CONSERVATION GENOMICS

WPSG
Český Krumlov
2025
Workshop on Population and Speciation Genomics

Emiliano Trucchi

Marche Polytechnic University

WHAT IS THE DIFFERENCE BETWEEN CONSERVATION GENETICS AND GENOMICS?

WHAT IS THE FIRST INGREDIENT OF CONSERVATION GENOMICS?

HOW CAN GENOMICS INFORM CONSERVATION BIOLOGY?

IN WHICH APPLICATION GENOMICS IS A GAME CHANGER?

with a close up on two topics:

Genetic load

ROHs

WHAT AM I DOING TO MAKE USE OF GENOMICS IN CONSERVATION?

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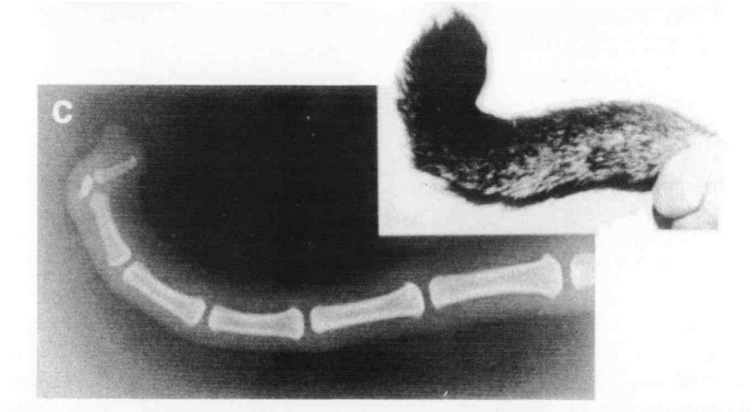
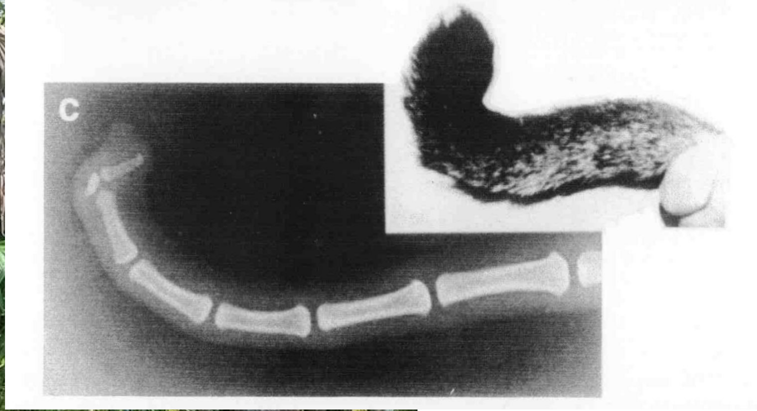
WHOSE TAIL IS THIS?

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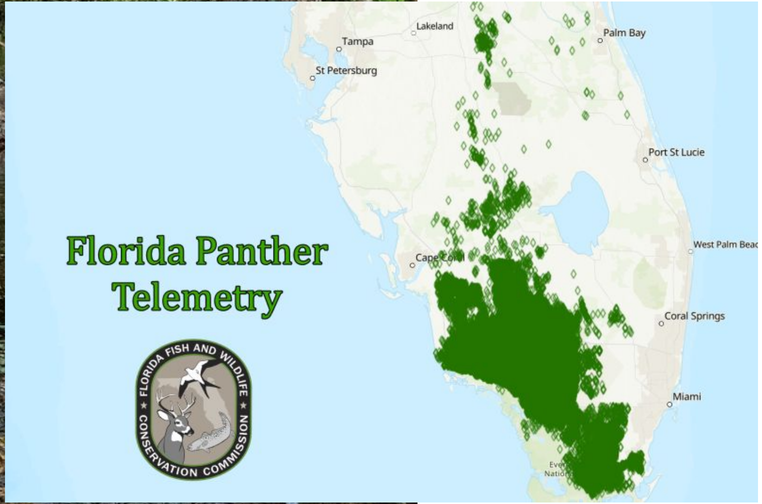
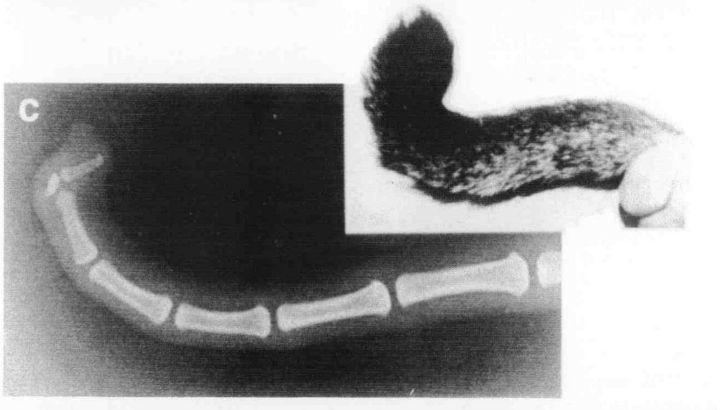

Florida Panther Telemetry
Lakeland
Palm Bay
Tampa
St Petersburg
Port St Lucie
West Palm Beac
Cape Co
Coral Springs
Miami
FLORIDA FISH AND WILDLIFE CONSERVATION COMMISSION

THE FLORIDA PANTHER

from genetics to genomics

Endangered since late ‘60 - early ‘70

Small relic population of 30 individuals in Big Cypress Swamp and Everglades NP



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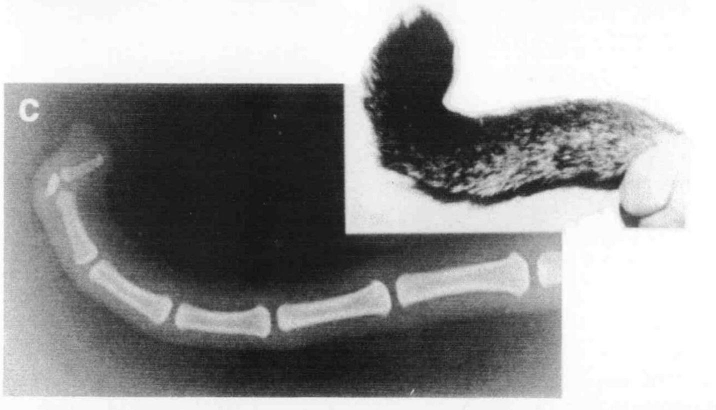

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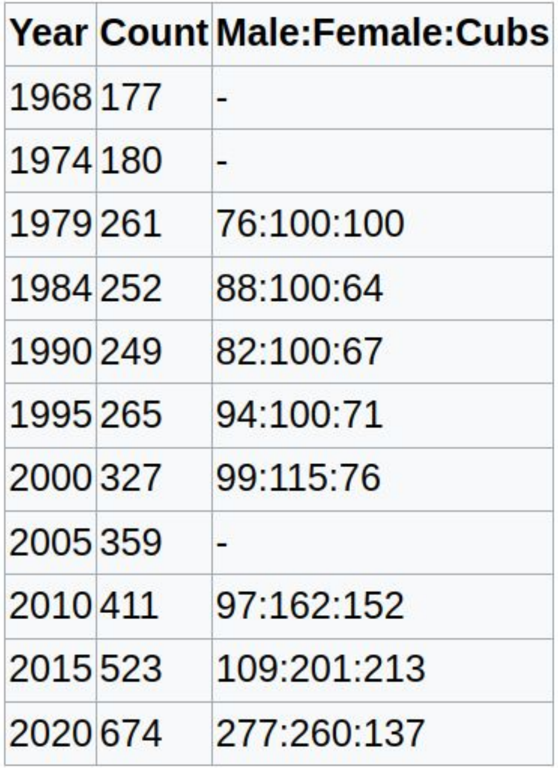
One mitochondrial haplotype, very low allozyme diversity, remarkably low minisatellite DNA diversity (as low as the Asian lions from the Gir Forest Sanctuary)

THE ASIATIC LION

Gir Forest Sanctuary



Year	Count	Male:Female:Cubs
1968	177	-
1974	180	-
1979	261	76:100:100
1984	252	88:100:64
1990	249	82:100:67
1995	265	94:100:71
2000	327	99:115:76
2005	359	-
2010	411	97:162:152
2015	523	109:201:213
2020	674	277:260:137

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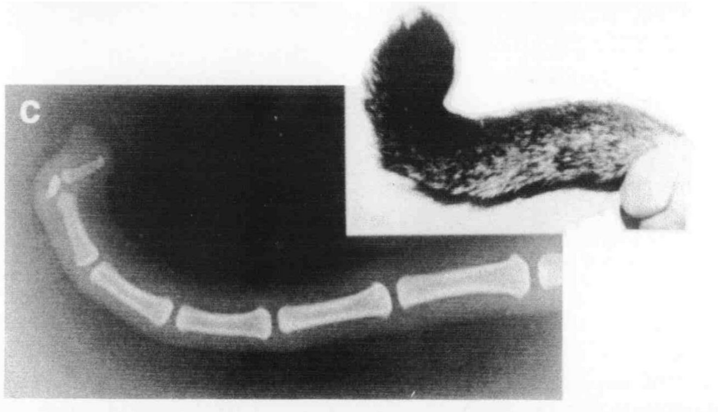

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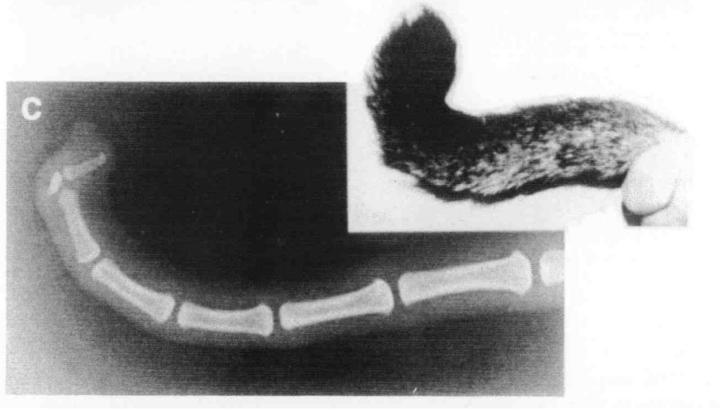

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RECOMMENDATION: immediate augmentation of the population with Texas pumas! (Roelke et al 1993)

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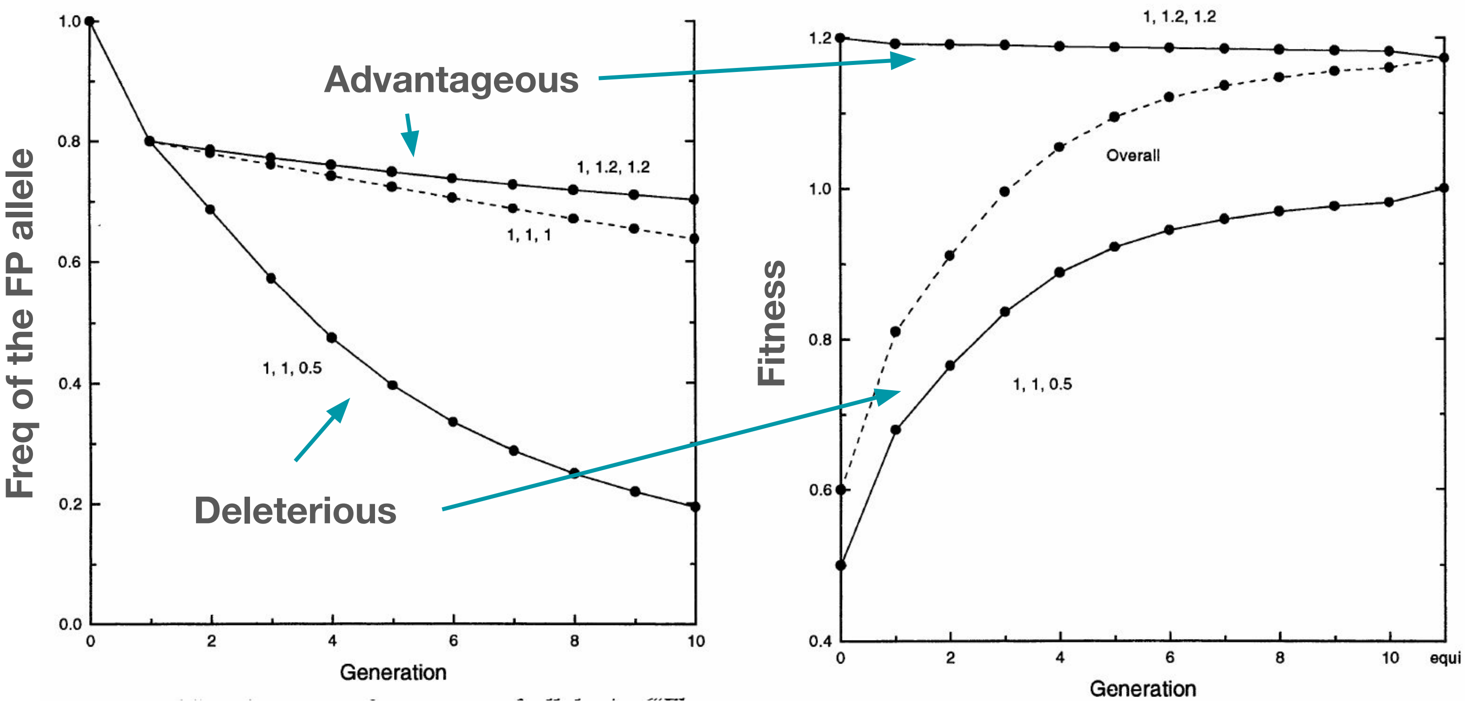
from genetics to genomics

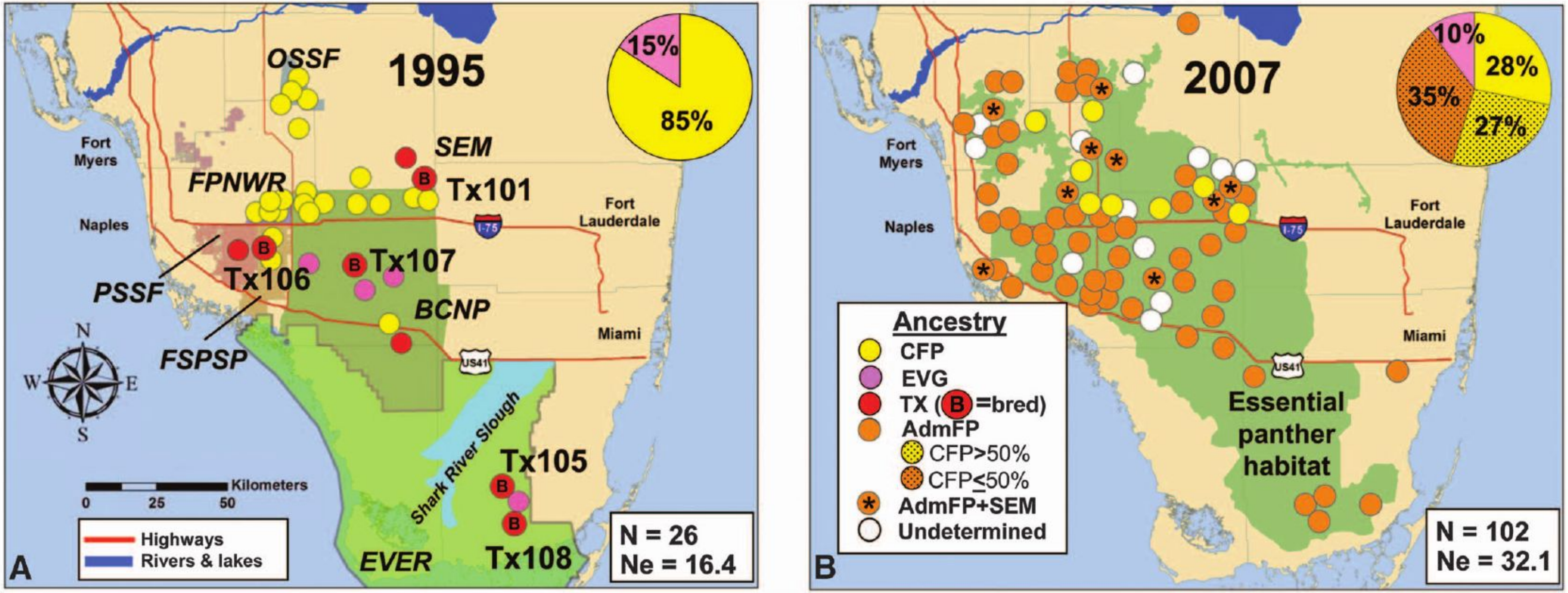


GENETIC RESCUE (m=0.2 females) will eliminate lowered fitness, restore genetic diversity, retain adaptive alleles (Hendrick 1995)

THE FLORIDA PANTHER

from genetics to genomics

Hybrid females did better! 3X survival!

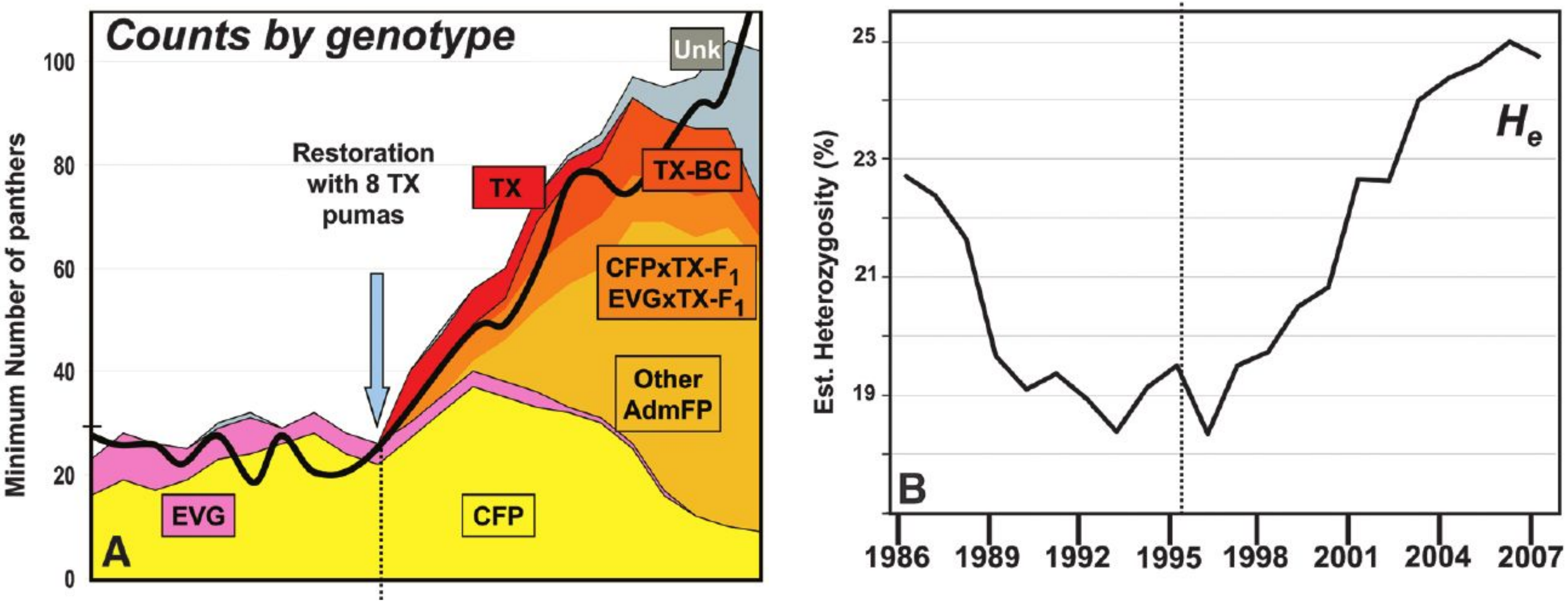


12 YEARS AFTER THE GENETIC RESCUE N, Ne and range increased due to hybrids individuals (Johnson et al 2010)

THE FLORIDA PANTHER

From genetics to genomics

Heterozygosity
estimated using **STR!**



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THE FLORIDA PANTHER

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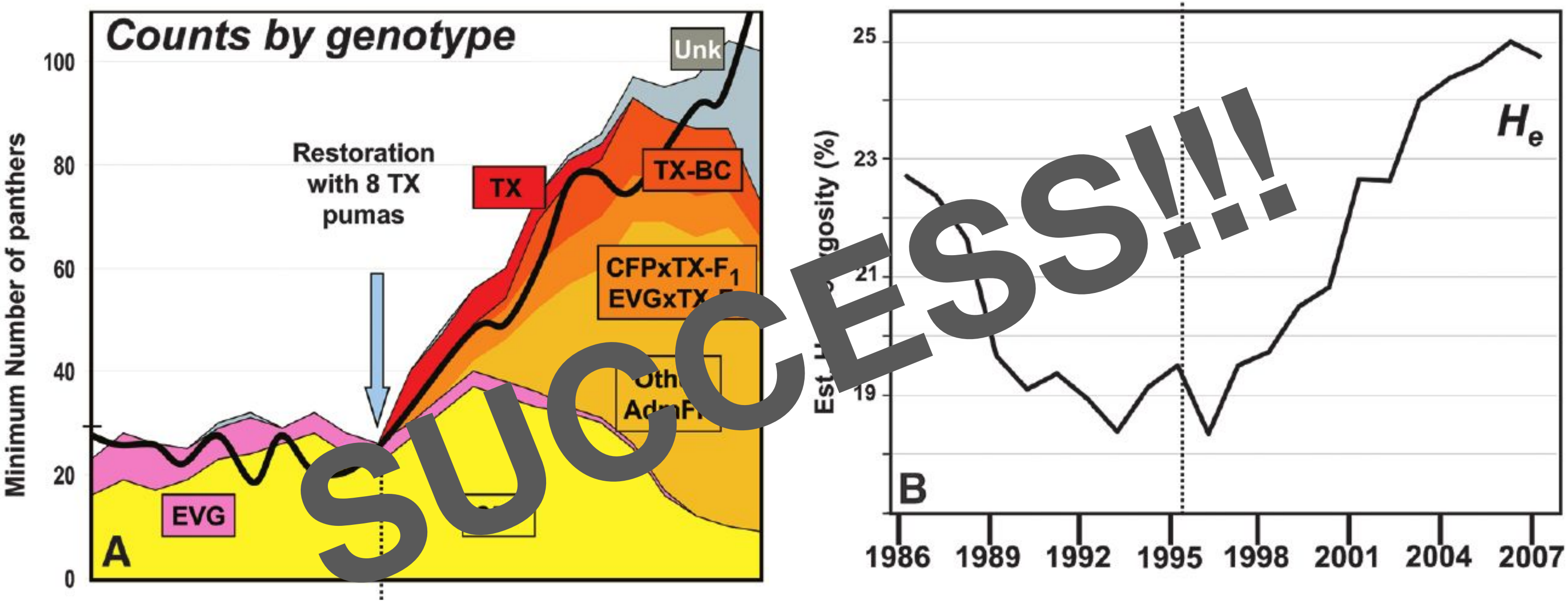


SUCCESS!!!

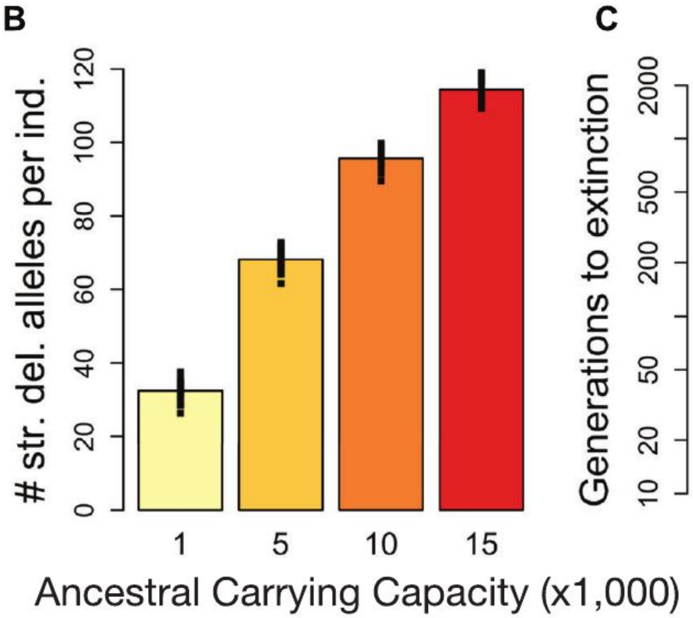
12 YEARS AFTER THE GENETIC RESCUE N, Ne and range increased due to hybrids individuals, and heterozygosity, of course! (Johnson et al 2010)

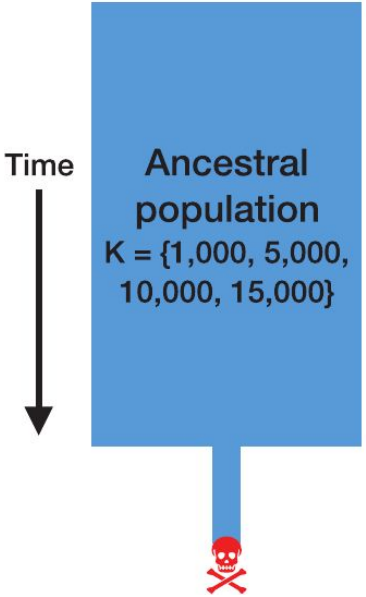
THE FLORIDA PANTHER
From genetics to genomics

BUT LET'S INVESTIGATE THIS SUCCESS STORY
THROUGH A GENOMIC LENS

THE FLORIDA PANTHER

From genetics to genomics





GENOMICS SIMULATIONS indicate that larger populations have larger risk of extinction in case of a sudden bottleneck (Kyriazis et al 2021)

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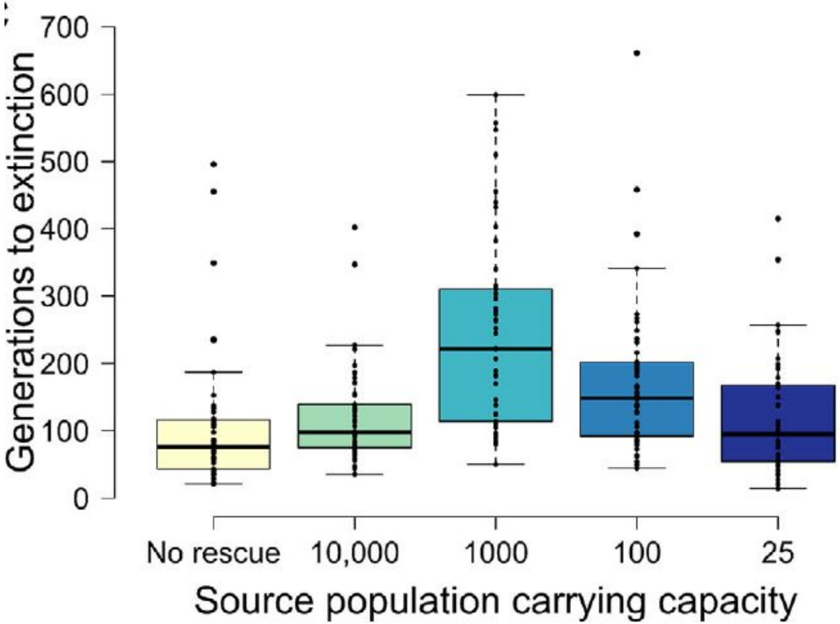

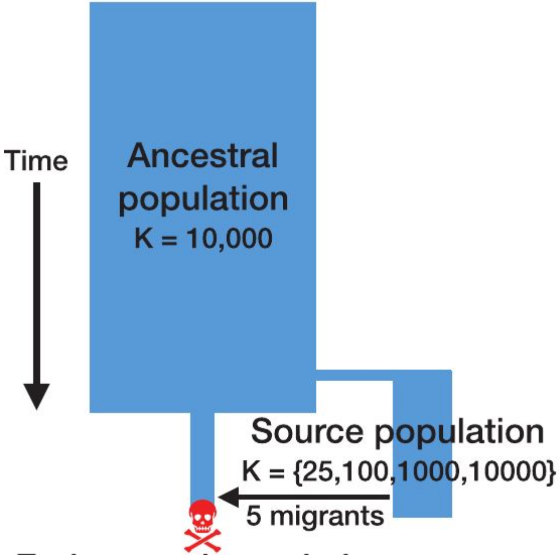

AND THAT RESCUES FROM LARGE POPS are expected to be less effective - if the receiving population stays small (Kyriazis et al 2021)

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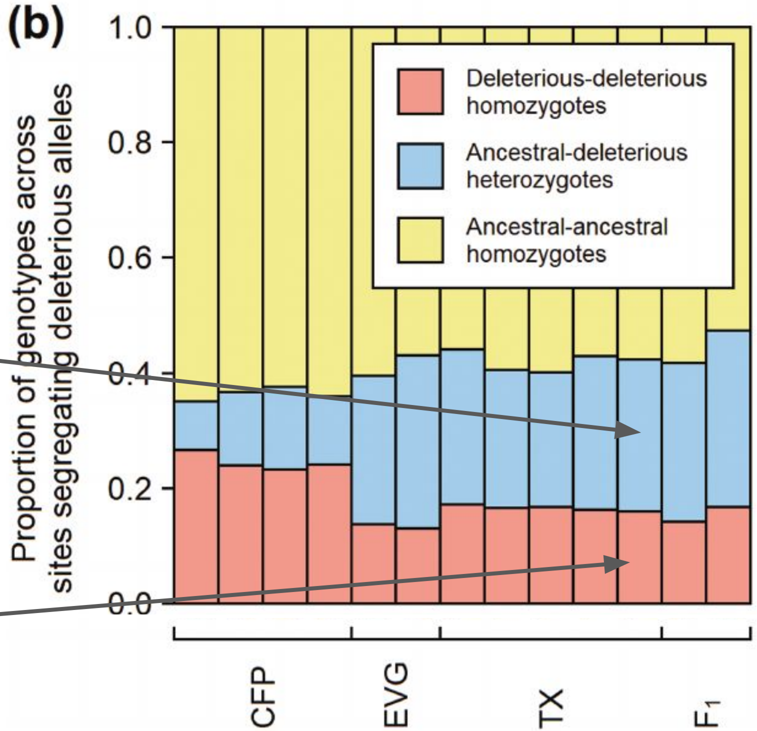

Masked genetic load
has increased

Realized genetic load
has decreased

AS IT IS THE CASE OF THE FLORIDA PANTHER which is accumulating deleterious alleles from the large donor Texas pop! (Ochoa et al 2022)

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All in all, it is still using **population genetics models** to inform conservation biology

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The first simplest advantage: using lots of loci (whole-genome) improves the accuracy of our estimates

ESTIMATING EFFECTIVE POPULATION SIZE (Ne)

Are more individuals better than more loci?

How to estimate N_e (e.g., stationary size) from one diploid individual?

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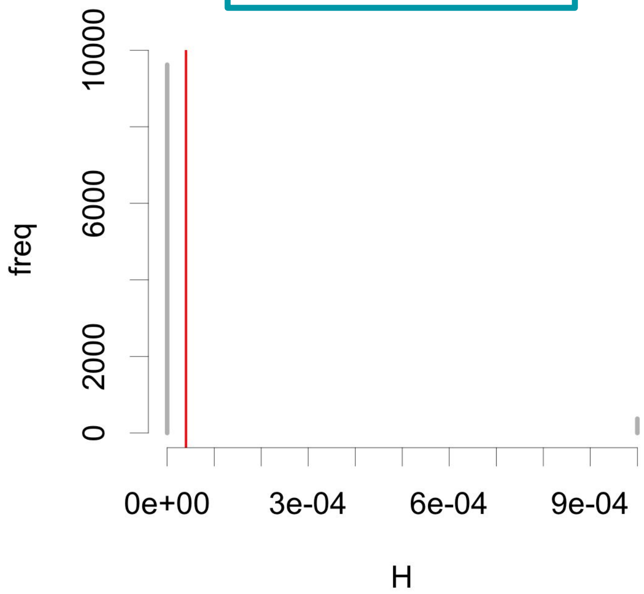

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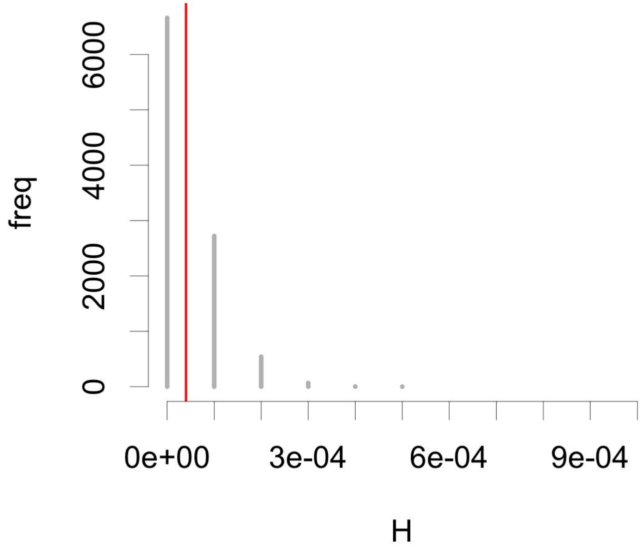

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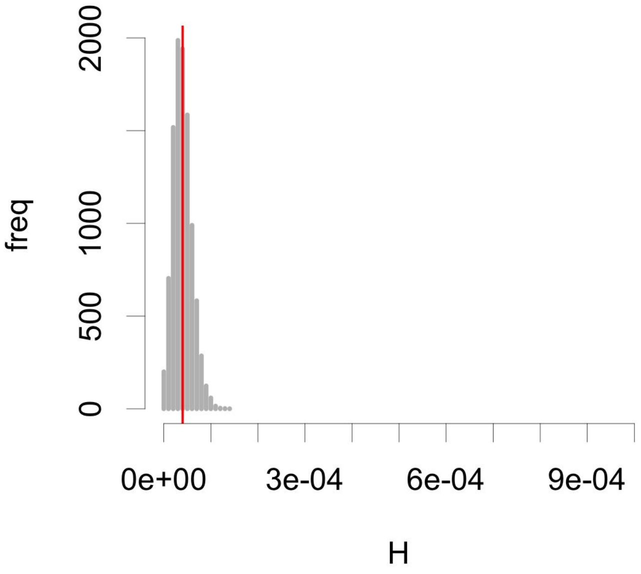

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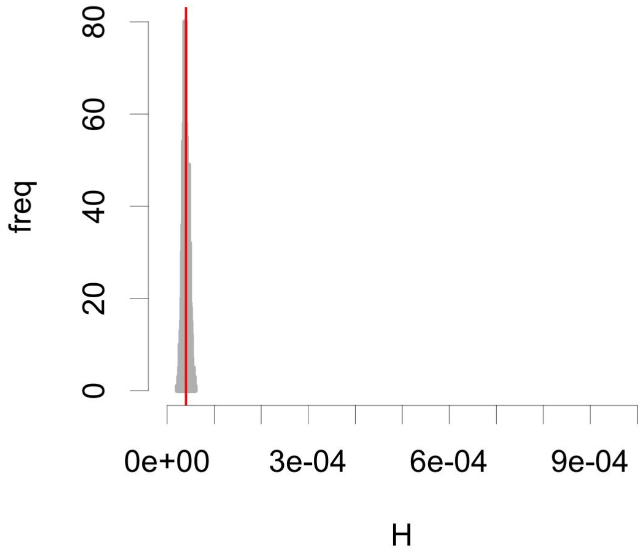

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$$\hat{\theta} = \frac{S}{\sum_{i=1}^{n-1} 1/i}$$

$$\hat{\theta} = \theta = 4N_e\mu$$

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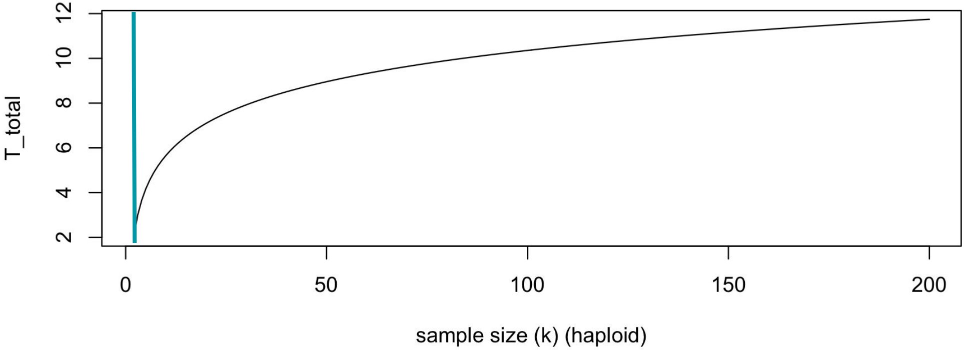
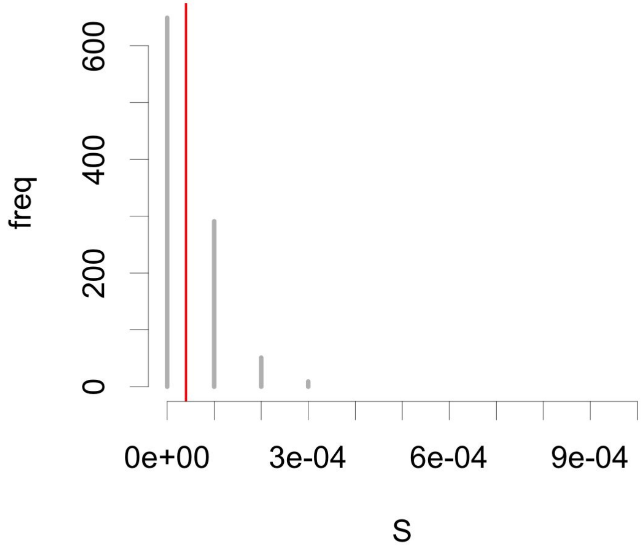


n. of sites = 10000 ; k = 2



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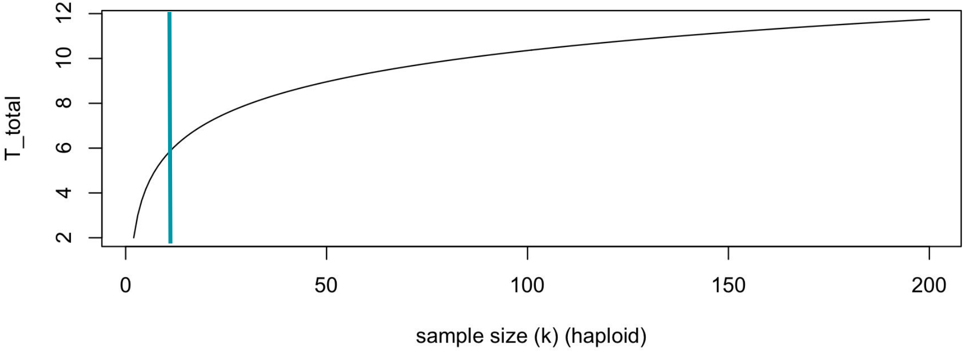
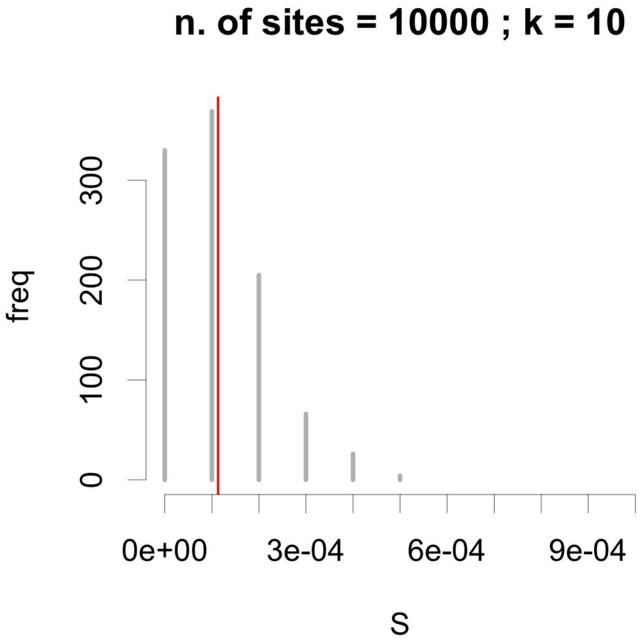
5 diploid individual





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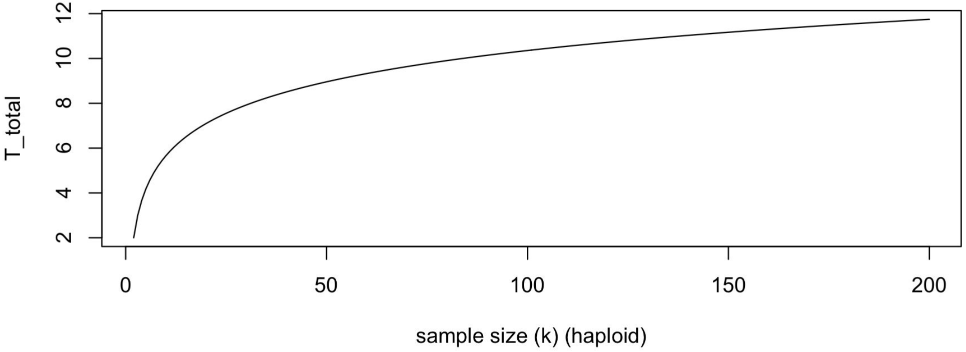
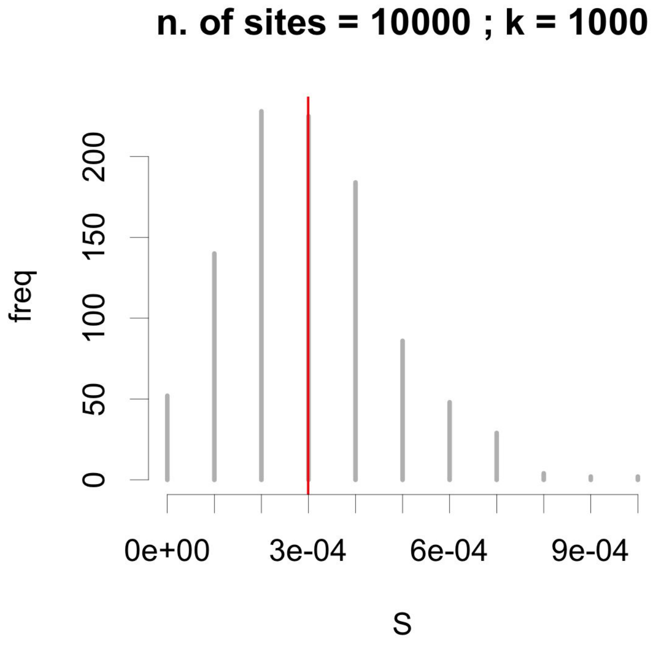
500 diploid individual

Not much really!!




n. of sites = 10000 ; k = 1000

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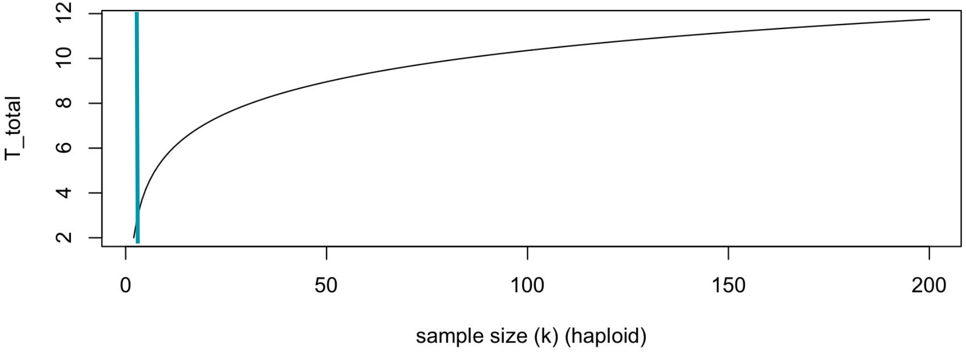
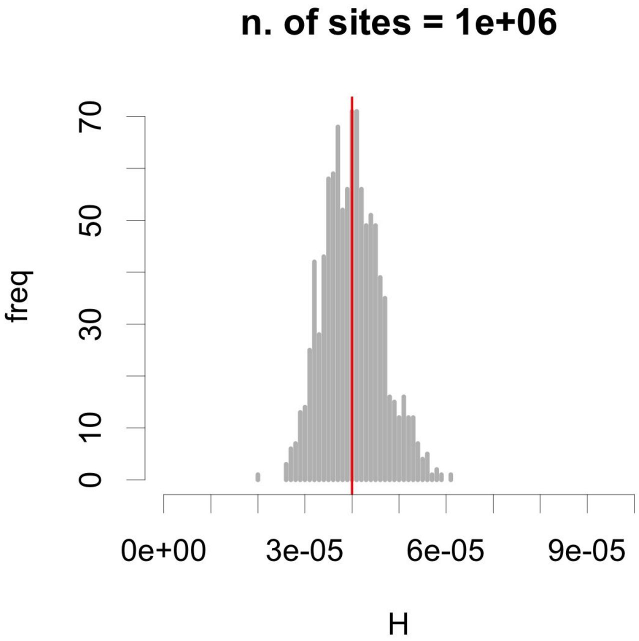
Better to increase the number of sites

(or both)





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FIRST, A WELL ANNOTATED REFERENCE GENOME

A blossoming of genome assembly initiatives

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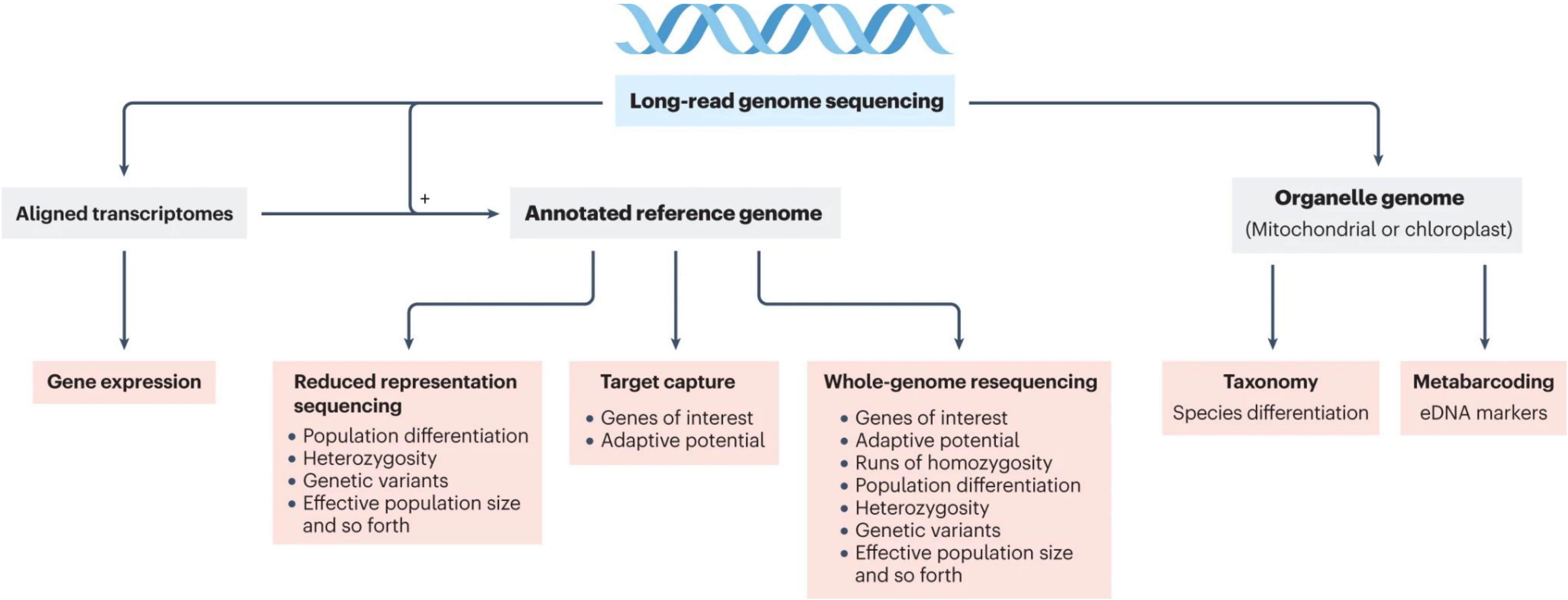


Long-read genome sequencing

Aligned transcriptomes

+

Annotated reference genome

Organelle genome
(Mitochondrial or chloroplast)

Gene expression

Reduced representation sequencing
- Population differentiation
- Heterozygosity
- Genetic variants
- Effective population size and so forth

Target capture
- Genes of interest
- Adaptive potential

Whole-genome resequencing
- Genes of interest
- Adaptive potential
- Runs of homozygosity
- Population differentiation
- Heterozygosity
- Genetic variants
- Effective population size and so forth

Taxonomy
Species differentiation

Metabarcoding
eDNA markers

A reference genome can lead to many downstream applications (Hogg 2023)

FIRST, A WELL ANNOTATED REFERENCE GENOME

A blossoming of genome assembly initiatives

EARTH
BIOGENOME
PROJECT
sequencing life for the future of life

Earth BioGenome Project: Sequencing life for the future of life

Harris A. Lewin, Gene E. Robinson, W. John Kress, +20, and Guojie Zhang Authors Info & Affiliations

Edited by John C. Avise, University of California, Irvine, CA, and approved March 15, 2018 (received for review January 6, 2018)

April 23, 2018 | 115 (17) 4325-4333 | https://doi.org/10.1073/pnas.1720115115

VGP
VERTEBRATE
GENOMES
PROJECT
A PROJECT OF THE G10K CONSORTIUM

Article | Open Access | Published: 28 April 2021

Towards complete and error-free genome assemblies of all vertebrate species

Arang Rhie, Shane A. McCarthy, Olivier Fedrigo, Joana Damas, Giulio Formenti, Sergey Koren, Marcela

FIRST, A WELL ANNOTATED REFERENCE GENOME

A community effort to set the golden standard in EU

ERGA

European Reference Genome Atlas

The era of reference genomes in conservation genomics

Giulio Formenti [29] • Kathrin Theissinger [29] • Carlos Fernandes [29] • ... Erich D. Jarvis • Miklós Bálint • European Reference Genome Atlas (ERGA) Consortium [30, 31] • Show all authors • Show footnotes

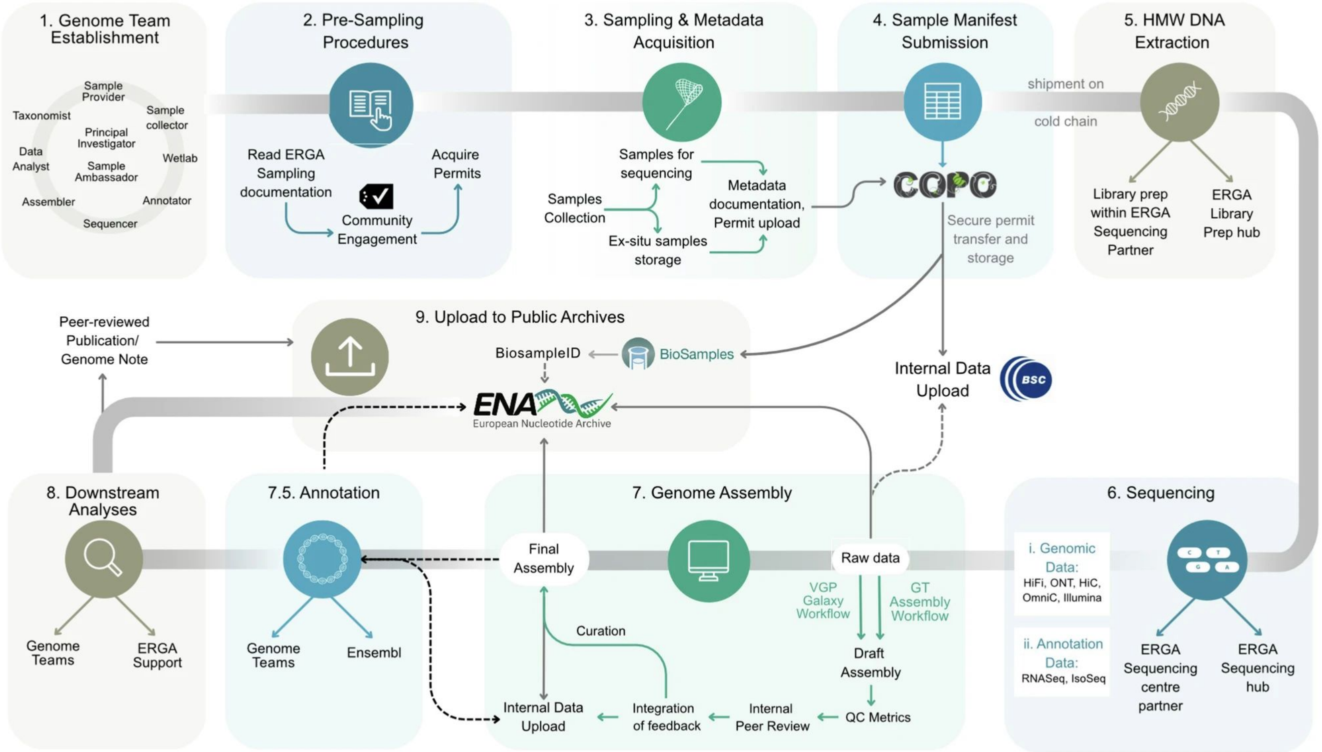
Open Access • Published: January 24, 2022 • DOI: https://doi.org/10.1016/j.tree.2021.11.008 •

OUR MISSION

Sequencing reference-quality genomes for all European species

FIRST, A WELL ANNOTATED REFERENCE GENOME

From sample to high quality genome assemblies



1. Genome Team Establishment

Sample Provider
Sample collector
Wetlab
Annotator
Sequencer
Assembler
Data Analyst
Taxonomist
Principal Investigator
Sample Ambassador

2. Pre-Sampling Procedures

Read ERGA Sampling documentation
Community Engagement
Acquire Permits

3. Sampling & Metadata Acquisition

Samples Collection
Samples for sequencing
Ex-situ samples storage
Metadata documentation, Permit upload

4. Sample Manifest Submission

COPO
Secure permit transfer and storage

shipment on cold chain

5. HMW DNA Extraction

Library prep within ERGA Sequencing Partner
ERGA Library Prep hub

6. Sequencing

i. Genomic Data:
HiFi, ONT, HiC, OmniC, Illumina

ii. Annotation Data:
RNASeq, IsoSeq

ERGA Sequencing centre partner
ERGA Sequencing hub

7. Genome Assembly

Raw data
VGP Galaxy Workflow
GT Assembly Workflow
Draft Assembly
QC Metrics
Internal Peer Review
Integration of feedback
Internal Data Upload
Curation
Final Assembly

Internal Data Upload
BSC

7.5. Annotation

Genome Teams
Ensembl

8. Downstream Analyses

Genome Teams
ERGA Support

9. Upload to Public Archives

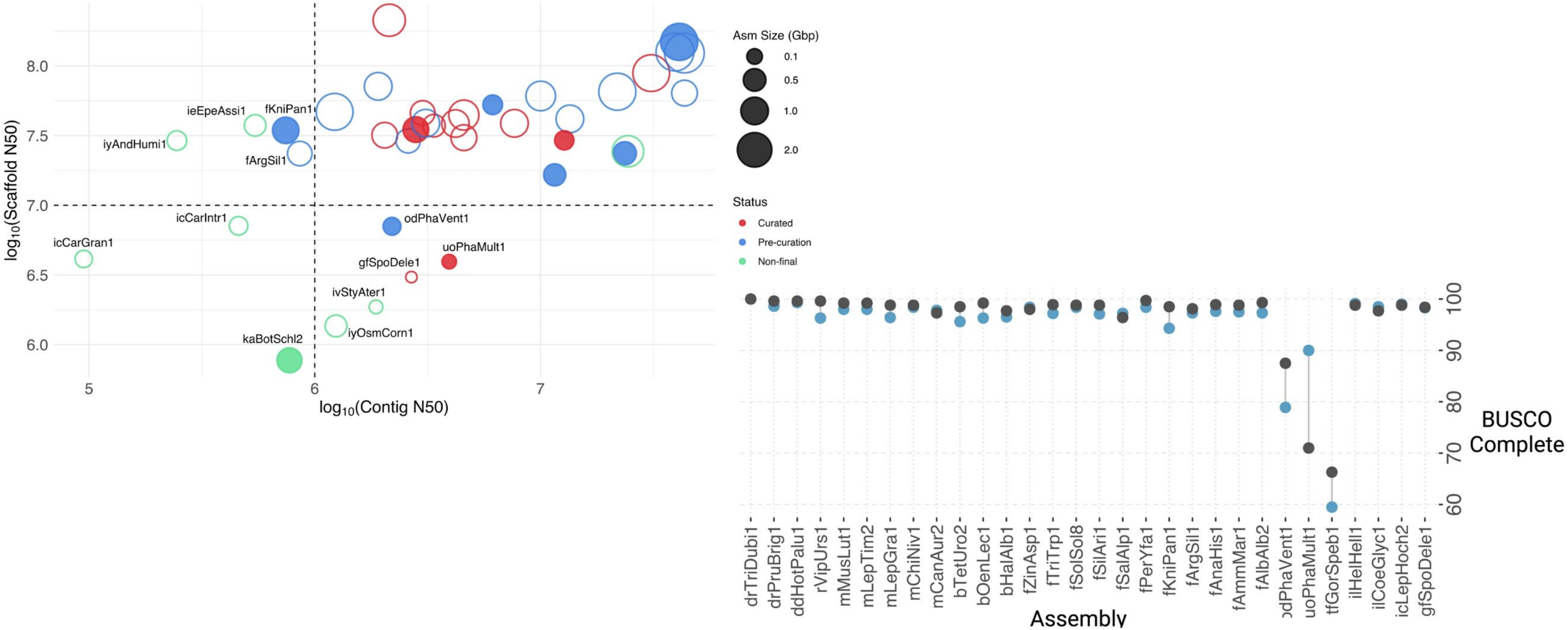
BiosampleID
BioSamples
ENA
European Nucleotide Archive

Peer-reviewed Publication/ Genome Note

BEST PRACTICE in the ERGA pilot study (Mc Cartney et al 2024)

FIRST, A WELL ANNOTATED REFERENCE GENOME

Scaffold length (up to chrs!), QVs, and BUSCO requirements



QUALITY THRESHOLDS in the ERGA pilot study (Mc Cartney et al 2024)

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The gold standard is a high-quality, highly-contiguous (e.g., chromosome level) reference genome assembly

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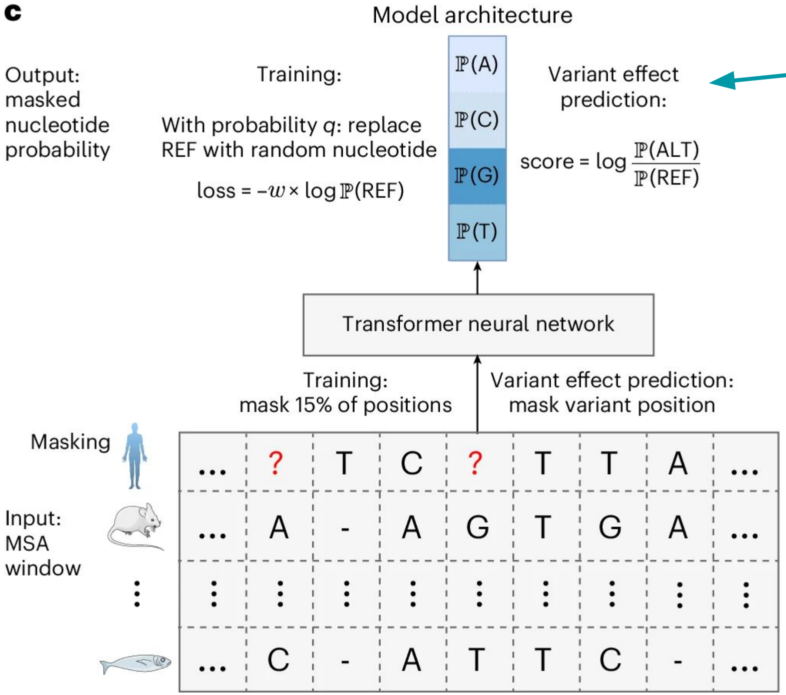
There's room for improvements: technological (e.g., PacBio Kinnex), methodological (e.g., machine learning approaches)

GENOMIC FEATURES ANNOTATION

New AI powered approaches



We'll talk more about it later

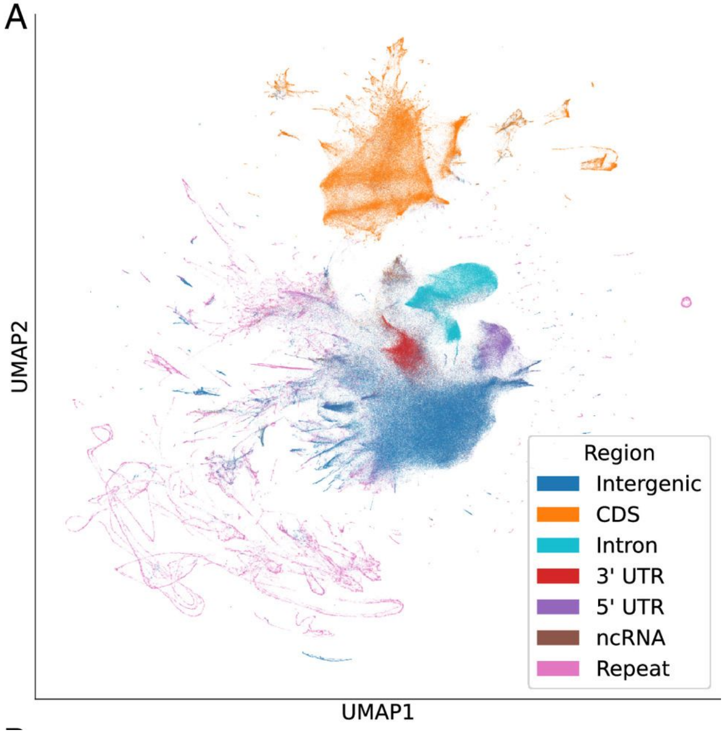
Genomic Pretrained Network /with multiple-sequence alignment

GPN/GPN-MSA (Benegas et al 2023 and 2025).

GENOMIC FEATURES ANNOTATION

New AI powered approaches

It can learn **gene structure …**



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GENOMIC FEATURES ANNOTATION

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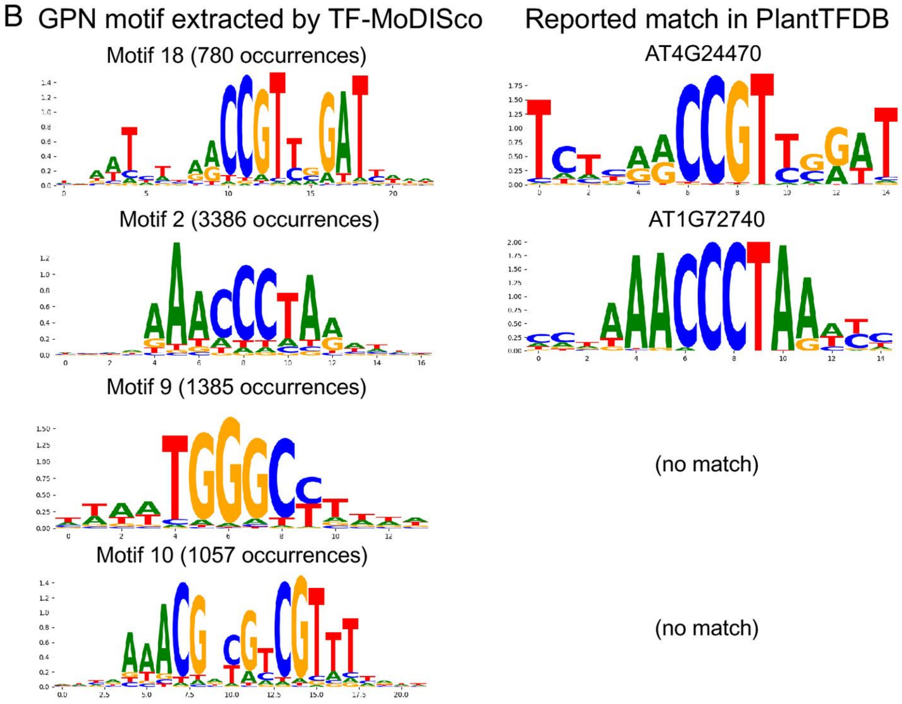


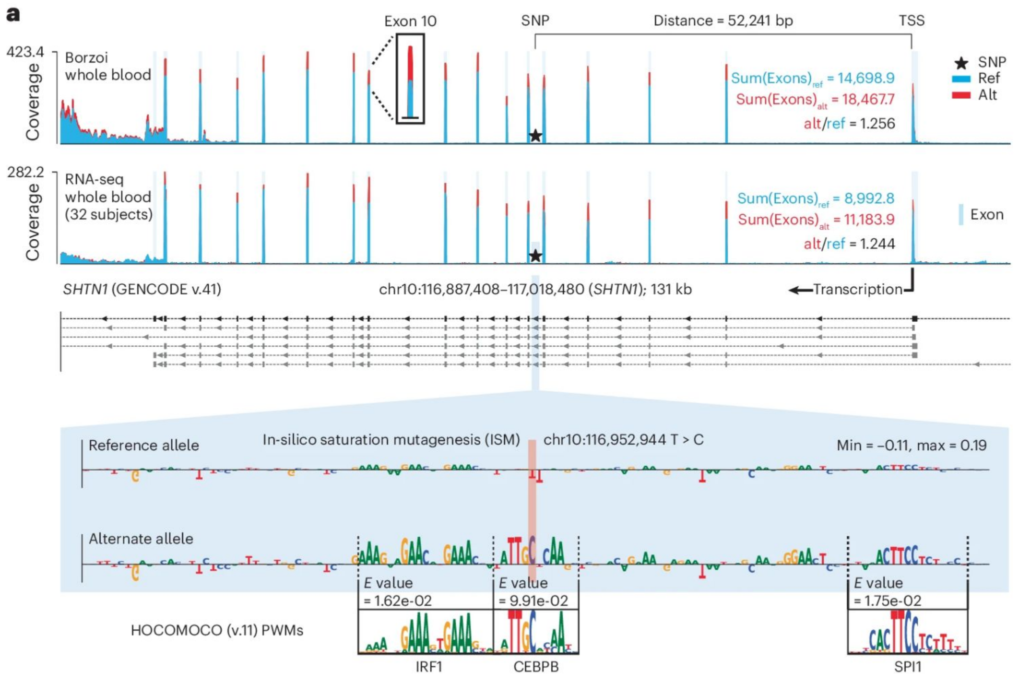
… and DNA motifs without supervision

Genomic Pretrained Network /with multiple-sequence alignment
GPN/GPN-MSA (Benegas et al 2023 and 2025).

GENOMIC FEATURES ANNOTATION

New AI powered approaches

It can predicts far away
TF variants effect on
gene expression

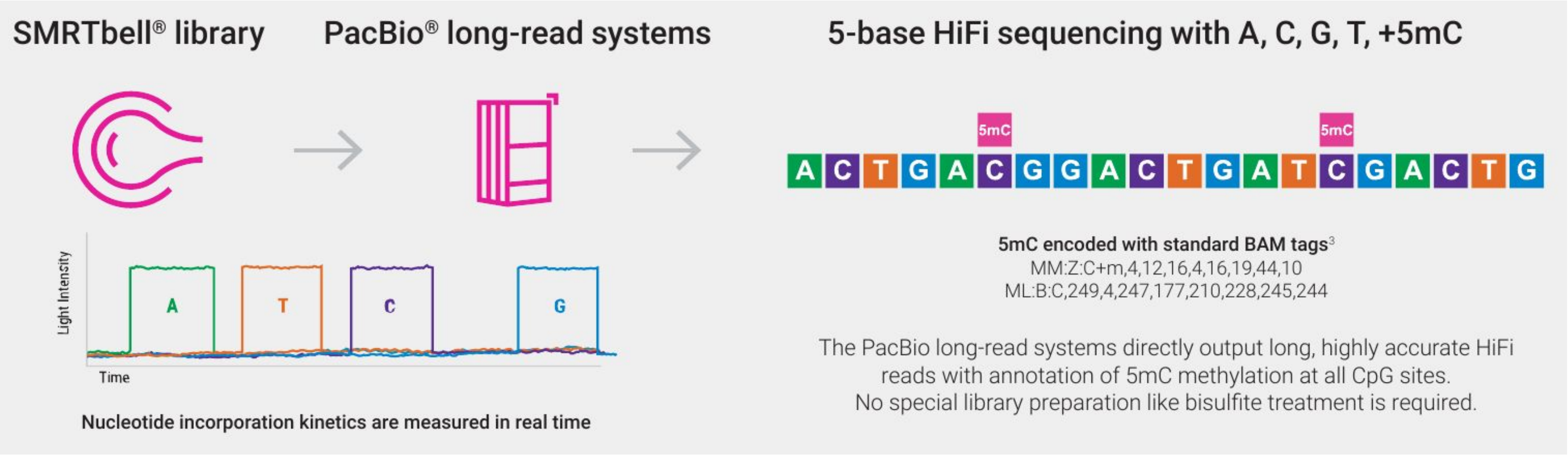


Borzoi derives TF motifs and a genome-wide map of nucleotide influence on gene structure and expression (Linder et al 2025).

GENOMIC FEATURES ANNOTATION

Technological improvements

Both ONT and PacBio can now provide accurate methylation data



Nucleotide incorporation kinetics are measured in real time

5mC encoded with standard BAM tags[3]

```
MM:Z:C+m,4,12,16,4,16,19,44,10
ML:B:C,249,4,247,177,210,228,245,244
```

The PacBio long-read systems directly output long, highly accurate HiFi reads with annotation of 5mC methylation at all CpG sites. No special library preparation like bisulfite treatment is required.

EPIGENETIC PROFILING from long-range sequencing data is much more affordable with falling costs of LRS

WHAT IS THE DIFFERENCE BETWEEN CONSERVATION GENETICS AND GENOMICS?

WHAT IS THE FIRST INGREDIENT OF CONSERVATION GENOMICS?

HOW CAN GENOMICS INFORM CONSERVATION BIOLOGY?

IN WHICH APPLICATION GENOMICS IS A GAME CHANGER?
with a close up on two topics:
Genetic load
ROHs

WHAT AM I DOING TO MAKE USE OF GENOMICS IN CONSERVATION?

PROGRESS IN ADOPTION OF GENOMICS IN CONSERVATION

Broadly used 🟩 Getting better 🟧 To be improved 🟥 (Hogg 2023)

Increase accuracy estimating parameters that require neutral markers

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🟩 Effective population size
🟩 Migration rates (gene flow)
🟧 Inferring haplotypes from linked loci to determine directionality of migration
🟩 Accuracy of kin estimates
🟩 Pedigree reconstruction of wild populations
🟩 Proportion of admixture to assess population delineations
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Estimate inbreeding depression (ID)

- 🟧 Selection coefficients (interactions between drift, selection and migration)
- 🟥 Molecular basis and genetic architecture of ID
- 🟥 Identifying loci contributing to ID by sequencing parents and offspring

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Detect local adaptation

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Captive breeding and assisted migration

- 🟩 Founder relationships in captive breeding programmes
- 🟩 Genome-wide heterozygosity to manage inbreeding depression
- 🟧 Genetic rescue
- 🟥 Minimizing adaptation to captivity

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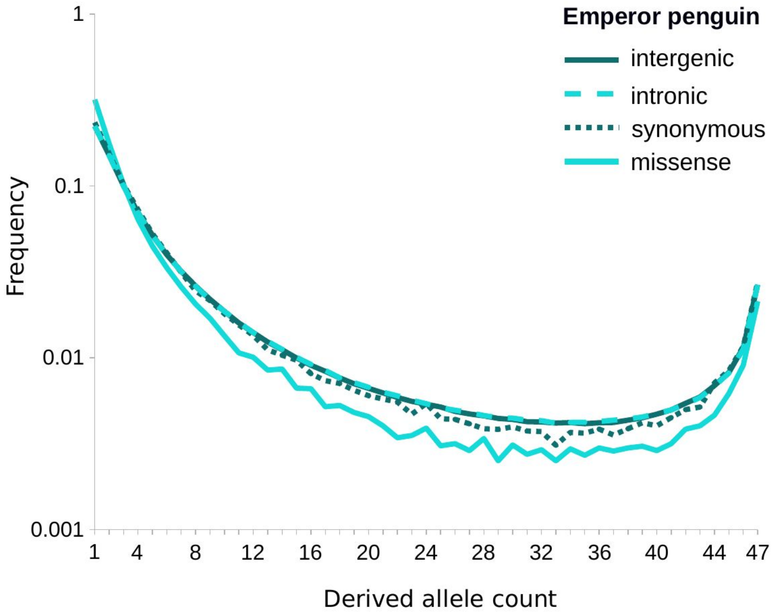
GETTING BEYOND NEUTRAL VARIATION

Investigating adaptive and maladaptive processes



ESTIMATE FUNCTIONAL DIVERSITY: nonsynonymous, exonic, in transcription factor binding sites, etc. (Trucchi et al 2024)

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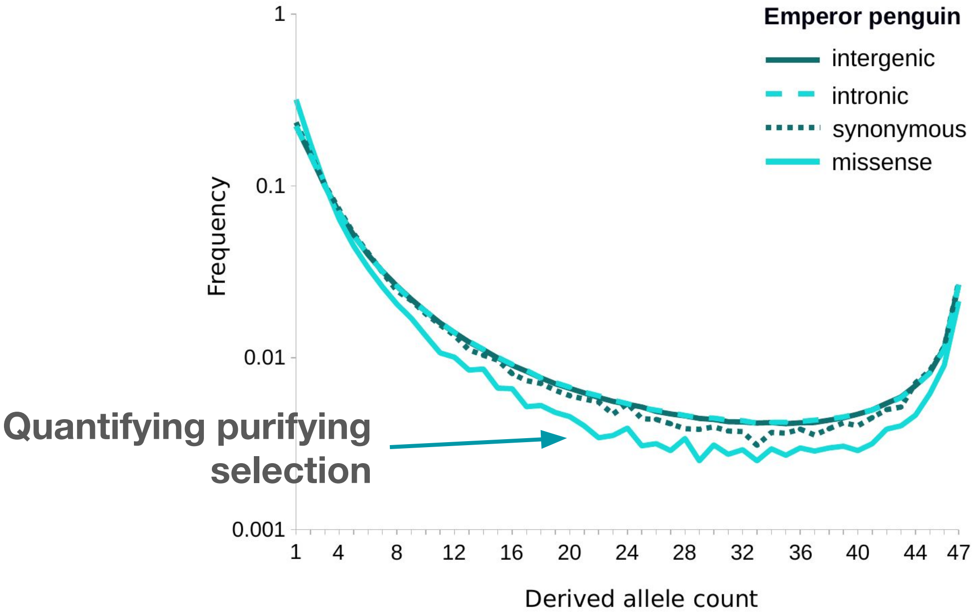

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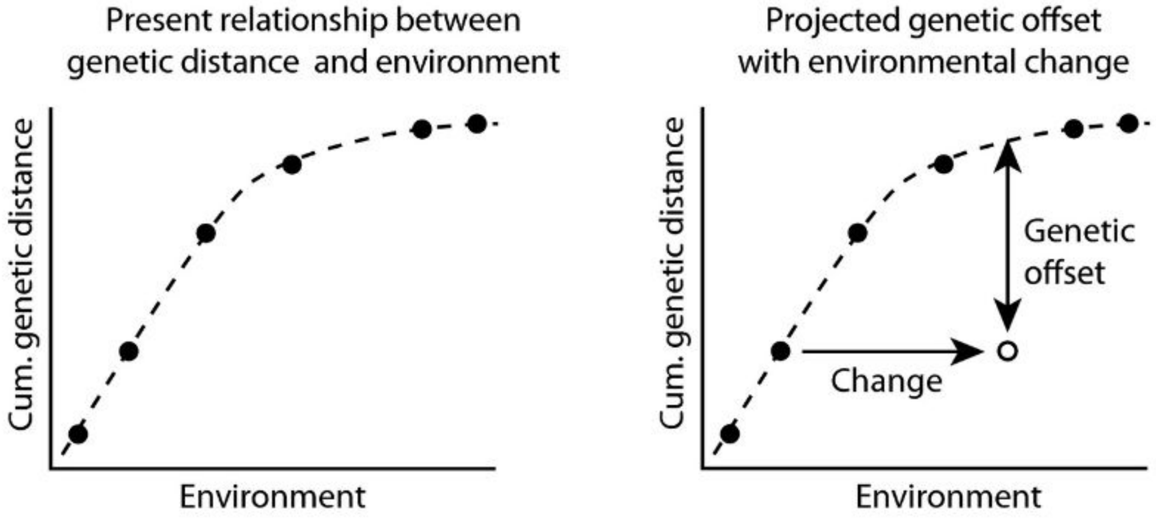

Rellstab et al 2012

DETECT LOCAL ADAPTATION: from selective differentiation and sweeps to genotype to environment association (GEA), to genomic offset or vulnerability.

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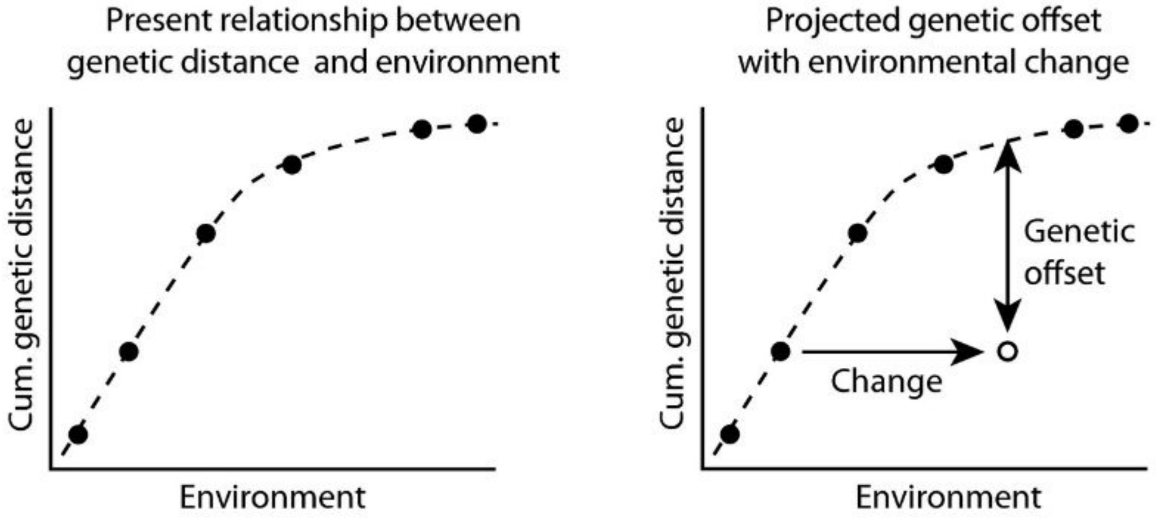

for **more details and more complex** approaches see
Hoffman et al 2021
or Lachmuth et al 2023

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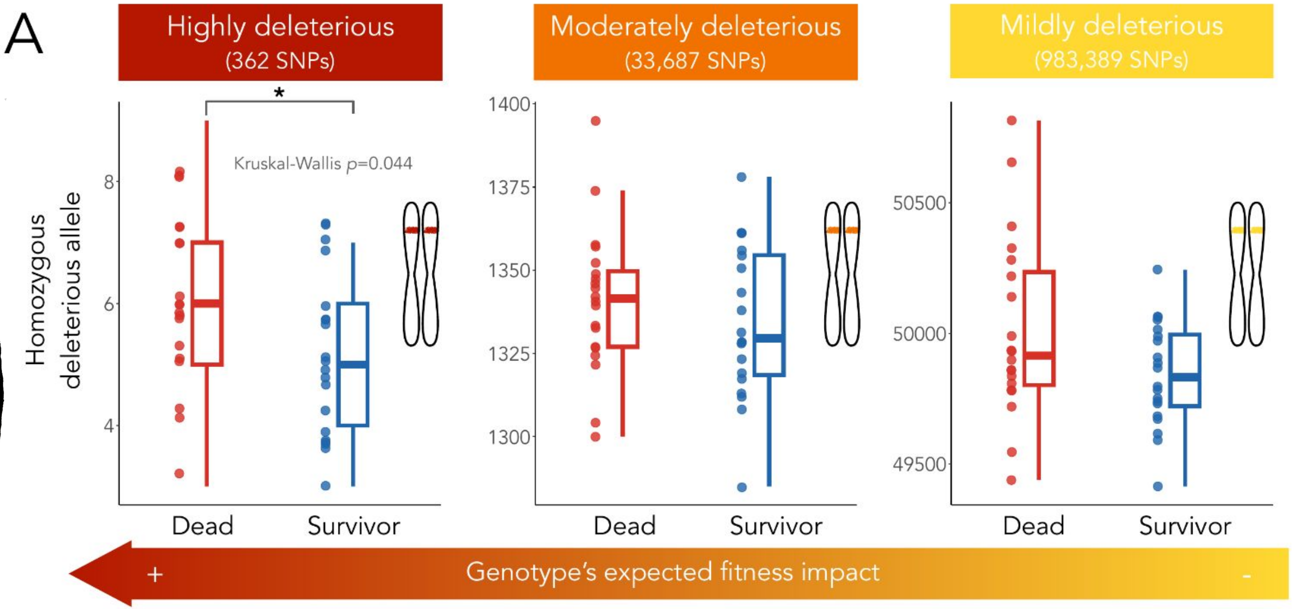

ESTIMATE GENETIC LOAD in relation to individual fitness (Fernandes et al *in prep*) or to compare different populations (Gabrielli et al *in review*)

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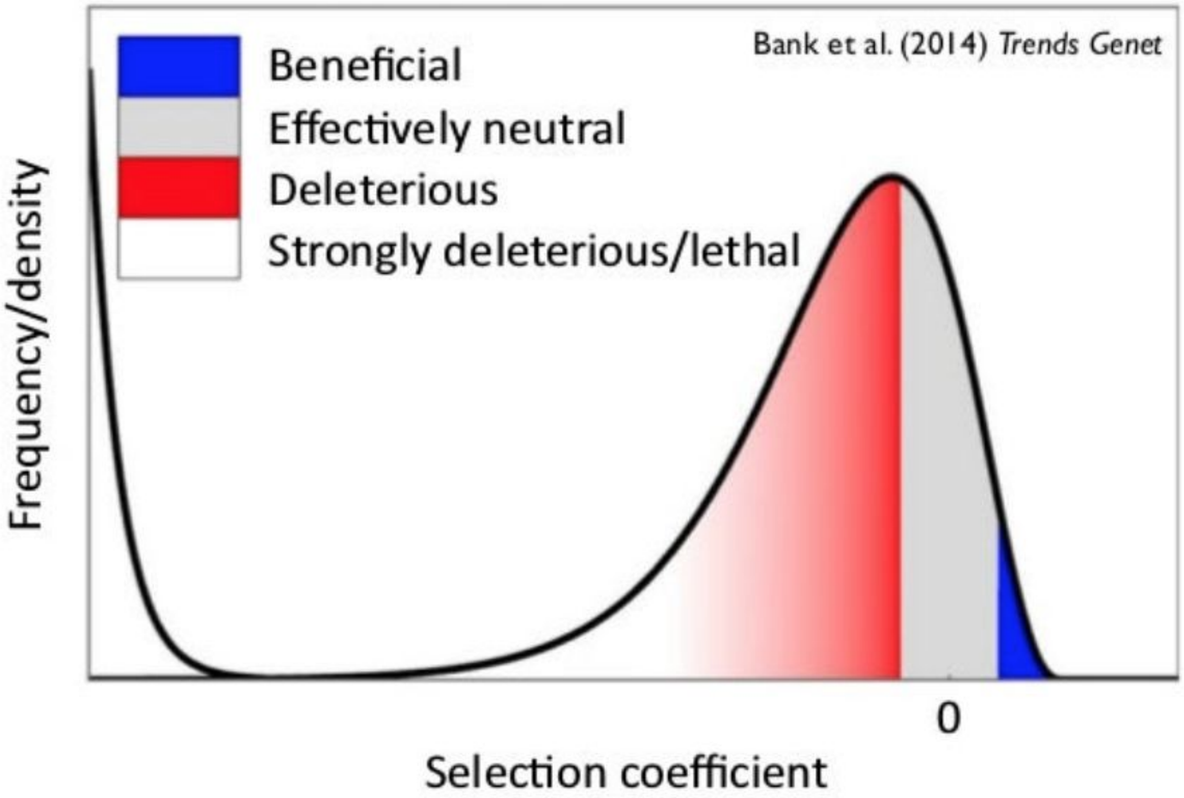
WHAT IS GENETIC LOAD?



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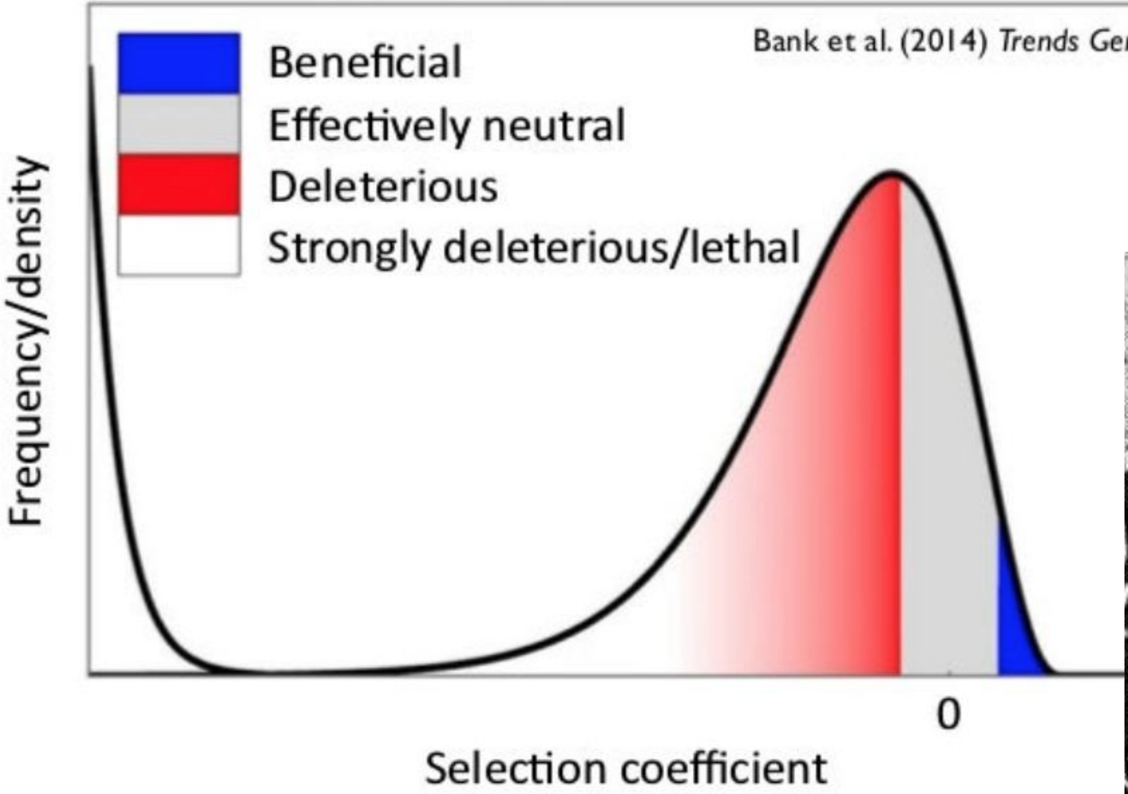
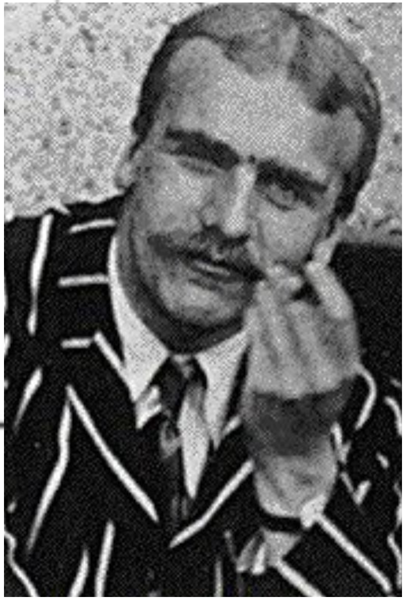

Bank et al. (2014) *Trends Genet*



COST OF NATURAL SELECTION: the price paid by a population for its capacity for further evolution

FITNESS AND GENETIC LOAD

GENETIC LOAD $= \dfrac{w_o - \bar{w}}{w_o}$

w_o	**Optimal fitness**
$\bar{w}$	**Average reduced fitness caused by mutation(s)**

FITNESS AND GENETIC LOAD

REDUCTION OF FITNESS DUE TO:

New deleterious mutations appearing -> **MUTATION LOAD**

Increase in frequency of deleterious mutations -> **DRIFT LOAD**

A more beneficial allele appearing -> **EVOLUTION LOAD**

Deleterious recessive mutations unmasked by inbreeding -> **INBREEDING LOAD**
(**masked** load or potential load)

Deleterious mutations introgressed from a different population -> **MIGRATION LOAD**
(hybrid load)

The break up of favourable combinations of alleles at different loci due to recombination -> **RECOMBINATION LOAD**

See Bertorelle et al 2022

FITNESS AND SELECTION COEFFICIENTS

	AA	Aa	aa
Frequency	$(1-q)^2$	$2q(1-q)$	q^2
Fitness (w)	1	1-hs	1-s

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REALIZED GENETIC LOAD $= \dfrac{1-\bar{w}}{1} = sq^2 + 2q(1-q)hs$

GENETIC LOAD AND SELECTION COEFFICIENTS

REALIZED GENETIC LOAD (n loci)

$$= \sum_{i=1}^{n} s_i q_i^2 + 2 \sum_{i=1}^{n} q_i[1 - q_i]h_i s_i$$

See Bertorelle et al 2022

INDIVIDUAL GENETIC LOAD PARTITION

TOTAL LOAD = REALIZED LOAD + MASKED LOAD

See Bertorelle et al 2022

INDIVIDUAL GENETIC LOAD PARTITION

TOTAL LOAD = **REALIZED LOAD** + **MASKED LOAD**

$$\sum_{i=1}^{L(hom)} s_i + \sum_{i=1}^{L(het)} 0.5\, s_i =$$

See Bertorelle et al 2022

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$$\sum_{i=1}^{L(hom)} s_i + \sum_{i=1}^{L(het)} 0.5\, s_i = \sum_{i=1}^{L(hom)} s_i + \sum_{i=1}^{L(het)} h_i s_i$$

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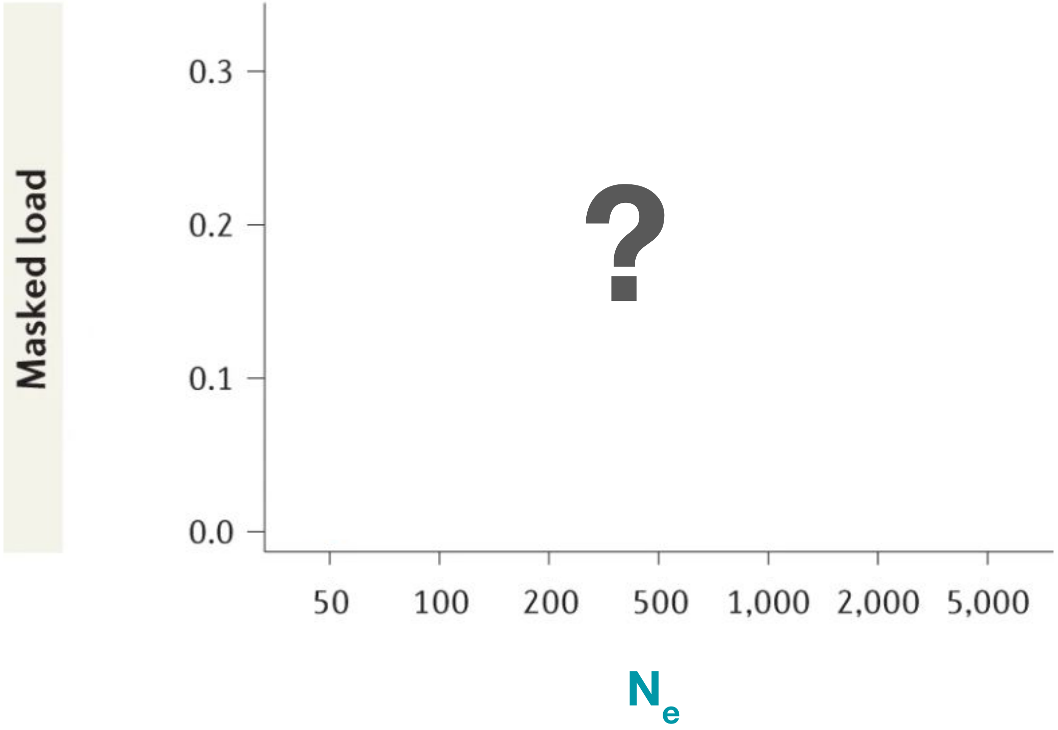
See Bertorelle et al 2022

INDIVIDUAL GENETIC LOAD AND POPULATION SIZE



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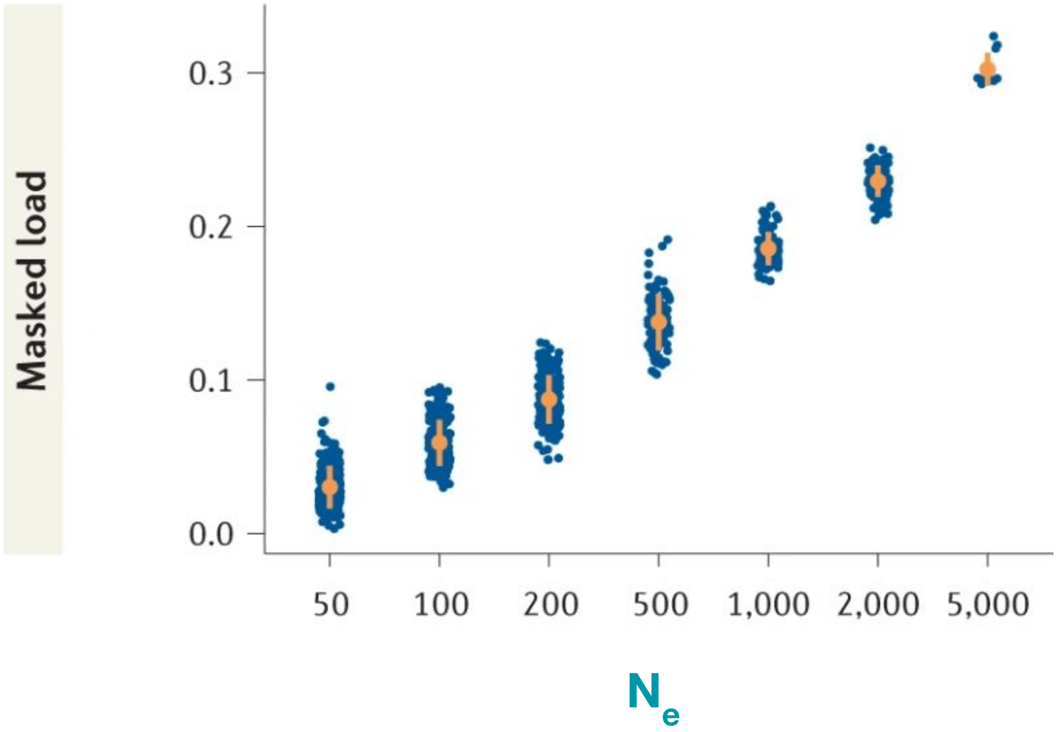

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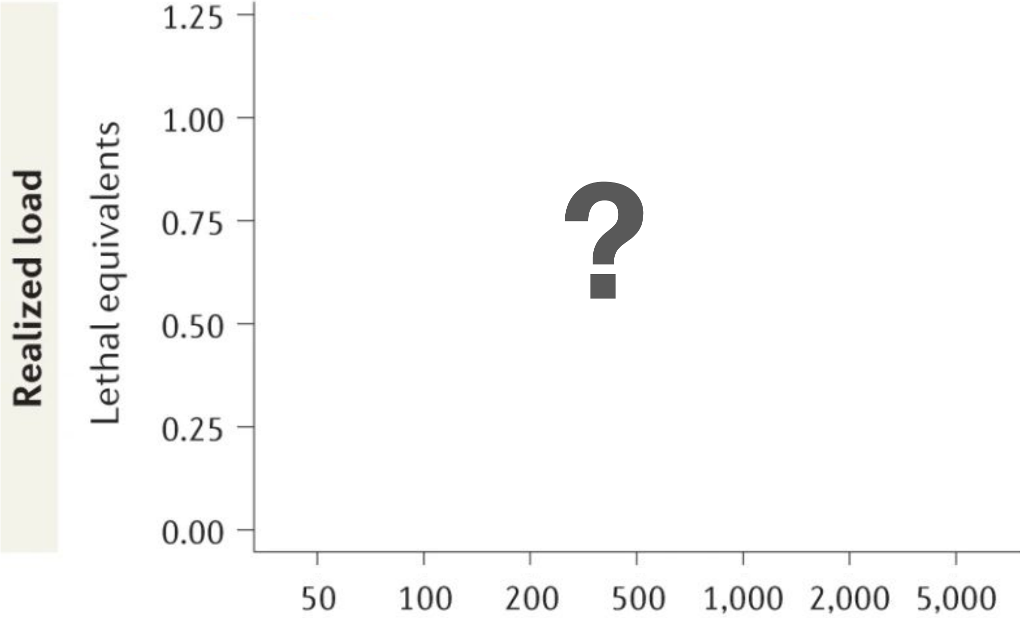

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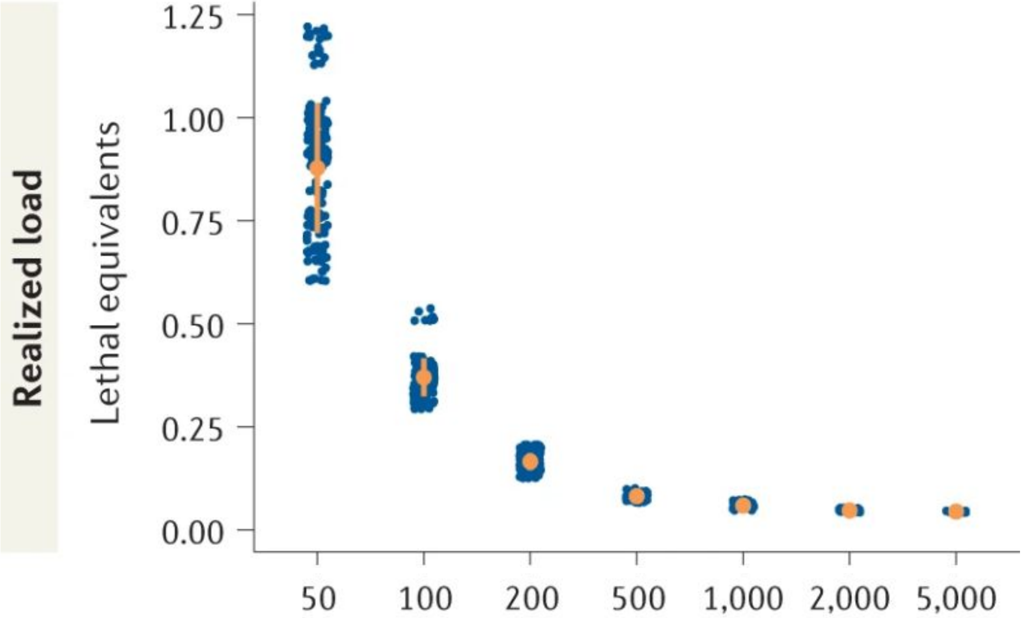

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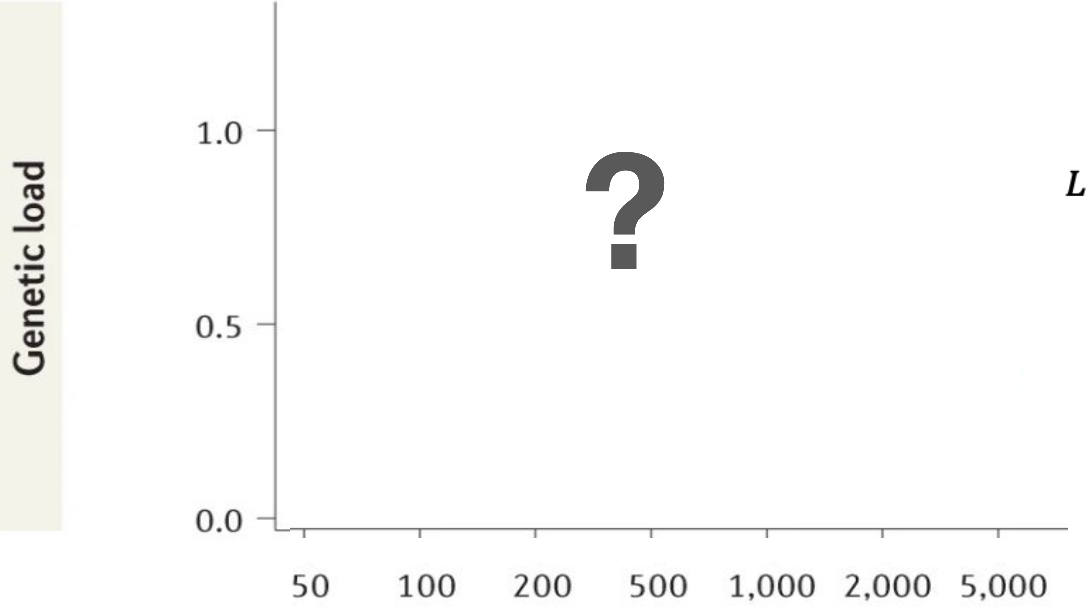

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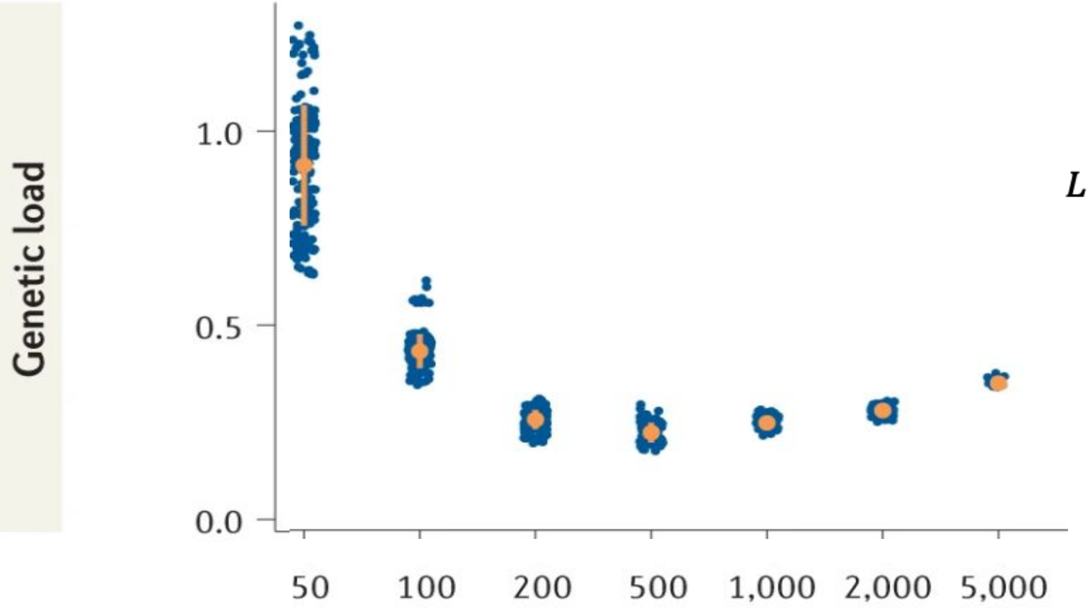

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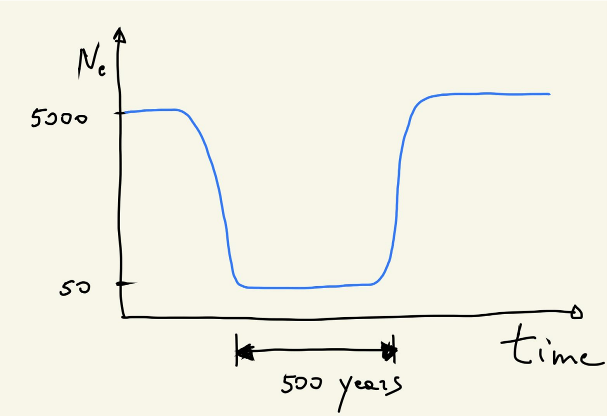

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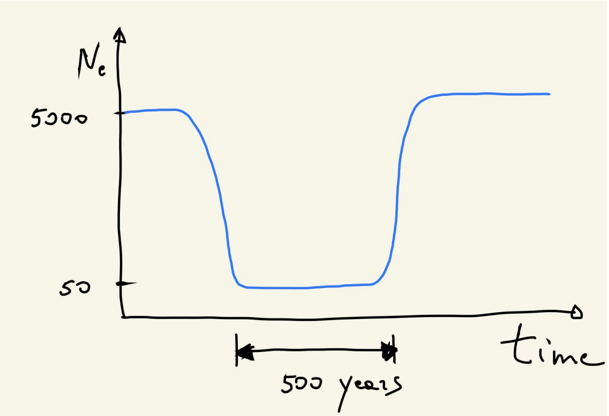

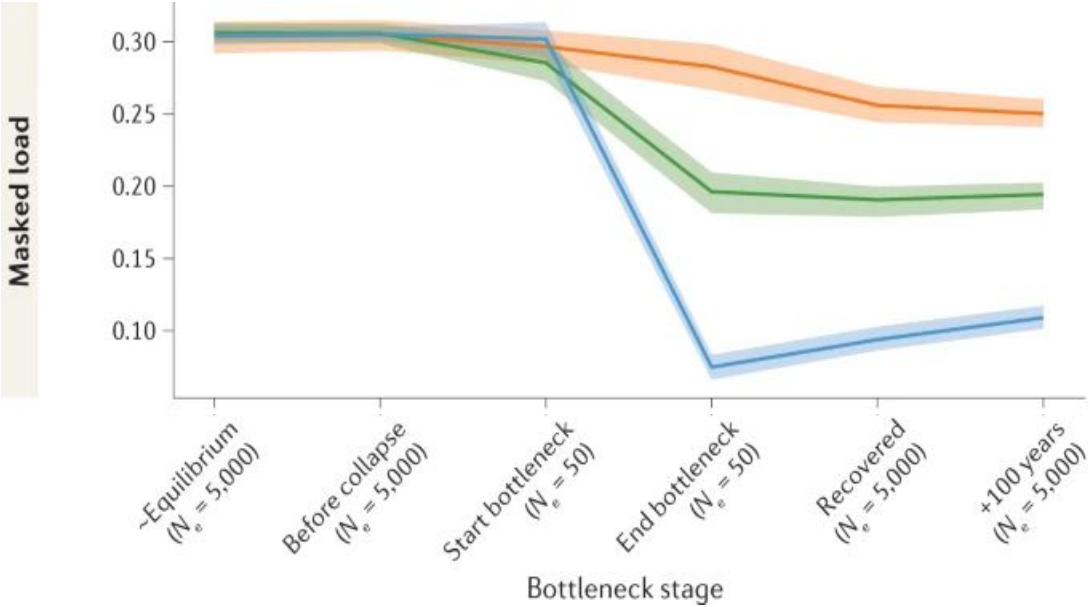

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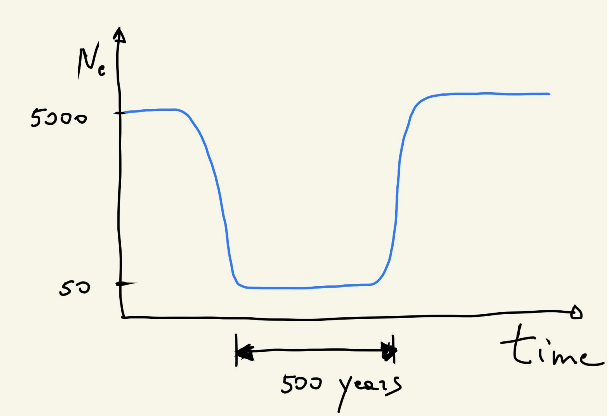

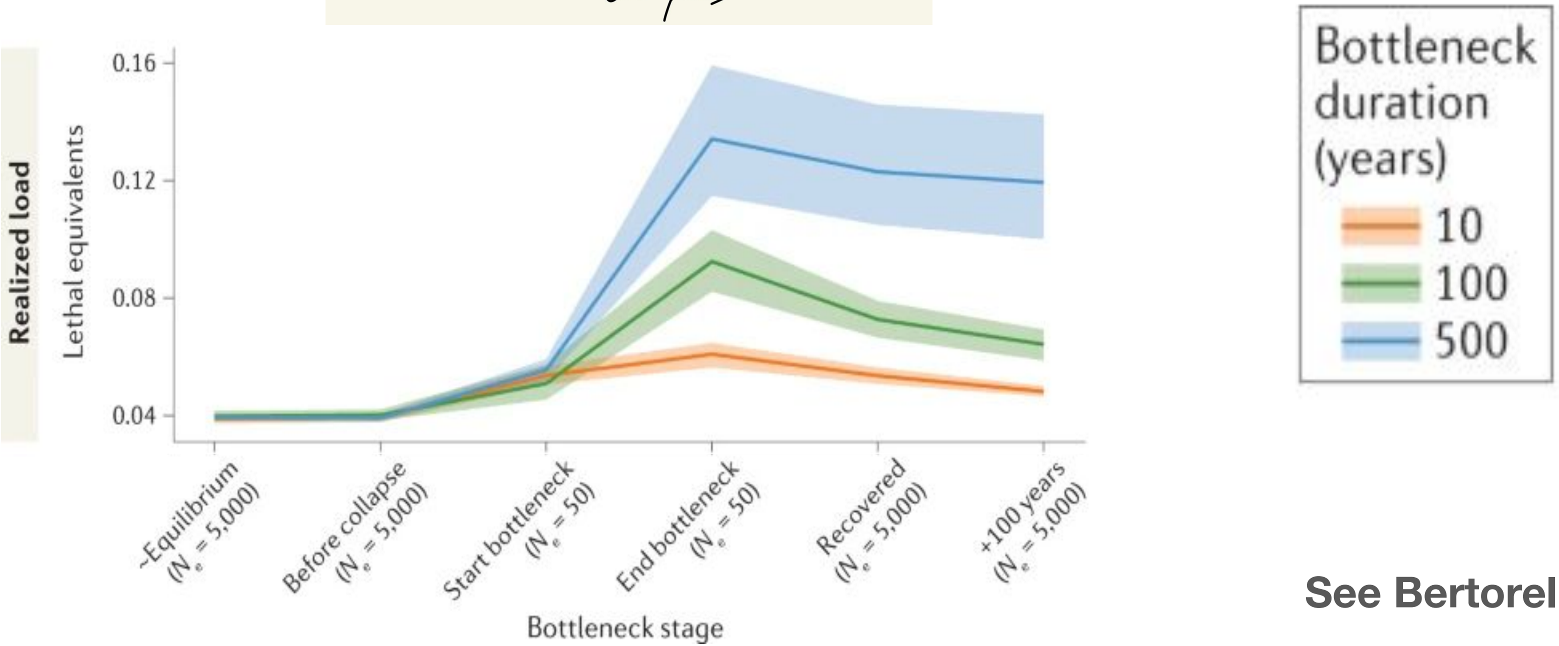

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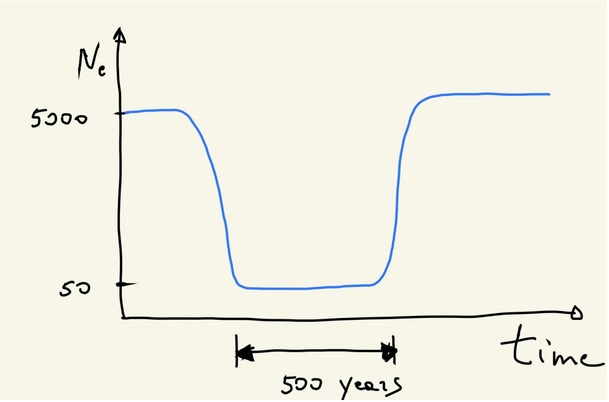

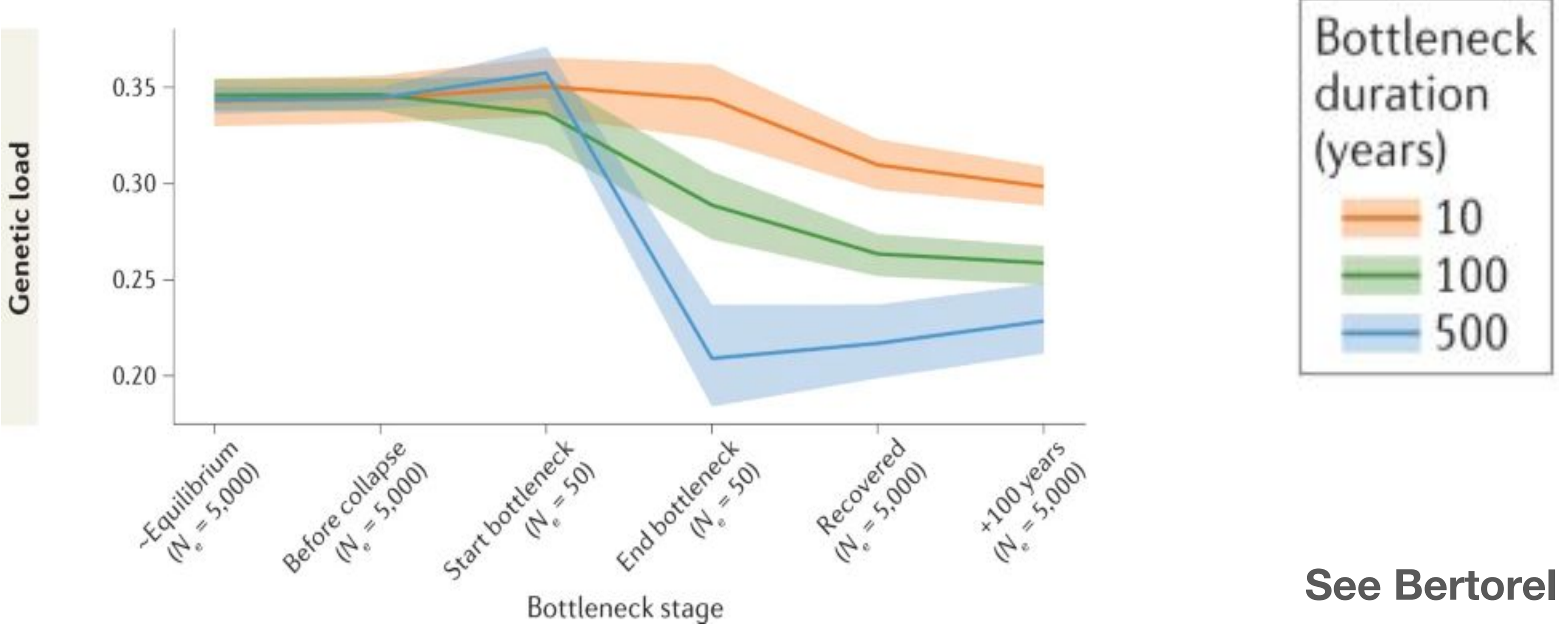

See Bertorelle et al 2022

ONE “LITTLE” PROBLEM:
HOW TO GET THE SELECTION COEFFICIENTS?

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PREDICTIONS BASED ON:

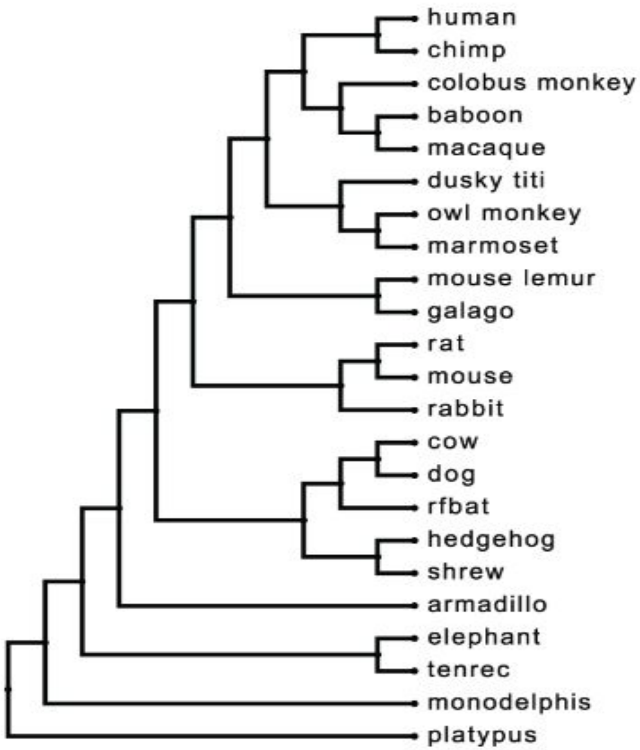
1) **CONSERVATION SCORES** through long evolutionary times (GERP, PhyloP, GPN-MSA*)

   Relies on **alignments of genomes** (e.g., Cactus)

Davydov 2010, Pollard et al 2010, Armstrong et al 2020

CONSERVATION SCORES

Phylogenetic Tree



Multiple Sequence Alignment

human	AATACGG	A	ACTTCATTCATT
chimp	AATATGG	A	ACTTCATTCATT
colobus monkey	AGTATGG	A	ACTTCATTCATT
baboon	AGTATGG	A	ACTTCATTCATT
macaque	AGTATGG	A	ACTTCATTCATT
dusky titi	AGTATGG	A	ACTTCATTCATT
owl monkey	AGTATGG	A	ACTTCATTCATT
marmoset	AGTATGG	A	ACTTCATTCATT
mouse lemur	AGTACGG	A	ACTTCATTCATT
galago	AGTACGG	A	ACTTCATTCATT
rat	AGTATGG	A	ACATCGTTCATT
mouse	AGTATGG	A	ACATCTTTCATT
rabbit	AGTATGG	A	ACATCATTCATT
cow	AGTATGG	A	ACATCATTCATT
dog	AGTACGG	A	ACATCATTCATT
rfbat	AGTATGG	A	ACATCGTTCATT
hedgehog	AGTATGG	A	ACATCATTCATT
shrew	AGTATGG	G	ACATCCTTCATT
armadillo	-------	-	------------
elephant	AGTATGG	A	ACATCGTTCATT
tenrec	AGTATGG	A	ACATCGTTCATT
monodelphis	AGTATGG	G	ACATCTTTCATT
platypus	AGTATGG	A	ACGTCATTCATT

Davydov 2010

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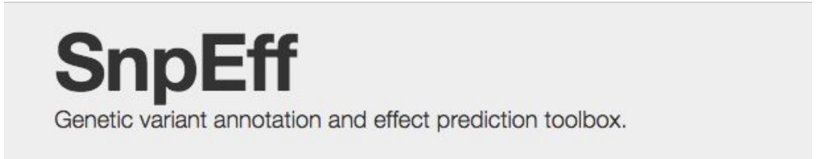
2) Impact of changes on coding sequence

   Relies on reference genome annotation (SNPEff)

Cingolani et al 2012

CODING SEQUENCE CHANGES



SnpEff

Genetic variant annotation and effect prediction toolbox.

Cingolani et al 2012

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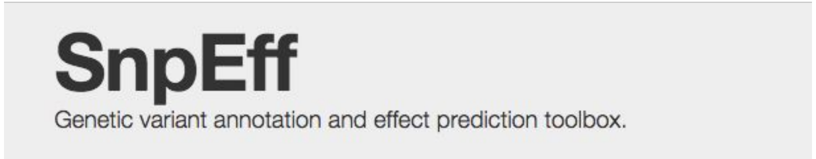

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putative impact

description of consequence
exon_loss_variant, stop_lost,
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estimation of level of impact
HIGH, LOW, MODERATE

Cingolani et al 2012

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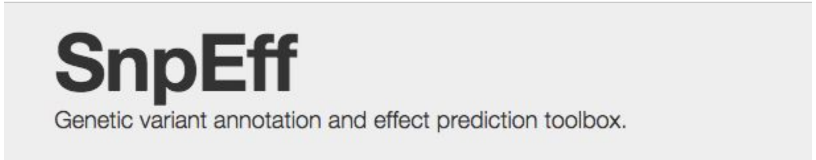

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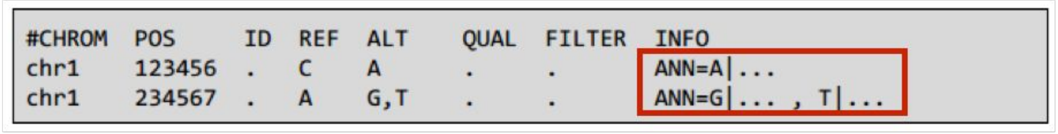

```
#CHROM  POS     ID  REF  ALT   QUAL  FILTER  INFO
chr1    123456  .   C    A     .     .       ANN=A|...
chr1    234567  .   A    G,T   .     .       ANN=G|... , T|...
```

ANN = Annotation aka effect or consequence

Cingolani et al 2012

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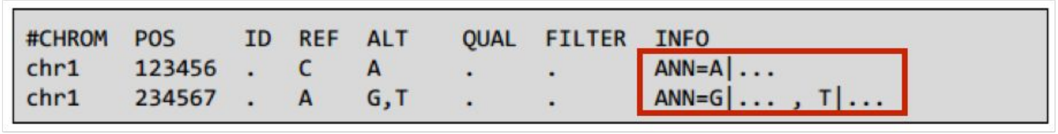

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Type (alphabetical order)	Count	Percent
HIGH	39	0.04%
LOW	395	0.407%
MODERATE	239	0.246%
MODIFIER	96,412	99.307%

Cingolani et al 2012

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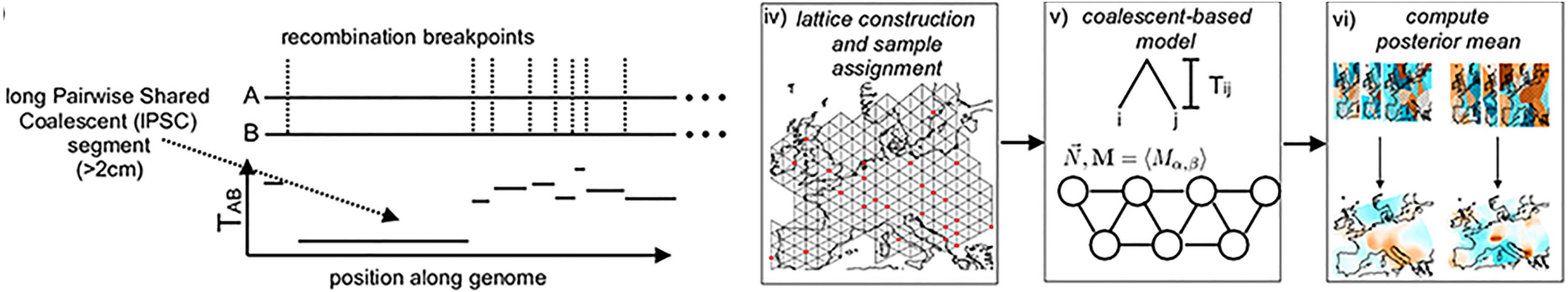
USING EXTENDED HAPLOTYPE INFORMATION

Which is getting more affordable with long-range sequencing

Sharing **Identity-By-Descent (IBD)** blocks to infer to infer recent migration (Al-Asadi 2019).

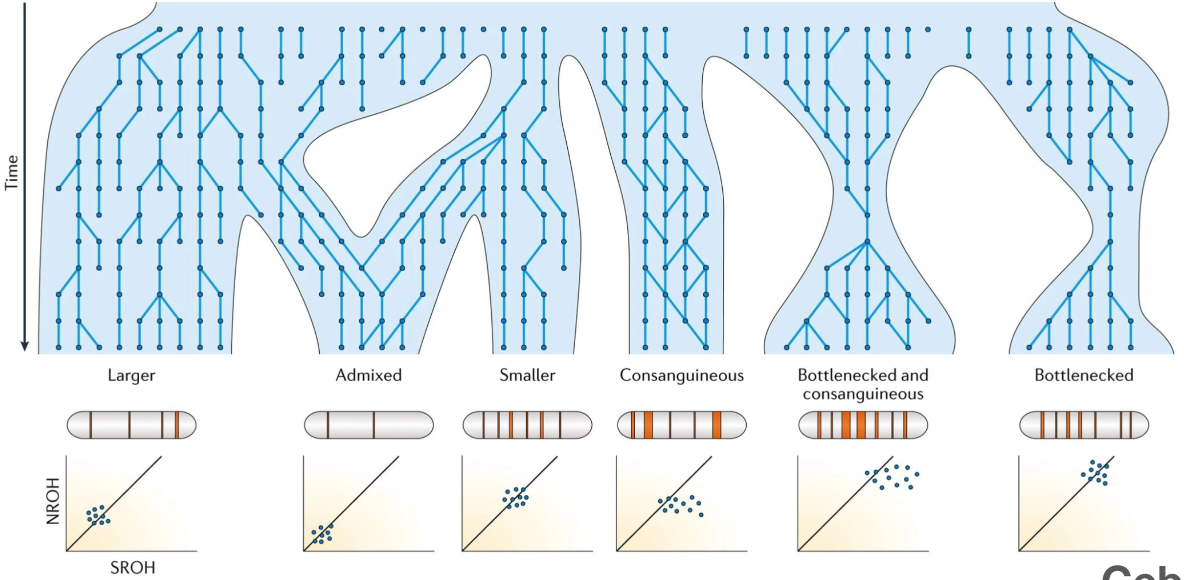


(but see AI non IBD block based alternative by Andy Kern (Smith et al 2024))

USING EXTENDED HAPLOTYPE INFORMATION

Which is getting more affordable with long-range sequencing

Using Runs-Of-Homozygosity (ROH) distribution length to infer recent inbreeding and population size changes



Ceballos et al 2018

WHAT IS THE DIFFERENCE BETWEEN CONSERVATION GENETICS AND GENOMICS?

WHAT IS THE FIRST INGREDIENT OF CONSERVATION GENOMICS?

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with a close up on two topics:

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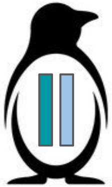
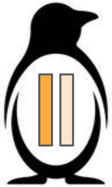
ROHs

WHAT AM I DOING TO MAKE USE OF GENOMICS IN CONSERVATION?

WHAT IS A RUN OF HOMOZYGOSITY?





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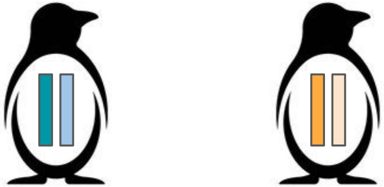

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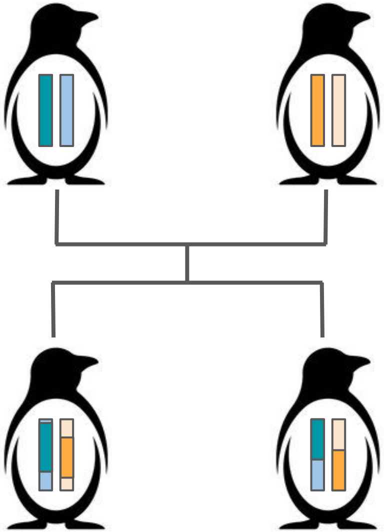

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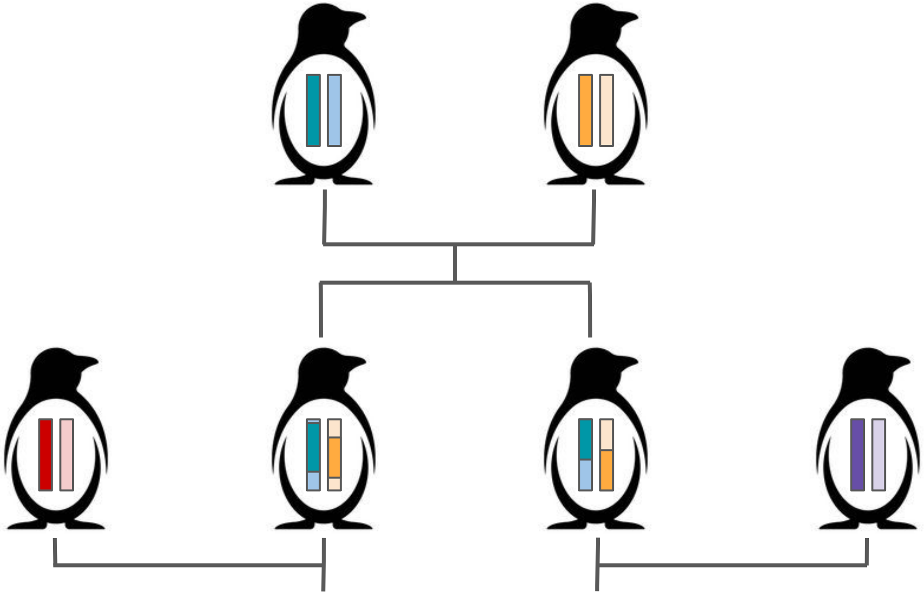

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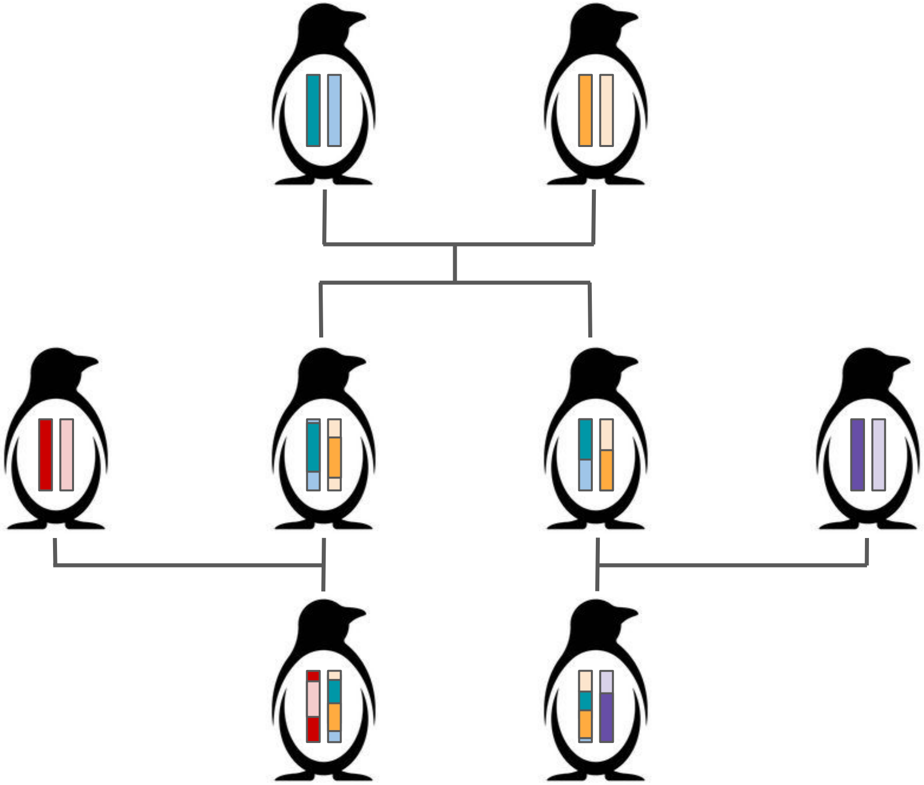

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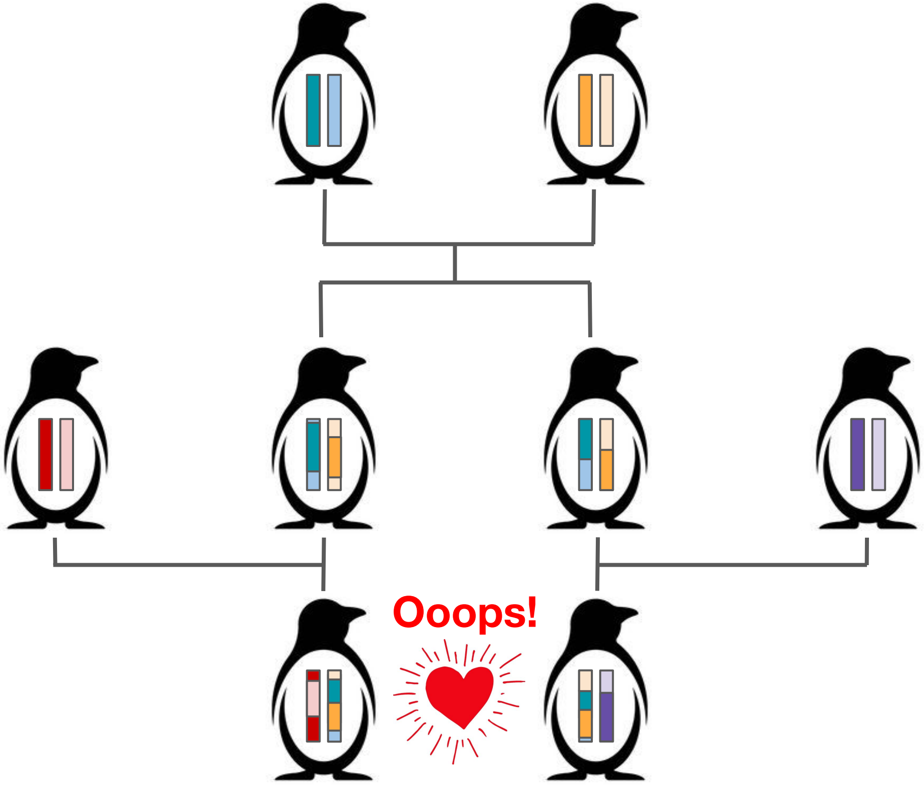

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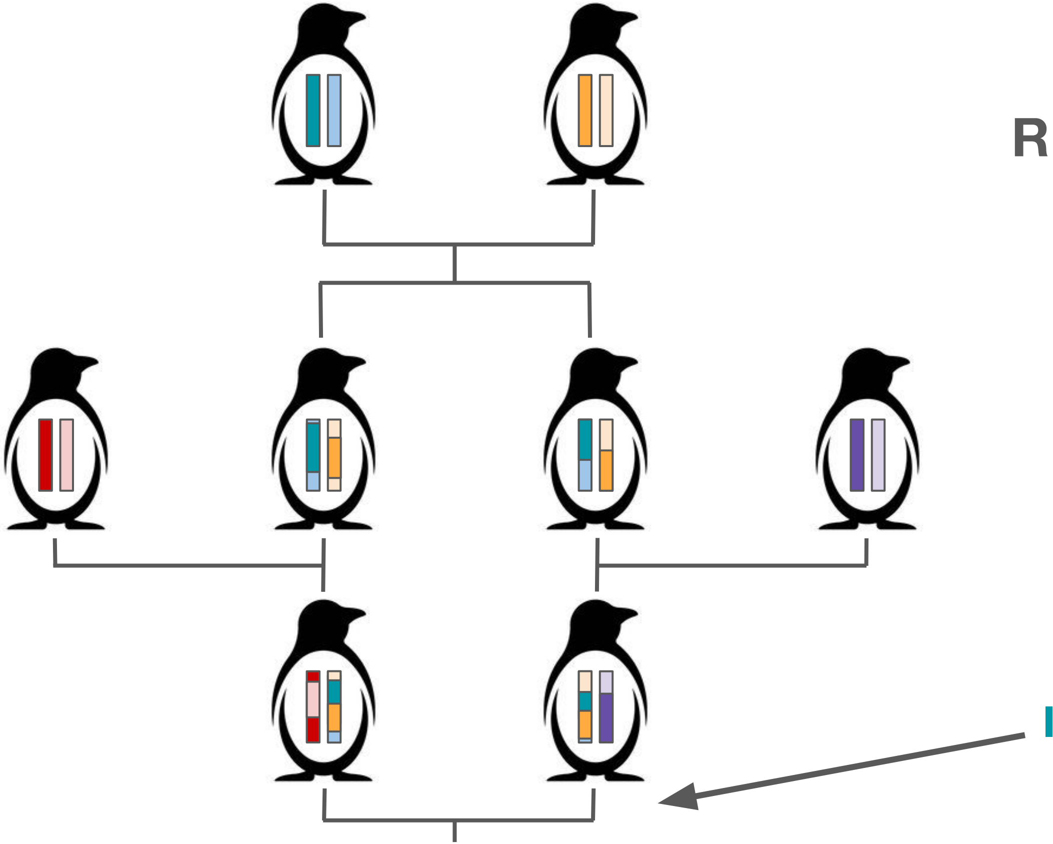
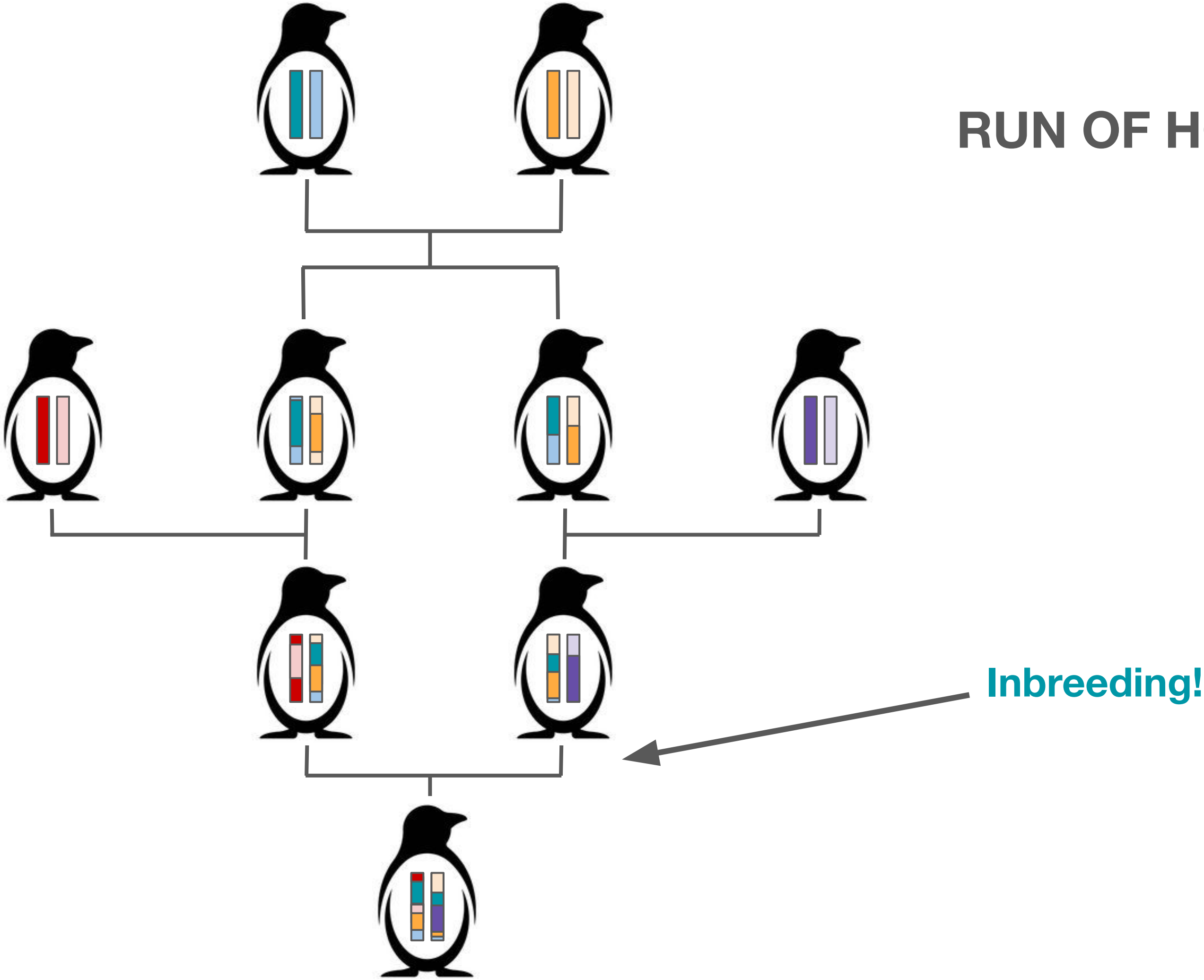


Inbreeding!

WHAT IS A RUN OF HOMOZYGOSITY?



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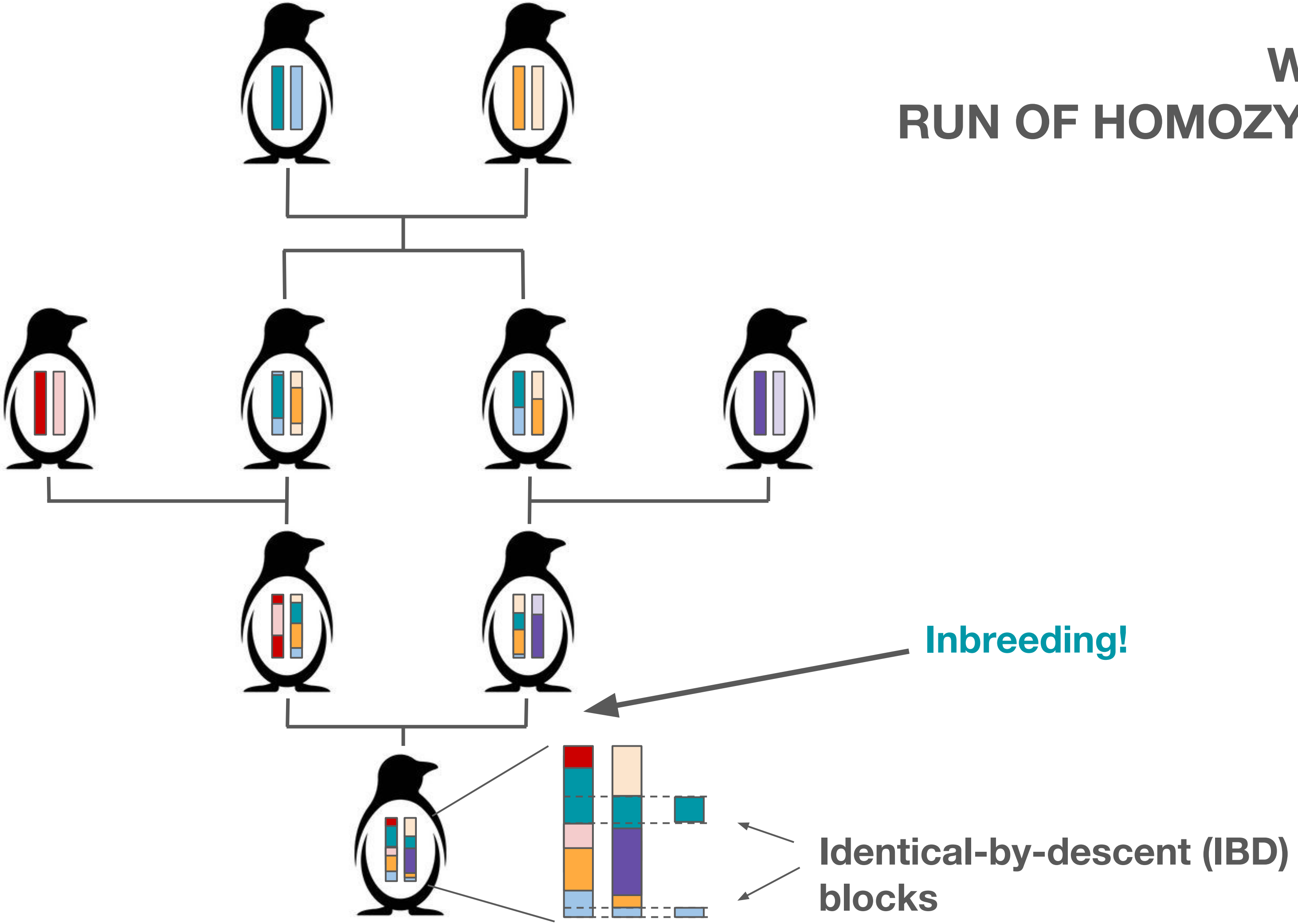


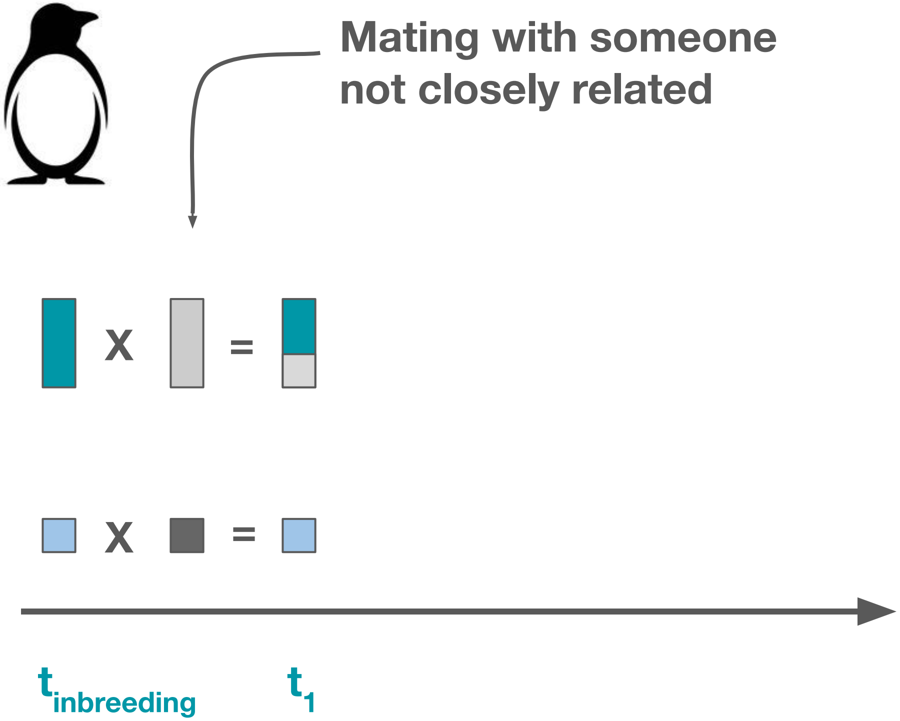
Inbreeding!

Identical-by-descent (IBD) blocks

WHAT'S THE FATE OF A RUN OF HOMOZYGOSITY?

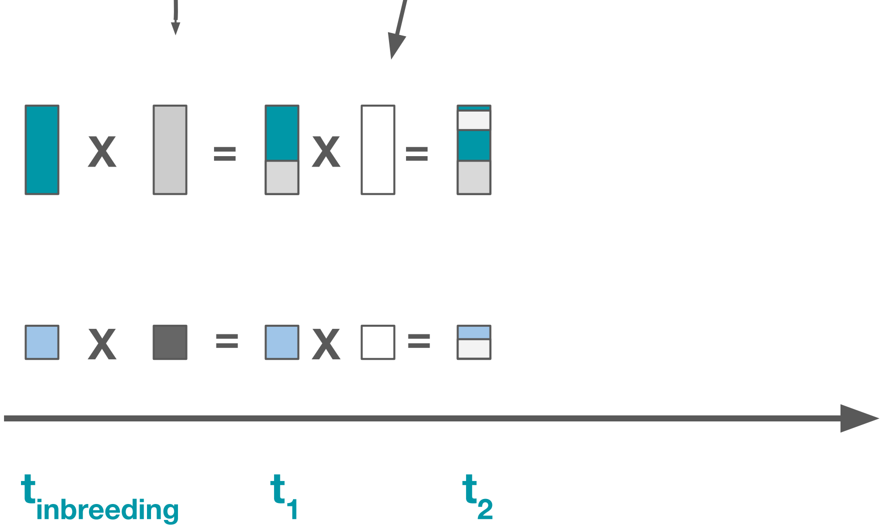
$t_{inbreeding}$

WHAT’S THE FATE OF A RUN OF HOMOZYGOSITY?



Mating with someone not closely related

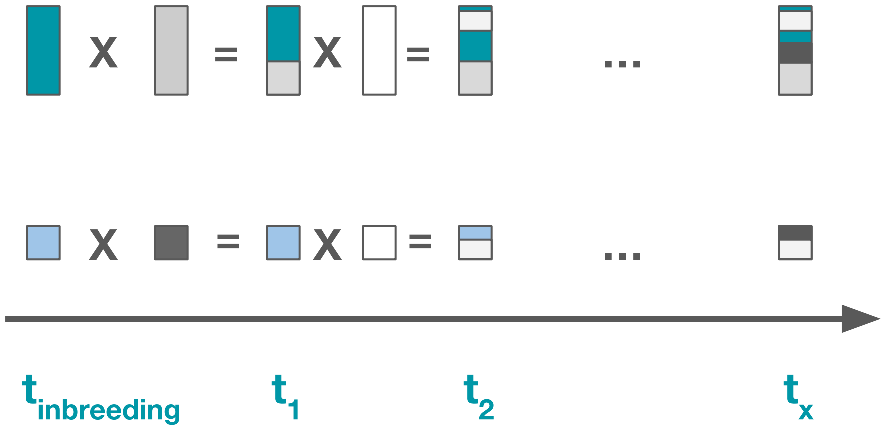
WHAT'S THE FATE OF A RUN OF HOMOZYGOSITY?


Mating with someone not closely related

$t_{inbreeding}$ t_1 t_2

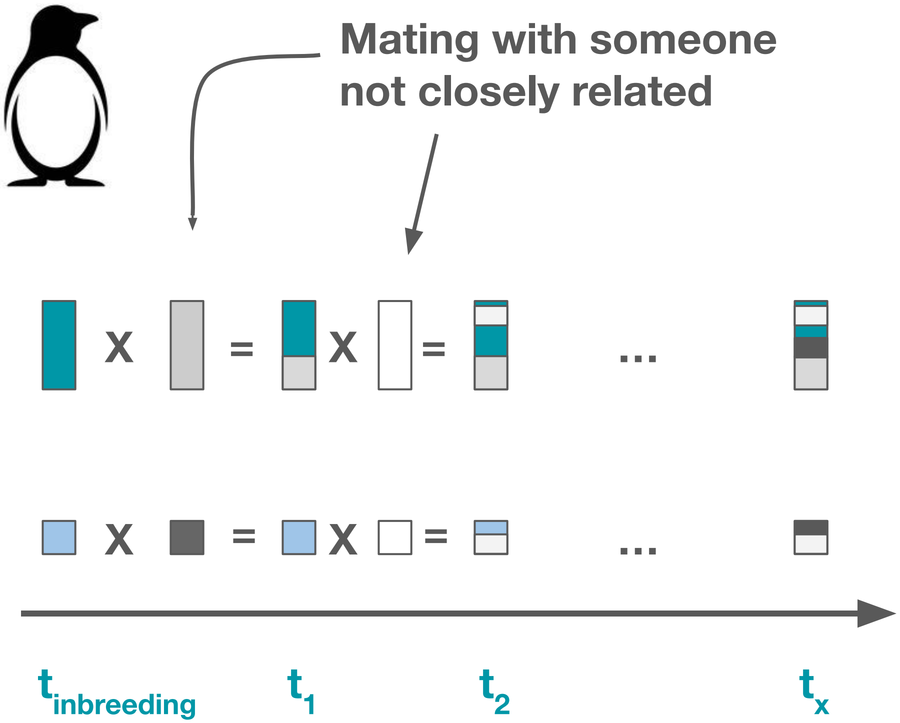
WHAT'S THE FATE OF A RUN OF HOMOZYGOSITY?

Mating with someone not closely related



Getting **shorter** and shorter

WHAT'S THE FATE OF A RUN OF HOMOZYGOSITY?


Mating with someone not closely related

Recombination rate (ρ)

Chromosome length (L)

Time (t)

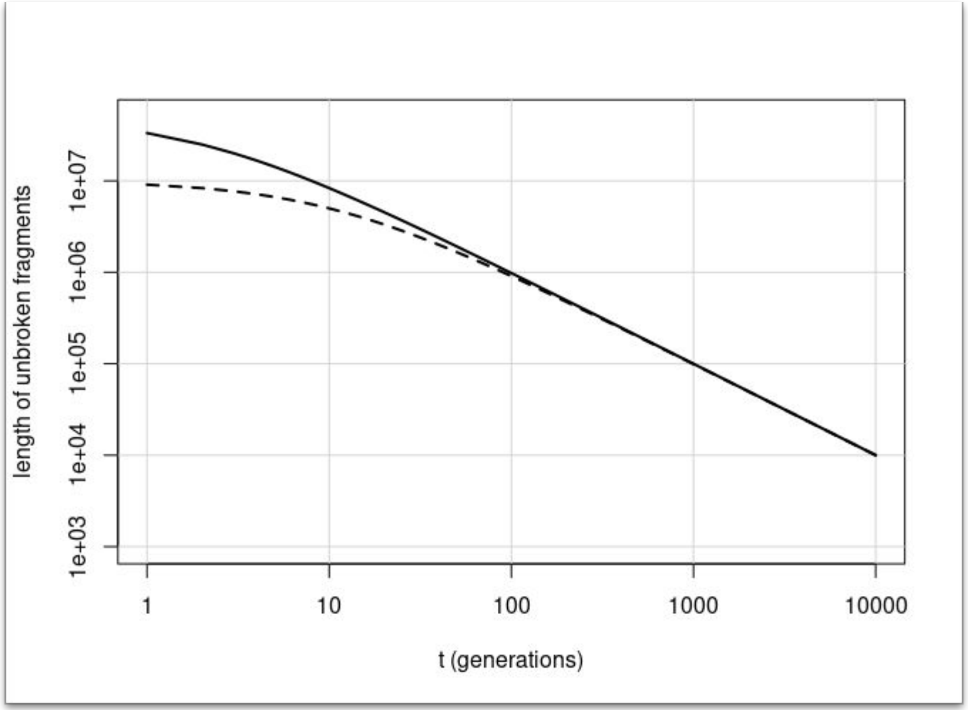
Expected number of breaks

$\rho L t$

Expected unbroken fragment length

$L/(1+\rho L t)$

WHAT'S THE FATE OF A RUN OF HOMOZYGOSITY?



Recombination rate (ρ): 1×10^{-8} gen^{-1} bp^{-1}

Chromosome length (L_0):

5×10^7 bp (solid) or 1×10^7 bp (dashed)

Time (t): 10000 generations

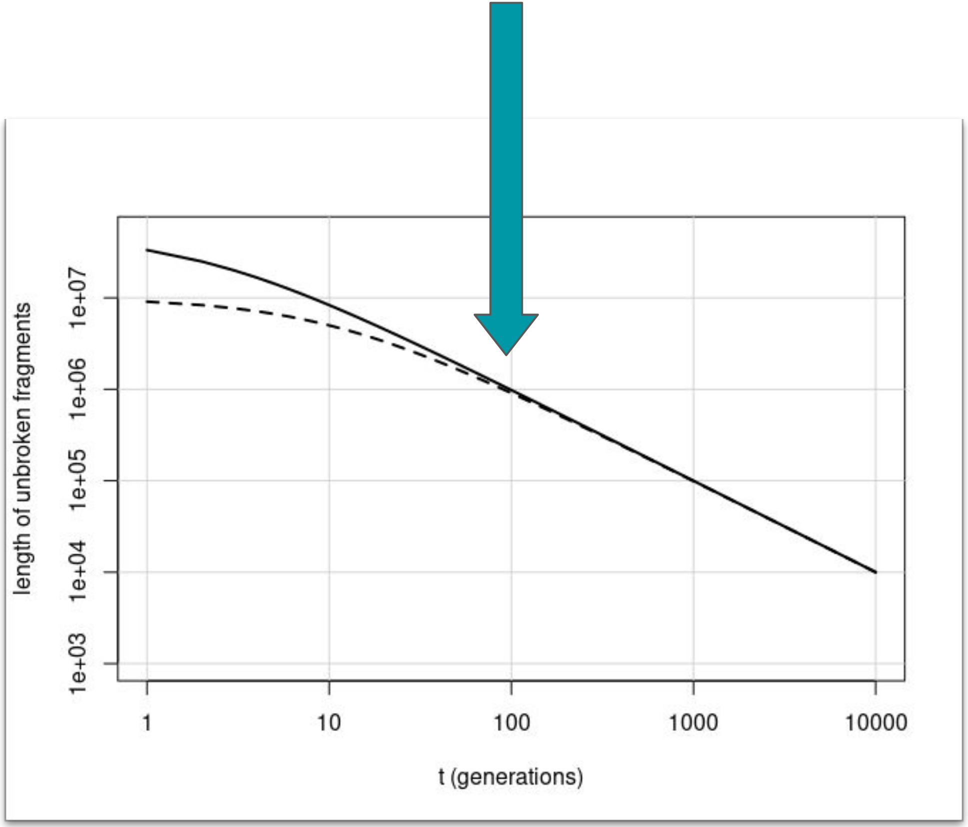
Expected number of breaks:

$\rho L_0 t$

Expected unbroken fragment length

$L_t = L_0/(1+\rho L_0 t)$

WHAT'S THE FATE OF A RUN OF HOMOZYGOSITY?



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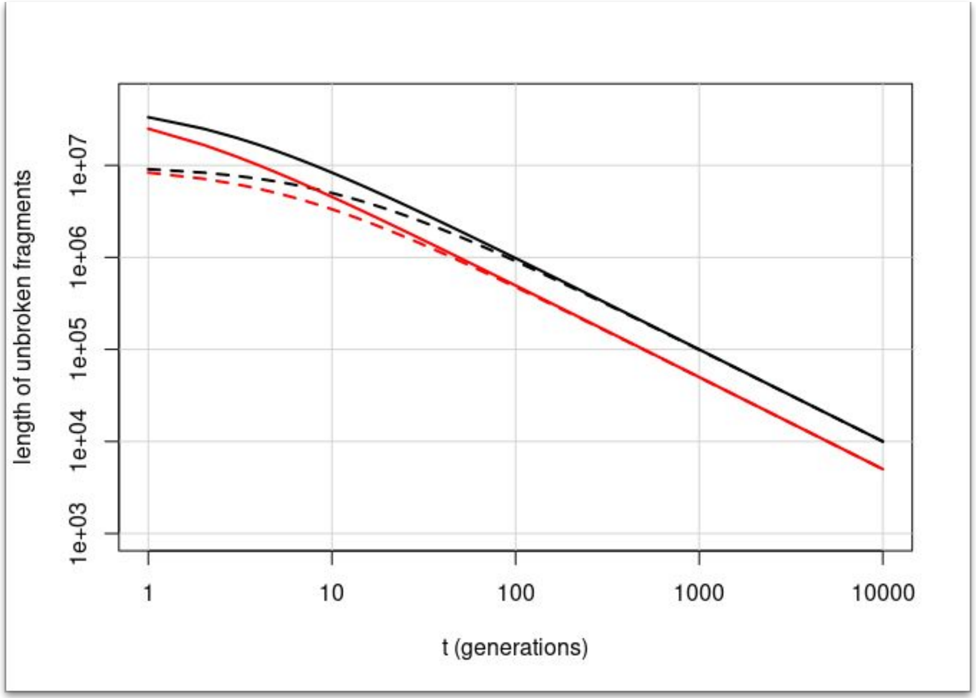
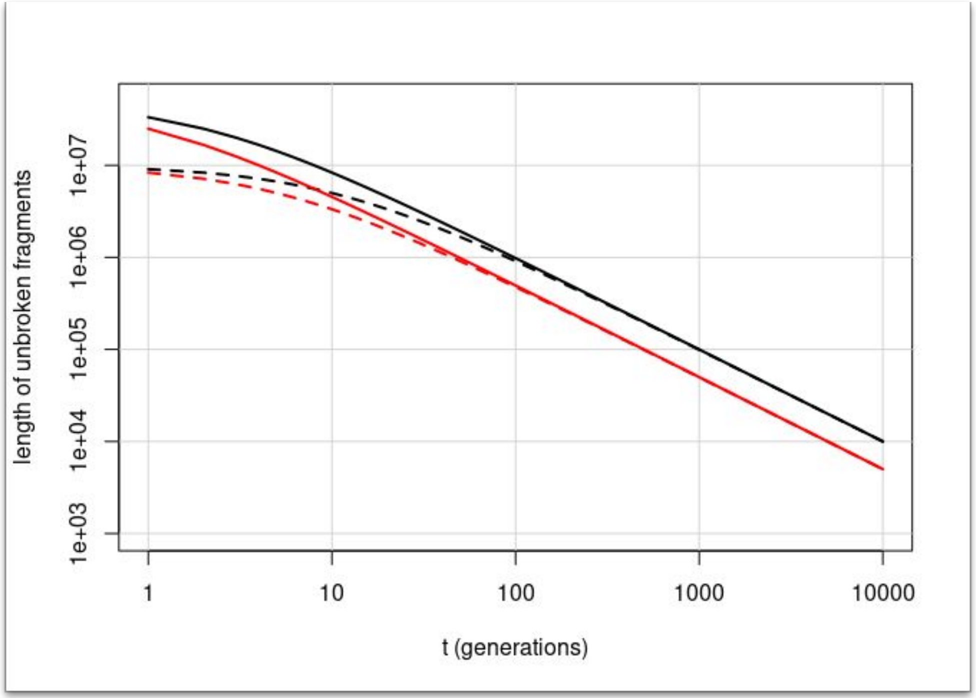
WHAT'S THE FATE OF A RUN OF HOMOZYGOSITY?



For **two lineages (ROH)**, the expected length of an Identity by descent (IBD) block is simply

$$L_t = L_0/(1+2\rho L_0 t)$$

WHAT'S THE FATE OF A RUN OF HOMOZYGOSITY?



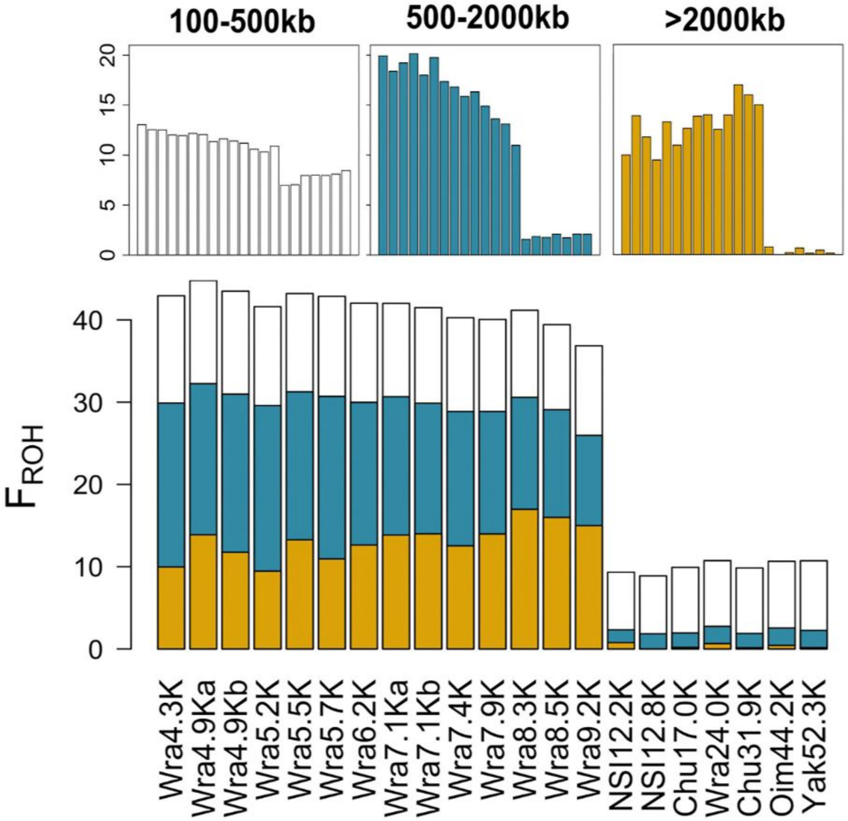
For **two lineages (ROH)**, the expected length of an Identity by descent (IBD) block is simply

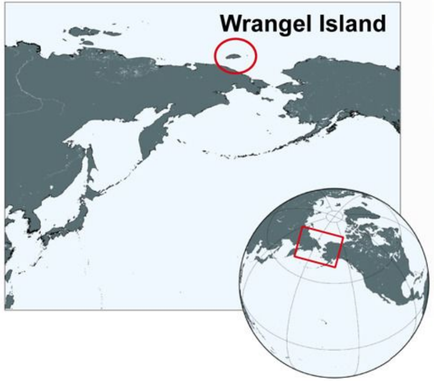
$$L_t = L_0/(1+2\rho L_0 t)$$

From this we can get an expectation on the time of the inbreeding loop (and on the population size at that time*)

$$t = 1/(2\rho L_t)$$

ROHS CAN INFORM ABOUT PAST **INBREEDING EVENTS**

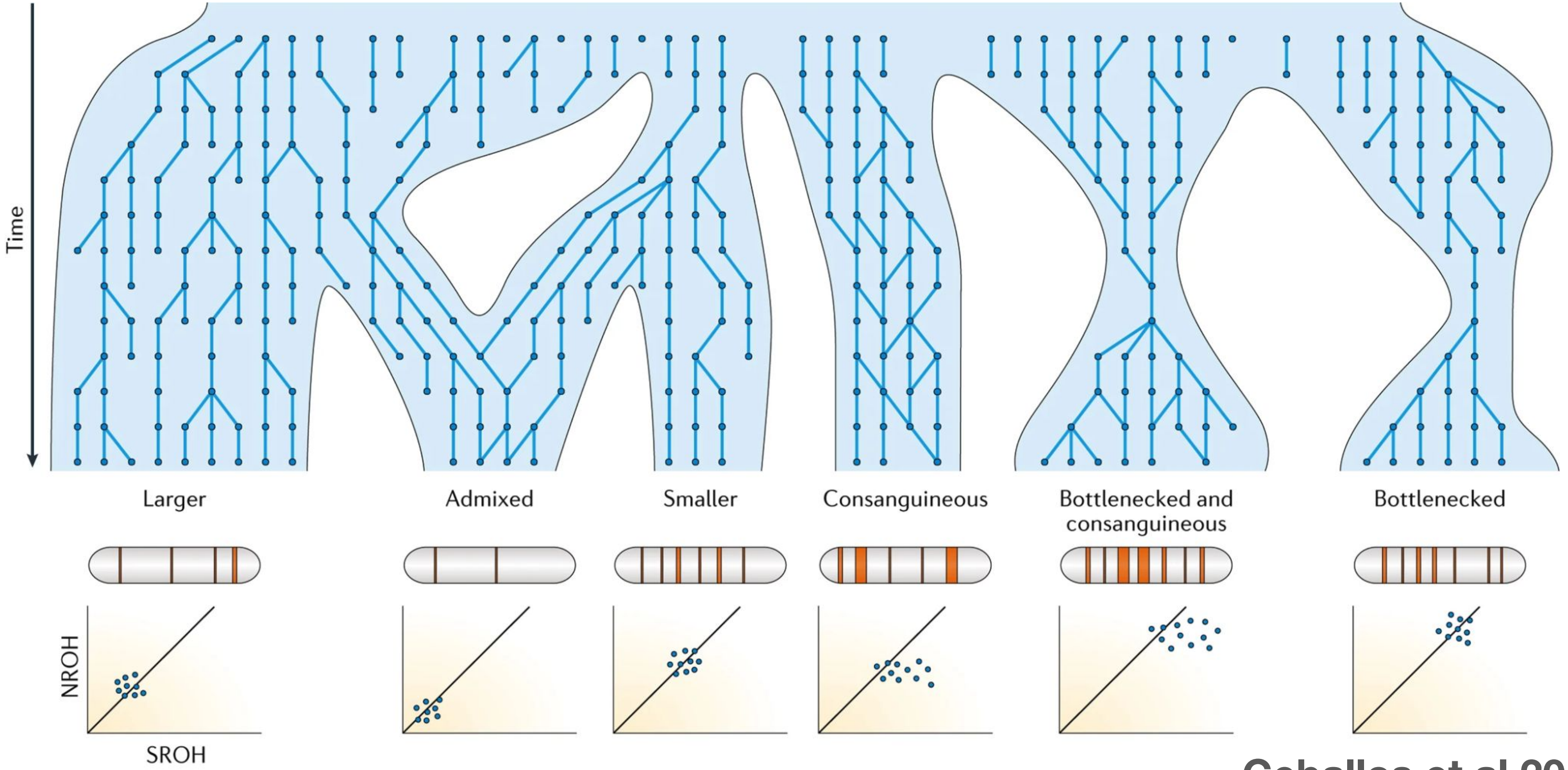




Infer when most recent inbreeding event happened by **ROHs of maximum length***

Dehasque et al 2024

ROHS CAN INFORM ABOUT PAST DEMOGRAPHY



Total number of ROH

Sum total length of ROH

Ceballos et al 2018

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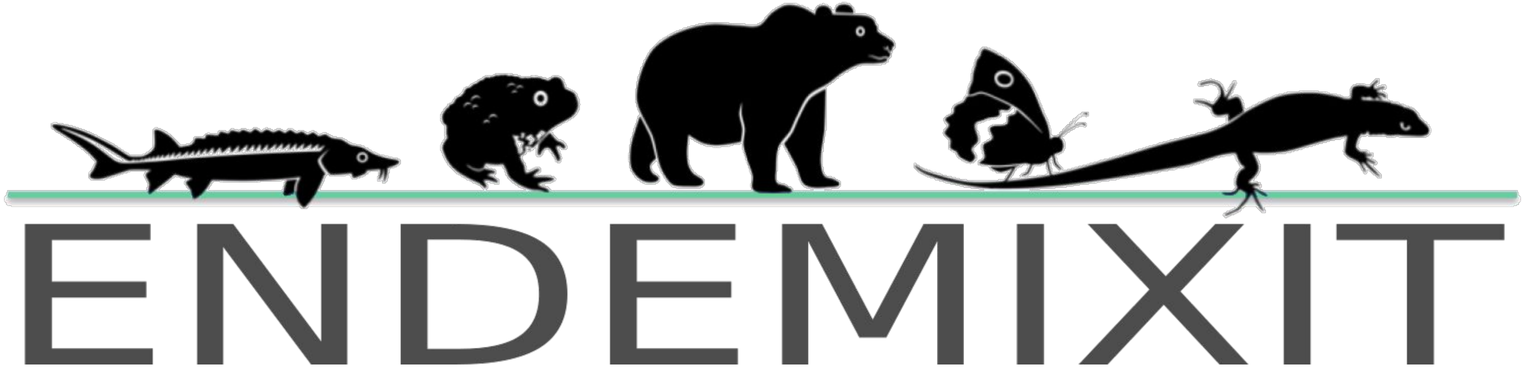
WHAT AM I DOING TO MAKE USE OF GENOMICS IN CONSERVATION?

GENOMIC SUSCEPTIBILITY TO EXTINCTION

A whole-genome approach to study and protect endangered Italian endemics



PI: **Giorgio Bertorelle**, University of Ferrara

https://endemixit.com/
https://youtu.be/mL_JzgOqk7c

GENOMICS OF FIVE ENDEMIC SPECIES

Very to very small population size

Marsican bear
(2.2Gb)

GENOMICS OF FIVE ENDEMIC SPECIES

Very to very small population size

Marsican bear
(2.2Gb)

Ponza grayling
(0.4Mb)

GENOMICS OF FIVE ENDEMIC SPECIES

Very to very small population size

Marsican bear
(**2.2Gb**)

Ponza grayling
(**0.4Mb**)

Aeolian lizard
(**1.4Gb**)

GENOMICS OF FIVE ENDEMIC SPECIES

Very to very small population size

Marsican bear
(2.2Gb)

Ponza grayling
(0.4Mb)

Aeolian lizard
(1.4Gb)

Apennine yellow-bellied toad (10Gb)

GENOMICS OF FIVE ENDEMIC SPECIES

Very to very small population size

Marsican bear
(2.2Gb)

Adriatic sturgeon
(1.4Gb, tetraploid)

Ponza grayling
(0.4Mb)

Aeolian lizard
(1.4Gb)

Apennine yellow-bellied toad (10Gb)

BEFORE ENDEMIXIT THERE WAS JUST THE APENNINE BEAR!

Survival and divergence in a small group: The extraordinary genomic history of the endangered Apennine brown bear stragglers

Andrea Benazzo[a,1], Emiliano Trucchi[a,b,1], James A. Cahill[c], Pierpaolo Maisano Delser[d,e,f], Stefano Mona[d,e], Matteo Fumagalli[g], Lynsey Bunnefeld[h,i], Luca Cornetti[j], Silvia Ghirotto[a], Matteo Girardi[k], Lino Ometto[l,m], Alex Panziera[a], Omar Rota-Stabelli[l], Enrico Zanetti[a], Alexandros Karamanlidis[n], Claudio Groff[o], Ladislav Paule[p], Leonardo Gentile[q], Carles Vilà[r], Saverio Vicario[s], Luigi Boitani[t], Ludovic Orlando[u], Silvia Fuselli[a], Cristiano Vernesi[k], Beth Shapiro[c], Paolo Ciucci[t], and Giorgio Bertorelle[a,2]

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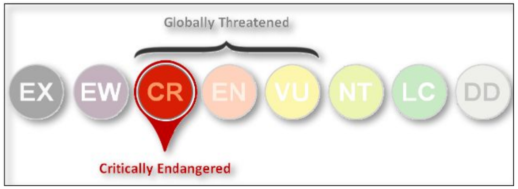
MOVE TO THE OLD PRESENTATION ON THE BEAR!

The extraordinary genomic history of the endangered Apennine Brown Bear

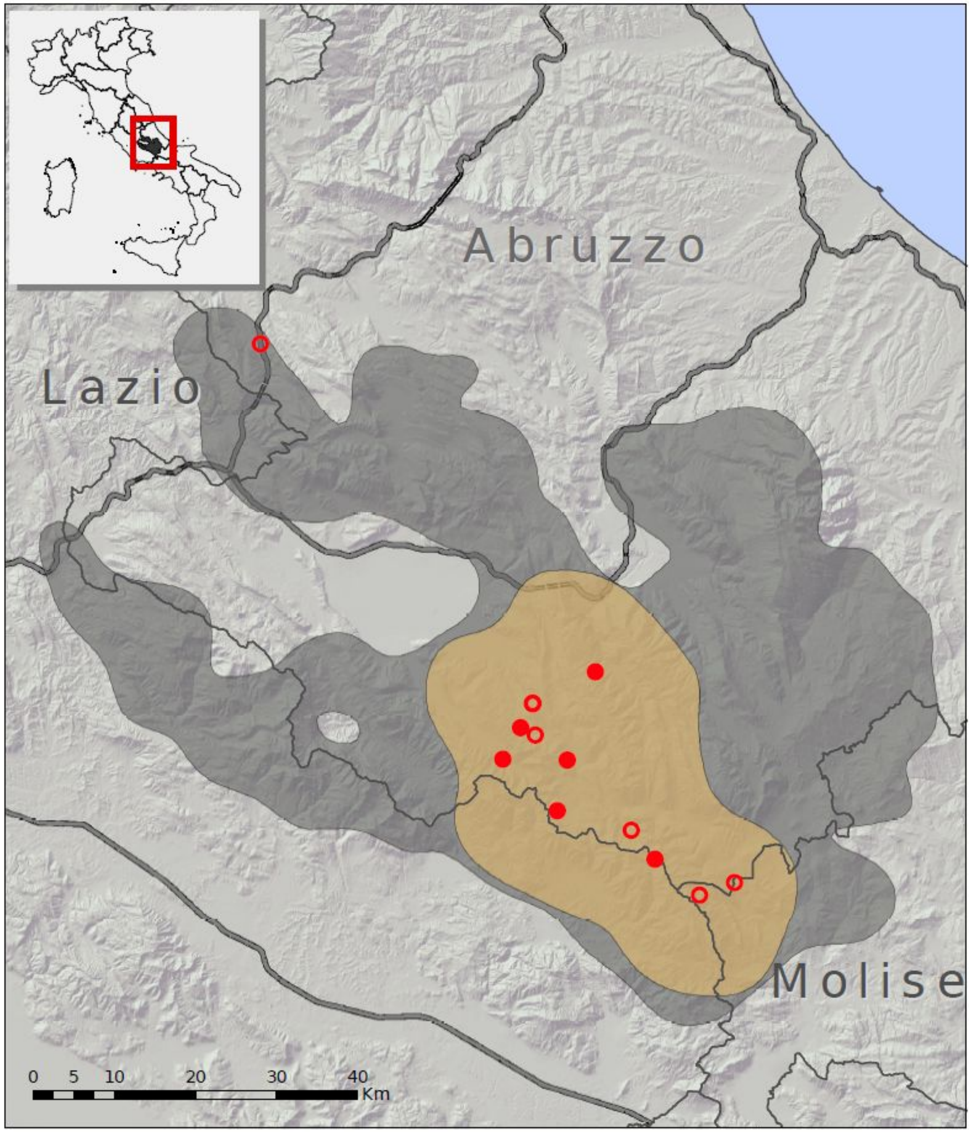
Brown bear distribution

A small and isolated population in Central Italy

Less than **100** individuals

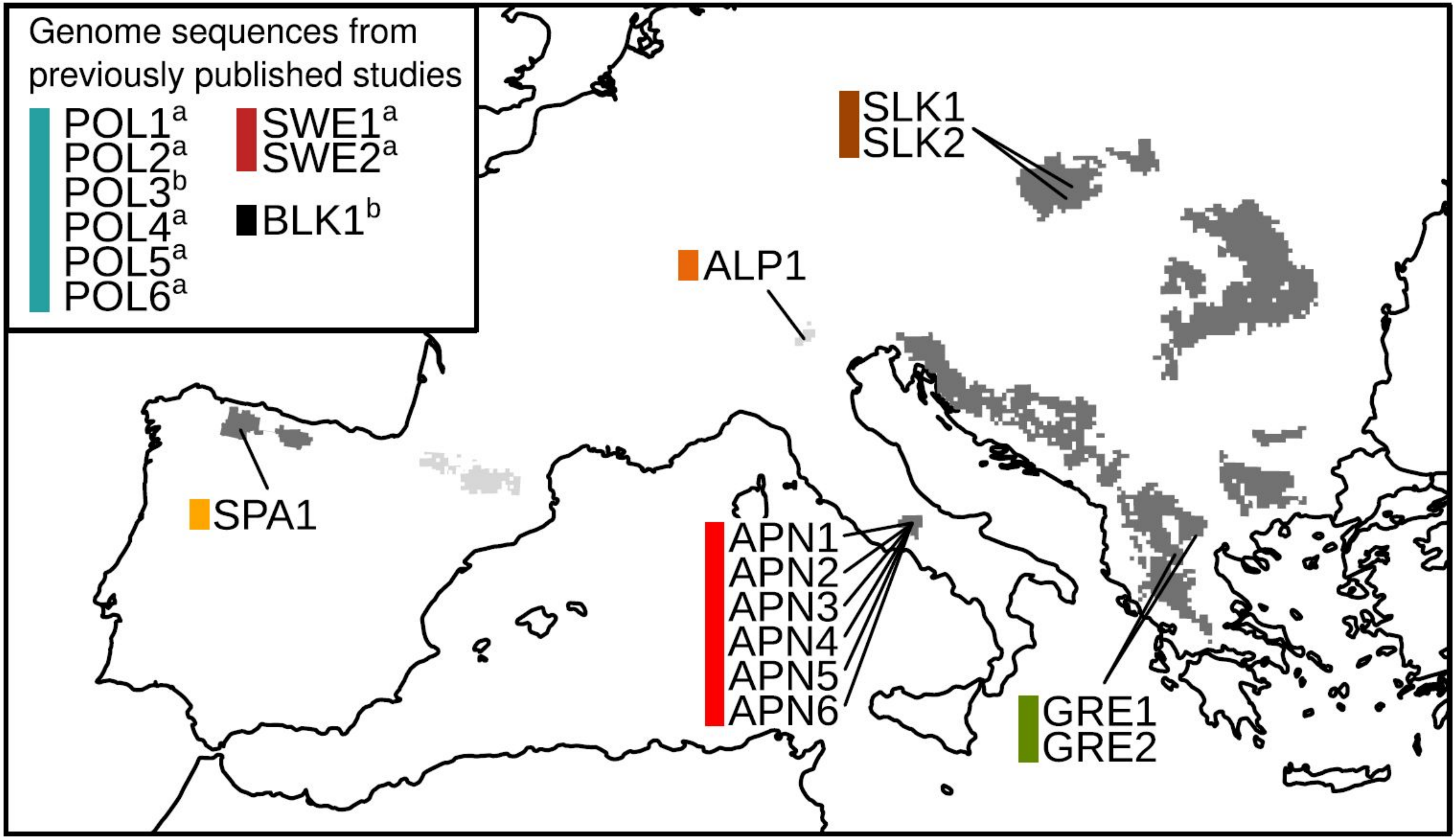


Parco
Nazionale
d'Abruzzo
Lazio e Molise



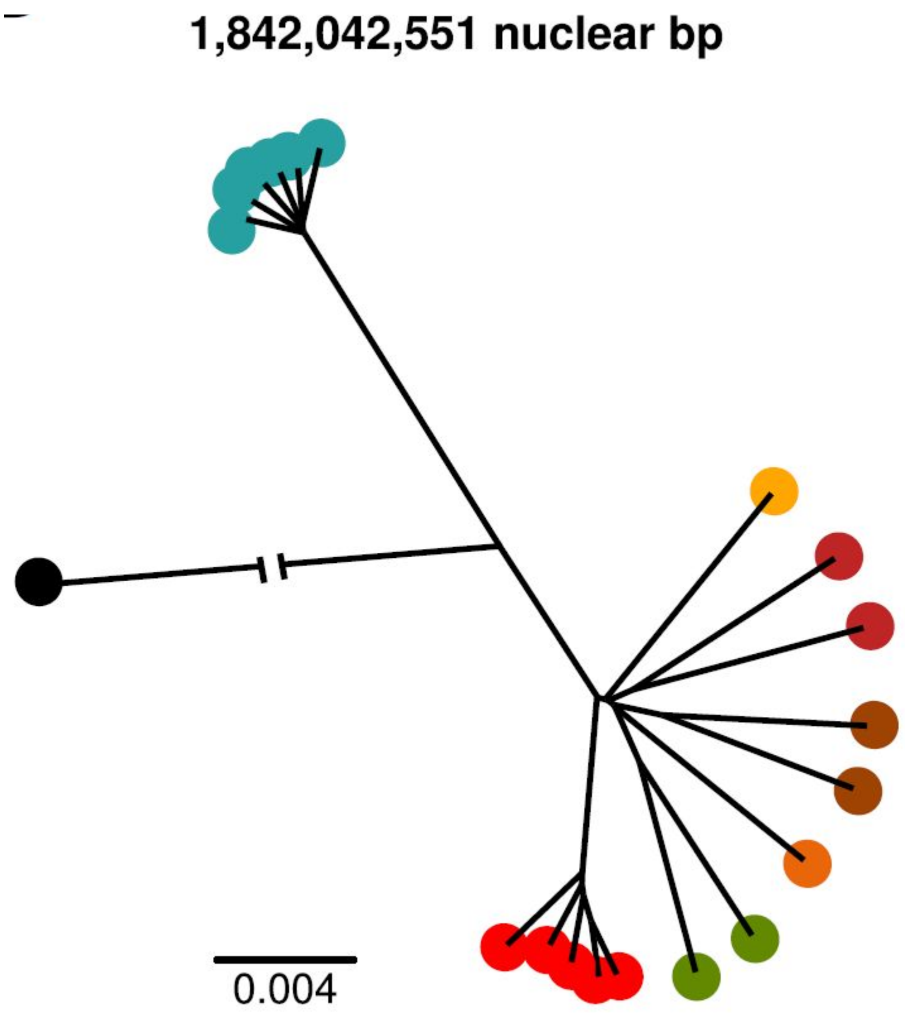
Whole-genome data

A few other samples from other European populations

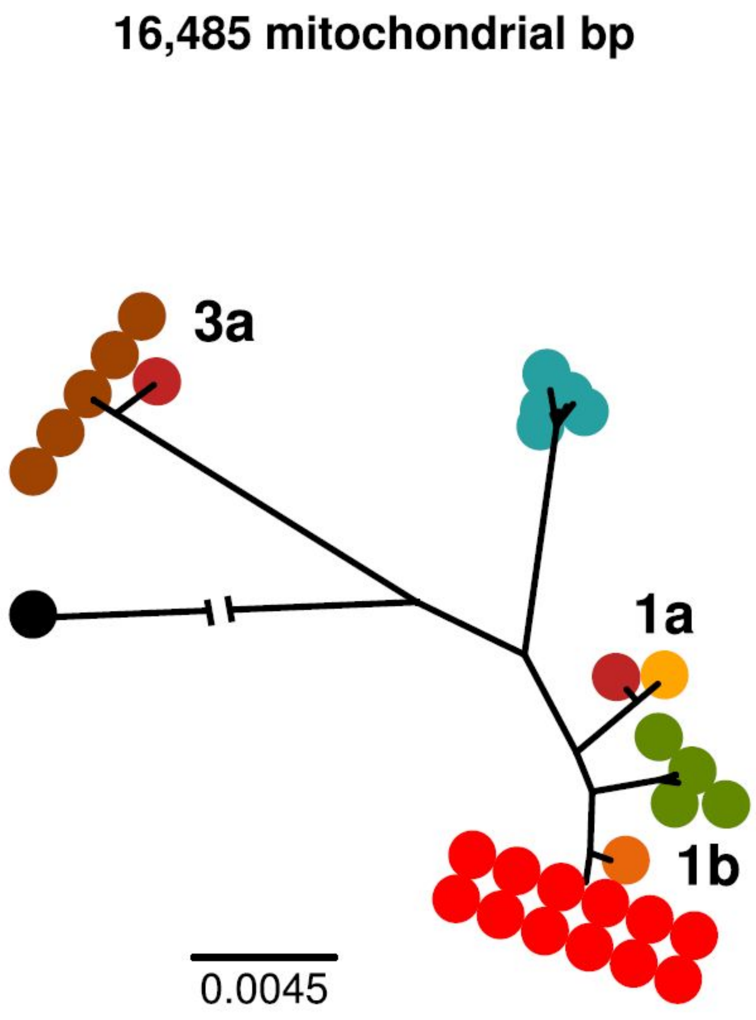


a: Liu et al 2014, b: Miller et al 2012

Structure of genomic diversity
Whole-genome and mitochondrion contrasting histories



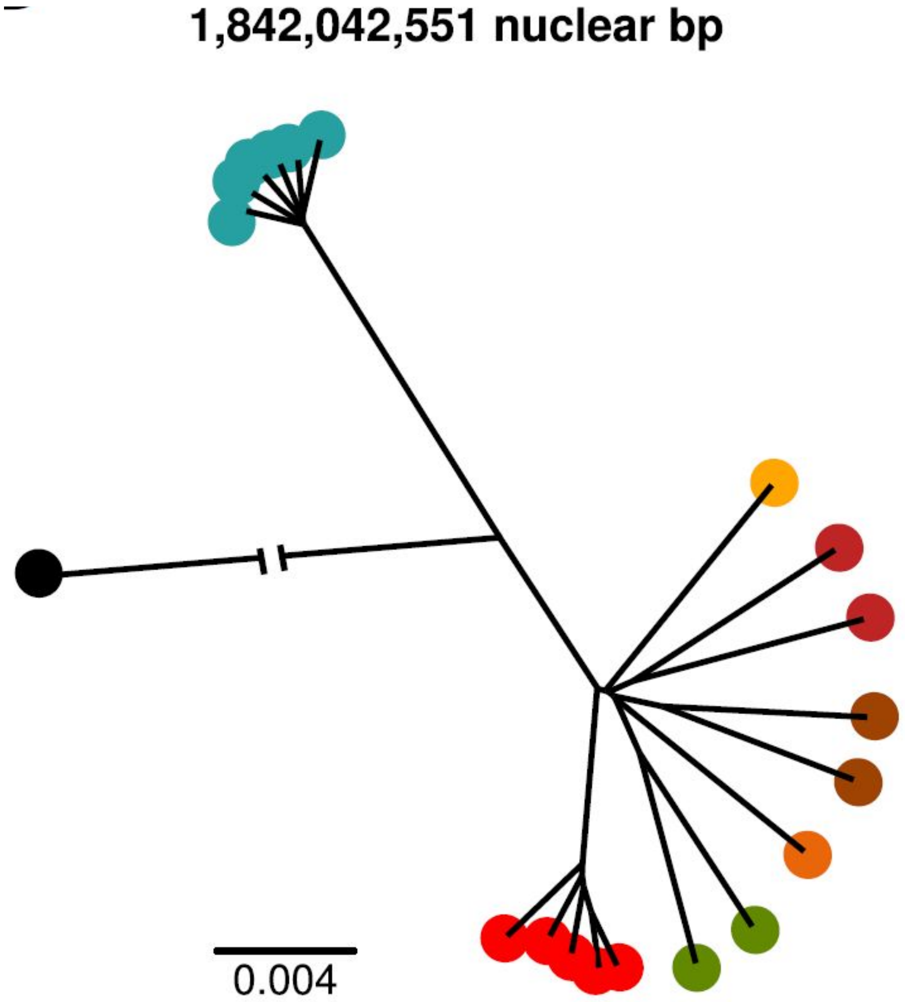
ANGSD, ngsDist, nj from ape *R* package



Geneious, MAFFT, genetic distance (HKY), NJ

Structure of genomic diversity

Y-chr agrees with the genome: sex-biased introgression?



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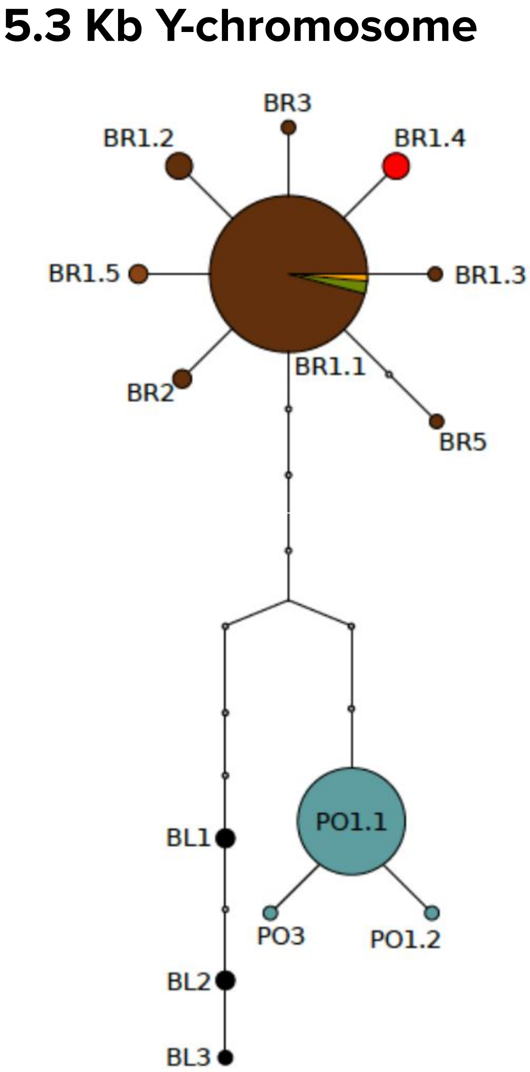
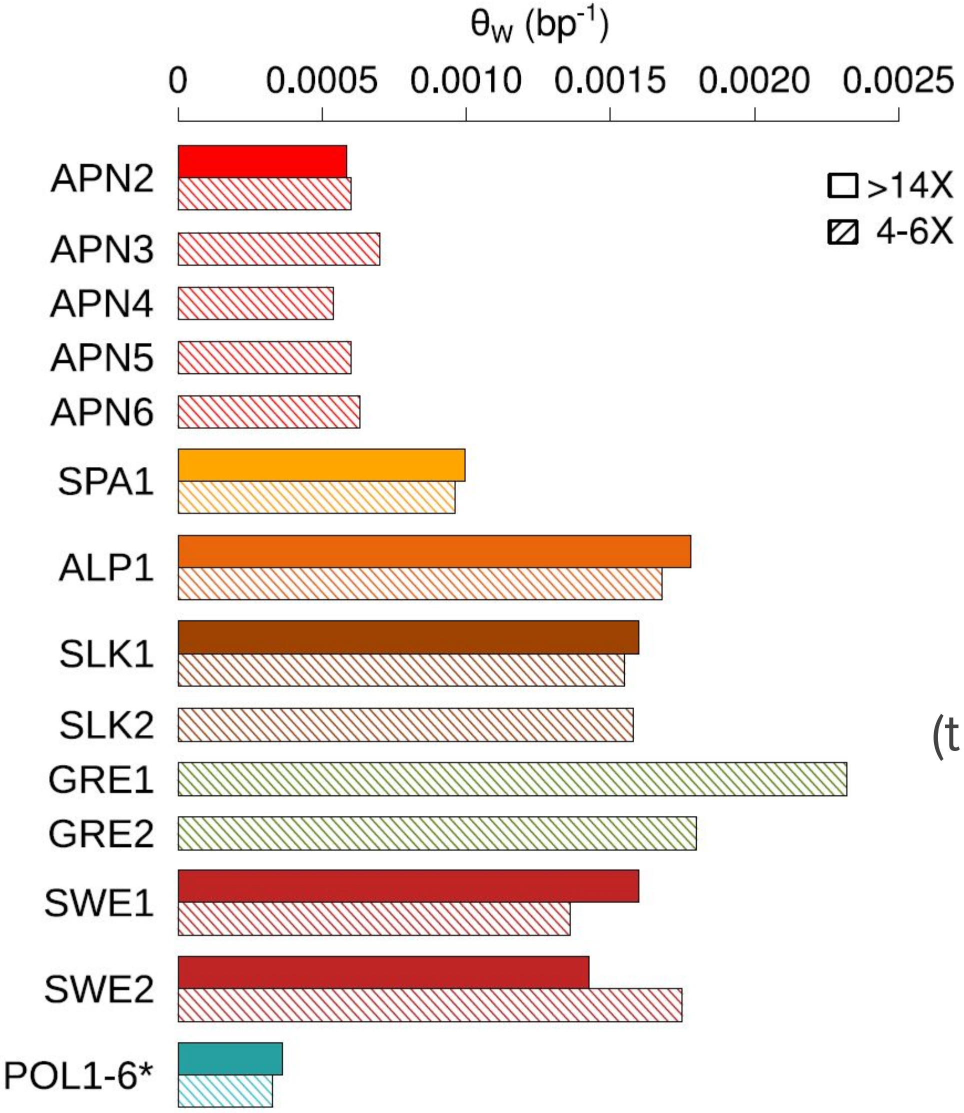


MAFFT, TCS

Genomic diversity - average

Low but the polar bear is worse

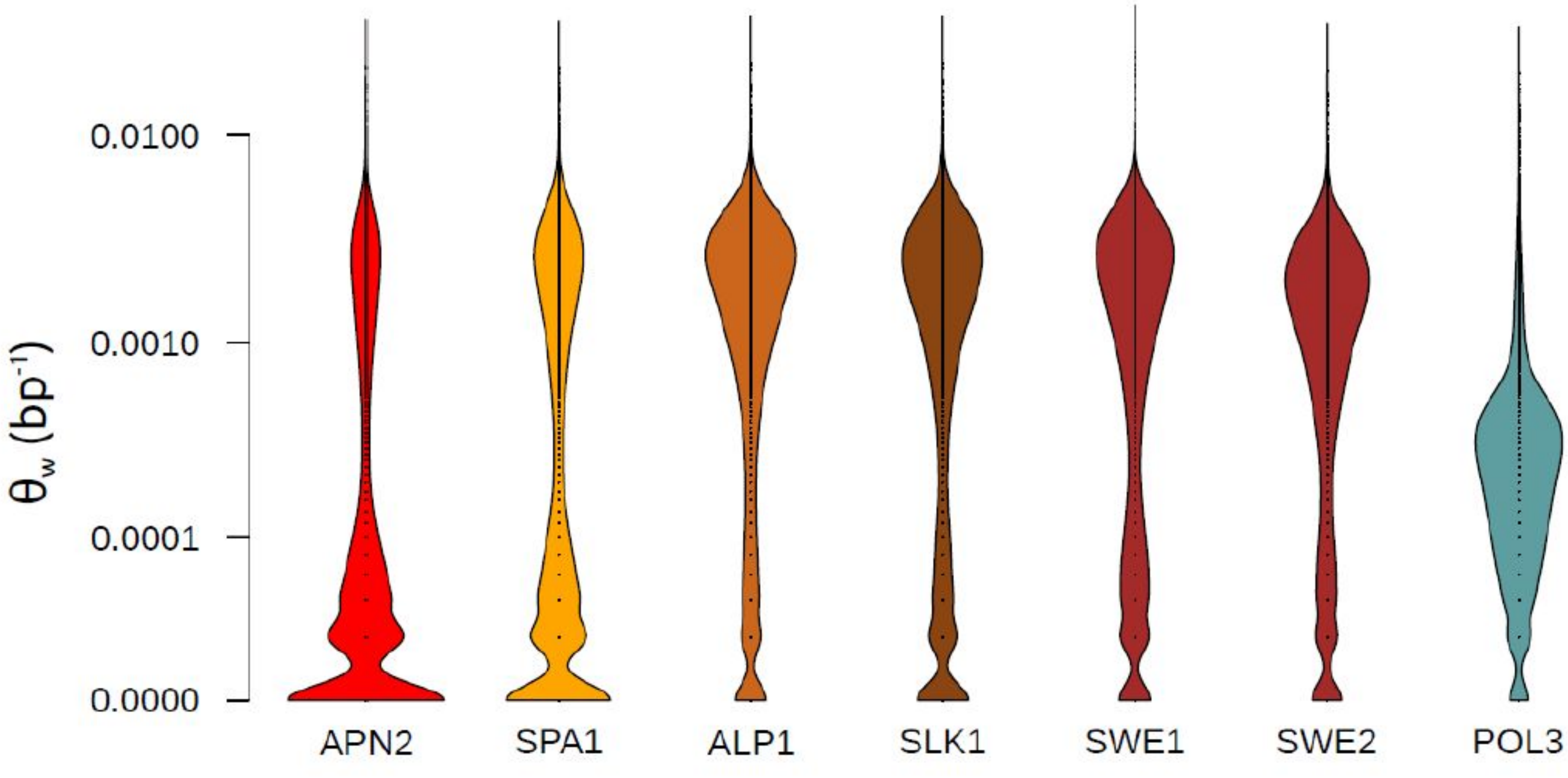


High coverage:
vcftools, SNP density, 50kb windows
(then downsampled and analyzed as low coverage)

Low coverage:
ANGSD, realSFS, do_theta, 50kb windows

Genomic diversity - distribution

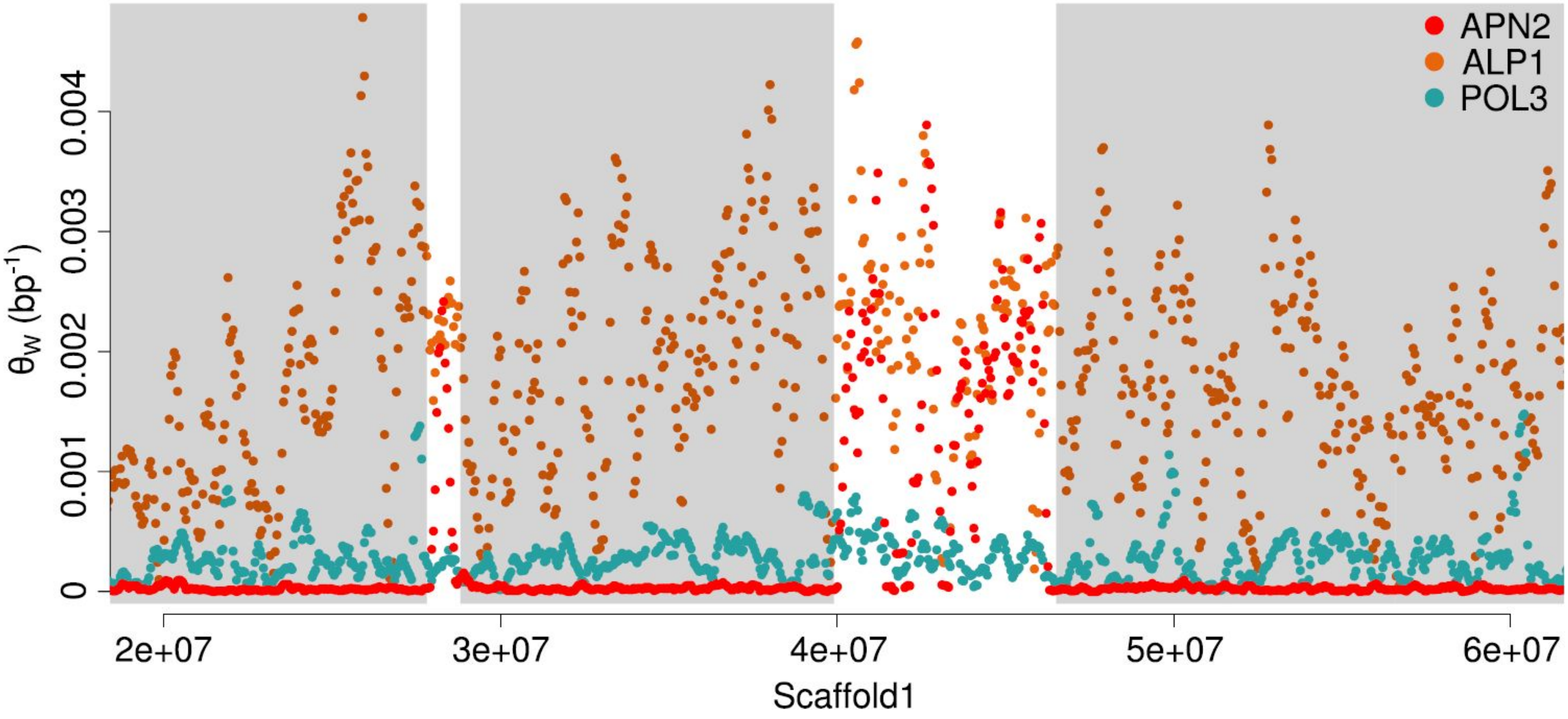
Low but the polar bear is worse…maybe not!



vcftools, SNP density in 50kb windows

Genomic diversity - distribution

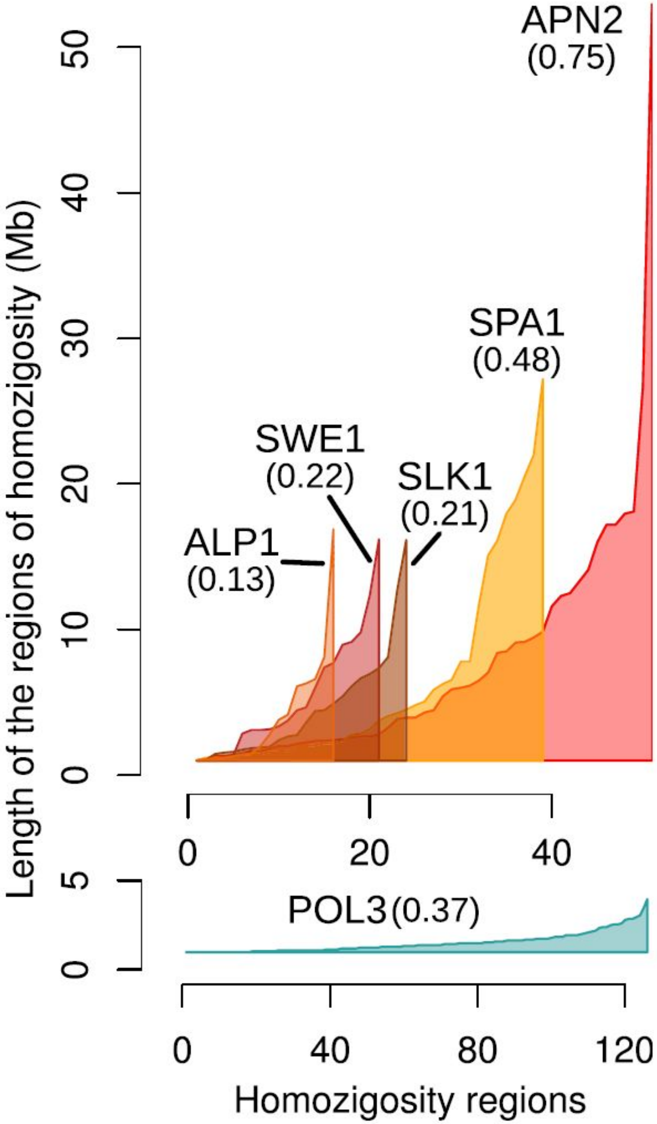
Long stretches with no diversity in the Apennine bear



vcftools, SNP density in 50kb windows

Inbreeding estimates

Much higher than any other European bear

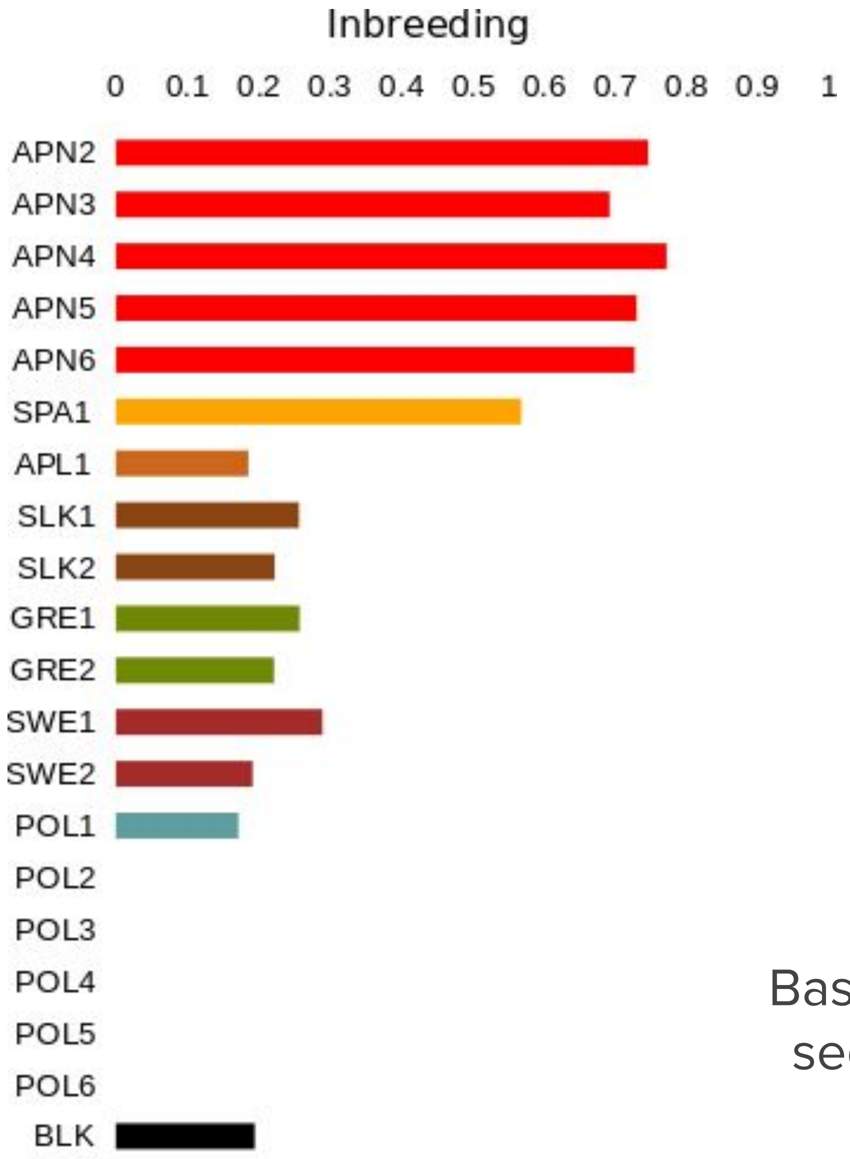


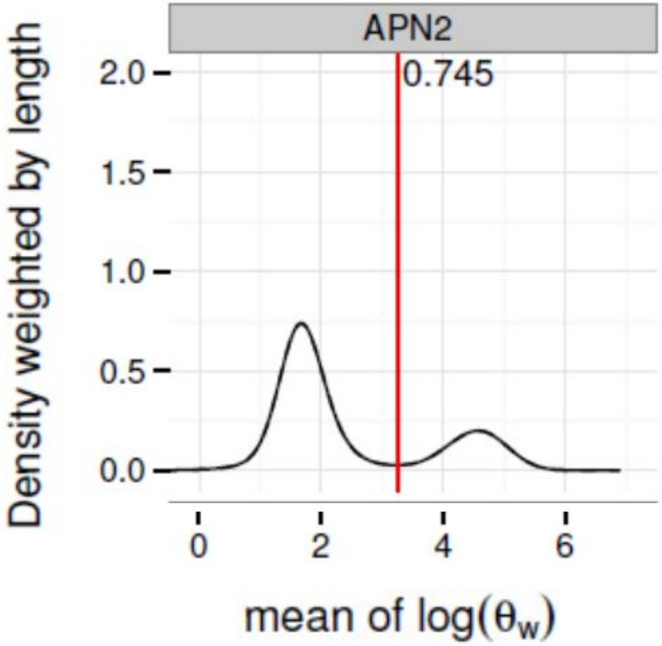
ROH: Regions longer than 1Mb with less than 25 segregating sites in the 13 longest scaffolds, ca. 0.5 Gb - sorted

(percent of the analyzed scaffolds)

Inbreeding estimates

Much higher than any other European bear

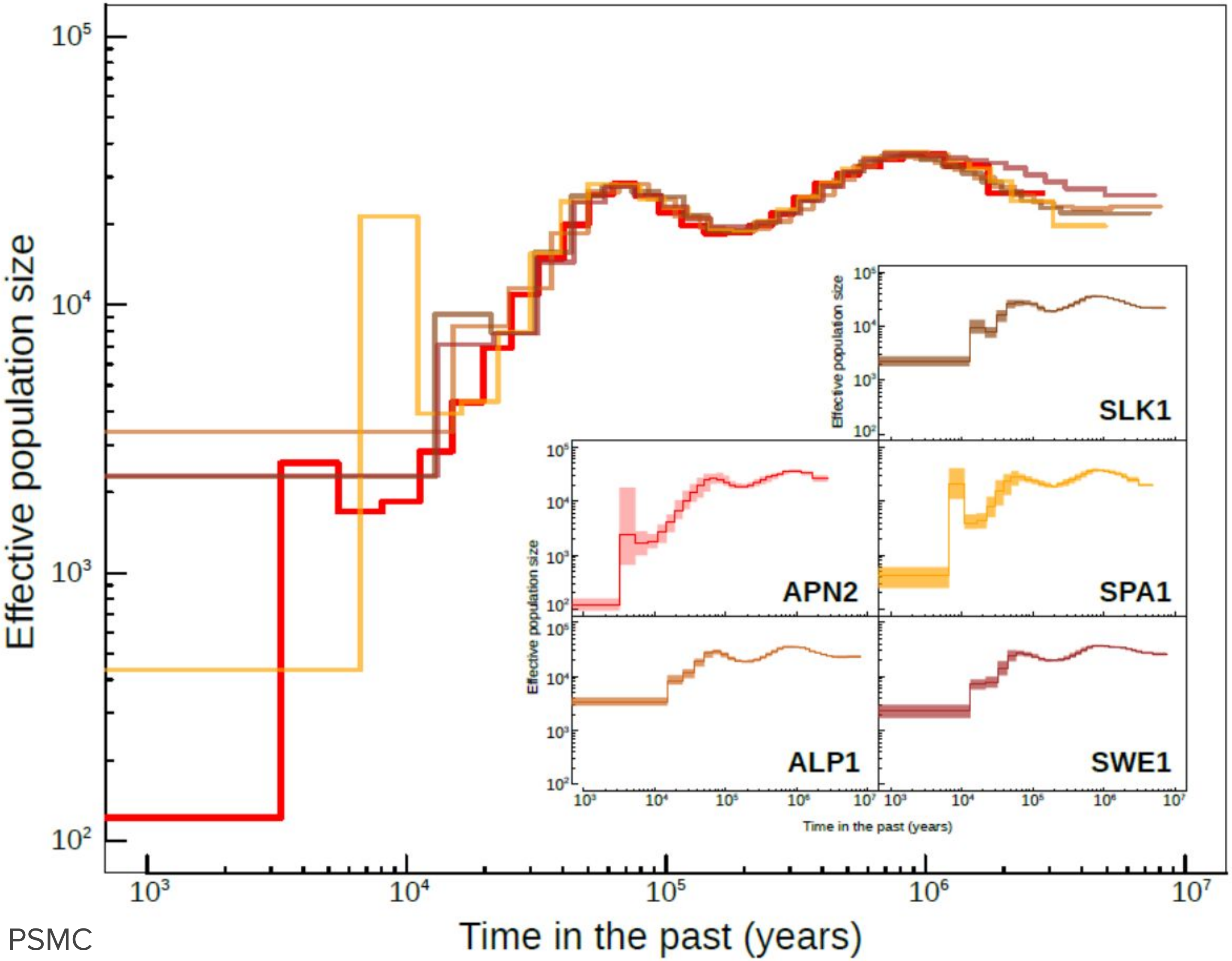




Based on the proportion of the genome segments that are mostly homozygous (Prüfer et al 2014)

Past demography

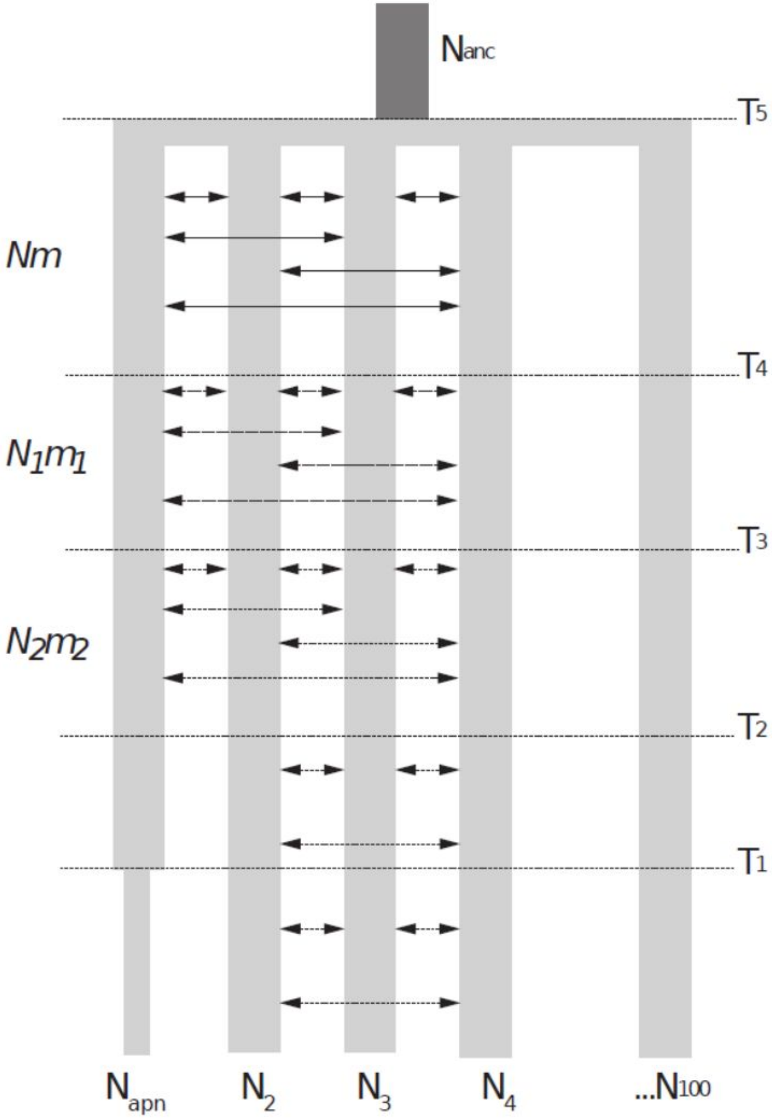
Apennine population declined more than other Eu pops



PSMC

Past demography

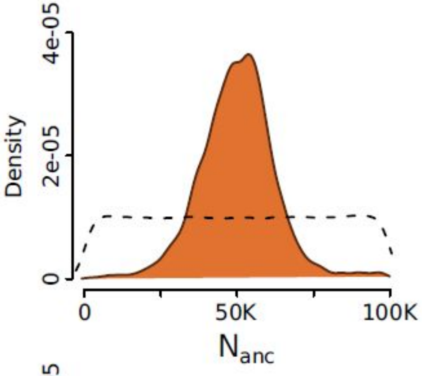
Fragmentation of a large European population

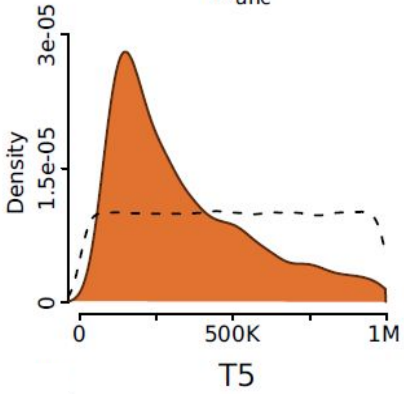


ABC metapopulation model

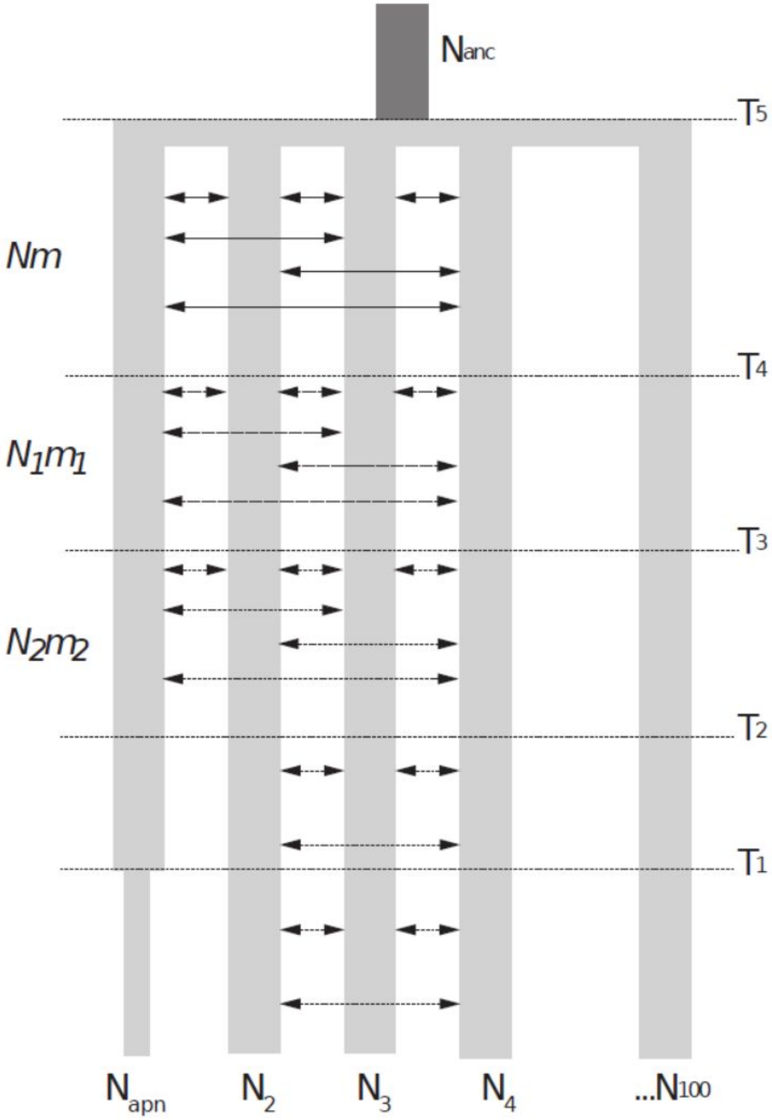
Ancestral population

Ancient European spread





Past demography
Fragmentation of a large European population



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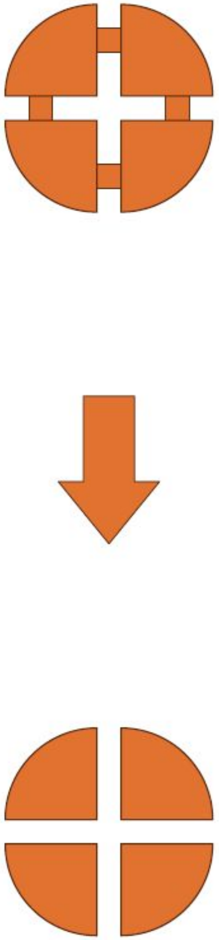
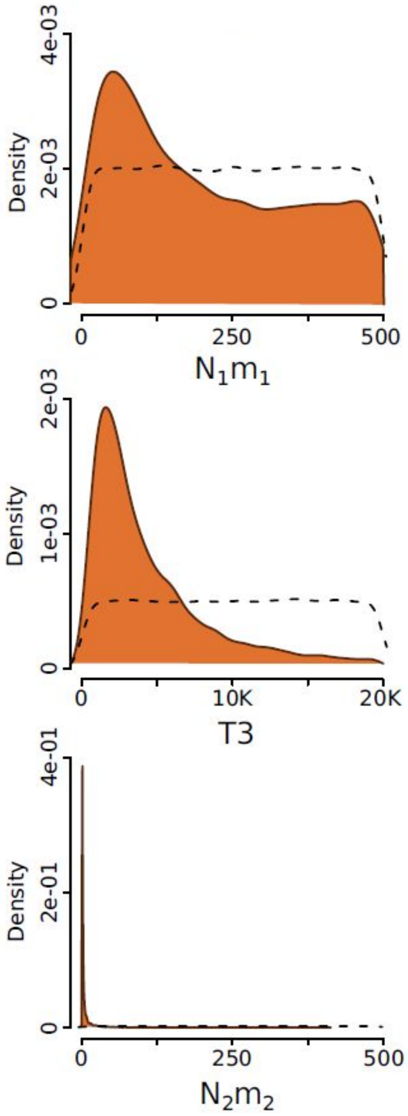


High connectivity among ancient populations

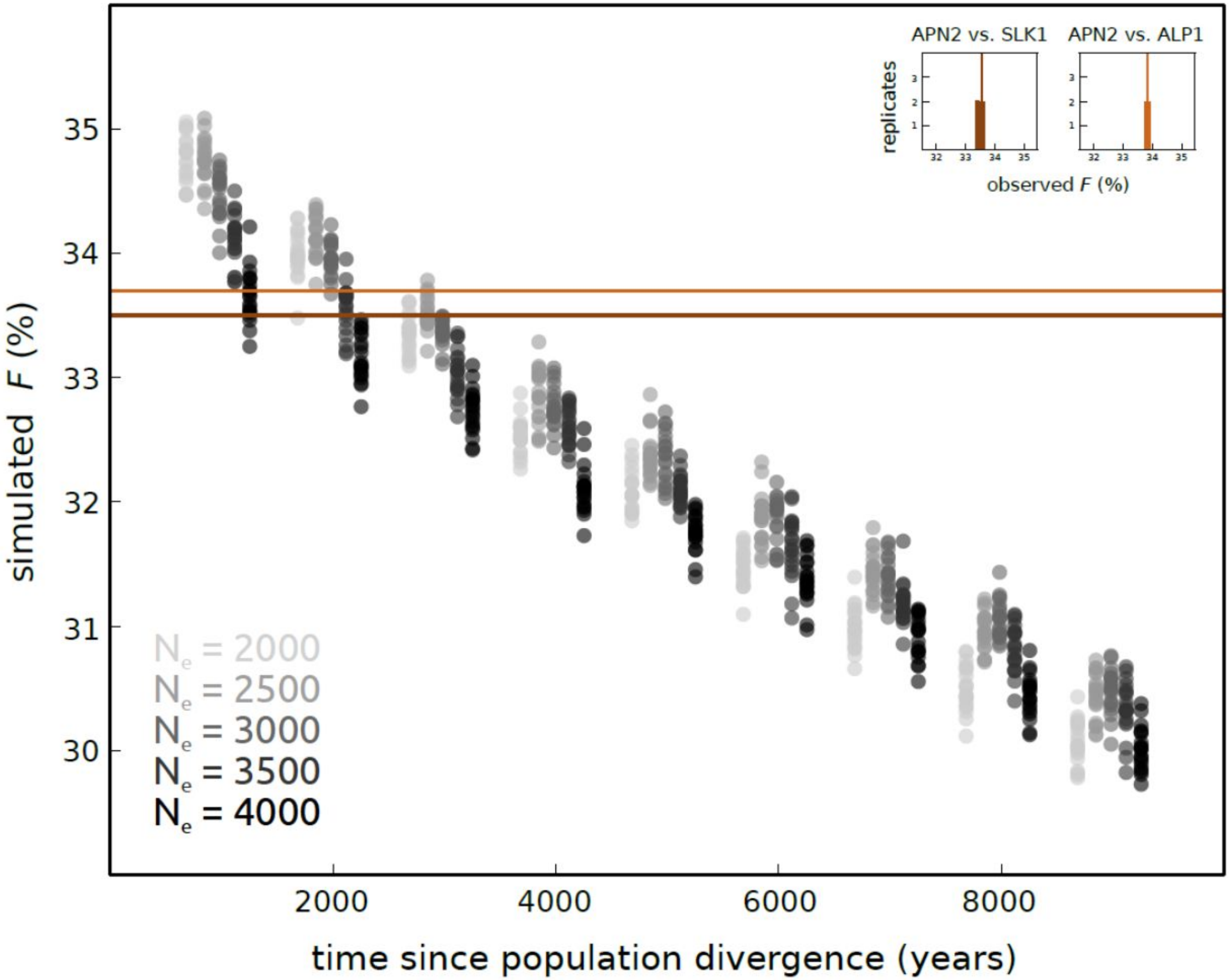
Recent fragmentation

Low connectivity among modern populations



Past demography

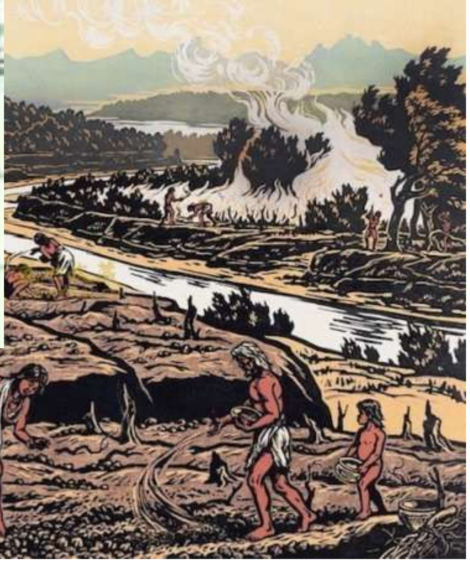
Apennine bears have been isolated since then



F statistic (Green et al 2010), simulation with ms

Past demography

Expansion of Neolithic farming in Europe burning forests



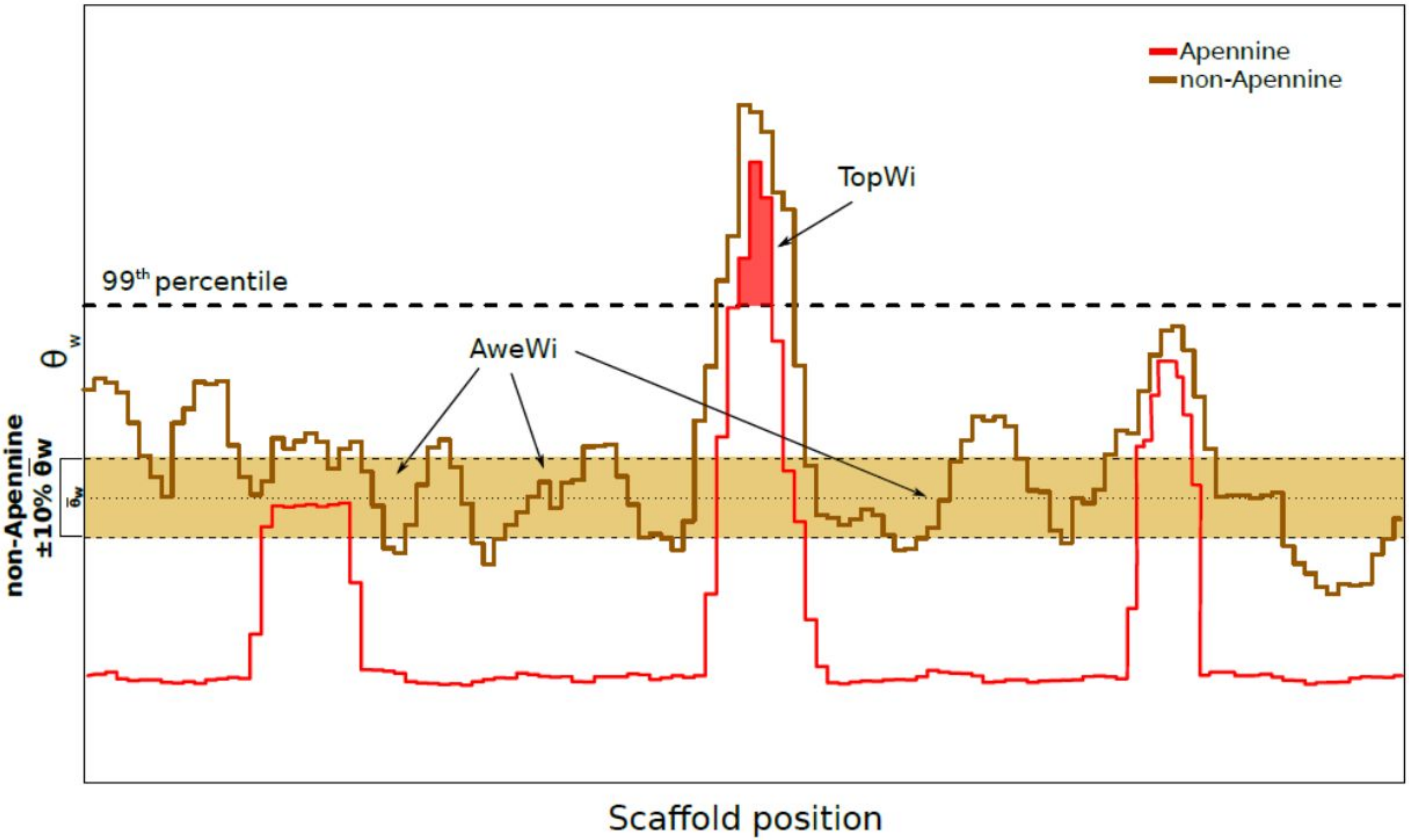
Why is this population still there?

Given its likely high extinction probability

Credits: Fabrizio Caputi

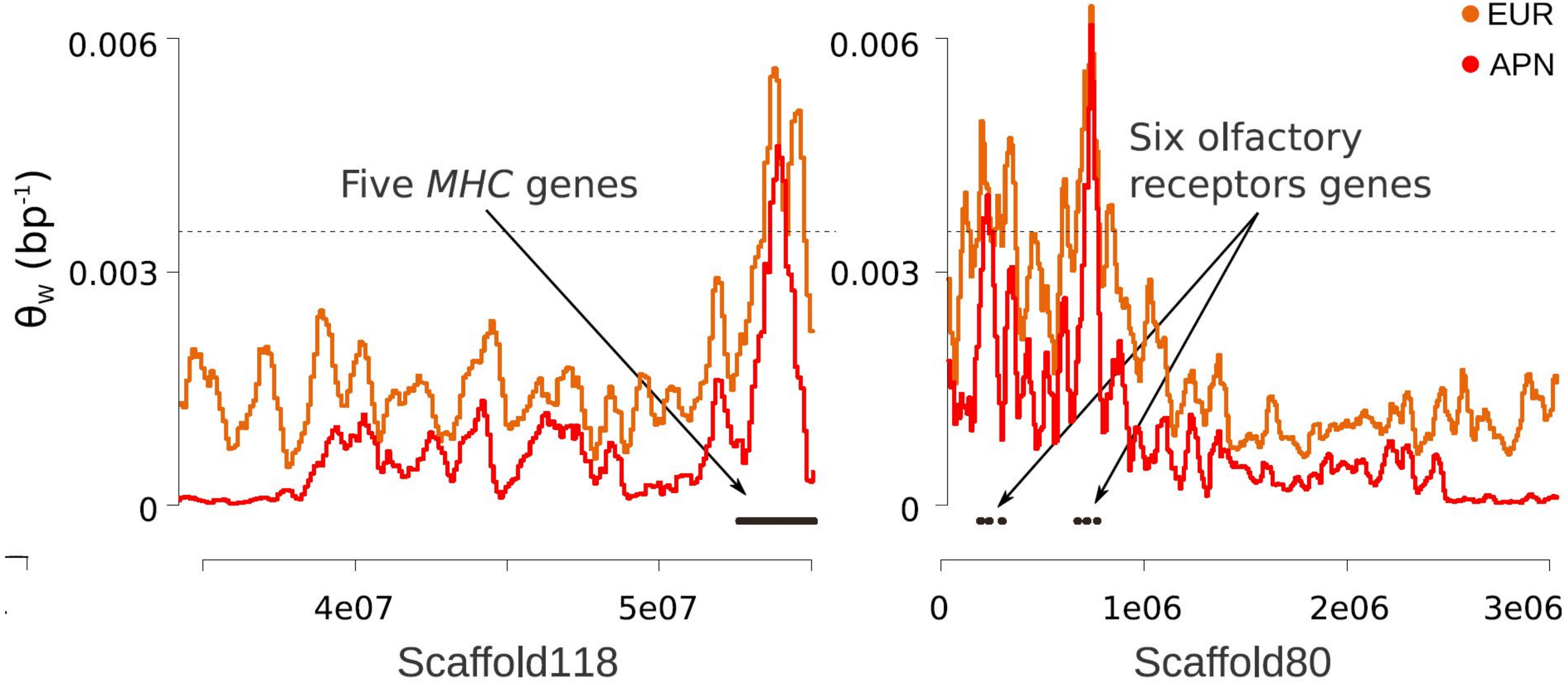
Selective processes: balancing

Retention of high diversity regions (HDR)



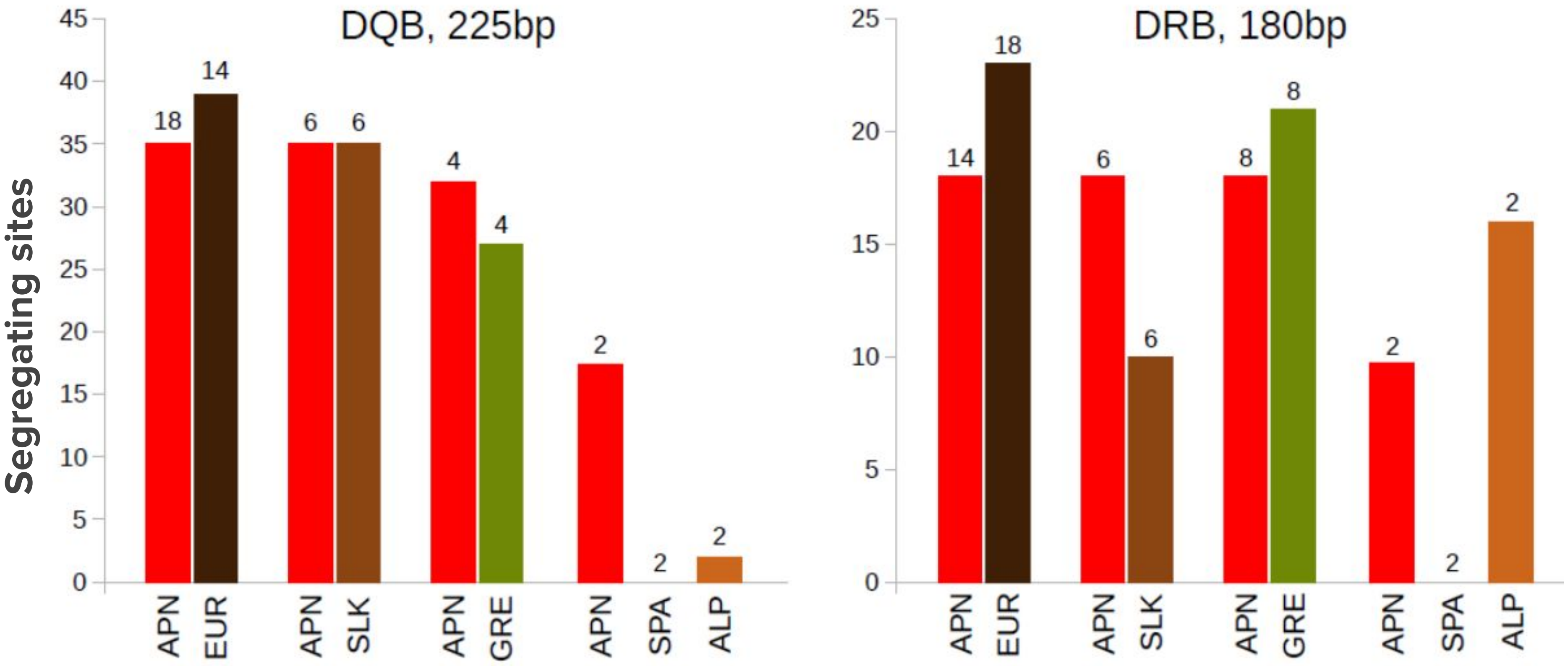
Selective processes: balancing

HDR are enriched in immune and olfactory genes



Selective processes: balancing

MHC genes are as diverse as in the rest of Europe



Sanger-sequencing of two *MHC* class II loci in additional individuals from Apennine and the rest of Europe

Genetic load

Accumulation of deleterious effects

Predicted deleterious substitution fixed in the Apennine bear genomes (by Panther + Polyphen):
40 + 4 stop codon

None in the other European bears!

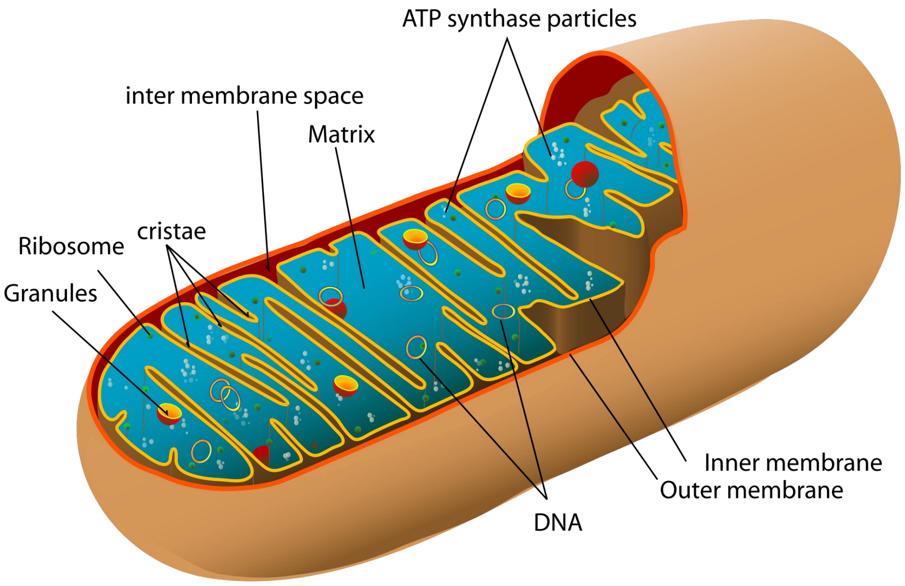
Genetic load

Accumulation of deleterious effects

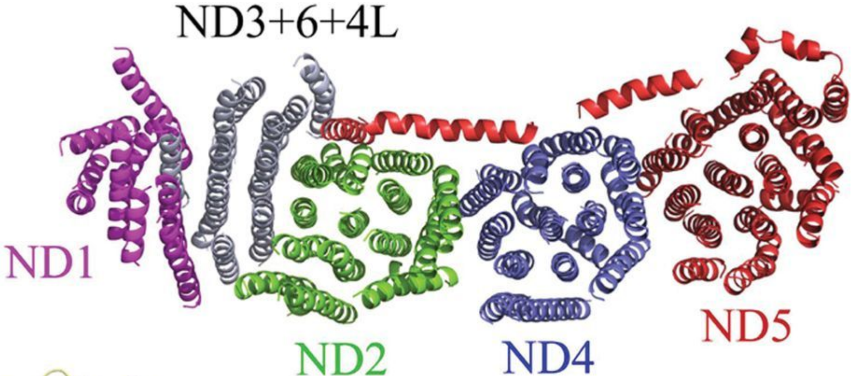
Predicted deleterious substitution fixed in the Apennine bear genomes (by Panther + Polyphen):
40 + 4 stop codon

None in the other European bears!

Five subs in the **mt ND5** of which the most deleterious one is not found elsewhere



Gene	Position	#APNs (out of 6)	#NonAPNs (out of 45)	Score
ND5	G526E	6	0	-4.28
ND5	P447S	6	5	-3.59
ND5	T555A	6	3	-2.52

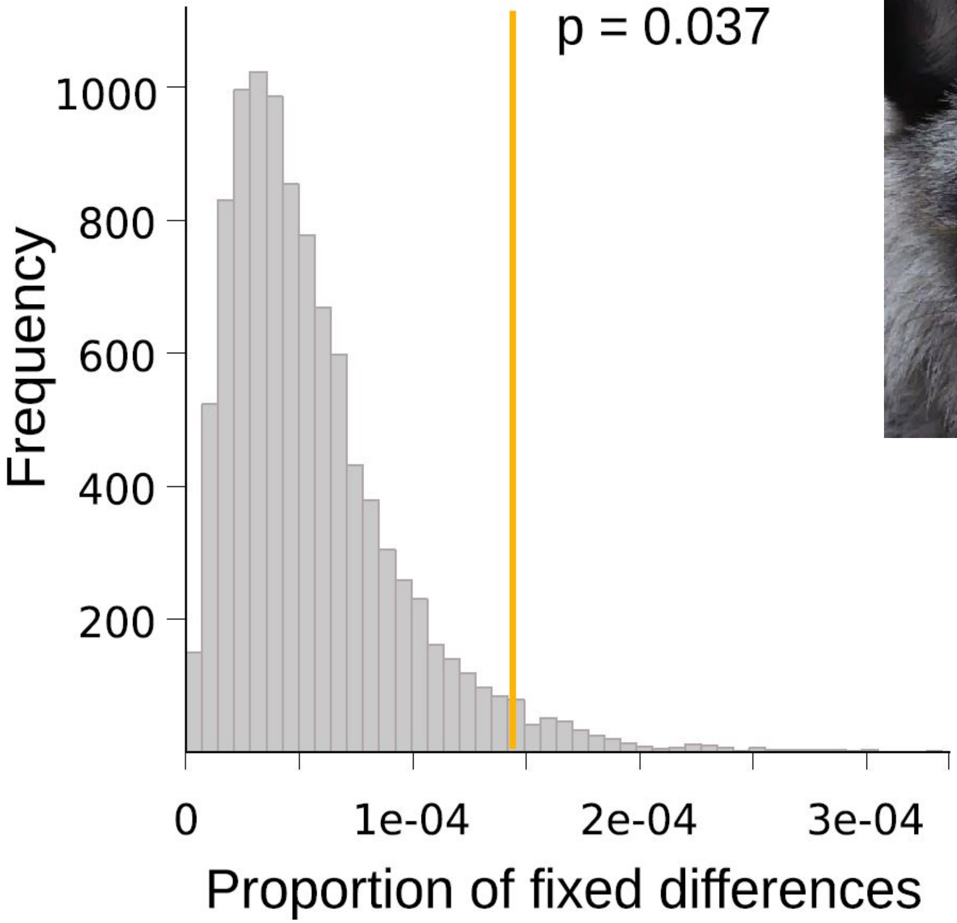


Genetic load in specific group of genes

Are Apennine bears less aggressive?

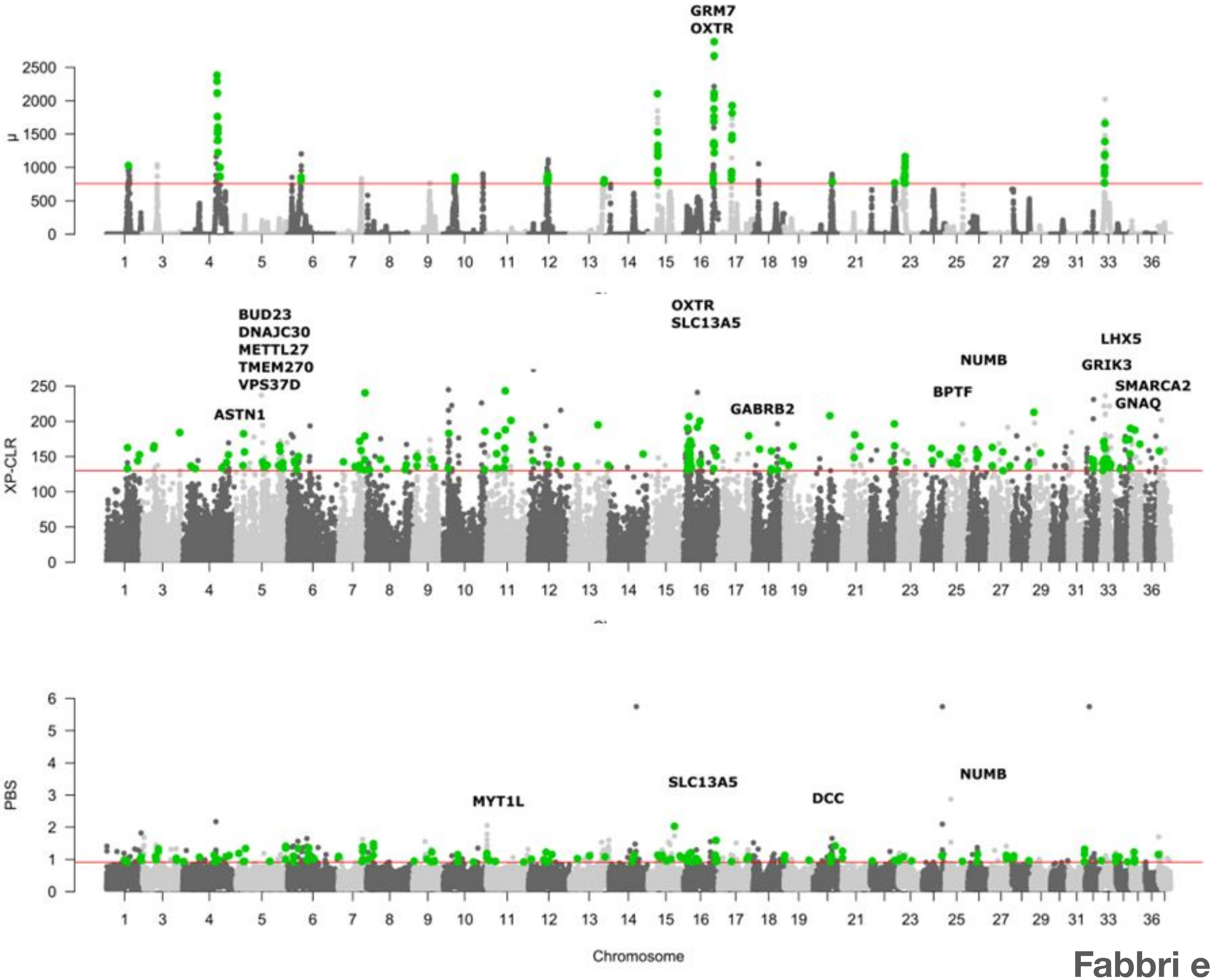
Fixed differences in 22 "tameness" genes

Not all deleterious fixations come to harm



Pattern confirmed with **additional individuals** in three of these genes: PLAXNB1, DCC, DLL3

FURTHER EVIDENCE OF DIVERGENCE AT BEHAVIORAL GENES WITH MORE RECENT DATA AND ANALYSES



Fabbri et al *in prep*

Future conservation strategies

Trade-off between increasing fitness without losing ancestry

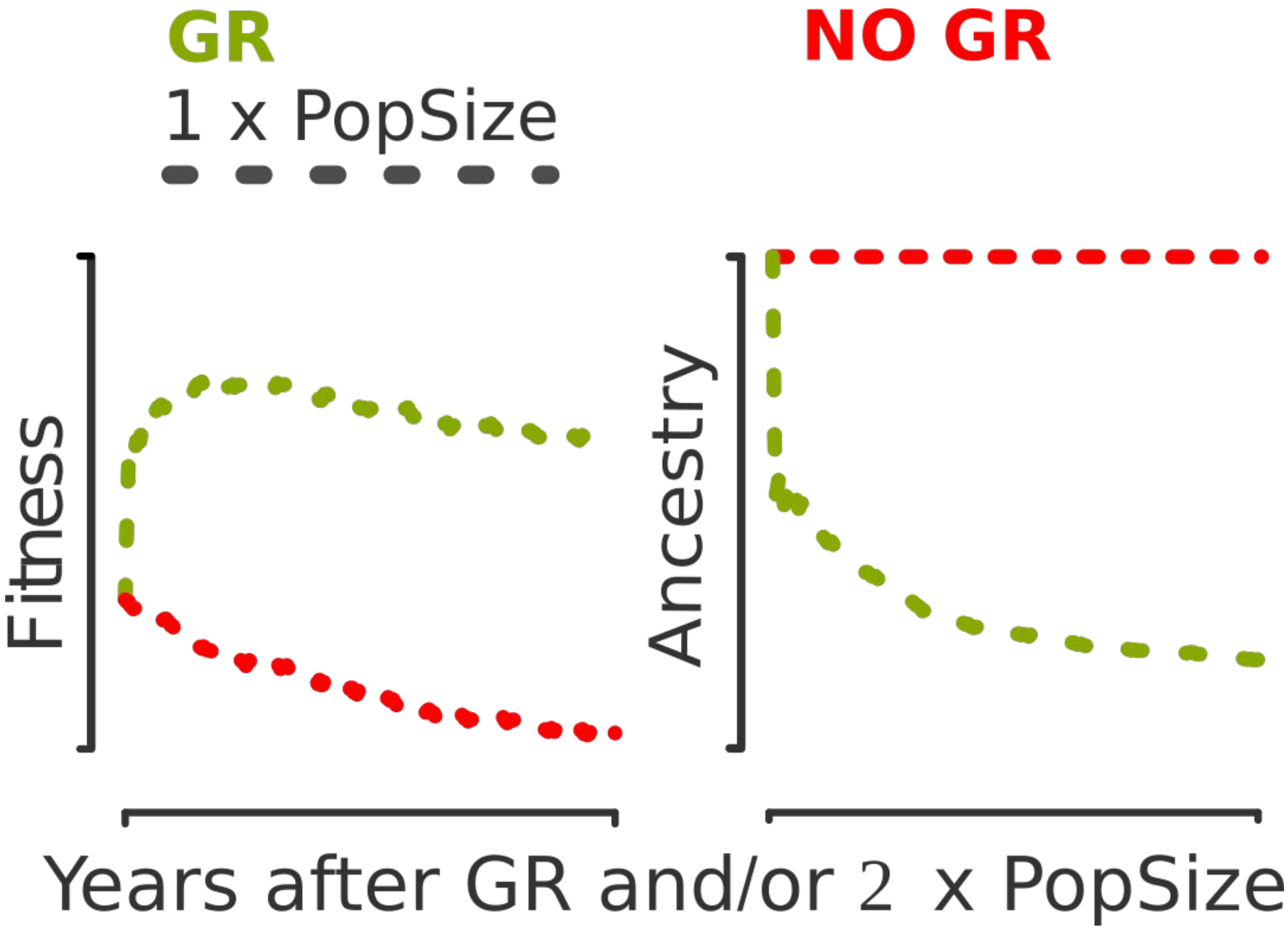
Genetic rescue vs. doubling population size

Realistic forward simulation using Slim

How many individuals? How to choose them? -> 5 with the lowest **genetic load** as proportion of deleterious alleles

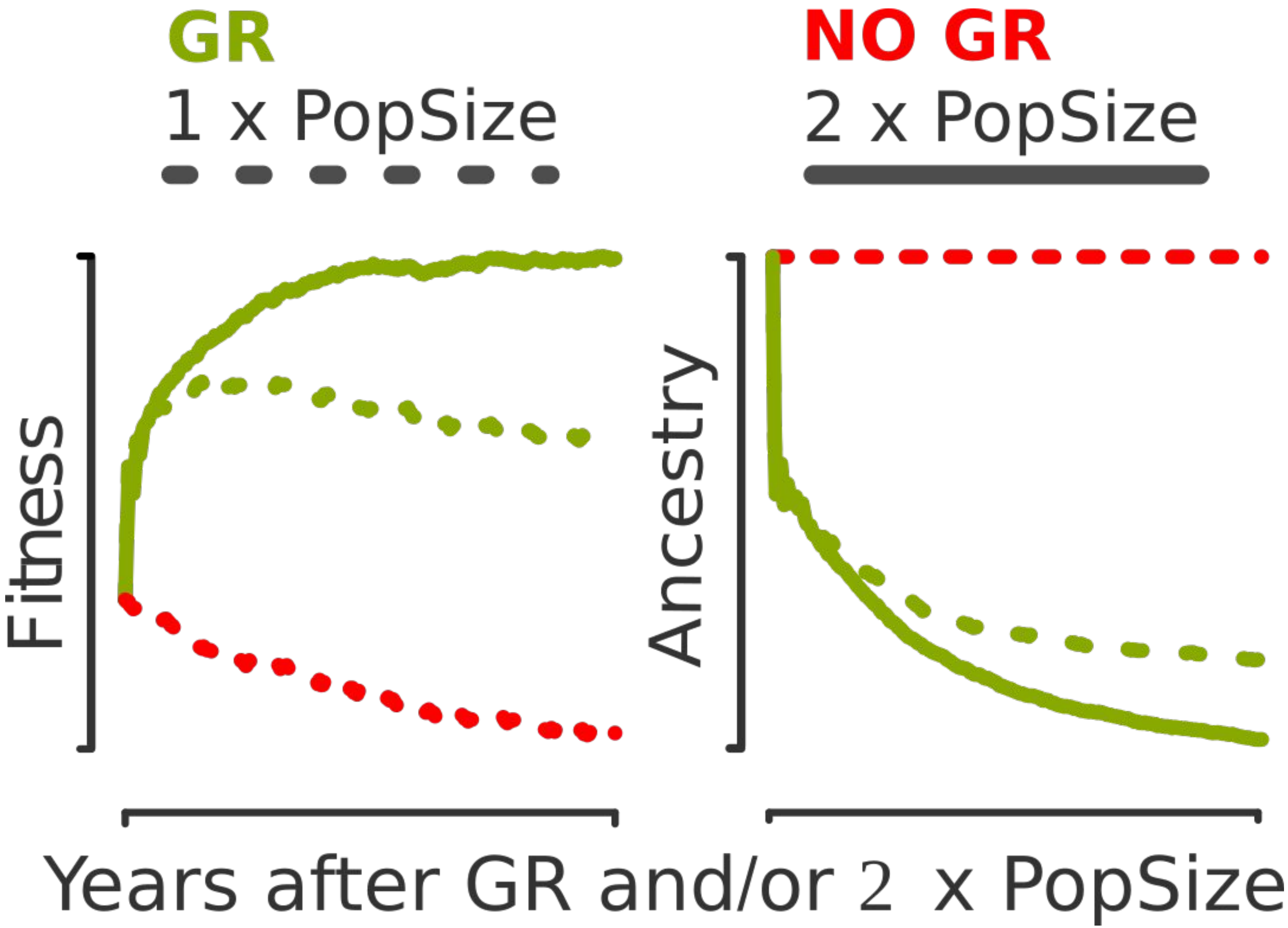
Future conservation strategies

Trade-off between increasing fitness without losing ancestry



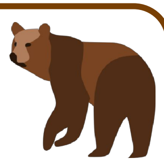
Future conservation strategies

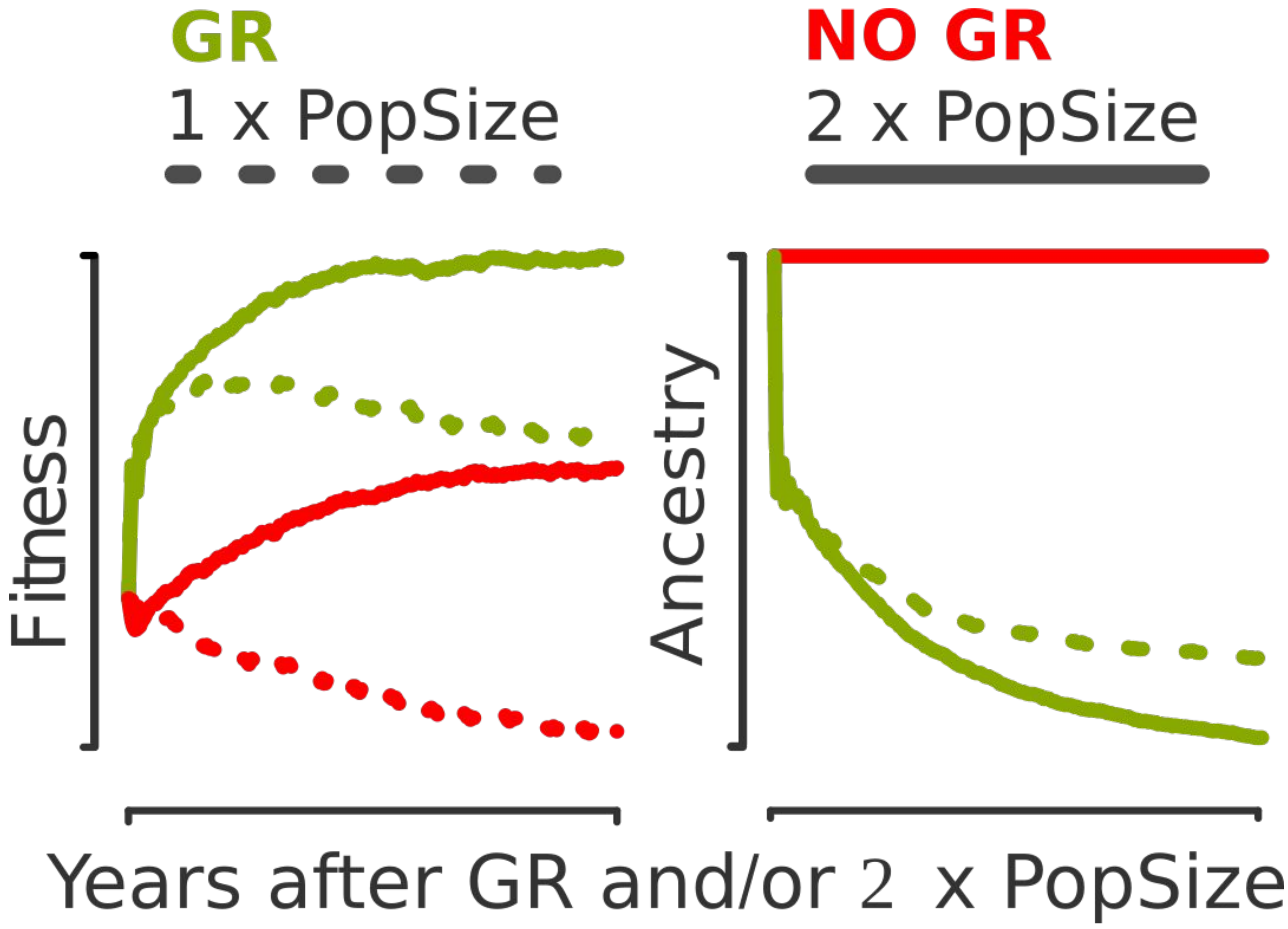
Trade-off between increasing fitness without losing ancestry



Future conservation strategies

Trade-off between increasing fitness without losing ancestry





GENOMICS OF FIVE ENDEMIC SPECIES

Very to very small population size

Marsican bear
(2.2Gb)

Adriatic sturgeon
(1.4Gb, tetraploid)

Ponza grayling
(0.4Mb)

Aeolian lizard
(1.4Gb)

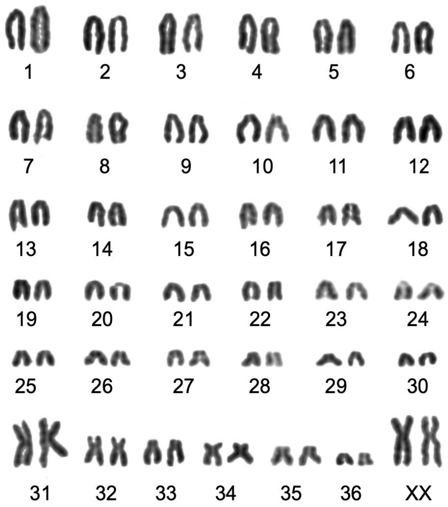
Apennine yellow-bellied toad (10Gb)

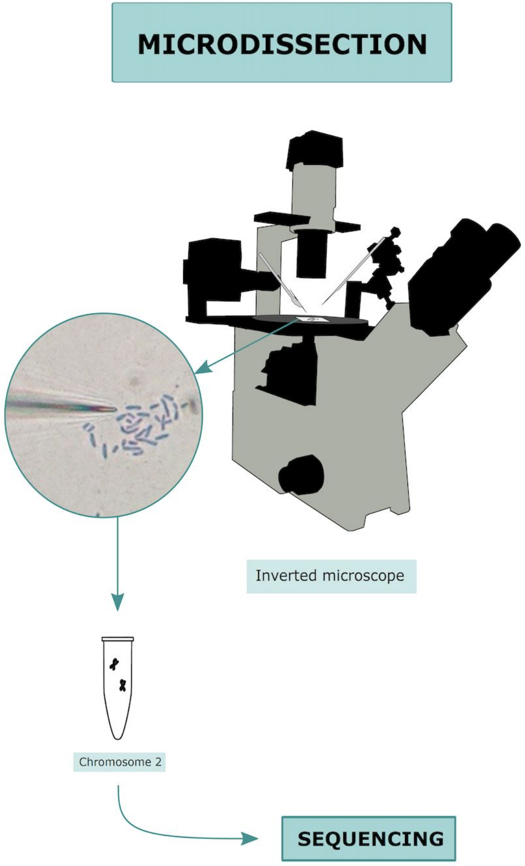
SAMPLING FOR GENOME ASSEMBLY AND RESEQUENCING

Small and large populations to compare

KARYOTYPE AND CHROMOSOME SORTING

The toad has few but huge chromosomes





MICRODISSECTION

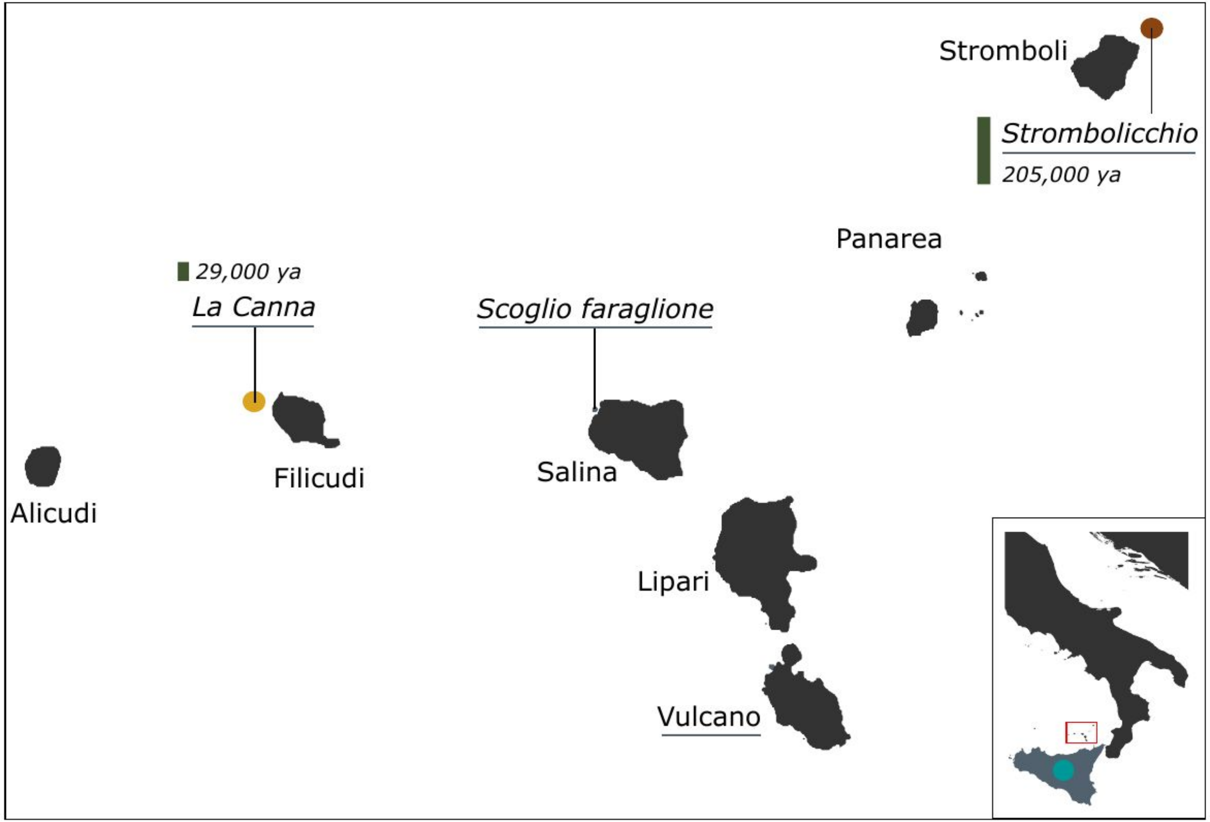
Inverted microscope

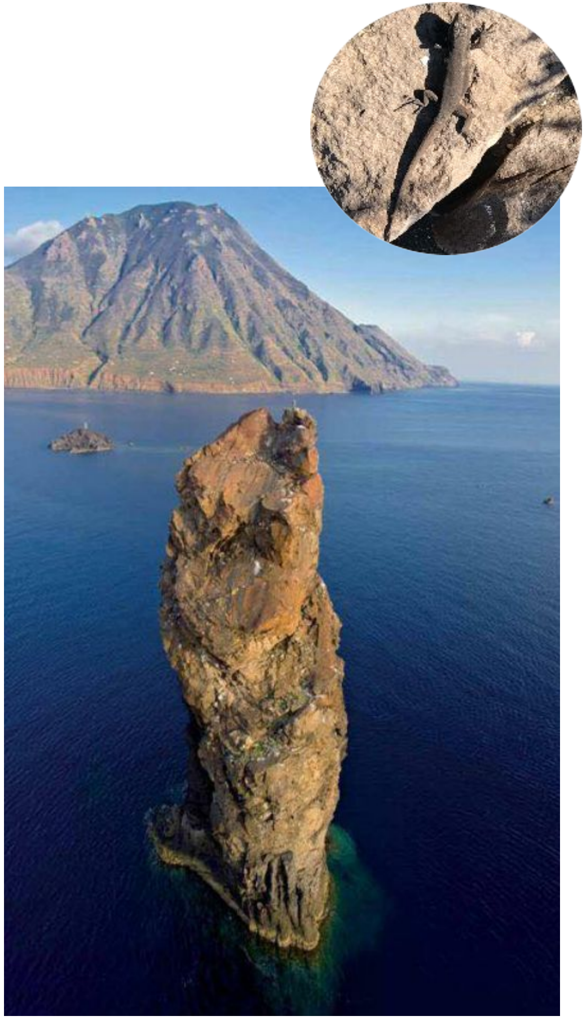
Chromosome 2

SEQUENCING

LIVING ON A ROCK AS A WILD INBRED STRAIN

Setting the limit for genomic variation and genetic load





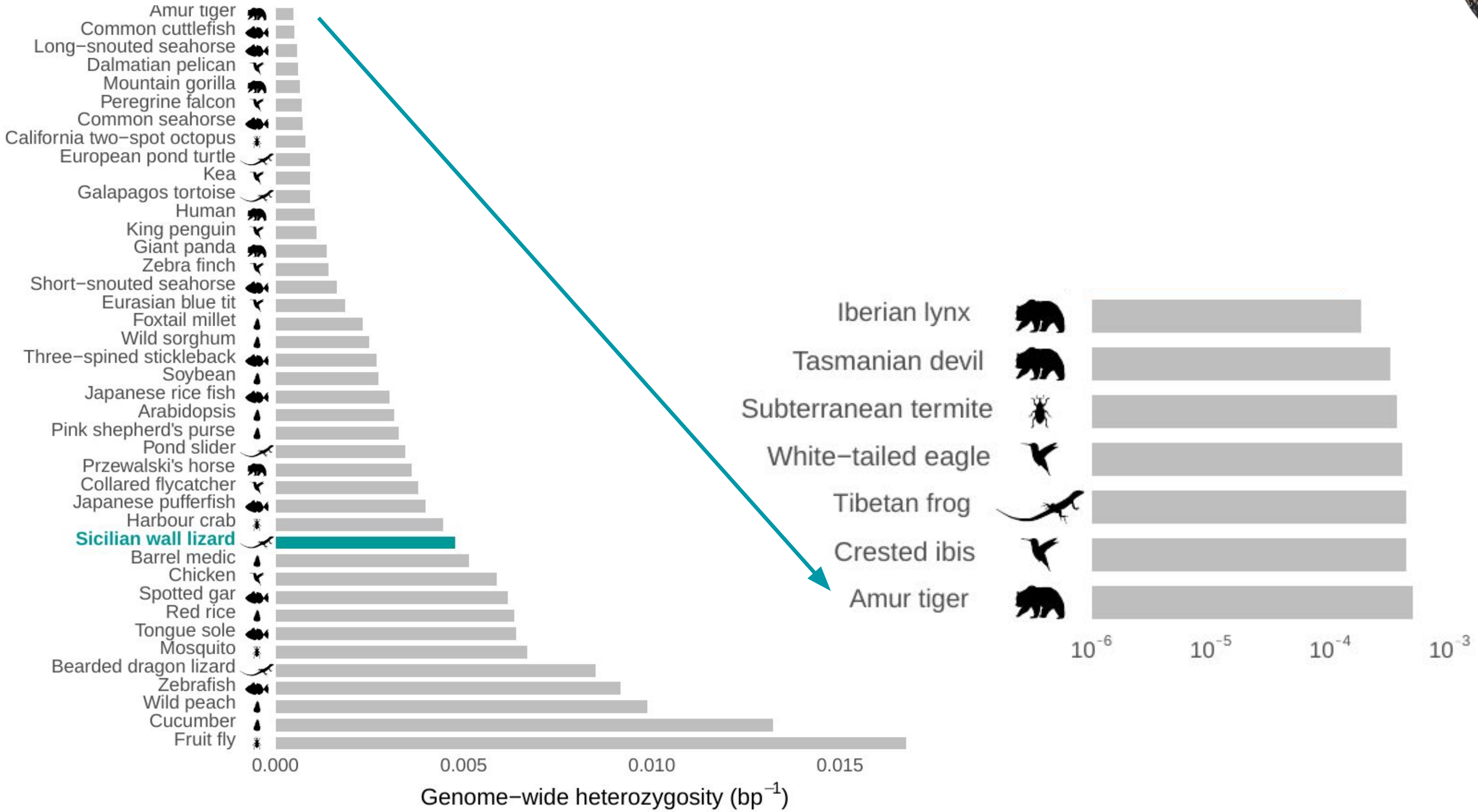
LIVING ON A ROCK AS A WILD INBRED STRAIN

Setting the limit for genomic variation and genetic load

From Sicily

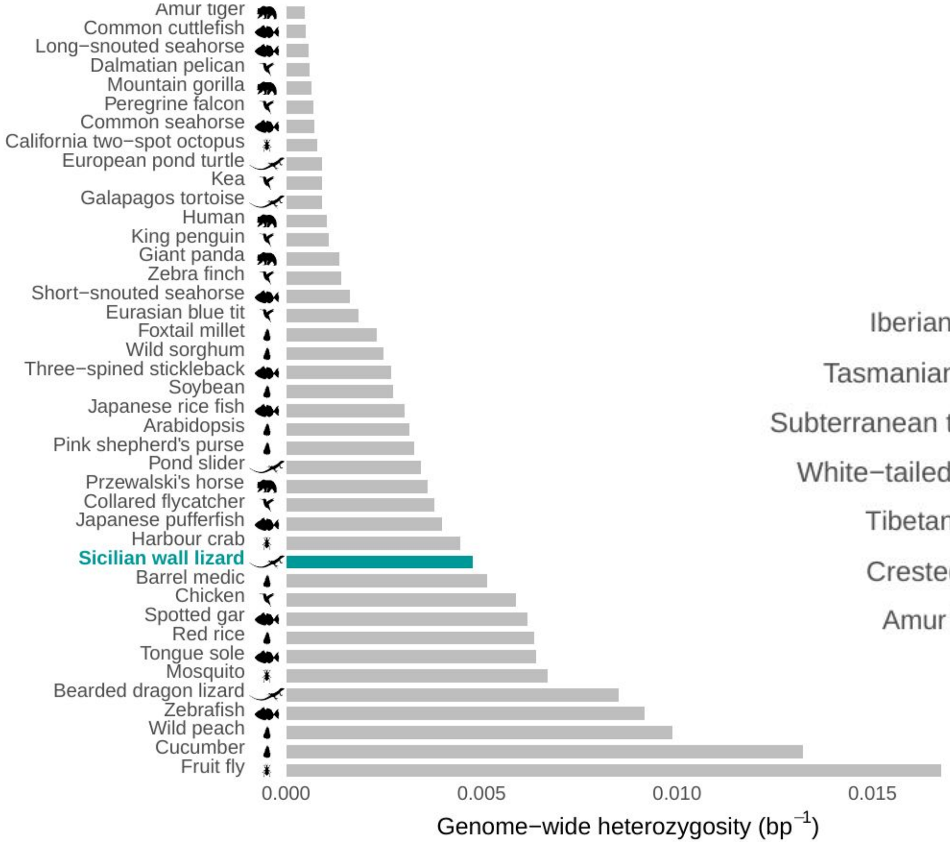
LIVING ON A ROCK AS A WILD INBRED STRAIN

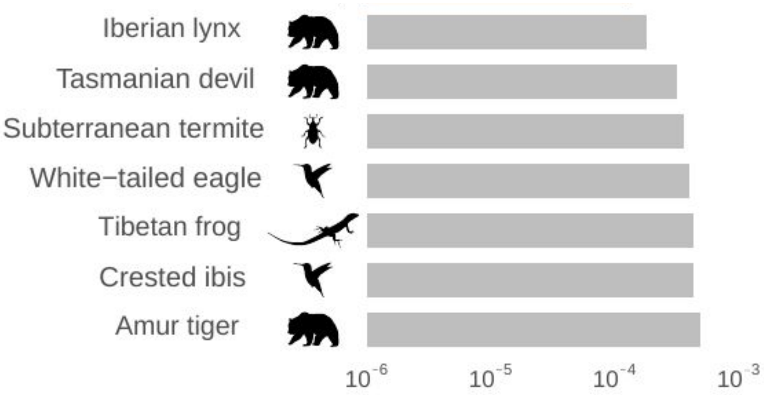
Setting the limit for genomic variation and genetic load



LIVING ON A ROCK AS A WILD INBRED STRAIN

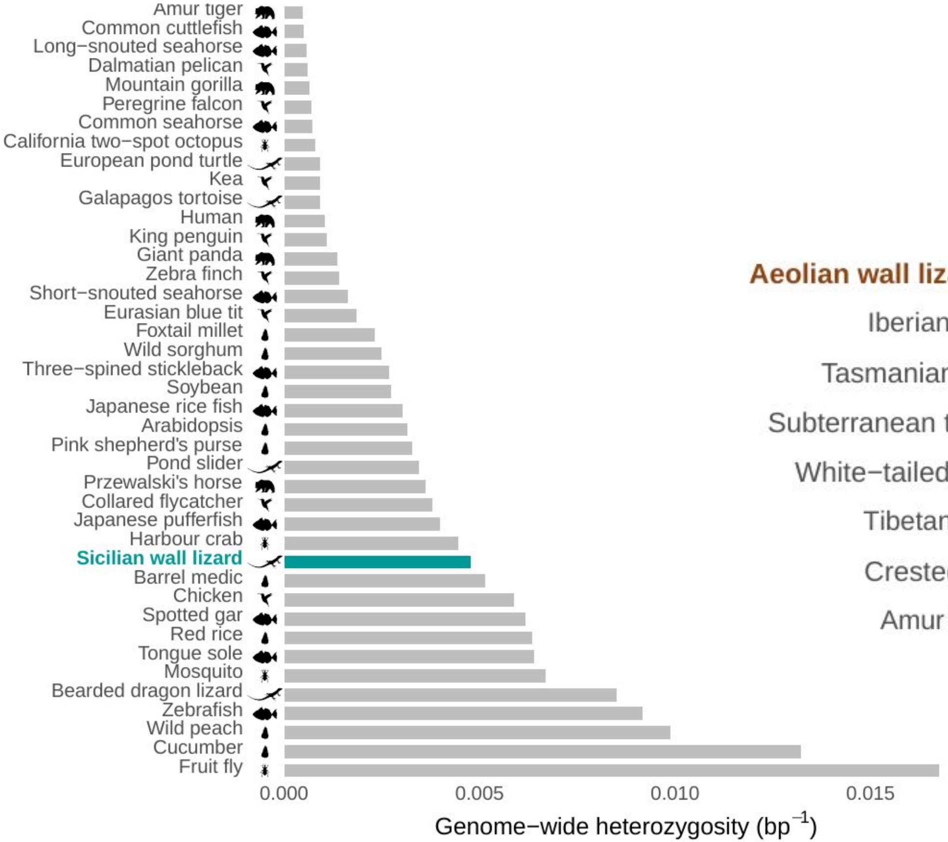
Setting the limit for genomic variation and genetic load

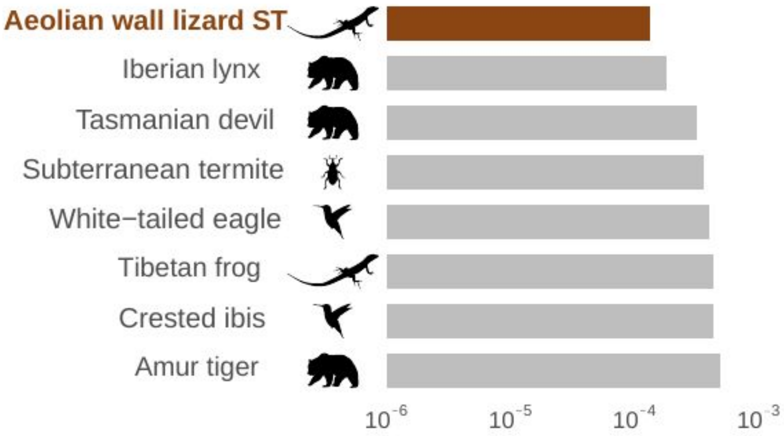




LIVING ON A ROCK AS A WILD INBRED STRAIN

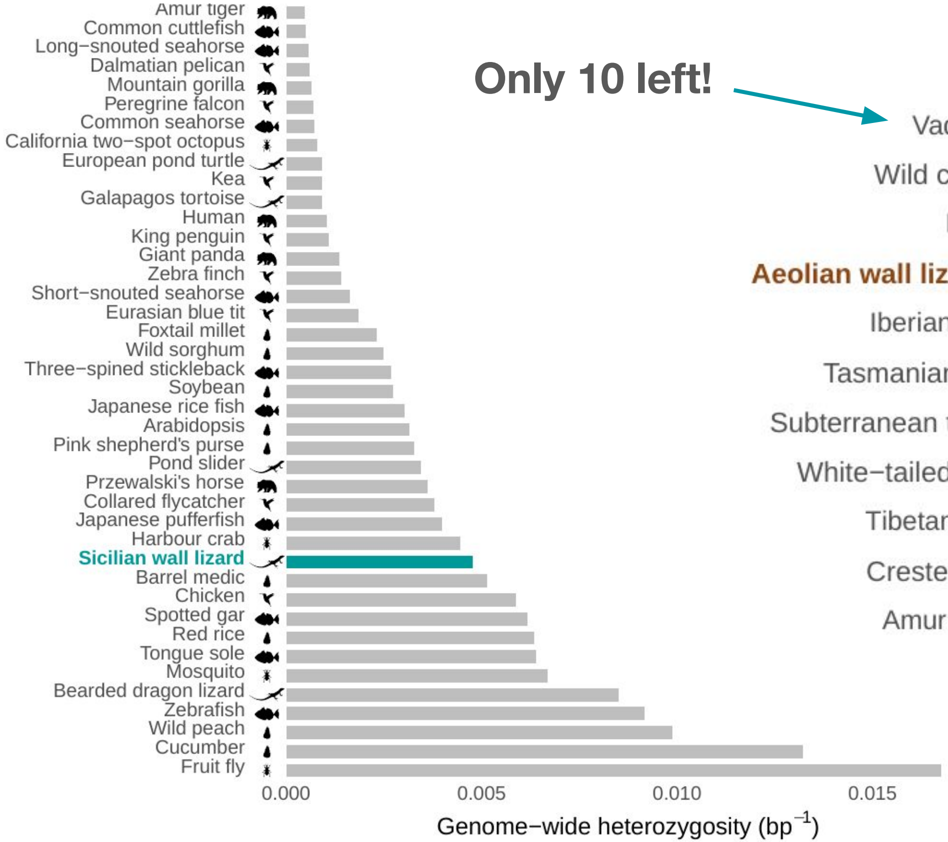
Setting the limit for genomic variation and genetic load



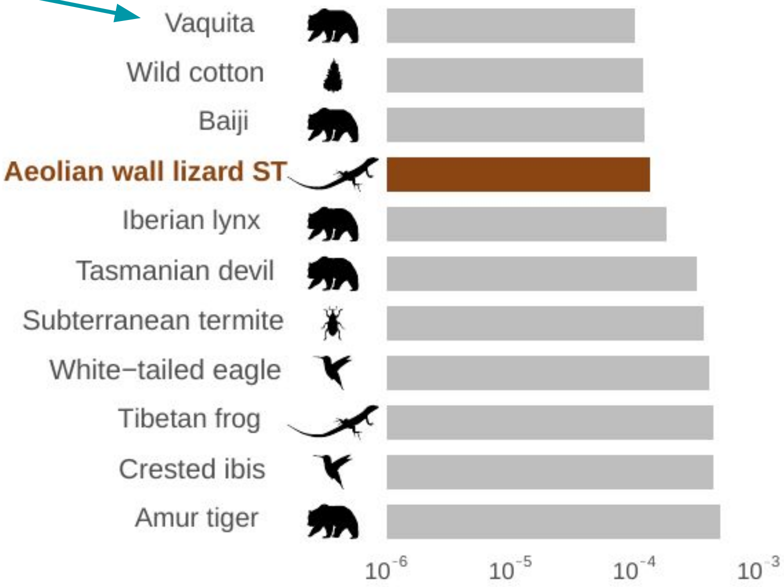


LIVING ON A ROCK AS A WILD INBRED STRAIN

Setting the limit for genomic variation and genetic load



Only 10 left!



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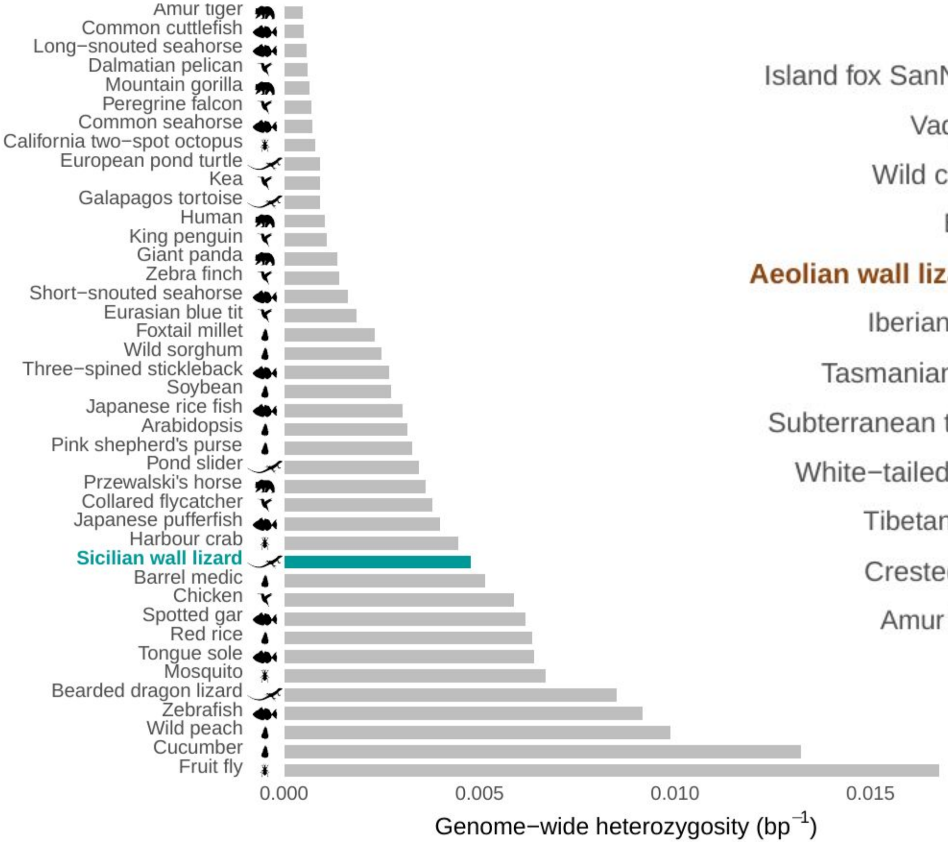
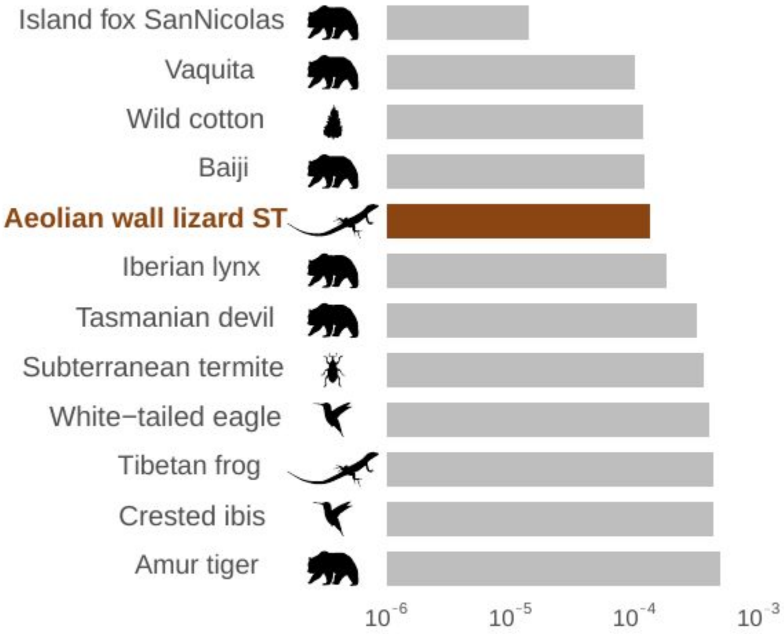
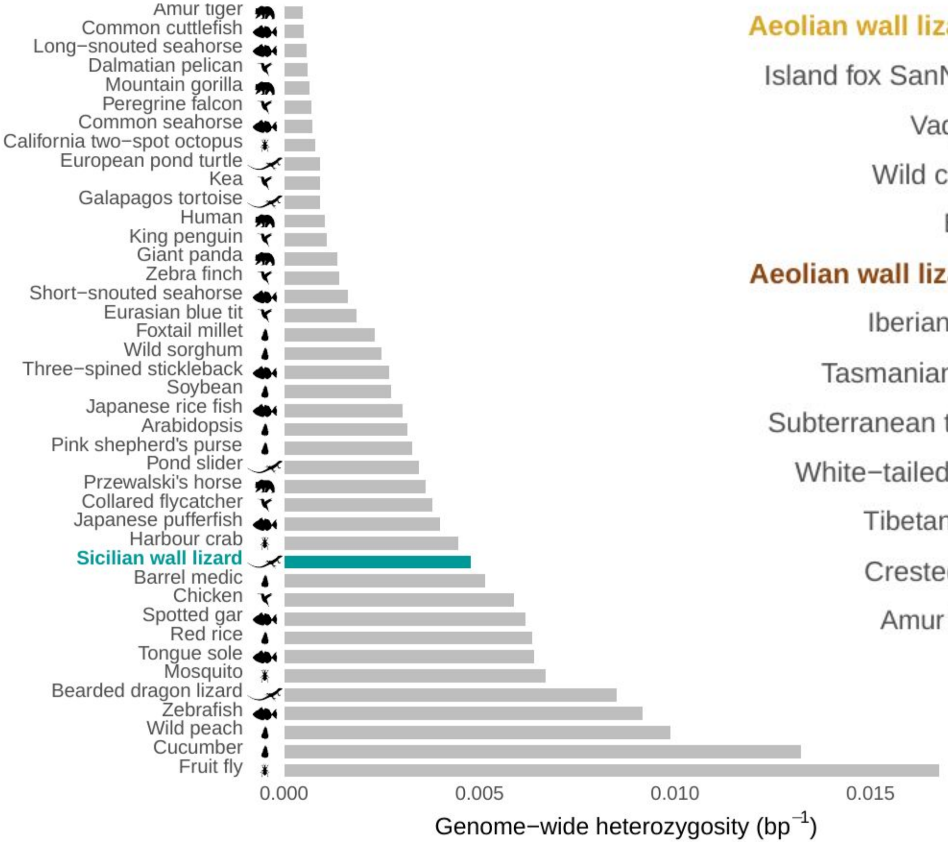
Setting the limit for genomic variation and genetic load

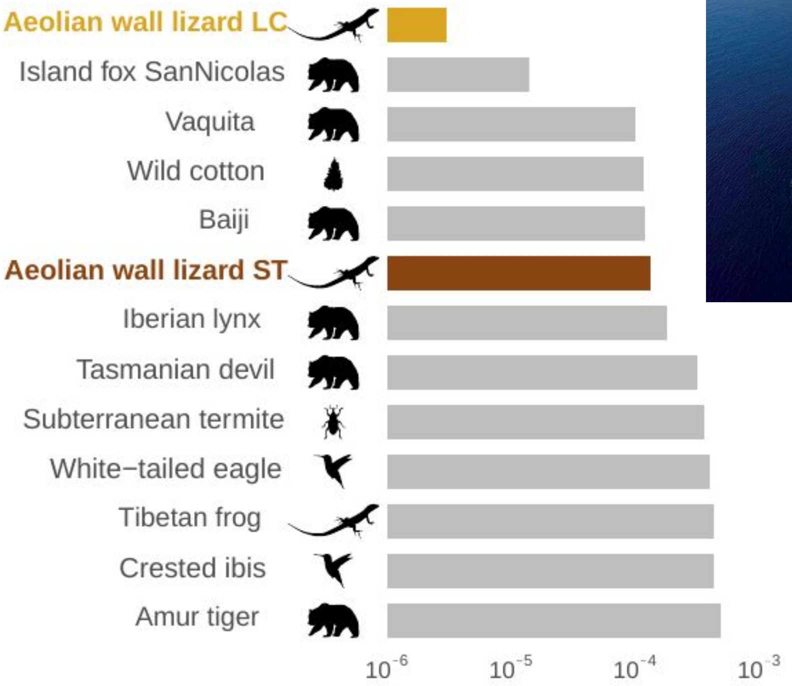


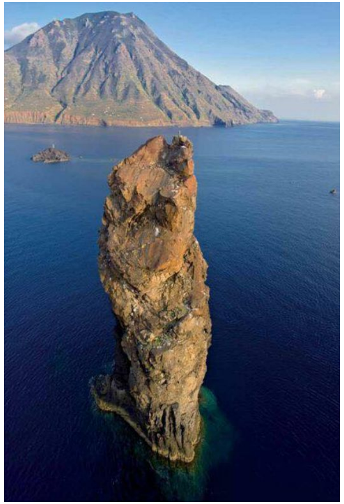


LIVING ON A ROCK AS A WILD INBRED STRAIN

Setting the limit for genomic variation and genetic load







LIVING ON A ROCK AS A WILD INBRED STRAIN

Setting the limit for genomic variation and genetic load

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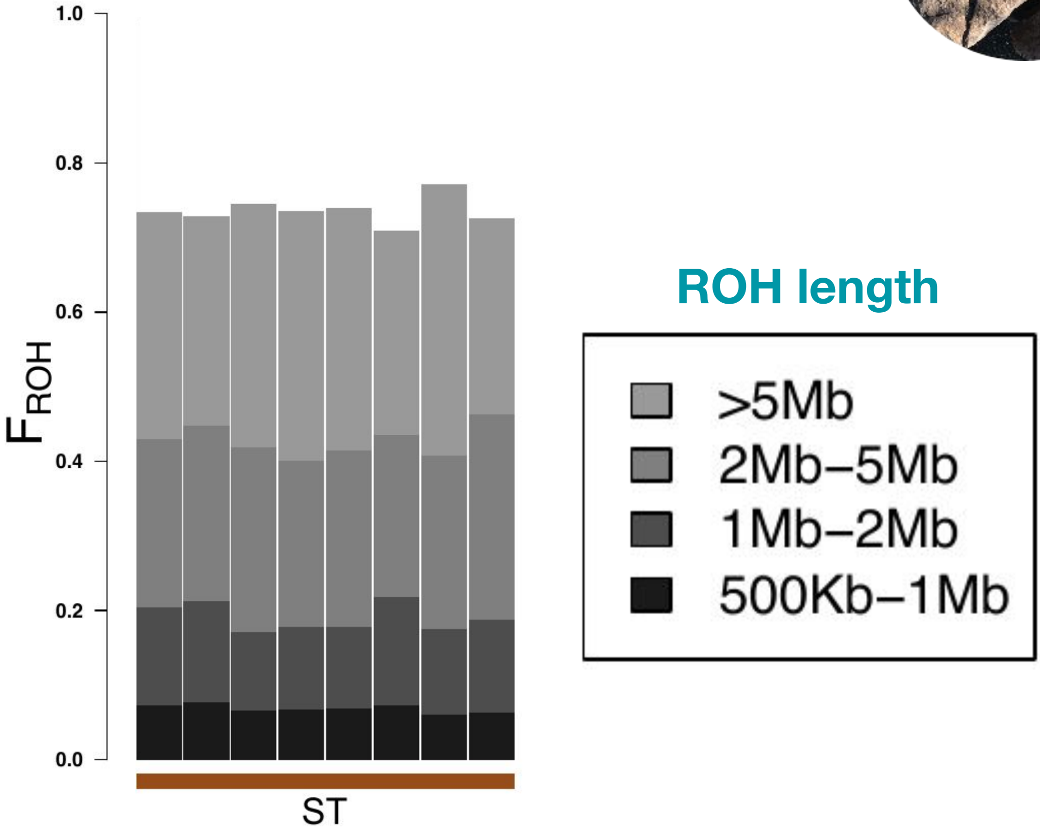

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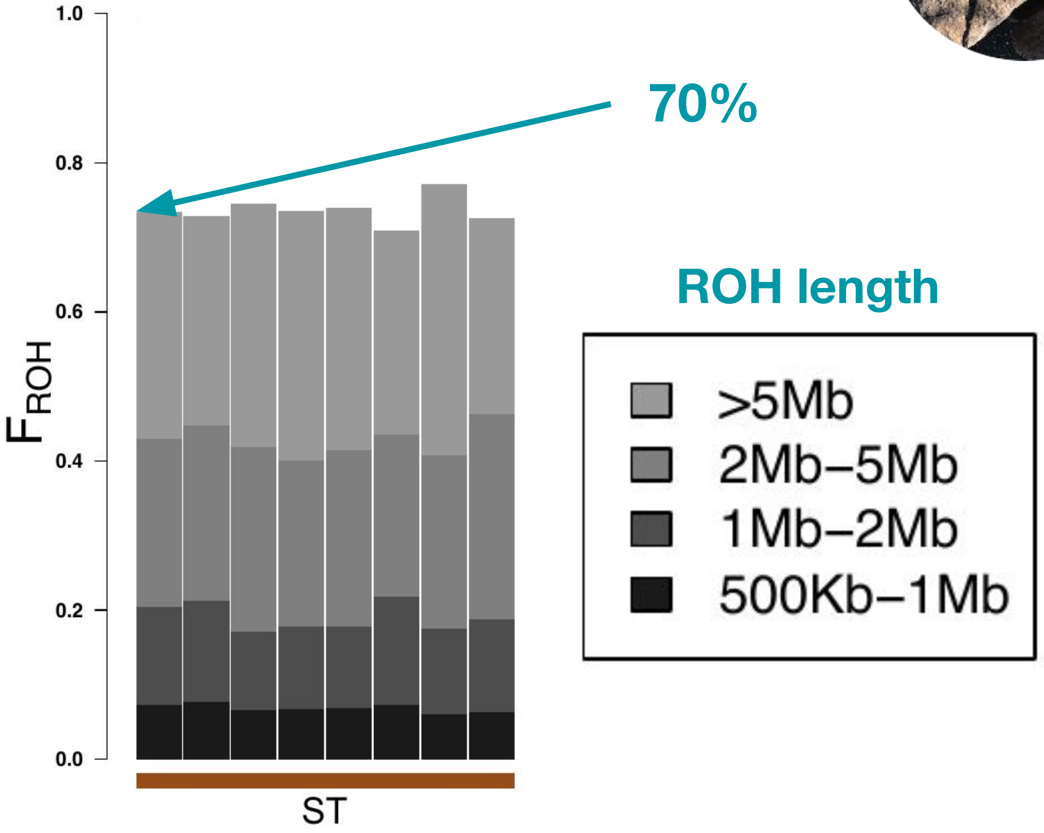
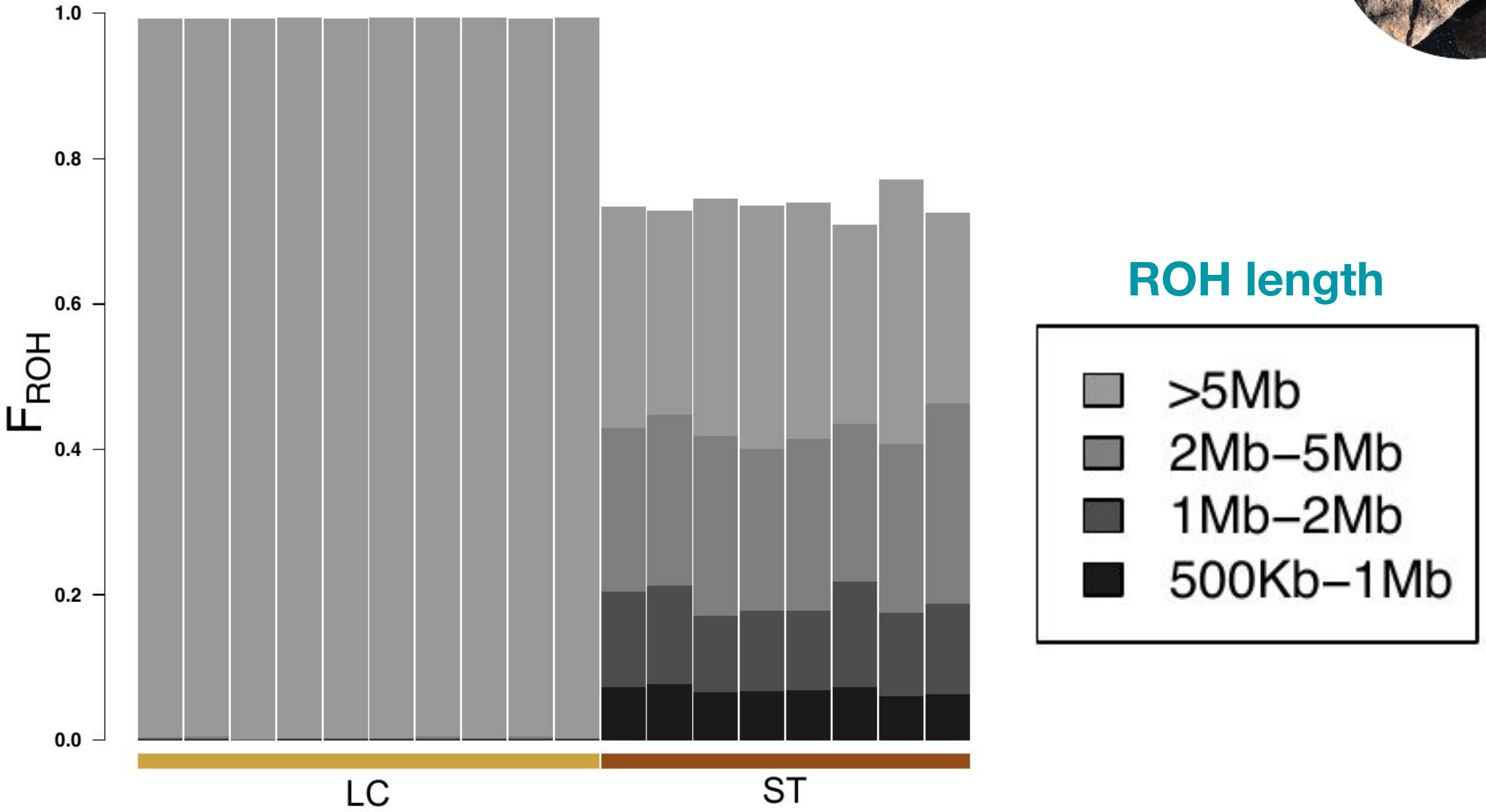


70%

ROH length

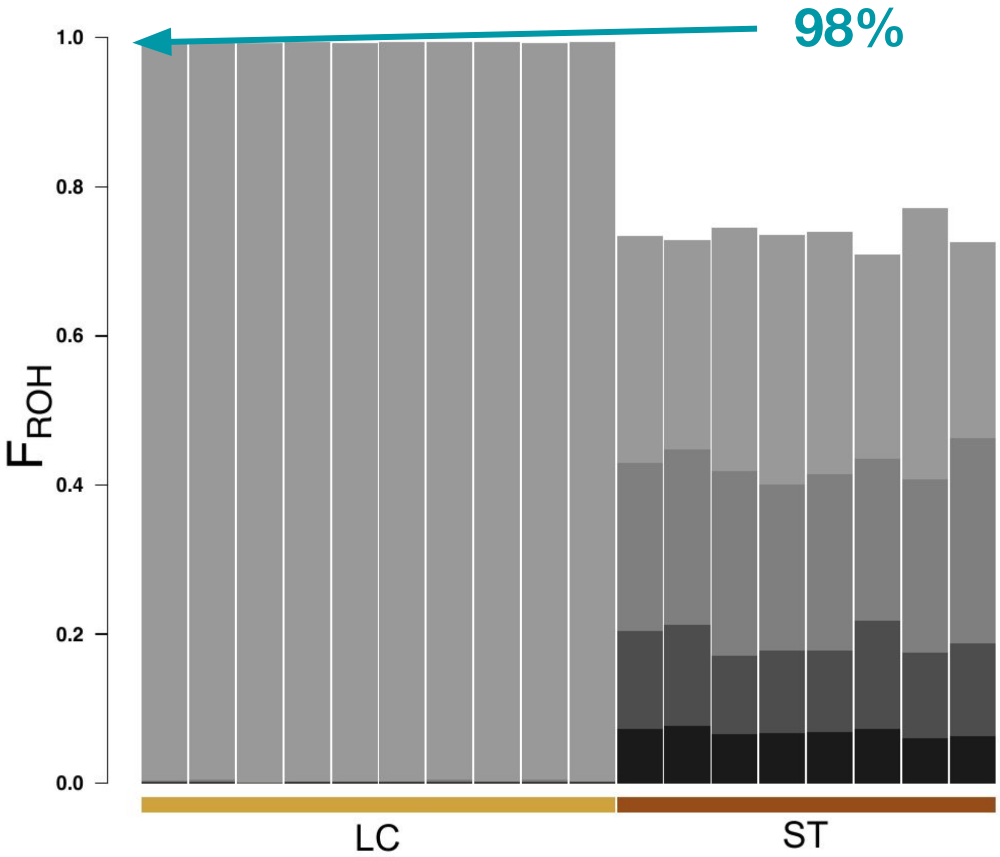
LIVING ON A ROCK AS A WILD INBRED STRAIN

Setting the limit for genomic variation and genetic load



LIVING ON A ROCK AS A WILD INBRED STRAIN

Setting the limit for genomic variation and genetic load

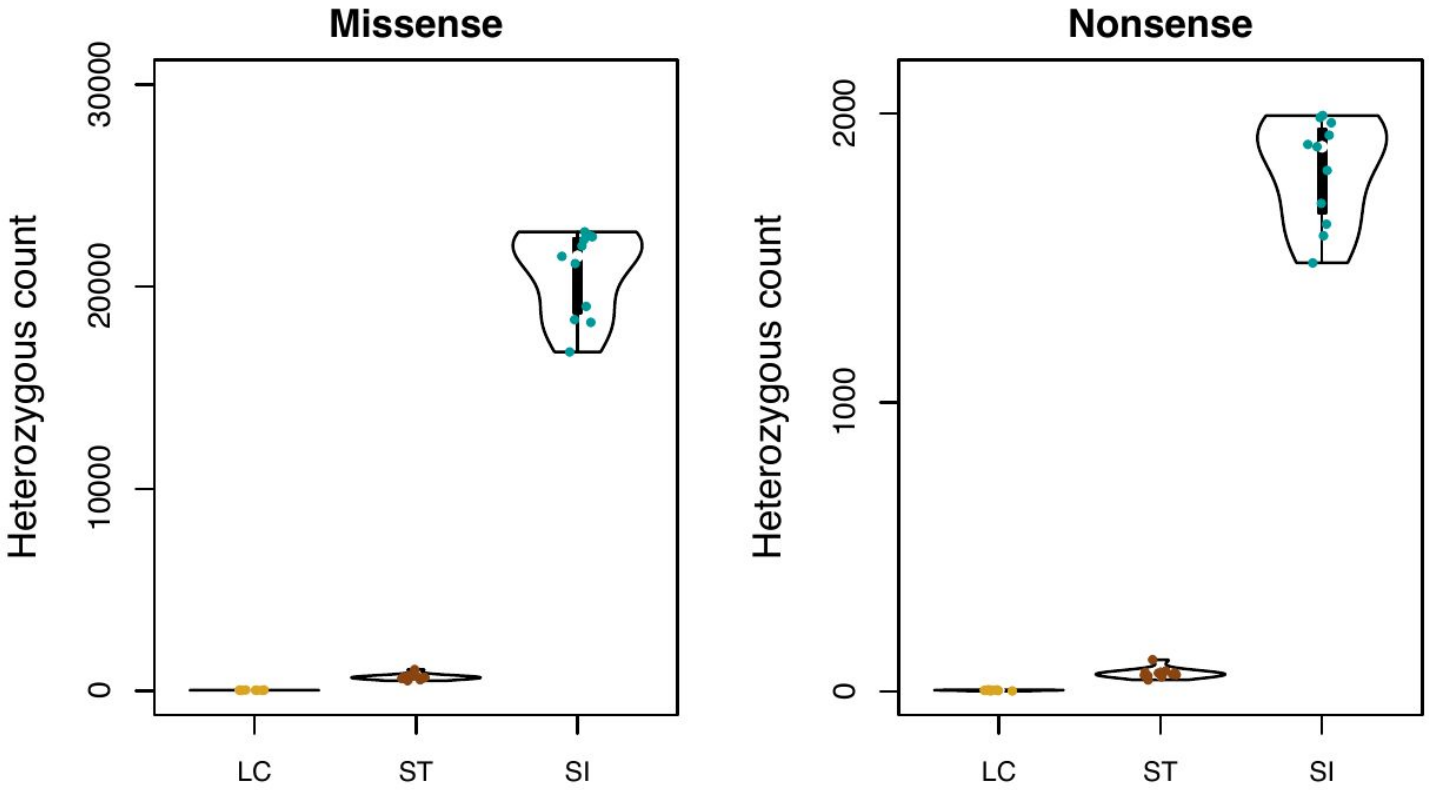


ROH length

- ▨ >5Mb
- ▨ 2Mb–5Mb
- ■ 1Mb–2Mb
- ■ 500Kb–1Mb

LIVING ON A ROCK AS A WILD INBRED STRAIN

Setting the limit for genomic variation and genetic load

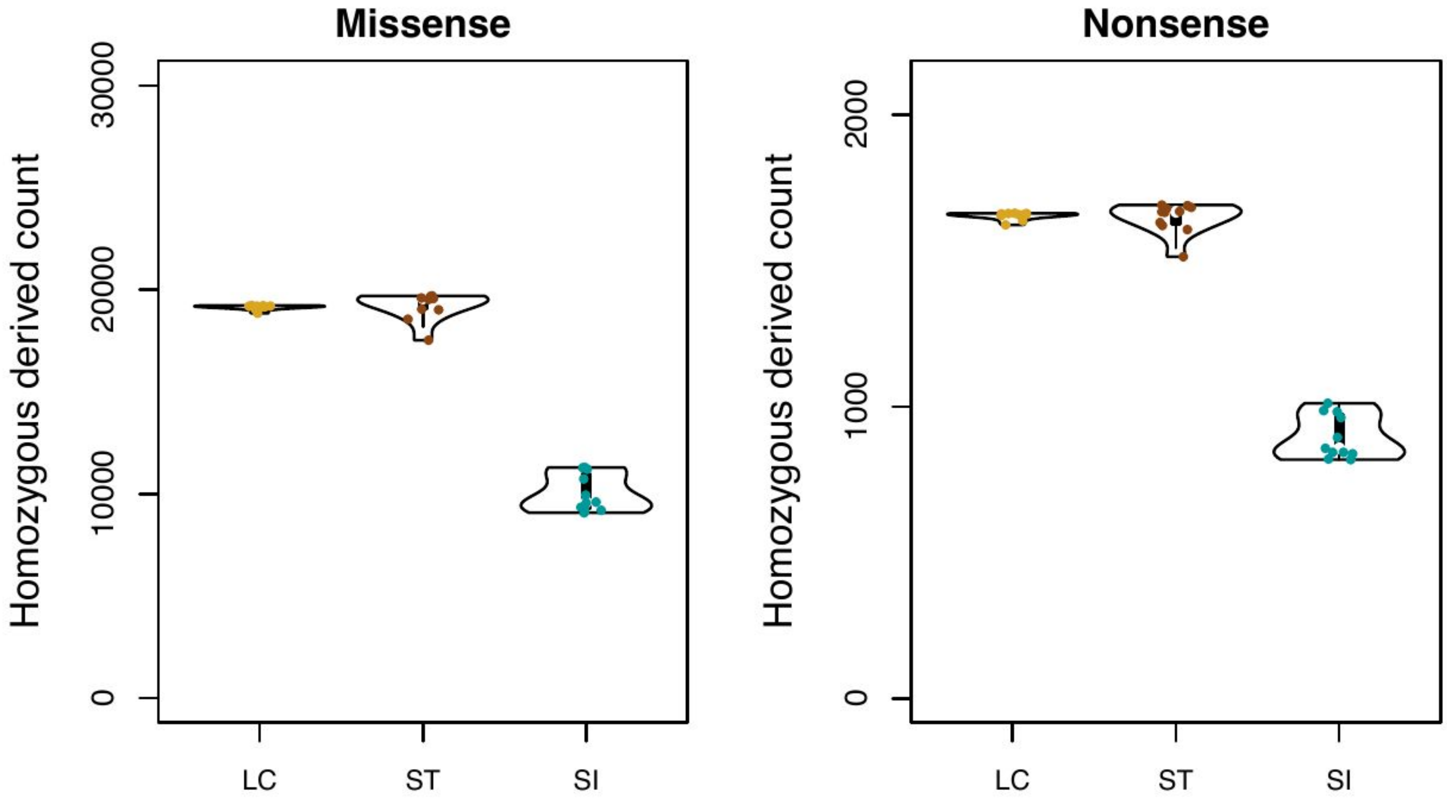


MASKED

GENETIC LOAD

LIVING ON A ROCK AS A WILD INBRED STRAIN

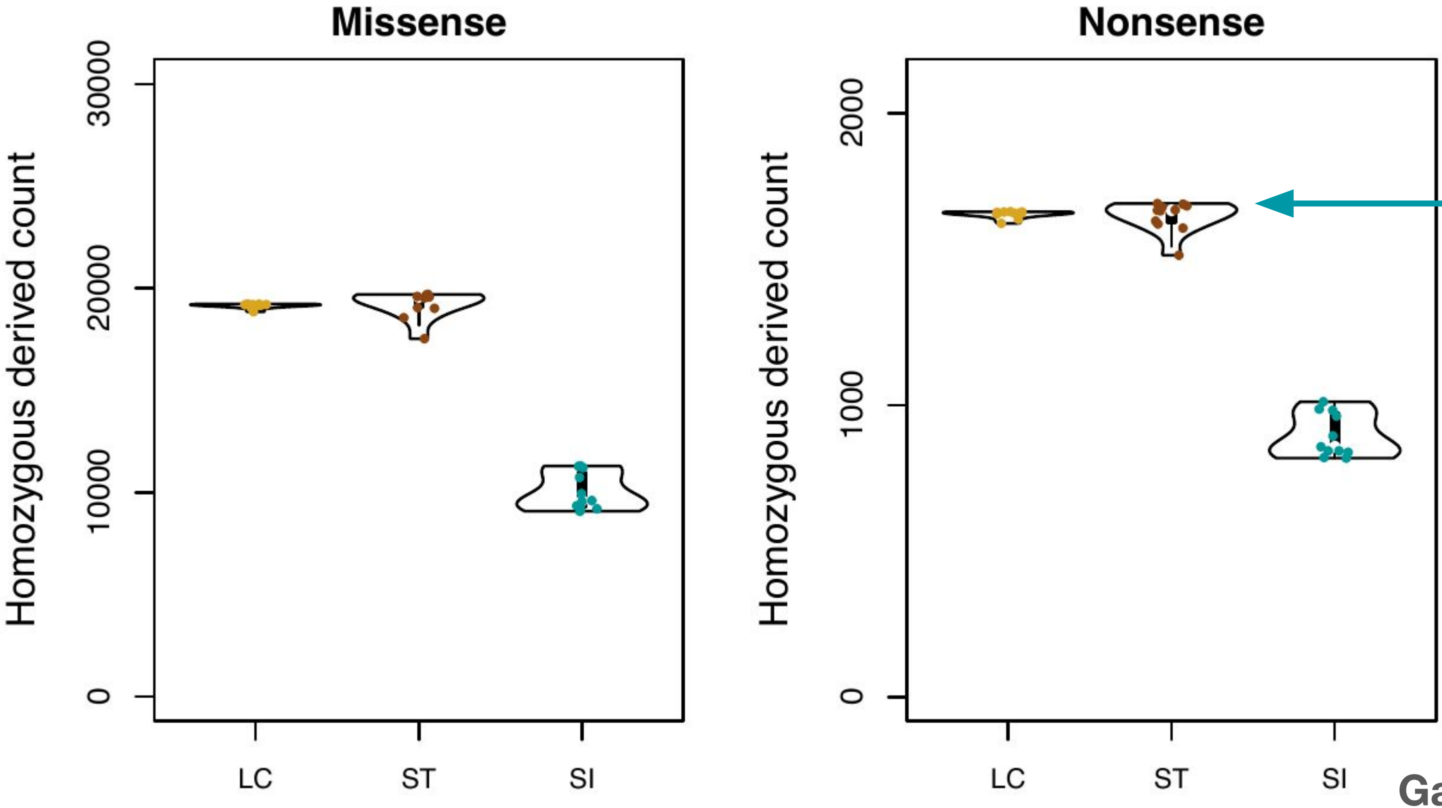
Setting the limit for genomic variation and genetic load



REALIZED
GENETIC LOAD

LIVING ON A ROCK AS A WILD INBRED STRAIN

Setting the limit for genomic variation and genetic load



MAX THRESHOLD?

REALIZED GENETIC LOAD

Gabrielli et al *submitte*

TESTING PREDICTIONS OF DELETERIOUSNESS

Impaired bioenergetics in the Apennine brown bear

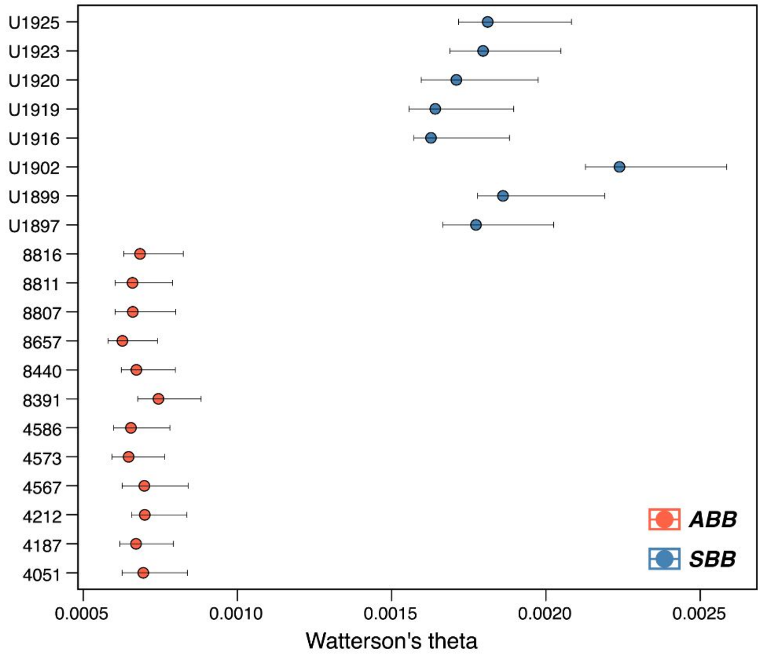
Slovakian

Apennine

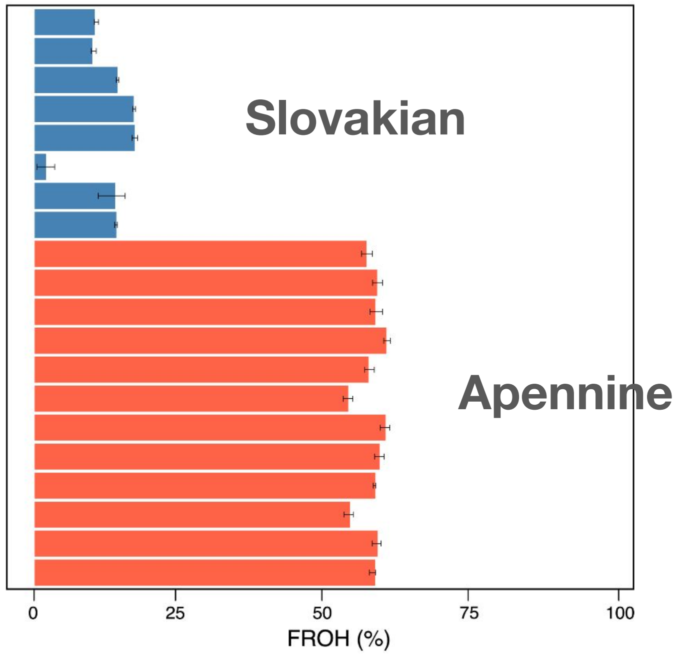
TESTING PREDICTIONS OF DELETERIOUSNESS

Impaired bioenergetics in the Apennine brown bear

a)

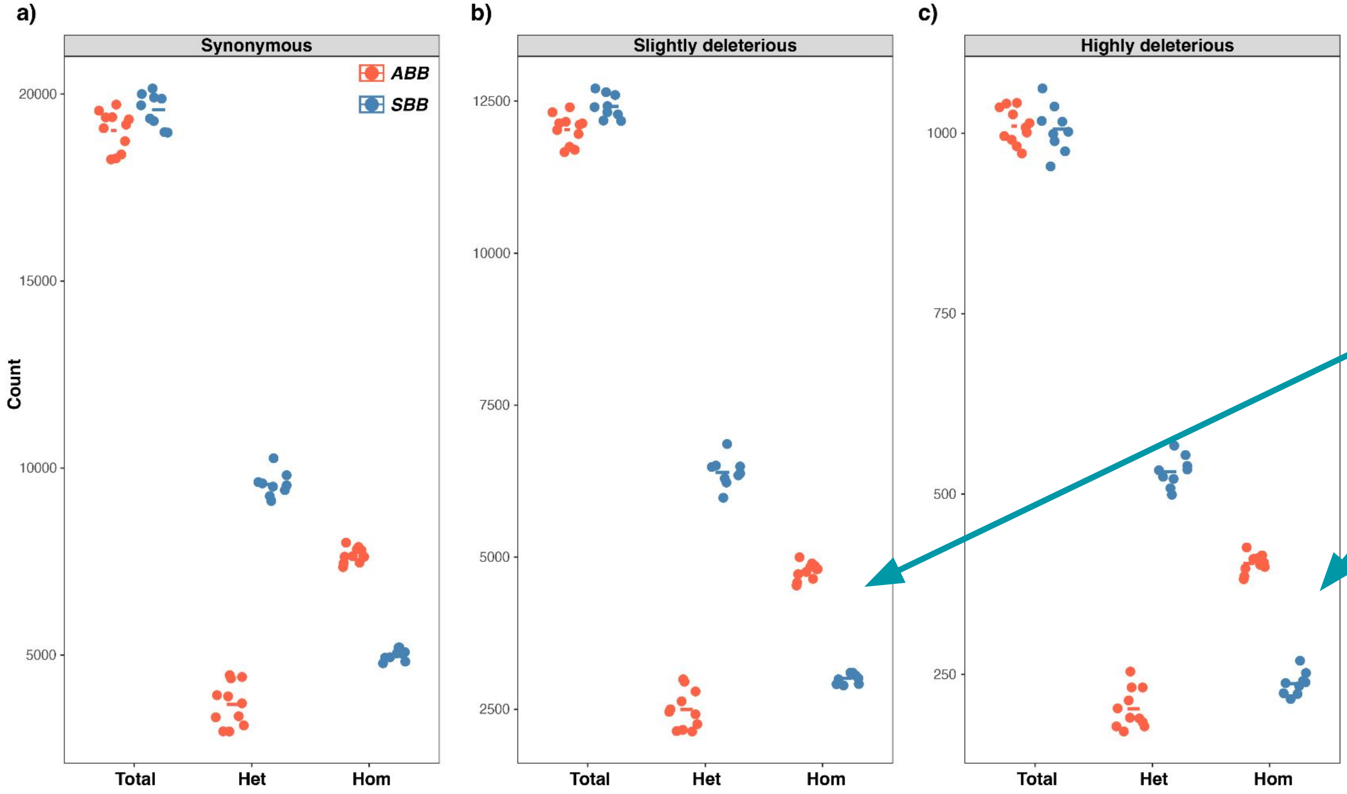


b)



TESTING PREDICTIONS OF DELETERIOUSNESS

Impaired bioenergetics in the Apennine brown bear



REALIZED
GENETIC LOAD

Fabbri et al *in prep*

TESTING PREDICTIONS OF DELETERIOUSNESS

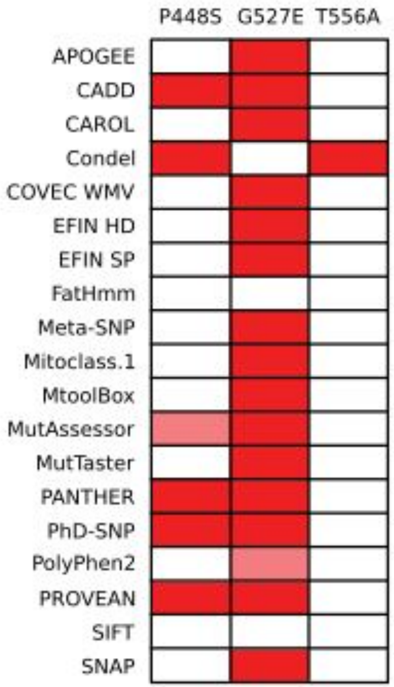
Impaired bioenergetics in the Apennine brown bear

Ca. 40 predicted deleterious substitution in the Apennine bears

TESTING PREDICTIONS OF DELETERIOUSNESS

Impaired bioenergetics in the Apennine brown bear

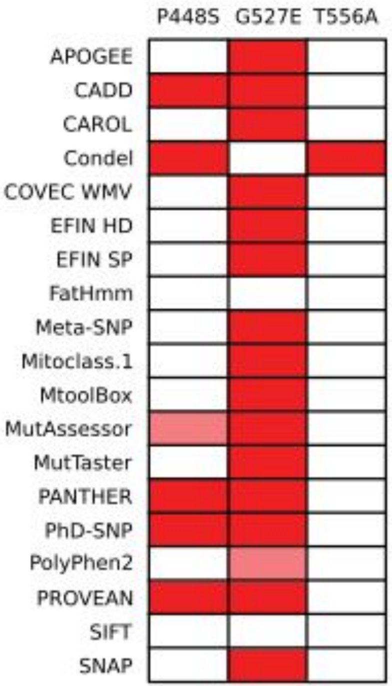
3 in the mitochondrial ND5 -> **RESPIRATORY COMPLEX I**



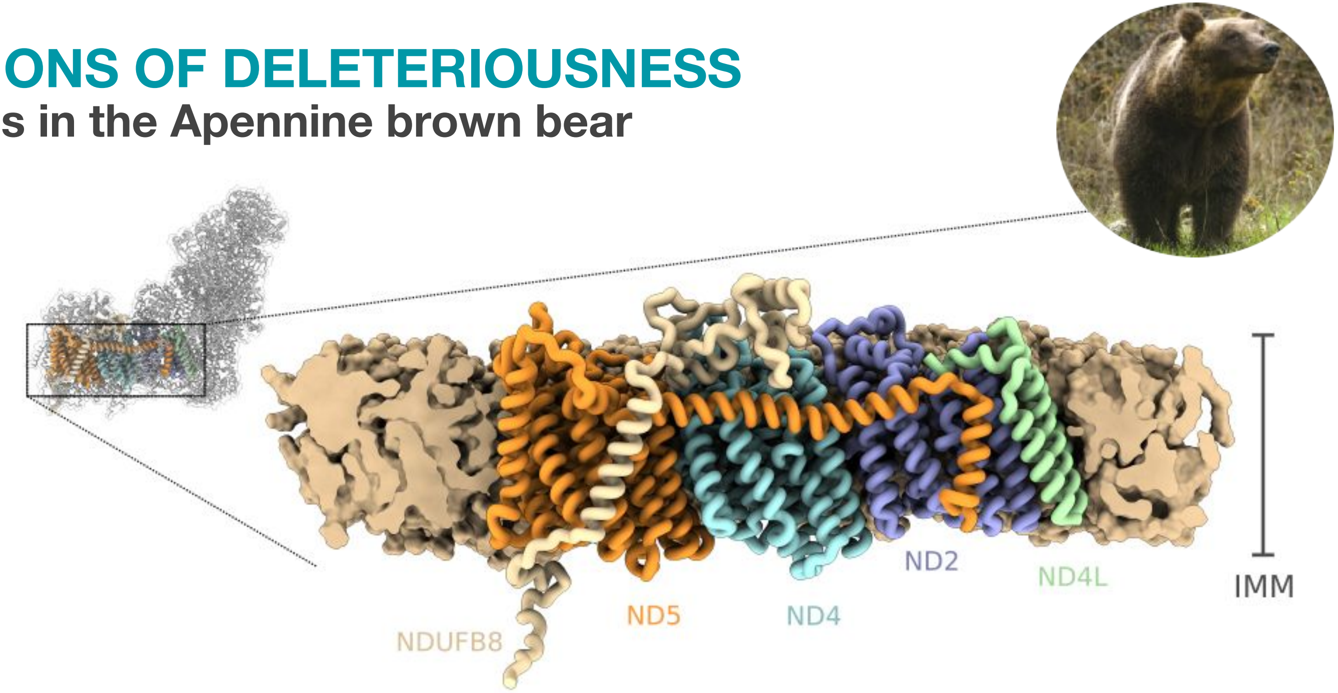
	P448S	G527E	T556A
APOGEE		🟥	
CADD	🟥	🟥	
CAROL		🟥	
Condel	🟥		🟥
COVEC WMV		🟥	
EFIN HD		🟥	
EFIN SP		🟥	
FatHmm			
Meta-SNP		🟥	
Mitoclass.1		🟥	
MtoolBox		🟥	
MutAssessor	▨	🟥	
MutTaster		🟥	
PANTHER	🟥	🟥	
PhD-SNP	🟥	🟥	
PolyPhen2		▨	
PROVEAN	🟥	🟥	
SIFT			
SNAP		🟥	

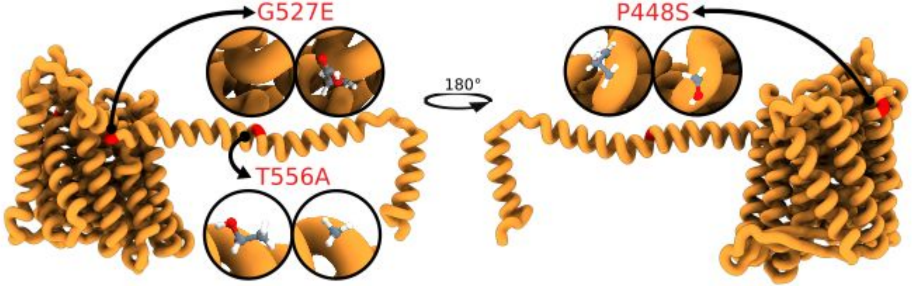
TESTING PREDICTIONS OF DELETERIOUSNESS

Impaired bioenergetics in the Apennine brown bear



	P448S	G527E	T556A
APOGEE		🟥	
CADD	🟥	🟥	
CAROL		🟥	
Condel	🟥		🟥
COVEC WMV		🟥	
EFIN HD		🟥	
EFIN SP		🟥	
FatHmm			
Meta-SNP		🟥	
Mitoclass.1		🟥	
MtoolBox		🟥	
MutAssessor	▨	🟥	
MutTaster		🟥	
PANTHER	🟥	🟥	
PhD-SNP	🟥	🟥	
PolyPhen2		▨	
PROVEAN	🟥	🟥	
SIFT			
SNAP		🟥	





TESTING PREDICTIONS OF DELETERIOUSNESS

Impaired bioenergetics in the Apennine brown bear

		ND5 positions			Experiments		
		448	527	556	MDCK cells	Fibroblasts	MD simulations
Apennine	🟪	S	E	A	✓	✓	✓
S448	🟨	S	G	T	✓		
E527	🟥	P	E	T	✓		✓
A556	🟩	P	G	A	✓		
CTRL	◼	P	G	T	✓		✓
Alpine	⬛	S	G	A		✓	

TESTING PREDICTIONS OF DELETERIOUSNESS

Impaired bioenergetics in the Apennine brown bear

		ND5 positions			Experiments		
		448	527	556	MDCK cells	Fibroblasts	MD simulations
Apennine	🟪	S	E	A	✓	✓	✓
S448	🟨	S	G	T	✓		
E527	🟥	P	E	T	✓		✓
A556	🟩	P	G	A	✓		
CTRL	◼	P	G	T	✓		✓
Alpine	⬛	S	G	A		✓	

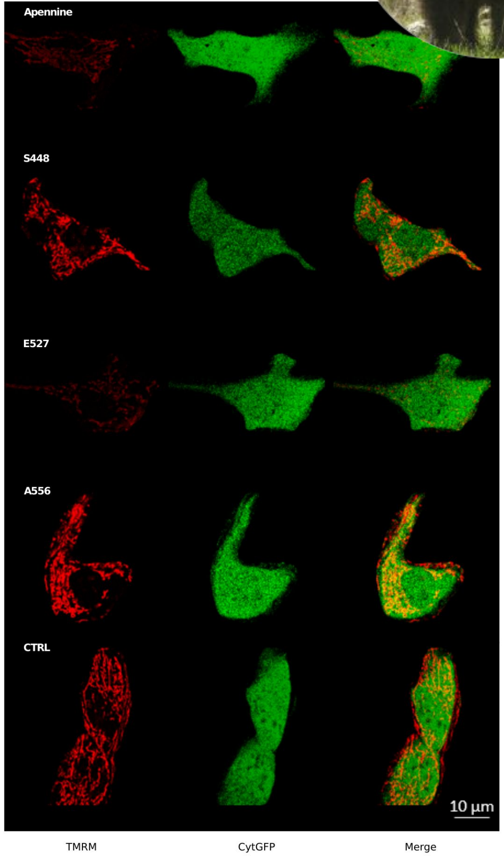
TESTING PREDICTIONS OF DELETERIOUSNESS

Impaired bioenergetics in the Apennine brown bear

		ND5 positions			Experiments		
		448	527	556	MDCK cells	Fibroblasts	MD simulations
Apennine	🟪	S	E	A	✓	✓	✓
S448	🟨	S	G	T	✓		
E527	🟥	P	E	T	✓		✓
A556	🟩	P	G	A	✓		
CTRL	▨	P	G	T	✓		✓
Alpine	⬛	S	G	A		✓	

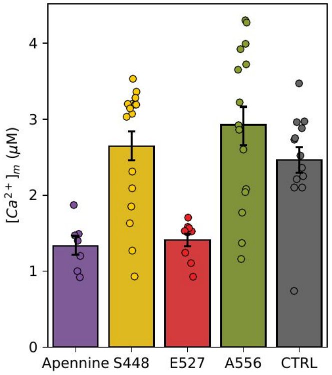
EXPERIMENT IN VITRO - MDCK CELLS

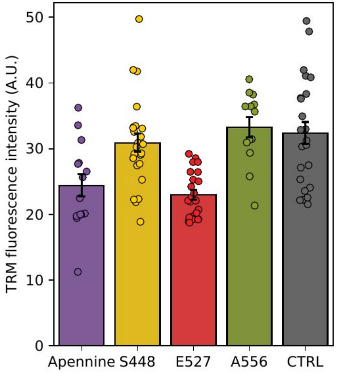
Impaired bioenergetics in the Apennine brown bear



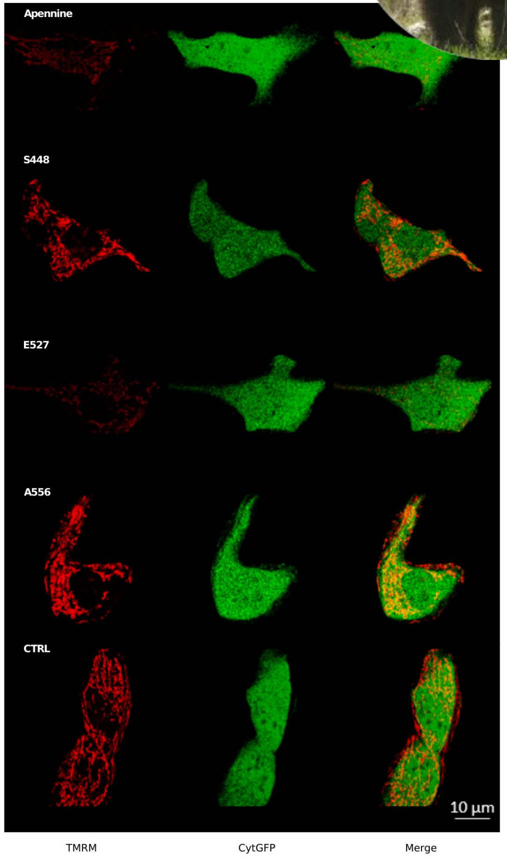
EXPERIMENT IN VITRO - MDCK CELLS

Impaired bioenergetics in the Apennine brown bear



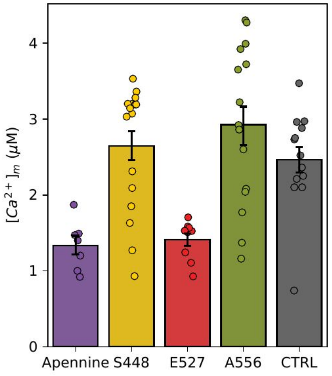


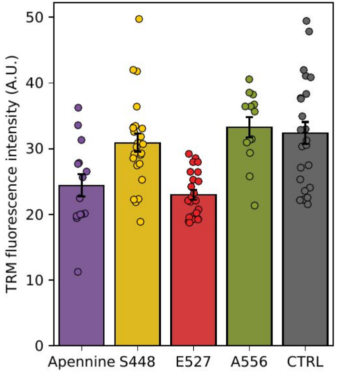
Transmembrane potential is lower



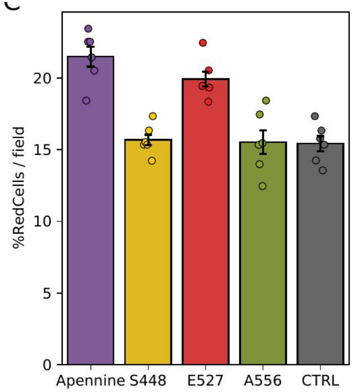
EXPERIMENT IN VITRO - MDCK CELLS

Impaired bioenergetics in the Apennine brown bear

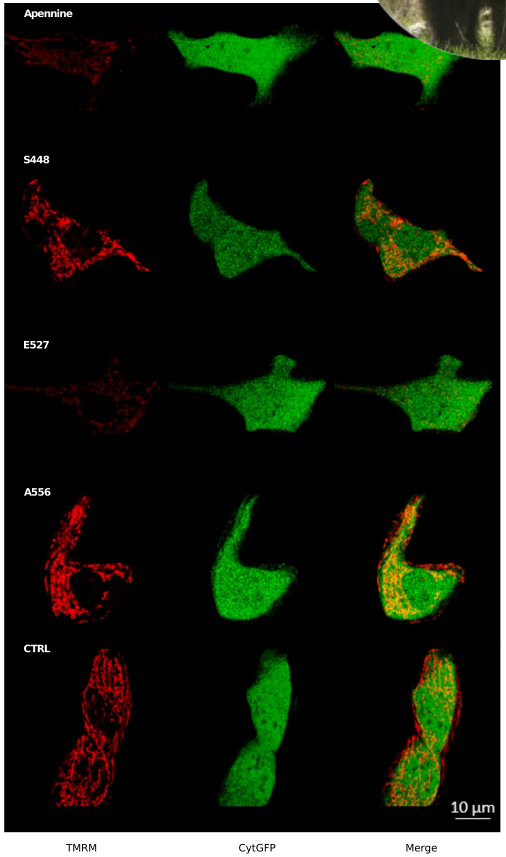




Transmembrane potential is lower



ROS production is higher

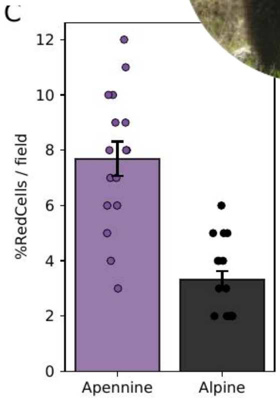


EXPERIMENT EX VIVO - FIBROBLASTS

Impaired bioenergetics in the Apennine brown bear

ROS

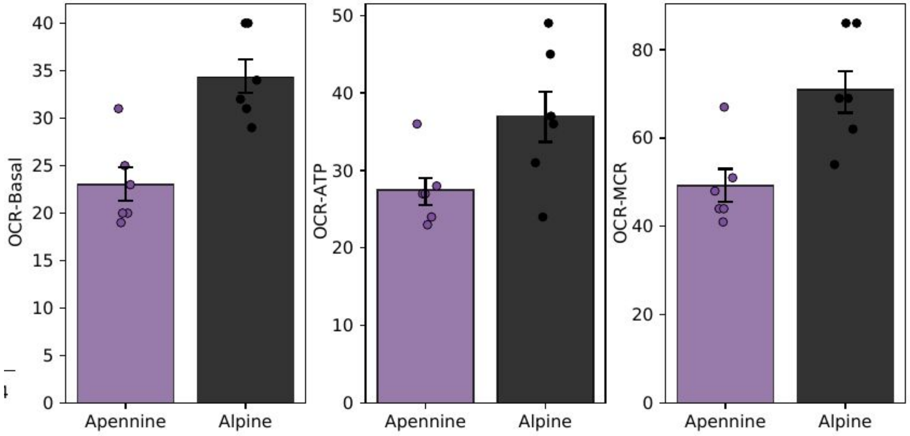
are higher



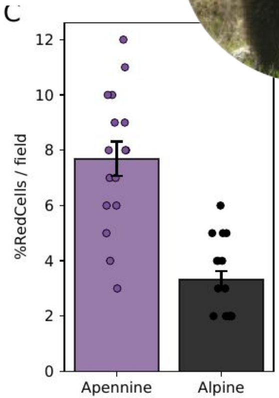
EXPERIMENT EX VIVO - FIBROBLASTS

Impaired bioenergetics in the Apennine brown bear

ATP production
is lower



ROS
are higher



EXPERIMENT EX VIVO - FIBROBLASTS

Impaired bioenergetics in the Apennine brown bear

ATP production
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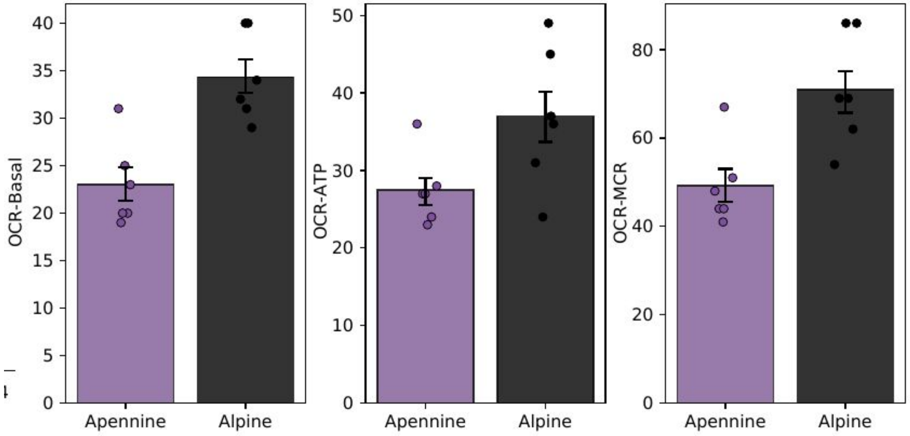
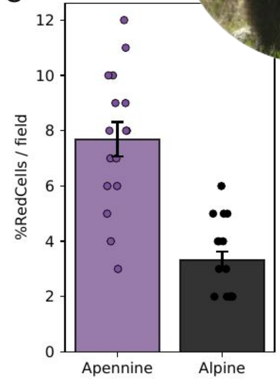
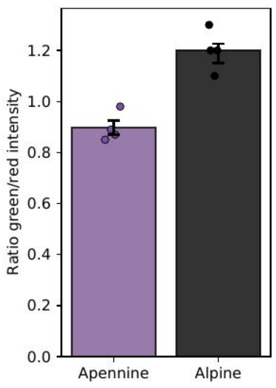


ROS
are higher



D

MT
are older

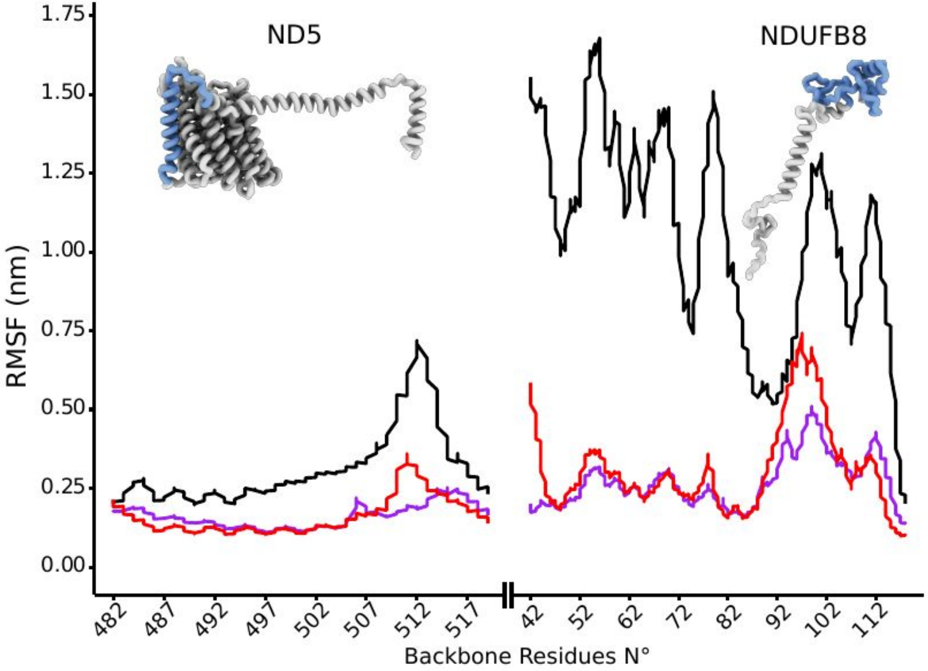


EXPERIMENT IN SILICO - MOLECULAR DYNAMICS

Impaired bioenergetics in the Apennine brown bear

EXPERIMENT IN SILICO - MOLECULAR DYNAMICS

Impaired bioenergetics in the Apennine brown bear

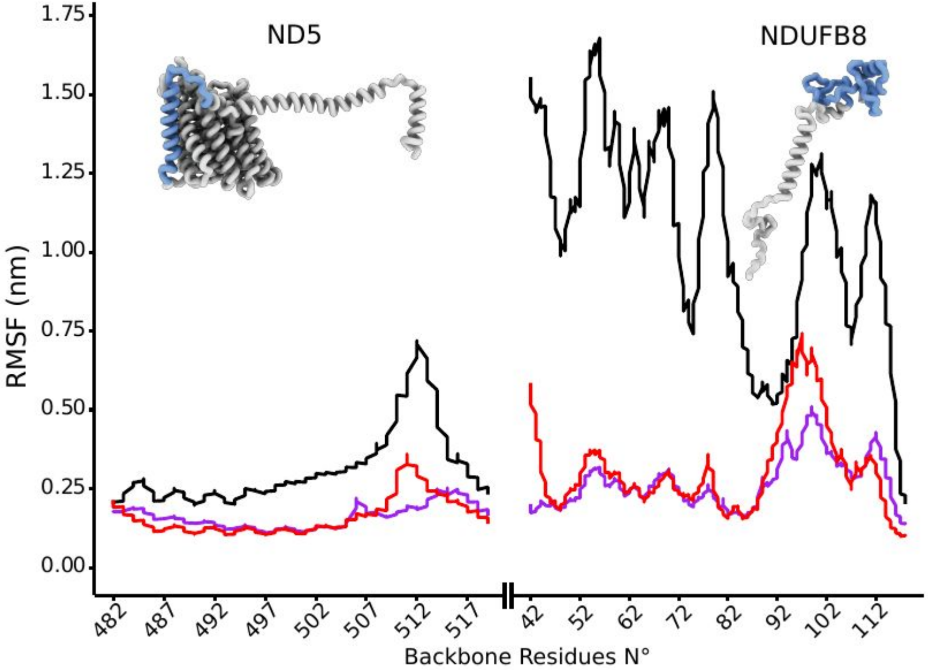


Rigidity

increases

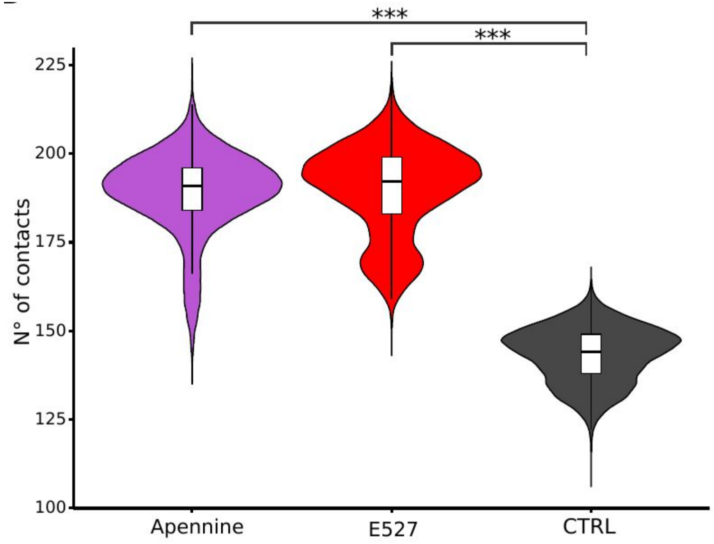
EXPERIMENT IN SILICO - MOLECULAR DYNAMICS

Impaired bioenergetics in the Apennine brown bear



Rigidity
increases

Number of contacts
Is higher



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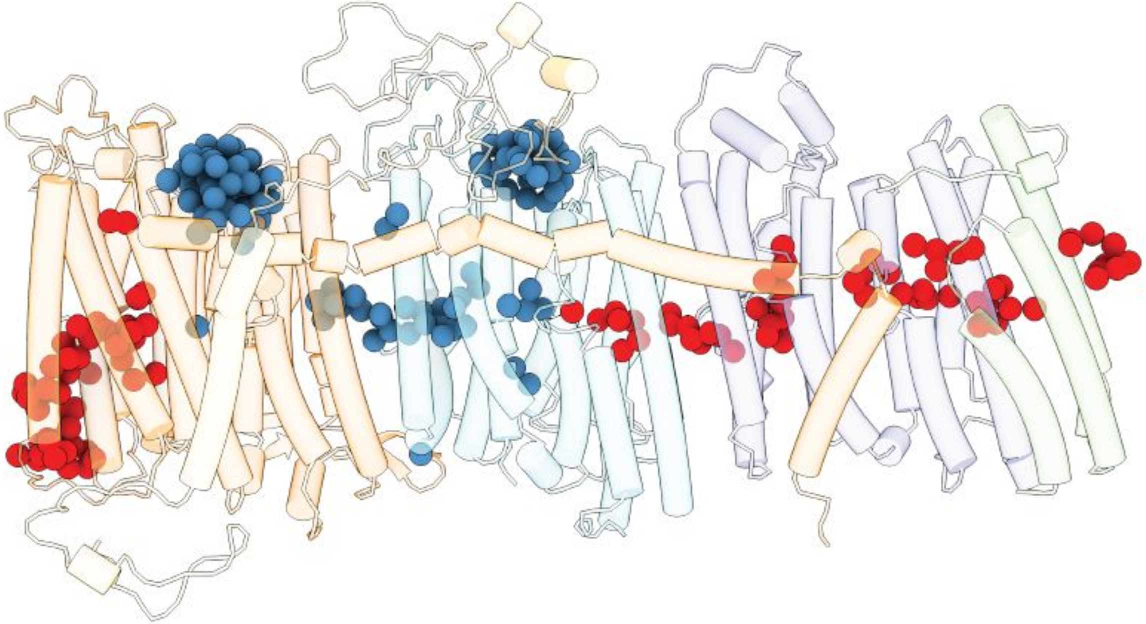

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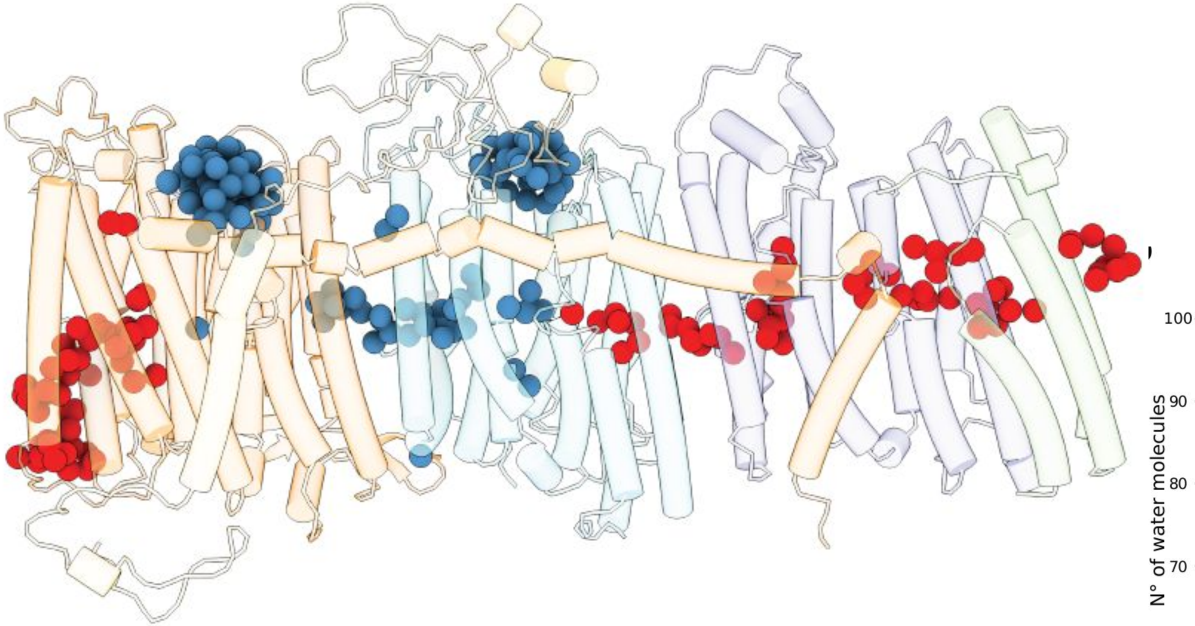
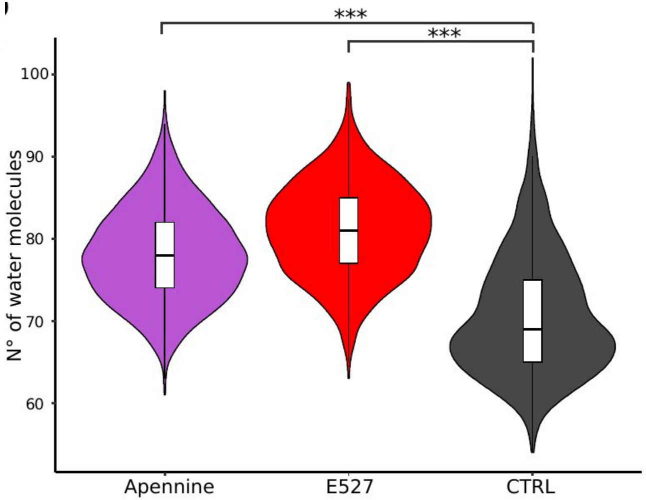


Hydration increases



Trucchi et al *submitted*

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FUTURE DIRECTIONS

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INTEGRATE ADVANCED MOLECULAR BIOLOGY METHODS UP TO GENETIC EDITING TO CORRECT FIXED DELETERIOUS MUTATIONS IN ENDANGERED SPECIES AND SYNTHETIC BIOLOGY UP TO DE-EXTINCTION

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IMPROVE PREDICTION OF GENOMIC FEATURES WITH AI

CONSERVATION GENOMICS

WPSG
Český Krumlov
2025
Workshop on Population and Speciation Genomics

QUESTIONS?