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?

incipient species?

ecological speciation
Why Sticklebacks?

Lateral Plate Evolution

- modified scales
- protection from piscine predators
Why Sticklebacks?

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
low plate morph

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

Colosimo et al. (2005)
Science 307:1928
A great example for undergrad textbooks...

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

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

\[ V_P = V_A + V_D + V_E + V_{G \times E} + V_{res} \]
- 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

Colosimo et al. (2005)
Science 307:1928
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

\[ +N \text{ generations of selection} \]
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
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
Why Sticklebacks?

Adaptation from Standing Genetic Variation
➢ replicate environments/colonizations more likely than replicate mutation?
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
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?
“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

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*Chan et al.* (2010)
Science 327:302
Does Transcription Reflect Expression?

Not a direct path to a functional protein!!!
Comparative Transcriptomics

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

Leder et al. (2009)
BMC Genomics 10:426
Comparative Transcriptomics

- 4×44k custom microarray
- Lab-reared fish (F₂)
  - 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

Adaptive transcriptome
Differential Transcription

- 1,834 transcripts ➔ 1,698 genes
- 924 transcripts ➔ 851 genes (down-regulated in VAT)
- 916 transcripts ➔ 857 genes (up-regulated in VAT)

Adaptive transcriptome
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>regulation of protein localization, transport &amp; secretion</td>
<td>2.34</td>
<td>14</td>
<td>63</td>
<td>2.50 (1.50 - 4.20)</td>
</tr>
<tr>
<td>detection of external stimuli</td>
<td>1.66</td>
<td>4</td>
<td>13</td>
<td>2.95 (2.05 - 4.28)</td>
</tr>
<tr>
<td>response to steroidal stimuli</td>
<td>1.36</td>
<td>4</td>
<td>12</td>
<td>2.83 (2.02 - 3.60)</td>
</tr>
<tr>
<td>regulation of cellular growth</td>
<td>1.11</td>
<td>10</td>
<td>24</td>
<td>1.76 (1.19 - 2.40)</td>
</tr>
<tr>
<td>regulation of GTPase activity</td>
<td>1.11</td>
<td>12</td>
<td>40</td>
<td>2.23 (1.10 - 3.90)</td>
</tr>
<tr>
<td>regulation of cell adhesion</td>
<td>1.08</td>
<td>3</td>
<td>12</td>
<td>2.17 (1.83 - 2.55)</td>
</tr>
<tr>
<td>glucose &amp; carbohydrate homeostasis</td>
<td>1.08</td>
<td>3</td>
<td>9</td>
<td>2.37 (2.20 - 2.65)</td>
</tr>
<tr>
<td>regulation of ion transport</td>
<td>1.08</td>
<td>6</td>
<td>12</td>
<td>2.40 (1.83 - 3.70)</td>
</tr>
<tr>
<td>nuclear organization</td>
<td>1.03</td>
<td>6</td>
<td>11</td>
<td>2.18 (1.55 - 2.68)</td>
</tr>
<tr>
<td>regulation of protein signaling</td>
<td>0.99</td>
<td>3</td>
<td>9</td>
<td>2.70 (2.70 - 2.70)</td>
</tr>
<tr>
<td>SMAD protein localization</td>
<td>0.91</td>
<td>3</td>
<td>11</td>
<td>2.50 (1.61 - 3.95)</td>
</tr>
<tr>
<td>glucose &amp; carbohydrate metabolism</td>
<td>0.87</td>
<td>27</td>
<td>26</td>
<td>2.37 (1.63 - 3.87)</td>
</tr>
<tr>
<td>membrane protein proteolysis</td>
<td>0.87</td>
<td>4</td>
<td>8</td>
<td>3.23 (1.44 - 5.82)</td>
</tr>
<tr>
<td>regulation of immune response</td>
<td>0.86</td>
<td>13</td>
<td>28</td>
<td>2.31 (1.04 - 4.80)</td>
</tr>
<tr>
<td>response to intra-cellular pathogens</td>
<td>0.85</td>
<td>7</td>
<td>10</td>
<td>2.49 (1.33 - 3.80)</td>
</tr>
<tr>
<td>mitochondrial organization</td>
<td>0.83</td>
<td>3</td>
<td>7</td>
<td>3.70 (2.00 - 4.70)</td>
</tr>
<tr>
<td>regulation of intra-cellular protein transport</td>
<td>0.81</td>
<td>8</td>
<td>19</td>
<td>1.68 (1.24 - 2.03)</td>
</tr>
<tr>
<td>water homeostasis</td>
<td>0.81</td>
<td>6</td>
<td>10</td>
<td>3.22 (1.65 - 4.70)</td>
</tr>
<tr>
<td><strong>response to oxidative stress</strong></td>
<td><strong>0.75</strong></td>
<td><strong>9</strong></td>
<td><strong>25</strong></td>
<td><strong>1.92 (1.22 - 2.62)</strong></td>
</tr>
<tr>
<td>regulation of lipid metabolism</td>
<td>0.74</td>
<td>14</td>
<td>17</td>
<td>2.33 (1.23 - 3.80)</td>
</tr>
<tr>
<td>regulation of macromolecular secretion</td>
<td>0.64</td>
<td>7</td>
<td>7</td>
<td>2.29 (1.76 - 2.70)</td>
</tr>
<tr>
<td>exocytosis</td>
<td>0.63</td>
<td>6</td>
<td>26</td>
<td>1.53 (1.13 - 2.03)</td>
</tr>
<tr>
<td><strong>glutathione, peptide &amp; sulfur metabolism</strong></td>
<td><strong>0.60</strong></td>
<td><strong>3</strong></td>
<td><strong>17</strong></td>
<td><strong>1.57 (1.24 - 1.87)</strong></td>
</tr>
<tr>
<td>regulation of cellular development</td>
<td>0.59</td>
<td>7</td>
<td>25</td>
<td>1.90 (1.22 - 2.70)</td>
</tr>
<tr>
<td>transport of organic acids</td>
<td>0.55</td>
<td>5</td>
<td>16</td>
<td>1.60 (1.14 - 2.52)</td>
</tr>
<tr>
<td>regulation of muscle development</td>
<td>0.53</td>
<td>10</td>
<td>10</td>
<td>1.86 (1.19 - 3.33)</td>
</tr>
<tr>
<td>DNA catabolism</td>
<td>0.51</td>
<td>7</td>
<td>14</td>
<td>1.71 (1.36 - 1.97)</td>
</tr>
</tbody>
</table>
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
- F1 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

- 8x15k 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
    - $h^2 = \text{posterior mode}$
    - 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

\[ V_D \]

\[ h^2 \] 74 - 98% of transcripts

\[ d^2 \] 41 - 99% of transcripts

Additive Genetic Variance Exceeds

- $V_D$
- direct temperature effects

Heritability of Transcription

Adaptive transcriptome
Response to Environmental Stress

Quantifying Environmental Effects

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

![Graph showing average treatment effect](image)
Signatures of Selection

- demonstrate that trait divergence exceeds that expected under neutral differentiation
  \( Q_{ST} > F_{ST} \)

\[
Q_{ST} = \frac{\sigma_{among}^2}{\sigma_{among}^2 + 2h^2(\sigma_{within}^2)}
\]

Spitze (1993)
Genetics 135:367
Signatures of Selection

15-17% transcripts

83-85% neutral

Leder, McCairns et al. (2015)
Mol. Biol. Evol. 32:674
Adaptation from Standing Genetic Variation

Selection
Contingency

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

- largely as expected...

Leinonen, McCairns et al. (2012)
Evolution 66:3866
Standing Genetic Variation

Genotype Frequencies

- but, some odd exceptions in Lapland

![Map of locations and images of fish](image)

- a) Marine (ancestral)
- b) Freshwater (low-plated)
- c) Freshwater (small-plated)

Scale: 10 mm
Standing Genetic Variation

- and the same phenotype reported elsewhere

**c) Freshwater (small-plated)**

![Map](image-url)
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

Contingency & standing genetic variation
Functional Convergence/Equivalency?

Contingency & standing genetic variation
Functional Convergence/Equivalency?

- Evidence is equivocal
  - Inverse relationship with plate area
  - But likely co-variation with body morphology

Contingency & standing genetic variation
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

Contingency & standing genetic variation
What’s in the Pipeline?

Transcriptional Profiling

- developmental time-series
  - during plate development

90d Post-Fertilization

BAR
KAR
PUL

Contingency & standing genetic variation
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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Mikko Nikinmaa

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