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Biomarker discovery for the black soldier fly (*Hermetia illucens*)

Elida Espinoza, PhD

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Session 50: Insect Genetics and Reproduction

Industrial scale, micro farming of the black soldier fly (BSF)

- EnviroFlight has spent the past 5 years rearing BSF as the first United States commercial-scale BSFL production facility
- A short generation time and high reproductive rate make the BSF a great insect to farm
- Highly palatable and nutritious, our feedstock ingredients are defined by AAFCO, registered with the FDA

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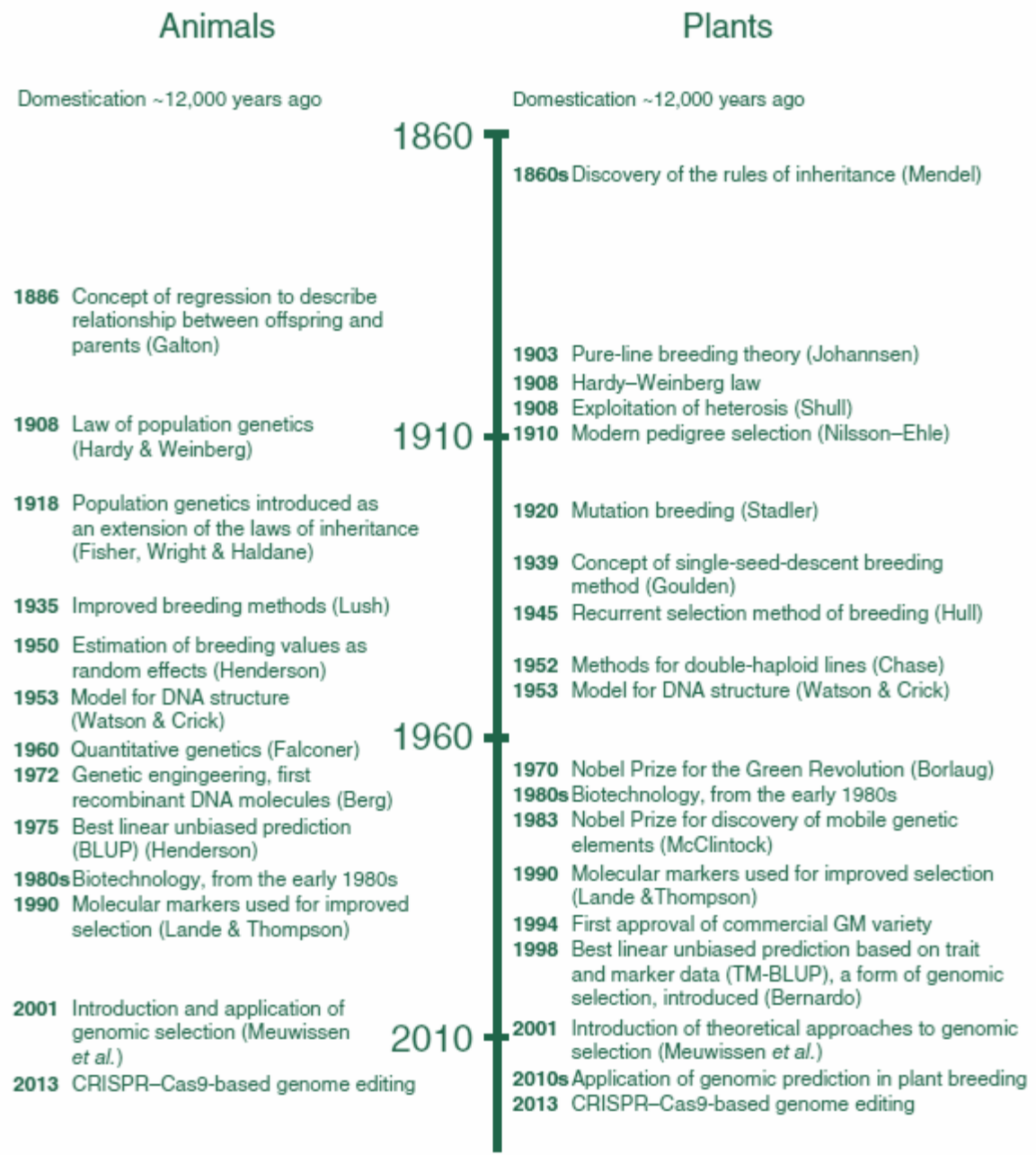

Figure 1 Some key milestones of selective animal and plant breeding.

From domestication to selective breeding

Today, breeders can target genes for selection and monitor their expression, establishing target values.

Hickey et al. 2017
Nature Genetics

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BSF Target Values for Commercial Production

External: The Customer, The Consumer

- Nutrition and Value-Added
  - Protein content
  - Lipid Content
  - Antimicrobial Peptides (AMP)

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BSF Target Values for Commercial Production

Internal: The Black Soldier Fly

- Growth, Development and Reproduction
  - Feeding efficiency
- Health and Immunology
  - Disease Susceptibility
  - Environmental Stressors

wikipedia.
com

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BSF Biomarker Pipeline

Discovery

- BSF Transcriptome
- BSF Proteome
- BSF Metabolome
- BSF Homologs

Analytical Validation and Testing

- Define performance characteristics
- Test conditions (biotic and abiotic)
- Specificity
- Sensitivity
- Accuracy
- Precision

Functional Validation and Testing

- Test populations (families/strains) and subpopulations (age, sex, life stage)
- Determine predictive values

Implementation and Monitoring

- When to use a biomarker?
- Optimizing use of biomarkers in experiments and breeding operations

BSF Homologs… what is available now?

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Arthropod species can serve as blueprint

- *Drosophila melanogaster* (fruit fly)
- *Apis mellifera* (European honey bee)
- *Bombyx mori* (Silkworm moth)
- *Litopenaeus vannamei* (Pacific white shrimp)

These species are well-studied and have a diverse literature focusing on growth, reproduction, behavior, health, and disease.

The fruit fly, *Drosophila melanogaster*, is one of the most comprehensively studied model organisms, supported by decades of research, advanced genetic tools and genomic resources.

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Discussion Outline

1. Growth, Development, and Reproduction Biomarkers
2. Nutritional Biomarkers
3. Health and Immunological Biomarkers
    - The Black Soldier Fly
4. Synteny and Gene Homology
    - The Fruit Fly and BSF

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Growth, Development and Reproduction Biomarkers

Efficient Resource Utilization

- By identifying developmental biomarkers related to feed efficiency and growth rate, animal husbandry practices can be optimized.

- This leads to reduced resource wastage, lower production costs, and increased productivity, ultimately benefiting both producers and the environment.

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Nutritional Biomarkers

Optimized Nutrition

- Dietary recommendations based on their unique biological responses.

- By identifying biomarkers that reflect specific nutrient needs and deficiencies, appropriate nutrition can be developed to optimize health and well-being.

The ideal nutritional needs of BSF are unknown.

As a decomposer, BSF can consume a wide variety of organic waste, but we're all too familiar with the variance that can bring about in their development.

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Health and Immunological Biomarkers

Health Monitoring

- Enables effective monitoring of a species health and well-being, especially during critical life stages and in various environmental conditions.

- These biomarkers can help track normal growth patterns and identify deviations that might indicate underlying health issues.

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Stressors can be detrimental to insect health

The environment (abiotic and biotic) plays a crucial role in insect rearing

- Feed substrate (as habitat/bedding also)
- Ambient temperature
- Container temperature (“microhabitat”)
- Microorganisms in substrate (e.g. bacteria)

Heat shock proteins (HSPs) are ubiquitous and conserved protein families in both prokaryotic and eukaryotic organisms, and they maintain cellular proteostasis and protect cells from stresses.

HSPs are commonly used as biomarkers of environmental stress.

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HSPs in BSF

Gianetto et. al. 2017 *Gene*

HSPs (*Hsp70* and *Hsp90,* respectively *Hihsp70 and Hihsp90*)

- 2nd instar larva and 5th instar larva BSF
- Differential expression, *Hihsp90* upregulation in 5th instar larva

Malawey et. al. 2021 *JIFF*

- Male and female adult BSF, 4 and 7 days old
- Temperature: 18℃, 27℃, 33℃
- Differential expression in older M only

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HSPs in BSF – What more to explore?

We have barely started to scratch the surface…

- Broader range of temperatures, and more specificity within the range of temperature, e.g. 27℃ - 38℃; and 36℃, 37℃, 38℃, 39℃, 40℃
- Longer duration or exposure to condition, e.g. days vs. hours
- Longitudinal, life history studies
  - Follow same cohort of individuals
  - Monitor impacts of condition throughout various life stages
  - Generational impacts, and lasting effects of stress

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Synteny and Gene Homology with other Diptera

- **Synteny** refers to the conservation of gene order and arrangement between different species' genomes.
- **Gene homology** indicates the presence of similar genes with shared ancestry in different species.
- Comparing synteny and gene homology between species helps reveal evolutionary relationships, identify functional genes, and understand genomic rearrangements.

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Synteny and Gene Homology with other Diptera

Example:

D. melanogaster and *Anopheles gambiae* (malaria mosquito)

- 113 pairs of putative orthologs of the two species
- 41-73% of the known orthologous genes remain linked in the respective homologous chromosomal arms

(Bolshakov et al. 2002 *Genome Res.*)

wikipedia.
com

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Gene homology of *H. illucens* vs. *D. melanogaster*

Assembly:
iHerIll2.2.curated.20191125 (GCF_905115235.1)

(Generalovic et al. 2021
Gene
Gene

Species	Gene	Perc. Ident.	BLAST Hill Accession #	Hill Gene ID
Drosophila melanogaster	*ple*	78.18%	XP_037905372.1	LOC119648026
Drosophila melanogaster	*Pu*	92.92%	XP_037914340.1	LOC119653634
Drosophila melanogaster	*Ddc*	80.47%	XP_037909367.1	LOC119650592
Drosophila melanogaster	*Vmat*	71.53%	XP_037918023.1	LOC119655925
Drosophila melanogaster	*Catsup*	61.14%	XP_037919987.1	LOC119657241
Drosophila melanogaster	*DAT*	78.63%	XP_037924324.1	LOC119660014
Drosophila melanogaster	*Trh*	75.14%	XP_037906744.1	LOC119648909
Drosophila melanogaster	*SerT*	82.53%	XP_037917149.1	LOC119655363
Drosophila melanogaster	*Tdc2*	82.08%	XP_037903401.1	LOC119646852
Drosophila melanogaster	*Tbh*	69.28%	XP_037919231.1	LOC119656744
Drosophila melanogaster	*Hdc*	74.68%	XP_037903092.1	LOC119646648
Drosophila melanogaster	*tadr*	51.47%	XP_037903554.1	LOC119646935
Drosophila melanogaster	*lovit*	63.72%	XP_037912607.1	LOC119652494
Drosophila melanogaster	*CarT*	68.97%	XP_037903774.1	LOC119647085
Drosophila melanogaster	*ebony*	60.25%	XP_037912663.1	LOC119652533
Drosophila melanogaster	*tan*	63.23%	XP_037919807.1	LOC119657119

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Final Remarks

It's important to note that the process of developing genetic biomarkers can vary depending on:

- the condition being studied
- the technology available
- the regulatory requirements of different regions

Additionally, advancements in genomics and technology continue to shape how genetic biomarkers are identified and utilized.

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Merci!

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Elida Espinoza, PhD
Entomologist – Genetics R&D
eespinoza@enviroflight.net

www.enviroflight.net