



# Evolutionary dynamics of FMDV in buffalo: a tale of quasi-species, selection, recombination and persistence

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and Bryan Charleston

GFRA, Seoul, 27/10/2017



Same experiment presented by Eva Pérez-Martín in the previous talk

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

Sequencing of SAT1 only (persistent serotype)

Inoculum:

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

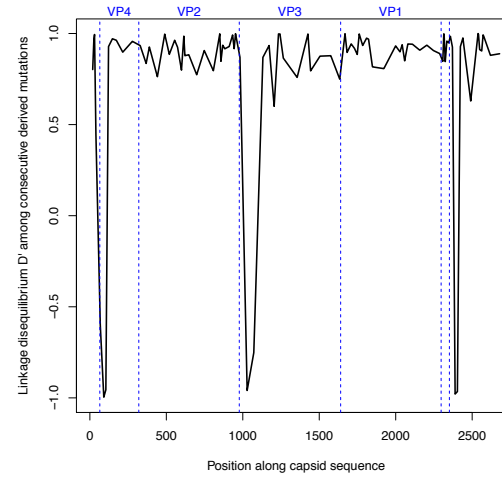
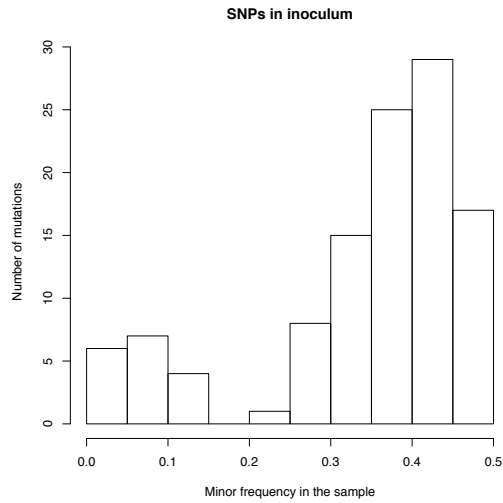
Virus from micro-dissections of 3 buffalos (2 at 35dpi, 1 at 400dpi):

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

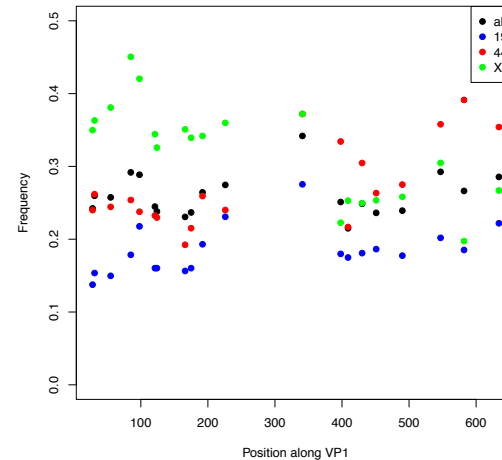
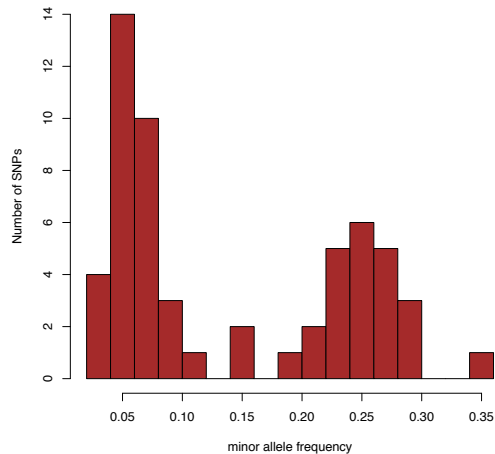
Furthermore, Sanger/NGS of probang and tonsil swabs from multiple individuals



# Quasi-species structure and selection

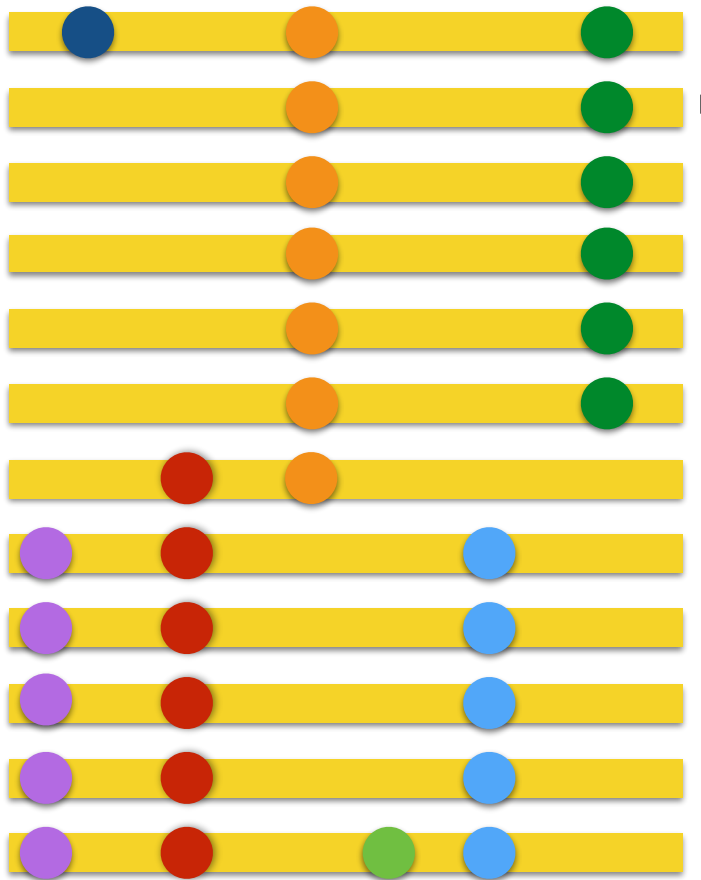


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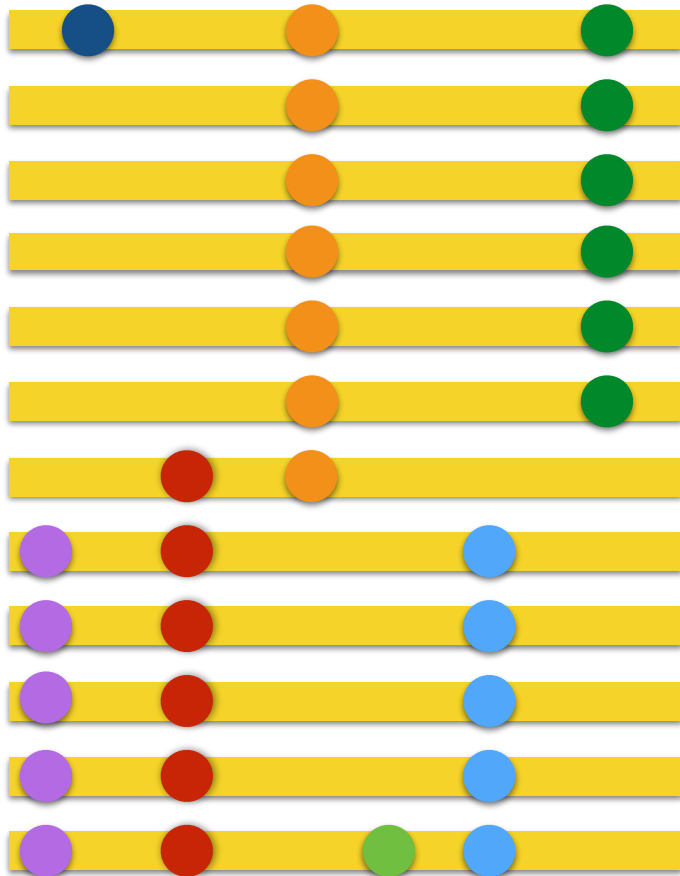




# Inoculum:



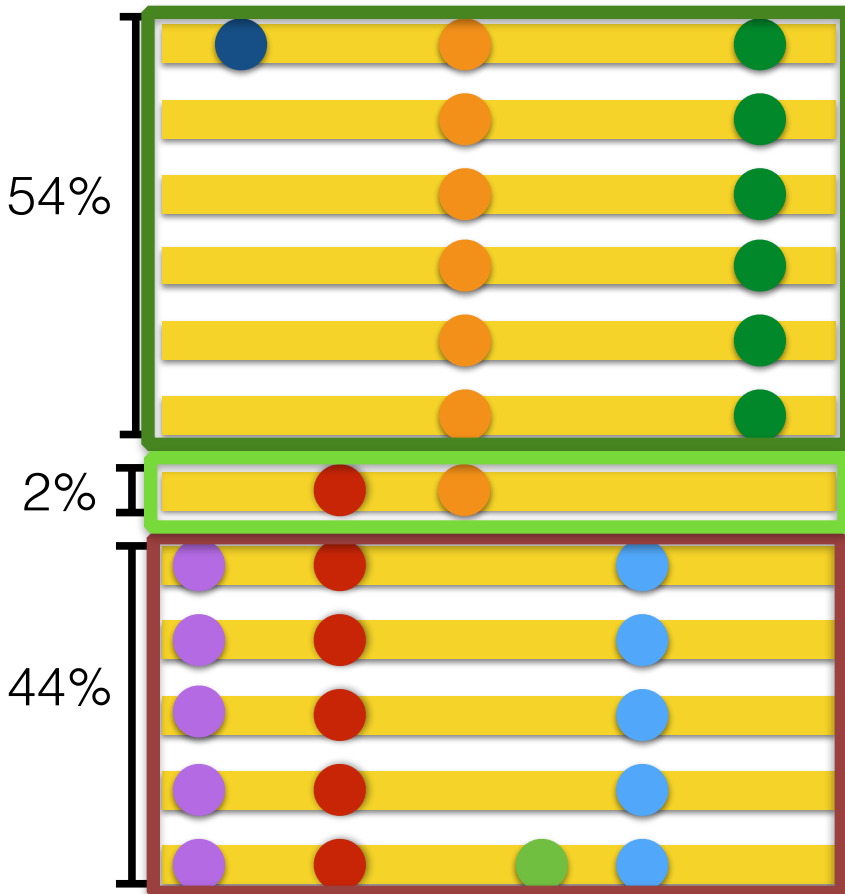
# Inoculum:



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

Expectation:  
either identical viruses,  
or a single quasi-species

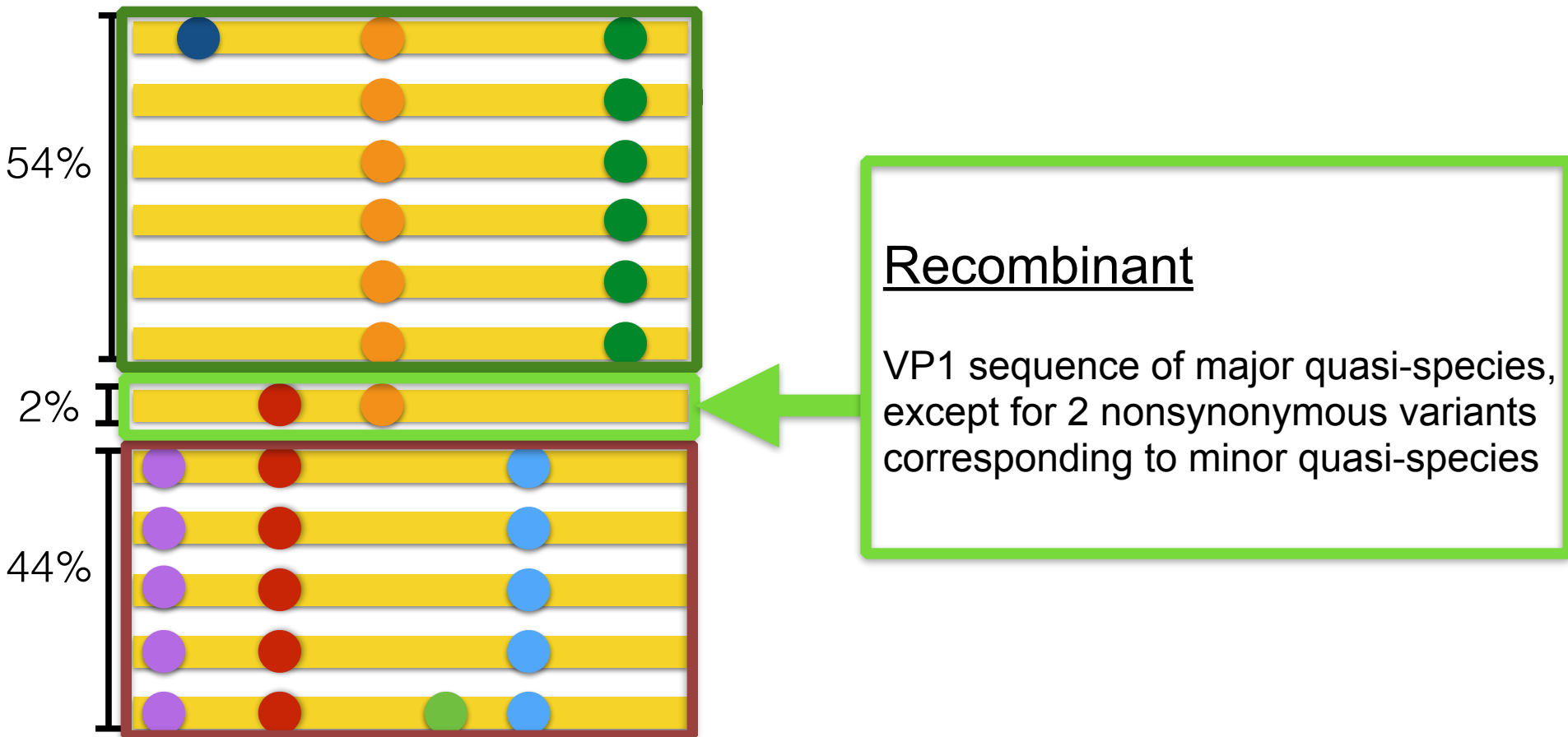
# Inoculum: two quasi-species (plus recombinants)



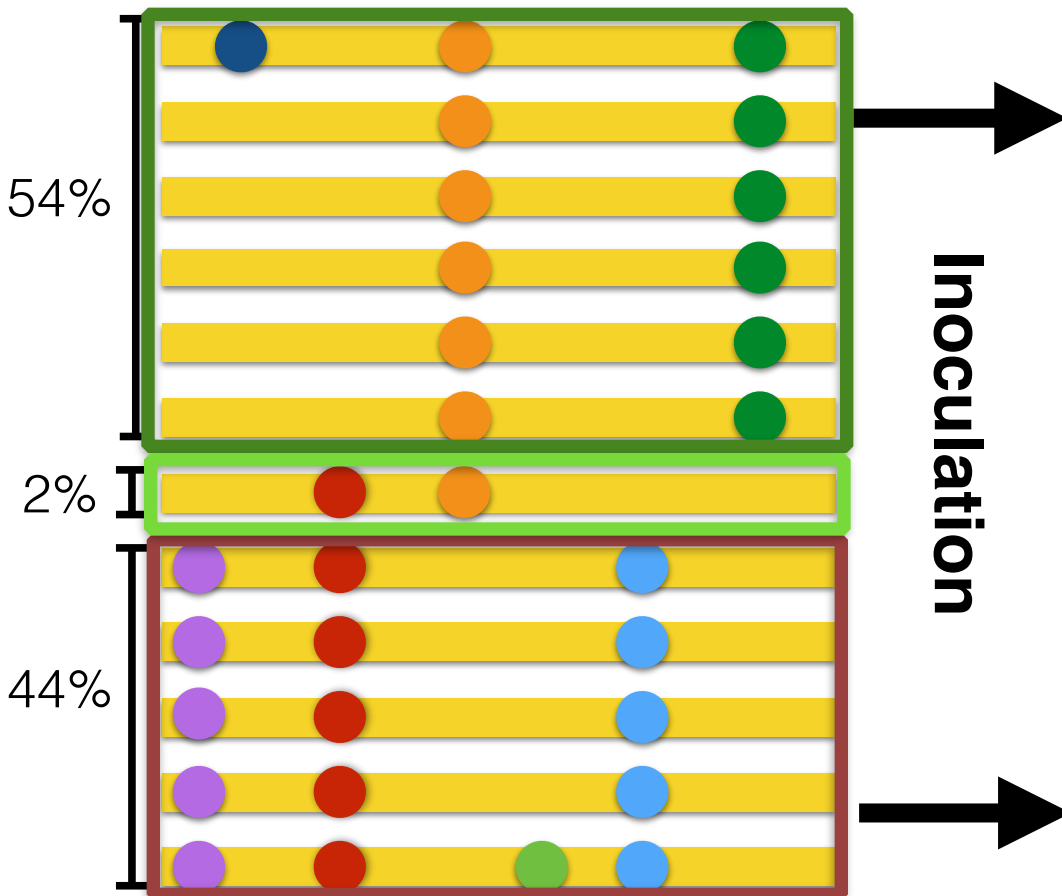
Two main quasi-species  
with 3% sequence divergence

Large fraction of recombinant  
sequences

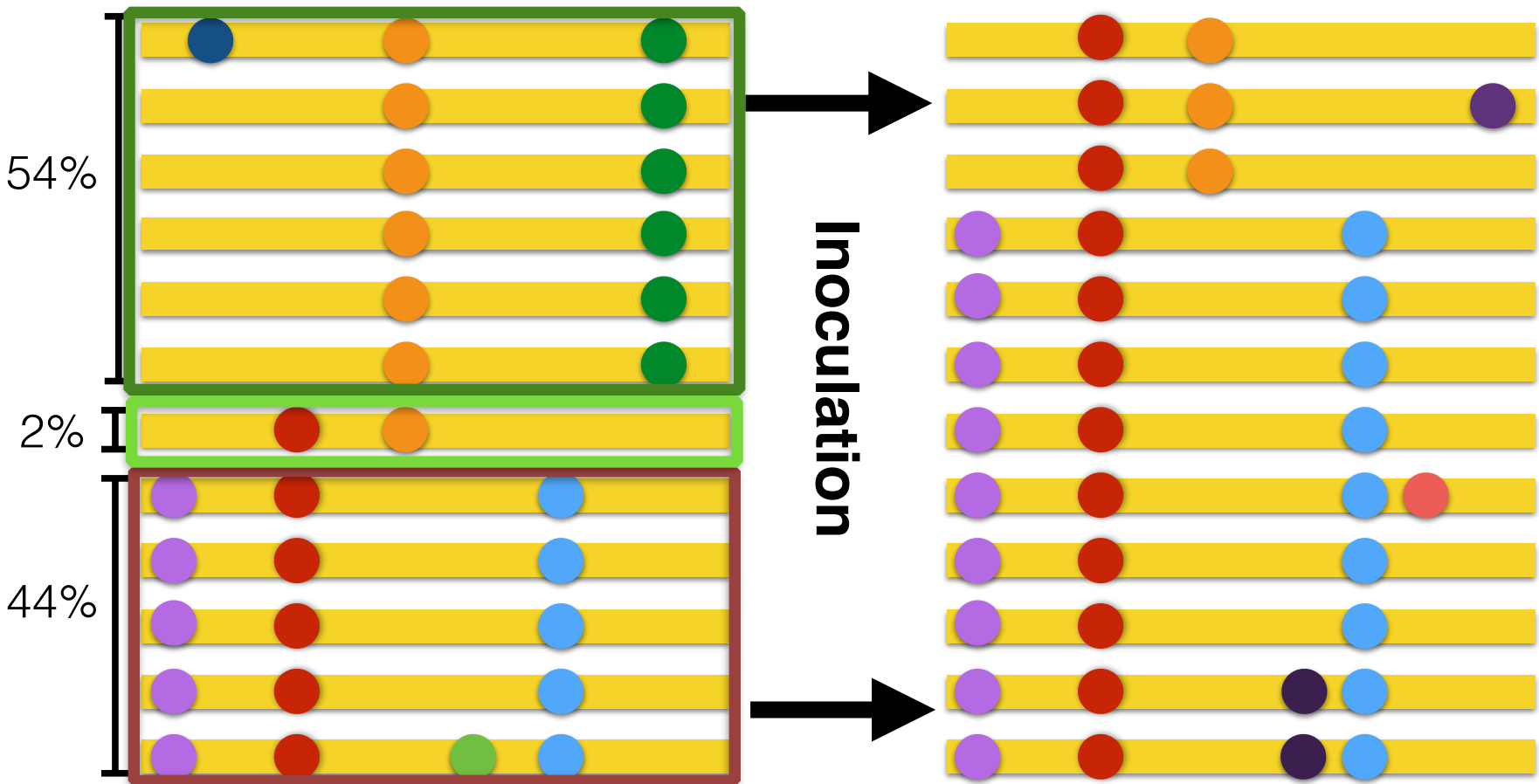
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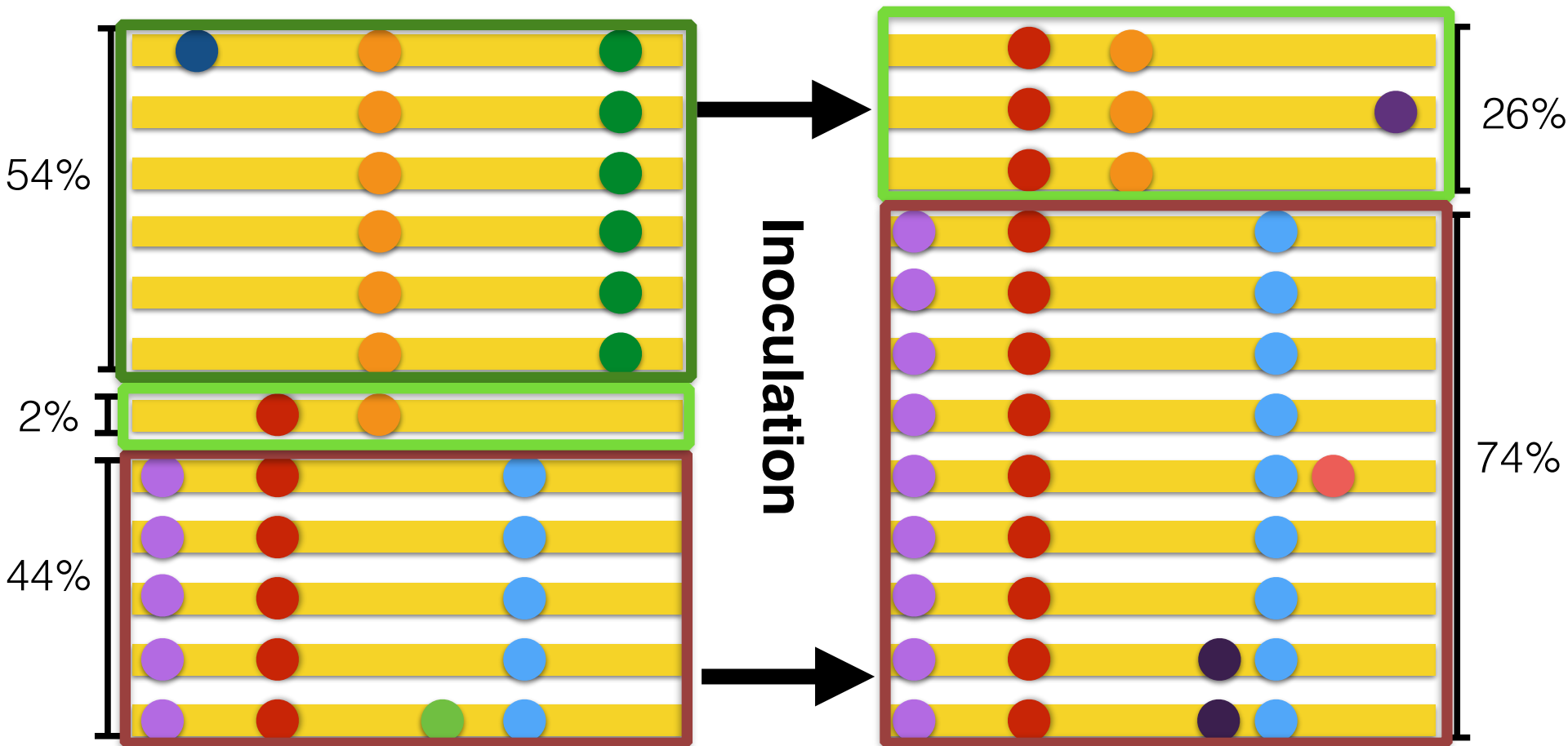
# Strong post-inoculation changes in quasi-species frequencies



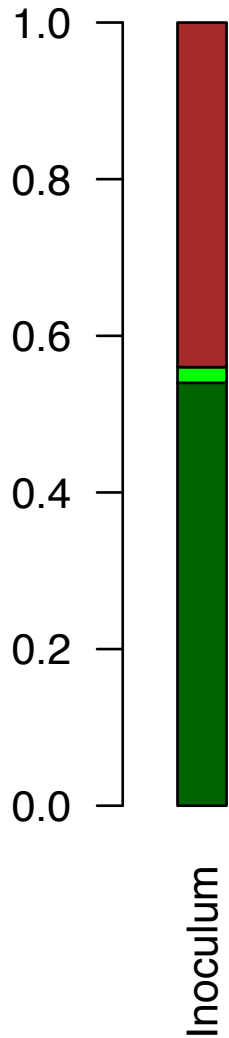
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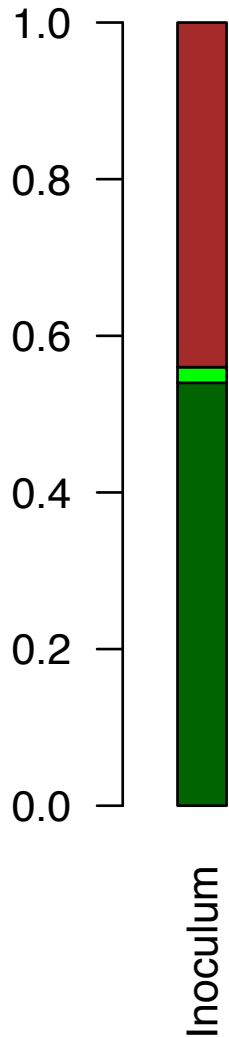


# Quasi-species structure across animals and tissues

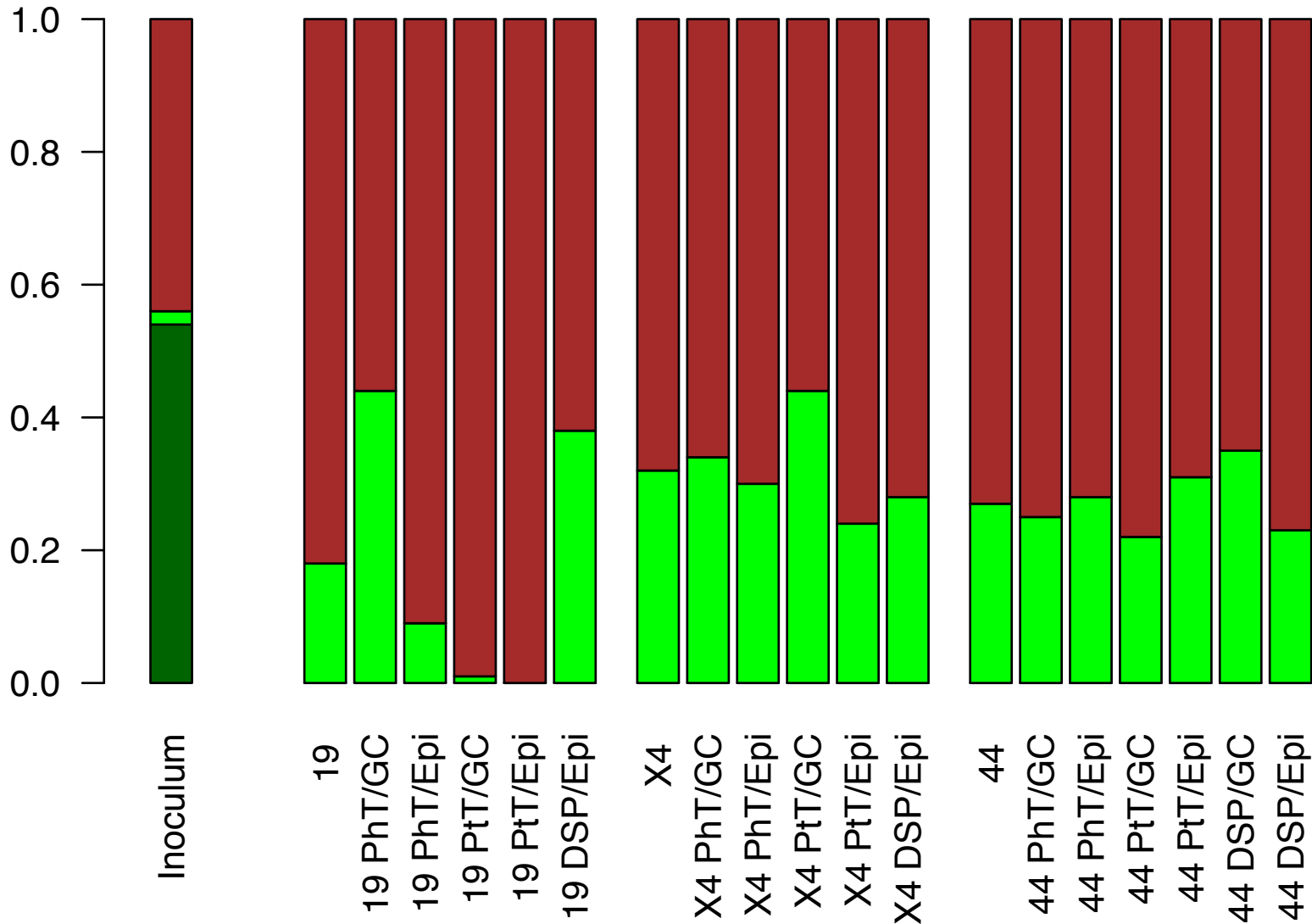




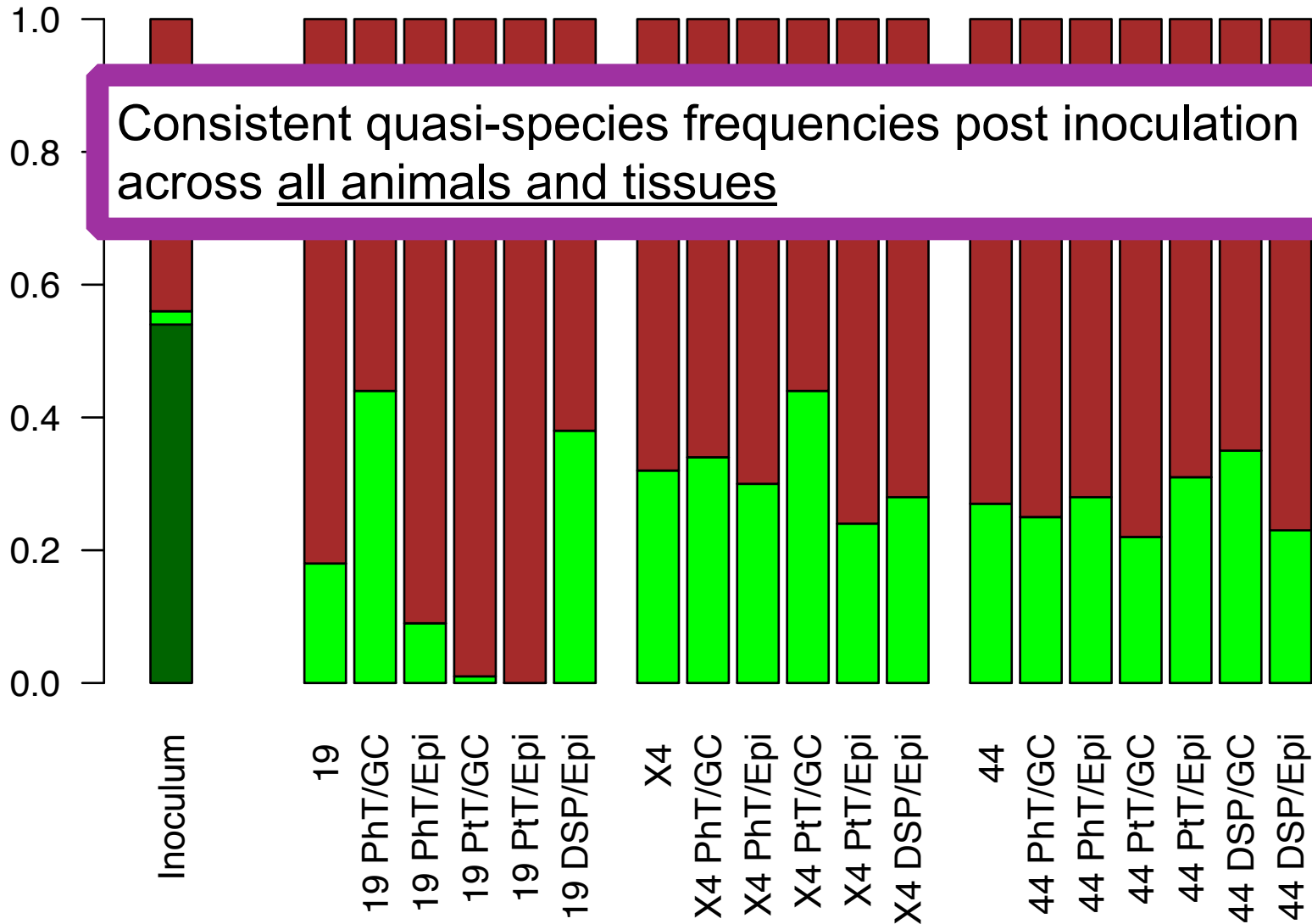
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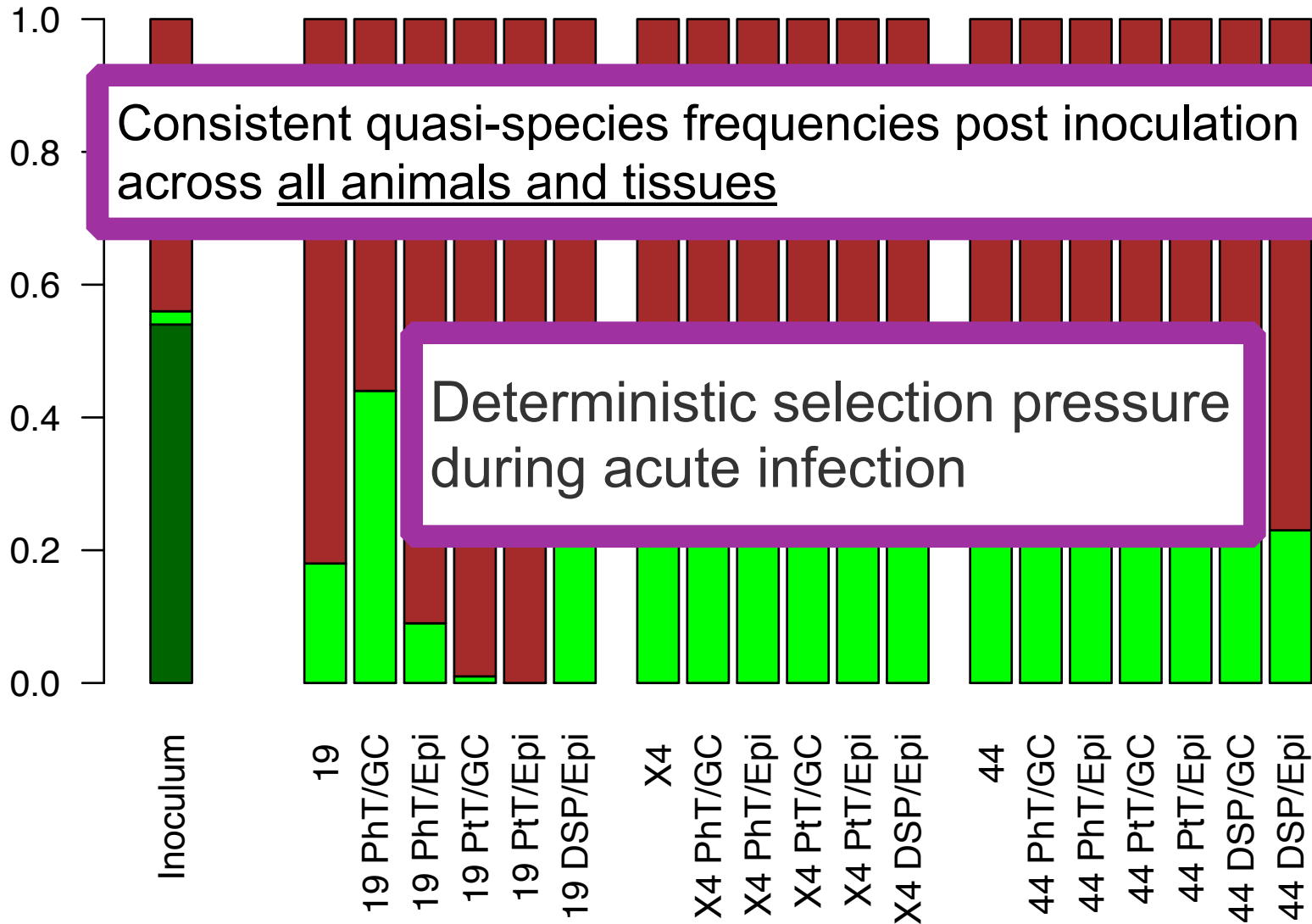
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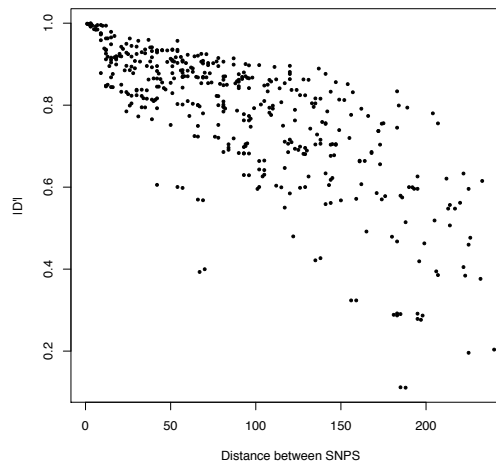
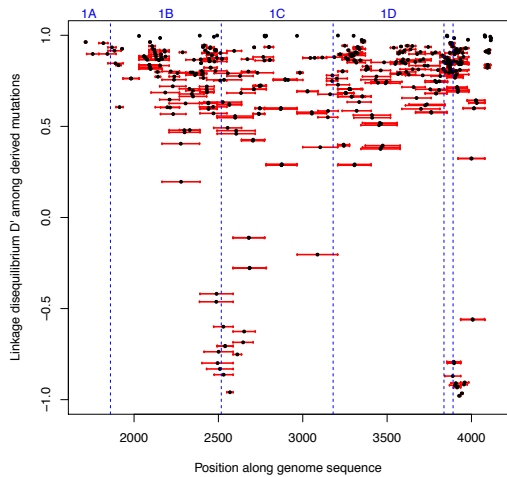


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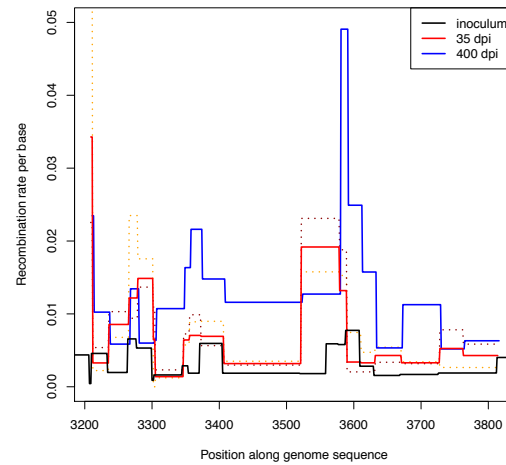
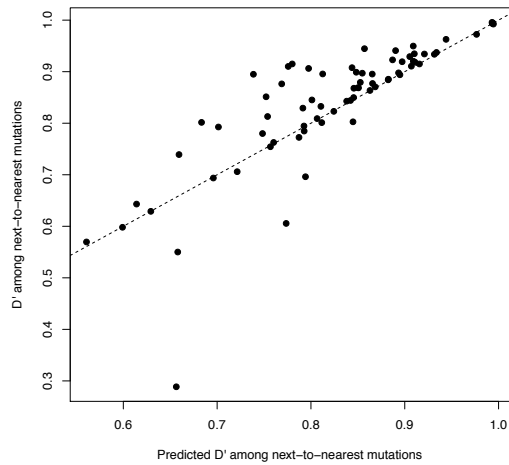
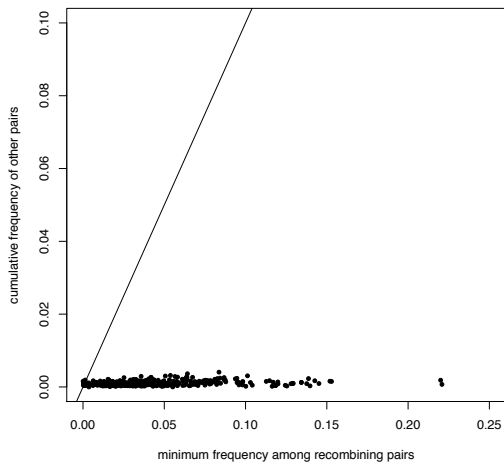




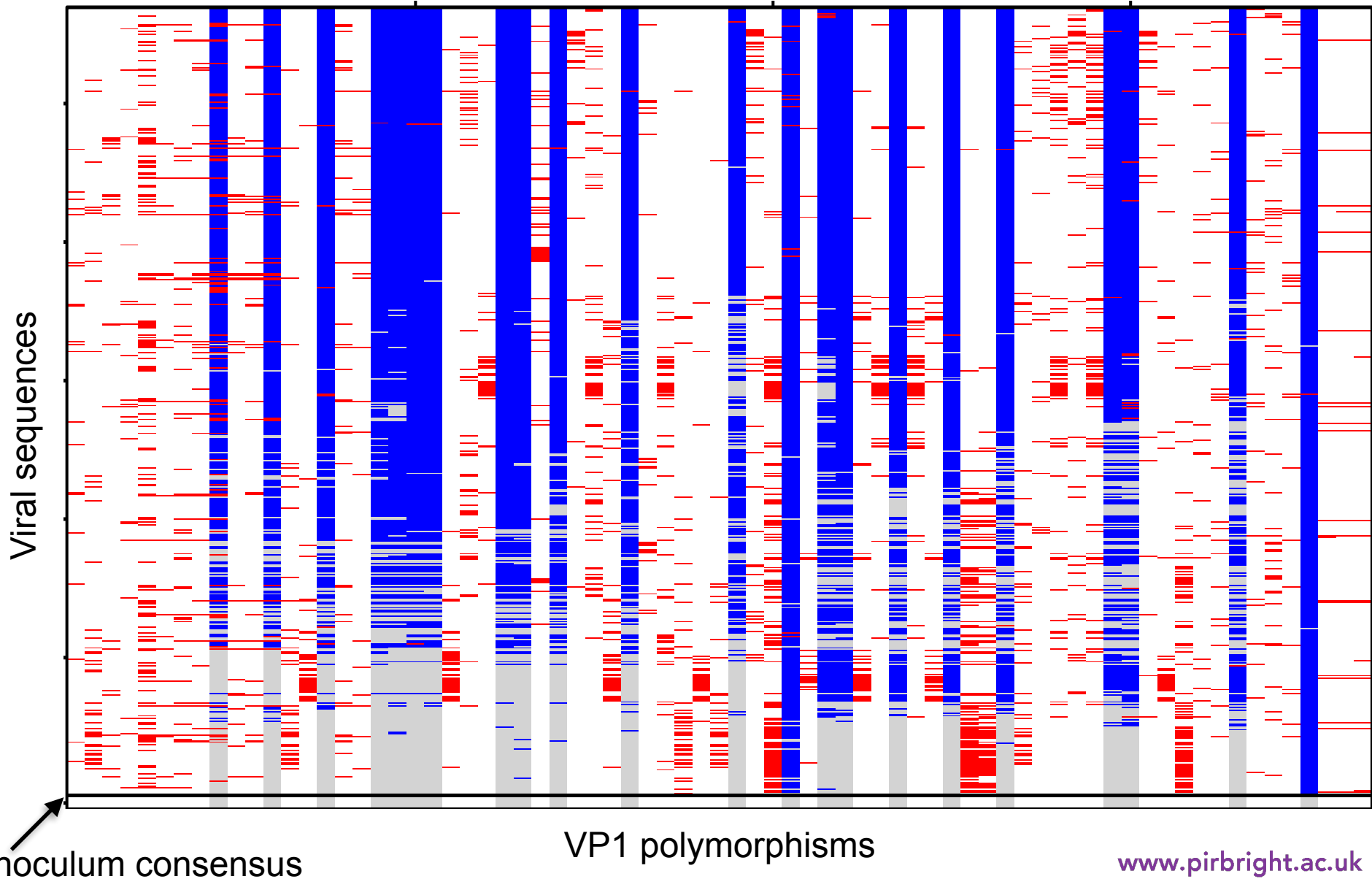
# Recombination



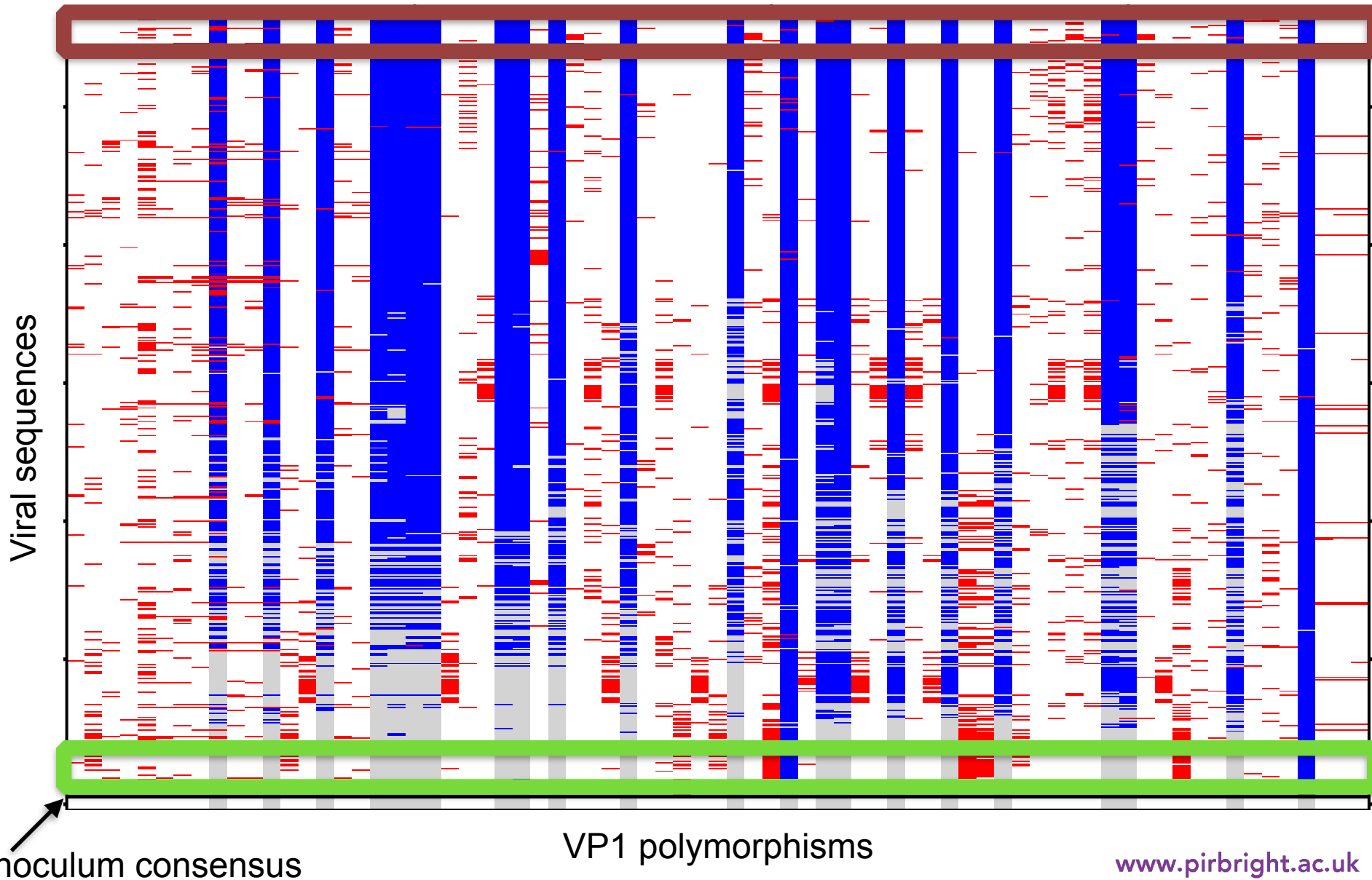
# Recombination



# Recombinants in buffalos

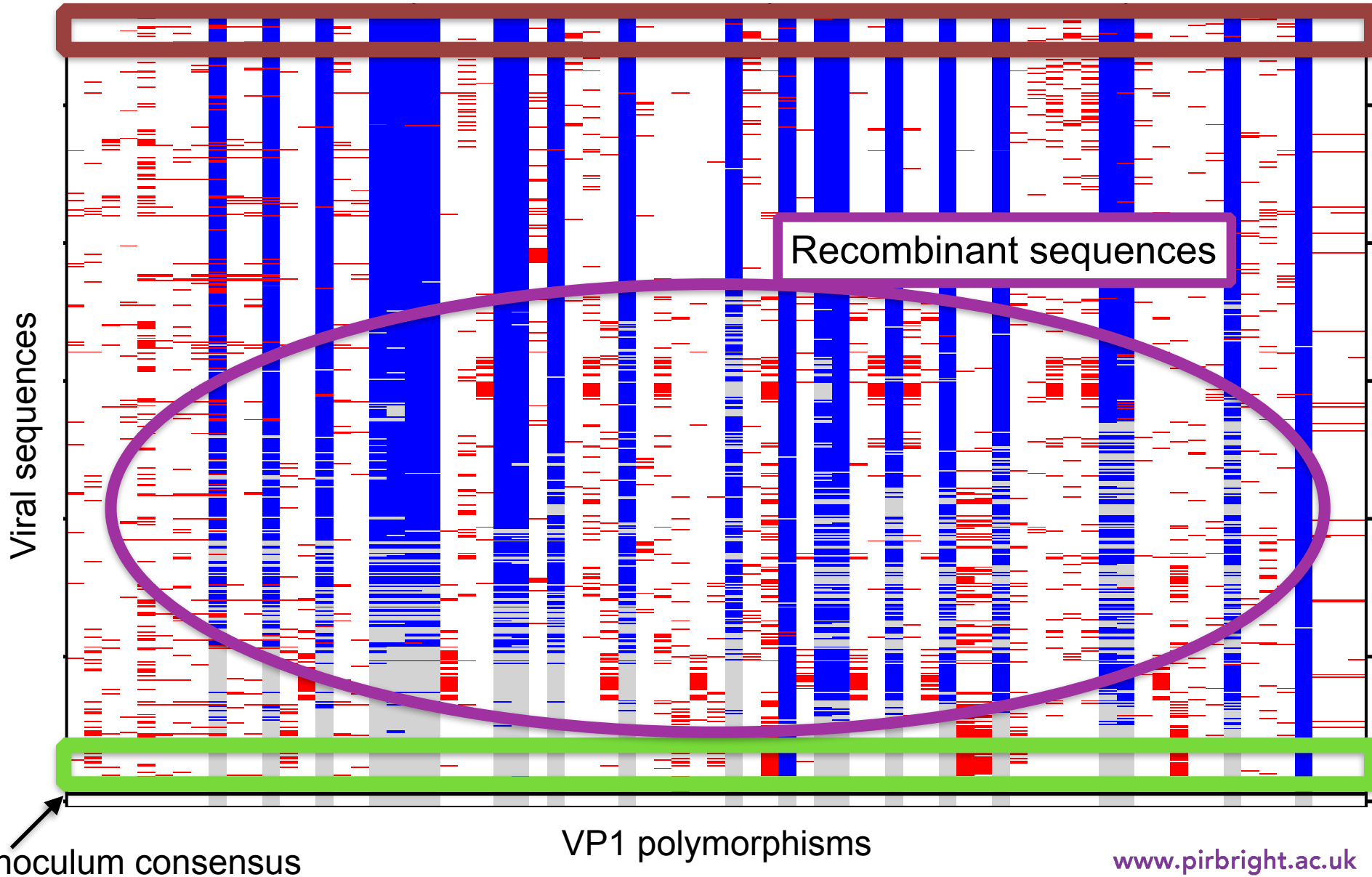


# Recombinants in buffalos



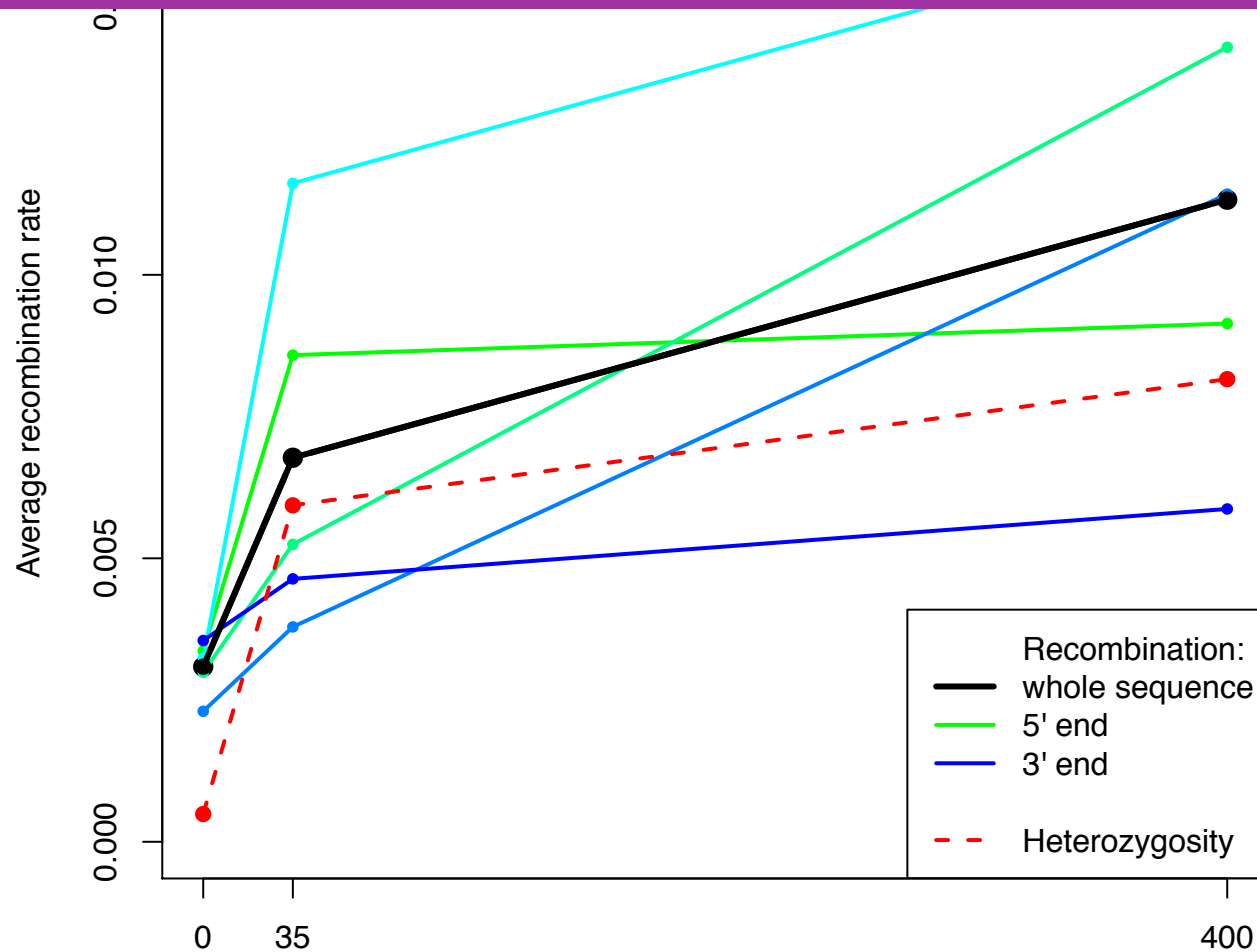


# Recombinants in buffalos



# Increase in recombinants and sequence diversity with time

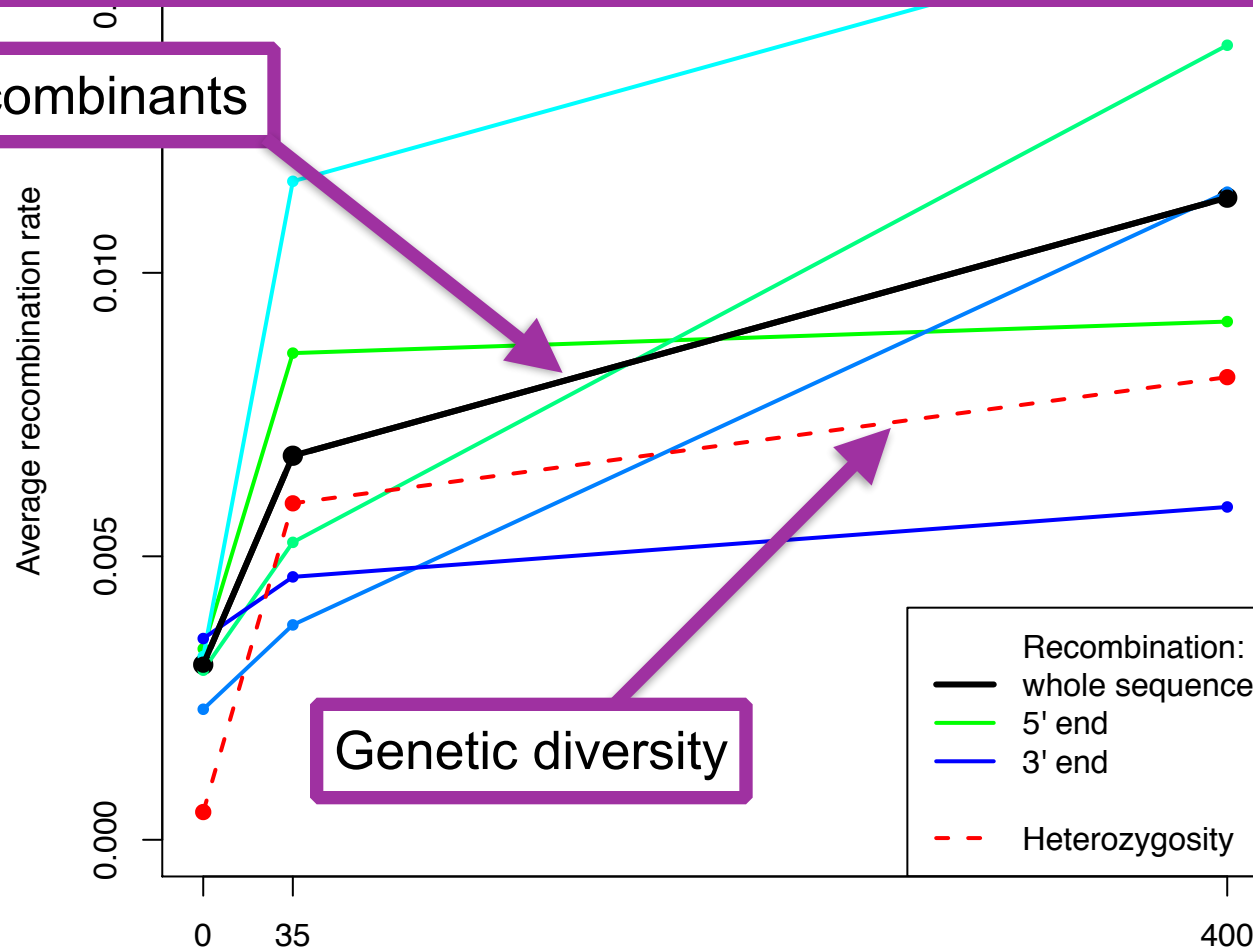
Recombination occurs during viral replication



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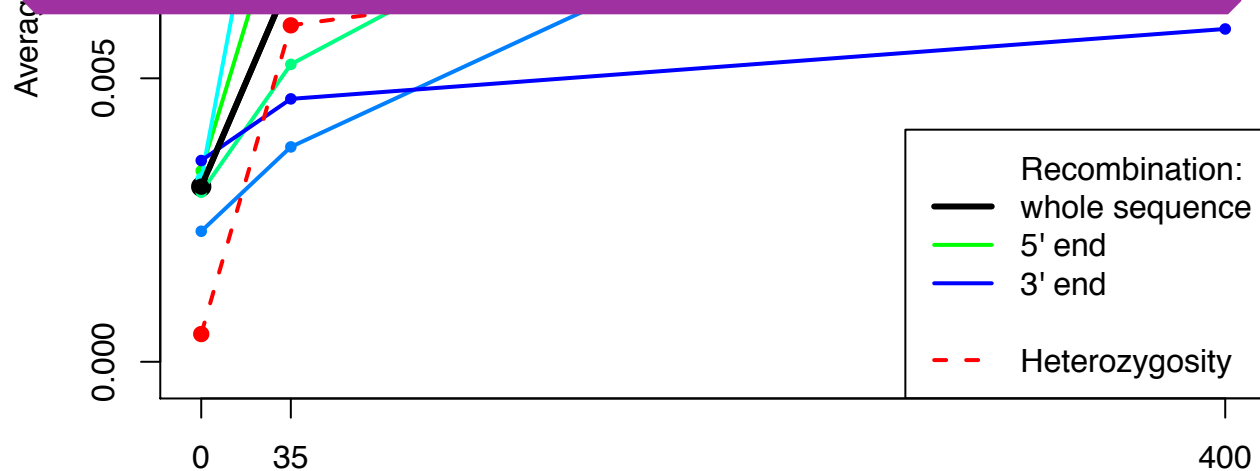
Recombinants



# Increase in recombinants and sequence diversity with time

Recombination occurs during viral replication

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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Recombination occurs during viral replication

Absolute recombination rates in VP1:  
acute phase:

~0.2 per base per year

persistent phase:

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Average

0.005

0.000

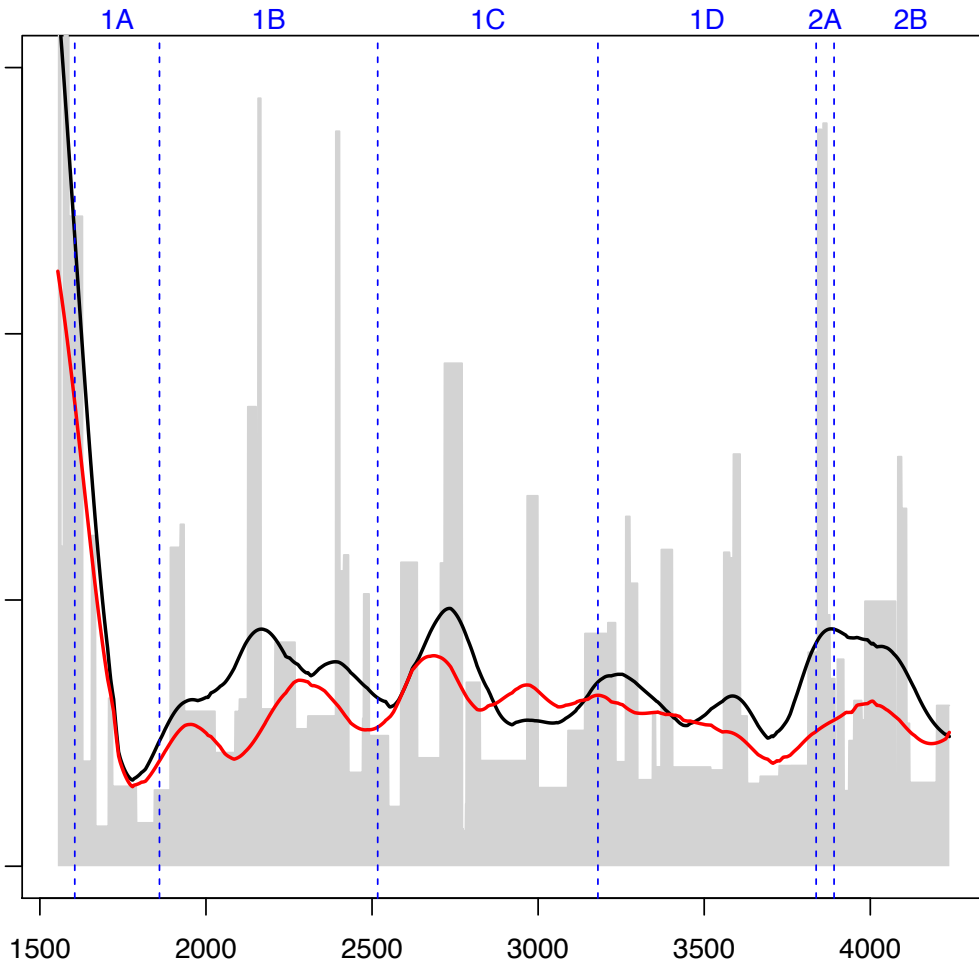
0 35

400

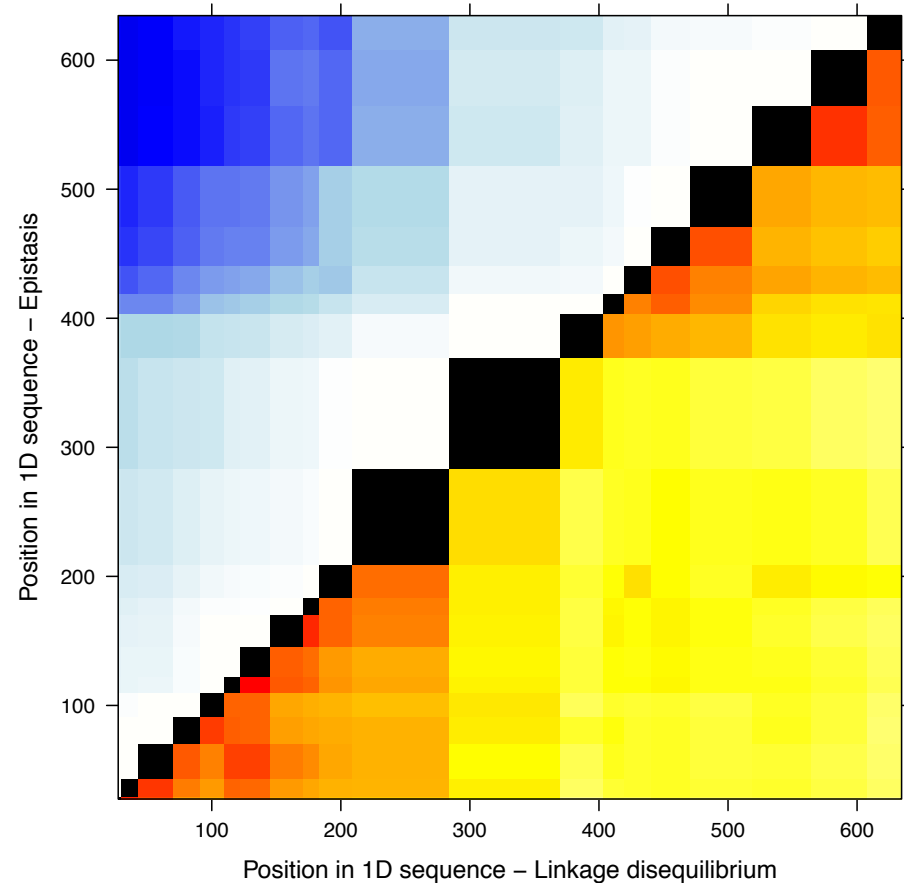
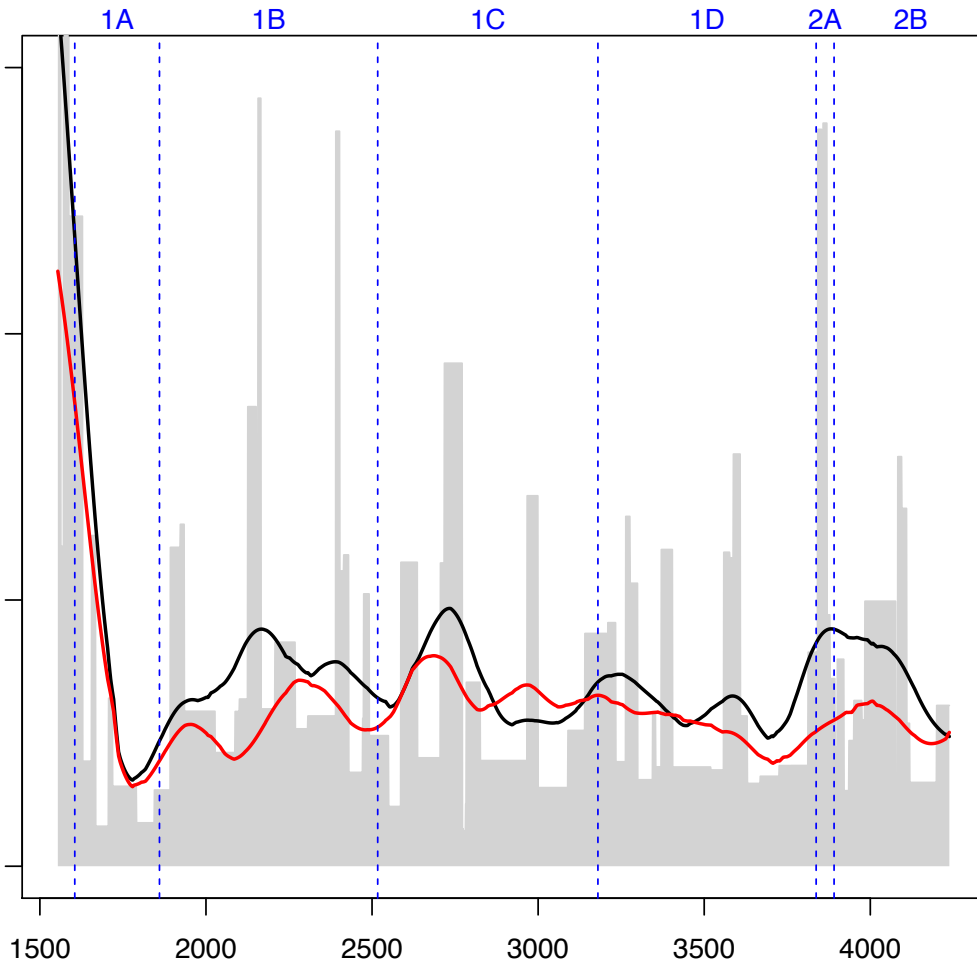
Replication during persistent phase  
is about 40 times slower than  
replication during acute phase of infection

-- Heterozygosity

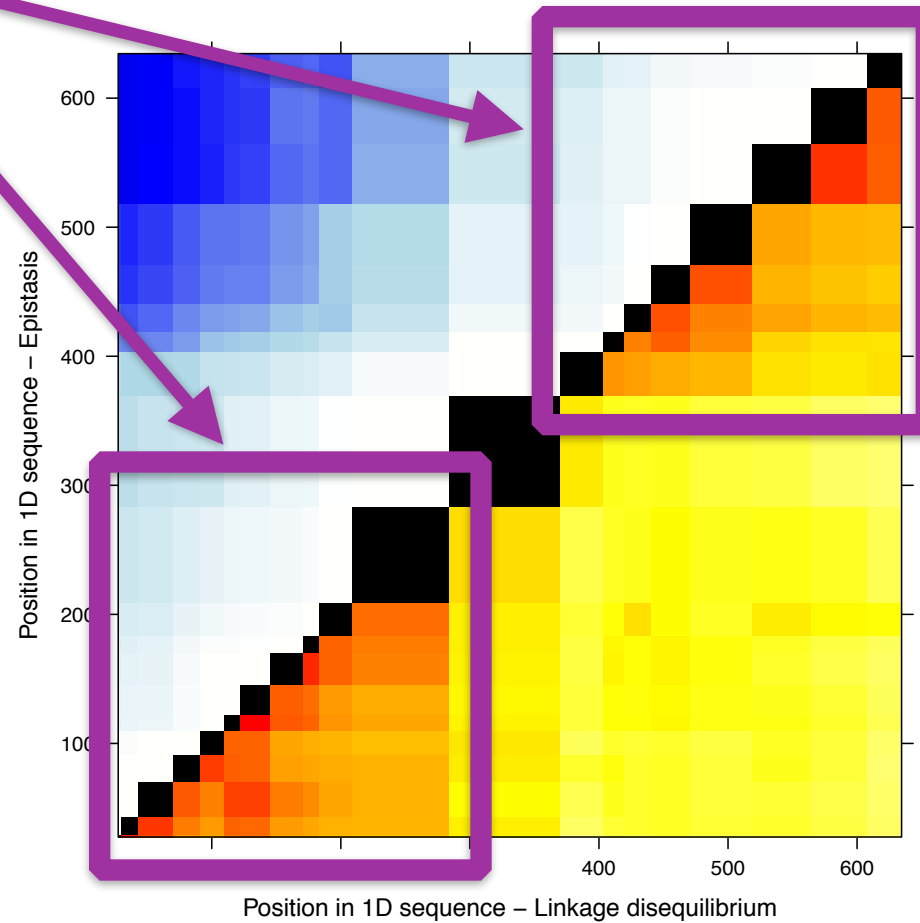
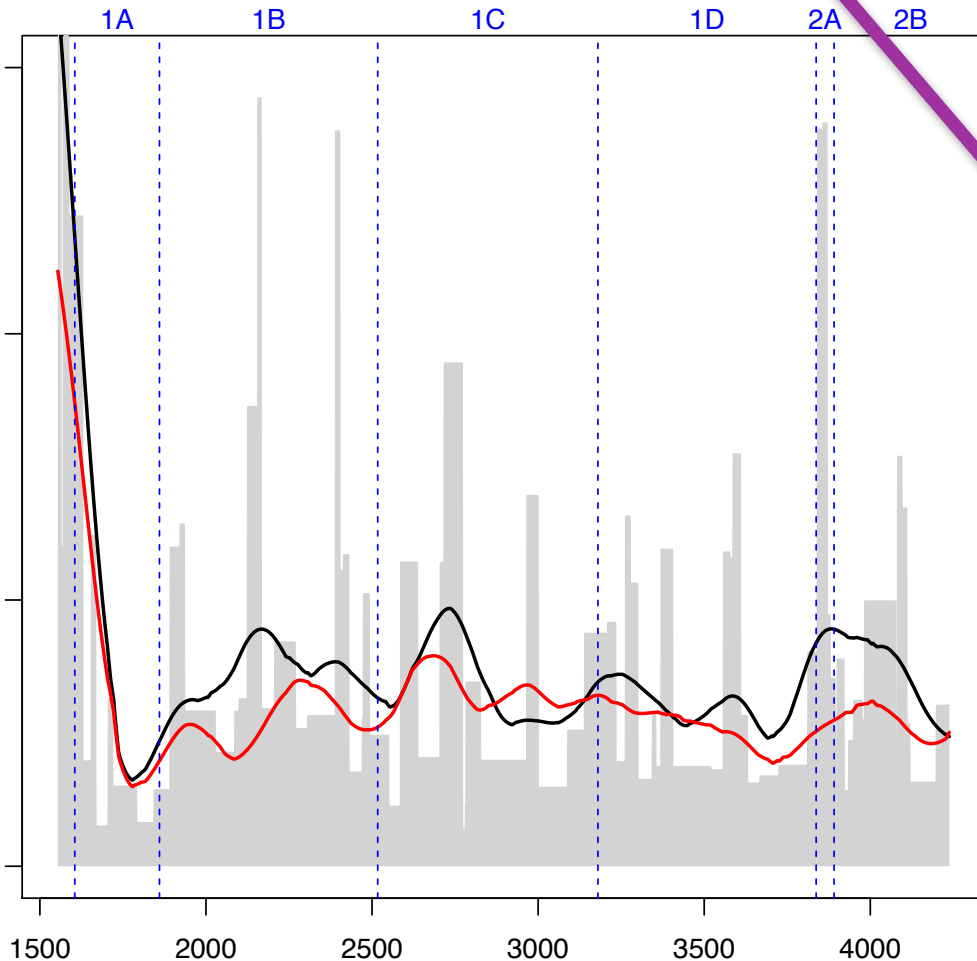
# Recombination map of capsid genes



# Recombination map of capsid genes and mosaic structure inside VP1

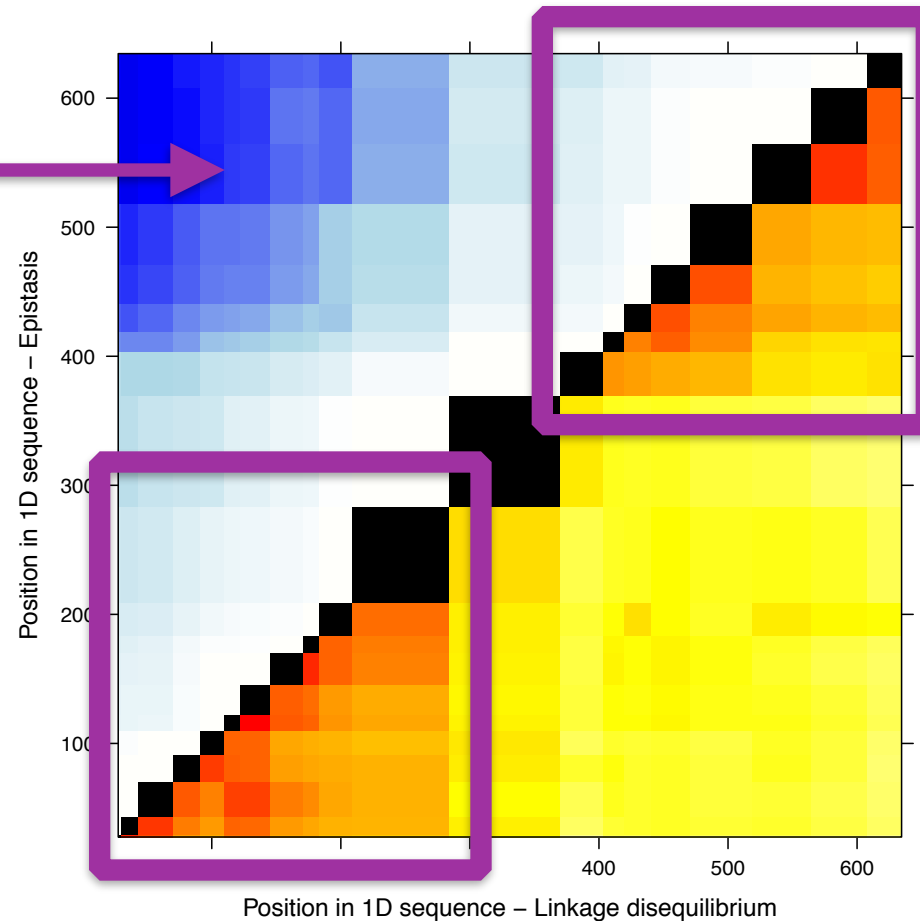
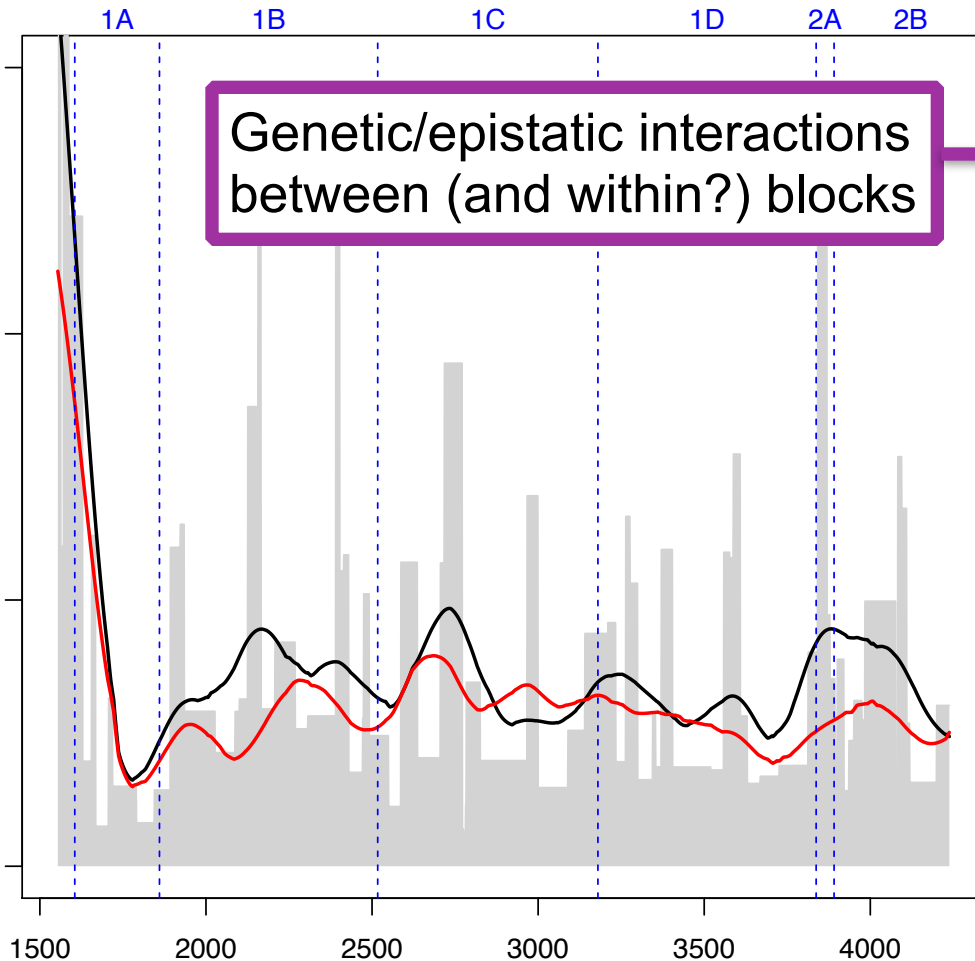


# Recombination map of capsid genes and mosaic structure inside VP1





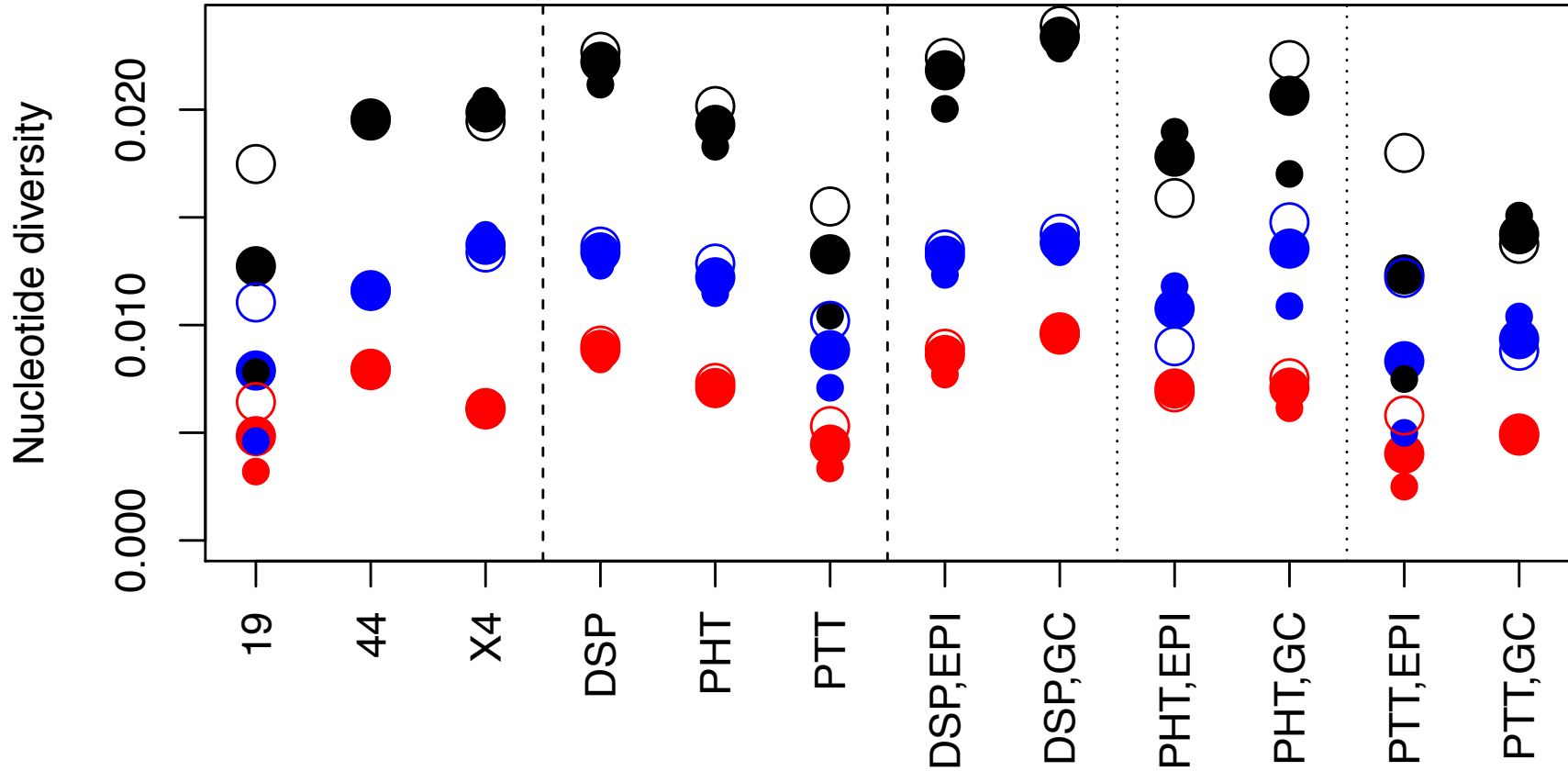
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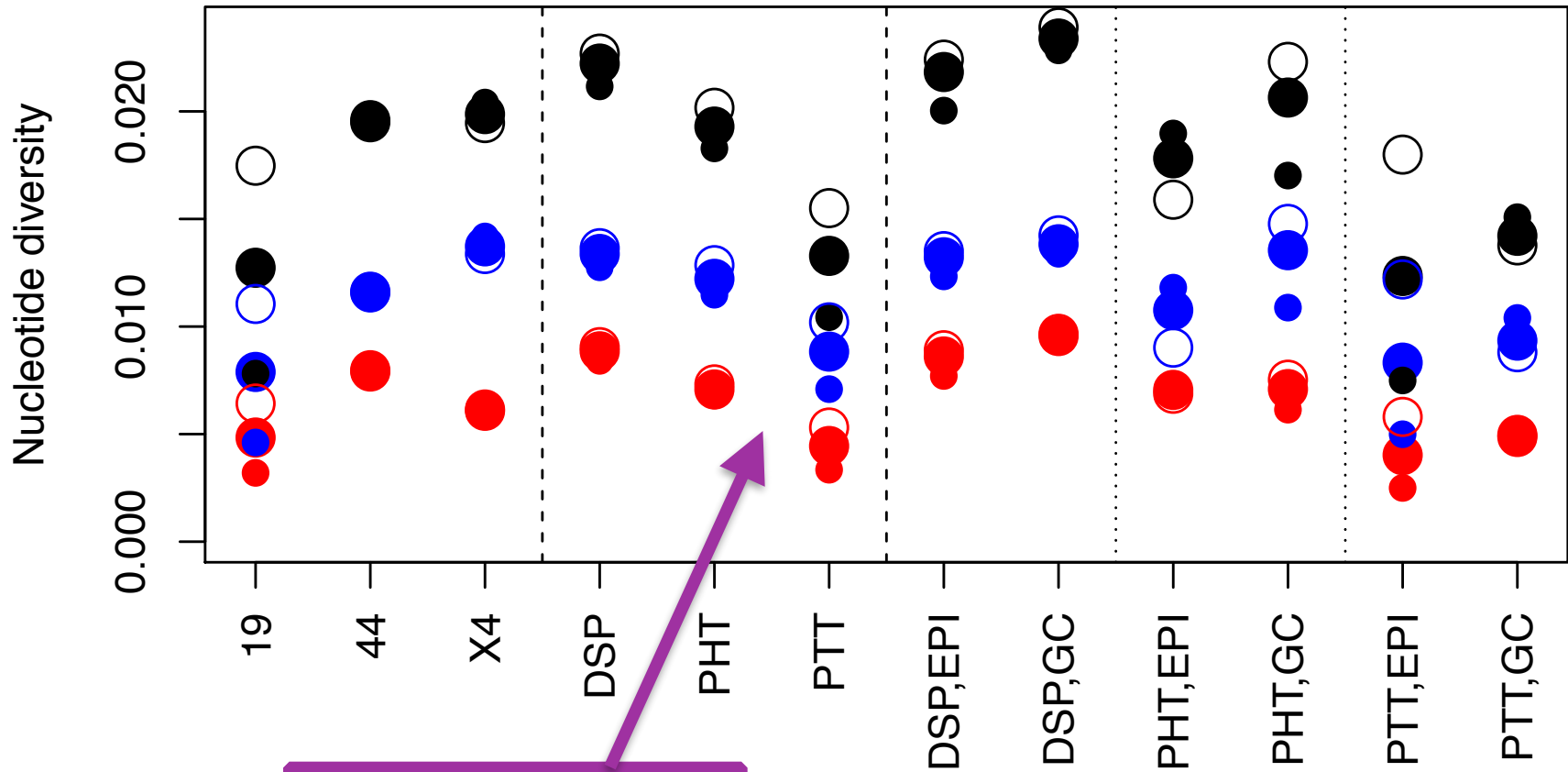


# Genetic diversity and differentiation

# FMDV genetic diversity within animals, tissues and locations in tissues

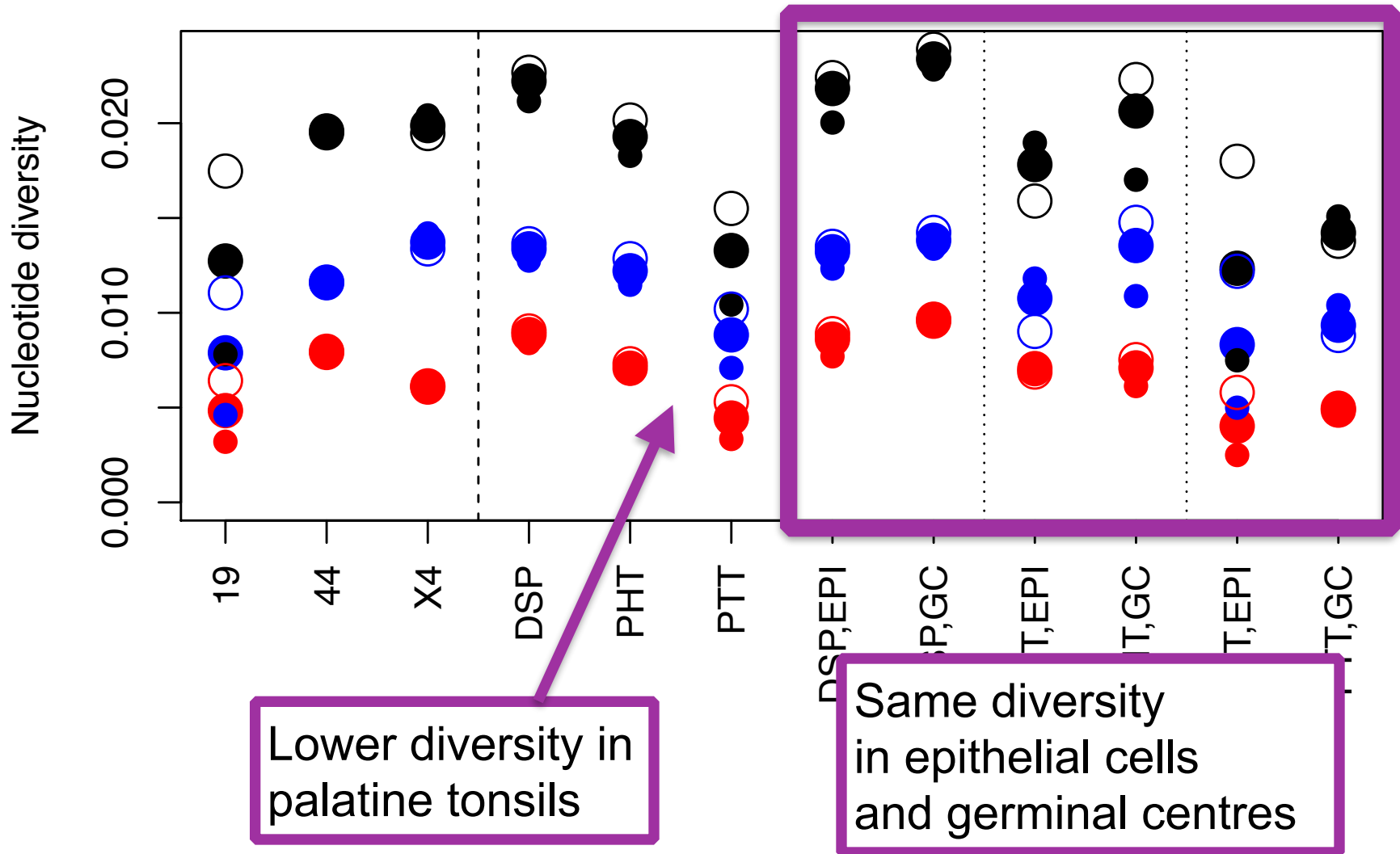


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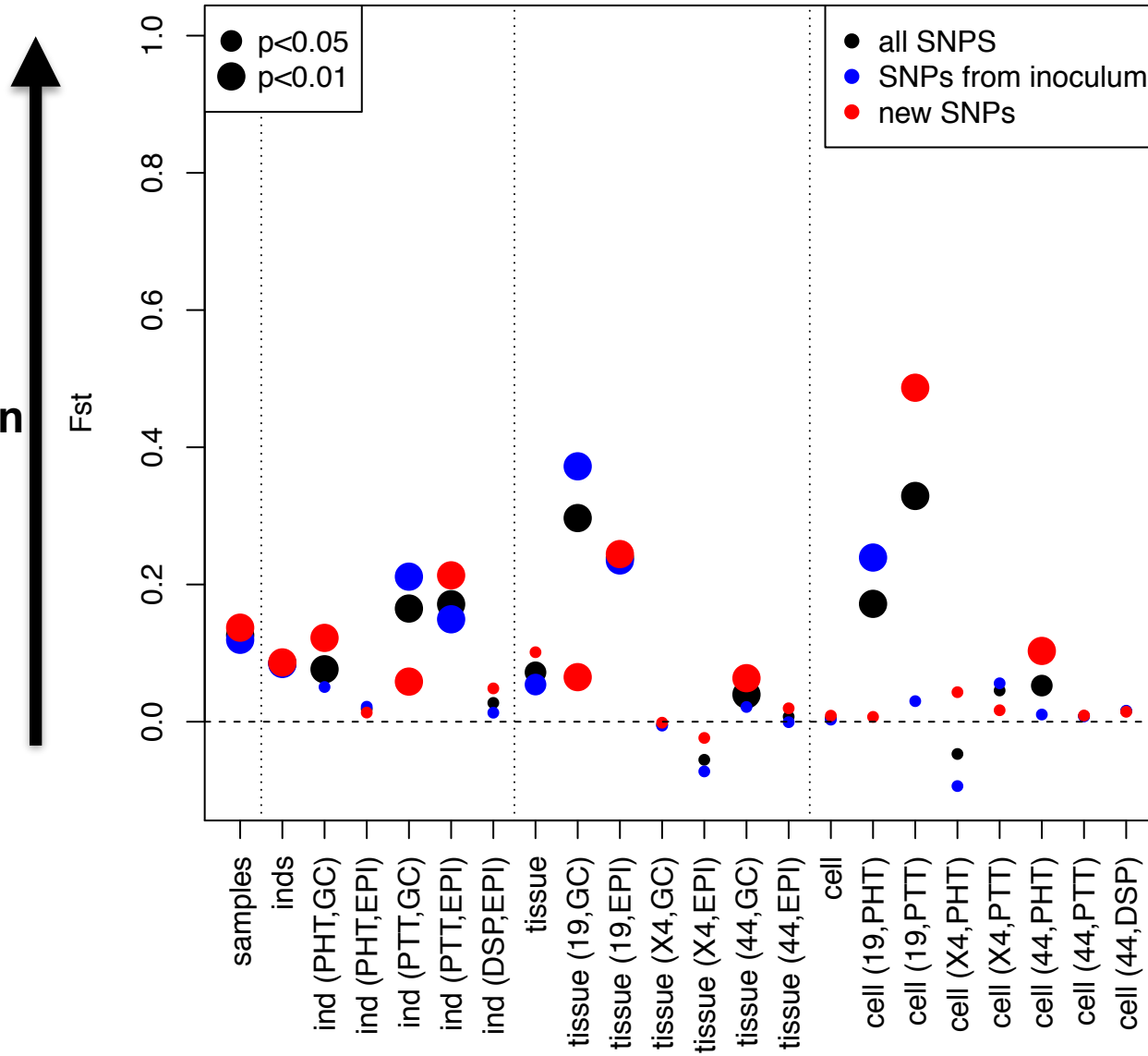
Lower diversity in palatine tonsils

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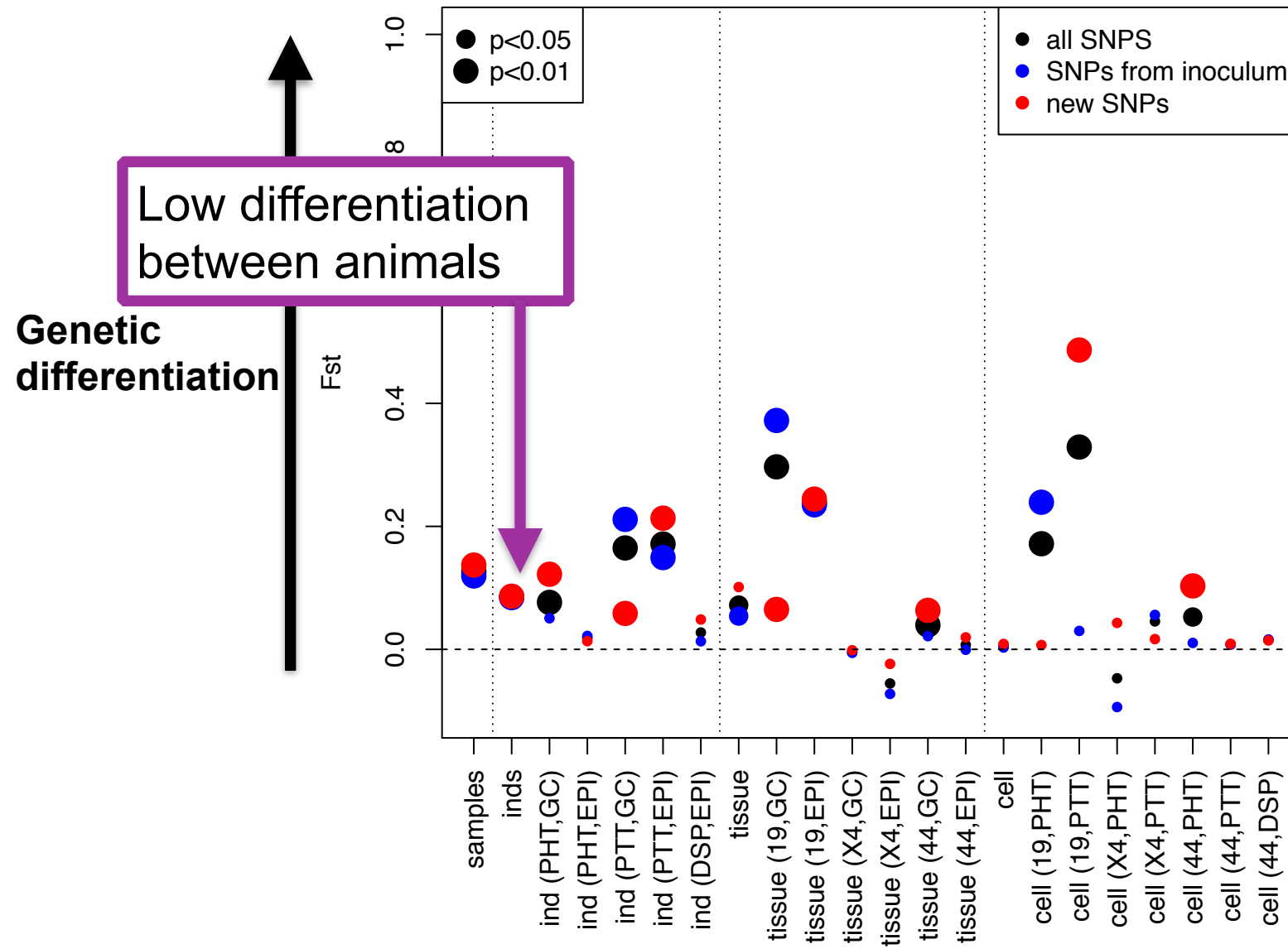


# Genetic differentiation between animals, tissues and locations in tissues

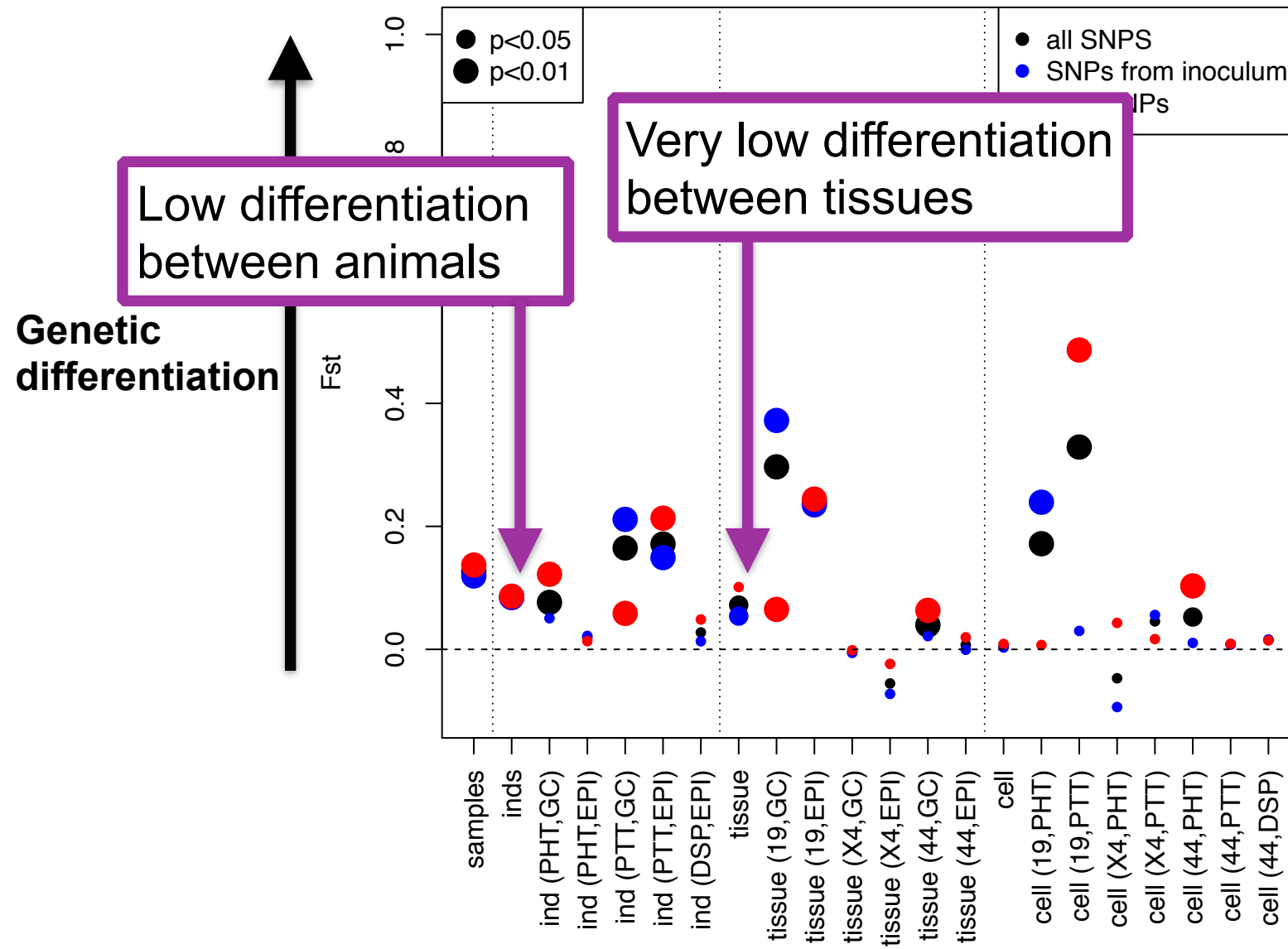
Genetic differentiation



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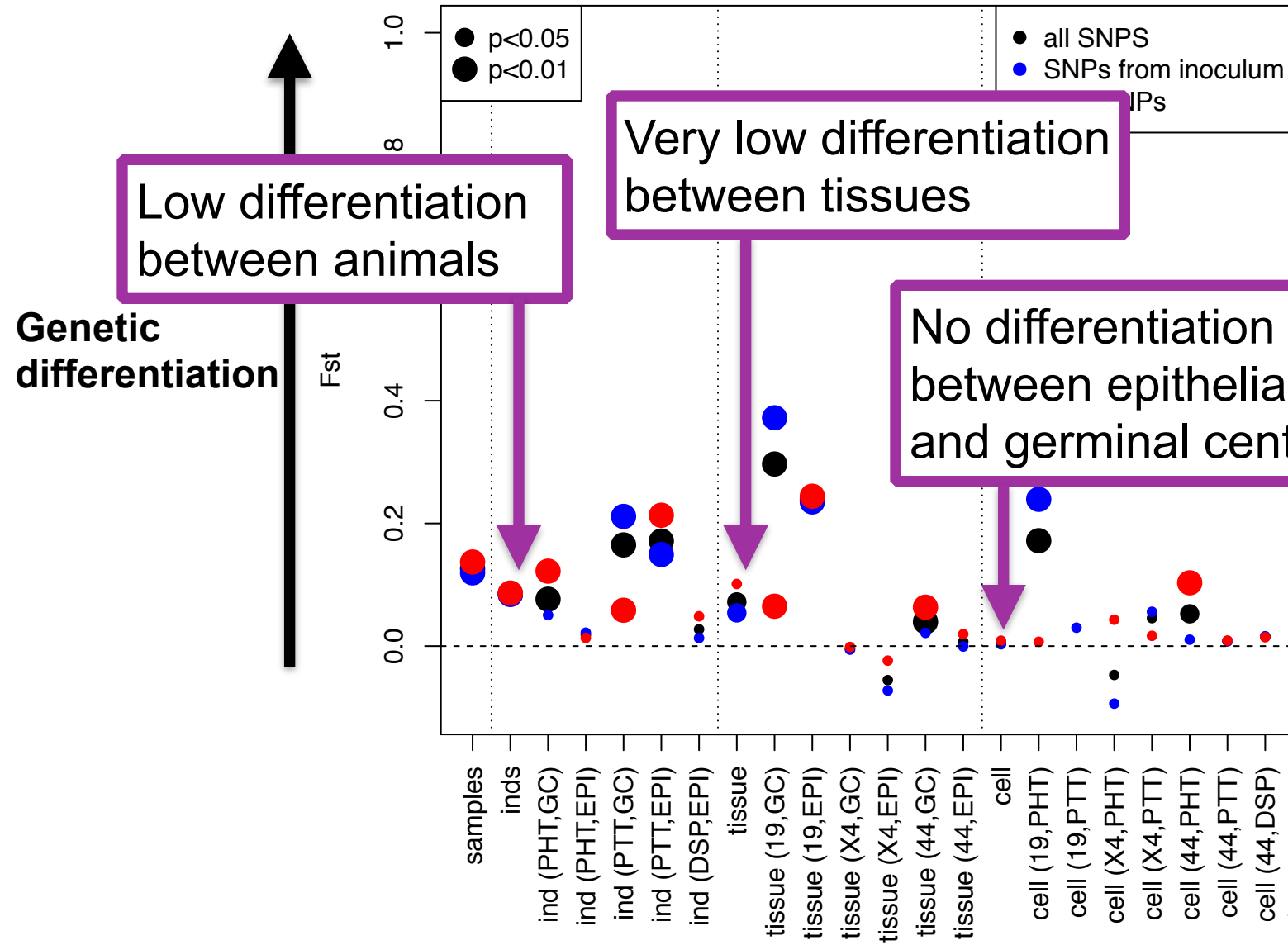


# Genetic differentiation between animals, tissues and locations in tissues





# Genetic differentiation between animals, tissues and locations in tissues



# 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 during acute infection
- Within-host recombination in capsid genes
  - Why not observed in large-scale phylogenies?*
- Replication in carrier state is ~40 times slower than during acute infection
- Similar levels of genetic diversity and low differentiation between animals/tissues
  - Genetic diversity was present or originated during acute infection phase*

# Acknowledgments

## Collaborators:

Eva Pérez-Martín

Fuquan Zhang

Francois Maree

Paolo Ribeca

Bryan Charleston

## The Pirbright Institute:

Nick Knowles

Antonello Di Nardo

