

# Museomics: a window to past population history

Jérémy Gauthier

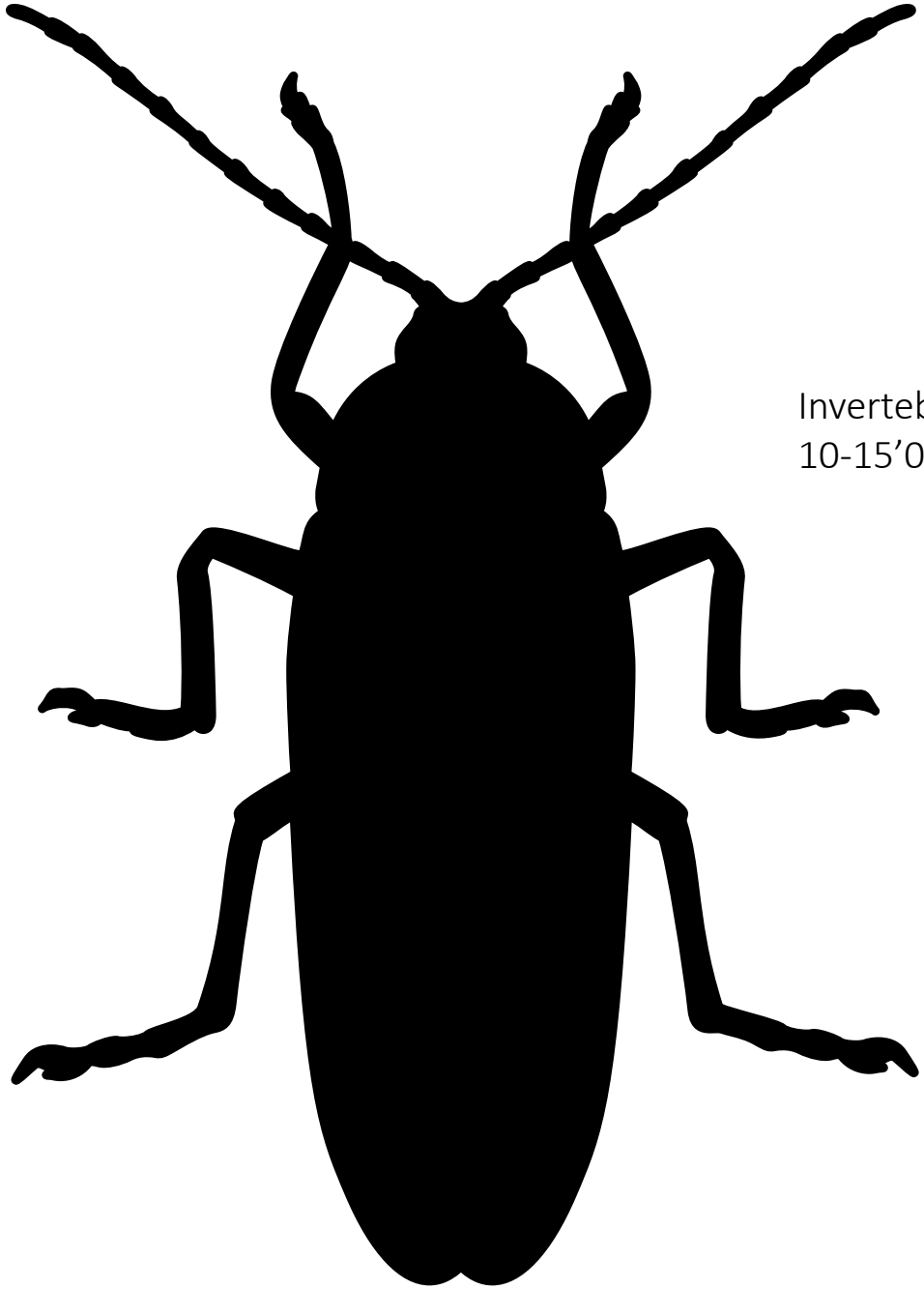


2-3 billion specimens in  
museums !

Muséum d'histoire  
naturelle de Genève  
(MHNG)

15 million specimens





Invertebrates  
10-15'000'000 (MHNG)



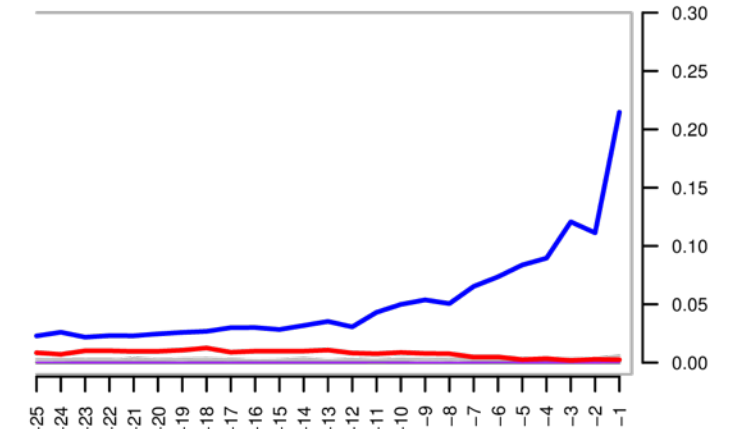
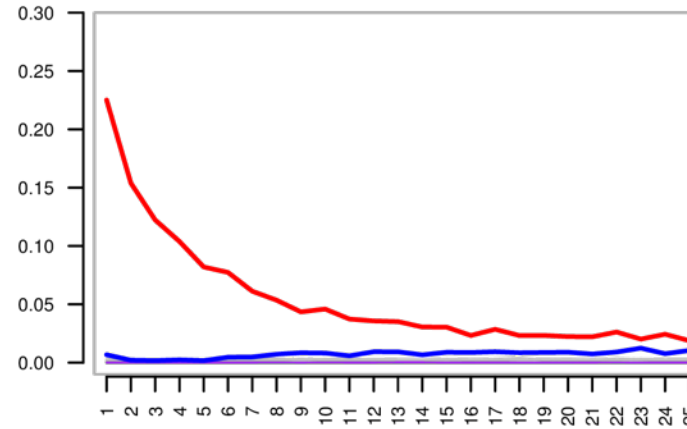
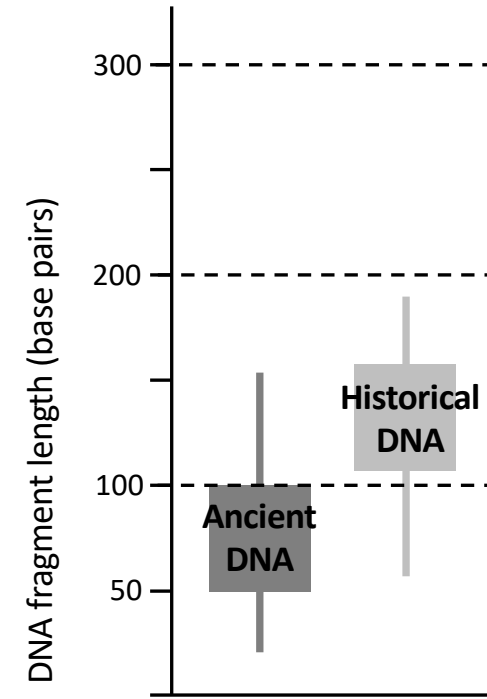
Vertebrates  
330'000 (MHNG)



Minerals and Fossils  
170'000 (MHNG)

# Main issues when working with historical DNA

- Low quantity of DNA
- Contaminations
- Small fragments
- Chemical modifications: deamination

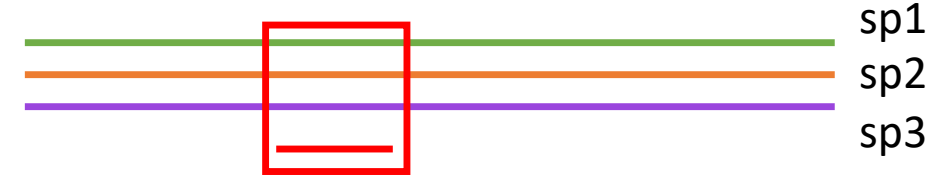


MapDamage2 results

# What approaches to circumvent these problems?

- *Sequence capture using probes (exons, genome, UCE):*

usually requires reference genomes  
in silico probe synthesis is expensive



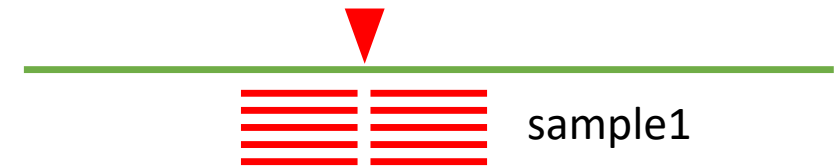
- *Shotgun sequencing, genome skimming:*

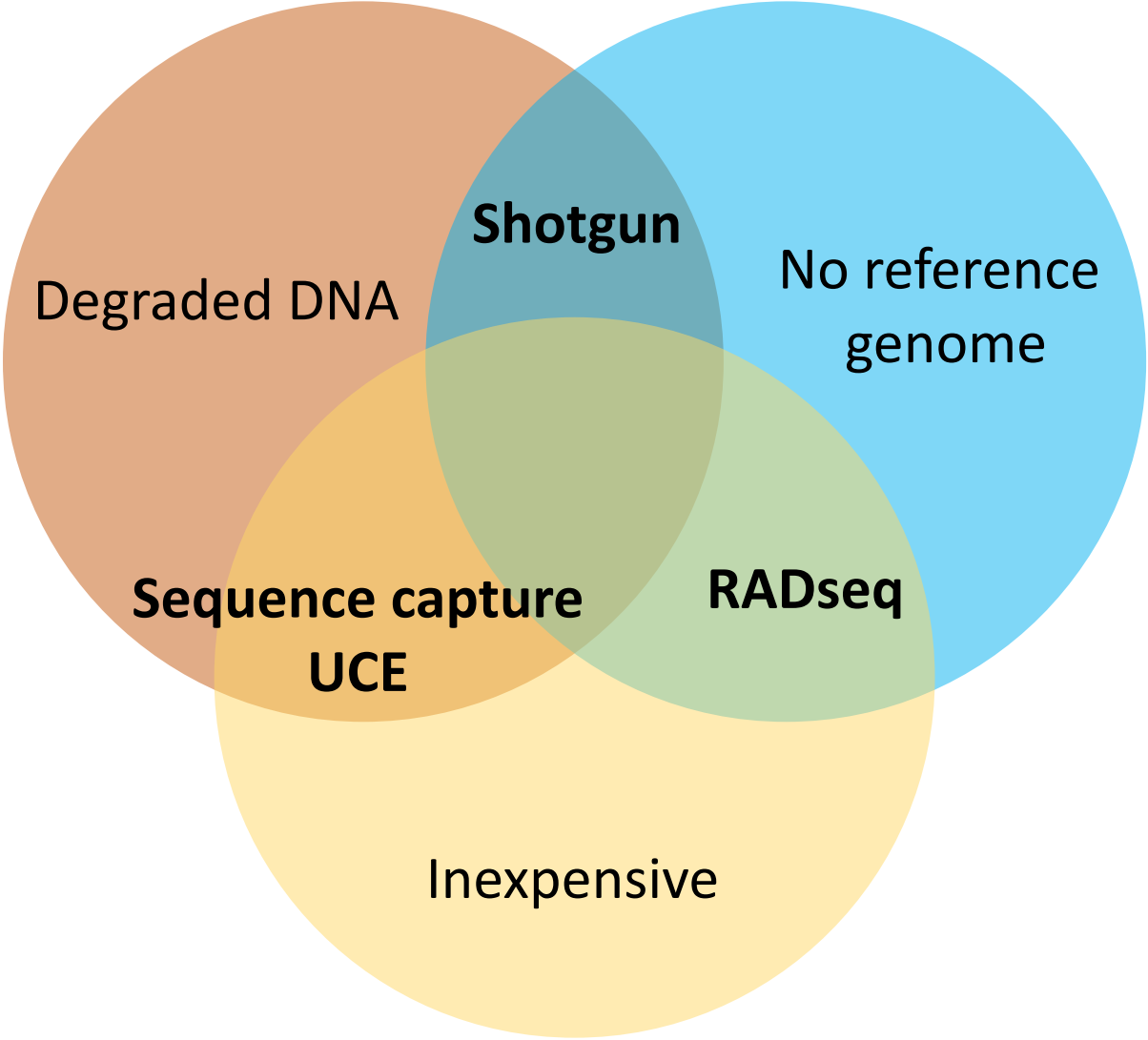
expensive for many samples  
number of shared loci is random



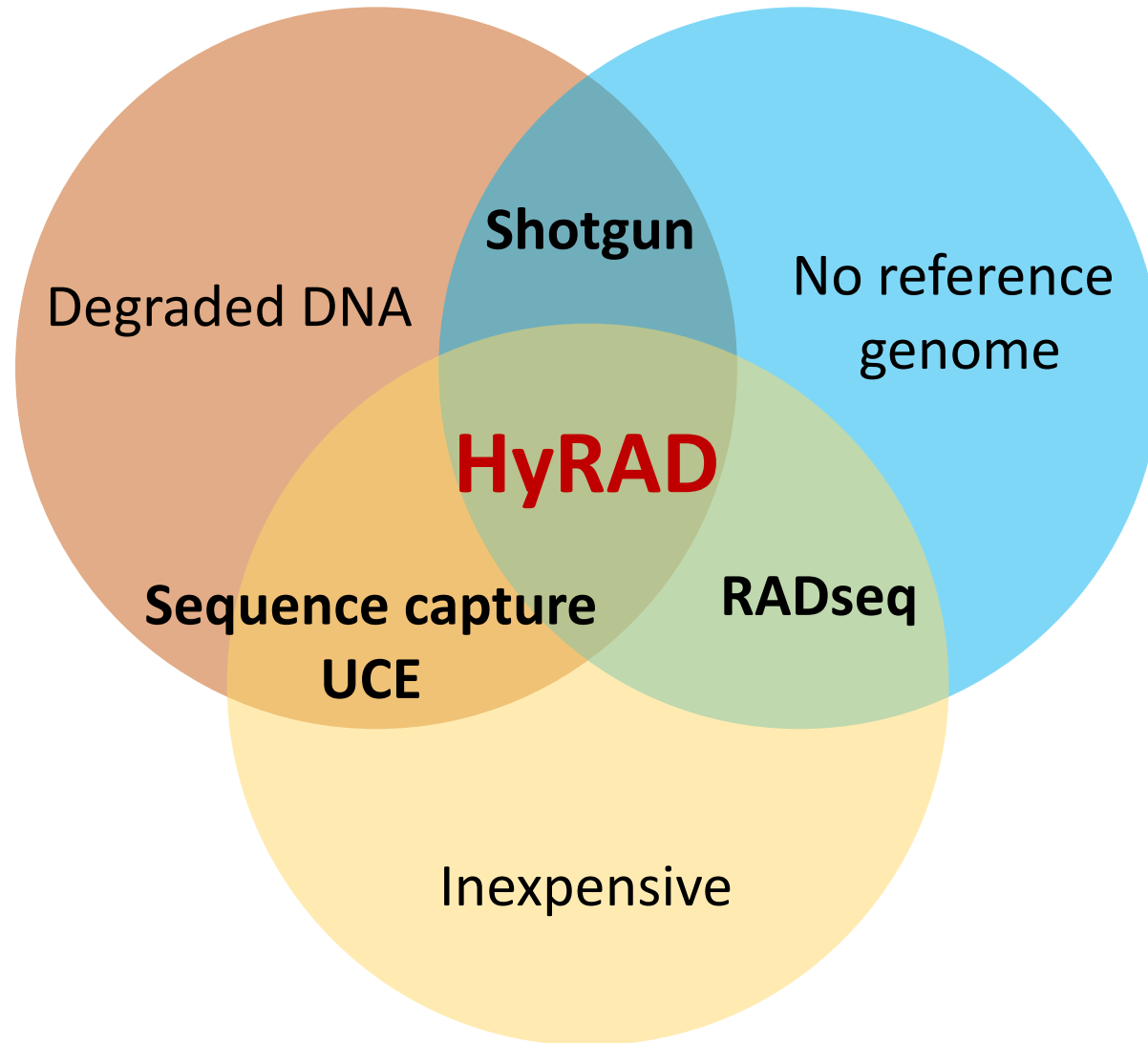
- *RADseq:*

problematic with degraded DNA





# Genome complexity reduction + hybridization capture



Synthesized commercial probes

**RAPTURE** (Ali et al. 2016)

**GBS-capture** (Barreiro et al. 2016)

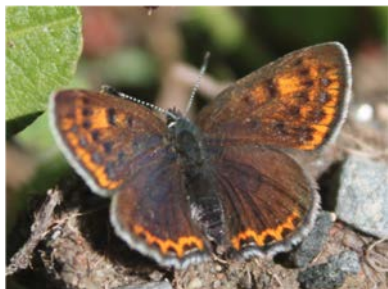
**RADcap** (Hoffberg et al. 2016)

Bench-top produced probes

**HyRAD** (Suchan et al. 2016)

**HyRAD-X** (Schmid et al. 2017)

# HyRAD



Fresh sample DNA  
extraction

genomic DNA

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# HyRAD



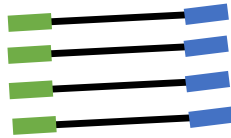
Fresh sample DNA  
extraction

genomic DNA

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ddRAD library



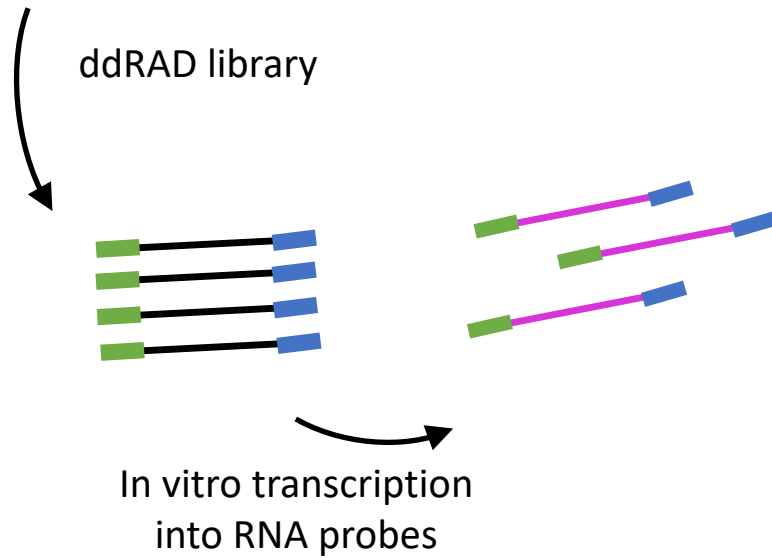
# HyRAD



Fresh sample DNA  
extraction

genomic DNA

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# HyRAD



Fresh sample DNA extraction

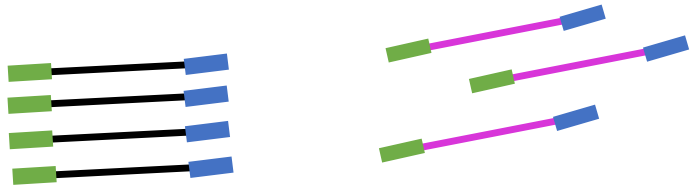


Historical DNA extraction

genomic DNA



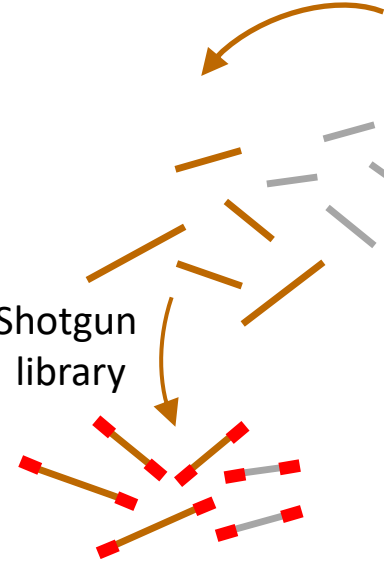
ddRAD library



In vitro transcription into RNA probes



Shotgun library



Including contaminant or untargeted loci

# HyRAD



Fresh sample DNA extraction

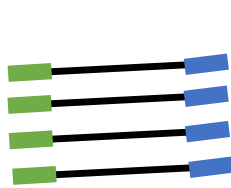


Historical DNA extraction

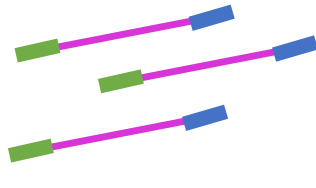
genomic DNA



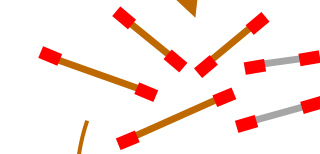
ddRAD library



In vitro transcription into RNA probes

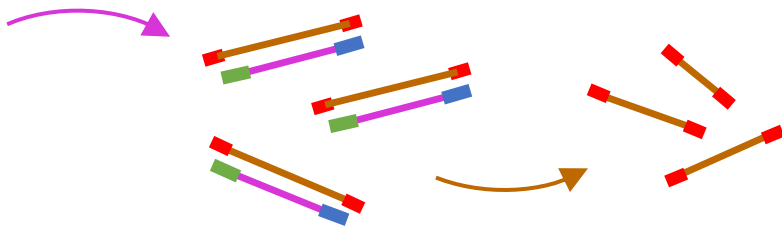


Shotgun library



Including contaminant or untargeted loci

Hybridization



Capture and wash

# HyRAD



Fresh sample DNA extraction

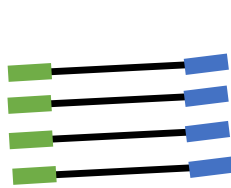


Historical DNA extraction

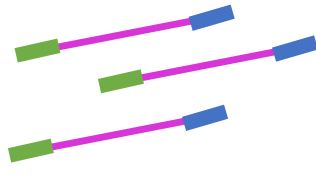
genomic DNA



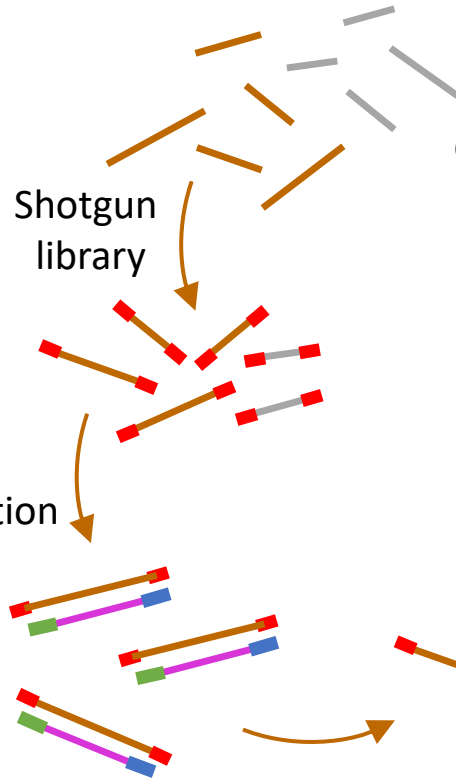
ddRAD library



In vitro transcription into RNA probes

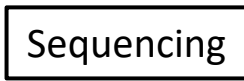


Hybridization



Capture and wash

Sequencing



# HyRAD



Fresh sample DNA extraction

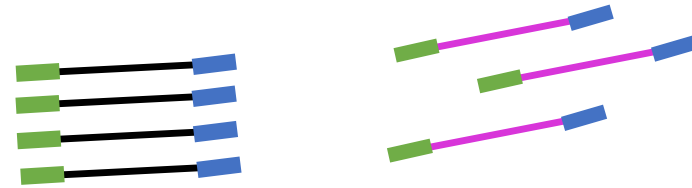


Historical DNA extraction

genomic DNA



ddRAD library



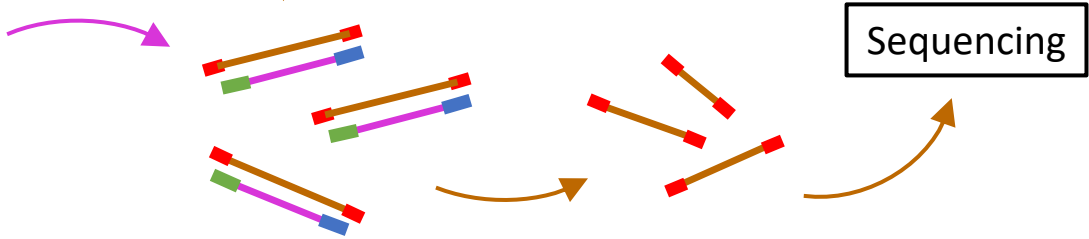
In vitro transcription into RNA probes

Probes sequencing

Shotgun library

Including contaminant or untargeted loci

Hybridization



Capture and wash

Sequencing

# Evolution of Carabinae: wing loss in Calosomes

Museomics

Collection samples

HyRAD-X protocol



*Haplothorax burchellii*

# Evolution of Carabinae: wing loss in Calosomes

## Museomics

Collection samples  
HyRAD-X protocol

Classic DNA extraction

one leg is removed



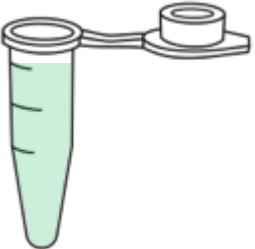
crushed



DNA extraction

Non-destructive DNA extraction

Dried and back  
on the specimen



DNA extraction



leg in buffer  
with proteinase K

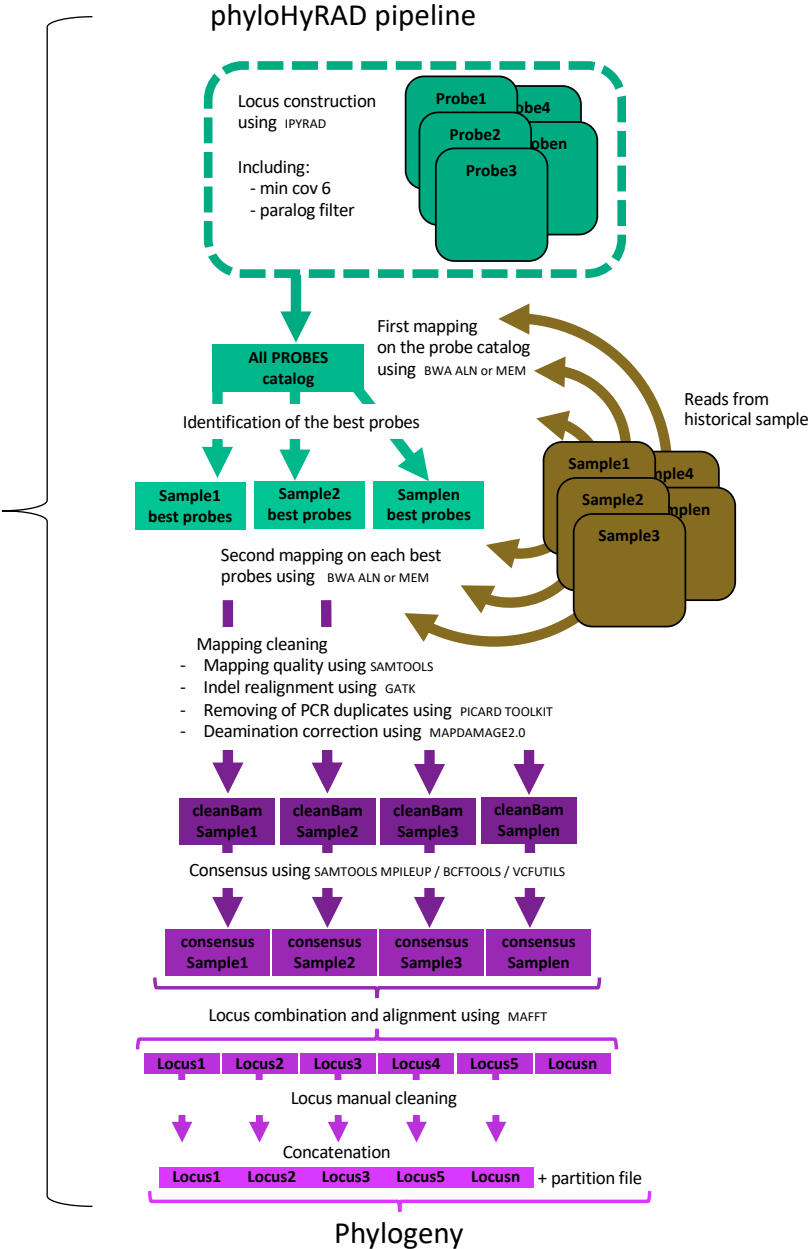


*Haplothorax burchellii*



# Evolution of Carabinae: wing loss in Calosomes

Museomics  
 Collection samples  
 HyRAD-X protocol  
 ↓  
 phyloHyRAD pipeline



# Evolution of Carabinae: wing loss in Calosomes

Museomics  
 Collection samples  
 HyRAD-X protocol

↓

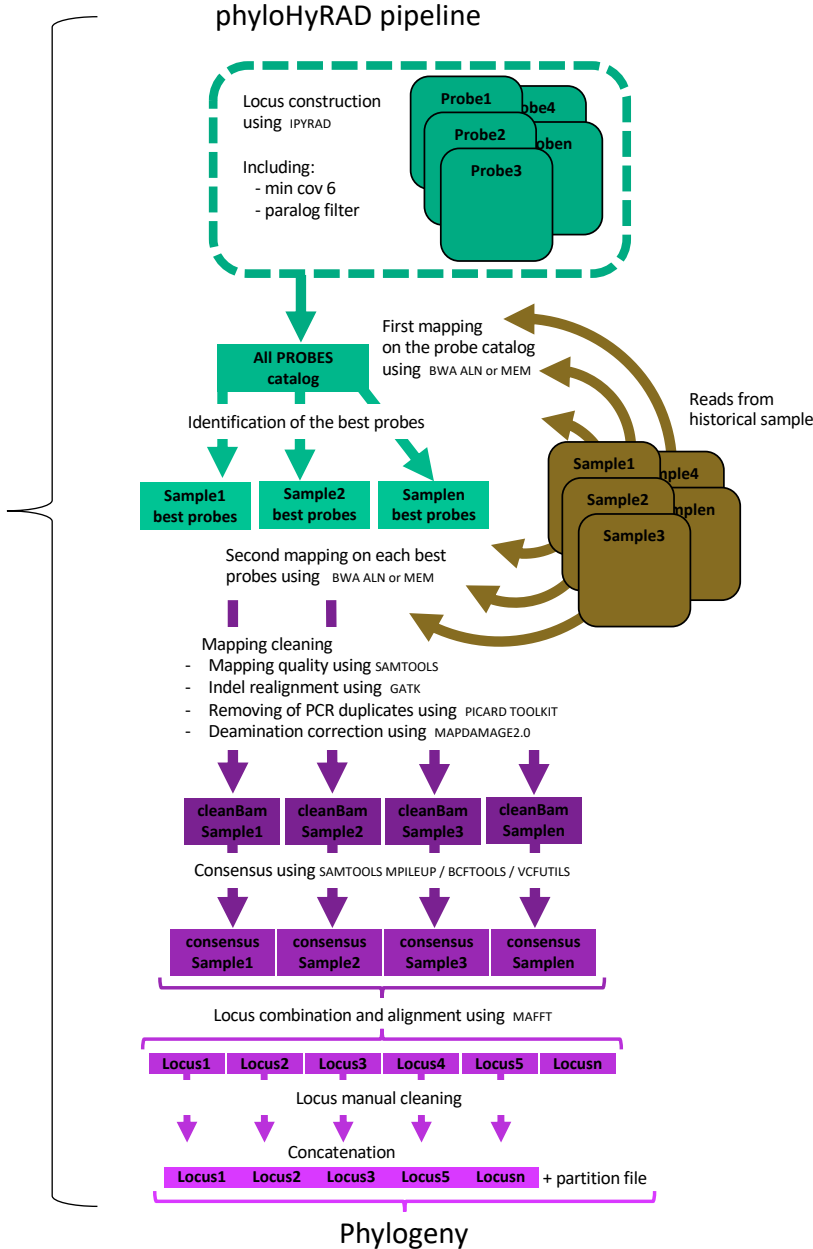
phyloHyRAD pipeline

↓

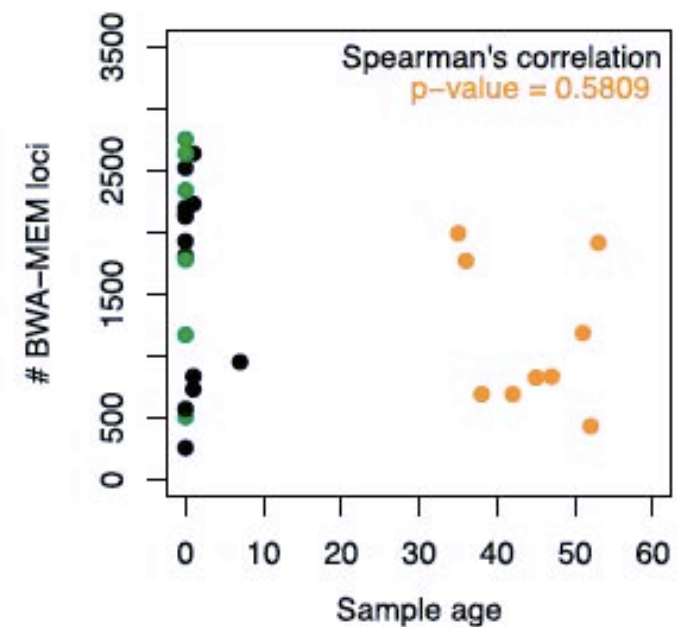
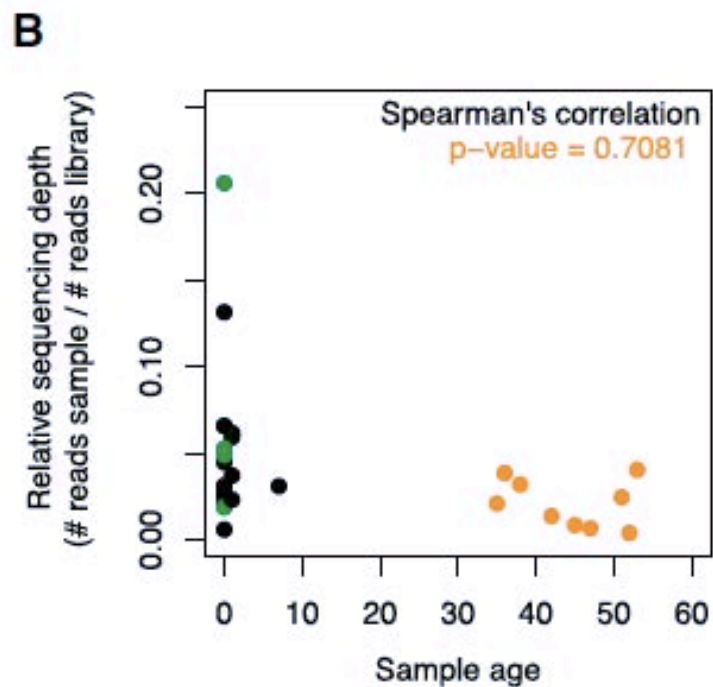
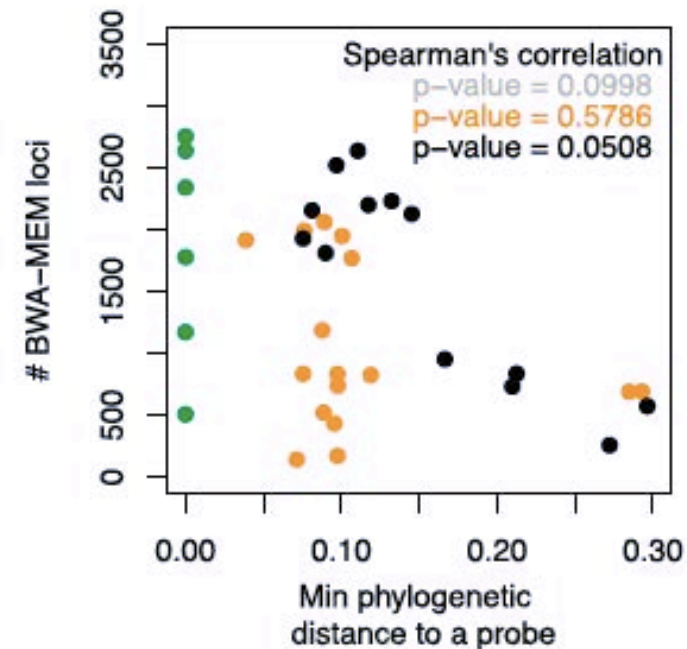
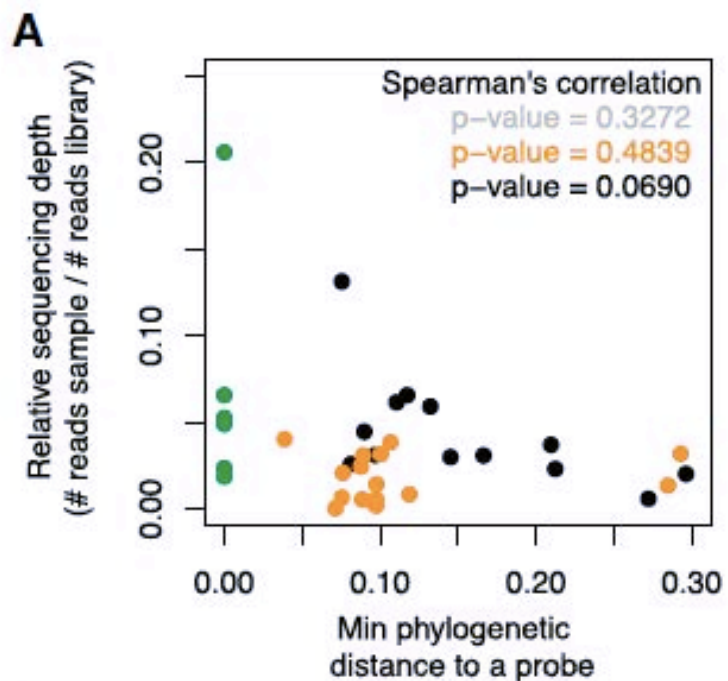
2945 shared loci

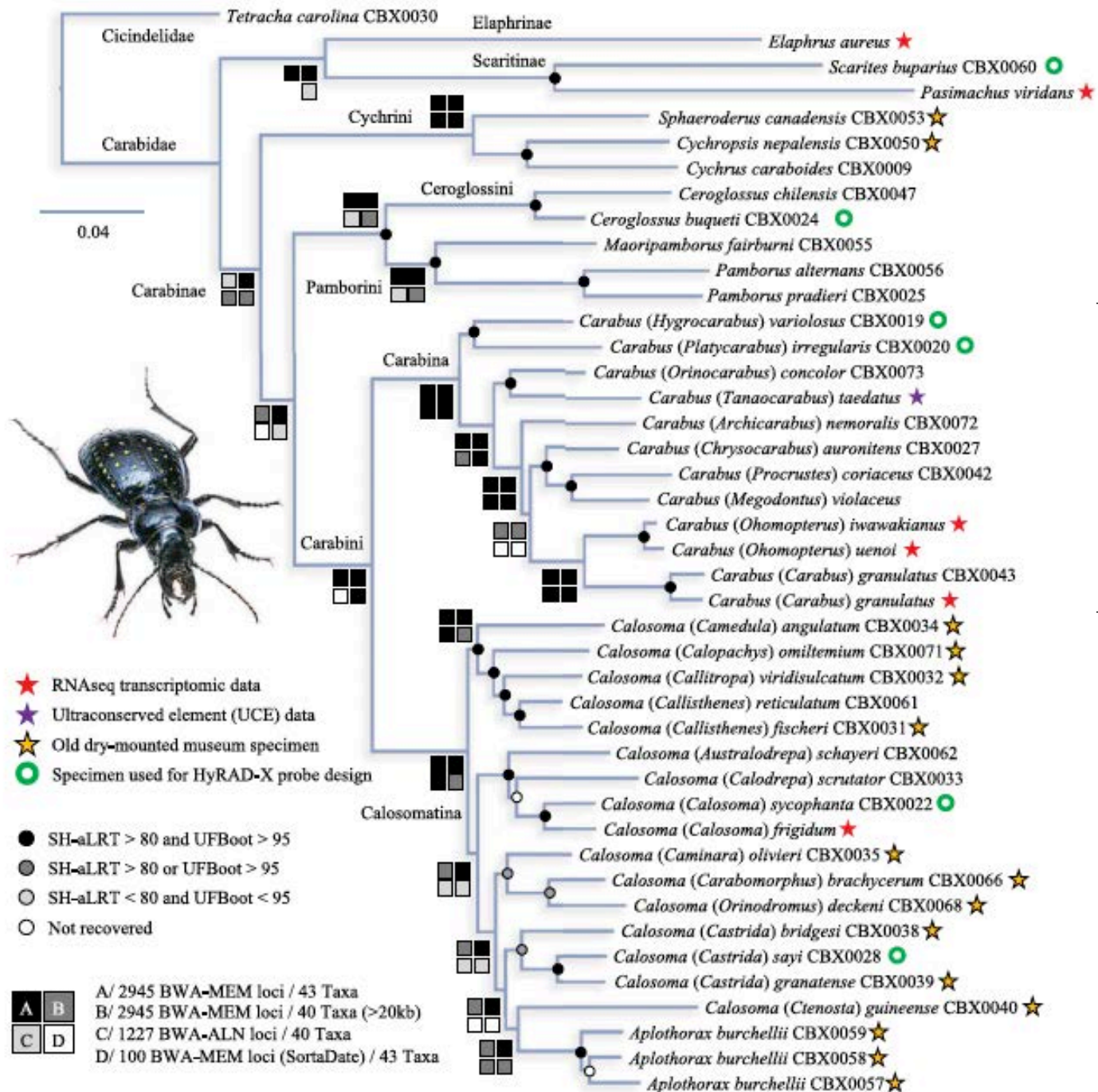
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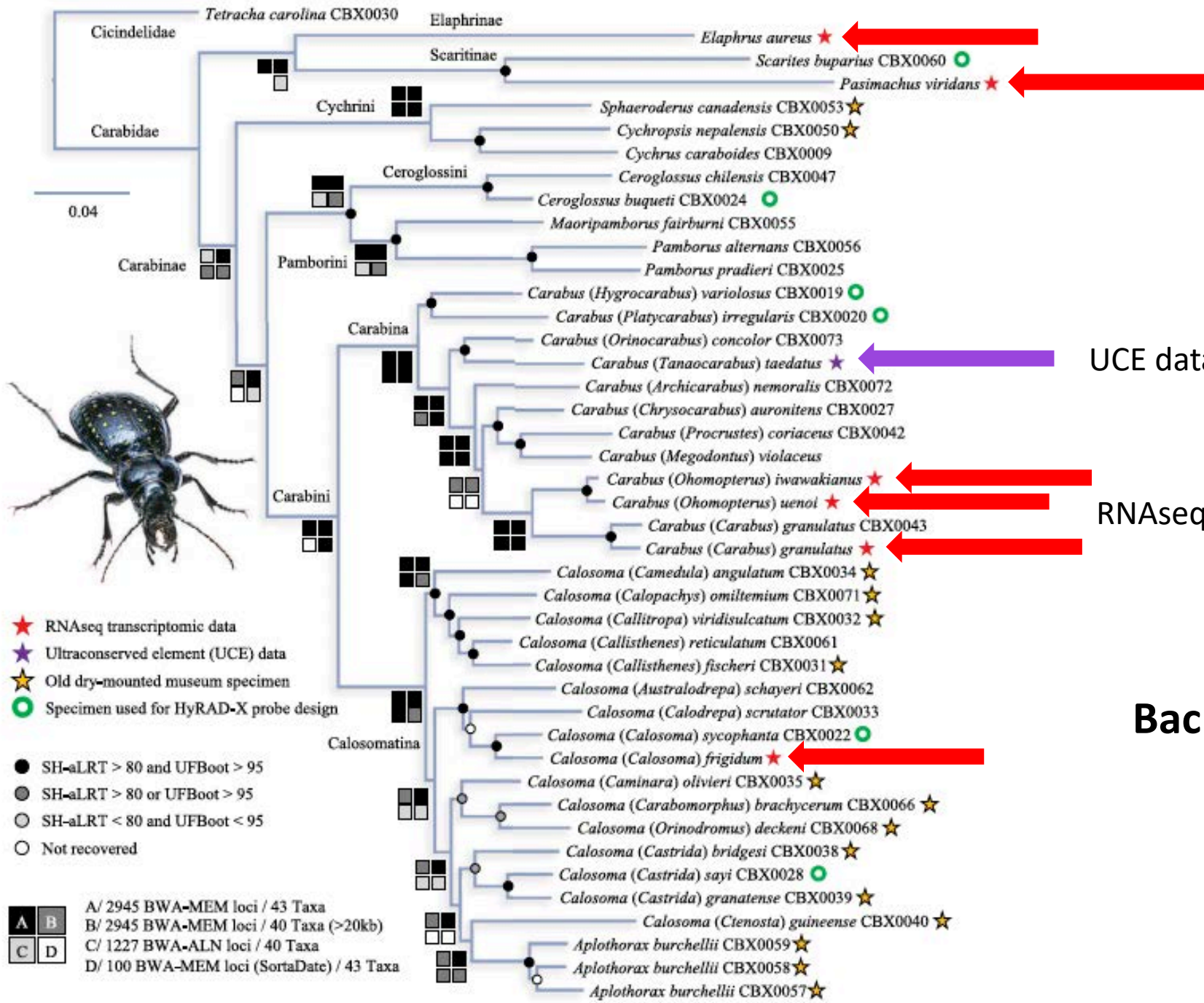
Phylogenomic inferences



# Putative biases ?



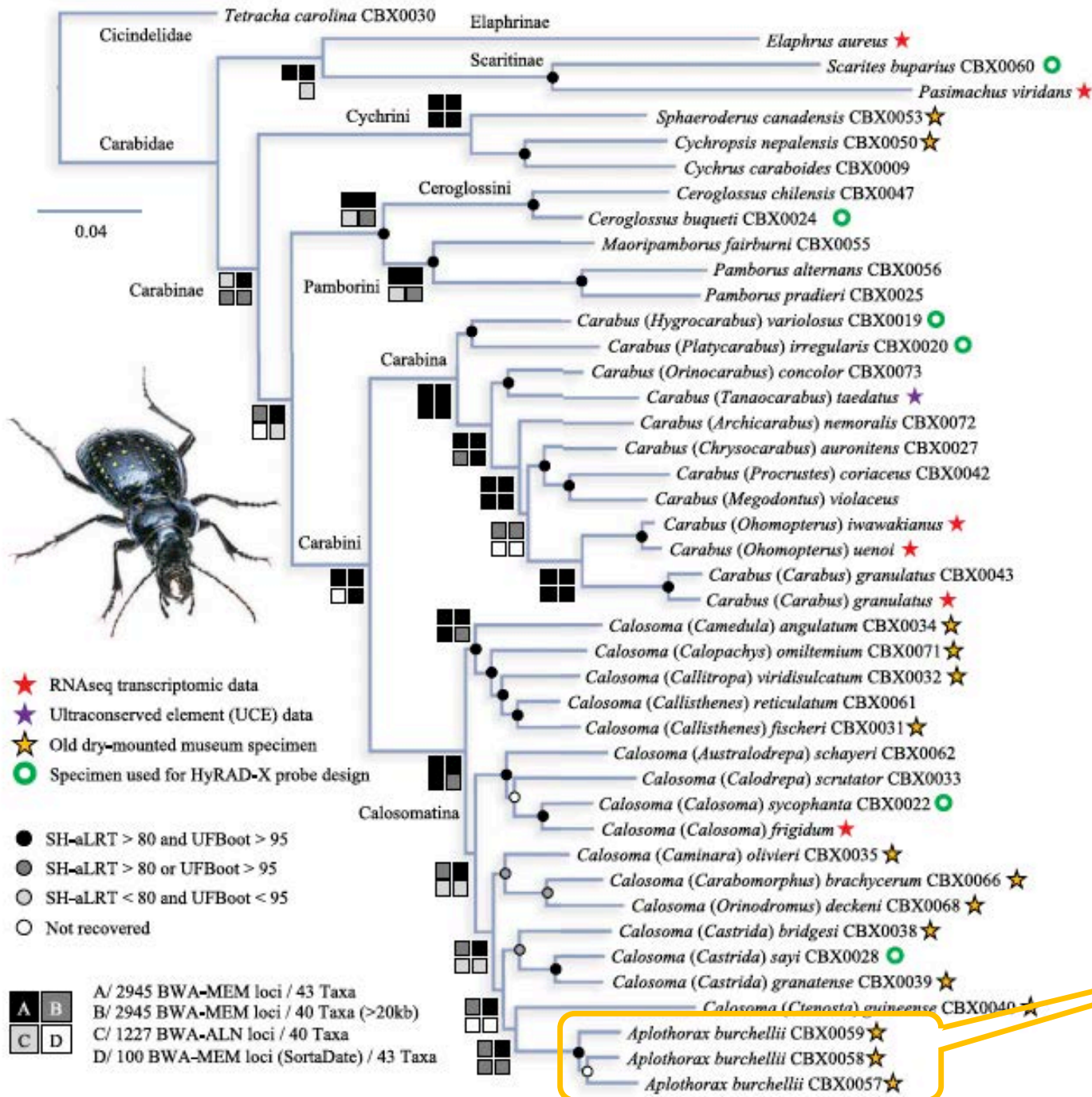




UCE data from Baca et al. 2017 Syst Entomol

RNAseq data from 1KITE project

**Backwards compatibility with existing data**



St Helena endemic species  
Extinct since 1968



*Haplothorax burchellii*

# Diversification of *Oreina* species

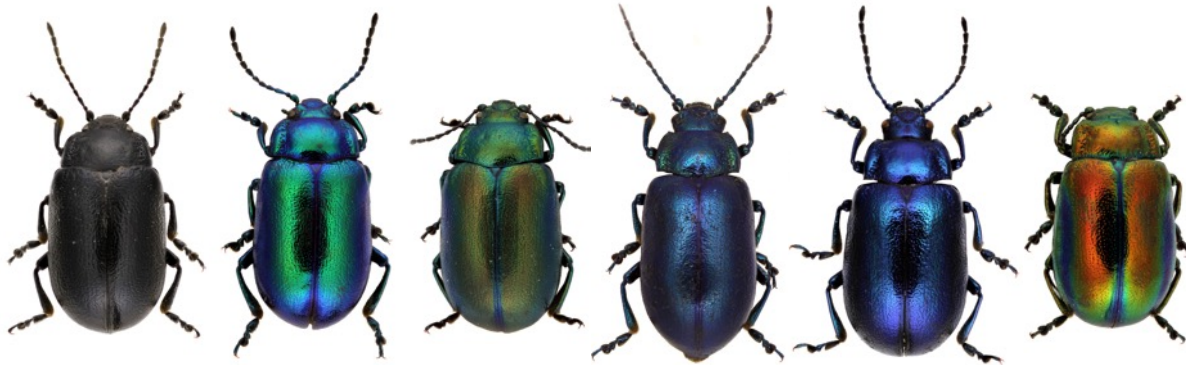
*Oreina alpestris*



*Oreina speciosa*



*Oreina speciosissima*



- Monophyly of *Oreina* ?
- Mechanisms involved in diversification ?

# Diversification of *Oreina* species



HyRAD (Hybridization RAD) protocol



# Diversification of *Oreina* species



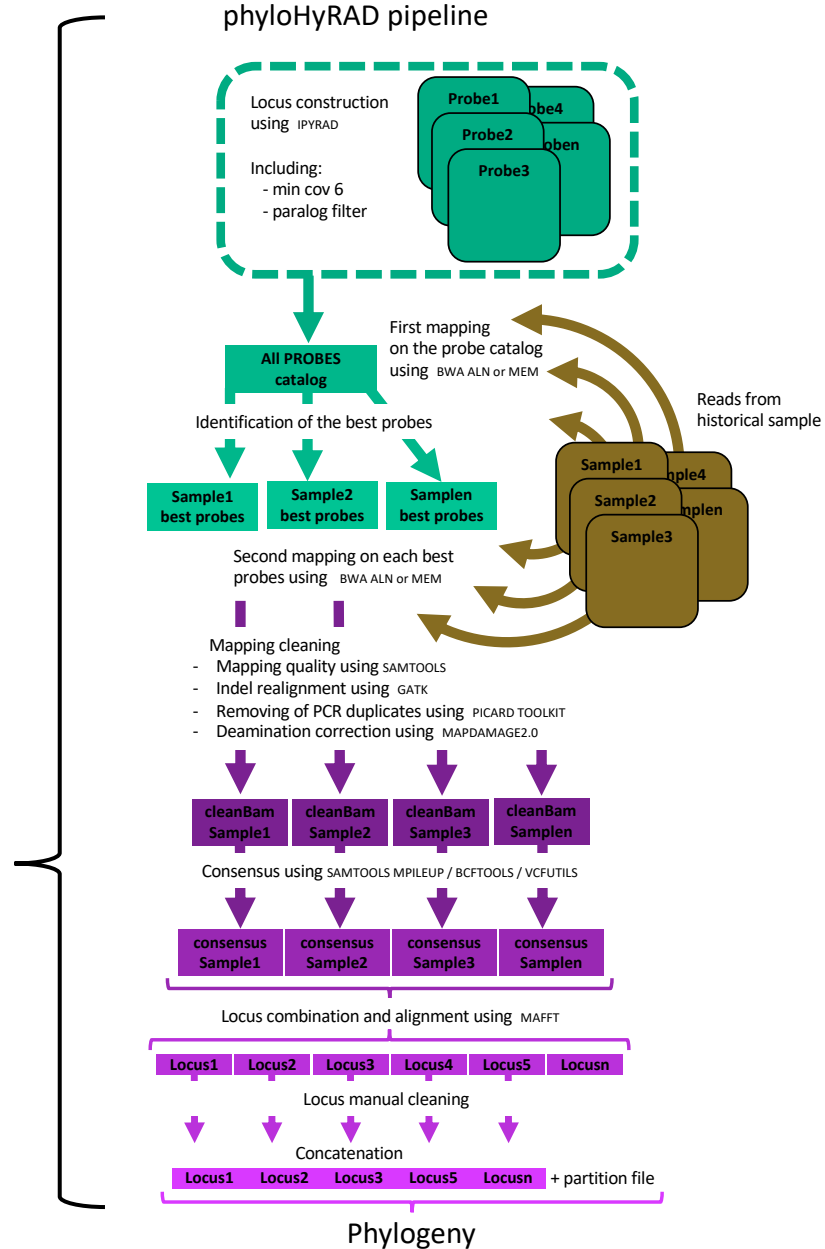
HyRAD (Hybridization RAD) protocol



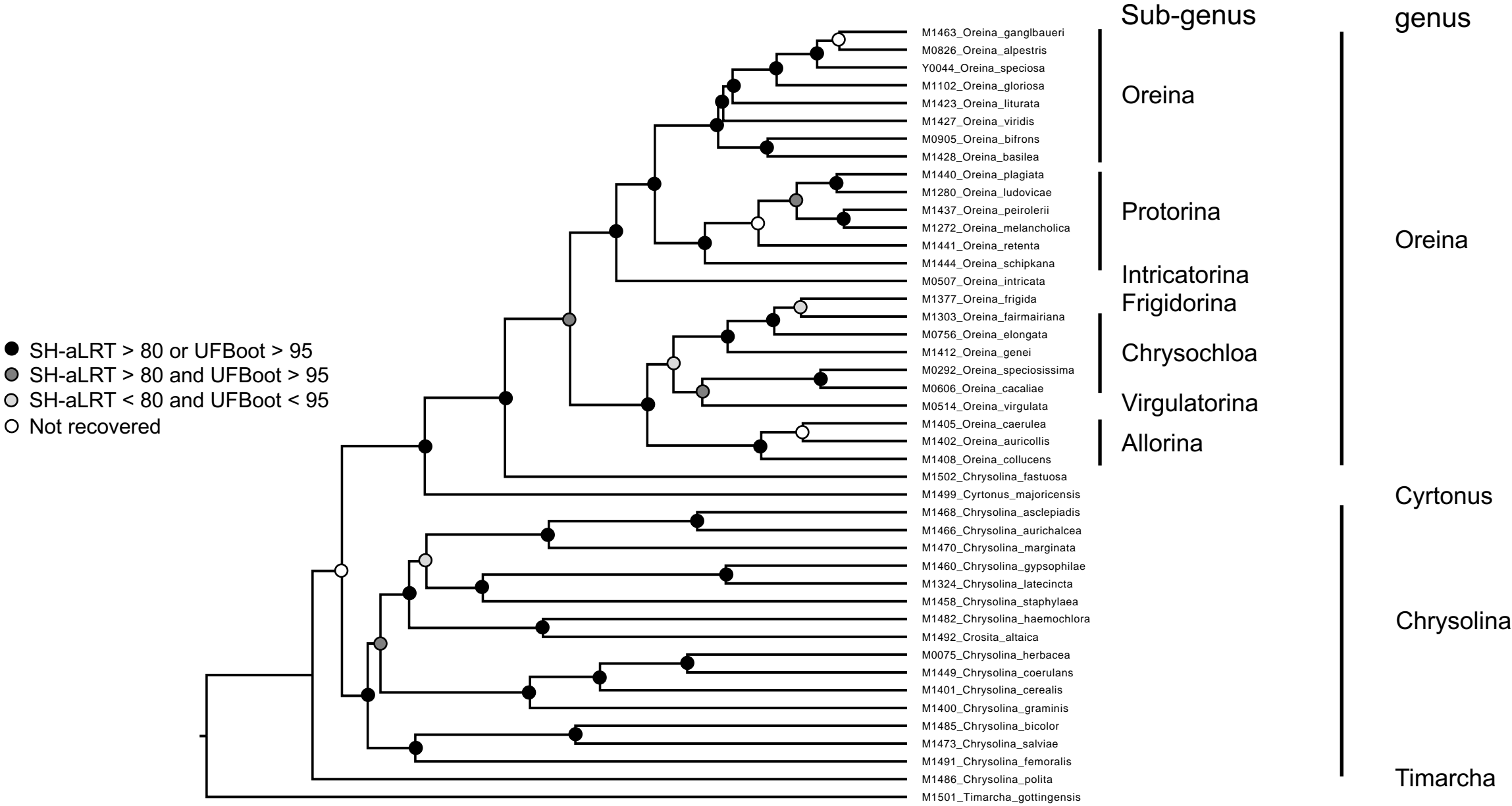
phyloHyRAD pipeline



2235 shared loci

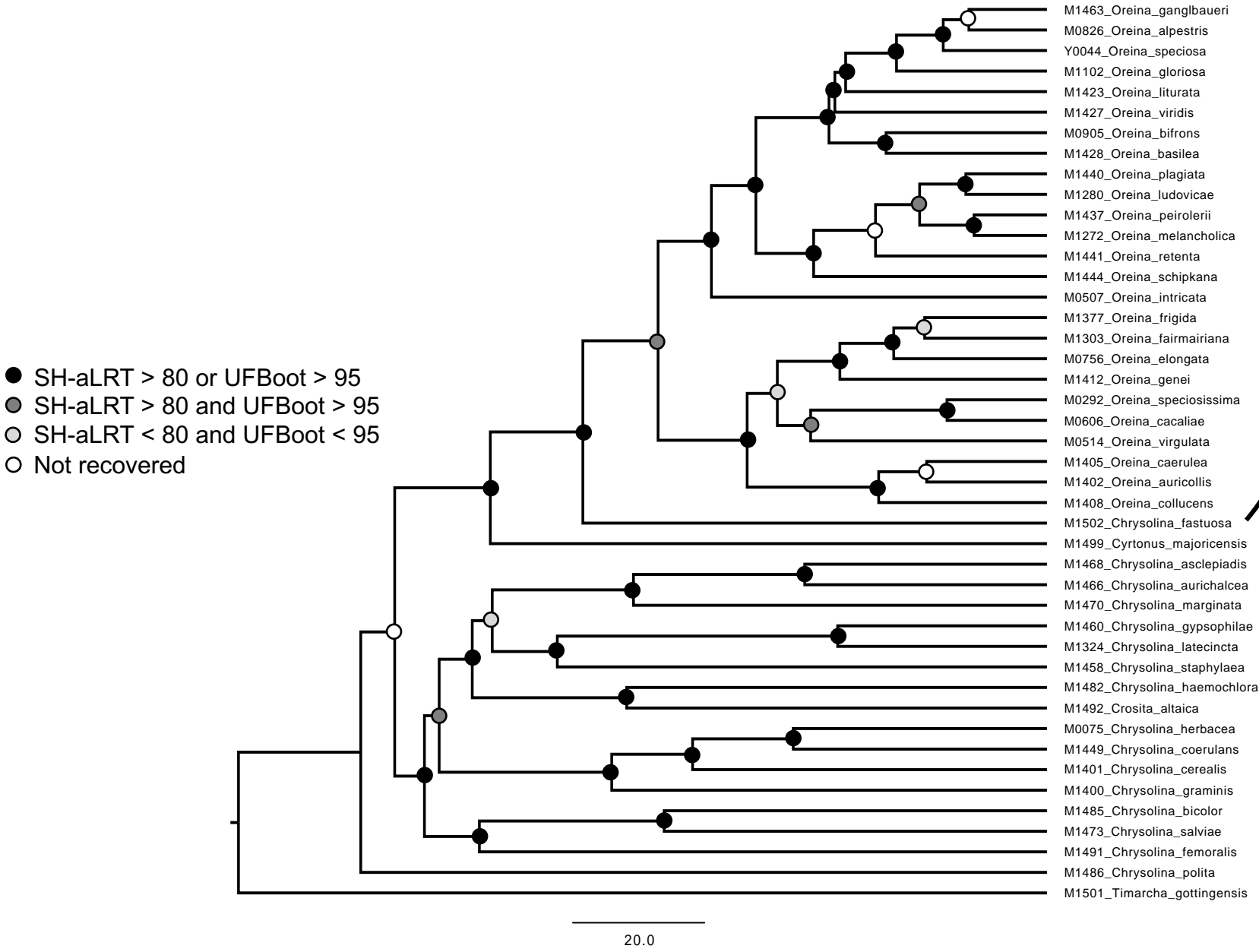


# Diversification of *Oreina* species



20.0

# Diversification of *Oreina* species



*Chrysolina fastuosa*



New genus : *Fasta fastuosa*

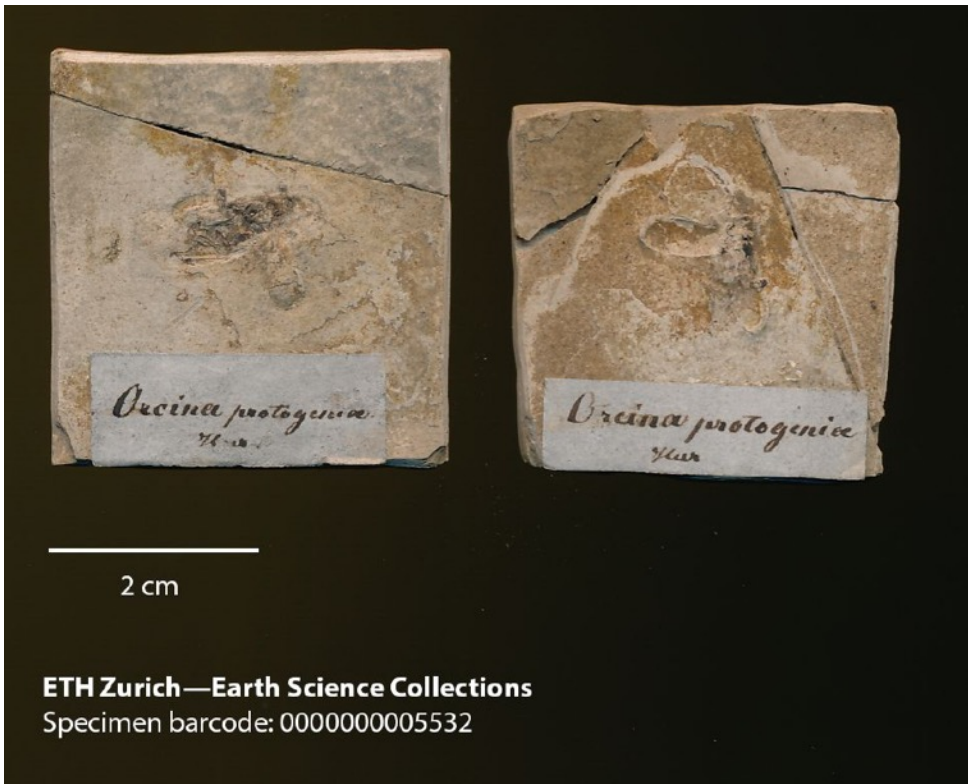
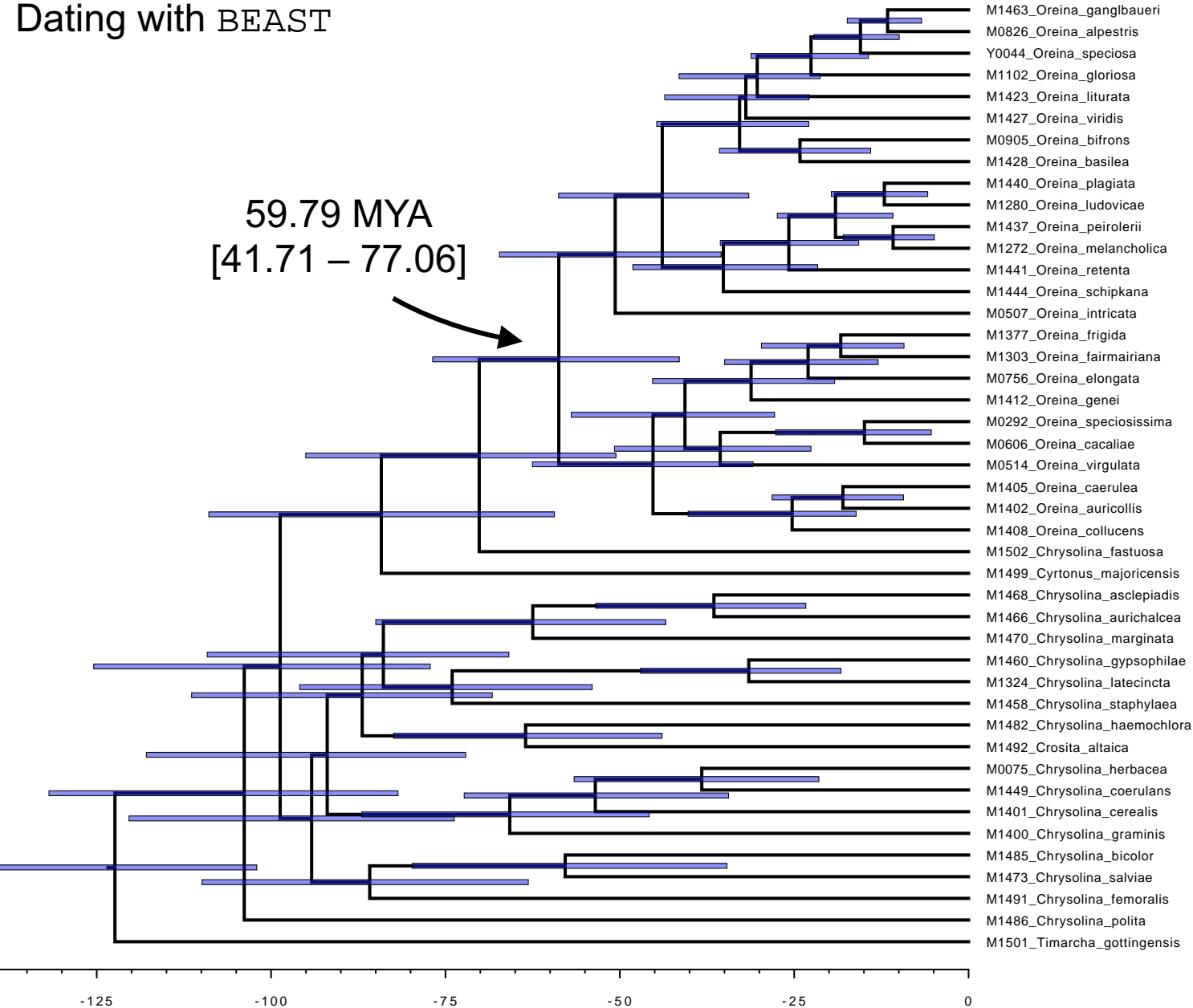
Cyrtonus

Chrysolina

Timarcha

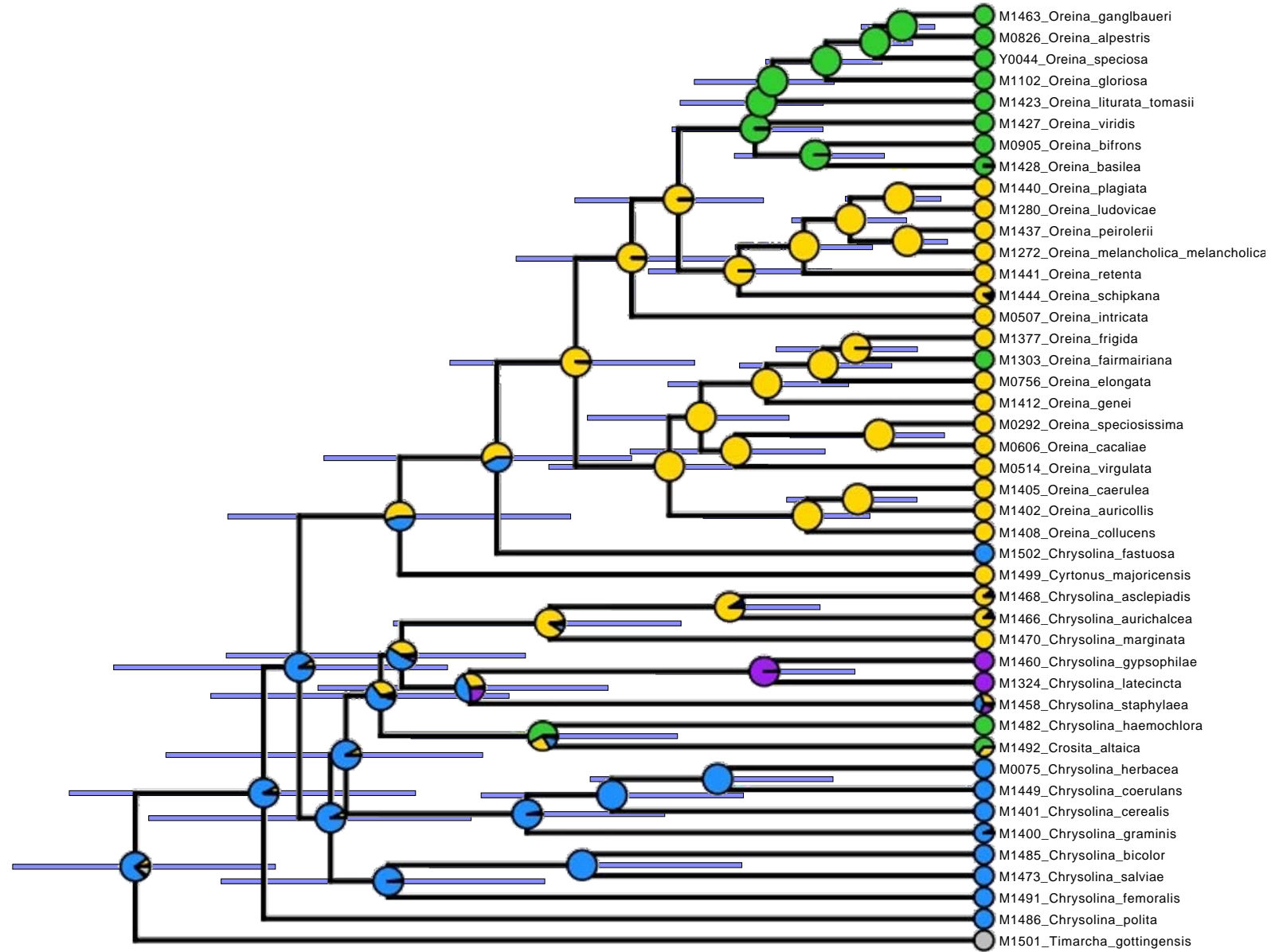
# Diversification of *Oreina* species

Dating with BEAST



*Oreina* fossils  
*Oreina amphyctionis* (Heer, 1847)  
*Oreina hellenis* (Heer, 1847)  
*Oreina protogeniae* (Heer, 1847)  
 Sarmatian deposits in Germany  
 dated to **11.6-12.7** Ma ago  
*Oreina pulchra* (Förster, 1891)  
 Brunstatt (France) and Kleinkembs (Germany)  
 dated to **28.4-33.9** Ma ago  
 Gauthier\*, Borer\* et al. in prep

# Diversification of *Oreina* species

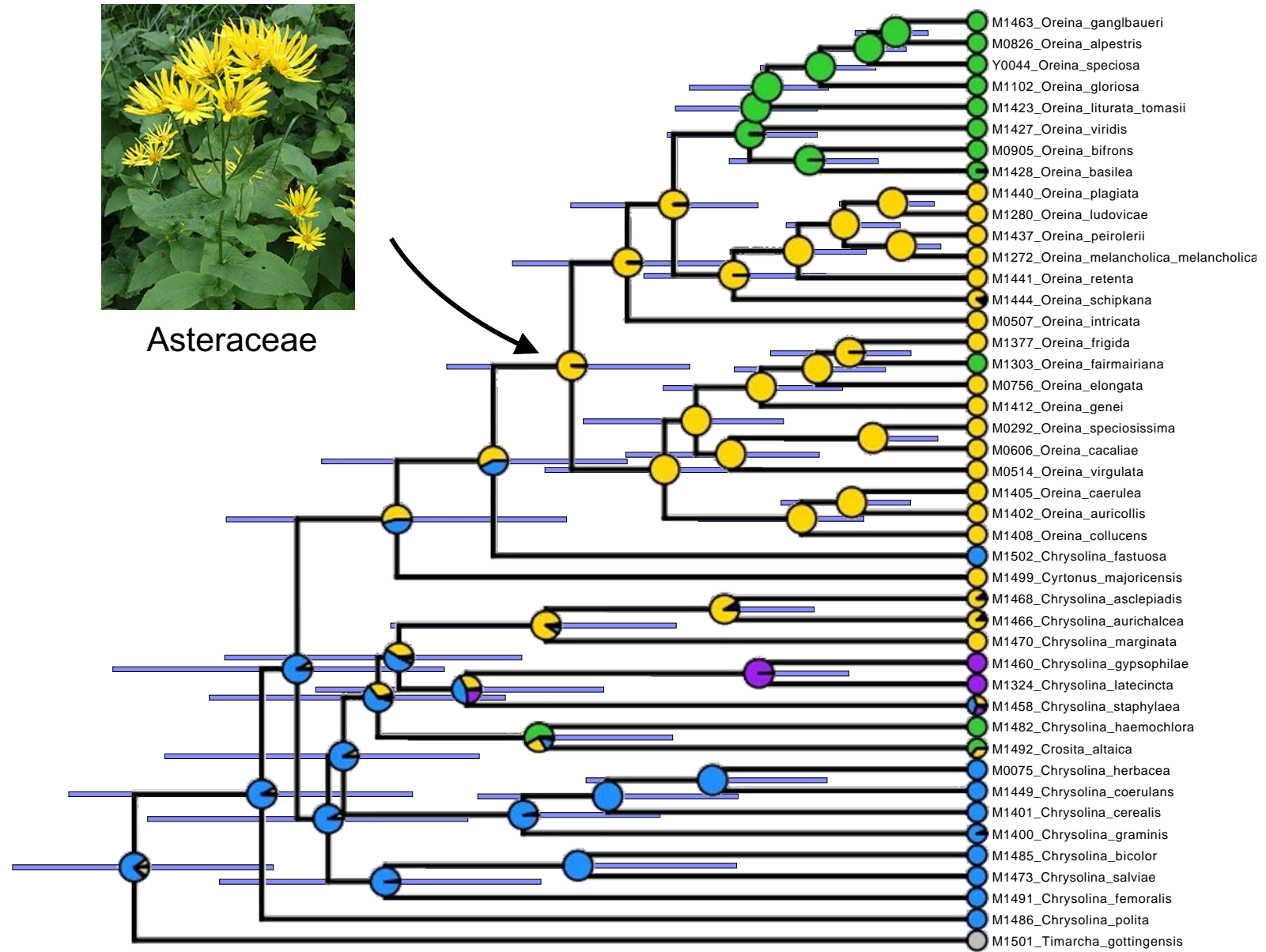


- Apiaceae
- Asclepiadaceae
- Asteraceae
- Lamiaceae
- Plantaginaceae
- Ranunculaceae
- Rubiaceae

# Diversification of *Oreina* species



Asteraceae



genus

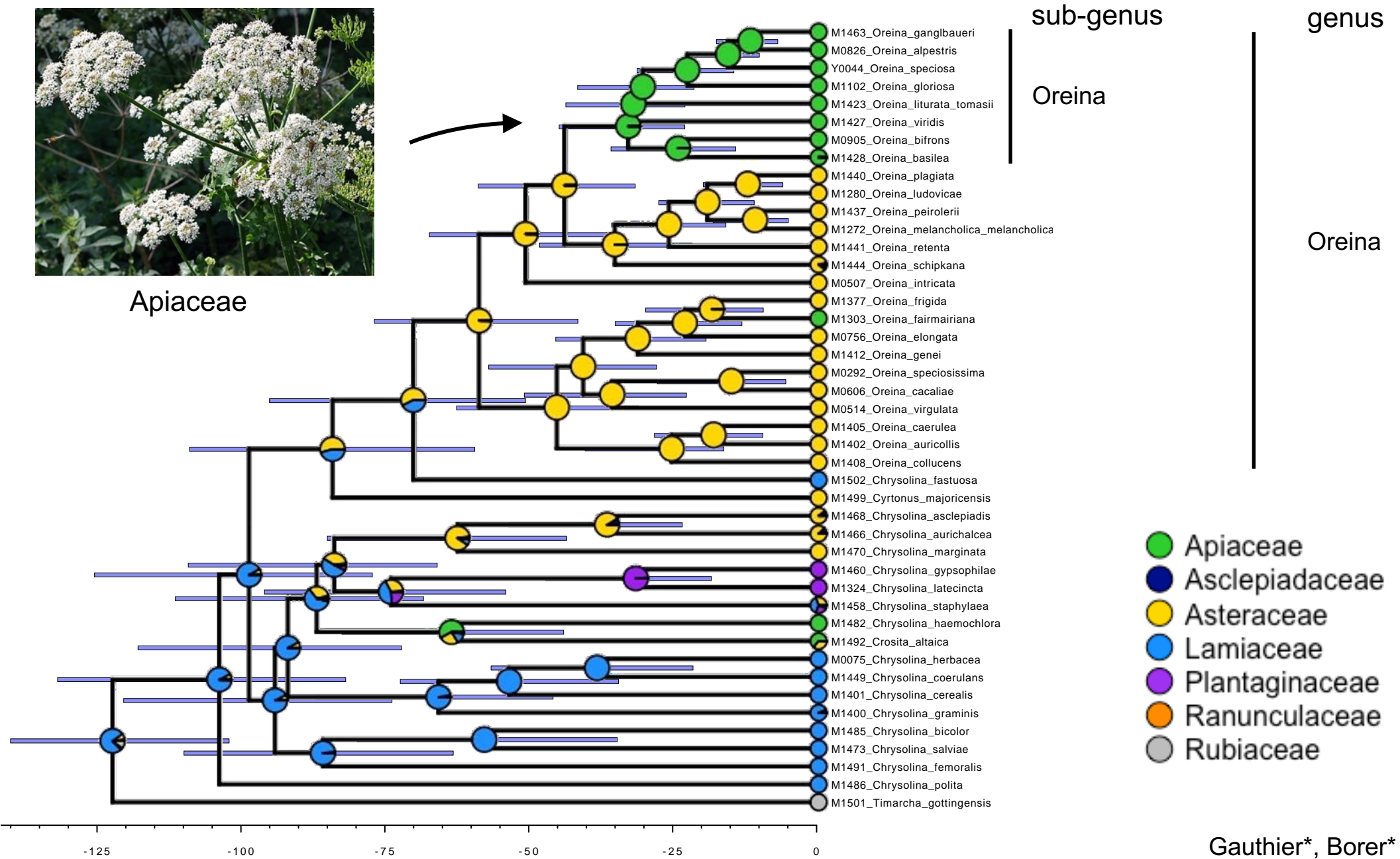
Oreina

- Apiaceae
- Asclepiadaceae
- Asteraceae
- Lamiaceae
- Plantaginaceae
- Ranunculaceae
- Rubiaceae

# Diversification of *Oreina* species



Apiaceae



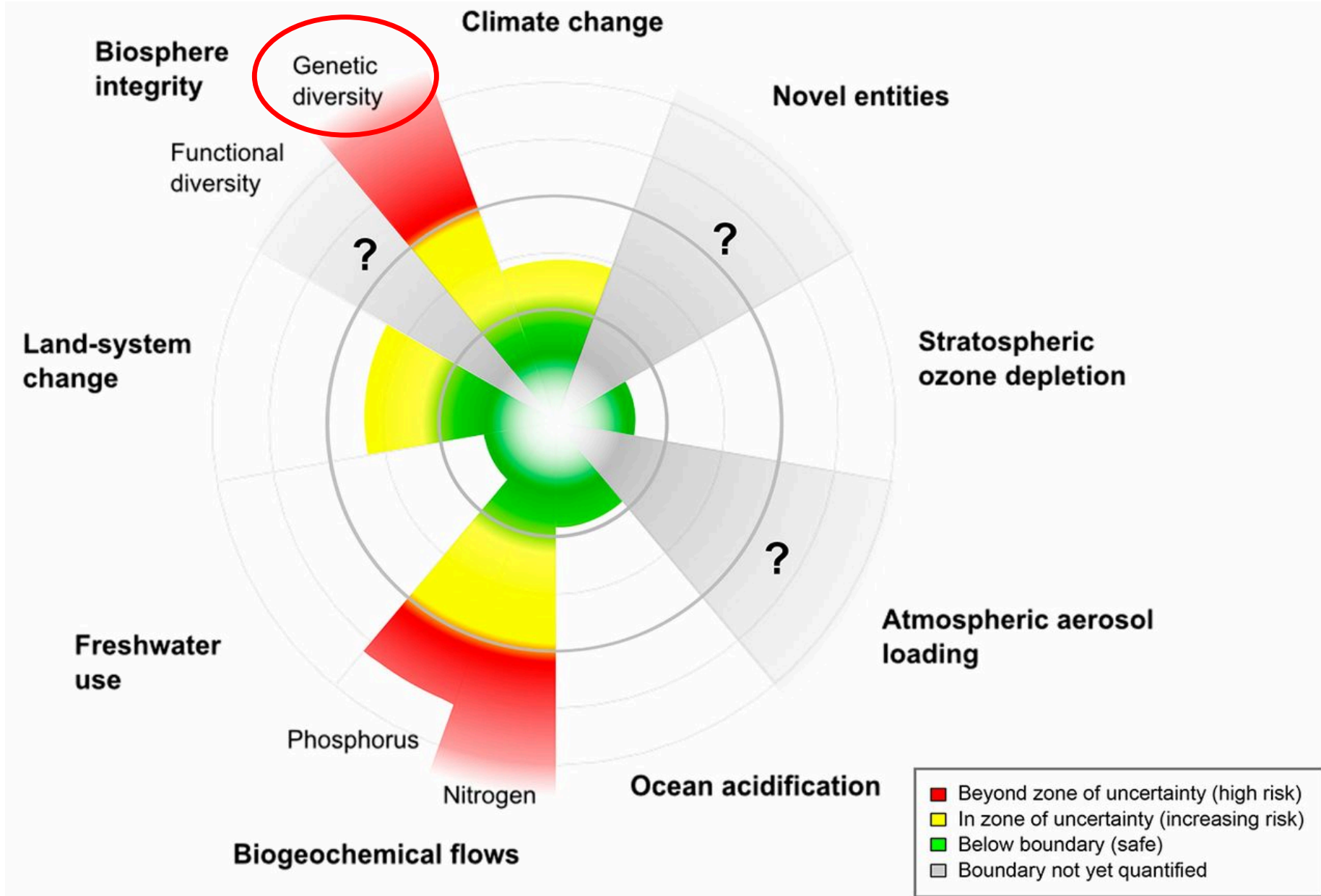




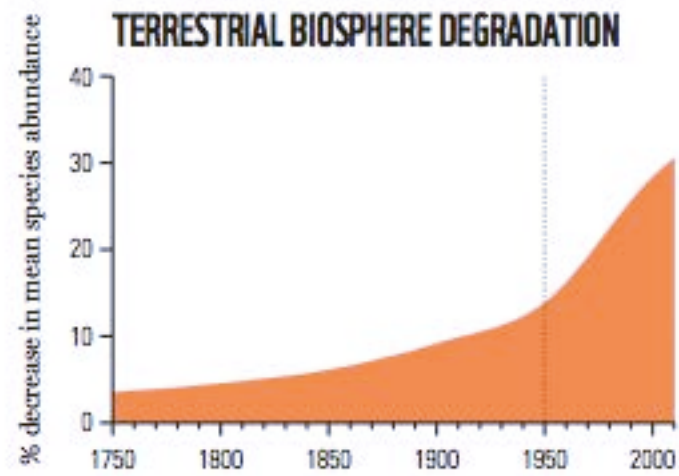
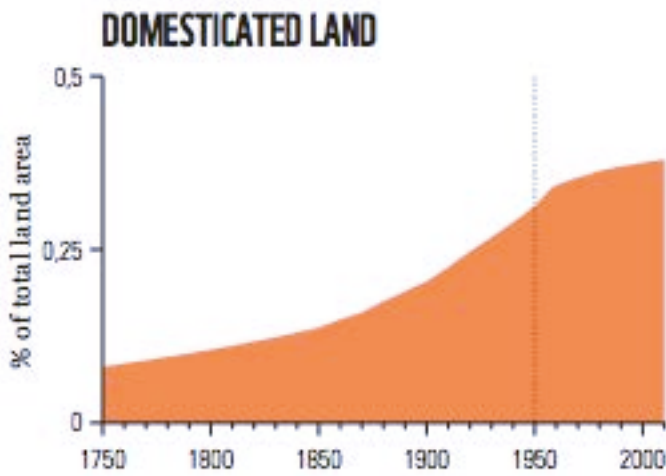
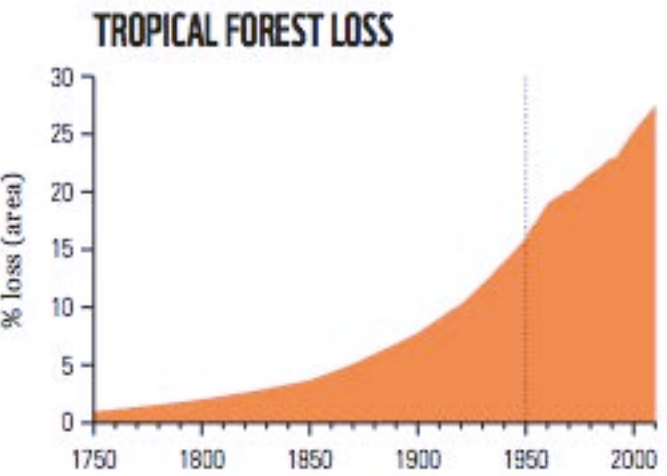
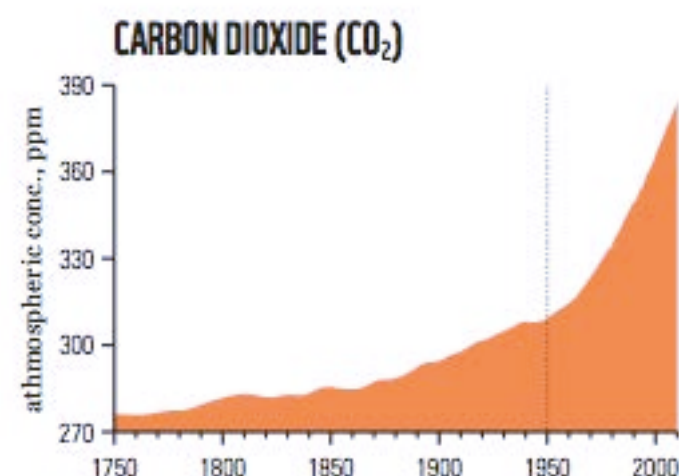
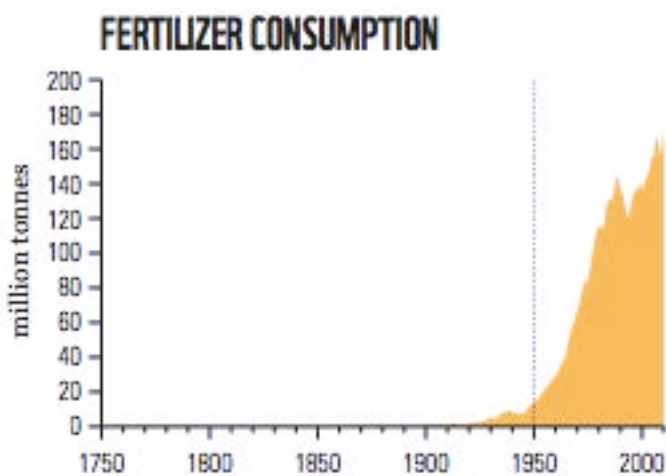
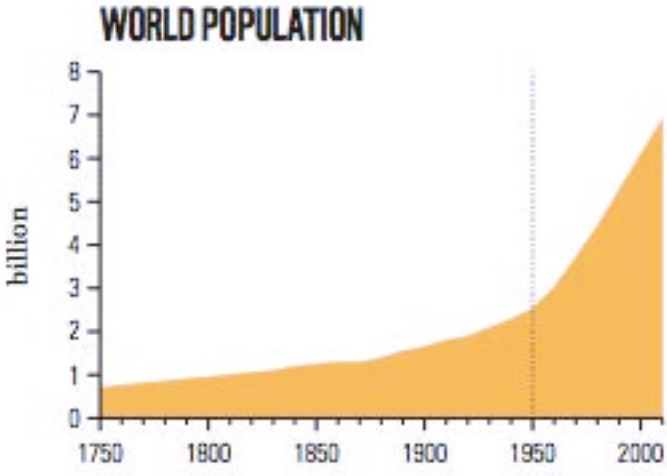


An incredible biobank to track  
the recent history of populations

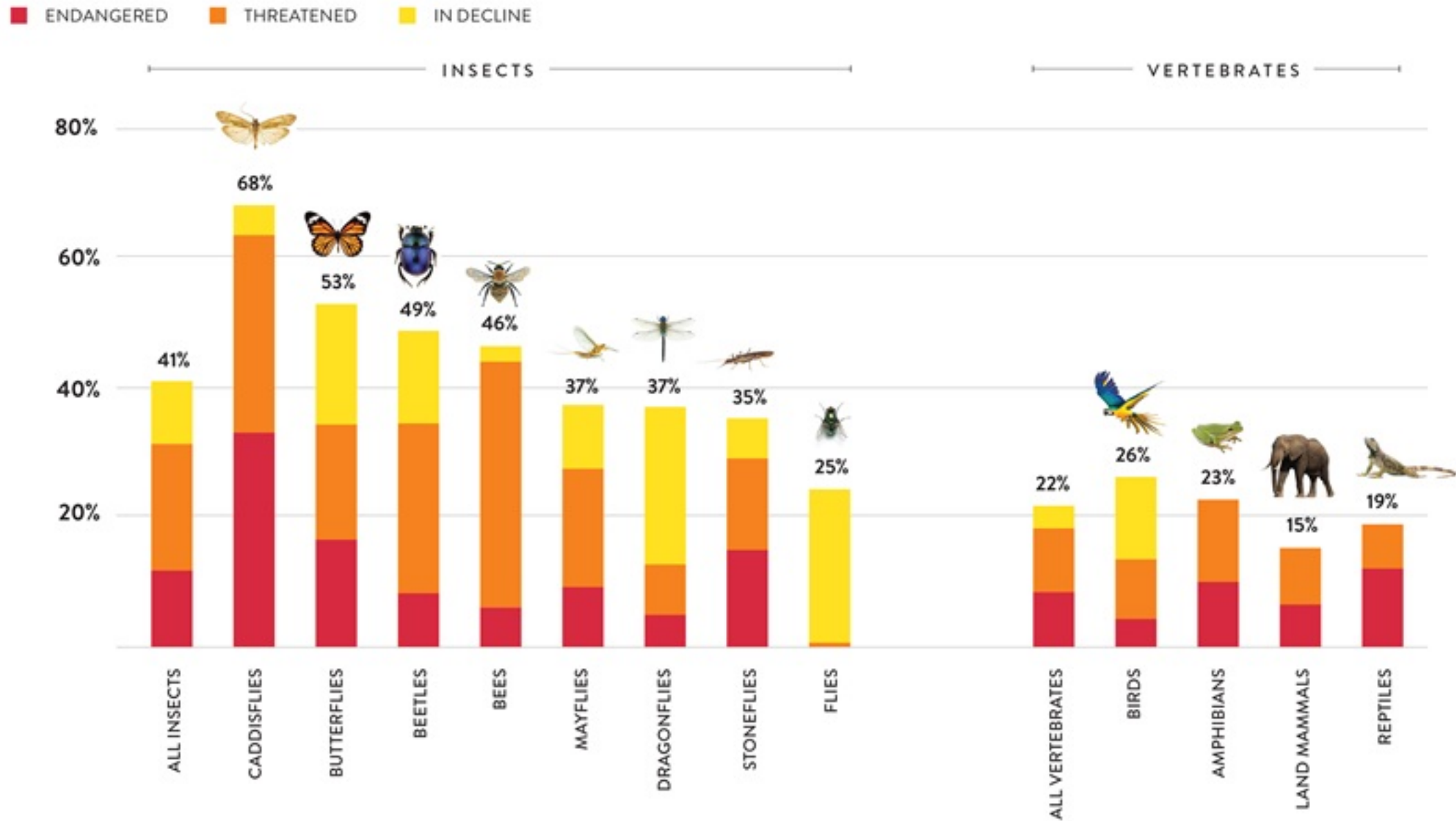
# Biodiversity crisis



# The Great Acceleration

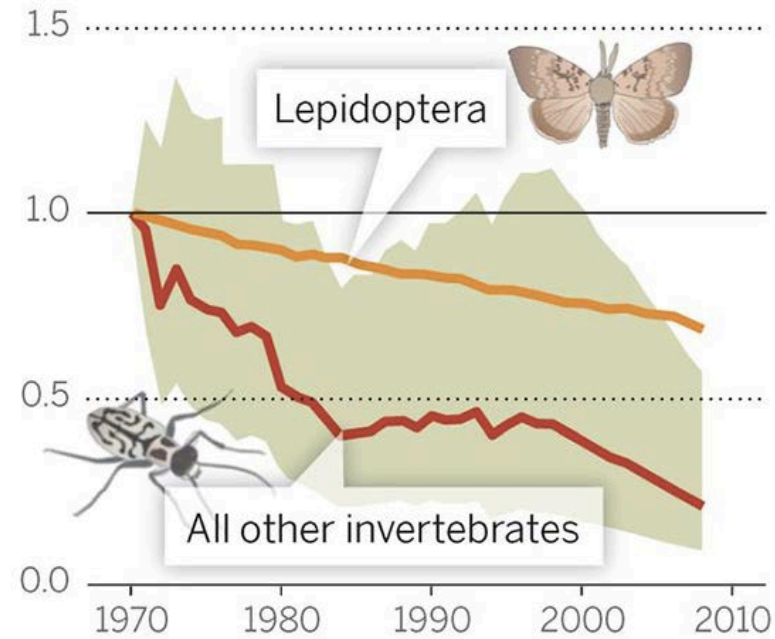


# Insect decline



# Insect decline

Global index of invertebrate abundance



Diszo et al. 2014 Science

RESEARCH ARTICLE

## More than 75 percent decline over 27 years in total flying insect biomass in protected areas

Caspar A. Hallmann<sup>1,\*</sup>, Martin Sorg<sup>2</sup>, Eelke Jongejans<sup>1</sup>, Henk Siepel<sup>1</sup>, Nick Hofland<sup>1</sup>, Heinz Schwan<sup>2</sup>, Werner Stenmans<sup>2</sup>, Andreas Müller<sup>2</sup>, Hubert Sumser<sup>2</sup>, Thomas Hörrn<sup>2</sup>, Dave Goulson<sup>3</sup>, Hans de Kroon<sup>1</sup>



Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Biological Conservation

journal homepage: [www.elsevier.com/locate/biocon](https://www.elsevier.com/locate/biocon)

## Over a century of data reveal more than 80% decline in butterflies in the Netherlands

Arco J. van Strien<sup>a,\*</sup>, Chris A.M. van Swaay<sup>b</sup>, Willy T.F.H. van Strien-van Liempt<sup>c</sup>, Martin J.M. Poot<sup>a</sup>, Michiel F. WallisDeVries<sup>b,d</sup>



Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Biological Conservation

journal homepage: [www.elsevier.com/locate/biocon](https://www.elsevier.com/locate/biocon)

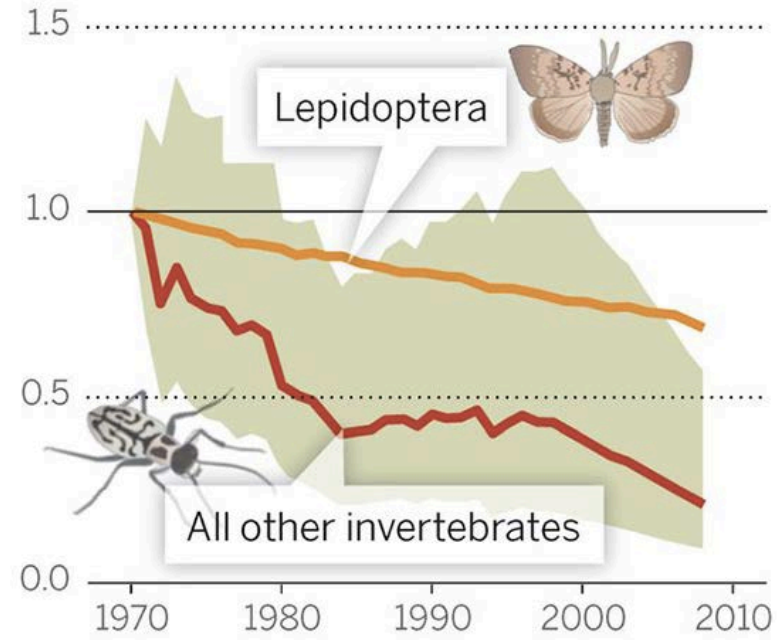
Review

## Worldwide decline of the entomofauna: A review of its drivers

Francisco Sánchez-Bayo<sup>a,\*</sup>, Kris A.G. Wyckhuys<sup>b,c,d</sup>

# Insect decline

## Global index of invertebrate abundance



Diszo et al. 2014 Science

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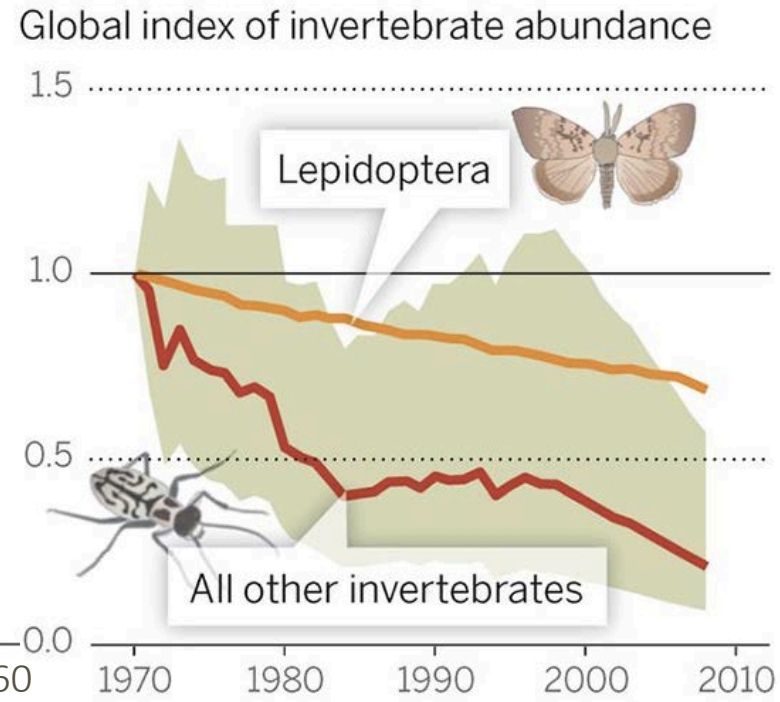
Caspar A. Hallmann<sup>1\*</sup>, Martin Sorg<sup>2</sup>, Eelke Jongejans<sup>1</sup>, Henk Siepel<sup>1</sup>, Nick Hofland<sup>1</sup>, Heinz Schwan<sup>2</sup>, Werner Stenmans<sup>2</sup>, Andreas Müller<sup>2</sup>, Hubert Sumser<sup>2</sup>, Thomas Hörrn<sup>2</sup>, Dave Goulson<sup>3</sup>, Hans de Kroon<sup>1</sup>

# Insect decline

RESEARCH ARTICLE

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Diszo et al. 2014 Science

?

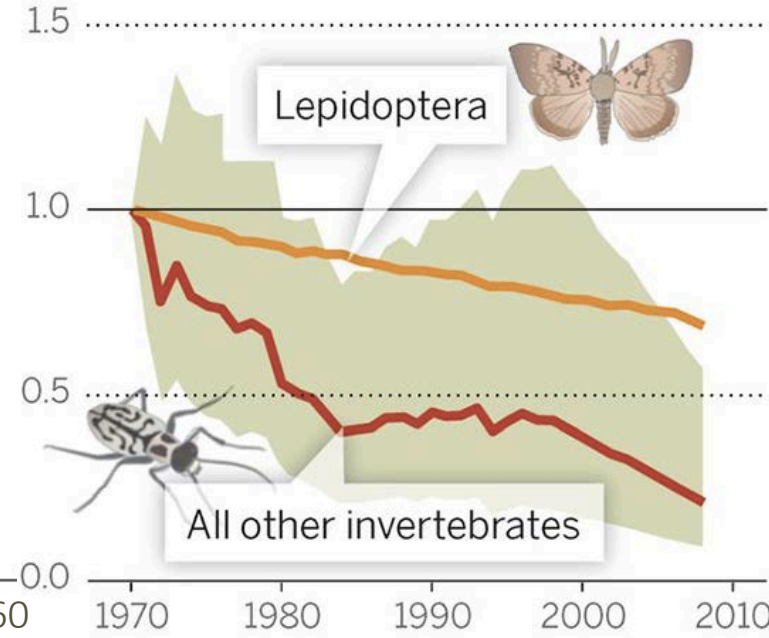
# Insect decline

RESEARCH ARTICLE

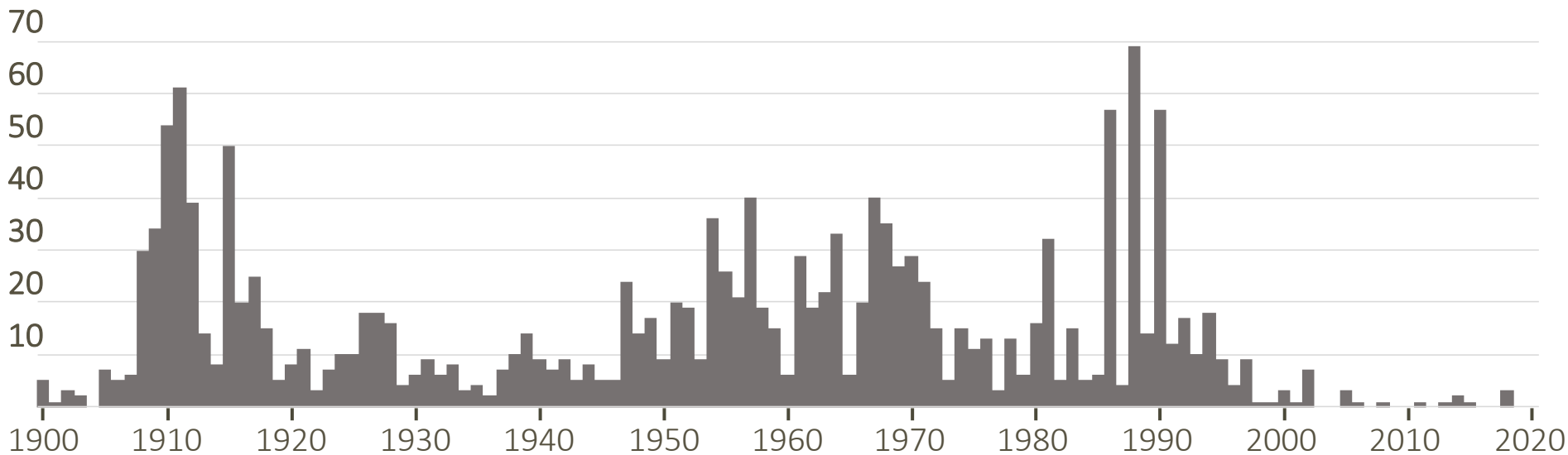
## More than 75 percent decline over 27 years in total flying insect biomass in protected areas

Caspar A. Hallmann<sup>1\*</sup>, Martin Sorg<sup>2</sup>, Eelke Jongejans<sup>1</sup>, Henk Siepel<sup>1</sup>, Nick Hofland<sup>1</sup>, Heinz Schwan<sup>2</sup>, Werner Stenmans<sup>2</sup>, Andreas Müller<sup>2</sup>, Hubert Sumser<sup>2</sup>, Thomas Hörrn<sup>2</sup>, Dave Goulson<sup>3</sup>, Hans de Kroon<sup>1</sup>

Global index of invertebrate abundance



Diszo et al. 2014 Science



*Pieris napi*  
in swiss collections







Representative of the genetic state  
of their population of origin at the time they were caught

# HyRAD

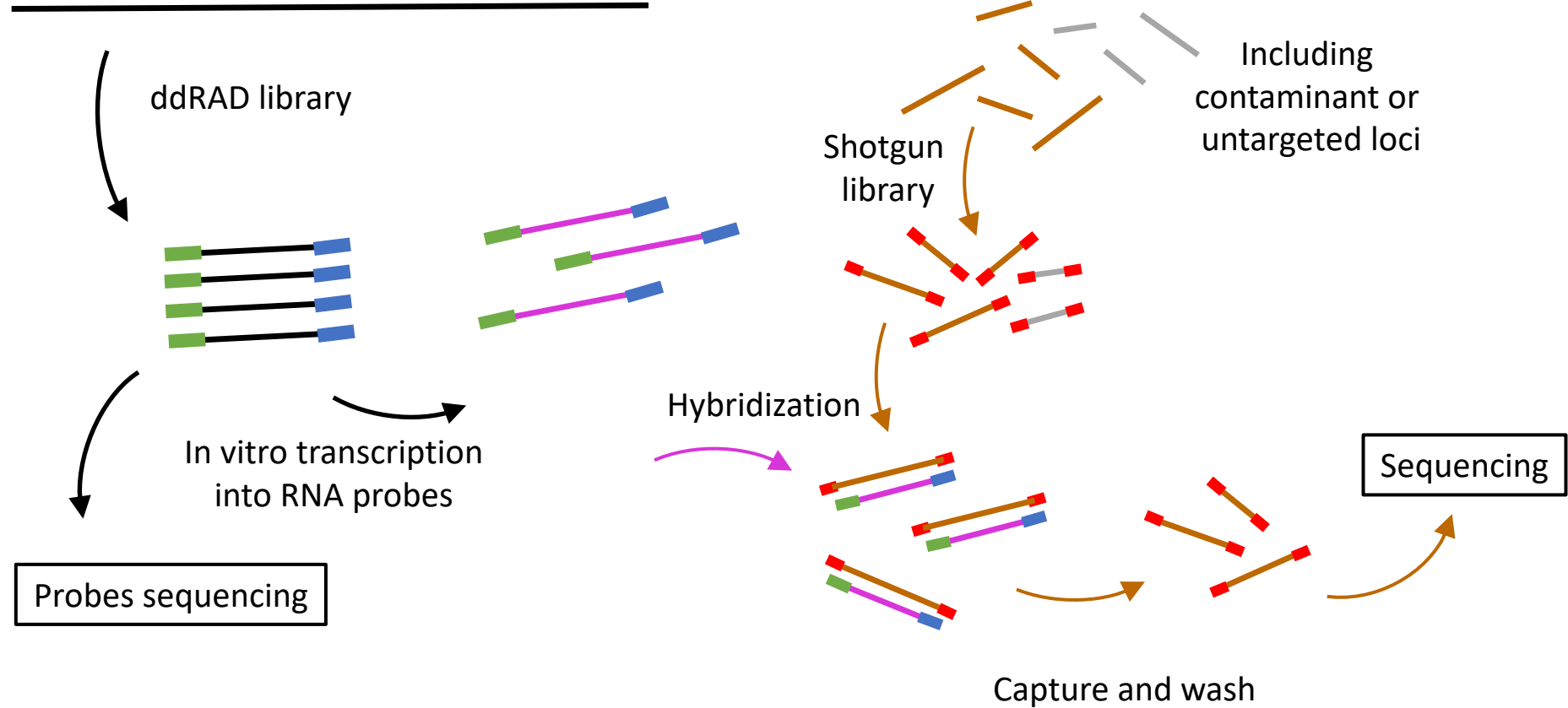


Fresh sample DNA extraction



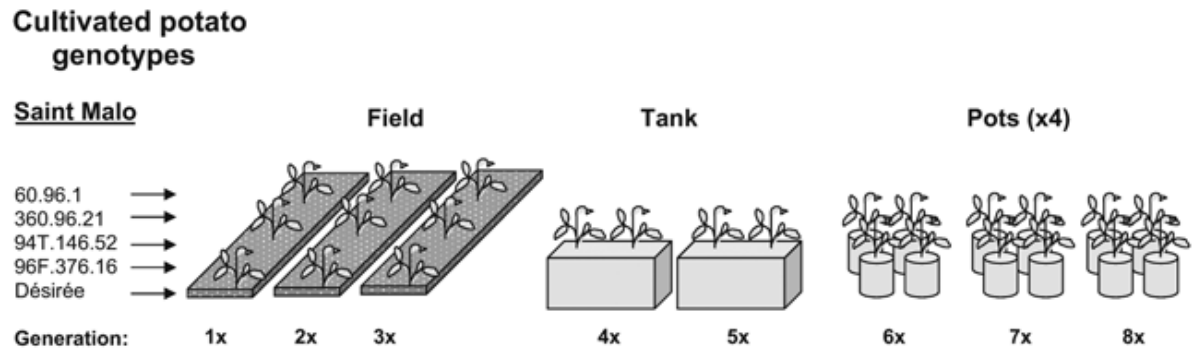
Ancient DNA extraction

genomic DNA



# A new way in evolutionary biology

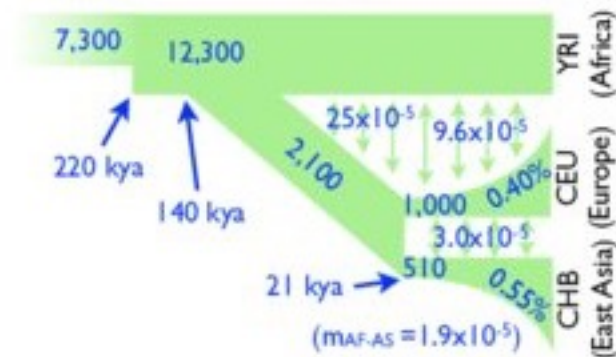
## Experimental evolution



Time series

Tracking allelic frequency changes

## Population genomics on current populations



Demographic inferences

Genome scan

## Museomics

Time series on natural populations



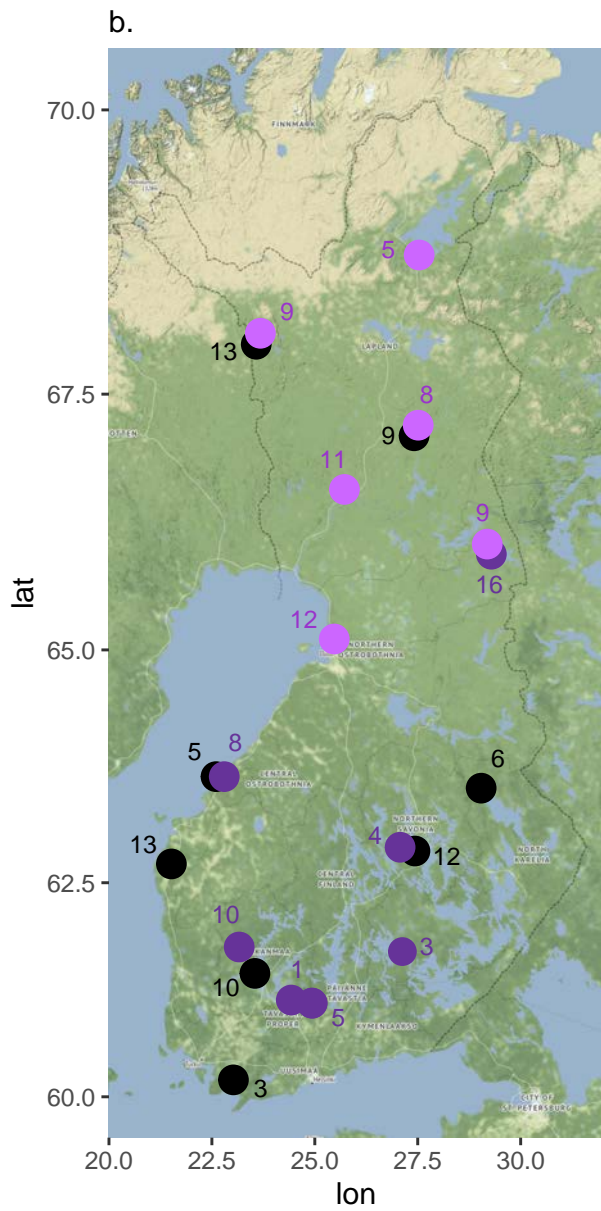
# Sampling

**LUOMUS**  
FINNISH MUSEUM OF NATURAL HISTORY

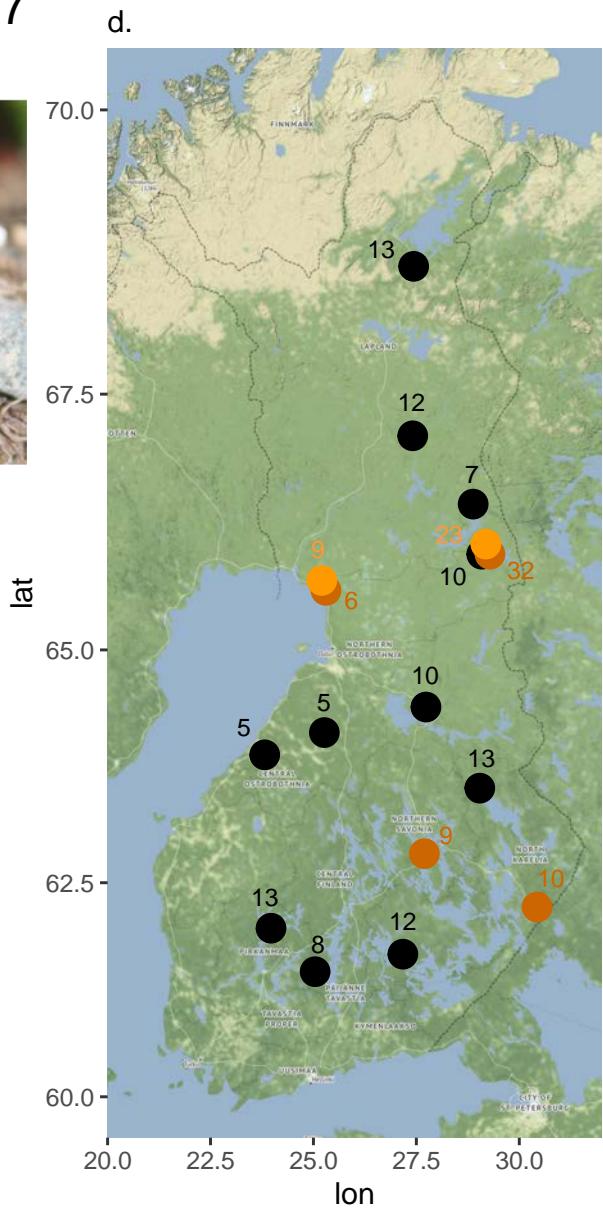
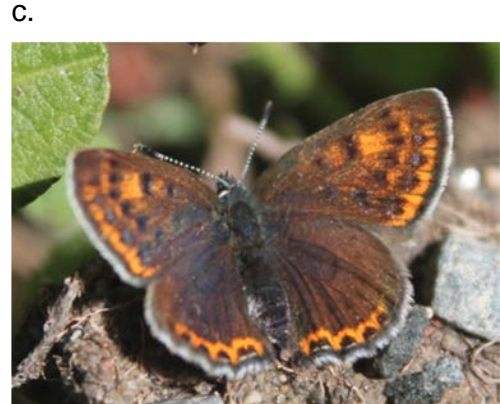


# Sampling

*Erebia embla* N = 169



*Lycaena helle* N = 197



# HyRAD

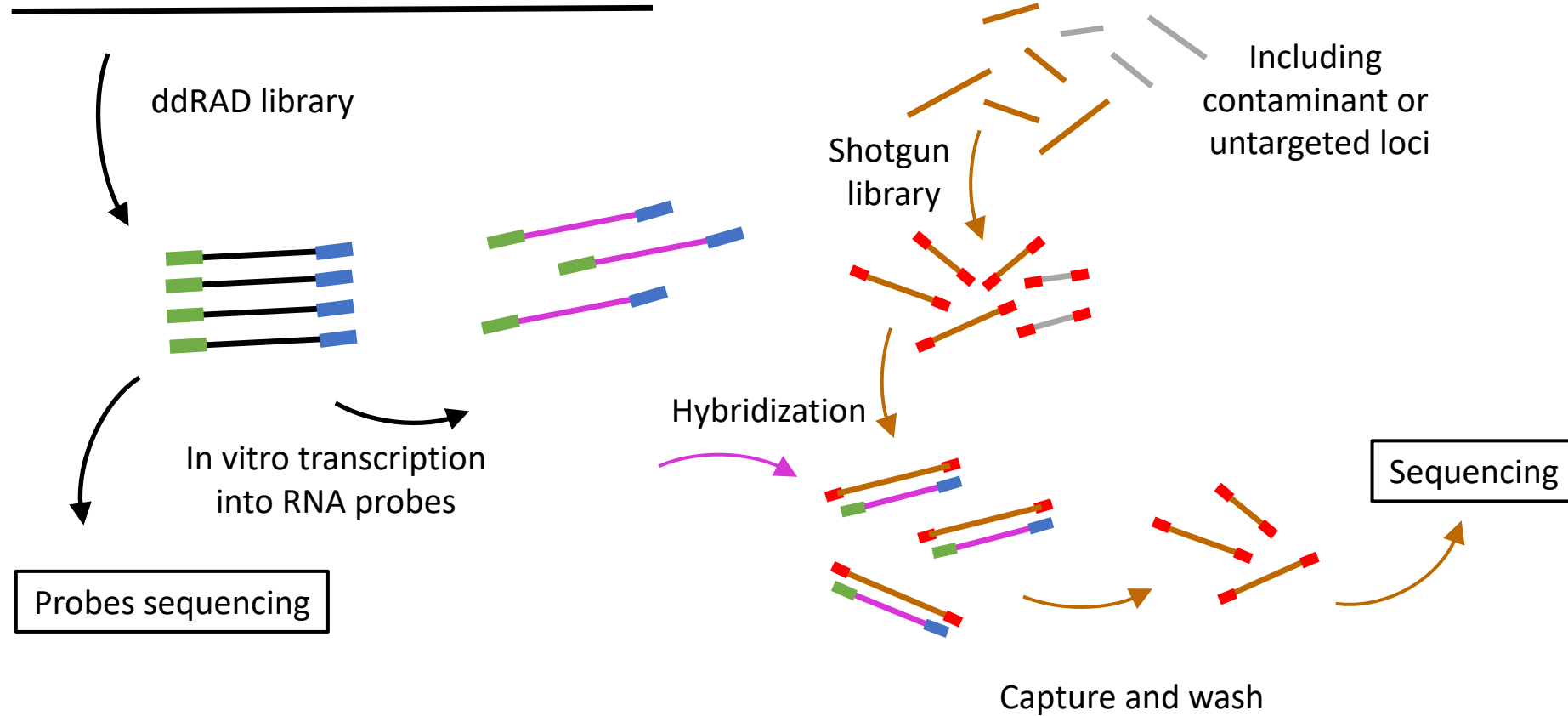


Fresh sample DNA extraction



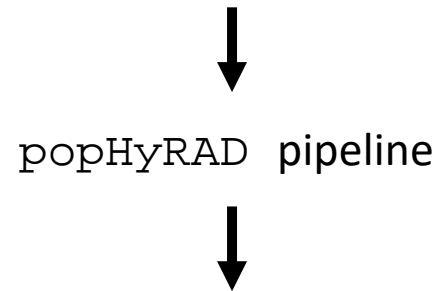
Ancient DNA extraction

genomic DNA

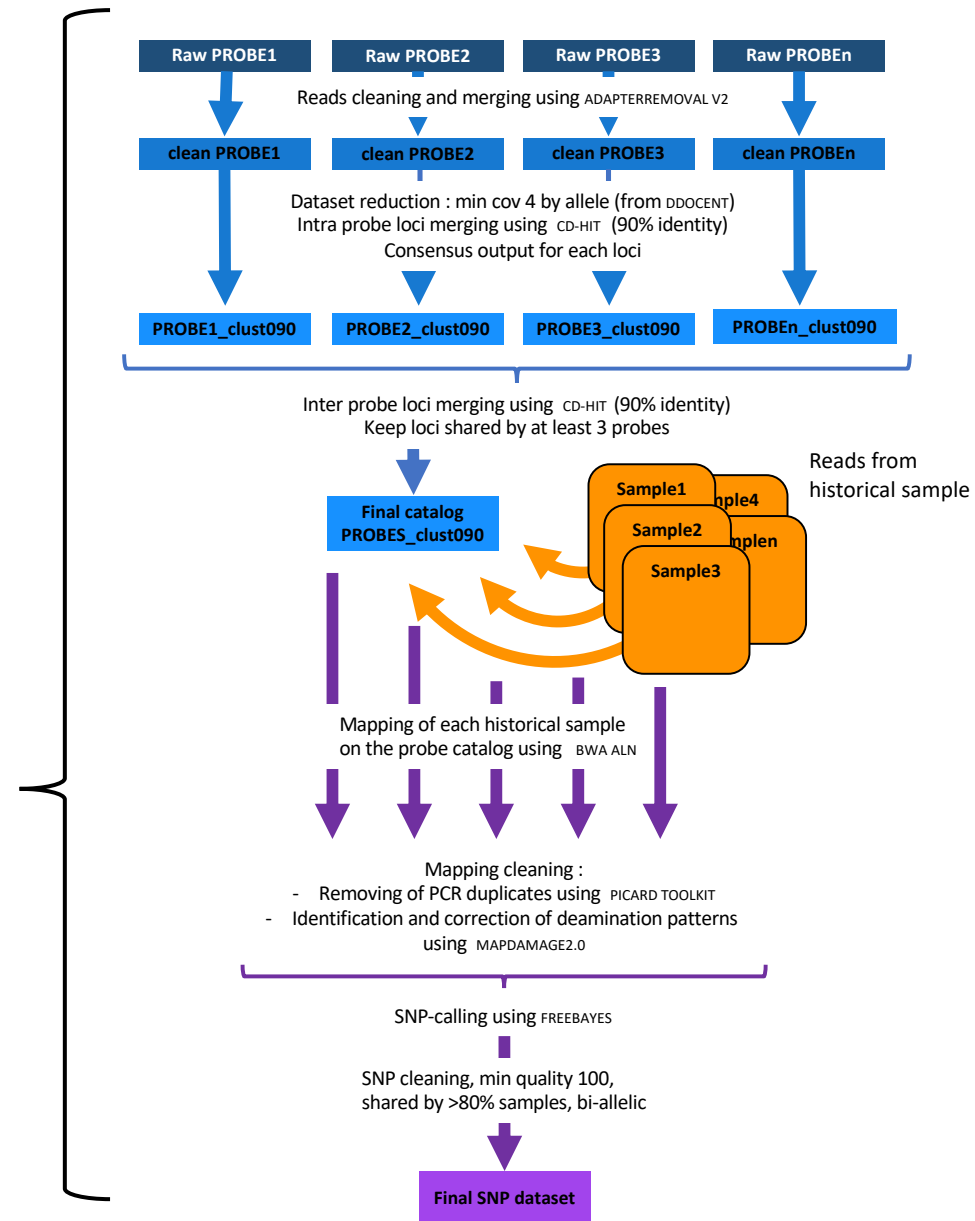




## HyRAD (Hybridization RAD) protocol



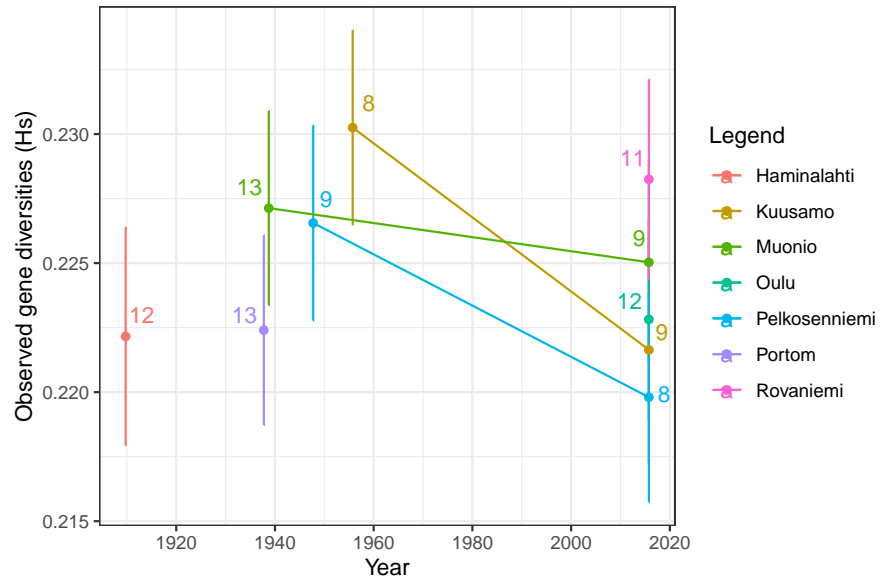
|                      |            |            |
|----------------------|------------|------------|
| <i>Erebia embla</i>  | 1,015 loci | 2,549 SNPs |
| <i>Lycaena helle</i> | 869 loci   | 2,742 SNPs |



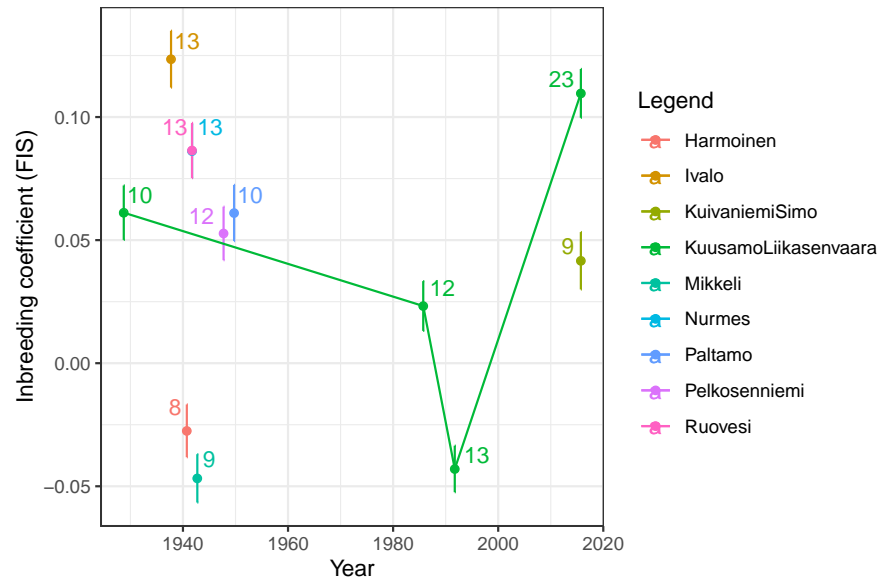
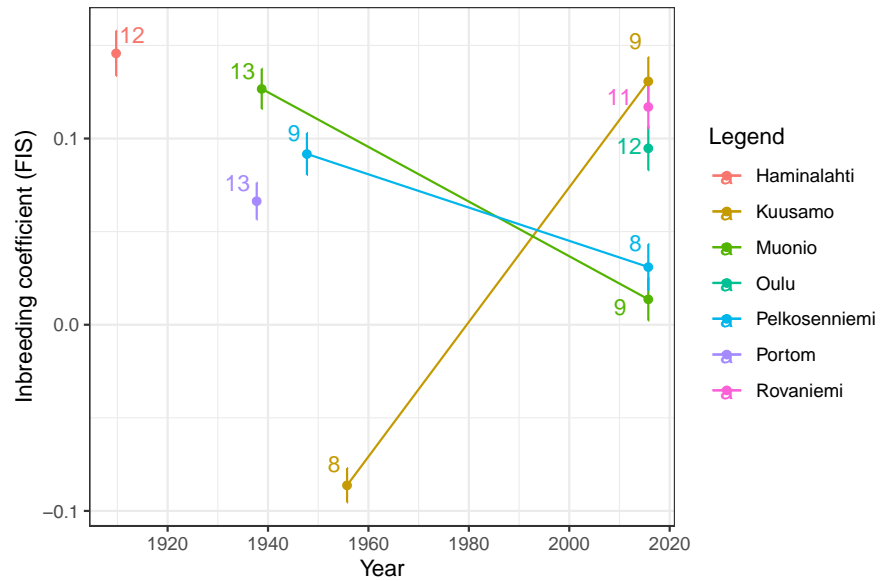
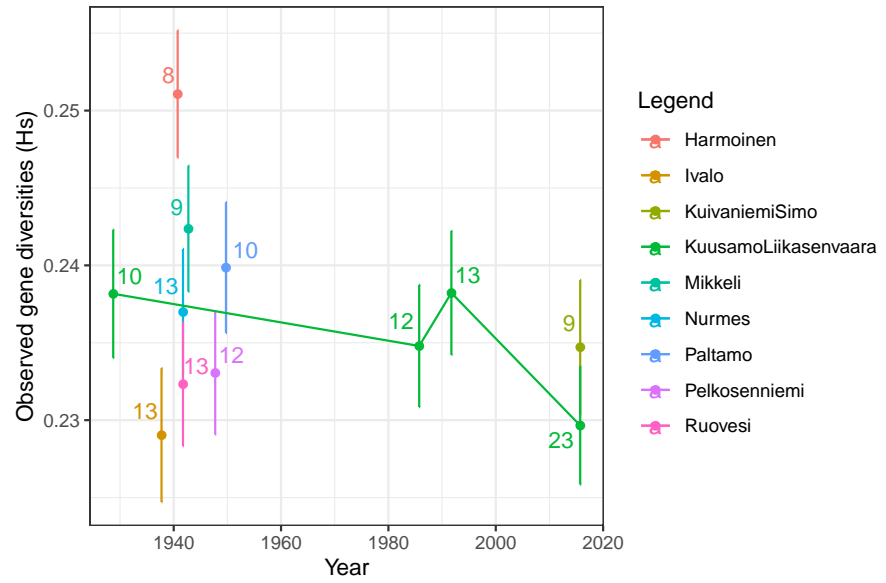


# Genetic statistics evolution

## *Erebia embla*

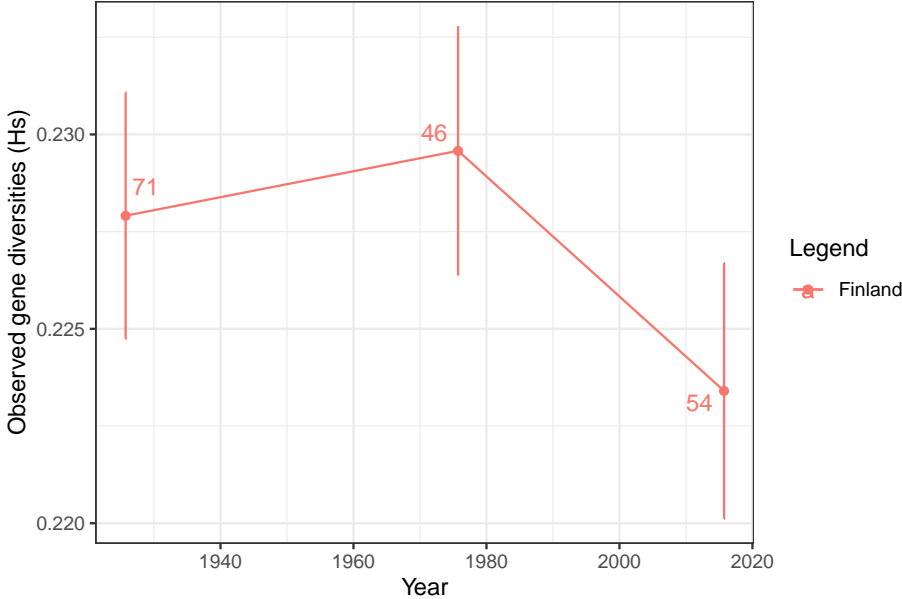


## *Lycaena helle*

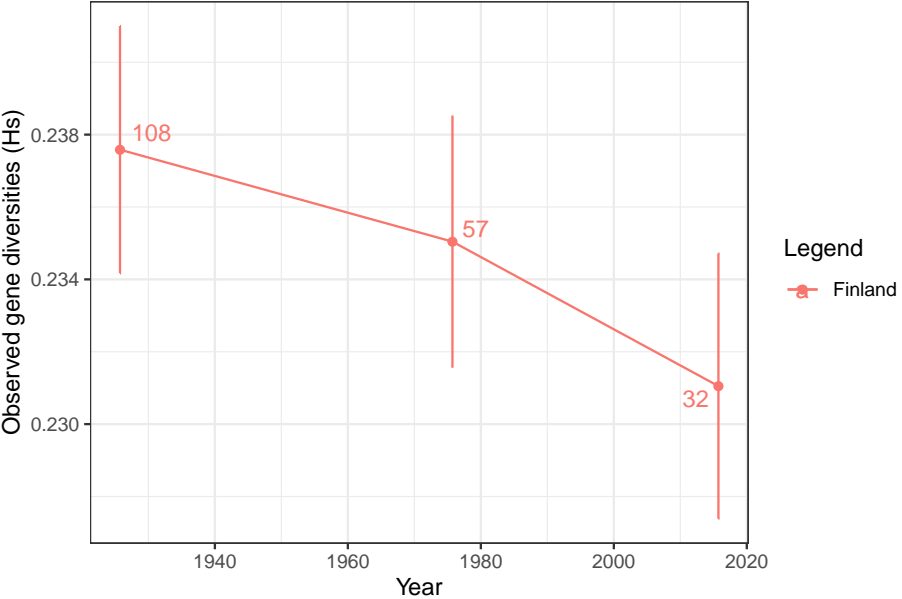


# Finland patterns

*Erebia embla*

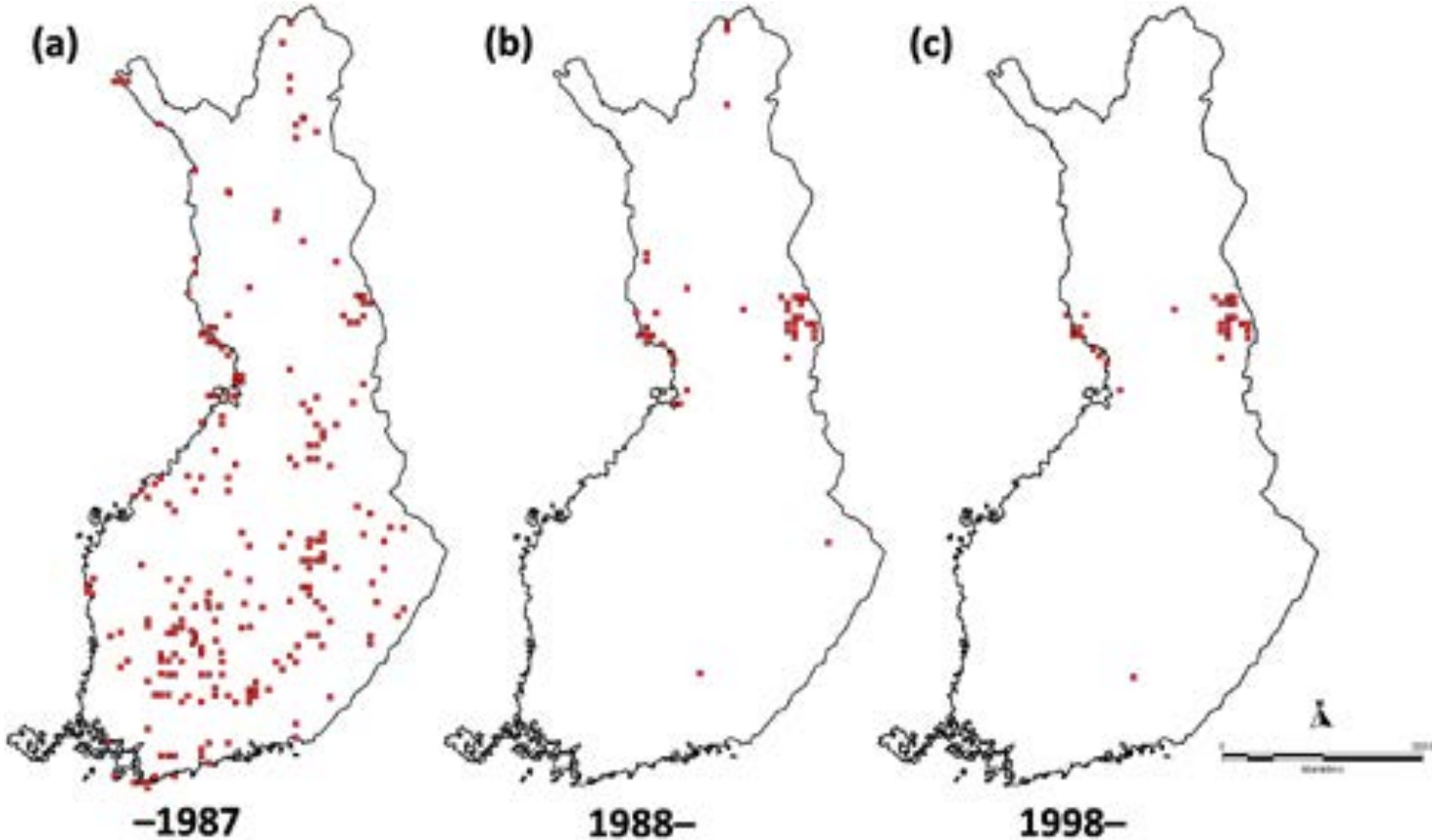


*Lycaena helle*

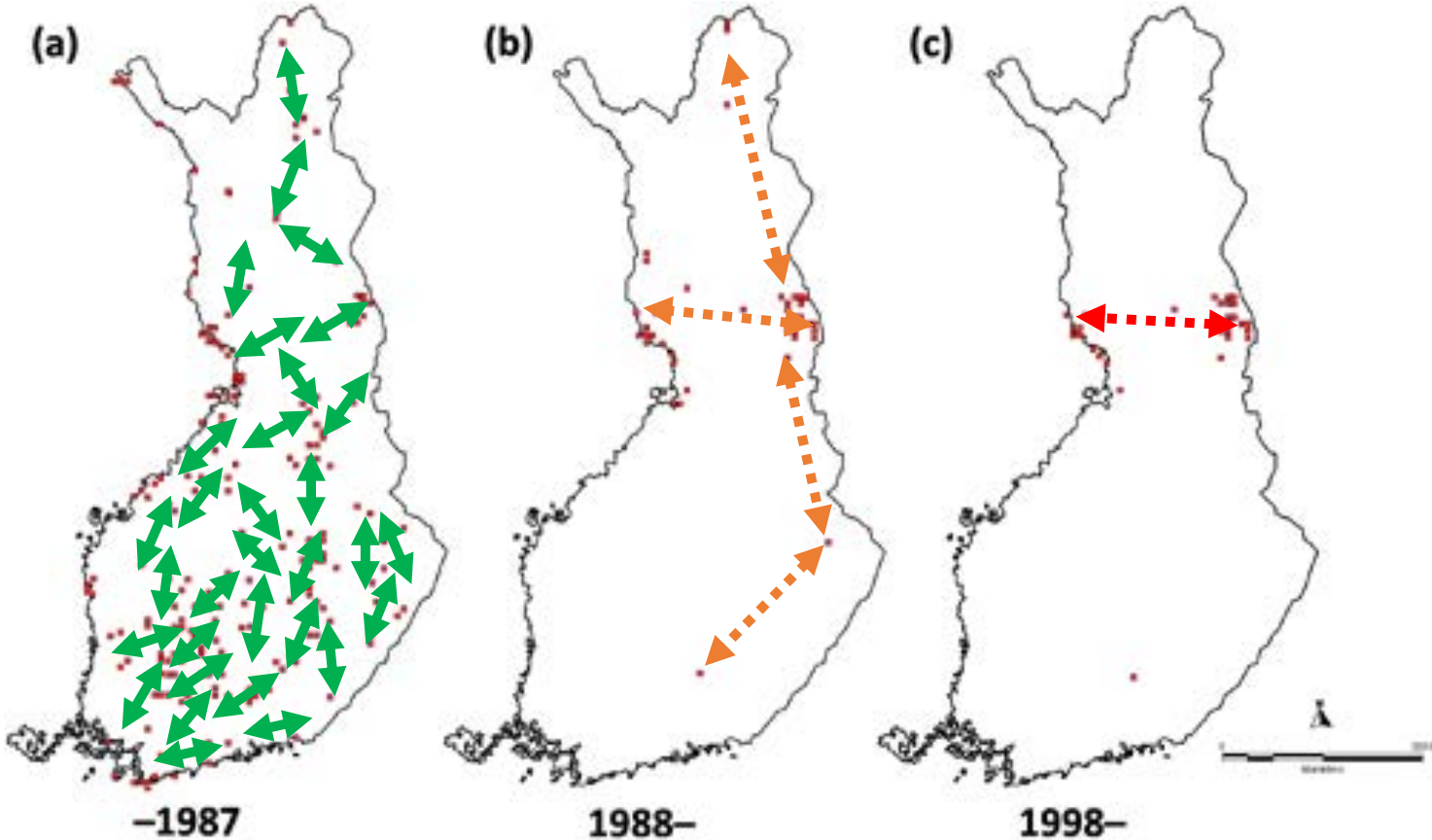


# Comparison with field records

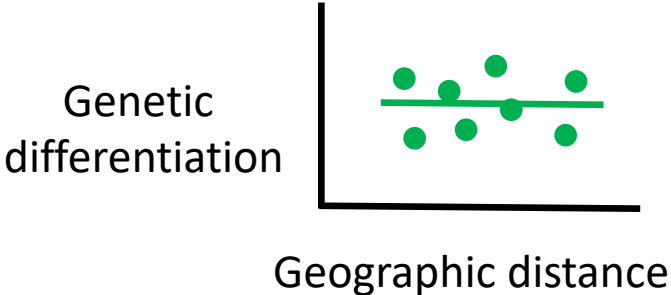
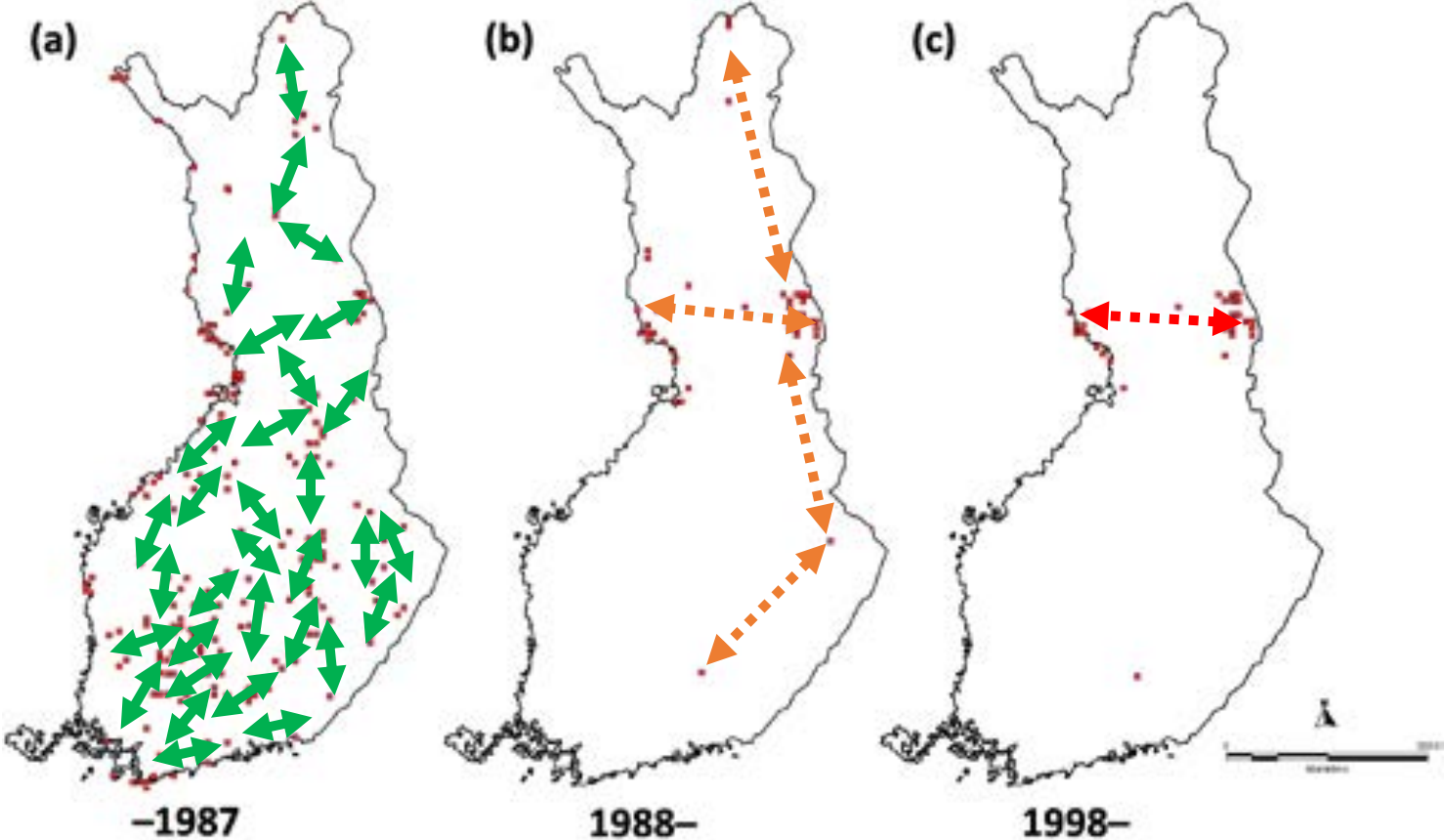
*Lycaena helle*



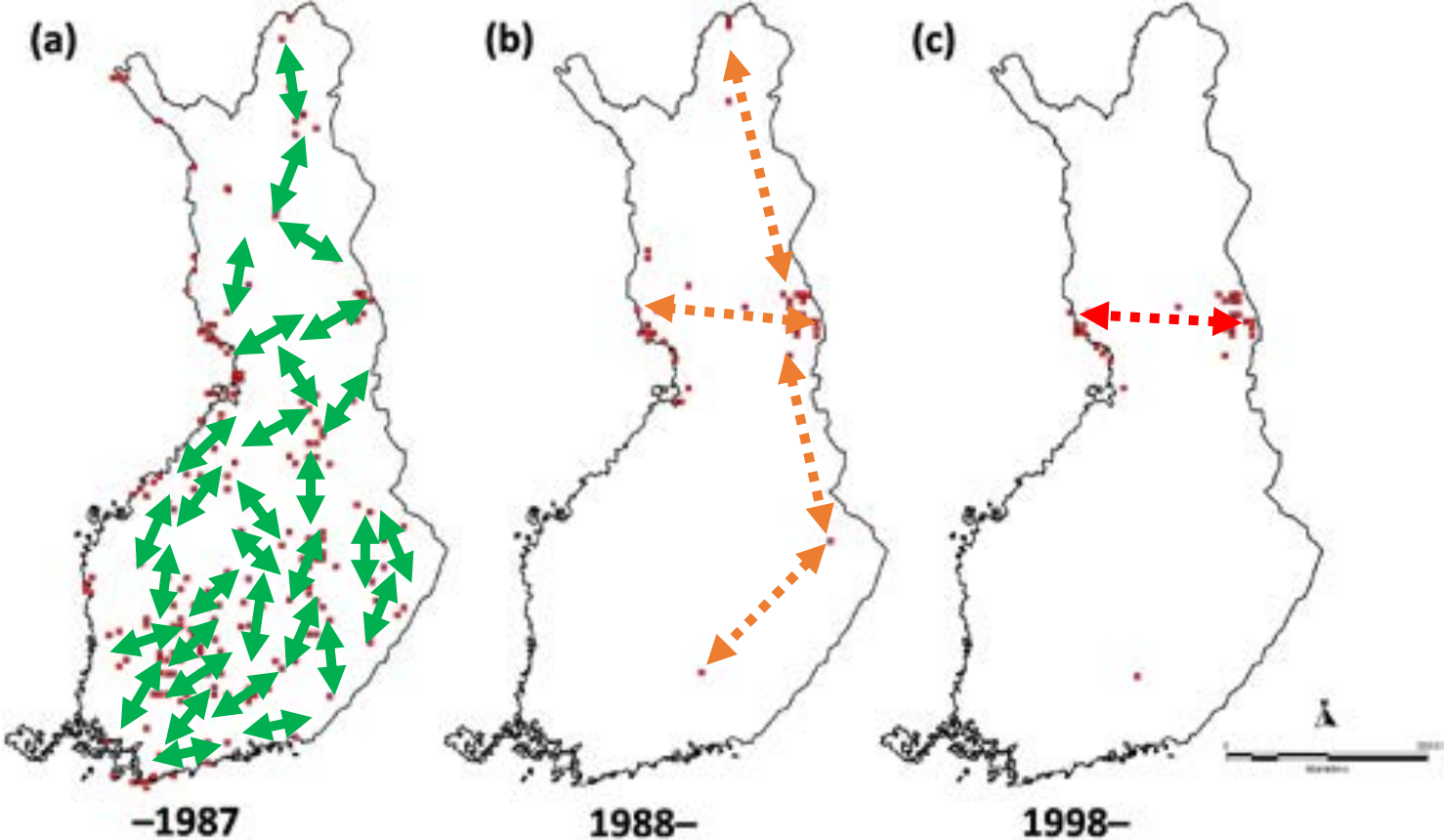
# Isolation by Distance



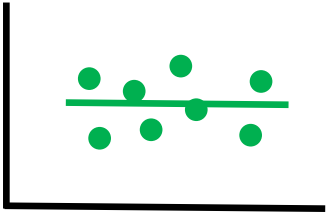
# Isolation by Distance



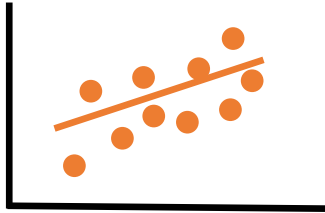
# Isolation by Distance



Genetic differentiation

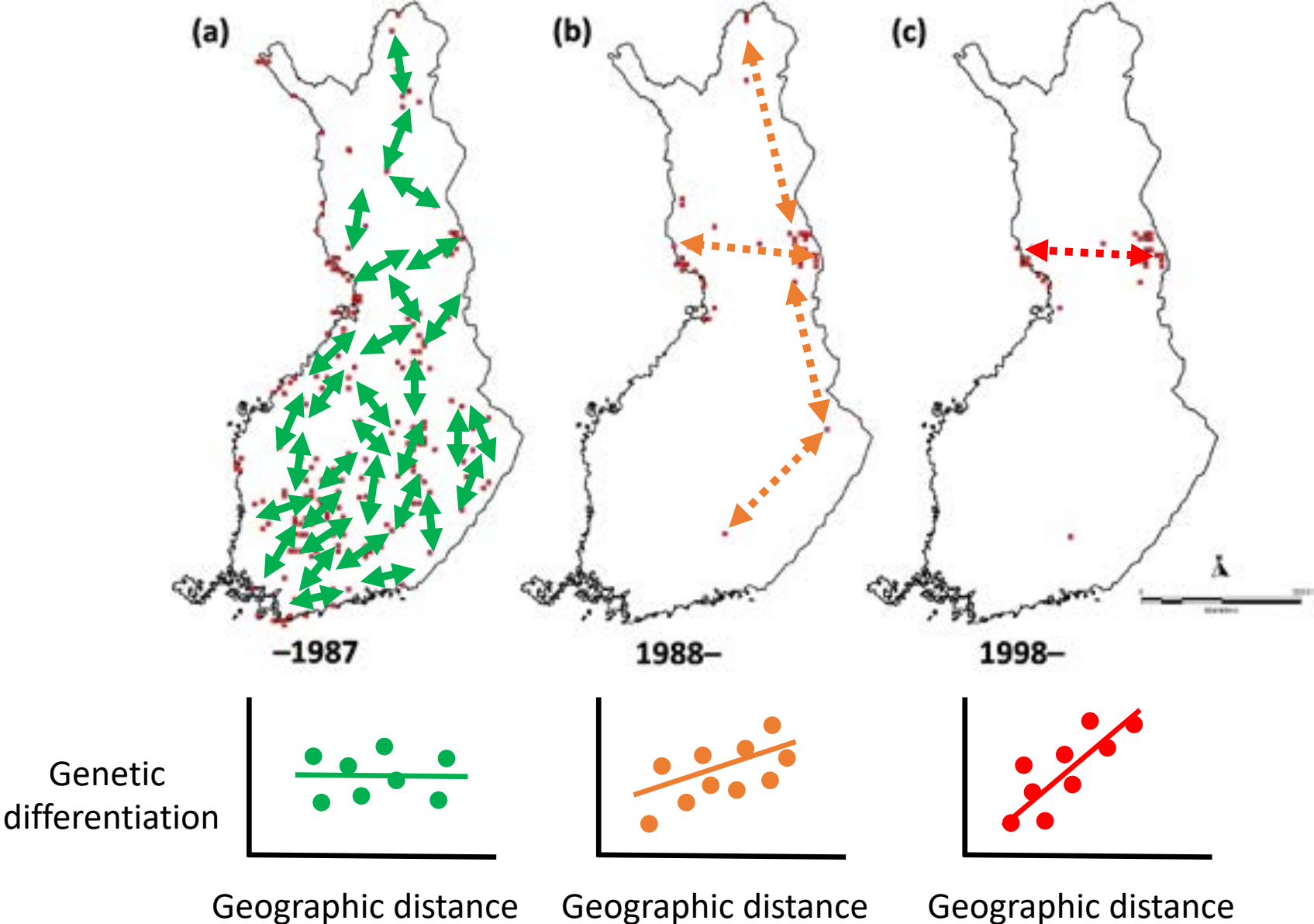


Geographic distance



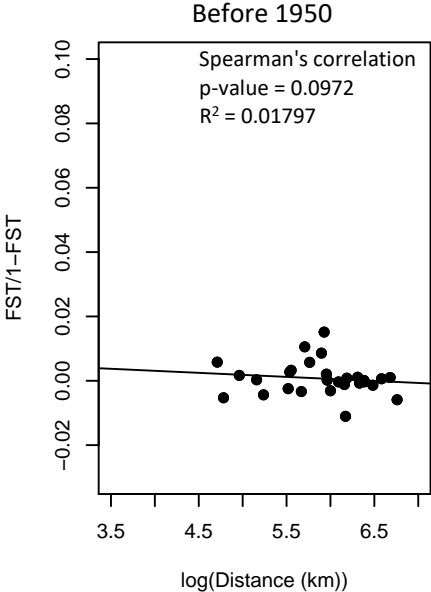
Geographic distance

# Isolation by Distance



# Isolation by Distance

*Erebia embla*

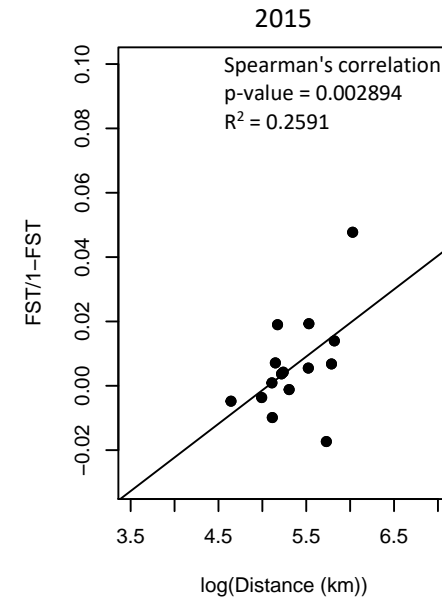
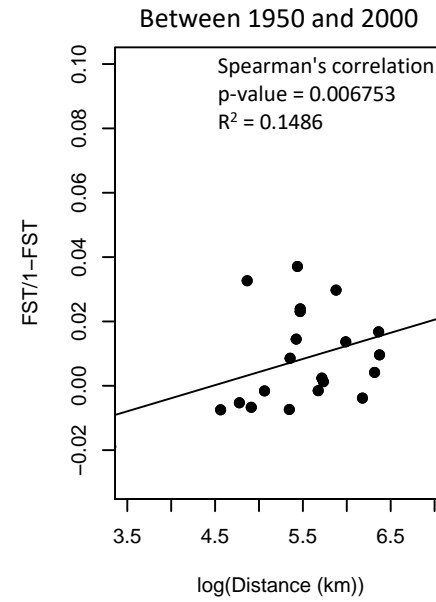
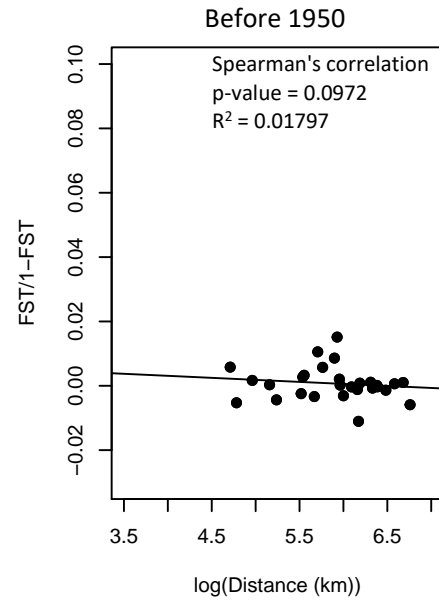




# Isolation by Distance

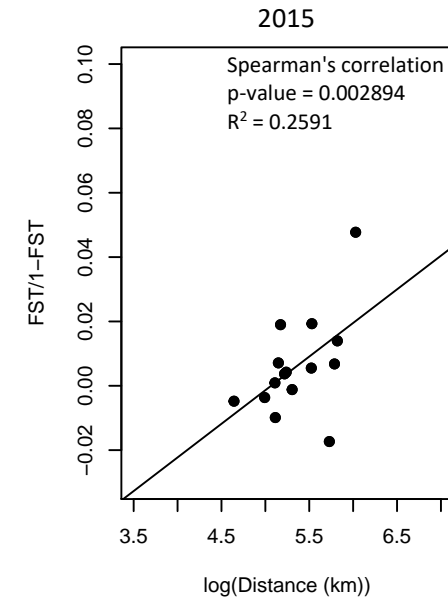
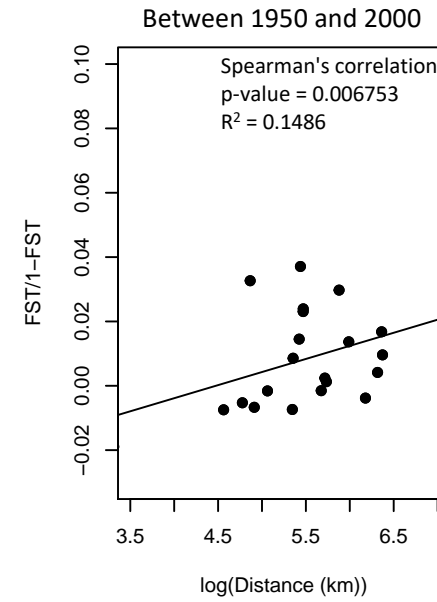
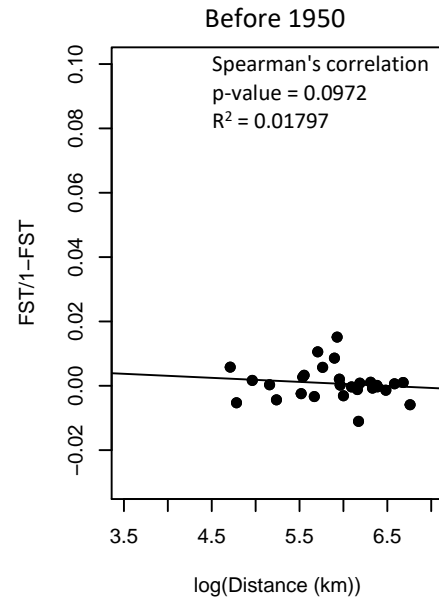


*Erebia embla*

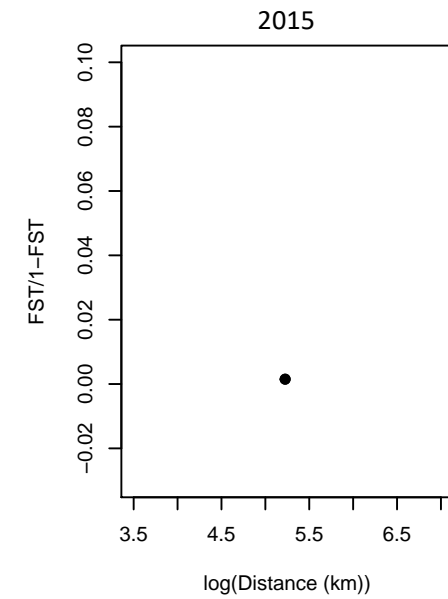
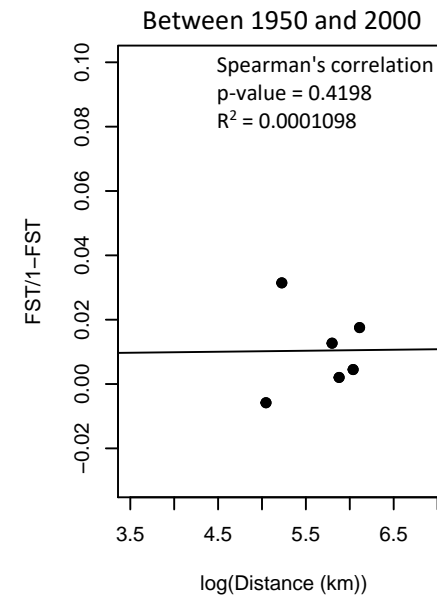
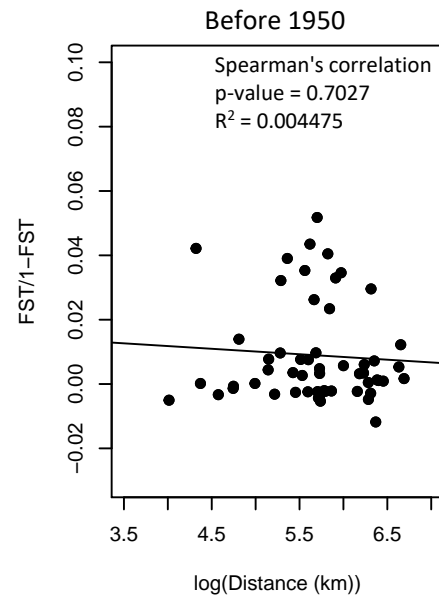


# Isolation by Distance

*Erebia embla*



*Lycaena helle*





Swiss Confederation

Federal Office for the Environment FOEN

## 10 insect species from different orders at the Swiss scale (Lepidoptera Coleoptera EPT Orthoptera Hymenoptera)



*Pieris napi*



*Polyommatus  
icarus*



*Cetonia  
aurata*



*Baetis  
alpinus*



*Serratella  
ignita*



*Miramella  
alpina*



*Chorthippus  
parallelus*



*Omocestus  
viridulus*



*Bombus  
humilis*



*Bombus  
pascuorum*

# Databasing

1. Gathering of extant databases
2. Digitalisation of collection specimens



Specimen  
Metadata  
DNA extraction



Final database of 12 769 entries  
8 608 (67%) entries newly produced

# Historic population identification

Year  
Location

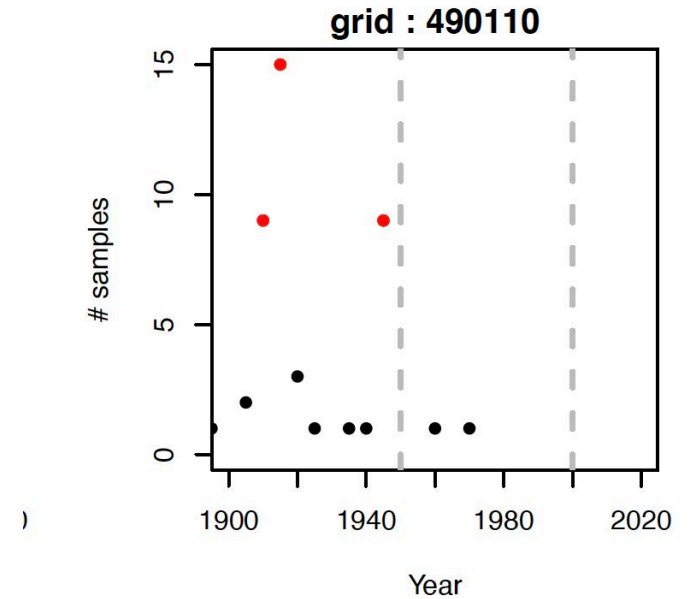
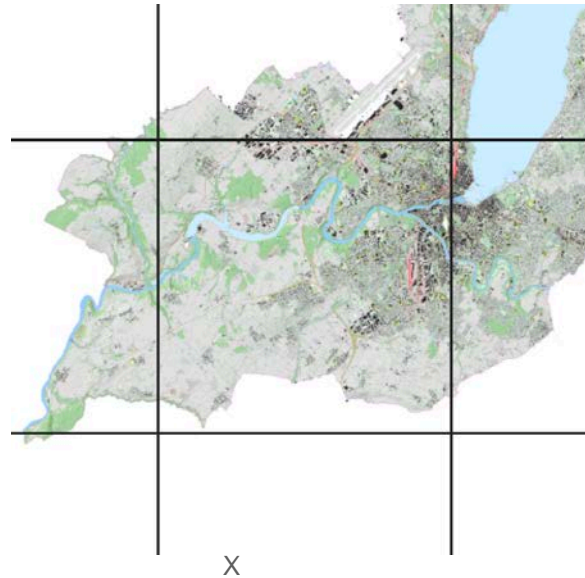
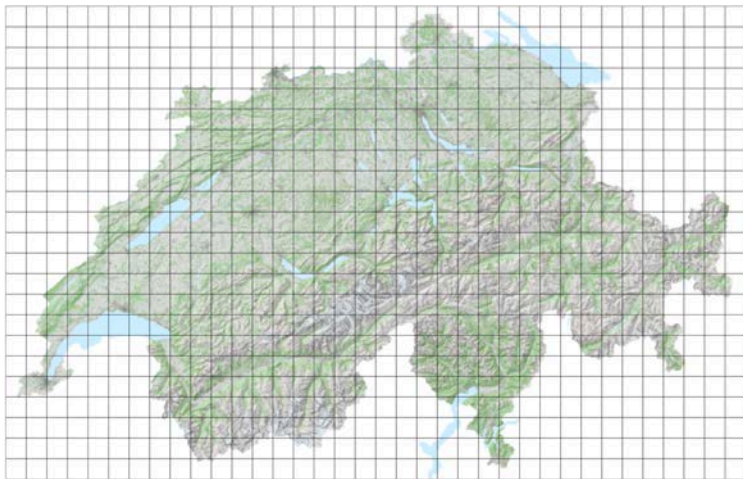


X, y

Grid cell (10x10km; infoFauna)

Location accuracy

Sum of individuals  
of the same grid  $\geq 6$  before 1950  
in a period of 5 years



# Collection's sampling

Total nb collection specimens: 2 739

Sampled so far: 1 301 (47.5%)

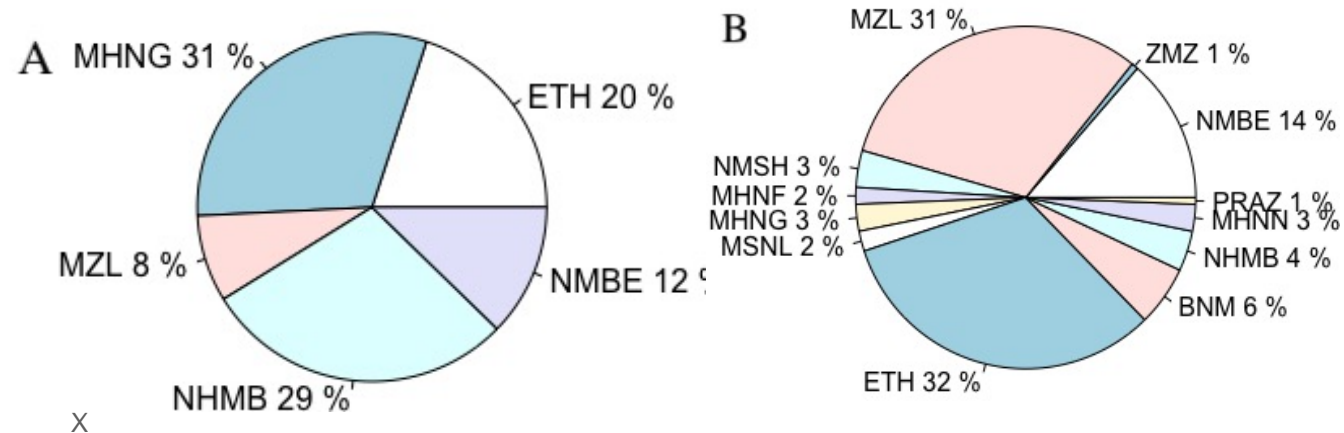


1 leg

Avoid cross contamination

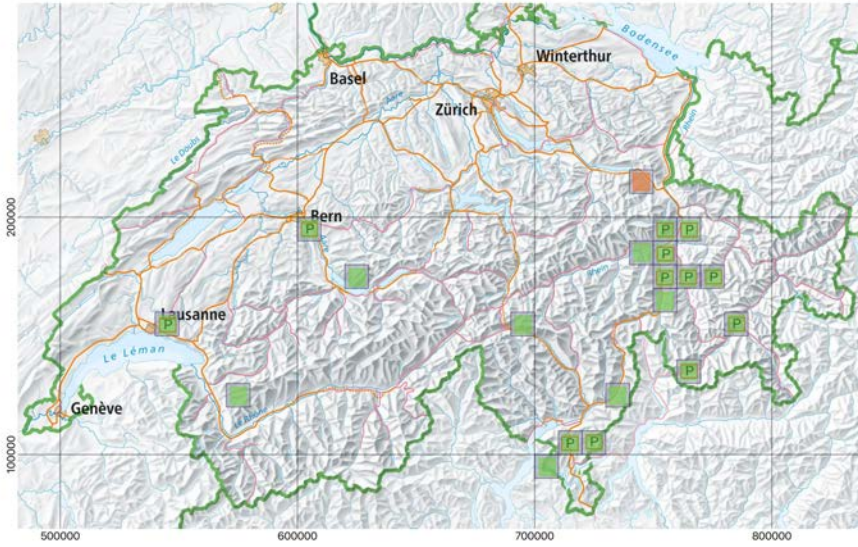
Non-Hymenoptera species

Hymenoptera species



# *Chorthippus parallelus*

DeciNe project  
SCR LV03  
June 2021



## Field campaigning

161 locations to sample

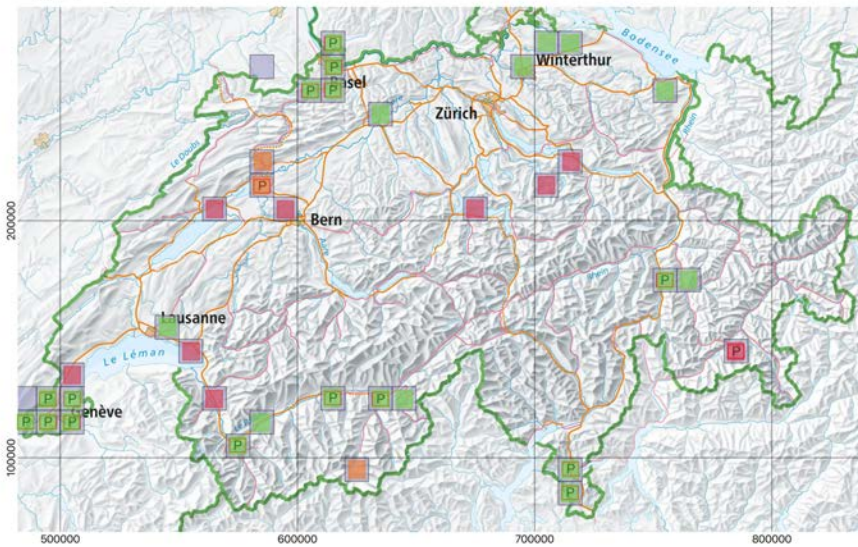
756 samples

72 localities

1 locality → 1 complete individual  
9 legs

# *Polyommatus icarus*

DeciNe project  
SCR LV03  
June 2021



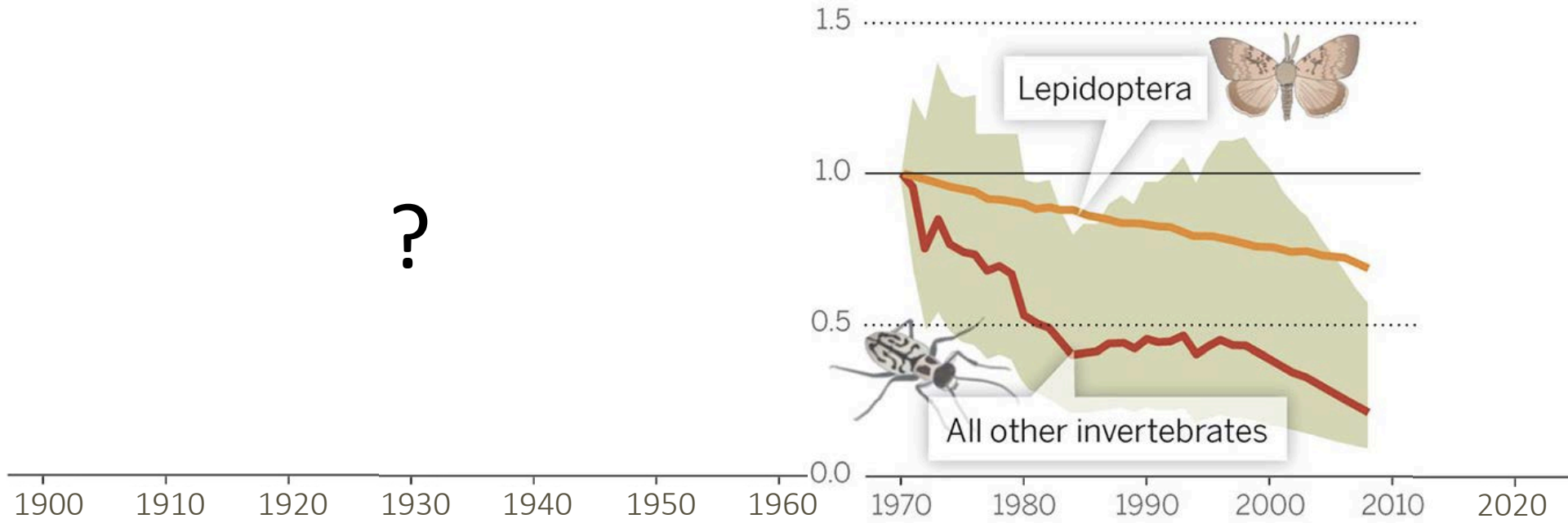
X



# Insect decline

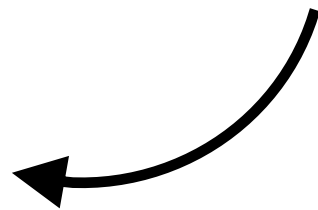
?

Global index of invertebrate abundance



Past diversity

Museomics

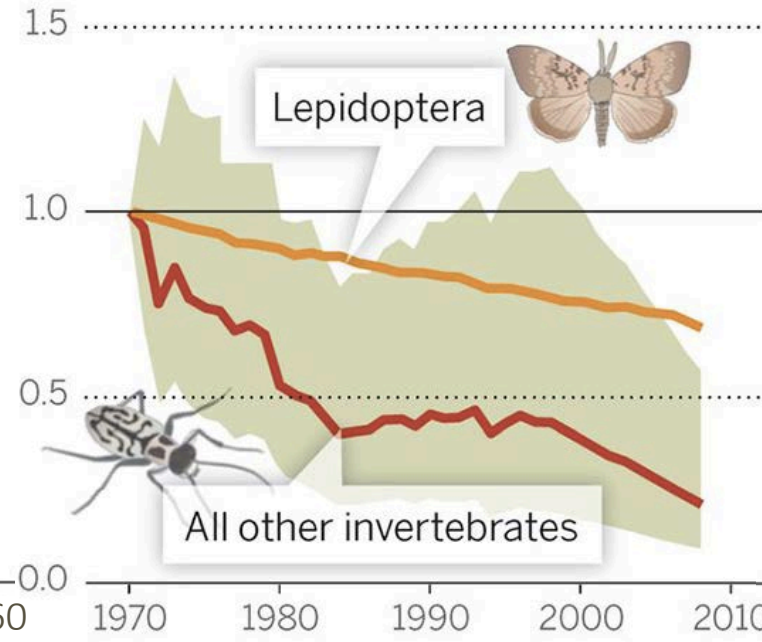




# Insect decline

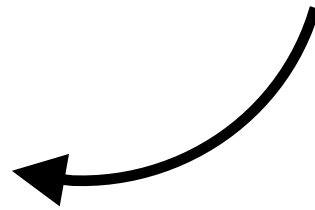
?

Global index of invertebrate abundance

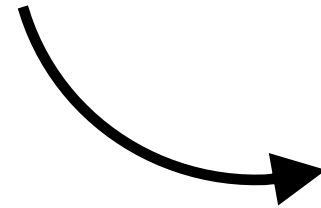


?

Past diversity  
Museomics

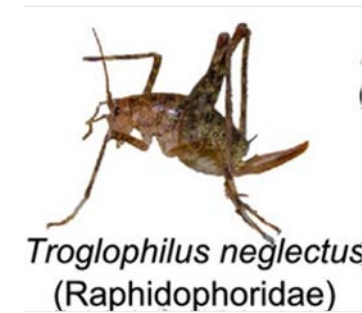
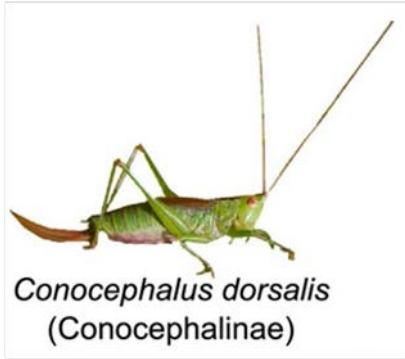


Tracking the evolution  
of the diversity

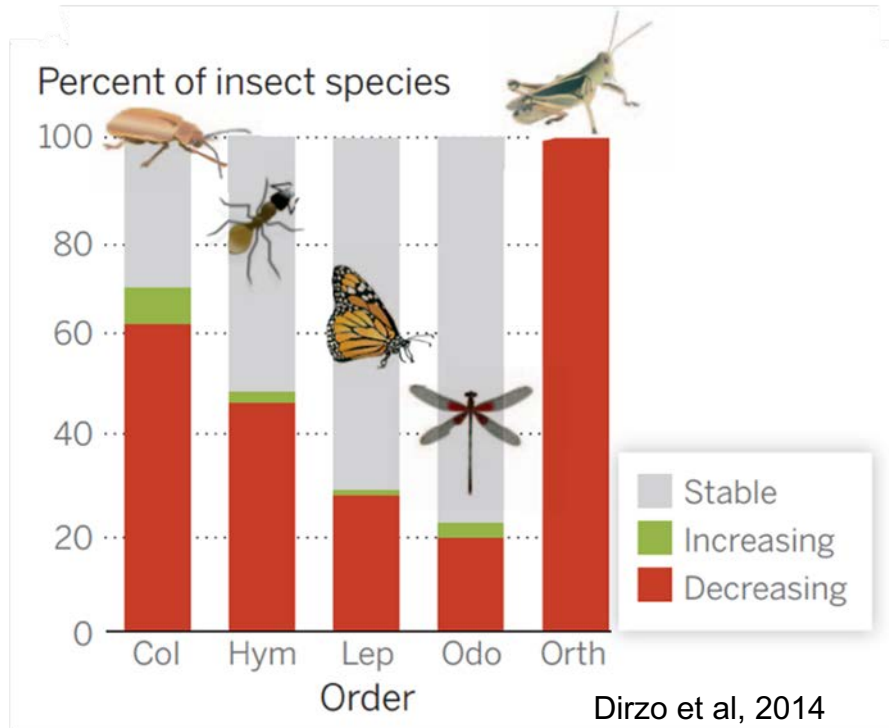


# Development of a multi-locus marker for swiss orthoptera

## OrthoSwissBOL project



# Orthopteran species



## Bioindicator species

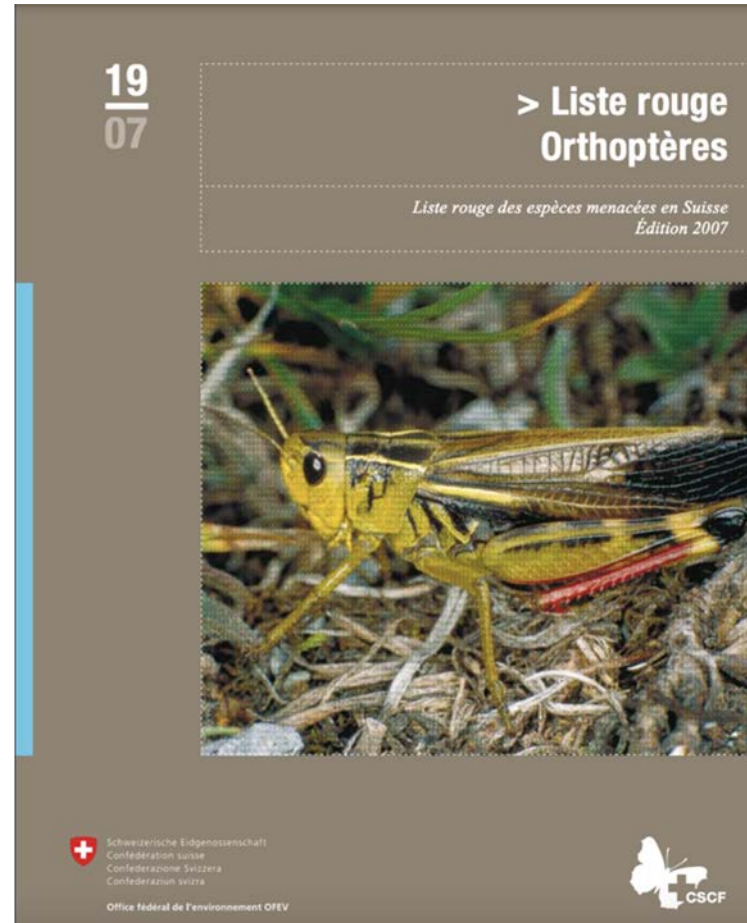
- pioneer areas in river alluvial zones
- secondary pioneer areas
- marshes and wet meadows
- scorched lawns, dry meadows and pastures
- semi-forest



# Swiss Orthoptera

105 species

7 families

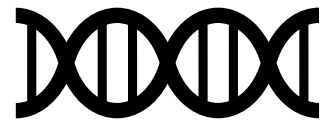
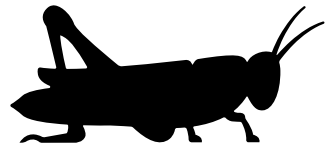


- Red List of Swiss Orthoptera 2007:  
40% are threatened
- Ongoing update

# Single-locus barcode

DNA barcoding of crickets, katydids and grasshoppers (Orthoptera) from Central Europe with focus on Austria, Germany and Switzerland

O. HAWLITSCHKEK,\*† J. MORINIÈRE,\* G. U. C. LEHMANN,‡ A. W. LEHMANN,§ M. KROPP,¶ A. DUNZ,\* F. GLAW,\* M. DETCHAROEN,\* S. SCHMIDT,\* A. HAUSMANN,\* N. U. SZUCSICH,\*\* S. A. CAETANO-WYLER†† and G. HASZPRUNAR\*,‡‡



Define trophic networks

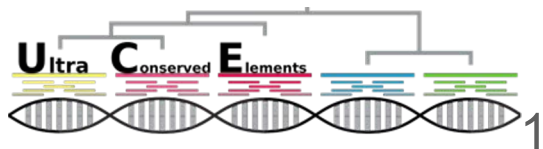
Define species boundaries

Species monitoring

Undercover cryptic species

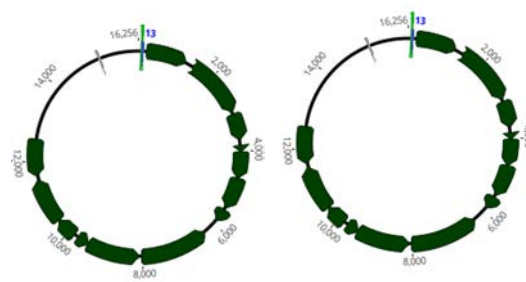
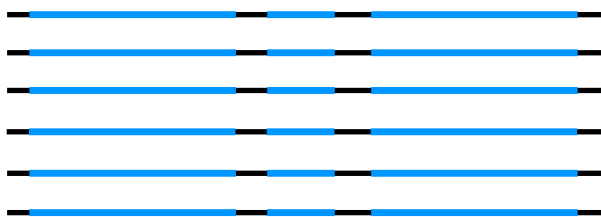
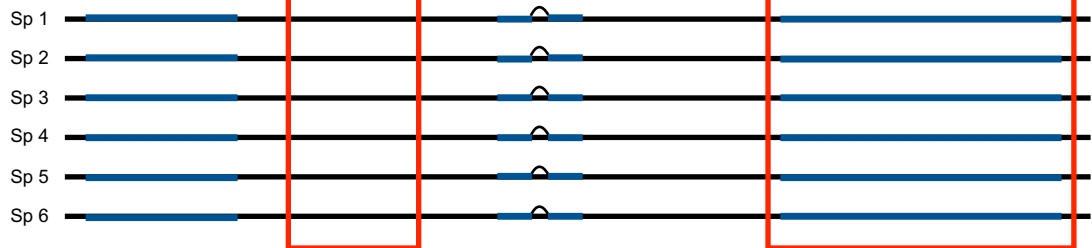
40% of Caelifera can not be identified using COI barcode

- Lack of variation at the interspecies level
- Incomplete lineage sorting
- Nuclear Mitochondrial DNA Segments (*numts*)
- Negligible variation within-species



Ribosomal DNA 3

Mitogenome 4



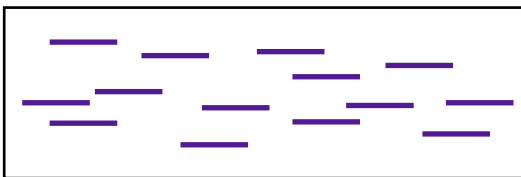
Alignment



Probes design



Probe set  
*de novo*  
synthesis



# Ribosomal DNA<sub>3</sub>



Complete rDNA sequences

2 - Caelifera  
0 - Ensifera

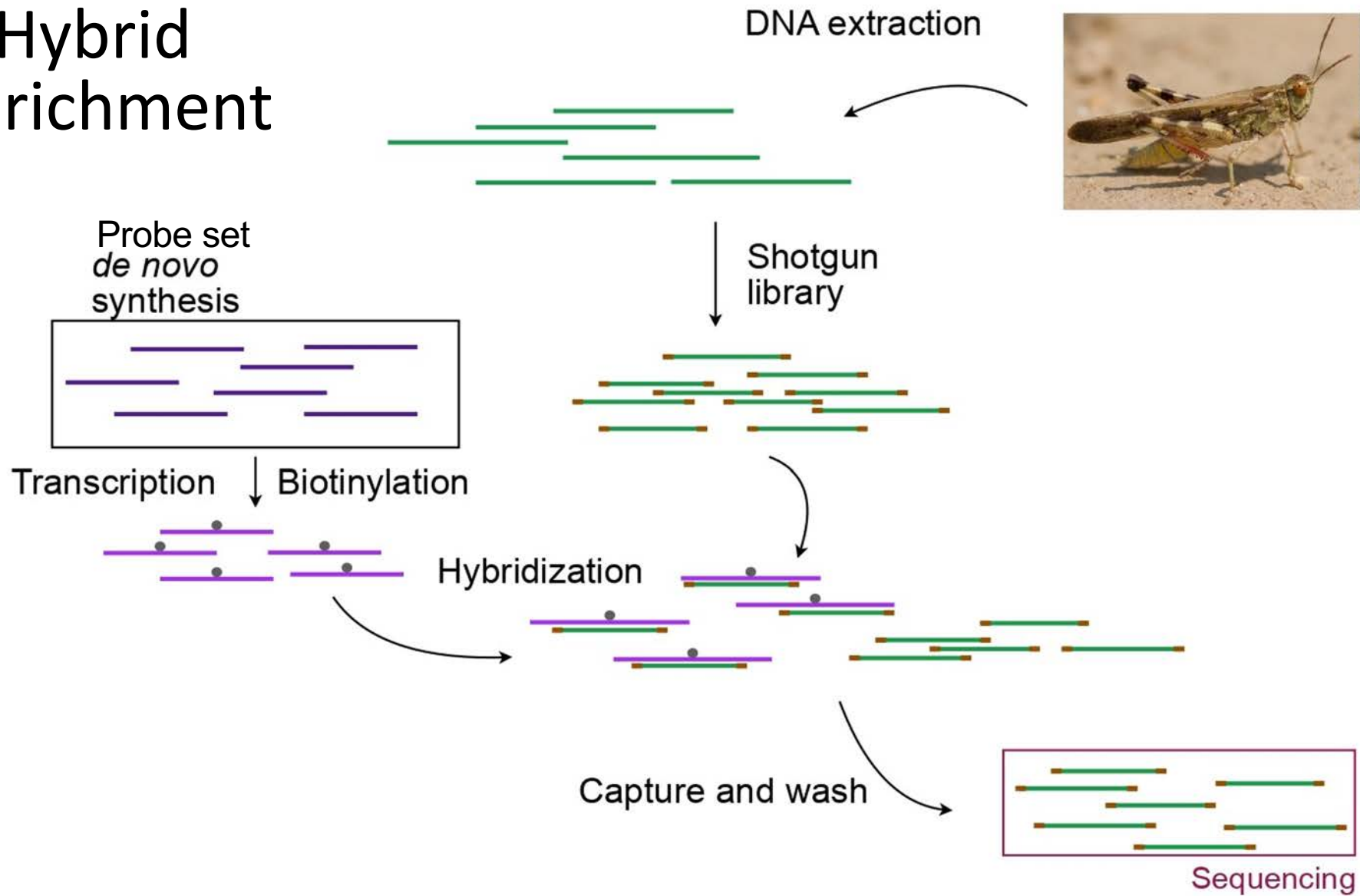
Universal eukaryote primers Krehenwinkel et al. 2018



Ribosomal DNA sequences for 13 new species!



# Anchored Hybrid Enrichment





# Perspectives


- Update of the Orthoptera Red List
- Application of the multi-locus marker approach to other groups

19  
07

## > Liste rouge Orthoptères

*Liste rouge des espèces menacées en Suisse*  
Édition 2007



 Schweizerische Eidgenossenschaft  
Confédération suisse  
Confederazione Svizzera  
Confederaziun svizra

Office fédéral de l'environnement OFEV



# Thank you for your attention



Inés Carrasquer  
Camille Pitteloud  
Julia Bilat  
Nadir Alvarez



# Monitoring



# Development of a multi-purpose and multi-locus marker for (Swiss) Orthoptera

## OrthoSwissBOL project



Schweizerische Eidgenossenschaft  
Confédération suisse  
Confederazione Svizzera  
Confederaziun svizra

Swiss Confederation

**Federal Office for the Environment FOEN**

# Multi-locus marker: Species delimitation

Groupe *Chorthippus biguttulus*

*C. brunneus* →

*C. mollis*

*C. biguttulus*

*C. eisentrauti* ↙



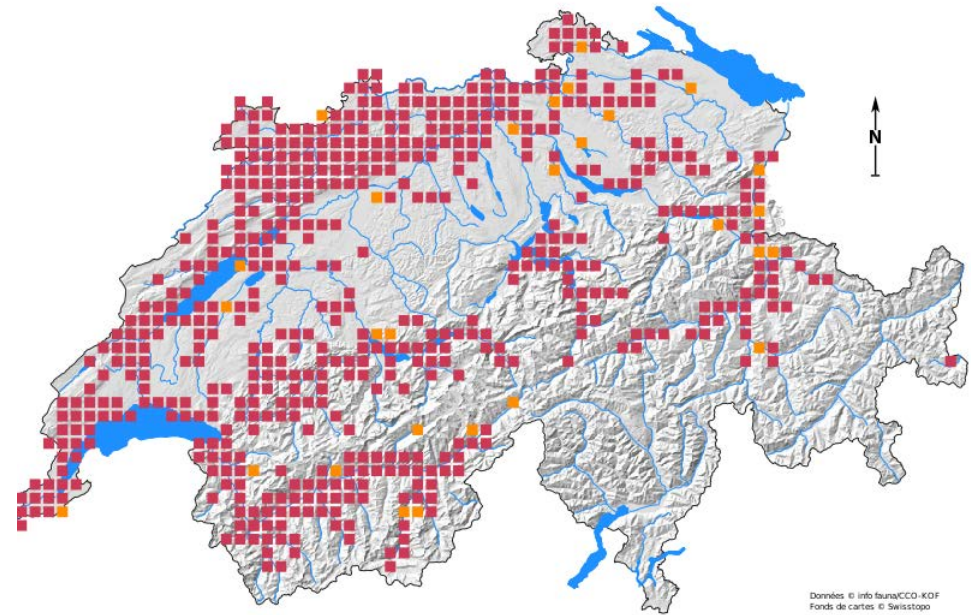
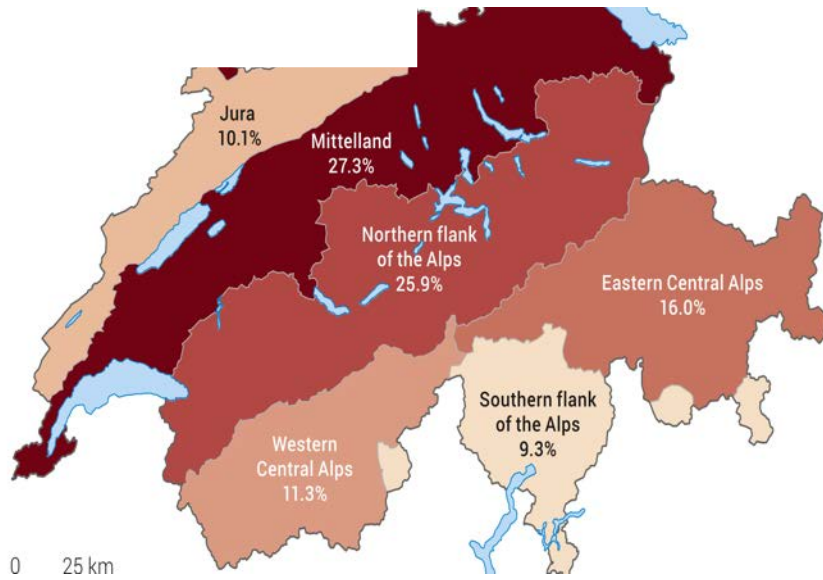
*Platycleis albopunctata*

ssp. *albopunctata*

ssp. *grisea*



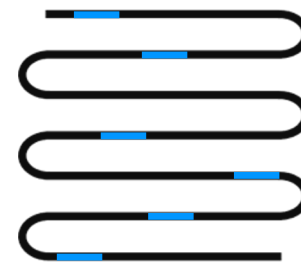
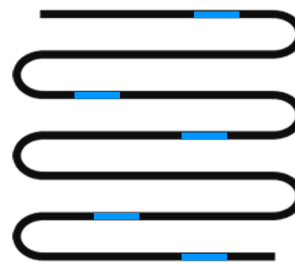
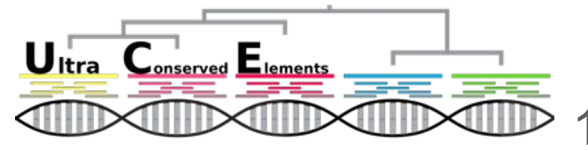
# Multi-locus marker: Genetic diversity inference



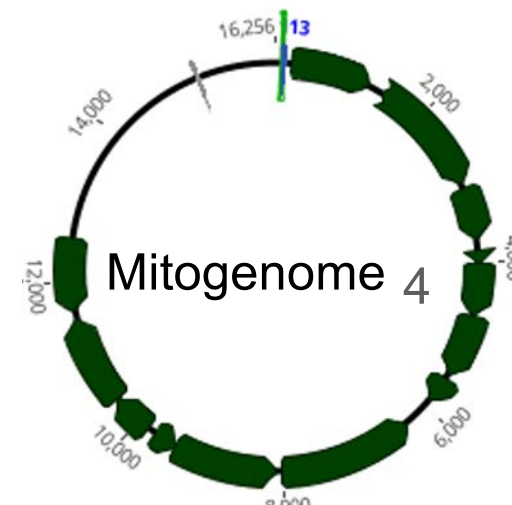
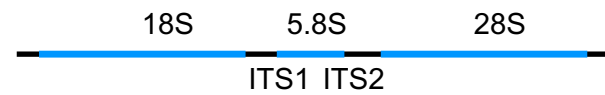
| ESPECE             | JU | PL | NA | AiOc | AiOr | SA | Total général |
|--------------------|----|----|----|------|------|----|---------------|
| pus barbarus       | 3  |    |    |      |      |    |               |
| pus italicus       | 4  | 6  |    |      | 4    | 1  | 3             |
| pus siciliae       |    |    |    |      |      |    | 3             |
| pus albomarginatus | 8  | 5  | 1  |      |      |    |               |
| pus apicarius      | 3  |    | 5  |      |      | 4  |               |
| pus biguttulus     | 8  | 5  | 9  | 2    | 3    |    |               |
| pus brunneus       | 6  | 5  | 1  | 2    | 3    |    | 6             |
| pus dorsatus       | 4  | 8  | 4  | 1    | 3    |    | 5             |
| pus eisentrauti    |    |    | 3  | 2    | 2    |    | 4             |
| pus mollis         | 2  | 8  |    | 3    | 3    |    | 3             |
| pus montanus       | 11 | 2  | 14 | 1    | 2    |    |               |
| pus parallelus     | 14 | 6  | 8  | 4    | 3    |    | 4             |
| pus pullus         |    |    |    | 1    | 3    |    |               |
| pus vagans         | 4  |    |    |      | 8    |    | 3             |

*Platycleis albopunctata*  
ssp. *albopunctata*

# Genomic components

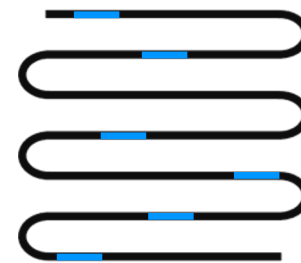
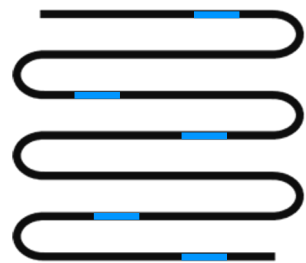
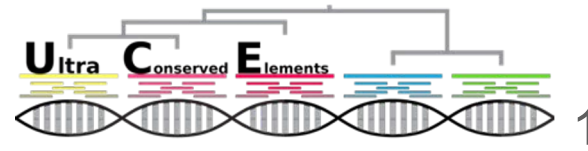


Ribosomal DNA 3

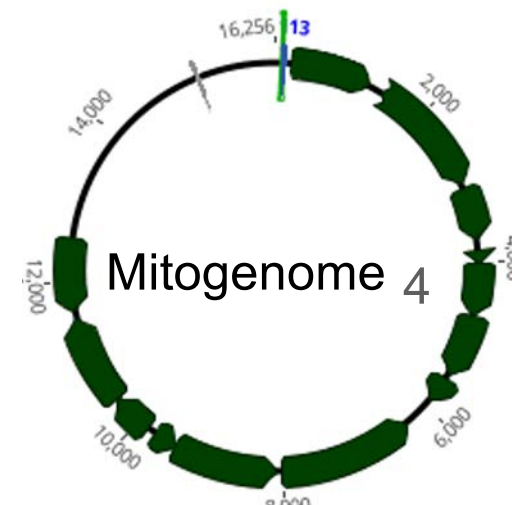
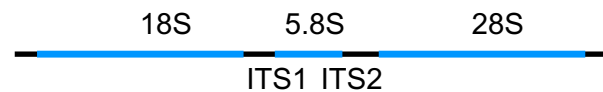


# Genomic components

- Informativeness
- Universality
- Backward compatibility
- Standardization

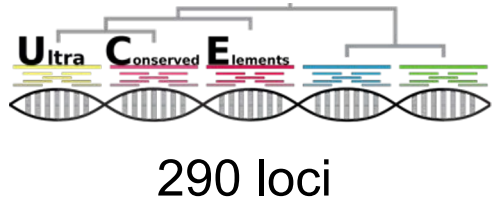


Ribosomal DNA 3





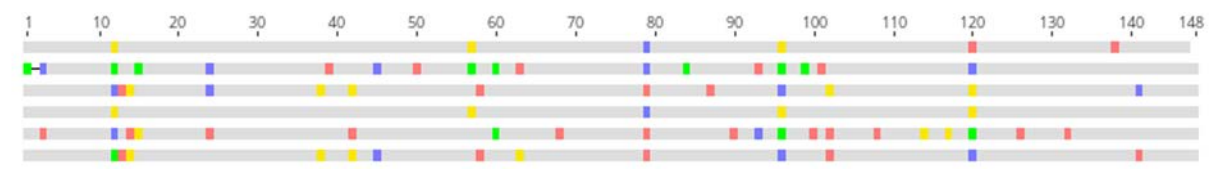
# Conserved genetic regions



PHYLUCE Faircloth, 2016

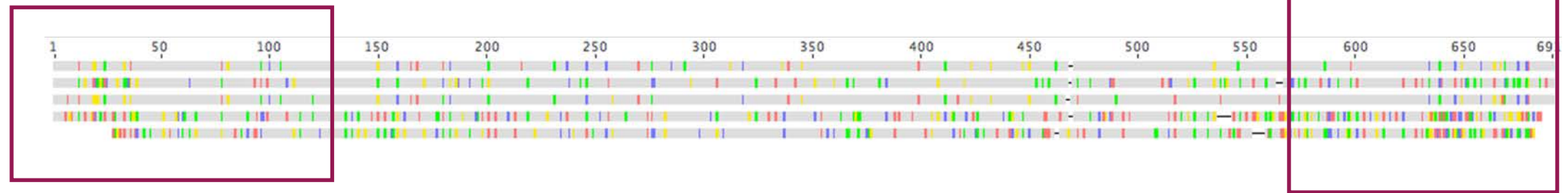


**Reference genomes**  
3 x Ensifera  
3 x Caelifera  
1 outgroup



BUSCO v.4 Manni et al., 2021

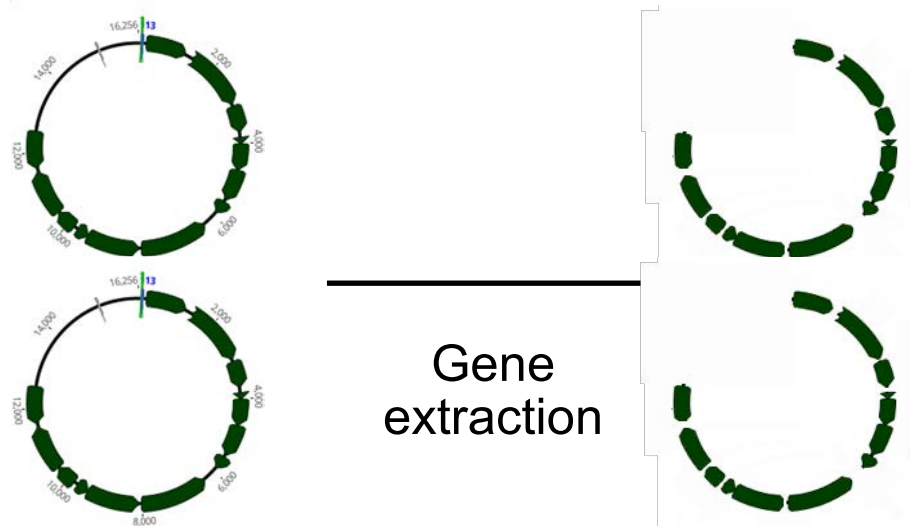
64 loci



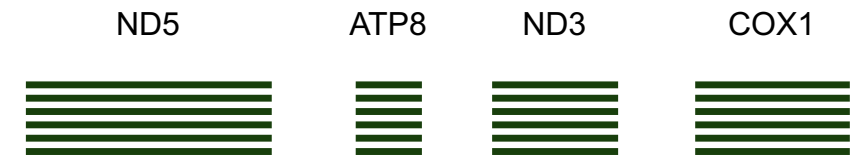
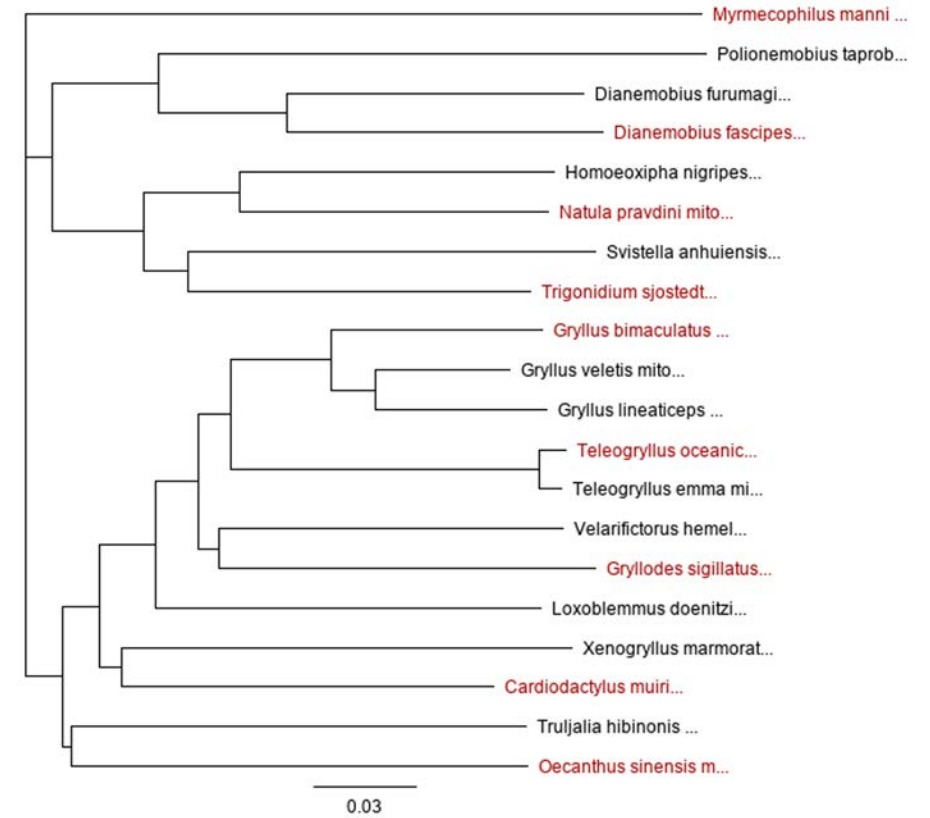
# Mitogenome<sub>4</sub>

 NCBI 240 complete mitochondrion

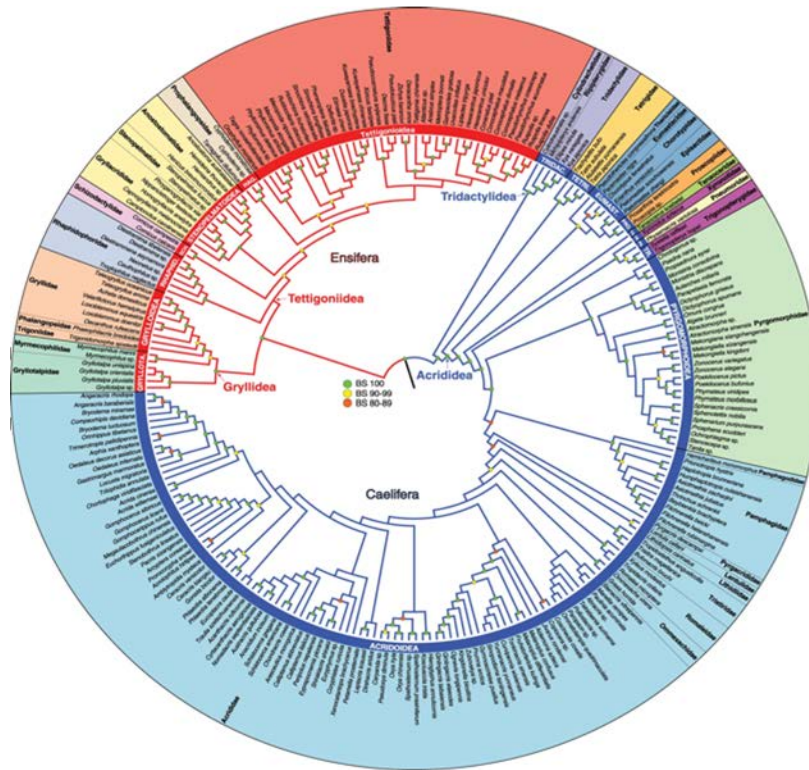
42 selected mitogenomes  
evenly distributed in the phylogeny



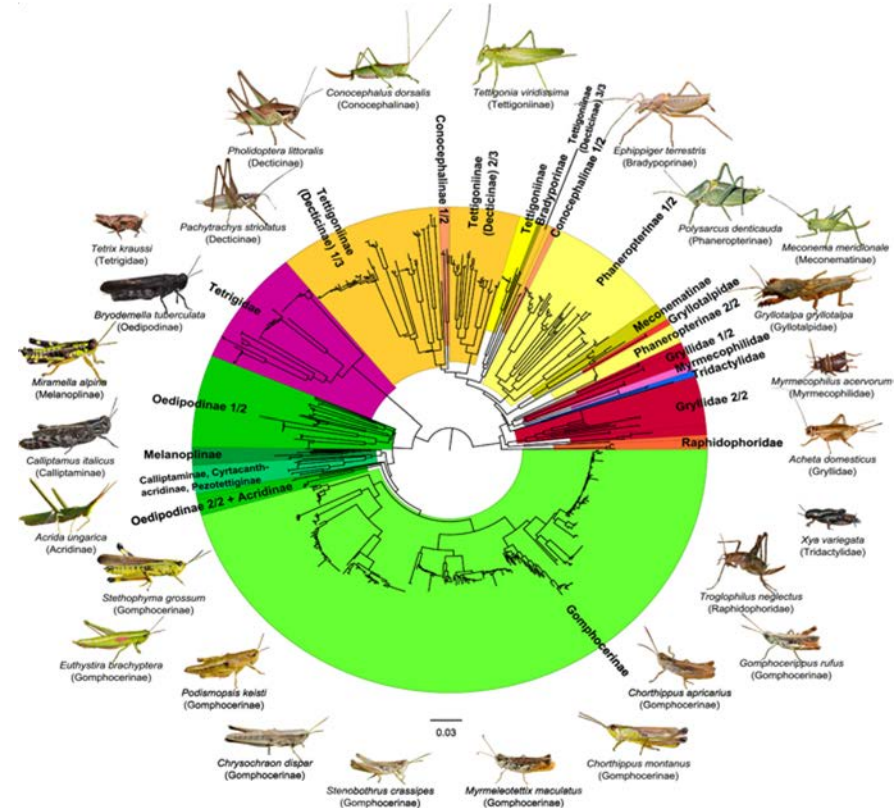
Alignment



# Multi-locus marker: Phylogenetic reconstruction

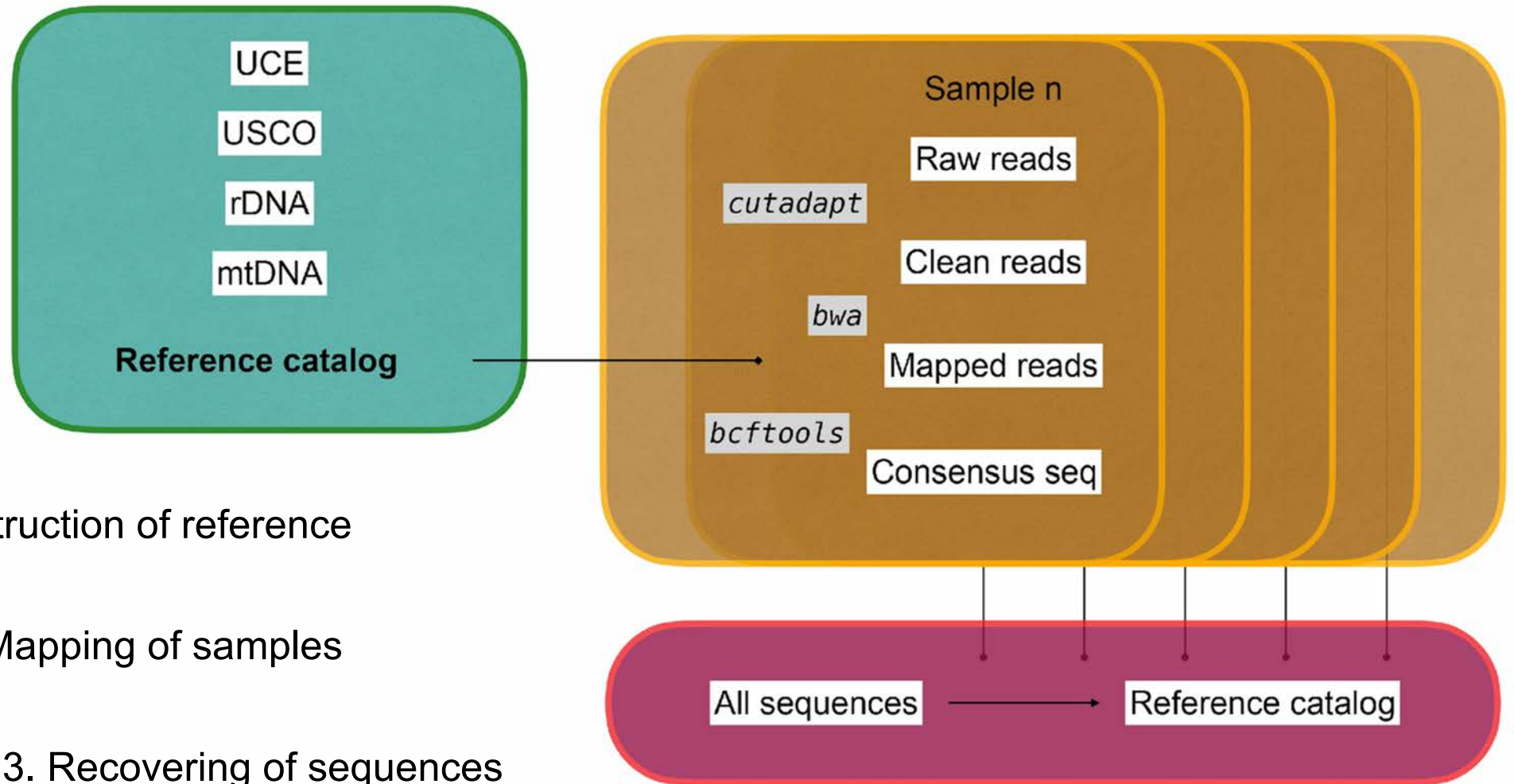


Transcriptomes  
Song et al., 2020



mtDNA  
Hawiltschek et al., 2020

# Data treatment



1. Construction of reference

2. Mapping of samples

3. Recovering of sequences

# Selection of the best combination of loci

Species delimitation  
Phylogenetic power  
Within-species diversity

**Multi-locus barcode**







# Extinction vortex

