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Frog Lab Report

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## **Cryptic vs. Aposematic Frog Influence on Habitat Use and Breeding Behavior**

### **Abstract**

In Bocas Del Toro, the poison dart frog is found in varying morphologies (morphs) across the islands and mainland. These different frog morphs exhibit distinct traits to achieve evolutionary success. Aposematic (red) and cryptic (green) frog morphs are already known to differ in behavior and poison level. We conducted a study to analyze whether variation across frog morphs affect habitat use and breeding behavior. We hypothesized that there would be significant variation among frog morphs regarding habitat use and breeding behavior. We selected two study sites in the Bocas Del Toro archipelago, one supporting an aposematic frog morph and the other supporting a cryptic morph. We then collected data on substrate type, color and vertical movement of the frog using male frog playback calls. The results of the study found that aposematic frogs displayed heightened responsiveness to playback calls compared to cryptic frogs. Although there was no statistically significant difference between the two morphs in terms of substrate type, substrate color, or vertical movement were found, the study suggests differing behavioral strategies based on frog morph in Bocas Del Toro.

### **Introduction**

The Bocas Del Toro archipelago is covered by dense tropical rainforests, creating an ideal home for numerous tropical frogs. Frogs from the Bocas Del Toro Province have extreme variance in color and pattern, sympatrically and allopatrically (Aguilar-Gómez et. al., 2024).

Selection drives new morphologies (morphs) to form within the evolutionary model resulting from the interplay between predation avoidance, intraspecific competition, and mate choice (Aguilar-Gómez et. al., 2024). Frog species adapt these different morphologies and traits to help them succeed evolutionarily. These traits can range from exhibiting unique behavioral patterns to utilizing distinct survival strategies to avoid predation. Different frog morphs are usually associated with different traits. For example, in Bocas Del Toro, poison dart frogs are found with different morphs across islands and the mainland, where the aposematic morphs were found to be more toxic and aggressive than cryptic morphs (Galeano et. al., 2016). Therefore, variation across frog morphs may also influence habitat use and breeding behavior. We conducted a study to determine whether variation across frog morphs would influence these behaviors. We hypothesized that variation across frog morphs would influence habitat use and breeding behavior. We predicted that cryptic (green) frogs would select for substrates that would reduce their visibility, aposematic (red) frogs would be more territorial and react at a higher rate to other males, aposematic frogs would react faster to signs of other males, and aposematic frogs would display more defensive behaviors as compared to cryptic frogs.

## **Materials and Methods**

The study was conducted in two sites, each corresponding to a different frog morph. For aposematic frogs, the study site was in the forest at Hospital Point, on Isla Solarte. The site was a disturbed forest near the beach, at an early stage of regeneration, with scattered large trees and a river embankment running through. For the cryptic frogs, the study site was the forest behind La Coralina, on Isla Colón. The site was a secondary forest, with an open understory and closed canopy.

Figure 1: Map Depicting the Two Study Sites in the Bocas Del Toro Archipelago

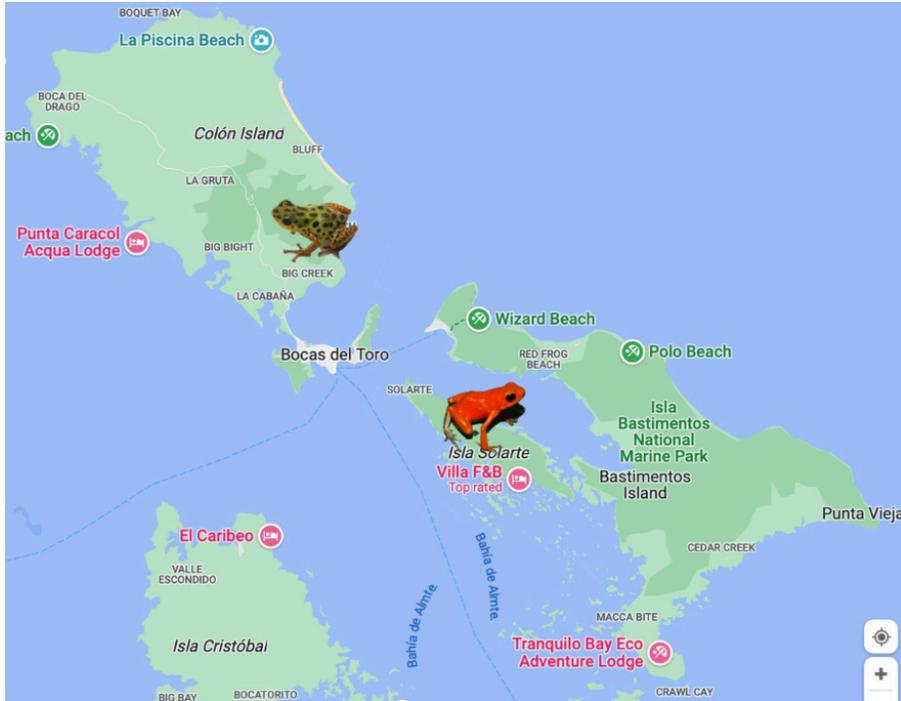


Figure sourced from Daniel Medina. Green areas (land) representing the different islands in the Bocas Del Toro Archipelago. Blue areas (water) representing the Caribbean Sea. Red frog marker representing the study site where field research was conducted on aposematic frogs. Green frog marker representing the study site where field research was conducted on cryptic frogs.

Data was collected on aposematic and cryptic frogs through a series of controlled steps. After spotting a frog, we placed a speaker one meter away from the frog. We then played the one minute long frog male call recording, waited thirty seconds, then played the call again. If the frog reacted within the first two plays of the recording, we moved on. If the frog did not react within the first two plays of the recording, we repeated the recording up to five times, moving on after observing a reaction or reaching the five play limit. After observing the frog's behavior, we recorded the substrate, if the frog reacted to the call, the distance moved either towards or away from the speaker, and the total time it took the frog to move either towards or away from the speaker. When we finished observing a frog, we collected that frog inside a plastic bag to try and minimize sampling error that could arise from sampling the same frog multiple times. We also

observed whether the frog was female or male. These steps were completed at each location for the distinct frog morph found there.

We analyzed the data we collected using software and statistical tests. Excel, Jamovi, and R were the software programs used to run tests and create figures. To compare substrate preferences between aposematic and cryptic morphs, we used a Chi-square test. To analyze vertical movement of the frogs (height), we used the Mann-Whitney U test. To evaluate territorial behavior in reaction to the male playback call, we created a comparative table.

## **Results**

In this study, 67 total frogs were found, of which, 54 were aposematic and 13 were cryptic.

### ***Substrate Preferences***

A Chi Square test was used to analyze the preferences in substrate color and substrate of the two frog morphologies. It was found that there was not statistically significant data to determine preference in substrate color ( $\chi^2 = 0.053$ ,  $p=0.82$ ), and there was not statistically significant data to determine preference in substrate ( $\chi^2= 2.335$ ,  $p= 0.51$ ).

Figure 1: Abundance of Substrate Color vs Frog Morph

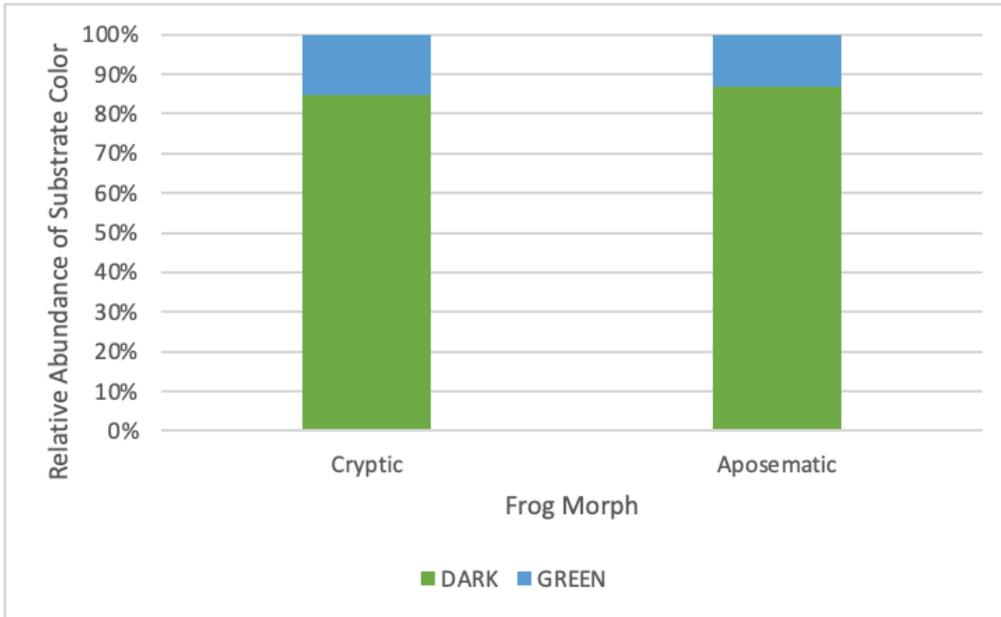


Figure sourced from Microsoft Excel created using data from field research. Relative abundance of the green and dark substrate color that the cryptic frogs and aposematic frogs were found on.

Figure 2: Abundance of Type of Substrate vs Frog Morph

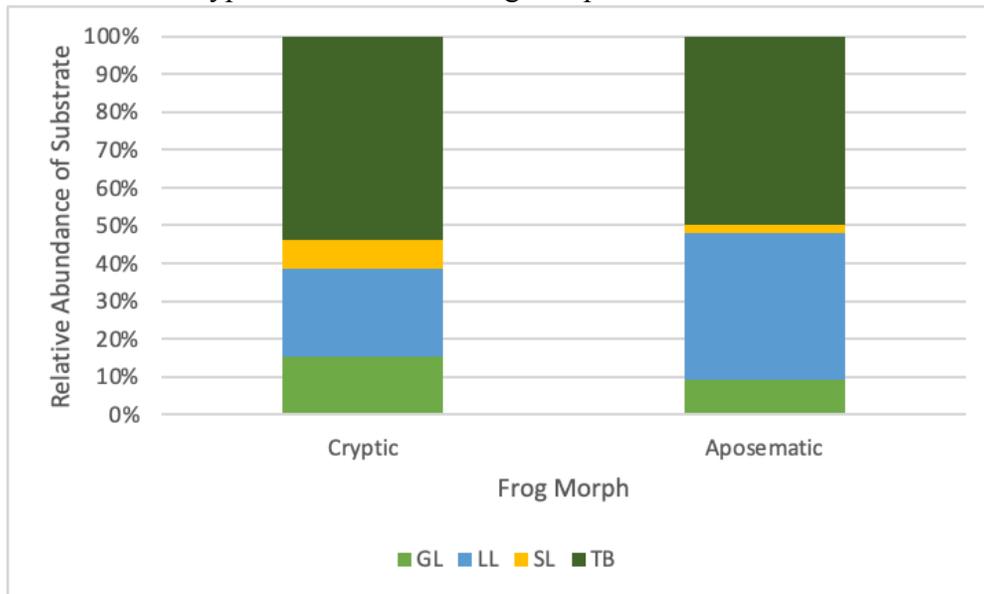


Figure sourced from Microsoft Excel created using data from field research. Relative abundance of four substrates (green leaf (GL), leaf litter (LL), soil (SL), and tree bark (TB)) that the cryptic frogs and aposematic frogs were found on.

Figure 3: Height vs Density of Frog Height

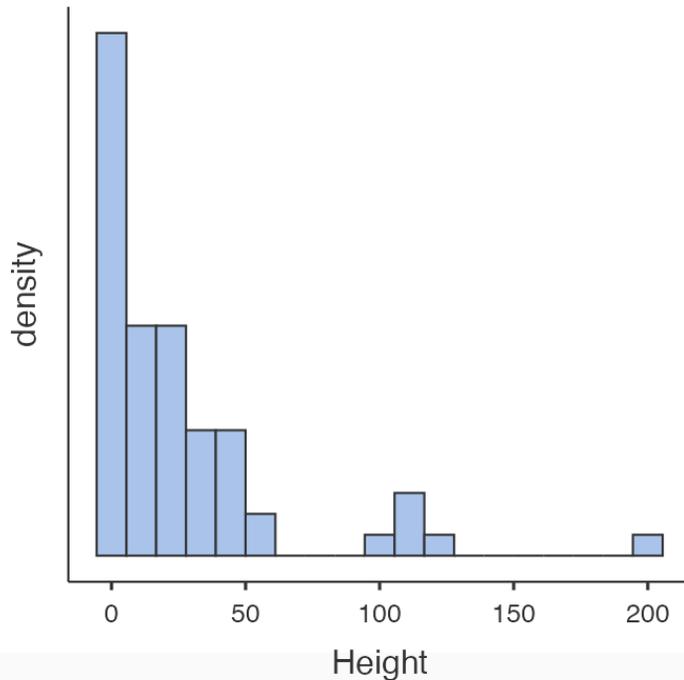


Figure sourced from Jamovi created using data from field research. A Q-Q plot comparing the found frog heights to a theoretical normal distribution. The plot is skewed to the right and contains multiple outliers.

Due to violations of the normality assumptions, a Mann-Whitney U test was used to test the relationship between morph type and height. It was found that there is no statistically significant difference between the green and red morph types ( $p=0.27$ ).

***Territorial Behavior***

It was found that both the cryptic and aposematic frogs both partook in calling and tracking while only aposematic frogs partook in charge and veer. Out of the total frogs collected, 23% of cryptic frogs performed territorial behaviors, and 21% of aposematic frogs performed territorial behaviors.

Table 1: Description and quantity of behaviors displayed by the Cryptic and Aposematic frogs.

Behavior	Explanation	Points	Cryptic	Aposematic
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Calling	Vocalize	1	1	8
Pushups	Extending the forelegs to elevate and low the anterior part of the body	1		
Body raising	Extending all four legs to elevate the entire body	1		
Limb Shaking	Rapid movement of a limb up and down	1		
Tracking	Orientation of the body in the direction of the call while staying static or moving forward	1	1	4
Charge and veer	Running toward the call and moving away prior to physical contact	2	1	
Charge and contact	Running toward the call and initiation in physical contact	3		
Escape	Escape in response to aggression	-1		
Statue	Freezing in one position	-1		
Body lowering	Lowering the body	-1		

Table 1 description, behavior names, and points follow Galeano and Harms (2016).

## Discussion

Overall, the results of our study determined a difference in agonistic behaviors between the two color morphs, suggesting evolutionary divergence based upon phenotypic color divergence. Aposematic frogs (red morphs) displayed heightened responsiveness to playback calls compared to cryptic frogs (green morphs). This finding is consistent with research by Galeano and Harms (2016), which found that more conspicuously colored morphs displayed greater aggressiveness in intraspecific interactions. Additionally, our findings aligned with Pröhl & Ostrowski (2011), which found that red males spent more time foraging and were more active

than green males, who spent more time hidden. These differences suggest that aposematic frogs are more aggressive and defensive, while cryptic frogs rely more on camouflage and evasion, supporting our hypothesis that aposematic frogs would display more defensive behaviors as compared to cryptic frogs.

However, our study found no statistically significant differences between the two morphs in terms of substrate preference, substrate color, or height. This is inconsistent with our hypothesis and may indicate that habitat use may not be as strongly influenced by phenotypic morphology or that other factors such as habitat availability and environmental conditions may impact these preferences.

Potential sources of error in our study include inconsistent volumes of the call played across trials, or possibly not meeting the one-meter distance requirement from the frog due to terrain (hills, trees, etc.) or the frog's positioning. Additionally, standing over frogs while observing their behavior may have influenced their natural responses.

Future research could be focused specifically on female behavior, as it would provide valuable insights into how morph specific behaviors influence mating strategies and reproductive success. Additionally, taking age into account when observing behavior could provide insights into age specific breeding behaviors between the different morphs. This research would contribute to the broader understanding of how phenotypic variation in frogs (cryptic and aposematic) can influence behavioral strategies related to survival and reproduction.

## Literature Cited

Aguilar-Gómez, D., Freeborn, L., Yuan, L., Smith, L. L., Guzman, A., Vaughn, A. H., Steigerwald, E., Stuckert, A., Yang, Y., Linderoth, T., MacManes, M., Richards-Zawacki, C., & Nielsen, R. (2024, January 1). Evolution and diversification of the aposematic poison frog, *Oophaga pumilio*, in Bocas del Toro. bioRxiv.

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