The role of Epigenetic Inheritance

- Lamarck Revisited

- Lamarck was incorrect in thinking that the inheritance of acquired characters is the main mechanism of evolutionary adaptation (Natural Selection more common)

- However, we now know that the inheritance of acquired characters does happen sometimes, through the inheritance of epigenetic modifications
Epigenetic Inheritance

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Epigenetic Inheritance

***Heritable changes in phenotypic frequency across generations, but with NO changes in genotypic or allele frequencies

→ affects what is being exposed to Natural Selection
**Historical perspective**

**Epigenetics**: term coined by C.H. Waddington in 1942. Alterations in gene expression, but where the DNA itself remains unchanged.

Epigenetic modifications have been widely known since the 1970s. Not much in textbook, but watch 13 minute NOVA clip on epigenetics: [http://www.pbs.org/wgbh/nova/body/epigenetics.html](http://www.pbs.org/wgbh/nova/body/epigenetics.html)

**Epigenetic inheritance**: However, that epigenetic modifications could sometimes be inherited is a very recent discovery.

We still do not know how widespread transgenerational epigenetic inheritance is in any organism.
Epigenetic Modifications

• “Epigenetic marks” (methylation, etc.) are made across the genome at each generation to define cell types and patterns of gene expression in the developing embryo. These “marks” define which genes are turned on and off.

• Marks from the previous generation are typically removed in the germline, to enable totipotency of cells in early embryos

• Epigenetic changes could be environmentally induced

• Occasionally the reprogramming is bypassed and some epigenetic marks get passed on → Lamarckian Evolution
Epigenetic Inheritance: Alterations in gene expression that are passed onto the next generation, but where the DNA itself remains unchanged.

NOVA clip on epigenetics if you want more background: http://www.pbs.org/wgbh/nova/body/epigenetics.html
DNA methylation is typically removed during zygote formation and re-established through successive cell divisions during development.
Implications:

**Epigenetic Effects:** The same genome could express different phenotypes: Epigenetic differences could result in phenotypic differences even among identical twins (clones)

**Epigenetic Inheritance:** Really rapid evolution could take place without changes in the genetic code
Outline

(1) Clarification of Concepts
(2) Epigenetic Modifications
(3) Epigenetic Inheritance
(4) Example of Epigenetic Inheritance
(1) Clarification of Concepts

• **Epigenetic effects**: typically not heritable changes in gene expression or cellular phenotype caused by mechanisms other than changes in the underlying DNA sequence. Examples: DNA methylation and histone deacetylation, both of which serve to suppress gene expression without altering the sequence of the silenced genes.

• **Transgenerational epigenetic effects**: These are regarded as effects on the phenotype (or on patterns of gene expression) that are detected across more than one generation and that cannot be explained by Mendelian genetics (or changes to the primary DNA sequence). Example: transgenerational plasticity, maternal effects.

• **Transgenerational epigenetic inheritance**: This term refers to effects on phenotype (or on patterns of gene expression) that are passed from one generation to the next by molecules in the germ cells and that cannot be explained by Mendelian genetics (or by changes to the primary DNA sequence).
Epigenetic Modification (Effect) *versus* Epigenetic Inheritance

Just the occurrence of **Epigenetic Modifications** alone is NOT a sign of **Epigenetic Inheritance**.

The changes that **MATTER** for evolution, whether they are epigenetic or genetic (mutations), **are those that get passed onto the next generation through the *germline*** (eggs or sperm).

Epigenetic modifications that occur in a single generation are one type of **phenotypic plasticity**.
Phenotypic Plasticity

Definition

• Differences in phenotype that a genotype exhibits across a range of environments, because of changes in gene expression

• Changes in gene expression could be caused by environmental cues instigating signal transduction...changes in gene expression could also be caused by epigenetic modifications

• Some traits with a plastic component: intelligence, height, temperature tolerance, salinity tolerance, muscle mass...
Acclimation (≠ Adaptation)

1) Result of **Phenotypic Plasticity**

2) Not heritable (Not inherited)

3) Short term or developmental response within a single generation

4) Arises through differential gene expression or other regulatory mechanism rather than natural selection
Example of phenotypic plasticity, caused by environmental change (not necessarily epigenetic)

Predator induced formation of helmets in Daphnia

Hebert and Grewe, 1985
(2) Epigenetic Modifications
Some Types of Epigenetic modifications
(incomplete list)

- **Chromatin Modification**: epigenetic alteration of chromatin structure, affecting gene expression
  
  **Examples**:
  - Methylation
  - Histone Modifications (acetylation, deacetylation)
  - Chromatin Remodelling

- **RNA-mediated Modifications**: post-transcriptional RNA modifications
  
  **Examples**:
  - RNA interference (RNAi) based mechanism - RNA strands interfere with the transcription of DNA or translation of mRNA
    - Often small RNAs, often those that target transposable elements
    - Seems to occur mainly at retrotransposons and other repeated elements.
Figure 2 Epigenetic machinery and interplay among epigenetic factors. Epigenetic marks are catalyzed by different epigenetic complexes, whose principal families are illustrated here. (a–c) Epigenetic regulation depends on the interplay among the different players: DNA methylation (a), histone marks (b) and nucleosome positioning (c). The interaction among the different factors brings about the final outcome. This figure illustrates selected examples of the possible interrelations among the various epigenetic players.
Consequences of Epigenetic Modifications:
Changes in Gene Expression

-- **Genomic imprinting**: where methylation and histone modifications alter gene expression without altering the genetic sequence. When inherited, these “epigenetic marks” are established in the germline and are maintained throughout all somatic cells of an organism.

-- **Gene Silencing**: could occur through several mechanisms, such as histone modification, mRNA destruction, or RNA interference (RNAi).

-- **Paramutation**: where interaction between two alleles at a single locus, results in a heritable change in expression of one allele that is induced by the other allele. Mechanism is not fully understood, but could occur via methylation or regulatory RNAs. Paramutation violates Mendel’s first law, which states that each allele remains completely uninfluenced by the other. (this is manifest at the phenotypic level, not affecting the genetic code)
Paramutations were first discovered and studied in maize (Zea mays) by R.A. Brink at the University of Wisconsin–Madison (Dept. of Genetics) in the 1950s.

Original Article:
**Epigenetic Modifications**

**Examples:**

**DNA Methylation:** methyl groups are enzymatically added and removed, through the action of methylases and demethylases.

The level of methylation generally correlates with the transcriptional state of a gene: active genes are less methylated than inactive genes.
One way in which DNA Methylation suppresses gene expression

- Methylation at the promoter site or upstream of the promoter

- Methylation of nucleotides in a regulatory element, such as the promoter, or upstream of the promoter, could lead to changes in gene expression.

- Usually, suppression of expression occurs due to interference with binding of the transcription factor.

- Typically more methylation leads to greater suppression of gene expression.
Epigenetic modifications

**DNA Methylation**

- Addition of a methyl group (CH₃) at the 5’ position of cytosine → reduce gene expression

- In adult somatic tissues, methylation usually occurs in the CpG islands, a CG rich region, upstream of the promoter region.

- In embryonic stem cells non-CpG methylation is prevalent (significant cytosine-5 methylation at CpA and CpT; Ramsahoye 2000).
Methyl groups are enzymatically added and removed to DNA, through the action of methylases and demethylases.

The level of methylation generally correlates with the transcriptional state of a gene: active genes are less methylated than inactive genes.

Example of a type of Epigenetic Modification:

**DNA Methylation**

Methyl groups are enzymatically added and removed to DNA, through the action of methylases and demethylases.

The level of methylation generally correlates with the transcriptional state of a gene: active genes are less methylated than inactive genes.
Epigenetic modifications

DNA Methylation

DNA methylation is carried out by a group of enzymes called DNA methyltransferases.

These enzymes not only determine the DNA methylation patterns during the early development, but are also responsible for copying these patterns to the strands generated from DNA replication.
Epigenetic modifications

DNA Methylation

• Crucial part of normal organismal development and cellular differentiation in higher organisms

• Alters gene expression pattern in cells

• Suppresses expression of viral genes and other deleterious elements that have been incorporated into the genome of the host over time

• Plays a crucial role in the development of nearly all types of cancer (hypermethylation, which represses transcription of the promoter regions of tumor suppressor genes)

• Might be the mechanism for learning and long term memory storage
Factors that could affect DNA Methylation

The Environment: such as diet toxins, vitamins, stress, affection (licking, hugging), etc. etc...
Example of DNA Methylation: DNA methylation of the *agouti* gene in mice have been found to cause brown skinny mice... demethylation of this gene (increased expression of *agouti*) results in yellow fat mice
• The Agouti viable yellow (Avy) allele is created by a retrotransposon insertion of an element (intracisternal A particle, IAP) upstream of the agouti locus.

• DNA methylation of the IAP promoter at Avy reduces transcription of the Avy gene (get brown skinny mice)
DNA Methylation (not inheritance): Childhood trauma

- Early childhood trauma can lead to DNA demethylation of the FKBP5 gene in human individuals that possess particular alleles at this gene.

- FKBP5 regulates glucocorticoid receptor activity and stress response. Demethylation of the “AA” genotype leads to excessive glucocorticoid receptor activation.

- Those homozygous for the “A” allele of the FKBP5 gene were more likely to suffer from depression, post-traumatic stress disorder, or anxiety disorders in adulthood, if they were abused as children (causing demethylation at this locus).

http://usrexp-sandbox.nature.com/neuro/journal/v16/n1/full/nn.3275.html
Figure 3 Histone modifications. All histones are subject to post-transcriptional modifications, which mainly occur in histone tails. The main post-transcriptional modifications are depicted in this figure: acetylation (blue), methylation (red), phosphorylation (yellow) and ubiquitination (green). The number in gray under each amino acid represents its position in the sequence.
Histone Deacetylases/ Histone Acetyltransferase

• The ability of a particular transcription factor to bind to its target gene is, in part, dependent on modifications that are made to the histone proteins

• Abnormal activity of HDACs has been observed in acute promyelocytic leukemia, acute myelogenous leukemia, non-Hodgkin lymphoma, and some types of colorectal and gastric carcinoma
Histone Deacetylases/ Histone Acetyltransferase

Histone Acetylation or Deacetylation: affects level of relaxation of the chromatin and level of gene transcription.

- **Histone acetyl transferases (HATs):** Acetylate Histones, **enhance transcription**; acetylation neutralizes positive charges on the histone by changing amines into amides and decreases the ability of the histones to bind to DNA, allowing gene expression.

- **Histone Deacetylases (HDACs):** Deacetylate Histones, **repress transcription**; remove acetyl groups from an $\varepsilon$-N-acetyl lysine amino acid on a histone.
Example of histone deacetylase: Aging

- **Sirtuin** or **Sir2 proteins** are a class of proteins that possess either **histone deacetylase** or mono-ribosyltransferase activity.

- As Histone Deacetylases, Sirtuins regulate transcription
- Sirtuins have been implicated in influencing aging, stress resistance, insulin signaling and metabolism (energy efficiency), and apoptosis and cancer suppression

- Hypothesized that: Caloric restriction $\rightarrow$ activate sirtuins $\rightarrow$ slow down aging
- Drugs that activate sirtuins might also slow down aging
SIRT1 regulates many functions involving apoptosis and cell survival

- For example, cooperates with HIC1 to regulate activity of p53 (tumor suppressor gene) and apoptosis.
DNA Methylation and Histone Deacetylation: Good parenting (transgenerational epigenetic effect)

In rats, mothers that engage in high or low amounts of licking/grooming of their pups

• Increased maternal care results in two types of epigenetic changes: (1) acetylation of histones H3-K9, and (2) demethylation of the transcription factor (NGFI-A) binding site to the promoter of the glucocorticoid receptor → higher GR activity

• Offspring that received high levels of licking/grooming show lower stress response (happy and calm) and become good mothers that lick their pups a lot… leading to the same epigenetic patterns

• Thus, the behavior of licking results in the same epigenetic pattern being passed on (but not Epigenetic Inheritance)

http://www.nature.com/neuro/journal/v7/n8/abs/nn1276.html
DNA Methylation and Histone Deacetylation: Good parenting

• This is an important example to illustrate the difference between a transgenerational epigenetic effect versus transgenerational epigenetic inheritance.

• This is a transgenerational epigenetic effect (maternal effect), but NOT transgenerational epigenetic inheritance.

• ***This is NOT Epigenetic Inheritance because the behavior is passed on, rather than the epigenetic mark itself (through the germ line)... Instead, the epigenetic mark is induced by the behavior of licking at each generation.
(3) Epigenetic Inheritance

- Only those epigenetic modifications that are heritable *via* the gametes (germ line) → lead to epigenetic inheritance.
(3) Epigenetic Inheritance

- Most epigenetic modifications, i.e. epigenetic marks, that are established in most tissues during an organism’s lifetime are irrelevant with respect to the next generation.

  ★ This is because epigenetic modifications are normally erased at each generation. For example, DNA methylation is typically removed (or possibly oxidized; Iqbal et al. 2011) during zygote formation and re-established through successive cell divisions during development.

  ★ And also because only epigenetic modifications of the mature gametes (and not in other tissues) have the potential to contribute to the phenotype of the offspring, the next generation.

- Only those epigenetic modifications that are heritable via the gametes (germ line) lead to epigenetic inheritance.
Epigenetic Inheritance

Alterations in gene expression *that are passed onto the next generation*, but where the DNA itself remains unchanged

Still a new frontier (this field is still extremely new), with many examples in plants and fewer concrete examples in animals
Childhood stress linked to transmission of miRNA levels to the next generation
(Dickson et al. 2018. Reduced levels of miRNAs 449 and 34 in sperm of mice and men exposed to early life stress. Translational Psychiatry. 8:101)

- Exposure of male mice and male humans to early life stress alters levels of specific sperm miRNAs that promote stress-associated behaviors in their offspring
- Increased childhood stress levels in both mice and men led to reduced levels of multiple miRNAs of the miR-449/34 family
- After mating stressed male mice, these sperm miRNA reductions persisted in both early embryos through at least the morula stage and in sperm of males derived from them, suggesting these miRNA changes contribute to transmission of stress phenotypes across generations (needs to be replicated)
Conclusive examples of Epigenetic Inheritance are more common in plants.

**Flower Symmetry**

In the toadflax *Linaria vulgaris*, radially symmetric flowers, rather than the typical bilaterally symmetric ones, is caused by DNA methylation of the CYCLOIDEA gene, which controls the formation of dorsa petals.
Conclusive examples of Epigenetic Inheritance are more common in plants

**Epigenetic Trigger for tomato ripening**

- Some tomatoes do not ripen and remain green.
- These tomatoes have a heritable cytosine hypermethylation of the Cnr gene promoter, which inhibits expression of the Cnr gene

http://usrexp-sandbox.nature.com/nbt/journal/v31/n2/full/nbt.2497.html
Hypermethylation of the Cnr promoter results in inhibition of RIN transcription factor binding, preventing Cnr gene expression and fruit ripening.

http://usrexp-sandbox.nature.com/nbt/journal/v31/n2/full/nbt.2497.html
Inconclusive Examples
(not conclusive; many studies do not clearly distinguish between transgenerational plasticity and epigenetic inheritance)

- Methylation at *FKBP5* gene in Holocaust survivors and their offspring. This gene affects glucocorticoid response (stress response), and is linked to PTSD and depression (Rachel Yehuda et al. 2015) → needs more study, as there is no evidence that the stress was passed through the germline rather than by parental behavior

- Results are not clearly interpretable (http://blogs.plos.org/biologue/2015/07/07/human-disease-epigenomics-2-0/)

- Mice exposed to toxins pass on fear of the odor of that toxin onto their offspring (not 100% conclusive)
  http://www.nature.com/news/fearful-memories-haunt-mouse-descendants-1.14272
• Examples of Epigenetic **Inheritance** are much more common in plants and more conclusive.

• Epigenetic inheritance itself is more common in plants than in animals likely because developmental programs are more flexible in plants; At least in mammals epigenetic marks tend to be more thoroughly removed at each generation.

• Studies need to be designed to more clearly distinguish between transgenerational epigenetic effects (e.g. transgenerational plasticity) and transgenerational **epigenetic inheritance**.
Which changes alleles frequencies?
Which changes genotype frequencies?

• Selection
• Genetic Drift
• Inbreeding
• Recombination
• Mutations
• Migration (Gene flow)
• Epigenetic modifications
Questions:

1. What is the difference between a transgenerational epigenetic effect versus epigenetic inheritance?

2. What is the necessary evidence required to conclude that Epigenetic Inheritance has actually occurred?

3. How does methylation/demethylation affect gene expression? What about histone acetylation/deacetylation?

4. What is the evidence for Epigenetic Inheritance in animals versus plants?

5. Is there conclusive evidence for Epigenetic Inheritance in animals?
1. Which of the following is **FALSE** regarding epigenetic modifications based on current evidence?

(a) Methylation typically suppresses gene expression  
(b) Acetylation of a histone increases expression of a gene  
(c) Most epigenetic modifications are added during development and are then erased at the next generation  
(d) Short strands of RNA can interfere with gene expression  
(e) The inheritance of epigenetic modifications has been found to be common in animals
2. Which of the following is an example of **transgenerational epigenetic effect**?

(a) An acetyl group is added to chromatin and increases expression of a gene
(b) Methyl groups are added to regulatory regions upstream of a gene, causing suppression of gene expression
(c) Small RNAs attach to DNA and suppress gene expression
(d) A male rat is exposed to a toxic chemical, leading to methylation of a gene that induces fear of its odor.
(e) A mother rat licks her pup, leading to methylation of a gene that leads to low stress and a positive disposition in the pup. The pup then grows up to become a mother that licks her pups, leading to methylation of the same gene in her pups.
Answers

• 1E

• 2E