

Mosquito Chronological Age Differentiation using Mid-Infrared Spectroscopy and Chemometrics

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Purpose

- Mosquito-borne diseases are responsible for significant morbidity and mortality on an international and domestic scale
- Many species of female mosquitoes require a blood meal for oogenesis
- Gap between subsequent blood meals is often days or weeks
- Older female mosquitoes are inherently more dangerous due to increased risk of infection

Current Status of Age Determination

- Present age grading is primarily based on parity
 - i.e. parous or nulliparous
- Chronologic age grading:
 - Quantitative Polymerase Chain Reaction (qPCR)
 - Near Infrared Spectroscopy (NIR)
- Limited Temporal Resolution
- Expensive

Infrared Spectroscopy Overview

- Mid-Infrared (IR) spectroscopy is a type of vibrational spectroscopy that is both sensitive and information rich
- Subtle changes in IR spectra correlate with changes in the biochemistry of mosquitoes as they age
- These changes in the IR spectra when coupled with advanced numerical analysis (chemometrics) may be used to predict the age of mosquitoes

Materials

- Eggs were reared from colony materials established from field materials or from existing strains
 - Samples frozen at different times after eclosion to represent different ages
- *Cx. quinquefasciatus*
 - young (<1 week) & old (>2 weeks)
- *Cx. tarsalis*
 - 5-day bins (e.g., 5, 10, 15, etc... ± 2 days)

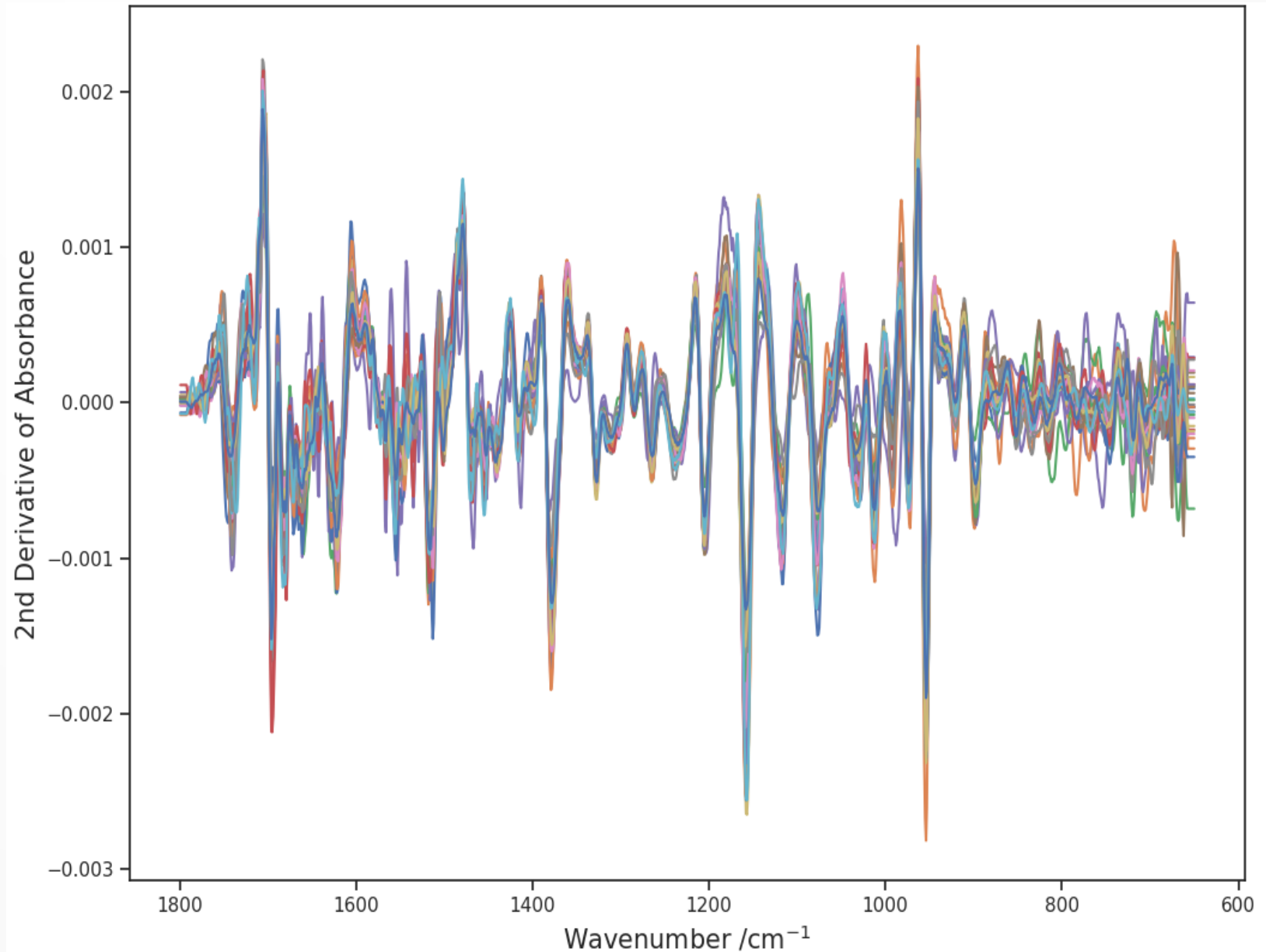
Instrumentation and Data-Processing

- ThermoNicolet™ model Centaurus IR Microscope attached to a ThermoNicolet™ iS10 bench
- OMNIC™ (version 9.8.372) & Python (version 3.6.3)
- Data are pre-processed by separating based on metadata
 - i.e. male vs female
- Cropping ($1800 - 650 \text{ cm}^{-1}$)
- Normalized by band height
- Second derivative function



Cx. quinquefasciatus Pre-Processing

Figure 1. *Cx. quinquefasciatus* spectra after cropping 1800 – 650 cm^{-1} , normalization by band height, and applying 2nd derivative function



Principal Components Analysis (PCA)

- Used to visualize the data and search for correlations with what we know about the data (i.e. young vs old)
 - Does not assume anything about the data
- Loading vectors (principal components) contain composite information of spectral features
- Scores (weights of the principal components)
- Scores plots
 - Separated by spectral features embedded in loading vectors and scores

Cx. quinquefasciatus Results

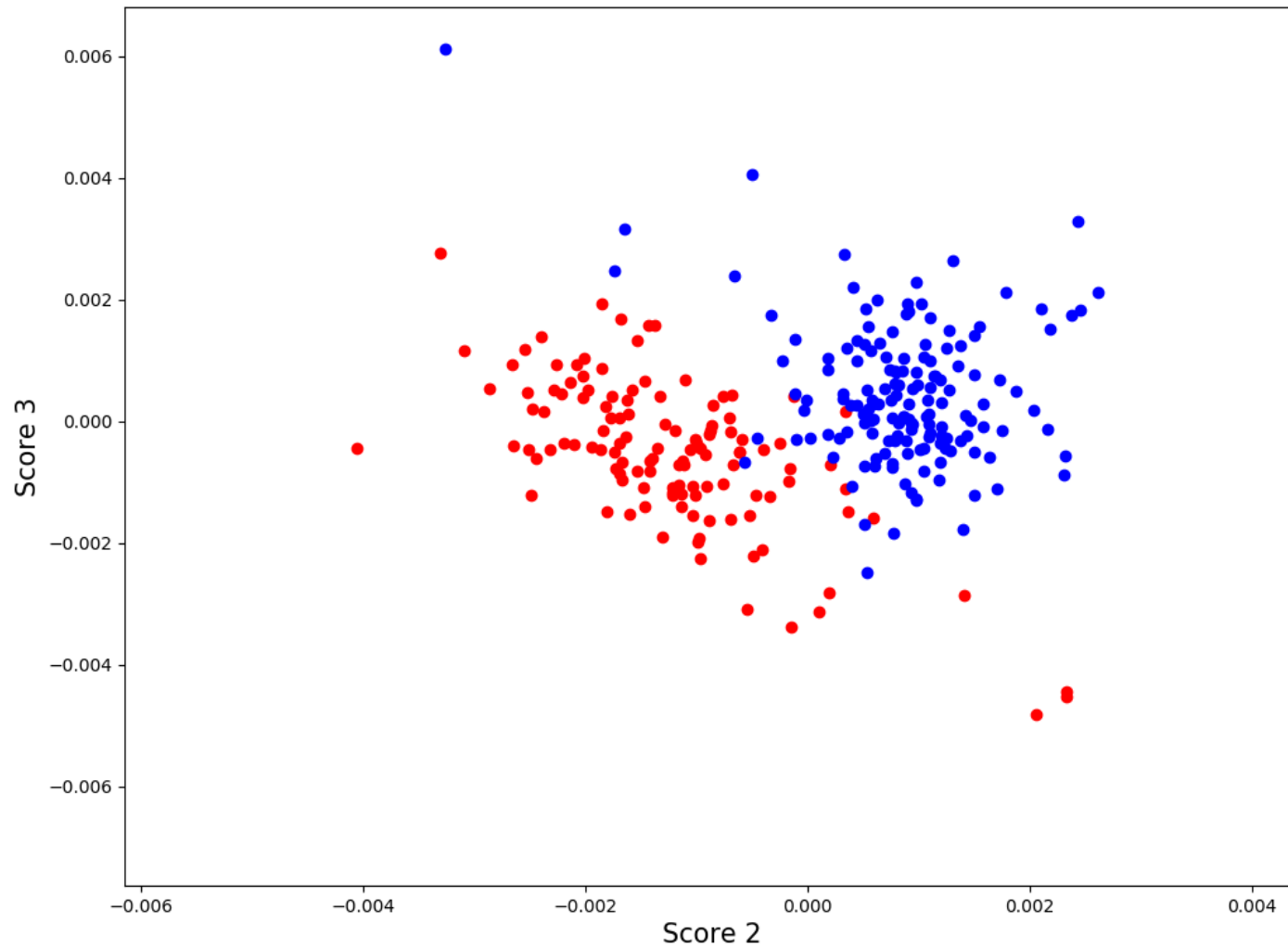
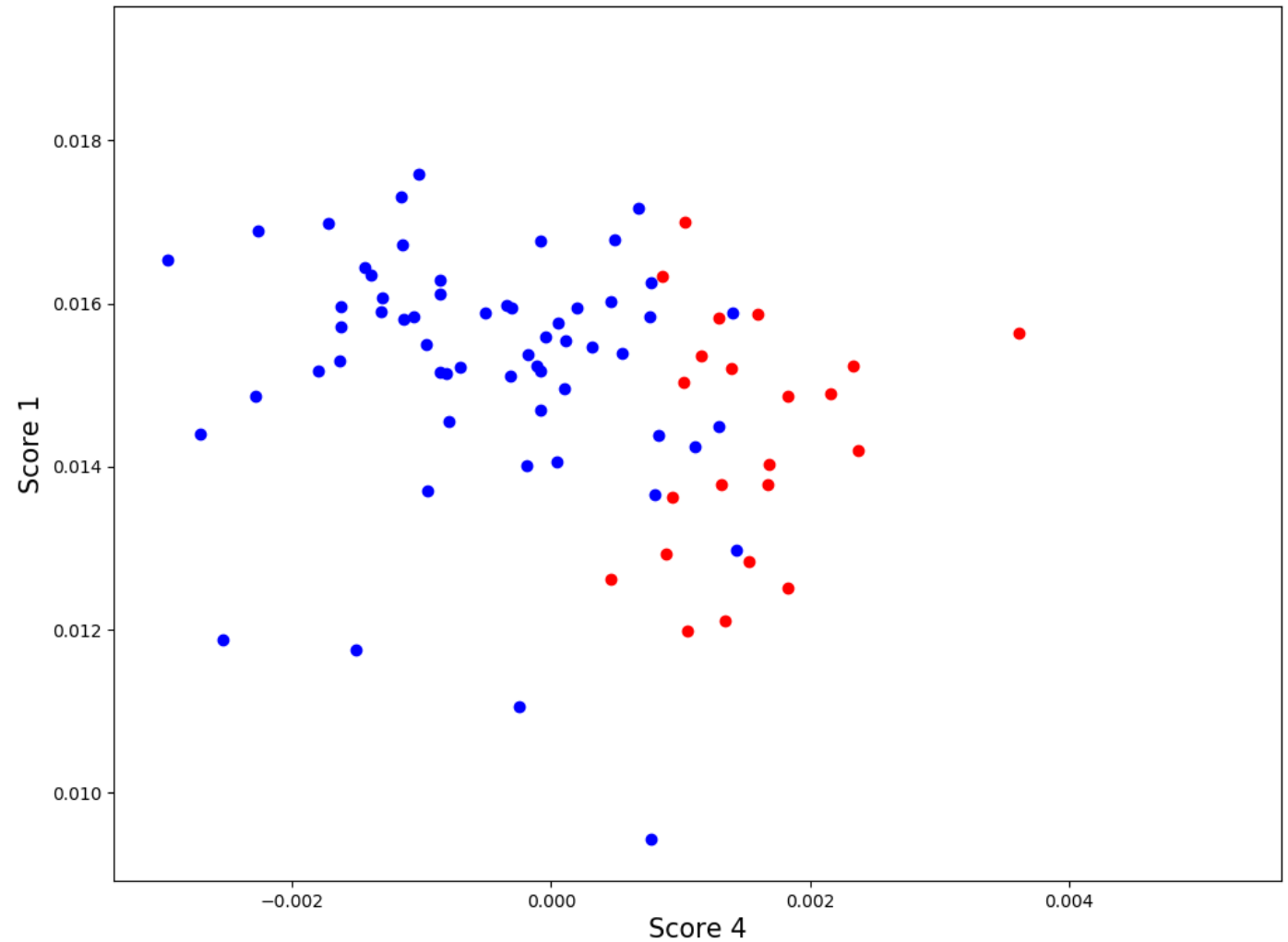


Figure 2. *Cx. quinquefasciatus* score plot <1 week (red) & >2 weeks (blue)

Cx. tarsalis Results

Figure 3. *Cx. tarsalis* score plot
5, 10 day bins (red); 15, 20, 25,
30, 40 day bins (blue)



Cx. tarsalis Results

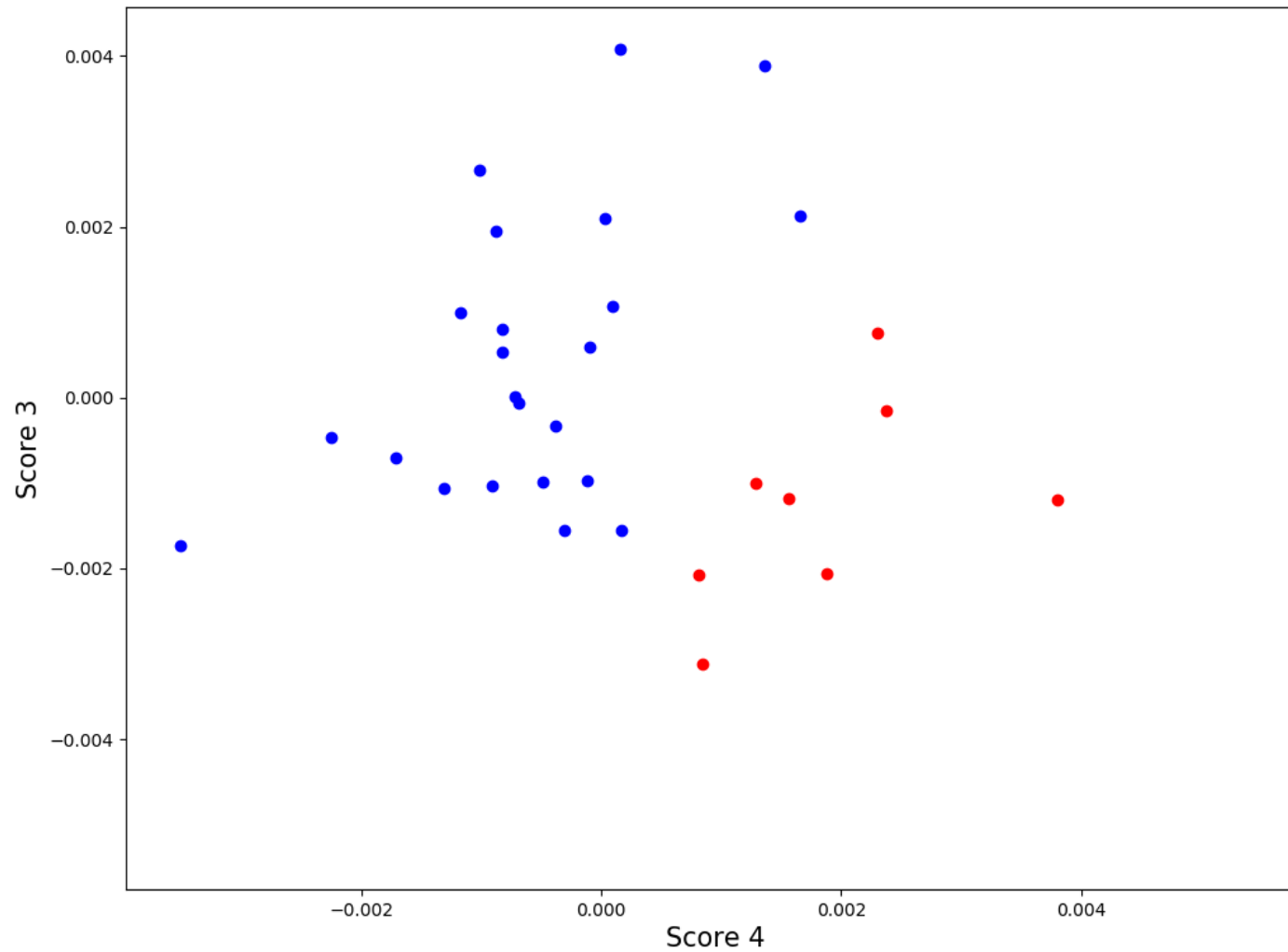


Figure 4. *Cx. tarsalis* score plot
5, 10 day bins (red); 15, 20, 25,
30, 40 day bins (blue); female

Takeaways and Next Steps

- Using PCA with only 2 dimensions, we can achieve good separation between groups of different ages
 - Add more dimensions to increase cluster separation
- Use PCA-Regression to predict ages with unknown samples
- *Ae. triseriatus* in 1-2 day age bins
 - Smaller time-scale
- Mid-IR spectroscopy could serve as new way to survey human health risk of mosquito populations