Preregistration

Species-Specific Differences in Stigma Contact: Evidence from Slow-Motion Videos and 3D Printed Bill Experiments in *Heliconia tortuosa*

Kotlyar Ferne[1]

[1] Department of Ecology and Evolutionary Biology, University of Toronto

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Study Information

Title	Species-Specific Differences in Stigma Contact: Evidence from Slow-Motion Videos and 3D Printed Bill Experiments in *Heliconia tortuosa*
Description	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, 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).

Hypotheses If our observations are true, specialized visitors should deposit nectar more frequently on the stigma and anthers of *H. tortuosa* flowers compared to non-specialized visitors.

Design Plan

Study type **Experiment**. A researcher randomly assigns bill types (treatments) to flowers (study subjects).

Blinding No blinding is involved in this study.

Study design This will be an experimental study with a two by two factorial comparison for anthers and stigma, separately. Each table will look like this:

Table 1: Final results table

	Yes	No
Specialized Visitors	-	-
Non-Specialized Visitors	-	-

Response variable: Nectar deposition on the stigma and anthers (binary outcome: Yes/No).

Predictor variable: Type of visitor (categorical with two levels: specialized vs. non-specialized). The specialized (traplining) level with be composed of green hermit (*Phaethornis guy),* and violet sabrewing (*Campylopterus hemileucurus)* hummingbirds. The non-specialized (territorial) level will be composed of

rufous-tailed (*Amazilia tzacatl)* and crowned woodnymph (*Thalurania colombica)* hummingbirds.

Randomization Simple randomization was used to assign treatments (bill type) to flowers.

Sampling Plan

Existing data **Registration prior to analysis of the data**.

As I am nearing the end of my degree, I have completed all my data collection and I am at the analysis and writing stage. Sadly, I only learned about preregistration after collecting all my data.

Data collection procedures **Study Location:** This study was conducted at the Las Cruces Biological Station in southern Costa Rica (Coto Brus Canton, Puntarenas Province).

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. 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.

Sample size Our target sample size was 13 flowers per species, for a total of 52 flowers (26 per bill type). In the end, we collected 55 flowers.

```
library(pwr)

pwr.chisq.test(
  w = 0.5,        # effect size (small=0.1, medium=0.3, large=0.5)
```

```
  df = 1,         # degrees of freedom
  sig.level = 0.05,
  power = 0.95)
```

Sample size rationale

```
##
##      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
```

Since we visually observed this effect and we are now testing it experimentally, we expect there to be a large effect size (w=0.5). We used a power analysis (`pwr.chisq.test` from the pwr package) with a large effect size of .50 at the standard .05 alpha and .95 power. We will need a sample size of at least 52 flowers.

Stopping rule

Since this is a small, complementary dataset to the larger study that does not take much time or resources, there will not be a set stopping point.

Variables

Manipulated variables

Bill Types: We used bills from 4 different hummingbird species that commonly visit *H. tortuosa* flowers, two specialized, traplining species and two non-specialized, territorial species. Bill replicas were generated from photogrammetry scans of each species.

Measured variables	**Dye on Stigma:** presence or absence of dye on stigma after having inserted and extracted a bill replica. This was either: no (N), yes (Y), little, lots, or maybe (M). Later, this was converted to the binary yes or no. **Dye on Anthers:** same as above, but on the anthers.
Indices	We will not be calculating any indices.

Analysis Plan

Statistical models	We will use a two by two chi-squared or a fishers exact test in the case of small counts. The independent variable is specialization (either specialized/trapliner or non-specialized/territorial), whereas the dependent variable is dye on stigma and separately, dye on anthers.
Transformations	This analysis does not require any additional transformations.
Inference criteria	We will use the standard $p<.05$ criteria for determining if the chi-square or fishers exact test suggest that the results are significantly different from those expected if the null hypothesis were correct.
Data exclusion	No checks will be performed to determine eligibility for inclusion. Outliers will be included in the analysis.
Missing data	If the stigma is missing, it will not be included in the analysis.