Genetic Drift and Selection in a Captive HSV Population

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HSV Background

- Extremely prevalent worldwide
- Wide range of disease severity
- $\alpha$-herpesvirus
- Large dsDNA genome

Genetic Diversity in HSV-1

- HSV-1 genomes are typically similar. (~3-4% variation between strains)
- Similar does not mean identical. (Dozens of AA changes)
- Genomic variation affects how the virus can escape selective pressures. (immune response, antiviral drugs, etc.)

Bowen et al., Virology, 2016
HSV Genetic Diversity Affects Observed Phenotypes

- HSV is well known to evolve in response to selective pressure.

  - Drug resistance mutations localized to TK and/or Polymerase.
  - Schnipper and Crumpacker, PNAS 1980
  - Hall et al., Virology 1984
  - Kit et al., AAC 1987
  - Burrel et al., AAC 2010

- Glycoprotein mutations affect immune response/clinical diagnosis.
  - Ruyechan et al., JVI 1979
  - Yuhasz and Stevens, JVI 1993
  - Rauch et al., JVI 2000
Research Questions

1. How readily does HSV evolve?
   • How fast do genetic variants arise?
   • Mechanism for genetic diversity?
   • Bottlenecking effects?

2. Where/when does genetic drift occur?
Sequential Passage of HSV-1 Populations

HSV-1 F Mixed
MOI 0.01
Passage 1

1. Titer
2. Plaque Morphology
3. Deep Sequence

HSV-1 F Purified
MOI 0.01
Passage 1

1. Titer
2. Plaque Morphology
3. Deep Sequence

Vero Cells (Primate Cell Line)
F Mixed Syncytial Population Increases over Passage

- F Purified virus population displayed CPE plaque morphology.
- Neither virus population’s titer changed substantially over passage.
Alignment of Full-Length Genomes Shows Limited Areas of Diversity

- Repeated regions are less conserved.
Minority Variants within Consensus Sequence

- Deep sequencing can reveal sequence variation at a sub-consensus level.
Minority Variants in a Mixed Population over Passage

Representative Sequencing Coverage (Read Depth)

Minority Variants (≥ 2%)

N=945 Minority Variants (10 passages)
Minority Variants in a Purified Population over Passage

Representative Sequencing Coverage (Read Depth)

Minority Variants (≥ 2%)

N=608 Minority Variants (10 passages)
Minority Variants in Coding Regions Vary in Frequency Over Passage

• This is a small subset of all observed minority variants in coding regions in the mixed population passages.
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• Mixed population took on a syncytial plaque phenotype over passage.
• Two minority variants in gB increased in frequency over passage.
• Arg858His and Leu817Pro are both known syncytial mutants (Gage et al., JVI 1993).
F Mixed population took on a syncytial plaque phenotype over passage.

Two minority variants in gB increased in frequency over passage.

Arg858His and Leu817Pro are both known syncytial mutants (Gage et al., JVI 1993).
gB Minority Variants Associated with Syncytia

- Variants located close enough to be located within the same sequencing read.
- We can then see how often each variant occurs on a given piece of DNA.
gB Minority Variants Associated with Syncytia

>2000 sequencing reads/locus

397 reads span both minority variants

<table>
<thead>
<tr>
<th>Variant</th>
<th>Reads (P8)</th>
<th>Plaque Morphology (P8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg858His</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L817P</td>
<td>37%</td>
<td>89% Syncytial</td>
</tr>
<tr>
<td>R858H</td>
<td>52%</td>
<td></td>
</tr>
<tr>
<td>Both</td>
<td>0.002%</td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>11%</td>
<td>11% CPE</td>
</tr>
</tbody>
</table>

Plaque Morphology (P8)

- CPE: Cytopathic effect
- Syncytial: Syncytia formation

338 reads span both minority variants
Conclusions

- Minority genetic variants can have major effects on virus biology.
Acknowledgements