Transgenerational Nutrition

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Overview of talk

- Research Program
- Observations – Ca/P conditioning
- Long term impacts of Environment (Diet)
- Epigenetics
- One Carbon Metabolism – Feeding the machinery
- Challenges in Poultry Production
- Can we feed the next generation(s)?

Nutrition and Performance

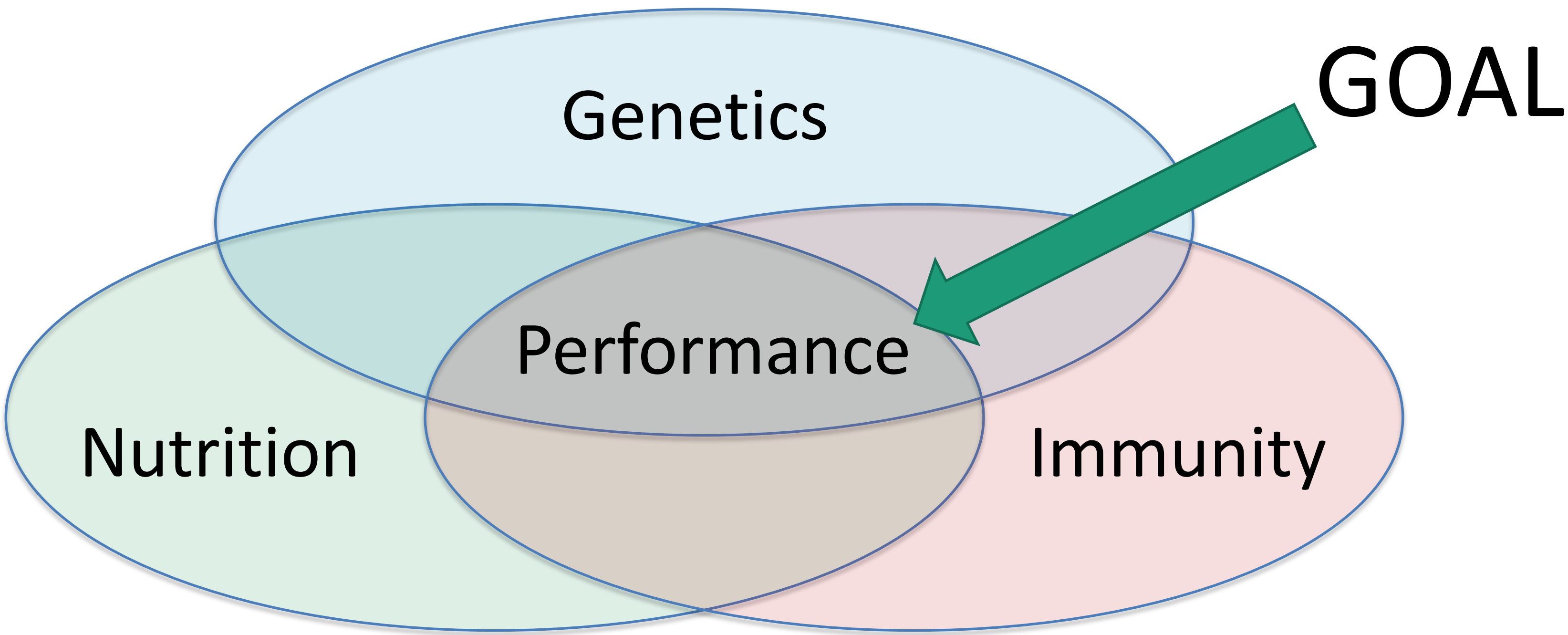
Diet B

Diet A

In life there is no such thing as a free lunch.

You can only cut the pie (genetic resources) into so many pieces.

Ultimately the “pie” is finite, there is a limit to the genetic potential of an organism.



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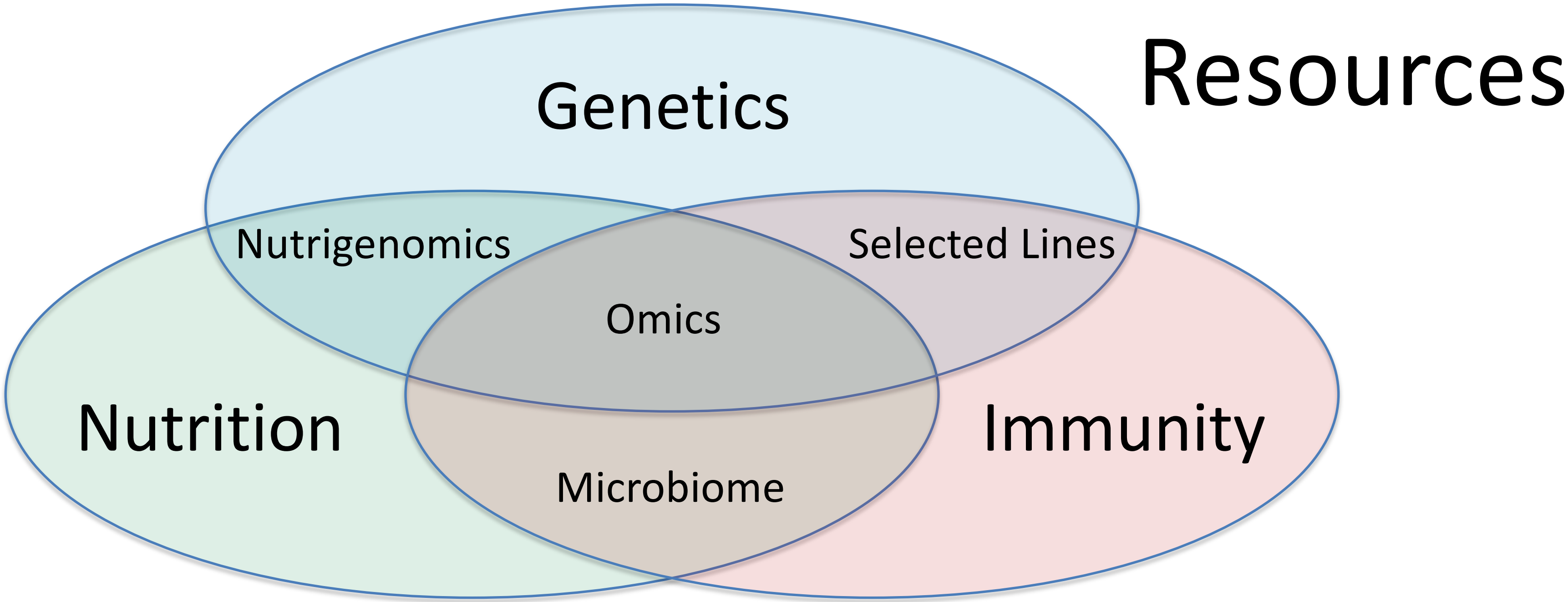
Professor of Animal Genomics, Biotechnology, Comparative Biomedical Sciences, Physiology, and Poultry Science



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PROGRAM

Resource allocation

GWAS

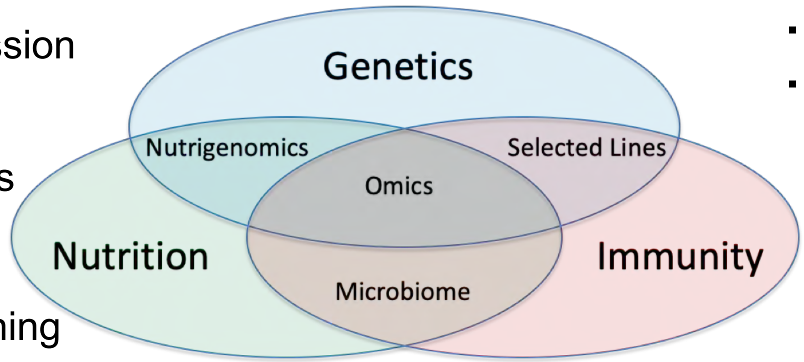
- Antibody response
- Comb/Feather/Polydact
- Pigmentation
- Heat Resistance

Nutrigenomics

- Feed additives
- Enzymes
- Gene Expression

Selected lines

- HAS/LAS – Ab
- HIM/LIM – Necrotic Enteritis
- RES/SUS - Arteriosclerosis



Feed Ingredients

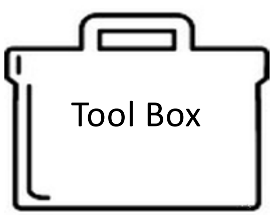
- Novel antimicrobials
- Hemp meal
- Protein
- Nutritional conditioning
- Methyl donors

Antibody Response

- Vaccine response
- Diet

Microbiome

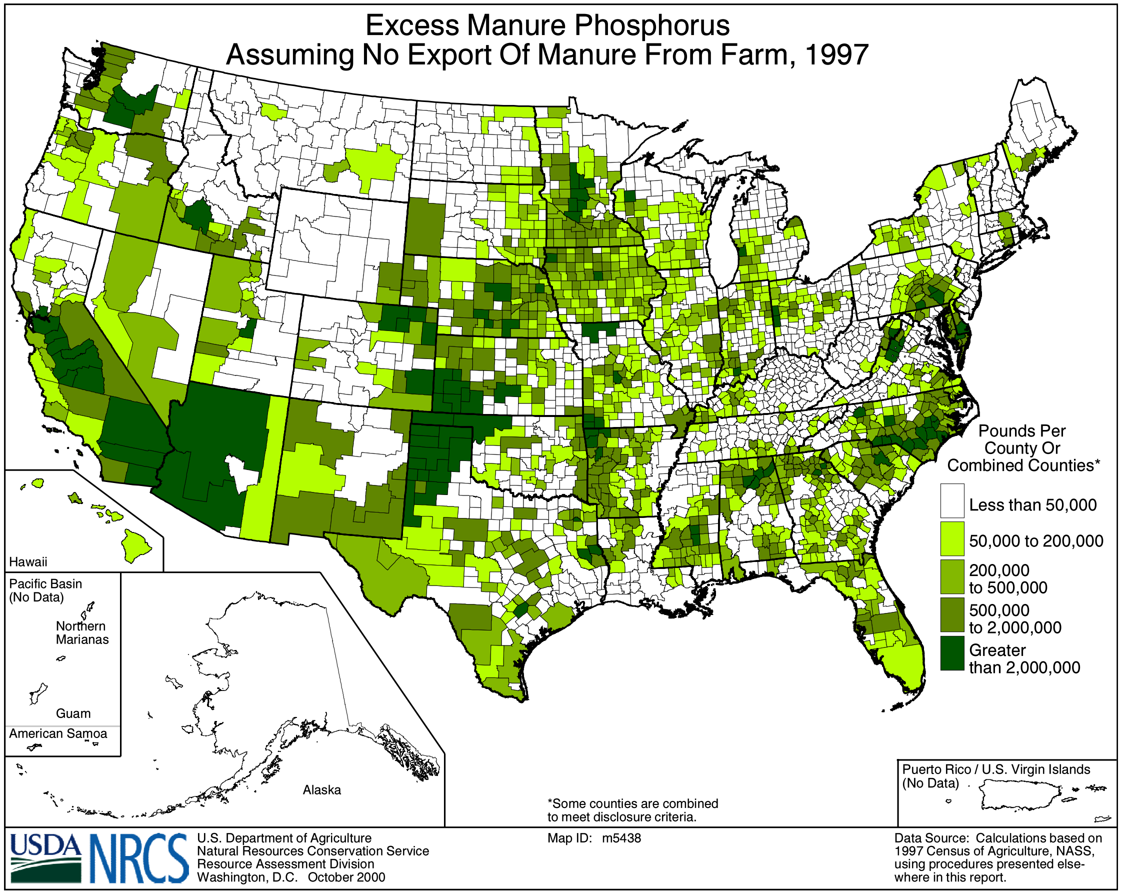
- Host interaction
- Diet



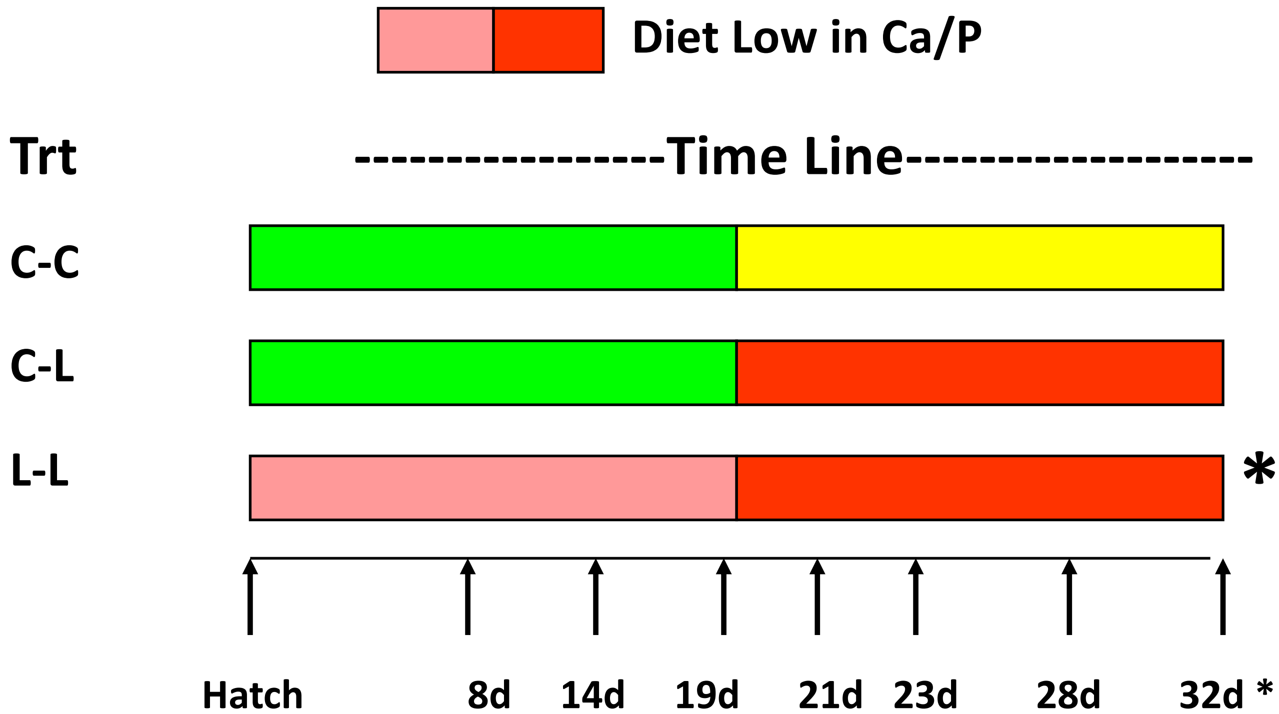
RNAseq
Genome Resequencing
Epigenetics

P = G + E + epi

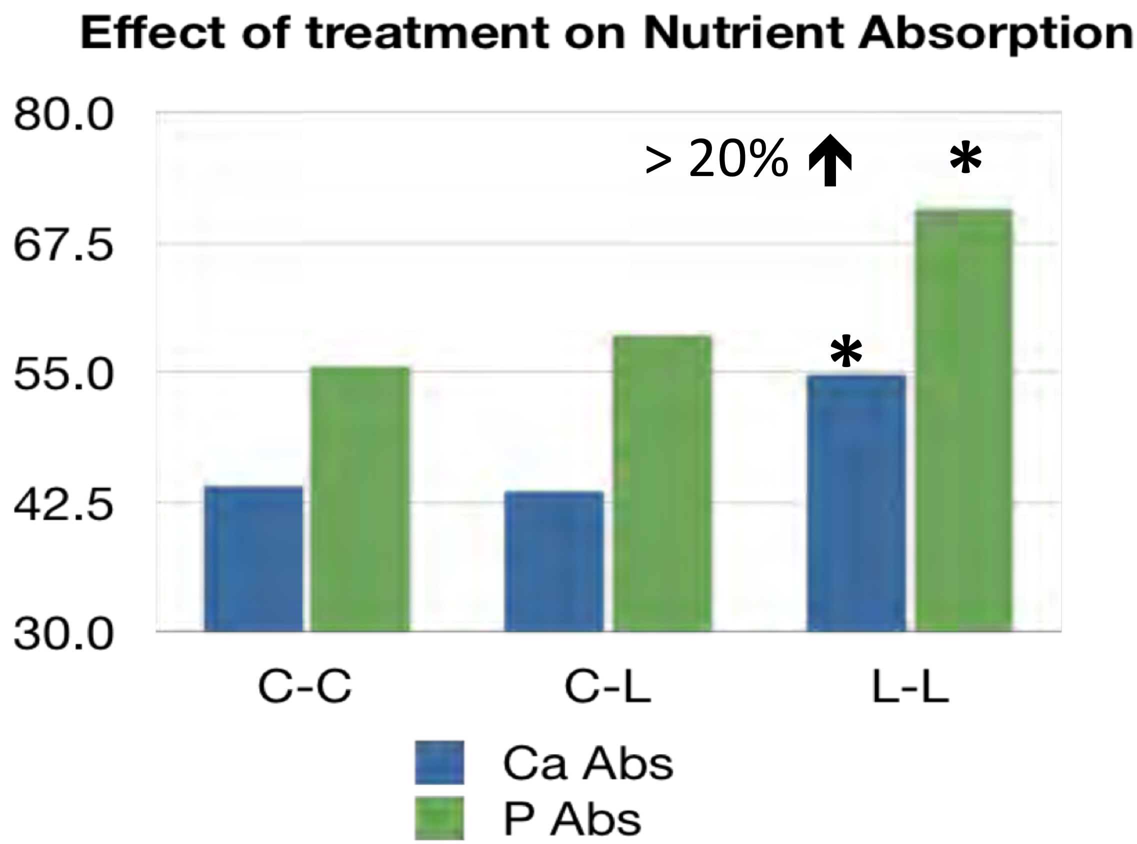
The practical problem: Manure Phosphorus



Early Nutrient Restriction

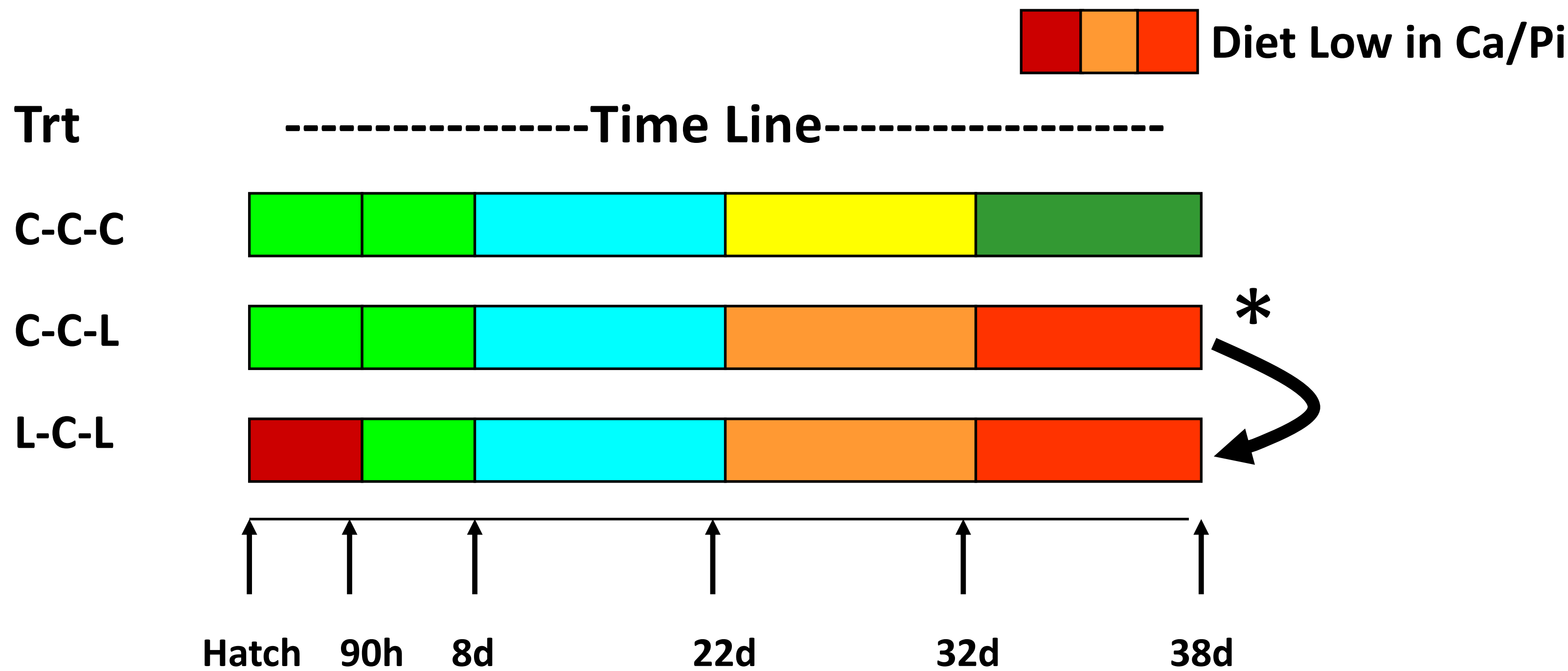


Yan et al, 2005



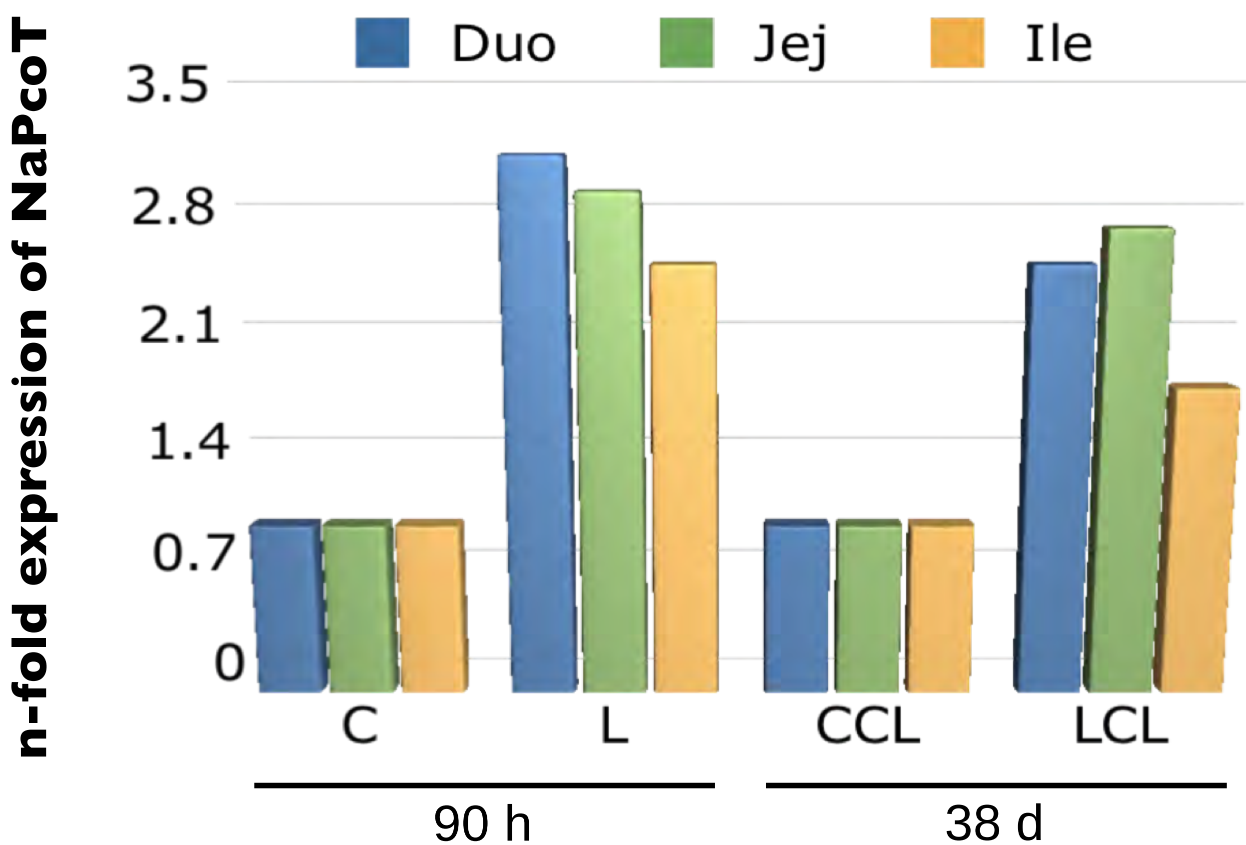
Yan et al, 2005

Long term impact of dietary conditioning



* ↑ BW ↓ feed/gain ↑toe ash ↑Ca abs ↑ P abs

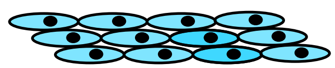
Long term impact of dietary conditioning

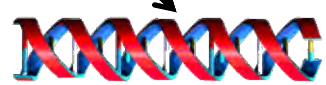


Mechanisms of Programming?

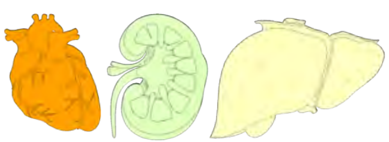
Nutritional Influence

Altered Cell Number or
intracellular organization





Reorganization of organ structure
Abnormal early cell-cell interactions?



Metabolic Differentiation
DNA Control?
DNA Environment?
Altered DNA methylation?
Mitochondrial DNA?

Epidemiological Observations:
Fetal origin of Adult Disease- The Barker Hypothesis

- 1989 David Barker found in inverse relationship between birth weight and death from heart disease in UK.

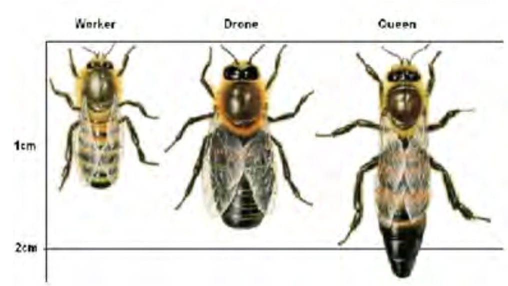
- Studies confirmed by "Dutch Hunger Winter" when food was cut off in occupied Netherlands. Individuals born during this time, and their offspring have high incidence of insulin-resistance as adults.
- "Fetal Origin of Adult Disease" confirmed for:
  - Coronary heart disease
  - Hypertension
  - Type II diabetes
  - Metabolic disorders, ect…

Epigenetics and Diet- Honeybee

- Larva destined to become a queen is fed large quantities of royal jelly by worker bees (nurse)
- Royal Jelly consumed by queen larva inactivates the Dnmt3 gene so genes can remain active and result in queen characteristics

- When Dnmt3 is active it silences genes in bee larva and inhibits them from developing into a queen

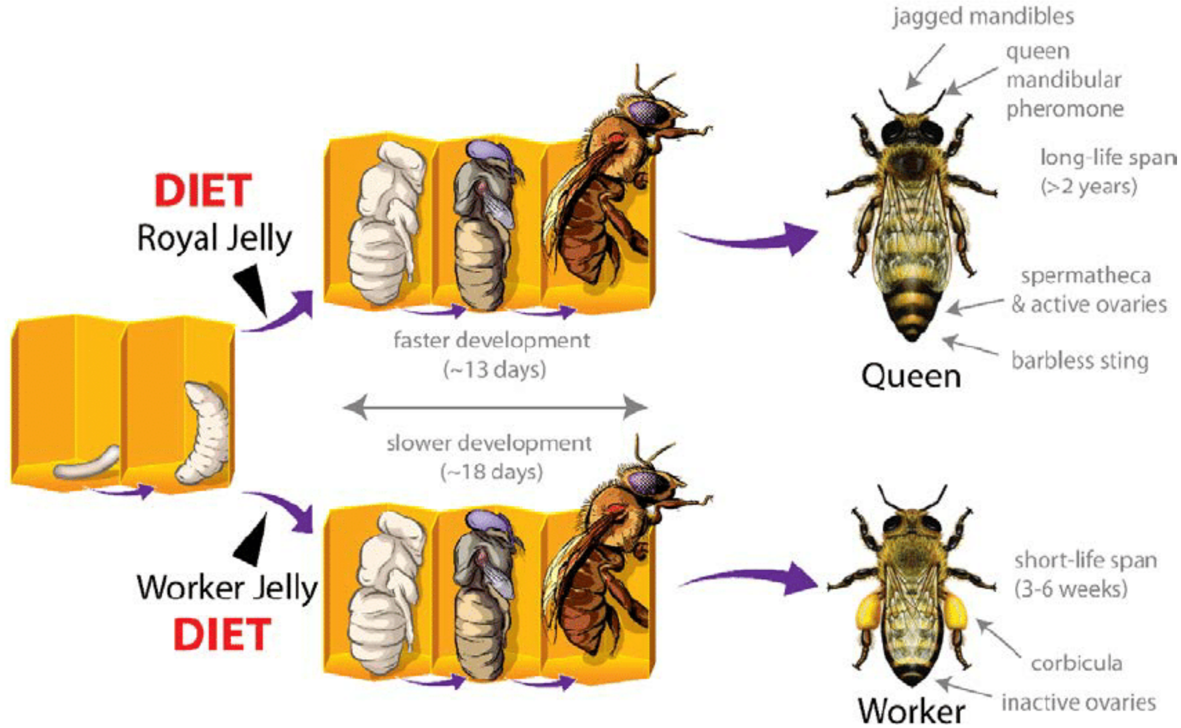
- Effects morphology, physiology, life span and behavior
  - Egg laying
  - Kills rival queens
  - Produce communication sounds
  - Pheromone production
  - Mating flights



Epigenetics - Transgenerational vs. Developmental Programming

Solitarius Gregarious

Ernst et al, 2015



Cridge et al, 2015

Transgenerational Epigenetics

Gregarious Solitarious

Schistocerca gregaria locust (left) and Schistocerca gregaria grasshopper (right).

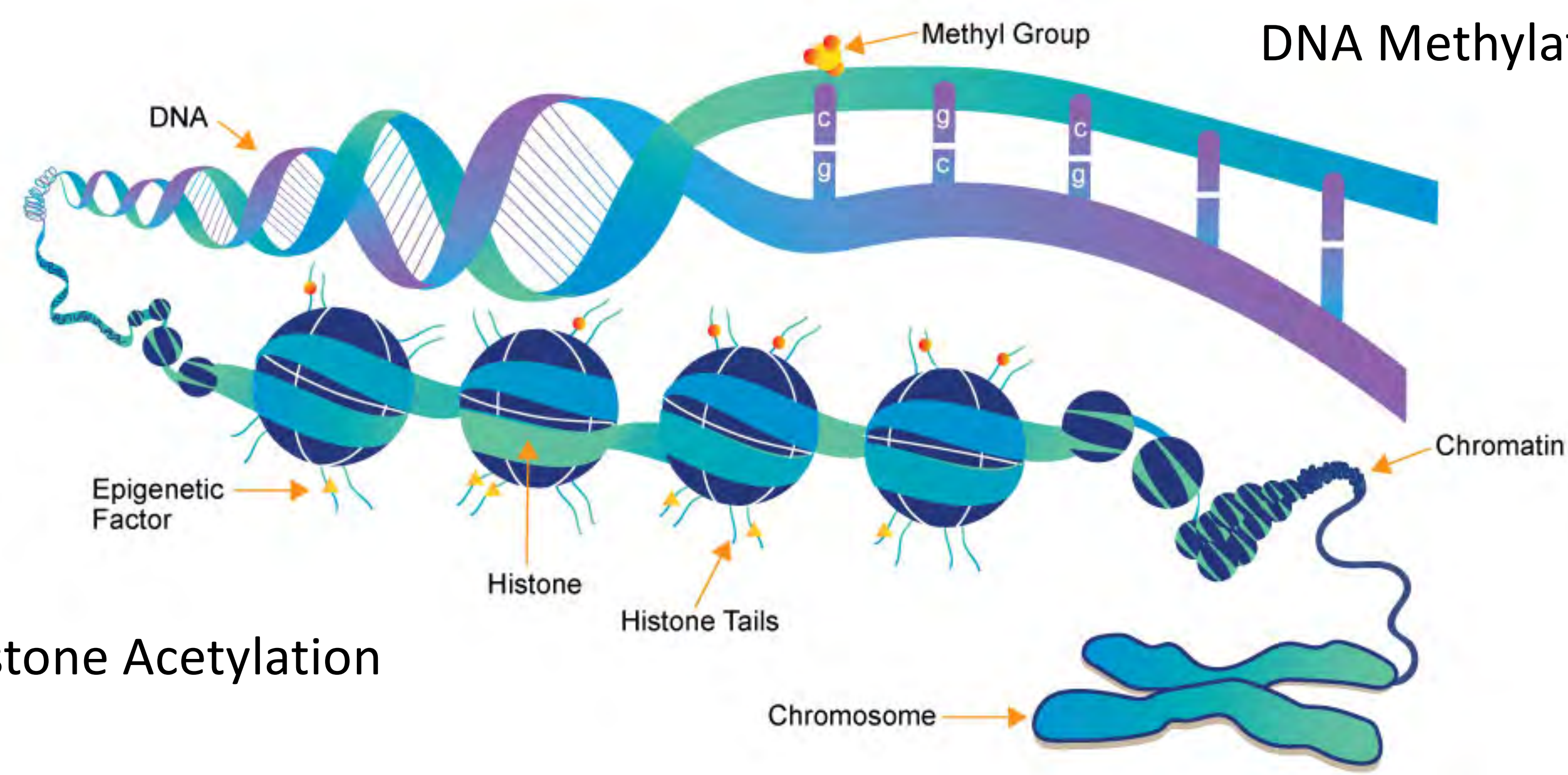
The hind legs of the locust are set at a different pitch. The wings are smaller while the brain is larger, and yet these two insects are the same species.

The grasshopper can transform into a locust in a matter of hours with the right stimulus (hormones, physical or visual stimulation), and will maintain this phenotype for 3-4 generations if returned to low density.

Pener & Yerushalmi

Epigenetic Modifications

DNA Methylation



Histone Acetylation

Dietary impact on DNA methylation status

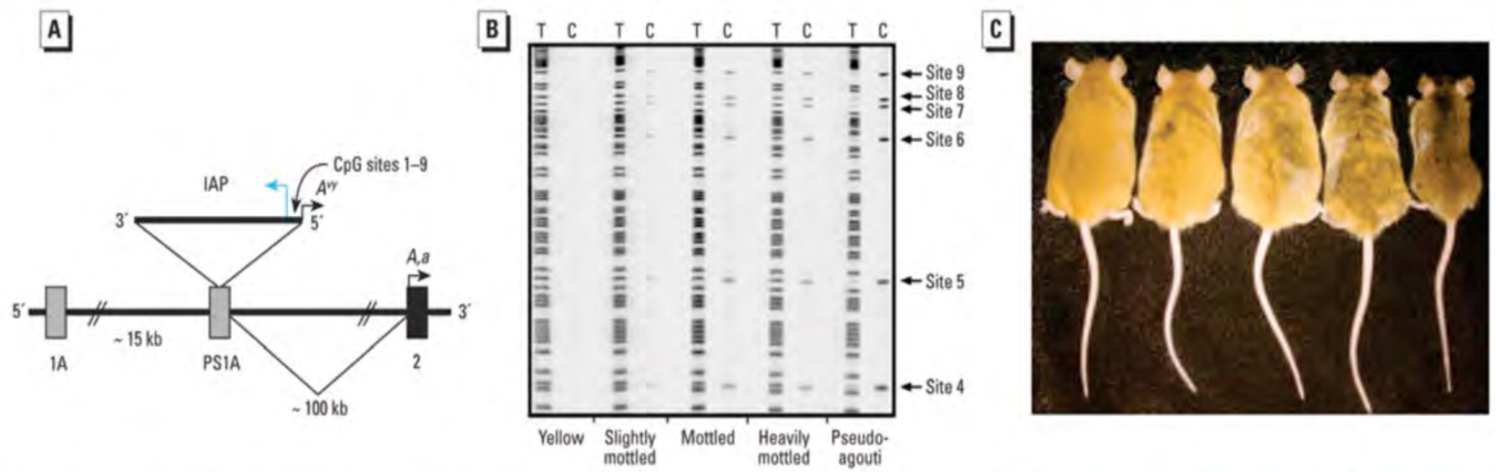
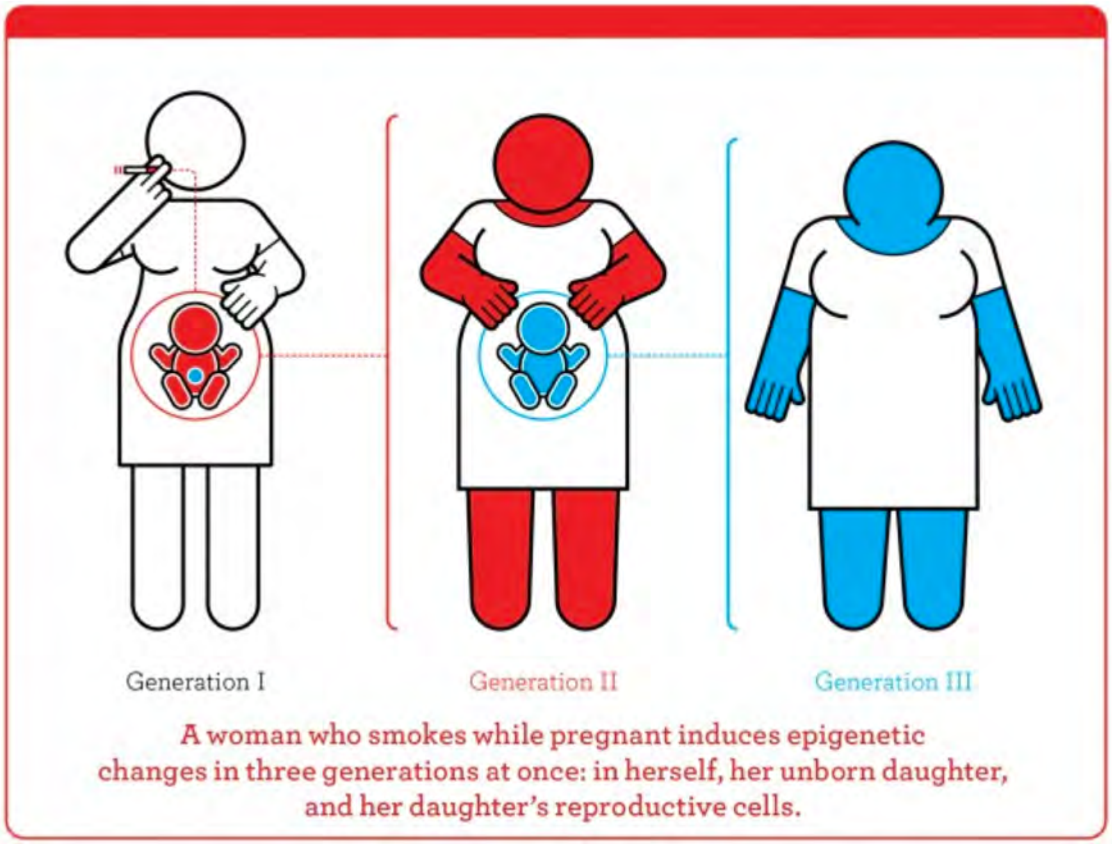


Figure 1. Methylation status of CpG sites within the A^{vy} IAP in genetically identical A^{vy}/a littermates. (*A*) A contraoriented IAP insertion within pseudoexon 1A (PS1A) of the murine *Agouti* gene. A cryptic promoter (short arrow labeled A^{vy}) drives ectopic *Agouti* expression. CpG sites 1–9 are oriented in the 3′ to 5′ direction with respect to the IAP insertion, as shown. Transcription of *A* and *a* alleles initiates from a hair-cycle–specific promoter in exon 2 (short arrow labeled *A,a*). (*B*) Pseudoagouti animals exhibit the highest degree of CpG methylation at sites 4–9. Bisulfite sequencing reveals increasing intensity of the cytosine lane at CpG sites 4–9 within the A^{vy} IAP in genetically identical A^{vy}/a animals representing the five coat classes. (*C*) Genetically identical week-15 A^{vy}/a mouse littermates representing the five coat-color phenotypes.

Transgenerational Nutrition



Environmentally induced epigenetic transgenerational inheritance

Environmental toxicants

Agricultural fungicides (Vinclozolin)

Agricultural pesticides (Methoxychlor)

Industrial contaminants (Dioxin/TCDD)

BPA and phthalates (Plastic compounds)

Herbicides (Atrazine and glyphosate)

Insect repellants (Permethrin and DEET)

Pesticides (DDT)

Industrial toxicants and biocides (Tributyltin)

Hydrocarbons (Jet fuel JP8)

Heavy metals (Mercury)

Other types of exposure

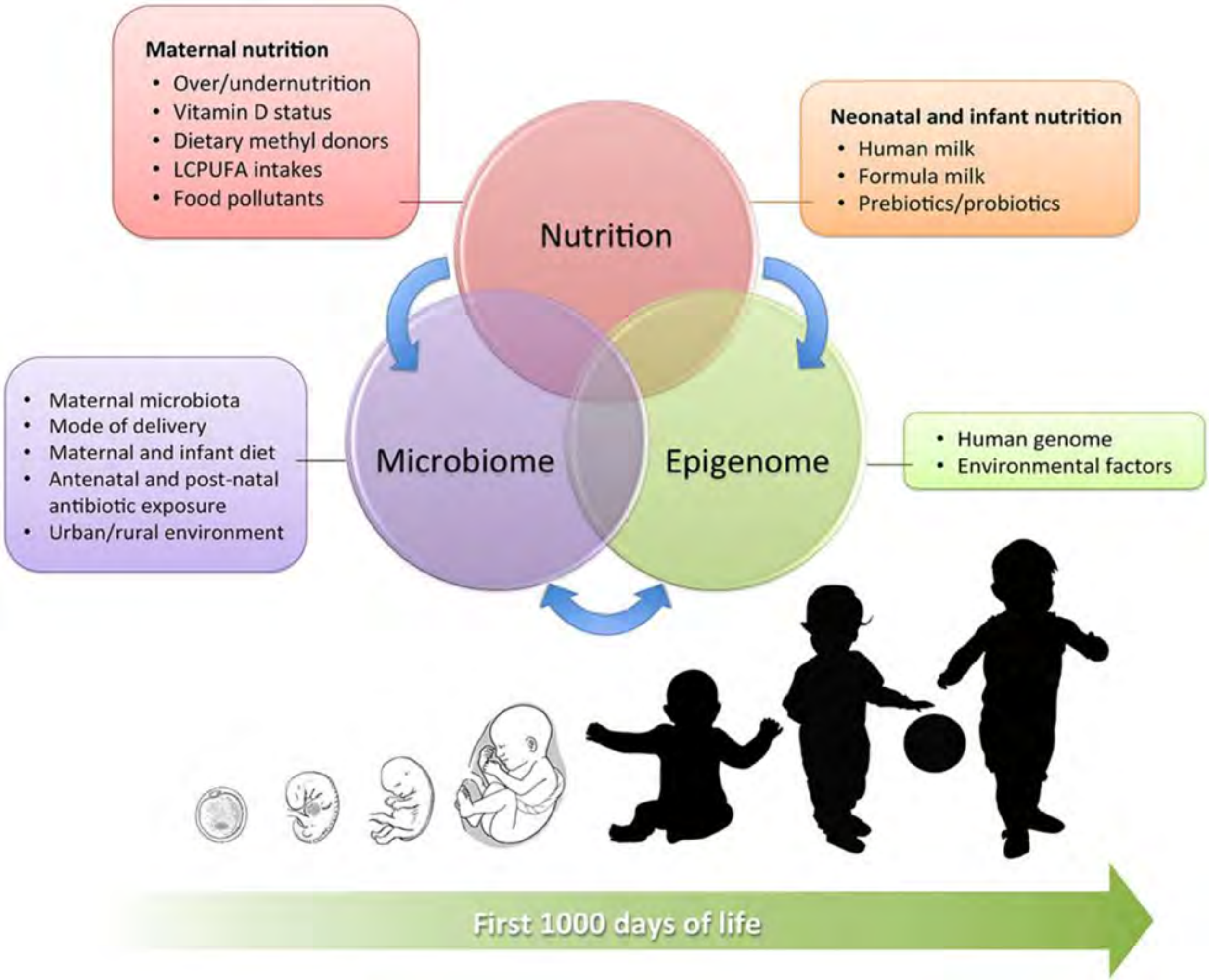
Nutrition (High fat or caloric restriction)

Temperature and drought (Plant health and flowering)

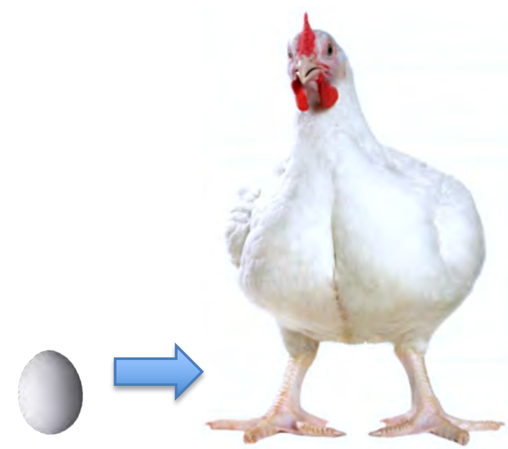
Smoking and alcohol

Stress and trauma (behavioral)

King and Skinner, 2020



Poultry as a model for probiotics, microbiota, and other factors effects on long term health



First ~63 days of life

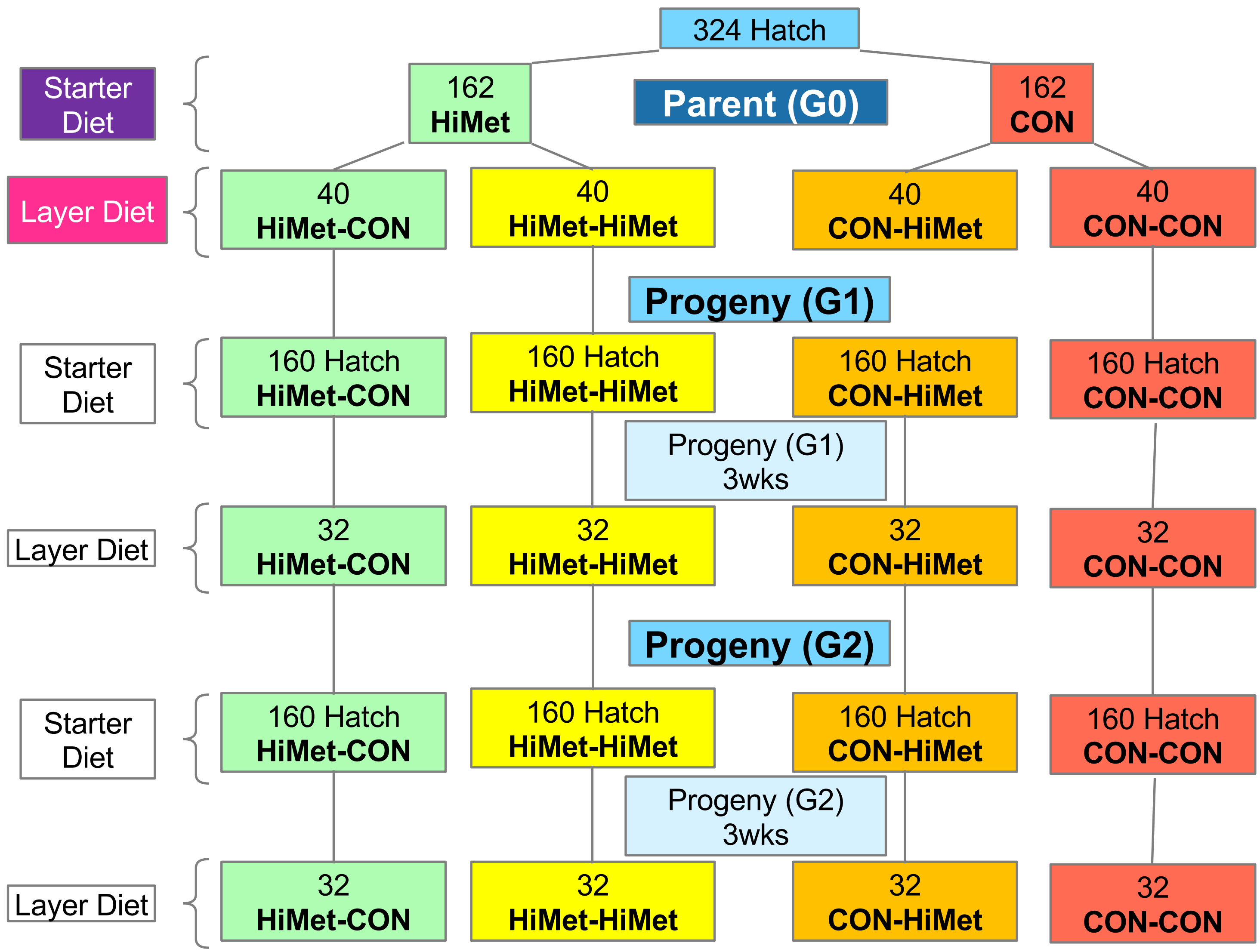
Looking for Transgenerational evidence

- Evaluate dietary methyl donor effects
  - Reproductive and Growth Performance
  - Gene Expression
  - Individual gene methylation patterns
  - Epigenetic effects of diet on progeny performance across multiple generations

PhD – Chelsea Phillips

Trial Design

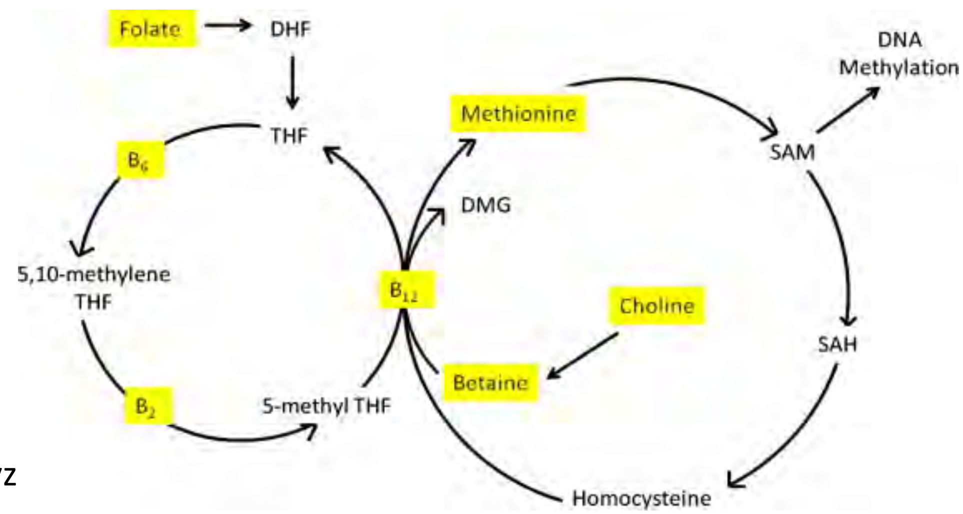
Japanese Quail were chosen as a model due to their short generation intervals



Starter Diet | Layer Diet

~ 10x of Choline Chloride, Betaine, B12, Folic acid, B6, Zn Sulfate

Dietary Methyl donors



- **<u>Choline</u>**: Oxidized to betaine as a source of methyl groups in forming methionine from homocystine *McGinnis et al. (1944)*
- **<u>Betaine</u>**: Methyl Donor
- **<u>B12</u>:** coenzyme of methionine synthase which catalyz
- **<u>Folic Acid</u>:** carrier of single carbon units as 5-methyl THF which is donated to homocystine for the formation of methionine *McKay et al. (2011)*
- **<u>B6</u>:** Coenzyme to hydroxymethyltransferase which converts THF to 5,10-methylene THF
- **<u>Zinc</u>:** cofactor required for amino acid metabolism and DNA modification

Nutrient Specifications

Methylation package (10X)	%	mg/kg
Choline Chloride 98%	0.5	7030
Betaine , 99%	0.5	5
B12, 98%	0.0015	1.5
Folic acid 95%	0.075	7.5
B6 (Pyridoxine 98%)	0.012	12
Zn Sulfate 99%	0.00987	99
Total methylation	1.09837	

Starter Nutrients	Amount
Crude Protein (%)	27.9
Met. Energy (KCAL/KG)	2,850
Calcium (%)	1.00
Avail. Phos. (%)	0.50
Met, total (%)	0.7392

Layer Nutrient	Amount
Crude Protein (%)	22.6
Met. Energy (KCAL/KG)	2,800
Calcium (%)	2.6
Avail. Phos. (%)	0.45
Met, total (%)	0.64

Trial Parameters

Phenotypes Collected for Each Generation					
G0 Parents	BW	# of Eggs	Egg Wt	Body Composition	DNA Methylation Seq Data, RNAseq
G1 Progeny	BW	# of Eggs	Egg Wt	Body Composition	DNA Methylation Seq Data, RNAseq
G2 Progeny	BW	# of Eggs	Egg Wt	Body Composition	DNA Methylation Seq Data, RNAseq

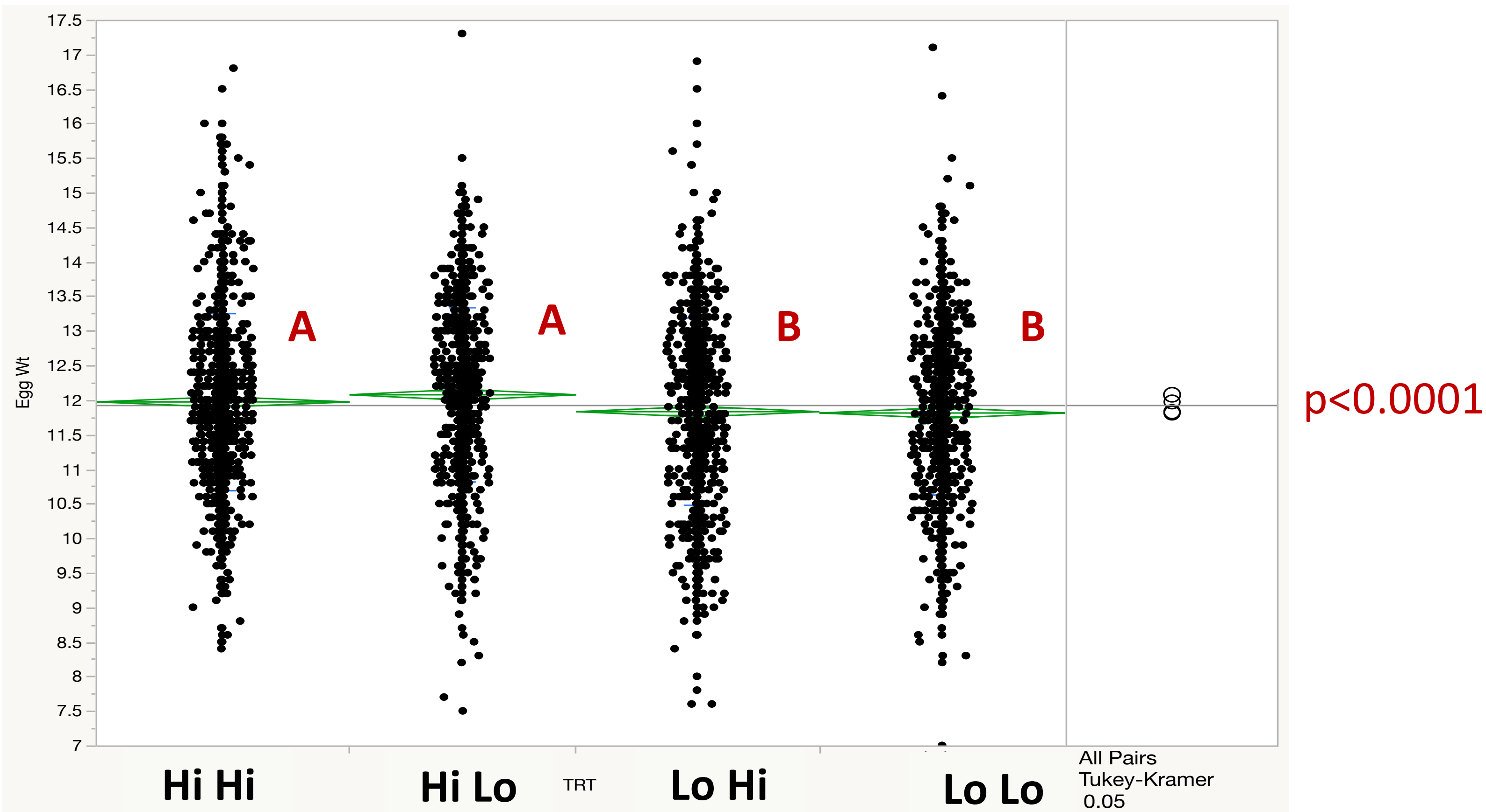
Phillips and Ashwell, unpublished

Trial Parameters

Significantly Different Phenotypes Collected for Each Generation			
G0 Parents	**21d BW** p<0.0001	**Egg Wt** p<0.0001	**Liver Wt as % BW** p<0.0028
G1 Progeny	**Hatch BW** p<0.0001	**Egg Wt** p<0.0001	
G2 Progeny		**Egg Wt** p<0.0001	

Phillips and Ashwell, unpublished

Progeny (G1) Egg Wt (g)

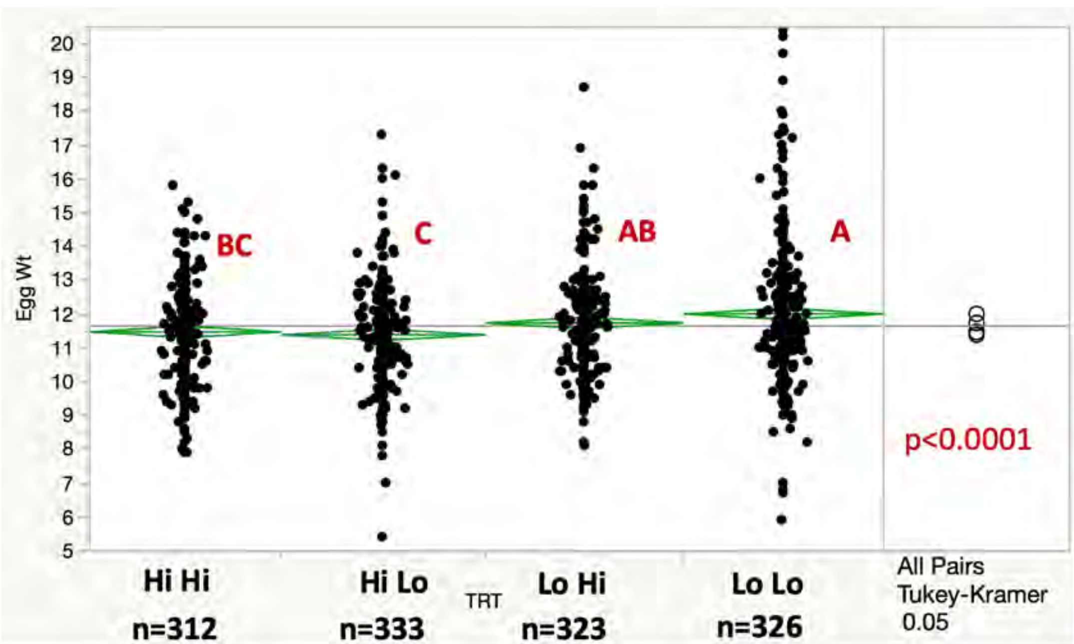


n=1106 **n=990** **n=1063** **n=1079**

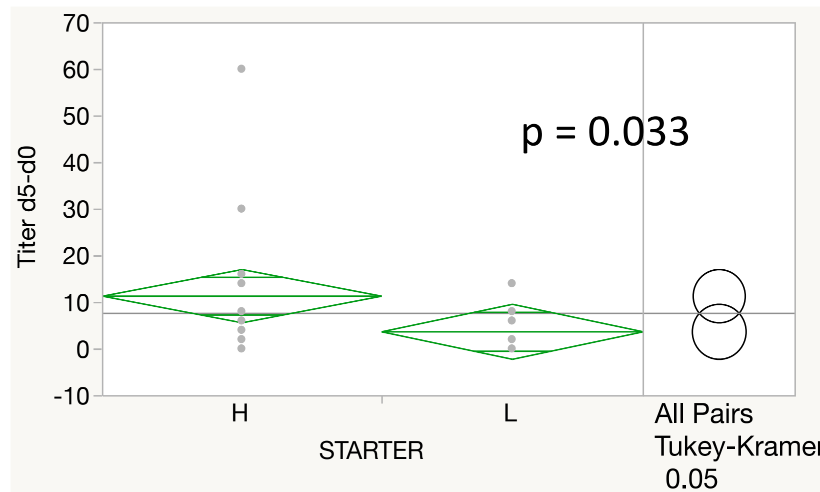
Phillips and Ashwell, unpublished

Transgenerational Nutrition

Progeny (G2) Egg Wt (g)



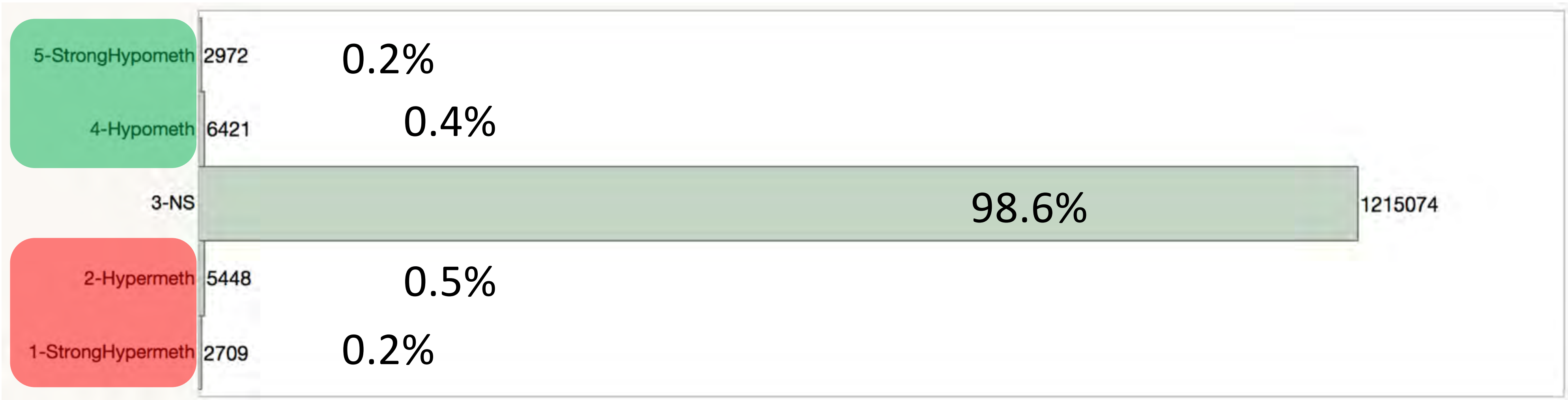
G0 HiMet Diet effects on
G2 SRBC d5 Ab Titer



HA - 0.1ml of 0.25% SRBC injected IV

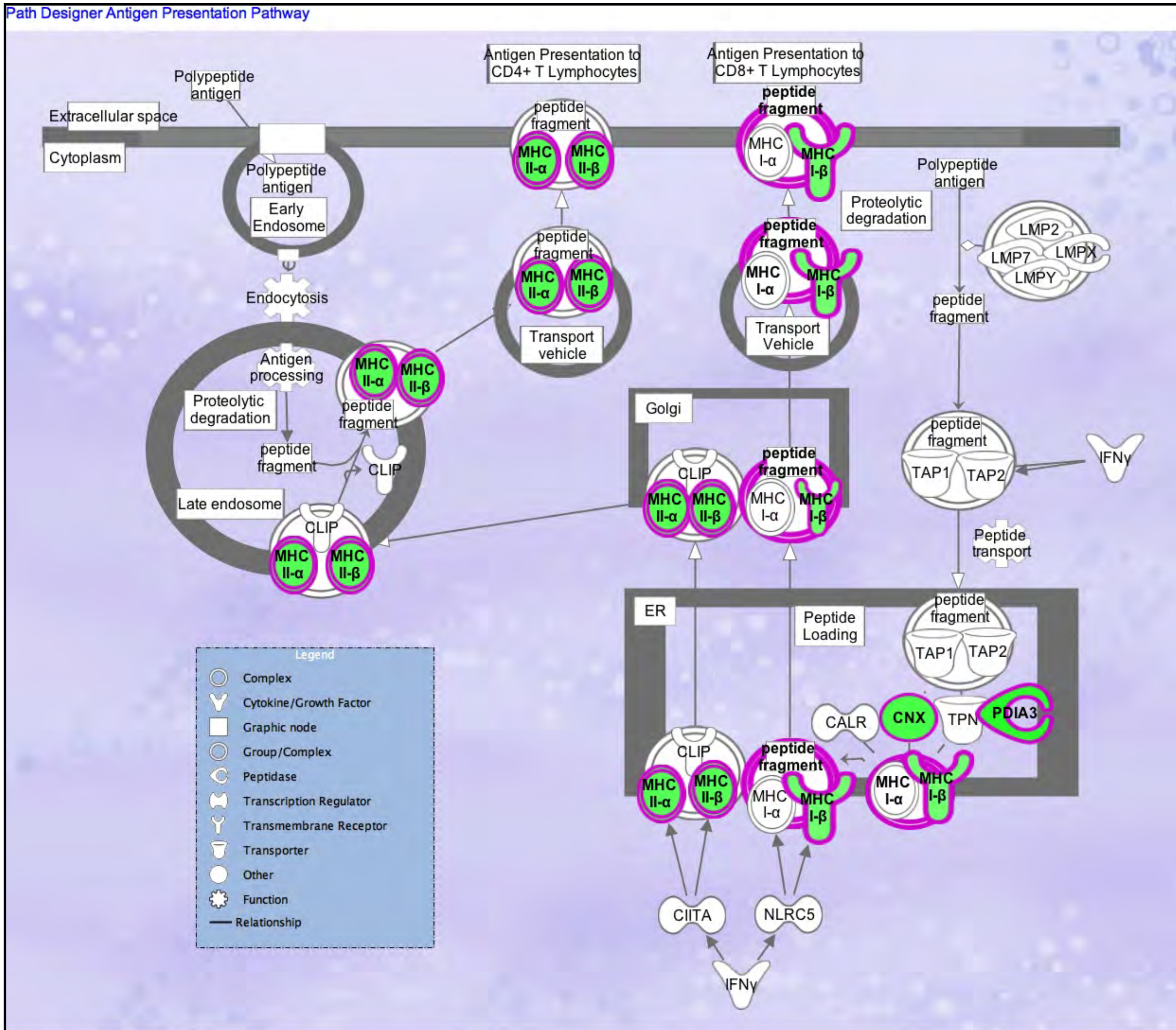
Phillips and Ashwell, unpublished

Classification of CpG changes resulting from diet – G0



- 1,232,624 CpGs had sufficient reads to make a statistical inference
- Average reads per CpG site - 39.3
- ~1.8 million CpGs predicted in chicken, ~70% coverage
- 98.6% of CpG sites are unchanged
- Pathway analysis of the ΔMeth loci – Ingenuity

Phillips and Ashwell, unpublished



Antigen Presenting Pathway Hypomethylation & Hypermethylation

MHC Class II Molecules

- Antigen Presenting Cells
- Initiate Immune Response

Common on antigen presenting cells but can be induced on other cells by interferon γ

MHC II expression is regulated by CIITA

INF γ triggers the expression of CIITA

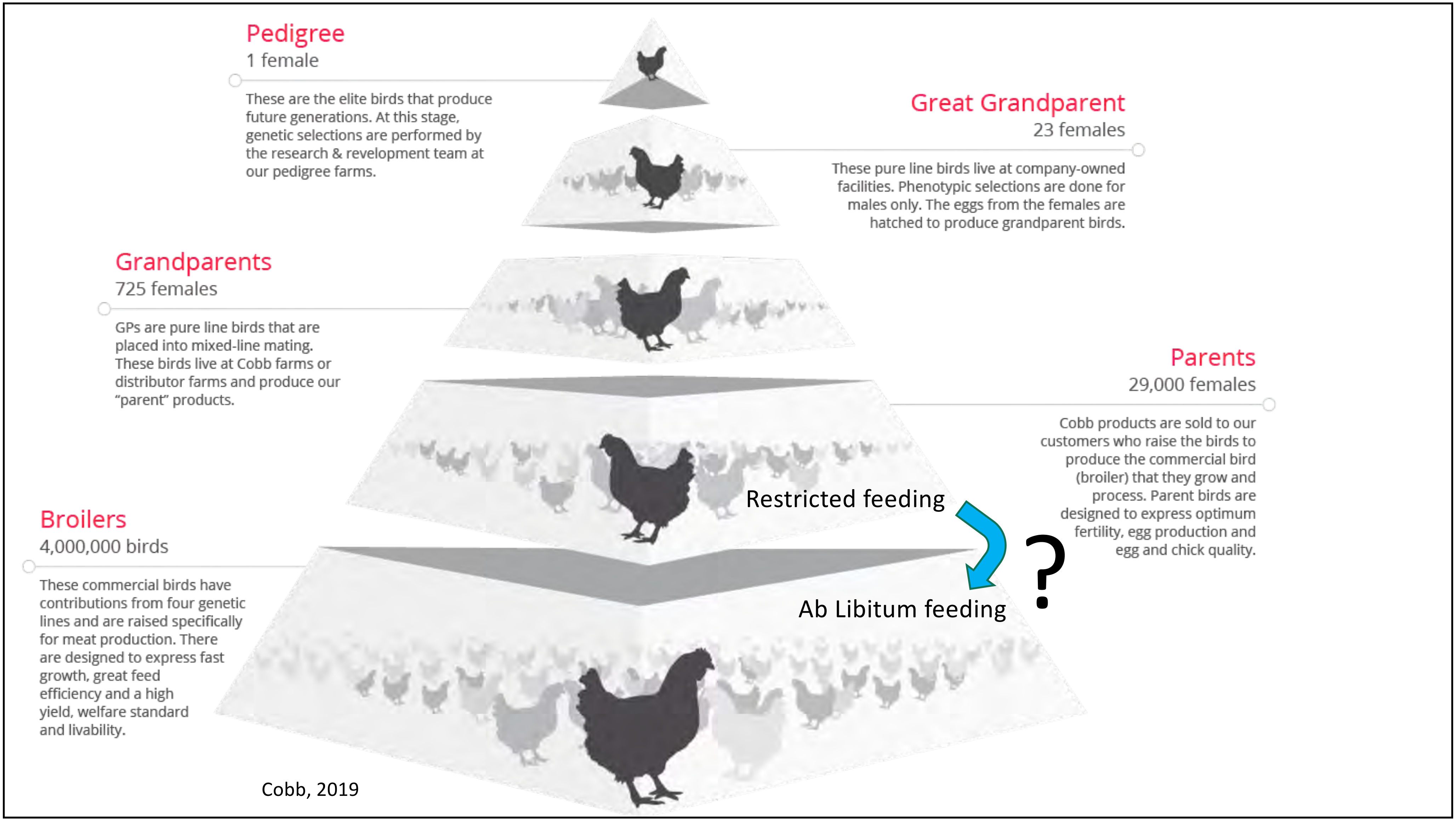
Triggered immune response

- Inflammation, swelling
- Possible B Cell activation

Insights….and future

- Diet impacts offspring for at least 2 generations
- Current industry practice must effect commercial broilers
- Investigate loci associated with egg size and determine if the methylation pattern correlates with the phenotypes
- Challenge or stress studies?
- Treatment effect in a single sex?

- **Current industry practice must effect commercial broilers**


Pedigree
1 female
These are the elite birds that produce future generations. At this stage, genetic selections are performed by the research & revelopment team at our pedigree farms.
Great Grandparent
23 females
These pure line birds live at company-owned facilities. Phenotypic selections are done for males only. The eggs from the females are hatched to produce grandparent birds.
Grandparents
725 females
GPs are pure line birds that are placed into mixed-line mating. These birds live at Cobb farms or distributor farms and produce our "parent" products.
Parents
29,000 females
Cobb products are sold to our customers who raise the birds to produce the commercial bird (broiler) that they grow and process. Parent birds are designed to express optimum fertility, egg production and egg and chick quality.
Restricted feeding
?
Ab Libitum feeding
Broilers
4,000,000 birds
These commercial birds have contributions from four genetic lines and are raised specifically for meat production. There are designed to express fast growth, great feed efficiency and a high yield, welfare standard and livability.
Cobb, 2019

Evaluate the impact of Precision Feeding

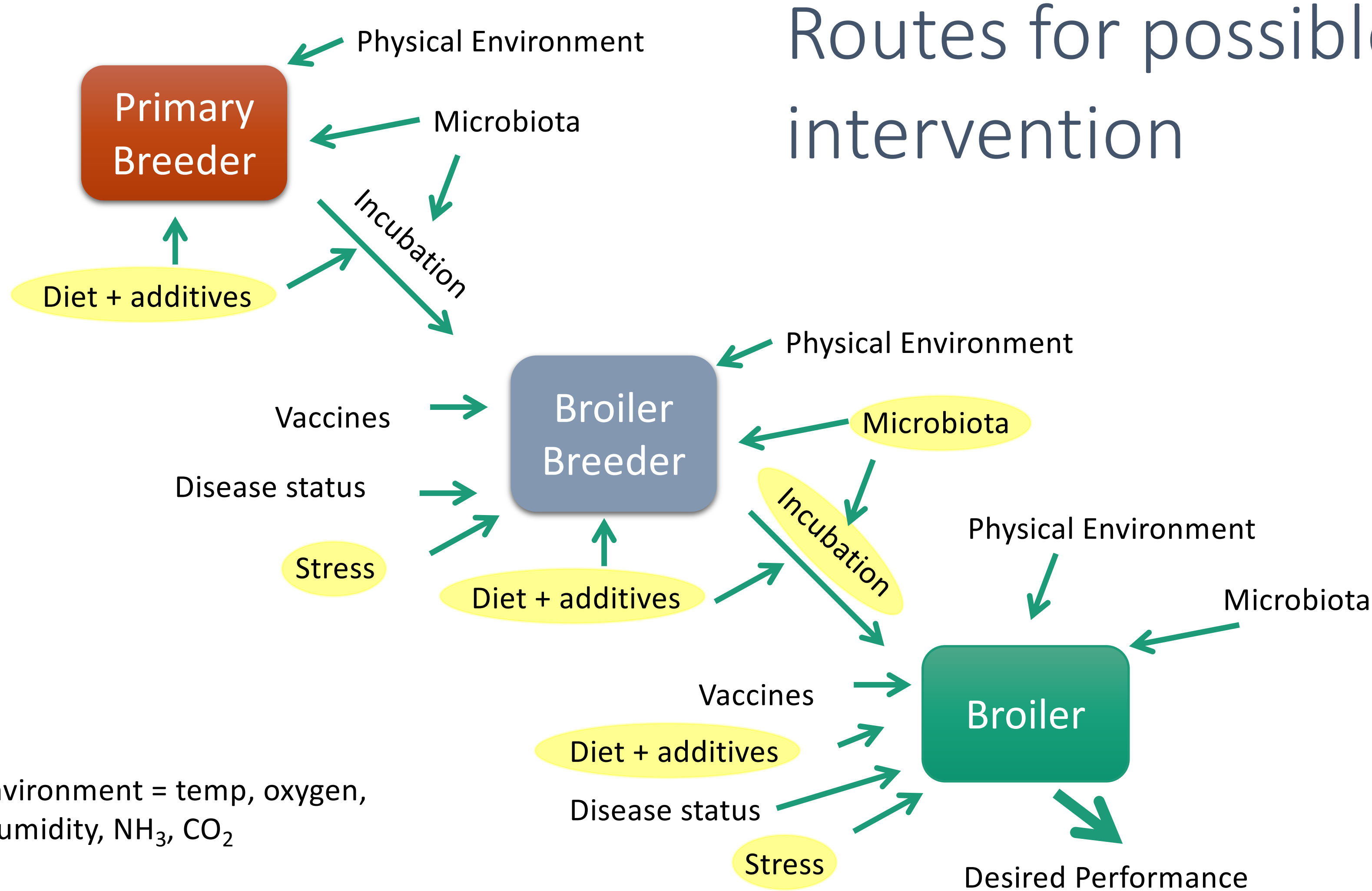
Breeders fed to increased body weights increases FCR in broiler progeny

Investigating the impact of graded feeding on breeder and broiler progeny gene expression patterns – altered stress pathways

Impact on DNA methylation?

Collaboration with Martin Zuidhof- University of Alberta

Routes for possible intervention



Physical Environment = temp, oxygen, housing, humidity, NH_3, CO_2

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- Chandler Keck
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Thank you for your attention

Questions?