Society for Conservation Biology

ICCB2023

Joint Introduction

Conservation Genomics

An Overview and Hands-on Training with
Genomic Analyses for Biodiversity Conservation (ID: 9)

&

Training to Improve Applications of Genetics and Genomics

for Biodiversity Conservation (ID:33)

Gordon Luikart
William Hemstrom
Jared Grummer

UNIVERSITY OF MONTANA

Purdue University

UNIVERSITY OF COPENHAGEN

1865 THE UNIVERSITY OF MAINE

University of Glasgow

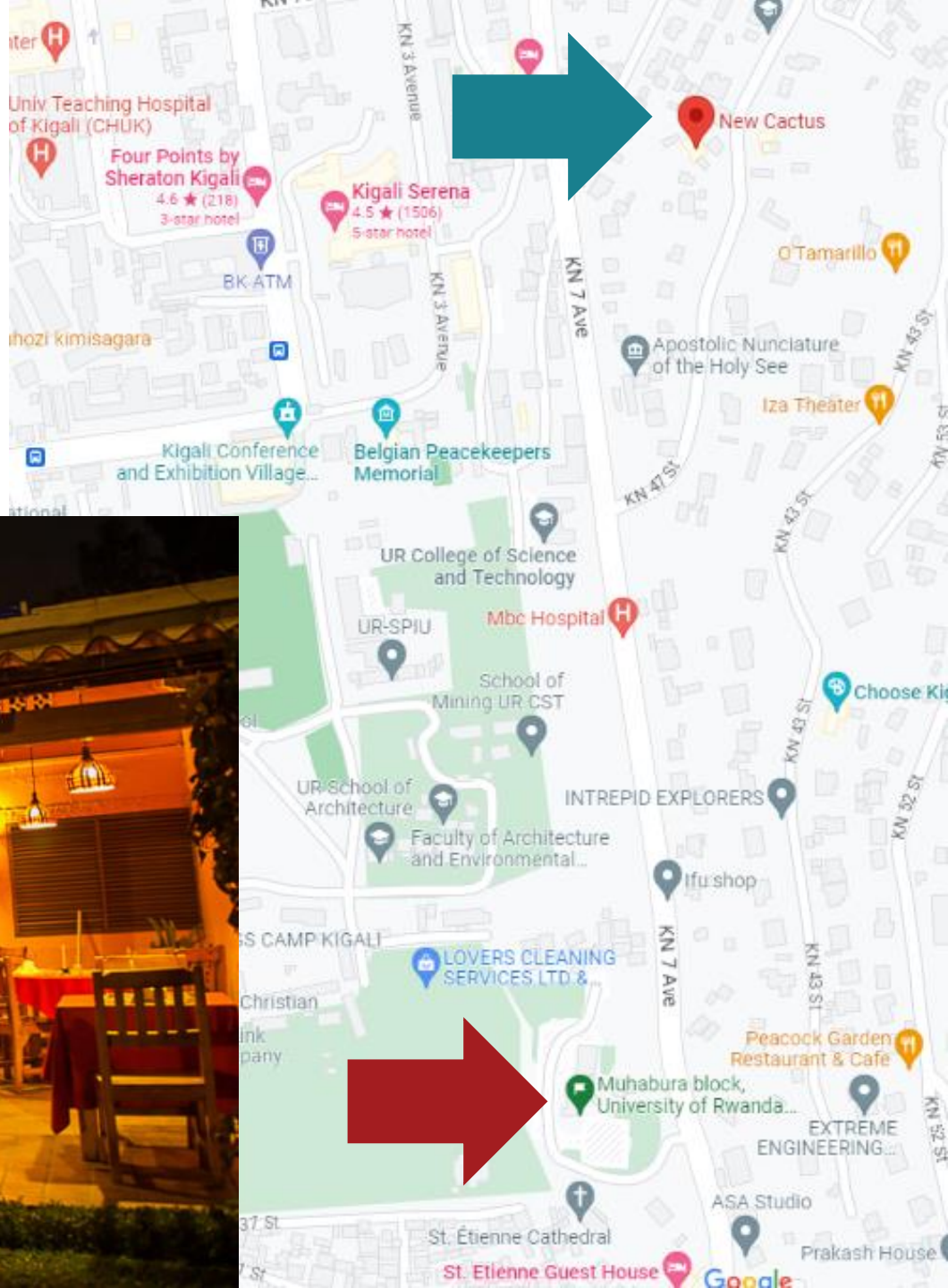
Laura Bertola
Isaac Overcast
Josiah Kuja
Anubhab Khan

Ice breaker: Join us!

New Cactus (KN 47 St)

~15 minutes walking

Pizza is on us!



The importance of genetic diversity

Laura Bertola

KØBENHAVNS UNIVERSITET

laura.bertola@gmail.com

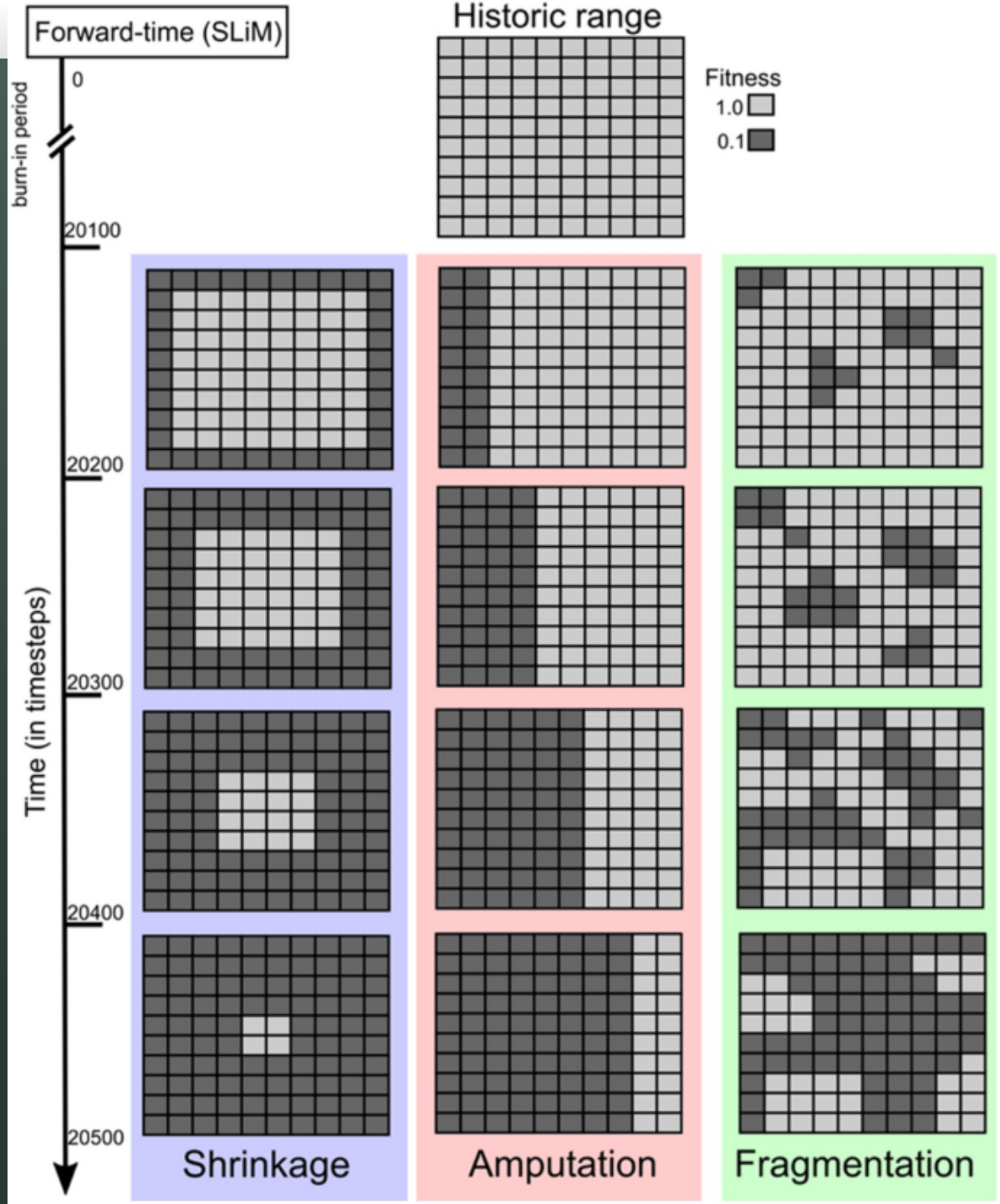
@LauraDBertola

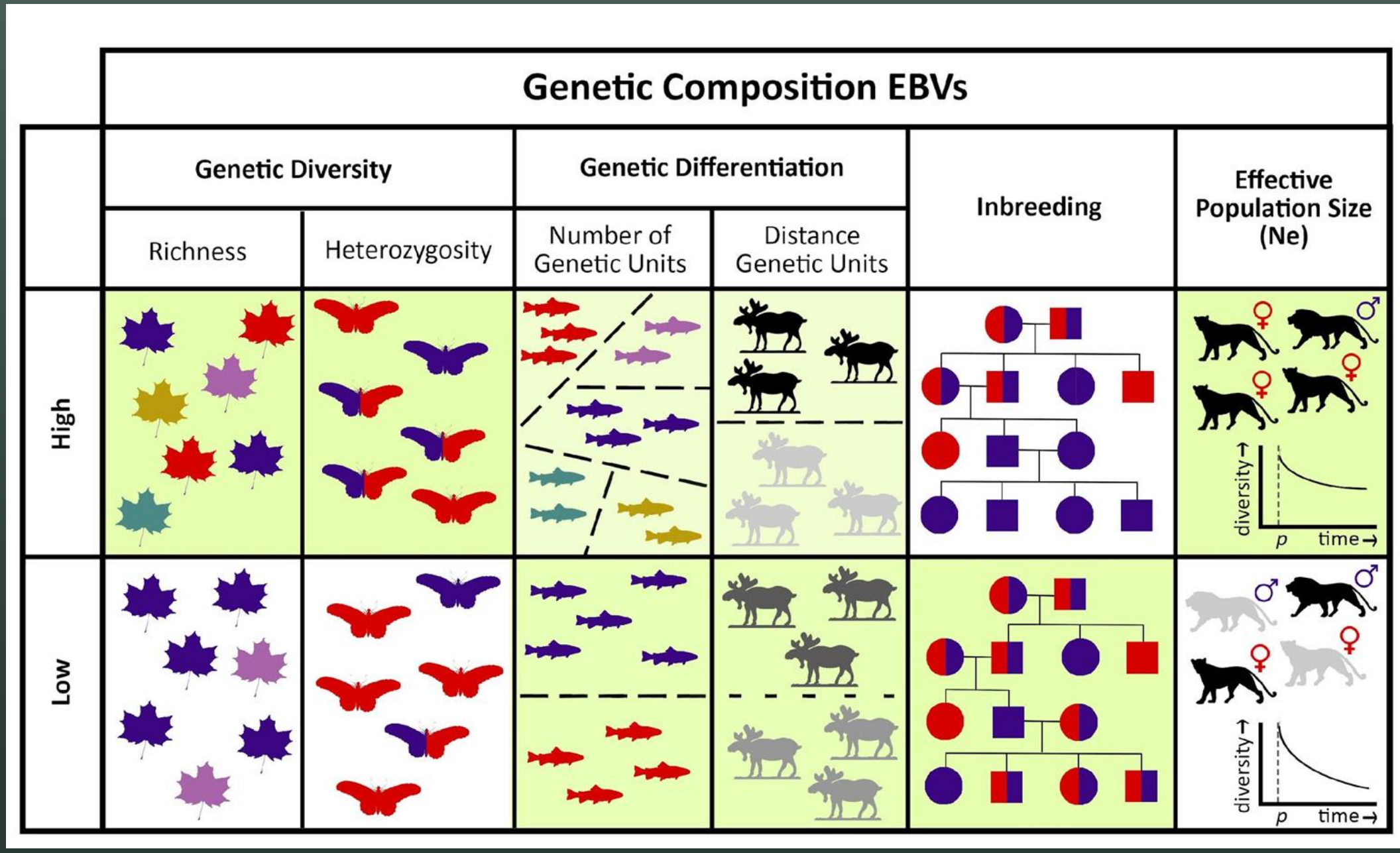
Loss of Biodiversity

- Primarily through loss of populations:
  mammals: loss of min. 30% (1900-2015)
  almost half: loss of >80%
  (Ceballos *et al.*, 2017)

- Not all range loss is equal
  (Rogan *et al.*, 2023)

Rogan *et al.*, 2023

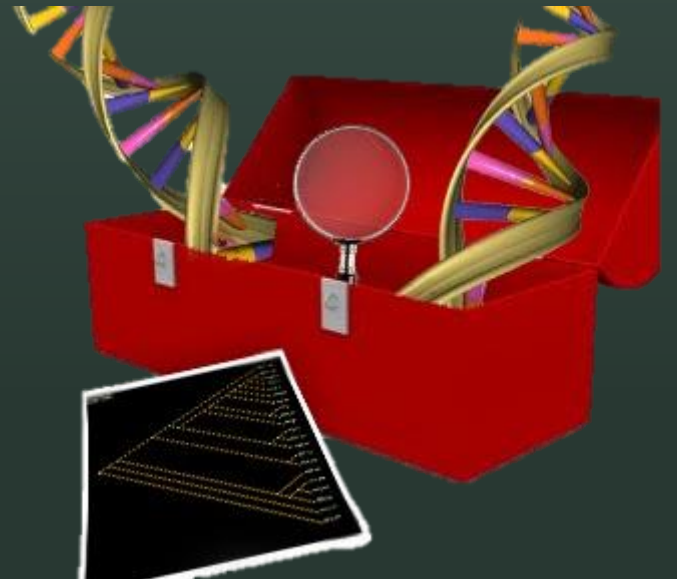




Hoban, *et al.*, Biological Reviews, 2022

Genetic data

- Diversity and connectivity
- Pedigrees, parentage
- Identify individuals (population estimates, individual home ranges)
- Hybridization
- Population history and future trajectory
- Prioritization, e.g. based on uniqueness or adaptive traits



The Expanding Role of Genetics & Genomics in Conservation

Gordon Luikart,
University of Montana, USA

Unprecedented Extinctions (2022)

Why be a conservation geneticist?

Approximately 10-30% of all vertebrate and plant species are threatened with extinction over the next few decades.

IUCN (1996, 2008, 2016)

It will take millions of years to correct the ongoing loss of genetic and species diversity. This is the folly our descendants are least likely to forgive us.

Edward O. Wilson (1984)

https://www.facebook.com/watch/?v=1561429063954907
https://earthsky.org/earth/extinct-bird-songs-woodpecker-warbler-kauai-oo/

LG1
LG2

Extinctions in 2021

Genetics has enormous untapped potential to help curb the extinction crisis!

This enormous potential of genetics results from...

Smooth handfish

Slide 3

LG1 Luikart, Gordon, 7/22/2023

LG2 smoot
Luikart, Gordon, 7/22/2023

A combination of advances in three areas

DNA sampling

Noninvasive field sampling, eDNA museum sampling

(Beja-Pereira et al. 2009)

+

Genotyping

Unlimited, informative DNA markers (lcWG-seq)

+

Data analysis

Statistical methods, models, & approaches

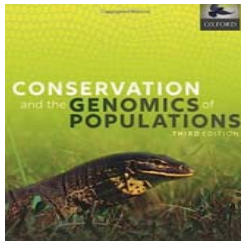
(Hemstrom et al. in review)

= Information explosion!

Improved conservation and management

Communication with managers, public, and policy makers is crucial!

(Chap 24 in Allendorf et al. 2022)



You & genetics can contribute in many ways!

1) Genetic diversity, inbreeding, & genetic rescue

2) Census & effective population size (past & present)

3) Population structure, units (CUs/MUs/ESUs)

4) Connectivity: geneflow & dispersal (past & present)

5) Local adaptation & selection

6) Phylogenetics, hybridization, outbreeding depression

7) Forensics, genetic identification, & law enforcement

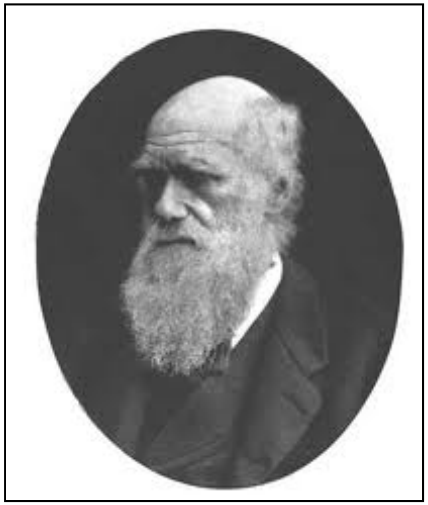
8) Disease ecology & transmission

9) Community genomics (interactions improve cons)

*Uses for ecology & demography versus gene diversity *per se*

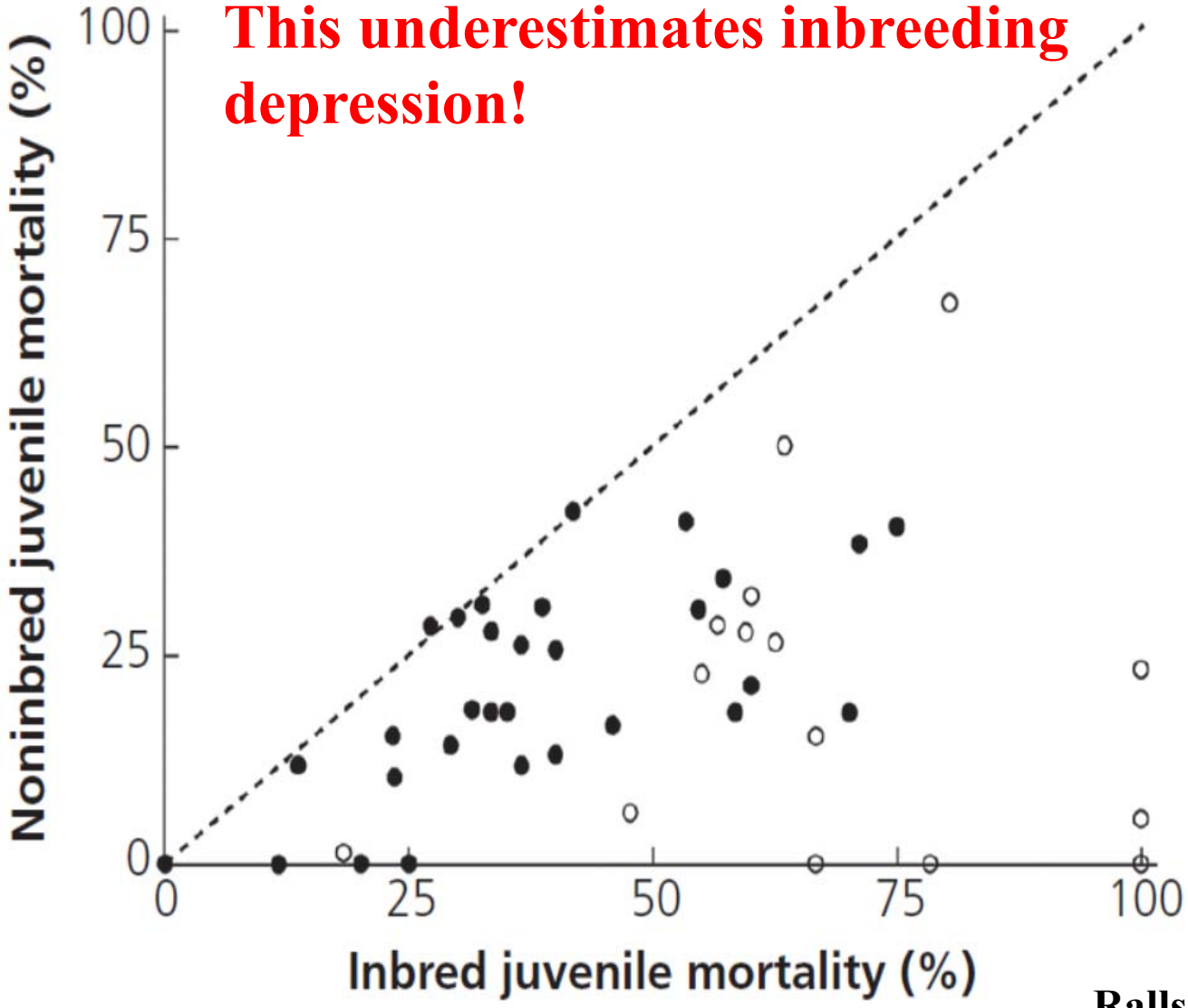
Conservation genetics and inbreeding avoidance has been practiced for >100 years!

As some of our British parks are ancient, it occurred to me that there must have been long-continued close interbreeding with the fallow-deer (*Cervus dama*) kept in them; but on inquiry I find that it is a common practice to infuse new blood by procuring bucks from other parks.



Married his cousin!

Inbreeding reduces individual fitness!



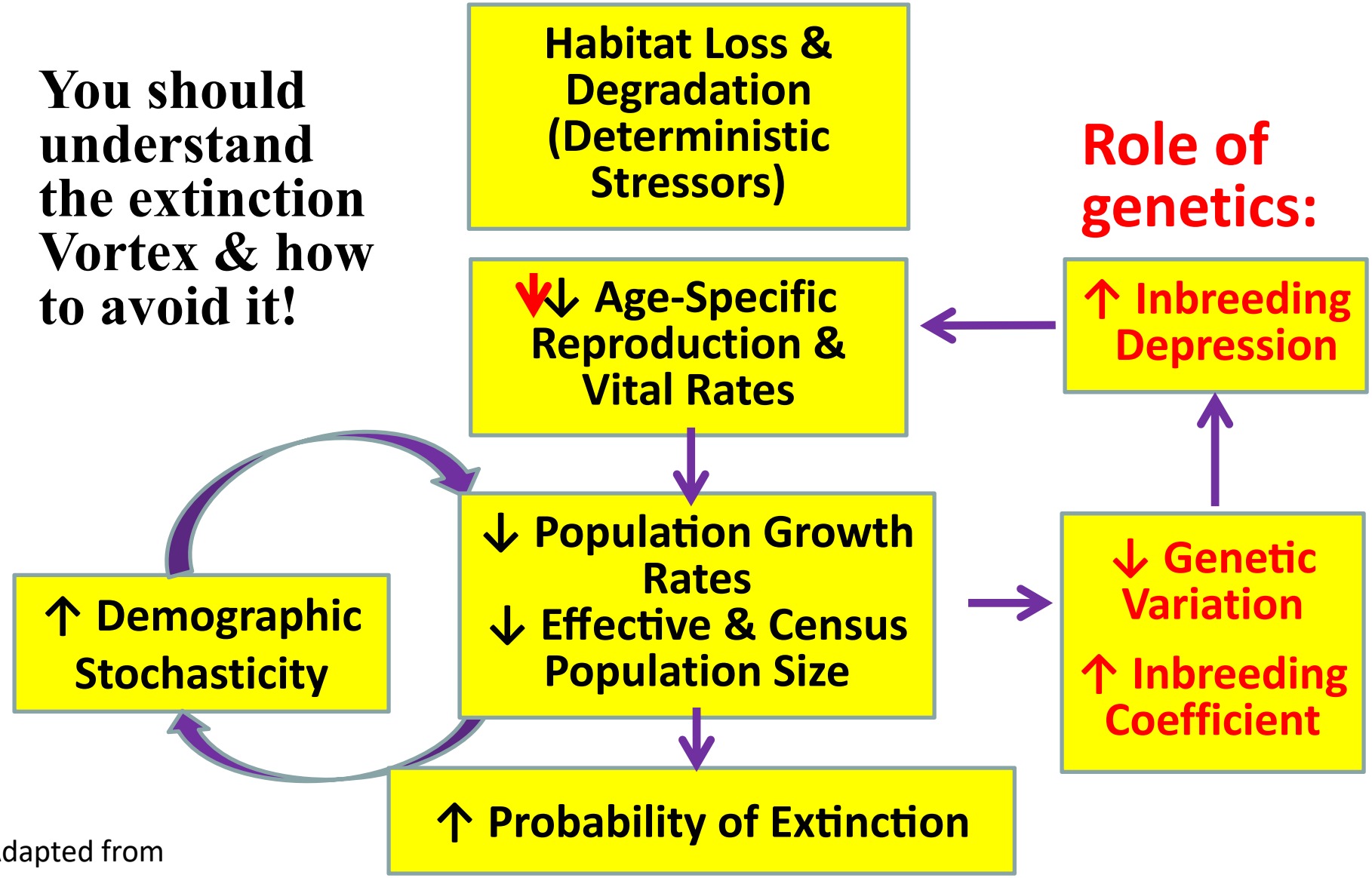
Each circle is one of 44 captive populations of mammals (16 ungulates, 16 primates, and 12 small mammals).

The line shows equal mortality in inbred and non-inbred progeny.

Ralls et al. 1979. Science.

Loss of variation reduces population persistence

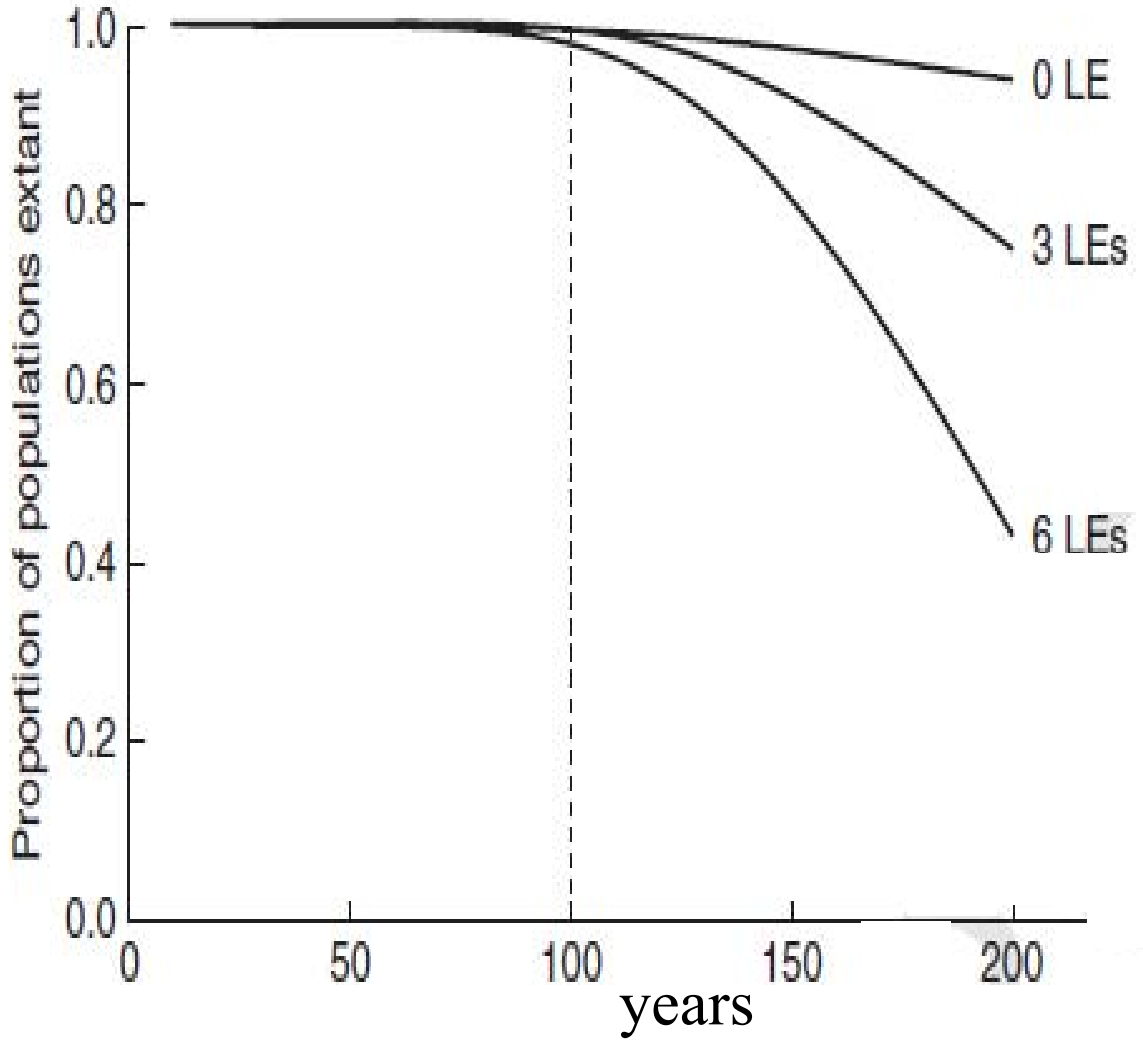
You should understand the extinction Vortex & how to avoid it!



Adapted from
Mills & Soule 1998, *Science*

PVA Simulations (with genetics!)

Inbreeding depression can reduce population persistence



No inbreeding

30% reduces Survival

60% reduces Survival

Allendorf et al. 2022
Parameterized with
Data from Ralls et al. 1979

Vortex sims of Yellowstone Grizzly population. Inbreeding reduced Juvie survival

nature ecology & evolution

ARTICLES

https://doi.org/10.1038/s41559-019-0968-1

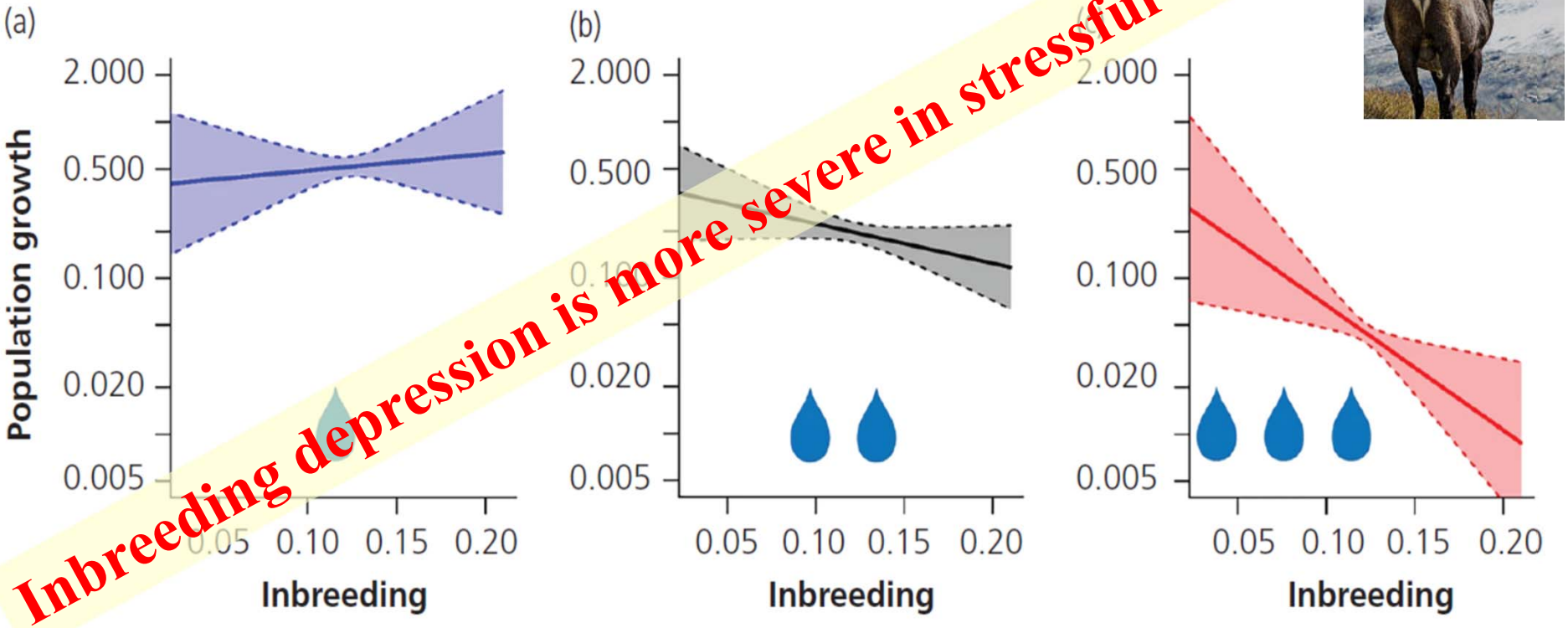
Empirical study **2019**

Inbreeding reduces long-term growth of Alpine ibex populations

Claudio Bozzuto[1,5,6], Iris Biebach[1,6], Stefanie Muff[1,2], Anthony R. Ives[3*] and Lukas F. Keller[1,4*]

Many studies document negative inbreeding effects on individuals, and conservation efforts to preserve rare species routinely employ strategies to reduce inbreeding. Despite this, there are few clear examples in nature of inbreeding decreasing the growth rates of populations, and the extent of population-level effects of inbreeding in the wild remains controversial. Here, we take advantage of a long-term dataset of 26 reintroduced Alpine ibex (*Capra ibex ibex*) populations spanning nearly 100 years to show that inbreeding substantially reduced per capita population growth rates, particularly for populations in harsher environments. Populations with high average inbreeding ($F \approx 0.2$) had population growth rates reduced by 71% compared with populations with no inbreeding. Our results show that inbreeding can have long-term demographic consequences even when environmental variation is large and deleterious alleles may have been purged during bottlenecks. Thus, efforts to

Inbreeding depression reduces population growth rates in alpine ibex



Inbreeding depression is more severe in stressful environments

Best-fitting statistical model relating population growth to average inbreeding in interaction with summer precipitation. From 26 reintroduced populations. Population-specific *F* (inbreeding) computed from microsatellites.

Bozzuto et al. 2019

Cautionary note:

PERSPECTIVE PNAS 2021

The inflated significance of neutral genetic diversity in conservation genetics

João C. Teixeira[a,b,1] and Christian D. Huber[a,1]

"We argue against the perceived importance of neutral genetic diversity of the conservation of wild populations and species"

The crucial role of genome-wide genetic variation in conservation

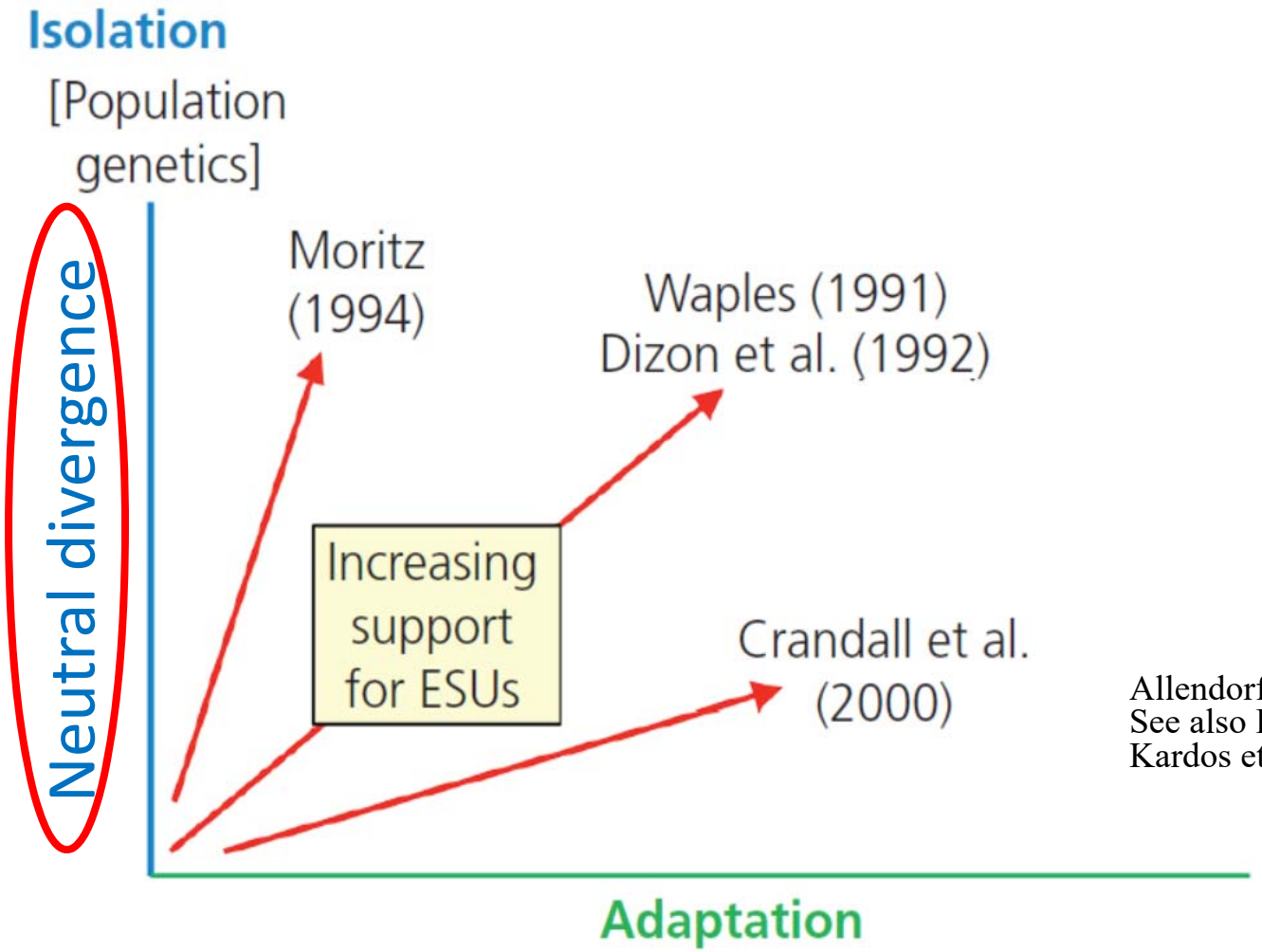
PNAS 2021

Marty Kardos[a,1], Ellie E. Armstrong[b], Sarah W. Fitzpatrick[c,d,e], Samantha Hauser[f], Philip W. Hedrick[g], Joshua M. Miller[h,i,j], David A. Tallmon[k], and W. Chris Funk[l]

The unprecedented rate of extinction calls for efficient use of genetics to help conserve biodiversity. Several recent genomic and simulation-based studies have argued that the field of conservation biology has placed too much focus on conserving genome-wide genetic variation, and that the field should instead focus on managing the subset of functional genetic variation that is thought to affect fitness. Here, we critically evaluate the feasibility and likely benefits of this approach in conservation. We find that population genetics theory and empirical results show that conserving genome-wide genetic variation is generally the best approach to prevent inbreeding depression and loss of adaptive potential from driving populations toward extinction. Focusing conservation efforts on presumably functional genetic variation will only be feasible occasionally, often misleading, and counterproductive when prioritized over genome-wide genetic variation. Given the increasing rate of habitat loss and other environmental changes, failure to recognize

Why conserve genome-wide neutral diversity first, then consider adaptive variation?

ESU or Distinct Population ID has 2 main requirements/axes



Allendorf et al. 2022;
See also **Hoffman et al. 2016**
Kardos et al. 2021

Why learn population genetics?

It's one of 3 key disciplines in Conservn. Biology

Evolution (= population genetics)

Ecology

Human dimensions

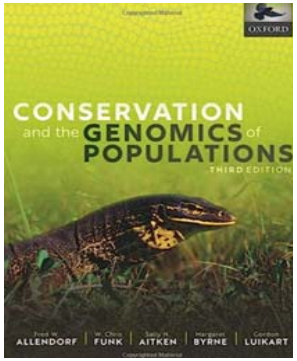
Groom, Meffe, Carroll 2007. Book

Why learn population genetics?

24.4.1 What is a conservation geneticist?

First and foremost, a good conservation geneticist is a population geneticist. To be effective in conservation genetics requires numerous other skills, but underlying all of those has to be a firm knowledge and understanding of genetic theory. This is non-negotiable and means that if you have an interest in applying genetics to conservation, it would be helpful to seek out a graduate training program featuring modules in population genetics. As a graduate student, you need at least one supervisor/advisor who specializes in the subject. Graduate students

Helen Taylor, Chapter 24 in Allendorf et al. (2022) book



Why learn population genetics theory?

Testing for Hardy–Weinberg Proportions: Have We Lost the Plot?

ROBIN S. WAPLES **2015**

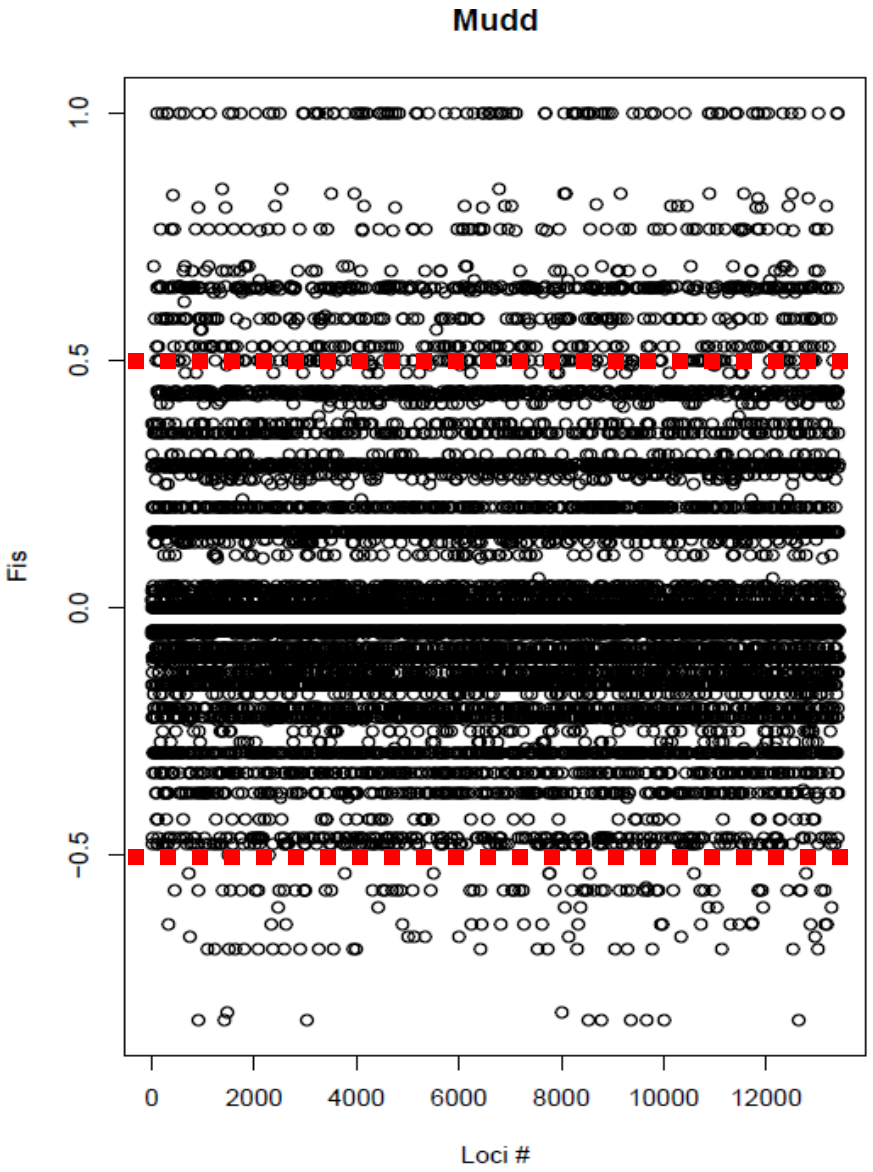
From the Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric

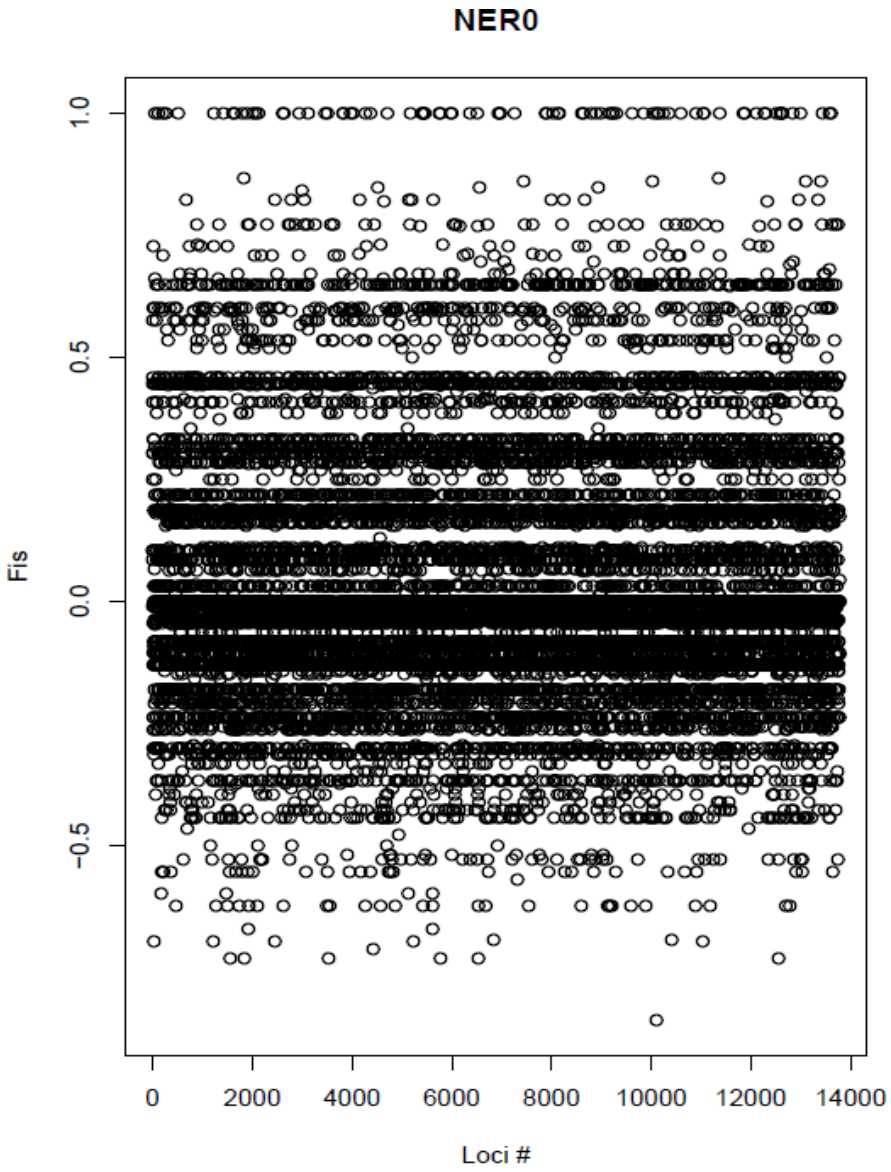
I am constantly appalled in reviewing manuscripts and even published papers to see people go through the motions of performing all the HW and LD tests, reporting the results, and then completely ignoring them, even when significant deviations are found. Few people know how to properly interpret multiple testing results, and most seem to have lost track of why it is essential to do these tests before using the data for other analyses.

See Sethuraman et al. 2019, Mol. Ecol. Res.

Why learn coding, line commands, & R?

To analyze NGS data (F_{IS} at 14,000 RAD SNPs in elk!)





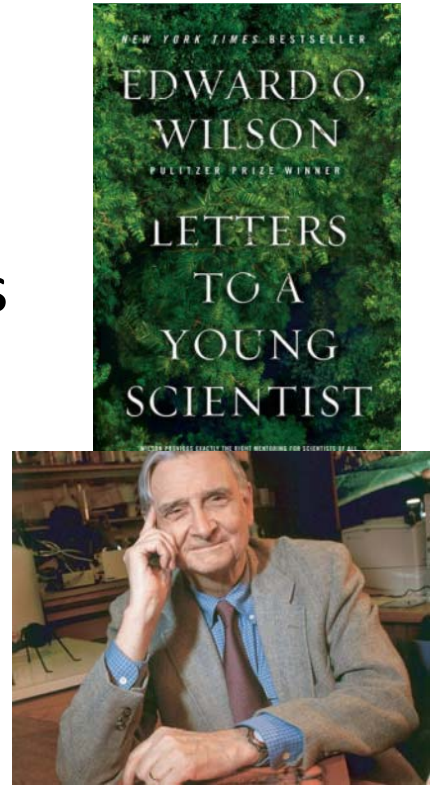
Do not be intimidated by math or coding

"Most successful scientists are only moderately literate in math." …Especially those who are most creative, visionary, who **advance their field.**

(Chapter 2 "Mathematics" in Wilson 2002)

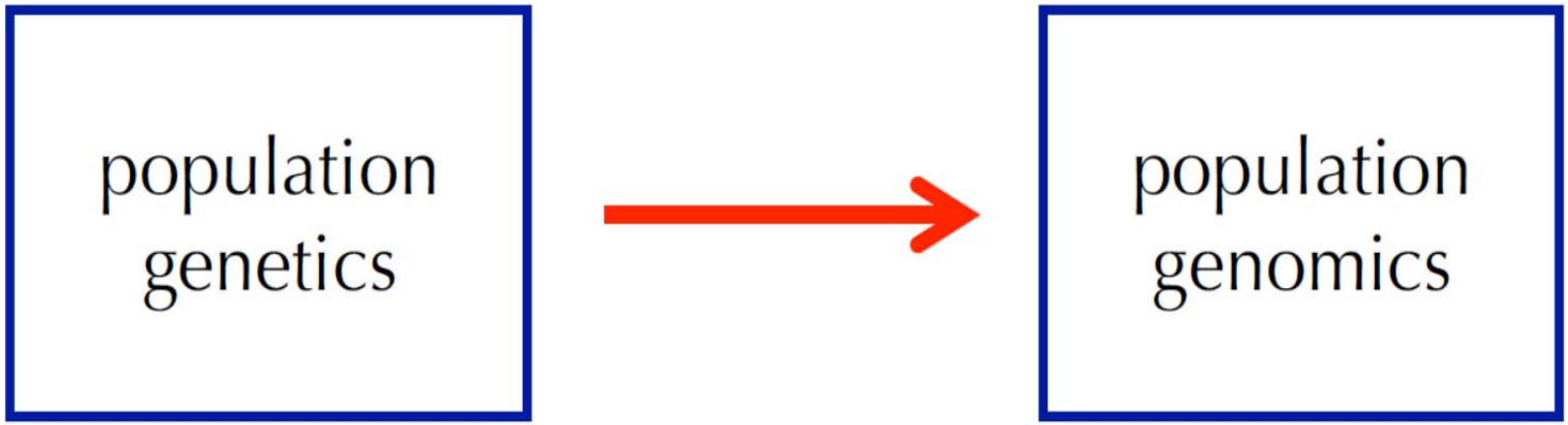
"Work ethic, passion, and collaboration are as important or more important…"

(Chapter 6 "What it takes"; Wilson 2002)



What is conservation genomics?

Application of genomic techniques in conservation



What is conservation genomics?

'Better' definition ("narrow-sense genomics")

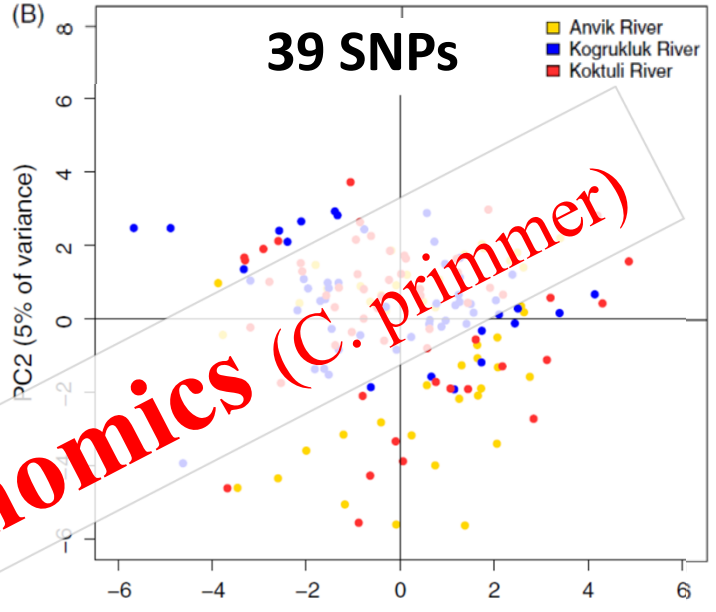
- address questions impossible with genetics alone
- functionally important loci

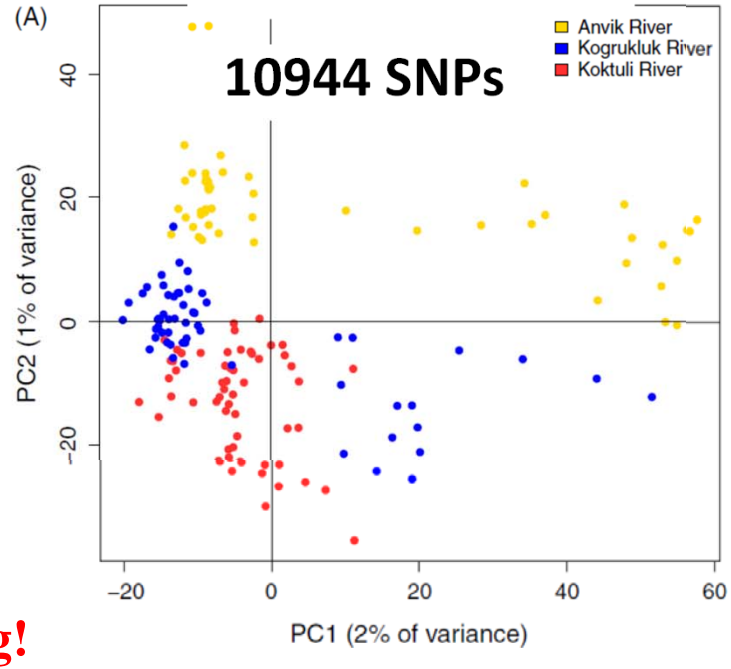
  -adaptive or deleterious vs neutral

  -associated with phenotypes (GWAS etc.)

Garner et al (2016) *TREE* 31: 81
Hohenlohe et al (2018) *Population Genomics* (Rajora OP, ed)

More loci improve substructure resolution
(often impossible w/o genomics)





Do genomics to not do genomics (C. primmer)

Using only 100 "informative" loci can also improve resolution!

So, won't need genomics for subsequent monitoring!

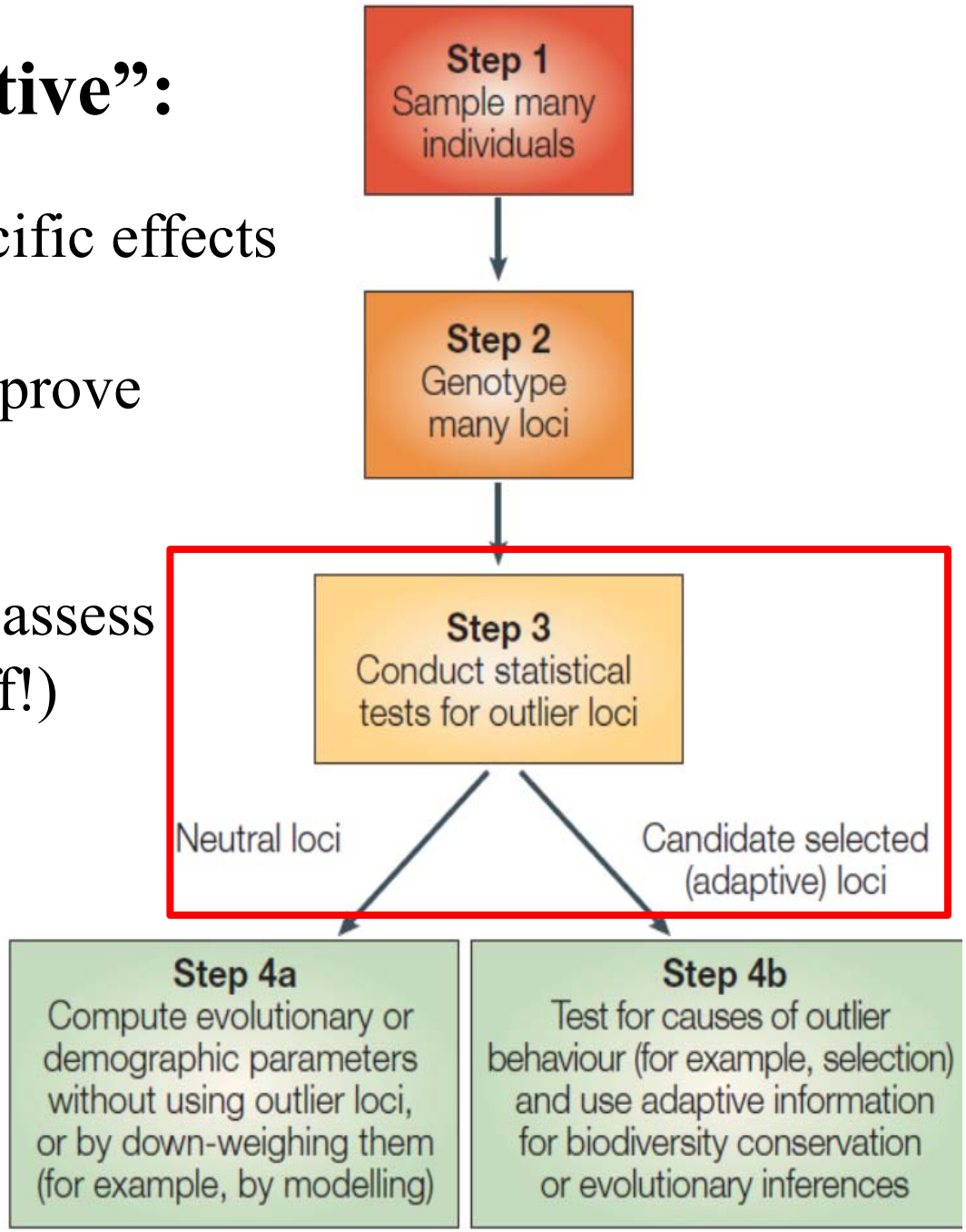
Larson et al. 2013. Evol Apps.

A “Genomics perspective”:

- Genome-wide vs locus-specific effects

- Remove adaptive loci to improve parameter estimation

- Use outlier/adaptive loci to assess function (the exciting stuff!)



Luikart et al. (2003) ***Nature Reviews Genetics. See Black et al. 2001***

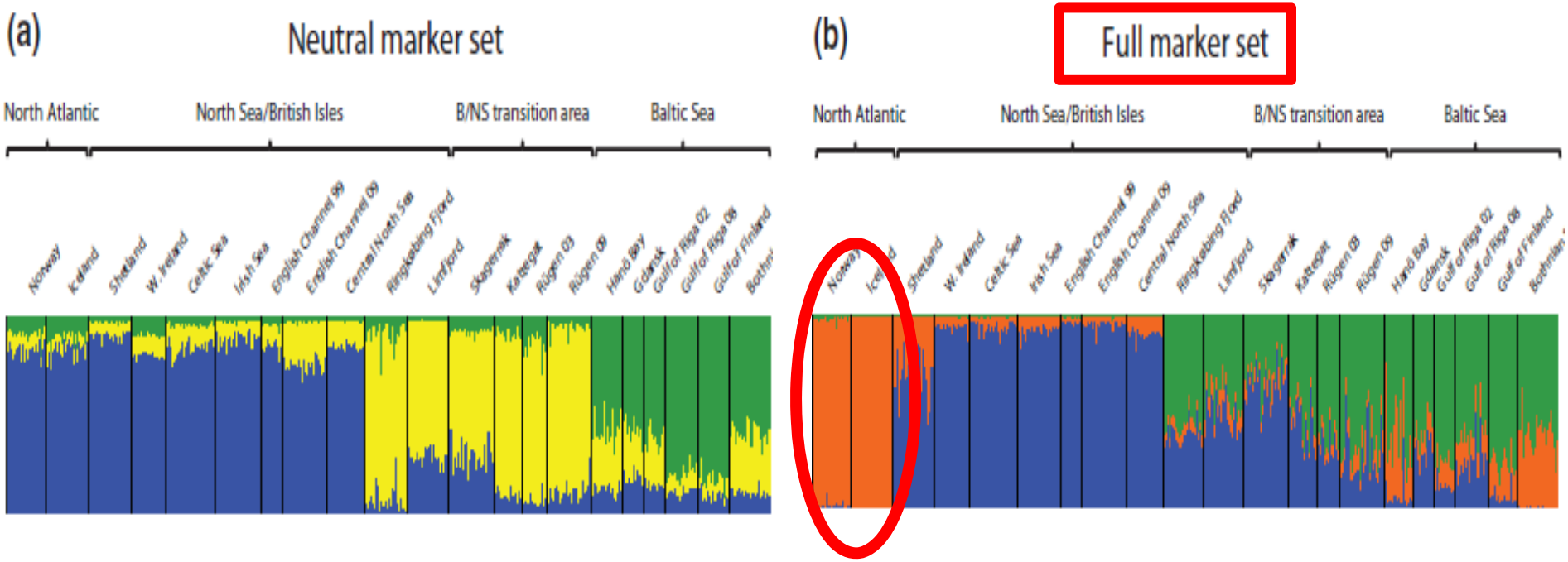
Use of Adaptive loci

MOLECULAR ECOLOGY

Molecular Ecology(2012) 21, 3686–3703

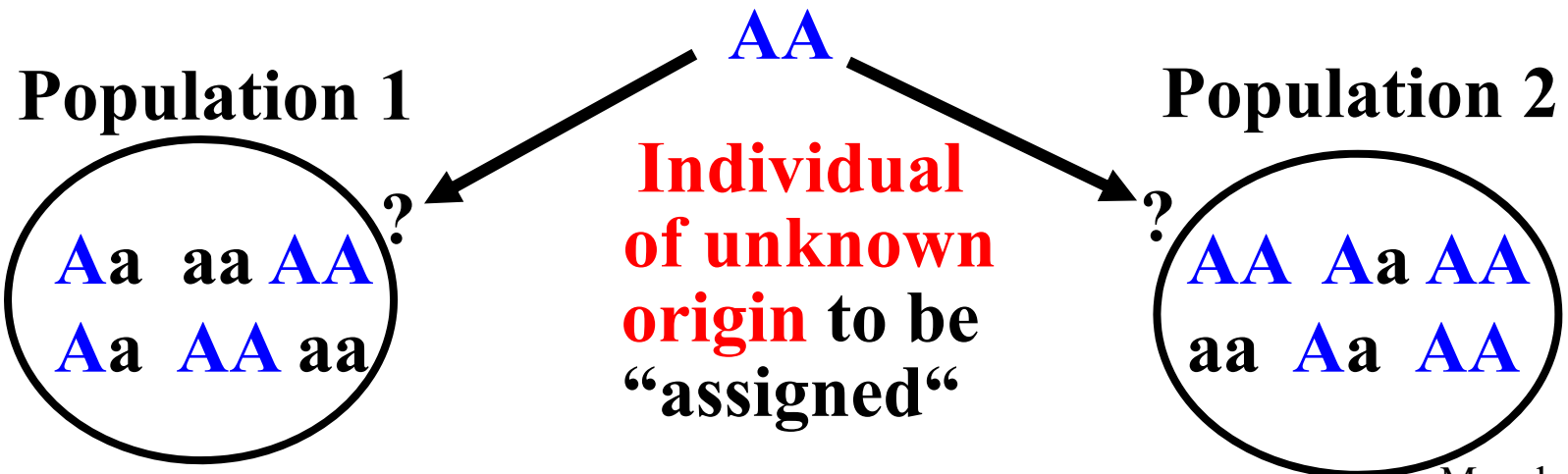
Environmental selection on transcriptome-derived SNPs in a high gene flow marine fish, the Atlantic herring (*Clupea harengus*)

MORTEN T. LIMBORG,*[1] SARAH J. HELYAR,†‡[1] MARK DE BRUYN,† MARTIN I. TAYLOR,†

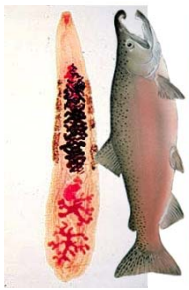


Genomics (adaptive loci) identify a cryptic adaptively-differentiated pop!

Assignment tests (using informative loci) help detect wildlife poaching and identify immigrants



Manel et al. 2002



AUTHOREA Pre-print 2023

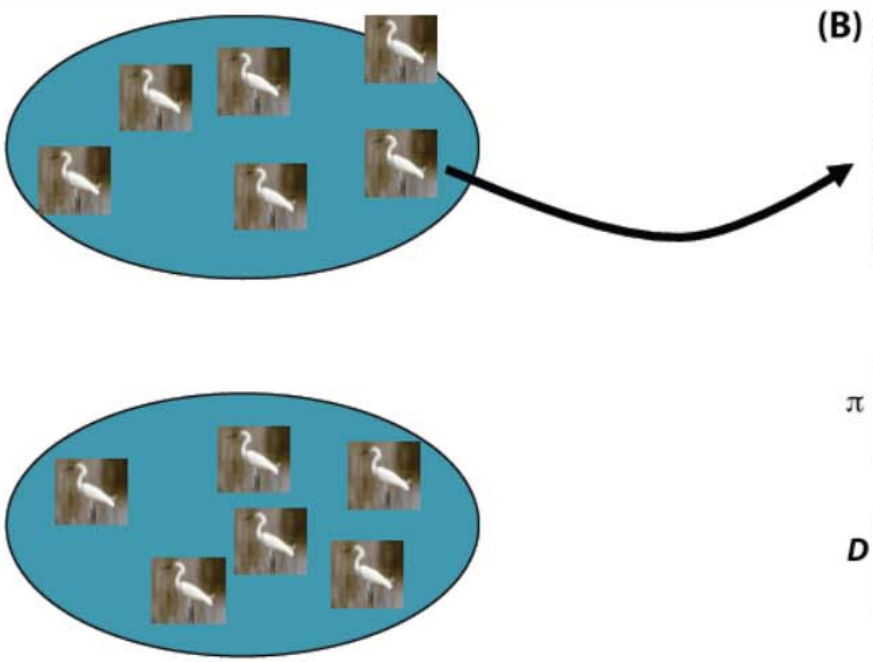
Low-coverage whole-genome sequencing for highly accurate population assignment: Mapping migratory connectivity in the American redstart (Setophaga ruticilla)

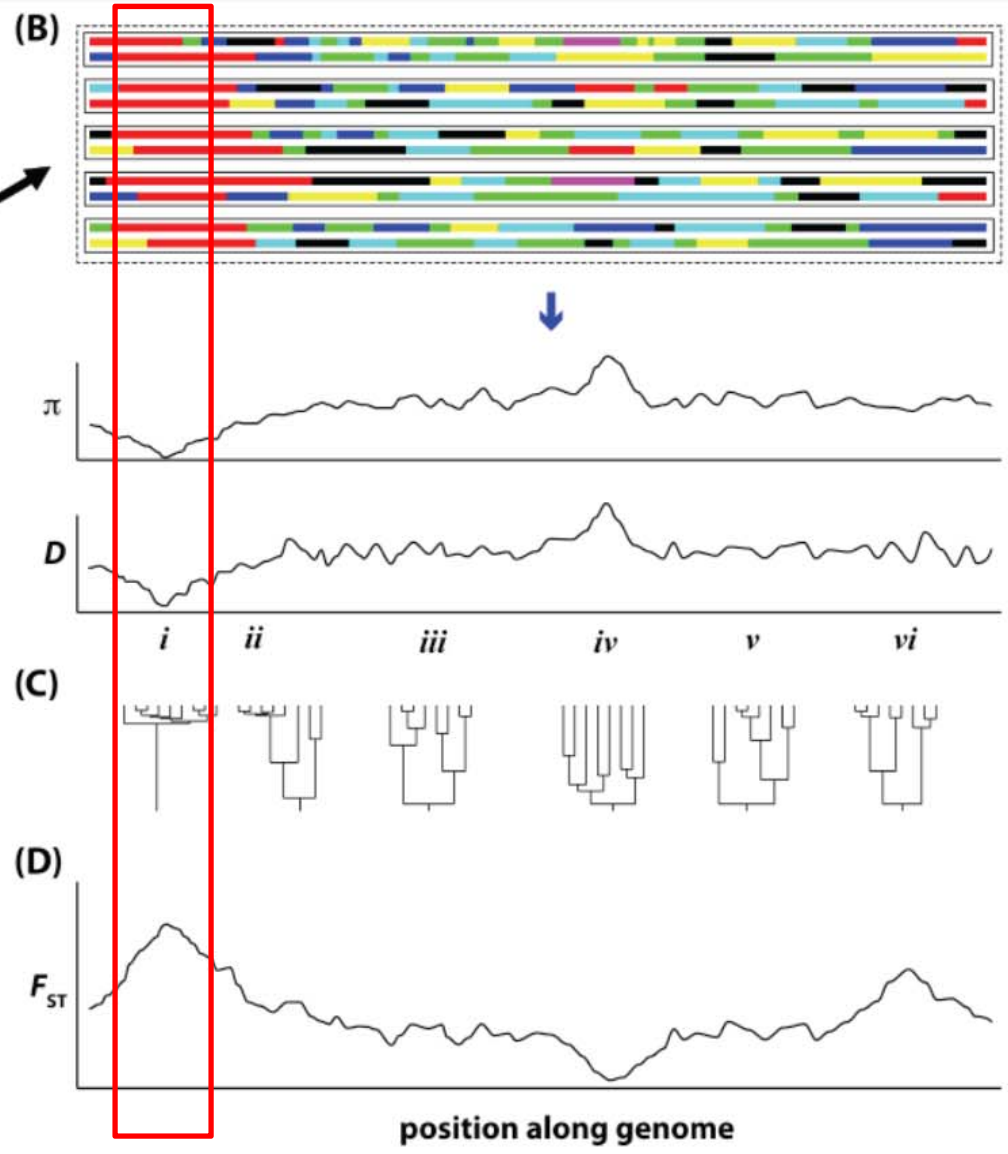
Matthew DeSaix[1], Eric Anderson[2], Christen Bossu[1], Christine Rayne[3], Teia Schweizer Nicholas Bayly[4], Darshan Narang[5], Julie Hagelin[6], H. Lisle Gibbs[7], James Saracco[8], Thomas W Sherry[9], Michael Webster[10], Thomas Smith[11], Peter Marra[12], and Kristen Ruegg[1]

"We use lcWGS to identify fine-scale population structure of the American Redstart, and accurately assign individuals (>98%) to breeding populations."

Down-sampling from 1.7x to 0.1x & 0.01x achieved high assignment accuracy!

A genomics perspective: sum stats along the genome





"Population genomics takes data on haplotypes within a population and calculates summary statistics as continuous variables along the length of the genome."

Hohenlohe et al (2010) ***Int J Plant Sci 171:1059***

Conclusions & summary

It’s an exciting time to be a conservation geneticist!

Use new methods with caution - evaluations are needed!

Know genetics theory & perspectives

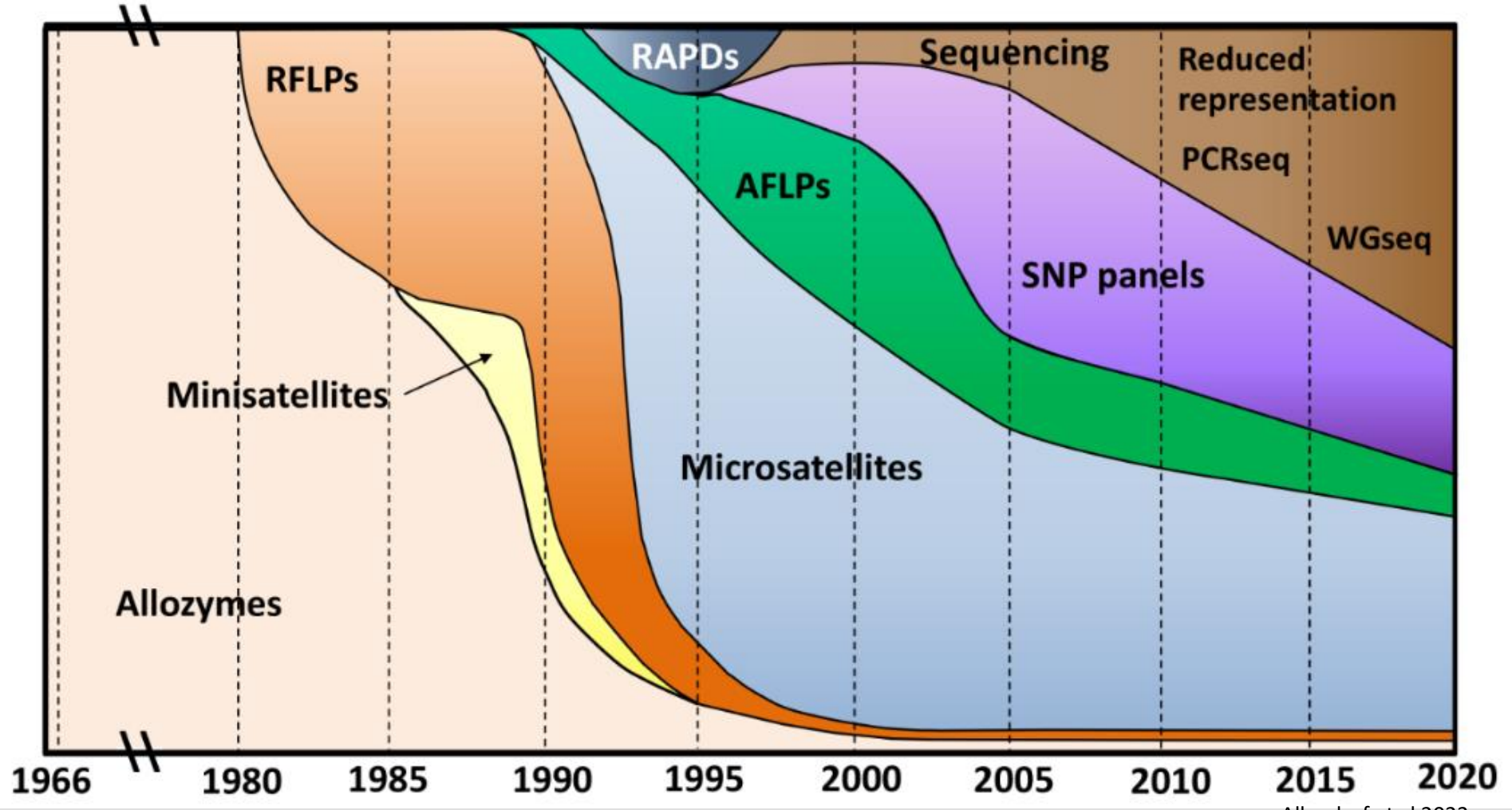
Biodiversity conservation needs you!

Thank you! Questions?

Genetics and Genomics: Data Types

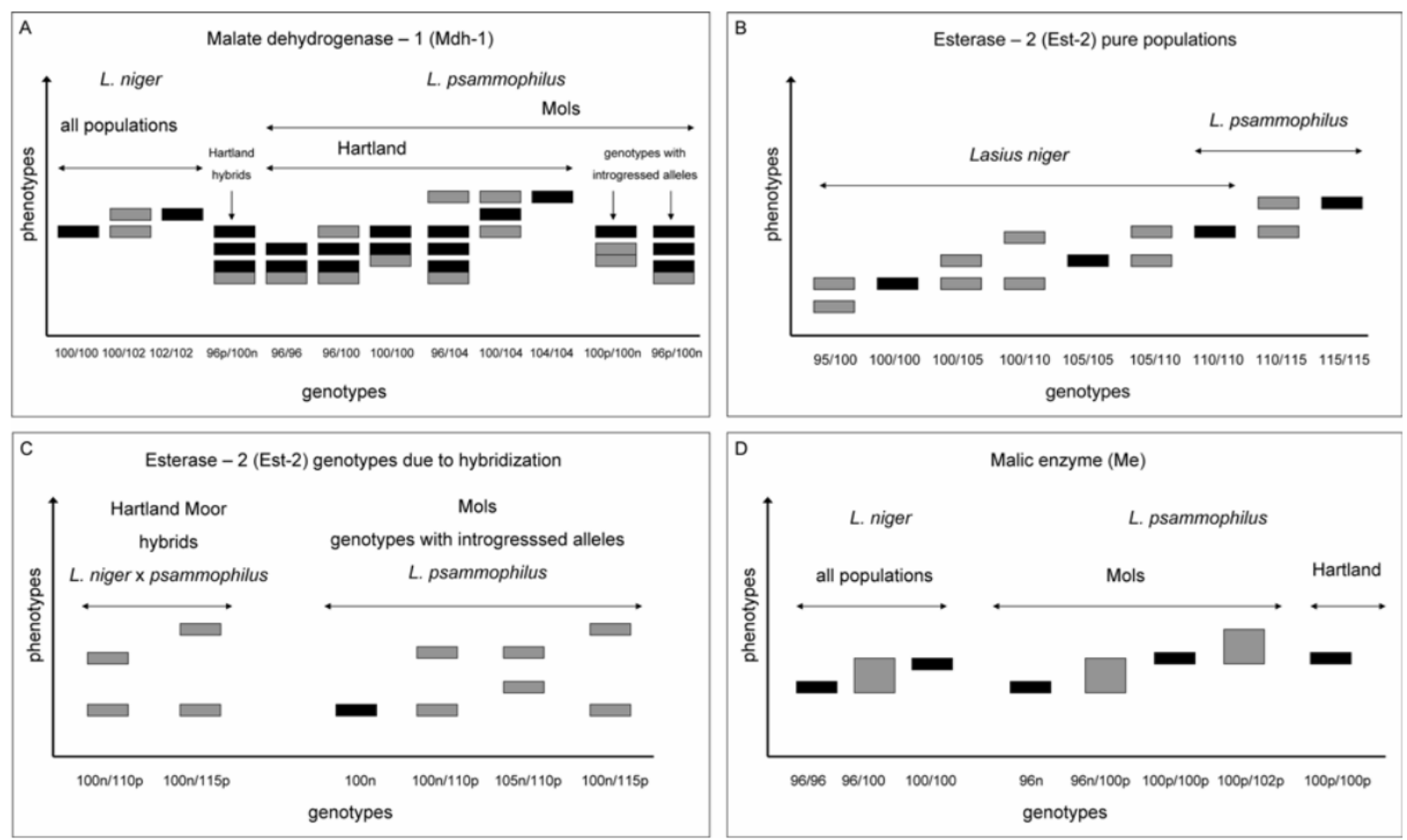
William Hemstrom

Genetics and Genomics: Data Types

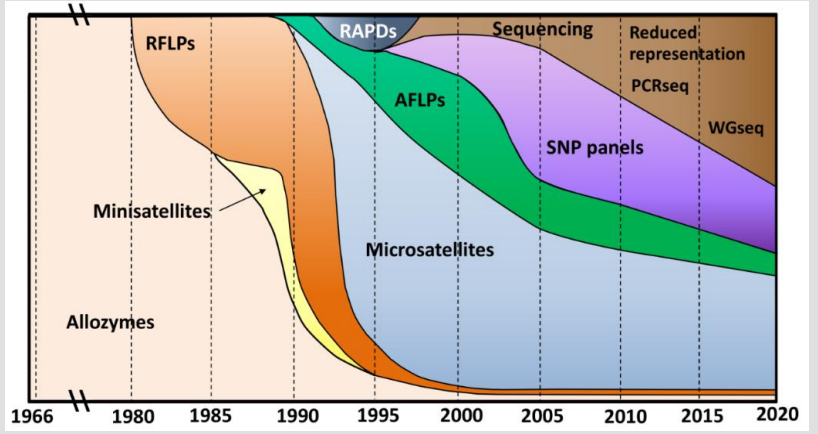


Allendorf et al 2023

Allozymes and RLFPs



Van der Have et al 2011



CTGACG
GCAGTC

- Enzyme configuration differences (allozymes)
- Restriction fragment length differences (RFLPs)
- Both old, rare today.
- Low power.
- 5-20 loci

Microsatellites

ATCATCATCAT

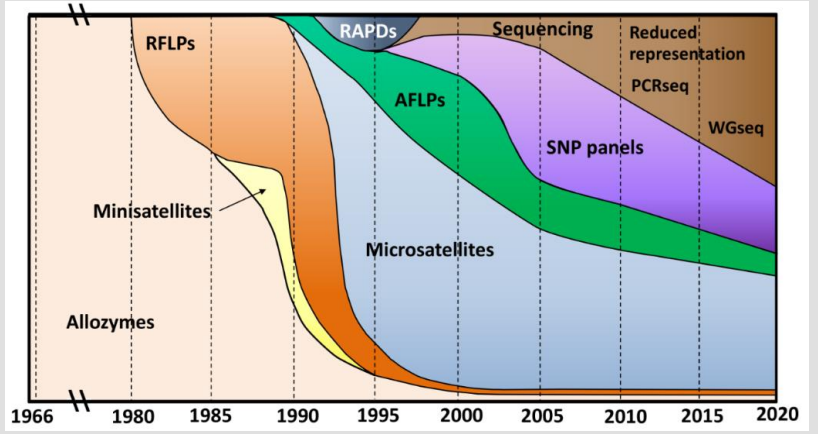
C x4

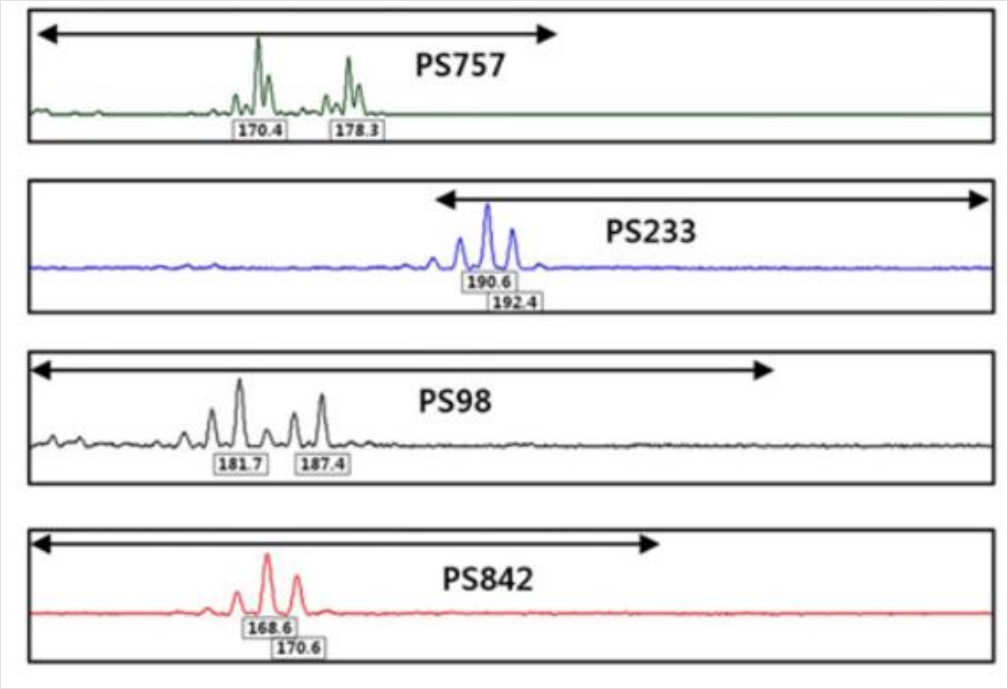
ATCATCATCATCAT

x5

ATCATCATCATCATATC

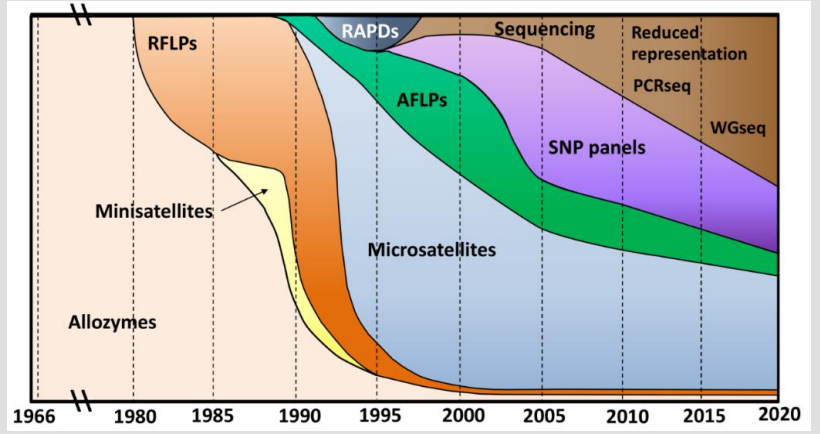
x6





Lee et al 2011

Microsatellites



ATCATCATCAT

C_{x4}

ATCATCATCATCAT

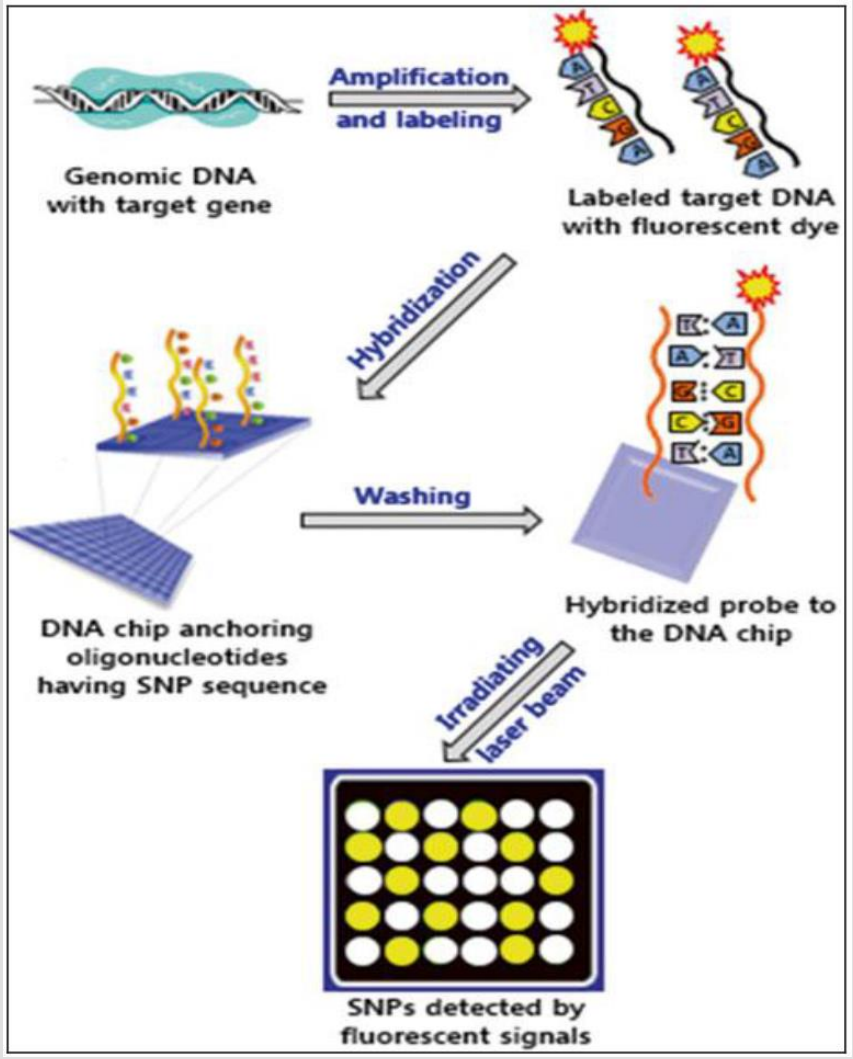
x5

ATCATCATCATCATATC

x6

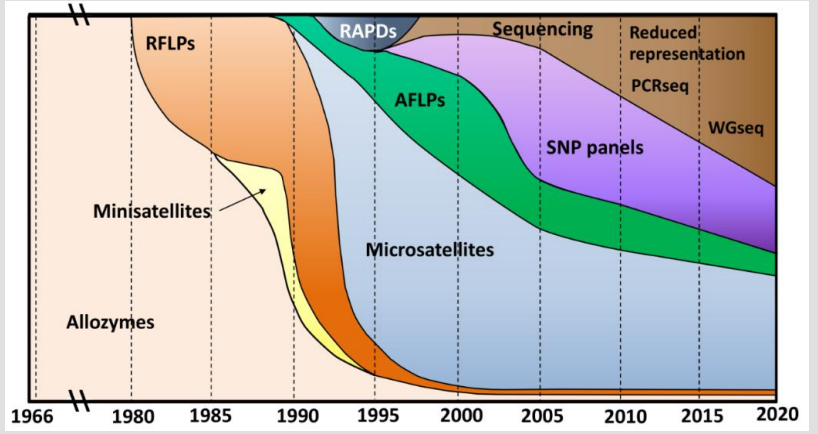
- Variable length repeats
- Highly polyallelic
- Easy
- Cheap
- 5-20 loci
- Still used today!

SNP panels/SNP chips



Mishra et al 2017

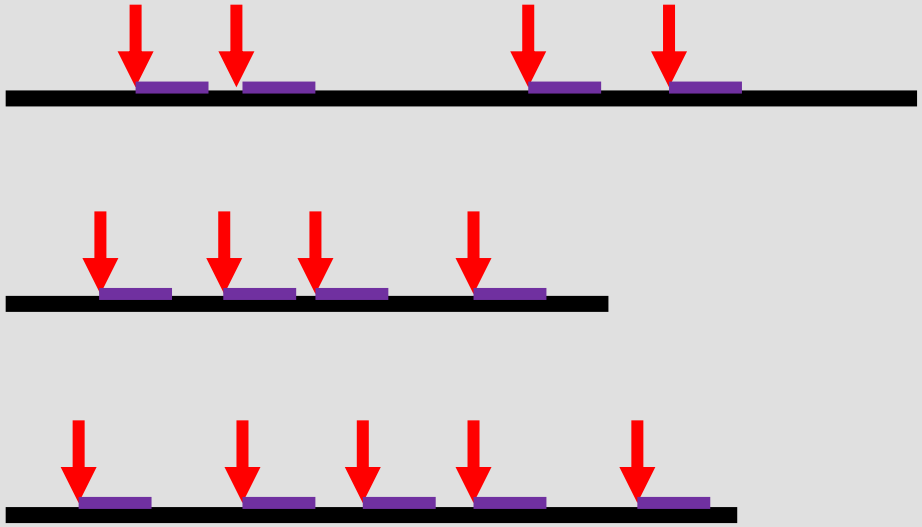
CGGACTACG
CGGAATACG

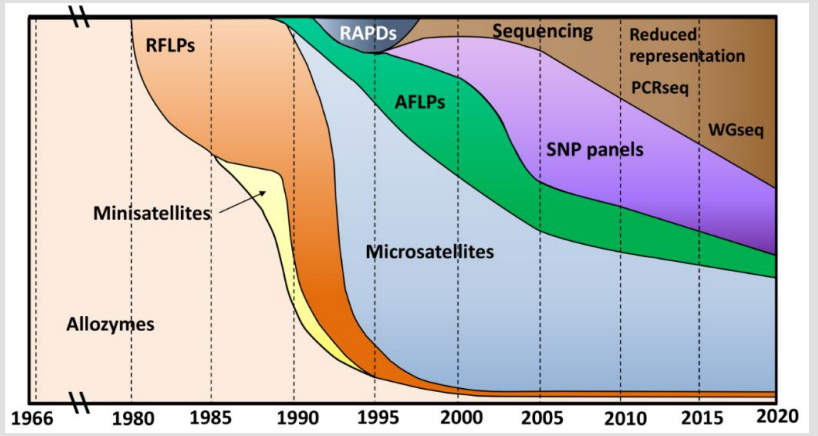


- Single-nucleotide Polymorphisms (SNPS)
- Only two alleles per locus
- Relatively cheap
- Need to be developed
- Higher level of technical expertise
- 1k – 100k+ loci

Reduced Representation

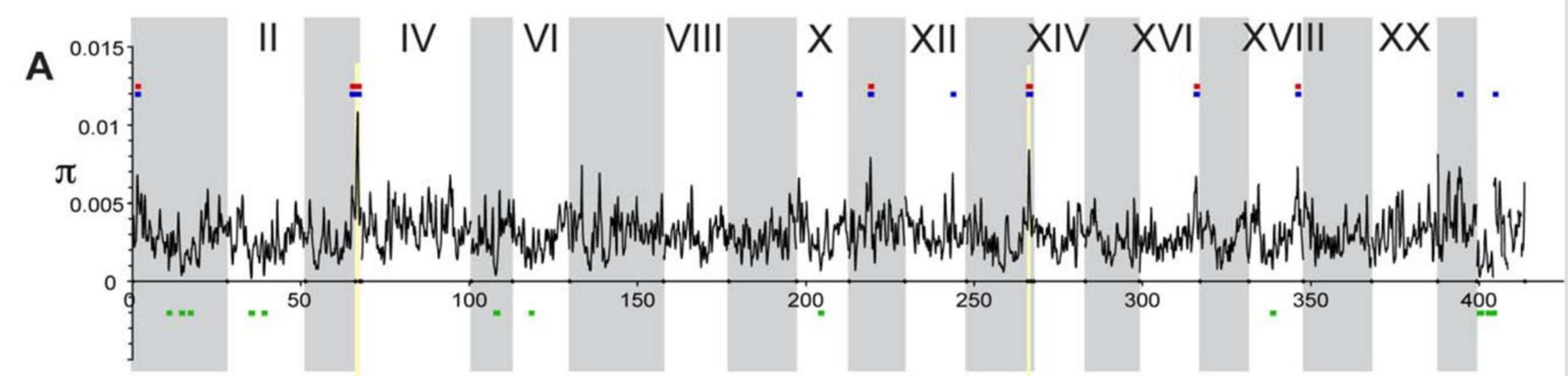
CTGACG
GCAGTC





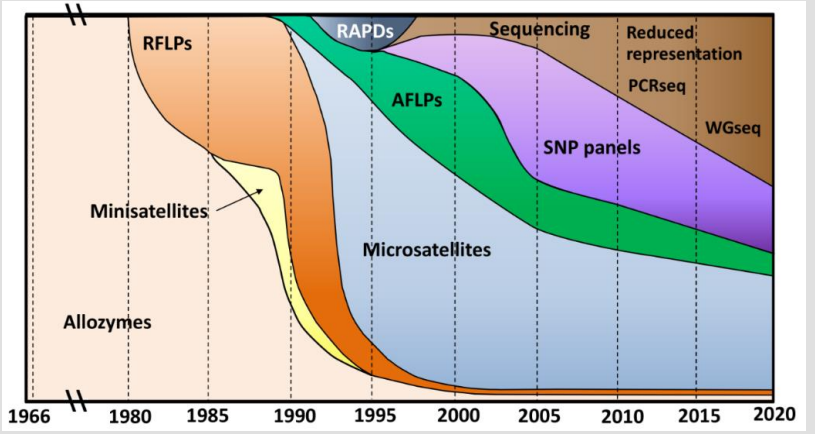
- SNPs/structural variants, etc.
- Can target random areas near cut sites (RAD)
  - Many exciting flavours!
- Can target expressed or specific areas (sequence capture)
- 1k-100k+ loci
- Fairly cheap
- No development needed for some types
  - RAD variants
  - Can benefit from a reference genome
- Great for studying patterns of diversity genome wide in non-model organisms
- Can pool individuals to save money
  - No individual data, just population level

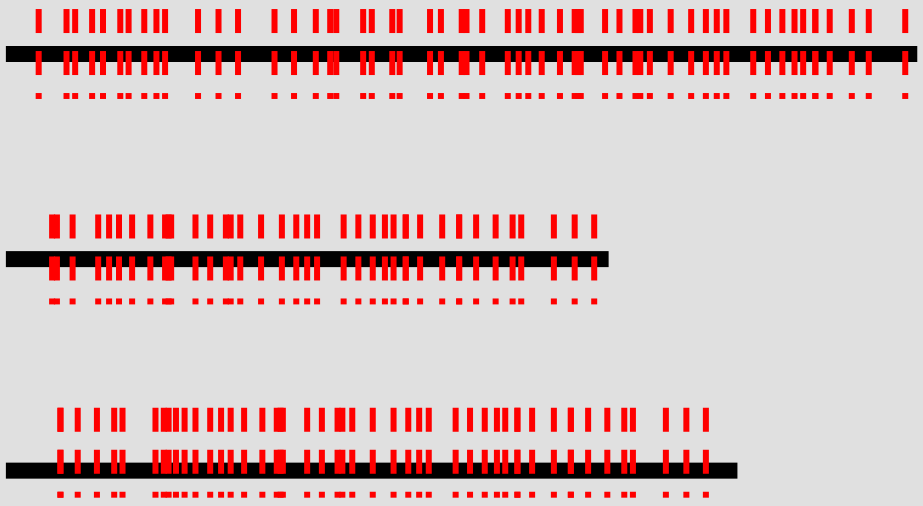
Reduced Representation



Hohenlohe et al 2010

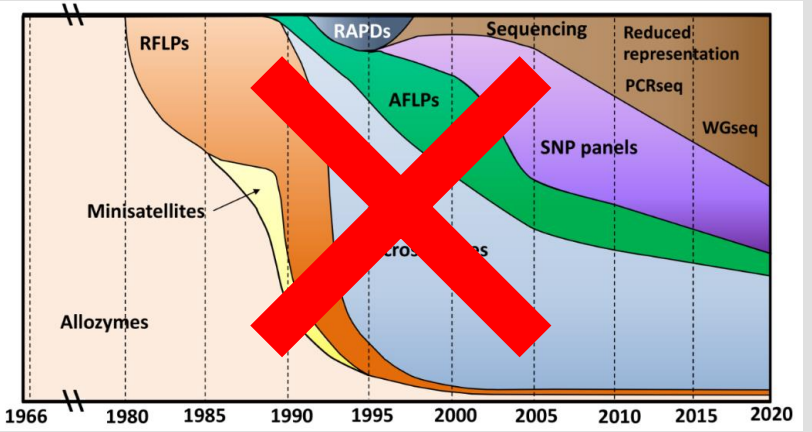
Whole-genome sequencing





- SNPs/structural variants, etc.
  - Essentially everything barring tough-to-sequence regions
- 100k – 10M+ loci
- Often expensive
  - getting cheaper over time!
- No development needed
  - Greatly benefits from a reference genome.
- Excellent for studying patterns of diversity genome wide
- The gold standard

RNA-seq



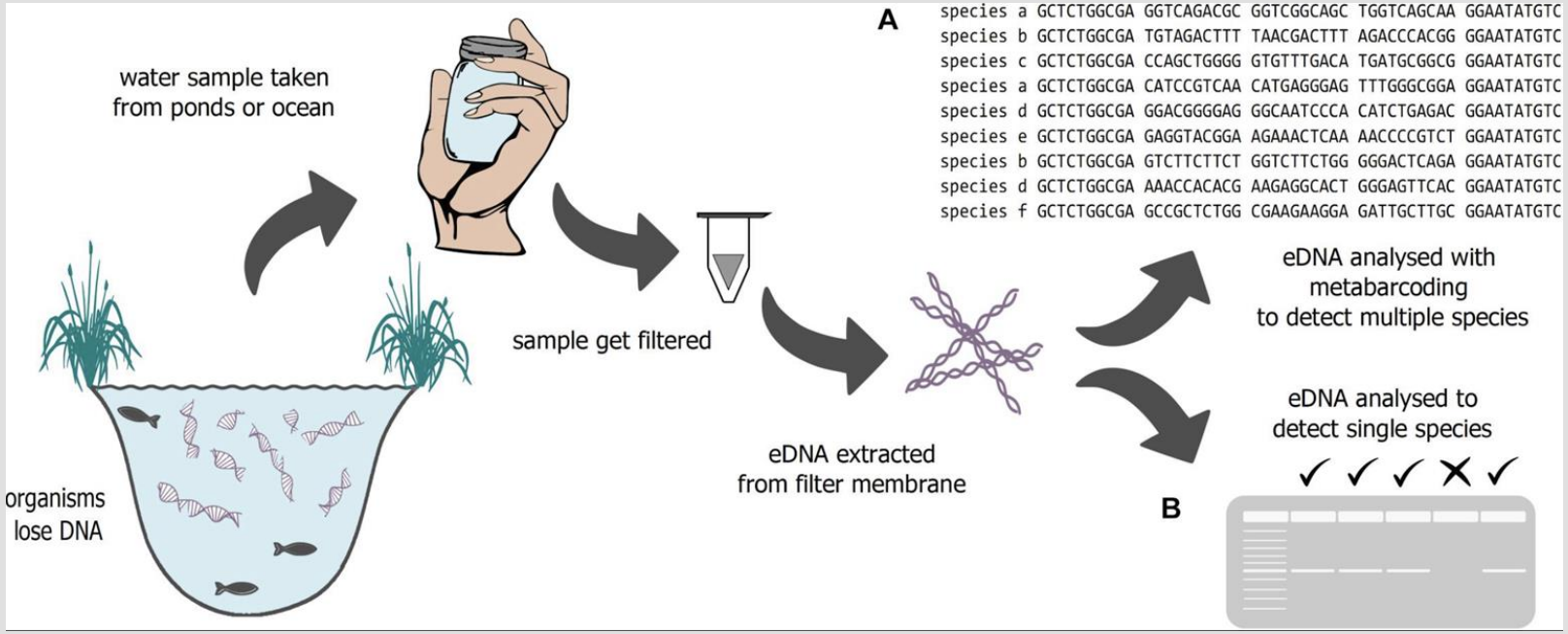
Central Dogma of Molecular Biology:

~~DNA~~ -> mRNA -> Protein -> phenotypes

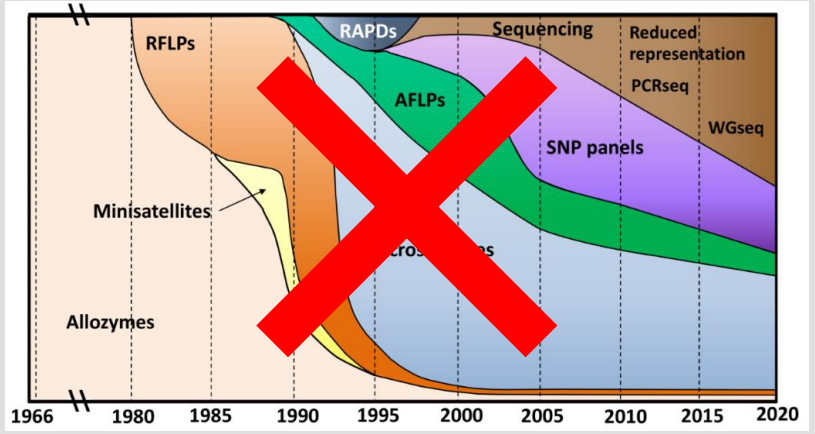
- Coding
- Regulatory
- Non-coding ("junk")

- Great for looking environmental effects on expression
- Great for looking for changes only in coding regions
- Can do a great job of population assignment in some cases
- Cannot assume things are neutral
  - Complicates population genetics
- Tricky, somewhat expensive

eDNA

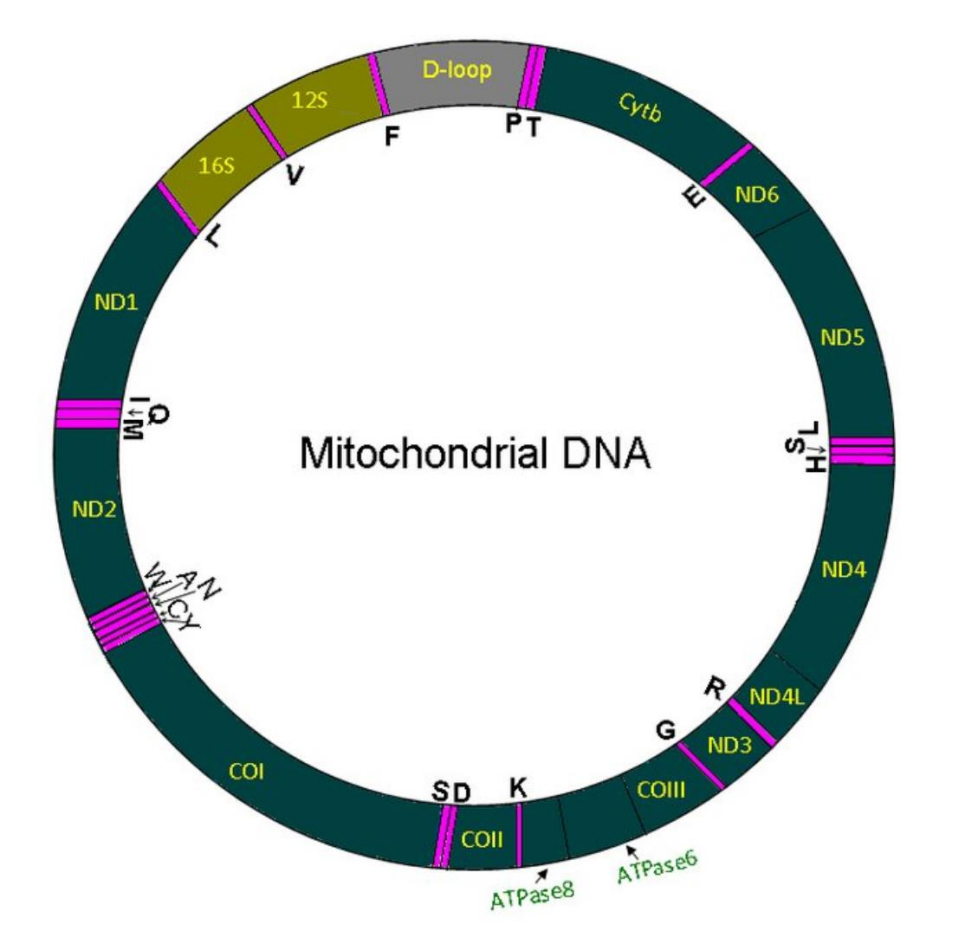


Schadewell and Adams 2021

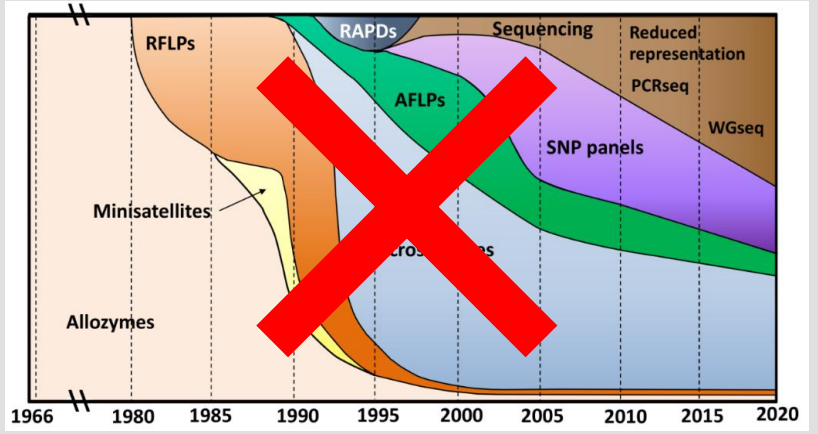


- Don’t need to catch your study species
- Can assess species composition
- Great for species detection
- Hard(er) to do population genetics
  - Depends on types of samples!
- Data quality is often lower

Mitochondrial DNA



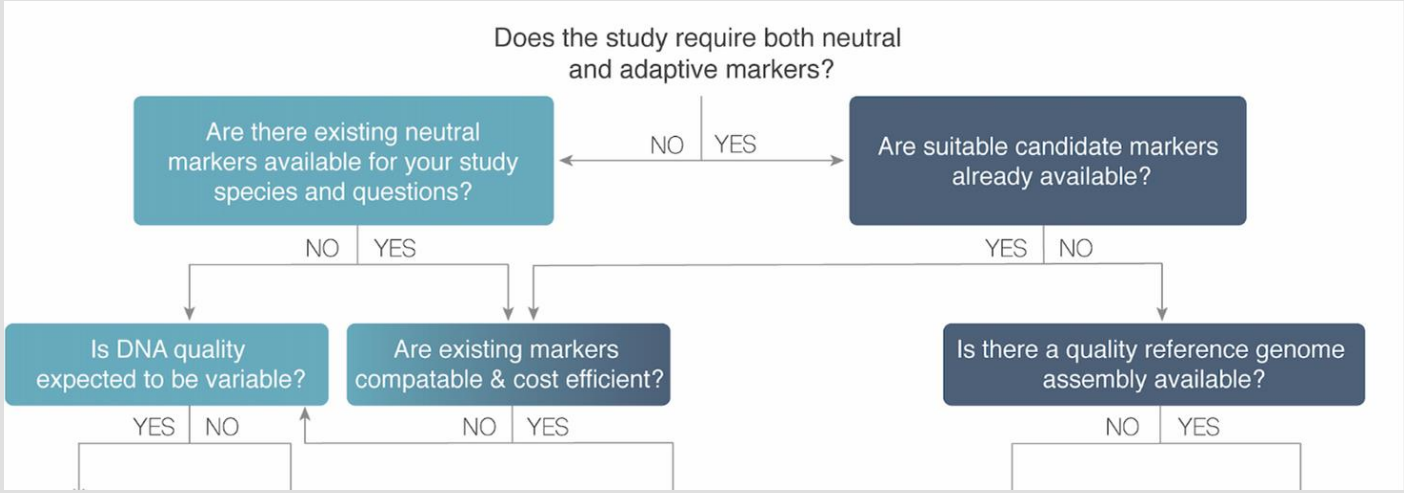
Raju et al 2011



- Cheap and fairly easy
- DNA lasts longer
  - Way more copies!
- Both well conserved regions and some with faster mutation rates.
- Excellent for species identification!

- Limited funding and tech?
  - Consider microsats
- Can’t sample your organism directly?
  - Consider eDNA
- Interested in expression?
  - Consider RNAseq
- Need neutral markers only?
  - Consider reduced representation/RAD
- Need neutral and adaptive?
  - Consider RAD (mid resolution) or WGS (high resolution)

Schiebelhut et al *in review: Practical guidance in conservation genomics: from study design to application*



Harnessing genomics for wildlife conservation

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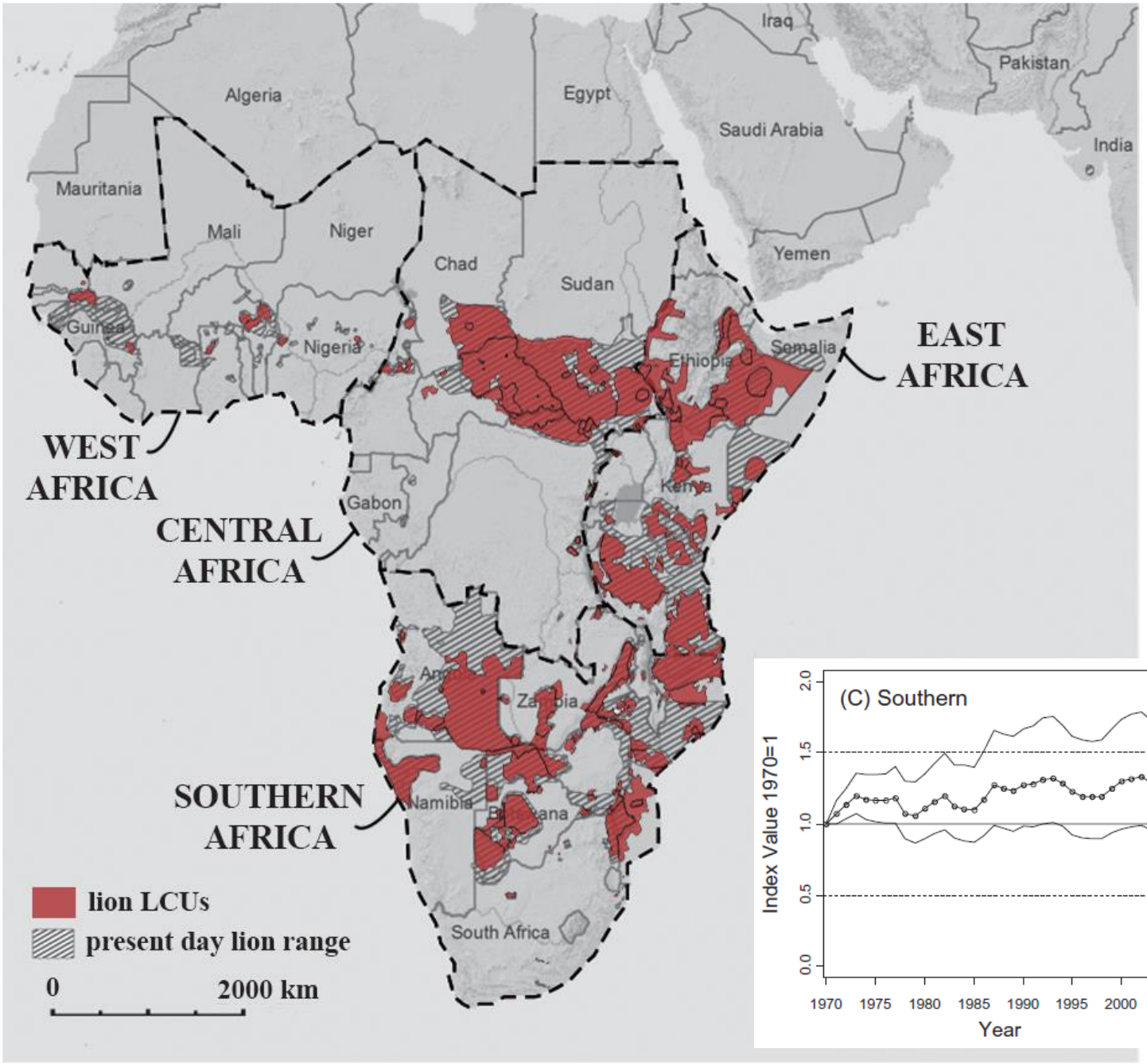
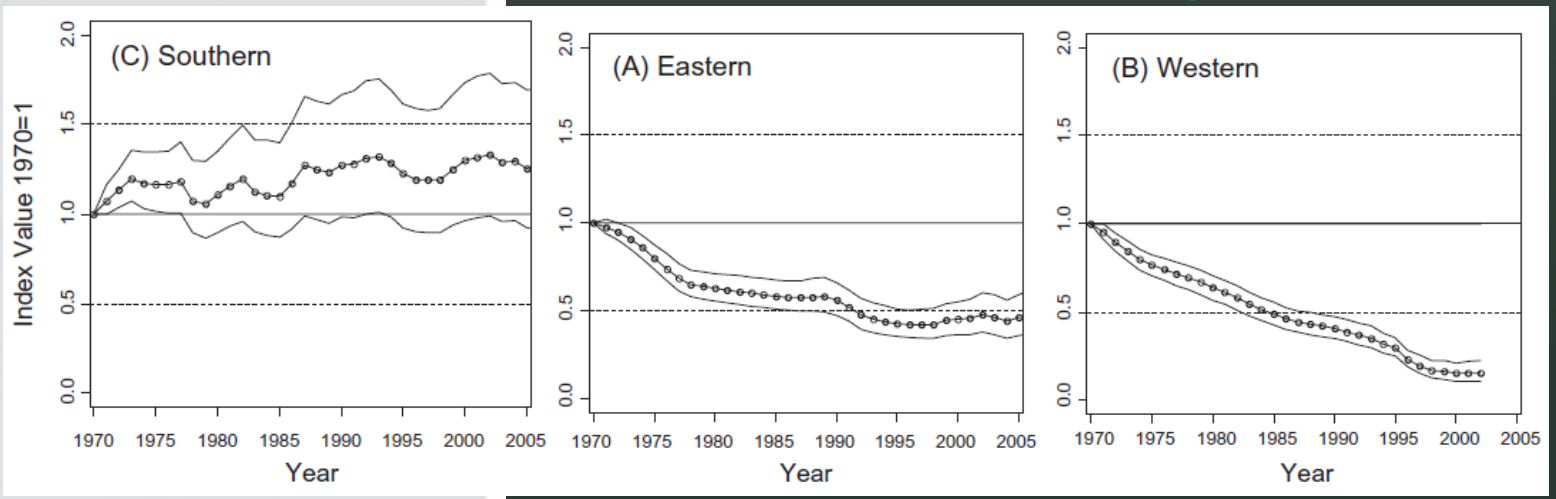


20,000-30,000 lions

~75% decline since 1970

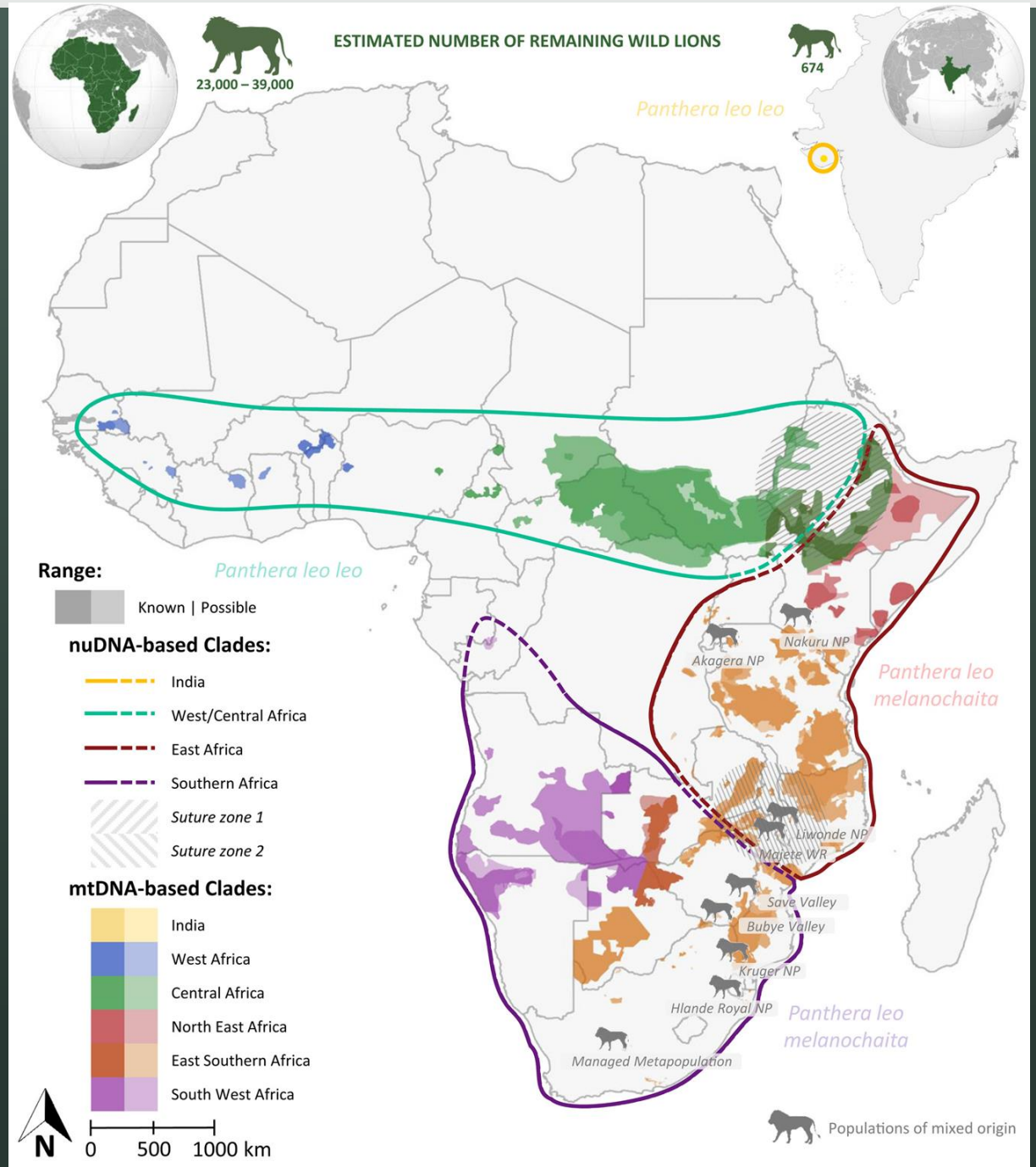
West and Central Africa strongest declines.

Craigie *et al.*, 2010



Generating genomic resources:

Baseline data

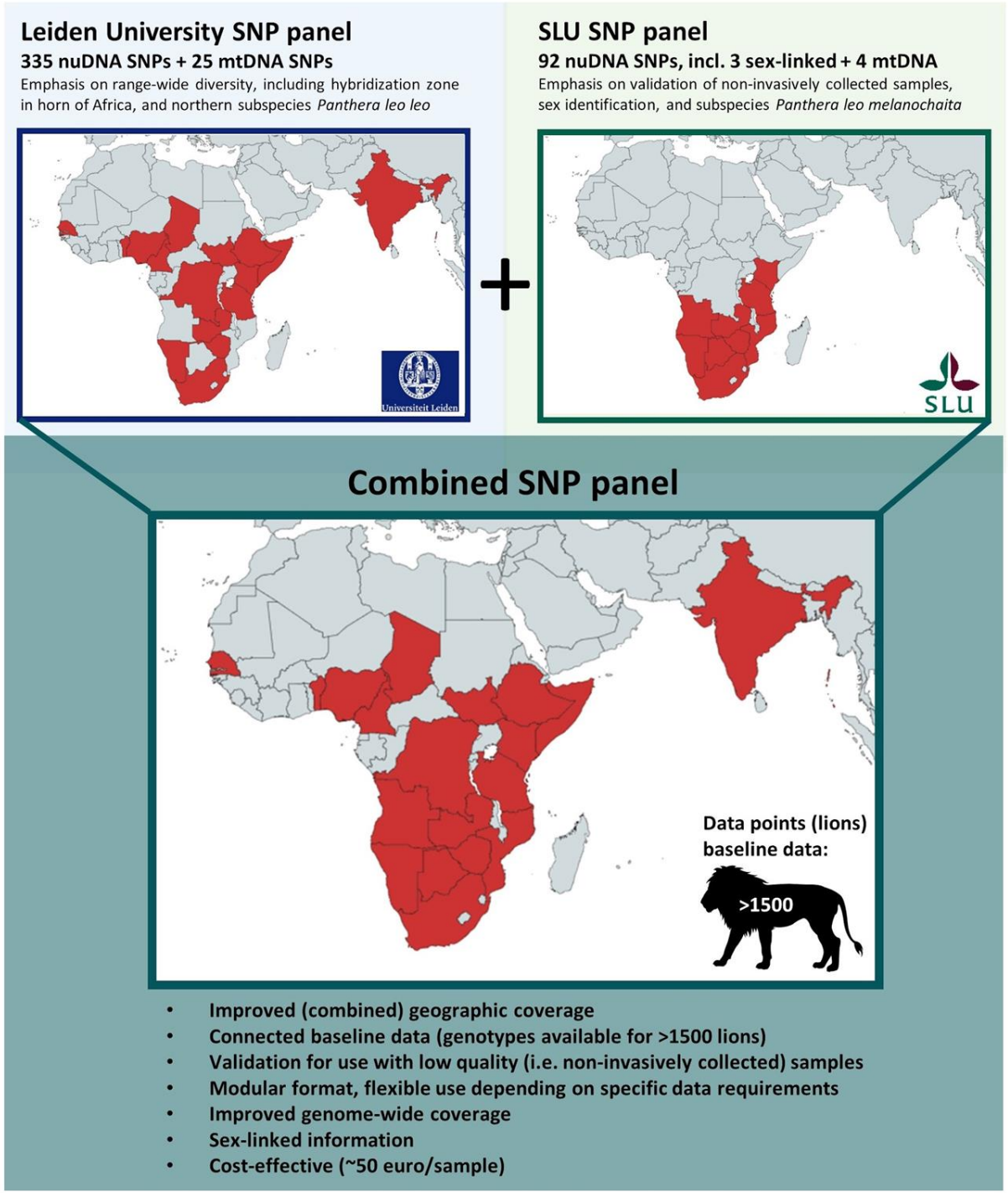


Bertola *et al.*, Evolutionary Applications 2022

A conservation genomics tool: SNP panels

- Leiden University:
  335 nuDNA SNPs + 25 mtDNA SNPs
- SLU (Göran Spong):
  92 nuDNA SNPs, incl. 3 sex-linked + 4 mtDNA

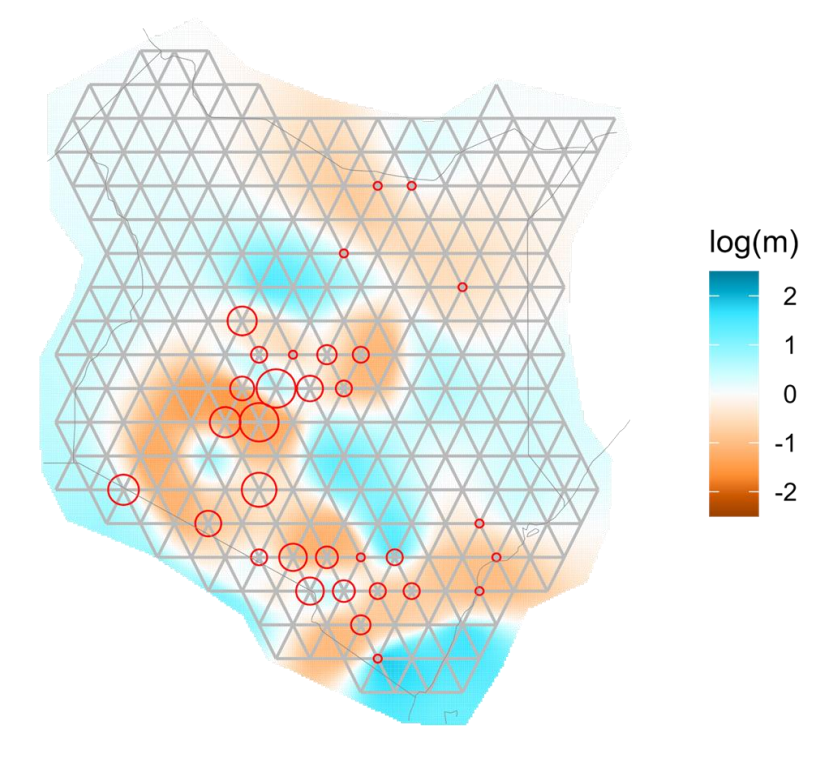
☐ **combined panel**

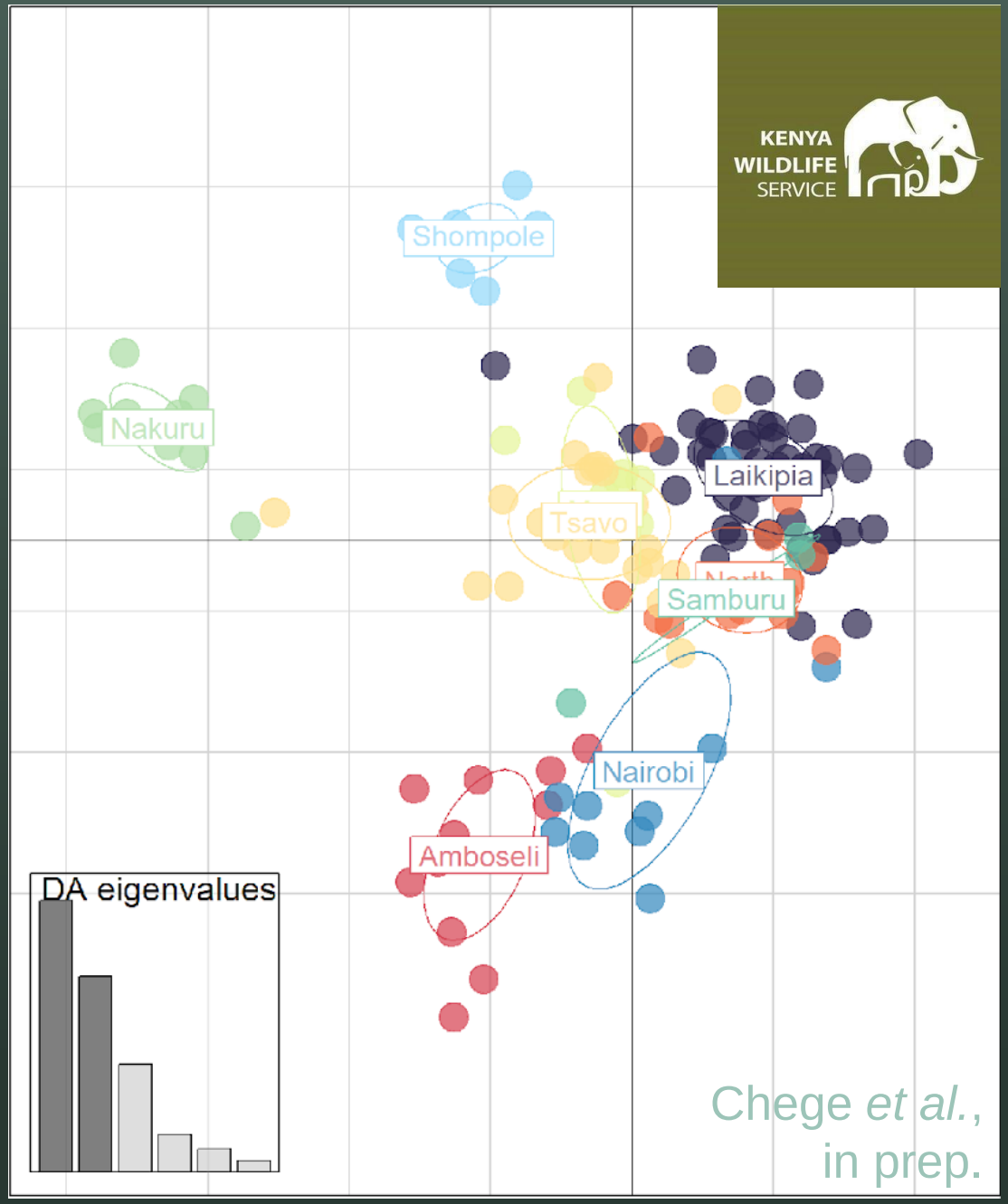


1) Local conservation decisions

~150 Kenyan lions genotyped

- Population structure
- Population diversity
- Translocation history



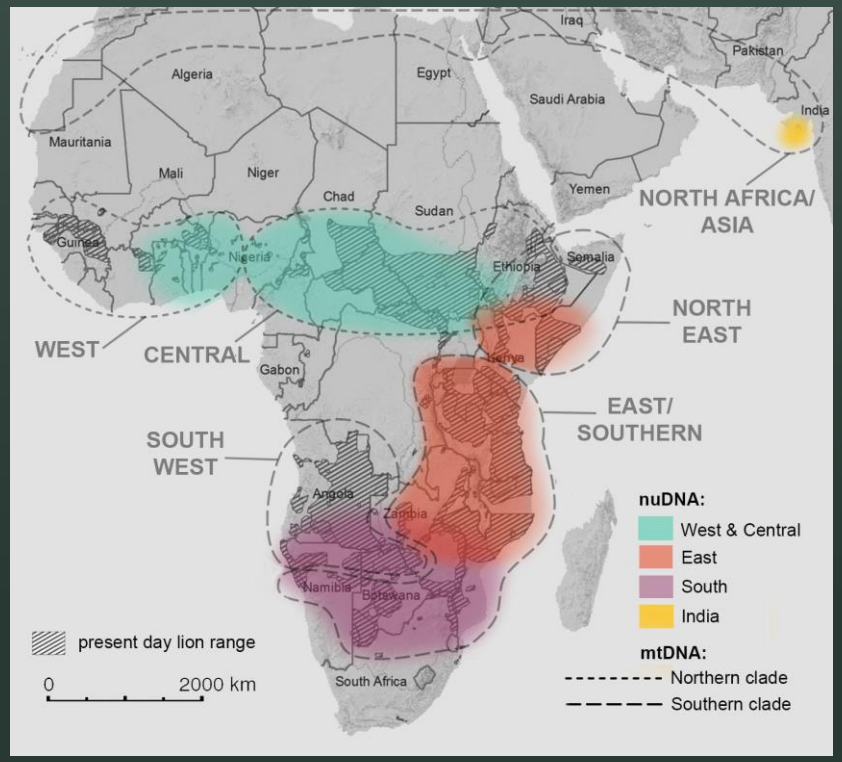


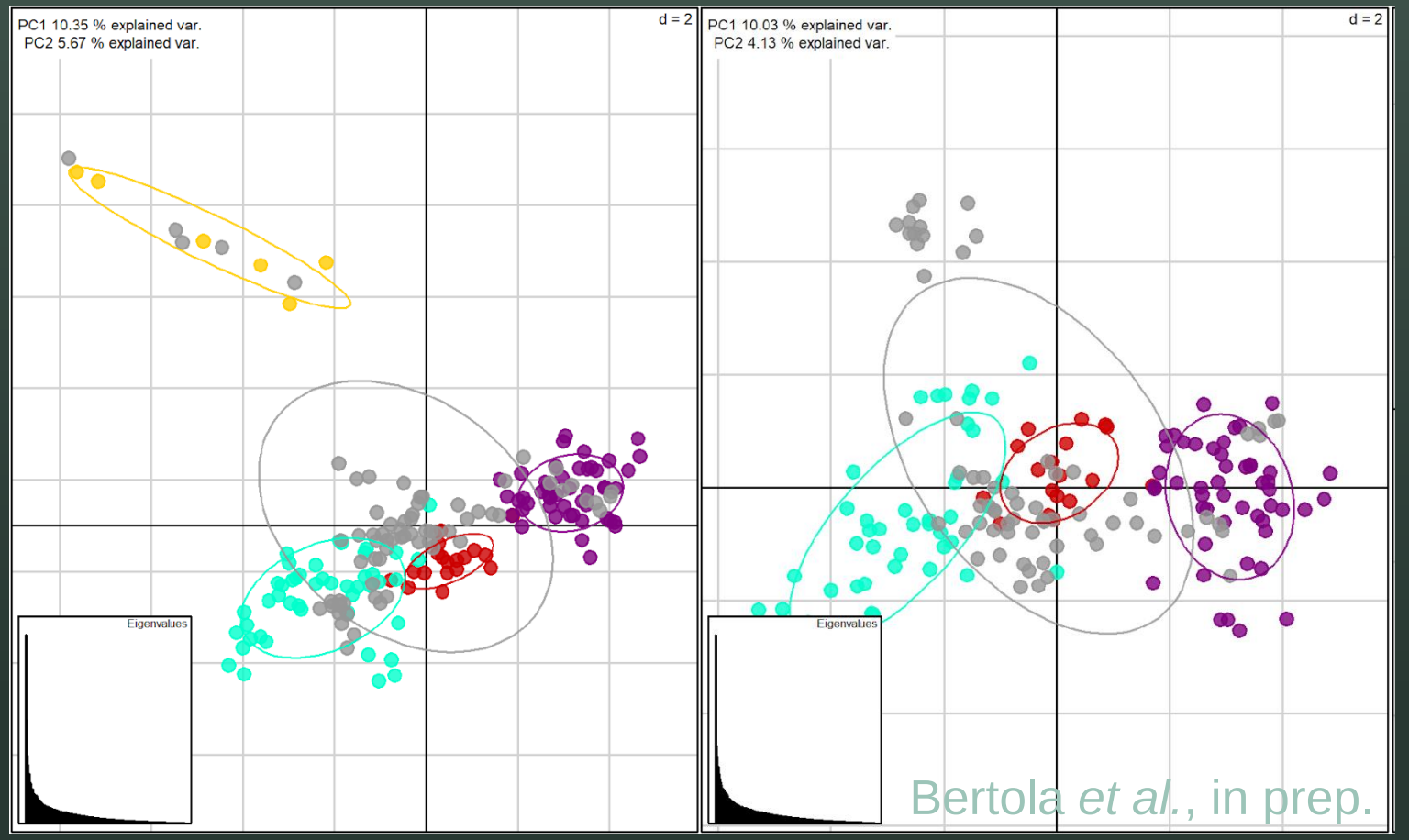
Chege *et al.*, in prep.

2) *Ex situ* population management

~70 zoo lions genotyped (in grey)

- Assignment to genetic cluster
- Level of genetic diversity
- Relatedness





Bertola *et al.*, in prep.

3) Forensics:

Traceability through Lion Localizer:
Source population, poaching hotspots, trade routes.
Currently based on mtDNA, SNP panel complimentary.

Lion Localizer

QUERY | INSTRUCTIONS | LABORATORY PROTOCOLS | FAQ | REFERENCES | ADMIN LOGIN

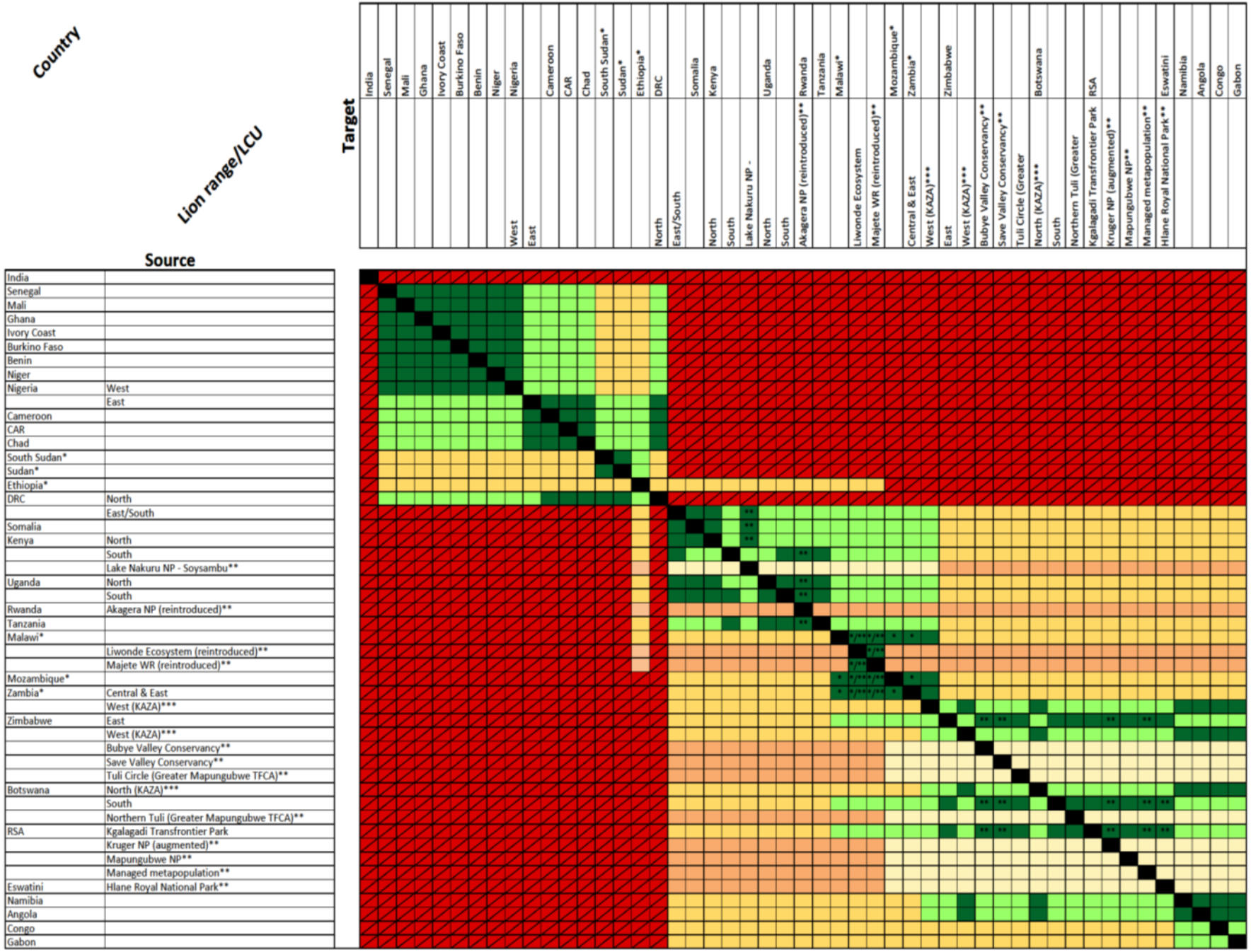
CAGCCTTCATAGGATATGTCTTACCGTGGGGCCAAATATCCTTTGAGGTGCAACTGTAATCACTAATCTCCTATCAGCAATCCCATA
GGCCGACCTAGTAGAGTGGATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCACTTCATCCTTCCA
ATCTCAGCCCTAGCAGCAGTCCACCTCCTGTTCCTCCATGAAACAGGATCTAATAACCCCTCAGGAATGGTATCTGACTCAGATAAAA
TCCATCCATACTATACAATCAAAGATATCCTAGGCCTTCTAGTACTAATCTTAACACTCATACTACTCGTCCTATTCTCACCAGACCT
AGATCCCGACAACTATACCCCCGCCAATCCTCTAAGCACCCCTCCCCATATCAAACCTGAATGGTACTTCCTATTTGCATATGCAATC
TCTATTCCCAATAAACTAGGAGGAGTTTTAGCCCTAGTTCTATCCATCTTAATCTTAGCAATTATCCCTGCCCTCCACACTTCCAAAC
GATTAATGTTTCGACCACTAAGTCAATGCTTATTCTGATTCCTAGTAGCGGACCTTCTGACCCTGACATGAATTGGTGGCCAACCTGT
CCCCTTCATCACCATCGGCCAACTAGCCTCCATCCTATACTTCTCCATTCTTCTAATCCTAATACCCATCTCAGGCATTATTGAAAAC
CTCAAATGAAGA

Locations may be shown or removed as pins on the map by clicking on the box next to each location name. However, this is enabled only for the closest-matching haplotype at each geographic location. Location and closest haplotype information may also be shown by clicking on an icon or pin on the map itself.

Haplot…	Mismatches	Match…	Locality	Country
LEO0002	0	1140	(11.6; -9.3)	Guinea
LEO0002	0	1140	(11.05; 1.517)	Benin
LEO0002	0	1140	Kainji NP	Nigeria

4) Translocations

Identifying suitable source-target population pairs.

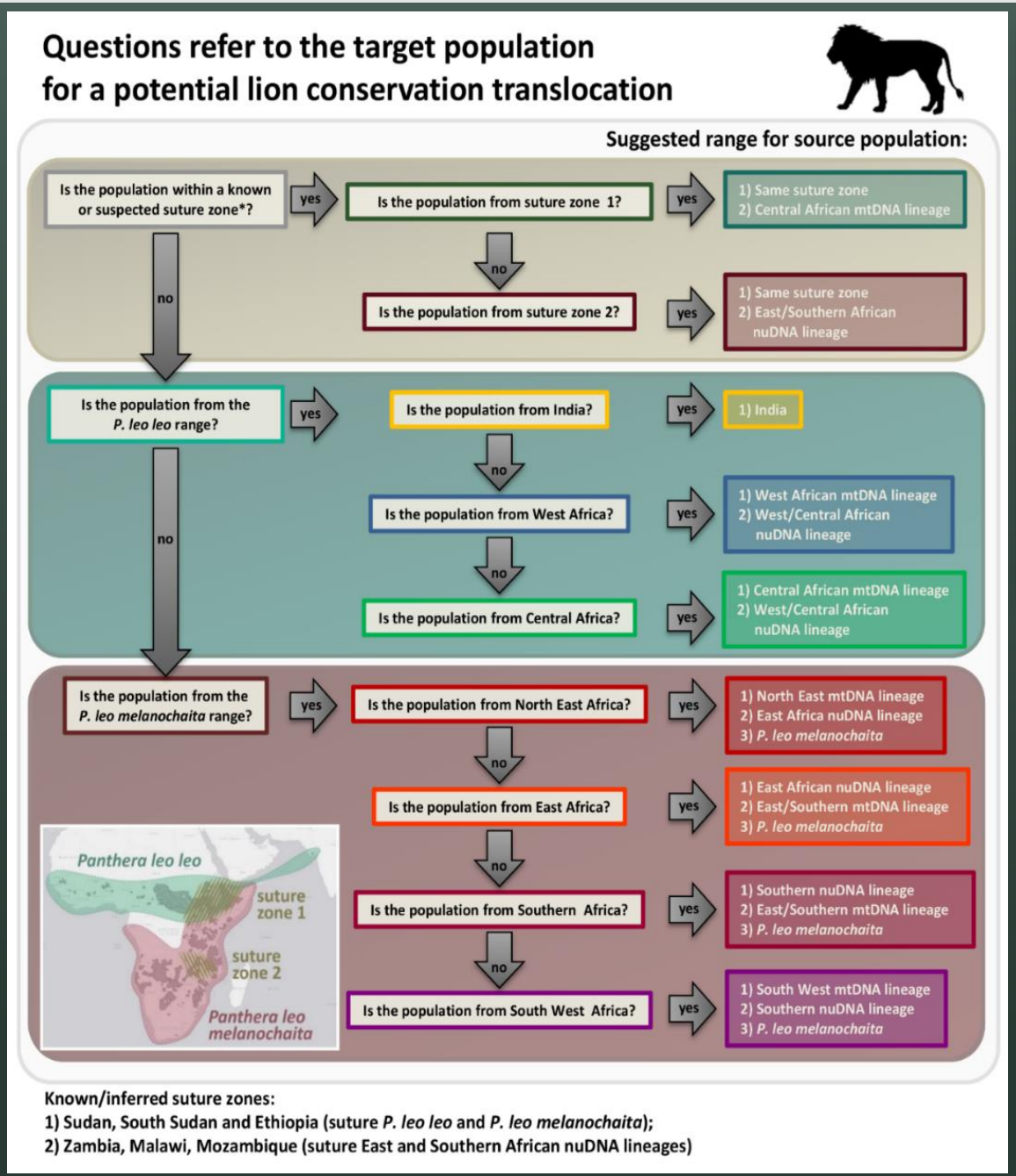


Bertola *et al.*, Evolutionary Applications 2022

Communicating genomic resources:

Decisions Guide.

Bertola *et al.*, Cat News 2022



IUCN

SPECIES SURVIVAL COMMISSION
CAT SPECIALIST GROUP