Manuscript

Ferne Kotlyar

2025-09-24

Introduction

According to 67 slow-motion videos (240 fps) of wild *Heliconia tortuosa* visitors, specialized hummingbirds appear to displace nectar and deposit it on the stigma as they exit the flower. This appears to occur less frequently with non-specialized hummingbirds. The goal is to compare the likelihood of nectar deposition on the stigma between specialized and non-specialized hummingbirds. To test this species-specific difference, we conducted nectar dye experiments using 3D printed bill replicas of *H. tortuosa*'s primary visitors[1,2], two specialized (traplining) hummingbirds: green hermits (*Phaethornis guy)*, and violet sabrewings (*Campylopterus hemileucurus)*, and two non-specialized (territorial) hummingbirds: rufous-tailed, (*Amazilia tzacatl*) and crowned woodnymphs (*Thalurania colombica*). In these dye experiments, we inject flowers with dye, use the 3D printed bills, then measure the likelihood of dye deposition on floral anthers and stigma (response = presence/absence of dye).

Methods

Preliminary Power Analysis

Calculating the sample size needed for data collection.

```
##
##      Chi squared power calculation
##
##               w = 0.5
##               N = 51.97884
##              df = 1
##       sig.level = 0.05
##           power = 0.95
##
## NOTE: N is the number of observations
```

Assuming there will be a large effect (w=0.5), we will need a sample size of at least 52 flowers.

Short Description

Study Location: This study was conducted at the Las Cruces Biological Station in southern Costa Rica (Coto Brus Canton, Puntarenas Province)[3].

Data Collection: Open *H. tortuosa* flowers were collected from the Las Cruces Biological Station grounds. ~0.05 mL of fuchsin dye was injected into the base of the corolla tube right above the nectar chamber of *H.*

tortuosa flowers[4]. We then inserted and extracted the bill replicas, while mimicking hummingbird behaviour, as per the slow-motion videos. Immediately after removing the bill from the flower, the anthers and stigma were placed under a field microscope (Carson MicroFlip 100x-250x LED), and the presence (or absence) of dye was recorded on the anthers and stigma separately. Each species was used 13-14 times, for a total of n = 55.

Data Overview

The final results table will have a two by two factorial comparison for anthers and stigma, separately, where each table will look like this:

	Yes	No
Territorial	-	-
Trapliner	-	-

Results

Display Contingency Tables

```
## [1] "Anthers"
```

```
##
##               No Yes
##   territorial 21   7
##   trapliner    2  25
```

```
## [1] "Stigma"
```

```
##
##               No Yes
##   territorial 26   2
##   trapliner   13  14
```

Chi-Squared Analysis

```
##
##  Pearson's Chi-squared test with Yates' continuity correction
##
## data:  table(ND_clean$specialization, ND_clean$dye_on_anthers_binary)
## X-squared = 23.108, df = 1, p-value = 1.532e-06
```

```
##
##  Pearson's Chi-squared test with Yates' continuity correction
##
## data:  table(ND_clean$specialization, ND_clean$dye_on_stigma_binary)
## X-squared = 11.24, df = 1, p-value = 0.0008004
```

All results are significant, suggesting that trapliners and territorial birds deposit different amount of nectar on both the anthers and stigma of *H. tortuosa* flowers.

Fisher Exact Test

I will also use the Fisher Exact Test due to the small count of territorials placing dye on the stigma in the contingency table.

```
##
##  Fisher's Exact Test for Count Data
##
## data:  table(ND_clean$specialization, ND_clean$dye_on_anthers_binary)
## p-value = 2.893e-07
## alternative hypothesis: true odds ratio is not equal to 1
## 95 percent confidence interval:
##    6.17072 368.24946
## sample estimates:
## odds ratio
##   34.11459
```

```
##
##  Fisher's Exact Test for Count Data
##
## data:  table(ND_clean$specialization, ND_clean$dye_on_stigma_binary)
## p-value = 0.0003067
## alternative hypothesis: true odds ratio is not equal to 1
## 95 percent confidence interval:
##    2.497094 138.112315
## sample estimates:
## odds ratio
##    13.2968
```

Once again, all results are significant, with large odd ratios. However, the confidence intervals are extremely large as well.

Plot Results

Anthers Graph

Although the stats were done based on hummingbird specialization, the graph will be shown based on species to include more information.

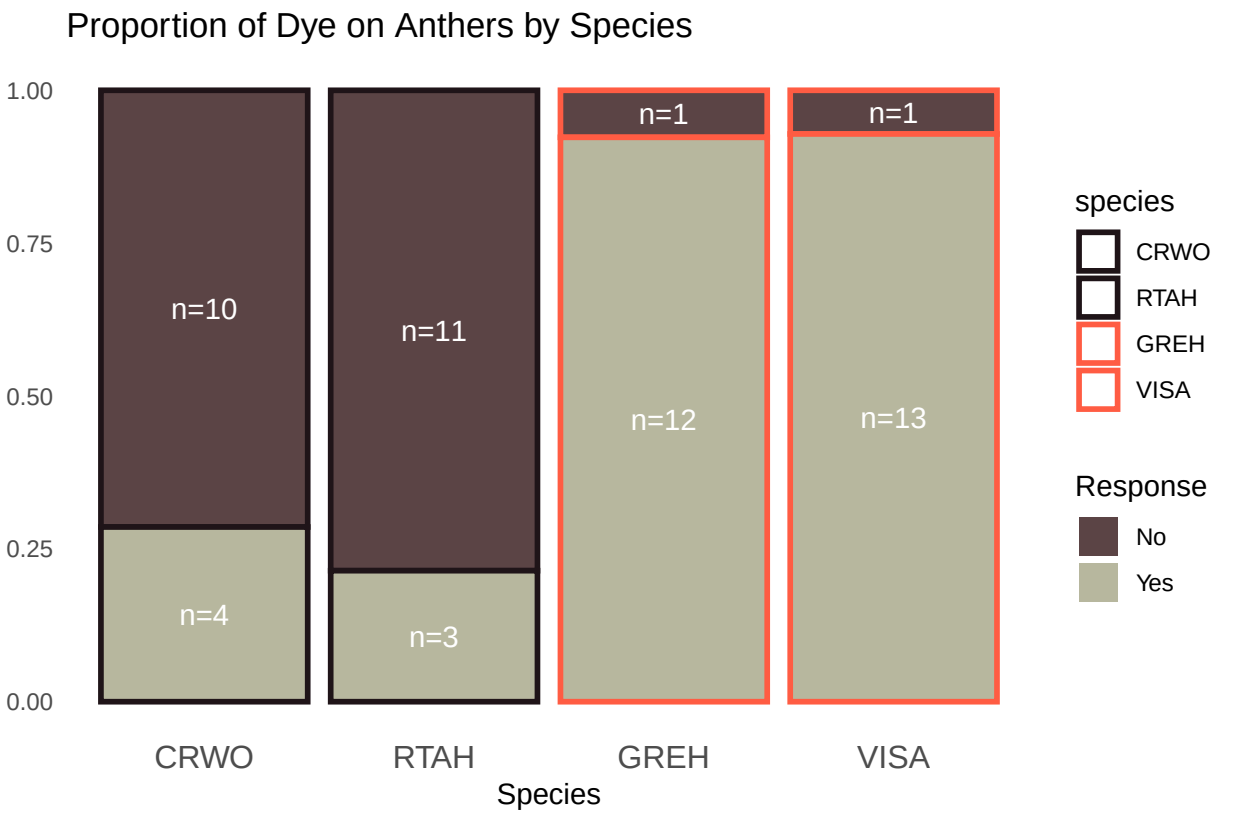
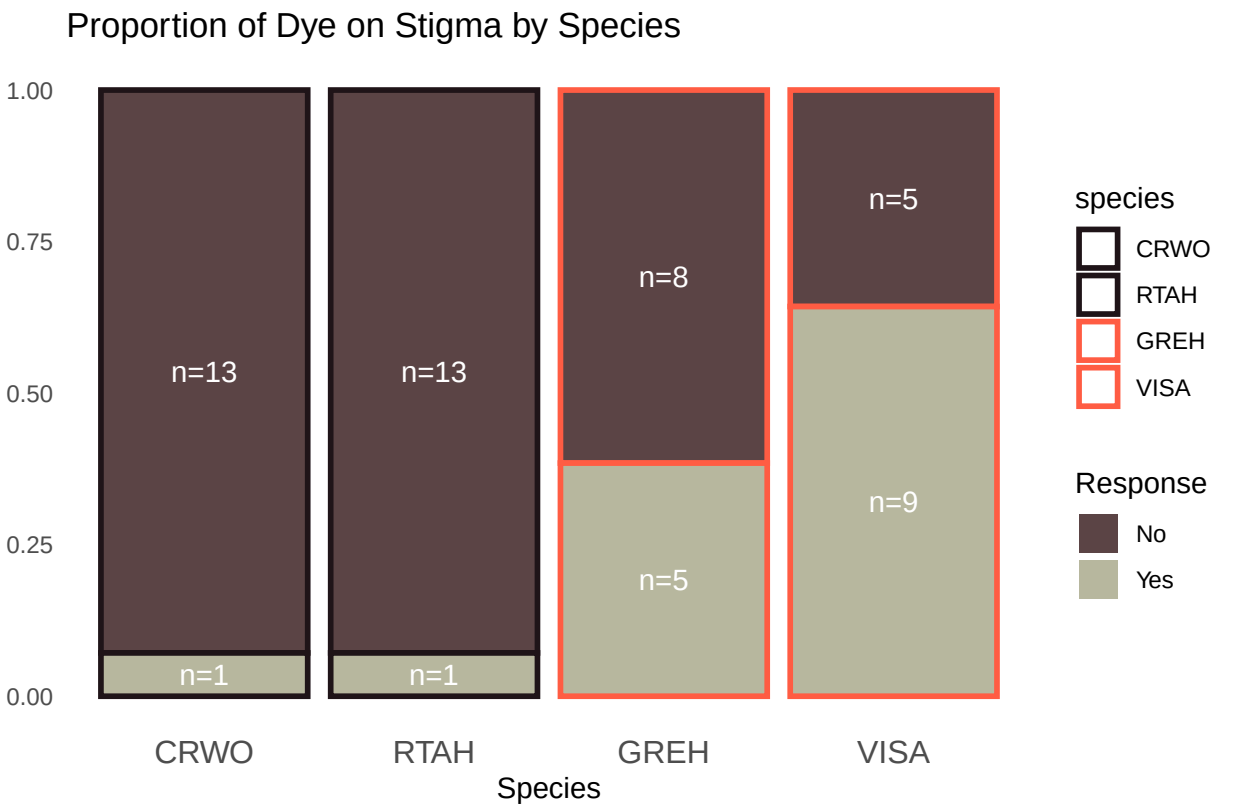


Figure 1. Dye deposition on anthers based on *H. tortuosa´*s primary visitors. This includes two specialized (trap

Stigma Graph

This graph is repeated for the dye on stigma results.



Dye deposition on stigma based on*H. tortuosa*'s primary visitors. This includes two specialized (traplining) hum

Combined Graphs

Here, we combine the graphs, then export the combined graph as a PNG into the 03_figures folder.

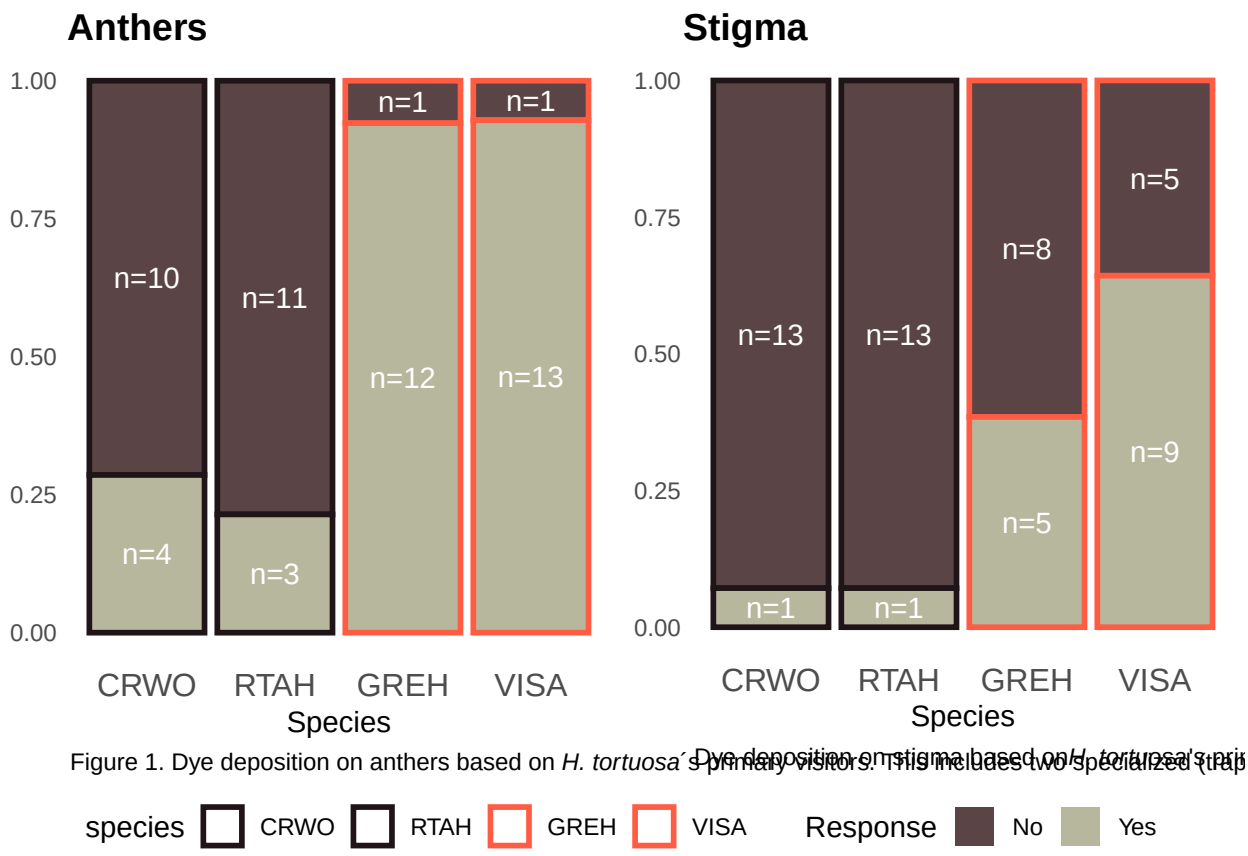


Figure 1. Dye deposition on anthers based on *H. tortuosa*´s primary visitors. This includes two specialized (trap) Dye deposition on stigma based on *H. tortuosa*'s pri

References

1. Betts, M. G., Hadley, A. S. & Kress, W. J. Pollinator recognition by a keystone tropical plant. *Proceedings of the National Academy of Sciences* **112**, 3433–3438 (2015).
2. Leimberger, K. G., Hadley, A. S. & Betts, M. G. Plant–hummingbird pollination networks exhibit limited rewiring after experimental removal of a locally abundant plant species. *Journal of Animal Ecology* **92**, 1680–1694 (2023).
3. Stiles, F. G. Ecology, Flowering Phenology, and Hummingbird Pollination of Some Costa Rican Heliconia Species. *Ecology* **56**, 285–301 (1975).
4. Kress, J. W. Self-incompatibility in central american heliconia. *Evolution* **37**, 735–744 (1983).