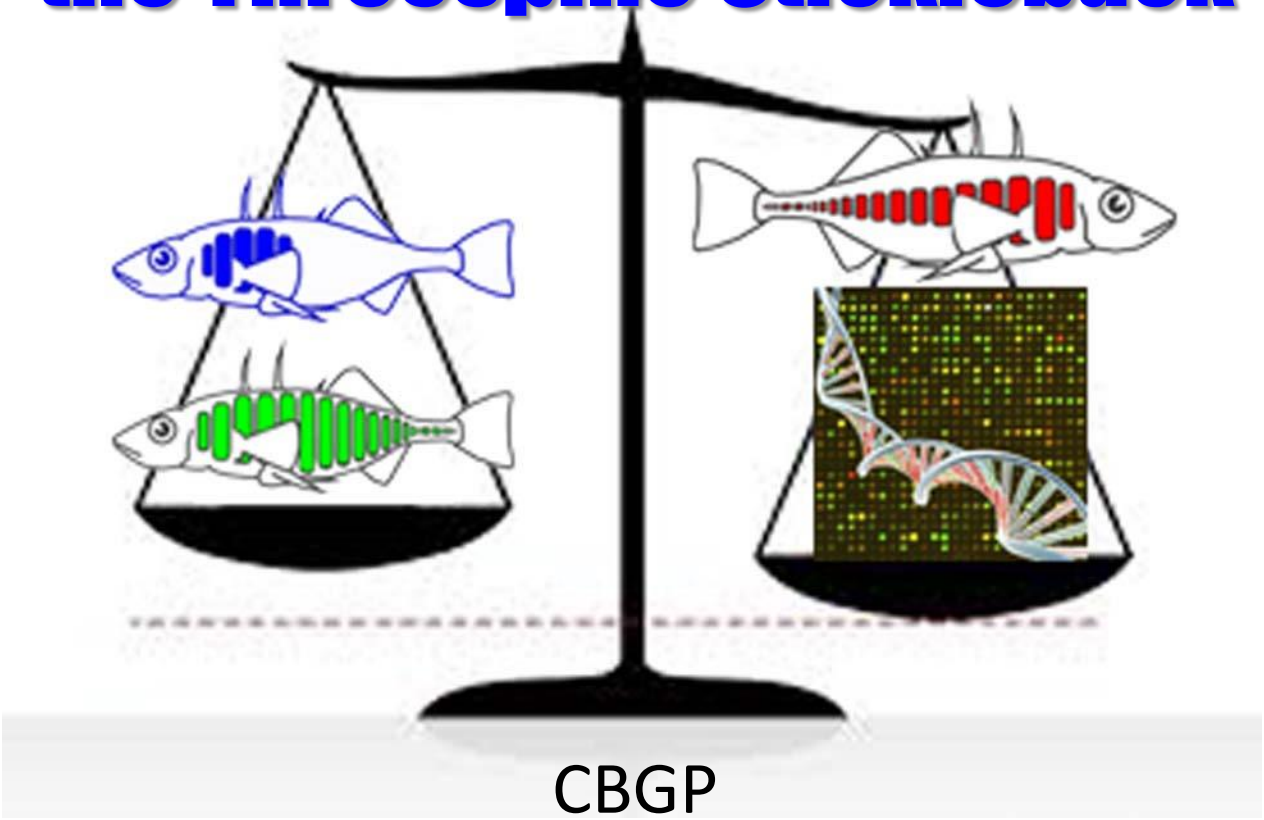
Tipping the Scales:

Other Lessons in Adaptive Evolution from the Threespine Stickleback



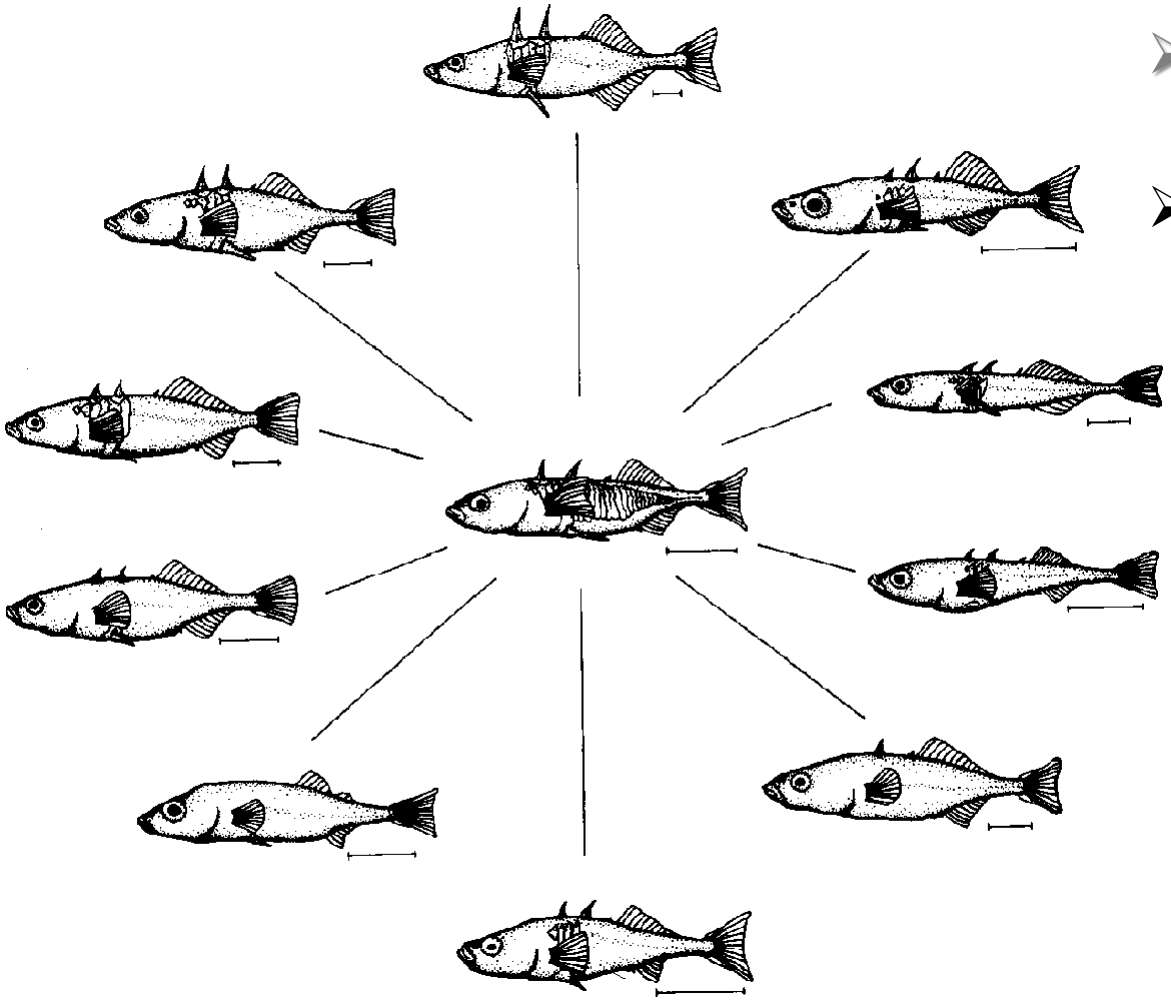
CBGP

29-Mar-2016

Why Sticklebacks?

- reproductive behaviour
  - ➡ one of Tinbergen's early models

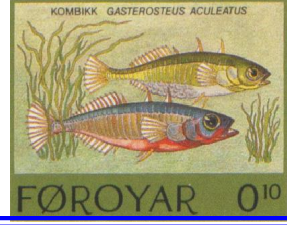
Why Sticklebacks?

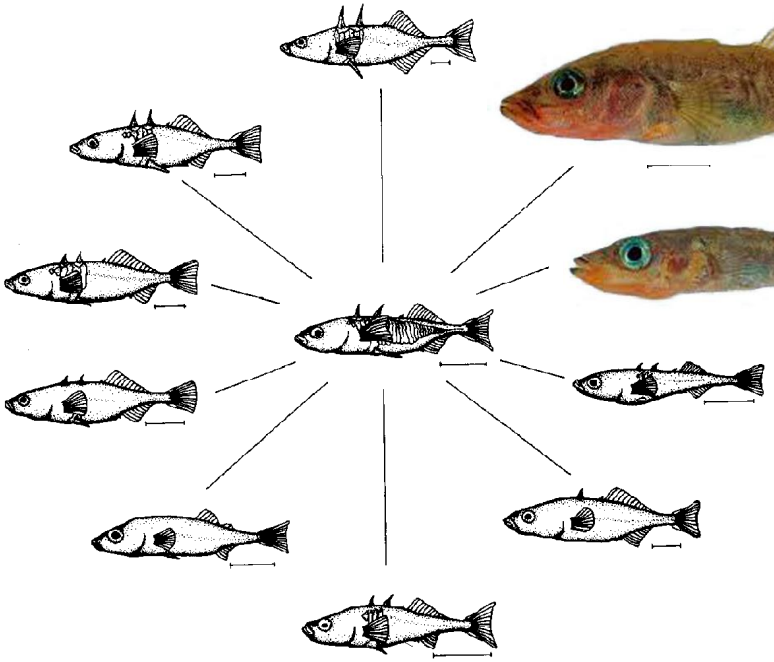


- reproductive behaviour
- morphological variation

Bell & Foster (1994)
Evolutionary Biology of the Threespine Stickleback

Why Sticklebacks?



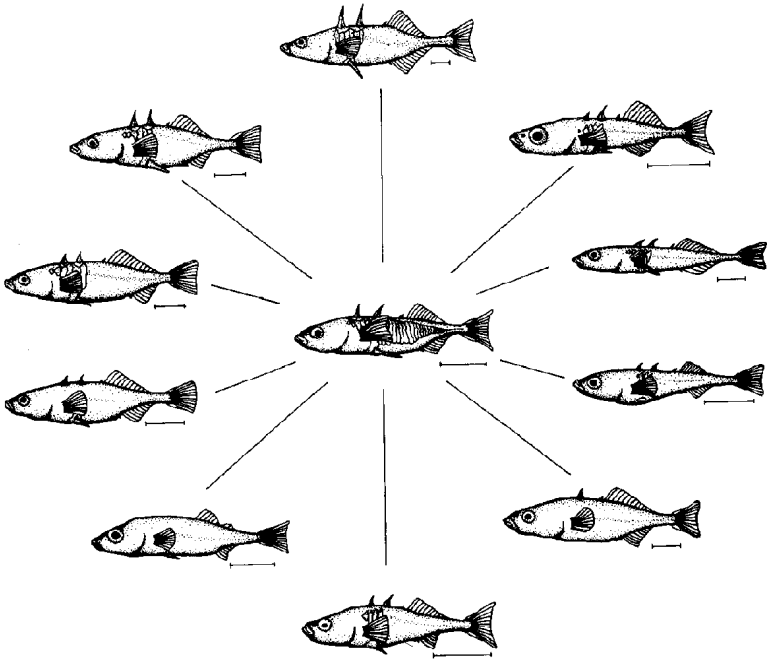


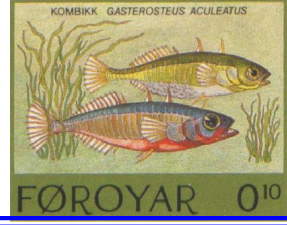
benthic

limnetic

- incipient species?
  - ➡ ecological speciation

Why Sticklebacks?

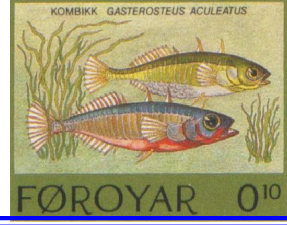


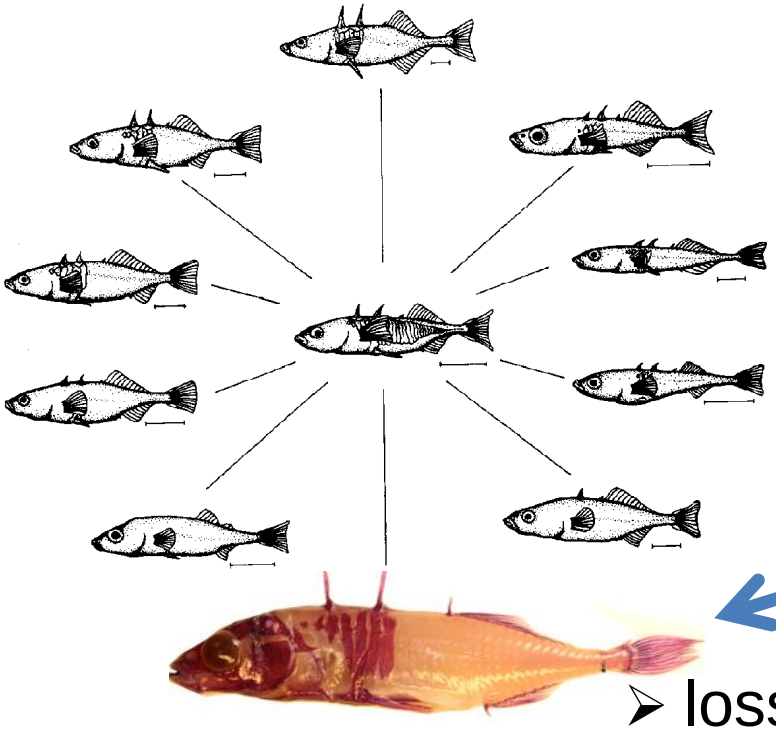


Lateral Plate Evolution

- modified scales
- protection from piscine predators

Why Sticklebacks?





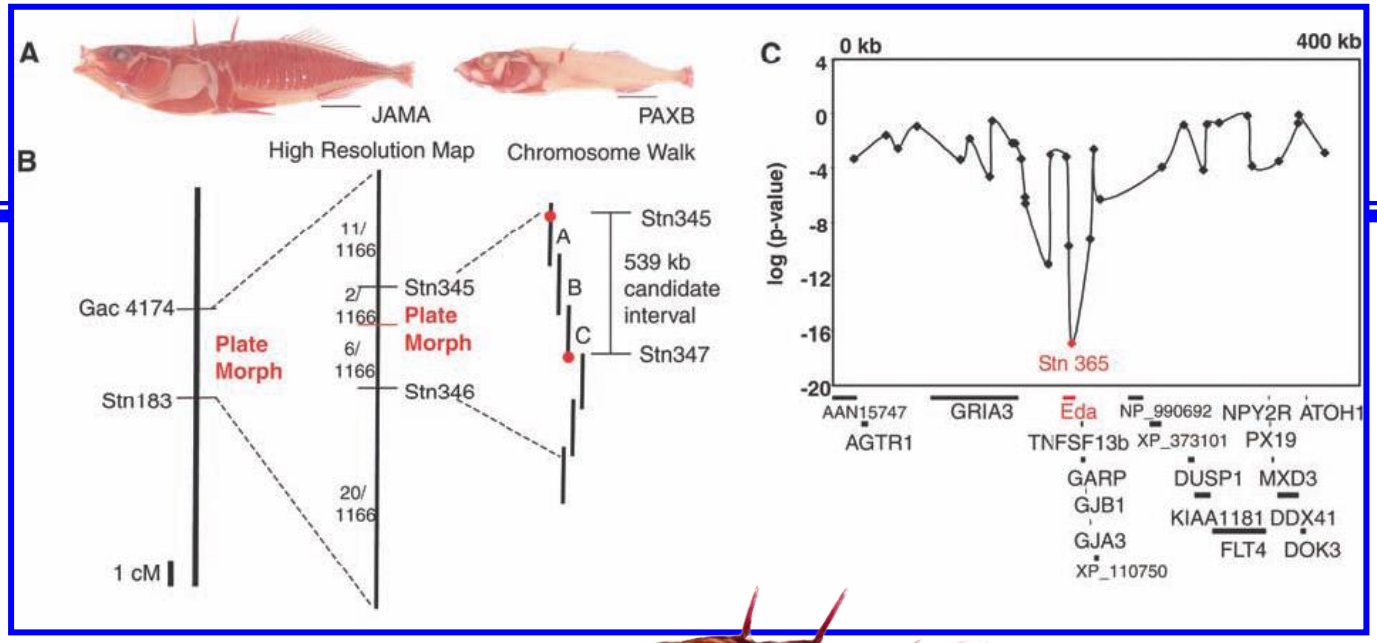
low plate morph

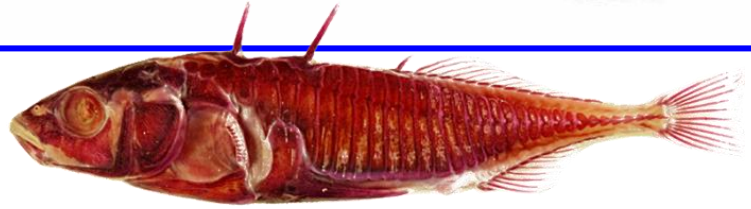
Lateral Plate Evolution

- modified scales
- protection from piscine predators

- loss of plates in freshwater
- selective advantage to different habitat use & predator types
  - ➡ increased flexibility results in greater burst swimming speed

Colosimo *et al*. (2005)
Science 307:1928



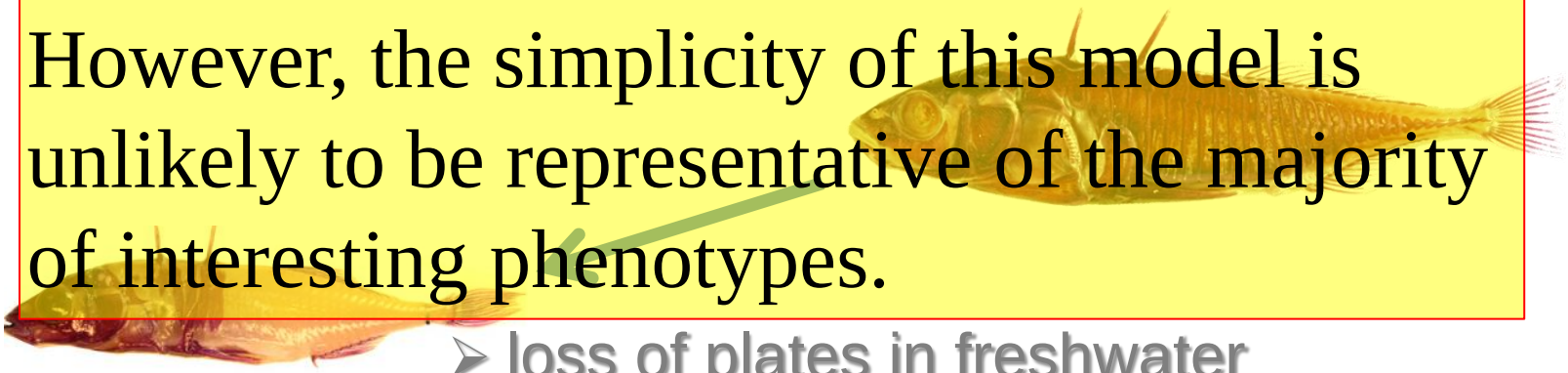


low plate morph

- loss of plates in freshwater
- selective advantage
- relatively simple genetic basis
  - ectodysplasin-a: *Eda*

A great example for undergrad textbooks...

However, the simplicity of this model is unlikely to be representative of the majority of interesting phenotypes.

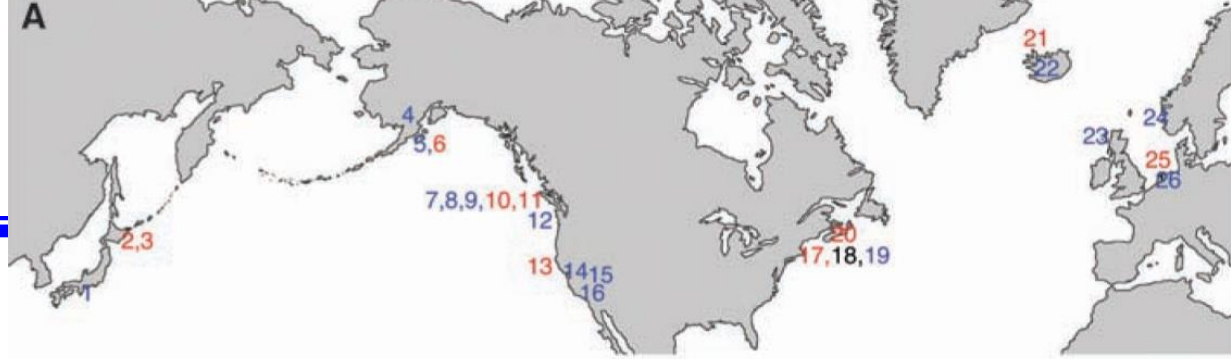


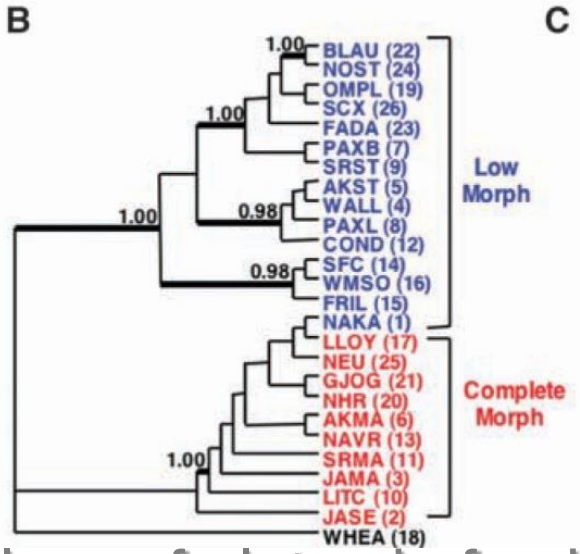
low plate morph

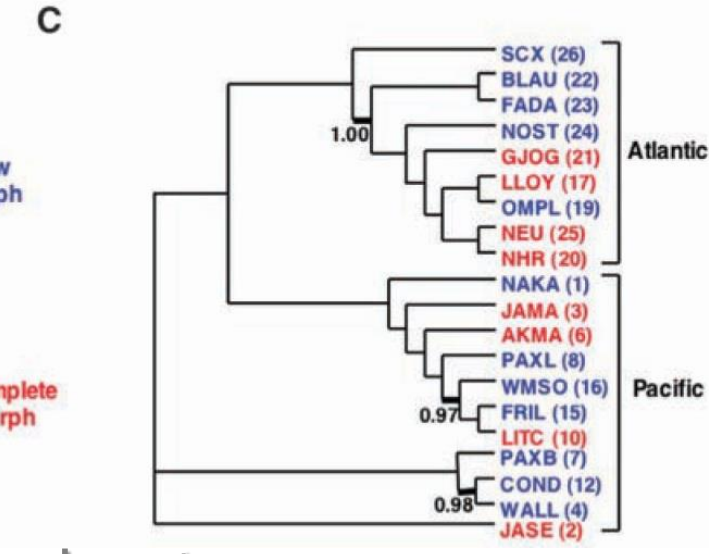
- loss of plates in freshwater
- selective advantage
- relatively simple genetic basis
  - ectodysplasin-a: *Eda*

$$V_P = V_A + V_D + V_E + V_{G\times E} + V_{res}$$

Colosimo *et al*. (2005)
Science 307:1928







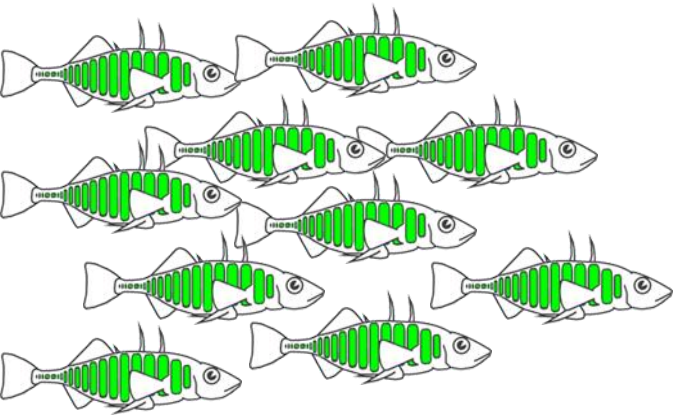
low plate morph

- loss of plates in freshwater
- selective advantage
- relatively simple genetic basis
- parallel evolution via shared *Eda* haplotypes
  - ➡ selection on standing genetic variation

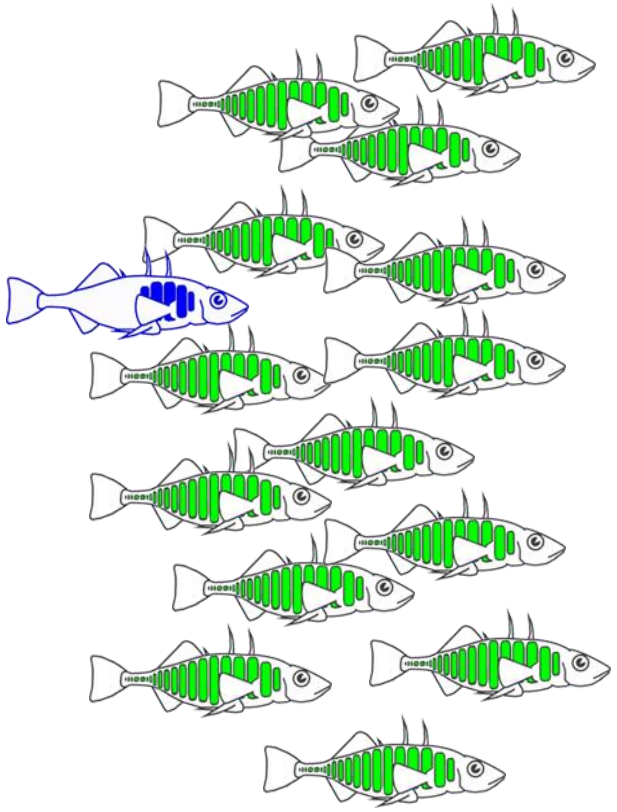
Why Sticklebacks?

Adaptation via *de novo* Mutation

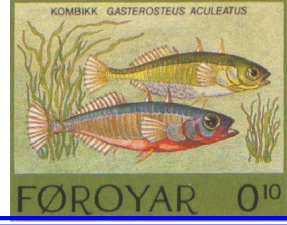
Colonizing Population



N generations in new habitat

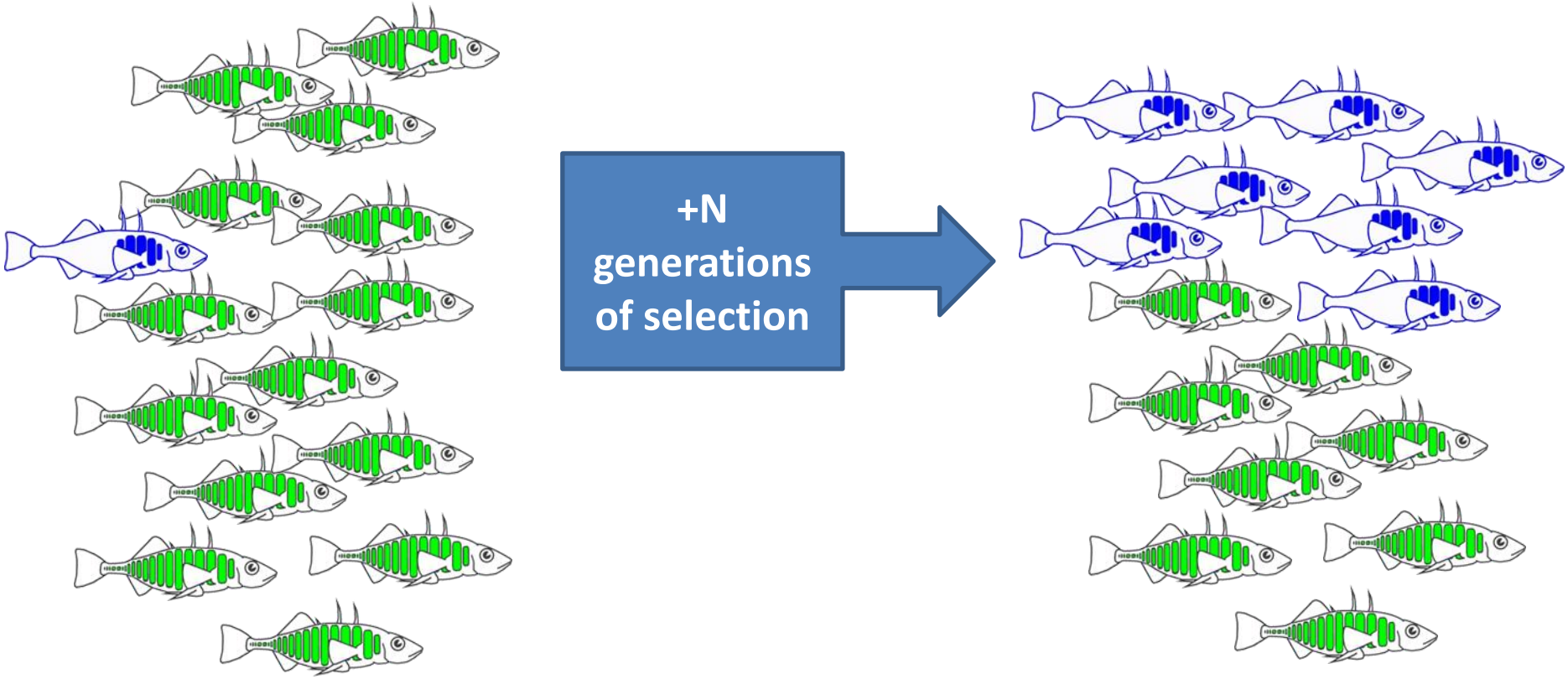


Why Sticklebacks?

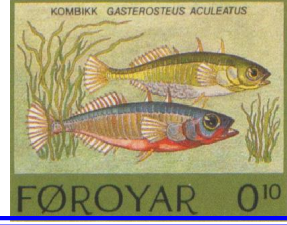


Adaptation via *de novo* Mutation

- if mutation is not lost to drift

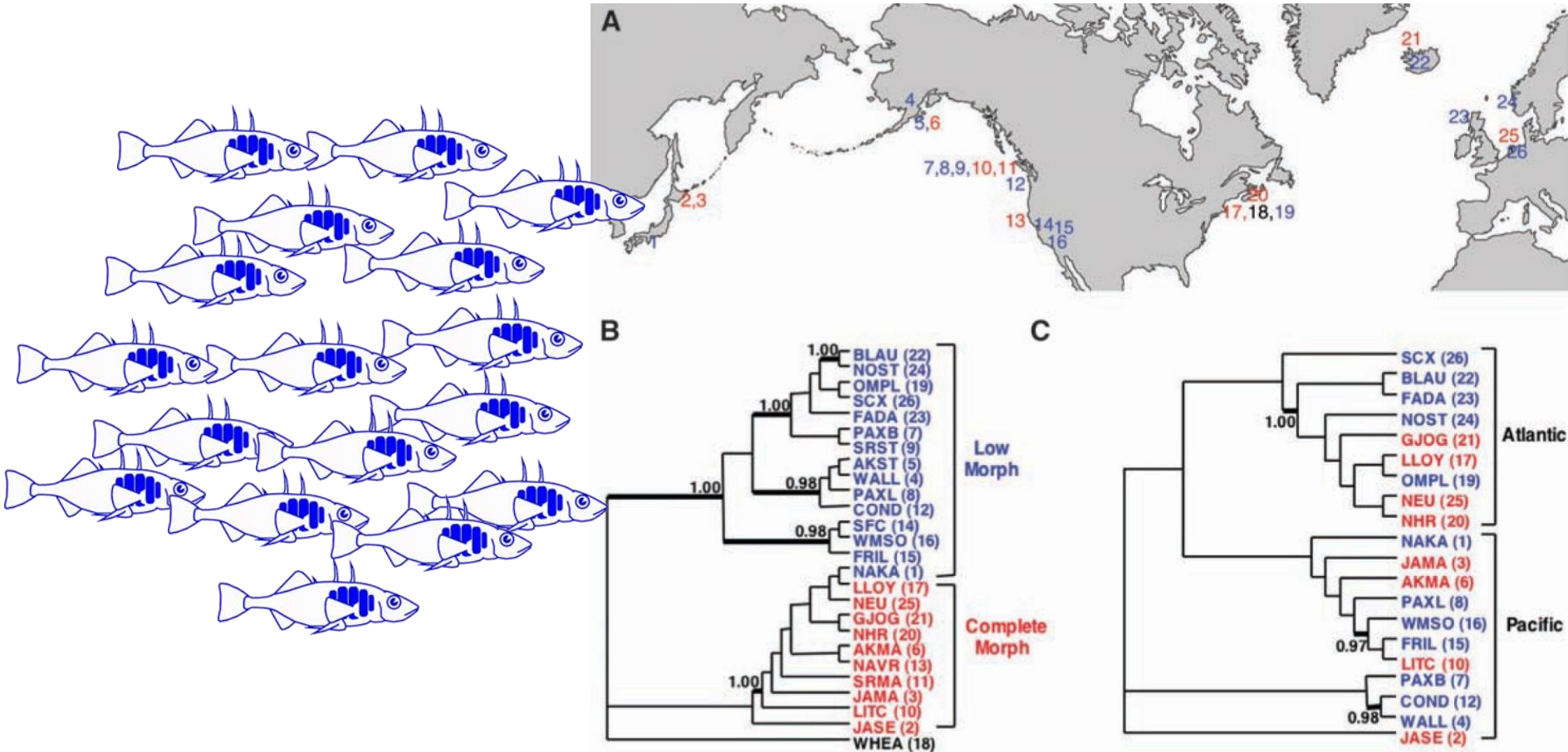


Why Sticklebacks?



Adaptation via *de novo* Mutation

- fixation may be rapid if selection is sufficiently strong
- probability of independent & parallel evolution?

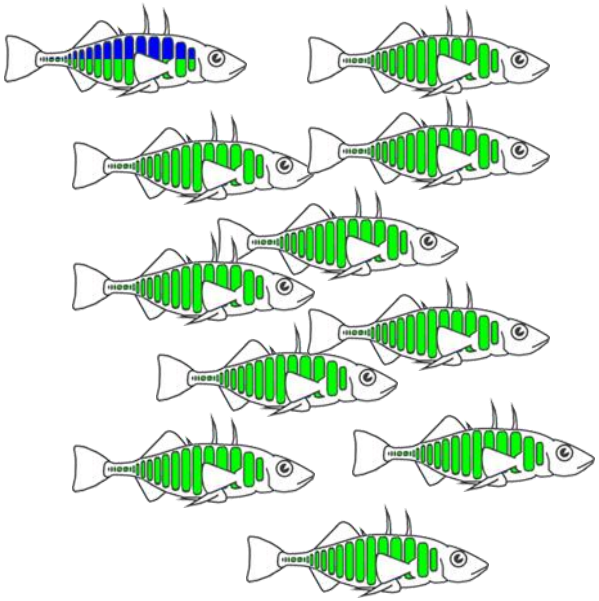


Why Sticklebacks?

Adaptation from Standing Genetic Variation

- full armour plating is dominant
- recessive *Eda* allele occurs at ca. 5% in marine populations

Ancestral Population

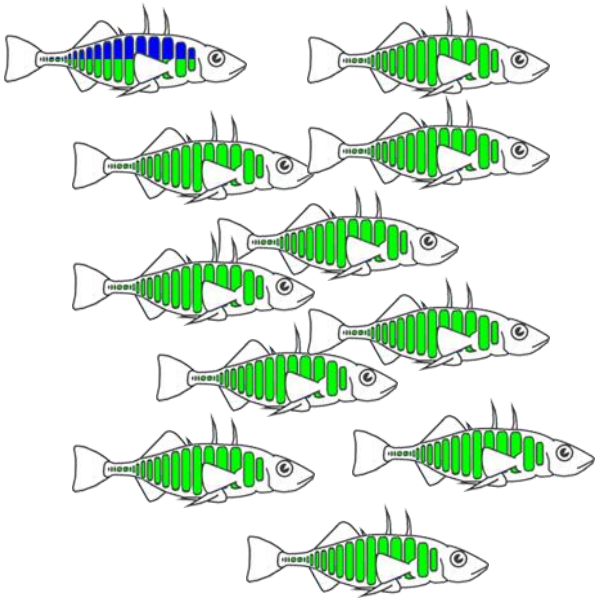


Why Sticklebacks?

Adaptation from Standing Genetic Variation

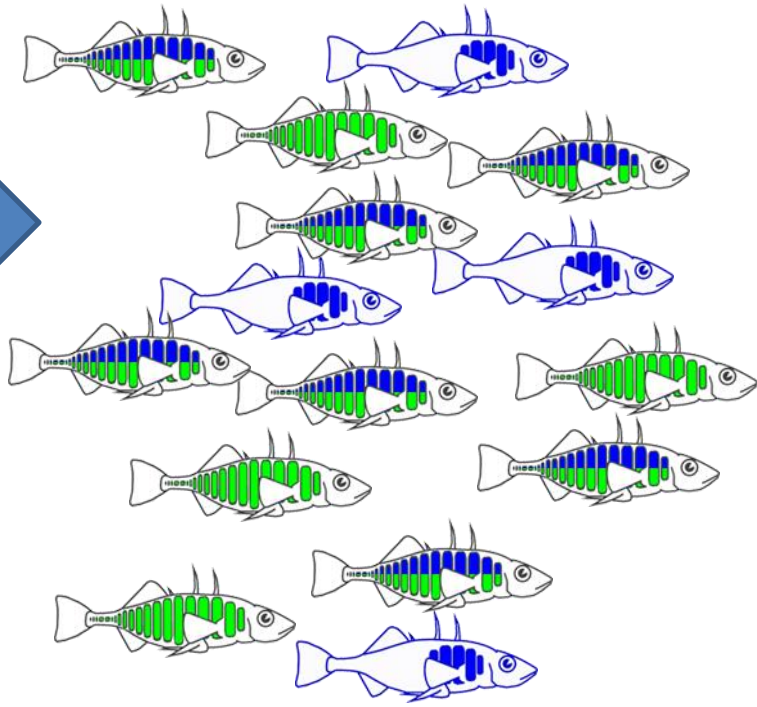
- genotype and phenotype frequency expected to change if selection is relaxed in new environment

Ancestral Population



N generations of relaxed selection

Novel Environment

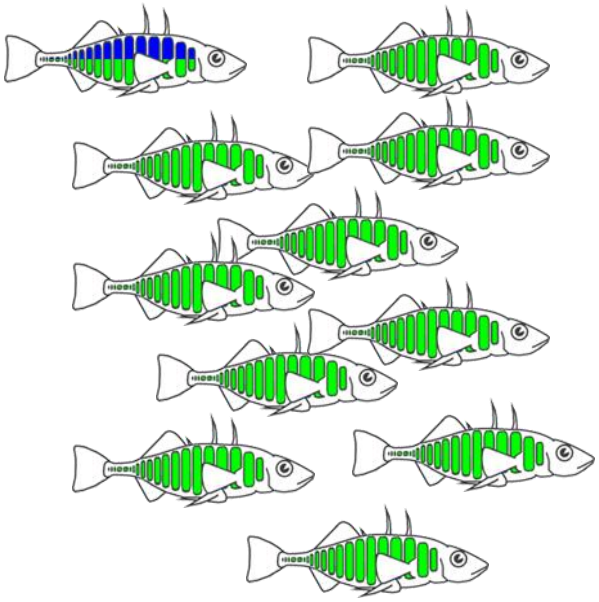


Why Sticklebacks?

Adaptation from Standing Genetic Variation

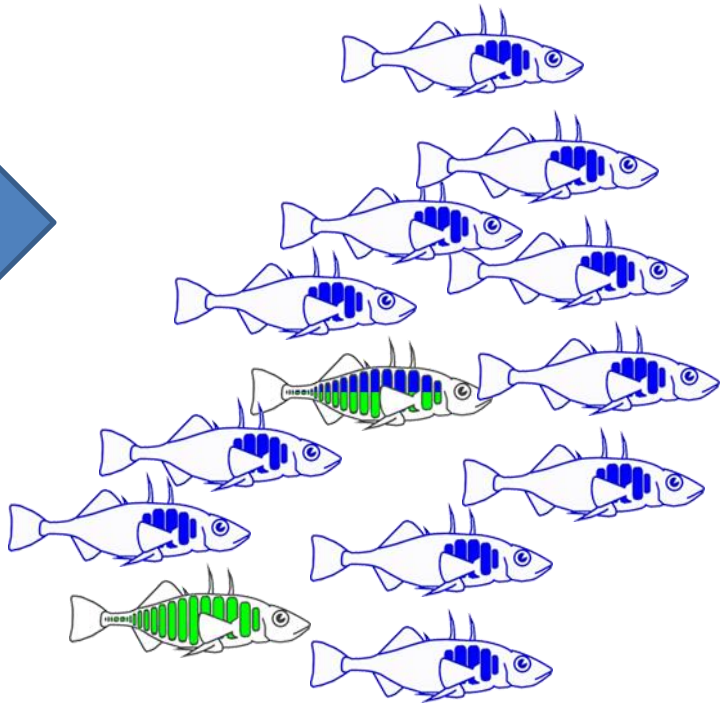
- if selection favours the recessive allele/trait

Ancestral Population

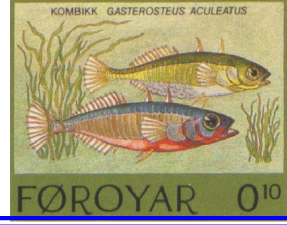


N generations of selection

Novel Environment

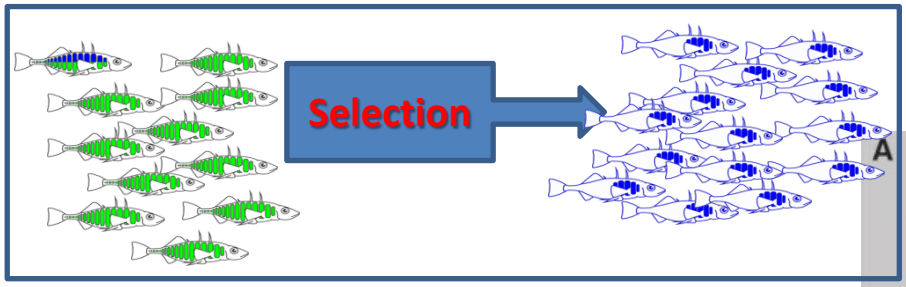


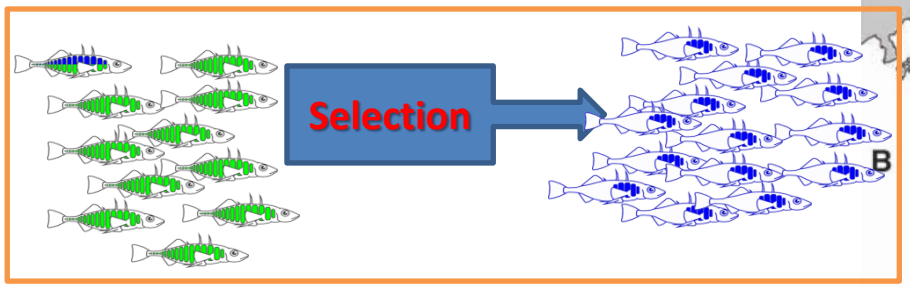
Why Sticklebacks?

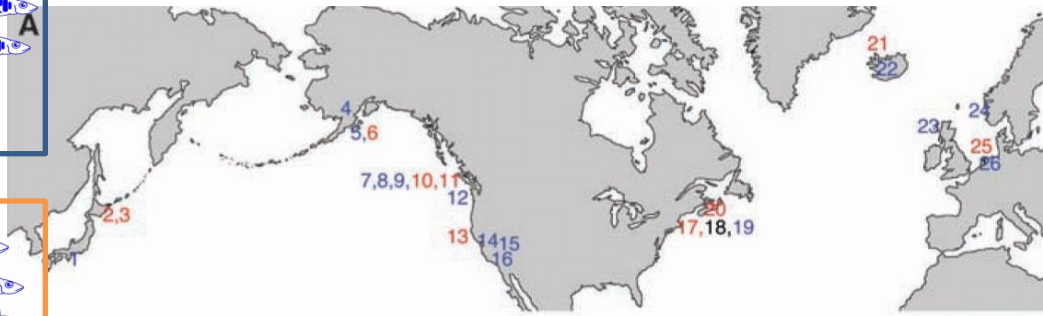


Adaptation from Standing Genetic Variation

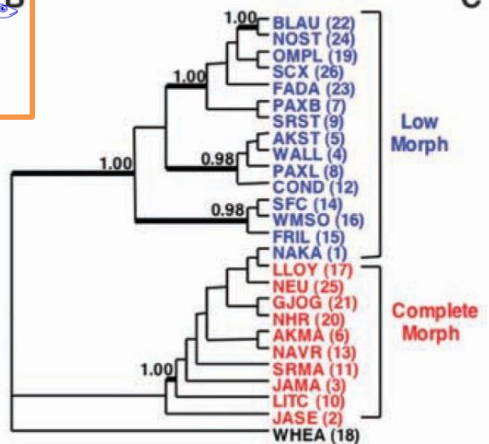
- replicate environments/colonizations more likely than replicate mutation?

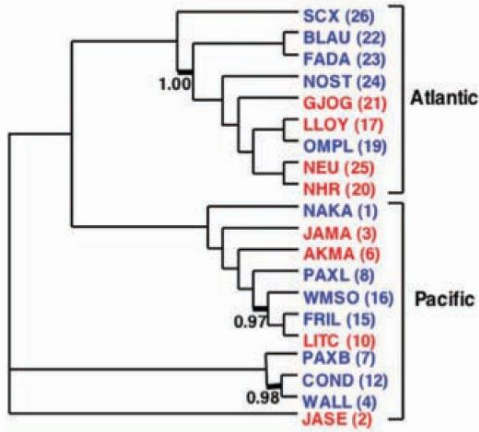






C

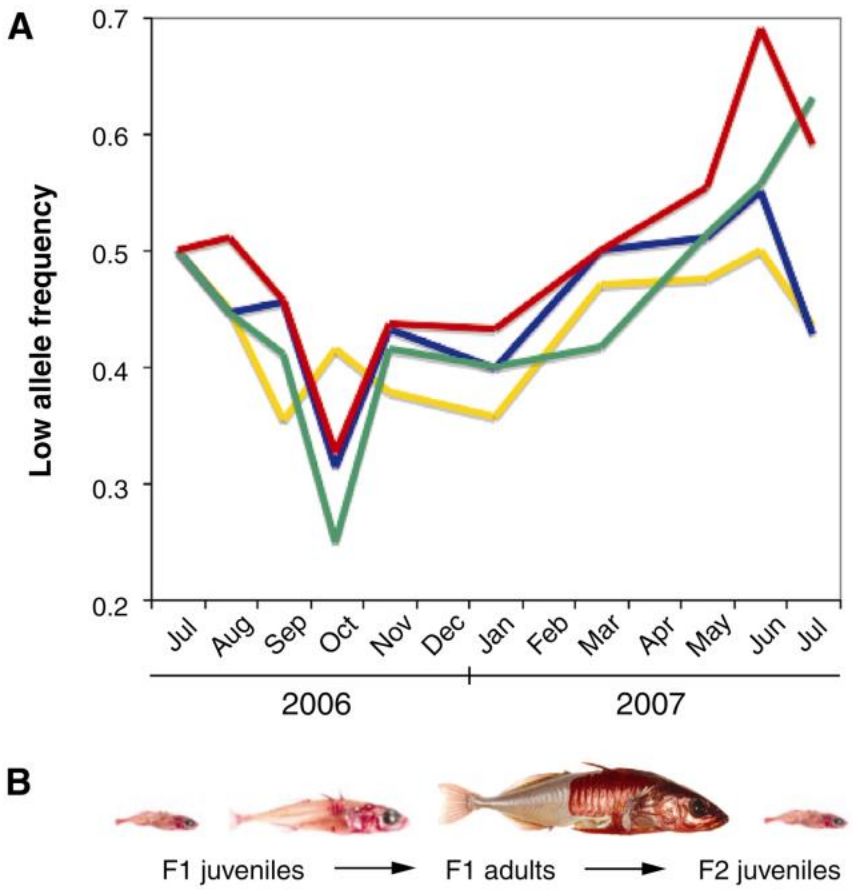




Why Sticklebacks?

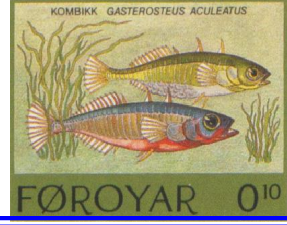
Adaptation from Standing Genetic Variation

- recessive allele frequency increased after 2 generations



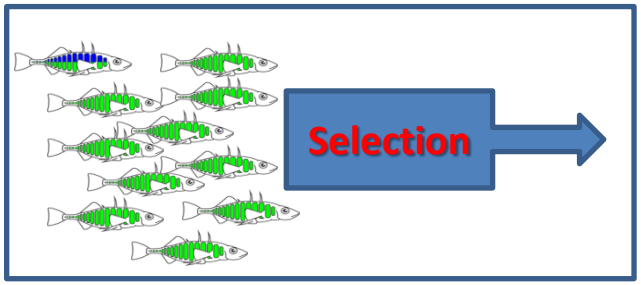
Barrett *et al*. (2008)
Science 322:255

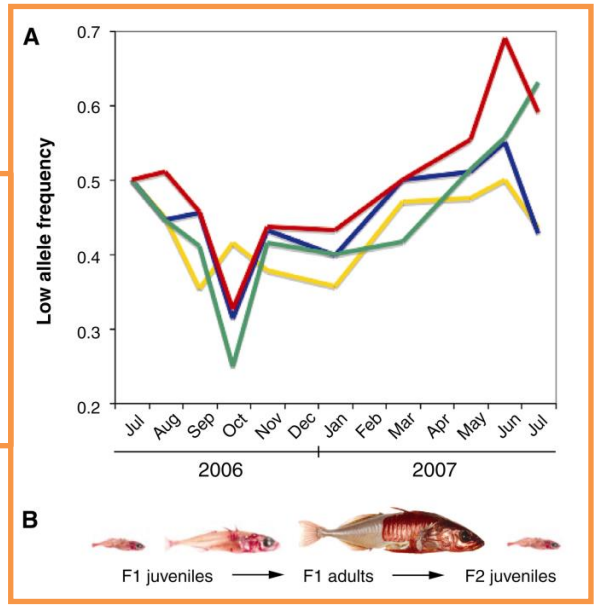
Why Sticklebacks?



Contingency

- the unspoken artefact

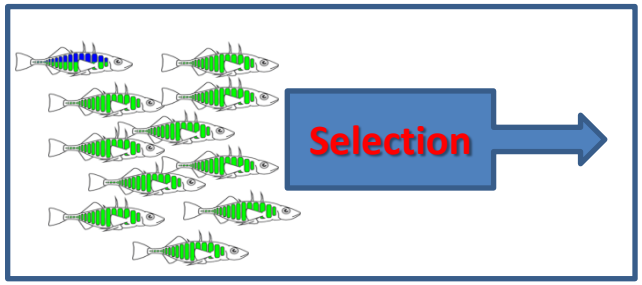


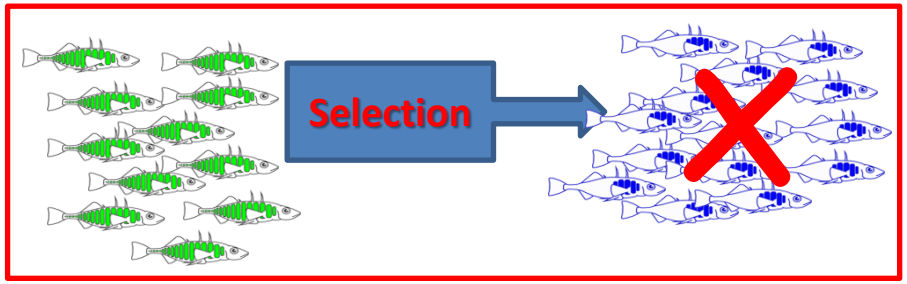


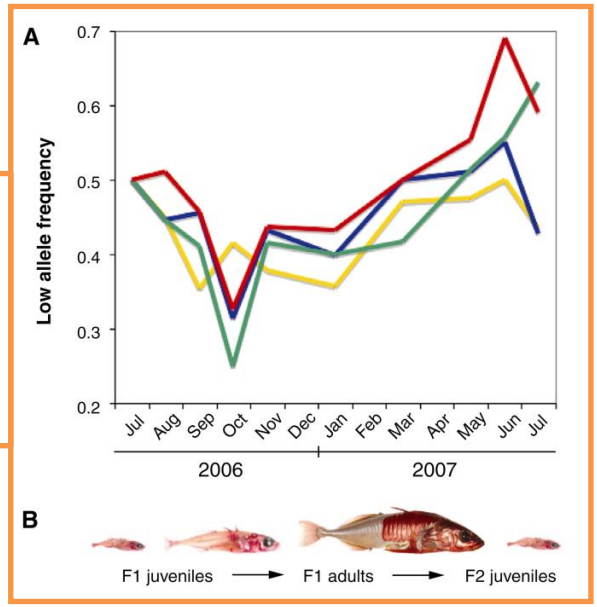
Why Sticklebacks?

Contingency

- the unspoken artefact
- informative of the limits of this model







Muddying the Waters…

- transcription as a complex phenotype
  - adaptive potential within the transcriptome
    - quantitative genetics
    - signatures of selection
- contingency & adaptation from standing genetic variation
  - what of populations lacking “pre-adaptive” variants?

Why Transcription?

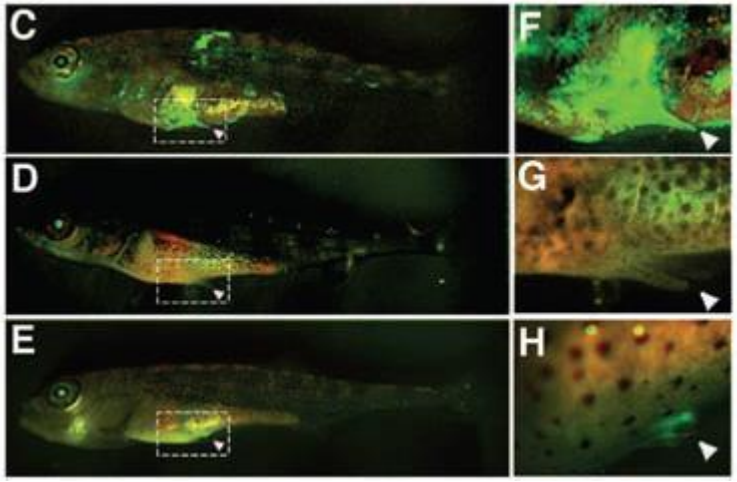
"*We suggest that evolutionary changes in anatomy and way of life are more often based on changes in the mechanisms controlling the expression of genes than on sequence changes in proteins.*"

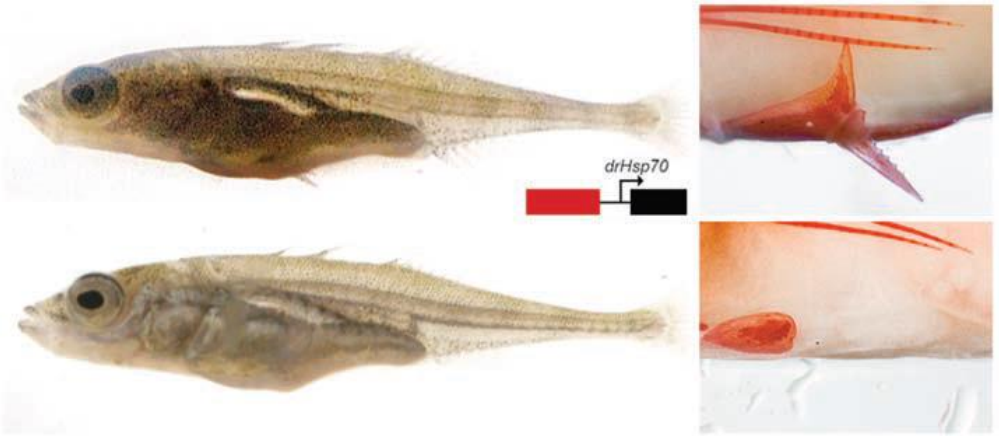
King & Wilson (1975)
Science 188:107

Why Transcription?

Gene Expression & Phenotypic Variation

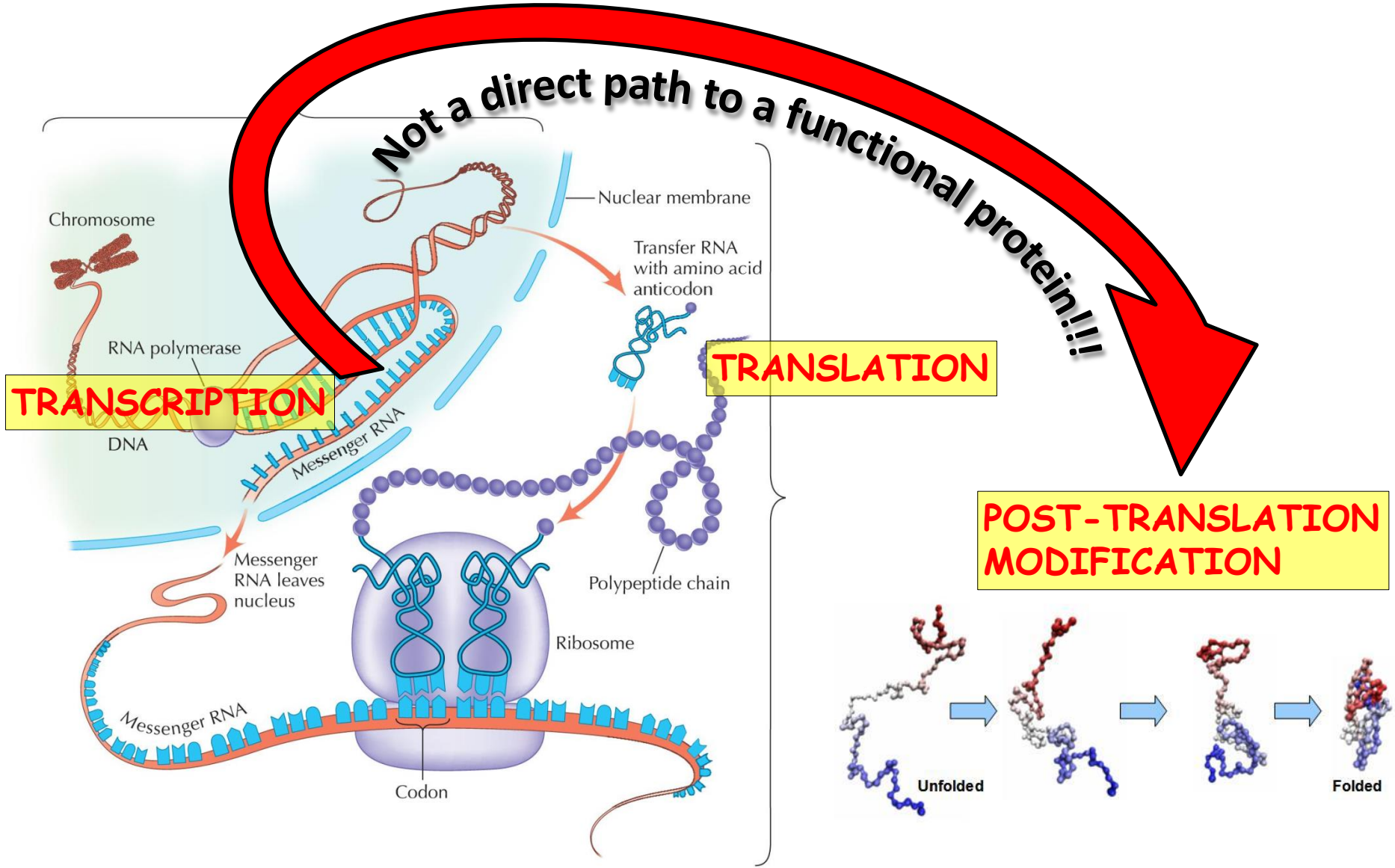
- a 'gold standard' example from sticklebacks
  - pelvic reduction associated with differential expression of *Pitx1* gene
  - evidence from:
    - sequence alignments
    - FISH
    - gene rescue





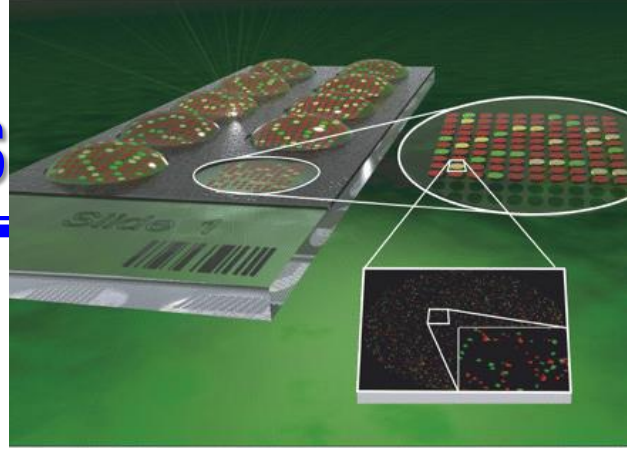
Chan *et al*. (2010)
Science 327:302

Does Transcription Reflect Expression?



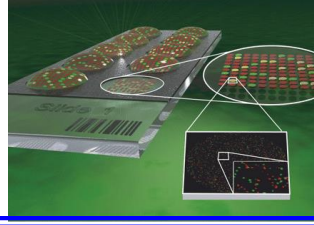
Comparative Transcriptomics

- 4×44k custom oligonucleotide microarray
  - 19,274 genes
    - ≈93% of genes in stickleback genome
    - 27,723 transcripts

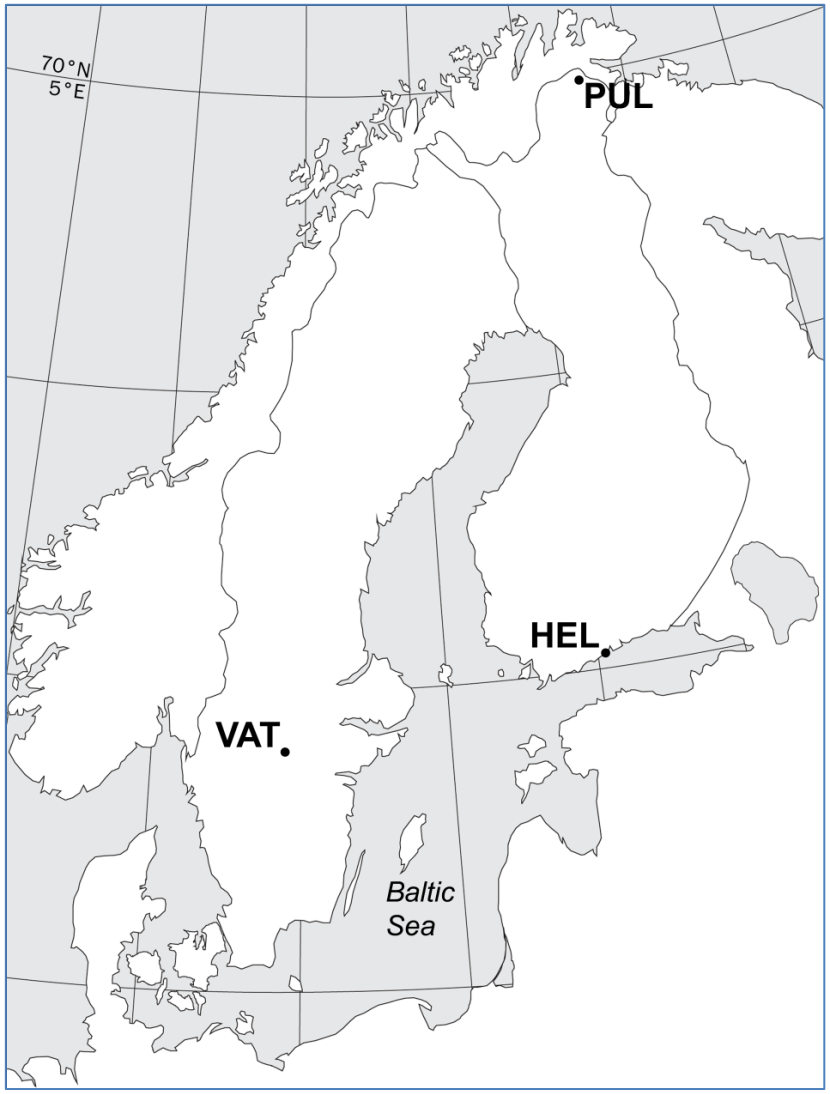


Leder *et al*. (2009)
BMC Genomics10:426

Comparative Transcriptomics

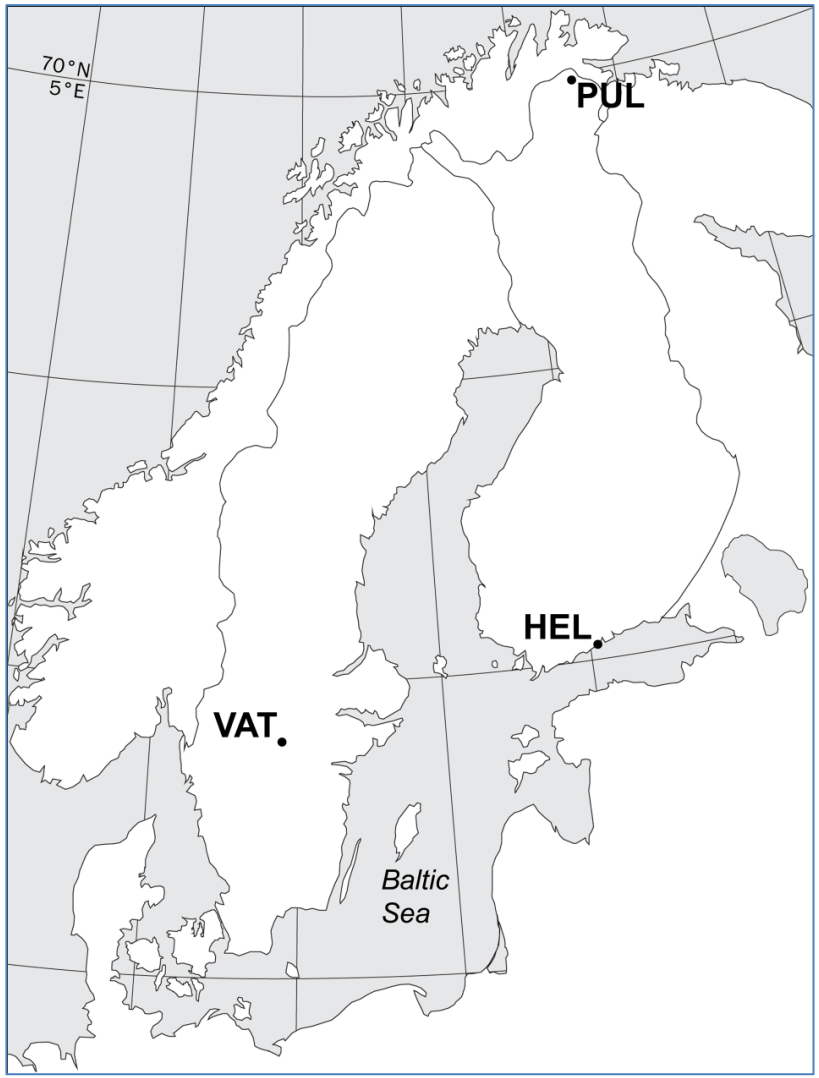


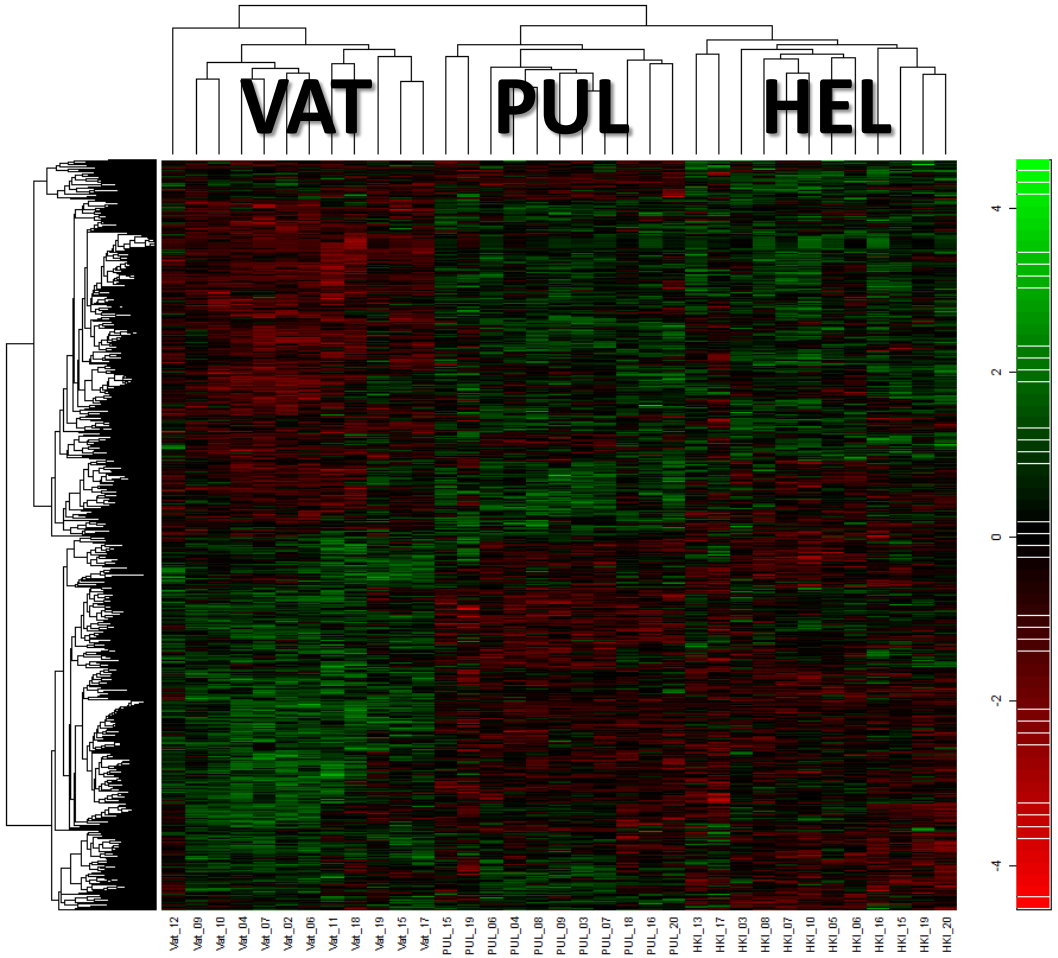
- 4×44k custom microarray
- lab-reared fish (F_2)
  - 1 ‘marine’ population
    - ancestral form
  - 2 derived freshwater populations
- thermal treatment
  - 17°C (control)
  - 23°C (over 6 hours)
- mRNA from liver tissue



Nikinmaa *et al*. (2013)
Proc. R. Soc. B 280:20122974

Differential Transcription





Differential Transcription

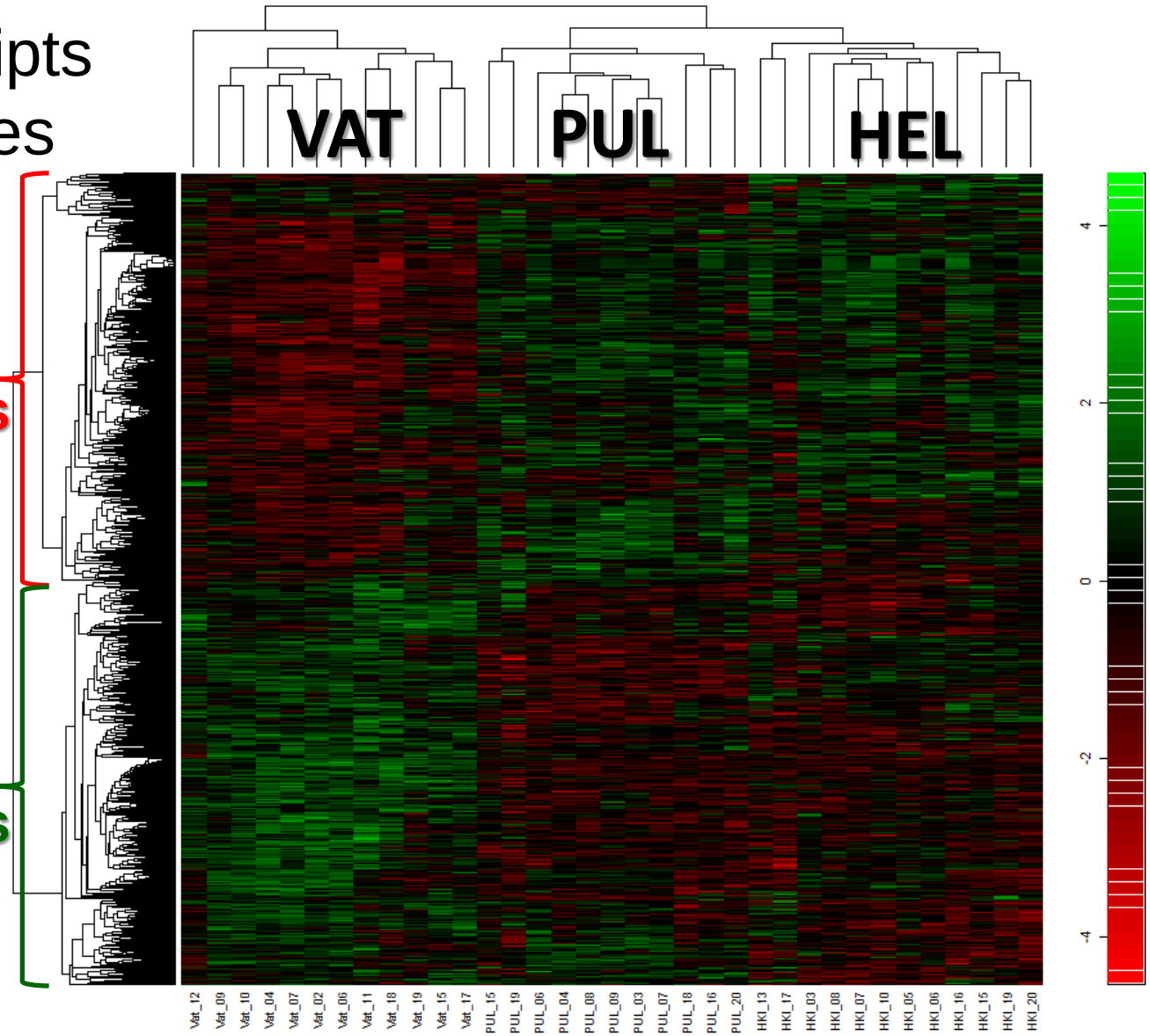
- 1,834 transcripts
  - ➡ 1,698 genes

down-regulated in VAT

- **924 transcripts**
  - ➡ **851 genes**

up-regulated in VAT

- **916 transcripts**
  - ➡ **857 genes**

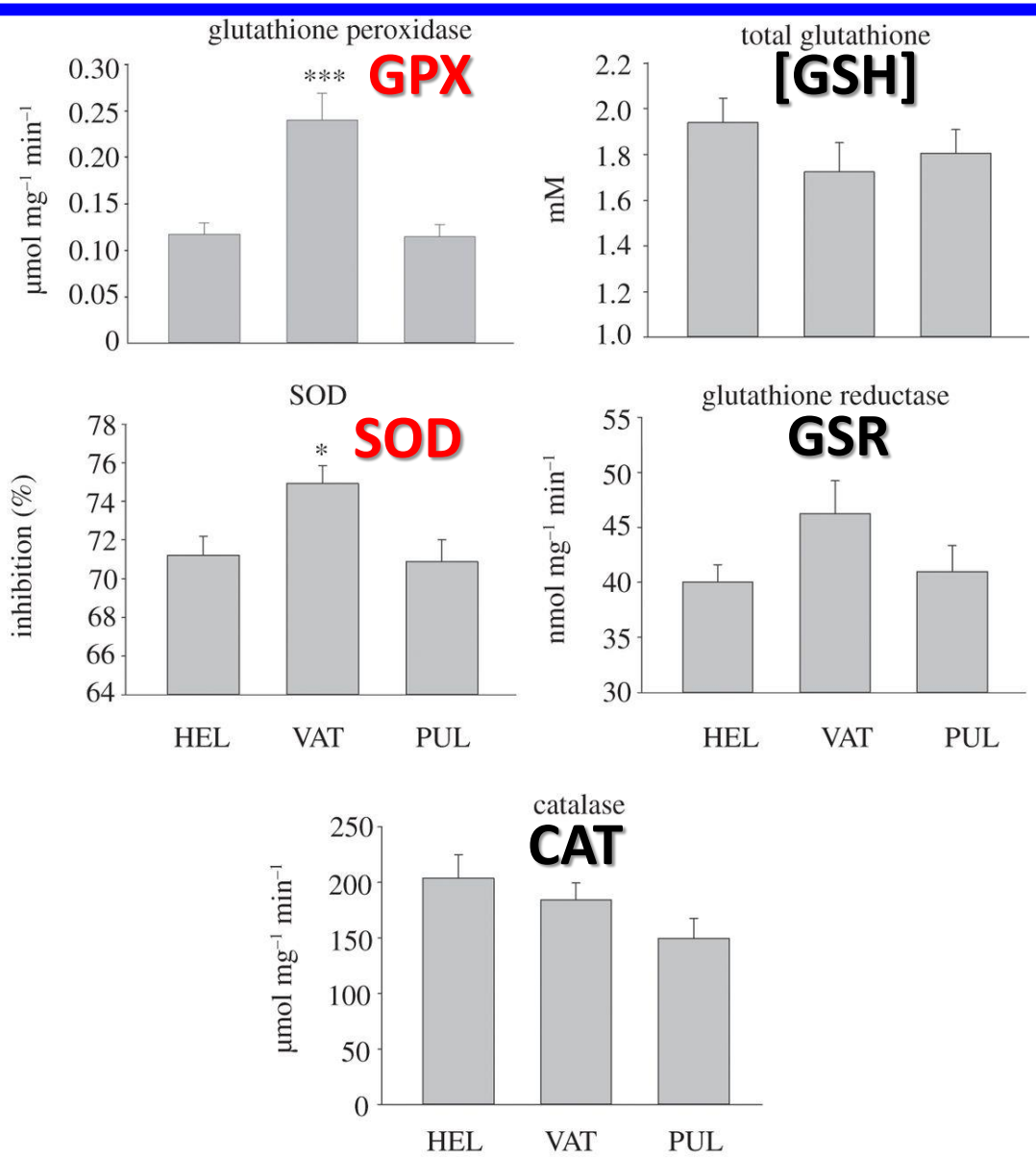


Functional Annotation

Functional Cluster	Enrich. Score	No. BP	No. Genes	Fold Enrich.
regulation of protein localization, transport & secretion	2.34	14	63	2.50 (1.50 - 4.20)
detection of external stimuli	1.66	4	13	2.95 (2.05 - 4.28)
response to steroidal stimuli	1.36	4	12	2.83 (2.02 - 3.60)
regulation of cellular growth	1.11	10	24	1.76 (1.19 - 2.40)
regulation of GTPase activity	1.11	12	40	2.23 (1.10 - 3.90)
regulation of cell adhesion	1.08	3	12	2.17 (1.83 - 2.55)
glucose & carbohydrate homeostasis	1.08	3	9	2.37 (2.20 - 2.65)
regulation of ion transport	1.08	6	12	2.40 (1.83 - 3.70)
nuclear organization	1.03	6	11	2.18 (1.55 - 2.68)
regulation of protein signaling	0.99	3	9	2.70 (2.70 - 2.70)
SMAD protein localization	0.91	3	11	2.50 (1.61 - 3.95)
glucose & carbohydrate metabolism	0.87	27	26	2.37 (1.63 - 3.87)
membrane protein proteolysis	0.87	4	8	3.23 (1.44 - 5.82)
regulation of immune response	0.86	13	28	2.31 (1.04 - 4.80)
response to intra-cellular pathogens	0.85	7	10	2.49 (1.33 - 3.80)
mitochondrial organization	0.83	3	7	3.70 (2.00 - 4.70)
regulation of intra-cellular protein transport	0.81	8	19	1.68 (1.24 - 2.03)
water homeostasis	0.81	6	10	3.22 (1.65 - 4.70)
response to oxidative stress	0.75	9	25	1.92 (1.22 - 2.62)
regulation of lipid metabolism	0.74	14	17	2.33 (1.23 - 3.80)
regulation of macromolecular secretion	0.64	7	7	2.29 (1.76 - 2.70)
exocytosis	0.63	6	26	1.53 (1.13 - 2.03)
glutathione, peptide & sulfur metabolism	0.60	3	17	1.57 (1.24 - 1.87)
regulation of cellular development	0.59	7	25	1.90 (1.22 - 2.70)
transport of organic acids	0.55	5	16	1.60 (1.14 - 2.52)
regulation of muscle development	0.53	10	10	1.86 (1.19 - 3.33)
DNA catabolism	0.51	7	14	1.71 (1.36 - 1.97)

Enzymatics

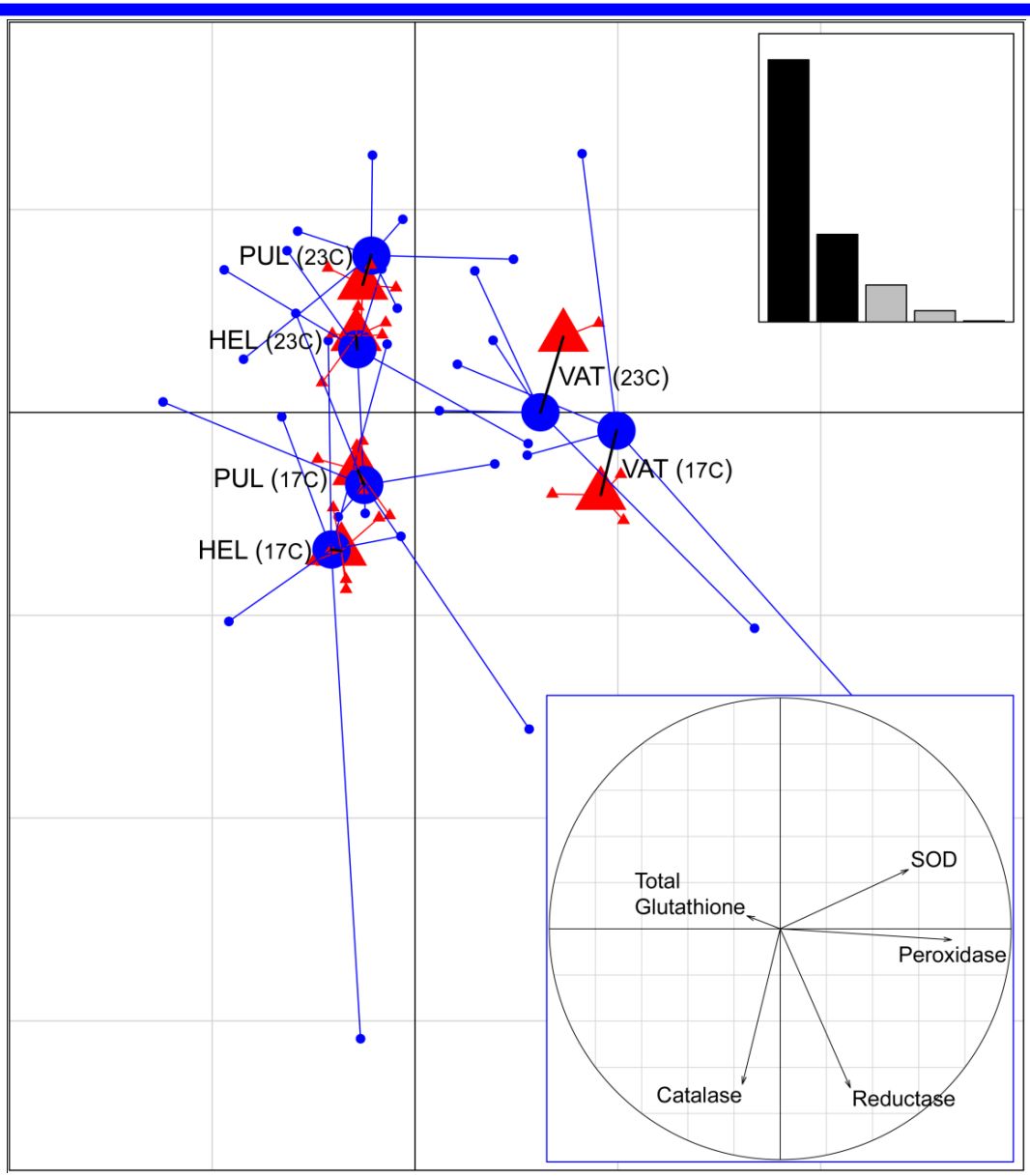
- enzymes & substrate in cellular redox reactions
  - ➡ response to oxidative stress
- substrate (GSH) concentration & enzyme activity data
- same population-specific trends observed



Nikinmaa *et al*. (2013)
Proc. R. Soc. B 280:20122974

Multivariate Similarity

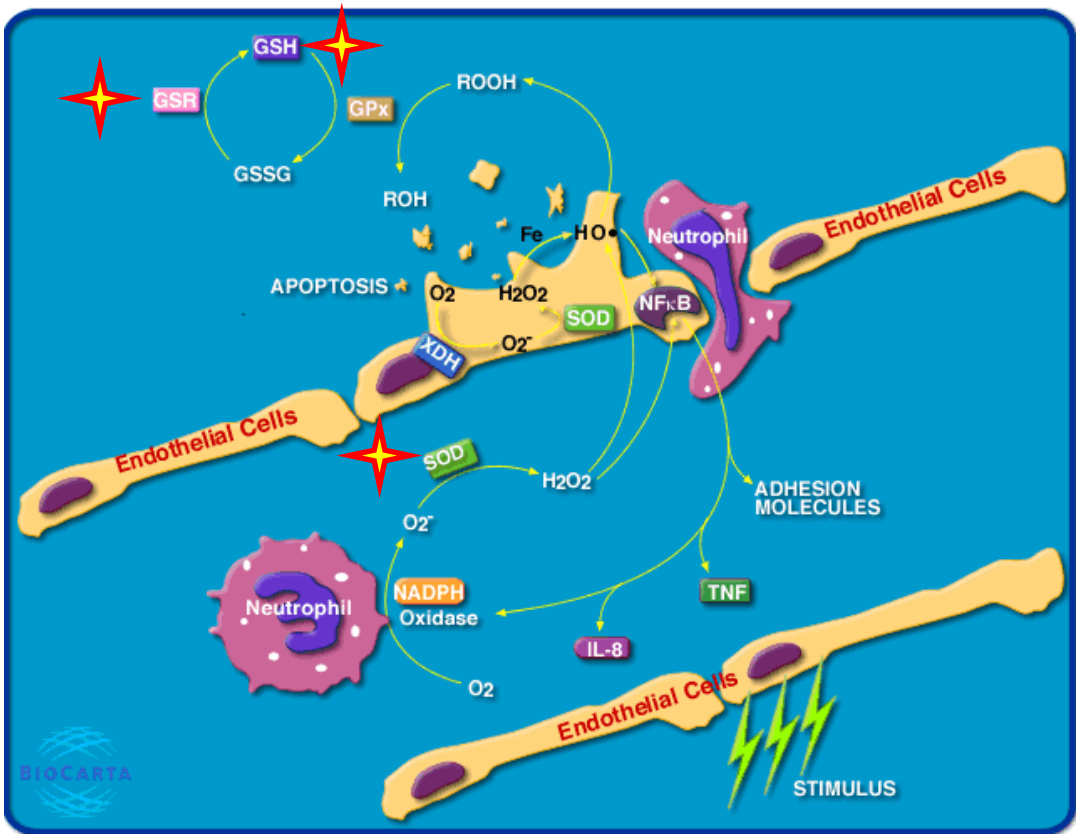
- co-inertia analysis (**CoIA**)
  - ordination of transcription and enzymatic data
- 35.7% 'co-variation' between datasets (p=0.002)
  - axis 1: 66%
  - axis 2: 22%

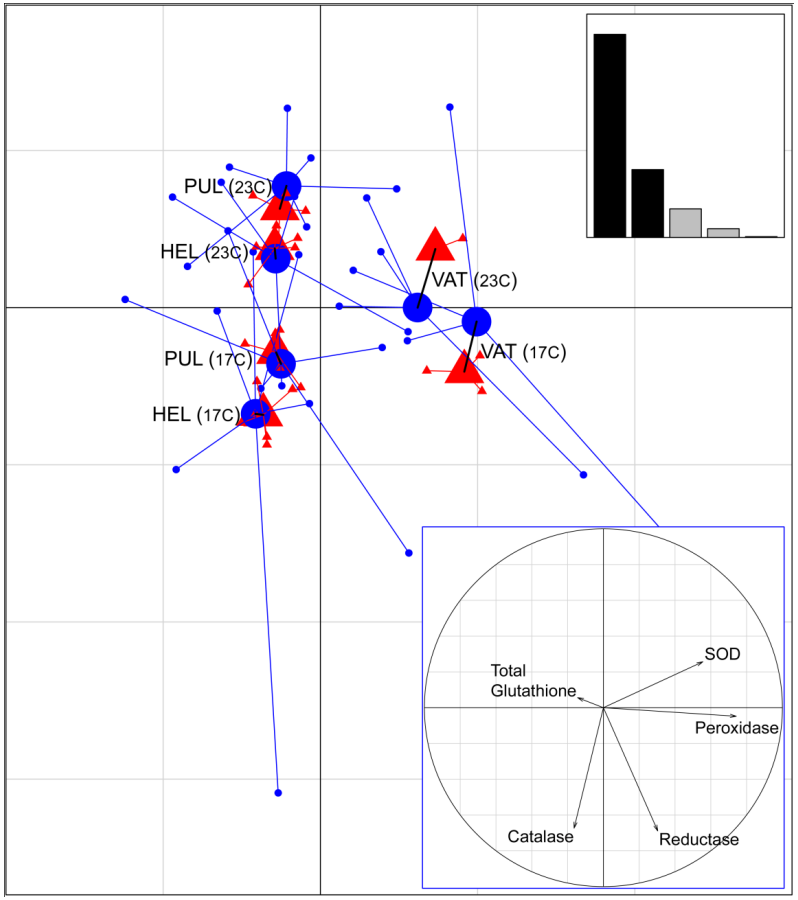


Nikinmaa *et al*. (2013)
Proc. R. Soc. B 280:20122974

Annotation of Col Axis 1 Probes

- response to oxidative stress
- 6.6 fold enrichment for genes associated with 'free radical induced apoptosis'
  - GSR, GPX1 & SOD1

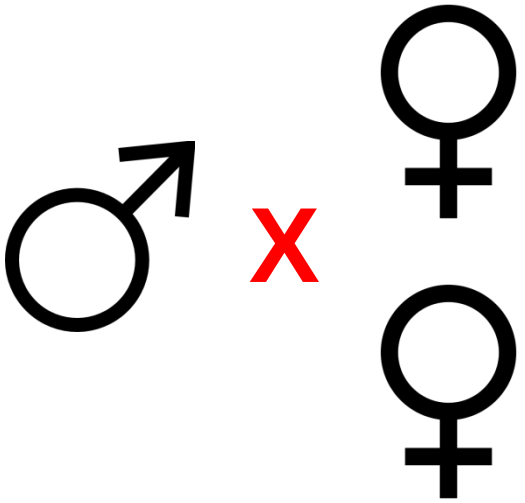




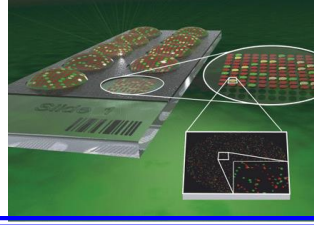
Heritability of Transcription

Breeding Design

- broodstock sampled from Baltic Sea
- 60 dams & 30 sires
  - 2 half-sib families per sire
- F_1 offspring
  - 60 families in total
    - 8-10 offspring per dam
    - 574 offspring total
- 80% chance of detecting $h^2 \geq 0.06$
  - power & FDR estimated by simulation



Heritability of Transcription



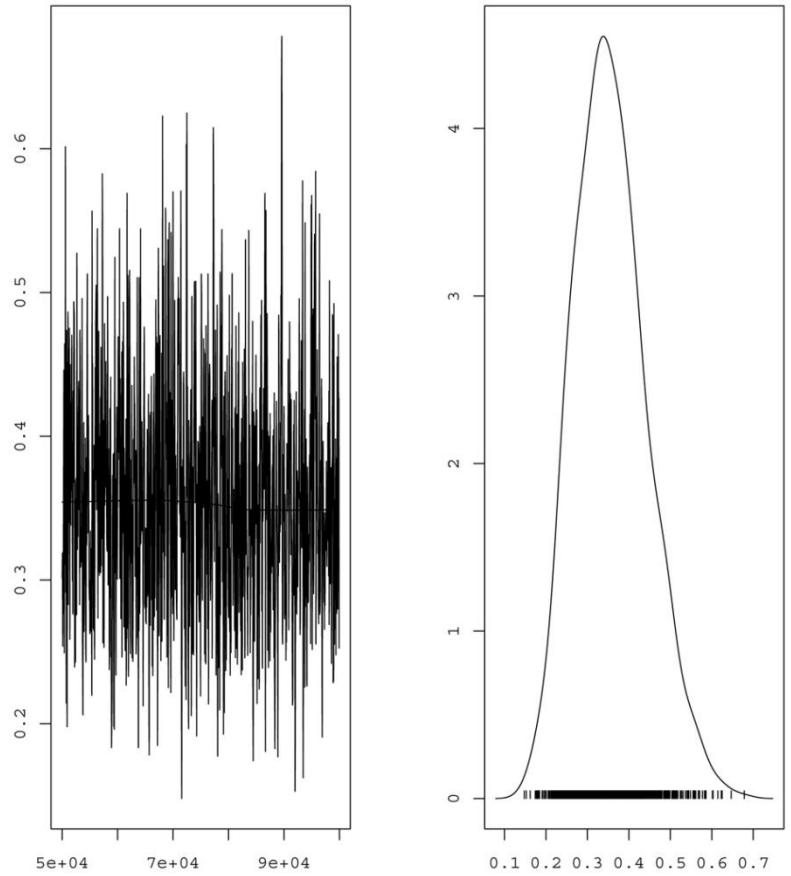
Transcriptional Profiling

- 8×15k custom microarray
  - 10,899 transcript-specific probes designed from *Gasterosteus* genome
    - 9,420 of 15,198 predicted genes
- adult fish (20 months)
  - sexually ‘immature’
- thermal treatment
  - each family divided in ½
    - 17°C (control)
    - 23°C (over 6 hours)
- total RNA extracted from liver

Heritability of Transcription

Bayesian Estimation of Variance Components

- ‘animal model’
  - removal of effects
    - dye
    - sex
    - temperature
- 100,000 iterations
  - 50,000 burn-in
  - 1,000 MCMC samples
    - h^2 = posterior mode
    - 95% PDI



MCMCglmm

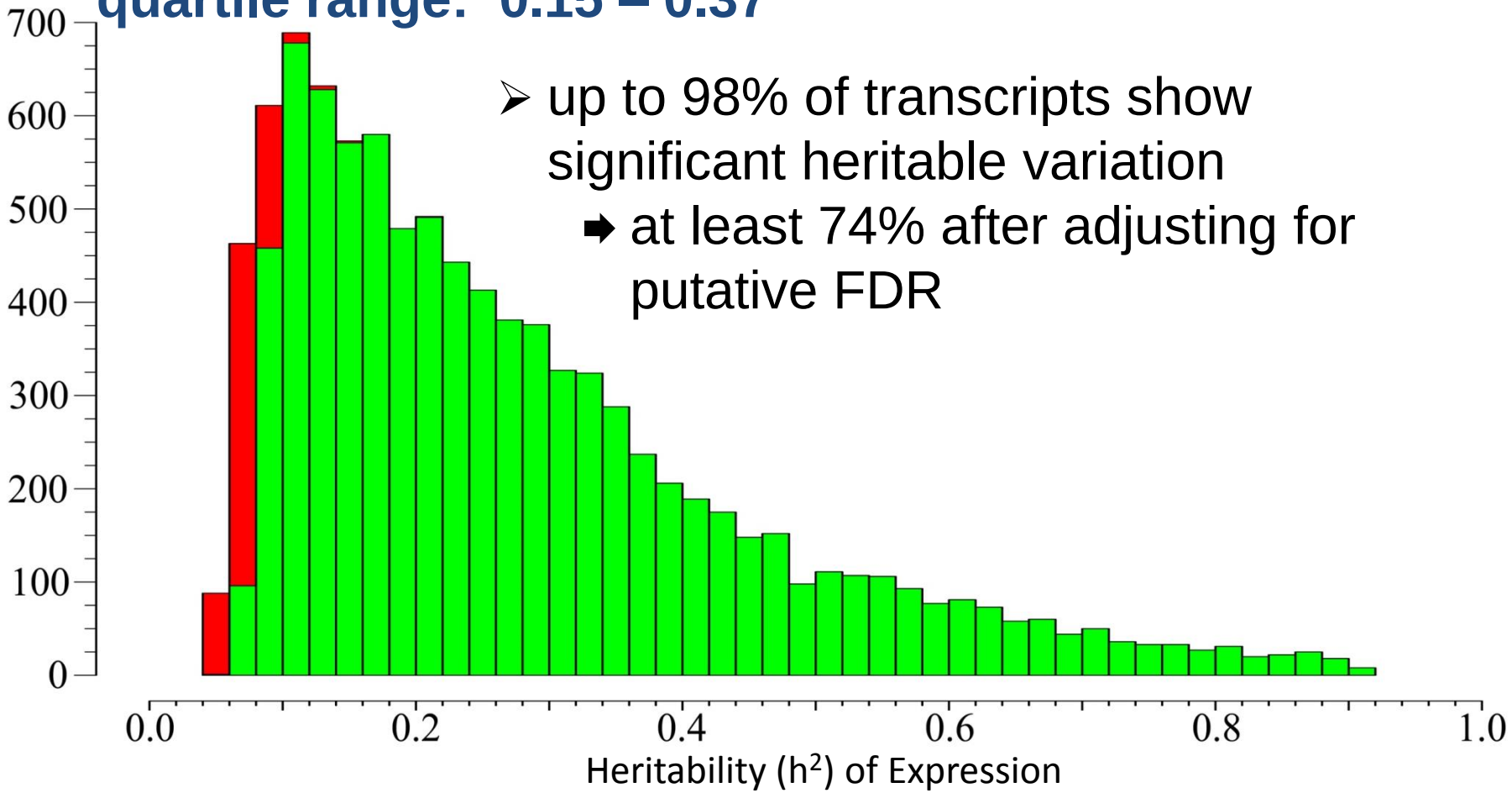
Hadfield (2010)
J. Stat. Software 33:1

Heritability of Transcription

Distribution of h^2 Estimates

median h^2 = 0.24

quartile range: 0.15 – 0.37

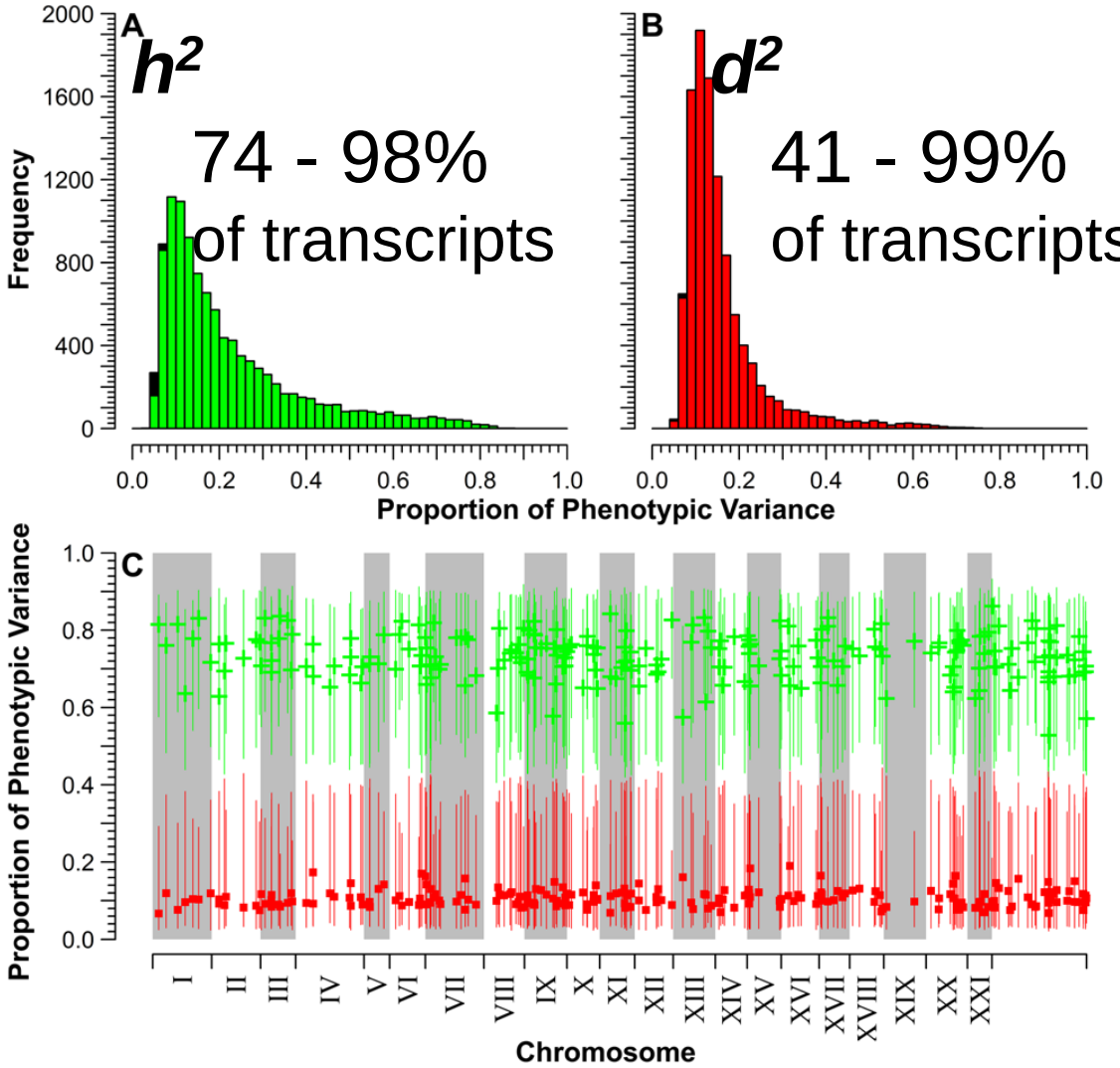


- up to 98% of transcripts show significant heritable variation
  - ➡ at least 74% after adjusting for putative FDR

Heritability of Transcription

Additive Genetic Variance Exceeds

- V_D

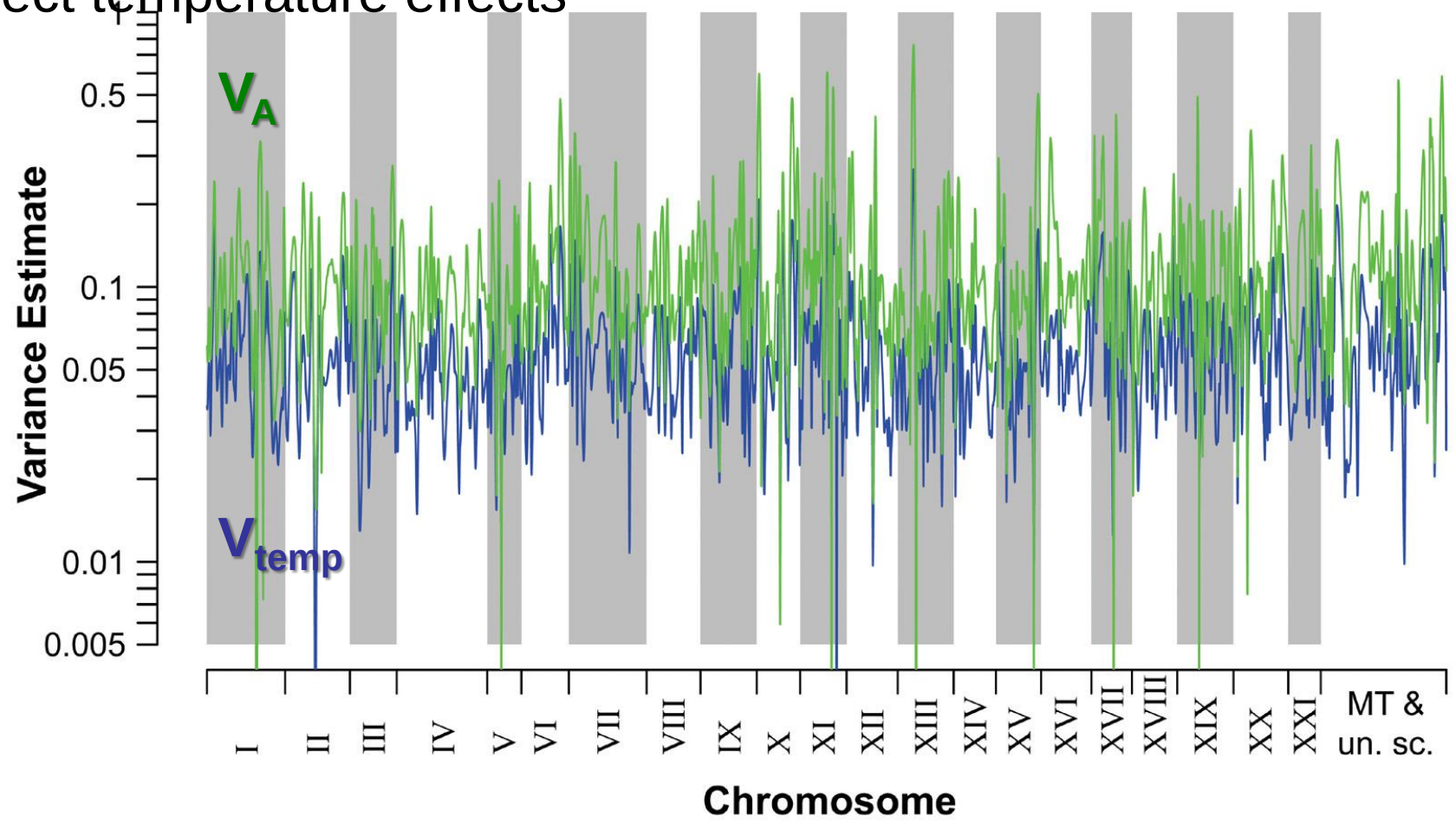


Leder, McCairns *et al*. (2015)
Mol. Biol. Evol. 32:674

Heritability of Transcription

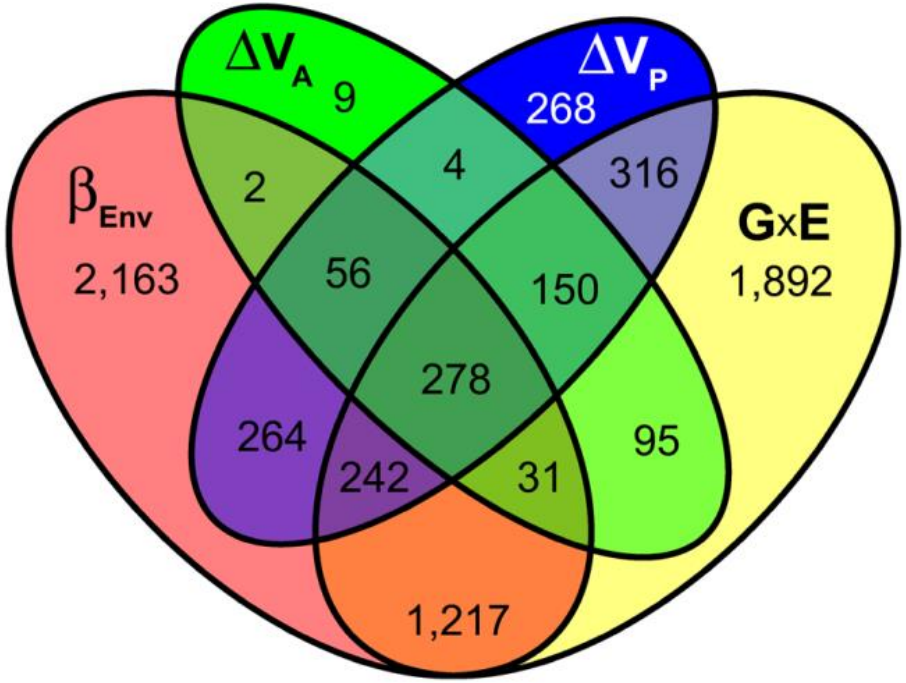
Additive Genetic Variance Exceeds

- V_D
- direct temperature effects



Response to Environmental Stress

Quantifying Environmental Effects



Leder, McCairns *et al*. (2015)
Mol. Biol. Evol. 32:674

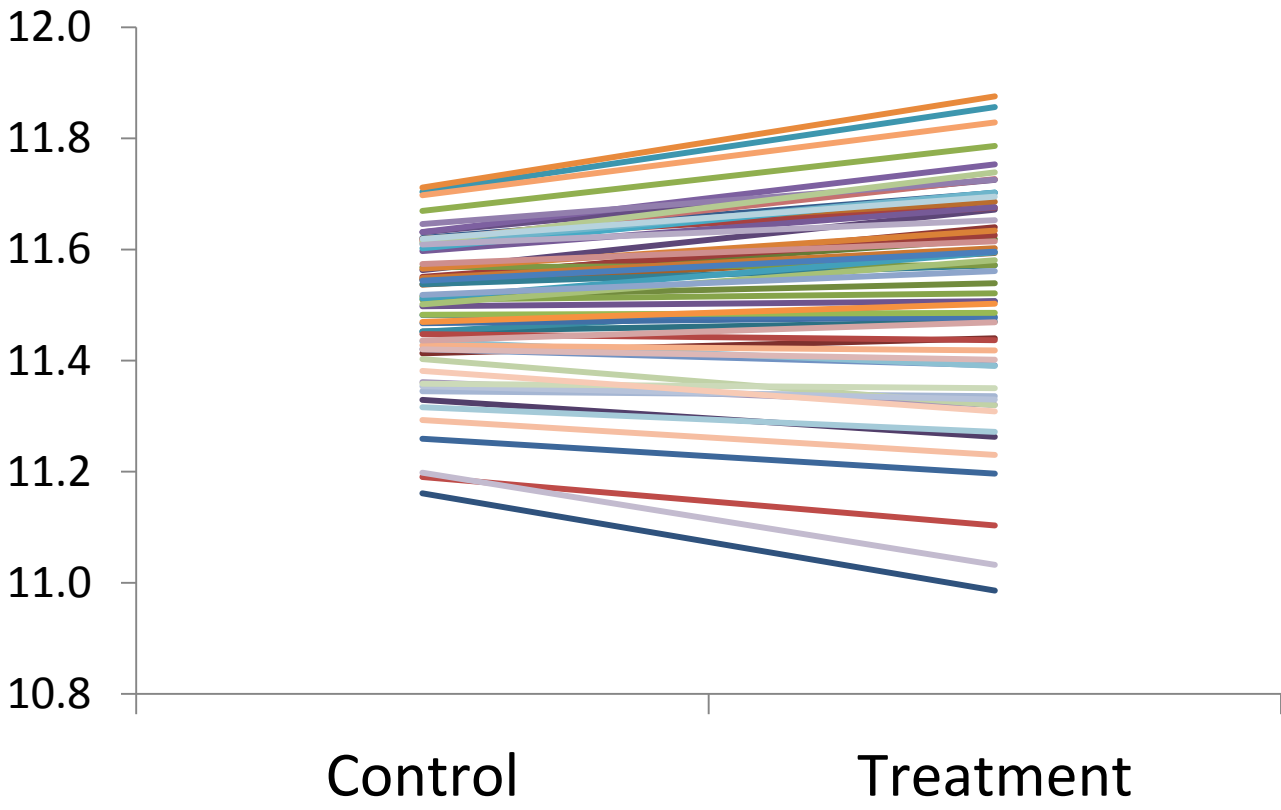
Response to Environmental Stress

Environmental Effects Mediated via G×E

- 41% of transcripts exhibit significant variation among families in treatment effect (random slopes)

***e.g.* PRKDC**

- protein kinase
- involved in cell cycle, apoptosis, telomere maintenance



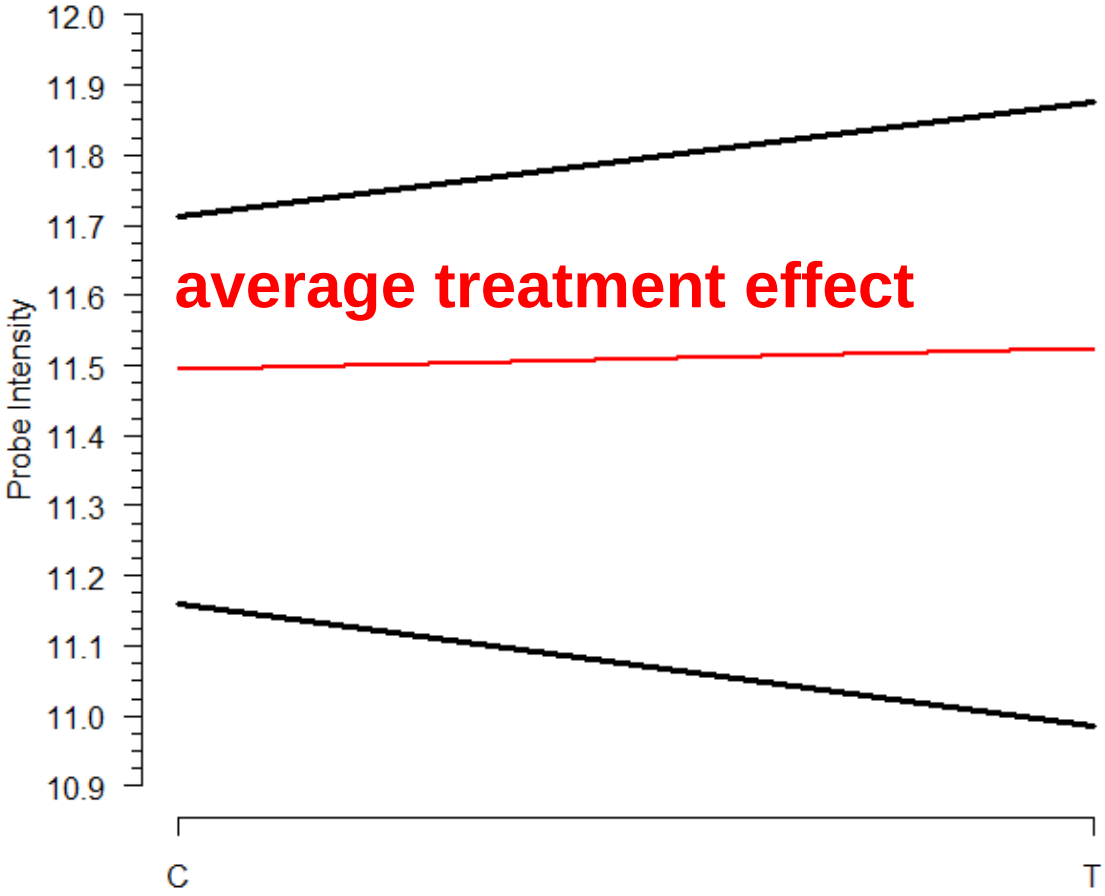
Response to Environmental Stress

Environmental Effects Mediated via G×E

- G×E may mask our ability to detect a thermal response

***e.g.* PRKDC**

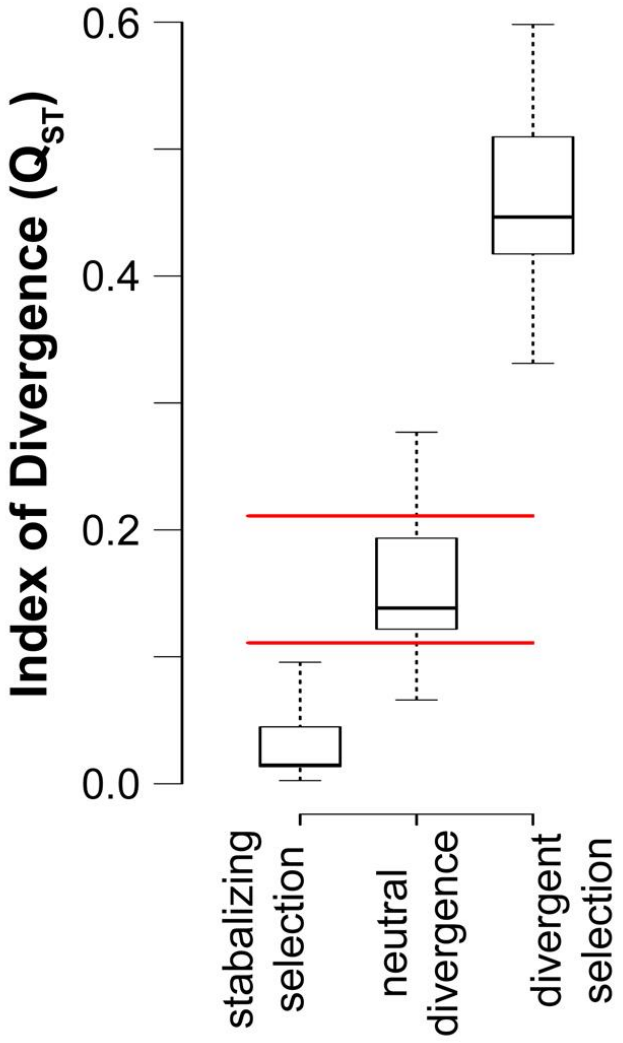
- protein kinase
- involved in cell cycle, apoptosis, telomere maintenance



Signatures of Selection

- demonstrate that trait divergence exceeds that expected under neutral differentiation
  - ➡ $Q_{ST} > F_{ST}$

$$Q_{ST} = \frac{\sigma^2_{among}}{\sigma^2_{among} + 2h^2(\sigma^2_{within})}$$

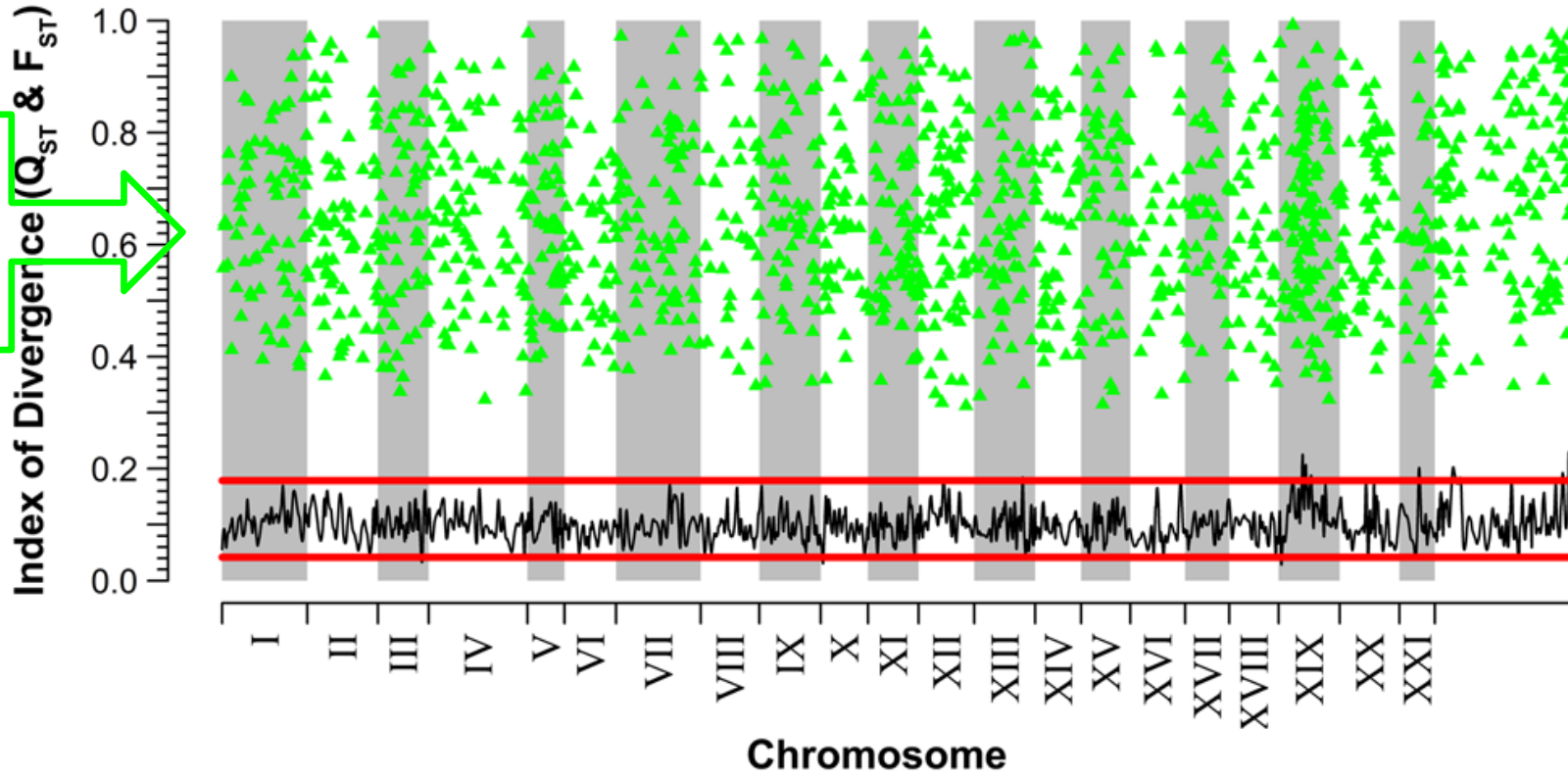


Spitze (1993)
Genetics 135:367

Signatures of Selection

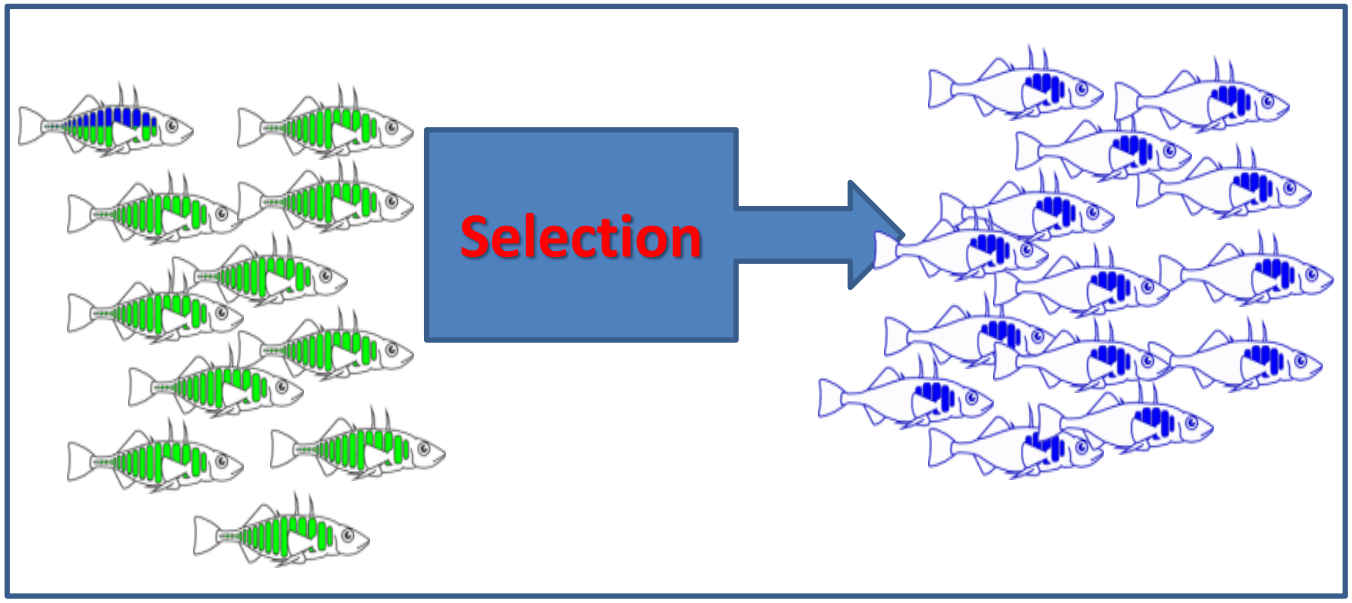
15-17%
1,411 transcripts

83-85% neutral



Leder, McCairns *et al*. (2015)
Mol. Biol. Evol. 32:674

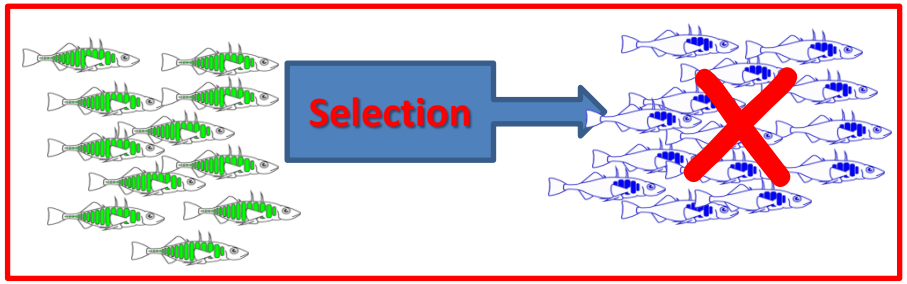
Adaptation from Standing Genetic Variation



Adaptation from Standing Genetic Variation

Contingency

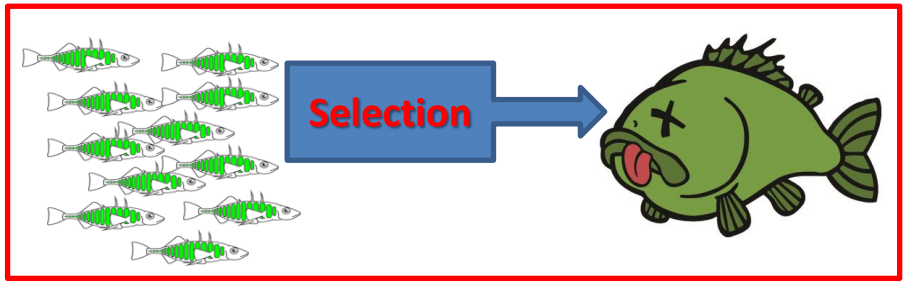
- recessive *Eda* allele may not be present in the colonizing group
  - ➡ frequency is low in marine populations (ca. 5%)



Adaptation from Standing Genetic Variation

Contingency

- recessive *Eda* allele may not be present in the colonizing group
- but what if selection against the dominant allele/phenotype is strong?

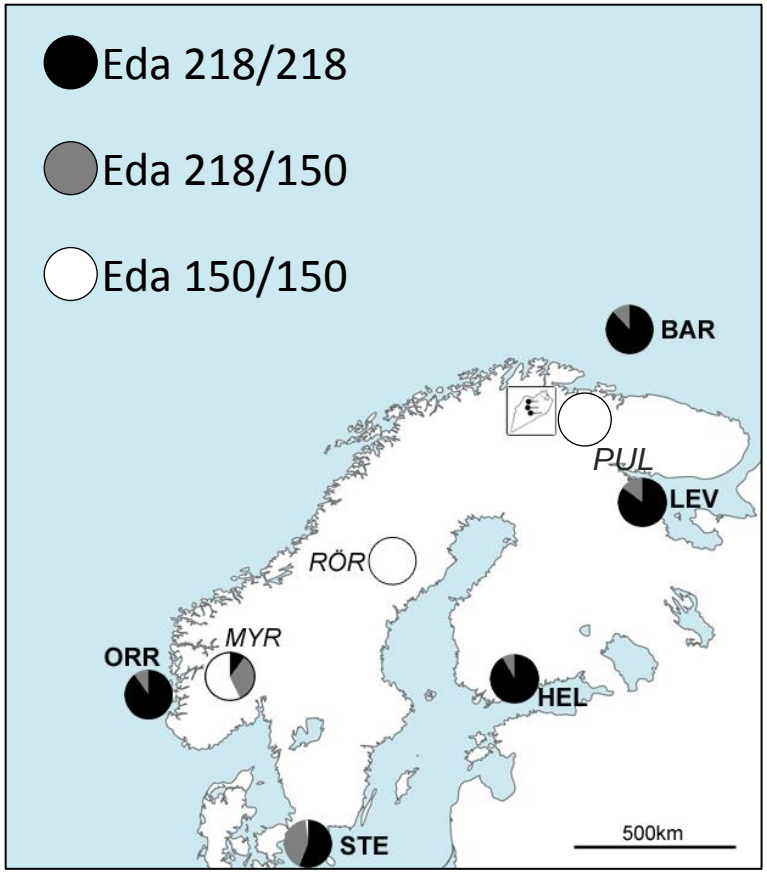


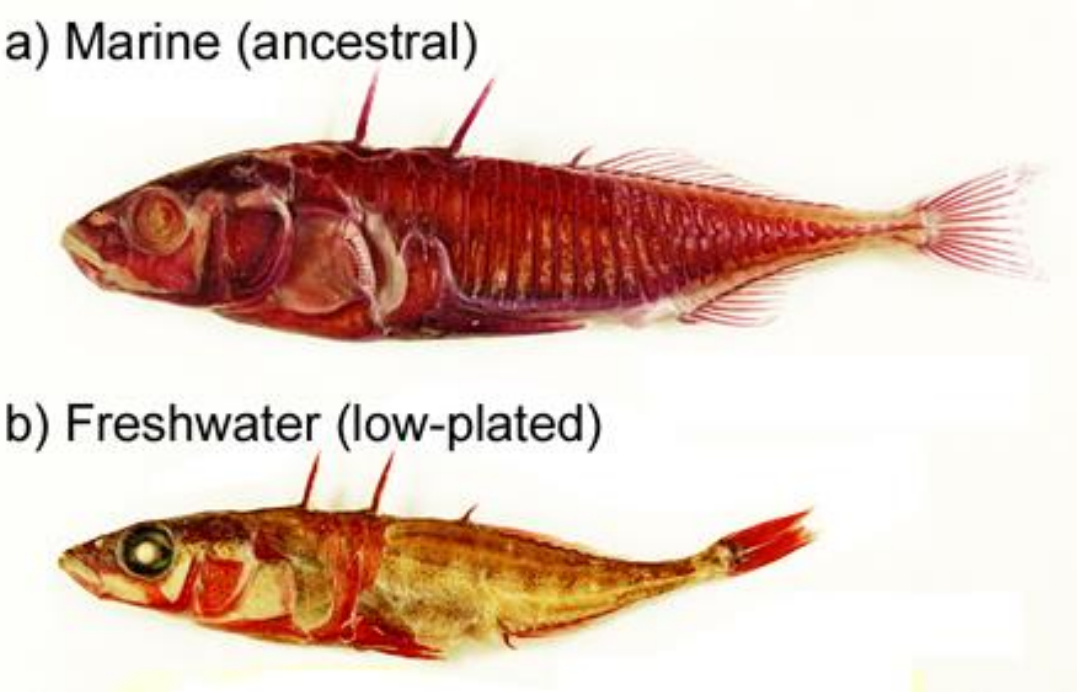
Is population extirpation the only outcome for the colonizing group?

Standing Genetic Variation

Genotype Frequencies

- largely as expected...



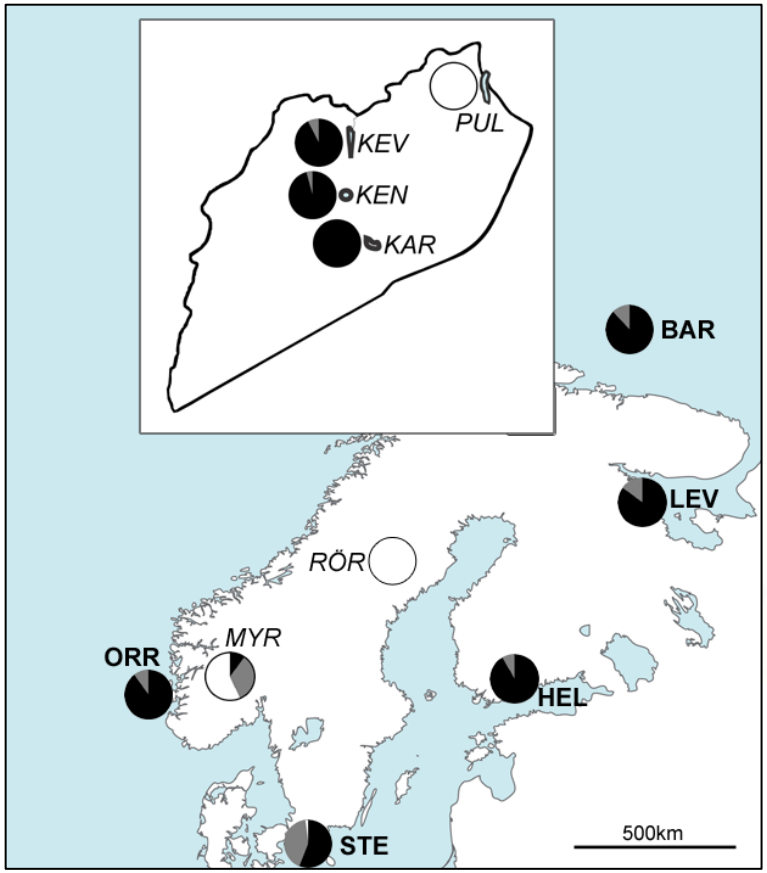


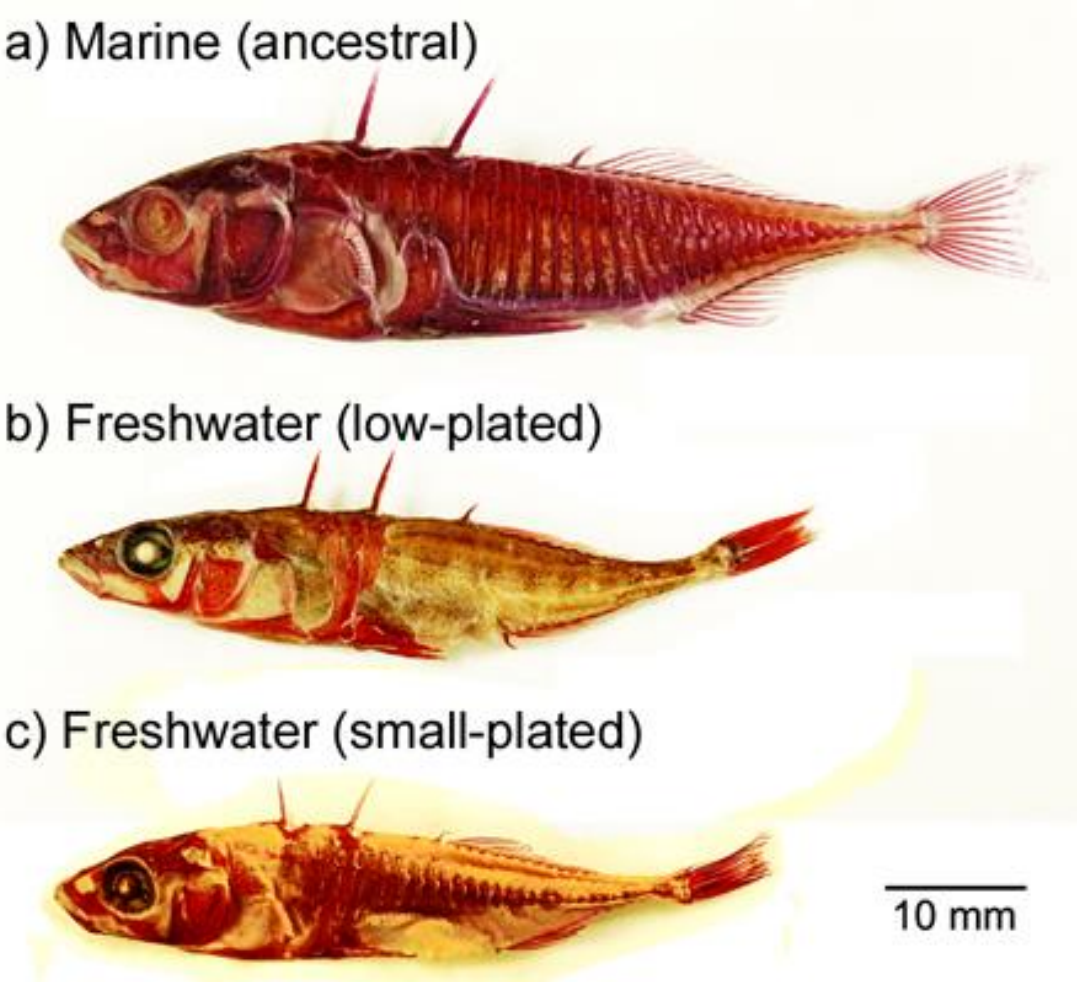
Leinonen, McCairns *et al*. (2012)
Evolution 66:3866

Standing Genetic Variation

Genotype Frequencies

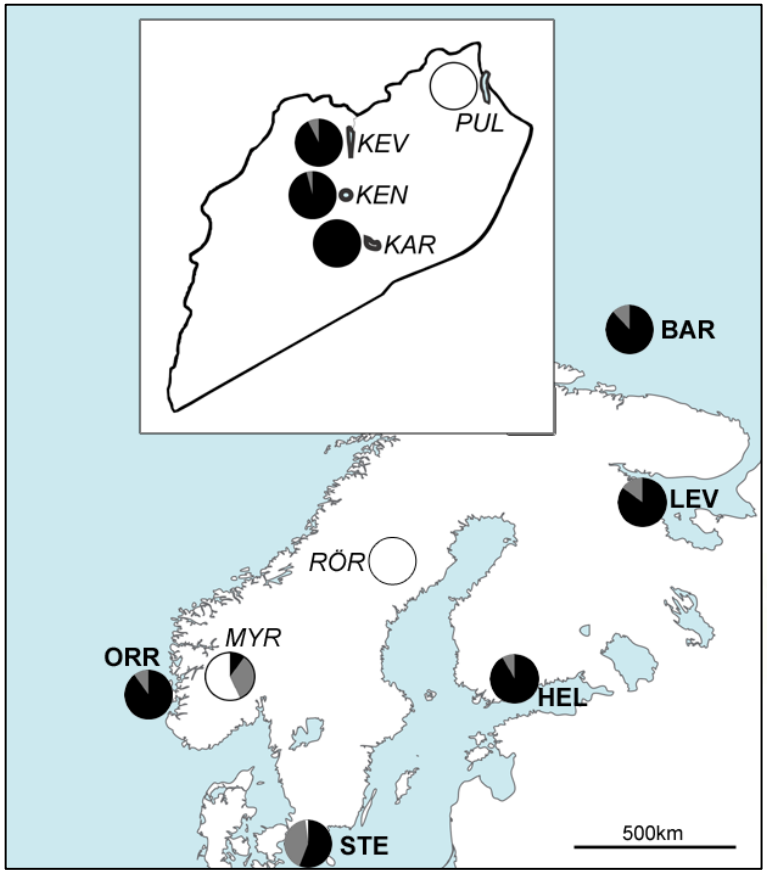
- ...but, some odd exceptions in Lapland





Standing Genetic Variation

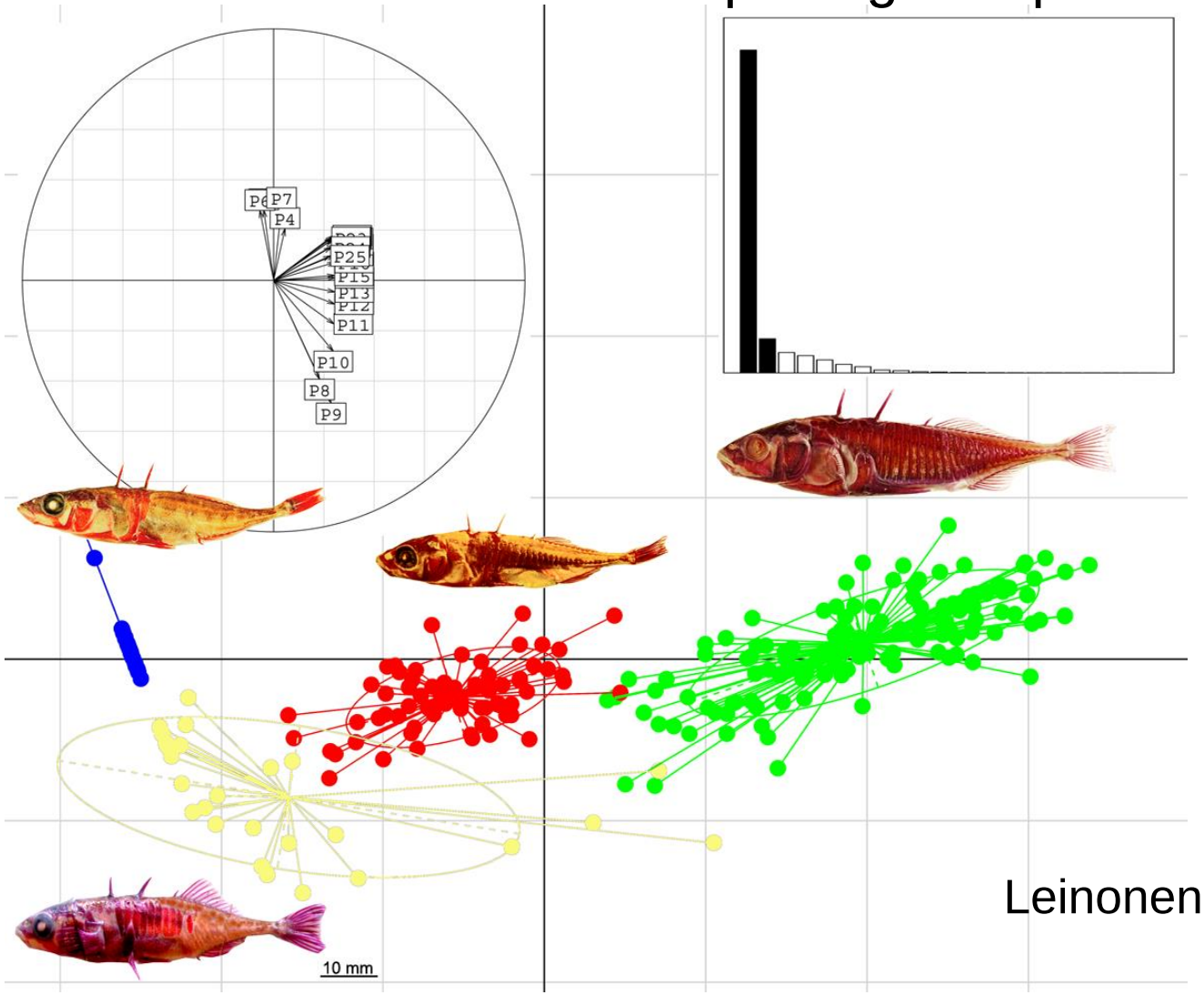
- and the same phenotype reported elsewhere



c) Freshwater (small-plated)

Novel/Atypical Freshwater Evolution

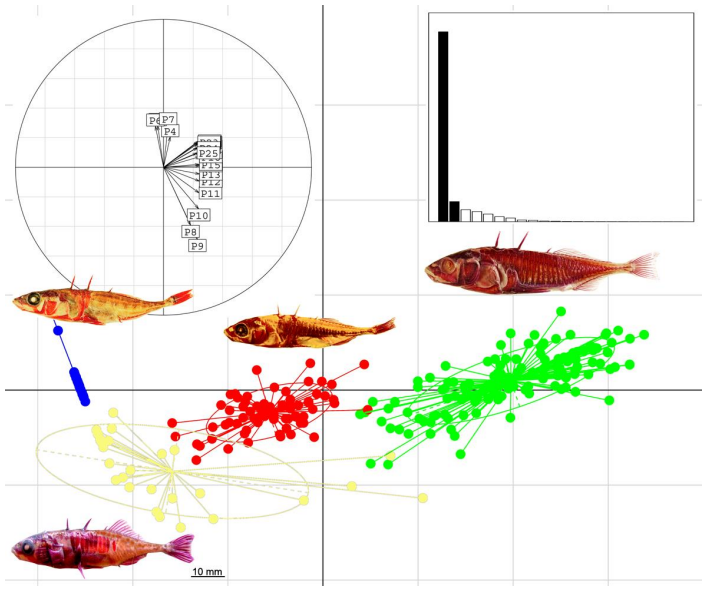
- discrete clusters in morphological space

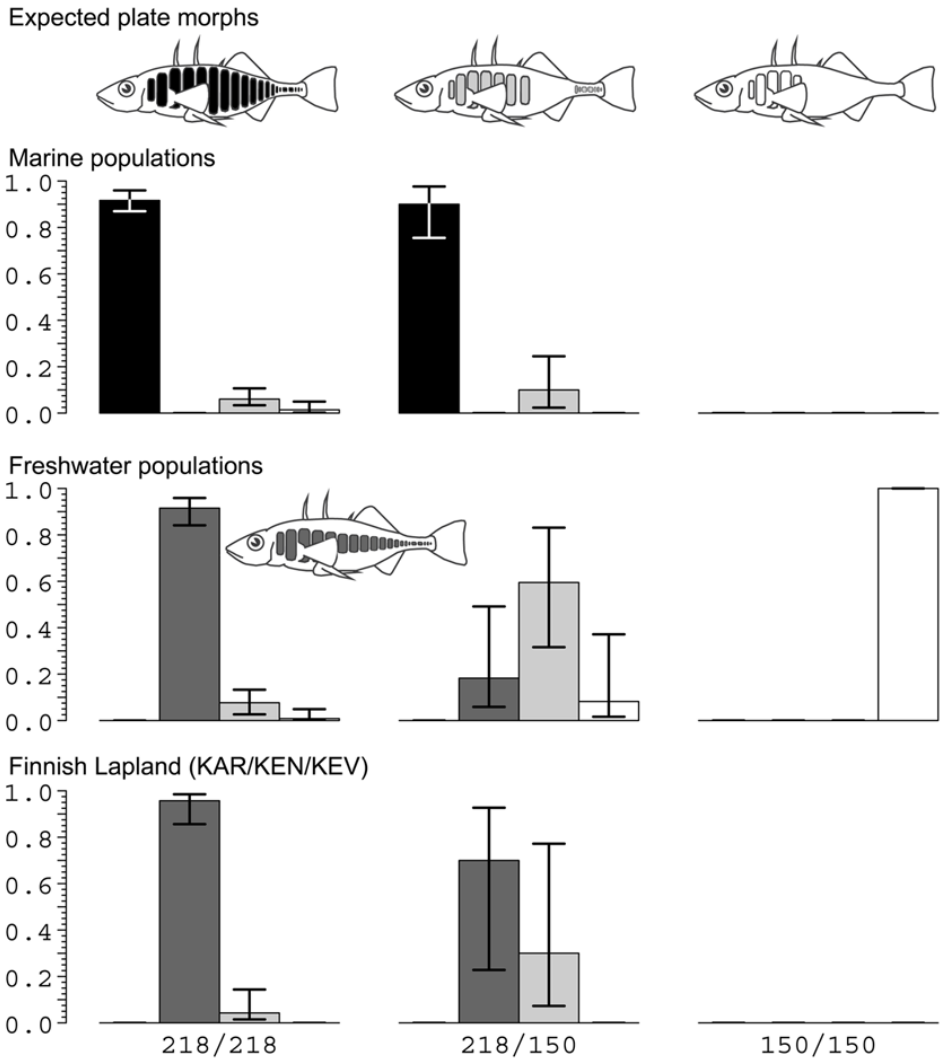


Leinonen, McCairns *et al*. (2012)
Evolution 66:3866

Novel/Atypical Freshwater Evolution

- discrete clusters in morphological space
- *Eda* genotypes not shared w/ "typical" FW morphotype
  - ➡ more "marine-like"

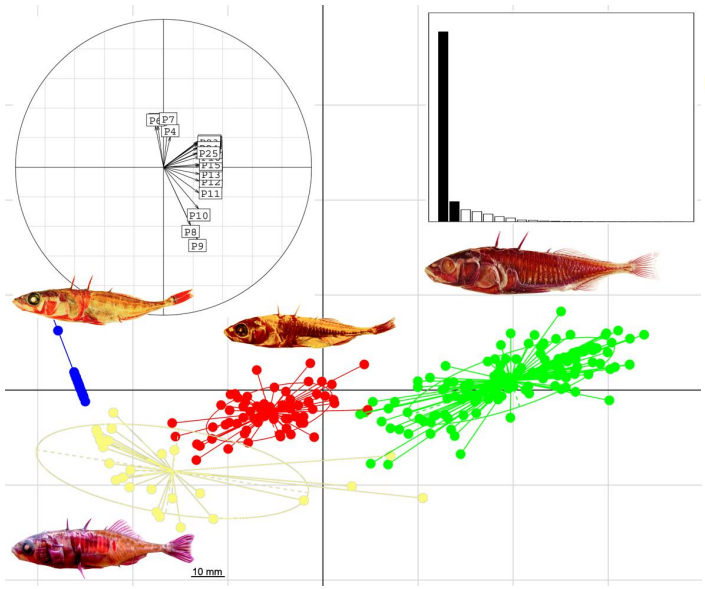


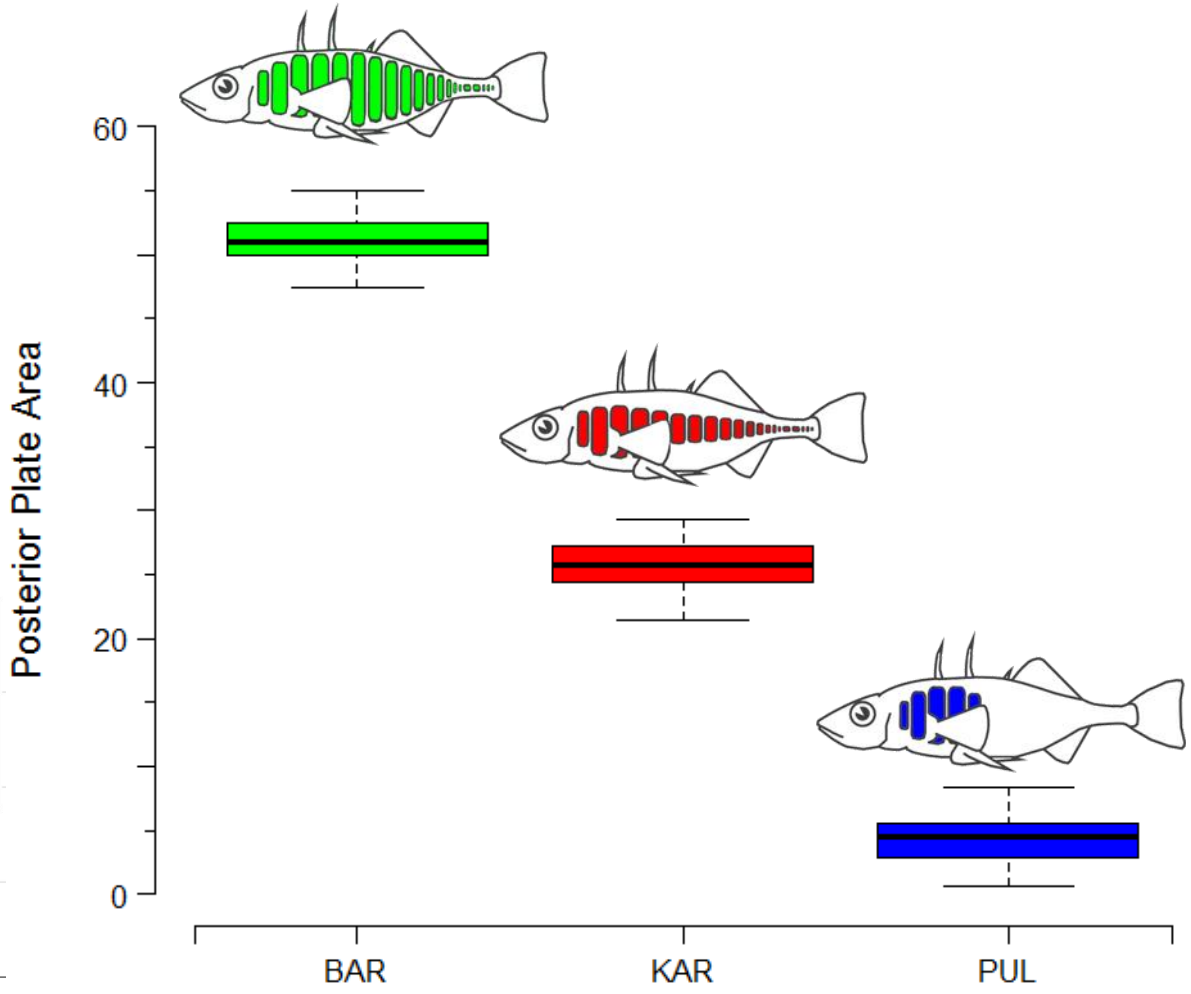


Novel/Atypical Freshwater Evolution

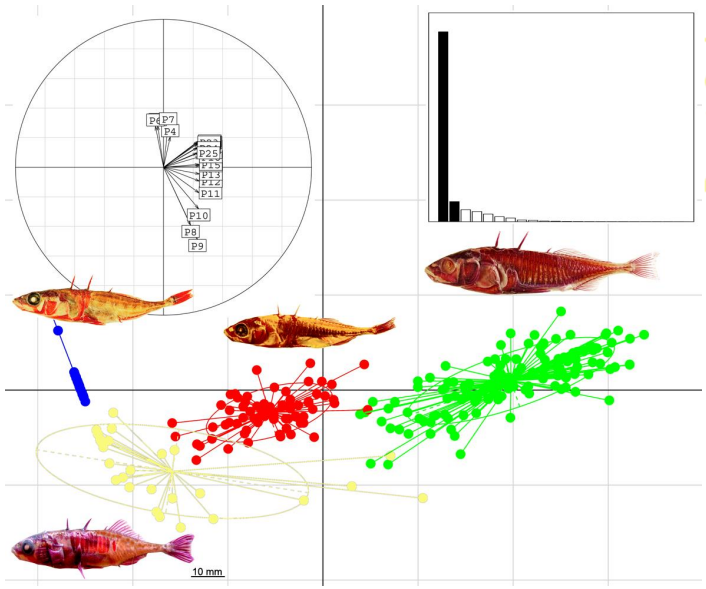
True Breeding

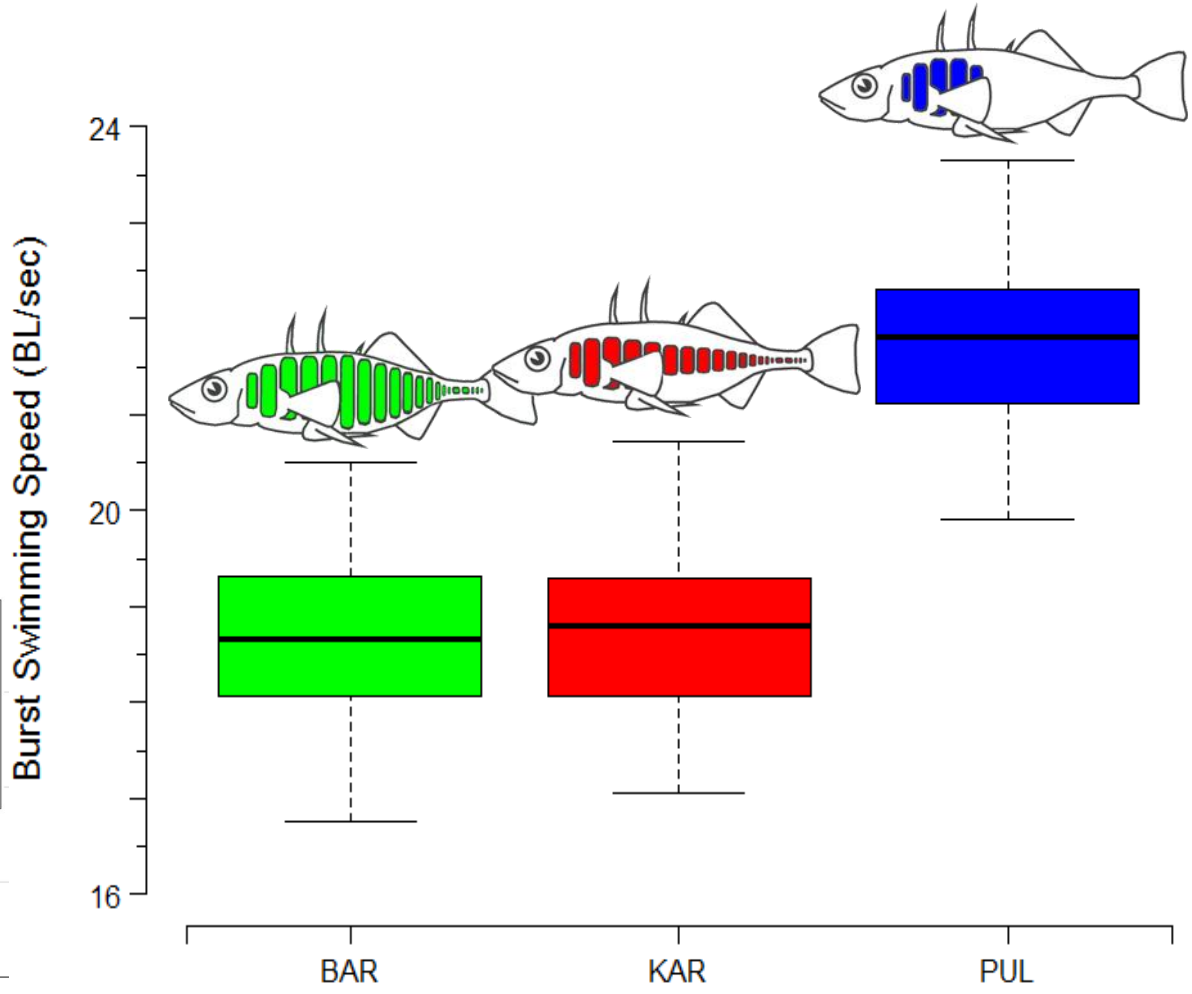
- F_2 lab crosses





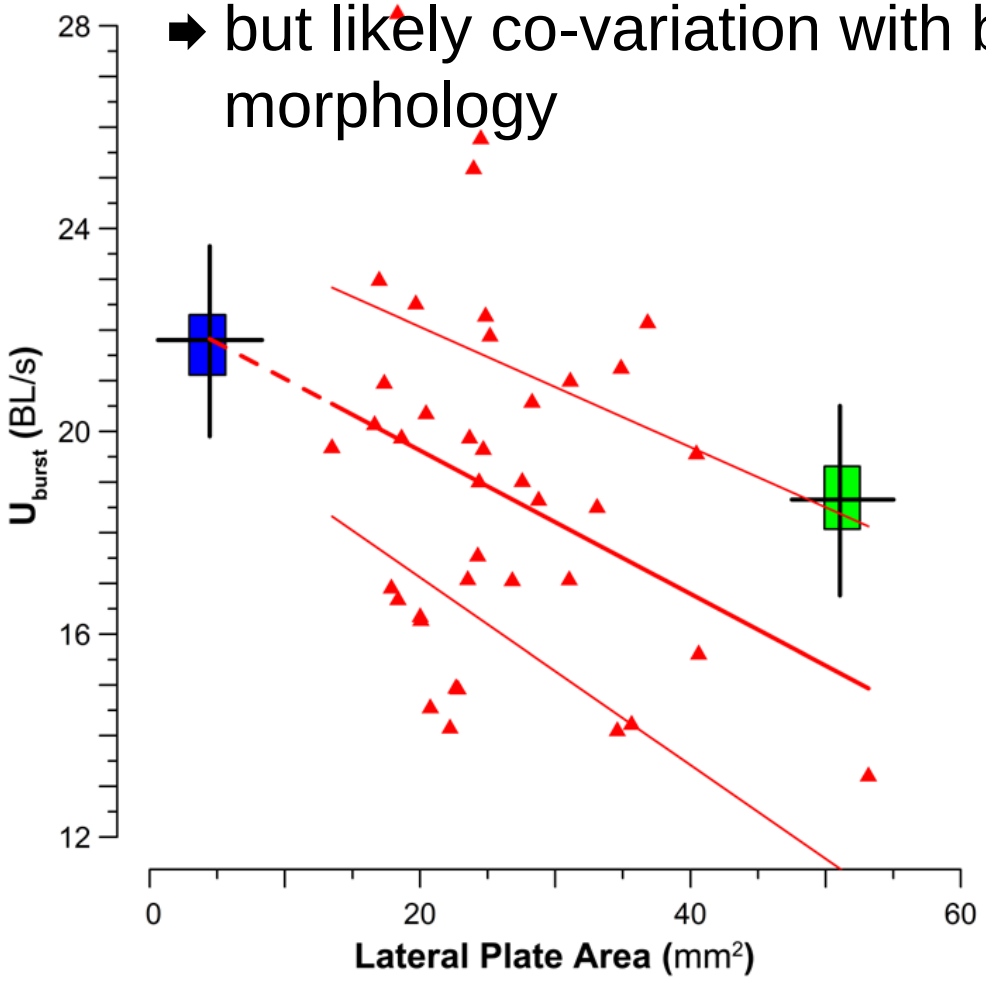
Functional Convergence/Equivalency?

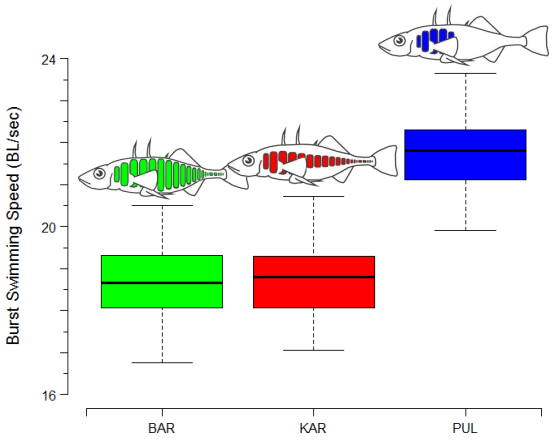


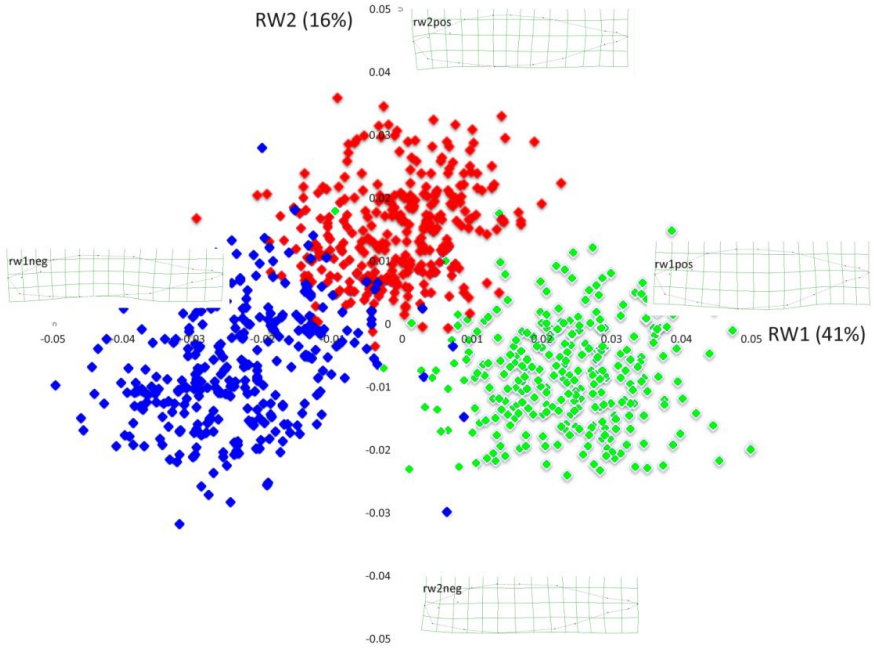


Functional Convergence/Equivalency?

- evidence is equivocal
  - inverse relationship with plate area
  - but likely co-variation with body morphology

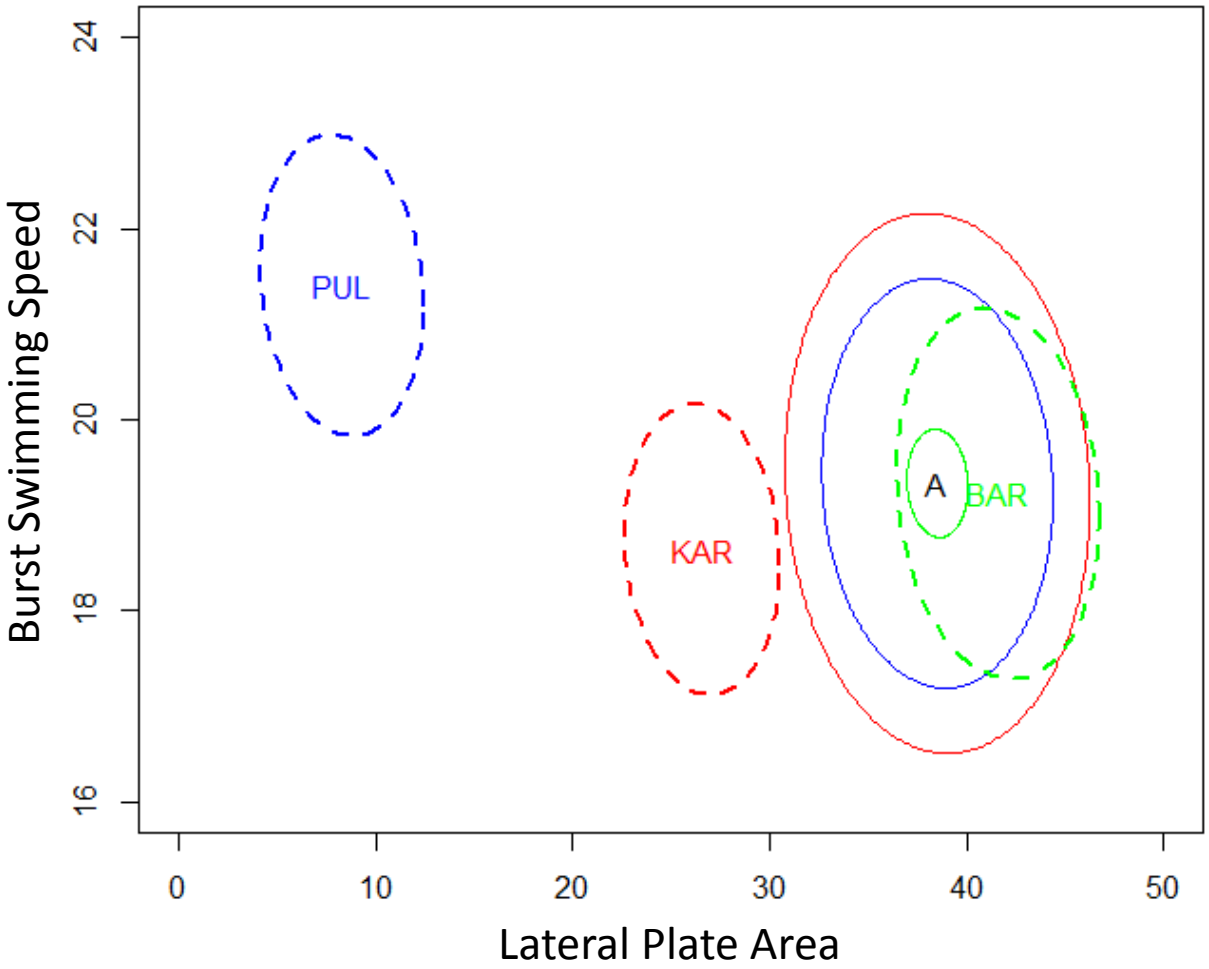


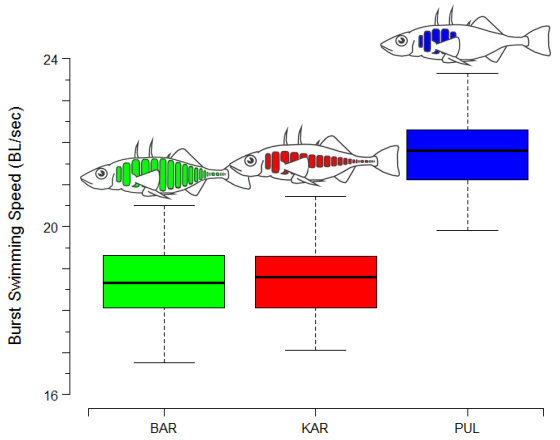


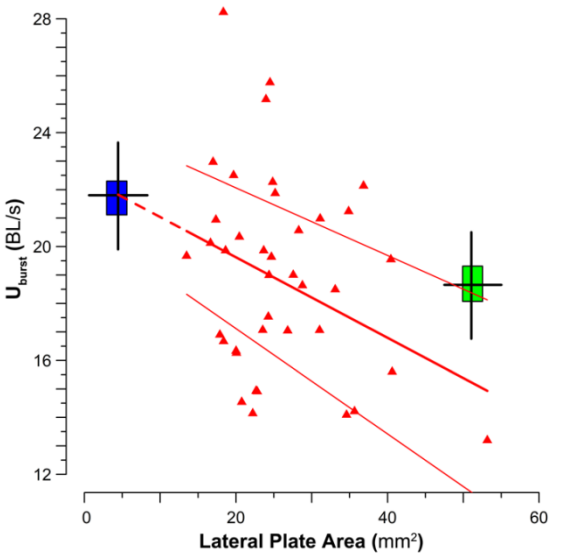


Functional Convergence/Equivalency?

- evidence is equivocal
- signature of correlational selection



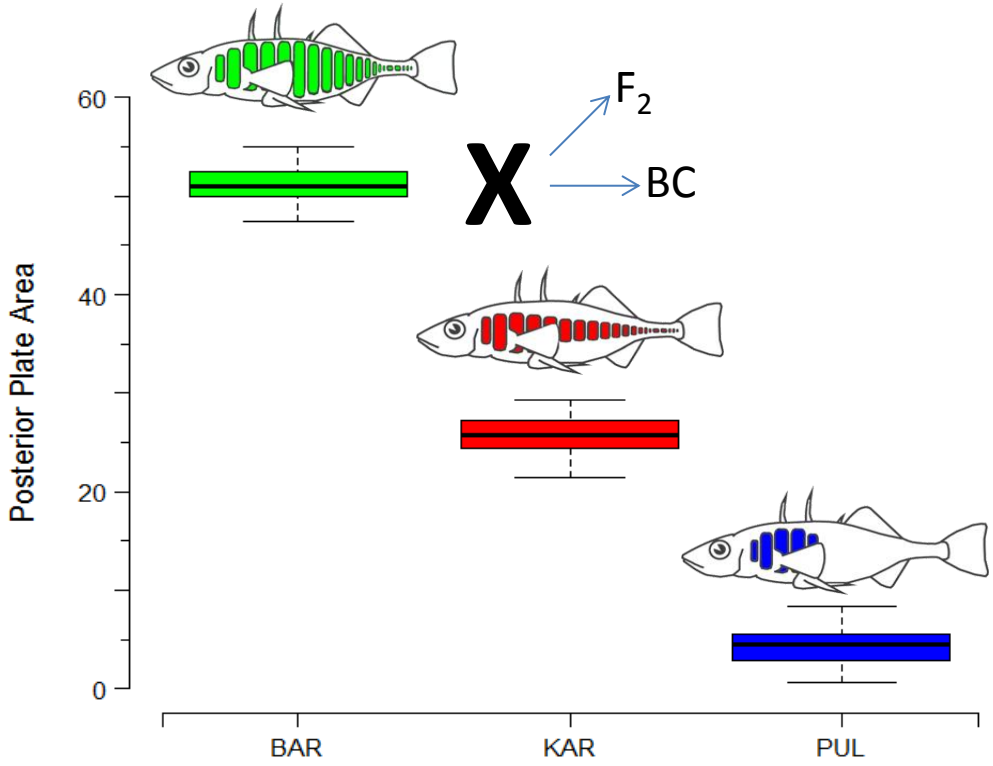




What's in the Pipeline?

Association Mapping

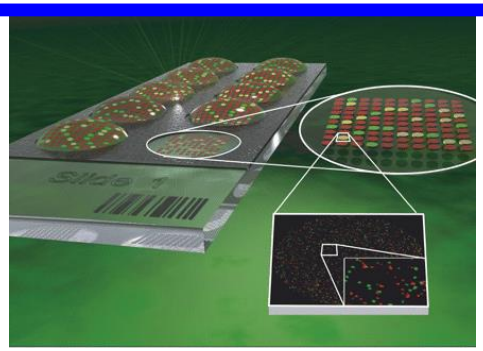
- experimental crosses
  - ➡ RAD-Seq
- shedding light on pleiotropy & "substrate" of correlational selection

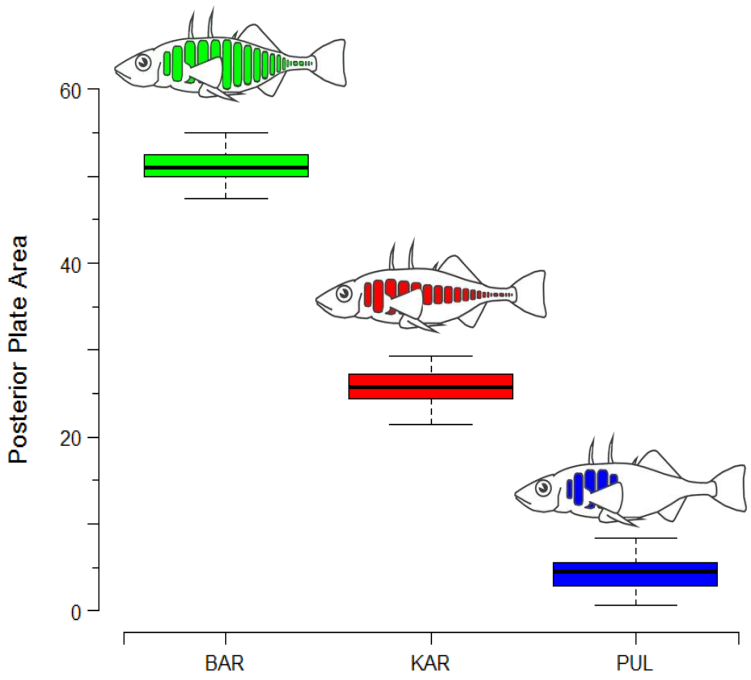


What's in the Pipeline?

Transcriptional Profiling

- developmental time-series
  - during plate development





90d Post-Fertilization

BAR

KAR

PUL

General Conclusions

- transcriptional variation: high signal-to-noise ratio
  - ➡ reflection of functional variance
  - ➡ substantial additive genetic variance
  - ➡ more evidence of selection than expected
- there's more ways to skin a stickleback: flexibility in the face of missing "essential" and/or "pre-adaptive" variation
  - ➡ developmental plasticity?
- let's not forget about contingency
  - ➡ model of adaptation from standing genetic variation relevant to invasion biology

Thanks, kiitos & спасибо

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CSC