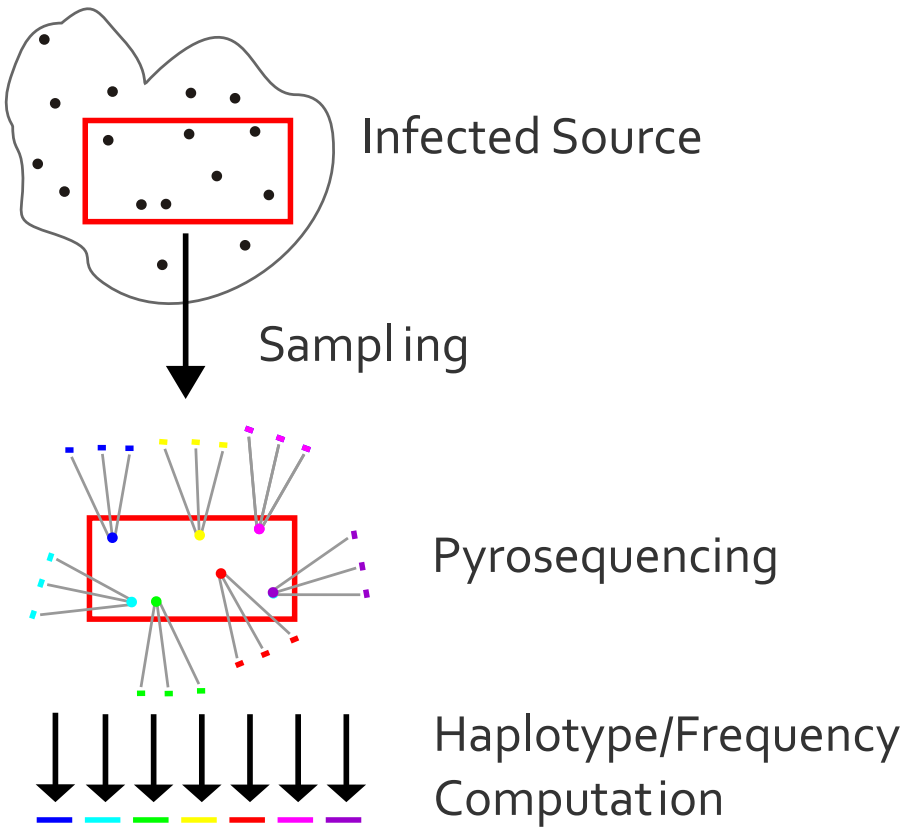
[a] Liu 1996

Limiting Dilution



- Isolate individual sequences via dilution, then amplify to get enough copies for sequencing
- Limited by number of individual genomes which can be sequenced in reasonable amount of time

Pyrosequencing



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- Computational techniques for haplotype/frequency estimation

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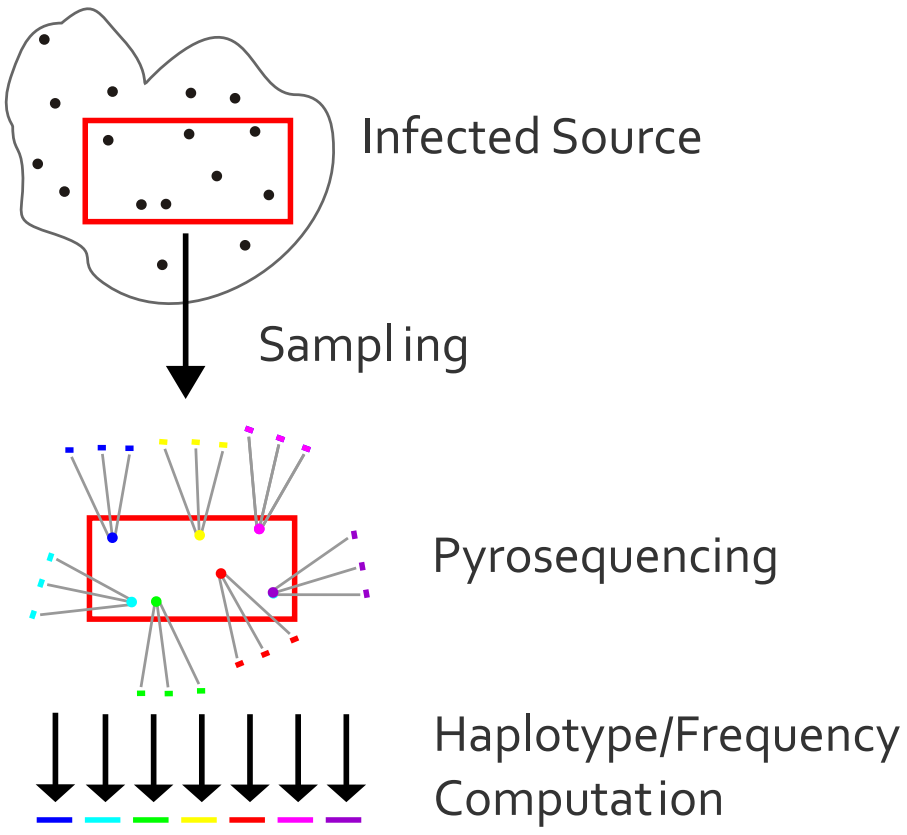

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Limitations

- Works best when known template sequence available
- Subject to quality of algorithms applied

Viral fitness

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  1. Infect cells
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- May differ from fitness *in vitro*

In vitro vs. in vivo

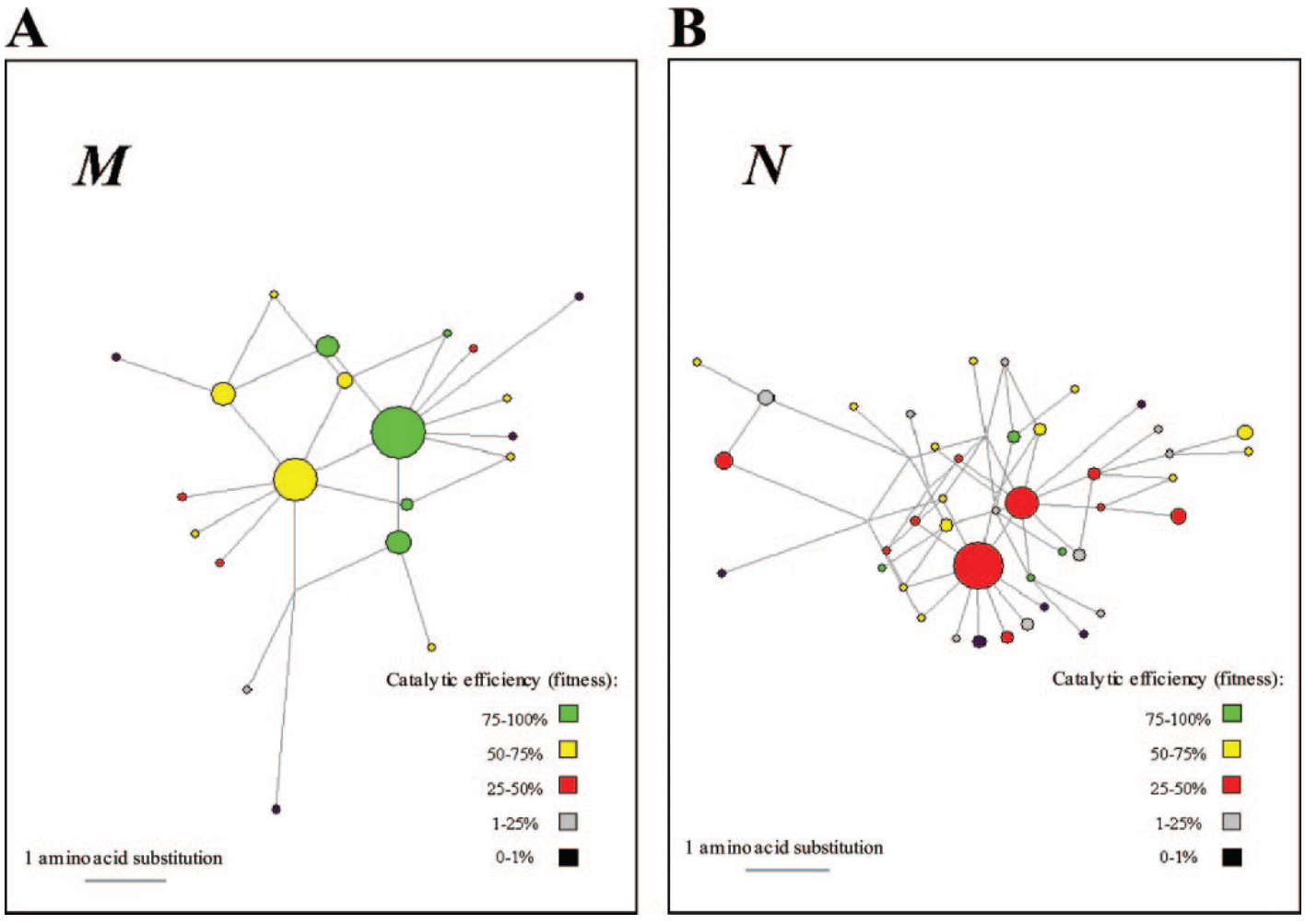

Figure: HIV quasispecies fitness maps for two individuals [Fernandez 2007]

Replication capacity / persistence

Characteristics which affect a virus' ability to survive and replicate *in vivo* over a long period of time include:

- Epitope sequences
- Tissue tropism
- Ability of viral proteins (eg. protease, reverse transcriptase) to function efficiently in presence of Abs/ART

Initial Infection

- Early quasispecies diversity limited by transmission bottleneck[1] (unrelated to replication capacity?)
- Quasispecies which become established are not necessarily fittest/most virulent of infecting population

[1] Karlsson1998
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- May decrease viral fitness over sequential transmissions[2]

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Selection Forces

After infection is established, three major selection forces act on HIV quasispecies:

1. The host's **natural immune response**, mediated by antibodies and T cells
2. **Competition** between quasispecies for limited resources
3. **Drug therapy**, if applicable

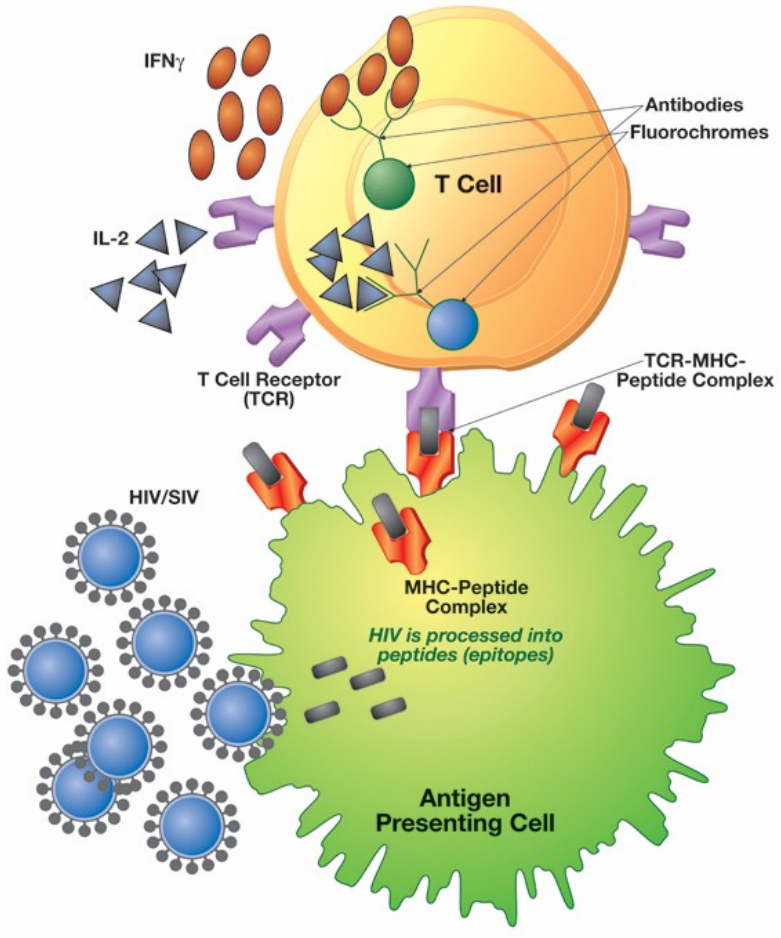
Natural Immune Response - Antibodies

- Antibodies attach to outer surface of circulating virus particles, neutralizing them
- In HIV, main target is viral envelope (encoded by *env* gene)
- Constant interplay[3] between
  - Quasispecies mutation to avoid detection by antibodies
  - Production of antibodies which recognize currently circulating quasispecies

[3] Richman 2003

Natural Immune Response - T cells

- T cells recognize **epitope**, small piece of viral protein presented on surface of infected cell
- Recognition of viral epitope triggers T-cell-mediated destruction of cell
- Viruses with "unrecognizable" epitopes can remain undetected in a cell
- Epitope presentation/recognition by CD8+ varies by individual's HLA type[a]



[a] Lichterfeld 2005

Viral Competition

- Fitness cost associated with escape mutations (CTL or HAART) can be high
- Viruses carrying these mutations may be out-competed by more efficient (eg. wild-type) virus in absence of selective pressure[4]
- Potential loss of resistance mutations forms the basis of "drug holiday" and "intermittent therapy" regimens

[4] Leslie 2004, Friedrich 2004

Drug Therapy and Quasispecies

Quasispecies theory[5] shows why single-drug regimens (eg. AZT) failed:

- Initial therapy causes reduction in viral load, increases supply of uninfected cells

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Idea

Raise barriers to escape so that viruses which carry necessary mutations are likely to have low replication capacity

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HAART

- Highly Active Anti-Retroviral Therapy now standard treatment in developed countries
- Involves a combination of three or more drugs from the following categories:
  1. Nucleoside Reverse Transcriptase Inhibitors (NRTI)
  2. Non-Nucleoside RTIs (NNRTI)
  3. Protease Inhibitors (PI)
  4. Integrase Inhibitors
  5. Entry Inhibitors
  6. Maturation Inhibitors
- Difficult for HIV to mutate to evade ≥ 3 drugs simultaneously without severe loss of replicative capacity

Breaking bottlenecks

HAART exerts extreme selective pressure on HIV, yet:

- "Drug holiday" and "intermittent therapy" regimes have had limited success in practice
- Recent studies[7] have found quasispecies resistant to multiple drugs

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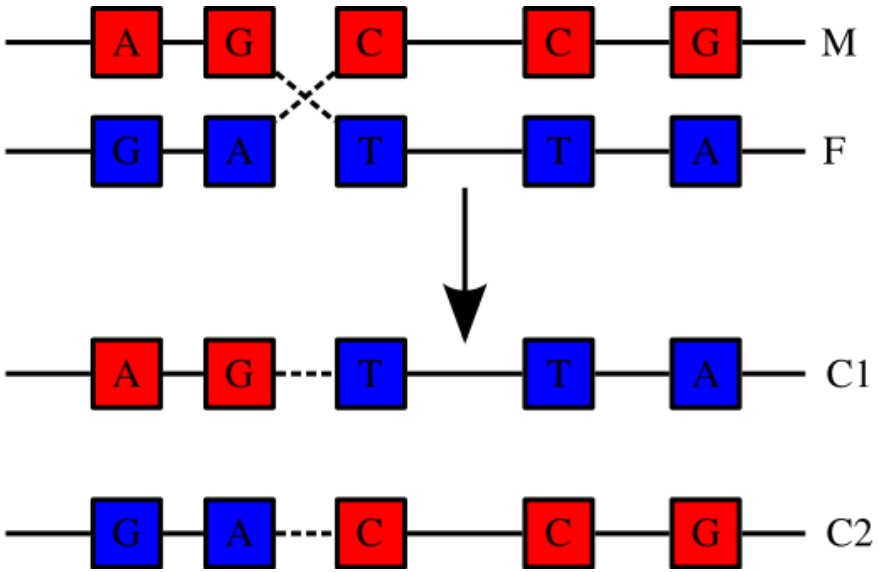
How is quasispecies diversity maintained?

[7] Quan 2008

Recombination



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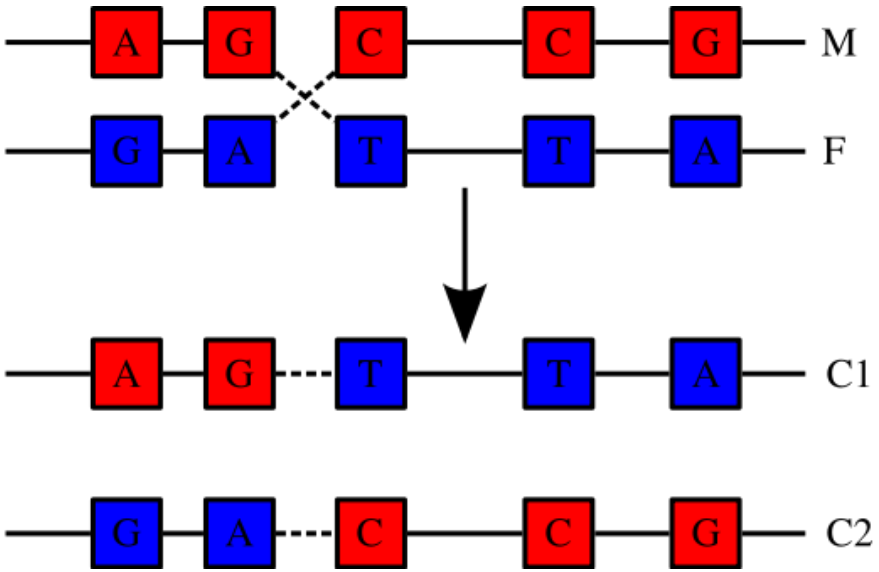

- A major force for introducing new (possibly resistant) quasispecies into viral population
- Possibly also a mechanism for avoiding evolutionary bottlenecks[8]:
  - Fitness "peak jumping"
  - Limit size of regions which are homogeneous across quasispecies

[8] Charpentier 2006

Compartmentalization

- Distinct quasispecies populations may become established in different parts of the body, eg. gut, blood, CSF[9], breast milk[10], genital tract[11], and CD4 cells[12]

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- Some suggestion that reservoirs may exchange genetic information (Diem, Harrington)

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- "Rewind" ability allows HIV to adapt quickly to change in biological conditions

[15] Ruff 2002

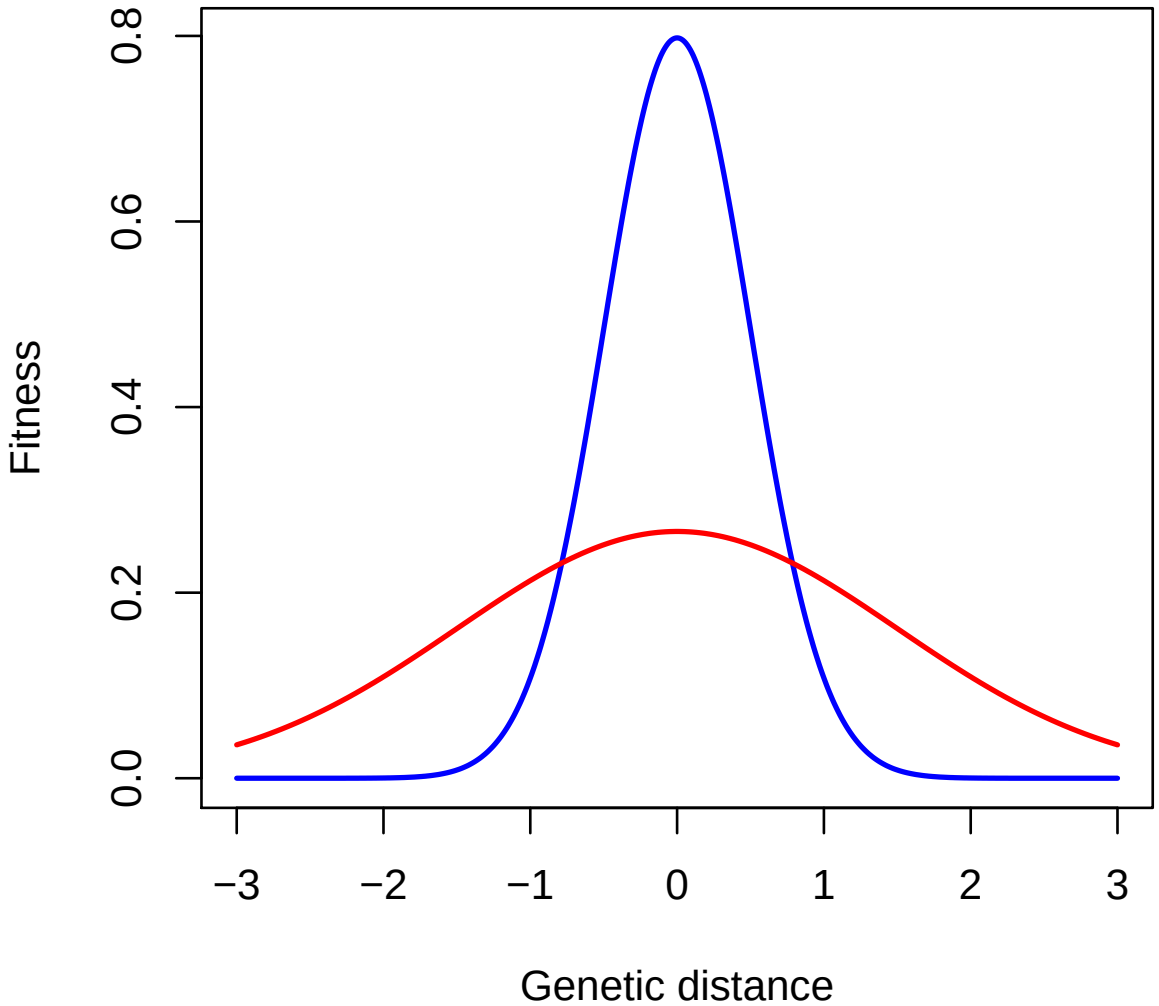
Impact of Diversity

- Quasispecies diversity plays an important role in ensuring HIV's survival by
  1. Ensuring the **stability** of the viral population
  2. Improving adaptability to different biological conditions through **cooperative interactions**
  3. Helping to thwart **drug therapy** regimes

Population Stability: Survival of the flattest?

- Theory based on ideas of Eigen and Schuster [16], "verified" *in silico* [17] and *in vivo* [18]
- **Basic idea:** Collection of quasispecies with higher *average* fitness will outcompete one with lower average fitness, even if latter contains individuals with very high fitness

[16] Schuster 1988, Eigen 1996
[17] Wilke 2001
[18] Elena 2008


Fitness
0.0
0.2
0.4
0.6
0.8
−3
−2
−1
0
1
2
3
Genetic distance

Cooperative interactions

Quasispecies theory predicts that viral populations, not individual variants, are the target of evolutionary selection.

Neat experiment [Vignuzzi 2006]

Compare growth of wild-type (WT) poliovirus to one with high-fidelity (HF) polymerase *in vitro* and *in vivo*

Cooperative interactions, cont'd.

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Conclusion

Sequence diversity **itself**, *not particular set of mutations, determines pathogenesis*

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- **Recombination** restricts evolutionary bottleneck effect to very specific genomic regions, maintains diversity at adjacent regions
- **Archiving** maintains a catalogue of viruses resistant to previous treatments, making previously-tried drugs ineffective

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- Quasispecies diversity is crucial to HIV's survival in face of these selection forces
- Future vaccine and therapy approaches will need to account for and/or exploit HIV intrahost population dynamics

Acknowledgements

- Prof. Peter Gilbert
- Prof. Julie Overbaugh
- Anne Piantadosi

Thanks!

Questions?