

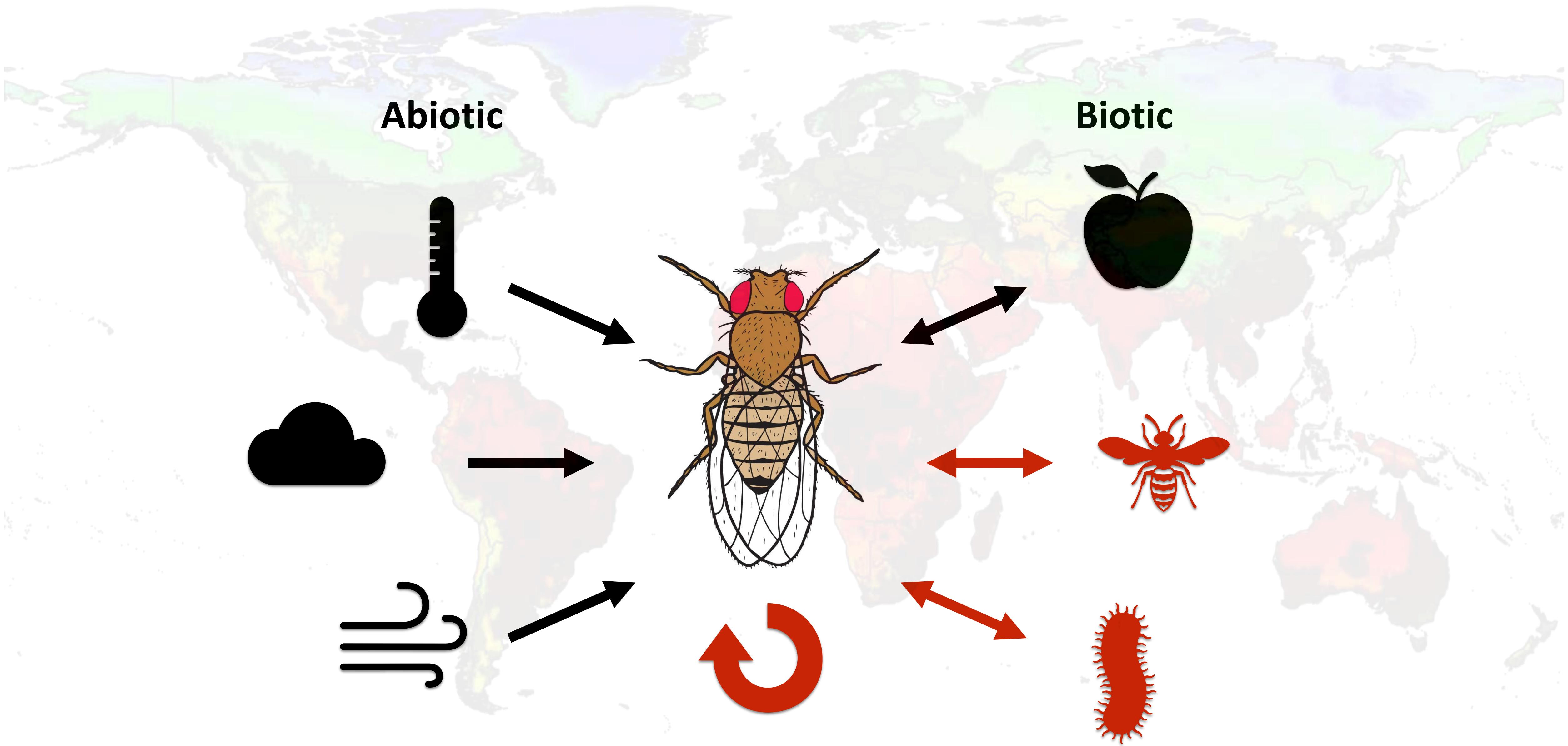
From Museums to Microbes:

A concise introduction to Museomics using *Drosophila melanogaster* and its symbionts as a model



Martin Kapun

Abiotic & Biotic factors trigger complex interactions



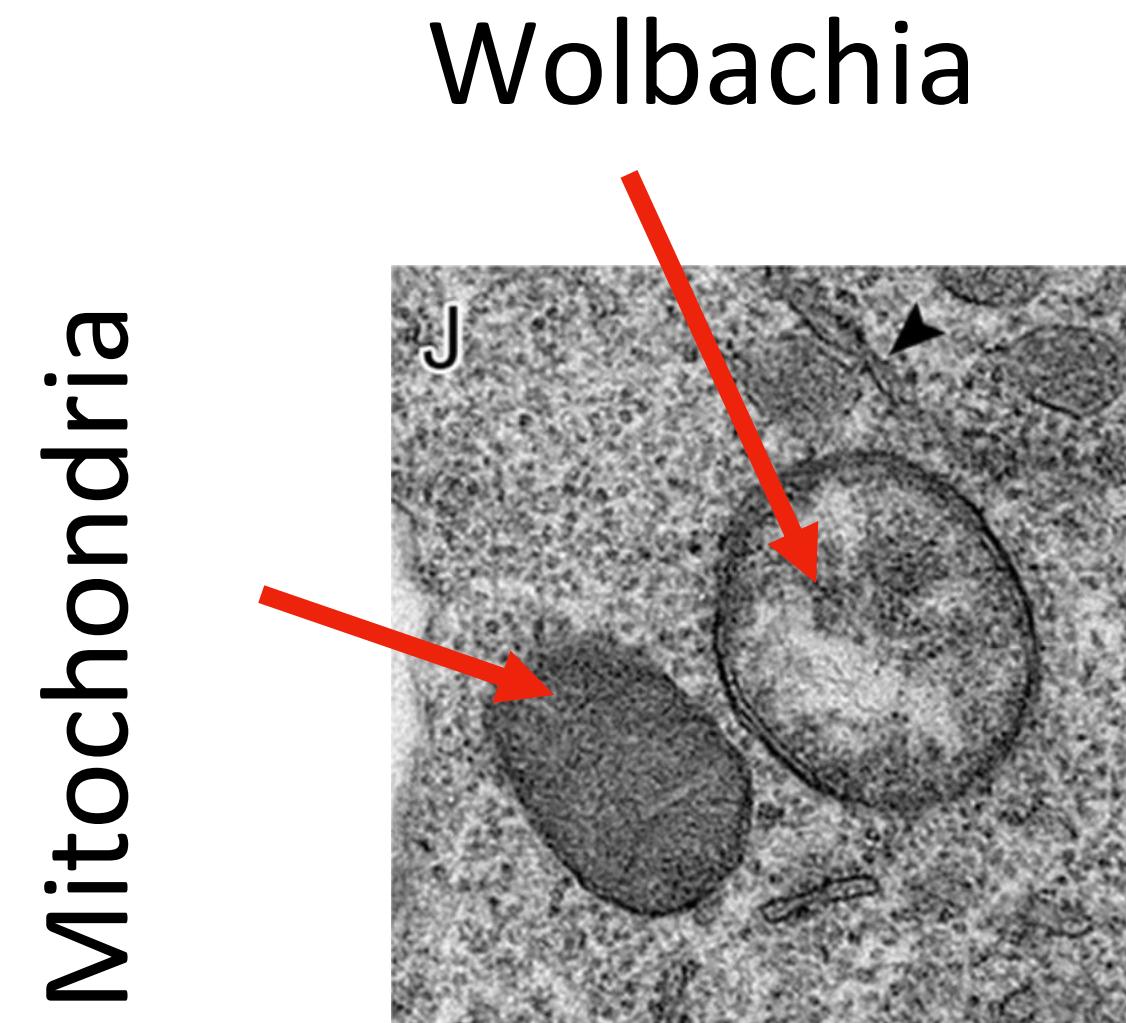
The *Wolbachia-Drosophila* system



Drosophila melanogaster

World-wide distribution as human
commensal

Long history as genetic model



Wolbachia pipiensis

Facultative/obligatory endosymbiont

Manipulates the host reproduction

Drosophila melanogaster



GTOGGCAATCCTAAAGATAGCCAAATTATTATTGTTAGATACTCAC
AGGACAGTCAGATGCGAGTGTGAAATCAGTGAATT
FB2024_02, released April 23, 2024

FlyBase

Tools ▾ Downloads ▾ Links ▾

Overview
Current Release
Archived Data
Map Conversion
FTP Site:
Releases (FTP)
Genomes (FTP)

Citing FlyBase

External Resources

- News and Outreach
- Drosophila Journals and Protocols
- Tool/Reagent Resources

Help FlyBase's significant
Questions? See

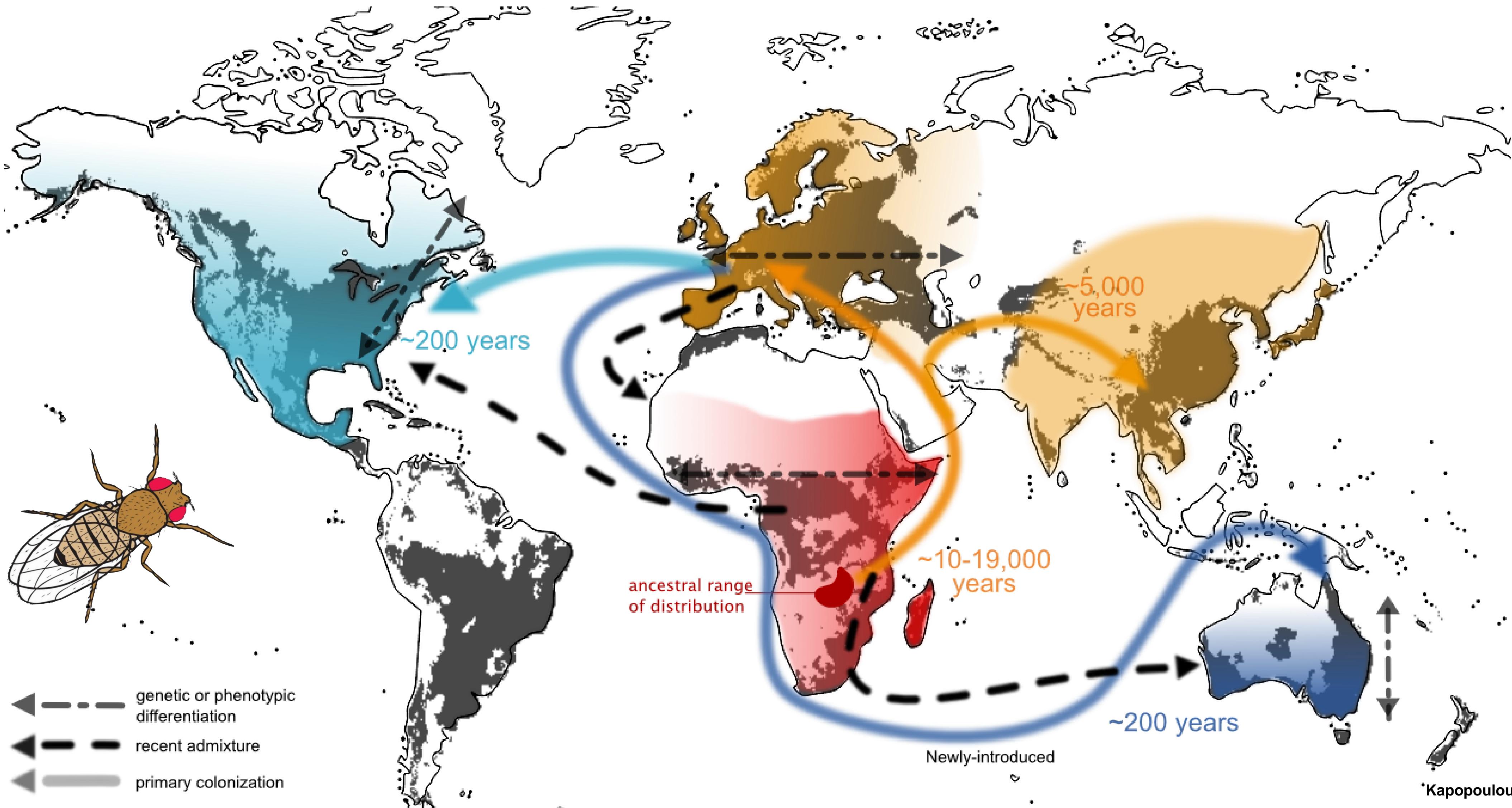
QuickSearch

Human Disease Protein Domains

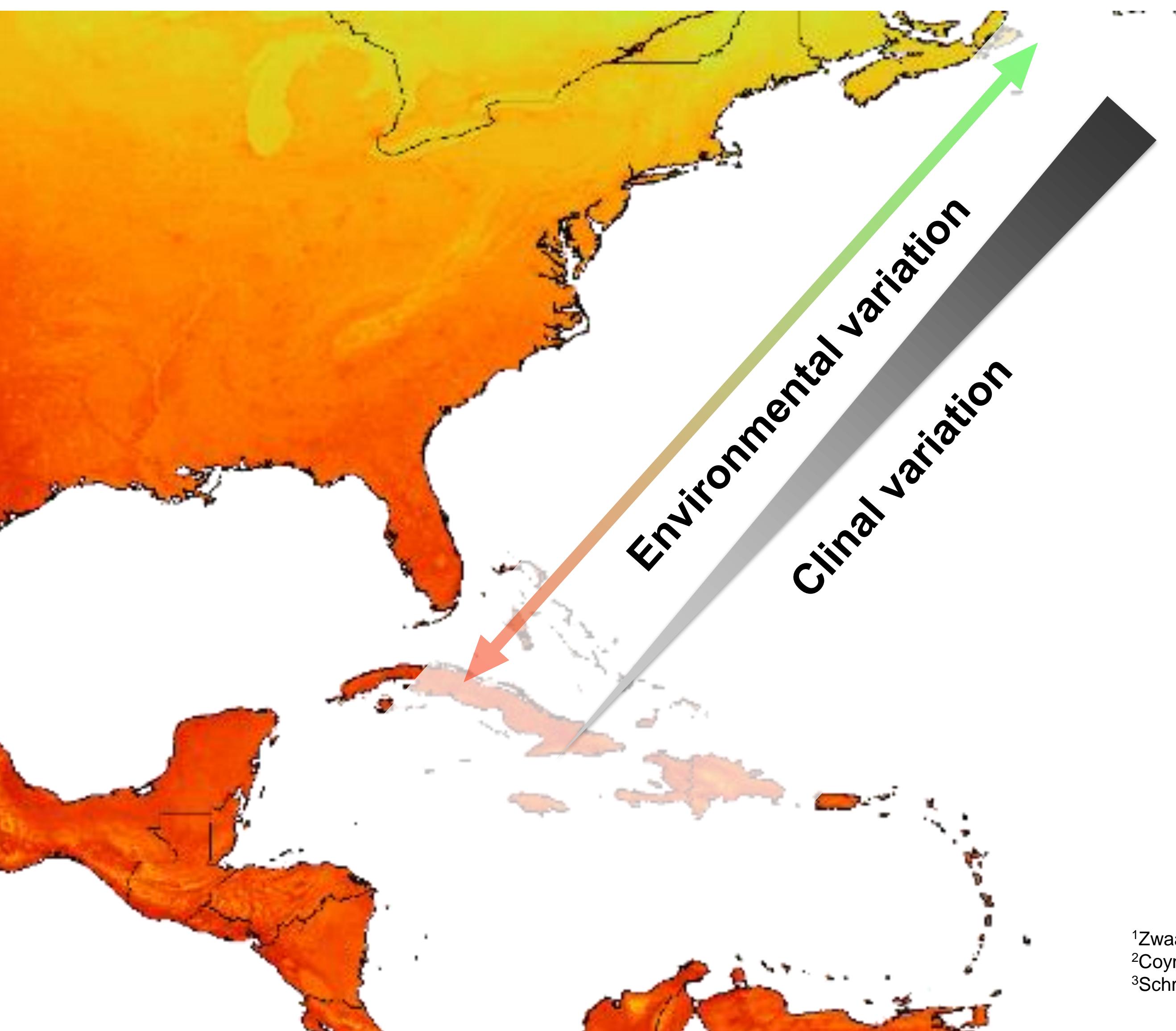
Search FlyBase Homologs GAL4

The FlyBase website interface is shown, featuring a header with a fly illustration and release information, followed by a main content area with links to tools like BLAST and JBrowse, and sections for external resources and quick search.

The world-wide distribution of *D. melanogaster*



Clinal variation along environmental gradients



Cinal variation

Phenotypes¹⁻⁴

Bodysize
Development time
Chill coma recovery
Immunity

Genotypes⁵⁻¹¹

Allozymes
microsatellites
SNPs
Inversions

¹Zwaan et al. (2000) *Heredity*
²Coyne et al. (1987) *Genetics*
³Schmidt et al. (2008) *Evolution*

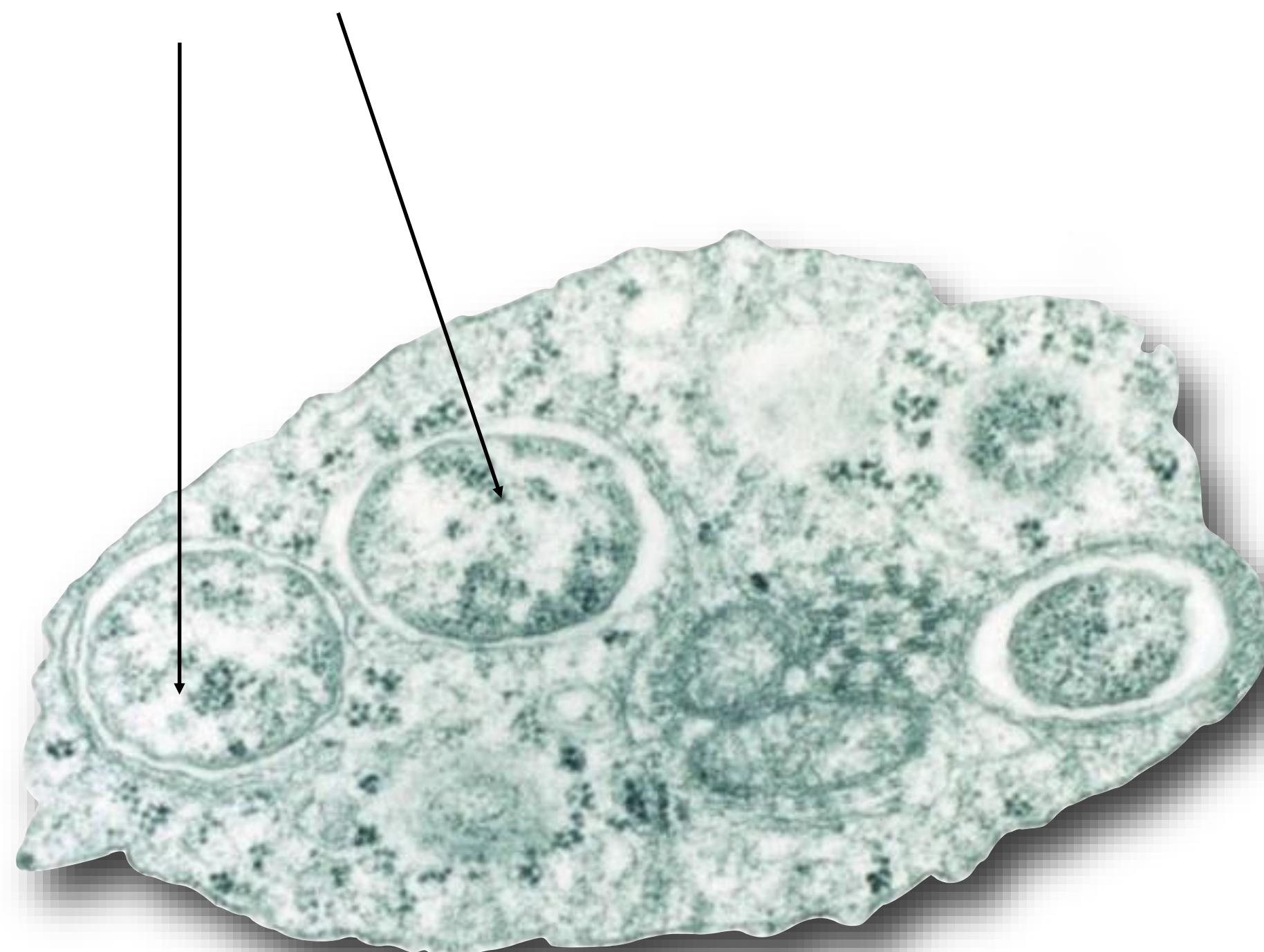
⁴Behrman et al. (2018) *Proc B*
⁵Fabian et al. (2012) *Mol Ecol*
⁶Caracristi et al. (2003) *MBE*

⁷Mettler et al. (1977) *Genetics*
⁸Sezgin et al. (1999) *Genetics*
⁹Kapun et al. (2011) *Mol Ecol*

¹⁰Kapun et al. (2016) *MBE*
¹¹Kapun et al. (2016) *JEB*

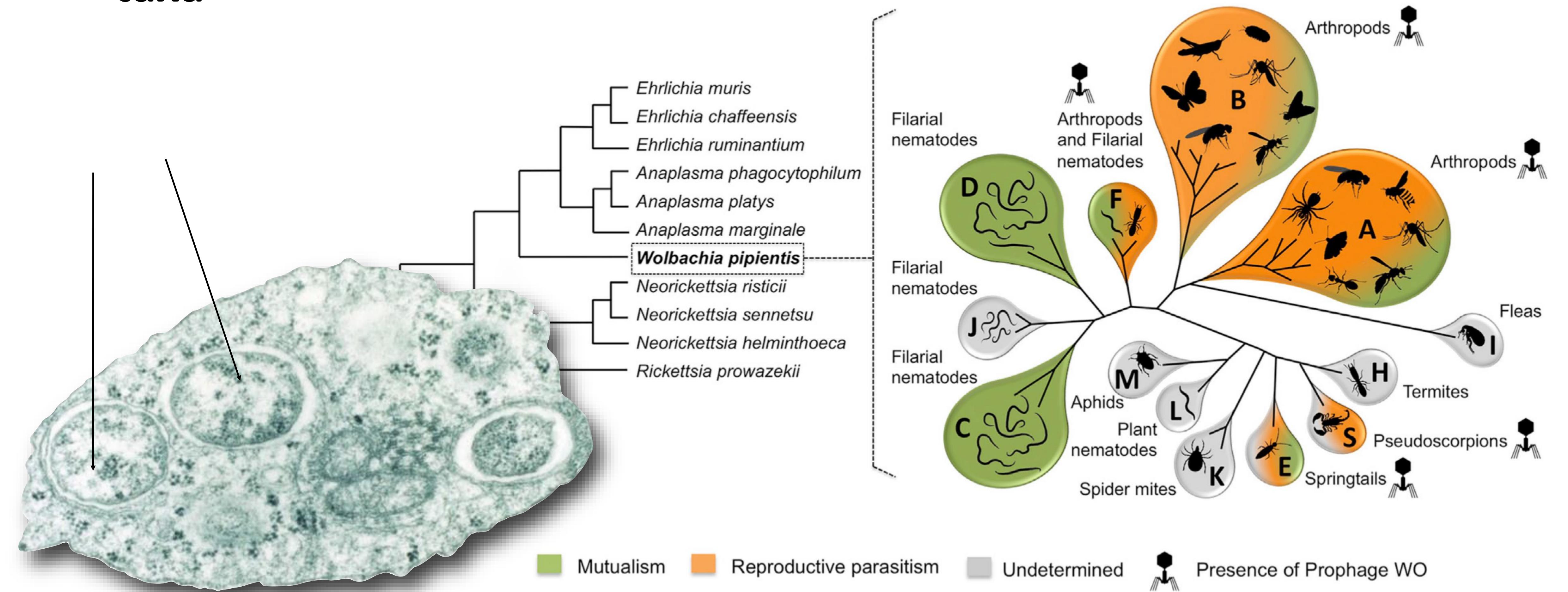
Wolbachia pipiens

- gram-negative bacterial endosymbiont in 40% of all arthropod taxa



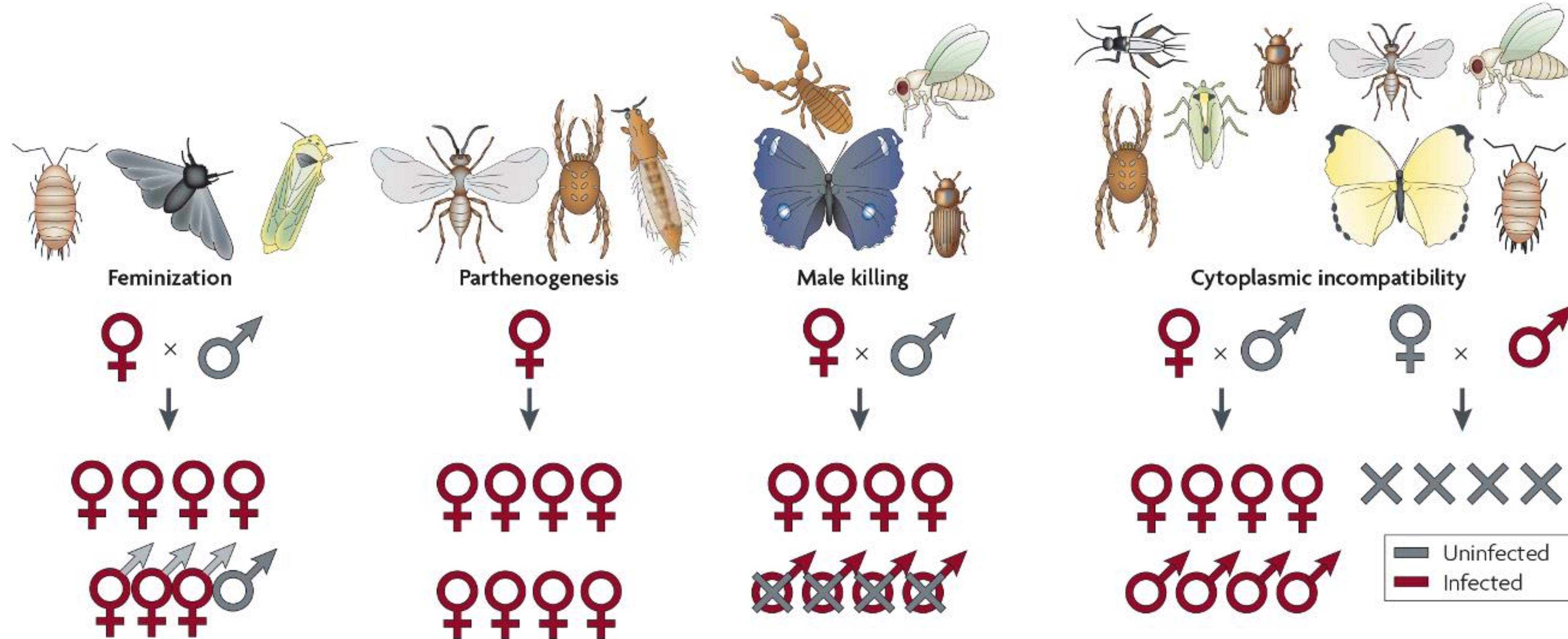
Wolbachia pipiens

- gram-negative bacterial endosymbiont in 40% of all arthropod taxa



Wolbachia pipiens

- Reproductive parasite

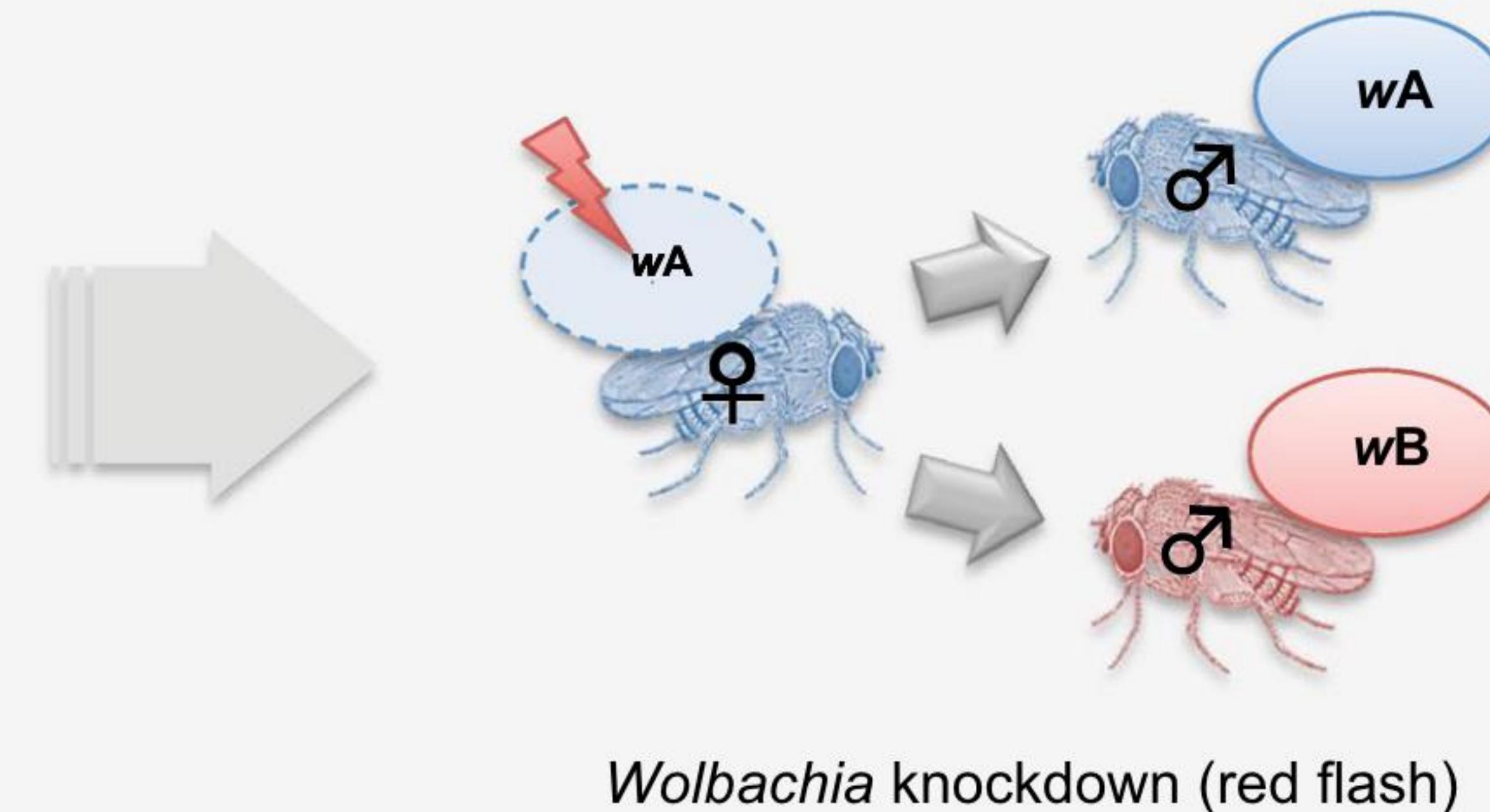
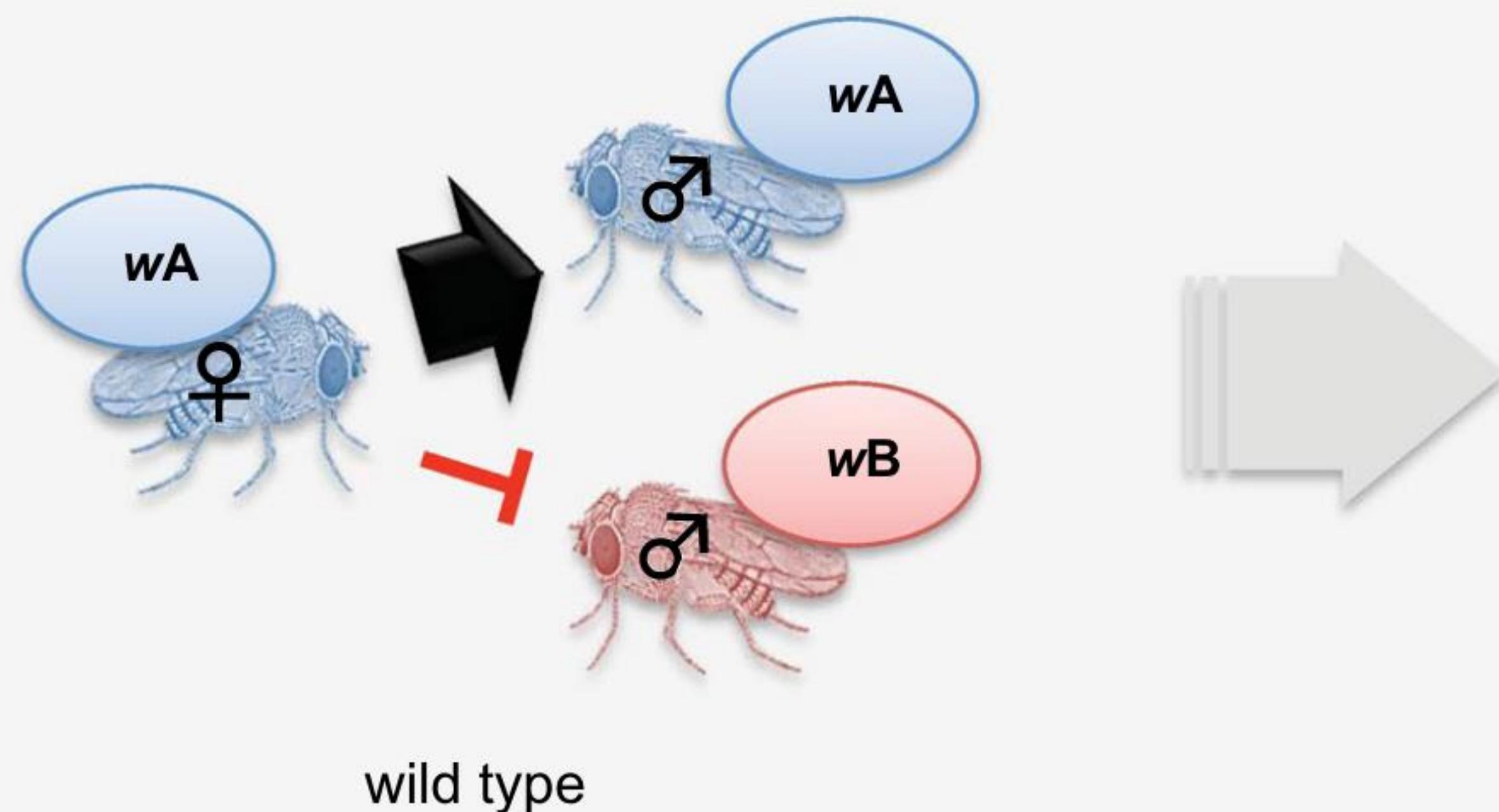


Wolbachia pipiens

- Barriers to gene flow in the host

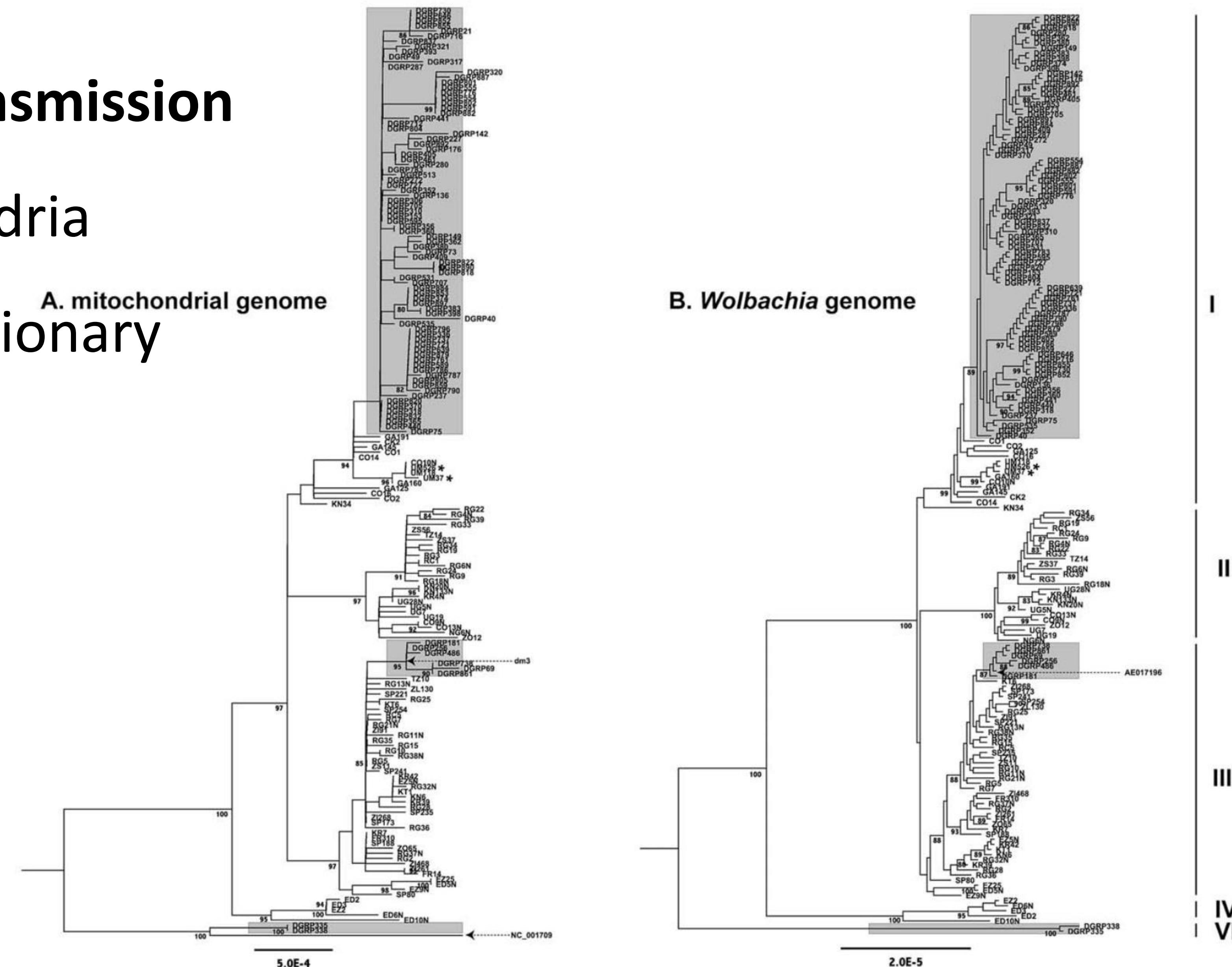


- a Mate choice in *D. paulistorum*: *Wolbachia*-knockdown females lose assortative mating behavior against heterogametic wild type males (Miller et al. 2010)



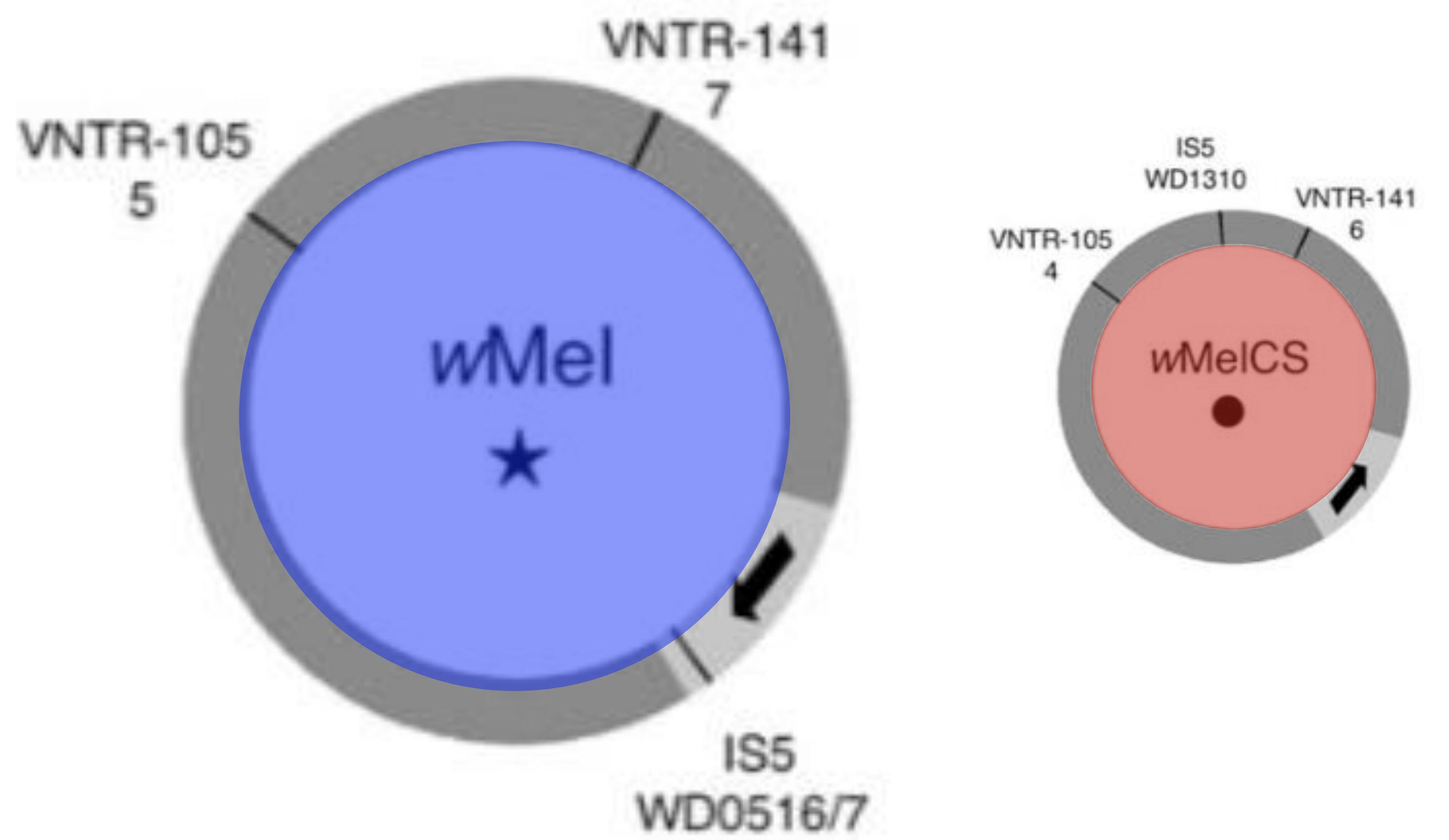
Wolbachia pipiens

- vertical maternal transmission
 - Similar to mitochondria
 - Often shared evolutionary history

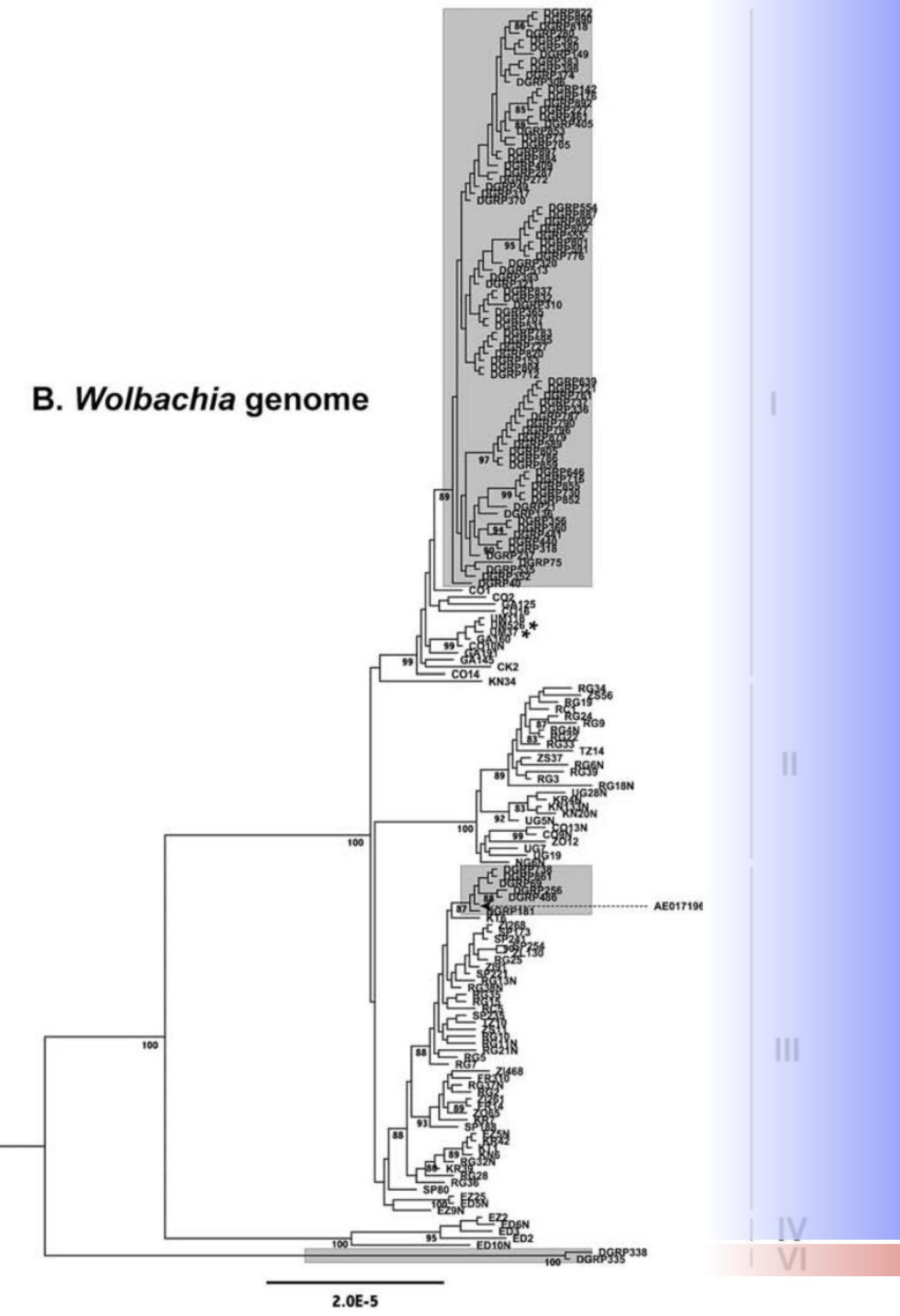


Wolbachia pipiens in *D. melanogaster*

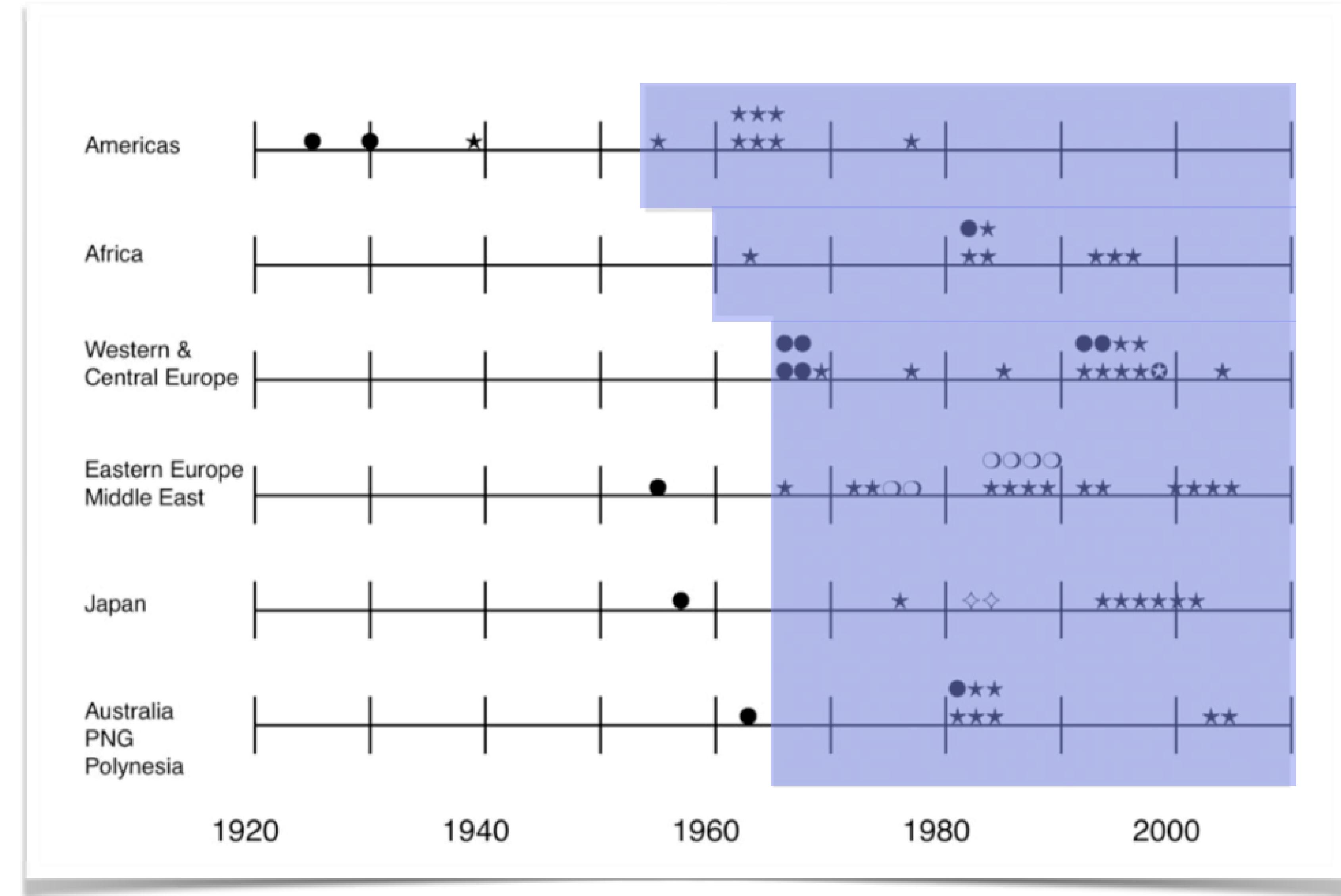
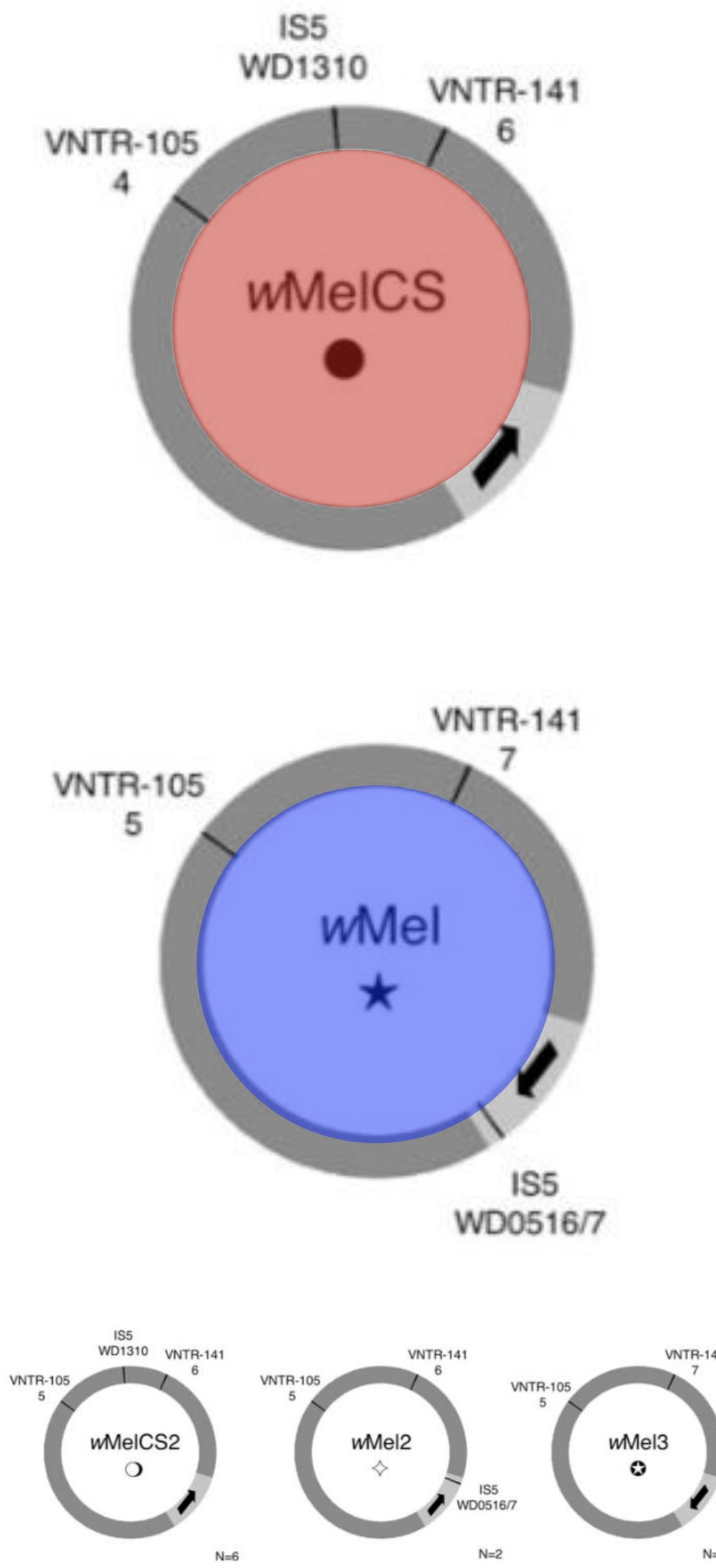
- Two main types



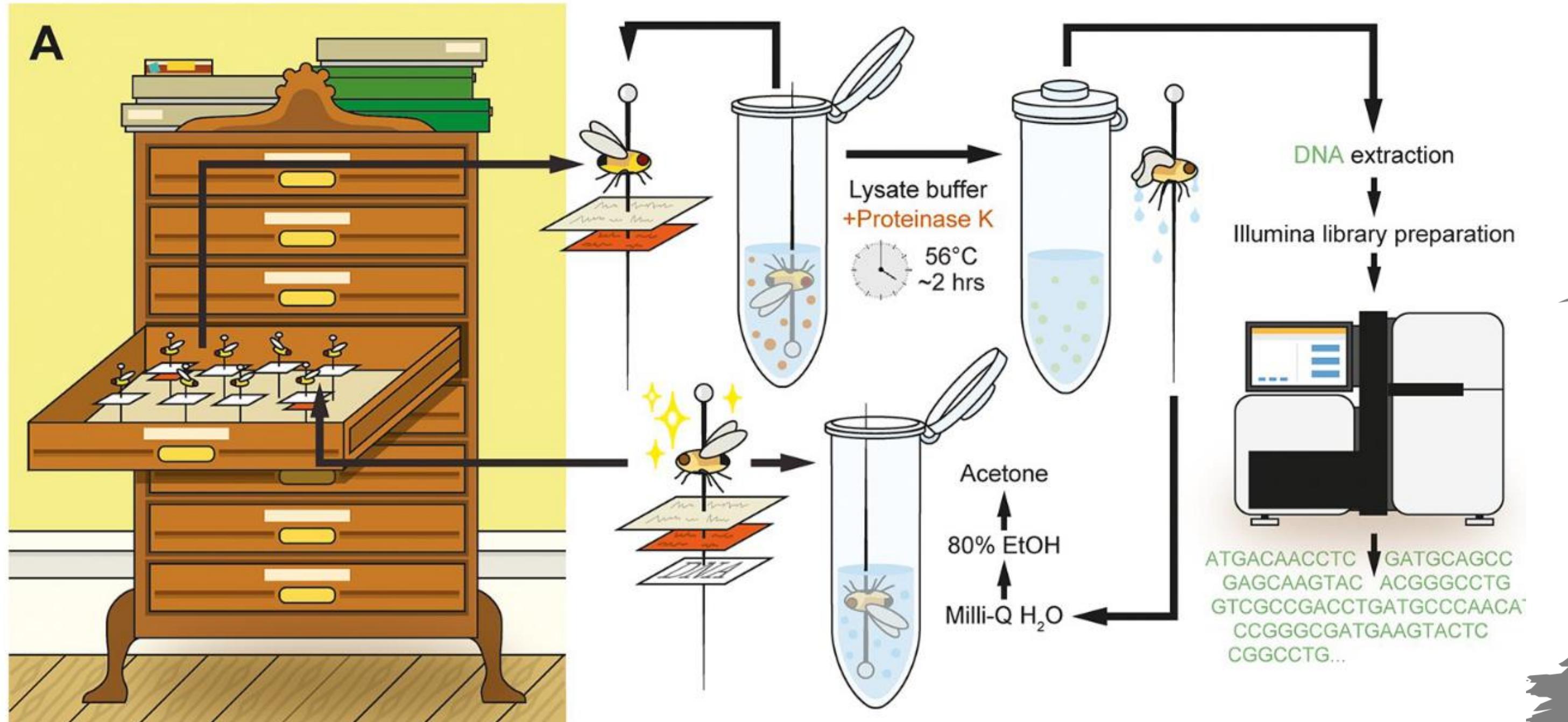
B. *Wolbachia* genome



Global *Wolbachia* replacement?



Museomics can help!

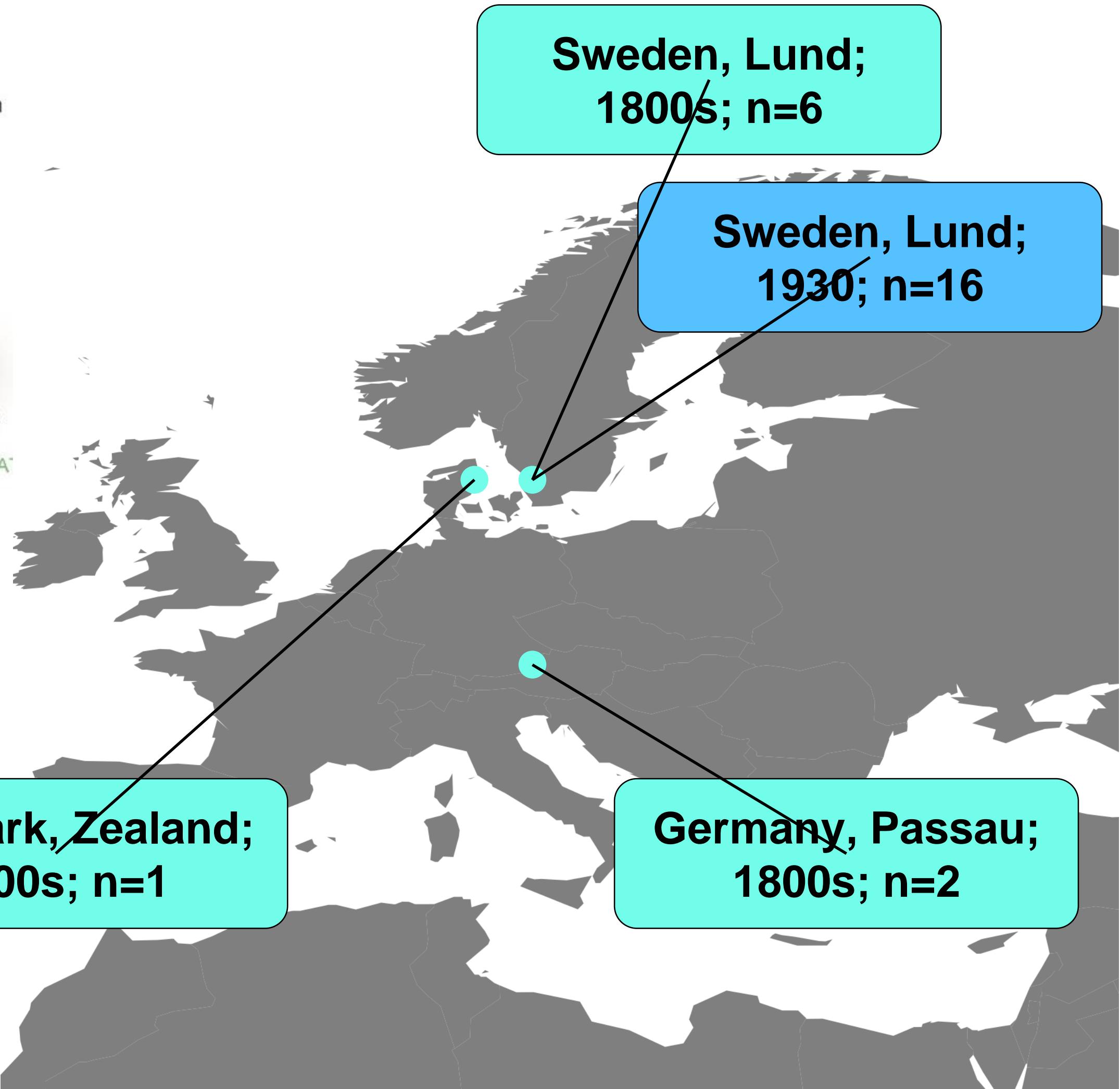


Denmark, Zealand;
1800s; n=1

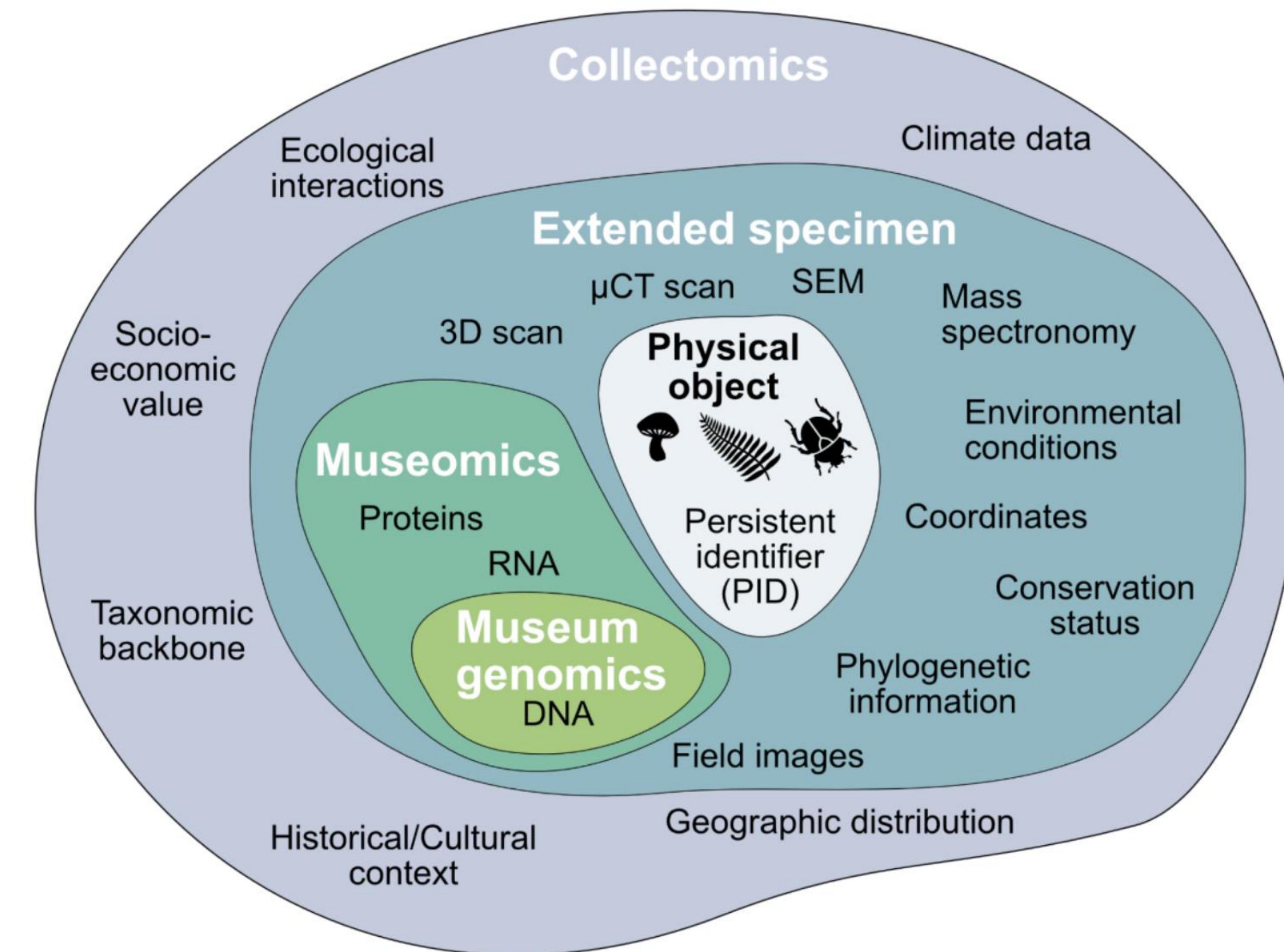
Sweden, Lund;
1800s; n=6

Sweden, Lund;
1930; n=16

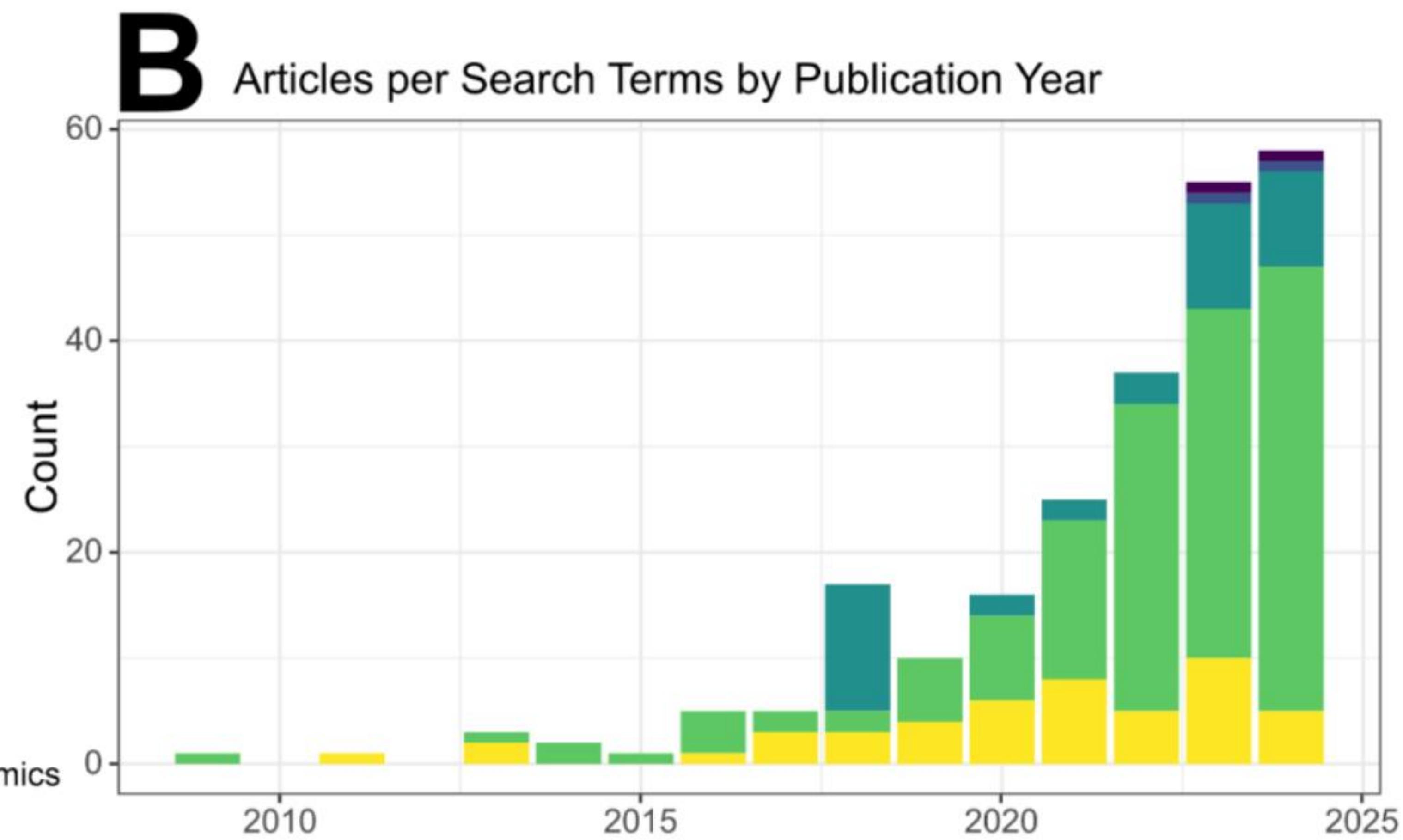
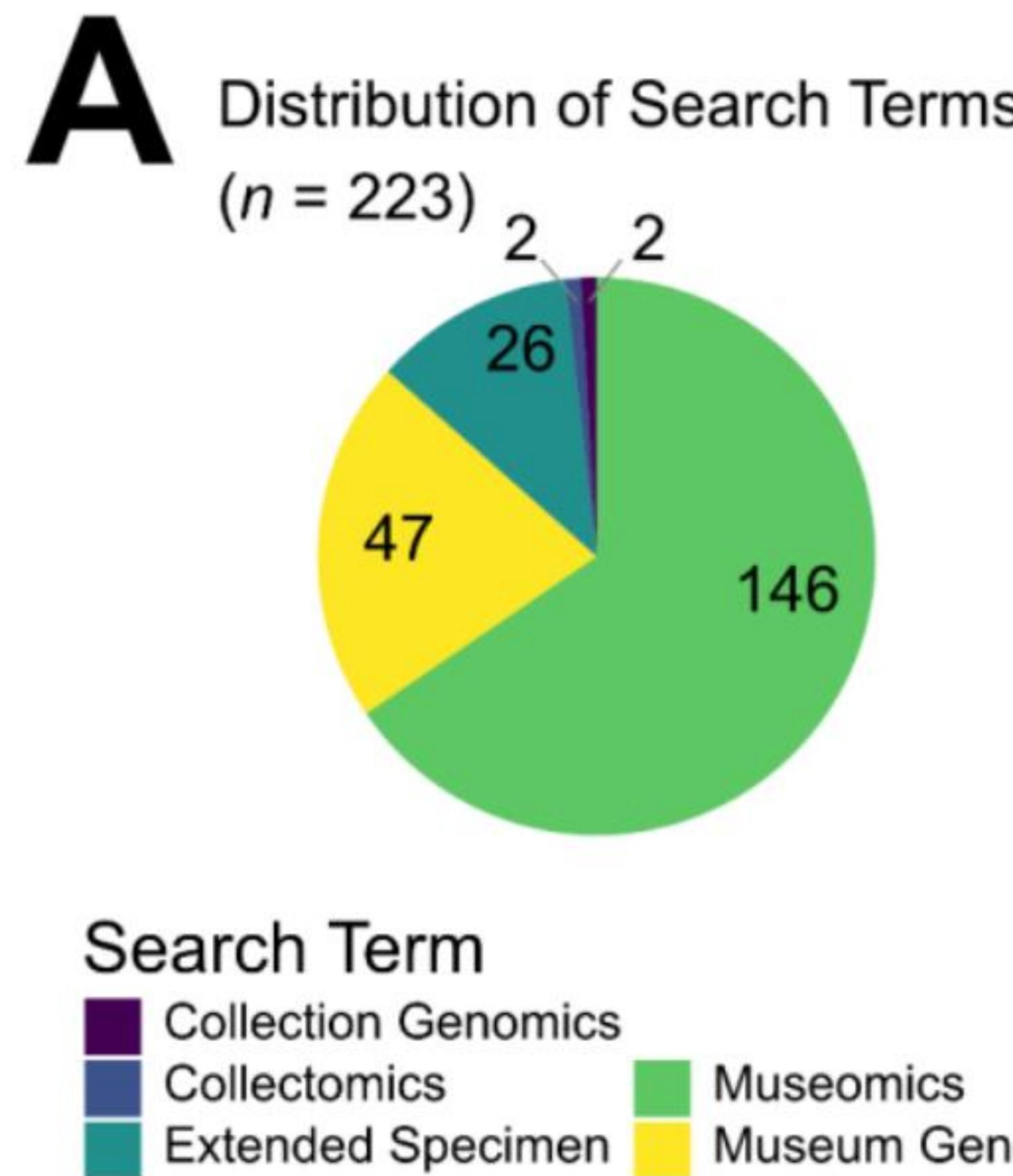
Germany, Passau;
1800s; n=2



But what is Museomics?

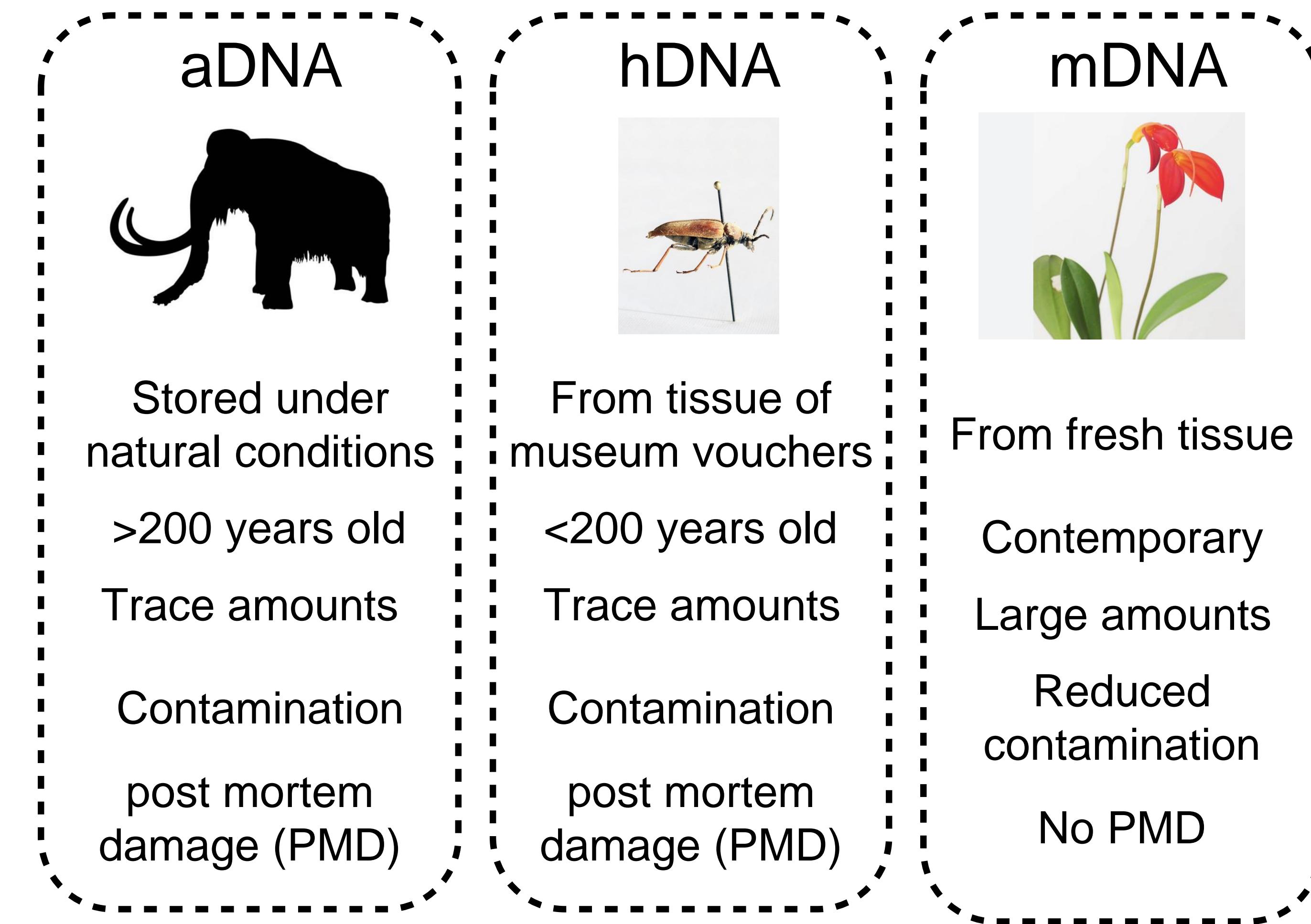


But what is Museomics?



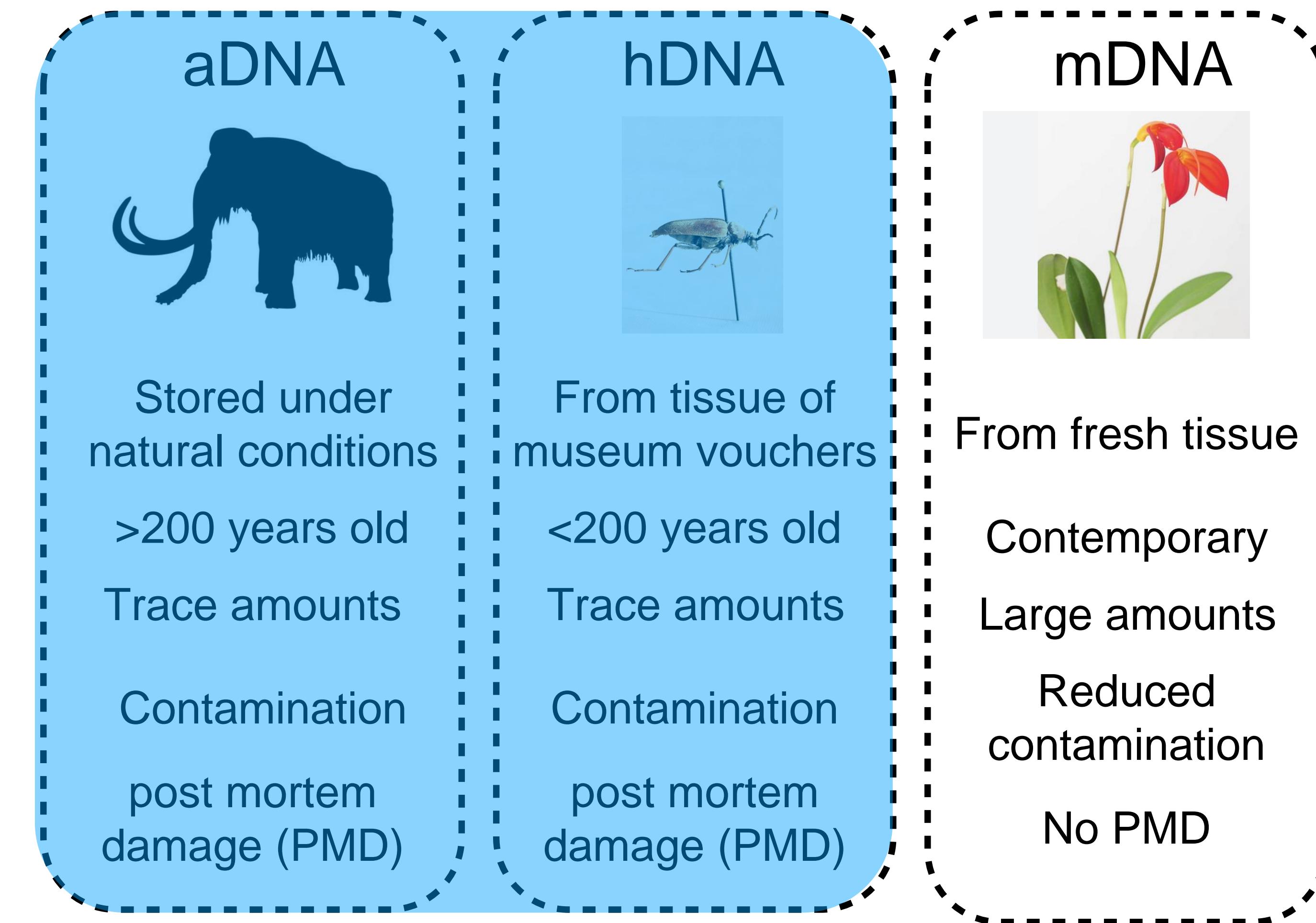
But what is Museomics?

“Archival DNA”

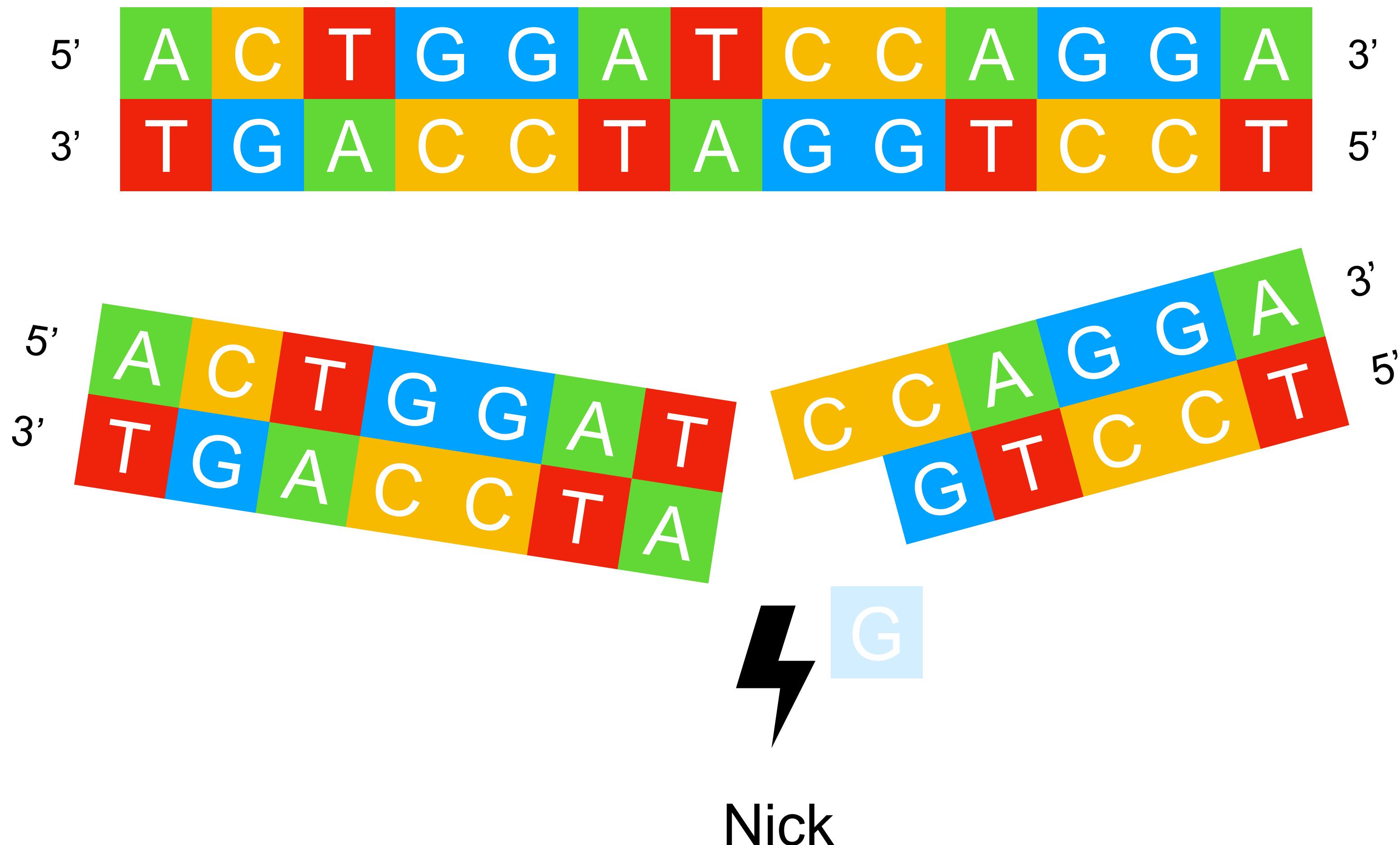


But what is Museomics?

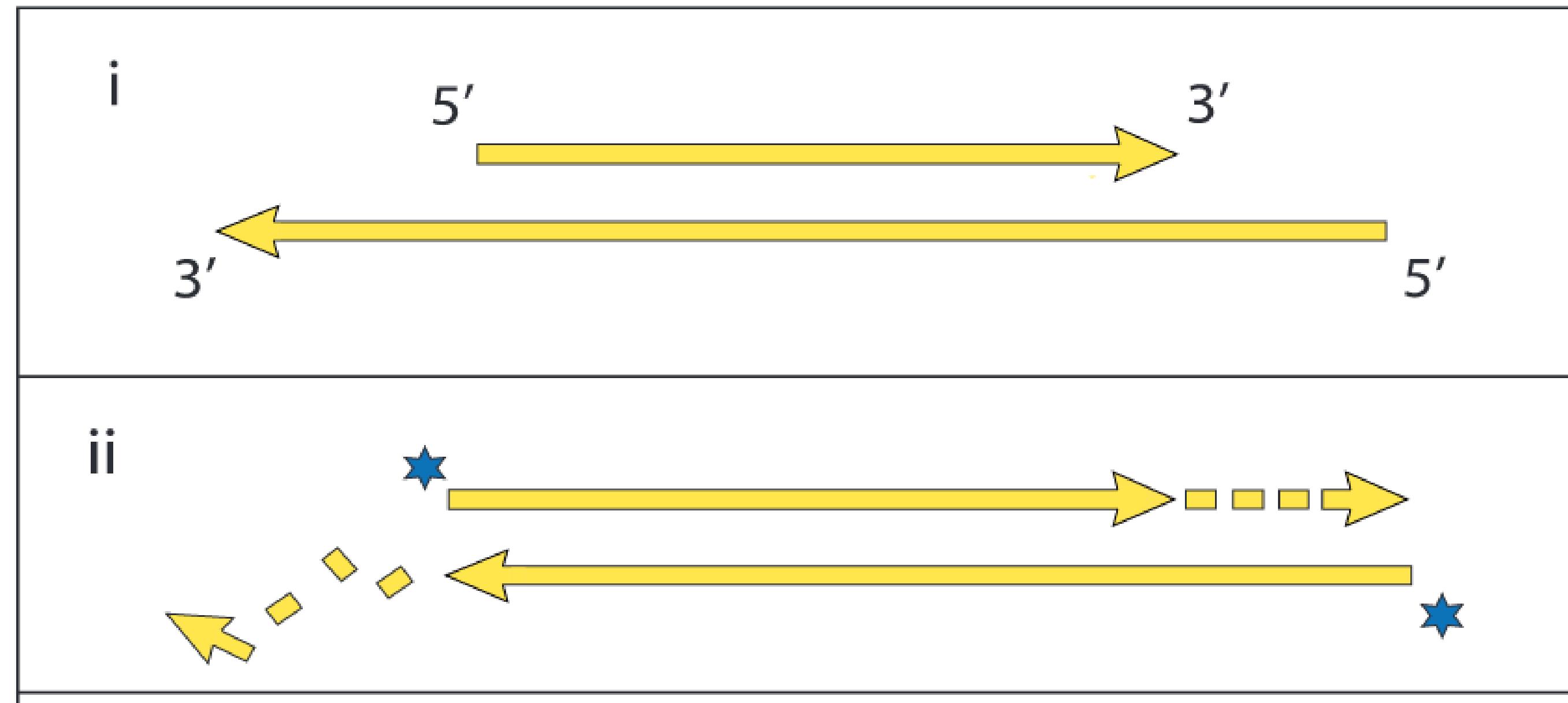
“Archival DNA”



Properties of “old” DNA!



Properties of “old” DNA!



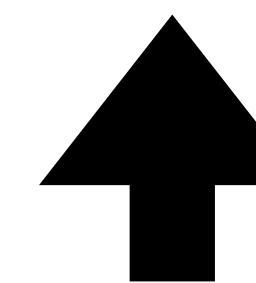
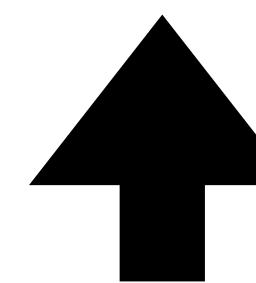
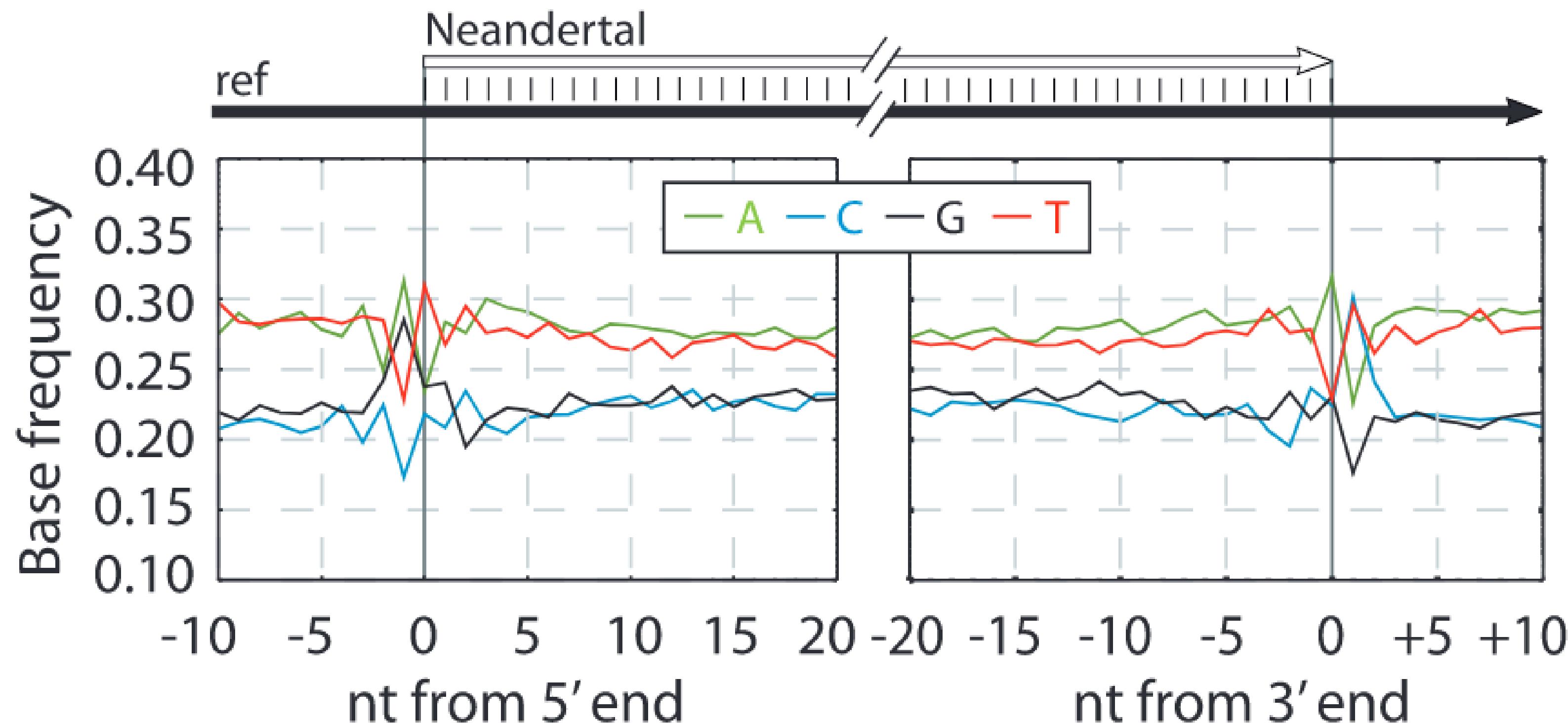
Depurination

- Loss of G or A
- Can result in nicking
- Can result in DNA fragmentation with single-strand overhang

How to detect?

- End-repair during library prep with T4 DNA polymerase
- 5' overhang filled (polymerase activity)
- 3' overhang removed (exonuclease activity)

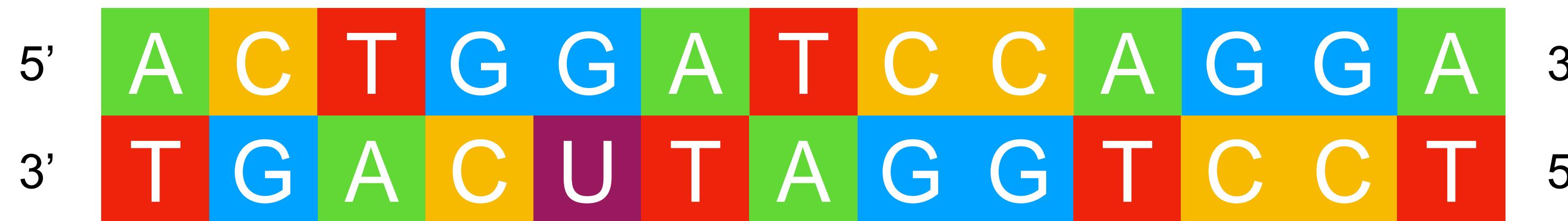
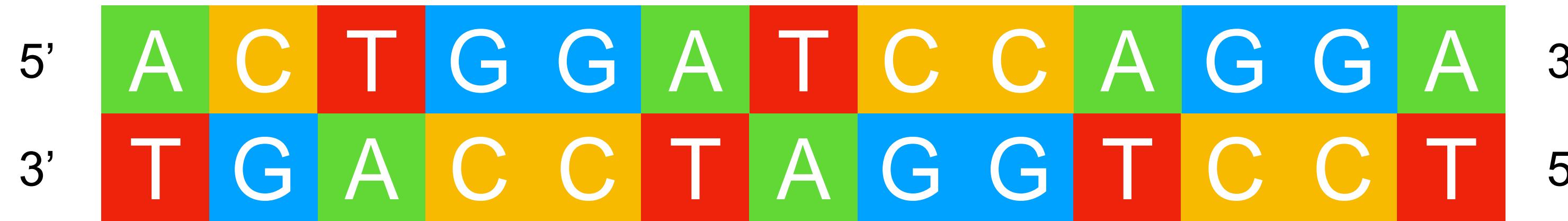
Properties of “old” DNA!



Depurination

- Loss of G or A
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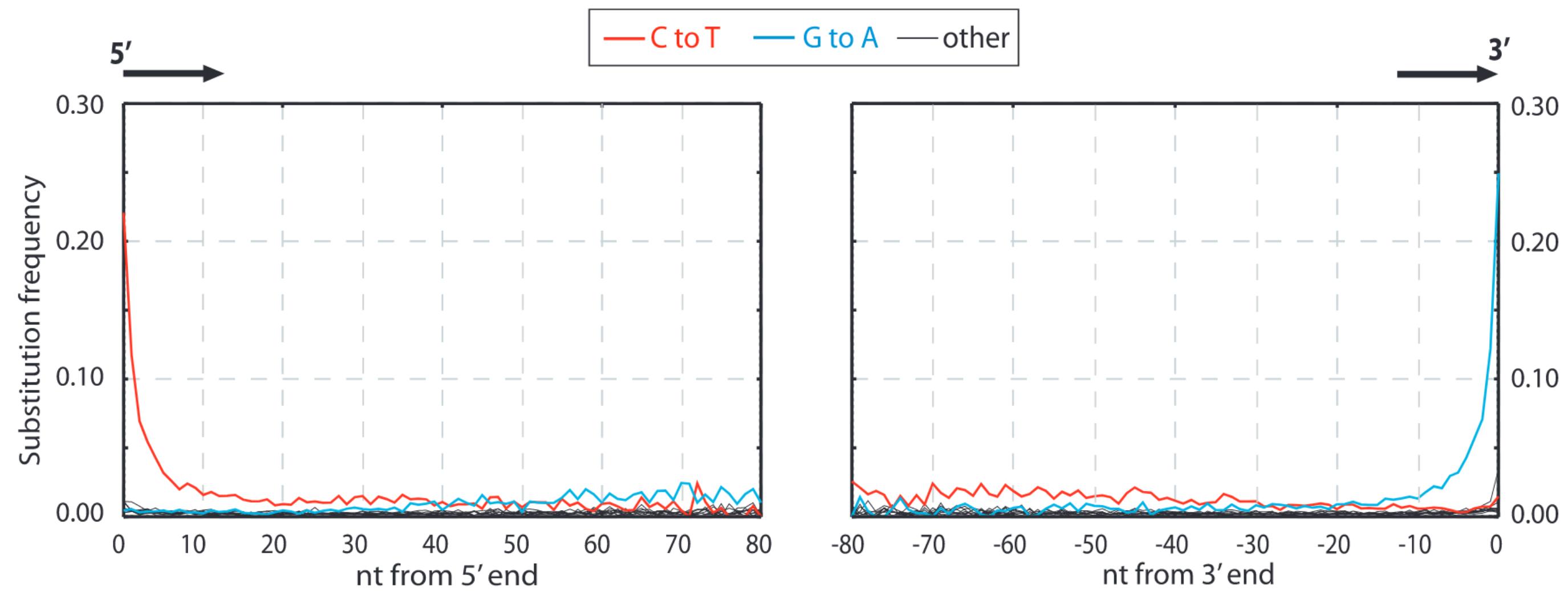
Properties of “old” DNA!



Deamination

- Degradation of C->U
- Uracil recognised as T during PCR and sequencing
- Most frequent in single-stranded DNA

Properties of “old” DNA!



Deamination

- Degradation of C->U
- Uracil recognised as T during PCR and sequencing
- Most frequent in single-stranded DNA

Properties of “old” DNA!

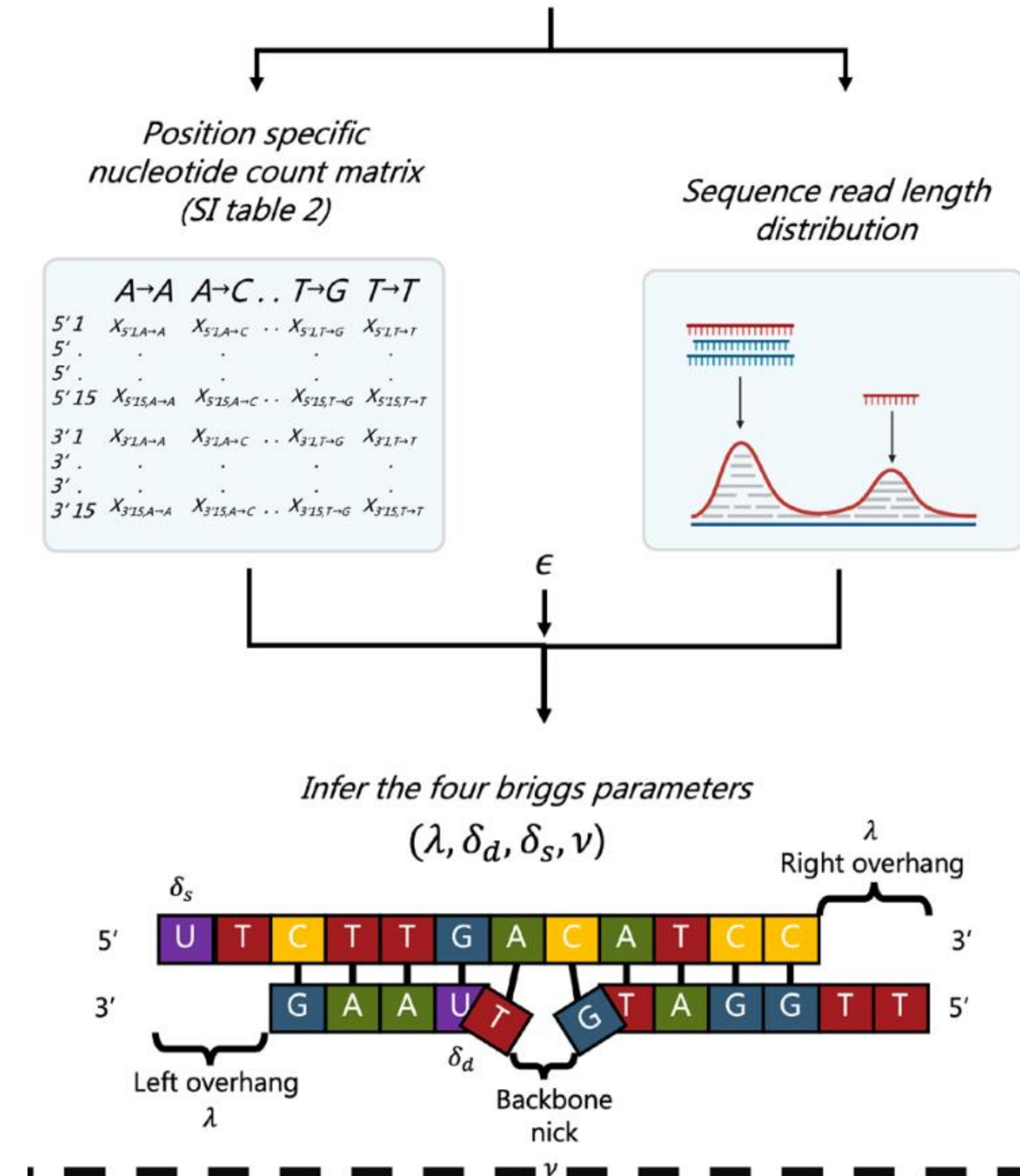
“Briggs”-Parameters

λ : The average length of overhangs

δ_d : Deamination rates in the double-stranded region.

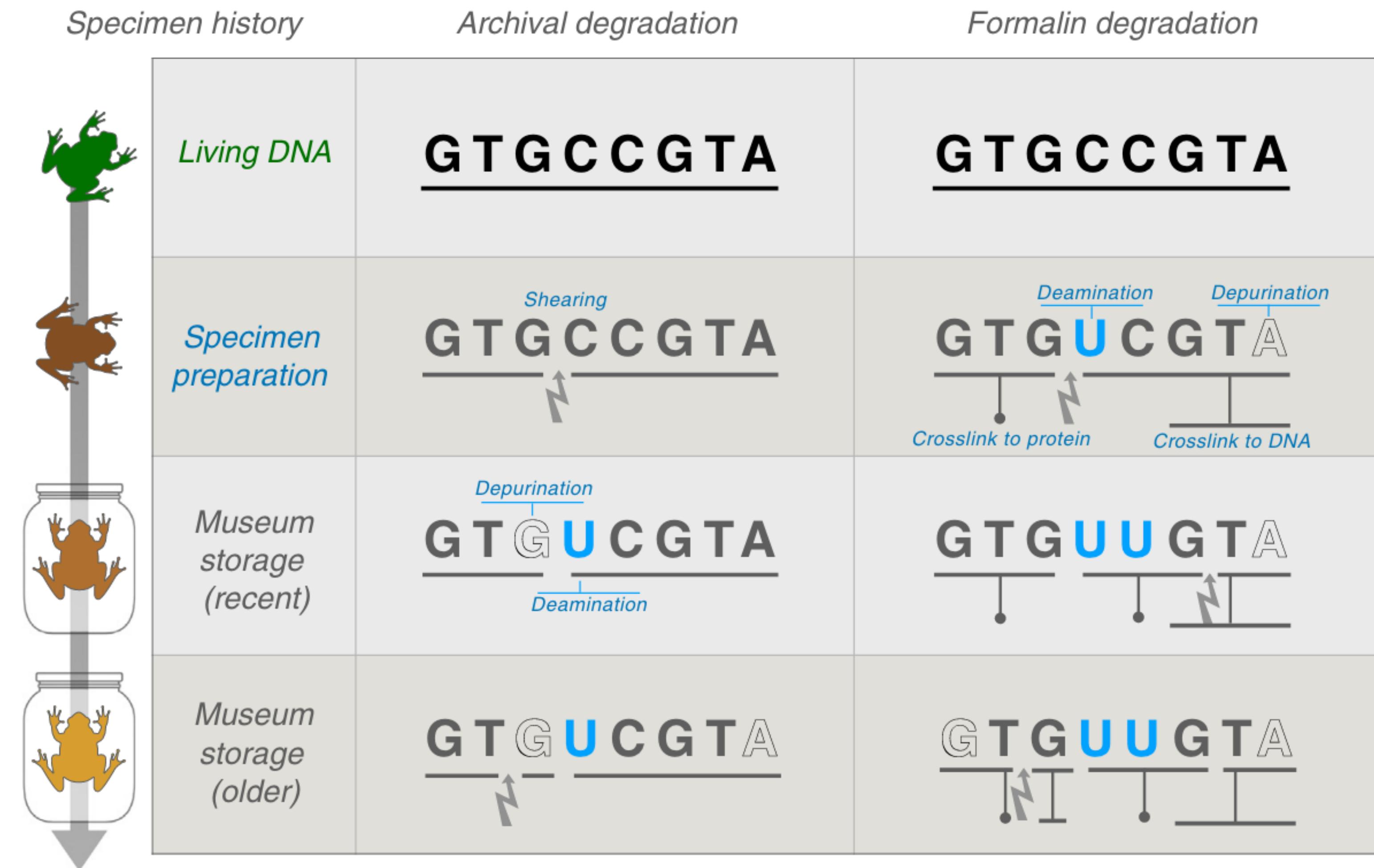
δ_s : Deamination level in the single-stranded (overhang) region.

ν : Nick frequency.



Properties of “old” DNA!

- Quality criteria
 - Fragment sizes
 - Reference Coverage
 - Read depth
 - Deamination C -> T
 - Depurination G -> “”



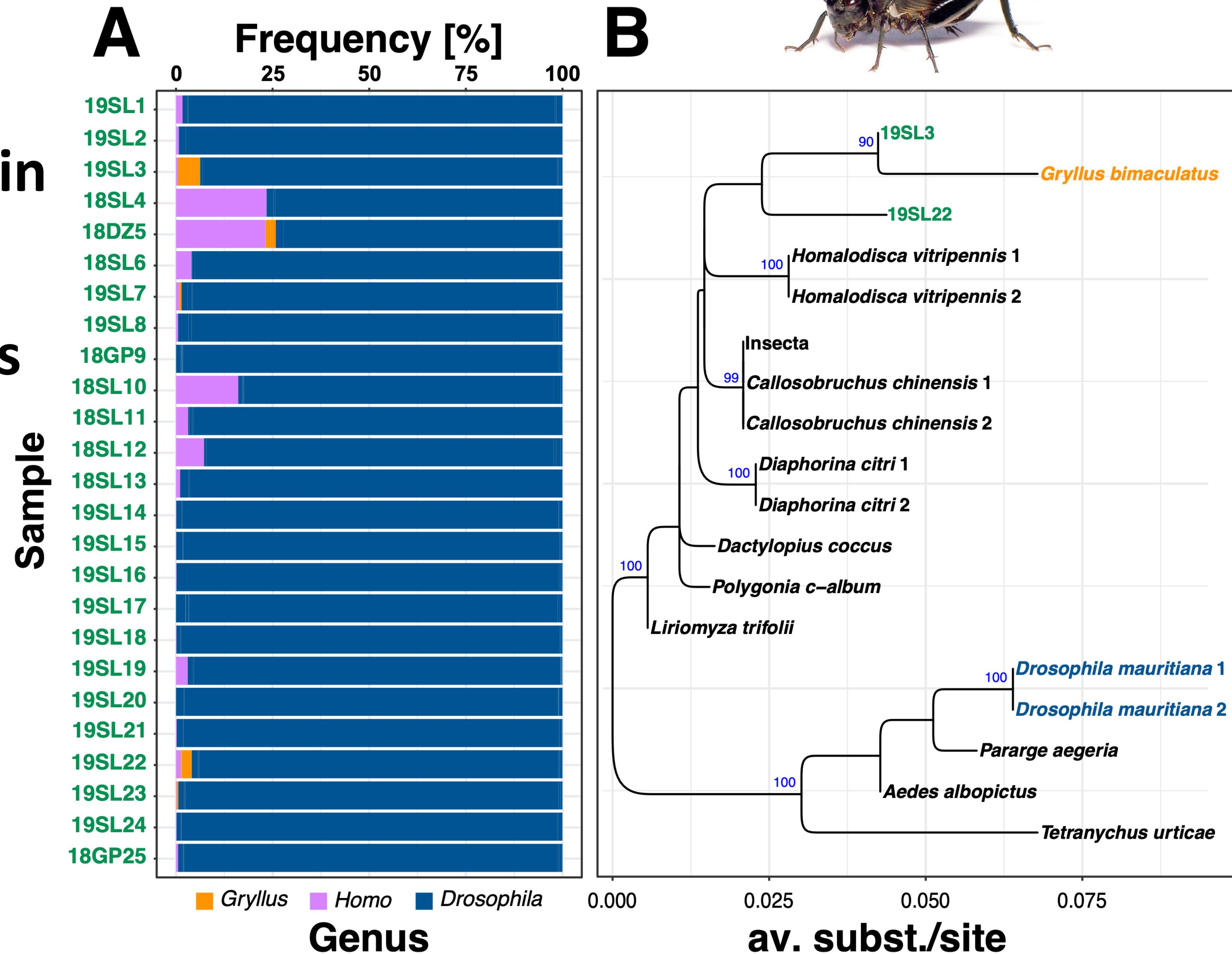
Let's do some analyses

The screenshot shows a web browser window with a tab titled "nhmvienna/Workshop_VI_Museomics". The URL in the address bar is "github.com/nhmvienna/Workshop_VI_Museomics?tab=readme-ov-file". The page displays the repository details for "Workshop_VI_Museomics", which is public and forked from "capoony/MuseomicsWorkshop2025". The repository has 1 branch and 0 tags. The "Code" tab is selected. The "About" section indicates no description, website, or topics are provided. Contributors listed are "capoony" and "bf35304". The repository has 24 commits.

https://github.com/nhmvienna/Workshop_VI_Museomics

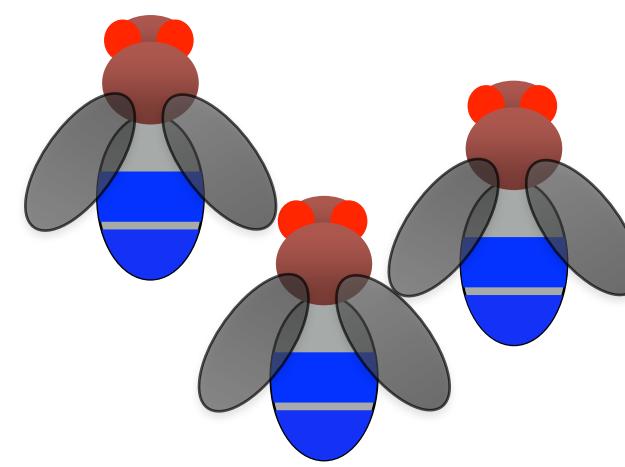
Contamination revisited!

- Consensus sequence of *Wolbachia*-surface protein (*wsp*)
- Comparison to sequences from Genbank with ML tree
- “Hyper”-contamination with endosymbiont of contaminant!



Which *Wolbachia*?

wMel



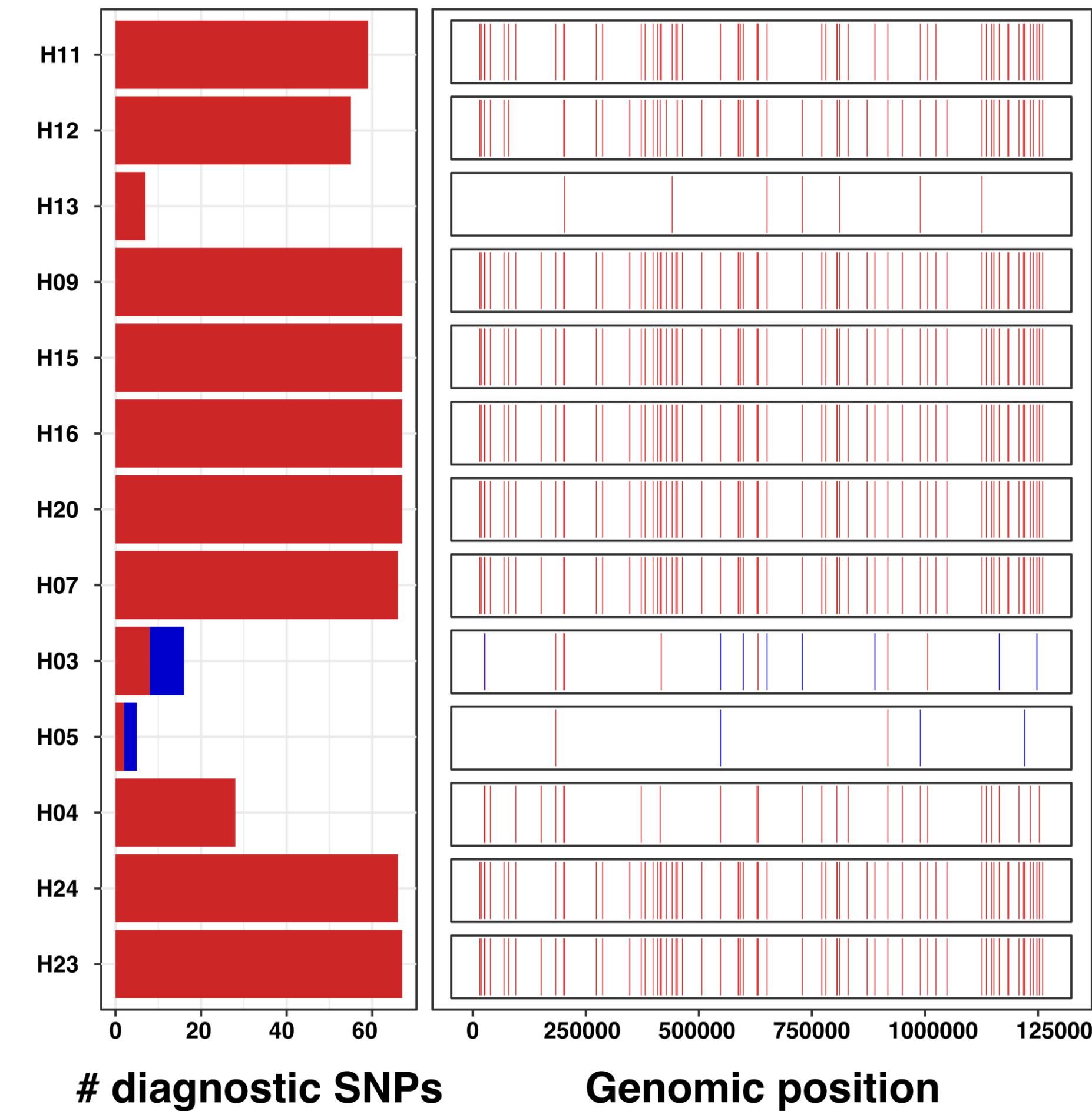
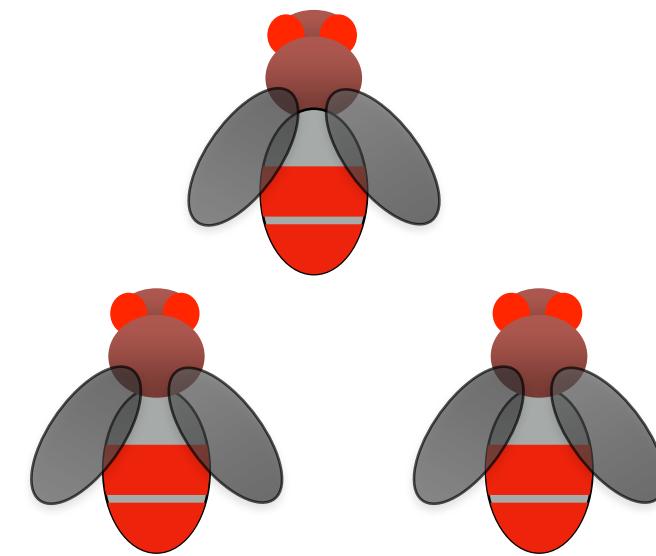
SNP1 A T

SNP2 G T

SNP3 C G

SNP4 A T

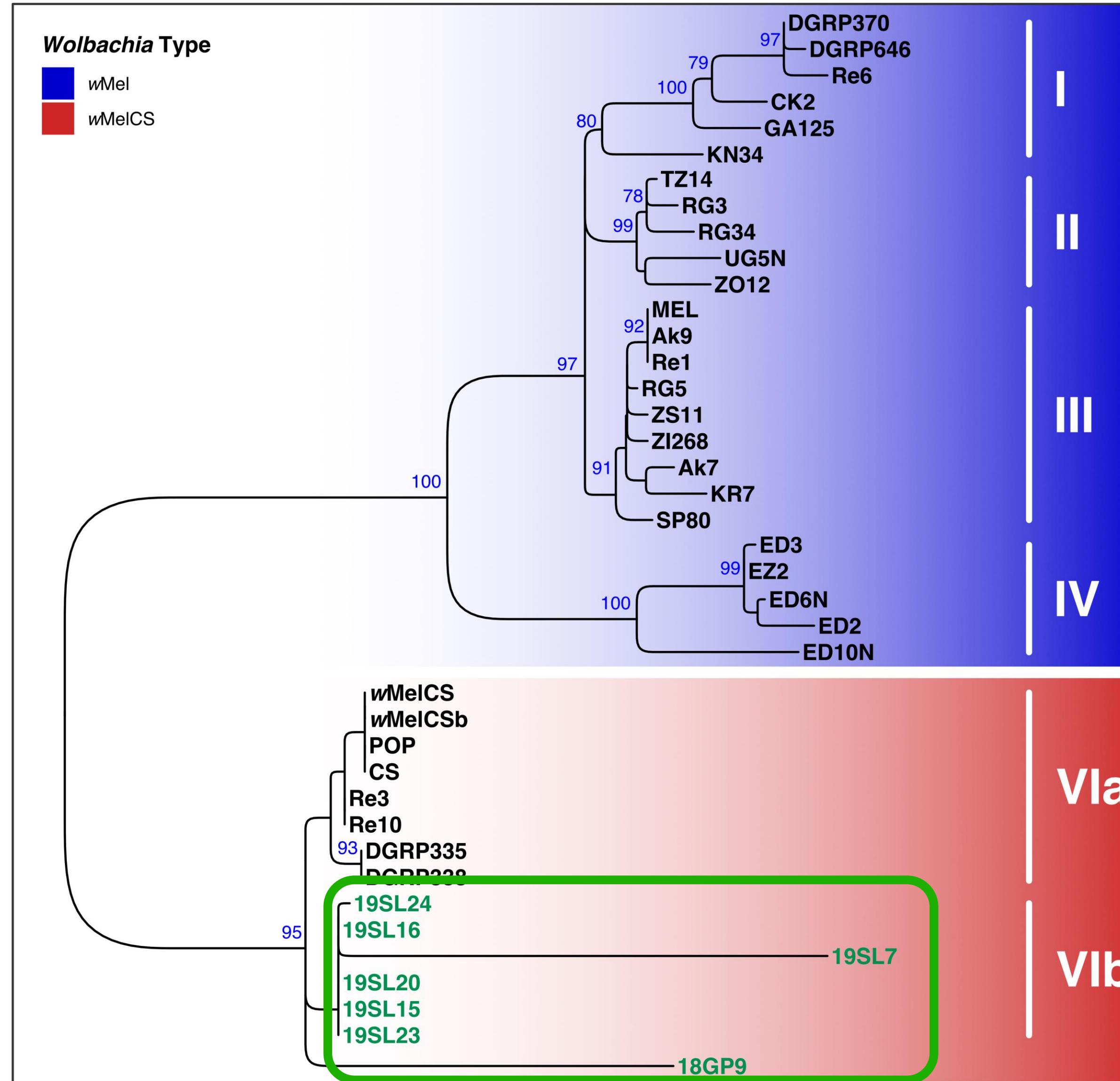
wMelCS



- 11 historic samples with wMelCS (except two??)

Phylogenetic reconstruction

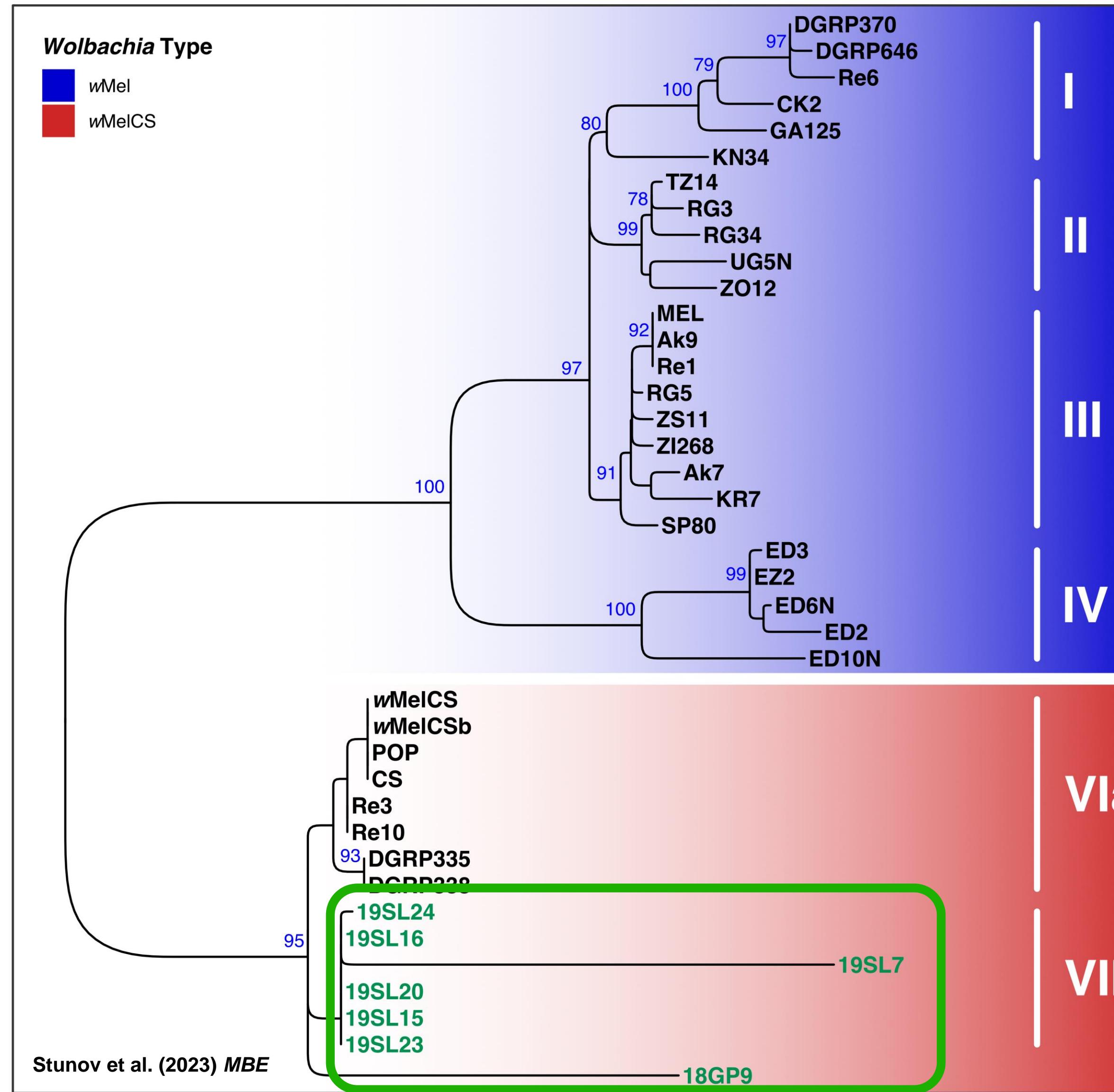
Genome-wide SNPs



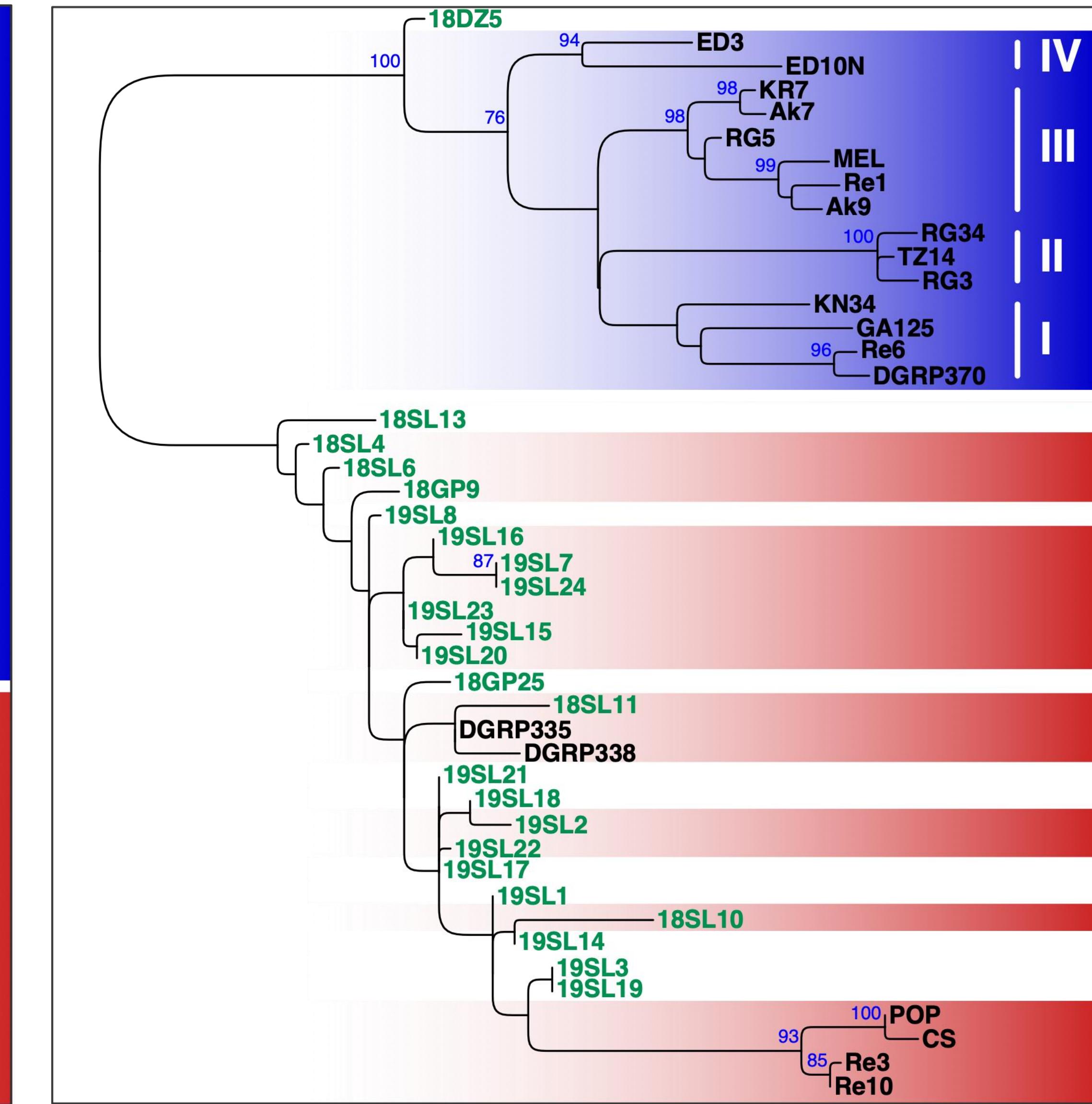
- 279 genome-wide SNPs used for ML tree reconstruction with RAxML.
- 40 samples
 - 7 historic
 - 33 recent

What about mitochondria?

Genome-wide SNPs

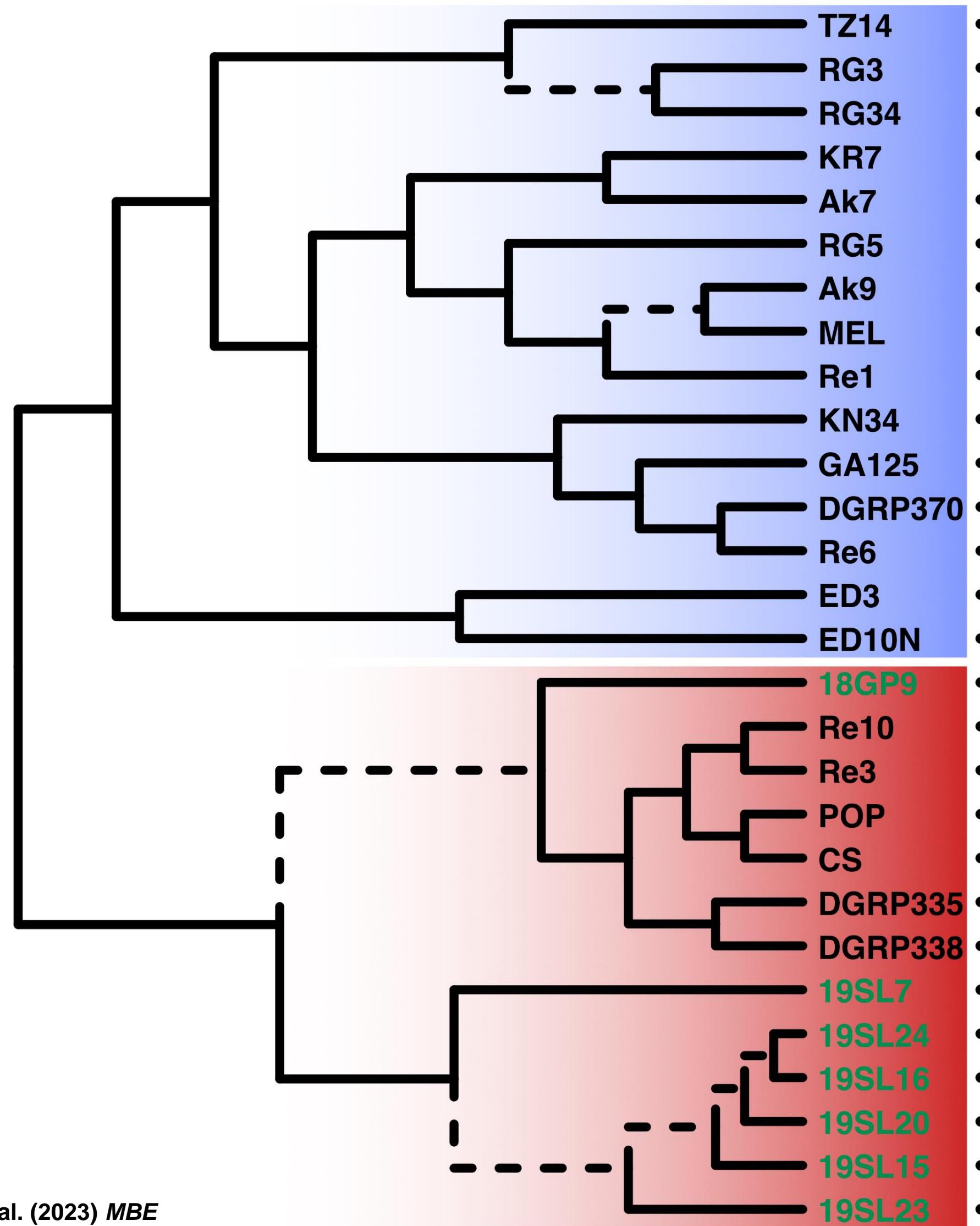


Mitochondria

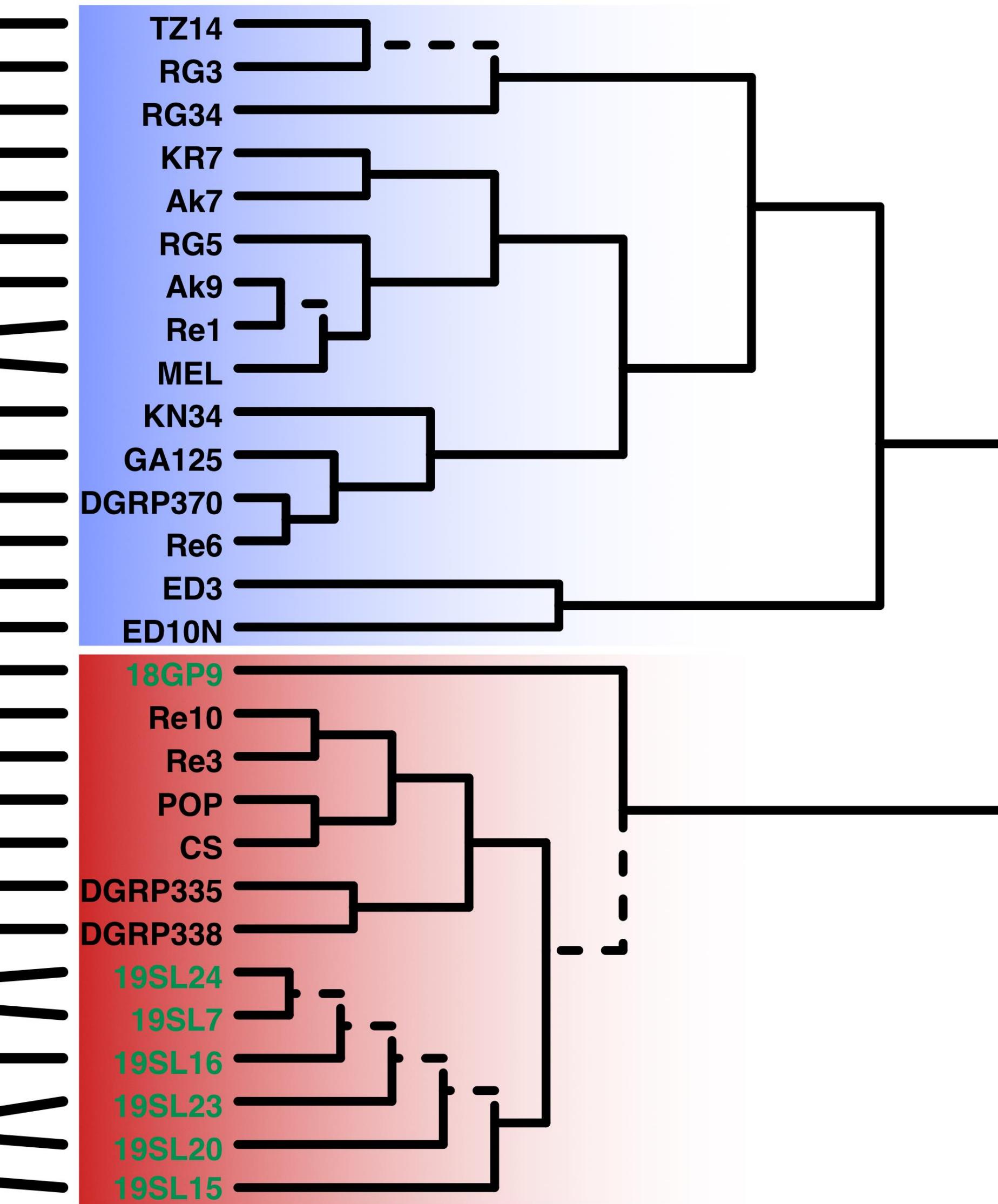


Tight relationship

Wolbachia



Mitochondria



Conclusions

- Historic DNA allows to explore temporal change
- Contamination may lead to wrong conclusions
- Sometimes it is the bycatch, that can help address evolutionary questions about the focal species

...about this project

Historic Museum Samples Provide Evidence for a Recent Replacement of *Wolbachia* Types in European *Drosophila melanogaster*

Anton Strunov,¹ Sandra Kirchner,² Julia Schindelar,² Luise Kruckenhauser,^{2,3} Elisabeth Haring,^{2,3} and Martin Kapun  ^{1,2,*}

¹Center for Anatomy and Cell Biology, Medical University of Vienna, Vienna, Austria

²Natural History Museum Vienna, Central Research Laboratories, Vienna, Austria

³Department for Evolutionary Biology, University of Vienna, Vienna, Austria

***Corresponding author:** E-mail: martin.kapun@nhm-wien.ac.at.

Associate editor: Daniel Falush

<https://doi.org/10.1093/molbev/msad258>

https://github.com/capoony/WolbachiaEvolHist_2023

Thank you very much...



FWF

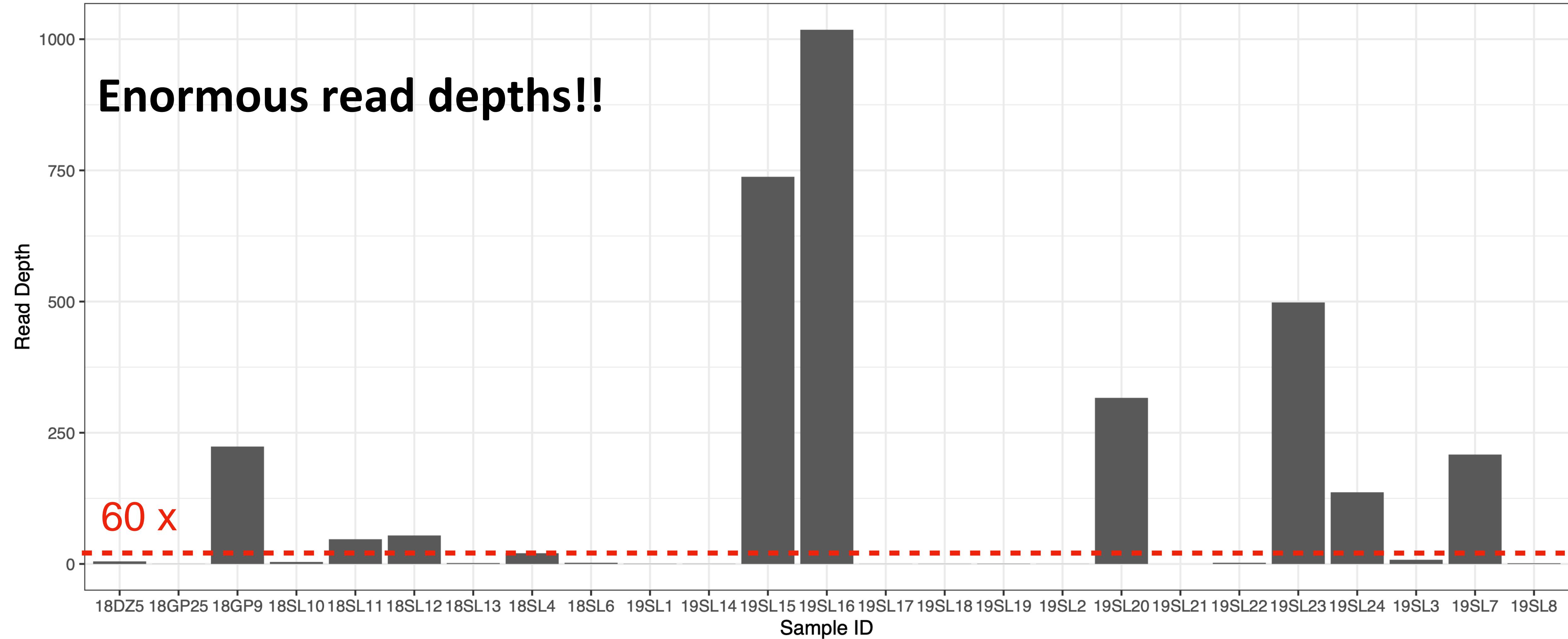
Der Wissenschaftsfonds.



MEDIZINISCHE
UNIVERSITÄT WIEN

naturhistorisches
museum wien **nhm**

Denovo *Wolbachia* assemblies



Denovo *Wolbachia* assemblies

- denovo assembly with SPAdes
- QC with BLOBtools
 - GC-content
 - Read-depth
 - Contig Size
 - BLAST hits

