Role of epigenetic variation in adaptation

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28.09.2018
1 Introduction
   • What is epigenetics?
   • Why care about epigenetics?

2 Models of epigenetic variation and adaptation
   • A simple model
   • More complex models

3 Testing the role epigenetic variation in adaptation empirically

4 Conclusions
Outline

1. **Introduction**
   - What is epigenetics?
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2. **Models of epigenetic variation and adaptation**
   - A simple model
   - More complex models

3. **Testing the role epigenetic variation in adaptation empirically**

4. **Conclusions**
Acknowledgements

- Sinéad Collins (Edinburgh)
- Tarmo Ketola (Jyväskylä)
- Hanna Johannesson (Uppsala)
- Eric Selker (Oregon)

Funding

- Academy of Finland
- Center of Excellence in Biological Interactions
- Emil Aaltonen Foundation
I’m currently working with the filamentous fungus *Neurospora crassa*

Role of epigenetic mechanisms in phenotypic plasticity and transgenerational effects

Epigenetic changes that occur during adaptation

Characterization of the properties of epigenetic changes (in the future)
This talk is about role of epigenetics and adaptation
We have investigated this with models and experiments
Mostly based on work with Sineád Collins in Edinburgh
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What is epigenetics?

- There are many types of non-genetic inheritance
  - I’m focusing on chromatin changes

**Definition**

*Changes in gene transcription through modulation of chromatin, which are not caused by changes in DNA sequence*

Allis et al. (Eds.) 2009. Epigenetics
Epigenetics

- DNA methylation
  - Cytosine methylation
  - Adenosine methylation (also in eukaryotes: flies, worms, and algae)
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Histone modifications / small RNA’s
- Histone methylation (different residues)
- Small RNA’s
Inheritance of DNA methylation

Mechanism is clear, most DNA methylation occurs in symmetric CG context, after DNA replication hemimethylated sites are recognized and methylated

- Not all methylation is reset in meiosis
- Lot of variation in resetting, plants vs. animals
Alleles based on DNA methylation

A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening

Kenneth Manning1, Mahmut Tör1, Mervin Poole2, Yiguoy Hong1, Andrew J Thompson1, Graham J King3, James J Giovannoni4 & Graham B Seymour2

An epigenetic mutation responsible for natural variation in floral symmetry

Pilar Cubas*, Coral Vincent & Enrico Coen

John Innes Centre, Colney Lane, Norwich NR4 7UH, UK

A metastable DWARF1 epigenetic mutant affecting plant stature in rice

Kotaro Miura*, Masakazu Agetsuma*, Hidemi Kita and Motoyuki Ashikari*1
Inheritance of histone modifications and RNA’s

- Mechanism of inheritance of histone marks is not clear
- Different hypotheses have been proposed
- There is evidence that histone modifications and small RNA’s form a positive feedback loop
Epigenetic inheritance

Paternal Diet Defines Offspring Chromatin State and Intergenerational Obesity

Anita Öst,1,6,8,* Adelheid Lempradl,1,8 Eduard Casas,2,3 Melanie Weigert,1 Theodor Tiko,1 Merdin Deniz,1

Öst et al. 2014 Cell

In flies, the diet of fathers influenced offspring triglyceride content, and histone methylation was required for this response.
Transgenerational inheritance

Within and between generation phenotypic plasticity in trichome density of *Mimulus guttatus*

L. M. HOLESKI
Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS, USA

Descendants of Primed Arabidopsis Plants Exhibit Resistance to Biotic Stress

Ana Slaughter, Xavier Daniel, Victor Flors, E

Transgenerational induction of defences in animals and plants

Anurag A. Agrawal*, Christian Laforsch† & Ralph Tollrian†
Summary: Epigenetic variation

Induced epigenetic variation

Environmental change

Pure epigenetic variation

Error in propagation

Next generation

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Spontaneous epigenetic variation

- Random with respect to fitness
- Changes happen because of biochemical errors in propagation
- Analogous to genetic mutations

For the remainder of this talk, I’ll focus on spontaneous epigenetic variation (aka ’pure epigenetic variation’).
At the moment, the best characterised epigenetic changes are DNA methylation changes in the plant *Arabidopsis thaliana*.

Becker et al. 2011; Schmitz et al. 2011; van der Graaf et al. 2015

**Rate of DNA methylation changes in *Arabidopsis***

- Forward mutation rate $2.56 \times 10^{-4}$ per site per generation
- Backmutation rate $6.30 \times 10^{-4}$ per site per generation
- Contrast to genetic mutation rate of $7 \times 10^{-9}$ per site per generation
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Why epigenetics?

Why is an evolutionary biologist interested in epigenetics?

- Some epigenetic changes can be inherited
- Thus they can potentially be under selection
Chemical basis of heredity itself does not matter in evolution. Epigenetics will only make a difference for evolution if:

- Epigenetic inheritance has different properties than genetic inheritance
  - There is some evidence that rates of DNA methylation change are very high
  - Different distribution of effects?
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A simple model

Let’s start with a very simple model, using the standard equations of population genetics.
A simple model

- Scenario: There is an environmental change that requires silencing of a formerly constitutively expressed gene
- 1 locus, 3 allele system

Assumption: previously reported *Arabidopsis* mutation rates used here, fitness of ancestral allele is $1 - s$, where $s = 0.05$
A simple model

Allele frequencies change due to mutation and selection, according to standard equations written in matrix form for multiple alleles:

$$\Delta \vec{p} = \Delta \vec{p}_{sel} + \Delta \vec{p}_{mut}$$
A simple model

- Two-phase adaptation with epigenetic variation
- Faster initial adaptation
- Eventual replacement of epigenetic allele with the genetic allele
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Adding some complexity

- Similar dynamics occurs with multiple loci on N-K fitness landscapes
- Epigenetic variation allows faster peak shifts
- Klironomos et al., 2013. How epigenetic mutations can affect genetic evolution: Model and mechanism. BioEssays
Adding some complexity

- Kronholm & Collins, 2016. Epigenetic mutations can both help and hinder adaptive evolution. Molecular Ecology
- Individual based simulations
- Arbitrary mutational effects using Fisher’s geometric model
Adaptive walks with epigenetic variation

- Similar dynamics observed in this model
- Epigenetic variation can speed up adaptation
Adaptive walks with epigenetic variation

- Epigenetic variation is used initially and then lost
Adaptive walks with epigenetic variation

Results

- Stability of epigenetic changes is important
  - Reversion rate has to be below 0.1 for epigenetic mutations to contribute
Adaptive walks with epigenetic variation

Results

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- **Phenotypic effects of epigenetic mutations are important**
  - If mutational supplies of genetic and epigenetic mutations are equal, epigenetic variation is only beneficial if phenotypic effects are smaller
Adaptive walks with epigenetic variation

Results

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- Phenotypic effects of epigenetic mutations are important
  - If mutational supplies of genetic and epigenetic mutations are equal, epigenetic variation is only beneficial if phenotypic effects are smaller

- Epigenetic changes tend to replaced by genetic changes
  - This process is driven by reversion rate of epimutations and is slow
Distribution of phenotypic effects

Are the distributions of phenotypic effects different for genetic and epigenetic mutations?

- Epigenetic mutations can
  - Change gene expression (silence, activate, fine-tune)
  - Change regulation (add or delete cis-regulatory elements)
  - Change protein structure
  - Change copy number (gene duplications, deletions, even whole genome duplication)
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Distribution of phenotypic effects

- Based on this argument, one would expect that phenotypic effects of epigenetic mutations are a *some* subset of the phenotypic effects of genetic mutations.

- We don’t know these distributions empirically.
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Experimental test

- Making models is fine, but does epigenetics actually matter in adaptation?
- We did an evolution experiment with the single celled algae *Chlamydomonas reinhardtii*
- Kronholm et al., 2017. Epigenetic and genetic contributions to adaptation in *Chlamydomonas*. Molecular Biology and Evolution
The experiment

- We let experimental populations of algae adapt to different environments
  - High NaCl
  - High CO$_2$
  - Low P
- We manipulated epigenetic variation chemically
  - Demethylating agents + deacetylation inhibitor
- We sequenced the genomes and methylomes for some adapted populations
The experiment

- Environments
  - Control
  - High CO$_2$
  - Low P
  - High NaCl

- Chemical treatments
  - Control
  - Methylation
  - Methylation + deacetylation

- ~ 200 generations of selection

Are there differences when we manipulate epigenetics?
The experiment

- Different environments, chemical treatments within environments, and different strains within chemical treatments
- No variation at the start of the experiment (single clone)
- Populations went through $\sim 200$ generations of batch transfer
- Control populations for increased mutation rate
- Growth rates assayed at the end of the experiment
  - Relative to populations evolved in the control environment and corresponding treatment
Phenotypic results: High NaCl environment

- Control populations adapted the most
- Chemical manipulation decreases the amount of adaptation
Phenotypic results: High CO$_2$ environment

- Growth rate of control populations evolved in high CO$_2$ goes down
  - This is actually adaptation!
  - This has been shown in multiple experiments and with different species of algae
- Thus chemical manipulation also decreases adaptation here
Phenotypic results: Low P environment

- There was adaptation to low P environment
- Chemical manipulation did not have an effect
- Many extinctions in this environment
Summary of results

- We could not explain our results by chemical toxicity
  - No differences in numbers of generations among chemical treatments
- Or increased mutation rate
  - Demethylating agents are mutagenic and adaptation in high NaCl is mutation limited, but treatments decrease adaptation
Sequencing results

- We sequenced the genomes (18 clones) and methylomes (13 clones) of clones sampled from the evolved populations.
Genetic mutations

- Multiple mutations were detected
- Many in genes of unknown function
- Adaptation to common laboratory environment
  - Mutations in flagellar genes
- In high NaCl environment, gene with a chloride channel domain
- In high CO$_2$ environment, nothing obviously related to CO$_2$
For DNA methylation, we focused on longer stretches of methylation:

- Differentially methylated regions (DMRs)
- Median length: 61 bp, range: 9 to 1150 bp
- These are more likely to be functional than changes in methylation of single cytosines
Clustering of DMRs

Methylation frequency (%)

0 20 40 60 80

Color Key

Demet.
Control
High CO2
High NaCl
Control
Evolved
Ancestor

Treatment Environment History

Control
Demet.
88 83
100
91
87
89
100 63
96
100
97
100
70

P11B4 P11B11 P12E4 P1B3 F5F6 P6E8 P4C5 P10C5 P6E2 P5B10 P1D2 P4C7 P2D9 Demet CC2937cont CC2937hco2 CC2937salt Control
Gene ontology term enrichment in DMRs

- Certain GO terms were enriched in DMRs that changed during the experiment
- Adaptation to common laboratory environment
  - Methylation changes in genes possibly involved aminoglycan metabolic processes
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  - Methylation changes in genes possibly involved aminoglycan metabolic processes
- In high NaCl environment
  - GO term: transmembrane transport ($p = 0.013$) enriched in DMRs
- In high CO$_2$ environment
  - GO term: membrane depolarization was enriched ($p = 3.2 \times 10^{-4}$) due to DMRs in genes that are voltage gated Ca$^{+2}$ channels
Summary

- Both genetic mutations and methylation changes occurred during the experiment
- Genetic mutations could not explain methylation changes
  - We would need to invoke unique *trans*-acting mutations in each clone

Some methylation changes could be plastic responses to environment, but they were not consistent.

Overall, results indicate that epigenetic changes contribute to adaptation in *Chlamydomonas*.

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Open questions

Many open questions remain:

- How much of epigenetic variation is under genetic control?
- How much affects the phenotype?

- (Large?) part of it must be neutral
- For DNA methylation: functional importance of individual cytosines vs. larger regions
- Differences between different epigenetic marks and different species

- Epigenetic mechanisms in fungi, plants, and animals do not work the same way

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Conclusions

- Epigenetic variation can influence adaptation
- This depends on the properties of epigenetic variation

Important properties of epigenetic variation

- Rate of epigenetic changes
- Stability of epigenetic changes
- Distribution of their phenotypic effects

To integrate epigenetic changes into evolutionary theory we need to understand these factors

End of presentation: What questions do you have?