# Role of epigenetic variation in adaptation

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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

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UNIVERSITY OF JYVÄSKYLÄ

ACADEMY OF FINLAND

EMIL AALTOSEN SÄÄTIÖ

# Current and future research interests

- 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)

# Introduction

- 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)

# Epigenetics

- DNA methylation
    - Cytosine methylation
    - Adenosine methylation (also in eukaryotes: flies, worms, and algae)
- 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

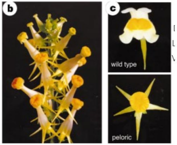
## A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening

Kenneth Manning[1], Mahmut Tör[1], Mervin Poole[2], Yiguo Hong[1], Andrew J Thompson[1], Graham J King[3], James J Giovannoni[4] & Graham B Seymour[2]

## 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*

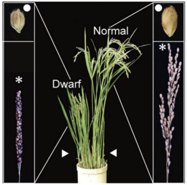


## A metastable *DWARF1* epigenetic mutant affecting plant stature in rice

Kotaro Miura[a], Masakazu Agetsuma[a], Hidemi Kita

tsuoka[a], Steven E. Jacobsen[c],

and Motoyuki Ashikari[a,1]



*Epi-d1*

# 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

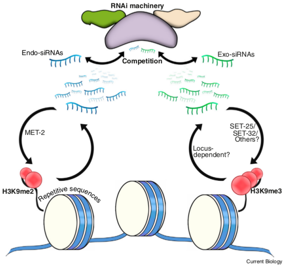
LETTER

https://doi.org/10.1038/s41586-018-0239-3

**Epigenetic inheritance mediated by coupling of RNAi and histone H3K9 methylation**

Ruby Yu[1], Xiaoyi Wang[1] & Danesh Moazed[1]*

Nature 2018



Rechavi & Lev 2017 Current Biology

# Epigenetic inheritance

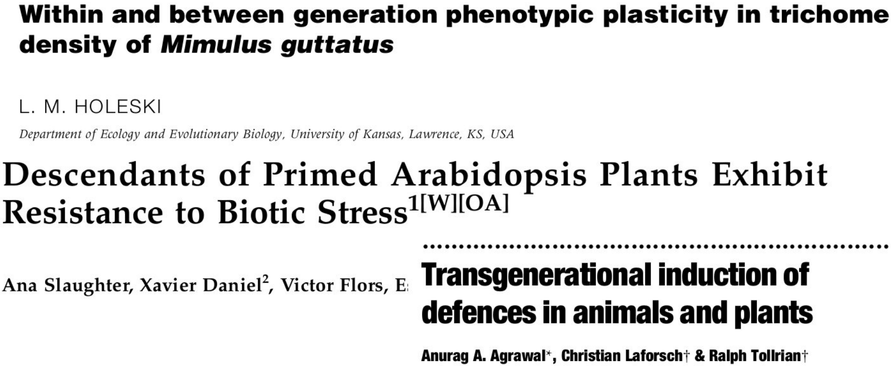
**Paternal Diet Defines Offspring Chromatin State and Intergenerational Obesity**

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*

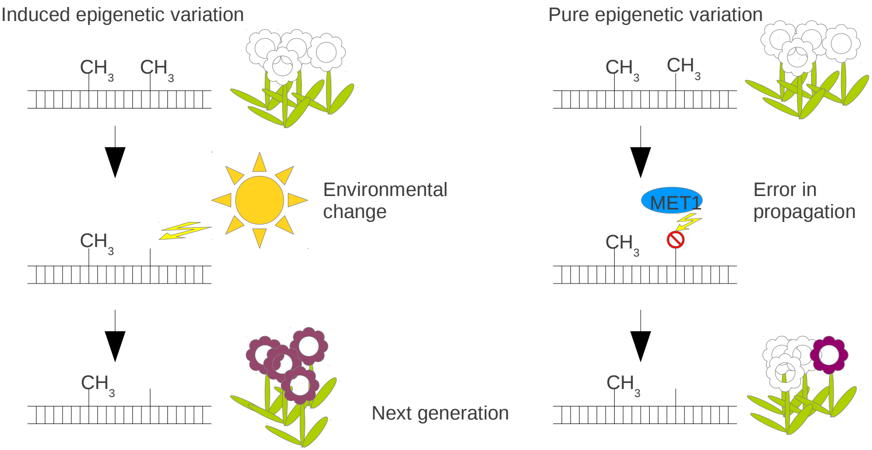
**Descendants of Primed Arabidopsis Plants Exhibit Resistance to Biotic Stress[1][W][OA]**

Ana Slaughter, Xavier Daniel[2], Victor Flors, E

**Transgenerational induction of defences in animals and plants**

**Anurag A. Agrawal*, Christian Laforsch† & Ralph Tollrian†**

# Summary: Epigenetic variation



# 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')

# DNA methylation changes in *Arabidopsis*

- 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

# Epigenetics and evolution

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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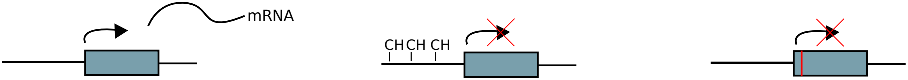
# A simple model

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



Ancestral allele

Epigenetic allele

Genetic allele

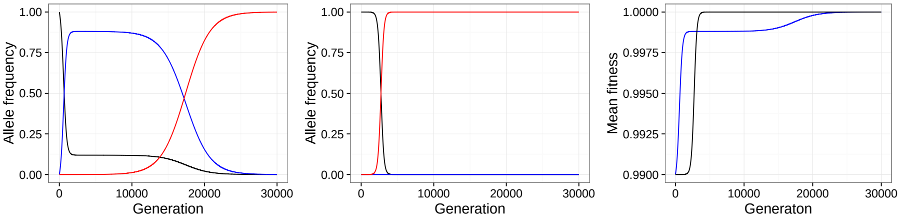
Assumption: previously reported *Arabidopsis* mutation rates used here, fitness of ancestral allele is $1 - s$, where $s = 0.05$

# 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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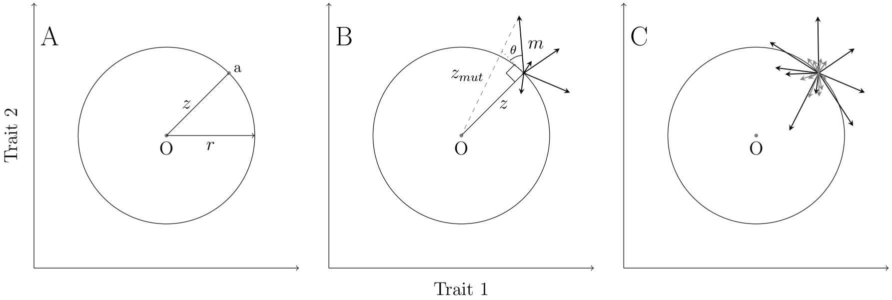
# Adding some complexity

- 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

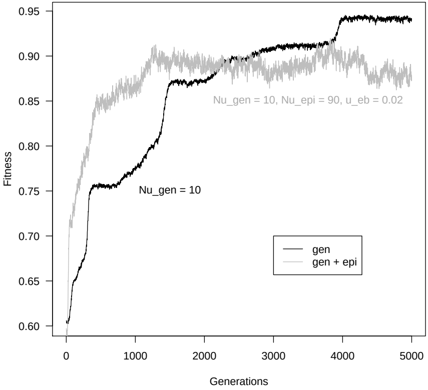
# Adding some complexity

- 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



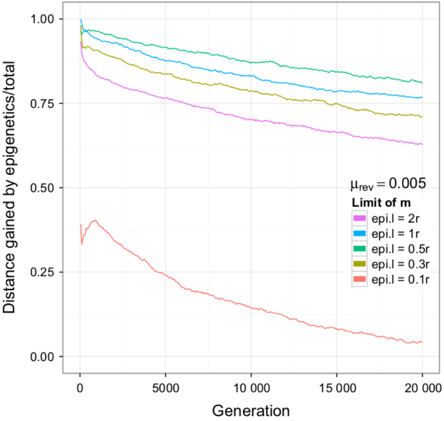
# Adaptive walks with epigenetic variation

- 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

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- Phenotypic effects of epigenetic mutations are important
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# 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
- 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)

# Distribution of phenotypic effects

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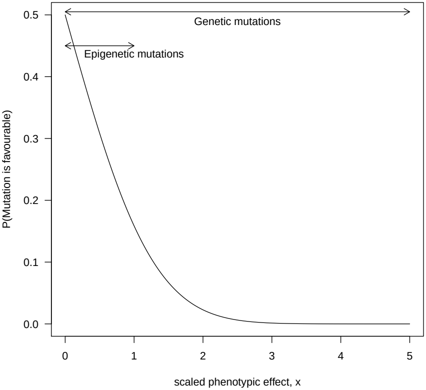
- Epigenetic mutations can
  - Change gene expression (silence, activate, fine-tune)
- Genetic mutations can
  - Change gene expression (silence, activate, fine-tune)
  - Change regulation (add or delete *cis*-regulatory elements)
  - Change protein structure
  - Change copy number (gene duplications,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

# 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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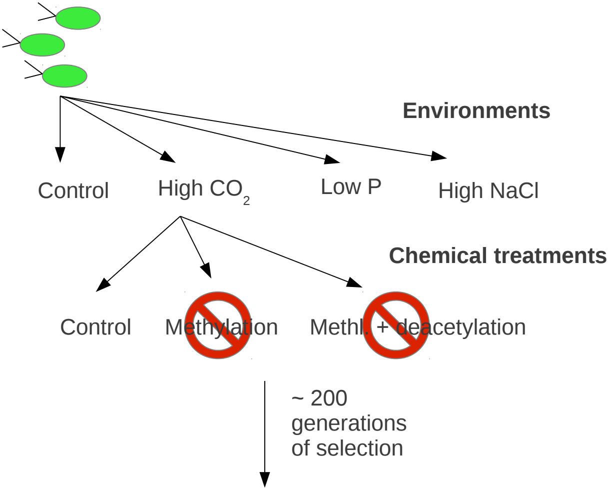
# Experimental test

- 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

Methl. + deacetylation

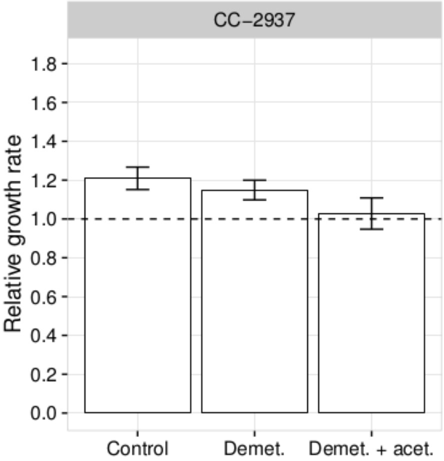
~ 200
generations
of selection

Are there differences when we manipulate epigenetics?

# The experiment

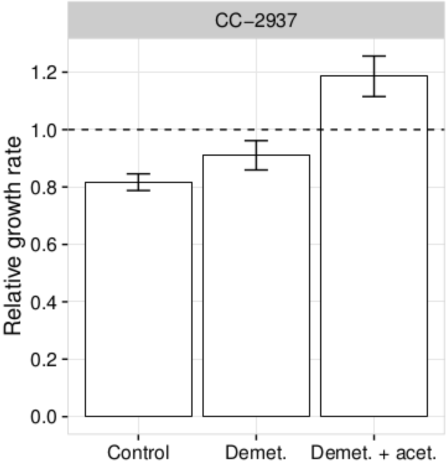
- Different environments, chemical treatments within environments, and different strains within chemical treatments
- No variation at the start of the experiment (single clone)
- Populations went through $\sim 200$ generations of batch transfer
- Control populations for increased mutation rate
- Growth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

# Phenotypic results: High NaCl environment



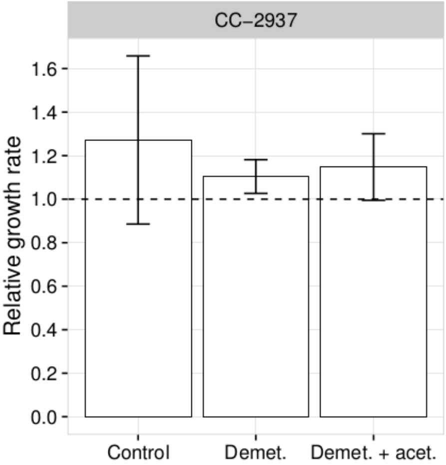
- Control populations adapted the most
- Chemical manipulation decreases the amount of adaptation

# 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

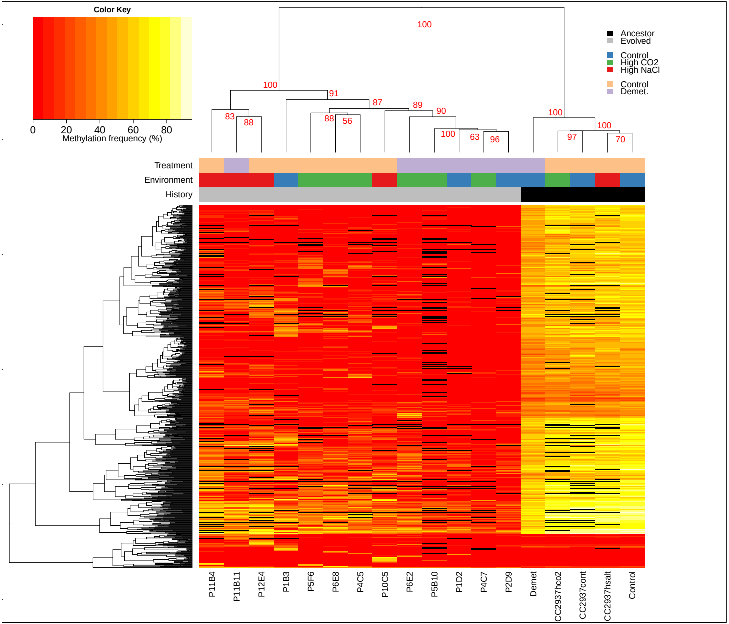
# Genetic mutations

- 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 $CO_2$

# DNA methylation

- For DNA methylation, we focused in 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



# 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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- Adaptation to common laboratory environment
  - 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

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- 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 question remain:
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- How much of epigenetic variation is under genetic control?
- How much of it 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

# 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?