Emerging Trends in Agricultural Diagnostics

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Talk Outline

Genetic Research Background
  • Reproductive biology - lizard
  • Conservation genetics – bird

Diagnostic Biotechnology
  • qPCR

Current NAGC research
  • Pathogens – honey bees
  • Pathogens – row crops
  • Traits – weeds
Undergraduate Interest - Reptiles

NDSU
Masters Research

Do females have genetically diverse clutches?
Masters Research

Do females have genetically diverse clutches? **Yes!**

Paternity Analysis (1 locus)

- **Mother**: 172, 182
- **Baby 1**: 182, 204
- **Baby 2**: 172, 204
- **Baby 3**: 182, 204
- **Baby 4**: 164, 172
- **Baby 5**: 154, 182
Ph.D. Research

Can translocated birds boost genetic diversity in an endangered population?

2006-2009
110 females

MN
WI
Ph.D. Research

Can translocated birds boost genetic diversity in an endangered population?

**mtDNA diversity restored to near historic levels**
Wildlife to Agriculture

**Graduate Work**

- Image of a lizard
- Image of a prairie chicken

**Career**

- Image of bees
- Image of soybeans
- Image of crops
NAGC At A Glance

“To translate scientific discoveries into solutions for production agriculture, functional foods, and bioenergy.”

Staff

40+ years of Lab Experience

ISO Accredited Testing Lab

Collaborators
What motivates us

• Bridging the gap between research and practical applications in biotechnology

• Diagnostics is lagging in agriculture compared to livestock & human health

• Pest & pathogens are top threats to crop supplies and products
PCR-based Diagnostics
Polymerase Chain Reaction (PCR)

Molecular copier for small segments of DNA

Diagnostic tool to:

- Detect and quantify pathogens
- Detect unique traits in organisms
Overview of PCR-based Test Development

| Identify genetic target | Find **unique** genomic regions | Design primers to amplify target with **PCR** | Use detection aids (probes) with light-sensitive equipment |

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**DNA Sequence Alignment**

Non-target

Target

Non-target

Arrows = DNA differences between target and non-targets
PCR-based Diagnostics

Collection → Extract → Amplify

Bees → Bees → Pathogen
PCR-based Diagnostics

Extract  
Amplify  
Analyze

Bee  
Pathogen 1  
Pathogen 2

Pathogen 1  
Pathogen 2  
Negative

Multiplex

Fluorescent Signal

Amplification Cycle
PCR-based Diagnostics

**Analyze (Cₜ)**

- **Fluorescent Signal**
  - High
  - Low

- **Amplification Cycle**
  - 0
  - 14
  - 30
  - 40

**Standard Curves**

- **Cycling Threshold (Cₜ)**
  - 0
  - 14
  - 30
  - 40

- **Pathogen Quantity**
  - 0
  - 100,000

- **Pathogen 1**
- **Pathogen 2**
High-throughput

Quantify
Diagnostic Research at NAGC

Bees

Crops

Weeds
Honey Bee Crisis

What’s Killing American Honey Bees?
Benjamin P. Oldroyd

Honey bee colony losses in Canada.

Declines of managed honey bees and beekeepers in Europe

Bees brought to their knees: microbes affecting honey bee health
Jay D. Evans and Ryan S. Schwarz
United States Department of Agriculture (USDA)—Agricultural Research Service (ARS) Bee Research Laboratory, Beltsville Agricultural Research Center (BARC) East Building 476, Beltsville, MD 20705, USA
Multiple factors contribute to colony persistence

Nutrition
Weather
Pesticides
Genetics
Pathogens
Mites
Management
Honey Bee Pathogen Panel

**Dicistroviridae**
- Acute Bee Paralysis Virus (ABPV)
- Black Queen Cell Virus (BQCV)
- Israeli Acute Bee Paralysis Virus (IABPV)
- Kashmir Bee Virus (KBV)

**Iflaviridae**
- Deformed Wing Virus-A (DWV-A)
- Deformed Wing Virus-B (DWV-B)
- Sacbrood Virus (SBV)
- Slow Bee Paralysis Virus (SBPV)

**Unclassified RNA viruses**
- Chronic Bee Paralysis Virus (CBPV)
- Lake Sinai Virus-1 (LSV1)
- Lake Sinai Virus-2 (LSV2)

**Bacteria**
- *Melissococcus plutonius*
- *Paenibacillus larvae*

**Fungi**
- *Nosema ceranae*
- *Nosema apis*
Beekeepers move colonies between states
Beekeepers move colonies between states
Beekeepers move colonies between states
Objectives. How is colony strength associated with pathogen loads in migrating honey bees?

Migration Routes
- ND-CA-ND Route
- ND-TX-CA-TX-ND Route
- ND-Storage-CA-*-ND Route
*location of storage facility varied among operations

Study Overview – Colony Visits
- Feb 2019 (CA)
- June/July 2019 (ND)
- Aug/Sept 2019 (ND)
- Feb 2020 (CA)
- June/July 2020 (ND)
- Aug/Sept 2020 (ND)

Collected at Visit
- Colony Strength
- Varroa Mite Counts
- Bees for Pathogen Panel

In partnership with
Objectives.

How is colony strength associated with pathogen loads in migrating honey bees?

In partnership with

Honey Bee Research Study Overview – Colony Visits

Migration Routes

Feb 2019 (CA)
June/July 2019 (ND)
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Collected at Visit

Colony Strength
Varroa Mite Counts
Bees for Pathogen Panel

Visits

ND-CA-ND Route
ND-TX-CA-TX-ND Route
ND-Storage-CA-*-ND Route

*location of storage facility varied among operations

Research Hive

In partnership with
Field Work

CA

ND
Lab Work
Lab Work
Data Collected in 2019-20

**Commercial Beekeeper Operations**

• Eight operations (2,000 – 20,000 colonies)
• Sampling Events (Visits) = 957
Data Collected in 2019-20

Commercial Beekeeper Operations
- Eight operations (2,000 – 20,000 colonies)
- Sampling Events (Visits) = 957

Variables
- Colony strength at visit (scale range 0-10)
- Colony loss (if known)
- Migration route (Direct CA, TX, Storage)
- Pathogen diagnosis and quantification at NAGC
  - Total Pathogen Tests \( 957 \times 15 = 14,355 \)
Pathogen diversity across 957 colony visits

Average Pathogens/Colony Sample = 5.3
## Top Seven Pathogens

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nosema ceranae</em></td>
<td>95</td>
</tr>
<tr>
<td>Sacbrood Virus</td>
<td>93</td>
</tr>
<tr>
<td>Black Queen Cell Virus</td>
<td>89</td>
</tr>
<tr>
<td>Deformed Wing Virus B</td>
<td>79</td>
</tr>
<tr>
<td>Deformed Wing Virus A</td>
<td>64</td>
</tr>
<tr>
<td>Israeli Acute Bee Paralysis Virus</td>
<td>25</td>
</tr>
<tr>
<td>Acute Bee Paralysis Virus</td>
<td>25</td>
</tr>
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</table>
# Factors Linked to Colony Strength

<table>
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<tr>
<th>Factor</th>
<th>Result</th>
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<tr>
<td>IABPV</td>
<td>Greater loads in <strong>Weak</strong> colonies</td>
</tr>
<tr>
<td>ABPV</td>
<td>Greater loads in <strong>Weak</strong> colonies</td>
</tr>
<tr>
<td>Route</td>
<td>Storage route had <strong>Weak</strong> colonies</td>
</tr>
<tr>
<td>Year</td>
<td><strong>Stronger colonies in 2020</strong> than 2019</td>
</tr>
</tbody>
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<table>
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<td>Pathogen Diversity</td>
<td>Greater diversity in <strong>Weak</strong> colonies</td>
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Conclusions

Most colonies have sub-clinical infections

**Weak colonies** more likely to be infected with two viral pathogens: **ABPV & IABPV**

**Weak colonies** also had greater pathogen diversity

**Transportation route** affects colony strength

Monitoring pathogen levels can be a **proxy** for colony health and persistence.
Submitted Bee Samples (2016-20)

Colonies Sampled

2000+

States 22
Pest and Pathogen Diagnostics in Crops
Pathogens & Pests contribute to dramatic worldwide yield losses

These 5 crops account for half of global human calorie intake!

Current issues with diagnostics of crop diseases

1) **Symptom-based diagnosis in the field is difficult**

“Symptoms may look similar to other common diseases, *sometimes causing confusion and misdiagnoses.*” – CropWatch, UNL

“Symptoms vary by hybrid susceptibility. Hybrids *may not experience the characteristic lesion.*” – Purdue University Ext.
Current issues with diagnostics of crop diseases

2) **Reliance on traditional lab techniques that are less sensitive**

- Fresh sample **required**
- Unable to test environment (soil or residue)
Surveillance for Disease risk relies on weather data

**Weather data**
- Easiest to collect
- Only 1 side of the disease triangle

**Pathogen data**
- Increases resolution of disease risk

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Disease Triangle

- Pathogen
- Environment
- Host Plant
National Predictive Modeling Tool Initiative

New in 2020

27 Collaborating University and Research Institutions

Inputs → Big Data Processing & Modeling → Output
National Predictive Modeling Tool Initiative

New in 2020

27 Collaborating University and Research Institutions

Inputs

Soil, residue, and spore samples
National Predictive Modeling Tool Initiative

New in 2020

27 Collaborating University and Research Institutions

Output

Heat Map
Weed Diagnostics
Herbicide resistance is an increasing issue

Unrestrained weed growth would reduce crop yield by 50% across US and Canada [The bill = $43 billion annually]
Amaranth (Pigweeds)

Prolific seed producer – 100,000s of seeds

Dispersed by wildlife, flooding, humans

Fast growth rate (up to 4 inches per day)

Up to 78% yield loss in soybeans

Herbicide Resistant
Pigweeds escaping herbicide treatments

Greenhouse Bioassays
Genetic basis for herbicide resistance

**PPX2L gene**

Missing Amino Acid in Mutant

ΔG210
Structure = Function

Wildtype - Only space for the herbicide molecule

Mutant – Large gap rendering the herbicide less effective
Genetic tests for herbicide resistance

**Palmer amaranth & Waterhemp (Pigweeds)**

PPO-Inhibitor Resistance (ΔG210)

Leaf samples

![Graph showing genetic tests for herbicide resistance]

- **Homozygous mutant**
- **Heterozygotes**
- **Homozygous wildtype**

*Bee Suit Optional*
Upcoming Statewide Survey

Develop distribution maps for herbicide resistance in pigweeds

NAGC comprehensive panel
  • PPO-inhibitors
  • Glyphosate
  • ALS-inhibitors

Help producers avoid application of ineffective herbicides
Conclusions

- **Learning molecular protocols** create career opportunities than span disciplines.
  - Wildlife to Agriculture

- **PCR-based tests** can answer many pressing questions in disease and pest management

- **Strong need** for a workforce in agriculture diagnostics
  - Pests and pathogens are not going away!