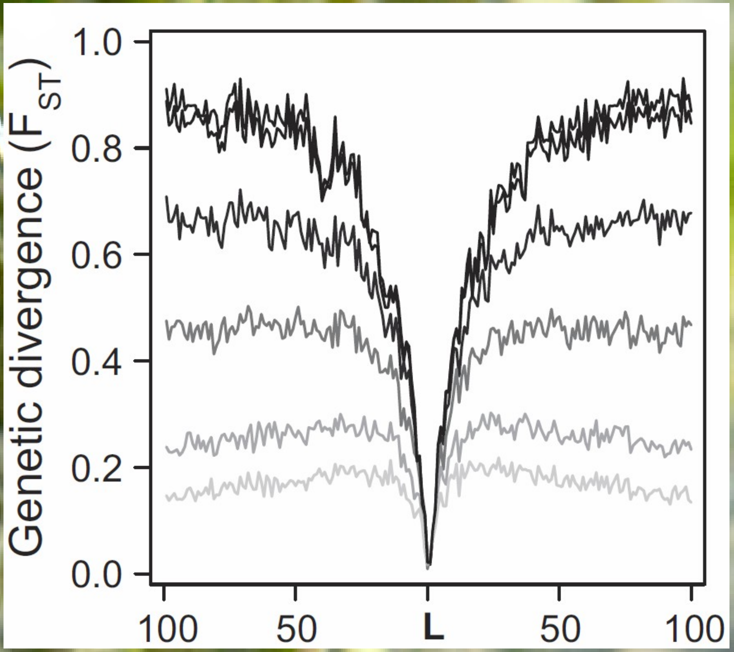
Evolutionary population genomics



- Background
- The number of loci
- Parallel evolution
- Recombination

Photo: Marius Roesti

Daniel Berner, University of Basel
daniel.berner@unibas.ch
http://www.salzburgerlab.org/team/daniel_berner

Cesky Krumlov
2 Feb 2016

Collaborators

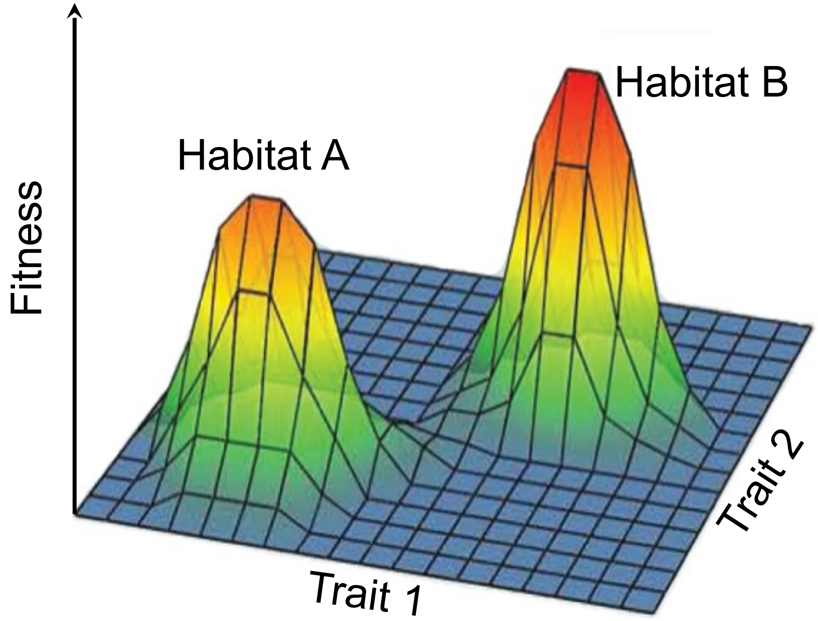
Marius Roesti

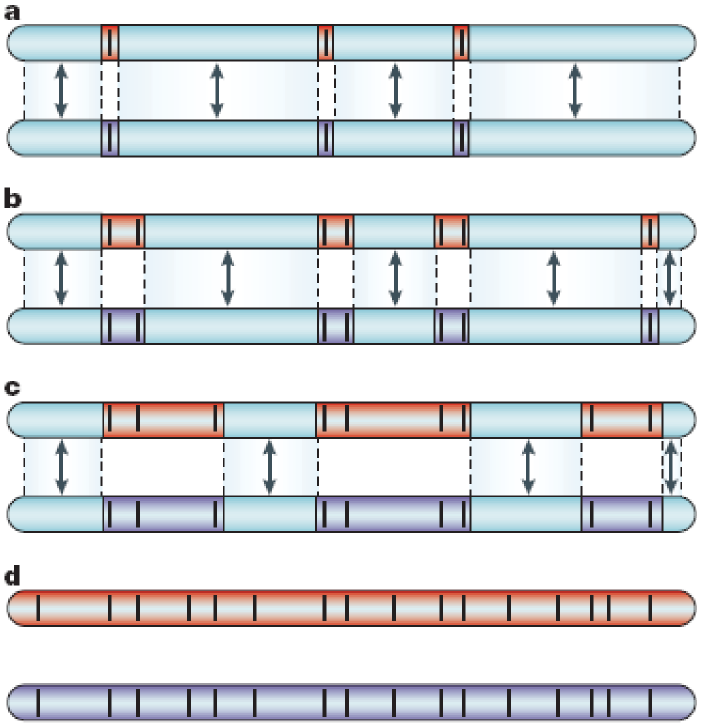
Dario Moser

Andrew Hendry
McGill, Montreal

Walter Salzburger
Uni Basel

Focus and questions

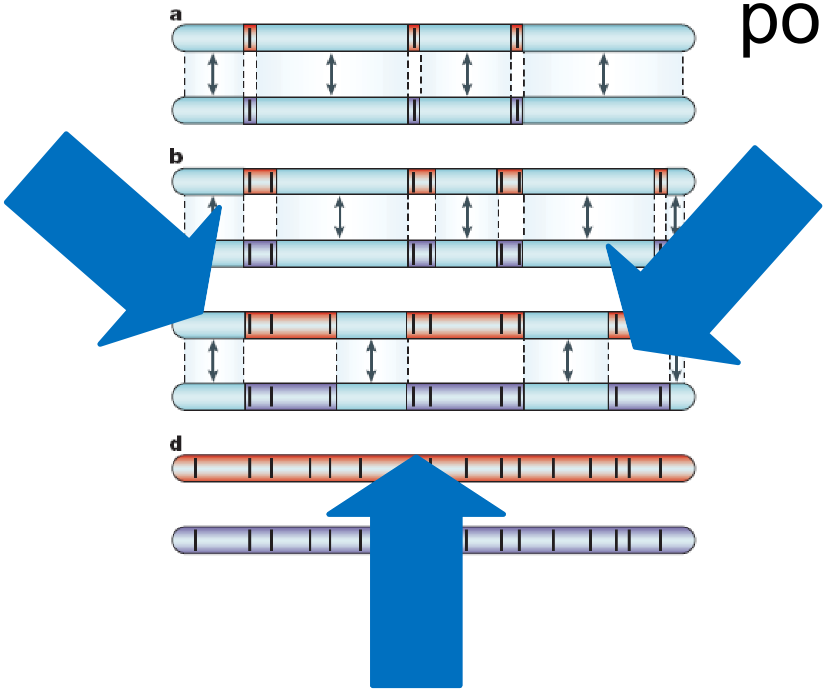




Wu & Ting 2004 Nat Rev Genet

- How many genes underlie adaptive divergence?
- How are they arranged in the genome?
- Where do the alleles come from?
- What is their function?
- What is their effect size?
- To what extent are alleles reused in multiple populations?

Background



Phenotypic information from natural populations and laboratory crosses

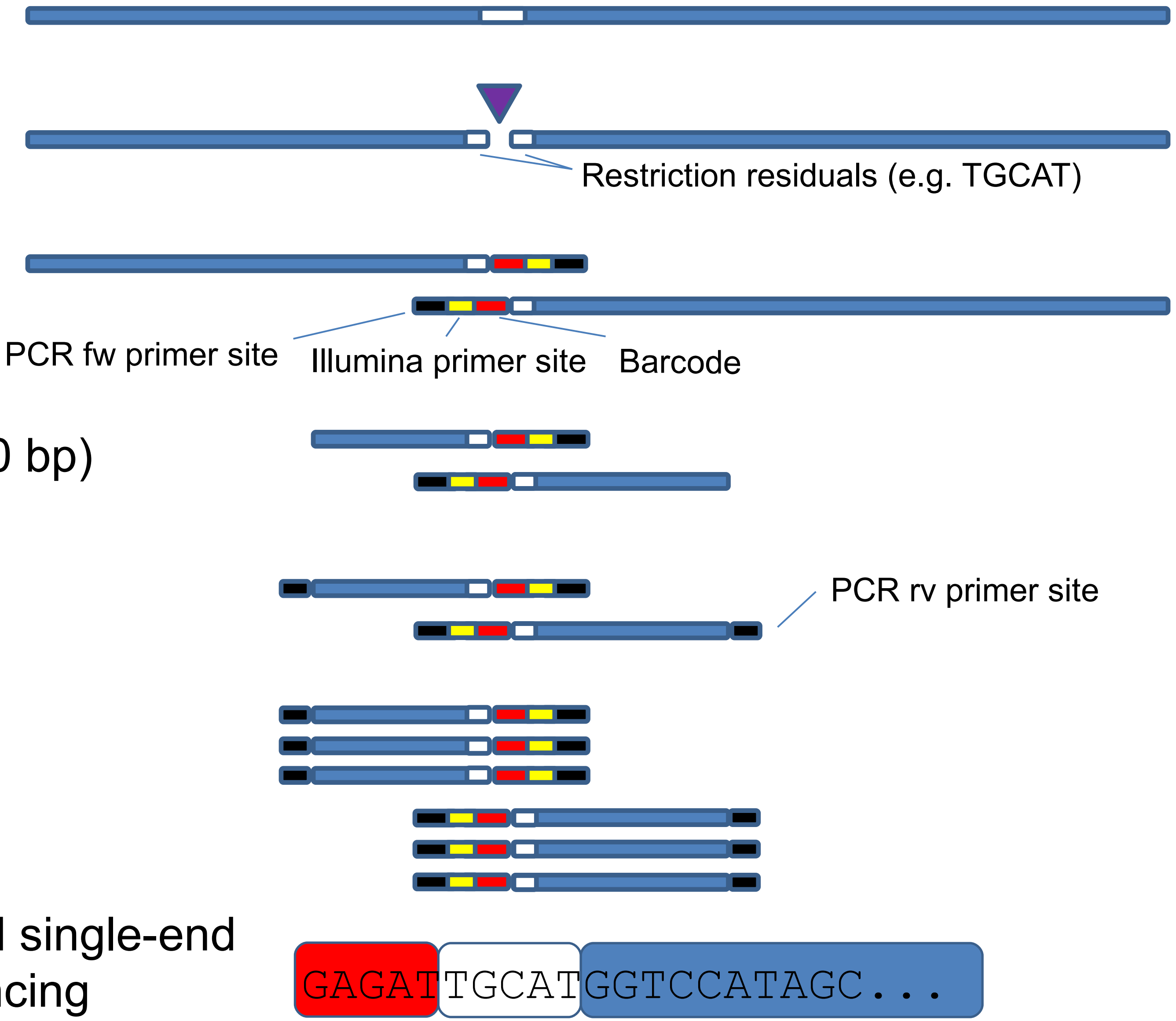
Genome-wide polymorphism data

Complexity reduction using *in silico* experiments

Background

RADseq as a tool for *de novo* SNP discovery

RAD = **R**estiction site-**a**ssociated **D**NA



Genomic DNA

Enzymatic restriction

Adaptor 1 ligation

Shearing (500 -700 bp)

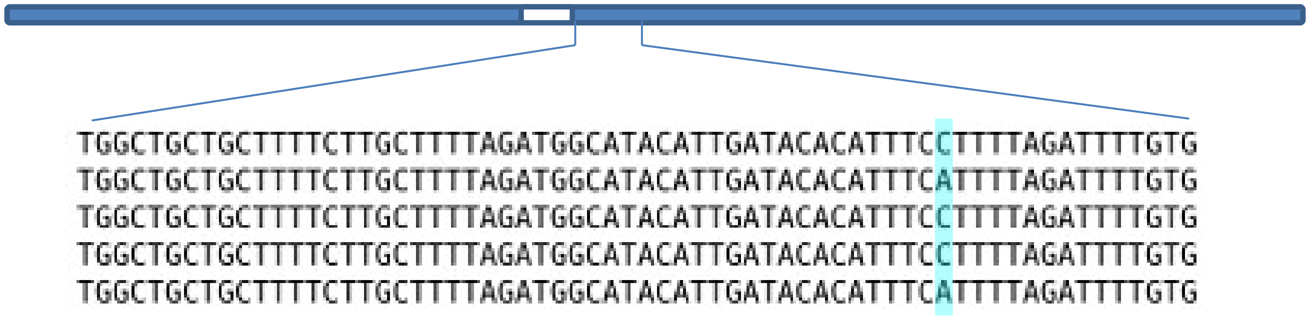
Adaptor 2 ligation

PCR Amplification

→ Multiplexing and single-end Illumina sequencing

From reads to SNPs

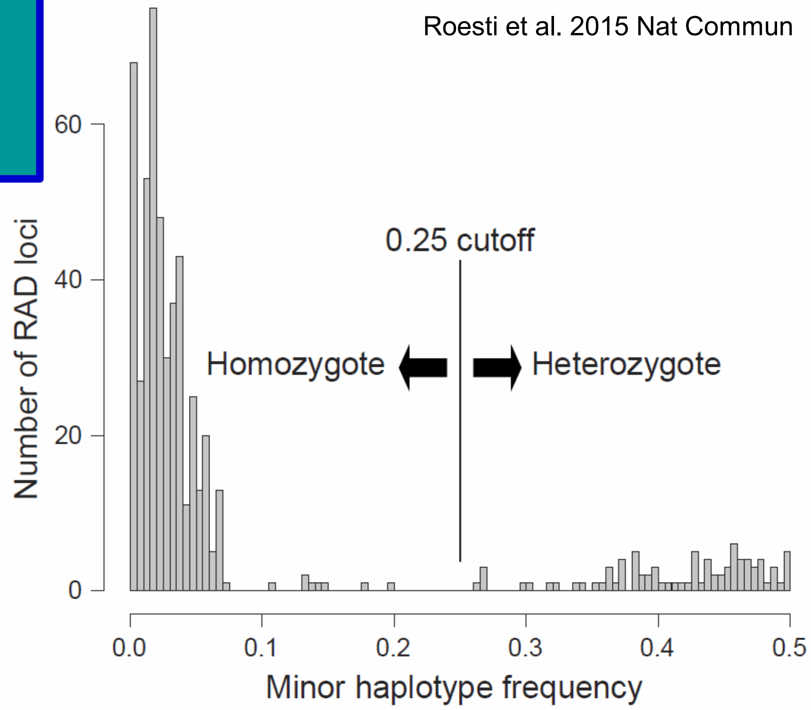
Demultiplexing, alignment to reference (typical coverage: >= 40x)



```
TGGCTGCTGCTTTTCTTGCTTTTAGATGGCATACATTGATACACATTTCCTTTTAGATTTTGTG
TGGCTGCTGCTTTTCTTGCTTTTAGATGGCATACATTGATACACATTTCATTTTAGATTTTGTG
TGGCTGCTGCTTTTCTTGCTTTTAGATGGCATACATTGATACACATTTCCTTTTAGATTTTGTG
TGGCTGCTGCTTTTCTTGCTTTTAGATGGCATACATTGATACACATTTCCTTTTAGATTTTGTG
TGGCTGCTGCTTTTCTTGCTTTTAGATGGCATACATTGATACACATTTCATTTTAGATTTTGTG
```

Consensus genotyping for each individual

Keep
It
Simple and
Stupid



Roesti et al. 2015 Nat Commun

Robust RAD genotyping based on the distribution of whole haplotypes

Background

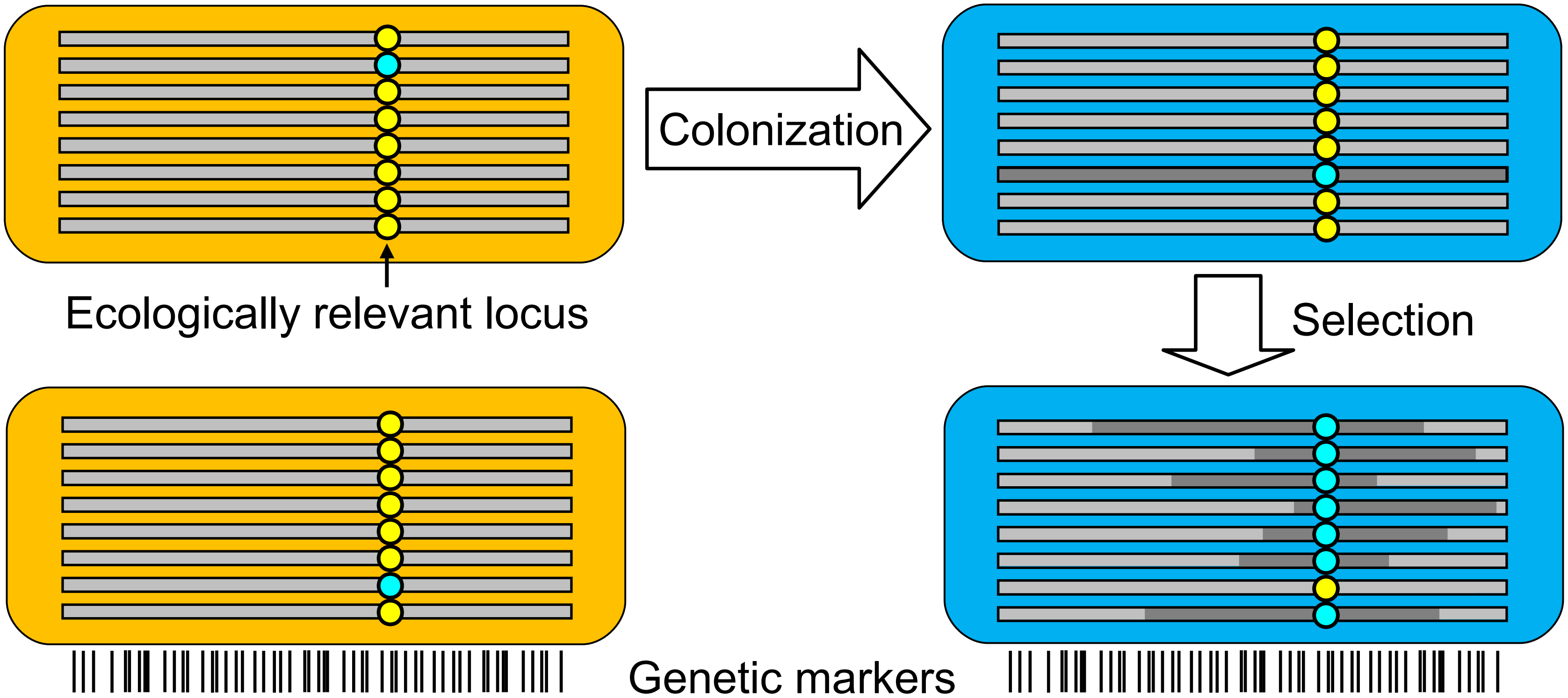
```
chrI 11229884 18 0 AGCTCGCTTCTCCATTCCAAGAGAAAAAGTTTCAAGCCCACCTGTGATCCCTCTAATTAAAGAA
chrI 11229884 18 0 AGCTCGCTTCTCCATTCCAAGAGAAAAAGTTTCAAGCCCACCTGTGATCCCTCTAATTAAAGAA
chrI 11237765 21 0 ATCAGCATCAGAACACATCTTCATCCCTTGGCTTACAGGCCGACTGAACTCTGTGGCTCTGCGG
chrI 11237765 21 0 ATCAGCATCAGAACACATCTTCATCCCTTGGCTTACAGGCCGACTGAACTCTGTGGCTCTGCGG
chrI 11245660 43 0 GCTGCTGCAGTGCAGGGAAGGAACAAGCGGCTGTAACATTATTTTCTTTCAACGACATATAGCA
chrI 11245660 43 0 GCTGCTGCAGTGCAGGGAAGGAACAAGCGGCTGTAACATTATTTTCTTTCAACGACATATAGCA
chrI 11245663 59 0 CTGACCAAAGCTCTGTGGTTTTCCCAGGGCAAGCTGTCTTGCTCGTCCGTCTGCGACTCATCTG
chrI 11245663 59 0 CTGACCAAAGCTCTGTGGTTTTCCCAGGGCAAGCTGTCTTGCTCGTCCGTCTGCGACTCATCTG
chrI 11344930 39 1 TGGCTGCTGCTTTTCTTGCTTTTAGATGGCATACATTGATACACATTTCCTTTTAGATTTTGTG
chrI 11344930 39 1 TGGCTGCTGCTTTTCTTGCTTTTAGATGGCATACATTGATACACATTTCATTTTAGATTTTGTG
```

SNP calling

```
GGTCAAAAAACAAGCGGTAGGCCCCCTCATACACAACAACACATTAGCAGGGTCGTCATGGAGC
GGTCAAAAAACAAGCGGTAGGCCCCCTCATACACAACAACACATTAGCAGGGTCGTCATGGAGC
GGTCAAAAAACAAGCGGTAGGCCCCCTCATACACAACAACACATTAGGAGGGTCGTCATGGAGC
GGTCAAAAAACAAGCGGTAGGCCCCCTCATACACAACAACACATTAGGAGGGTCGTCATGGAGC
GGTCAAAAAACAAGCGGTAGGCCCCCTCATACACAACAACACATTAGGAGGGTCGTCATGGAGC
GGTCAAAAAACAAGCGGTAGGCCCCCTCATACACAACAACACATTAGGAGGGTCGTCATGGAGC
...
GGTCAAAAAACAAGCGGTAGGCCCCCTCATACACAACAACACATTAGGAGGGTCGTCATGGAGC
GGTCAAAAAACAAGCGGTAGGCCCCCTCATACACAACAACACATTAGCAGGGTCGTCATGGAGC
GGTCAAAAAACAAGCGGTAGGCCCCCTCATACACAACAACACATTAGCAGGGTCGTCATGGAGC
GGTCAAAAAACAAGCGGTAGGCCCCCTCATACACAACAACACATTAGCAGGGTCGTCATGGAGC
GGTCAAAAAACAAGCGGTAGGCCCCCTCATACACAACAACACATTAGCAGGGTCGTCATGGAGC
```

```
                 ID    POP AL chrI_11814_11931 chrI_49709_49790 chrI_89249_89330
1 Ga_lib_33_AACCC random  X                G                C                C
2 Ga_lib_33_AACCC random  Y                A                C                T
3 Ga_lib_33_AAGGG random  X                G                C                C
4 Ga_lib_33_AAGGG random  Y                G                C                C
5 Ga_lib_33_AATTT random  X                G                T                T
```

Basic logic of SNP-based genome scans for selection



Needed:

- genome-wide high-density markers
- Reference genome

How many genes underlie adaptive divergence?

Threespine stickleback

- Ancestrally marine
- Fossil record back to 13 My

Truckee formation, Nevada (USA), 10my

- Colonization of freshwater, mainly postglacial
- Repeated and predictable divergence from marine ancestor

Lake-stream divergence in stickleback

- Parallel evolution - lake-stream divergence is replicated many times



Berner et al. 2010
Mol Ecol

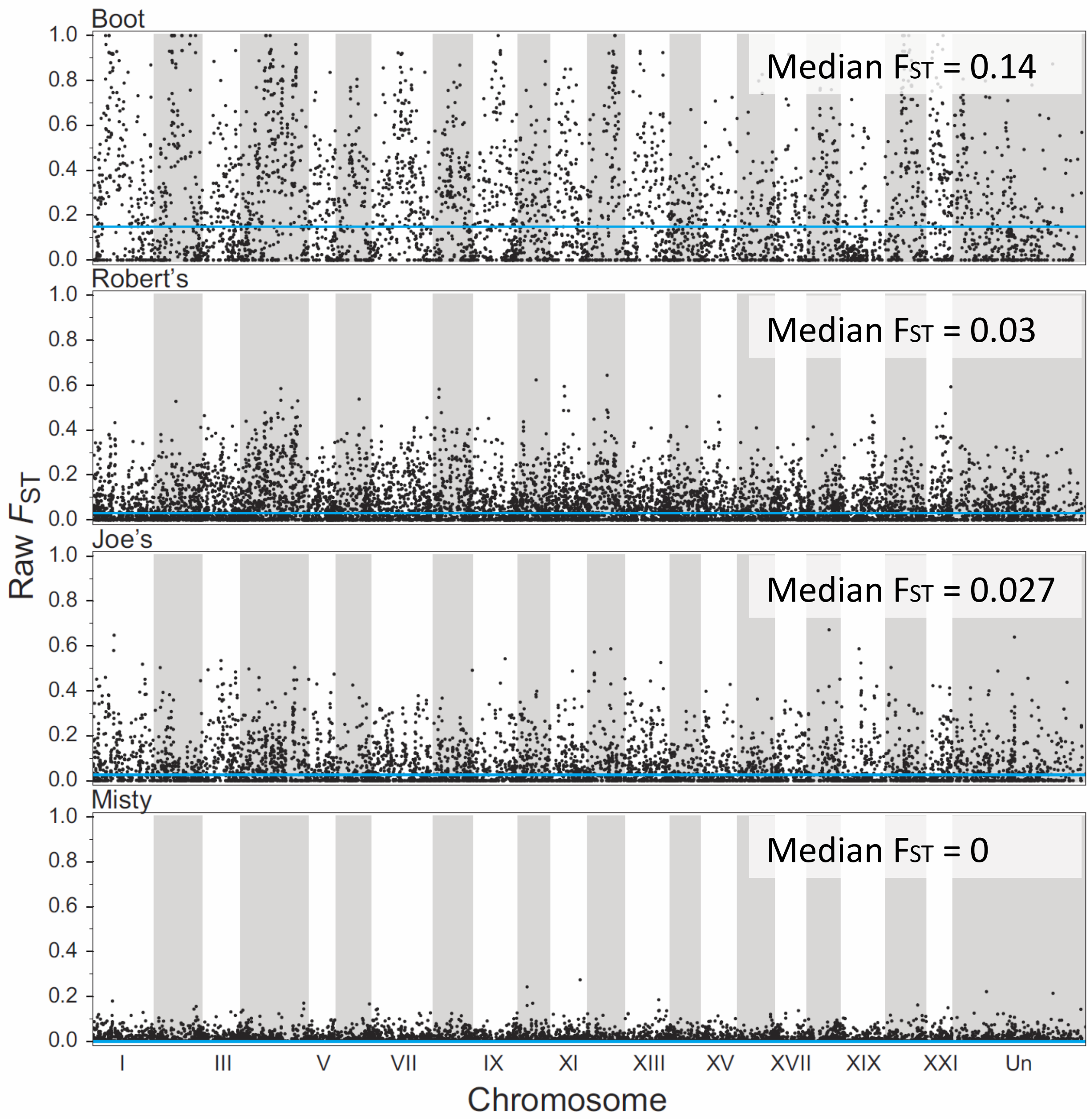
- Predictable niche differentiation (pelagic vs. benthic prey)


Lake


Stream

Early divergence mapping in lake-stream stickleback Roesti et al. 2012 Mol Ecol

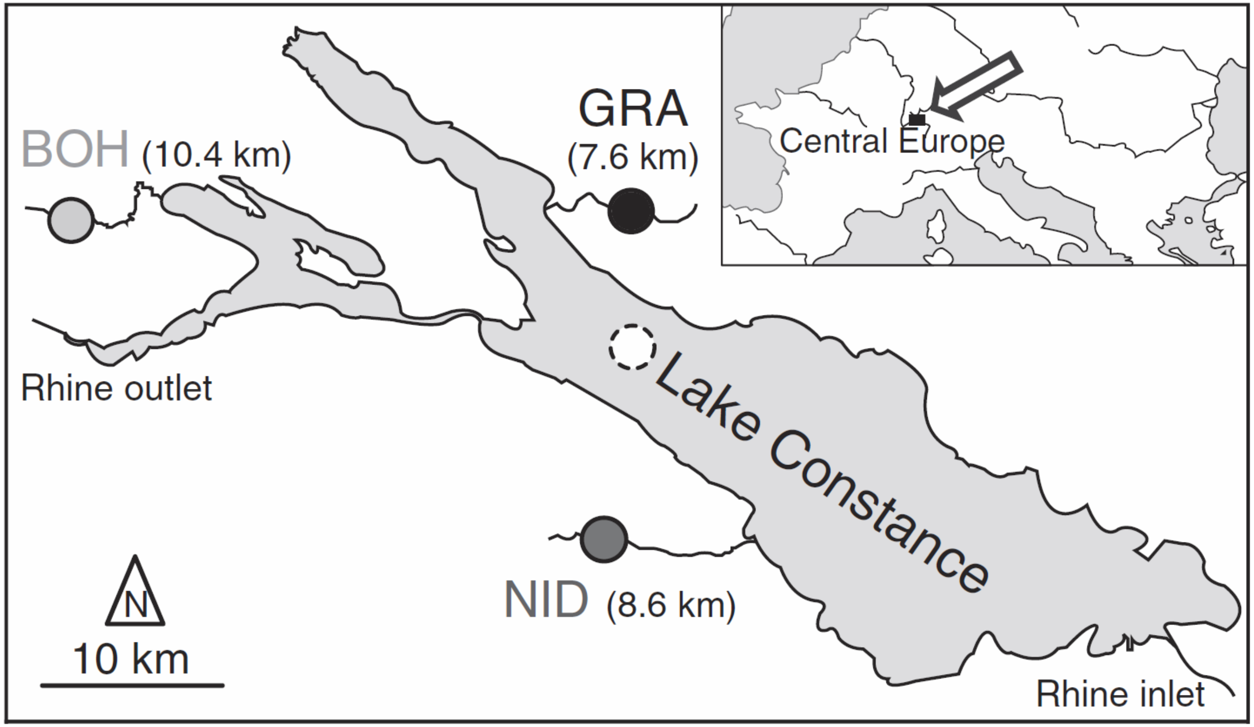
- 4 lake-stream population pairs from Canada, 27 individuals from each habitat
- SNPs generated by RADseq (Sbf1 cutter, c. 20k restriction sites across the genome)
- Calculation of F_{ST} at all SNPs



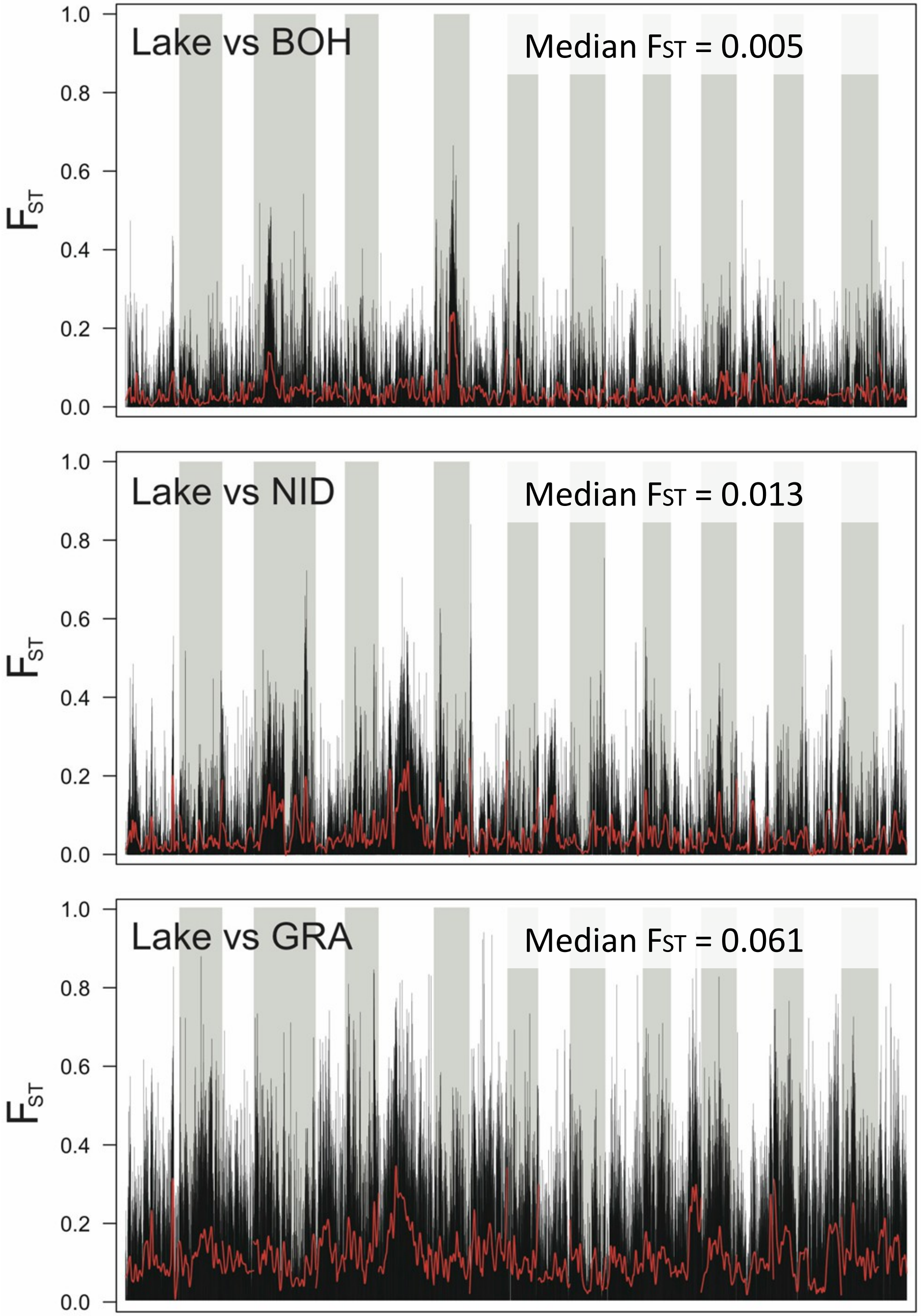
- Variable magnitude of baseline genomic differentiation
- Divergence is heterogeneous across the genome
- Very many loci seem to be under divergent selection

Higher-resolution divergence mapping

Roesti et al. 2015 Nat Commun



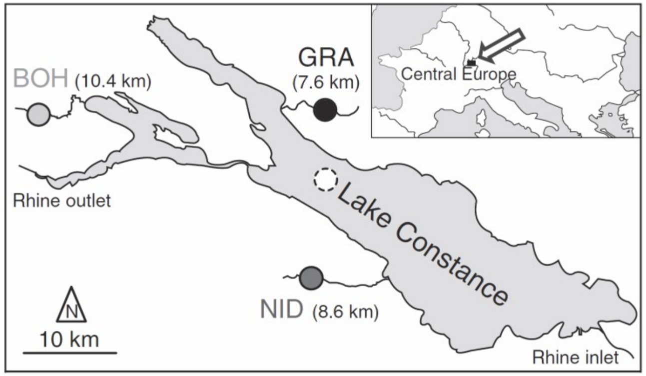
- Lake Constance and 3 stream populations, 22-25 individuals from each habitat
- SNPs generated by RADseq (Nsi1 cutter, c. 140k restriction sites across the genome)
- Calculation of F_{ST} at all SNPs

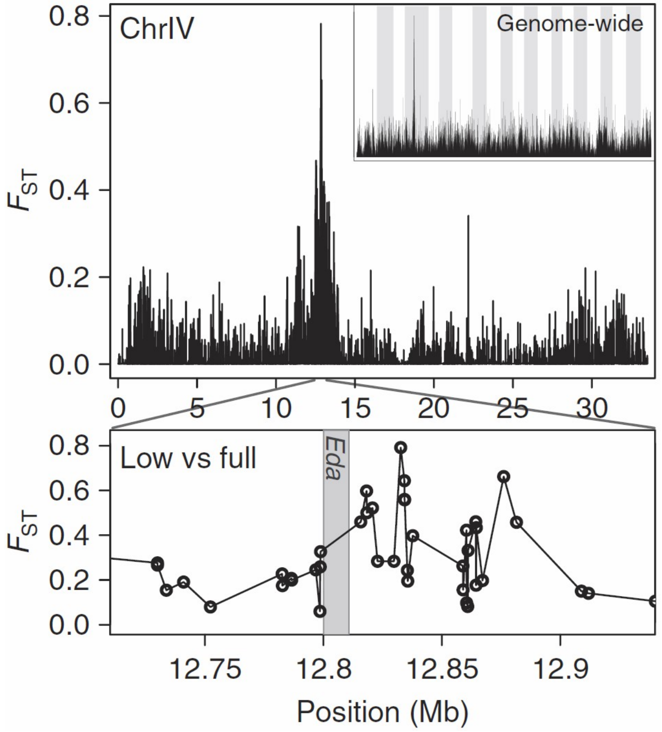


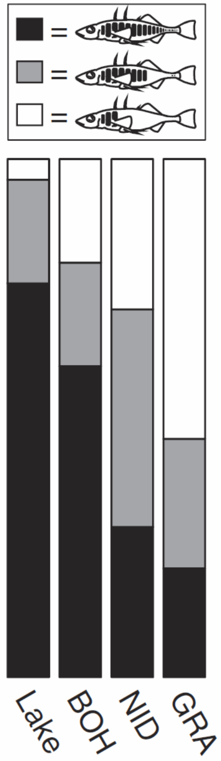
Similar findings:
variable magnitude of baseline genomic differentiation; highly polygenic, heterogeneous divergence

Support for highly polygenic selection offered by the *Eda* locus

Roesti et al. 2015 Nat Commun



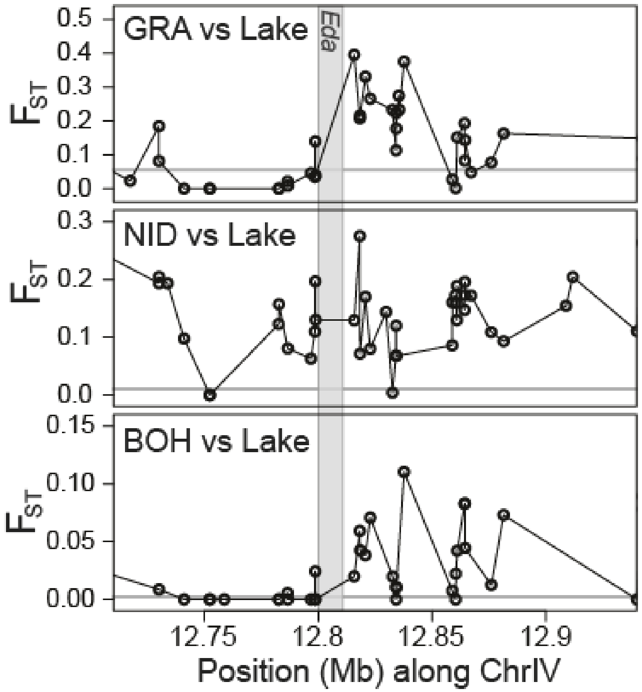





Dario Moser

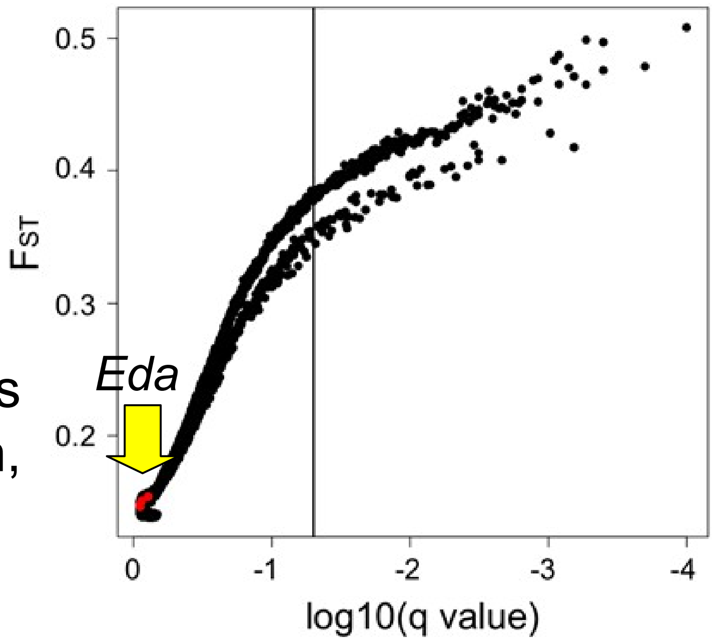
Adaptive polymorphism in lateral plating

Association mapping (low- vs. fully plated) confirms that *Ectodysplasin* (*Eda*) variants influence lateral plating

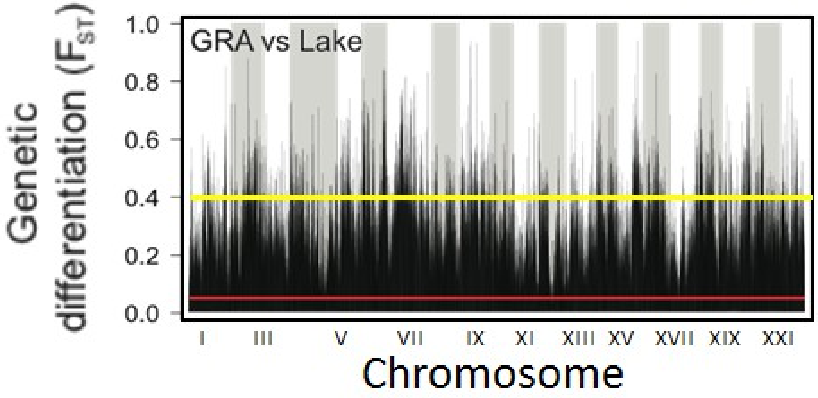


F_{ST} profiles around *Eda*, based on the populations

‘Outlier’ analysis using BayeScan, GRA vs. lake



F_{ST} genome-wide, GRA vs Lake



Eda

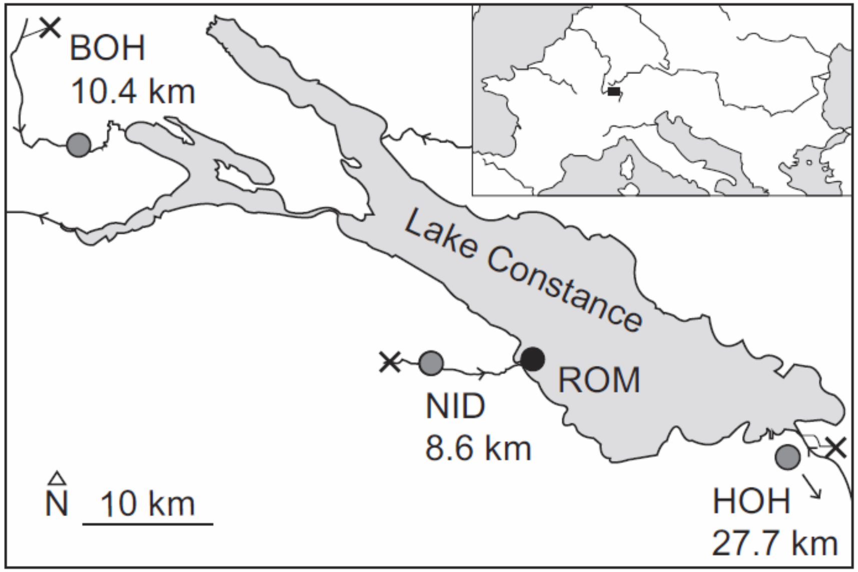
Genome-wide baseline

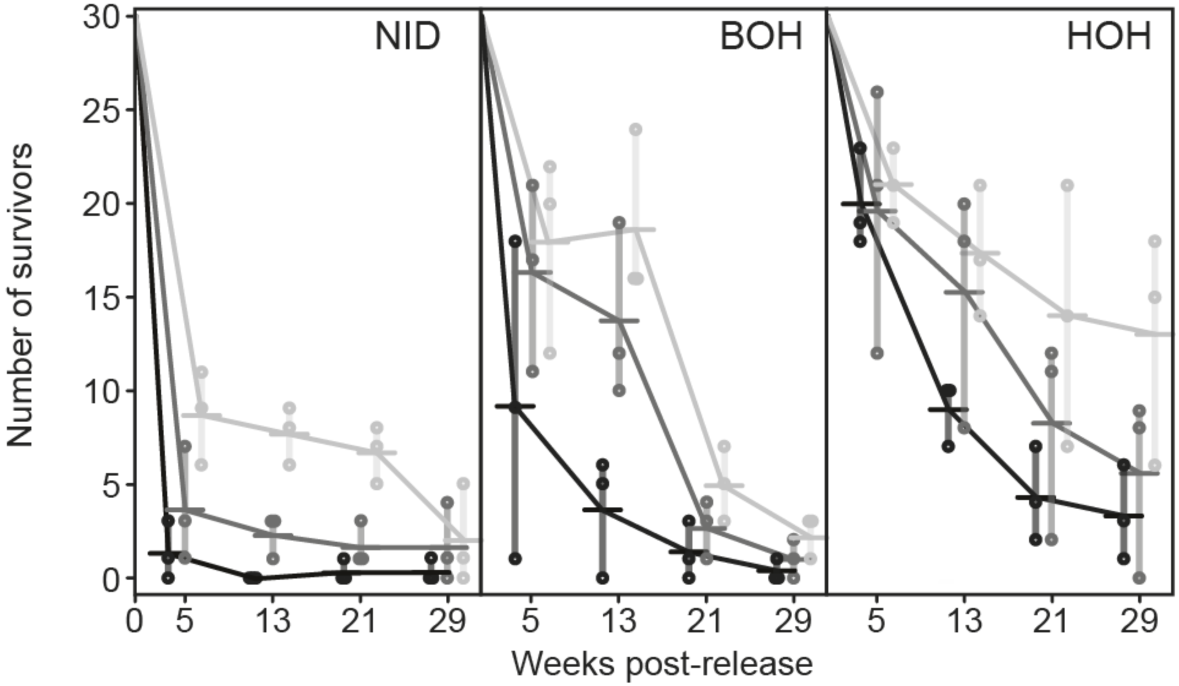
- Hundreds of loci are involved in lake-stream divergence
- Detection issue...

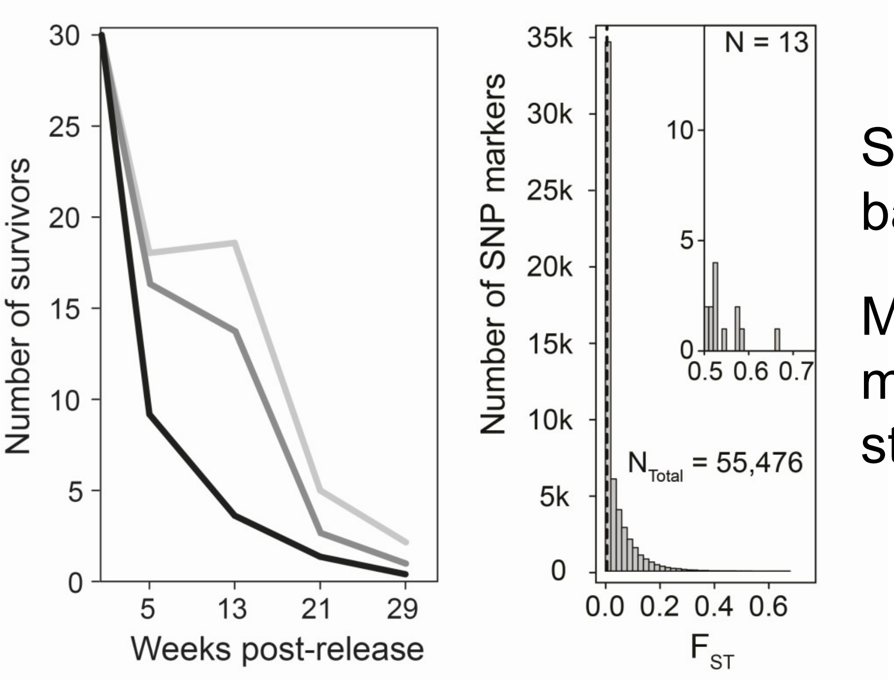
What is the fitness correlate of genomic divergence?

Moser et al. 2016 J Evol Biol

- Field enclosure experiment (3x3)
- Stocking with 90 lab-reared, marked individuals (lake, stream, F1 hybrid)
- Tracking of survival by recapture over 29 weeks





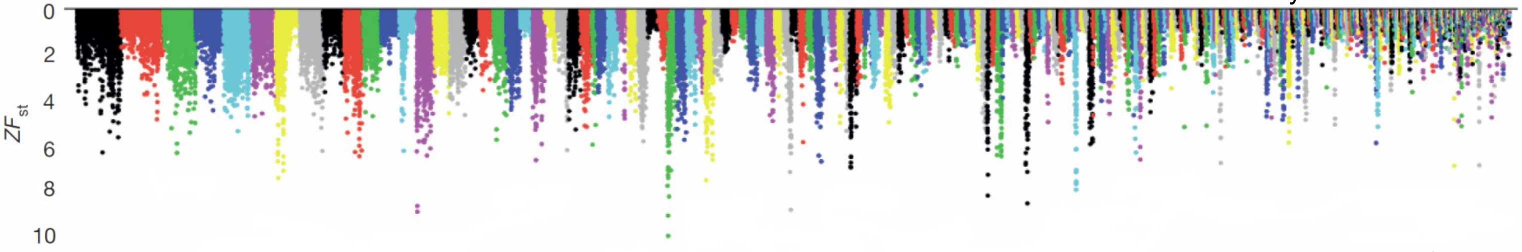


Strong fitness differences despite weak baseline genomic differentiation

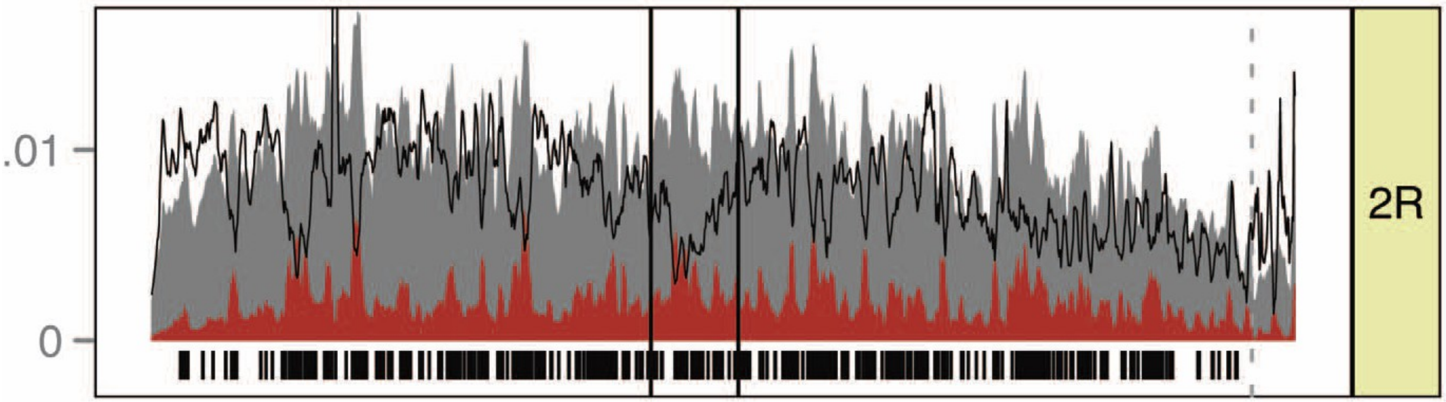
Modest allele frequency differences at many ecologically important loci allow strong adaptive divergence

Evidence on the number of loci from other systems

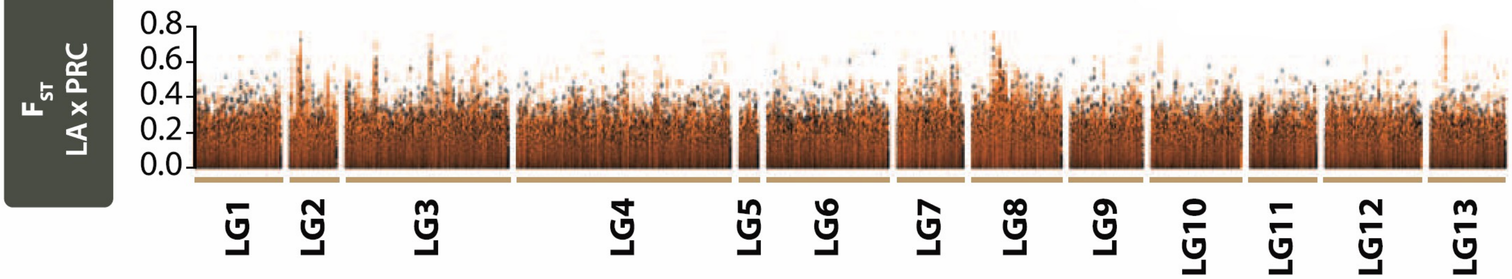
Lamichhaney et al. 2015 Nature



Lawniczak et al. 2010 Science



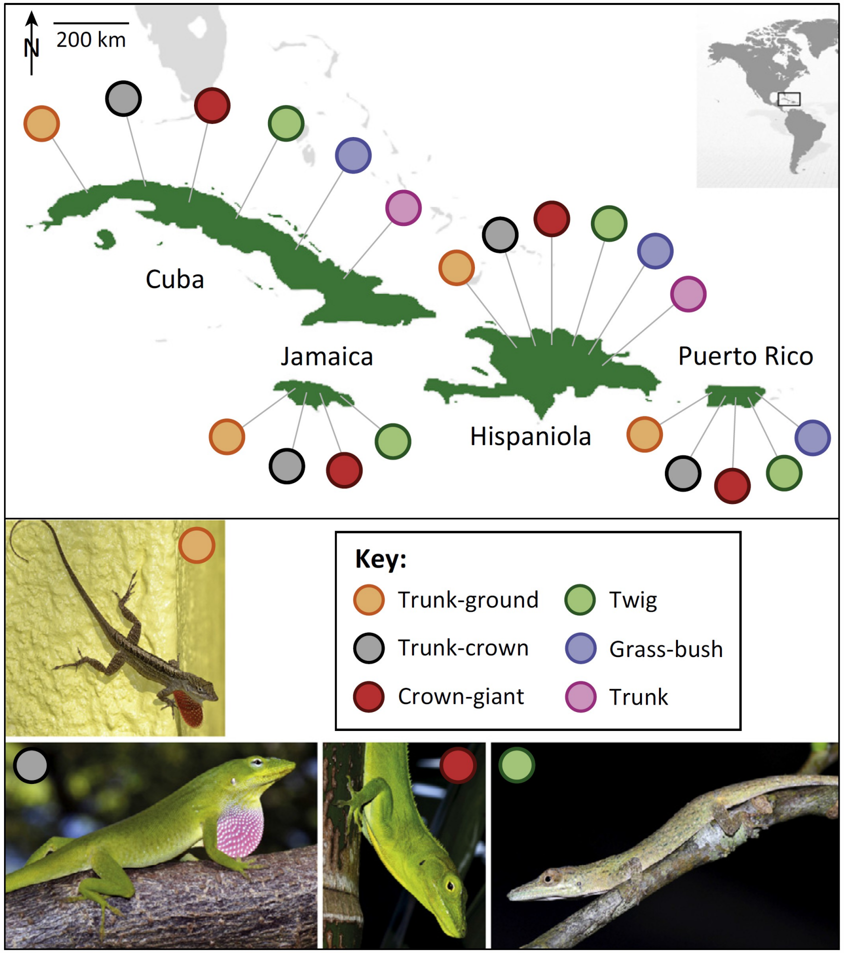
Soria-Carrasco et al. 2014 Science



Conclusions

- Evolutionary population genomics benefits from integrative research approaches
- It does not always have to be full-genome sequencing
- A robust genome assembly is the key to informative genomic investigations
- Divergence into different ecological niches generally involves a great number of allele frequency shifts
- We should abandon the idea that we can count or characterize these shifts comprehensively

Parallel evolution



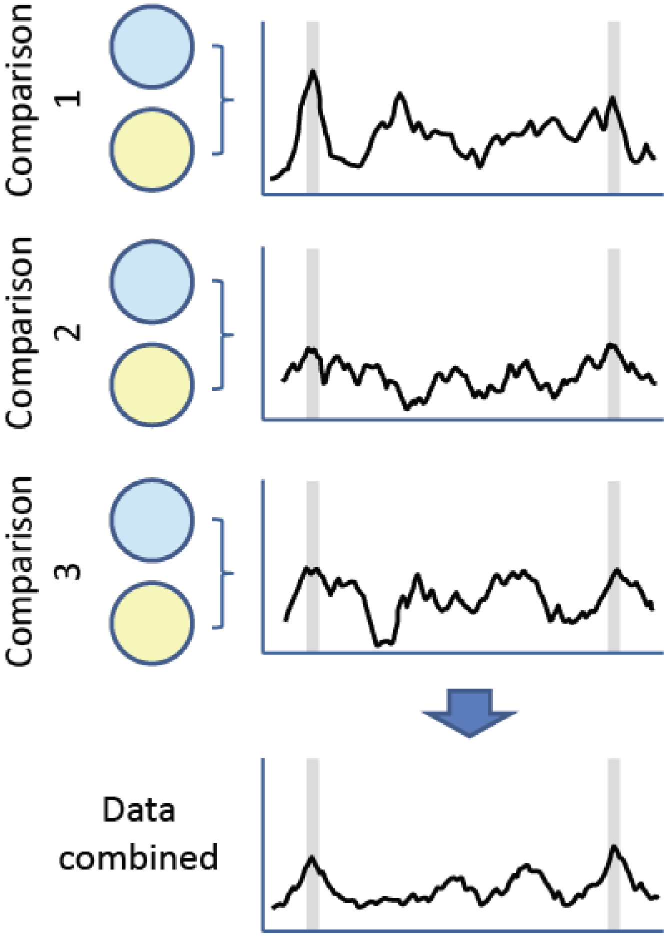
Berner & Salzburger 2015 Trends Genet

- Recurrent evolution of habitat-associated phenotypes
- Deterministic outcome of natural selection
- Focus initially phenotypic, now often genetic

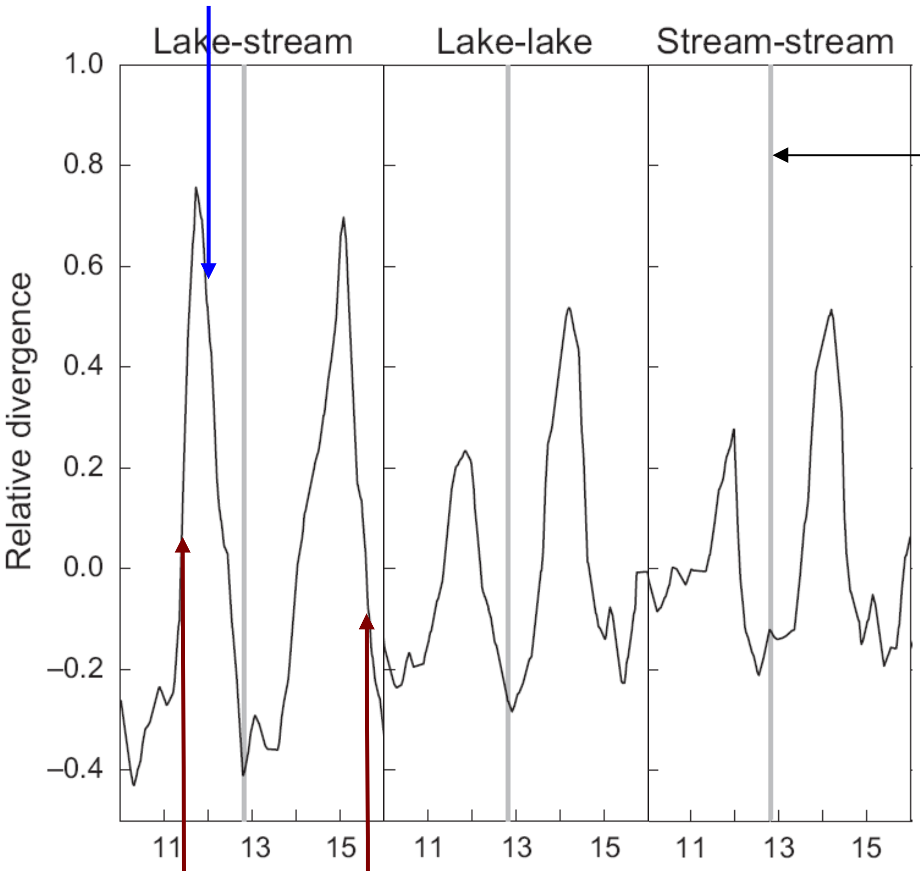
1. Searching for adaptation loci using parallel evolution in Canadian lake-stream stickleback pairs

Roesti et al. 2012, 2014 Mol Ecol

Logic: screening for high-differentiation genome regions shared among the replicate lake-stream pairs



Other lake-stream genome scan
Deagle et al. 2012 Proc R Soc B



Position on chromosome 4

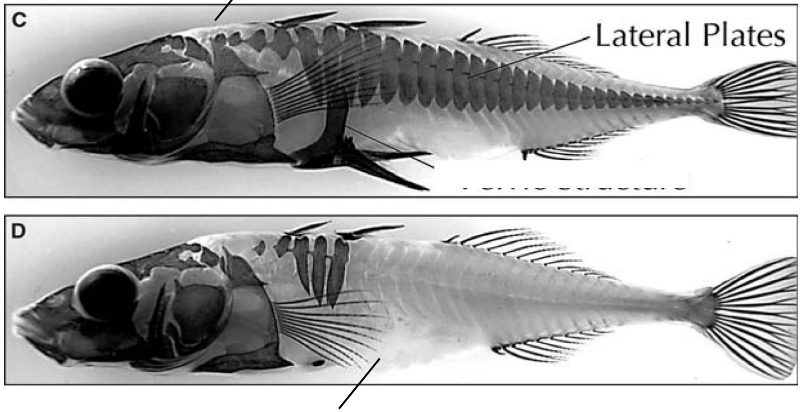
Benthic-pelagic genome scan Jones et al. 2012 Curr Biol

Can we be certain that F_{ST} peaks reflect loci under parallel divergent selection?

Ectodysplasin (*Eda*) gene

Oceanic (ancestral) morph

Cresko et al. 2007 PNAS

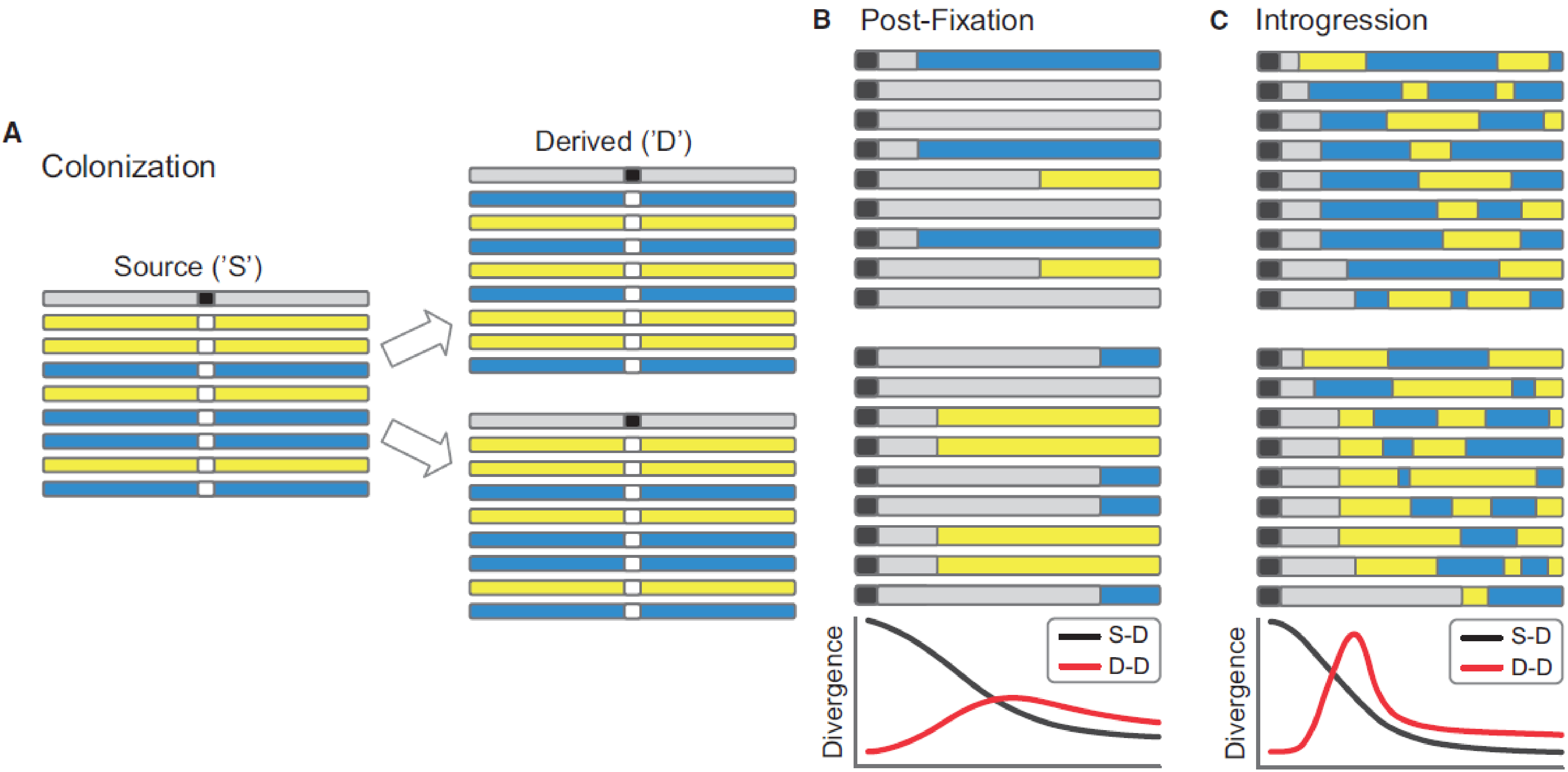


Freshwater morph

Freshwater populations share the low-plated phenotype, driven by *Eda* variants

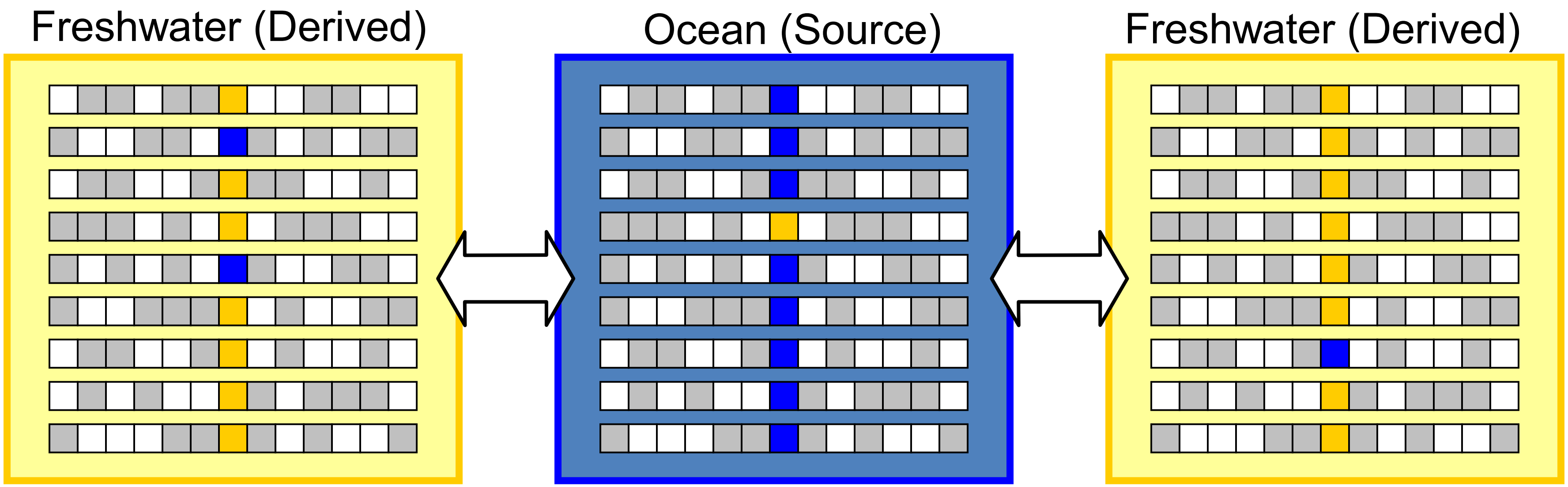
Hypothesis

Peak-valley-peak pattern among derived populations is driven by repeated adaptation from shared genetic variation at a single locus



Simulation study

- Multiple independent colonization of new habitats from ancestral source with standing variation at selected locus

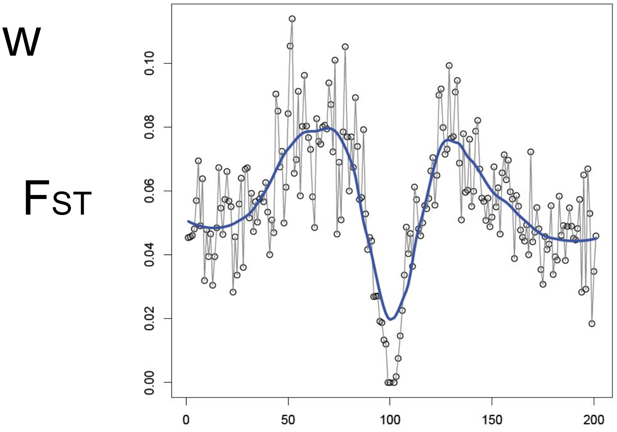


Freshwater (Derived)

Ocean (Source)

Freshwater (Derived)

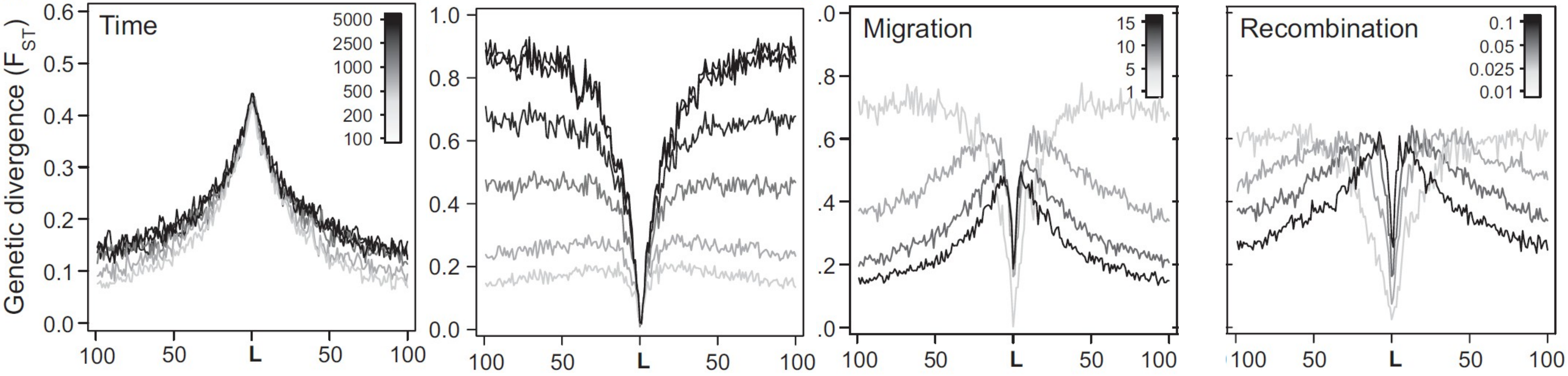
- Adaptation with gene flow




Sergey Gavrilets

- Parameters explored: time, N colonizers, carrying capacity, migration rate, selection strength, recombination rate

Source-derived Derived-derived



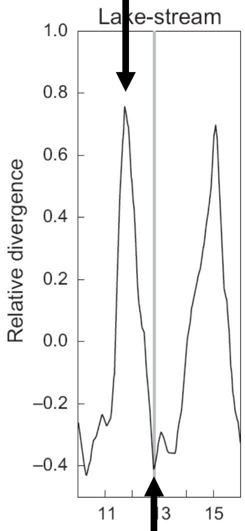
The selected locus acts as a localized barrier to gene flow

Source-derived contrasts reveal the classical signature of divergence with gene flow

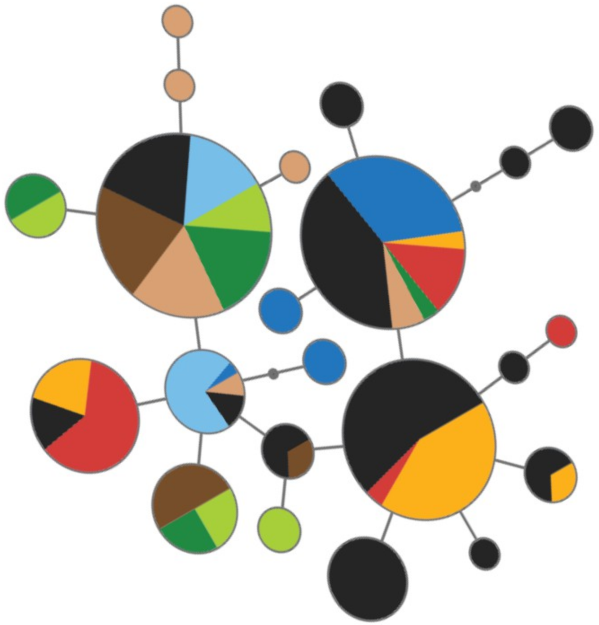
Derived-derived contrasts: repeated adaptation from shared variation drives a peak-valley-peak pattern

Confirming the model through targeted sequencing

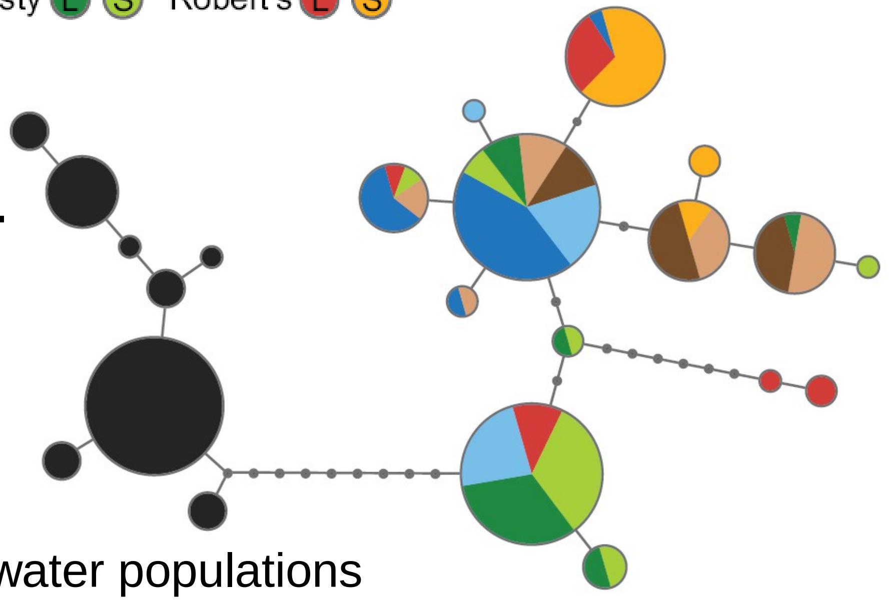
on the peak



within *Ectodysplasin*



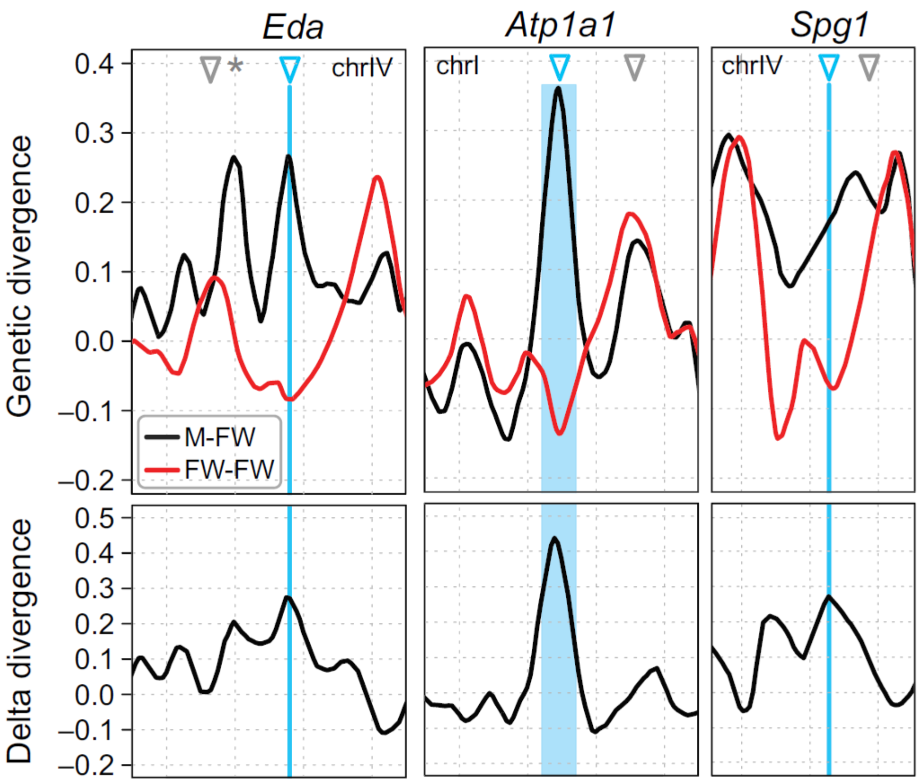
Marine ● Boot L S Joe's L S Misty L S Robert's L S

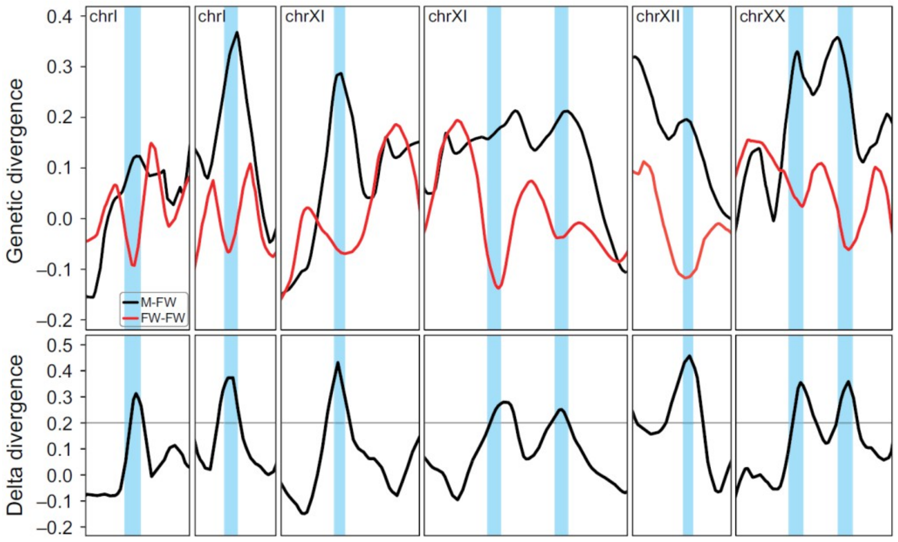


Complete marine-freshwater isolation at *Eda* but not away from it – localized barrier to gene flow

Extensive haplotype sharing among the freshwater populations – adaptation from shared genetic variation

The peak-valley-peak signature as a discovery tool

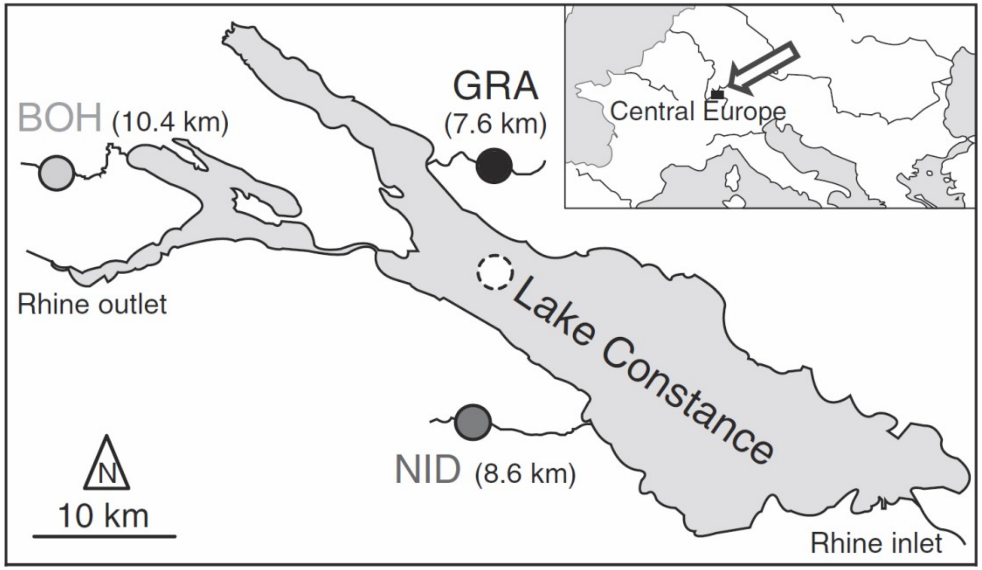


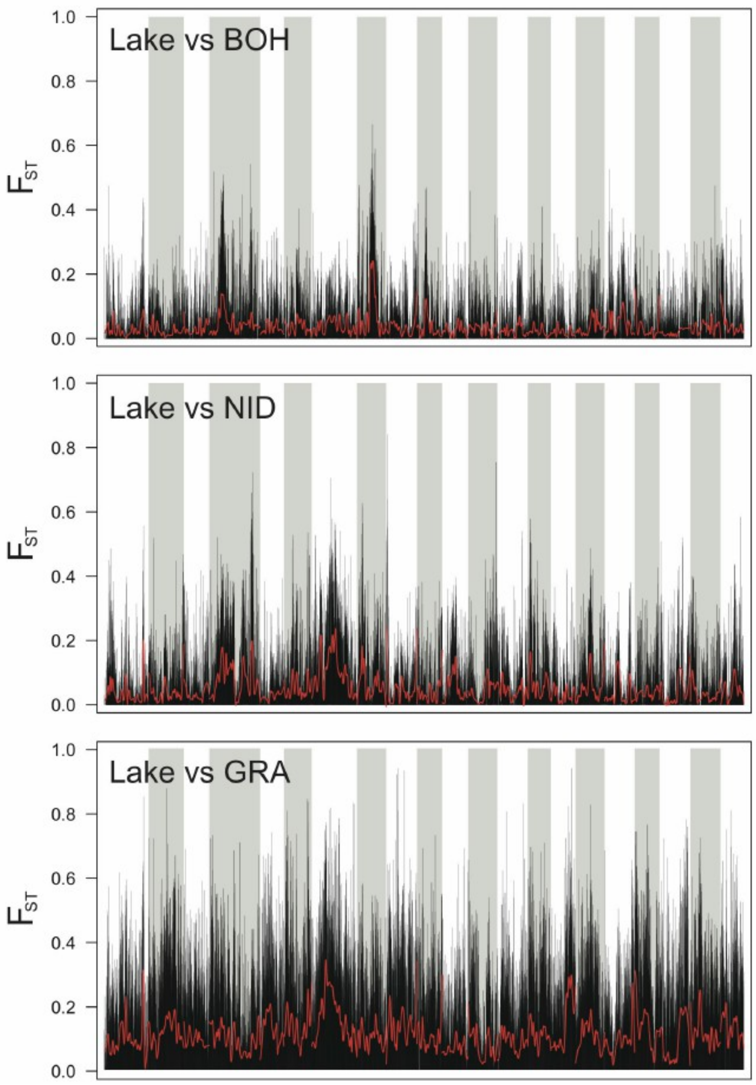


- Marine-freshwater divergence is highly polygenic and involves extensive allele recycling
- Derived freshwater populations still carry the footprints of the ancestral marine-freshwater divergence
- Parallel evolution is useful for the detection of adaptation loci – but one must look for the right signature at the right ecological level

2. Searching for adaptation loci using parallel evolution in lake-stream stickleback from the Lake Constance region Roesti et al. 2015 Nat Commun

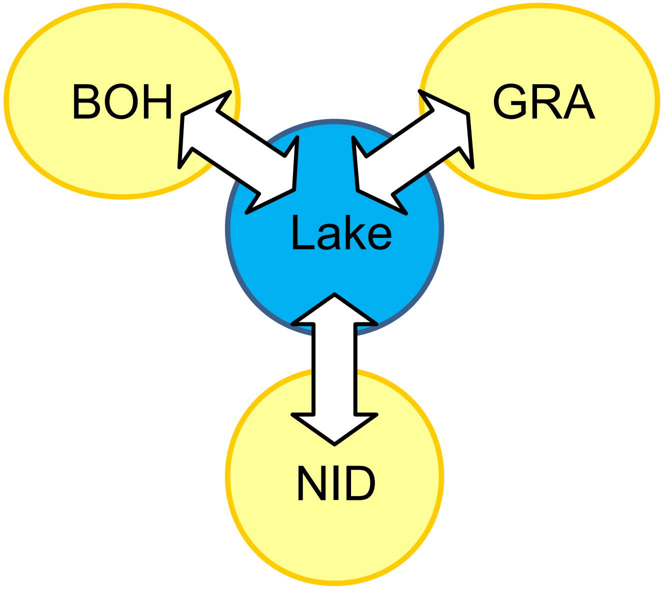
Logic: screening for high-differentiation genome regions shared across multiple lake-stream contrasts



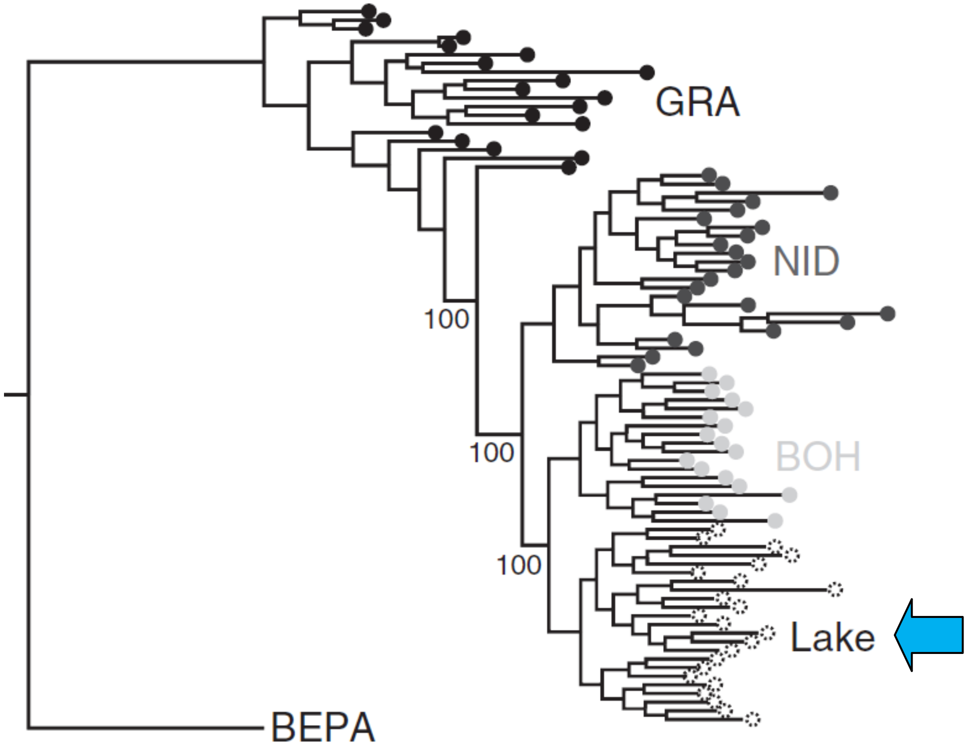


Assumptions ...

- The lake population is the ancestor
- Stream adaptation has occurred three times in parallel

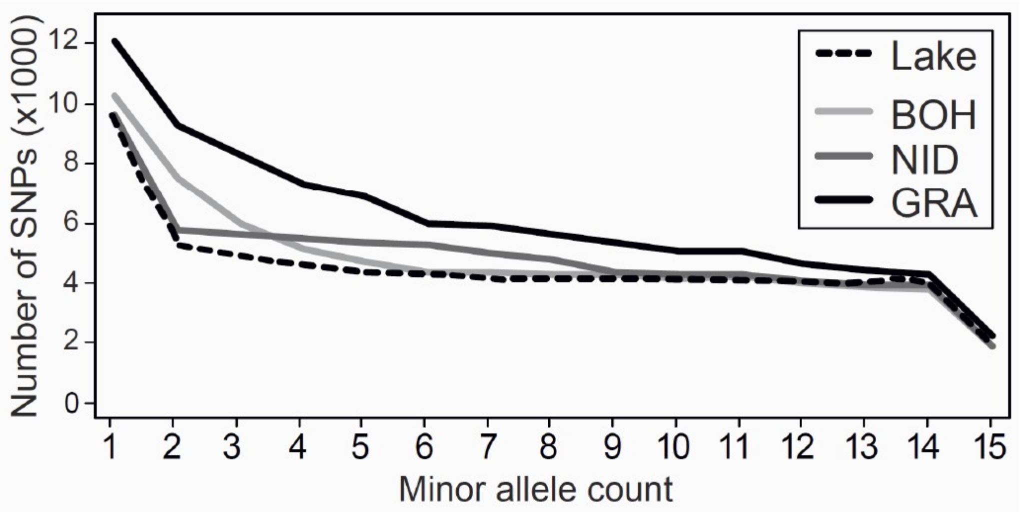


... and reality



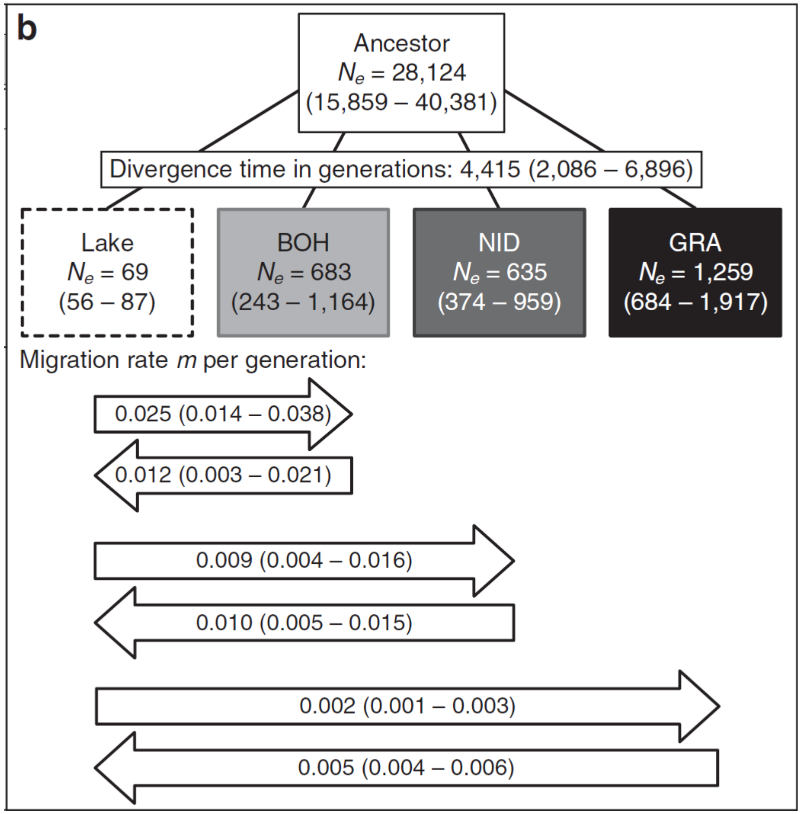
Phylogeny
(51 k SNPs)

The lake population seems derived from stream fish



Site frequency spectra from the four populations

Demography with fastsimcoal, 14.8 million positions

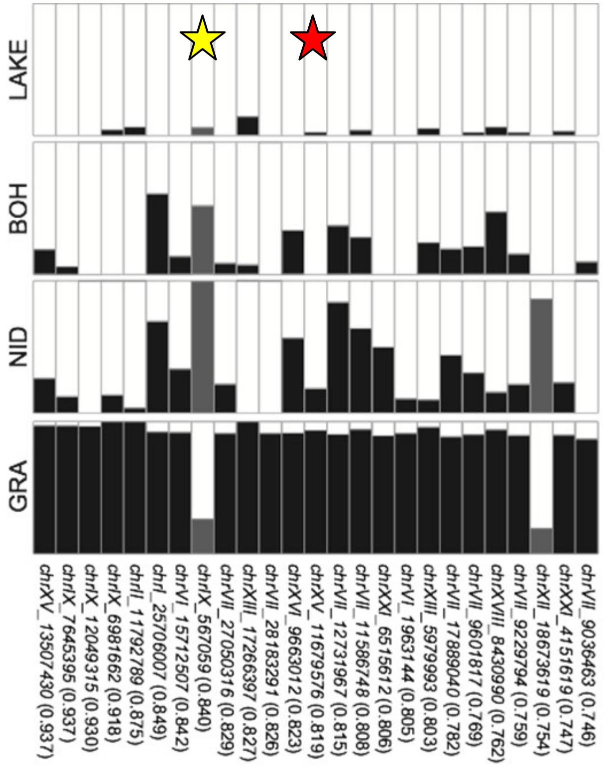


The lake population has the lowest genetic diversity

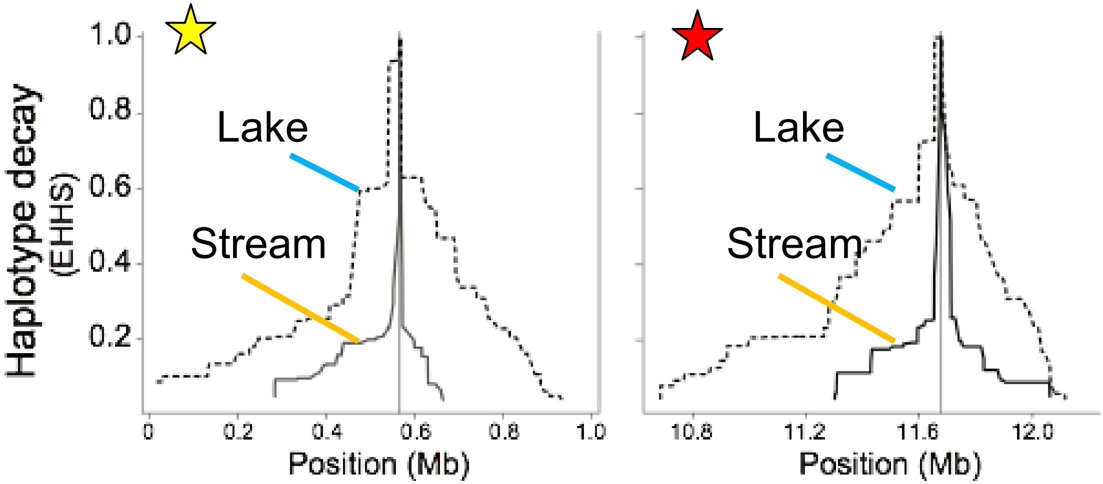
Hypothesis: The lake population has diverged from an ancestral stream form

Evidence from selected regions

F_{ST} extremes from all lake-stream comparisons



Haplotype tract length around F_{ST} extremes

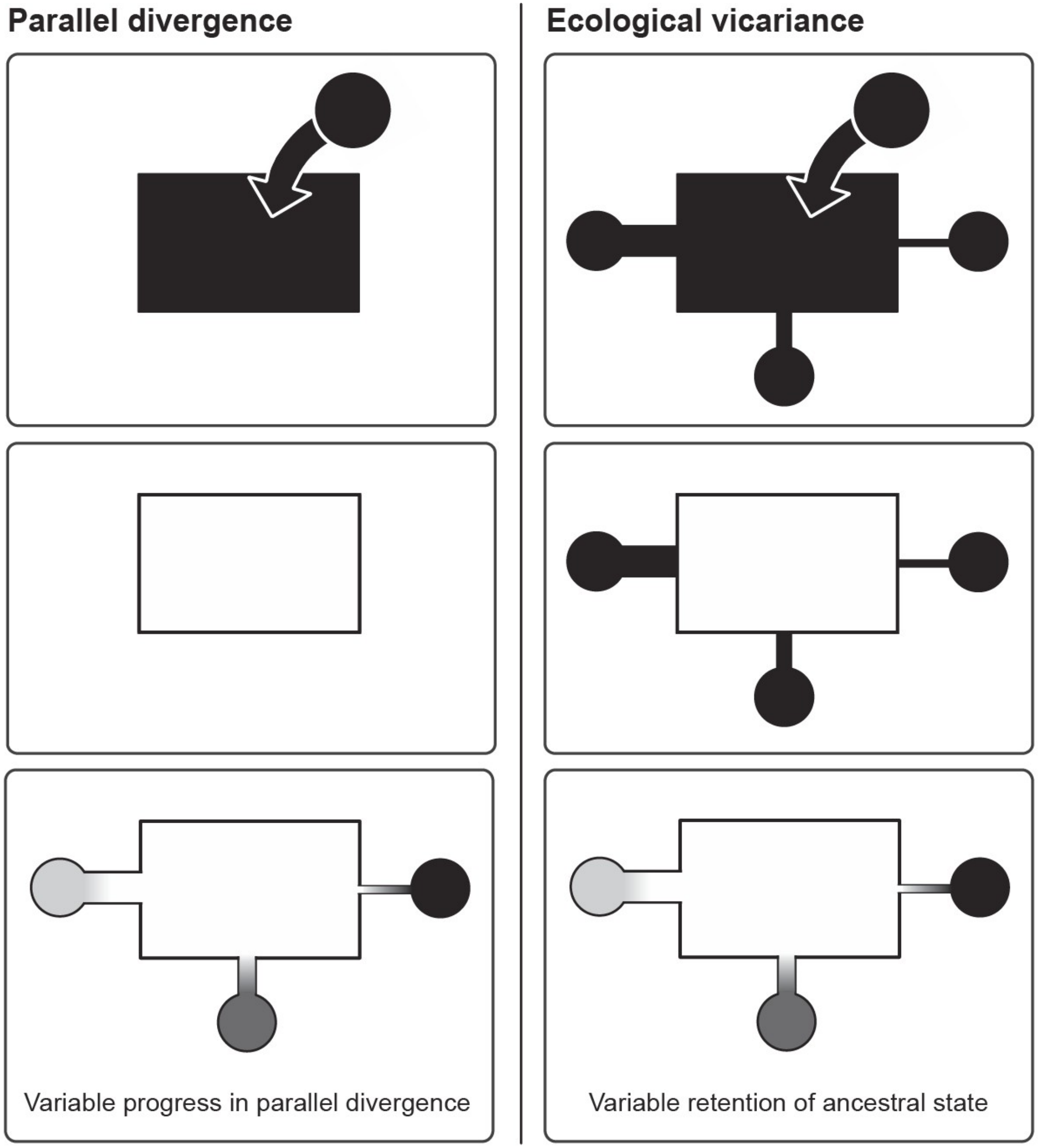


Selection has occurred mainly in the lake

Selective sweeps have eliminated genetic variation

Resolving the history of adaptive divergence in Lake Constance stickleback

- Two alternative processes can result in similar patterns of population differentiation
- The population structure in the Lake Constance region is consistent with ecological vicariance
- Adaptation has occurred only once; conclusions about the determinism of natural selection are flawed

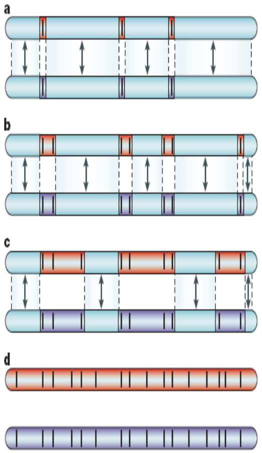


Conclusions

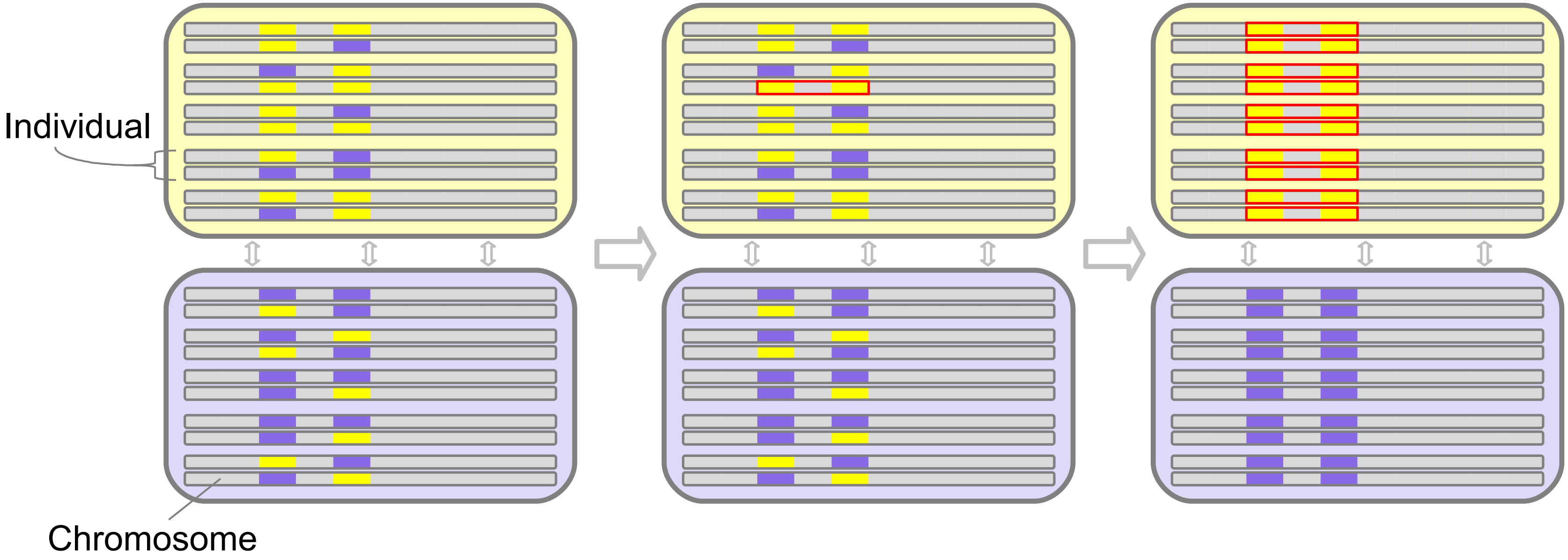
- Studying parallel evolution can be tricky: signatures in the genome might reflect ancient selection unrelated to the focal population contrast
- Parallel evolution can be mimicked by alternative evolutionary histories
- Robust ecological and demographic information, and sequence data from loci under selection, are valuable when inferring parallel evolution

Recombination and genomics

- Divergence with gene flow – a migration-selection antagonism
- The recombination rate influences this antagonism



Wu & Ting 2004 Nat Rev Genet



Migration-selection balance at two recombining loci

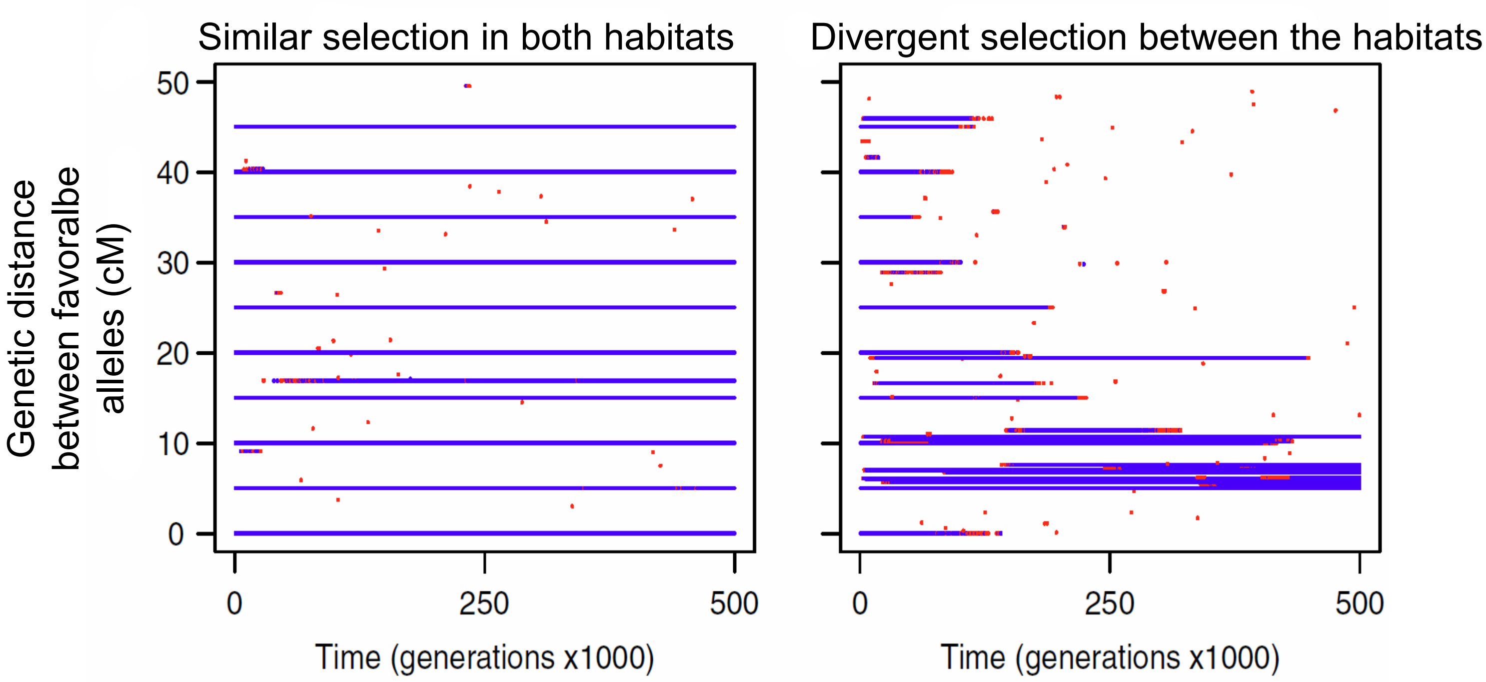
Recombination suppression captures two favorable alleles

The two alleles now act as single locus of larger effect and reach stronger divergence

Theoretical evidence of the adaptive benefit of reduced recombination

Yeaman 2013 PNAS

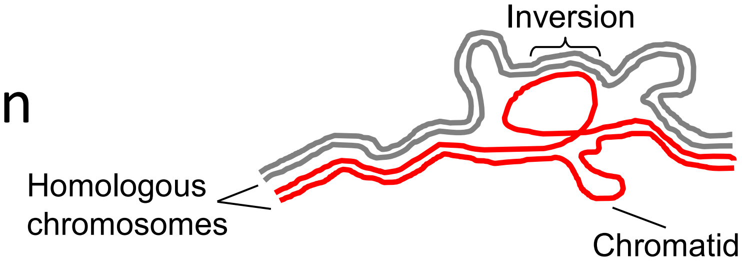
- Two habitats, 10 loci under migration-selection balance along a single chromosome
- Mutation: cut-paste positional relocation of the different alleles



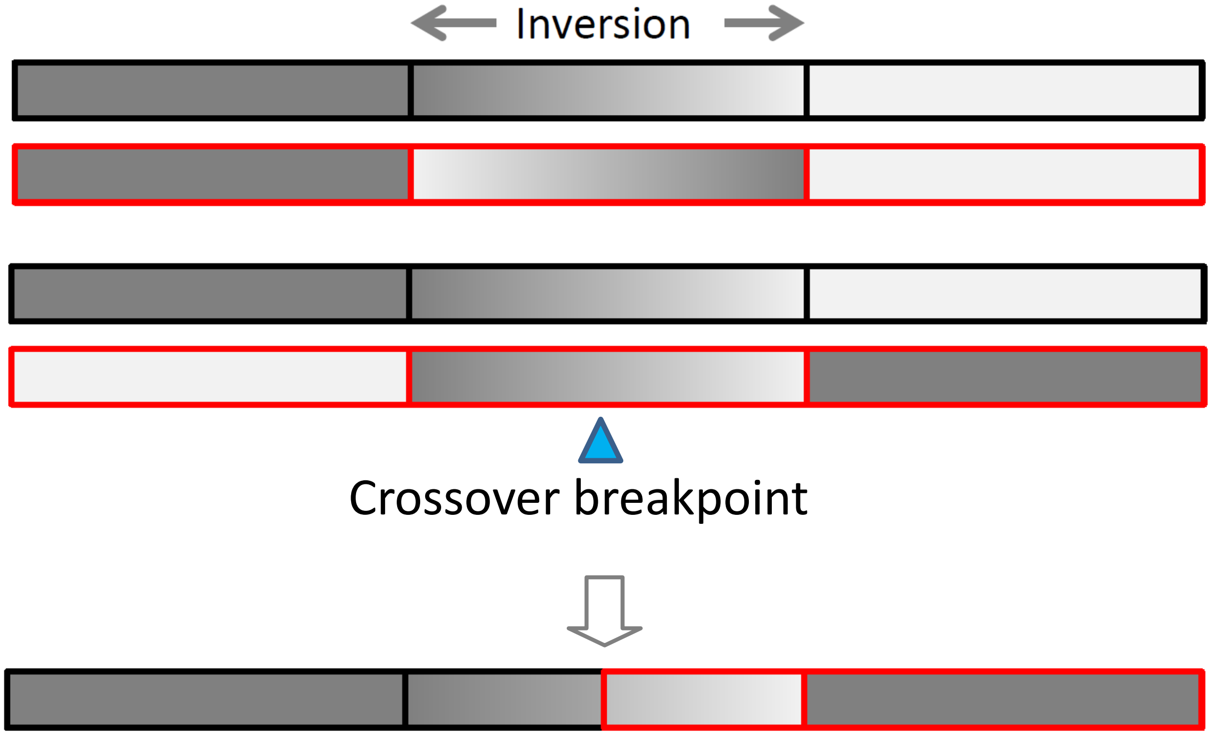
Selection favors a more compact genomic architecture (physical proximity = lower recombination frequency)

An easy way to suppress recombination: inversion

The inversion loop hinders recombination



When recombination still occurs within the inversion, the recombined gametes display gene duplications/deletions



Inversion

Inversion-heterozygous individual

Standard

Inverted

Crossover within the inversion

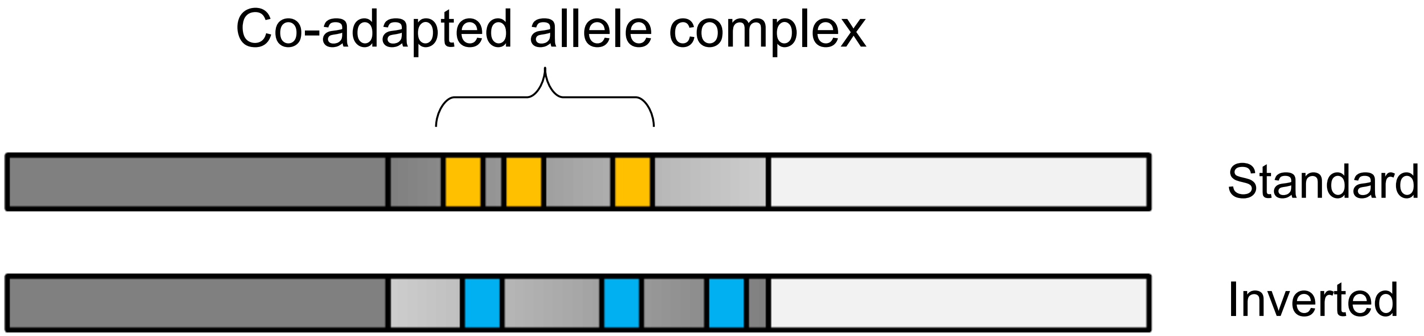
Standard

Inverted

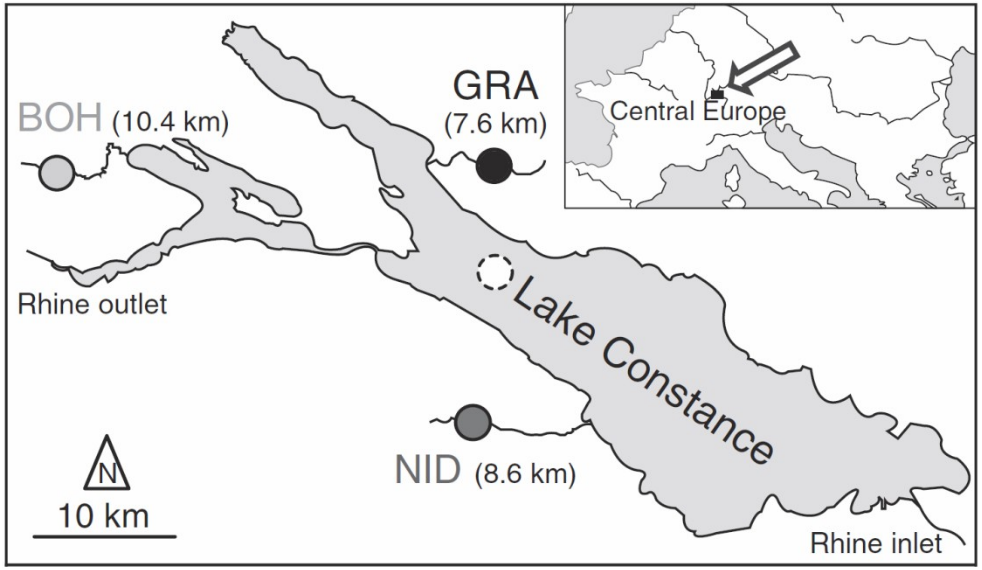
Crossover breakpoint

Gamete with unbalanced chromatid

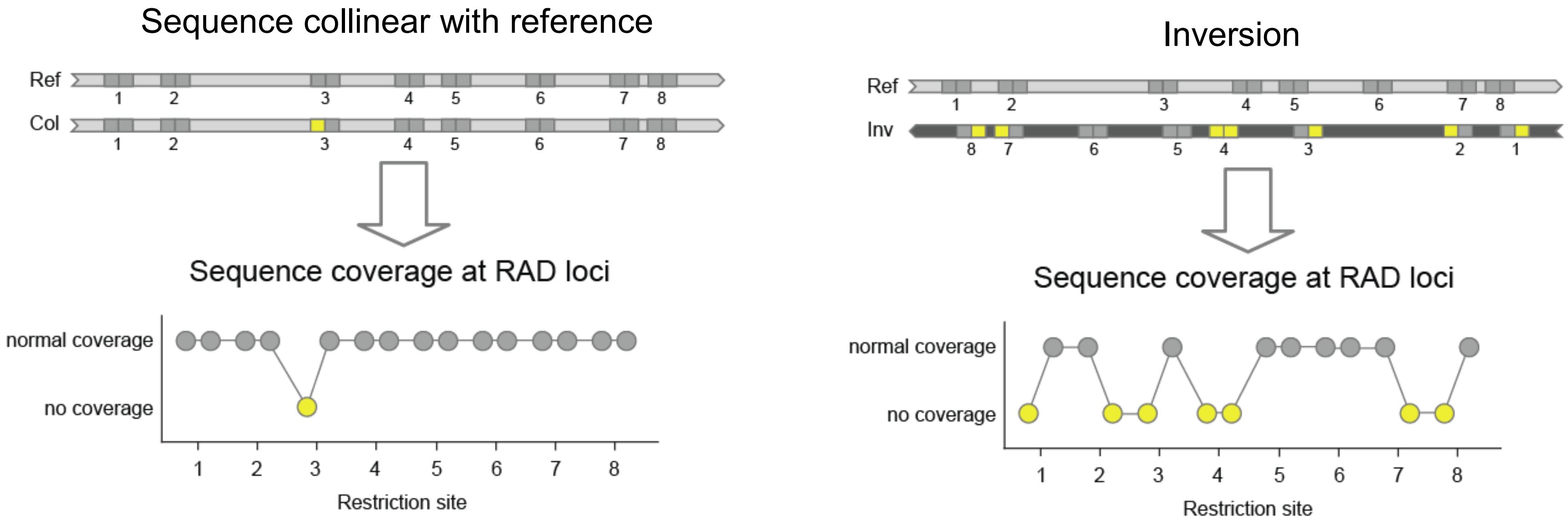
Prediction: inversions coupling adaptive alleles should be important to adaptive divergence



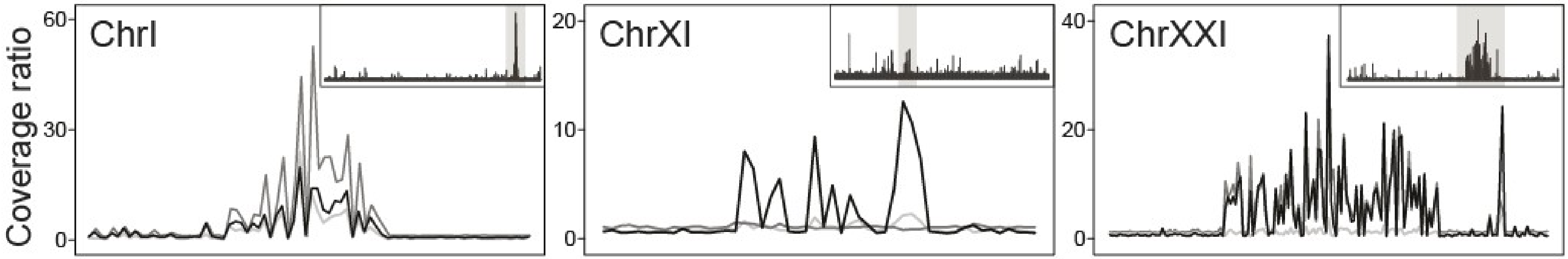
Exploring the significance of inversions in lake and stream stickleback Roesti et al. 2015 Nat Commun



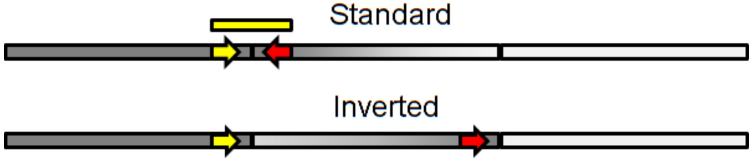
- Screen for inversions in Lake Constance stickleback
- Method: distortion in sequence coverage among RAD loci



Coverage scans in lake-stream stickleback



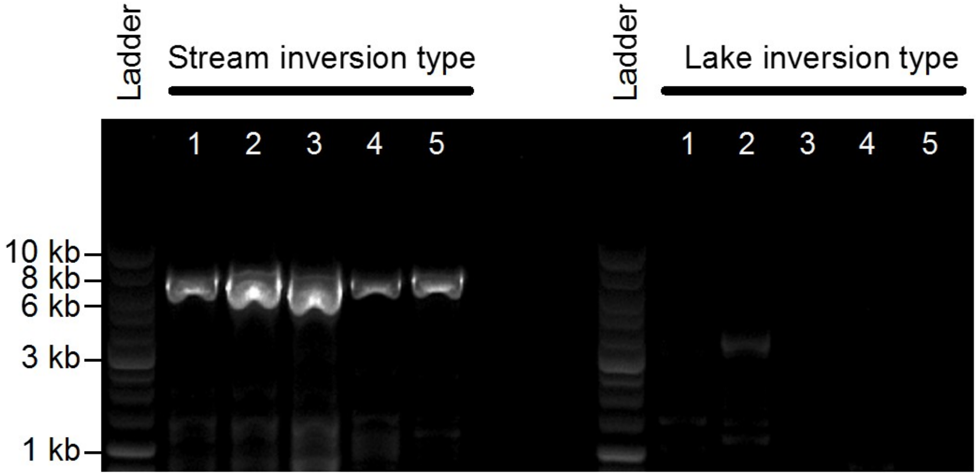
Confirming the inversions using PCR primers across breakpoints



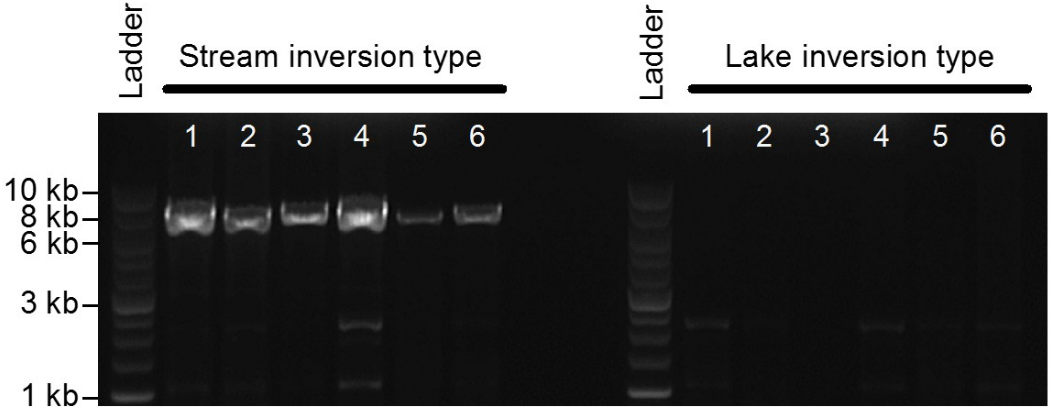
Three major inversions segregate in the Lake Constance basin

Detected previously in marine-freshwater stickleback Jones et al. 2012 Nature

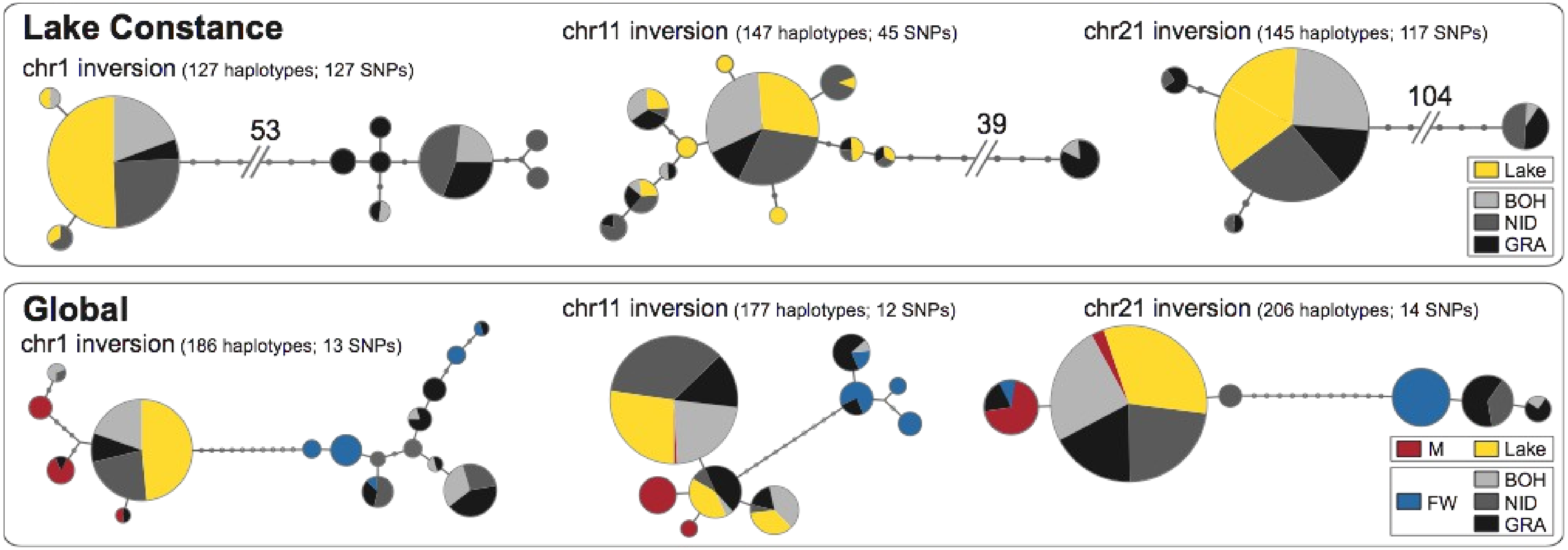
ChrI inversion

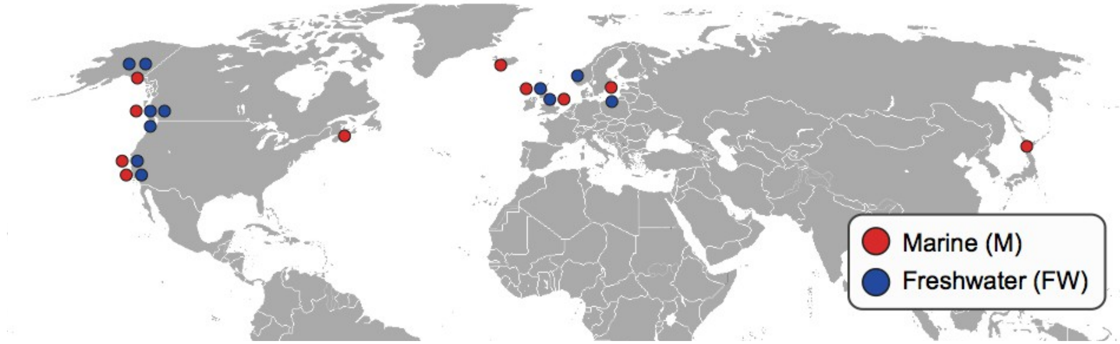


ChrXI inversion



Exploring the ecology of the inversions based on inversion-specific haplotype genealogies



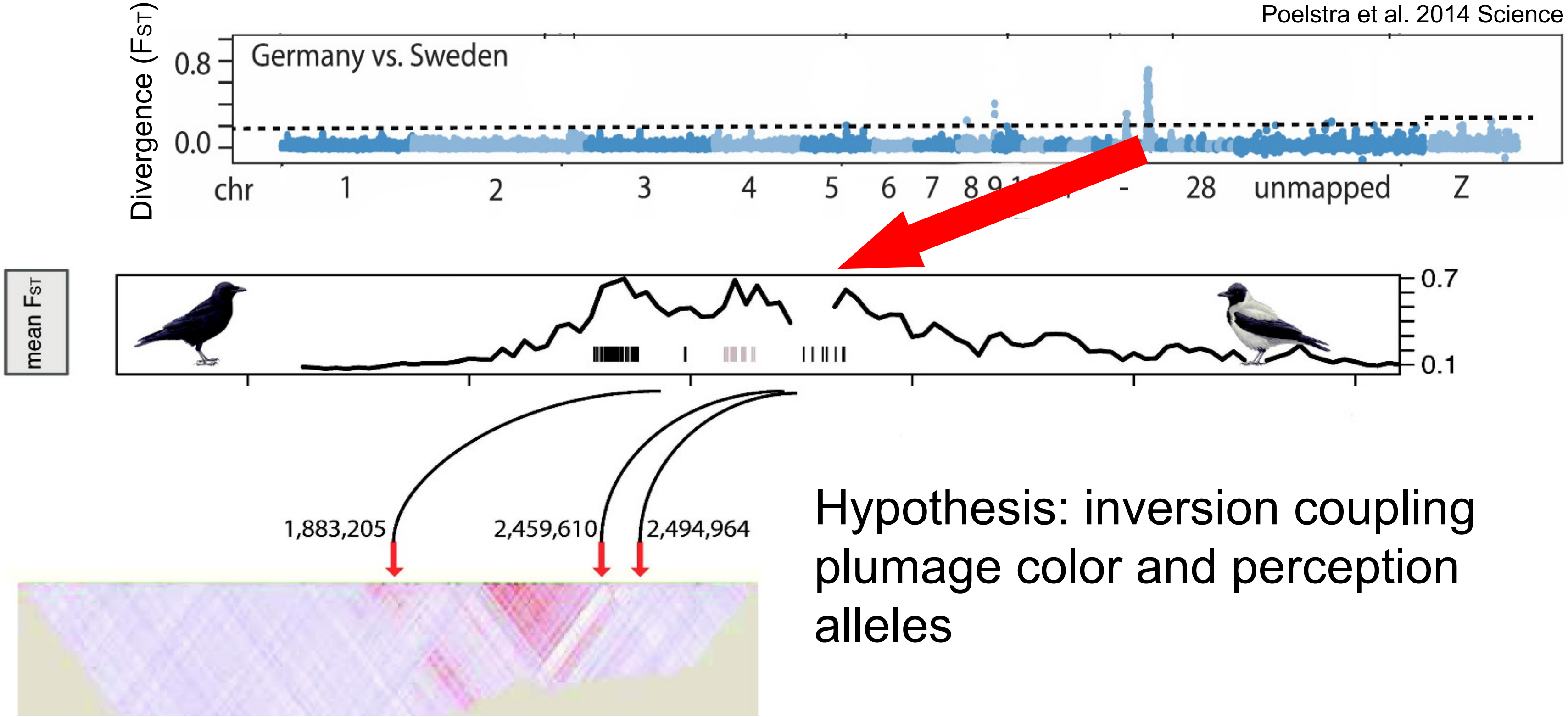


Jones et al. 2012 Nature

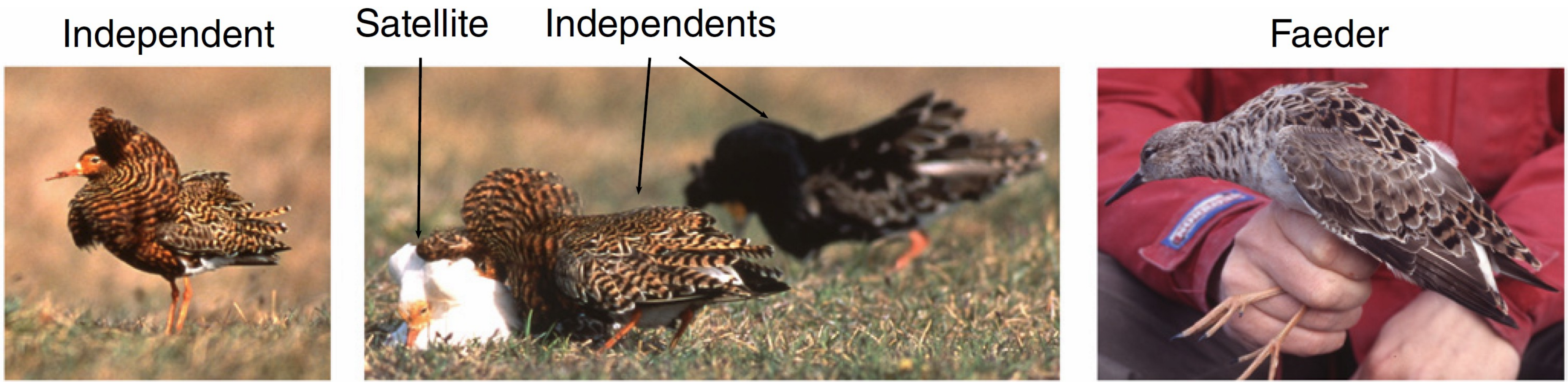
- Ancient inversions have been re-cycled for lake-stream divergence
- Likely ecological factor: pelagic vs. benthic life style
- Parallel evolution at a global scale

Evidence from other systems for a role of inversions in adaptive divergence

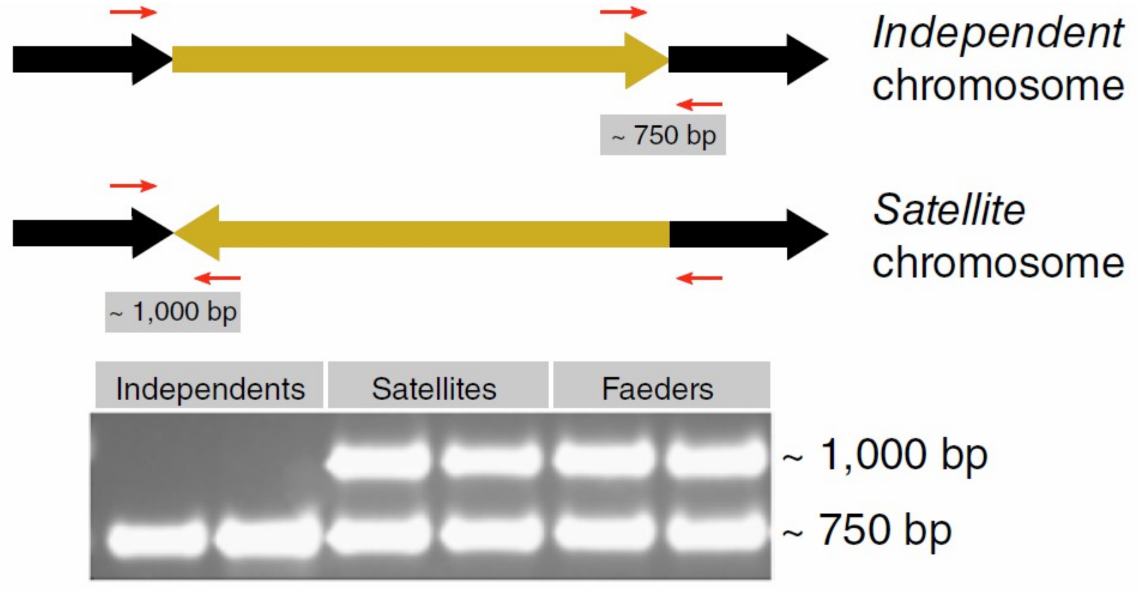
Poelstra et al. 2014 Science



Hypothesis: inversion coupling plumage color and perception alleles

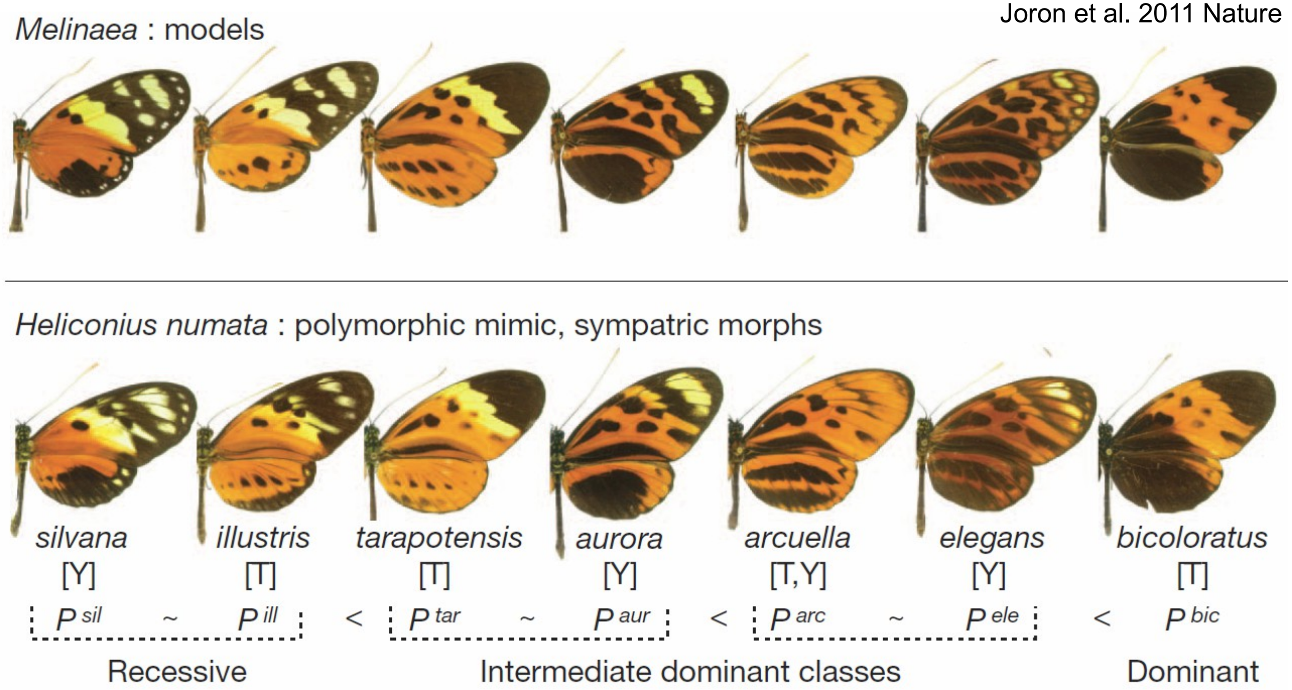


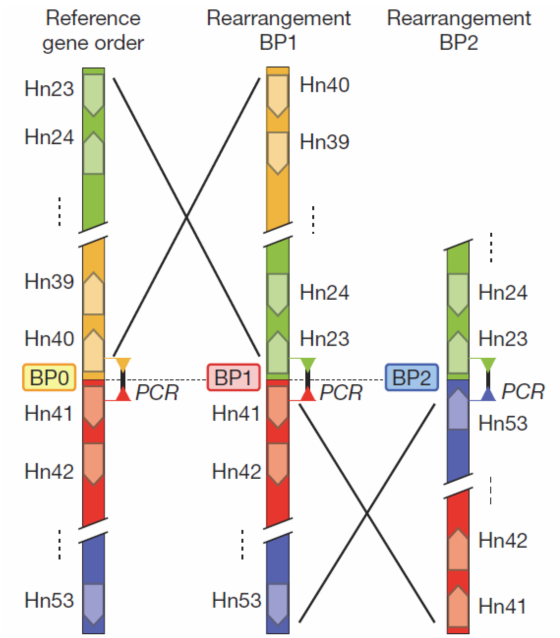
Lamichhaney et al. 2015 Nat Genet



Inversion coupling behavior and plumage color alleles

Joron et al. 2011 Nature

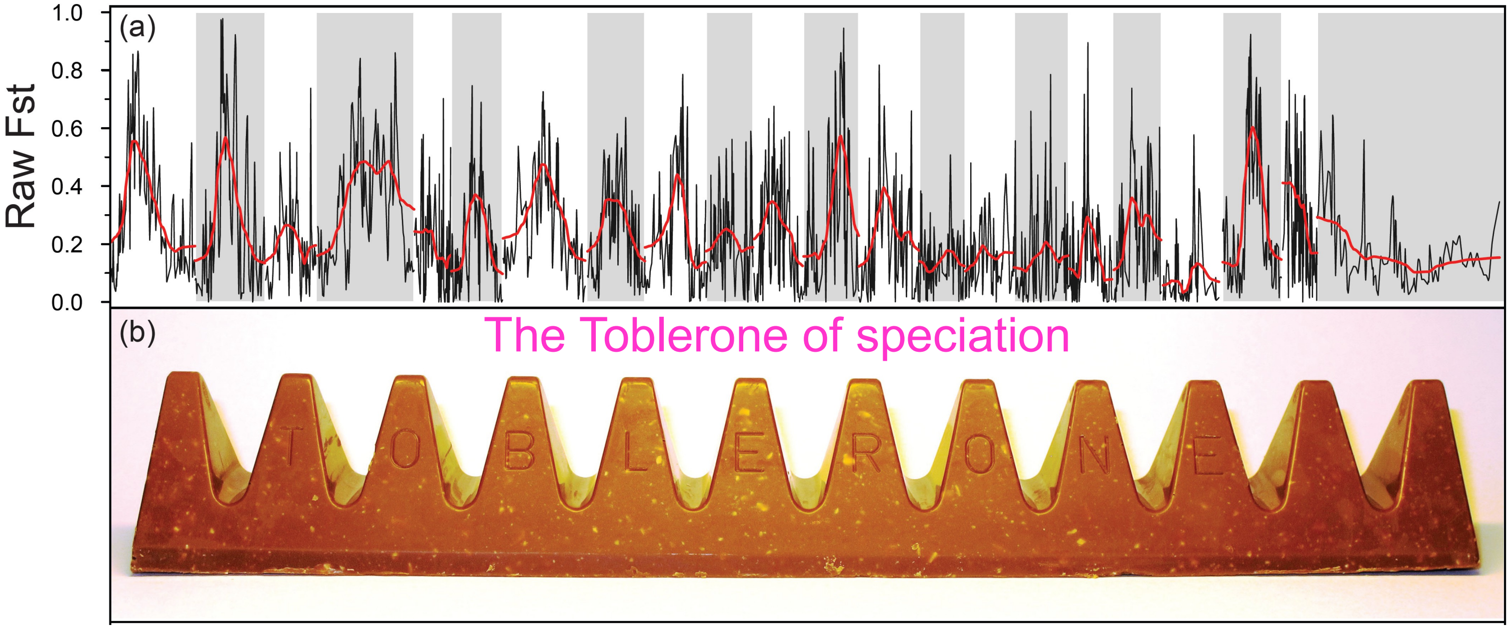




Wing color elements coupled in adjacent inversions

Aren’t we overlooking something rather important?

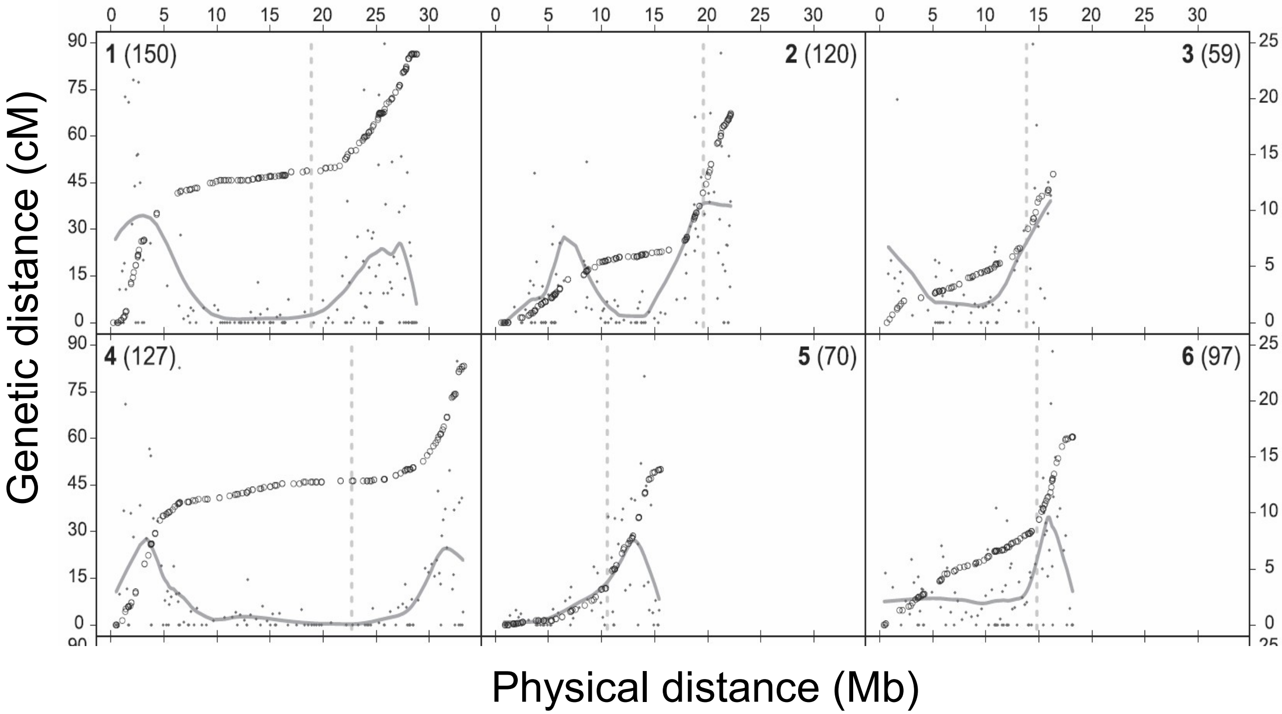
Genomic divergence between lake and stream stickleback from Vancouver Island is biased toward chromosome centers Roesti et al. 2012 Mol Ecol



Let’s call this CCBD for **C**hromosome **C**enter-**B**iased **D**ivergence

A recombination-based explanation for CCBD Roesti et al. 2012, 2013 Mol Ecol

Linkage mapping (F2 intercross, N = 280) reveals reduced recombination rate in stickeback chromosome centers

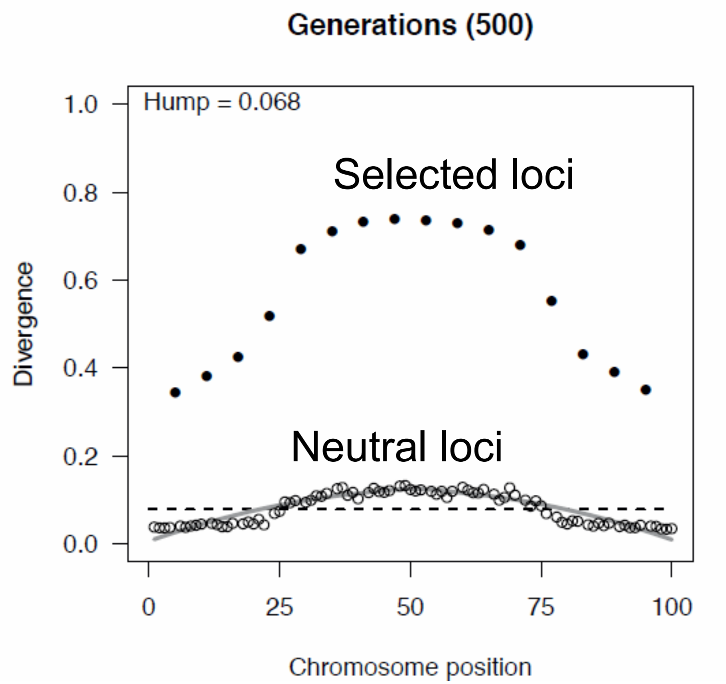


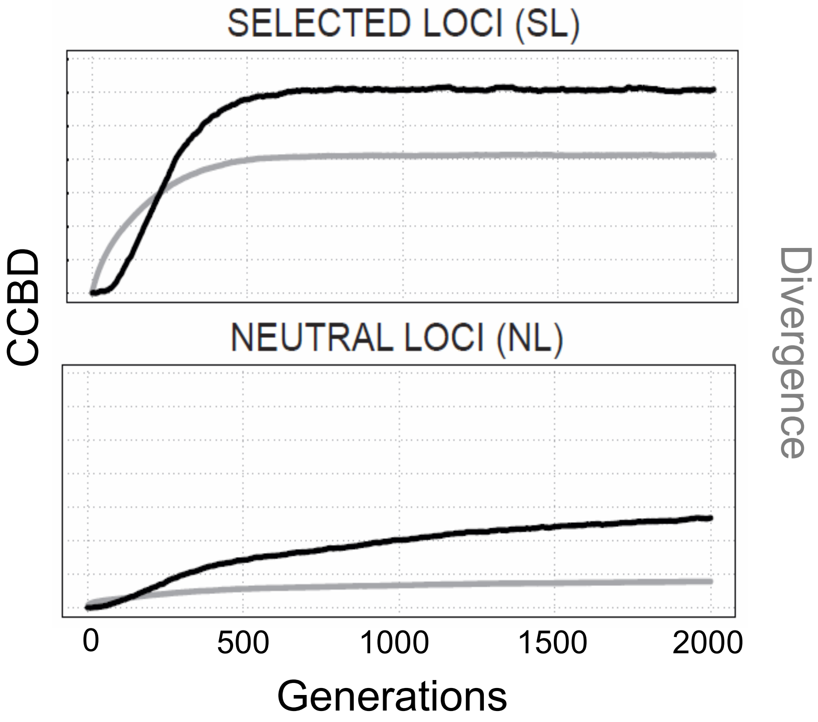
Genomic divergence in lake-stream stickleback is highly polygenic

Hypothesis: The coupling of alleles in chromosome centers makes the introgression of DNA in chromosome centers more difficult than in the peripheries

Validation using adaptive divergence *in silico* Berner & Roesti, in progress

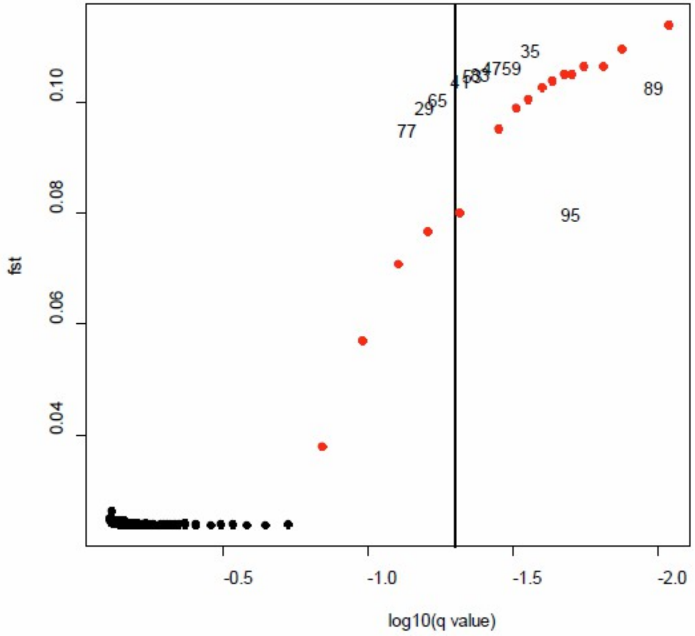
- Adaptive divergence with gene flow between two populations, using standing variation at many loci under selection
- Single chromosome; neutral loci interspersed between the selected ones
- Recombination is biased to the peripheries
- Parameters: Pop size, time, selection strength, migration rate, recombination bias, etc



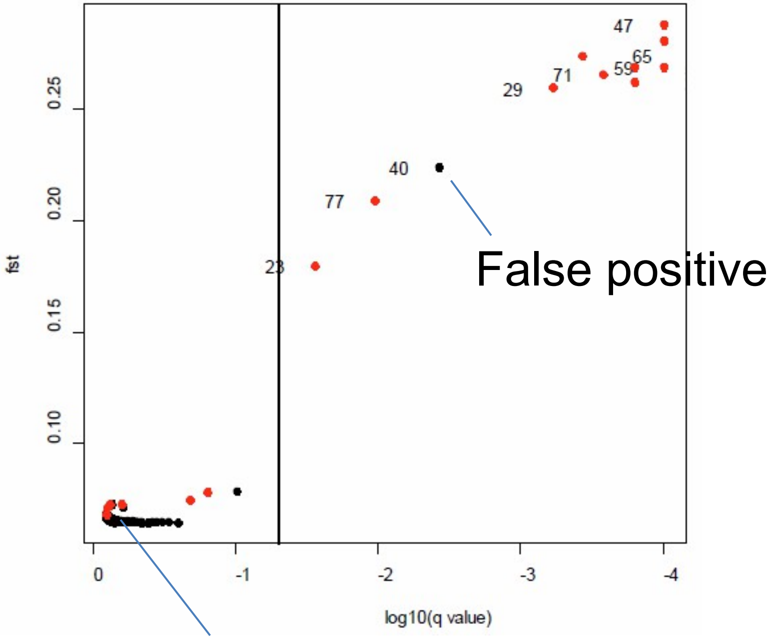


One of many consequences of CCBD

Biased outlier detection: standard F_{ST} outlier scan using BayeScan



Recombination rate homogeneous along chromosome



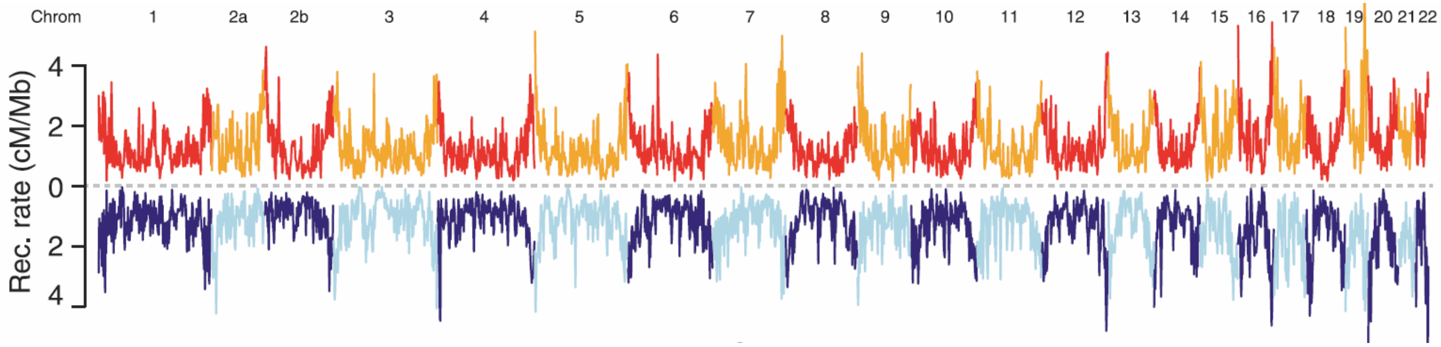
Recombination rate biased toward chromosome center

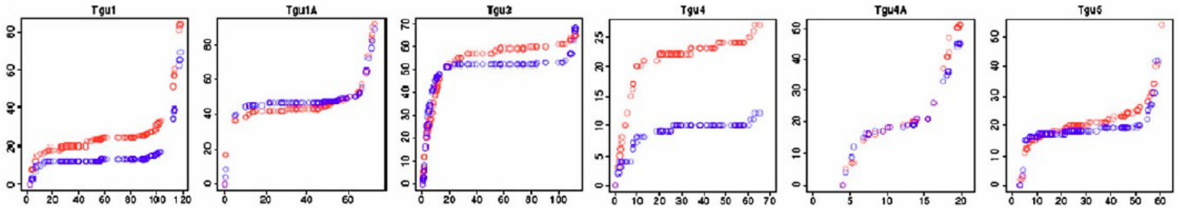
False positive

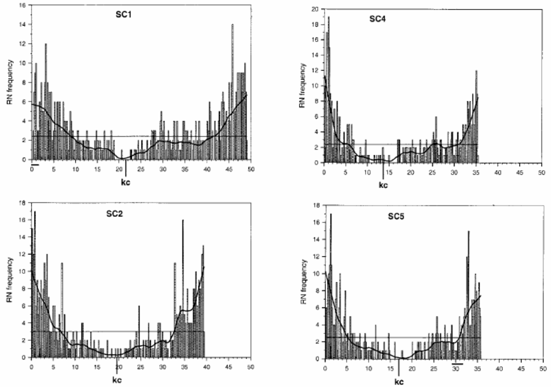
False negatives

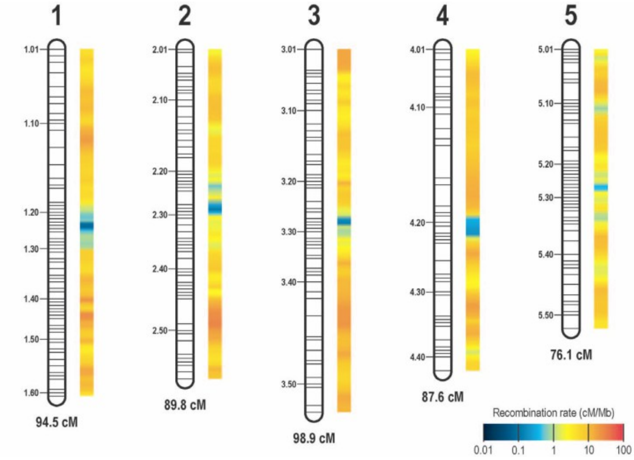
We ARE overlooking something rather important!

Elevated recombination in chromosome peripheries seems taxonomically universal









Conclusions

- Recombination suppression via inversions clearly contributes to adaptive divergence
- Our perspective on the general adaptive significance of inversions, however, is biased by simple large-effect systems
- The consequences broad-scale heterogeneity in recombination rate along chromosomes is largely ignored in current genomics (theory coming up!)


Photo: Dario Moser

Thanks

- Marius Roesti, Dario Moser, Benjamin Kueng, Anja Frey, Walter Salzburger, Andrew Hendry, Sergey Gavrilets
- Christian Beisel, Ina Niessen (Department of Biosystems Science and Engineering, D-BSSE Basel)

- SNF
- University of Basel

UNI BASEL

FNSNF
Swiss National Science Foundation