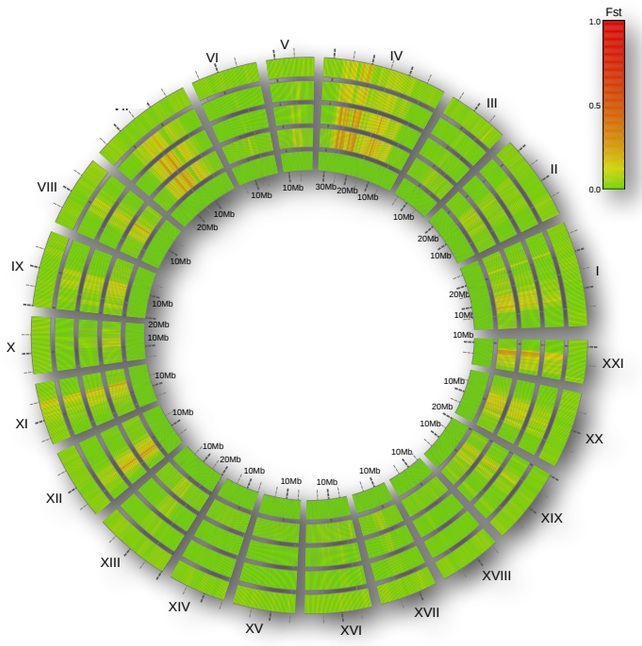


Ecological & evolutionary genomic analyses using RADseq

2017 Workshop on Genomics
Český Krumlov

Bill Cresko
Institute of Ecology and Evolution
Department of Biology
University of Oregon

UNIVERSITY OF OREGON

Outline for today's lecture

RADseq for ecological and evolutionary genomics

Primer on Population Genomics

Evolutionary genomics of stickleback fish

- Population genomics of rapid adaptation
- Using long read RAD-seq for coalescent analyses
- Genome Wide Association Studies using RAD-seq

Genomically enabling the Gulf pipefish

RAD-seq experimental and statistical considerations

Stacks software pipeline (this afternoon & evening)

January 2016 Issue

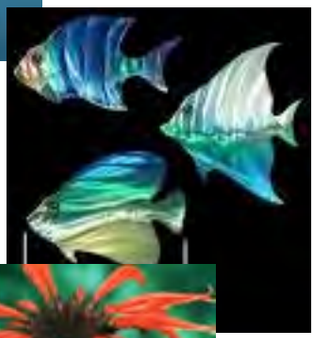
STUDY DESIGNS

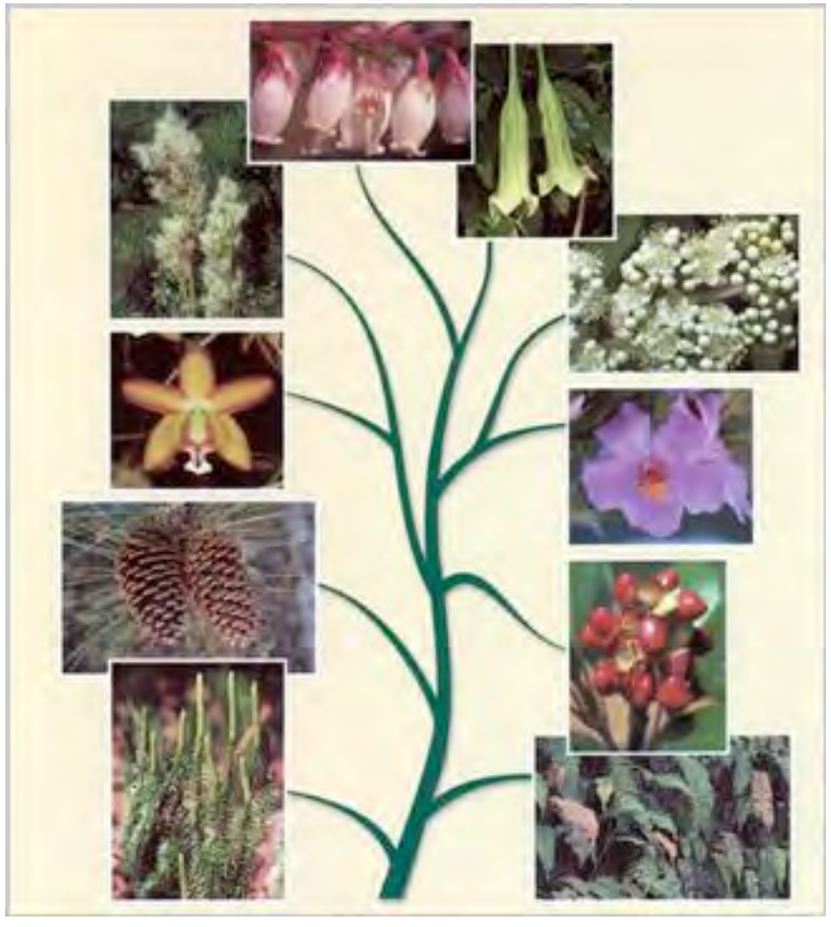
Harnessing the power of RADseq for ecological and evolutionary genomics

Kimberly R. Andrews[1], Jeffrey M. Good[2], Michael R. Miller[3], Gordon Luikart[4] and Paul A. Hohenlohe[5]

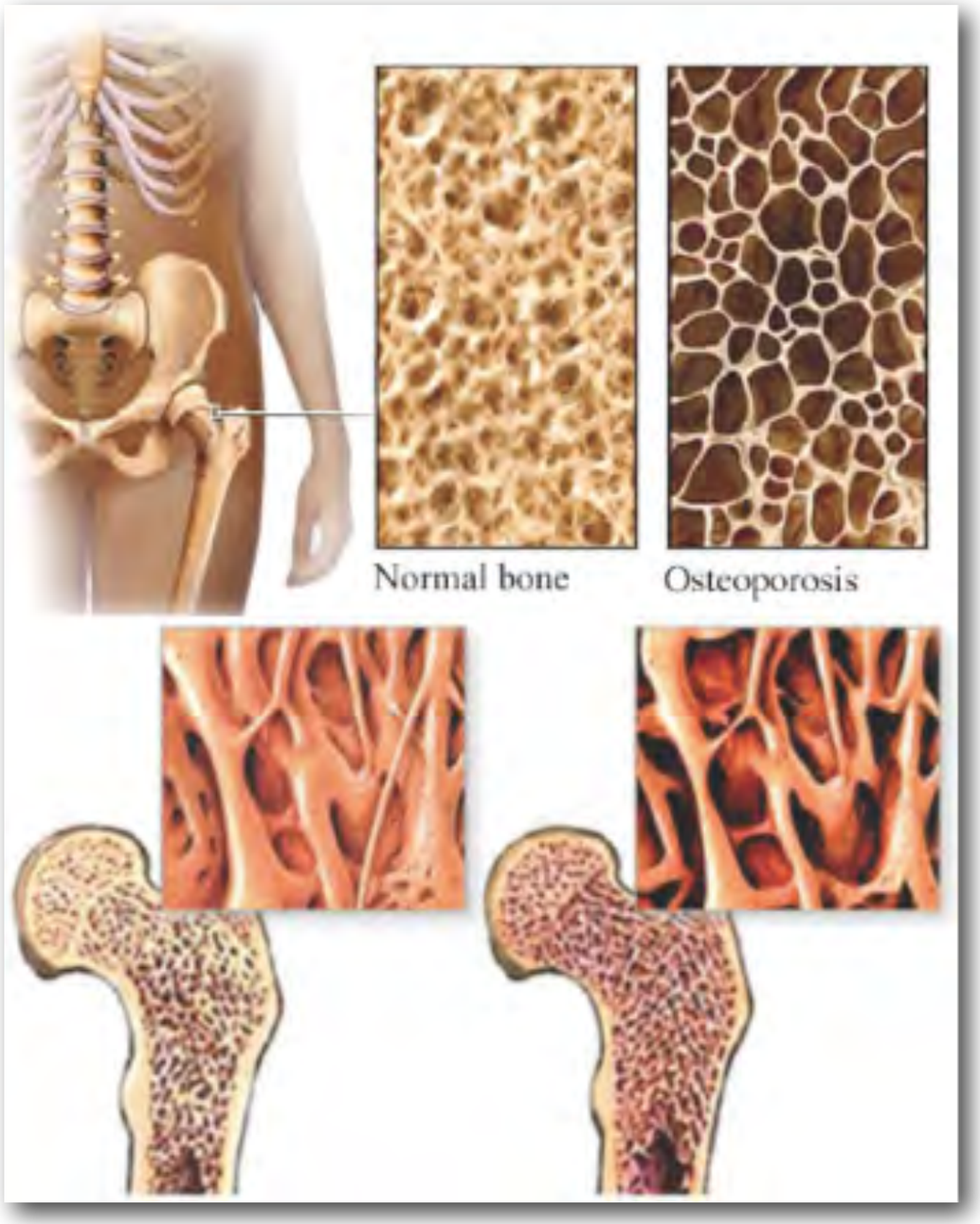
nature REVIEWS GENETICS

Why do species look the way that they do?

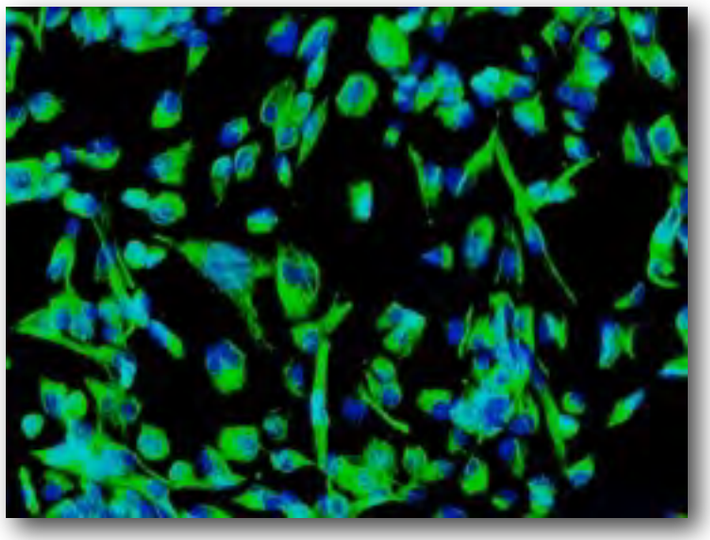


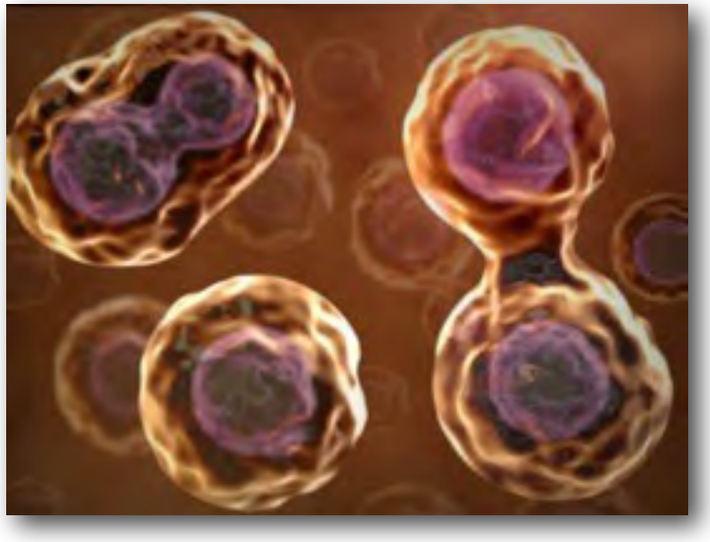


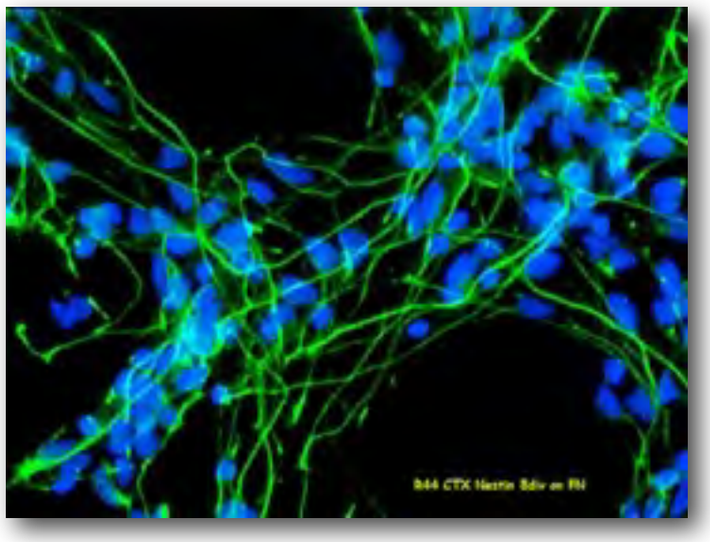
Why do organisms vary?

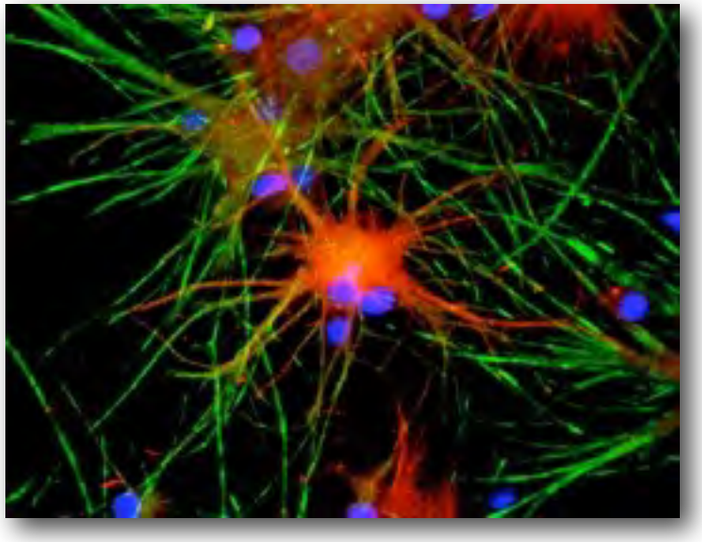


How is cellular functional diversity created?

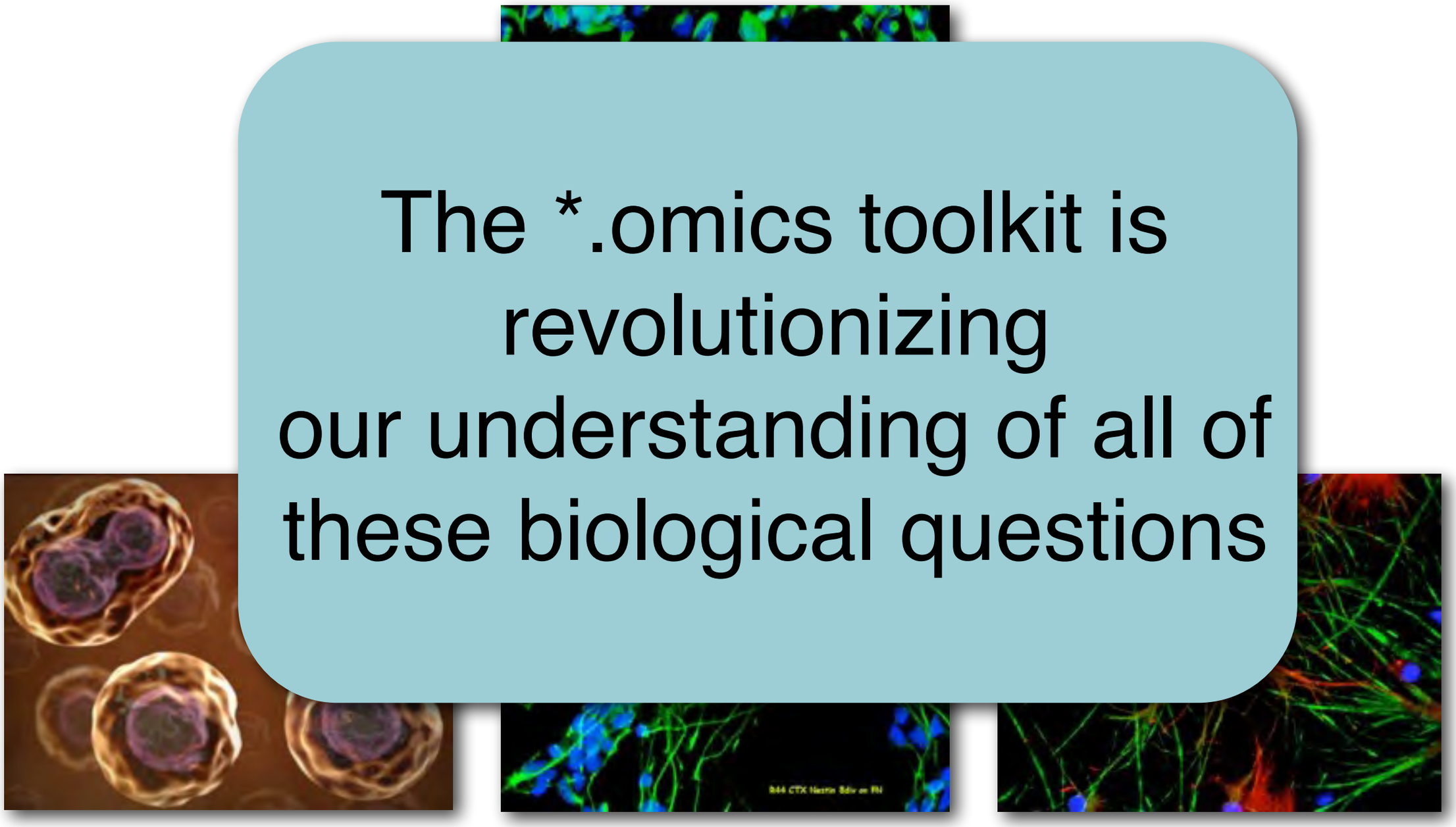








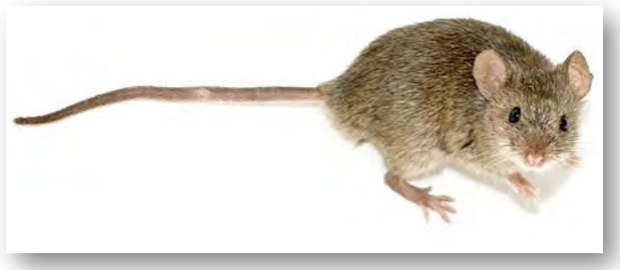
How is cellular functional diversity created?



The *.omics toolkit is revolutionizing our understanding of all of these biological questions

(1998)

(2000)



(2002)

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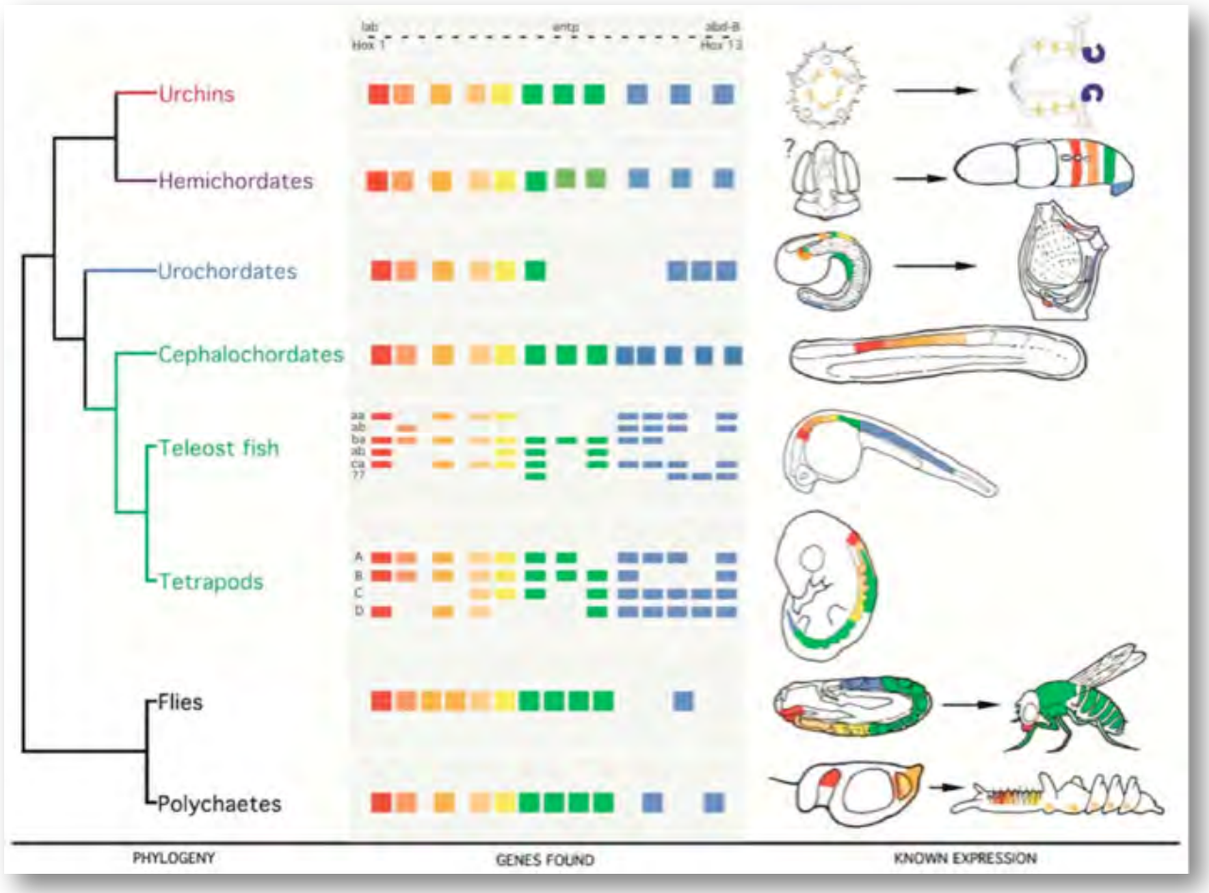
(2006)



Comparative Genomics

Vertebrate **zygotes** or embryos

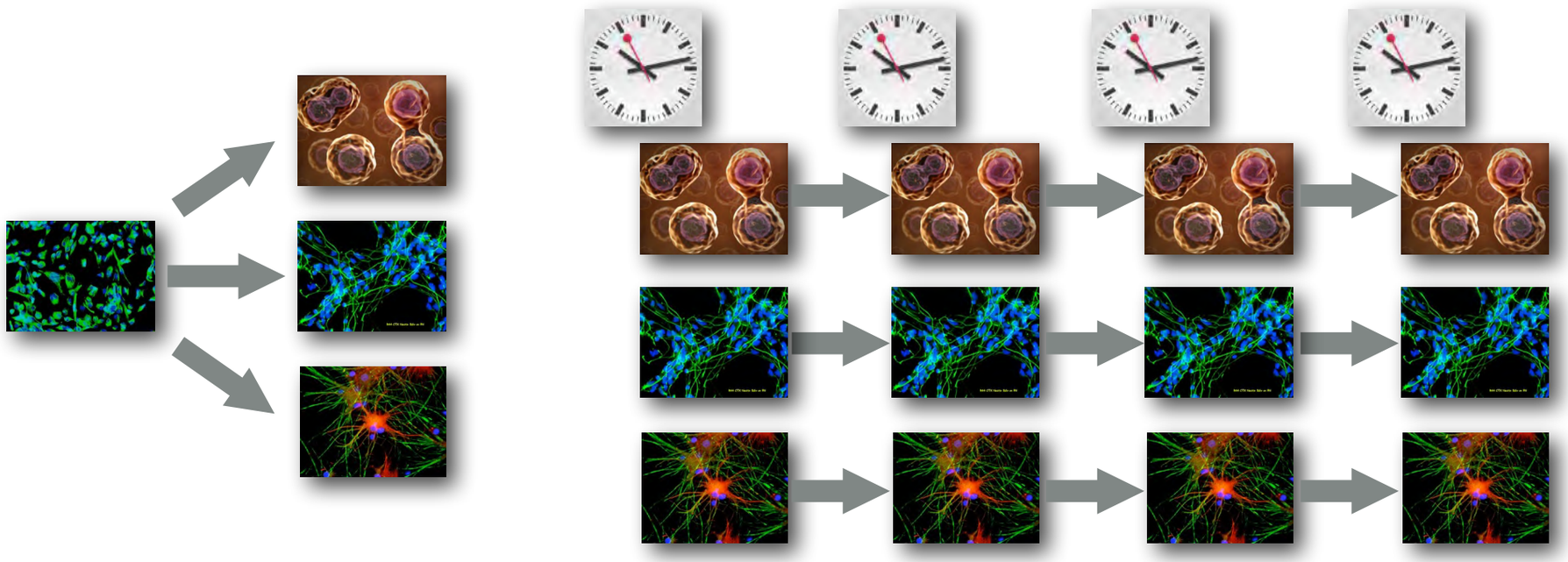
28 day human

19h zebrafish

Vertebrate **zygotes** or embryos

28 day human

19h zebrafish

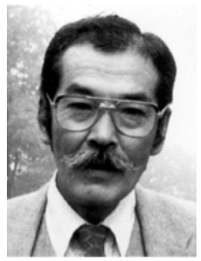


Functional Genomics











Population Genomics

How do we ‘genomically enable’ research studies of non-model (and model) organisms?

1. Genetic markers & genetic maps
2. Physical maps (genomes)
3. Transcriptomes
4. Gene expression analyses
5. Epigenetic & functional analyses

Assaying genetic variation:
Shouldn't we just sequence everything?

Why not just sequence entire genomes??

- Still prohibitively expensive for many studies

- For many studies a full sequence isn't necessary
    - genomes of many organisms are organized in linkage blocks
    - well spaced markers will provide the necessary coverage

- Genetic maps are very useful in genomic studies
    - a high density genetic map can facilitate genome assembly
    - genomes may be segregating structural variation

Alternative - Reduced representation sequencing

- Use restriction enzyme digestion to focus sequencing of multiple samples on homologous regions across the genome
- Simultaneous identification and typing of single nucleotide polymorphisms (SNPs) and haplotypes
- The cost is a fraction of the cost of re-sequencing the genome
- Thousands of genomes can be assayed in just a few weeks

What is RADseq?

(Restriction-site Associated DNA)

2007

Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers

Michael R. Miller,[1] Joseph P. Dunham,[2] Angel Amores,[3] William A. Cresko,[2] and Eric A. Johnson[1,4]

[1]Institute for Molecular Biology, University of Oregon, Eugene, Oregon 97403, USA; [2]Center for Ecology & Evolutionary Biology, University of Oregon, Eugene, Oregon 97403, USA; [3]Institute of Neuroscience, University of Oregon, Eugene, Oregon 97403, USA

2008

OPEN ACCESS Freely available online

PLoS one

Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers

Nathan A. Baird[1*], Paul D. Etter[1*], Tressa S. Atwood[2], Mark C. Currey[3], Anthony L. Shiver[1], Zachary A. Lewis[1], Eric U. Selker[1], William A. Cresko[3], Eric A. Johnson[1*]

1 Institute of Molecular Biology, University of Oregon, Eugene, Oregon, United States of America, 2 [illegible], Eugene, Oregon, United States of America, 3 The Center for Ecology and Evolutionary Biology, University of Oregon, Eugene, Oregon, United States of America

What is RADseq?

(Restriction-site Associated DNA)

22,830 *SbfI* sites in threespine stickleback genome
~ 45,000 RAD-Tags

HiSeq2500 Illumina Lane:
160 million reads

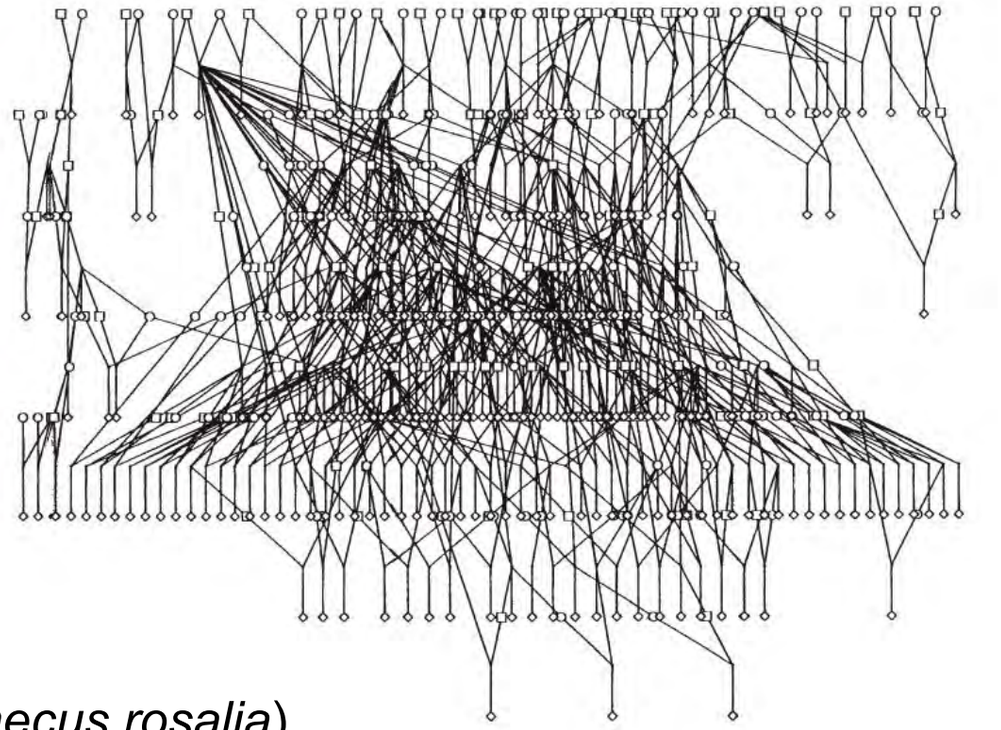
HiSeq4000 Illumina Lane:
350 million reads

For what types of studies can RADseq be useful?

Identifying genetically distinct individuals and estimating genetic diversity

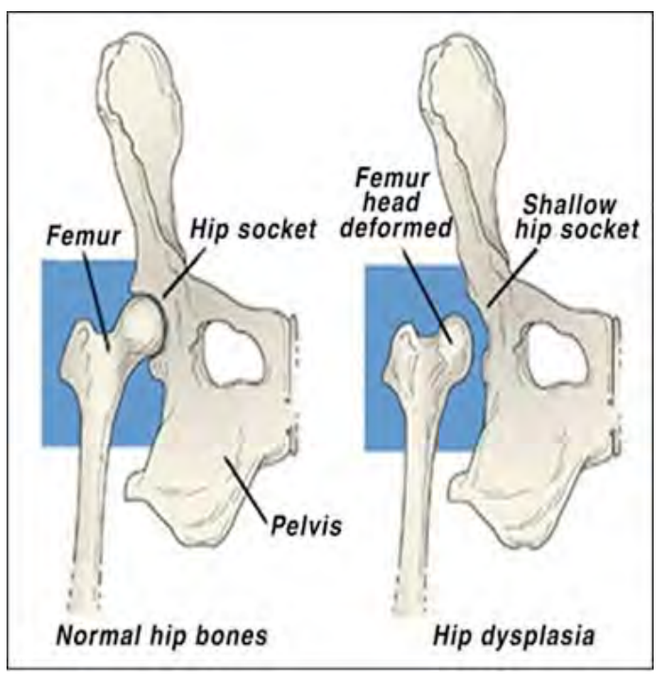
Quaking Aspens

Defining the relationships among individuals and populations

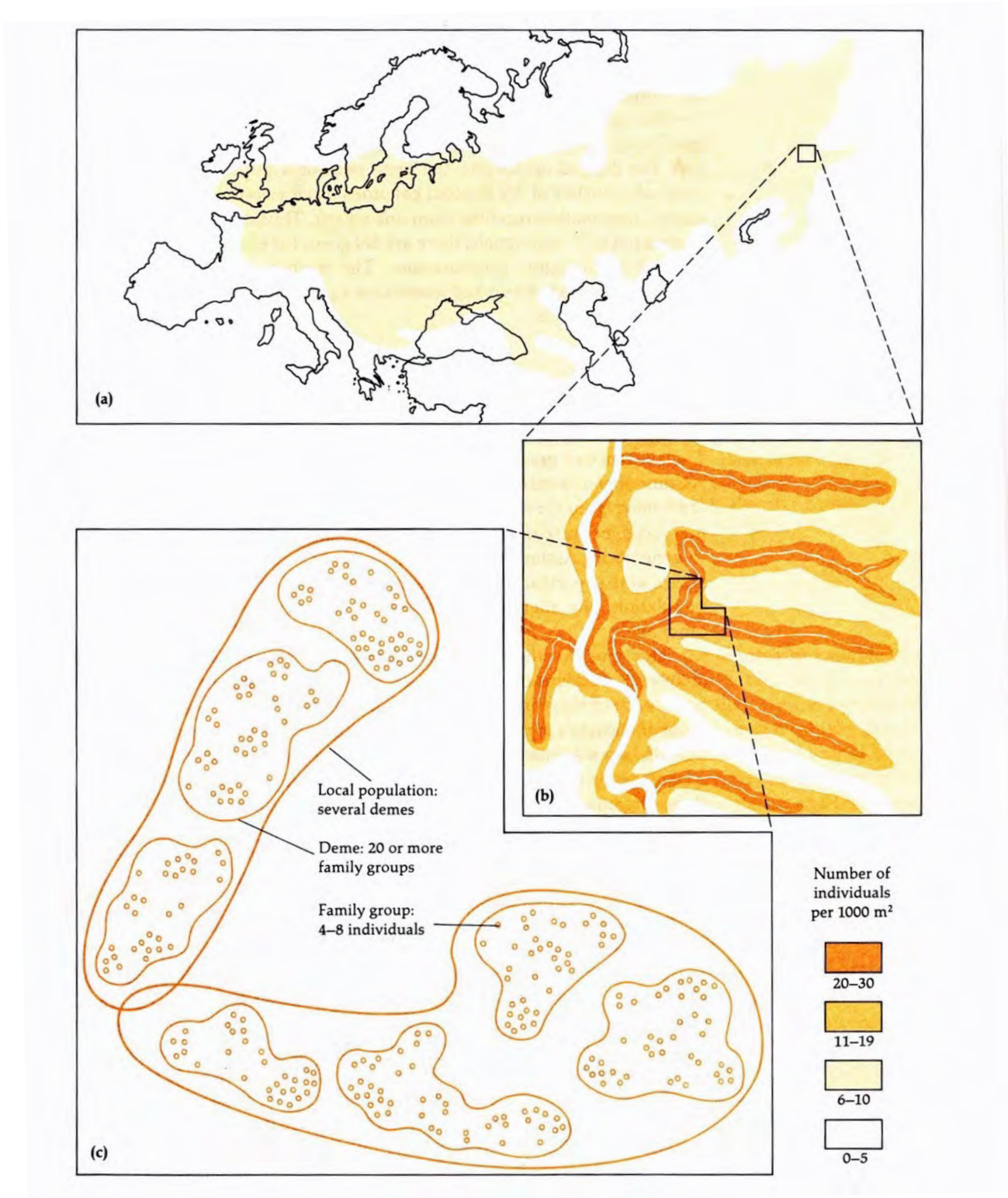


The golden lion tamarin (*Leontopithecus rosalia*).

Precisely quantifying the amount of inbreeding in wild and captive populations



Defining the relationships among individuals and populations



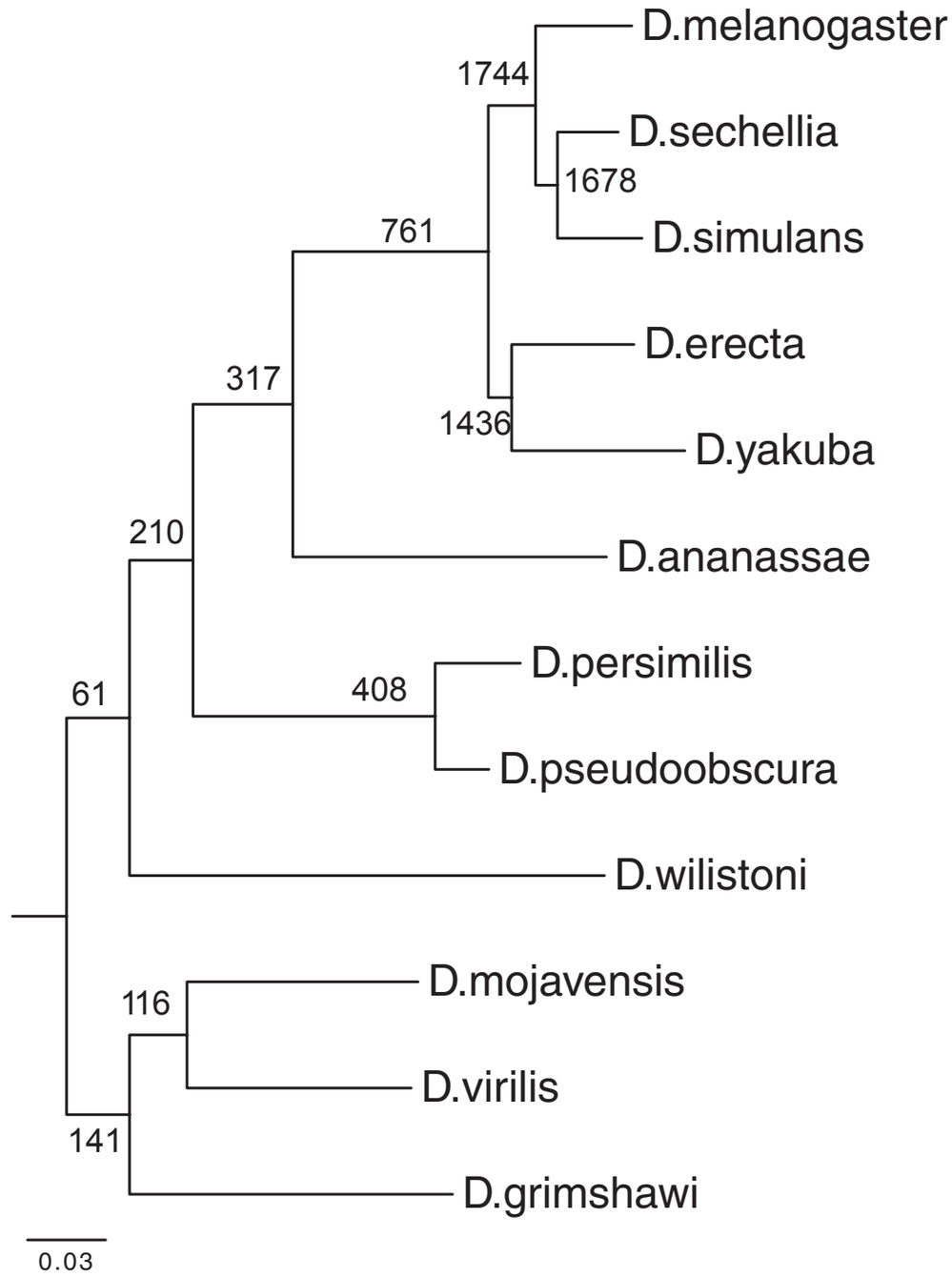
Ecology and Evolution

Open Access

Is RAD-seq suitable for phylogenetic inference? An in silico assessment and optimization

Marie Cariou, Laurent Duret & Sylvain Charlat

Université de Lyon, Université Lyon 1, CNRS, UMR 5558, Laboratoire de Biométrie et Biologie Evolutive, 43 bou Villeurbanne F-69622, France



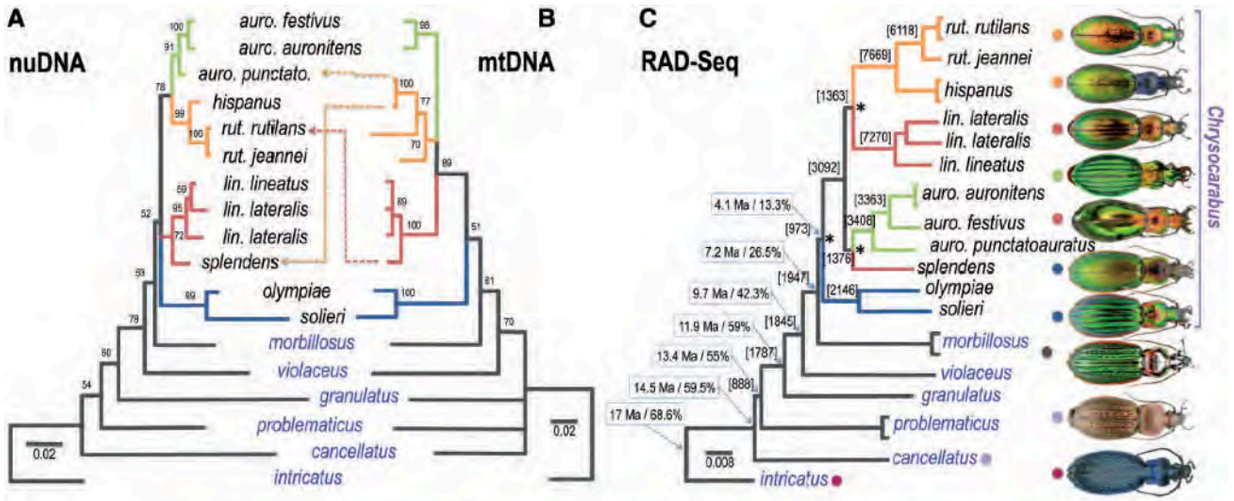
Phylogenetic relationships

Species pair *D. melanogaster*	Node depth (My)	Orthologous tags	Retrieved orthologous tags (%)	In clusters including paralogs (%)
D.sechellia	5.4	2978	99	5
D.simulans	5.4	2892	99	4
D.erecta	12.6	2390	97	3
D.yakuba	12.8	2314	97	8
D.ananassae	44.2	916	68	9
D.persimilis	54.9	648	65	9
D.pseudoobscura	54.9	648	66	9
D.wilistoni	62.2	242	49	6
D.grimshawi	62.9	290	60	8
D.virilis	62.9	286	59	5
D.mojavensis	62.9	298	59	8

Empirical Assessment of RAD Sequencing for Interspecific Phylogeny

Mol. Biol. Evol. 31(5):1272–1274

Astrid Cruaud,*,†,1 Mathieu Gautier,†,1,2 Maxime Galan,1 Julien Foucaud,1 Laure Sauné,1 Gwenaëlle Genson,1 Emeric Dubois,3 Sabine Nidelet,3 Thierry Deuve,4 and Jean-Yves Rasplus1



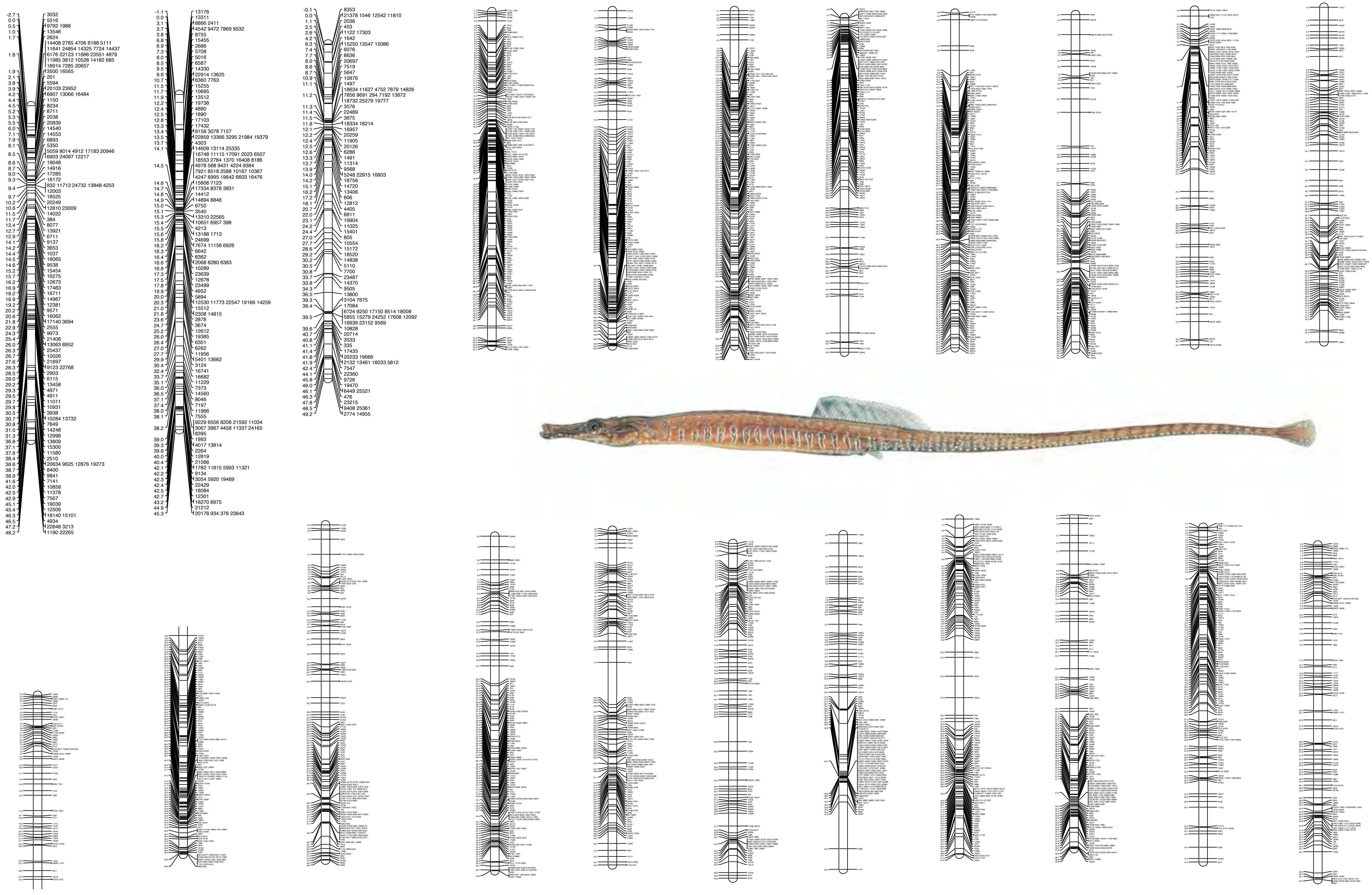
D

	Sanger		RAD ($n_{cstacks}$ = 10)	
Matrix :	*Carabus* (18 ind./13 spp.)	*Chrysocarabus* (12 ind./7 spp.)	*Carabus* (18 ind./13 spp.)	*Chrysocarabus* (12 ind./7 spp.)
Number of loci :	9		25 425	
Alignment length :	5 584 bp		2 262 825 bp	
Data set size :	100 512 nt	67 008 nt	40 730 850 nt	27 153 900 nt
Variable sites :	1030 (18.4%)	535 (9.6%)	367 813 (16.3%)	216 300 (9.6%)
Informative sites :	495 (8.9%)	264 (4.7%)	132 186 (5.8%)	91 420 (4.0%)
Missing data :				
without old DNA sample	21.8%	17.9%	26.9%	13.3%
of old DNA sample (*C. olympiae*)	65.5%		75.4%	
Sample preparation and sequencing :	1 week / ca. 1 200 USD (without marker screening)		3 weeks / ca. 3 600 USD	
Data Analysis (CBGP cluster) :	2-3 days		1-2 weeks	

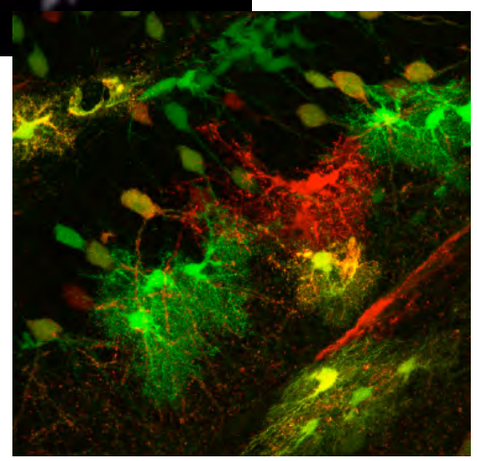
Abstract

Next-generation sequencing opened up new possibilities in phylogenetics; however, choosing an appropriate method of sample preparation remains challenging. Here, we demonstrate that restriction-site-associated DNA sequencing (RAD-seq) generates useful data for phylogenomics. Analysis of our RAD library using current bioinformatic and phylogenetic tools produced 400× more sites than our Sanger approach (2,262,825 nt/species), fully resolving relationships between 18 species of ground beetles (divergences up to 17 My). This suggests that RAD-seq is promising to infer phylogeny of eukaryotic species, though potential biases need to be evaluated and new methodologies developed to take full advantage of such data.

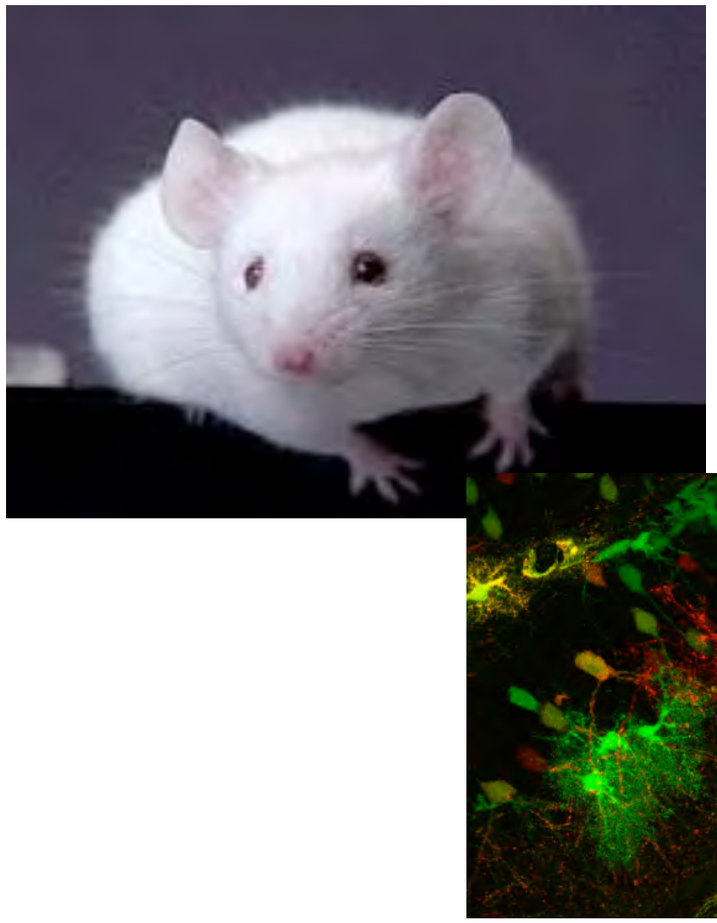
Improve genome assemblies with genetic maps

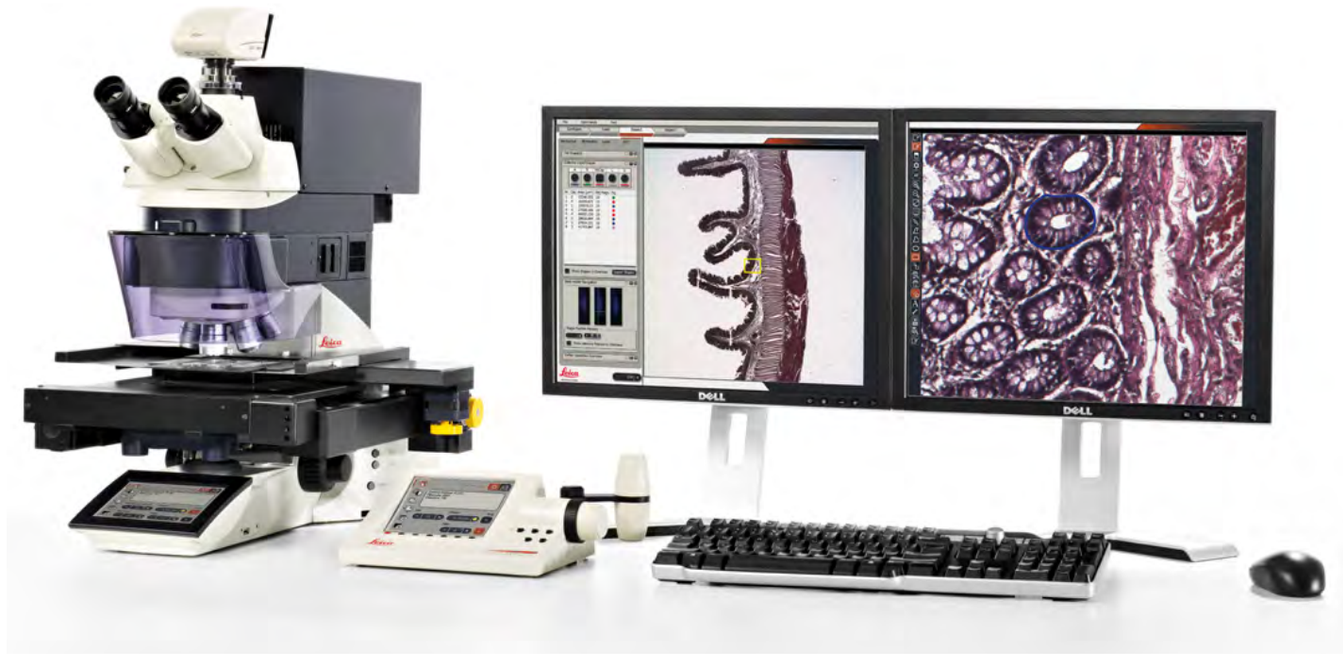


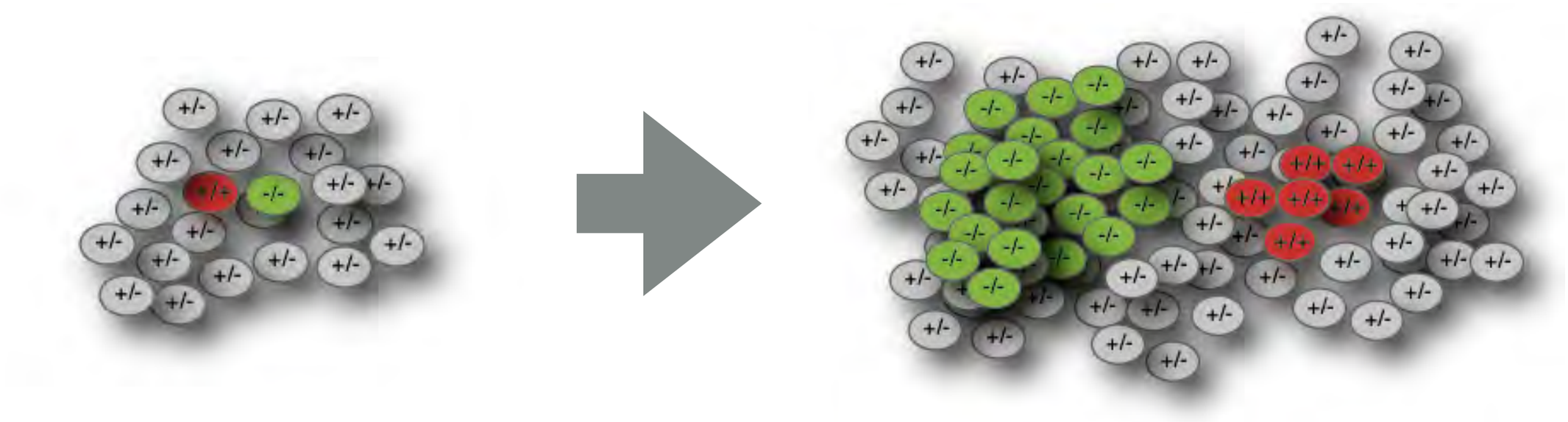
Studying cancer as an evolutionary process



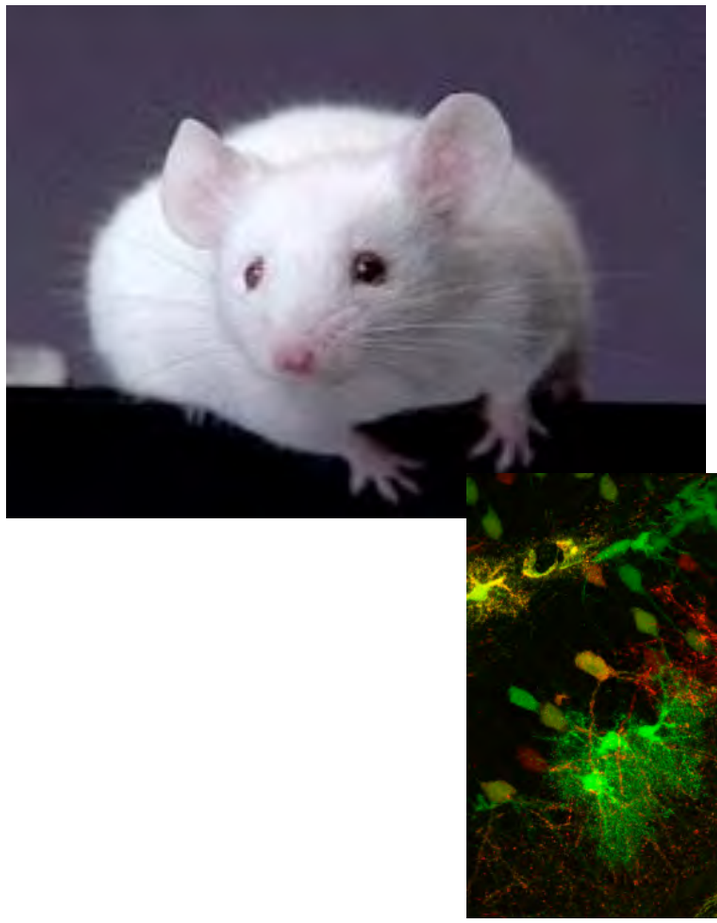
Studying cancer as an evolutionary process



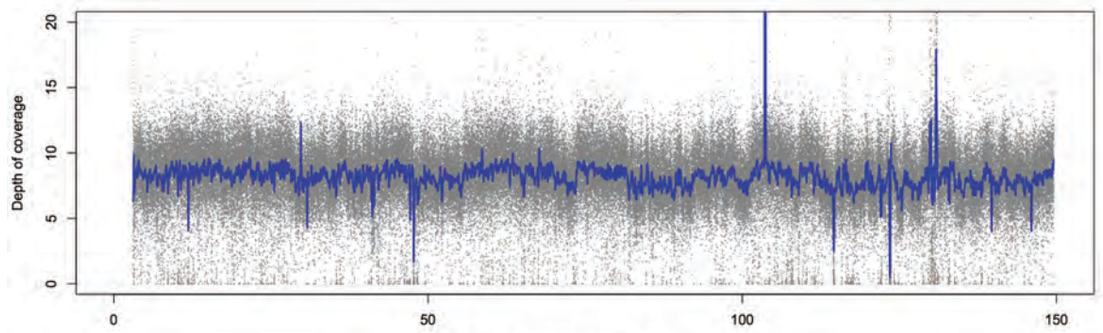




Studying cancer as an evolutionary process

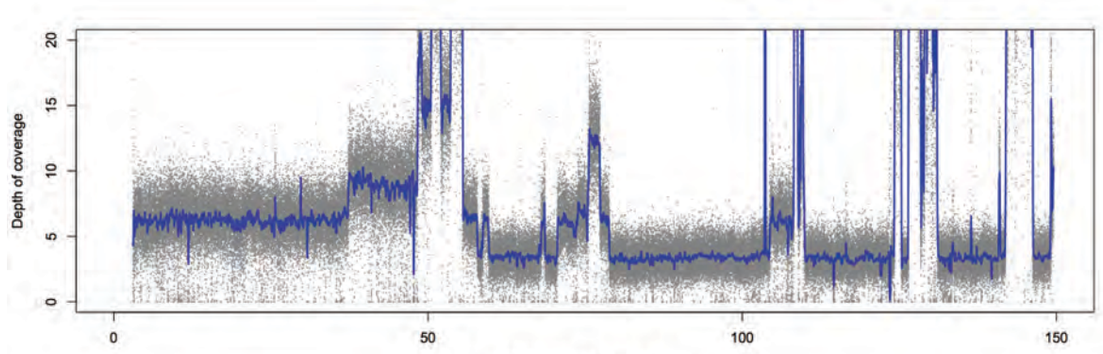


Wild Type



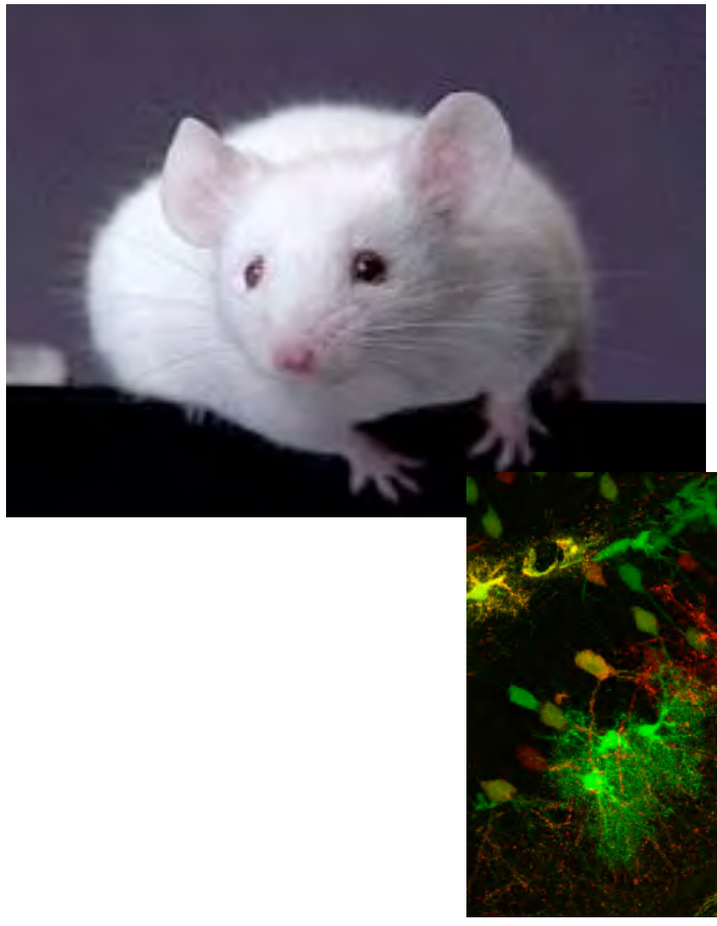
WGS

Mutant

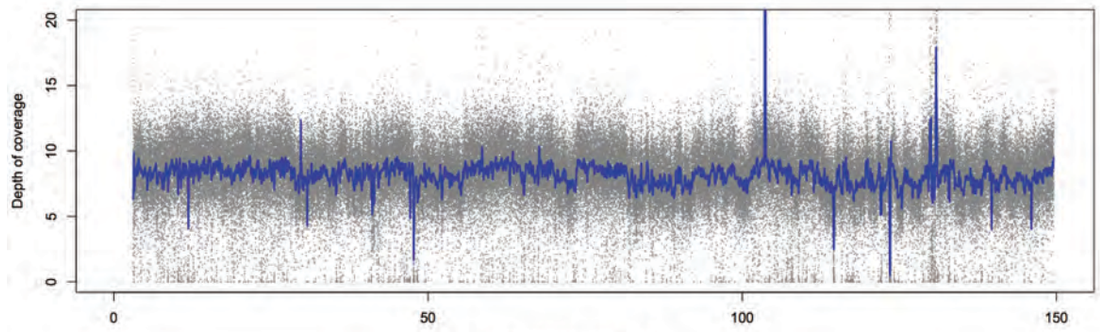


WGS

Studying cancer as an evolutionary process



Wild Type



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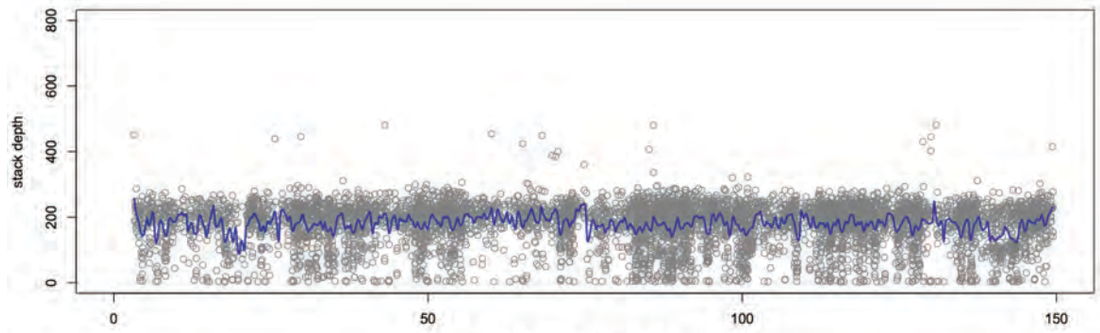
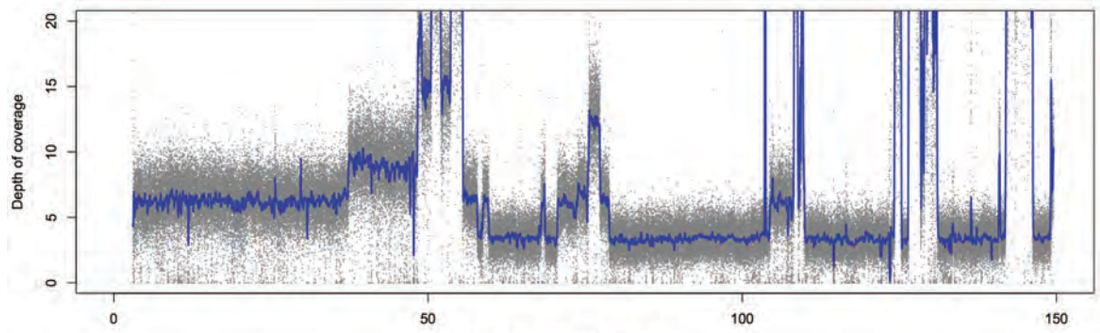
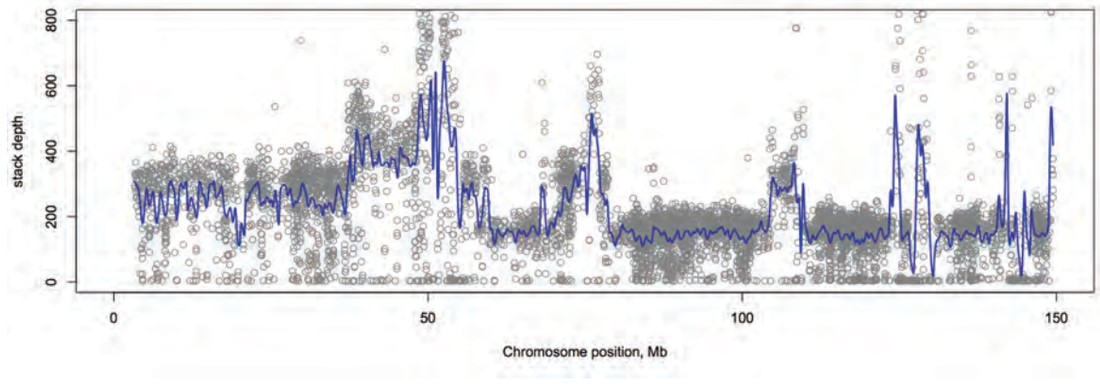


RAD

Mutant



WGS

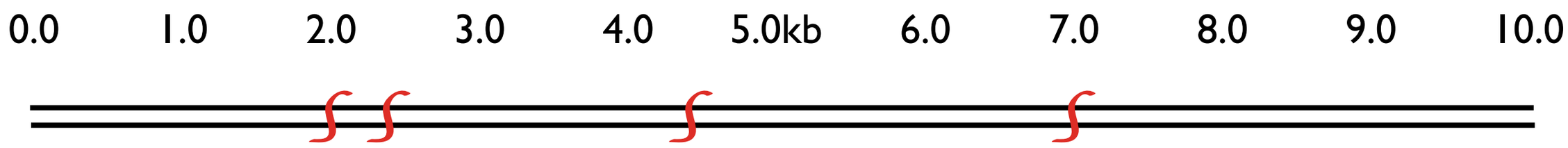


RAD

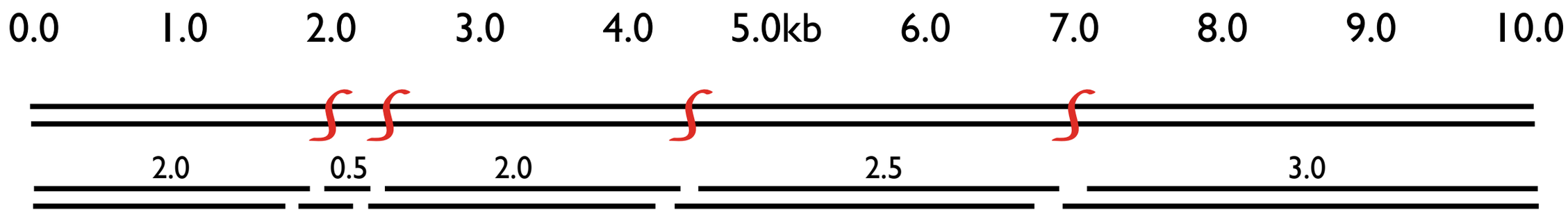
Let’s go into a little more detail

Restriction Enzyme (RE) digestion and first adaptor ligation

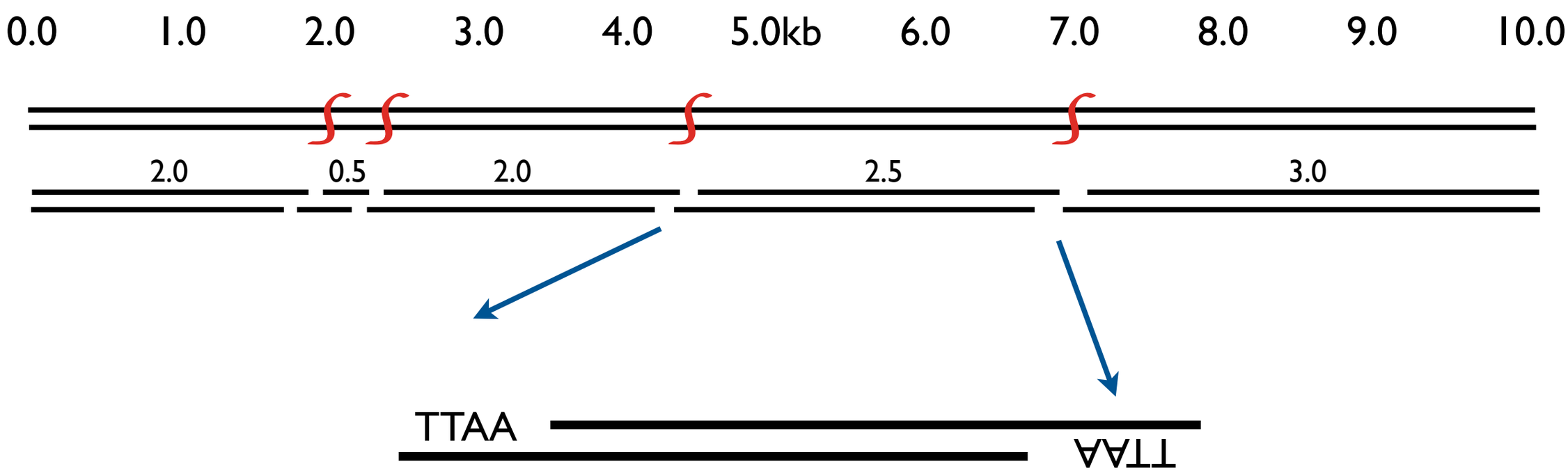
Restriction Enzyme (RE) digestion and first adaptor ligation



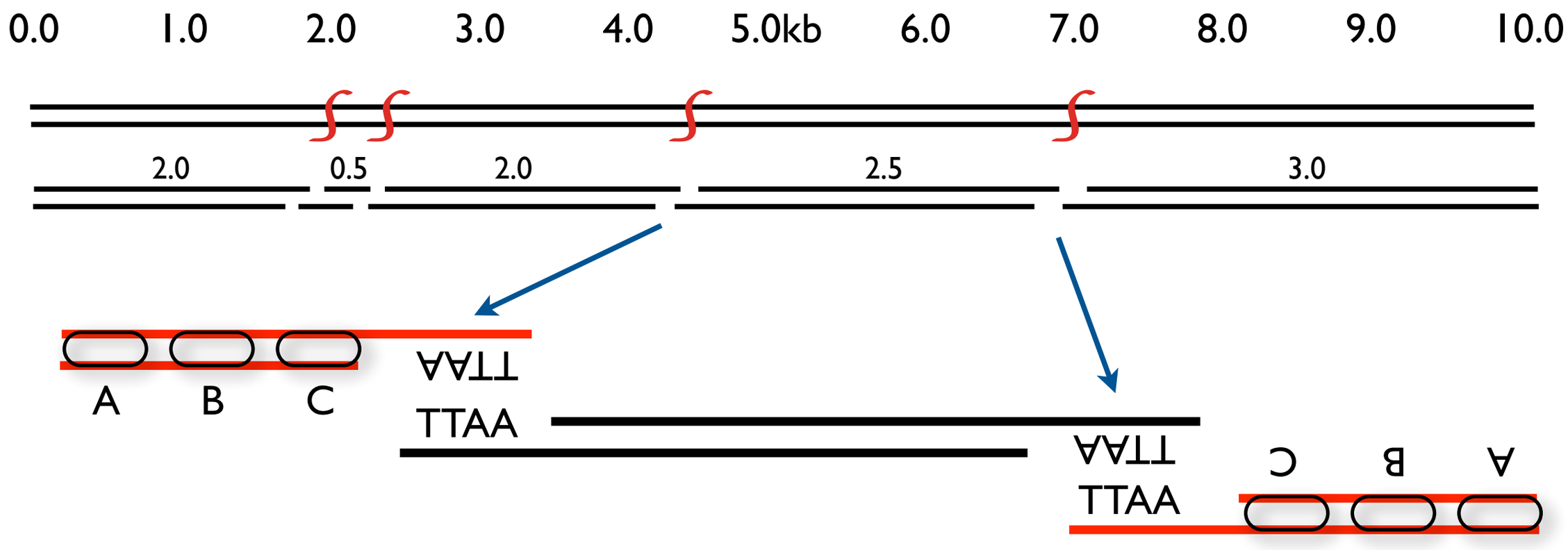
Restriction Enzyme (RE) digestion and first adaptor ligation



Restriction Enzyme (RE) digestion and first adaptor ligation

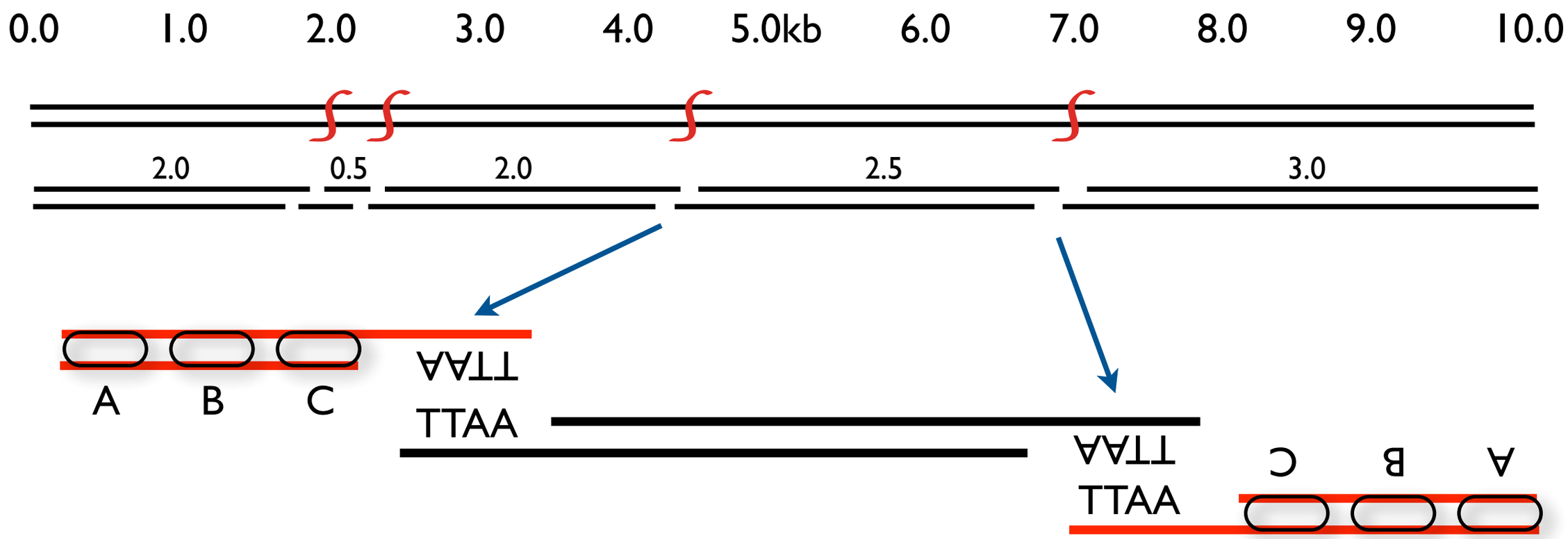


Restriction Enzyme (RE) digestion and first adaptor ligation



A = Amplification primer
B = Sequencing primer
C = Barcode

Restriction Enzyme (RE) digestion and first adaptor ligation

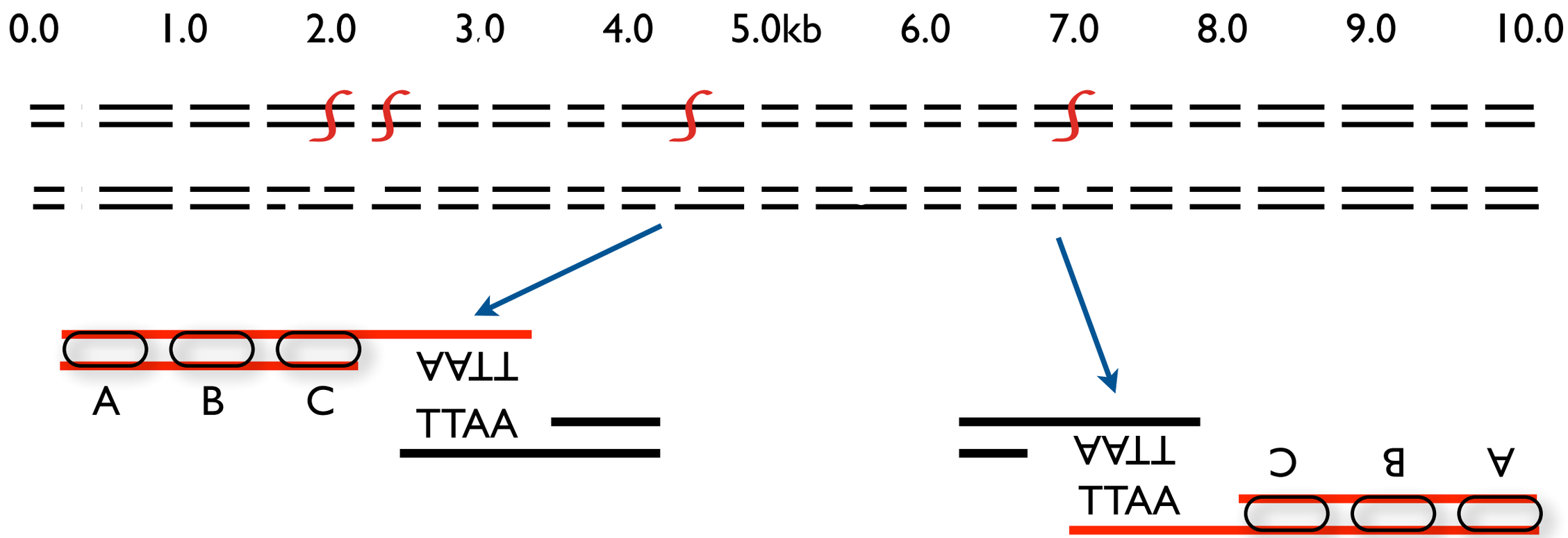


A = Amplification primer
B = Sequencing primer
C = Barcode

Note - there are now commonly two levels of barcodes used:

Sample Barcodes and
Molecular Identification Barcodes (MIPs)

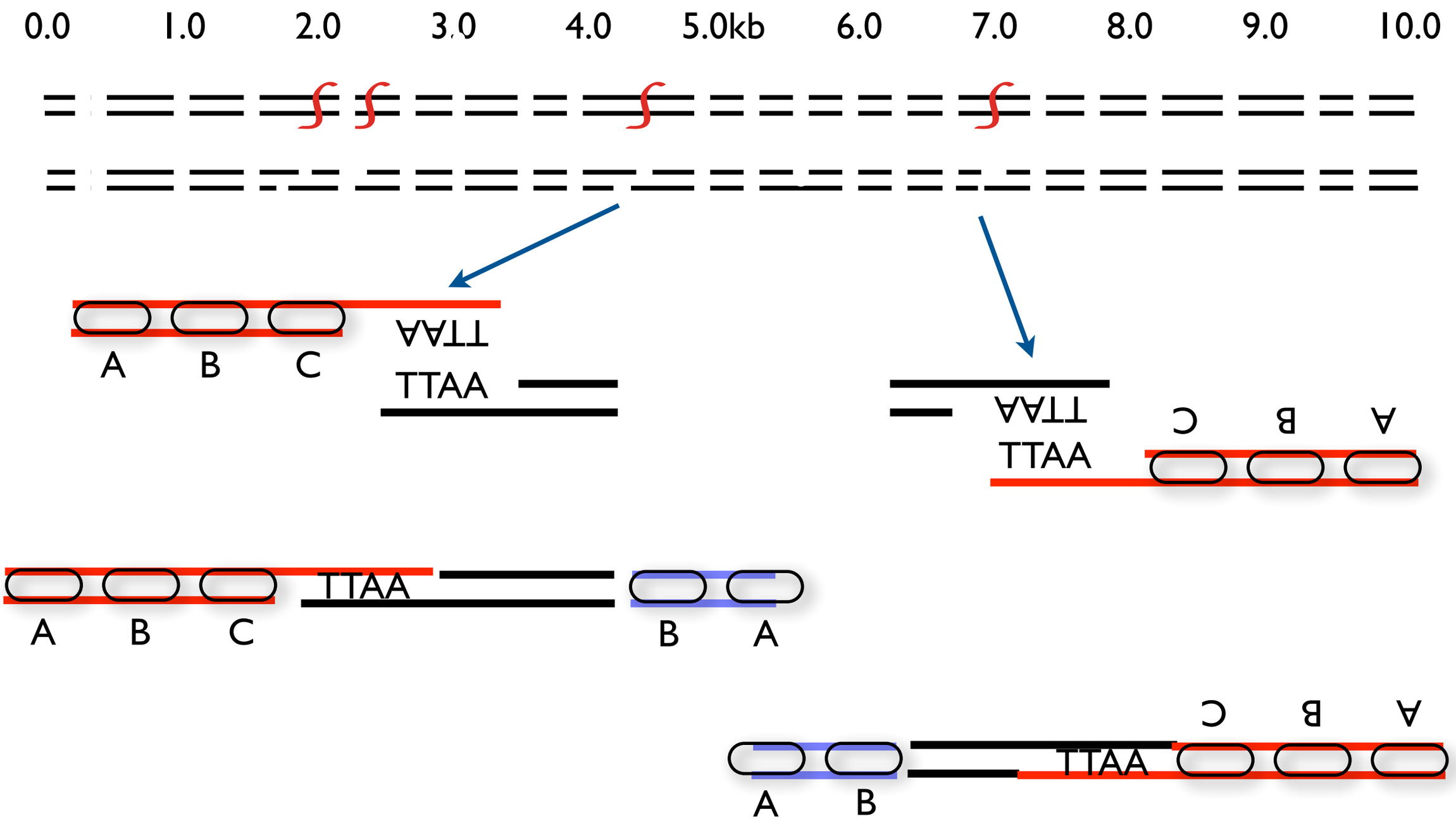
Shearing and second adaptor ligation



* Defining difference between original RAD and other approaches*

A = Amplification primer
B = Sequencing primer
C = Barcode

Shearing and second adaptor ligation



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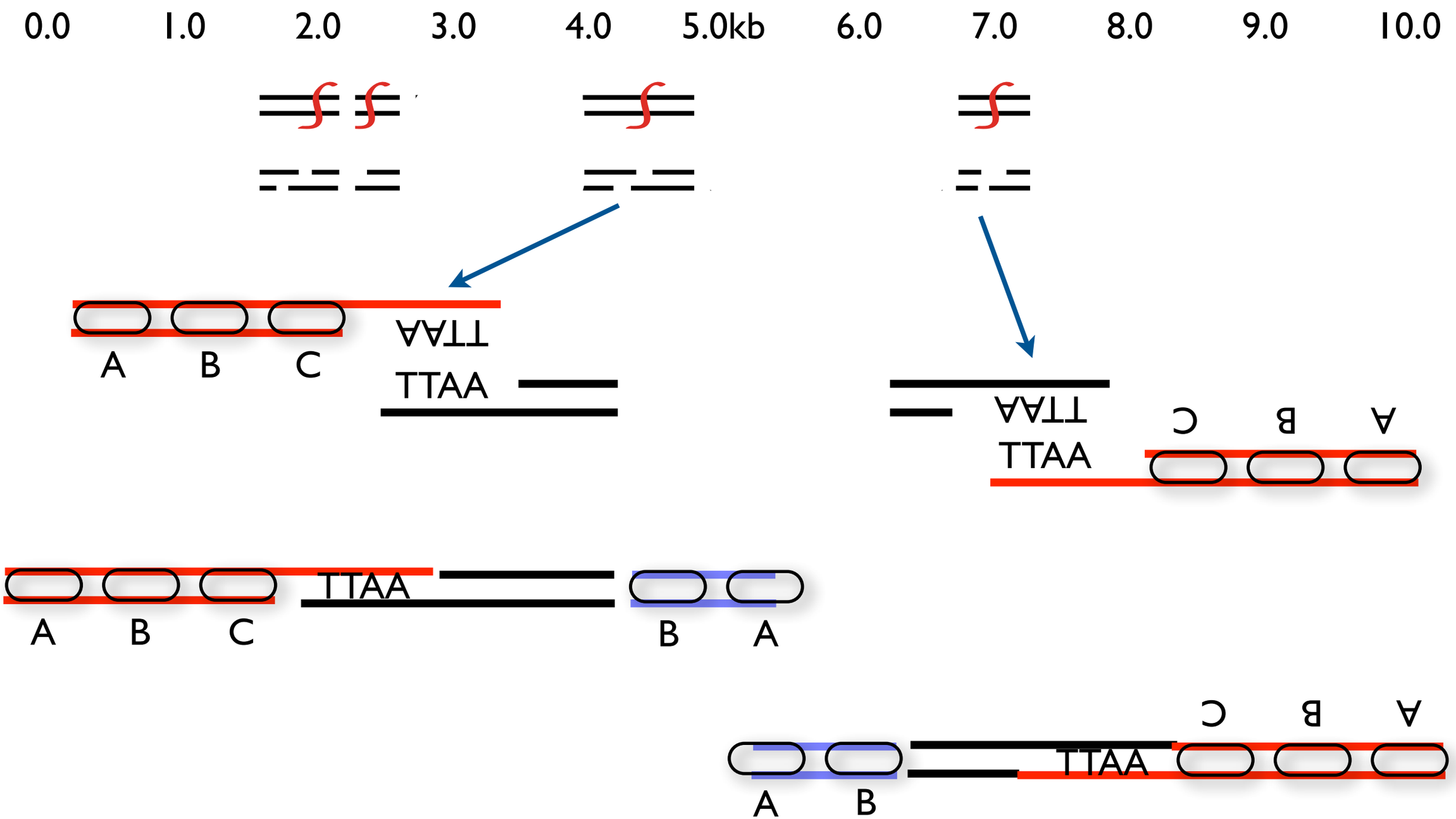
Shearing and second adaptor ligation



A = Amplification primer
B = Sequencing primer
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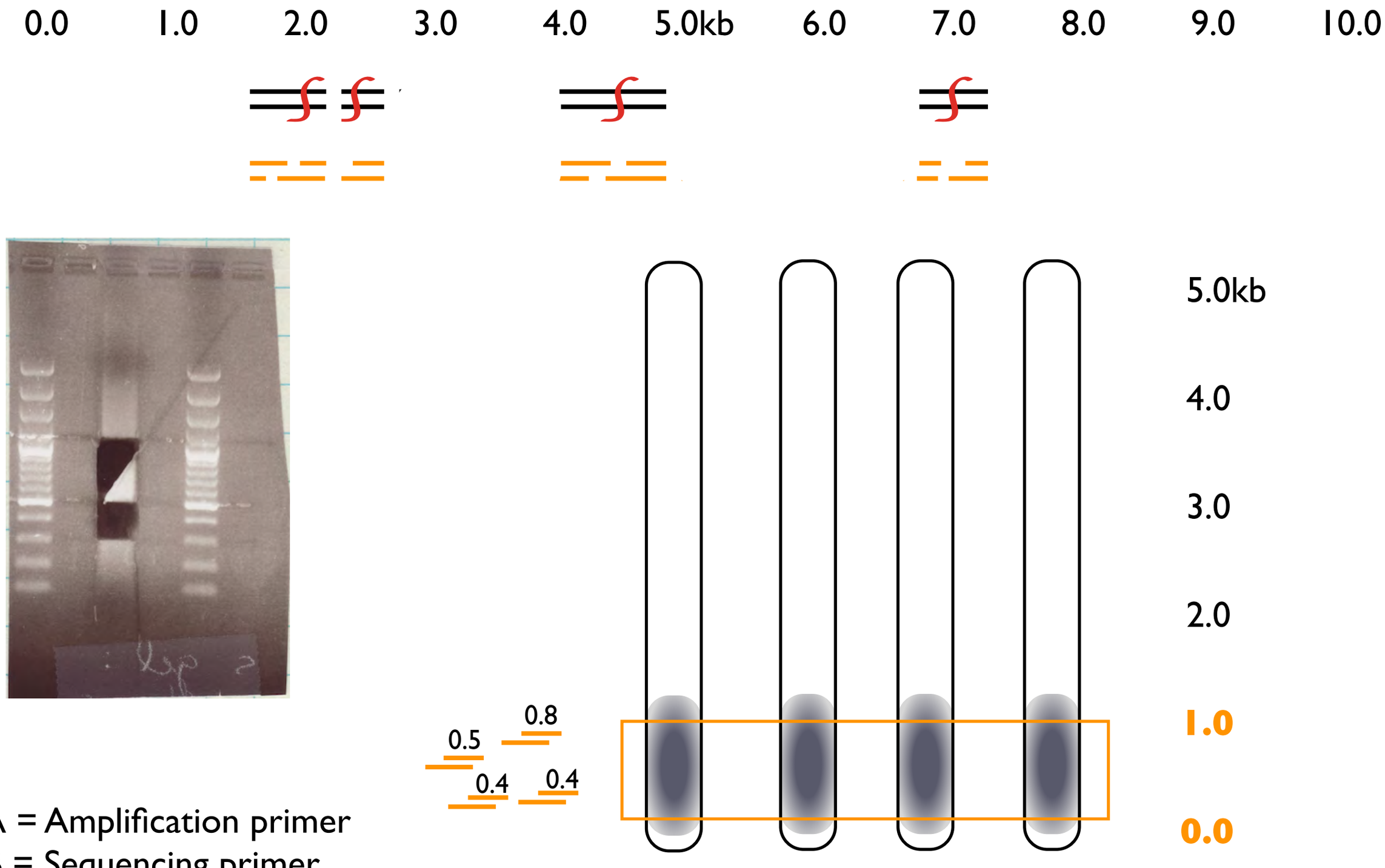
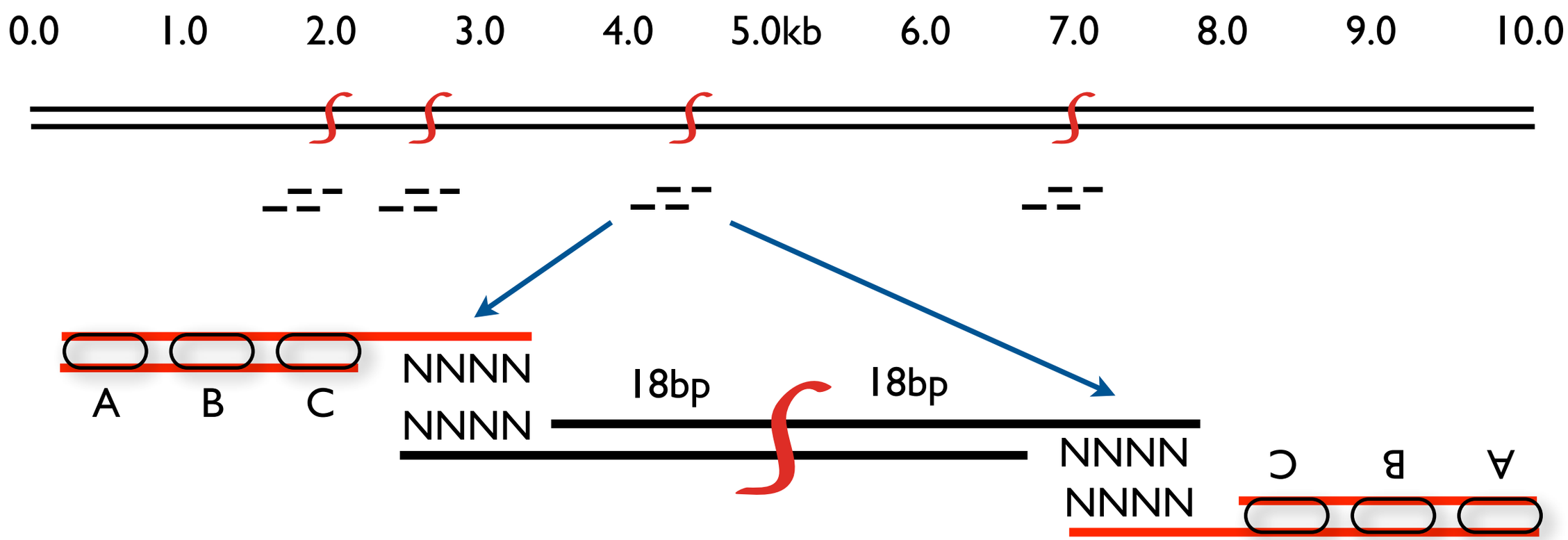
Shearing makes consistent fragments for sequencing



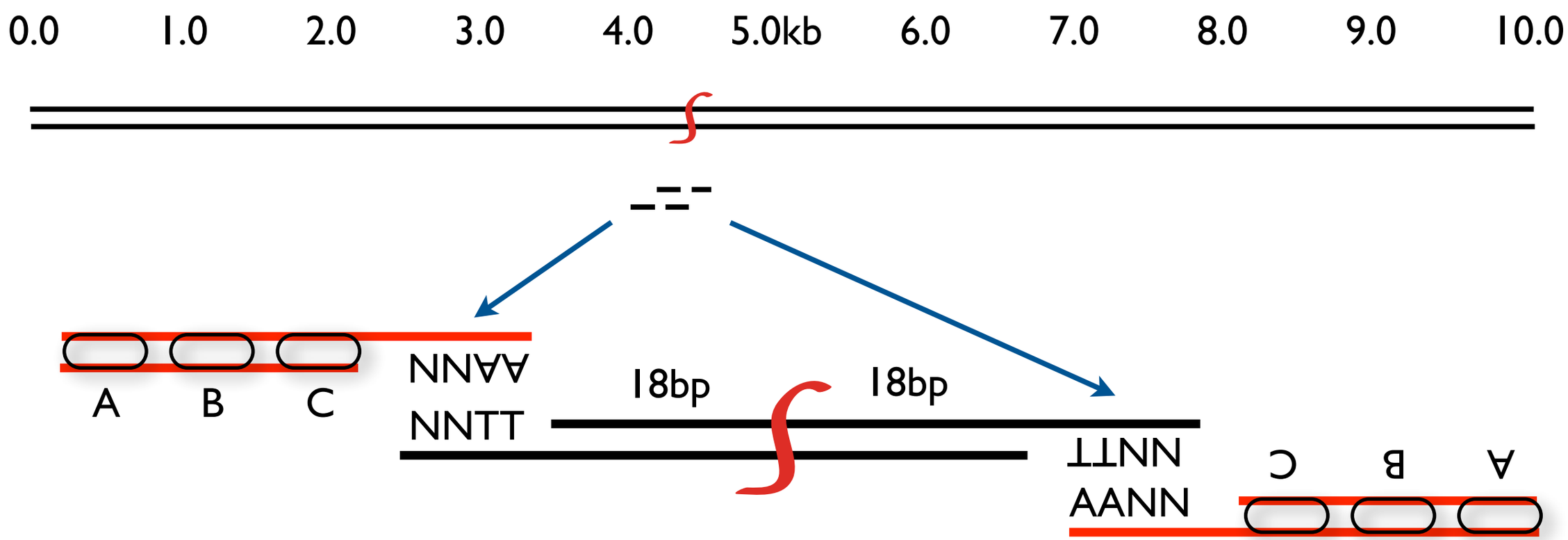
A = Amplification primer
B = Sequencing primer
C = Barcode

2bRAD - type 2b restriction enzyme



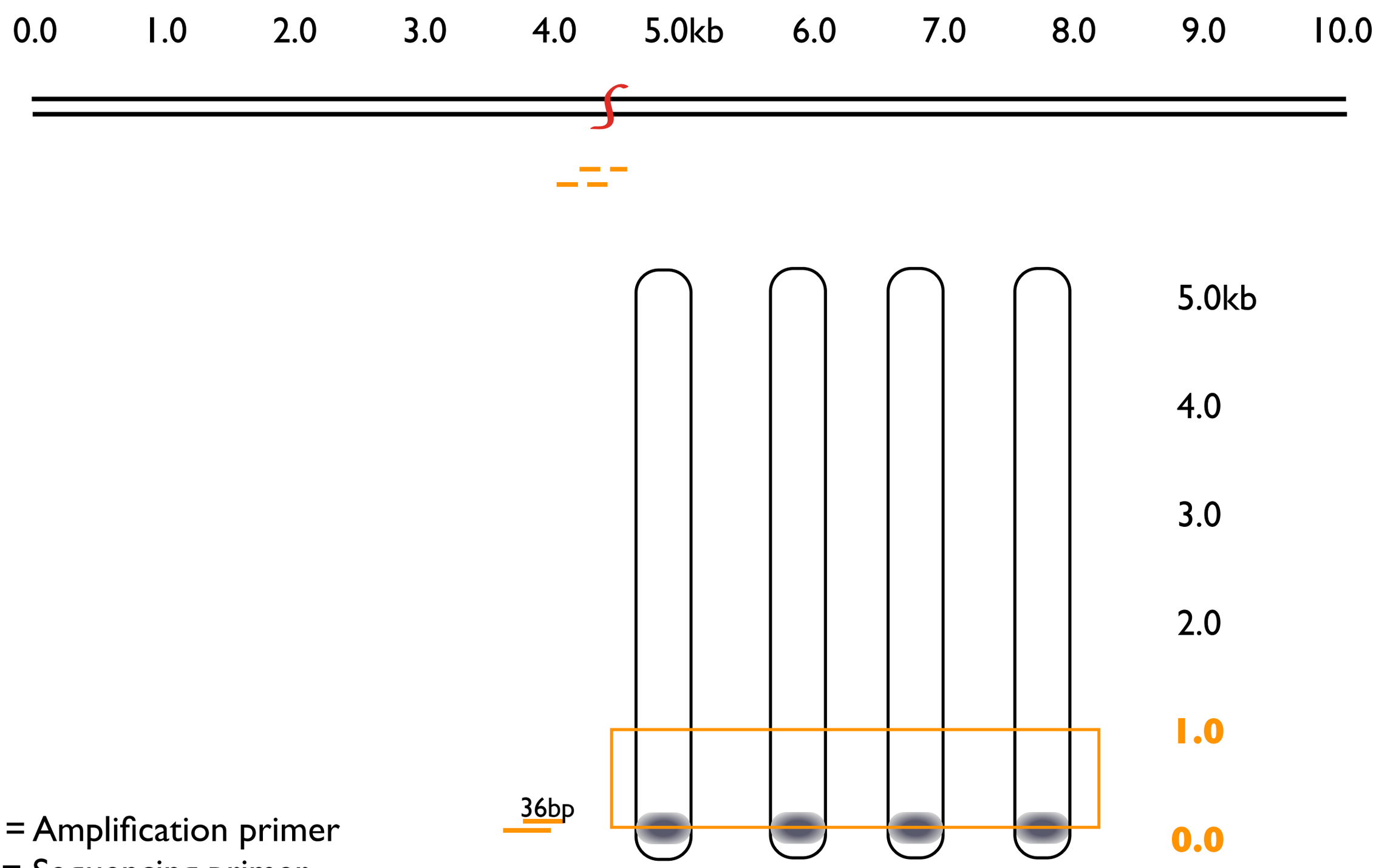
A = Amplification primer
B = Sequencing primer
C = Barcode

2bRAD - can scale number of markers easily



A = Amplification primer
B = Sequencing primer
C = Barcode

2bRAD - size selection is difficult



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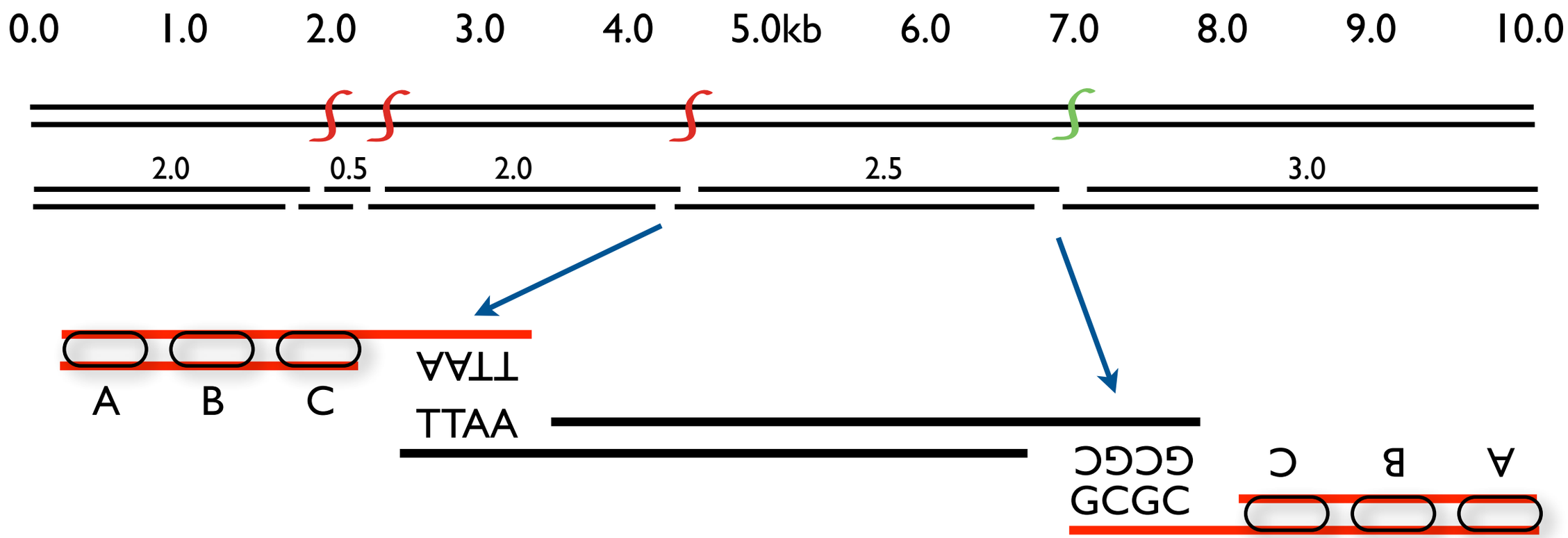
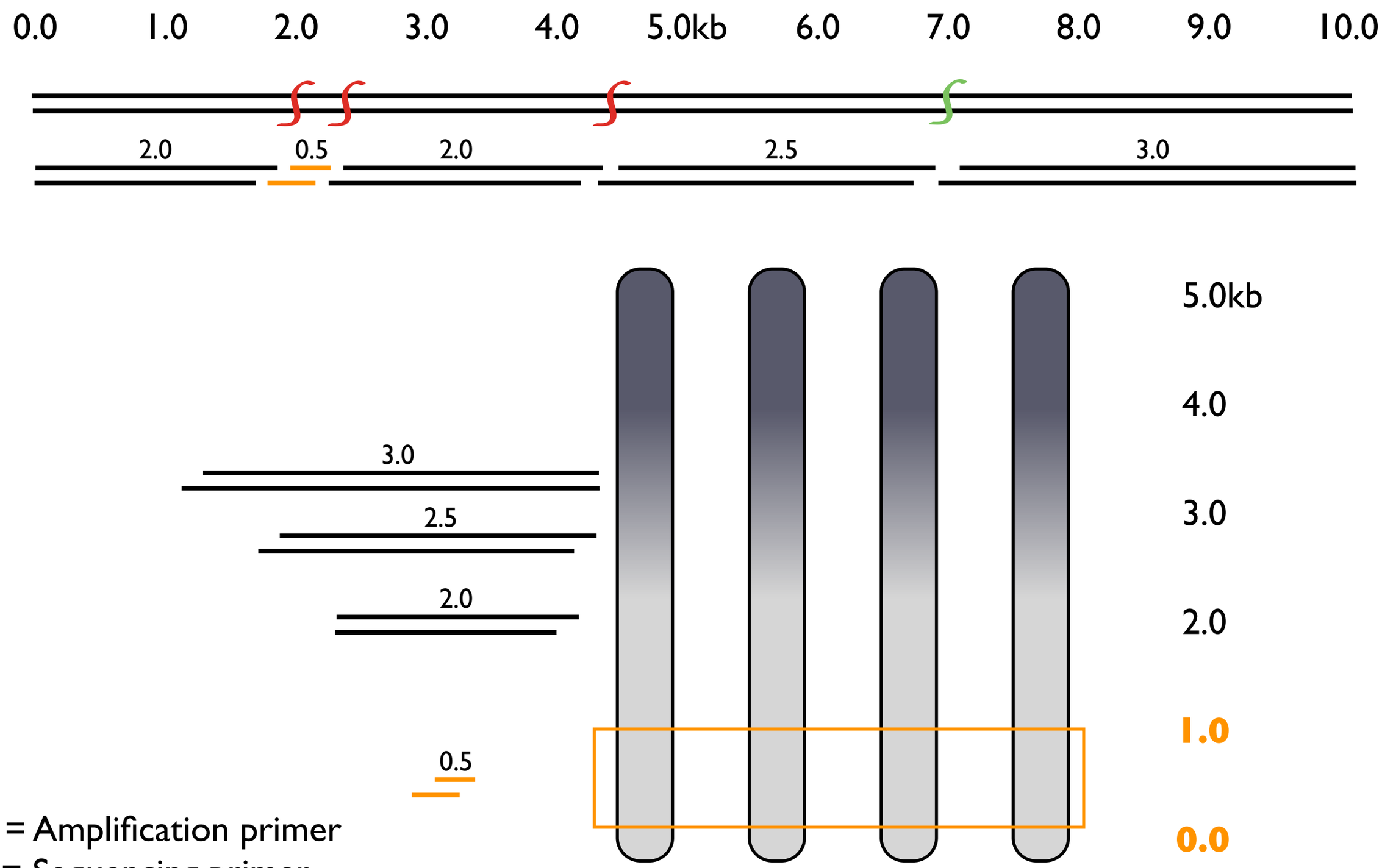
B = Sequencing primer
C = Barcode

Single (GBS) or Double Digest RAD (ddRAD)



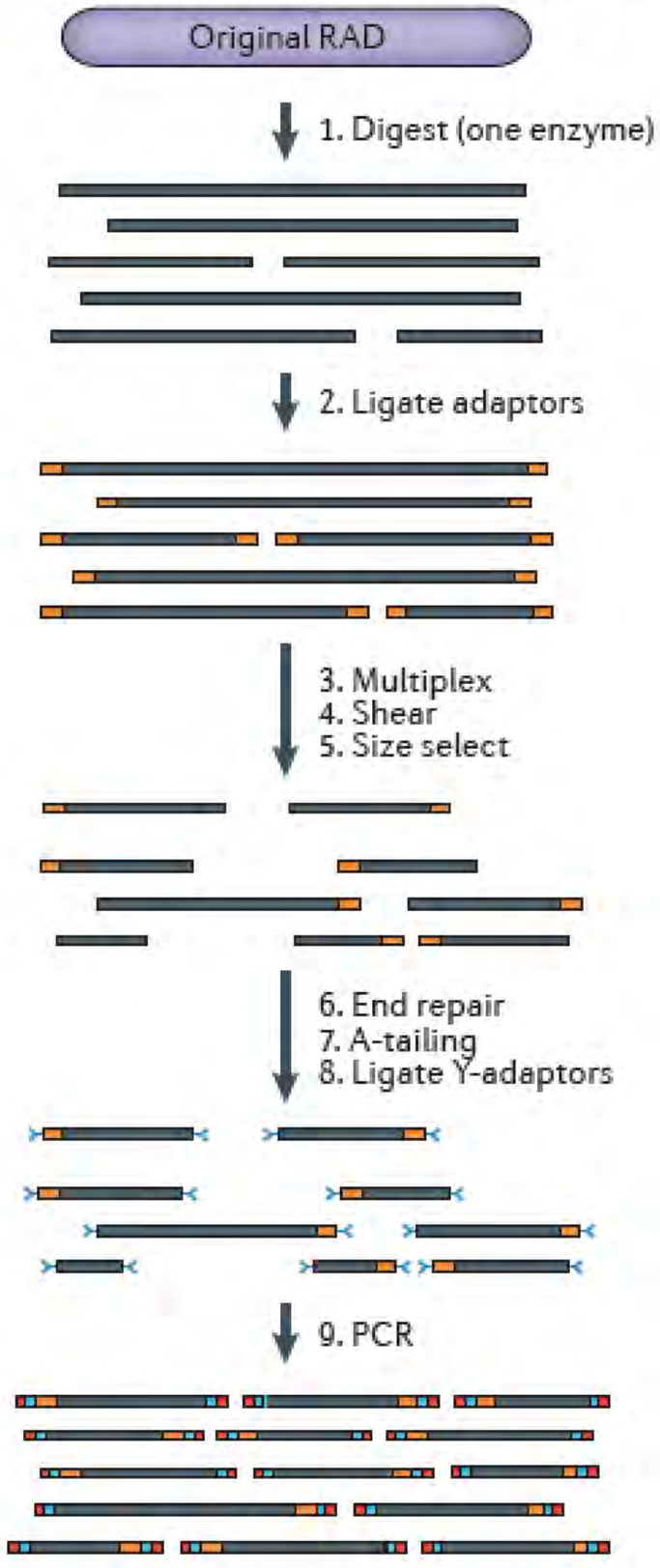
A = Amplification primer
B = Sequencing primer
C = Barcode

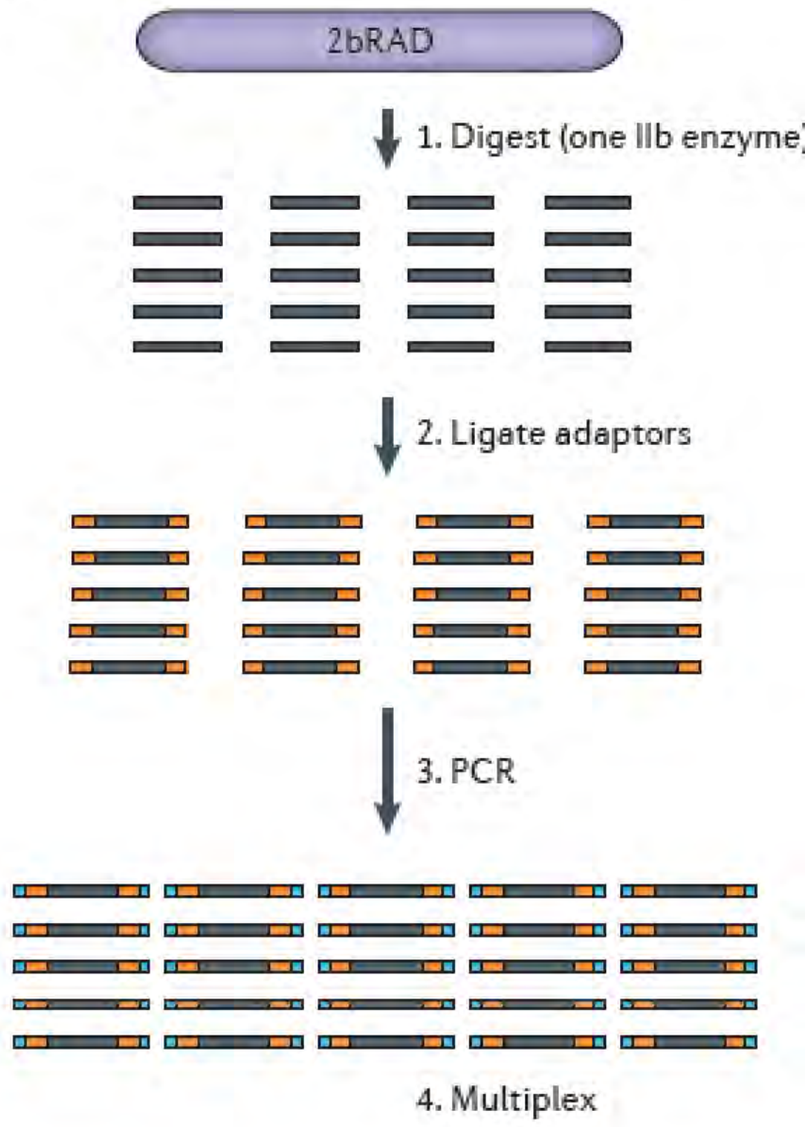
Size selection is more problematic without shearing



A = Amplification primer
B = Sequencing primer
C = Barcode

RADseq with one enzyme digestion & shearing



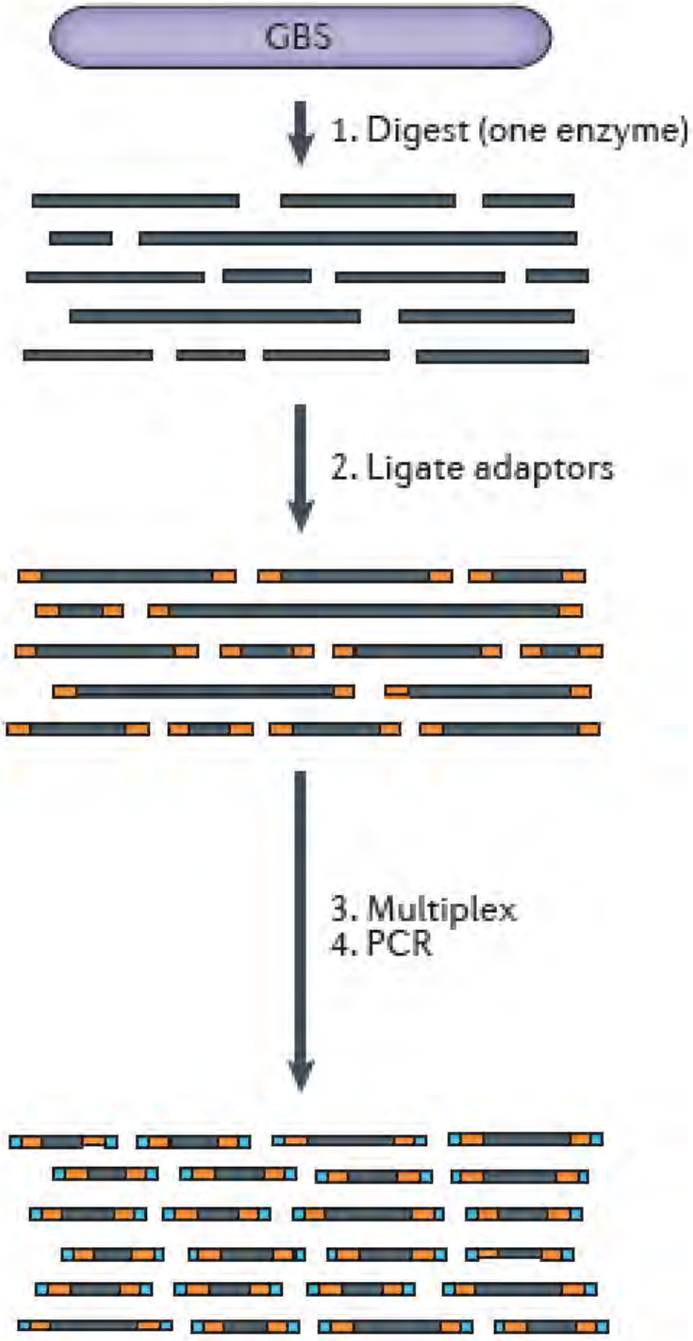


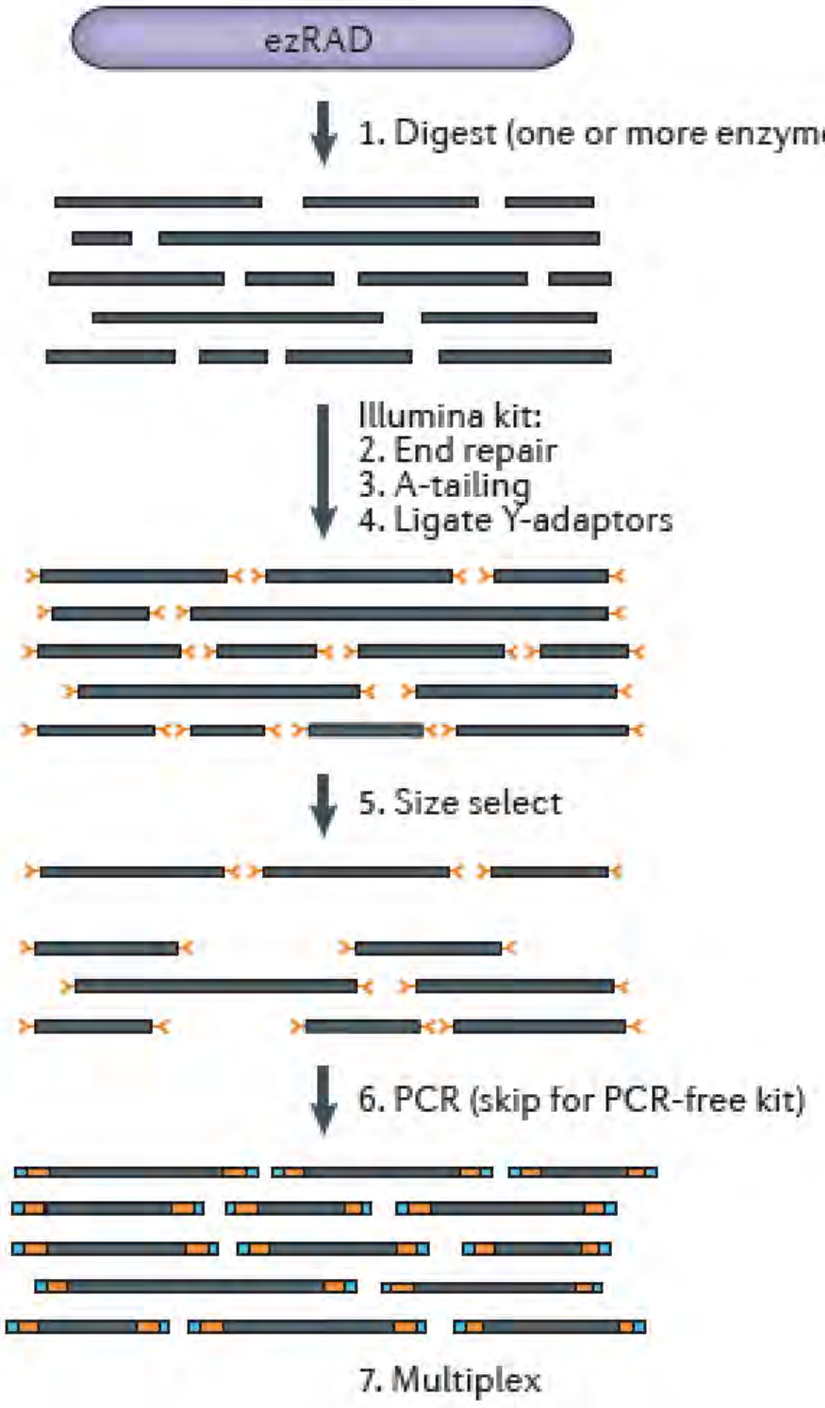
STUDY DESIGNS

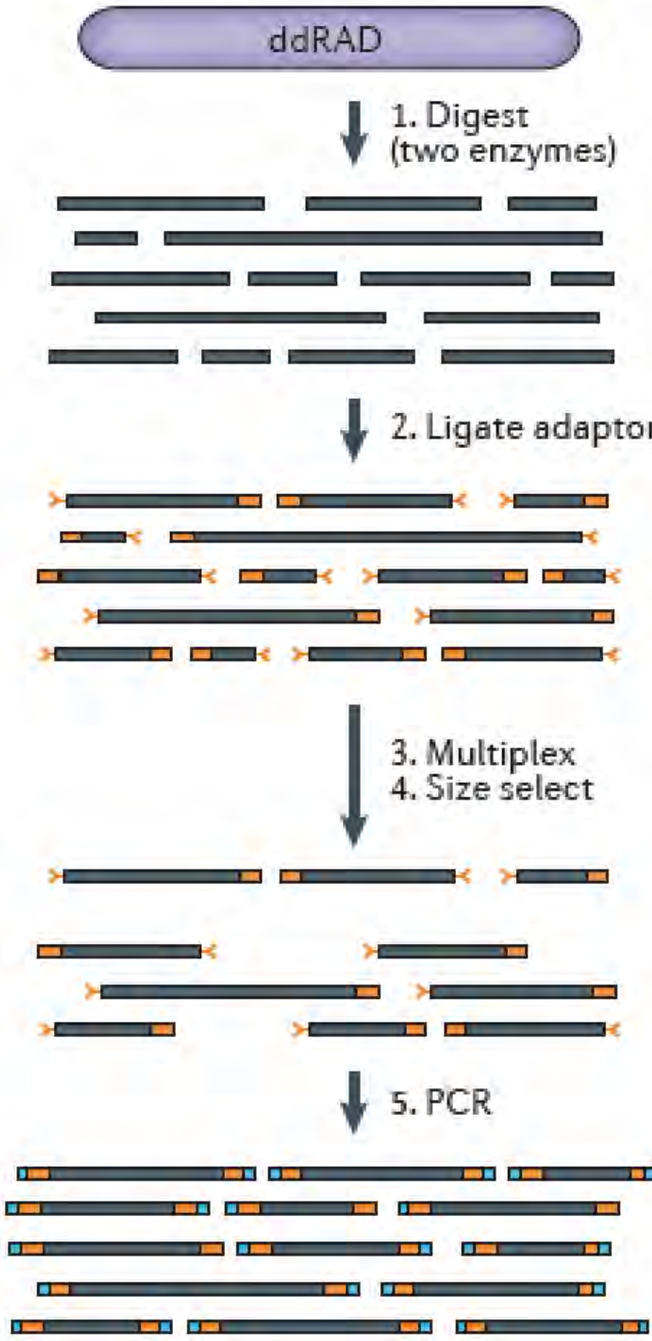
Harnessing the power of RADseq for ecological and evolutionary genomics

Kimberly R. Andrews[1], Jeffrey M. Good[2], Michael R. Miller[3], Gordon Luikart[4] and Paul A. Hohenlohe[5]

RADseq with one or two enzyme digestion







Summary of plusses and minuses of RAD family

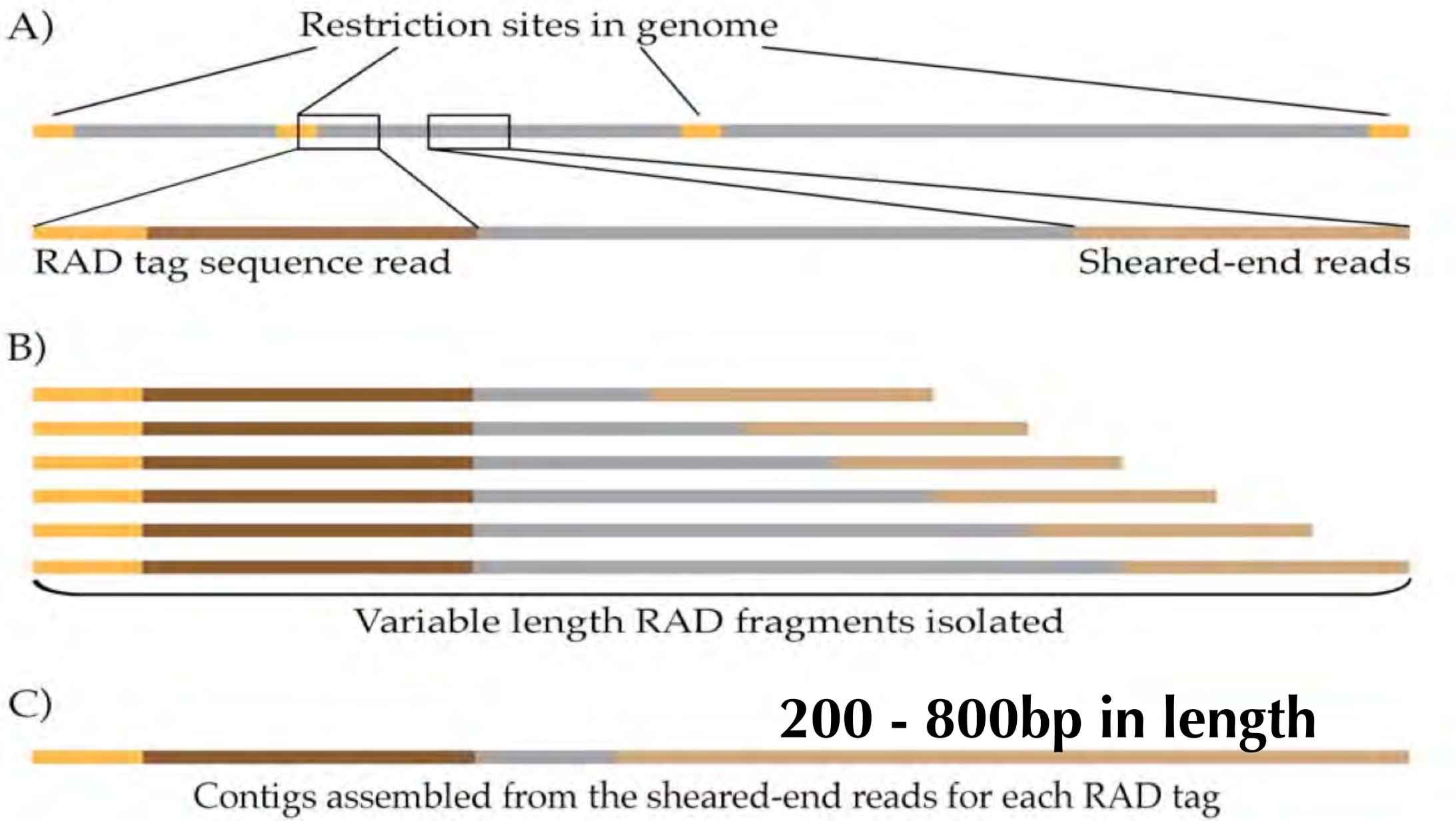
STUDY DESIGNS

Harnessing the power of RADseq for ecological and evolutionary genomics

Kimberly R. Andrews[1], Jeffrey M. Good[2], Michael R. Miller[3], Gordon Luikart[4] and Paul A. Hohenlohe[5]

	Original RAD	2bRAD	GBS	ddRAD	ezRAD
Options for tailoring number of loci	Change restriction enzyme	Change restriction enzyme	Change restriction enzyme	Change restriction enzyme or size selection window	Change restriction enzyme or size selection window
Number of loci per 1 Mb of genome size*	30–500	50–1,000	5–40	0.3–200	10–800
Length of loci	≤1kb if building contigs; otherwise ≤300 bp‡	33–36 bp	<300 bp‡	≤300 bp‡	≤300 bp‡
Cost per barcoded or indexed sample	Low	Low	Low	Low	High
Effort per barcoded or indexed sample§	Medium	Low	Low	Low	High
Use of proprietary kit	No	No	No	No	Yes
Identification of PCR duplicates	With paired-end sequencing	No	With degenerate barcodes	With degenerate barcodes	No
Specialized equipment needed	Sonicator	None	None	Pippin Prep‖	Pippin Prep‖
Suitability for large or complex genomes¶	Good	Poor	Moderate	Good	Good
Suitability for *de novo* locus identification (no reference genome)#	Good	Poor	Moderate	Moderate	Moderate
Available from commercial companies	Yes	No	Yes	Yes	No

Random shearing in original RAD - Local Paired End (PE) Assemblies

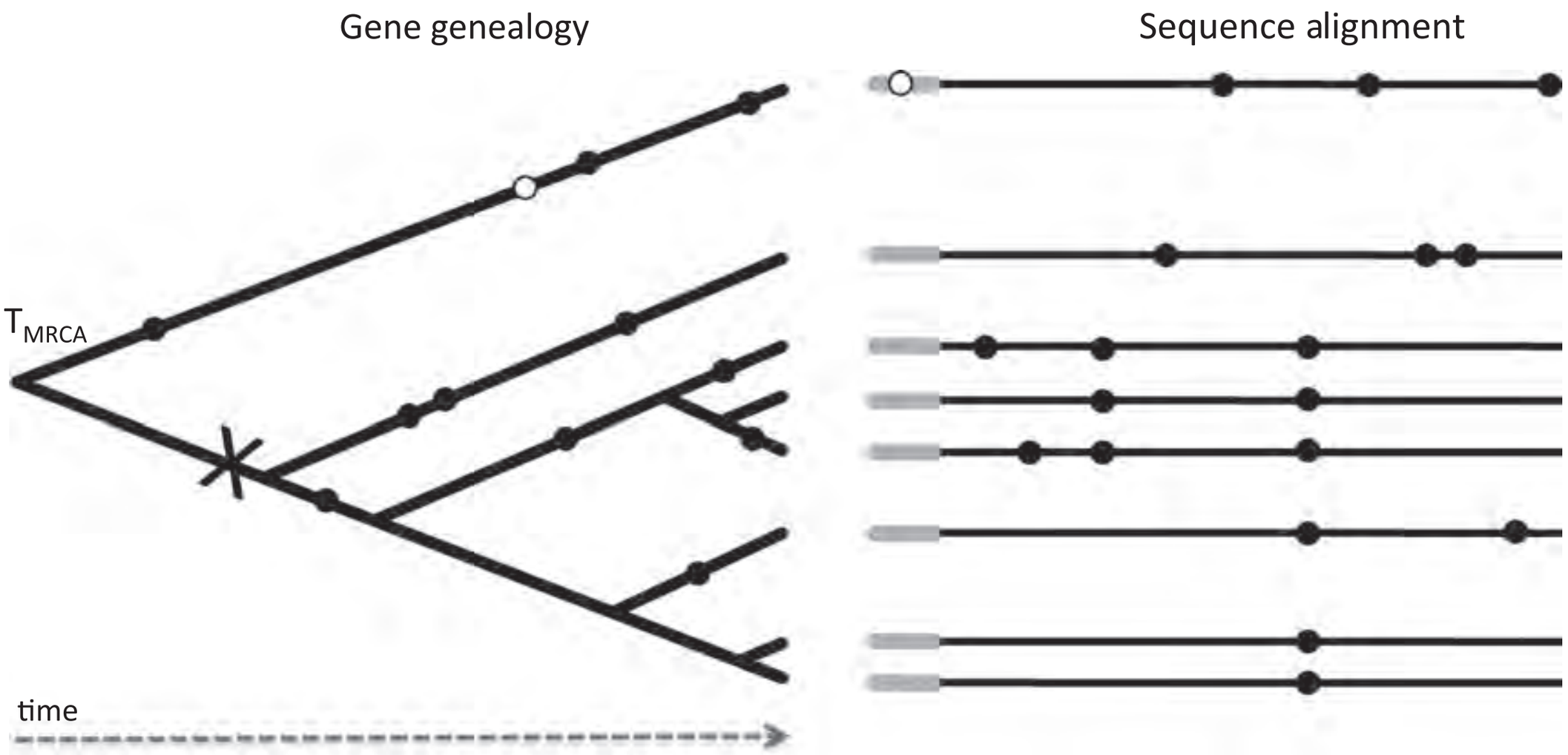


‘Bias’ in RADseq

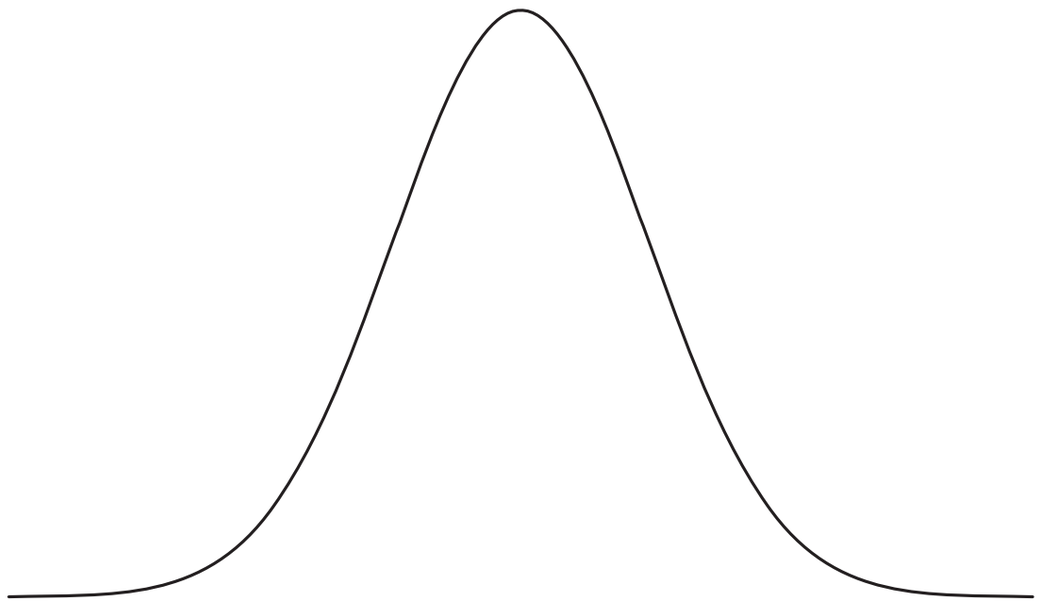
MOLECULAR ECOLOGY
Molecular Ecology (2013) **22**, 3179–3190 doi: 10.1111/mec.12

RADseq underestimates diversity and introduces genealogical biases due to nonrandom haplotype sampling

B. ARNOLD,[1] R. B. CORBETT-DETIG,[1] D. HARTL and K. BOMBLIES
Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA



‘Bias’ in RADseq

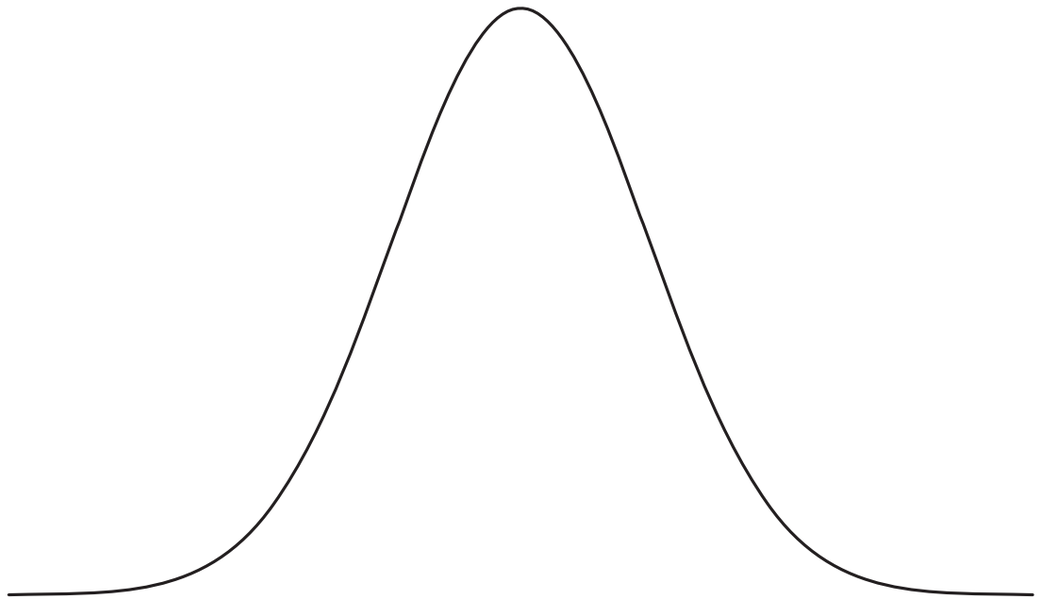


$$f(x) = \frac{1}{\sqrt{2\pi\sigma}} e^{\frac{-(x-\mu)^2}{2\sigma^2}}$$

$e = 2.7182....$

$\pi = 3.1415....$

‘Bias’ in RADseq



$$\overline{x} = \frac{1}{n}\sum_{i-1}^{n} x_i$$

$$s^2 = \frac{1}{n-1}\sum_{i-1}^{n} (y_i - \overline{y})^2$$

‘Bias’ in RADseq is increased in some RAD protocols

		Mean Recombination	
Protocol	θ per bp	θ_{we}/θ_{wa}	π_e/π_a
Standard	0.0001	0.994	0.995
	0.001	0.987	0.982
	0.01	0.956	0.933
Double digest	0.0001	0.835	0.836
	0.001	0.858	0.851
	0.01	0.829	0.797

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Biological studies that benefit from whole genome approaches powered by RAD-seq

- Defining individuality, parentage and pedigrees
- Performing quantitative genetic studies in outbred populations
- Fine scale estimates of population structure
- Identifying the genetic basis of inbreeding depression
- Making management decisions for biological populations
- Genome Wide Association Studies (GWAS) studies of traits
- Building genetic maps to genetically enable non model organisms
- Estimating species and higher level phylogenetic relationships
- Population genomics - identifying the signatures of natural selection

Biological studies that benefit from whole genome approaches powered by RAD-seq

- Defining individuality, parentage and pedigrees
- Performing quantitative genetic studies in outbred populations
- Fine scale e
- Identifying
- Making ma
- Genome W
- Building ge anisms
- Estimating species and higher level phylogenetic relationships
- Population genomics - identifying the signatures of natural selection

Any study where improving biological sample size would be beneficial

Outline for today's lecture

RAD-seq for ecological and evolutionary genomics

Primer on Population Genomics

Evolutionary genomics of stickleback fish

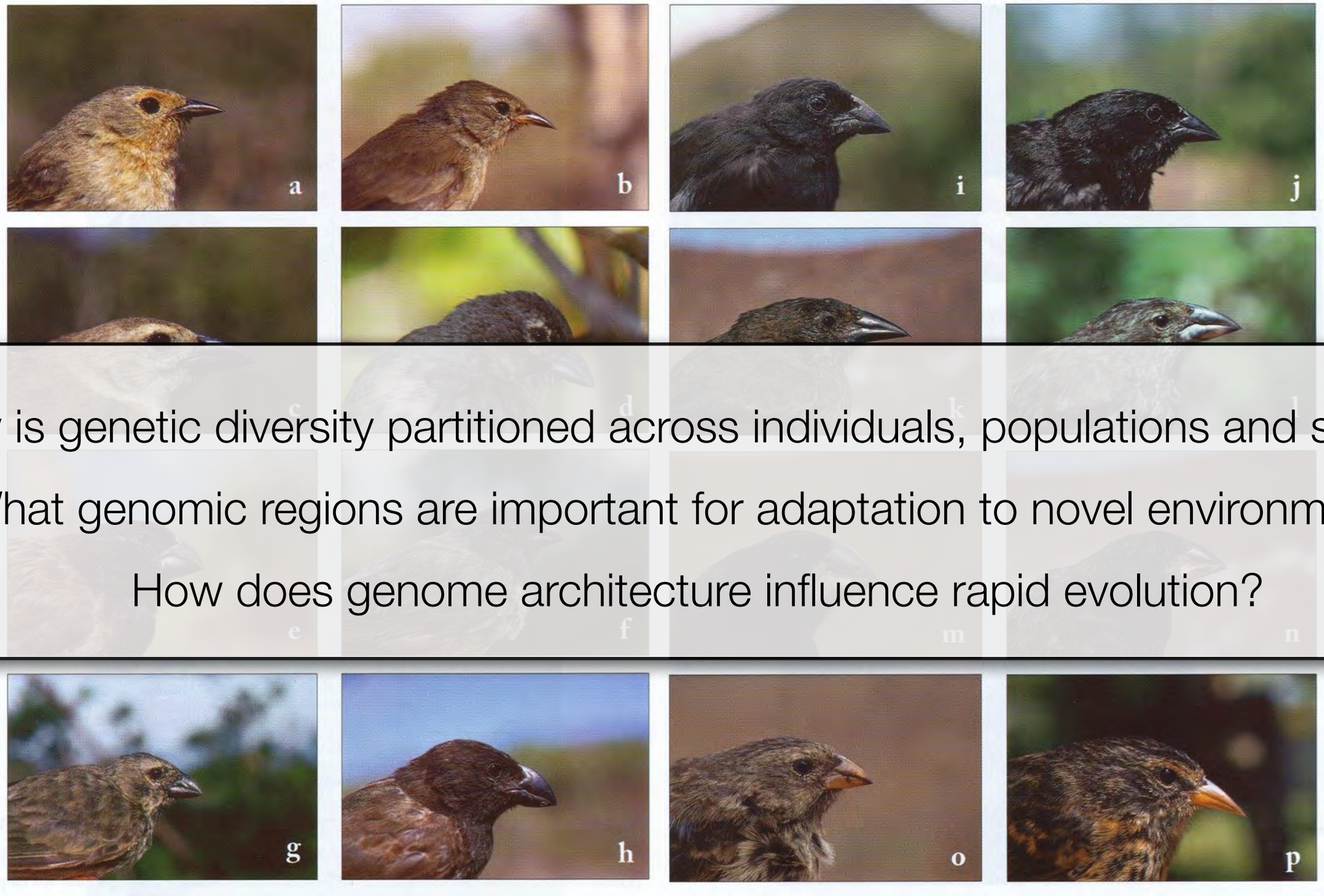
- Population genomics of rapid adaptation
- Using long read RAD-seq for coalescent analyses
- Genome Wide Association Studies using RAD-seq

Genomically enabling the Gulf pipefish

How do organisms adapt to novel environments?

from Grant and Grant. 2007. How and why species multiply: The radiation of Darwin's finches. Princeton University Press

How do organisms adapt to novel environments?



How is genetic diversity partitioned across individuals, populations and species?

What genomic regions are important for adaptation to novel environments?

How does genome architecture influence rapid evolution?

from Grant and Grant. 2007. How and why species multiply: The radiation of Darwin's finches. Princeton University Press

Four fundamental processes in evolution

Origin of genetic variation

mutation

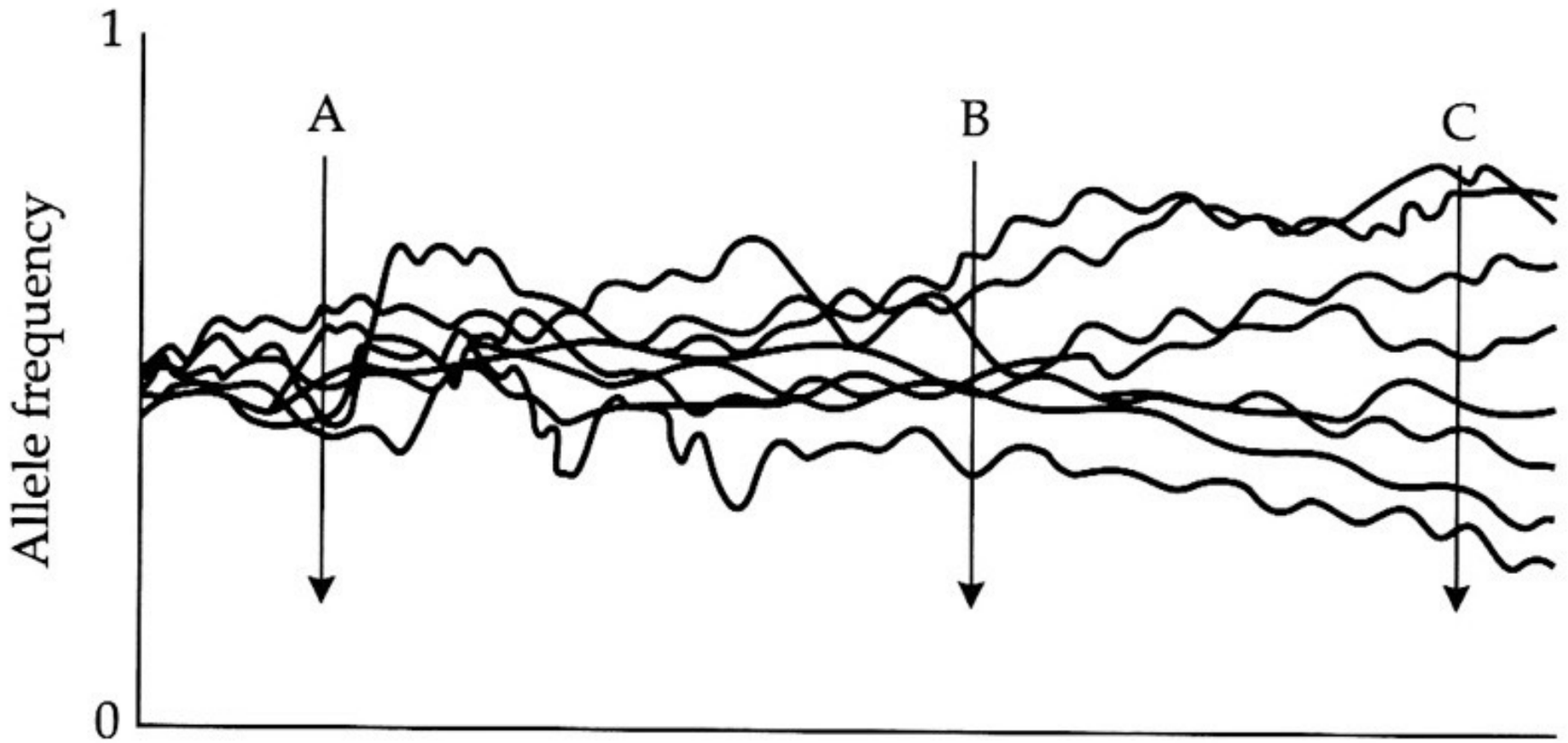
migration

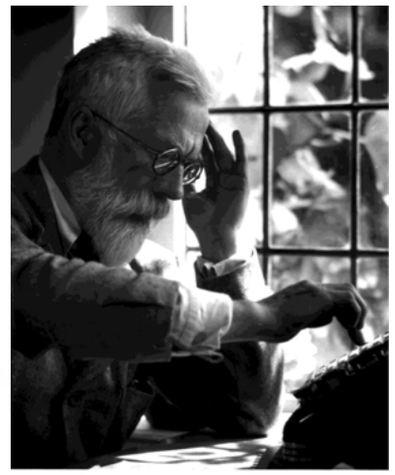
Sorting of variation

genetic drift

natural selection

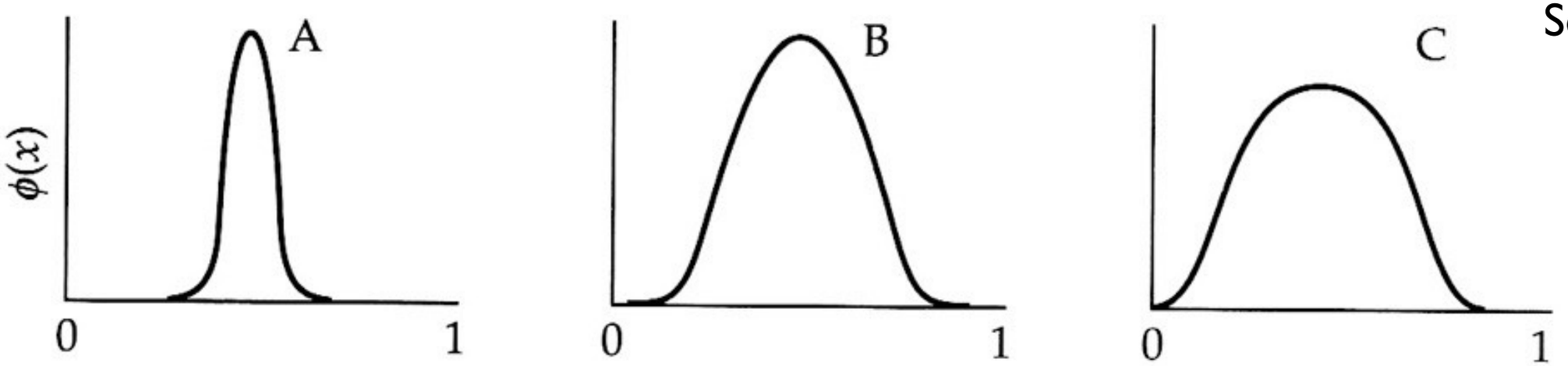
Genetic drift is a null model

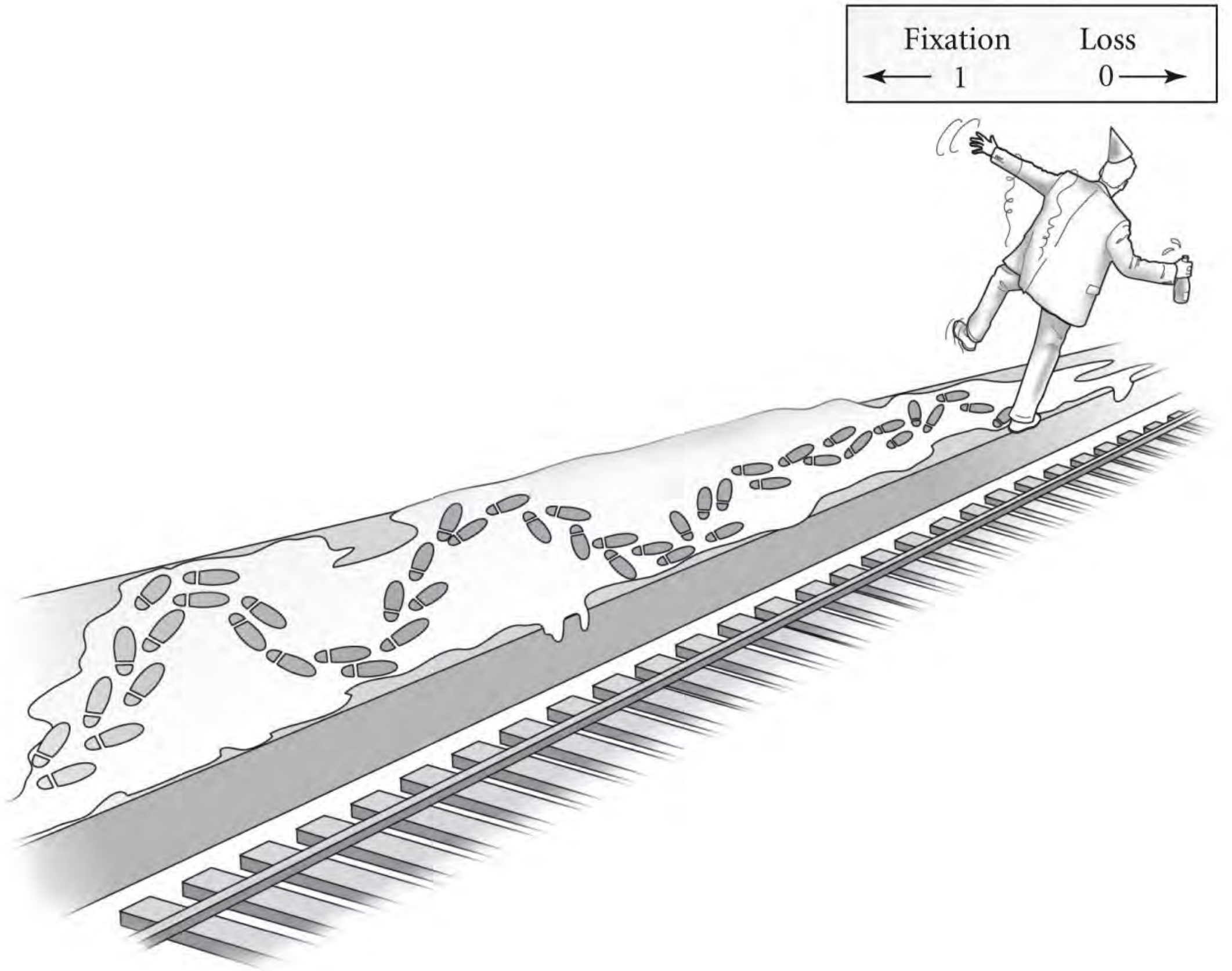




R. A. Fisher

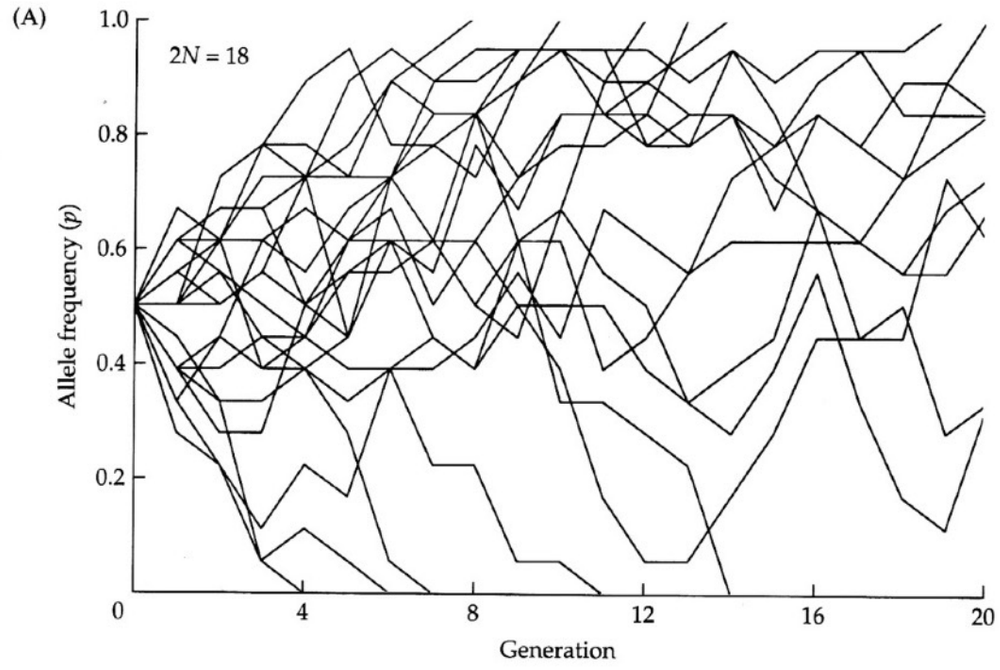
Sewall Wright

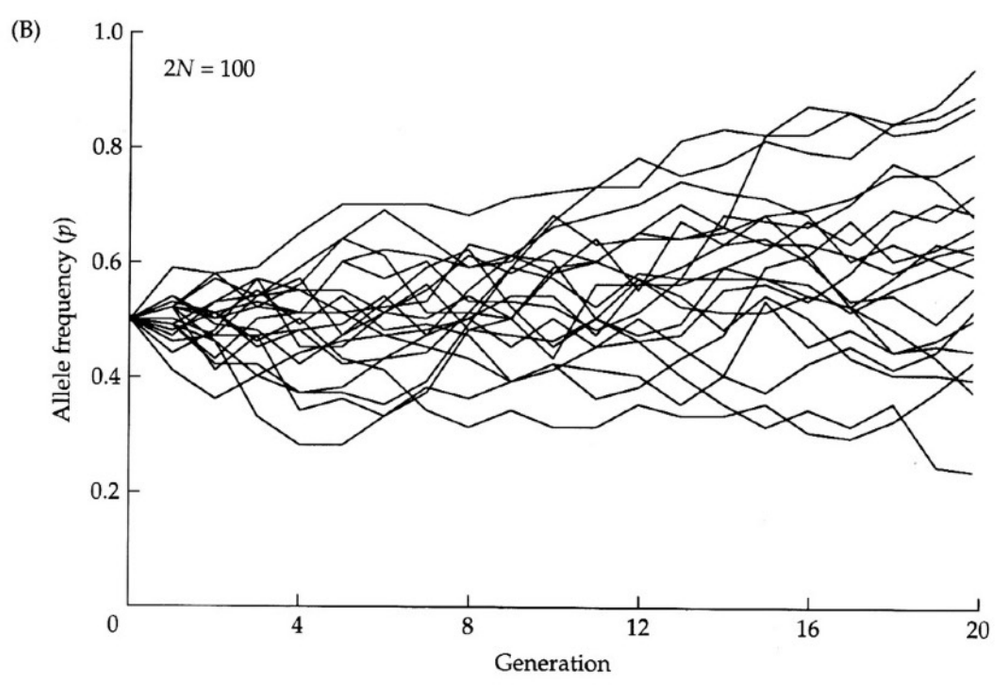




***EVOLUTION 2e*, Figure 10.2**

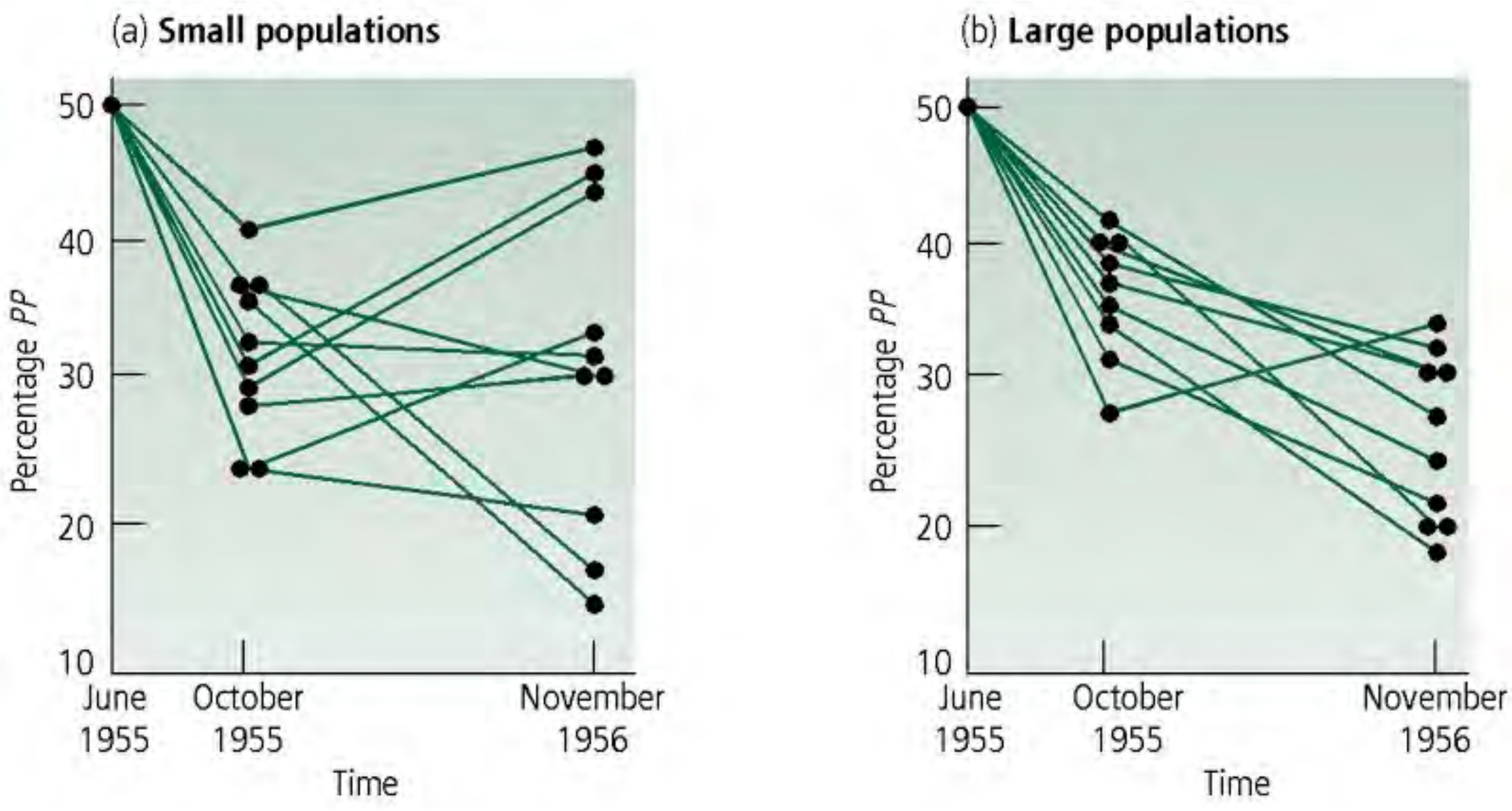
Size of population (Ne) affects rate of spread (diffusion)





Affects the entire genome equally (on average)

Natural selection biases the allele frequency change, but drift is still occurring



*** The effects of selection can be genomically localized ***

Population genomics

Simultaneous genotyping of **neutral** and **adaptive** loci

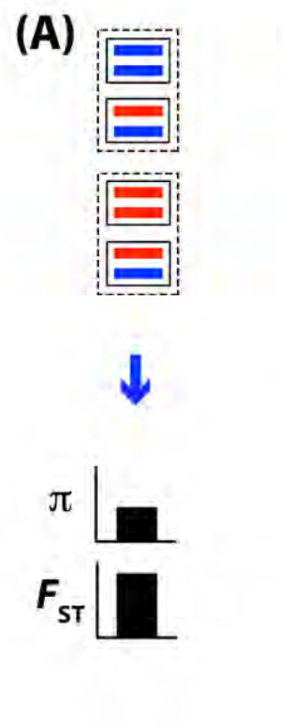
Genome-wide background provides more precise estimates:

- Demographic processes (e.g. N_e)
- Phylogeography

Outliers from background indicate:

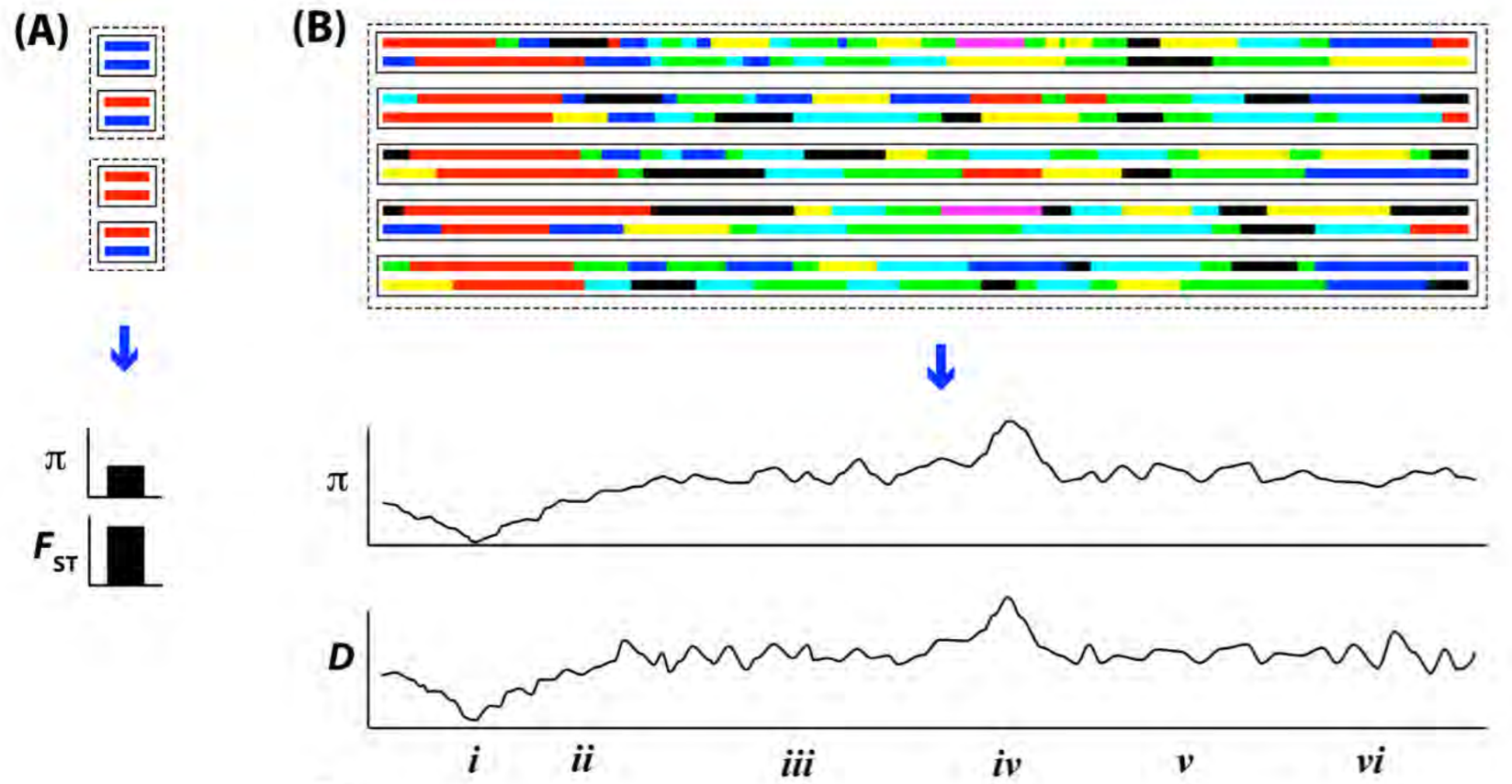
- Selective sweeps
- Local adaptation

Population genomics of ordered markers



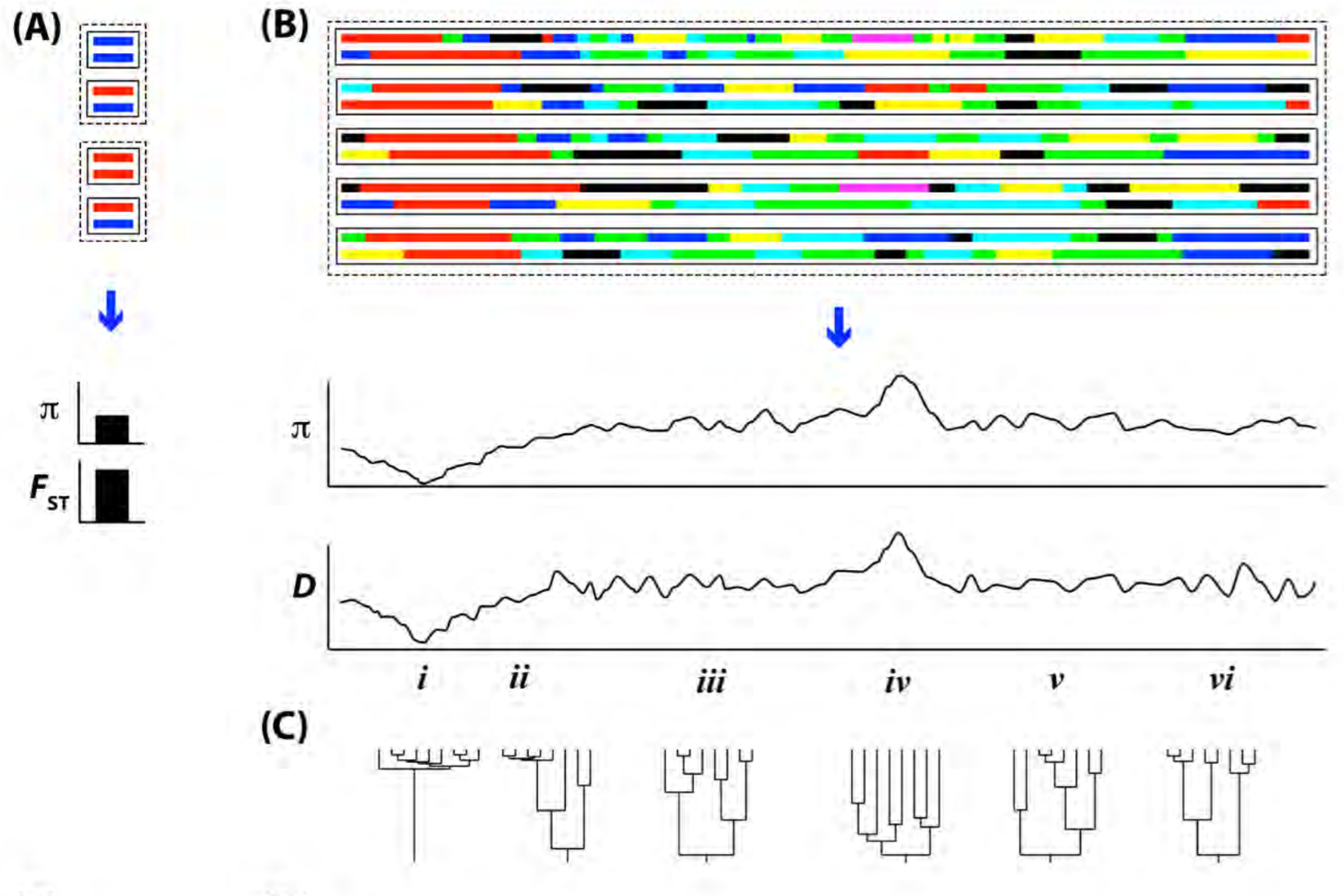
Hohenlohe et al. 2010. Int J Plant Sciences

Population genomics of ordered markers



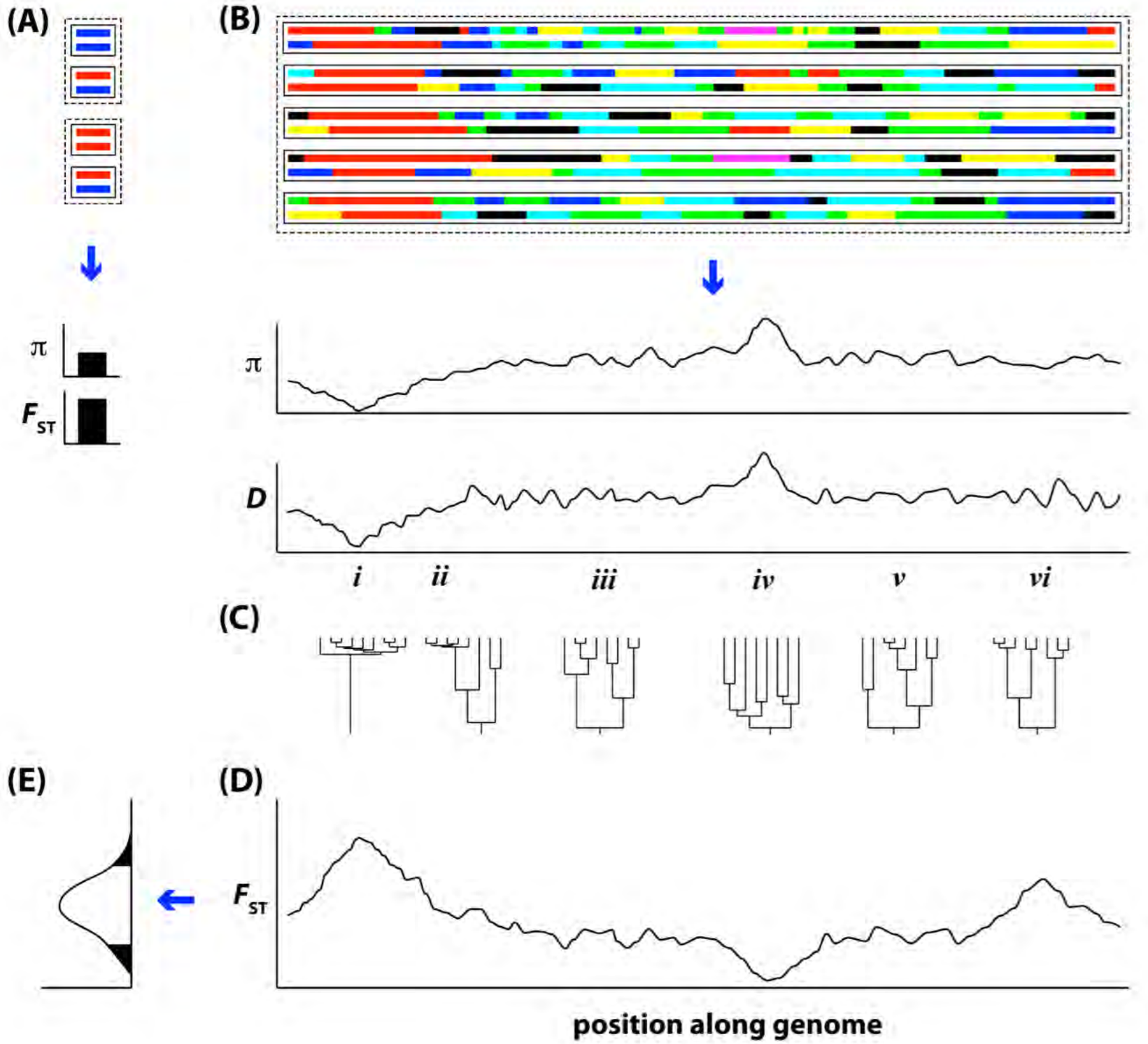
Hohenlohe et al. 2010. Int J Plant Sciences

Population genomics of ordered markers



Hohenlohe et al. 2010. Int J Plant Sciences

Population genomics of ordered markers

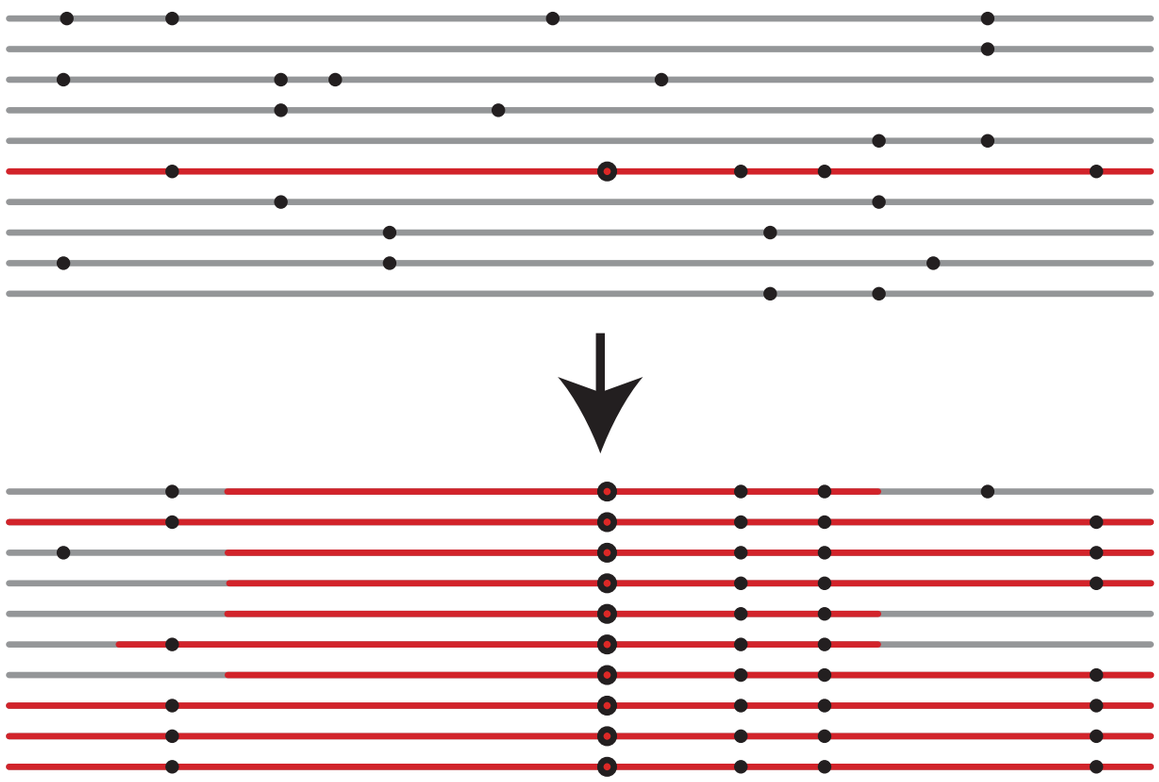


Hohenlohe et al. 2010. Int J Plant Sciences

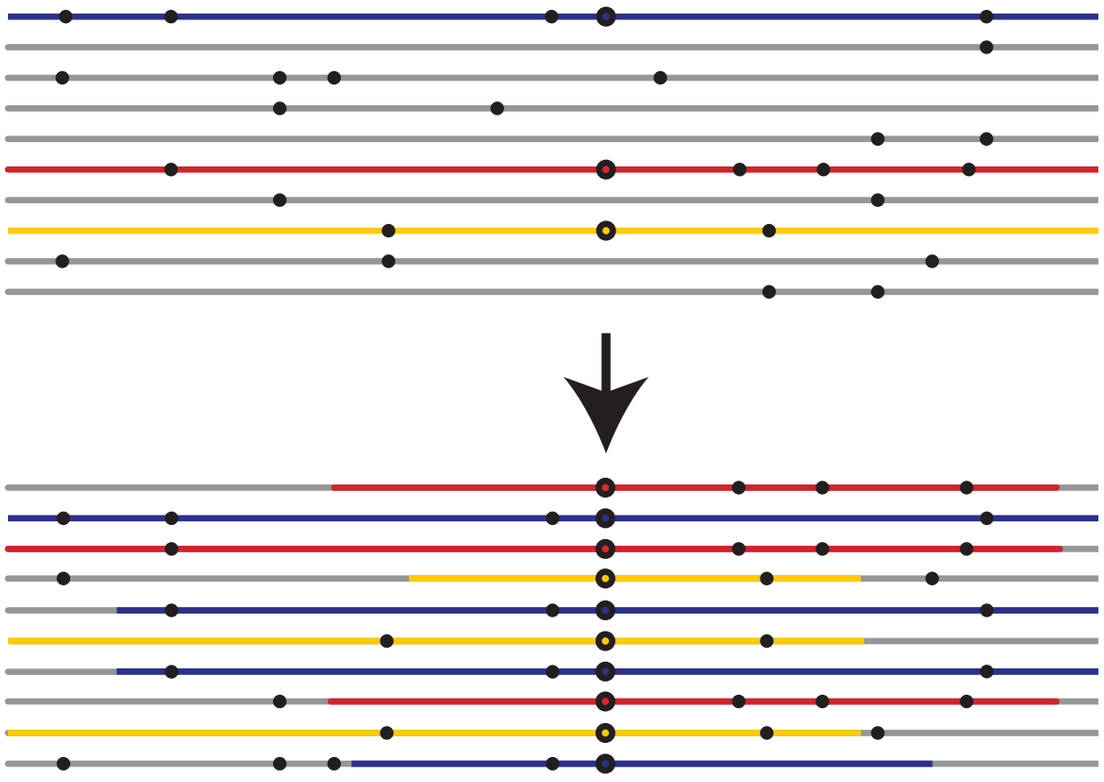
Sweeps of directional selection across genomes

Past

Hard Sweep

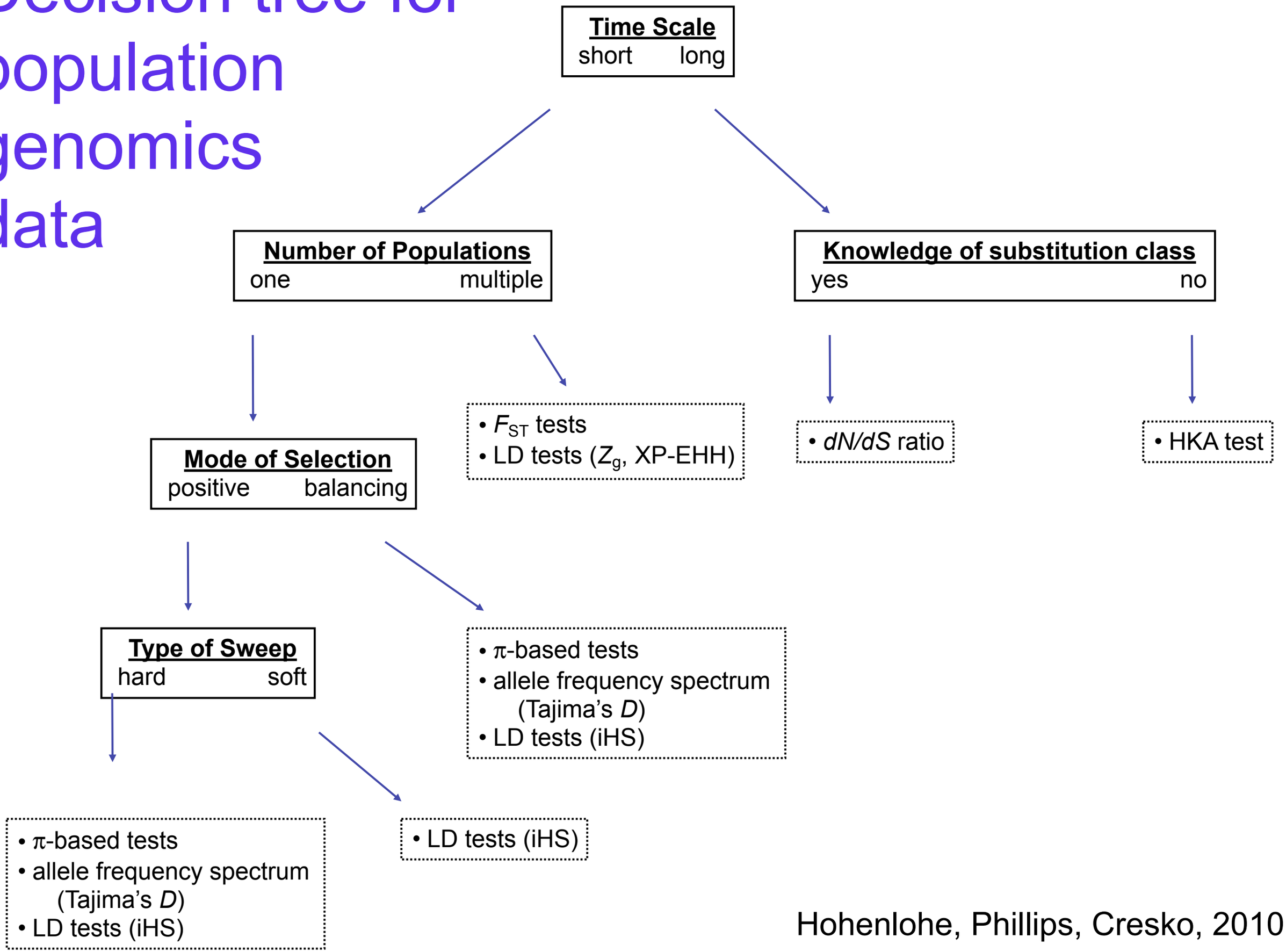


Soft Sweep



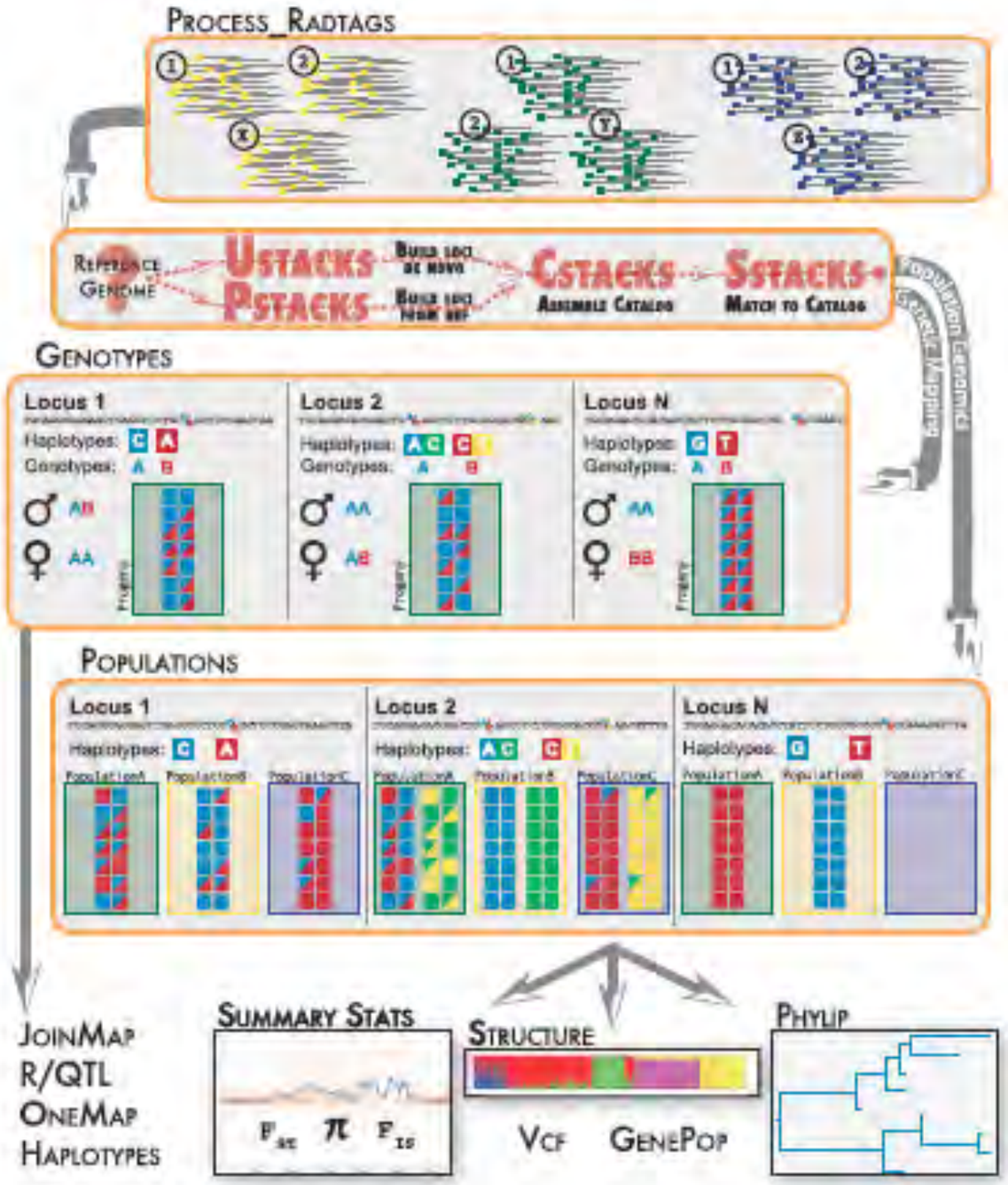
Present

Decision tree for population genomics data



Hohenlohe, Phillips, Cresko, 2010

Stacks analysis pipeline for RAD-seq



Stacks: Building and Genotyping Loci *De Novo* From Short-Read Sequences

Julian M. Catchen,* Angel Amores,† Paul Hohenlohe,* William Cresko,* and John H. Postlethwait†,1

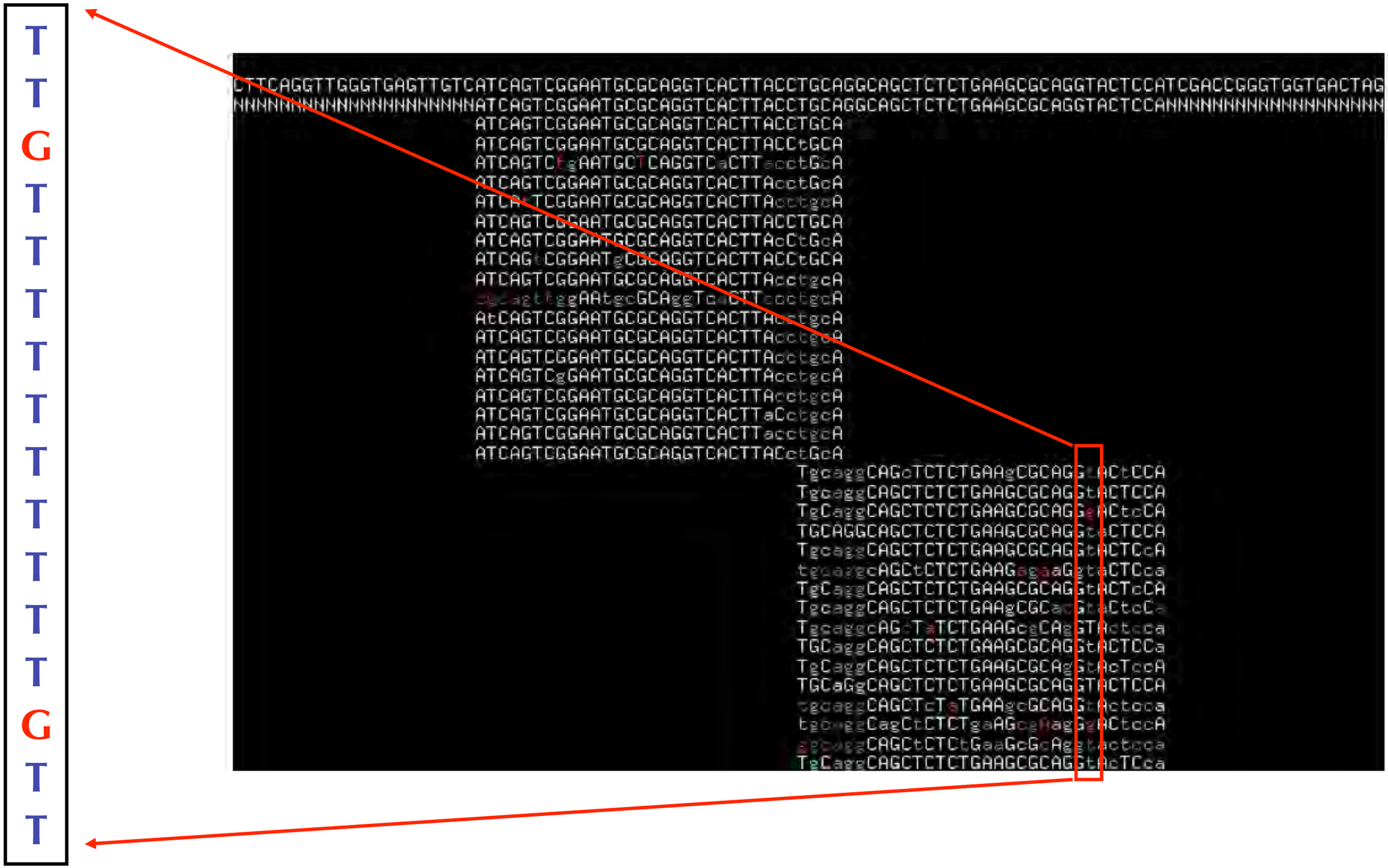
*Center for Ecology and Evolutionary Biology and †Institute of Neuroscience, University of Oregon, Eugene, Oregon 97403

Stacks: an analysis tool set for population genomics

JULIAN CATCHEN,* PAUL A. HOHENLOHE,*† SUSAN BASSHAM,* ANGEL AMORES‡ and WILLIAM A. CRESKO*

*Institute of Ecology and Evolution, University of Oregon, Eugene, OR 97403-5289, USA, †Biological Sciences, University of Idaho, Moscow, ID 83844-3051, USA, ‡Institute of Neuroscience, University of Oregon, Eugene, OR 97403-1254, USA

Stacks



Stacks

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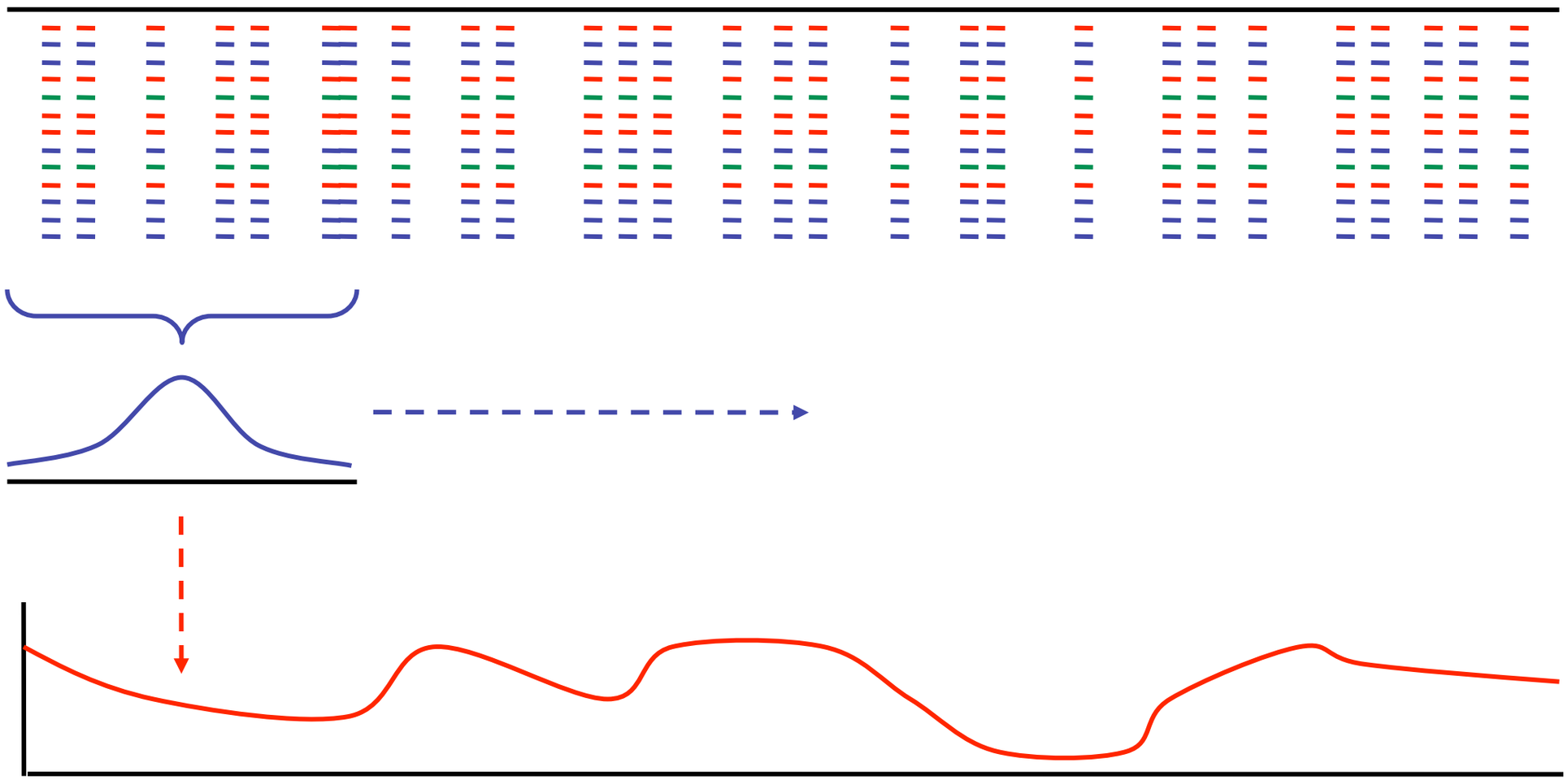
$$L(n_1\,\text{hom}) = P(n_1,n_2,n_3,n_4) = \frac{n!}{n_1!n_2!n_3!n_4!}\left(1-\frac{3\varepsilon}{4}\right)^{n_1}\left(\frac{\varepsilon}{4}\right)^{n_2}\left(\frac{\varepsilon}{4}\right)^{n_3}\left(\frac{\varepsilon}{4}\right)^{n_4}$$

$$L(n_1 n_2\,\text{het}) = P(n_1,n_2,n_3,n_4) = \frac{n!}{n_1!n_2!n_3!n_4!}\left(0.5-\frac{\varepsilon}{4}\right)^{n_1}\left(0.5-\frac{\varepsilon}{4}\right)^{n_2}\left(\frac{\varepsilon}{4}\right)^{n_3}\left(\frac{\varepsilon}{4}\right)^{n_4}$$

Maximum likelihood genotyping based on multinomial distribution of nucleotide reads

Making statistics continuous across the genome

Kernel-smoothing average of summary statistics along genome



Bootstrap re-sampling to estimate significance of moving average

Stacks

7.4.2 batch_X.sumstats_summary.tsv: Summary of summary statistics for each population

Column	Name	Description
1	Pop ID	Population ID as defined in the Population Map file.
2	Private	Number of private alleles in this population.
3	Number of Individuals	Mean number of individuals per locus in this population.
4	Variance	
5	Standard Error	
6	P	Mean frequency of the most frequent allele at each locus in this population.
7	Variance	
8	Standard Error	
9	Observed Heterozygosity	Mean obsered heterozygosity in this population.
10	Variance	
11	Standard Error	
12	Observed Homozygosity	Mean observed homozygosity in this population.
13	Variance	
14	Standard Error	
15	Expected Heterozygosity	Mean expected heterozygosity in this population.
16	Variance	
17	Standard Error	
18	Expected Homozygosity	Mean expected homozygosity in this population.
19	Variance	
20	Standard Error	
21	Π	Mean value of π in this population.
22	Π Variance	
23	Π Standard Error	
24	F_{IS}	Mean measure of F_{IS} in this population.
25	F_{IS} Variance	
26	F_{IS} Standard Error	

Stacks

7.4.4 batch_X.hapstats.tsv: Haplotype-based summary statistics for each locus in each population

Column	Name	Description
1	Batch ID	The batch identifier for this data set.
2	Locus ID	Catalog locus identifier.
3	Chromosome	If aligned to a reference genome.
4	Basepair	If aligned to a reference genome.
5	Population ID	The ID supplied to the populations program, as written in the population map file.
6	N	Number of alleles/haplotypes present at this locus.
7	Haplotype count	
8	Gene Diversity	
9	Smoothed Gene Diversity	
10	Smoothed Gene Diversity P-value	
11	Haplotype Diversity	
12	Smoothed Haplotype Diversity	
13	Smoothed Haplotype Diversity P-value	
14	Haplotypes	A semicolon-separated list of haplotypes/haplotype counts in the population.

Stacks

7.4.3 batch_X.fst_Y-Z.tsv: F_{ST} calculations for each pair of populations

Column	Name	Description
1	Batch ID	The batch identifier for this data set.
2	Locus ID	Catalog locus identifier.
3	Population ID 1	The ID supplied to the populations program, as written in the population map file.
4	Population ID 2	The ID supplied to the populations program, as written in the population map file.
5	Chromosome	If aligned to a reference genome.
6	Basepair	If aligned to a reference genome.
7	Column	The nucleotide site within the catalog locus, reported using a zero-based offset (first nucleotide is enumerated as 0).
8	Overall π	An estimate of nucleotide diversity across the two populations.
9	F_{ST}	A measure of population differentiation.
10	FET p-value	P-value describing if the F_{ST} measure is statistically significant according to Fisher's Exact Test.
11	Odds Ratio	Fisher's Exact Test odds ratio.
12	CI High	Fisher's Exact Test confidence interval.
13	CI Low	Fisher's Exact Test confidence interval.
14	LOD Score	Logarithm of odds score.
15	Corrected F_{ST}	F_{ST} with either the FET p-value, or a window-size or genome size Bonferroni correction.
16	Smoothed F_{ST}	A weighted average of F_{ST} depending on the surrounding 3σ of sequence in both directions.
17	AMOVA F_{ST}	Analysis of Molecular Variance alternative F_{ST} calculation. Derived from Weir, Genetic Data Analysis II, chapter 5, "F Statistics," pp166-167.
18	Corrected AMOVA F_{ST}	AMOVA F_{ST} with either the FET p-value, or a window-size or genome size Bonferroni correction.
19	Smoothed AMOVA F_{ST}	A weighted average of AMOVA F_{ST} depending on the surrounding 3σ of sequence in both directions.
20	Smoothed AMOVA F_{ST} P-value	If bootstrap resampling is enabled, a p-value ranking the significance of F_{ST} within this pair of populations.
21	Window SNP Count	Number of SNPs found in the sliding window centered on this nucleotide position.

Notes: The preferred version of F_{ST} is the **AMOVA F_{ST}** in column 17, or the corrected version in column 18 if you have specified a correction to the **`populations`** program (option `-f`)

7.4.6 batch_X.phistats_Y-Z.tsv: Haplotype-based F_{ST} calculations for each pair of populations

Column	Name	Description
1	Batch ID	The batch identifier for this data set.
2	Locus ID	Catalog locus identifier.
3	Population ID 1	The ID supplied to the populations program, as written in the population map file.
4	Population ID 2	The ID supplied to the populations program, as written in the population map file.
5	Chromosome	If aligned to a reference genome.
6	Basepair	If aligned to a reference genome.
7	Φ_{ST}	
8	Smoothed Φ_{ST}	
9	Smoothed Φ_{ST} P-value	
10	F_{ST}'	
11	Smoothed F_{ST}'	
12	Smoothed F_{ST}' P-value	
13	D_{EST}	
14	Smoothed D_{EST}	
15	Smoothed D_{EST} P-value	

Outline for today's lecture

RAD-seq for ecological and evolutionary genomics

Primer on Population Genomics

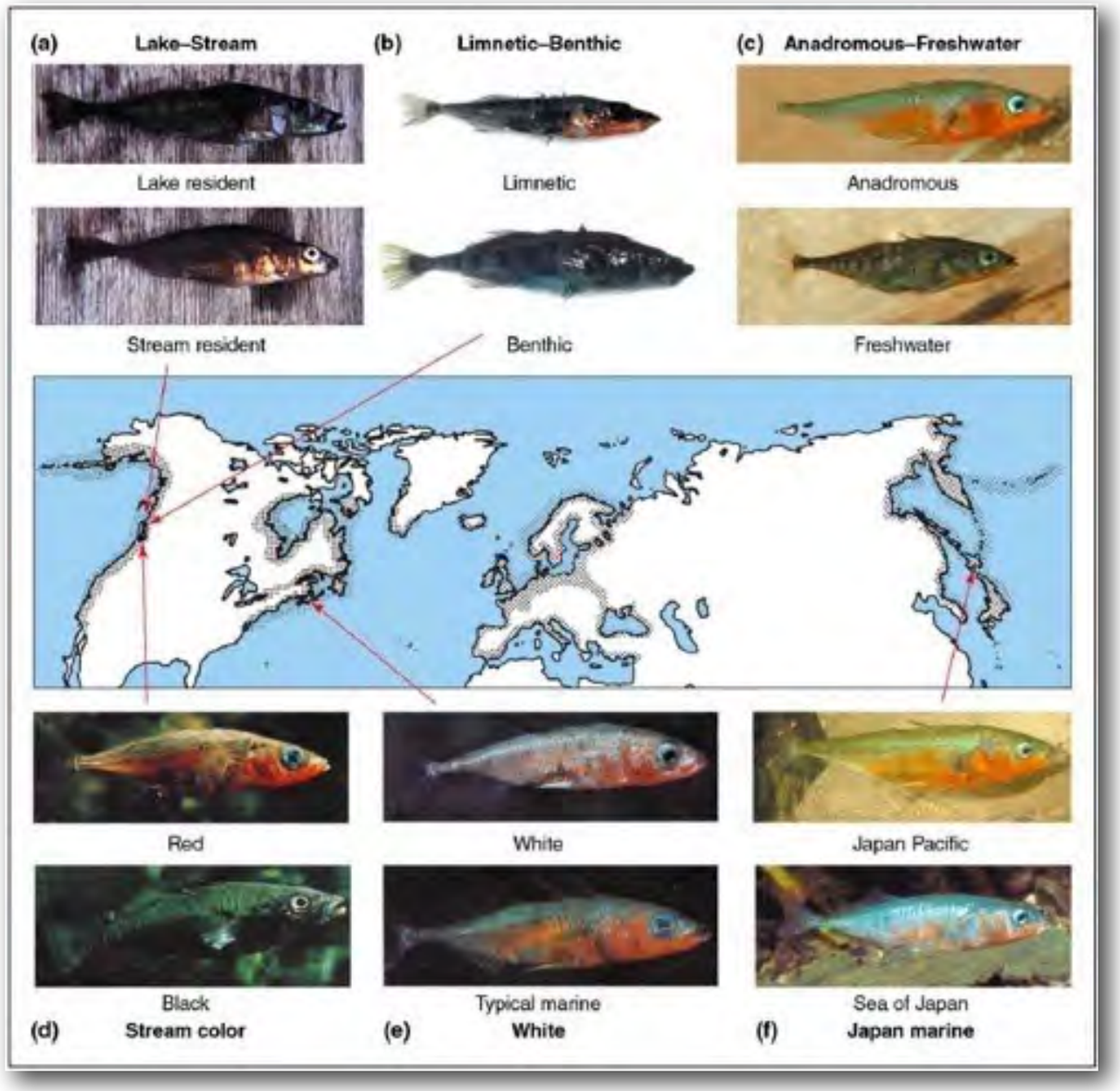
Evolutionary genomics of stickleback fish

- Population genomics of rapid adaptation
- Using haplo-RAD-seq for coalescent analyses
- Genome Wide Association Studies using RAD-seq

Genomically enabling the Gulf pipefish

Threespine stickleback, *Gasterosteus aculeatus*

Threespine stickleback, *Gasterosteus aculeatus*

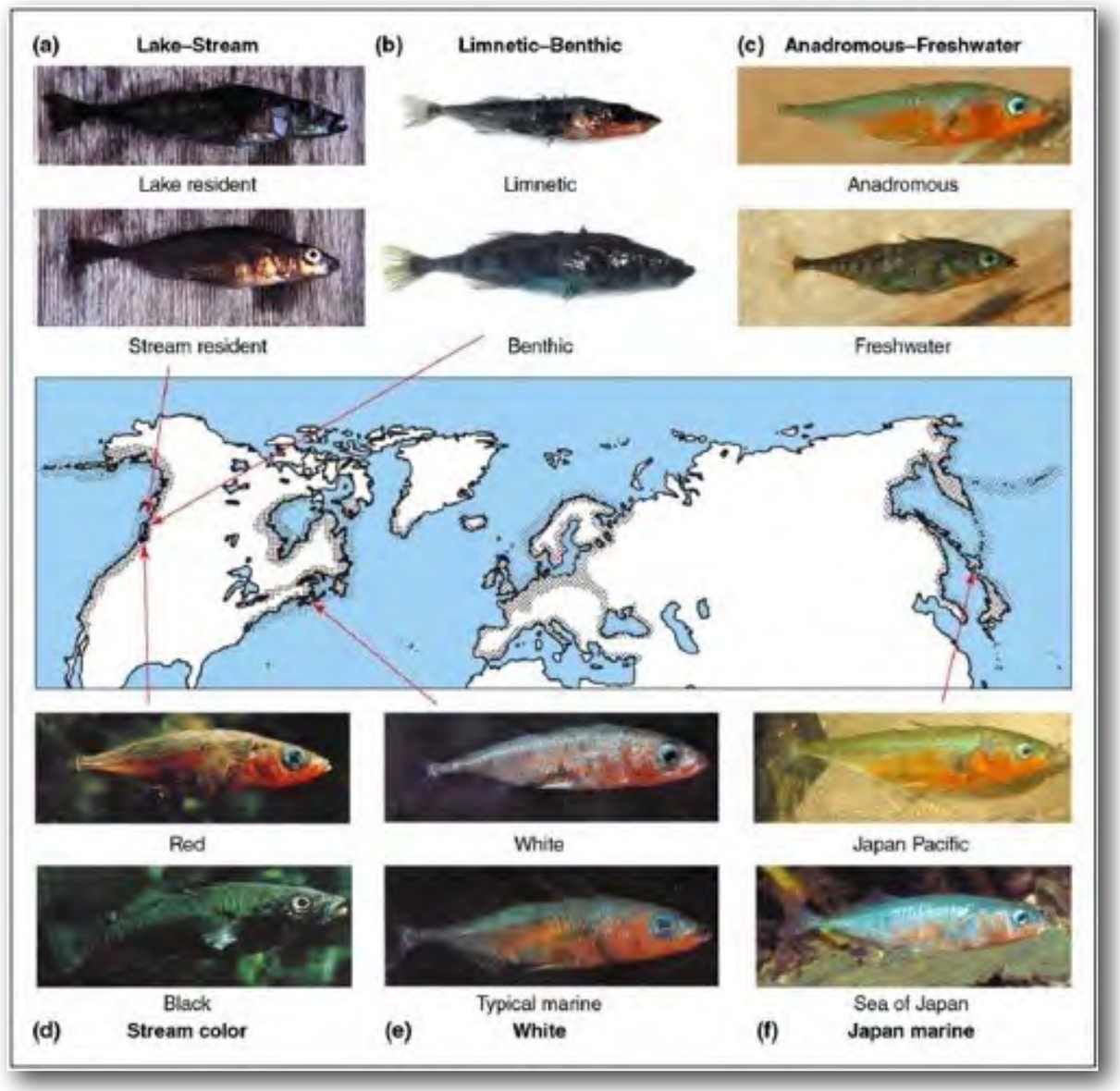


Rundle and McKinnon 2002

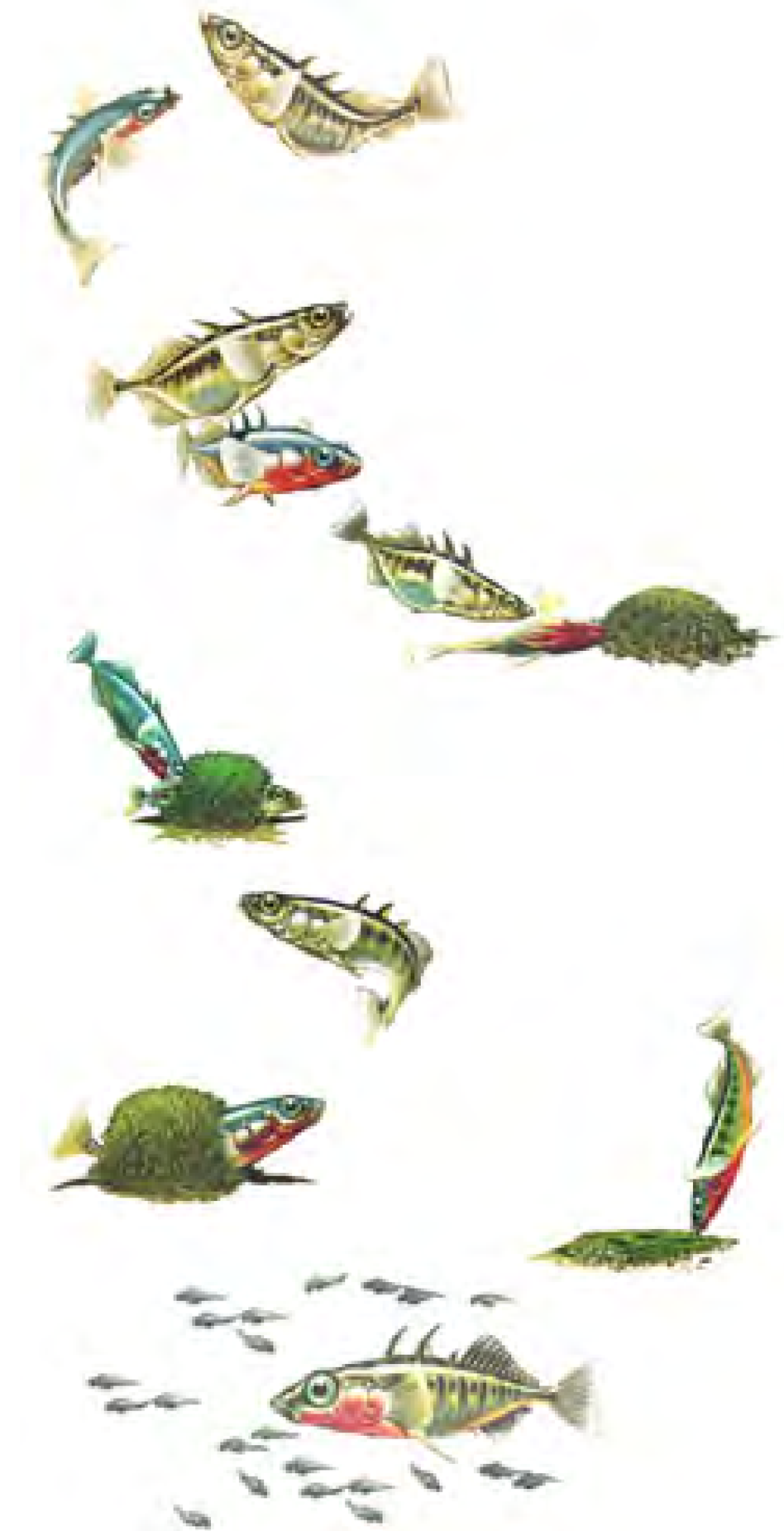
Threespine stickleback, *Gasterosteus aculeatus*

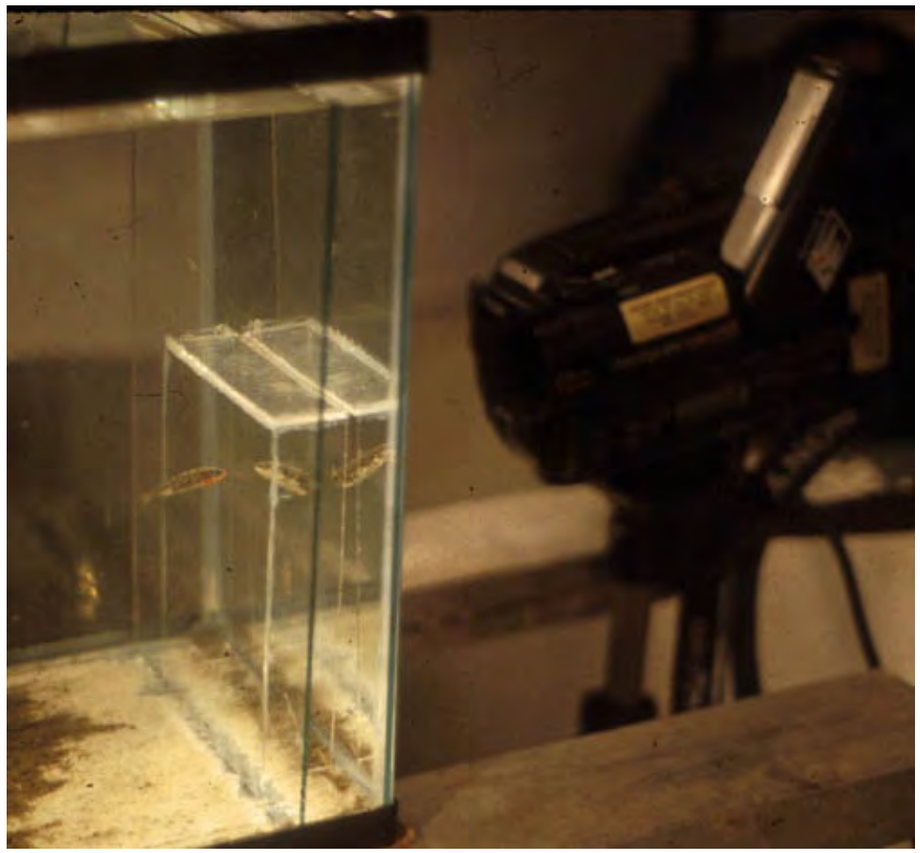
Pelvic Structure

Lateral Plates



Rundle and McKinnon 2002





Stickleback phenotypes mapped in the lab so far.....

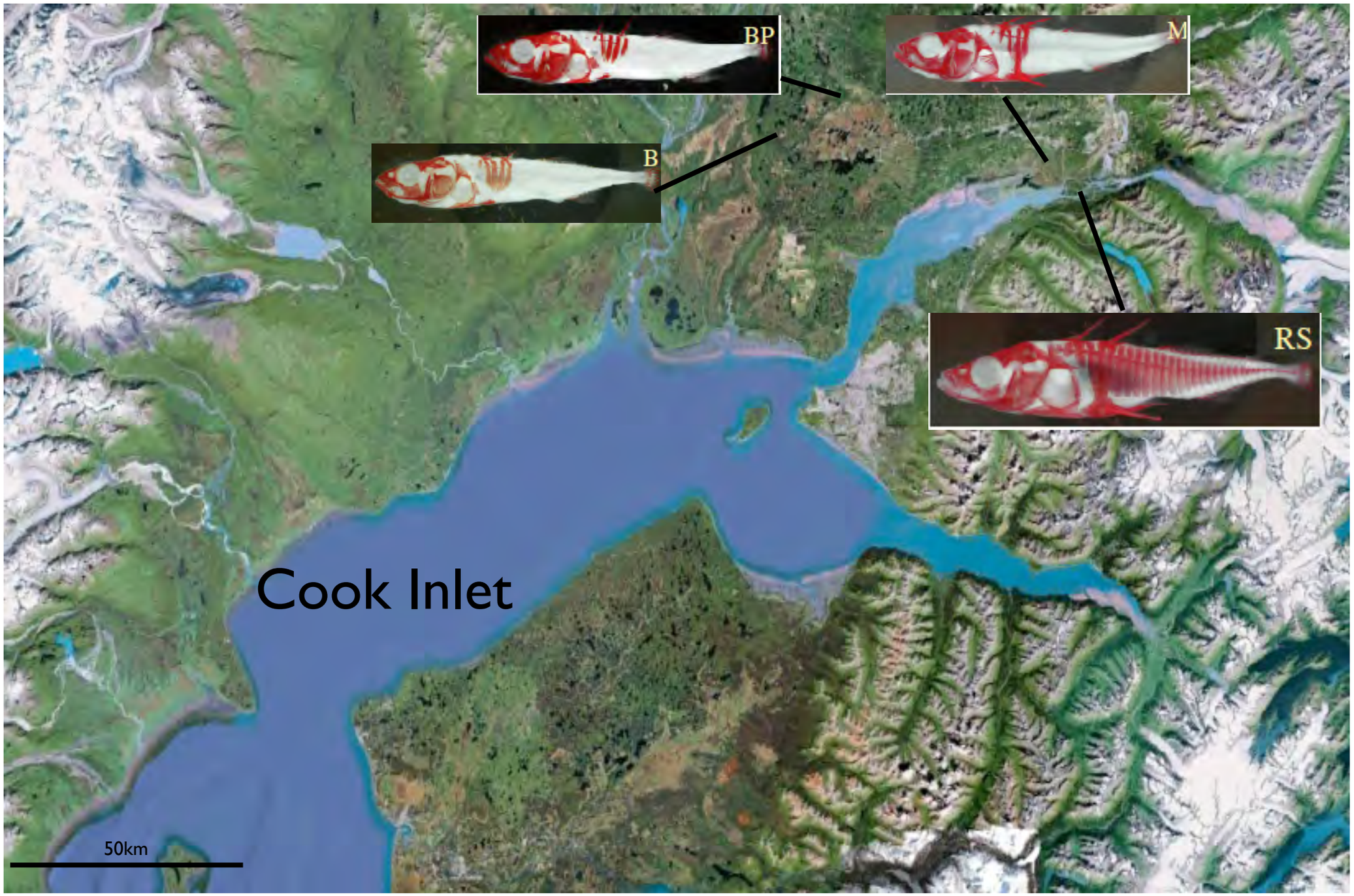
Pelvic structure size and shape *** (*Eda*)
Lateral plate number *** (*Pitx1*)
Body coloration *** (*KitL*)
Opercle bone shape
Pelvic spine length
Body shape
Courtship behavior
Gill raker size
Dorsal spine length

- A trend of large effect loci identified in the laboratory

- Similar genomic regions and sometimes alleles mapped in independent populations

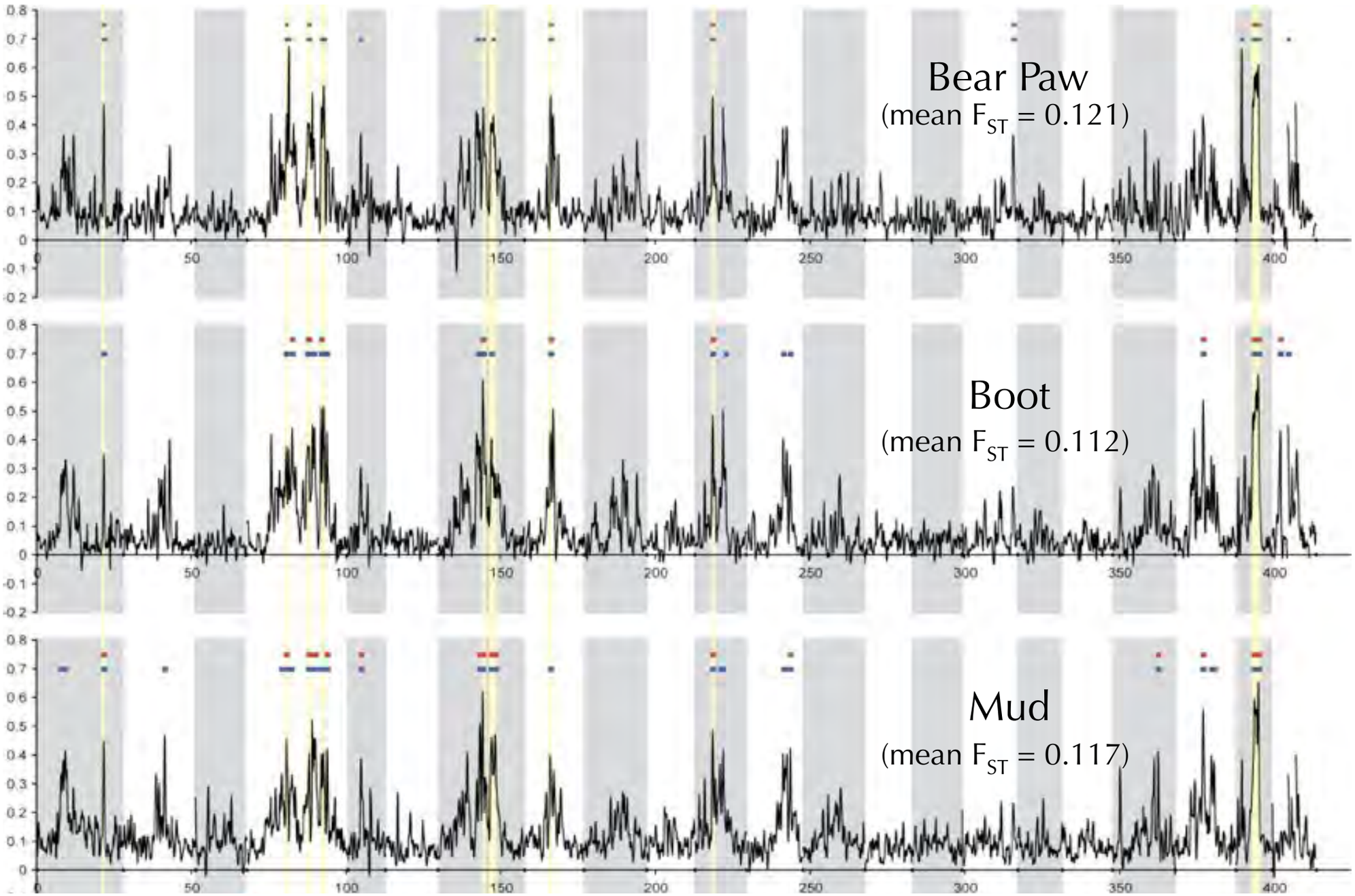
- A question is whether population genomics studies can provide complementary and more complete information.

Signatures of natural selection in 13,000 years



Hohenlohe, Bassham et al. 2010. PLoS Genetics

Signatures of natural selection in 13,000 years



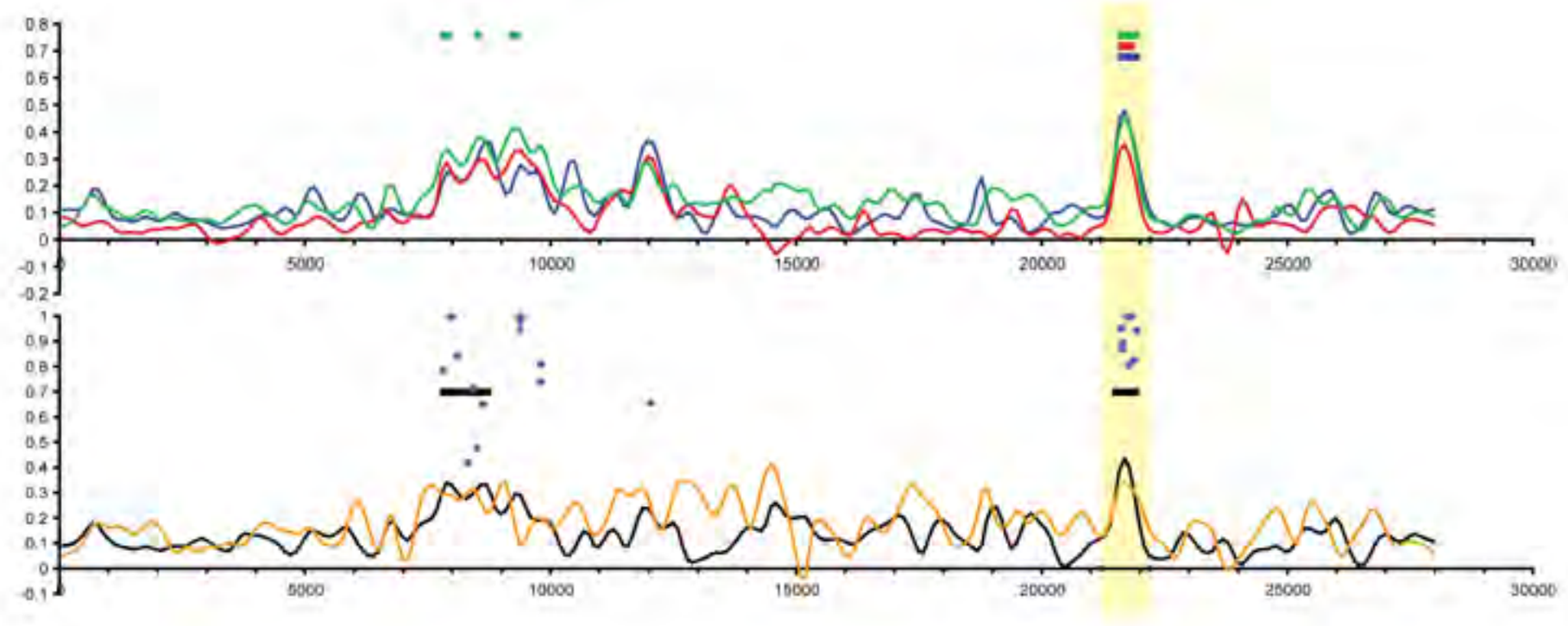
F_{st}

Genomic location (mBases)

Hohenlohe, Bassham et al. 2010. PLoS Genetics

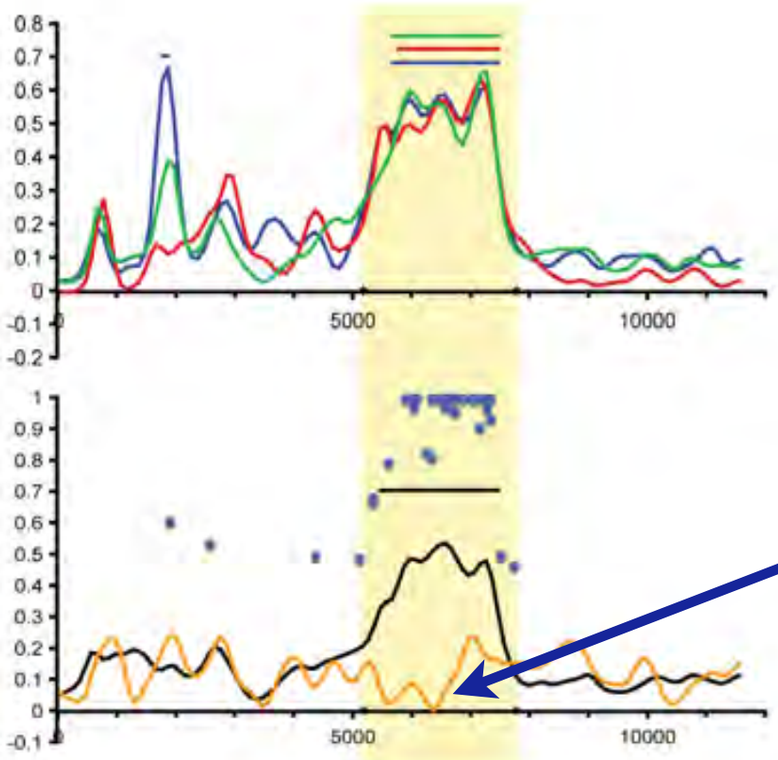
Numerous novel regions identified

LGI



Different alleles

LGXXI



More often the same alleles

Emily Lescak

Julian Catchen

Susan Bassham

Mary Sherbick

Frank von Hippel

PNAS

Evolution of stickleback in 50 years on earthquake-uplifted islands

Emily A. Lescak[a,b], Susan L. Bassham[c], Julian Catchen[c,d], Ofer Gelmond[b,1], Mary L. Sherbick[b], Frank A. von Hippel[b], and William A. Cresko[c,2]

[a]School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Fairbanks, AK 99775; [b]Department of Biological Sciences, University of Alaska Anchorage, Anchorage, AK 99508; [c]Institute of Ecology and Evolution, University of Oregon, Eugene, OR 97403; and [d]Department o University of Illinois at Urbana–Champaign, Urbana, IL 61801

Edited by John C. Avise, University of California, Irvine, CA, and approved November 9, 2015 (received for review June 19, 2015)

NATIONAL SCIENCE FOUNDATION

Middleton Island - 50 year old populations

1955

2008


Independent

Giant Quake Smashes Alaska Cities; 100 Feared Dead, Tidal Waves Roll

Photo Credits: BLM, E-Terra

Middleton Island - 50 year old populations

1955

2008

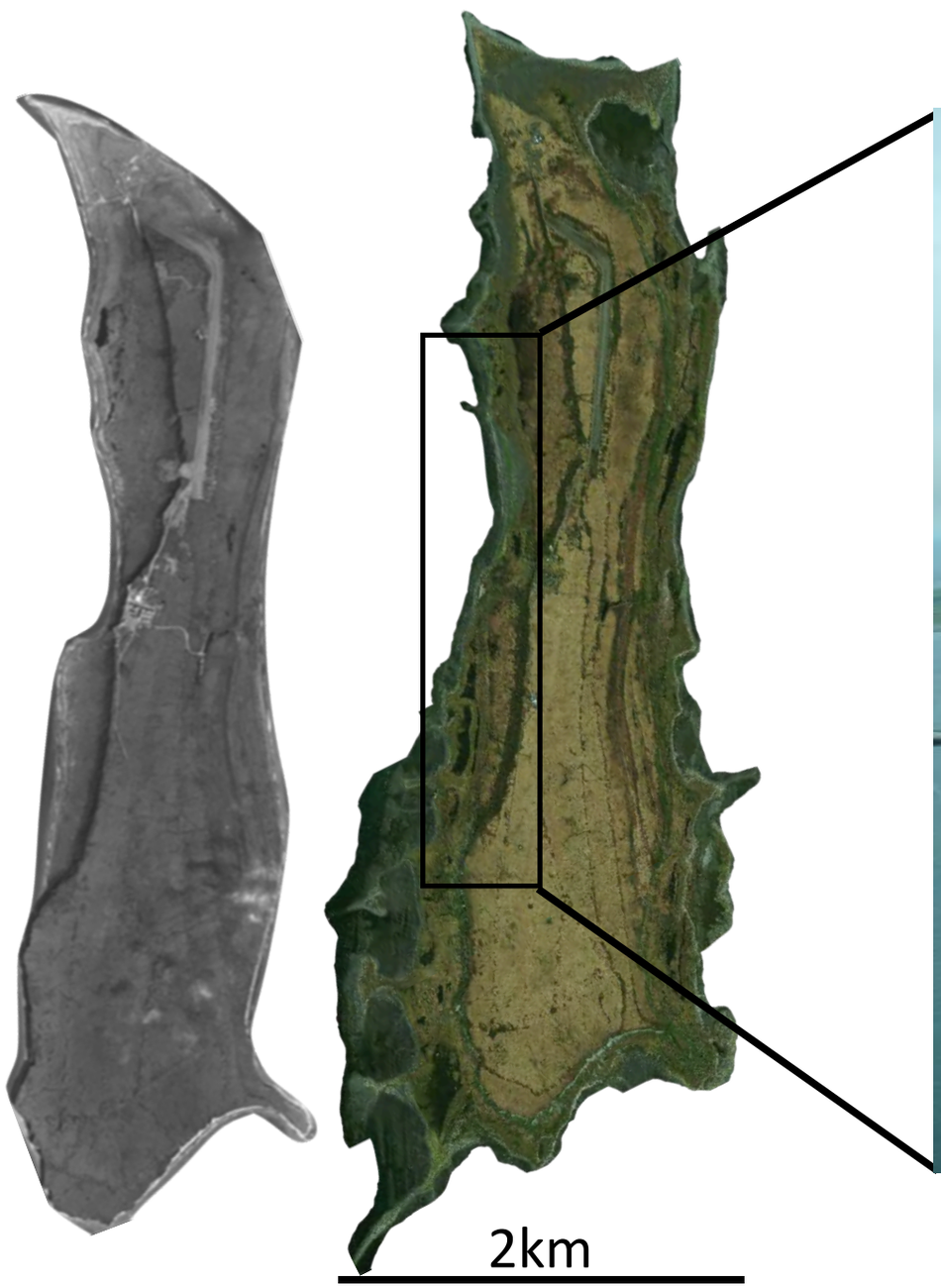


Photo Credits: BLM, E-Terra

Middleton Island - 50 year old locations

1955

2008

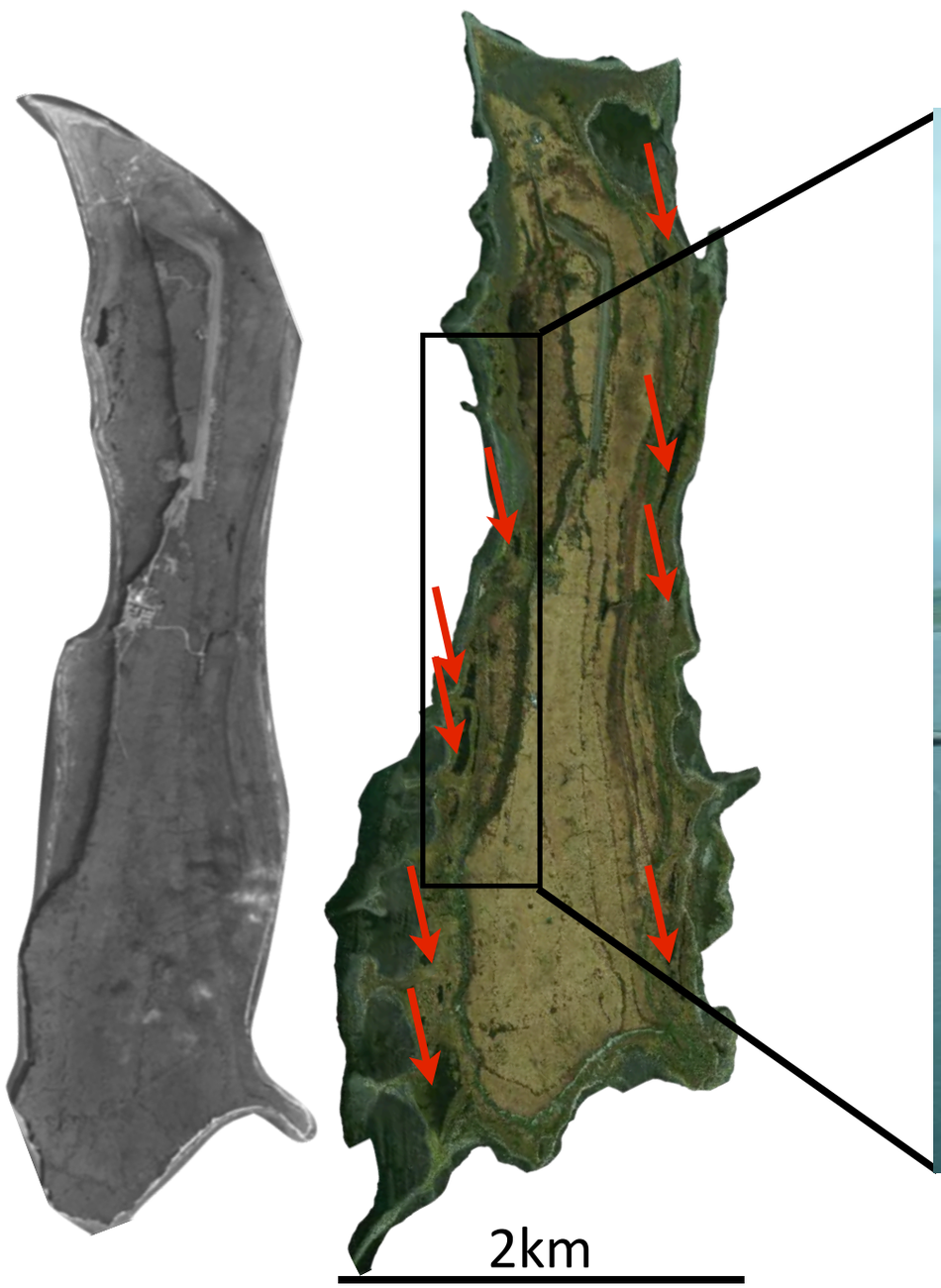


Photo Credits: BLM, E-Terra


MPD SITE 13 BAY


N34WM


DANGER
HIGH
VOLTAGE

B)

Danger Island

Da02

Da04

0.5 km

Oceanic Habitat

Freshwater Habitat

Completely Plated

Low Plated

C)

Montague Island

Mo30

Mo33

Mo35

Mo36

Mo37

40 km

N

D)

Middleton Island

Mi15

Mi07

Mi23

Mi06

Mi16

Mi28

Mi13

Mi09

Mi08

Mi12

Mi14

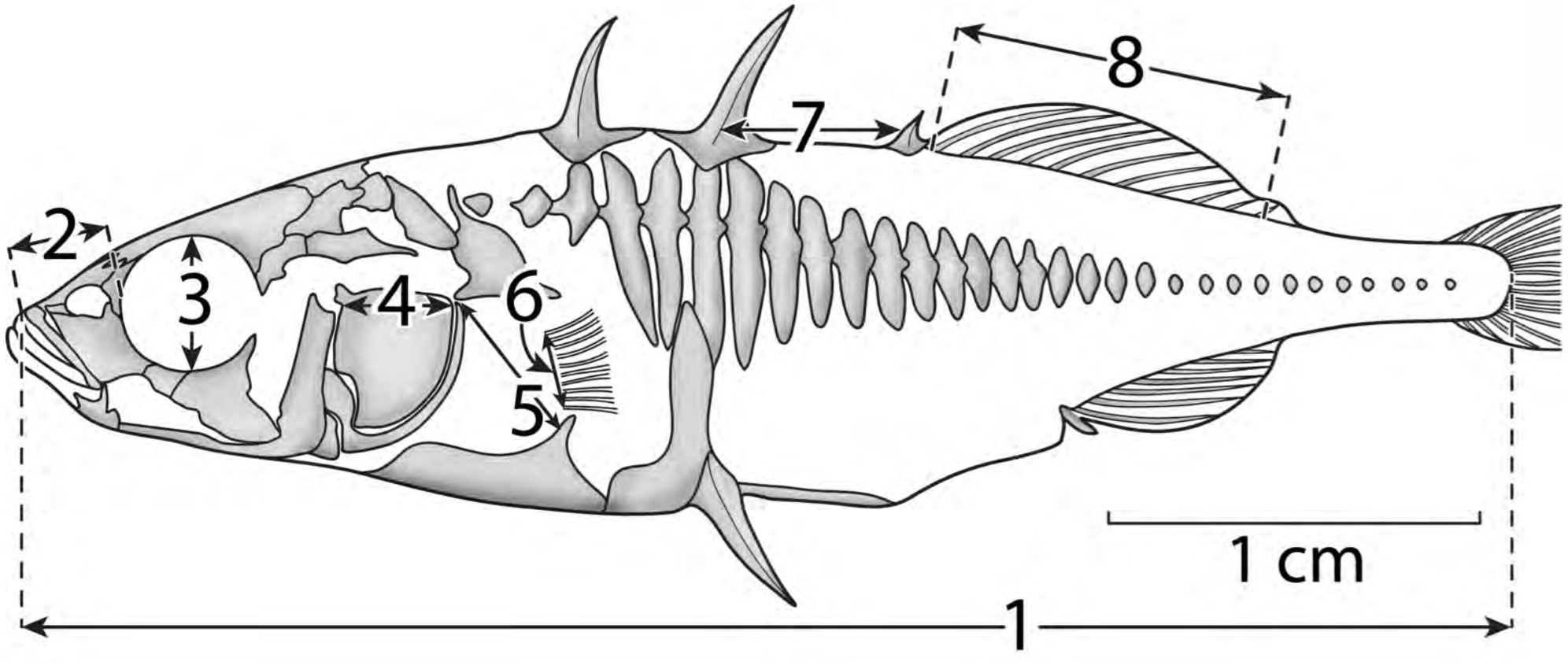
Mi17

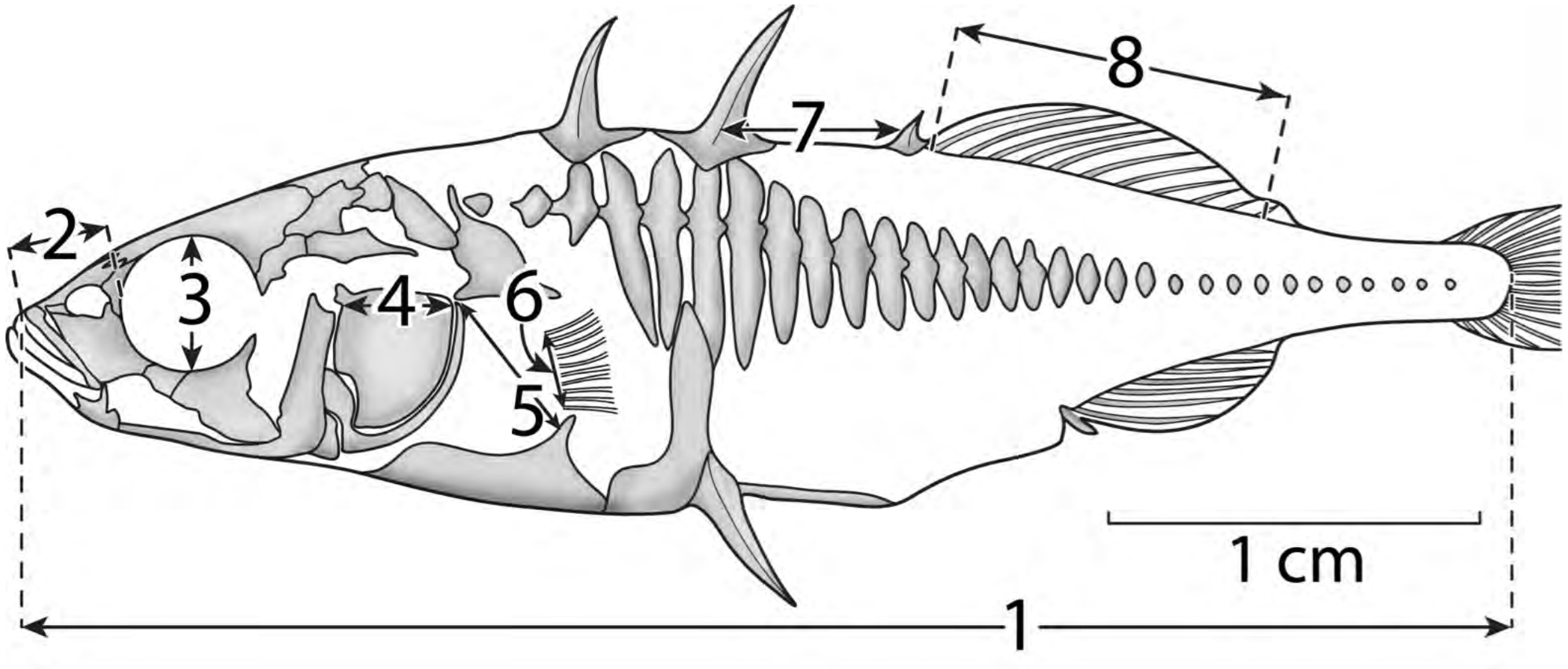
Mi22

Mi19

Mi11

2 km

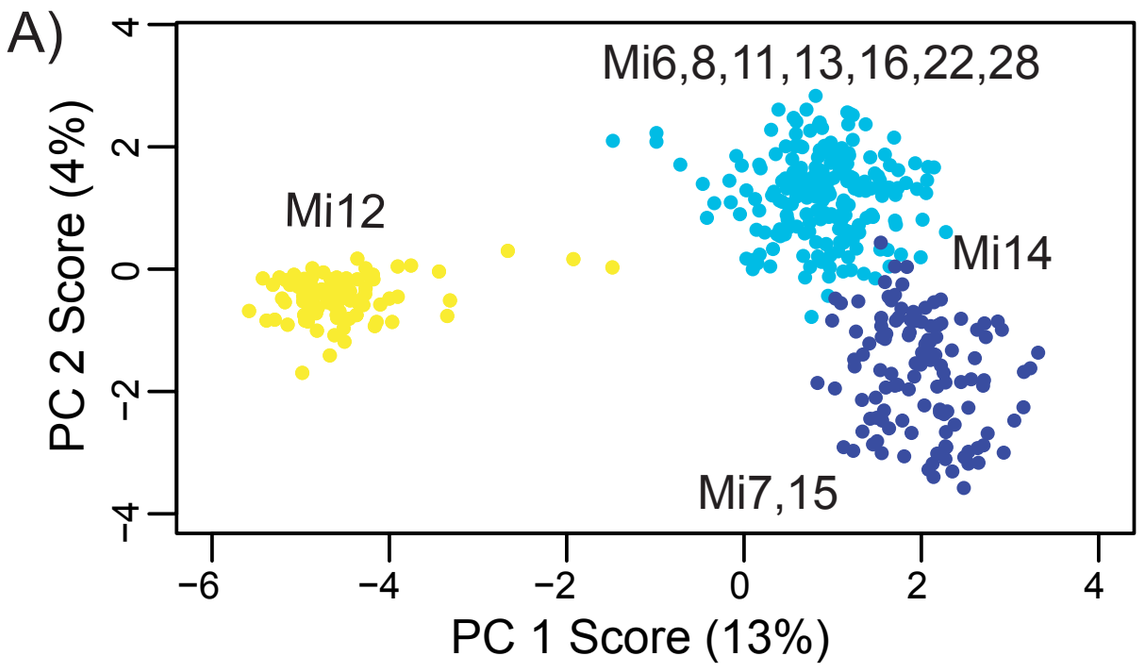

8
7
2
3
4
6
5
1 cm
1

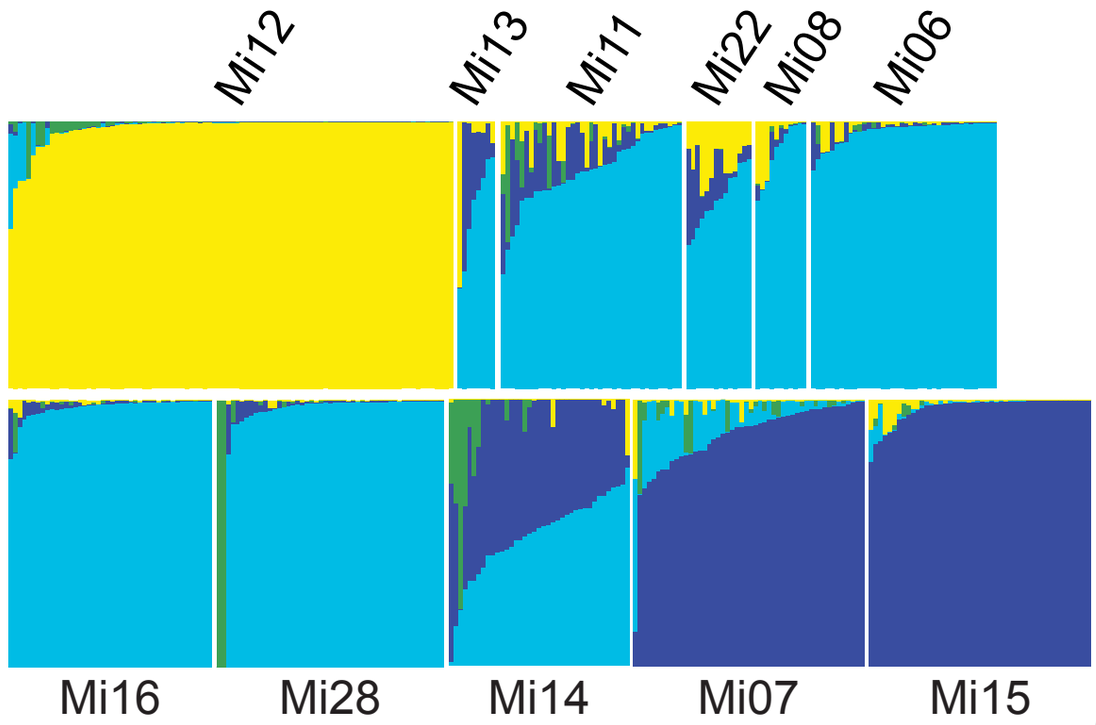


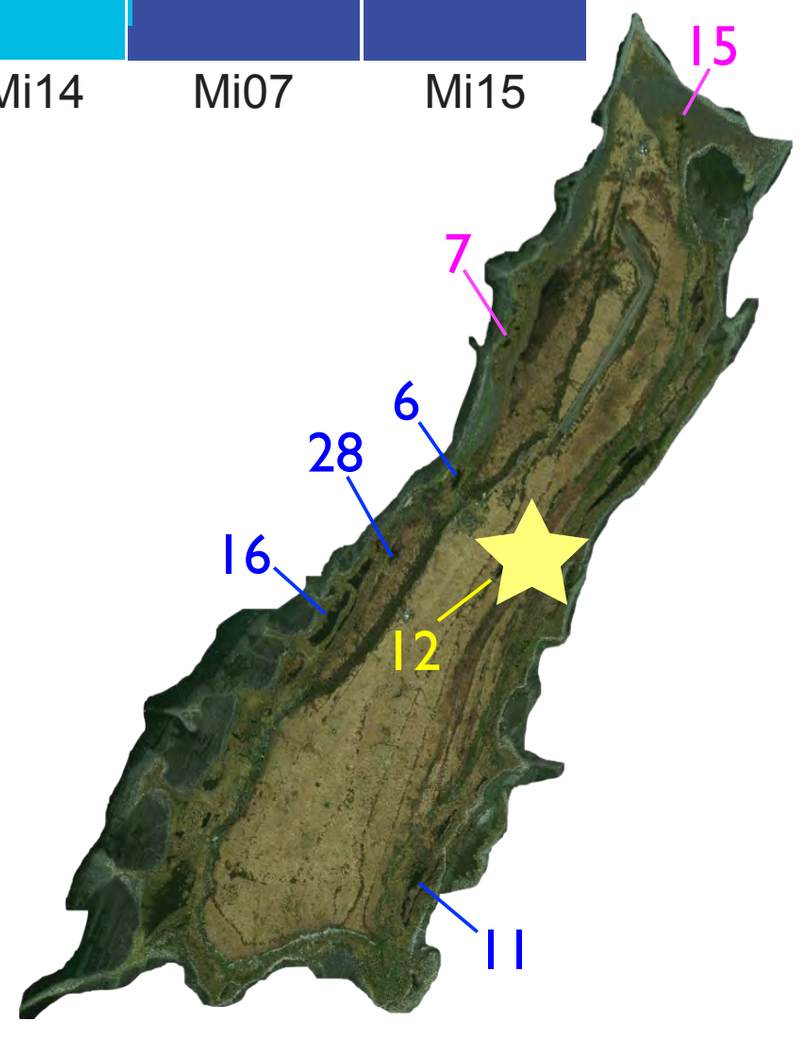
RAD-seq analysis

110,000 SNPs per individual
>1000 Individuals
20 million genotypes

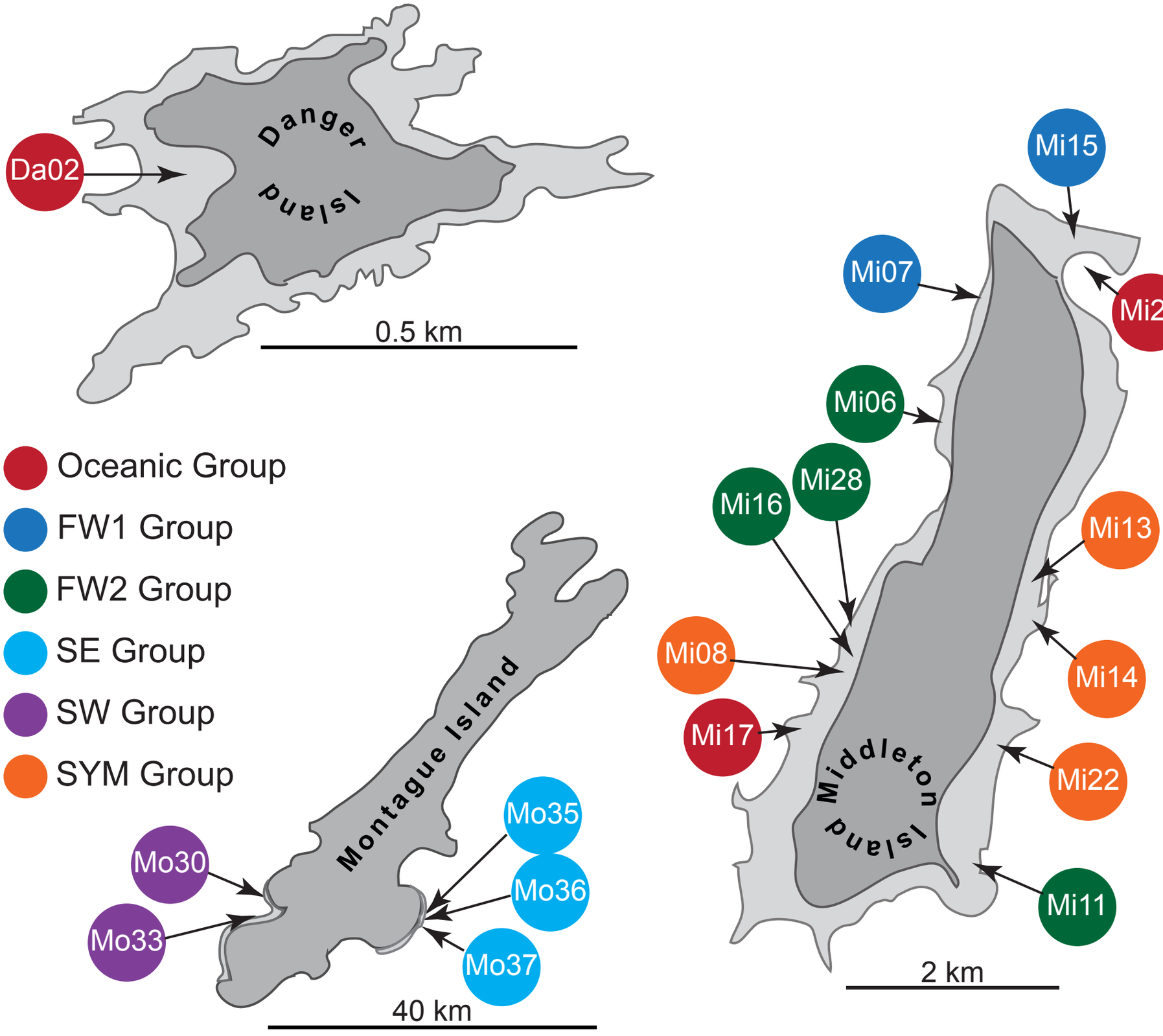
Structure analysis shows independent evolution even among populations on a single island







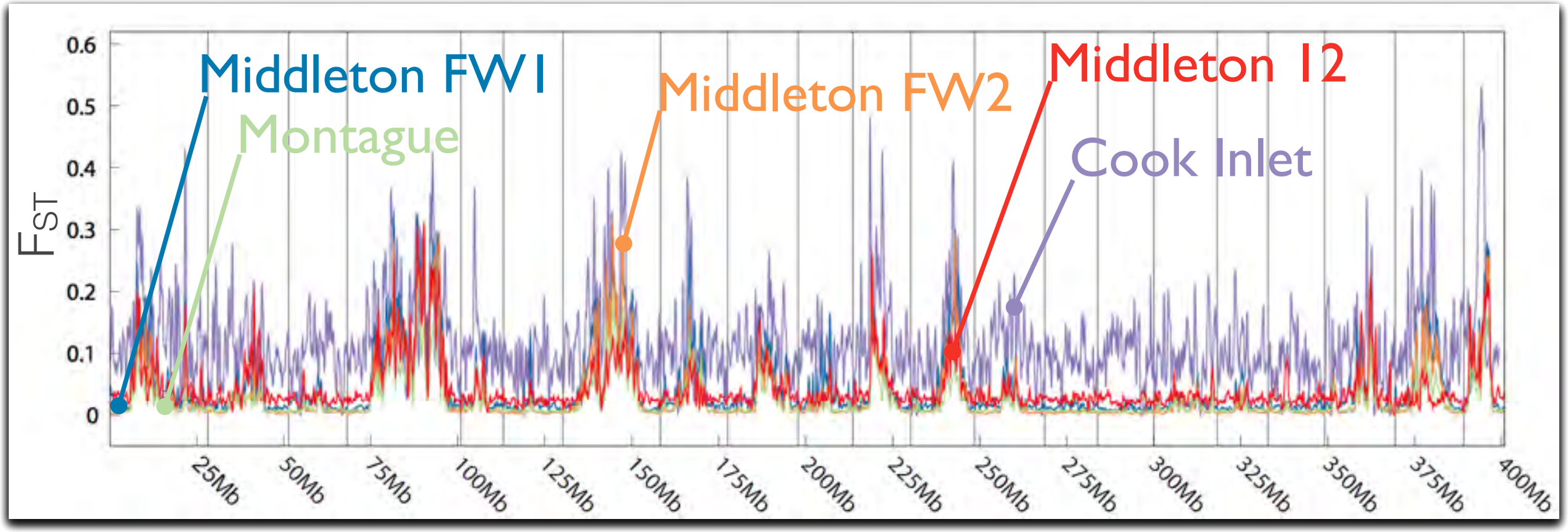
At least six independent evolutionary events in freshwater in the last 50 years



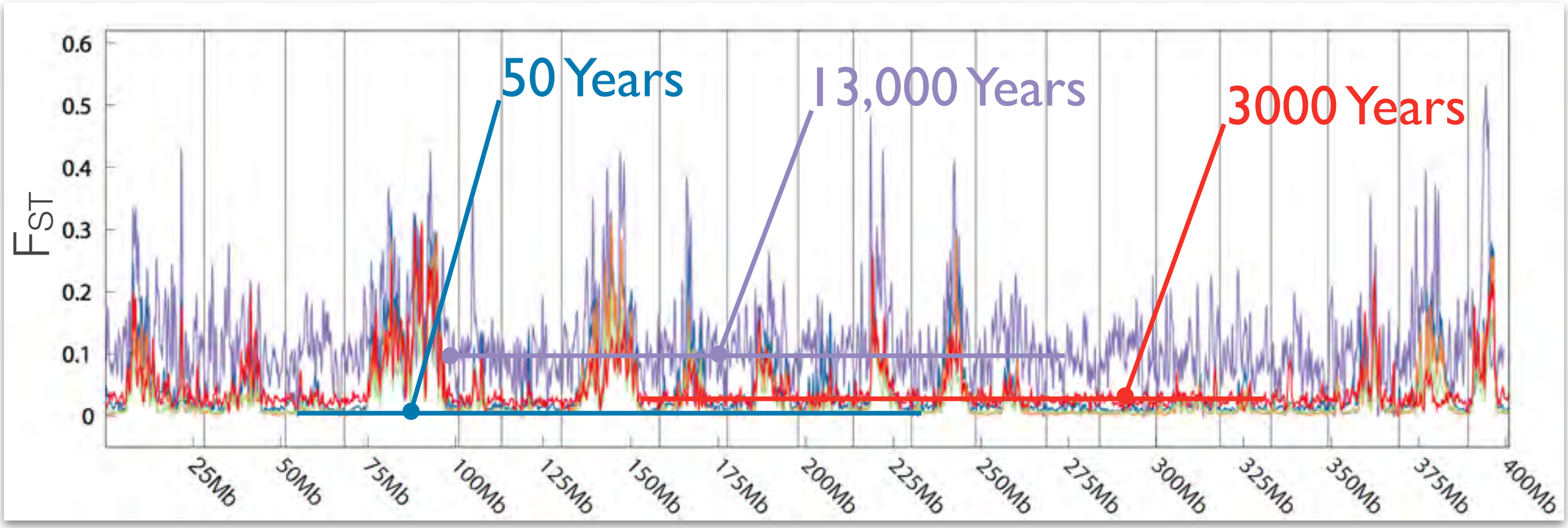
How much of the genome is differentiated?

How similar are the genomic patterns of differentiation?

Bassham, Catchen et al. *in prep*

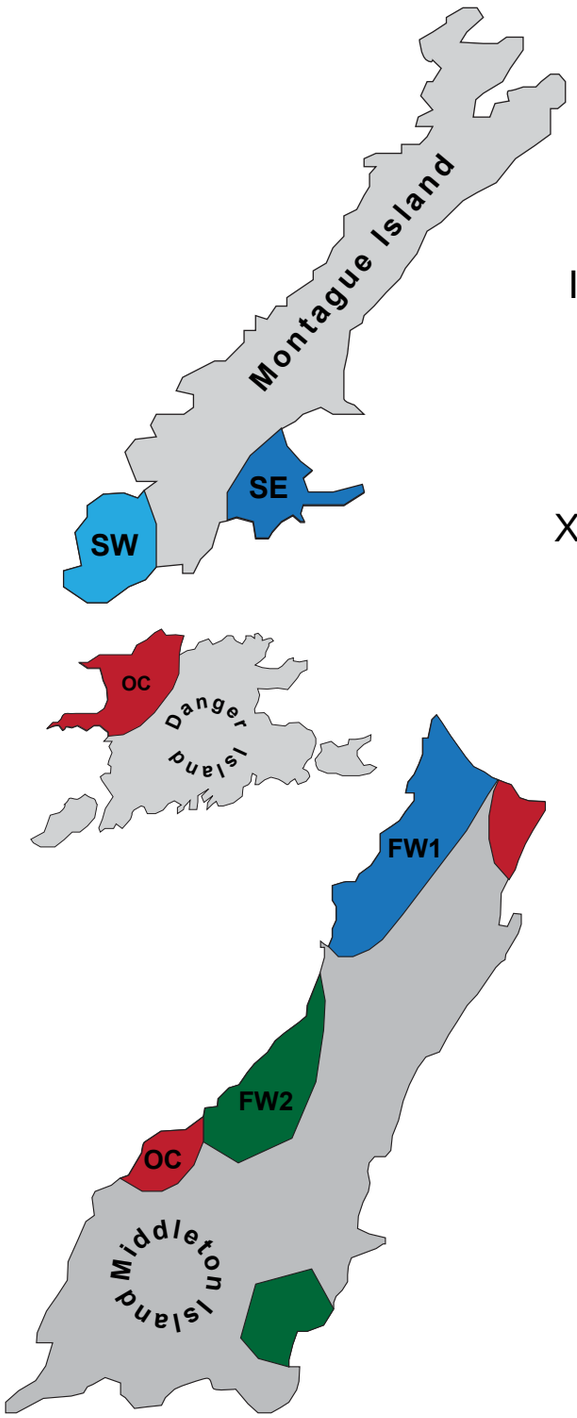


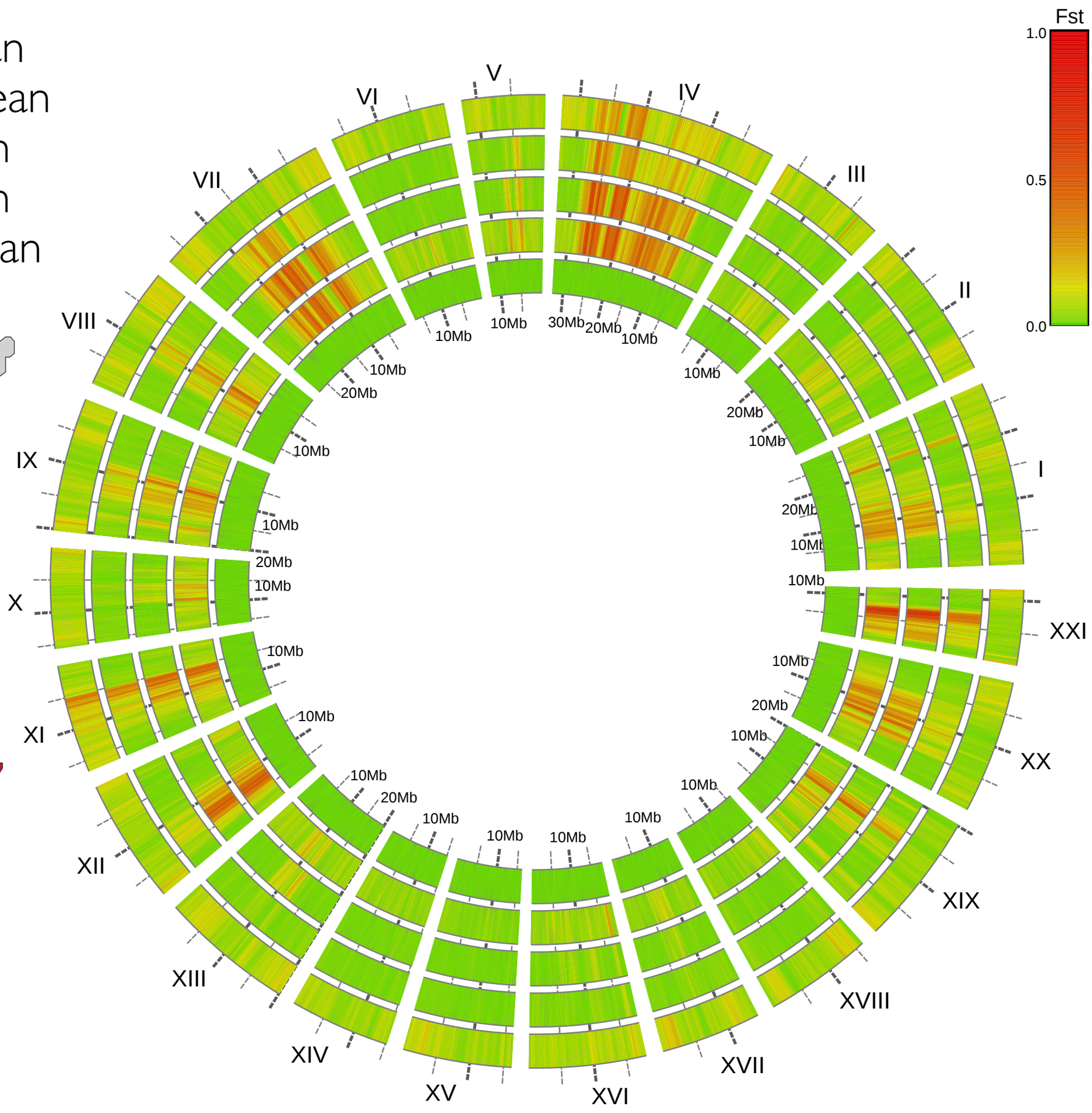
Freshwater Populations (grouped) vs. Marine



Freshwater Populations (grouped) vs. Marine

MoSe vs. Ocean
MoSW vs. Ocean
FW2 vs. Ocean
FW1 vs. Ocean
Ocean vs. Ocean





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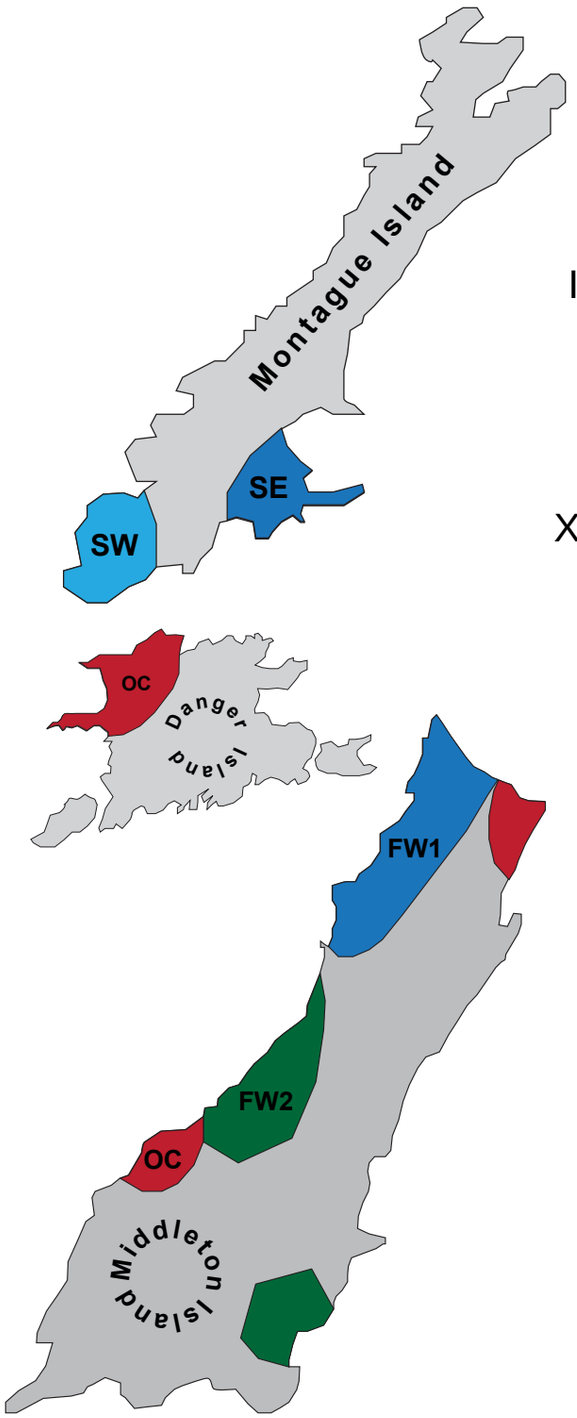
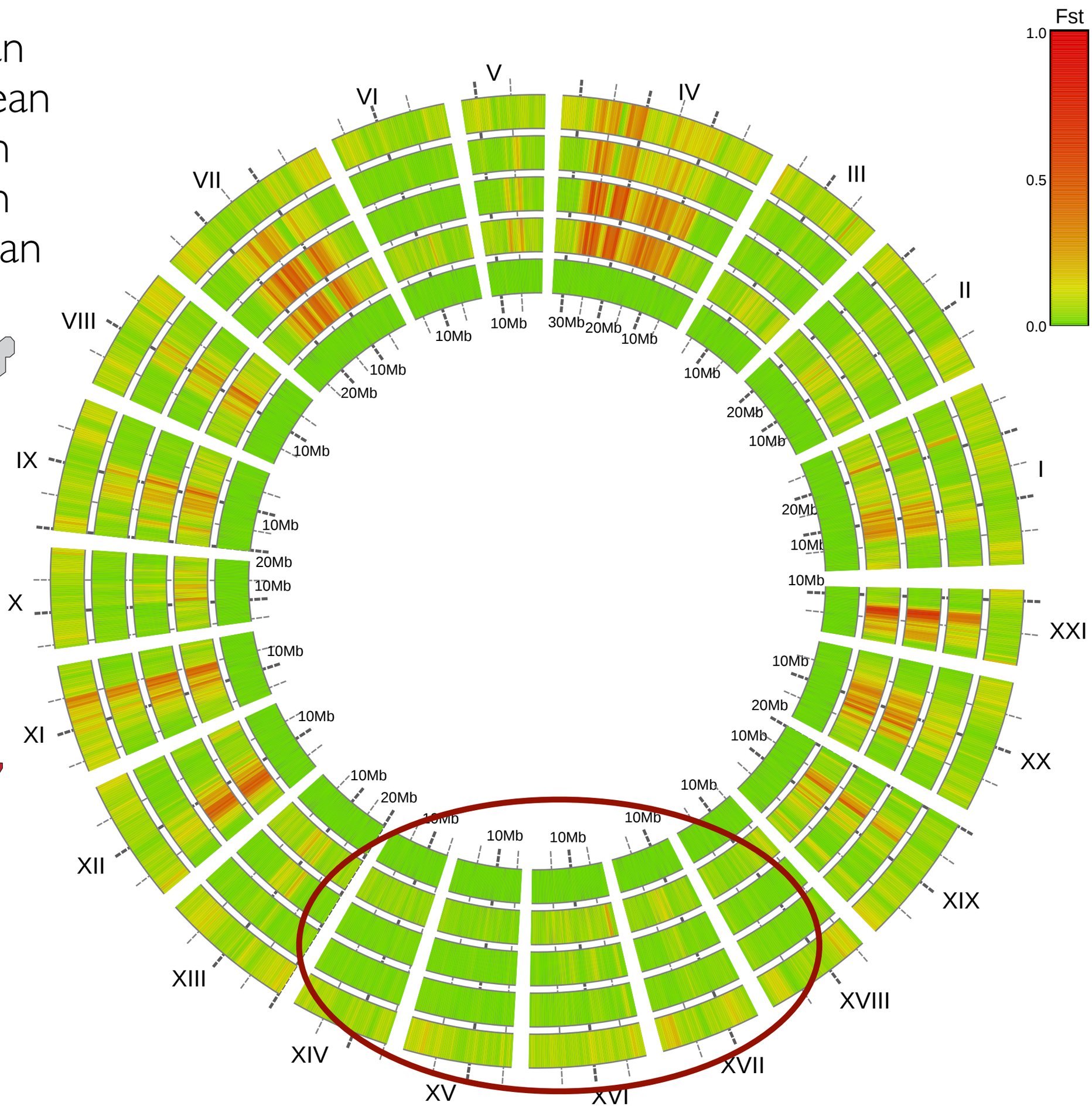
Ocean vs. Ocean





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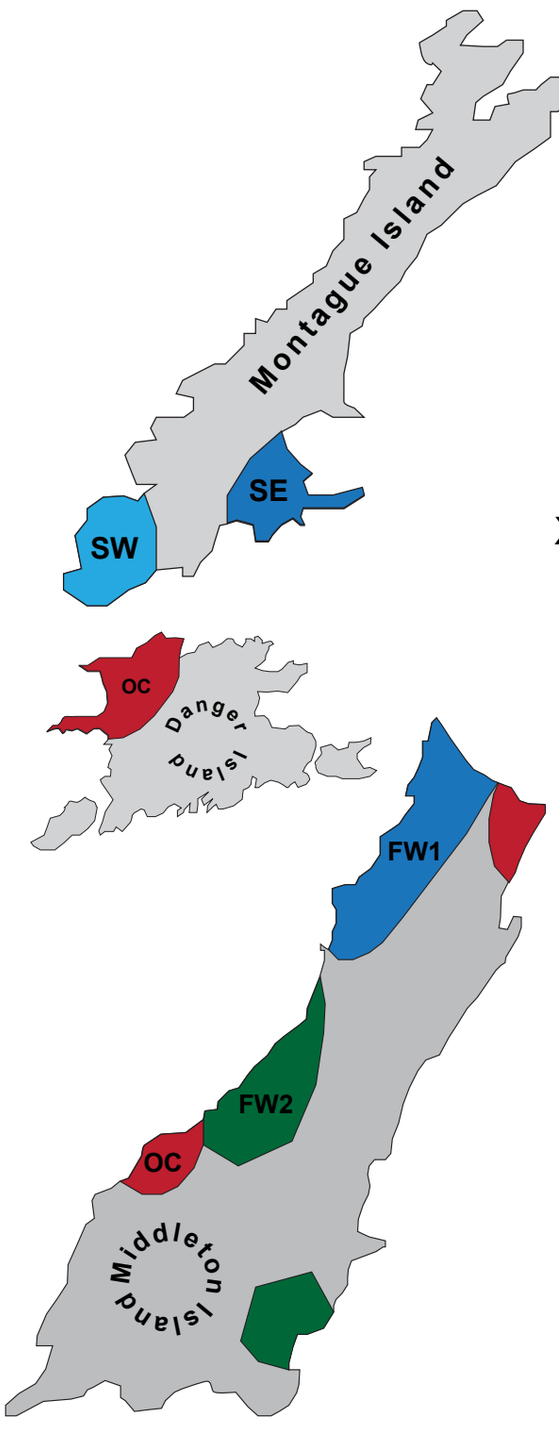
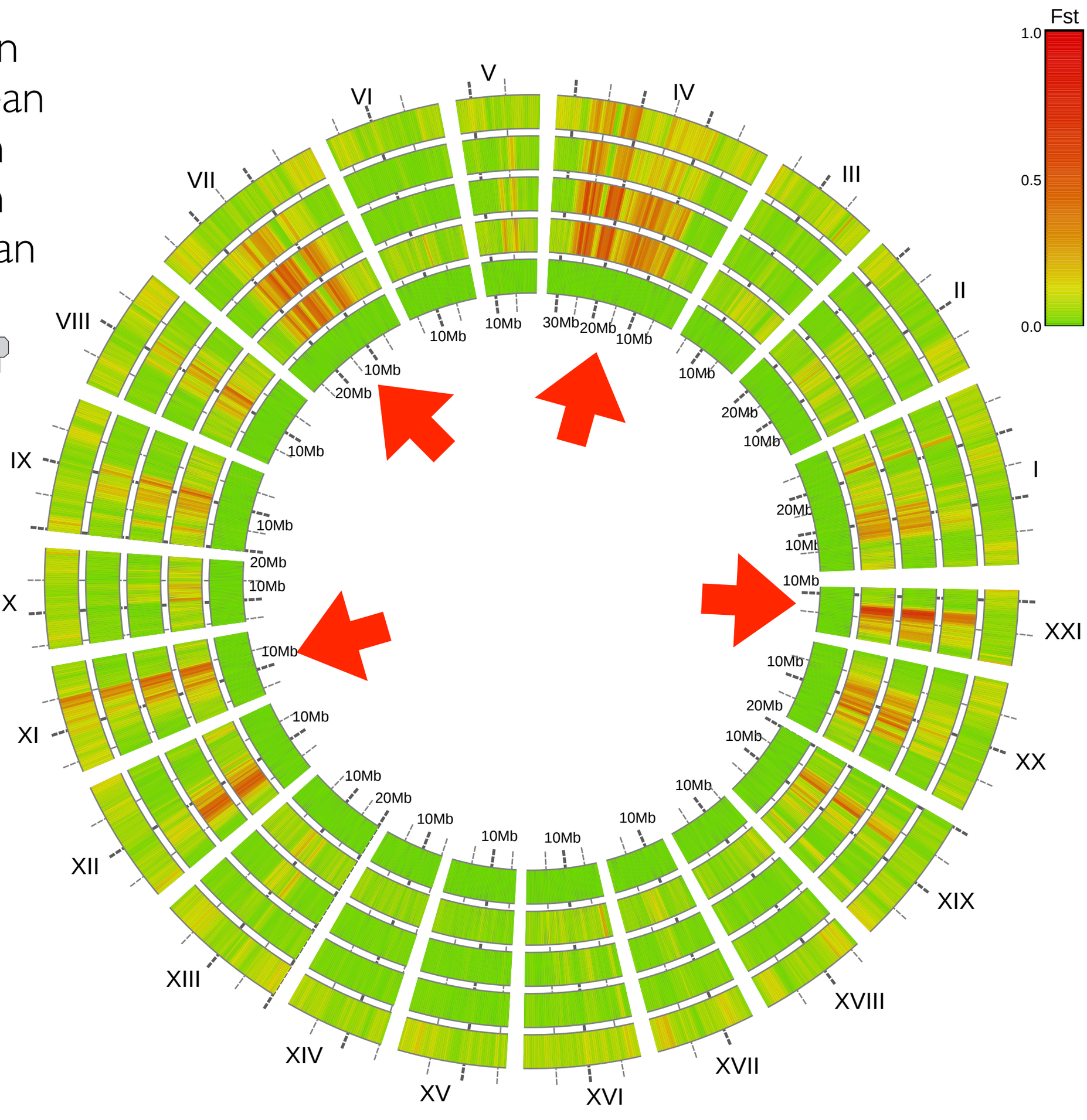
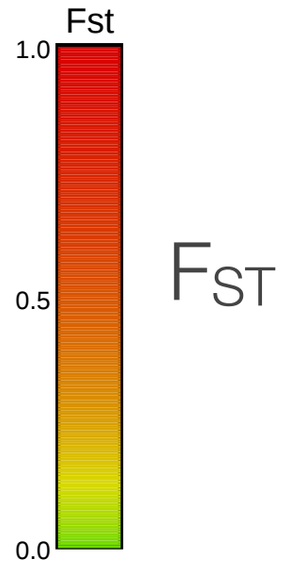


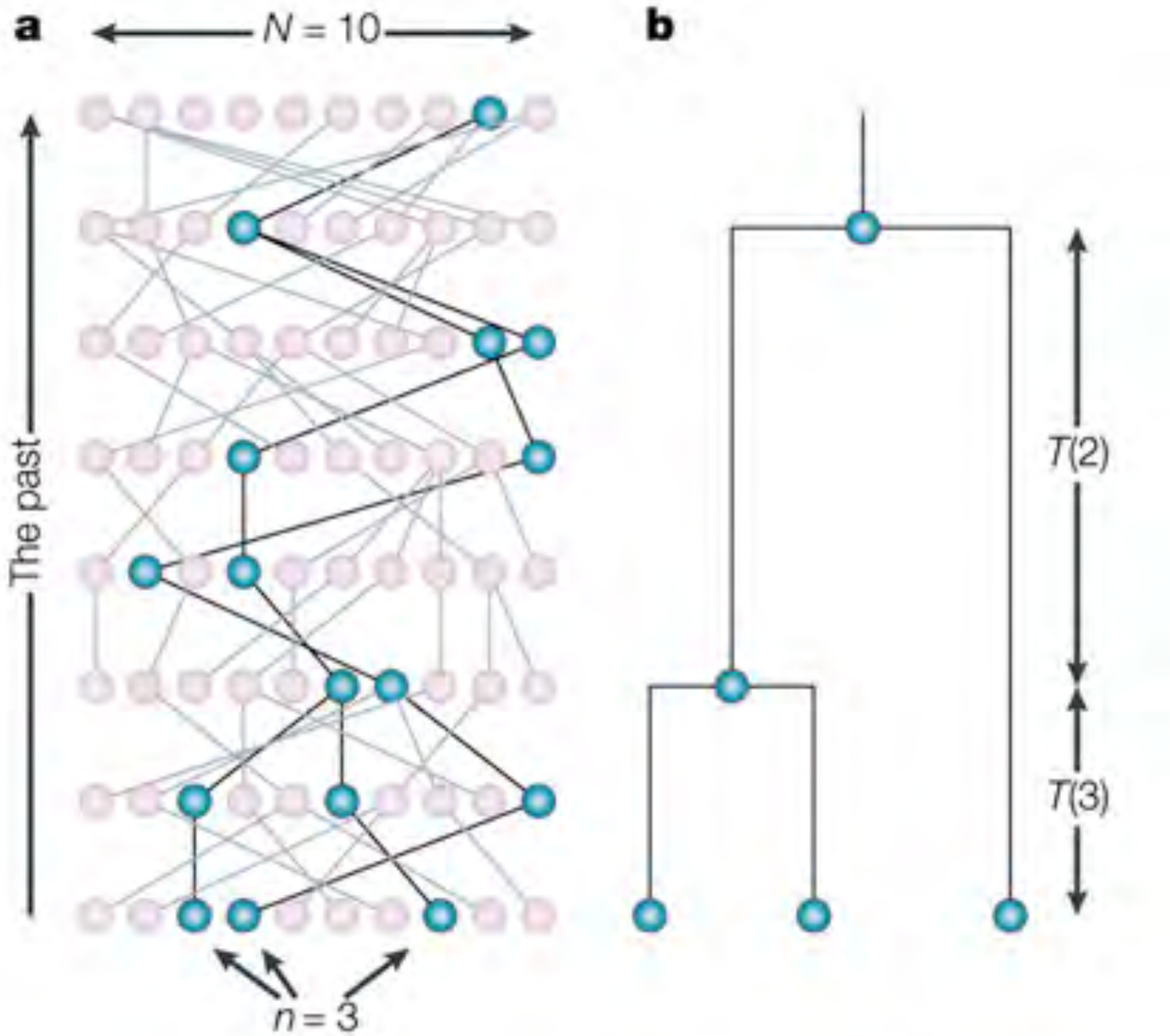




Coalescent analyses using RADseq

Thom Nelson

The coalescent in population genetics

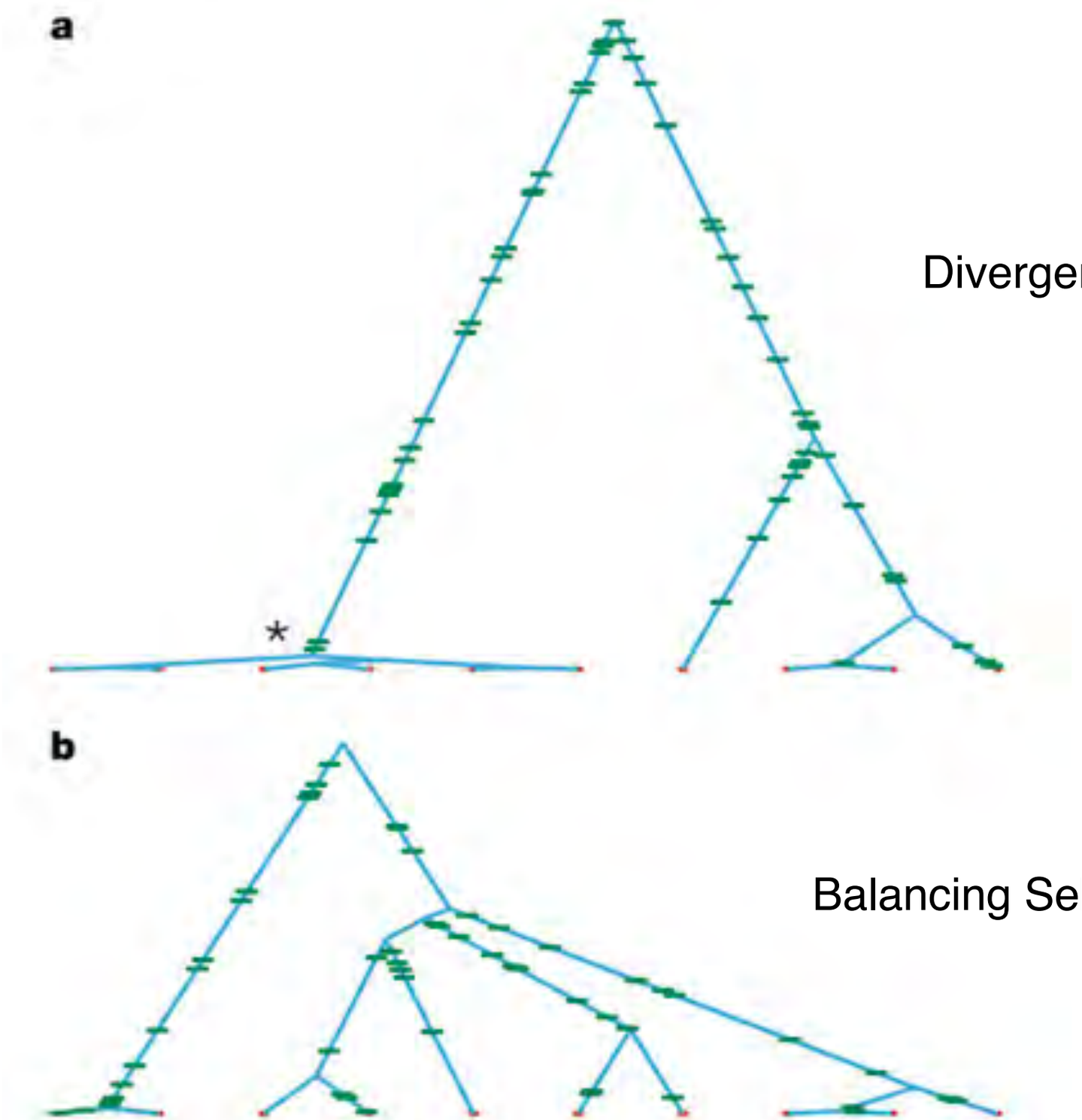


Noah A. Rosenberg & Magnus Nordborg

Nature Reviews | Genetics

Neutral coalescent expectations

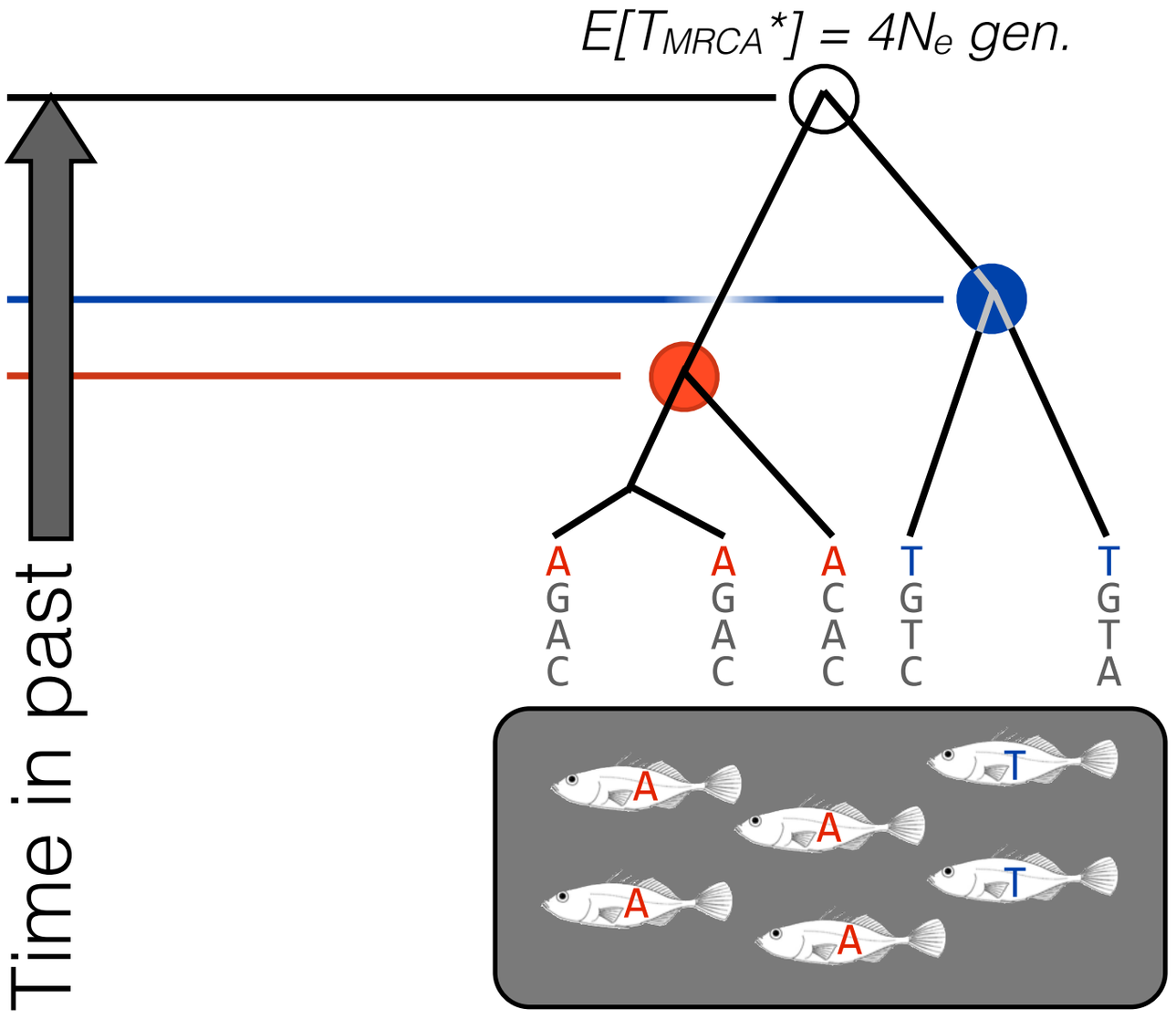
Natural selection and the coalescent



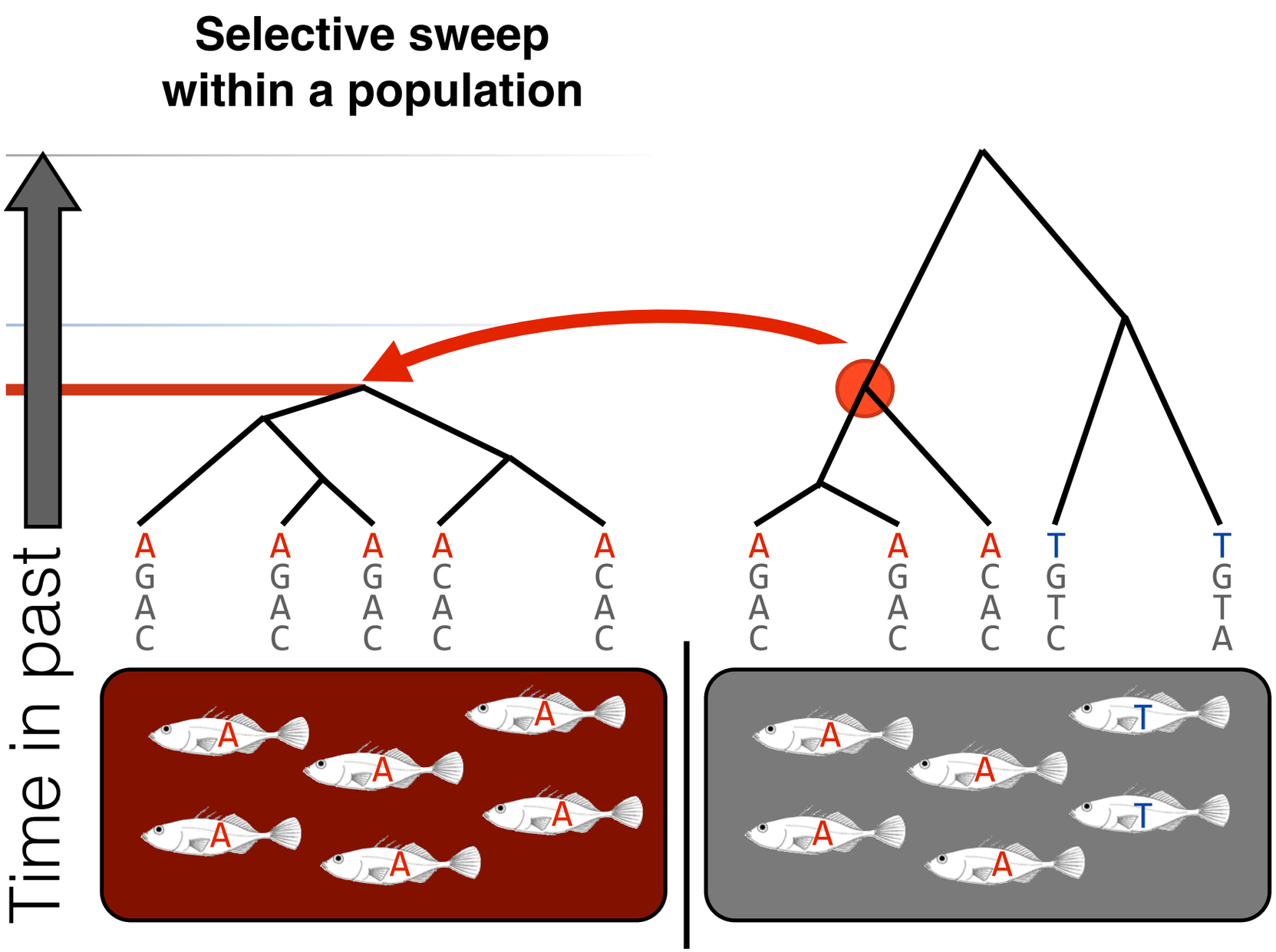
Divergent Selection

Balancing Selection

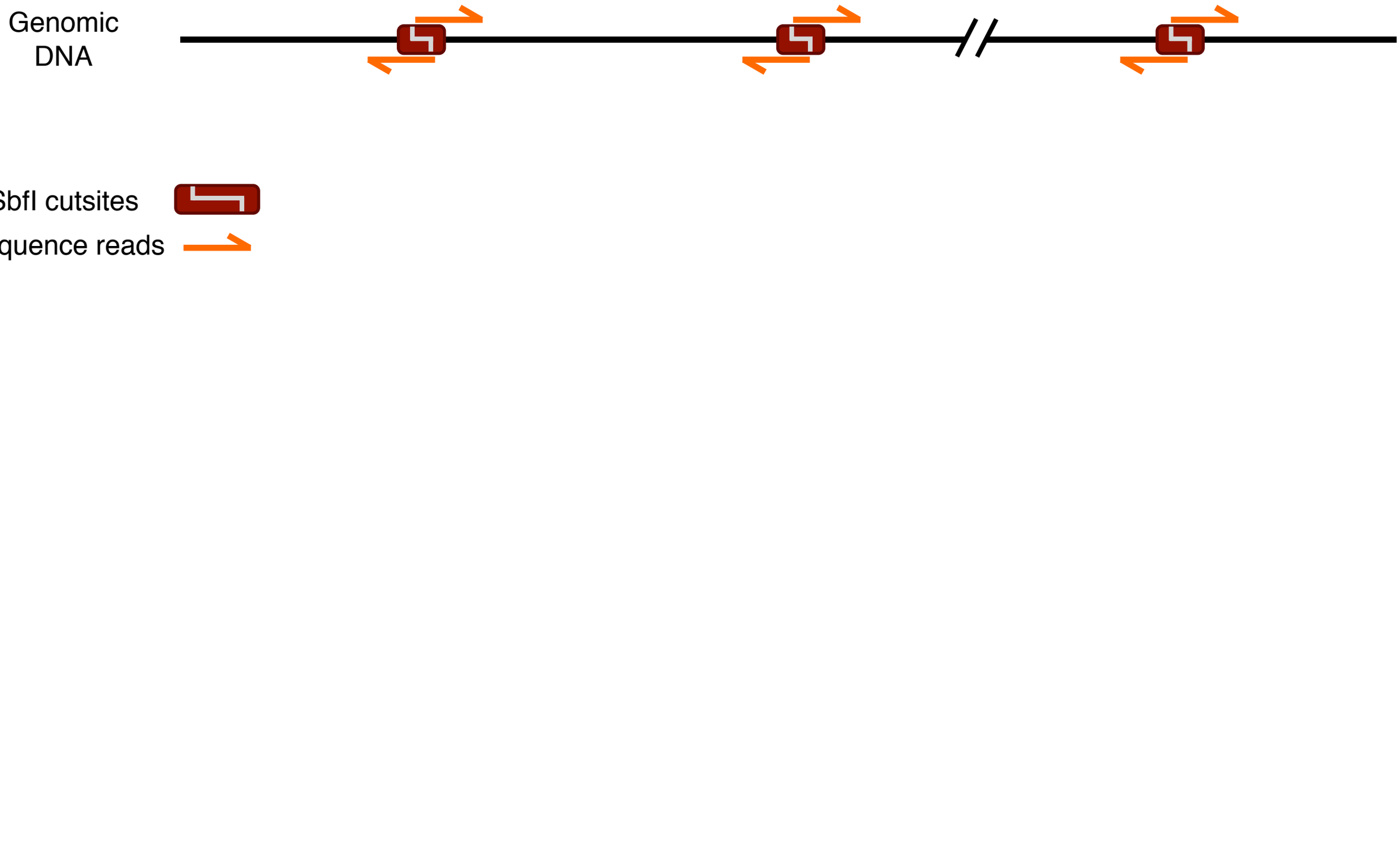
Coalescent analyses in stickleback



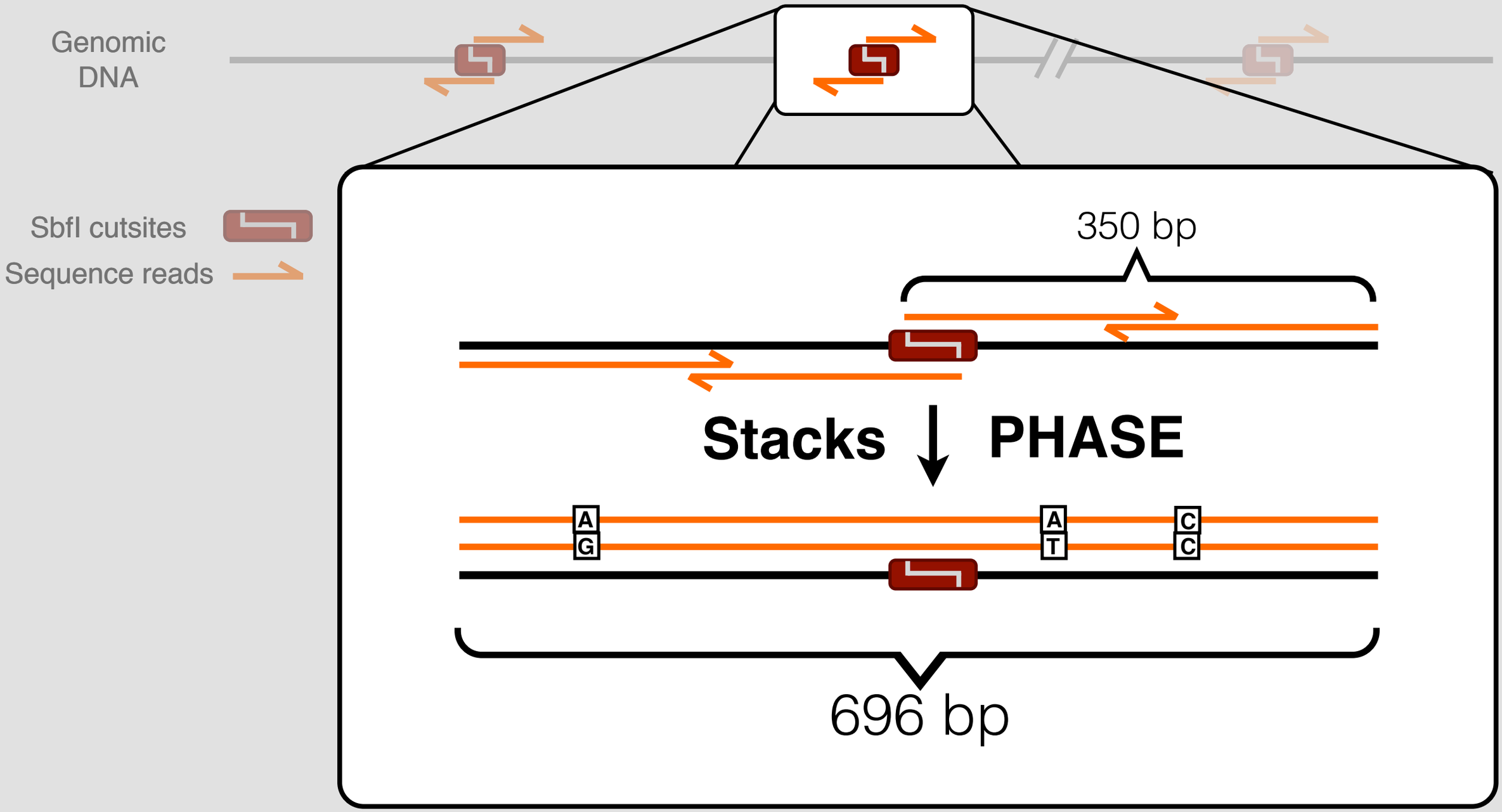
Coalescent analyses in stickleback



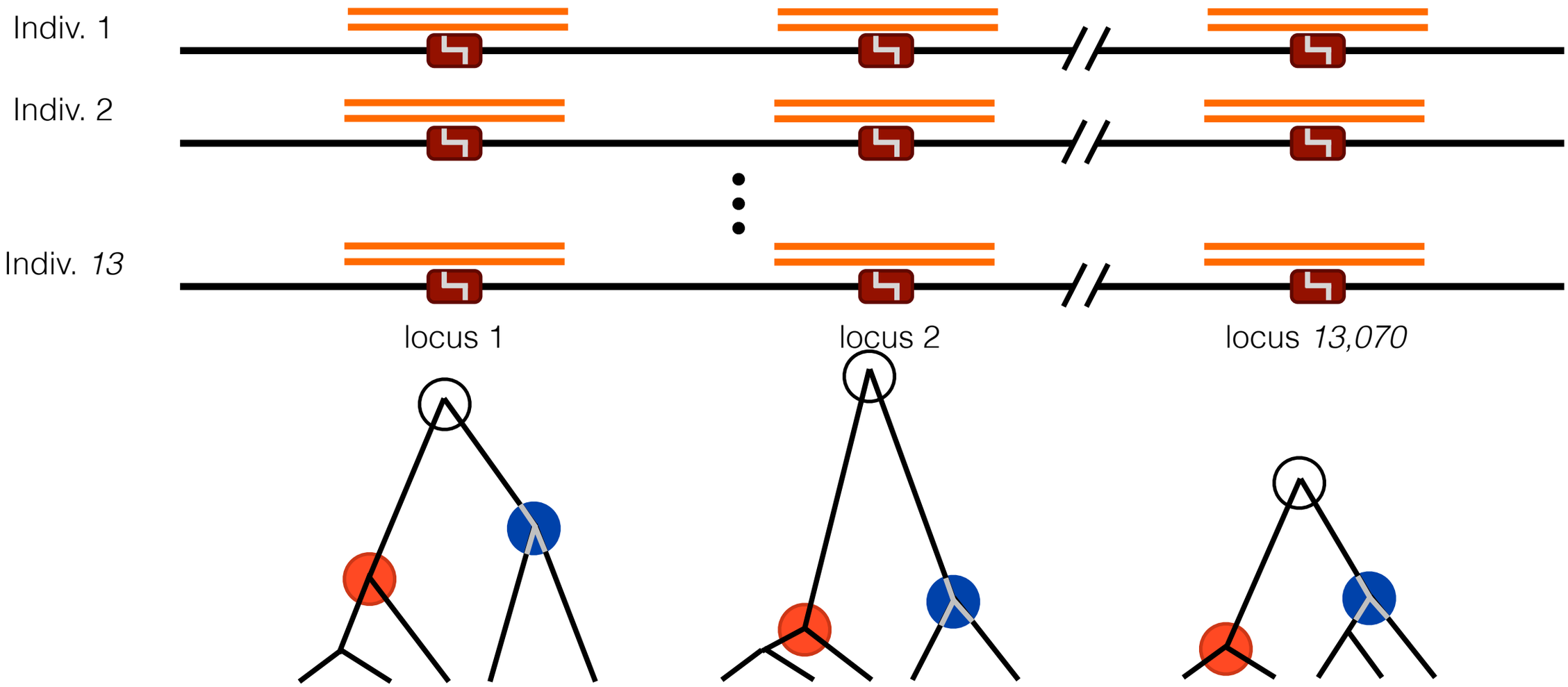
Coalescent analyses with RAD-seq



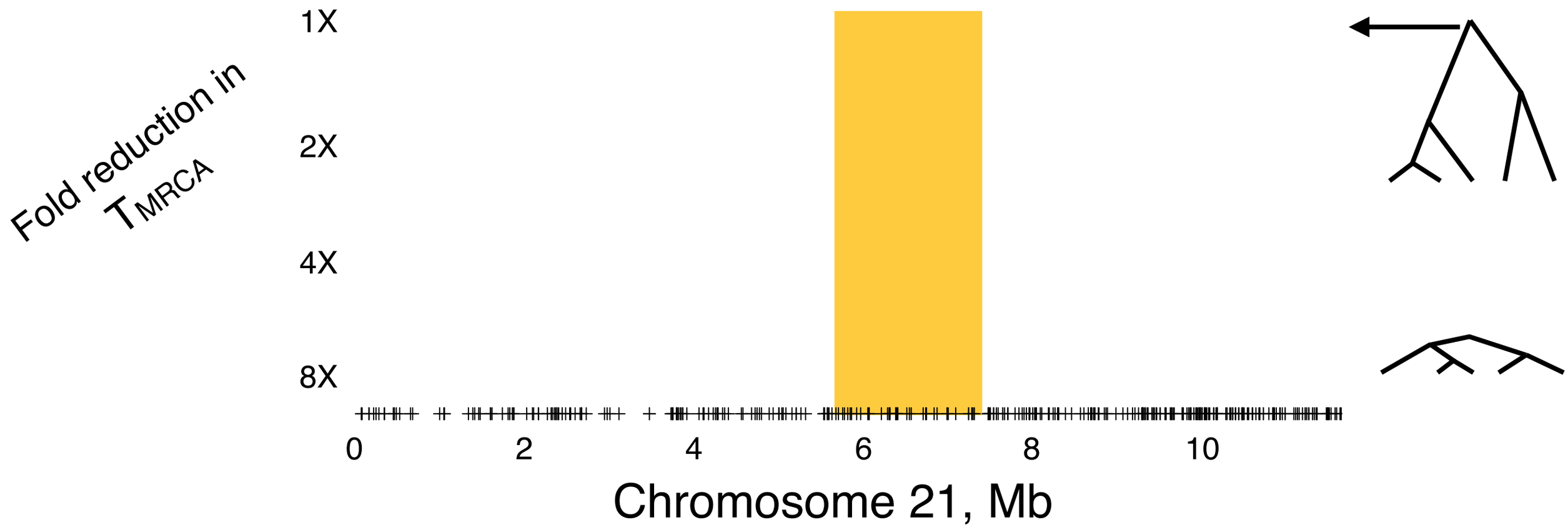
Coalescent analyses with RAD-seq



Coalescent analyses with RAD-seq

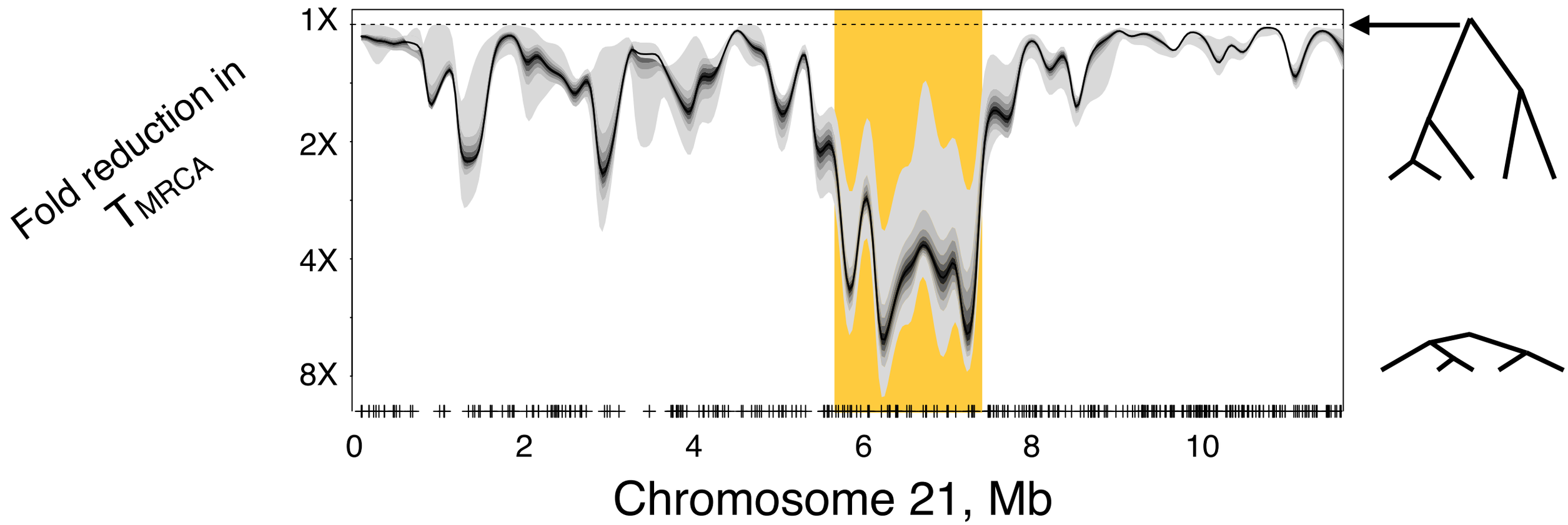


Selection in one population reduces coalescence time



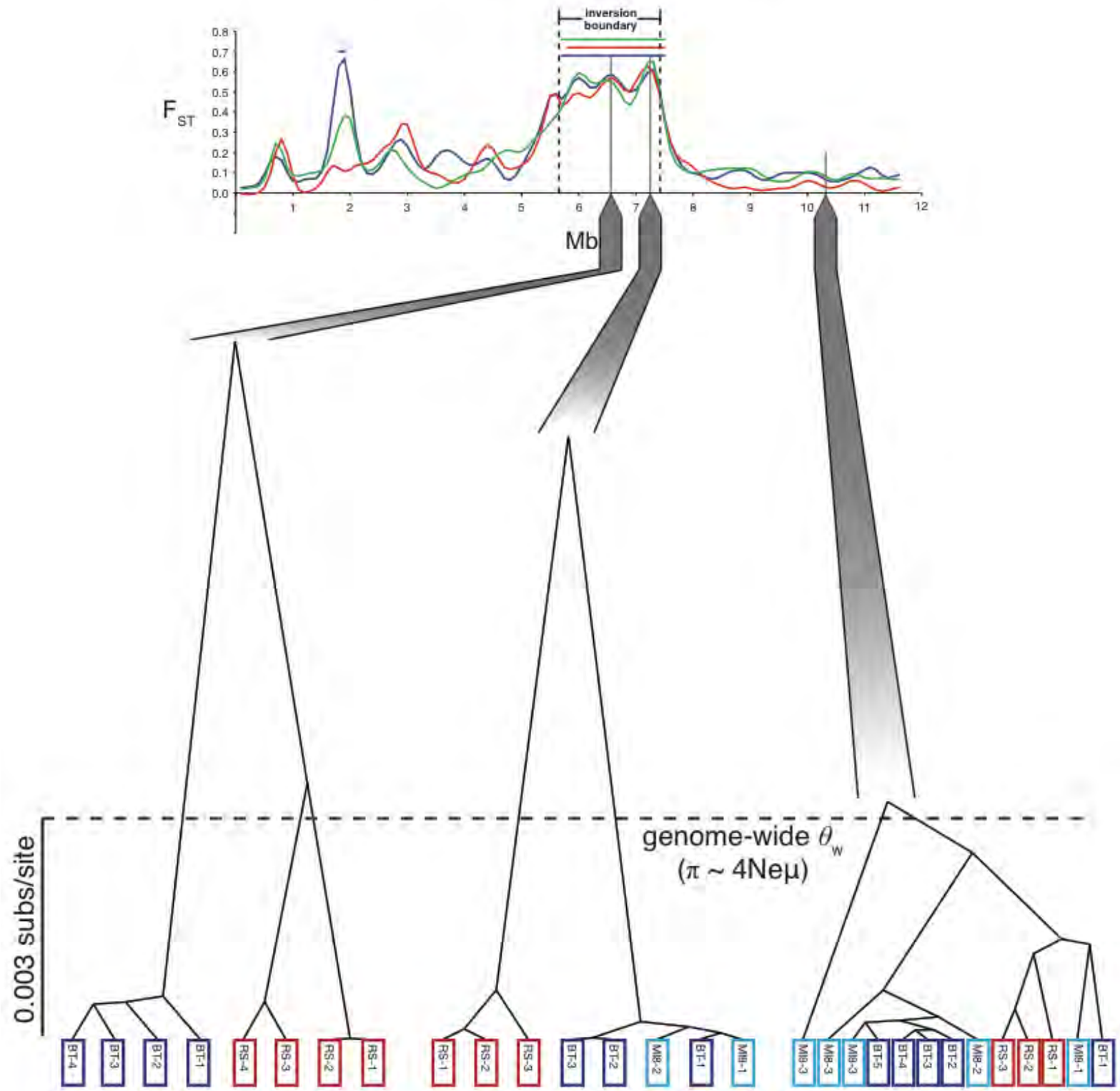
$$\log_2\left(\frac{T_{MRCA}\mathit{FW}}{T_{MRCA}\mathit{ALL}}\right)$$

Selection in one population reduces coalescence time

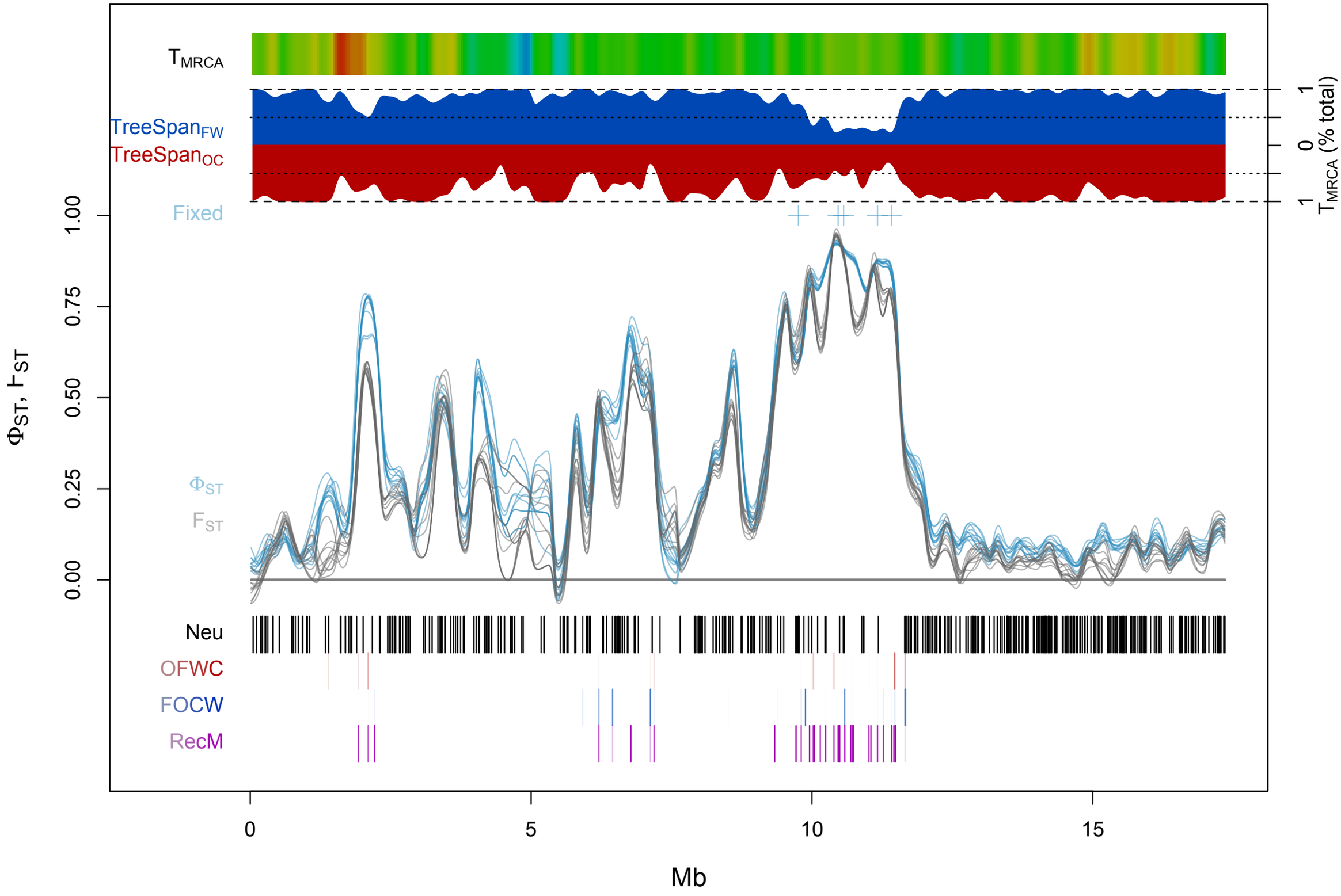


$$\log_2\left(\frac{T_{MRCA}\mathit{FW}}{T_{MRCA}\mathit{ALL}}\right)$$

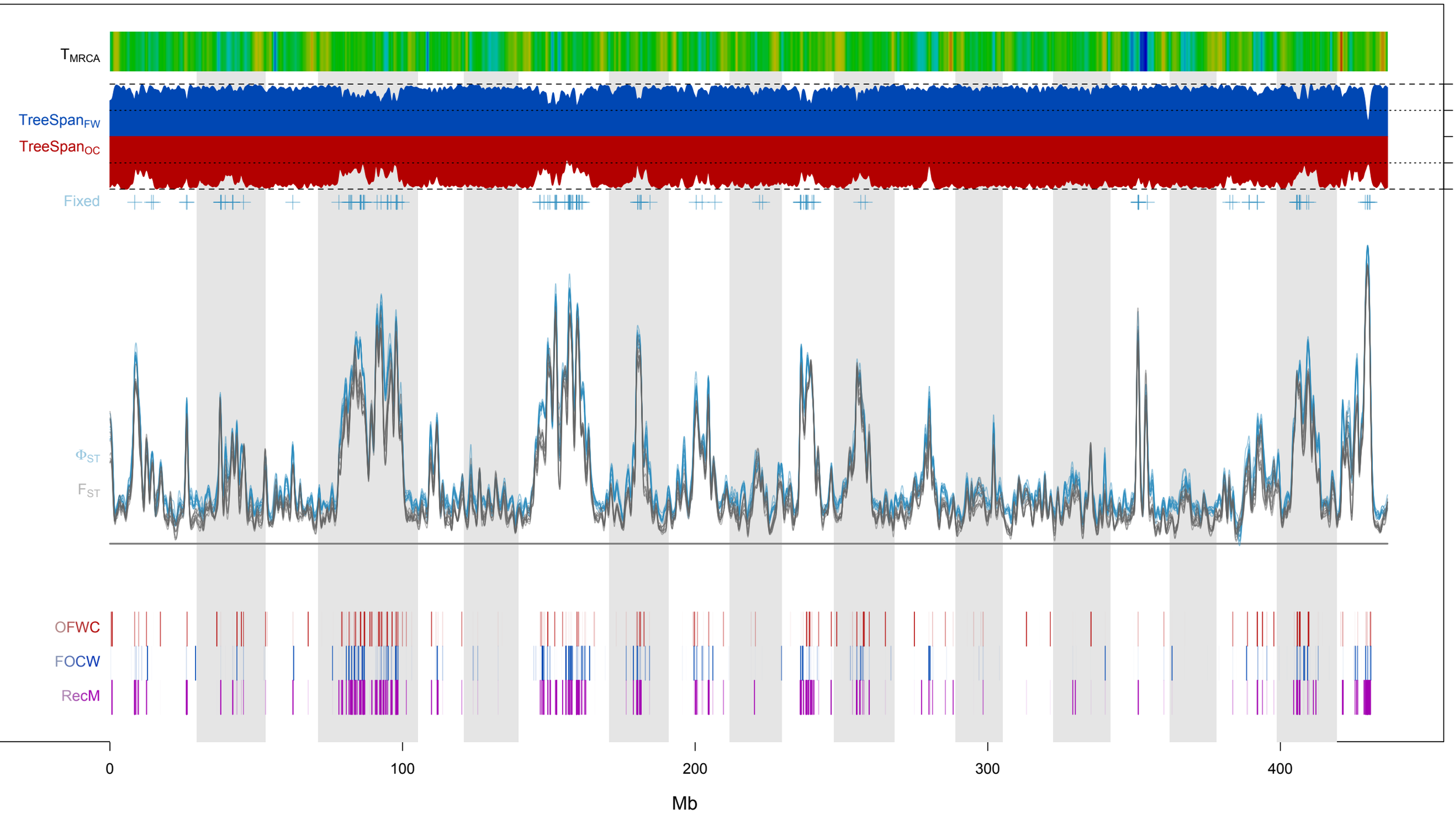
However, across populations the coalescence time can increases significantly in a genomic region



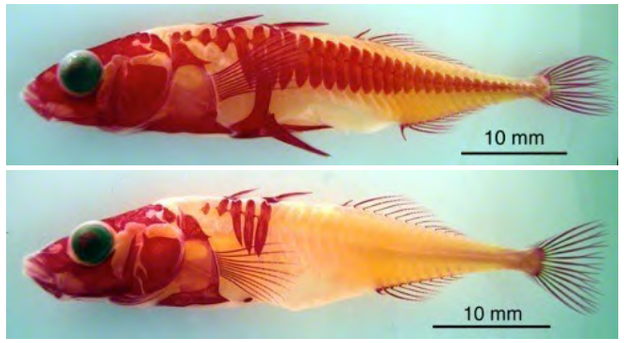
Relative proportion of T_{mrca} across different habitats LG21



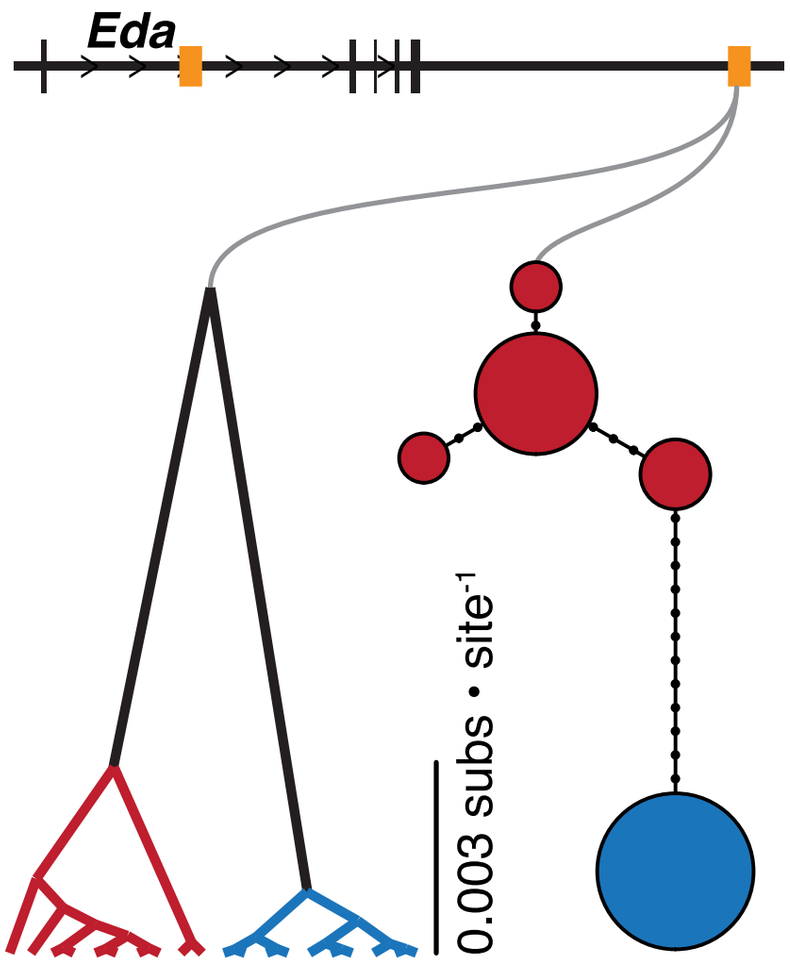
Relative proportion of T_{mrca} across different habitats
Entire genome



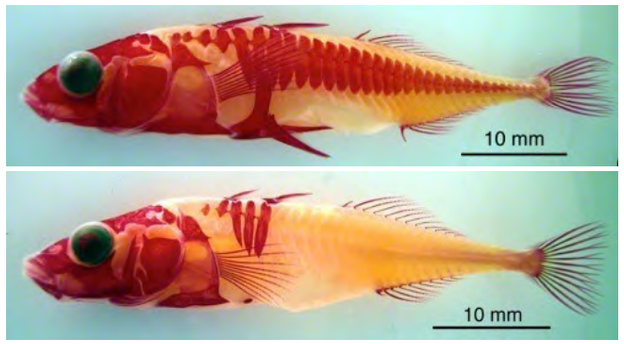
Increased absolute divergence between habitats

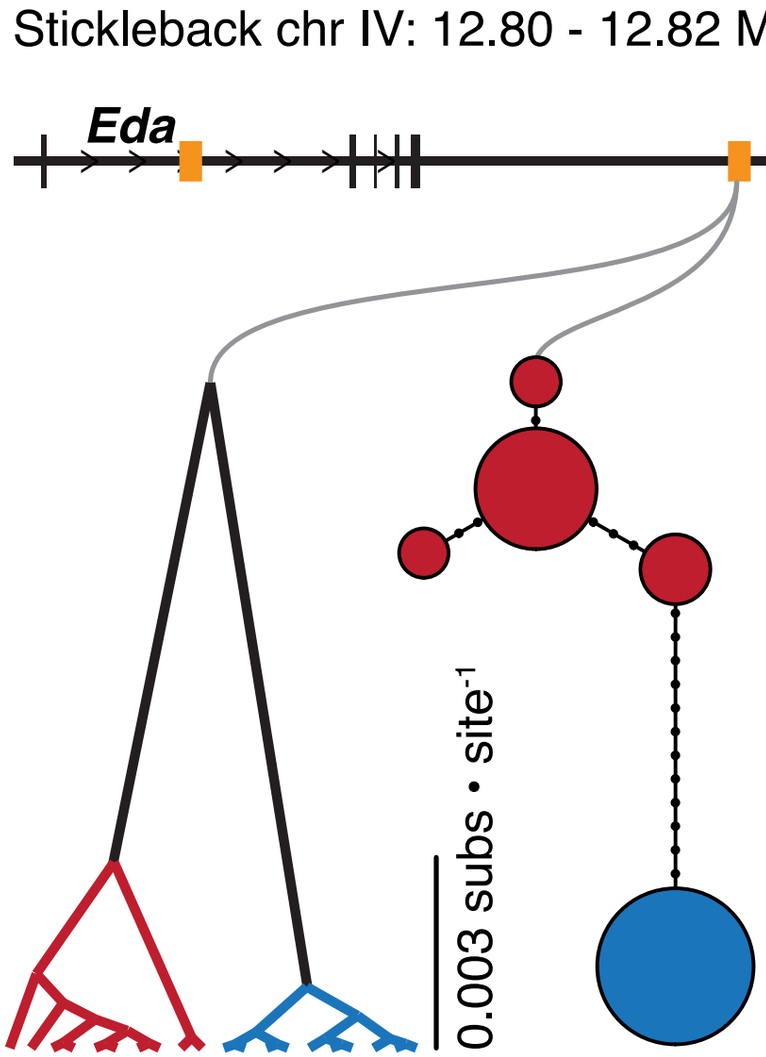


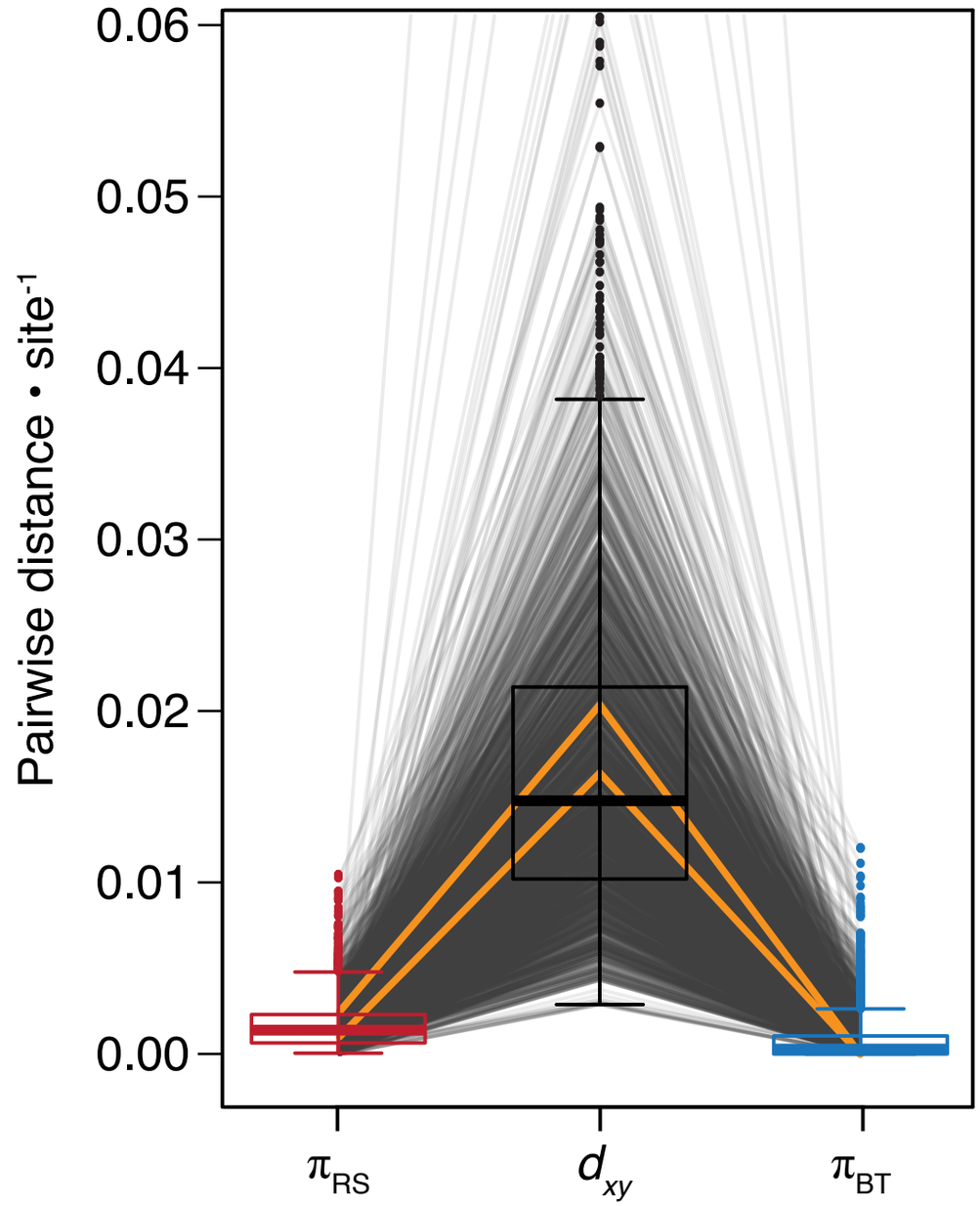
Stickleback chr IV: 12.80 - 12.82 Mb



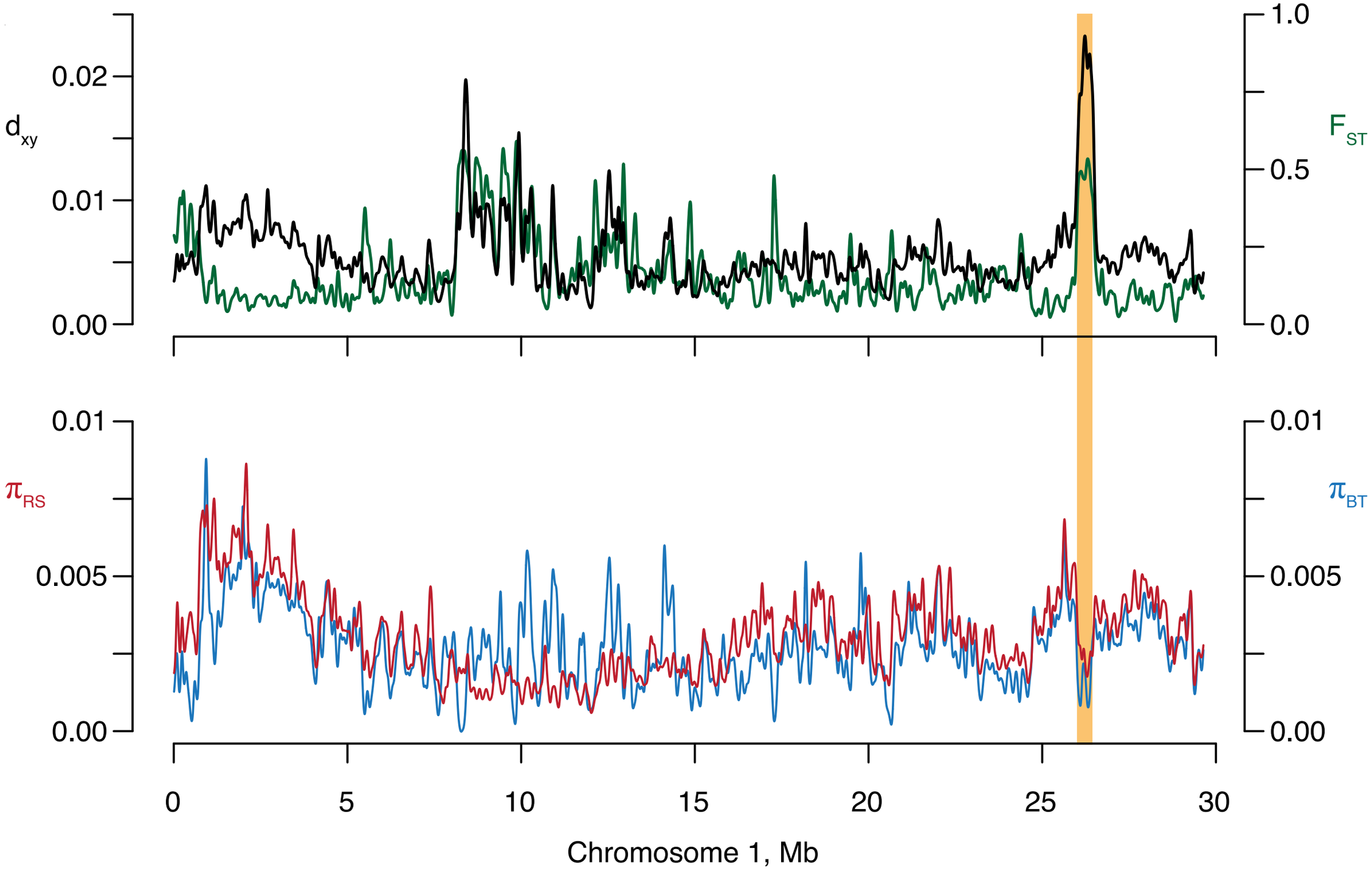
Increased absolute divergence between habitats







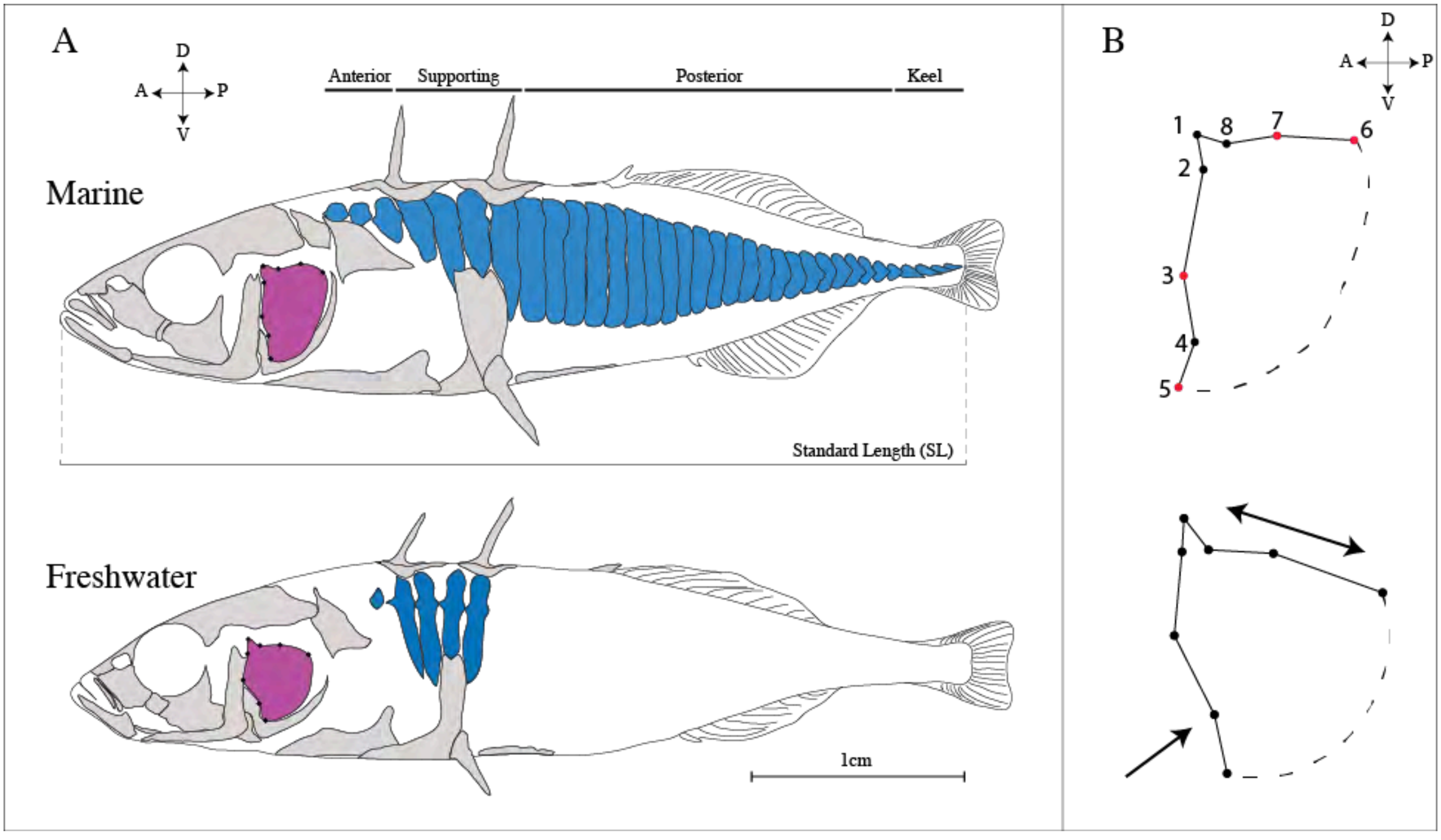
Absolute divergence co-localizes with inversions



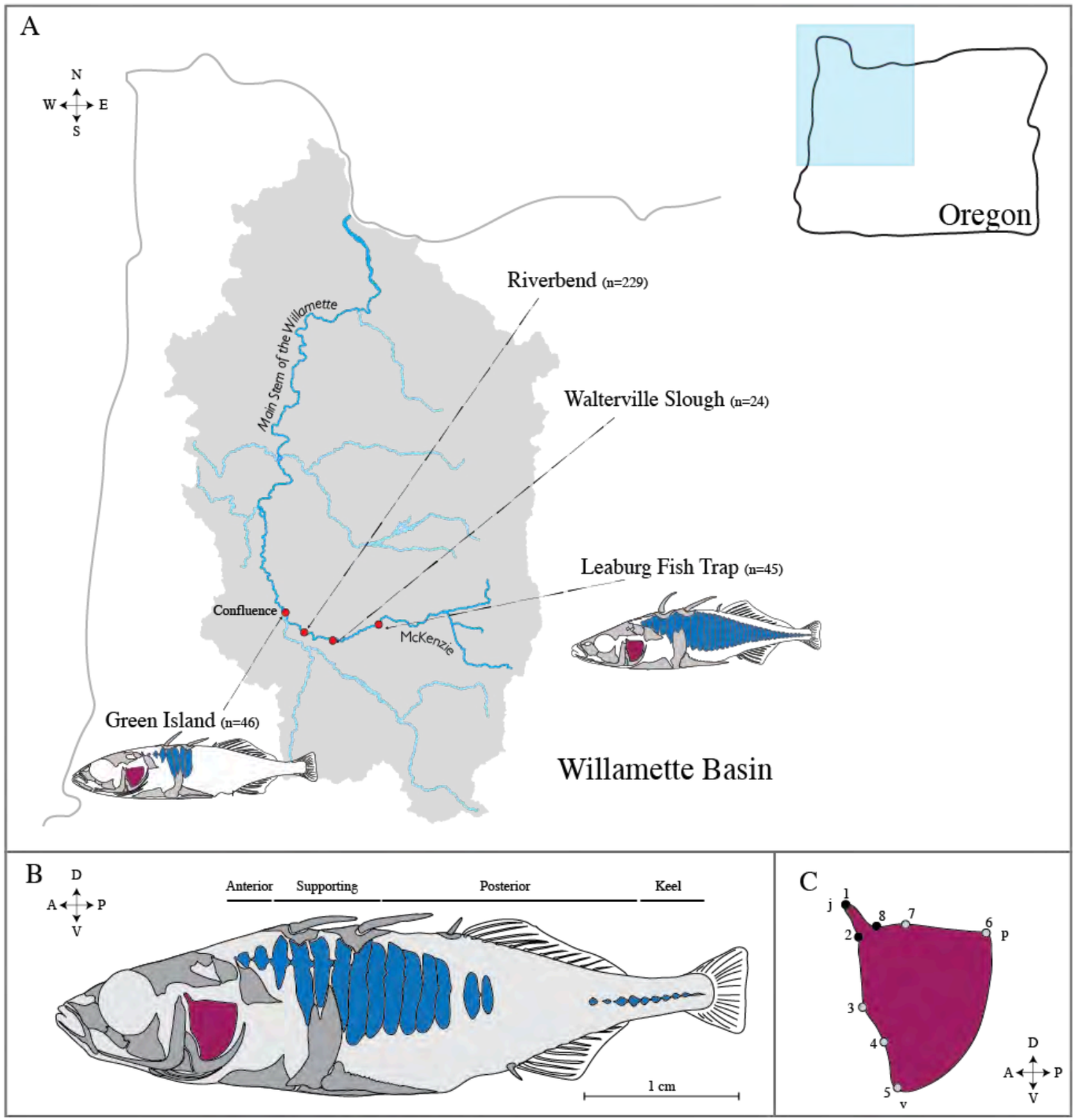
How do the genomic patterns of divergence link to phenotypic diversification?

Kristin Alligood

Lateral plate and opercle shape co-vary in the wild



An interesting stickleback hybrid population in Oregon



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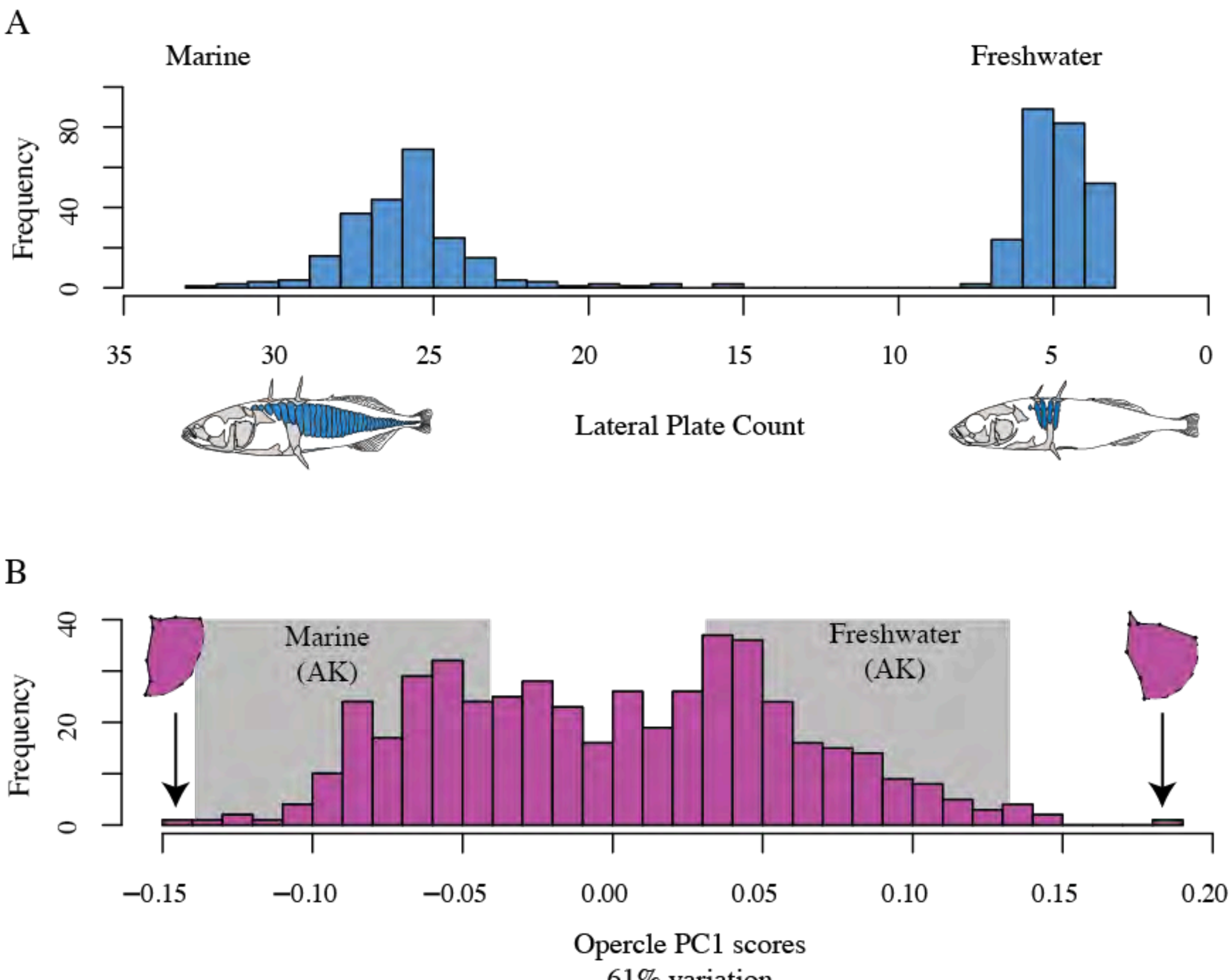

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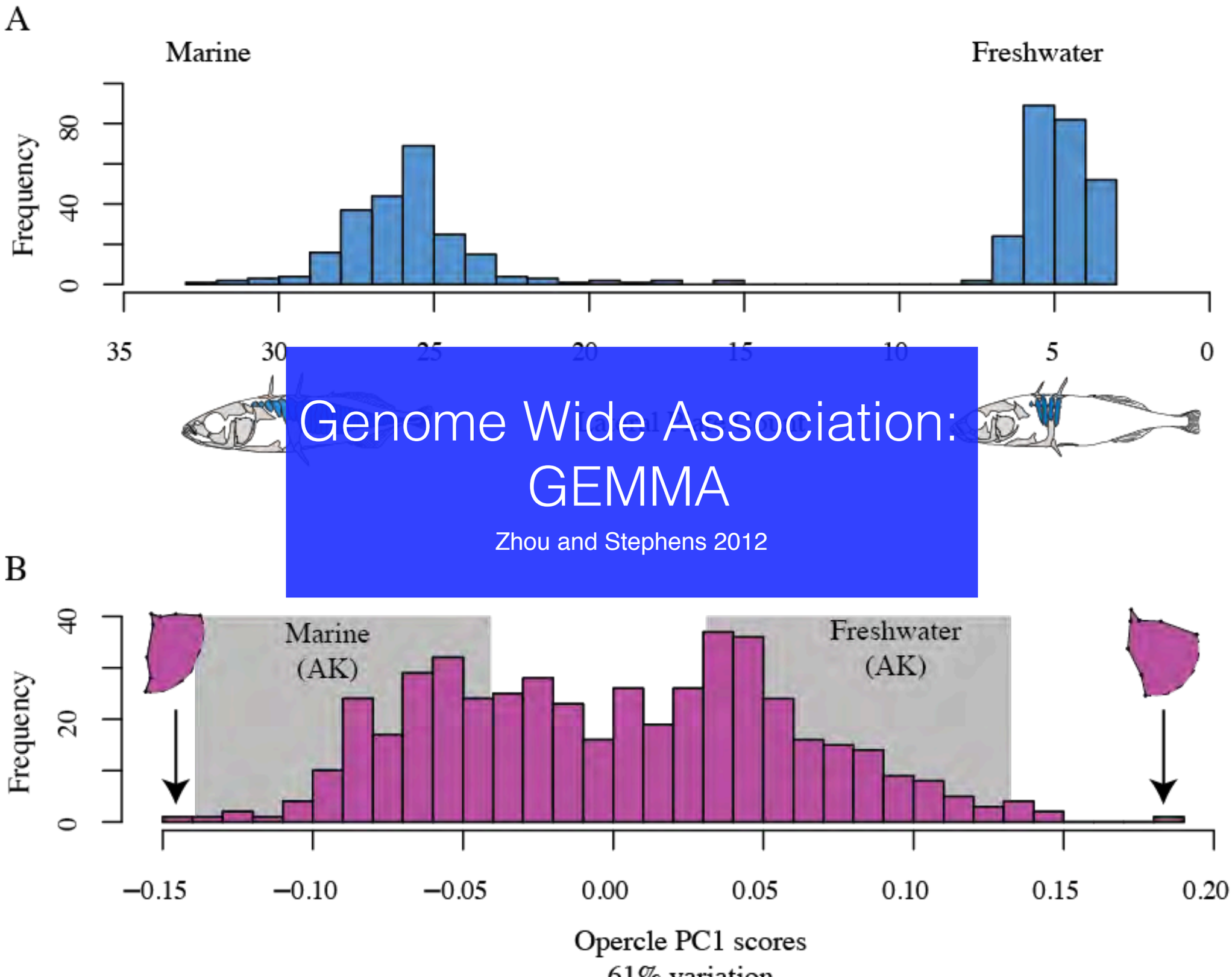
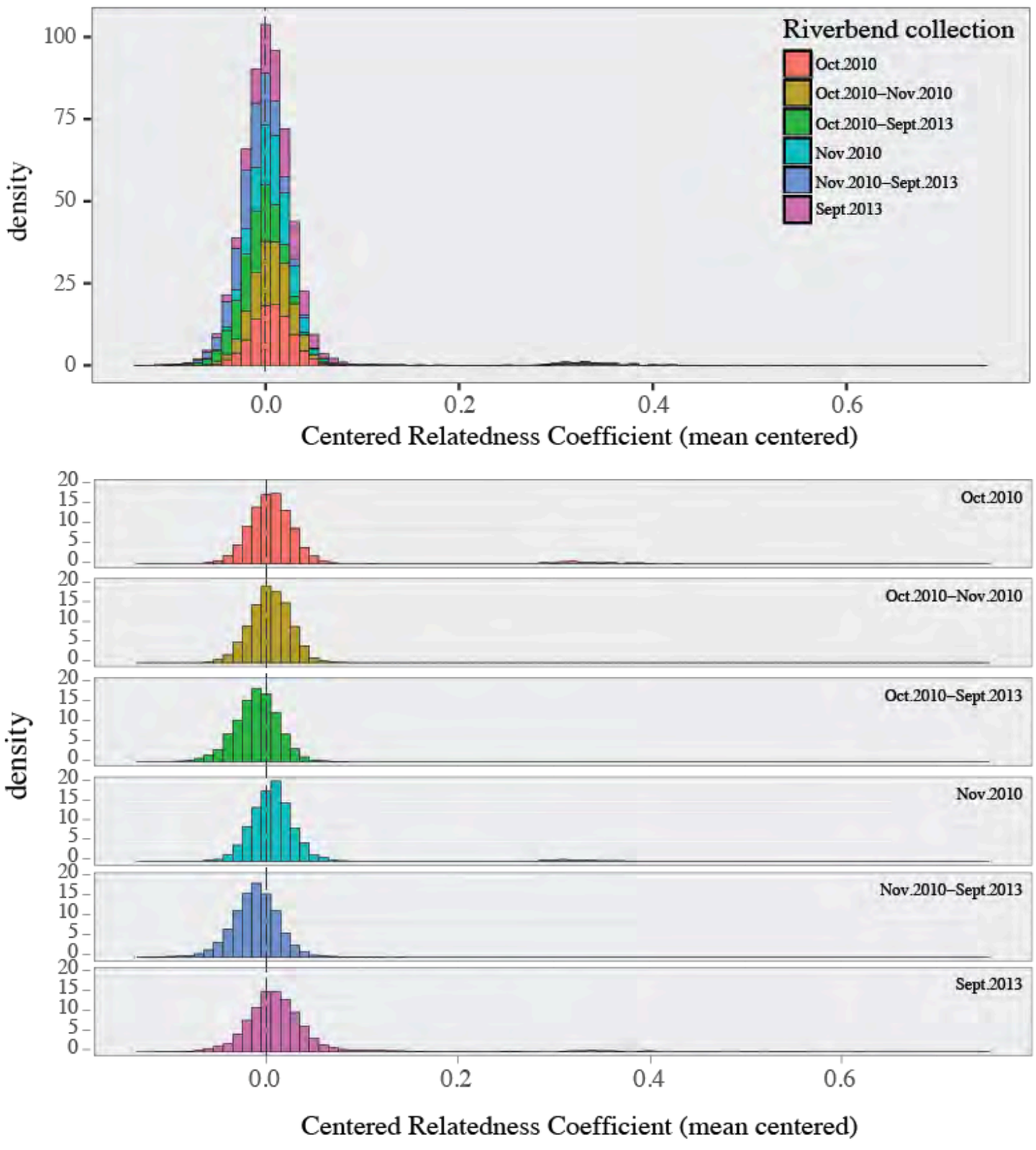


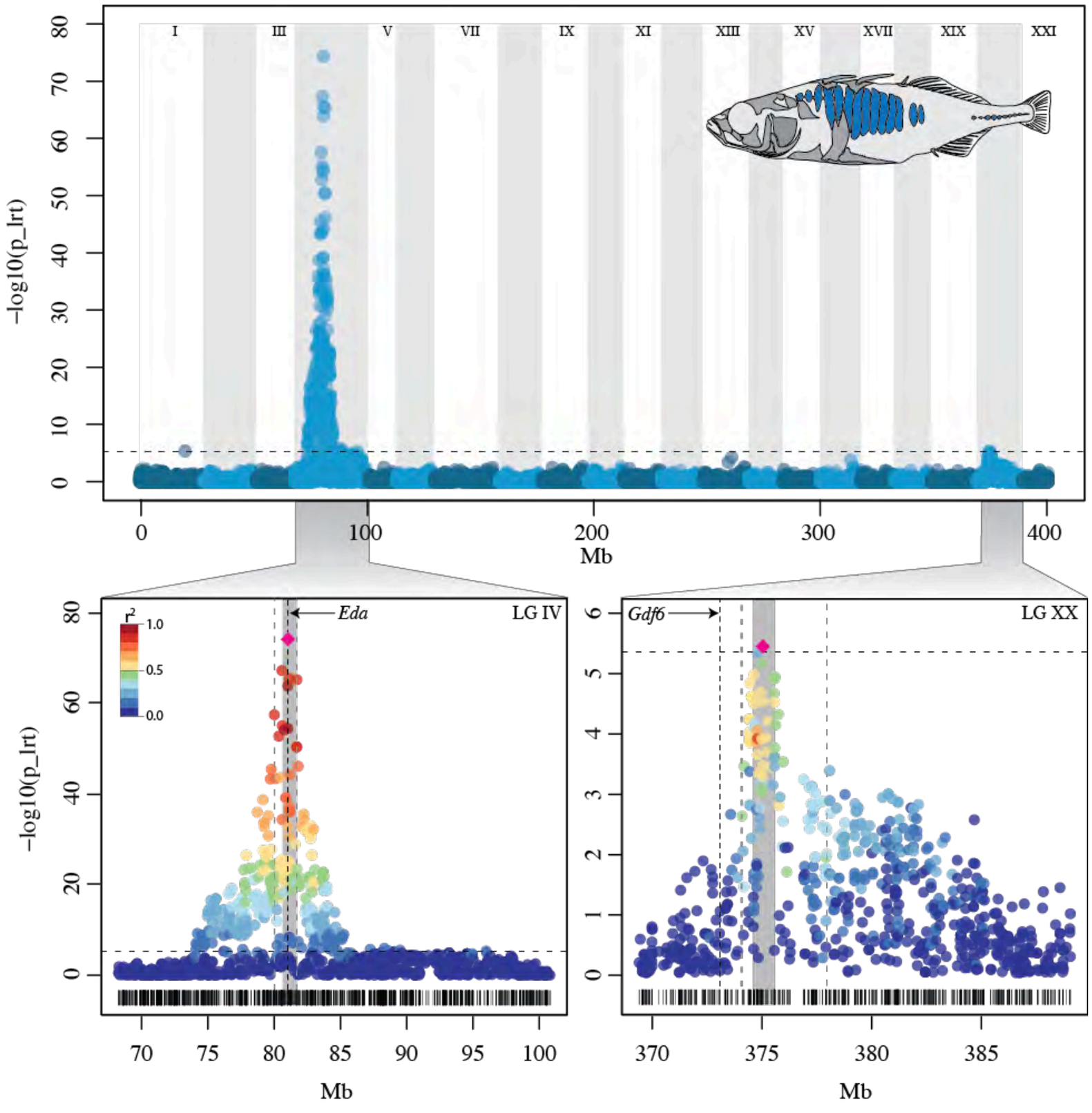
Genome Wide Association: GEMMA

Zhou and Stephens 2012

Pairwise relatedness among individuals



Lateral plate count:
A good hit to a novel locus on LGXX



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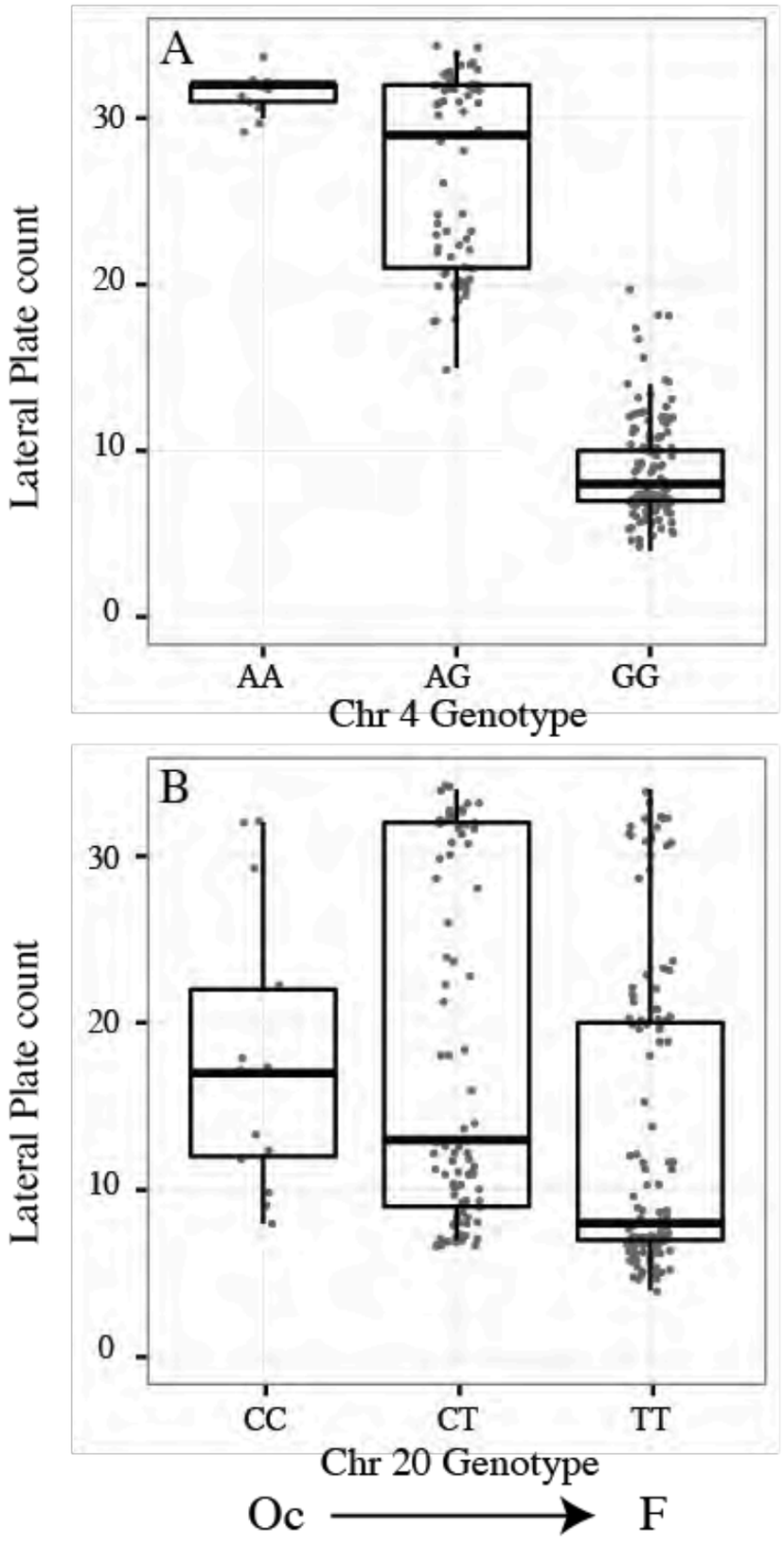
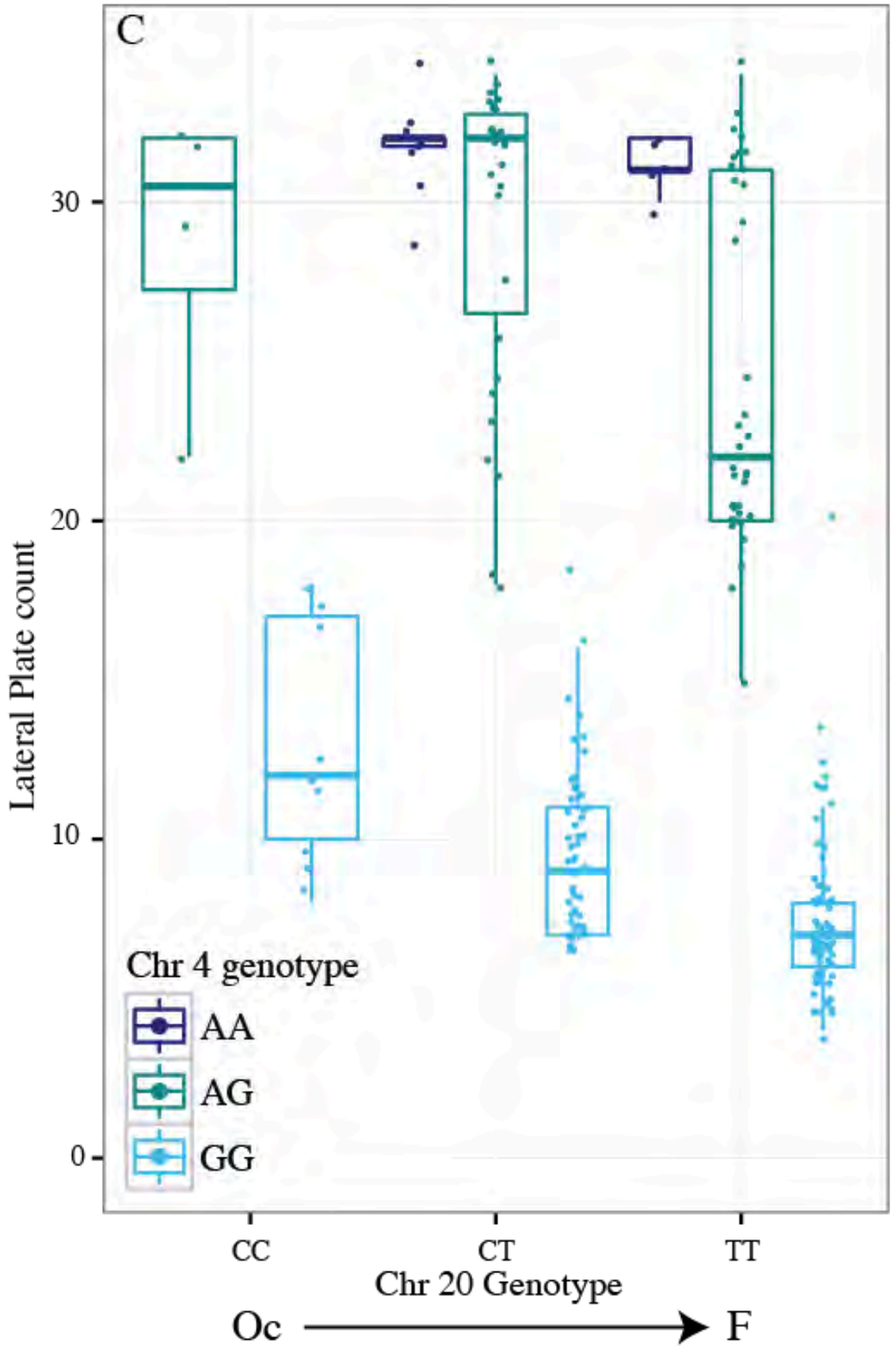
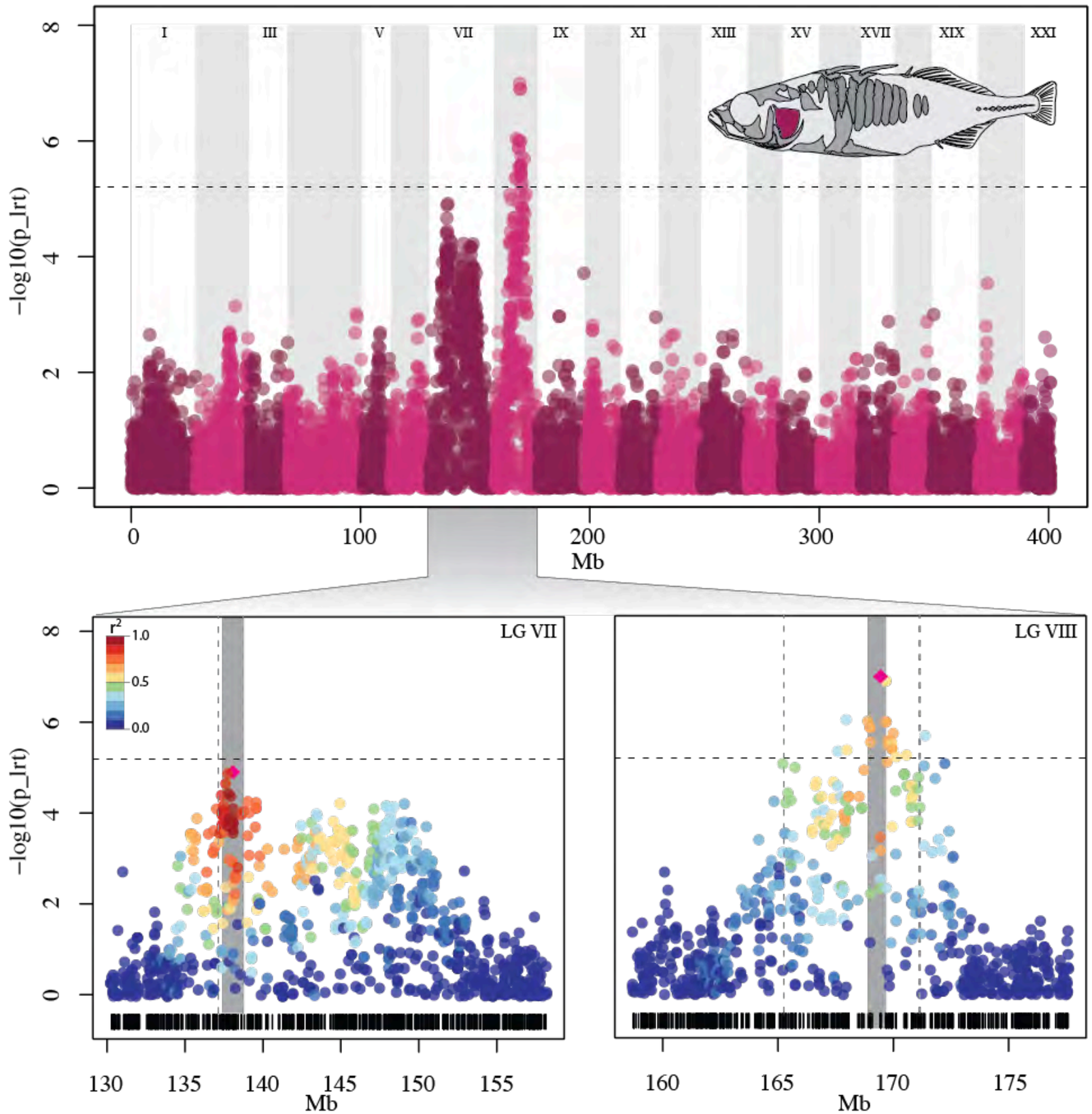
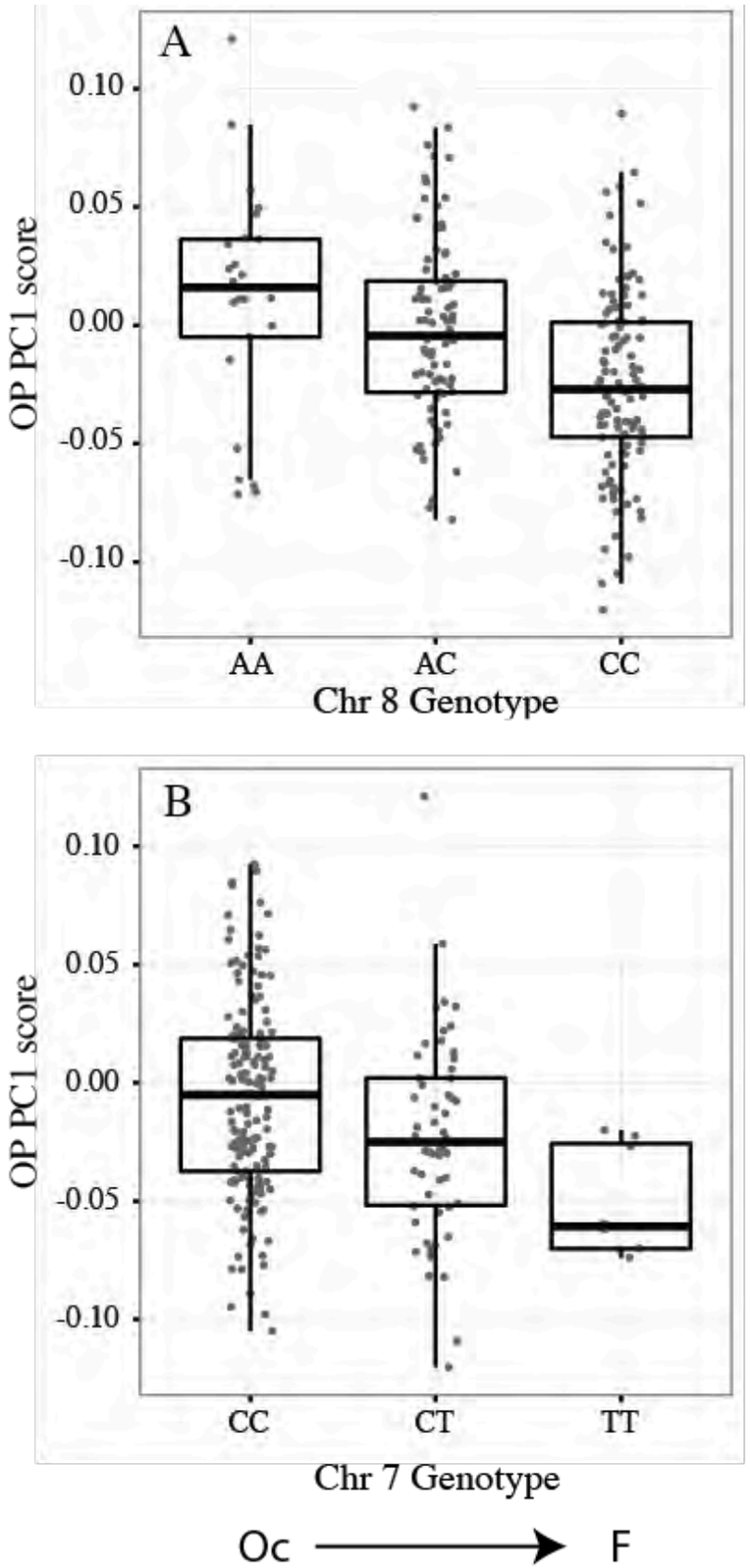


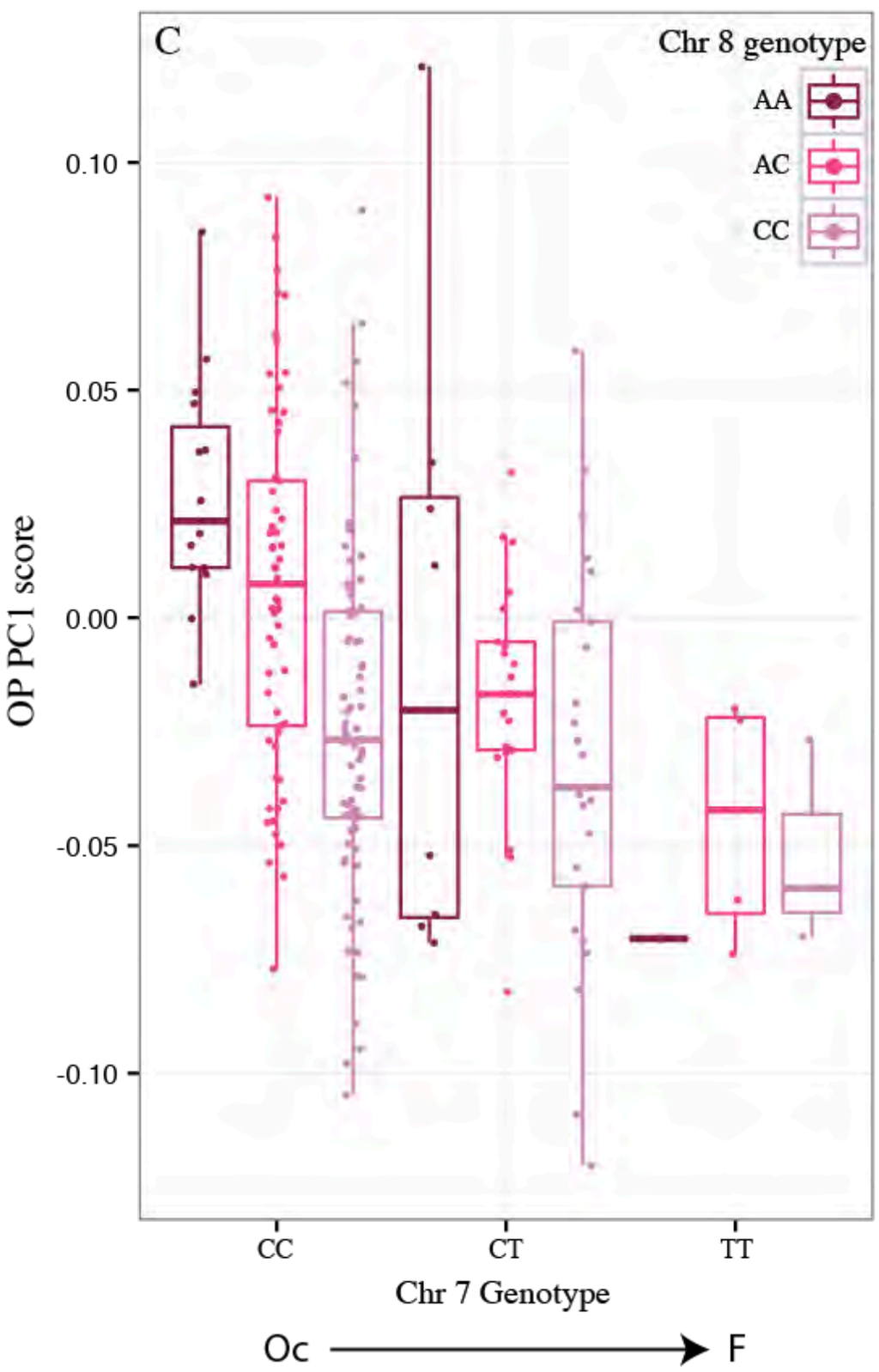


Opercle shape:
Good hits on LGVII and LGVIII

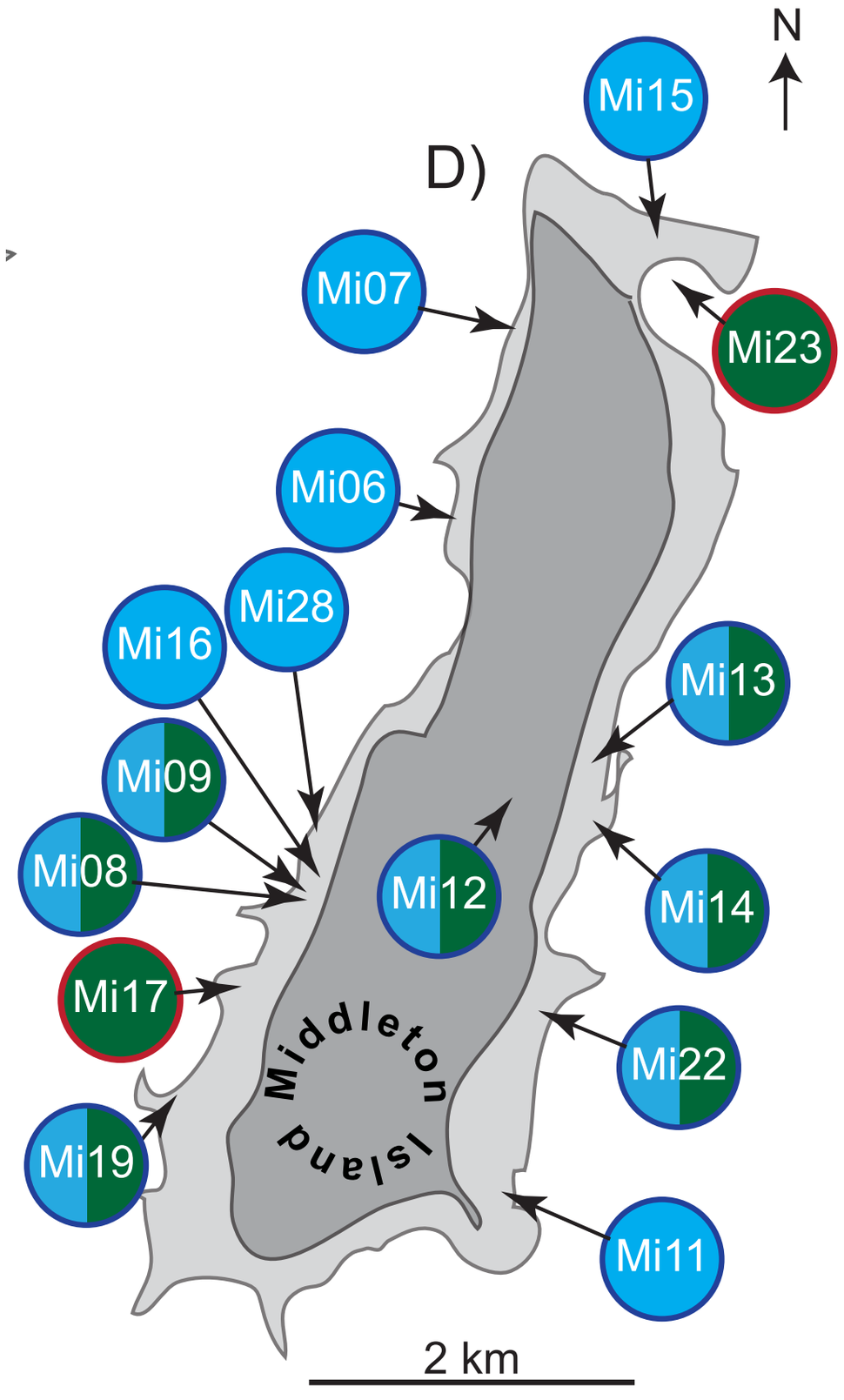


Opercle shape:
Good hits on LGVII and LGVIII

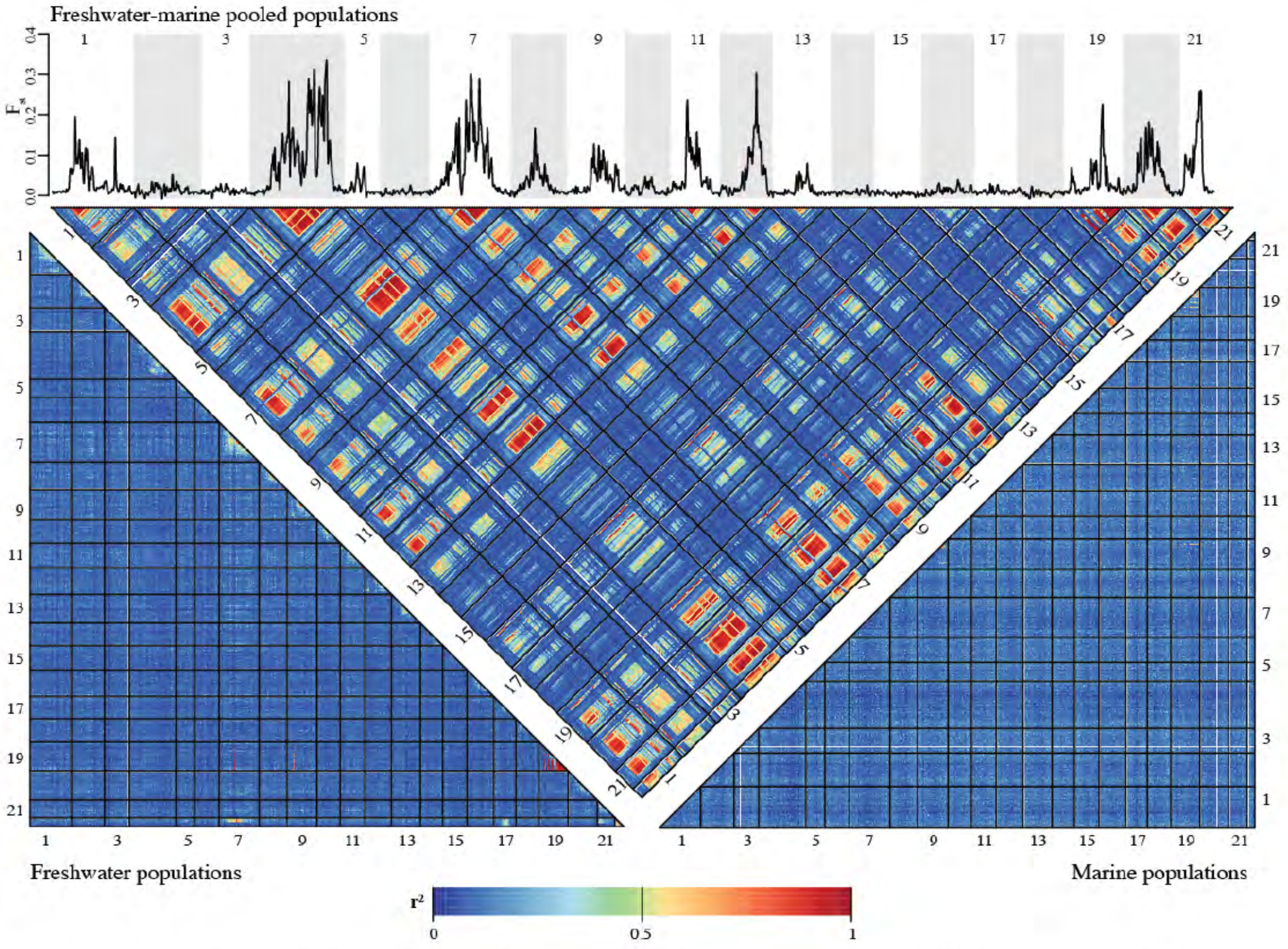




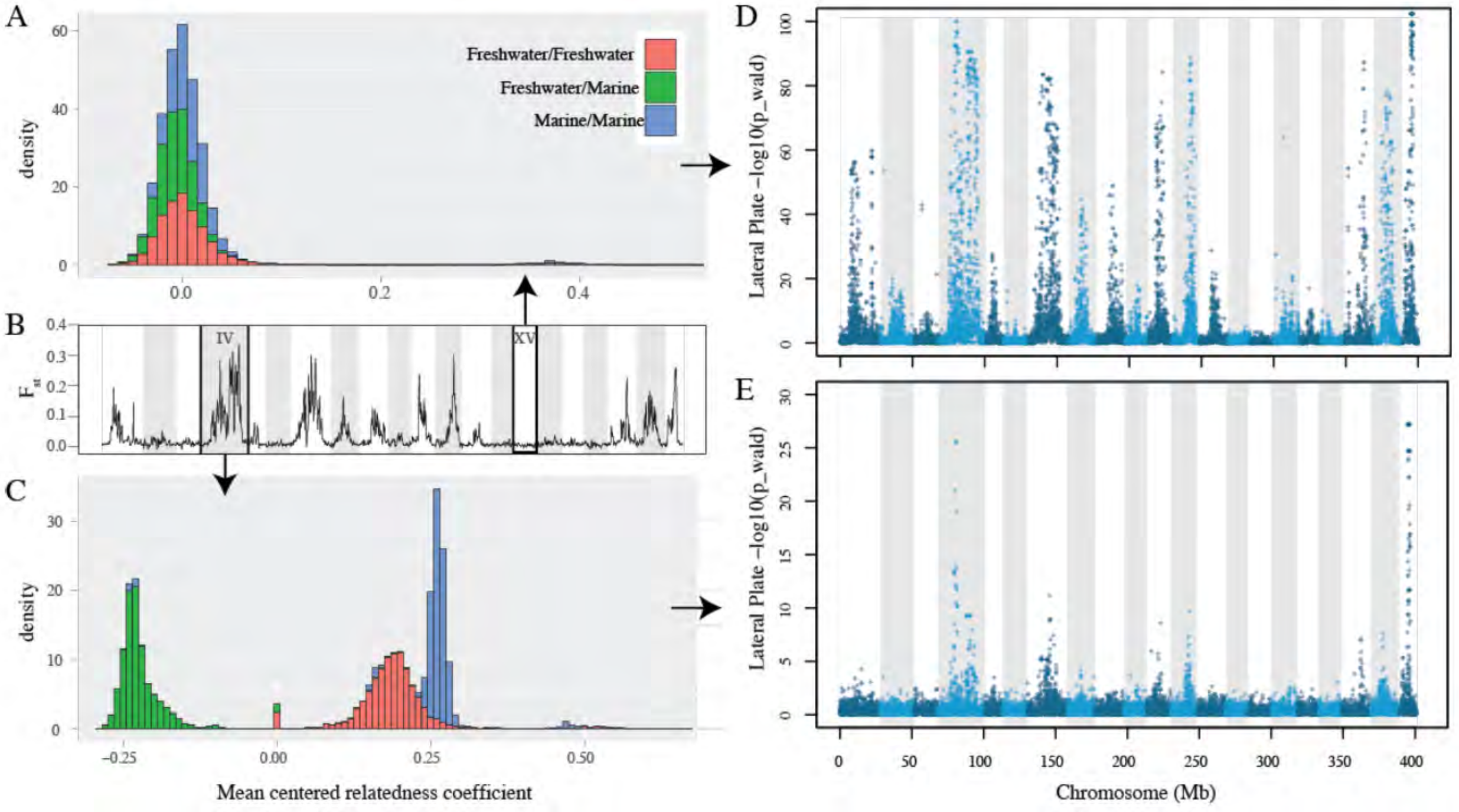
Can’t we just pool together young divergent populations and do GWAS?



Linkage disequilibrium created by natural selection

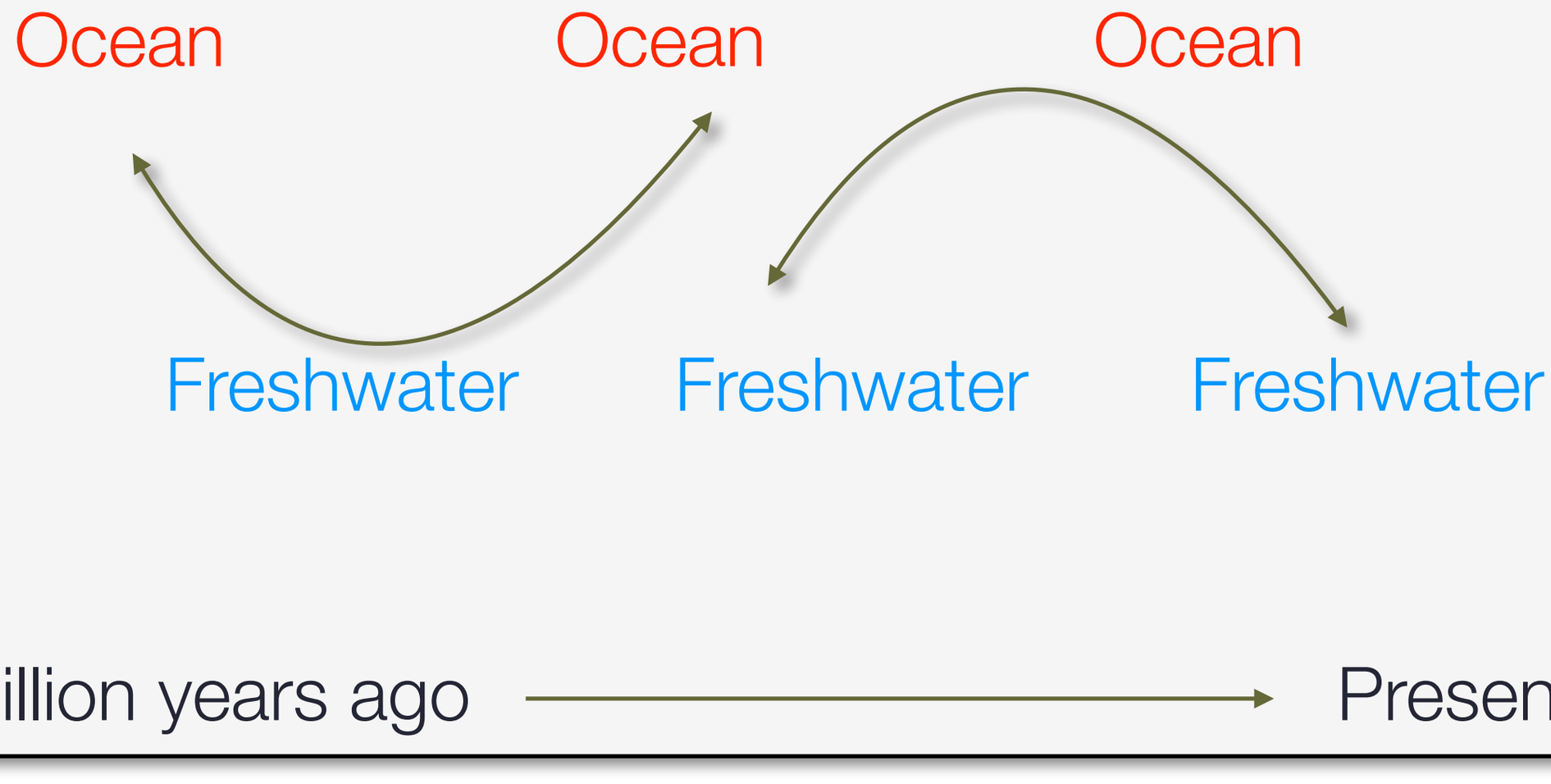


This sort of population structure is difficult to control



- Previous work has shown that the freshwater genomes evolve in 13,000 years.
- These new Middleton Island data shows that the phenotype can appear in as little as *50 years*.
- Much of the divergence involves soft sweeps.
- This could represent thousands of haplotypes reassembling, but the genome appears *chunkier*.
- Many haplotypes are *habitat specific*, quite *ancient* and coincide with *structural variation*
- Diverging phenotypes *map* to these same genomic regions

Hypothesis: Old genomic architecture variation is a product of the metapopulation structure of stickleback, and this architecture strongly influences subsequent rapid evolution.



Outline for today's lecture

RAD-seq for ecological and evolutionary genomics

Primer on Population Genomics

Evolutionary genomics of stickleback fish

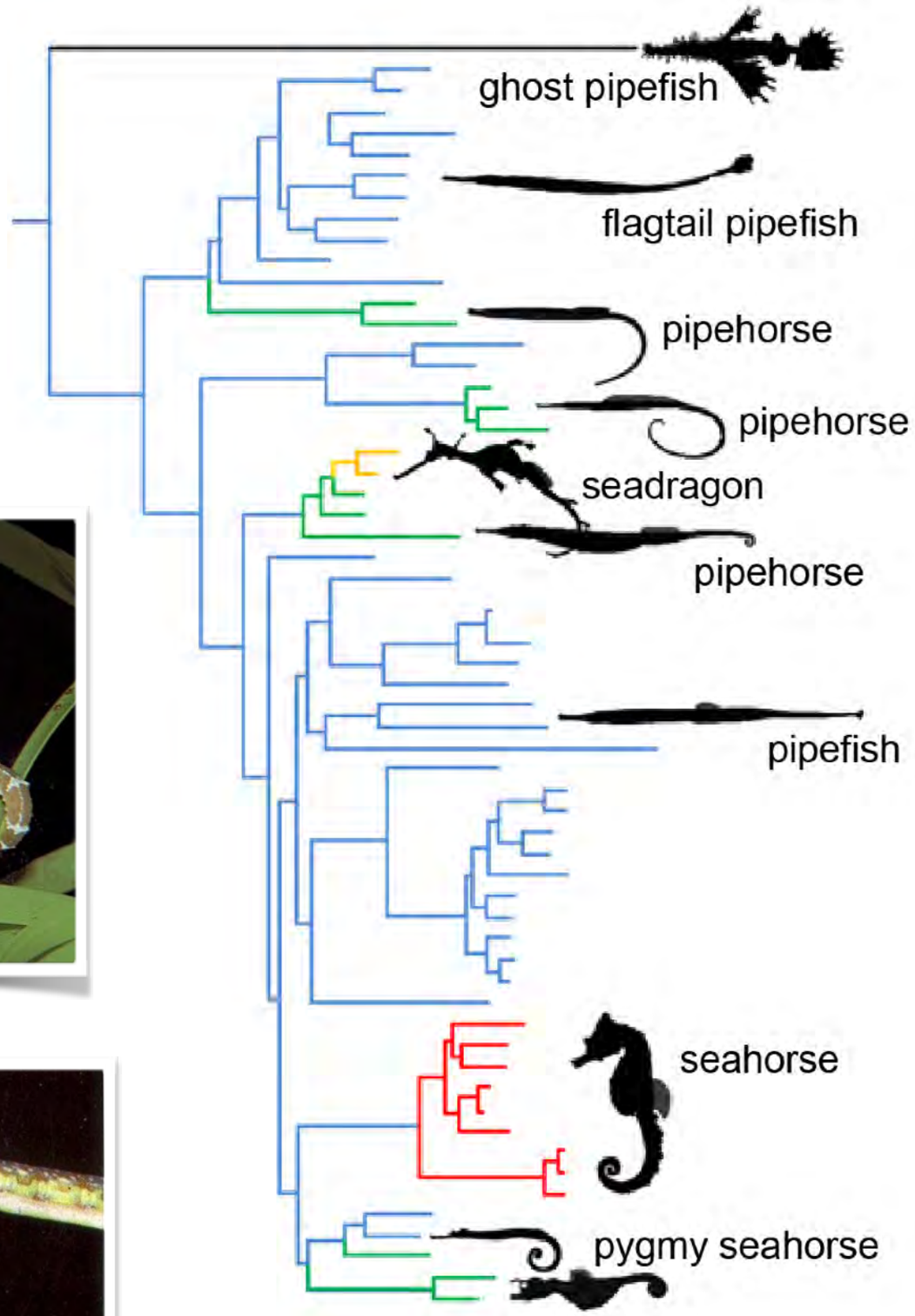
- Population genomics of rapid adaptation
- Using long read RAD-seq for coalescent analyses
- Genome Wide Association Studies using RAD-seq

Genomically enabling the Gulf pipefish

What if you don’t have a genome sequence?

A case study of RAD-seq and genome assembly

Seahorses, sea dragons and pipefishes



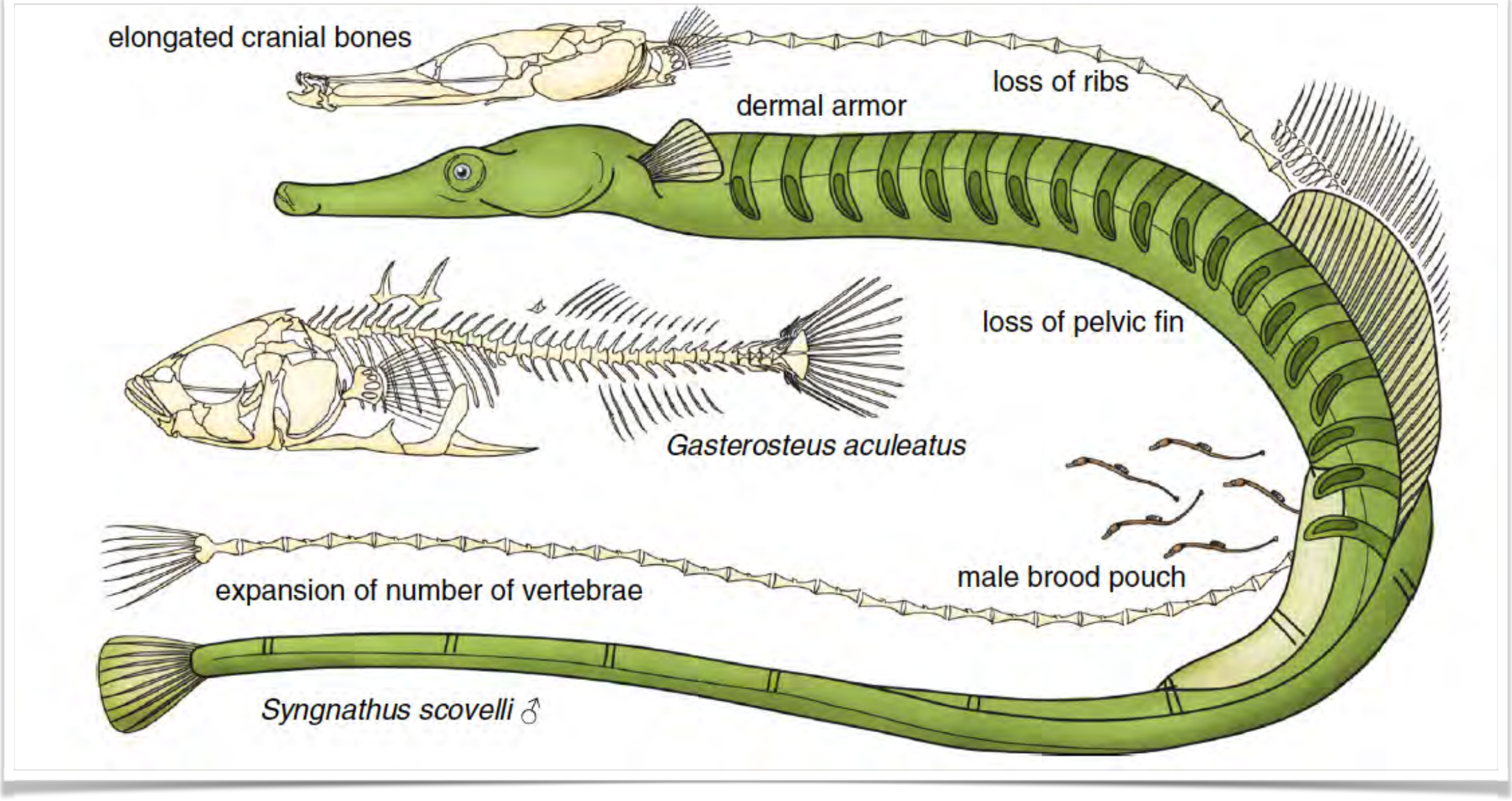
Small *et al. Genome Biology* (2016) 17:258
DOI 10.1186/s13059-016-1126-6

Genome Biology

RESEARCH Open Access

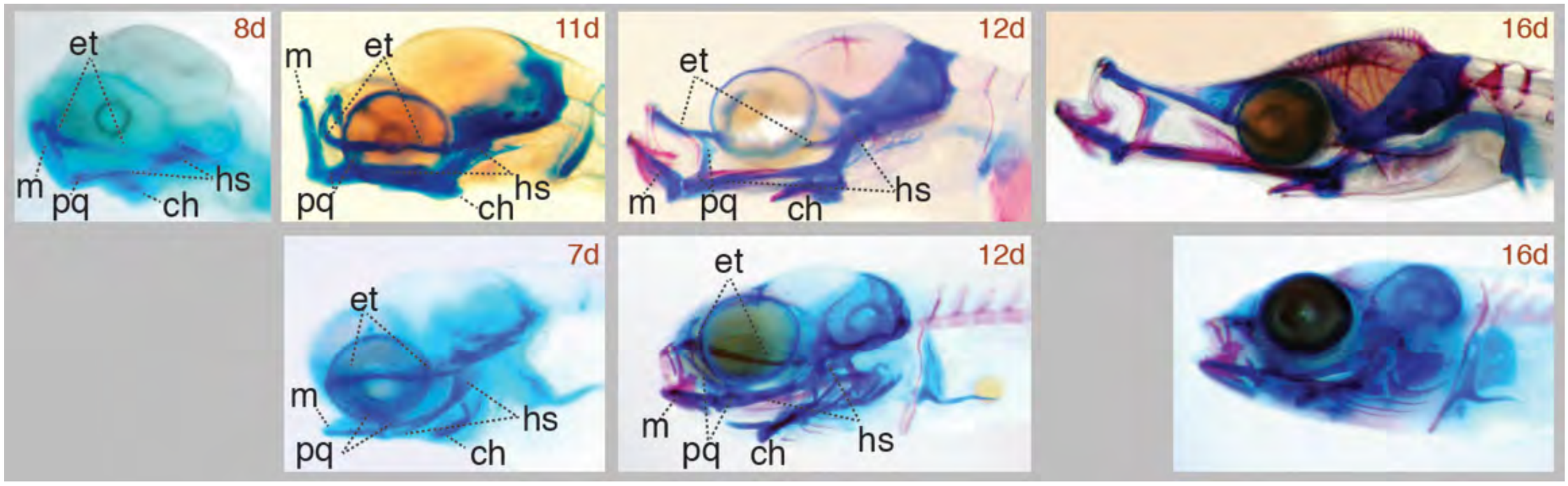
The genome of the Gulf pipefish enables understanding of evolutionary innovations

C. M. Small[1†], S. Bassham[1†], J. Catchen[1,2†], A. Amores[3], A. M. Fuiten[1], R. S. Brown[1,4], A. G. Jones[5] and W. A. Cresko[1*]



We're really interested in head development

Pipefish



Stickleback

How did we genomically enable pipefish

1) A high quality transcriptome

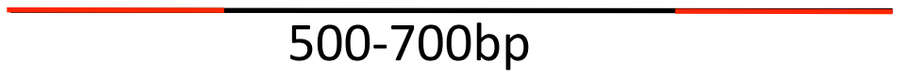
2) Very dense RAD genetic map

3) Deep shotgun sequencing of the genome

4) Order contigs against the RAD reference map

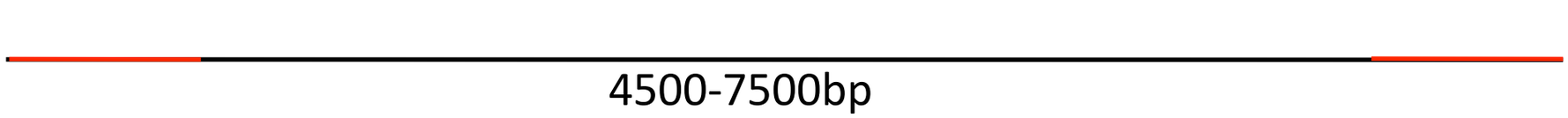
Illumina genomic libraries for pipefish genome

paired end 101bp



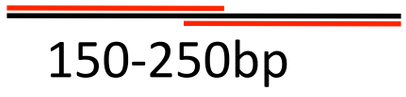
25x

mate pair



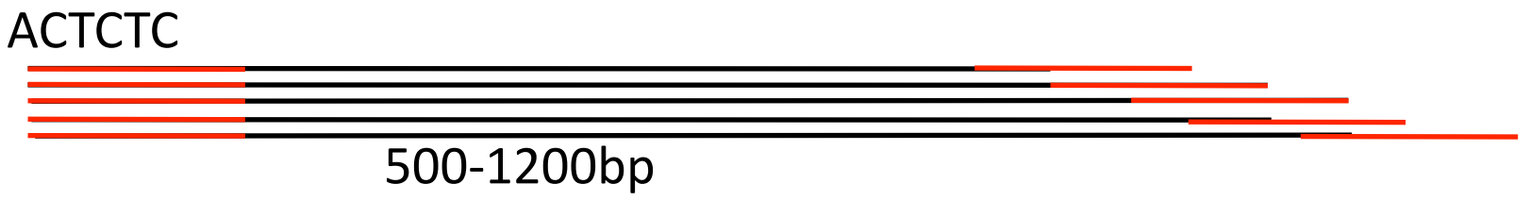
2x

overlapping



40x

paired end RAD



15-25x of 3% of the genome

Nearly the whole genome is covered

Table 1 Scaffold-level assembly statistics for the Gulf pipefish genome

Genome	Scaffolds (n)	Longest scaffold	Scaffold N50	Contig N50	Assembly length	Gaps in assembly (%)	CEGs complete (%)
Gulf pipefish (*Syngnathus scovelli*)	2104	6.7 Mb	640.4 kb	32.2 kb	307.0 Mb	6.6	98.8
African turquoise killifish (*Nothobranchius furzeri*)	29,054	0.7 Mb	119.7 kb	8.7 kb	1010.9 Mb	7.7	94.8
Blind cave fish (*Astyanax mexicanus*)	10,542	9.8 Mb	1775.3 kb	14.7 kb	1191.1 Mb	19.1	87.9
Spotted gar (*Lepisosteus oculatus*)	2105	21.3 Mb	6928.1 kb	68.3 kb	945.8 Mb	8.1	90.7

Created a genetic map

Generated an F1 family of 103 individuals

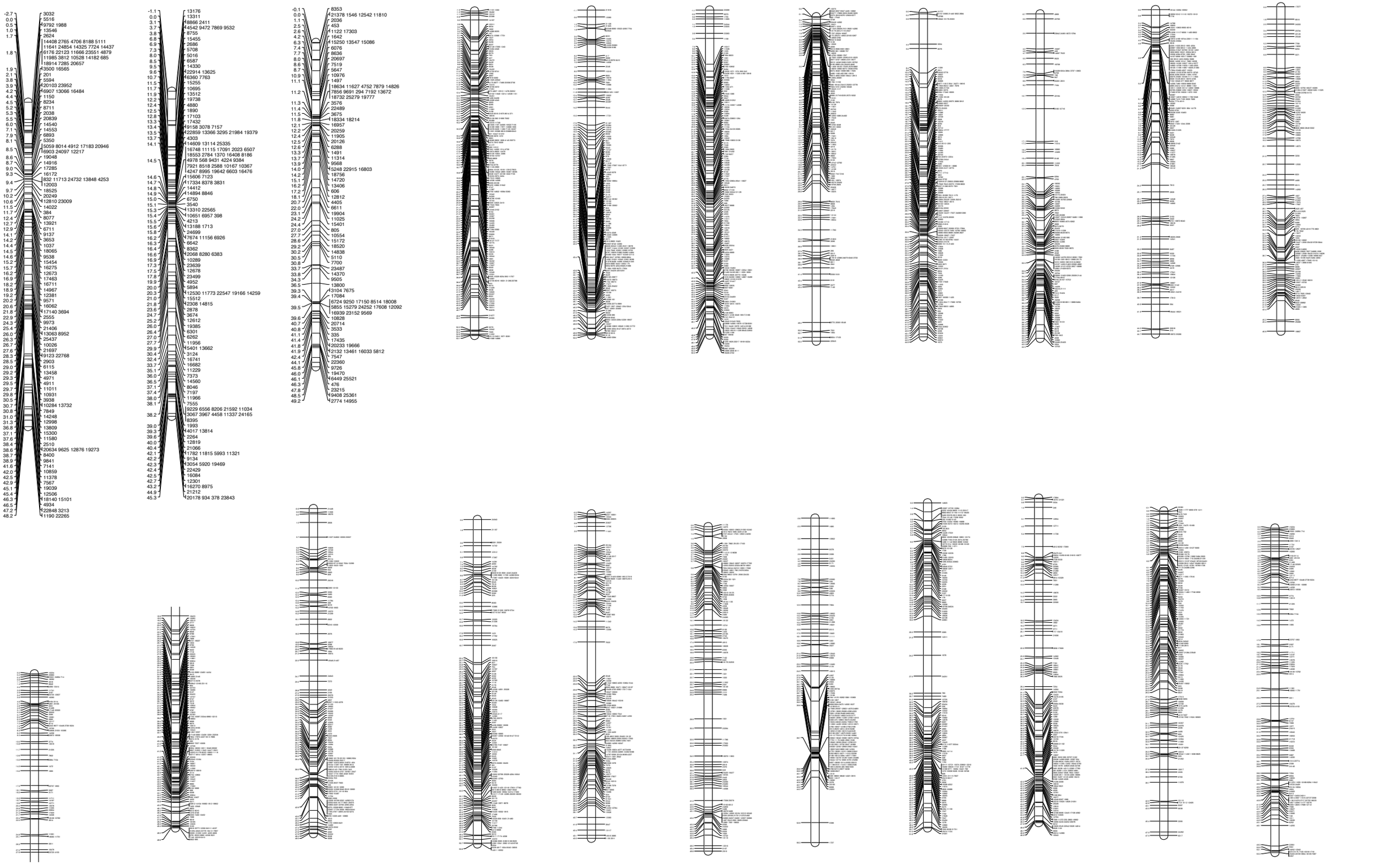
RAD sequenced the parents and offspring

Analyzed the data using *Stacks*

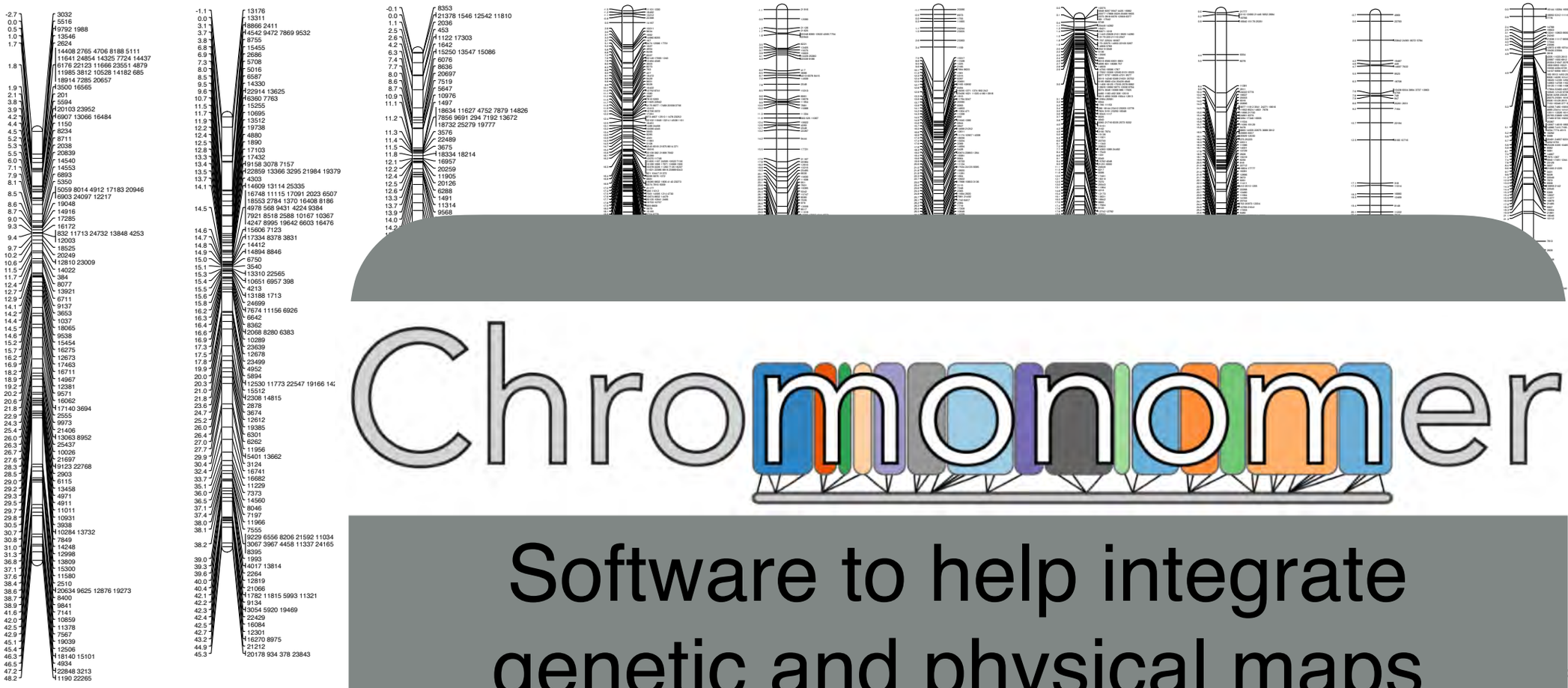
Output to JoinMap format

Created Linkage map

22 LGs; 6000 segregating SNPs; 30,000 RAD sites



The pipefish genetic map and genome together

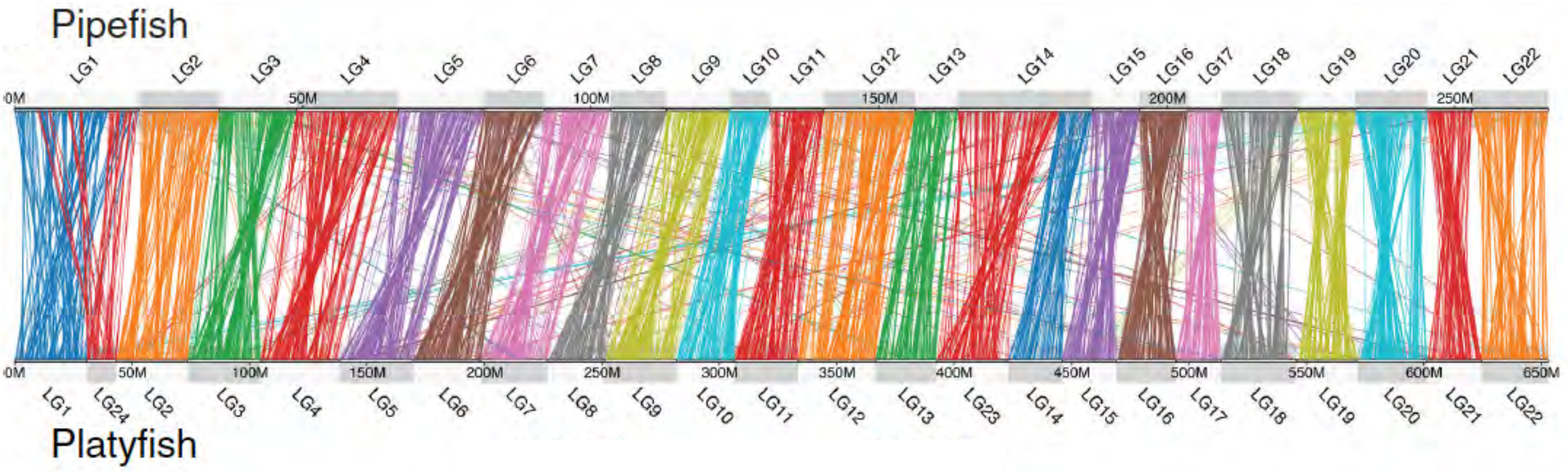


Chromonomer

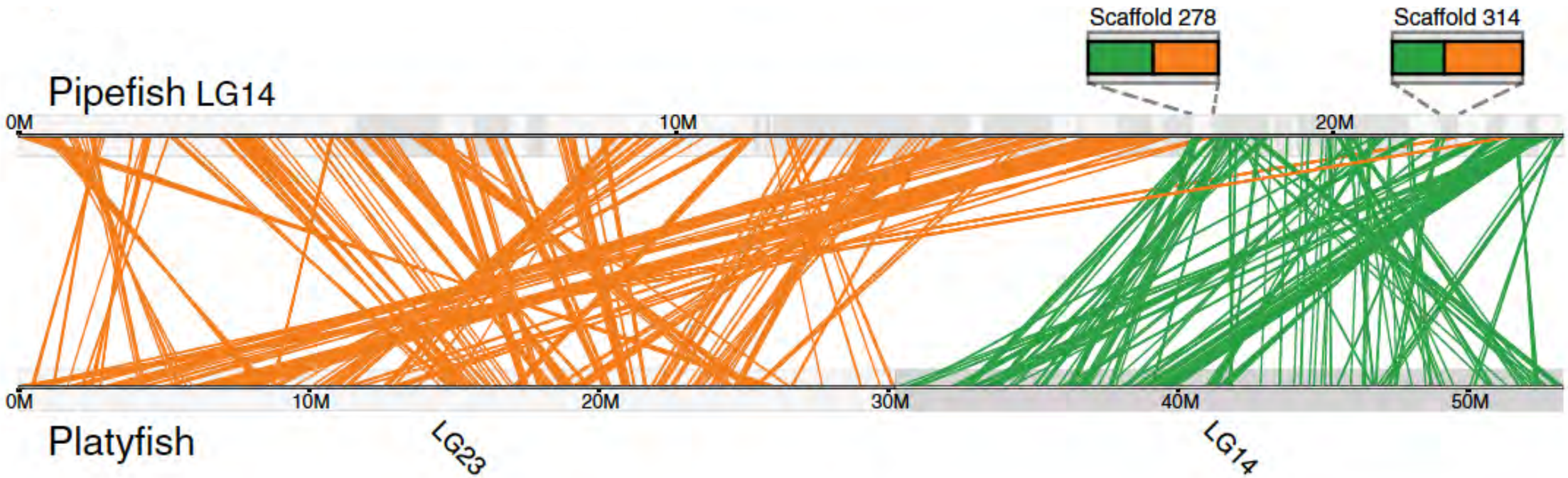
Software to help integrate genetic and physical maps

http://catchenlab.life.illinois.edu/chromonomer/

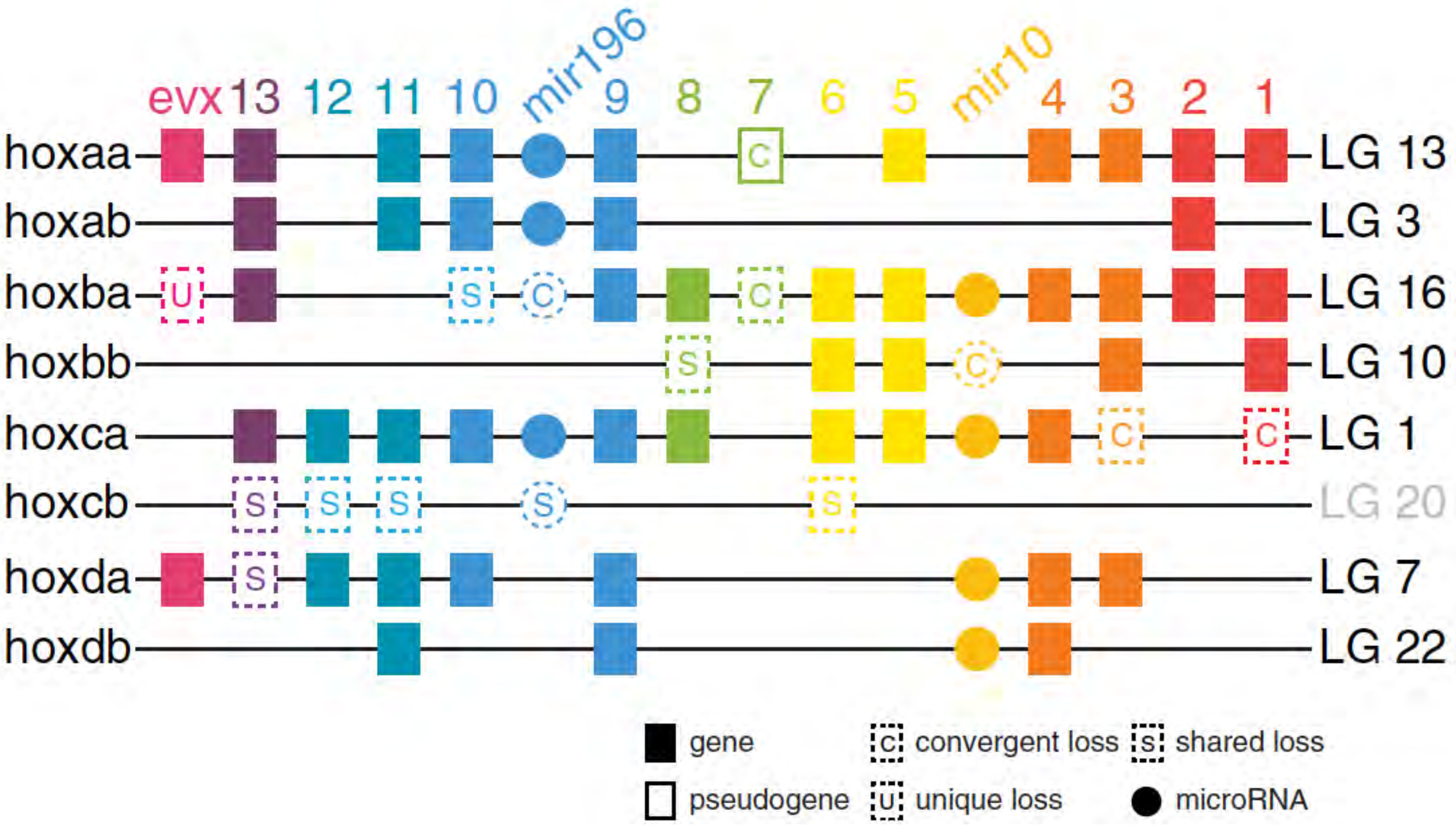
Two instances of chromosome fusion in pipefish



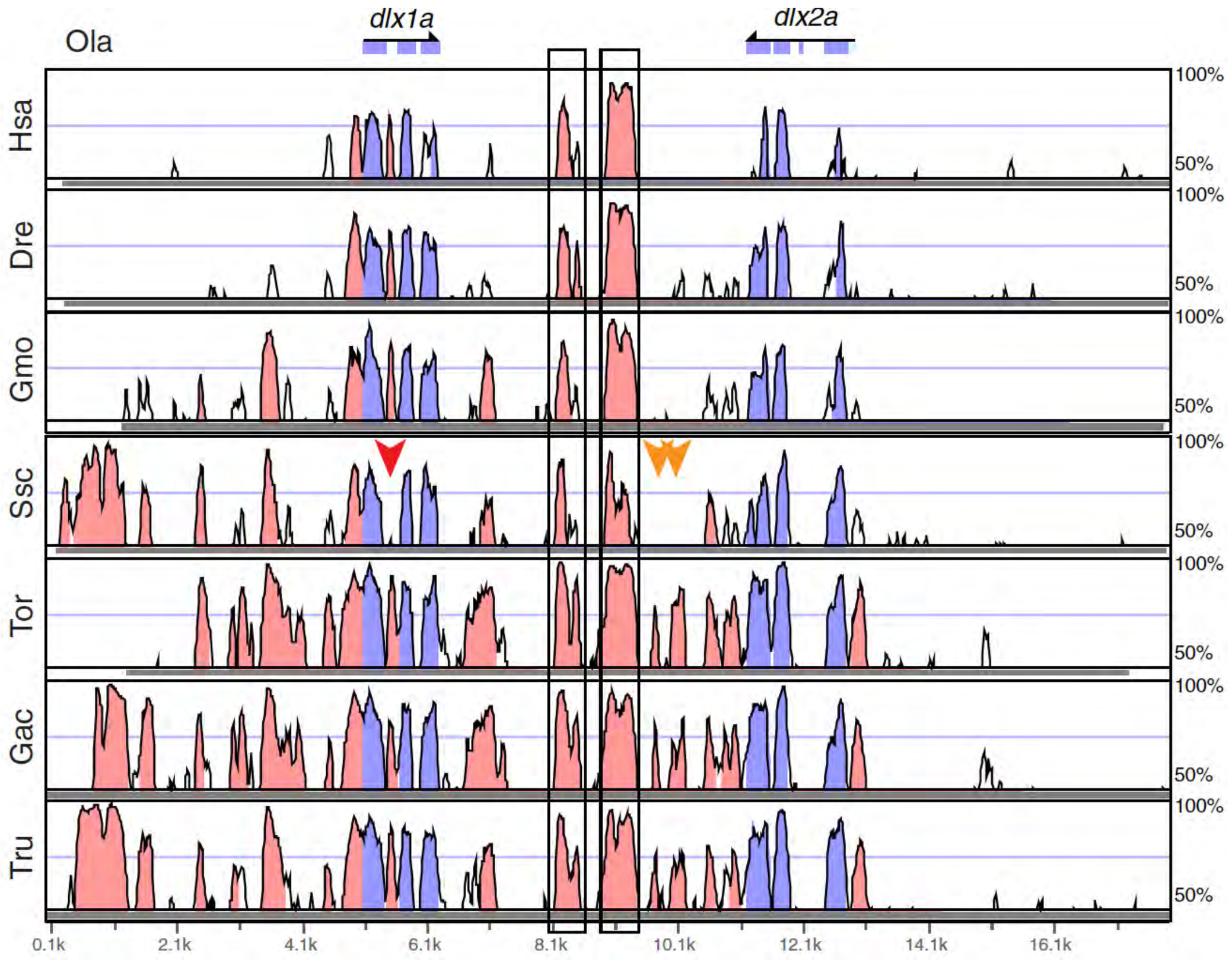
Two instances of chromosome fusion in pipefish



Key losses in *Hox* gene clusters



Key losses in conserved non-coding elements



Disruption of core hind fin patterning program

No evidence of *Tbx4* in the assembly

Disruption of core hind fin patterning program

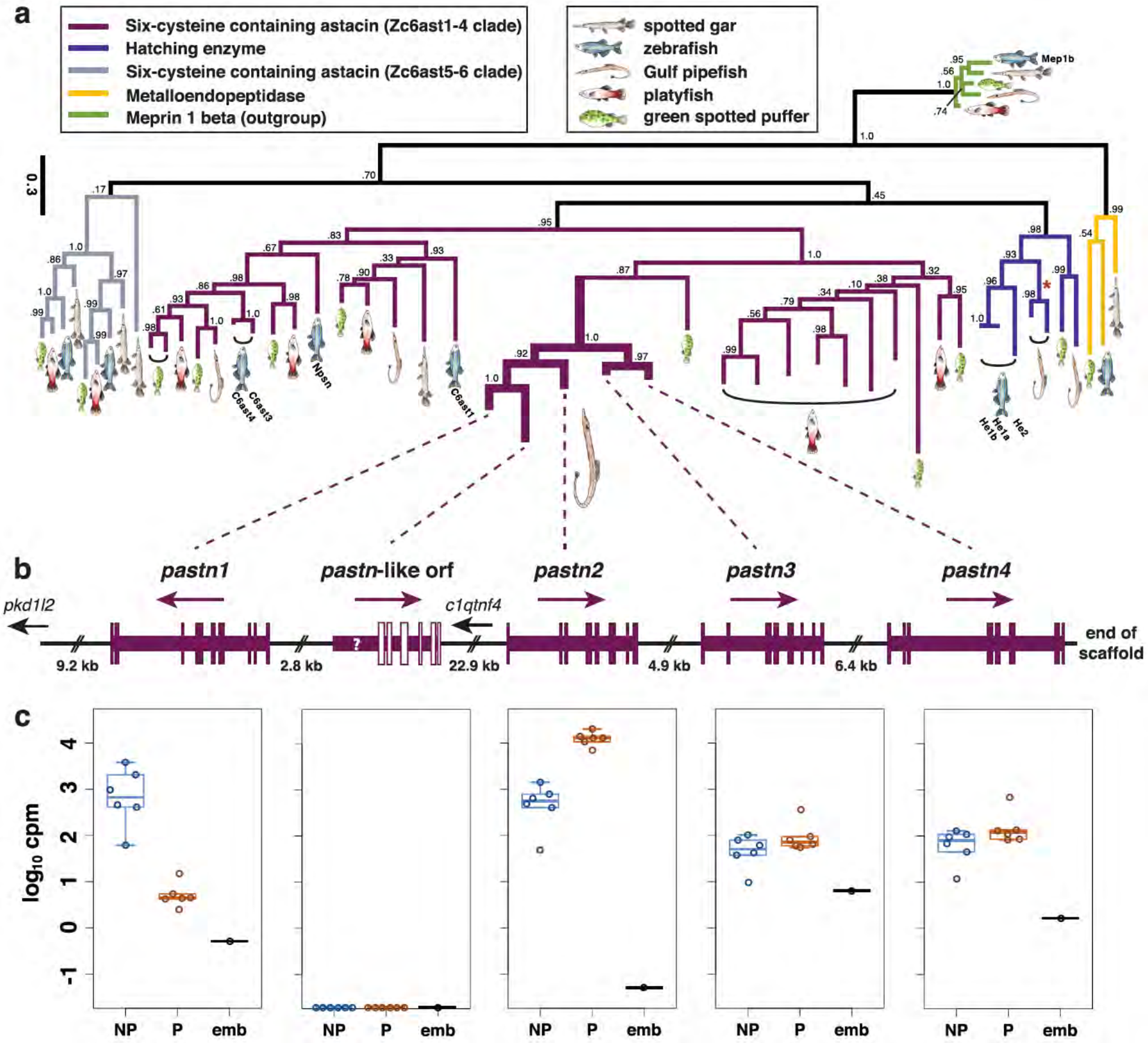
Disruption in coding sequence of *Pitx1*

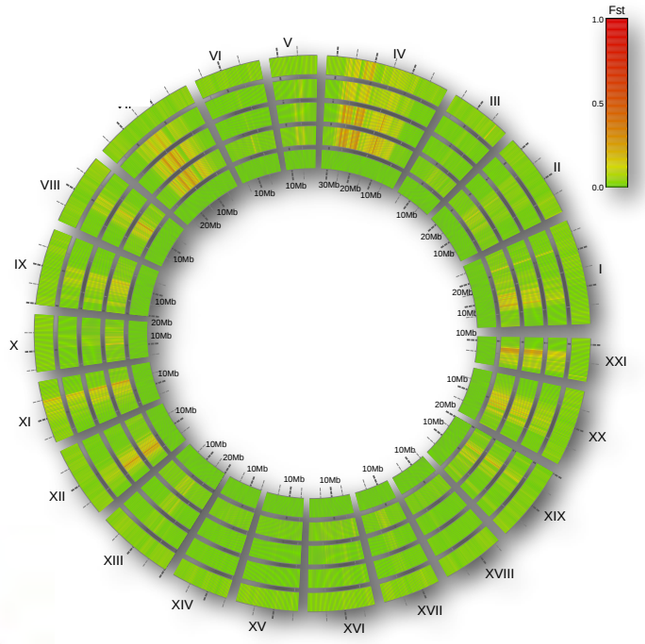
```
Pitx1
WRKRERNQQLDLCKGGYVPQFSGLVQPYED-VYA------AGYS-YNNWAAKSL-APAPLSTKSFTF-FNSM--SPLSSQ--SMF-SA-PSSISSMTM-------PSSMGPGAV  human
WRKRERNQQMDLCKNGYVPQFNGLMQSYDE-MYA-------GYH-YNNWATKSL-TPAPLSTKGFTF-FNSM--SPLPSQ--SMF-SA-PSTISSMNM-------SSTMGHSGV  coelacanth
WRKRERNQQMDLCKNSYLPQFSGLVQPYDD-MYP-------AYT-YNNWTNKGL-APAPLSTKNFTF-FNSM--SPLTSQ--SMF-SA-PNSISSMNM-------PPTMAHTAV  gar
WRKRERNQQMDLCKNSYLPQFSGLMQPYDD-VYP-------TYT-YNNWTNKGL-TPAPLSTKNFTF-FNSM--SPLTSQ--SMF-SA-PSSISSMSM-------ASGMGHSAV  zebrafish
WRKRERNQQMDLCKSGYLPQFSGLVQPYED-MYP-------PYT-YNNWTNKGL-GPAPLSTKSFPF-FNSM--SPLTSQ--SVF-SA-PSSISSMTM-------ASGMAHSAV  cavefish
    ERNQQMDLCKNSYLPQFSGLMQPYDD-MYP-------AYT-YNNWTNKGL-APAPLSTKNFTF-FNSM--STLTSQ--SMF-SA-PNSISSMSM-------SSGMGHSAV  ghost pipefish
WRKRERNQQMDLCKNAYLPQFSGLMQPYDDPMYPAAAAAAAAYT-YNNWPNKSLHGP-----KNFPF-FNSM--SPLTSQPVTMFSSS-PAPITTMSVQAAAAAAAAAHAHGGM  Gulf pipefish
    ERNQQMDLCKNTYLPQFSGLMQPYED-MYP------AAYT-YNNWTNKGLHGPTAPLAAKNFPFFNSM--SPLASQ--SVFSSSPTSISGMSMQHAAAAAASAGMAHSGV  messmate pipefish
WRKRERNQQMDLCKNSYLPQFSGLMQPYDD-MYP-------AYT-YNNWTNKGL-APAPLSTKNFTF-FNSM--SPLTSQ--SMF-SA-PNSISSMTM-------ASGMGHSAV  medaka
WRKRERNQQMDLCKNSYLPQFSGLMQPYDD-MYP-------AYT-YNNWTNKGL-TPAPLSTKNFTF-FNSM--SPLTSQ--SMF-SA-PNSISSMTM-------ASGMGHSAV  tilapia
WRKRERNQQMDLCKNSYLPQFSGLMQPYDD-MYP-------AYT-YNNWTNKGL-APAPLSTKNFTF-FNSM--SPLTSQ--SMF-SA-PNSISSMTM-------ASGMGPSAV  pufferfish
WRKRERNQQMDLCKSSYLPQFSGLMQPYED-MYP-------TYS-YNNWPNKGL-APAPLSSKNFTF-FNSM--SPLTSQ--SMF-SA-PSSISSMTM-------APGMGPPAA  stickleback

Pitx2
WRKRERNQQAELCKNGFGPQFNGLMQPYDD-MYP-------GYS-YNNWAAKGL-TSASLSTKSFPF-FNSMNVNPLSSQ--SMF-SP-PNSISSMSM-------SSSMVPSAV  human
WRKRERNQQAELCKNGFGPQFNGLMQPYDD-MYP-------SYS-YNNWAAKGL-TSASLSTKSFPF-FNSMNVNPLSSQ--TMF-SP-PNSISSMSM-------SSSMVPS-V  coelacanth
WRKRERNQQAELCKNGFGPQFNGLMQPYDD-MYP-------SYT-YNNWAAKGL-TSASLSTKSFPF-FNSMNVNPLSSQ--TMF-SP-PNSISSMSM-------SSSMVPSAV  gar
WRKRERNQQAELCKNGFGPQFNGLMQPYDD-MYP-------SYT-YNNWAAKGL-TSASLSTKSFPF-FNSMNVNPLSSQ--TMF-SP-PNSISSMSM-------SSSMVPSAV  zebrafish
WRKRERNQQAELCKGGFGAQFNGLVQPYED-MYA-------SYPPYNNWAAKSL-APASLSAKSFPF-FNSVNVSPLSSQ--AVF-SP-PTSISSMSV-------SSGMVPT--  cavefish
WRKRERNQQAELCKNGFGPQFNGLMQPYDD-MYP-------GYT-YNNWAAKGL-TSASLSTKSFPF-FNSMNVNPLSSQ--AMF-SP-PNSISSM---------TSGMVPSAV  Gulf pipefish
WRKRERNQQAELCKNGFGPQFNGLMQPYDD-MYP-------SYT-YNNWAAKGL-TSASLSTKSFPF-FNSMNVNPLSSQ--TMF-SP-PNSISSM---------TSSMVPAAV  medaka
WRKRERNQQAELCKNGFGPQFNGLMQPYDD-MYP-------SYT-YNNWAAKGL-TSASLSTKSFPF-FNSMNVNPLSSQ--TMF-SP-PNSISSM---------TSSMVPSAV  tilapia
WRKRERNQQAELCKNGFGPQFNGLMQPYED-MYP-------SYT-YNNWAAKGL-TPASLSTKSFPF-FNSMNVNPLSSQ--TMF-SPAPNSISSM---------TSGMVPSAV  pufferfish
WRKRERNQQAELCKNGFGPQFNGLMQPYED-MYP-------SYT-YNNWAAKGL-TSASLSTKSFPF-FNSMNVNPLSSQ--TMF-SP-SNSISSM---------TSSMVPSAV  stickleback

Pitx3
WRKRERSQQAELCKGSFAAPLGGLVPPYEE-VYP-------GYS-YGNWPPKAL-A-PPLAAKTFPFAFNSVNVGPLASQ--PVF-SP-PSSIAASMV-------PSAAAAPGT  human
WRKRERNQQAELCKNGFGAQFNGLMQPYDD-MYS-------GYS-YNNWATKGL-ATSPLSAKSFPF-FNSMNVSPLSSQ--PMF-SP-PSSIASMTM-------PSSMVPSAV  coelacanth
WRKRERNQQAELCKNGFGAQFNGLMQPYDD-MYS-------GYS-YNNWATKSL-ATSPLSAKSFPF-FNSMNVSPLSSQ--PMF-SP-PSSISSMNM-------ASSMVPSAV  gar
WRKRERNQQAELCKNGFGAQFNGLMQPYDD-MYS-------GYS-YNNWATKSL-ASSPLSAKSFPF-FNSMNVSPLSSQ--PMF-SP-PSSIPSMNM-------ASSMVPSAV  zebrafish
WRKRERNQQAELCKNGFGAQFNGLMQPYDD-MYS-------GYS-YNNWATKSL-ASSPLSAKSFPF-FNSMNVSPLSSQ--PMF-SP-PSSIPSMNM-------ASSMVPSAV  cavefish
WRKRERNQQAELCKNGFGAQFNGLVQPYDD-MYA-------GYS-YNNWASKSL-AGGQLSAKSFPF-FNSMNVSPLSSQ--PMF-SP-PSSMPSMNM-------ASGMVPSAV  Gulf pipefish
WRKRERNQQAELCKNGFGAQFNGLMQPYDD-MYT-------GYS-YNNWATKSL-ASSPLSTKSFPF-FNSMNVSPLSTQ--PMF-SP-PSSIPSMNM-------ASSMVPSAV  medaka
WRKRERNQQAELCKNGFGAQFNGLMQPYDD-MYT-------GYS-YNNWATKSLAASSPLSAKSFPF-FNSMNVSPLSSQ--PMF-SP-PSSIPSMNM-------ASSMVPTAV  tilapia
WRKRERNQQAELCKNGFGAQFNGLMQPYDD-VYS-------GYS-YNNWAAKGL-ASSPLSAKSFPF-FNSMNVSPLSSQ--SMF-SP-PSSLPSMNM-------ASSMVPSAV  pufferfish
WRKRERNQQAELCKNGFGSQFNGLMQPYDD-MYT-------GYS-YNNWATKSL-ASSPLSAKSFPF-FNSMNVSPLSSQ--PMF-SP-PSSIPSMNM-------ASSMVPSAV  stickleback
```

Expansion of male pregnancy specific gene family





Ecological & evolutionary genomic analyses using RAD-seq - what have we learned?

Overall Conclusions

RAD-seq can be a tool for enabling new research in models & nonmodels

- SNP identification and genotyping
- documenting patterns of genetic variation
- identifying the molecular genetic basis of important phenotypic variation
- assessing how ecological processes structure this genetic variation in genomes
- analytical and computational approaches are challenging but manageable

Not your father's (or your mother's) genome assembly

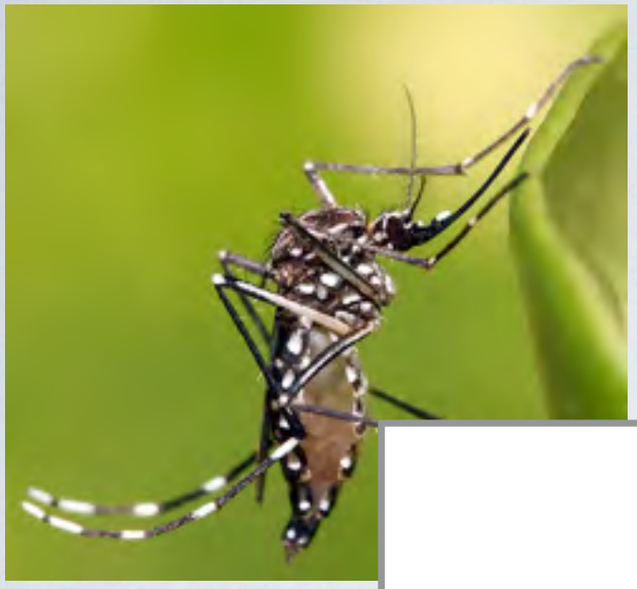
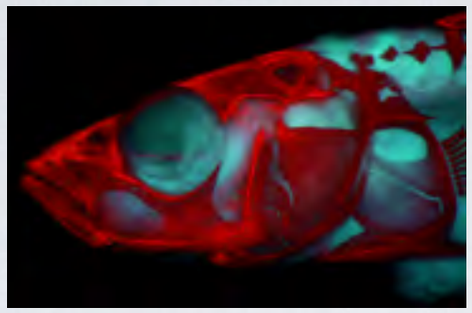
- a mixture of data types can be efficiently combined
- a genetic map is extremely useful for pulling it all together
- having a tiled genome of smaller contigs is often good enough

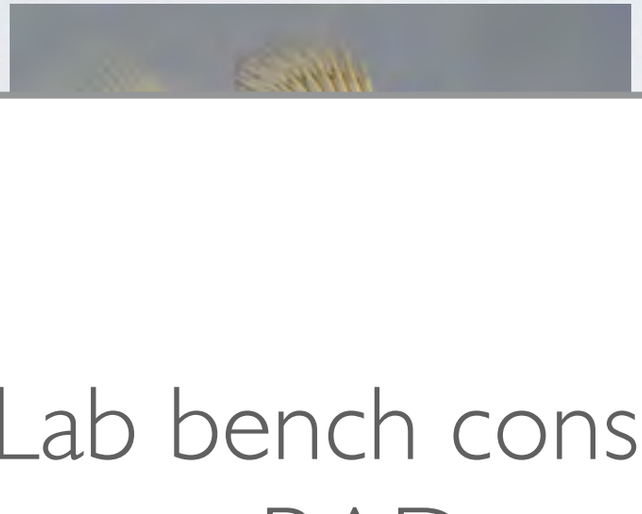
Open Source Genomics provides a suite of breakthrough technologies

- the molecular approaches are not as daunting as they first appear
- analytical and computational approaches are challenging
  - **New software tools can help, but knowledge of Unix and Scripting is essential**
  - **Also essential to be comfortable with classical and modern statistics**

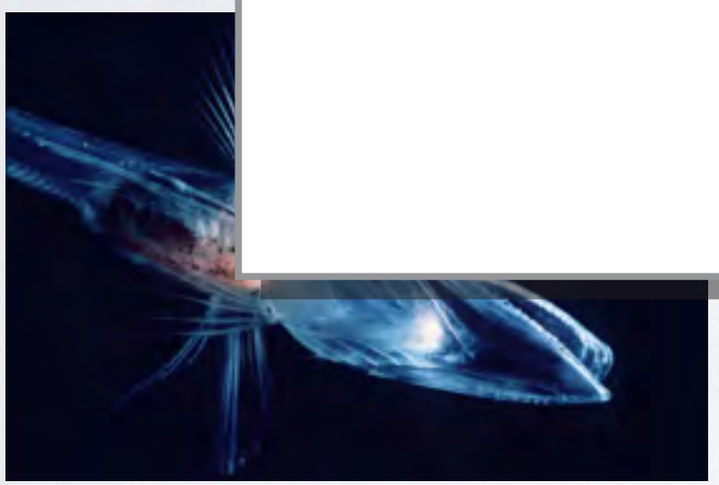
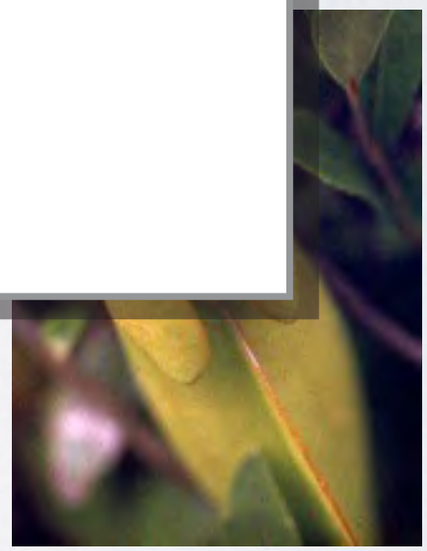
Acknowledgments

- *Past and present lab members* **Paul Hohenlohe**, **Thom Nelson**, Joe Dunham, Nicole Nishimura & **Mark Currey**
- *Collaborators* **Eric Johnson**, Patrick Phillips, **Chuck Kimmel**, **John Postlethwait**
- *Funding* from NSF & NIH, as well as Keck & Murdock Foundations

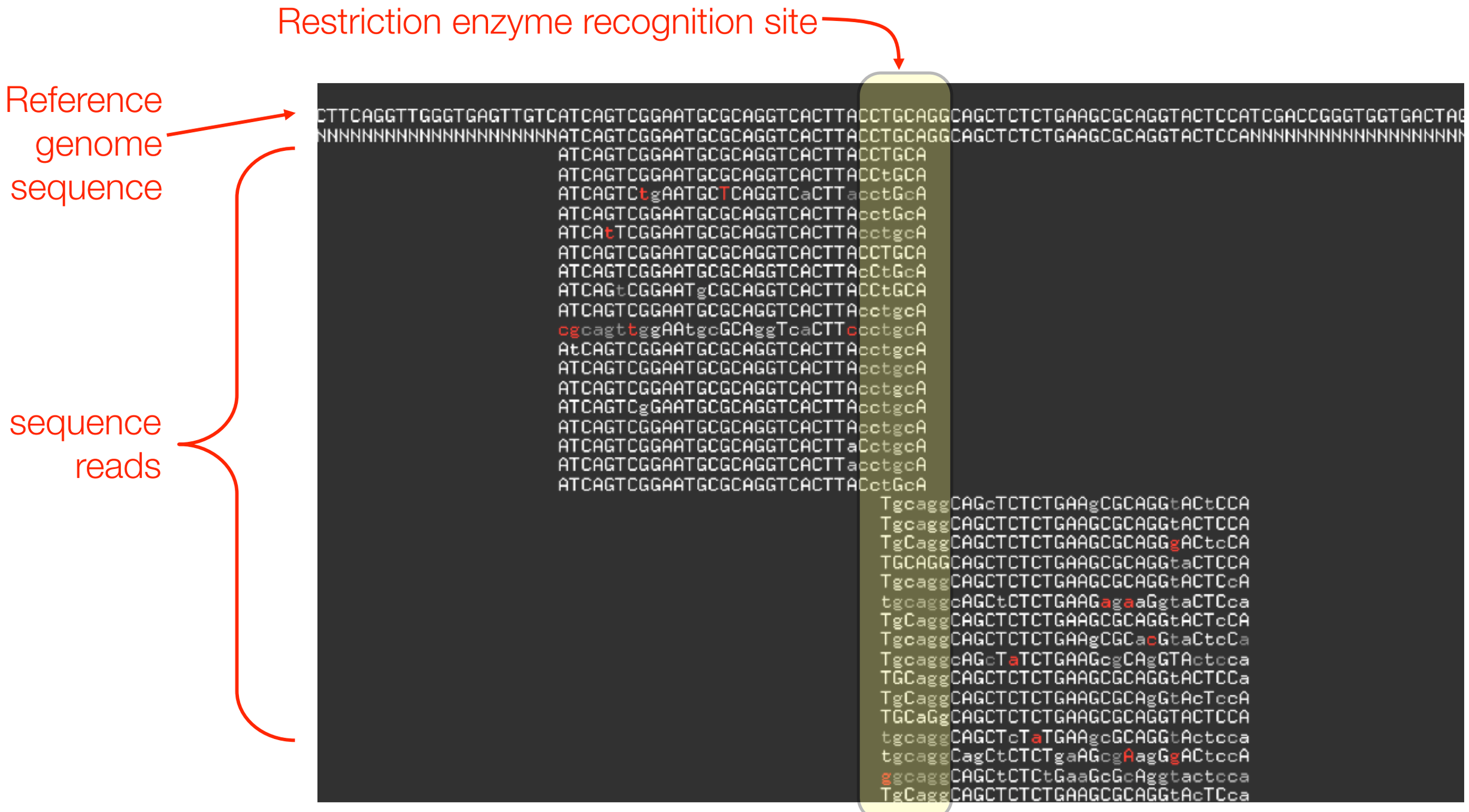



Lab bench considerations for RADseq studies


Statistical considerations in RAD-seq



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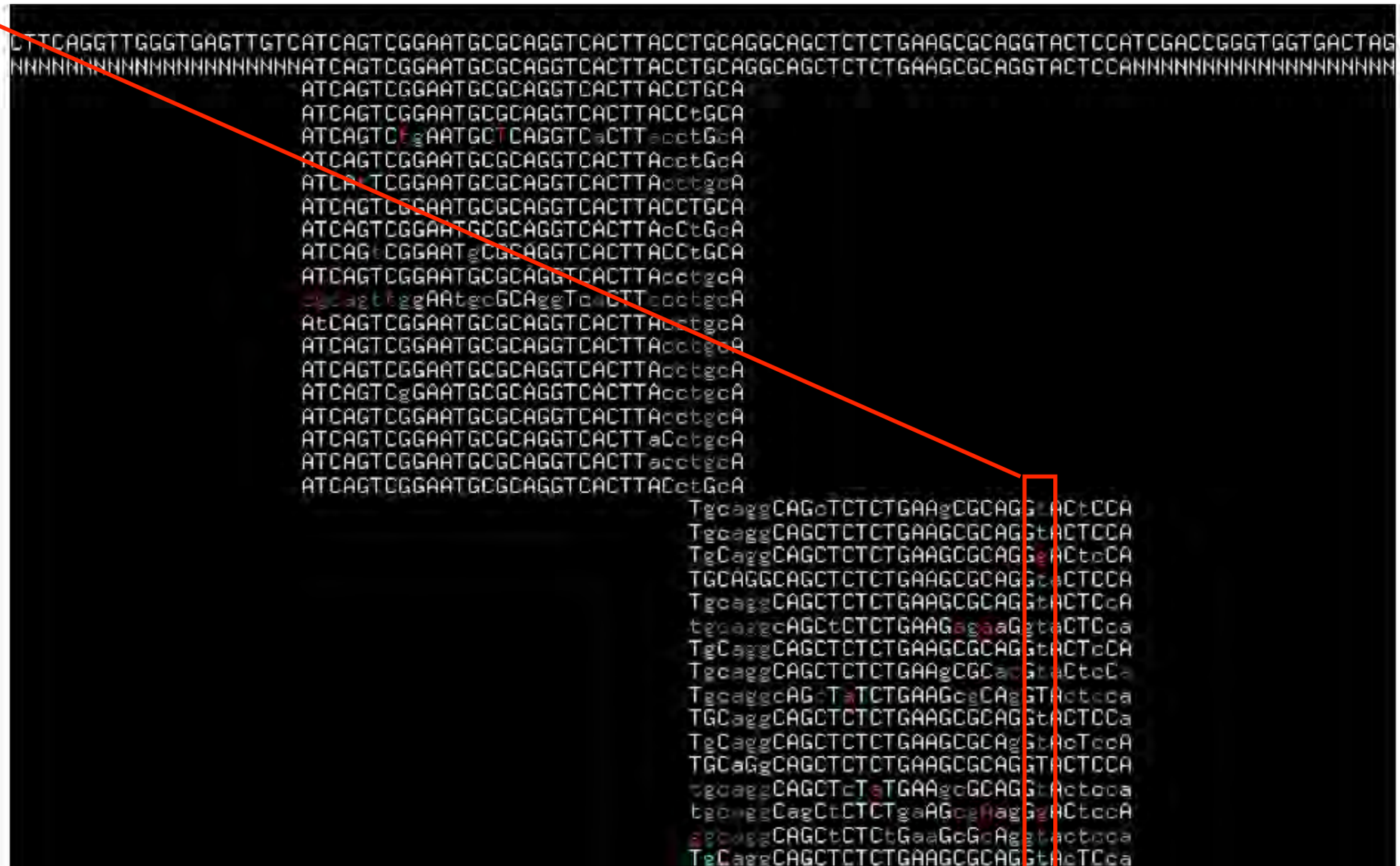

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The reads are 14 T and 2 G:

GT heterozygote?

GG homozygote with error?

AA homozygote with lots of error?

Needed a rigorous method to call genotypes

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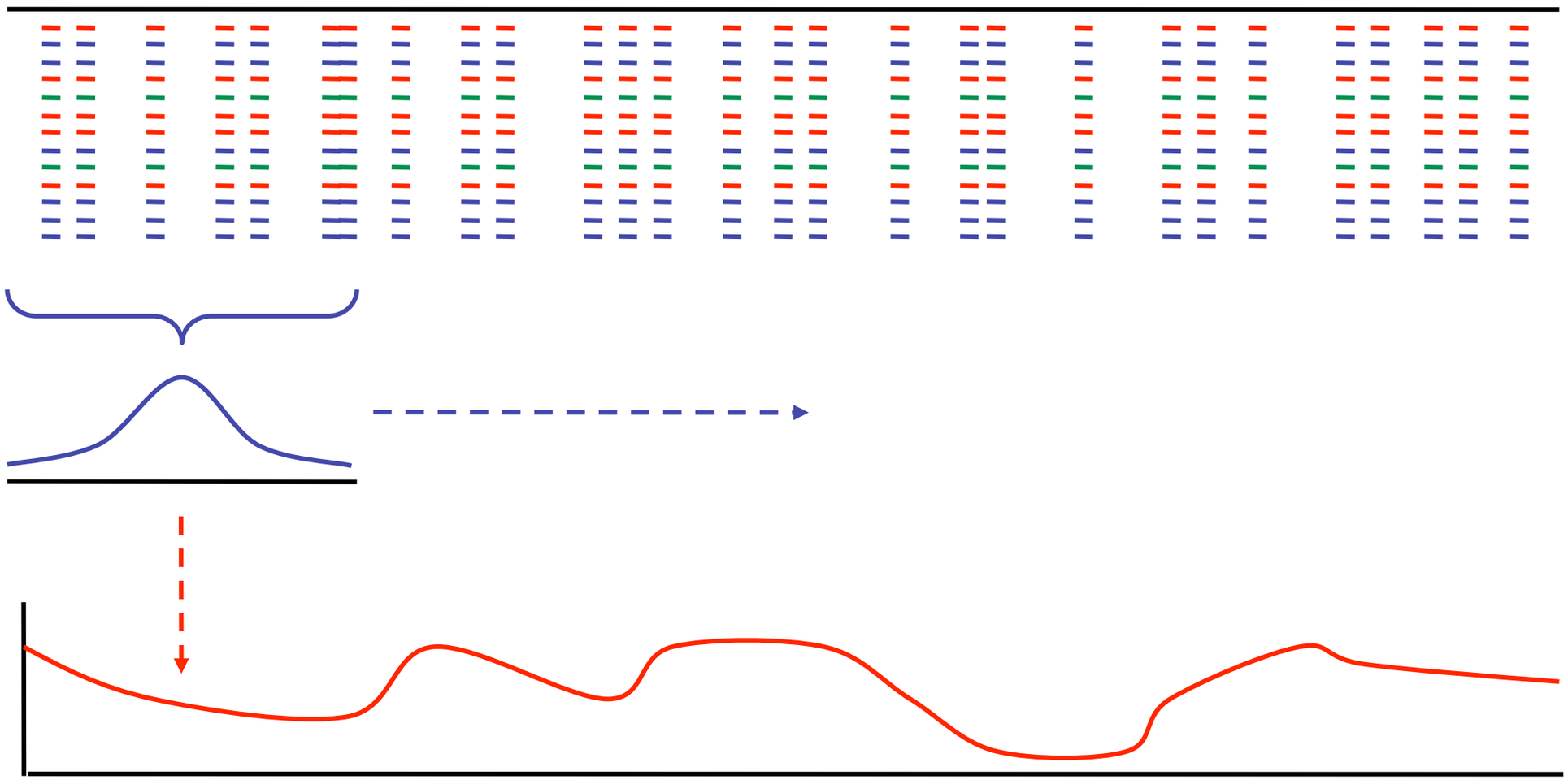
$$L(n_1\,\text{hom}) = P(n_1,n_2,n_3,n_4) = \frac{n!}{n_1!n_2!n_3!n_4!}\left(1-\frac{3\varepsilon}{4}\right)^{n_1}\left(\frac{\varepsilon}{4}\right)^{n_2}\left(\frac{\varepsilon}{4}\right)^{n_3}\left(\frac{\varepsilon}{4}\right)^{n_4}$$

$$L(n_1 n_2\,\text{het}) = P(n_1,n_2,n_3,n_4) = \frac{n!}{n_1!n_2!n_3!n_4!}\left(0.5-\frac{\varepsilon}{4}\right)^{n_1}\left(0.5-\frac{\varepsilon}{4}\right)^{n_2}\left(\frac{\varepsilon}{4}\right)^{n_3}\left(\frac{\varepsilon}{4}\right)^{n_4}$$

Maximum likelihood genotyping based on multinomial distribution of nucleotide reads

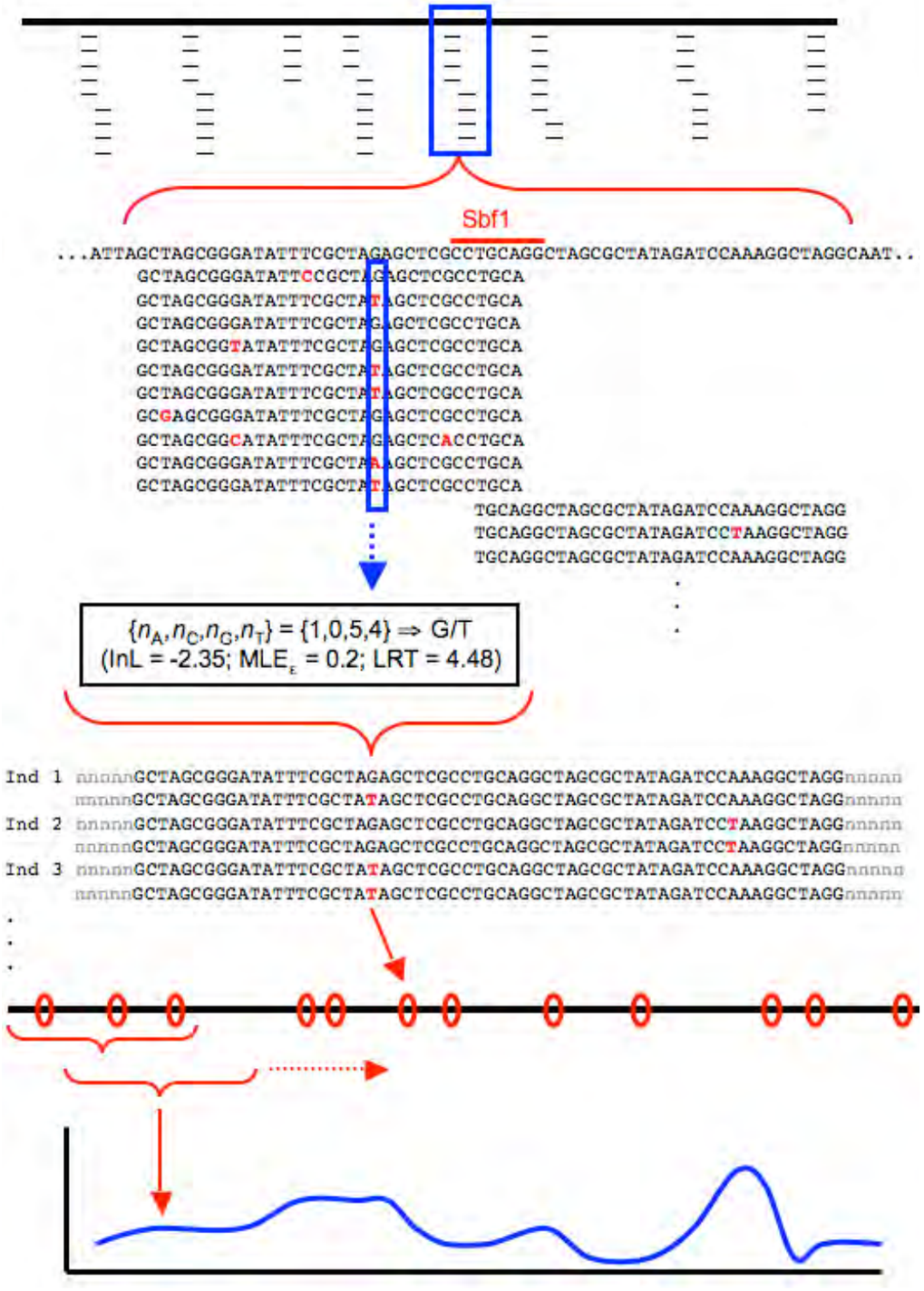
Making statistics continuous across the genome

Kernel-smoothing average of summary statistics along genome

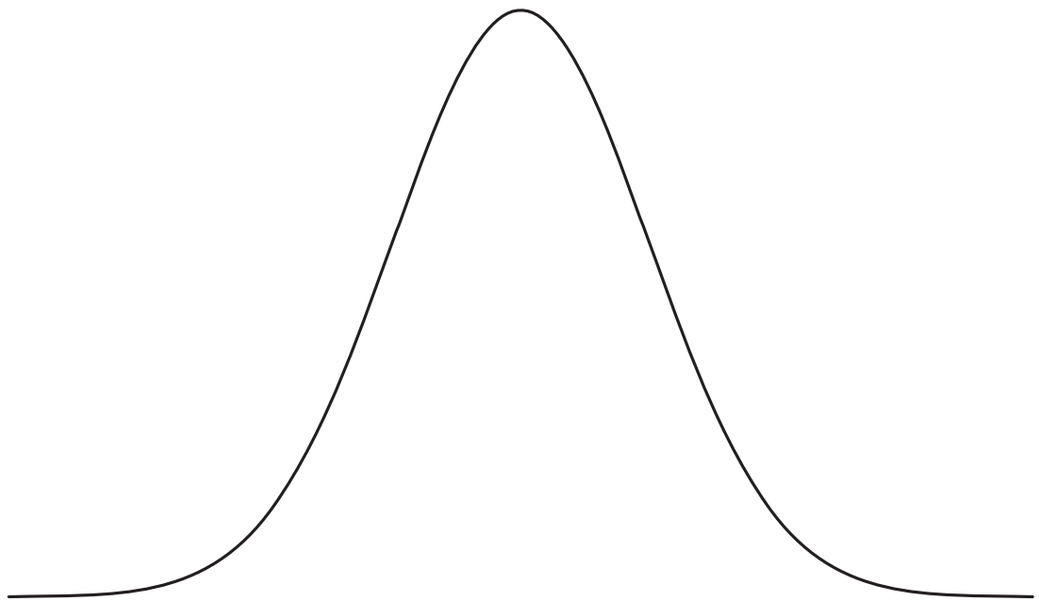


Bootstrap re-sampling to estimate significance of moving average

Overall pipeline



‘Bias’ in RAD-sequencing

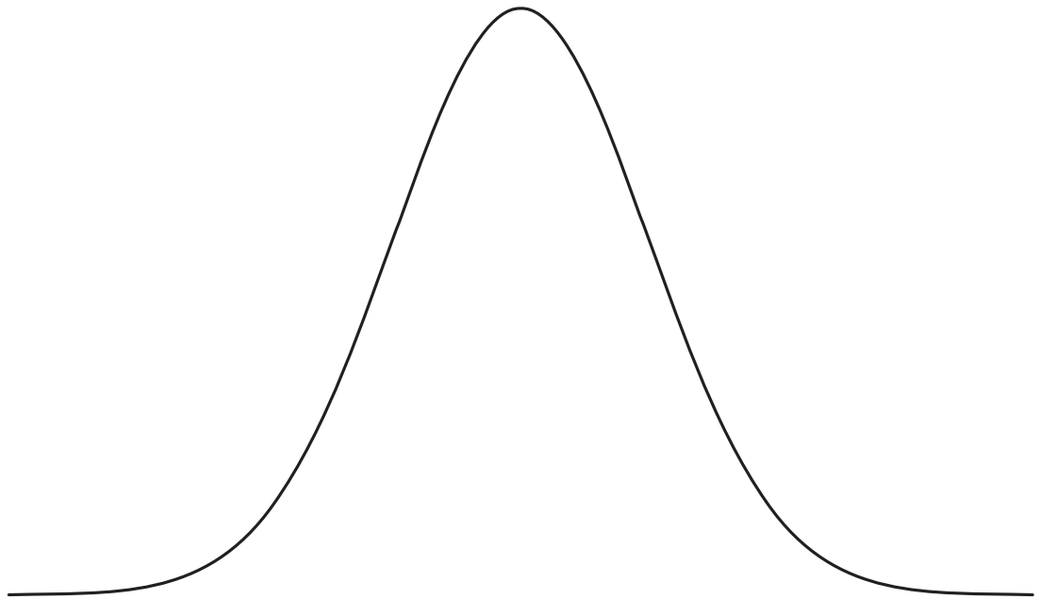


$$f(x) = \frac{1}{\sqrt{2\pi\sigma}} e^{\frac{-(x-\mu)^2}{2\sigma^2}}$$

$e = 2.7182....$

$\pi = 3.1415....$

‘Bias’ in RAD-sequencing



$$\overline{x} = \frac{1}{n}\sum_{i-1}^{n} x_i$$

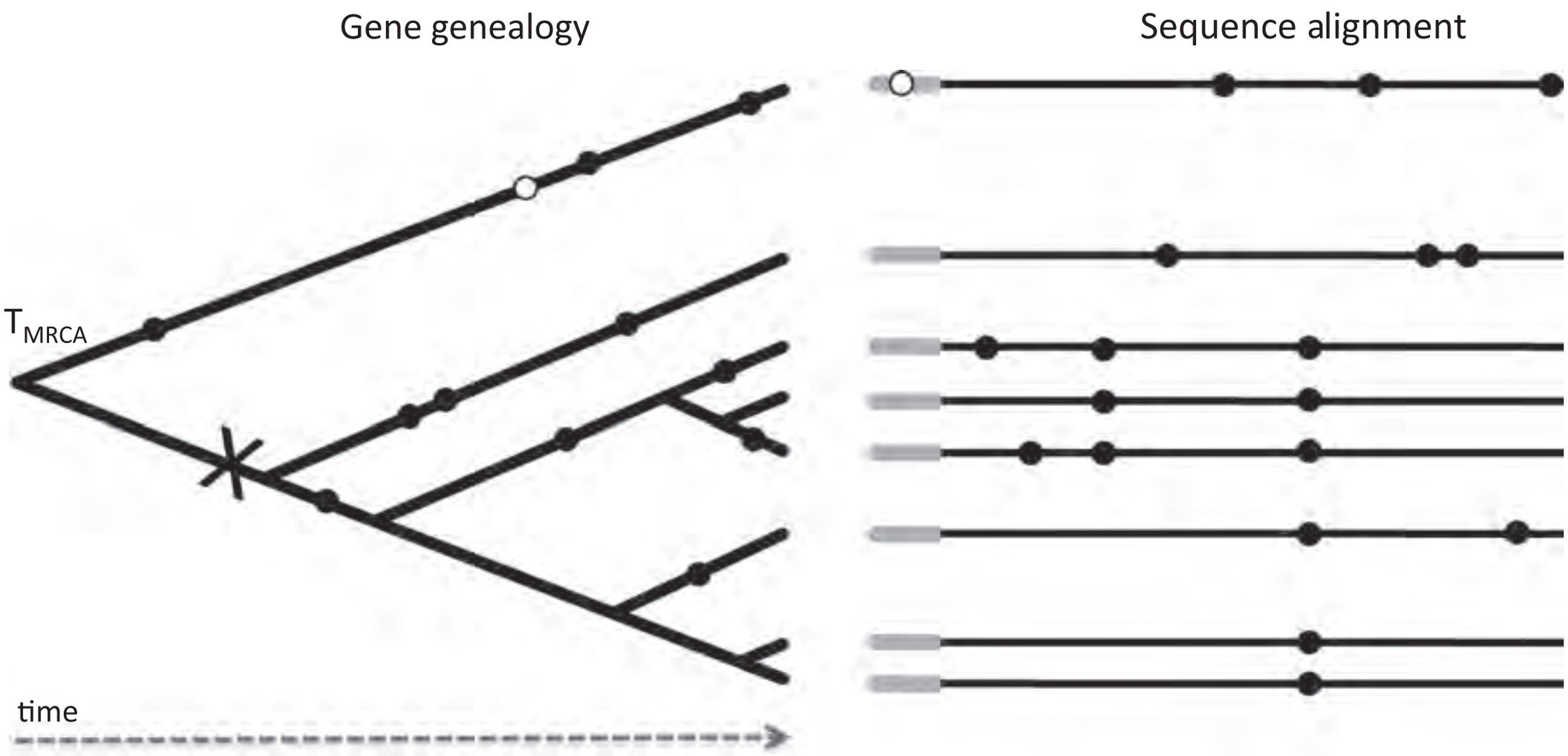
$$s^2 = \frac{1}{n-1}\sum_{i-1}^{n} (y_i - \overline{y})^2$$

Bias in RAD-sequencing

MOLECULAR ECOLOGY
Molecular Ecology (2013) **22**, 3179–3190 doi: 10.1111/mec.12

RADseq underestimates diversity and introduces genealogical biases due to nonrandom haplotype sampling

B. ARNOLD,[1] R. B. CORBETT-DETIG,[1] D. HARTL and K. BOMBLIES
Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA



Bias in RAD-sequencing summary

		Mean	
		Recombination	
Protocol	θ per bp	θ_{we}/θ_{wa}	π_e/π_a
Standard	0.0001	0.994	0.995
	0.001	0.987	0.982
	0.01	0.956	0.933
Double digest	0.0001	0.835	0.836
	0.001	0.858	0.851
	0.01	0.829	0.797

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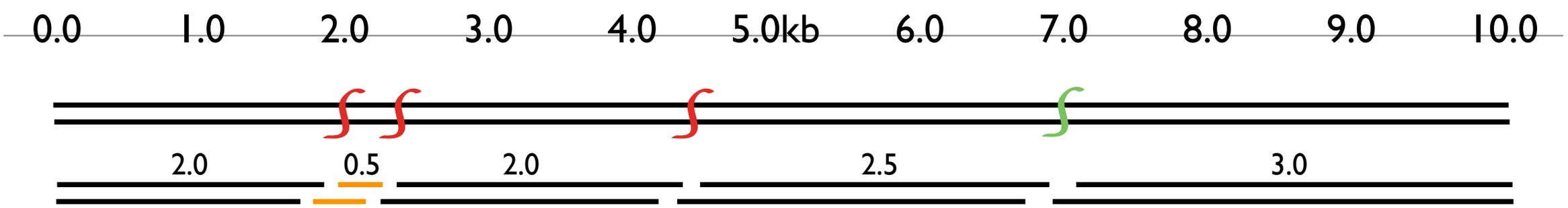
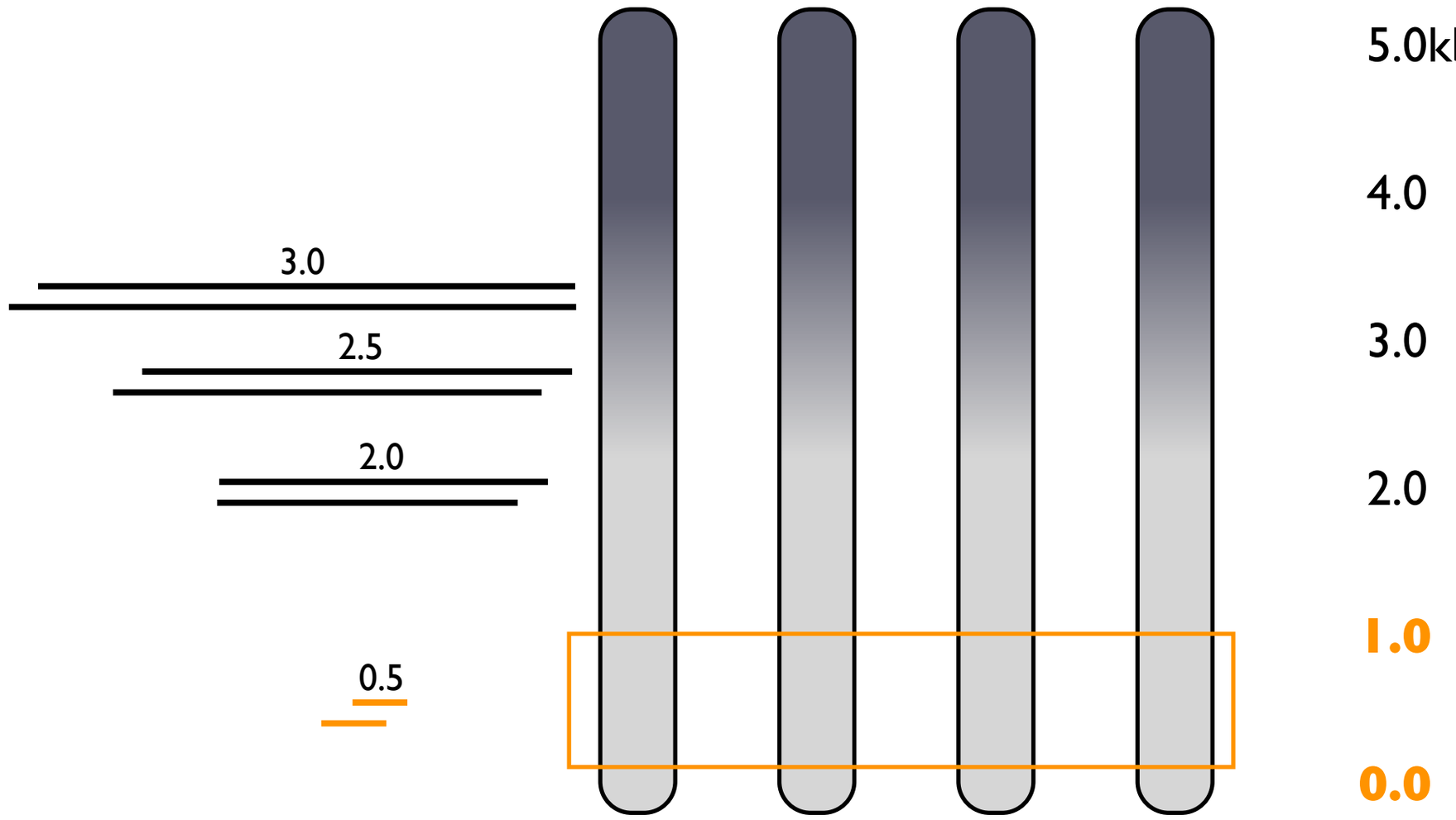
Why is ddRAD so much more biased?





Experimental design considerations for RAD

Tradeoffs:
Number of sites versus **Depth** of sequencing per site versus **Number of samples**

Experimental design considerations for RAD

Tradeoffs:
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```
raw reads / samples / sites = coverage at each RAD locus

        1,000,000 / 100 / 1,000 = 10x coverage

25 to 50x average coverage per RAD locus is a good goal
```

Experimental design considerations for RAD

Tradeoffs:
Number of sites versus **Depth** of sequencing per site versus **Number of samples**

How many tags do I need?

Things to consider

Choice of enzyme and genome size $(0.25)^n$ x genome size = expected # sites

Genomes are biased:

expect 112,300 six-cutter sites in stickleback (460 Mb)	actual ***EcoRI*** sites = 90,000
expect 7000 eight-cutter sites in stickleback	actual ***SbfI*** sites = 22, 800
expect 32,900 six-cutter sites in *C. remanei* (135 Mb)	actual ***EcoRI*** sites = 73,200

Experimental design considerations for RAD

Tradeoffs:
Number of sites versus **Depth** of sequencing per site versus **Number of samples**

How many tags do I need?

Things to consider

Choice of enzyme and genome size

Polymorphism and read length

Nucleotide polymorphism rate = 0.01 to 0.001 for most vertebrates

Stickleback populations: 0.01 to 0.02. At least 1 SNP every 100 bp, on average

Experimental design considerations for RAD

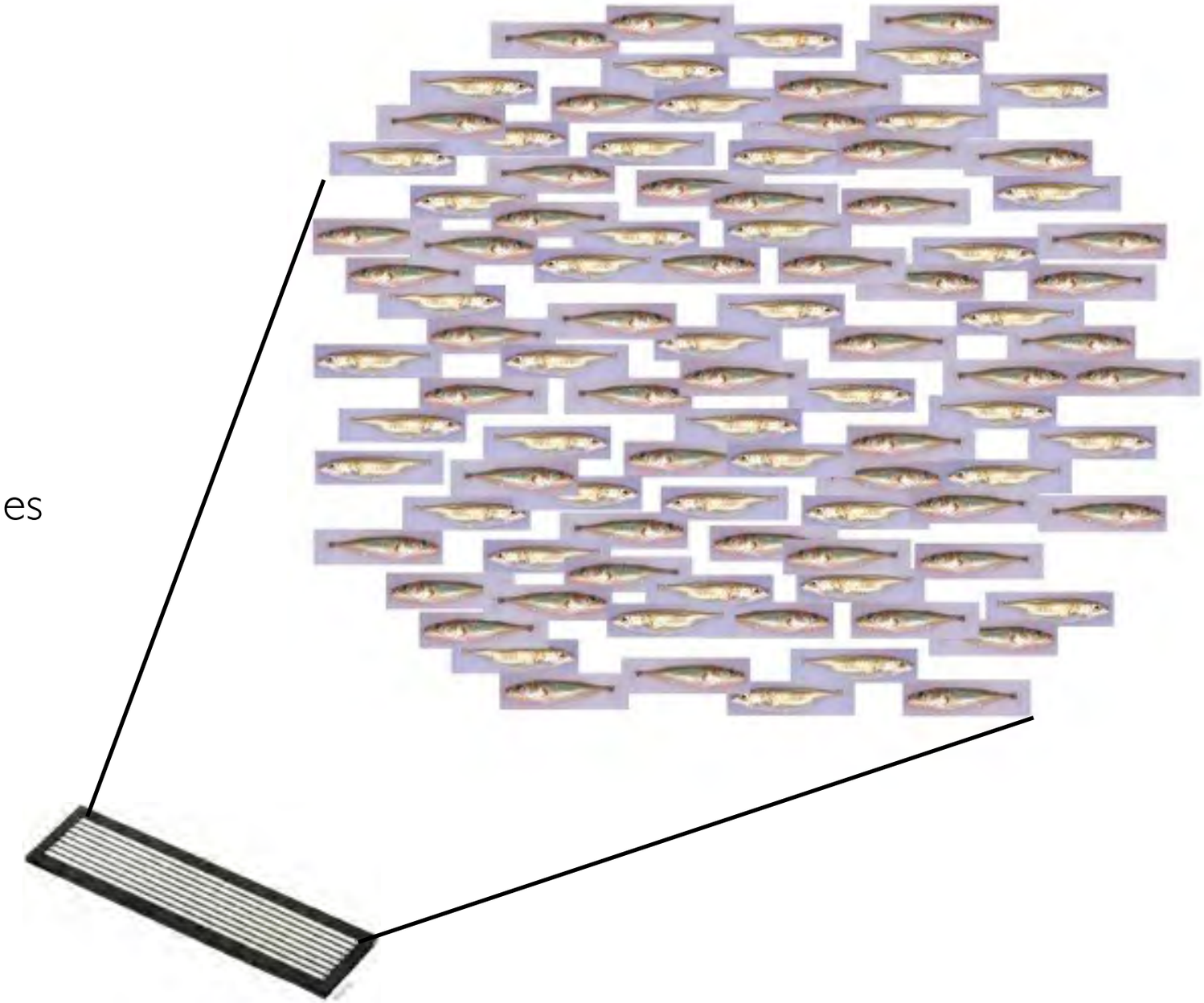
Tradeoffs:
Number of sites versus **Depth** of sequencing per site versus **Number of samples**

How many samples should be multiplexed?

Things to consider

Barcoded adapters
- 5 to 8nt barcodes
- Variable length barcodes
- Combinatorial barcodes (PE)

Barcode distance - two mismatches



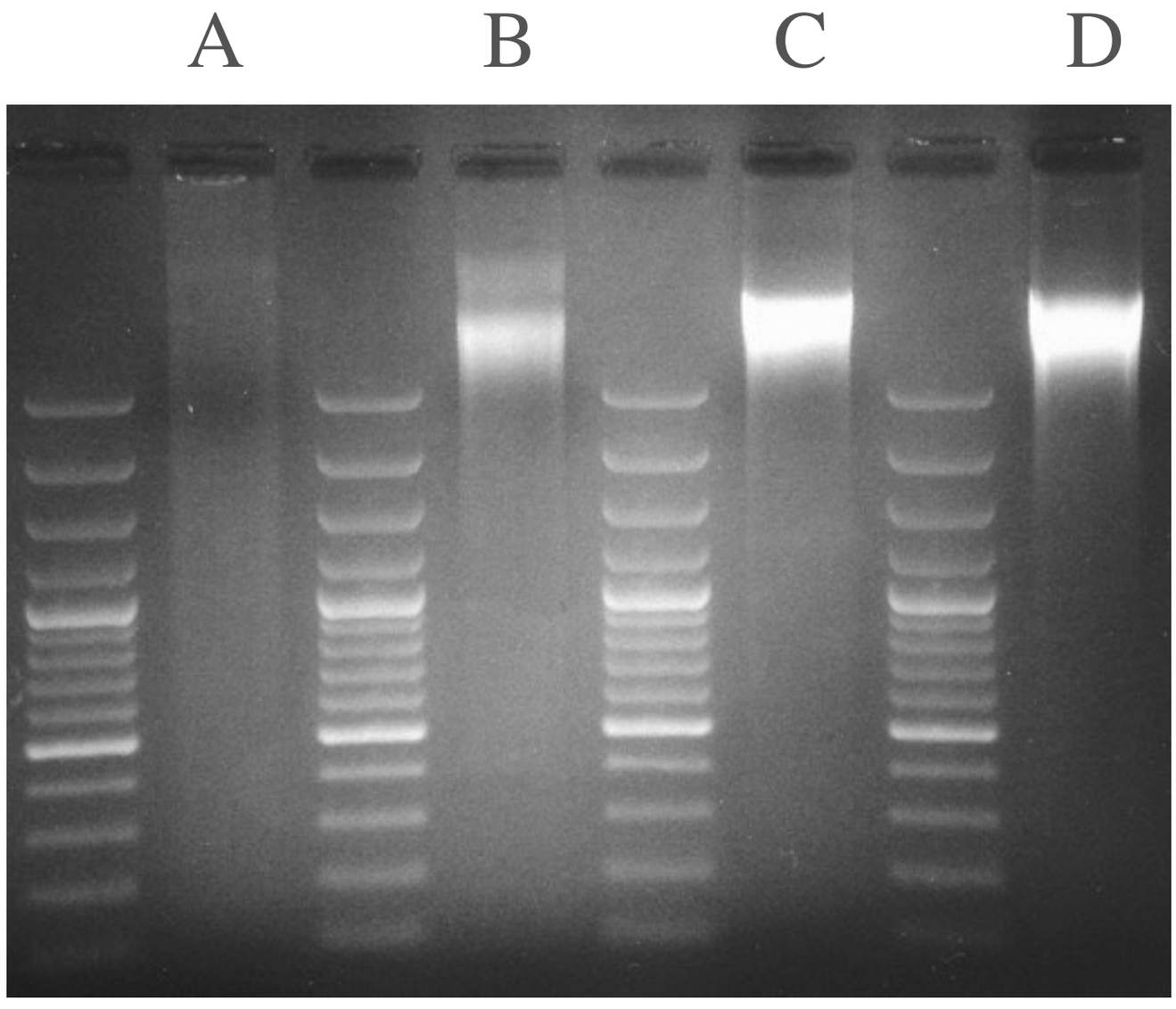
Molecular considerations in library building

How many samples should be multiplexed?

Things to consider

DNA Quality

Multiplex only like samples to help equalize representation of poor quality samples



Molecular considerations in library building

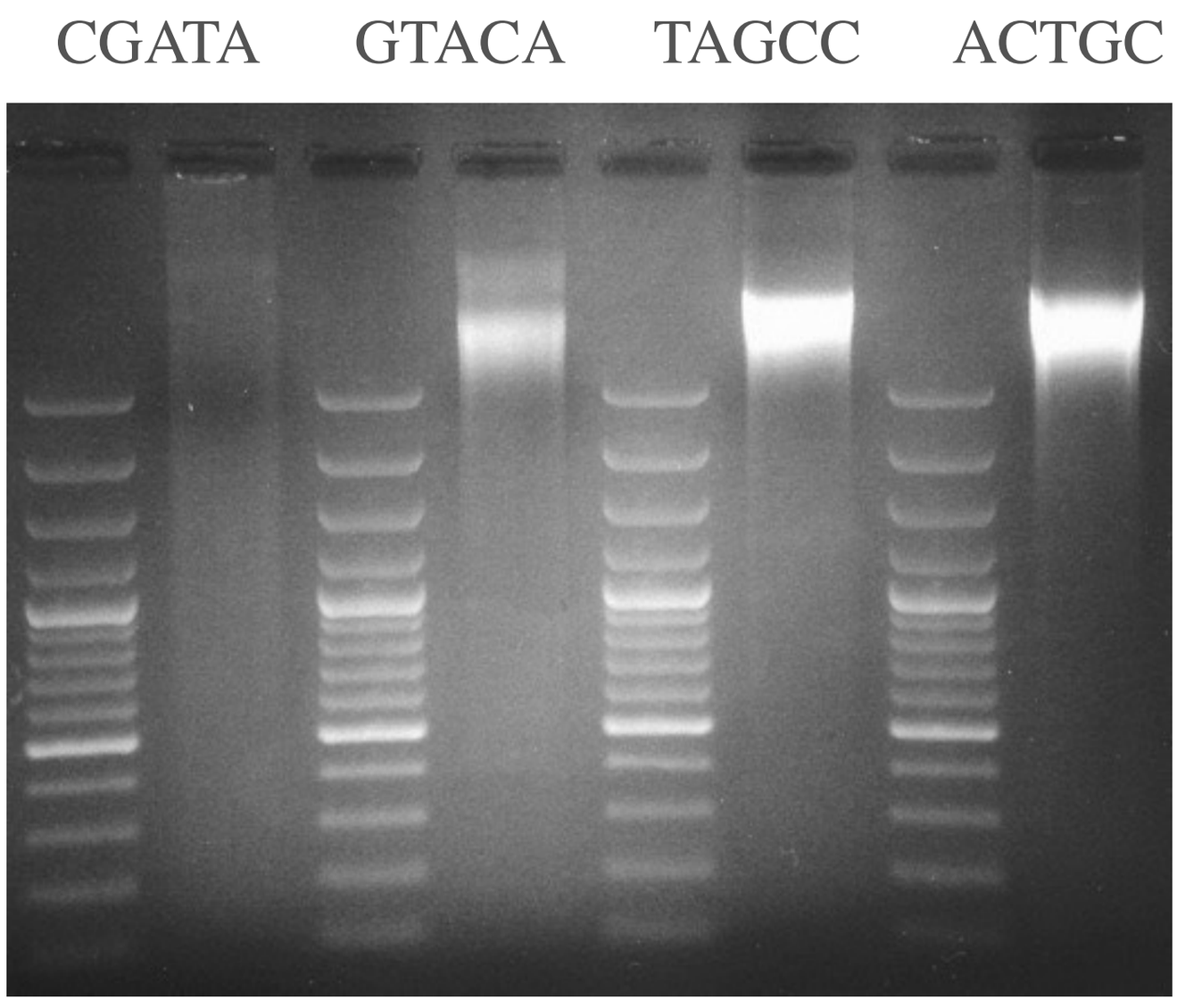
How many samples should be multiplexed?

Things to consider

DNA Quality

Diversify barcodes

Illumina cluster calling is confused by repetition in first 4 bases - can offset barcodes



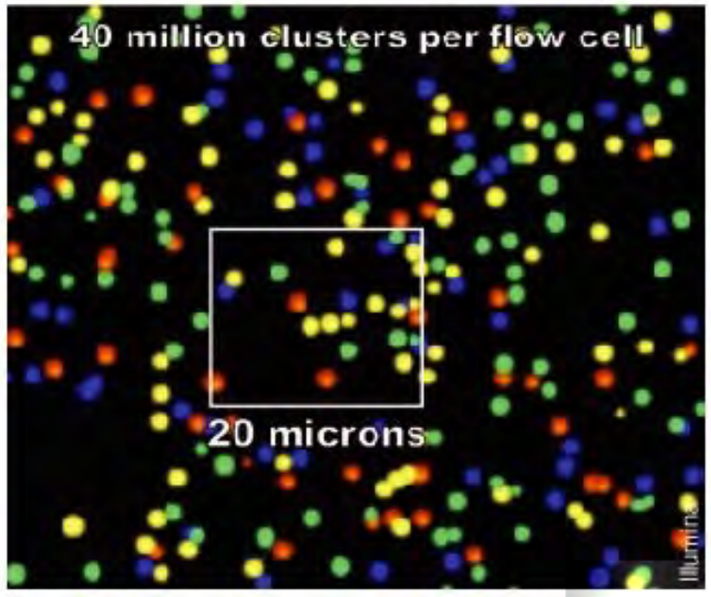
Molecular considerations in library building

How can I get the best depth of coverage?

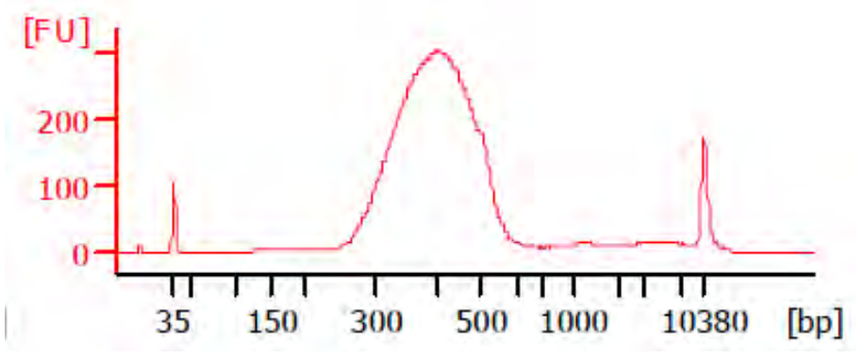
Things to consider

Fragment size

Smaller/tighter is better



Agilent Bioanalyzer



Molecular considerations in library building

How can I get the best depth of coverage?

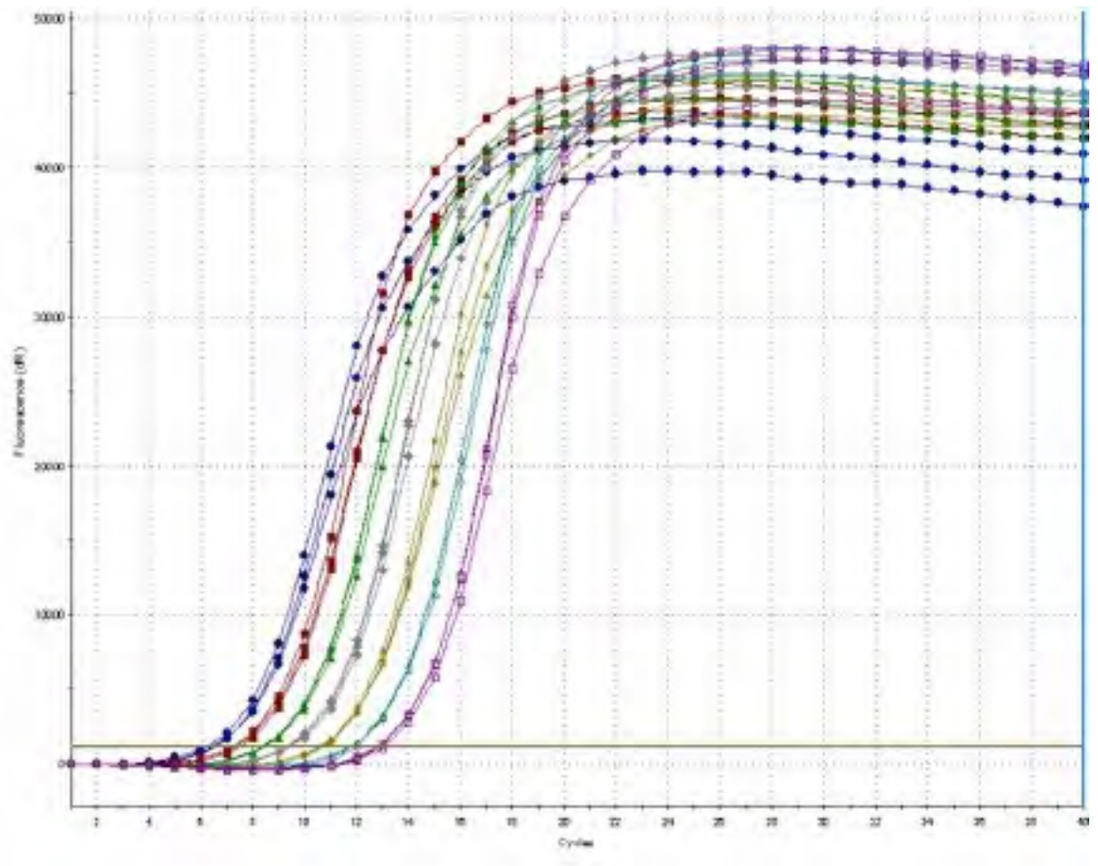
Things to consider

Fragment size

Library quality

qPCR

qPCR control should be similar to measured sample:



Molecular considerations in library building

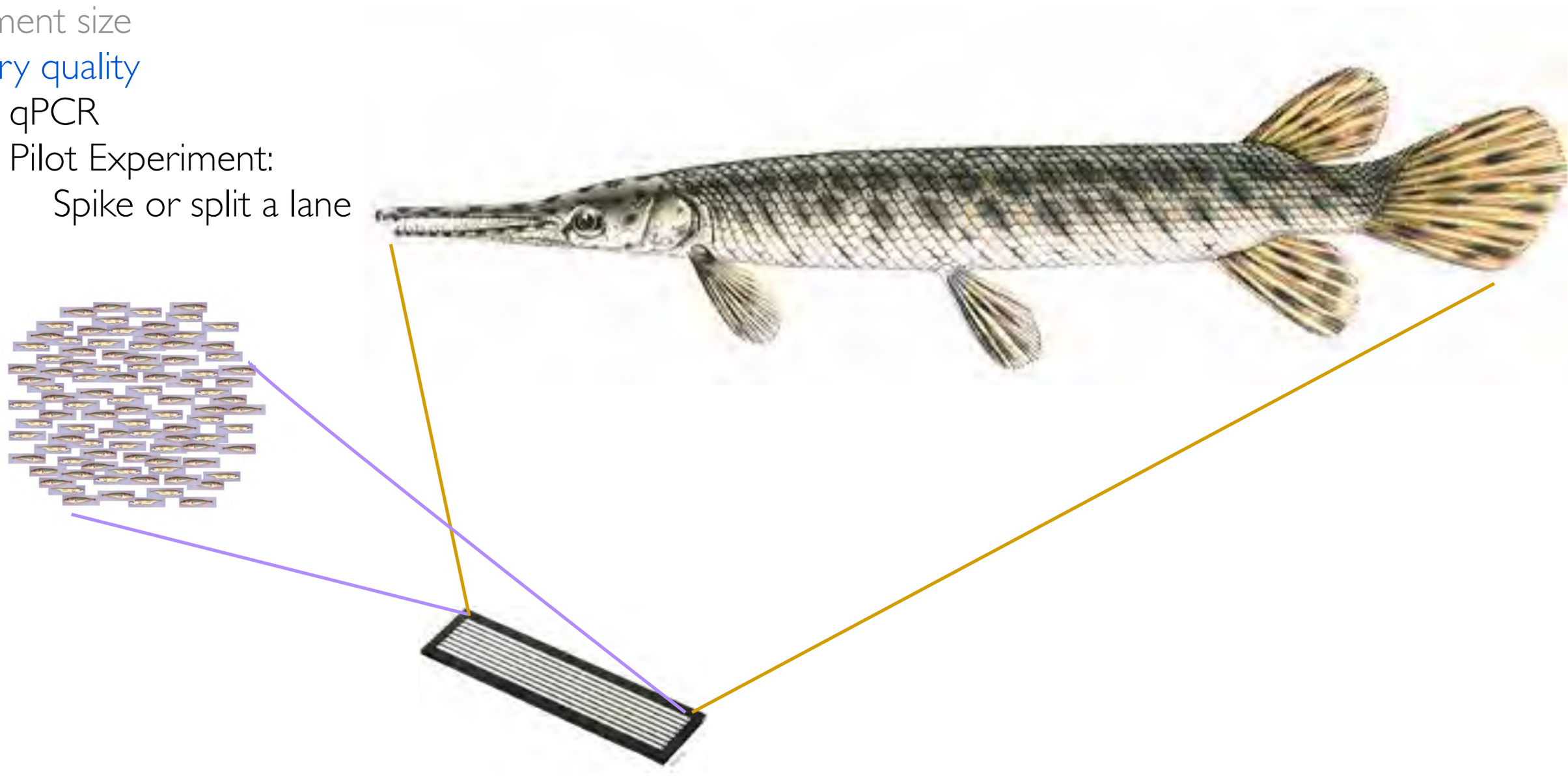
How can I get the best depth of coverage?

Things to consider

Fragment size

Library quality

- qPCR
- Pilot Experiment:
  - Spike or split a lane



The pipefish genetic map is closed; 22 LGs 6000 segregating SNPs; 30,000 RAD sites