truly responding to male vocalizations, there must be an alternative purpose for male chirping during the dry season.

Future research should quantify how habitat refuge influences the territorial displays of male frogs. Additionally, it would be informative to investigate variations between call types to further understand the role of vocalizations in how *D. pumilio* establishes and maintains territories. Studies of responses to playbacks during the breeding season would indicate if male behavior is more strongly territorial, which might help resolve the puzzle of why *D. pumilio* maintain territories during the non-breeding season.

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Relationships between the coloration and vertical distribution of butterflies

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Abstract: Adult butterflies exhibit a remarkable diversity of coloration, though the mechanism of natural selection by which many of these color patterns have evolved remains unclear. We tested the hypotheses that butterfly color differs along a vertical gradient within forests and has evolved as a predator-avoidance strategy. We predicted that light colored butterflies would preferentially occupy the mid- and upper-canopy while darker butterflies would occupy the forest understory, due to differences in light intensity and substrate color. We further predicted that butterflies with aposematic coloration would be equally distributed throughout the canopy due to low predation pressure. As predicted, mean height of dark butterflies was lower than light butterflies and the mean height of aposematic individuals was intermediate between the two. Light butterflies had a wider vertical distribution than dark or aposematic butterflies. To test whether the wing coloration was differentially cryptic at varying heights, we photographed each of the three butterfly types in both forest understory and canopy. We measured the time it took 14 human subjects to locate the butterfly in each photo. Response time did not differ between habitat types for aposematic or light colored butterflies, but time to locate darker butterflies was longer in the understory. These results suggest that predation pressure may cause selection for dark butterfly coloration and vertical distribution. The forces selecting for light coloration remain unknown, but sexual selection may play an important role.

Key Words: aposematic coloration, butterflies, Lepidoptera, predation

Introduction

The order Lepidoptera includes some of the most distinctive and colorful organisms in the world. The Neotropics contain an especially high diversity of butterfly colorations, though the mechanism of natural selection by which many of these patterns have evolved remains unclear. Many butterfly species use cryptic coloration as a defense against visual predators (DeVries 1987).

The predator-avoidance hypothesis predicts that butterfly coloration should differ between the forest canopy and understory due to changes in light intensity and substrate color. We expected darker butterflies (black, gray, brown) to be most abundant in the dark forest understory and lighter butterflies (white, yellow, light-blue) to be most abundant in the bright mid- and upper-canopy. Based on Devries' (1987) hypothesis that butterflies treat edge habitat as canopy habitat, we predicted that the color distribution of butterflies on the edge would be similar to the distribution in the canopy. An important exception to the predator-avoidance hypothesis is the warning coloration of aposematic butterfles, which are selectively avoided by avian predators due to batesian or mullerian mimicry (Chai and Srygley 1990). We predicted that butterflies with aposematic coloration would be evenly distributed throughout the canopy, understory, and edge because of low predation pressure.

Methods

The study was conducted at Estación Biológica La Selva from 13 - 16 February 2002. On 13 and 15 February we sampled five 400 m transects in the forest interior, between 09:30 - 12:30, on the Sendero Sura, Sendero Oriental, Sendero Tres Ríos and the Camino Experimental Norte trails. On 16 February we sampled two 400 m transects along the forest edge surrounding the La Selva biological lab area and the dining/residential area. We recorded the predominant color and vertical position of all visible butterflies during walking censuses and during 2 min stationary ob-

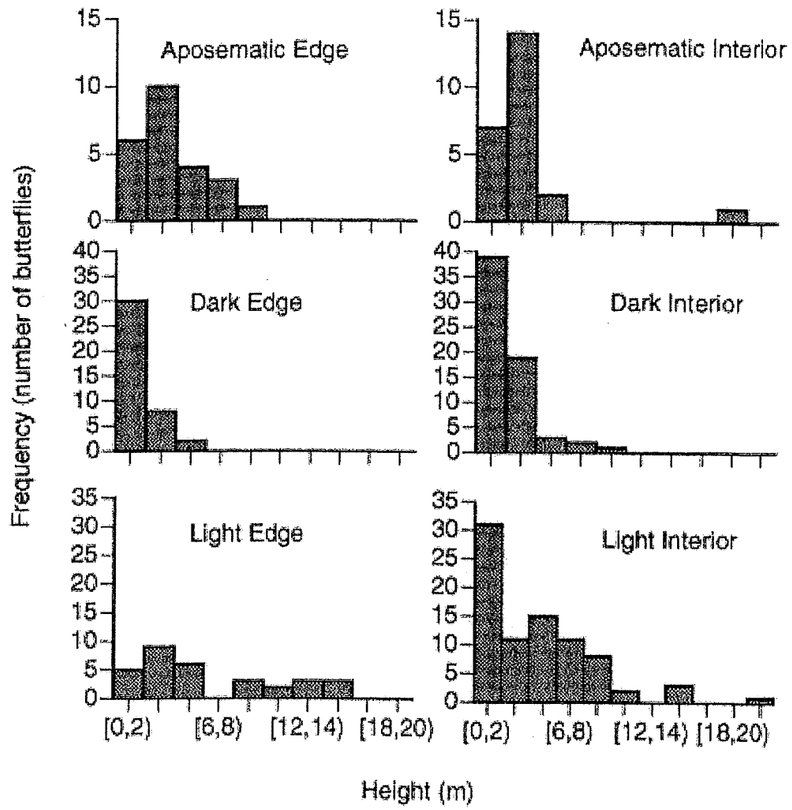

FIG. 1. Frequency of heights of butterflies observed in forest interior (n = 5) and edge habitat (n = 2). Butterflies were categorized by wing coloration as either aposematic, dark, or light.

servations made every 50 m.

We used a Nikon (Coolpix 990) digital camera to photograph dark, light and aposematic butterflies in the forest understory near the Sendero Tres Ríos and Sendero Sura trails and the canopy accessible from a bridge over the Rio Puerto Viejo. The setting for each photo was determined randomly, and pictures were taken ~2 m away from the focal butterfly. We controlled for differences in butterfly size by slightly adjusting the distance from the butterfly. We showed these images in random order to 14 participants and instructed them to find the butterfly as quickly as possible. Twelve pictures were shown, with each butterfly coloration type (light, dark, and aposematic) presented four times, twice each in the understory and canopy. We recorded the time for each observer to correctly locate the butterfly in each photograph.

We used a two-way ANOVA model to compare butterfly height distribution with respect to location (understory and edge), color (light, dark and aposematic), and the interaction between location and color. Similarly, we used a two-way ANOVA to analyze whether response time, transformed as $\log_{10}(x+1)$, varied with location, color, the interaction between location and color, and all possible interactions; observer was included as a block.

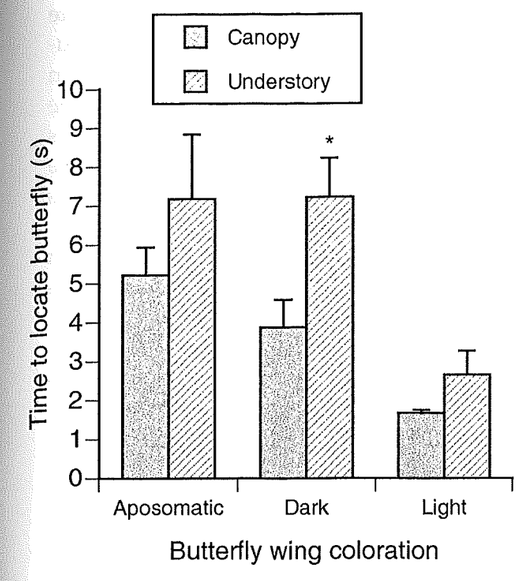

FIG. 2. Average time for human subjects (n = 14) to locate aposomatic, dark or light butterflies in photographs with a background of canopy or understory (±SE, *indicates P < 0.001).

Results

Over the three-day sampling period, we observed 284 total individuals in edge and forest habitats. Butterfly color varied significantly with height ($F_{2,284} = 70.1$, $P < 0.001$; Fig. 1). Light colored butterflies were distributed vertically throughout the entire forest. However, they were observed at greater average heights (mean ± SE = 5.99 ± 0.36 m; Fig. 1) than dark colored or aposematic butterflies. The vertical distribution of dark colored butterflies was smaller and these butterflies were observed at lower average heights than aposematic butterflies (mean ± SE = 1.36 ± 0.12 m and 2.85 ± 0.21 m, respectively; Fig. 1). Light butterflies had a wider vertical distribution than dark or aposematic butterflies (Table 1). The vertical distribution of individuals did not vary significantly with habitat, indicating that butterflies ranged over similar heights in edge and interior habitats ($F_{1,284} = 0.07$, $P = 0.80$). There was no significant interaction between habitat and color with vertical distribution ($F_{1,284} = 0.07$, $P = 0.80$).

TABLE 1. Mean height in m (±SD) of aposematic, dark and light colored butterflies across 2 habitat types (edge = 2, interior = 5, where sample size indicates number of 30 min samples on 400 m transects).

	Edge	Interior
Aposematic	3.12 ± 1.99	2.71 ± 2.62
Dark	0.93 ± 1.27	1.54 ± 1.42
Light	5.97 ± 4.61	6.01 ± 3.92

The amount of time it took a participant to find a butterfly (response time) depended upon butterfly coloration and the location or backdrop of the photograph ($F_{2,284} = 24.8$, $P < 0.0001$ and $F_{1,284} = 6.58$, $P = 0.01$, respectively; Fig. 2). Response time was shortest for light colored butterflies, indicating that they were the easiest to see. Dark colored and aposematic butterflies were equally difficult to see. Participants took longer to locate a dark butterfly within a photograph taken in the understory than within a photograph taken in the canopy (Fig. 2). There was low variation among observers, indicating that these patterns were similar for all observers.

Discussion

As predicted, butterfly coloration showed significant trends in vertical distribution in forest habitat. Dark butterflies occupied significantly lower positions than aposematic butterflies which, in turn, occupied lower positions than light butterflies. That the aposematic butterflies did not show the highest degree of variation was contrary to our original hypothesis that, because of the reduced risk of bird predation, this group would be more evenly distributed than either light or dark butterfles. Also, contrary to DeVries' hypothesis that butterfly distribution would be the same in edge habitat as in the forest canopy, the vertical distribution of coloration was similar between interior and edge habitats (Devries 1987).

Regarding the predation-avoidance hypothesis, dark butterflies were harder to

locate in understory photographs than in canopy photographs. This result indicates that dark butterflies are indeed more cryptic in the understory than either light or aposematic butterflies, and therefore, may be better suited to avoid predation at that height. Aposematic butterflies were equally difficult to locate in canopy and understory, which supports the hypothesis that aposematic coloration does not confer a greater cryptic advantage in either location. Contrary to our hypothesis that light butterflies would be more cryptic in the brighter forest canopy, light butterflies were equally difficult to locate in canopy and understory photographs. This could explain the high degree of variation observed in the vertical position of this group. Alternatively, variation in height may be explained by similar coloration and host-plant specificity in phylogenetically related species.

Though the butterfly selection test had some limitations, such as the small numbers of photographs used and the inability to incorporate butterfly cryptic behavioral adaptations, it supported the hypothesis that the vertical distribution of coloration is influenced by predator-avoidance. A future study could employ video technology, which might more accurately represent butterfly predator-avoidance behavior than the photographs used in this study. For instance, we observed that the flight pattern of many light colored butterflies appeared to mimic a falling leaf though we were unable to test whether this behavior was a cryptic defense.

While predation pressures may explain the coloration of dark butterflies, the forces influencing the light coloration of many butterflies remain unknown. Sexual selection may explain the abundance of light butterflies even in the face of increased predation risk, and therefore, mate preference may be the driving force behind the brilliant butterfly coloration so common in the tropics.

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