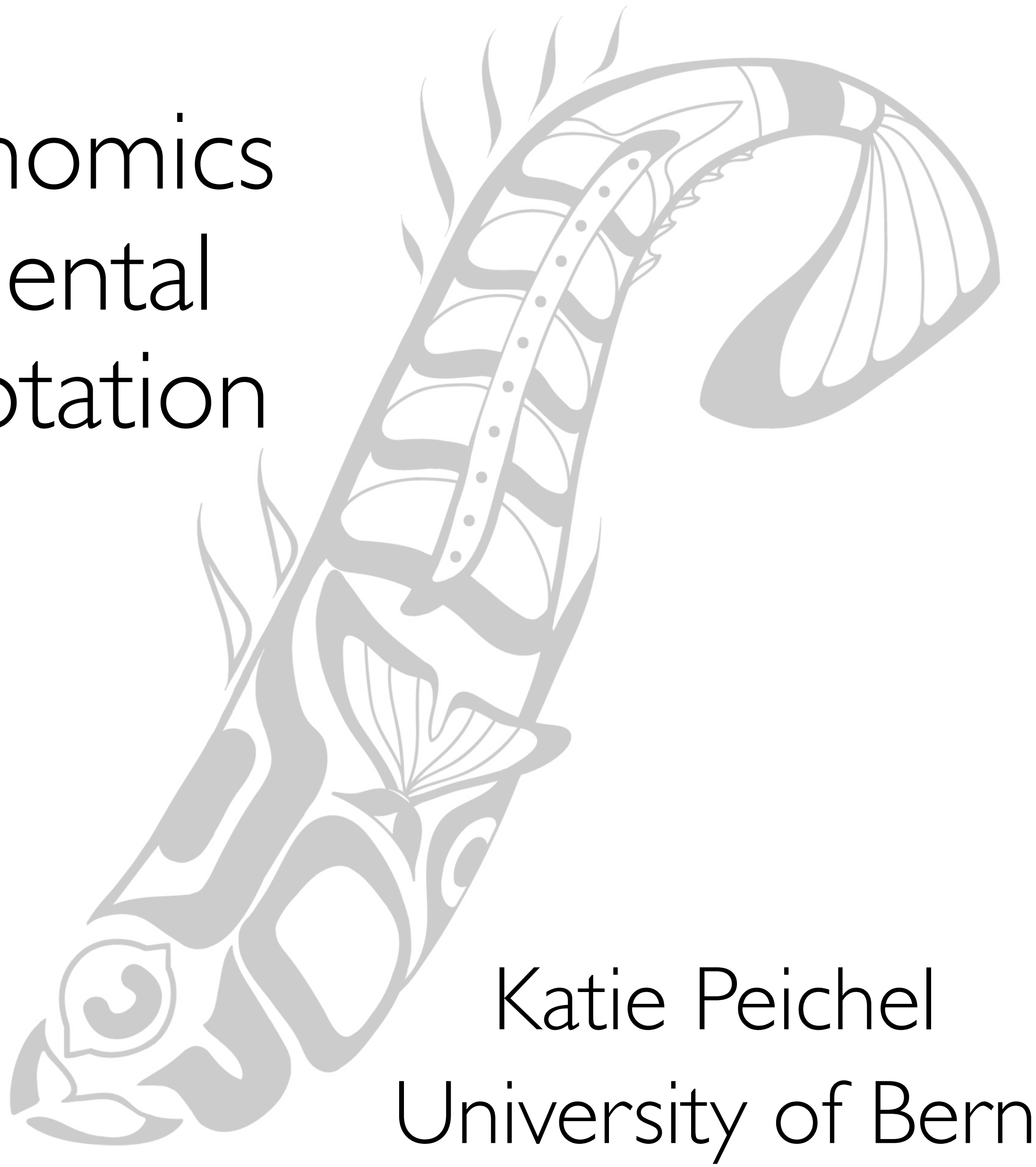
Combining genomics with experimental studies of adaptation



Katie Peichel
University of Bern

Disclaimer: I don't really know how to do anything with genomic data!

Peichel group fall 2024

But, the talented people that I have had in my group over the years do!

You will hear more from the amazing Majda Bohutínská this afternoon!

Plan for this morning*

- Part 1: Introduction to forward and reverse genetic approaches and QTL mapping (~45 min including an exercise)
  - Short break

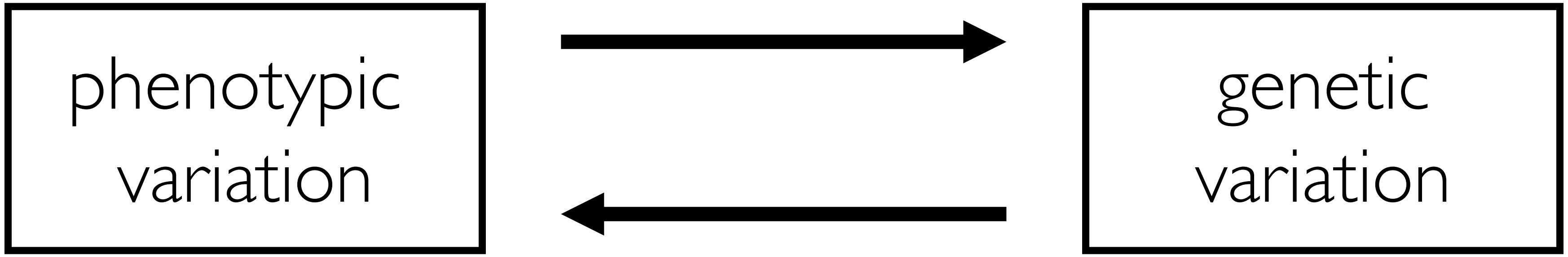
- Part 2: Combining genomics and experimental studies to understand the role of chromosomal rearrangements in adaptation to divergent environments (~45 min)
  - Short break

- Part 3: Combining genomics and experimental studies to determine why evolution is repeatable (~45 min)

*Please always interrupt with questions as you have them!!!

How do organisms adapt to different environments?

Genetics of adaptation



natural selection

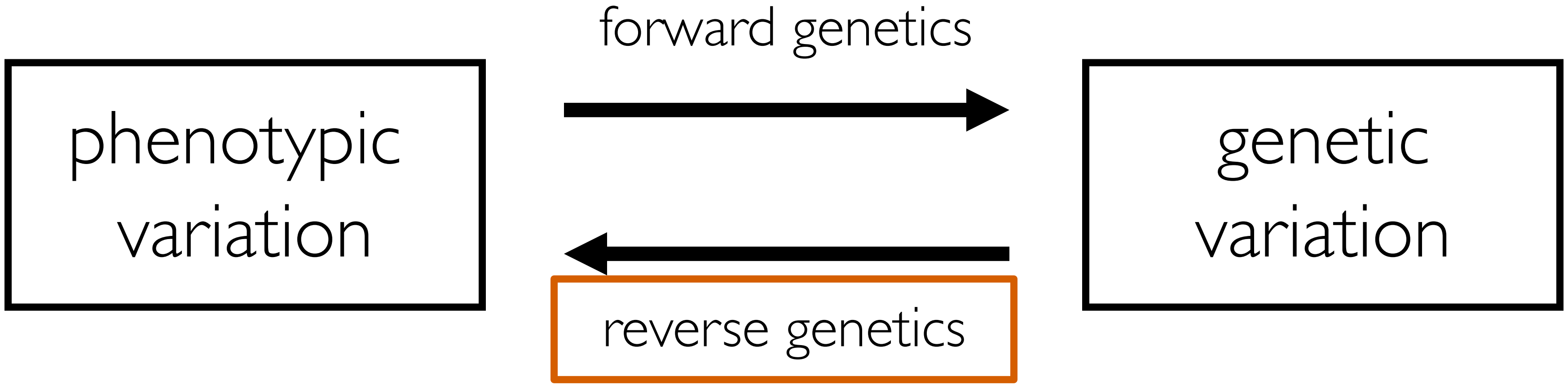
phenotypic variation

genetic variation

evolutionary change

How do we make these connections?

Two complementary approaches



forward genetics

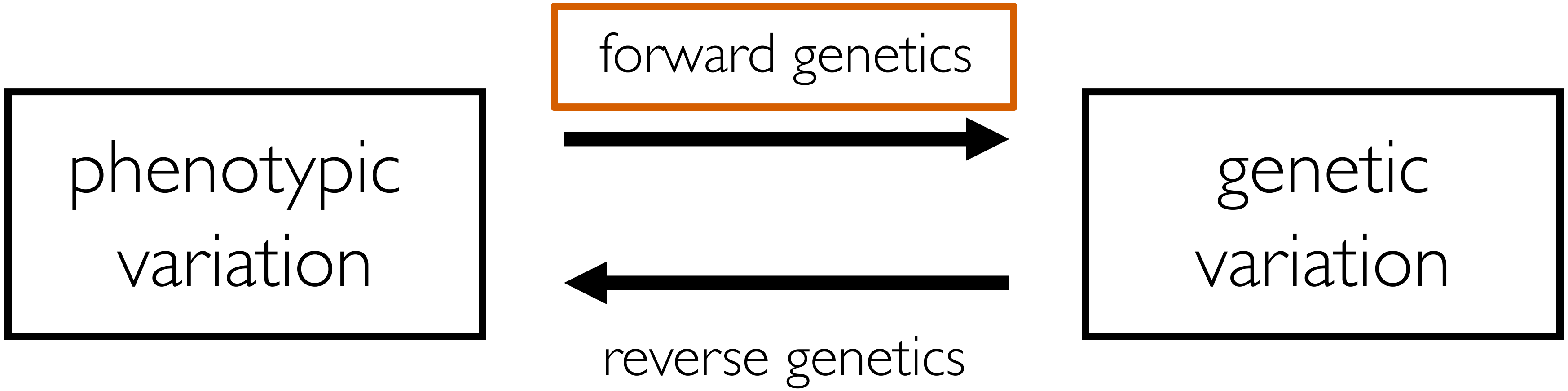
phenotypic variation

genetic variation

reverse genetics

What phenotypes are affected by this genotype?

Two complementary approaches



forward genetics

phenotypic variation

genetic variation

reverse genetics

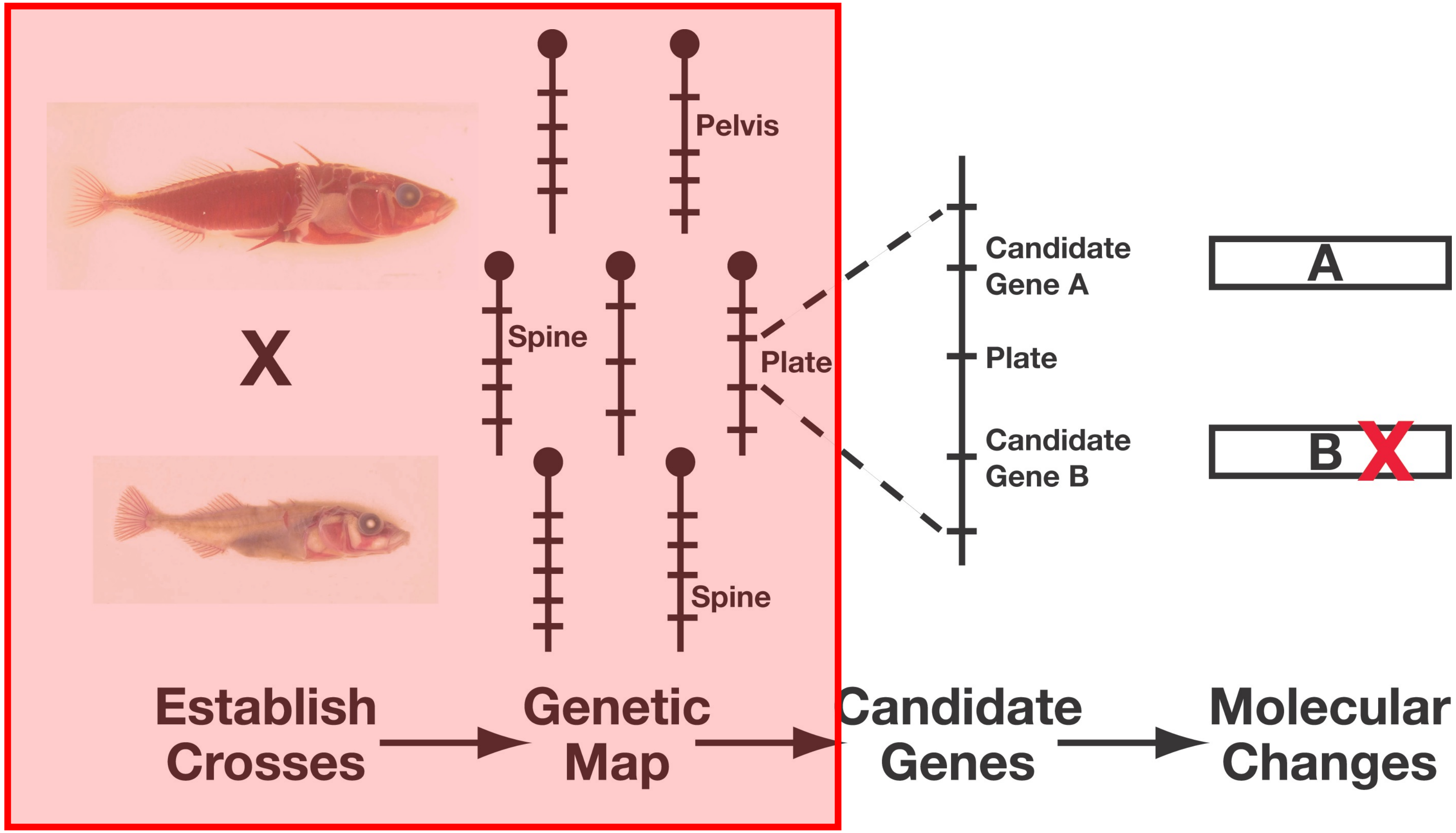
What genotypes underlie this phenotype?

Two complementary approaches

- Reverse genetics
  - Genotype driven: what is the function of the genes or genomic variants identified as targets of selection?
    - This is what you will learn from Majda this afternoon

- Forward genetics
  - Phenotype driven: what genes or genomic variants contribute to this adaptive phenotype?
    - This is what I will focus on for the first part of the morning

Forward genetics: quantitative trait loci (QTL) mapping approach



Establish Crosses → Genetic Map → Candidate Genes → Molecular Changes

Quantitative traits

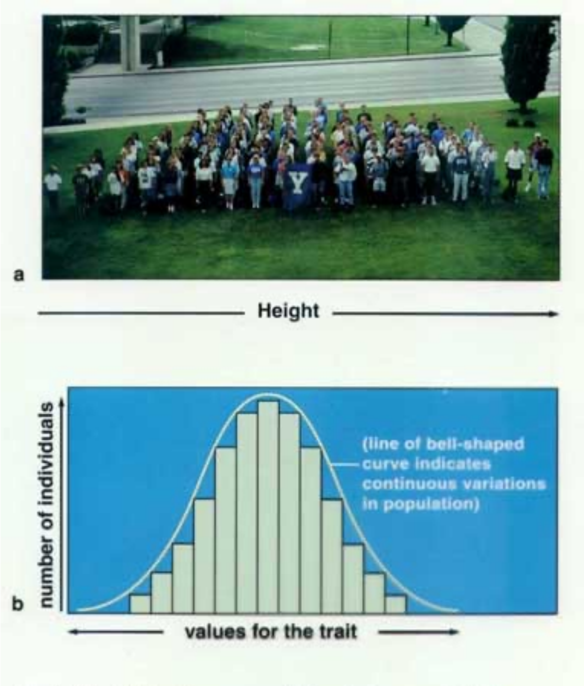
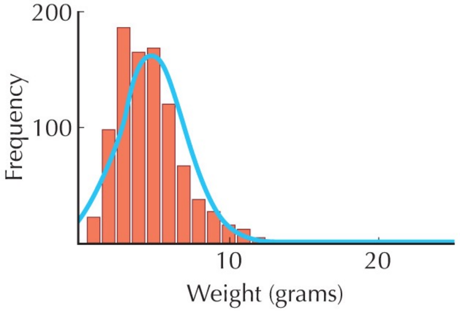
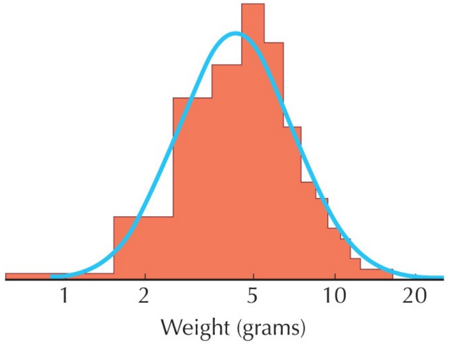
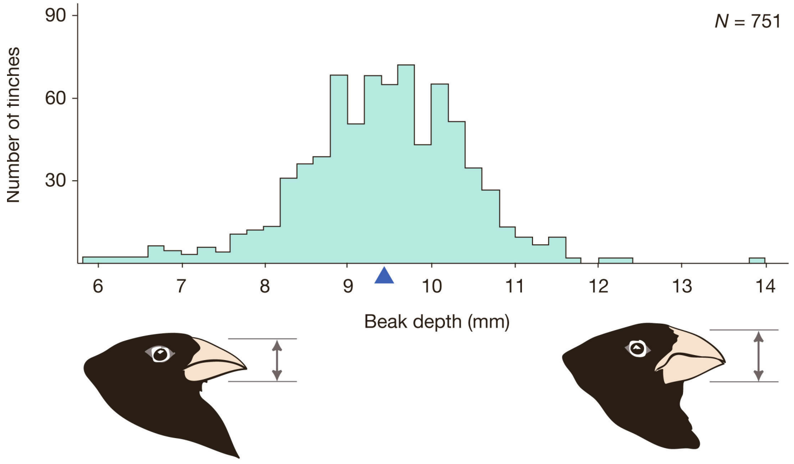


Fig. 9.18 (a) Continuous variation in human height. (b) Bell-shaped curve typical of continuous variation in a trait.







Human disease, domesticated crops and animals,
and most traits in natural populations!

Quantitative traits

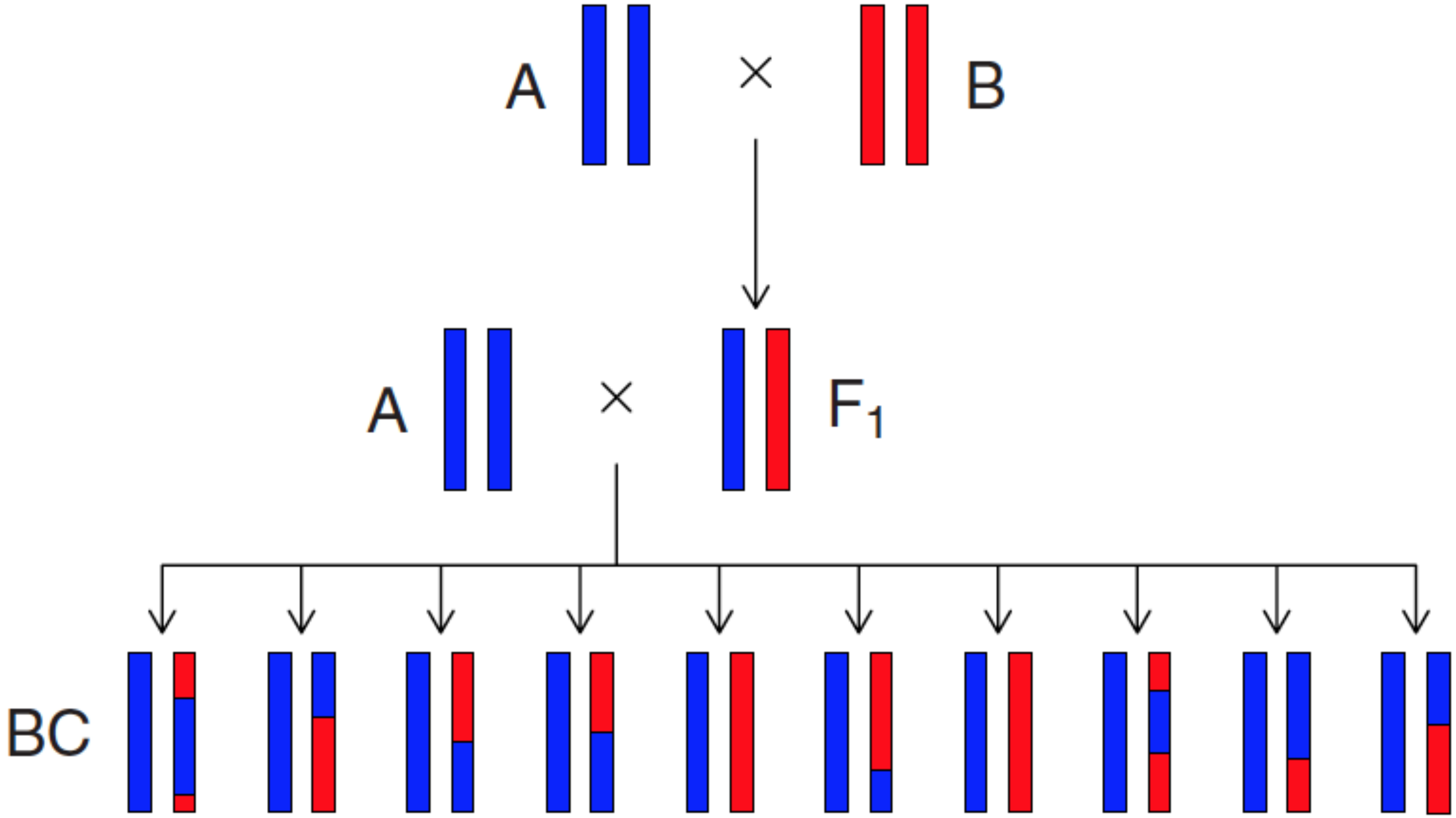
- Most phenotypic traits
- NOT a perfect correlation between phenotype and genotype
- Multiple genetic factors as well as environmental factors contribute
- Example:
  - Human height

Quantitative trait locus (QTL) mapping

What do you need for QTL mapping?

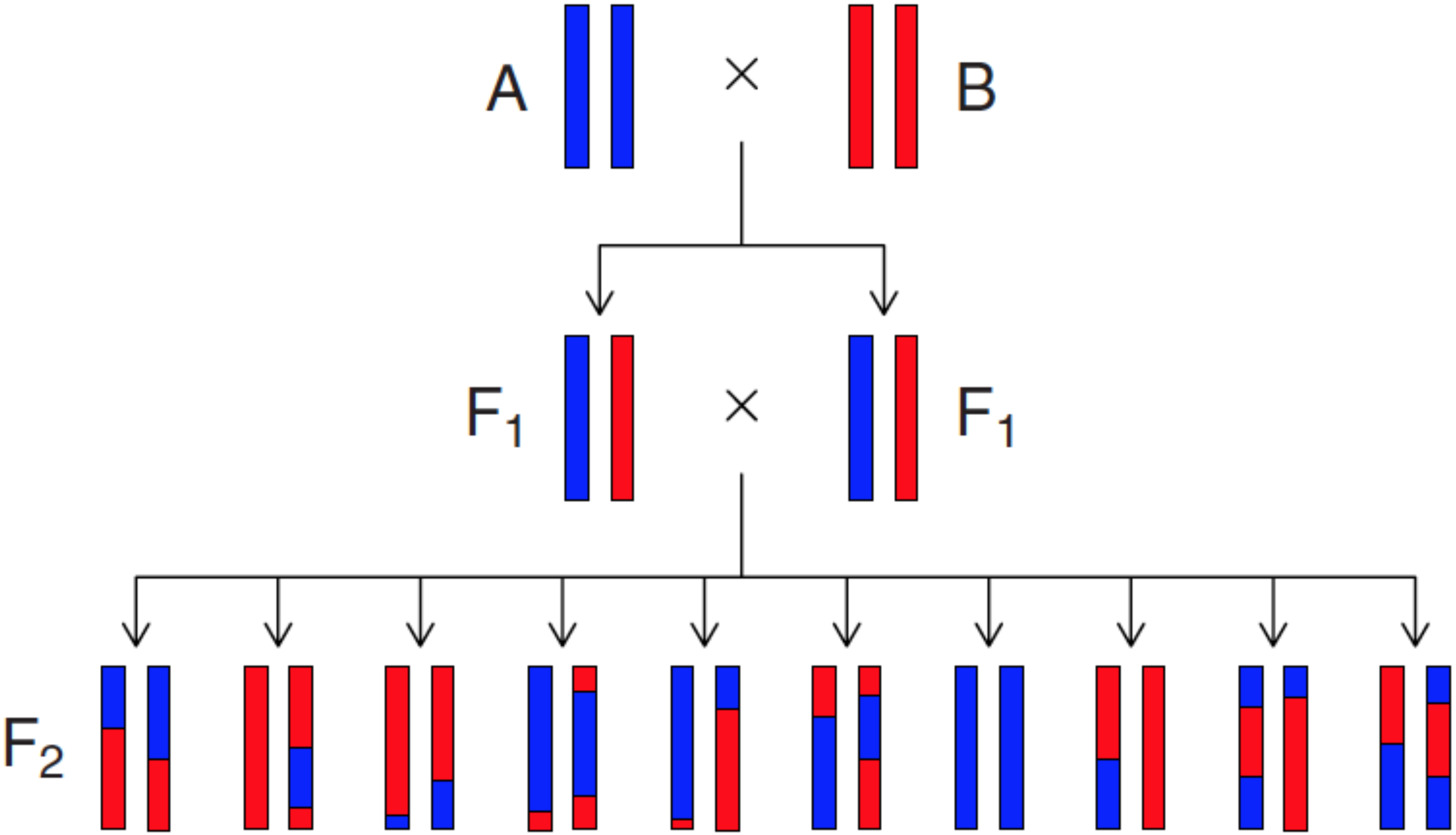
1. Genetic cross
2. Good phenotypic assays = phenotypes
3. Genetic markers = genotypes
4. Linkage map
5. Software for analyses

Genetic cross: backcross



Which genotype class is missing?

Genetic cross: intercross



Why might it not be possible to do an F1 intercross?

Genetic crosses

- Backcross vs F2 intercross
- Number of individuals is crucial
  - More individuals provide more power to detect loci of relatively small effect on phenotype
  - At least 500 backcross or F2 individuals is ideal, though not always feasible
  - 100 individuals is enough to detect loci with a moderate effect and often a good start!

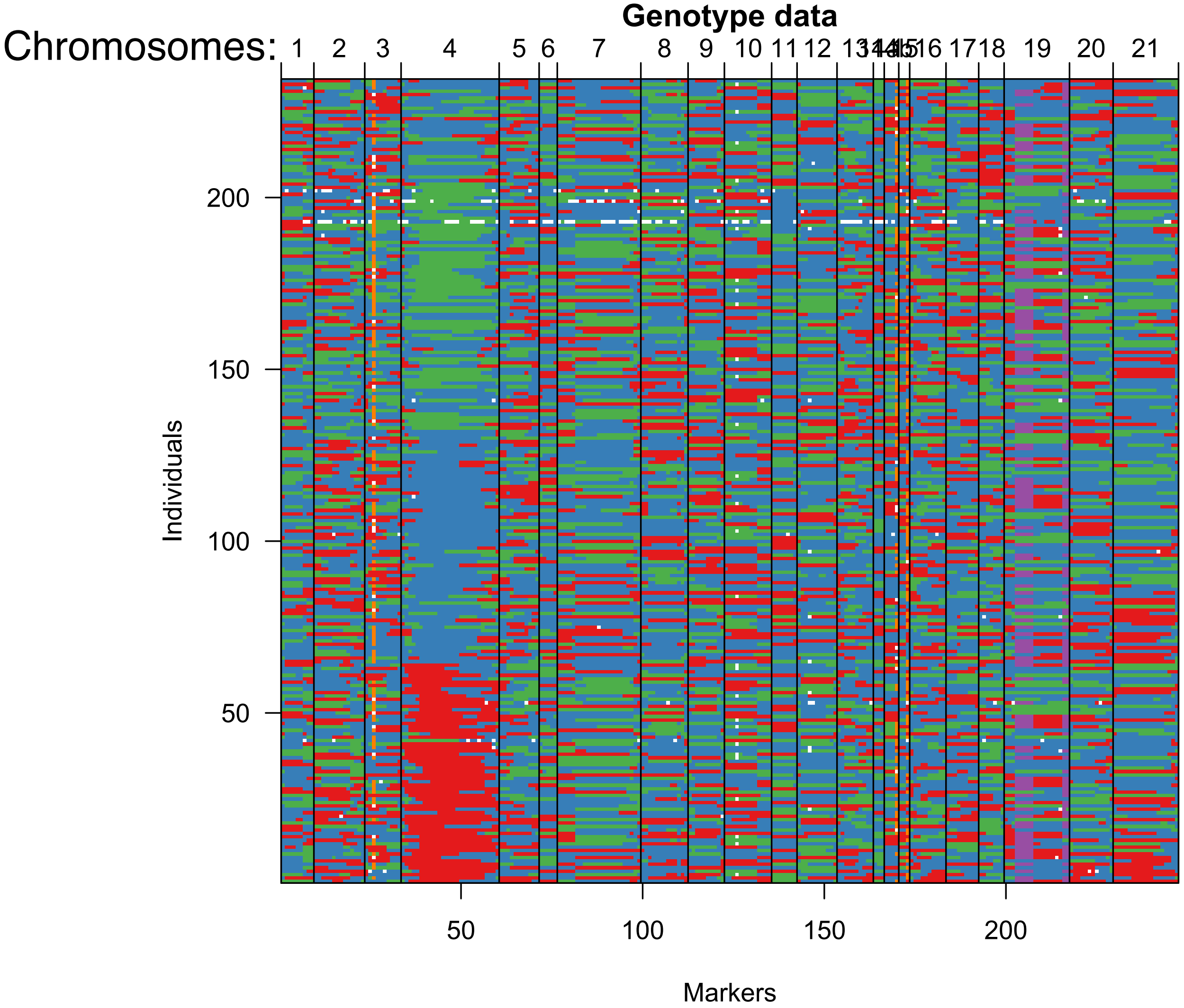
Phenotypes

- Careful phenotypic analyses is one of the most important and under-looked components of linkage mapping!
- Measure "component" traits of complex phenotypes
- Want to minimize sources of variation to isolate genetic component of variation
  - environmental effects
  - measurement error

Genotypes

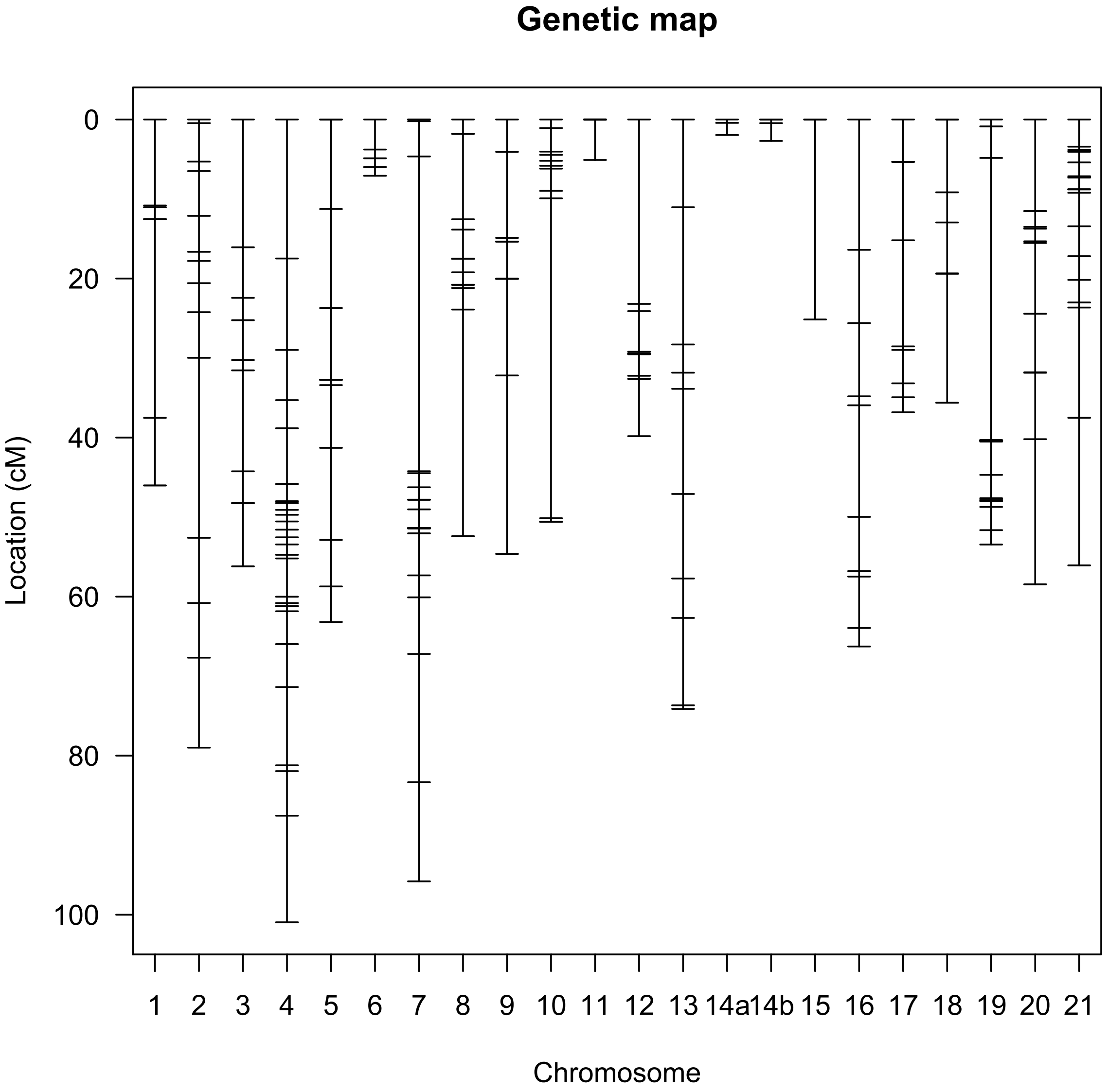
- Need genetic markers that allow you to determine whether an individual inherited two alleles from one grandparent (AA), two alleles from the other grandparent (BB), or is heterozygous (AB)
- Need many markers per chromosome
- Next-generation sequencing has provided a relatively easy way to identify these markers

Genotypes



Red = AA
Blue = AB
Green = BB

Linkage map

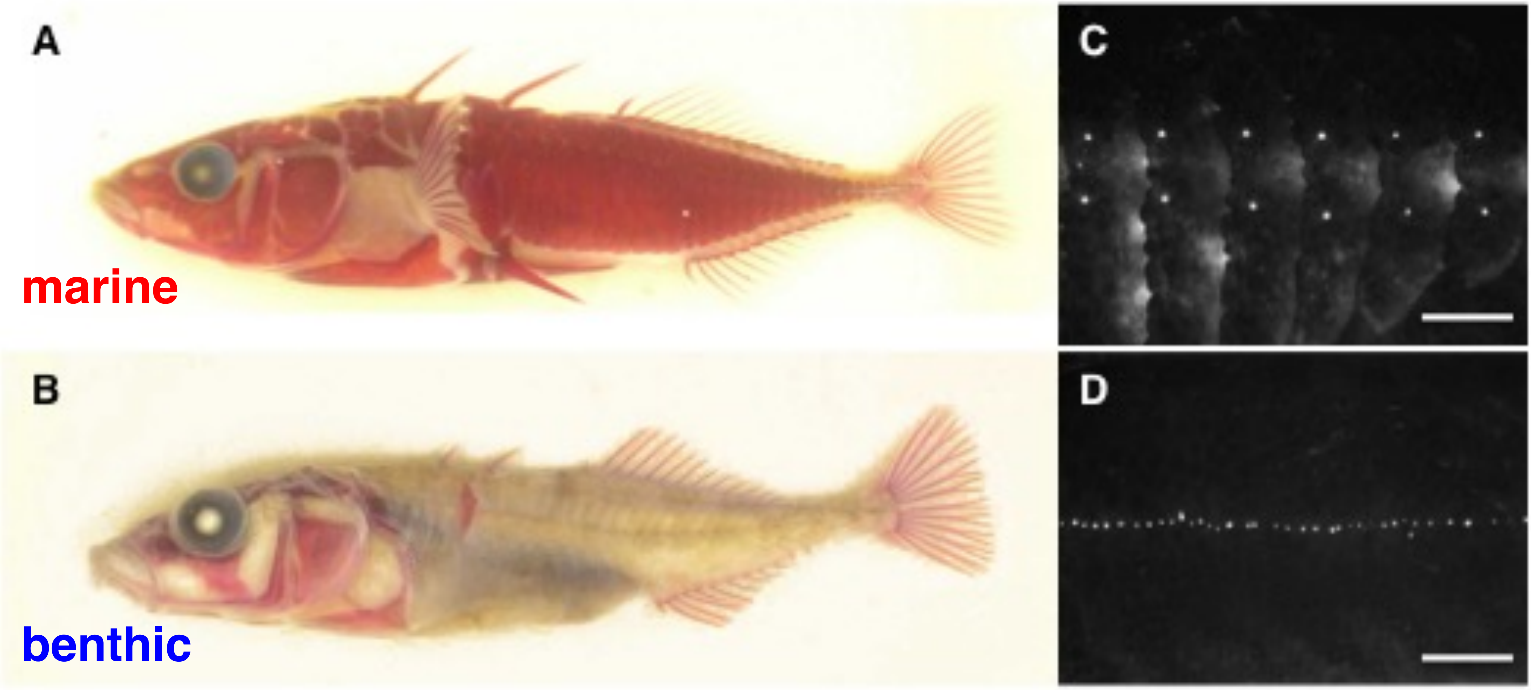

Genetic map
Location (cM)
0
20
40
60
80
100
1
2
3
4
5
6
7
8
9
10
11
12
13
14a
14b
15
16
17
18
19
20
21
Chromosome

Analyses software

- R/qtl is the best!
- Broman and Sen (2009) A guide to QTL mapping with R/qtl
- Open source!
- https://rqtl.org/

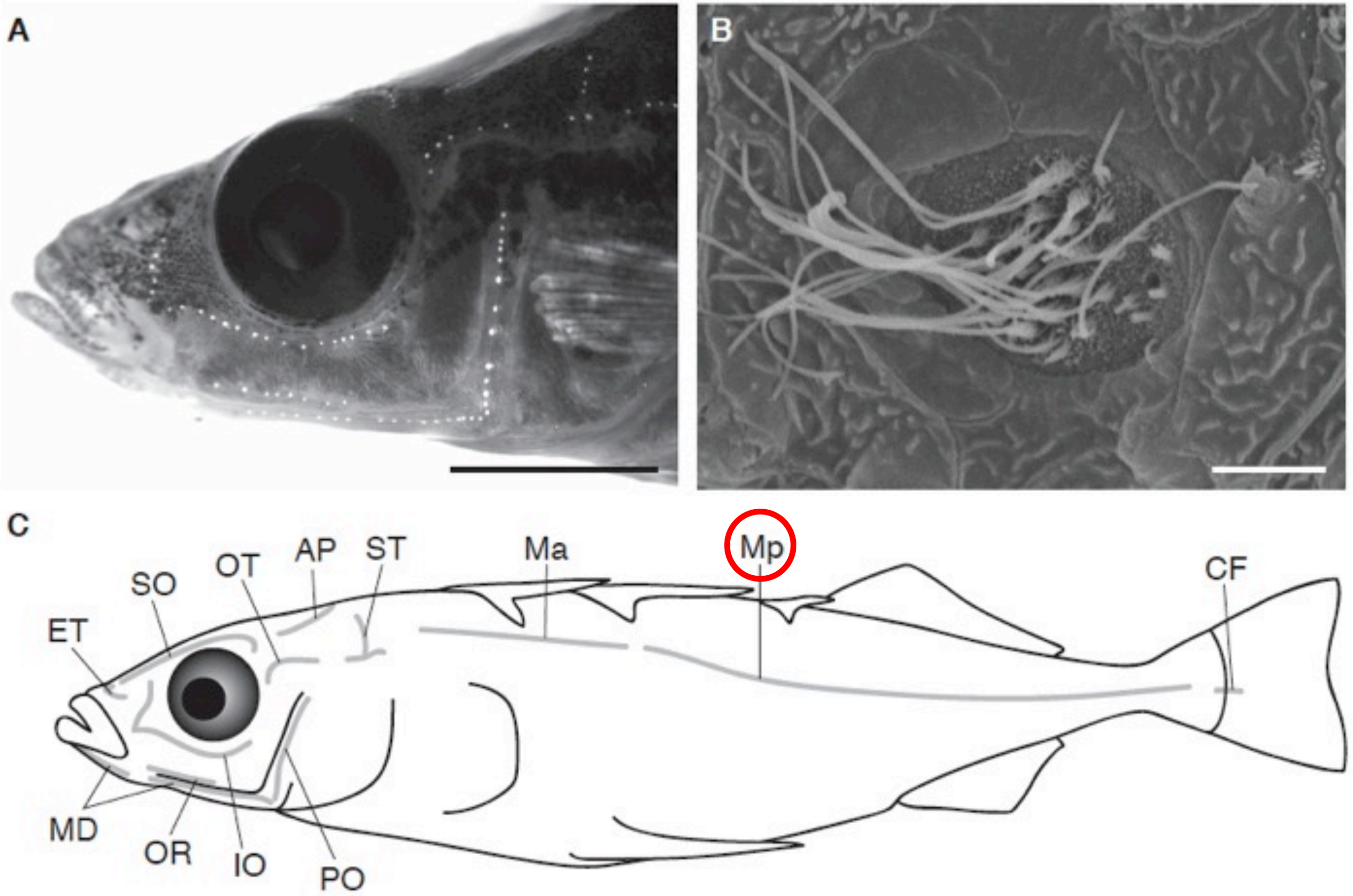
QTL mapping exercise

Lateral plate and lateral line differences between marine and benthic sticklebacks

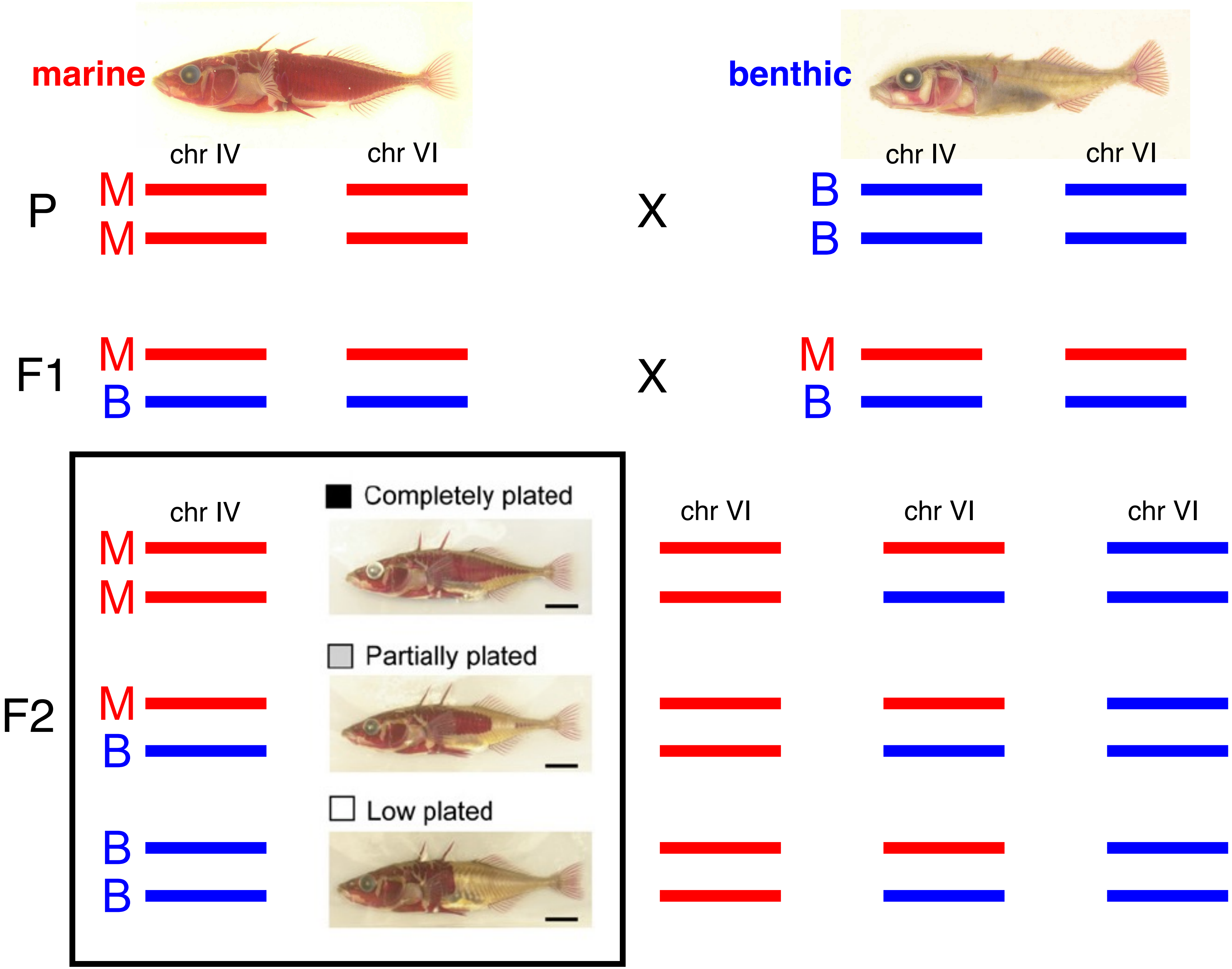


Wark *et al* 2012 *G3*

Mechanosensory lateral line



Wark & Peichel 2010 *J Exp Biol*



marine

chr IV

chr VI

benthic

chr IV

chr VI

P

M

M

X

B

B

F1

M

B

X

M

B

F2

chr IV

M

M

■ Completely plated

M

B

▨ Partially plated

B

B

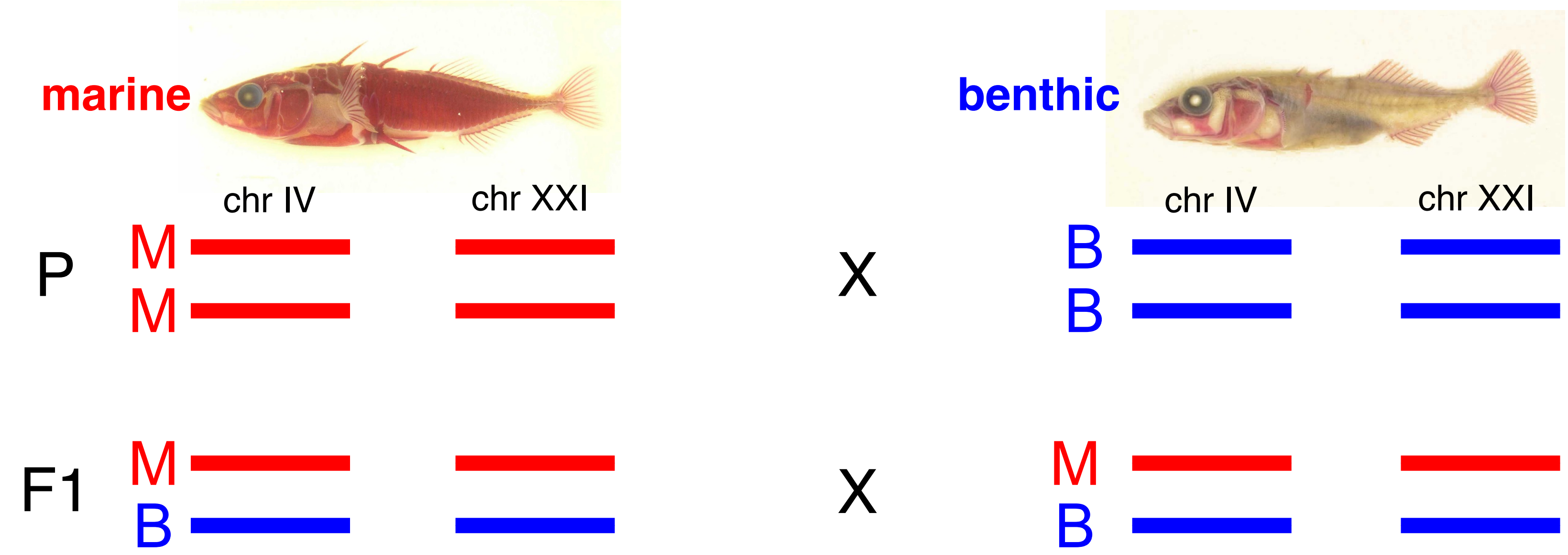
☐ Low plated

chr VI

chr VI

chr VI

Gene for plates is on chr IV



marine

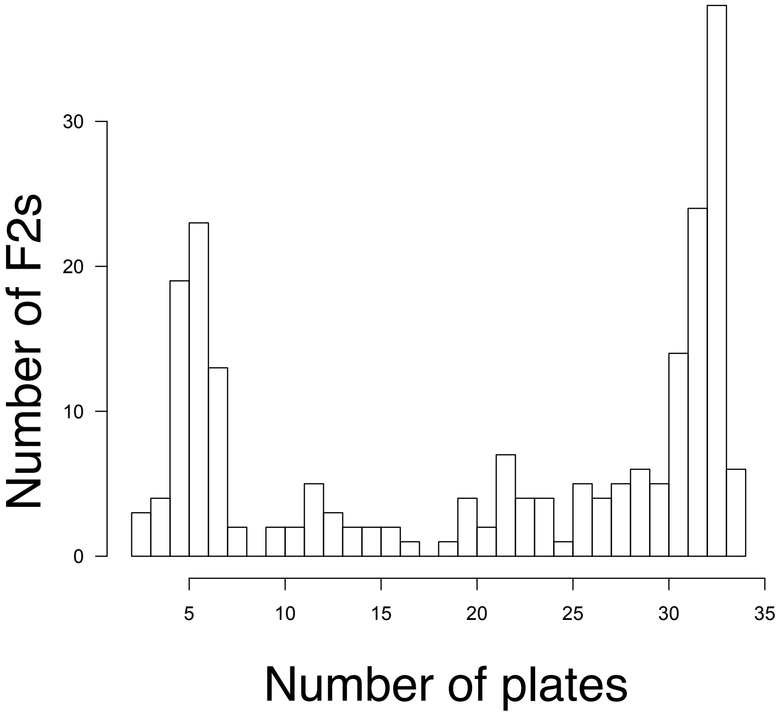
benthic

chr IV chr XXI

P M M X B B

F1 M B X M B

F2



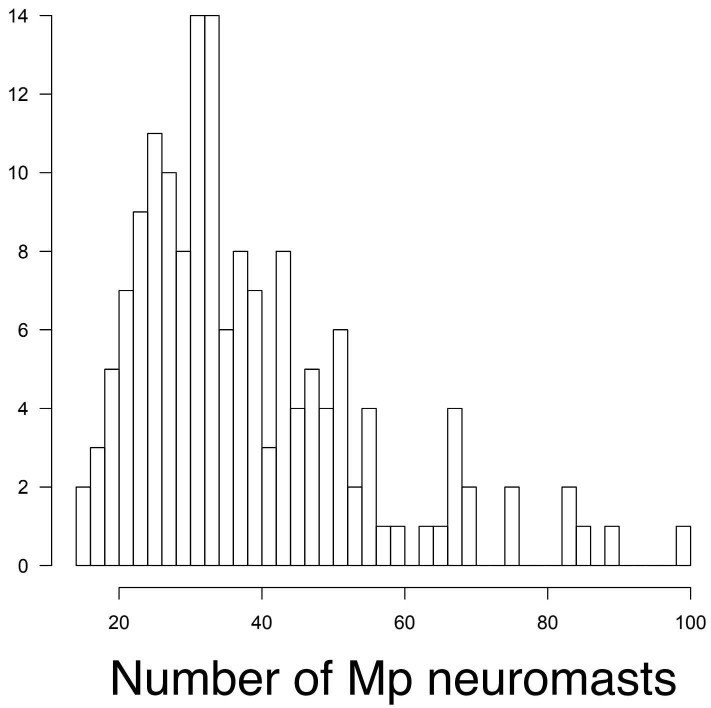


Plate number: no genotype-phenotype association at chr VI marker

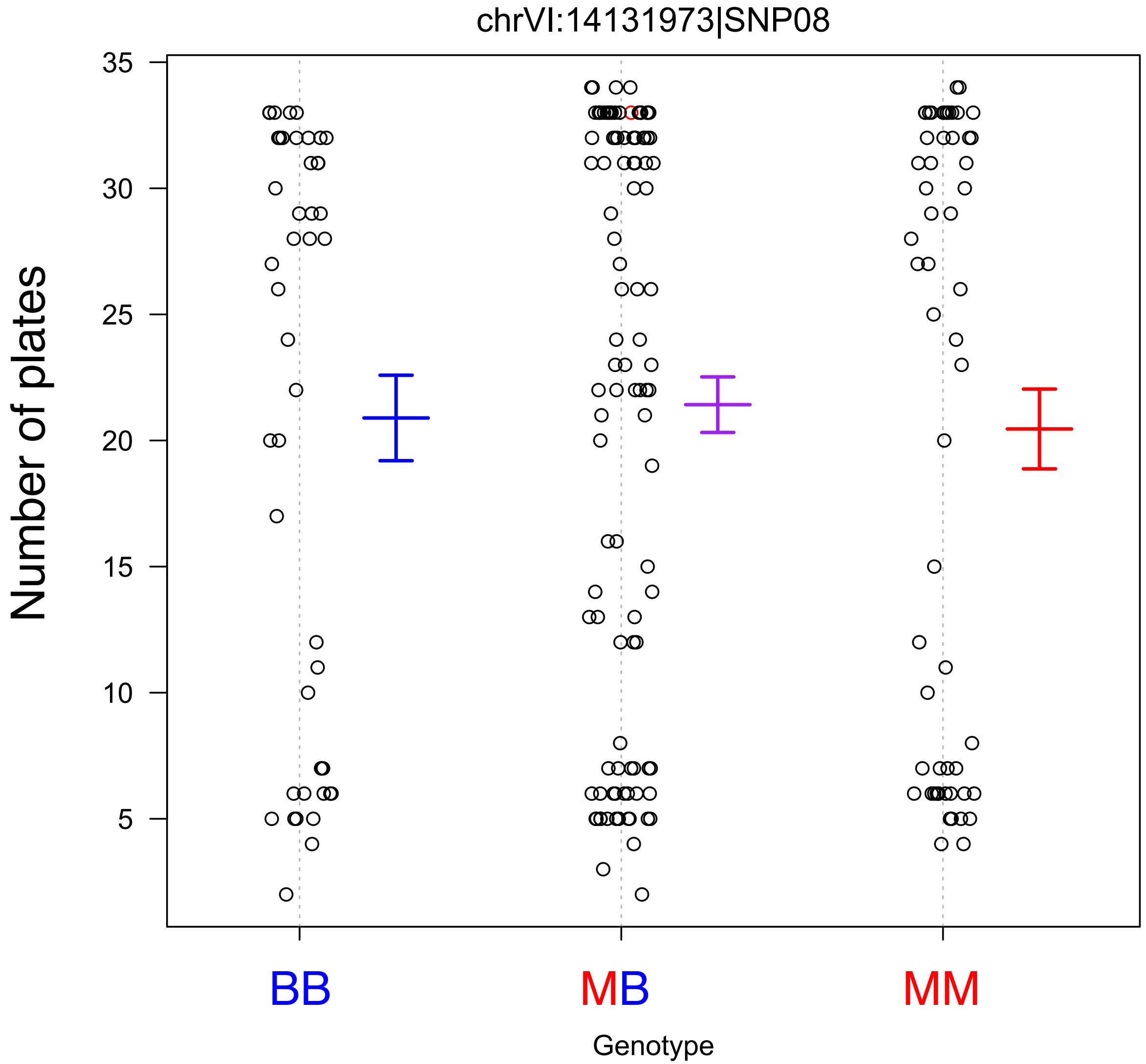


Plate number: genotype-phenotype association at chr IV marker

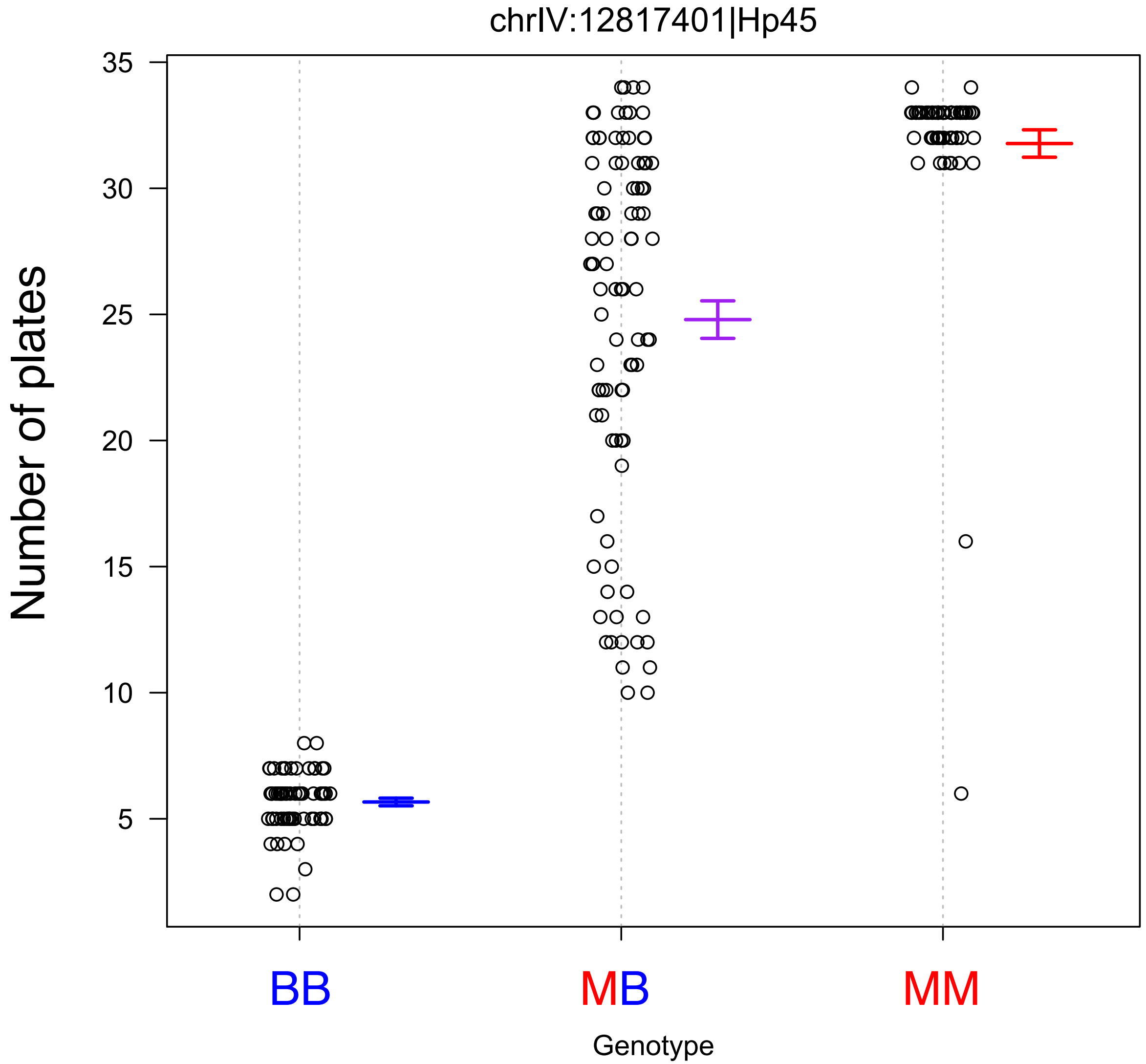


Plate number: genotype-phenotype association at chr XXI marker

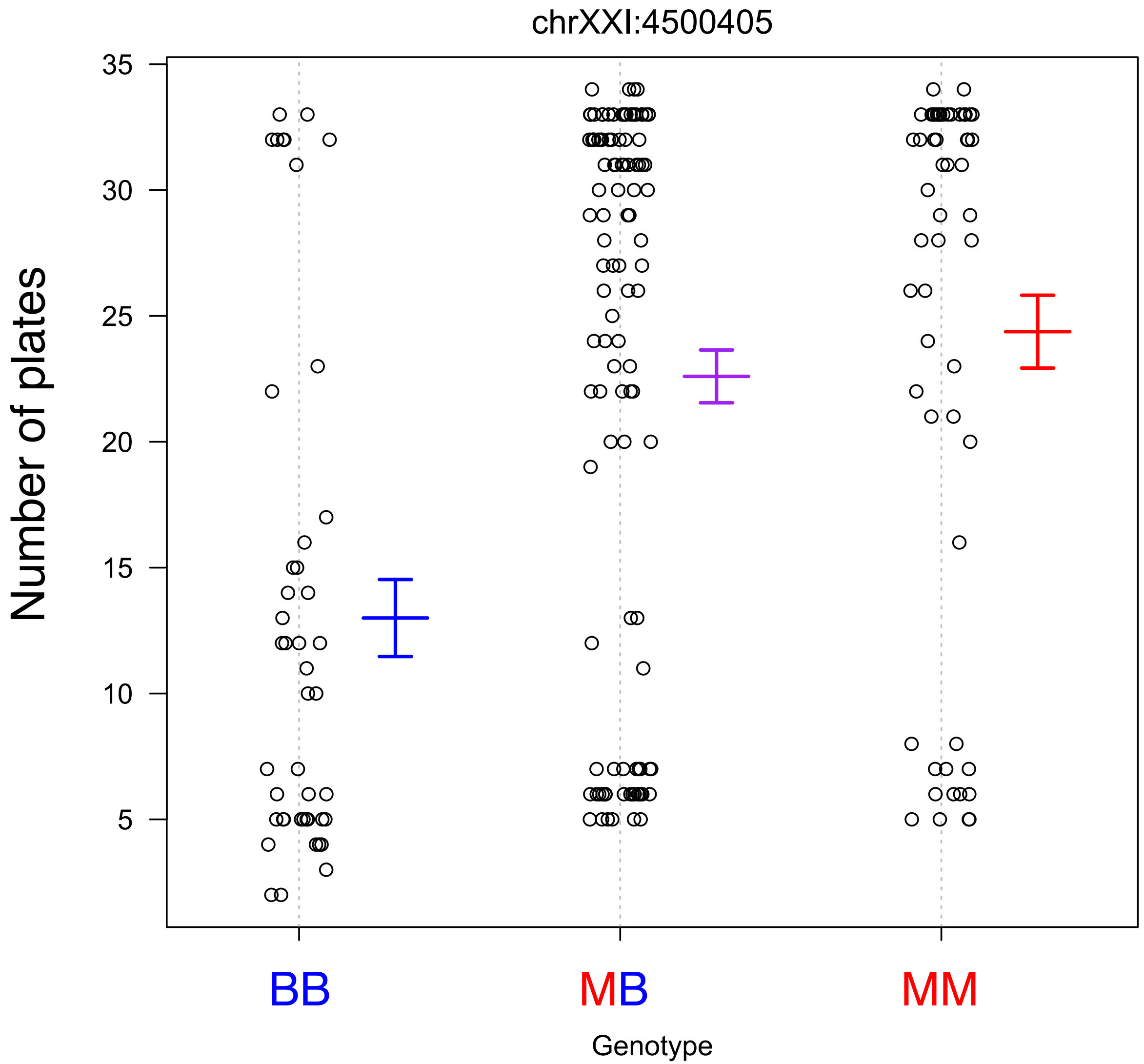
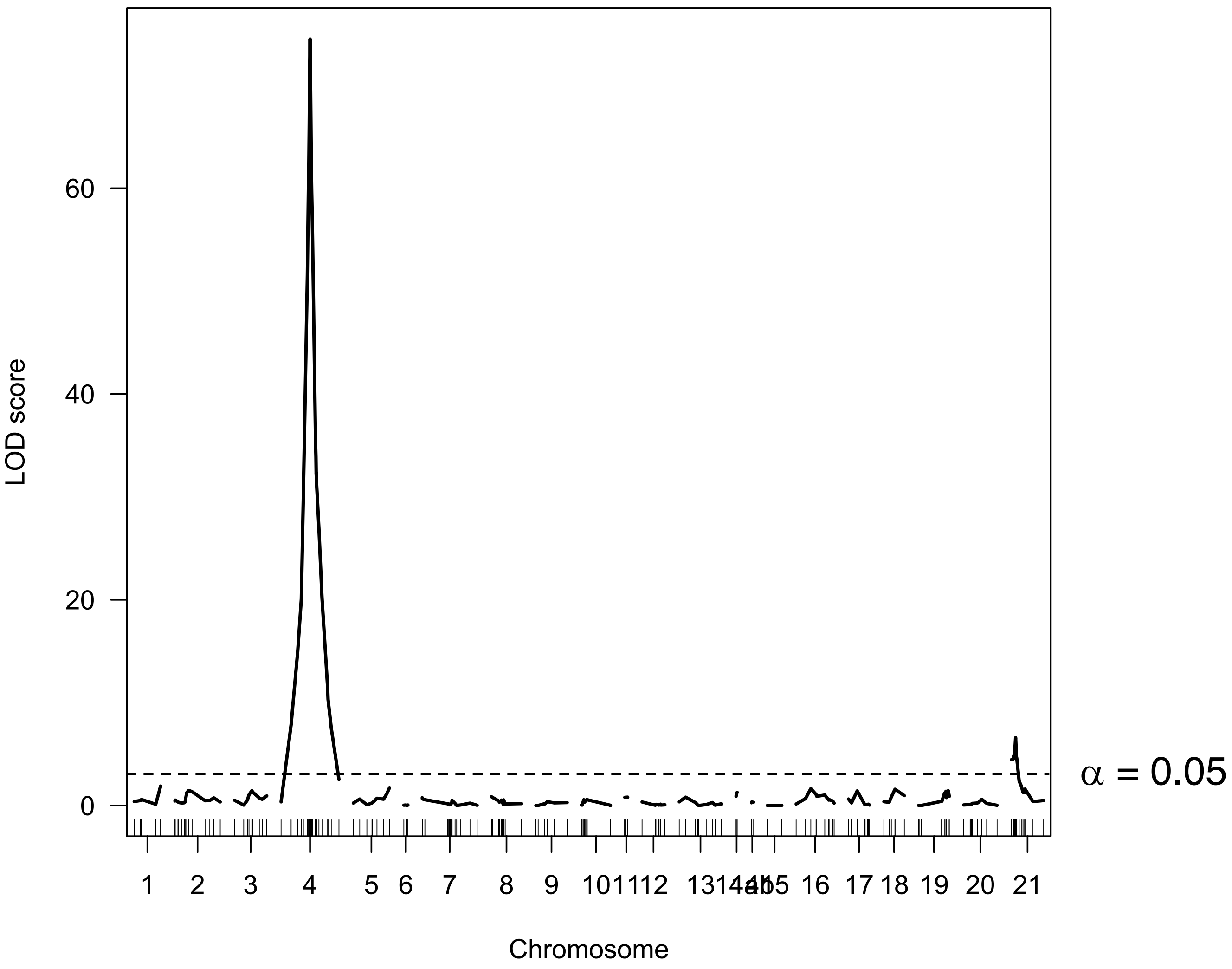


Plate number: QTL mapping

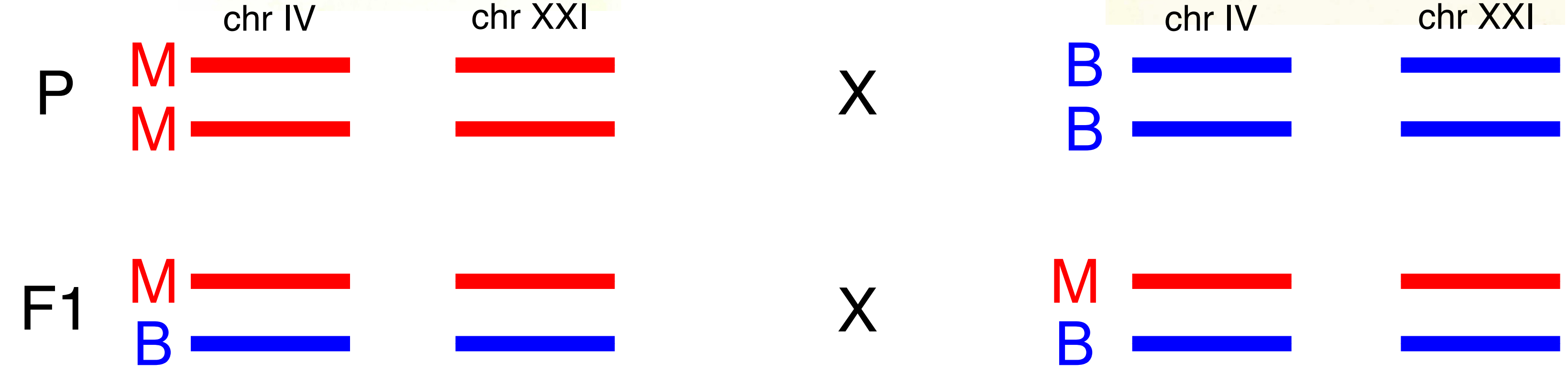


What is a LOD score?

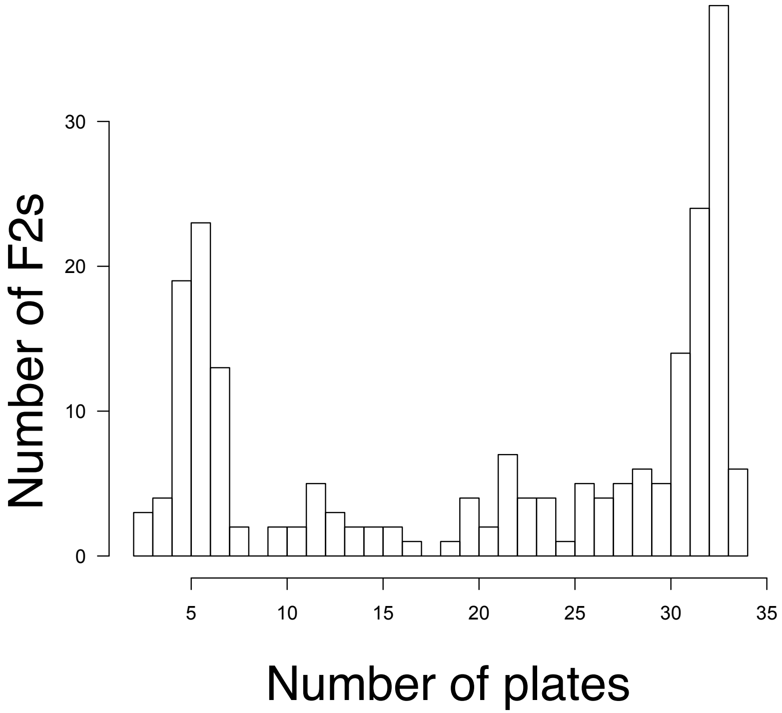
- LOD score is the strength of evidence for the presence of a genotype-phenotype association at a particular locus
- LOD = $\log_{10}$ likelihood ratio comparing the hypothesis of an association at a locus versus the null hypothesis of no association
- LOD of 3 is generally considered significant
  - probability of only 1 in 1000 that there is no association at a single locus
- But, we test many loci so we use permutation tests to empirically determine the significance threshold
  - Usually $\alpha = 0.05$ (5% false positive rate)

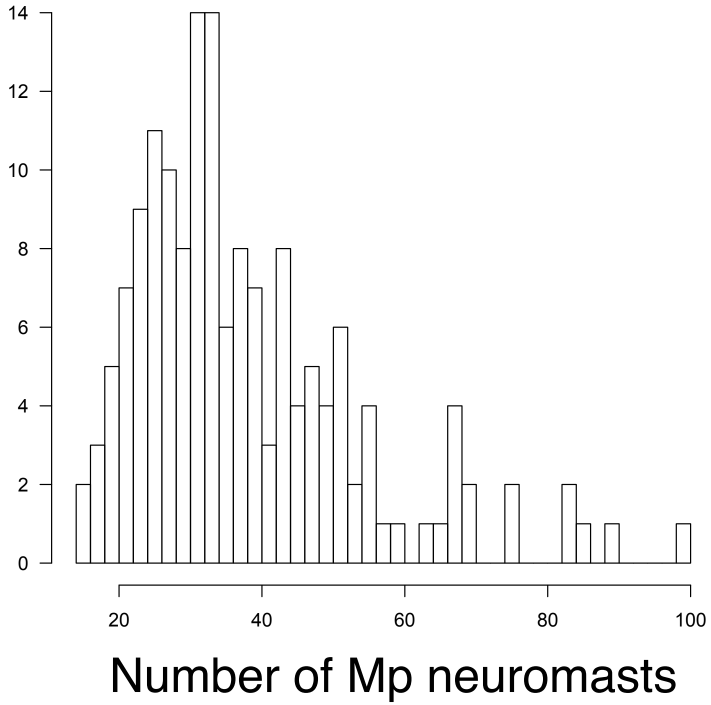
marine

benthic

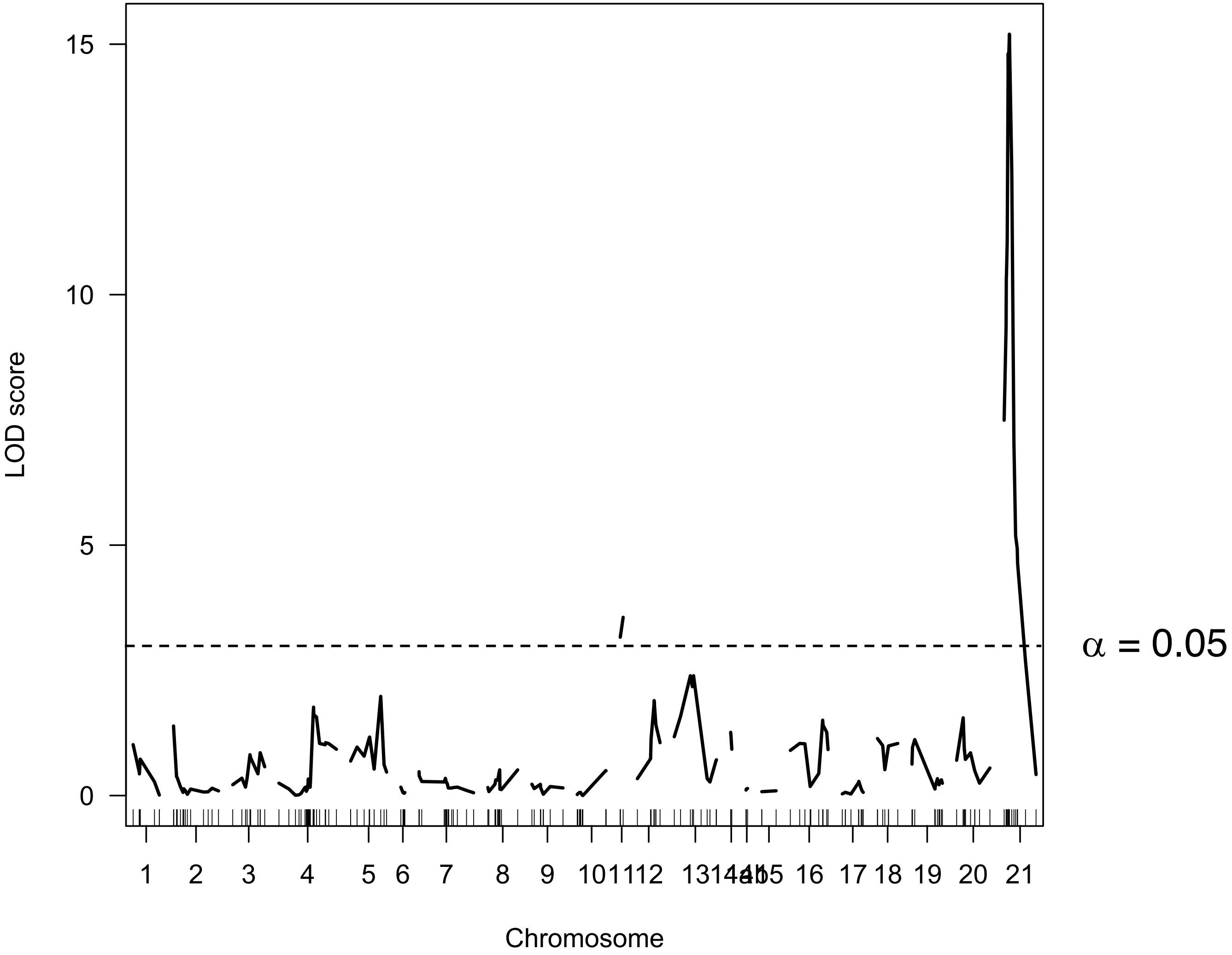


F2

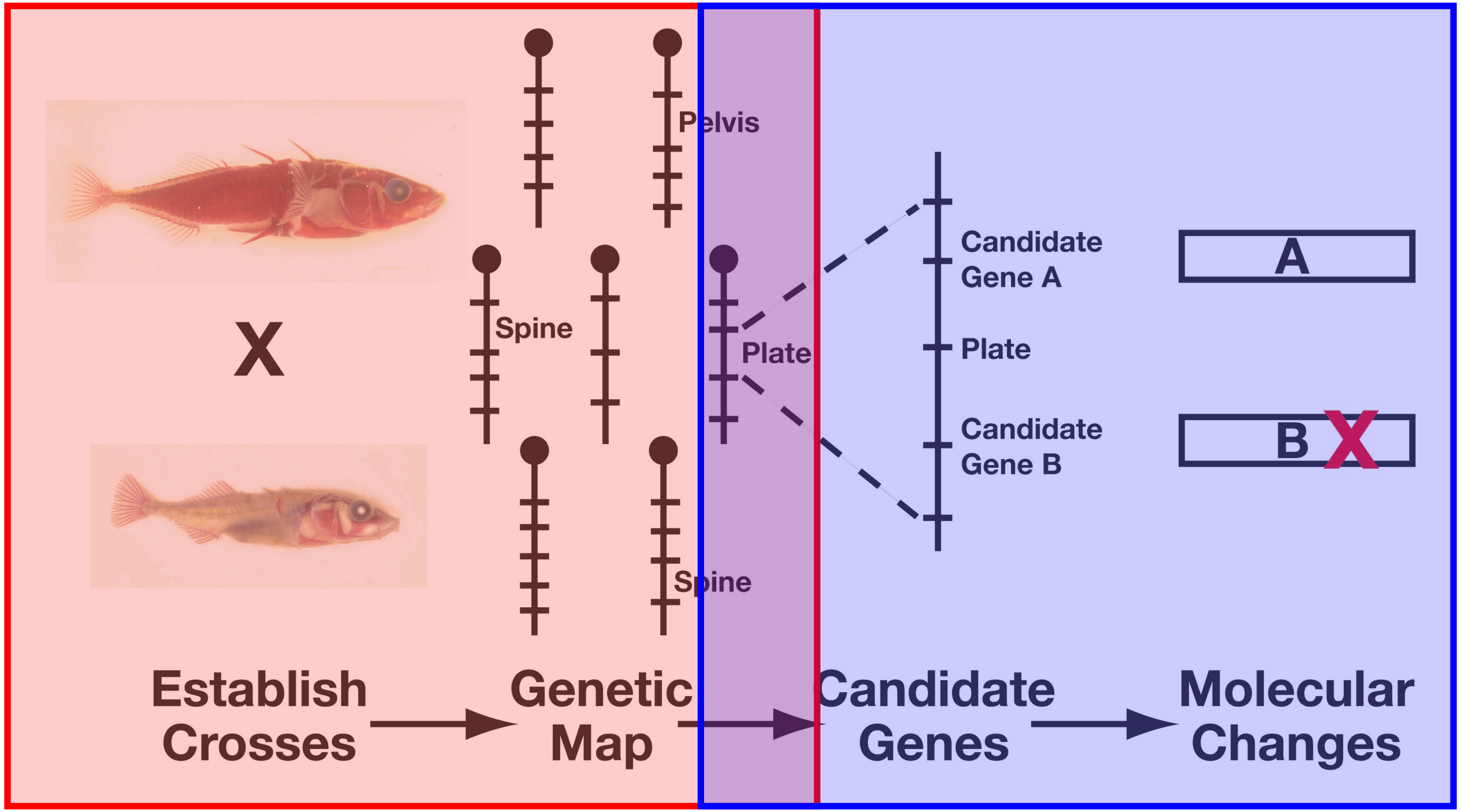




Mp neuromast number: QTL mapping



Forward genetics: quantitative trait loci (QTL) mapping approach



Establish Crosses → **Genetic Map** → **Candidate Genes** → **Molecular Changes**

We have a QTL: now what?

1. Use additional recombination mapping to further narrow QTL interval
2. Identify candidate genes in QTL interval
3. Look for molecular differences in candidate genes between populations with different phenotypes
4. Use genetic manipulation to show that a molecular difference is necessary and sufficient for phenotypic difference

Forward genetic approach

- Strengths?
  - Identifies the specific genes and mutations that underlie phenotypic traits

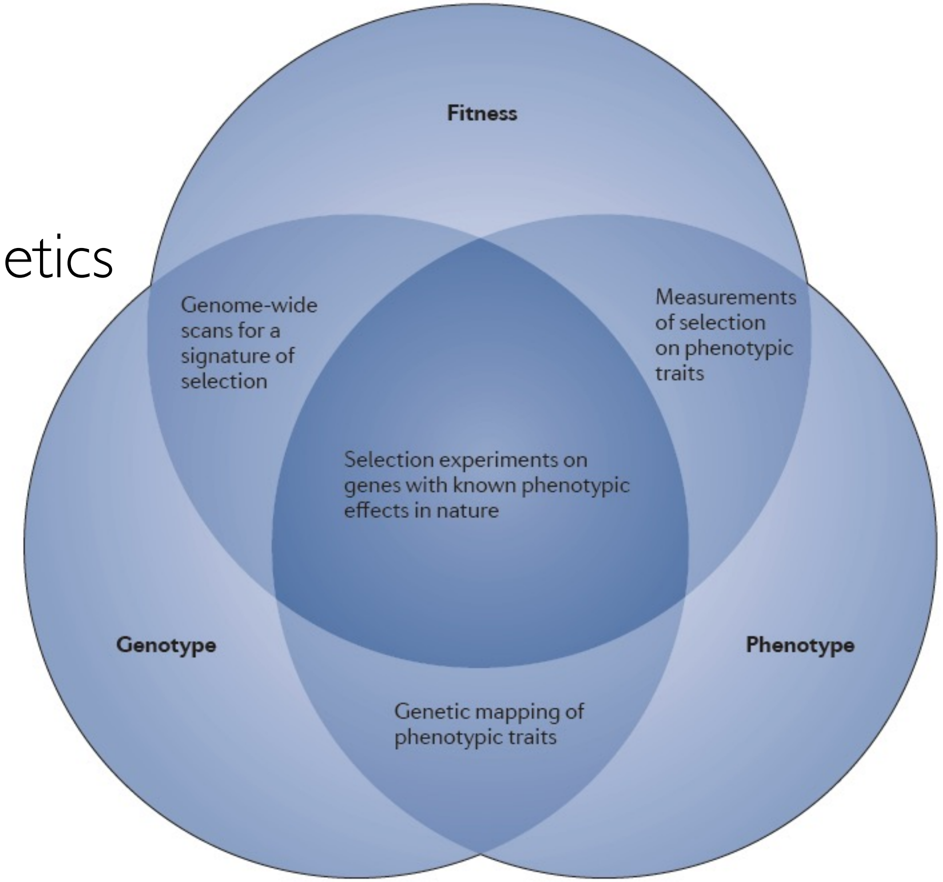
- Limitations?
  - Path from phenotype to genotype is long
  - Ability to cross the populations of interest
  - Limited by recombination events in crosses
  - Best to focus on traits that you know are adaptive

Reverse genetic approach

- Strengths?
  - Next generation sequencing makes this approach feasible in any natural population
  - Can survey entire genome
  - Identifies genes that are targets of selection

- Limitations?
  - Can be difficult to link genetic variation to phenotypic variation

Integrated conceptual framework to understand the genetics of adaptation



Reverse genetics

Forward genetics

Barrett & Hoekstra 2011 *Nature Reviews Genetics*

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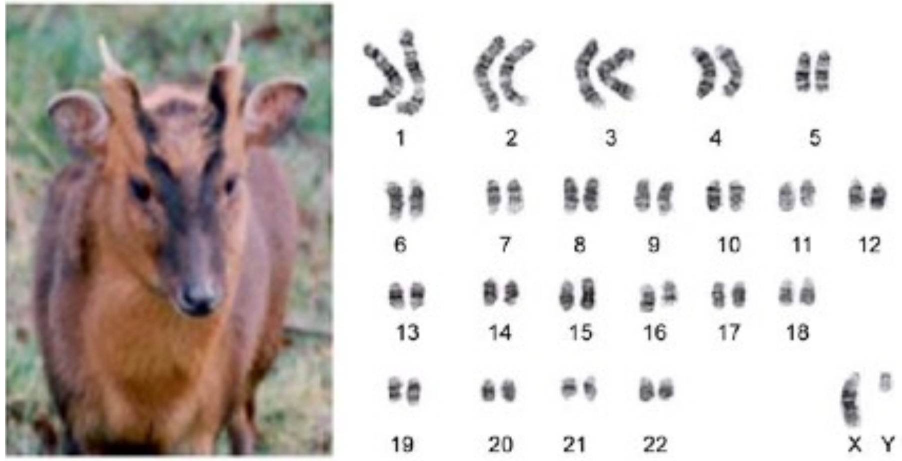
*Please always interrupt with questions as you have them!!!

Combining genomics and experimental studies to understand the role of chromosomal rearrangements in adaptation to divergent environments

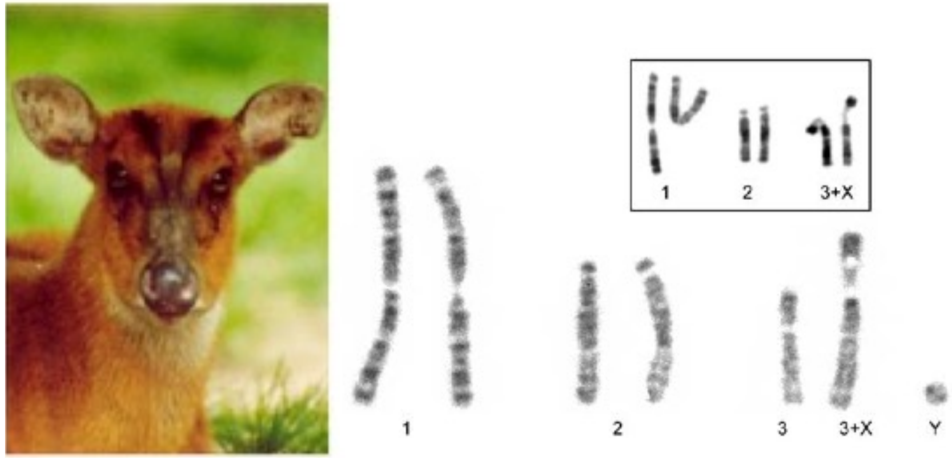
Katie Peichel
University of Bern

Chromosome number and structure can vary dramatically between species

Chinese muntjac
2n = 46

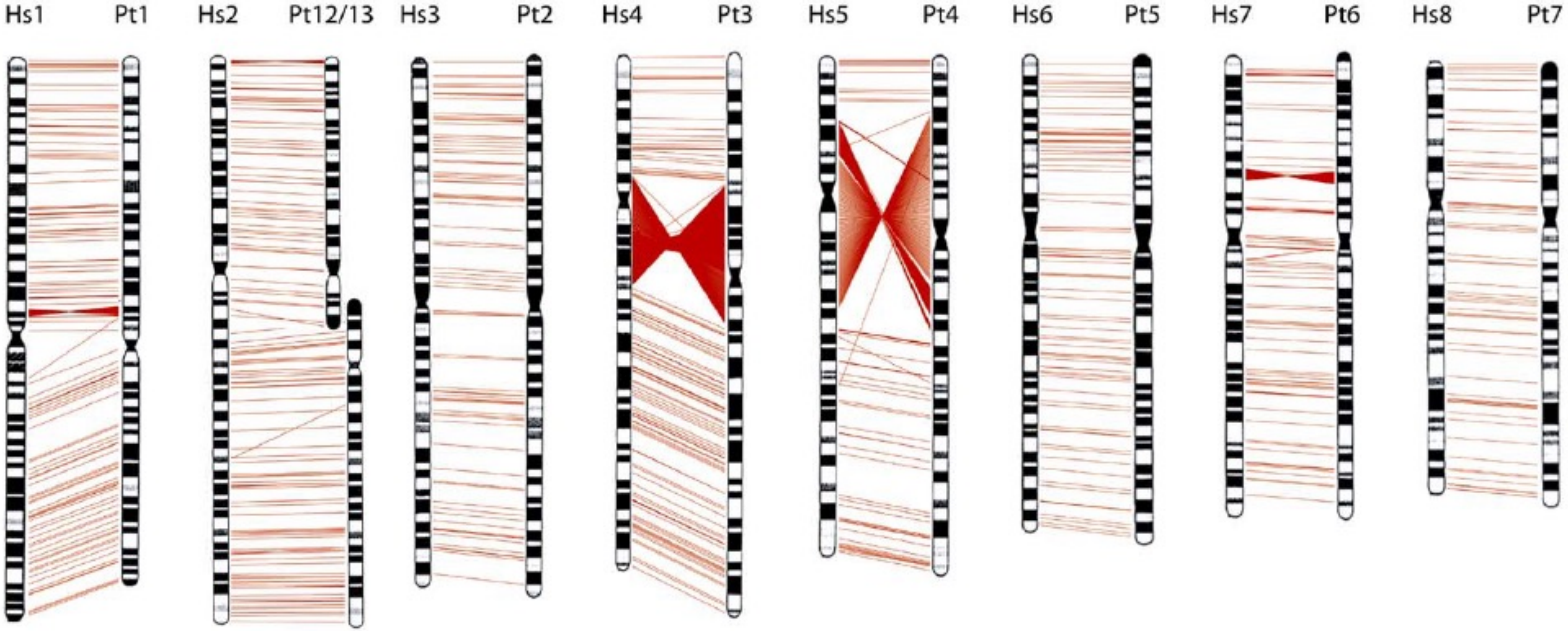


Indian muntjac
2n = 6,7



Scheuermann *et al* 2005

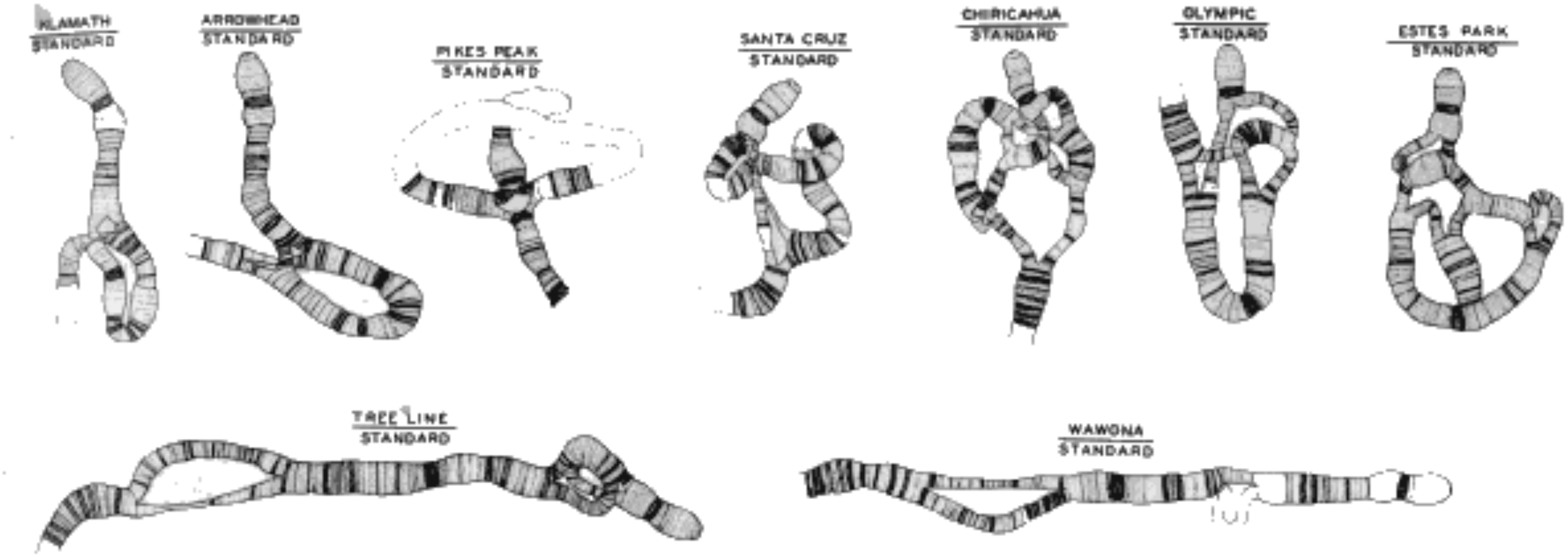
Chromosome number and structure can vary dramatically between species



Human *vs* chimpanzee: fusion and inversions

Feuk *et al* 2005

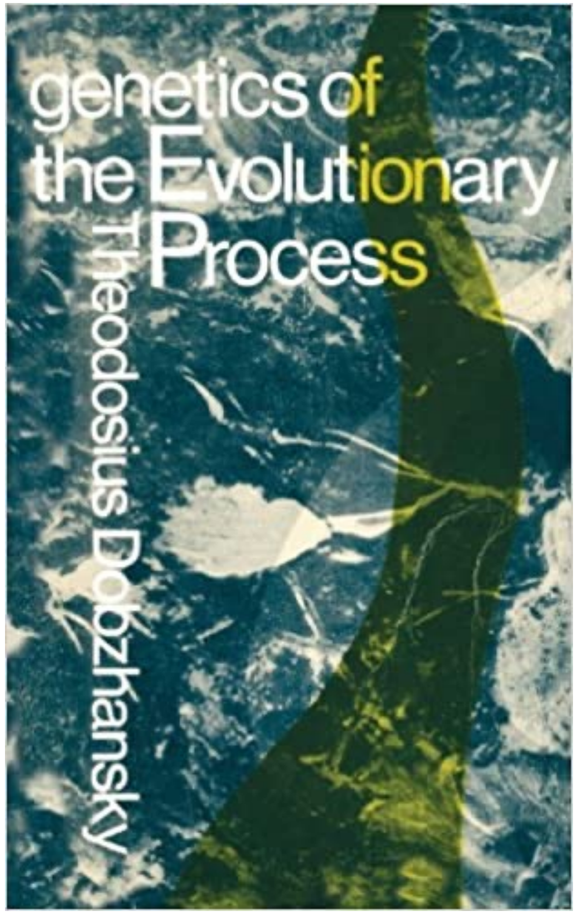
Chromosome number and structure can even vary dramatically within species



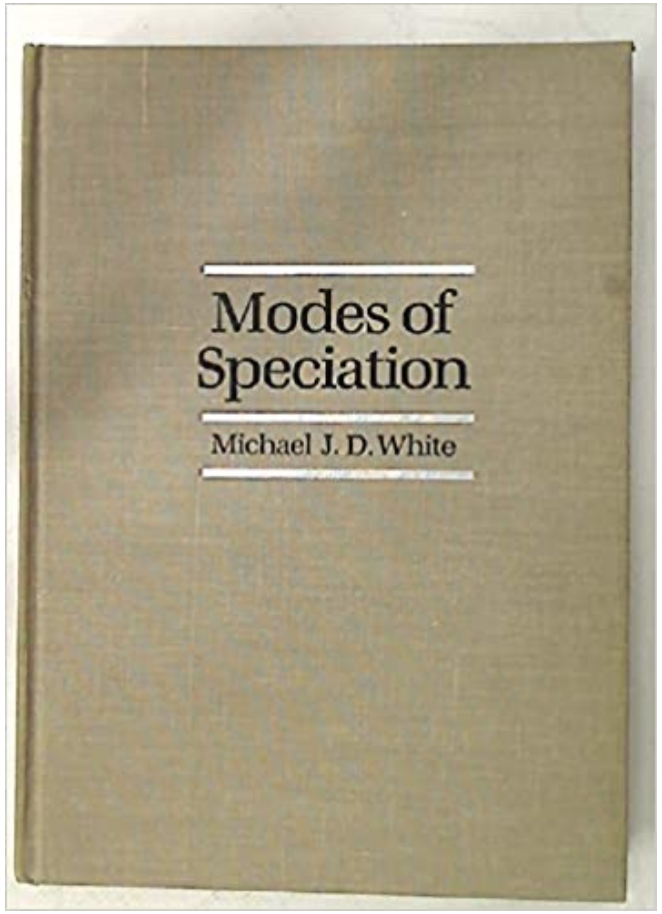
Drosophila pseudoobscura inversion polymorphisms

Dobzhansky & Sturtevant 1938

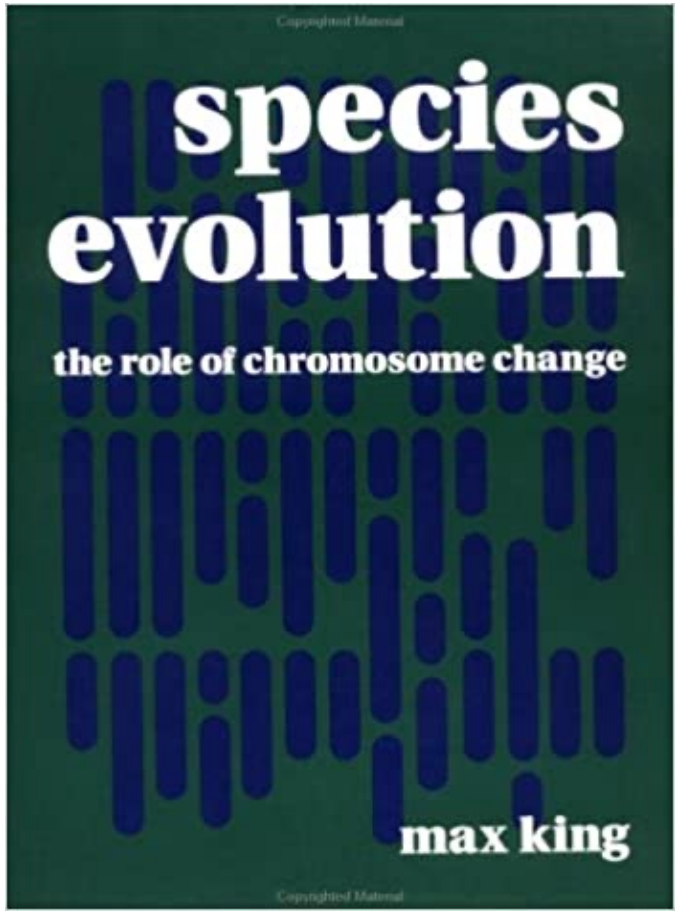
Chromosomal changes as drivers of adaptation and speciation?



Dobzhansky 1970



White 1978



King 1993

Chromosomal changes as drivers of adaptation and speciation?

Chromosomal rearrangements and speciation

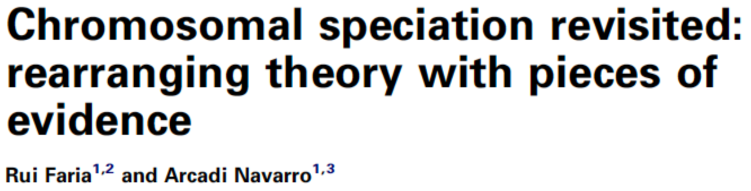
Loren H. Rieseberg

Genomic rearrangements and the evolution of clusters of locally adaptive loci

Sam Yeaman[a,b,1]

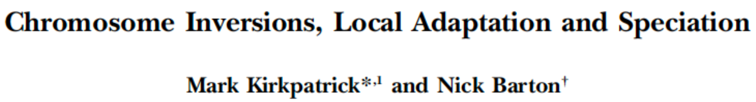
Chromosomal inversions and the reproductive isolation of species

Mohamed A. F. Noor*, Katherine L. Grams, Lisa A. Bertucci, and Jane Reiland



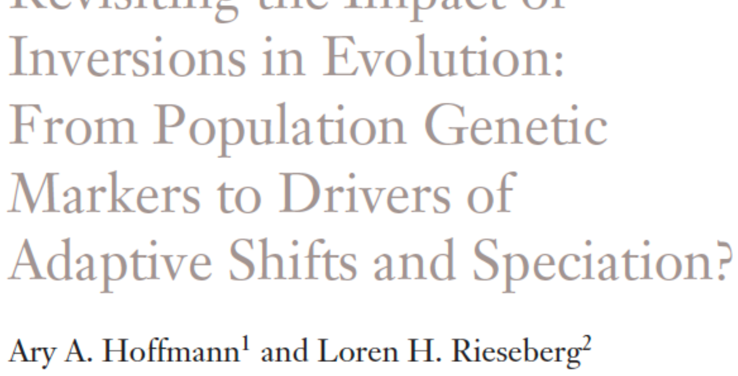
Chromosomal speciation revisited: rearranging theory with pieces of evidence

Rui Faria[1,2] and Arcadi Navarro[1,3]



Chromosome Inversions, Local Adaptation and Speciation

Mark Kirkpatrick*[,1] and Nick Barton[†]



Revisiting the Impact of Inversions in Evolution: From Population Genetic Markers to Drivers of Adaptive Shifts and Speciation?

Ary A. Hoffmann[1] and Loren H. Rieseberg[2]

Eco-Evolutionary Genomics of Chromosomal Inversions

Maren Wellenreuther[1,3,*] and Louis Bernatchez[2]

LOCAL ADAPTATION AND THE EVOLUTION OF CHROMOSOME FUSIONS

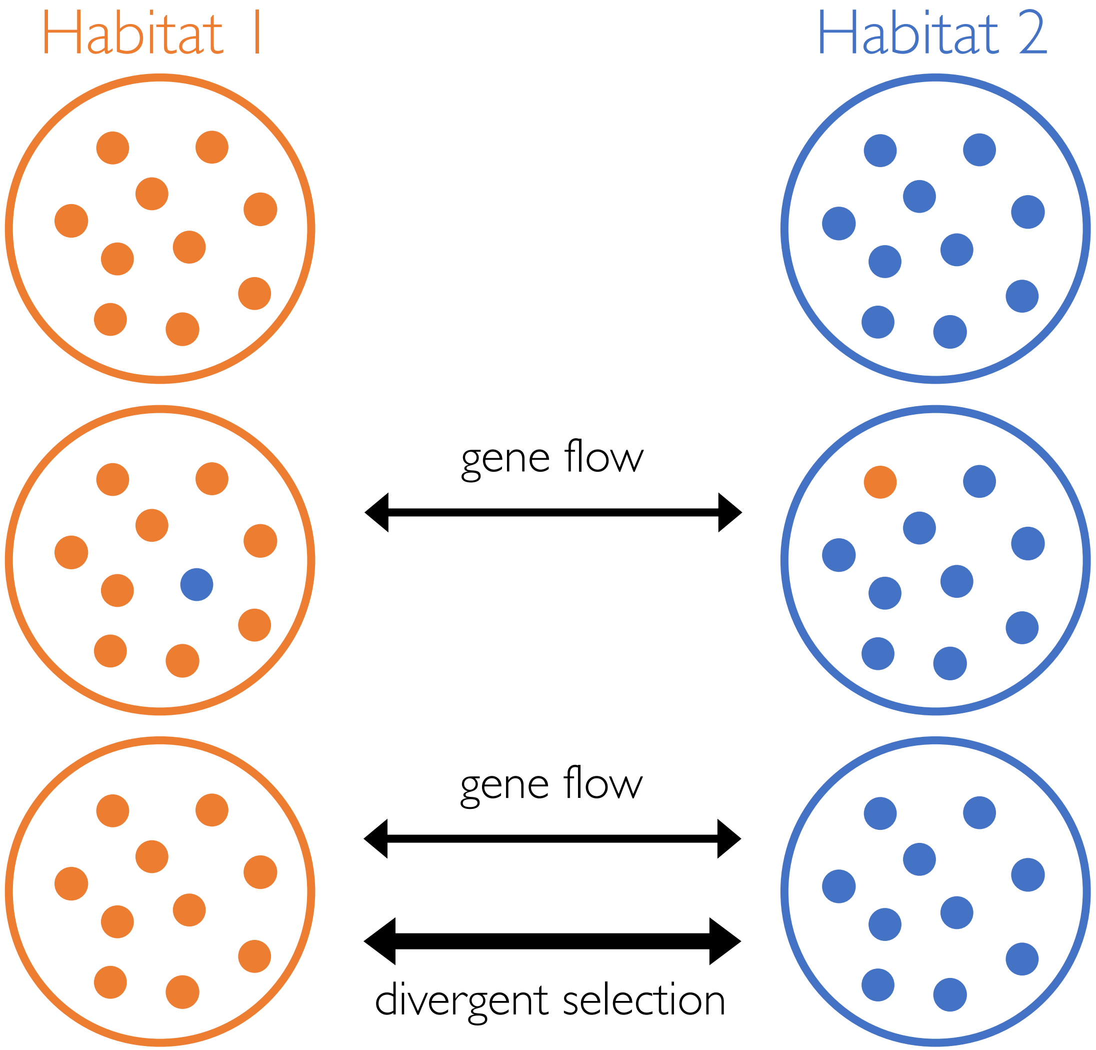
Rafael F. Guerrero[1,2] and Mark Kirkpatrick[1]

Chromosomal changes as drivers of adaptation and speciation?

- Many current sequencing studies are revealing evidence for changes in chromosome number and structure within and between species

- But, there is relatively little data directly linking these chromosomal changes to **adaptation** and speciation
  - Kitano *et al* 2009 *Nature*: chromosomal fusions and speciation
  - Peichel *et al* 2020 *Genome Biology:* inversions and sex chromosome evolution

Local adaptation to divergent environments



Habitat 1

Habitat 2

predators
prey
parasites
abiotic factors

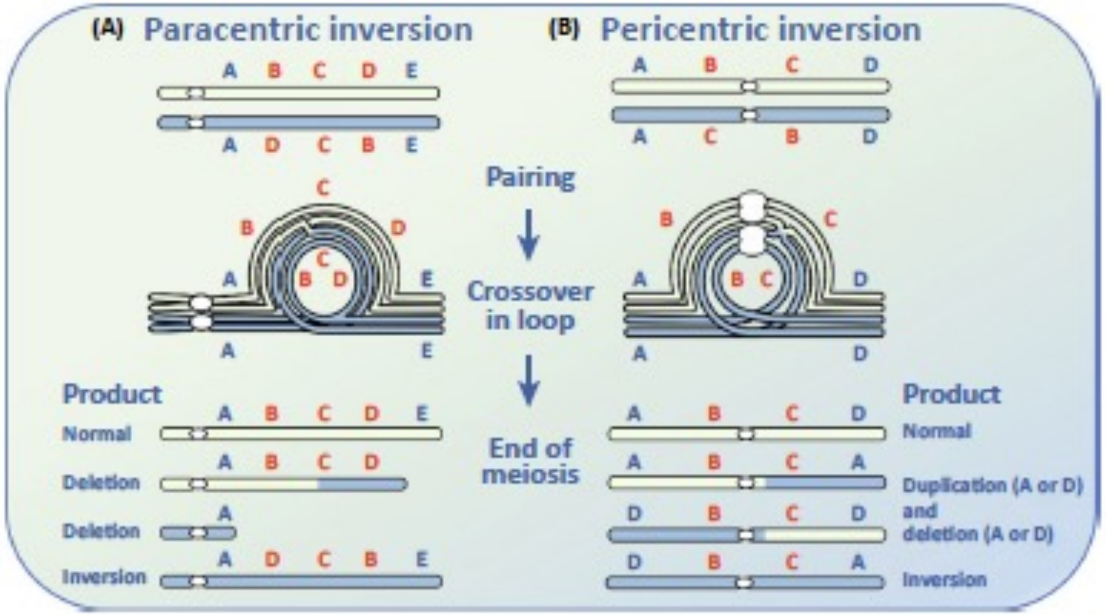
gene flow

predators
prey
parasites
abiotic factors

gene flow

divergent selection

Chromosomal changes could facilitate local adaptation by linking together adaptive alleles



Wellenreuther and Bernatchez 2018 *TREE*

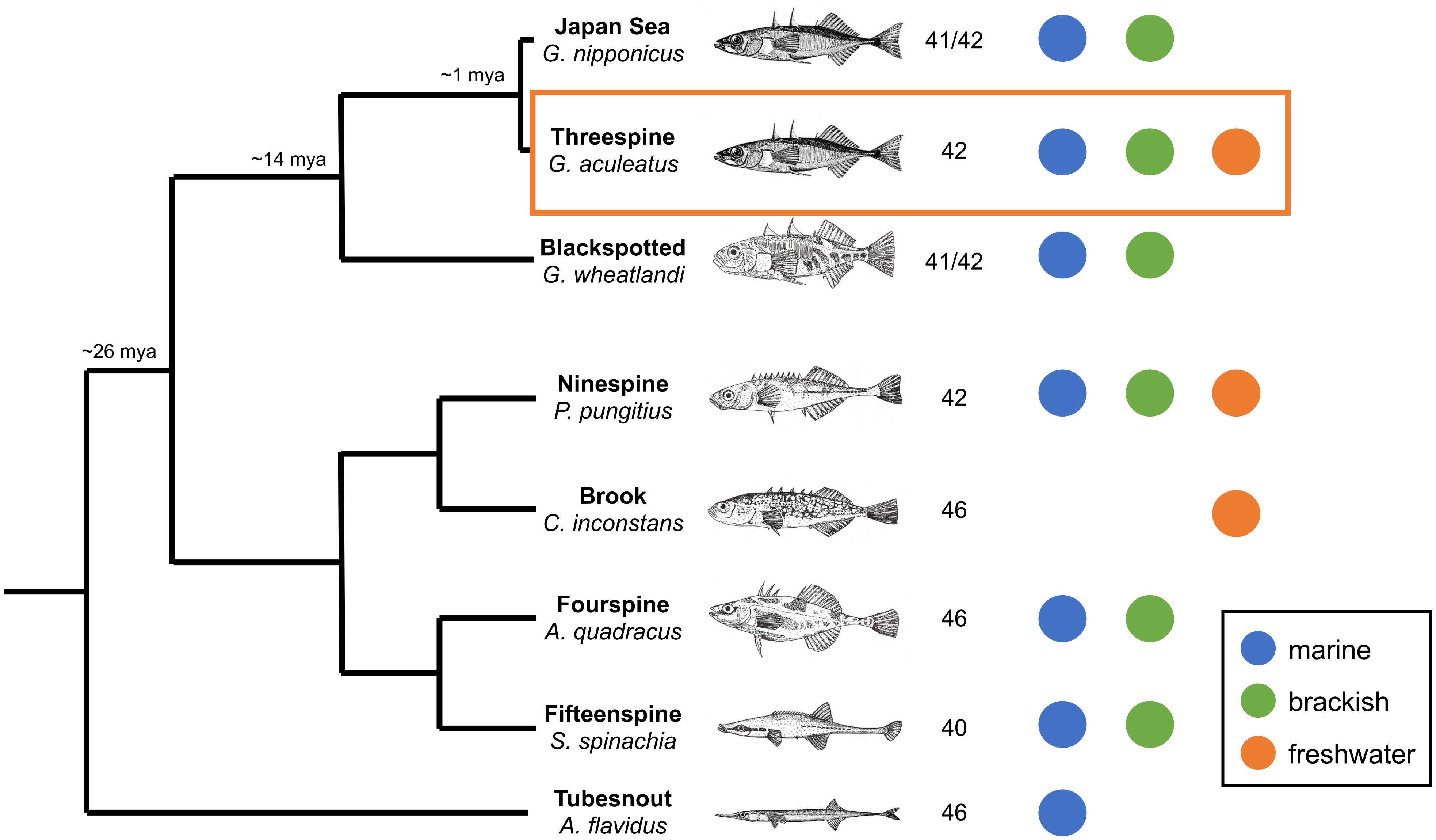
- If recombination happens within an inversion heterozygote, recombinant gametes are inviable
- Thus, recombination is effectively suppressed within inversions
- Inversions might be particularly important to link multiple adaptive alleles in cases of local adaptation with gene flow, where heterozygotes can be formed
- This theory predicts that we will find linkage of multiple adaptive traits to chromosomal inversions (or fusions*)

*Chromosomal fusions also lead to a local reduction of recombination but through a different mechanism

Do chromosomal changes facilitate local adaptation?

- Are chromosomal changes, such as inversions or fusions, under divergent selection in nature?
- Are multiple adaptive traits linked to chromosomal inversions or fusions?
- Do chromosomal inversions or fusions harbor multiple adaptive alleles?

Stickleback family of fish (Gasterosteidae)

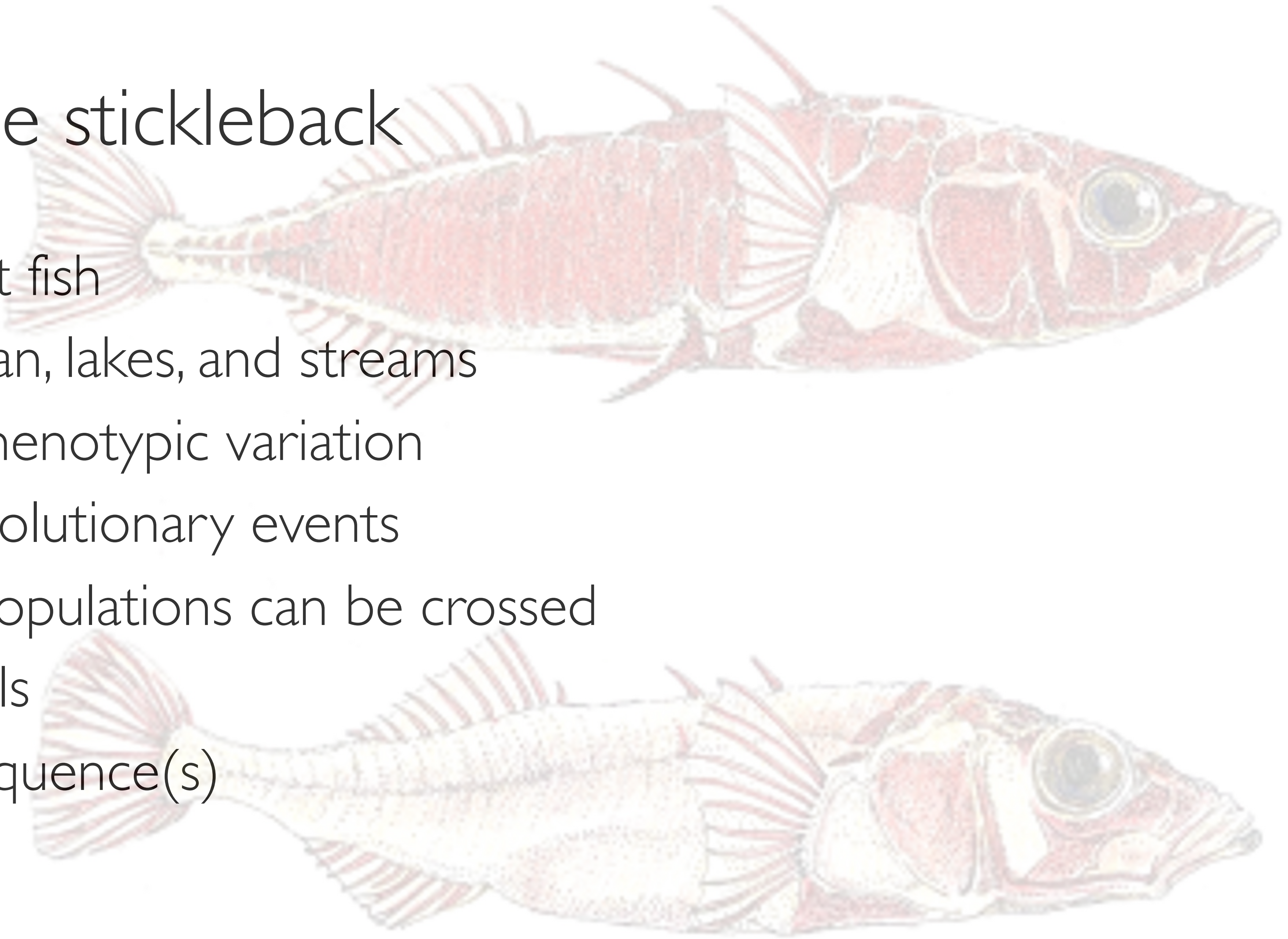


~1 mya
~14 mya
~26 mya

Japan Sea	*G. nipponicus*	41/42	🔵	🟢	
Threespine	*G. aculeatus*	42	🔵	🟢	🟠
Blackspotted	*G. wheatlandi*	41/42	🔵	🟢	
Ninespine	*P. pungitius*	42	🔵	🟢	🟠
Brook	*C. inconstans*	46			🟠
Fourspine	*A. quadracus*	46	🔵	🟢	
Fifteenspine	*S. spinachia*	40	🔵	🟢	
Tubesnout	*A. flavidus*	46	🔵		

🔵 marine
🟢 brackish
🟠 freshwater

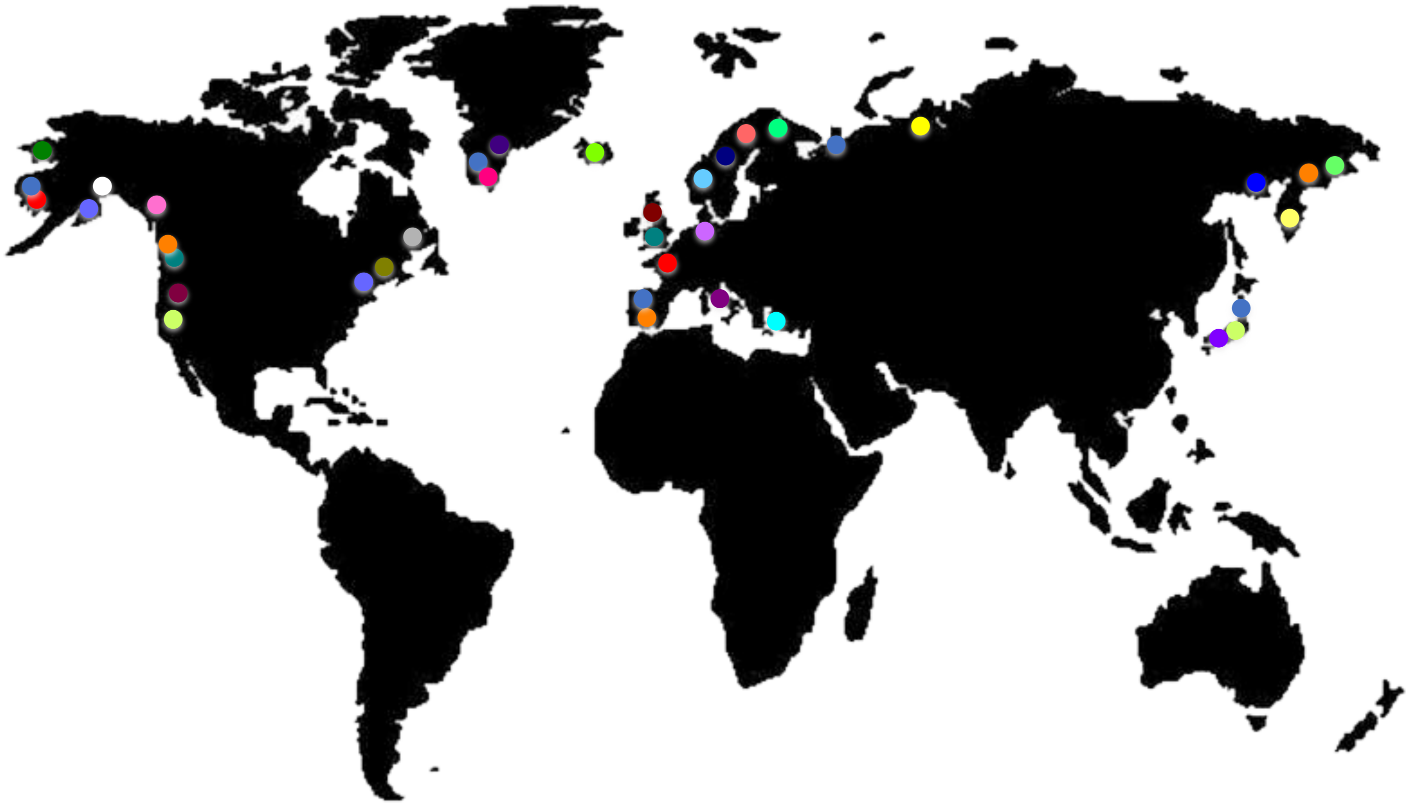
Threespine stickleback



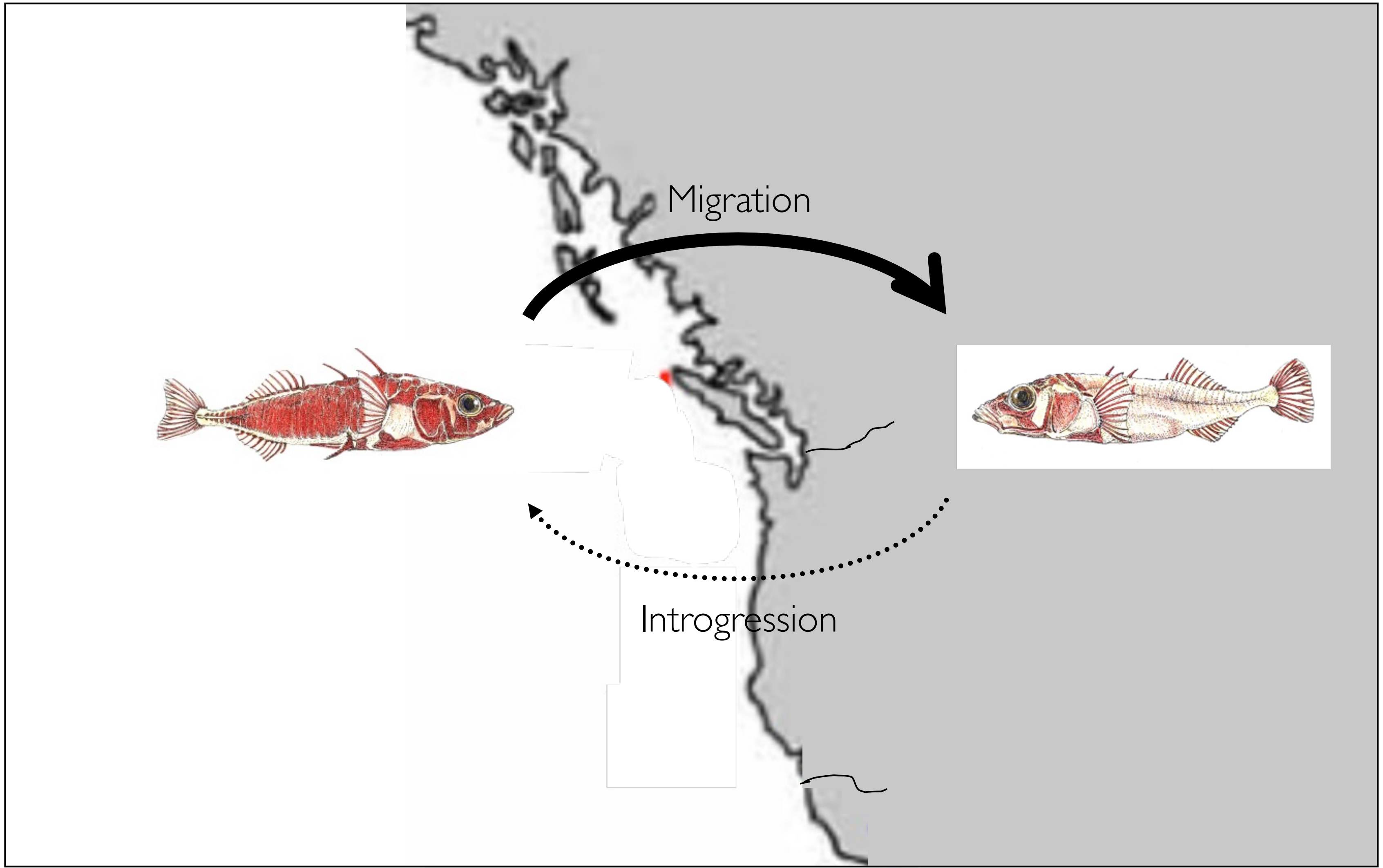
- Small teleost fish
- Lives in ocean, lakes, and streams
- Extensive phenotypic variation
- Replicate evolutionary events
- Divergent populations can be crossed
- Genetic tools
- Genome sequence(s)

Ancestral marine populations

Derived freshwater populations



Gene flow between marine and freshwater populations



Marine vs freshwater sticklebacks

marine

freshwater

Drawings by Kirsten Bomblies
Photos by Seiichi Mori and Jun Kitano

Marine vs freshwater sticklebacks

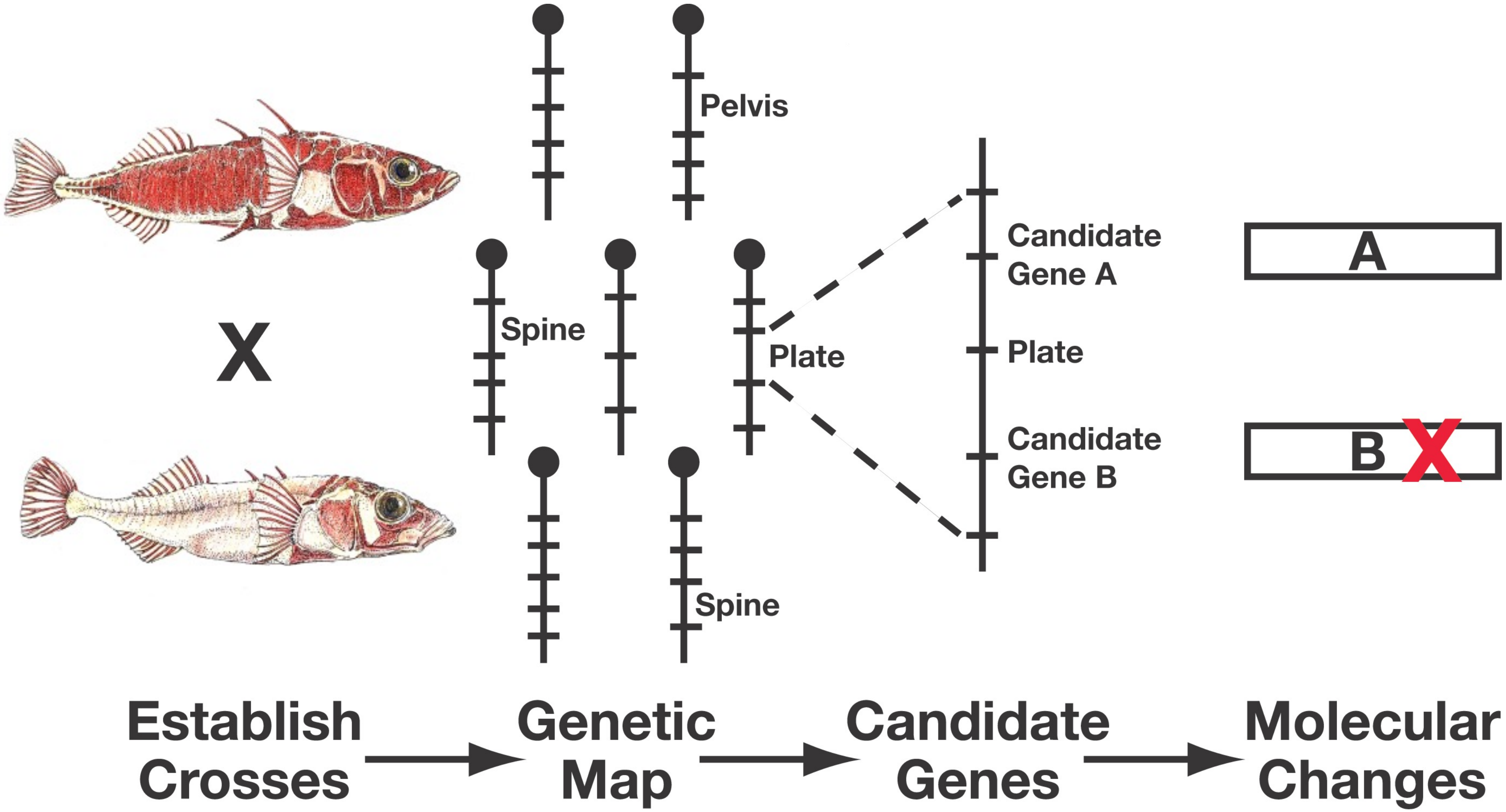
Large, silvery, plated
Migratory, schooling
Saltwater & freshwater tolerant
Lives 2 years

Small, striped, unplated
Resident, non-schooling
Saltwater intolerant
Lives 1 year

Photos by Seiichi Mori and Jun Kitano

Is there genetic linkage of these multiple adaptive traits?

Quantitative trait locus (QTL) mapping

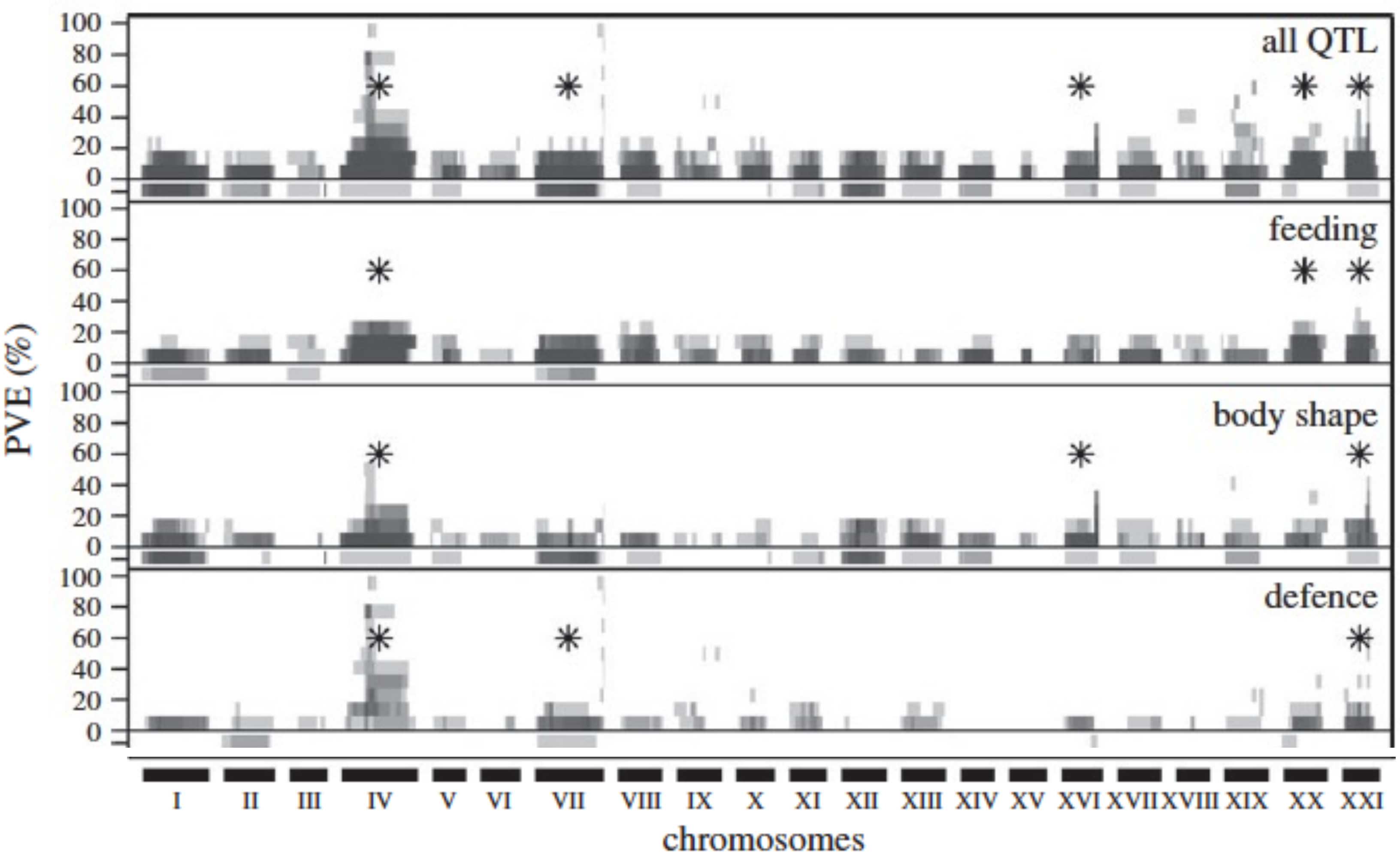


Are QTL clustered in the genome?

- 28 quantitative trait locus (QTL) mapping studies
- 1034 QTL identified in 9 trait categories
    - Morphology
        - Feeding, defense, body shape
        - Swimming, pigment, body size, respiration
    - Reproduction
    - Behaviour

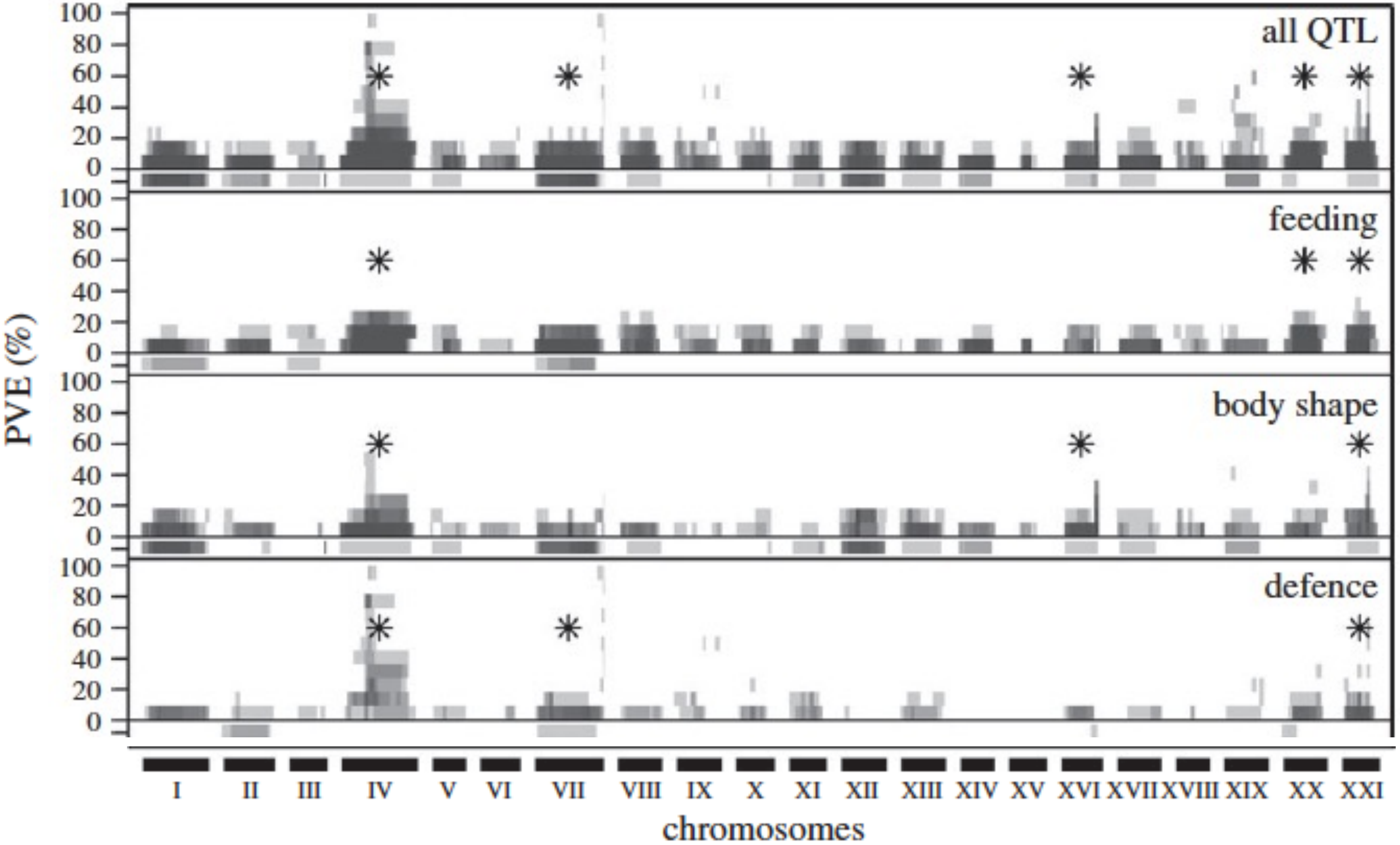

David Marques

QTL are clustered in the genome!



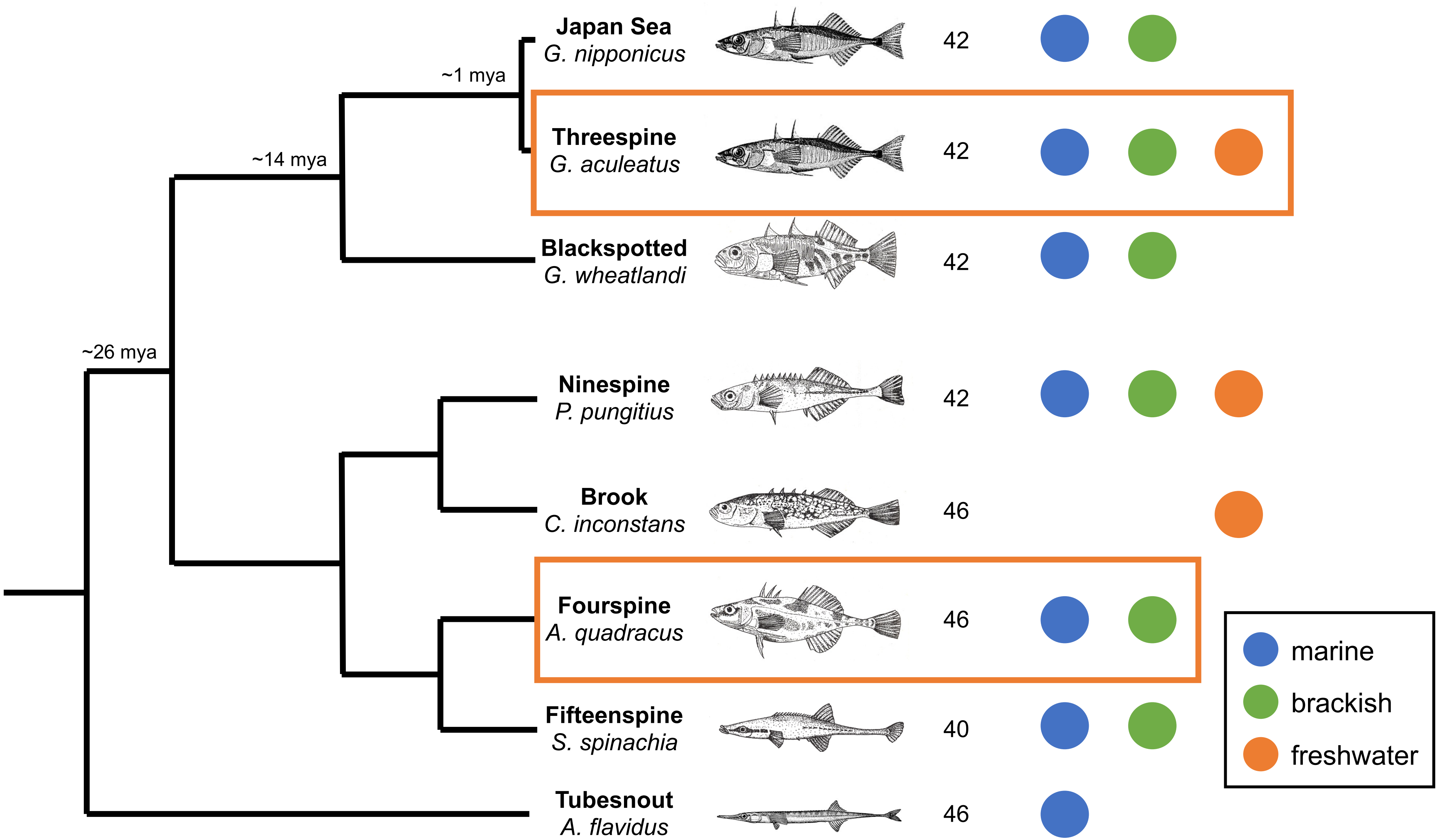
Peichel & Marques 2017 *Philosophical Transactions of the Royal Society London B*

Are these QTL clusters associated with chromosomal rearrangements?



Peichel & Marques 2017 *Philosophical Transactions of the Royal Society London B*

Stickleback family of fish (Gasterosteidae)



~1 mya

~14 mya

~26 mya

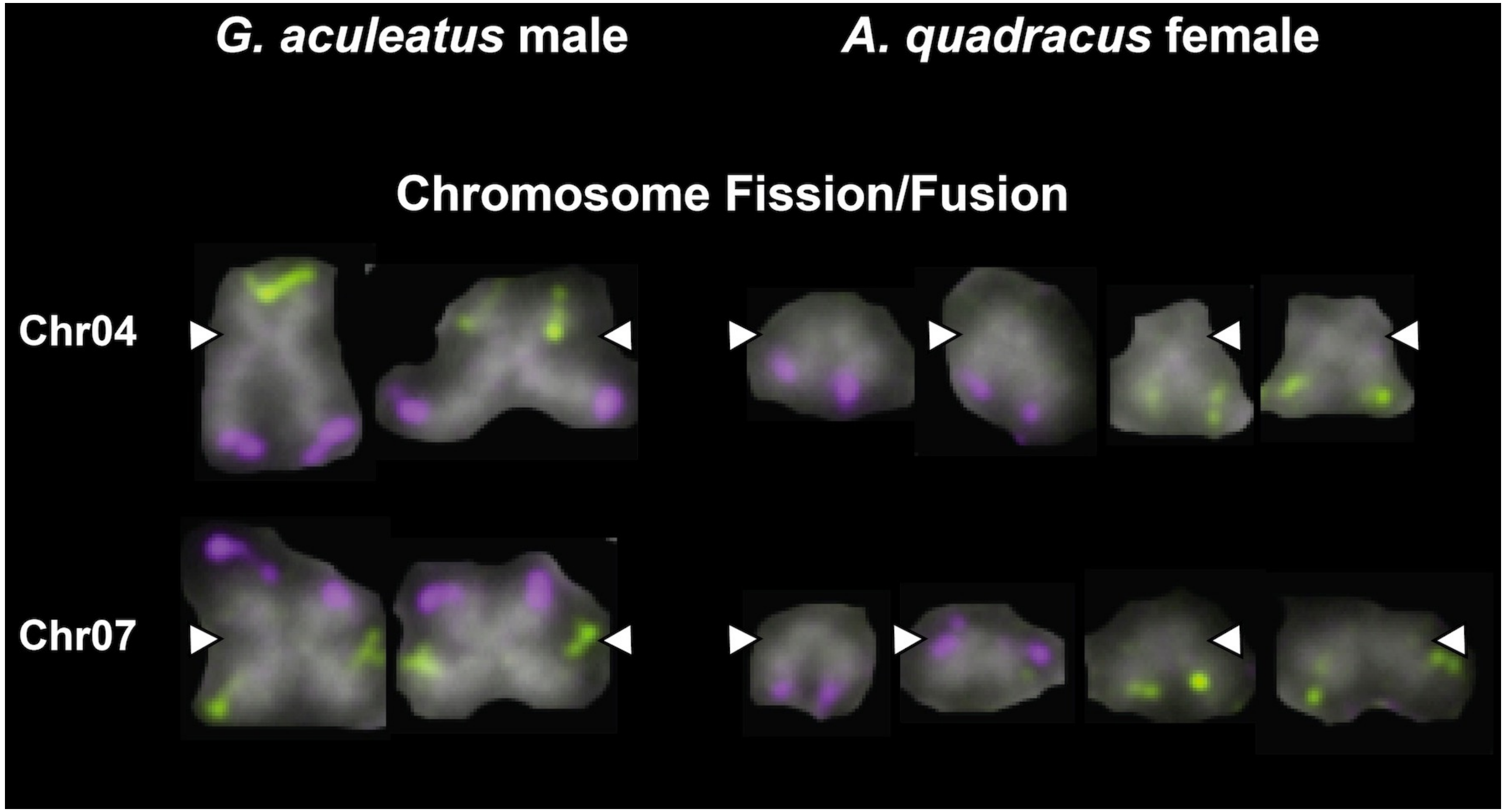
Japan Sea *G. nipponicus*	42	🔵	🟢	
Threespine *G. aculeatus*	42	🔵	🟢	🟠
Blackspotted *G. wheatlandi*	42	🔵	🟢	
Ninespine *P. pungitius*	42	🔵	🟢	🟠
Brook *C. inconstans*	46			🟠
Fourspine *A. quadracus*	46	🔵	🟢	
Fifteenspine *S. spinachia*	40	🔵	🟢	
Tubesnout *A. flavidus*	46	🔵		

🔵 marine

🟢 brackish

🟠 freshwater

Chromosomes 4 and 7 are rearranged



Urton *et al* 2011 *Cytogenetic and Genome Research*

Did chromosomal fusions facilitate local adaptation in threespine stickleback?

LOCAL ADAPTATION AND THE EVOLUTION OF CHROMOSOME FUSIONS

Rafael F. Guerrero[1,2] and Mark Kirkpatrick[1]

Fusions are proposed to facilitate adaptation by:

1. Bringing together previously unlinked adaptive alleles
2. Creating a region of reduced recombination where adaptive alleles can accumulate

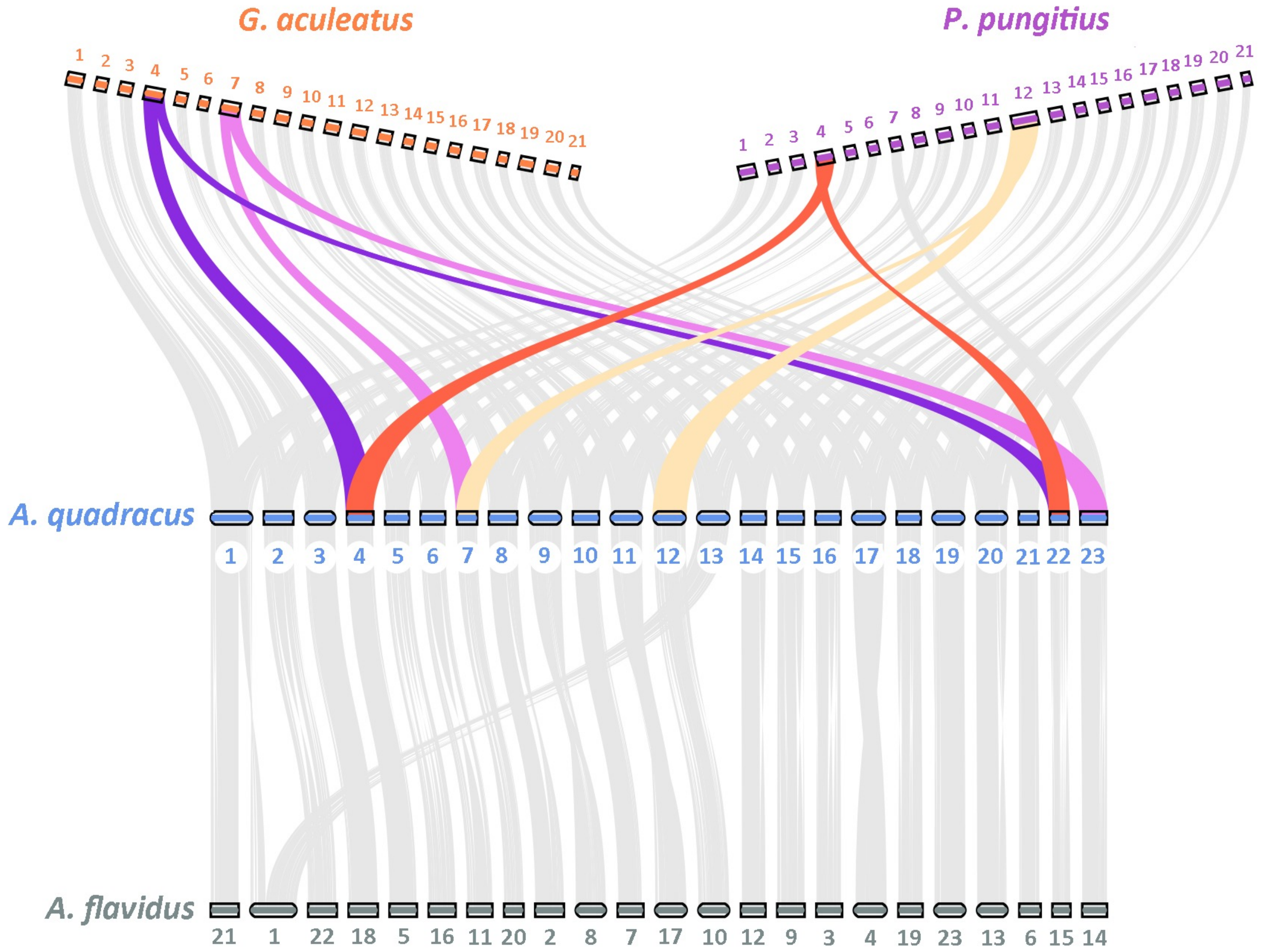
Zuyao Liu

Did chromosomal fusions facilitate local adaptation in threespine stickleback?

1) Is the difference in chromosome number between threespine stickleback and fourspine stickleback due to chromosomal fusion in threespine or chromosomal fission in fourspine?
   - Built a high-quality genome assembly of fourspine stickleback based on PacBio and Hi-C data

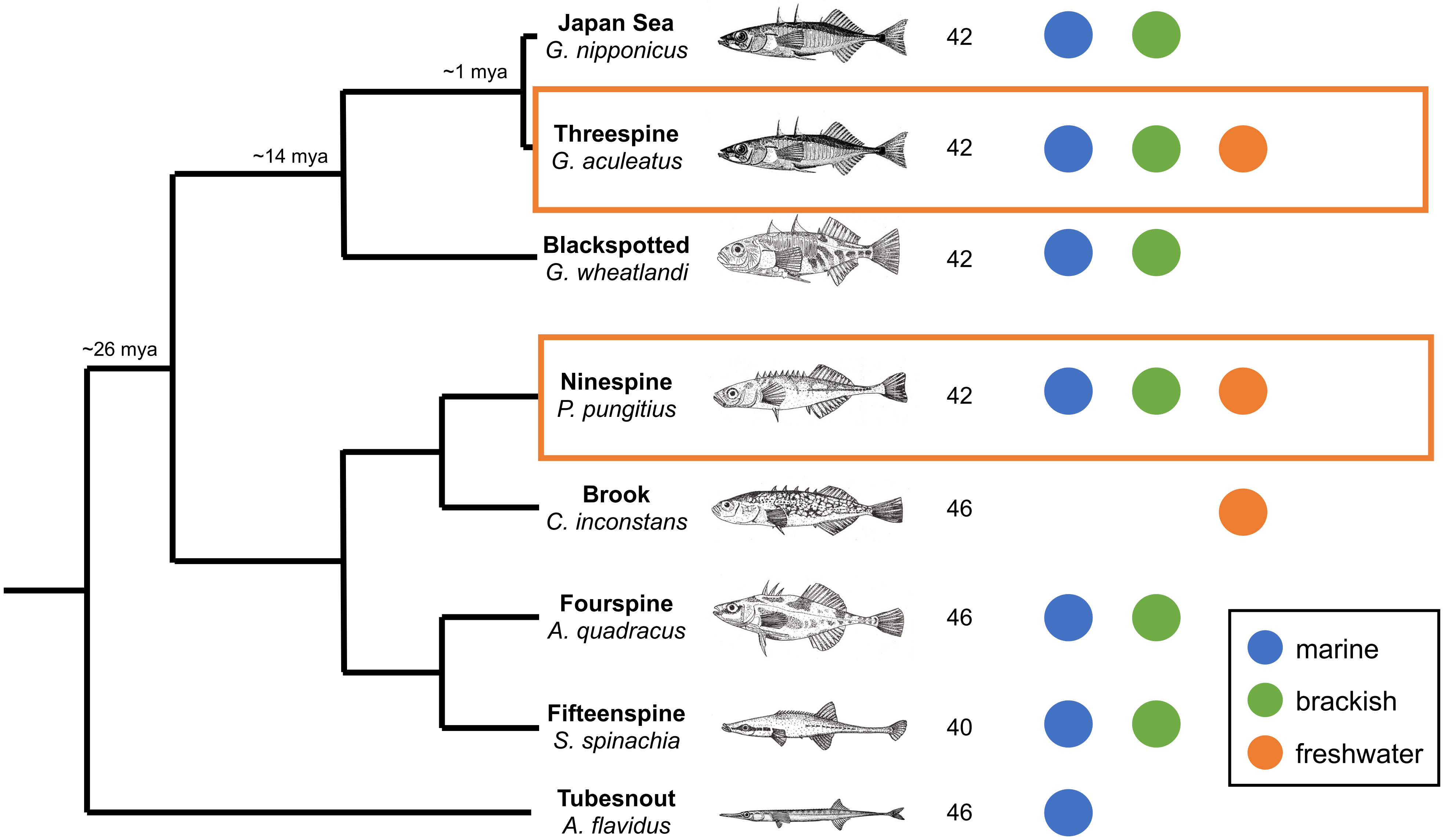
Liu *et al* 2022 *Molecular Biology and Evolution*

Independent fusions of the same chromosomes in threespine and ninespine stickleback



Liu *et al* 2022 *Molecular Biology and Evolution*

Stickleback family of fish (Gasterosteidae)



Did chromosomal fusions facilitate local adaptation in threespine stickleback?

1) Is the difference in chromosome number between threespine stickleback and fourspine stickleback due to chromosomal fusion in threespine or chromosomal fission in fourspine?
    - Fusion!
2) Is there an enrichment of QTL contributing to adaptive divergence in traits on chromosomes 4 and 7 in threespine stickleback?
    - YES!
3) Is there an enrichment of molecular signatures of divergent adaptation on chromosomes 4 and 7 in threespine stickleback?
    - YES!
4) How did chromosomal fusions facilitate adaptation to divergent habitats in threespine stickleback?

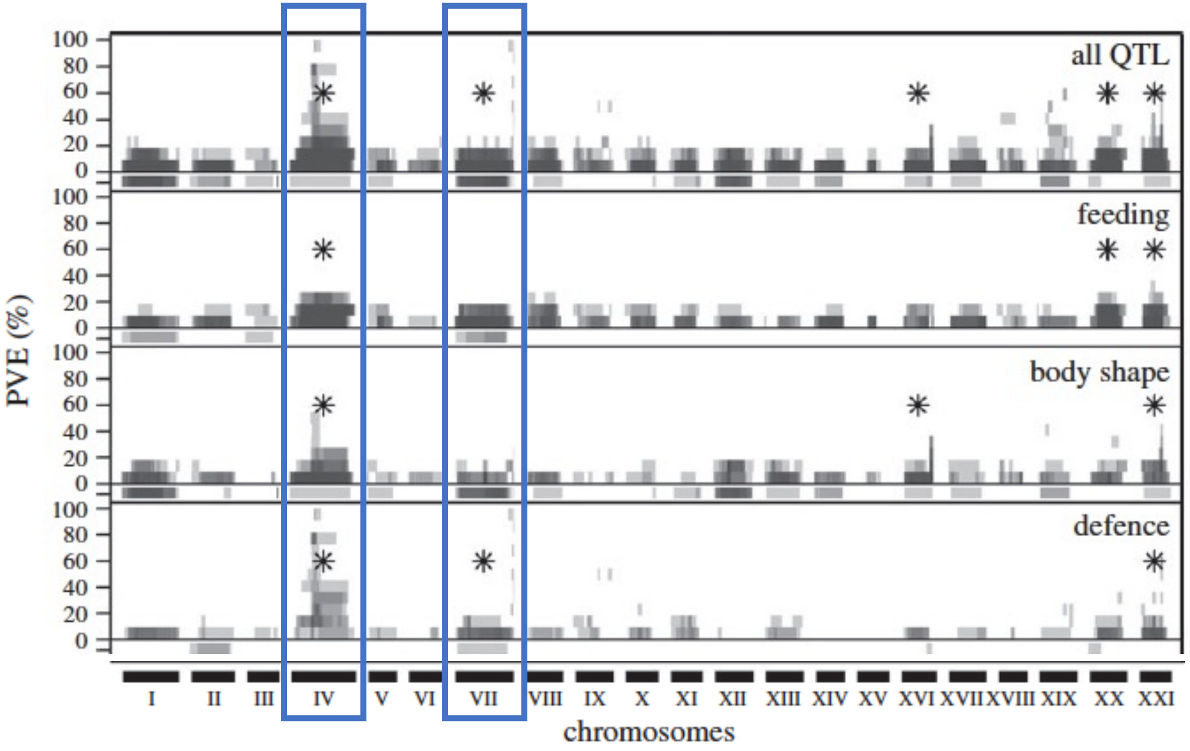
Liu *et al* 2022 *Molecular Biology and Evolution*

How did chromosomal fusions facilitate local adaptation in threespine stickleback?

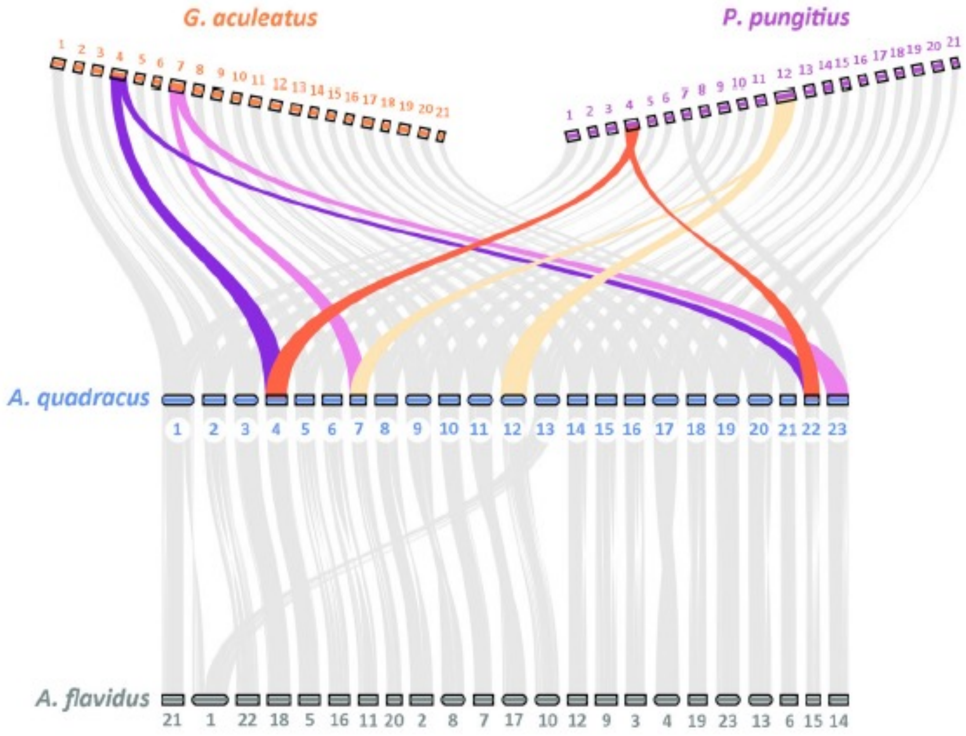
1. Bringing together previously unlinked adaptive alleles?
    - Probably not but difficult to test because the fusions are fixed in Gasterosteus genus, but only threespine stickleback can inhabit freshwater
2. Creating a region of reduced recombination where adaptive alleles can accumulate?
    - Probably!

Liu *et al* 2022 *Molecular Biology and Evolution*

Linked QTL clusters are associated with chromosomal **fusions** and inversions



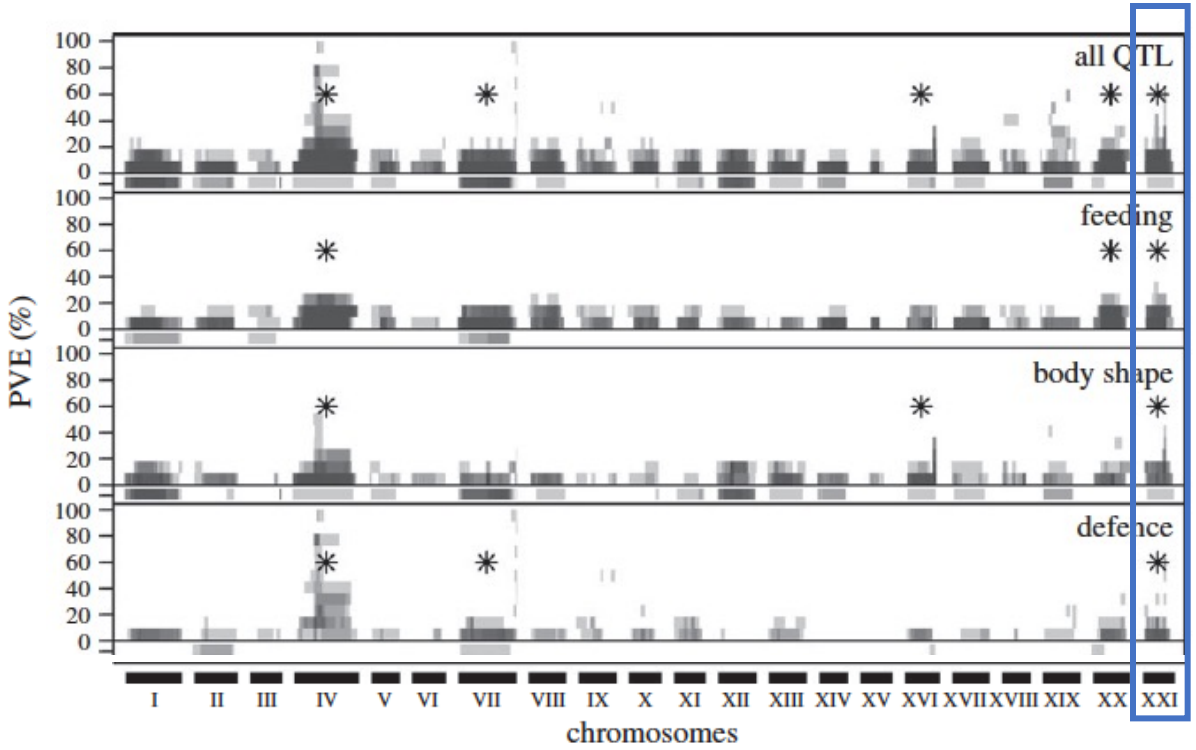
Fusions on chromosomes 4 and 7



Liu *et al* 2022 *Molecular Biology and Evolution*

Linked QTL clusters are associated with chromosomal fusions and **inversions**

Inversions on chromosomes 1, 11 & 21



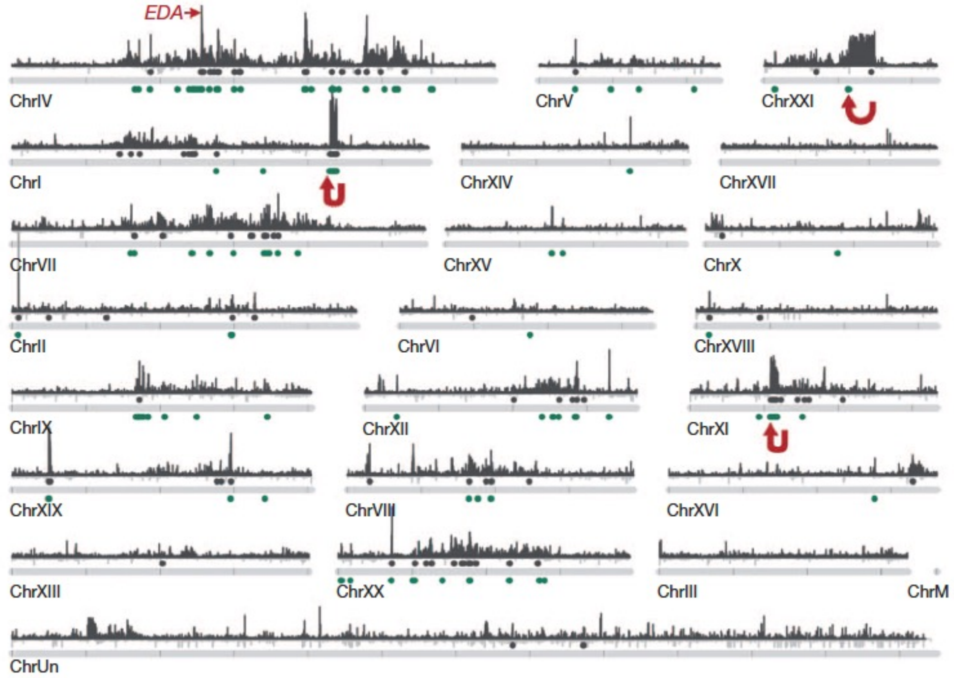
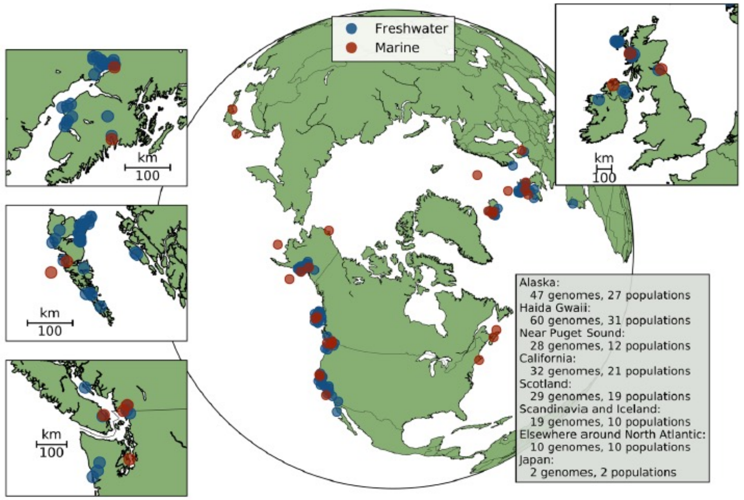


Figure from Jones *et al* 2012 *Nature*

Three inversions distinguish global marine and freshwater threespine stickleback populations



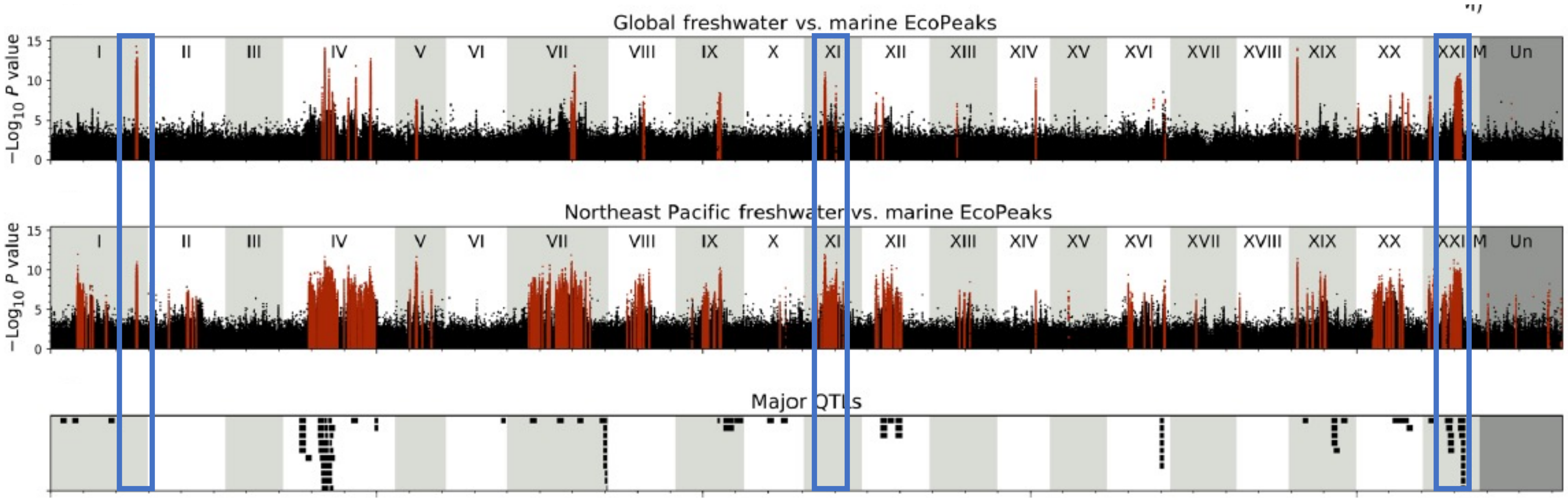


Figure from Roberts Kingman *et al* 2021 *Science Advances*

How do these inversions contribute to local adaptation?

Chr	Size in Mbp	Number of genes	Gene at breakpoint	QTL hotspot	TMRCA	Ancestral orientation
1	0.5	24	no	no	7 mya	marine
11	0.46	25	yes	no	6 mya	freshwater
21	2.2	109	no	yes	8 mya	freshwater

Swiss National Science Foundation

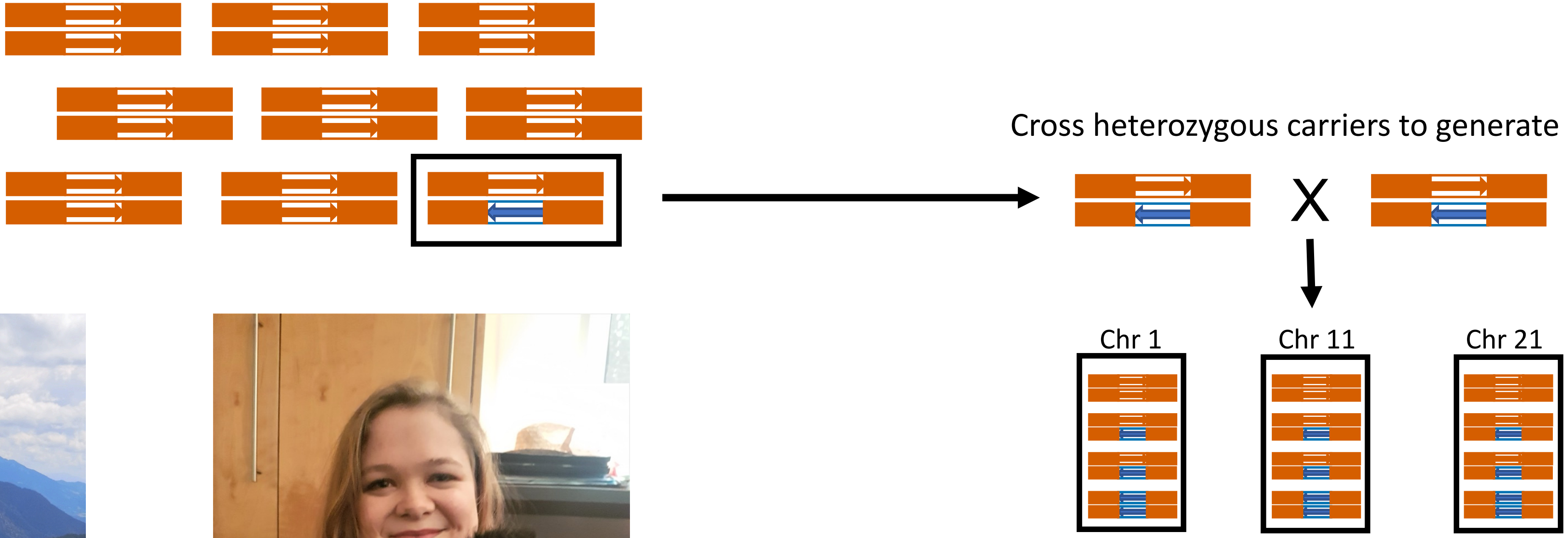
- What are the phenotypic effects of these inversions?
- Are these inversions under selection in freshwater?
- Can we identify the targets of selection within these inversions?

What are the phenotypic effects of inversions?

- Association mapping in wild populations that are polymorphic for the inversions
    - Freshwater sticklebacks from Lake Constance, Switzerland
    - Extensive phenotyping of morphology, physiology, and behavior
- Genetic mapping in crosses between marine inversion heterozygotes
    - Marine sticklebacks from British Columbia, Canada
    - Extensive phenotyping of morphology, physiology, and behavior

Genetic mapping in crosses between marine inversion heterozygotes

Screen marine fish to find heterozygous carriers of 3 freshwater inversions (2022)


Cross heterozygous carriers to generate F1s

F1 family
x 6 families

F1 family
x 6 families

F1 family
x 7 families

Ship 21 F1 families to Bern

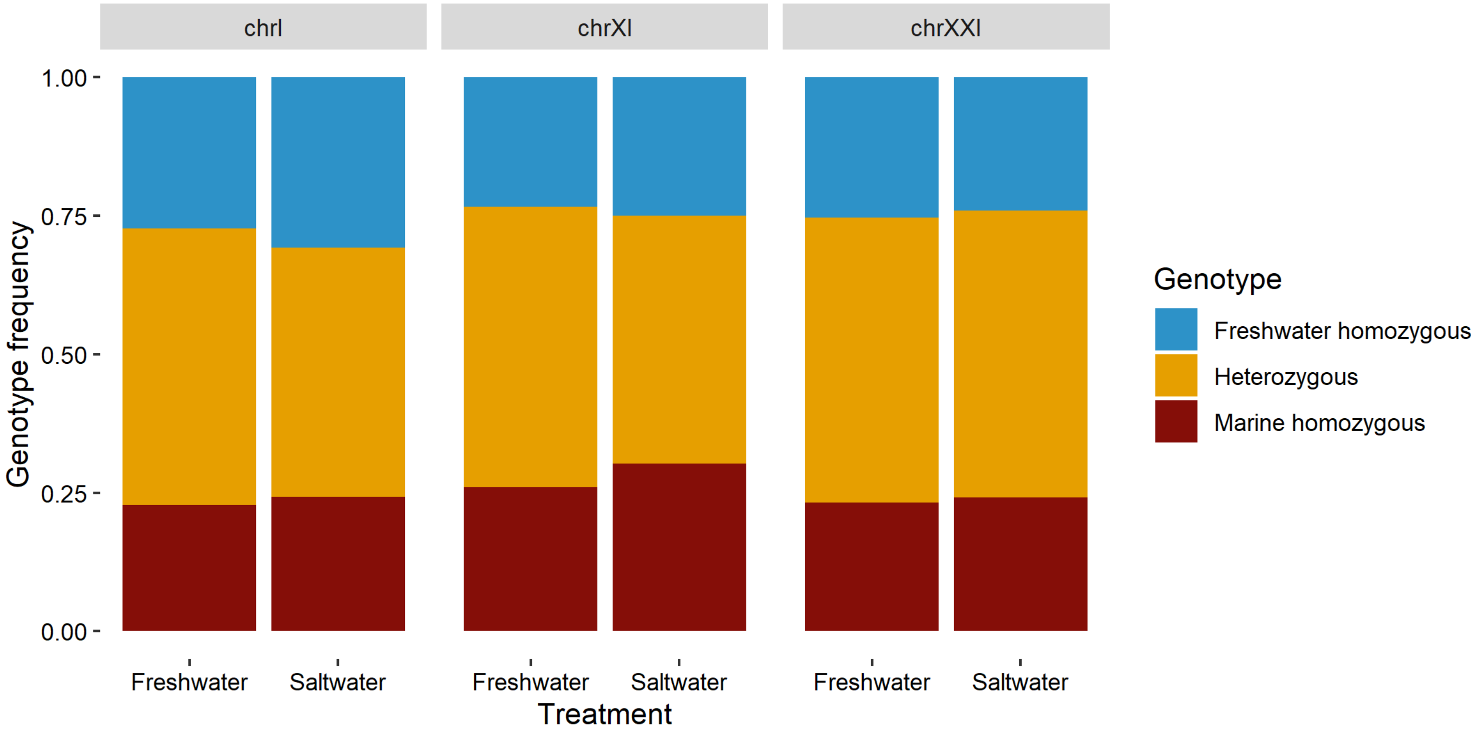
Grow half the family in saltwater and half in freshwater

Genotype and phenotype


Juliana Rodriguez Fuentes

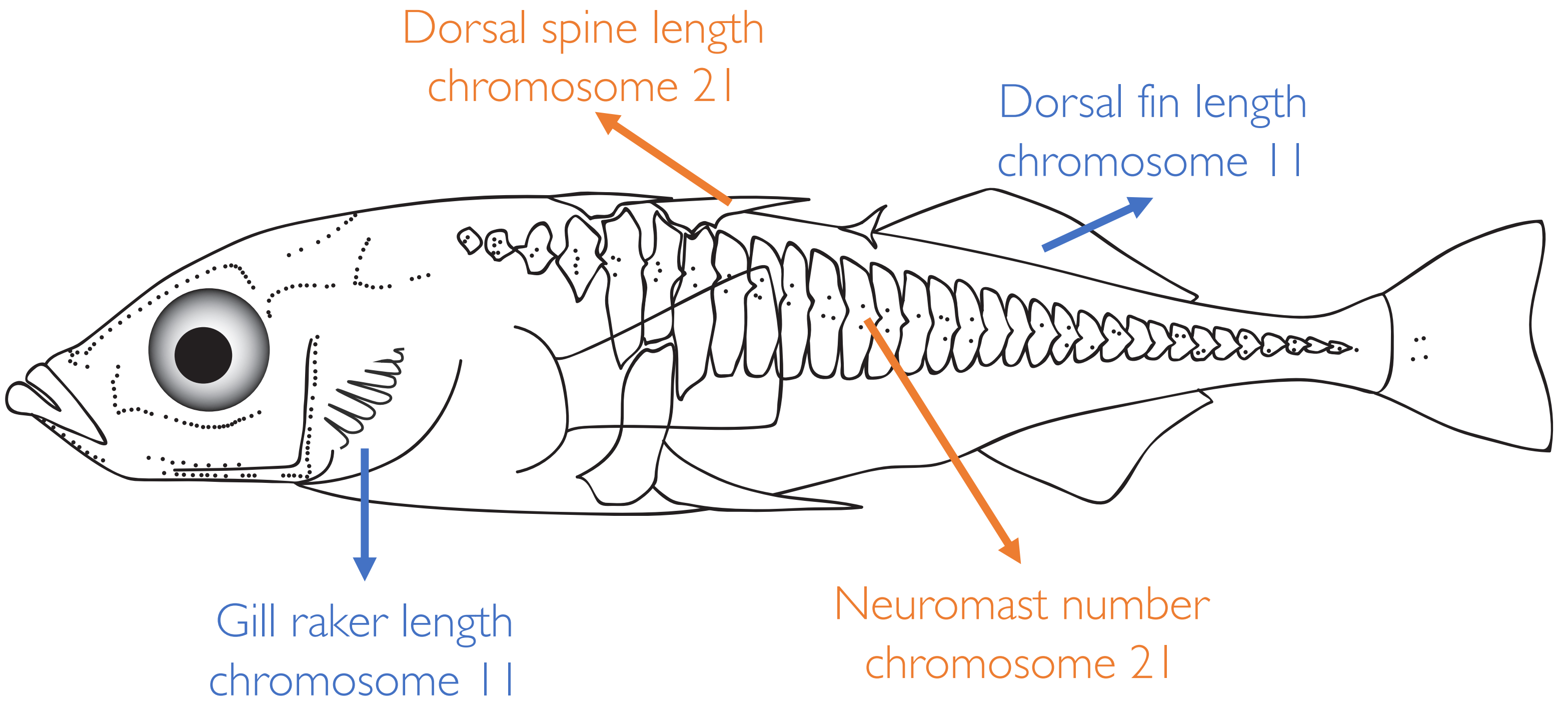

Nicole Nesvadba

No intrinsic lethality associated with inversion genotype in saltwater or freshwater



Juliana Rodriguez Fuentes

Preliminary results: multiple morphological traits map to chromosome 11 and 21 inversions



Dorsal spine length
chromosome 21

Dorsal fin length
chromosome 11

Gill raker length
chromosome 11

Neuromast number
chromosome 21

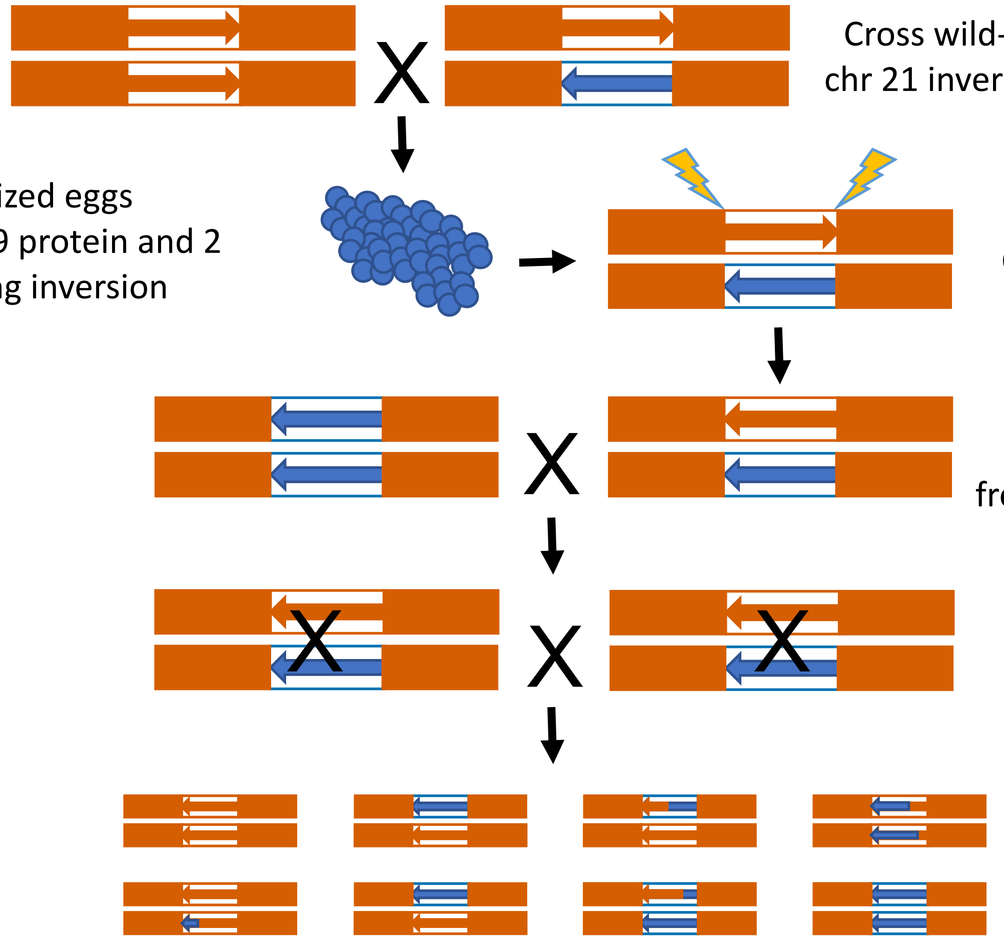
Juliana Rodriguez Fuentes

Do multiple traits map to the inversions because there are linked adaptive alleles?

- Problem: we cannot do fine mapping within inversions because there is no recombination between the marine and freshwater inversions in heterozygotes!

- Solution: "flip" the freshwater inversion to (hopefully) restore recombination in marine-freshwater heterozygotes!

We flipped the inversion on chromosome 21!



Cross wild-type marine with chr 21 inversion heterozygotes

Inject fertilized eggs with CRISPR/Cas9 protein and 2 gRNAs flanking inversion

Create mosaic F0

Cross mosaic F0 to marines with freshwater inversion

Intercross heterozygous F1s

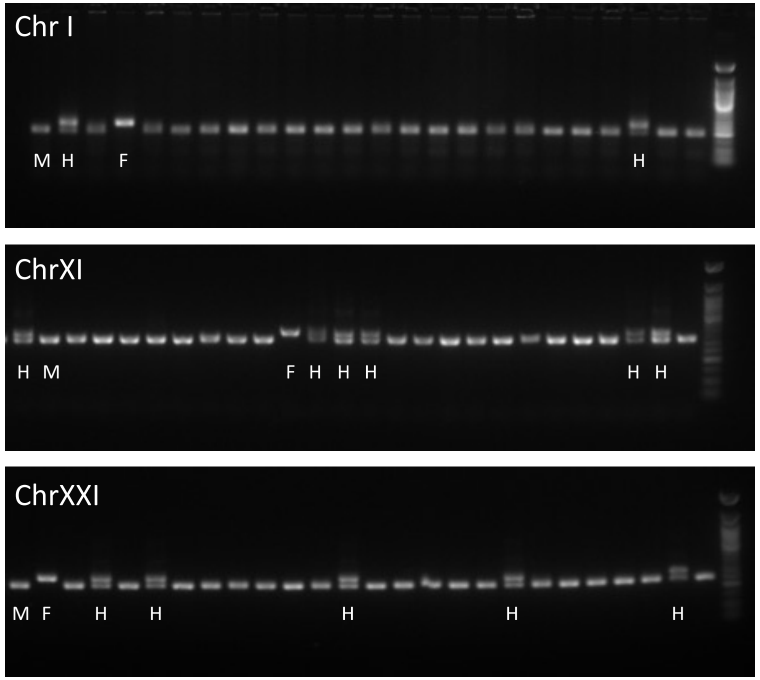
We are here!

Genotype and phenotype F2s

Juliana Rodriguez Fuentes & Nicole Nesvadba

What are the phenotypic effects of inversions?

- Worldwide association mapping project
  - Inversion frequencies: PCR genotyping of inversions in ~600 populations from across the entire stickleback distribution (~20,000 samples)
  - Phenotype data (population averages)
  - Ecological data (biotic/abiotic variables)




Delia Sclabas


Marius Roesti

How do these inversions contribute to local adaptation?

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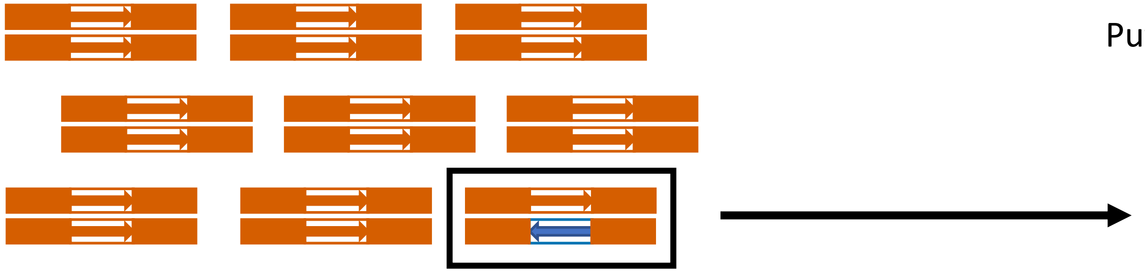
Swiss National Science Foundation

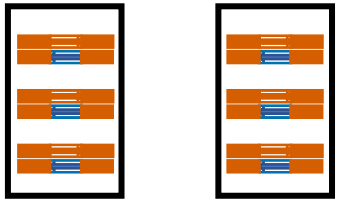
- What are the phenotypic effects of these inversions?
- **Are these inversions under selection in freshwater?**
- Can we identify the targets of selection within these inversions?

Are the inversions under selection in freshwater?

Screen marine fish to find heterozygous carriers of 3 freshwater inversions (2022)



Put heterozygotes in UBC ponds



~35 hets x3 ponds

~35 hets x3 ponds

Sample F1s spring 2023

Sample F2s spring 2024

Sample F3s in spring 2025

Lethally sample F4s spring 2026

Preliminary results: increase in frequency of freshwater allele at chromosome 11 inversion!

chr	pond	year	M	HET	FW	Total fish	P-value
11	2	2023	28	57	21	106	NS
11	2	2024	23	78	58	159	0.0004
11	7	2023	0	0	0	0	ND
11	7	2024	1	2	4	7	ND
11	14	2023	26	65	32	123	NS
11	14	2024	2	27	23	52	0.0002

How do these inversions contribute to local adaptation?

Chr	Size in Mbp	Number of genes	Gene at breakpoint	QTL hotspot	TMRCA	Ancestral orientation
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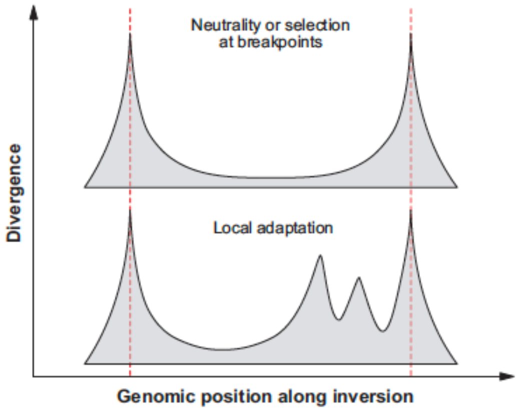
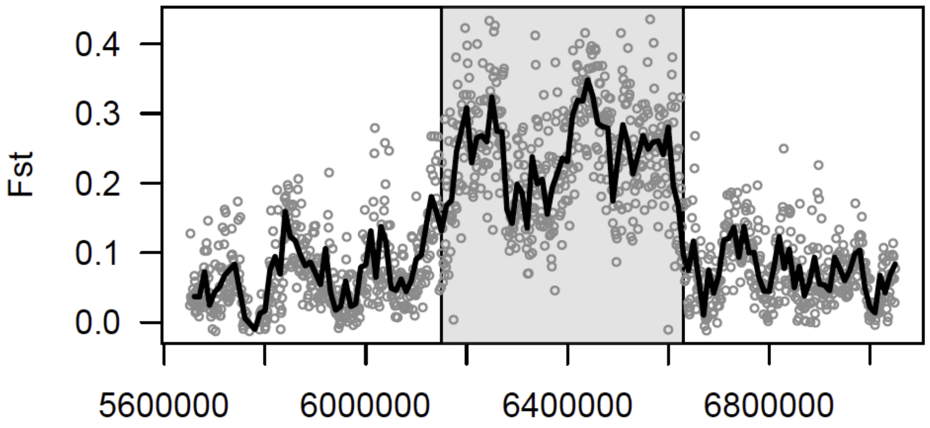
Swiss National Science Foundation

- What are the phenotypic effects of these inversions?
- Are these inversions under selection in freshwater?
- Can we identify the targets of selection within these inversions?

What are the molecular signatures of selection within inversions?

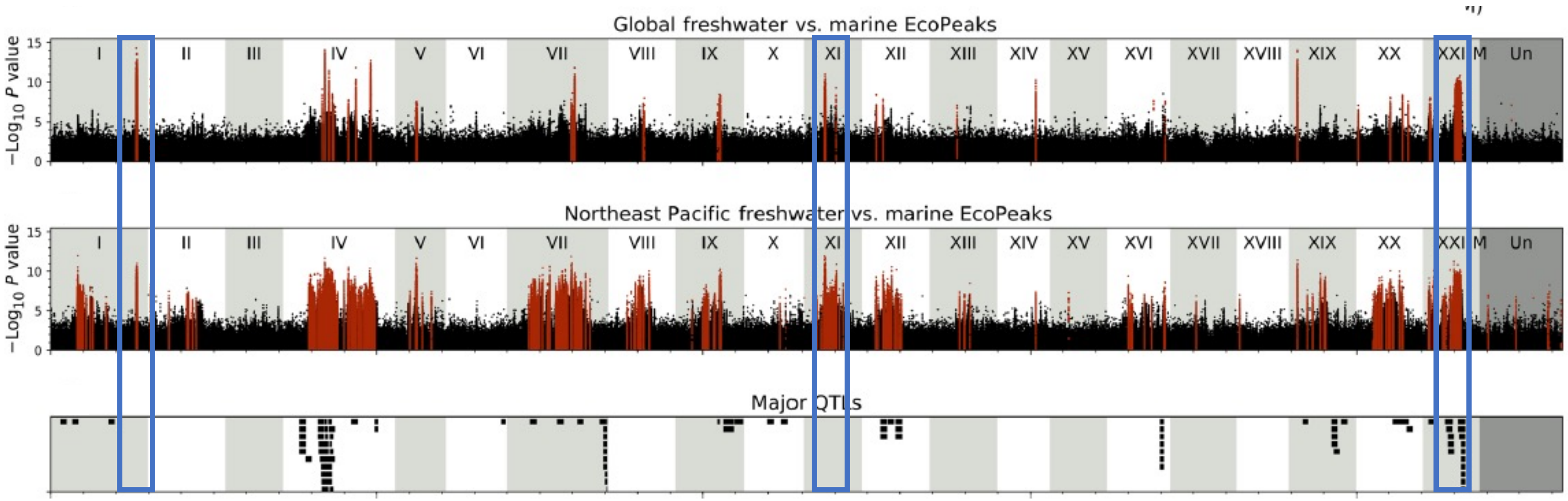
- Generate phased sequencing data (Swiss and Canadian populations)
  - Question 1. What form(s) of selection are acting on the inversions?
  - Question 2. Is there evidence for selection on multiple loci within the inversion or only on the inversion itself?
  - Question 3. Did selection for linkage of adaptive alleles contribute to the establishment of the inversion, or did adaptive alleles accumulate after the establishment of the inversions? (i.e. capture vs gain?)

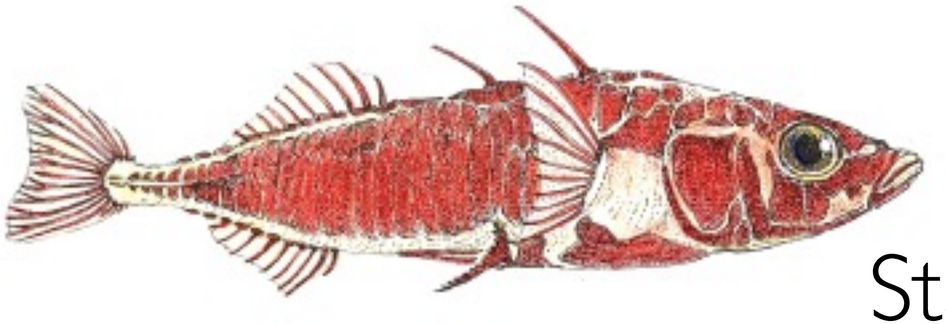





Stephan Peischl

How do these inversions contribute to local adaptation?





Stay tuned!

Do chromosomal changes facilitate local adaptation?

- Are chromosomal changes, such as inversions or fusions, under divergent selection in nature?
  - Preliminary results say yes (for chromosome 11)

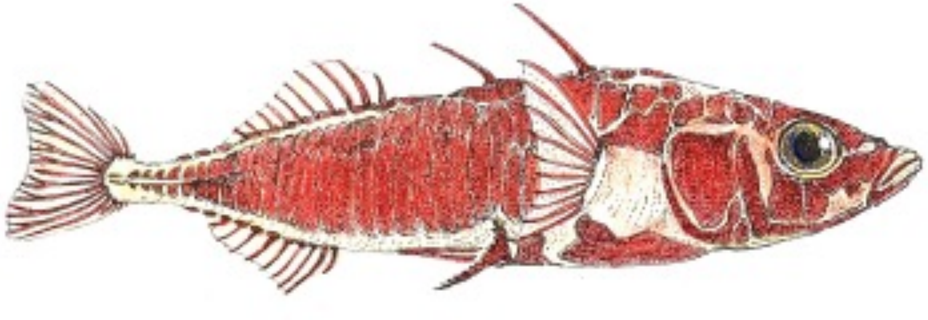
- Are multiple adaptive traits linked to chromosomal inversions or fusions?
  - Preliminary results say yes (for chromosomes 11 and 21)

- Do chromosomal inversions or fusions harbor multiple adaptive alleles?
  - Flipped inversions will help us find out!

Chromosomal changes as drivers of adaptation and speciation?

- I think so!

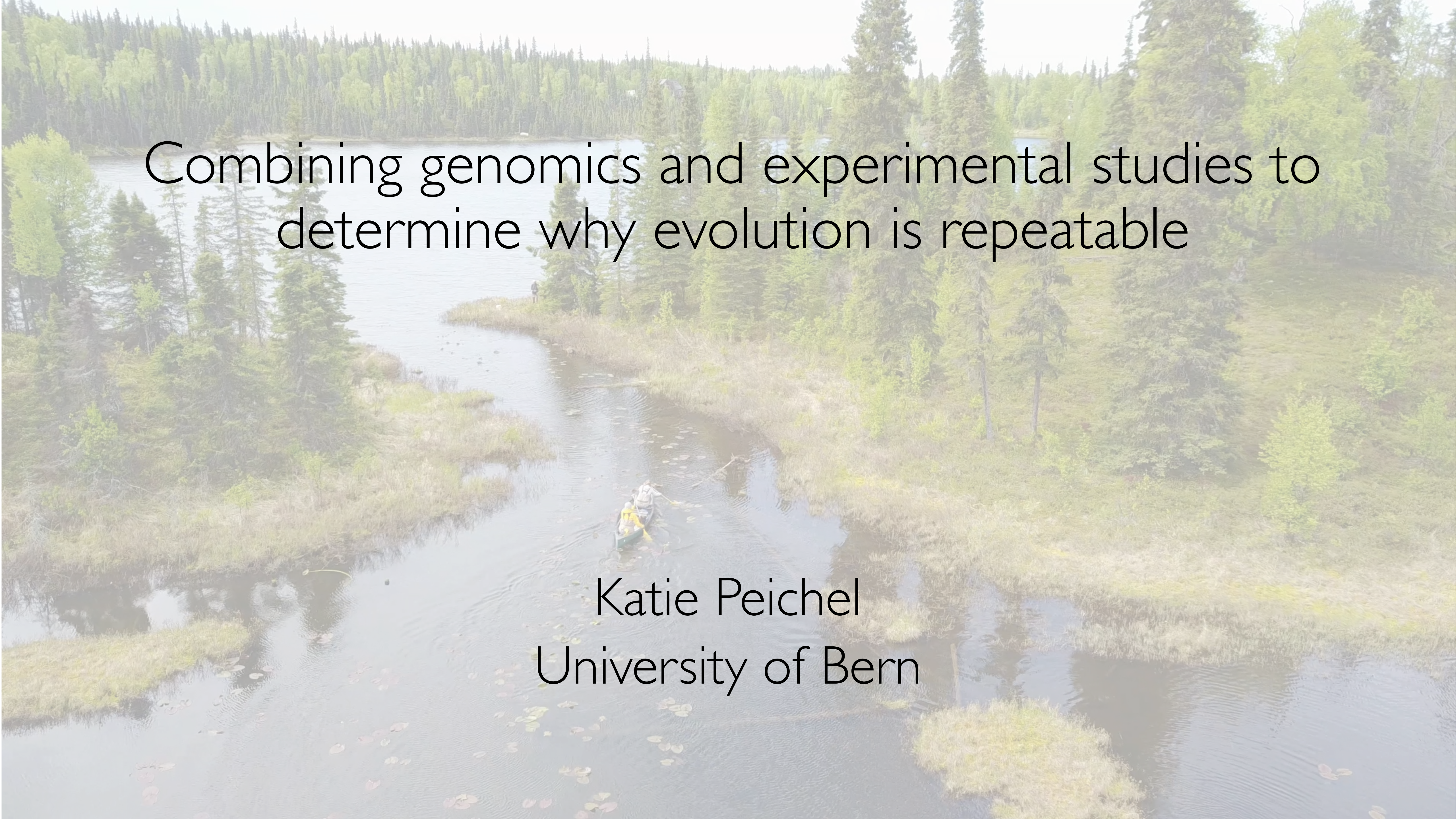
- But, an integration of lab and field studies are really needed to directly link these chromosomal changes to adaptation and speciation!



Thanks!

Zuyao Liu
Nicole Nesvadba
Marius Roesti
Juliana Rodriguez Fuentes
Delia Sclabas

David Marques
Stephan Peischl
Dolph Schluter
Stickleback community
Uni Bern sequencing center



Combining genomics and experimental studies to determine why evolution is repeatable

Katie Peichel
University of Bern

Is evolution repeatable?

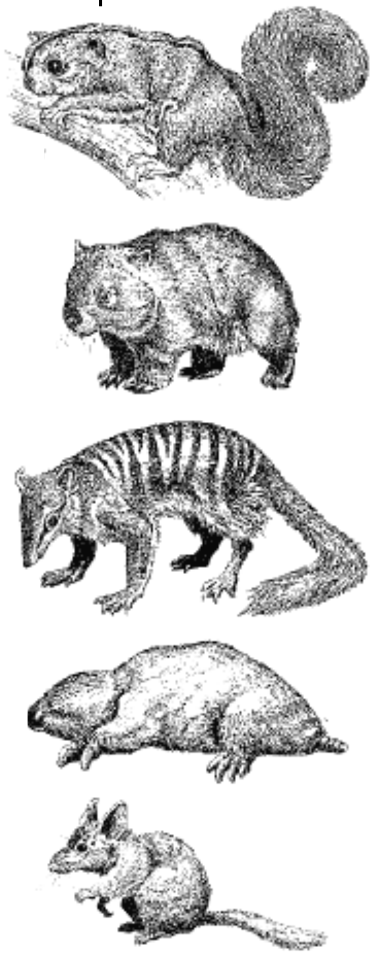
If we replayed the tape of life, would evolution repeat itself?

Gould (1989) *Wonderful Life*

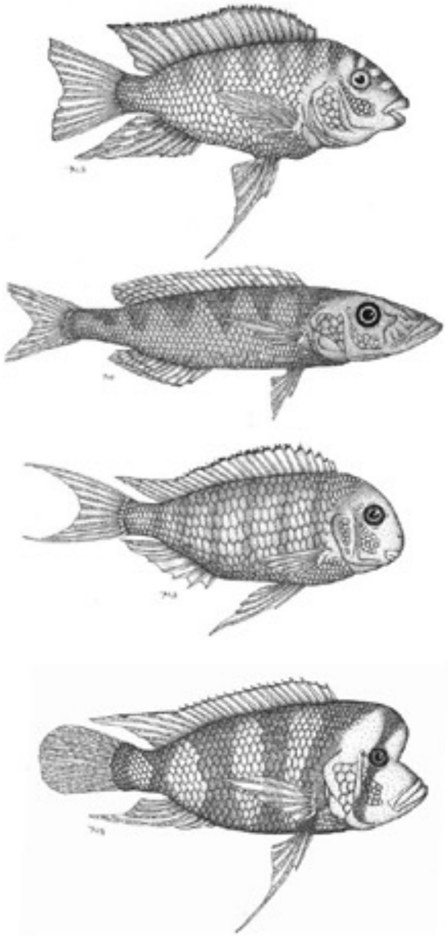
Evolution repeats itself!

Placentals

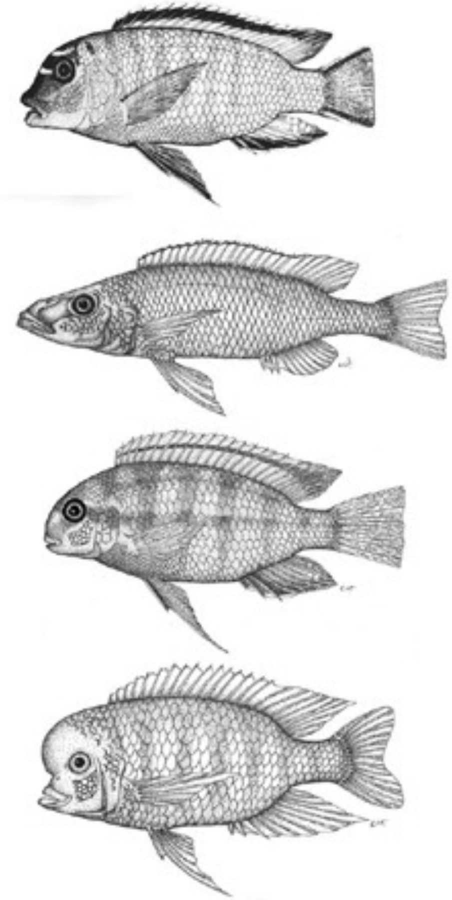
Marsupials



Lake Tanganyika

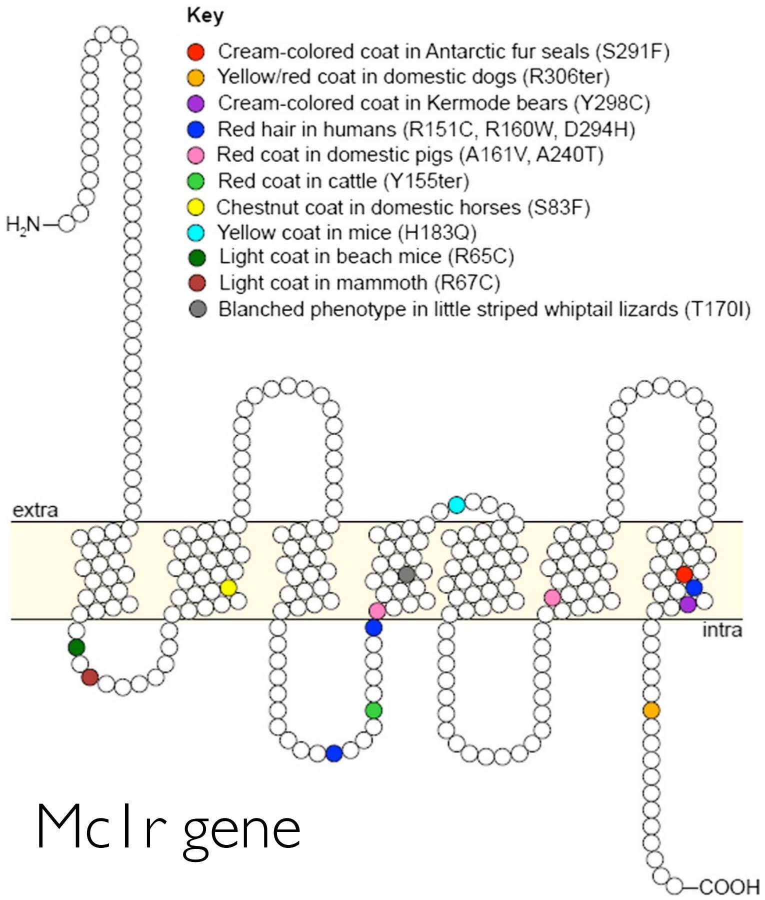


Lake Malawi



Evolution also repeats itself at the genetic level!

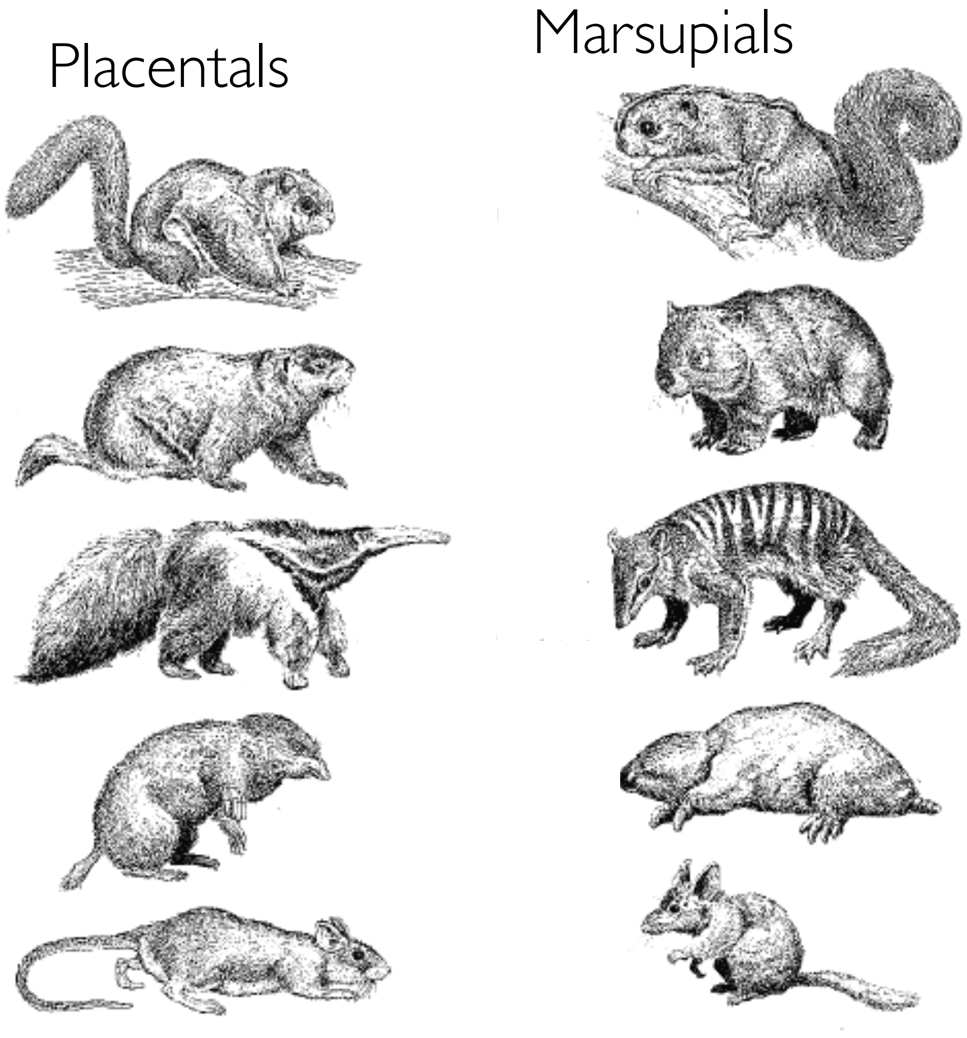
Hoekstra (2006) *Heredity*



Mc1r gene

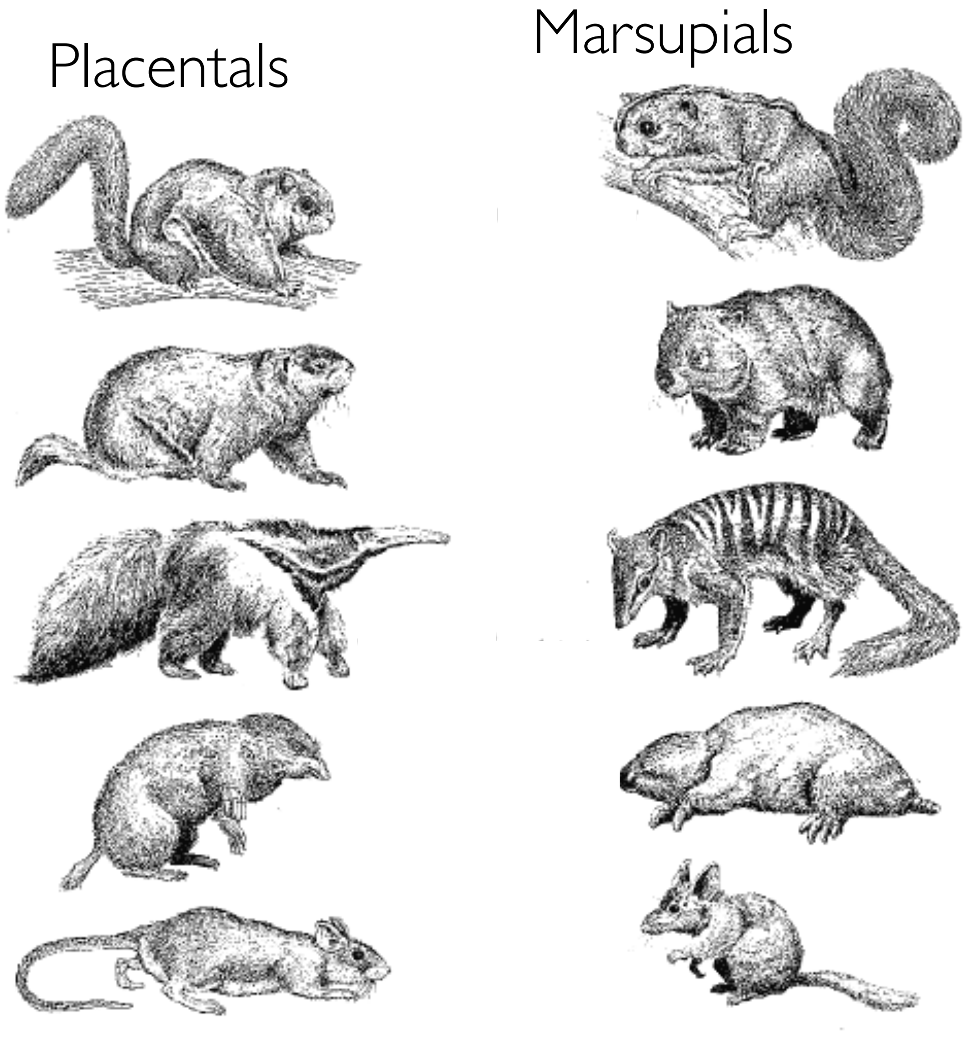
Peters et al (2016) *Ecology & Evolution*

Why is evolution repeatable?



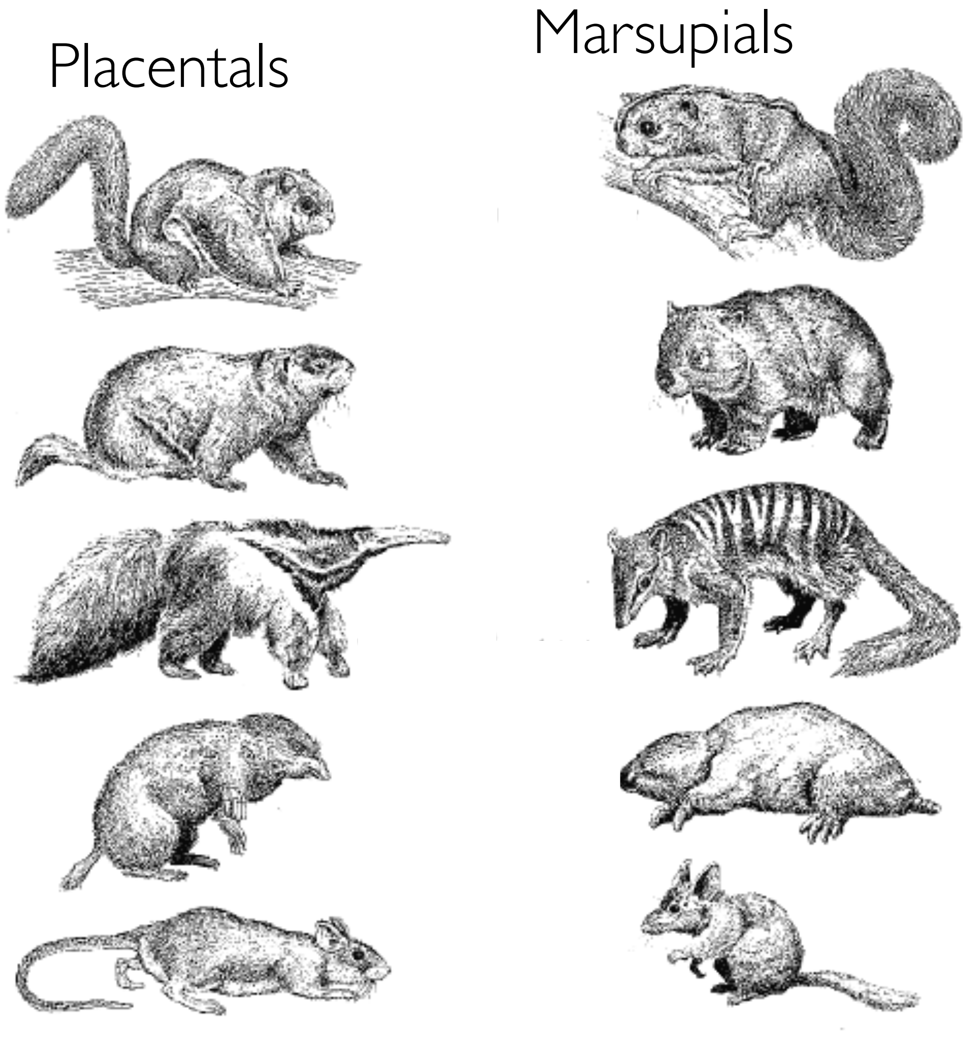
If we can understand why evolution repeats itself (or why it doesn't), maybe we can predict evolutionary responses in the future

Is evolution predictable?



- Medicine
  - When and how will a virus evolve to escape a vaccine?
- Agriculture
  - When and how will a pest evolve to resist a pesticide?
- Conservation
  - Will a species survive as a result of climate change?

Why is evolution repeatable?



Why is evolution repeatable at the phenotypic level?

Why is evolution repeatable at the genotypic level?

Questions

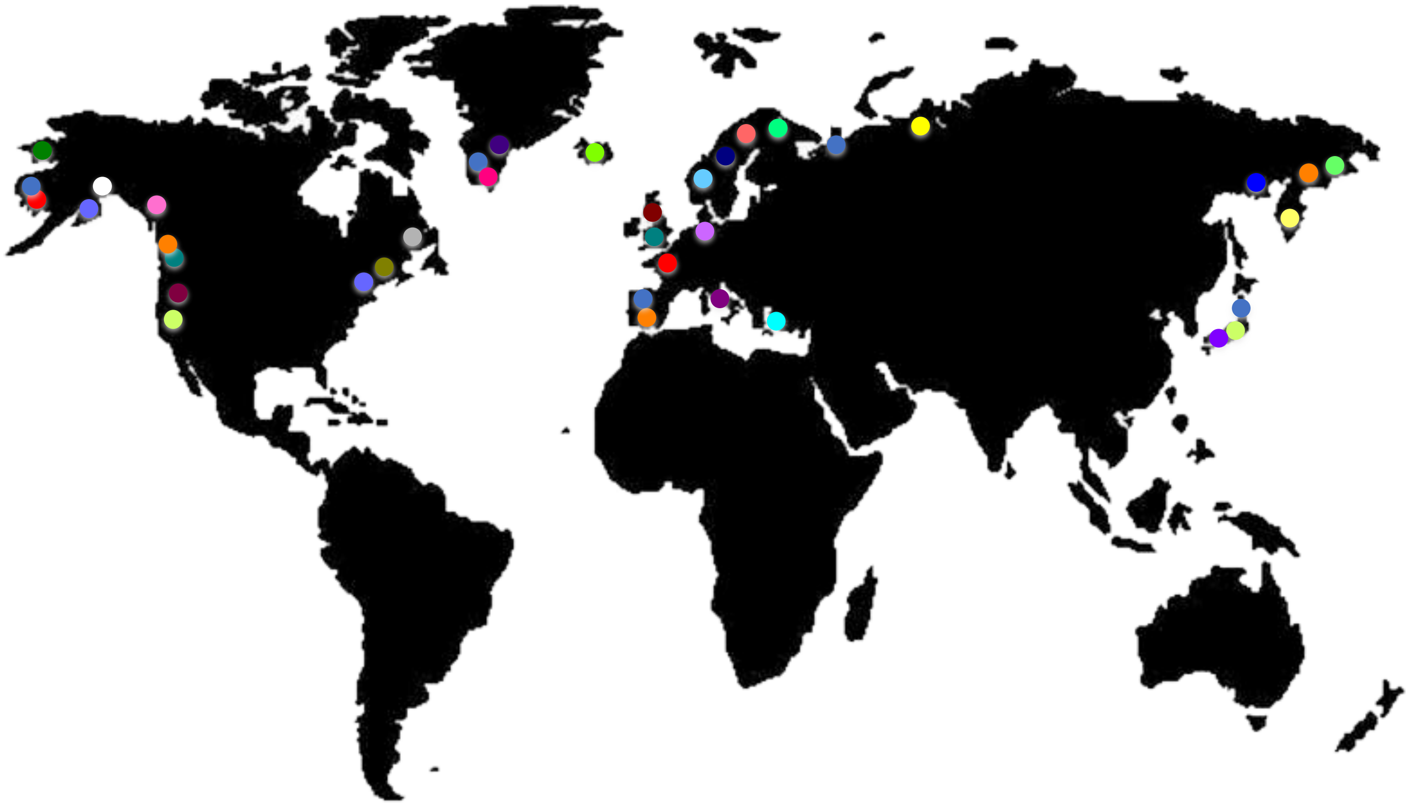
- How often do the same genetic changes underlie repeated phenotypic evolution and adaptation?
- Why might some genetic changes be used more frequently during repeated phenotypic evolution and adaptation?

Stickleback fish are a model system to study phenotypic and genotypic repeatability

- Lives in ocean, lakes, and streams
- Well-studied biology in wild and in lab
- Extensive phenotypic variation
- Genetic resources
  - Peichel et al (2001) *Nature*
- High quality genome assembly
  - Jones et al (2012) *Nature*
  - Peichel et al (2020) *Genome Biology*
- Evolutionary "supermodel"
- Repeated phenotypic evolution

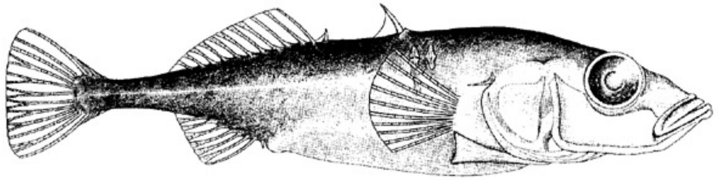
Repeated evolution of freshwater sticklebacks from the marine ancestor

Repeated evolution of freshwater sticklebacks from the marine ancestor

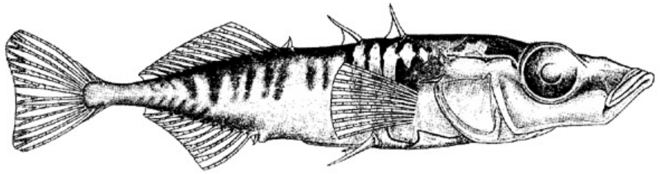


Repeated evolution of benthic and limnetic forms

benthic



limnetic



Repeated evolution of stream and lake forms

stream

lake

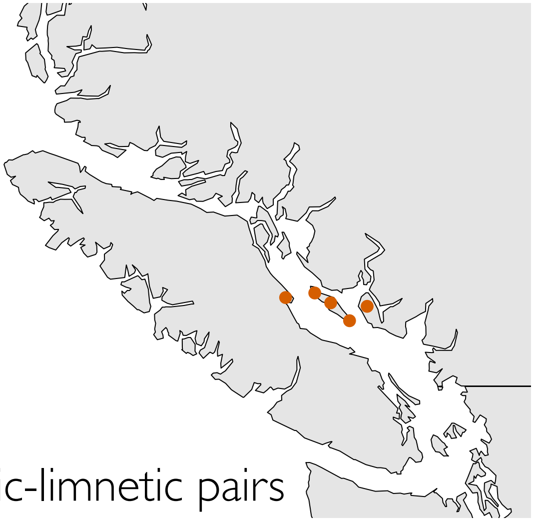
Questions

- How often do the same genetic changes underlie repeated phenotypic evolution and adaptation?
- Why might some genetic changes be used more frequently during repeated phenotypic evolution and adaptation?

Questions

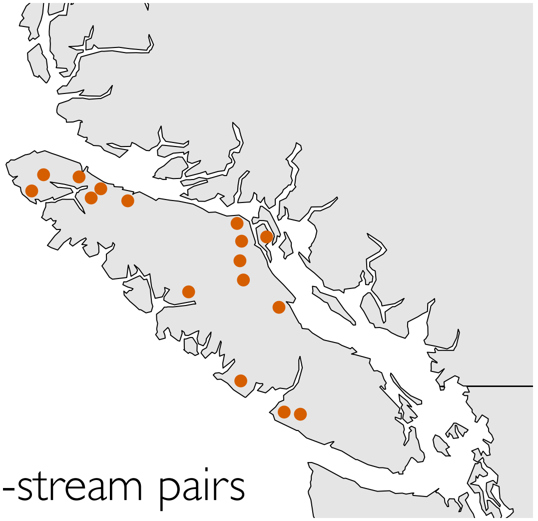
- How often do the same genetic changes underlie repeated phenotypic evolution and adaptation?
  - Forward genetics: QTL mapping
  - Reverse genetics: Population genomics
- Why might some genetic changes be used more frequently during repeated phenotypic evolution and adaptation?

Freshwater stickleback species pairs



Benthic-limnetic pairs

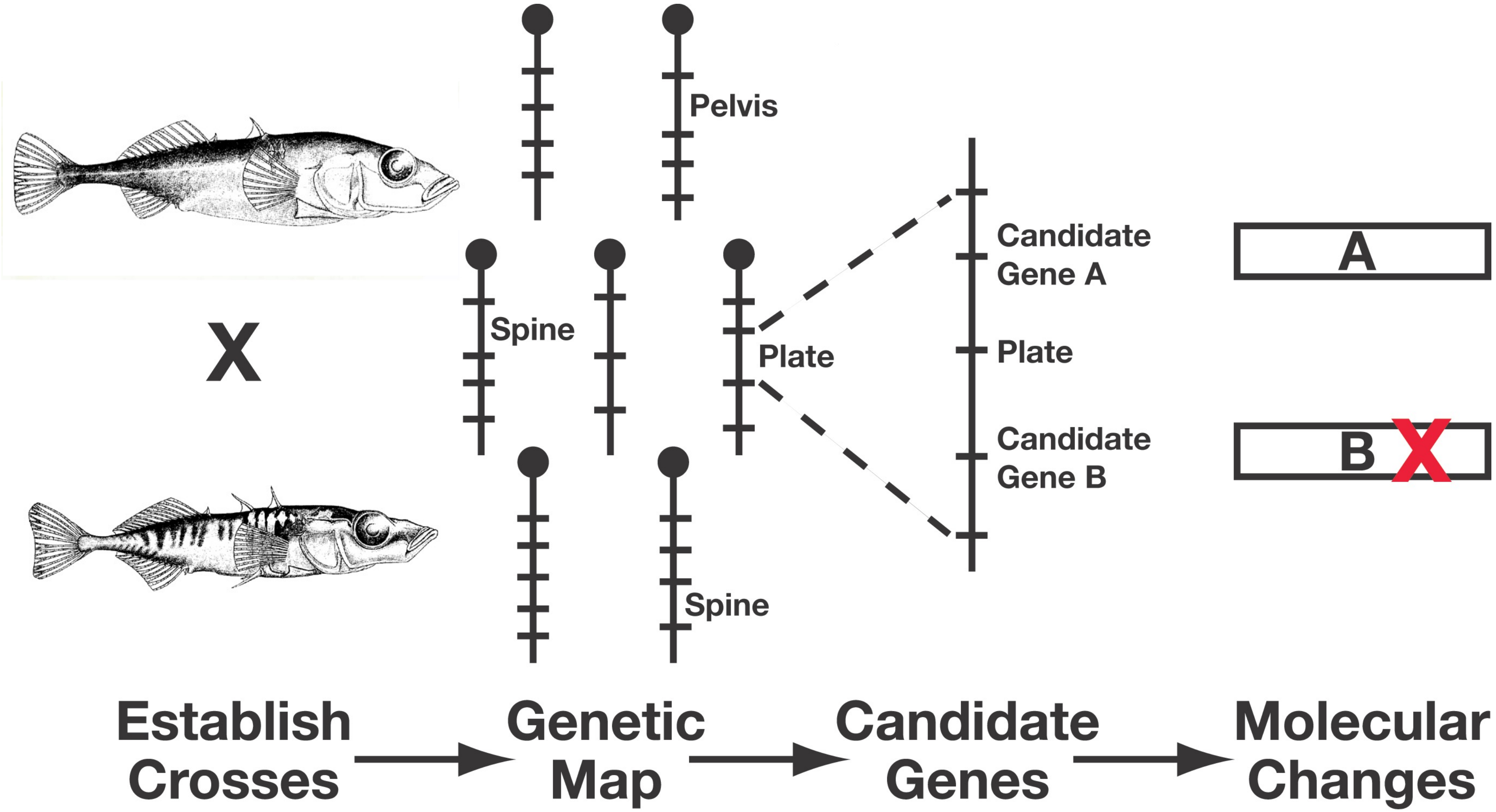
Collaboration with Dolph Schluter



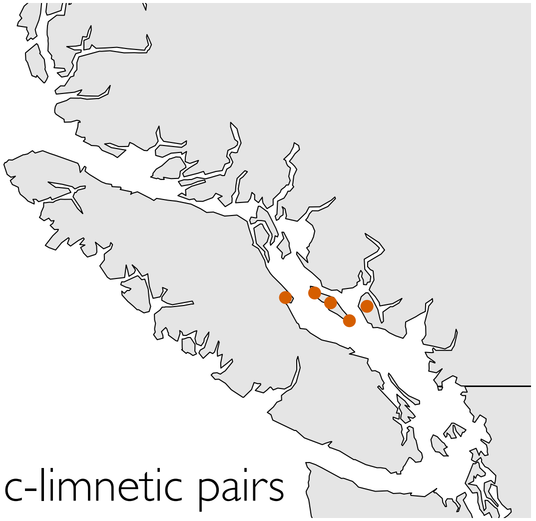
Lake-stream pairs

Collaboration with Yoel Stuart, Dan Bolnick, Andrew Hendry

Quantitative trait locus (QTL) mapping: which genotypes underlie repeated phenotypes?

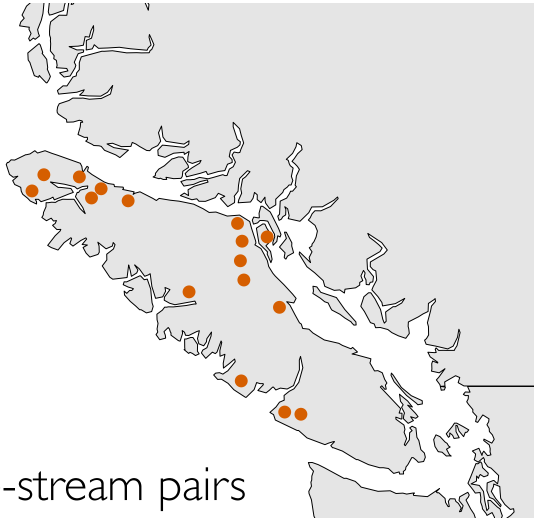


Repeated phenotypic evolution is not always repeated at the genetic level



Benthic-limnetic pairs

50% of QTL are shared between benthic-limnetic pairs
Conte et al (2015) *Genetics*



Lake-stream pairs

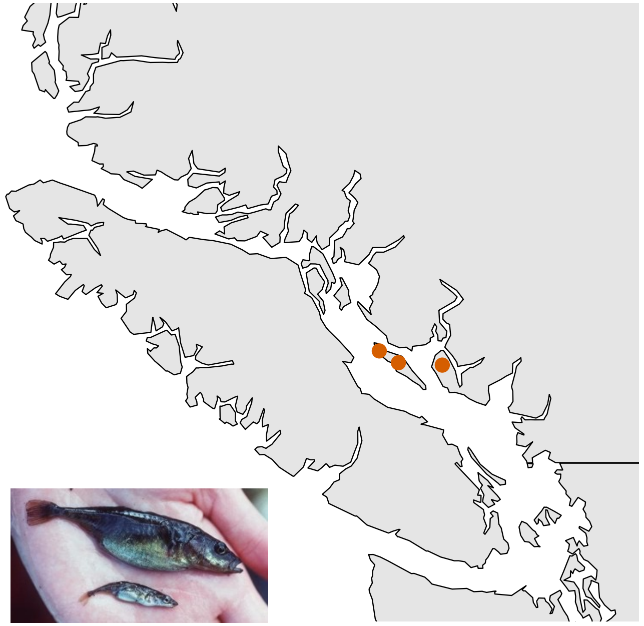
15% of QTL are shared between lake-stream pairs
Poore et al (2022) *Evolution*

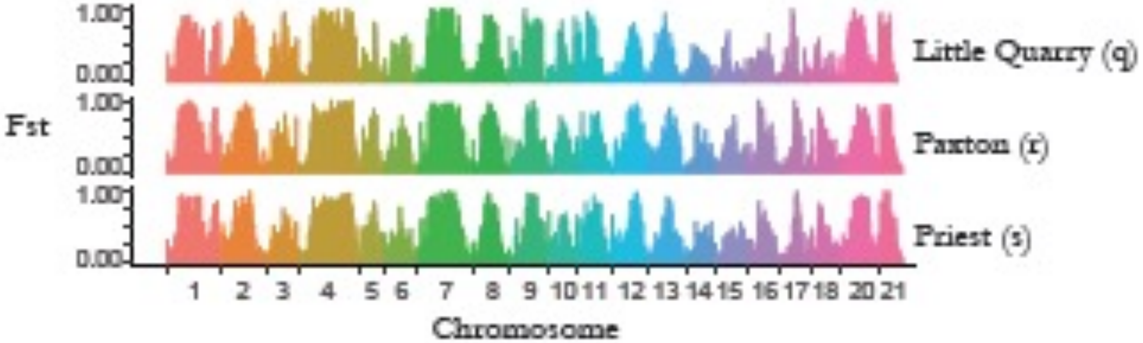
Population genomics: which genotypes are associated with repeated adaptation?

Diana Rennison
UC San Diego

- Lake-stream data
    - 16 lake-stream pairs (32 populations)
    - Sequencing for 24 individuals/population
- Benthic-limnetic data
    - 3 benthic-limnetic pairs (6 populations)
    - Sequencing for 20 individuals/population
- Fst between each lake-stream or benthic-limnetic pair was calculated in 50 kbp windows
    - 2513 windows in 16 lake-stream pairs
    - 5733 windows in 3 benthic-limnetic pairs

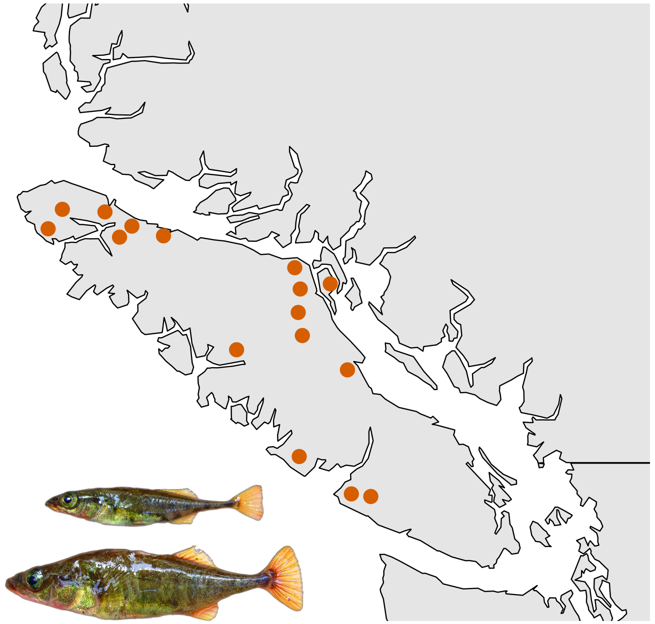
Highly repeatable genomic differentiation in benthic-limnetic pairs

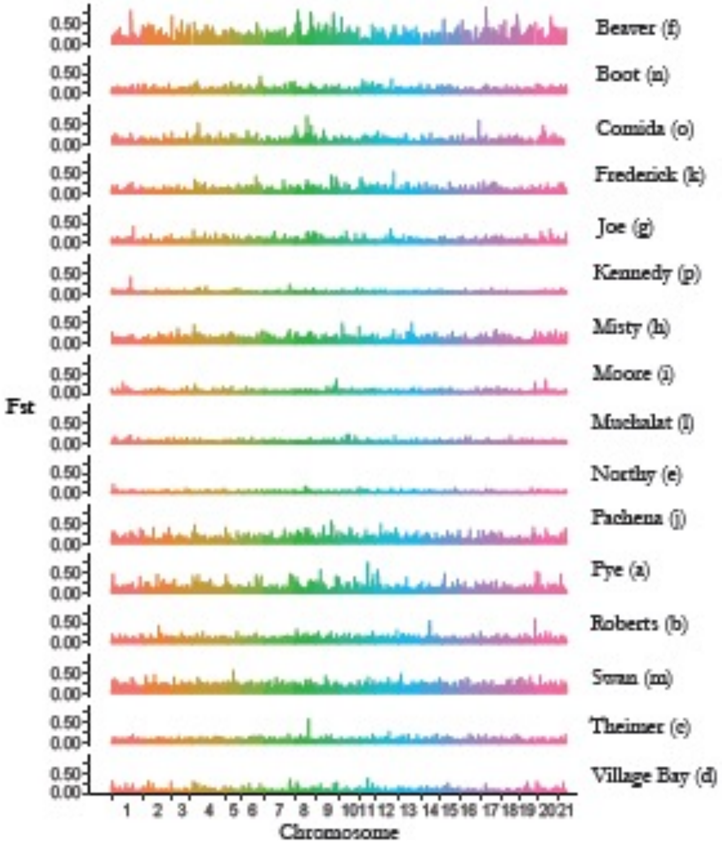




Rennison & Peichel (2022) *Molecular Ecology*

Lower repeatability of genomic differentiation in lake-stream pairs





Rennison et al (2019) *Philosophical Transactions of the Royal Society B*

Questions

- How often do the same genetic changes underlie repeated phenotypic evolution and adaptation?
    - Sometimes, but not always, and differs between systems!

- Why might some genetic changes be used more frequently during repeated phenotypic evolution and adaptation?

Questions

- How often do the same genetic changes underlie repeated phenotypic evolution and adaptation?
    - Sometimes, but not always, and differs between systems!

- Why might some genetic changes be used more frequently during repeated phenotypic evolution and adaptation?

Why is evolution repeatable at the genetic level?

1) Only one gene or mutation can produce the phenotype favoured by selection

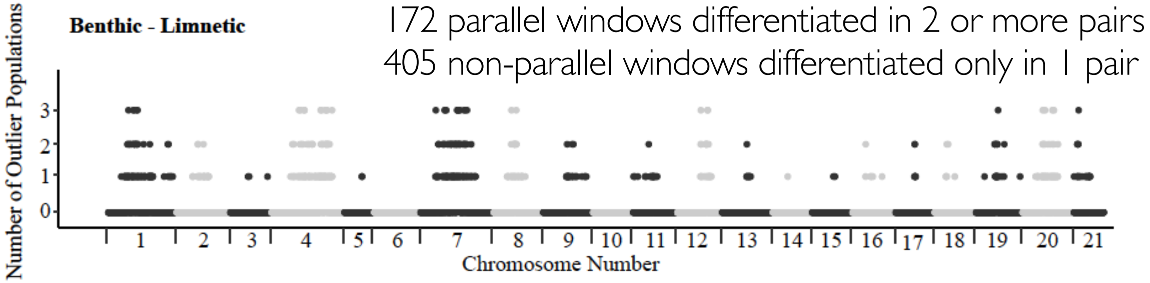
2) Many genes or mutations can produce the phenotype but some:
   a) are less pleiotropic and have fewer fitness constraints
   b) have higher mutation rates
   c) are in regions of low recombination

Population genomics: which genotypes are associated with repeated adaptation?

Diana Rennison
UC San Diego

- Lake-stream data
  - 16 lake-stream pairs (32 populations)
  - Sequencing for 24 individuals/population
- Benthic-limnetic data
  - 3 benthic-limnetic pairs (6 populations)
  - Sequencing for 20 individuals/population
- Fst between each lake-stream or benthic-limnetic pair was calculated in 50 kbp windows
  - 2513 windows in 16 lake-stream pairs
  - 5733 windows in 3 benthic-limnetic pairs

Parallel *vs* non-parallel 50 kb windows



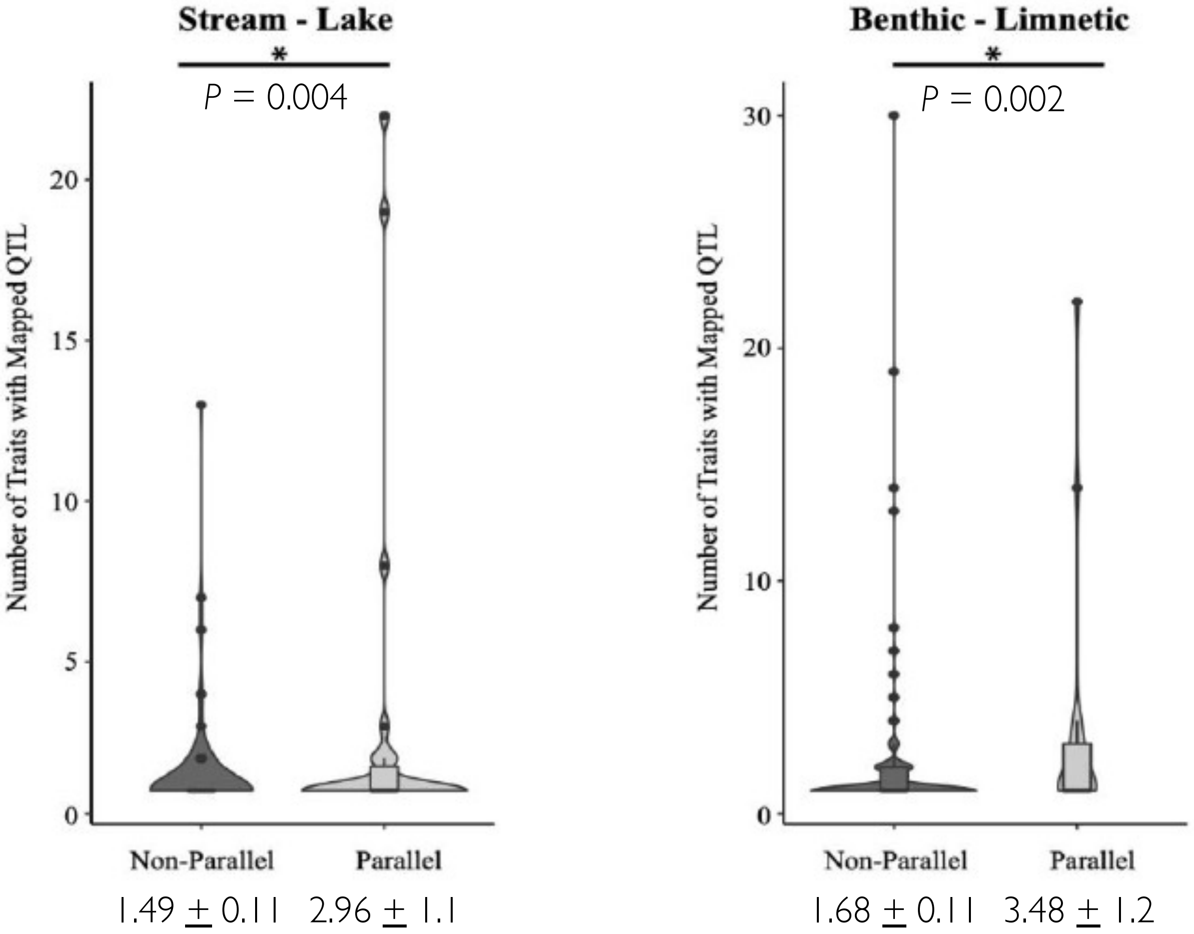
172 parallel windows differentiated in 2 or more pairs
405 non-parallel windows differentiated only in 1 pair

Rennison & Peichel (2022) *Molecular Ecology*

Do parallel and non-parallel windows differ?

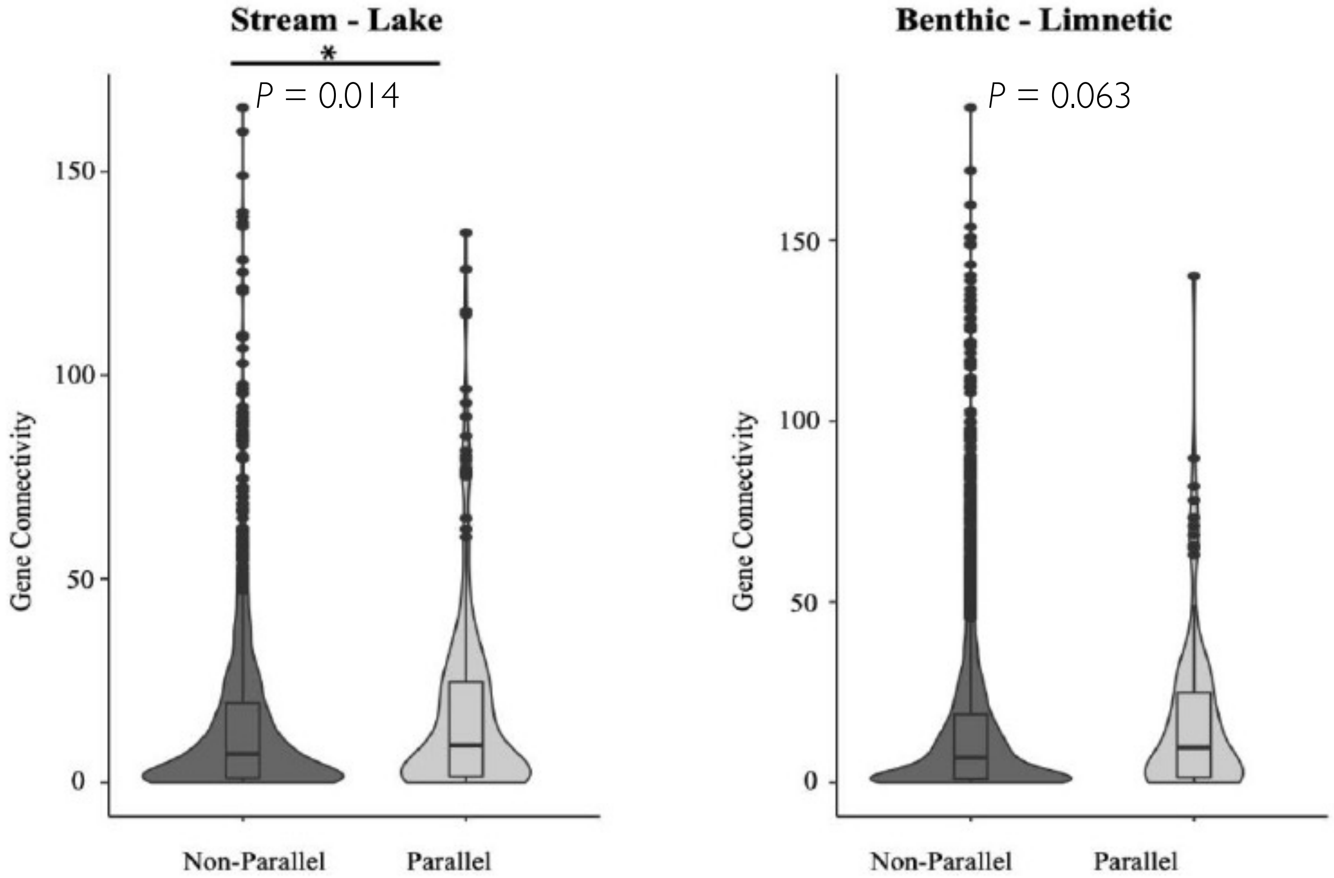
- Level of pleiotropy?
  - Number of QTL (Marques & Peichel 2017)
  - Gene connectivity (RNAseq co-expression network)
- Gene number?
- Recombination rate?
- Mutation rate?

Parallel windows contain more QTL



Rennison & Peichel (2022) *Molecular Ecology*

Parallel windows contain genes with higher connectivity



P = 0.014

P = 0.063

14.9 ± 0.6 18.1 ± 1.5

14.2 ± 1.0 18.1± 1.7

Rennison & Peichel (2022) *Molecular Ecology*

Do parallel and non-parallel windows differ?

- Level of pleiotropy?
    - Parallel windows have more QTL and higher connectivity
    - But, non-parallel windows are the most pleiotropic
- Gene number?
    - No difference
- Recombination rate?
    - Parallel windows have a lower recombination rate, but only in benthic-limnetic pairs where there is high gene flow
    - If we only consider outlier windows, there is no difference between parallel and non-parallel windows
- Mutation rate?
    - No difference if account for difference in recombination rate

Rennison & Peichel (2022) *Molecular Ecology*

Conclusions

- Pleiotropy does not always seem to be a constraint
- Rather, intermediate (and synergistic) levels of pleiotropy might be adaptive
- Old alleles with synergistic pleiotropic effects might be maintained as standing variation in systems like stickleback in which repeated adaptation has occurred many times

Rennison & Peichel (2022) *Molecular Ecology*

Questions and answers from sticklebacks

- How often do the same genetic changes underlie repeated phenotypic evolution and adaptation?
  - Sometimes, but not always, and differs between systems!

- Why might some genetic changes be used more frequently during repeated phenotypic evolution and adaptation?
  - Pleiotropy and selection on standing variation?

Maybe sticklebacks are special?

Do we see similar patterns in other systems?

Questions

- How often do the same genetic changes underlie repeated phenotypic evolution and adaptation?
- Why might some genetic changes be used more frequently during repeated phenotypic evolution and adaptation?

How often do the same genes underlie repeated phenotypic evolution?

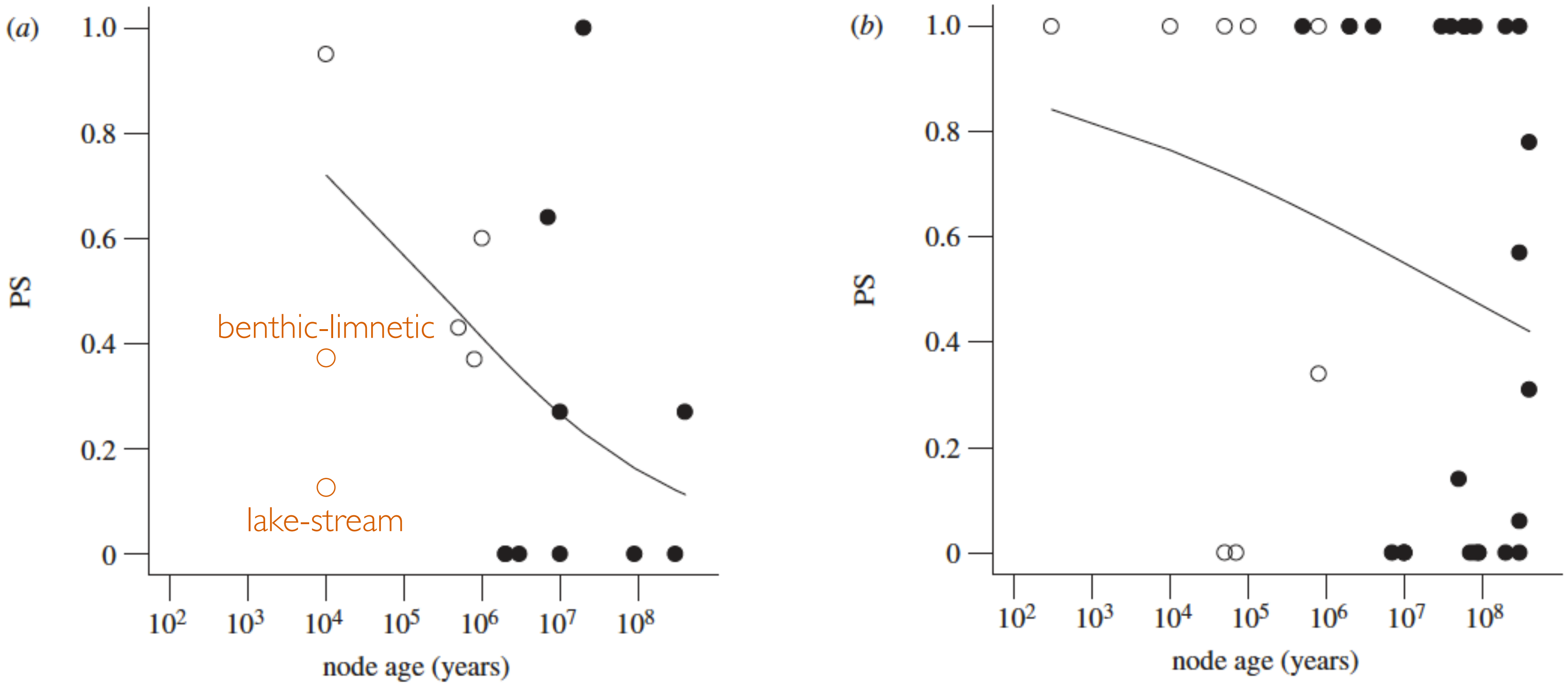
Conte et al (2012) *Proceedings of the Royal Society B*

How often do the same genes underlie repeated phenotypic evolution?

- Objective literature search revealed 25 case studies of two types:
    - genetic mapping studies
    - candidate gene studies
- Diverse taxa
    - fungi, plants, invertebrates, vertebrates
- Diverse traits
    - morphology, life history, toxins and toxin resistance, ability to utilize specific food sources

Conte et al (2012) *Proceedings of the Royal Society B*

Probability of gene reuse is high!



Genetic mapping

PS = 0.32 ± 0.10 s.e.

○ PS = 0.47 ± 0.15 s.e.

● PS = 0.24 ± 0.12 s.e.

Candidate gene

PS = 0.55 ± 0.08 s.e.

○ PS = 0.67 ± 0.17 s.e.

● PS = 0.51 ± 0.09 s.e.

Conte et al (2012) *Proceedings of the Royal Society B*

Caveats

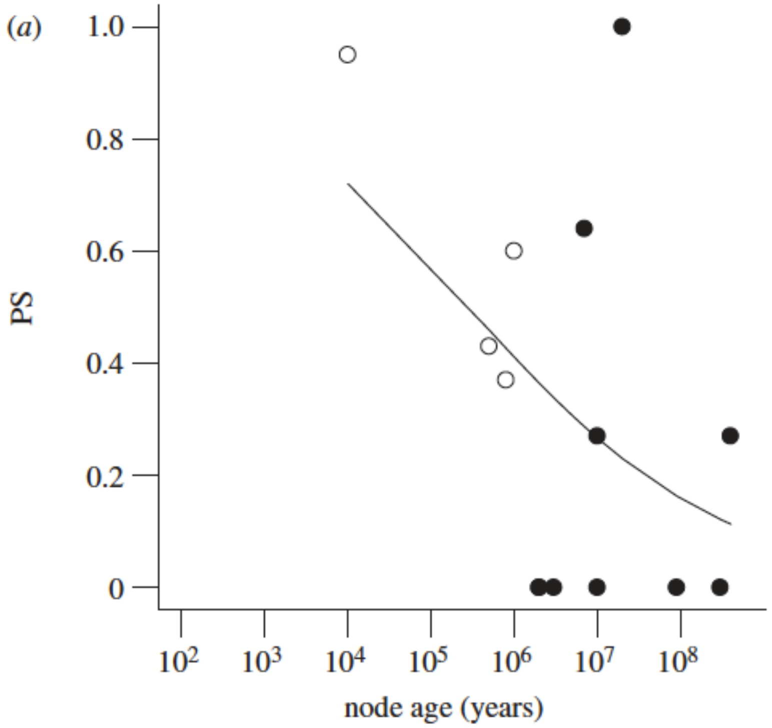
- Publication bias
- Small number of traits
- Detecting genes of small effect
- Different studies were done at different times in different ways
- Currently revisiting this meta-analyses
    - Stay tuned!

Magdalena Bohutínská

Questions

- How often do the same genetic changes underlie repeated phenotypic evolution and adaptation?
- Why might some genetic changes be used more frequently during repeated phenotypic evolution and adaptation?

Gene reuse decreases with divergence time



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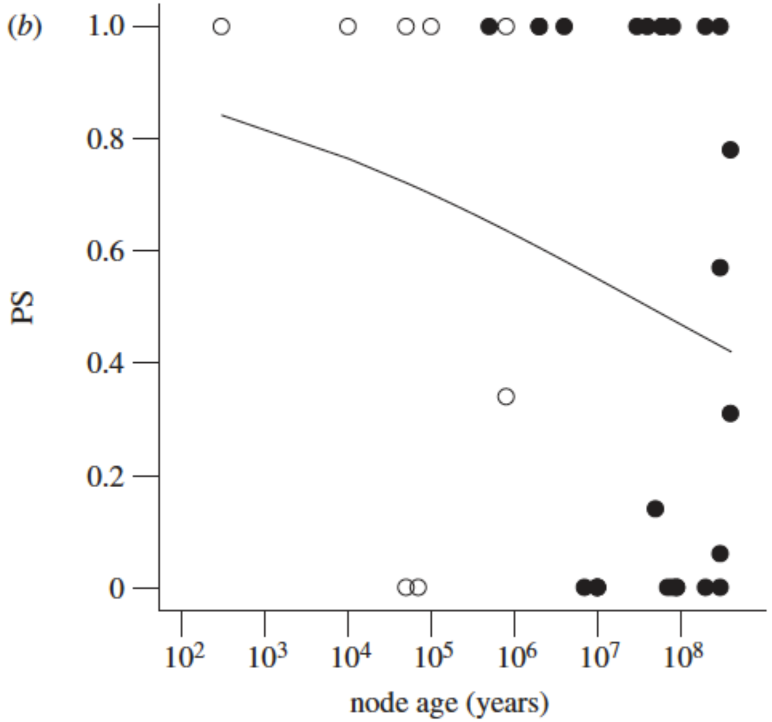

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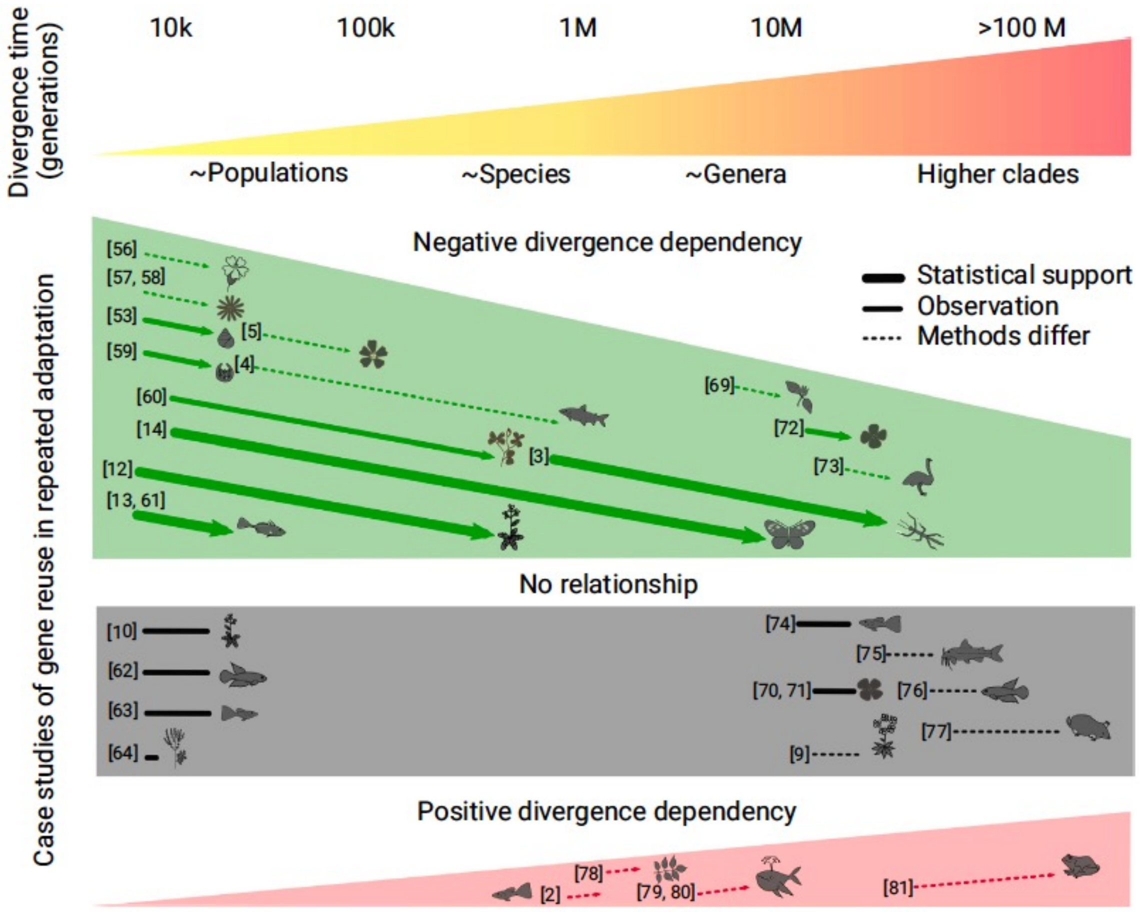
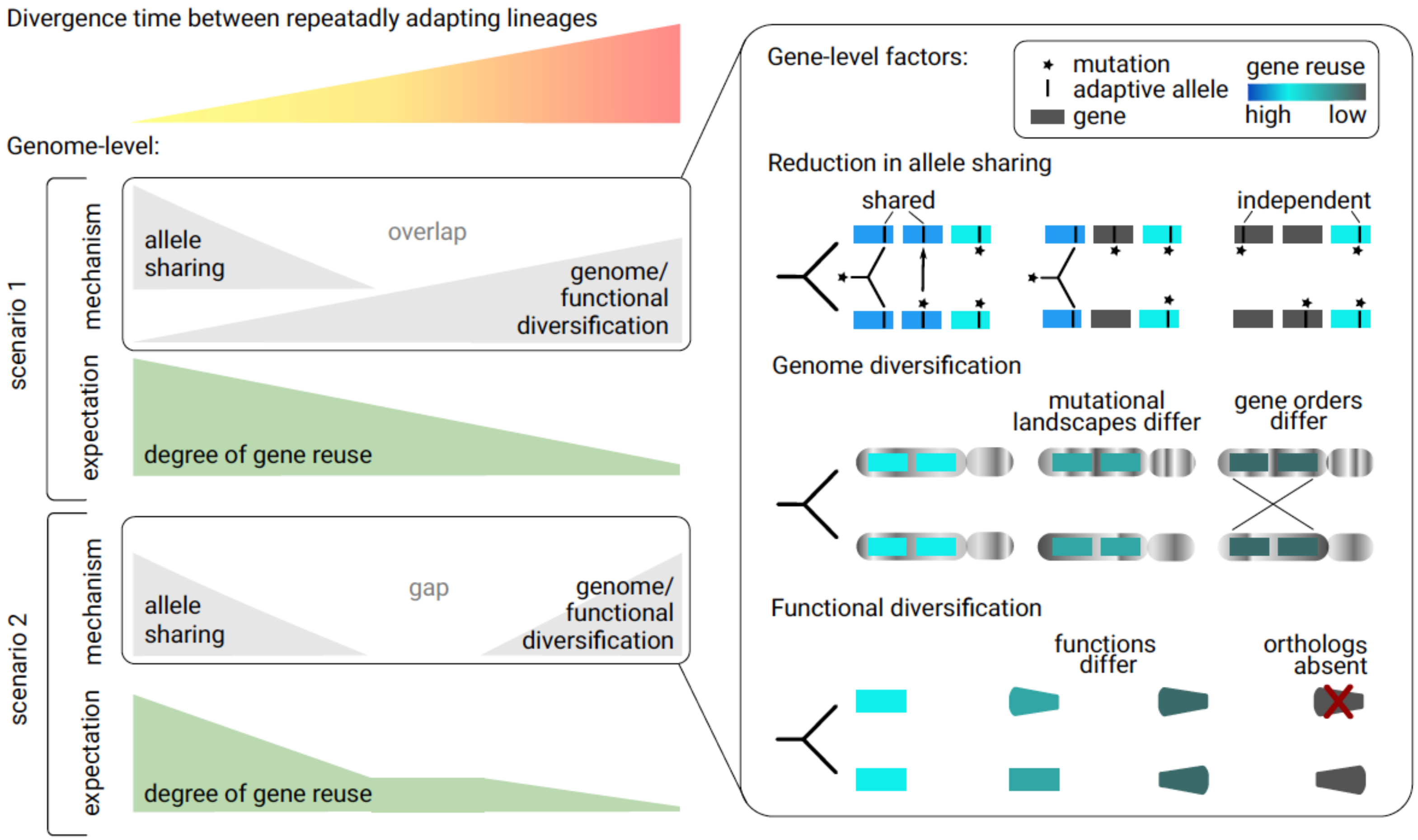


Bohuntínská & Peichel (2024) *Trends in Ecology & Evolution*

Possible genetic mechanisms



Bohuntínská & Peichel (2024) *Trends in Ecology & Evolution*

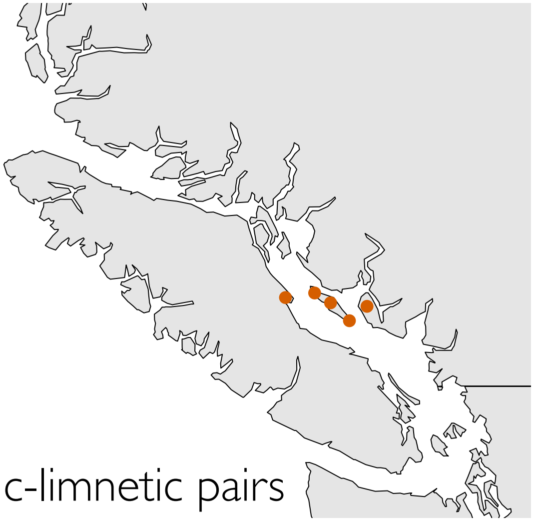
Questions and some answers

- How often do the same genetic changes underlie repeated phenotypic evolution and adaptation?
  - Sometimes, but not always!
- Why might some genetic changes be used more frequently during repeated phenotypic evolution and adaptation?
  - Synergistic pleiotropy (Rennison and Peichel 2022)
  - Gene flow (Bohutínská and Peichel 2024)
  - Other mechanisms?

Back to sticklebacks…

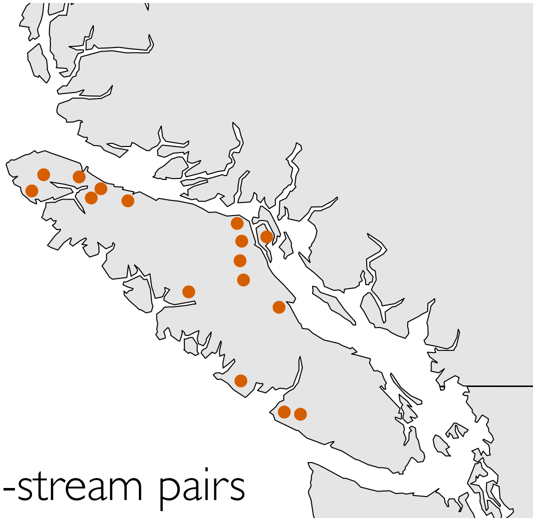
They are special☺

Why do the benthic-limnetic and lake-stream pairs differ?



Benthic-limnetic pairs

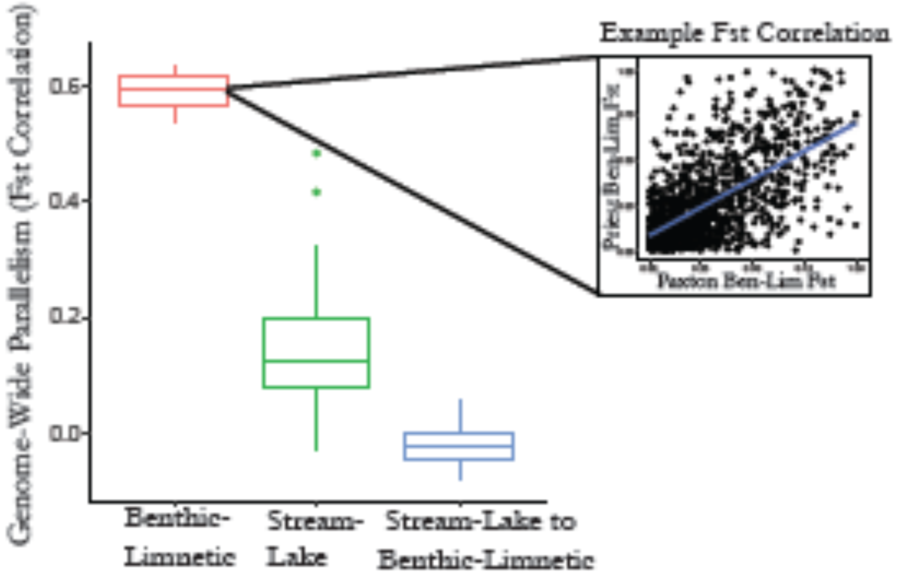
High genetic repeatability



Lake-stream pairs

Low genetic repeatability

Why do the benthic-limnetic and lake-stream pairs differ?



- Source of standing genetic variation?
- Evolutionary history?
- Extent of gene flow?
- Strength of repeated selection?

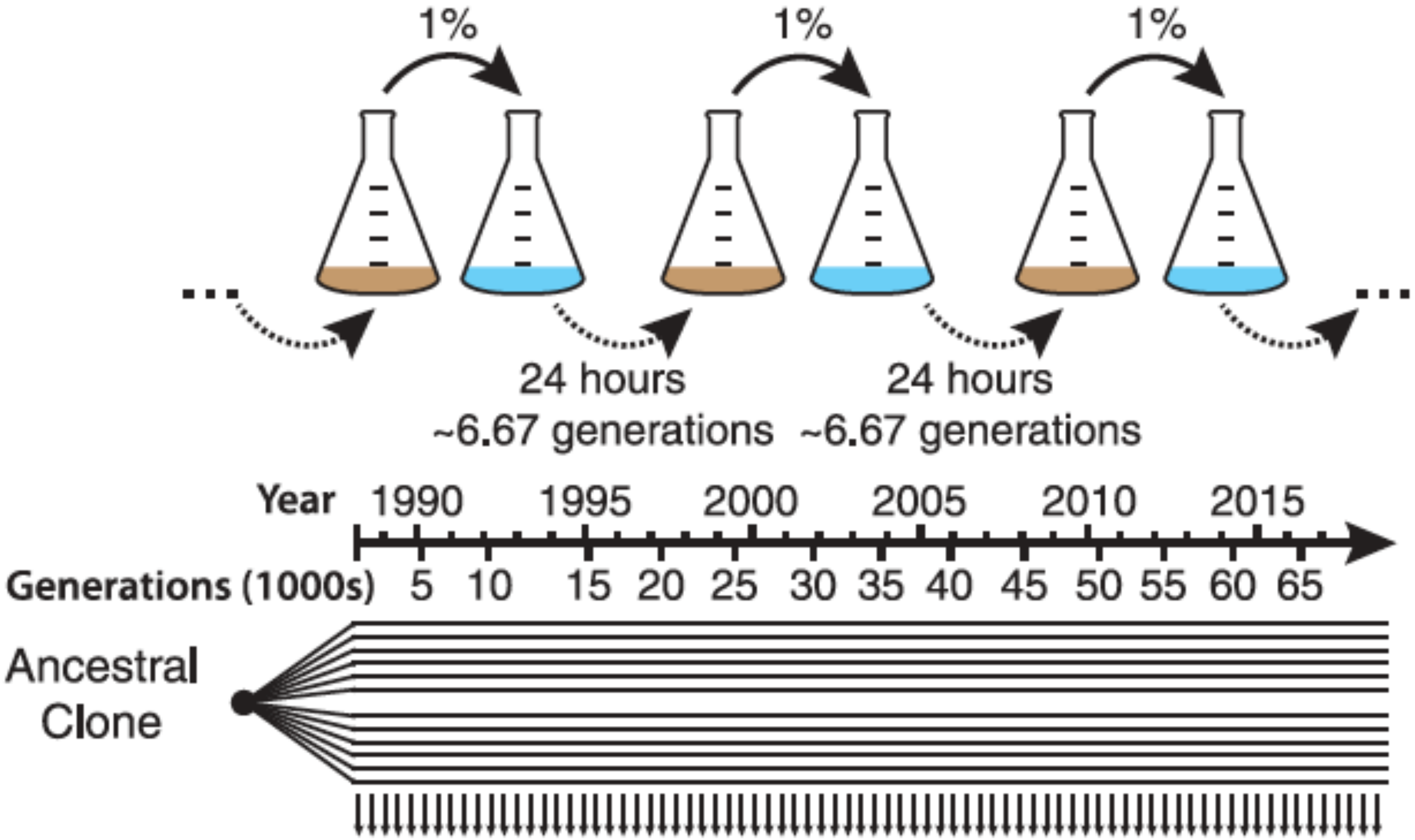
Problem!

We are only examining extant populations and lack information on the founding ancestral populations and ecosystem changes over time

What if we could do Gould's thought experiment?

If we replayed the tape of life, would evolution repeat itself?

What if we could do Gould's thought experiment?

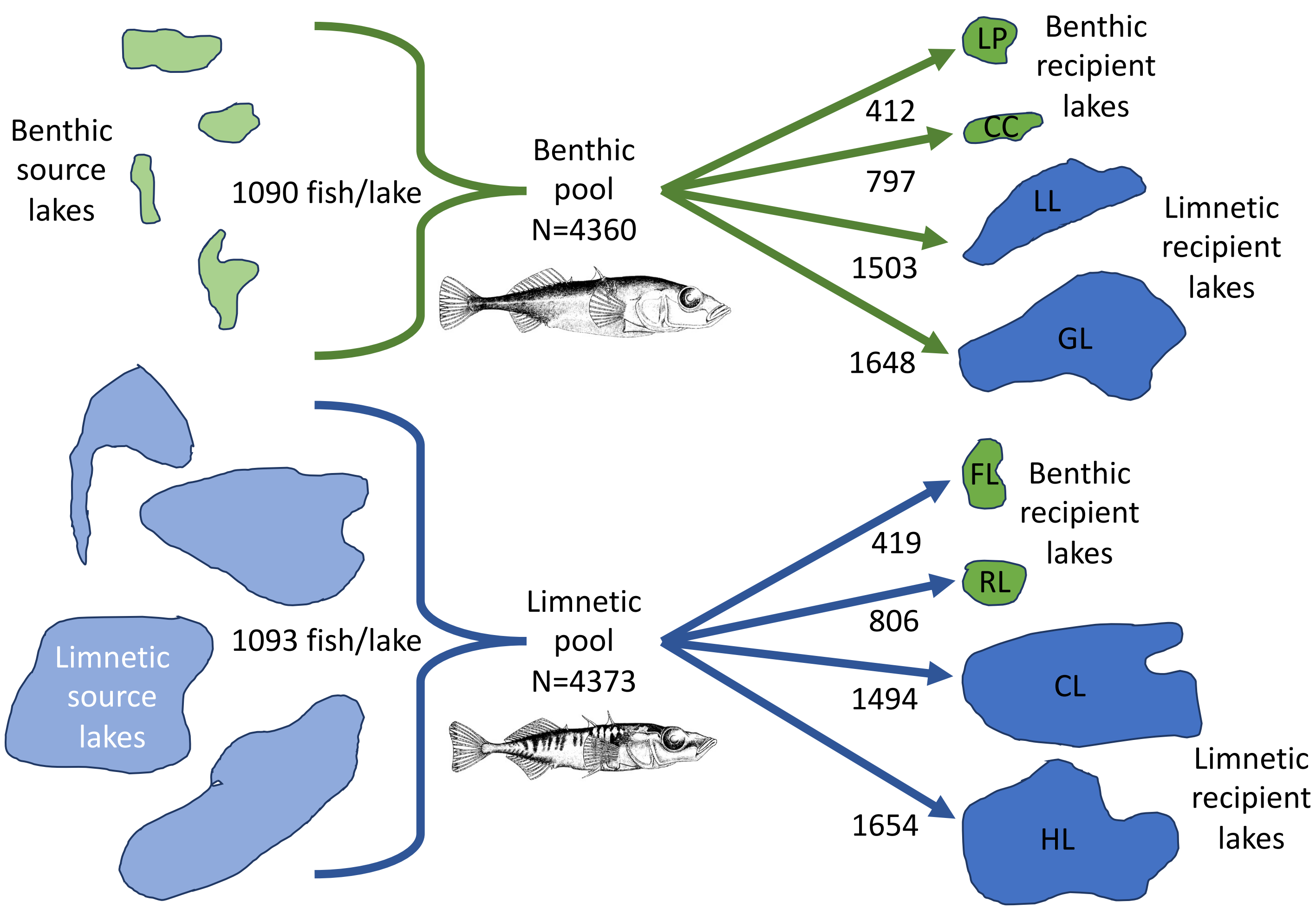


Blount et al (2018) *Science*

What if we could do Gould’s thought experiment **in natural ecosystems**?

Hope Lake, Alaska

FITNESS: Forward-In-Time Natural Experimental Study of Selection



Benthic source lakes

1090 fish/lake

Benthic pool
N=4360

412
797
1503
1648

LP
CC
Benthic recipient lakes

LL
GL
Limnetic recipient lakes

Limnetic source lakes

1093 fish/lake

Limnetic pool
N=4373

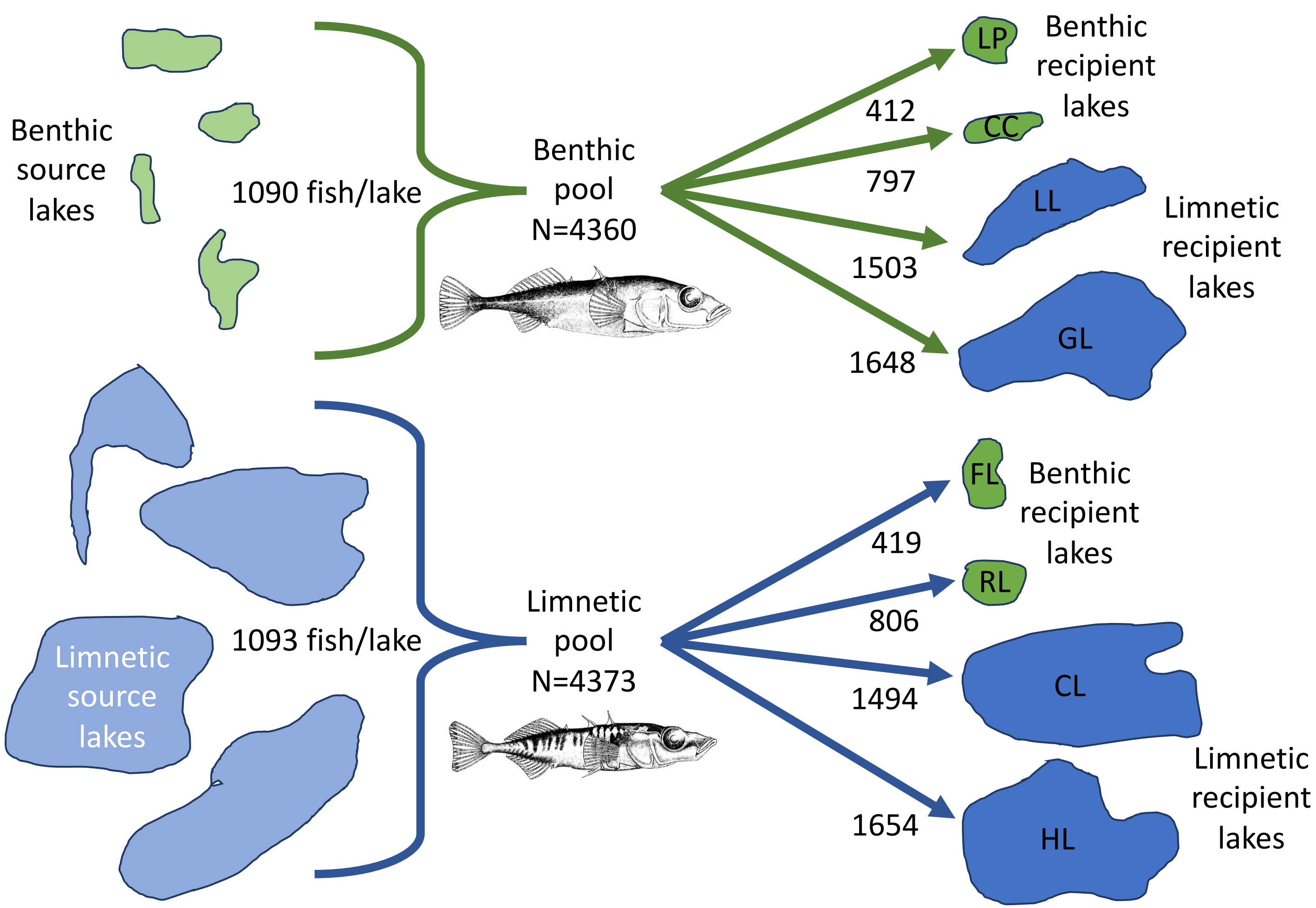
419
806
1494
1654

FL
RL
Benthic recipient lakes

CL
HL
Limnetic recipient lakes

Swiss National Science Foundation

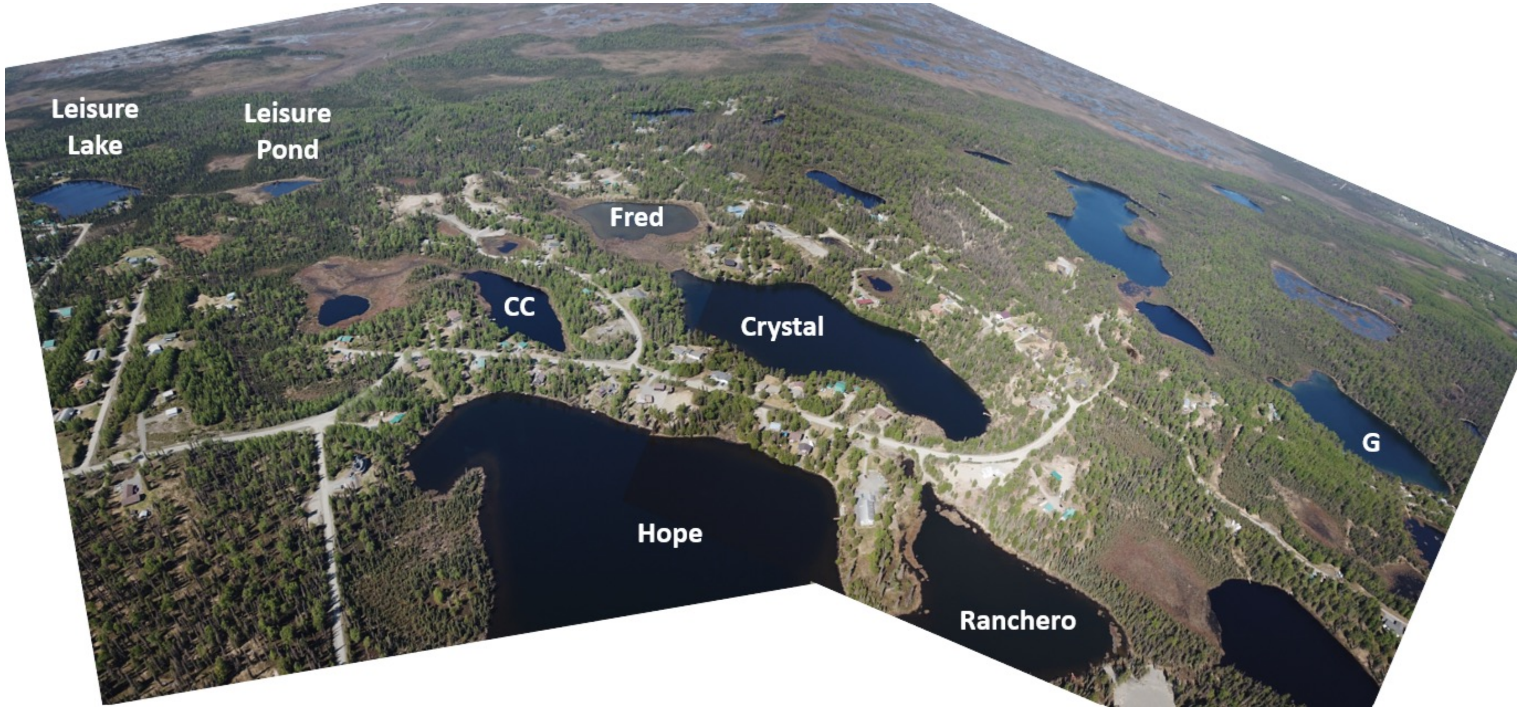
FITNESS: Experimental overview



- Whole genome sequences and phenotypes from 8733 founding fish
- Follow evolutionary trajectories of genotypes and phenotypes in recipient lakes for 8 generations (9216 fish)

FITNESS: Forward-In-Time Natural Experimental Study of Selection

Swiss National Science Foundation



How repeatable are genotypic and phenotypic trajectories?
Can we predict the evolutionary trajectories we see?

Today’s workshop: detecting positive selection

- Majda will use sequencing data generated by Milan from some of the founding indiviudals used for the FITNESS experiment!

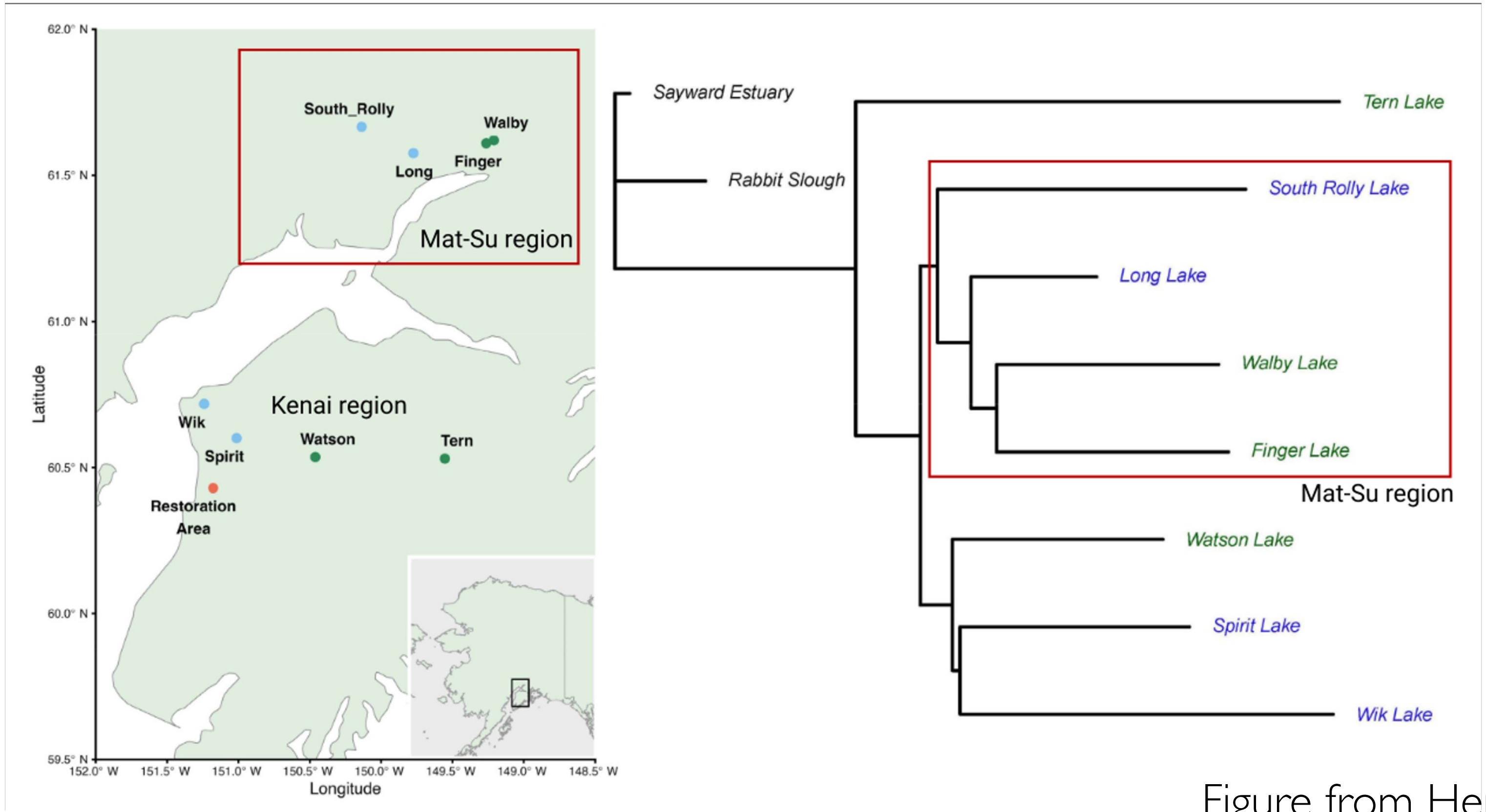


Figure from Hendry et al (2024) *Ecol Evol*

THANKS!

Benthic-limnetic pairs

Dolph Schluter (U British Columbia)
Gina Conte (U British Columbia)
Matt Arnegard (Fred Hutch)
Diana Rennison (UC San Diego)

Lake-stream pairs

Dan Bolnick (U Connecticut)
Andrew Hendry (McGill)
Rowan Barrett (McGill)
Yoel Stuart (Loyola University)
Diana Rennison (UC San Diego)
Hilary Poore (U Bern)

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Metanalyses

Magdalena Bohutínská

FITNESS project

Andrew Hendry (McGill)
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Dan Jeffries (U Bern)
Ben Sulser (U Bern)