FMDV evolutionary dynamics within infected buffaloes and its large-scale consequences

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African buffaloes experimentally infected with SAT1/2/3 inoculum

(Maree et al, *Differential persistence of foot-and-mouth disease virus in African buffalo is related to virus virulence*, Journal of Virology, 2016)

Aim: study within-host genetic variability and evolution post inoculation

Sequencing of <u>SAT1 only</u> (persistent serotype)

Inoculum:

- deep next-generation sequencing of capsid region
- average coverage ~30000

Virus from micro-dissections of 3 buffalos (2 at <u>35dpi</u>, 1 at <u>400dpi</u>):

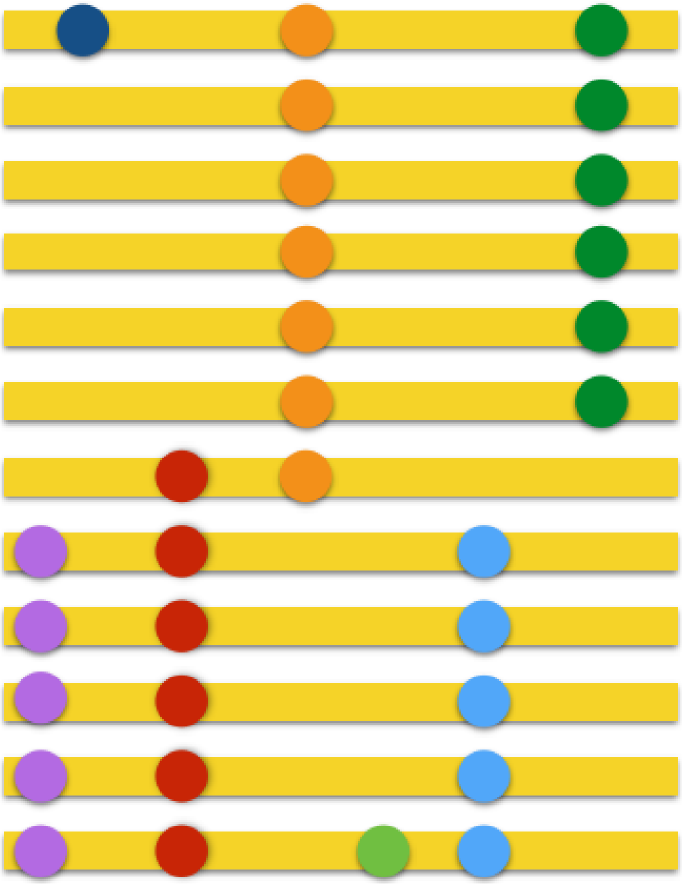
- Sanger sequencing of VP1
- viruses from dorsal soft palate, palatine and pharyngeal tonsils (both epithelium and germinal center)

Furthermore, Sanger/NGS of probang and tonsil swabs from multiple individuals

Quasi-species and selection

Inoculum:

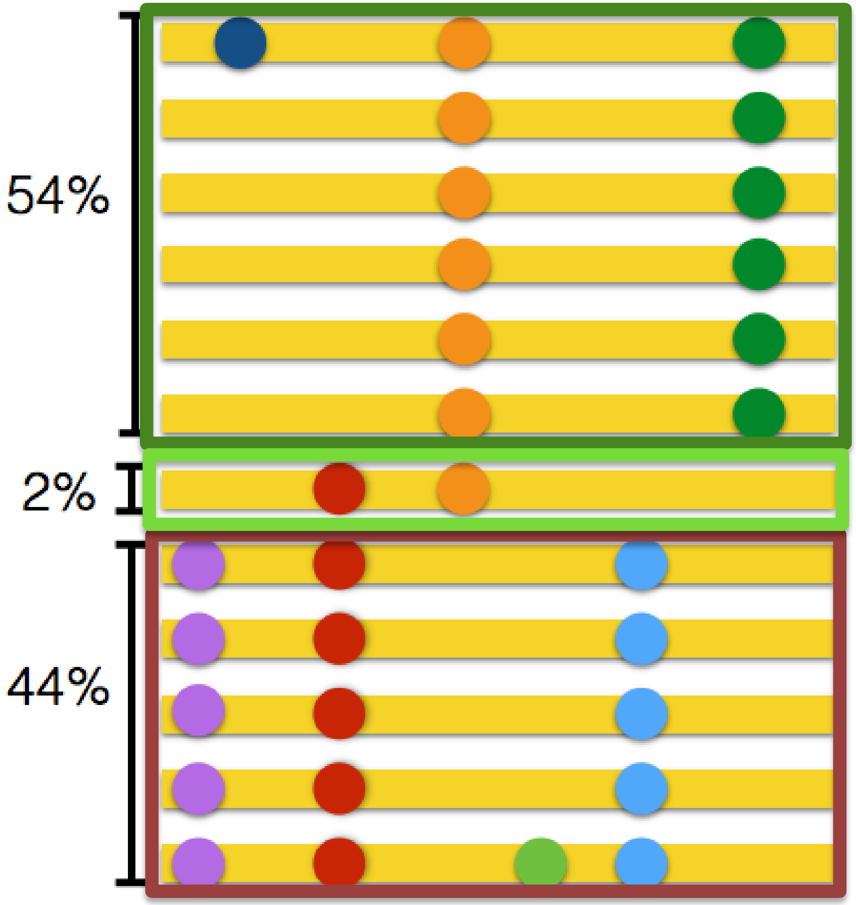
SAT1 sequences



Quasi-species or viral swarms:
population of viruses with similar sequences
(differing only by a few mutations)
evolving under high mutation rates

Expectation:
identical viruses
or a single viral swarm

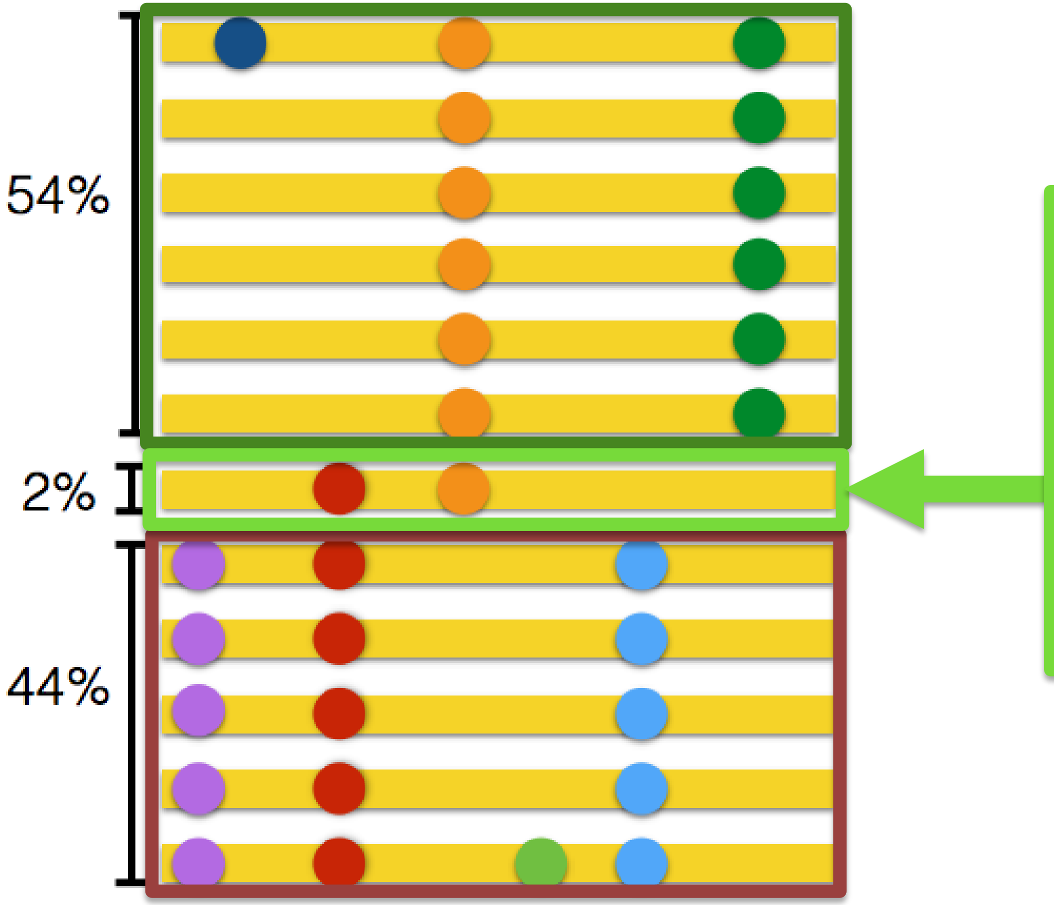
Inoculum:
two quasi-species (plus recombinants)



Two main quasi-species with 3% sequence divergence

Large fraction of recombinant sequences

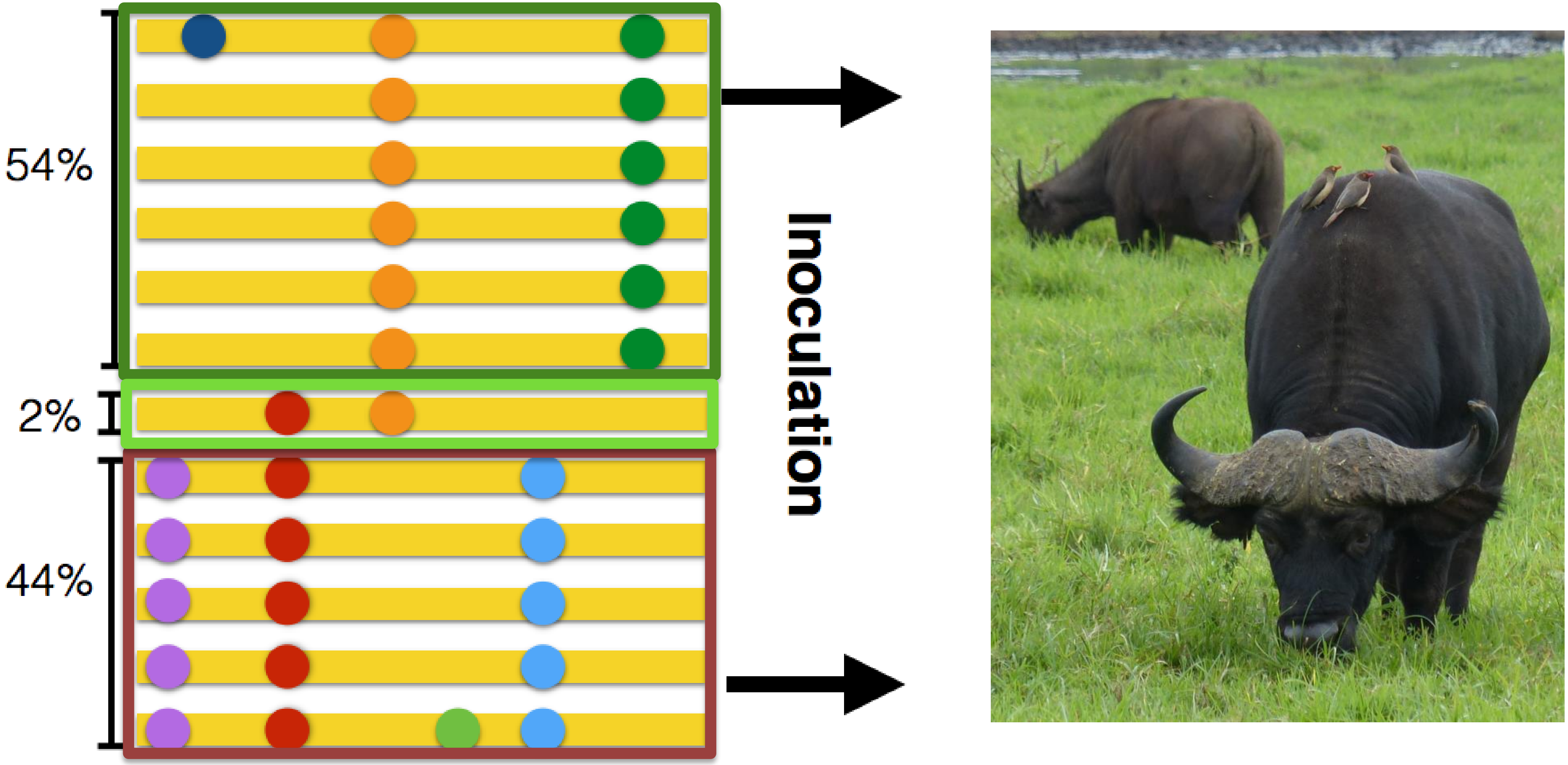
Inoculum:
two quasi-species (plus recombinants)



Recombinant

VP1 sequence of major quasi-species, except for 2 nonsynonymous variants corresponding to minor quasi-species

Strong post-inoculation changes in quasi-species frequencies



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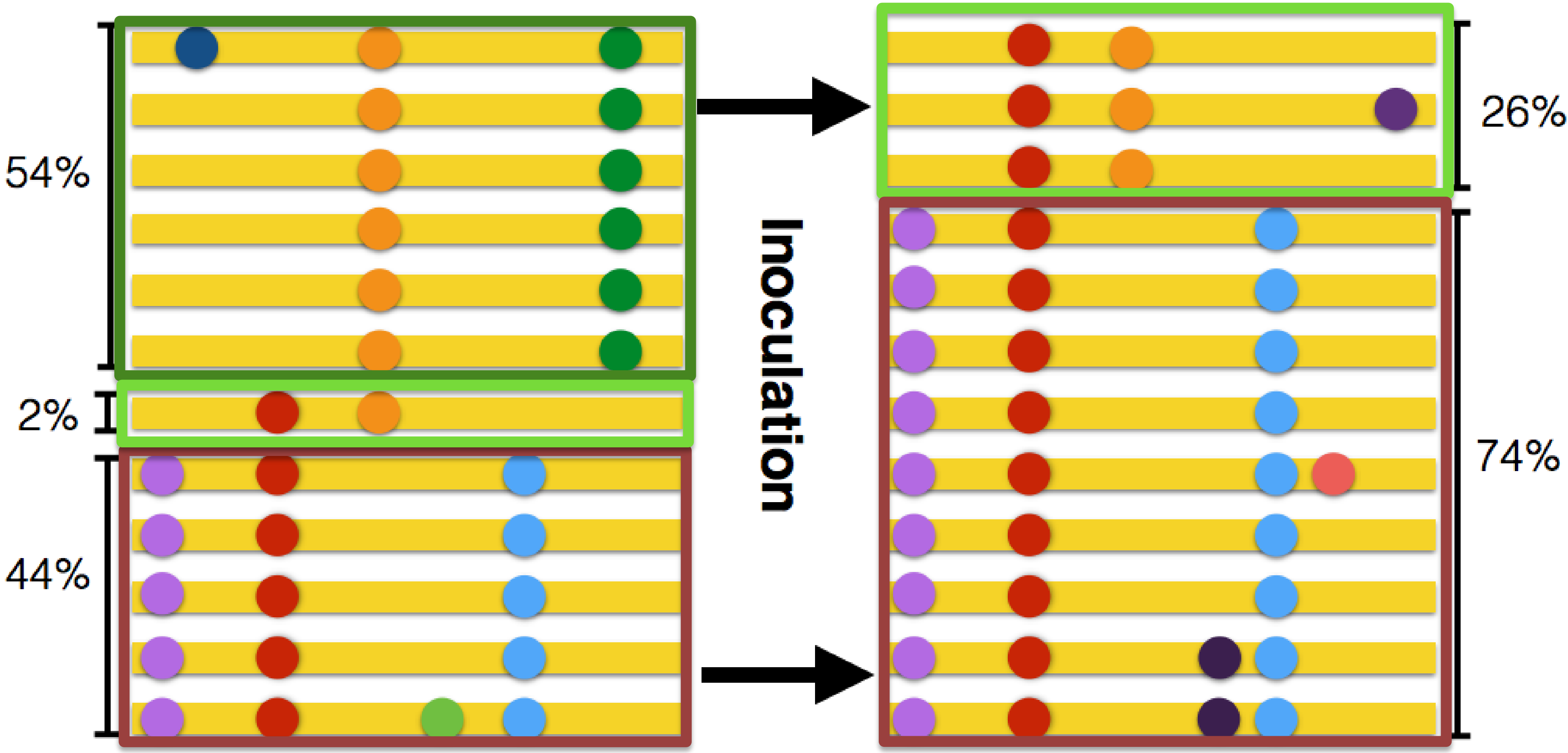
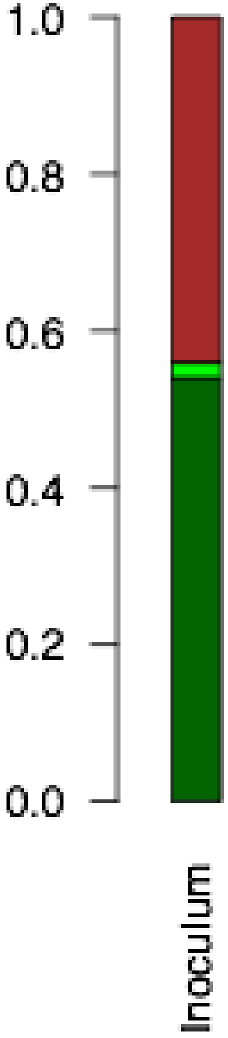


Quasi-species structure across animals and tissues



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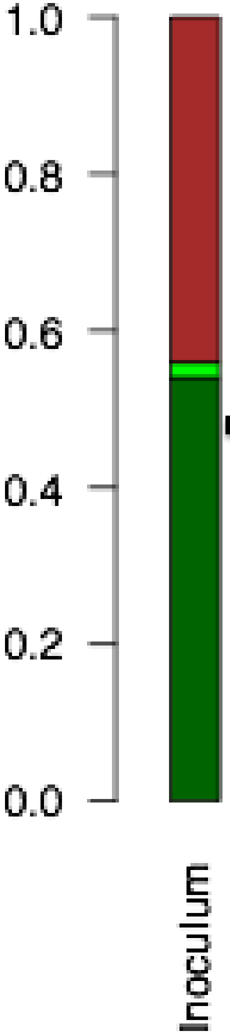

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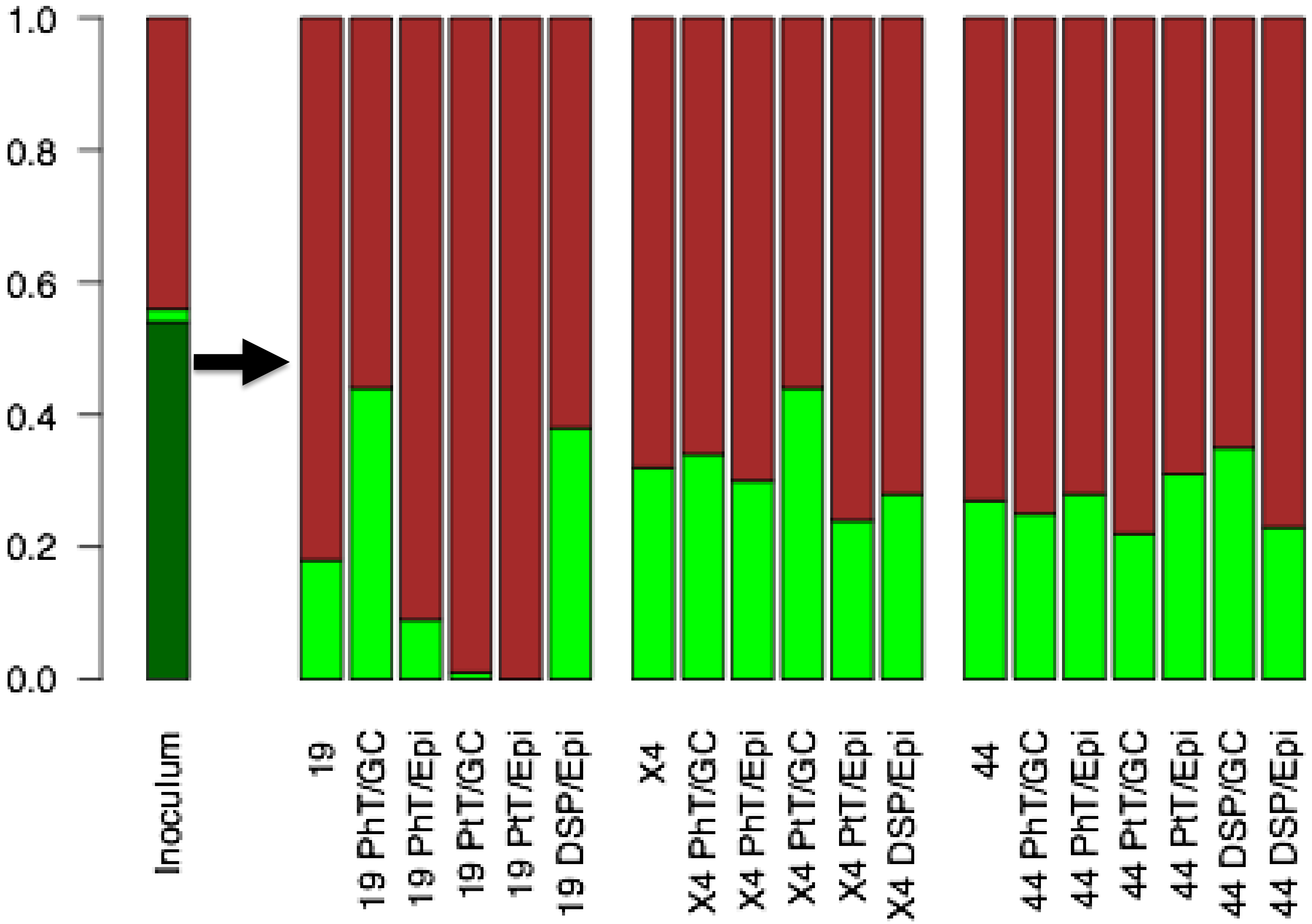

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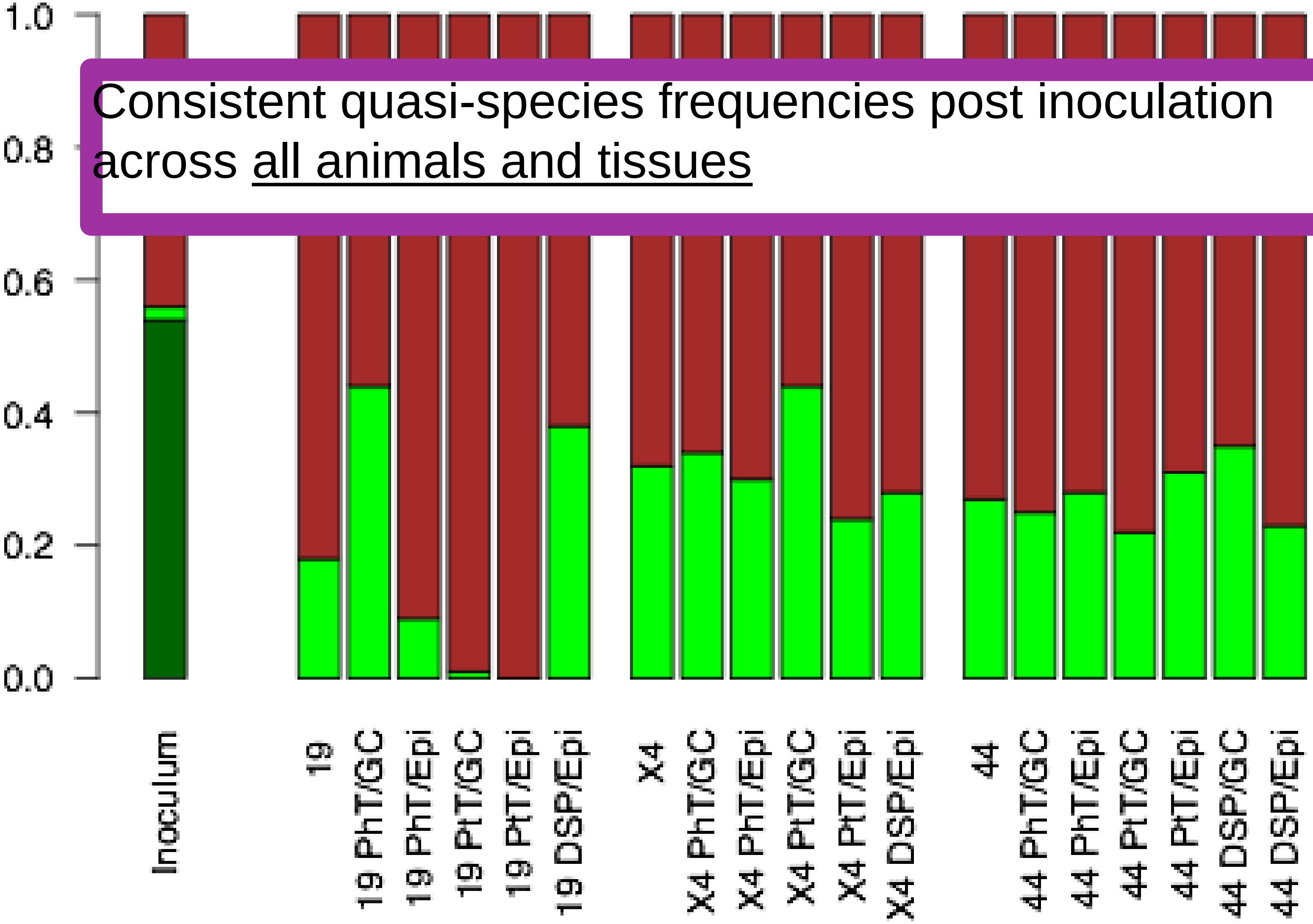
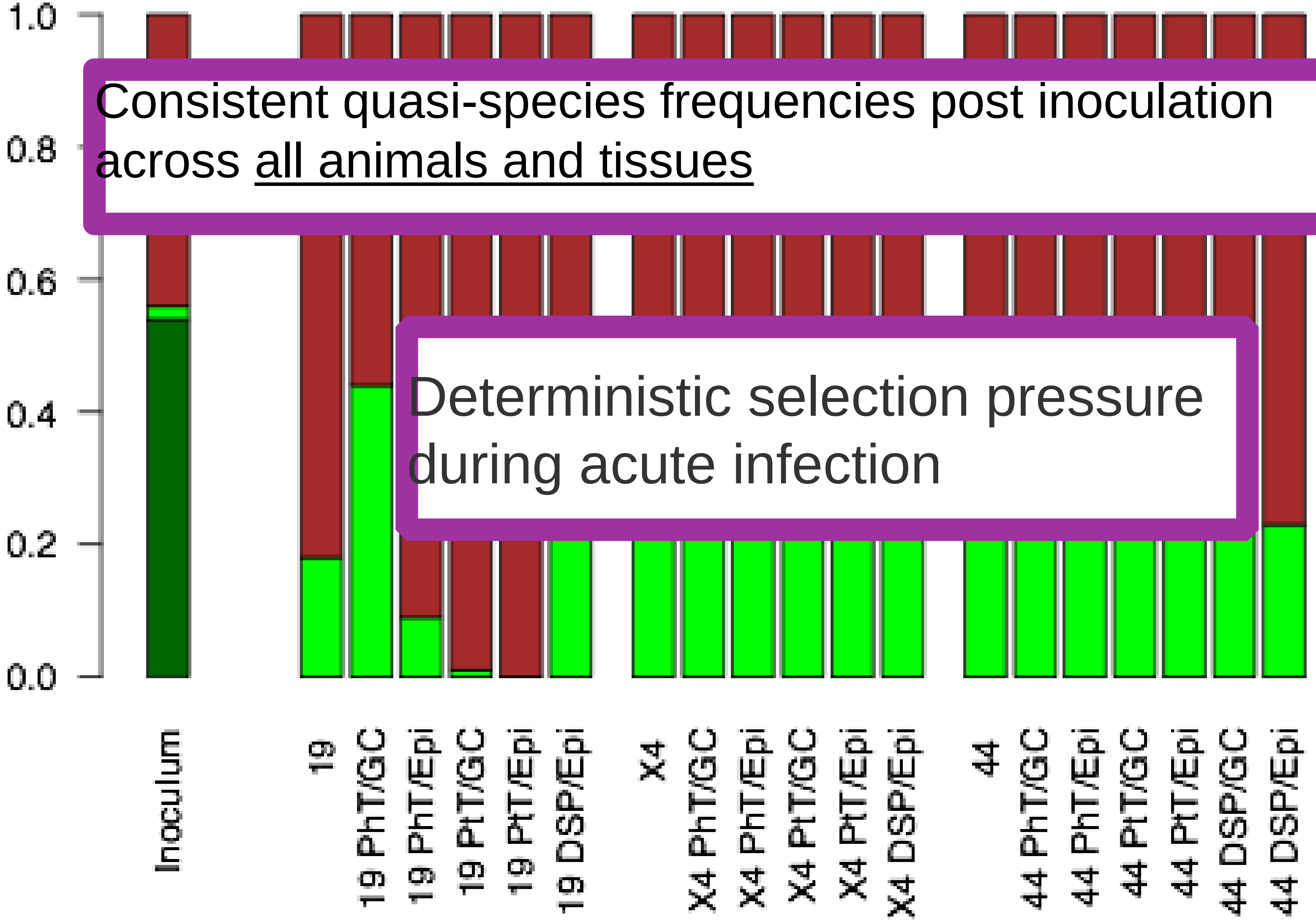


Consistent quasi-species frequencies post inoculation across all animals and tissues

Quasi-species structure across animals and tissues

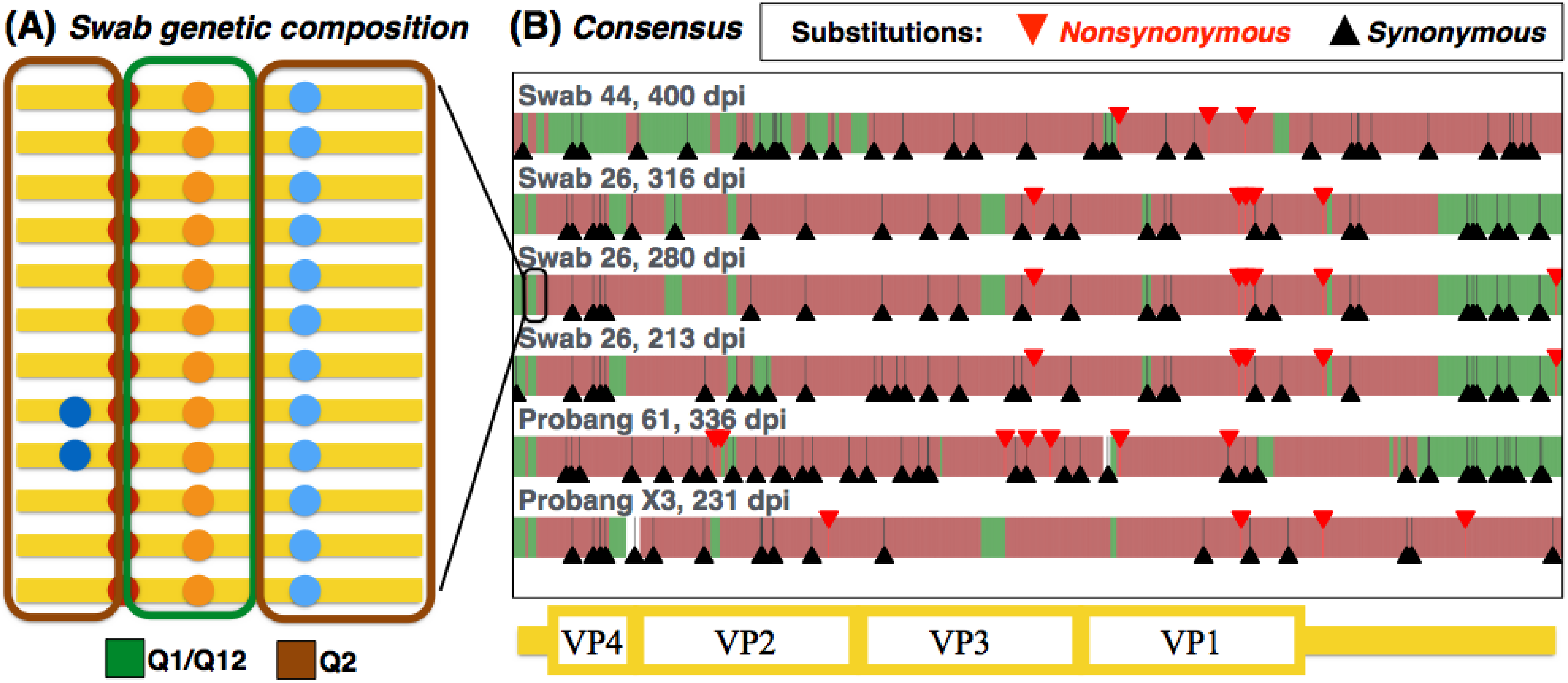


Consistent quasi-species frequencies post inoculation across <u>all animals and tissues</u>

Deterministic selection pressure during acute infection

But what about the tonsil swabs?

Consensus-level recombinants with little genetic variability!



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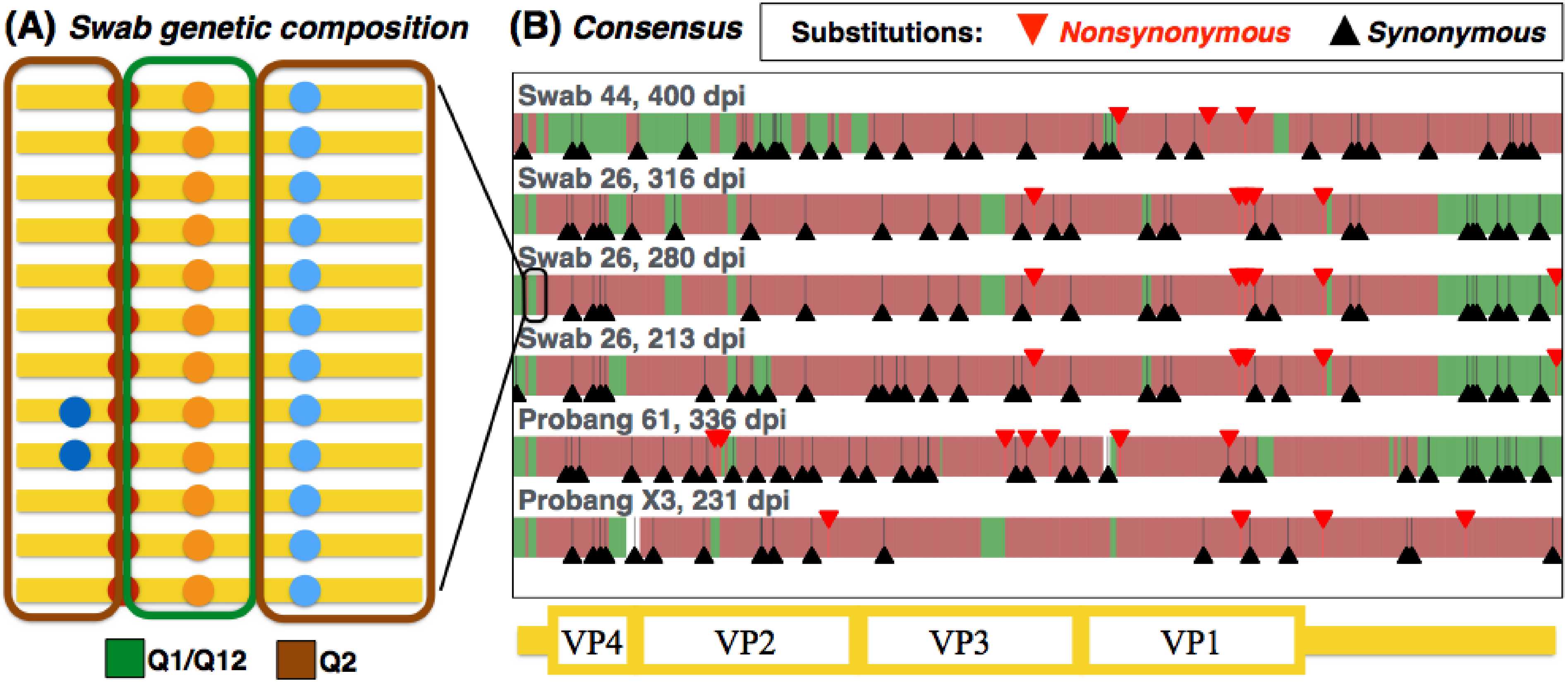
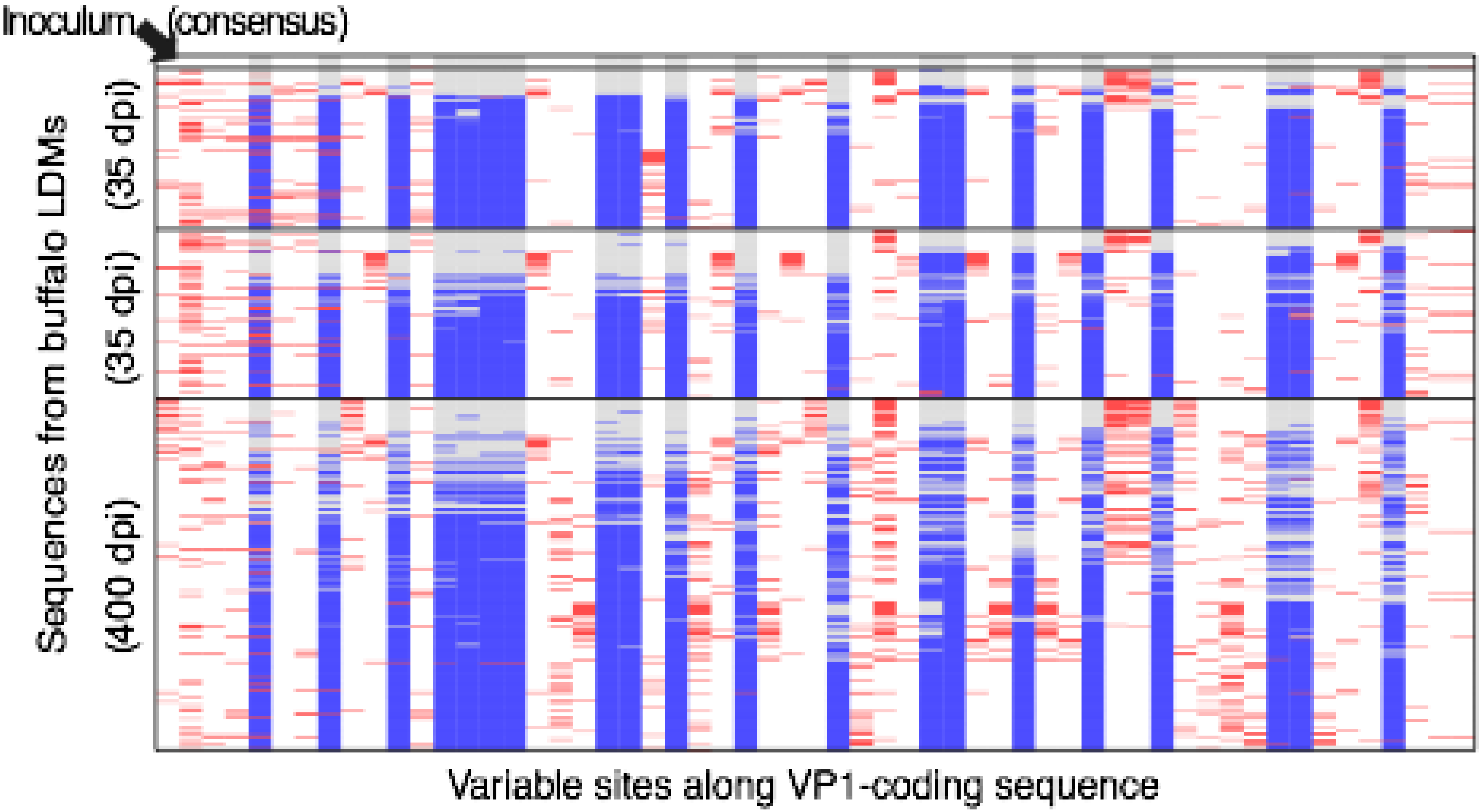


and high substitution rates (comparable to phylogenetic rates) but no evidence of immune escape!

Hypothesis: fast viral replication within a small reservoir

Within-host recombination and epistatic interactions

Recombinants in buffalos



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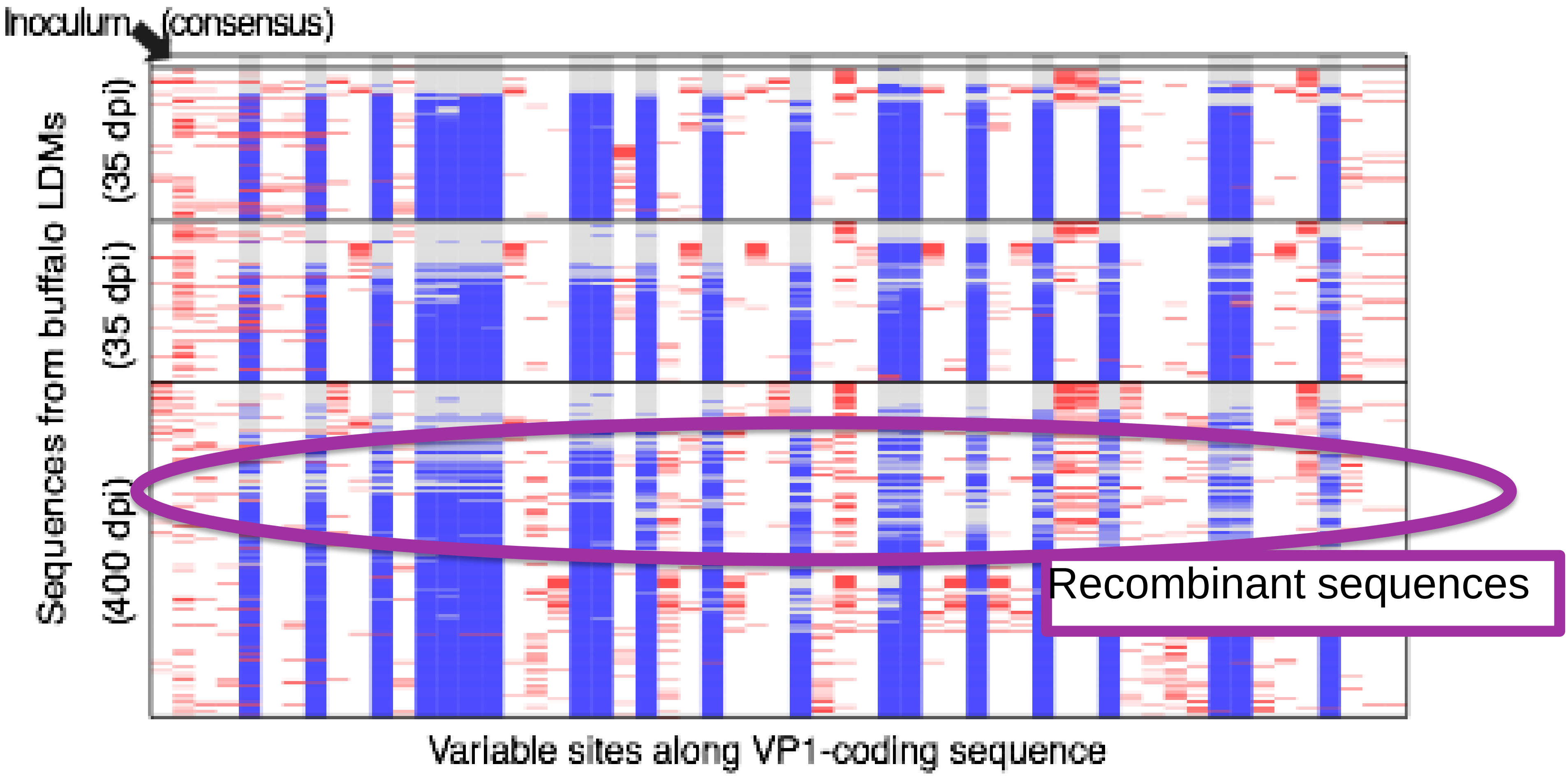
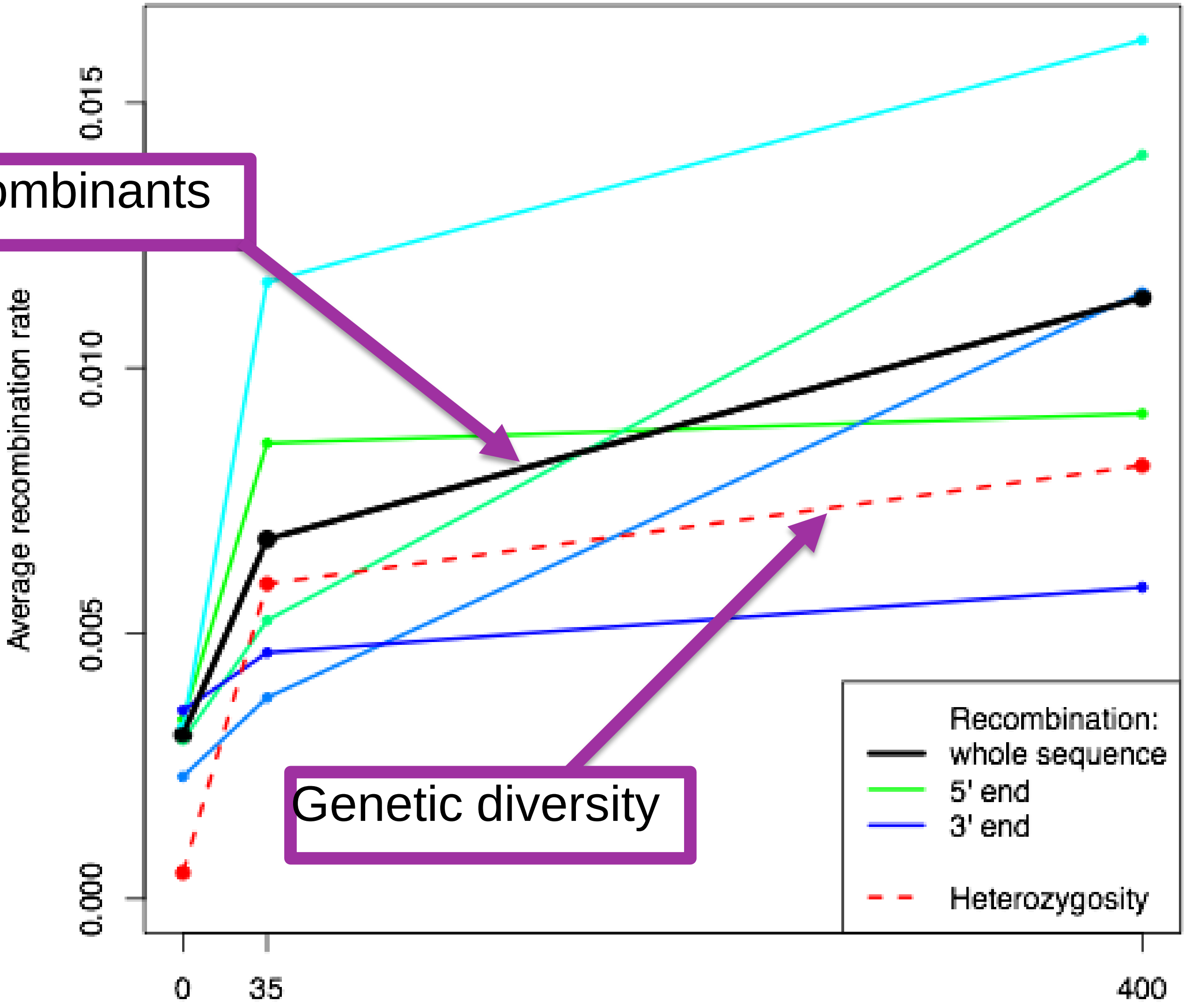


Recombinant sequences

Increase in recombinants and sequence diversity with time



Recombinants

Genetic diversity

Increase in recombinants and sequence diversity with time

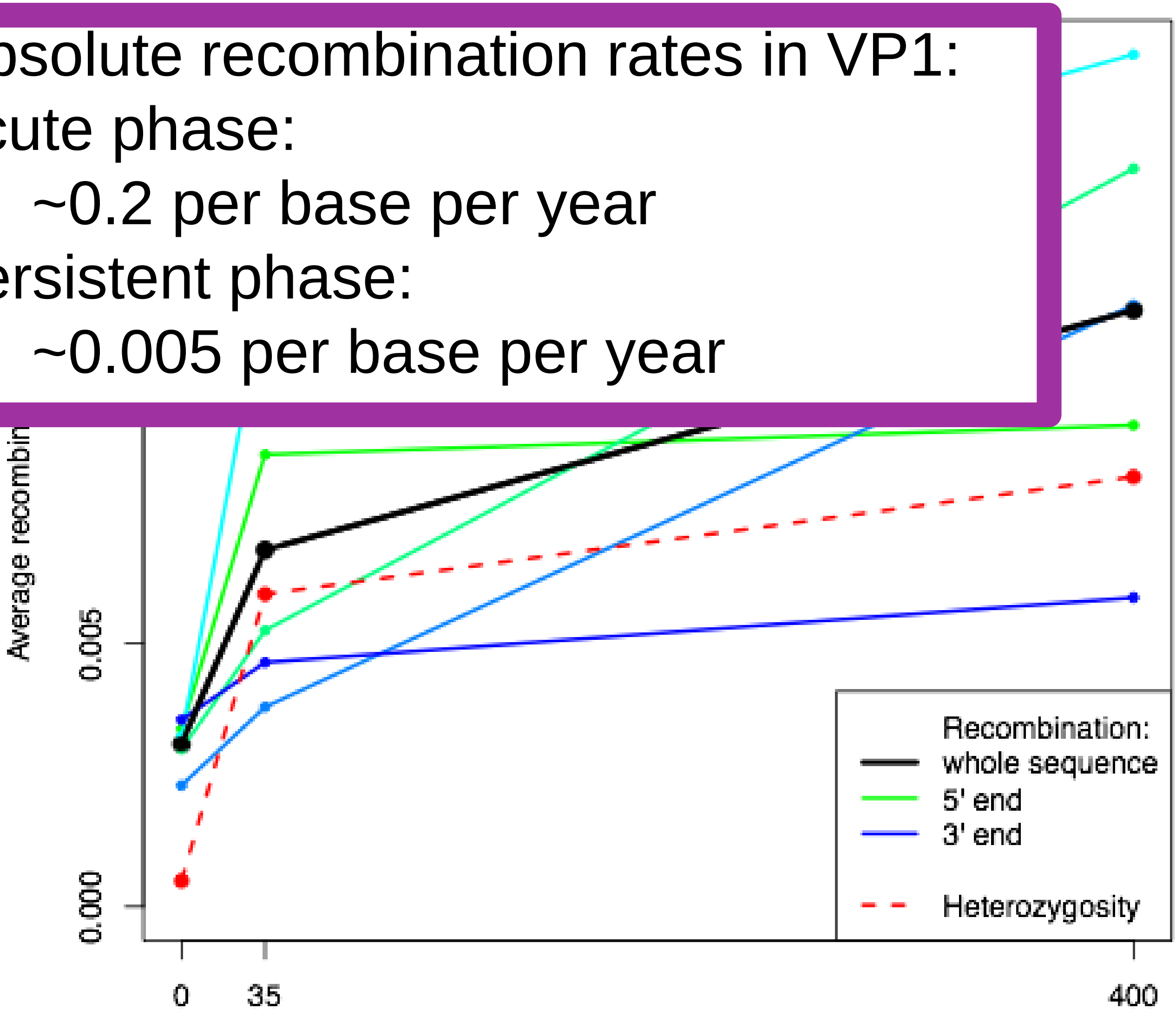
Absolute recombination rates in VP1:

acute phase:

~0.2 per base per year

persistent phase:

~0.005 per base per year



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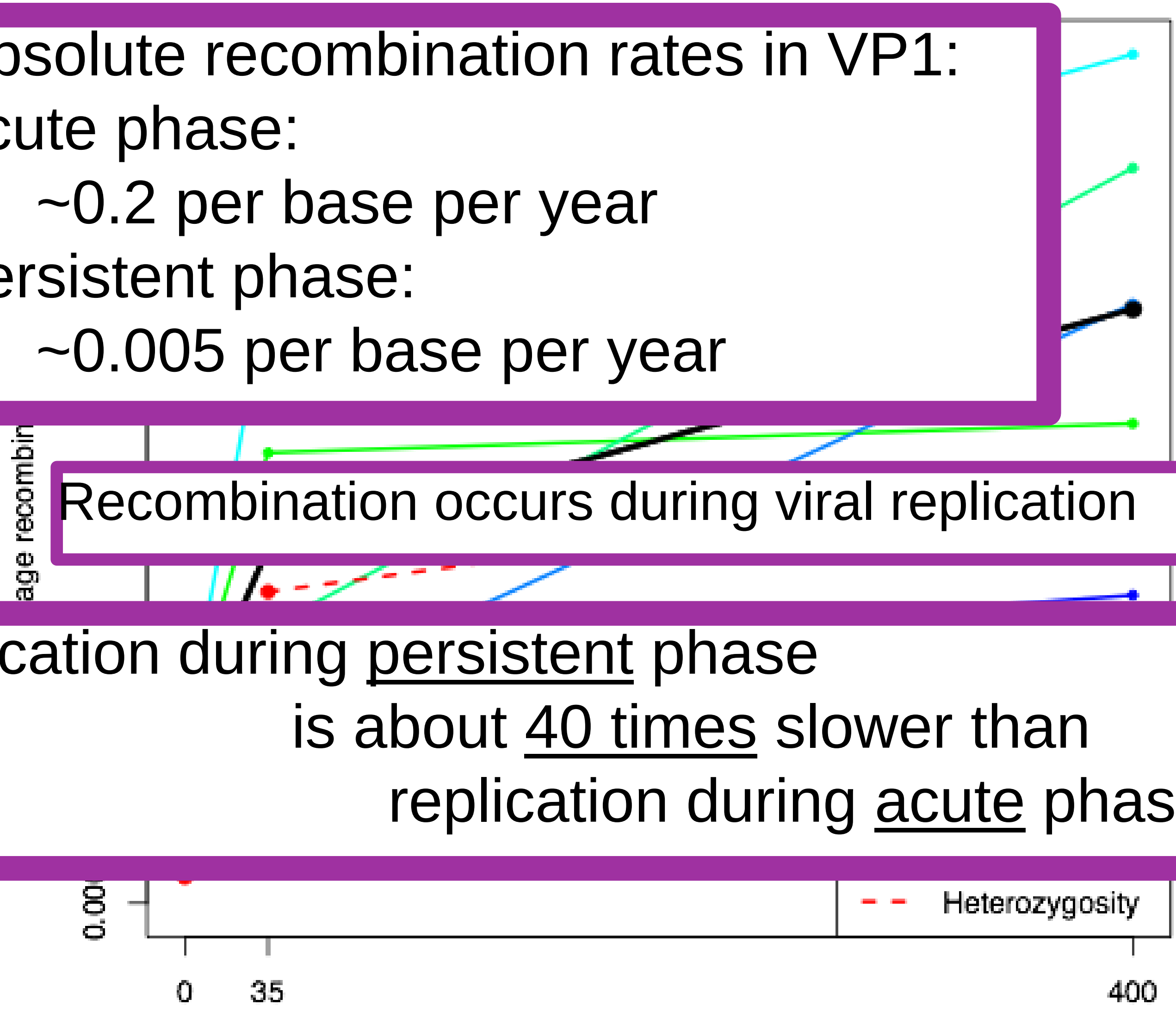

Absolute recombination rates in VP1:

acute phase:

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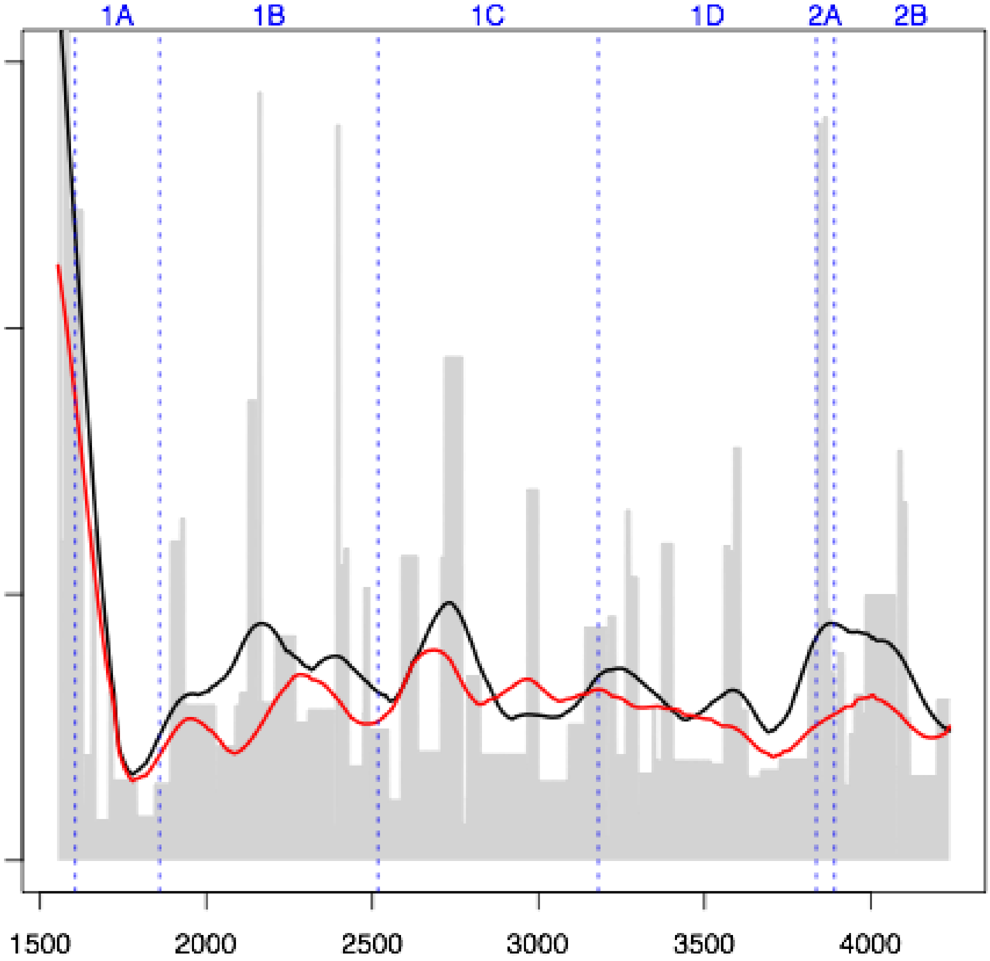
~0.005 per base per year

Recombination occurs during viral replication

Replication during <u>persistent</u> phase is about <u>40 times</u> slower than replication during <u>acute</u> phase of infection

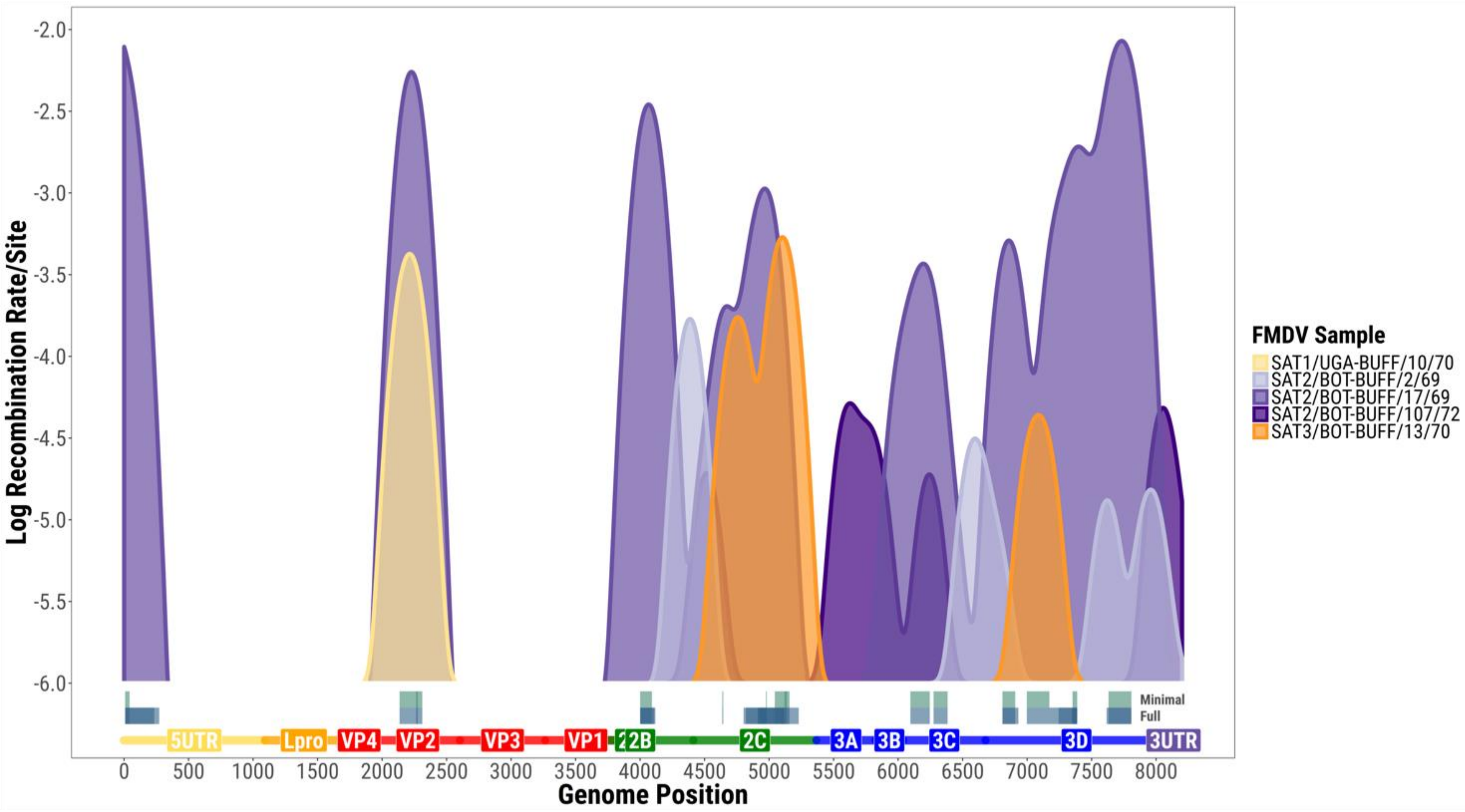
Recombination map of capsid genes



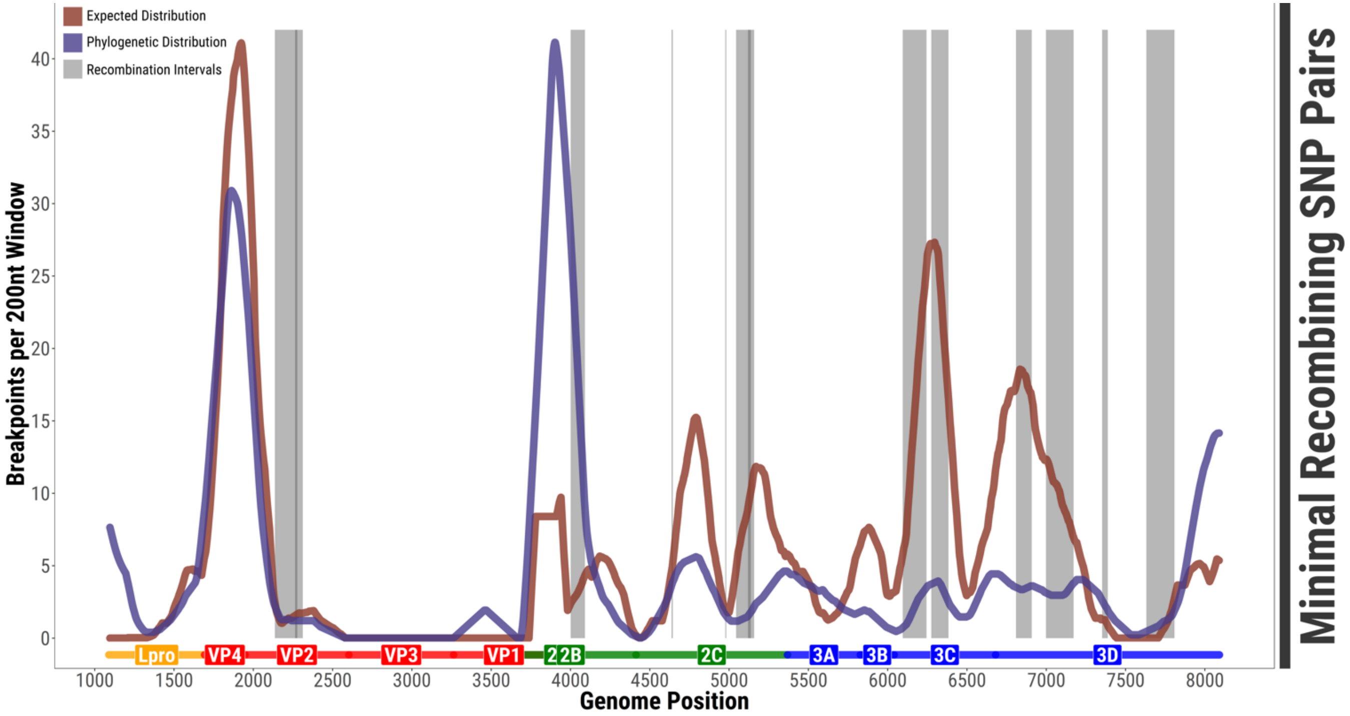
High recombination rates in the capsid-coding region

Are they compatible with usual phylogenetic estimates of recombination?

Full-genome intra-host recombination from SAT1/2/3 samples from wild buffaloes



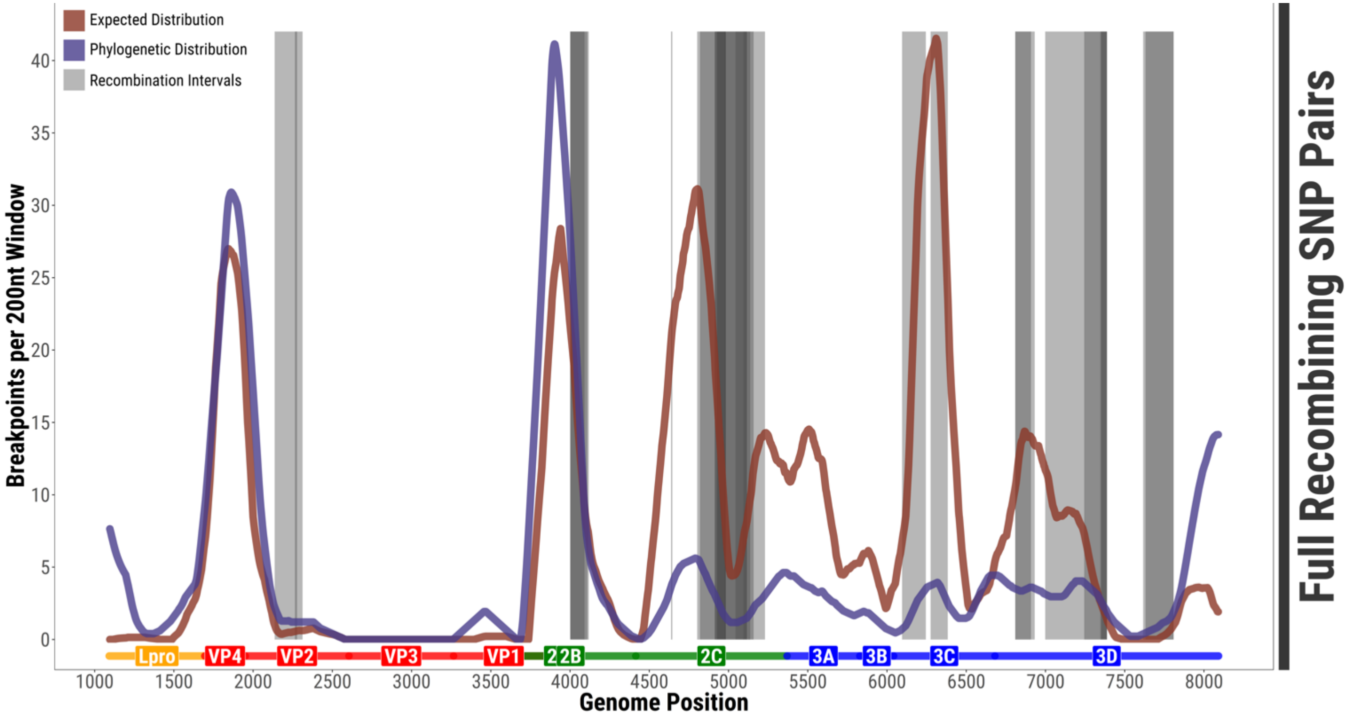
Mismatch between recombination rates at intra-host and phylogenetic scales



Ferretti et al, *Within-Host Recombination in the Foot-and-Mouth Disease Vi*

Mismatch between recombination rates at intra-host and phylogenetic scales

Cause? Selection and epistasis?



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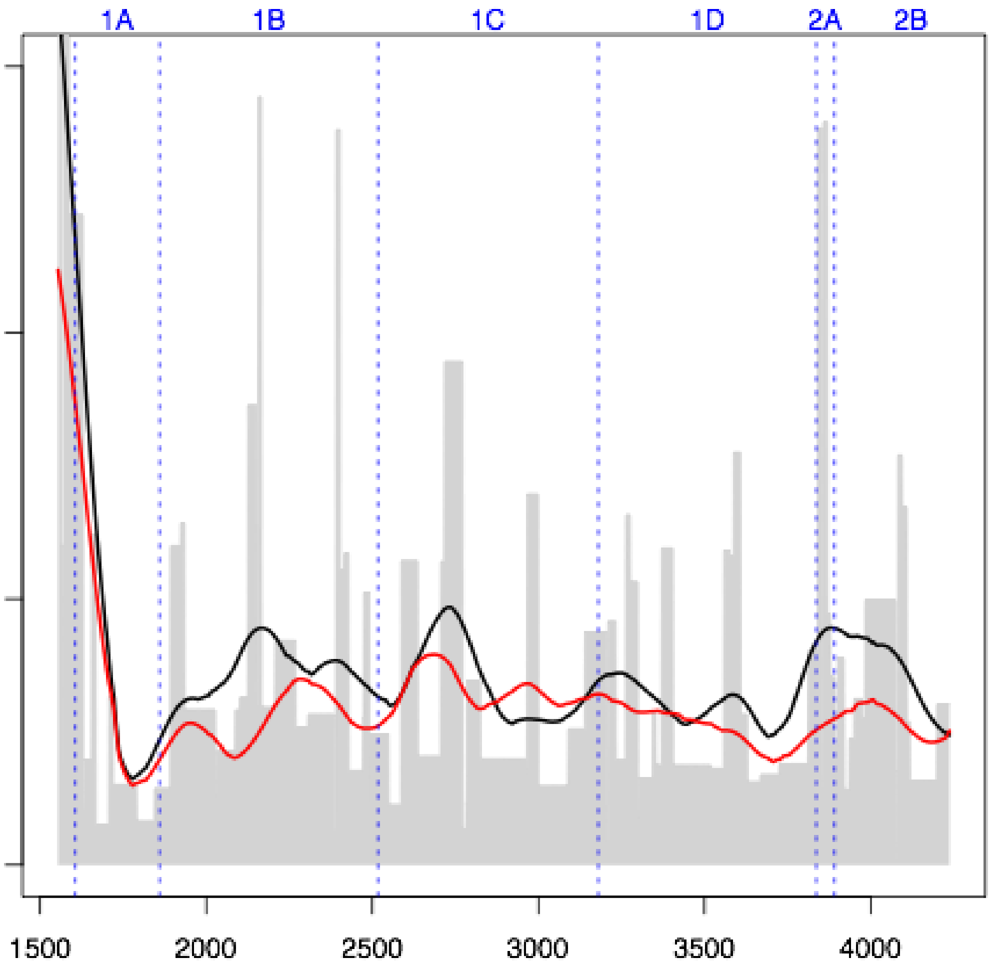
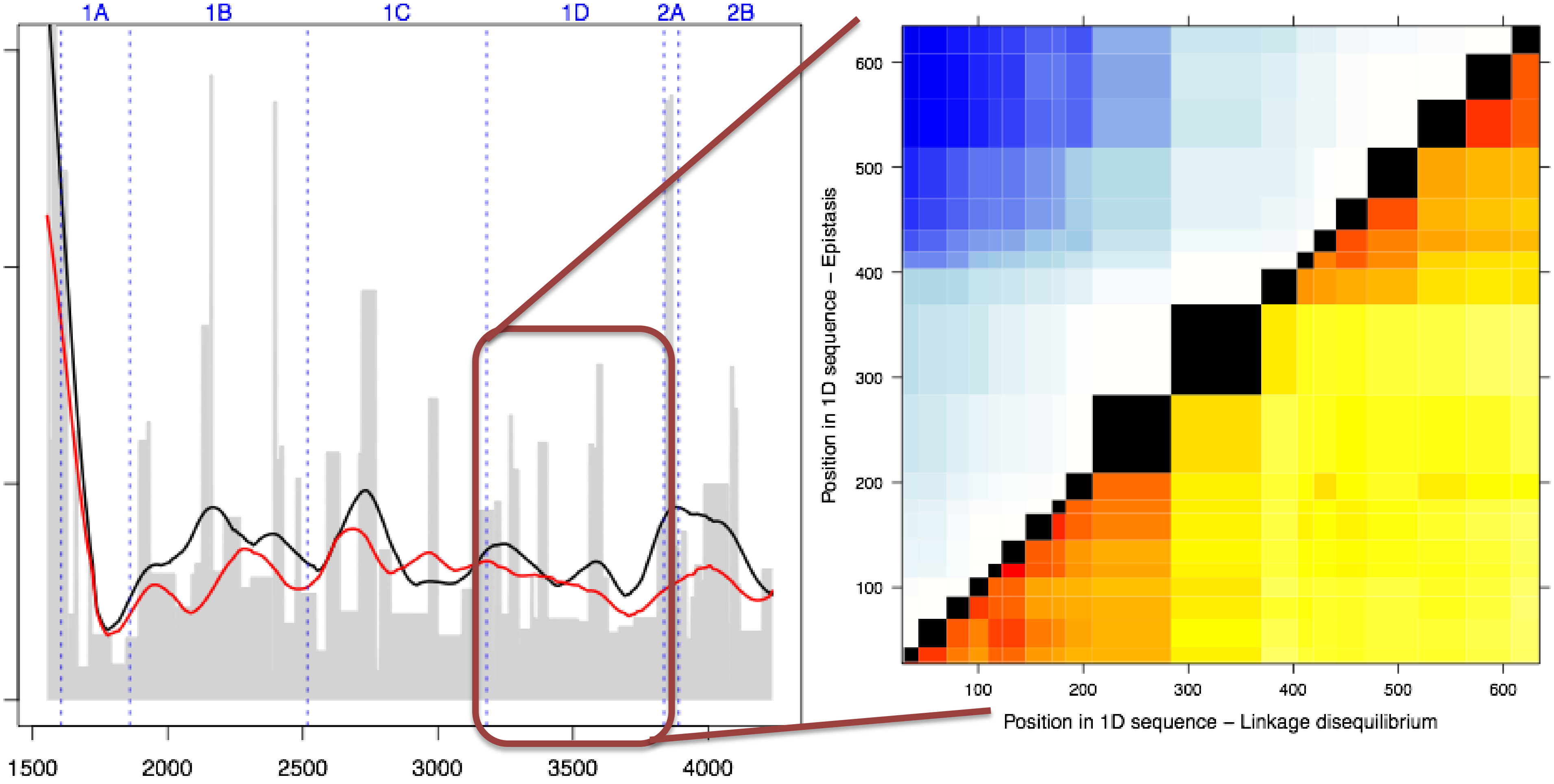
Recombination map of capsid genes



Recombination map of capsid genes and mosaic structure inside VP1



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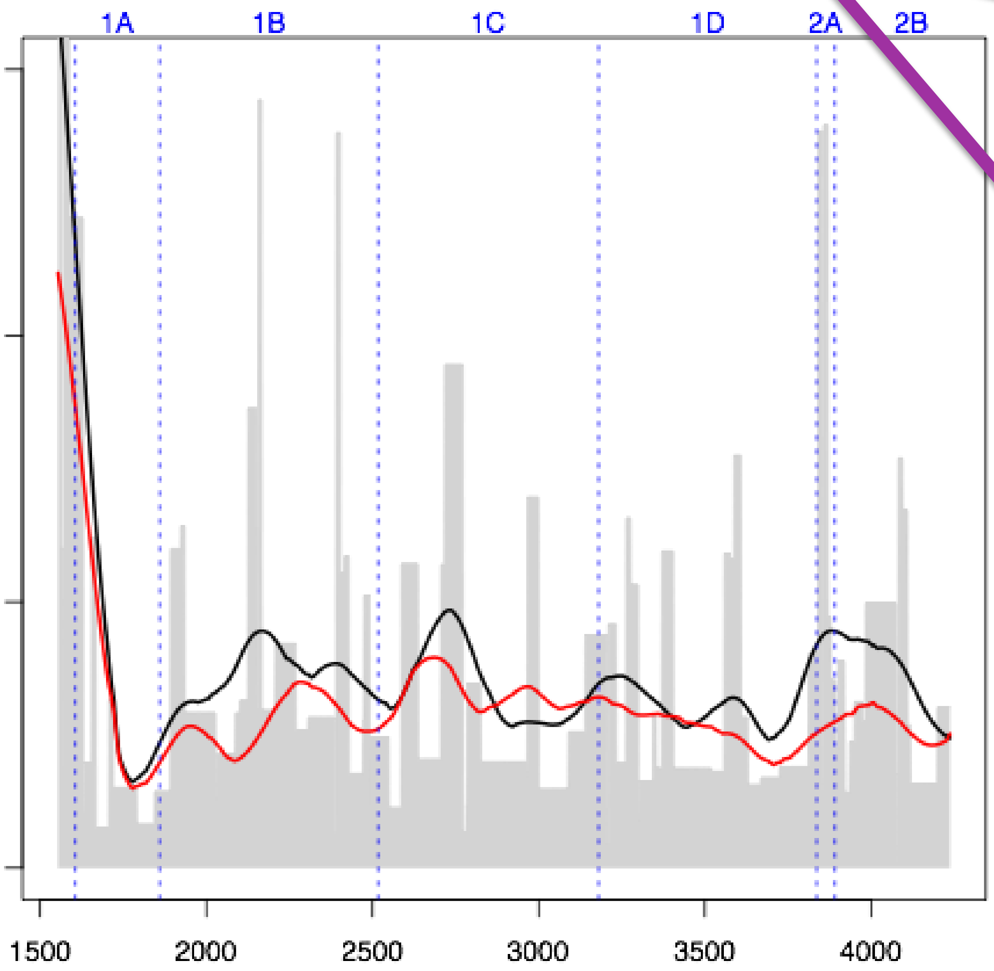

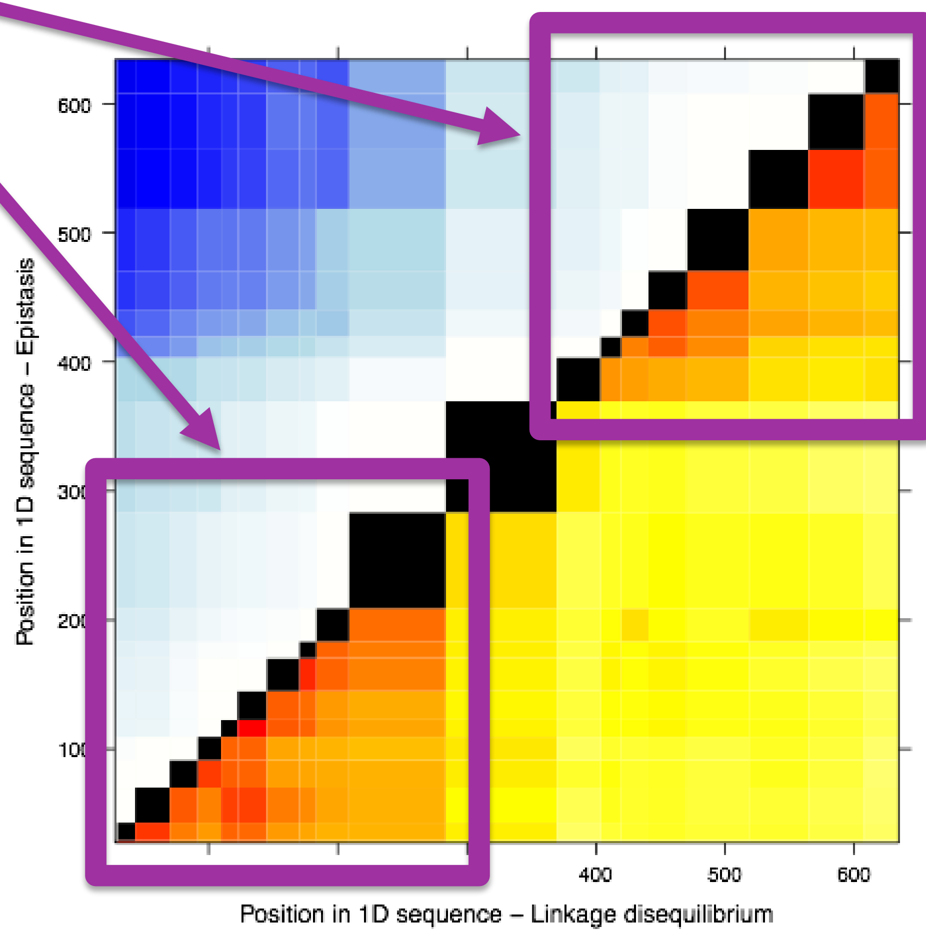

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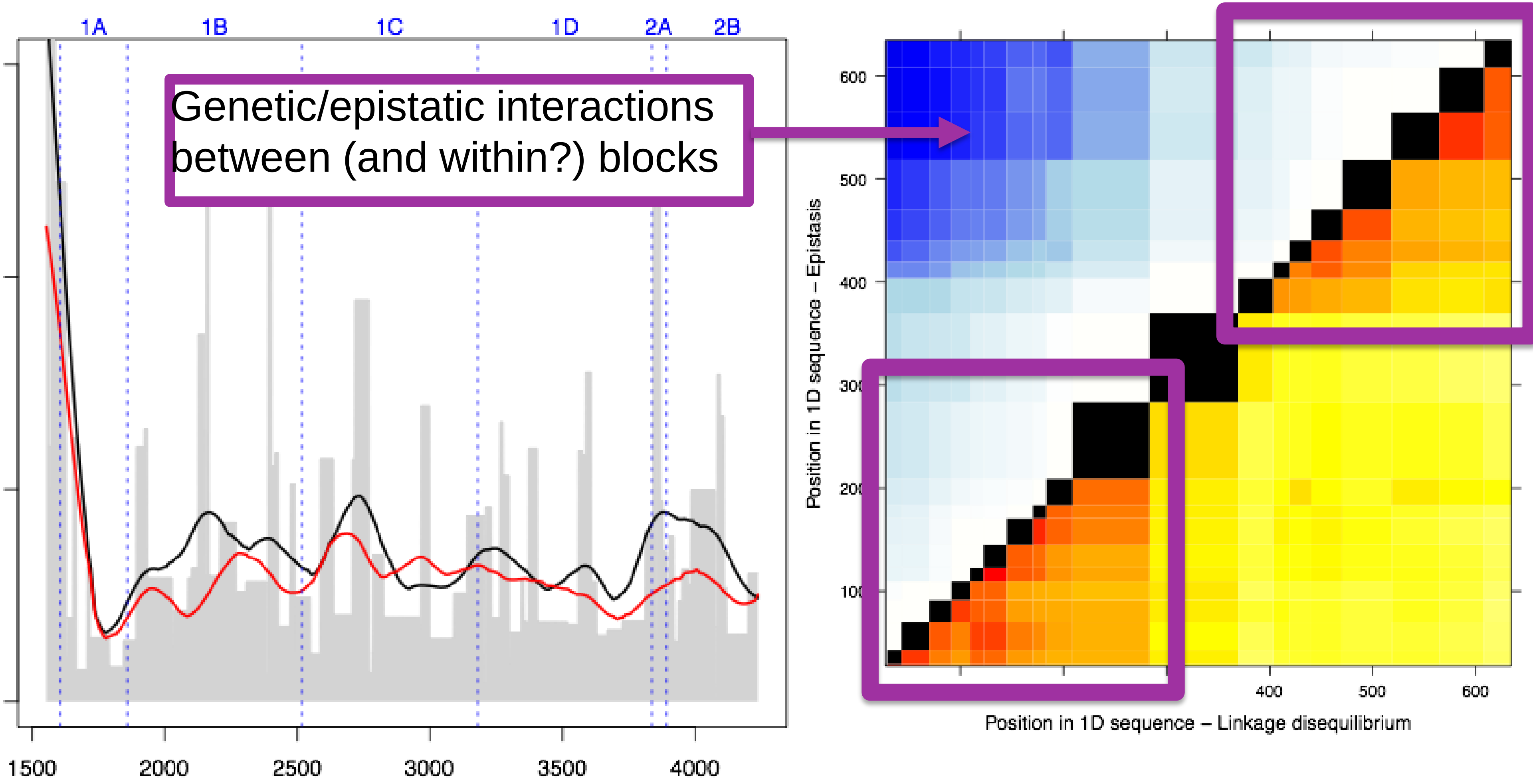
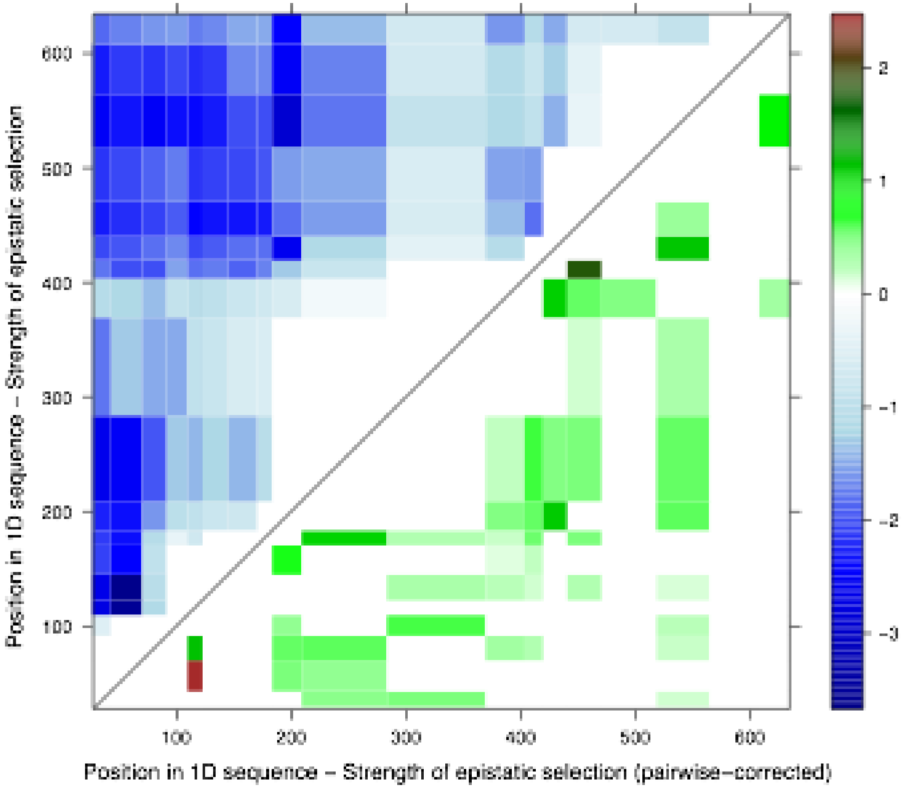
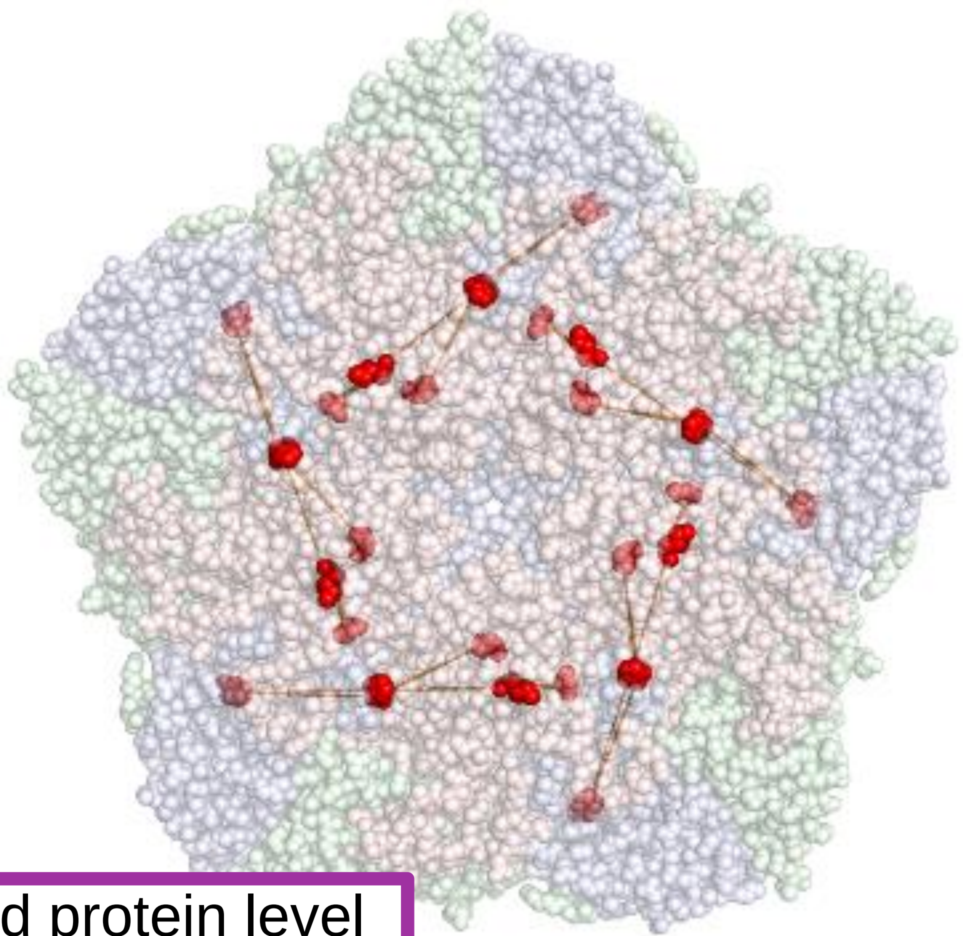


Genetic/epistatic interactions between (and within?) blocks

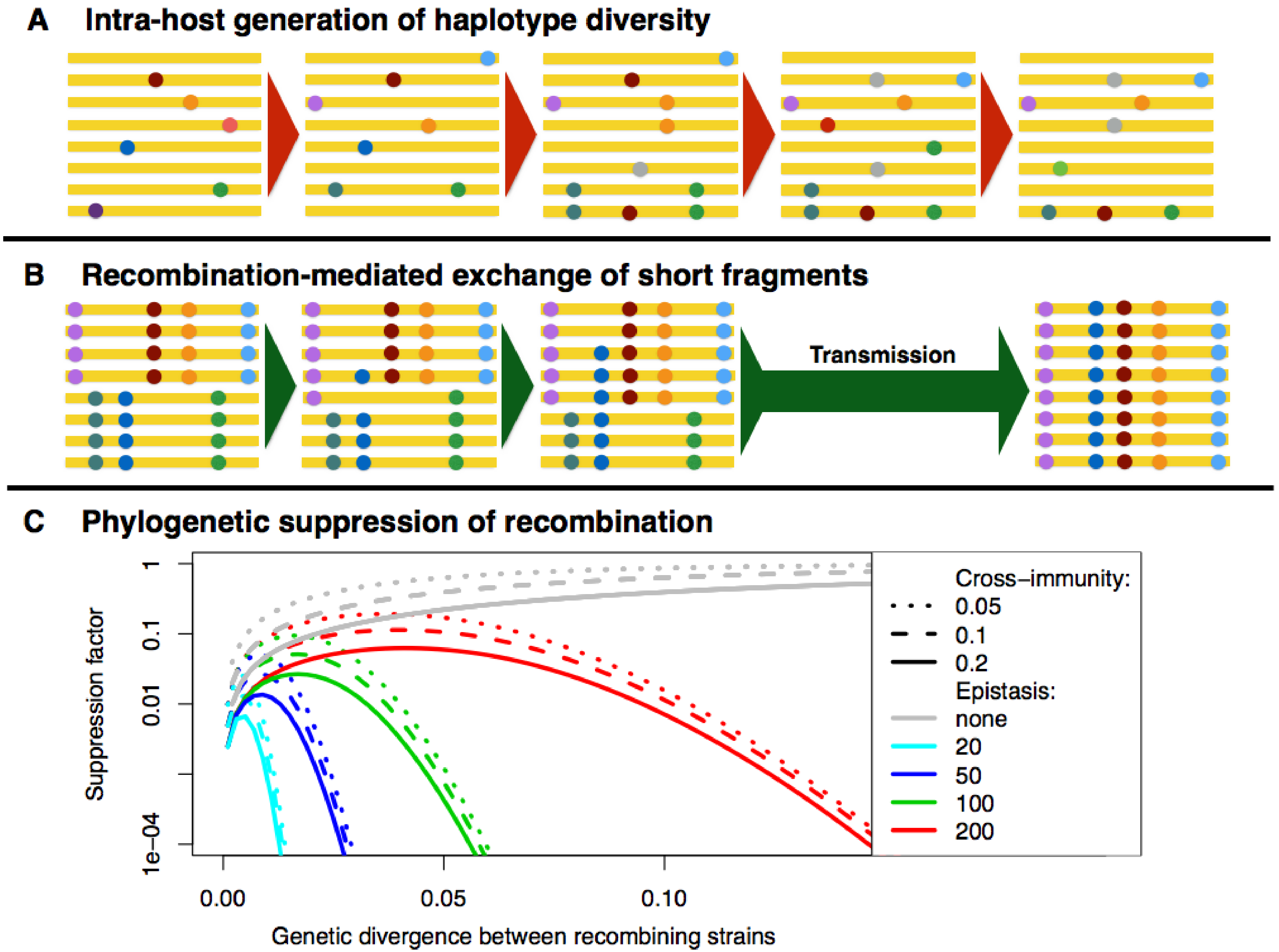
Intra-host selection and epistatic interactions inside VP1





Epistatic selection both at RNA and protein level

Large-scale consequences



Summary: surprises from deep sequencing

- Interesting and non-trivial quasi-species structure
  - *How often does it occur? Relevant for viral dynamics/evolution?*
- Systematic selection on quasi-species and viral sequences during acute infection *(but little or no immune escape)*

- High rates of within-host recombination in capsid genes
  - *Why not observed in large-scale phylogenies?*
  - *Selection, epistasis and epidemiological reasons as well…*
- Replication in carrier state is ~40 times slower than during acute infection

- Epistatic interactions within hosts and probably also during transmission/infection processes
  - *Large-scale consequences for generation of diversity and speciation*

Acknowledgments

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