The Molecular Basis of Evolutionary Change: Genomic Insights from Fish and Humans

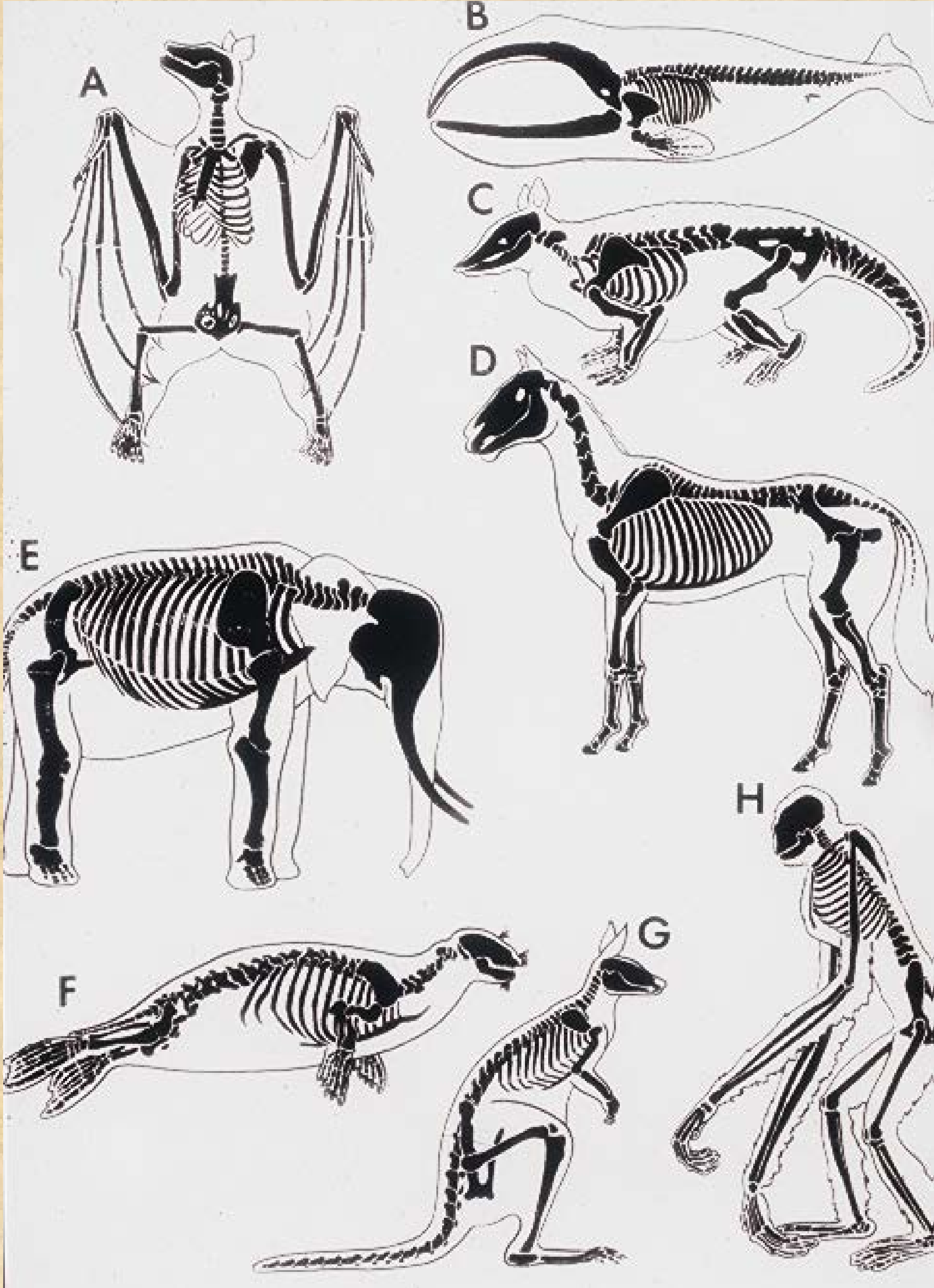
HGP10 symposium

April 25th, 2013

David Kingsley
HHMI and Stanford University

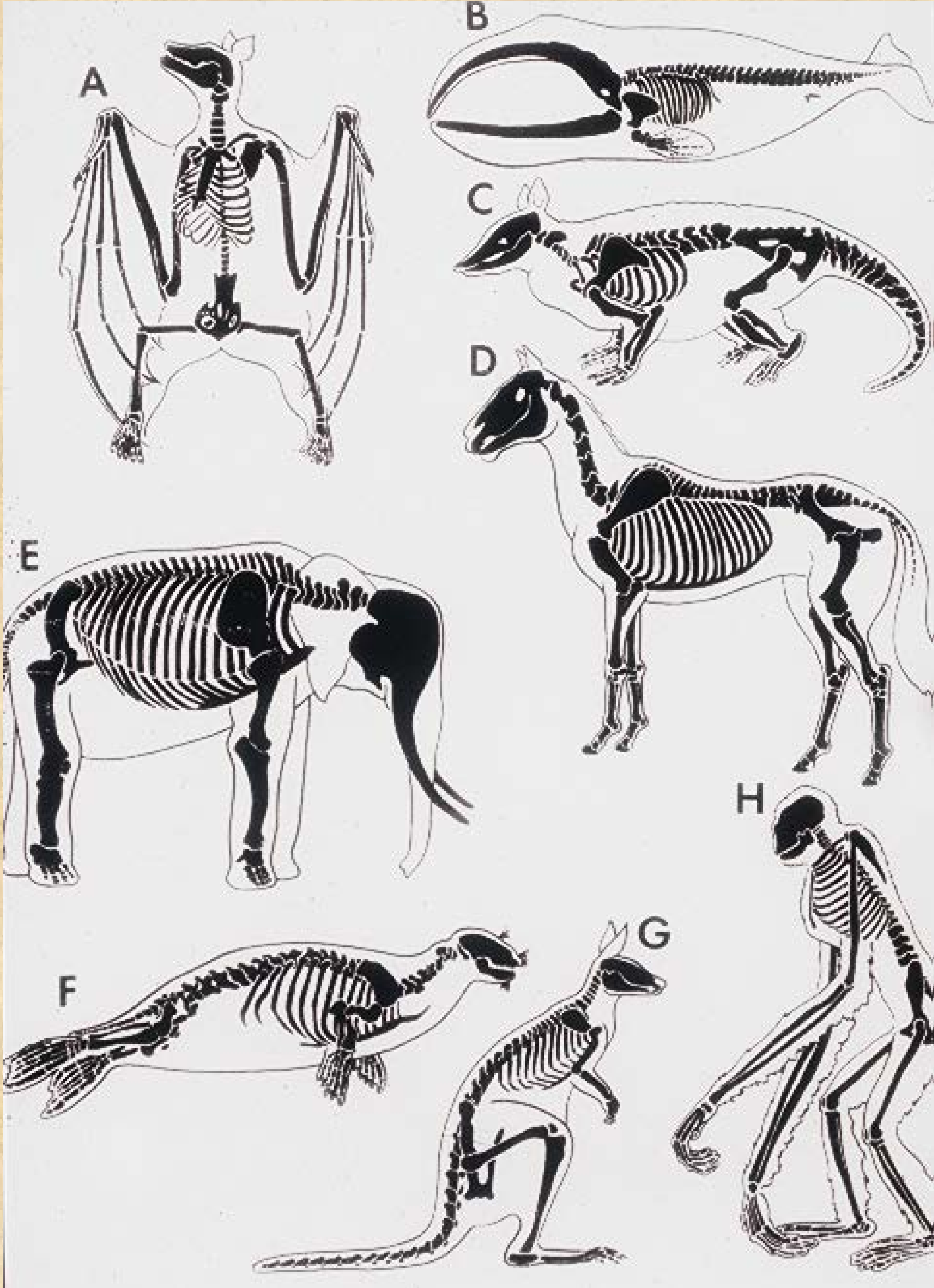
Animals differ!

- Morphology
- Physiology
- Feeding
- Locomotion
- Reproduction
- Behavior
- Intelligence
- Disease susceptibility
- Lifespan

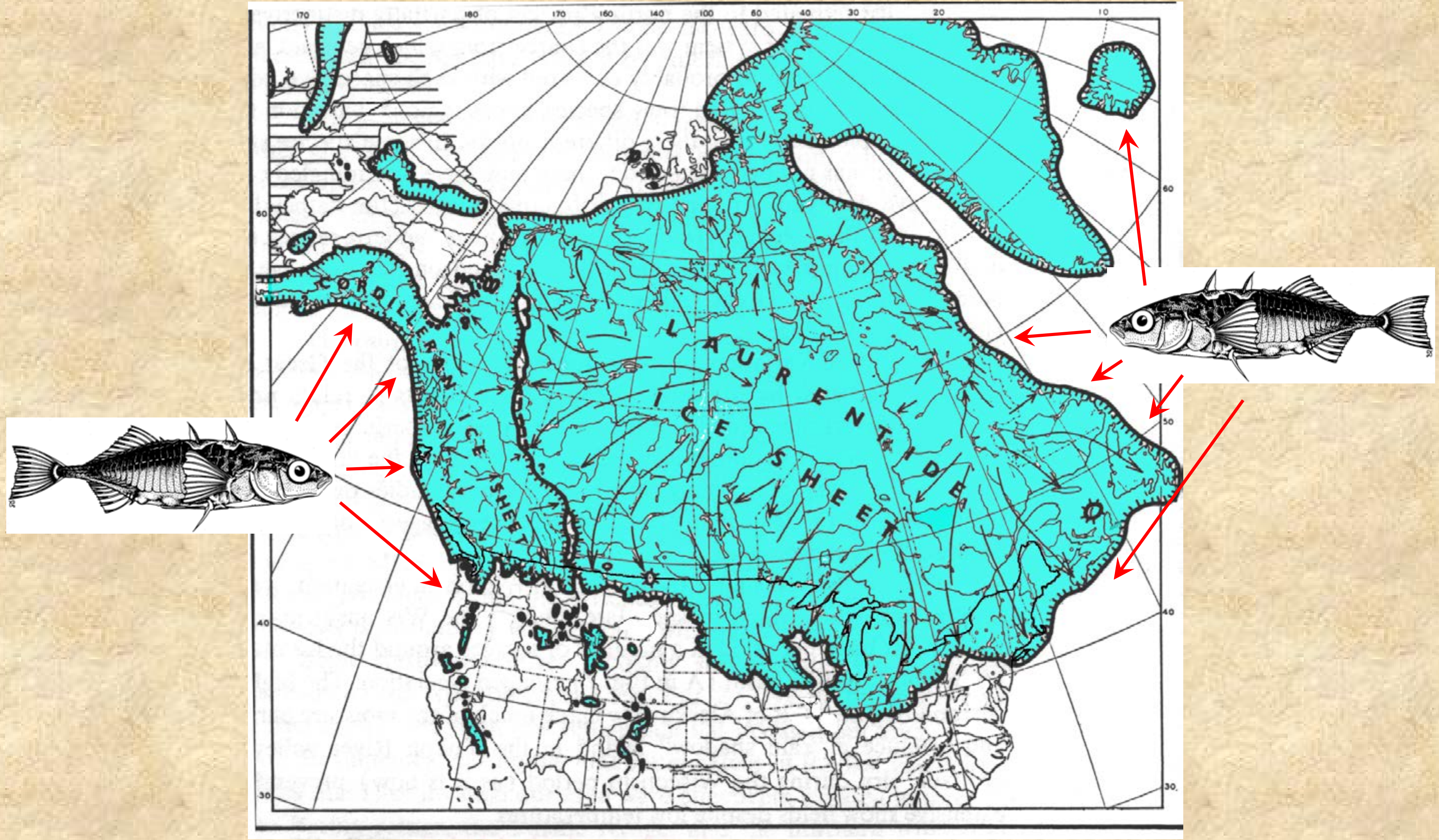


What is the molecular basis of these obvious differences?

- How many genetic changes?
- Which genes?
- What kinds of mutations?
- Few or many ways to evolve a given trait?



Very *recent*, and *repeated*, natural evolutionary experiments

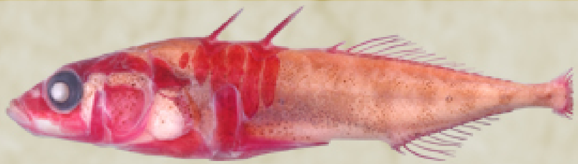


Adaptive Radiation

Paxton Lake (Benthic)
Vancouver, Canada

Friant, CA

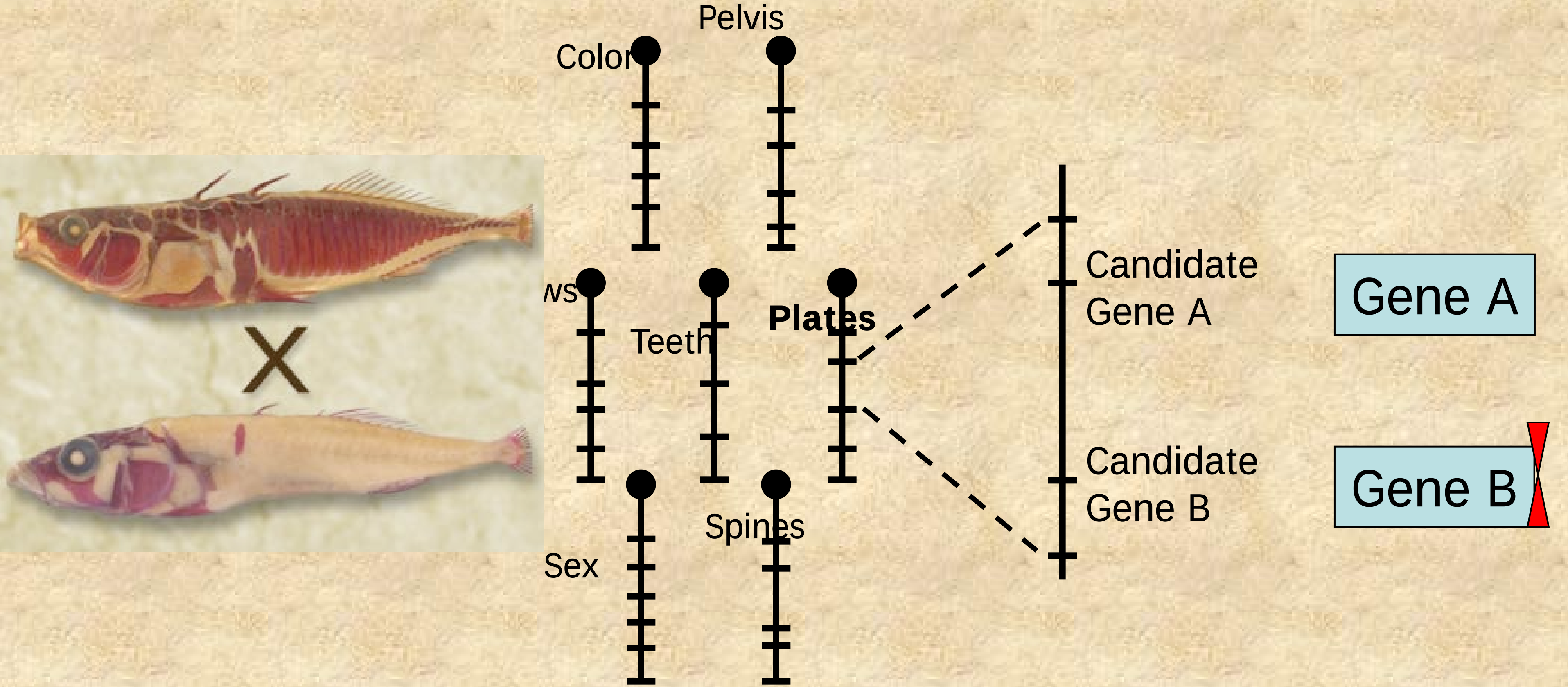
Ancestral Marine Phenotype



San Francisquito Creek, CA

Vifilsstadavatn, Iceland

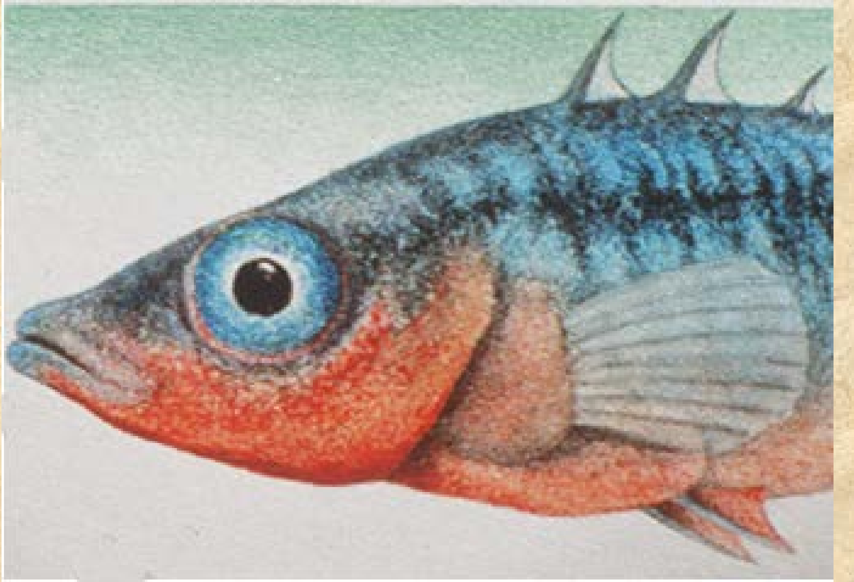
Experimental approach to finding the genes that control evolutionary change in natural populations



Establish Crosses → Build Genetic Map → Clone interval, identify candidates → Find Molecular Changes

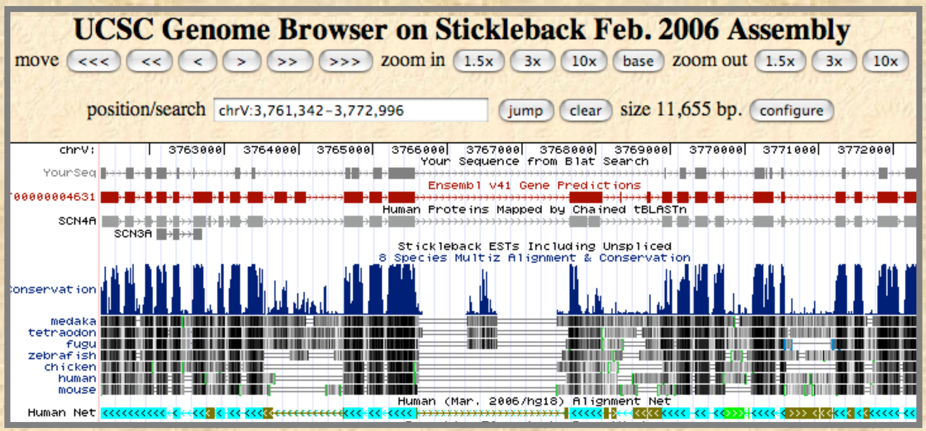
Prospects for genetics & cloning (1998)

- No sequences
- No clone libraries
- No genetic markers
- No linkage maps
- No transgenic methods



Today

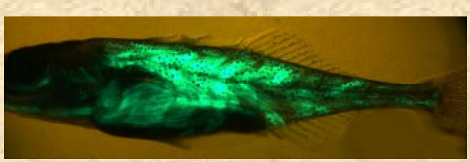
- Dense genetic and physical maps
- Transcriptome arrays
- Genome-wide SNP surveys
- Transgenics and TALEN targeting
- High-quality reference genome
- Resequencing of many natural populations



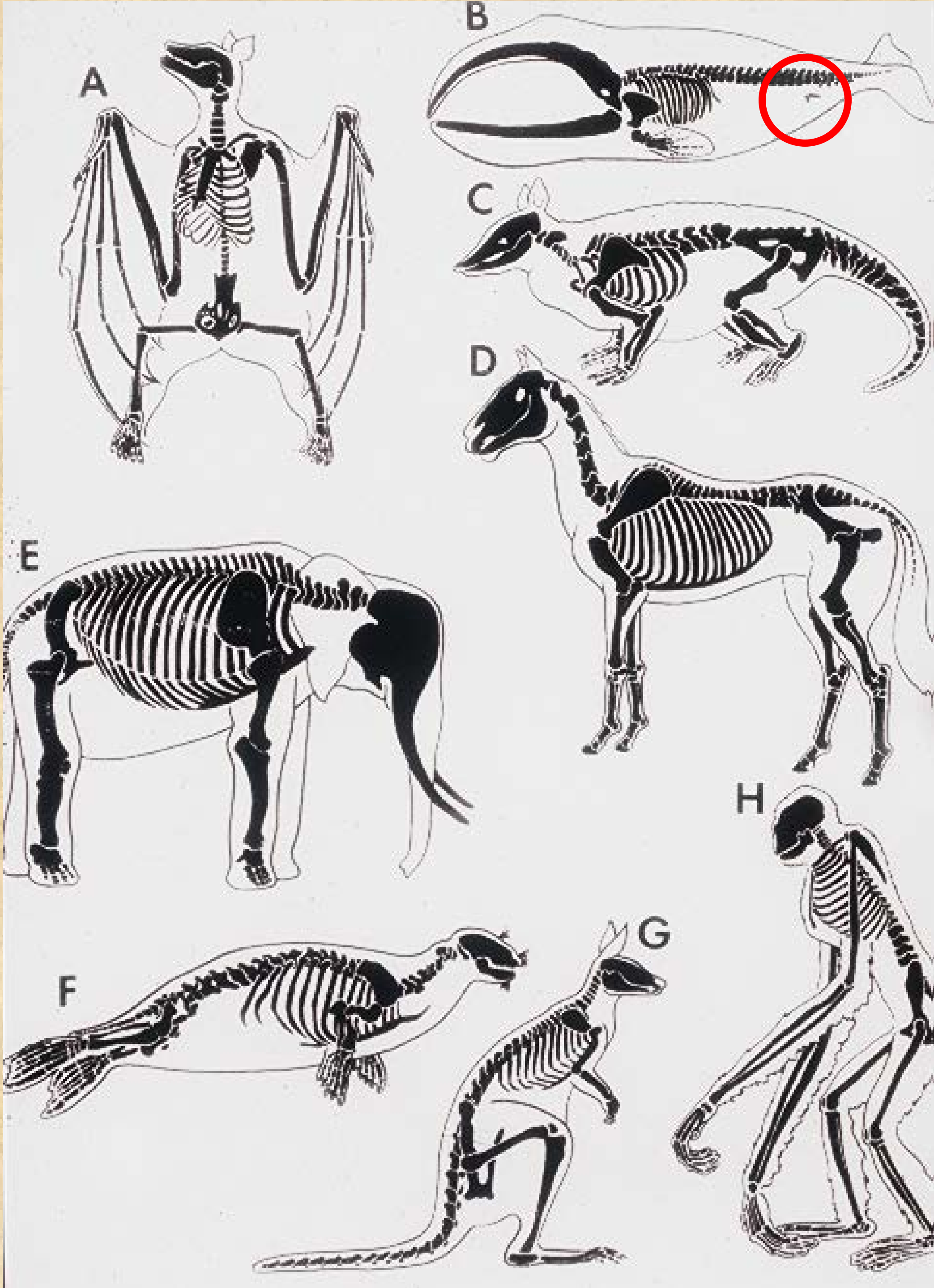
UCSC Genome Browser on Stickleback Feb. 2006 Assembly

position/search chrV:3,761,342-3,772,996

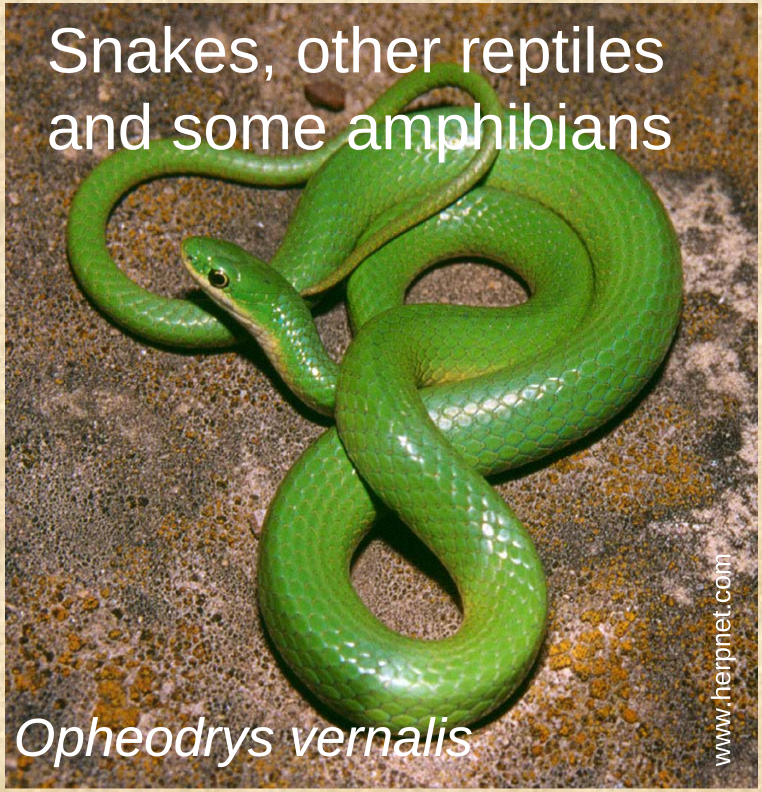
size 11,655 bp.



Hindlimb reduction occurs in many vertebrates



Hindlimb reduction occurs in many vertebrates


Snakes, other reptiles and some amphibians

Opheodrys vernalis

www.herpnet.com


Whales and aquatic mammals

Megaptera novaeangliae

www.learner.org

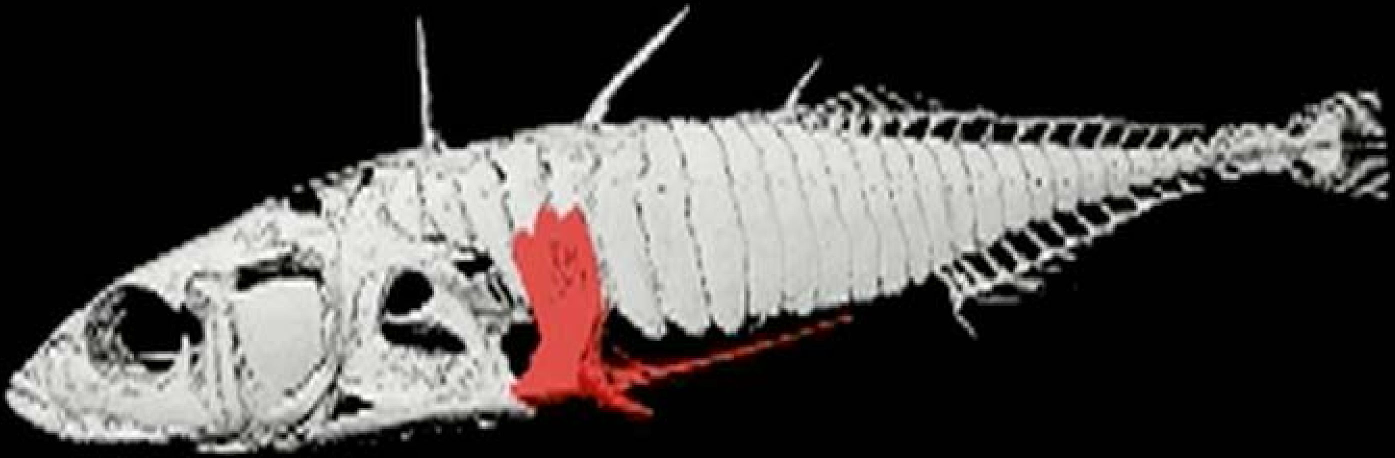

Many species of sticklebacks!

Gasterosteus dorssyus

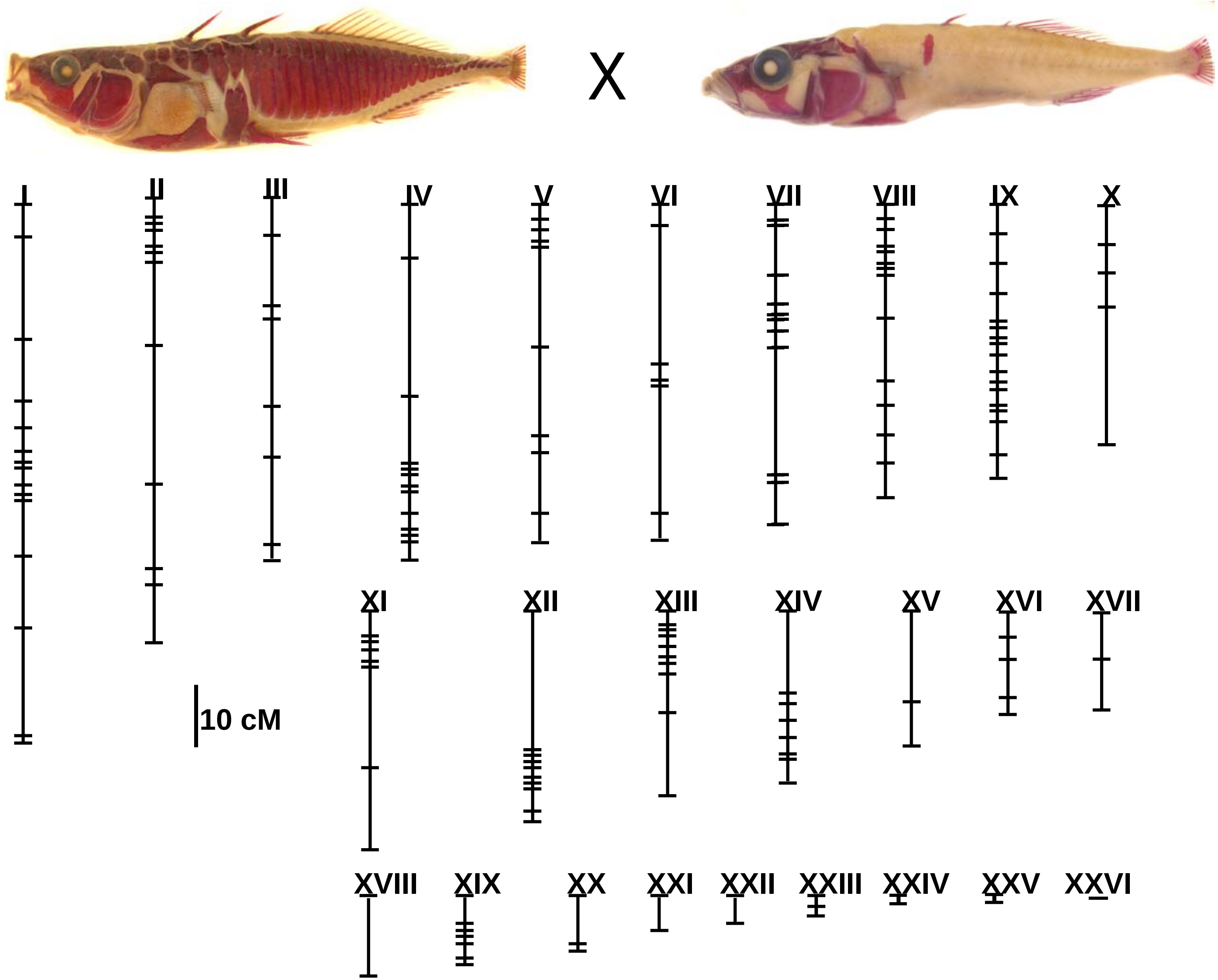
Mike Bell


Gasterosteus aculeatus

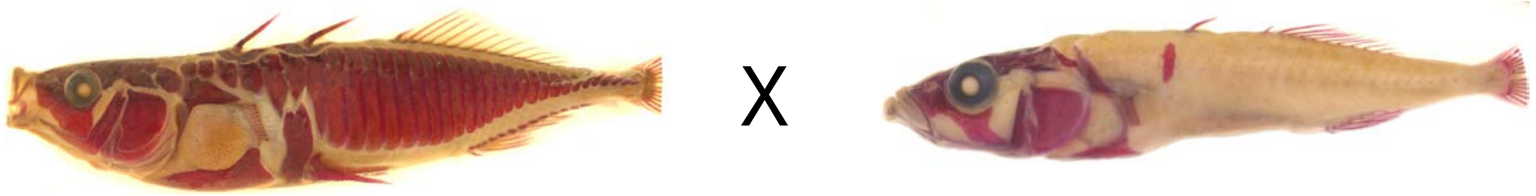
Matt Mcleod

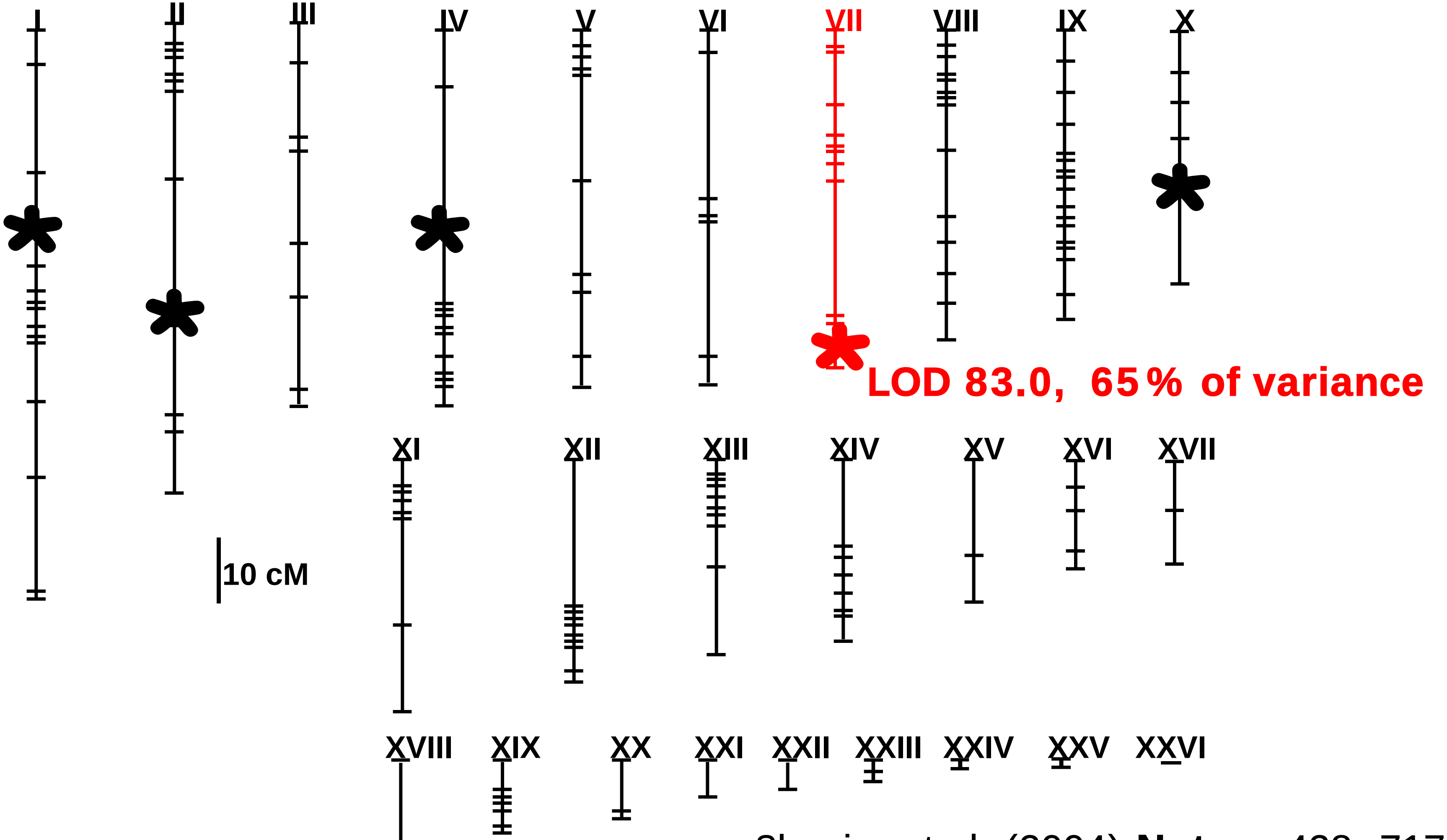


Genetic architecture of pelvic reduction



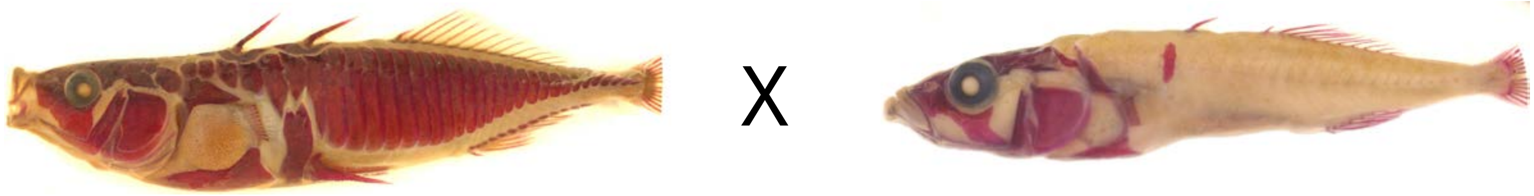
Genetic architecture of pelvic reduction

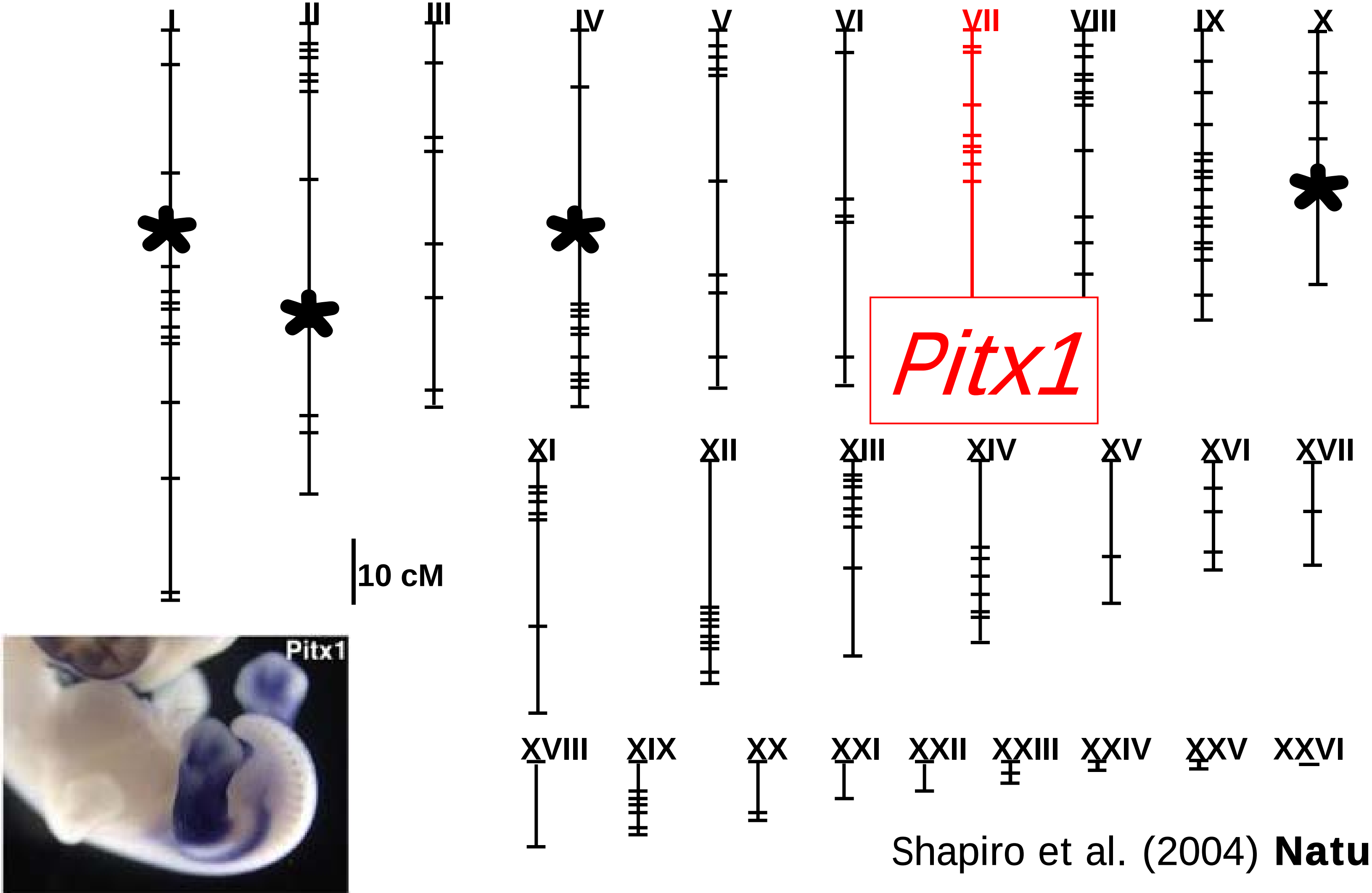




Shapiro et al. (2004) **Nature** 428: 717.

Genetic architecture of pelvic reduction





Shapiro et al. (2004) **Nature** 428: 717.

Pitx1

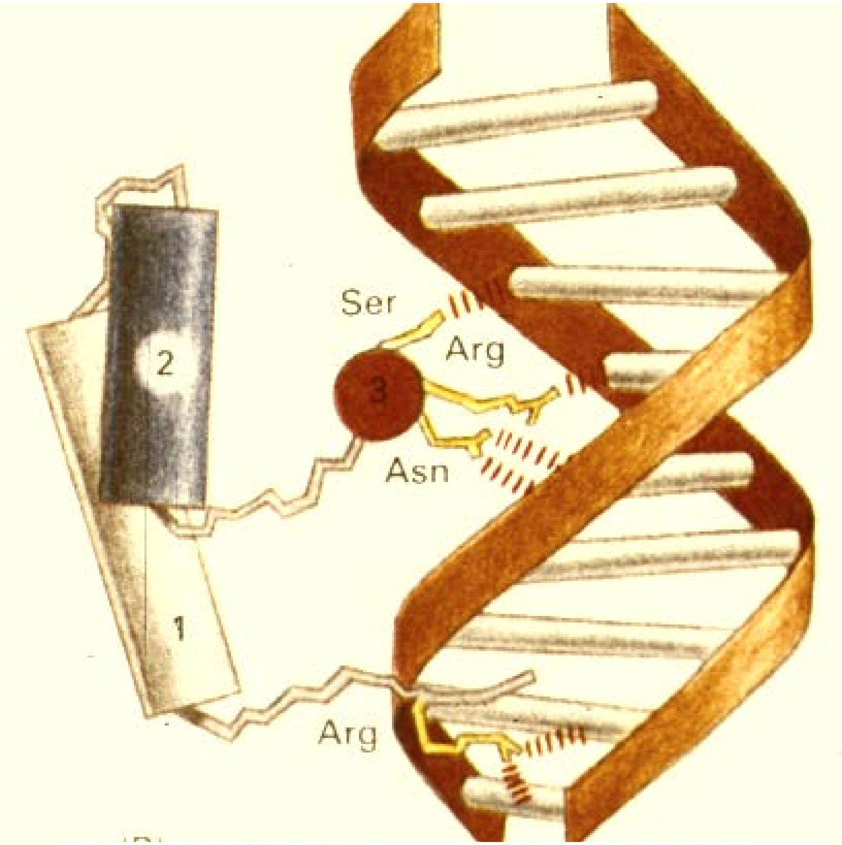
- Homeodomain protein that binds DNA and controls expression of multiple target genes

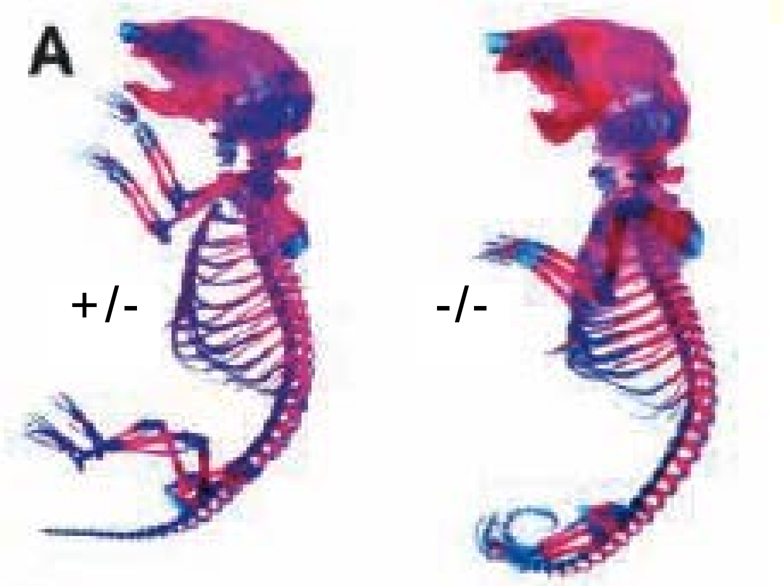
- Named *Pitx1* for important role in pituitary development

- Also called “*Backfoot*” gene, because of remarkable expression pattern in hindlimbs but not forelimbs of most vertebrate embryos

- Mouse knockouts have small hindlimbs, but die at birth with pituitary defects, craniofacial malformations, cleft palate

Lanctot et al. (1999); Szeto et al. (1999)





Hopeless monsters?

It is a general rule, of which every geneticist and breeder can give numerous examples, that the more drastically a mutation affects the phenotype, the more likely it is to reduce fitness. To believe that such a drastic mutation would produce a viable new type, capable of occupying a new adaptive zone, *is equivalent to believing in miracles*."

Ernst Mayr Populations, Species, and Evolution (1970)

$Pitx1^{+/-}$ $Pitx1^{-/-}$

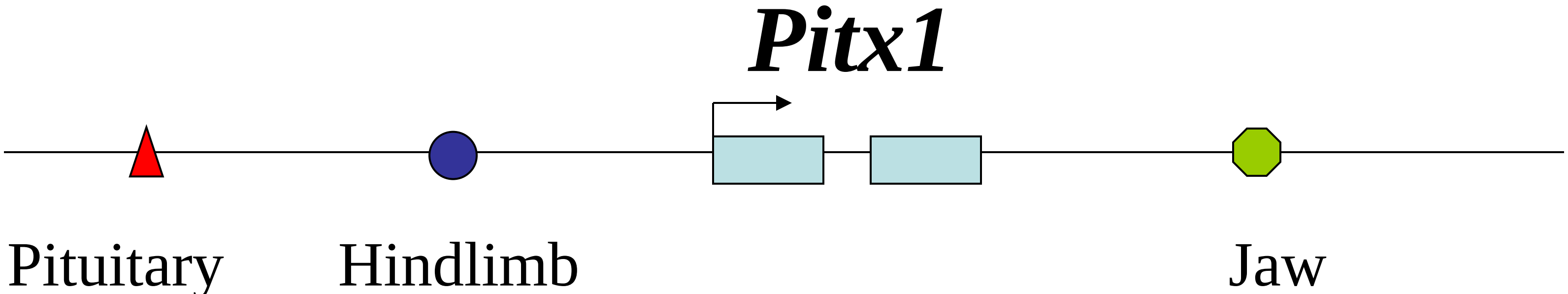
Lanctôt et al. 1999

What has happened in natural populations?

- PITX1 has identical amino acid sequence in marine and pelvic-reduced sticklebacks

- Clear change in expression at specific body site:

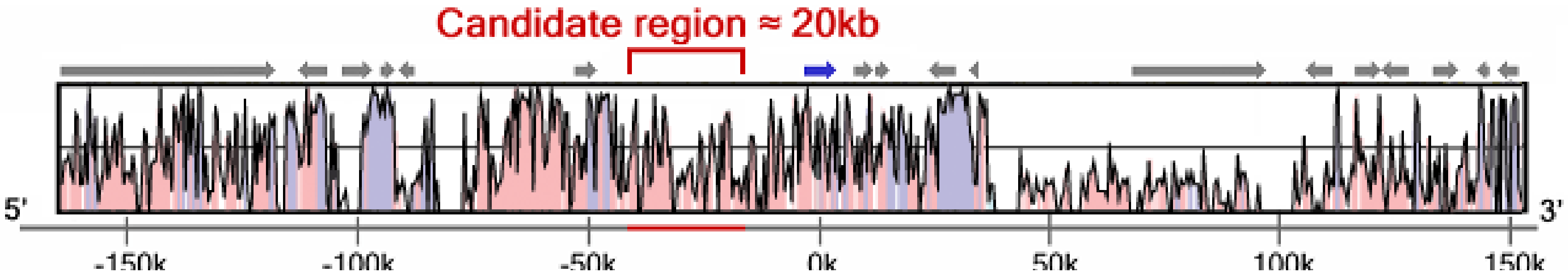
Mike Shapiro

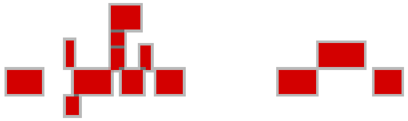


Pitx1

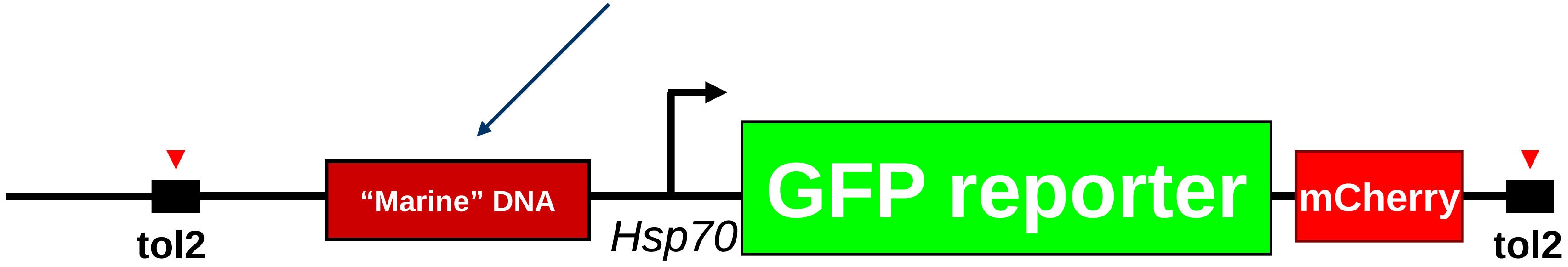
Pituitary Hindlimb Jaw

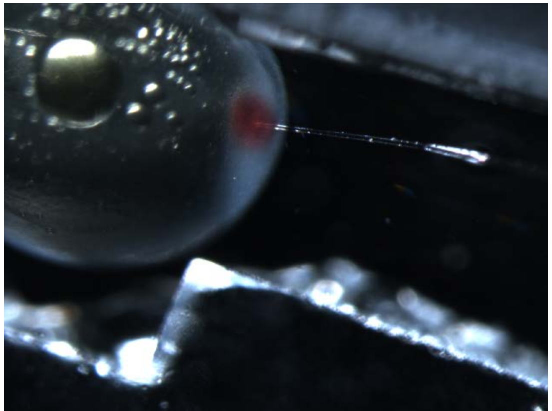
Can we find the proposed regulatory change?

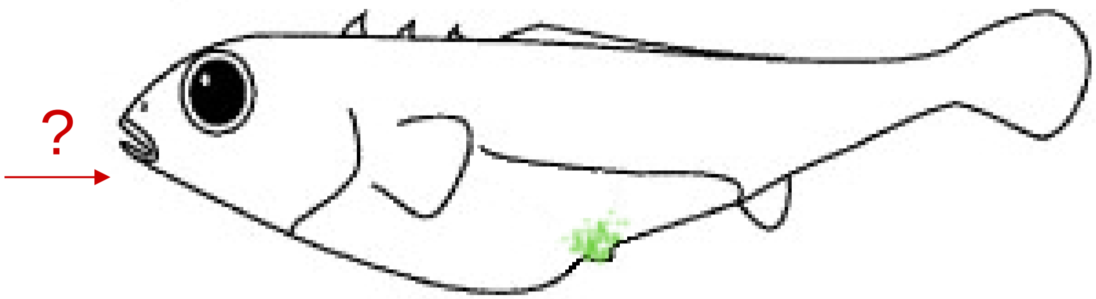




Transgenic Constructs

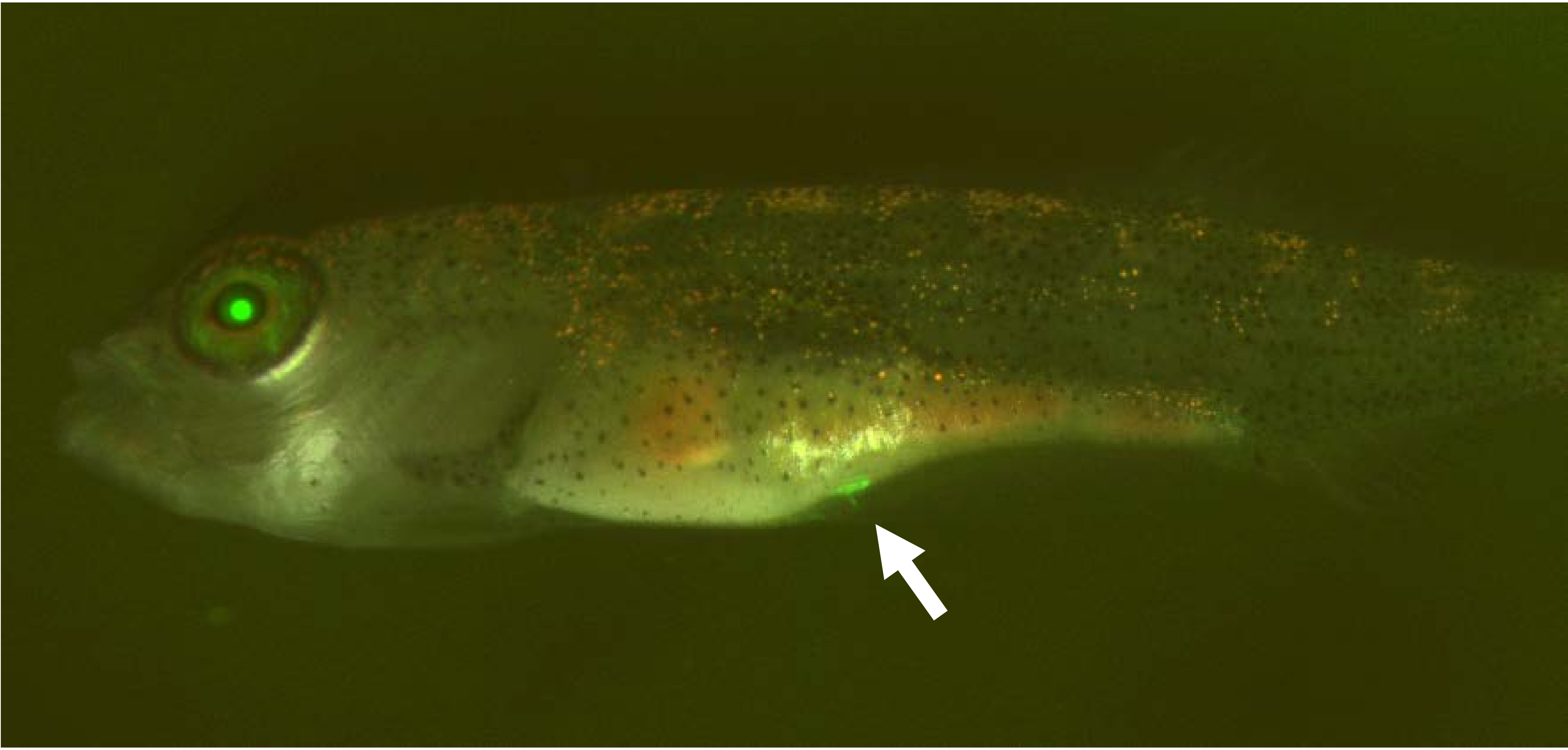






Frank Chan

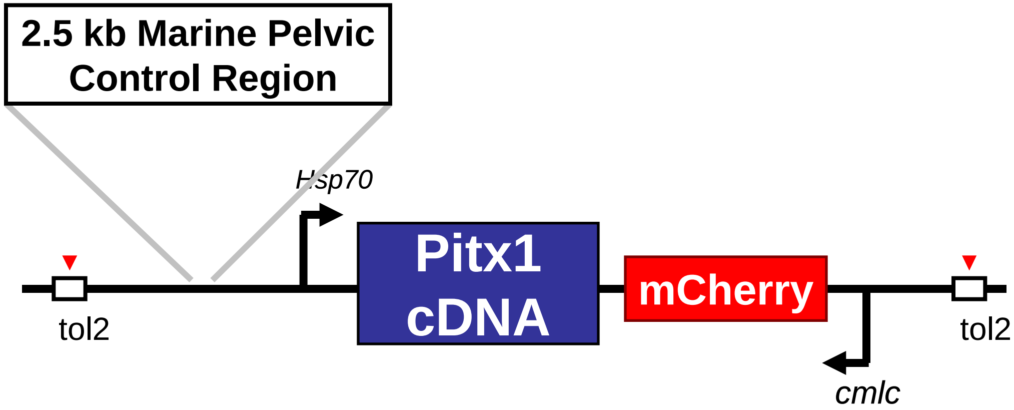
Key genetic interval for pelvic reduction contains a tissue specific pelvic enhancer of the *Pitx1* gene

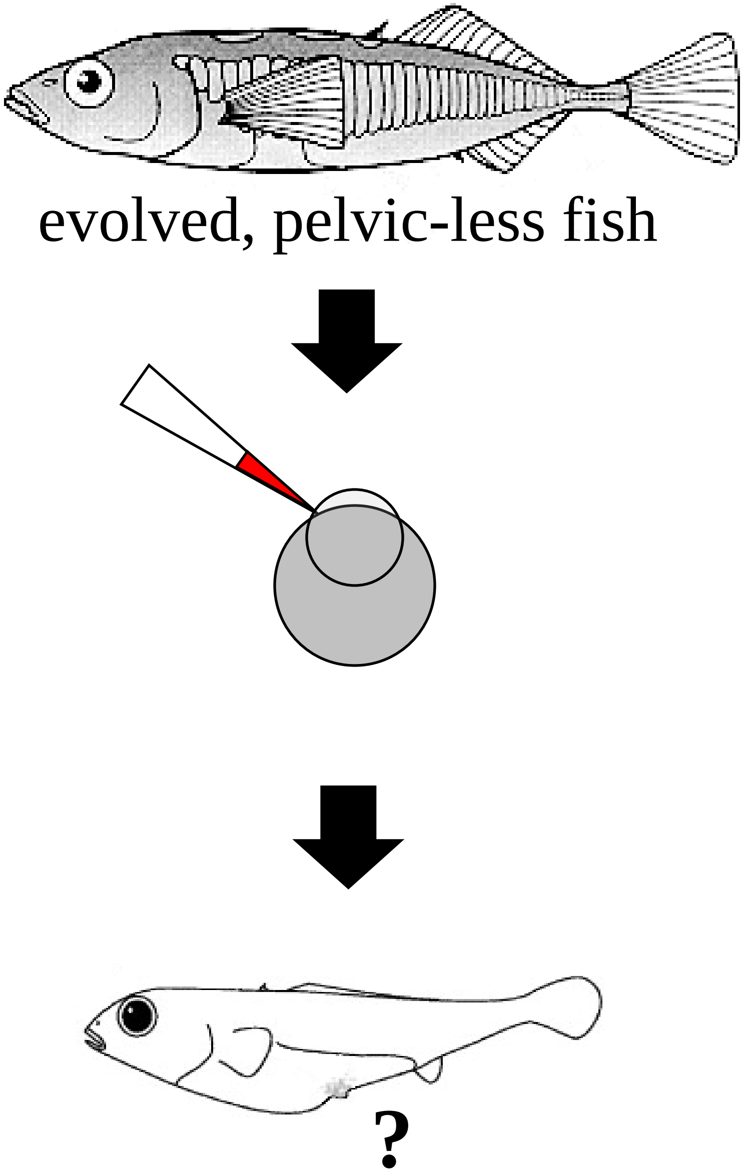


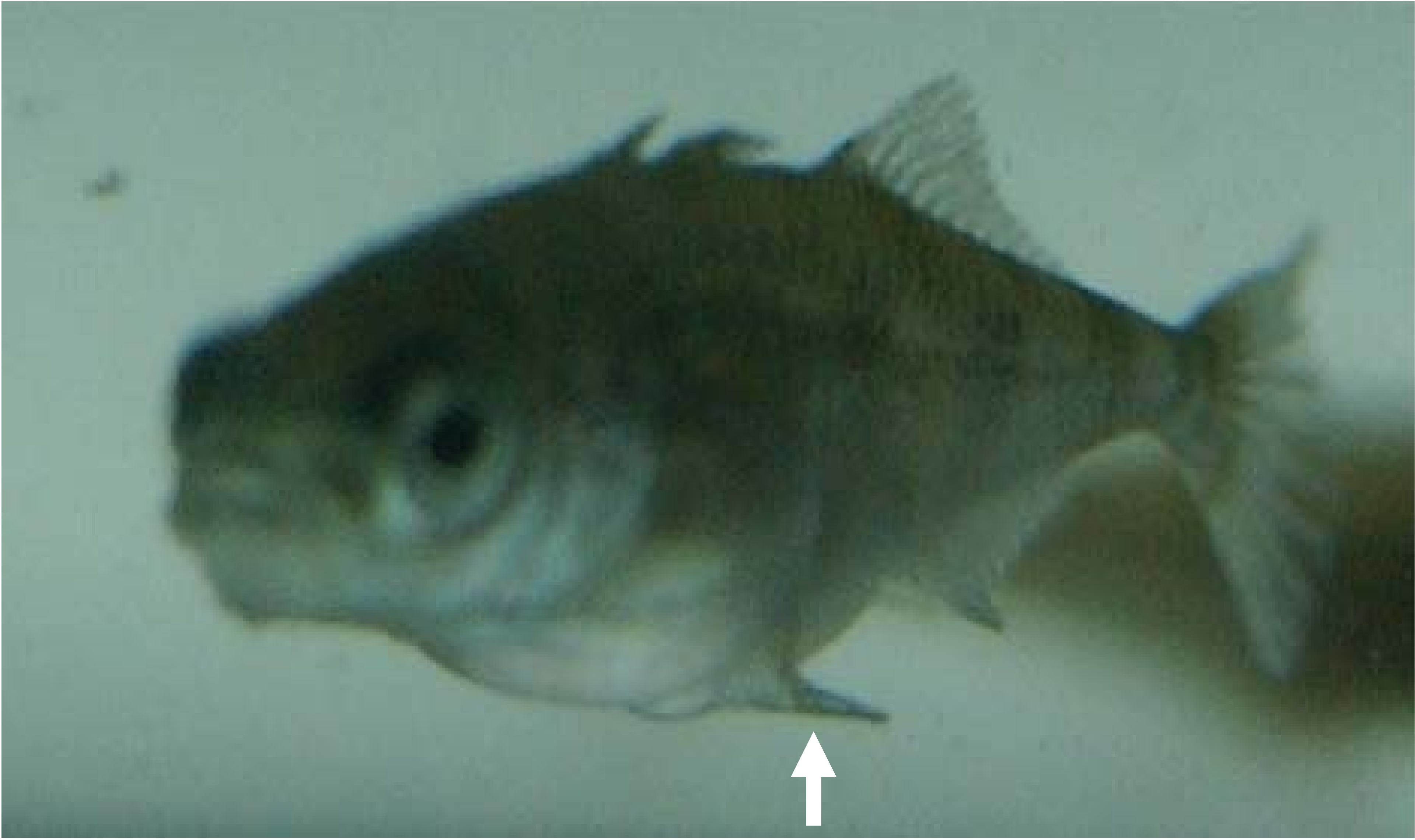
495 bp *Pitx1* non-coding sequence

Chan et al. (2010) Science

Using marine information to reverse evolutionary change



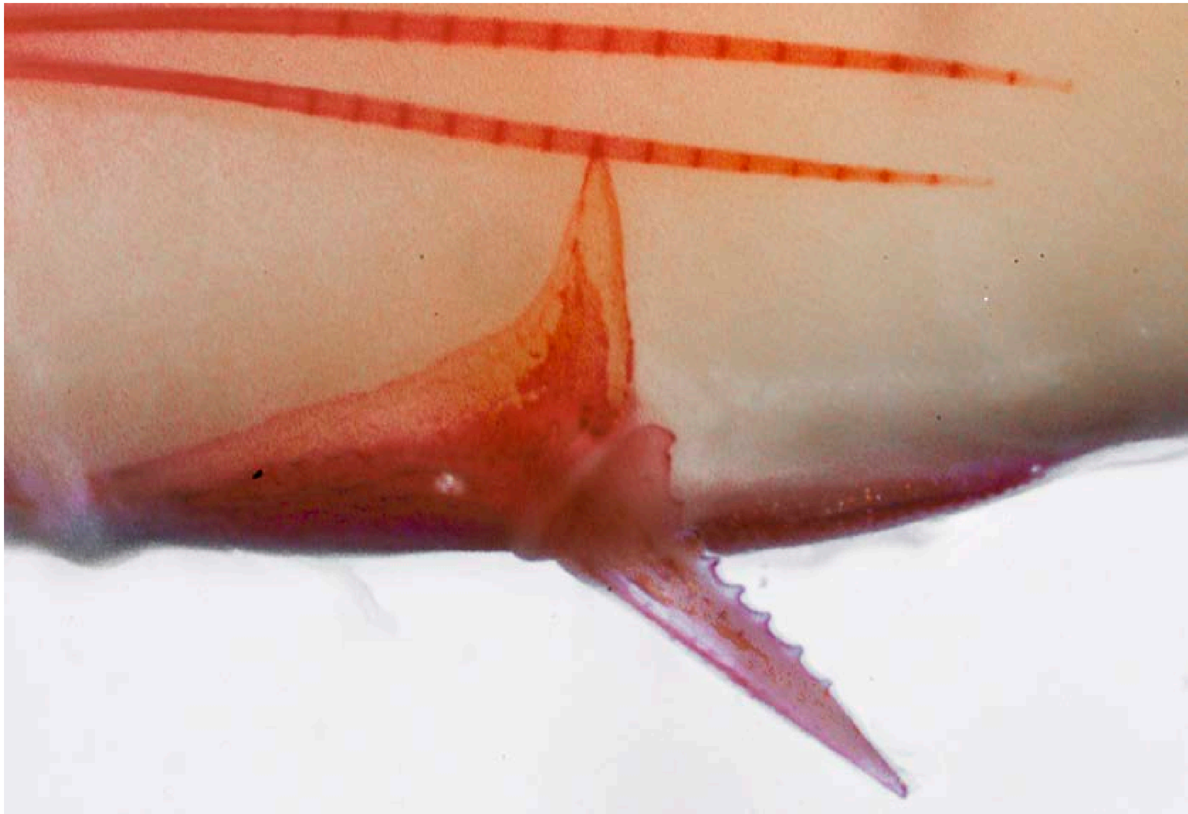




Chan et al. (2010) Science

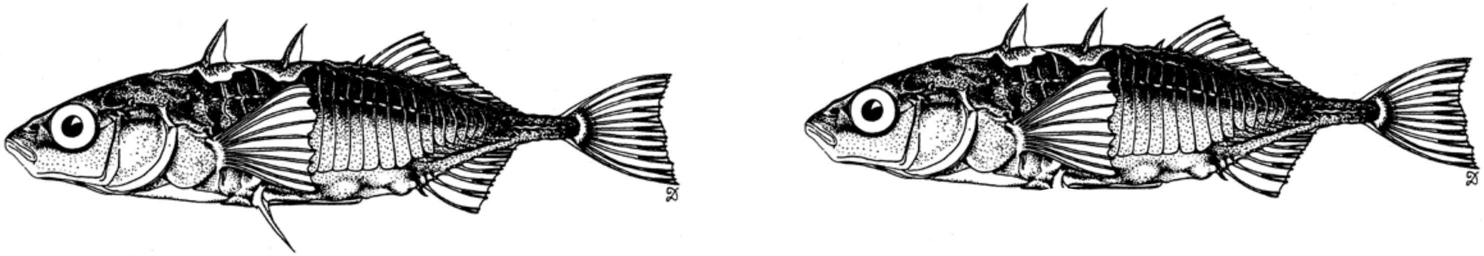
Vestigial pelvis in original evolved population

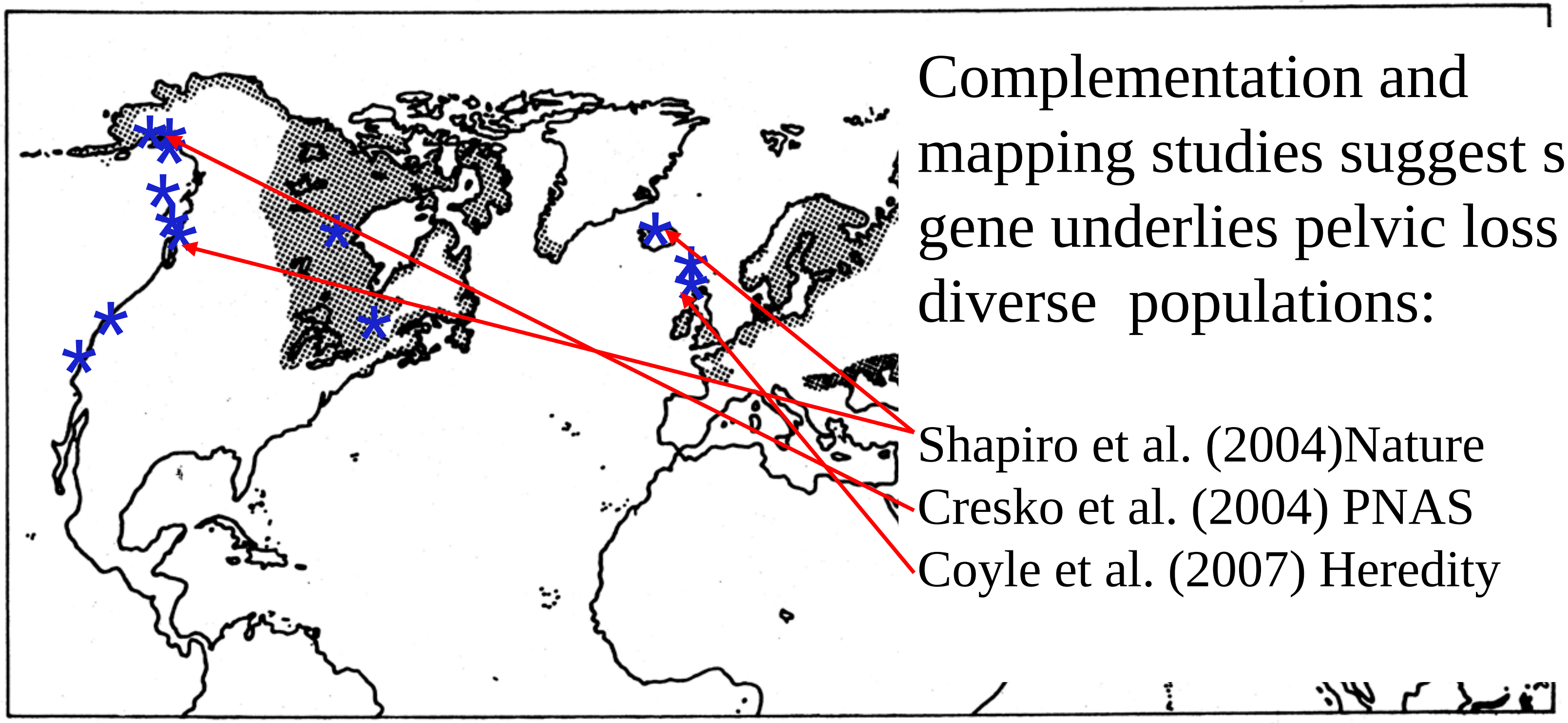
Transgenic rescue fish



Chan et al. (2010) Science

What are the regulatory DNA lesions in natural populations with pelvic reduction?

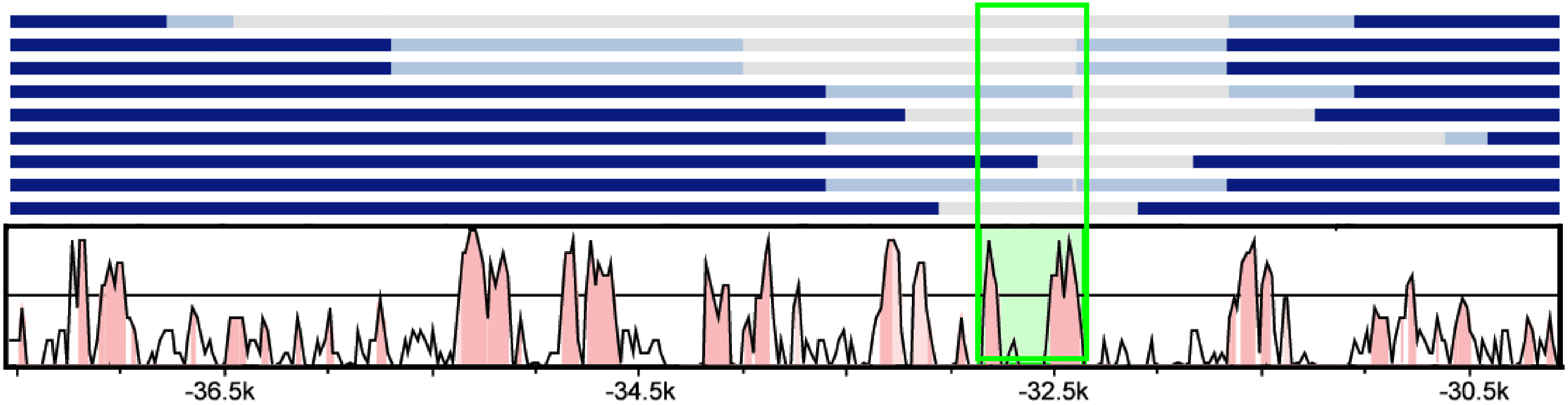




Complementation and mapping studies suggest same gene underlies pelvic loss in diverse populations:

Shapiro et al. (2004)Nature
Cresko et al. (2004) PNAS
Coyle et al. (2007) Heredity

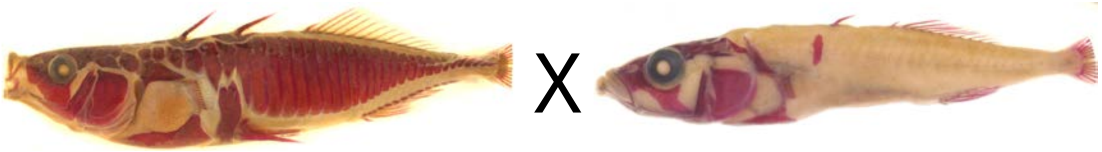
Multiple, independent deletions that overlap or completely remove pelvic control element

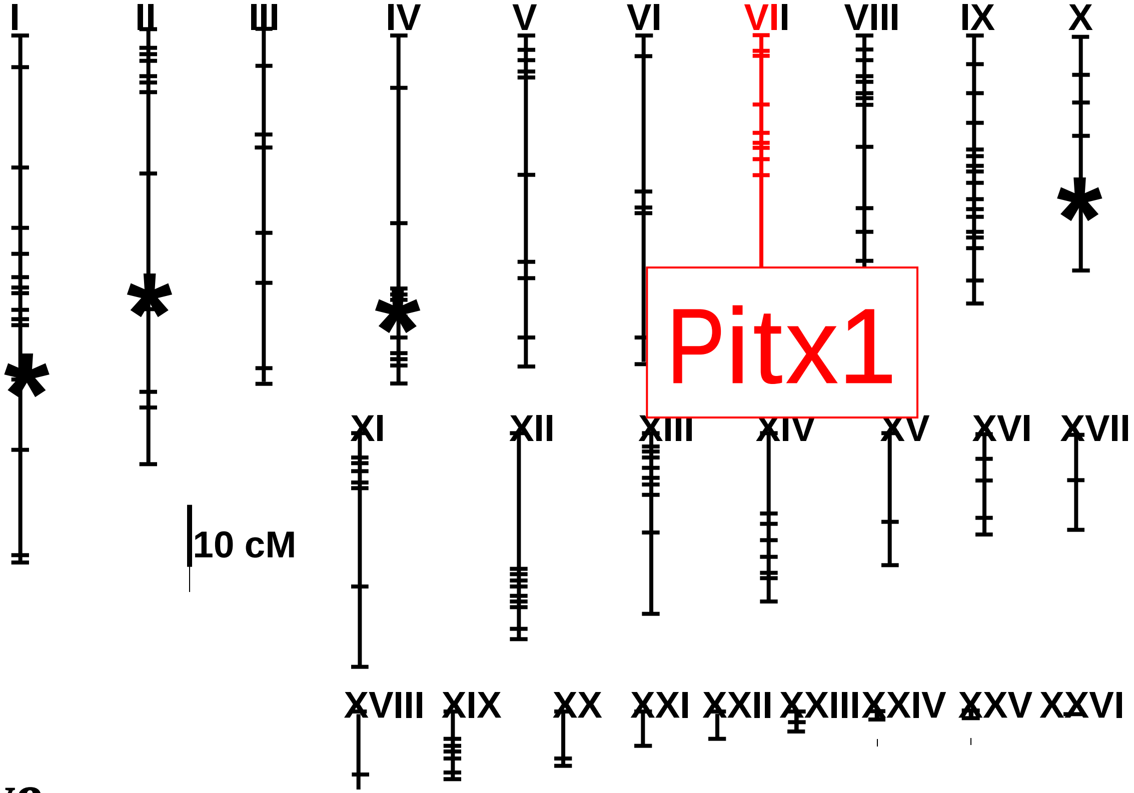


495 bp
pelvic
enhancer

Frank Chan

What is the molecular basis of major skeletal change in natural populations ?





- **How many genes?**

  Few, with large effects

- **What types of genes?**

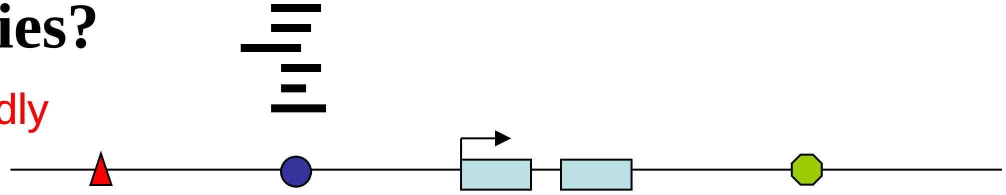
  Key developmental gene

- **What types of mutations?**

  Regulatory

- **Few or many ways to evolve particular morphologies?**

  Same mechanism used repeatedly



Traits Now Segregating in Crosses

Body skeleton

Pattern of lateral plates
Number of lateral plates
Number of dorsal spines
Size of dorsal spines
Number of fin rays
Presence or absence of hindfin

Feeding modifications

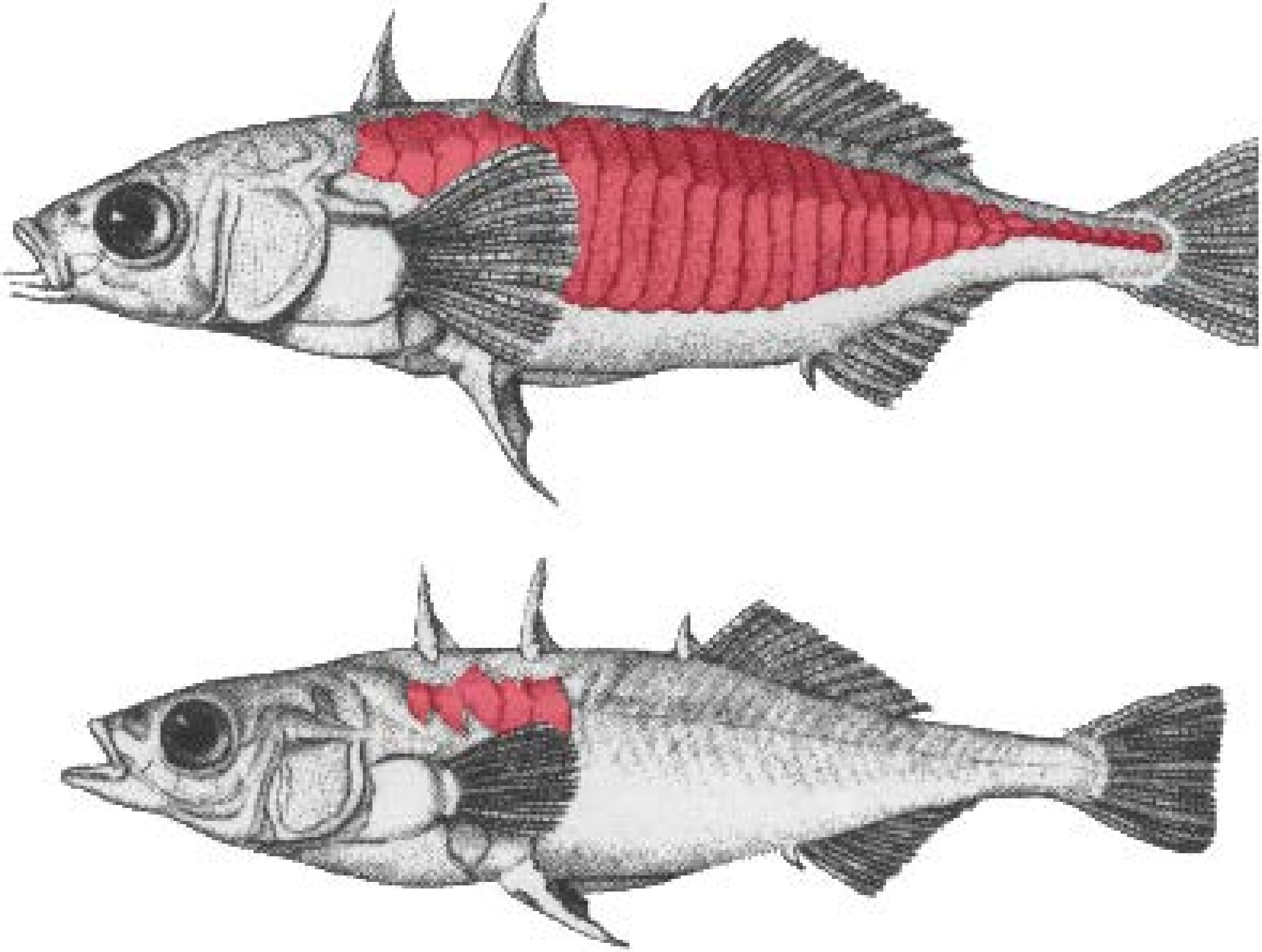
Jaw length
Size and number of teeth
Number of gill rakers

Mating and speciation

Body color
Color pattern
Color recognition
Mate preference
Sex determination

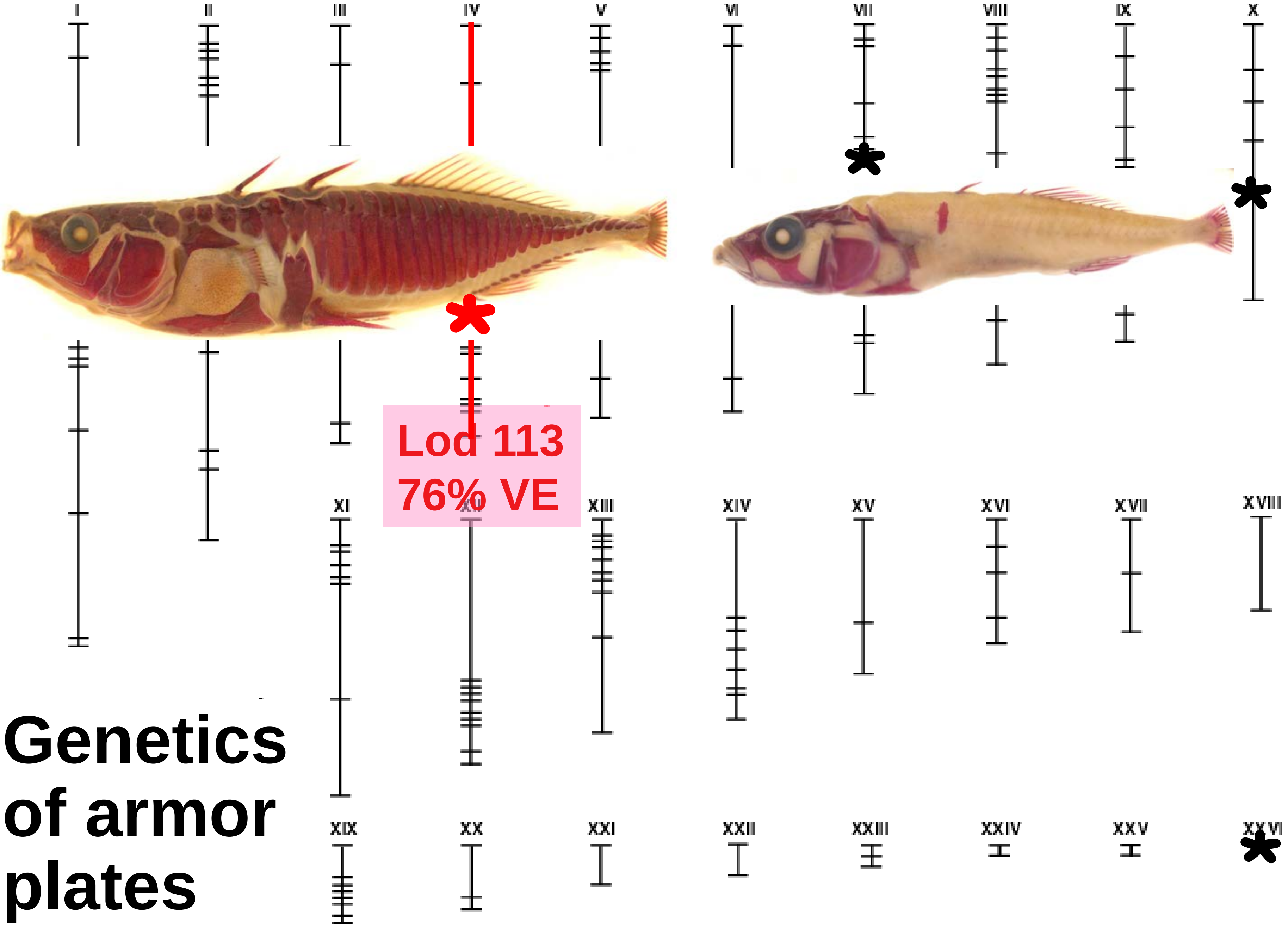
Other

Temperature preference
Salinity tolerance
Anti-predator behavior
Body size
Longevity



Cuvier and Valenciennes (1829)

Genetics of armor plates

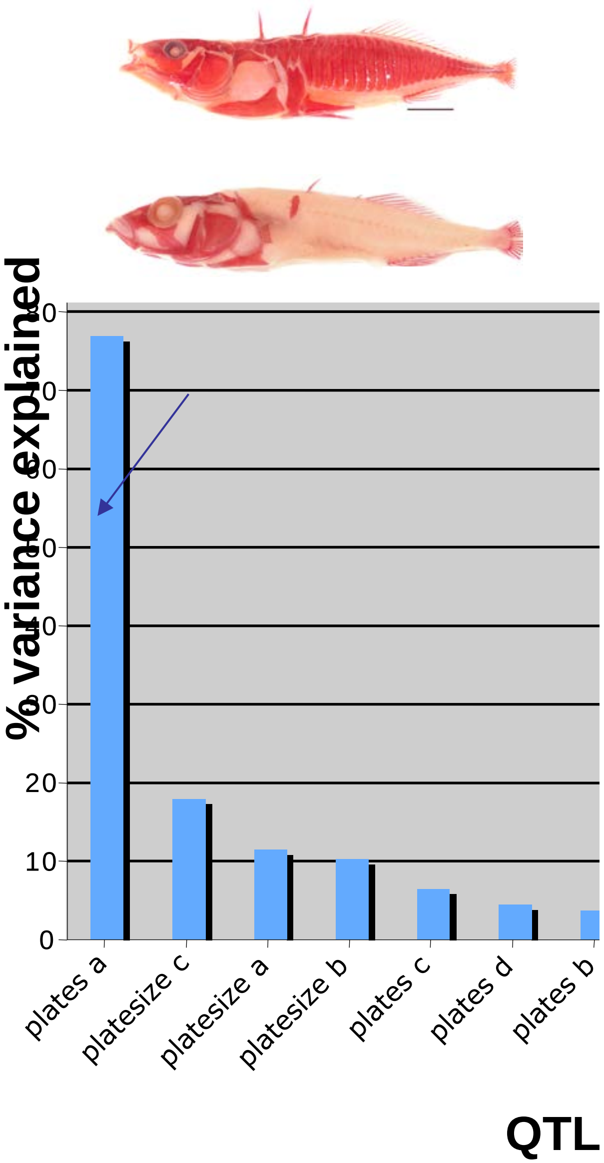


Lod 113
76% VE

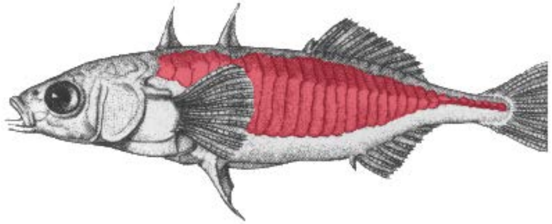
Colosimo et al. (2004) **PLoS Biol** 2: 635.

Evolutionary differences can be mapped to major loci

Armor plates

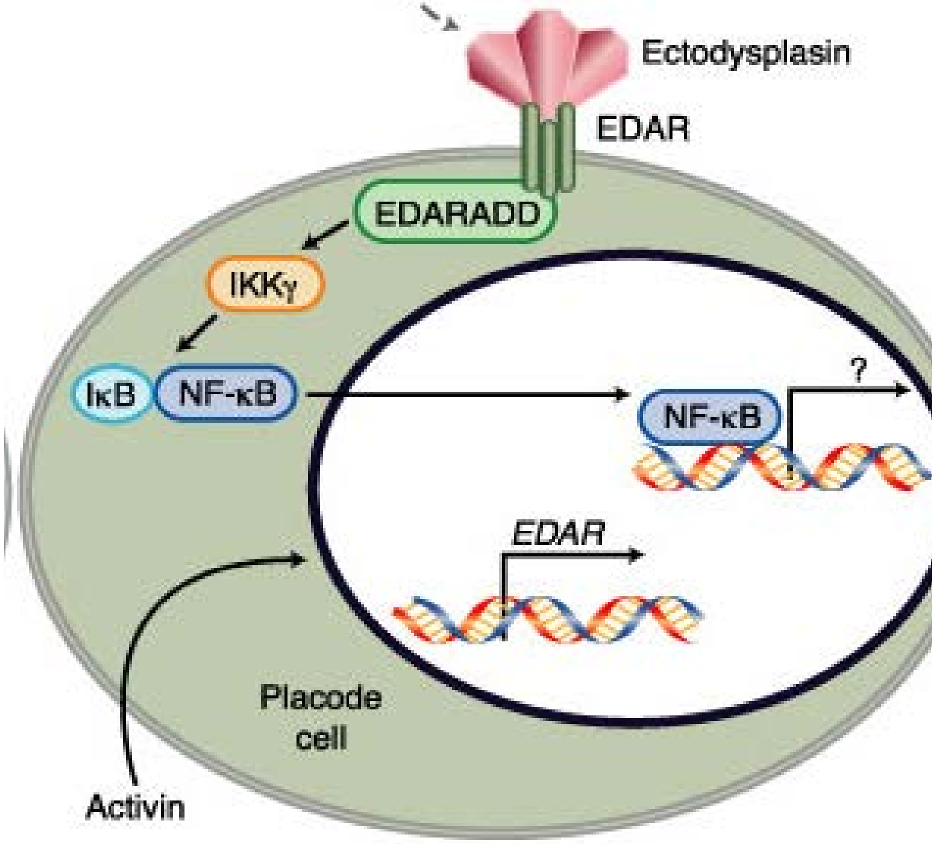


Colosimo, *PLoS Biology* (2004)



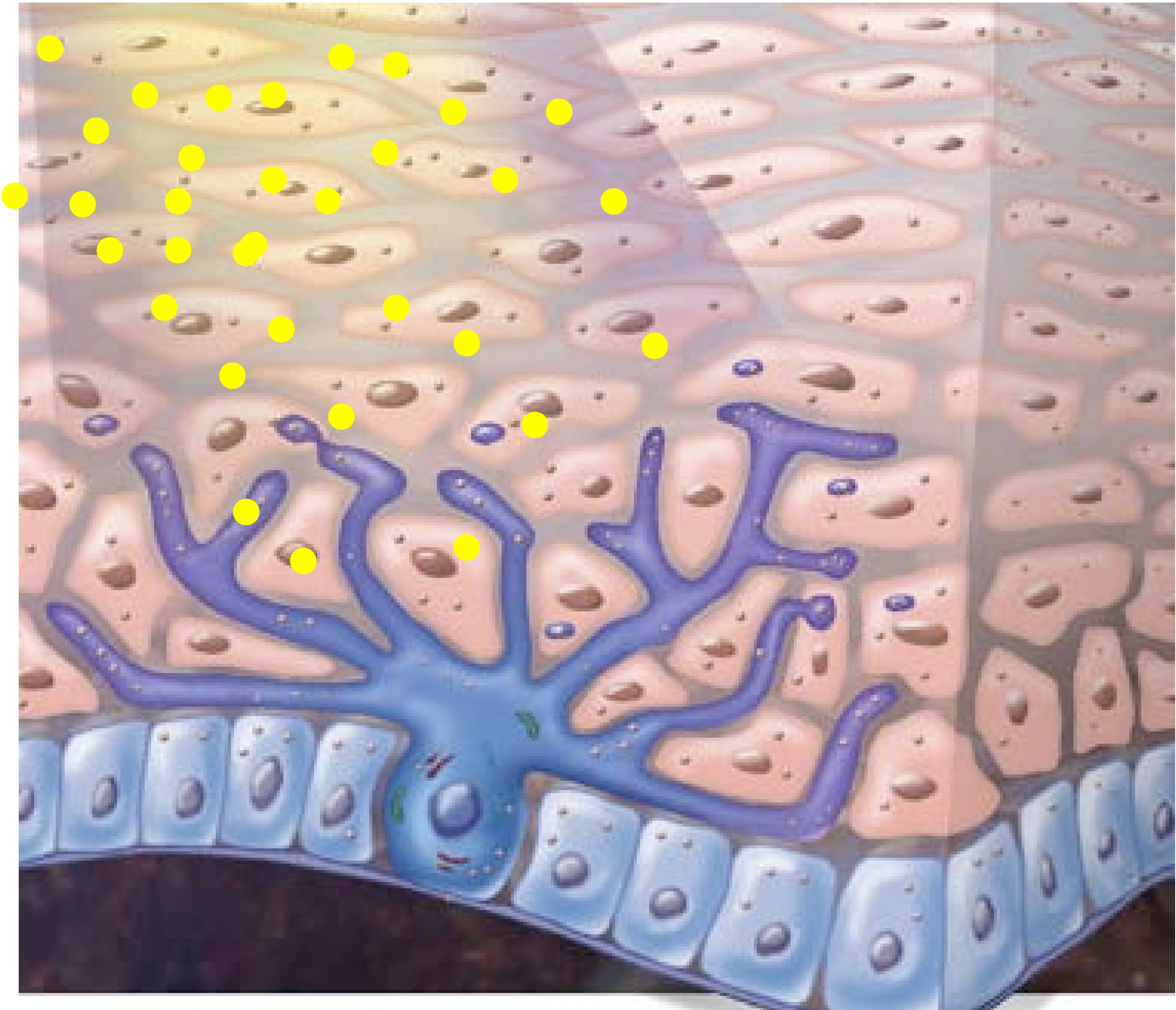
Ectodysplasin (EDA)

Secreted signal
in TNF family



KITLG

Stem Cell Factor
secreted signal



Receptors on:
pigment cells,
blood cells,
germ cells,
many others

Similar themes for all three traits and genes

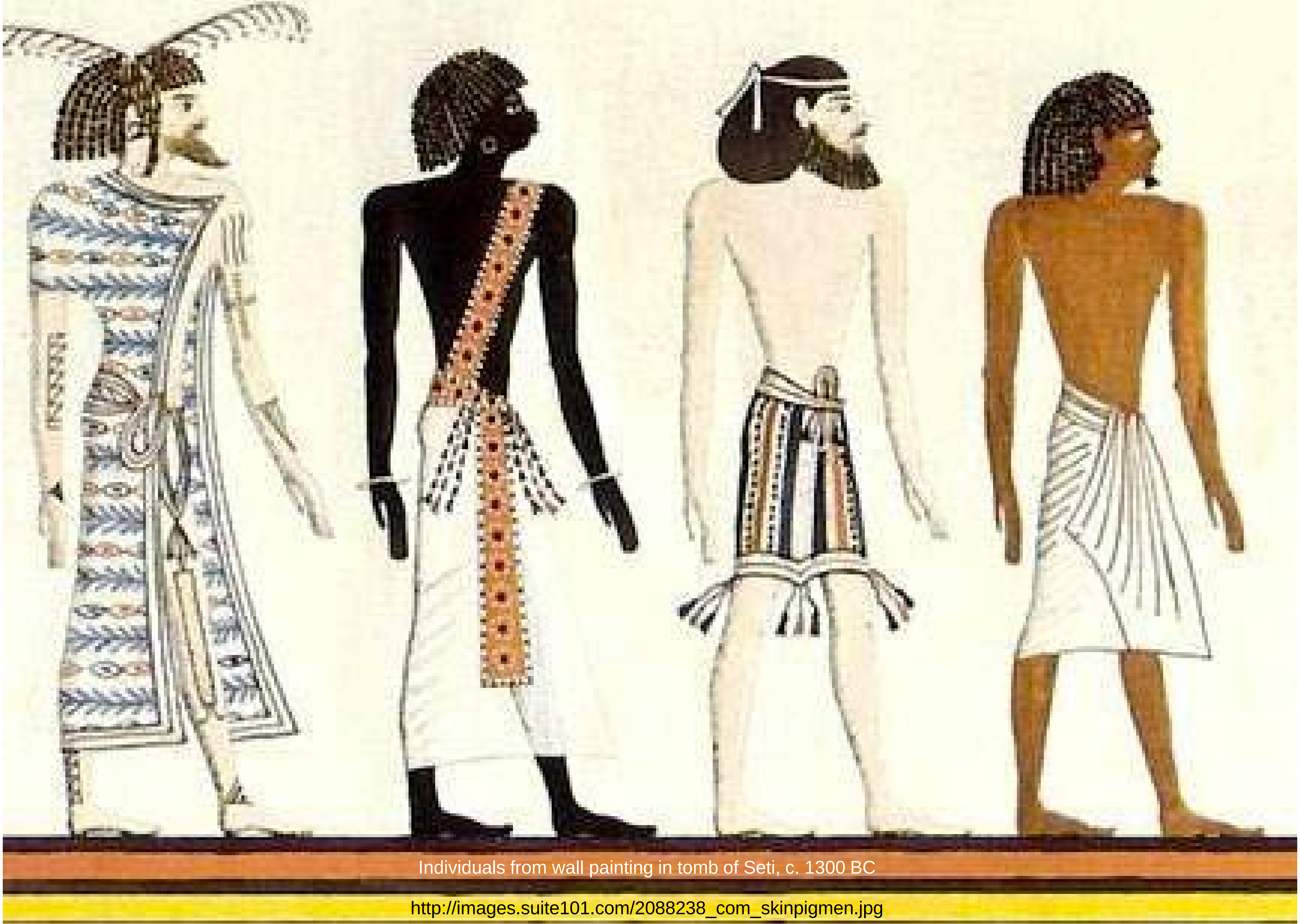
Pelvic reduction --> PITX1

Armor plate patterning --> EDA

Pigmentation --> KITLG

- Big phenotypic differences can map to small # loci
- Major regions = key developmental control genes
- Loss of function mutations deleterious, but regulatory mutations can be advantageous in nature
- Same genes used repeatedly when similar traits evolve in different populations

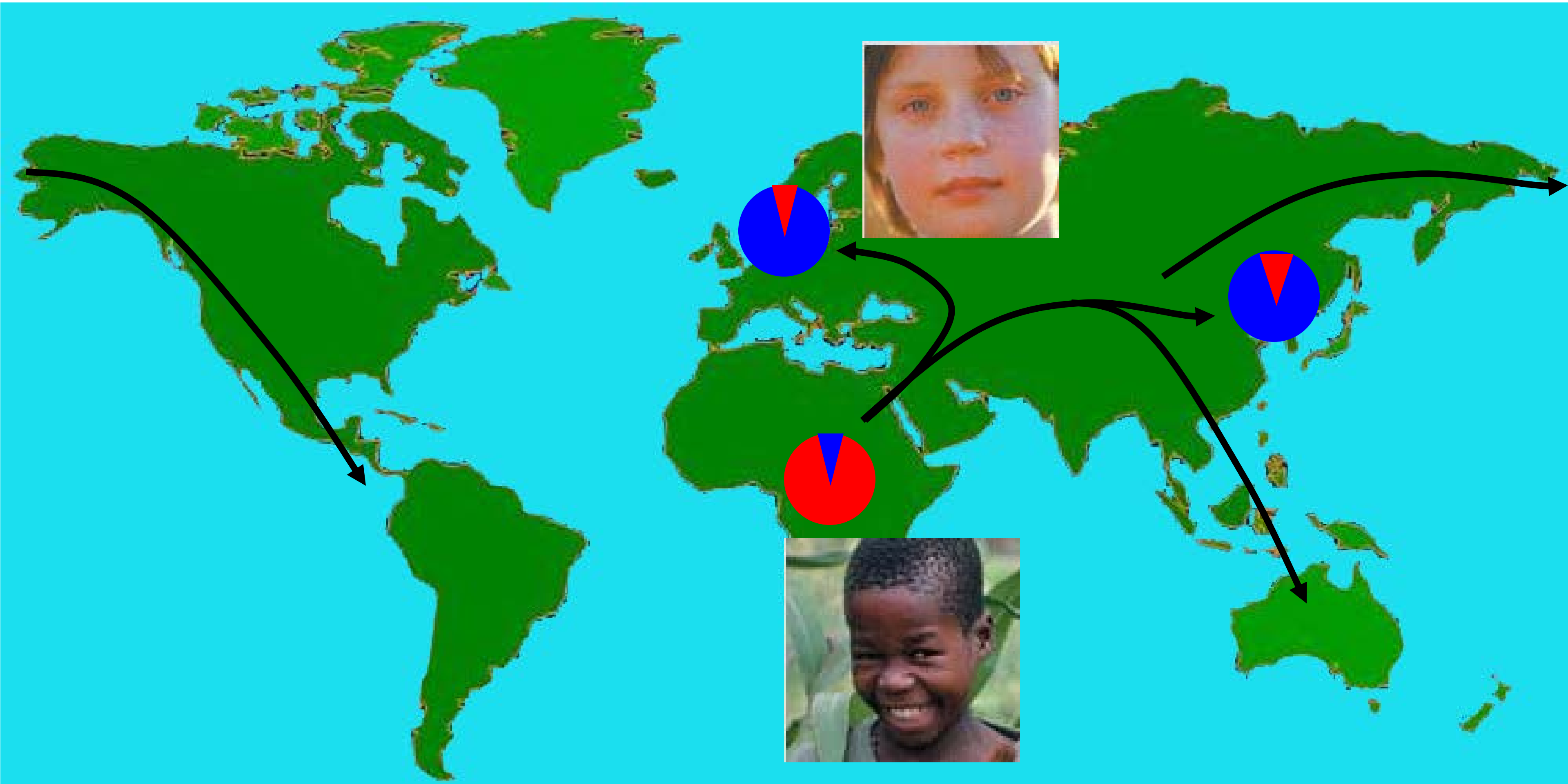
How far might reuse of evolutionary mechanisms extend?



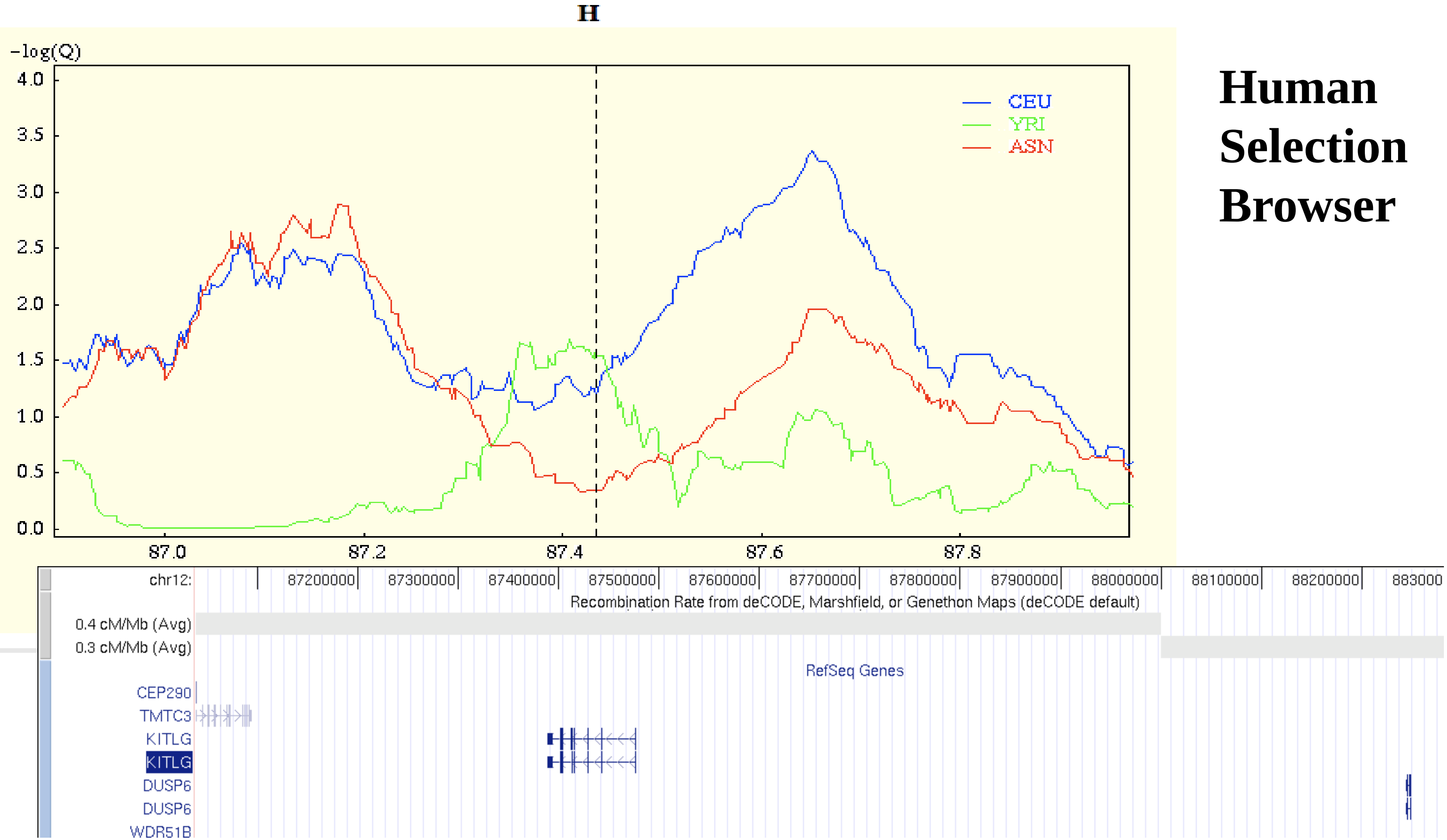
Individuals from wall painting in tomb of Seti, c. 1300 BC

http://images.suite101.com/2088238_com_skinpigmen.jpg

Same gene selected for pigment changes during both stickleback and human migrations



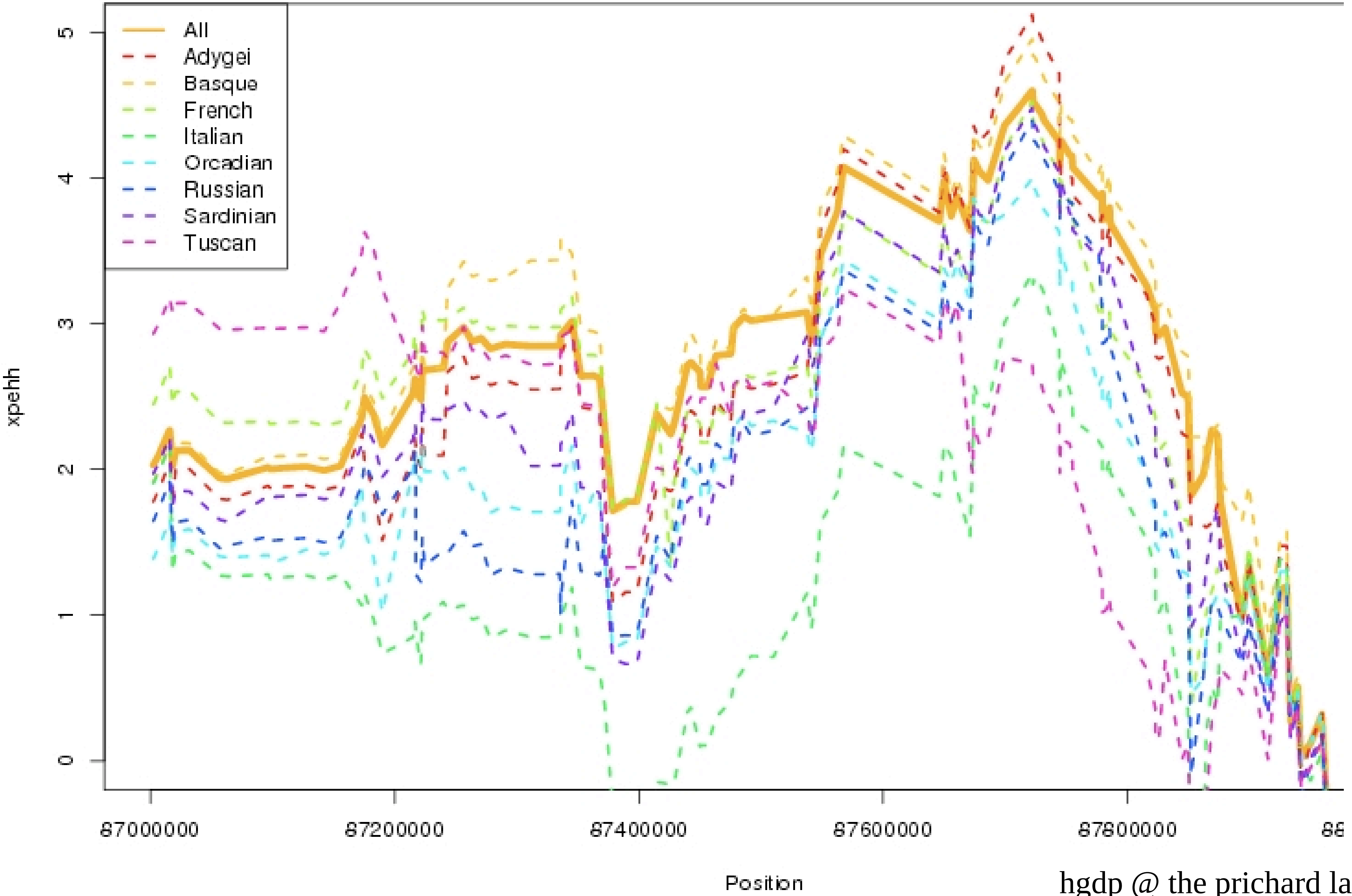
Miller et al. (2007) Cell



Human Selection Browser

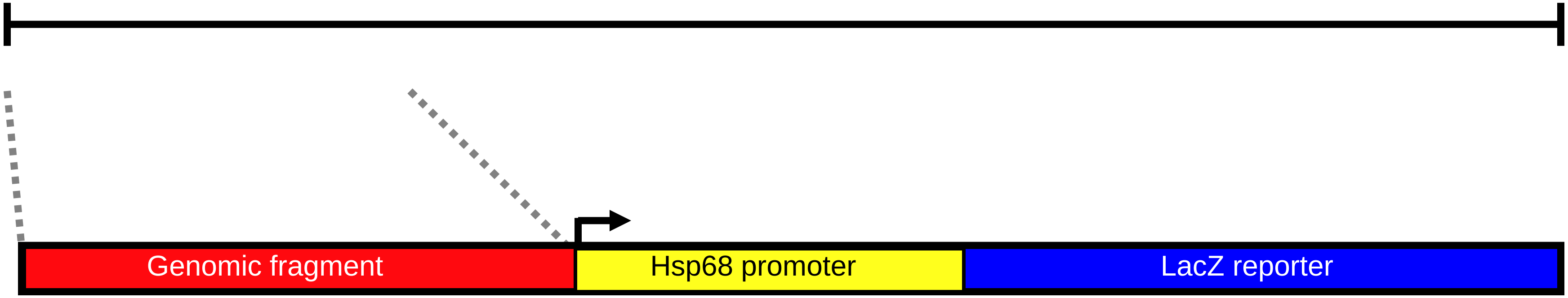
http://hg-wen.uchicago.edu/selection/haplotter.htm

Now searching for causative changes using molecular signatures of selection in humans



hgdp @ the prichard lab

Transient transgenic surveys for regulatory elements



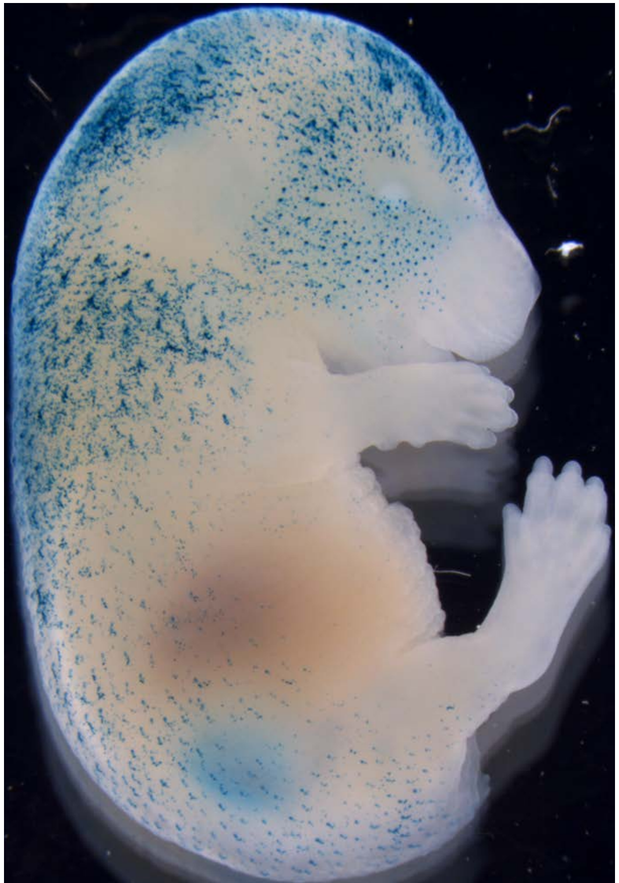
Genomic fragment

Hsp68 promoter

LacZ reporter

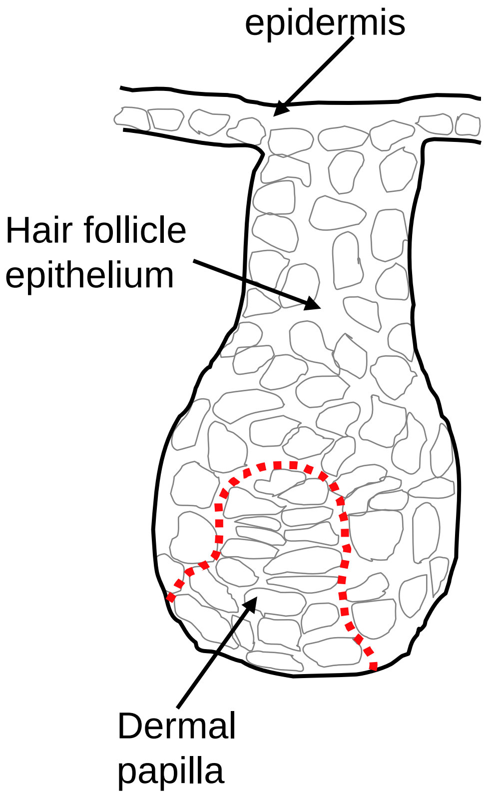
Microinjection of a reporter construct into newly fertilized mouse embryos

Look in transgenics for lacZ expression

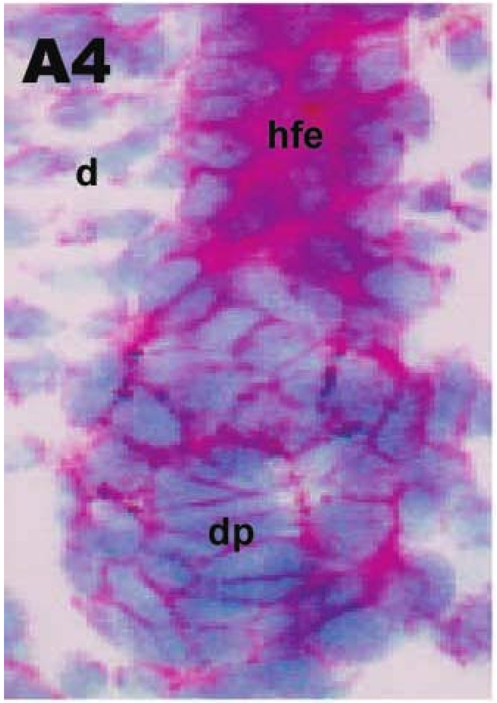


Enhancer mimics known KITLG expression in skin/hair

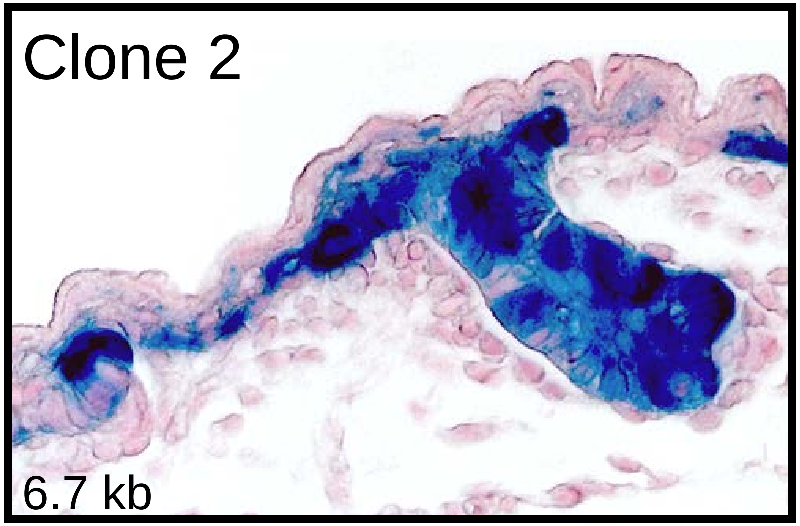
Schematic of developing hair follicle

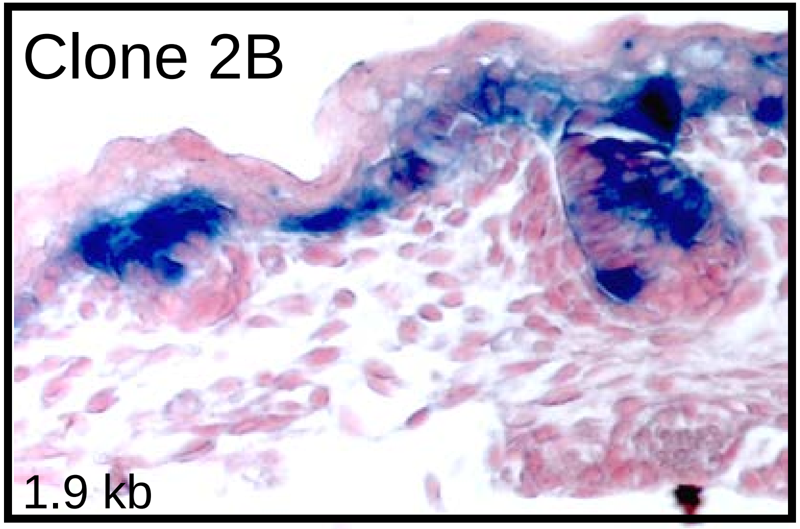


KITLG Ab

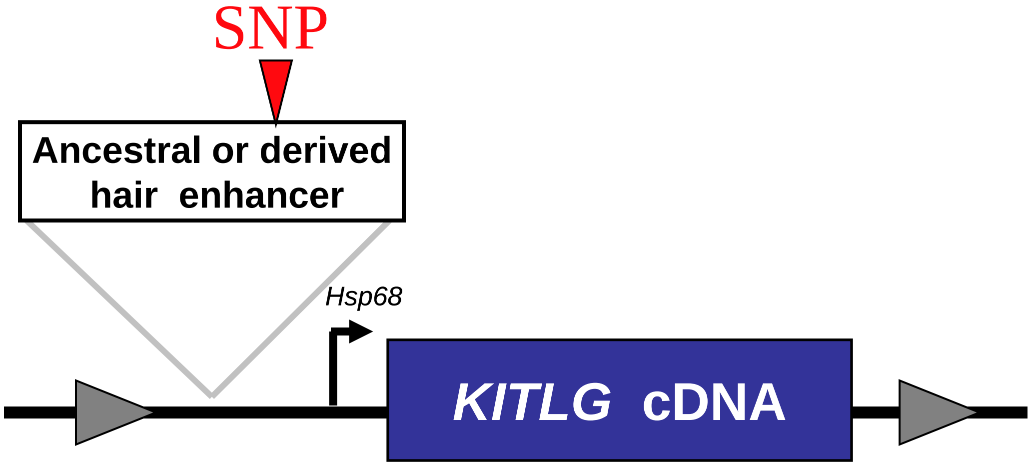


Peters et al., 2002

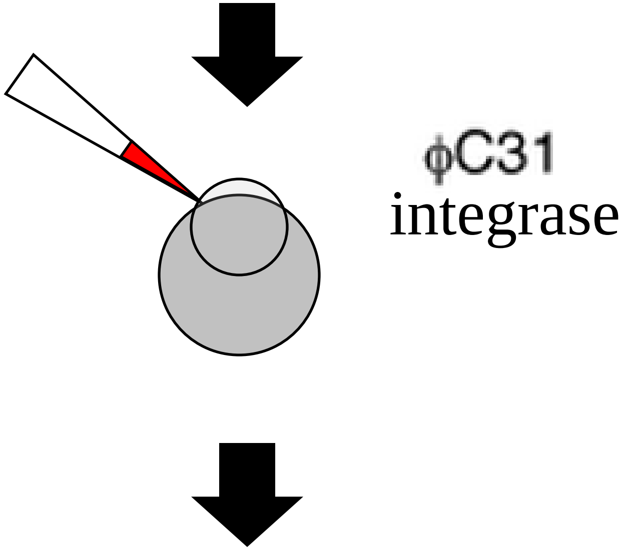




Testing effects of single human base pair differences in mice



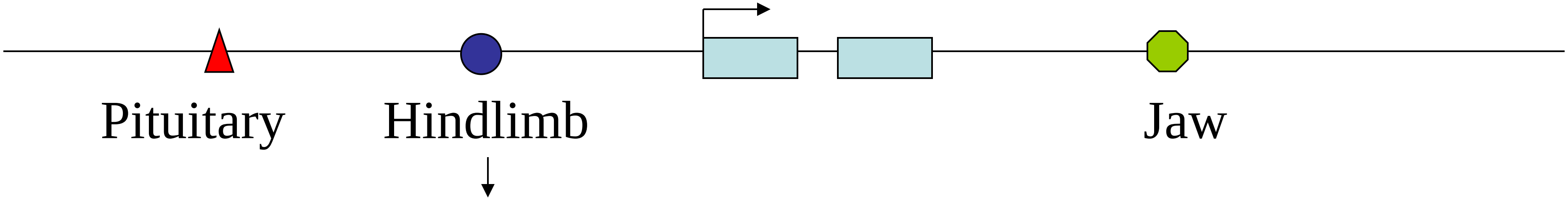
Recipient mice with *attP* recombination sites
at predefined Chr11 locus
(Tasic et al. (2011) <u>PNAS)</u>



Site-specific integrants, with matched copy number, position, and orientation in genome


Ancestral human SNP
Derived human SNP

PITX1

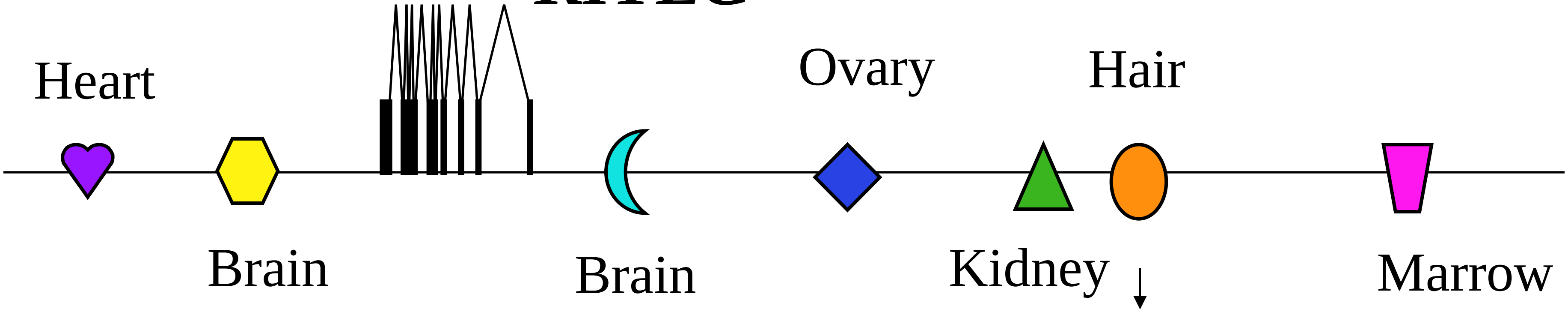


Pituitary

Hindlimb

Jaw

KITLG



Heart

Brain

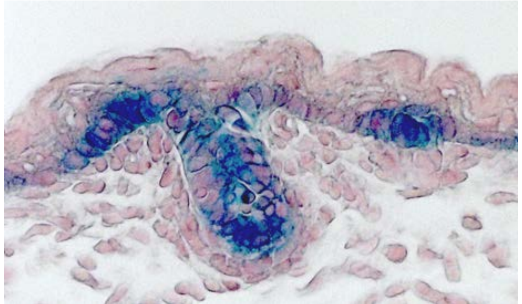
Brain

Ovary

Hair

Kidney

Marrow

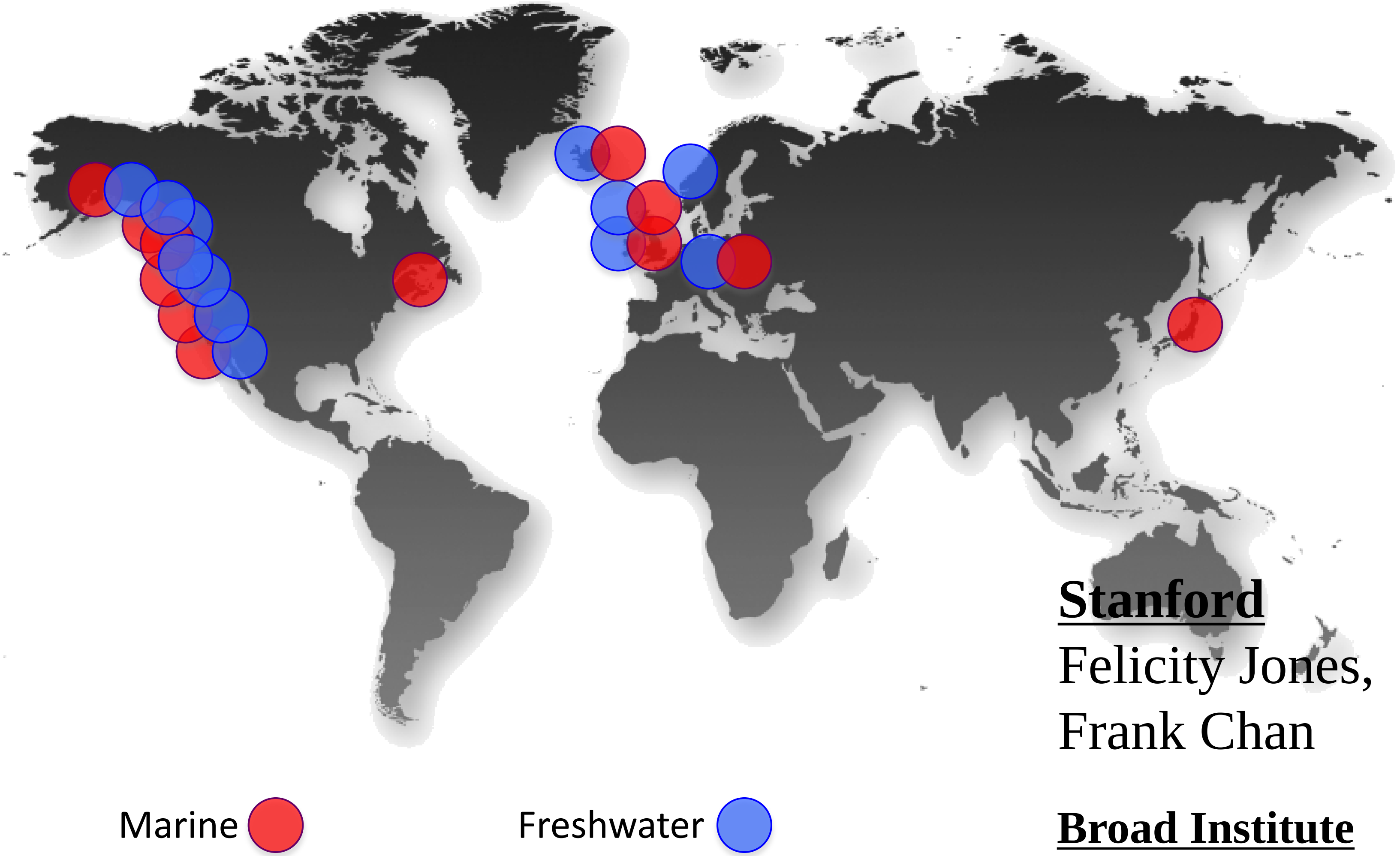


Case Histories

vs.

General Patterns

Using repeated evolution to find genome-wide set of adaptive loci



Stanford

Felicity Jones,
Frank Chan

Broad Institute

Kerstin Lindblad-Toh
Eric Lander

ARTICLE

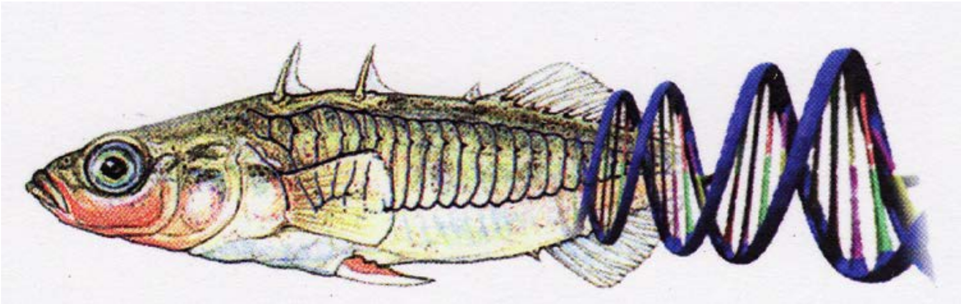
(2012) Nature 484:55-61

doi:10.1038/nature10944

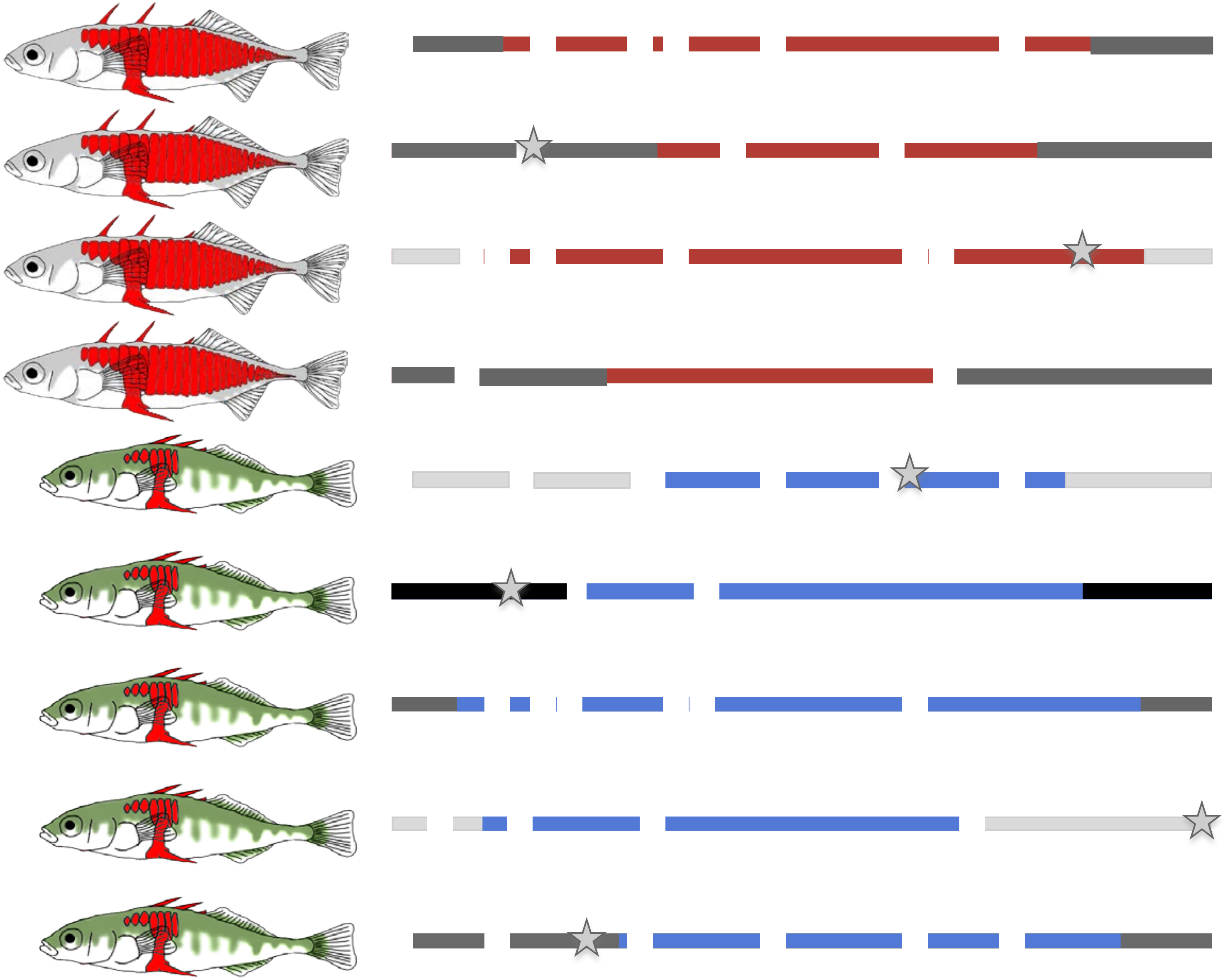
The genomic basis of adaptive evolution in threespine sticklebacks

Felicity C. Jones[1]*, Manfred G. Grabherr[2,3]*, Yingguang Frank Chan[1]†*, Pamela Russell[2]*, Evan Mauceli[2]†, Jeremy Johnson[2], Ross Swofford[2], Mono Pirun[2]†, Michael C. Zody[2], Simon White[4], Ewan Birney[5], Stephen Searle[4], Jeremy Schmutz[6], Jane Grimwood[6], Mark C. Dickson[6], Richard M. Myers[6], Craig T. Miller[1]†, Brian R. Summers[1], Anne K. Knecht[1], Shannon D. Brady[1], Haili Zhang[1], Alex A. Pollen[1], Timothy Howes[1], Chris Amemiya[7], Broad Institute Genome Sequencing Platform & Whole Genome Assembly Team‡, Eric S. Lander[2], Federica Di Palma[2], Kerstin Lindblad-Toh[2,3] & David M. Kingsley[1,8]

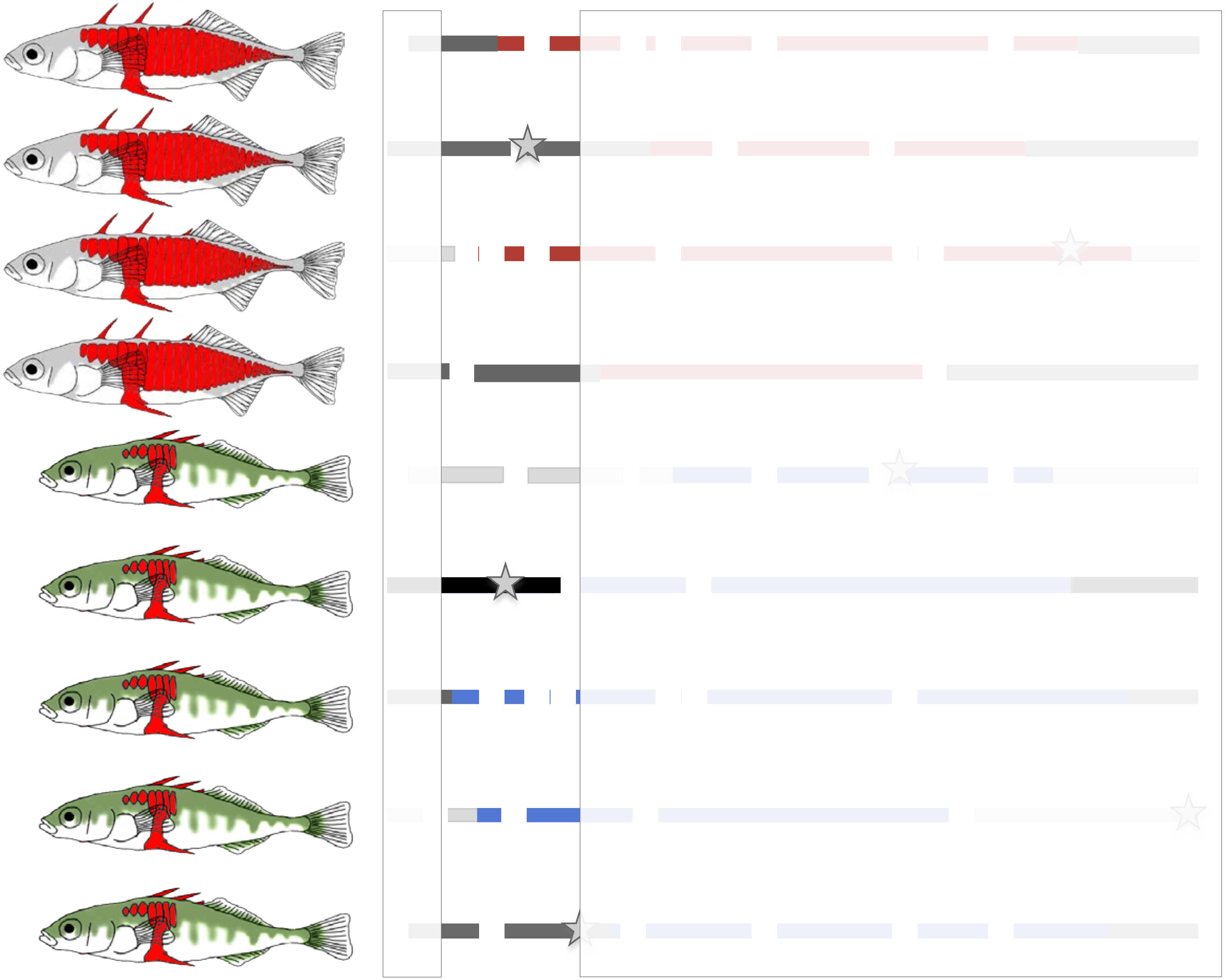
http://sticklebrowser.stanford.edu



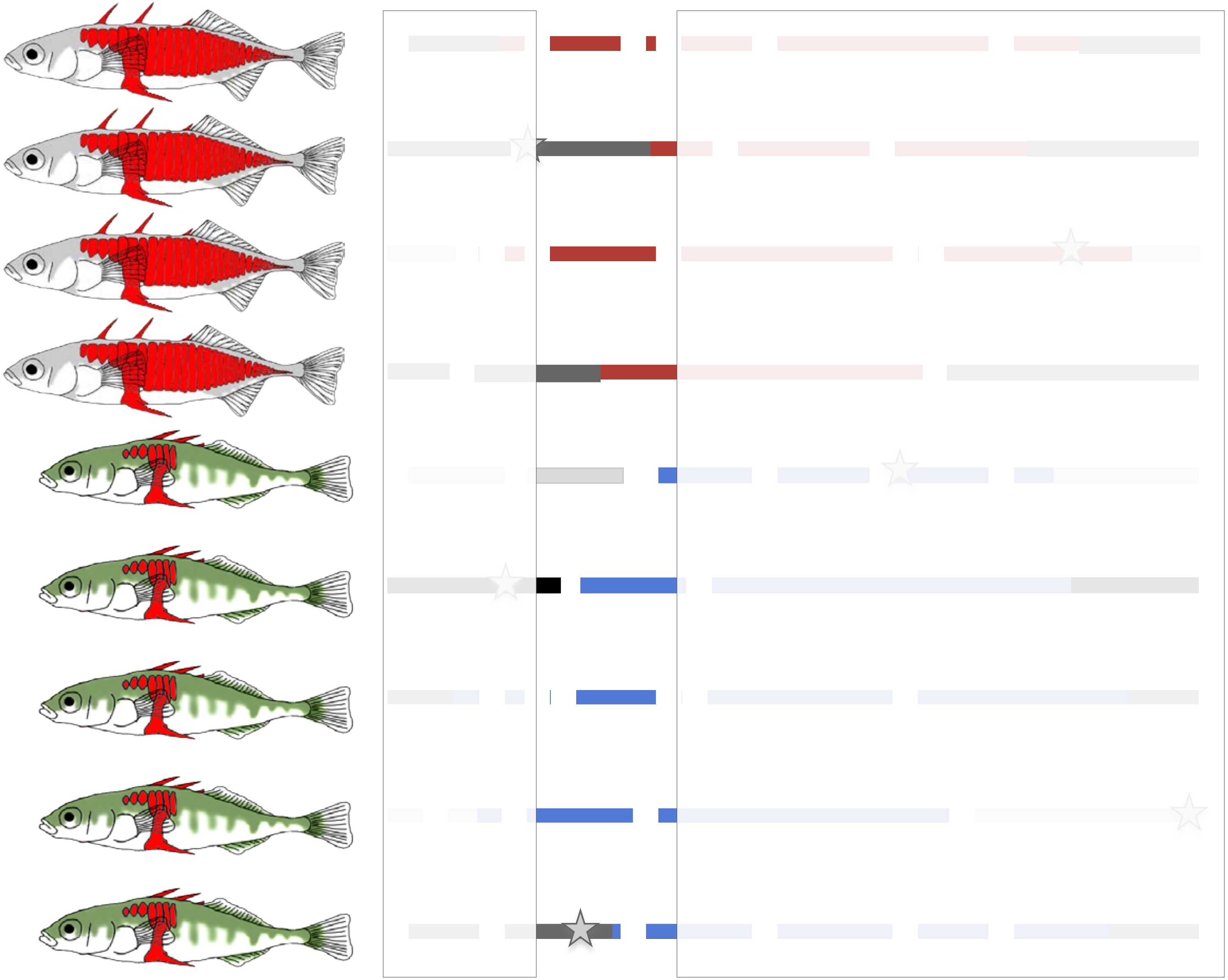
Comparing 21 genomes to find signatures of Parallel Evolution



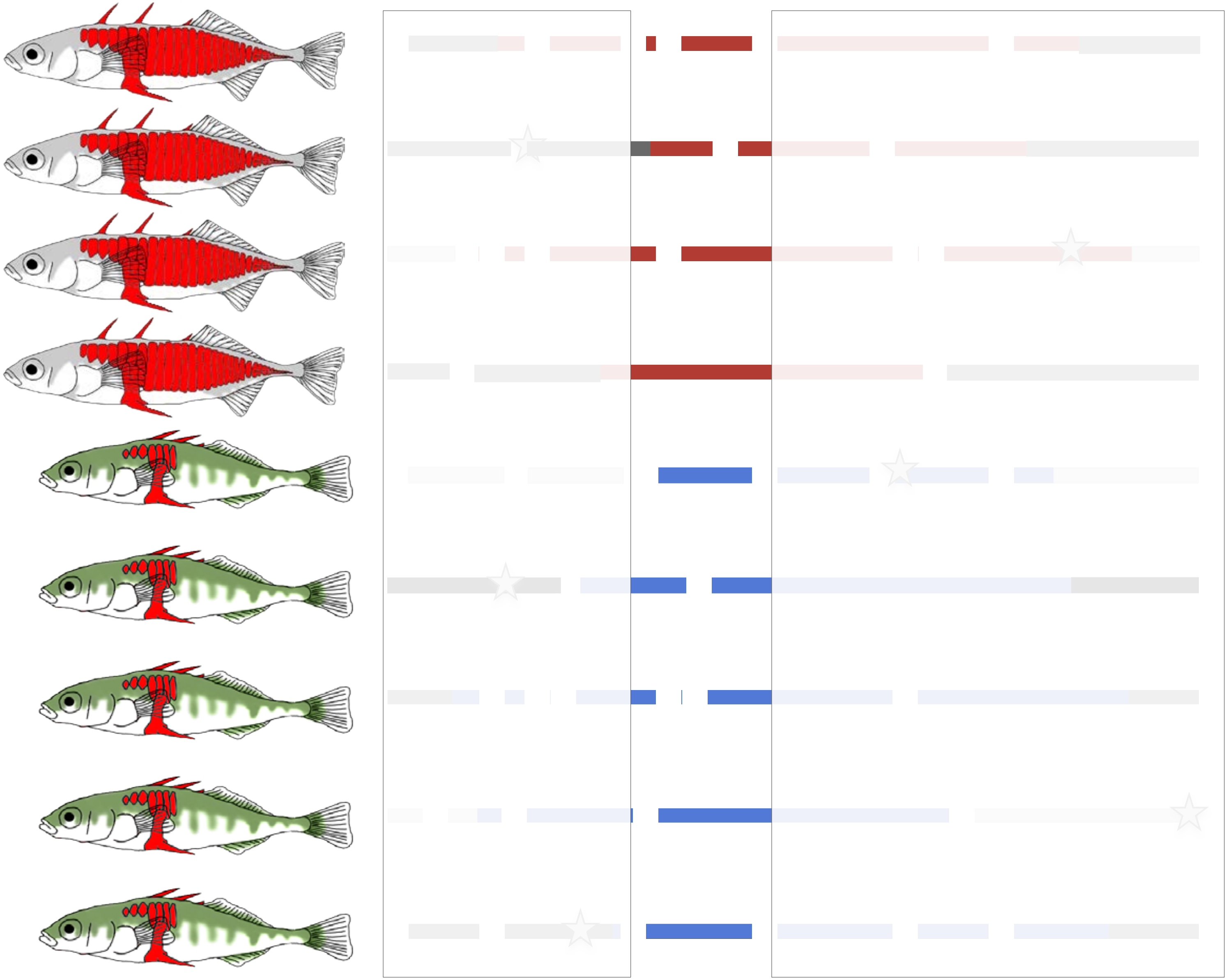
Comparing 21 genomes to find signatures of Parallel Evolution



Comparing 21 genomes to find signatures of Parallel Evolution

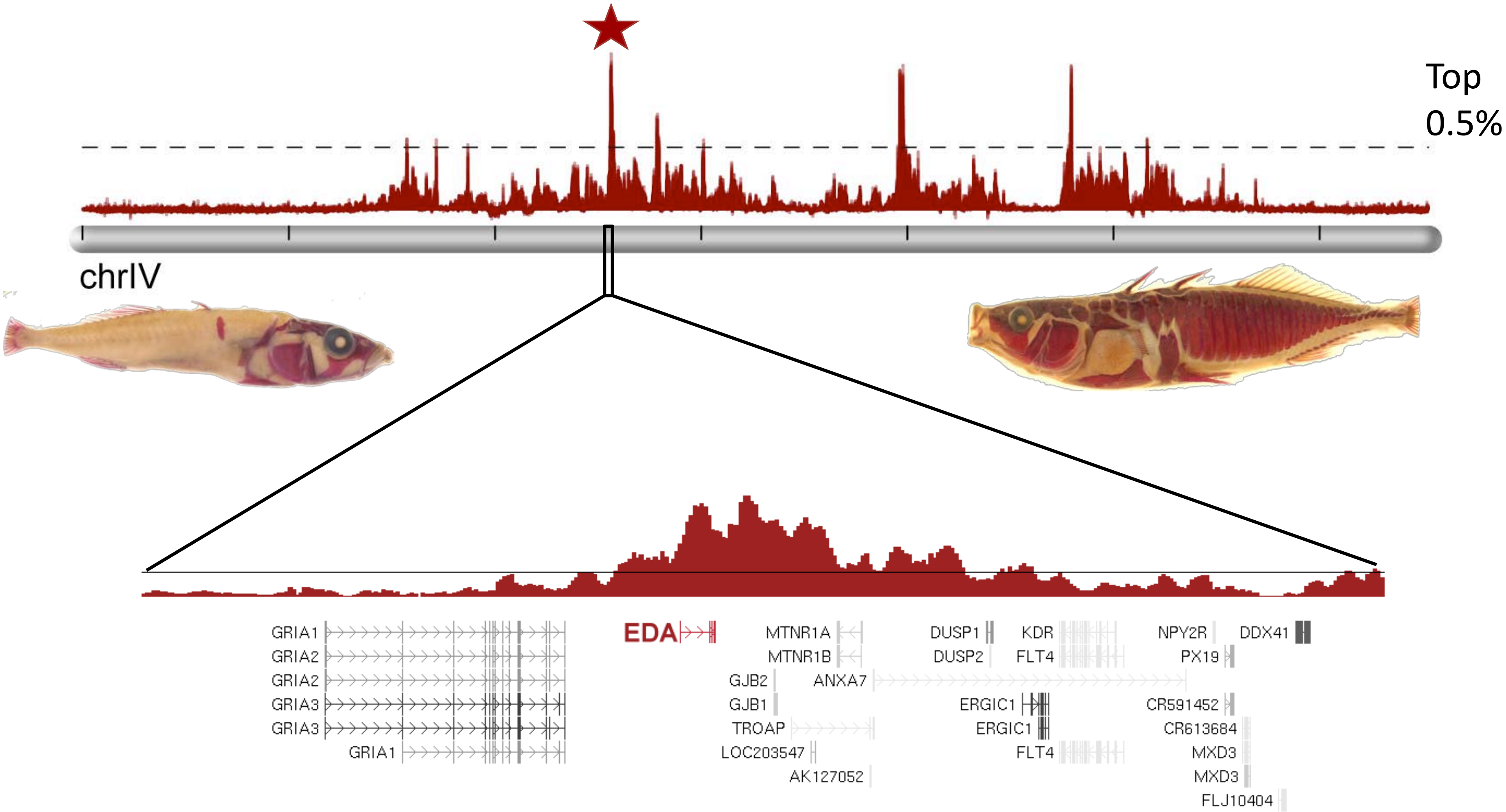


Comparing 21 genomes to find signatures of Parallel Evolution



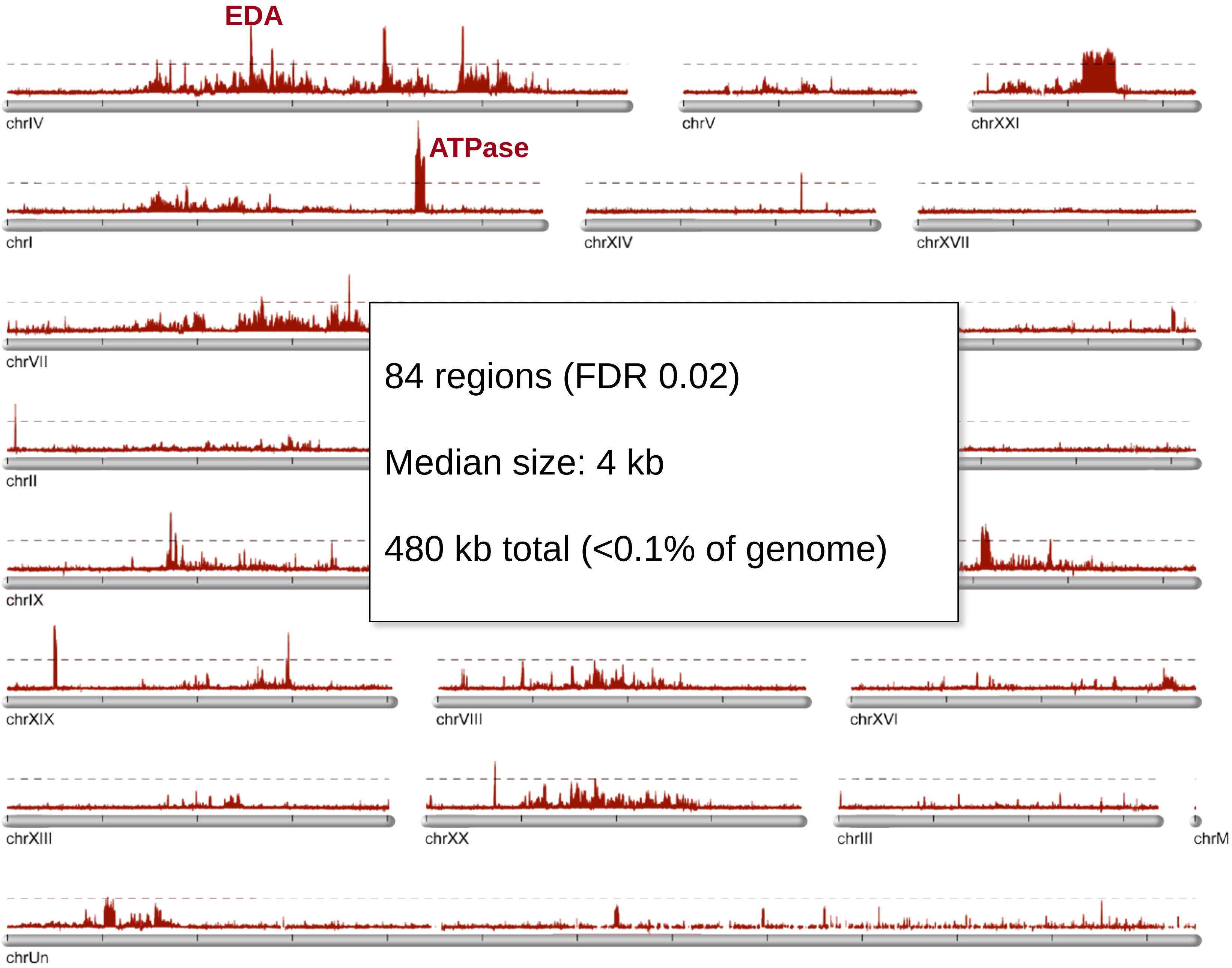
Whole genome sequence analysis rediscovers the EDA locus

EDA



Jones et al. (2012) *Nature*

A genome-wide set of regions contributing to repeated evolution



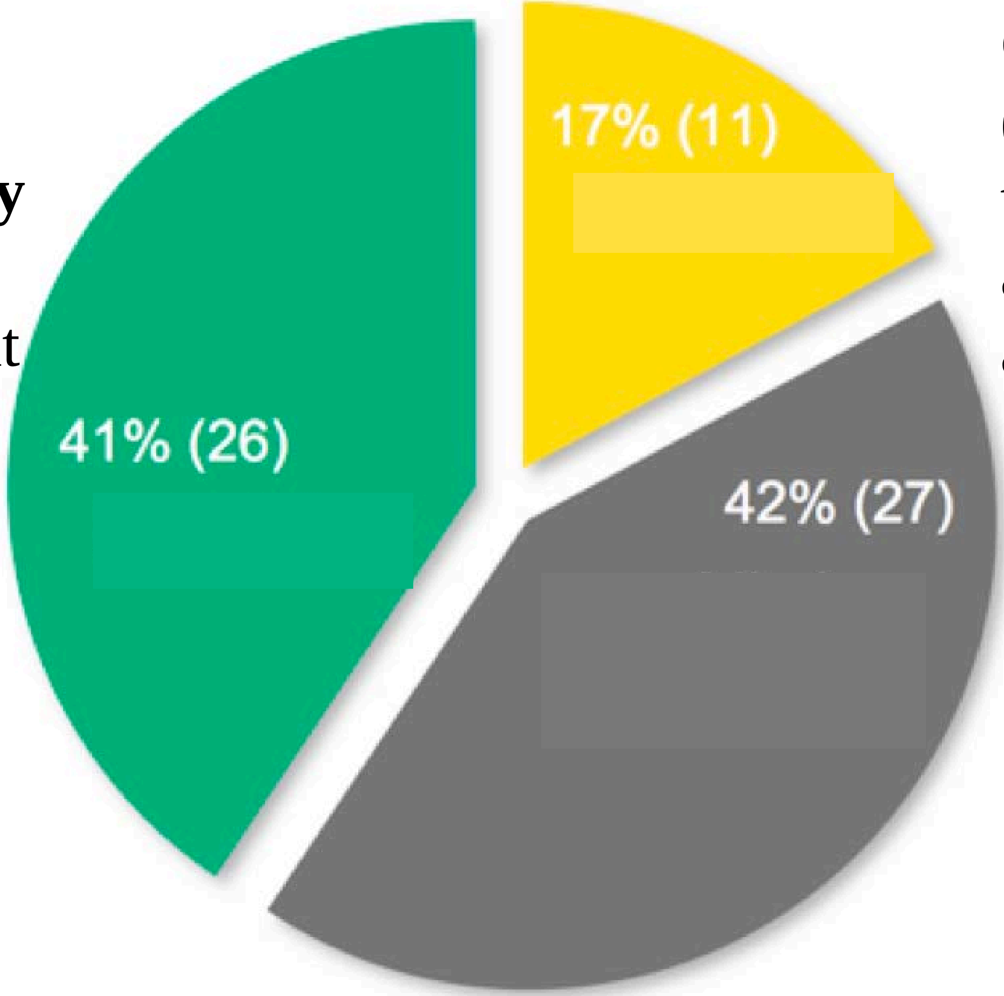
84 regions (FDR 0.02)

Median size: 4 kb

480 kb total (<0.1% of genome)

Jones et al. (2012) Nature

Does evolution occur primarily by coding or regulatory changes?



Regulatory
(Mar-FW regions that map entirely outside protein coding exons)

Coding
(Mar-FW regions that contain exons, and show consistent amino acid changes)

Likely Regulatory
(Mar-FW regions that contain both coding & noncoding regions, but only have changes in non-coding regions)

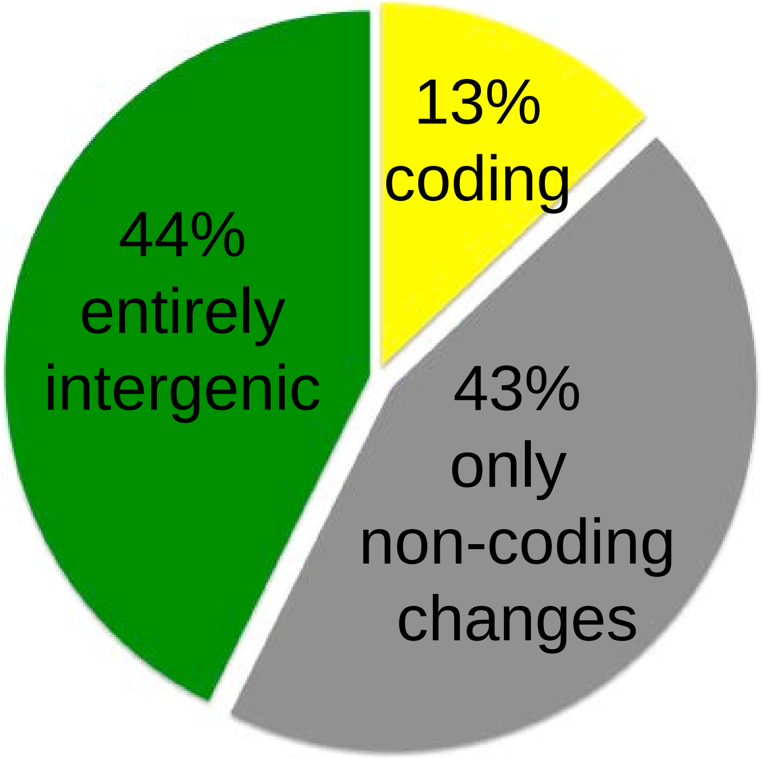
Jones et al. *Nature* (2012)

Recent surveys also identifying loci with strong molecular signatures of positive selection in human genome

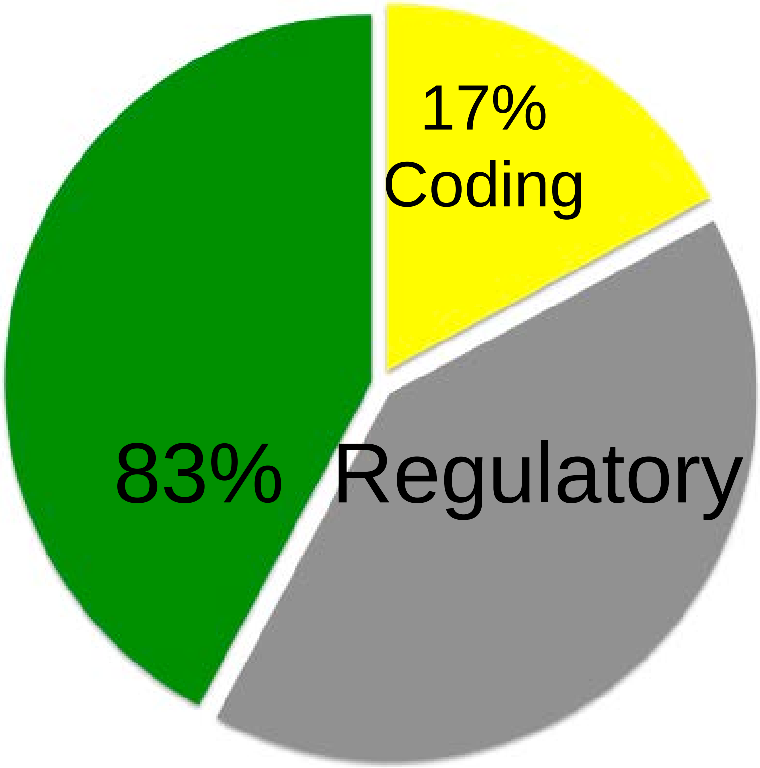
178 loci defined using patterns of:

- high Fst
- reduced polymorphism
- extended haplotypes
- increased frequency of derived allel

Grossman et al. Science (2010)

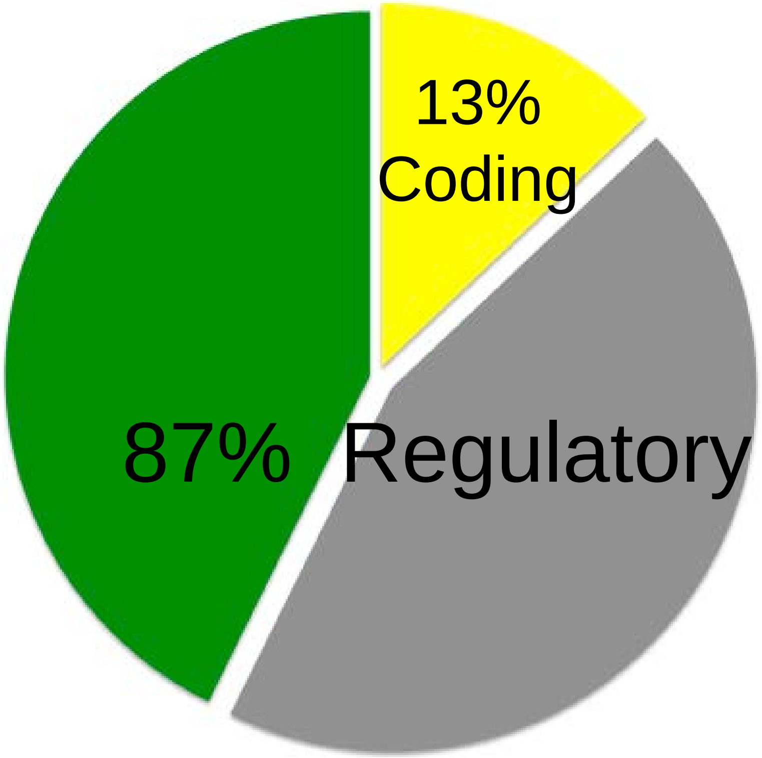


Two genome-wide sets of adaptive loci



Sticklebacks

Marine –> Freshwater
transition



Humans

Selection during establishment
of modern populations

Summary:

- Evolutionary differences are mappable
- Major differences can come from relatively few changes
- Similar mechanisms used repeatedly
- Regulatory changes the predominant basis for adaptive evolution in both fish and humans

Stanford

Frank Chan
Mike Shapiro
Melissa Marks
Craig Miller
Kate Guenther
Felicity Jones

Haili Zhang
Alex Pollen
Tim Howes
Vahan Indjeian
Brian Summers
Kathy Xie

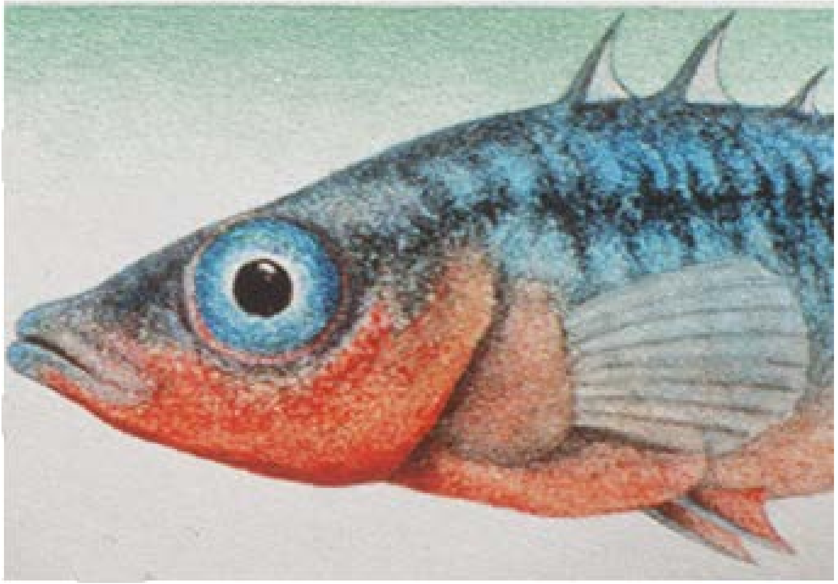
Dmitri Petrov
James Chai

Hudson Alpha

Rick Myers
Jeremy Schmutz
Jane Grimwood
Devin Absher

Fred Hutch

Katie Peichel
James Urton
Shaun McCann



Broad Institute

Manfred Grabbher, Pamela Russell, Evan Mauceli, Jeremy Johnson, Ross Swofford, Mono Pirun, Michael Zody, Federica DiPalma, Eric Lander, Kerstin Lindblad-Toh

Ensembl

Simon White
Ewan Birney
Steve Searle (Sanger)

BACs

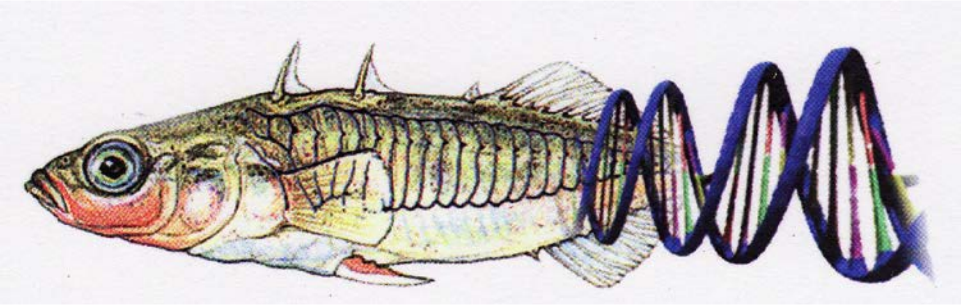
Chris Amemiya (Virginia Mason)
Pieter DeJong (CHORI)
Marco Marra (UBC Vancouver)

Stickleback collaborators

Dolph Schluter (UBC, Vancouver)
Mike Bell (NYU, Stony Brook)
Tom Reimchen (UBC, Victoria)
Bjarni Jonson (Iceland)

Human pigmentation studies

Mark Shriver (PennState)
Sandra Beleza
Rick Kittles
Liqun Luo, Bosiljka Tasic



NHGRI CEGS Funding