

Targeted population genomics in non-model species



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GENOME 10K[®]

Unveiling animal diversity

Genome 10K Project

To understand how complex animal life evolved through changes in DNA and use this knowledge to become better stewards of the planet.

The Genome 10K project aims to assemble a genomic zoo—a collection of DNA sequences representing the genomes of 10,000 vertebrate species, approximately one for every vertebrate genus. The trajectory of cost reduction in DNA sequencing suggests that **this project will be feasible within a few years.**

Capturing the genetic diversity of vertebrate species would create an unprecedented resource for the life sciences and for worldwide conservation efforts.

The growing Genome 10K Community of Scientists (G10KCOS), made up of leading scientists representing major zoos, museums, research centers, and universities around the world, is dedicated to coordinating efforts in tissue specimen collection that will lay the groundwork for a large-scale sequencing and analysis project.

Accomplishments

- ▶ Inspired partly by the Genome 10K project, the **i5K initiative** to sequence 5,000 insect genomes began in March 2011.
- ▶ G10K announces the first **101 species** for sequencing. These add to **120 vertebrate species** already being sequenced in public-sector genome projects. See them in **phylogenetic trees**.

Join us

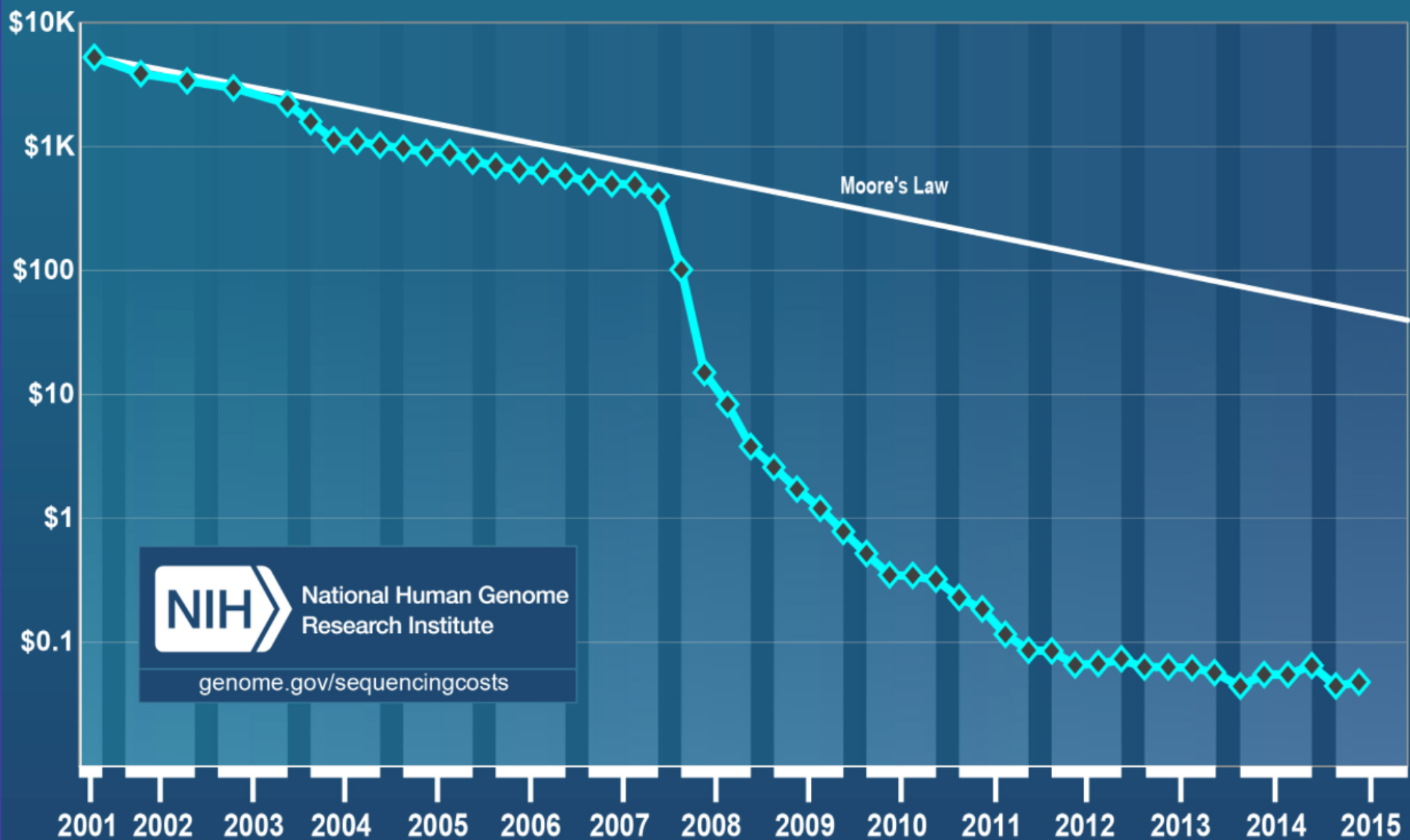
[Become a G10K affiliate](#)

Co-directors

David Haussler,
Howard Hughes Medical
Institute Investigator
Professor of Biomolecular
Engineering
UC Santa Cruz

Oliver A. Ryder
Director of Genetics
Kleberg Chair
San Diego Zoo Institute for
Conservation Research
Adjunct Professor, Division of
Biology

Cost per Raw Megabase of DNA Sequence



Outline

1. Rationale for genome partitioning
2. Approach for exon capture in non-model species
3. Two brief case studies

Genomic
DNA

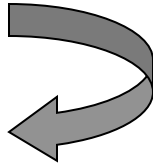
Bioinformatic challenges of species
without existing genomic resources



- WG sequencing still expensive for *populations*
- *de novo* WG assembly difficult
- WG data not necessary for many questions

Millions of
short sequences
higher error rate

Genomic
DNA

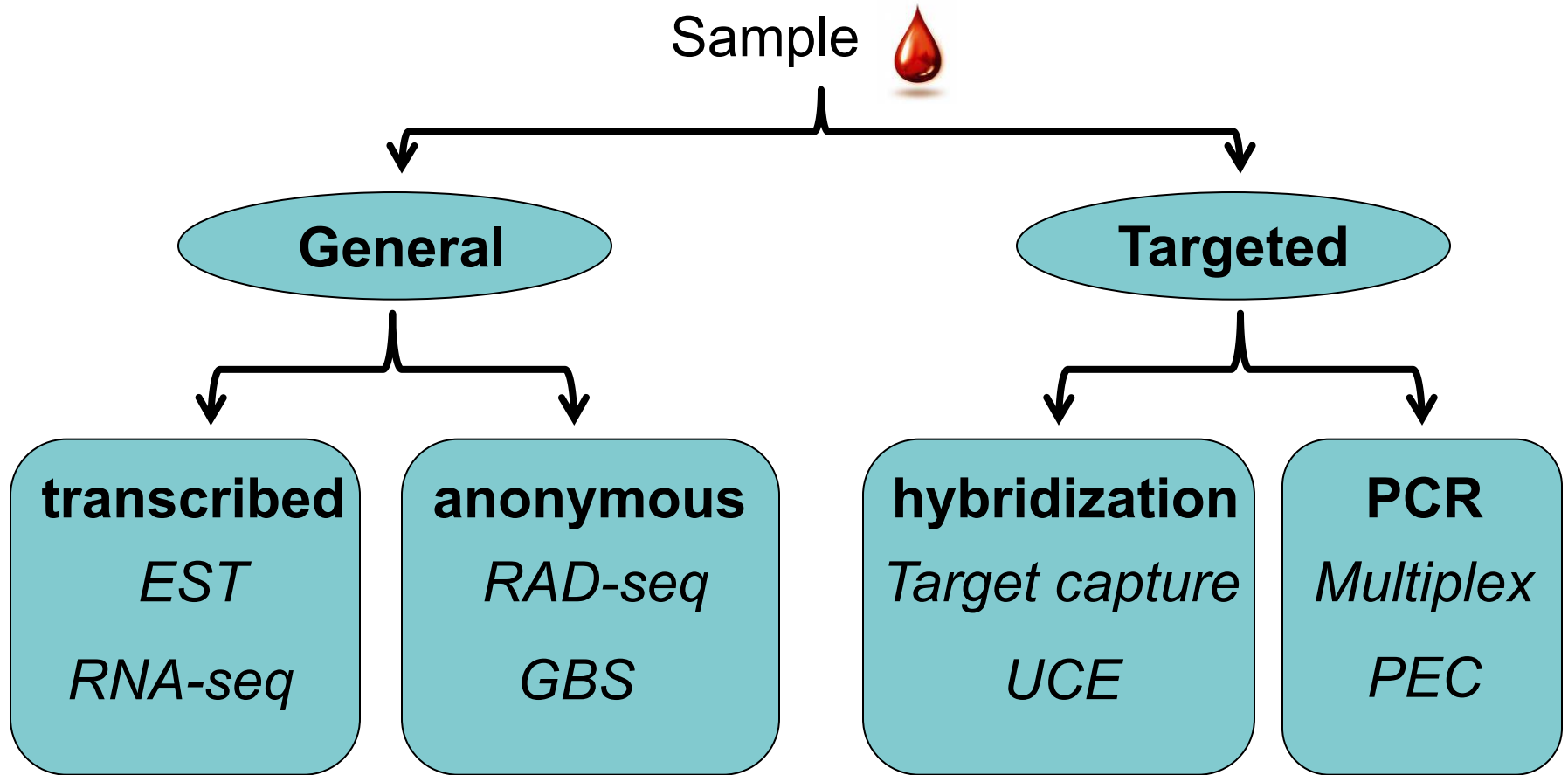


Genome partitioning through targeted enrichment offers an efficient and tractable solution



Millions of targeted reads
high-quality consensus for
part of the genome

Genome partitioning



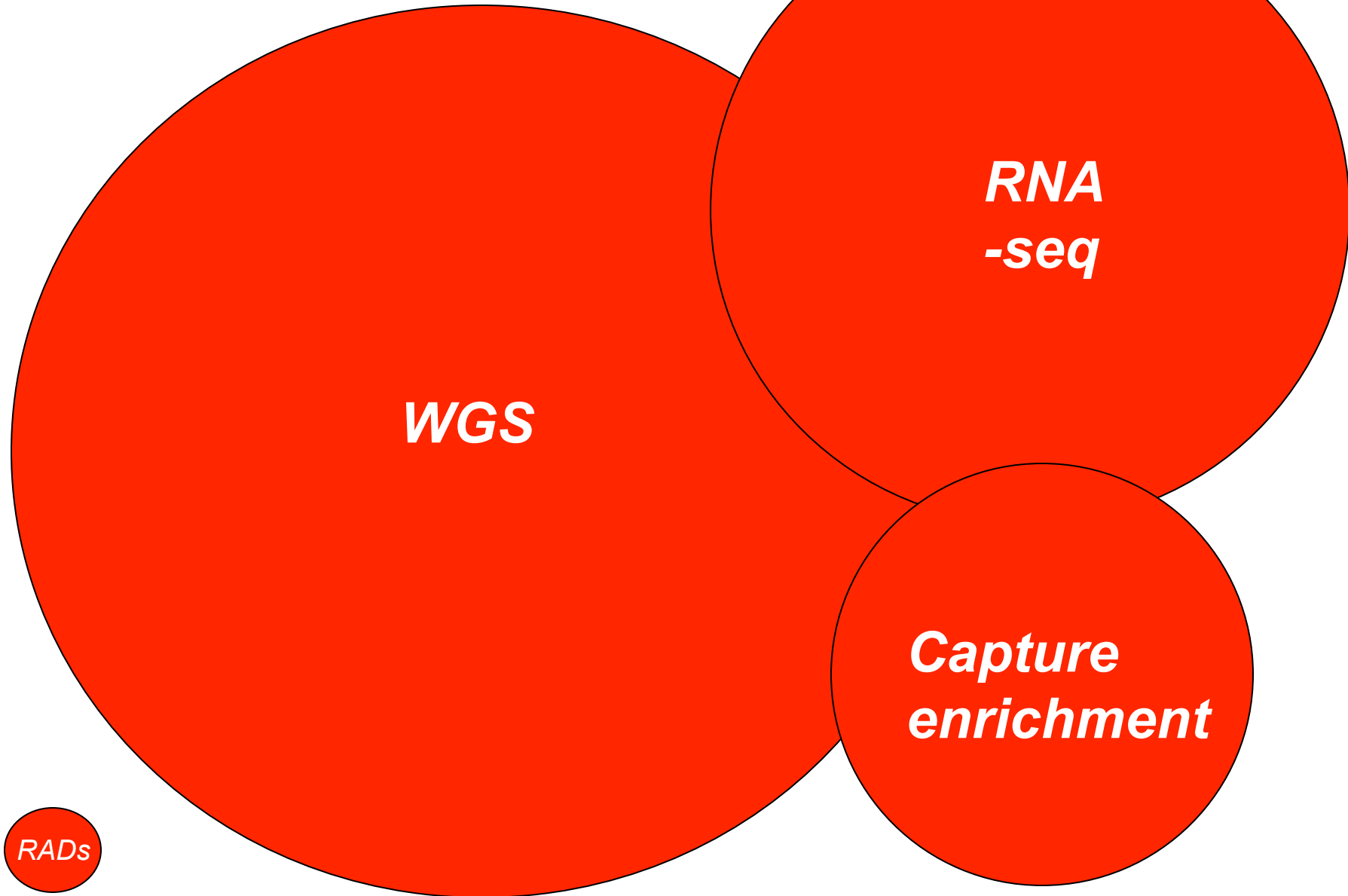
Genome partitioning is all about systematically reducing the size of the sequenced genome without reducing complexity



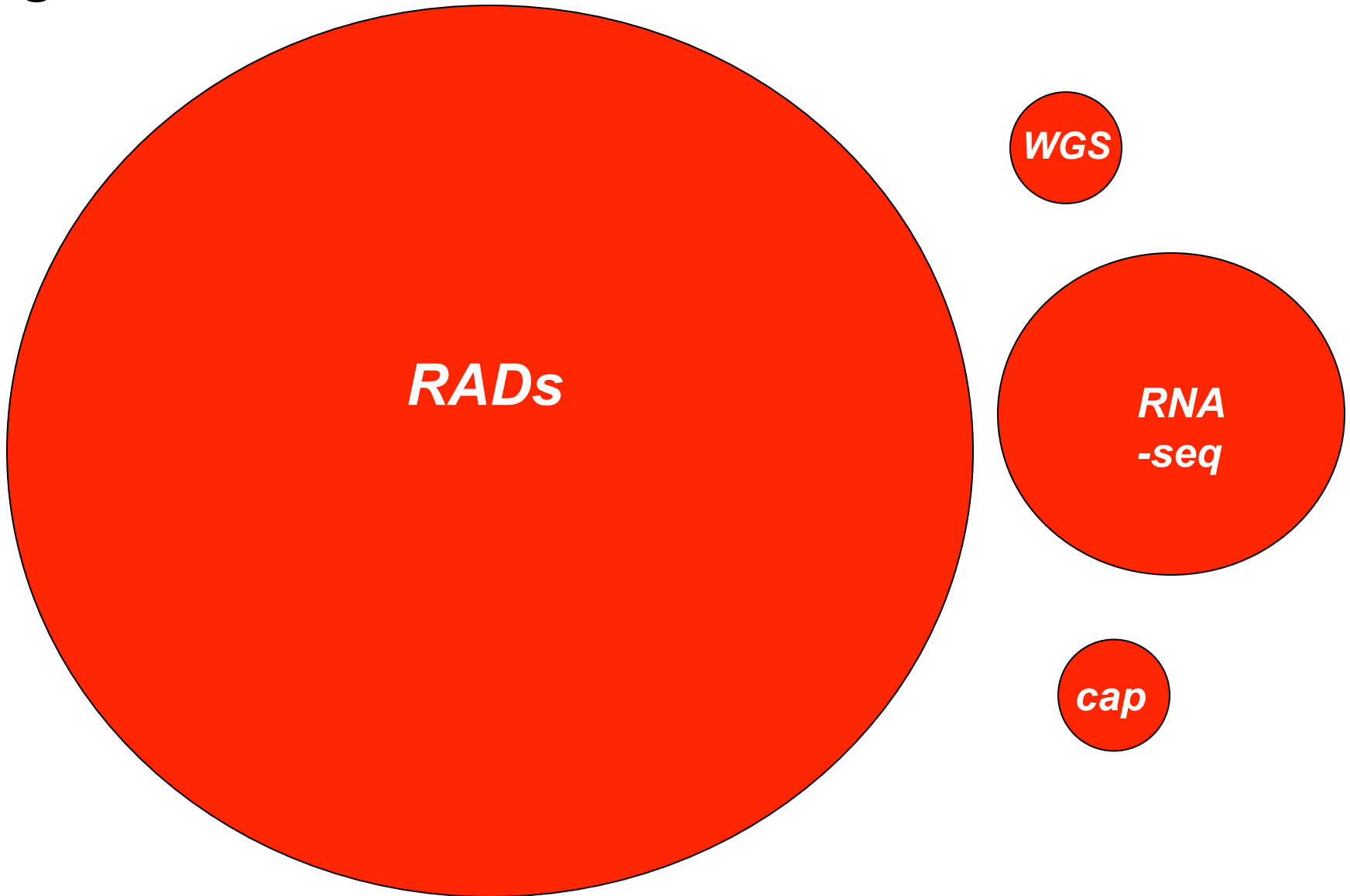
Approximately how many copies of the human genome (3Gb) are in 1 ng of genomic DNA?

300 haploid copies

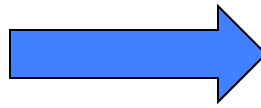
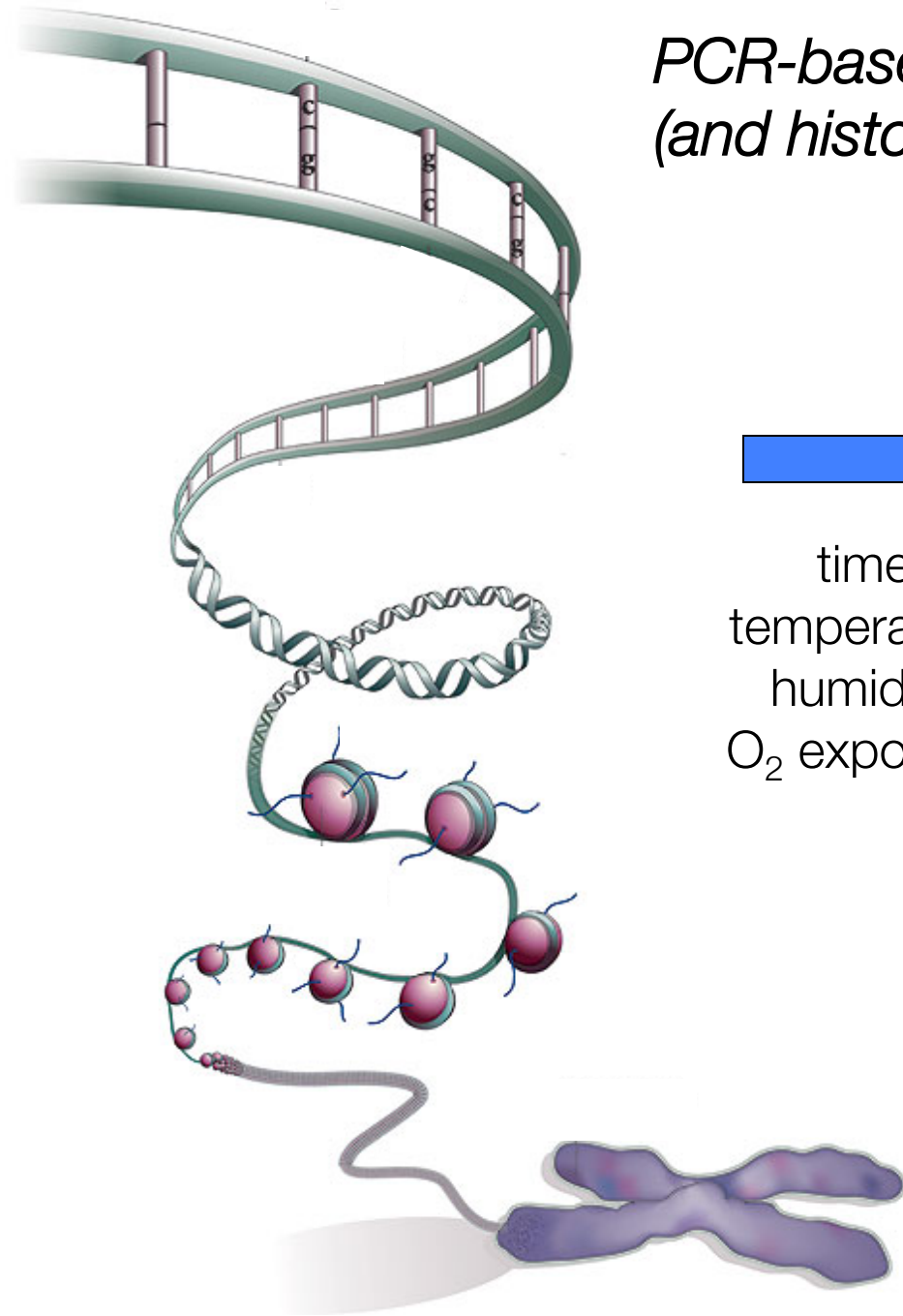
NGS genomics broadly



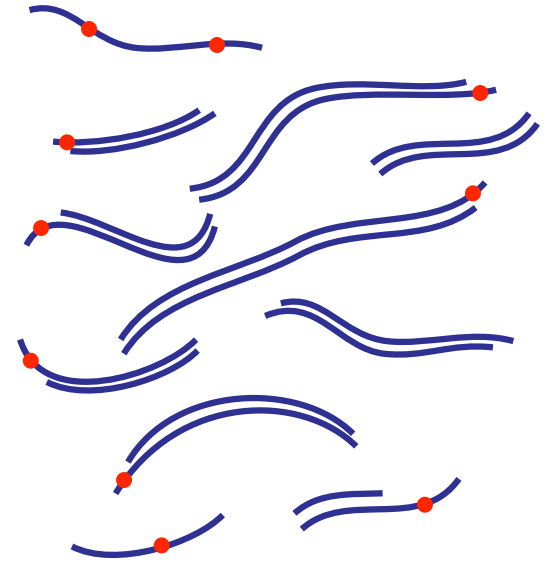
NGS genomics in conservation & evolution of genomic models



*PCR-based genetic analyses of ancient
(and historic) DNA are severely limited*



time
temperature
humidity
O₂ exposure



fragmented (short)
degraded (low quantity)
chemical modifications (error-prone)

Next-generation sequencing technologies overcome many of the biochemical limitations presented by ancient DNA

Issue presented by aDNA

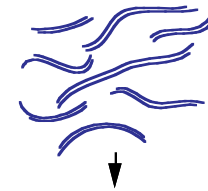
fragmented (short)

degraded (low quantity)

chemical modifications (error-prone)

NGS solution

Fragmentation of DNA
(200 - 300 bp)



Blunt-end repair of DNA fragments



Ligation of universal adapters

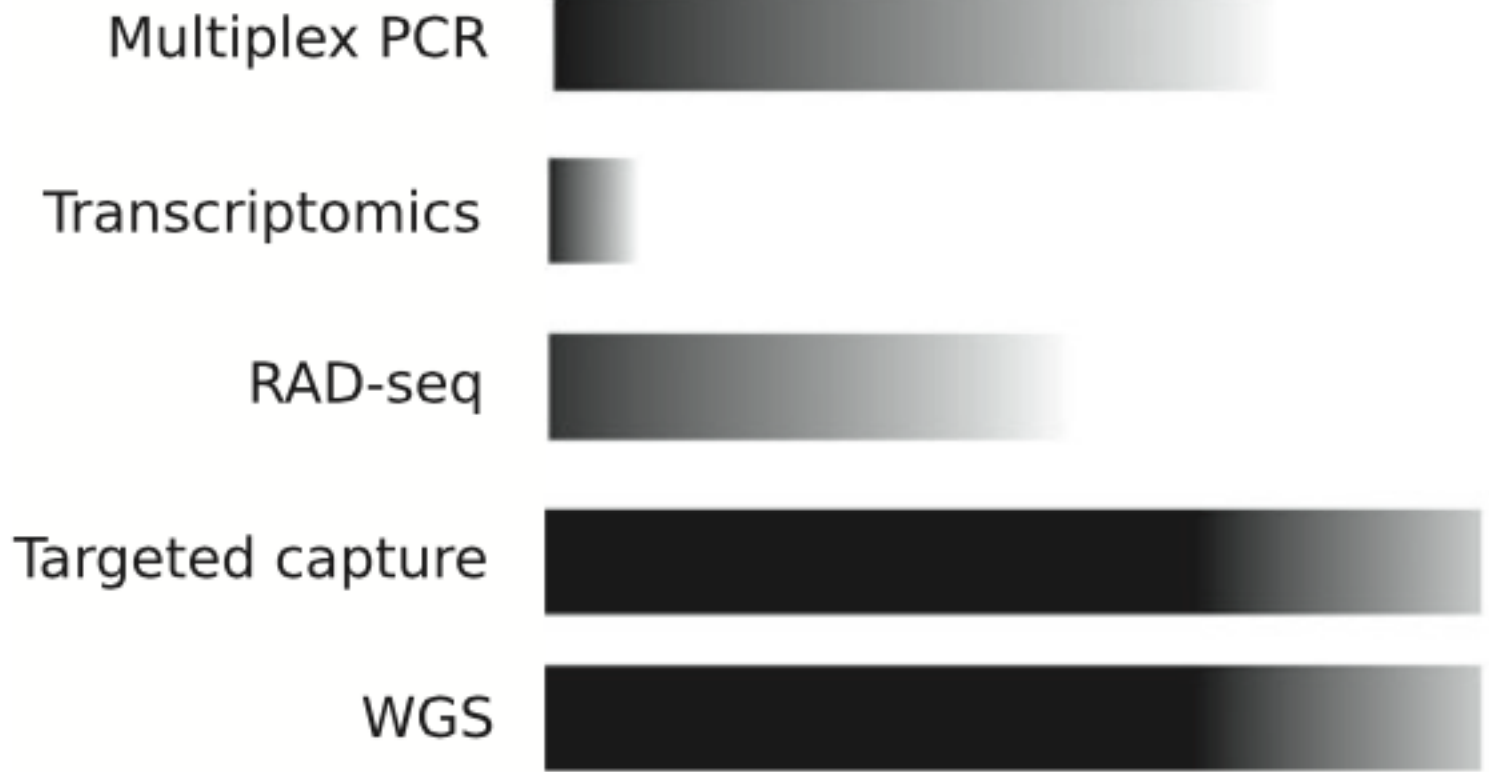


PCR amplification

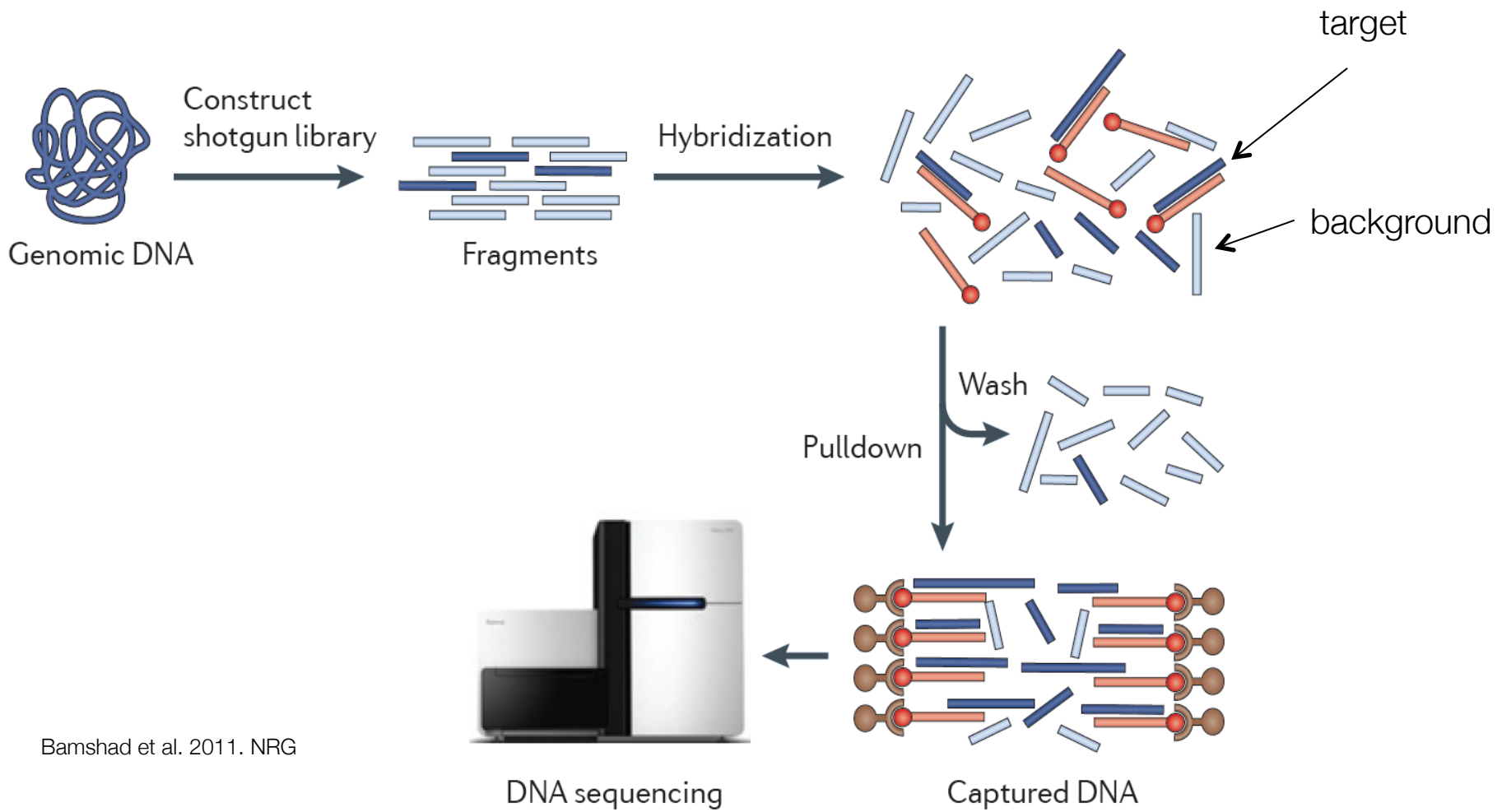


Deep sequencing

Degradation

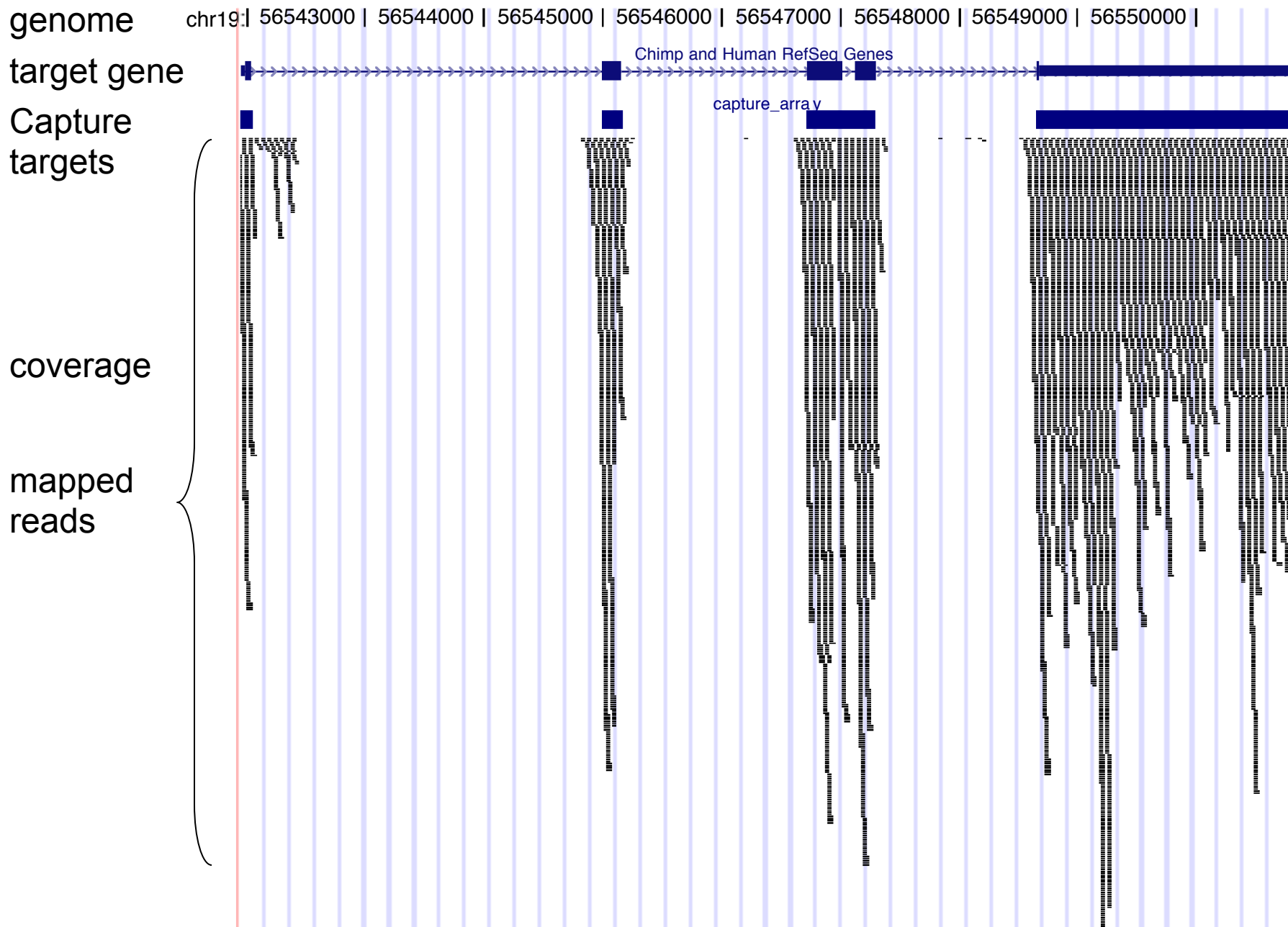


Target enrichment by in-solution capture



Bamshad et al. 2011. NRG

Sequence data from a single individual



**How do you conduct targeted genome partitioning
in species without existing genomic resources?**

How do you conduct targeted genome partitioning in species without existing genomic resources?

1. Utilize a general enrichment to recover genomic data in a limited sample to build a reference

- RNA-seq
- RAD-seq
- Shotgun sequencing

How do you conduct targeted genome partitioning in species without existing genomic resources?

2. Utilize a divergent reference

- Hybridization based capture is robust to evolutionary divergence (10% or greater)
- Exons tend to be conserved
- Captures spanning millions of years of divergence work with high to moderate success...

I. population genomics of a declining alpine species



II. evolution of seasonal crypsis



Collaborators & Funding – part I



Craig Moritz



Rasmus Nielsen



The Gordon & Betty Moore Foundation



Ke Bi



Dan Vanderpool

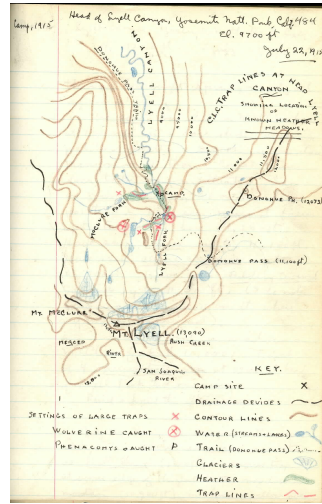
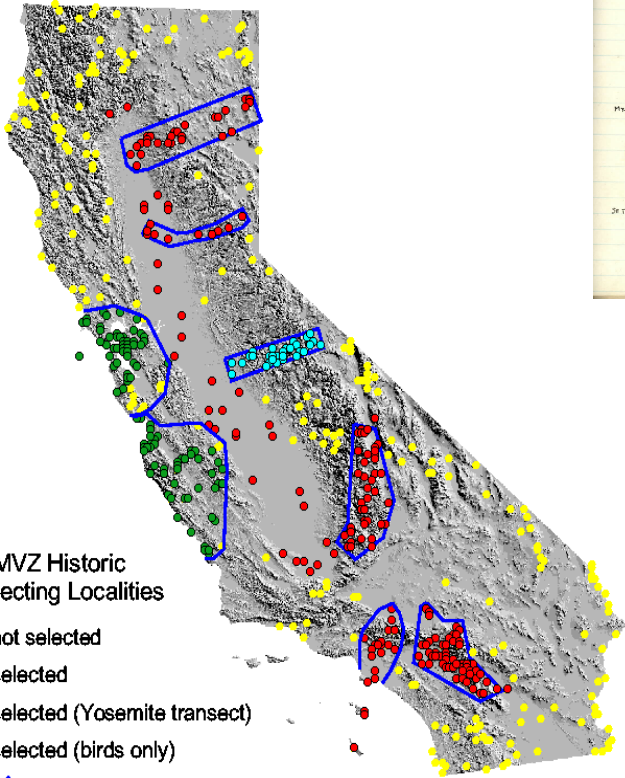


Tyler Linderoth



Grinnell museum surveys (early 1900s)

Joseph Grinnell



Maps

Diary 1914
 Fresno, Fresno Co. Cal. 546
 Aug. 20-1916

✓ 5204 ♂ Junco. Immature 105-23-11-9. wt. 10 gm. (5300 ft)
 ✓ 5205 ♂ Contaminia 3 imm. 225-20-35-15. wt. 57.7 gm.
 ✓ 5206 ♀m. Oreogastrea 212-121-22-9. wt. 27.8 gm.
 ✓ 5207 ♀ Gopher 173-42-26-5. ... 21.2 ...
 ✓ 5208 ♀ " 210-60-26-5. ... 197. ...
 ✓ 5209 ♀ " 190-56-23-5. ... 95. ...
 ✓ 5210 ♀ " 192-58-27-5. ... 90.6 ...

15 traps set in the ~~thick~~ tangle of willow and fallen timber along the margin of the lake caught *Oreogastrea gambeli*, *Junco* and *Oreogastrea* during the night and *Junco* and *Microtus* during the day. The *Microtus* were all young of the year.

The 3 rat traps caught *Contaminia* and the 20 gopher traps caught seven gophers. I was at loss to explain the taking of the *Perognathus* in such a favorable place as it was much more adapted to the woods of *Neotoma* than anything else. The *Perognathus* was an immature however and that might help account for his strange association.

Field notes



Specimens



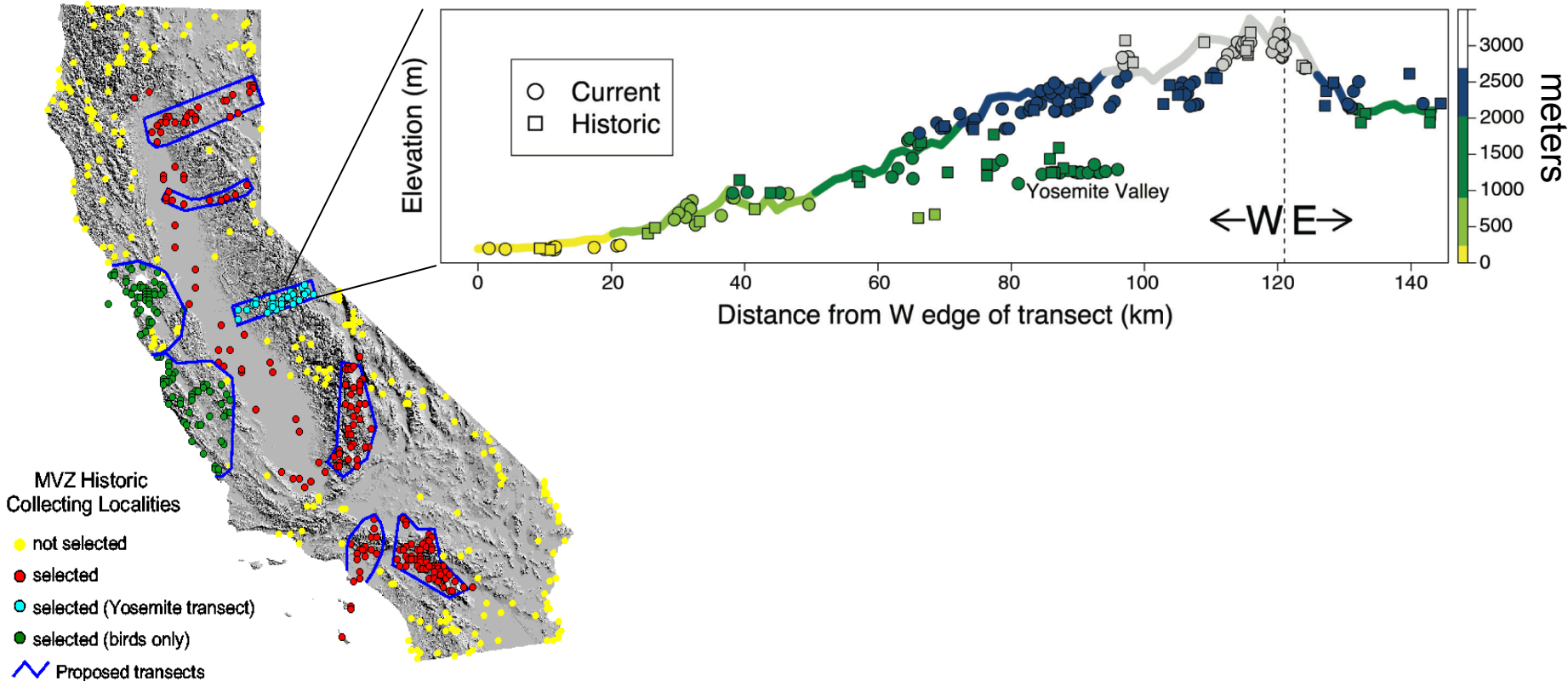
Impact of a Century of Climate Change on Small-Mammal Communities in Yosemite National Park, USA

Craig Moritz,^{1,2*} James L. Patton,^{1,2} Chris J. Conroy,¹ Juan L. Parra,^{1,2}
Gary C. White,³ Steven R. Beissinger^{1,4}

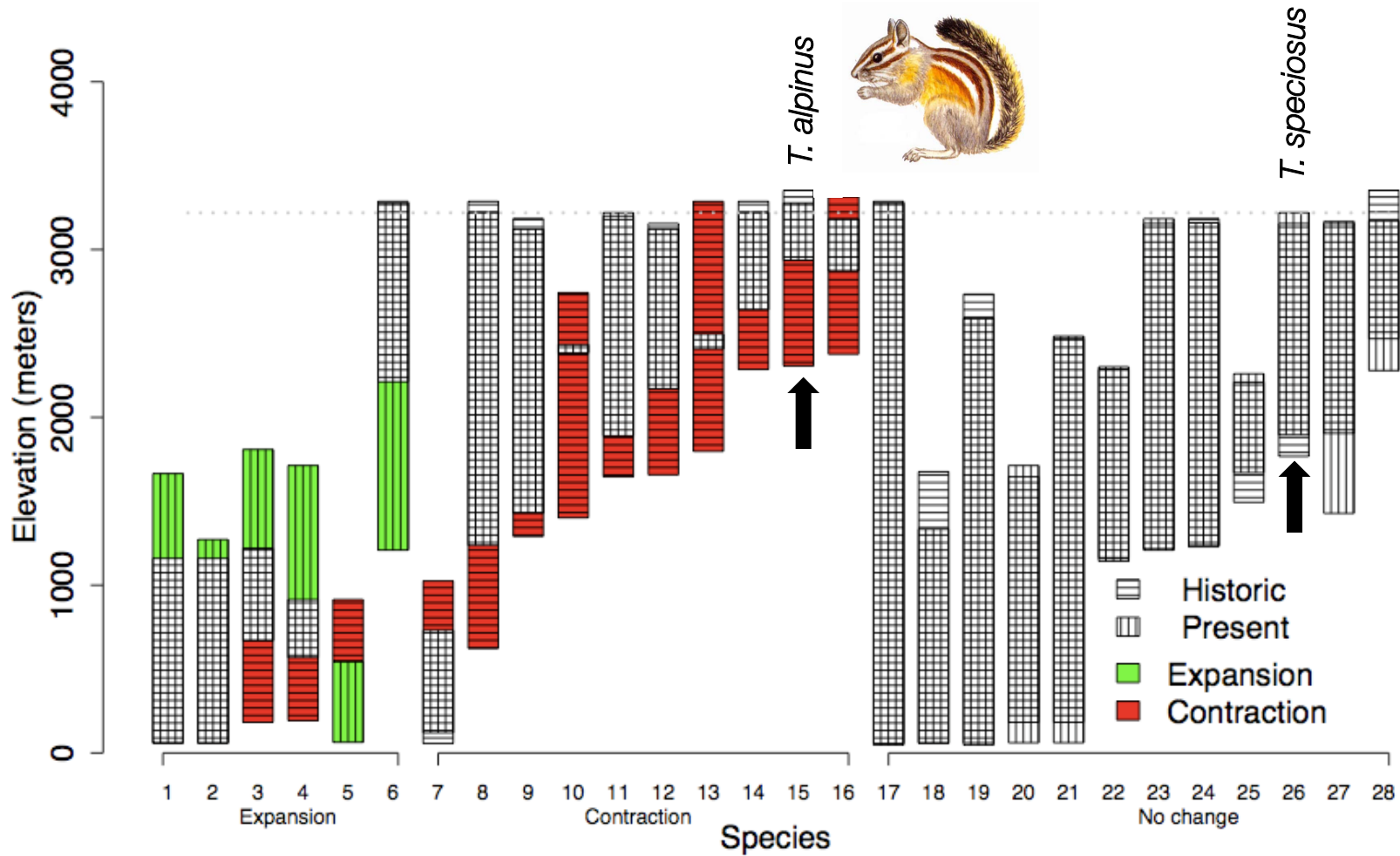
SCIENCE VOL 322 10 OCTOBER 2008

Joseph Grinnell

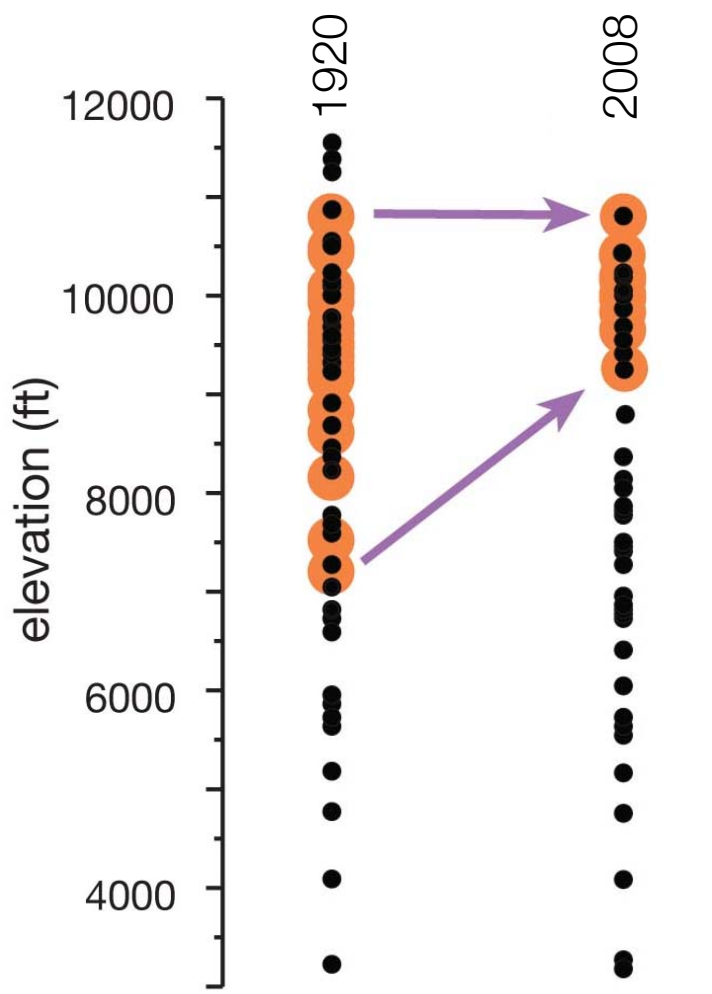
Grinnell resample project



- High elevation species mostly **contract**
- Low elevation species **expand/shift**
- Some species remain stable



Alpine chipmunk
(*Tamias alpinus*)



tree-line - 10,300 ft (3,140 M)



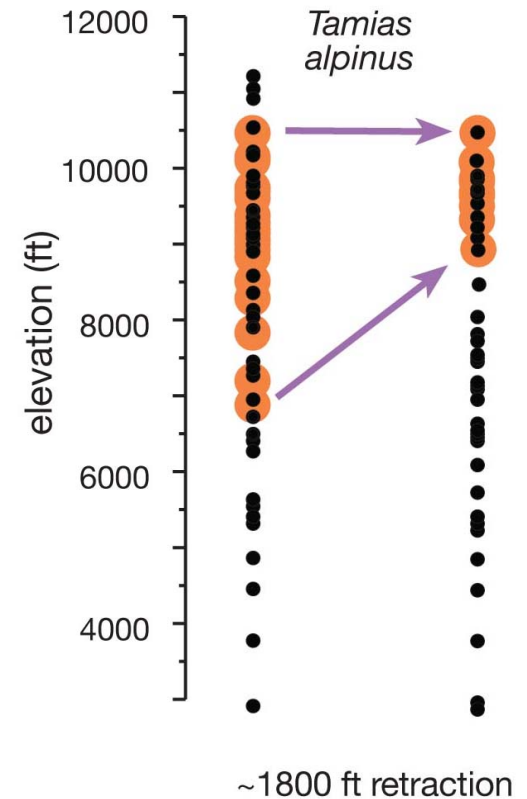
● surveyed ● detected



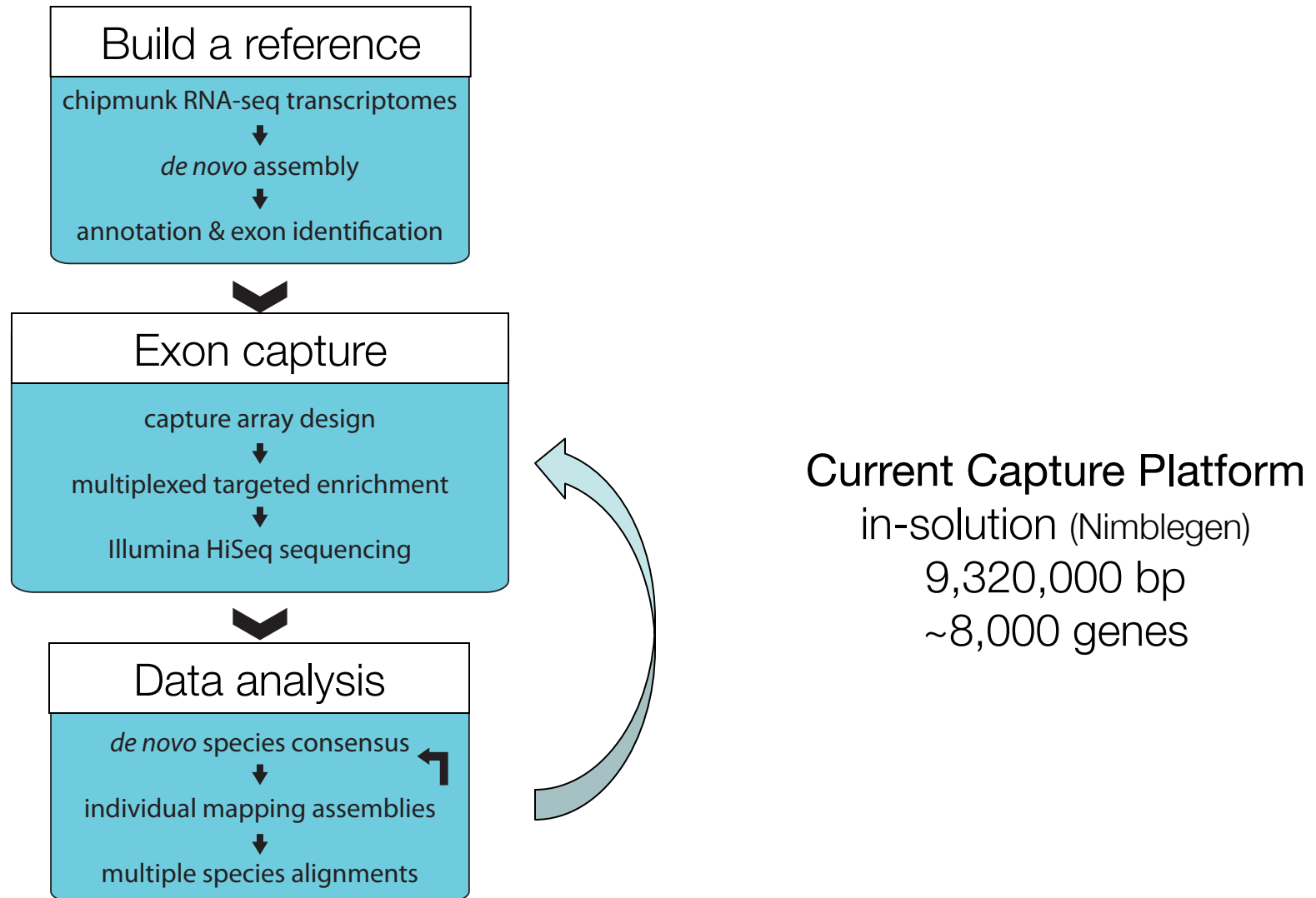
In the face of a severe range reduction, are there....

Significant changes in the level and pattern of genetic variation?

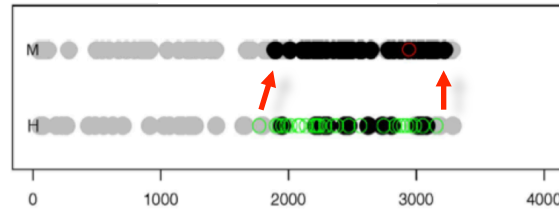
Genomic signatures of positive/negative selection associated with shrinking versus stable populations?



Population genomics in chipmunks?



Samples surveyed: N = 303 + outgroups

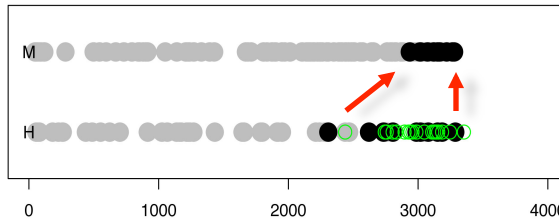


Modern: N=48

Historic: N=56

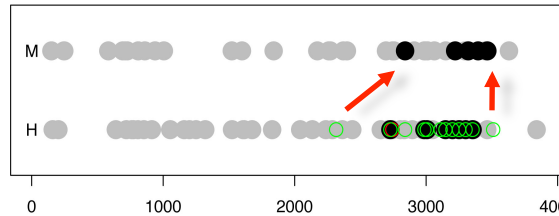


T. alpinus
(Alpine chipmunk)



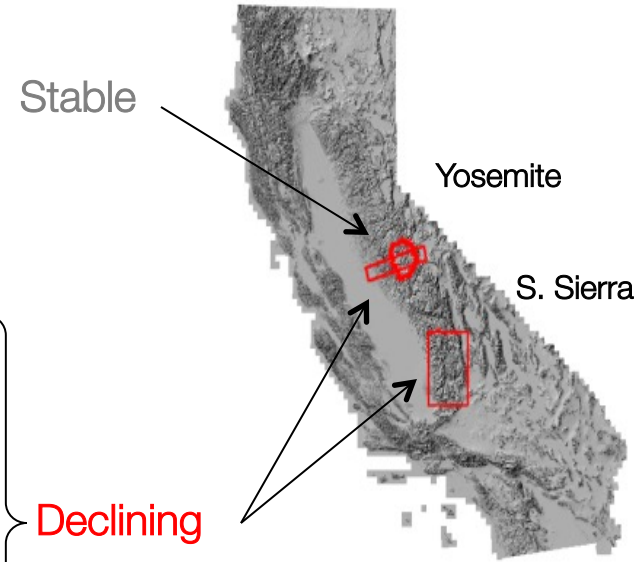
Modern: N=48

Historic: N=55



Modern: N=41

Historic: N=55



Six capture reactions: 1 population (barcoded) / reaction
Illumina HiSeq2000, 100 bp PE, 6 lanes: 1 population / lane

High specificity & coverage

(90% reads mapped to the target exons)

Recover >99% of targets (plus flanking introns)

Efficiency was as high for historical samples

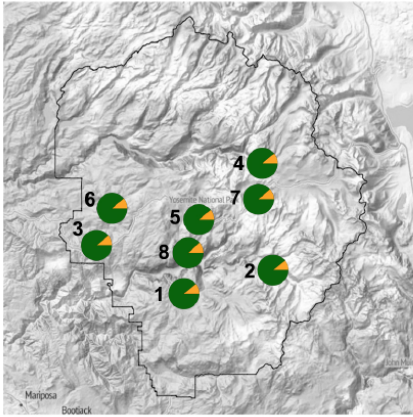
Low variance across individuals (no drop out)

9,000,000 bp sequenced to 25X per individual

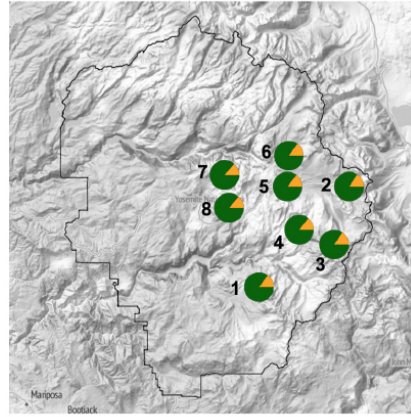
Increased population structure in the Alpine chipmunk – decreased pop size (N_e) & migration, increased genetic drift

Historic

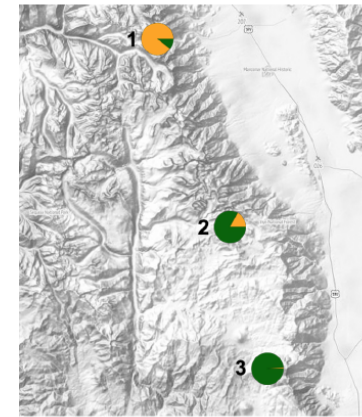
T. speciosus Yosemite



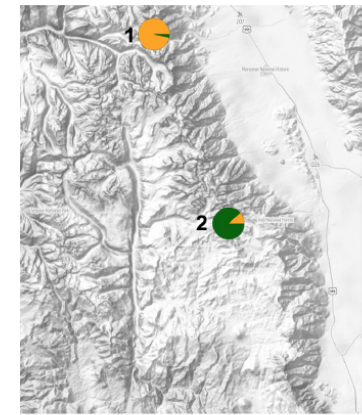
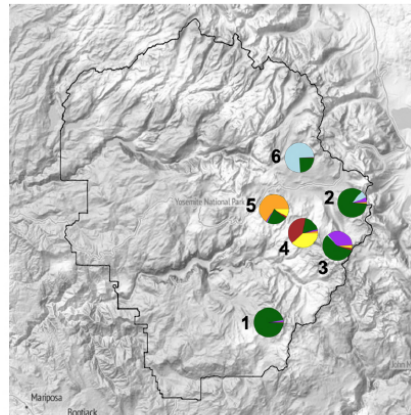
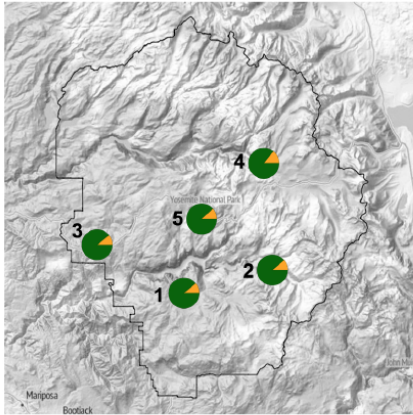
T. alpinus Yosemite



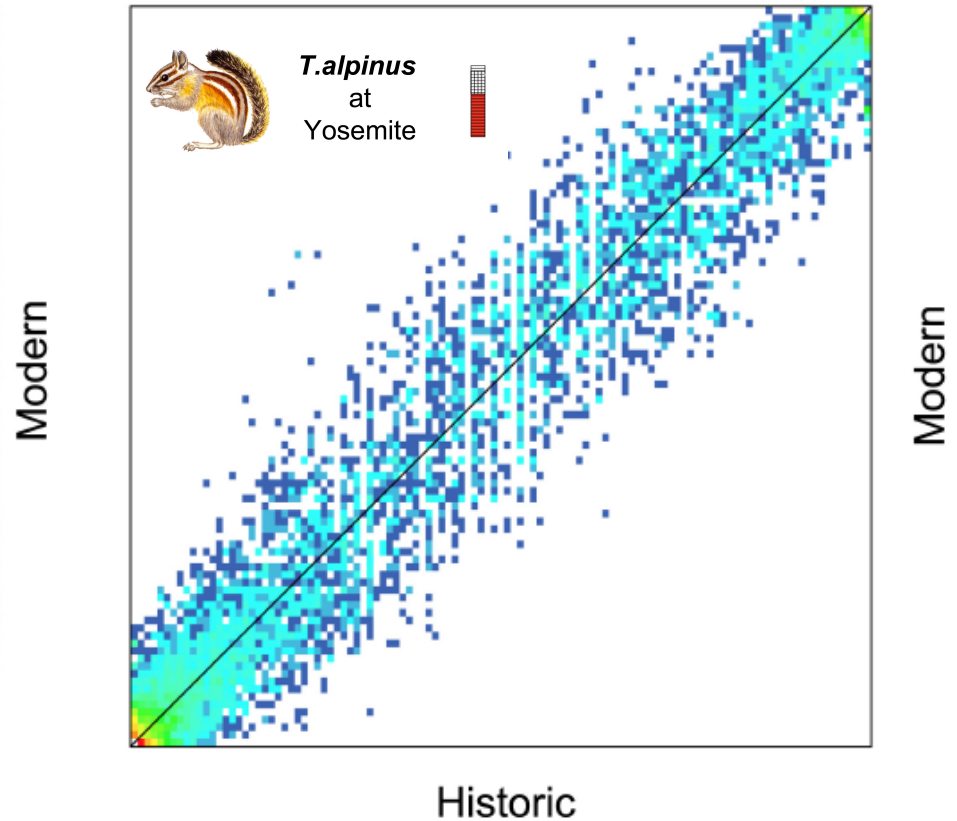
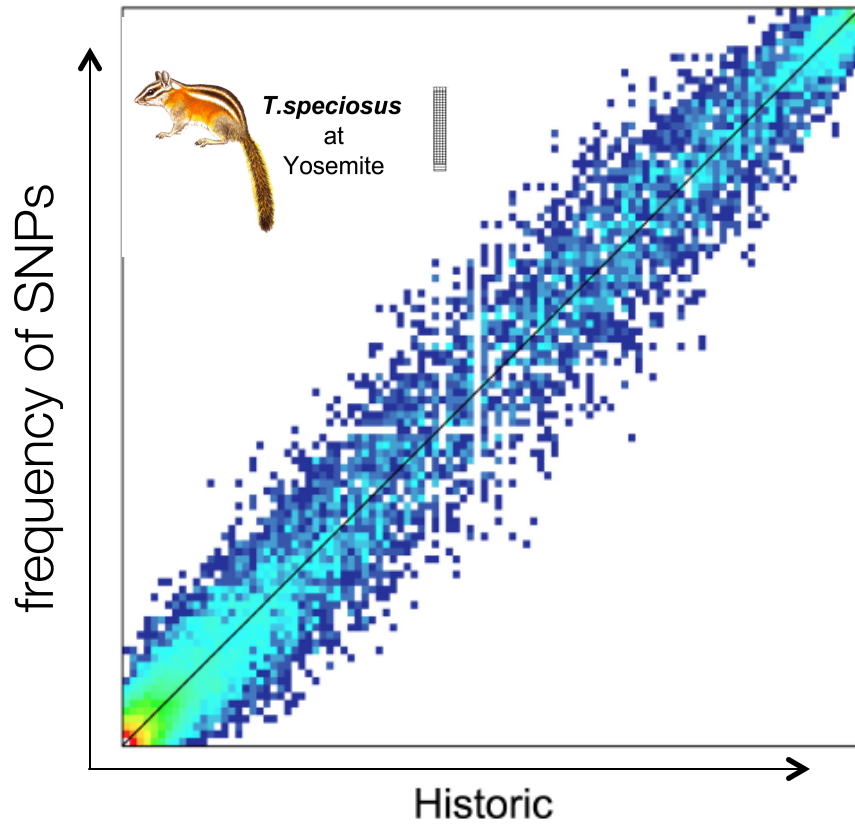
T. alpinus southern Sierra



Modern



Change in allele frequencies through time



Increased variance in the alpine chipmunk

Large allele frequencies shifts reveal potential targets of selection

I. population genomics of a declining alpine species



II. evolution of seasonal crypsis



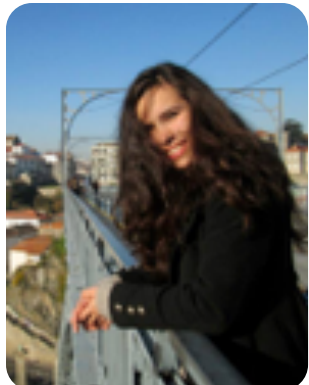
Collaborators & Funding



Paulo Alves



Jose Melo-Ferreira



Mafalda Ferreira



Matt Jones



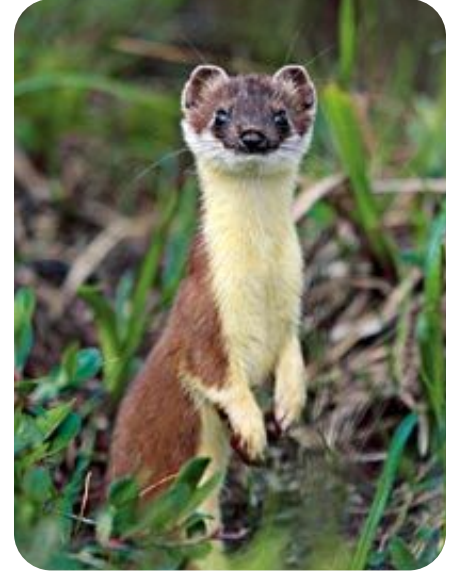
Scott Mills



Marketa Zimova

Seasonal change in coat color has independently evolved in several lineages


Controlled by photoperiod



Potential challenge of climate change:
Snow cover is changing, photoperiod is not.

A photograph of a Snowshoe Hare (Lepus americanus) sitting in a nest of pine needles and twigs in a forest. The hare has white fur with brown patches on its face and ears. It is surrounded by tree trunks and branches.

Snowshoe Hare (*Lepus americanus*)

A photograph of a mountain hare (Lepus timidus) sitting in a forest. The hare has white fur on its face and chest, with grey fur on its back and ears. It is surrounded by tree trunks and pine needles. The background is dark and out of focus.

no plasticity in onset of fall & spring molt
no plasticity in fall molt phenology
no behavioral plasticity to avoid mismatch

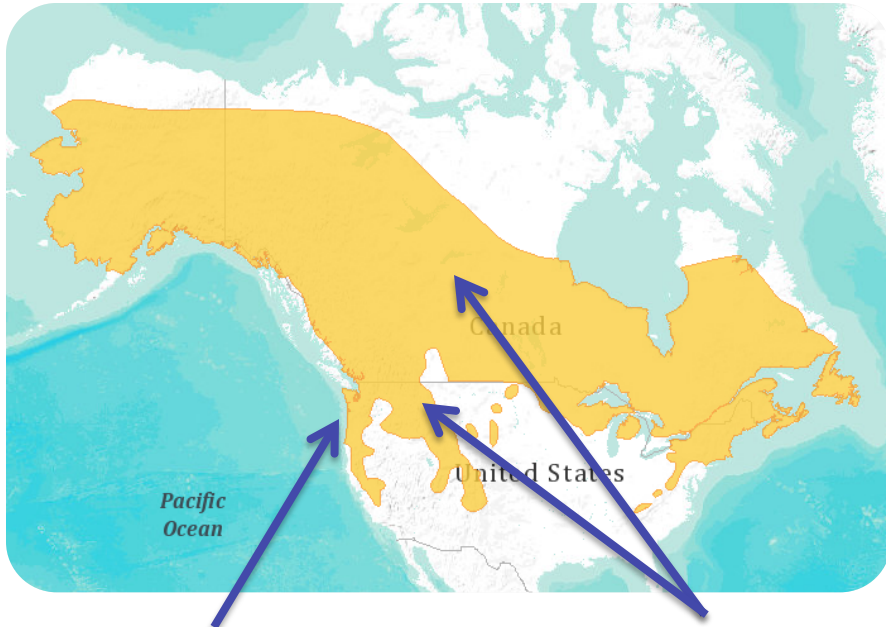
Mills *et al.*, 2013 PNAS
Zimova & Mills, 2014 Proc Roy Soc.

Large fitness cost to mismatch



Zimova & Mills, in review

Geographic variation in winter molts – not all populations turn white



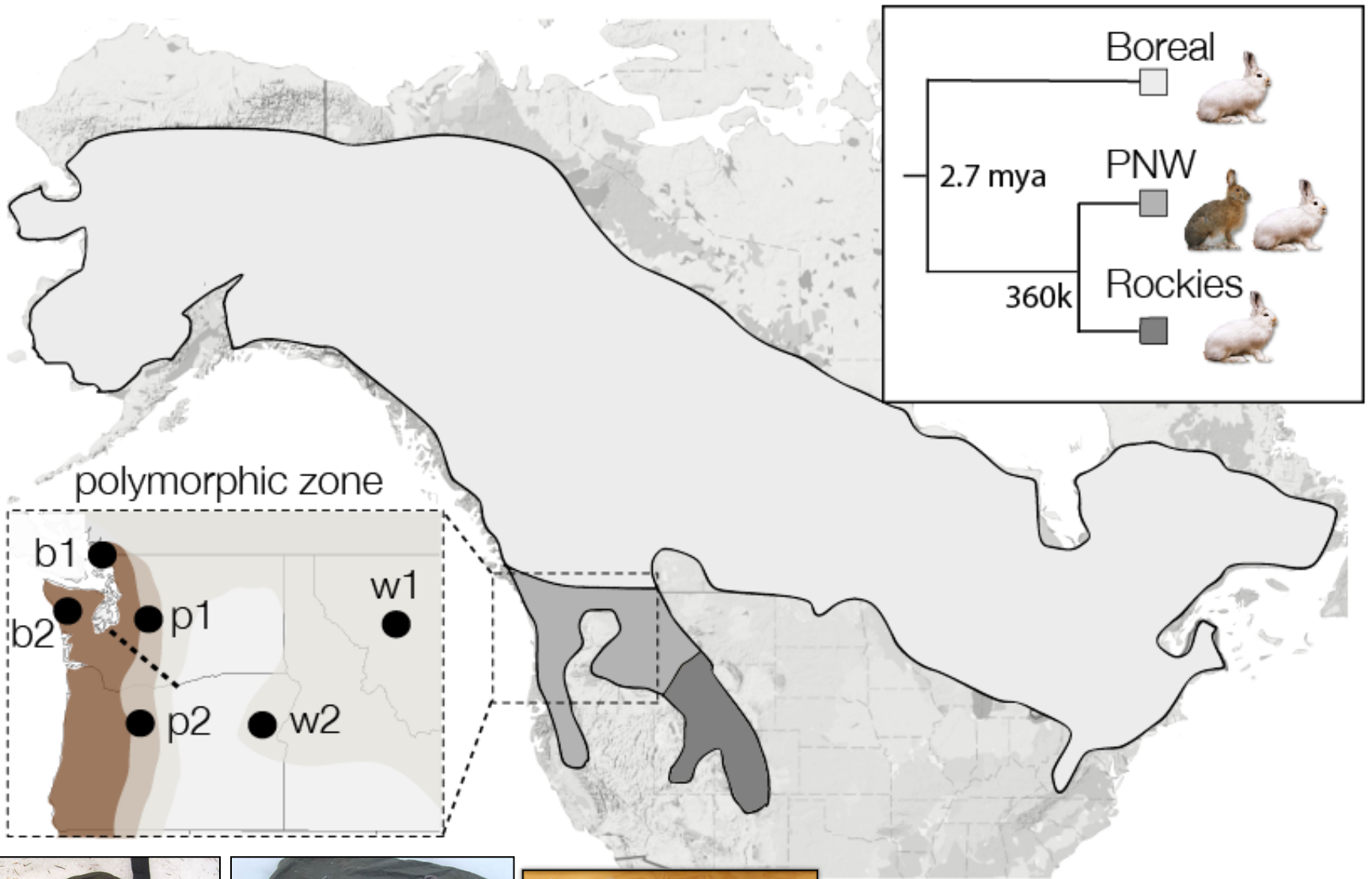
What is the genetic basis of seasonal molts to white winter coats?

Two components:

- *If* to change
- *When* to change



Winter molts



Discrete variation with no intermediates. Implies simple genetic basis

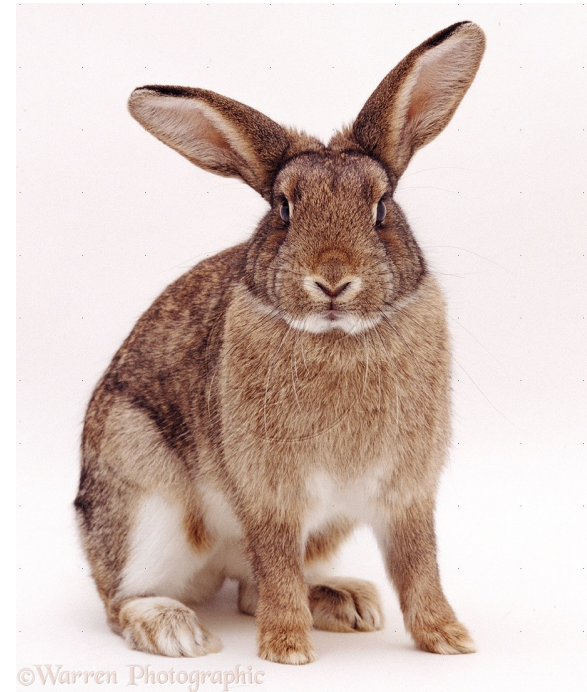
Next step: genome-wide **association study** of genetic variants with winter coat color in the polymorphic zone.

Snowshoe hare

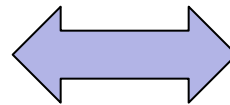


No genome

Rabbit



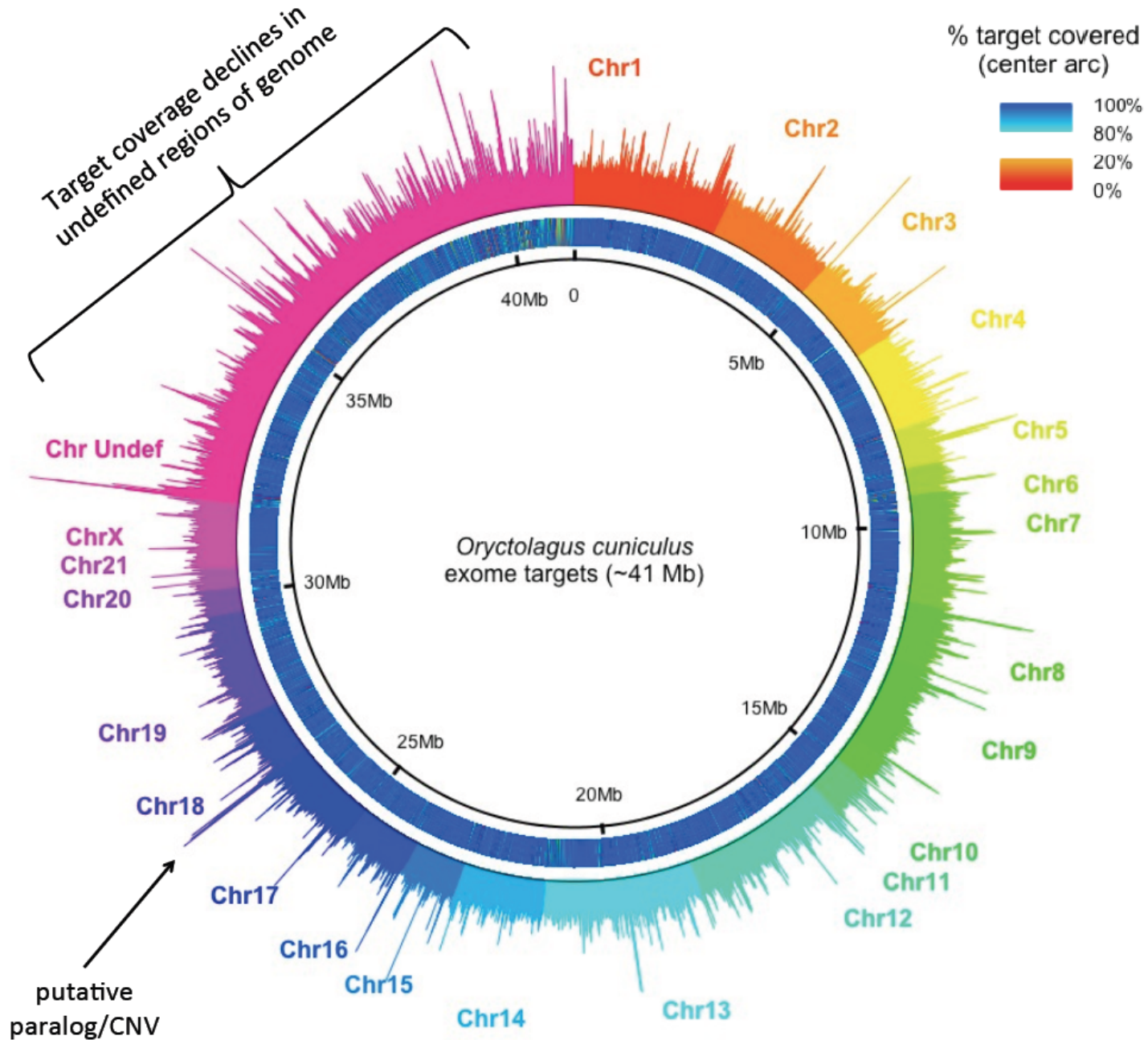
Genome



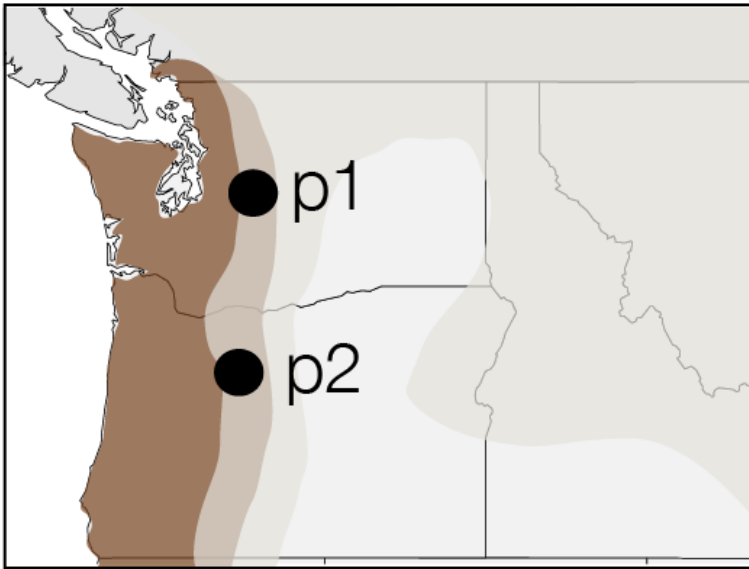
5% div

12-18 MY

Genome-wide coverage of snowshoe hare exome (all genes)



polymorphic zone



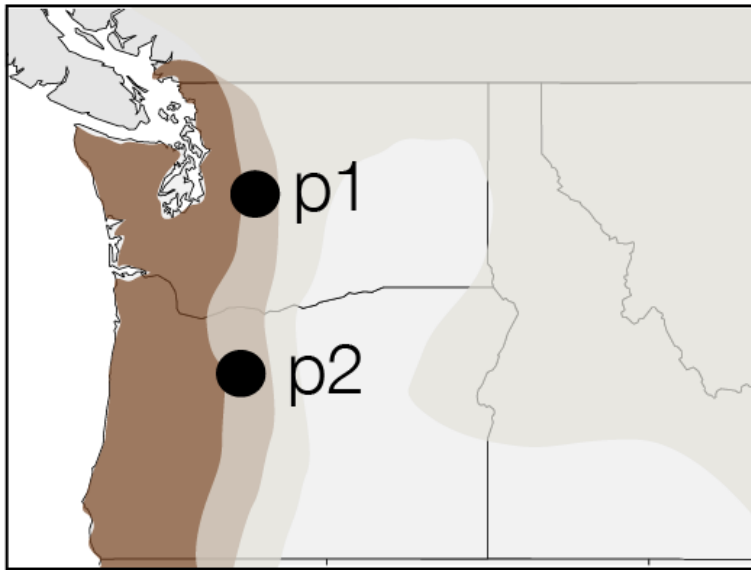
Hare-specific exome capture

60 Mb, coding & non-coding

15 white, 15 brown hares

~15X coverage / hare

polymorphic zone



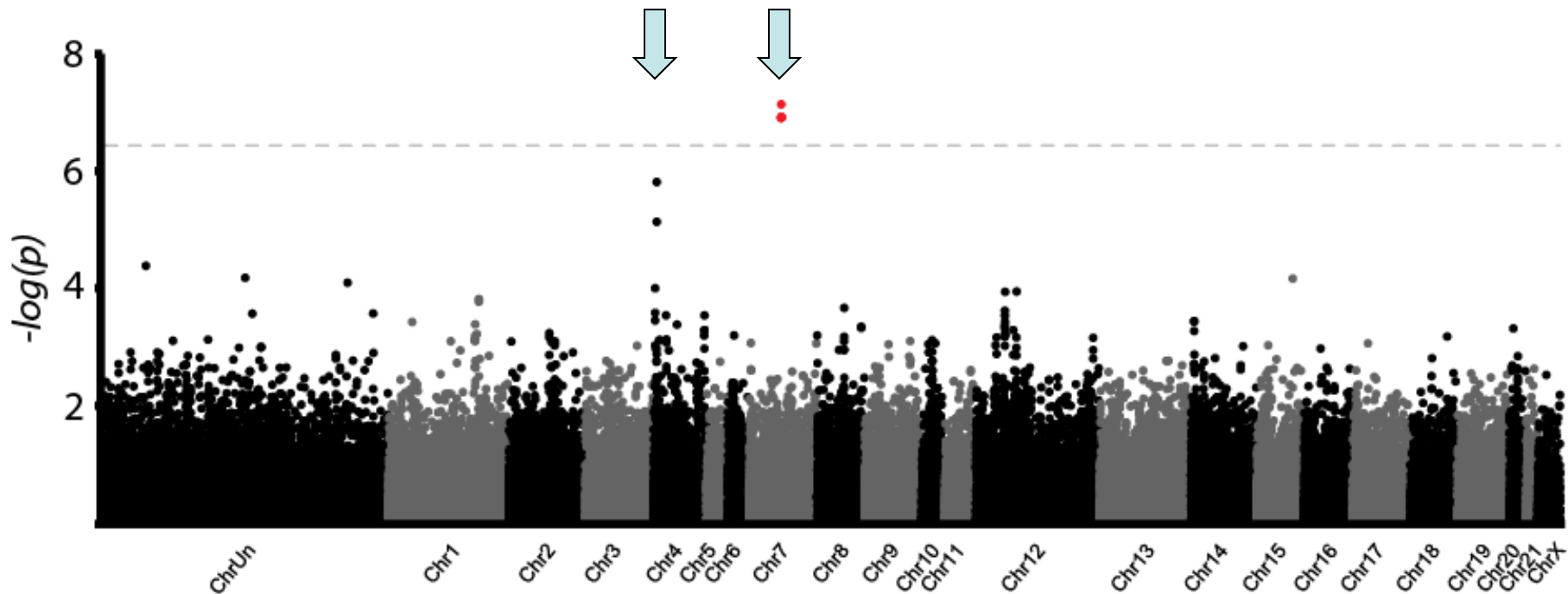
Hare-specific exome capture

60 Mb, coding & non-coding

15 white, 15 brown hares

~15X coverage / hare

2 genes w/ perfect association



Summary – case studies

NGS + genome partitioning provides a quick and efficient means to collect genomic data in non-model species

Efficient incorporation of both contemporary and historic samples with targeted capture

Rapid insight into standard population genetic questions, including dissection of ecologically relevant traits.



Genomics and the future of conservation genetics

Fred W. Allendorf^{**}, Paul A. Hohenlohe^{§||} and Gordon Luikart^{†#}

