

## A Closer Look at Insects

# Bug BLASTer: Exploring insect diversity in the soybean field using DNA analysis

*Are all insects found in soybean fields bad?*

*Insecta* is the most diverse Class of organisms. Estimates of insect species reach 750,000 or more, far outnumbering the combined number of all remaining species in Kingdom Animalia. *Insecta* is a highly successful group of organisms, found in nearly all ecological niches described within the biosphere (land, water, and air). Their success and ability to adapt to new environments has been both negative and positive in agriculture. Some have taken advantage of the bountiful food resources in agriculture, becoming serious pests for our livestock and crop production. Others have been beneficial to agriculture, where some species can help reduce the chances of population explosions of pest species.

Insect classification can be challenging, especially if using only visual characteristics (morphology). Insect species can have multiple life stages as they grow and those species that undergo complete metamorphosis (holometabolism) have dramatic changes in morphology. During the life cycle, some stages (or their damage) are more visible in the field, whereas the adult stage may be short-lived and occur elsewhere. For example, the larval stage may cause the most damage to crops in the field, whereas an adult moth has no mouthparts and is **cryptic** or camouflaged.

Genetic analysis using barcodes can help to classify Insects at any life stage. Barcodes are described as regions within the genome that differ among closely related species. These barcodes tend to be either genes or non-coding regions that flank genes critical for life processes, such as RNA translation and cellular respiration.

This activity provides an introduction to barcode or DNA analysis using a popular tool called BLAST (Basic Local Alignment Tool) that allows users to search for similarities between a sequence of interest (query) and sequences within the database. This search and find program allows researchers to identify new and related species across the diversity of life.

### Scenario

Crooked Lane Farm is having trouble with production of soybeans and scouting has revealed considerable insect damage in the field. The farm manager elects to survey the field for insects by sweeping nets through the foliage. All netted insects were preserved in a jar of ethanol and submitted to a diagnostic laboratory for identification. Unfortunately, many insects were smashed during the collection, making visual identification difficult. Instead, the laboratory performed a DNA extraction and barcode sequencing on the bulk insect sample. The laboratory produced a report that contained the most common DNA sequences from insects within the bulk sample. Your job as a DNA analyst (bioinformatician) is to help the farm manager identify the species, using the given sequences of DNA, through the use of public databases and basic bioinformatic tools.

### Materials

Computer or tablet with access to internet  
Insect DNA sequences at <https://grownnextgen.org/go/bugs>

### Procedure

Search the NCBI database to identify one or more of the DNA sequences using the following steps:

- a. Visit: <https://www.ncbi.nlm.nih.gov>
- b. Click on 'BLAST' in the right column.
- c. Click on 'Nucleotide blast'; paste in the DNA sequence you've been assigned in the rectangle; submit; wait for result

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### Reflection - Genetic Analysis

1. What is the name of the barcode region used in this analysis (hint: see description of similar sequences)?
2. This particular barcode is used in what critical life process?
3. In which organelle is this barcode gene located?

### Reflection - Insect ID and Management

4. What species of insect was the DNA sequence from? Provide both scientific and common names.
5. Is the insect native to the United States?
6. Is this insect harmful or beneficial to soybeans? Explain.
7. What do the experts recommend for management of the insect (if any)?

Have all groups discuss the insects found within the sample and how they may be impacting Crooked Lane Farm. Help create a plan to help the farmer address the issue by consulting one of the following guides.

North Carolina University Extension

<https://soybeans.ces.ncsu.edu/soybean-insect-pests/>

<https://soybeans.ces.ncsu.edu/soybean-beneficial-insects/predators-in-soybeans/>

<https://soybeans.ces.ncsu.edu/soybean-beneficial-insects/parasitoids-in-soybeans/>

Additional Reading on DNA analysis and Insect ID

<https://www.scientificamerican.com/article/dna-in-air-can-catalog-hidden-insects-all-around-us/>

<https://news.uoguelph.ca/2023/02/20-years-of-dna-barcoding-u-of-g-developed-technology-hits-milestone/>

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| Sequence Name | Insect ID                     | Full Description                                                                                                                                   |
|---------------|-------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| Seq1          | Lady Beetles                  | GU013586-b_extraction Coccinella septempunctata voucher BIOUG<CAN>:DS-Test-045 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial |
| Seq2          | Three cornered alfalfa hopper | MN888501_extraction Spissistilus festinus isolate ParlierCA cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial                 |
| Seq3          | Soybean aphid                 | MF830578_extraction Aphis glycines voucher BIOUG19928-F03 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial                      |
| Seq4          | Assassin Bugs (Wheel Bug)     | HQ985192-b_extraction Arilus cristatus voucher BIOUG<CAN>:10BBHEM-165 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial          |
| Seq5          | Stink bug                     | MZ872463_extraction Halyomorpha halys voucher Haha-2094 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial                        |
| Seq6          | Spined Soldier Bug            | MF939003-b_extraction Podisus maculiventris voucher BIOUG22593-F05 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial             |
| Seq7          | Japanese beetle               | KT708361_extraction Popillia japonica voucher BIOUG24009-A02 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial                   |
| Seq8          | Soybean stem borer            | MW983051_extraction Dectes texanus voucher USNM:ENT:01587216 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial                   |
| Seq9          | Seed corn maggot              | MF762780_extraction Delia platura voucher BIOUG20140-B11 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial                       |
| Seq10         | Green Lacewing                | MG514547-b_extraction Chrysoperla rufilabris voucher BIOUG07121-B06 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial            |

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|       |                       |                                                                                                                                                   |
|-------|-----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| Seq11 | Bean leaf beetle      | HQ582719_extraction Cerotoma trifurcata voucher<br>BIOUG<CAN>:09BBCOL-0433<br>cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial |
| Seq12 | Soybean looper        | GU799708_extraction Chrysodeixis includens voucher<br>BIOUG<CAN>:MDOK-1505<br>cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial |
| Seq13 | Corn earworm          | JF855191_extraction Helicoverpa zea voucher BGS04198<br>cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial                       |
| Seq14 | Green cloverworm      | KM547597_extraction Hypena scabra voucher BIOUG03390-A11<br>cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial                   |
| Seq15 | Saltmarsh caterpillar | HQ561156_extraction Estigmene acrea voucher<br>BIOUG<CAN>:DMLEP-0104<br>cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial       |
| Seq16 | Fall armyworm         | MT324067_extraction Spodoptera frugiperda voucher am14016<br>cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial                  |