Prey composition of *Nepenthes* pitchers in relation to pitcher morphology and habitat type

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Harvard University Biodiversity of Borneo Course, 1 August 2007

Abstract

The genus *Nepenthes* inhabits a wide range of habitats across the island of Borneo. This study focuses on one species, *N. gracilis* growing in two habitats in Lambir Hills National Park (one at the entrance to a plot of mixed dipterocarp forest and the other on a busy roadside*). N. gracilis* has dual pitcher morphology, an aerial (hanging) and terrestrial (growing along the ground) growth from; each morphology was observed growing at both sites. We were interested in the possibility that aerial and terrestrial pitchers could catch different types of prey; to test this, we measured prey composition of the two morpholigies across both habitats. Using R for statistical analysis and field sampling methods, we were able to conclude that aerial pitchers tend to catch winged insects while terrestrial catch predominantly terrestrial insects. We found no evidence of a difference in prey composition across habitats.

Introduction

Borneo is the global center of diversity for pitcher plants of the genus *Nepenthes*[1]. These climbing plants grow on nutrient-poor soils and supplement their nutritional intake with modified leaves which trap mainly insects as prey[1]. Generally, *Nepenthes* have two differing morphologies: aerial and terrestrial. Aerial pitchers climb like vines to hang from elevated locations and lack the ribs of the terrestrial pitchers; color changes with height as well. Higher aerial pitchers become a lighter green to match their habitat while the terrestrial remain a mottled red-green [1]. Presumably, morphological differences are intended to attract and catch different types of prey. To test this assumption we analyzed the prey contents of twenty terrestrial and twenty aerial pitchers in two different habitats: the entrance to the CTFS plot (PT: plot terrestrial, PA: plot aerial) and the roadside to the plot (RT: road terrestrial, RA: road aerial).

Hypotheses

(1)We predict that aerial pitchers will catch more winged prey while terrestrial pitchers will attract more wingless prey.
(2)We predict that the overall pitcher catch compositions will differ by habitat type and pitcher type (aerial vs. terrestrial).
(3)We predict that primarily small ants will become prey.

Methods

Two habitats were analyzed: the first occurred along the road to the CTFS Lambir plot; the second along the of the beginning of the trail to the plot. The same species of Nepenthes was studied at each site (See Figure 2 in Appendix). The exposed roadside habitat was comprised of taller grasses which housed the aerial pitchers and short mowed grass where the terrestrial pitchers resided. The second site was located within a disturbed area of a secondary forest where ferns, other vines, and small trees grew thickly, and leaf litter covered the ground.

To test for differences in prey composition between aerial and terrestrial pitchers we removed the pitcher contents of ten terrestrial and ten aerial pitchers at each site for a total number of forty pitchers. Pitcher contents were collected via filtration through a butterfly net into plastic vials. We washed out any remaining contents into the vials and returned the liquid to the pitcher after filtration.

Pitchers were sampled on two different days: 7/29 and 7/31. Pitchers collected on 7/29 contained the full accumulation of undisturbed prey, while those collected on 7/31 contained prey collected after a two day period.

Pitcher contents were analyzed using microscopes, identification of the insect prey to order and abundance of each prey type was noted. Upon the appearance of mass amounts of sample, a grid method was used to estimate sample size. We made distinctions between flying and non-flying prey within orders, i.e. flying and non-flying ants.

We analyzed our data with the statistical platform R. To compare the abundance of winged prey found between aerial and terrestrial pitchers we performed Fisher exact tests within and across habitats. In order to test if aerial and terrestrial pitchers varied in their overall prey-type composition, we analyzed our data with a permutation(ANOSIM) test.

Discussion/Results

The permutation test (ANOSIM) used to calculate the variation in species composition between aerial and terrestrial pitcher types across both habitats resulted in a statistic R of 0.08022 and a significance of 0.03 based on 100 permutations (see Figure 1 in Appendix), indicating that there is a significant difference between the prey captured in aerial and terrestrial pitchers. Permutation results also show that species diversity within terrestrial pitchers is much higher than that of the more uniform array of prey caught by aerial pitchers (see Figure 1 in Appendix). Fisher tests confirmed permutation results by showing a statistically significant difference between the capture of winged and wingless prey in each habitat (RT vs. RA: p-value 4.066×10^{-10}; Pt vs. PA: p-value 2.2×10^{-16}) as well as across both habitats(p-value 2.0×10^{-16})(see Table 1 in appendix). Aerial pitchers catch a larger amount of winged prey than terrestrial in each habitat implying that aerial pitchers function to catch winged prey. This function is maintained regardless of the habitat diversity surveyed in this project. Terrestrial pitchers catch predominantly wingless prey although the species diversity of prey caught in the terrestrial pitchers does vary greatly as compared to aerial pitchers, the function does not change between habitats.

As predicted, Formicidae (ants) make up the vast majority of the abundance of prey found within the pitchers (see Table A in Appendix). This may be due to the fact that, of the taxa found within the pitchers, ants are one of the only groups (among other Hymenoptera) that are attracted to the nectar on the pitcher surface. The effect of their response is compounded by the large number of individuals in a colony who respond to a forager's scent trail.

Although not necessarily relevant to the motivating questions of this study, it is important to note that there organisms observed in the pitchers which could not be classified as prey, such as live mosquito larvae, mites, and annelid worms. These organisms act as parasites of the *Nepenthes* eating caught prey and using the relatively stable environments to grow and mature in. Interestingly, after the rain, the aerial pitcher plants of the road saw a marked increase in the amount of live mosquito larvae while the aerial pitchers of the plot did not. Aerial pitchers residing on the tall grasses of the road-side are more open to the elements than those on the plot, it is possible that more standing water is held in aerial pitchers creating an ideal environment for the maturation of larvae. Although habitat difference did not reveal any differences in pitcher-prey relationships, it would be worth investigating the repercussions of habitat difference for other aspects of the *Nepenthes* life-cycle.

References

[1] Clarke, C. & Lee, C. Lyons, K., ed. (2004), *Pitcher Plants of Sarawak*, Natural History Publications (Borneo),Malaysia.

[2] R version 2.51 (2007-06-27) copyright © 2007 the R foundation for Statistical Computing.

ISBN 3-900051-07-0

Appendix

Position / Winged / Wingless PA / 24 / 21 PT / 1 / 765.5 **P-value:** 2.2x10^ -16 Position / Winged / Wingless RA / 26 / 6 RT / 5 / 40 **P-value:** 4.0x10^ -10		
Across Habitats	**Winged**	**Wingless**
Aerial	99	62
Terrestrial	15	1063.5
P-value: 2.0x10^ -16		

Table 1: Fisher tests investigating the difference in the abundance of winged and wingless prey caught by both aerial and terrestrial pitcher types. Tests were run for each habitat and across habitats ("Across Habitats"). The data for this table was taken on the first day of data collection (7.29.07), to see the fisher tests for the second please refer to the appendix.

Code	Diptera	Winged Ants	Ants	Coleoptera	Veined Wings	Hymenoptera	Blattodea	Arachnid	Millipede	Non-insect Arthropod	Isopod
P1A	1	0	1	1	0	0	0	0	0	0	0
P2A	3	0	1	0	0	0	2	0	0	0	0
P3A	0	0	1	1	1	0	0	0	1	1	0
P4A	0	0	1	0	2	0	0	0	0	0	0
P5A	0	0	1	0	0	0	0	0	0	0	0
P6A	0	0	2	0	1	0	0	0	0	0	0
P7A	0	1	3	0	4	0	0	0	0	0	0
P8A	0	0	3	0	3	0	0	0	0	0	0
P9A	0	0	3	1	0	0	0	0	0	0	0
P10A	0	0	3	0	0	0	2	0	0	0	0
P1T	0	0	3	0	0	0	0	0	0	0	1
P2T	0	0	4	0	0	0	0	0	0	0	0
P3T	0	0	4	0	0	1	0	0	0	0	0
P4T	0	0	5	0	0	0	0	0	0	0	0
P5T	0	0	6	0	0	0	0	0	0	0	0
P6T	0	0	37	0	0	0	0	0	0	0	0
P7T	0	0	706.5	0	0	0	0	0	0	0	0
P9T	0	0	0	0	0	0	0	0	0	0	1
P10T	0	0	0	0	0	0	0	0	0	2	0
R1A	0	0	1	0	5	0	0	0	0	0	0
R3A	1	0	1	0	0	0	0	0	0	0	0
R4A	0	0	0	0	1	0	0	0	0	0	0
R5A	0	2	1	1	0	0	0	0	0	0	0
R6A	0	2	0	1	0	1	0	0	0	0	0
R7A	0	0	1	0	1	0	0	0	0	0	0
R8A	3	0	2	1	0	0	0	0	0	0	0
R9A	0	0	0	0	1	0	0	0	0	0	0
R10A	0	0	0	0	6	0	0	0	0	0	0
R1T	0	0	5	0	0	0	0	0	0	0	0
R2T	0	3	0	0	1	0	0	0	0	0	0
R3T	0	0	3	1	0	0	0	0	0	0	0
R4T	0	0	4	0	0	0	0	0	0	0	0
R5T	0	0	3	0	0	1	1	0	0	0	0
R6T	0	0	18	1	0	0	0	0	0	0	0
R7T	0	0	1	0	0	0	0	0	0	0	0
R8T	0	0	0	0	0	0	0	1	0	0	0
R9T	0	0	312	0	0	0	0	0	0	0	0
R10T	0	0	1	0	0	0	0	0	0	0	0

Table 2: Insect Abundance by Pitcher Type and Location

Key:

P = Plot sample site **T** = Terrestrial pitcher

R = Road sample site **A** = Aerial pitcher

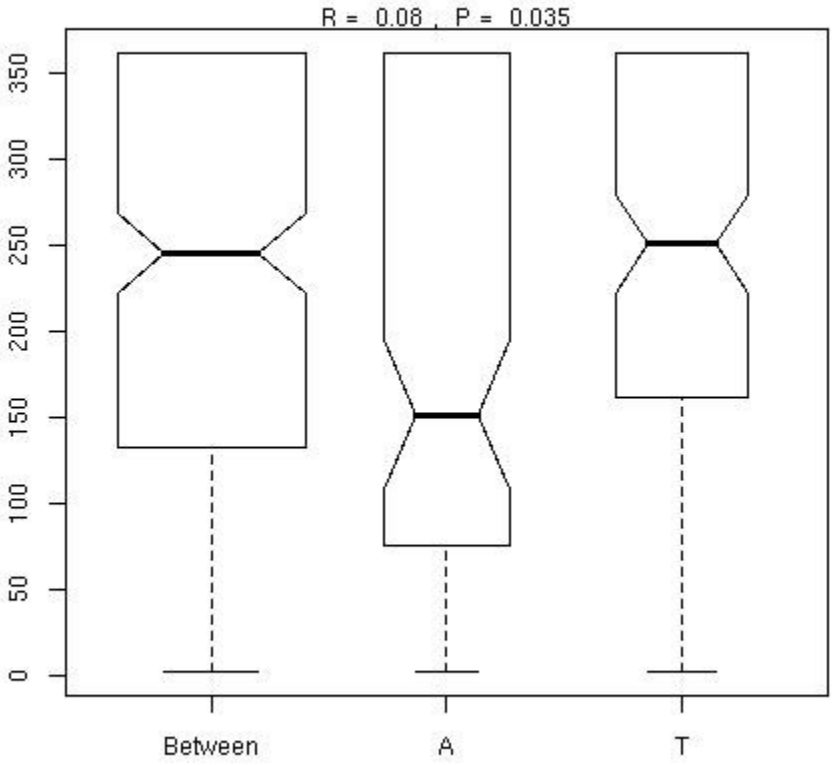

Figure1: ANOSIM of aerial (A) vs. terrestrial (T) pitchers in relation to type of prey capture using R statistical platform. Bars indicate that there is a significant difference between the prey captured in aerial and terrestrial pitchers.

Figure 2:

Aerial (left) and terrestrial (right) pitchers of study species.