

From Museums to Microbes:

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



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MLSpeciationGenomics
St. Andrews - 25/06/2025

Who am I?

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<https://github.com/capoony>

<https://www.researchgate.net/profile/Martin-Kapun>

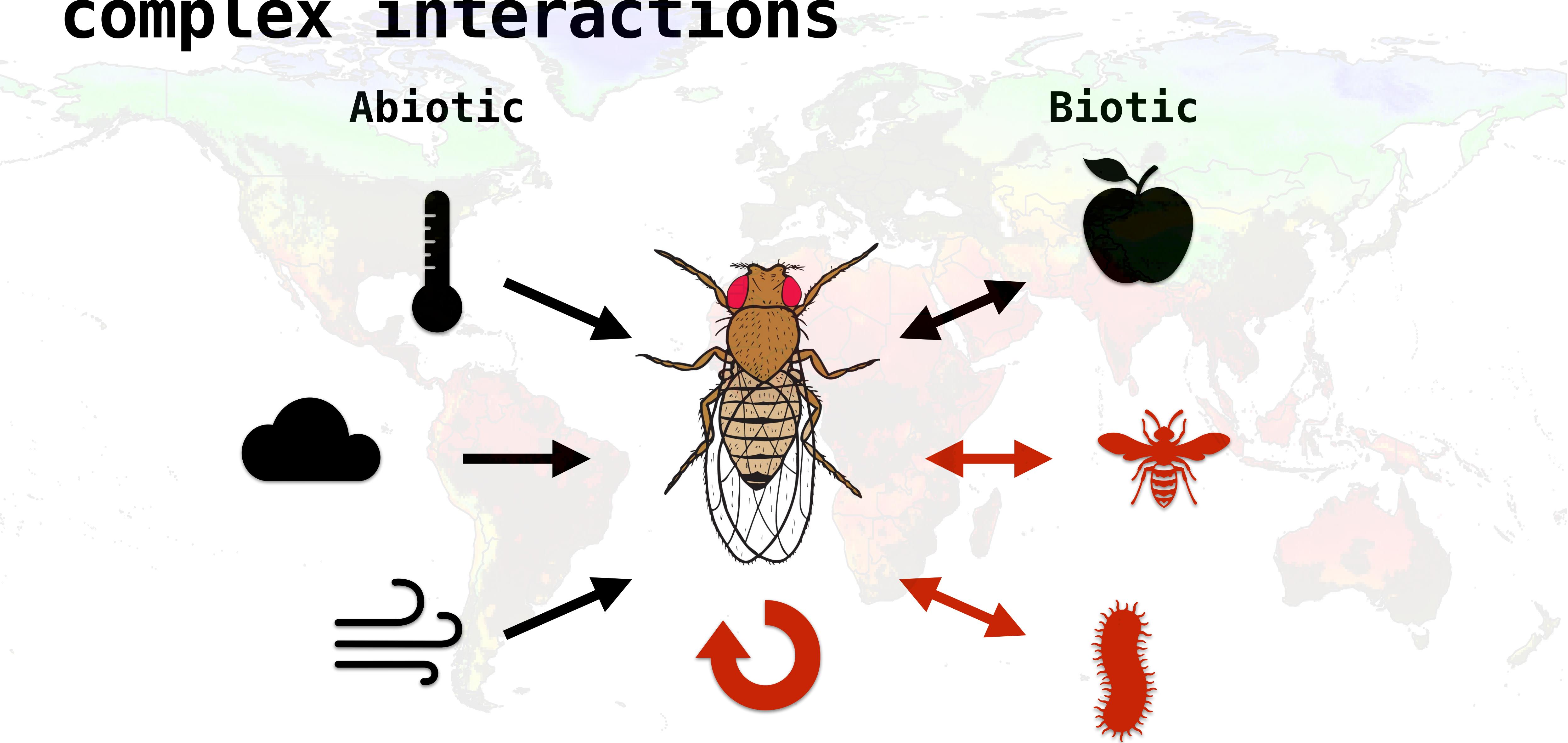
@capoony

Microevolution

Macroevolution

Eco-evolutionary
interactions

Abiotic & Biotic factors trigger complex interactions



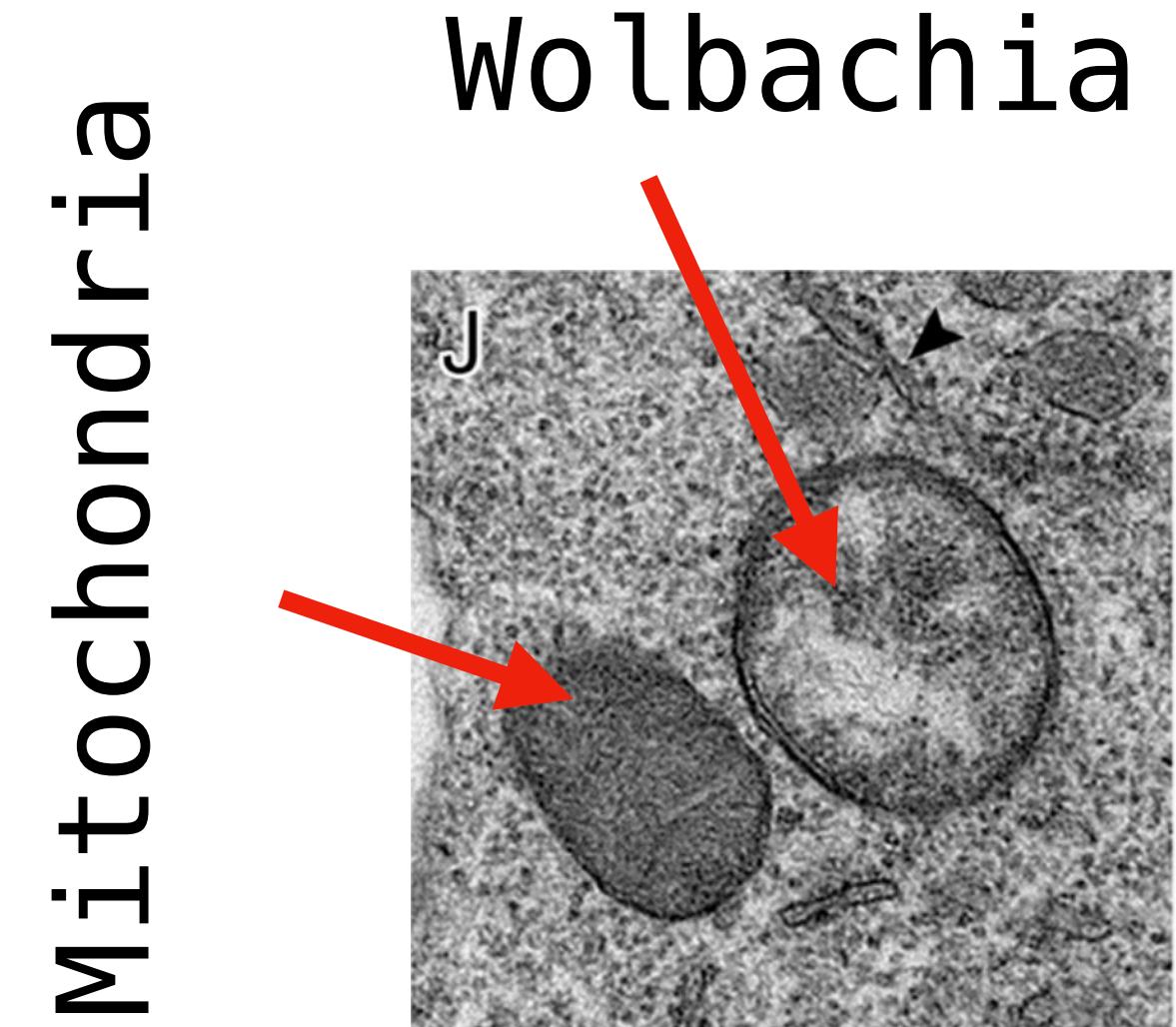
The *Wolbachia-Drosophila* system



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
AGGACAGTCAGATGCGAGACTGGAATCAGTGAATT
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

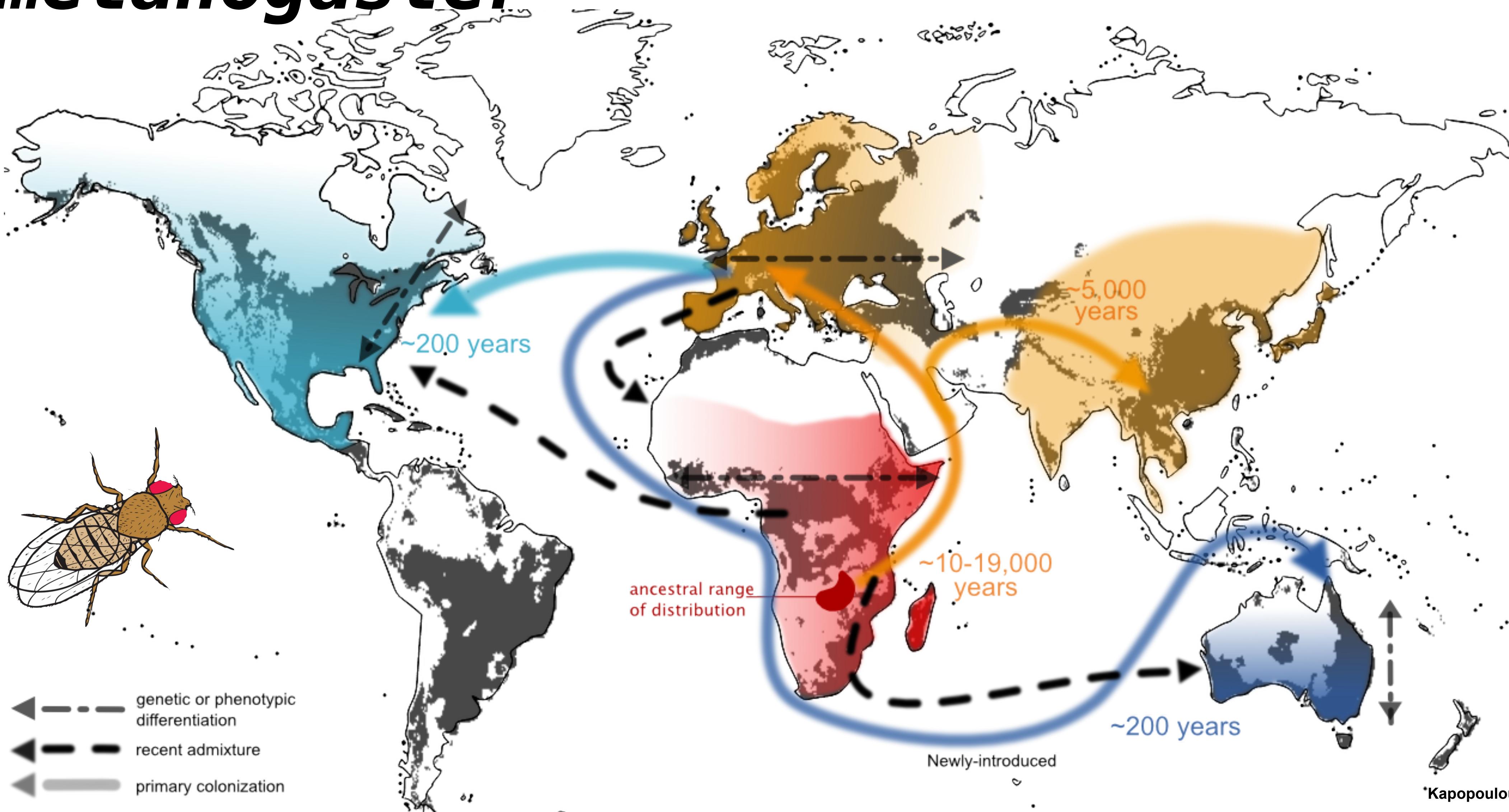
QuickSearch

Human Disease Protein Domains

Search FlyBase Homologs GAL4

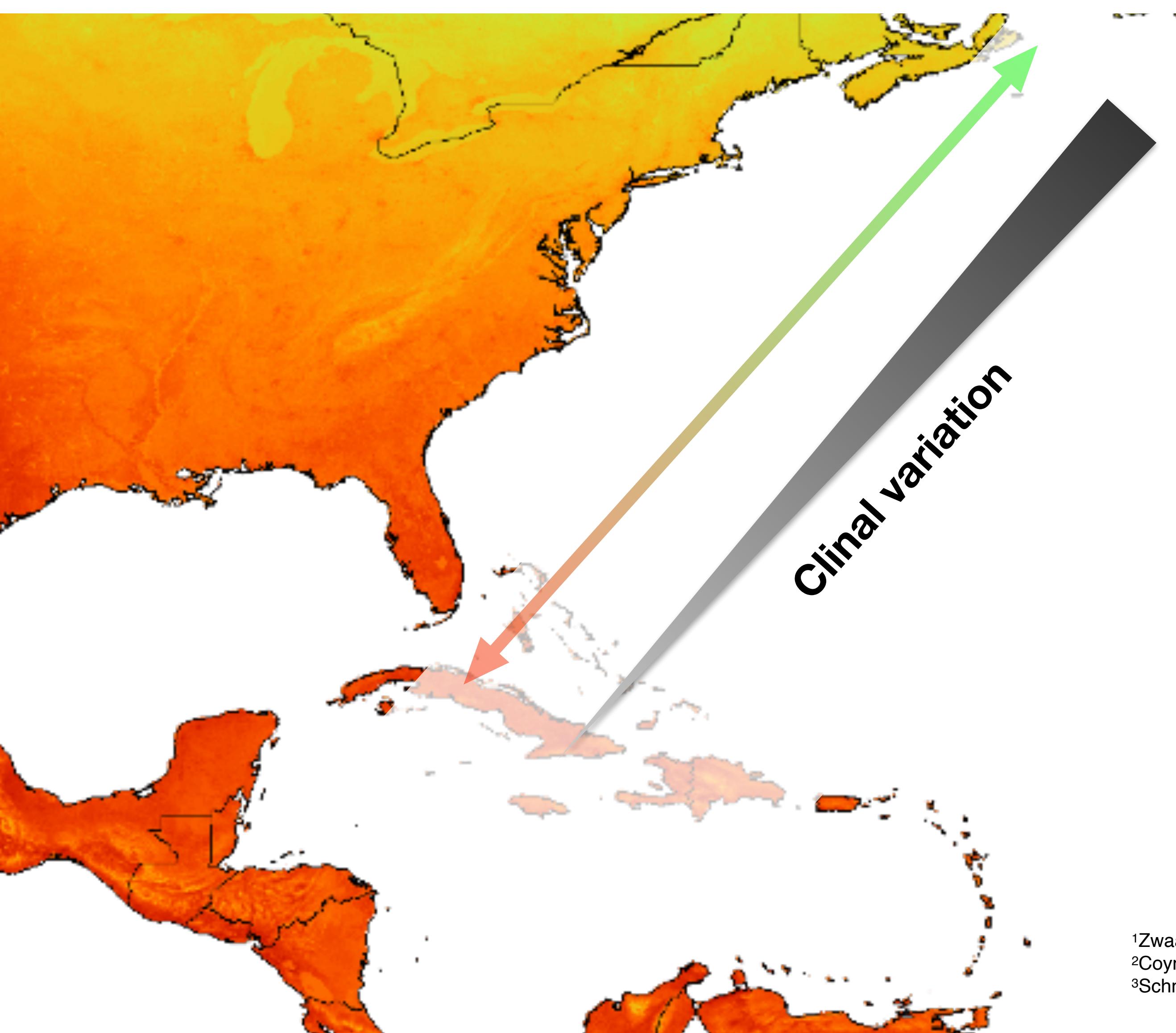
The FlyBase website interface is shown on the right. It features a header with the FlyBase logo, a menu bar with 'Tools', 'Downloads', and 'Links' dropdowns, and a sidebar with links to 'Overview', 'Current Release', 'Archived Data', 'Map Conversion', and 'FTP Site' (with options for 'Releases (FTP)' and 'Genomes (FTP)'). The main content area includes sections for 'Citing FlyBase', 'External Resources' (with links to 'News and Outreach', 'Drosophila Journals and Protocols', and 'Tool/Reagent Resources'), and a 'QuickSearch' bar at the bottom.

The world-wide distribution of *D. melanogaster*



Kapopoulou, Kapun et al. (2020) Sci Rep

Haudry, Laurent & Kapun (2020) in Statistical Population Genomics



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*

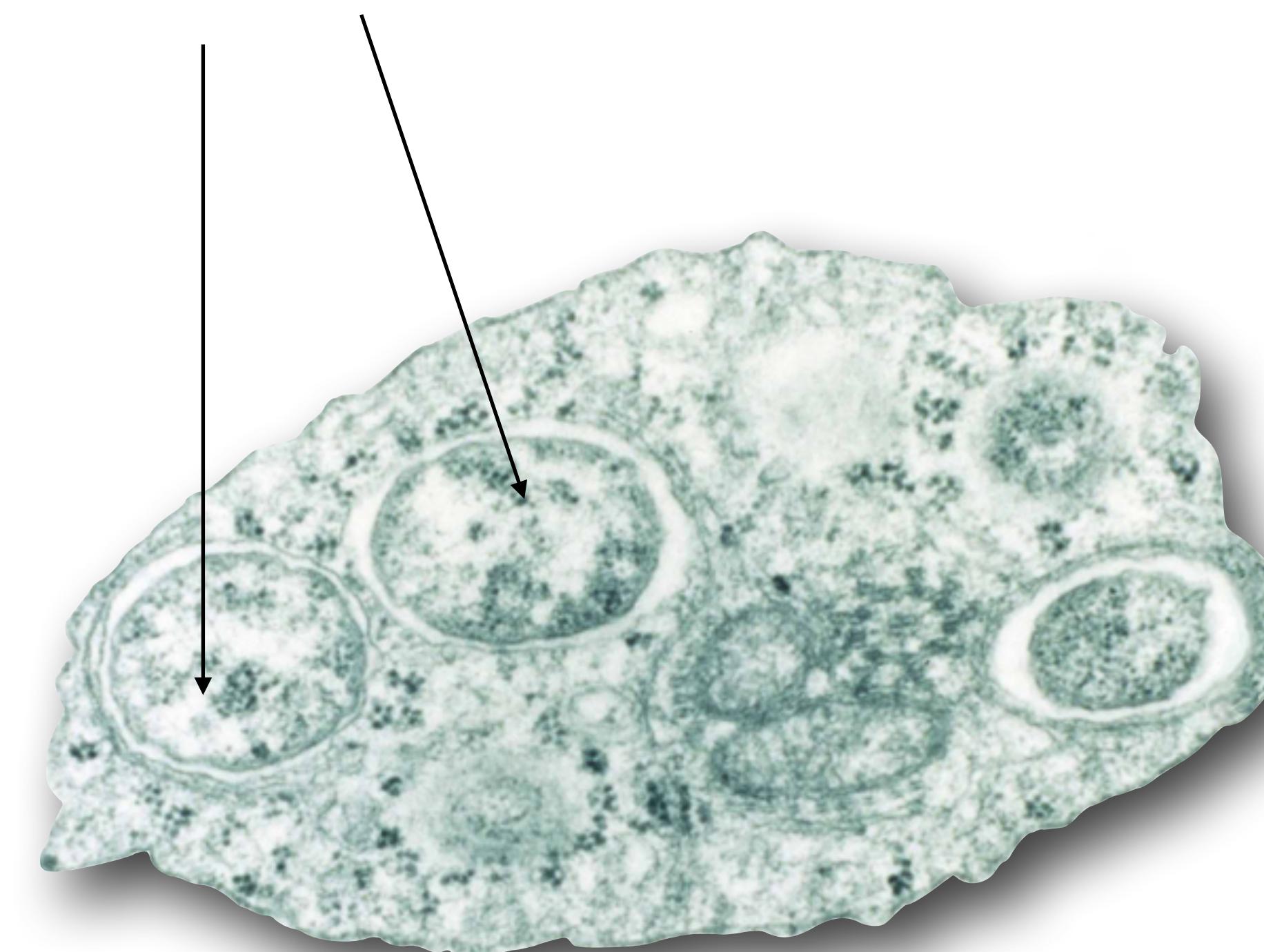
⁹Kapun *et al.* (2011) *Mol Ecol*

¹⁰Kapun *et al.* (2016) *MBE*

¹¹Kapun *et al.* (2016) *JEB*

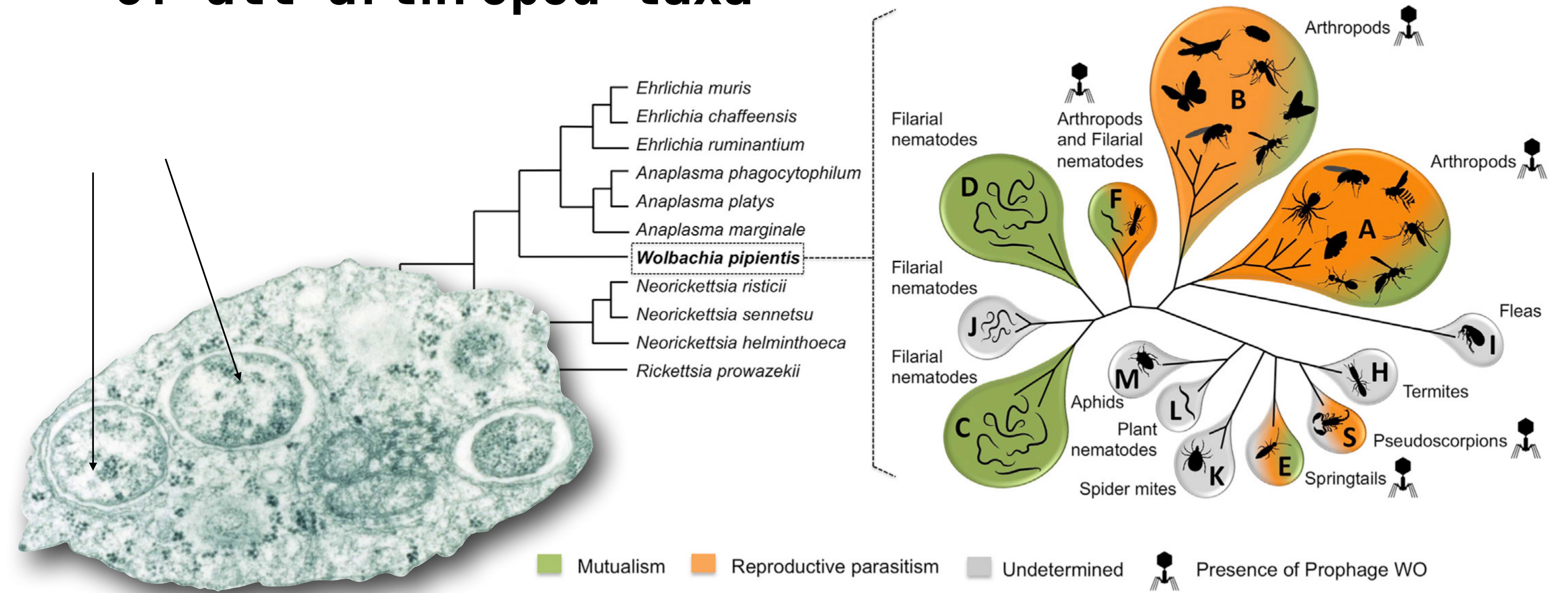
Wolbachia pipiens

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



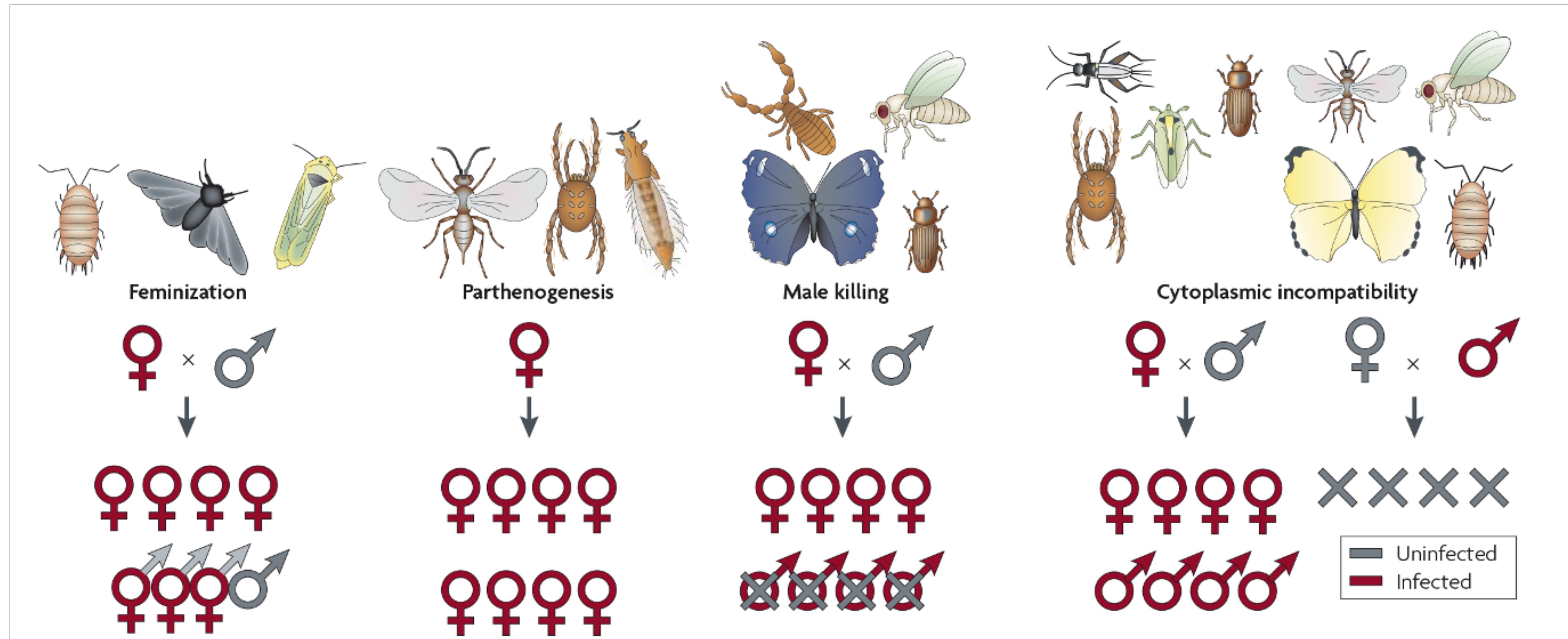
Wolbachia pipiens

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Wolbachia pipiens

- Reproductive parasite

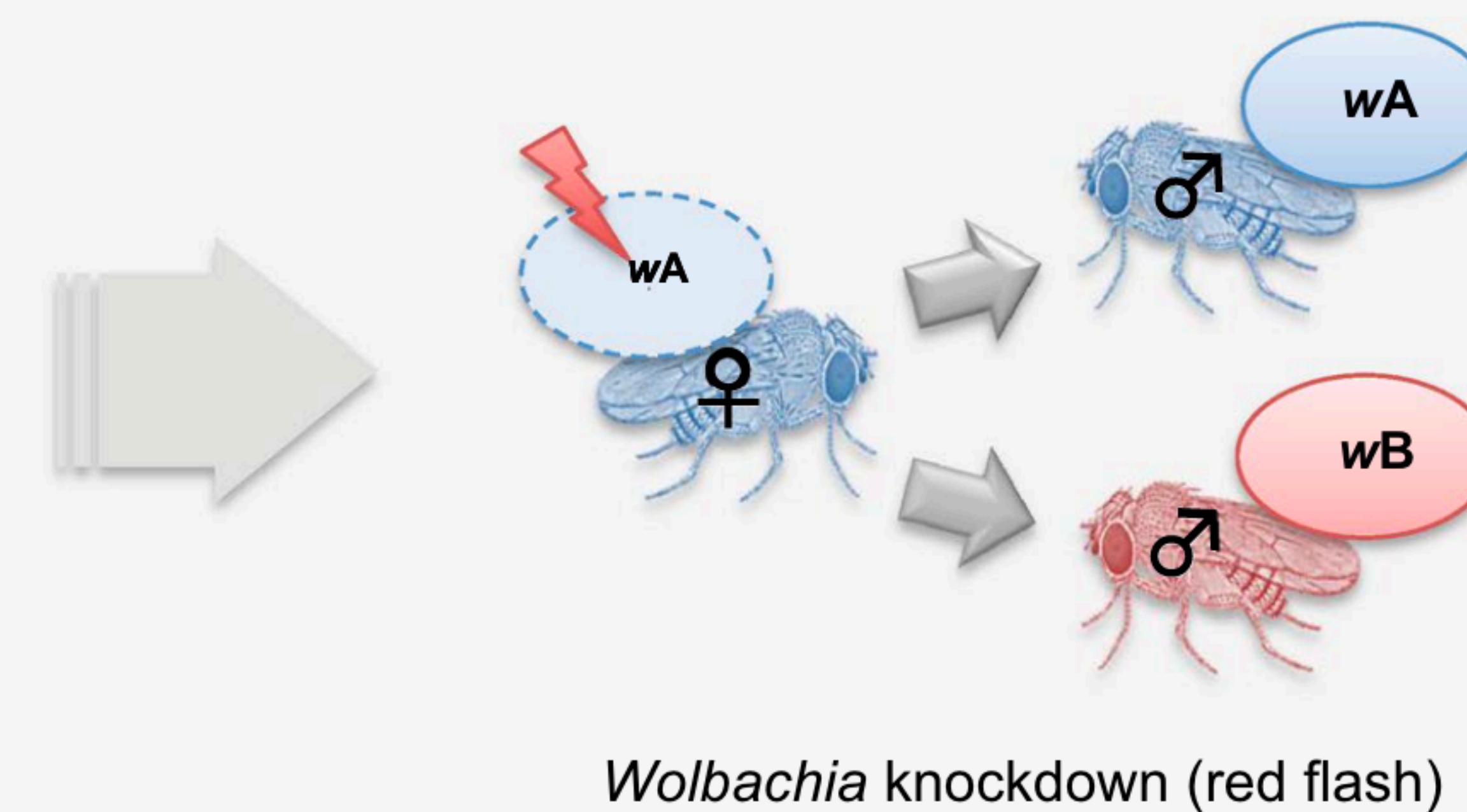
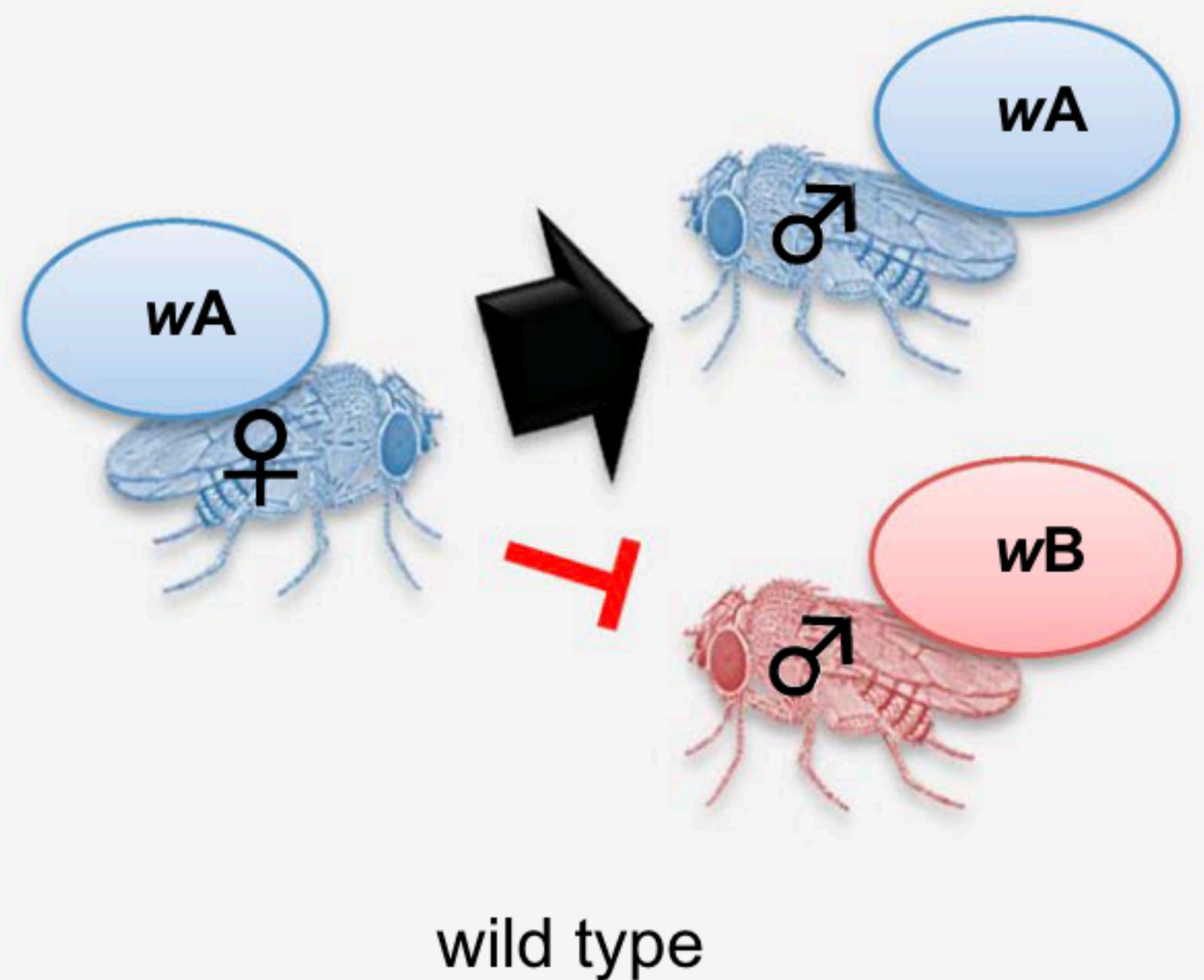


Wolbachia pipiens

- Barriers to gene flow in the host

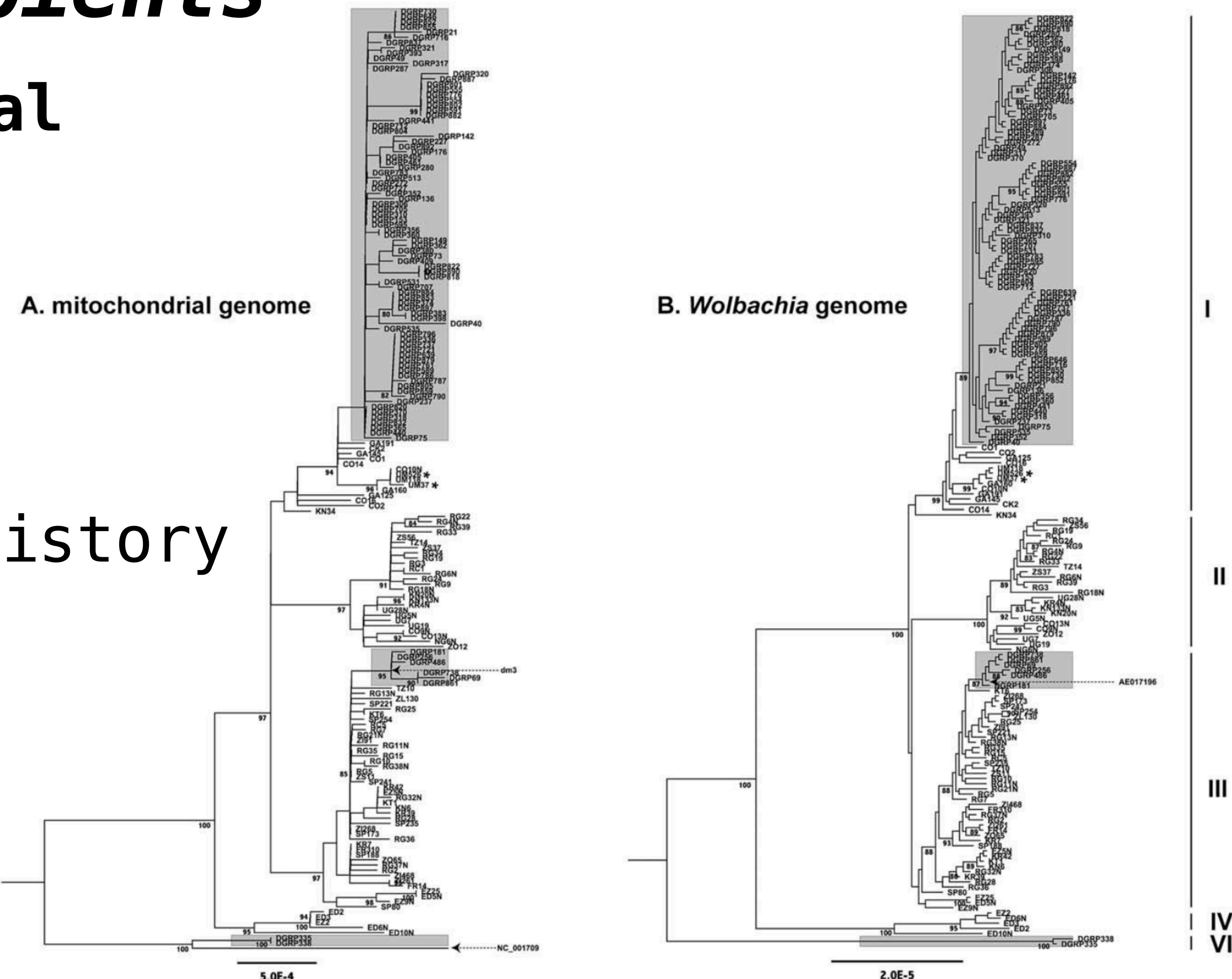


a Mate choice in *D. paulistorum*: *Wolbachia*-knockdown females lose assortative mating behavior against heterogametic wild type males (Miller et al. 2010)



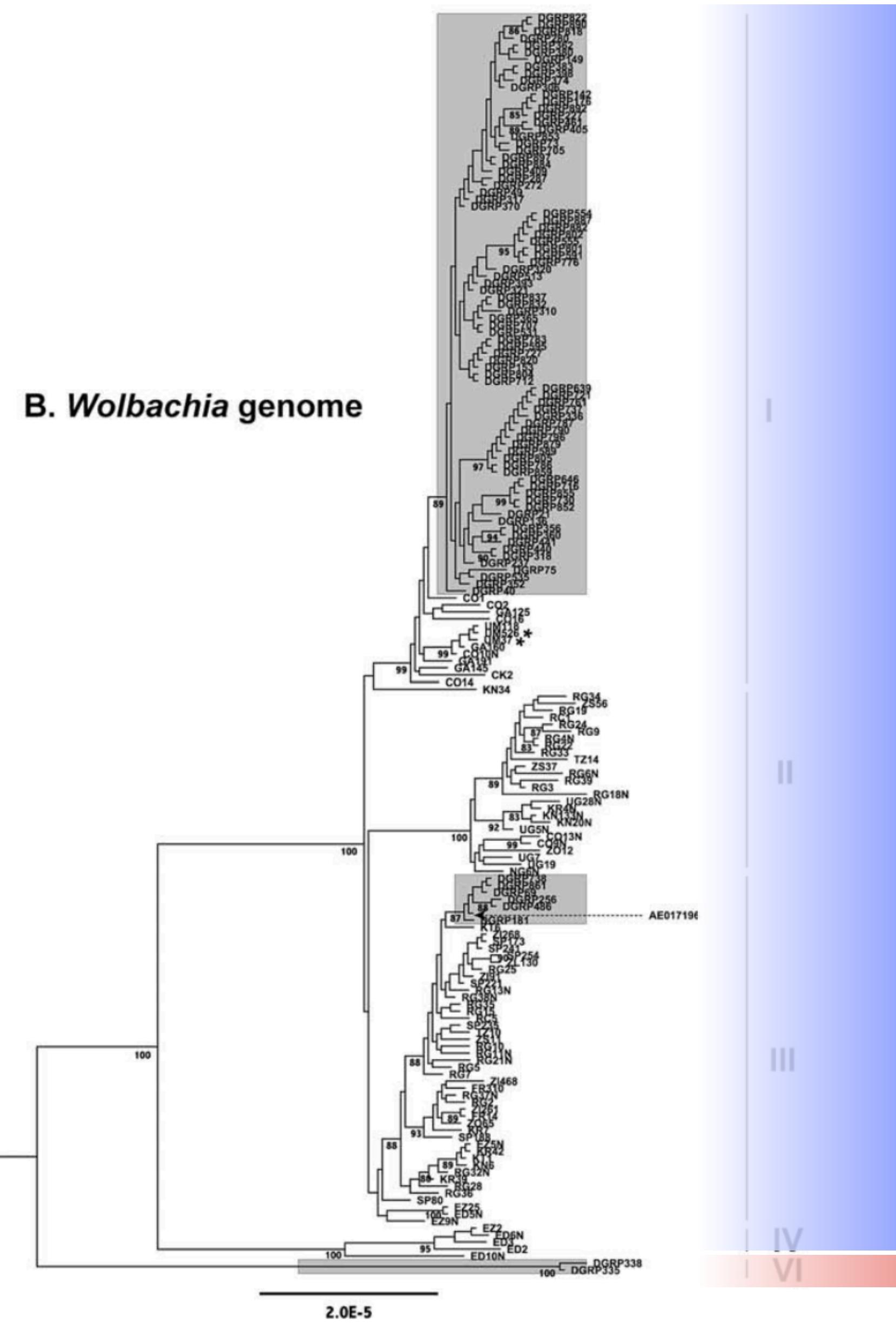
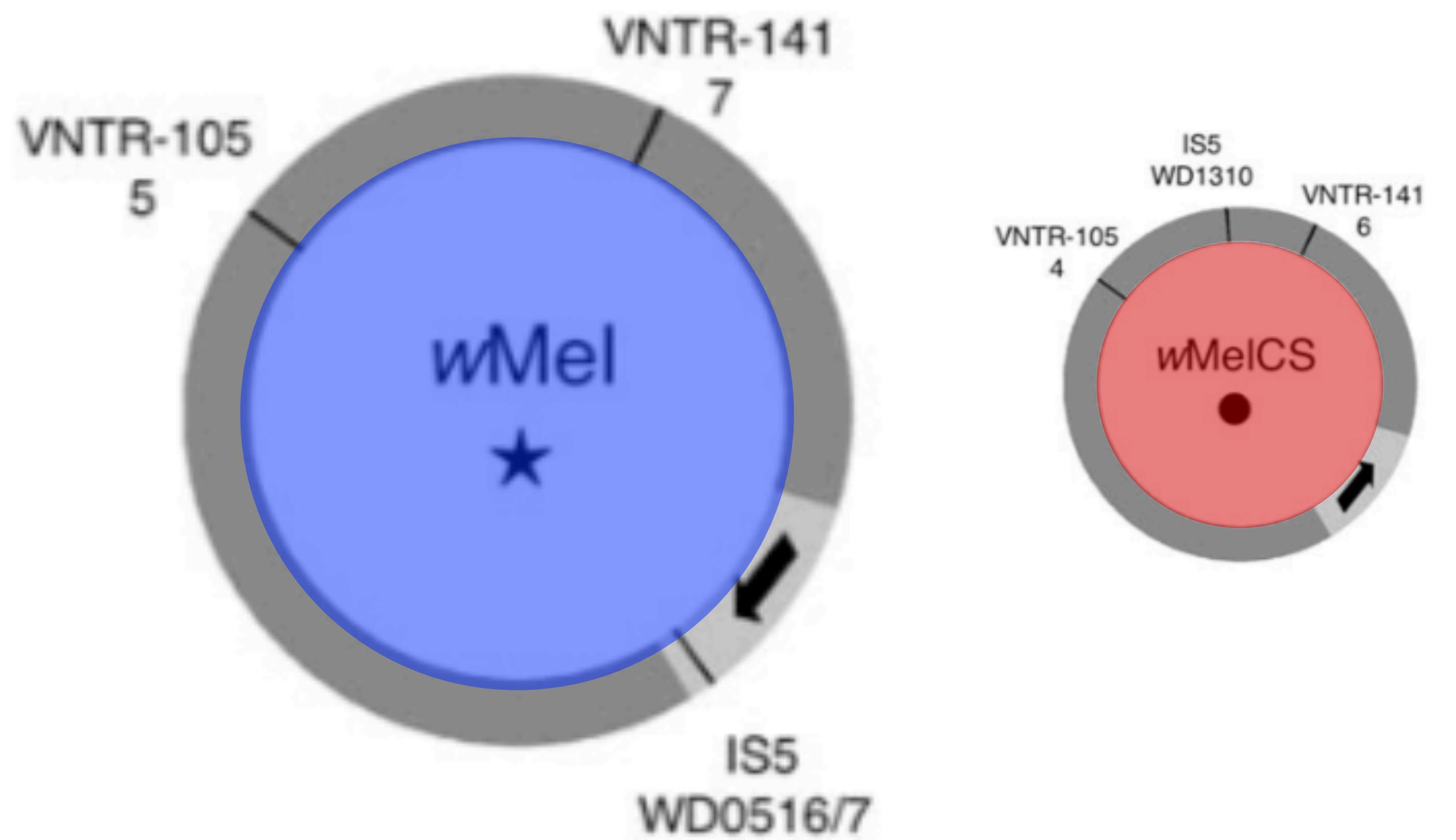
Wolbachia pipiens

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

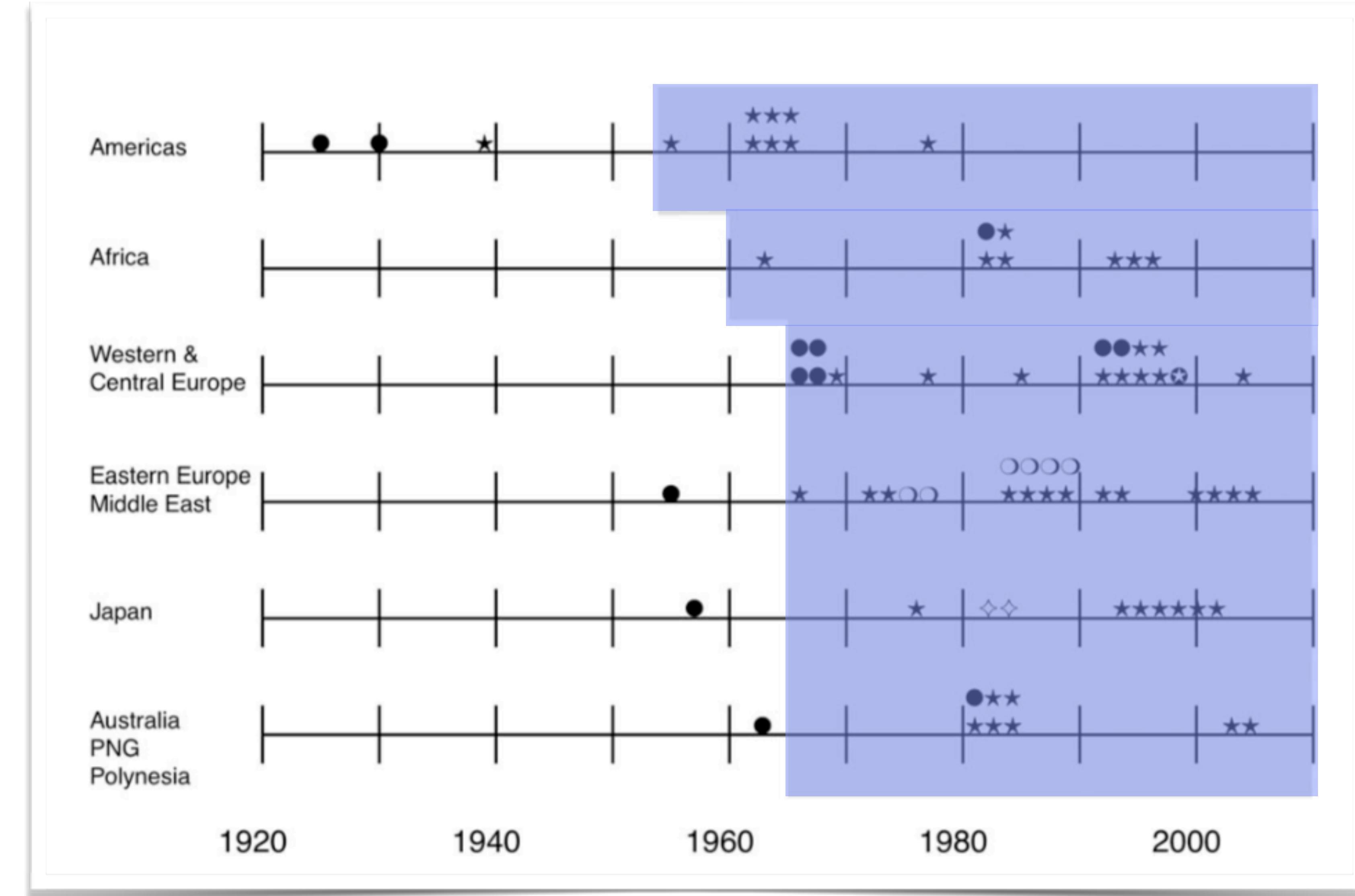
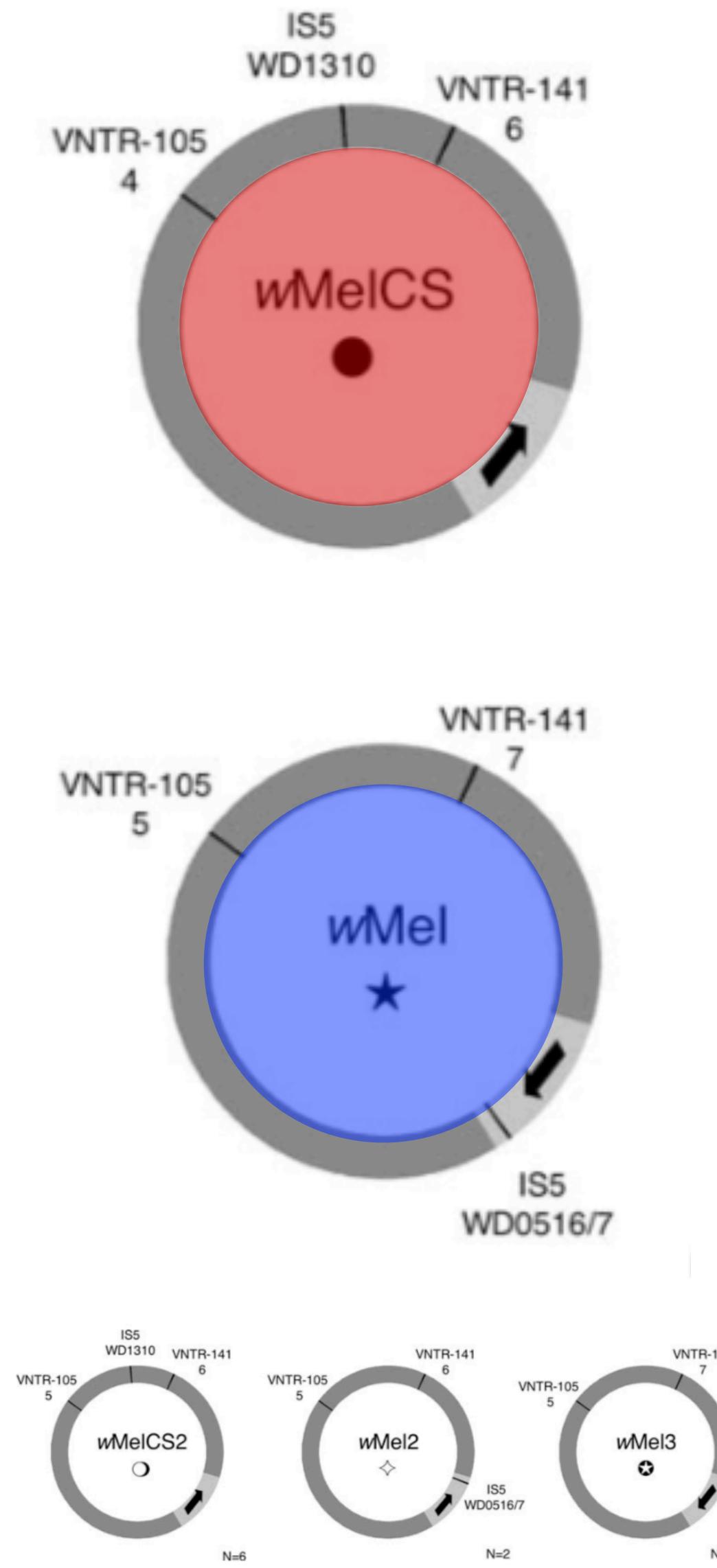


Wolbachia pipiens in *D. melanogaster*

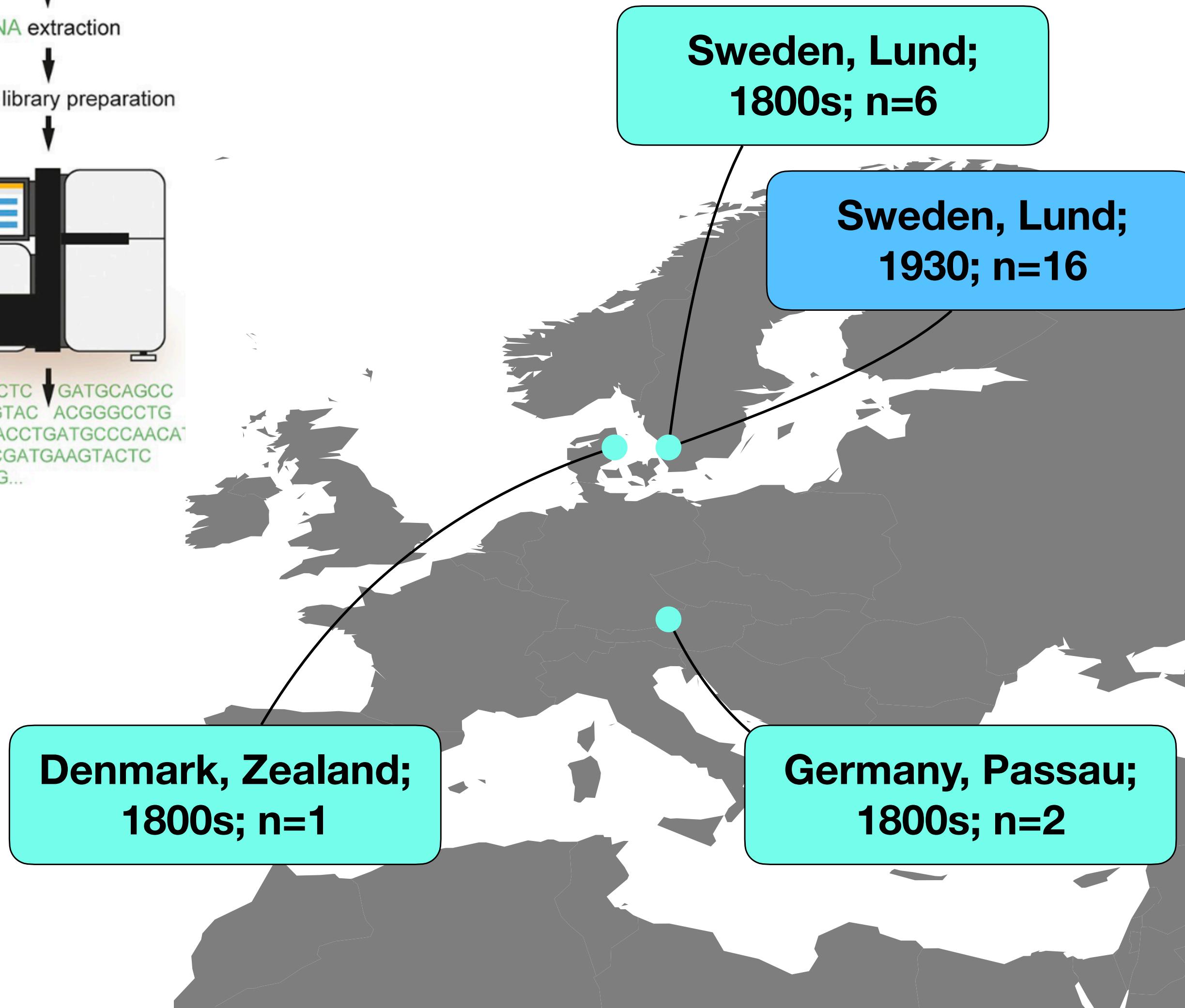
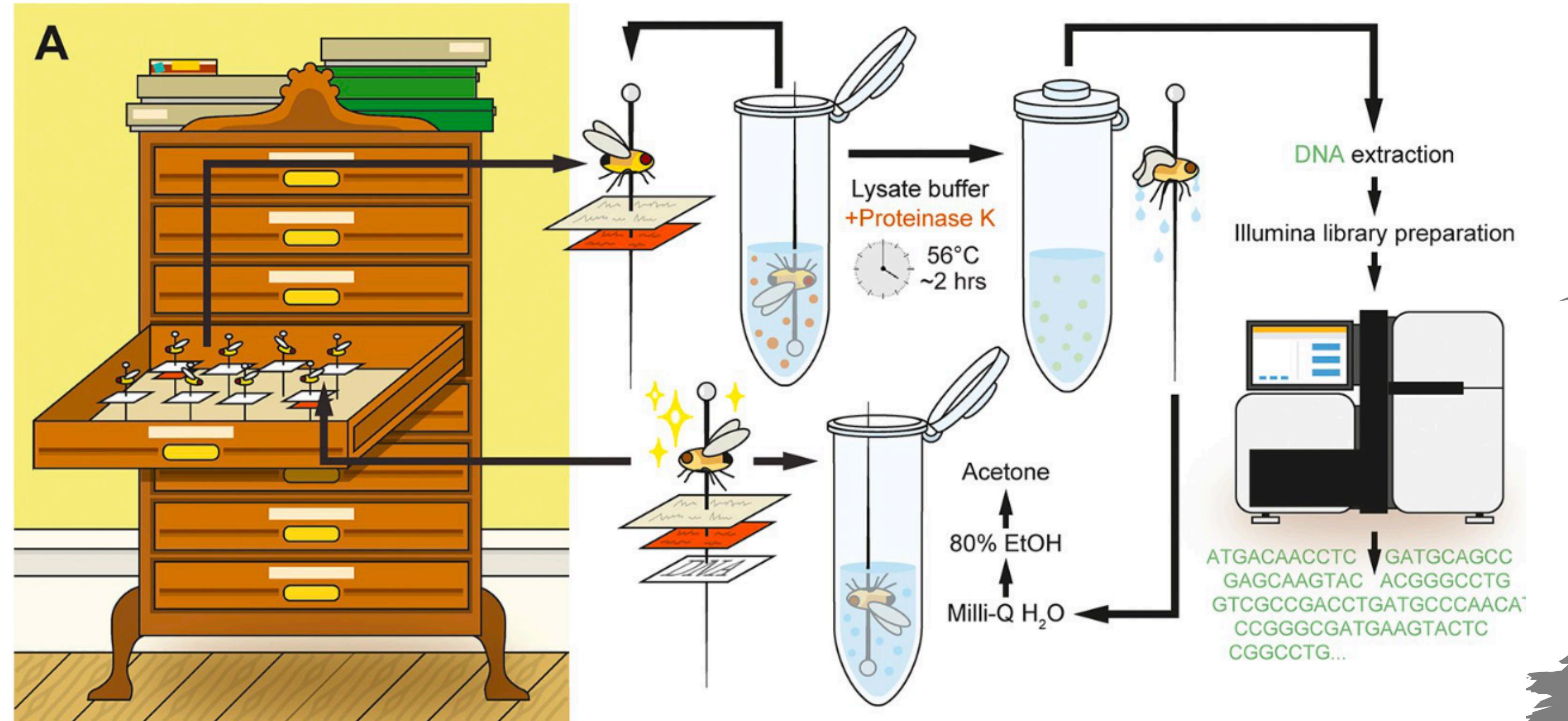
- Two main types



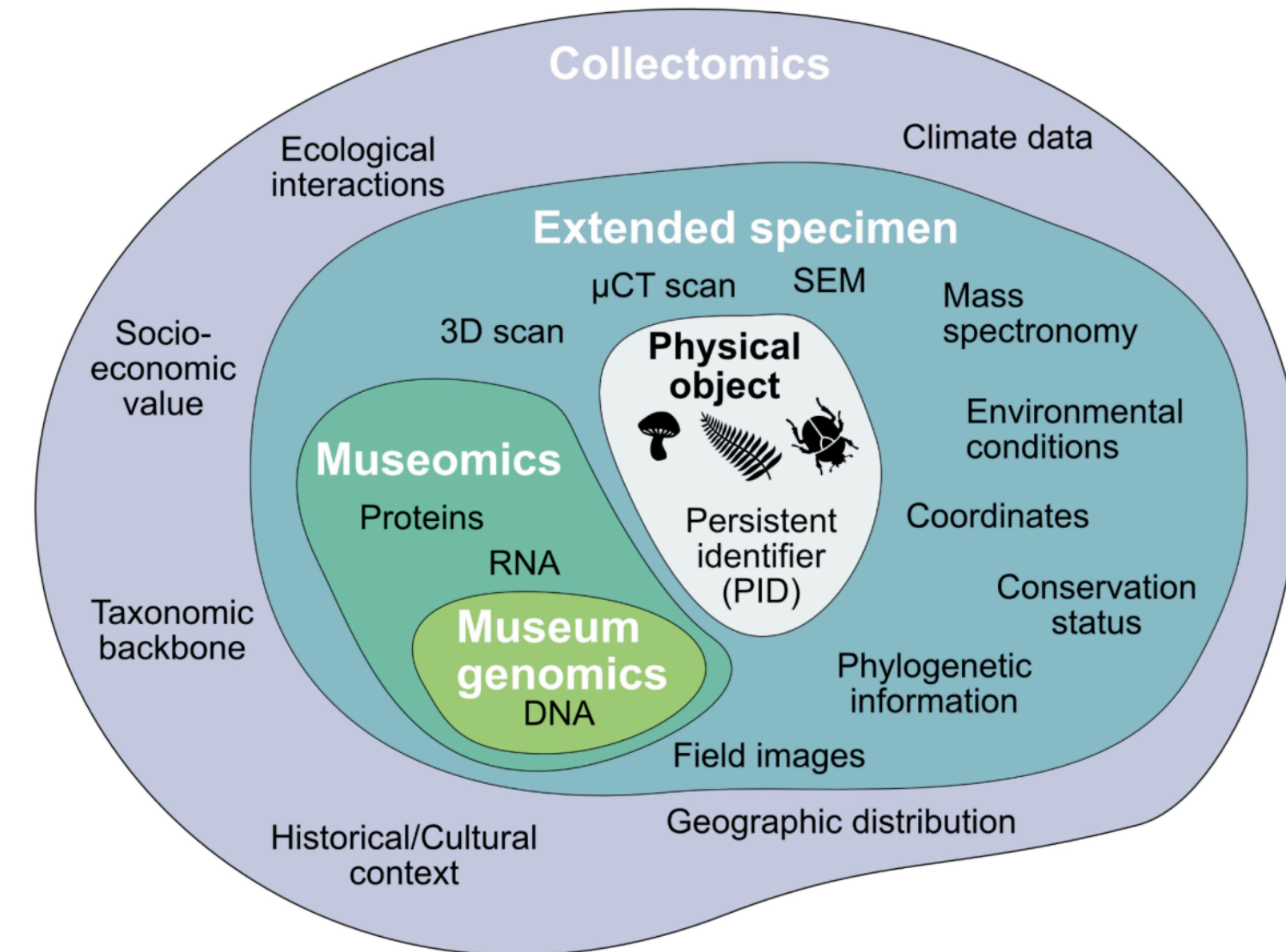
Global *Wolbachia* replacement?



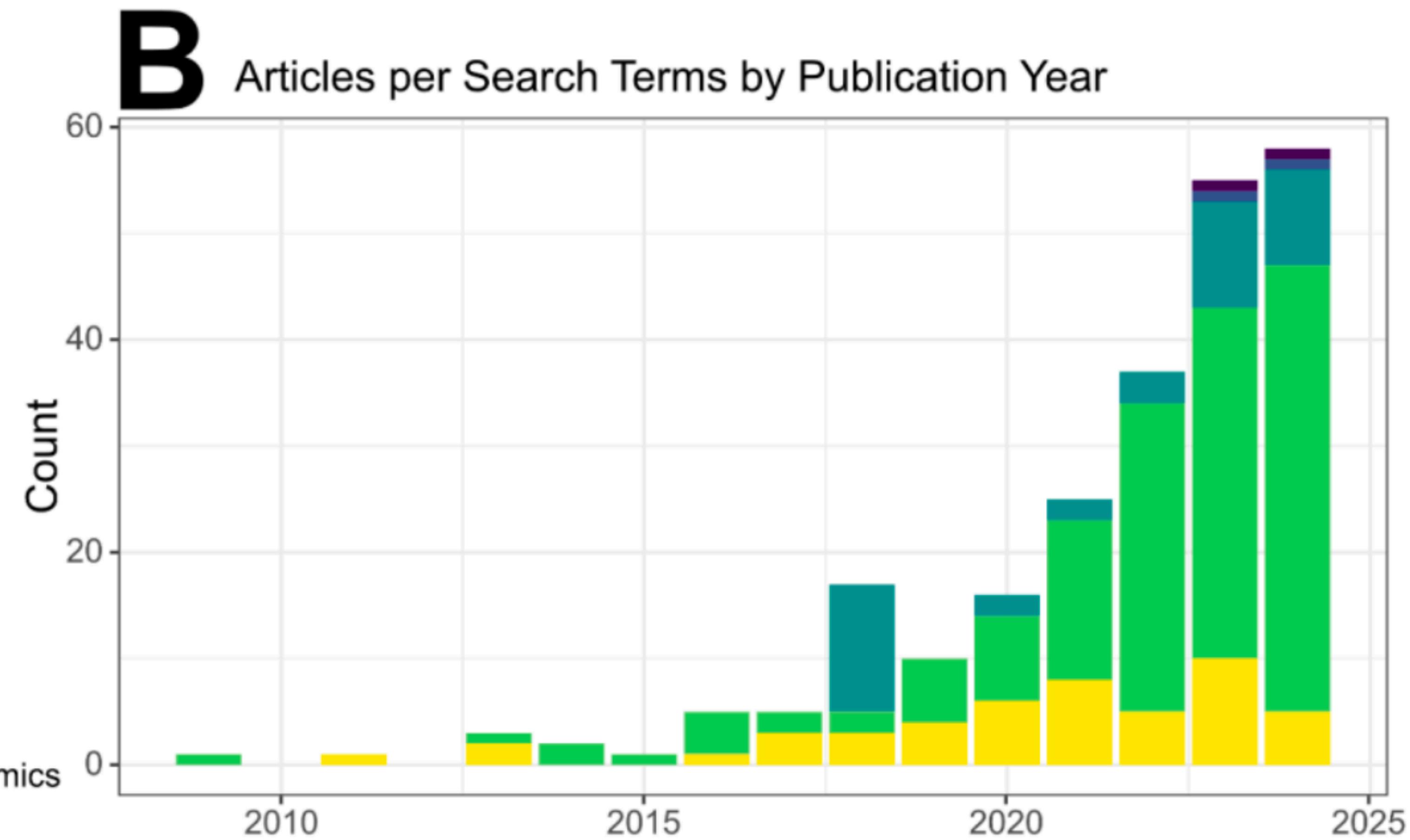
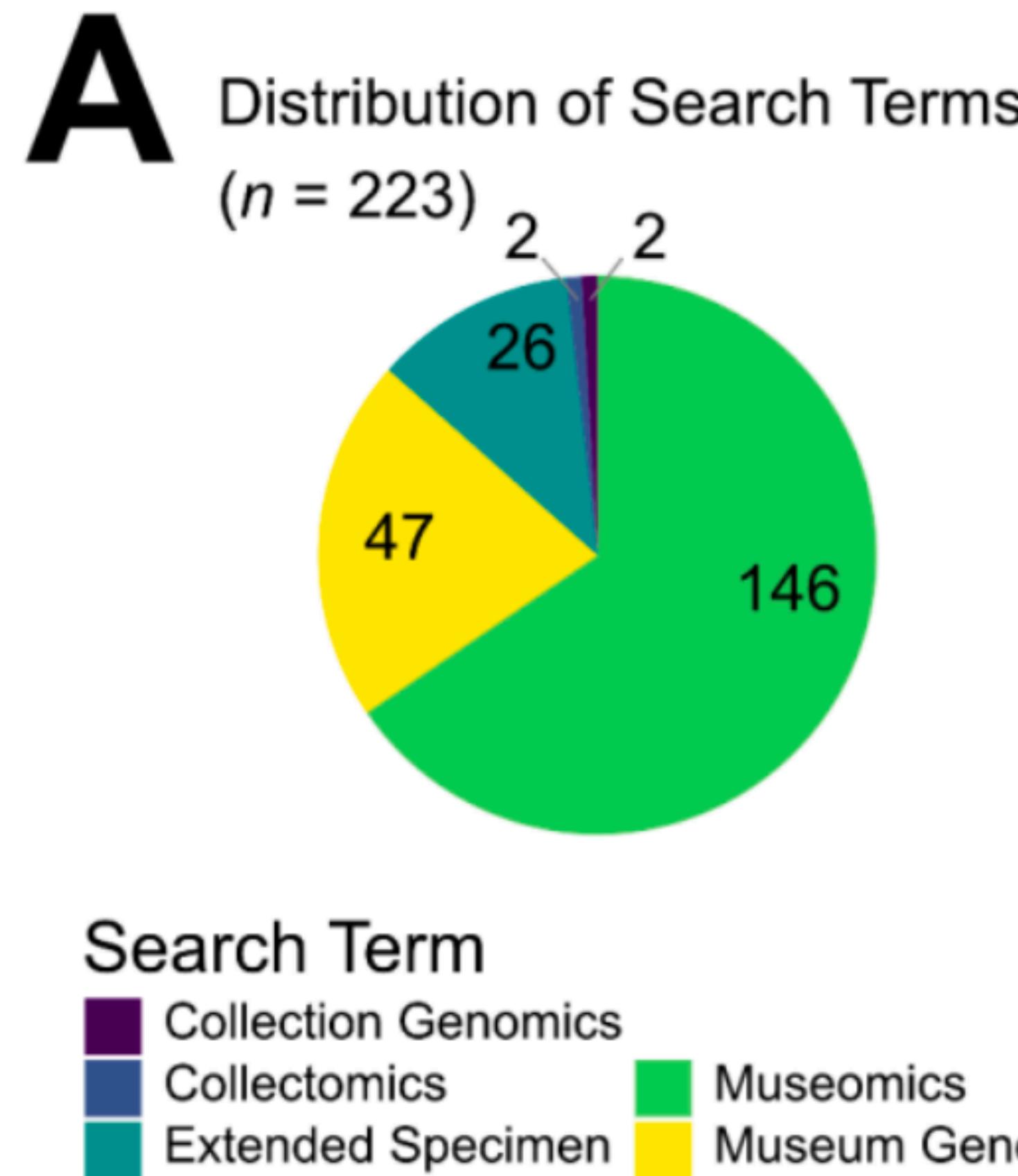
Museomics can help!



But what is Museomics?

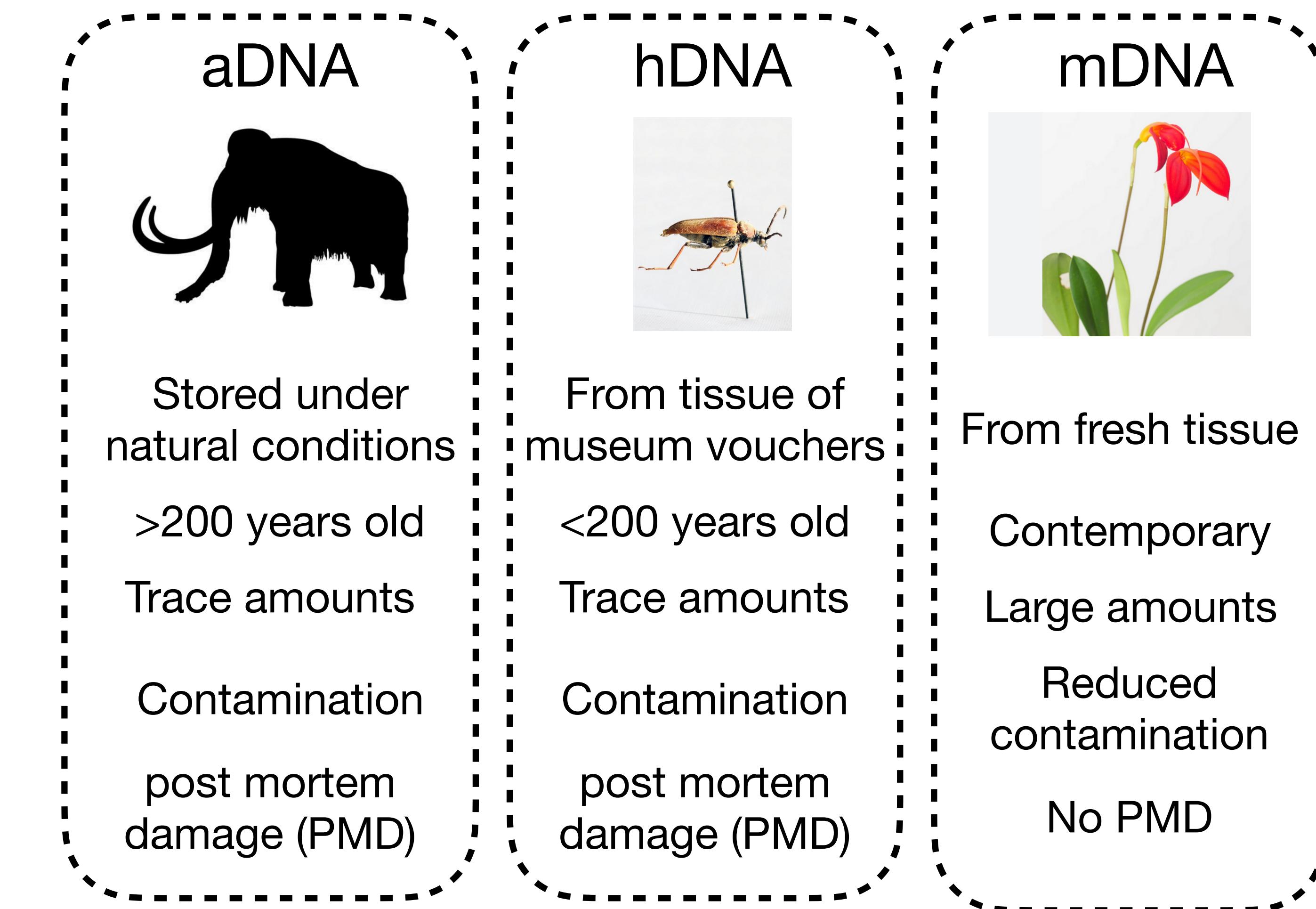


But what is Museomics?



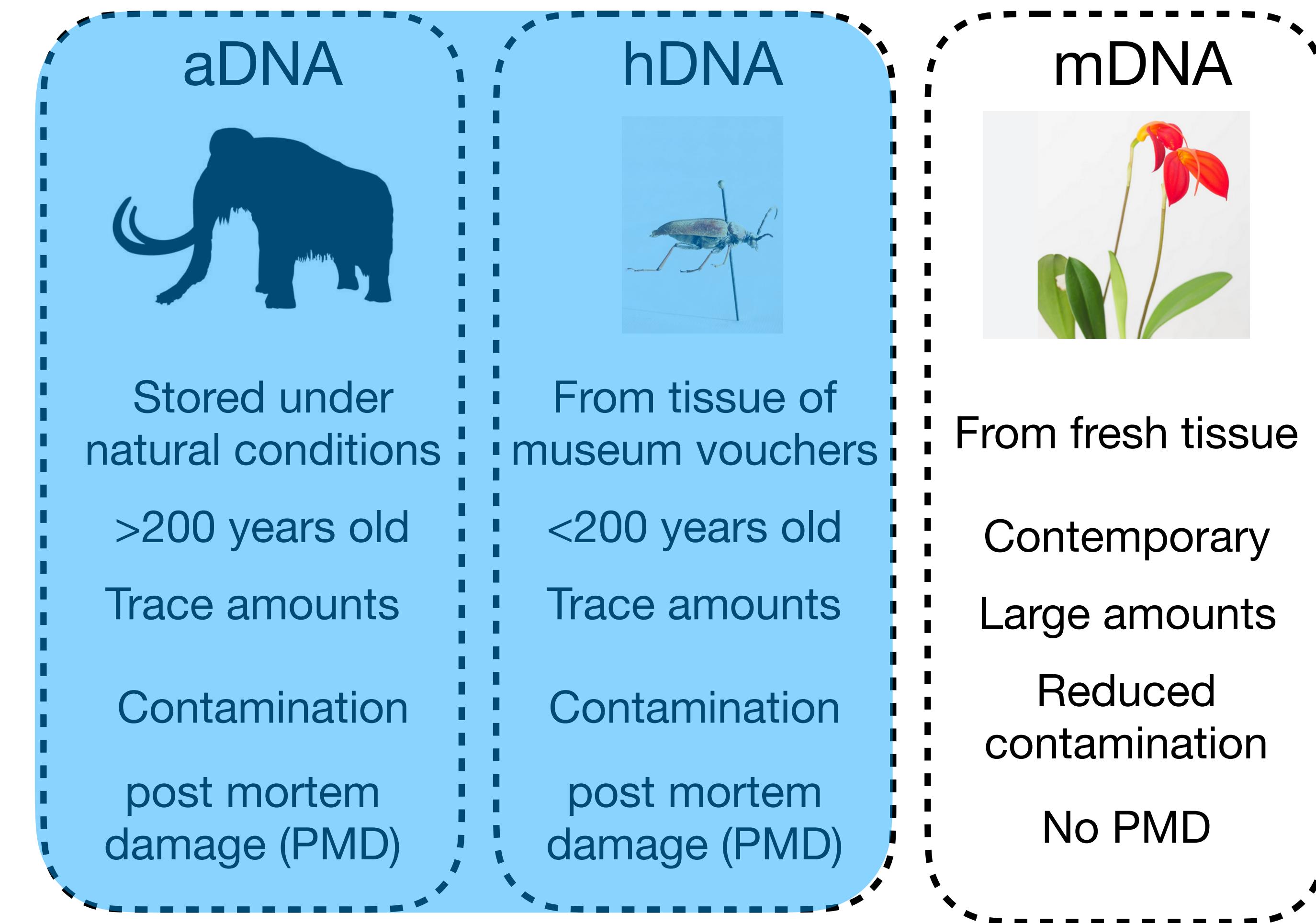
But what is Museomics?

“Archival DNA”

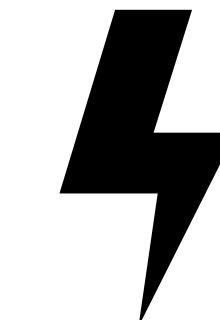


But what is Museomics?

“Archival DNA”



Properties of “old” DNA!

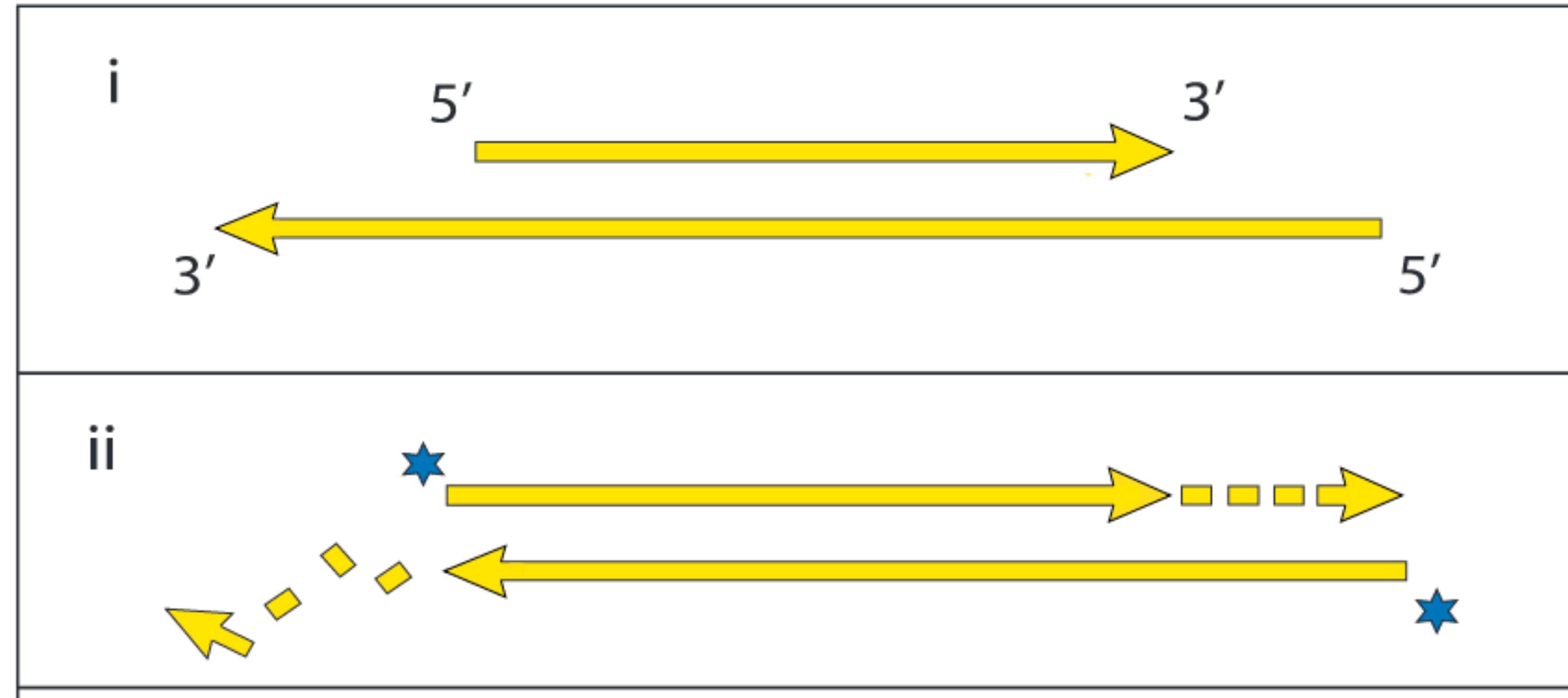


Nick

Depurination

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

Properties of “old” DNA!



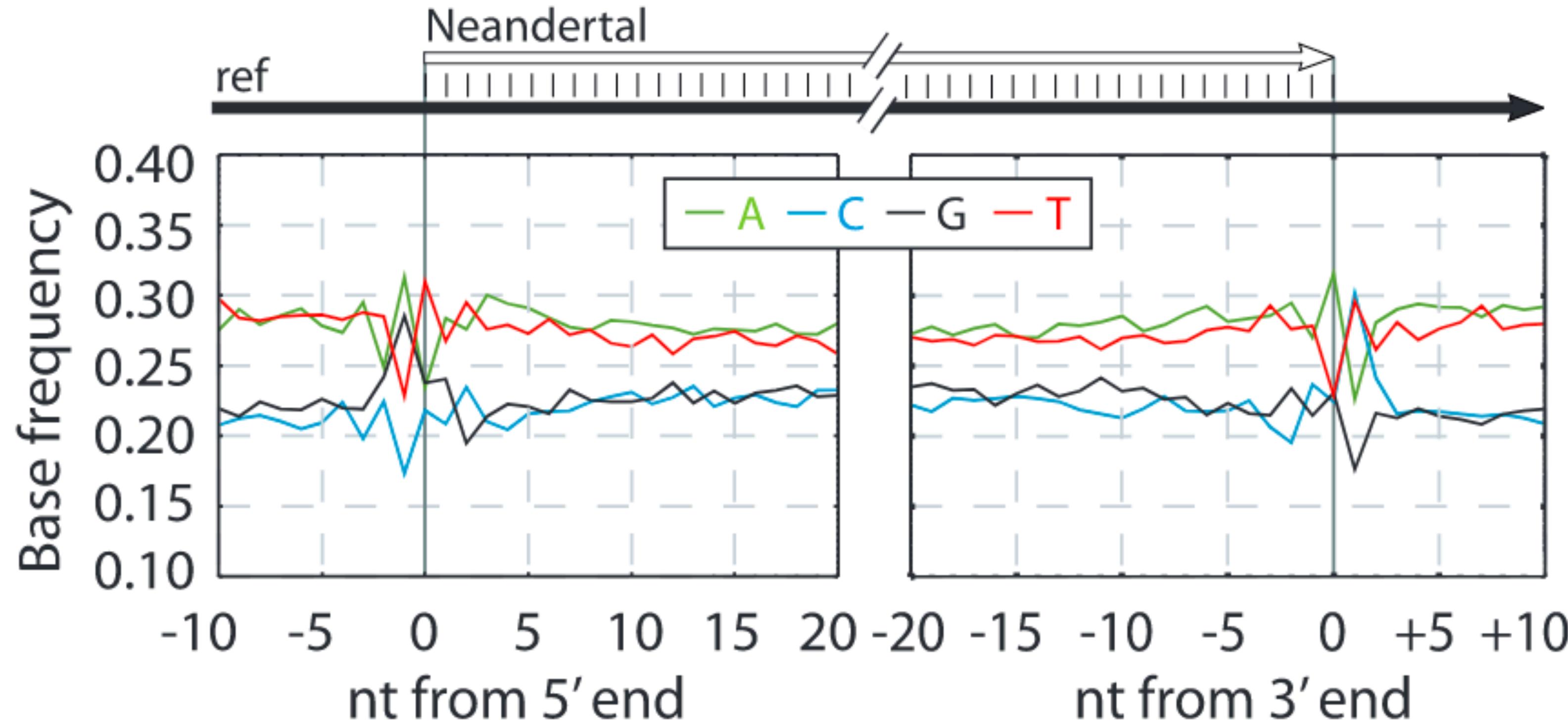
Depurination

- 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)

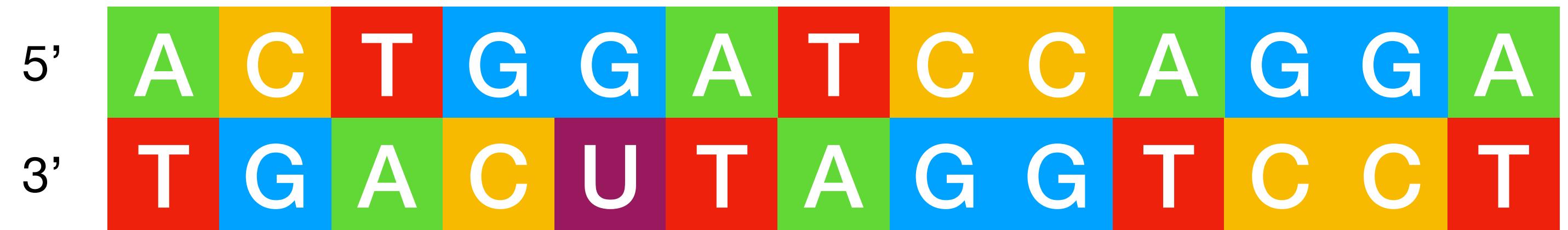
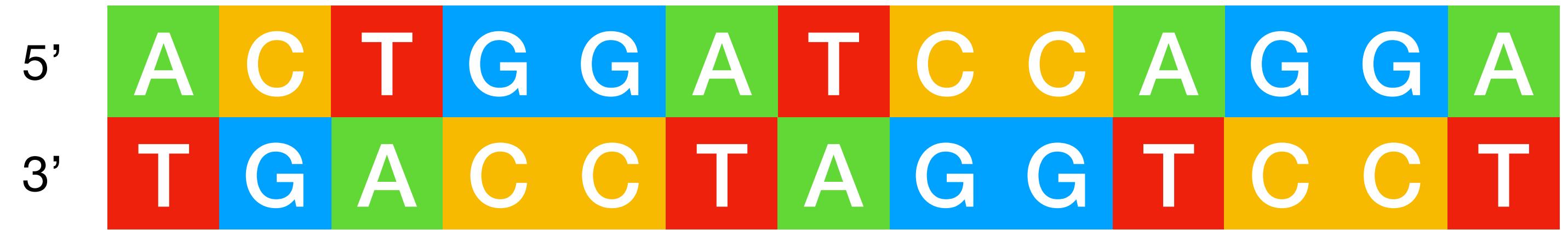
Properties of “old” DNA!



Depurination

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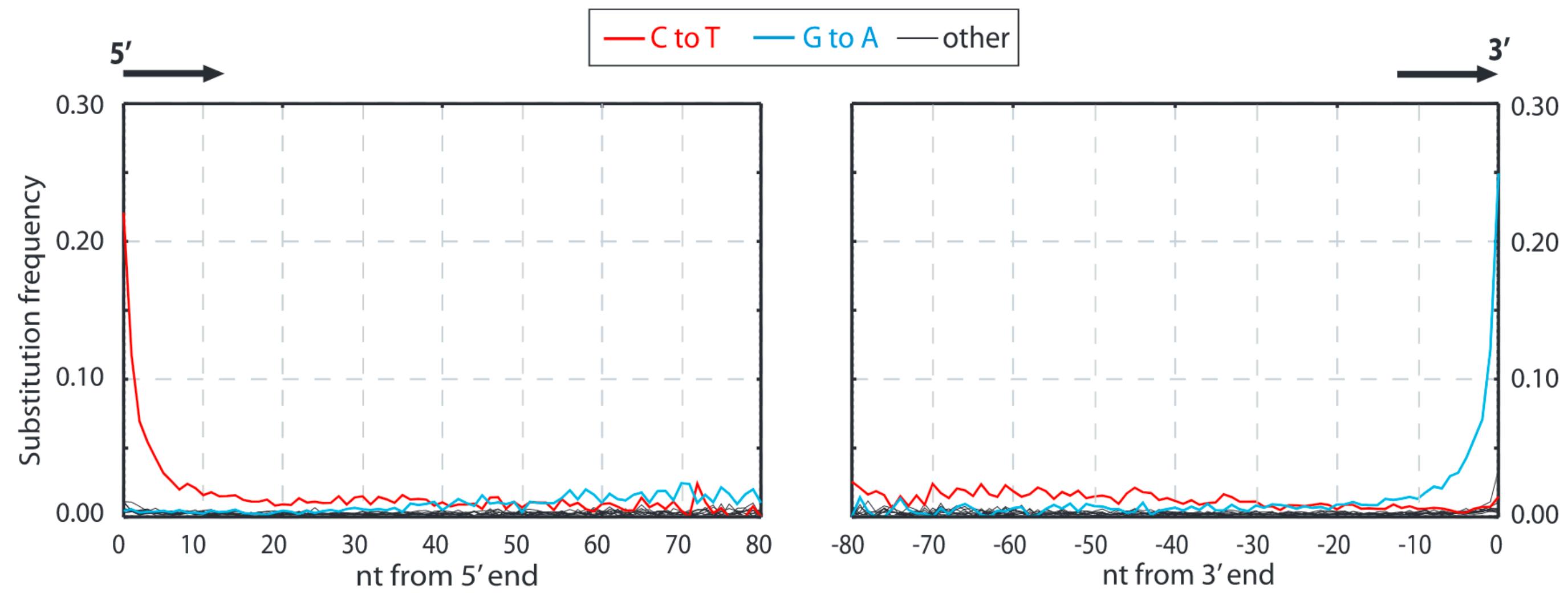
Properties of “old” DNA!



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 !

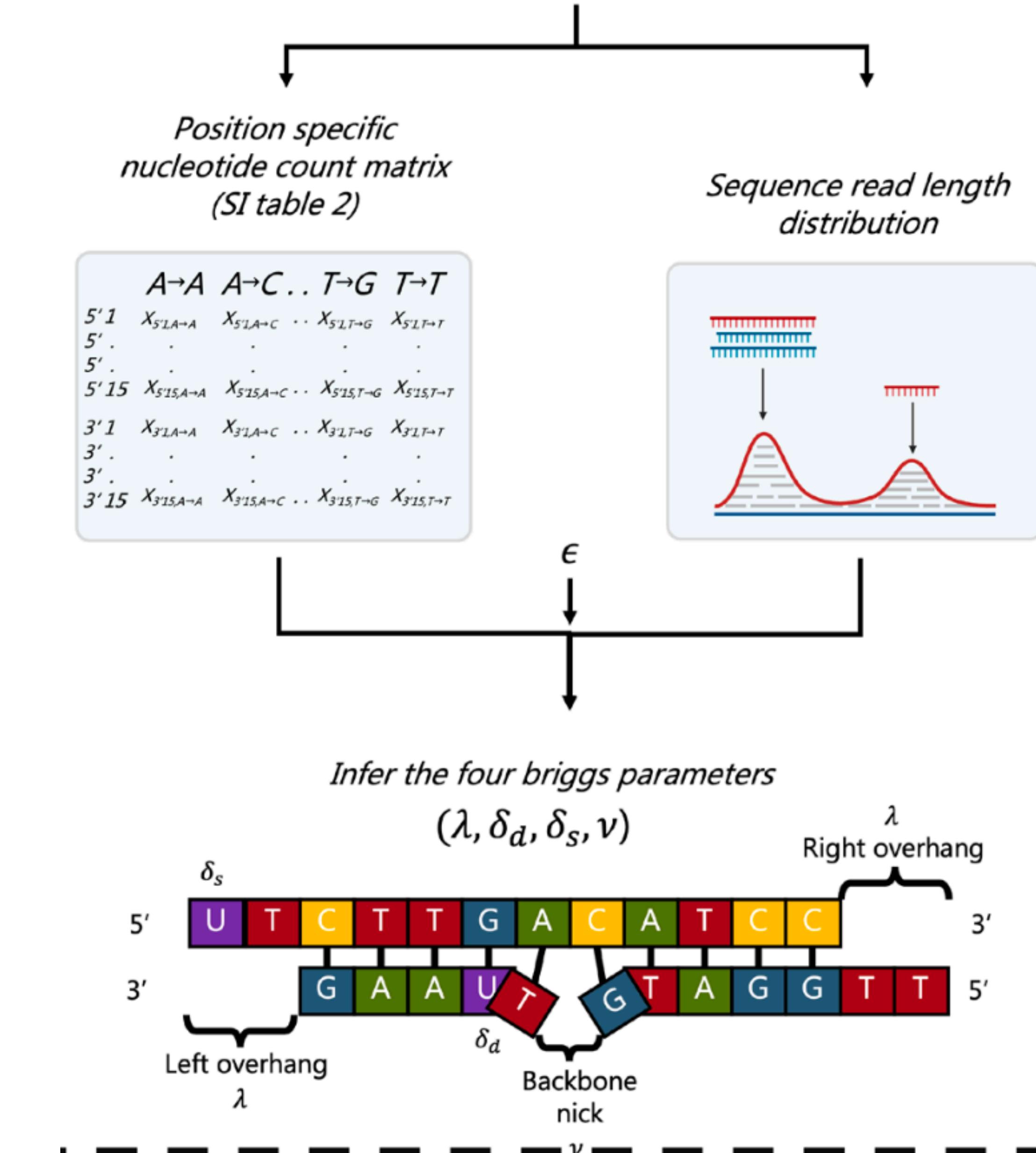
“Briggs”-Parameters

λ : The average length of overhangs

δ_d : Deamination rates in the double-stranded region.

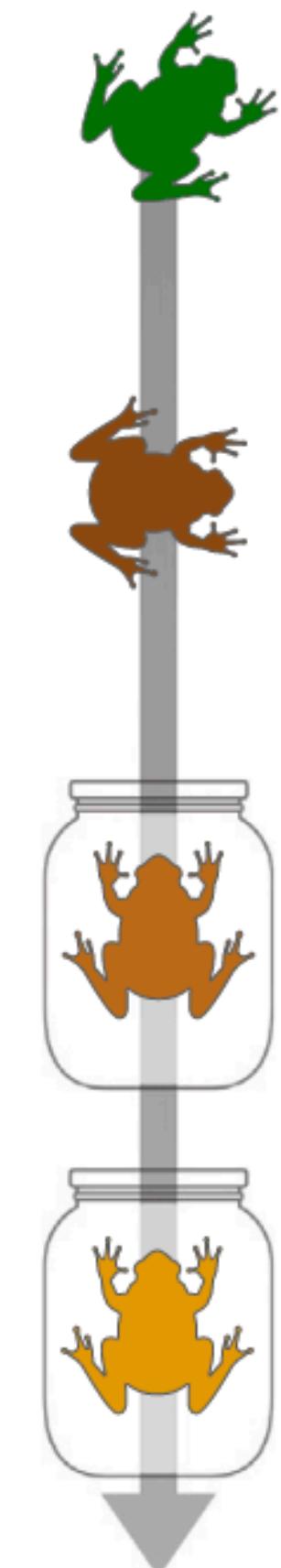
δ_s : Deamination level in the single-stranded (overhang) region.

v : Nick frequency.



Properties of “old” DNA!

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



<i>Specimen history</i>	<i>Archival degradation</i>	<i>Formalin degradation</i>
<i>Living DNA</i>	<u>GTGCCGTA</u>	<u>GTGCCGTA</u>
<i>Specimen preparation</i>	<u>GTGCCGTA</u> <i>Shearing</i>	<u>GTGUCGTA</u> <i>Deamination</i>
<i>Museum storage (recent)</i>	<u>GTGUCGTA</u> <i>Depurination</i>	<u>GTGUUGTA</u> <i>Depurination</i>
<i>Museum storage (older)</i>	<u>GTGUUCGTA</u> <i>Deamination</i>	<u>GTGUUGTA</u> <i>Depurination</i>

Let's do some analyses

The screenshot shows a GitHub repository page titled 'capoony/MuseomicsWorkshop2025'. The page includes a 'README' file and an 'MIT license' file. The main content is titled 'Museomics Workshop 2025: Bioinformatics Pipeline' and describes the bioinformatics workflow for processing and analyzing sequencing data for the Museomics Workshop 2025. A '1. Preparation' section is listed. On the right side, there are sections for 'Packages' (No packages published, Publish your first package), 'Languages' (Shell 66.5%, Python 33.5%), and 'Suggested workflows' (Based on your tech stack).

README MIT license

Museomics Workshop 2025: Bioinformatics Pipeline

This document describes the bioinformatics workflow for processing and analyzing sequencing data for the Museomics Workshop 2025.

1. Preparation

Packages

No packages published [Publish your first package](#)

Languages

Shell 66.5% Python 33.5%

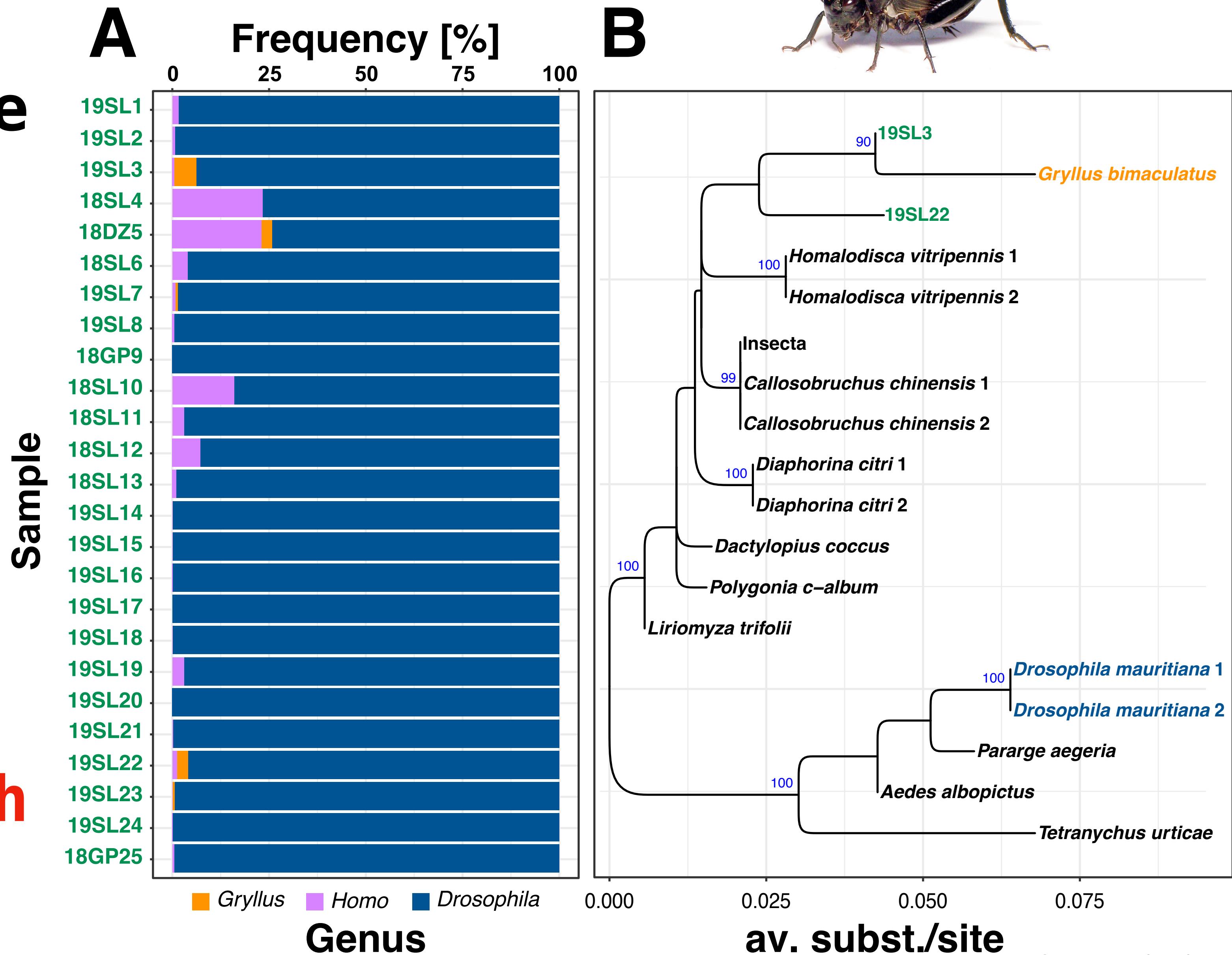
Suggested workflows

Based on your tech stack

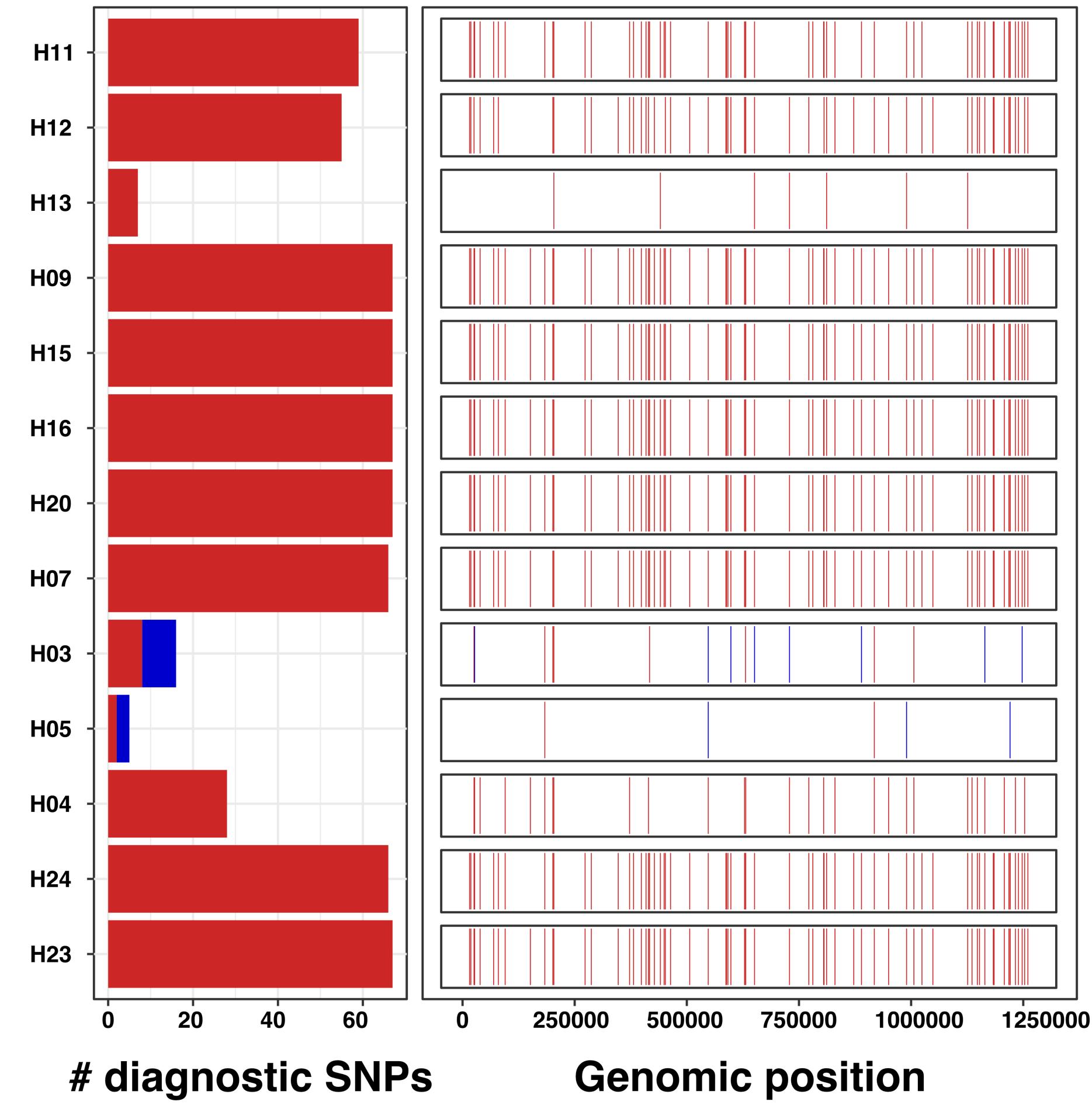
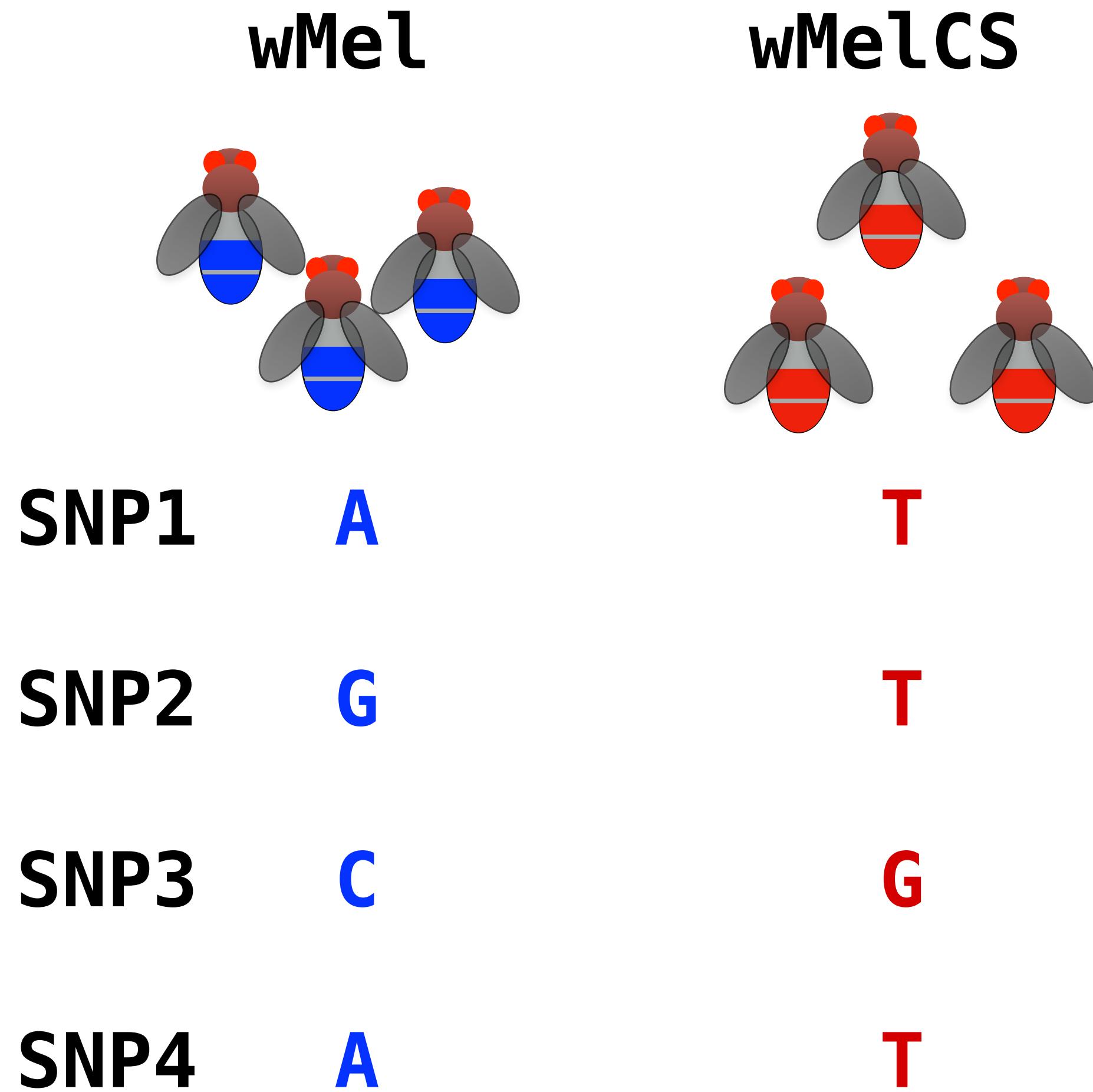
<https://github.com/capoony/MuseomicsWorkshop2025>

Contamination revisited!

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



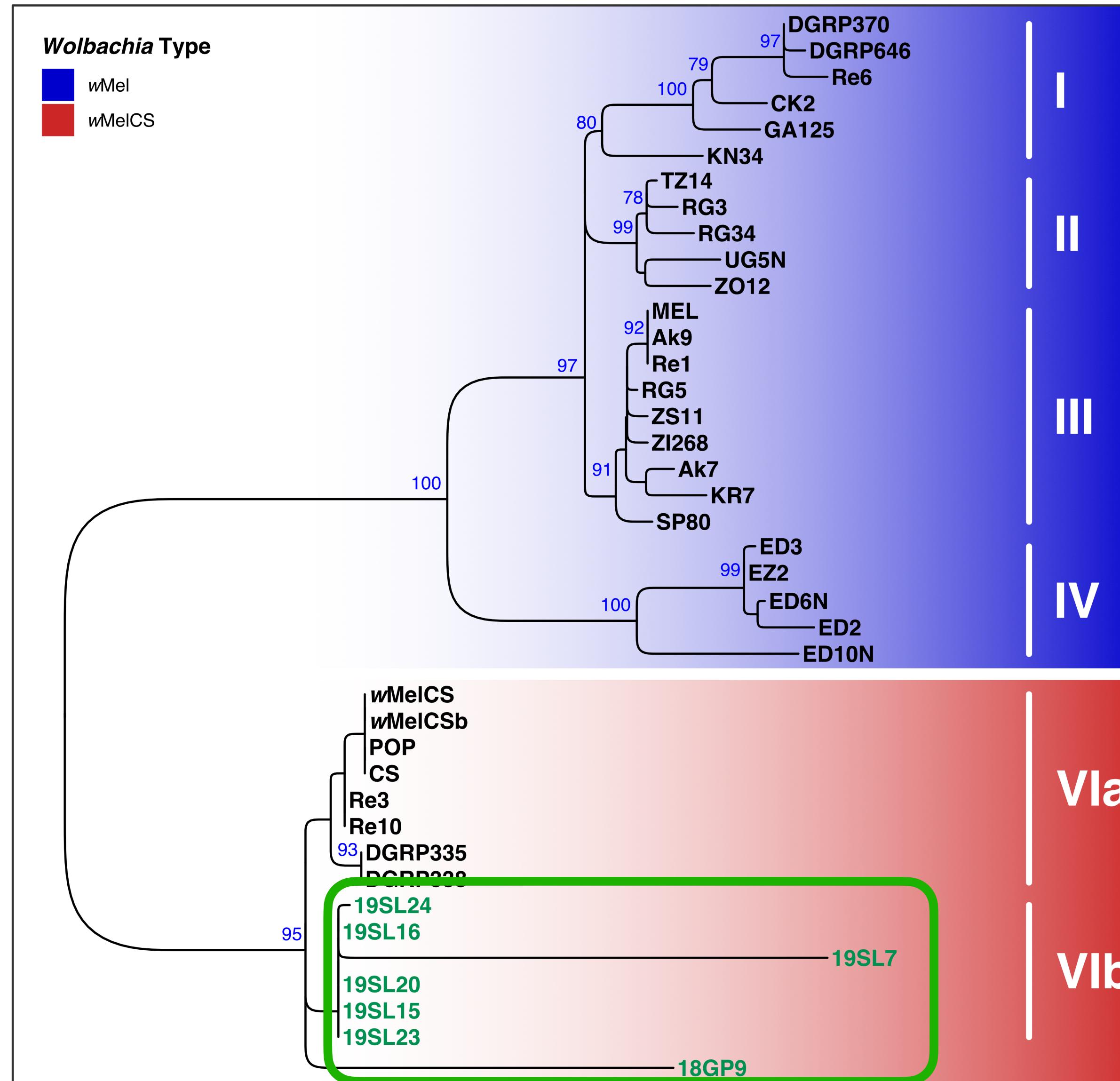
Which *Wolbachia*?



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

Phylogenetic reconstruction

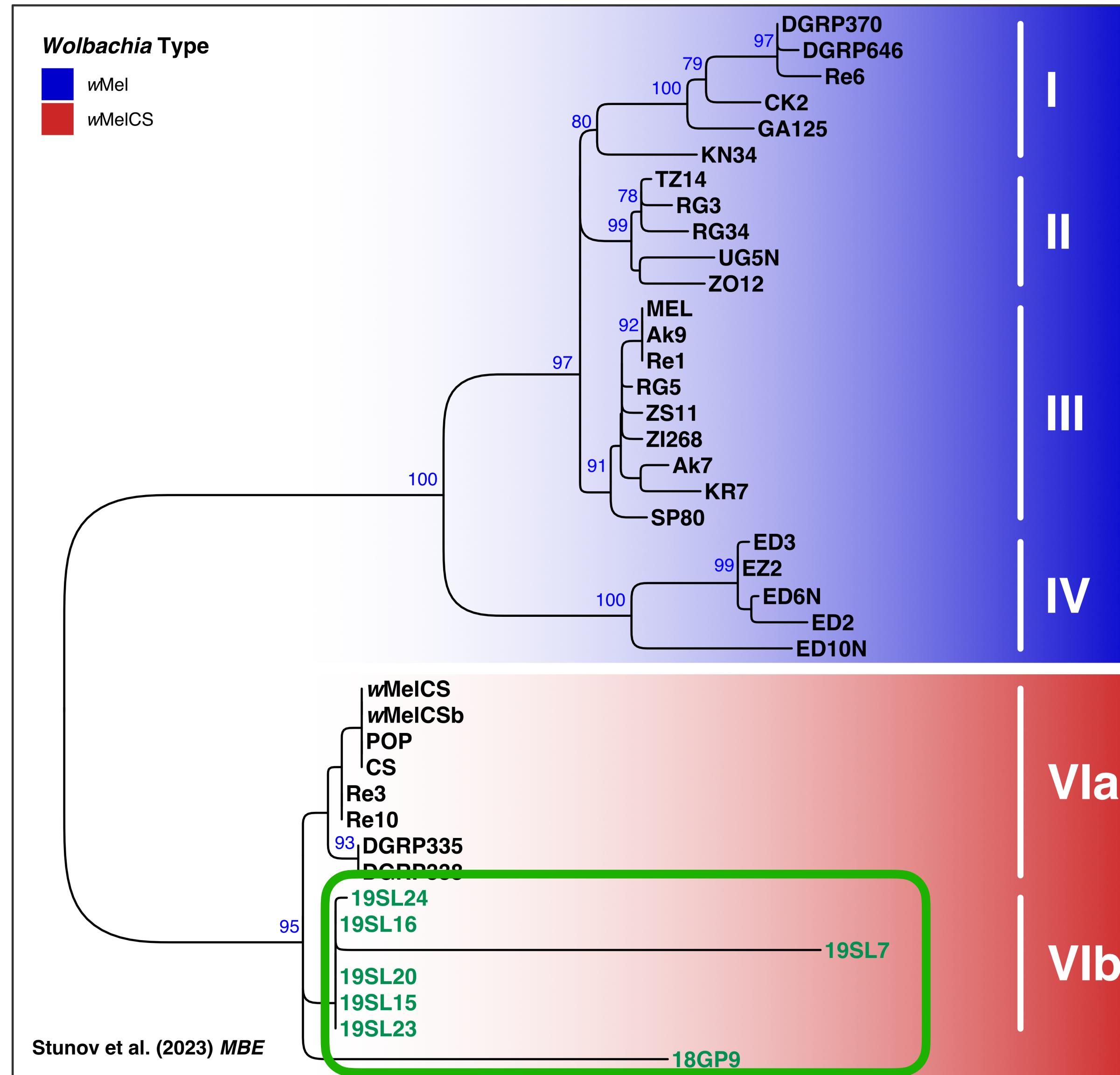
Genome-wide SNPs



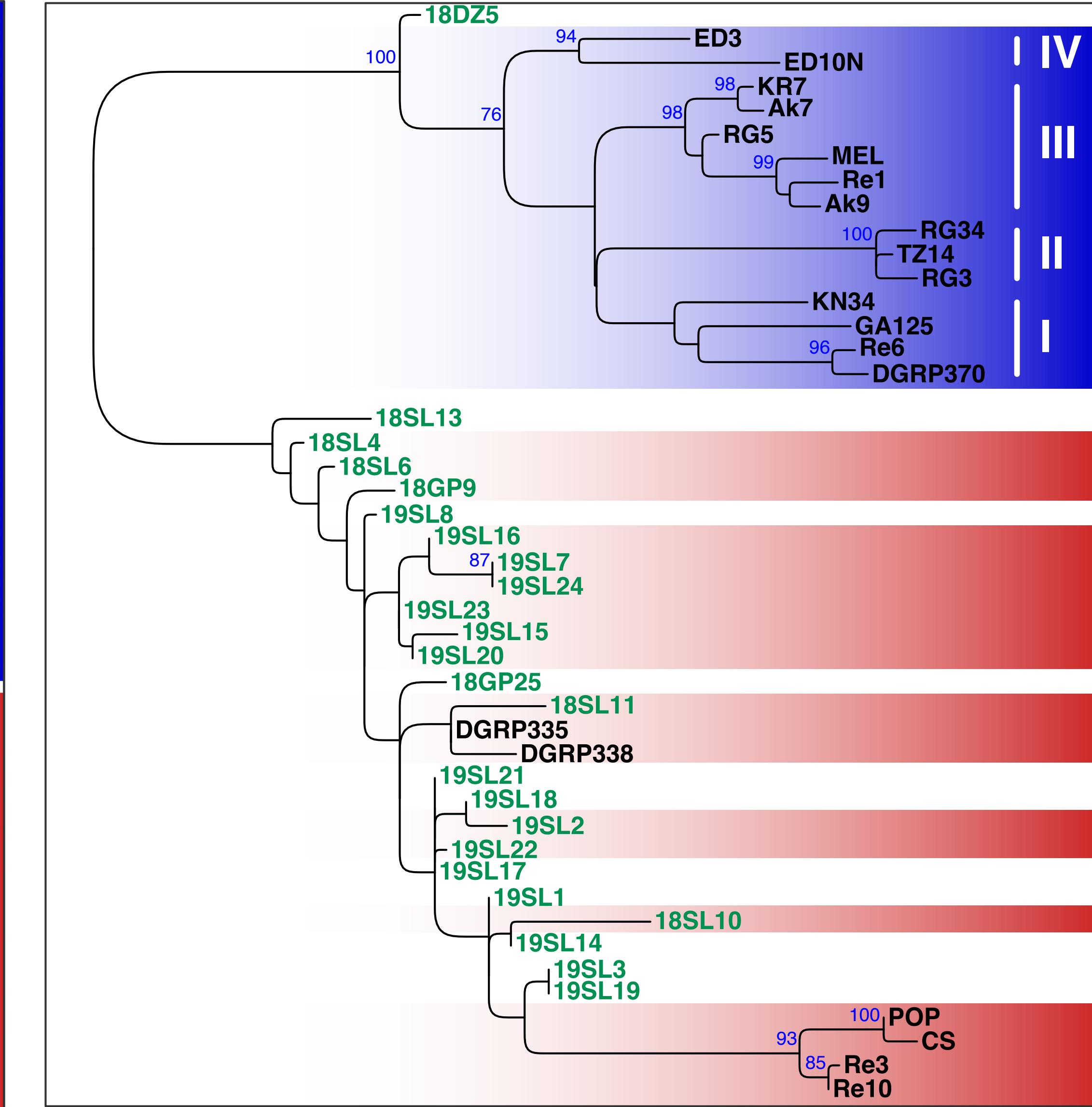
- 279 genome-wide SNPs used for ML tree reconstruction with RAxML.
- 40 samples
 - 7 historic
 - 33 recent

What about mitochondria?

Genome-wide SNPs

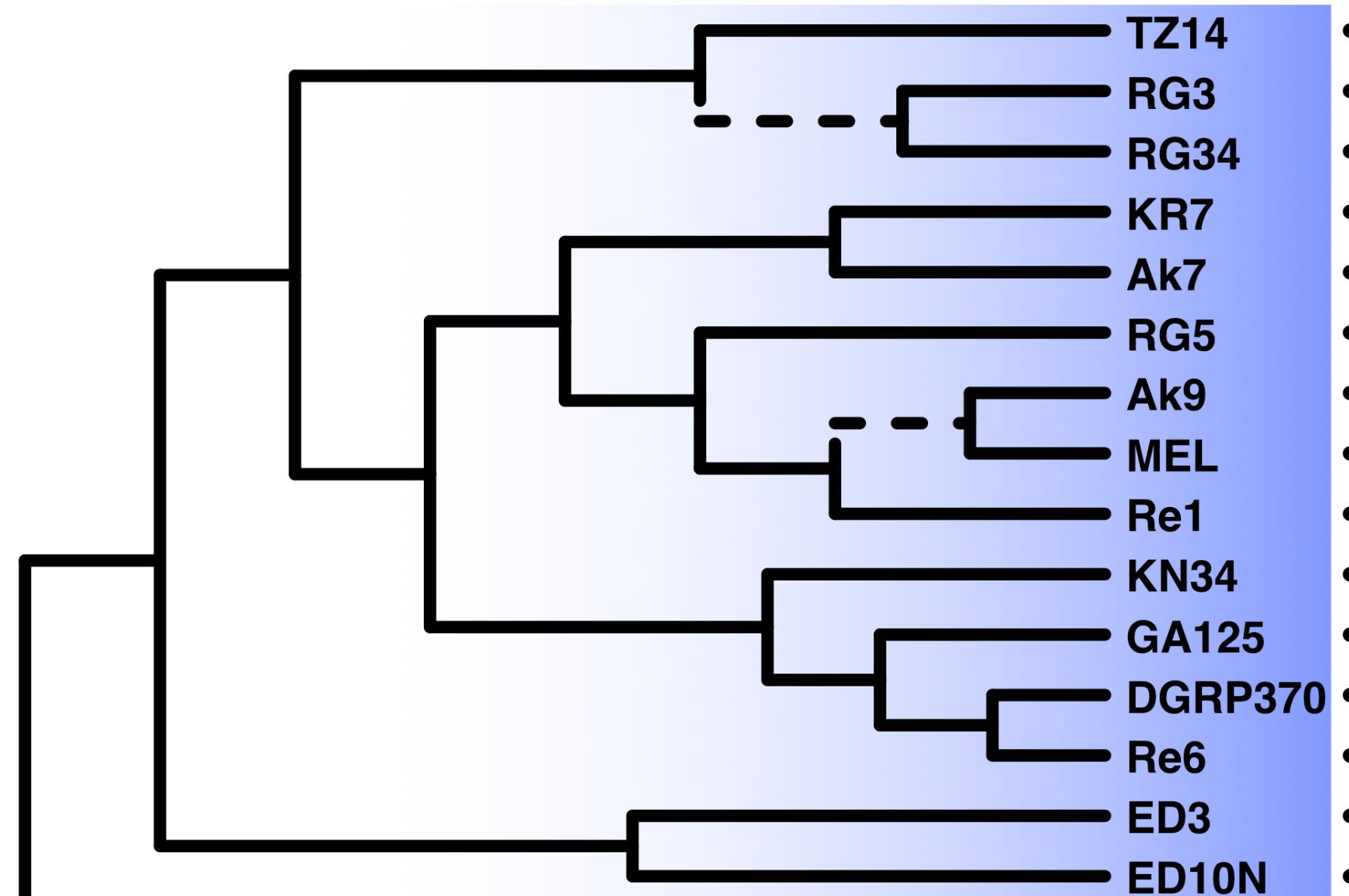


Mitochondria

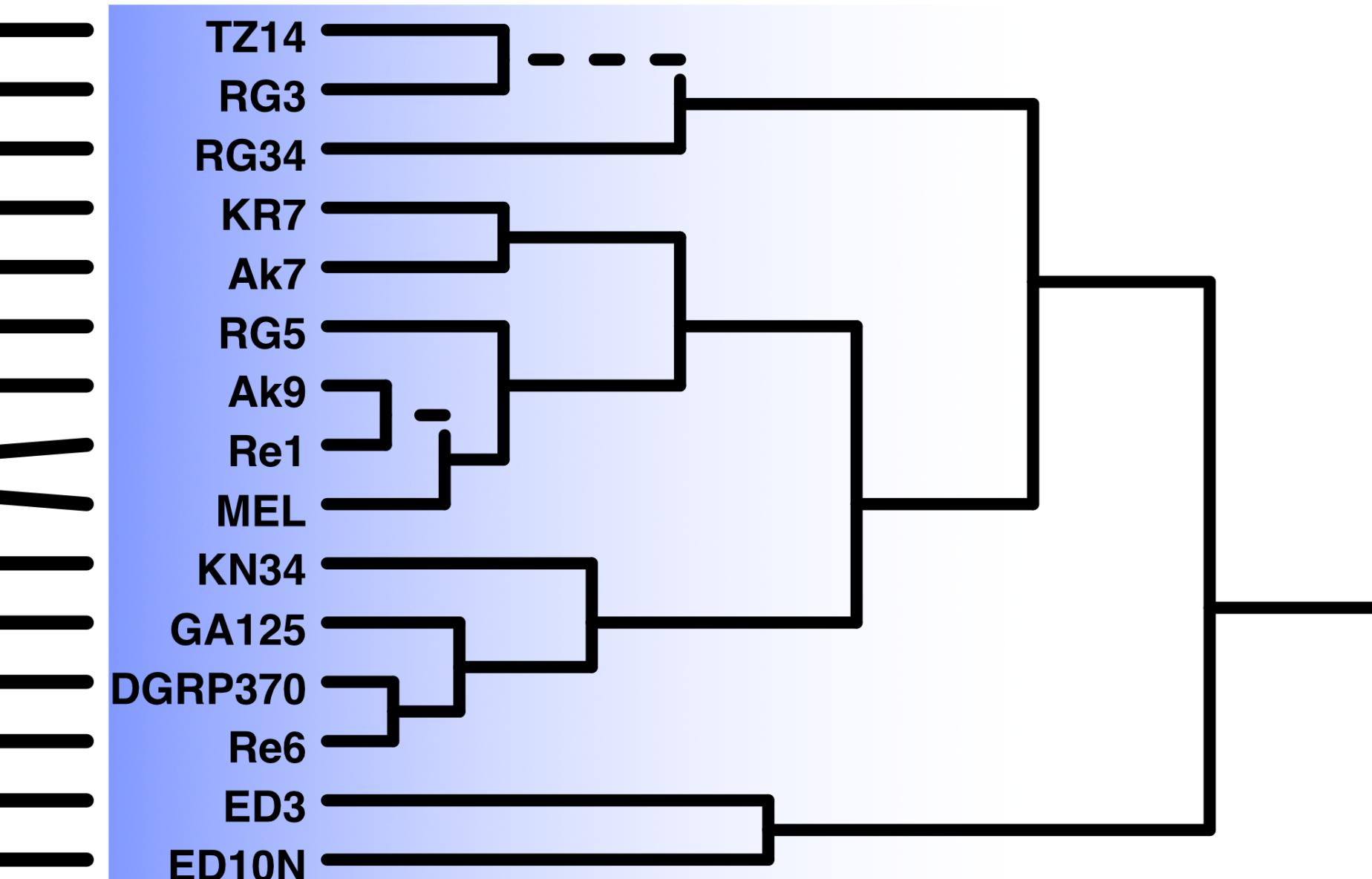


Tight relationship

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

Thank you very much . . .



FWF

Der Wissenschaftsfonds.

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NHM Vienna
Elisabeth Haring
Alexandra Wanka
Sandra Kirchner
Luise Kruckenhauser



MEDIZINISCHE
UNIVERSITÄT WIEN

naturhistorisches
museum wien **nhm**

... 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

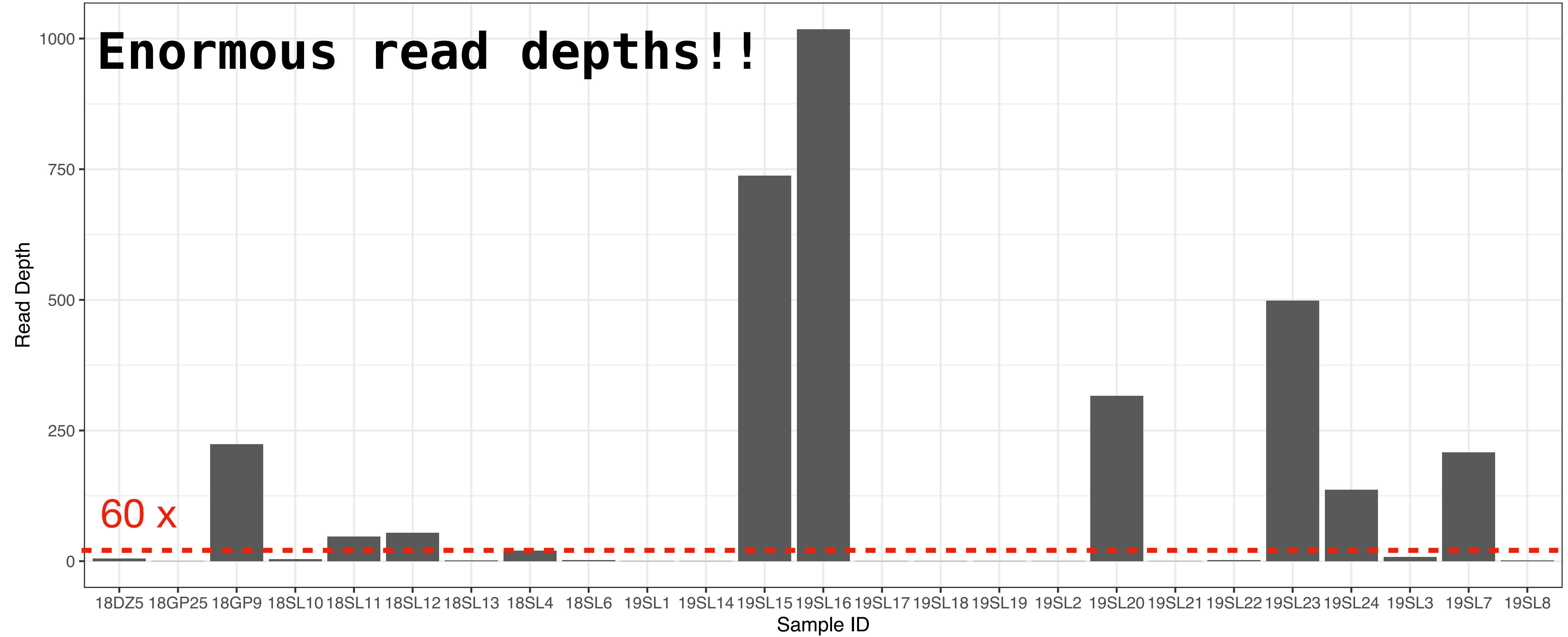
***Corresponding author:** E-mail: martin.kapun@nhm-wien.ac.at.

Associate editor: Daniel Falush

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

https://github.com/capoony/WolbachiaEvolHist_2023

Denovo *Wolbachia* assemblies



Denovo *Wolbachia* assemblies

- denovo assembly with SPAdes
- QC with BL0Btools
 - GC-content
 - Read-depth
 - Contig Size
 - BLAST hits

