Poison Frog Livers

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Project Overview

1. Evaluate Literatures
2. Investigation Questions
3. Data analysis and interpretation
4. Challenges and Setback

Background Information

- Aposematic/Cryptic
- Liver metabolism
- Skin toxins

Aposematic

E. anthonyi

E. tricolor

Hypothesis

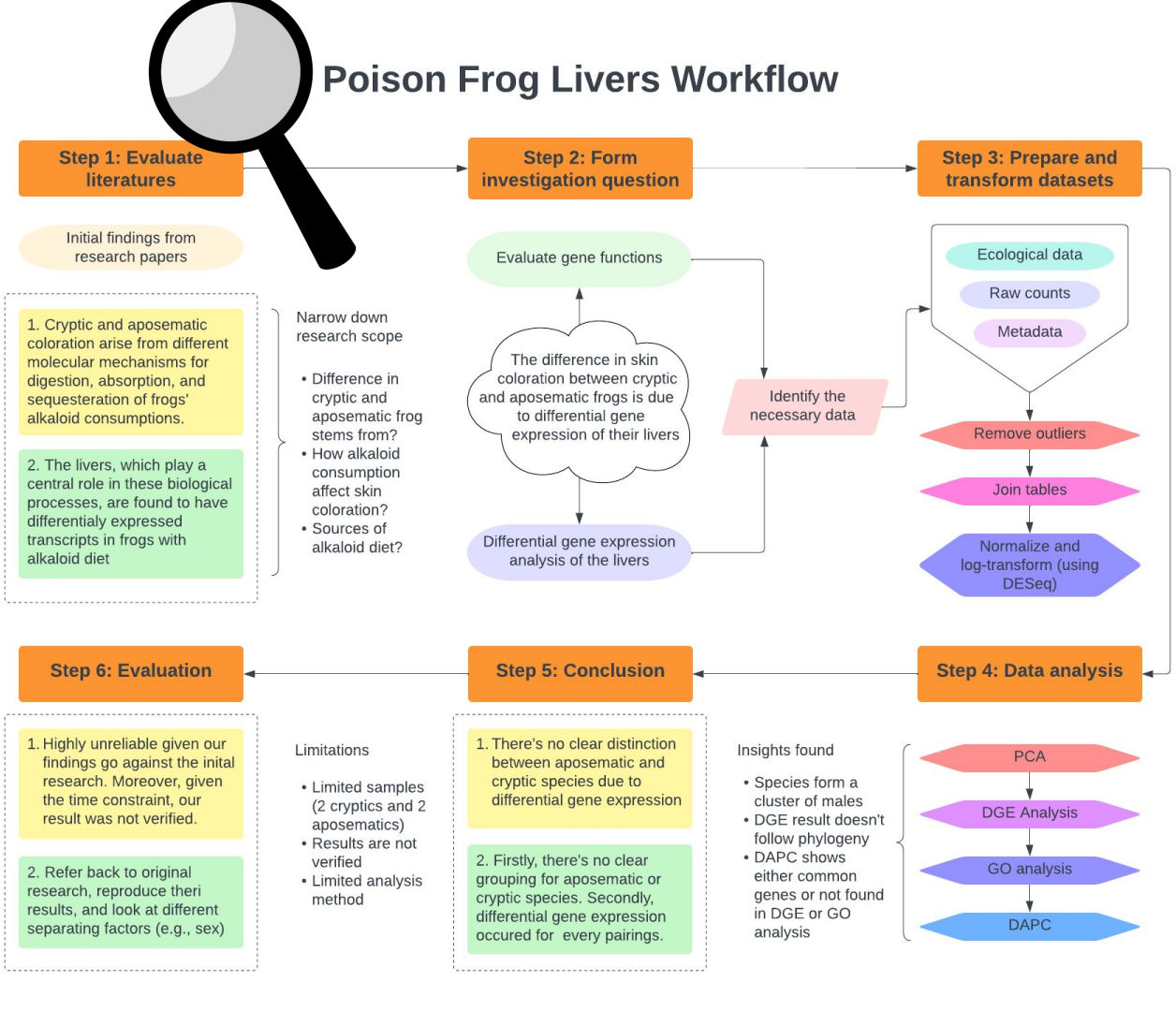
Cryptic vs aposematic coloration of poison frogs is due to differential gene expression of frogs' livers

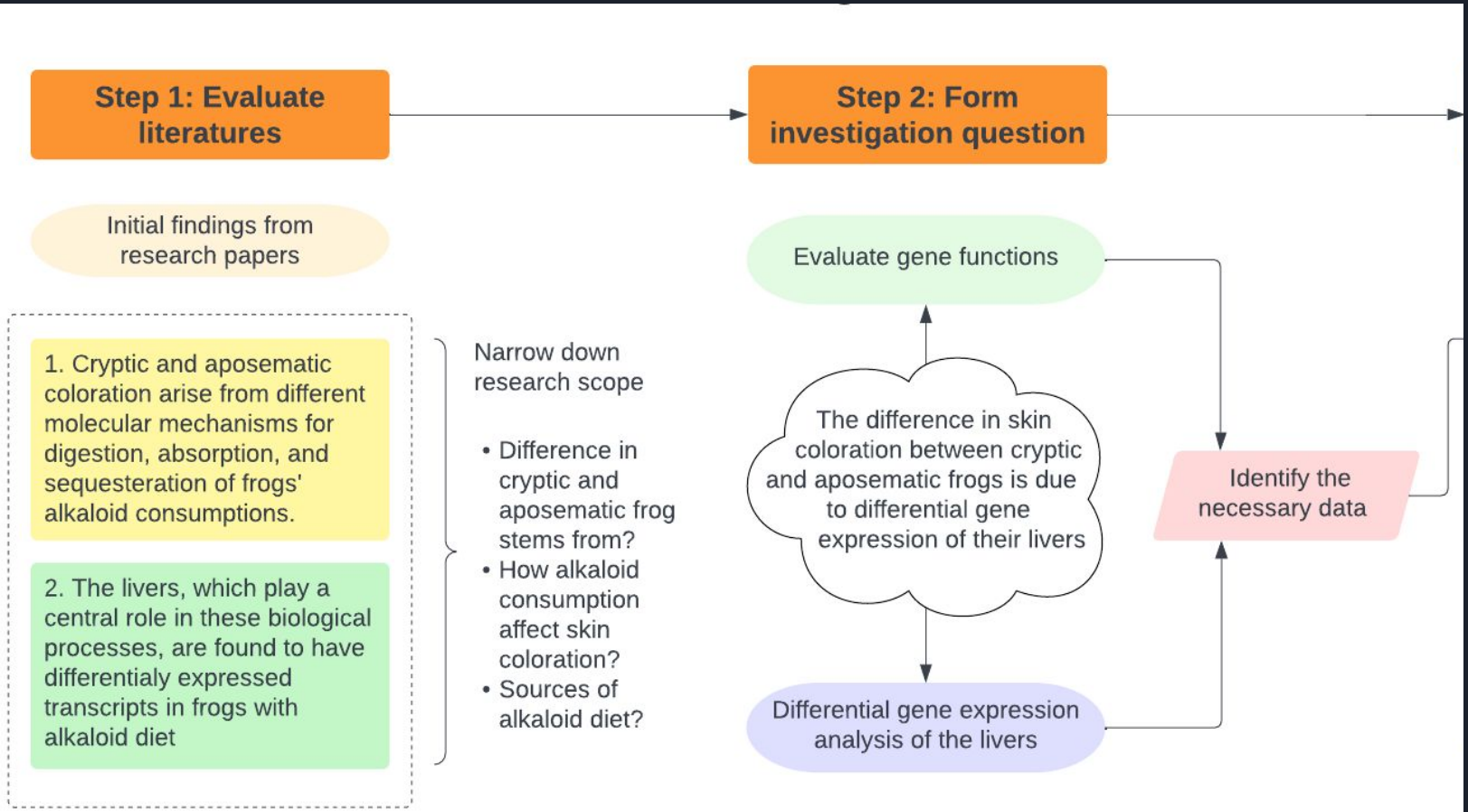
Cryptic

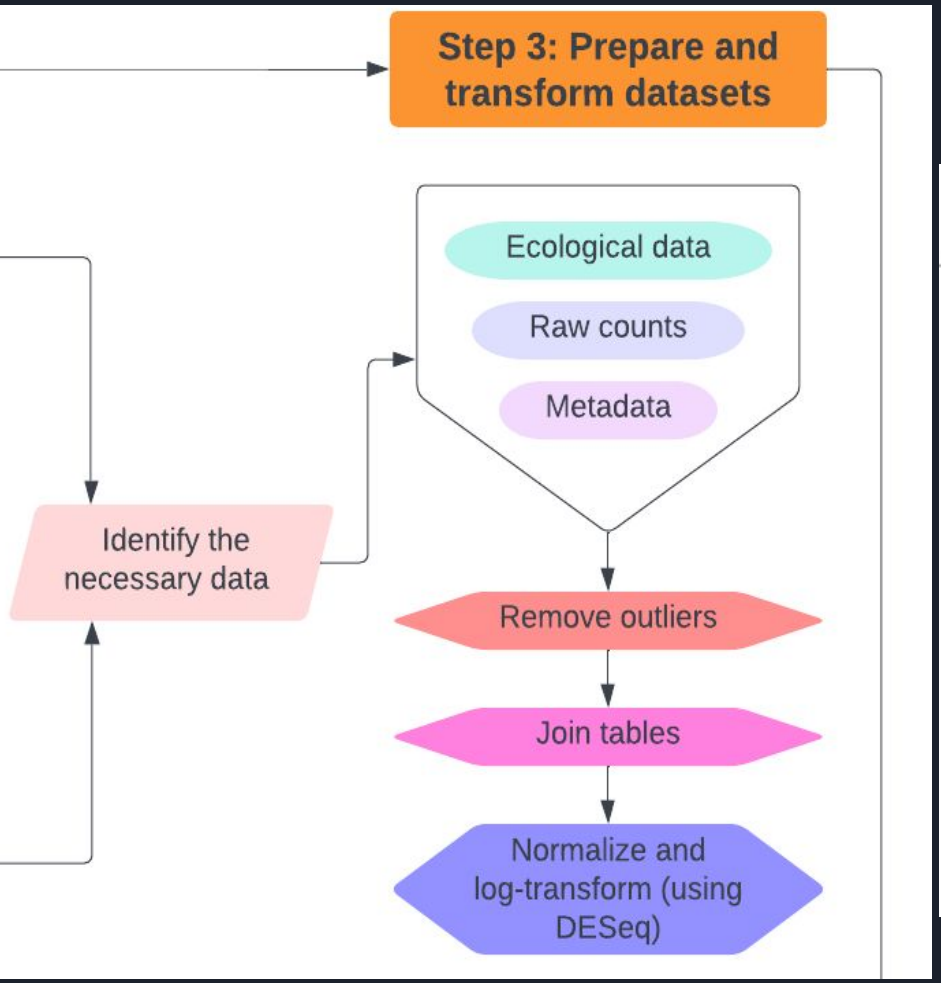
E. boulengeri

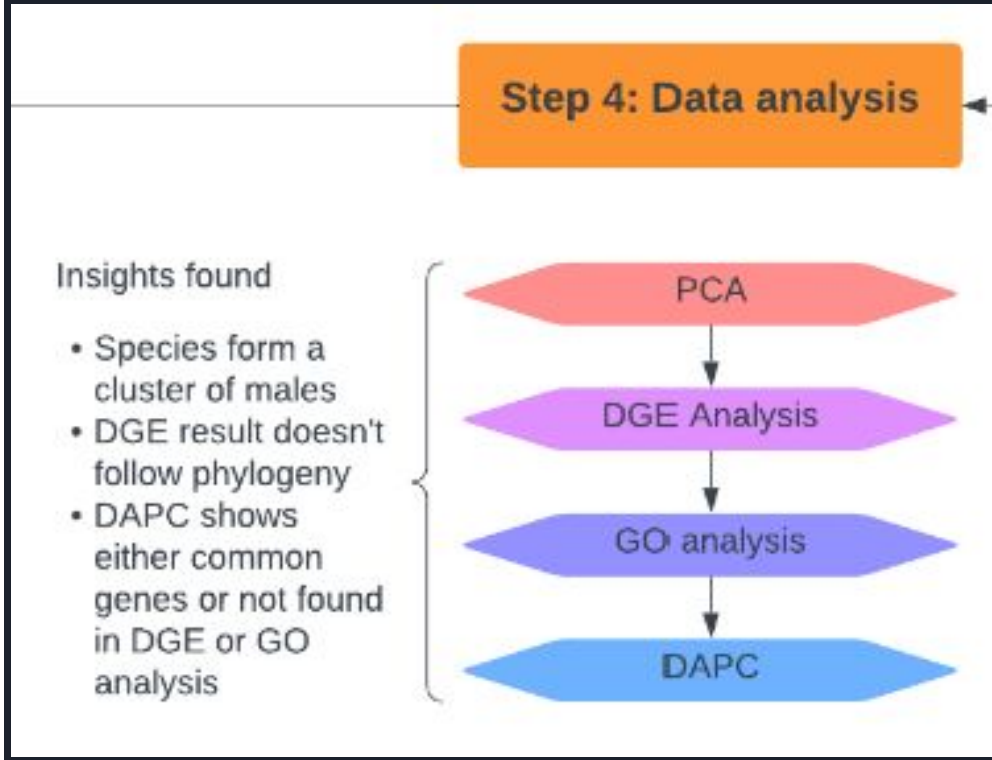
E. machalilla

Poison Frog Livers Workflow




Step 1: Evaluate literatures
Step 2: Form investigation question
Initial findings from research papers
1. Cryptic and aposematic coloration arise from different molecular mechanisms for digestion, absorption, and sequesteration of frogs' alkaloid consumptions.
2. The livers, which play a central role in these biological processes, are found to have differentialy expressed transcripts in frogs with alkaloid diet
Narrow down research scope
• Difference in cryptic and aposematic frog stems from?
• How alkaloid consumption affect skin coloration?
• Sources of alkaloid diet?
Evaluate gene functions
The difference in skin coloration between cryptic and aposematic frogs is due to differential gene expression of their livers
Differential gene expression analysis of the livers
Identify the necessary data


Step 3: Prepare and transform datasets
Ecological data
Raw counts
Metadata
Identify the necessary data
Remove outliers
Join tables
Normalize and log-transform (using DESeq)


Step 4: Data analysis
Insights found
• Species form a cluster of males
• DGE result doesn't follow phylogeny
• DAPC shows either common genes or not found in DGE or GO analysis
PCA
DGE Analysis
GO analysis
DAPC

Step 6: Evaluation

1. Highly unreliable given our findings go against the inital research. Moreover, given the time constraint, our result was not verified.

2. Refer back to original research, reproduce theri results, and look at different separating factors (e.g., sex)

Limitations

- Limited samples (2 cryptics and 2 aposematics)
- Results are not verified
- Limited analysis method

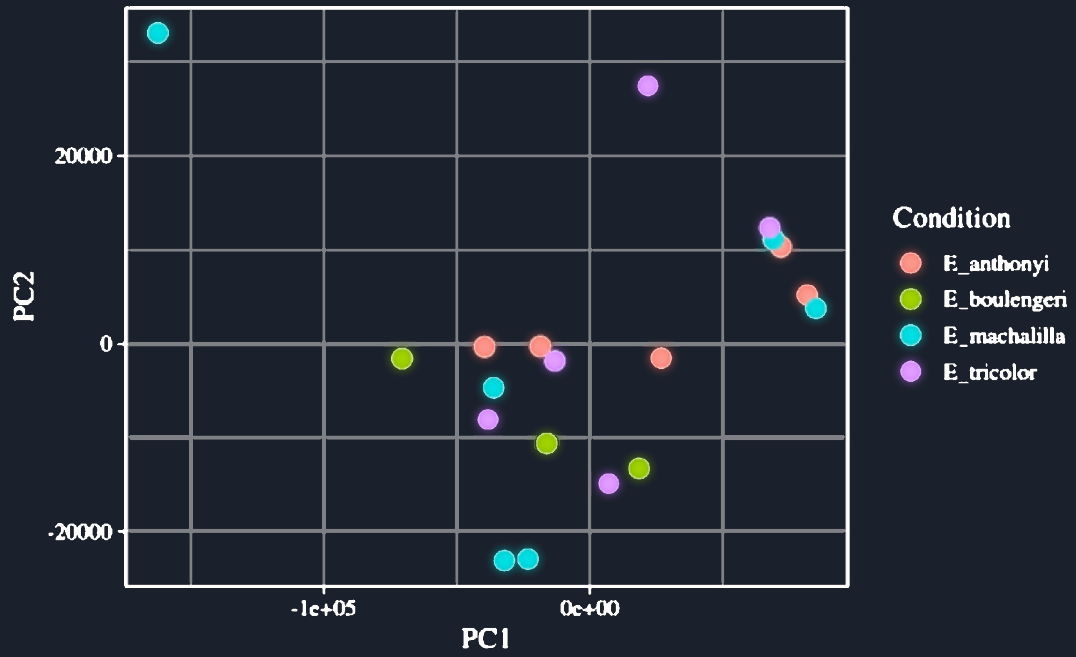
Step 5: Conclusion

1. There's no clear distinction between aposematic and cryptic species due to differential gene expression

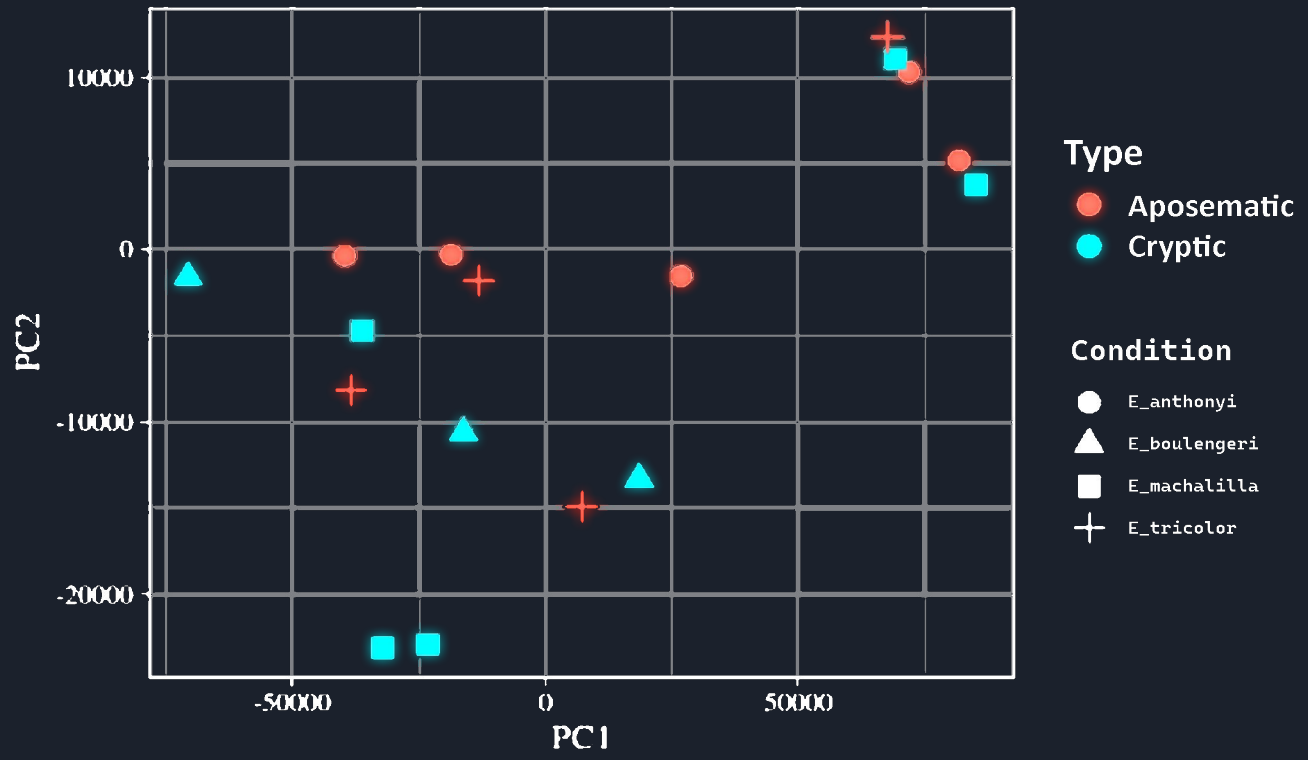
2. Firstly, there's no clear grouping for aposematic or cryptic species. Secondly, differential gene expression occured for every pairings.

PCA 1 - Grouped by different species

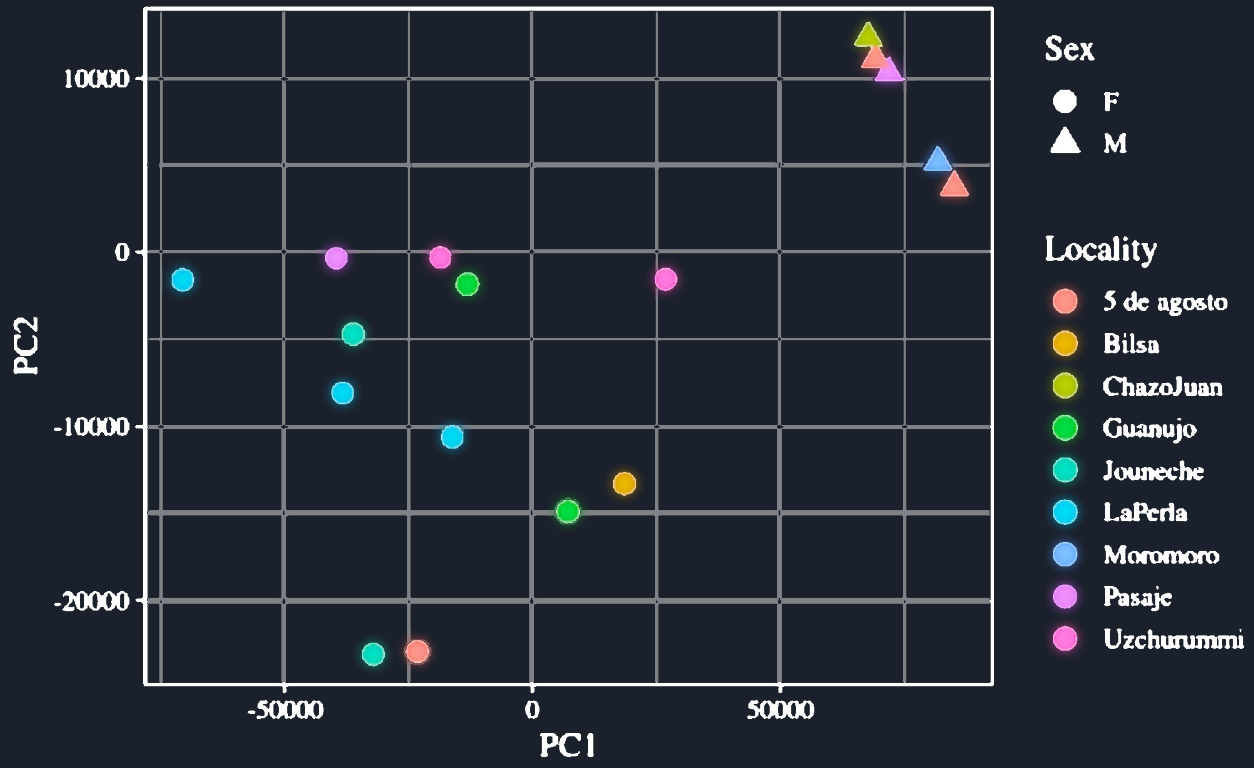
- PC1 explains 92.5 % of the variation
- We also need to take out the outliers (H2.6848_S16 and A3.6830_S17)



PCA 2 - Aposematic or Cryptic

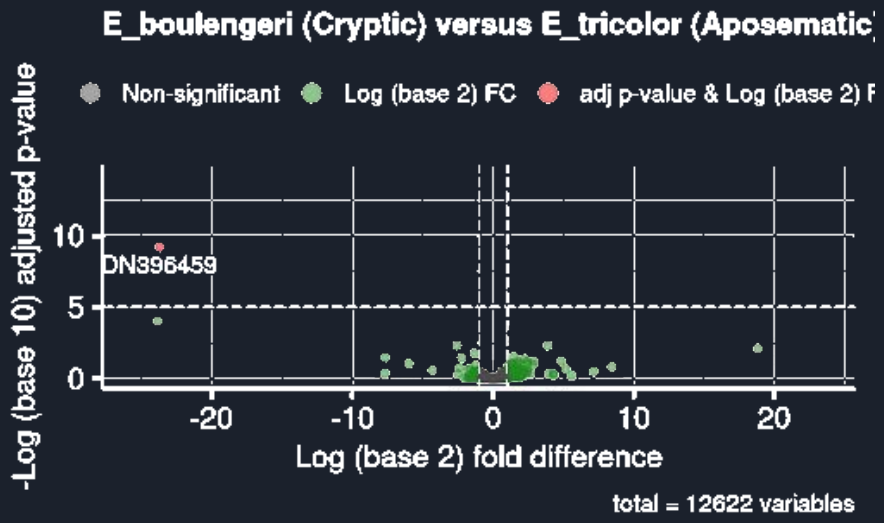


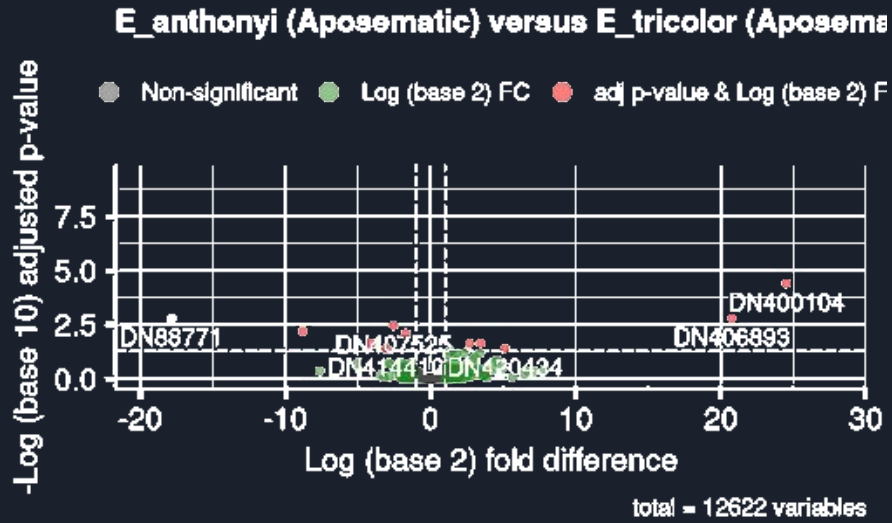
PCA 3 - Grouped by sex



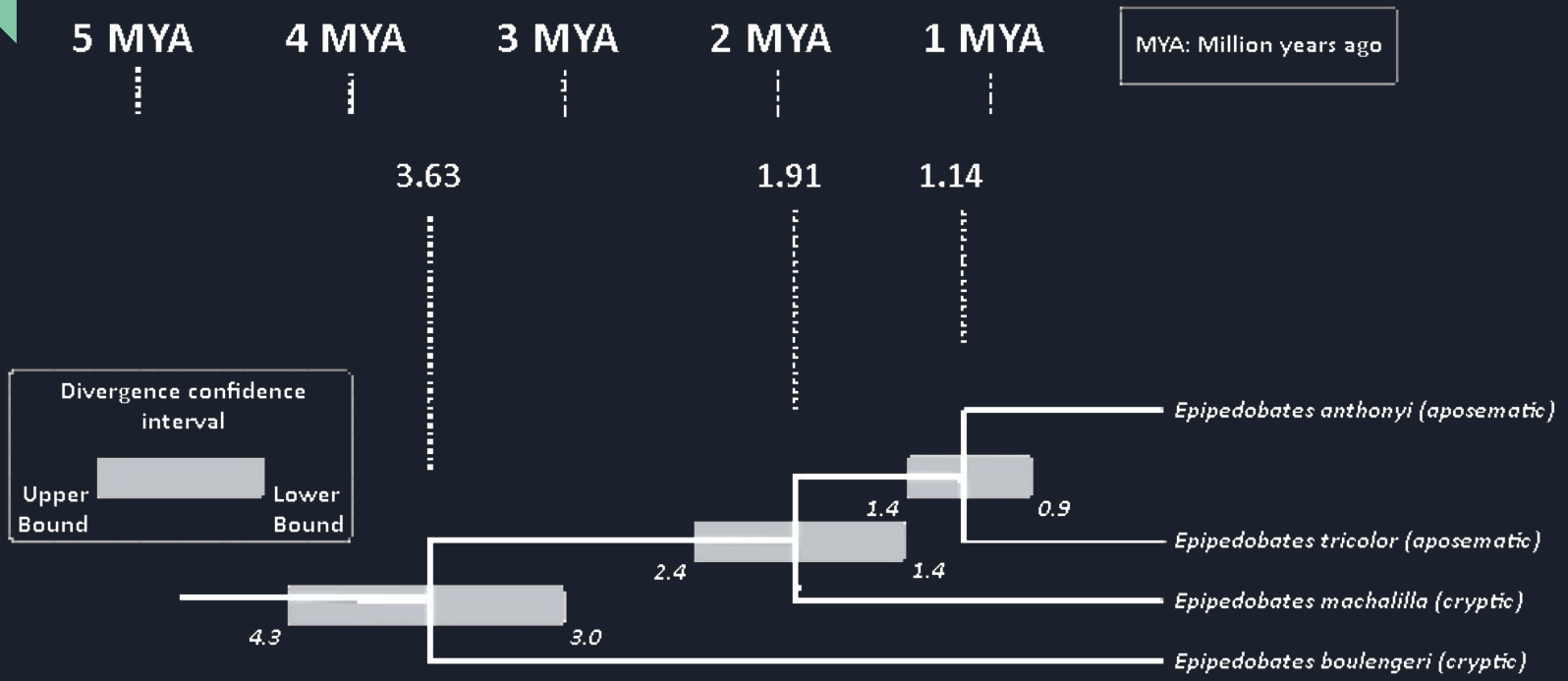
Differential Gene Expression Analysis

- DGE Analysis for every species combinations
- Default cutoff: Only one statistically significant differentiated gene
- 0.05 cutoff: More statistically significant differentiated (and repeating) genes

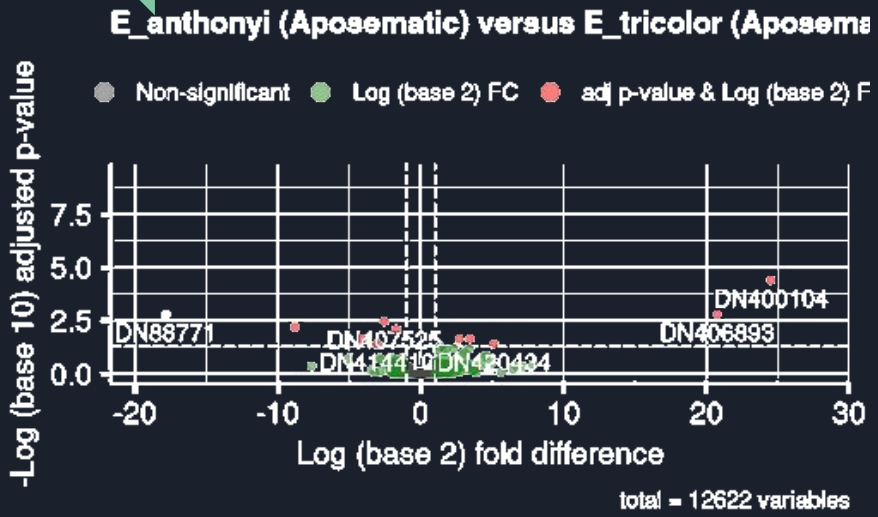


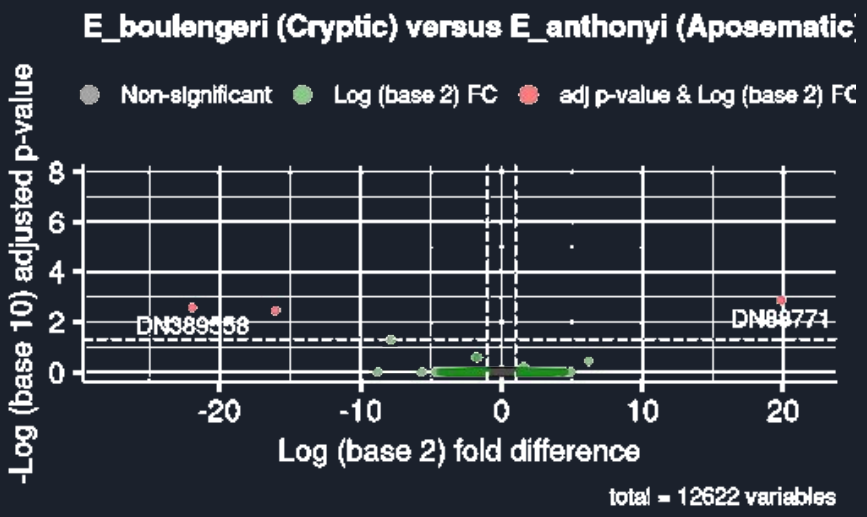


Phylogenetic Time Tree

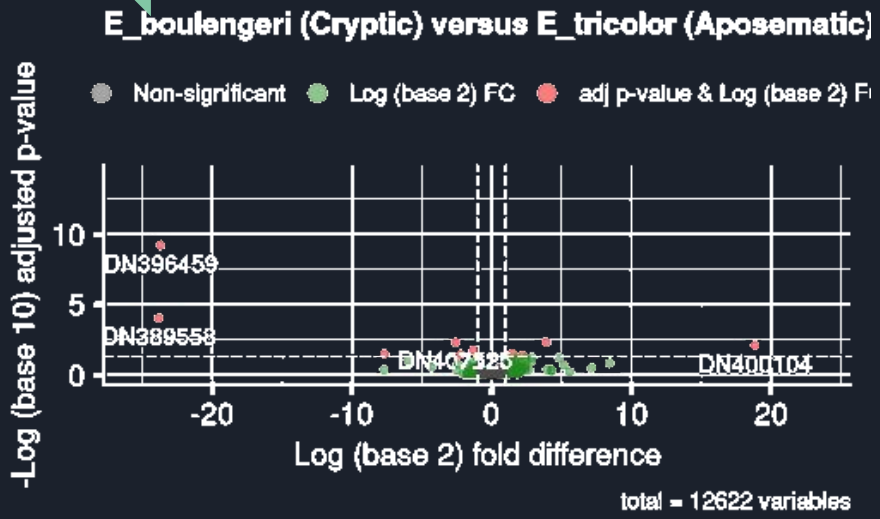


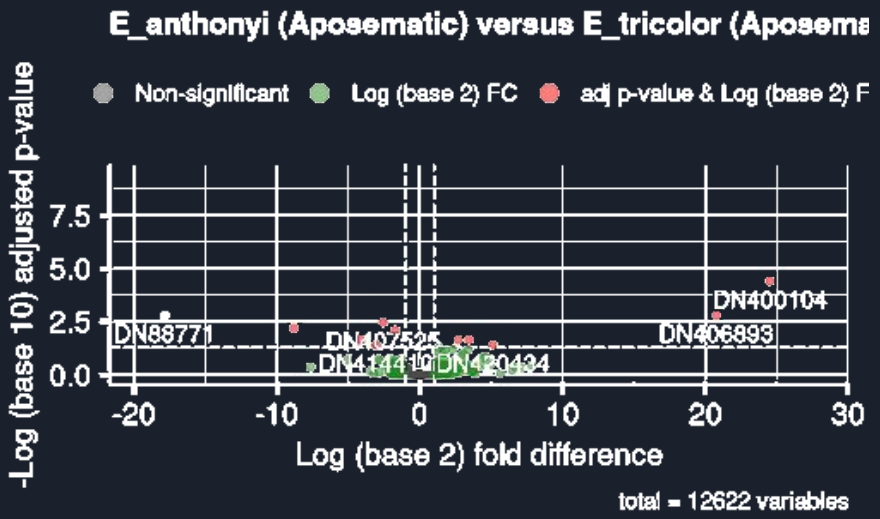
Doesn’t follow phylogenetic relationship

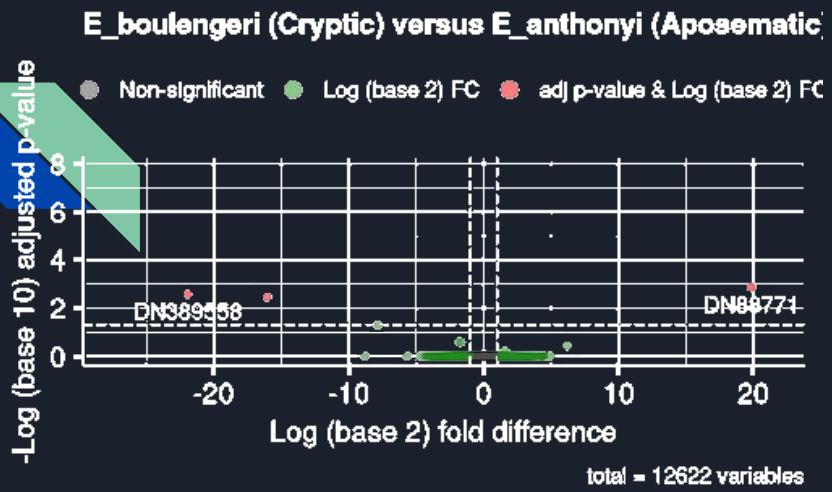


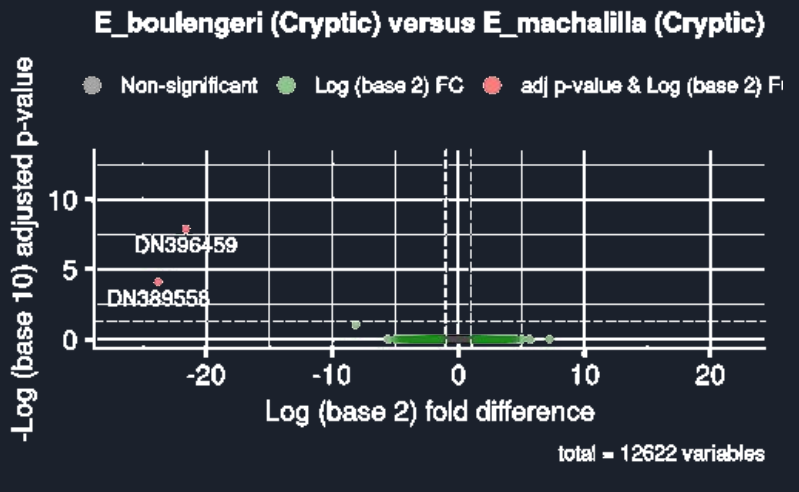


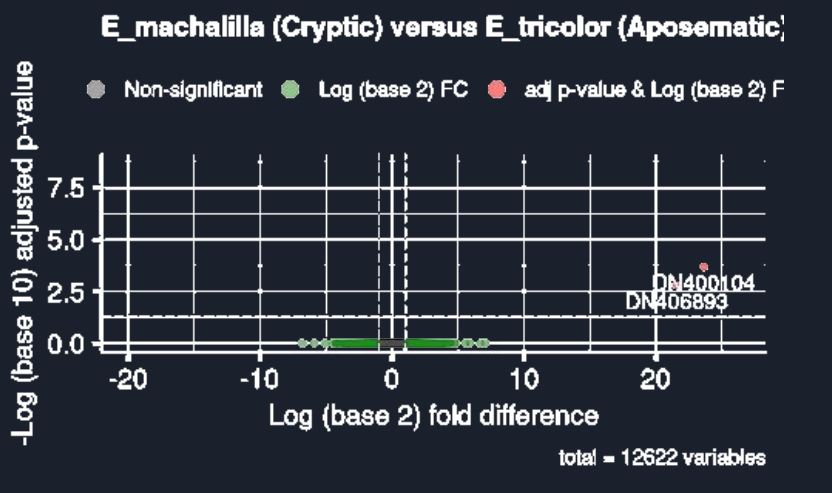
Number of differentially expressed genes






E_boulengeri (Cryptic) versus E_anthonyi (Aposematic)
Non-significant
Log (base 2) FC
adj p-value & Log (base 2) FC
-Log (base 10) adjusted p-value
DN389558
DN88771
Log (base 2) fold difference
total = 12622 variables


E_boulengeri (Cryptic) versus E_machalilla (Cryptic)
Non-significant
Log (base 2) FC
adj p-value & Log (base 2) FC
-Log (base 10) adjusted p-value
DN396459
DN389558
Log (base 2) fold difference
total = 12622 variables


E_machalilla (Cryptic) versus E_tricolor (Aposematic)
Non-significant
Log (base 2) FC
adj p-value & Log (base 2) FC
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Log (base 2) fold difference
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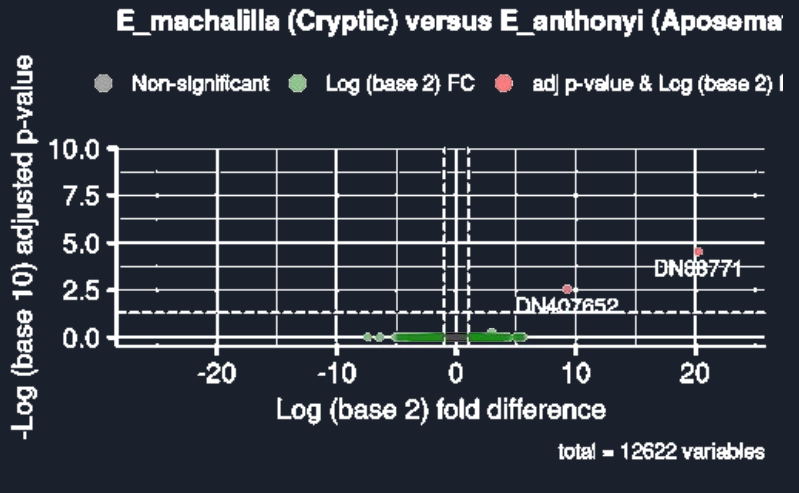
E_machalilla (Cryptic) versus E_anthonyi (Aposema
Non-significant
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adj p-value & Log (base 2) FC
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DN407652
Log (base 2) fold difference
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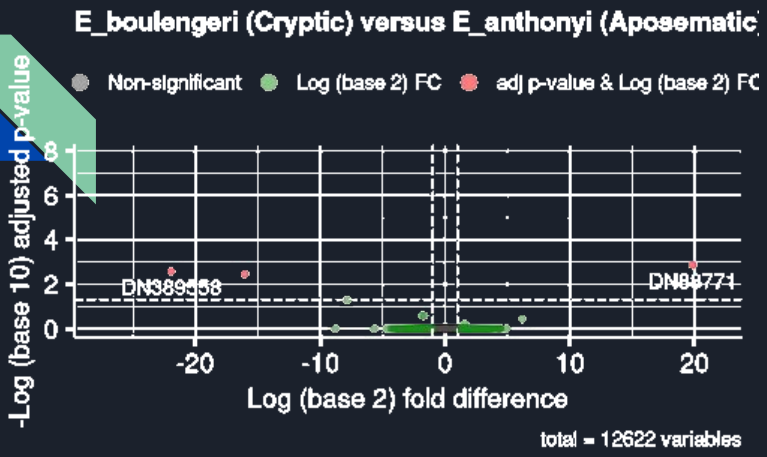
E_boulengeri (Cryptic) versus E_anthonyi (Aposematic
Non-significant
Log (base 2) FC
adj p-value & Log (base 2) FC
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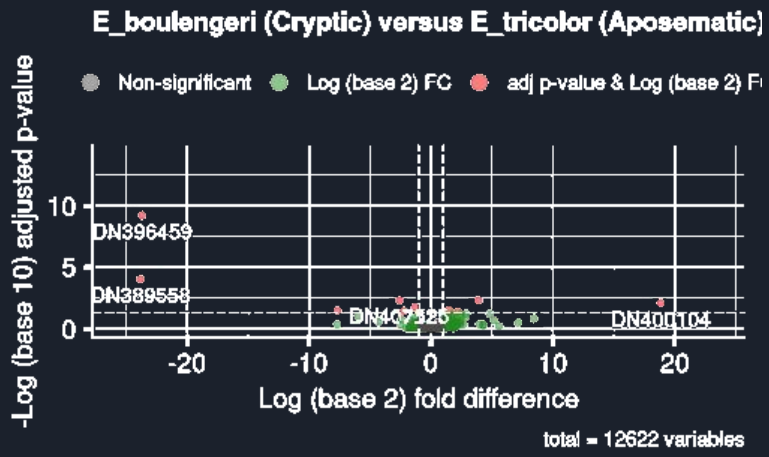
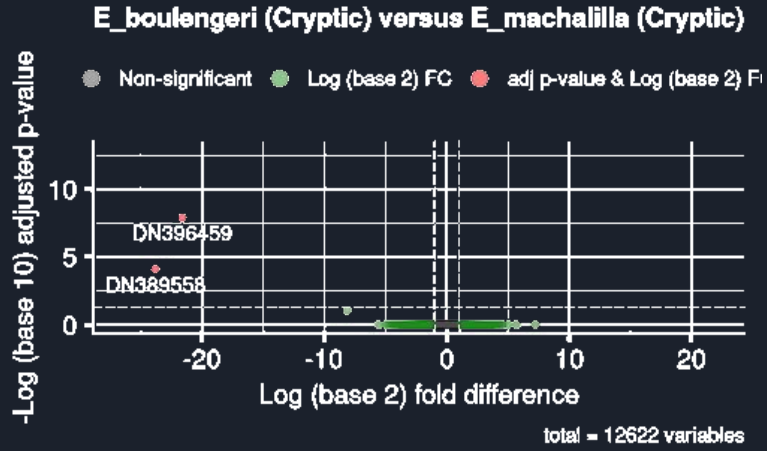
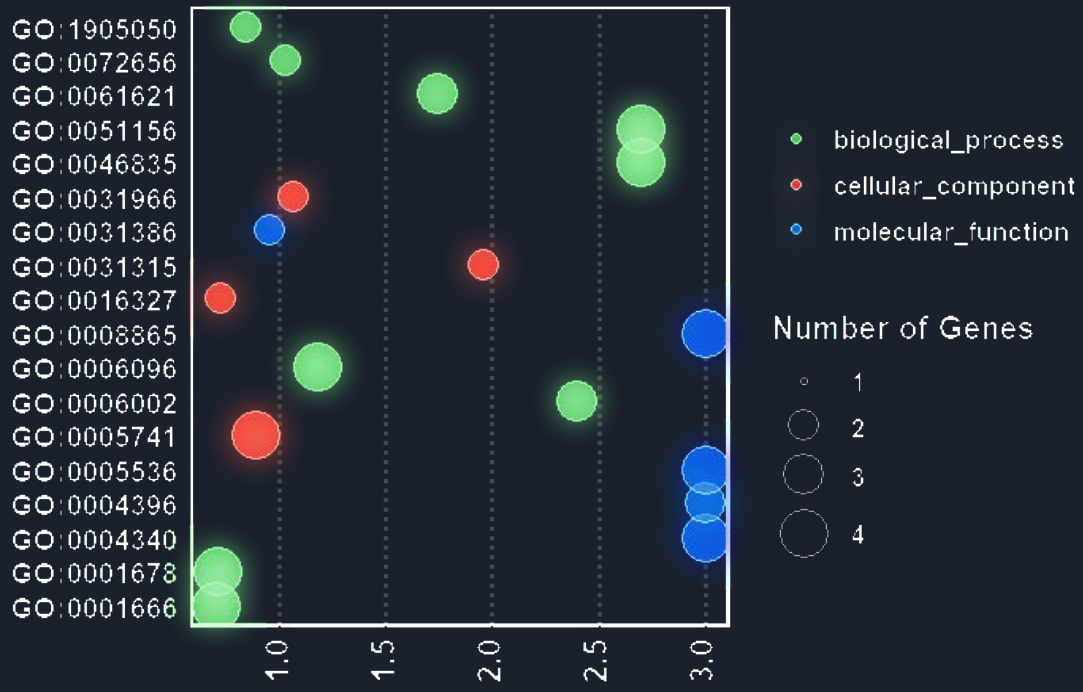
DN88771
Log (base 2) fold difference
total = 12622 variables


E_boulengeri (Cryptic) versus E_tricolor (Aposematic)
Non-significant
Log (base 2) FC
adj p-value & Log (base 2) F
-Log (base 10) adjusted p-value
DN396459
DN389558
DN400104
Log (base 2) fold difference
total = 12622 variables


E_boulengeri (Cryptic) versus E_machalilla (Cryptic)
Non-significant
Log (base 2) FC
adj p-value & Log (base 2) F
-Log (base 10) adjusted p-value
DN396459
DN389558
Log (base 2) fold difference
total = 12622 variables

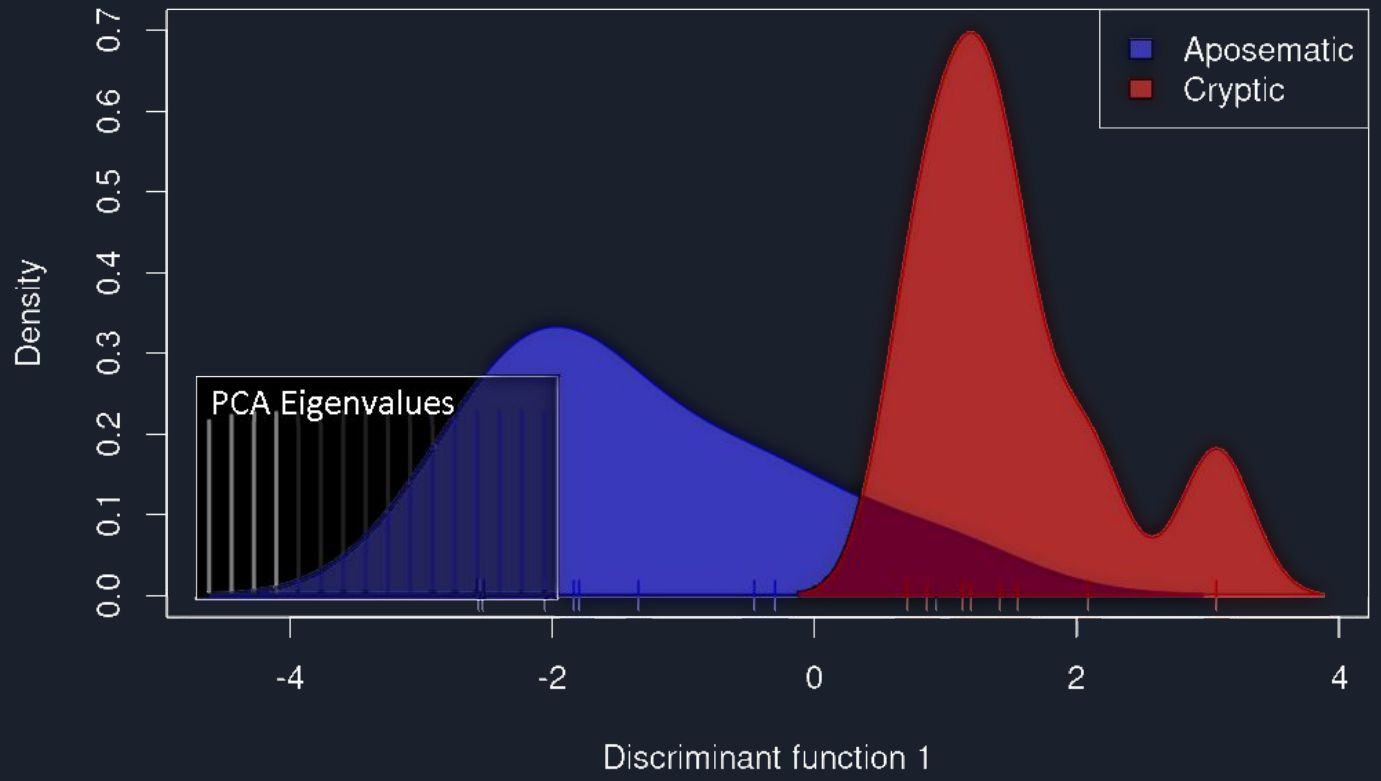
Gene Ontology Analysis

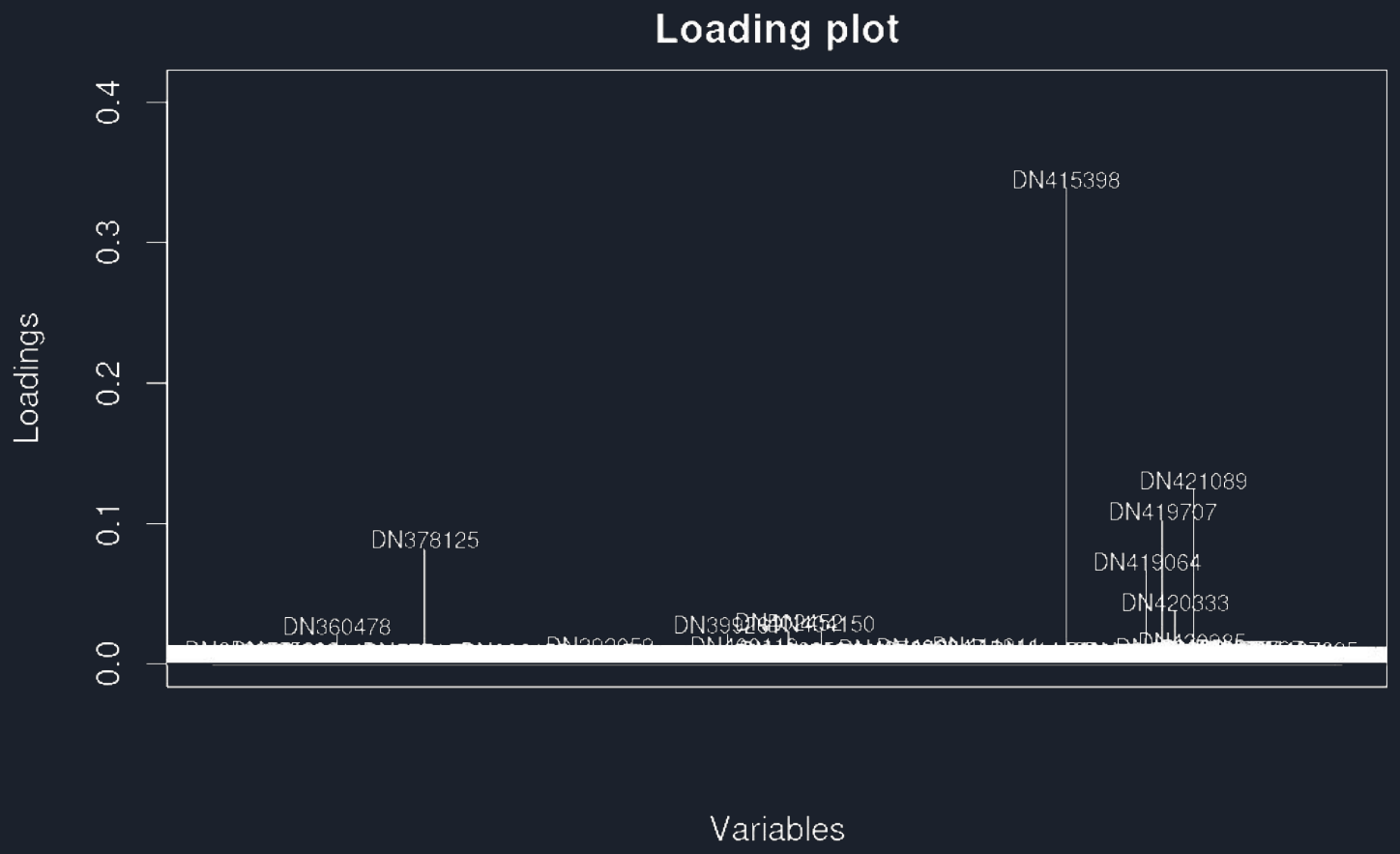


DAPC - Discriminant Analysis of PCs

- PCs are submitted to a Linear Discriminant Analysis
- Focus on between-group variation
- Steps:
  - Data frame manipulation
  - Determine how many PCs to retain
  - Run DAPC
  - Visualize

DAPC Aposematic vs Cryptic

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Loadings
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0.1
0.2
0.3
0.4
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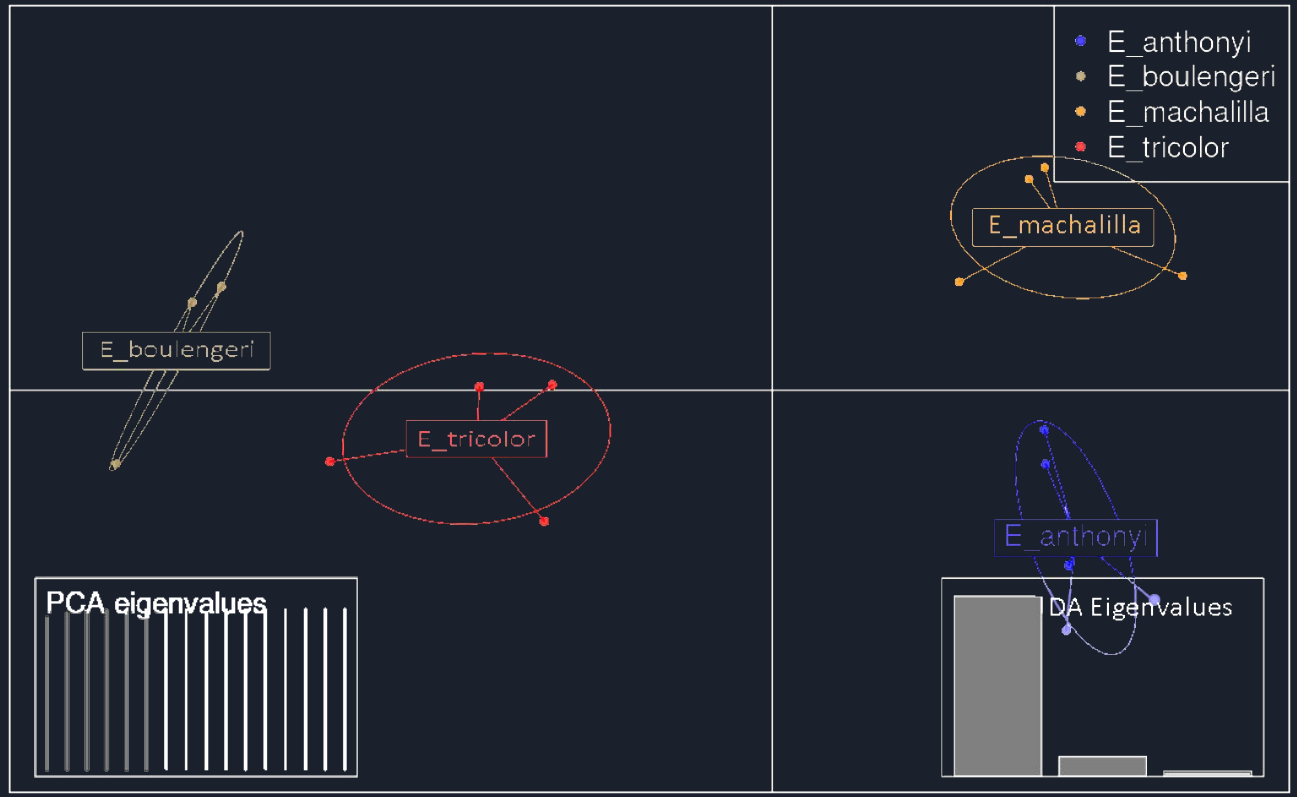
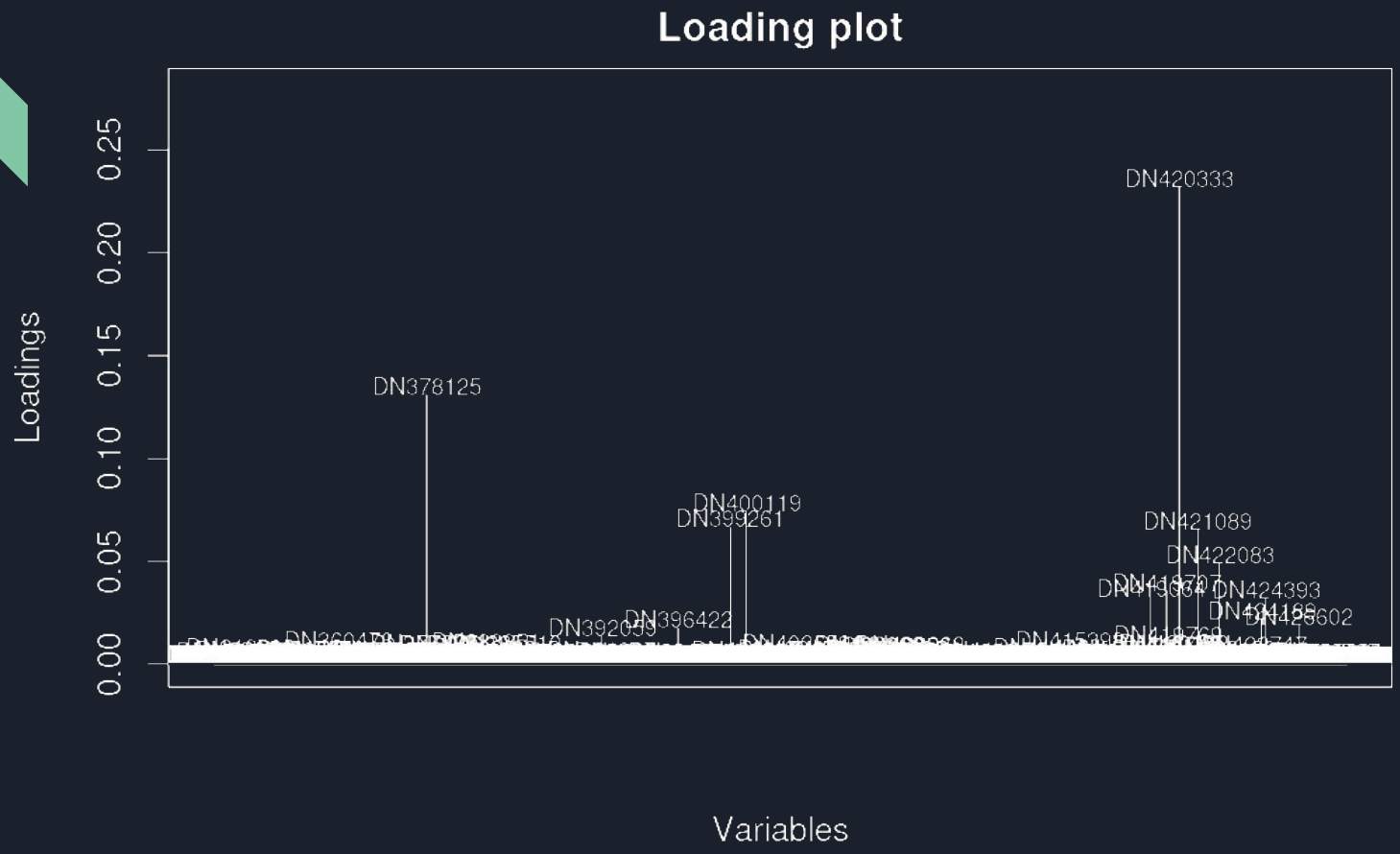
DN421089
DN419707
DN378125
DN419064
DN420333
DN360478
Variables

DAPC by Species




Loading plot
Loadings
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0.10
0.15
0.20
0.25
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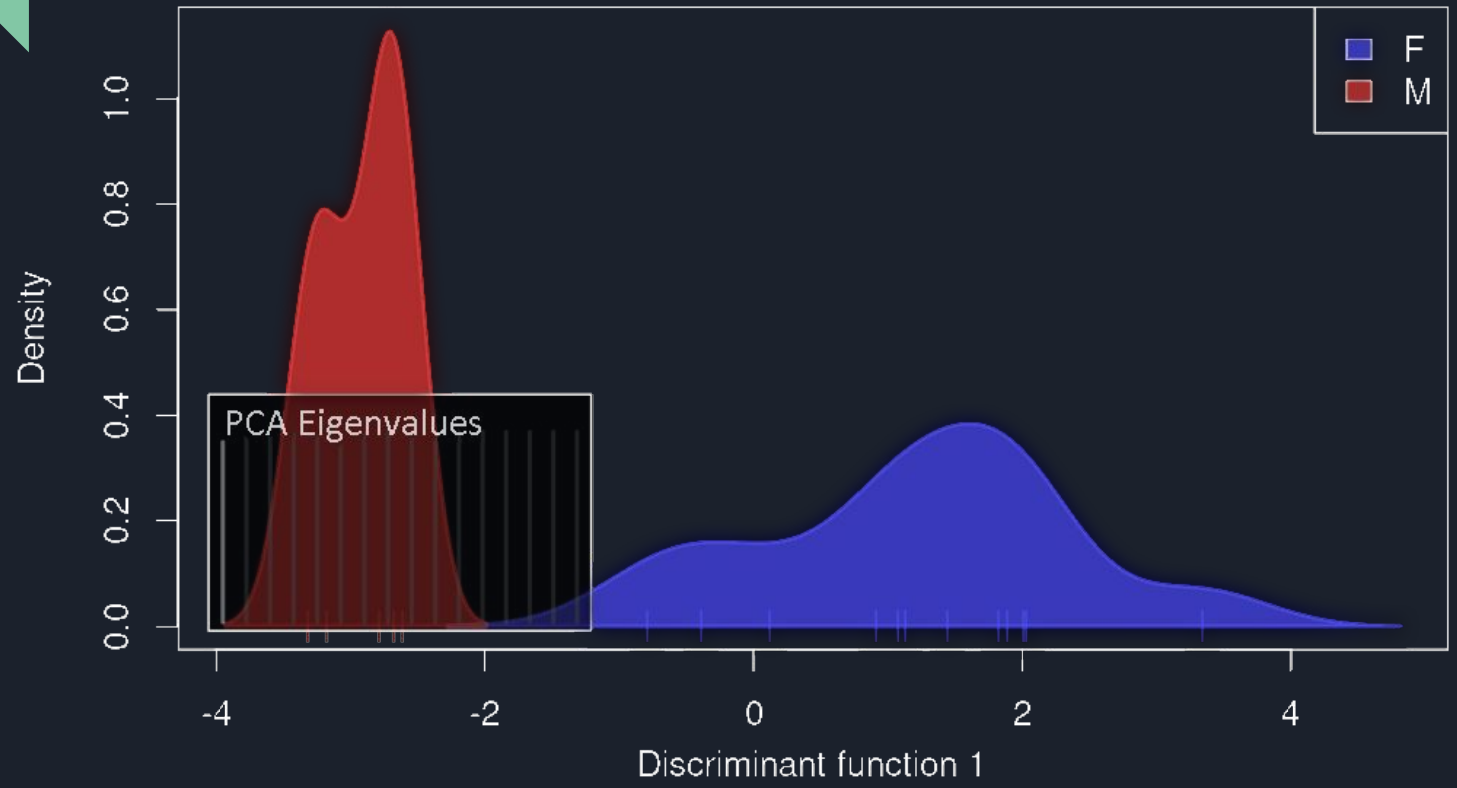
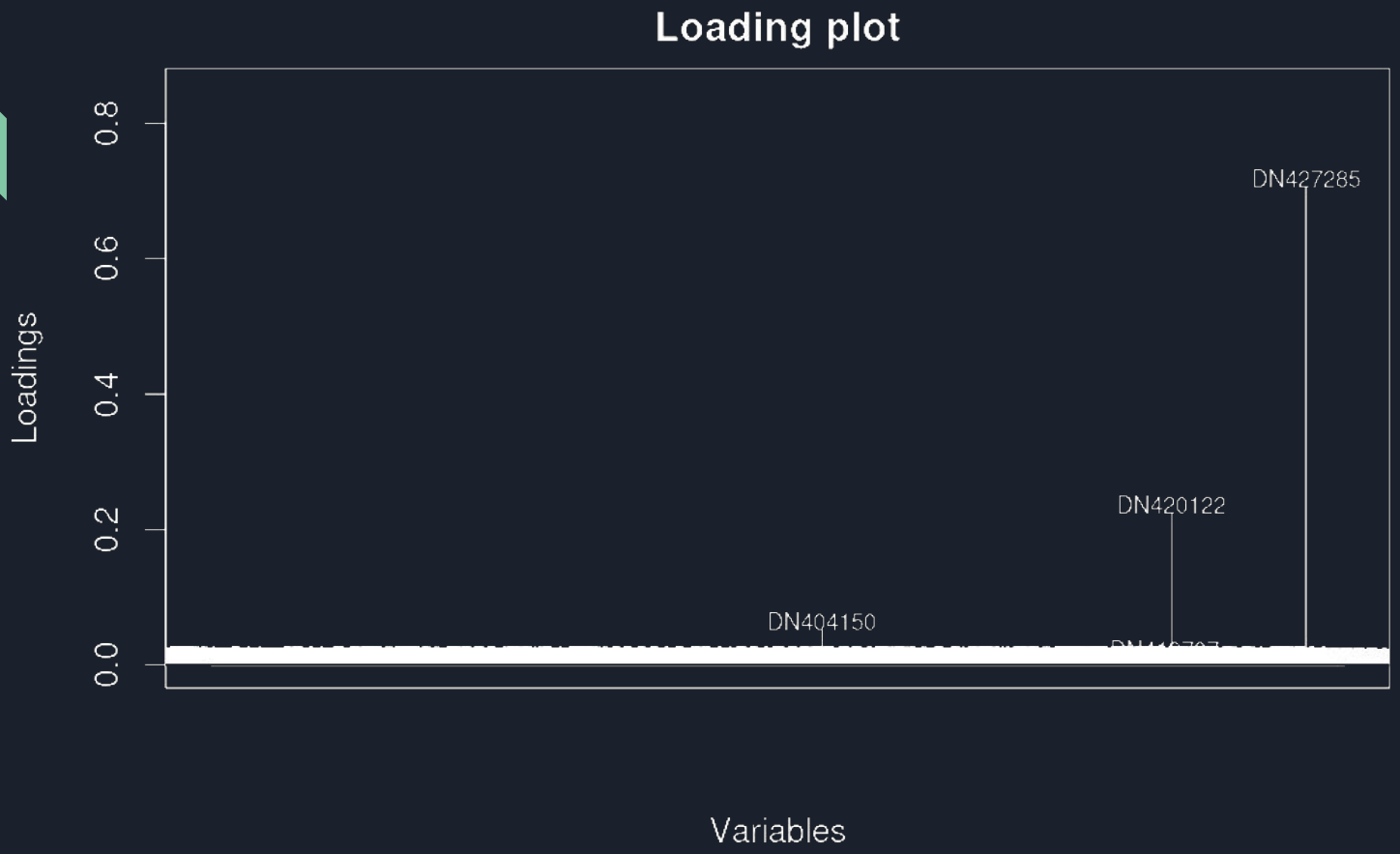
DN378125
DN400119
DN399261
DN421089
DN422083
DN424393
DN396422
DN392059
DN428602
Variables

DAPC Male vs Female




Loading plot
Loadings
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0.2
0.4
0.6
0.8
DN427285
DN420122
DN404150
Variables

Findings and Conclusions

- No clear clustering of aposematic vs cryptic species
- Few significant differentially expressed genes regardless of pairings
- Genes that drive separation aren’t notable
- Hypothesis not supported!

Limitations

- Limited samples: 2 cryptics and 2 aposematics
- Time constraint
  - Didn’t verify result
  - Didn’t test out different approaches (e.g., sex)
- Limited access to data

Next Steps

- Explore males and females separately
- Differences in how the sexes excrete alkaloids
- What else may contribute to the coloration differences (as opposed to looking towards gene expression in the liver).