

Preregistration

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

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Study Information

Title	Species-Specific Differences in Stigma Contact: Evidence from Slow-Motion Videos and 3D Printed Bill Experiments in <i>Heliconia tortuosa</i>
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Description	According to 67 slow-motion videos (240 fps) of wild <i>Heliconia tortuosa</i> 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
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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 <i>H. tortuosa</i> flowers compared to non-specialized visitors.
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Design Plan

Study type	Experiment. A researcher randomly assigns bill types (treatments) to flowers (study subjects).
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Blinding	No blinding is involved in this study.
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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:
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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 will 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.
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Sampling Plan

Existing data	Registration prior to analysis of the data.
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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).
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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.
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Sample size rationale	Since we visually observed this effect and we are now testing it experimentally, we expect there to be quite a large effect size. We used a power analysis (<code>pwr.chisq.test</code> from the <code>pwr</code> package) with a large effect size of .50 at the standard .05 alpha and .95 power, the desired sample size was 52.
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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.
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Variables

Manipulated variables	Bill Types: We used bills from 4 different hummingbird species that commonly visit <i>H. tortuosa</i> flowers, two specialized, traplining species and two non-specialized, territorial species. Bill replicas were generated from photogrammetry scans of each species.
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Measured variables	<p>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.</p> <p>Dye on Anthers: same as above, but on the anthers.</p>
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Indices	We will not be calculating any indices.
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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.
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Transformations	This analysis does not require any additional transformations.
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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.
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Data exclusion	No checks will be performed to determine eligibility for inclusion. Outliers will be included in the analysis.
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Missing data	If the stigma is missing, it will not be included in the analysis.
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