Towards Quantitative Insect Metabarcoding for Agroecosystem Monitoring

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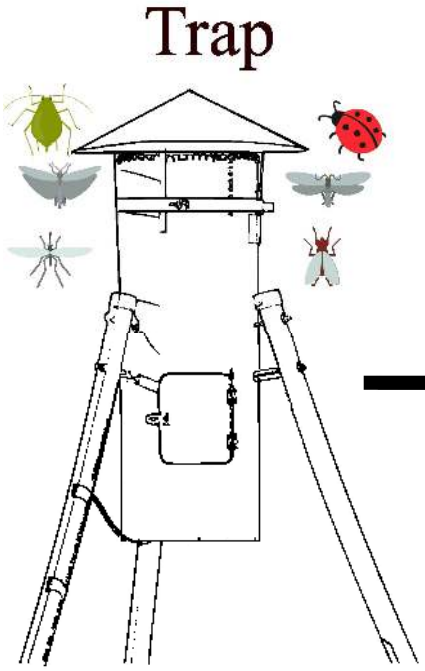
A major threat to grain production - insect pests

- The grains industry represents **34% of Australia's agriculture**, farm production was worth **$91.5 billion** in 2022-2023
- **Pest insects** significantly **lower crop yield** via **crop consumption** and the introduction of **plant pathogens**
- Key pests include aphids, moths, locusts, beetles, etc.

Pest surveillance – an important but time-consuming process

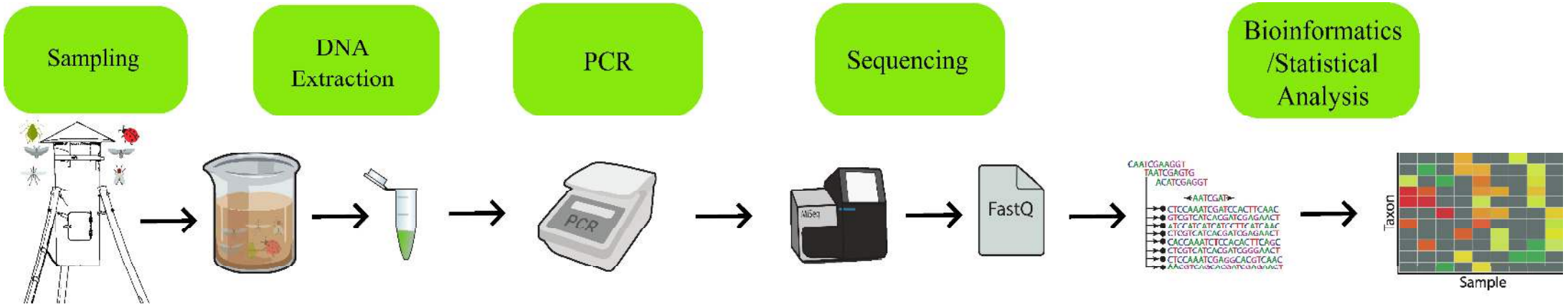
- Pest surveillance is a **difficult process** to maintain on a large scale
- Requires **numerous taxonomists** to distinguish every species collected from **large and diverse** samples



Potentially thousands of individual insects per sample

Metabarcoding – promising but imperfect prospect

- **Metabarcoding** can **reduce this strain** by increasing the throughput of samples
- Can **identify every species** within a bulk sample without taxonomic skills

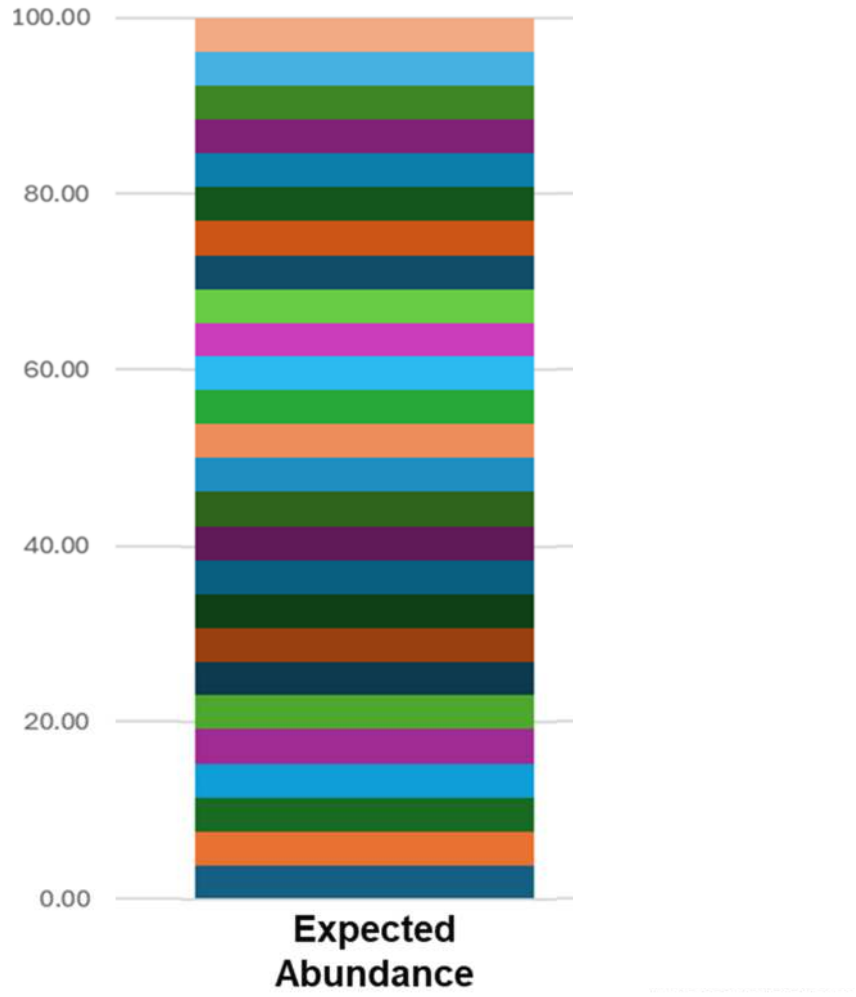


The importance of quantitative information in an agricultural context

- However, the data produced by existing protocols is **semi-quantitative:** produces **relative read abundances, not equivalent** to **insect counts**
- Cannot accurately monitor changes in population sizes of important **pest and beneficial** species
- Unable to provide key information to growers
  - i.e. **when/if** they should use **preventative measures** like spraying pesticides or releasing biocontrol species.

Bias in Metabarcoding data

- The **expected proportion of species** (DNA/individuals) per sample inputted into a metabarcoding protocol is **not reflected in the observed abundance** at the end of the protocol



The many potential sources of bias in metabarcoding

Metabarcoding data is **not well suited** for quantitative measures due to a **range of biases** that are **introduced** throughout the protocol

Absolute abundance:
# of insects per species per sample

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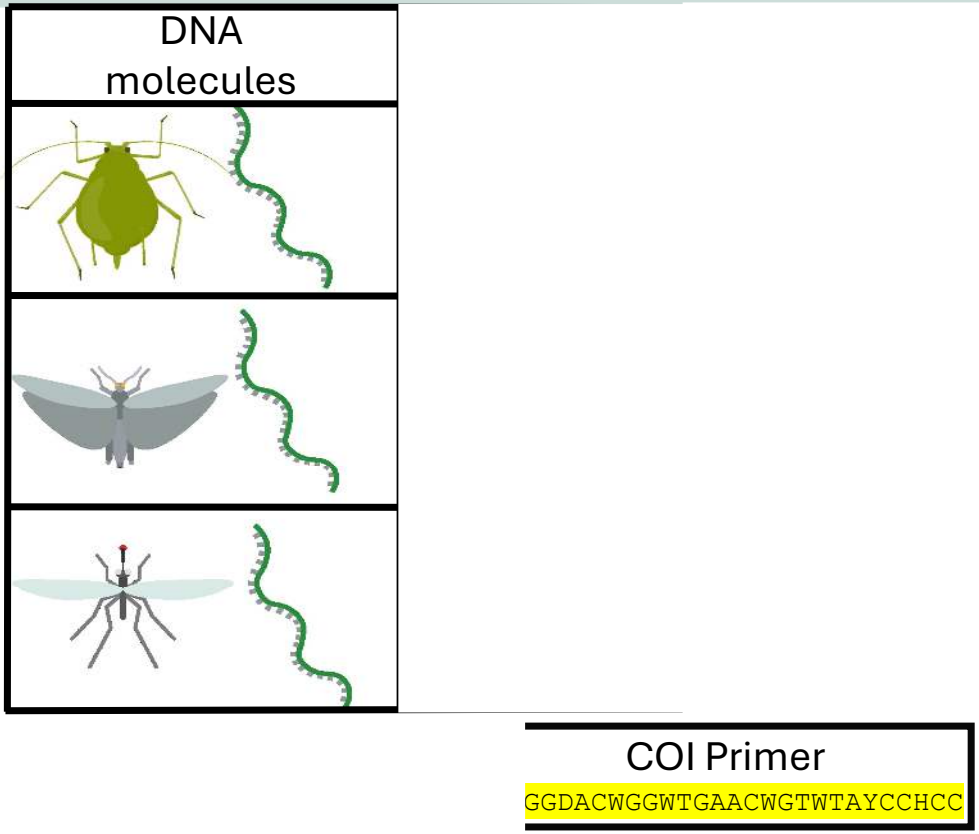
Relative abundance:
Proportion of reads produced per species per sample

Molecular Bias

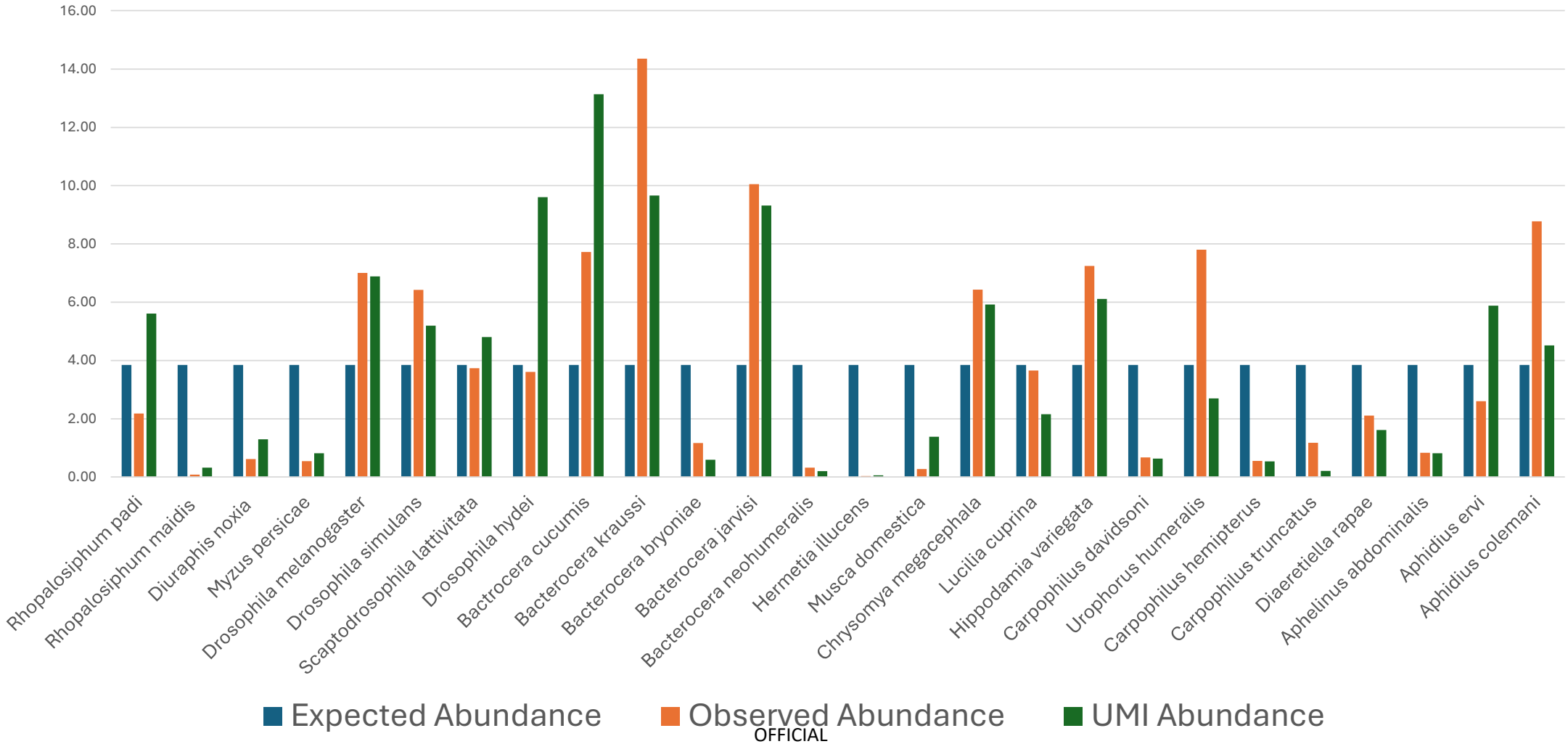
- Preferential amplification
  - Primer mismatch
  - Copy number variation
  - Polymerase/GC content

Solving amplification bias - Unique Molecular Identifiers (UMIs)



- In theory UMIs can determine **starting amounts of DNA molecules** per species

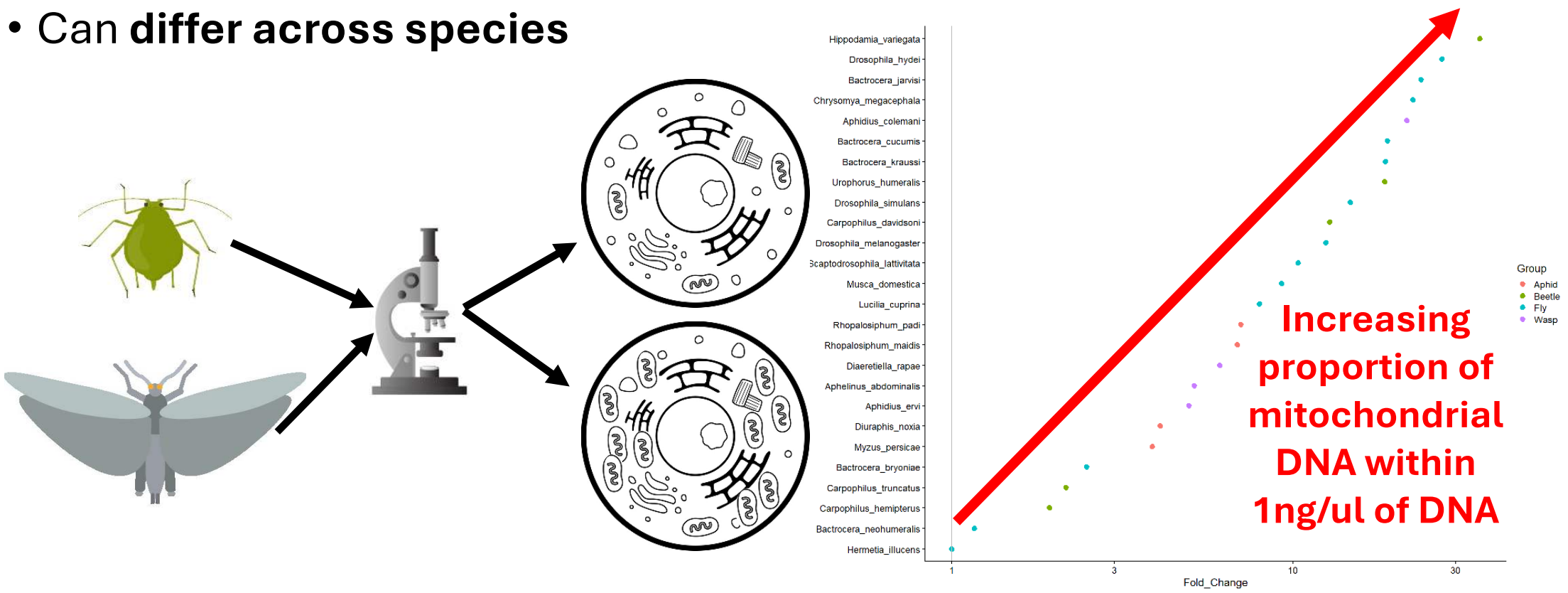
Unique Molecular Identifiers (UMIs) – A work in progress



Mitochondrial Copy Number (MCN)

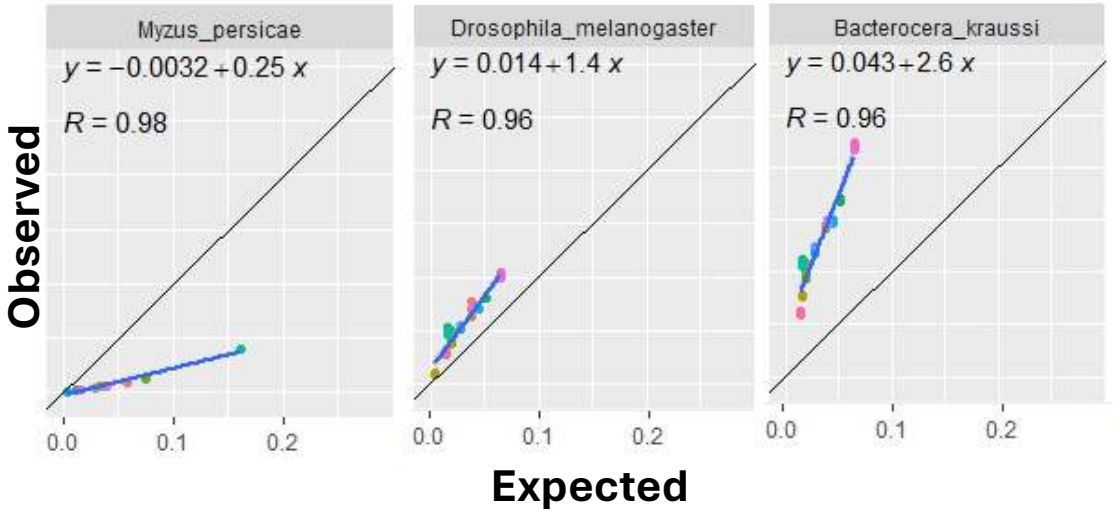
- MCN is an **under-addressed** source of bias
- Can **differ across species**

All samples were diluted to 1 ng/ul



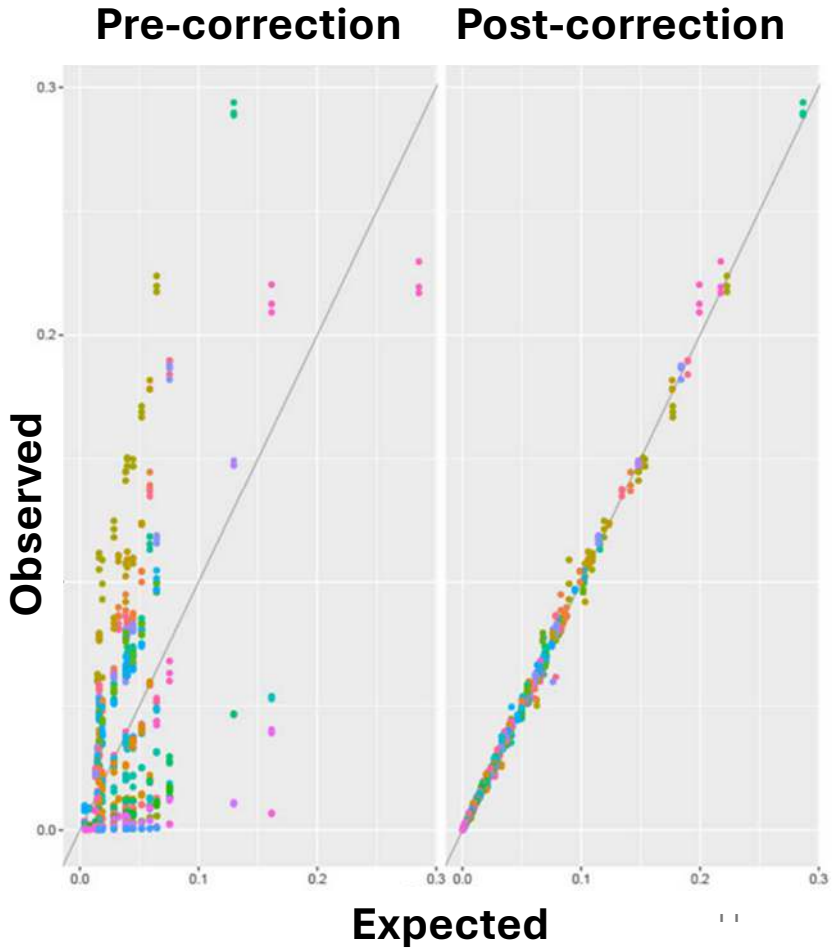
Consistent taxon-specific bias

- **Bias is consistent** within species **across samples**



Black line = 1:1 relationship between expected and observed data

Blue line = Linear trend of bias across samples



Correcting bias in real samples

- Can **correct** for **taxon-specific bias** in samples with **known quantity**
- Need to test how well this can be extrapolated to **unknown/physical samples**
- Additional factors to take into consideration include:
  - Physical characteristics of insects
  - Extraction-based biases

Next steps – questions to answer

- Can we develop standardised correction factors for species of interest?
- Can we incorporate a method to transform read proportions (relative abundance) into insect counts (absolute abundance)? (i.e. biomass, imaging or spike-ins)
- Can these methods be applied to real samples to inform grain pest management?

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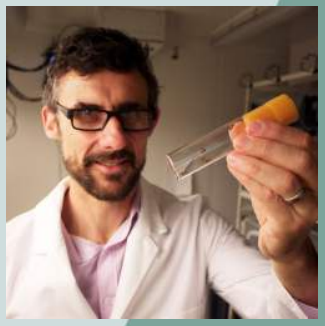

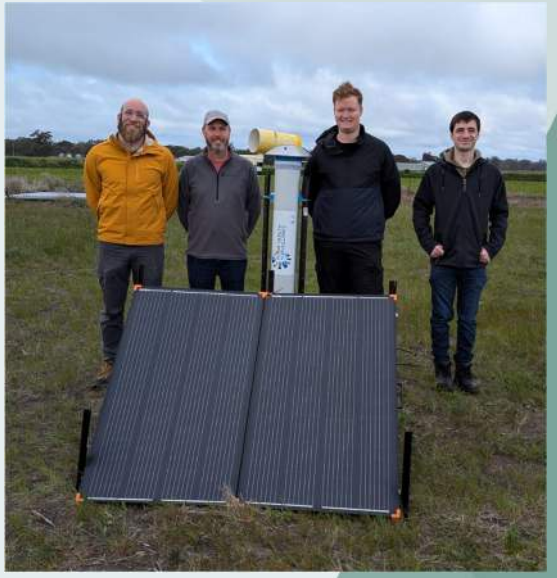

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