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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Basic aquatic communities in *Heliconia wagneriana*

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Abstract: Species of *Heliconia* with erect floral bracts are one of many types of plants that exhibit phytotelmata, plant "containers" which harbor aquatic communities. Skutch (1933) proposed that the water, a mixture of rainwater and plant secretions, protects the flowers and seeds from terrestrial insect predation, and though it may serve well in this regard, entire communities of aquatic invertebrates exploit the bracts as habitat. The impact of these organisms on *Heliconia* is not well studied, but the abundance of certain species has been shown to be negatively correlated with set seed (Seifert 1982). Broughton (1994) found that the water inside *H. wagneriana* bracts is significantly more basic than rainwater, which prompted me to address three questions in this study: what causes this pH change, what impact (if any) does it have on invertebrate populations (examined using *Paramecium* abundance), and how does this environment compare to other phytotelmata such as bromeliad tanks? Invertebrates filtered from the bract water and placed in rainwater did not change the pH of the water, indicating the alkalinity is due to plant secretions. When bract fluid was extracted and replaced with rainwater, the rainwater was either reabsorbed by the plant or evaporated within a 24 h time period. This may indicate that plant additives increase viscosity of surface tension to reduce evaporation or reduce reabsorption. One side effect of the additive(s) may be the increased pH of the water. *Paramecium* grown in pH-altered bract water showed equal growth rates in both rainwater pH-levels and bract water pH-levels, illustrating that the basic environment does not directly affect the *Paramecium* population, but effects on other invertebrates are not known. Bromeliad tank water had a pH equal to that of rainwater, and, surprisingly, no *Paramecium* populations, though it harbored its own aquatic community. Further tests are necessary to assess the effects of pH and additionally, if the presence of phytotelmata afford the plant net fitness gains or losses.

Key Words: fly larvae, Heliconiaceae, invertebrates, mosquito larvae, Paramecium *spp., phytotelmata*

Introduction

Many plants, such as bromeliads and pitcher plants, have phytotelmata, plant-formed "containers" which create an aquatic community. *Heliconia* species with erect inflorecences create phytotelmata with their cup-shaped bracts, filled with a mixture of both rainwater and water actively transported from the plant (Seifert 1982). Skutch (1933) proposed that the water "... may prevent the access of destructive insects or their larvae to the essential organs...." Though the water may protect the plant from terrestrial insects, aquatic invertebrate communities exist within these bracts. Species found in the bracts include beetle, fly and mosquito larvae, as well as protozoans and other microinvertebrates (Seifert 1982). These organisms feed on plant host tissue, floral parts, nectar, organic detritus, microorganisms and other insects in the bracts (Janzen 1983).

The aquatic invertebrates may have a negative effect on plant fitness. For example, syrphid fly larvae enter the flower and feed on nectar, and their physical presence prevents hummingbirds from getting nectar and pollinating the plant. The number of syrphid larvae has been shown to be inversely correlated with seed set, indicating that they negatively affect plant fitness (Seifert 1982). Alternatively, plants may receive supplemental nutrients from excreta and waste of the invertebrates.

Broughton et al. (1994) found a substantial difference between the pH of the water in the bracts and rainwater. Neither the cause of this differentiation nor the effects of pH on the associated aquatic invertebrate community have been studied. I chose to address these two topics by studying the population of *Paramecium* spp. within bract water of *H. wagneriana*. *Paramecium* were used as the study subject due to fast response to environmental changes (short generation time),

high abundance and its position in the middle of the food web in this community.

The source of the alkalinity of the bract water is likely due to plant secretions, the presence of the aquatic invertebrate communities within the bracts or a combination of the two. I hypothesize that the change in pH of the bract water is due to plant secretions, in part because Broughton et al. found no correlation between invertebrate abundance and pH. Additionally, I hypothesize that pH will affect *Paramecium* growth rate and that the pH at which *Paramecium* growth rate is highest will be different from the pH of the *H. wagneriana* bract water. If the growth rate is less than optimal at the bract water pH-level, the plant may be attempting to make the environment unfavorable for *Paramecium*. Alternatively, an overlap of bract pH and optimal growth rate pH may indicate that there are benefits gained by *Paramecium* presence (and perhaps associated invertebrate communities) such that the plant is providing an environment conducive to their growth.

Methods

All experiments were conducted on 13 - 16 February 2002 at La Selva Biological Station, Costa Rica. *Heliconia wagneriana* plants located approximately 50 m W of the dining facilities at the station were used for the experiments.

Measurements of the pH of the bract water were taken from the second-oldest bract on each of four inflorescences. A t-test was used to compare pHs of bract water and rainwater.

Two tests were performed to try to identify the source of the alkalinity in the water. In the first experiment, bract water was withdrawn with a pipette from the second-oldest bract on each of four inflorescences using a 10 mL glass pipet with a 2 mm tip, and the volume was recorded. The inside of the bract was rinsed clean three times with tap water (pH = 6.0), filled with 15 mL of filtered rainwater (pH = 6.3), then covered with a fine mesh to prevent the entry of invertebrates. The pH of the water in the bracts was tested after 24 h using pH paper (range 1 - 12).

In the second experiment, bract fluid was extracted in the manner described above from 5 bracts of various ages. Thirty mL of bract fluid was filtered through filter paper, and the filtrate (including invertebrates) was lightly rinsed from the paper with ~3 mL of tap water into two 100 mL plastic vials each filled with 20 mL of filtered rainwater. The vials were capped and measurements of the pH were taken 24 and 48 h after introduction of the invertebrates. A t-test was used to compare initial and resulting pH levels.

To find the optimal pH conditions for *Paramecium* growth, I experimentally altered the pH of the bract water in the lab, using pooled bract water from ~10 bracts of varying age on different plants. Macro-invertebrates were removed from the water and baseline counts of the number of *Paramecia* per drop of fluid were made using a dissecting scope with magnification 60 – 180X (n = 18). The number of *Paramecia* in each drop was counted twice and averaged to control for movement of the *Paramecia* within the drop. Five mL of the pooled bract water was then added to each of fifteen 20 mL glass vials. The pH of this water was then altered by the addition of diluted 1M HCl or NH_3. Concentrations of acid and base were prepared such that no more than 5 drops were added to the water to avoid diluting the sample. Measurements of resulting pH were taken using pH paper (range 1 - 12); the tested pH values ranged from 4.0 – 9.2. The vials were capped and allowed to sit ~24 h, at which point the population of *Paramecia* per drop was counted. The populations of 12 drops from each vial were counted.

Growth rate was calculated by subtracting the mean baseline number of *Paramecia* per drop from the number present 24 h after pH alteration. Both absolute numbers of *Paramecia* and growth rate were regressed

against pH using a second-degree polynomial. Growth rates at pH values similar to rainwater pH and bract water pH were compared using a paired t-test.

To compare the *Heliconia* community to another type of phytotelmata, I took pH measurements from six bromeliads and examined the invertebrate community from the water of three bromeliads using a dissecting microscope.

Results

There was a significant difference in the pH of bract water and the rainwater collected during the sample period ($t = 5.60$, $df = 20$, $P < 0.001$). Mean pH of bract water was 7.57 ± 0.15 and mean pH of rainwater was 6.27 ± 0.09. A wide variety of aquatic organisms were found in bract water, including small protozoans, *Paramecia*, mosquito larvae and a large spittle-bug-type homopteran which was not mentioned in literature on *Heliconia* bract communities.

Volumes of extracted bract water ranged from 15 - 22 mL. The four bracts that had been emptied and refilled with rainwater no longer contained fluid 24 h after they had been filled. Refilling the bracts with another 15 mL of rainwater resulted in similarly dry bracts after 24 h. Thus, pH could not be measured in these bracts.

The aquatic invertebrates added to rainwater did not cause the pH of the water to change ($t = 0.64$, $df = 10$, $P = 0.54$).

Alteration of the pH did have an effect on the growth rate of *Paramecia*, with the highest growth rates occurring between pH 6.0 and 8.0 (Fig. 1). The growth rates exhibited for a pH range similar to that of rainwater (6.2 - 6.5) and similar to that of the bract water (7.4 - 7.7) did not differ significantly ($t = 1.72$, $df = 55$, $P = 0.09$).

Water extracted from bromeliad tanks had a mean pH of 6.1 ± 0.04. This was not significantly different from the pH of rainwater ($t = 0.48$, $df = 8$, $P = 0.65$). No *Paramecia*

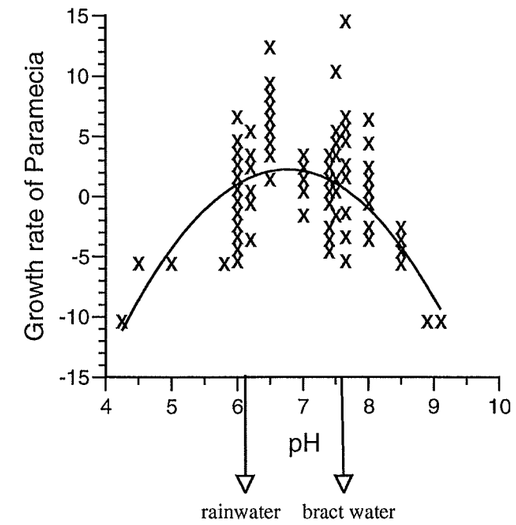

FIG. 1. Growth rate of Paramecium in pH-altered bract water ($r^2 = 0.55$).

were found in 12 drops of bromeliad tank water; however, comparable levels of detritus and mosquito larvae were present. Smaller protozoans were present but not as abundant as in *Heliconia* samples, and an additional type of macro-invertebrate (Gastropoda?) was abundant in the bromeliad water.

Discussion

The results provide strong evidence that *H. wagneriana* is changing the pH of the water within its floral bracts. The difference in pH of the water in the bracts and the external source of the water (rainwater) is high enough (1.3 pH units) to likely have biological effects on either the plant or the aquatic communities within the bracts. Due to the lack of pH change in the rainwater to which invertebrates had been added, it is likely that the alkalinity is due entirely to effects of the plant.

One interesting and unexpected finding was the failure of the added rainwater to remain within an emptied bract after a 24-h period. The water must have been reabsorbed

by the plant or evaporated. Because water remained in surrounding, unmanipulated bracts during the testing period, the latter possibility would necessitate that there is some additive to the bract water that is slowing evaporation. If the rainwater is being reabsorbed by the plant, the disappearance of the rainwater could be due to the relative osmolarities of the bract fluid and rainwater, promoting diffusion of rainwater into plant tissues. Alternatively, there could be other compounds added to bract water by the plant or invertebrate community over time that prevent its reabsorption into the plant.

Because the growth rate of *Paramecia* is approximately the same at rainwater-pH levels and bract water-pH levels, it is unlikely that the alkalinity of the bract water has an effect, positive or negative, on *Paramecium* populations. However, the effect of the alkalinity on other aquatic invertebrates in the bract community is not known.

The increased pH of bract water may simply be a side-effect of some compound which is added to the water by the plant. One possibility might be a plant additive that increases viscosity or surface tension, thus reducing evaporation of the bract water and secondarily affecting pH. By personal observation, the bract water seemed more viscous than either bromeliad water or rainwater and surface tension seemed higher, as the droplets of bract fluid were rounder than bromeliad/rainwater droplets. Additionally, a plant additive that affects the osmolarity of the water (thus keeping it from being reabsorbed by the plant) may have the effect of increasing the pH of the water.

The finding that bromeliad-tank water has a pH similar to that of rainwater further supports the hypothesis that the alkalinity is affected by the *Heliconia*-plant and not a result of the aquatic communities. The absence of *Paramecium* in the bromeliads could be due to a variety of factors, but the pH-alteration experiment provides strong evidence that it is not a pH limitation that is excluding *Paramecium*.

Future studies could investigate the evaporation rates of *Heliconia* bract water versus rainwater or bromeliad-tank water (by simply setting out pans of each in the sun) to determine if there is a *Heliconia* additive slowing evaporation. Additionally, water could be osmotically altered using salts and added to emptied *Heliconia* bracts to investigate possible osmotic effects. A more careful comparison of bromeliad-tank and *Heliconia* communities might shed light on differences in the abiotic environments, species composition and dispersal limitations which would affect the aquatic populations.

Increased alkalinity may have an effect on the bract community, and similar pH-alteration experiments could be performed using other invertebrate types, such as mosquito larvae or syrphid fly larvae, which have a more direct effect on plant fitness. If aquatic insects are capable of decreasing plant fitness as Seifert reported and their optimal pH is closer to that of rainwater, it is possible that the increased alkalinity of the bract water is an adaptation of the plant to reduce aquatic insect populations.

Based on the observation of the perseverance of bract water in relation to rainwater, it is likely there is some evolutionary advantage to having water in these bracts. This could be tested with a comparison of seed sets in *Heliconia* species with erect bract and those with hanging bracts. If water is successful at reducing flower and seed predation and increases plant fitness as a result, why do species with hanging inflorescences still exist? It is possible that there is a tradeoff between seed protection and aquatic insect predation which allows for the presence of these two morphologies, but further research would be needed to clarify the relative costs and benefits of each.

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