Population Genomics

Stories of bears, penguins and maybe beans

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Workshop on Genomics 2020
Cesky Krumlov

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Research on: Inferences of demographic and adaptive processes in natural populations of (mostly) non-model species.

Keywords: population genetics, genomics, molecular ecology, ***conservation genetics***, phylogeography, phylogenetics, bioinformatics.

My academic record: University of Roma3 (Master), Tor Vergata (PhD), Oslo (postdoc 1 - Marie Curie), Vienna (postdoc 2), Ferrara (researcher), Marche Polytechnic (assistant professor).

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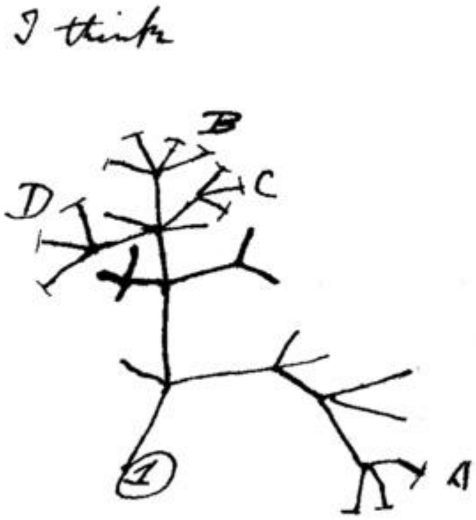
What is population genetics? **(back to basics)**

“The study of the **genetic composition** of natural populations and its evolutionary causes and consequences” *(Coop 2019)*

Genetic drift
Migration
Recombination
Mutation
Selection



What is population genetics? **(back to basics)**

A set of **theoretical models** to understand how these forces interact

Simple as any model simplifying reality

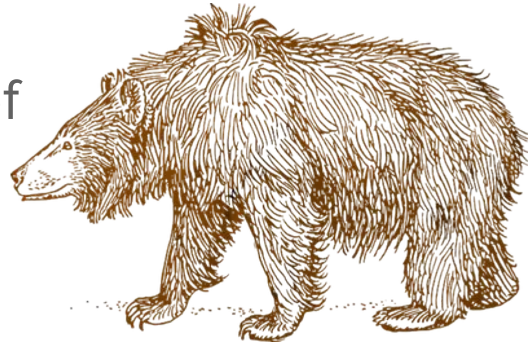
(... all models are wrong but some are useful, Box 1979)

Useful for understanding real patterns - give accurate predictions

(*e.g.*, medical genetics, crop improvements, species conservation)

Powerful as the basic rules of genetic transmissions are simple and universal

Population genomics as a tool in conservation biology



SIDE A: The extraordinary genomic history of the endangered Apennine Brown Bear

SIDE B: Climate-driven range shifts in fragmented ecosystems

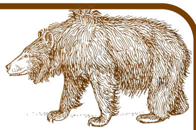
Bonus track: Ancient genomes reveal early farmers selected common bean while preserving diversity

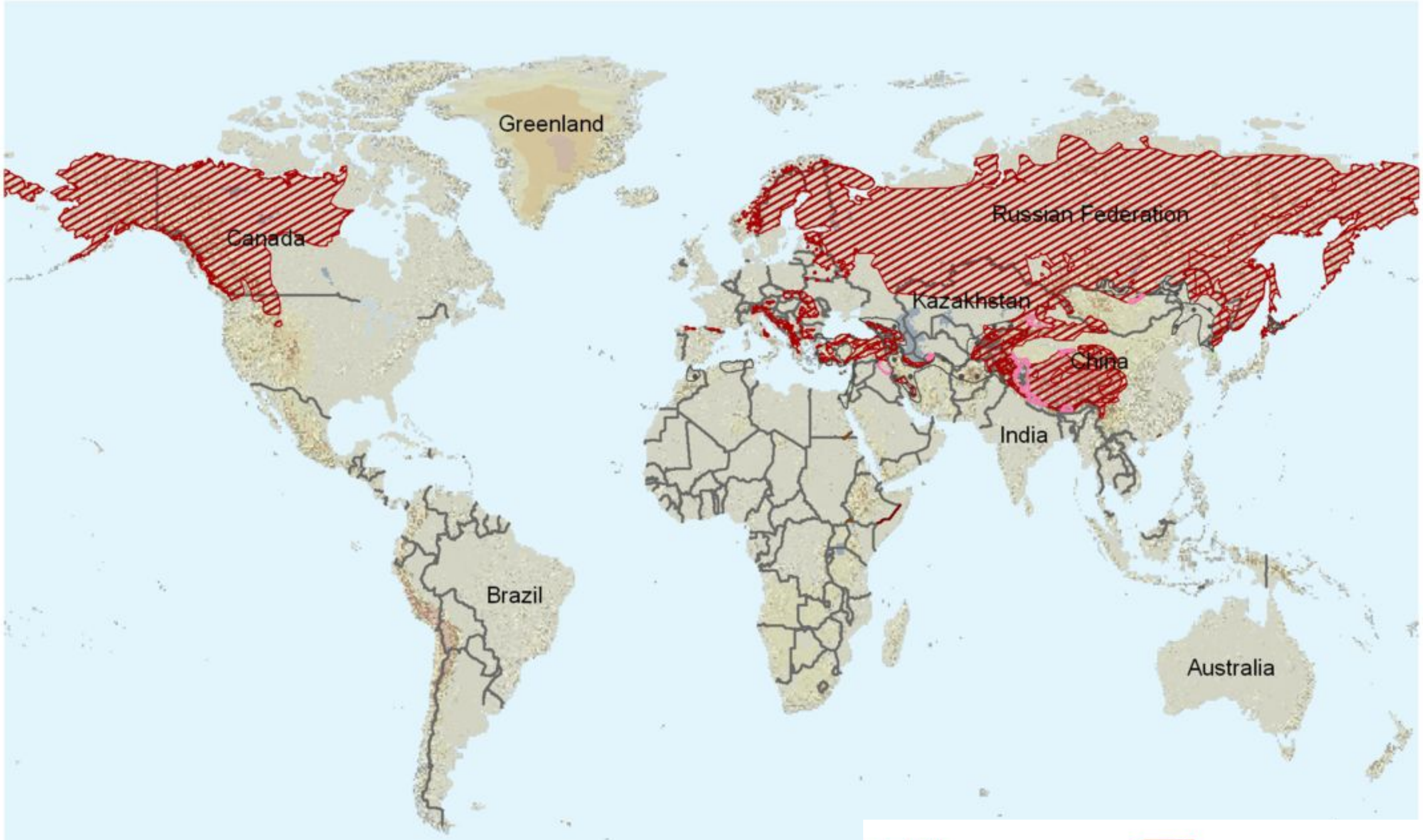
SIDE A

The extraordinary genomic history of the endangered Apennine Brown Bear

Brown bear distribution

Generally holarctic with a more southern range in Asia





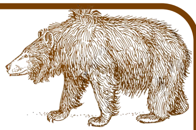
No global conservation issue

RED LIST
THE IUCN RED LIST OF THREATENED SPECIES™

NE DD **LC LEAST CONCERN** NT VU EN CR EW EX

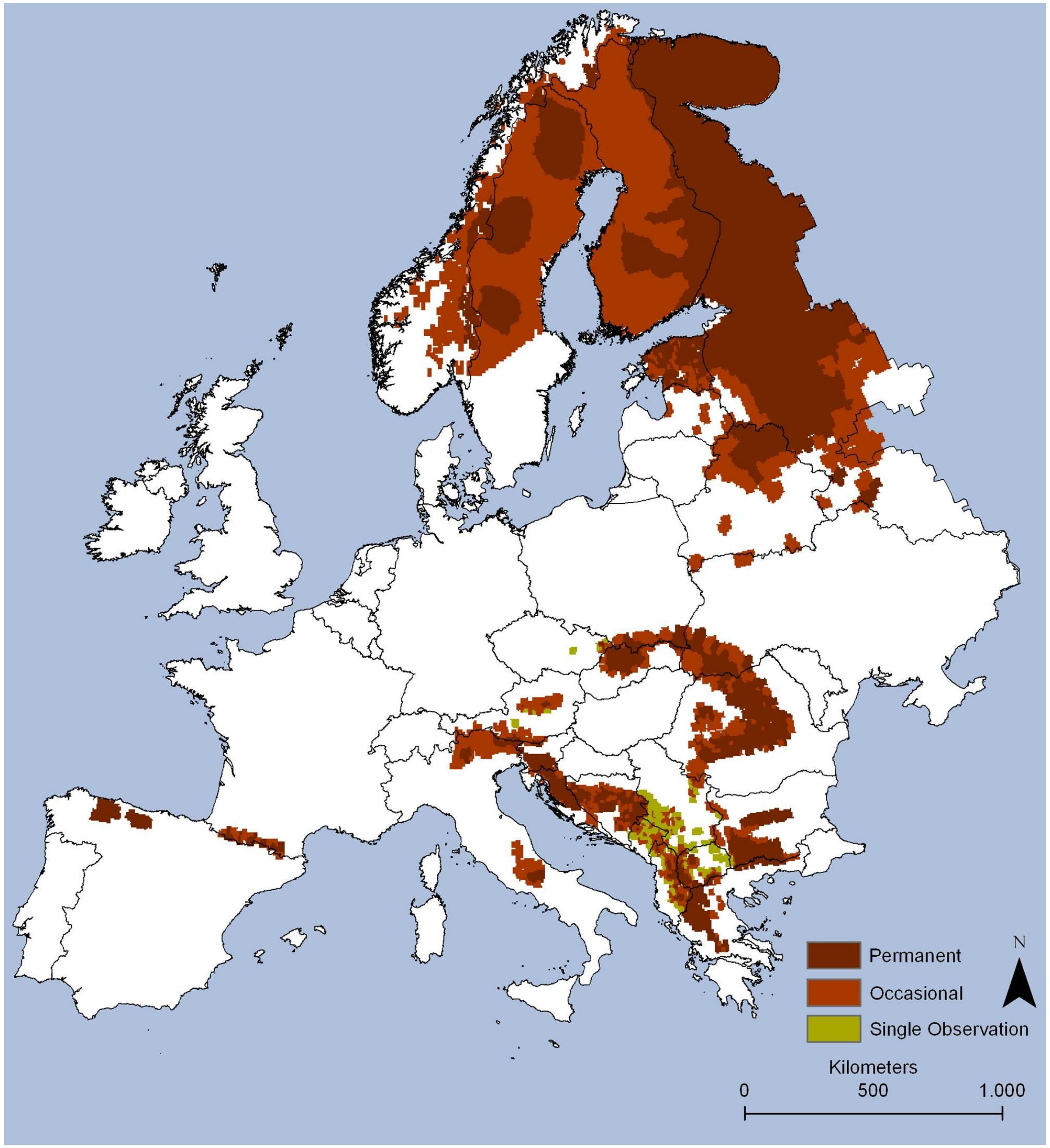
Brown bear distribution

Fragmented in South-Eastern Europe



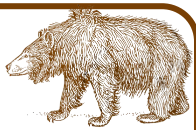
More or less stable population, ca. 17,000 individuals

(Status of Large Carnivore Populations in Europe 2012-2016)

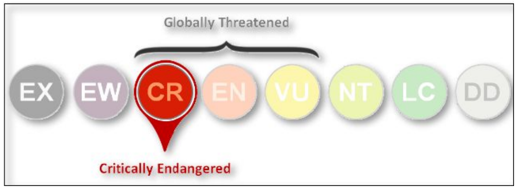


Brown bear distribution

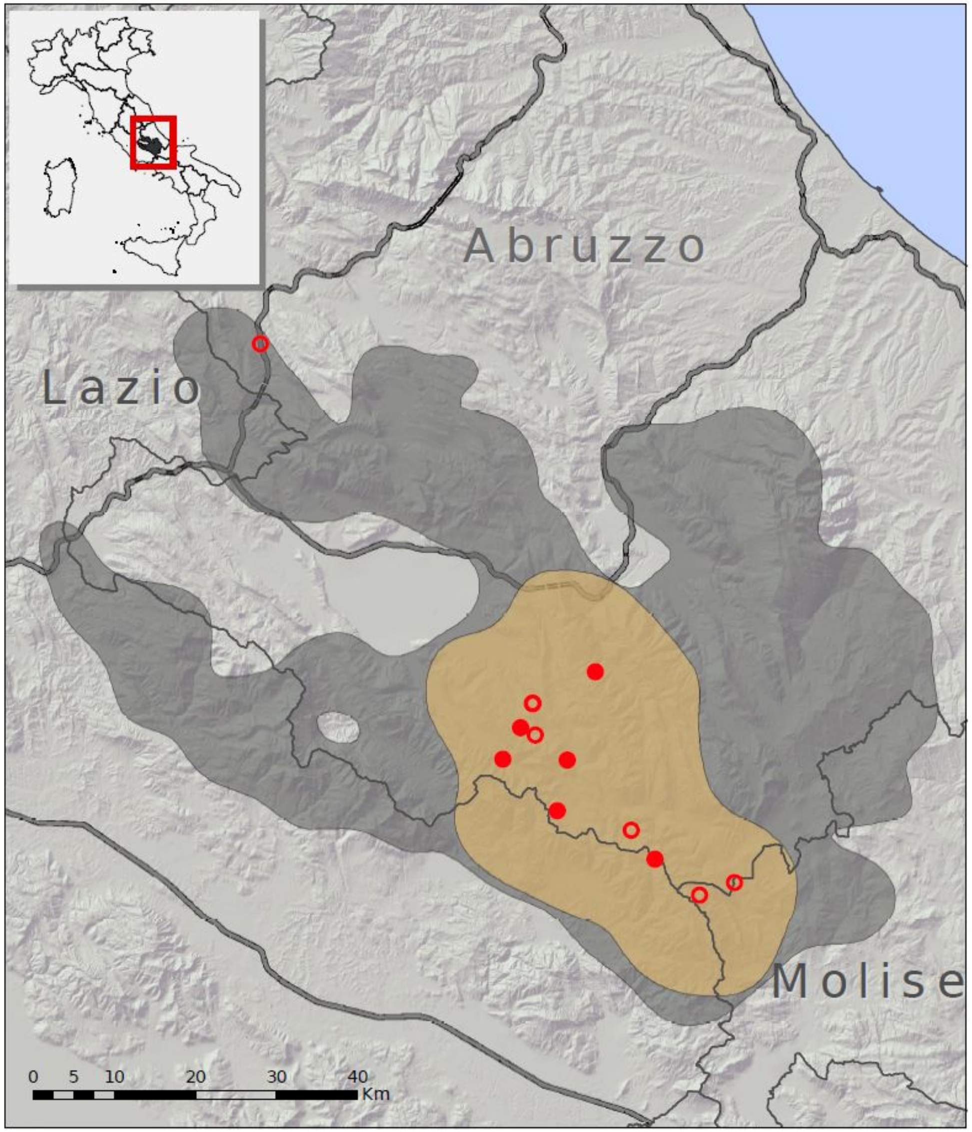
A small and isolated population in Central Italy



Less than 100 individuals since it the population has been checked (last century). Now ca. **50** individuals



National park since 1923



Brown bear distribution

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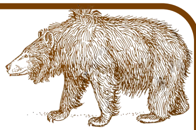

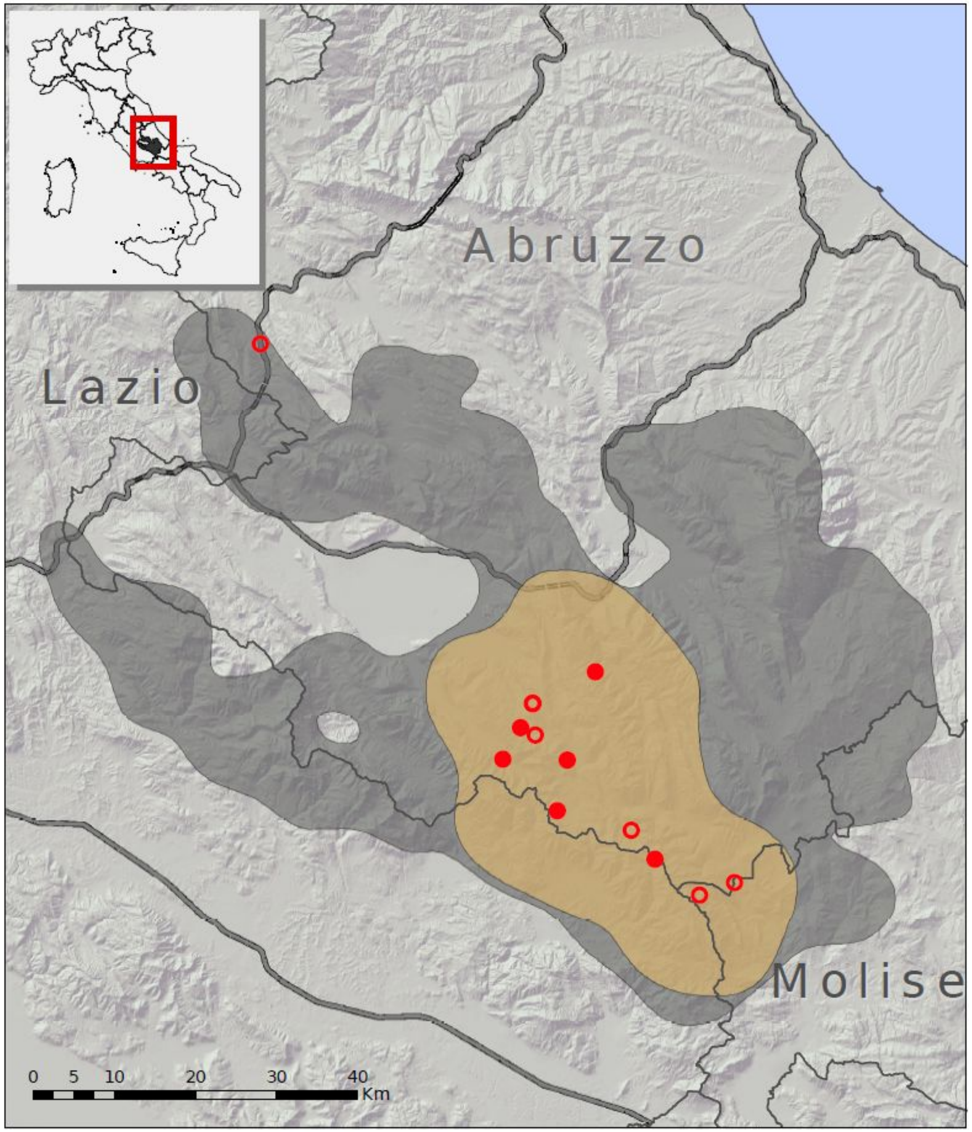

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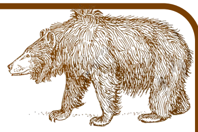
How long has this population been so small and isolated?

Brown bear distribution

A small and isolated population in Central Italy



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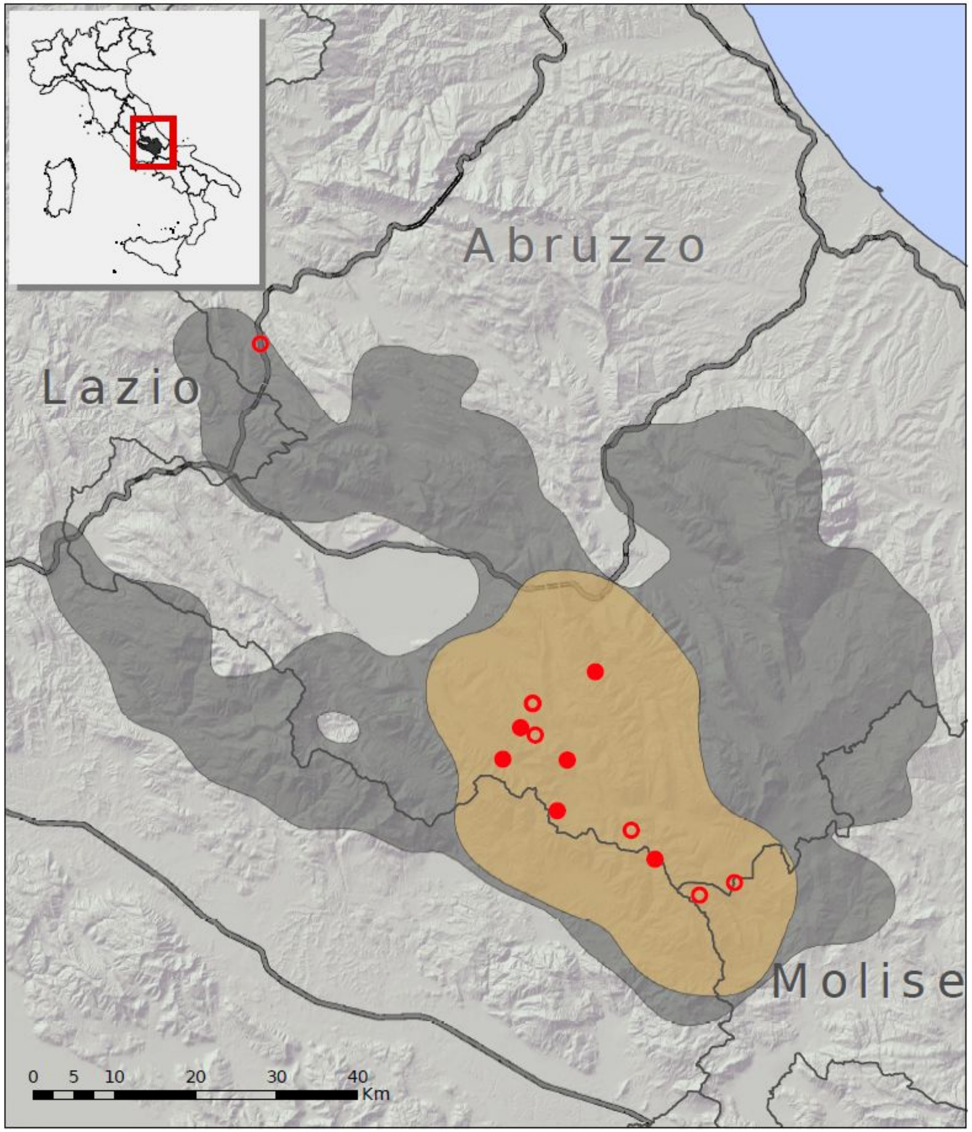
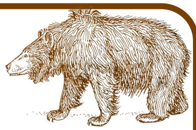
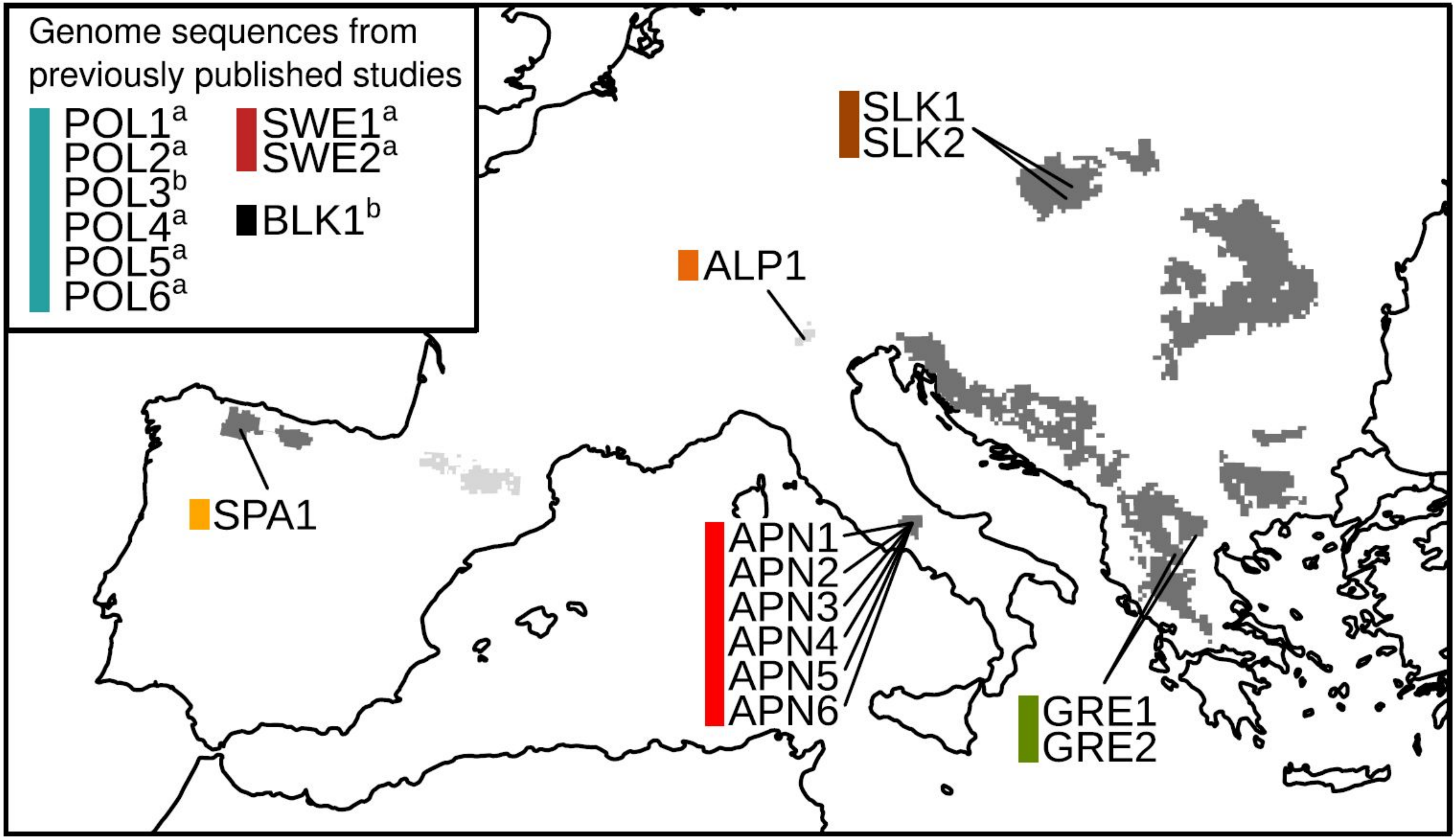


6 WGS (●),
12 whole mt genome sequenced (○)

Whole-genome data

A few other samples from other European populations …

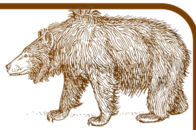




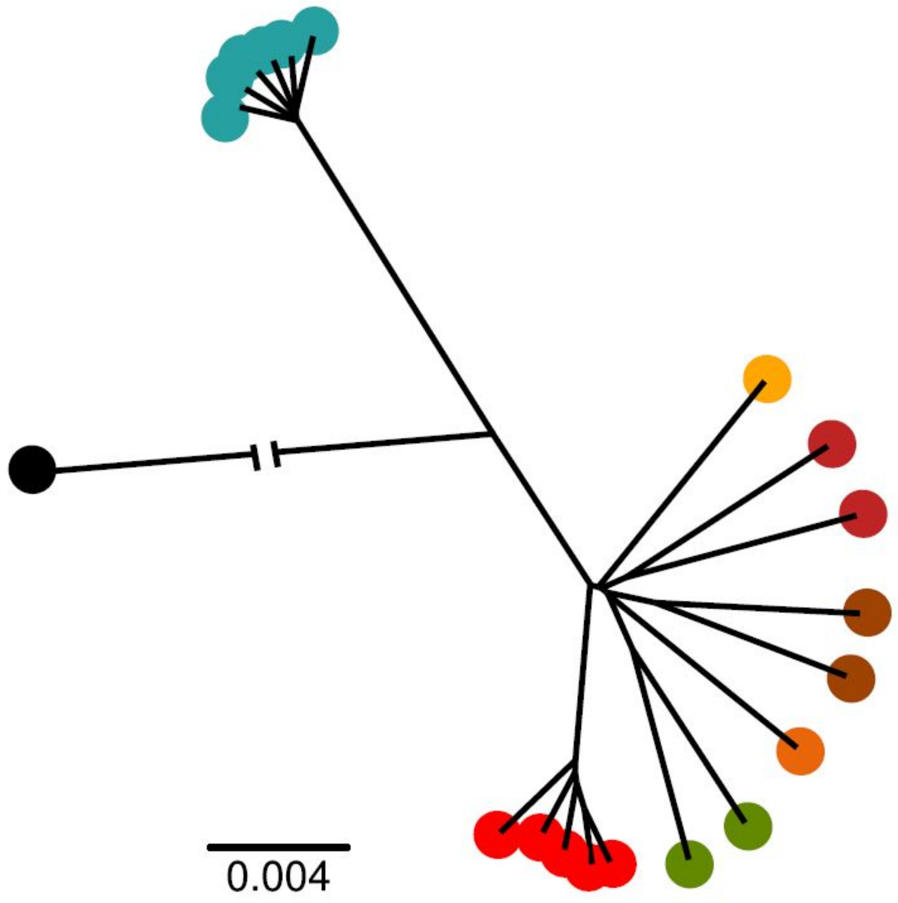
a: Liu et al 2014, b: Miller et al 2012

Structure of genomic diversity

Whole-genome and mitochondrion contrasting histories

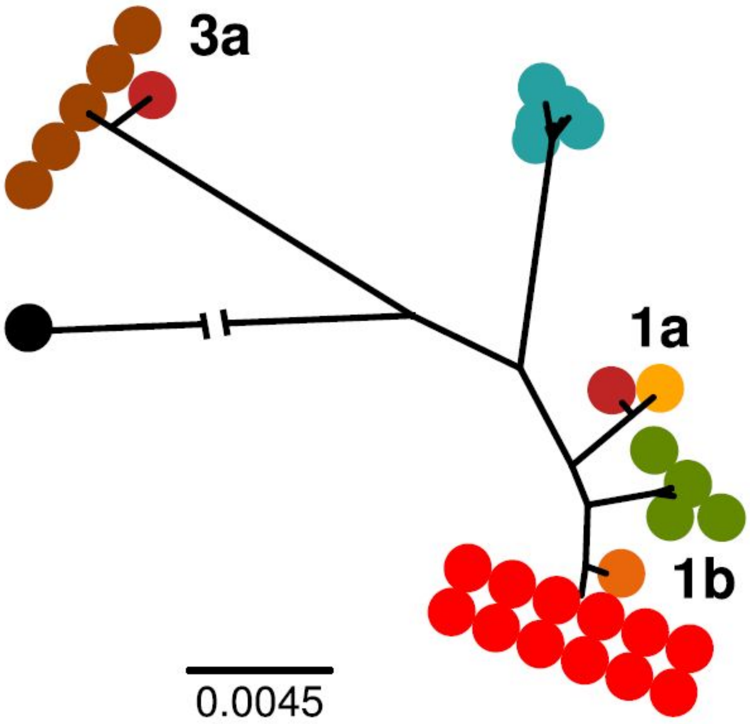


1,842,042,551 nuclear bp



ANGSD, ngsDist, nj from ape *R* package

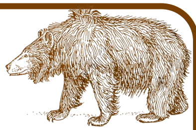
16,485 mitochondrial bp

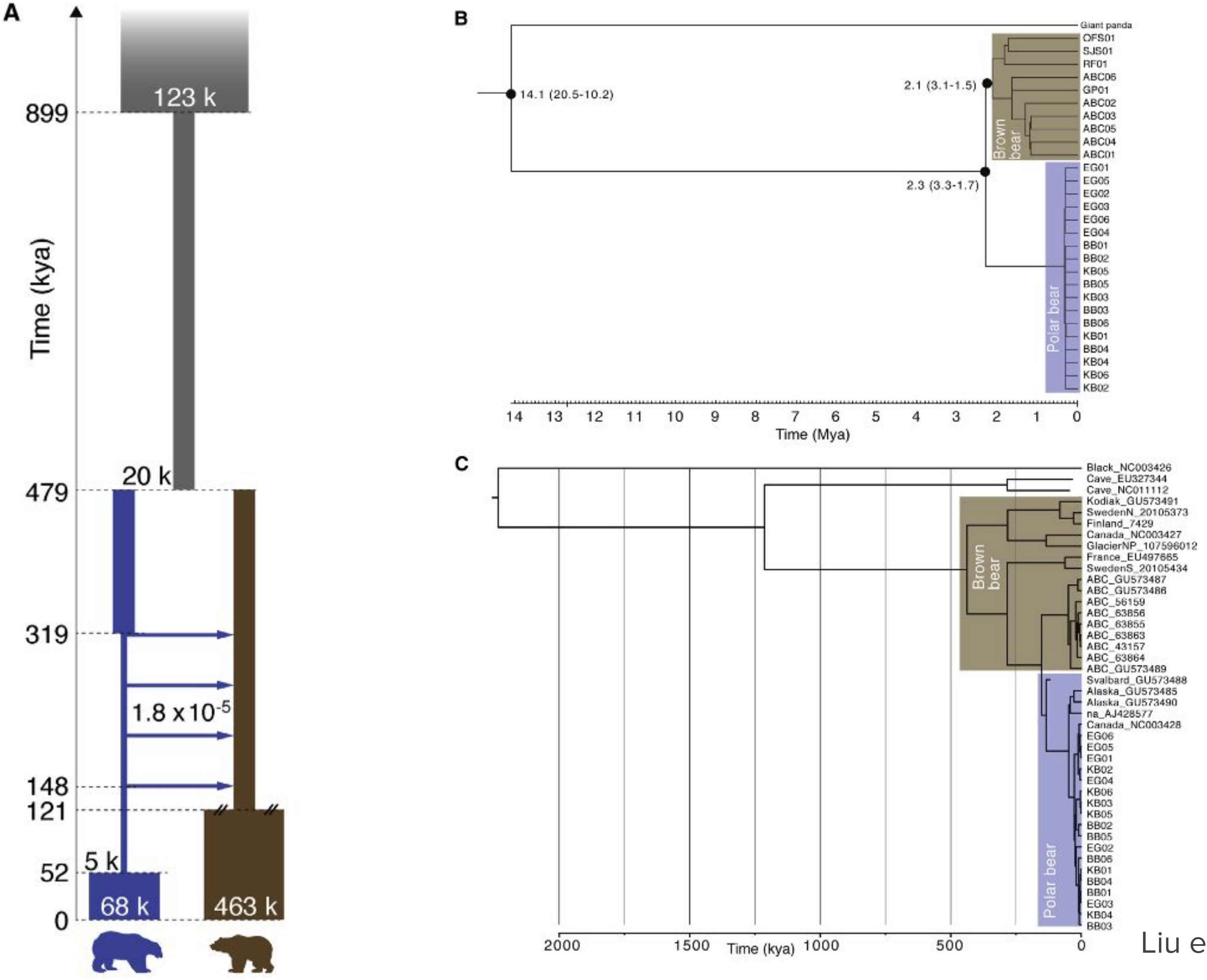


Geneious, MAFFT, genetic distance (HKY), NJ

Structure of genomic diversity

Whole-genome and mitochondrion contrasting histories

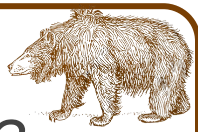




Liu et al 2014

Structure of genomic diversity

Y-chr agrees with whole-genome: sex-biased dispersal?



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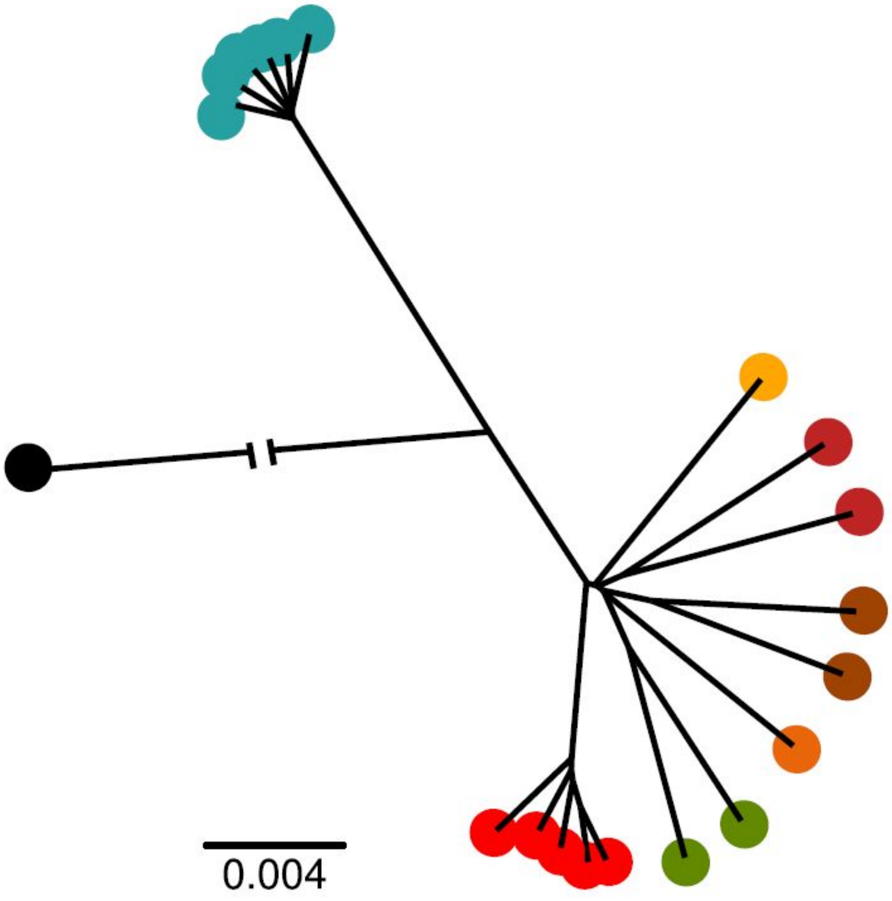
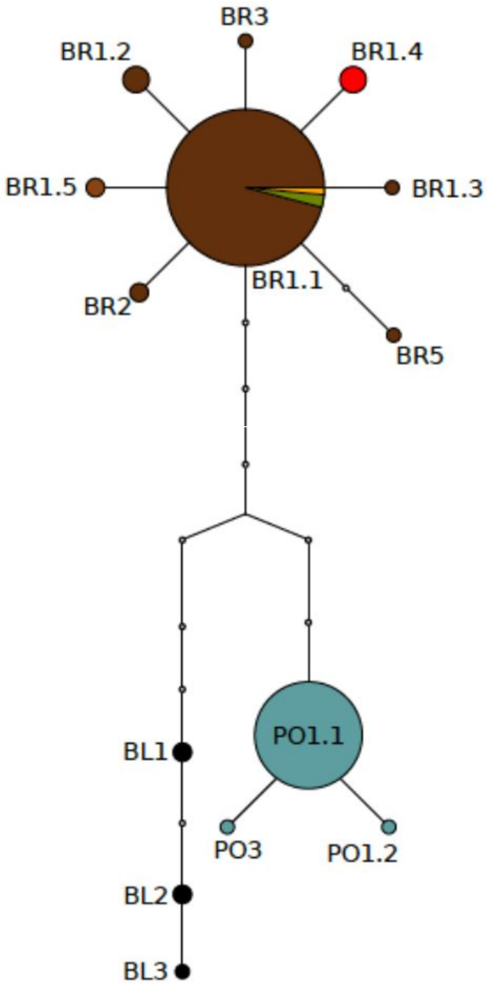


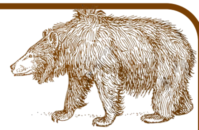
ANGSD, ngsDist, nj from ape *R* package

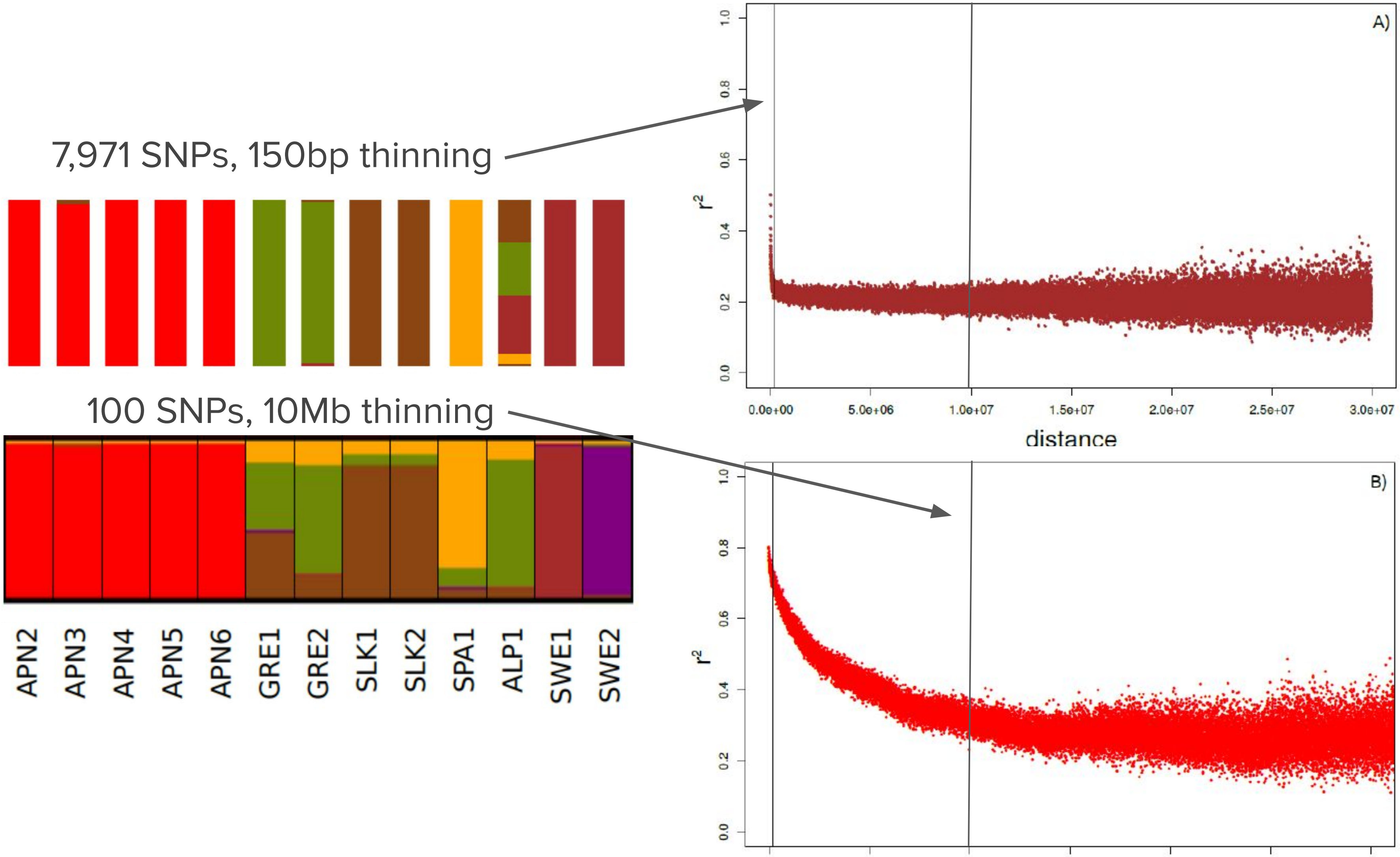
5.3 Kb Y-chromosome



MAFFT, TCS

Structure of genomic diversity
Robust to decay of linkage disequilibrium




7,971 SNPs, 150bp thinning

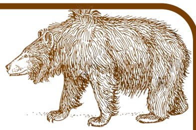
100 SNPs, 10Mb thinning

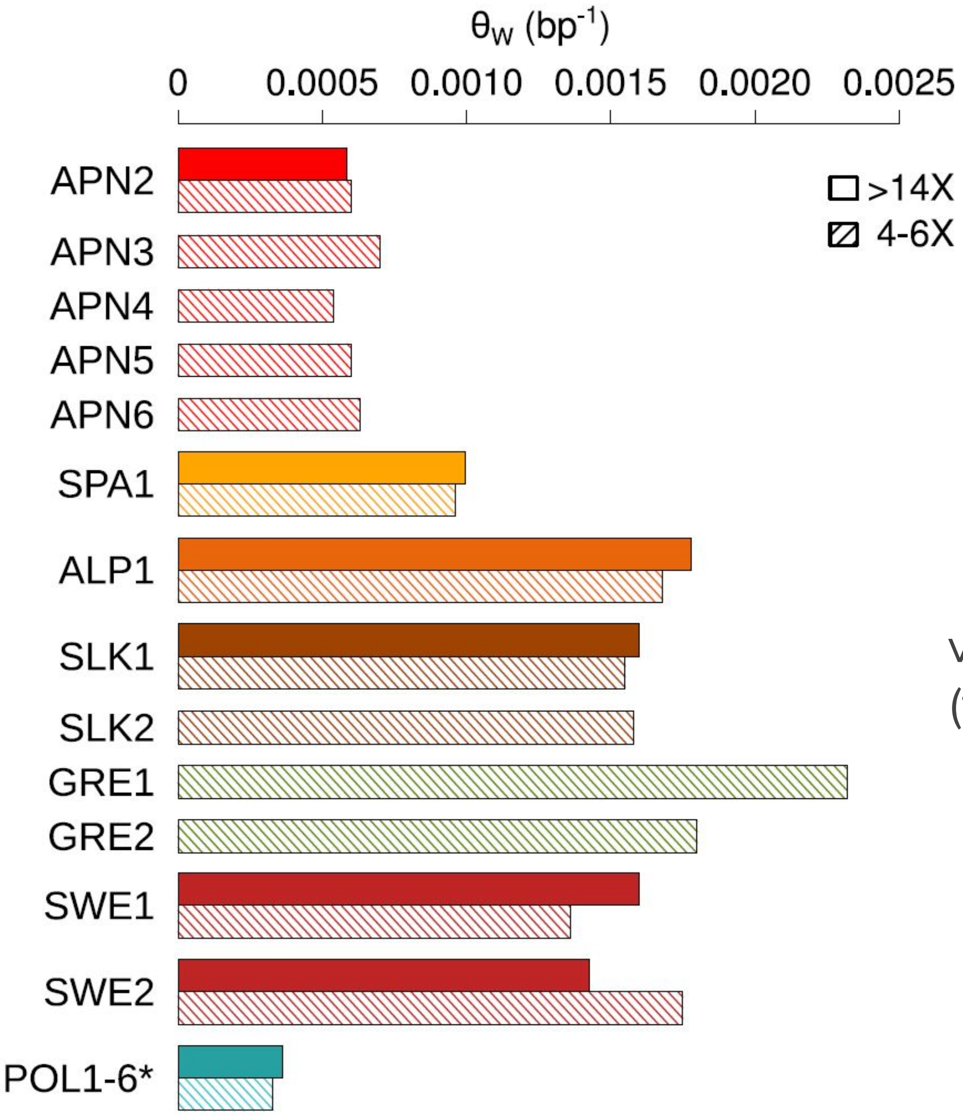
STRUCTURE, non-cds, non-rep

ANGSD, ngsLD

Genomic diversity - average

Low but the polar bear is worse



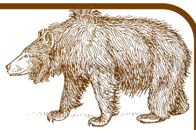


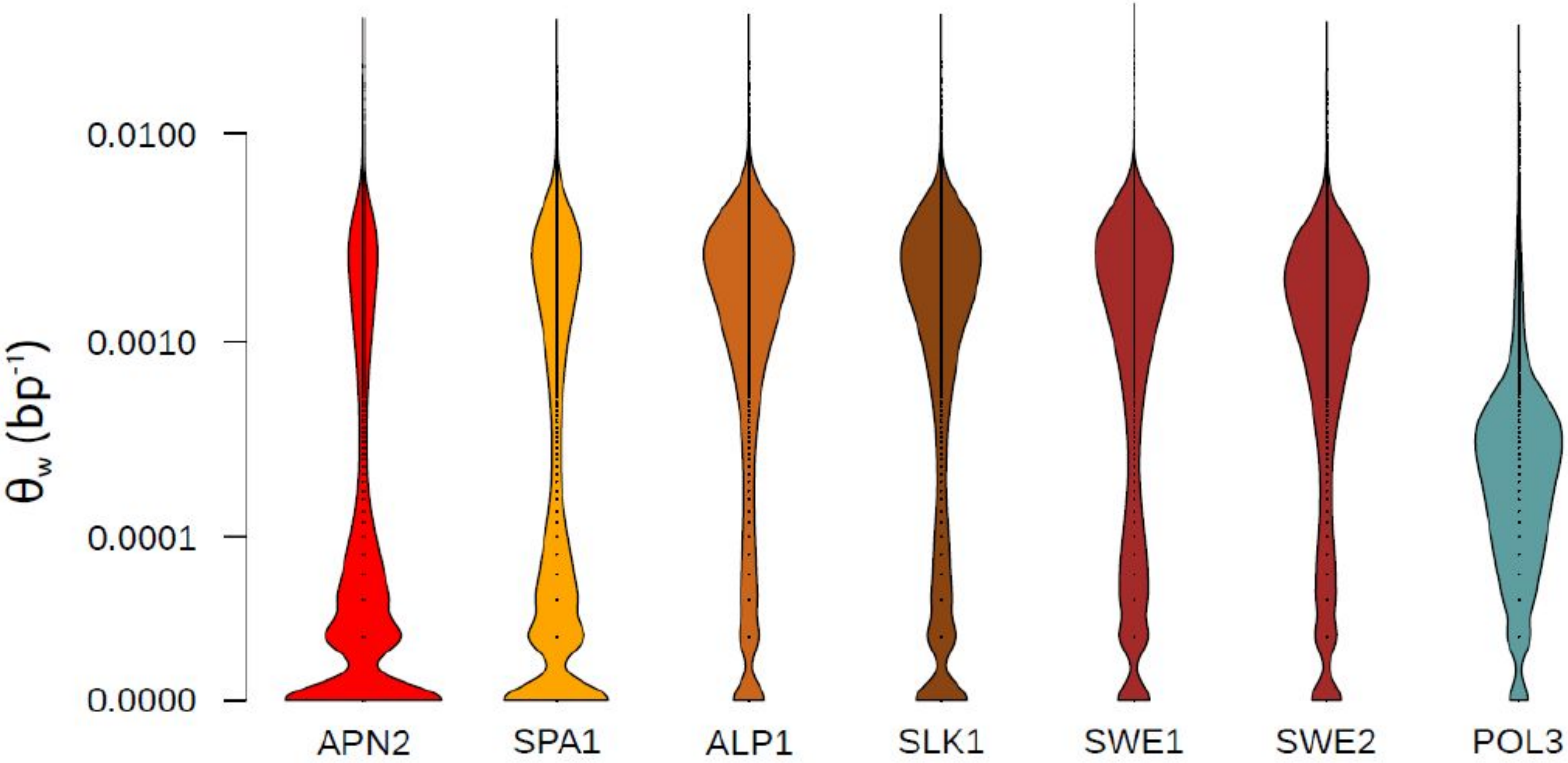
High coverage:
vcftools, SNP density, 50kb windows
(then downsampled and analyzed as
low coverage)

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

Genomic diversity - distribution

Low but the polar bear is worse...maybe not!

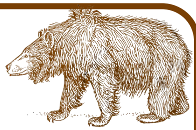


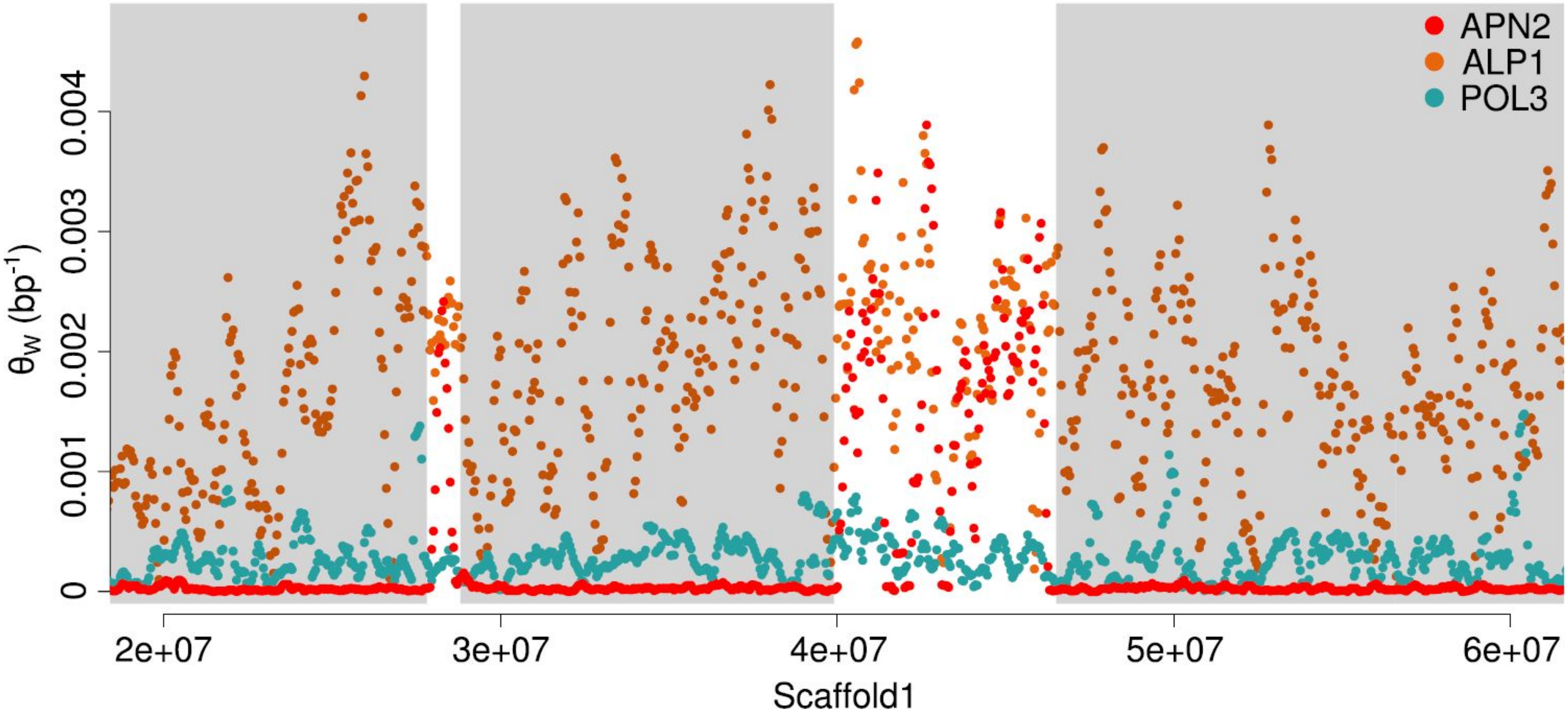


vcftools, SNP density in 50kb windows

Genomic diversity - distribution

Long stretches with no diversity in the Apennine bear

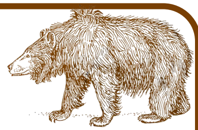


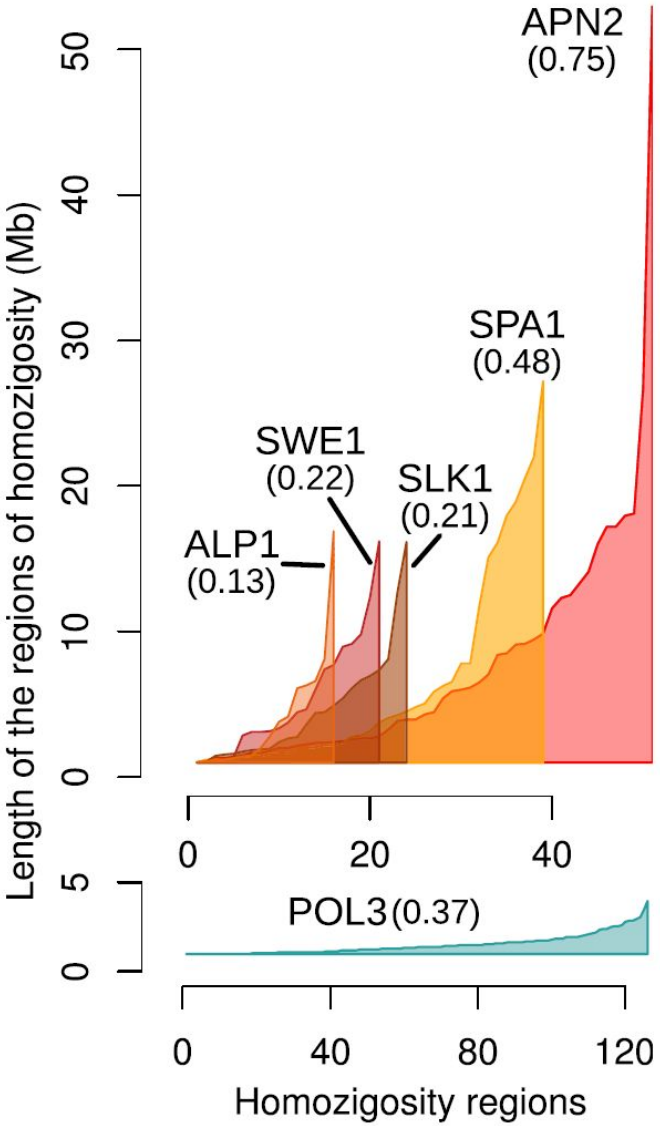


vcftools, SNP density in 50kb windows

Inbreeding estimates

Much higher than any other European bear



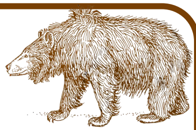


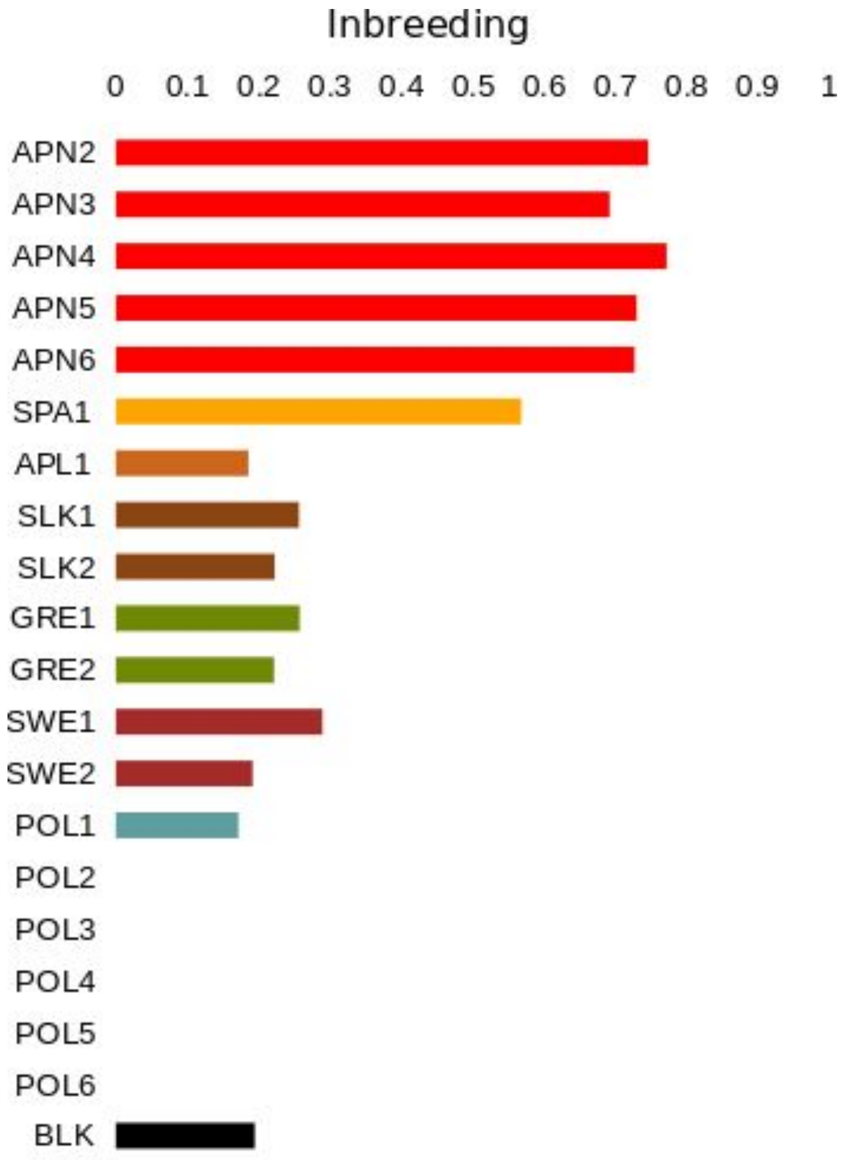
Regions longer than 1Mb with less than 25 segregating sites in the 13 longest scaffolds, ca. 0.5 Gb - sorted

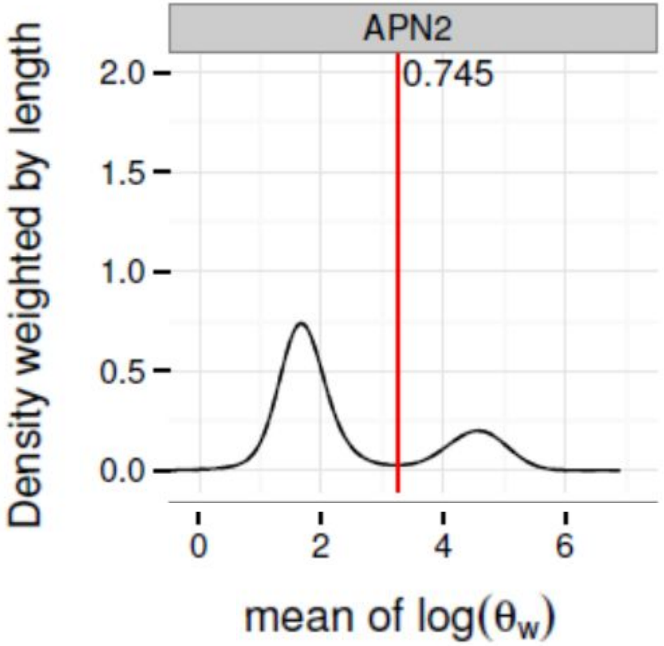
(percent of the analyzed scaffolds)

Inbreeding estimates

Much higher than any other European bear



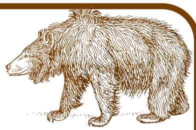


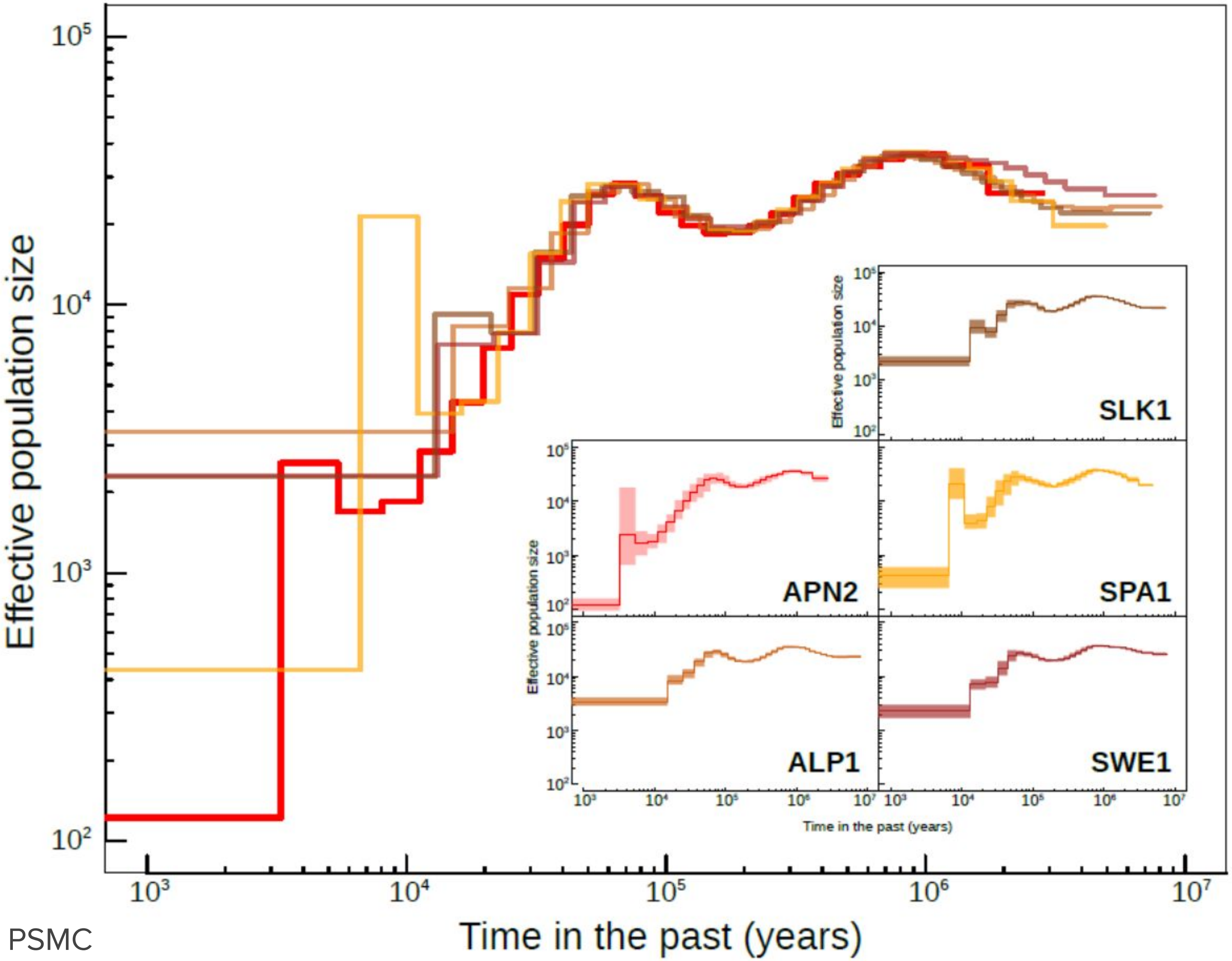


Based on the proportion of the genome segments that are mostly homozygous (Prüfer et al 2014)

Past demography

Apennine population declined more than other Eu pops

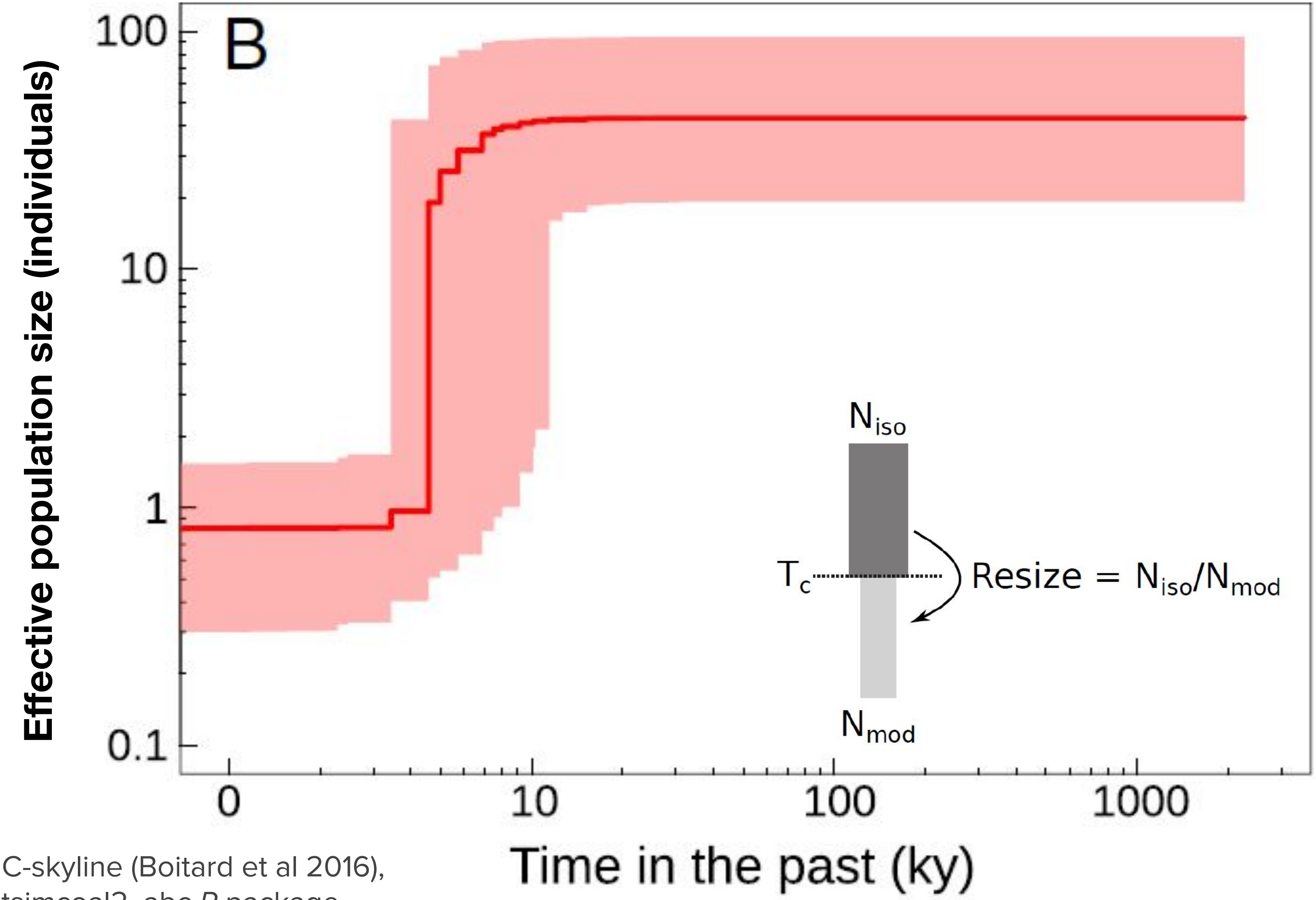




PSMC

Past demography

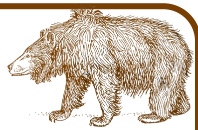
…and to very low Ne

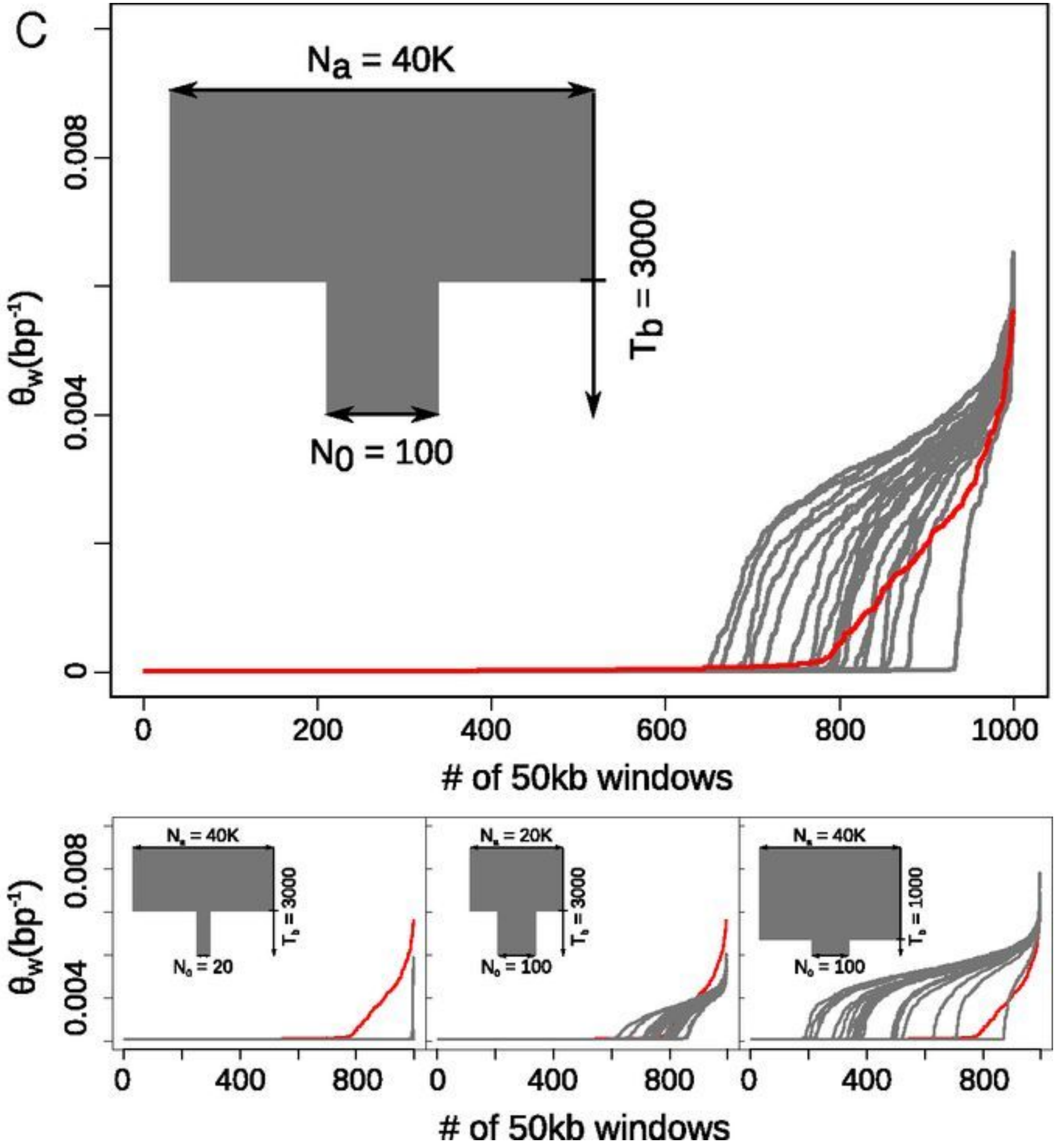


ABC-skyline (Boitard et al 2016), fastsimcoal2, abc *R* package

Past demography

Decline 3,000 years ago to about 100 individuals

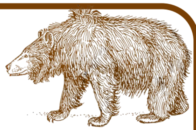


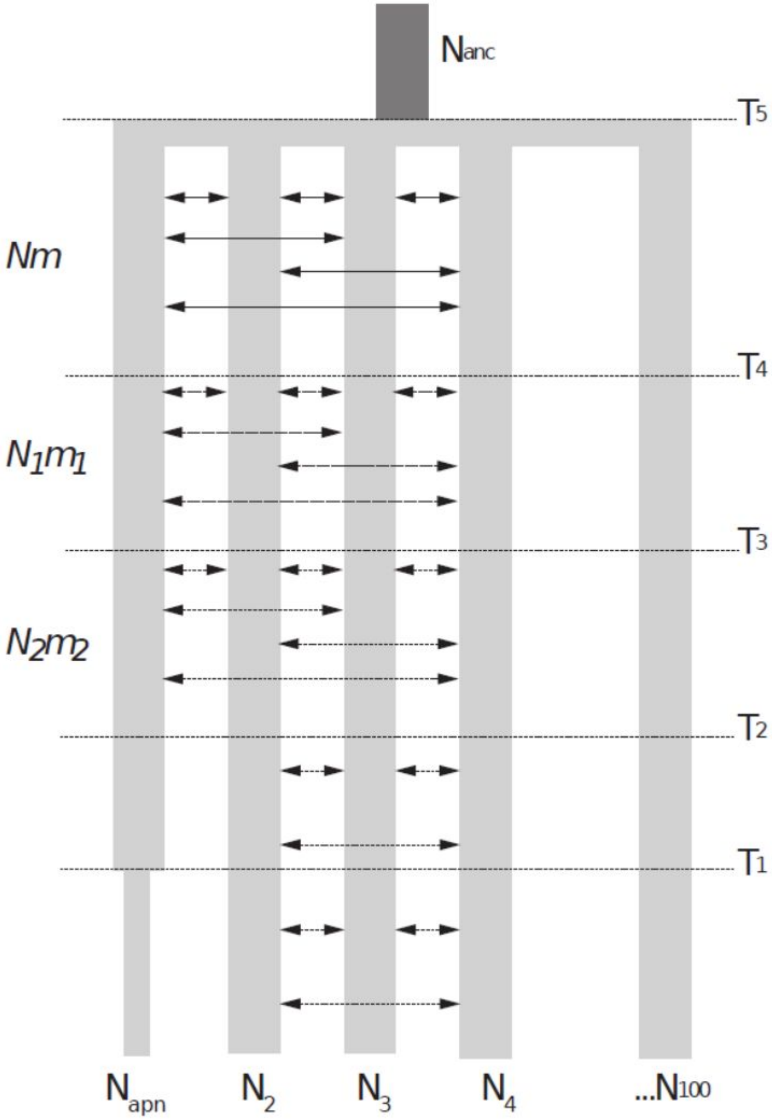


Simulation of expected pattern of homozygosity regions using *ms*

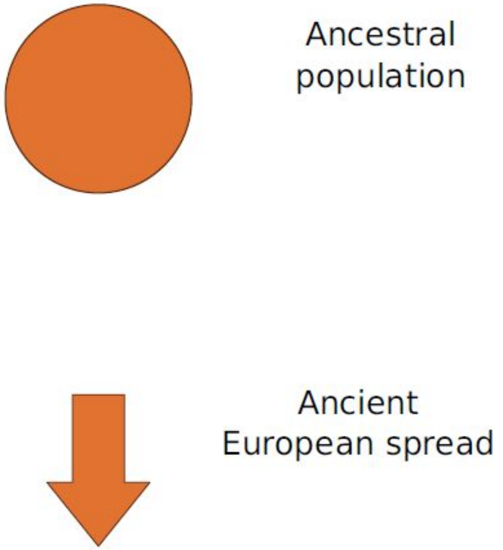
Past demography

Fragmentation of a large European population



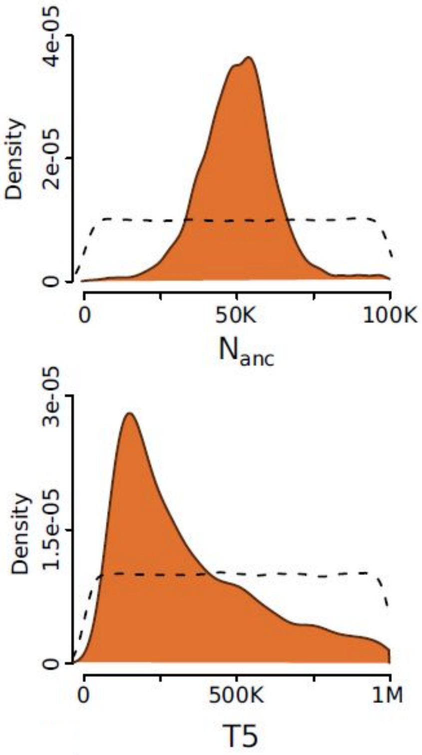


ABC metapopulation model



Ancestral population

Ancient European spread



Past demography

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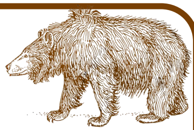

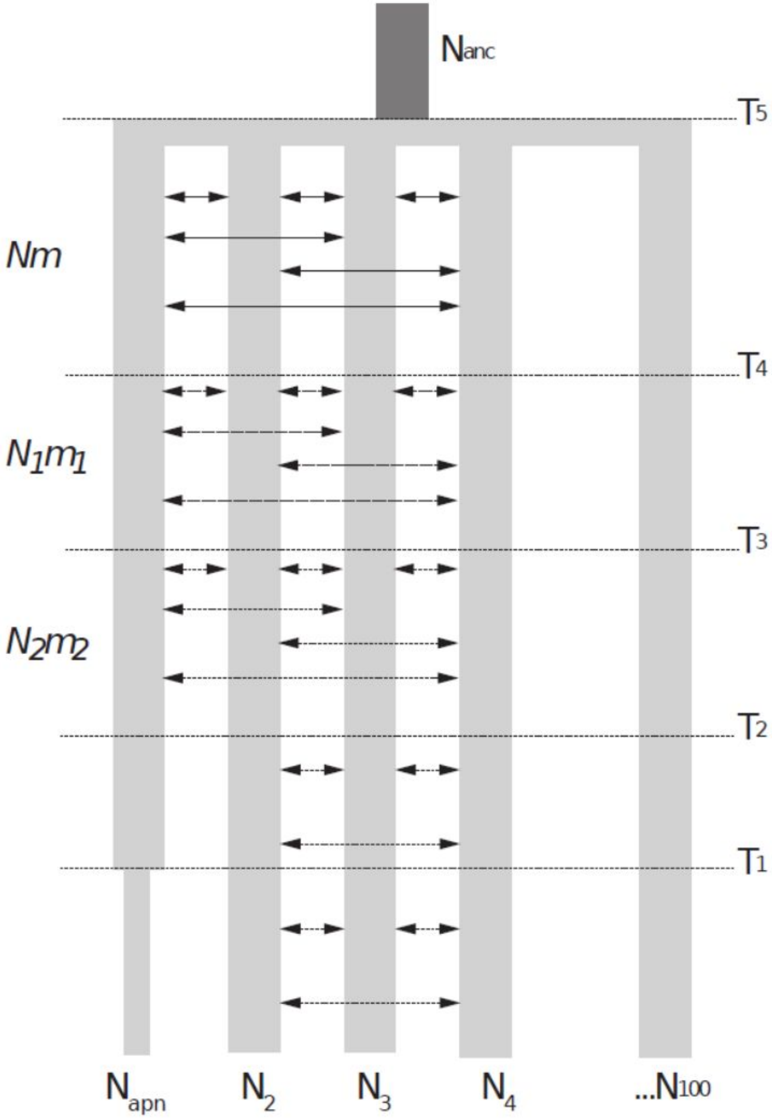

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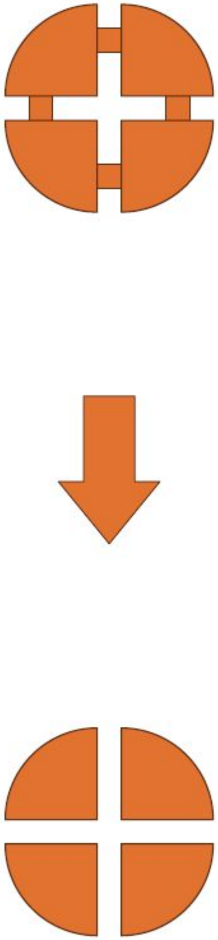
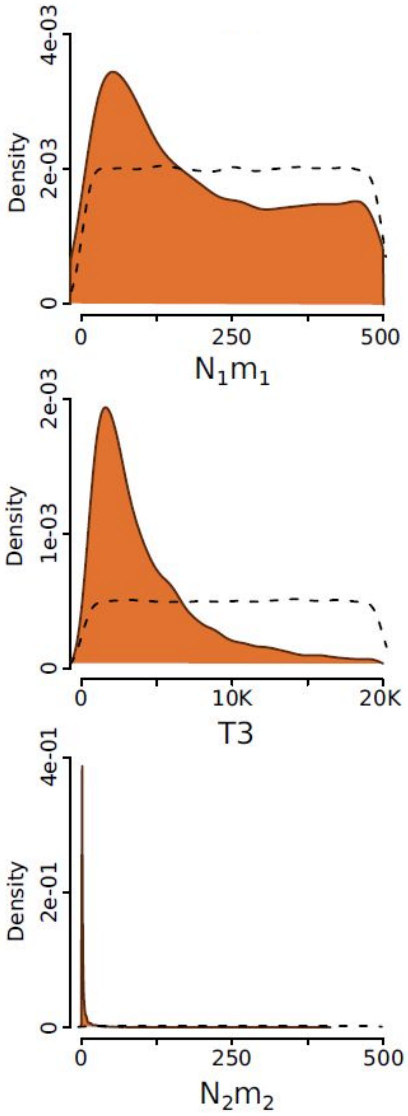


High connectivity among ancient populations

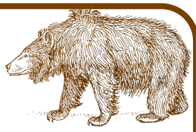
Recent fragmentation

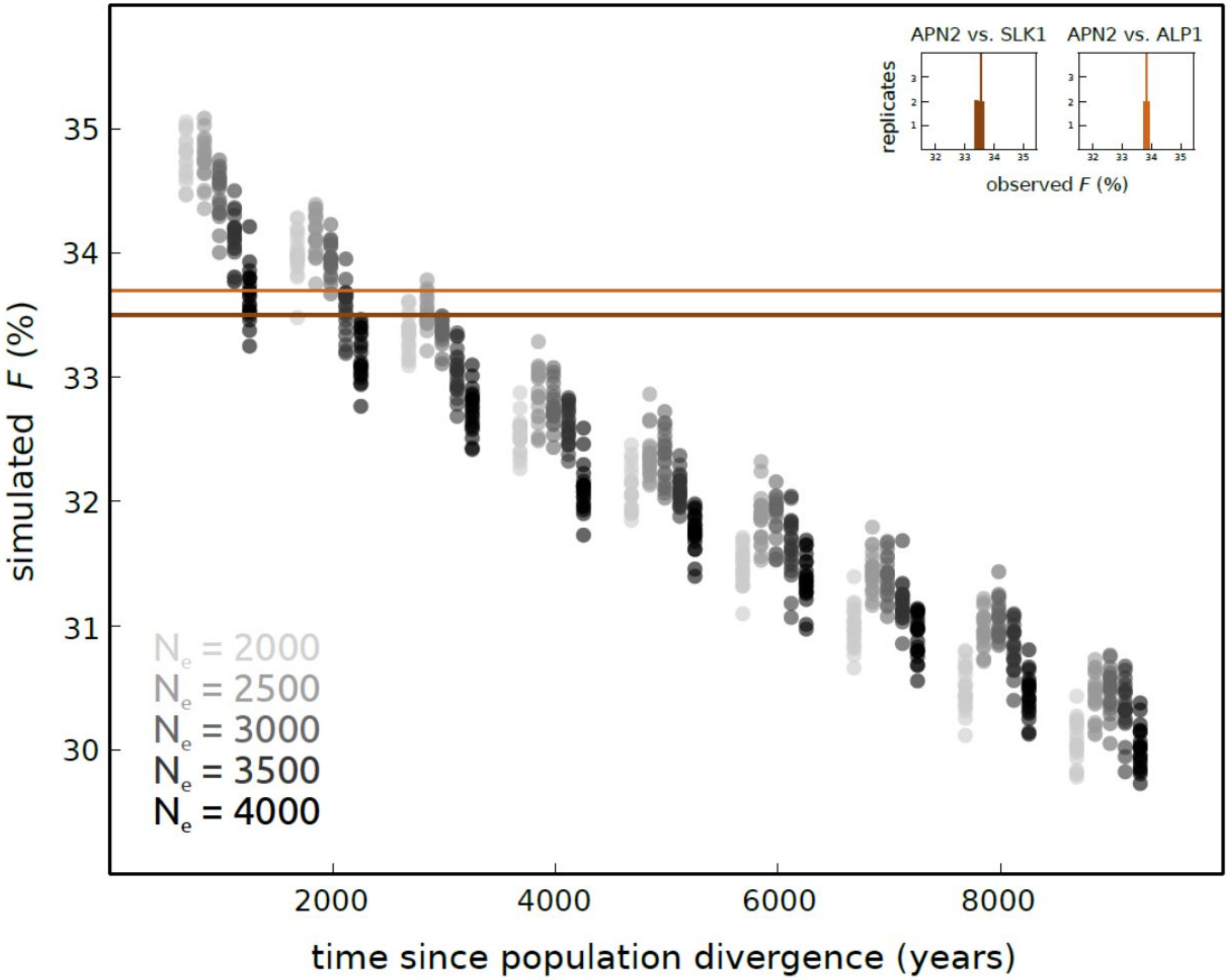
Low connectivity among modern populations



Past demography

Apennine bears have been isolated since then

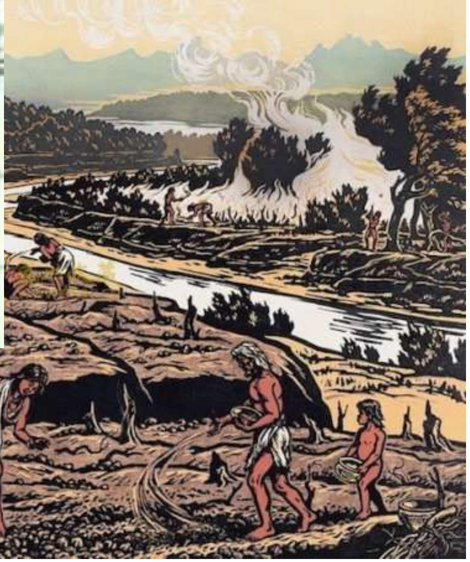




F statistic (Green et al 2010), simulation with ms

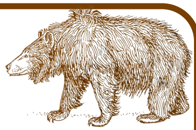
Past demography

Expansion of Neolithic farming in Europe burned forests



Why is this population still there?

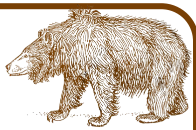
Given such high extinction probability

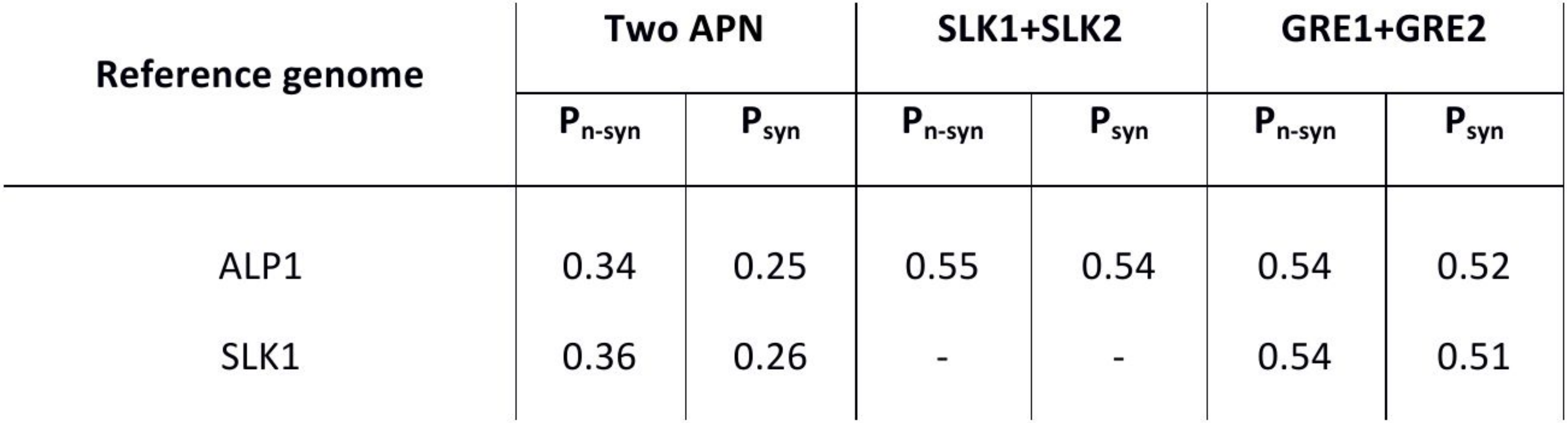


Credits: Fabrizio Caputi

Selective processes

Retention of non-synonymous polymorphisms





Reference genome	Two APN		SLK1+SLK2		GRE1+GRE2	
	$P_{n\text{-}syn}$	P_{syn}	$P_{n\text{-}syn}$	P_{syn}	$P_{n\text{-}syn}$	P_{syn}
ALP1	0.34	0.25	0.55	0.54	0.54	0.52
SLK1	0.36	0.26	-	-	0.54	0.51

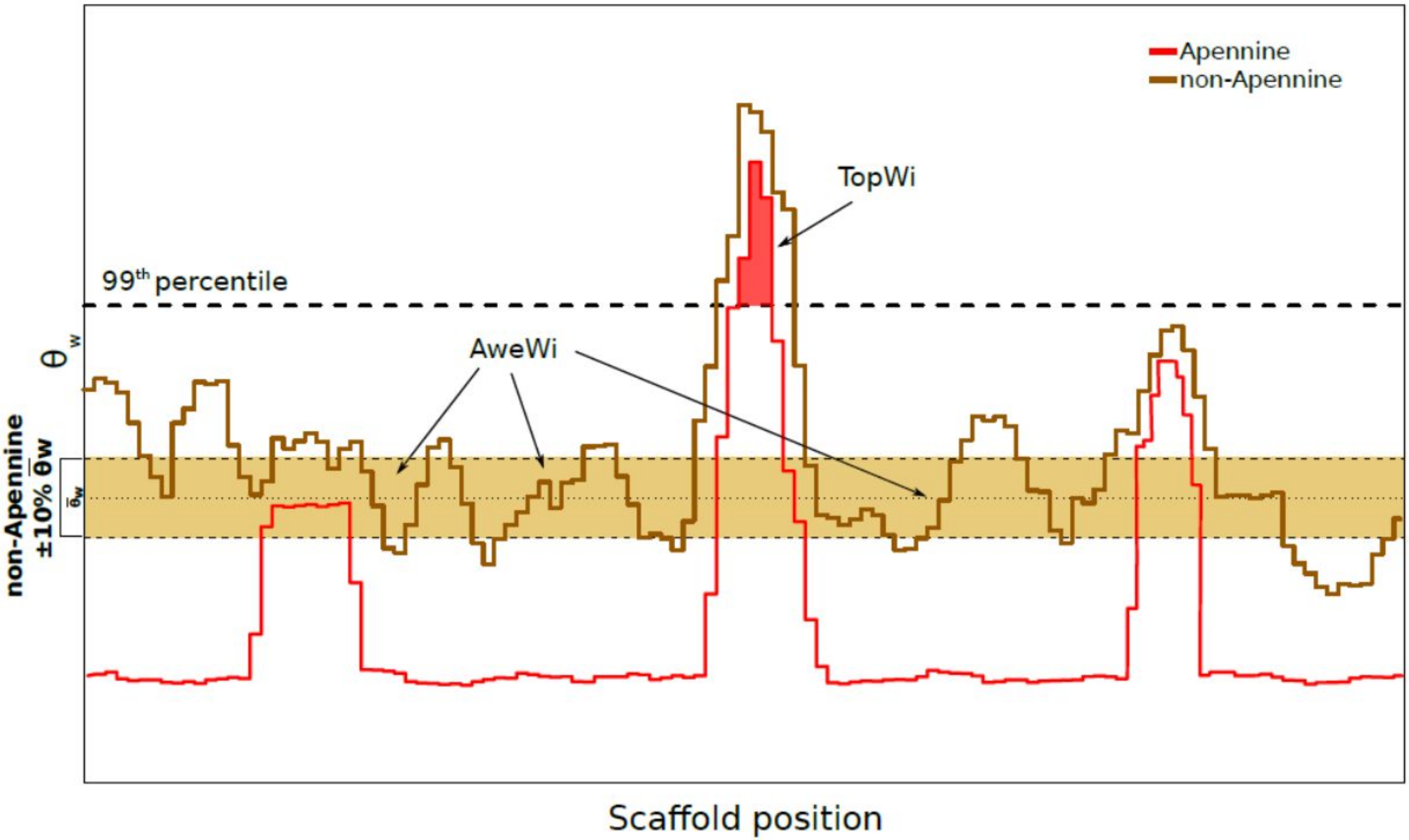
McDonald-Kreitman like test

Fraction of heterozygous sites in a European bear that are still heterozygote in the Apennine bear, for synonymous and nonsynonymous sites

Pn-syn = Psyn: drift
Pn-syn > Psyn: **balancing selection**
Pn-syn < Psyn: directional selection

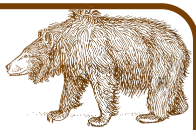
Selective processes: balancing

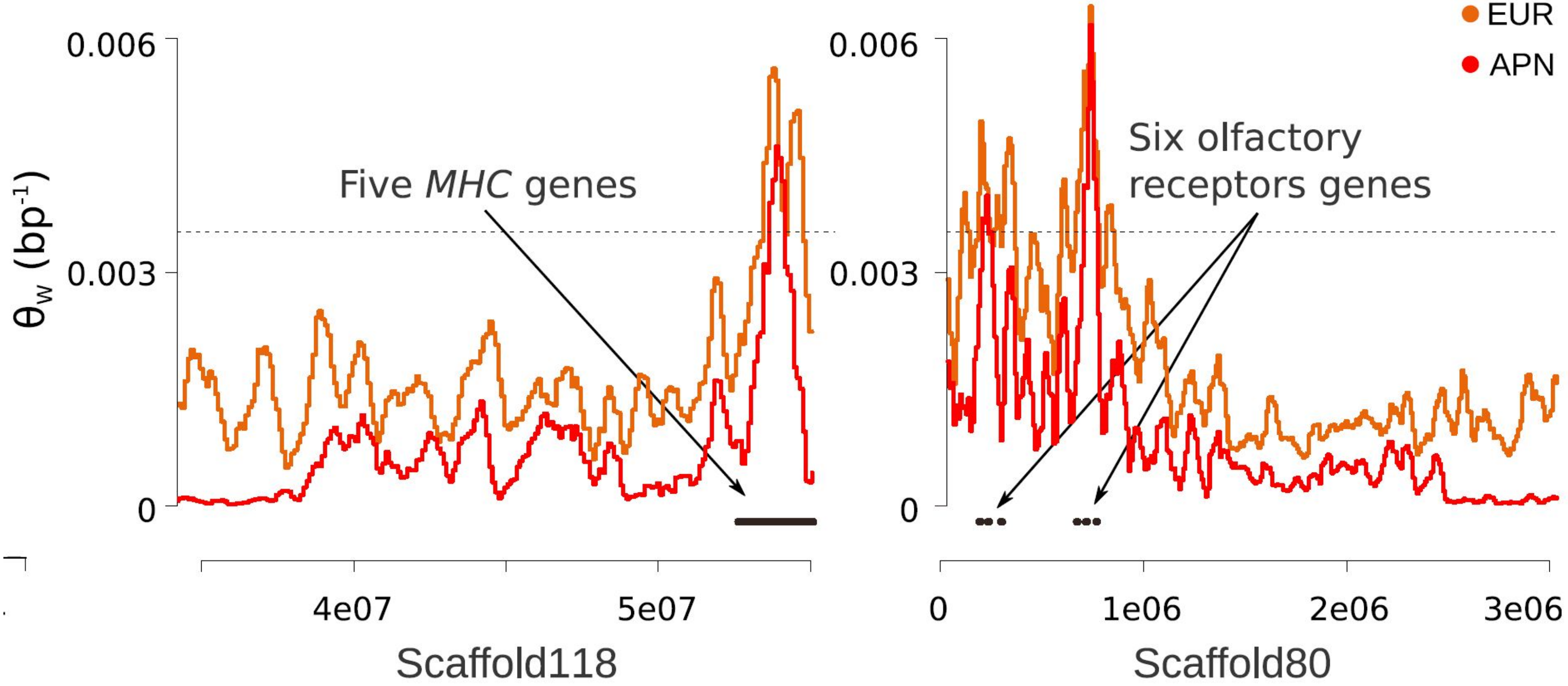
Retention of high diversity regions (HDR)



Selective processes: balancing

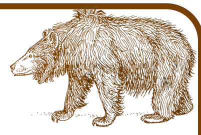
HDR are enriched in immune and olfactory genes

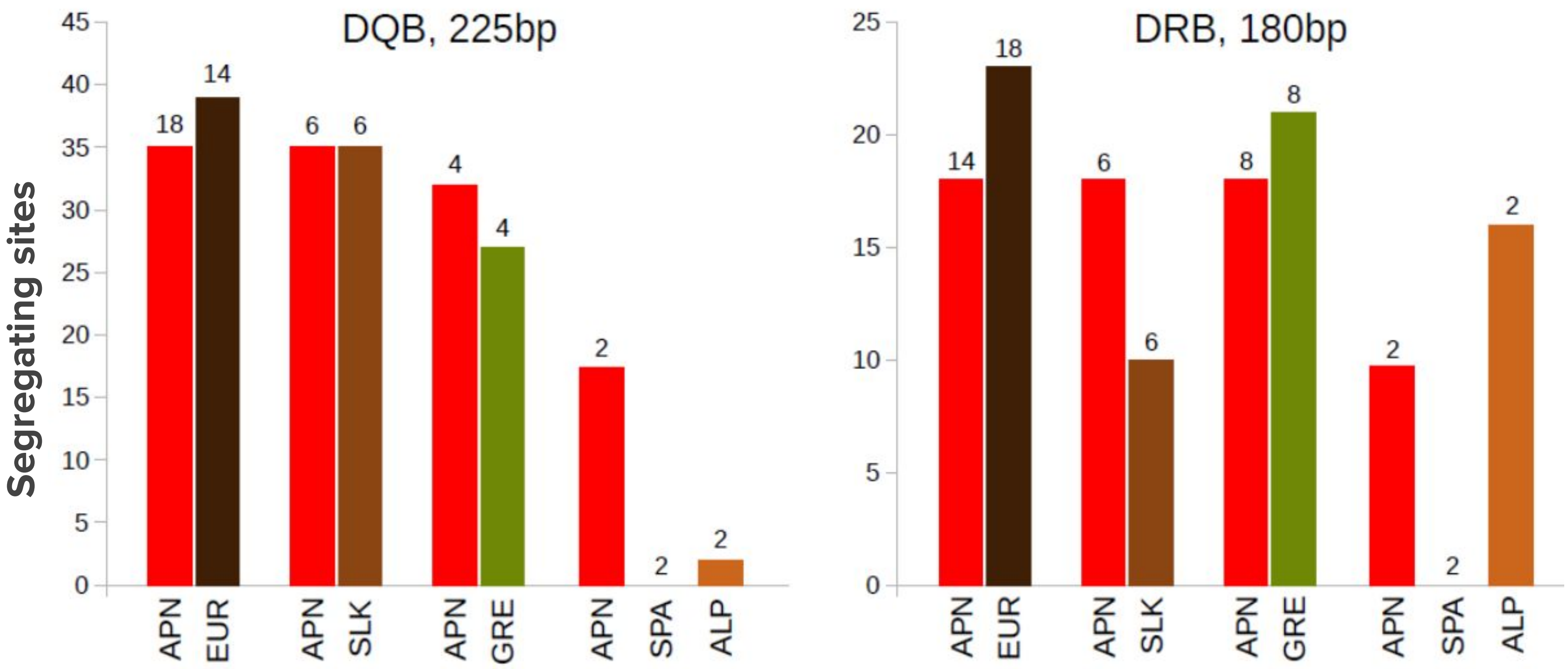




Selective processes: balancing

MHC genes are as diverse as in the rest of Europe

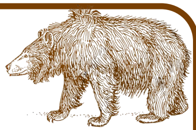




Sanger-sequencing of two *MHC* class II loci in additional individuals from Apennine and the rest of Europe

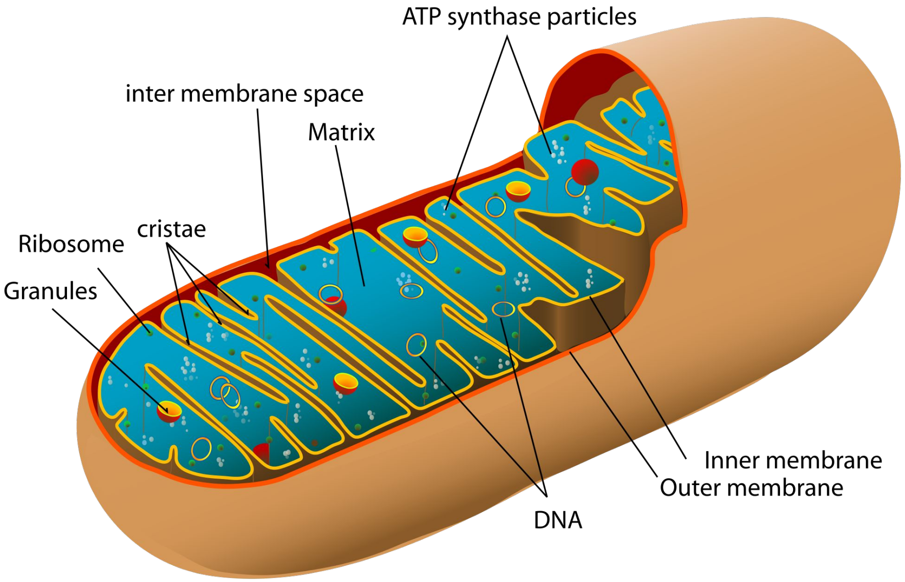
Genetic load

Accumulation of deleterious effects

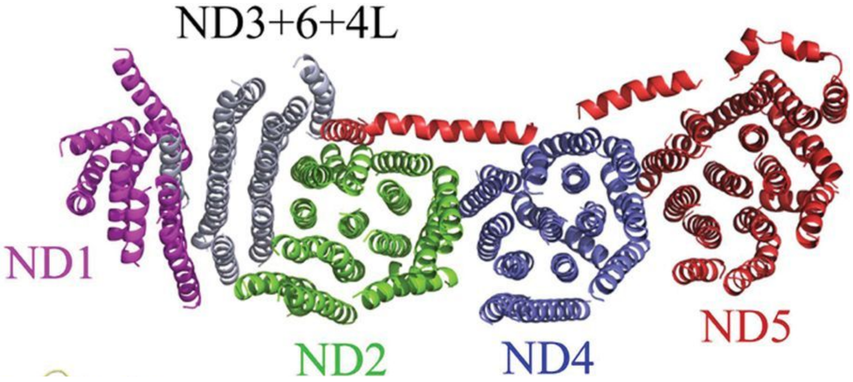


Predicted deleterious substitution fixed in the Apennine bears (by Panther + Polyphen): 40 + 4 stop codon

None in the other European bears!



5 in the **mt ND5** of which the most deleterious is not found elsewhere



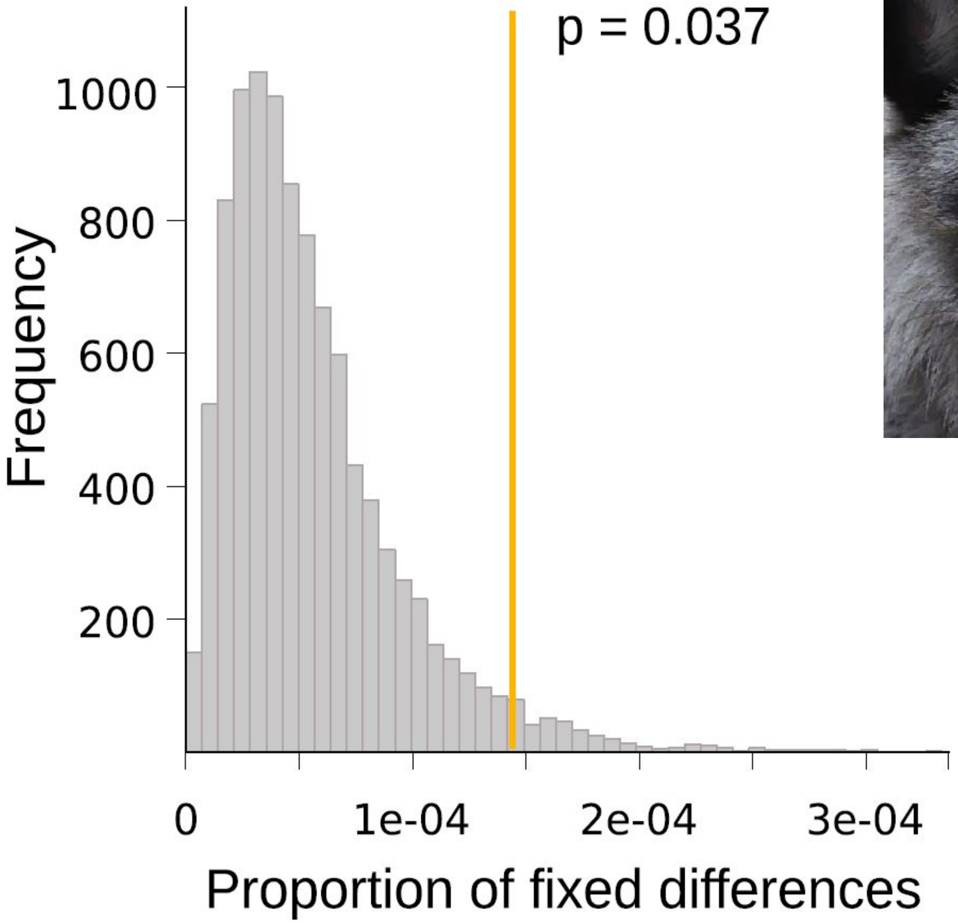
Gene	Position	#APNs (out of 6)	#NonAPNs (out of 45)	Score
ND5	G526E	6	0	-4.28
ND5	P447S	6	5	-3.59
ND5	T555A	6	3	-2.52

Genetic load in a specific group of genes

Are Apennine bears less aggressive?

Fixed differences in 22 “tameness” genes

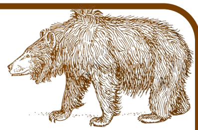
Not all deleterious fixations come to harm

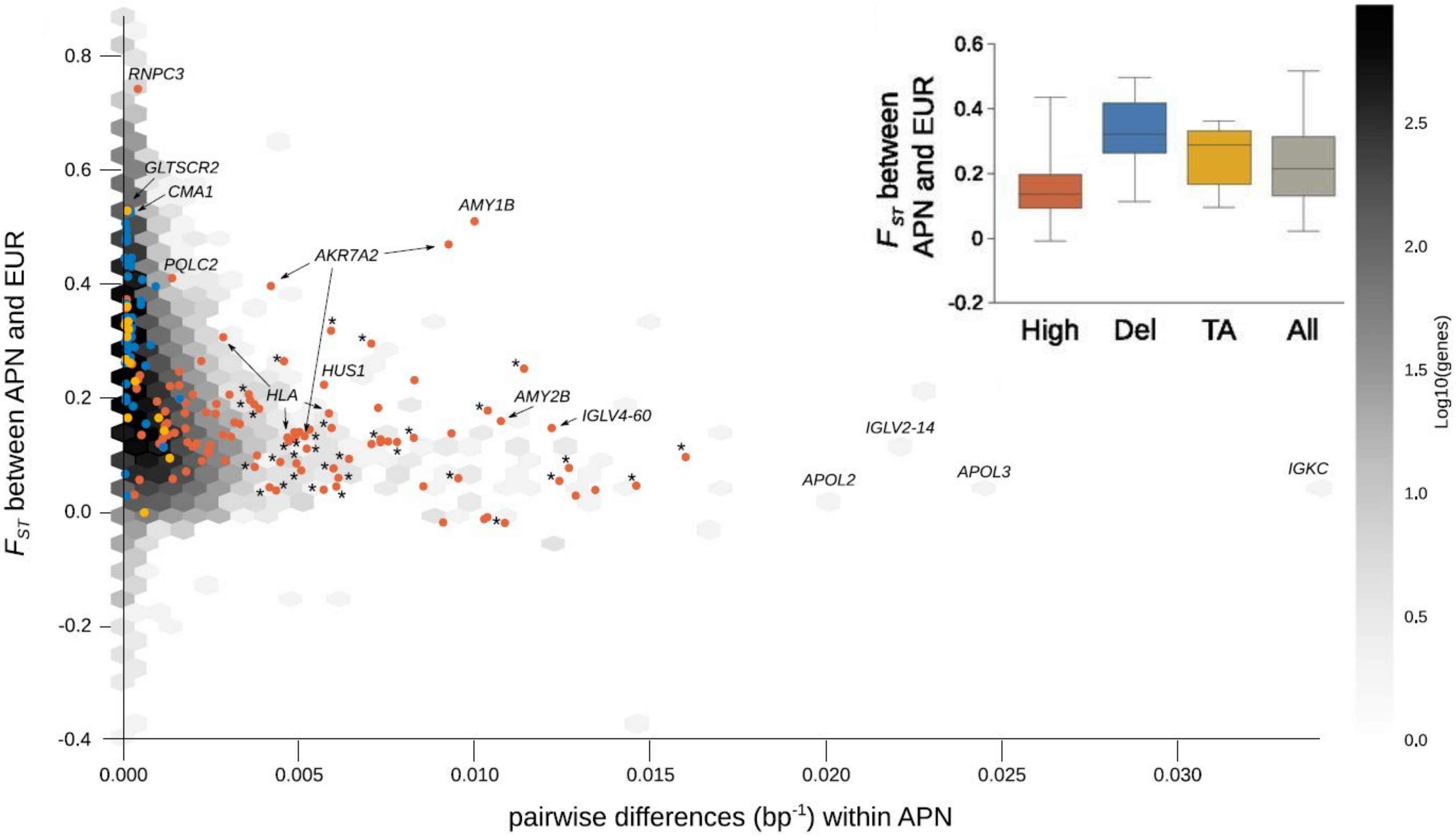


Pattern confirmed with **additional individuals** in three of these genes: PLAXNB1, DCC, DLL3 (not yet checked in the others)

Maintaining and losing diversity in genes

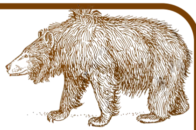
Balancing selection and deleterious alleles fixation by drift





Concluding

Surviving and diverging at very small population size



Apennine brown bear population is isolated since 300 generations (3,000 years) likely due to slash-and-burn agriculture during Neolithic expansion in Europe.

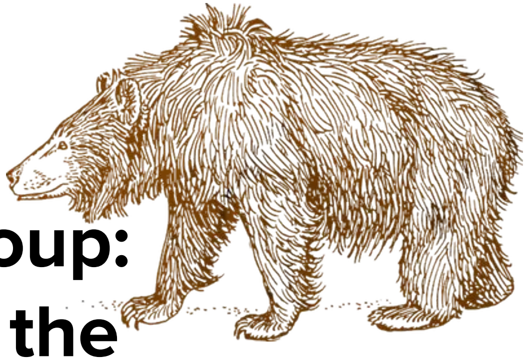
Population size has been rather small (less than 100-300) since then causing **high inbreeding and massive loss of diversity genome-wide**.

Genetic load of this population is high due to **fixation of deleterious substitutions** by genetic drift.

Some regions retaining **high genetic diversity due to functional or structural reasons** (duplicated loci, ectopic recombination) could have favoured survival.

Some alleles fixed by drift (or selected by hunting?) could have **changed behavior-related traits** decreasing human persecution of this population and, again, favouring survival.

(This work was done with just a handful of whole-genomes but about three years of work of quite some people!!)



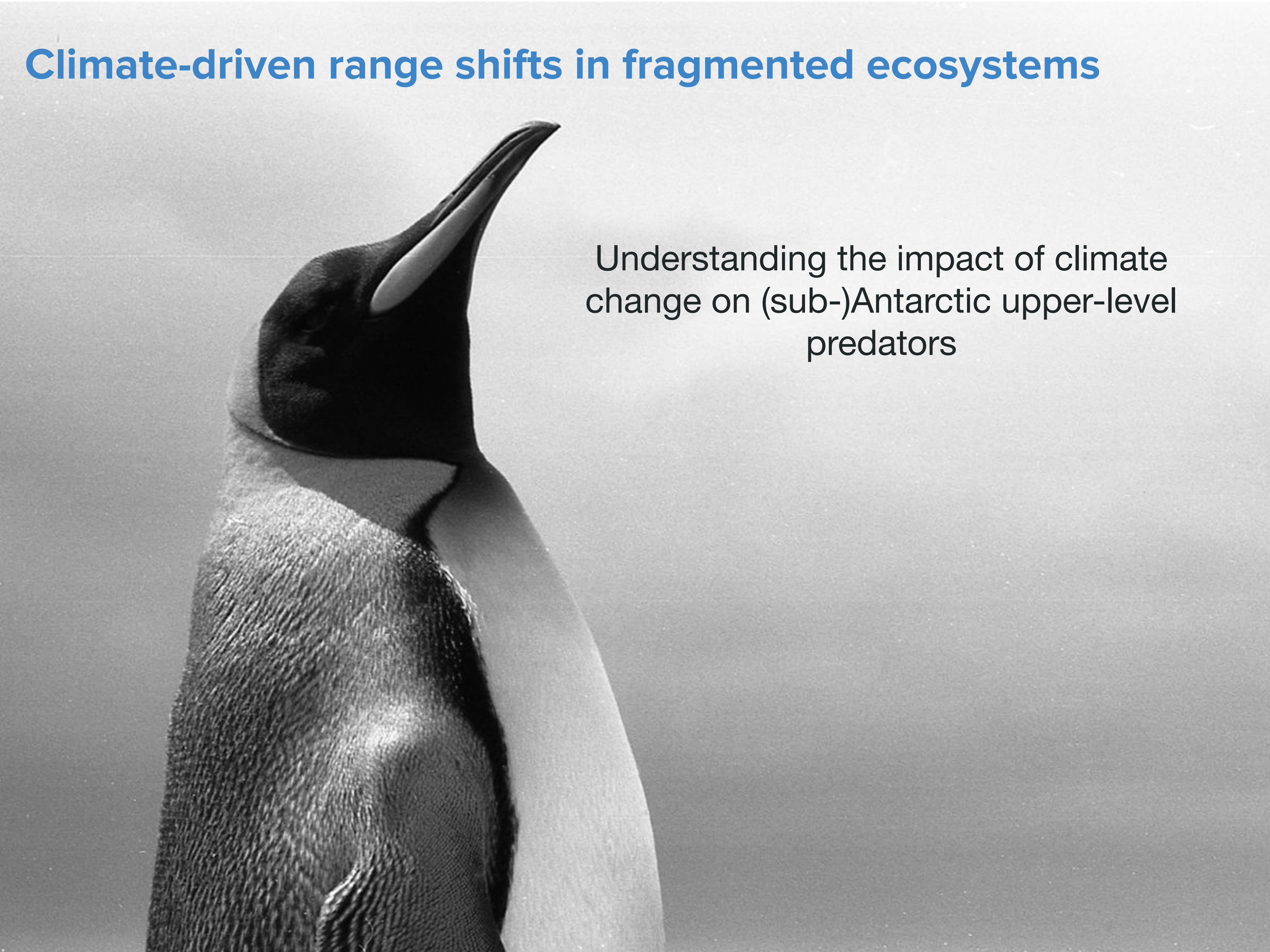
Survival and divergence in a small group: The extraordinary genomic history of the endangered Apennine brown bear stragglers

Andrea Benazzo*, Emiliano Trucchi*, James A. Cahill, Pierpaolo Maisano Delser, Stefano Mona, Matteo Fumagalli, Lynsey Bunnefeld, Luca Cornetti, Silvia Ghirotto, Matteo Girardi, Lino Ometto, Alex Panziera, Omar Rota-Stabelli, Enrico Zanetti, Alexandros Karamanlidis, Claudio Groff, Ladislav Paule, Leonardo Gentile, Carles Vilà, Saverio Vicario, Luigi Boitani, Ludovic Orlando, Silvia Fuselli, Cristiano Vernesi, Beth Shapiro, Paolo Ciucci, and Giorgio Bertorelle (2017) ***PNAS***, 114, E9589-E9597

UNIFE

SIDE B

Climate-driven range shifts in fragmented ecosystems

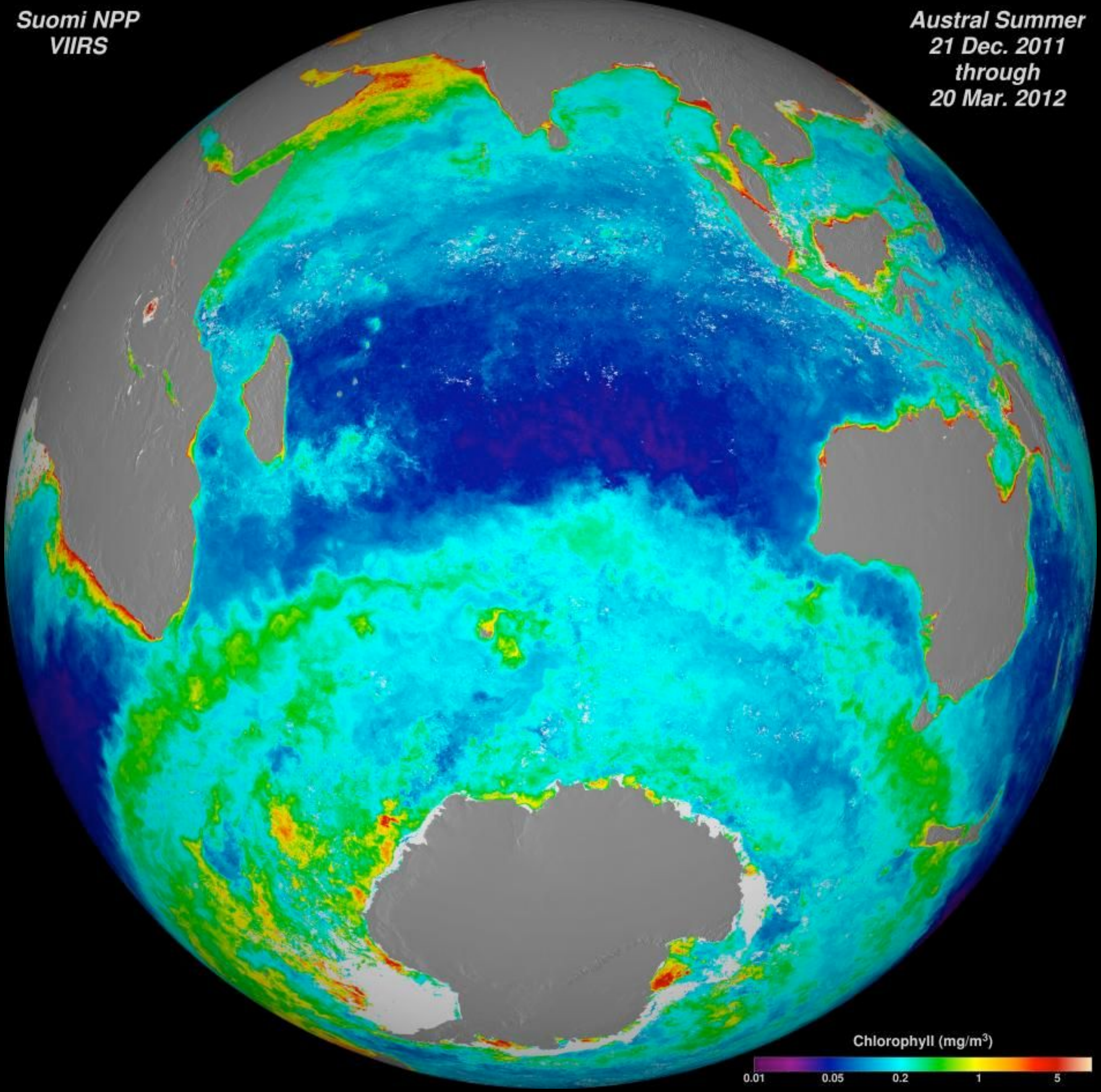


Understanding the impact of climate change on (sub-)Antarctic upper-level predators

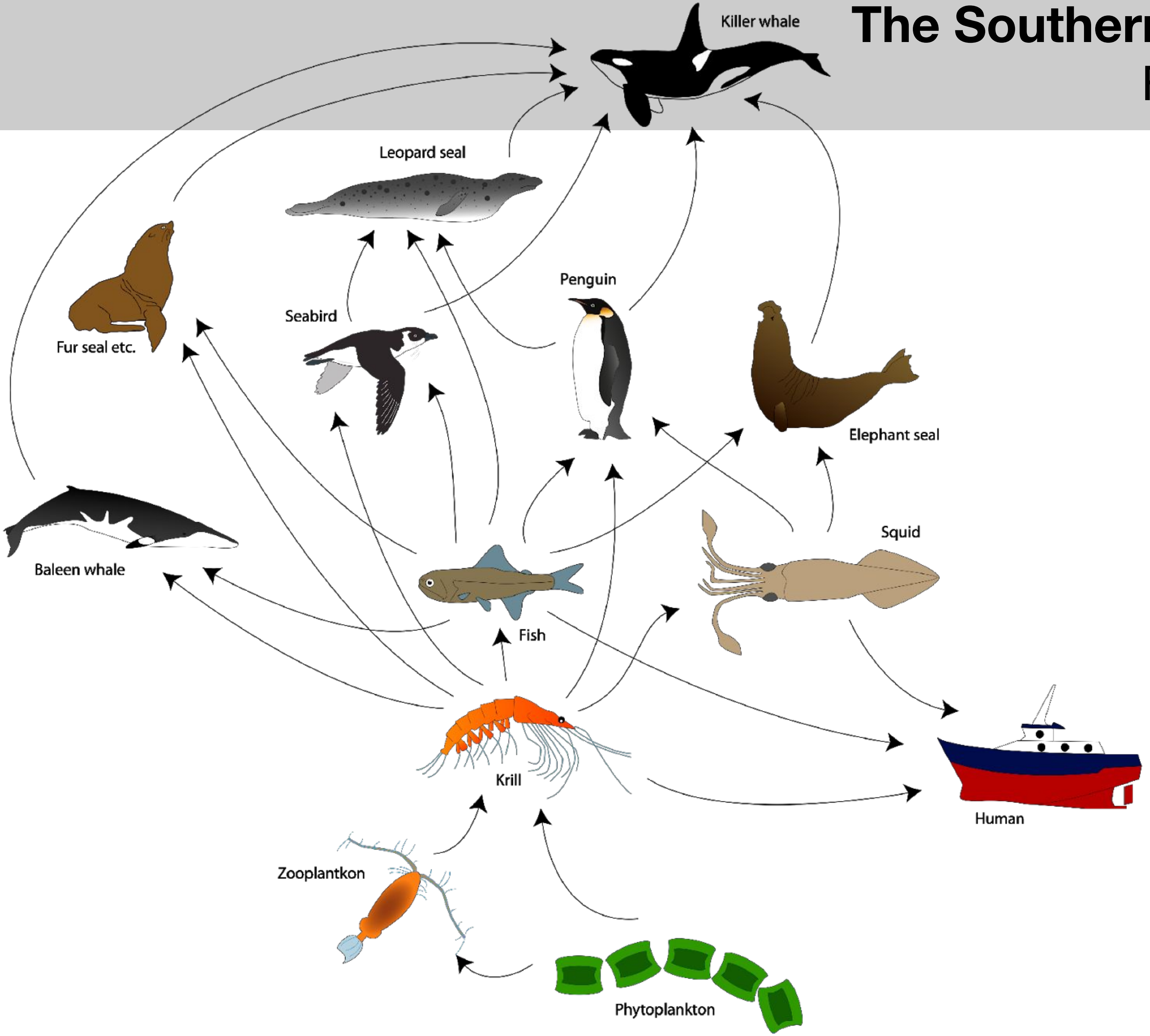
The Southern Ocean:
The Antarctic Polar Front

The Southern Ocean:

Chlorophyll and marine productivity



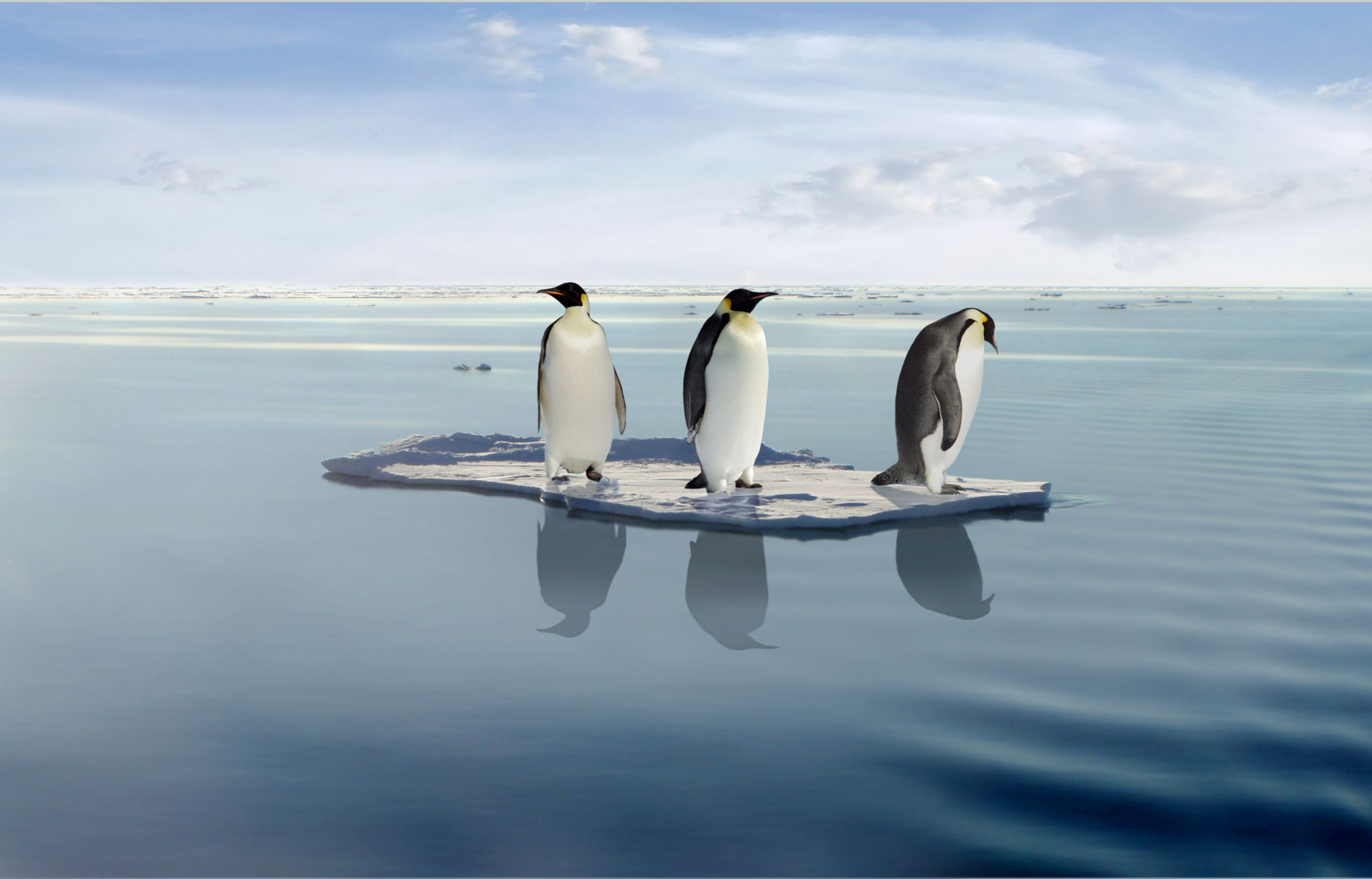
The Southern Ocean:
Food web



Global warming:

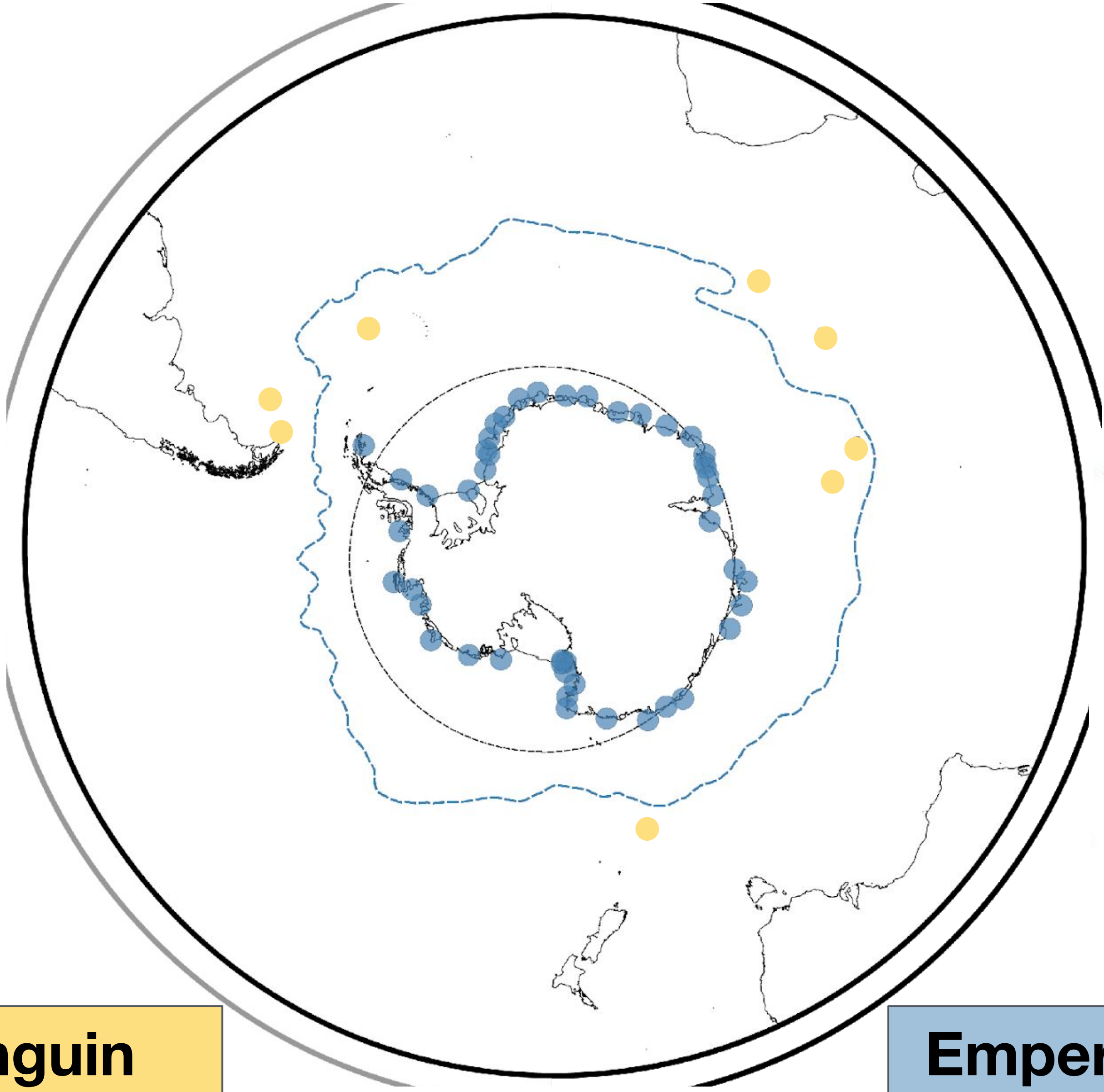
A fact

Global warming:
Which is the effect in Antarctic ecosystems?



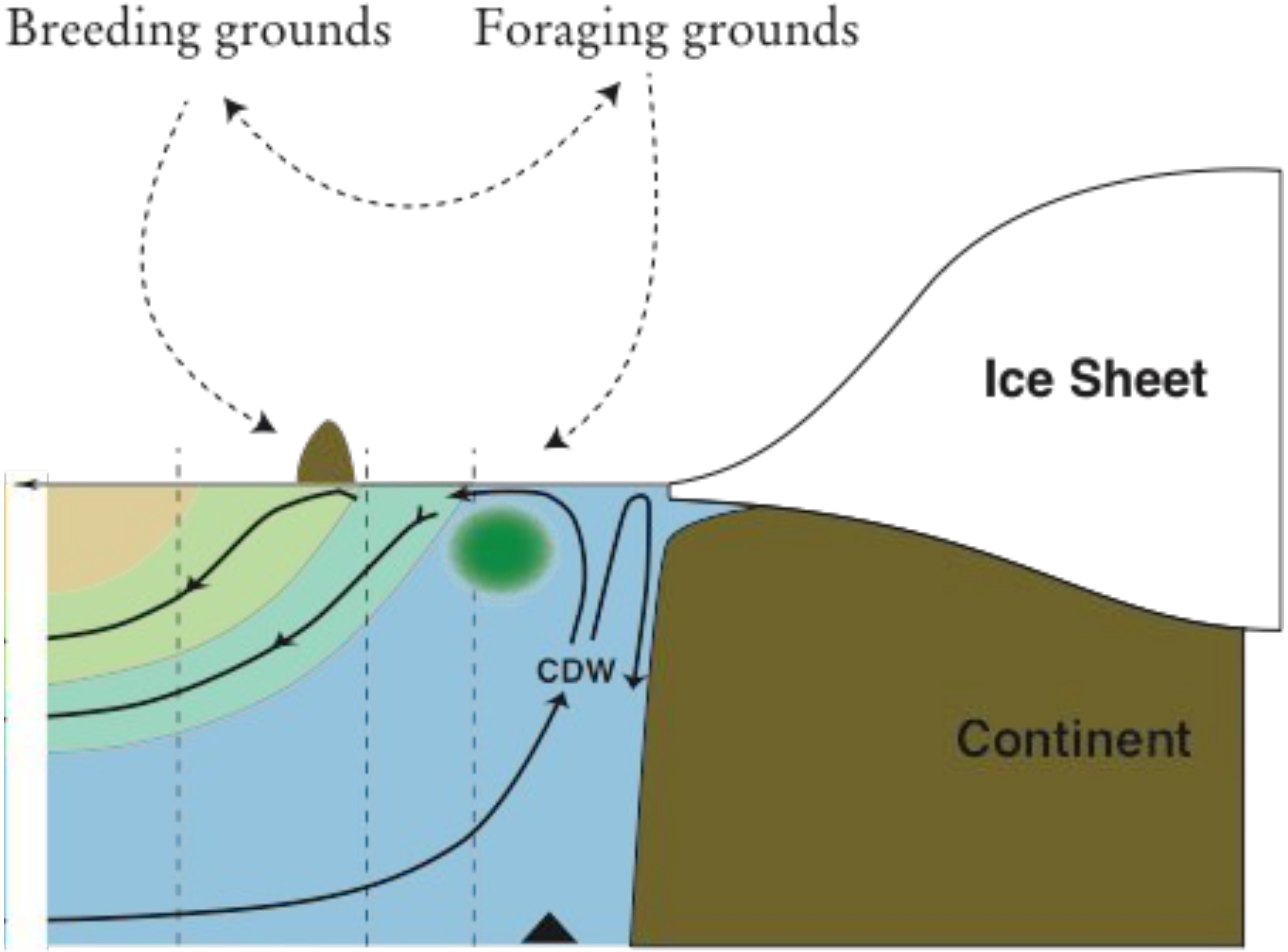
The Southern Ocean:
Penguins’ cradle

King penguin



Emperor penguin

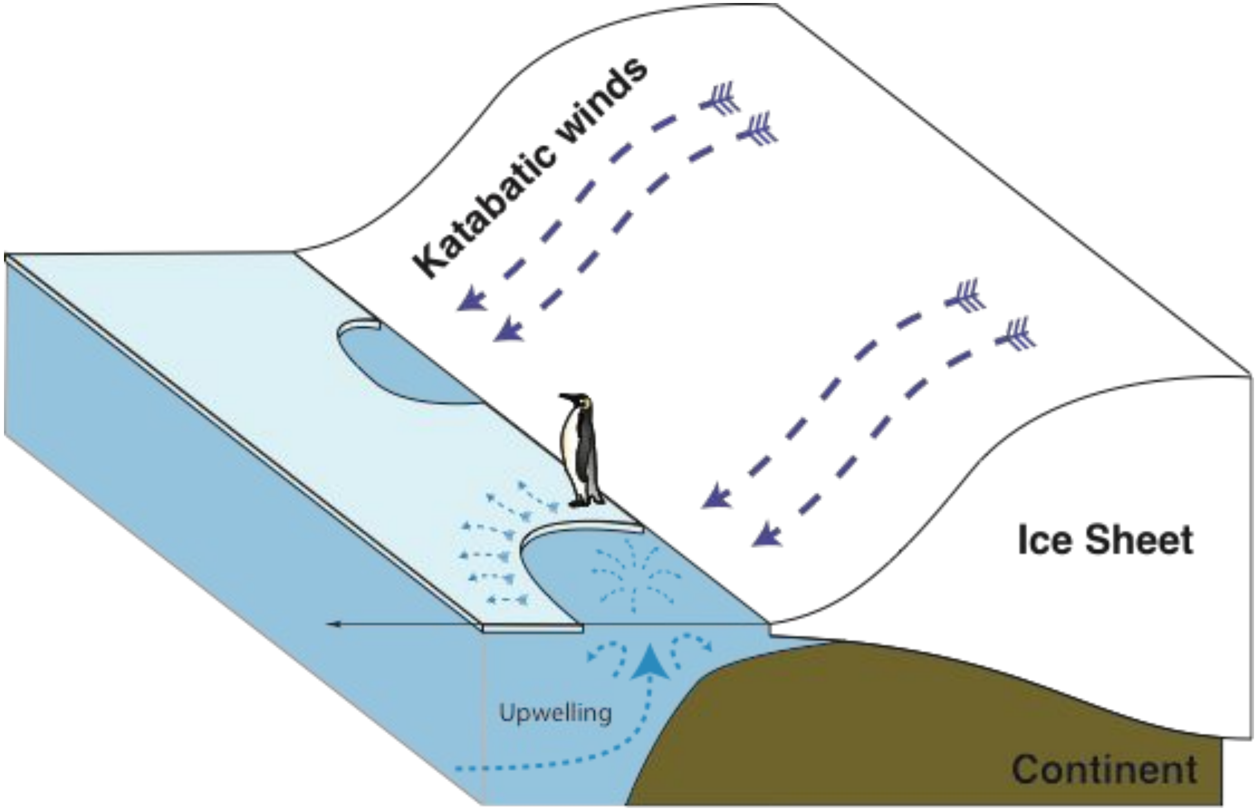
The King penguin’s foraging strategy:
The polar front area

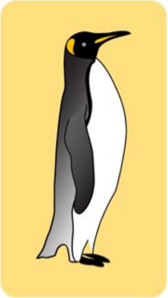

Breeding grounds

Foraging grounds

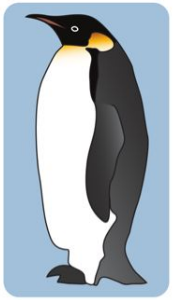
The Emperor penguin’s foraging strategy:

The coastal polynyas

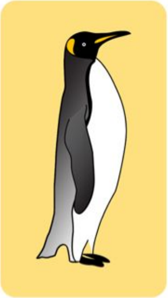




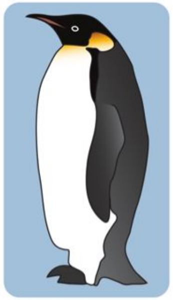
Question



Which will be the **effects of climate change** on **demography and distribution** of the two species?



Methods

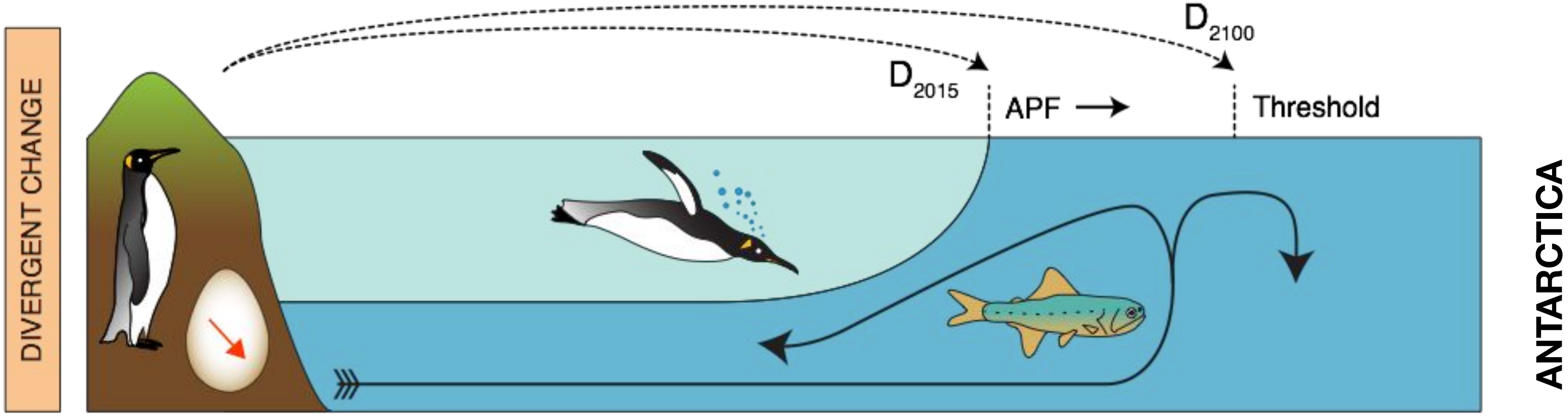


Species distribution modeling…

...coupled with **genomics** (RADseq + WGS) to validate model assumptions and hindcasting results

Our model of habitat suitability

Increasing temperature and shifting Polar Front



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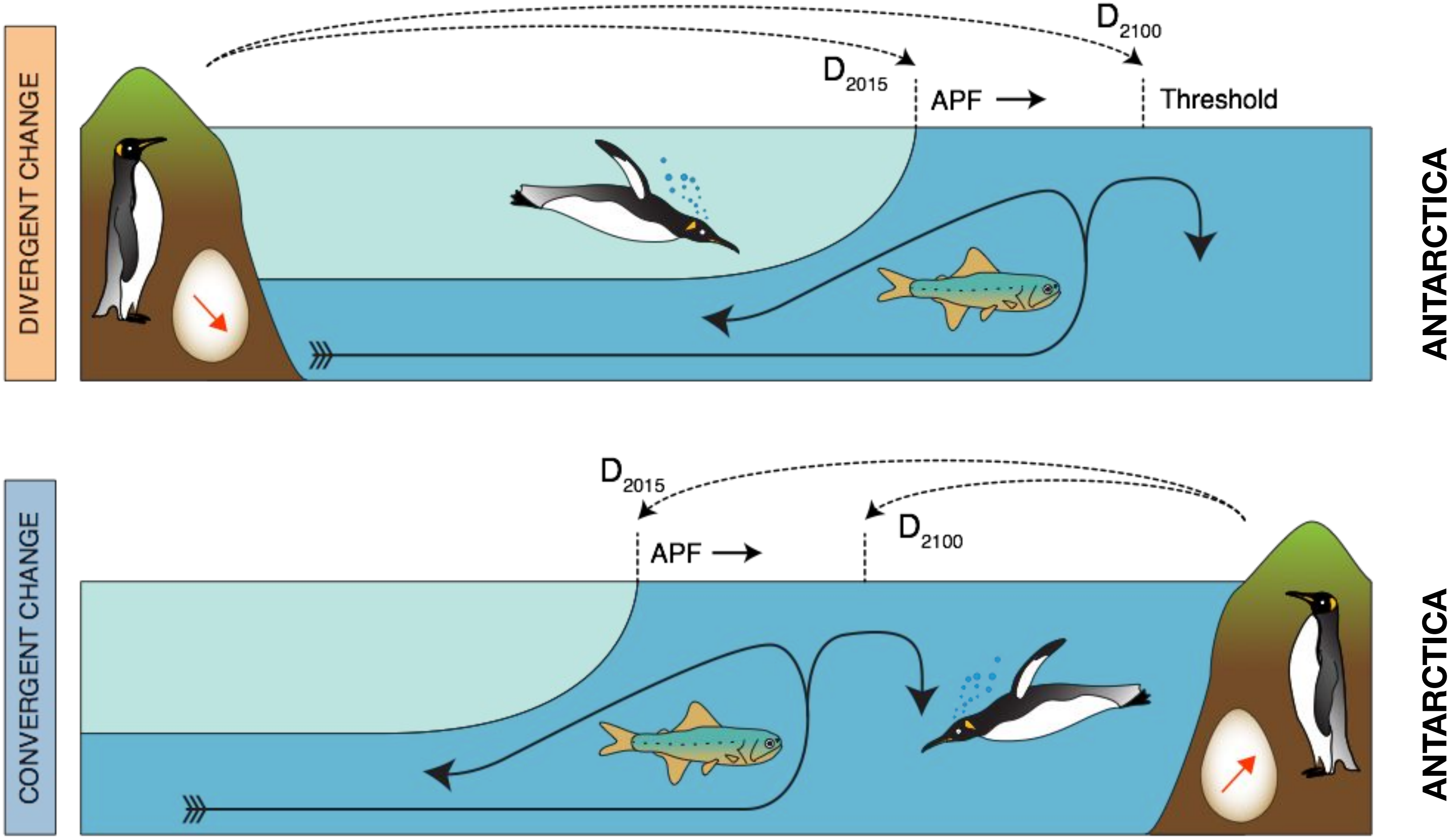
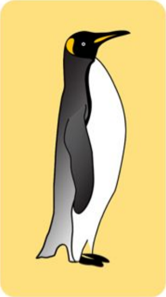
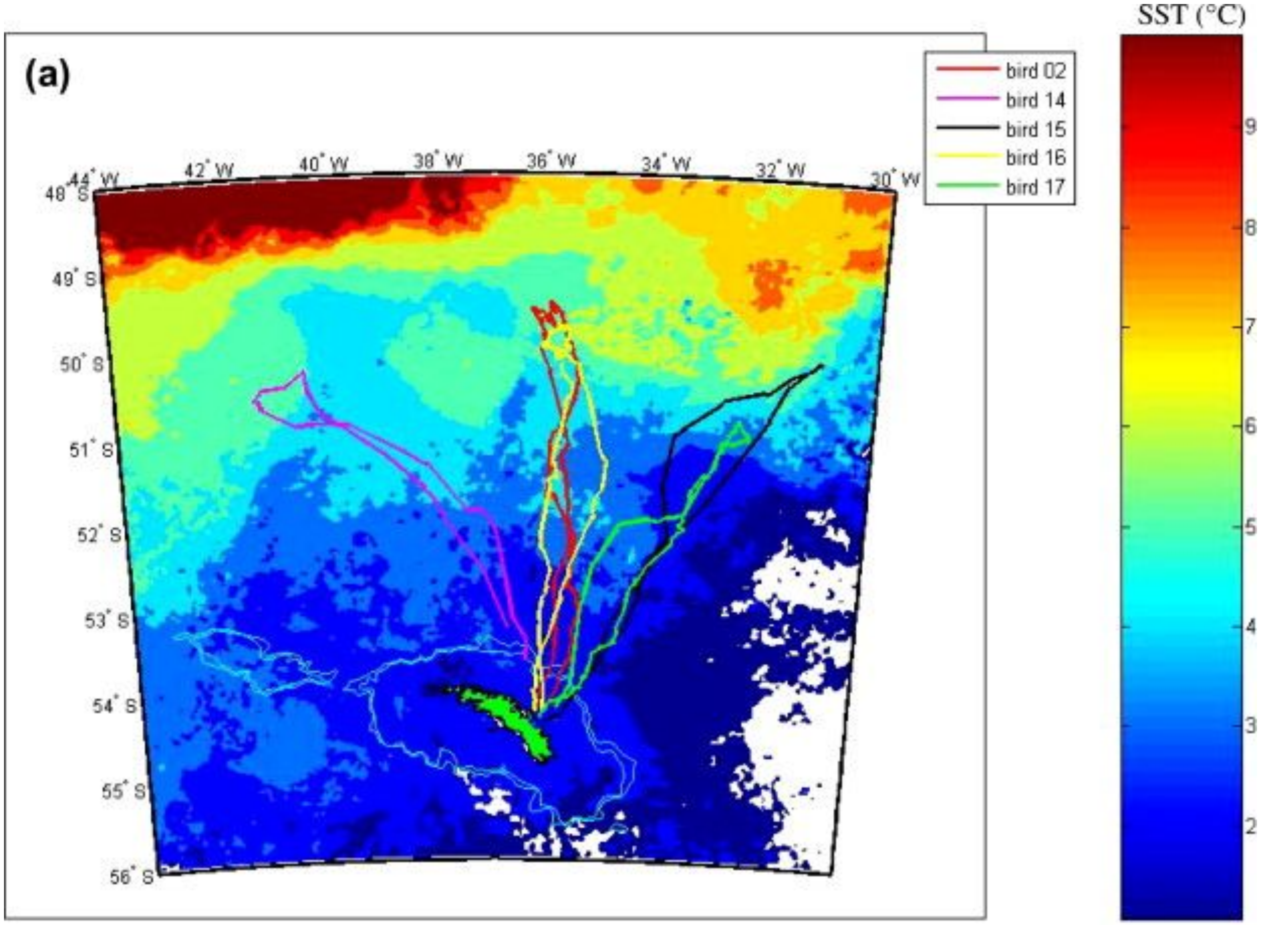




Bio-physical ecological niche model

Major constraints

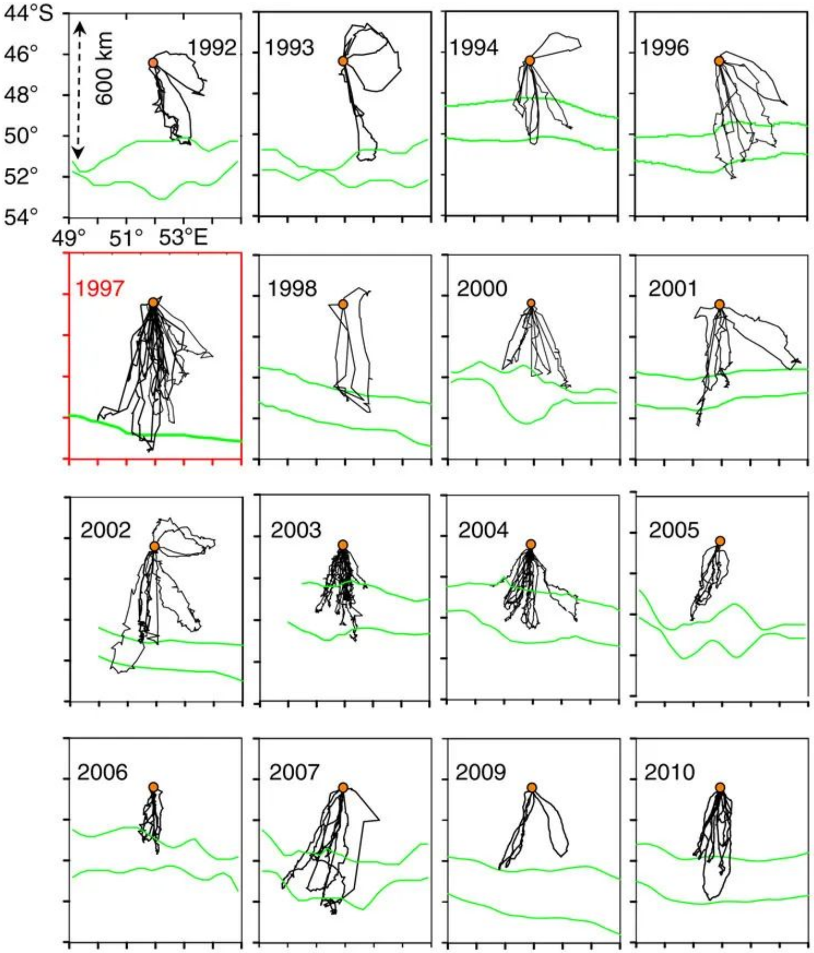


Scheffer *et al.* 2010

Sea Surface Temperature (SST) and efficient foraging

Bio-physical ecological niche model

Major constraints



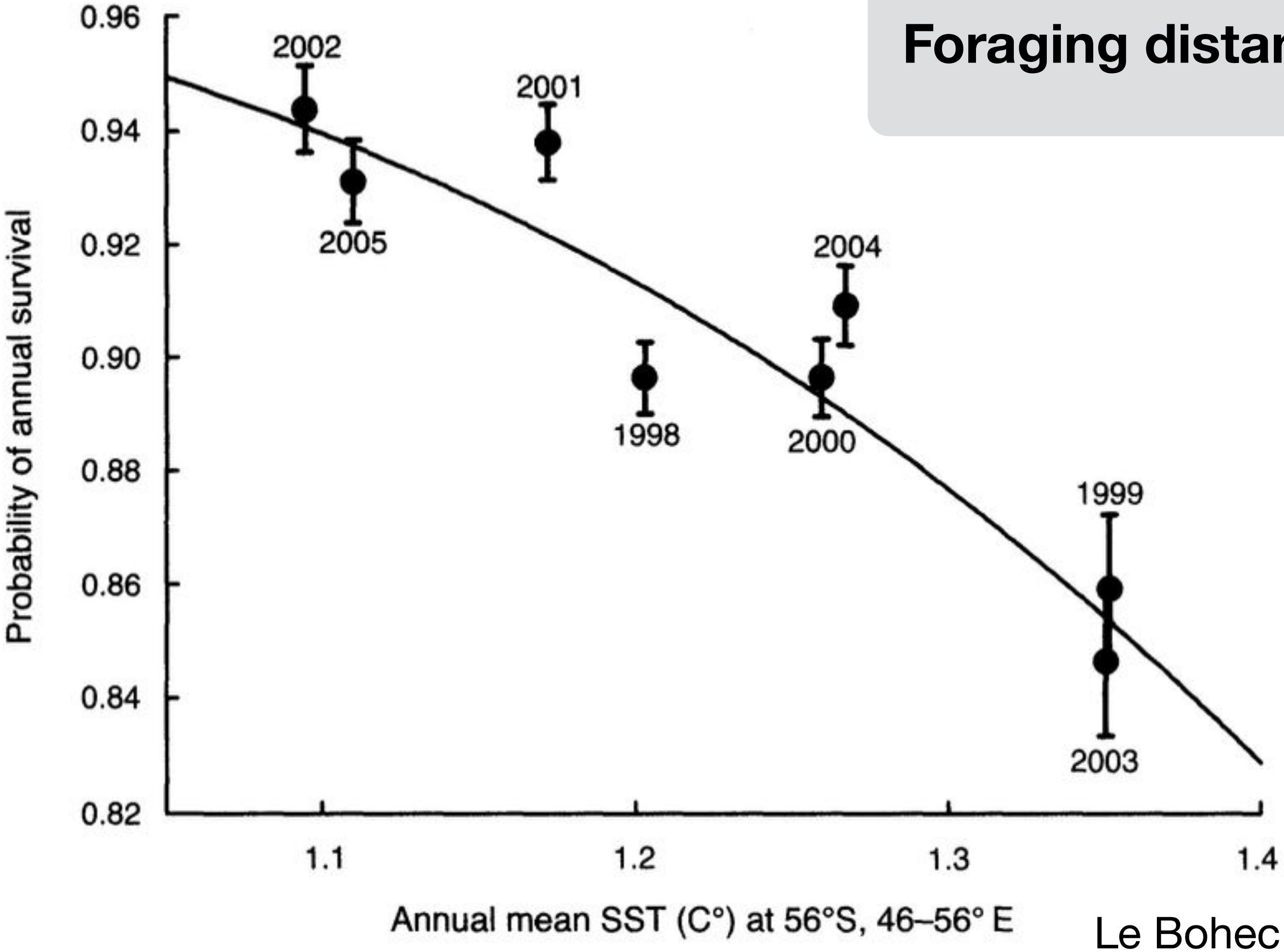
Sea Surface Temperature (SST) and efficient foraging

Bost *et al.* 2015

Bio-physical ecological niche model

Major constraints

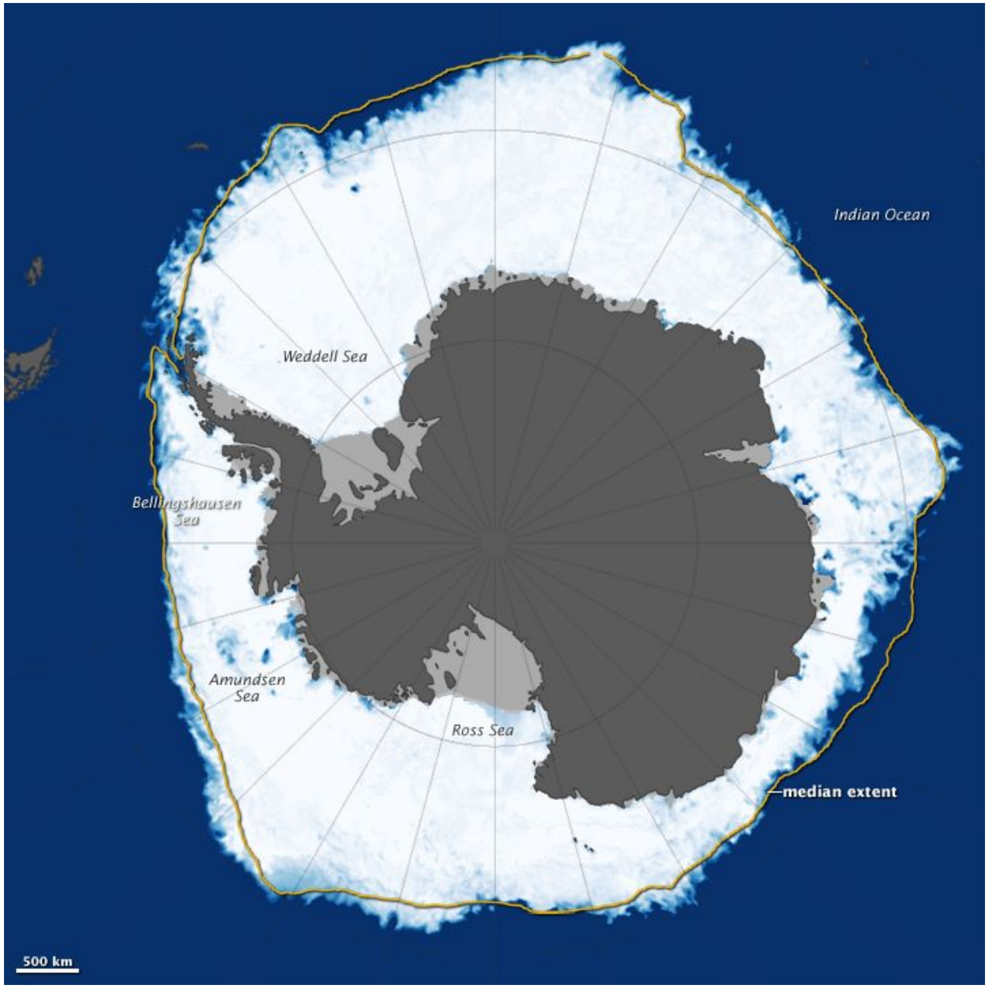
Foraging distance -> 700 km limit



Le Bohec *et al.* 2008

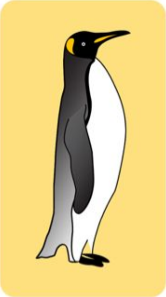
Bio-physical ecological niche model

Major constraints



NASA Earth Observatory

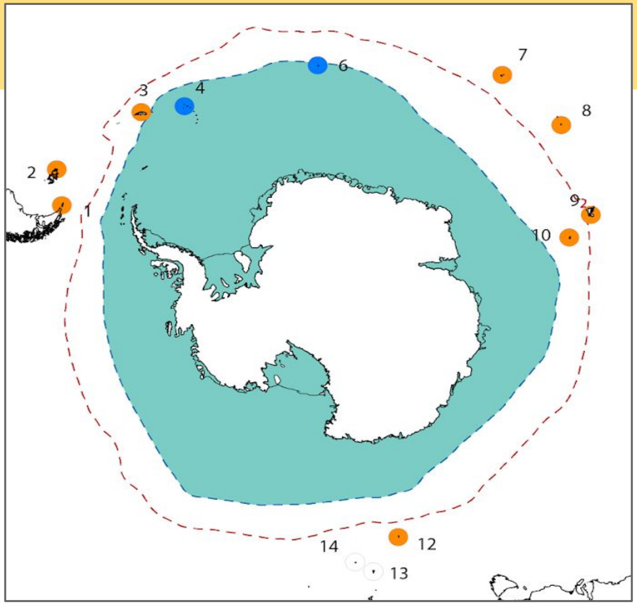
Temperature -> year-round ice-free breeding grounds

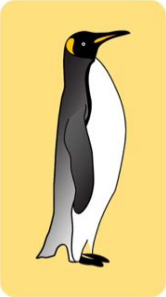


Our model finds all suitable islands

Accurate description of current distribution

PRESENT

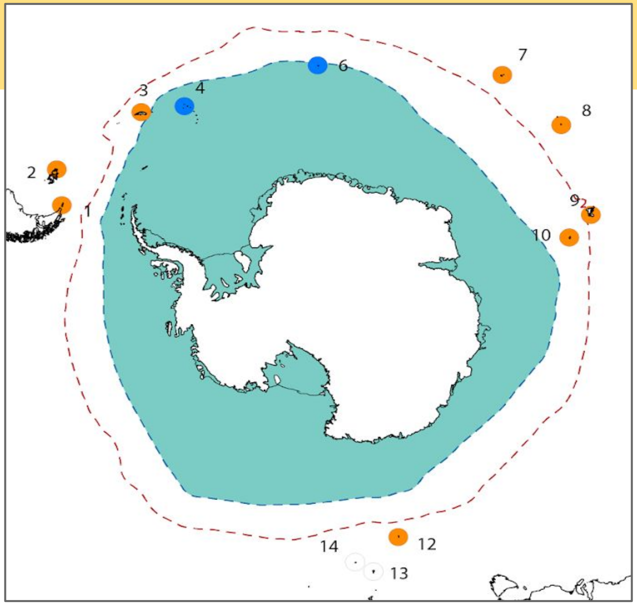




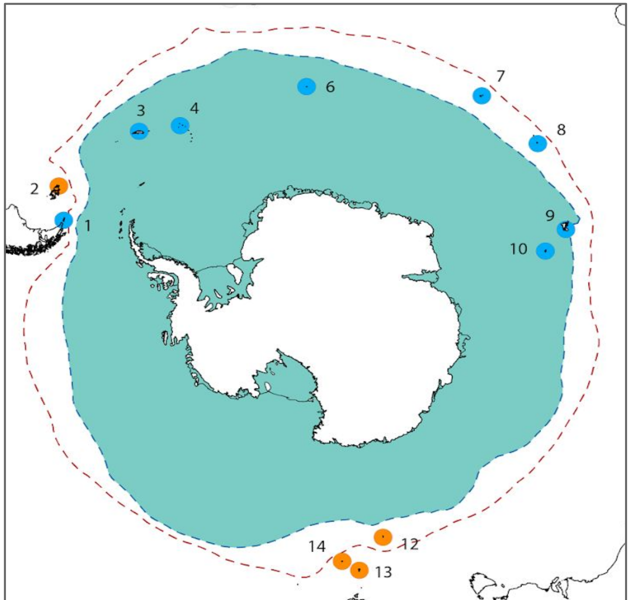
Our model suggests cold ice age refugia

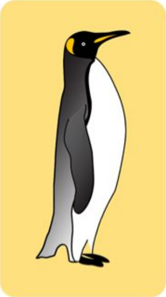
Hindcasting very few suitable islands at the LGM

PRESENT



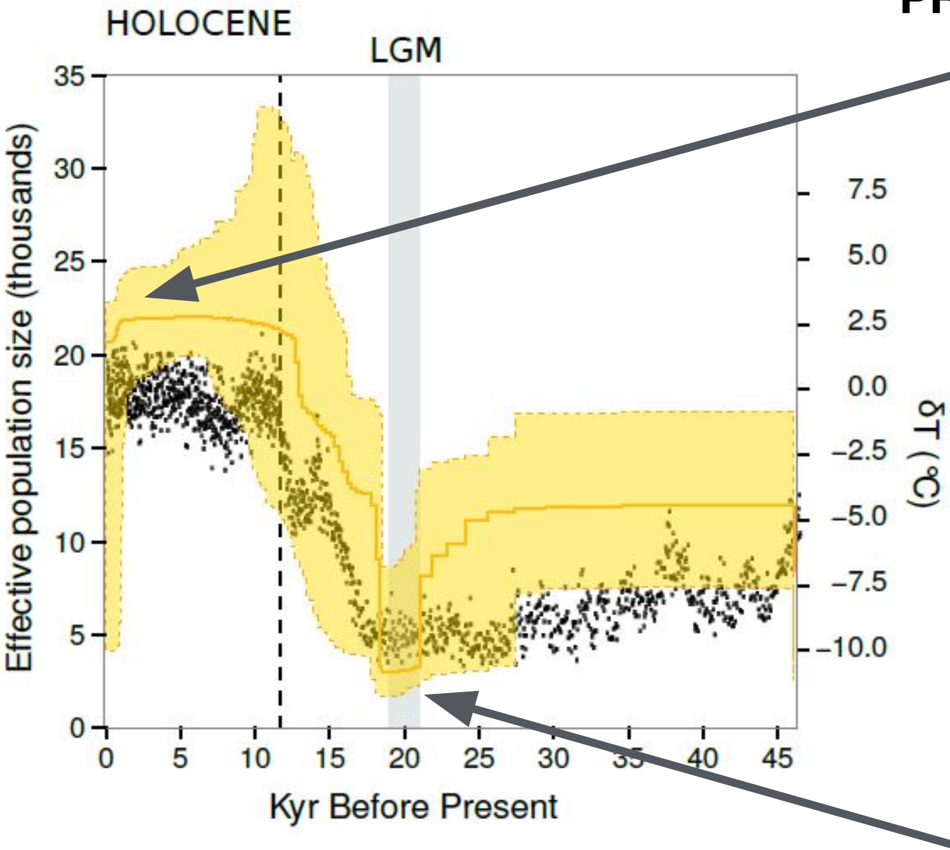
PAST





Genomic inference of past demography

In agreement with the species distribution model



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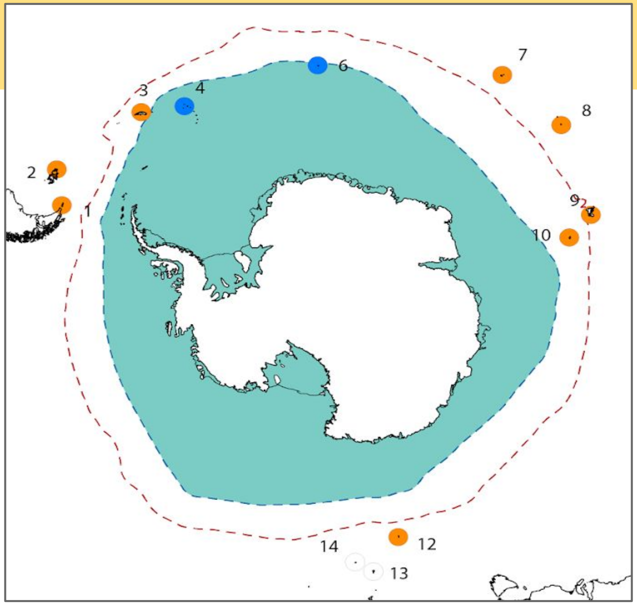
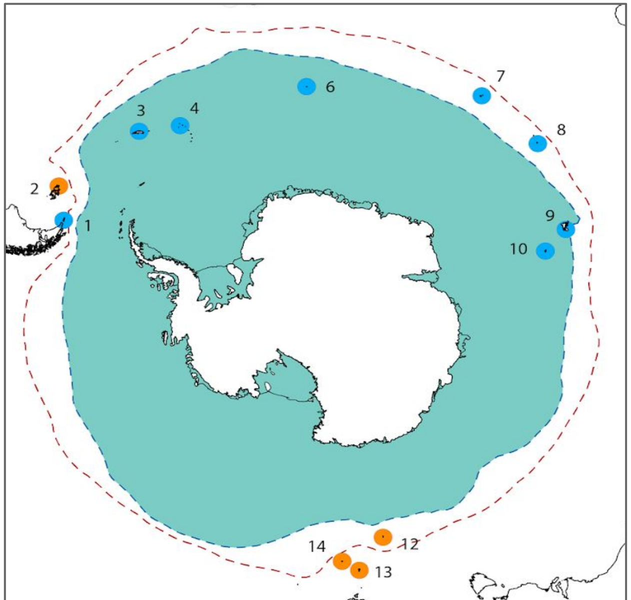


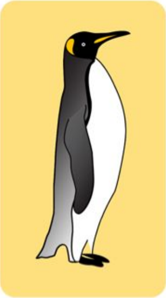
PAST



Stairway plot using unfolded site frequency spectrum estimated by ANGSD

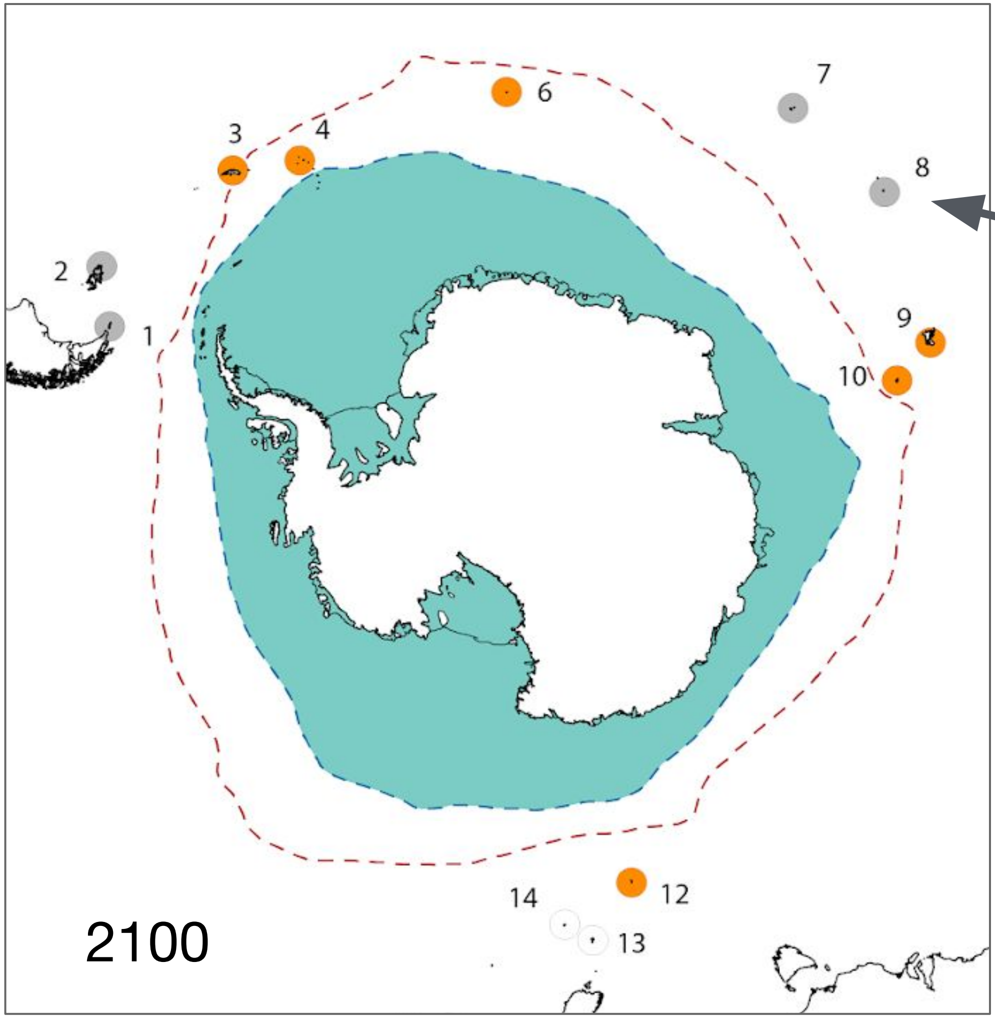
What is our prediction for the future?

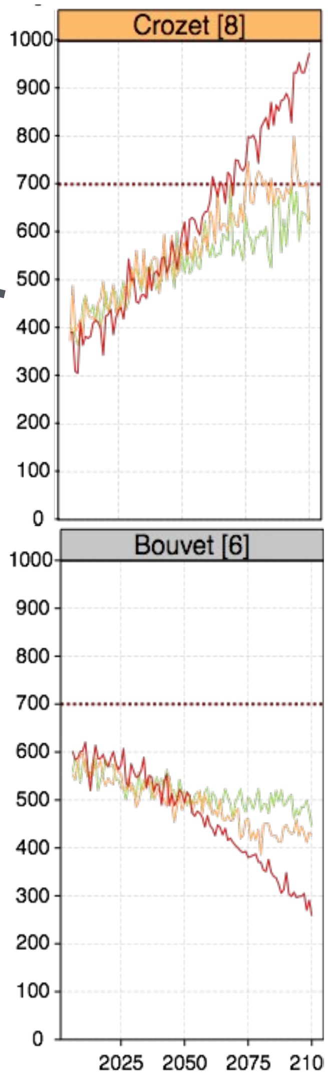
Under ongoing climate change scenario (up to 2100)



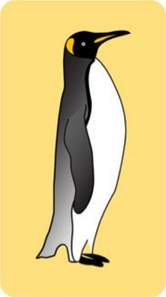
Our model predicts largest colonies will be lost

Few islands will become suitable





PROJECTED FORAGING DISTANCE



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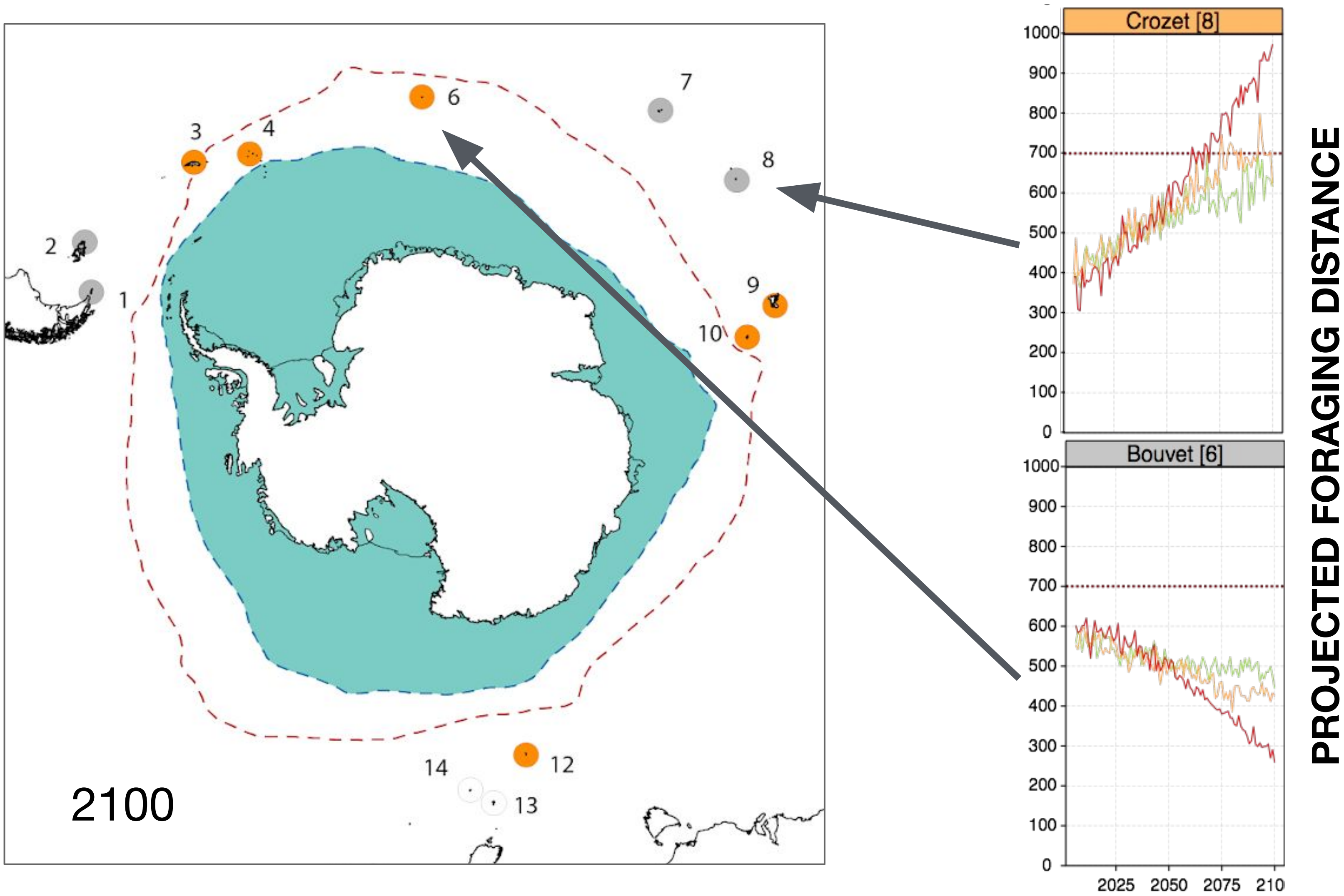
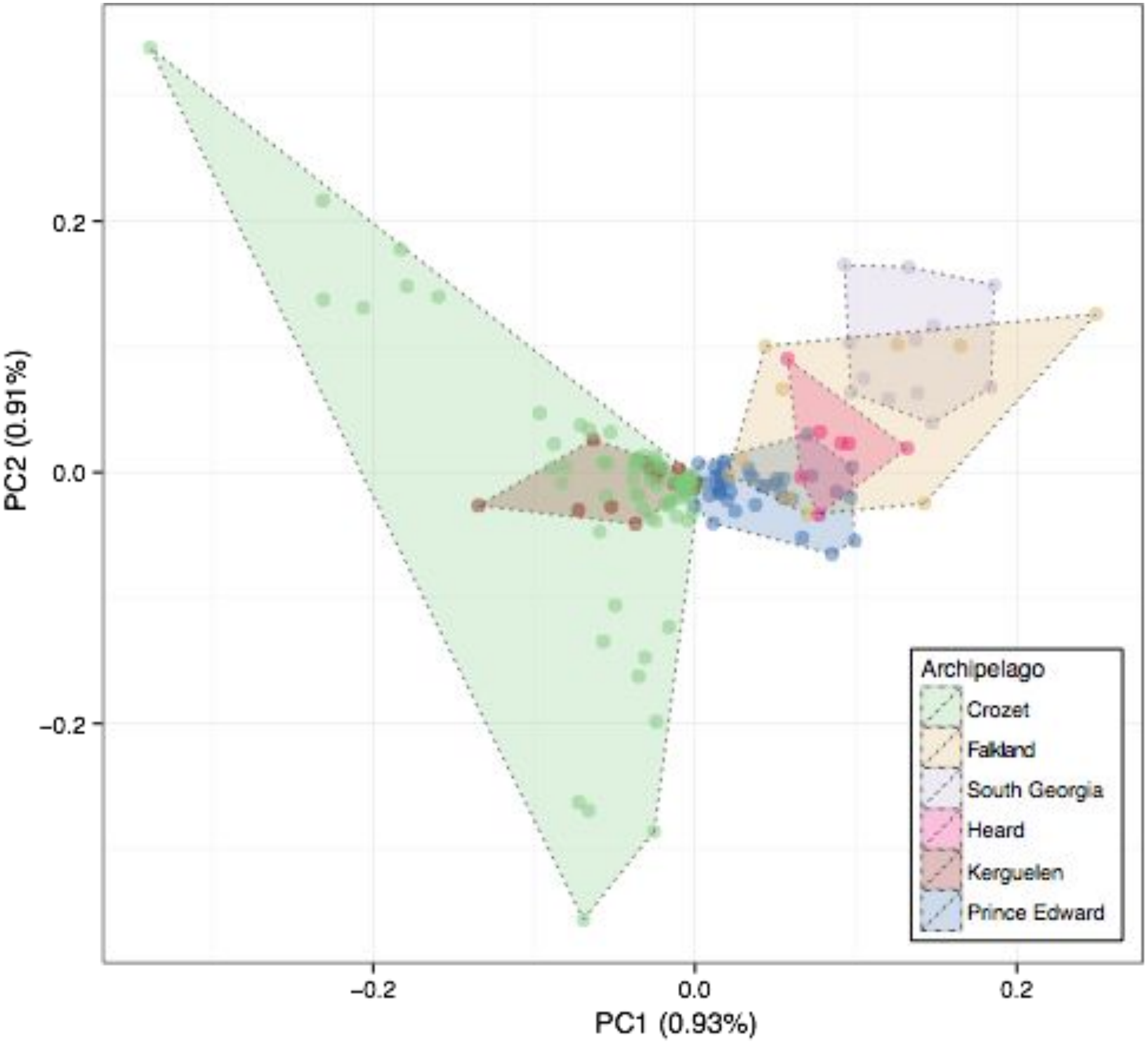


Genomic inference of high gene flow
World-wide connectivity in the King penguin

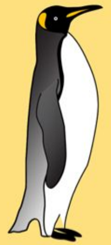


One single **highly connected** metapopulation

Long-range dispersal is not an issue

About **50,000 loci** genotyped in ca. **160 individuals** from **13 colonies** in **6 archipelagos.**

PCA



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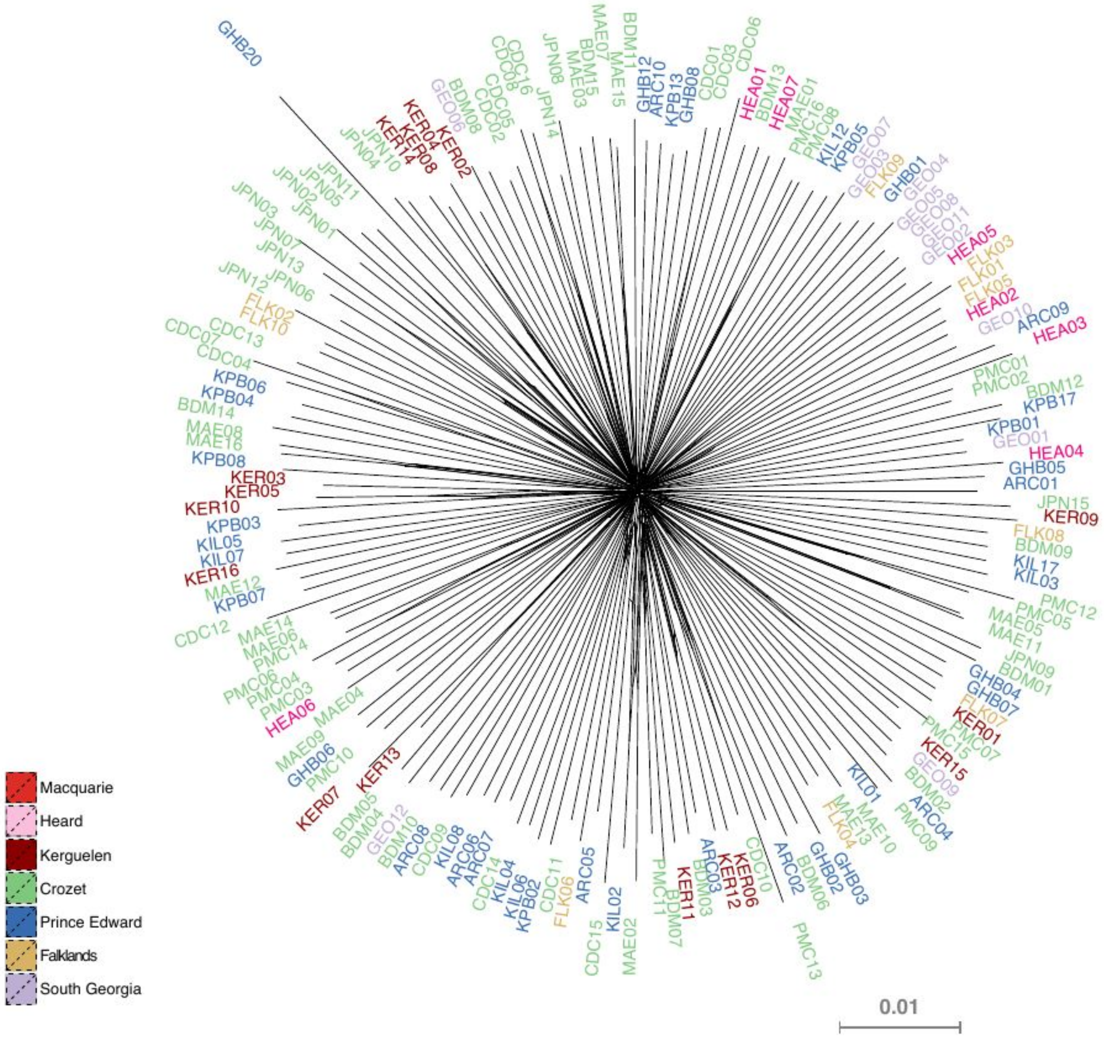

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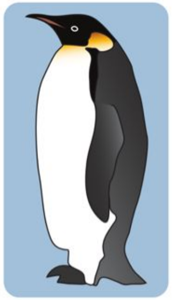
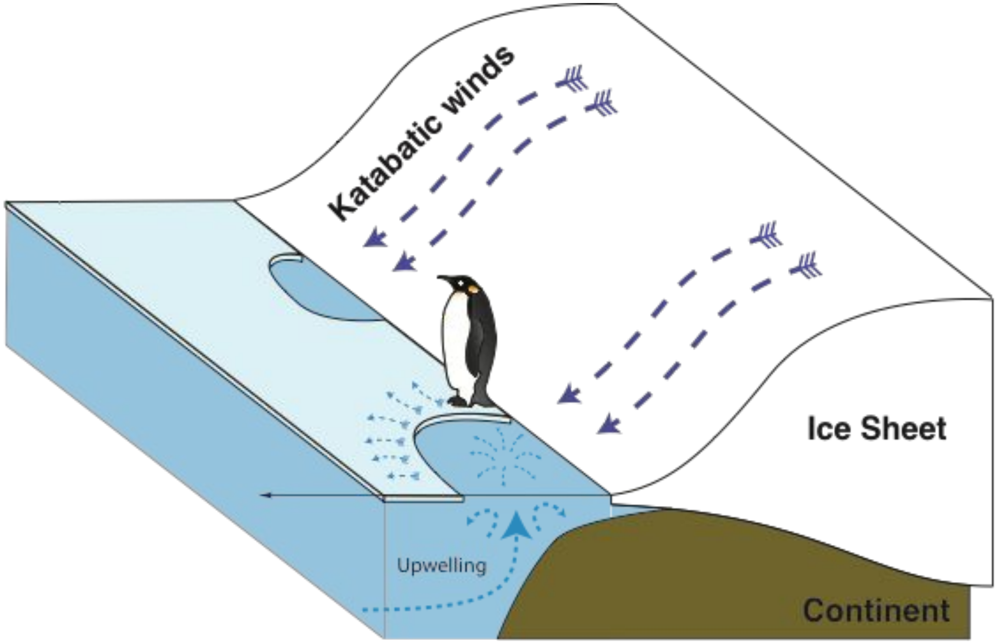
NJ tree

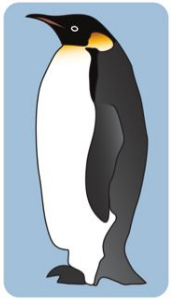


The Emperor penguin's foraging strategy:

Coastal polynyas are maintained by wind stress

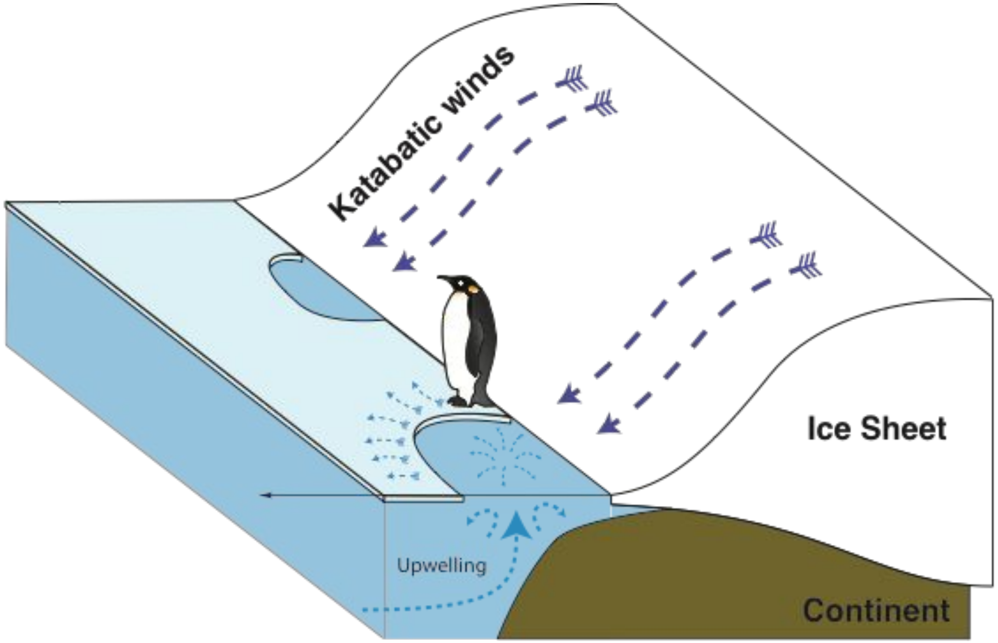


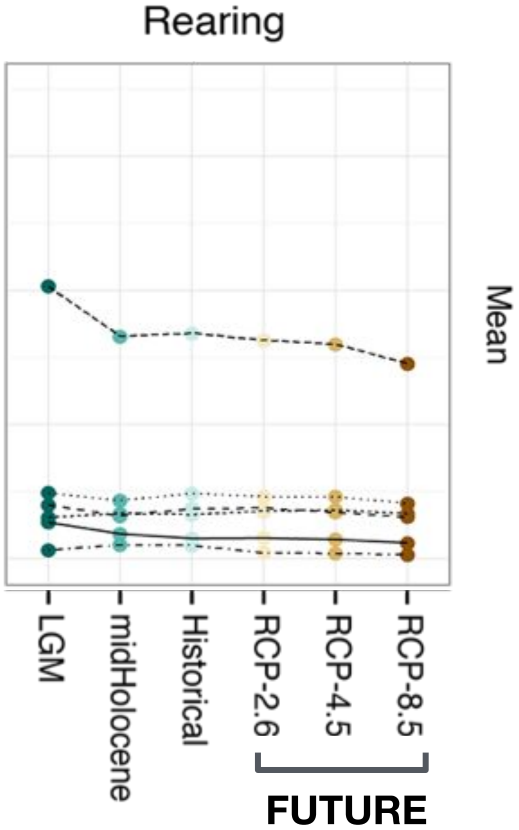
Modelling wind stress on coastal ice area



The Emperor penguin's foraging strategy:
Coastal polynyas are maintained by wind stress

6 models assessed for **4 variables,**
under **6 « forcing periods »**

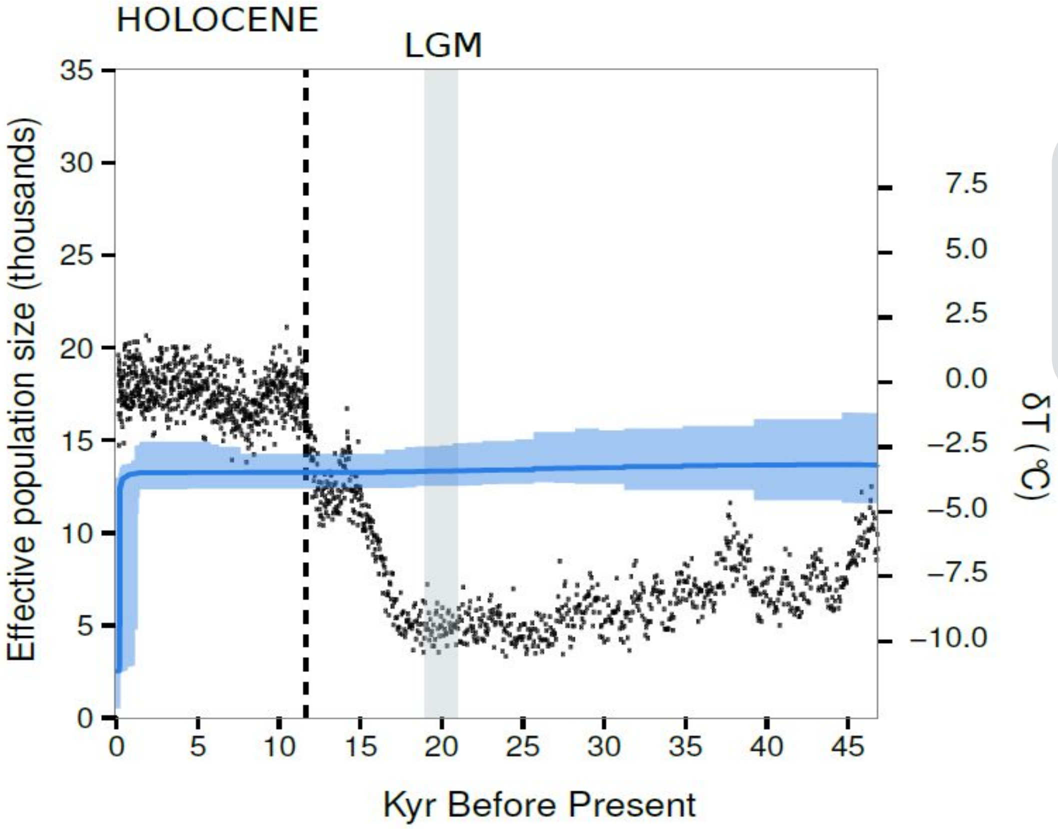




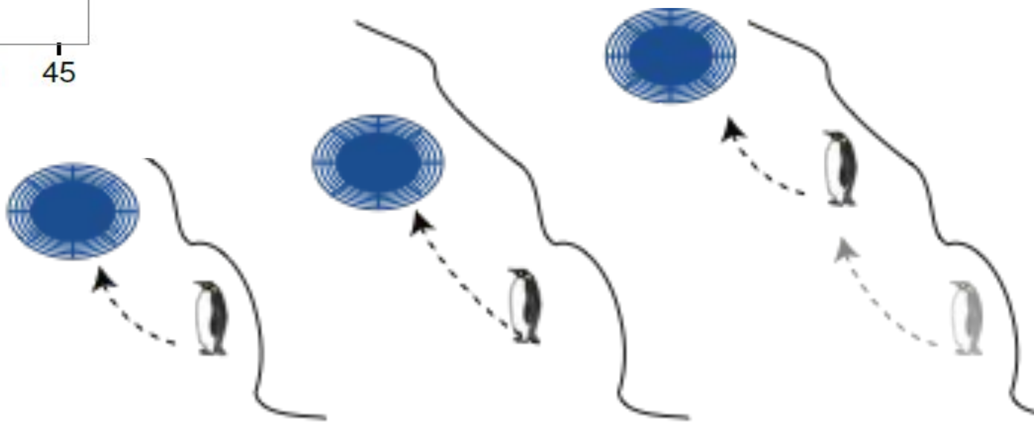
Modelling wind stress on
coastal ice area

Genomic inference shows a constant population

No detectable effect of past climatic shifts

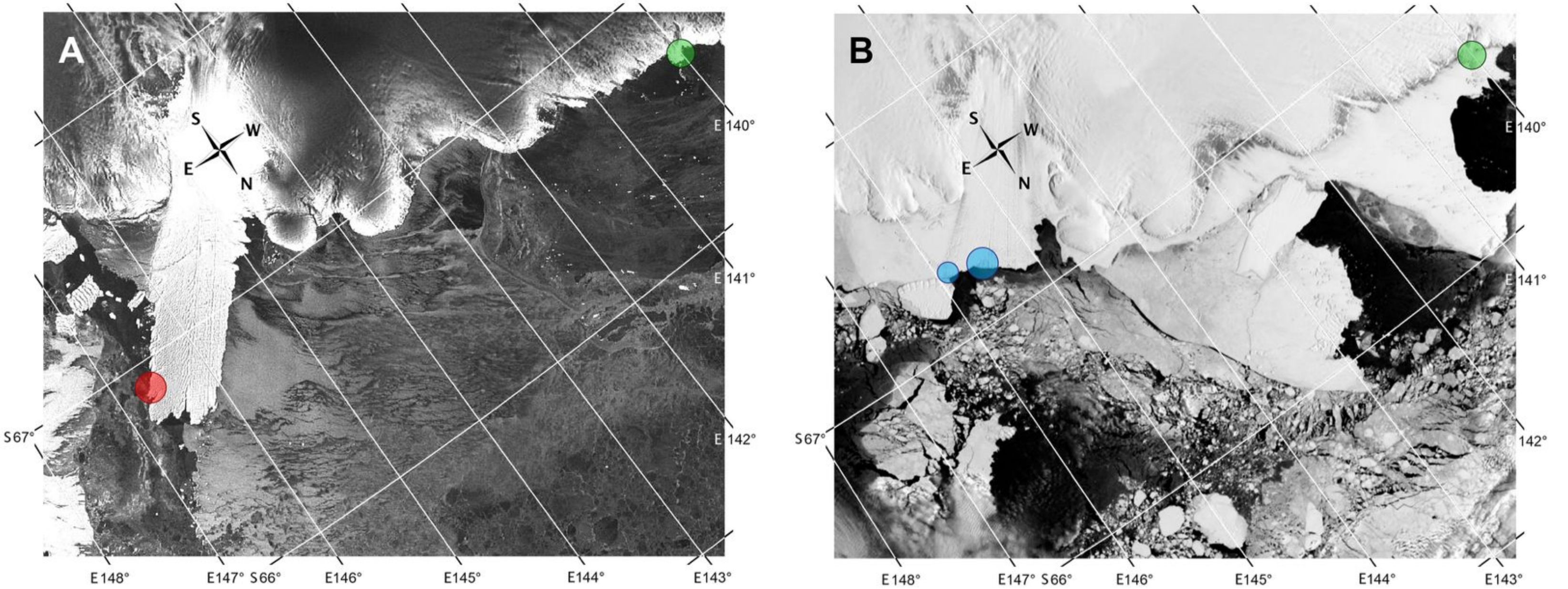


Polynyas move along the coast but were (and likely will be) always present



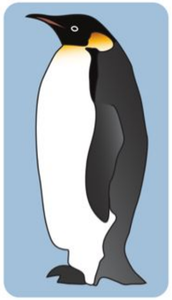
Stairway plot using unfolded site frequency spectrum estimated by ANGSD

« Catastrophic pulse dispersal »?



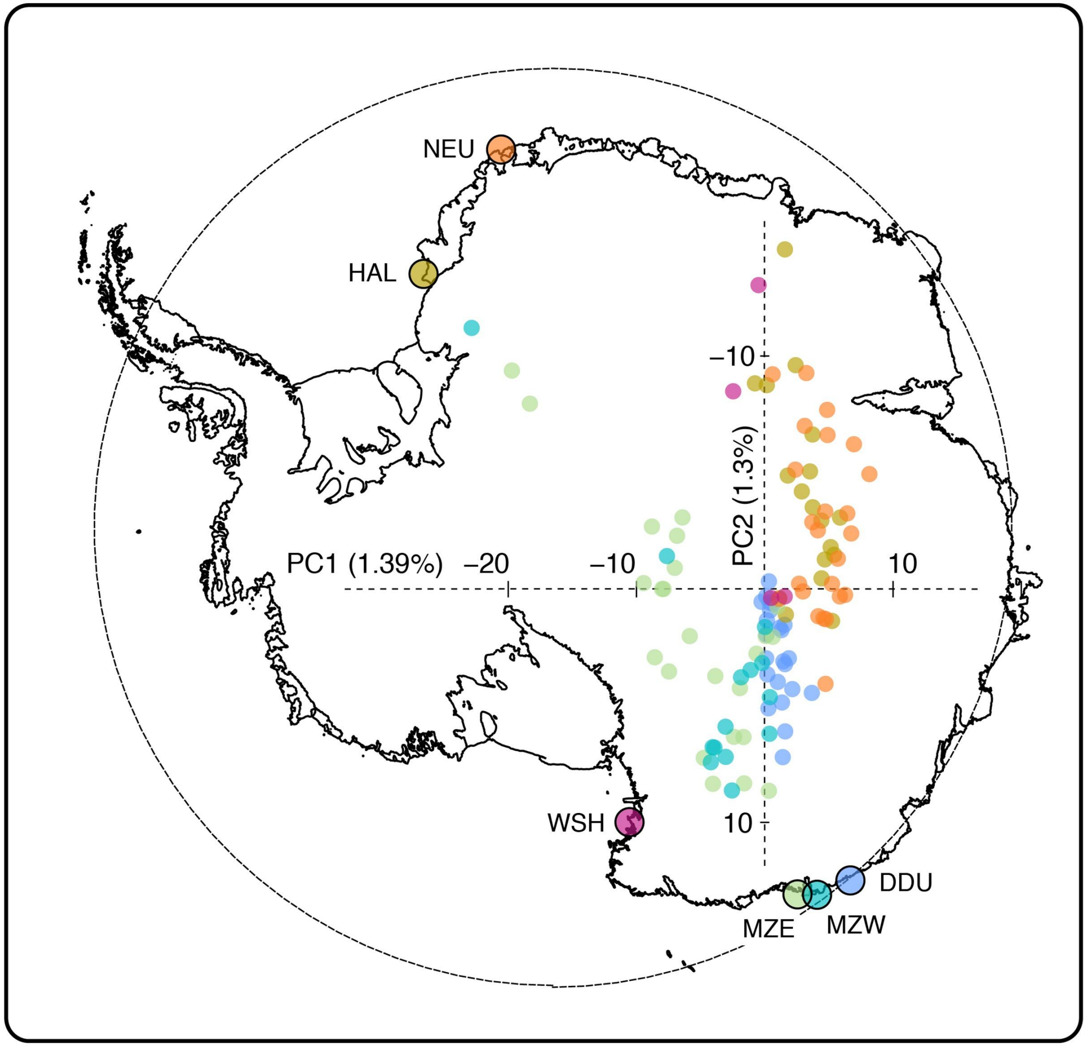
Mertz glacier, Adélie Land, before & after 2010

« Mass dispersal » events: Emperors need to stay flexible



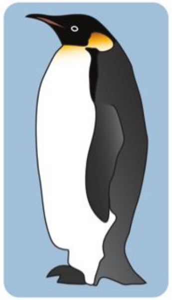
Genomic-based inference of high gene flow

Full circumpolar migration in the Emperor penguin



Very **weak** signal of **genetic differentiation** between colonies from the opposite site of Antarctica.

About **35,000 loci** genotyped in ca. **110 individuals** from **6 colonies**.



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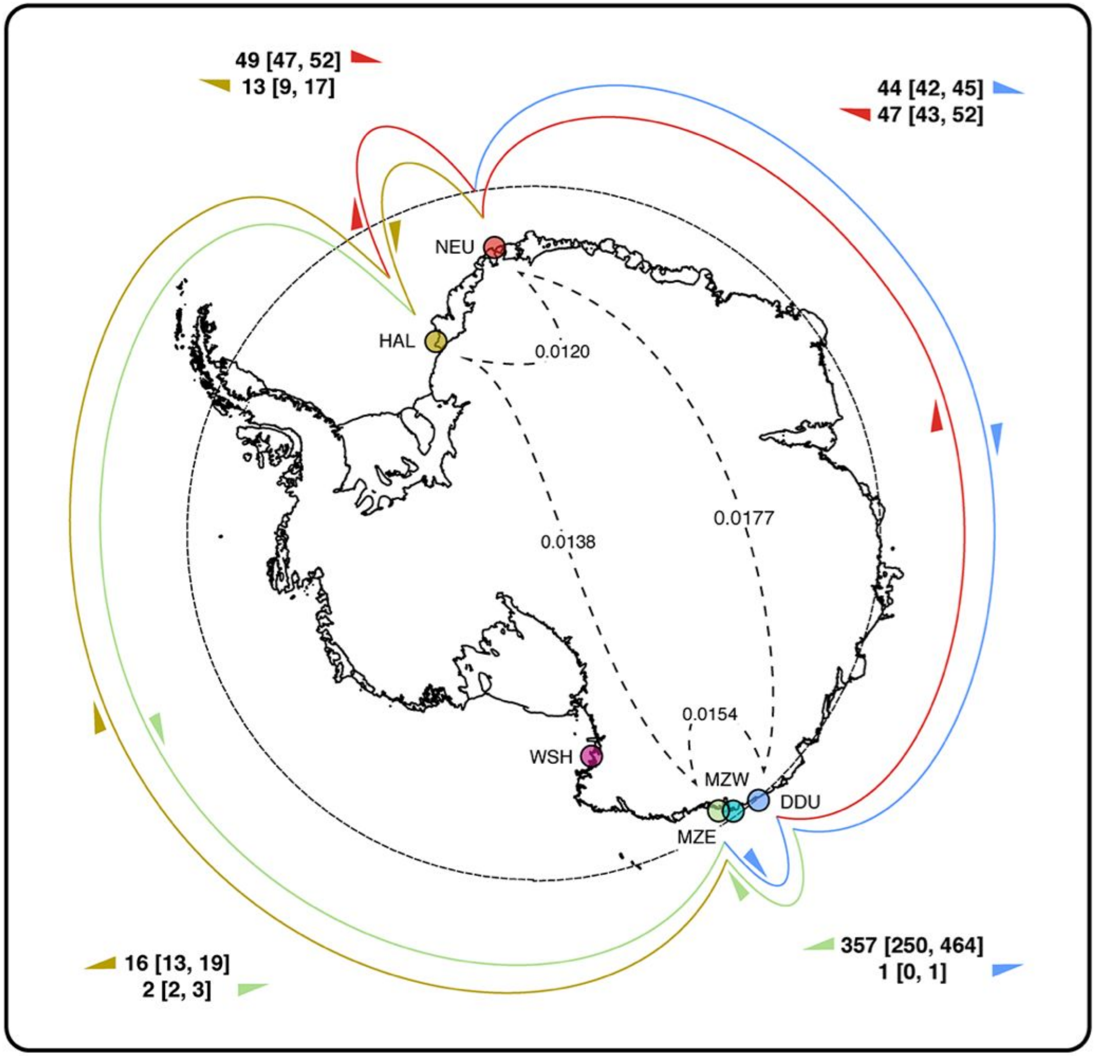

One single **highly connected** metapopulation

Long-range dispersal is not an issue

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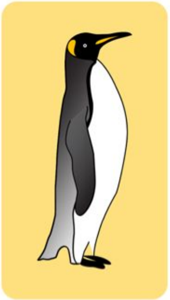
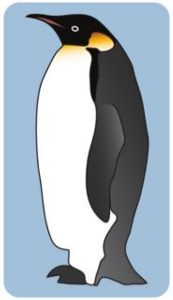
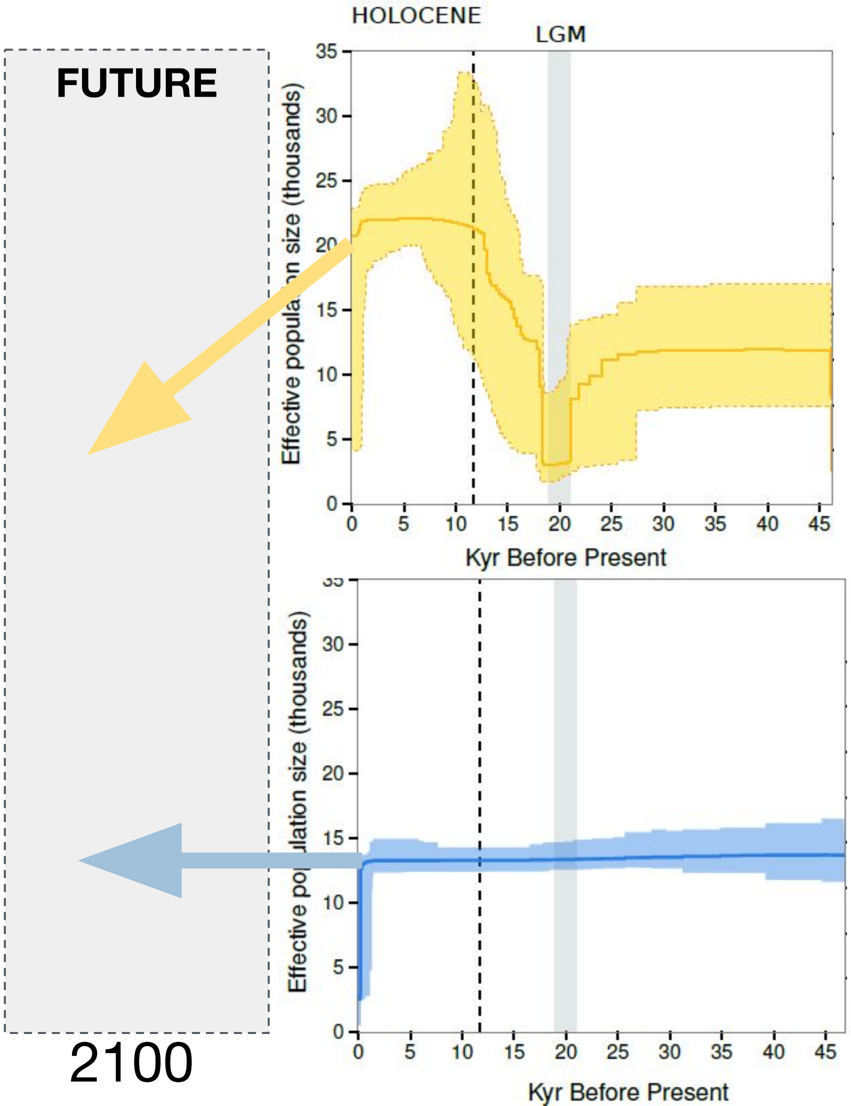
Migration rate (around) and Fst (inside) estimates

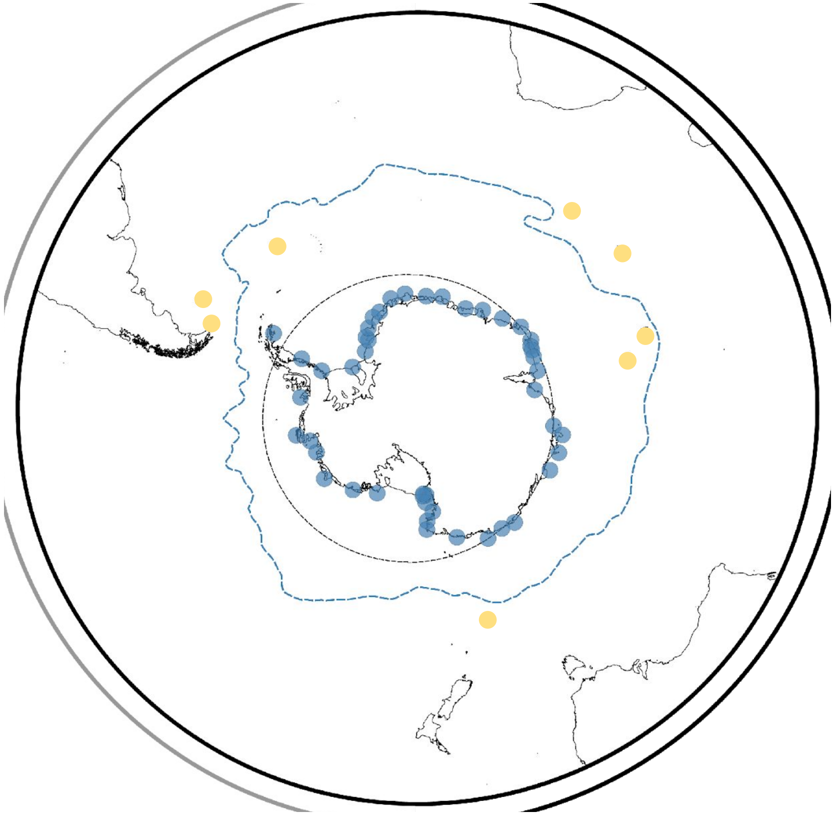


Conclusions



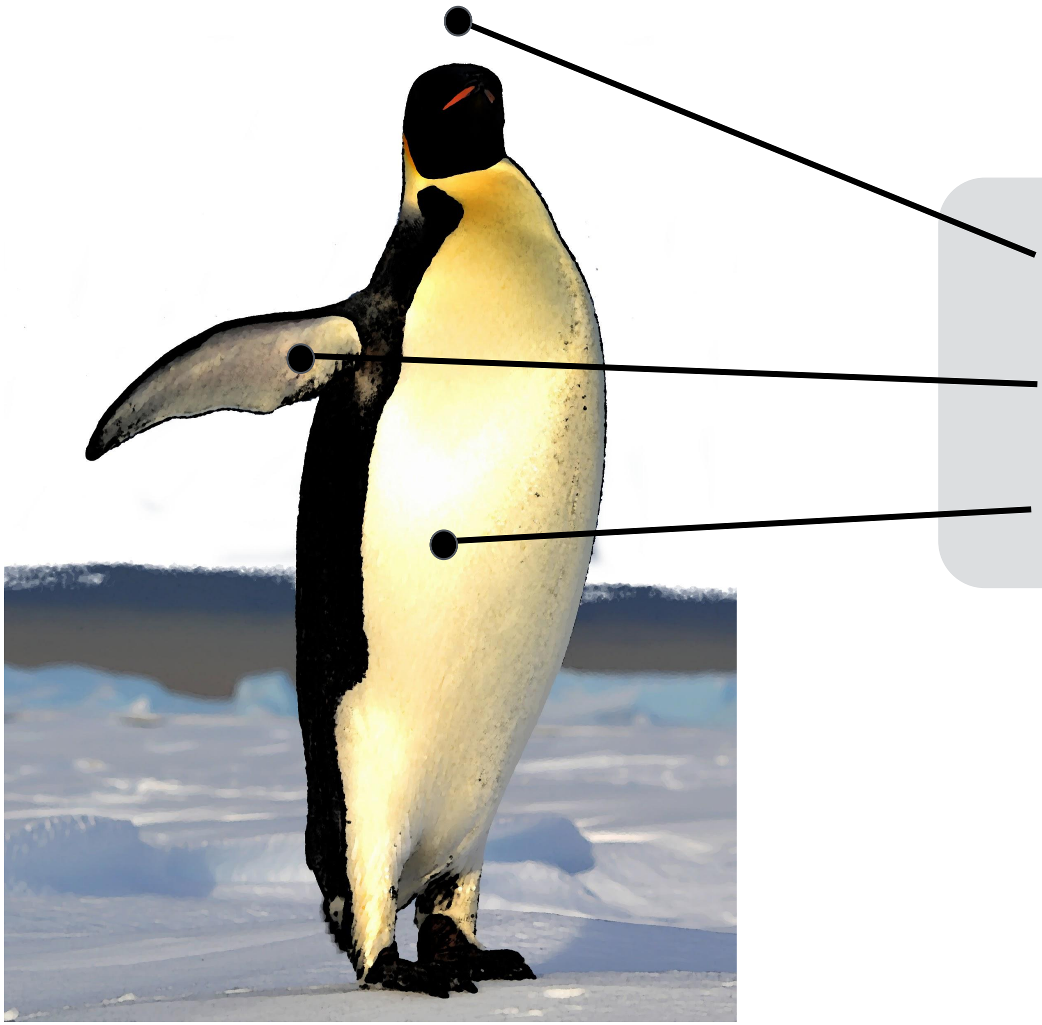


Habitat fragmentation more than extreme-cold adaptation drives response **to global warming**



Research in progress

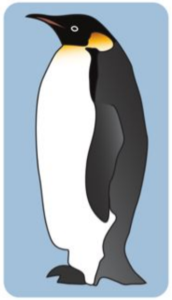
Understanding the genetic basis of the adaptations to the coldest environment on Earth



Behaviour

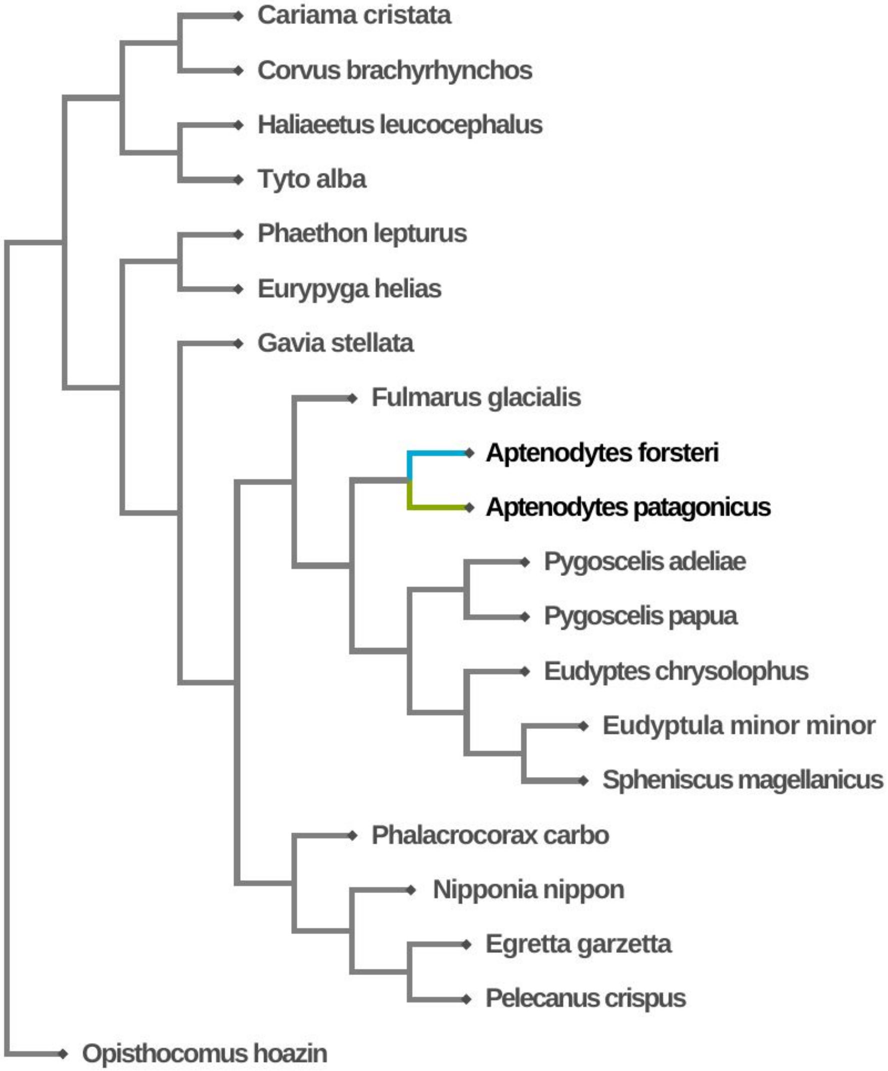
Insulation

Metabolism



Research in progress

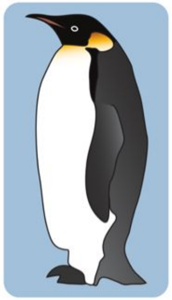
Understanding the genetic basis of the adaptations to the coldest environment on Earth



Long term selection

genes with significantly different dN/dS along the target branches

Using published reference genome CDS data within a custom pipeline to **find orthologs and run PAML**



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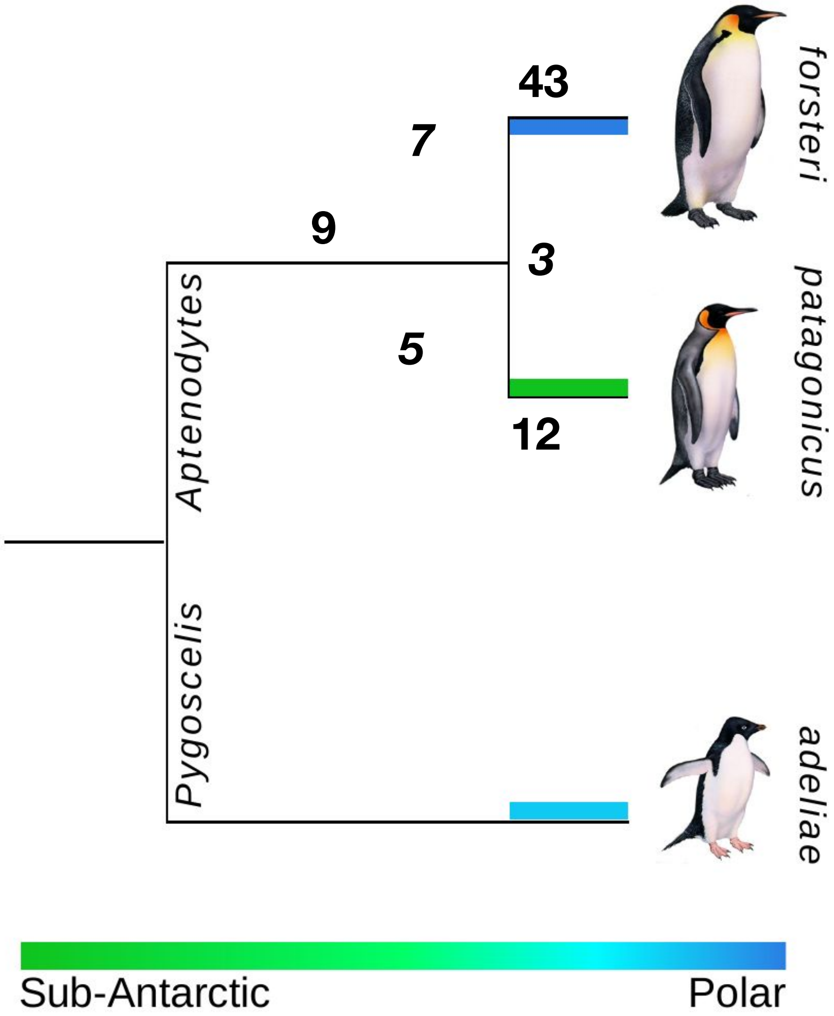
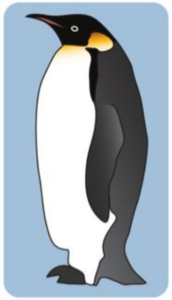


Long term selection

Testing ca. 7,500 orthologs, 3.5X more genes show a signal in the Emperor branch



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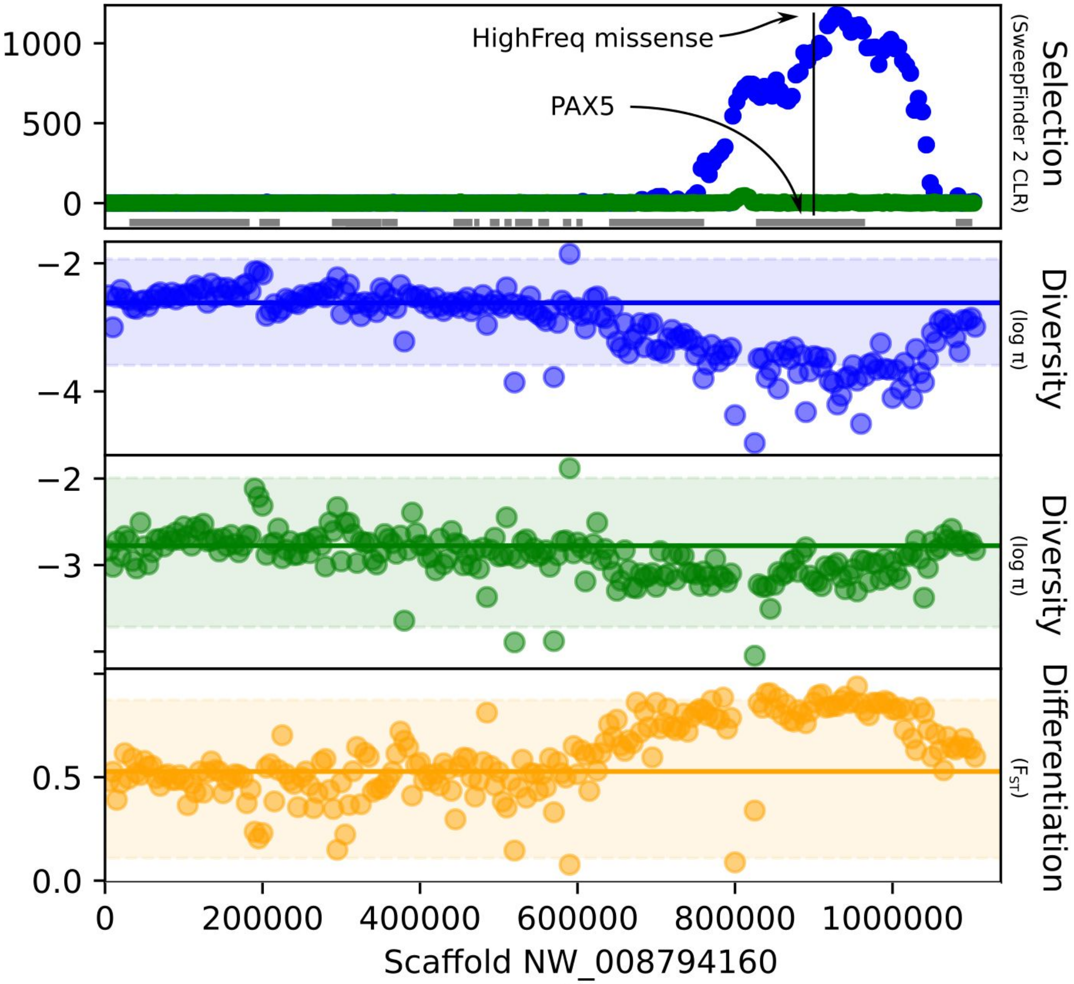
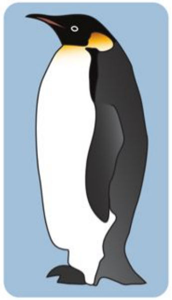


Clear differential selection signature in the Emperor (blue) vs the King (green) penguin

Whole-genome sequencing of 24 Emperor, 24 King, 3 Adelie and 3 Gentoo for **selection scan** with different approaches



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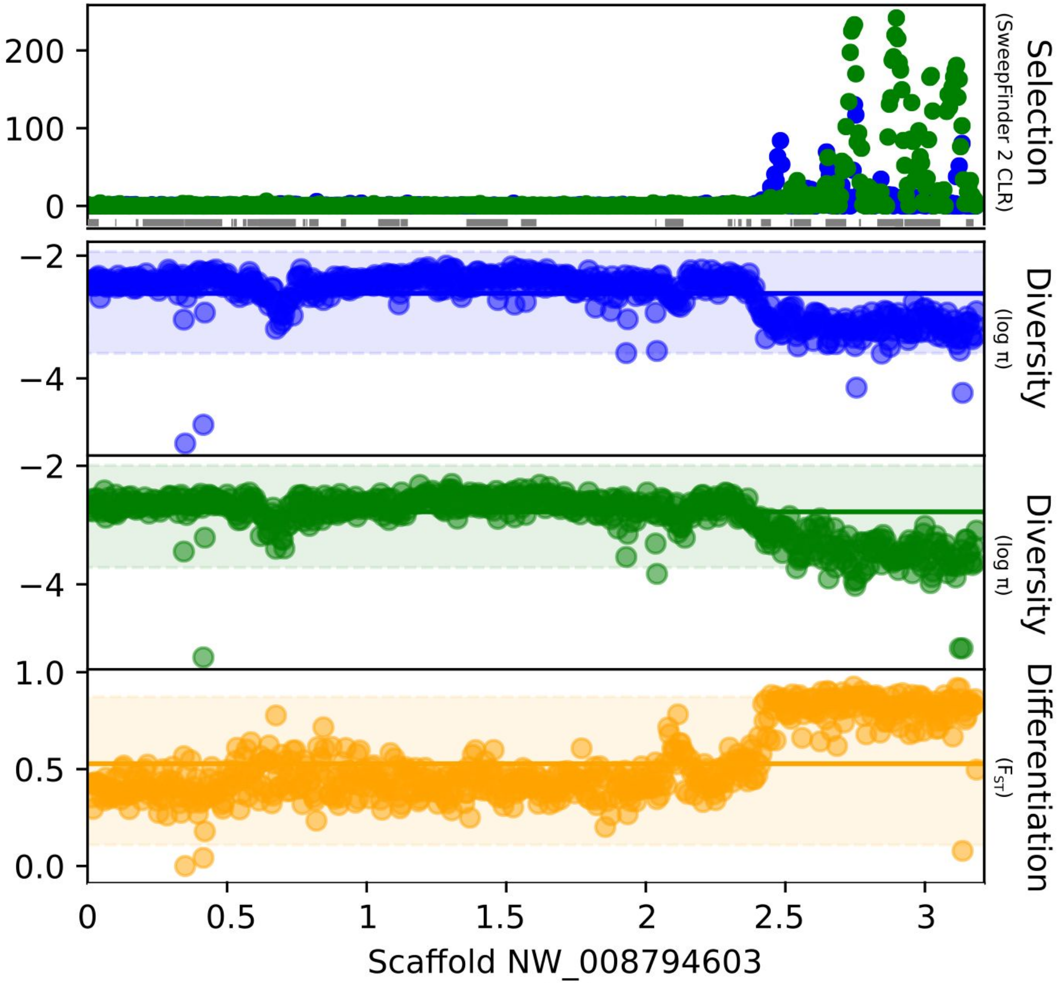

Overlapping selection signature in the Emperor (blue) vs the King (green) penguin

How **recombination rate** landscape constraints adaptation?

Acknowledgements

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Cristofari et al 2016 Full circumpolar migration ensures evolutionary unity in the Emperor penguin. *Nature communications* 7

Cristofari et al 2018 Climate-driven range shifts of the king penguin in fragmented ecosystems. *Nature Climate Change* 8, 245-251

Trucchi et al 2019 Reply to:'The role of ocean dynamics in king penguin range estimation'. *Nature Climate Change* 9, 122

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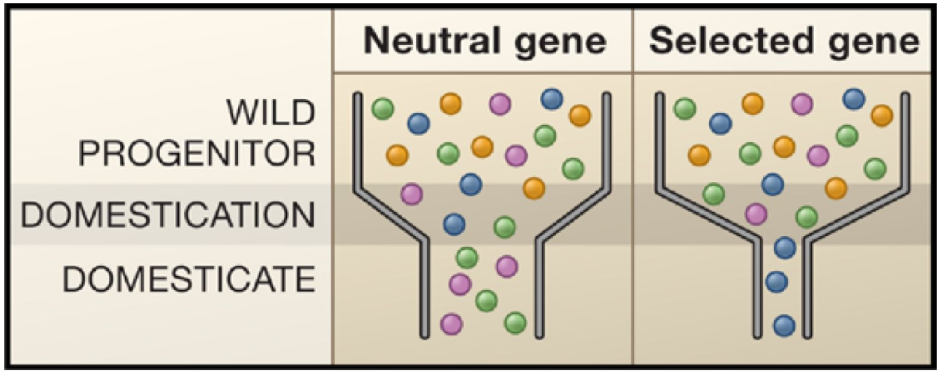
BONUS TRACK

Ancient genomes reveal early farmers selected common beans while preserving diversity

Understanding tempo and mode of domestication

Selection on traits of interests

Genetic **drift** due to
the domestication bottleneck



Doebley and Gaut 2006

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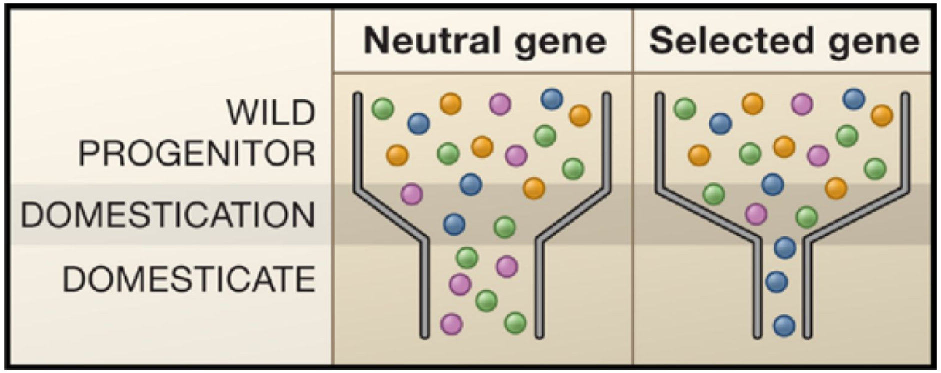
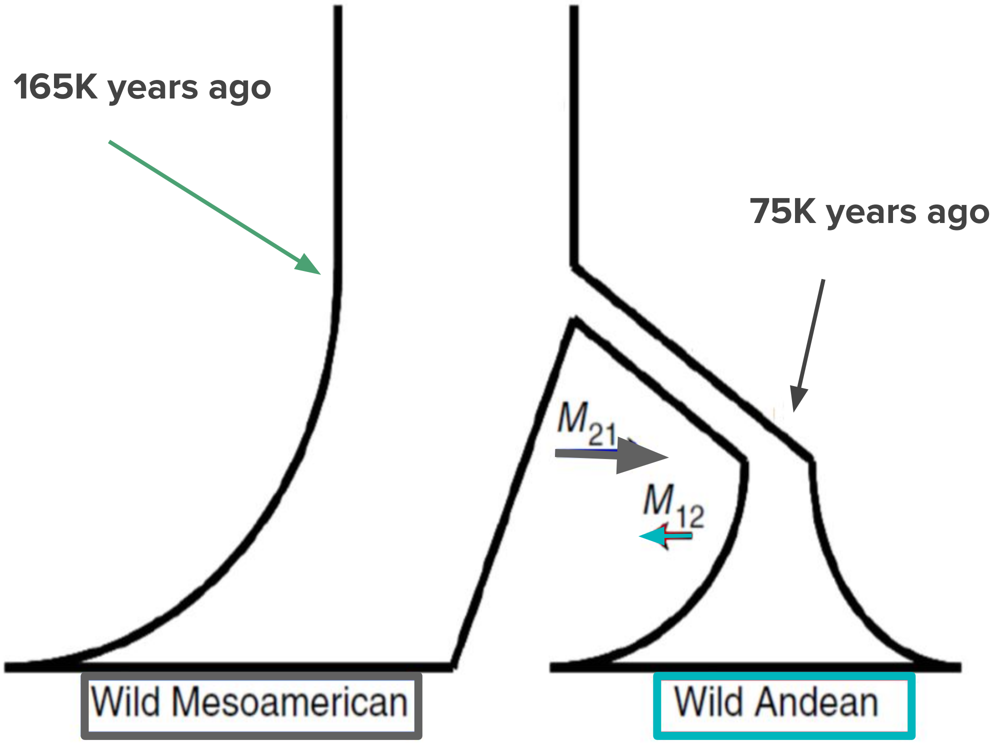


Doebley and Gaut 2006

Which is the underlying genetics of selected phenotypic traits?

What is the temporal dynamics of both selection and loss of genetic diversity (genomic erosion)?

Common bean double independent domestication in meso and south America



165K years ago

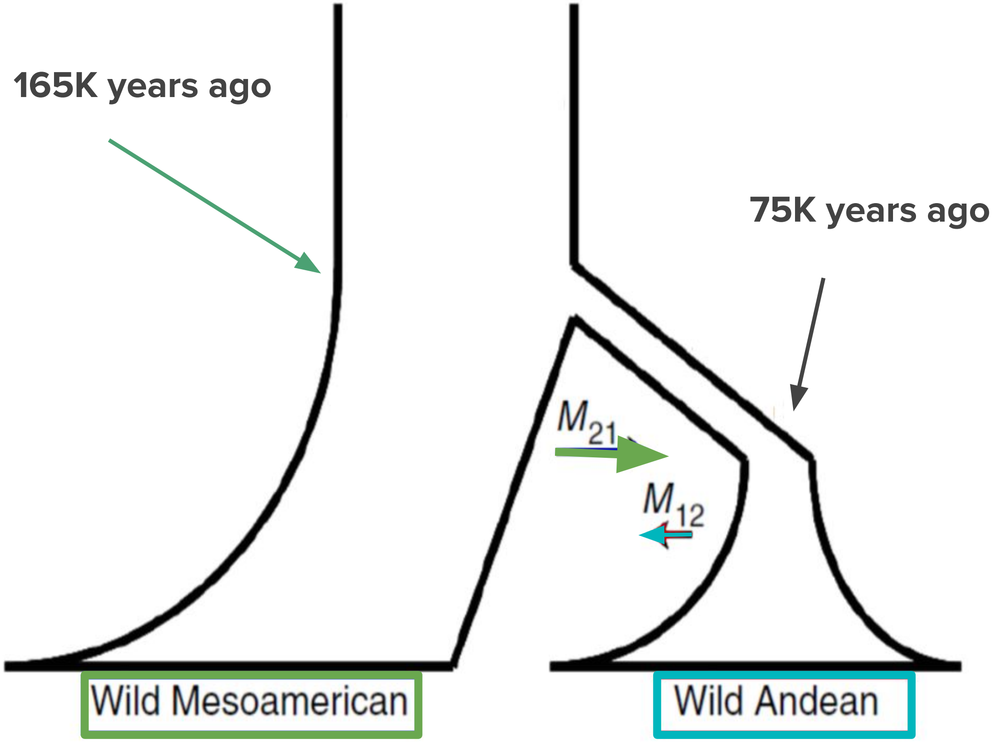
75K years ago

Wild Mesoamerican

Wild Andean

Schmutz et al 2014

Common bean double independent domestication in meso and south America



Schmutz et al 2014

Bitocchi et al 2012; Gaut 2014

Ancient genomics focus on south America domestication

5 museum collections

7 geographic area

9 archaeological sites
in **North-West Argentina**

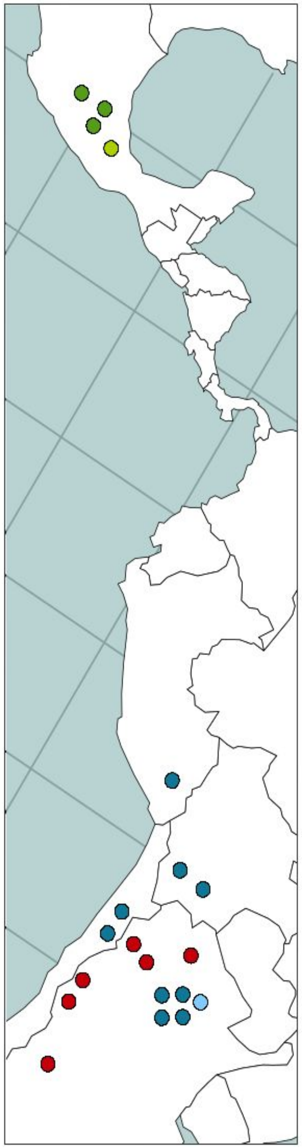
150 bean seeds collected
(*and 16 pod fragments*)

Ancient genomics focus on south America domestication

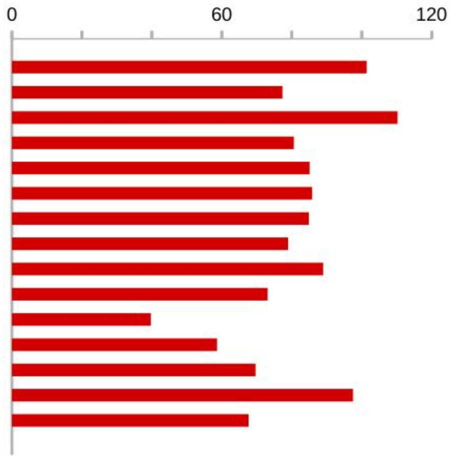
Preliminary low coverage sequencing of 30 ancient seeds

15 ancient seeds whole-genome sequenced at **4-18X** coverage

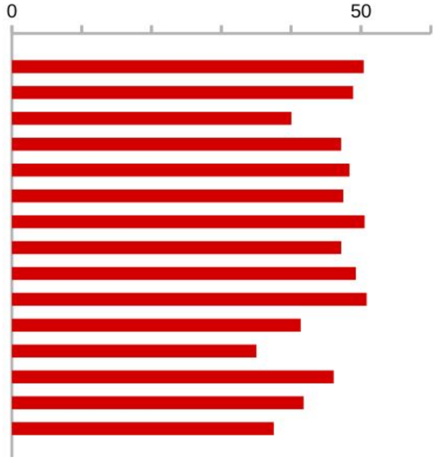
14 modern seeds, wild and domesticated



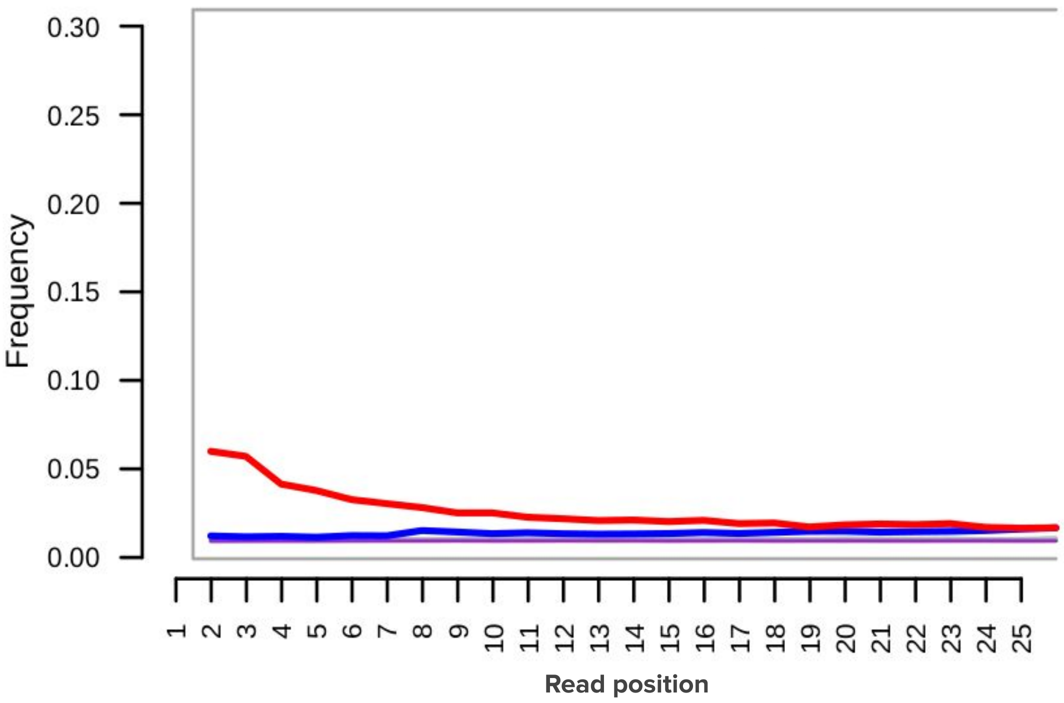
High quantity and quality of endogenous DNA in ancient common bean seeds



Average read length (bp)

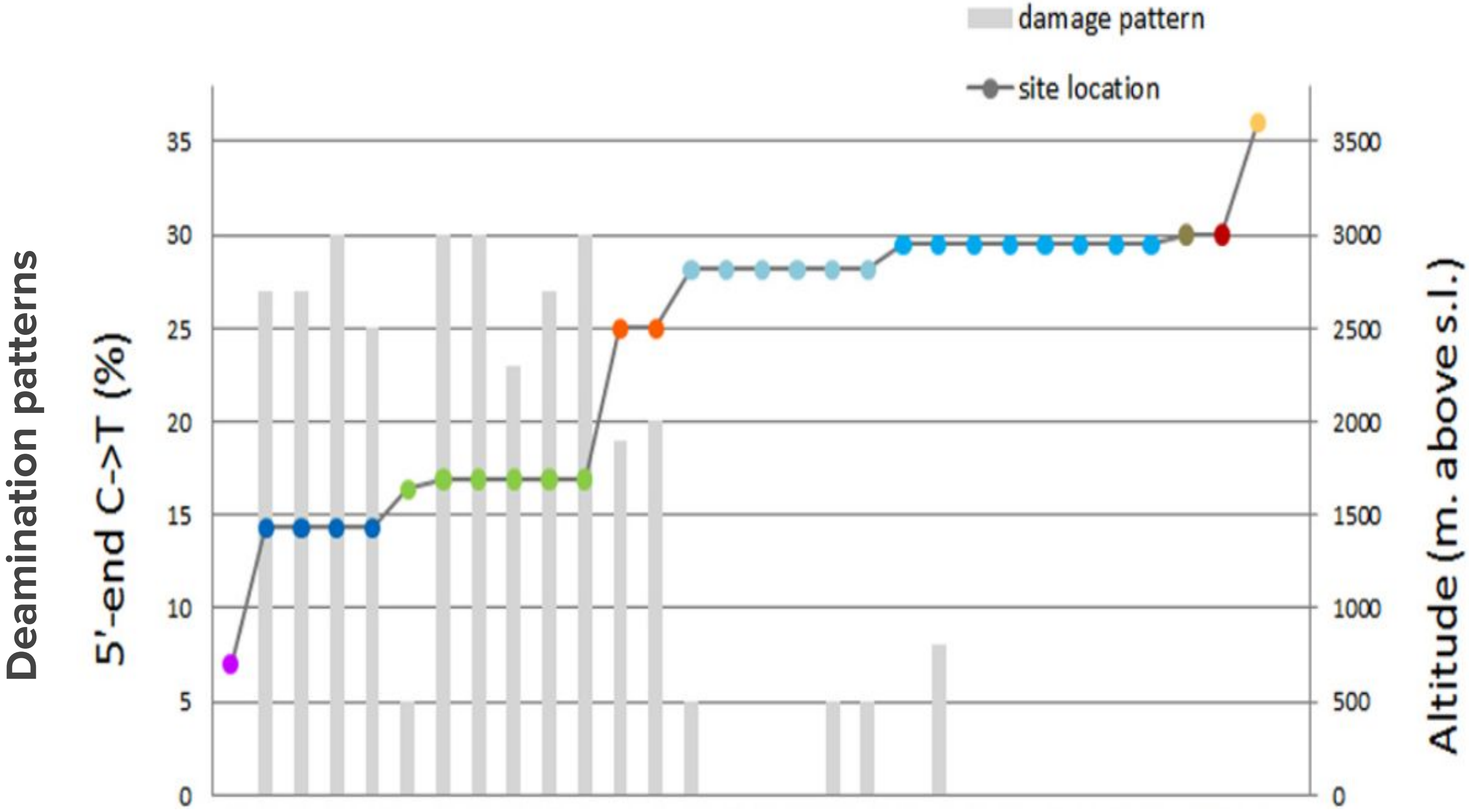


Endogenous DNA (%)

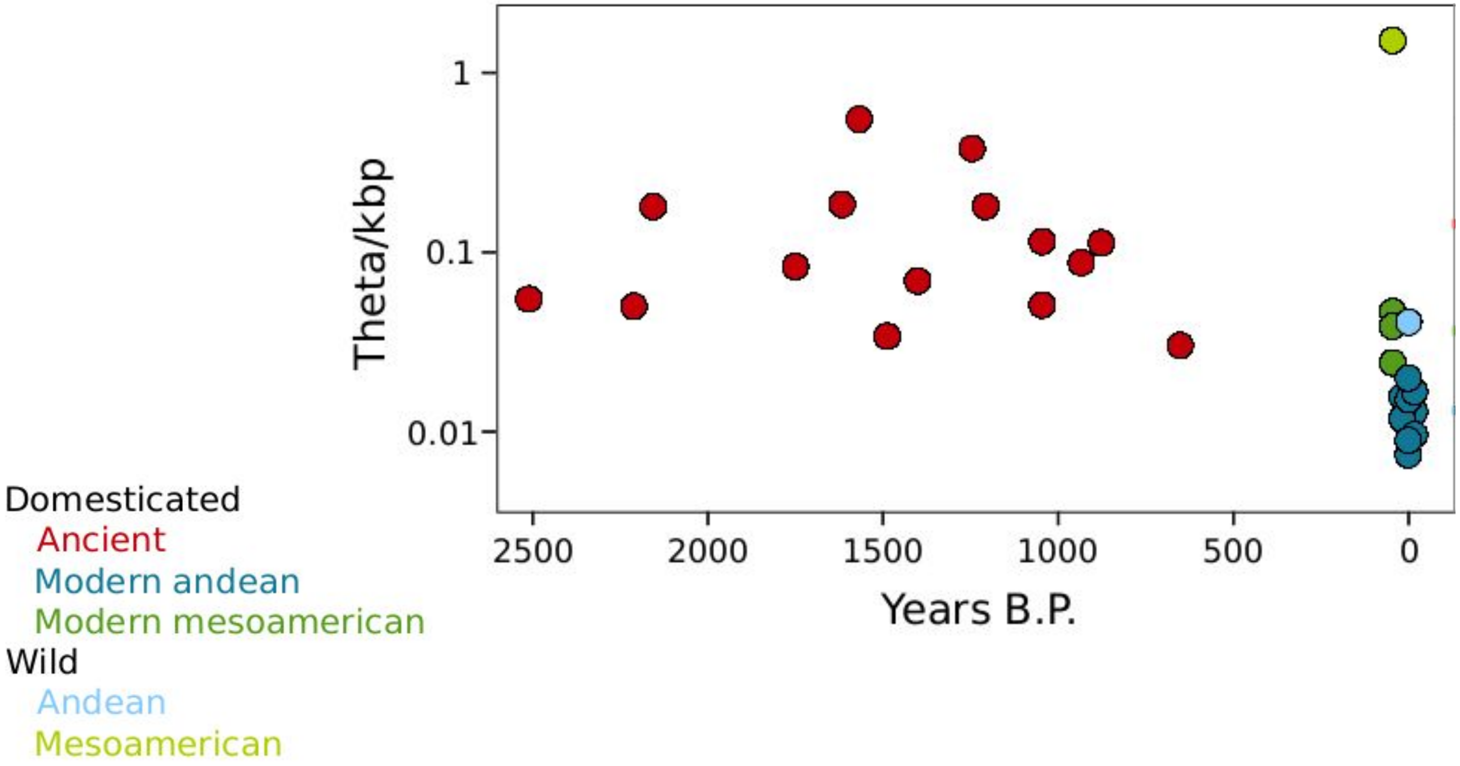


Deamination patterns typical of ancient DNA

Better preservation of ancient DNA at high altitude sites



Loss of genetic diversity is recent in common bean domestication



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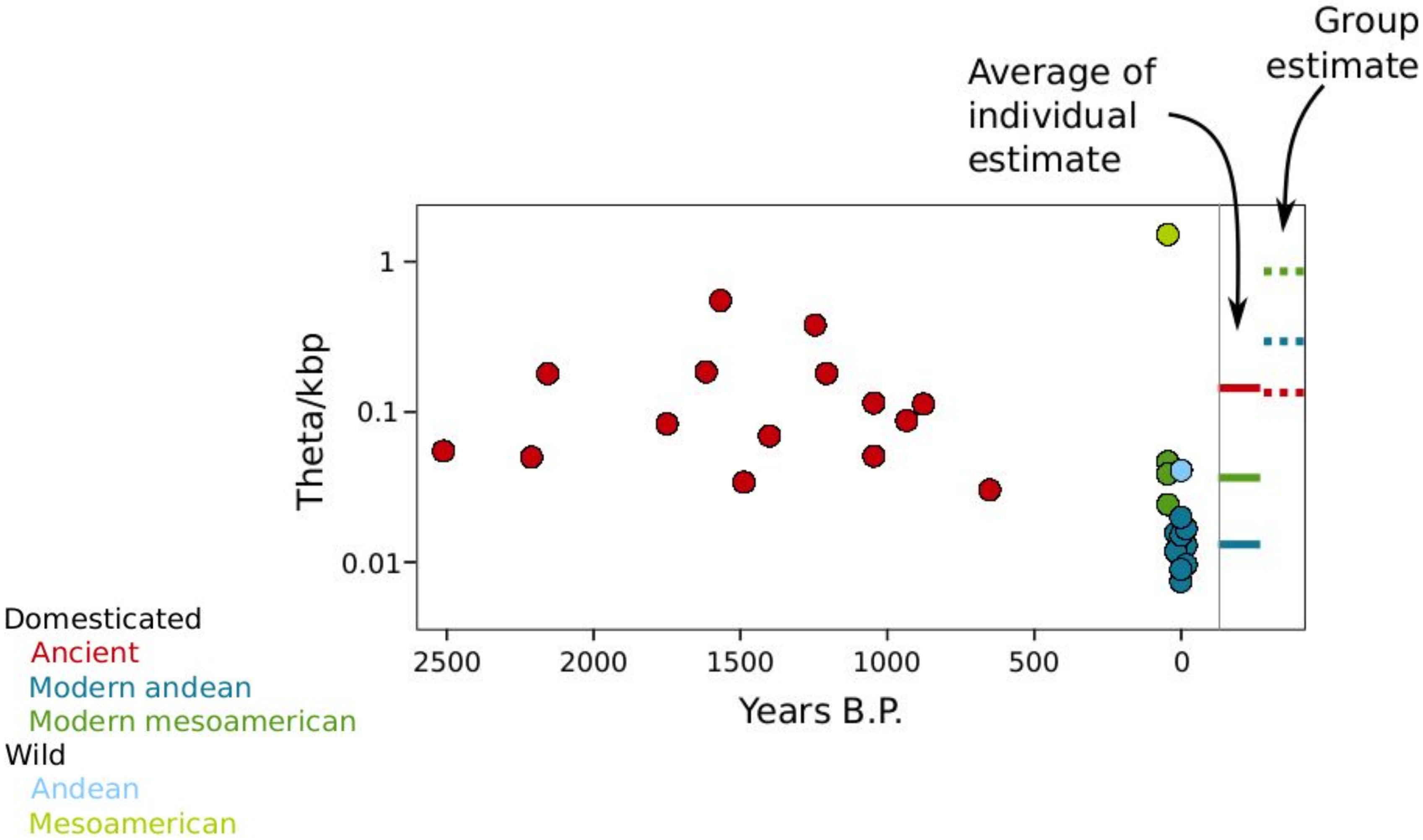
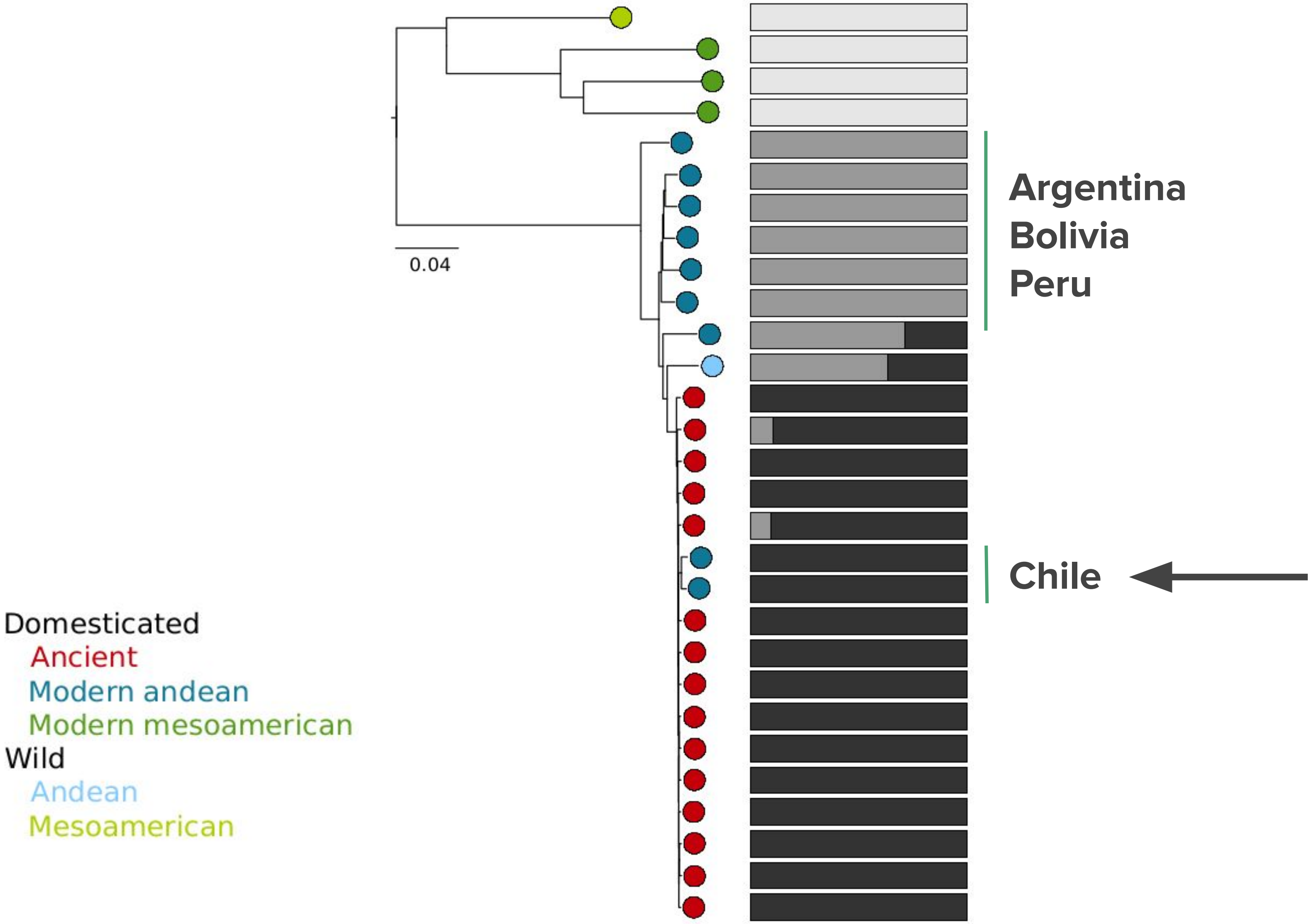
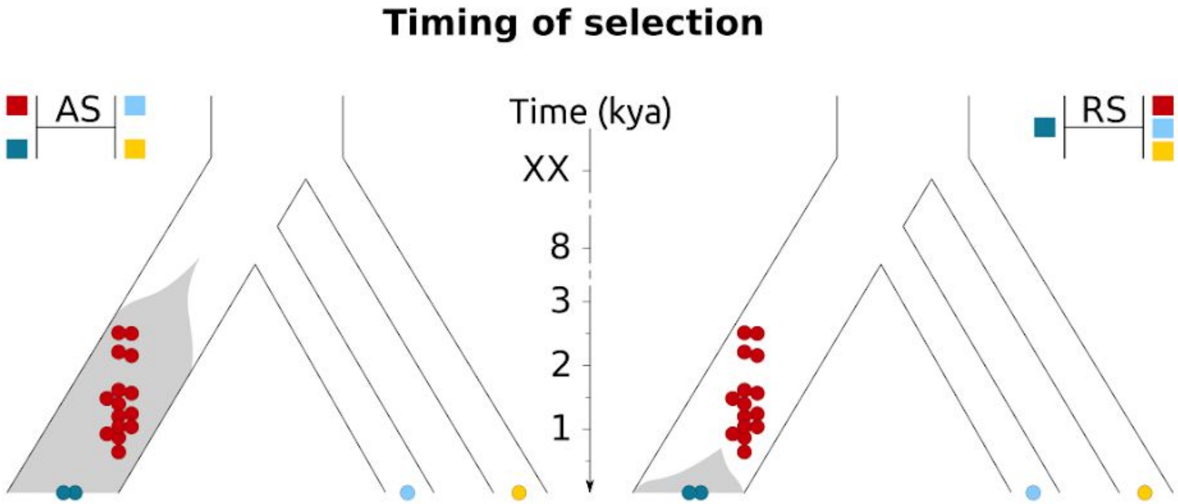


All ancient seeds belong to the same genomic clade

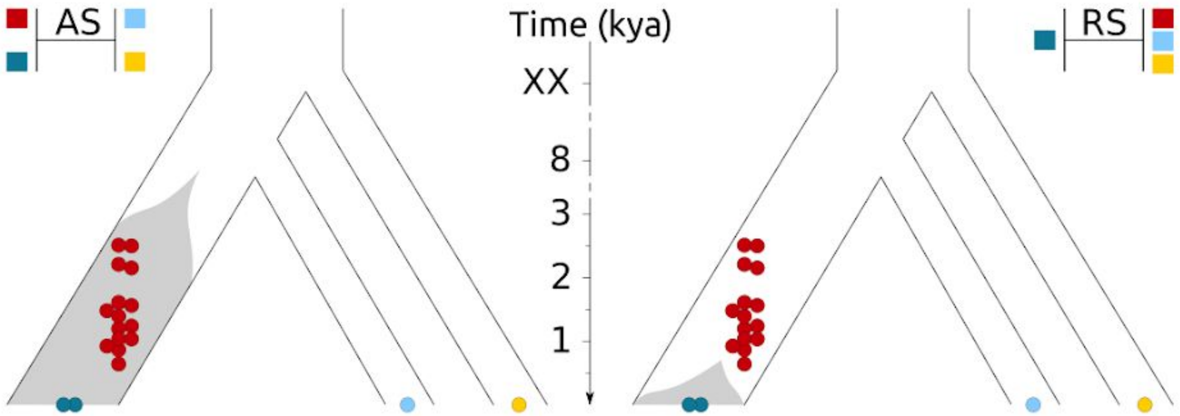


Gene-by-gene scan of enrichment in fixed alternative alleles

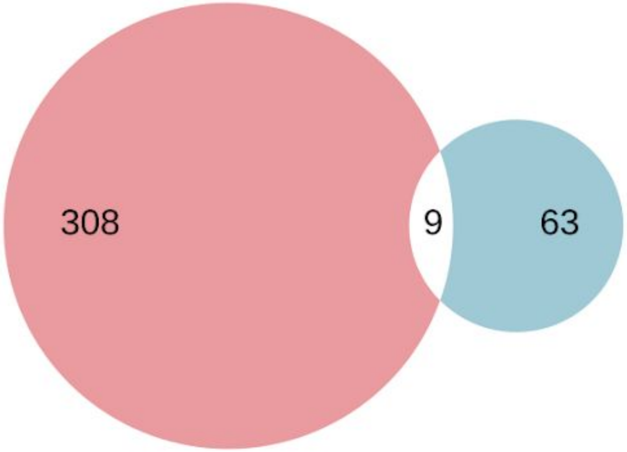


Selection affected 4.5X more genes in ancient than in recent times

Timing of selection



Selected genes

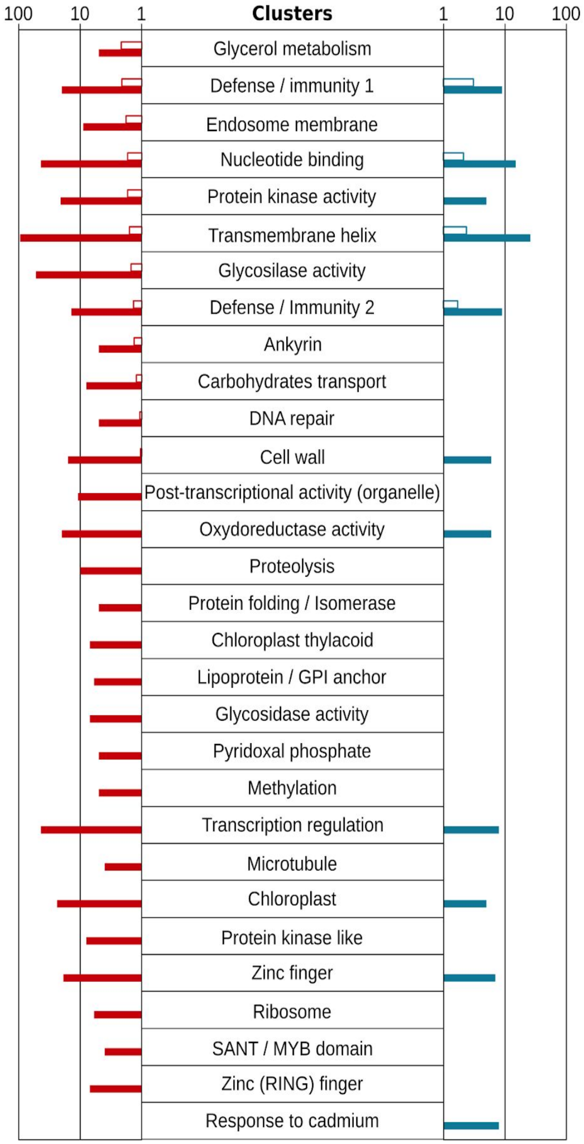


Different functional gene groups were selected by ancient vs. recent selection

Ancient selection targets: glycerol metabolism, carbohydrate and sugar transport and metabolism, intracellular transport, regulatory elements, modification of proteins, glycosylation

Recent selection targets: immunity and defense, regulatory elements and transmembrane transport

A few immune genes show signature of both ancient and recent selection

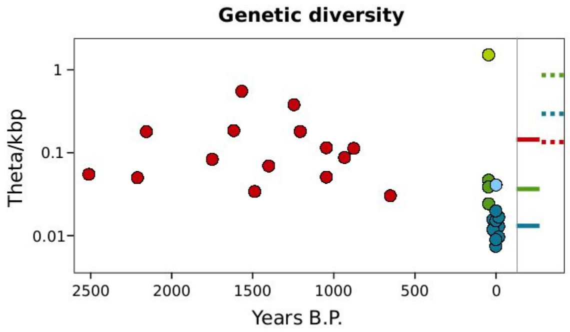


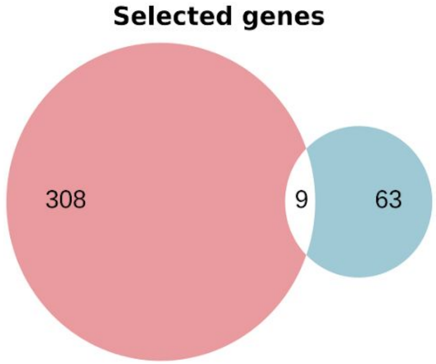
Concluding, ancient selection but recent ge erosion characterize bean domestication

Early selection was probably based on **larger number** of seeds

Seeds or cultivars **exchange** was common

More sustainable than modern breeding programs (since Green revolution)





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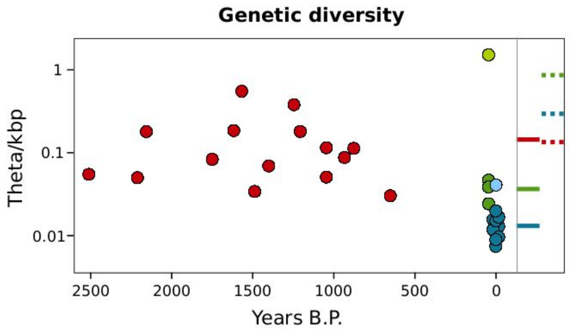
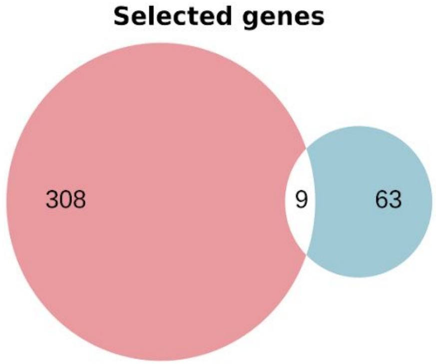
Modern landraces from Chile are the most similar to the ancient Andean cultivars





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BEAN ADAPT

Trucchi et al (2019) Ancient genomes reveal early farmers selected common beans while preserving diversity. ***BioRxiv***, https://doi.org/10.1101/791806

Take home message?

Any species can be a ***model species***

Analyses are more important than data

Get many ***collaborators***

Enjoy Genomics!

Emiliano Trucchi

Workshop on Genomics 2020
Cesky Krumlov