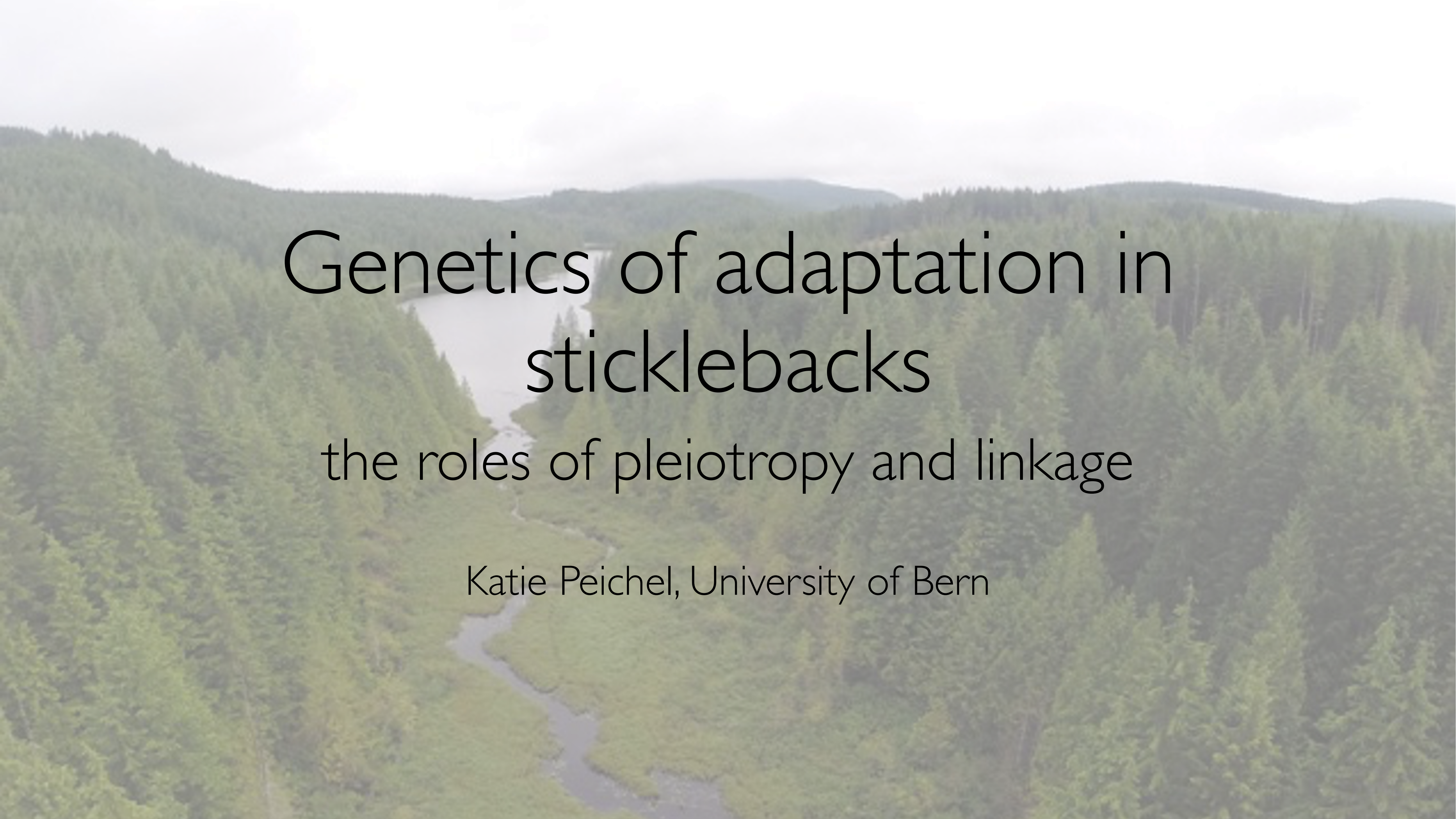


Genetics of adaptation in sticklebacks

the roles of pleiotropy and linkage

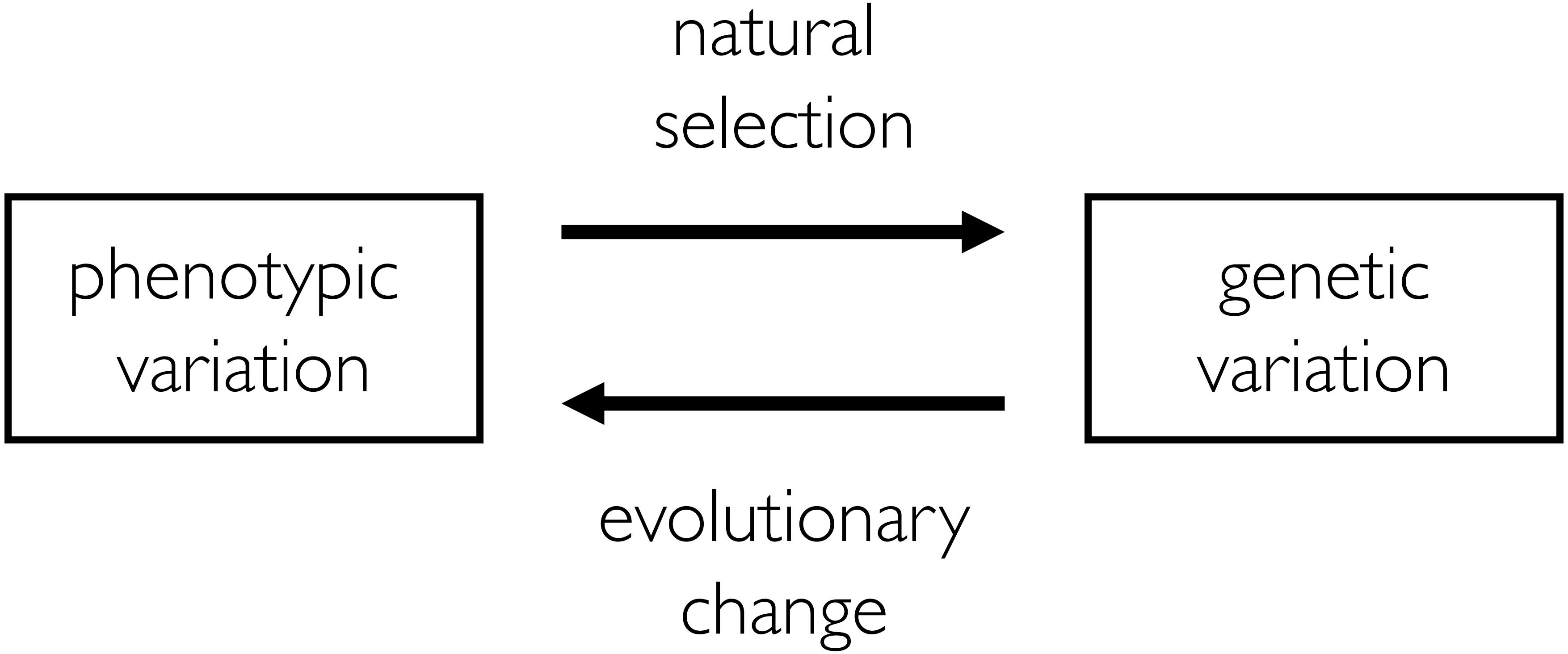
Katie Peichel, University of Bern

Long-term research interests

- What are the genetic and genomic changes that underlie the processes of adaptation and speciation?
- How does the genome itself evolve, and how does this affect the processes of adaptation and speciation?
  - Sex chromosome evolution
  - Chromosomal fusions
  - Chromosomal inversions

How do organisms adapt to divergent environments?

Genetics of adaptation



natural selection

phenotypic variation

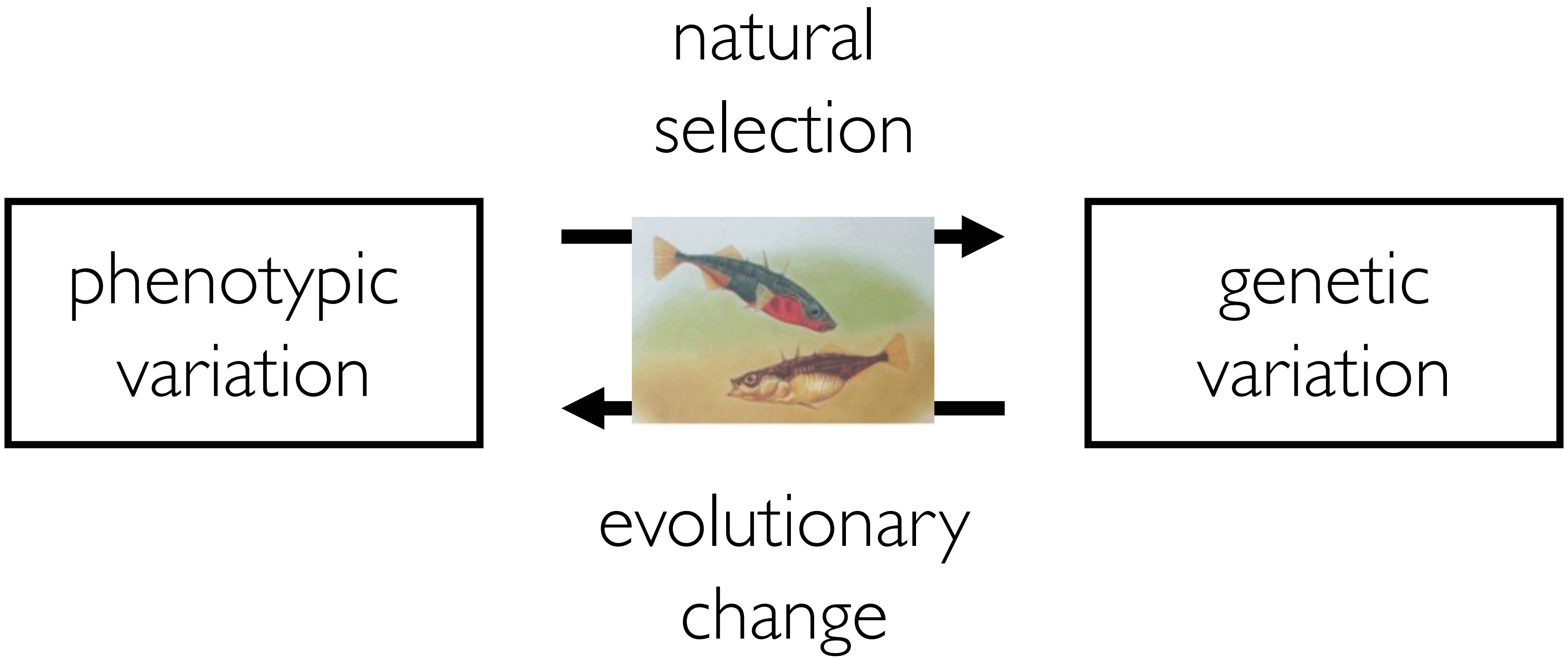
genetic variation

evolutionary change

Specific questions

- Does adaptation occur via a few mutations of large effect, or many mutations of small effect?
- What genes and mutations contribute to adaptation?
- Are the same genes and mutations involved in adaptation to the similar environments?
- Does genetic linkage and/or pleiotropy facilitate or constrain adaptation?

Genetics of adaptation

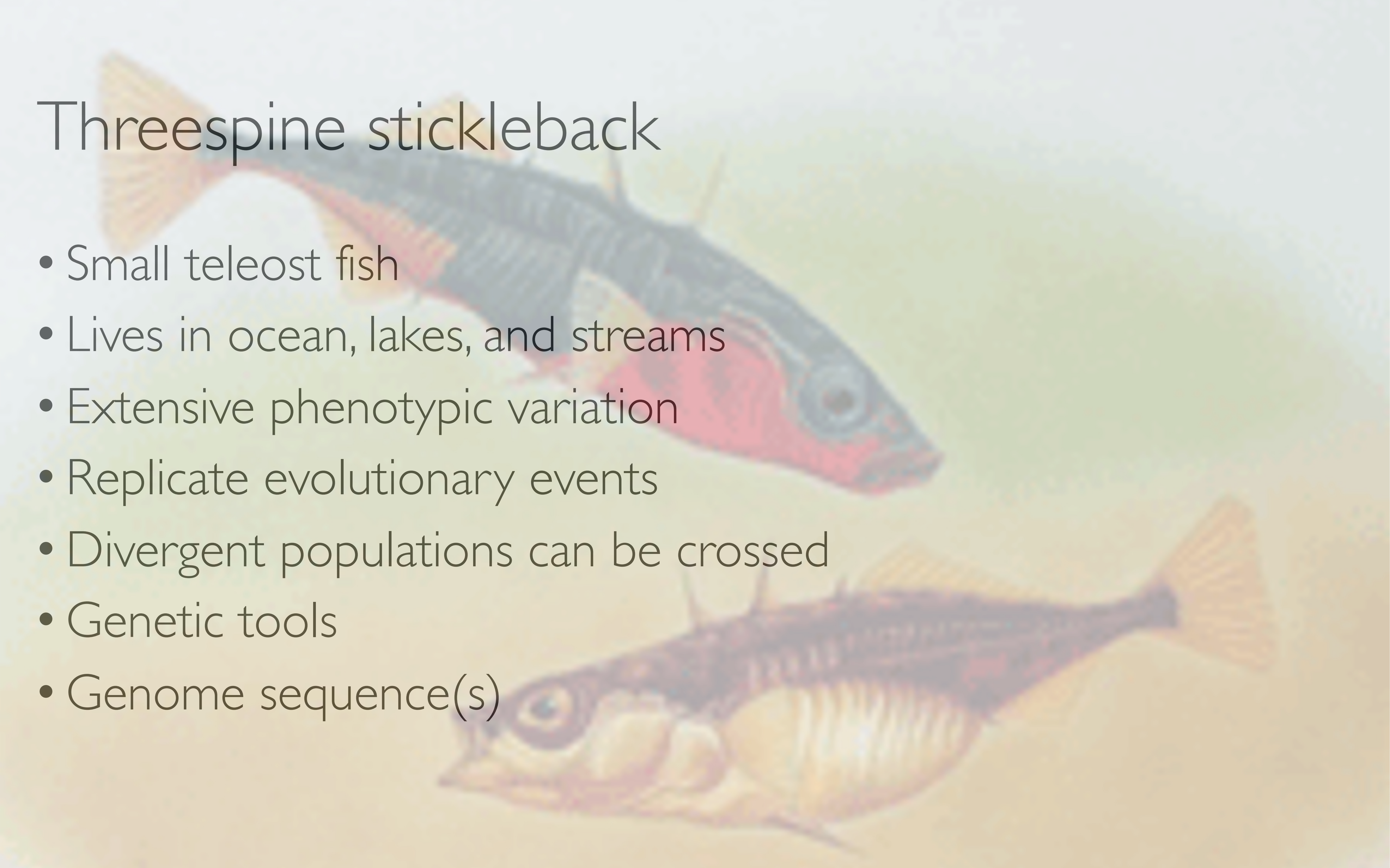


phenotypic variation

natural selection

evolutionary change

genetic variation

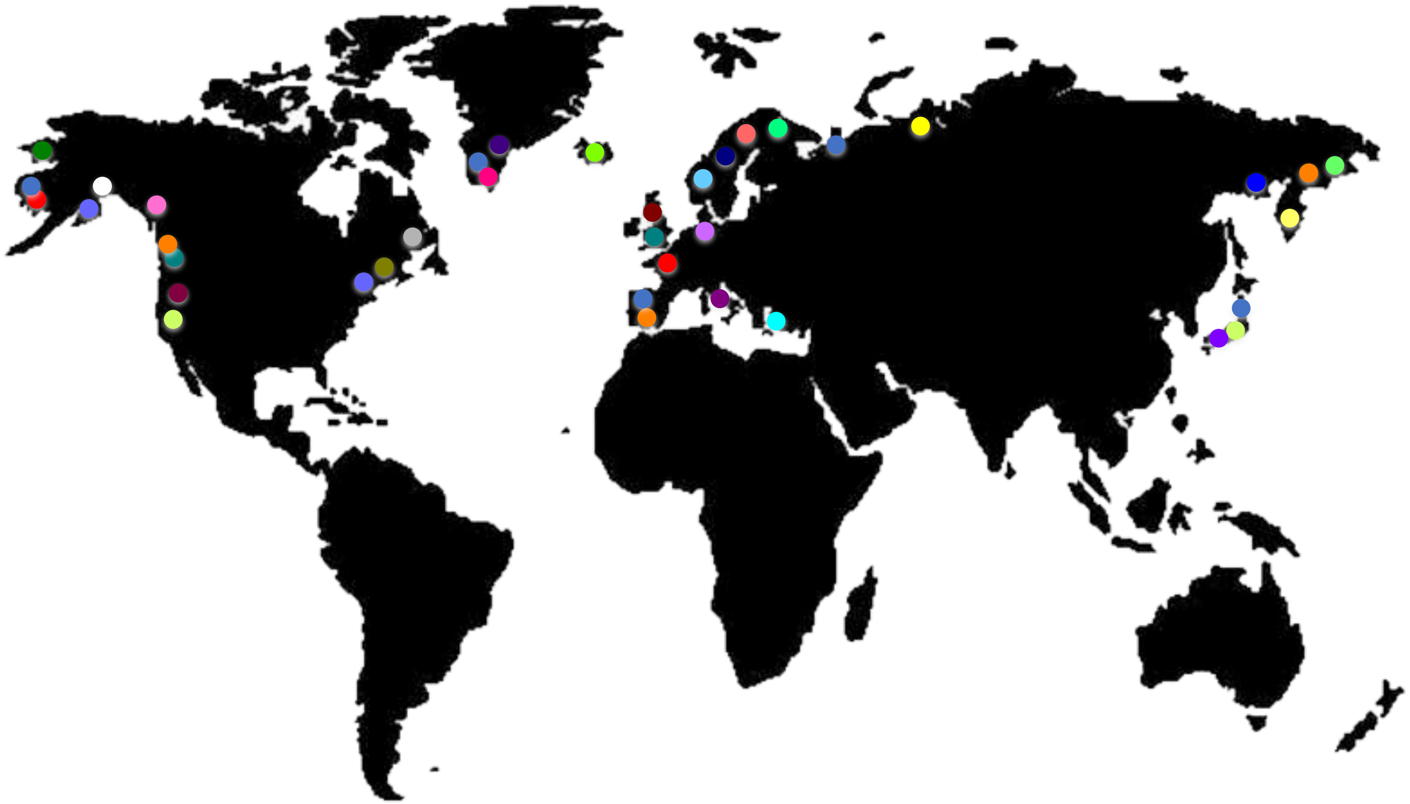


Threespine stickleback

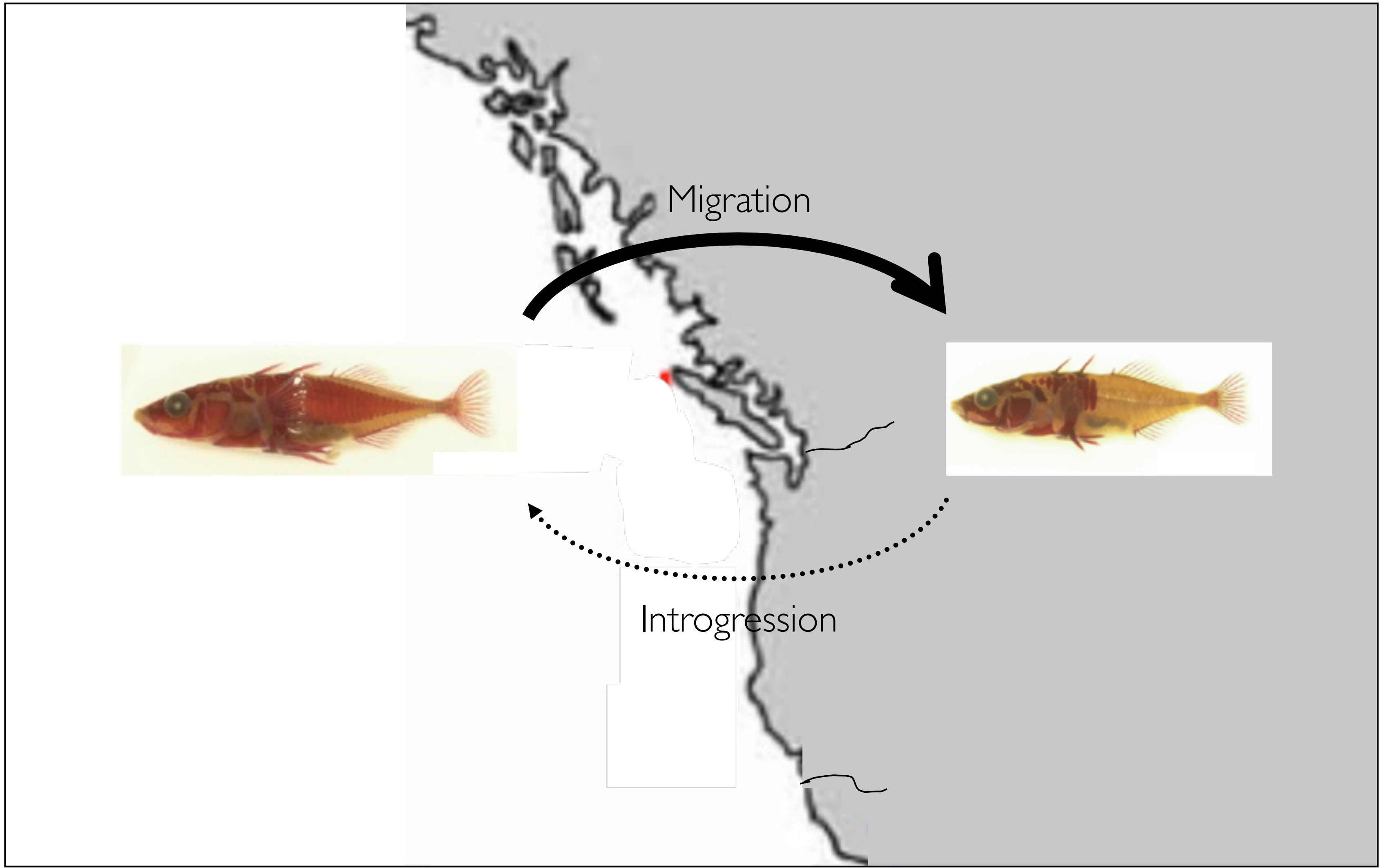
- Small teleost fish
- Lives in ocean, lakes, and streams
- Extensive phenotypic variation
- Replicate evolutionary events
- Divergent populations can be crossed
- Genetic tools
- Genome sequence(s)

Ancestral marine populations

Derived freshwater populations



Gene flow between marine and freshwater populations



Marine vs freshwater sticklebacks

Photos by Seiichi Mori and Jun Kitano

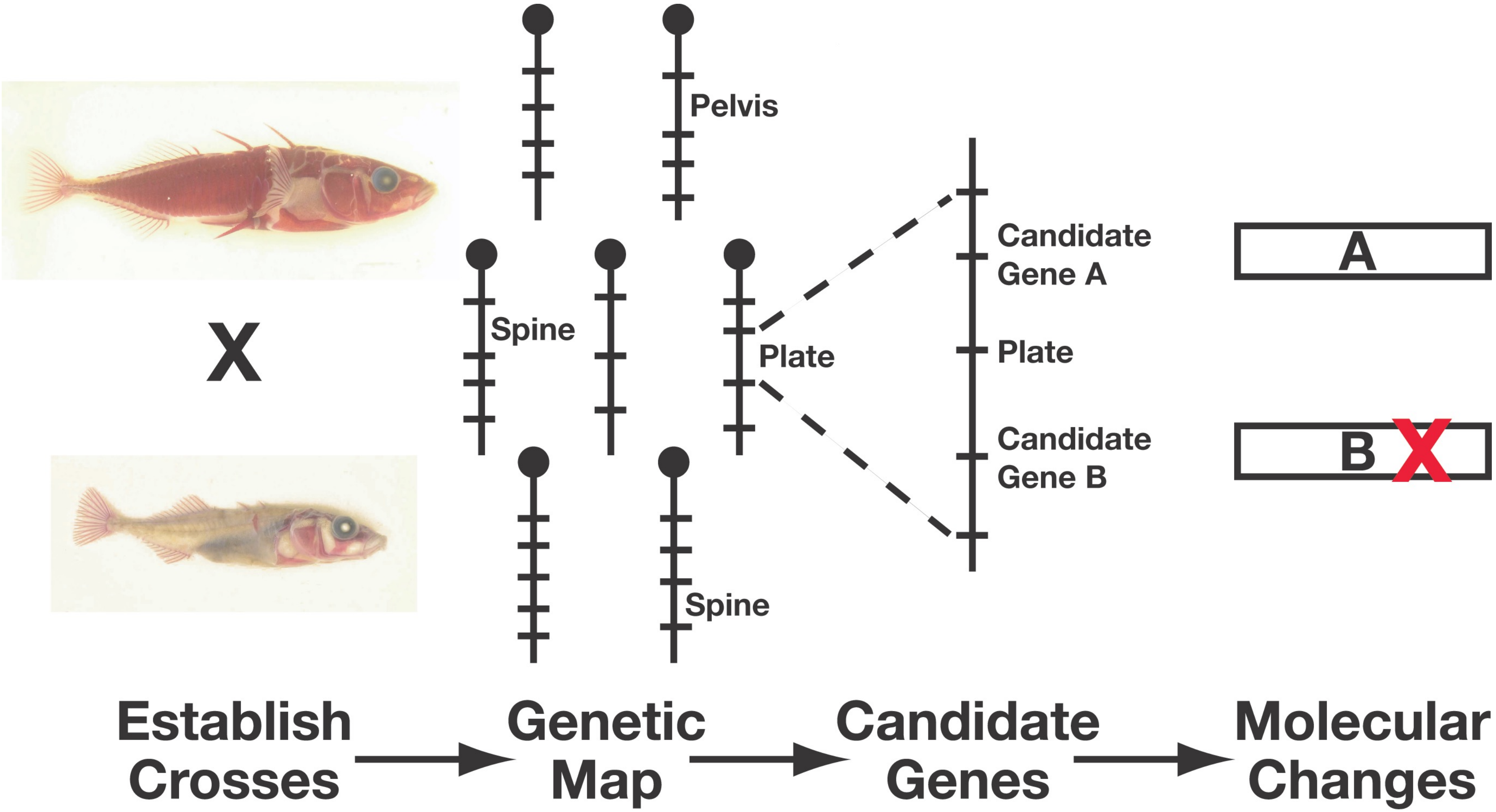
Marine vs freshwater sticklebacks

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

Quantitative trait locus (QTL) mapping



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

Stickleback QTL database

- 28 quantitative trait locus (QTL) mapping studies until 2017
- 1034 QTL identified in 9 trait categories
  - Morphology
    - Feeding, defense, body shape
    - Swimming, pigment, body size, respiration
  - Reproduction
  - Behaviour

David Marques

Specific questions

- Does adaptation occur via a few mutations of large effect, or many mutations of small effect?
- What genes and mutations contribute to adaptation?
- Are the same genes and mutations involved in adaptation to the similar environments?
- Does genetic linkage and/or pleiotropy facilitate or constrain adaptation?

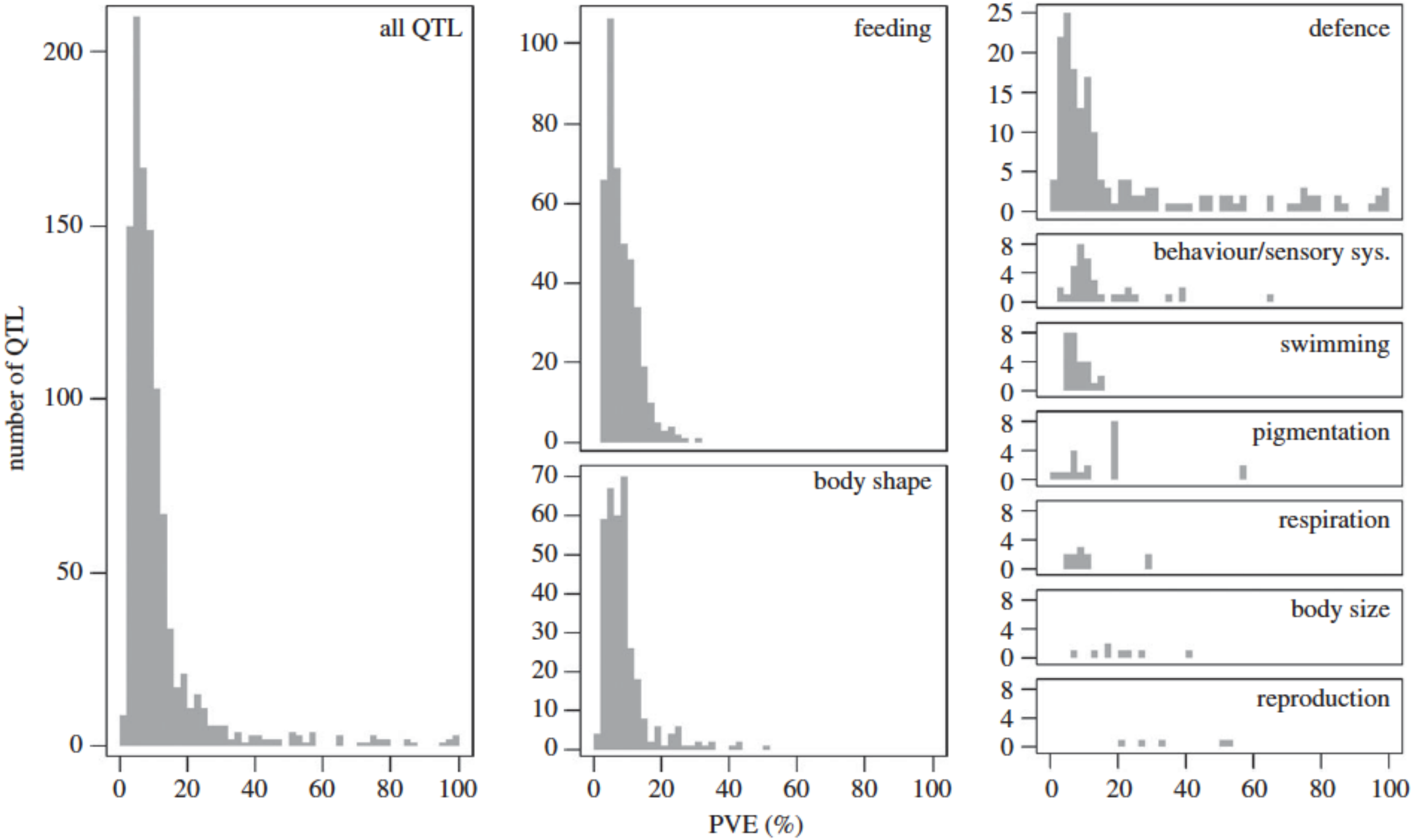
Specific questions

- Does phenotypic evolution occur via a few mutations of large effect, or many mutations of small effect?
- What genes and mutations contribute to phenotypic evolution?
- Are the same genes and mutations involved in the evolution of similar phenotypes?
- Does genetic linkage and/or pleiotropy facilitate or constrain phenotypic evolution?

Specific questions

- Does adaptation occur via a few mutations of large effect, or many mutations of small effect?
- What genes and mutations contribute to adaptation?
- Are the same genes and mutations involved in adaptation to the similar environments?
- Does genetic linkage and/or pleiotropy facilitate or constrain adaptation?

Many loci of small effect, few of large effect

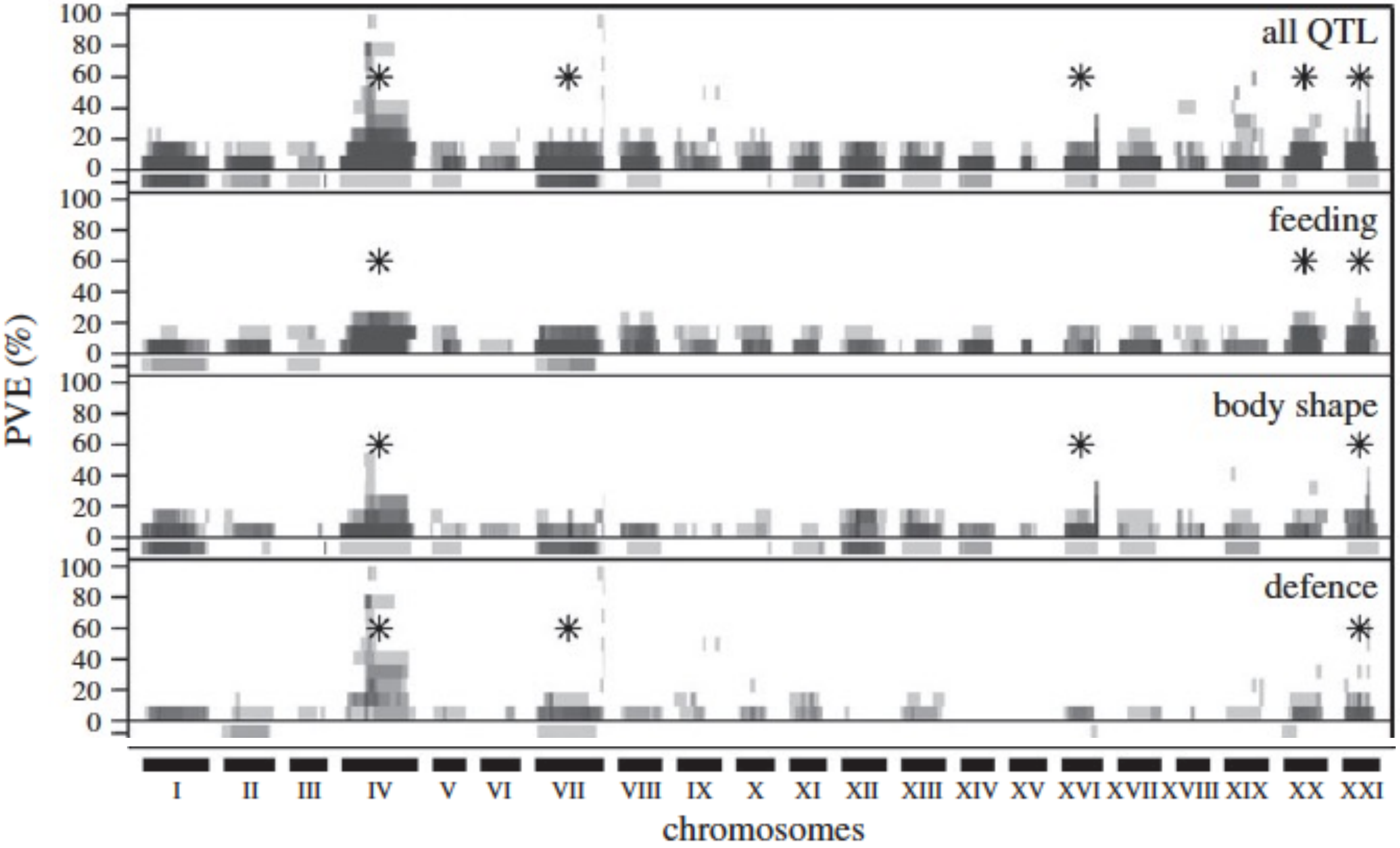


Peichel & Marques (2017) *Philosophical Transactions of the Royal Society London B*

Specific questions

- Does adaptation occur via a few mutations of large effect, or many mutations of small effect?
- What genes and mutations contribute to adaptation?
- When the same phenotype evolves, are the same genes and mutations involved?
- Does genetic linkage and/or pleiotropy facilitate or constrain adaptation?

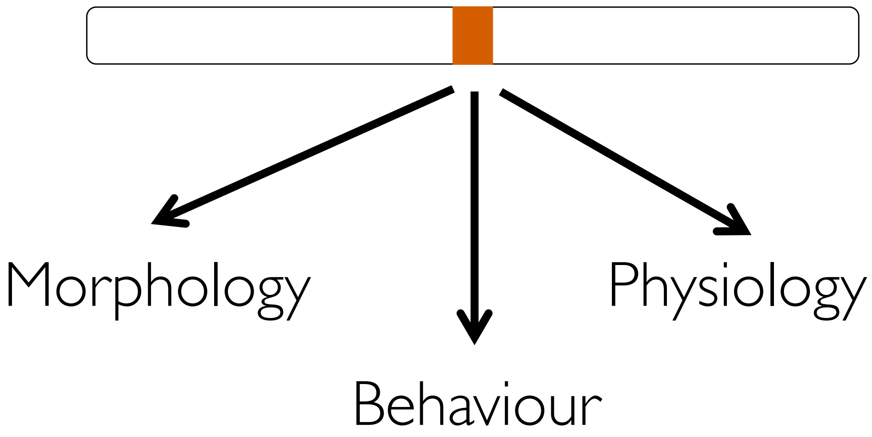
QTL are clustered in the genome



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

Are QTL clusters due to pleiotropy or linkage?

Pleiotropy

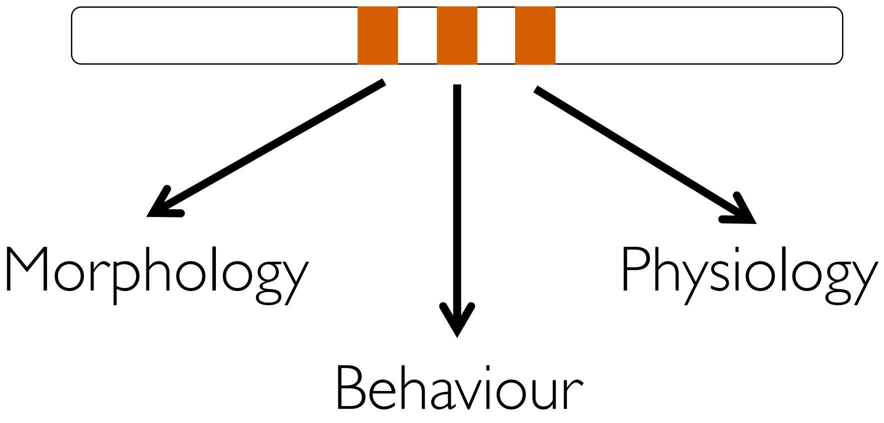


Morphology

Physiology

Behaviour

Linkage

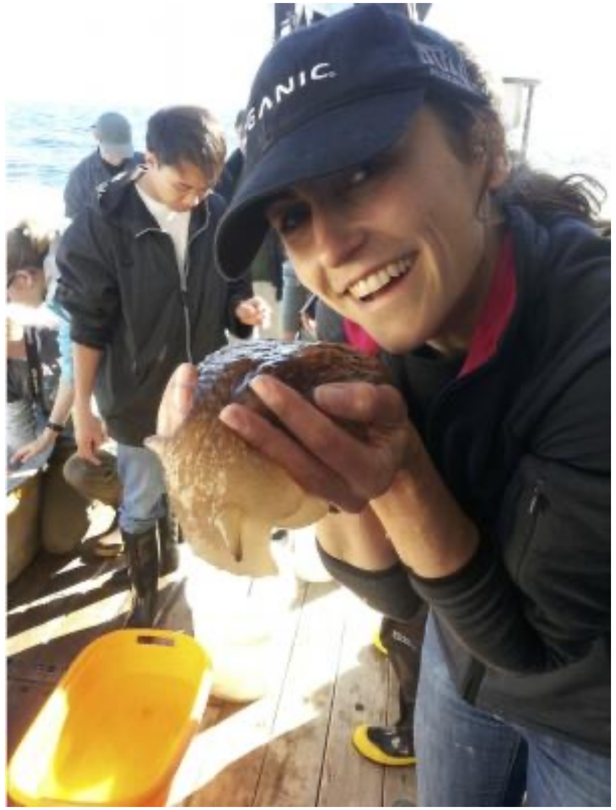


Morphology

Physiology

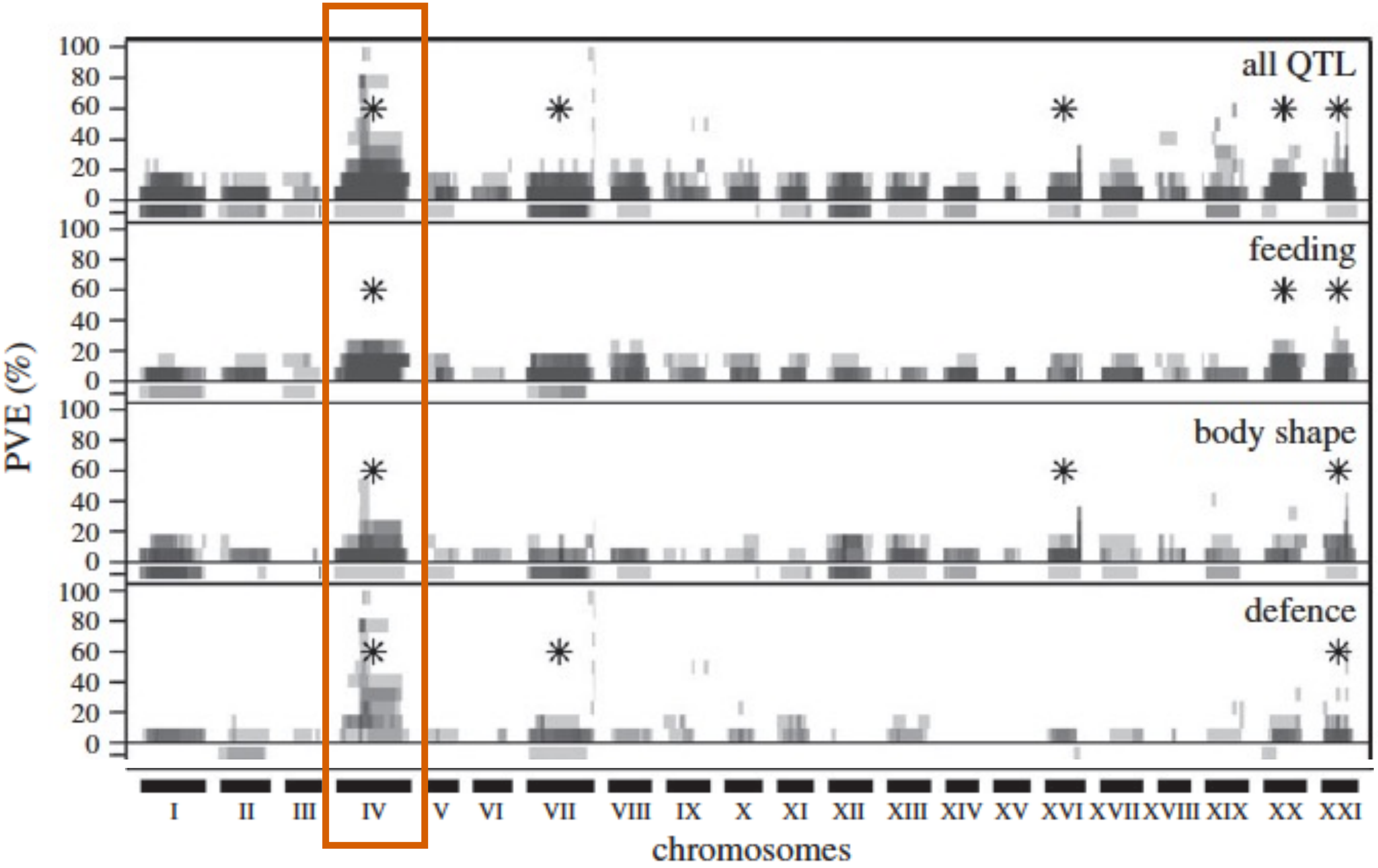
Behaviour

Are QTL clusters due to pleiotropy or linkage?



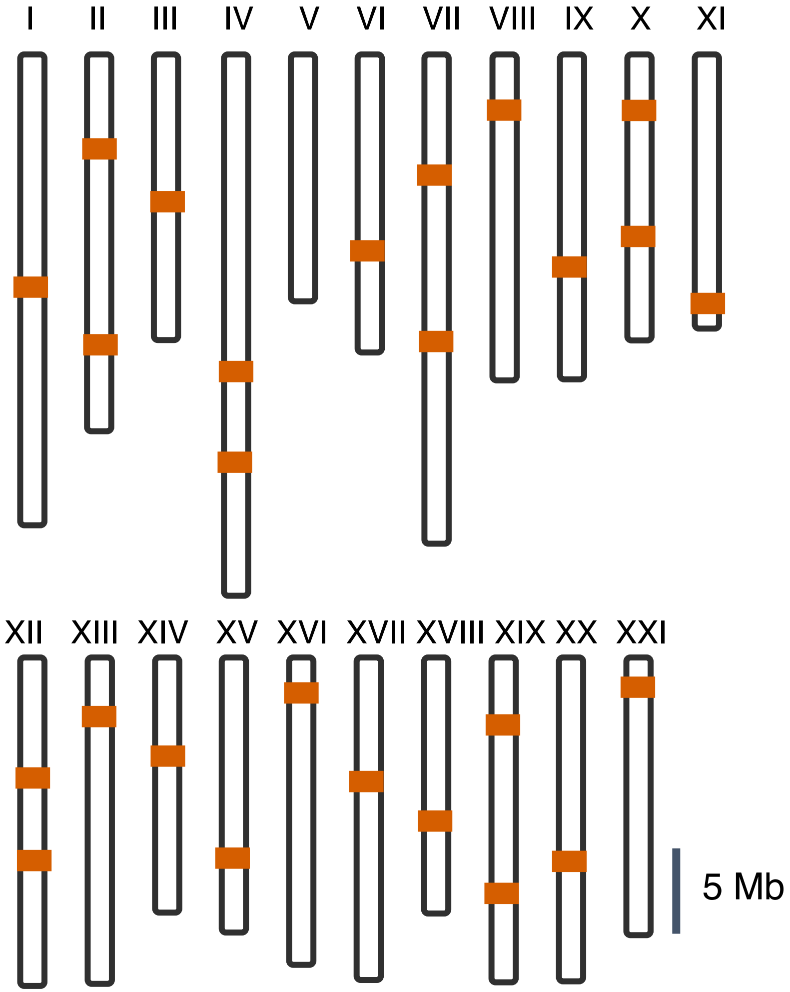
Sophie Archambeault

QTL cluster on chromosome IV



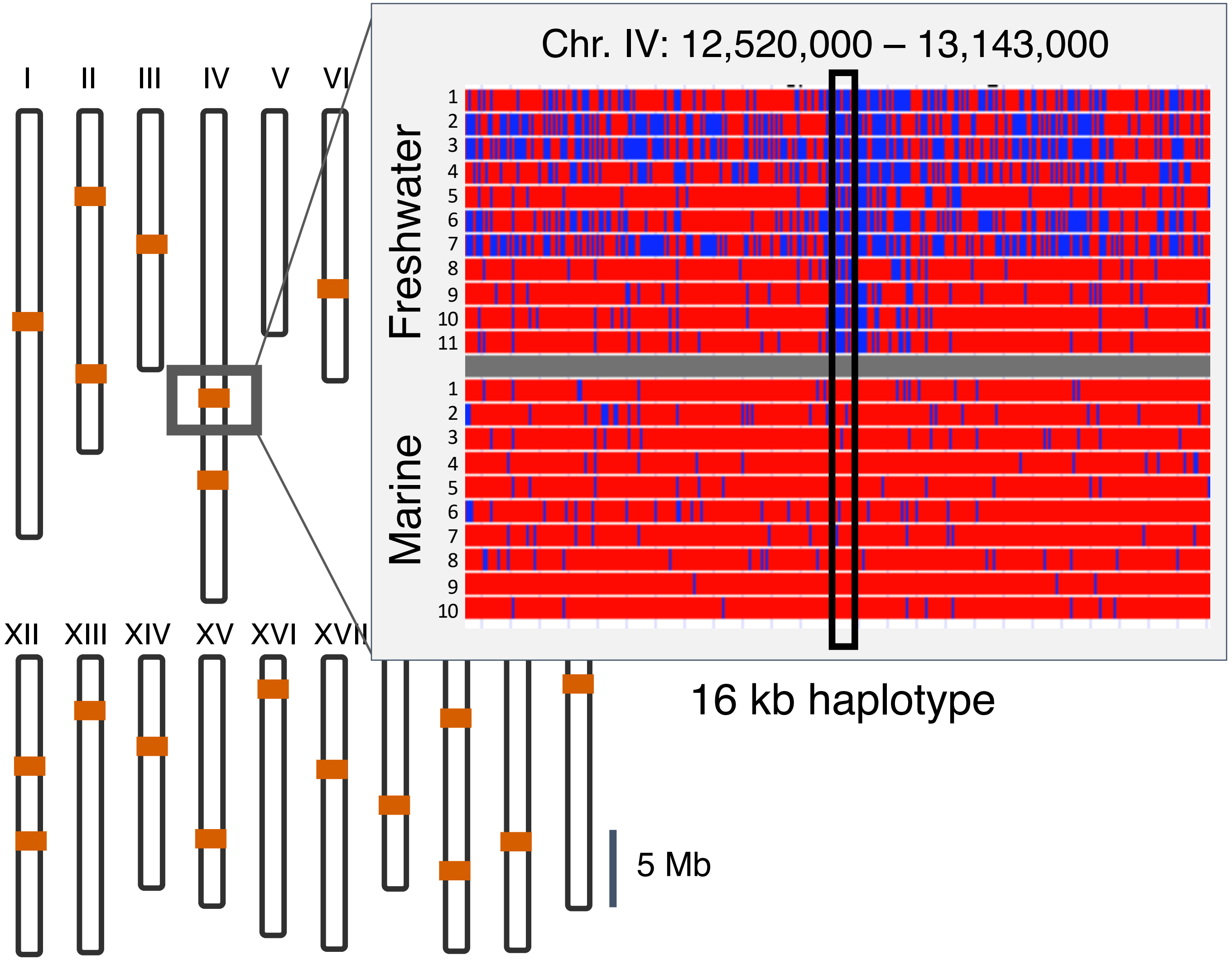
Peichel & Marques (2017) *Philosophical Transactions of the Royal Society London B*

Genomic divergence on chromosome IV



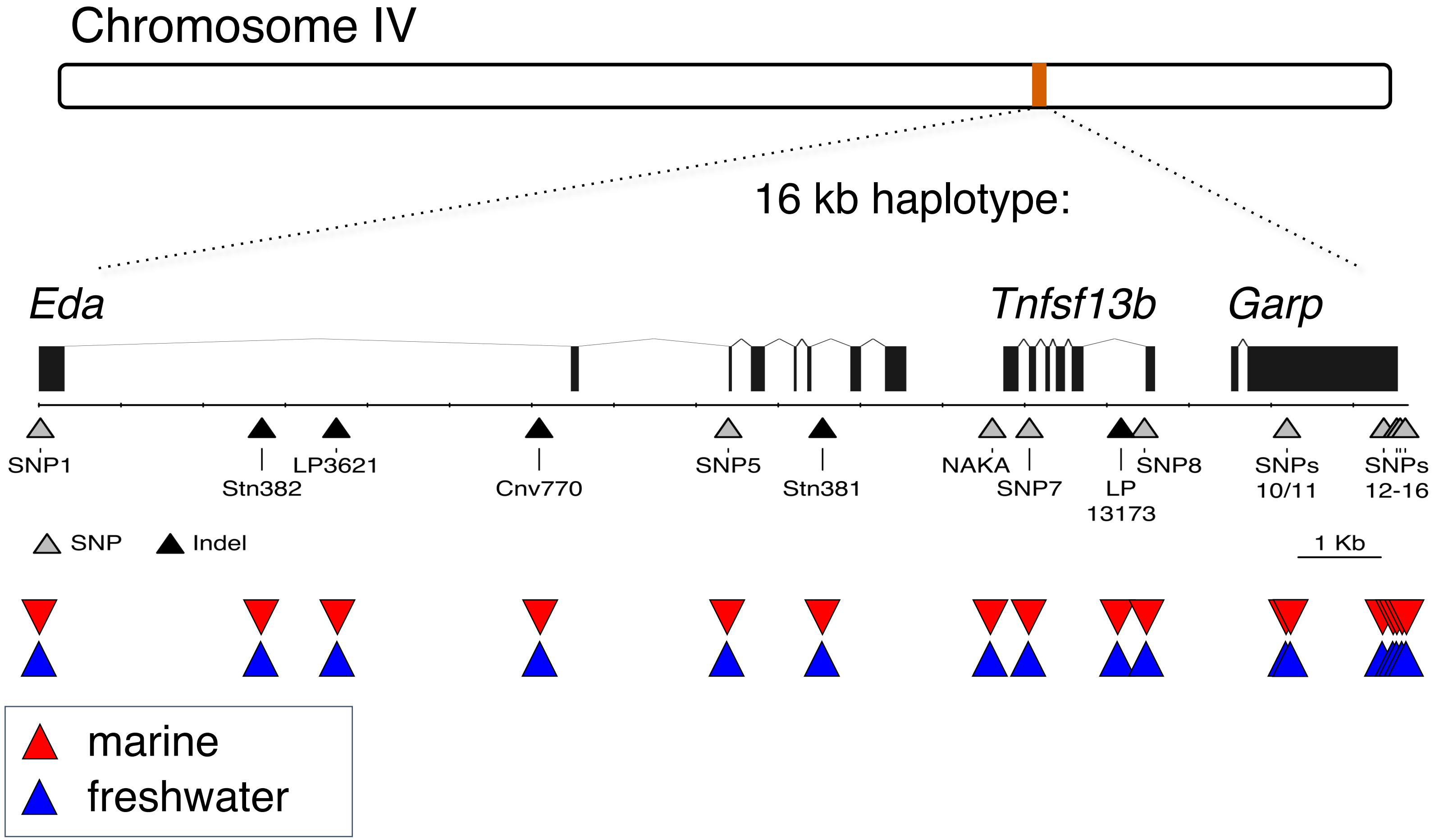
Jones et al. (2012) *Nature*

Genomic divergence on chromosome IV



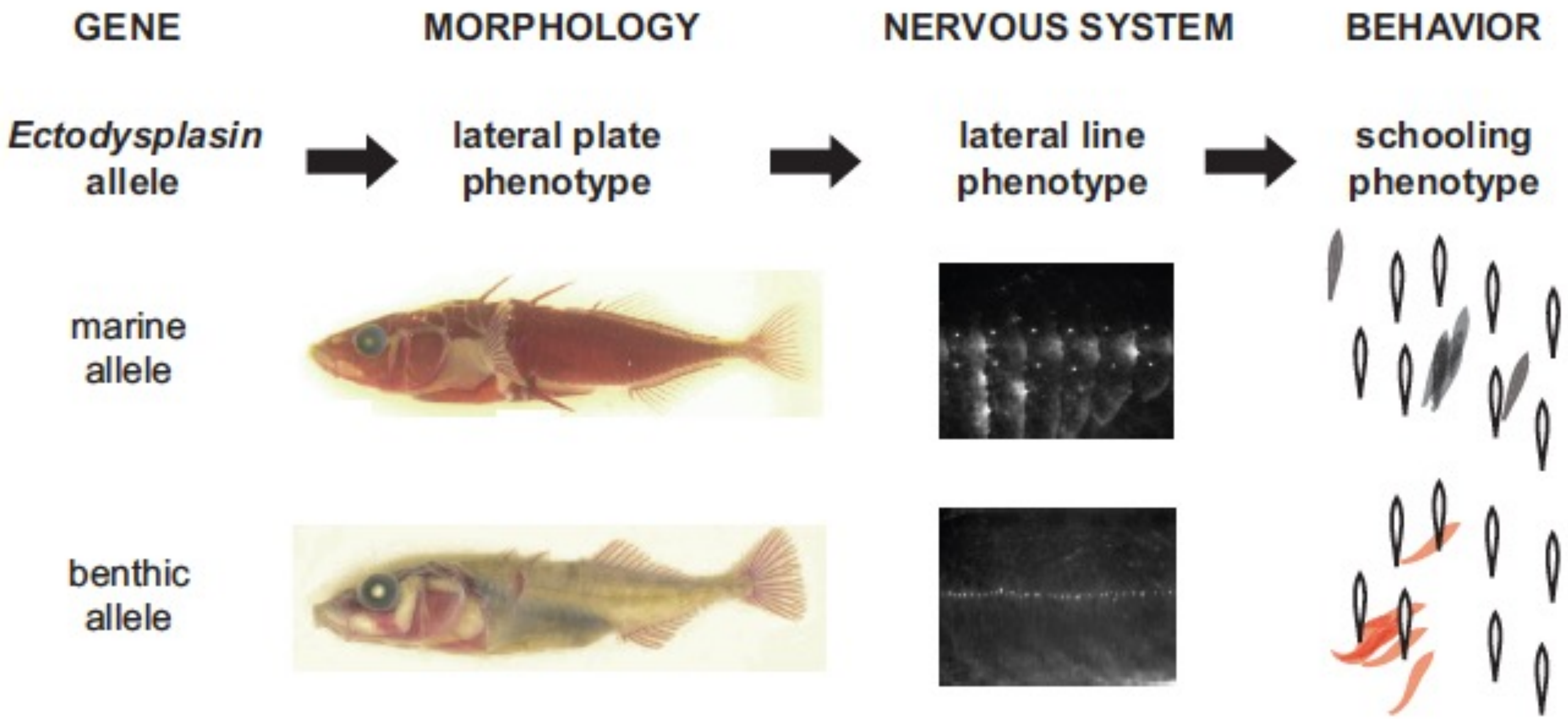
Jones et al. (2012) *Nature*

The 16 kb haplotype contains three protein coding genes and 17 polymorphisms



Colosimo et al. (2005) *Science*; O'Brown et al. (2015) *eLife*; Lowe et al. (2018) *Genome Research*

Transgenic studies show that the *Eda* gene affects three phenotypes



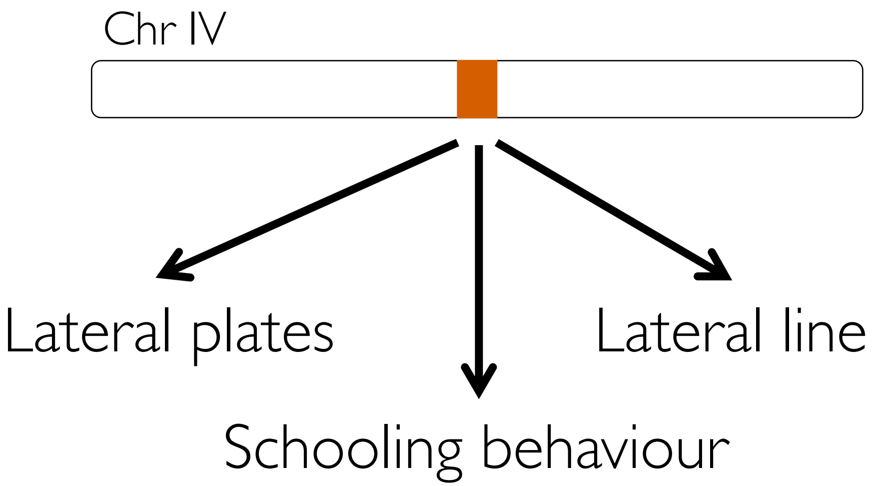
Colosimo et al. (2005)
Science

Mills et al. (2014)
EvoDevo

Greenwood et al. (2016)
Genetics

Master mutation or mass of mutations in *Eda*?

Pleiotropy



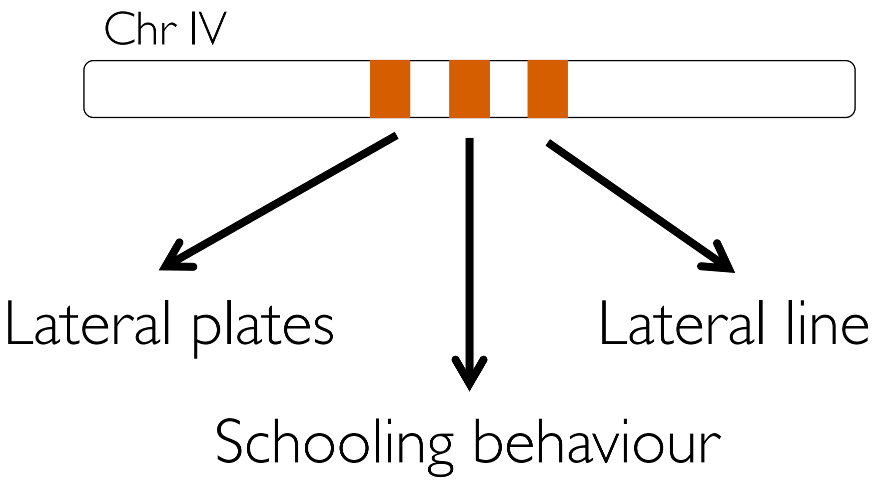
Chr IV

Lateral plates

Lateral line

Schooling behaviour

Linkage



Chr IV

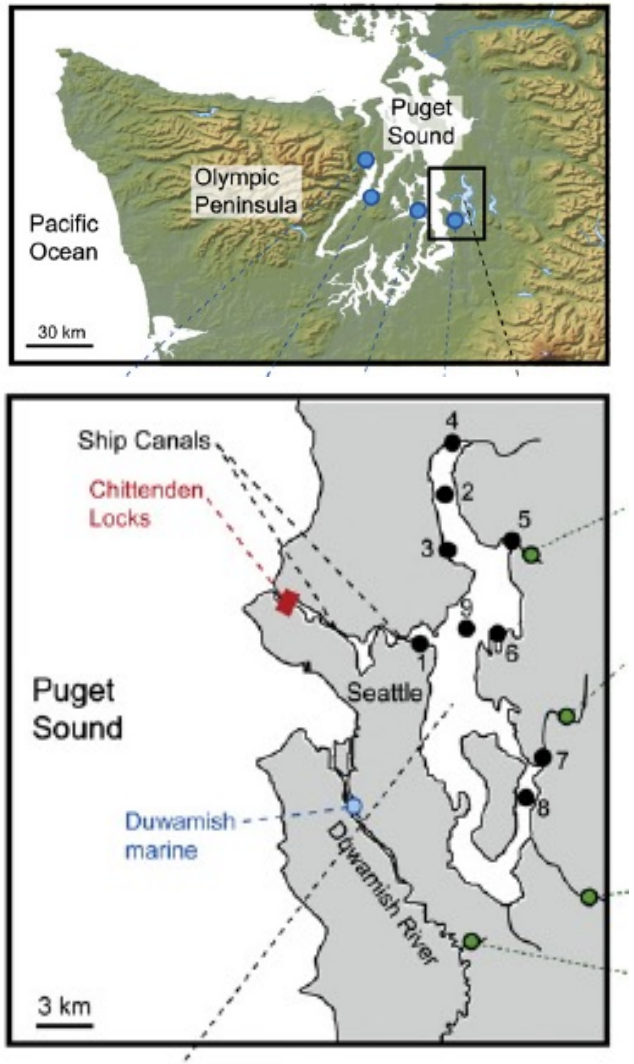
Lateral plates

Lateral line

Schooling behaviour

Approach:
finer recombination mapping, followed by functional experiments

Association mapping in Lake Washington



■ Completely plated

MM = CC

□ Partially plated

MF = CL

□ Low plated

FF = LL

Kitano et al. (2008) *Current Biology*

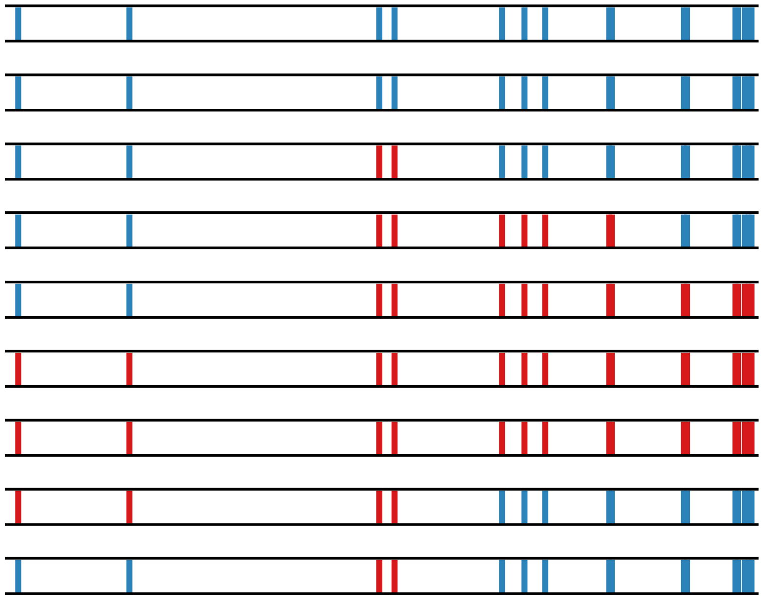
Association mapping in Lake Washington

Plate phenotypes:

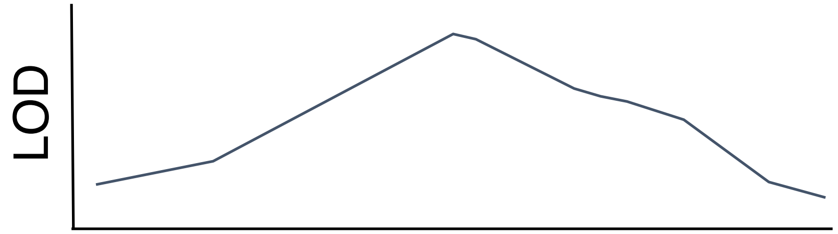
Alleles

🟥 marine = complete
🟦 freshwater = low

haplotype markers:



Lateral plates:



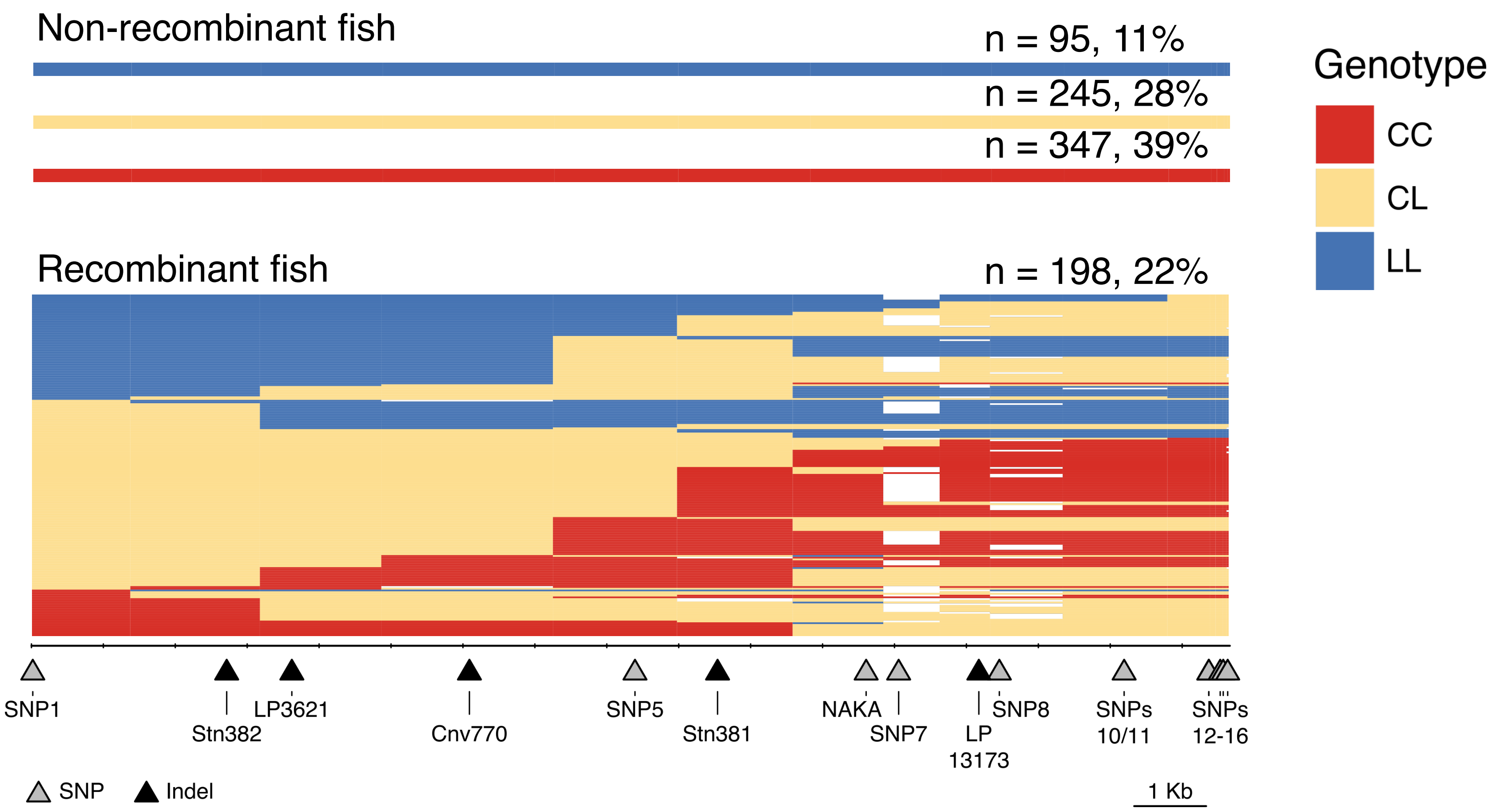
Collecting in Lake Washington

- Randomly sampled ~900 fish in 2015 and 2016
- Genotyped with markers in the 16kb haplotype
- Phenotyped for all traits known to map to QTL cluster on chr IV


Hilary Poore

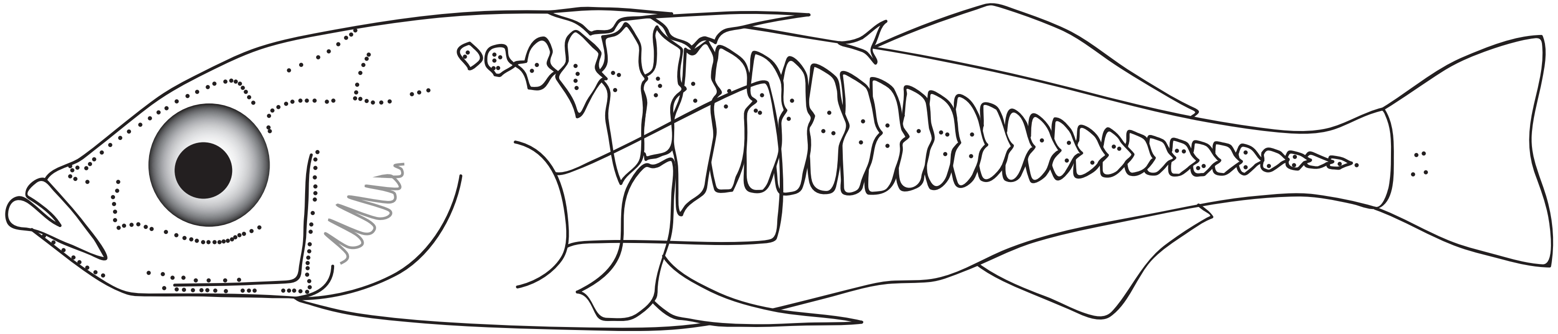
Archambeault et al. (2020) *Evolution Letters*

Recombination in the 16 kb haplotype



Archambeault et al. (2020) *Evolution Letters*

Phenotyping traits that map to chromosome IV

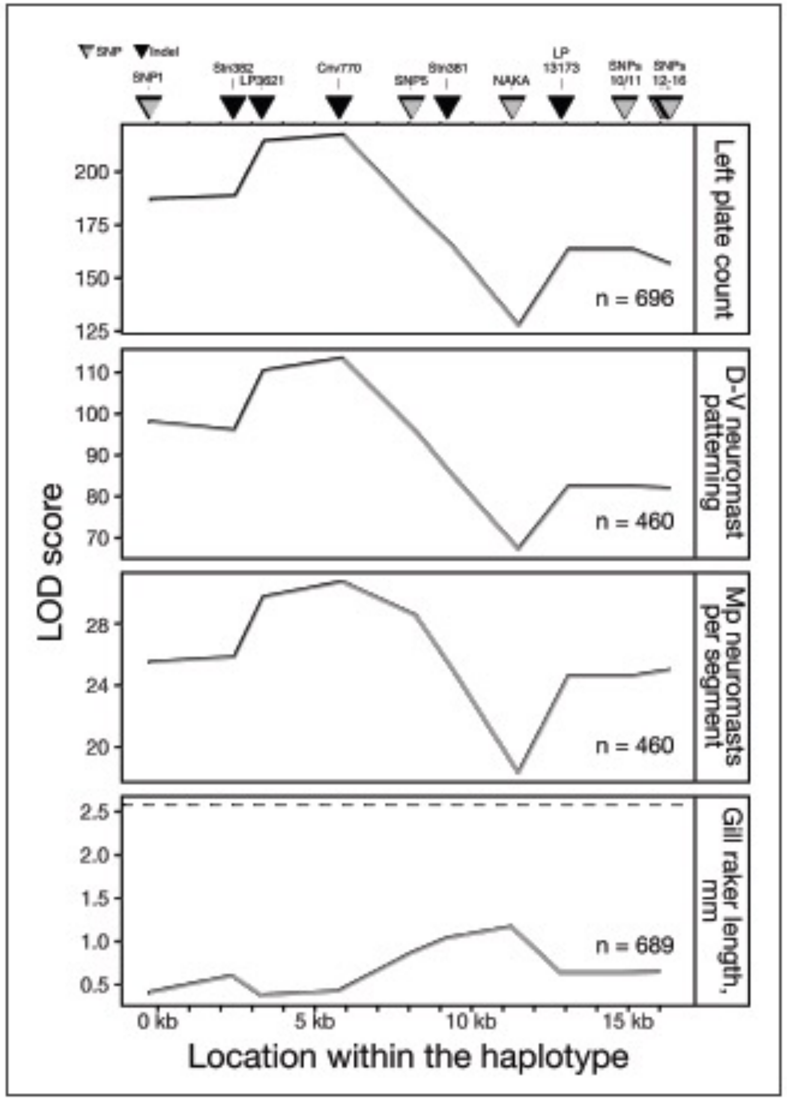


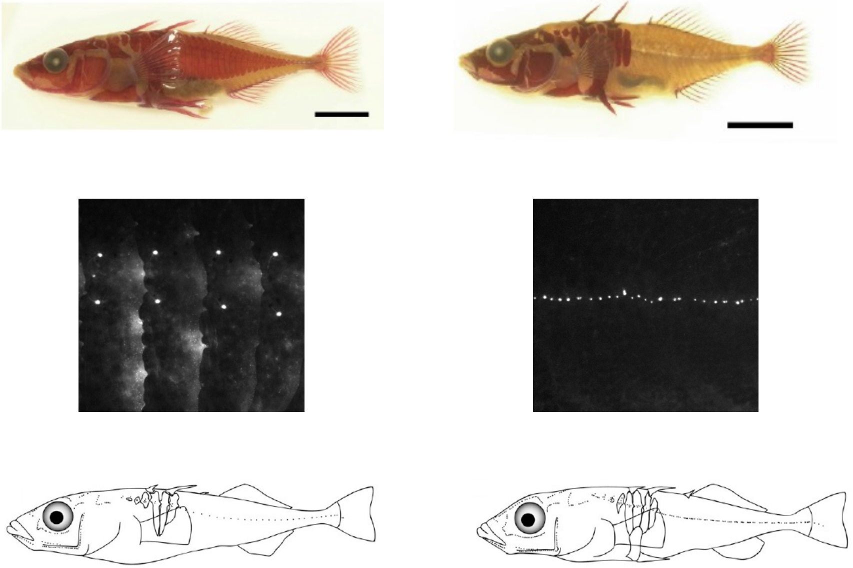
124 total traits (41 previously mapped to chr IV):

- Lateral bony plate number
- Neuromast patterning
- Neuromast numbers in various lateral lines
- Dorsal, anal & pelvic spine lengths
- Dorsal & anal fin ray numbers
- Gill raker number & length
- Tooth number
- Body shape
- Jaw size and shape
- Ectodermal bony plate sizes

Luis Bärtschi

Pleiotropic effects on lateral plates and lateral line

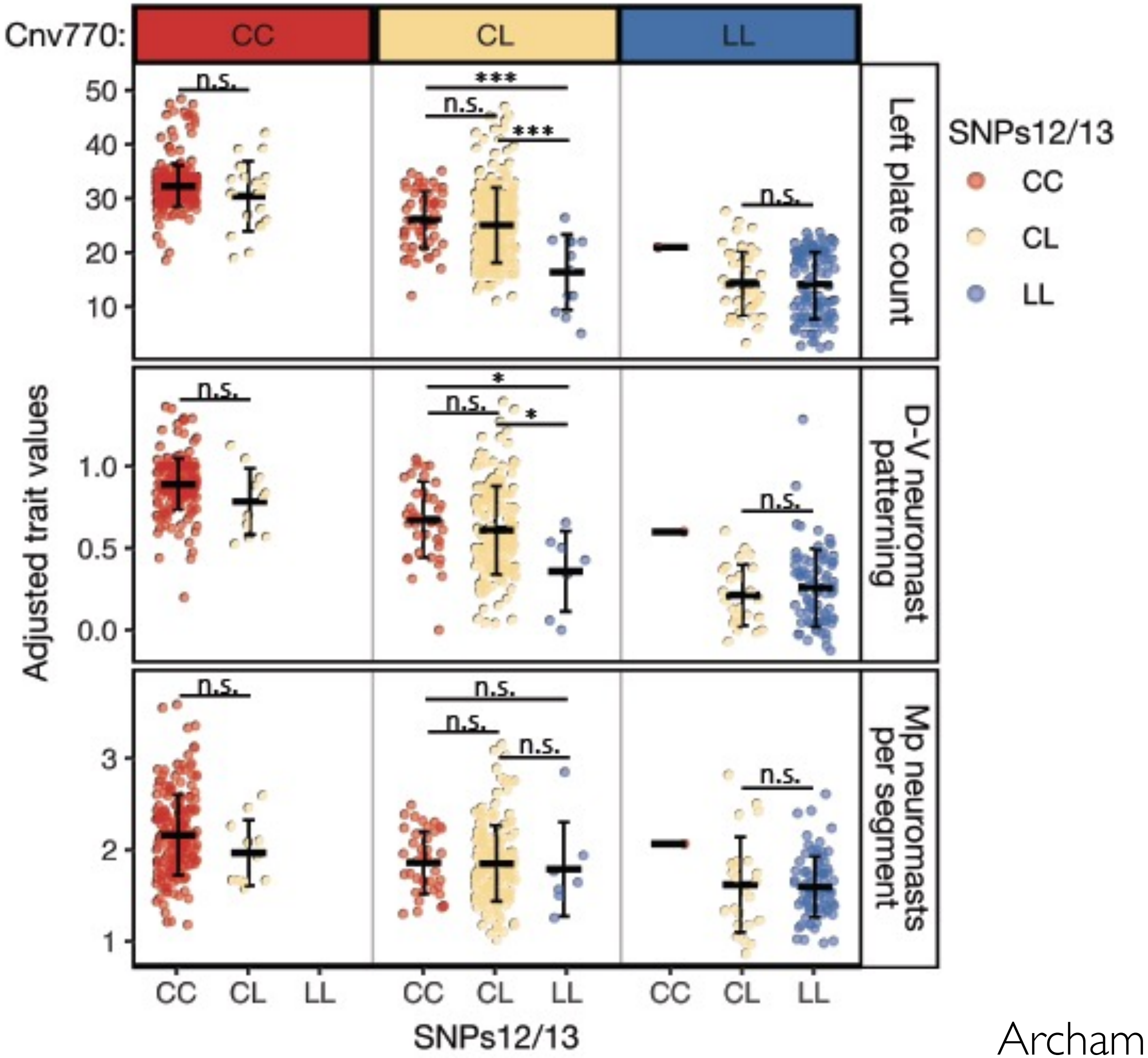




No other traits mapped!

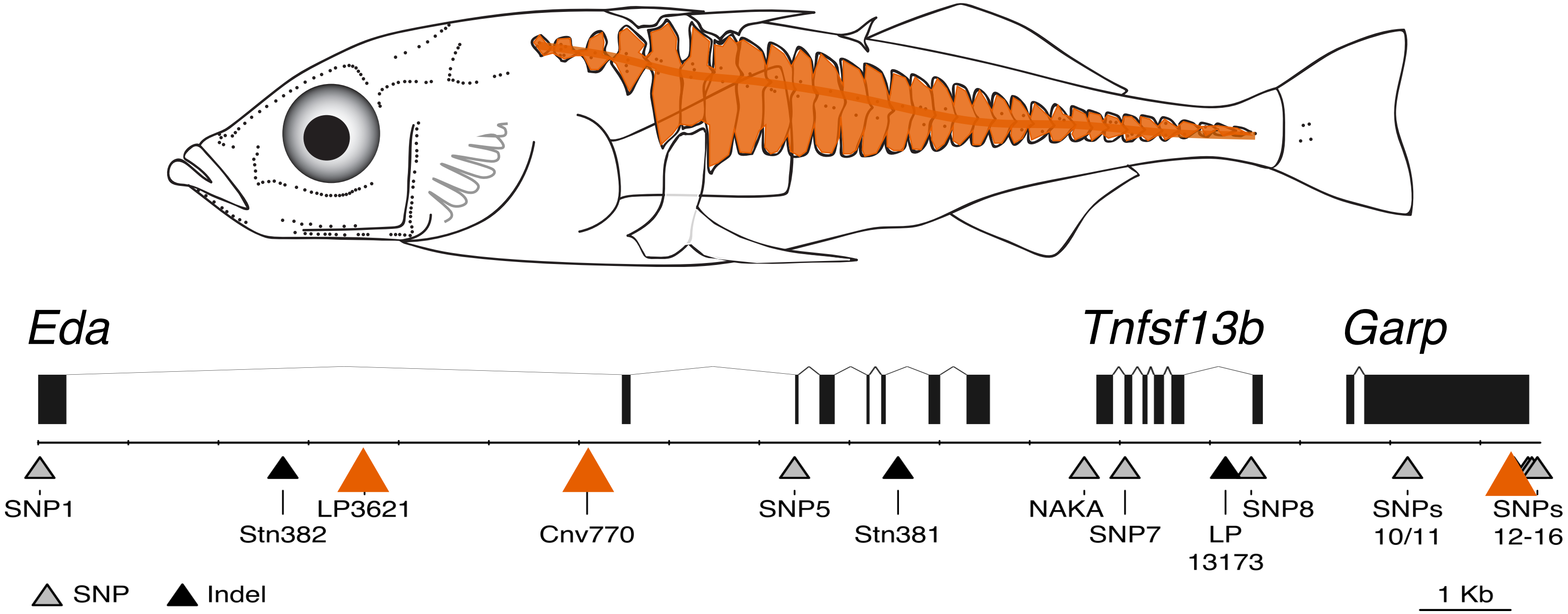
Archambeault et al. (2020) *Evolution Letters*

Linked effects on lateral plates and lateral line



Archambeault et al. (2020) *Evolution Letters*

Pleiotropy! and a little linkage

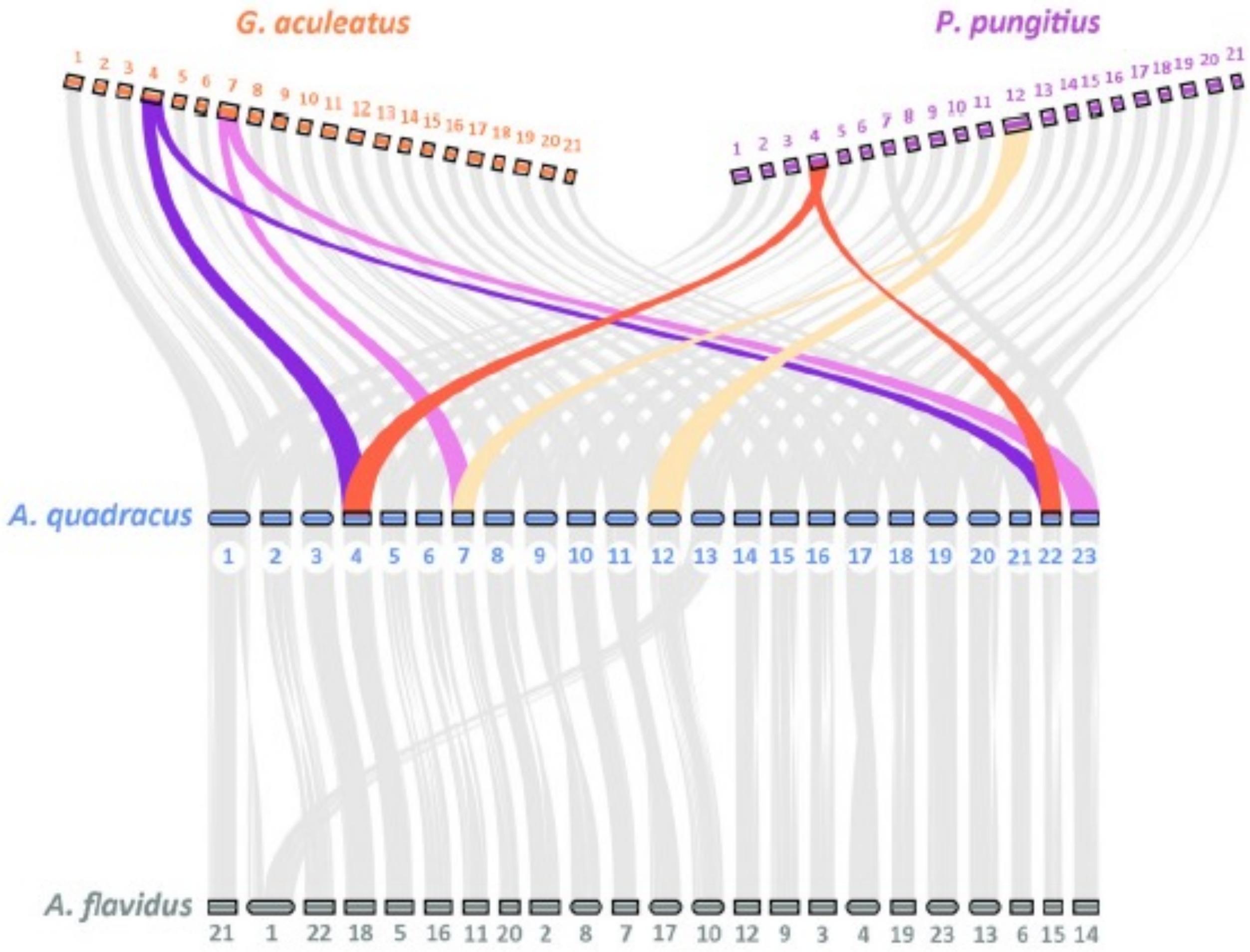


Archambeault et al. (2020) *Evolution Letters*

Conclusions and open questions

- Evidence for pleiotropic effects of a single mutation in *Eda*
  - What is the causative mutation?
    - CRISPR/Cas9 experiments ongoing
  - Are other phenotypes associated with variation in the *Eda* locus?
    - Currently investigating immune/mucous phenotypes
- But the QTL cluster on chromosome IV is not only due to pleiotropic effects of *Eda* locus
  - How are such adaptive clusters of linked alleles built?

Clusters on chromosomes 4 and 7 result from chromosomal fusions in the threespine lineage

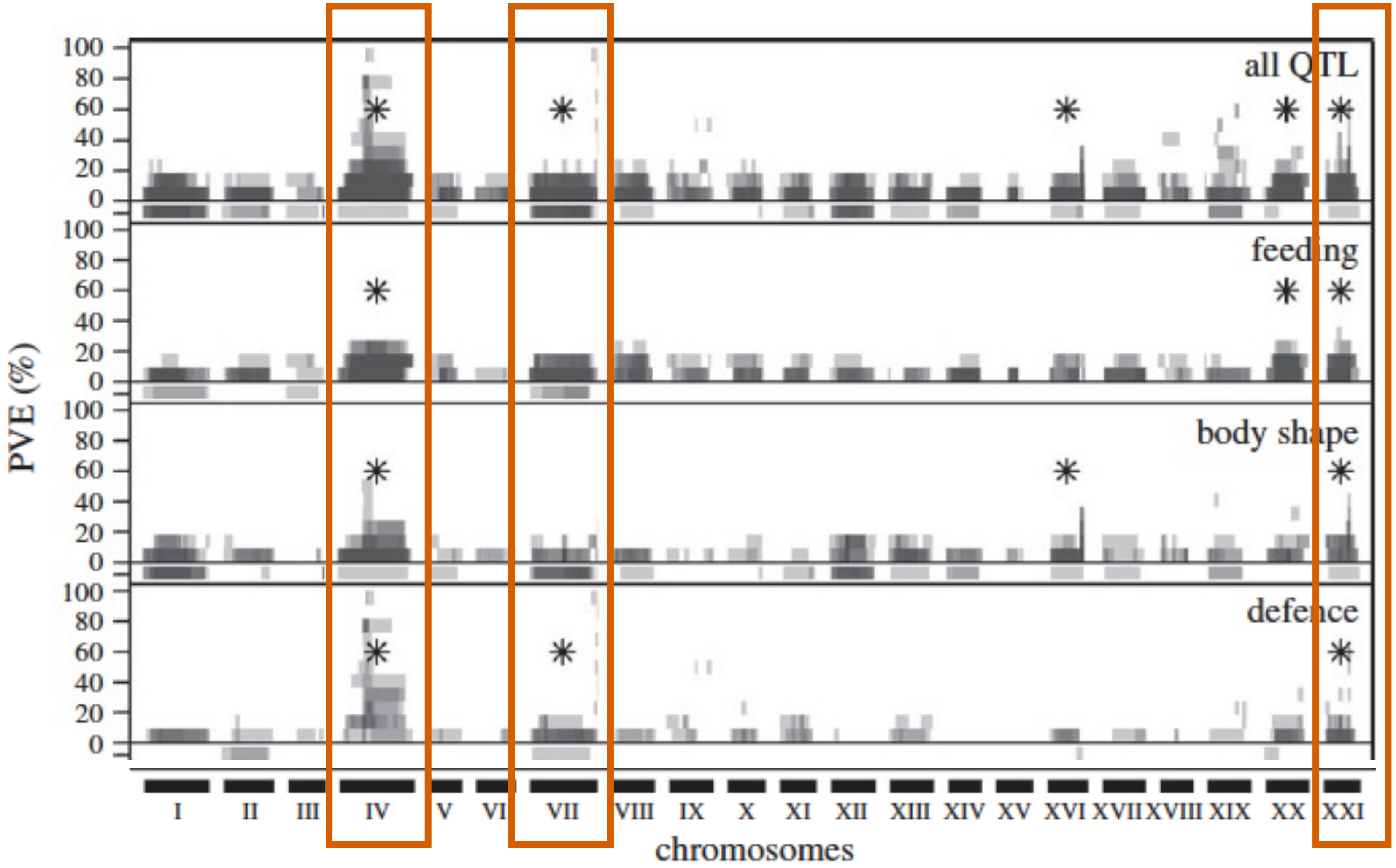


Liu et al. (2022) *Molecular Biology and Evolution*

QTL clusters overlap with chromosomal fusions and inversions

Inversion!

Swiss National Science Foundation



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

Specific questions

- Does adaptation occur via a few mutations of large effect, or many mutations of small effect?
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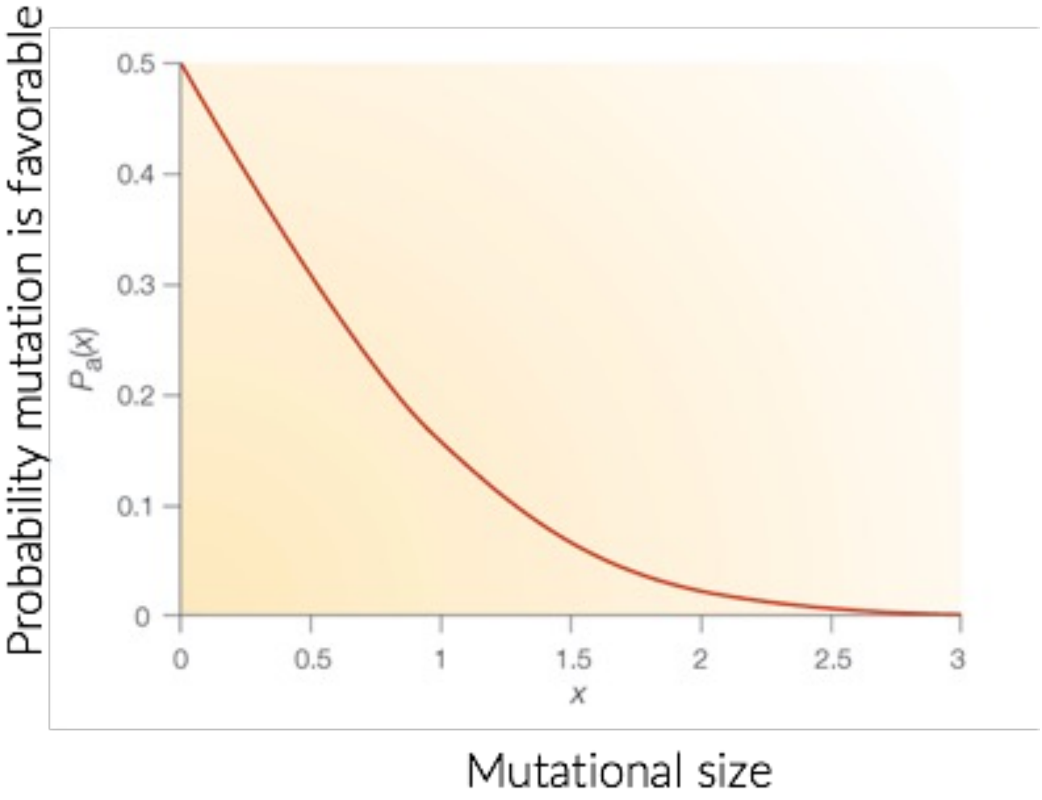
Pleiotropy is proposed to be a constraint in adaptation



Mutational size is dependent on the number of characters affected = pleiotropy

RA Fisher (1930) *The Genetical Theory of Natural Selection*

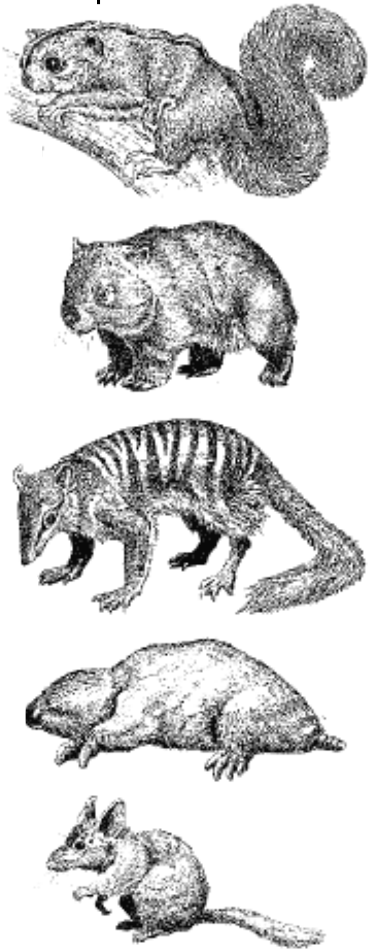
Does pleiotropy facilitate or constrain adaptation?

Diana Rennison
Asst Prof, UC San Diego

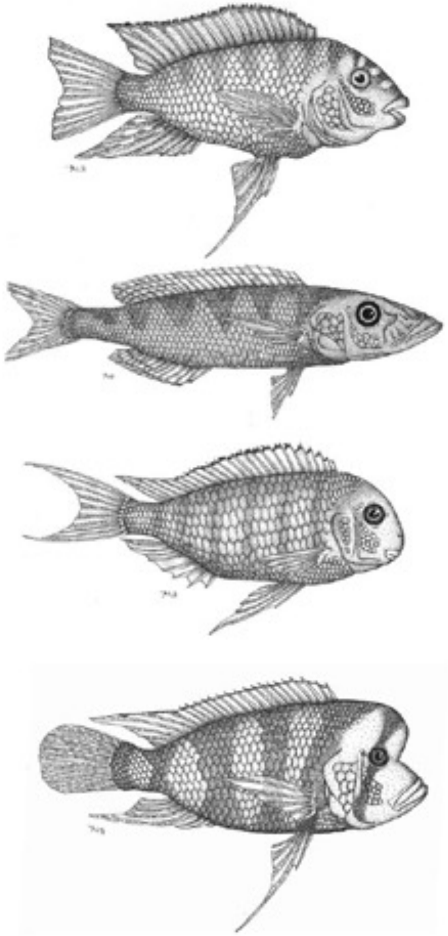
Repeated evolution provides a test

Placentals

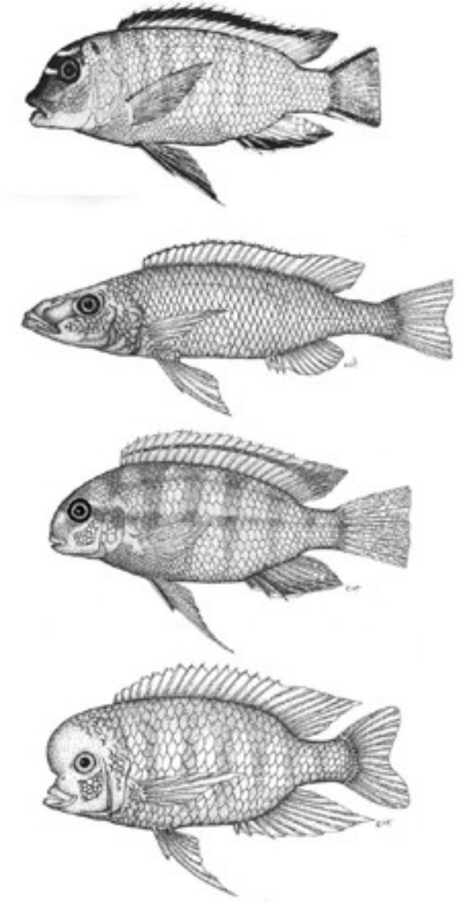
Marsupials



Lake Tanganyika

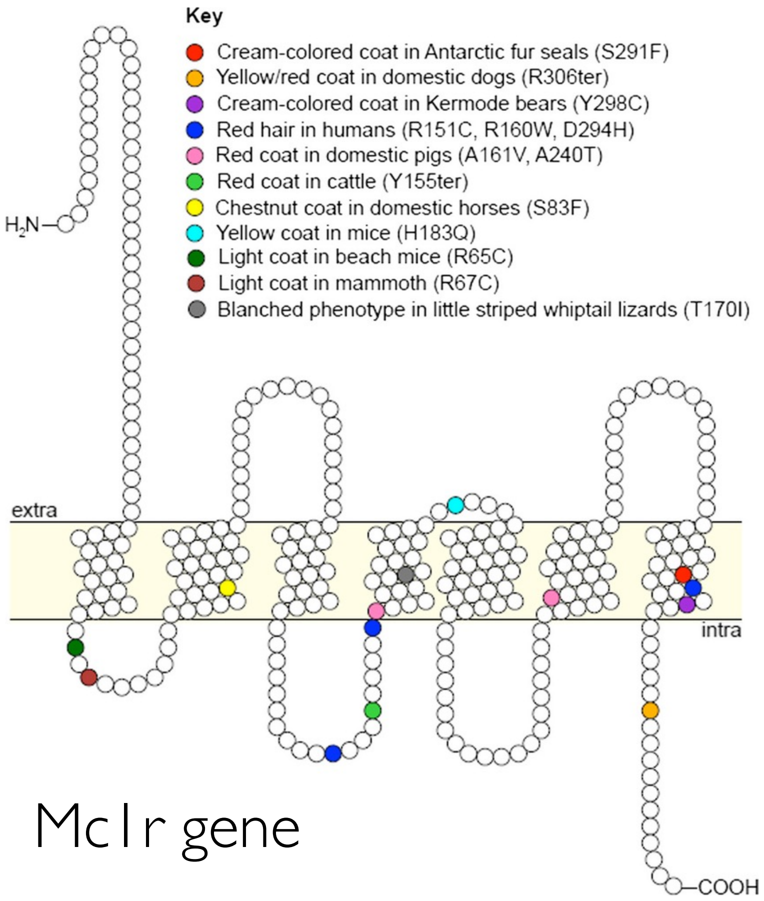


Lake Malawi



Evolution also repeats itself at the genetic level

Hoekstra *Heredity* 2006



Mc1r gene

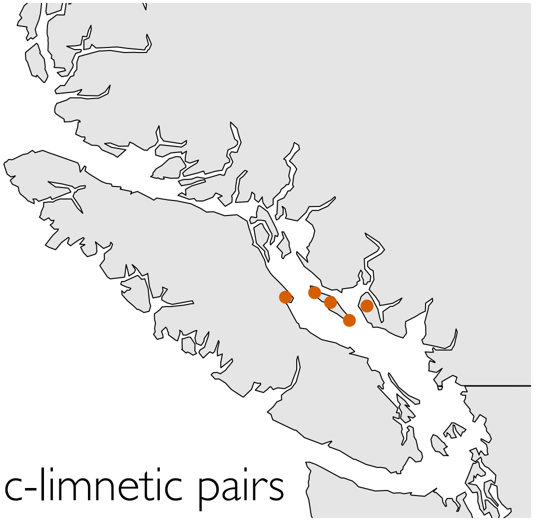
Peters et al. *Ecology & Evolution* 2016

Why is evolution repeatable at the genetic level?

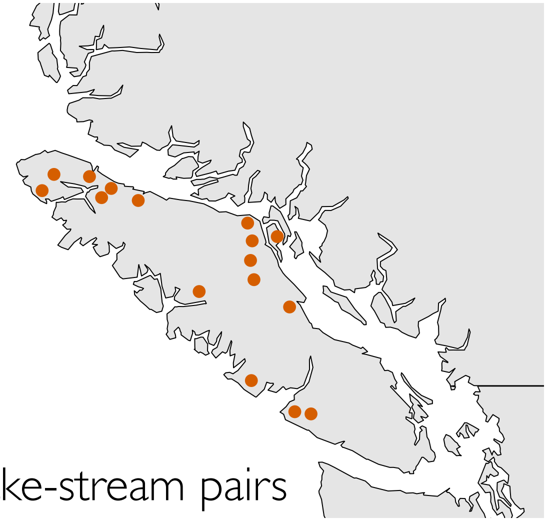
1) Only one gene or mutation can produce the phenotype favoured by selection

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

Repeated evolution of freshwater species pairs



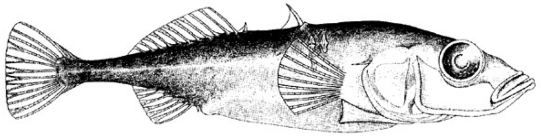
Benthic-limnetic pairs



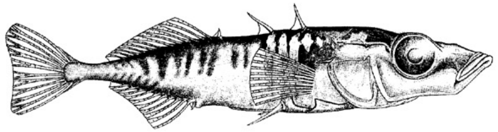
Lake-stream pairs

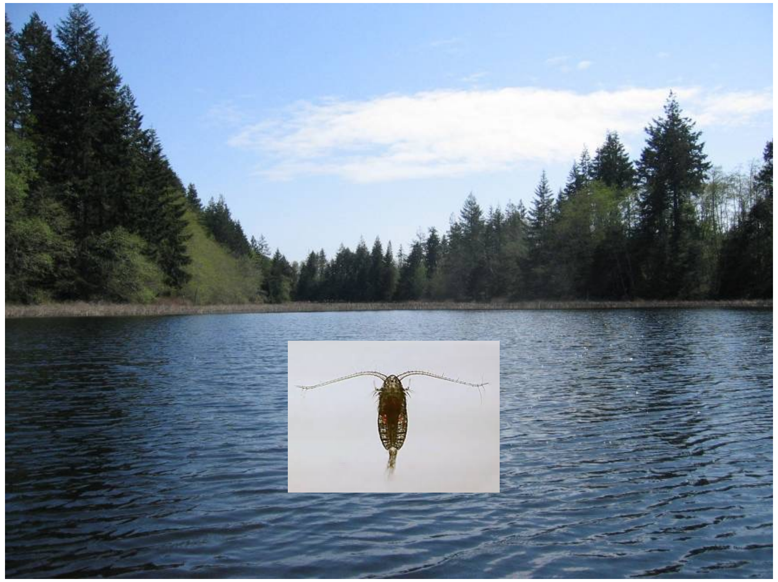
Benthic-limnetic pairs

benthic



limnetic



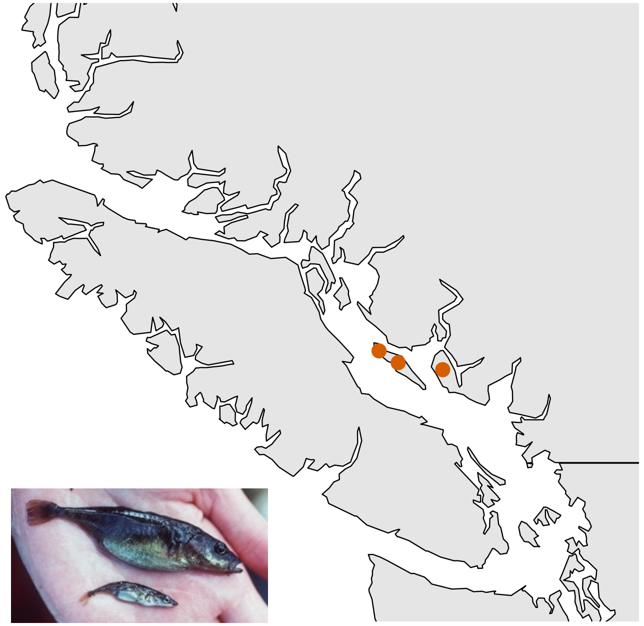


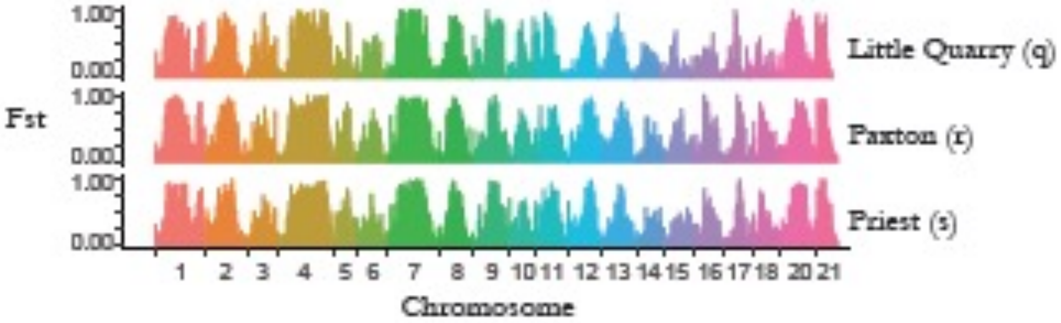
- Benthic-limnetic stickleback from 3 watersheds
- Genotyping-by-sequencing data from 20 individuals/population
- Estimated genome-wide F_{st} from ~35,000 SNPs

Lake-stream pairs

- Lake-stream stickleback from 16 watersheds
- Double enzyme RAD-seq from 24 individuals/population
- Estimated genome-wide F_{st} from ~68,000 SNPs

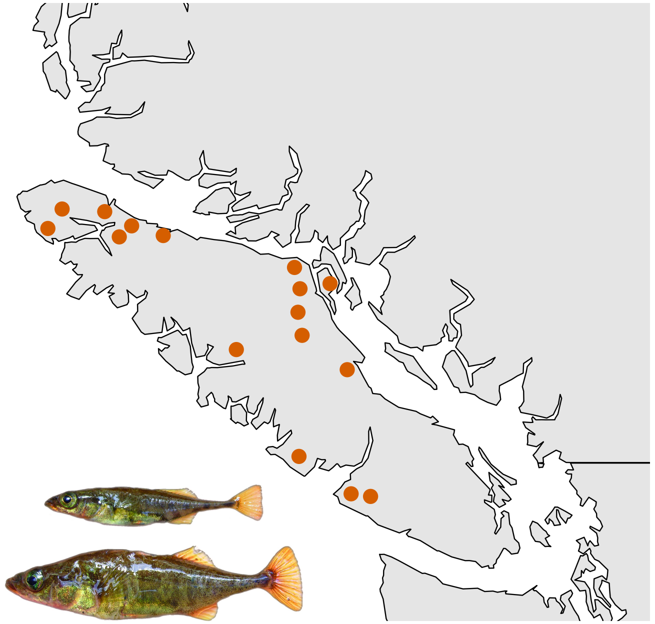
Repeatable genomic differentiation in benthic-limnetic pairs

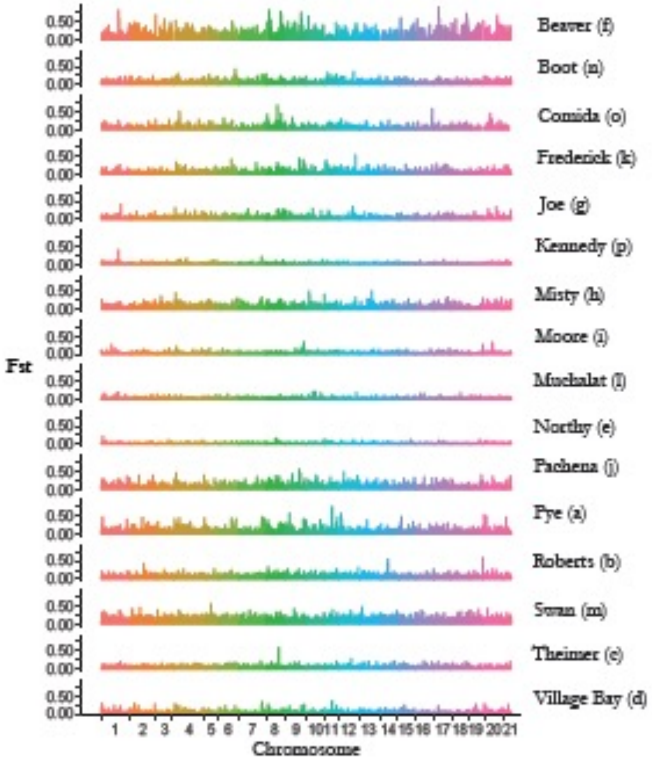




Rennison & Peichel (2022) *Molecular Ecology*

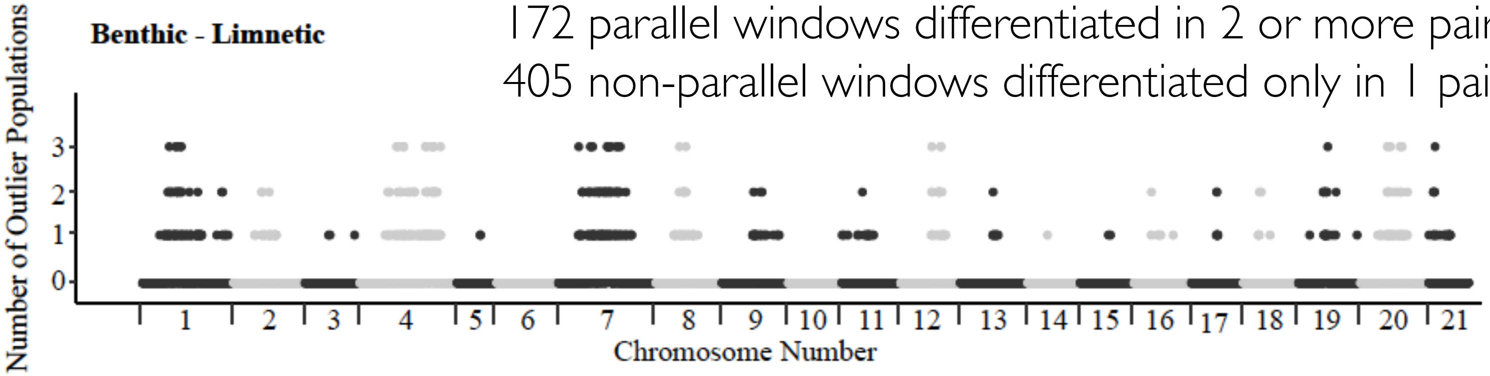
Repeatable genomic differentiation in lake-stream pairs





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

Parallel *vs* non-parallel windows (50 kbp)



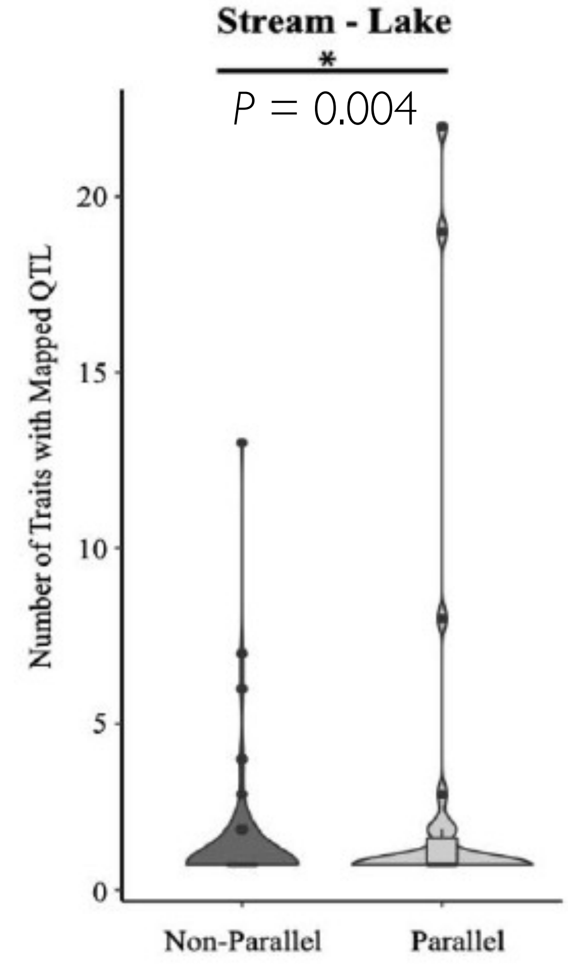
172 parallel windows differentiated in 2 or more pairs
405 non-parallel windows differentiated only in 1 pair

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?

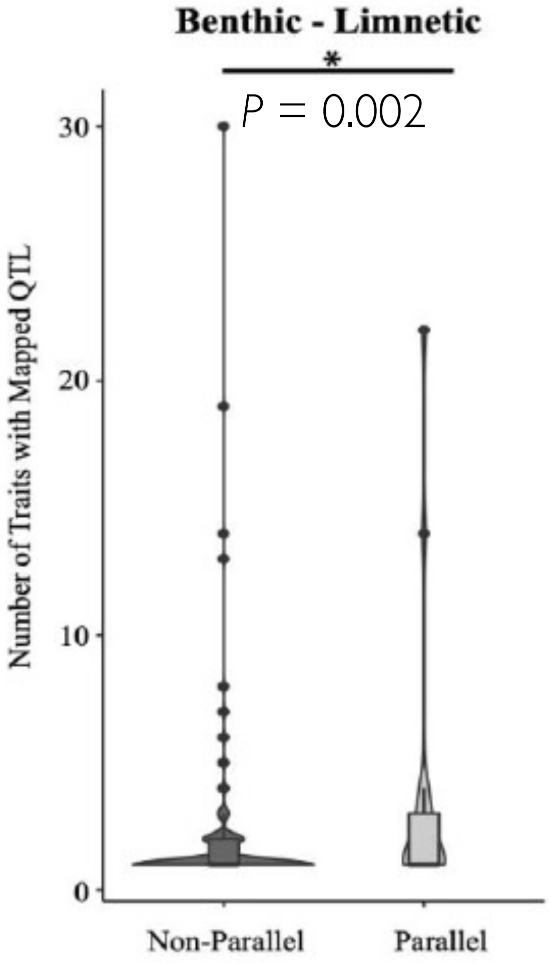
Parallel windows contain more QTL



Stream - Lake

P = 0.004

1.49 ± 0.11 2.96 ± 1.1



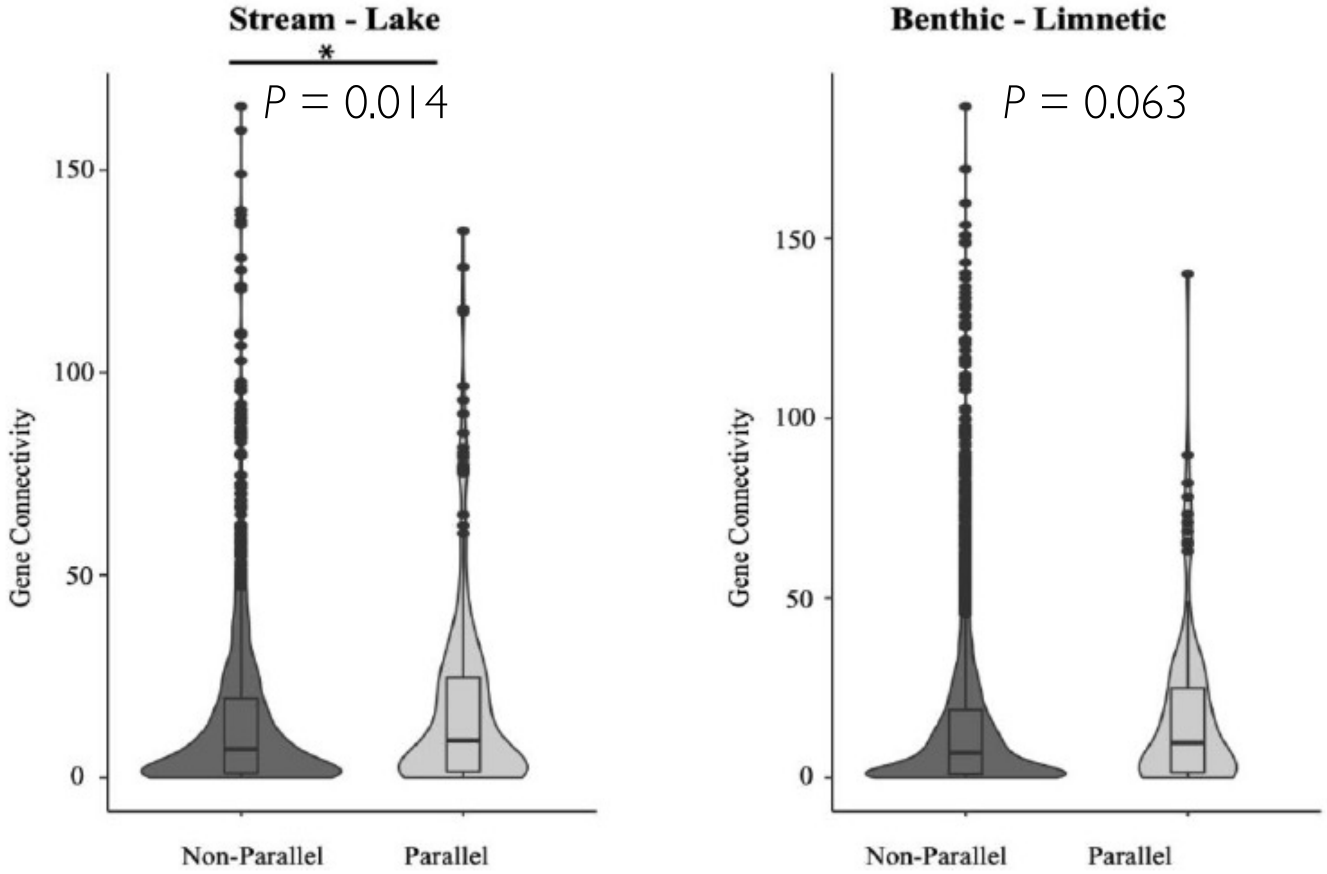
Benthic - Limnetic

P = 0.002

1.68 ± 0.11 3.48 ± 1.2

Rennison & Peichel (2022) *Molecular Ecology*

Parallel windows contain genes with higher connectivity



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 (like *Eda*) might be maintained as standing variation in systems like stickleback in which repeated adaptation has occurred many times

Challenges and questions

- Directly measuring whether mutations have a pleiotropic effect on phenotypes is hard!
- Directly measuring whether mutations have a pleiotropic effect on fitness is even harder!
- Are there other approaches that might allow us to more broadly address the role of pleiotropy in adaptation?

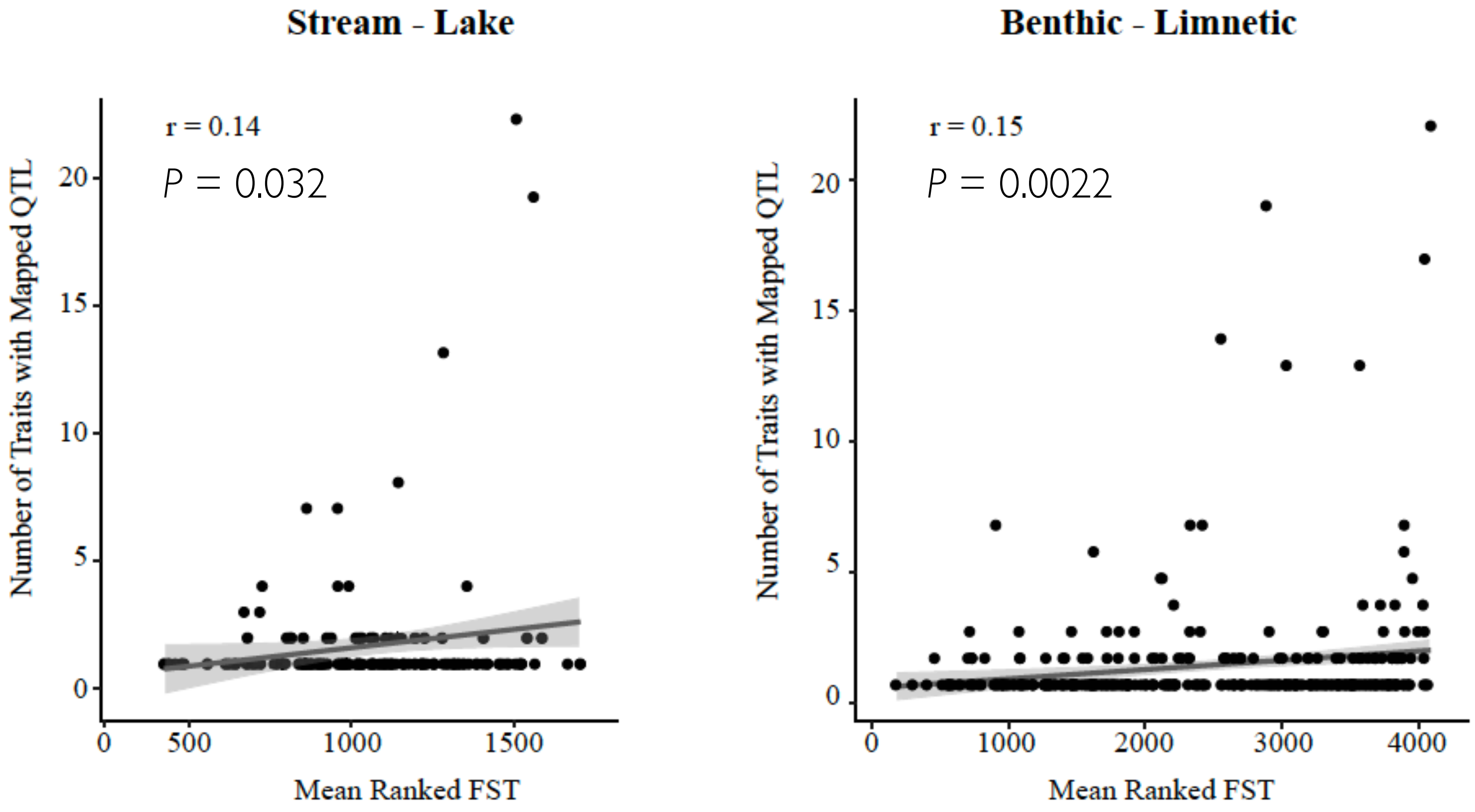
University of Bern
David Marques

Sophie Archambeault
Luis Bärtschi
Aurélie Merminod

Diana Rennison (now UCSD)

University of Washington
Dave Beauchamp
Casey Clark
Madi Gamble
Ted Pietsch

Parallel windows contain more QTL



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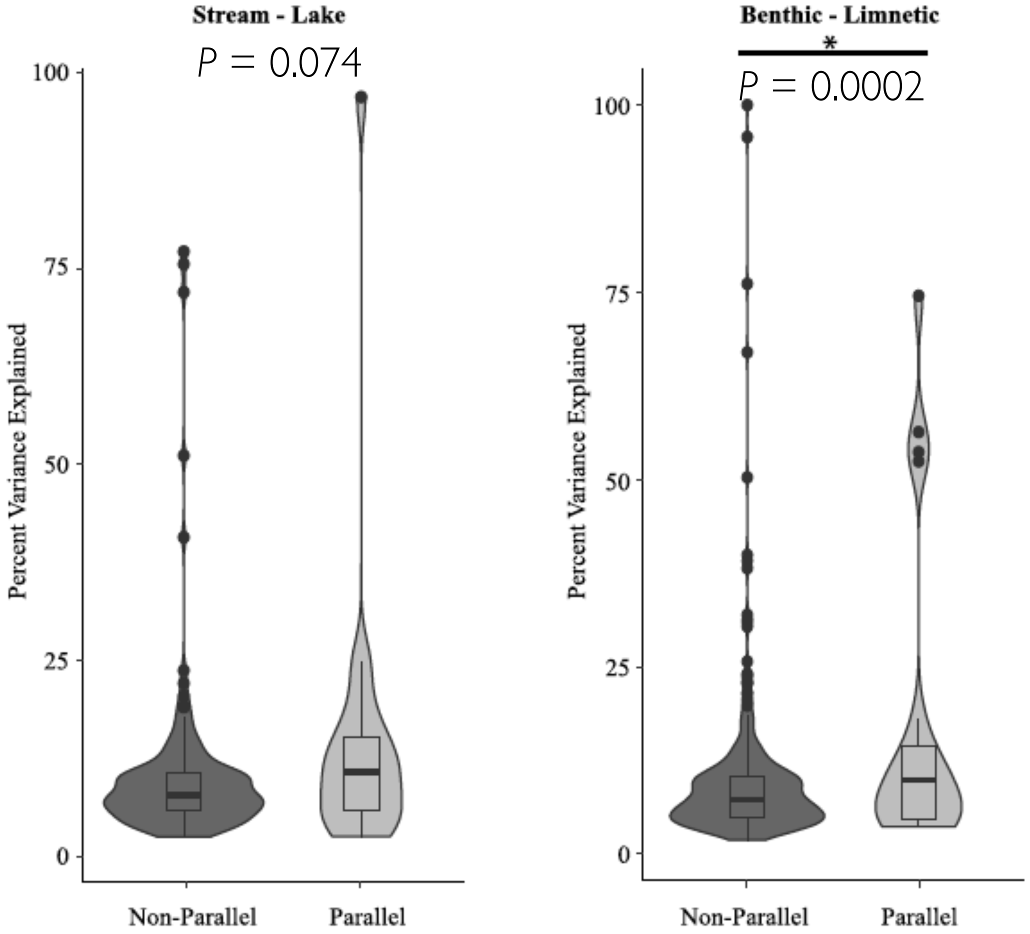
Parallel windows have a larger effect size



P = 0.074

P = 0.0002

9.48 ± 0.95

13.68 ± 4.14

9.02 ± 0.48

17.37 ± 4.7

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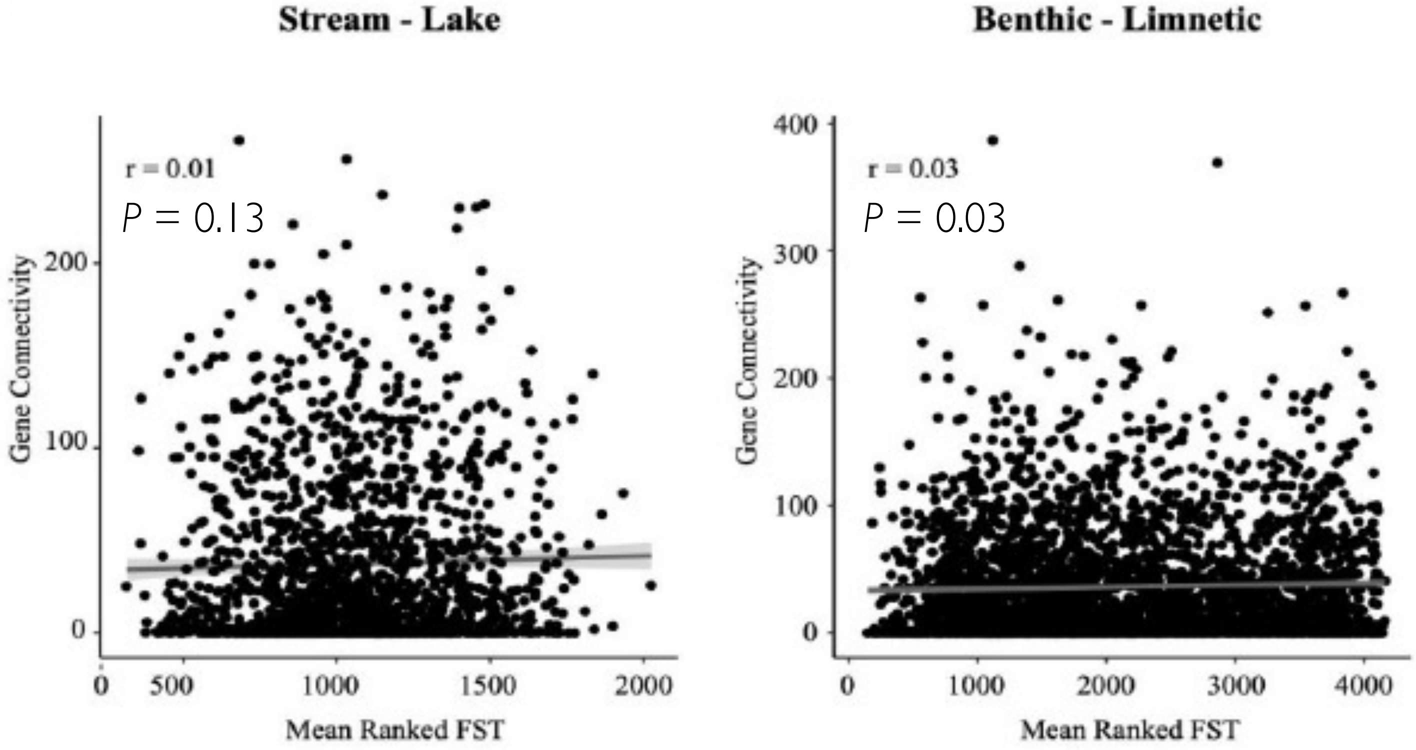
Parallel windows contain genes with higher connectivity



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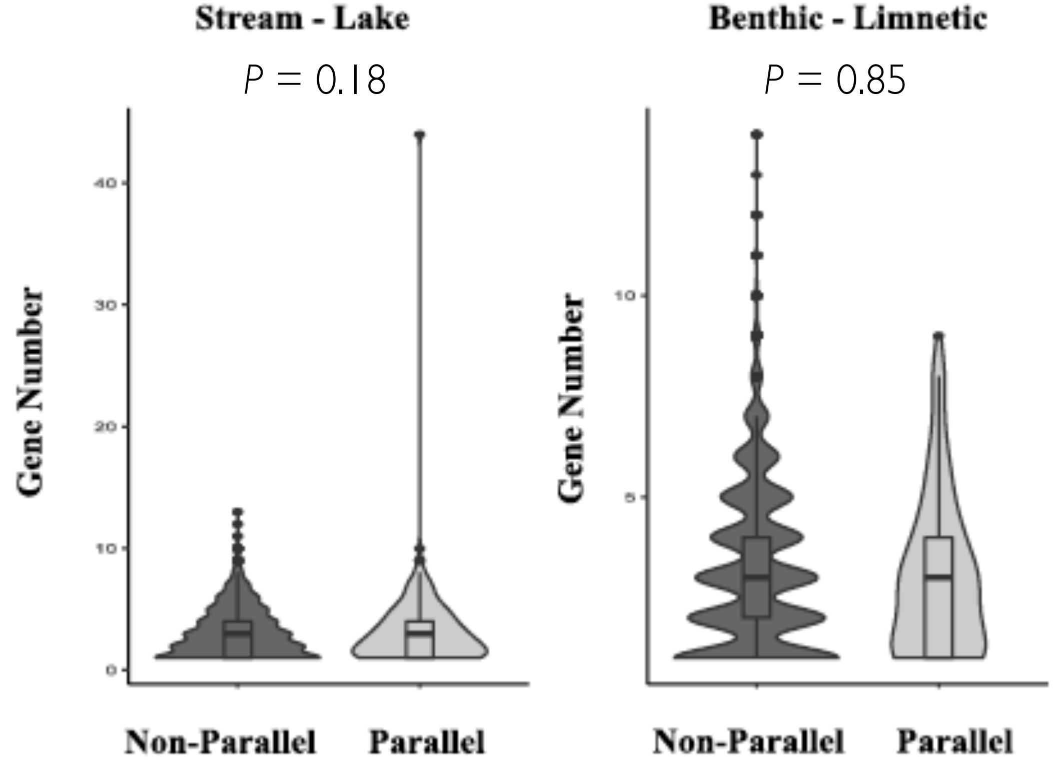
Parallel windows do not differ in gene number



$P = 0.18$

$P = 0.85$

Non-Parallel	Parallel	Non-Parallel	Parallel
3.1 ± 0.1	3.2 ± 0.2	3.2 ± 0.03	3.0 ± 0.2

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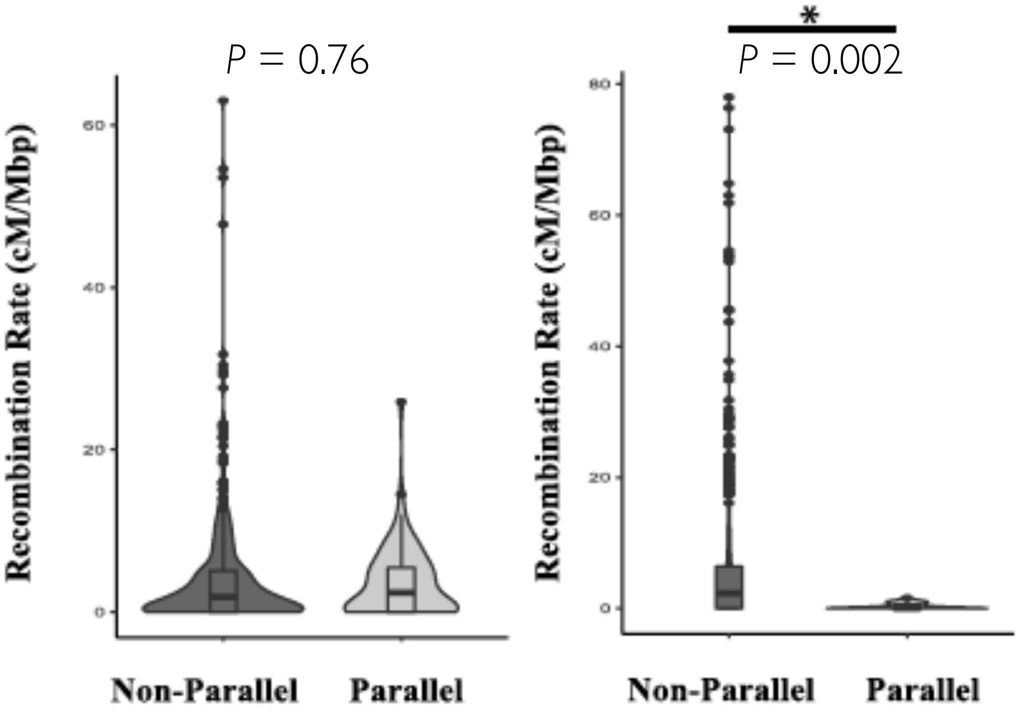
Parallel windows have lower recombination rate in benthic-limnetic pairs



P = 0.76

P = 0.002

4.24 ± 0.34

3.6 ± 0.51

4.86 ± 0.2

0.30 ± 0.1

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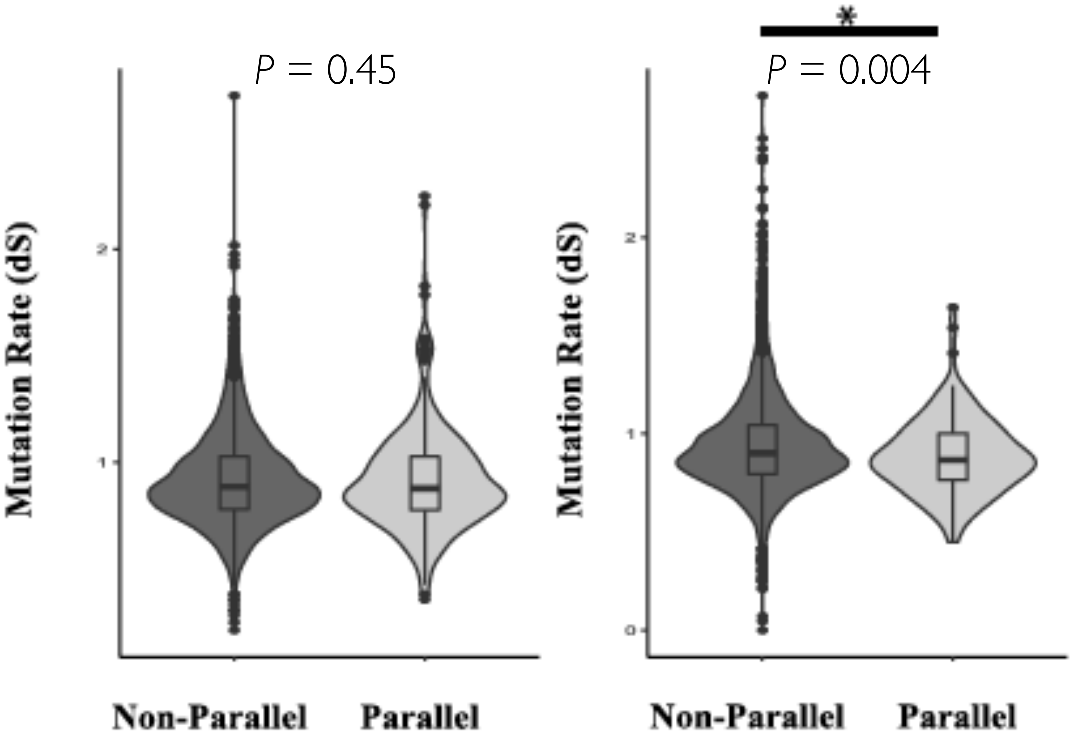
Parallel windows have lower mutation rate in benthic limnetic pairs



P = 0.45

0.92 ± 0.01
0.92 ± 0.01

P = 0.004

0.94 ± 0.003
0.89 ± 0.02

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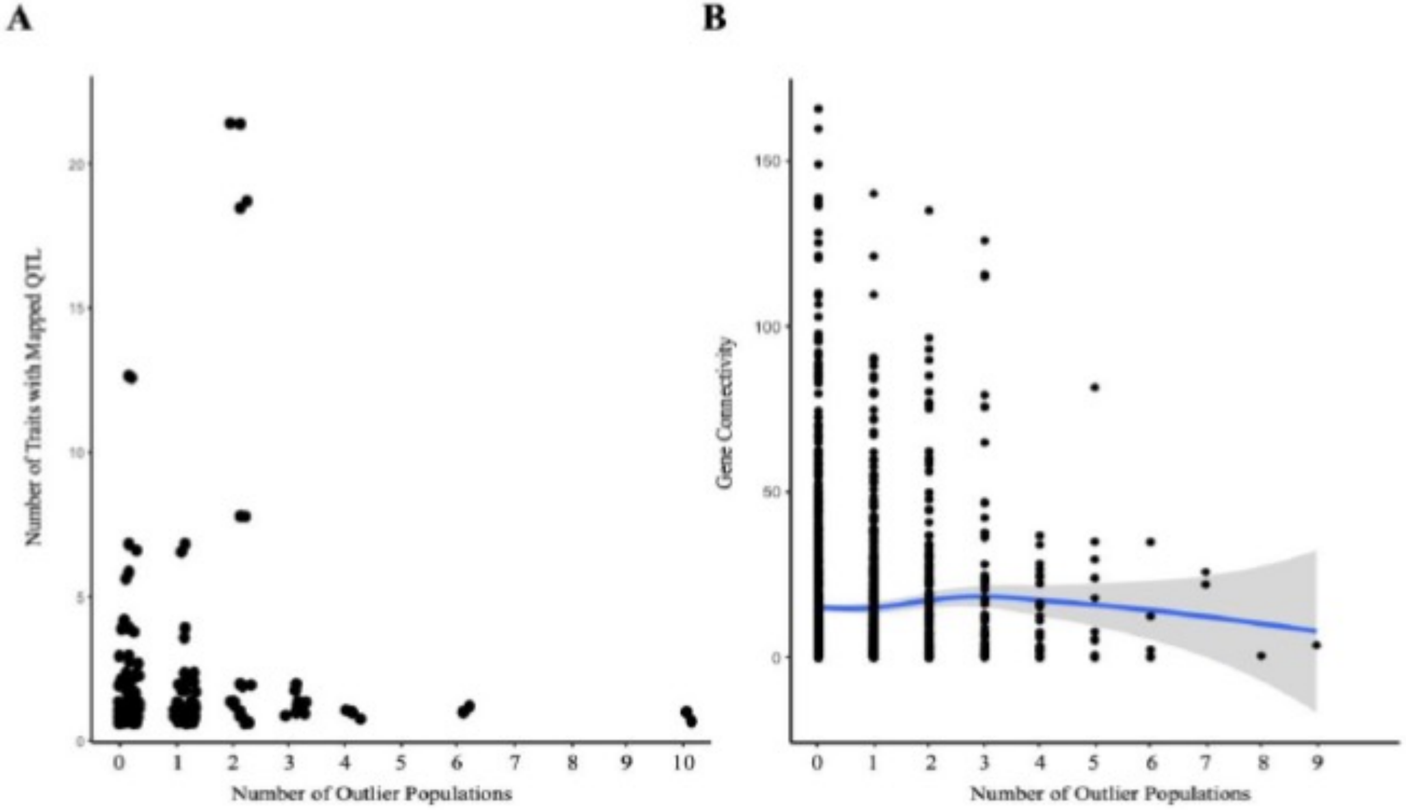
Pleiotropy is highest at intermediate levels of parallelism



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