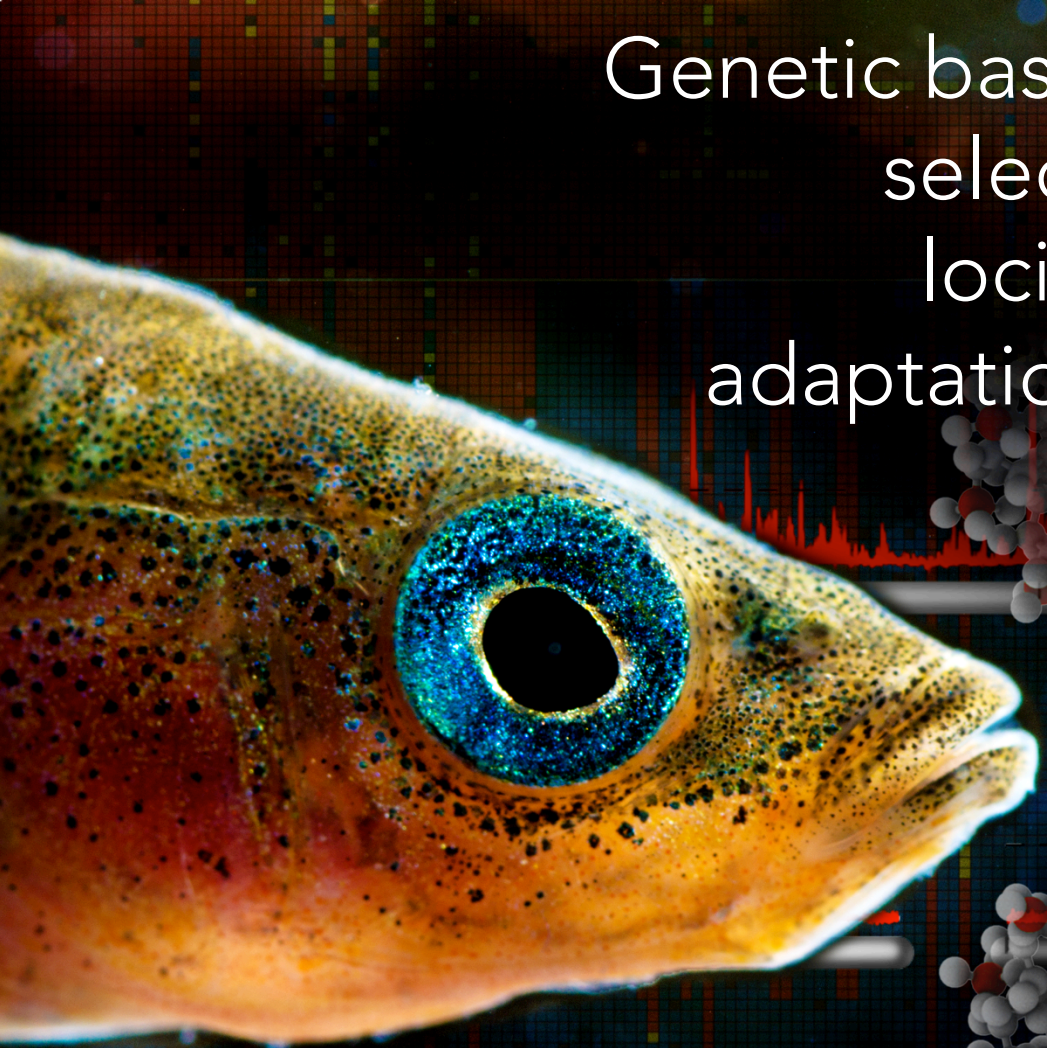
Genetic basis and signatures of selection around loci underlying adaptation and speciation

Felicity Jones

Friedrich Miescher Laboratory of Max Planck Society

Tübingen, Germany



Male threespine stickleback

DFG Deutsche Forschungsgemeinschaft

erc European Research Council Consolidator Grant

MAX-PLANCK-GESELLSCHAFT

fml

What is the molecular basis of adaptive divergence?

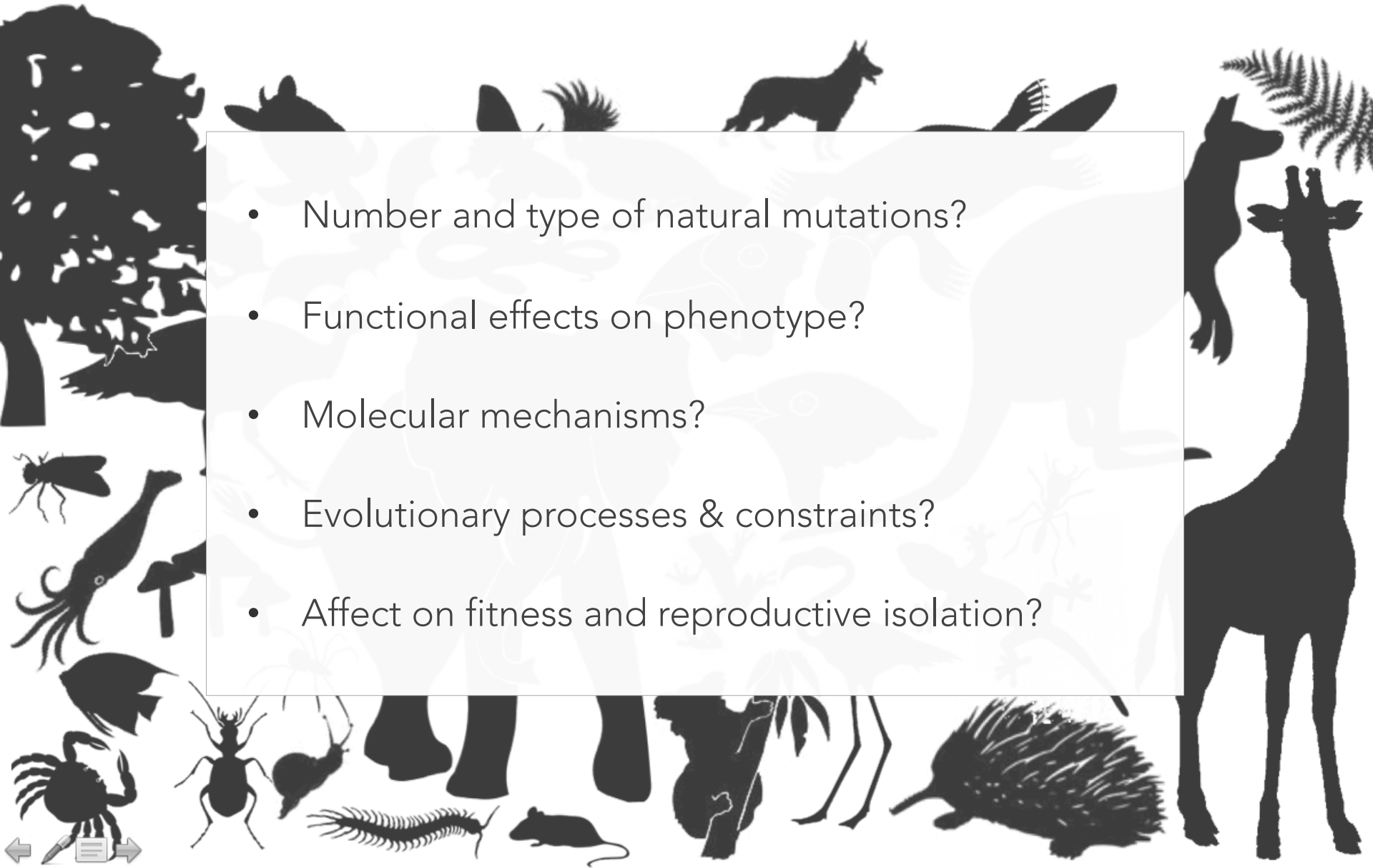
Two parts:

1. Methods used to identify loci underlying adaptive divergence
   - Forward genetic mapping
   - Population genomics

2. CASE STUDIES of molecular signatures of selection around known loci
   - EDA
   - Pitx1

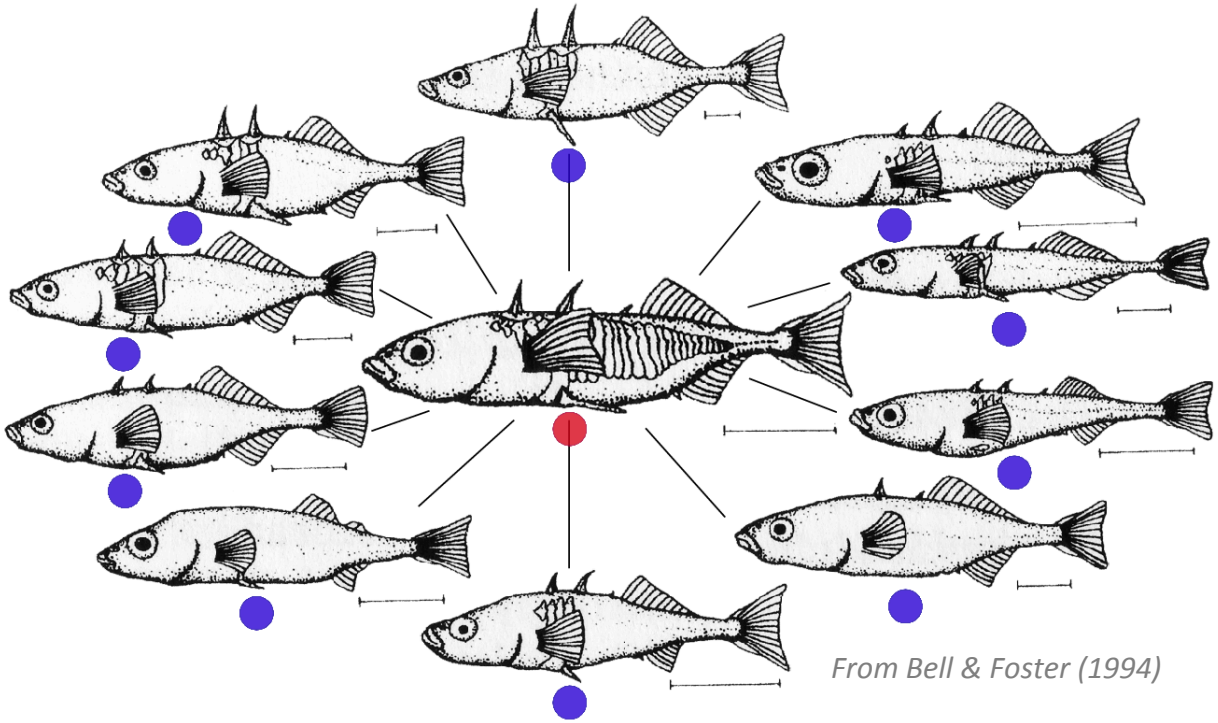
   (what patterns might we expect to see at other adaptation loci?)

How to identify and test the loci and alleles underying adaptation?



- Number and type of natural mutations?
- Functional effects on phenotype?
- Molecular mechanisms?
- Evolutionary processes & constraints?
- Affect on fitness and reproductive isolation?

Threespine sticklebacks have undergone a recent adaptive radiation



From Bell & Foster (1994)

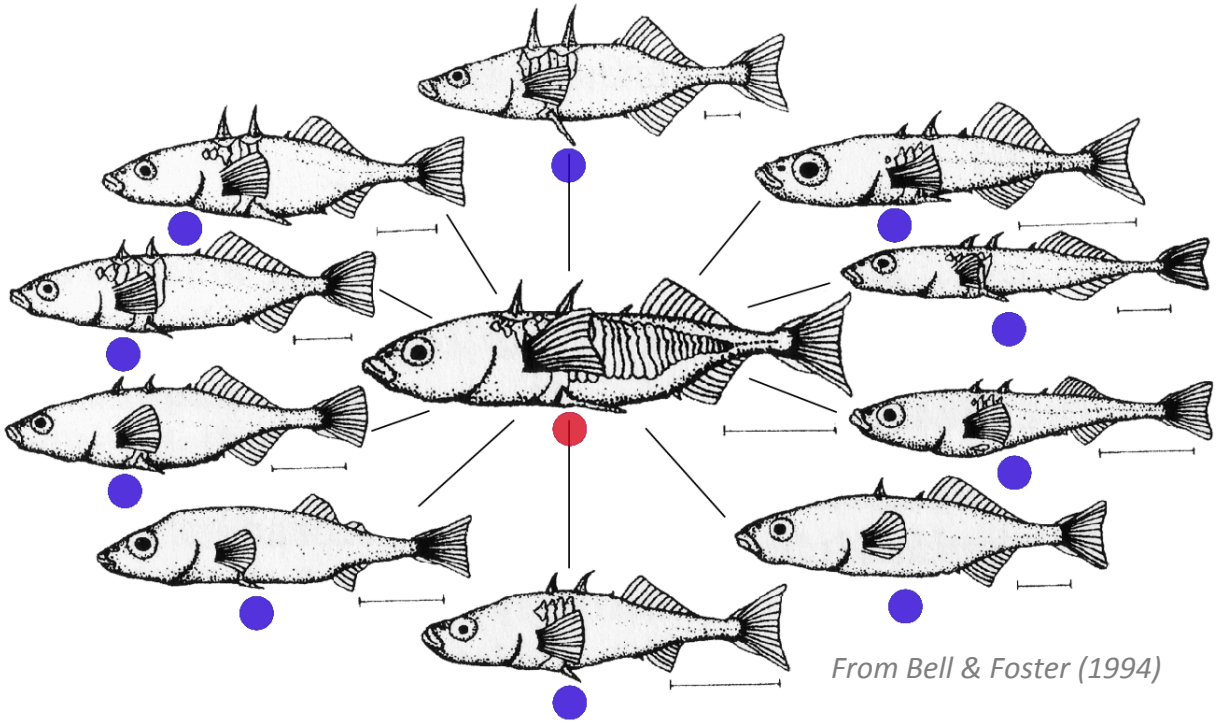
Marine
Freshwater

Marine and freshwater sticklebacks differ in many traits

Migratory Marine

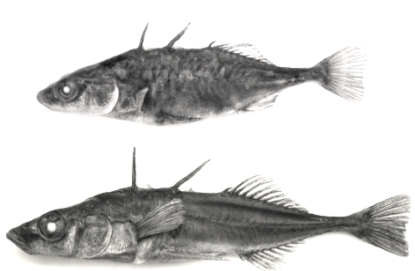
Resident Freshwater

Different types of sympatric "Species-Pairs" have evolved



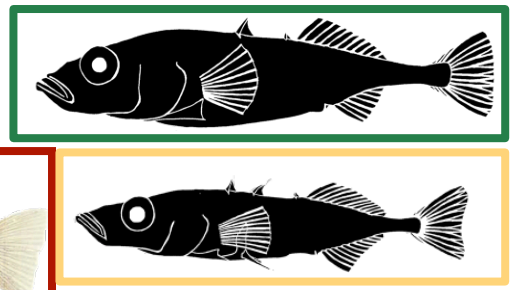
From Bell & Foster (1994)

Marine
Freshwater

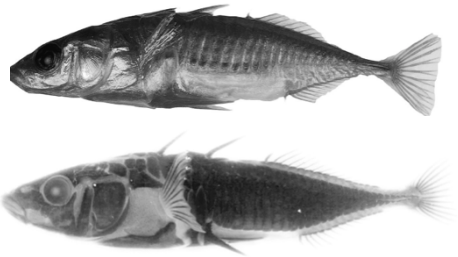


Lake — Stream

Marine — Freshwater



Benthic — Limnetic



Japan Sea — Pacific Marine

Different species-pairs have evolved (and are at different 'stages' of speciation)

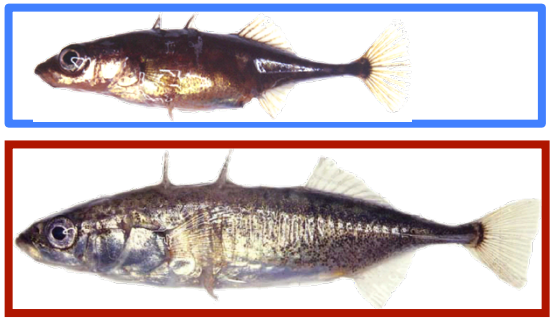
Strength of Reproductive Isolation

Weak

LAKE-STREAM
(100's of independent events)

Weak-Medium

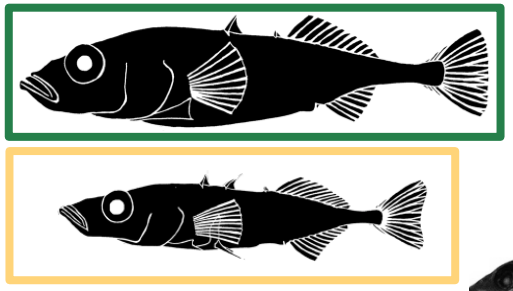
Hagen 1967; Jones et al 2006, 2008



MARINE – FRESHWATER
(10000's of independent events)

Medium

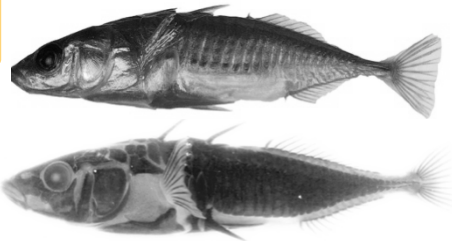
McPhail, Schluter et al (many pubs)



BENTHIC – LIMNETIC
(5 independent events)

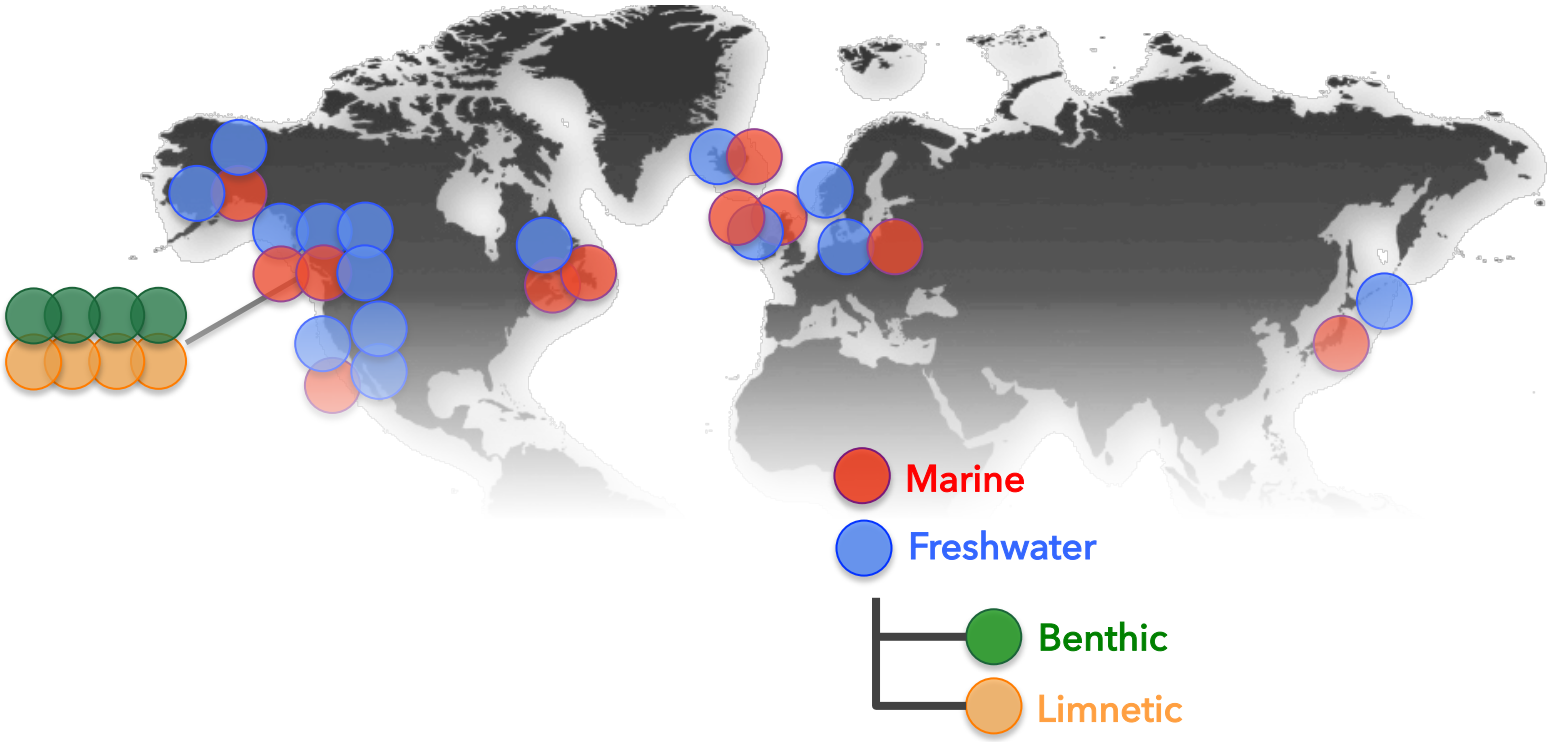
Strong

(Kitano et al 2009)

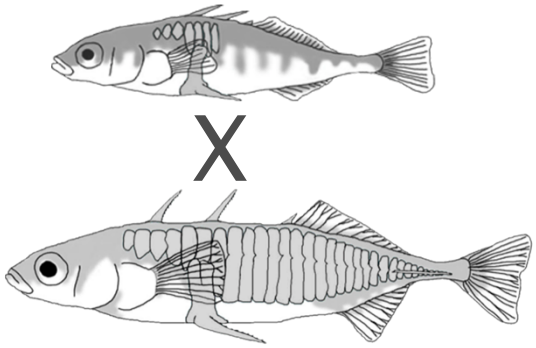


JAPAN SEA – PACIFIC MARINE
(1 independent event)

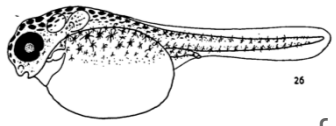
Repeated & independent evolution provides biological replicates of the evolutionary process



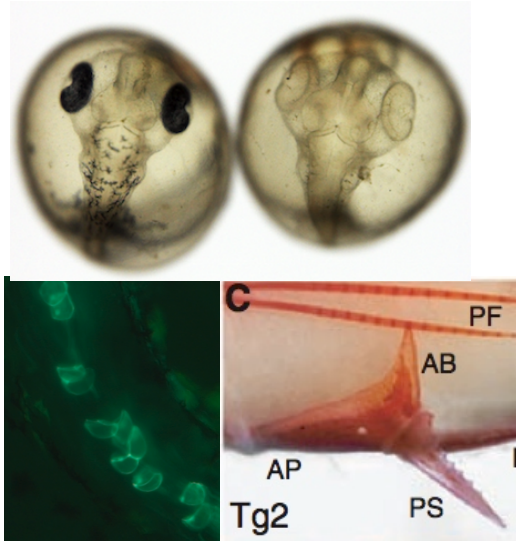
A broad suite of genetic and genomic tools in sticklebacks



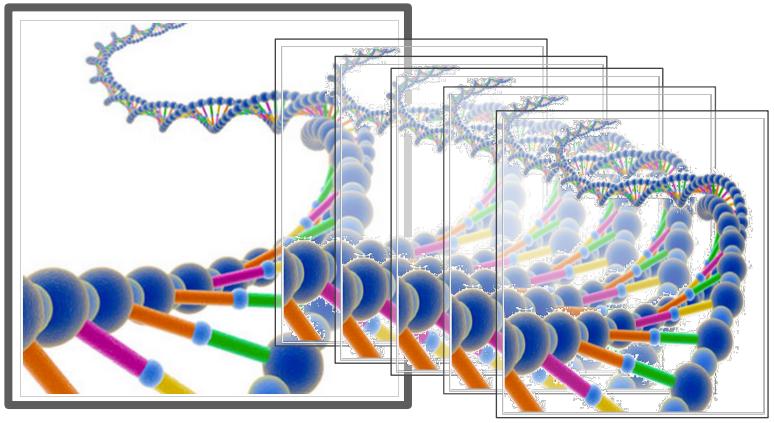
Easy husbandry, viable inter-crosses



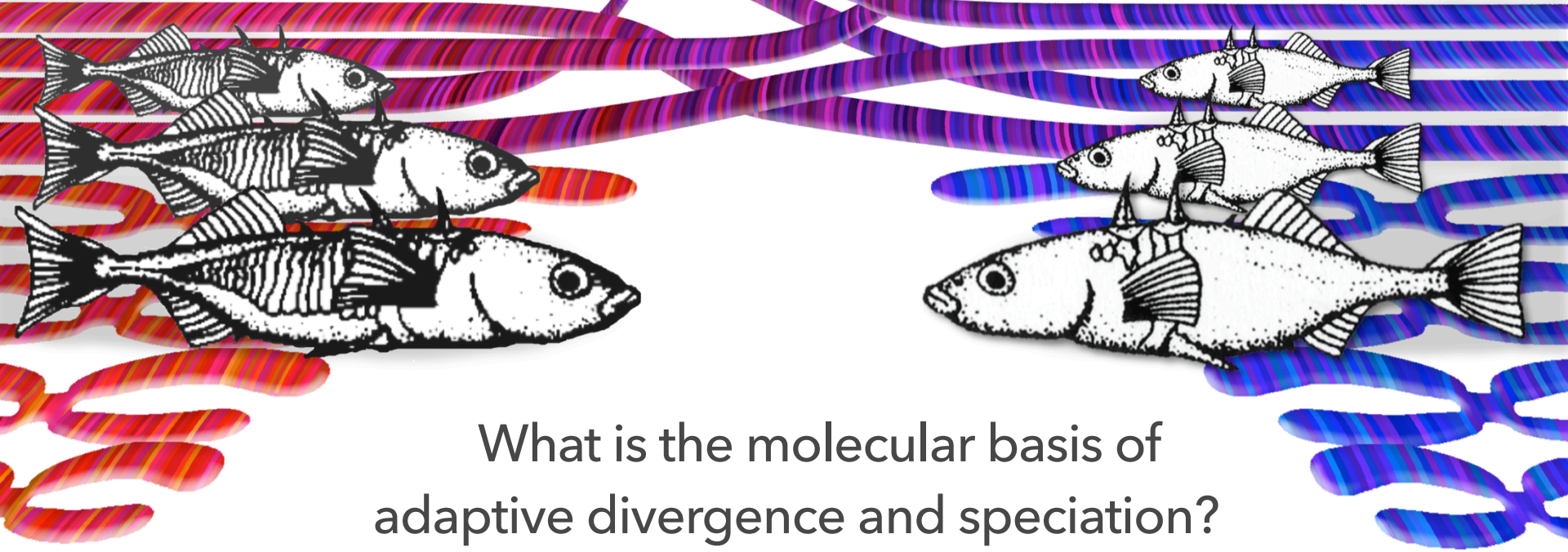
Transparent embryos for developmental studies



Transgenic techniques for reporter assays, knock-outs, knock-ins, & genome editing



Small (0.45GB), well-assembled Ref Genome >> hundreds of short-read sequenced genomes



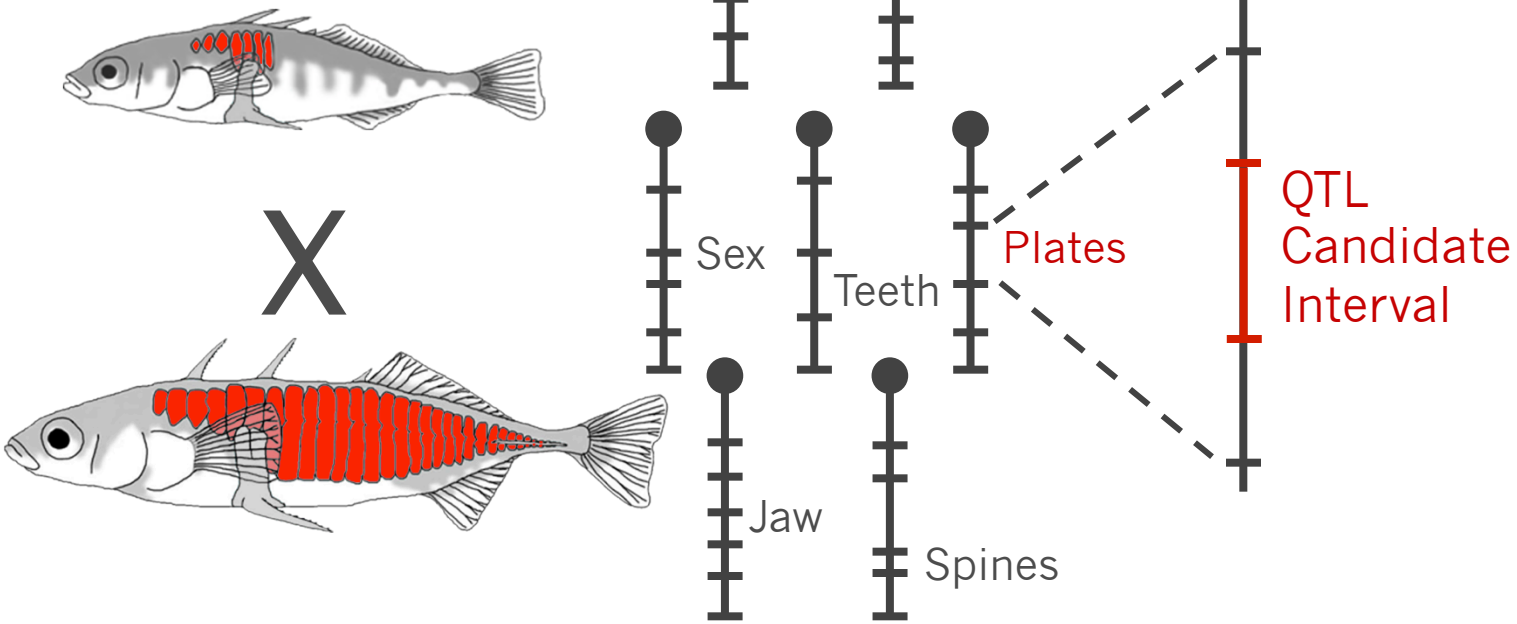
What is the molecular basis of adaptive divergence and speciation?

Identifying and functionally testing adaptive loci

1. Forward genetics: from trait to basepairs

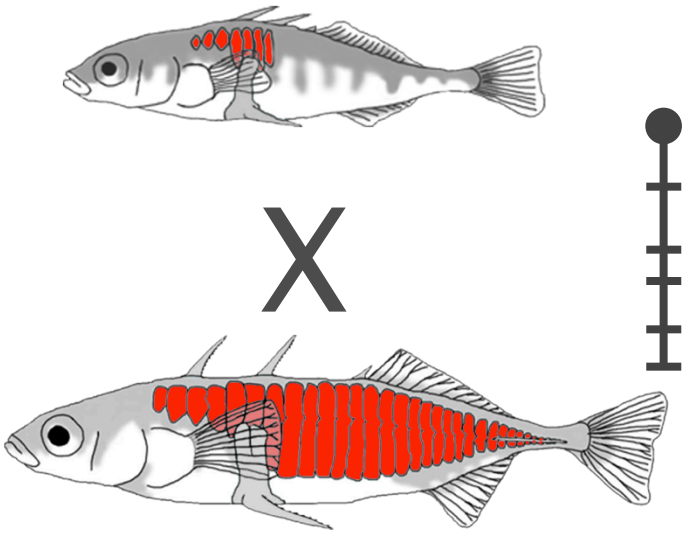
2. Population genomics

Finding the genes using forward genetics: Quantitative Trait Locus (QTL) mapping in sticklebacks

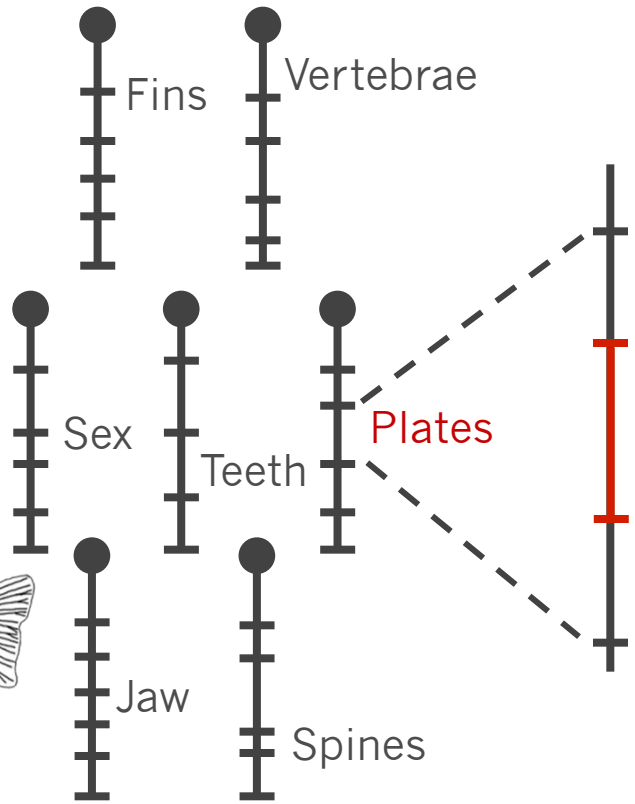


Cross divergent forms → Establish F2 generation, Build Genetic Map → Identify 2LOD Interval

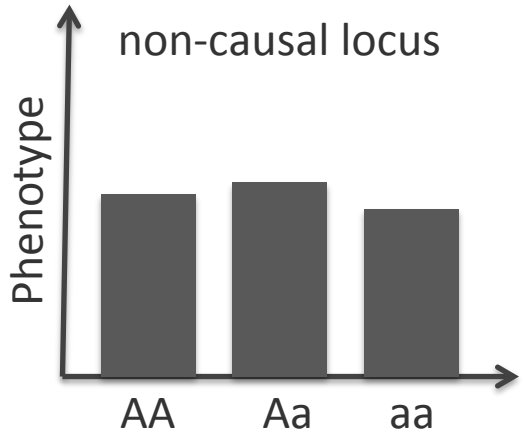
Finding the genes using forward genetics: Quantitative Trait Locus (QTL) mapping in sticklebacks

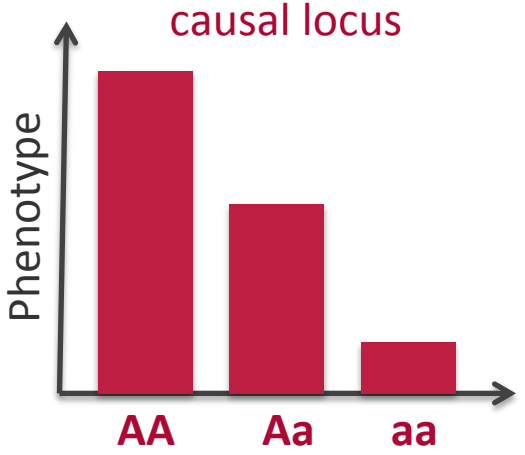


X



QTL Candidate Interval



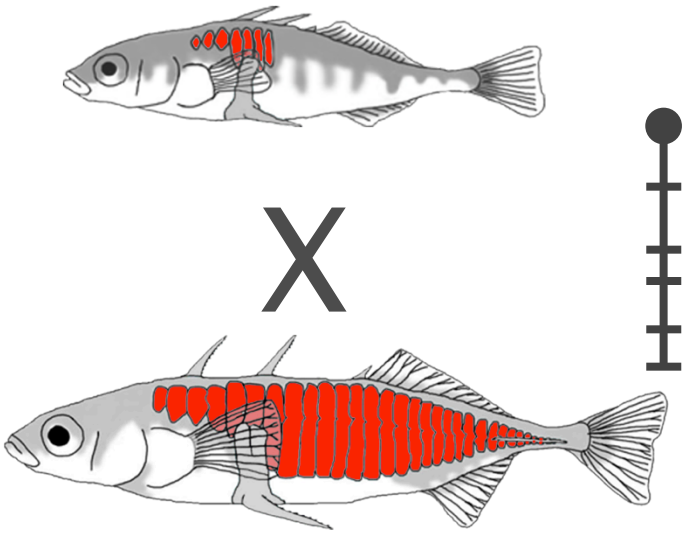


Cross divergent forms → Establish F2 generation, Build Genetic Map → Identify 2LOD Interval

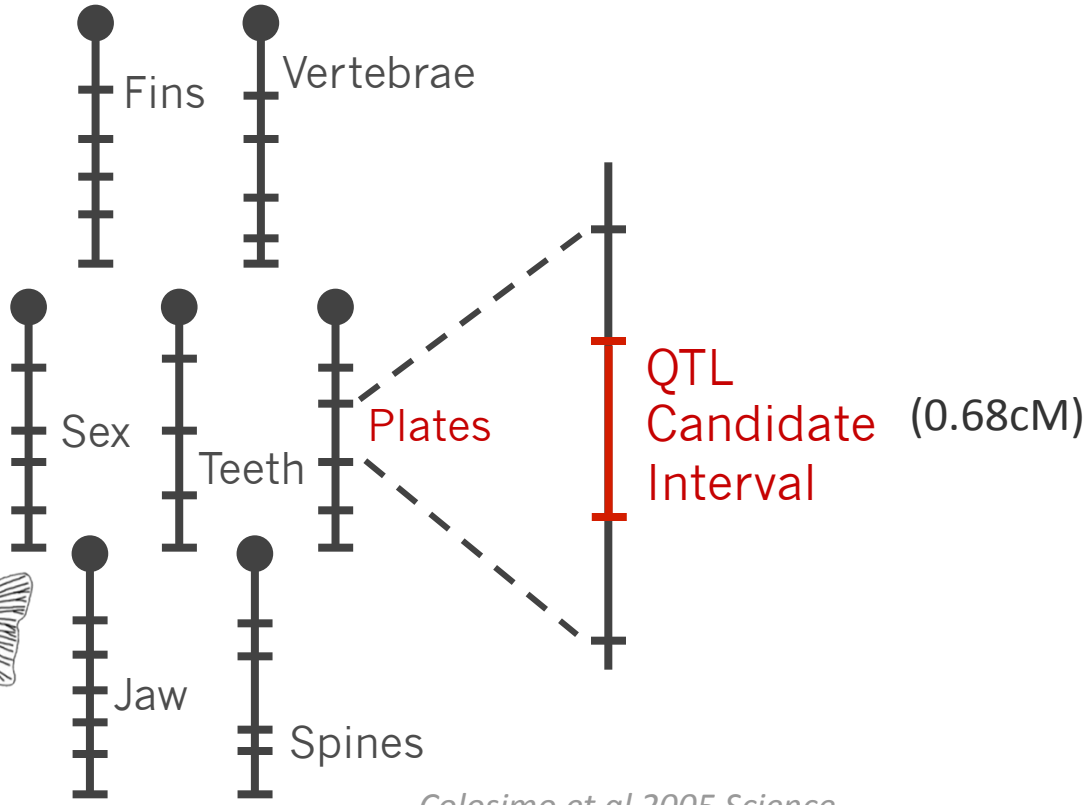
Finding the genes using forward genetics: QTL mapping in sticklebacks has been very successful

Arnegard et al (2014), Miller et al (2014), Greenwood et al (2011), Kitano et al (2009), Miller (2007), Colosimo et al (2004), Shapiro et al (2004) …

Forward genetics:
Narrowing QTL intervals to causal base pairs or haplotypes



X

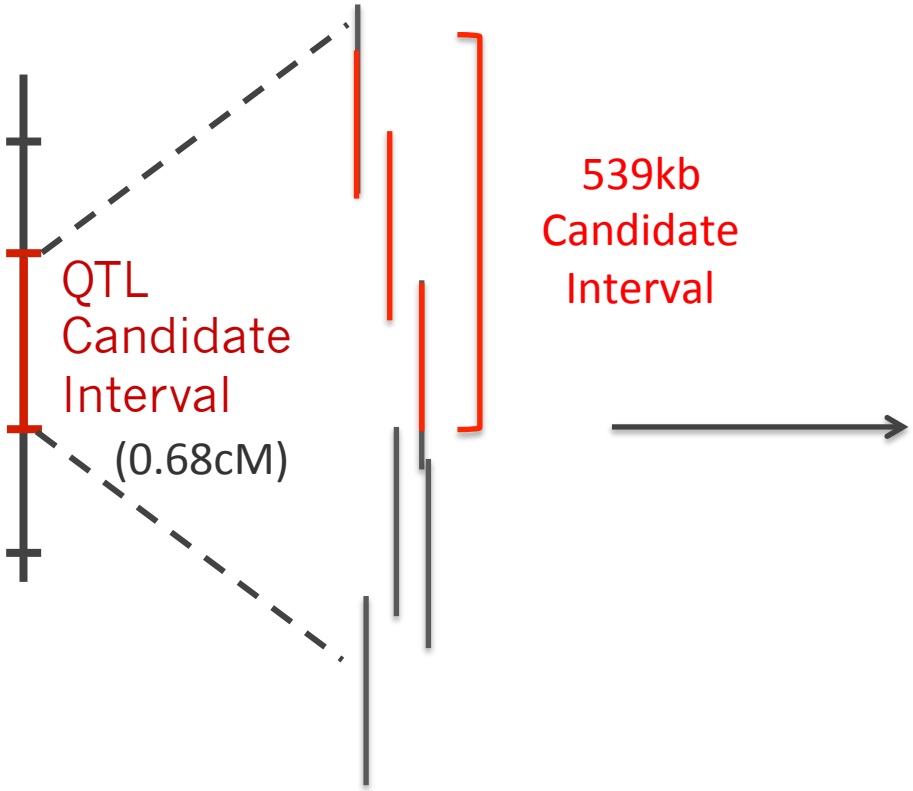


Colosimo et al 2005 Science

Cross divergent forms → Establish F2 generation, Build Genetic Map → Identify 2LOD Interval

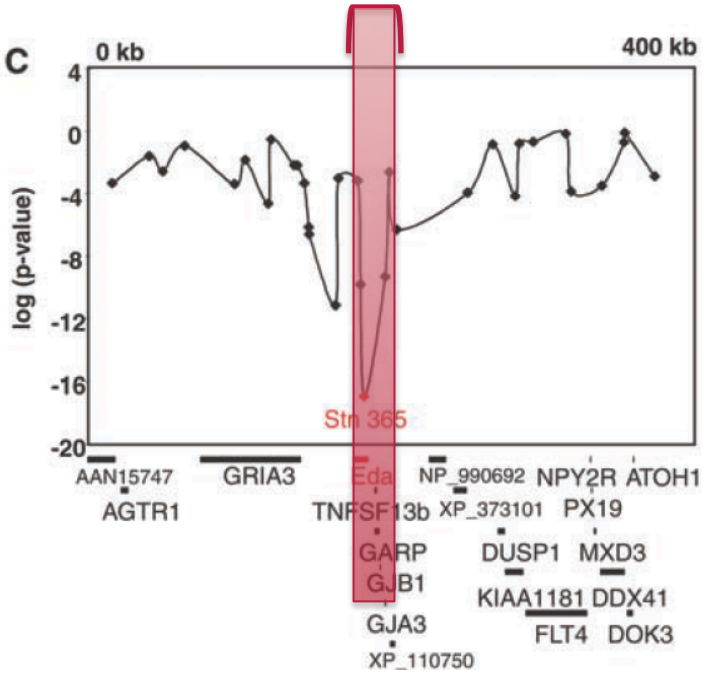
Forward genetics: Narrowing QTL intervals using association mapping in natural populations

Chromosome walking



Association mapping using a polymorphic natural population (Friant River, California)

16kb candidate interval



Colosimo et al 2005 Science

Forward genetics: Narrowing QTL intervals by demonstrating functional significance of genes

Transgenic Rescue using candidate gene (EDA) within interval

Rescuing plates on low plated fish

Colosimo et al 2005 Science

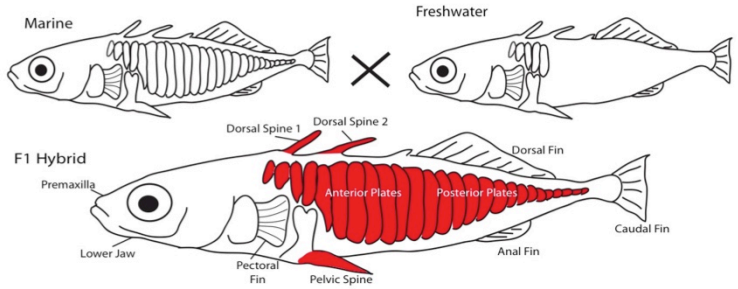
Forward genetics:
Narrowing QTL intervals using Allele-Specific Expression tests for cis-regulatory element

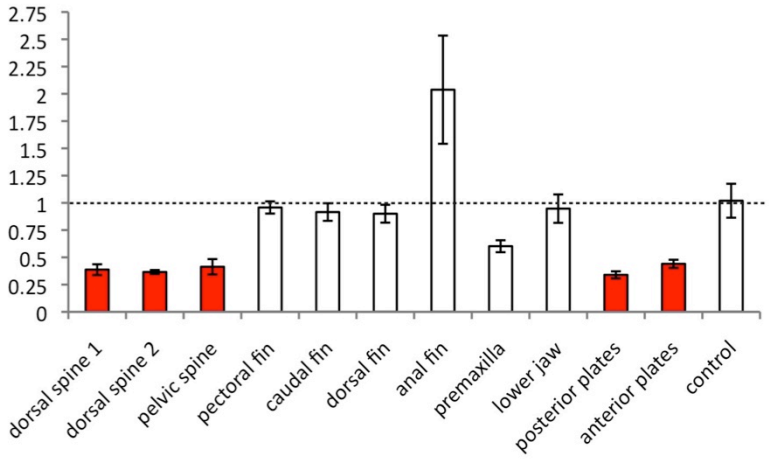
Transgenic Rescue using candidate gene (EDA) within interval

Rescuing plates on low plated fish

Colosimo et al 2005 Science

Allele specific expression reveals a cis-acting regulatory mutation

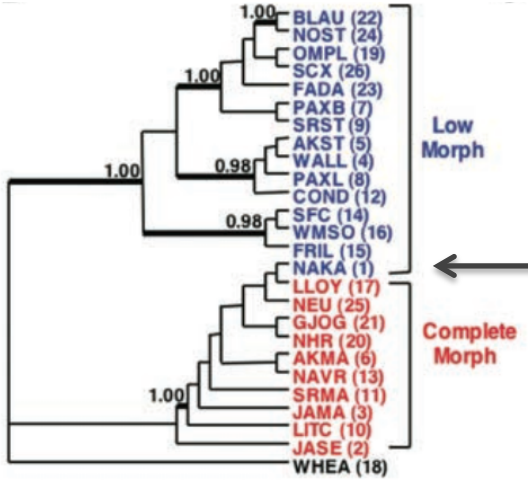




O'Brown et al 2015 ELife

Forward genetics:
Narrowing QTL intervals to causal base pairs or haplotypes using exceptional / unusual populations

EDA gene tree



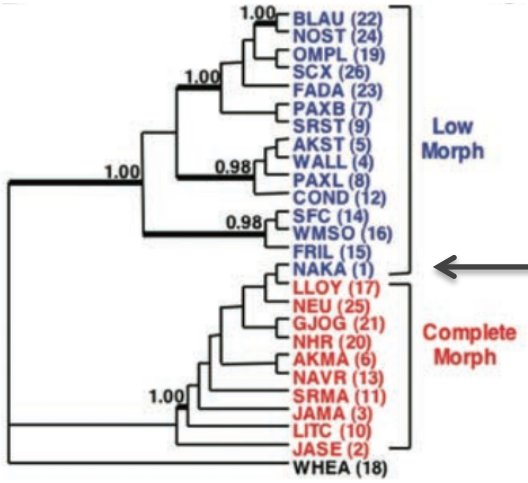
"NAKA - An unusual exception"

Low plated Japanese fish with high plated EDA haplotype

Colosimo et al 2005 Science

Forward genetics:
Narrowing QTL intervals to causal base pairs or haplotypes

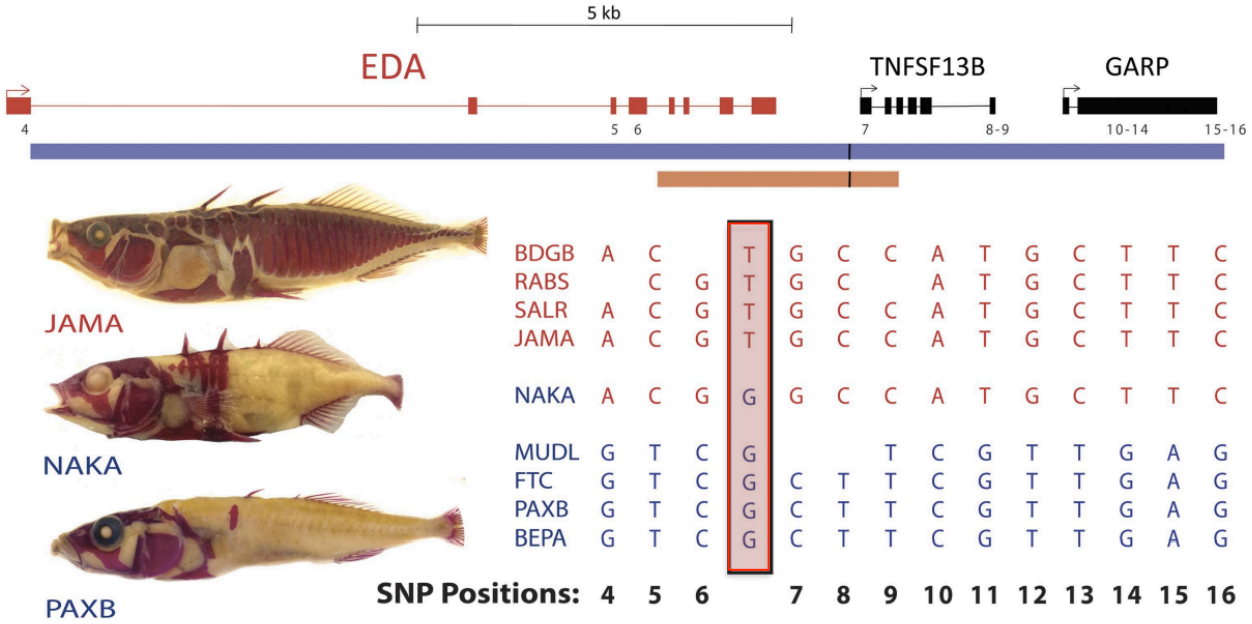
EDA gene tree



"NAKA - An unusual exception"
Low plated Japanese fish
with high plated EDA haplotype

Colosimo et al 2005 Science

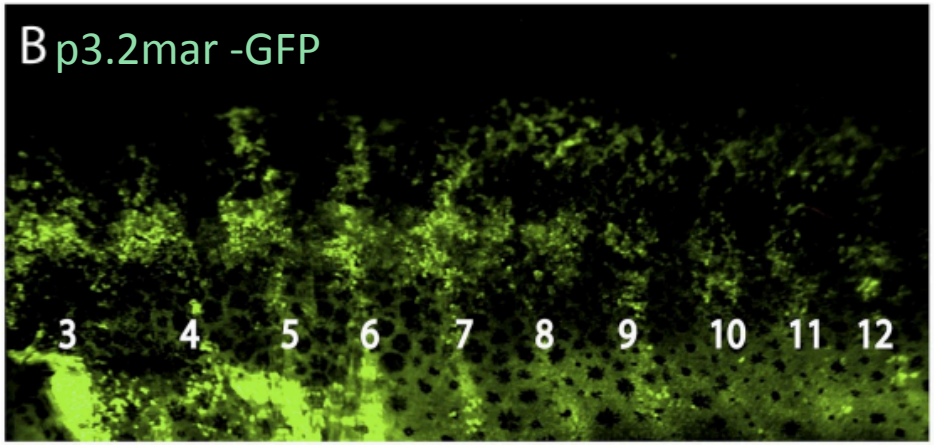
Sequencing of an unusual exception (Low Plated Japanese Fish) identifies a single 3' substitution



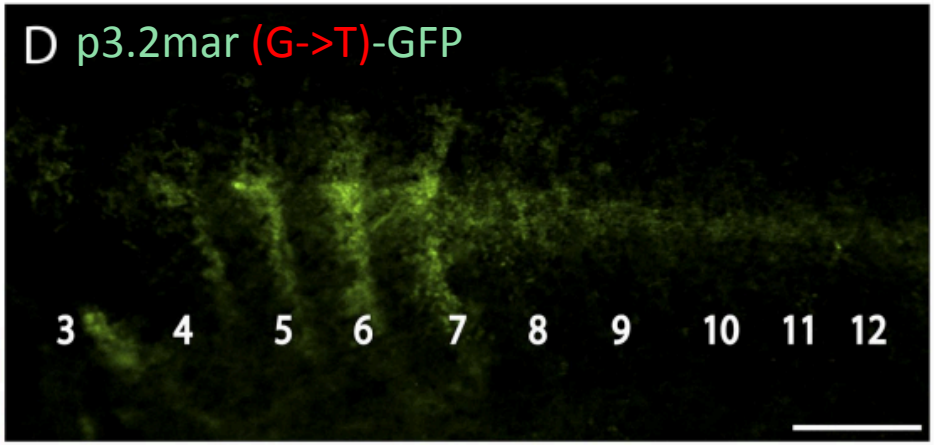
O'Brown et al 2015 ELife

Forward genetics:
Narrowing QTL intervals using reporter assays to demonstrate the functional significance of a single 1bp mutation

Regulatory enhancer assays functionally demonstrate loss of expression caused by 1bp change.



Wildtype marine enhancer expression pattern.



Single bp substitution reduces expression pattern.

O'Brown et al 2015 ELife

Forward genetics:
Narrowing QTL intervals to causal base pairs or haplotypes

1. QTL mapping	0.63cM ~500kb (many genes)
2. Association mapping in naturally polymorphic population	16kb (3 genes)
3. **Functional Assay:** Rescue of plates using transgenics	1 gene (EDA)
4. Allele specific expression assay reveals cis-acting element	Cis-acting element underlies differences in EDA expression.
5. Sequencing of a population that is an unusual exception (low plated phenotype but high plated haplotype)	1bp substitution, 3' of EDA
6. **Functional Assay:** Regulatory enhancer assays show sufficiency of substitution	1bp substitution, 3' of EDA

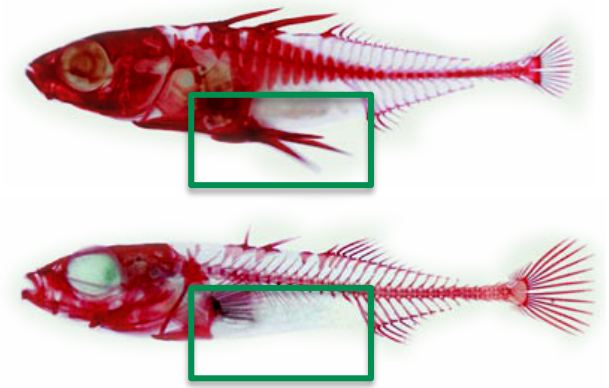
Forward genetics:
Narrowing QTL intervals to causal base pairs or haplotypes

1. QTL mapping	0.63cM ~500kb (many genes)
2. Association mapping in naturally polymorphic population	16kb (3 genes)
3. **Functional Assay:** Rescue of plates using transgenics	1 gene (EDA)
4. Allele specific expression assay reveals cis-acting element	Cis-acting element underlies differences in EDA expression.
5. Sequencing of a population that is an unusual exception (low plated phenotype but high plated haplotype)	1bp substitution, 3' of EDA
6. **Functional Assay:** Regulatory enhancer assays show sufficiency of substitution	1bp substitution, 3' of EDA

Fine mapping, Allele-specific expression & Transgenic assays identify *cis*-regulatory elements controlling adaptive traits

Plates (*EDA*)

Colosimo et al (2005) Science
O'Brown et al (2015) ELife



Pelvis (*Pitx1*)

Shapiro et al (2004) Nature
Chan et al (2010) Science

Single bp mutation

in a 3′ *cis*-regulatory element causes loss of EDA expression

O'Brown et al (2015) ELife

488bp deletion

of a 5′ *cis*-regulatory element causes loss of Pitx1 expression

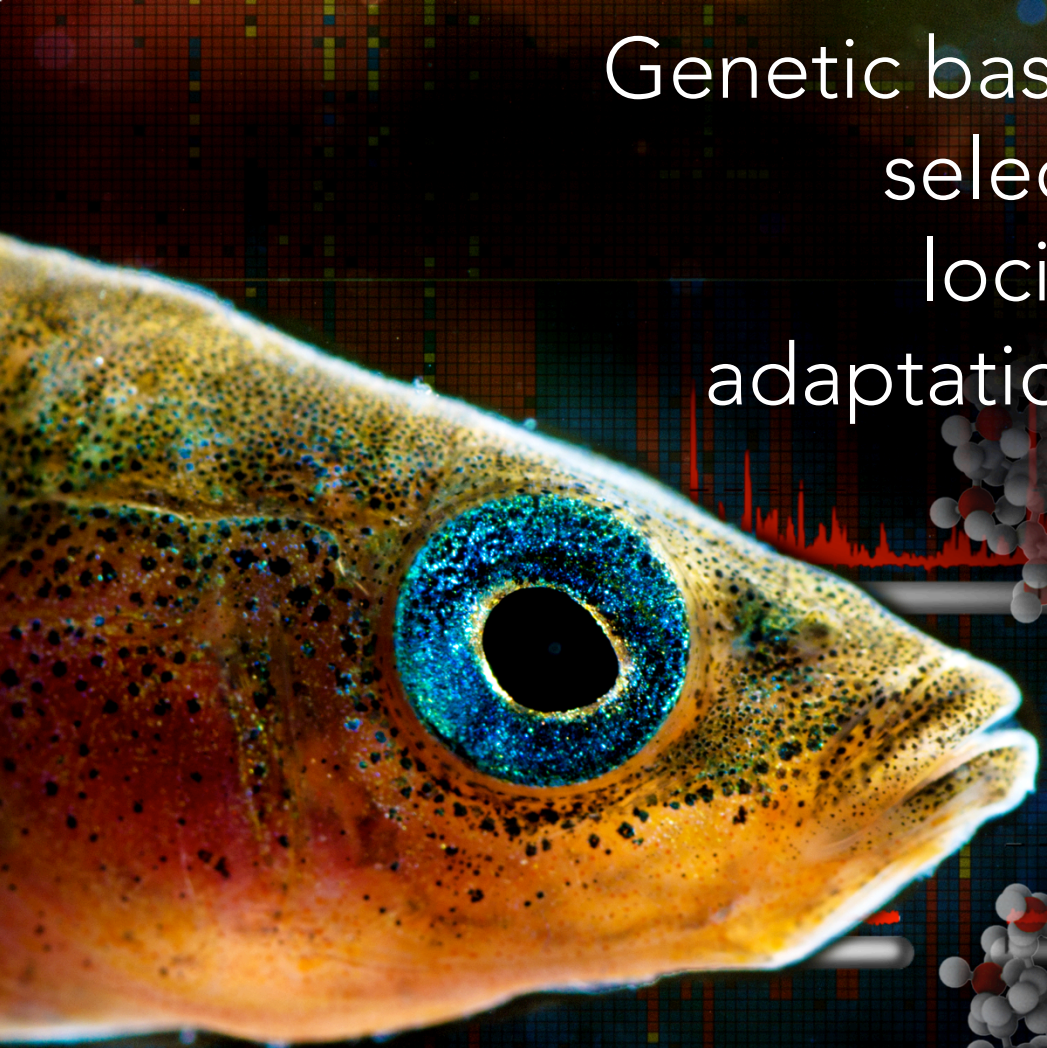
Chan et al (2010) Science

Genetic basis and signatures of selection around loci underlying adaptation and speciation

Felicity Jones

Friedrich Miescher Laboratory of Max Planck Society

Tübingen, Germany



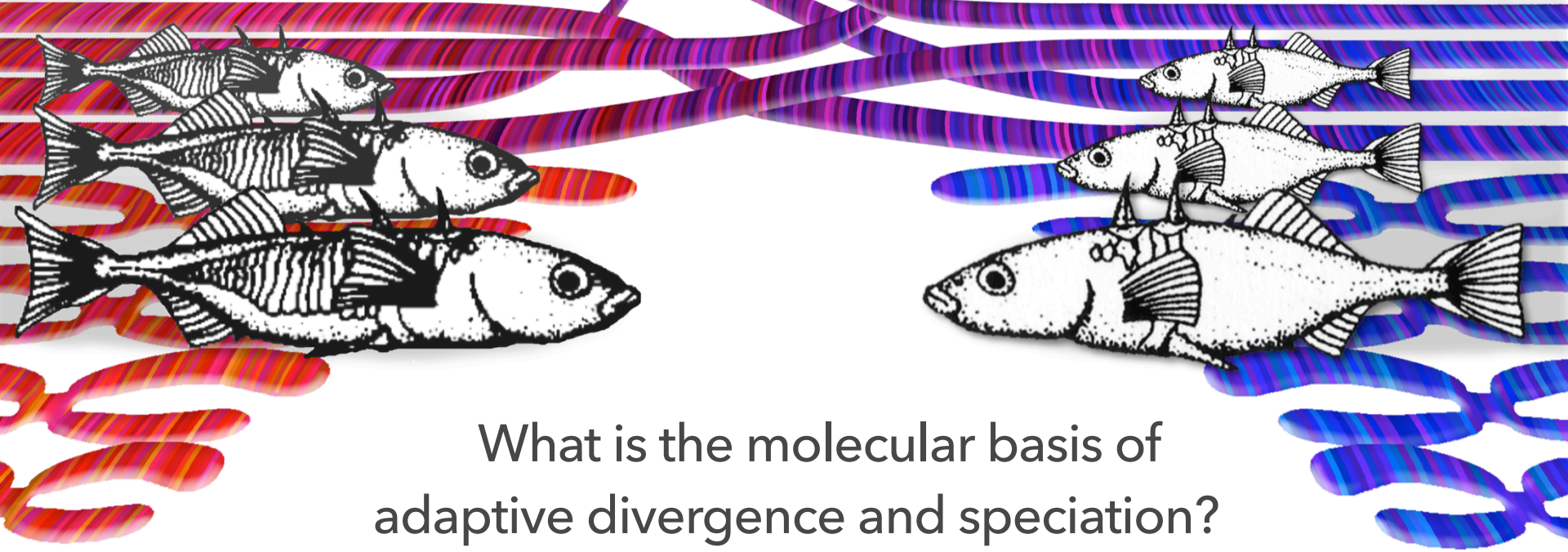
Male threespine stickleback

DFG Deutsche Forschungsgemeinschaft

erc European Research Council Consolidator Grant

MAX-PLANCK-GESELLSCHAFT

fml



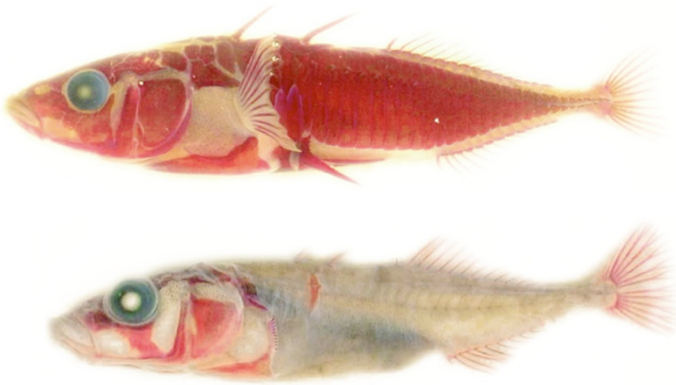
What is the molecular basis of adaptive divergence and speciation?

Identifying and functionally testing adaptive loci

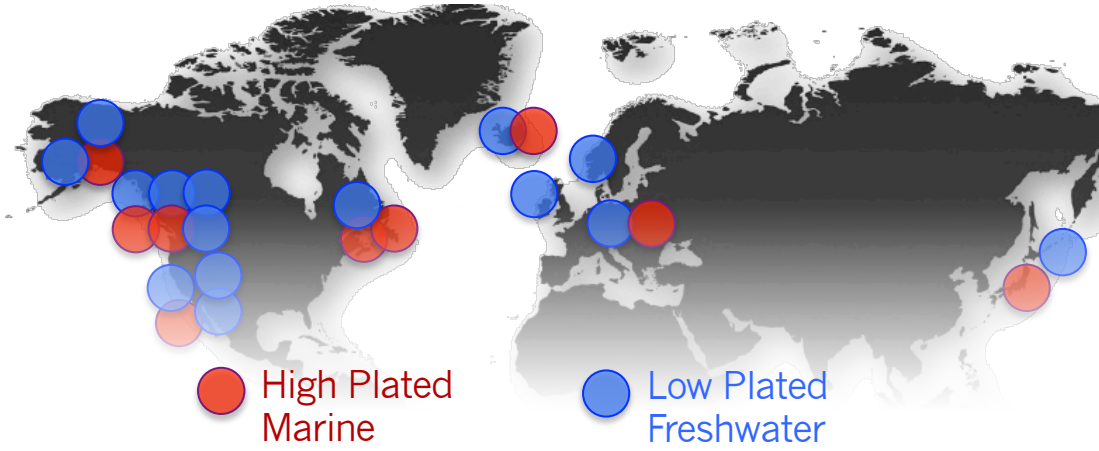
1. Forward genetics: from trait to basepairs

2. Population genomics

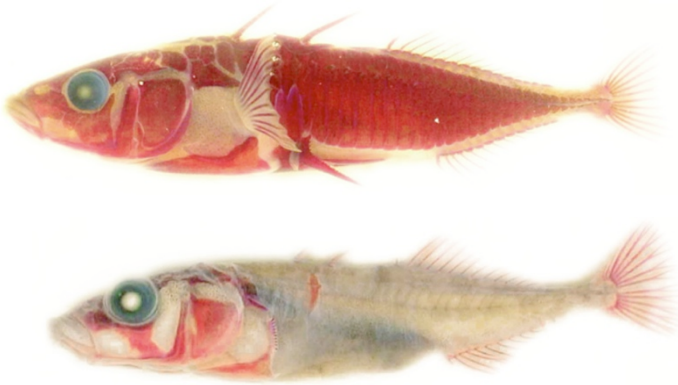
Loss of lateral plates has evolved repeatedly via re-use of an ancient EDA haplotype



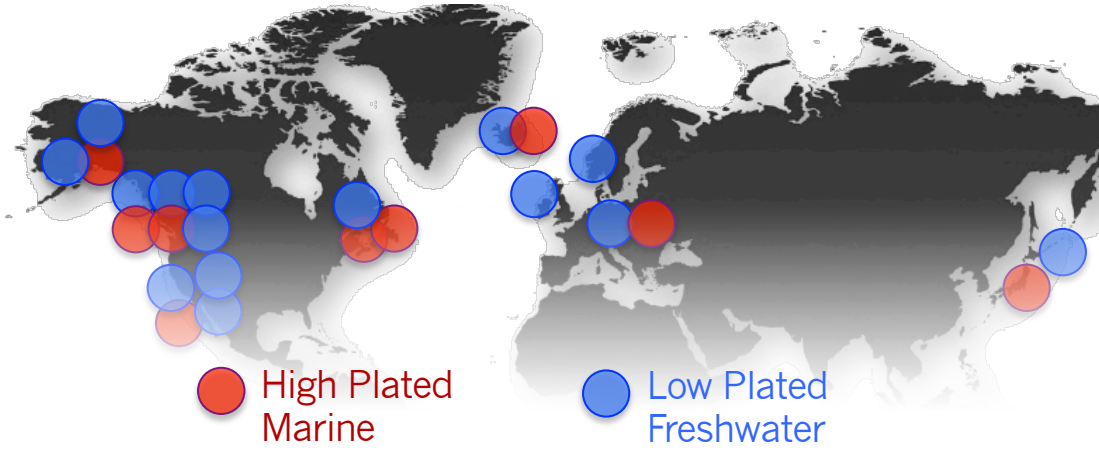
Plates (*EDA*)

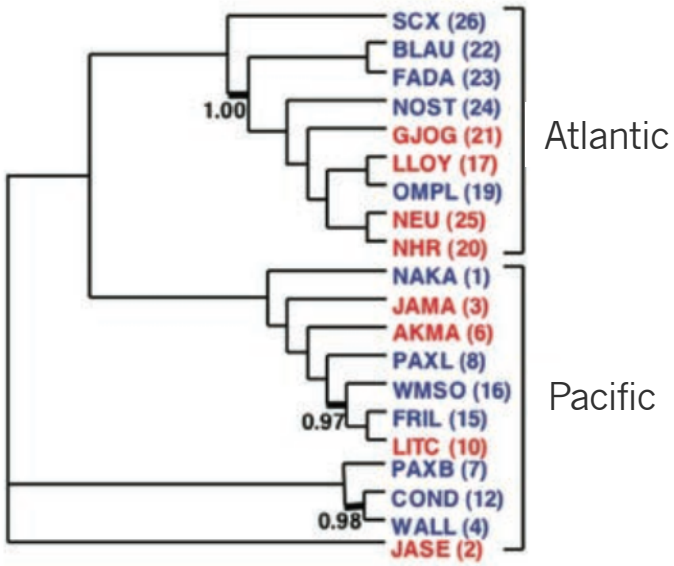


Loss of lateral plates has evolved repeatedly via re-use of an ancient EDA haplotype

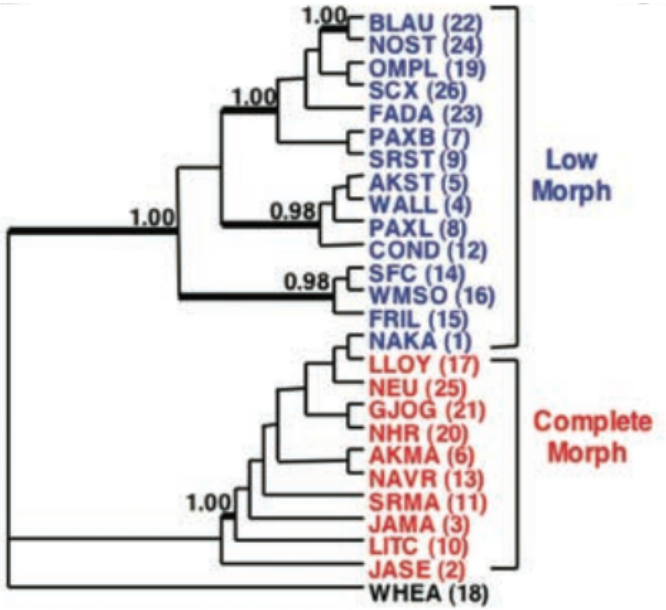


Plates (*EDA*)





Genome-Wide Tree (neutral markers)



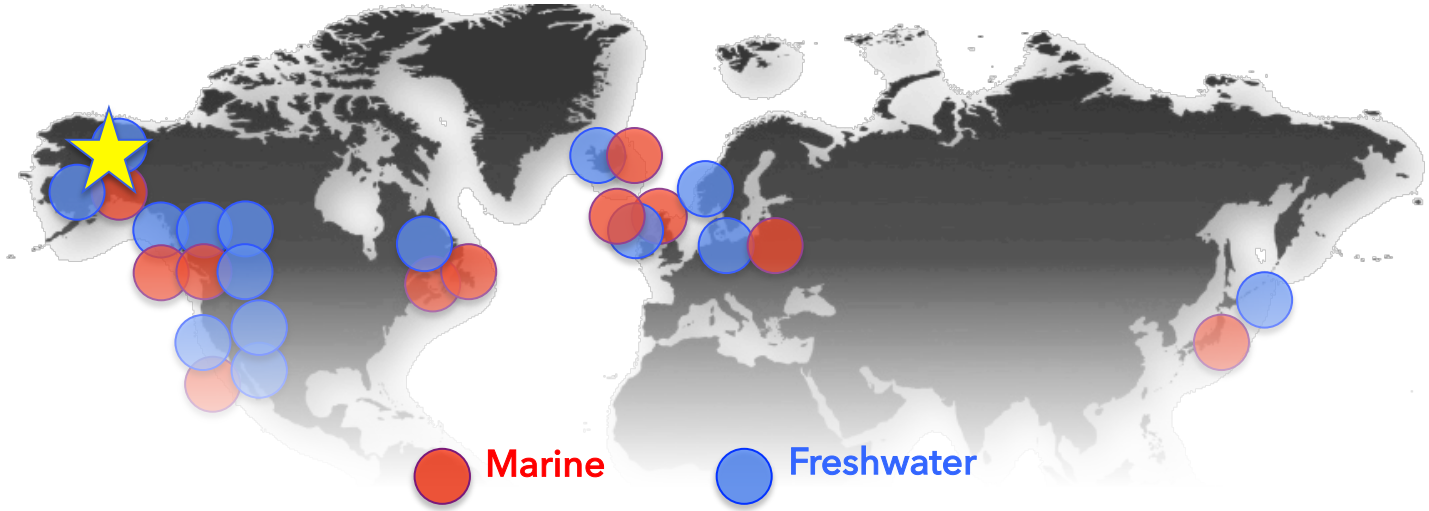
EDA Tree

Colosimo et al (2005) Science

Leveraging parallelism to identify adaptive loci

[Whole genome sequencing of replicate marine – freshwater species pairs]

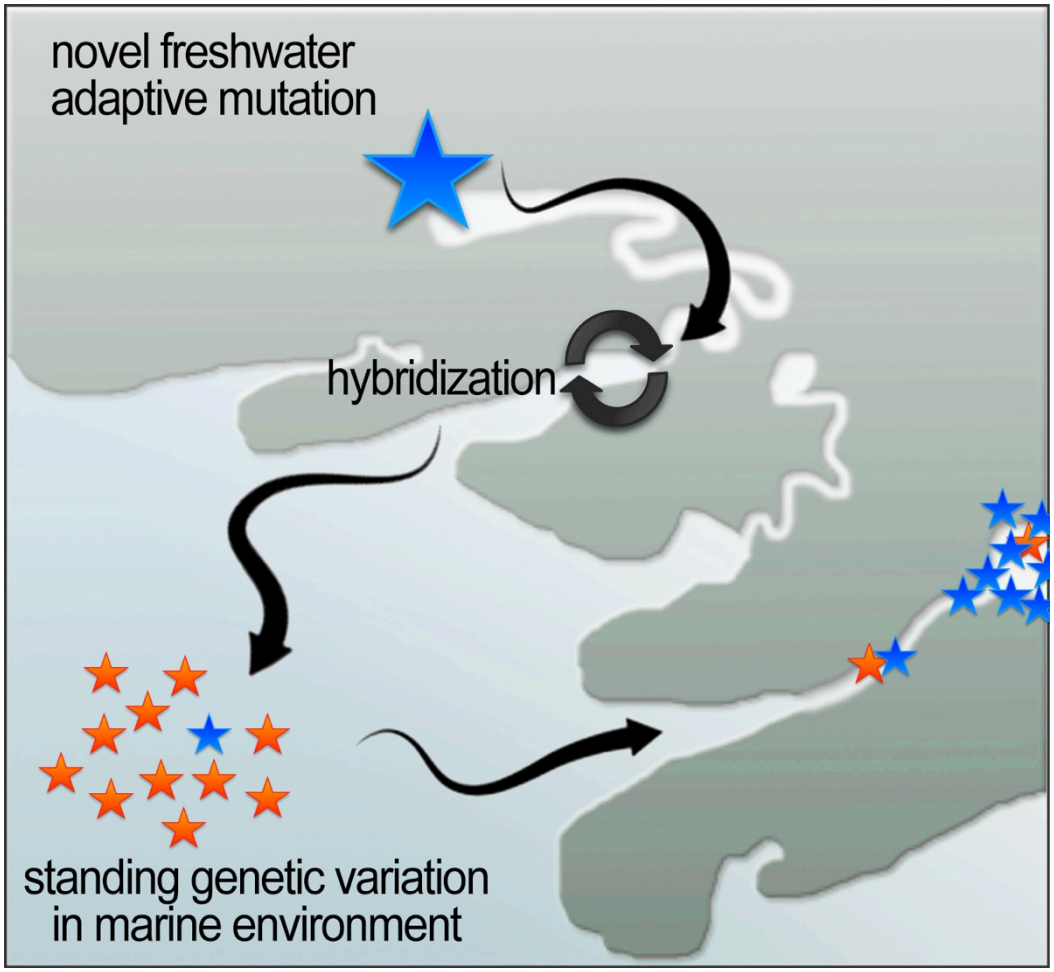
(21 stickleback genomes, Jones et al 2012 Nature)



Marine

Freshwater

Parallel evolution via reuse of pre-existing genetic variation



Low Plated EDA alleles 'hide' at low frequency (0-6%) in the High Plated marine population

Selective sweep of "pre-screened" adaptive variation

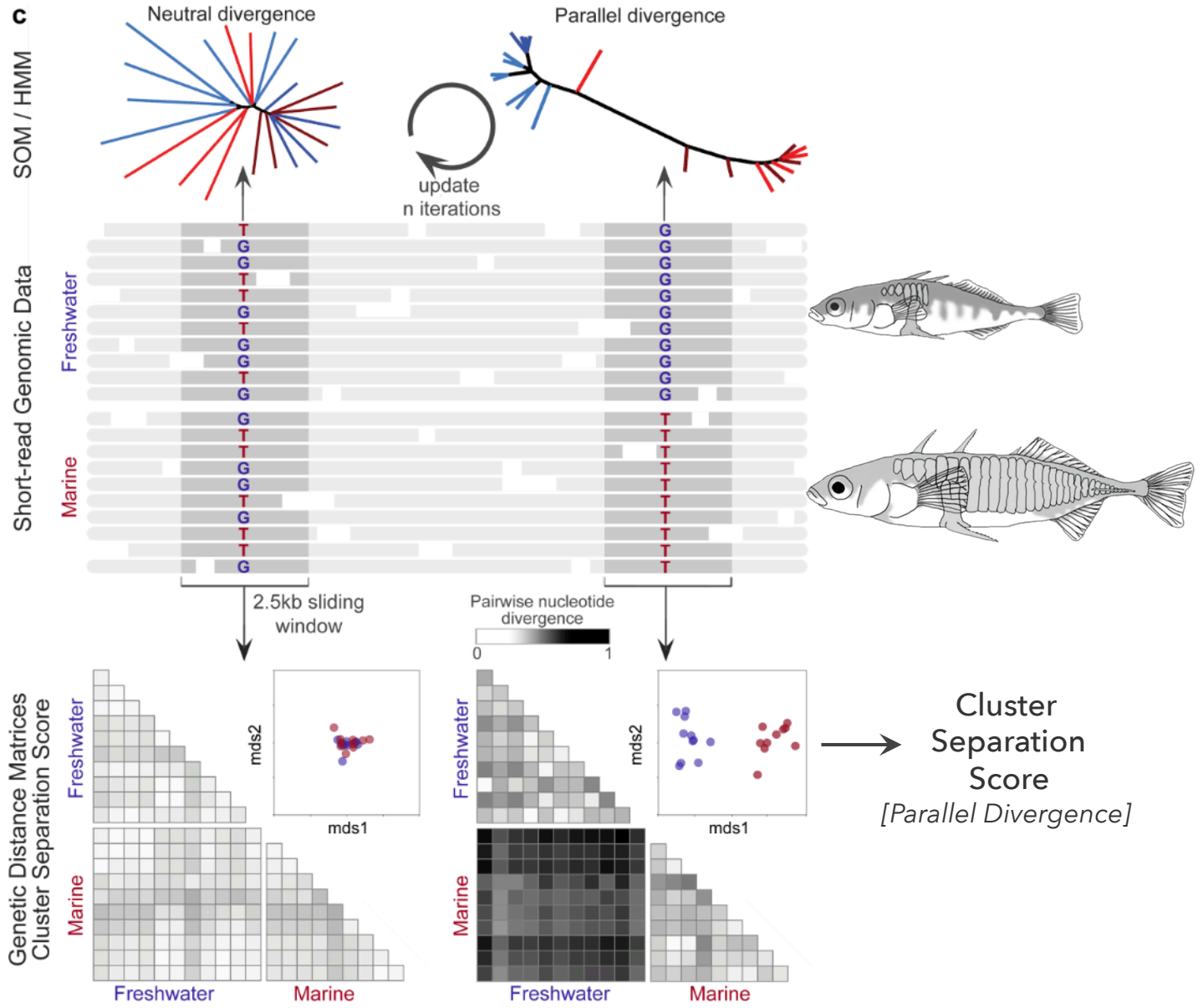
Colosimo *et al* (2005) Science
O'Brown et al (2015) ELife

Two different statistical approaches to identify parallel adaptive loci

Self-organized Map / Hidden Markov Model

Illumina short-read genomic sequencing

Genetic distance matrices

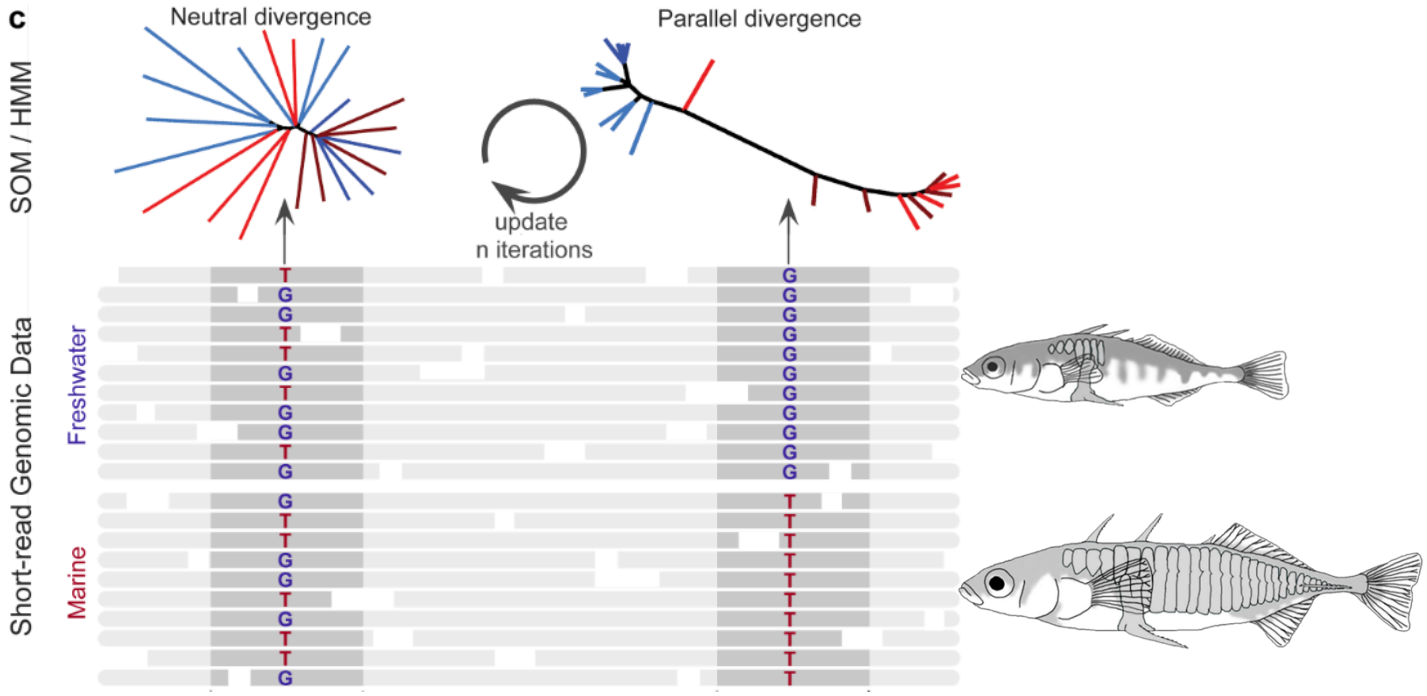


Jones et al (2012) Nature

Two different statistical approaches to identify parallel adaptive loci

"Saguaro"
Self-organized Map /
Hidden Markov Model

Illumina short-read
genomic sequencing

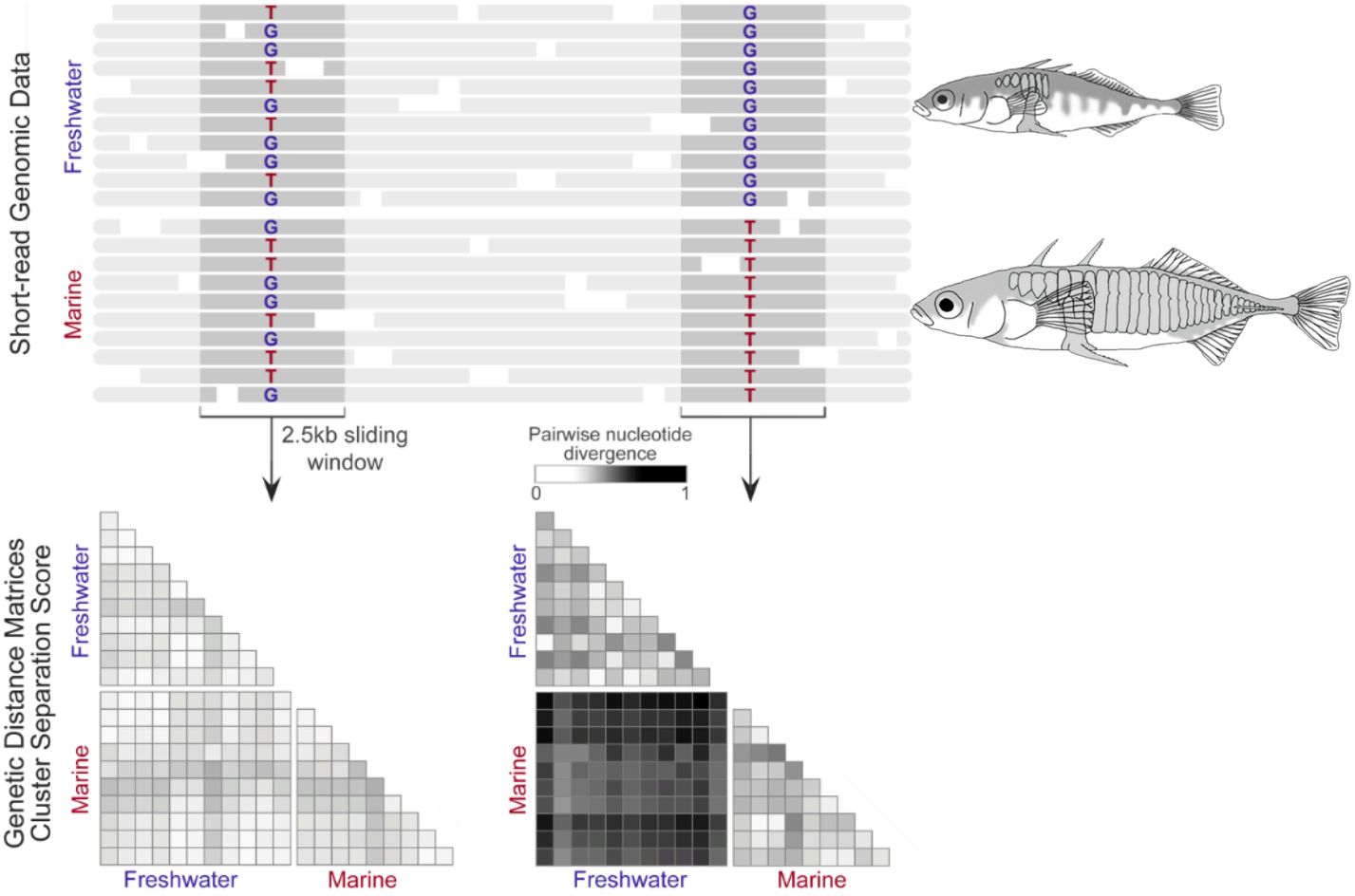


Jones et al (2012) Nature

Two different statistical approaches to identify parallel adaptive loci

Illumina short-read genomic sequencing

Genetic distance matrices

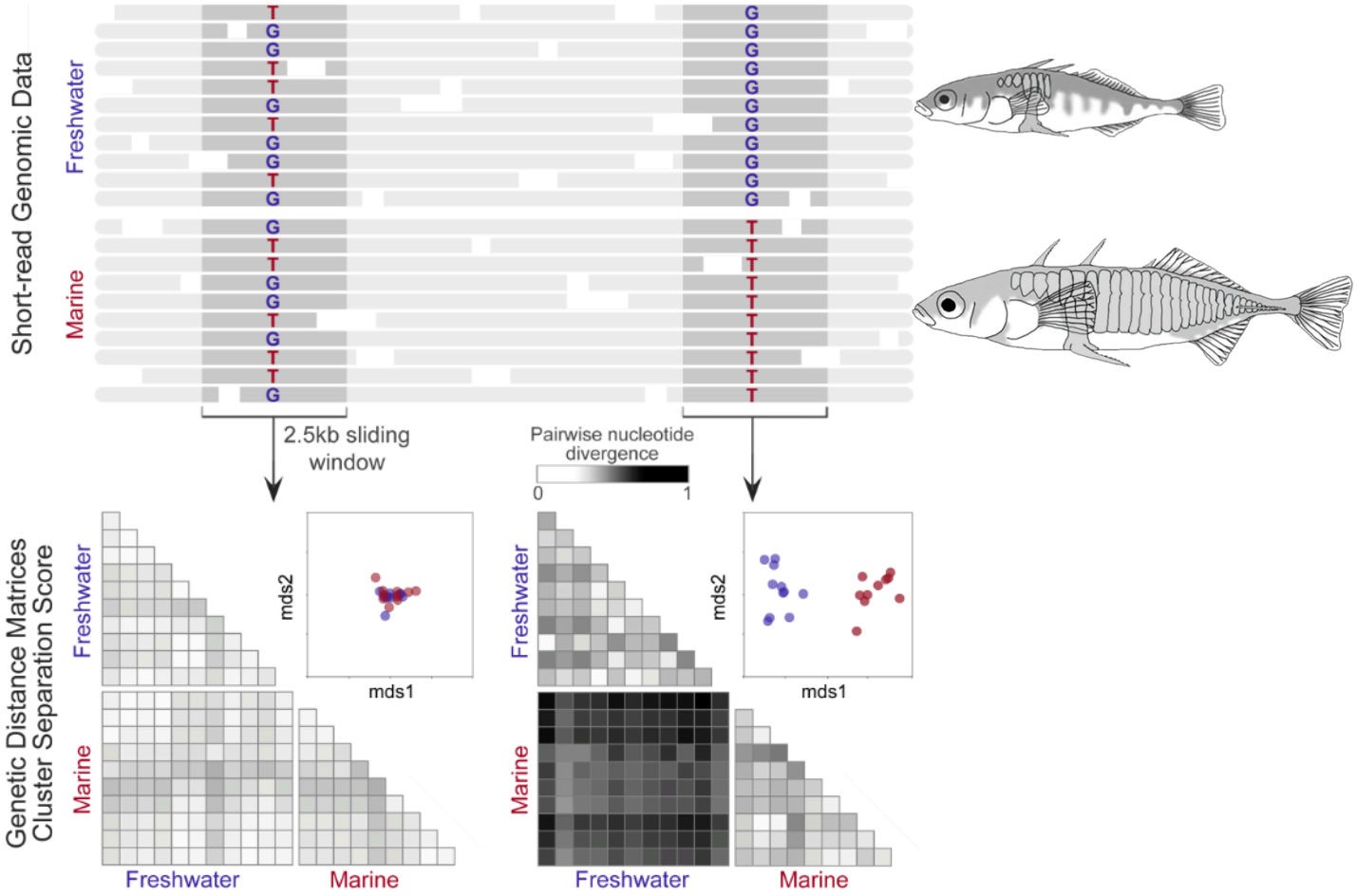


Jones et al (2012) Nature

Two different statistical approaches to identify parallel adaptive loci

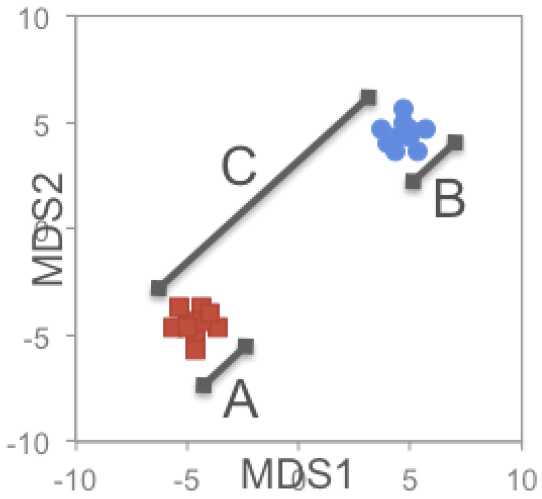
Illumina short-read genomic sequencing

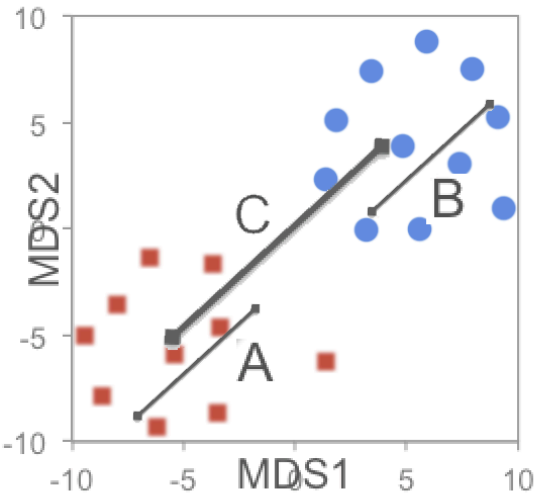
Genetic distance matrices

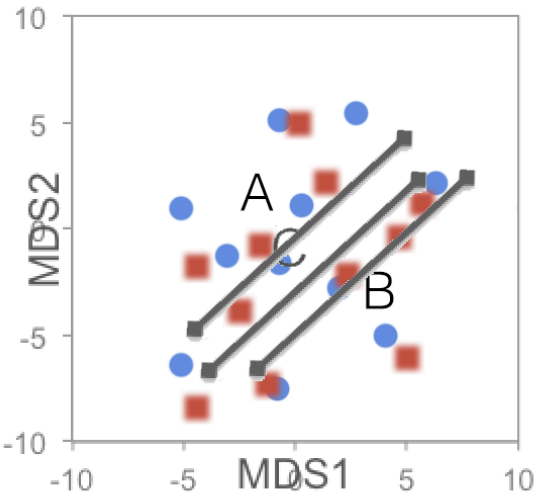


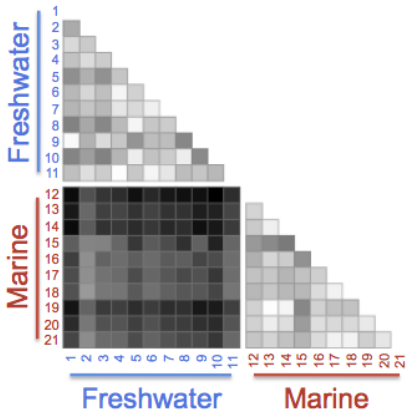
Jones et al (2012) Nature

P-values calculated by permutation tests

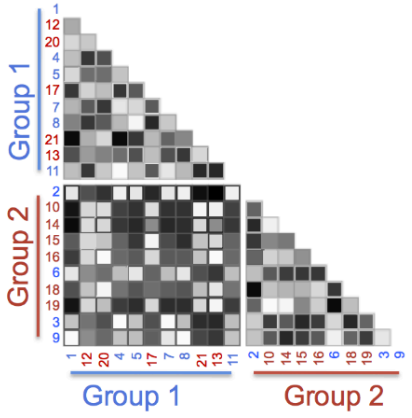




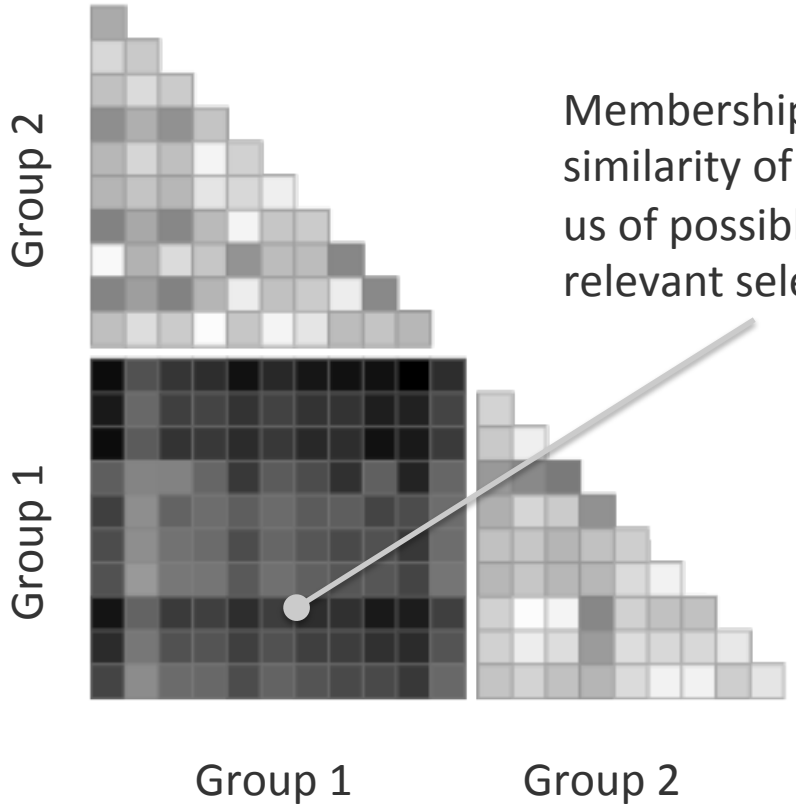




Shuffle group membership >350,000 times



Bayesian 'Data-Driven' Clustering to identify other types of clustering patterns in the genome



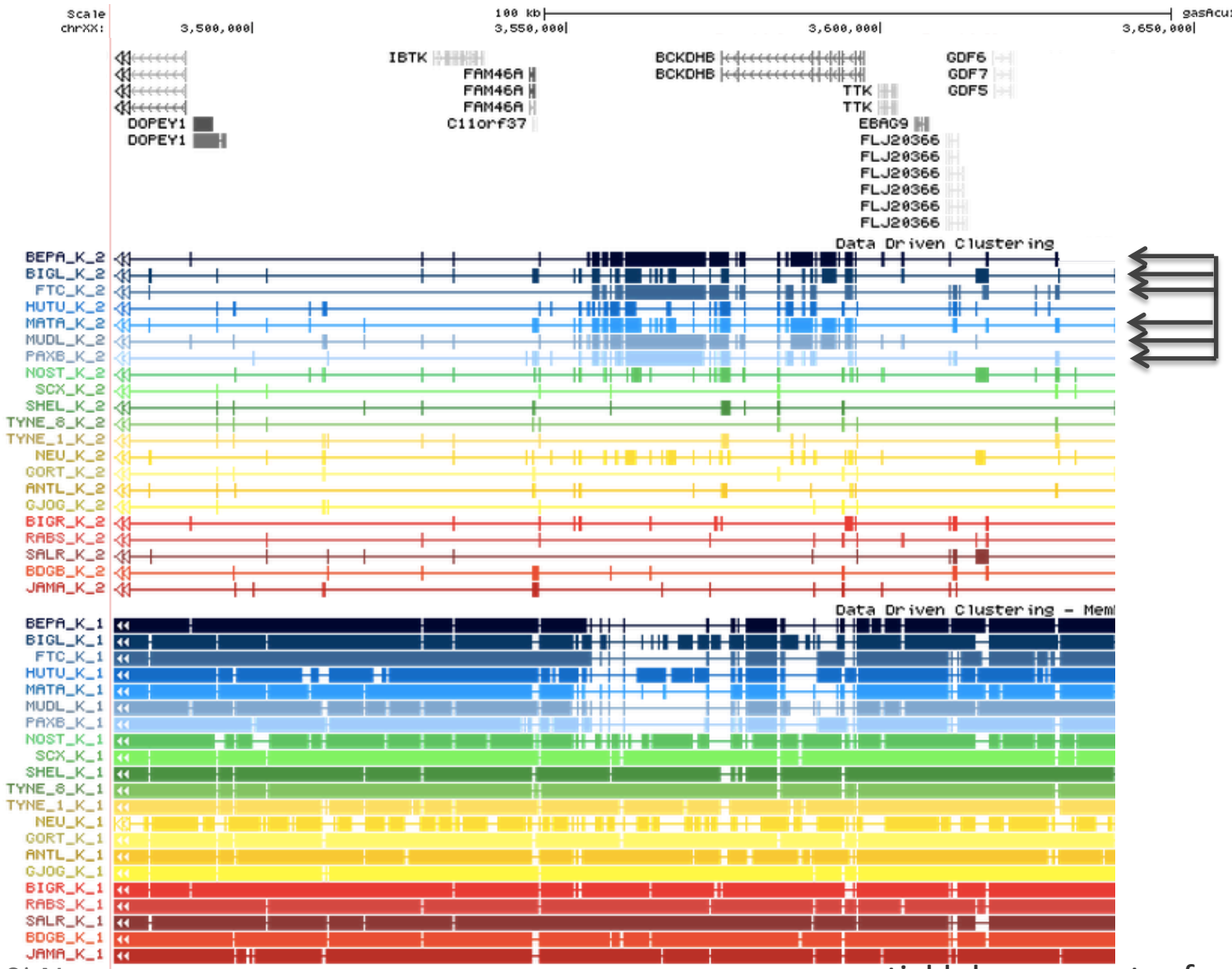
Membership and phenotypic/ecological similarity of group members may inform us of possible genetic function and relevant selection pressures

Jones et al (2012) Nature

Bayesian 'Data-Driven' Clustering to identify other types of clustering patterns in the genome

Fish falling in cluster K2

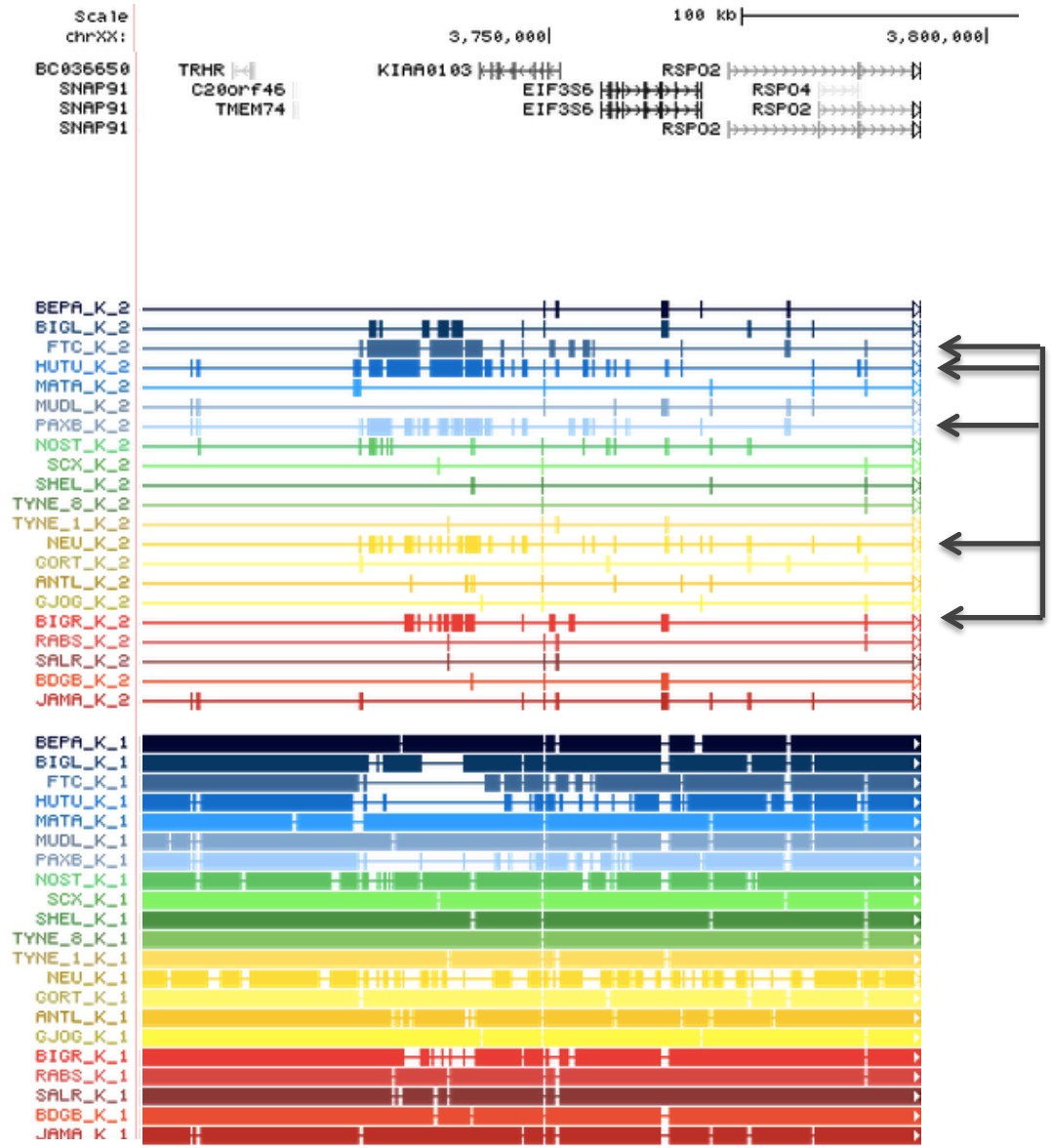
Fish falling in cluster K1



Jones et al (2012) Nature

sticklebrowser.stanford.edu

Bayesian 'Data-Driven' Clustering to identify other types of clustering patterns in the genome



Fish falling in cluster K2

Fish falling in cluster K1

Jones et al (2012) Nature

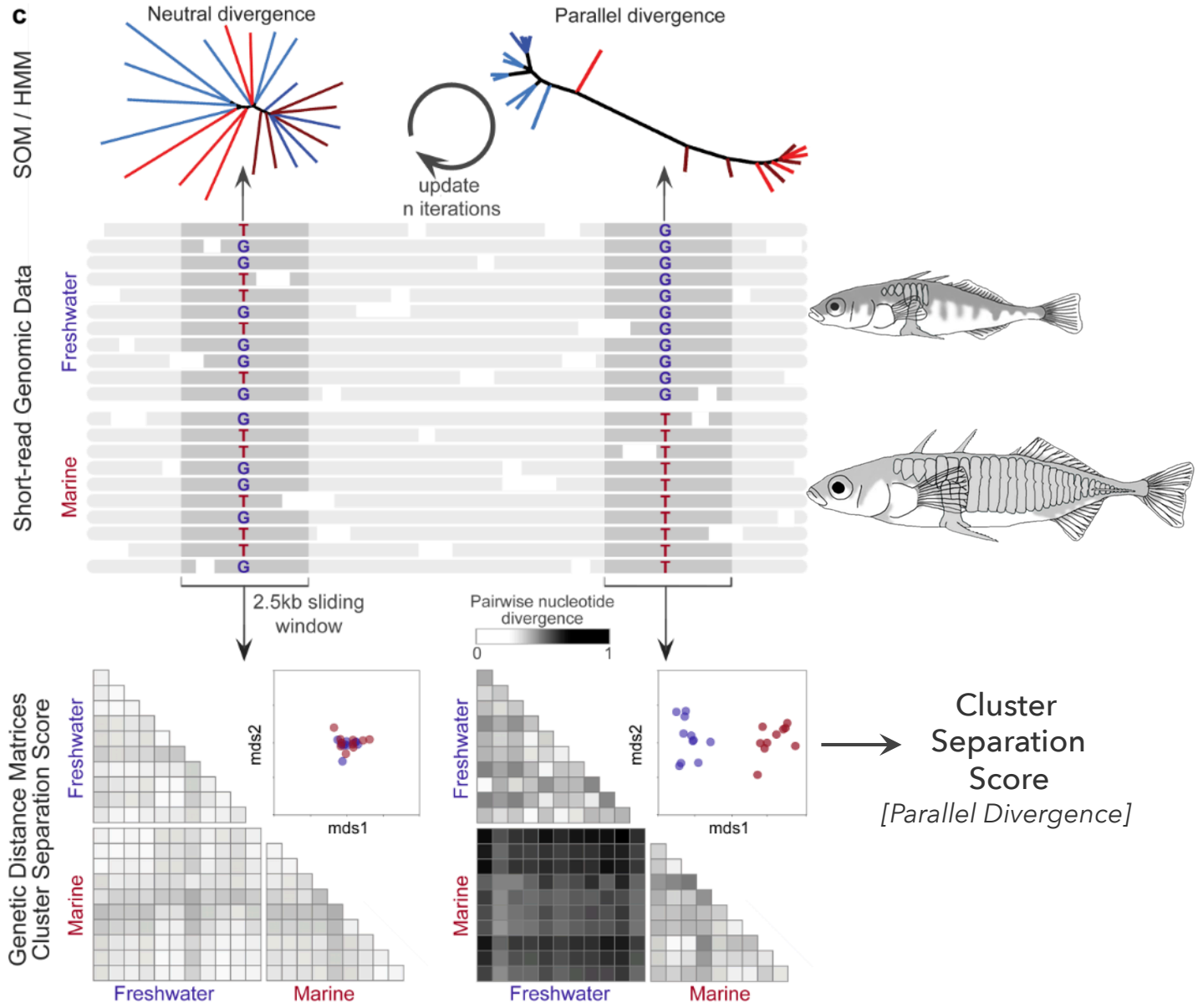
sticklebrowser.stanford.edu

Two different statistical approaches to identify parallel adaptive loci

Self-organized Map / Hidden Markov Model

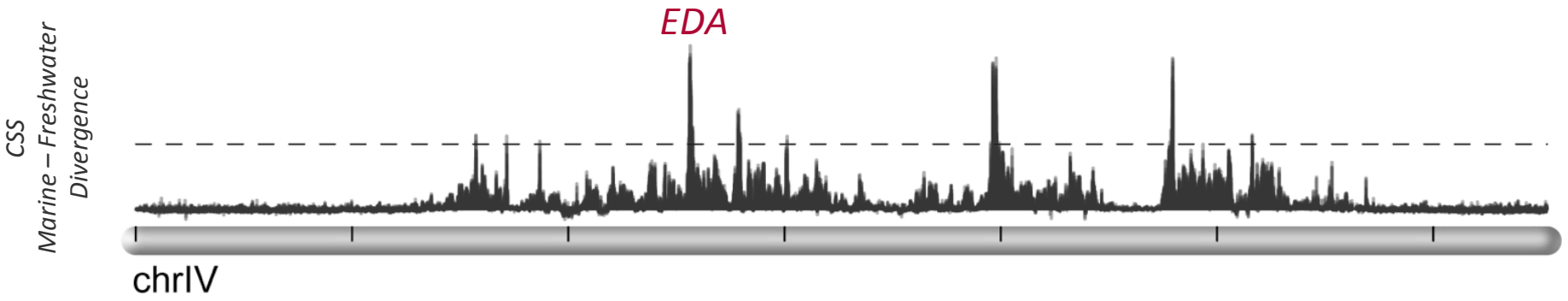
Illumina short-read genomic sequencing

Genetic distance matrices

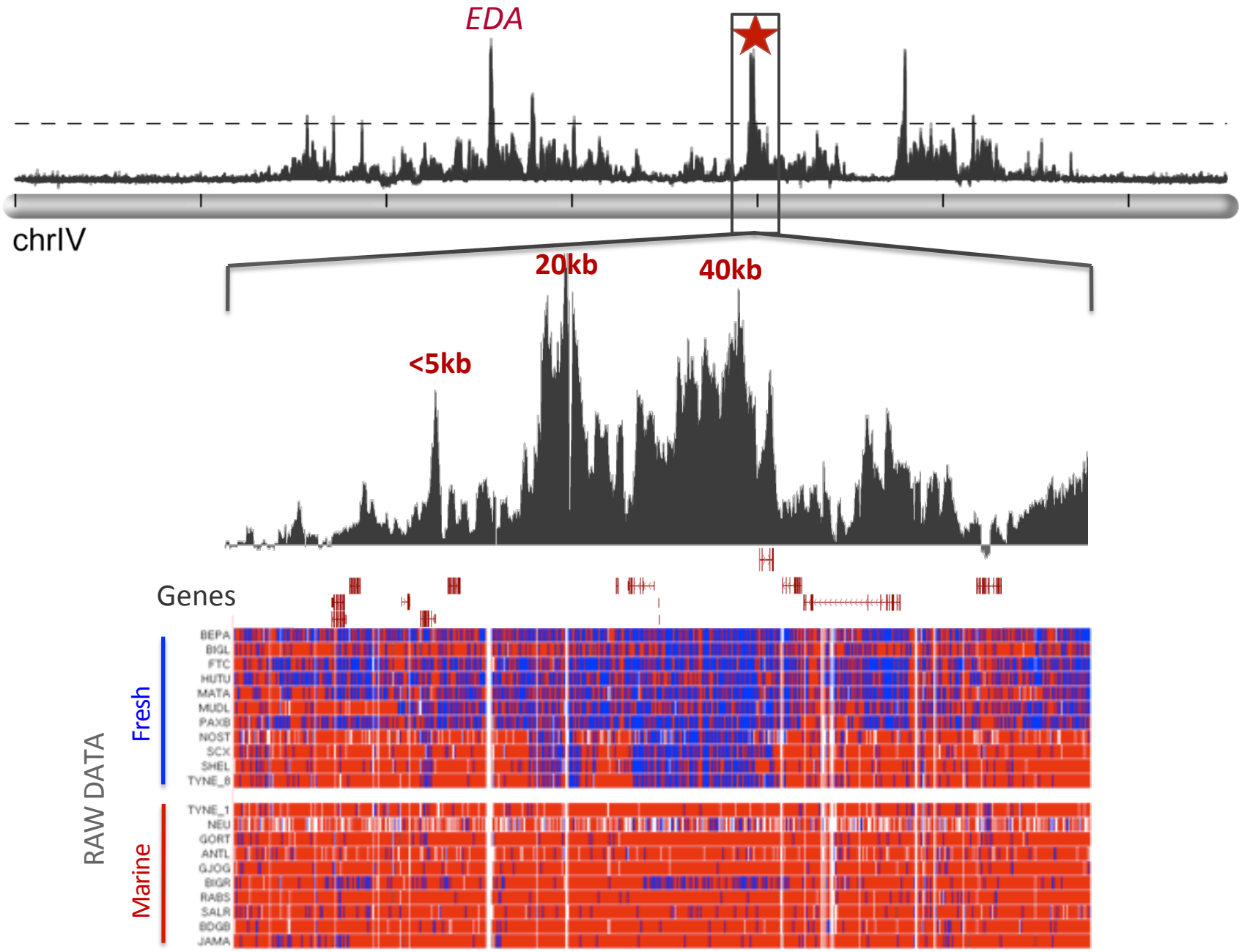


Jones et al (2012) Nature

Clear signals of parallel marine-freshwater divergence

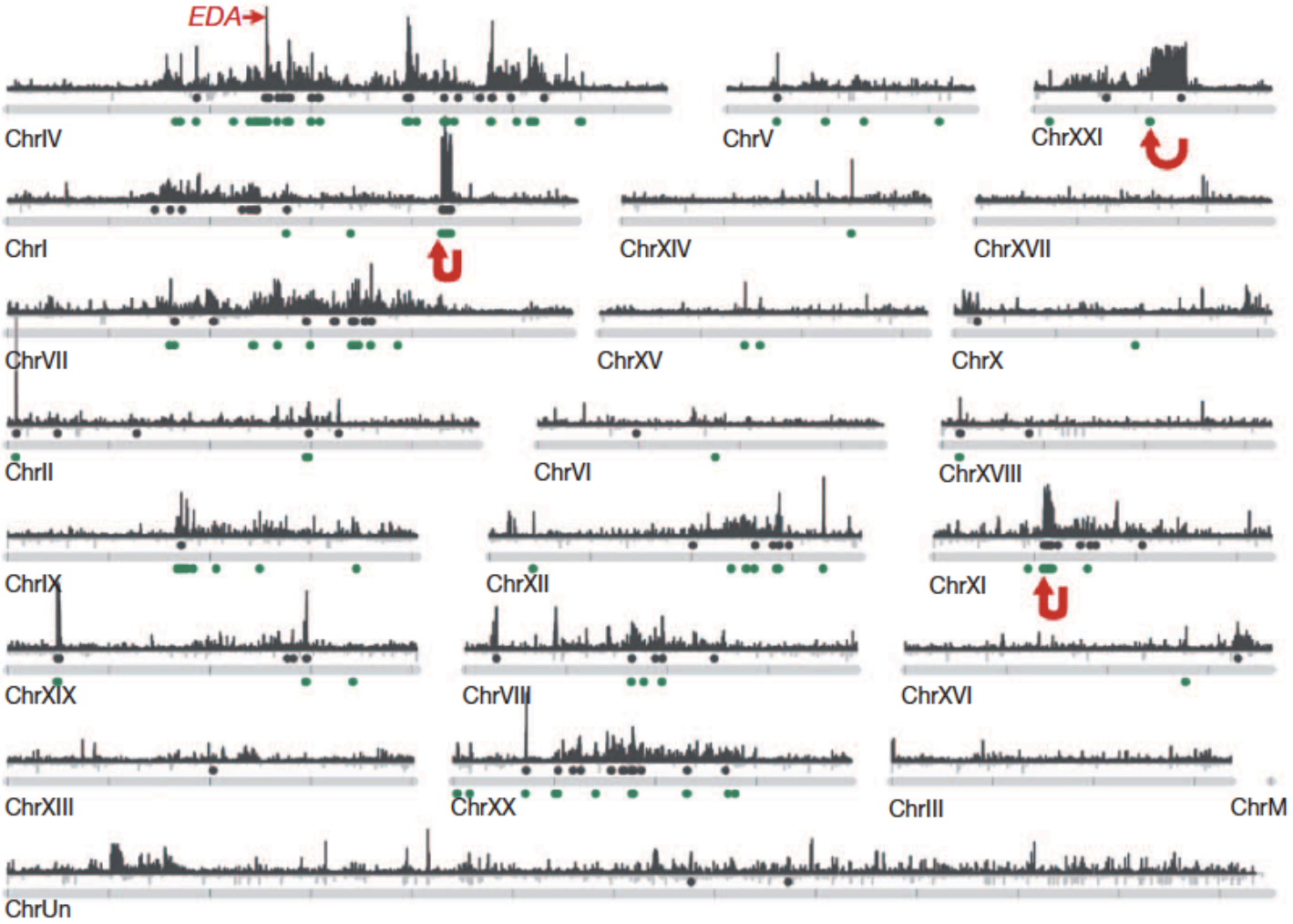


High resolution signals = good for functional follow-up

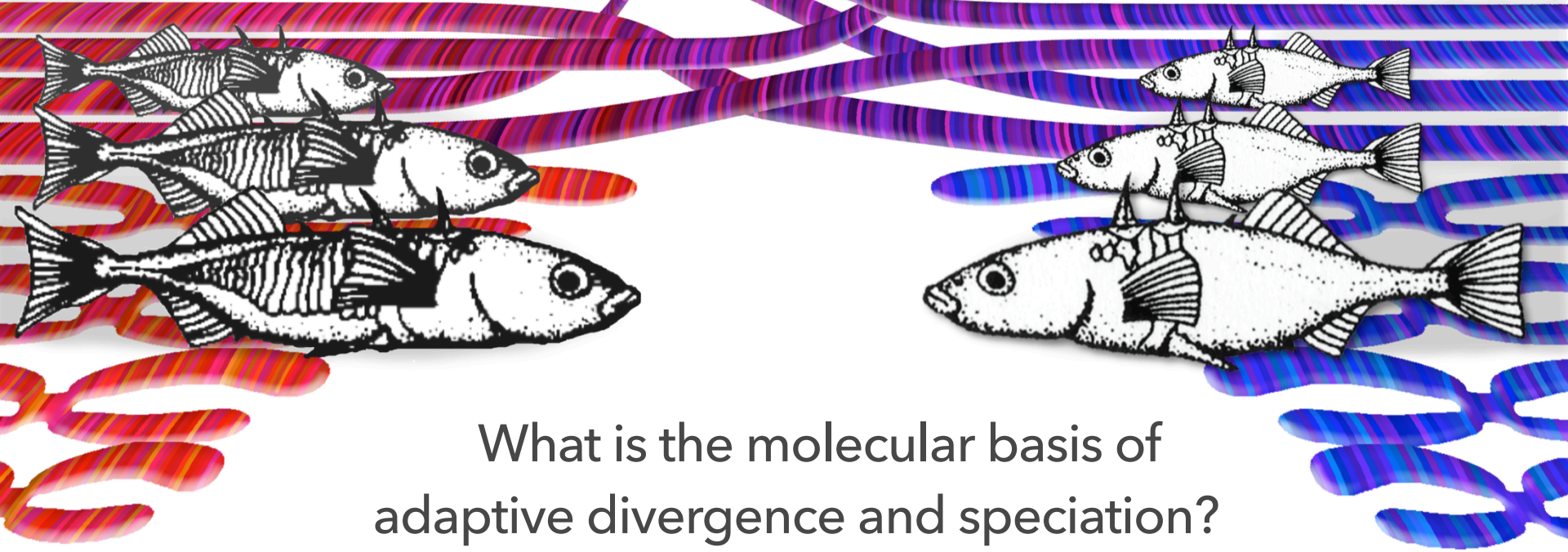


Jones et al (2012) Nature

Whole genome sequencing of marine – freshwater species pairs revealed parallel adaptive divergence at ~81 genomic loci



Jones et al (2012) Nature



What is the molecular basis of adaptive divergence and speciation?

Identifying and functionally testing adaptive loci

1. Forward genetics: from trait to basepairs

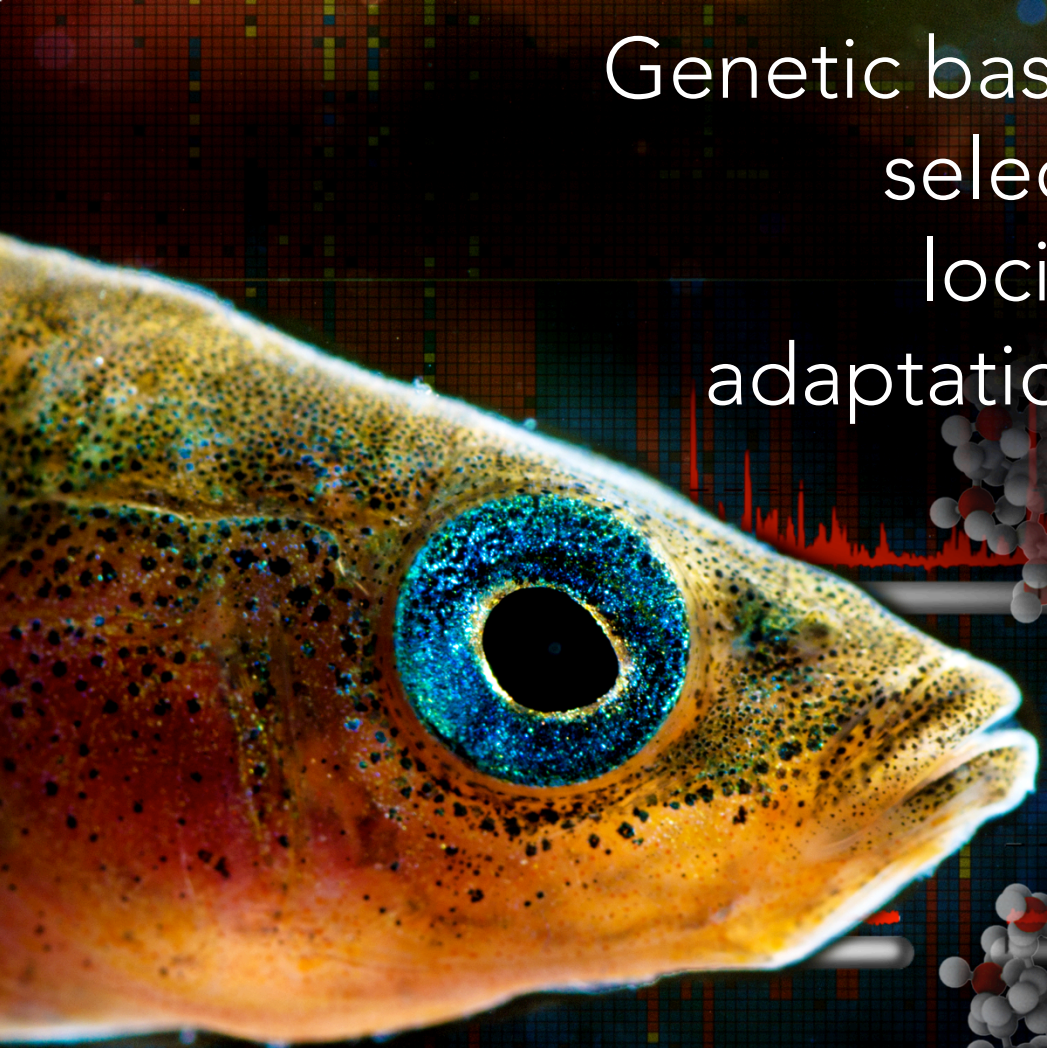
2. Population genomics

Genetic basis and signatures of selection around loci underlying adaptation and speciation

Felicity Jones

Friedrich Miescher Laboratory of Max Planck Society

Tübingen, Germany



Male threespine stickleback

DFG Deutsche Forschungsgemeinschaft

erc European Research Council Consolidator Grant

MAX-PLANCK-GESELLSCHAFT

fml

What is the molecular basis of adaptive divergence?

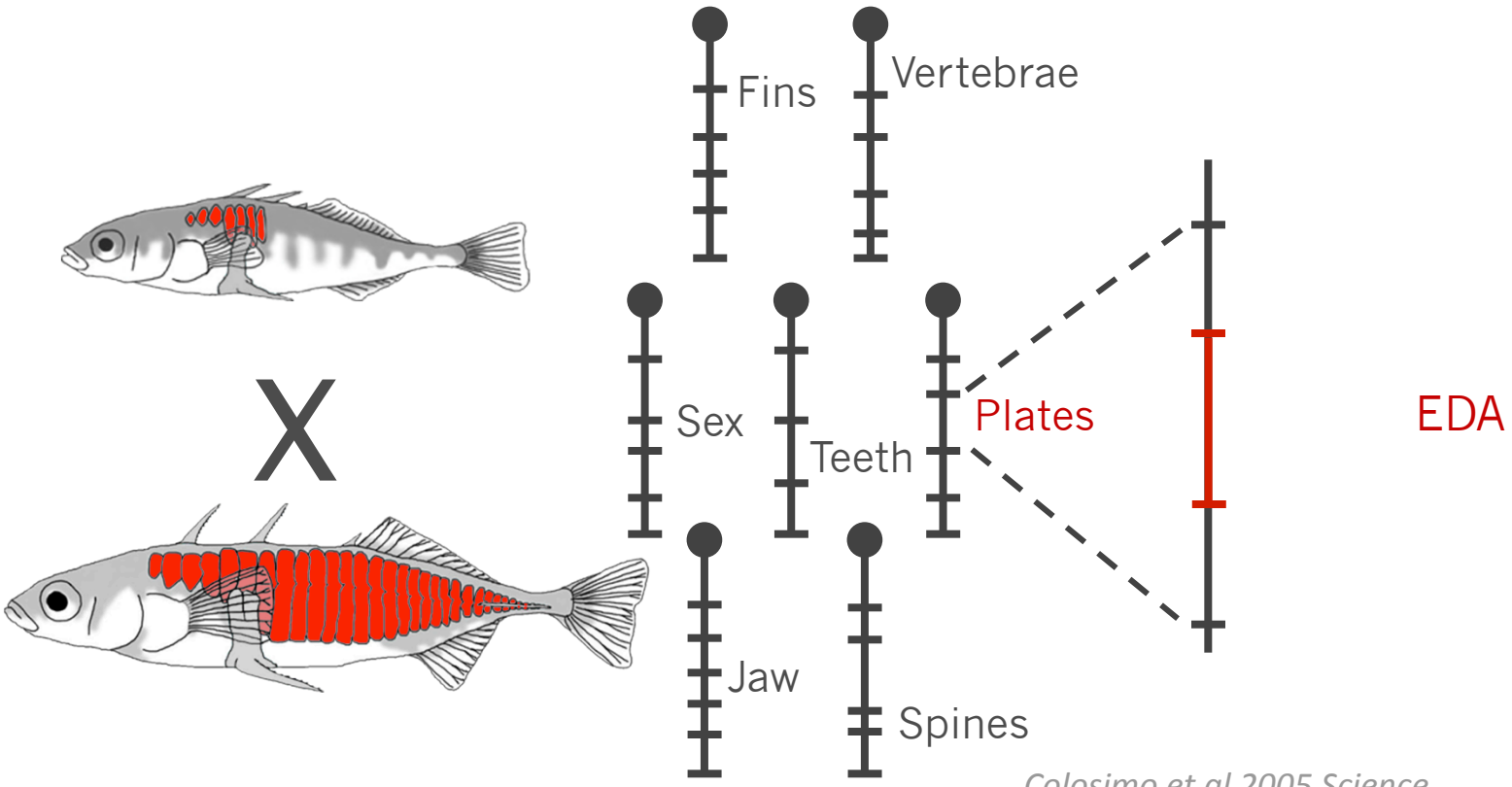
Two parts:

1. Methods used to identify loci underlying adaptive divergence
   - Forward genetic mapping
   - Population genomics

2. CASE STUDIES of molecular signatures of selection around known loci
   - EDA
   - Pitx1

   (what patterns might we expect to see at other adaptation loci?)

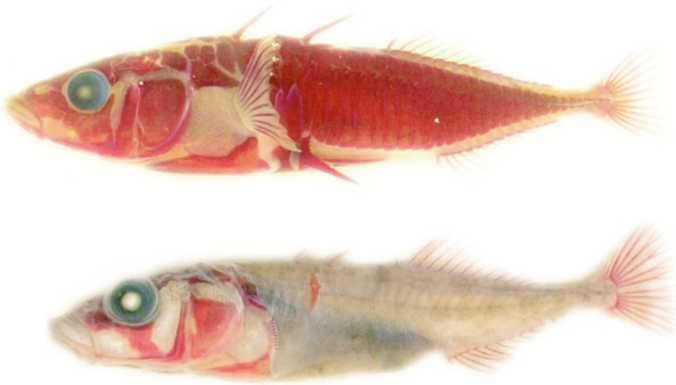
The EDA locus explains as much as 70% of variation in plate number



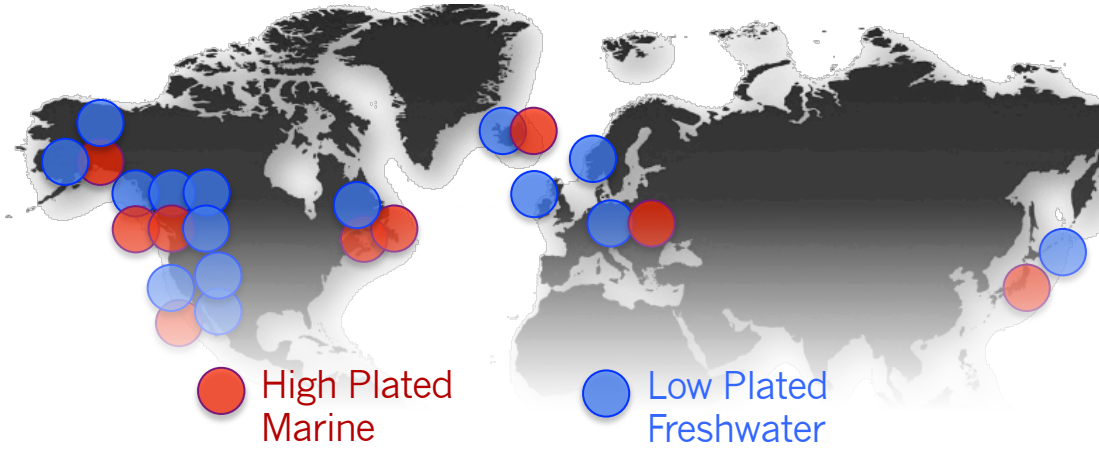
Colosimo et al 2005 Science

Cross divergent forms → Establish F2 generation, Build Genetic Map → Identify 2LOD Interval

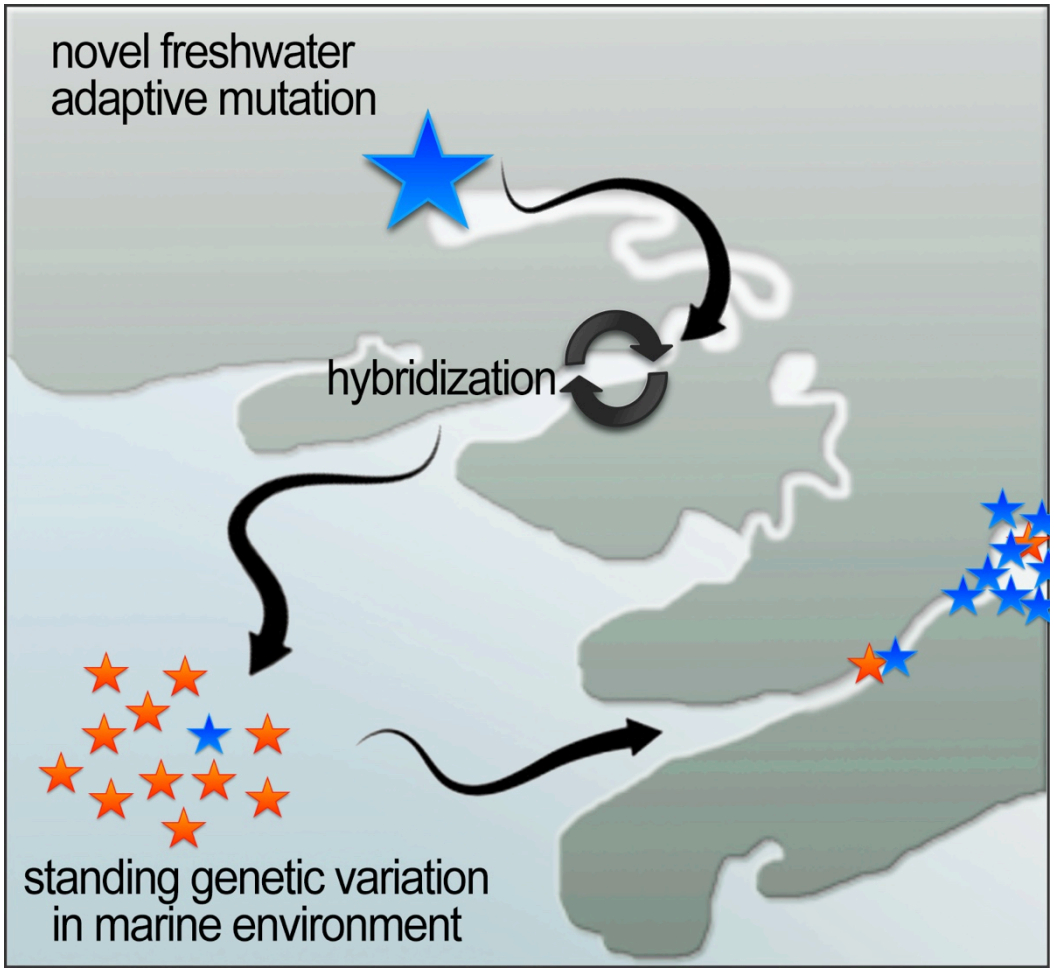
Loss of lateral plates has evolved repeatedly via re-use of an ancient EDA haplotype



Plates (*EDA*)



Parallel evolution via reuse of pre-existing genetic variation

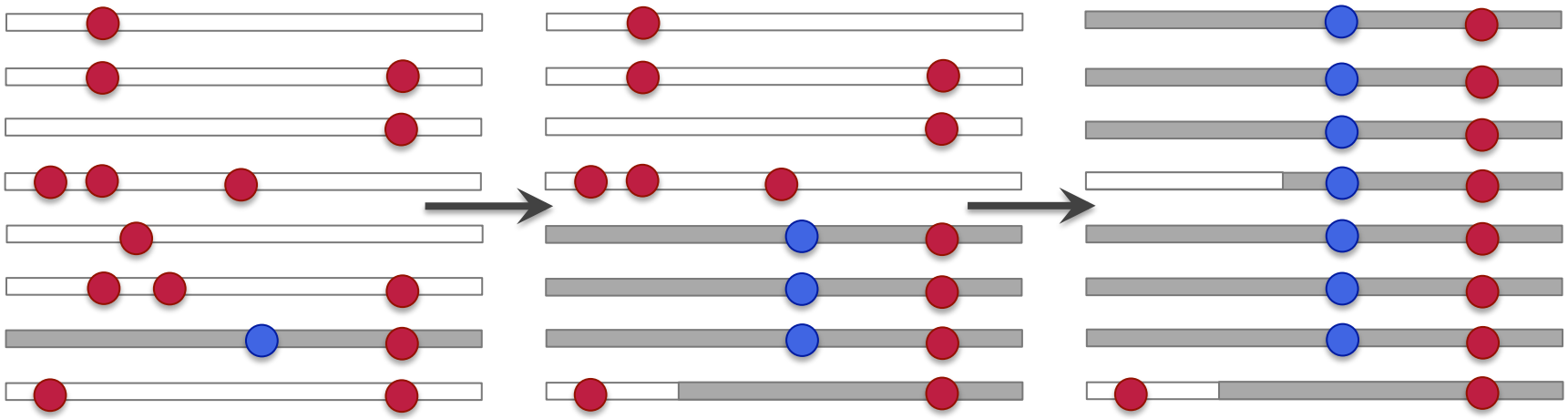


Low Plated EDA alleles 'hide' at low frequency (0-6%) in the High Plated marine population

Selective sweep of "pre-screened" adaptive variation

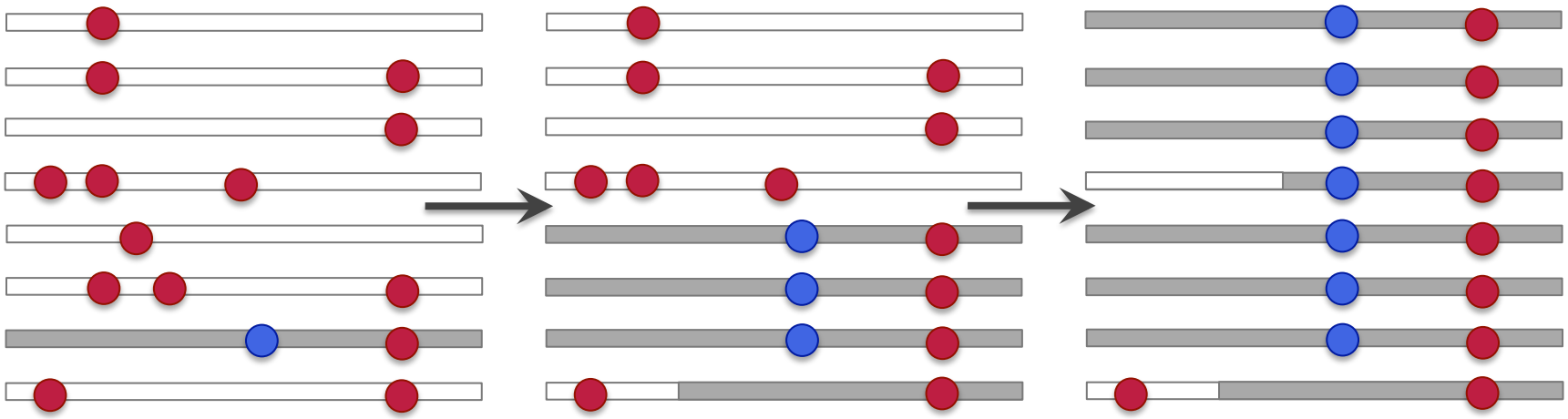
Colosimo *et al* (2005) Science
O'Brown et al (2015) ELife

Molecular signatures of selection



Neutral allele
Adaptive allele

Molecular signatures of selection

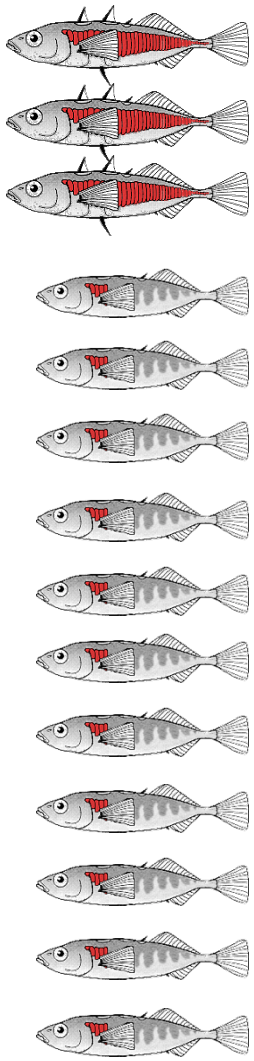


Neutral allele
Adaptive allele

Within population statistics:
Reduction in genetic diversity
Excess of derived alleles
Aberrant site frequency spectrum
Extended haplotype block size
(Elevated rates of protein evolution)

Cross population statistics:
Elevated genetic divergence
Difference in haplotype size

Molecular signatures of selection around EDA using high density genotyping arrays



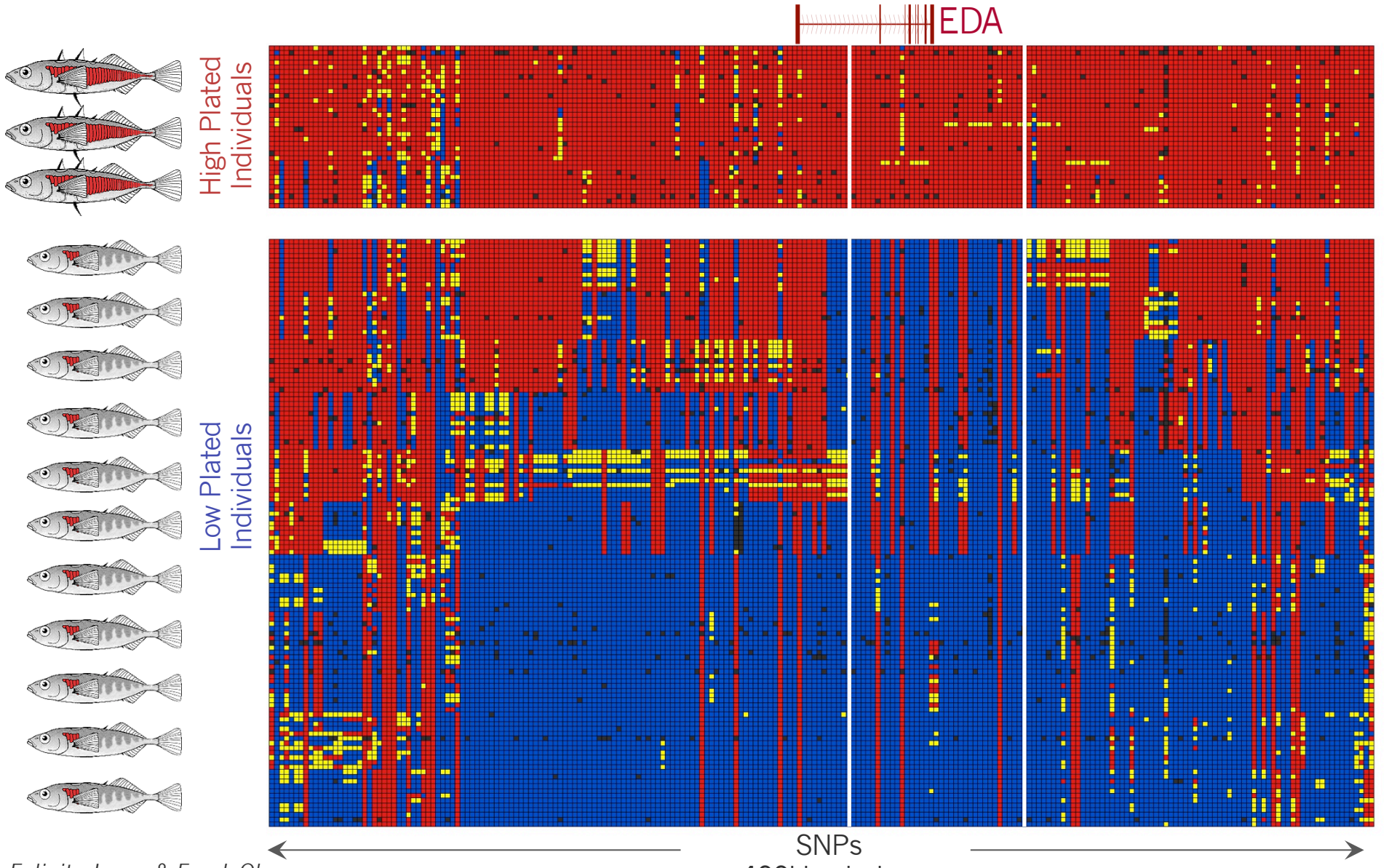
High Plated Individuals

Low Plated Individuals

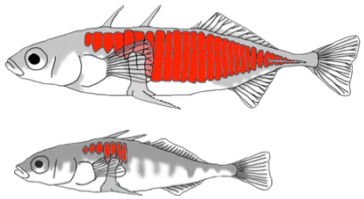
SNPs
400kb window

Felicity Jones & Frank Chan

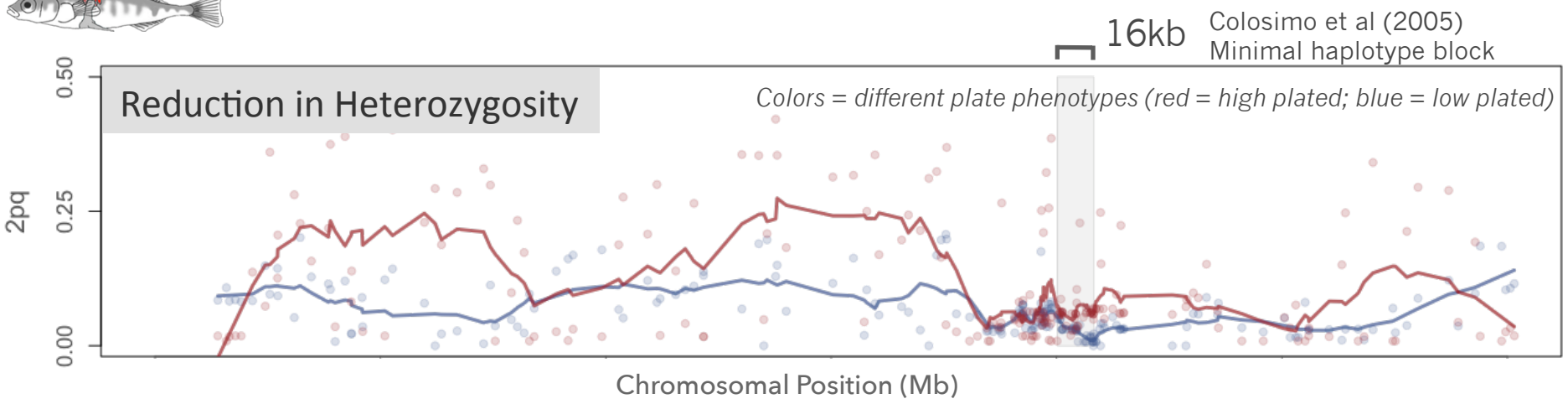
Molecular signatures of selection around EDA using high density genotyping arrays

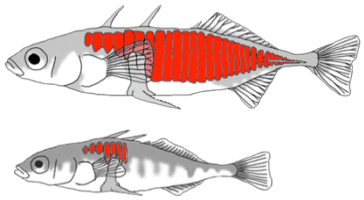


Felicity Jones & Frank Chan



Molecular signatures of selection around *EDA* using high density genotyping arrays





Molecular signatures of selection around *EDA* using high density genotyping arrays

16kb

Colosimo et al (2005)
Minimal haplotype block

Reduction in Heterozygosity

Colors = different plate phenotypes (red = high plated; blue = low plated)

2pq

0.50
0.25
0.00

Excess of Derived Alleles

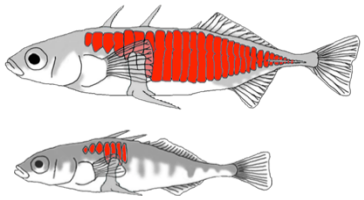
Fay & Wu's H

0.000
-0.005
-0.010

Fay & Wu's H (low plated populations)

Colors = different geographic regions (AK, CA, BC, AT)

Chromosomal Position (Mb)



Molecular signatures of selection around *EDA* using high density genotyping arrays

16kb

Colosimo et al (2005)
Minimal haplotype block

Reduction in Heterozygosity

Colors = different plate phenotypes (red = high plated; blue = low plated)

2pq

0.50
0.25
0.00

Excess of Derived Alleles

Fay & Wu's H (low plated populations)

Colors = different geographic regions (AK, CA, BC, AT)

Fay & Wu's H

0.000
-0.005
-0.010

Increased Genetic Divergence

Average F_{ST} between high and low plated populations

Fst

1.0
0.5
0.0

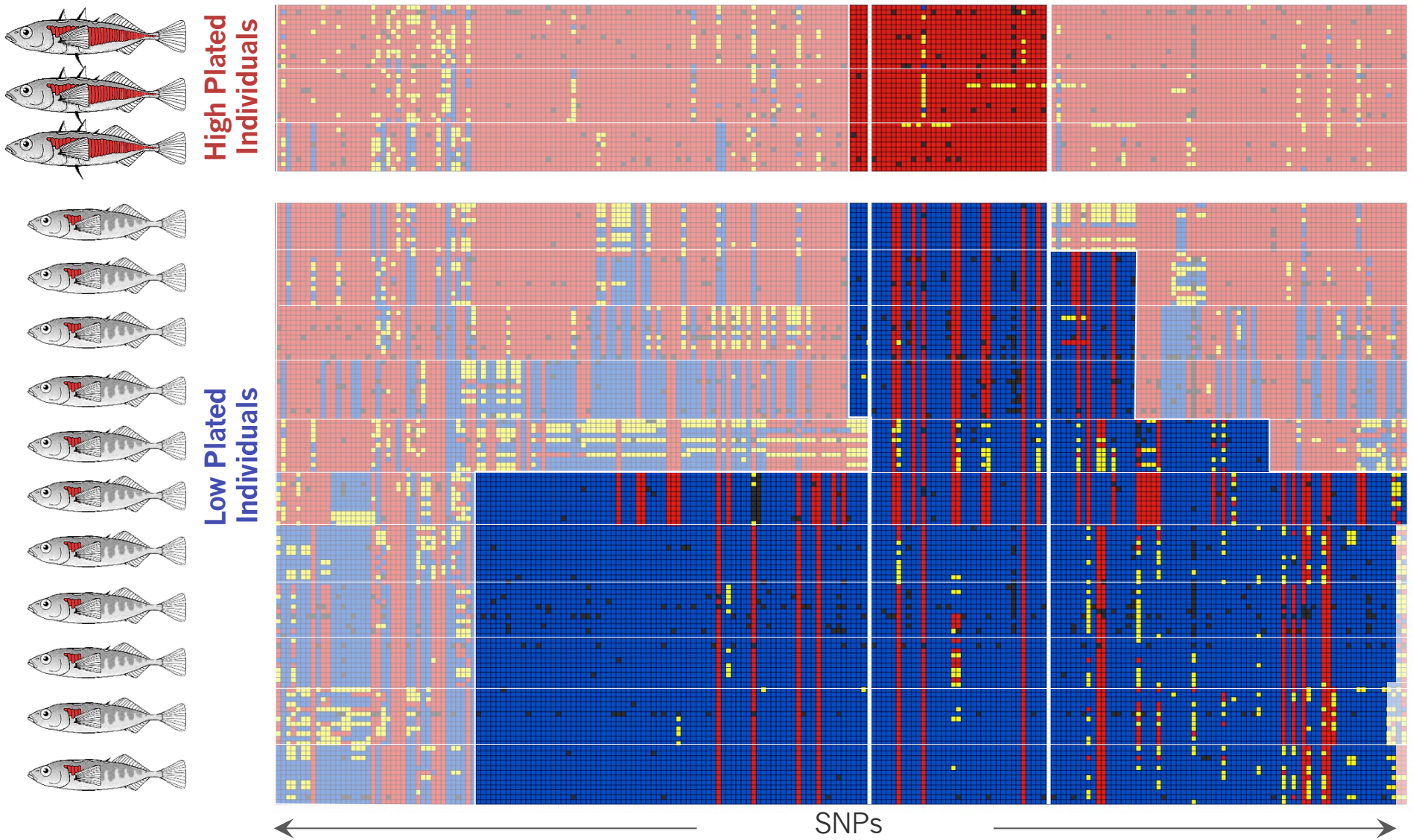
12.4 12.5 12.6 12.7 12.8 12.9 13.0

Chromosomal Position (Mb)

How is high resolution achieved?

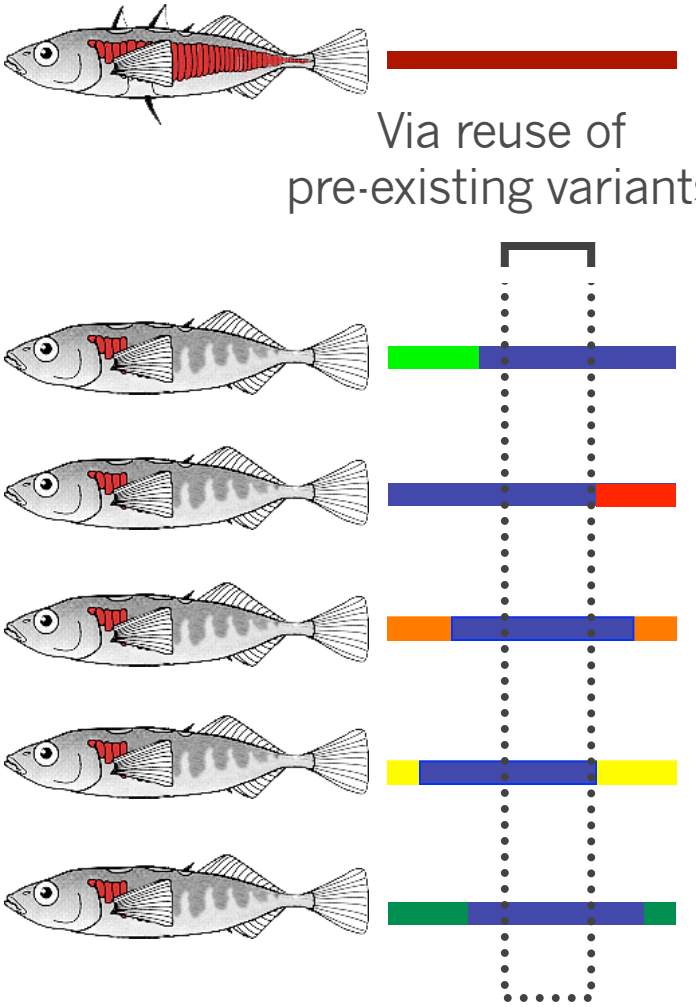
Comparisons *across* populations help narrow the EDA region

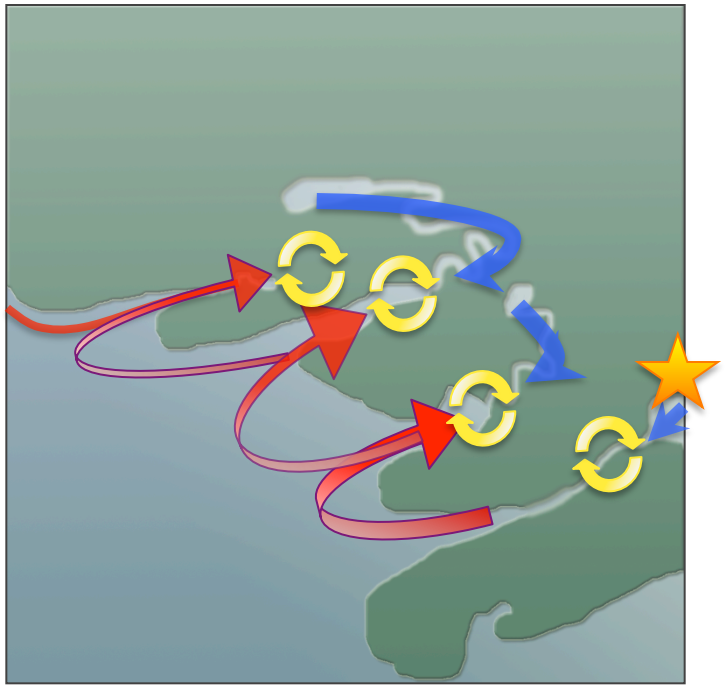
< 6.7kb



Felicity Jones & Frank Chan

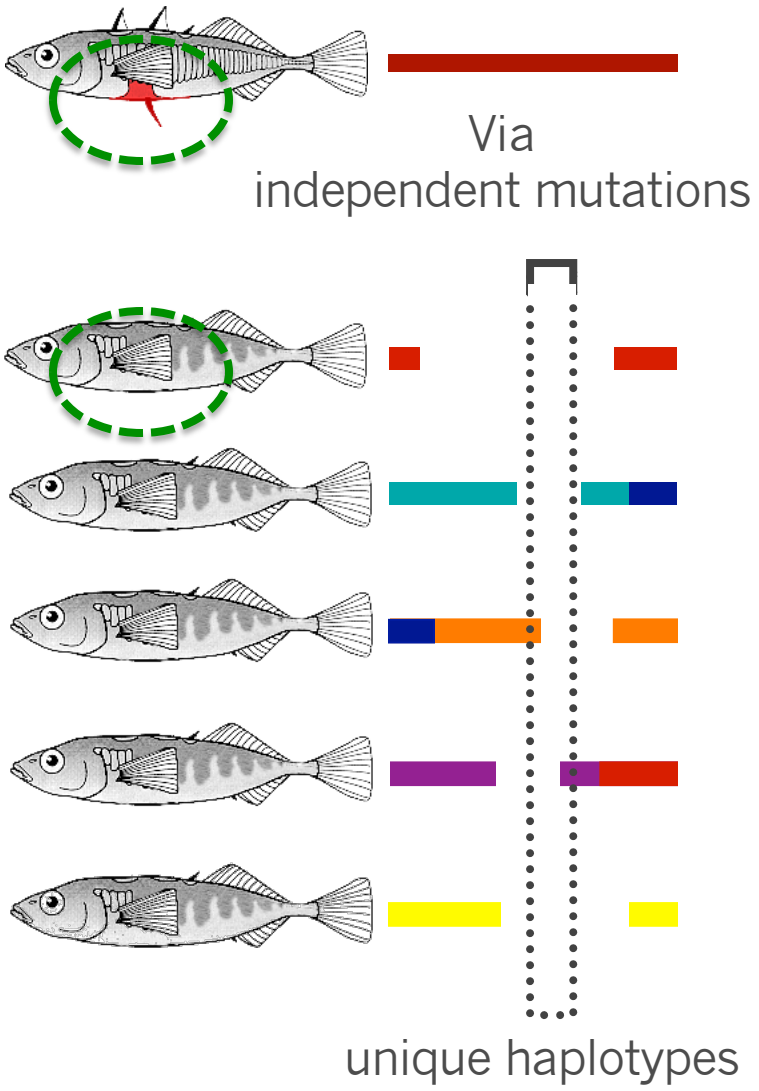
Parallel evolution via reuse of pre-existing variation





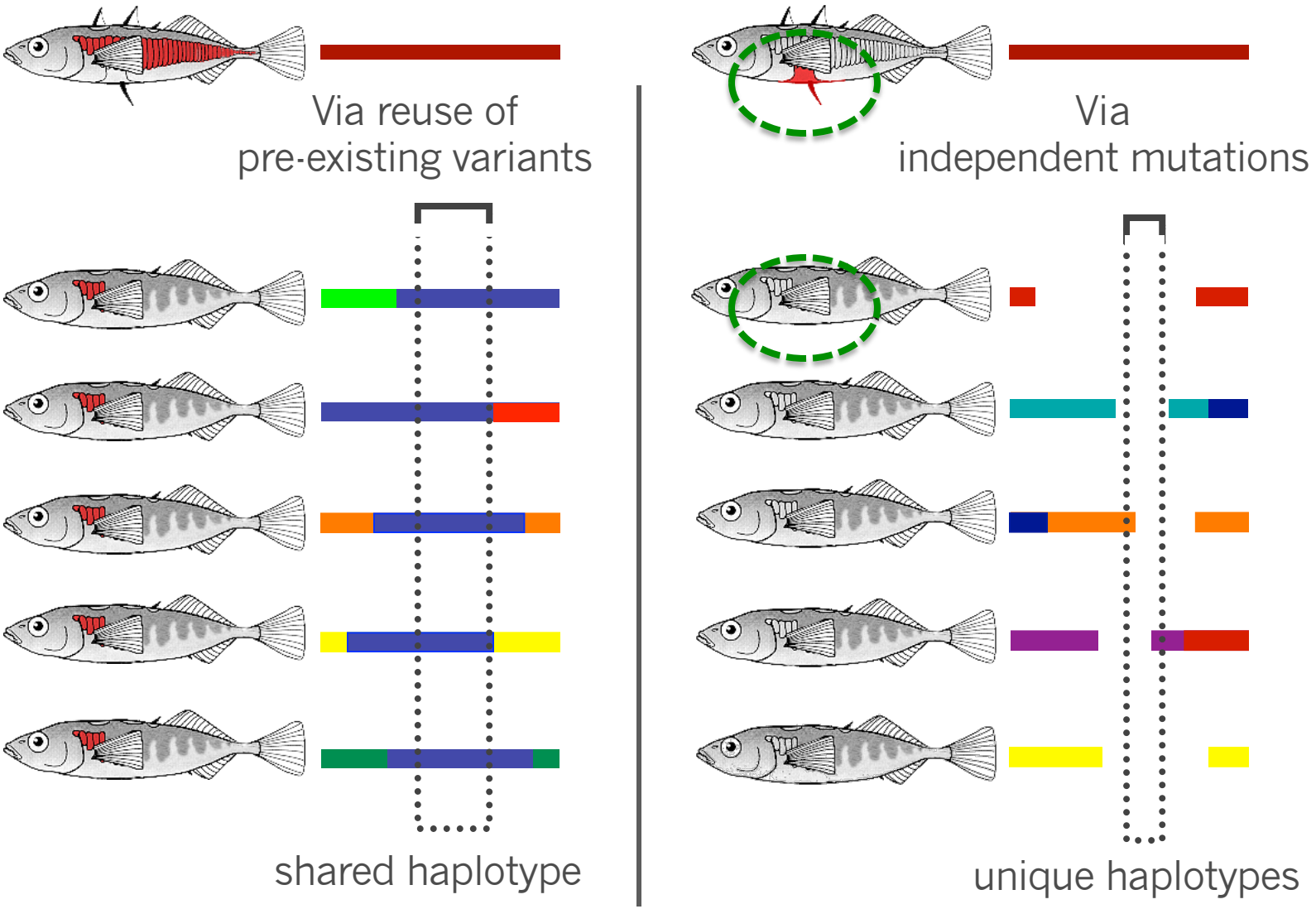
Plates (*EDA*) *Colosimo et al (2005) Science*
Pigmentation (*KITLG*) *Miller et al (2009) Cell*
81 new genomic loci *Jones et al (2012) Nature*

An alternative mode of parallel evolution



Pelvis (*PITX1*) *Chan et al (2010) Science*

An alternative mode of parallel evolution



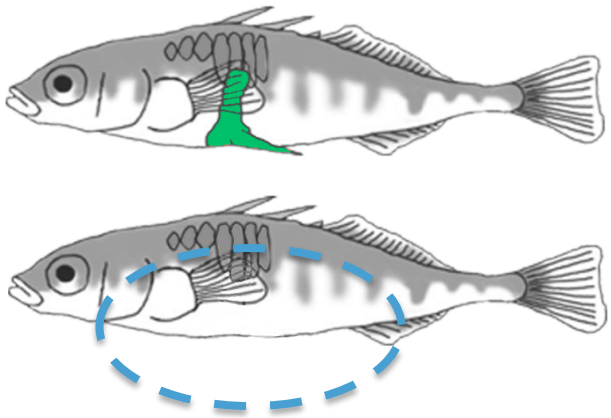
Plates (*EDA*) *Colosimo et al (2005) Science*

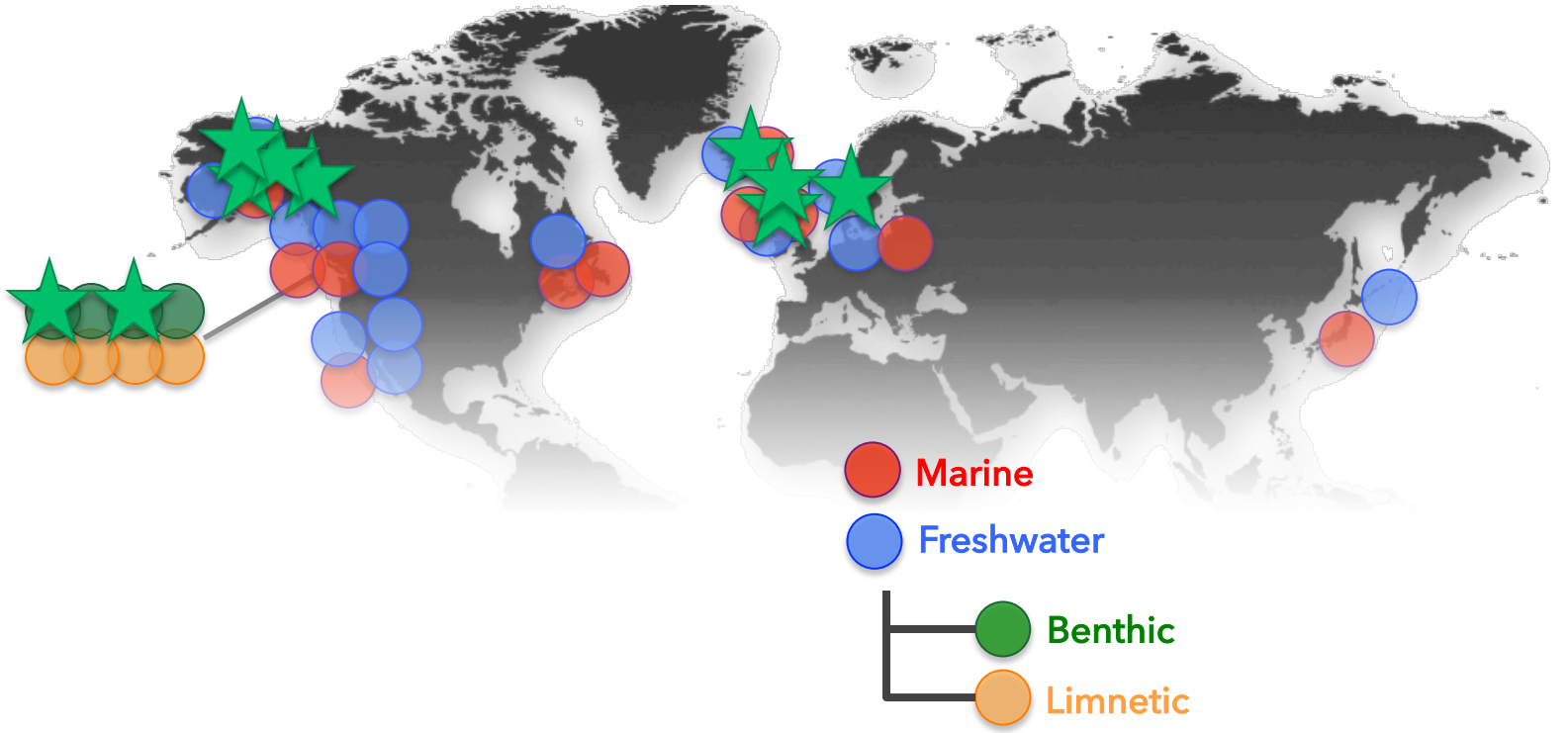
Pigmentation (*KITLG*) *Miller et al (2009) Cell*

Pelvis (*PITX1*) *Chan et al (2010) Science*

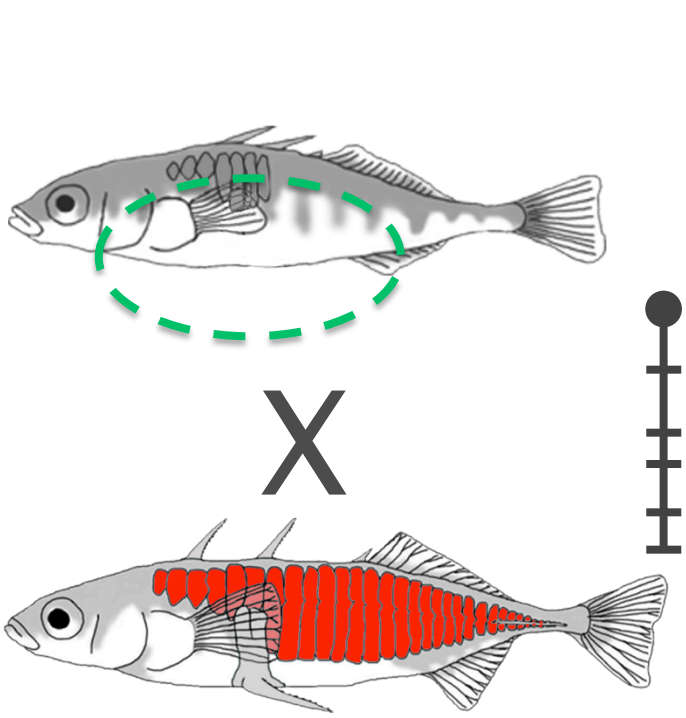
81 new genomic loci *Jones et al (2012) Nature*

Pelvic spine loss has evolved repeatedly





A major QTL explaining close to 80% variation on linkage group VII



X

Fins
Vertebrae
Sex
Teeth
Pelvis
Jaw
Plates

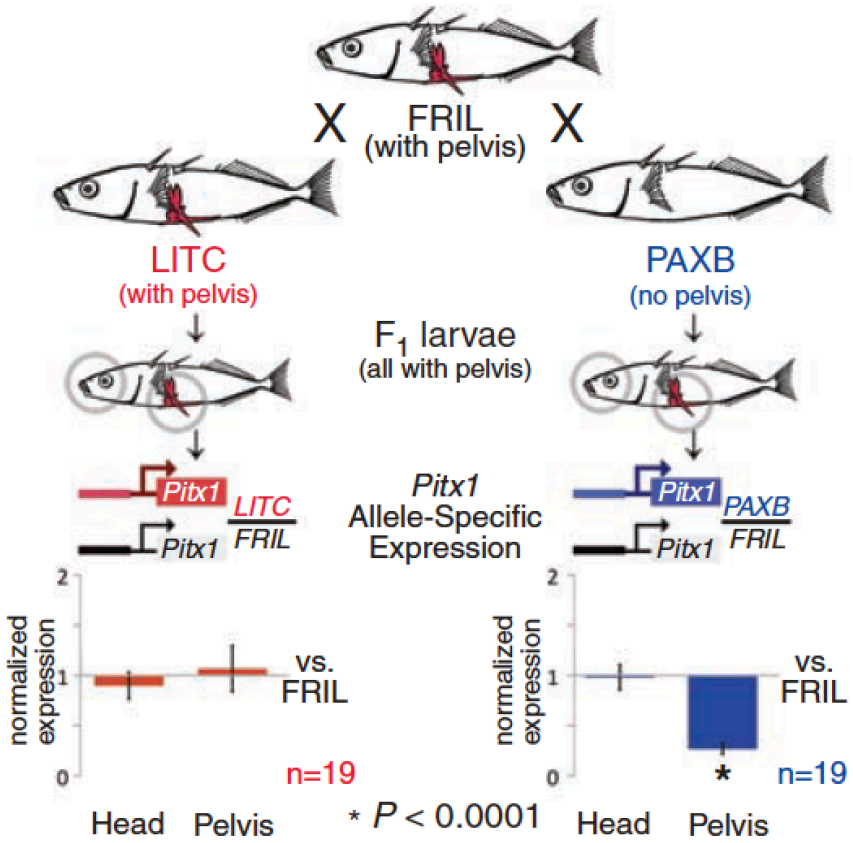
QTL Candidate Interval (including *Pitx1* gene: a key hindlimb developmental regulator)

Cross divergent forms → Establish F2 generation, Build Genetic Map → Identify 2LOD Interval

Shapiro et al 2004 Nature

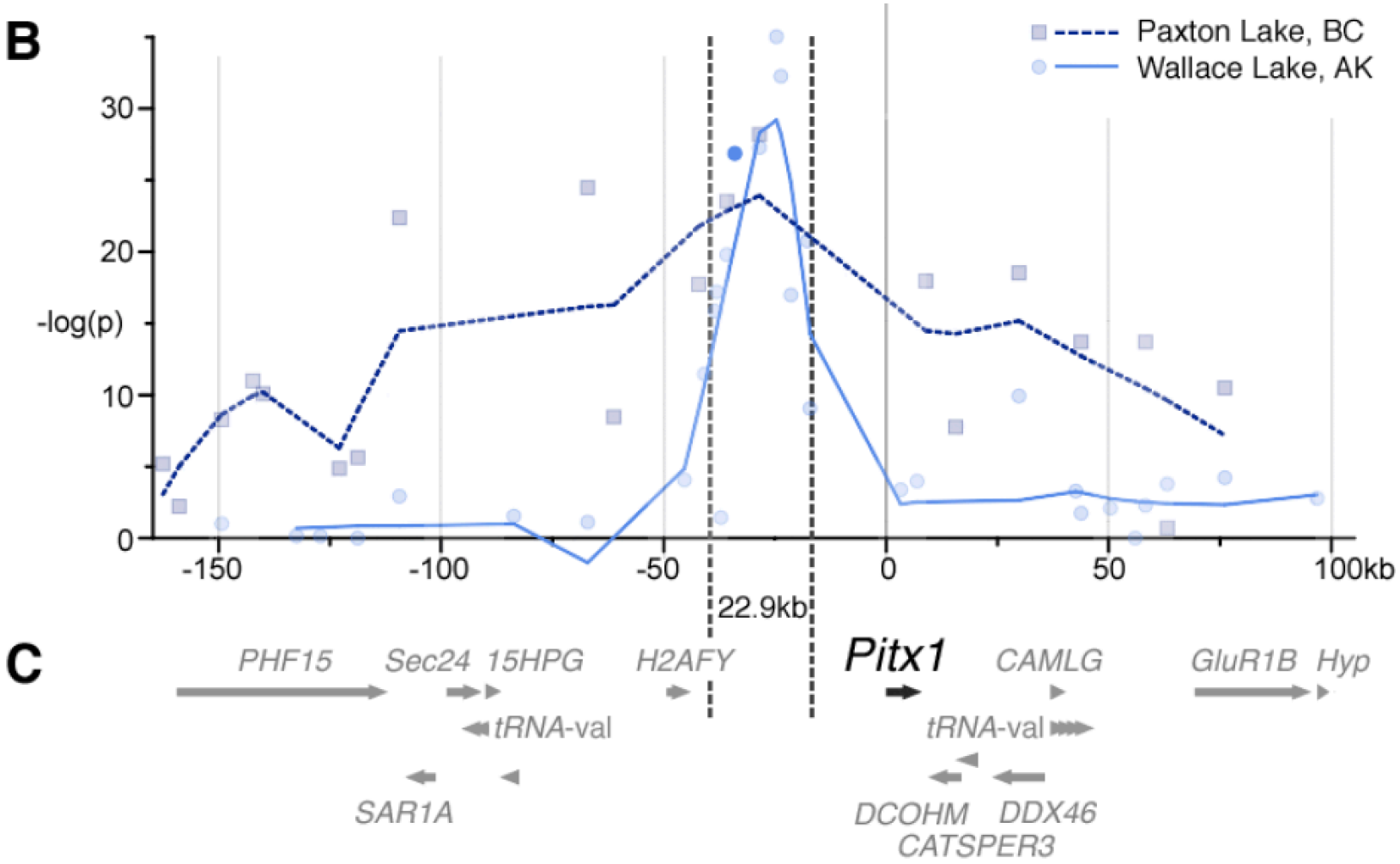
Pitx1 allele-specific expression assay in F1 hybrids

- Higher expression of complete-pelvis Pitx1 allele than pelvic-reduced allele
- Cis-regulatory element, rather than transacting factor



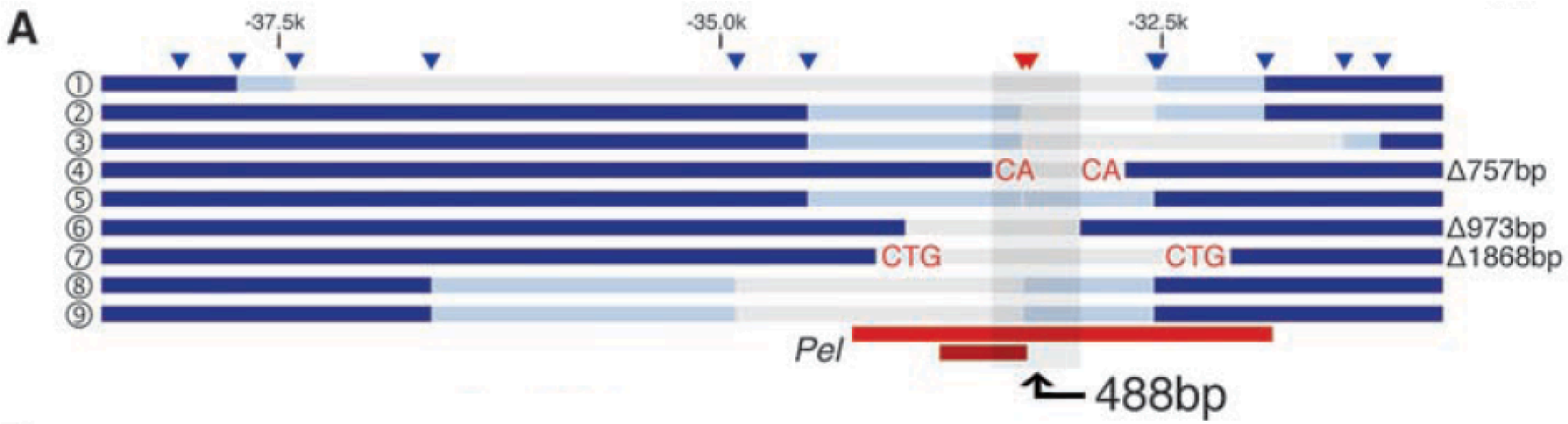
Chan et al 2010 Science

Association mapping in two populations polymorphic for pelvic-reduction identifies a 23kb region 5′ of Pitx1



Chan et al 2010 Science

A high density genotyping array reveals deletions in pelvic-reduced populations with a minimum shared interval of 488bp



Chan et al 2010 Science

Transgenic assays reveal a regulatory element and phenotypic rescue

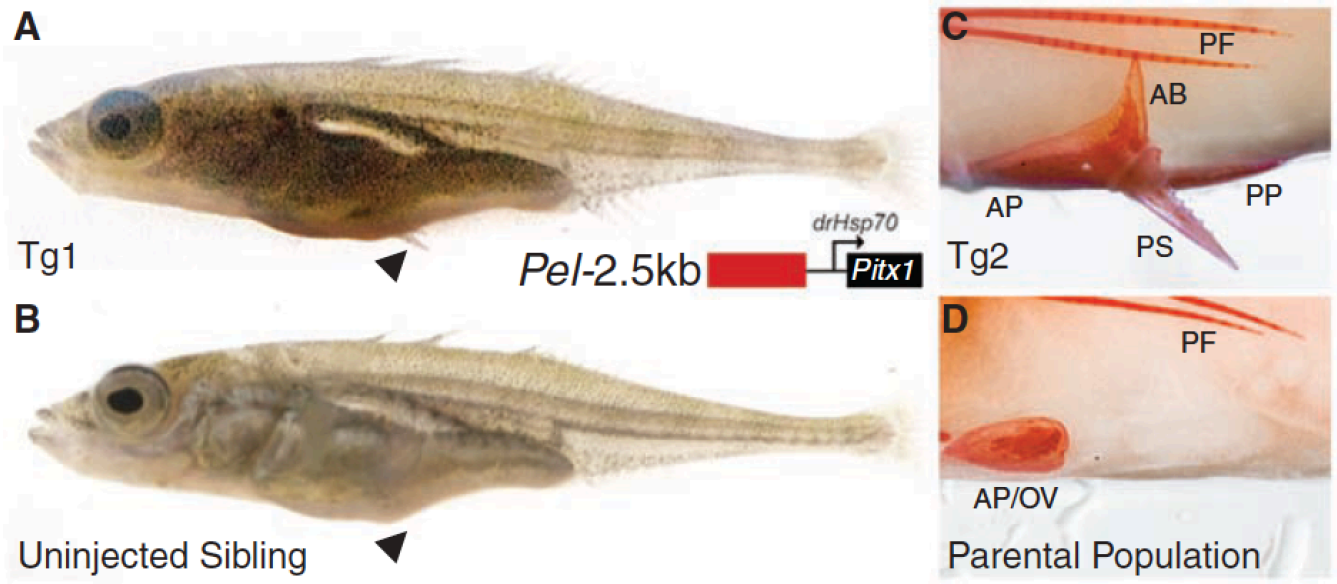
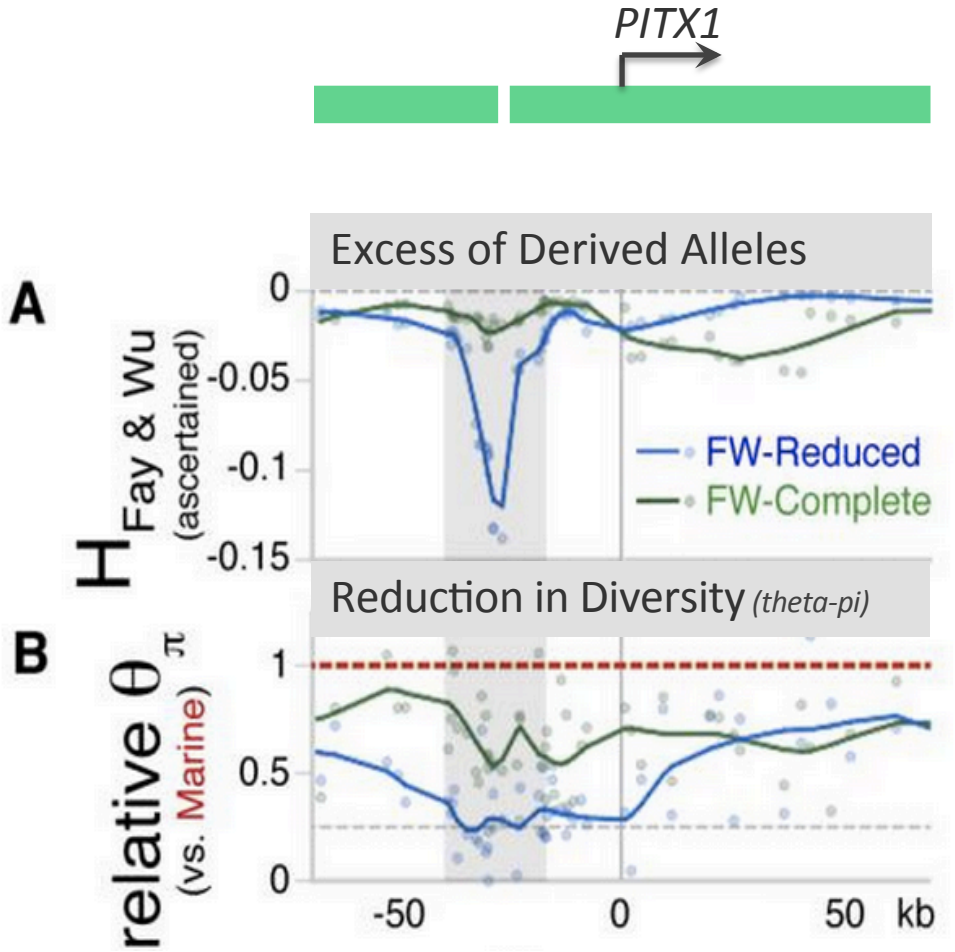


Fig. 3. (**A**) Juvenile pelvic-reduced BEPA stickleback expressing a *Pitx1* transgene driven by the *Pel*-2.5-kb^{SALR} enhancer compared with (**B**) uninjected sibling. External spines form only in transgenic fish (arrowhead). (**C** and **D**) Alizarin red–stained pelvic structures of adult transgenic fish compared with BEPA parental phenotype. BEPA fish normally develop only a small ovoid vestige (OV) of the anterior pelvic process (AP). Transgenic fish show clear development of the AP, ascending branch (AB), and posterior process (PP) of the pelvis, and a prominent serrated pelvic spine. Pectoral fin (PF) rays develop in both fish.

Chan et al 2010 Science

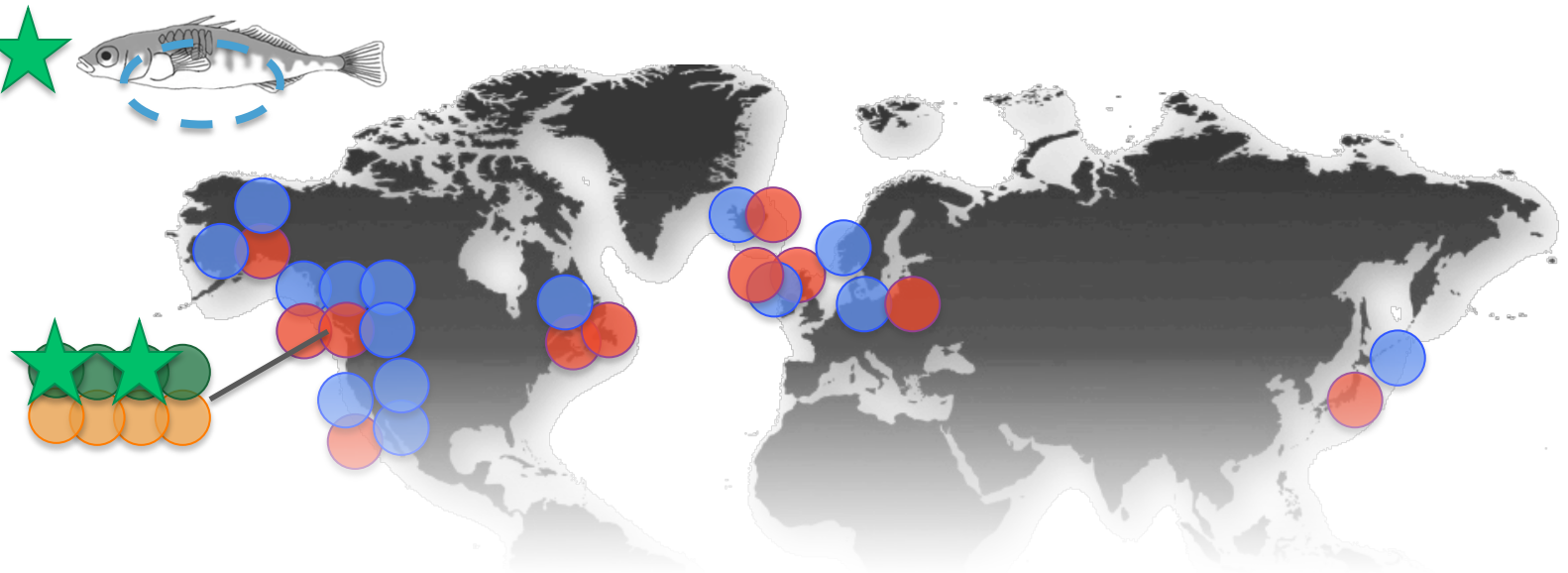
Molecular signatures of selection at the Pel regulatory element in pelvic reduced populations

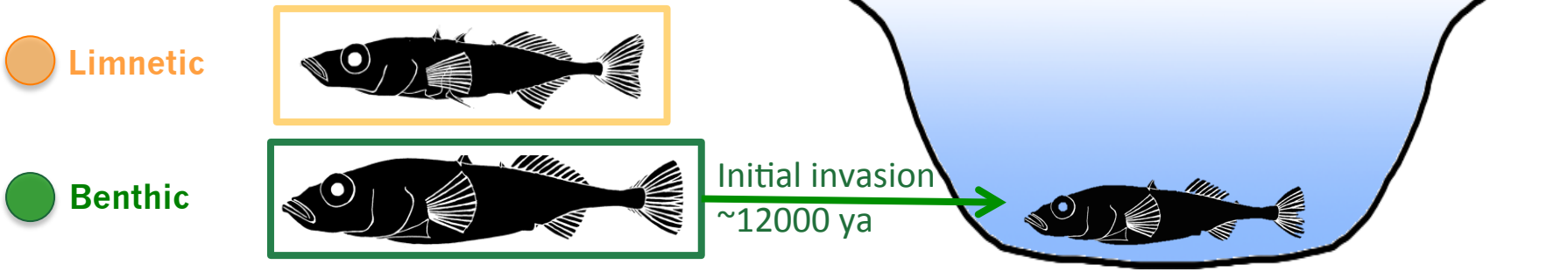


Chan et al (2010) Science

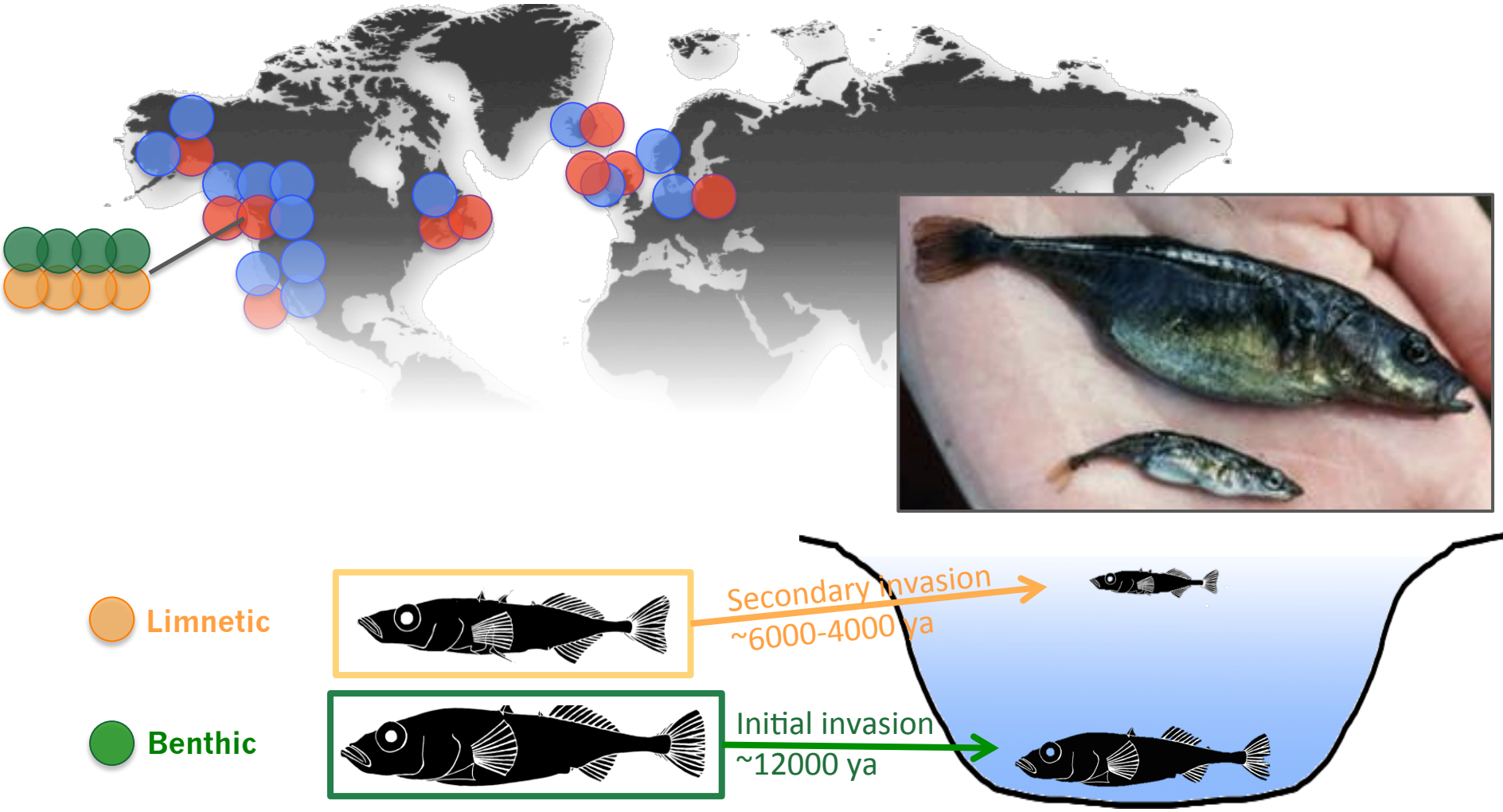
Chan et al 2010 Science

Pelvic spine loss has evolved repeatedly (including in two benthic populations)



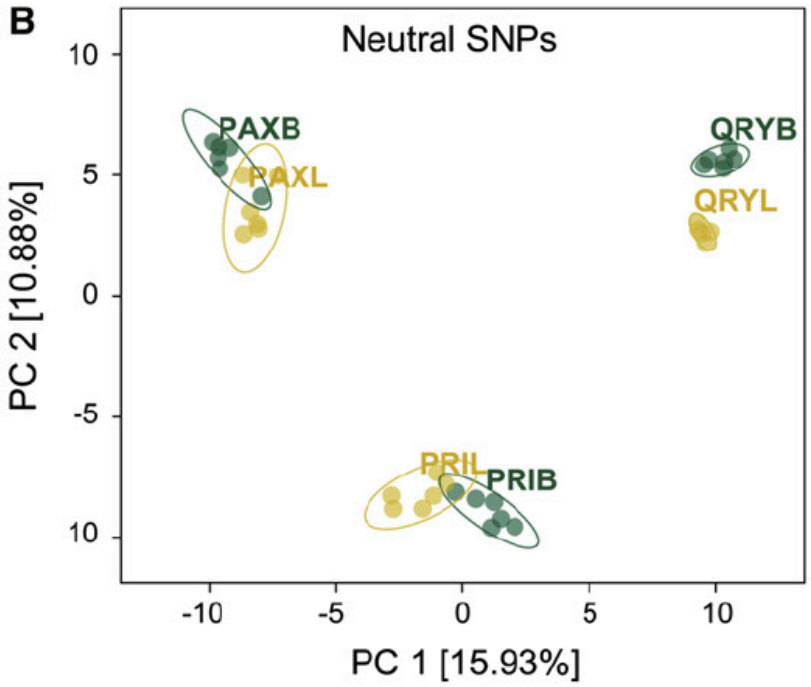


Benthic and limnetic ecotypes evolved following a "Double Invasion" event caused by isostatic rebound

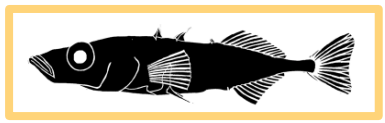


What is the genetic basis of the divergence?
At neutral loci, fish cluster together by lake

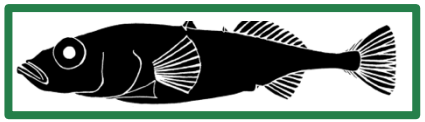
Genome wide SNP genotyping array
(~1000 SNPs)



Limnetic



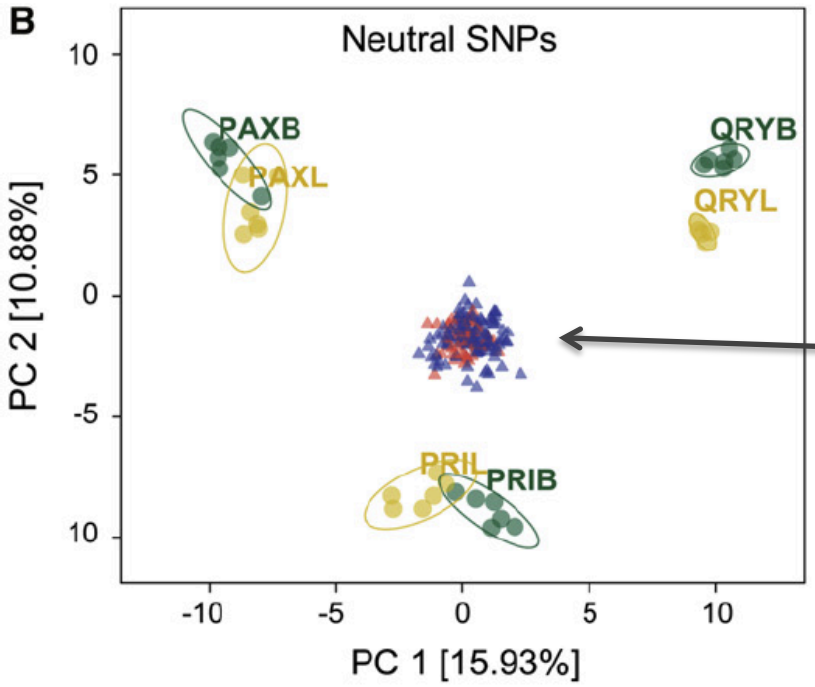
Benthic



Jones et al (2012) Current Biology

What is the genetic basis of the divergence?
At neutral loci, fish cluster together by lake

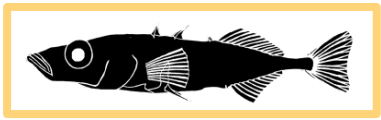
Genome wide SNP genotyping array
(~1000 SNPs)



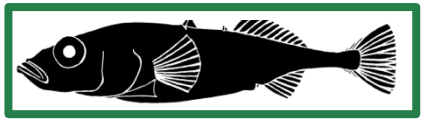
Projection of global populations onto PCA using loadings from Benthic Limnetic eigenvectors

Global marine & freshwater fish populations are not predictive of the source of neutral variation

Limnetic



Benthic

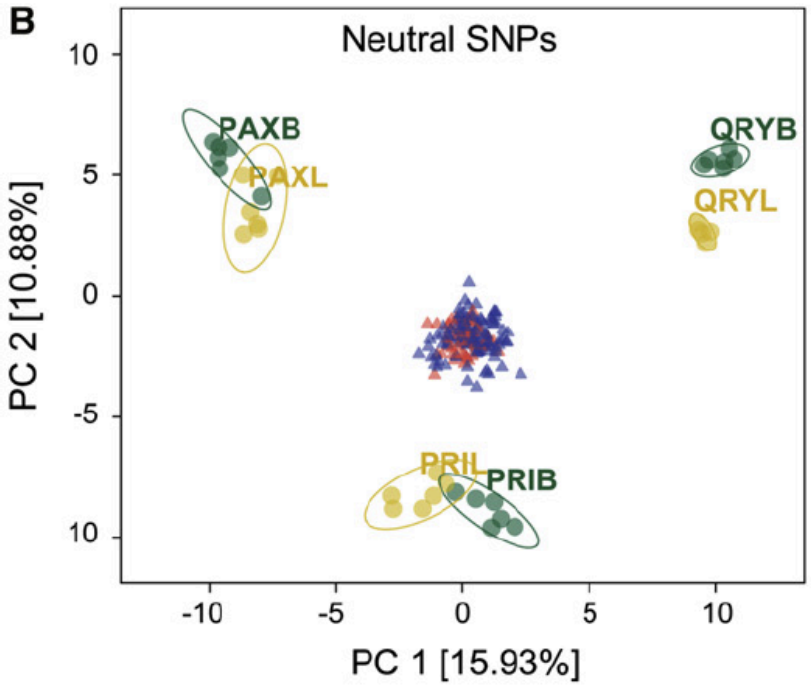


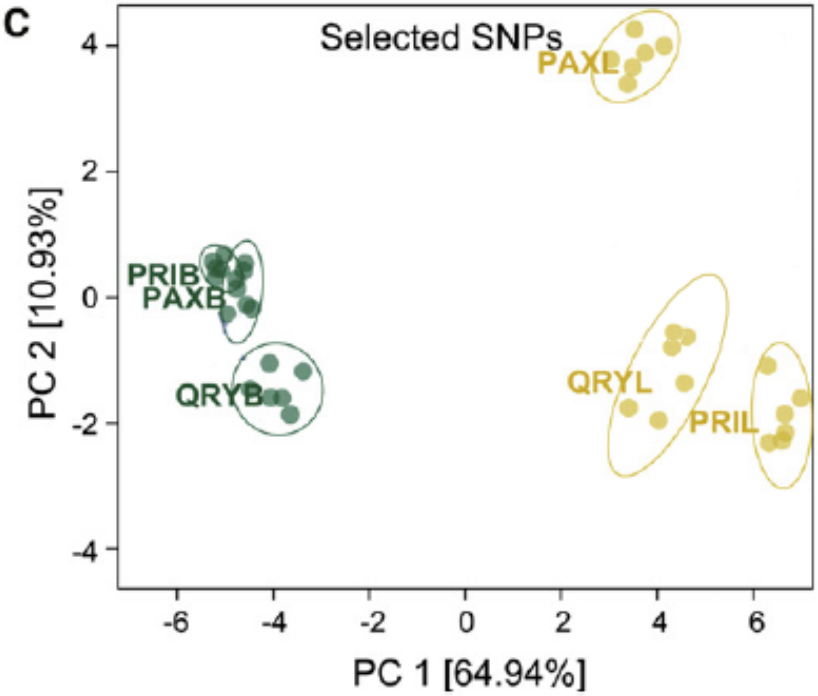
Jones et al (2012) Current Biology

But at selected loci, fish cluster by ecotype

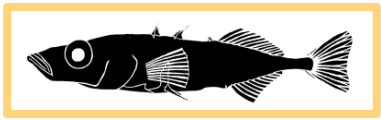
Genome wide SNP genotyping array
(~1000 SNPs)

F_{ST} Outlier SNPs:

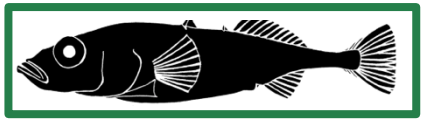




Limnetic



Benthic

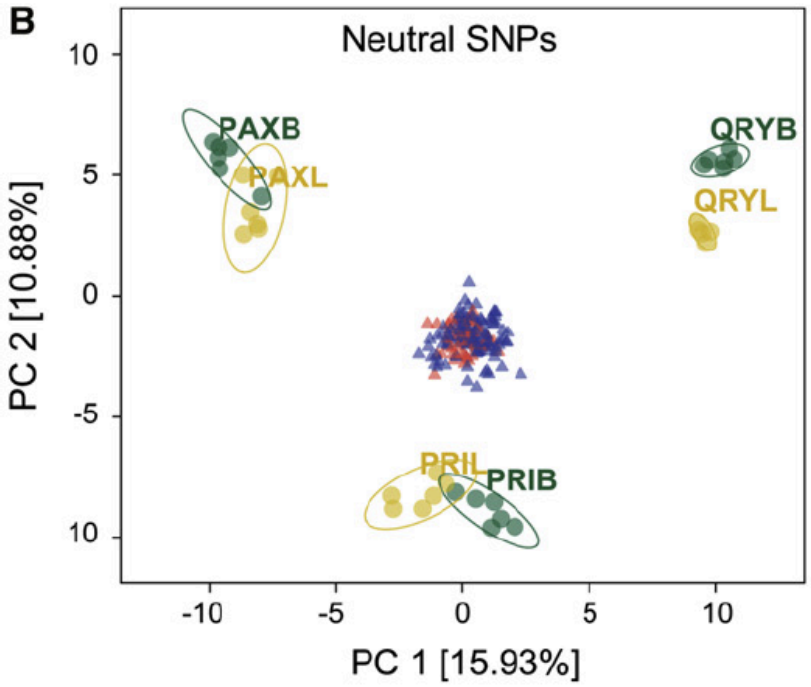


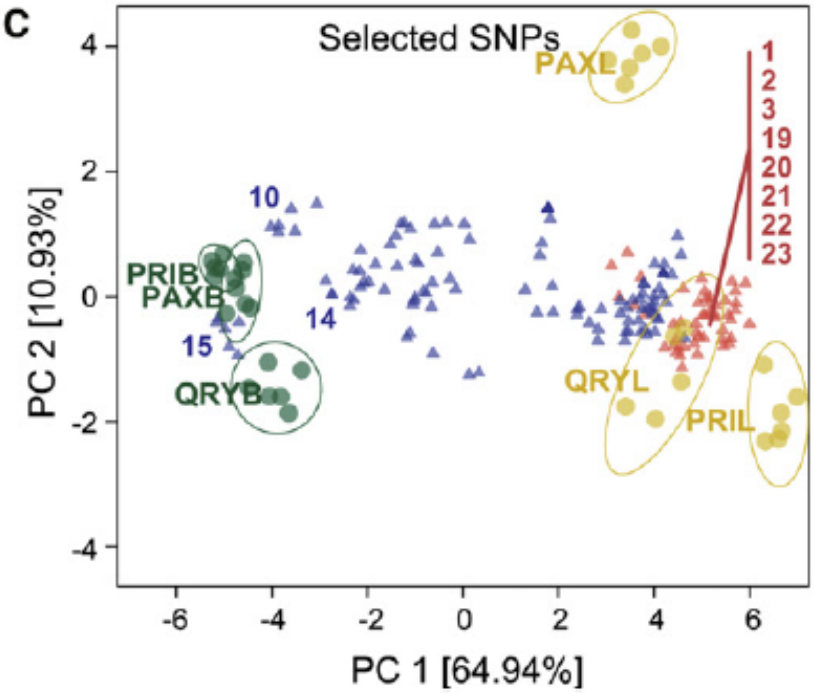
Jones et al (2012) Current Biology

But at selected loci, fish cluster by ecotype

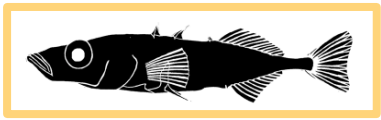
Genome wide SNP genotyping array
(~1000 SNPs)

F_{ST} Outlier SNPs:

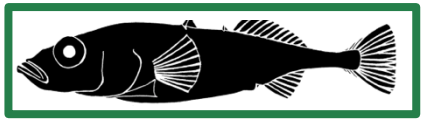




Limnetic



Benthic

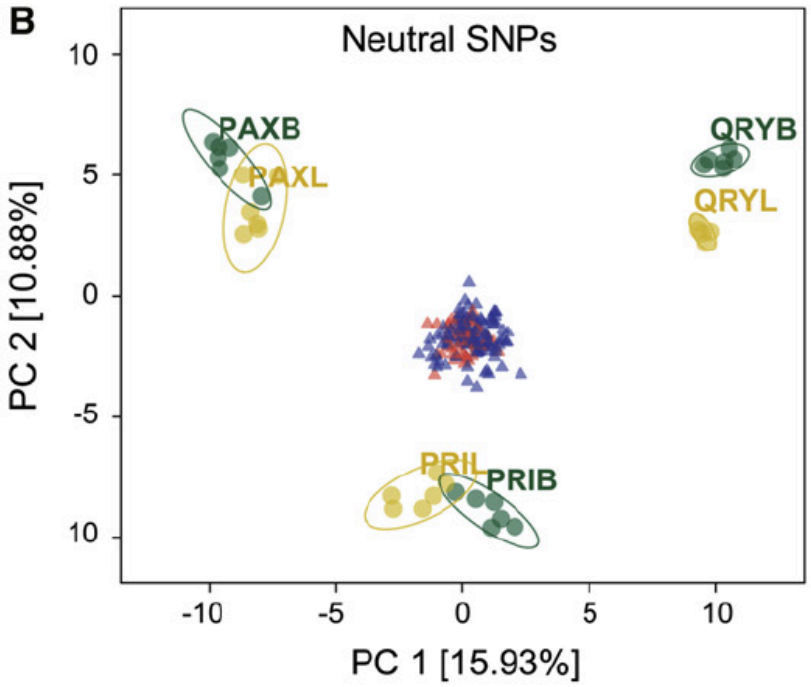


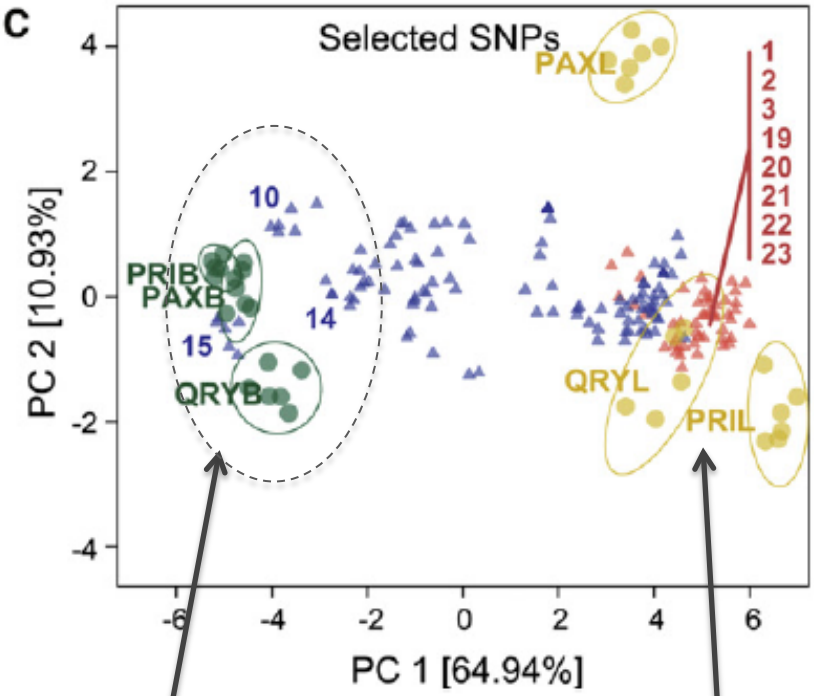
Jones et al (2012) Current Biology

At selected loci, benthic fish carry freshwater alleles, limnetic carry marine alleles

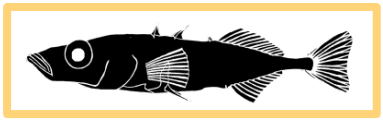
Genome wide SNP genotyping array
(~1000 SNPs)

F_{ST} Outlier SNPs:

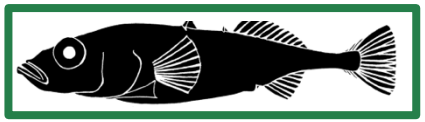




Limnetic



Benthic

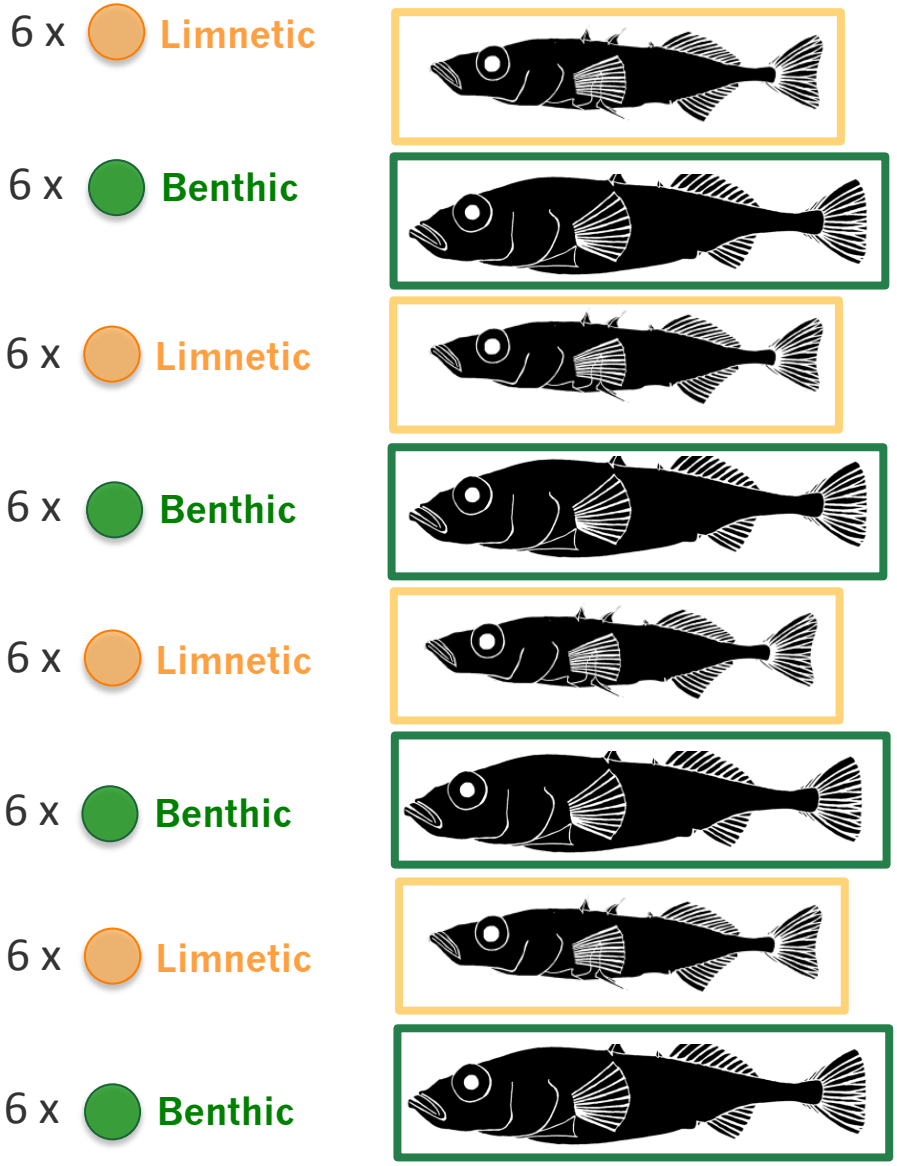


At divergent loci, benthic fish carry alleles similar to those carried by nearby freshwater populations.

Limnetic fish carry marine alleles

Jones et al (2012) Current Biology

Whole genome sequencing of 48 benthic & limnetic ecotypes from 4 lakes



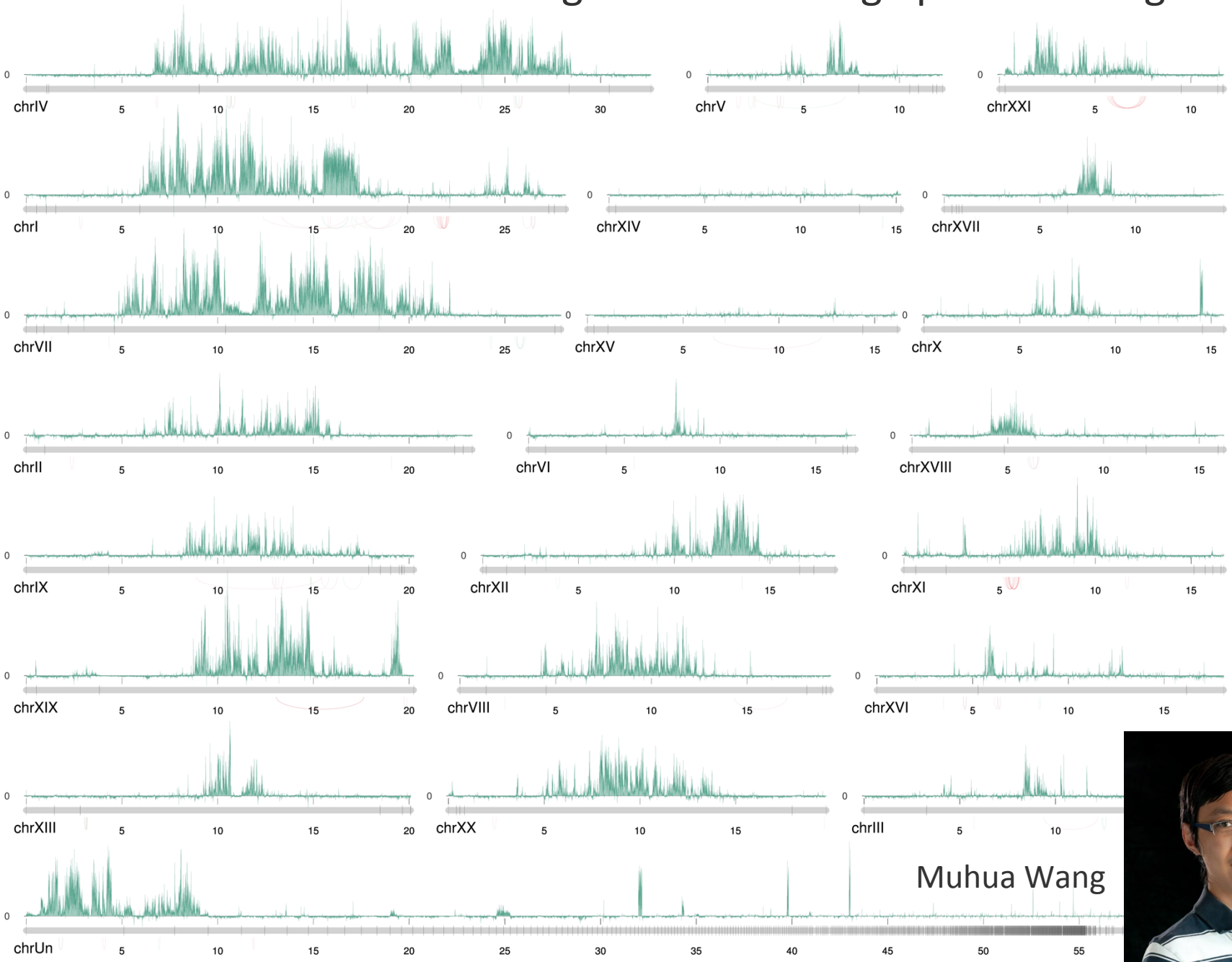
PAXTON LAKE

PRIEST LAKE

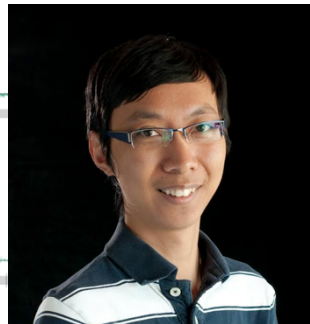
QUARRY LAKE

ENOS LAKE (“collapsed”)
[Historical samples sequenced]

Parallel benthic-limnetic divergence across large parts of the genome



Muhua Wang

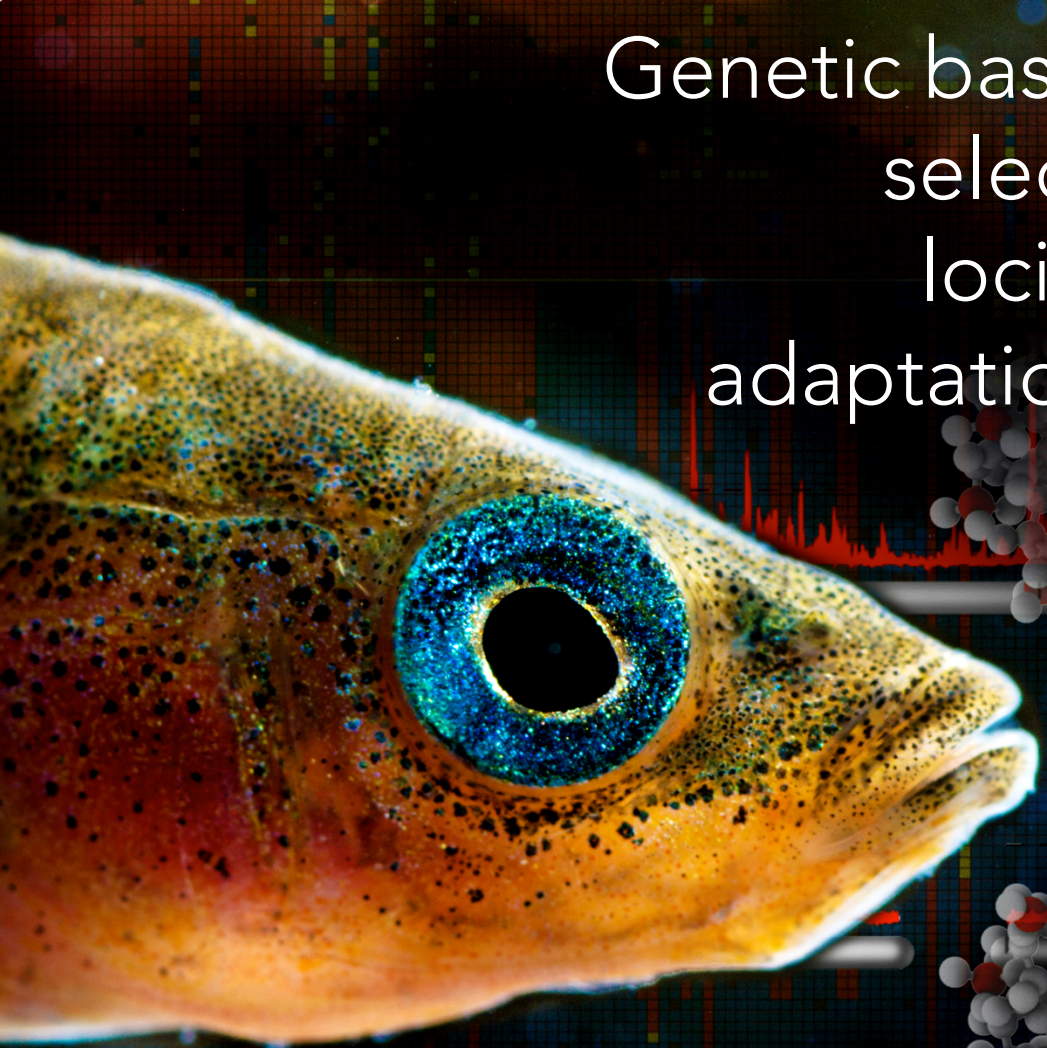


Genetic basis and signatures of selection around loci underlying adaptation and speciation

Felicity Jones

Friedrich Miescher Laboratory of Max Planck Society

Tübingen, Germany



Male threespine stickleback

DFG Deutsche Forschungsgemeinschaft

erc European Research Council Consolidator Grant

MAX-PLANCK-GESELLSCHAFT

fml

TODAY's EXERCISE: Molecular signatures of selection around Pitx1 in Benthic fish?

Lessons from case studies of known adaptive loci: EDA and PITX1

Association mapping in natural populations is extremely helpful at narrowing the relevant interval (EDA ~20kb; PITX1 ~23kb)

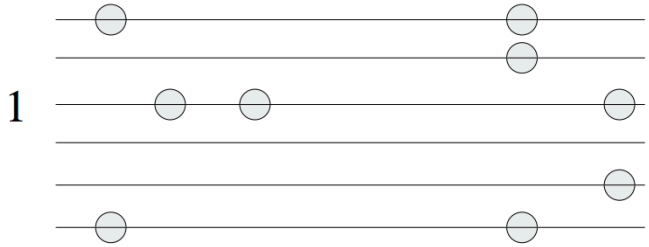
The signature of phenotype-genotype association is so small that reduced representation sequencing methods (eg GBS, RAD) may miss it entirely.

Whole genome sequencing (including low pass "skim" sequencing) can give excellent high resolution signal

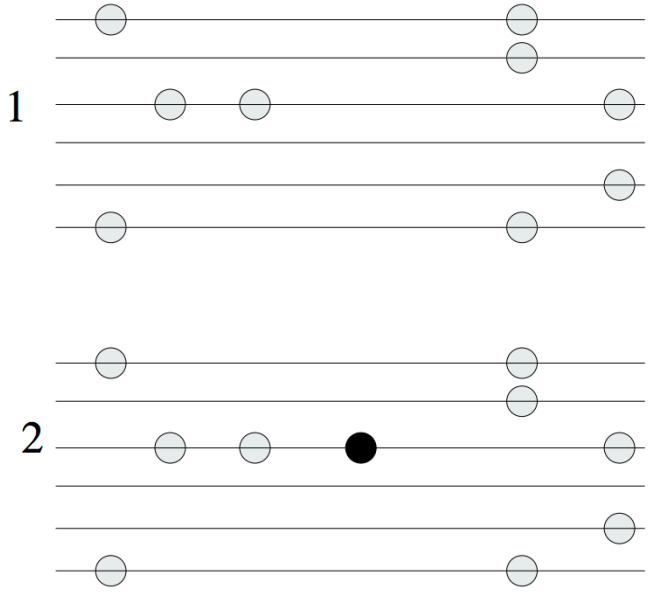
In the case of EDA, whole genome sequencing of individuals from different populations (as opposed to many individuals from a single population) helped narrow the boundaries of the region.

Signatures of selection can be narrow narrow: 20-50kb wide are not unusual.

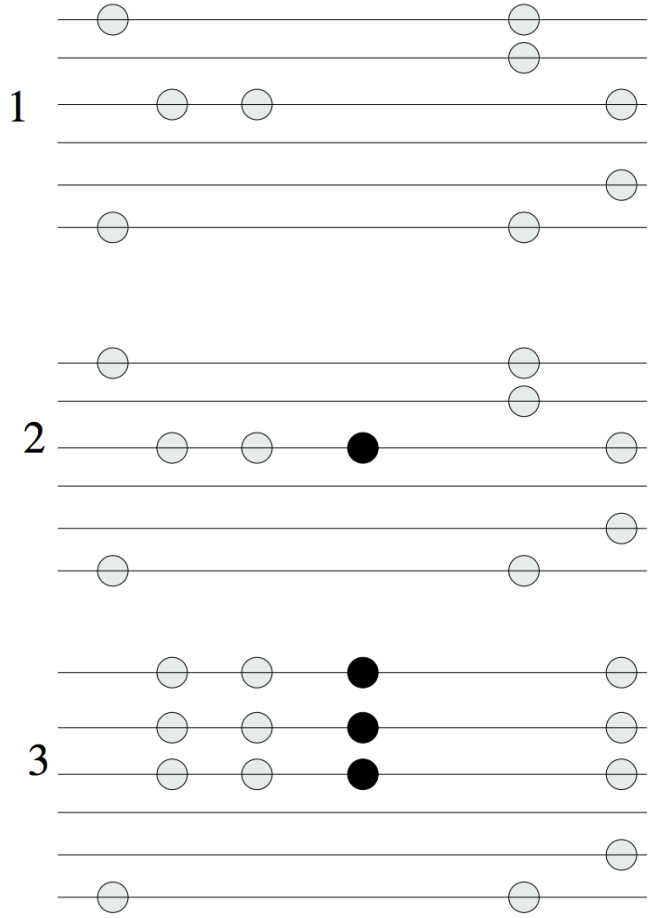
Molecular signatures of a selective sweep



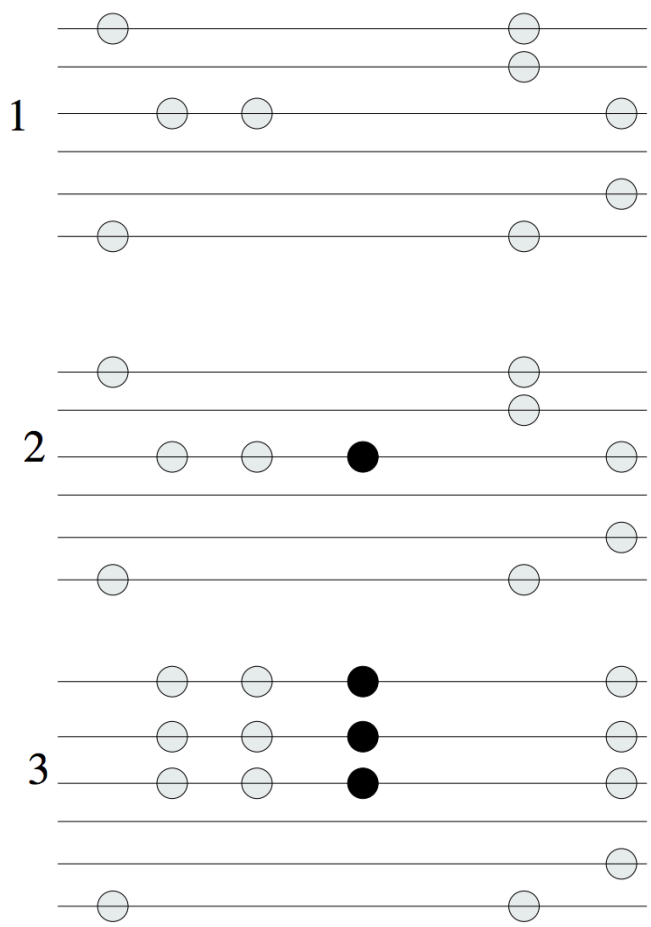
Molecular signatures of a selective sweep

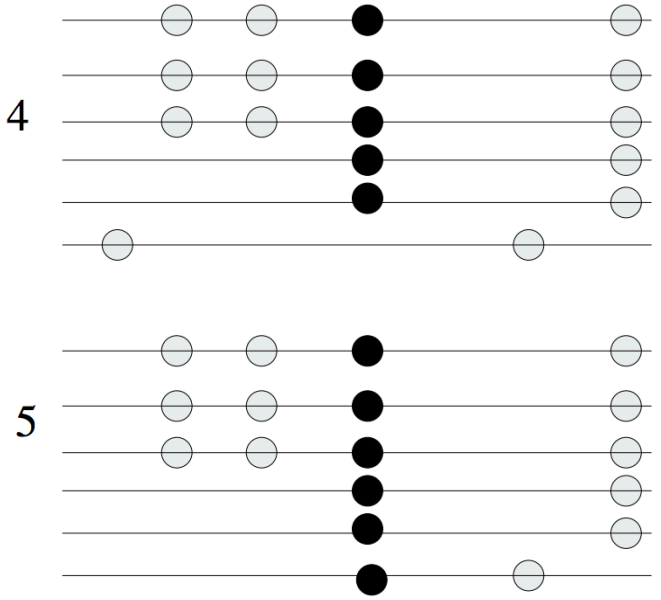


Molecular signatures of a selective sweep

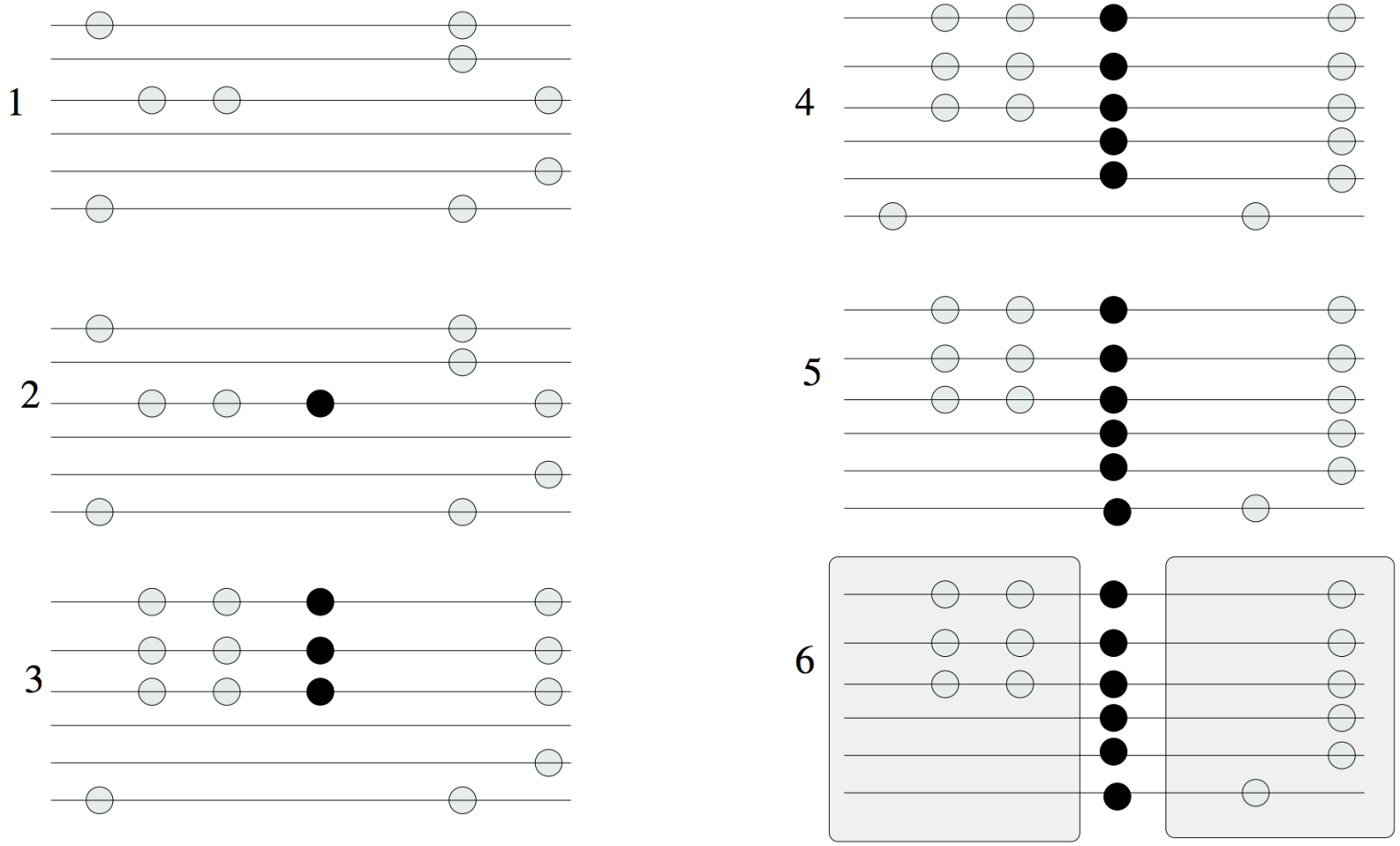


Molecular signatures of a selective sweep

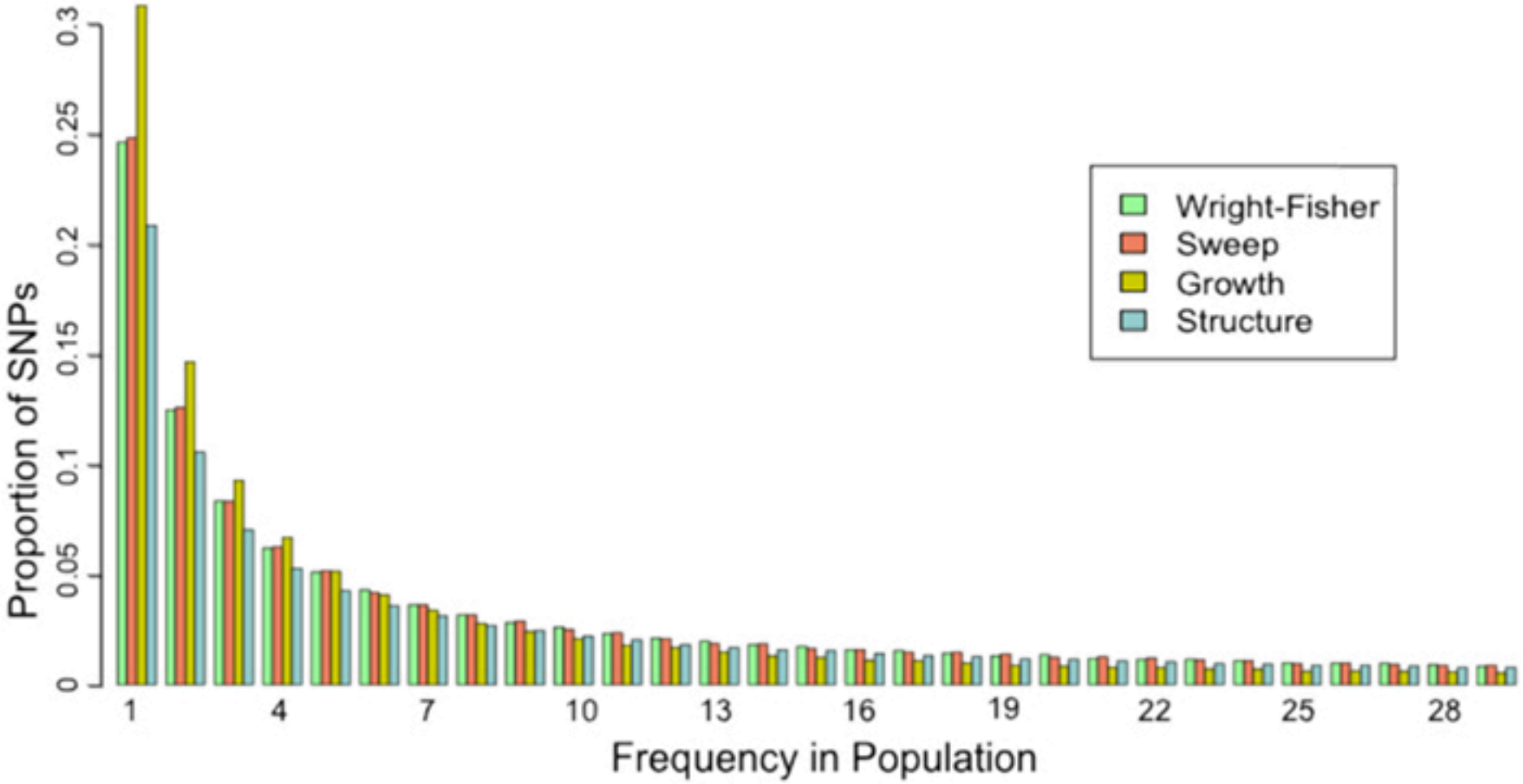




Molecular signatures of a selective sweep



Summarising Polymorphism Data with a Site Frequency Spectrum



Summarising Polymorphism Data with a Site Frequency Spectrum