Response to Reviewer Feedback

Formatting, grammar, and p-value rounding corrections were made. We have adjusted the language of the introduction to provide a clearer statement of the aims of our analysis. We received one comment indicating we needed to include more references, but as there was no specifics cited and we could not find unsubstantiated claims, we ignored this comment. Figure1 and 2 were remade with shapes and new color palettes for more accessible viewing. We opted to keep point data on figure 1A in an effort to show how data changed from the original data set. Figures 1A, 1B, and 2 are now referenced within the relevant results section. We also resized the axes on what is now Figure 2. To address the comment discussing spatial and temporal variability owing to the fact this dataset was compiled from multiple global studies across a wide timespan, a section was added in the discussion section of the paper stating how the limited scope of the data provided as well as un-standardized descriptions of study sites, biased collecting of count data over time and for different locations, and unstandardized or lack of descriptions of main ecological factors would have made such an analysis difficult and unreliable. Two additional paragraphs were added to the discussion to further discuss the ecological role of carnivorous plants, the potential evolutionary implications for carnivorous plants and insects as a result of prey dynamics, and why these studies may be important in relation to an uncertain ecological future providing a clear conclusion for the study. We also attempted to clarify our methodology and process regarding richness of prey capture.

Reviewer 1

Major comments:

Lines 87-90: Authors should explain what the richness metric is measuring. I think authors are talking about insect prey order richness, but this is not clear. Authors should also clarify this in Figure 3. Authors compare prey order richness, but from Figure 2, it looks like the counts of prey belonging to some groups was much greater than others. Was there any difference in prey order richness within each group? I also suggest calculating and modeling prey order diversity as a function of predation method as this would consider the number of different prey orders as well as the number of individuals within each order.

Reviewer 2

Major comments- L92-93 – What was the sample size for dipterans? Although it is technically insignificant, I think it is fair to mention how close it is to significance and look more closely at effect size before coming to a set conclusion about it.

Reviewer 4

Major comments

L136-L140: This is a bit unclear. If the plants are generalist predators for flying insects, do you mean that they are targeting flying insects? If this is the case, the conclusion that the plants catch more flightless insects does not clearly follow.

Entomological Techniques and Data Analysis Group Project

Prey-Trapping Mechanisms in Carnivorous Plants: Analyzing Differences in Prey Capture Dynamics Across Arthropod Taxa

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Abstract:

Carnivorous plants rely on a variety of methods for prey capture, and we may expect trap types to vary in the taxa of prey they collect. Using a historical data set that compiles more than 20 studies, we examined overall prey capture and taxa richness for a variety of arthropod orders to determine if there was a notable difference between predation method. Analysis of the dataset indicated that carnivorous plant predation methods, categorized as “sticky trap,” “pitcher,” and “active trapping types,” significantly influence prey counts of formicidae, Acarina, Thysanoptera, Lepidoptera, and Orthoptera. No notable effect of predation method was seen on prey counts of Diptera, Coleoptera, Hemiptera, Hymenoptera, or Araneae. Specifically, plants using "sticky traps" captured fewer formicidae and Lepidoptera compared to "pitcher" methods, while "pitcher" methods resulted in higher Orthoptera counts. A significant difference in Acarina counts was also observed, with "sticky traps" yielding higher numbers than "pitchers." Additionally, no significant effect of predation type on prey richness was found. The known intimate relationship between formicidae (ants) and carnivorous plants as well as the flightless nature of insects such as Acarina (ticks and mites) may influence their susceptibility as prey, unlike other insect groups such as Diptera. Limitations in the dataset, particularly the skewed sample sizes toward pitcher and sticky trap methods, highlight the need for further research to address gaps in understanding the diet of carnivorous plants and improve sampling methods, especially for active traps.

Introduction:

Carnivorous plants have "captured" the attention of the public and scientists since the 1800s, with Charles Darwin leading foundational research into the subject. While few examples of flora consuming fauna existed in western society, Darwin's experiments in the late 19th century proved not only that carnivorous plants could capture prey, but also that they could provide nourishment to the plant (Darwin 1895). Now, over a century later, more than 20 genera of carnivorous plants are known globally (Ellison & Gotelli 2009). To be considered a carnivorous plant, individuals must have the ability to absorb nutrients from dead prey (typically invertebrates) across plant surfaces, obtain a growth or reproductive advantage from this unique character, and have specific anatomical features to lure, capture, and digest prey (Givnish 2015). These qualities allow carnivorous plants to thrive in nutrient-poor environments, accounting for the multiple evolutions of this trait among angiosperms (Ellison & Gotelli 2009).

Despite the varied evolutionary history of carnivorous plants, there is a high degree of convergence among taxa with regards to prey capture (Ellison & Gotelli 2009). Some of these methods of prey capture may have adapted from one another, such as sticky venus-fly traps like those in the genus *Drosera* that resemble the "sticky leaves" seen in *Pinguicula*. There is some speculation on the evolutionary relationship of these similar traps, with some signs indicating this may be simply a nested example of convergent evolution driven by prey type and availability. Other prey capture techniques, such as "pit-fall" traps of the tropical pitcher plant (*Nepenthes spp.*), are unique and isolated phylogenetically, likely representing a more specialized approach to prey capture (Ellison & Gotelli 2009). Additionally, differences in prey capture may exist within genera, such as different volatile compounds correlating to differences in prey type between *Sarracenia spp.*, pointing to an incredibly nuanced evolution of prey capture (Dupont et al. 2023). A deeper understanding of prey capture in these plants will not only shed light on their ecological roles but may also provide insights into the evolution of their adaptations.

Despite their intriguing nature, little is known about the relationship between prey and plant. Different capture mechanisms—such as pitfall traps, active traps, and sticky traps—may attract distinct invertebrate communities, leading to variations in capture efficiency and taxa richness. This project, therefore, aims to investigate the invertebrate capture counts across various genera of carnivorous plants and assess taxa richness in relation to different prey capture methods. This will be accomplished through the analysis of a comprehensive data set of more than 20 studies, with data recording prey capture data across several decades (Ellison & Gotelli 2023). The analyses will contribute to the broader understanding of the arthropod as plant prey dynamic, potential prey preferences, and the evolutionary pressures shaping the fascinating world of carnivorous plants.

Materials & Methods:

Dataset:

The dataset used for analysis was sourced from the open-access EDI Data Portal repository and titled “Prey Capture by Carnivorous Plants Worldwide 1923-2007” (Ellison & Gotelli 2023). Data from the prey content samples in multiple global studies of carnivorous plants from 1923-2007 were compiled into a single data file by the authors. The dataset contains multiple types of data, predominantly count and proportion data. We focused our efforts specifically on count data due to uncertainty as to how the original authors had calculated their proportion data.

Data preparation:

Species of carnivorous plants were first assigned into one of three predation method groups at the genus level: pitcher, active trapping, and passive sticky traps (**Table 1**). Total arthropod counts per taxonomic order (or family, in the case of formicidae) were grouped by predation type. Observations totaling zero were removed from the dataset, considering that zeros may not represent true cases of no arthropods in a group being caught by a plant, but rather may be due to what arthropod groups original authors recorded in their methodology. Lastly, due to small sample sizes in several available arthropod groups, we restricted analyses to several major arthropod groups (**Table 2**).

Analysis:

All statistical analyses were conducted using R 4.3.2 (R Core Team 2023). We used the “lme4” package (Bates et al. 2015) to fit generalized linear mixed effect models for analyses. Counts for each arthropod group (**Table 2**) were fitted as a response variable against predation type. We fitted genus as a random intercept to account for any variation within species and assumed a Poisson error distribution structure. The relative effect of each factor was assessed using type III Wald chi-square analysis of deviance conducted with the “car” package (version 3.1-2;Fox 2019).When there was a significant difference between counts by predation method, we conducted pairwise comparisons using the “emmeans” package in R (Lenth 2024).

This dataset was also examined for taxa richness, performed with base R program packages. Richness is reported as the number of unique insect orders captured, as that is the furthest taxonomic resolution offered by the dataset. This was obtained by summing the number of unique orders captured by plants of each predation type in each study. A linear model was fitted with predation type as the predictor, and richness the response variable.

Results:

Due to small sample sizes, the individual insect count data of the major arthropod groups (Table 2) were totaled and organized by carnivorous plant predation method (Fig. 1A). The mean number (+/- SE) of insects captured per each arthropod group were organized by carnivorous plant predation method (Fig. 1B). Most notably, the group Hymenoptera does not include formicidae, which were treated separately for analyses.

Predation methods do not appear to have a significant effect on the number of Diptera (df = 2, p = 0.057), Coleoptera (df = 2, p = 0.16), Hemiptera (df = 2, p = 0.12), Hymenoptera (df = 2, p = 0.78), and Araneae (df = 2, p = 0.90) captured.

Predation methods have a significant effect on formicidae prey counts (df = 2, p = 0.01), Pitcher traps captured significantly higher (z = 63.4, p < 0.01) amounts of formicidae prey (mean 645 +/- 235.4) compared to plants employing "sticky trap" predation methods (mean 8.50 ± 2.75). The contrast between the two predation methods showed an estimated average difference of 3.22 ± 1.27 counts of formicidae prey (p = 0.01). ~~The genus intercept in the model showed a variance of 1.93 and a standard deviation of 1.39, indicating that there was variability in counts of formicidae prey across genera~~.

Predation methods appear to have a highly significant effect on Acarina prey counts (df = 2, p < 0.01). Carnivorous plants employing "sticky traps" as their predation method were associated with higher counts (average of 0.75 counts higher (SE = 0.18, z = -4.3, p = 0.0001) compared to carnivorous plants employing "pitcher" predation methods. Plants employing "active trapping" as their predation method did not show any significant different in average counts of Acarina captured when compared to either "sticky traps" (-0.38 counts ± 0.20, z = -1.9, p= 0.14) or "pitchers" (0.38 counts ± 0.22, z = 1.8, p = 0.18). The genus intercept in the

~~model showed a very slight amount of variance (0.023 ± 0.15) indicating that there is not a lot of variability in counts of Acarina across plant genera~~.

Predation methods also have a significant effect on Thysanoptera prey counts (df = 1, p < 0.001). No Thysanoptera data were reported in the dataset for plants employing "active trapping" predation methods, but plants employing "sticky traps" had significantly higher average counts of Thysanoptera (2.19 ± 0.50, z = -4.4, p < 0.0001) when compared to plants employing "pitcher" predation methods. No counts of Lepidoptera from plants employing "active trapping" were reported, however there was an effect of predation method on counts between plants employing "sticky traps" versus those employing "pitchers" (z = 2.4, p = 0.014). Plants using "sticky traps" caught a much lower number (1.75 ± 0.72, z = 2.4, p = 0.014) of Lepidoptera prey on average when compared to those using "pitcher" methods. Predation method had a significant effect on the average number of Orthoptera prey caught (z = 5, p < 0.0001). Like with Thysanoptera and Lepidoptera, Orthoptera counts were not available for plants employing "active trapping." Plants using "pitcher" predation methods caught significantly higher average counts of Orthoptera prey (average of 1.54 ± 0.31 counts higher, z = 5, p <0.0001) when compared to plants using "sticky trap" methods.

Predation type was found to have no significant effect on richness of prey capture (F = 1.16, p = 0.32), with the mean richness for each predation type being roughly 10 (Fig. 2).

Discussion:

Predation methods are often tailored to the predator's preferred prey. Our analysis shows that this holds true for predatory plants, even when prey capture is examined at a very broad taxonomic level. We found significant effects of predation method on both Acarina (mites and ticks) and formicidae (ants). While we expected some effect of ants, mites are a surprise. Ants have been observed to have an intimate relationship with carnivorous plants (Moon et al. 2010, Bazile et al. 2012), with some exhibiting mutualistic behaviors and even tending them. Some ants even directly interact with the traps without adverse effects due to this mutualism (Bazile et al. 2012), which may have been an oversight in some of the earlier data collection. This same relationship is not substantiated for mites, who, in a cursory search of literature, cannot immediately be found to interact appreciably with carnivorous plants. One possibility is that carnivorous plants may not be specialized to capture flying insects. We see no support of the other groups in these models, and these remaining groups are insects capable of flight. By contrast, both ants and mites typically are flightless, which may contribute to their susceptibility to predation by carnivorous plants.

A limitation of this dataset is the skew towards pitcher plant (n = 11) and sticky trap (n = 26) types, with much less data provided for active trapping (n = 5). This variation in samples per predation type is a result of i.) our decision to investigate differences in arthropod catch between these groups of carnivorous plants and ii.) the nature of the data - gathered from multiple differing studies. Moreover, our observations were further limited by not being able to compare proportional data with total count data. Potentially, it is much more difficult to sample prey capture from active traps, as in the case of Venus fly traps (*Dionaea muscipula* Sol.) where the prey is surrounded by plant tissue for digestion. It is unclear how better measures of capture could be obtained for these groups, but future research should focus on expanding our knowledge of arthropod predation in relation to carnivorous plant species.

It is worth noting that several factors may also influence the diets of carnivorous plants such as temporal and spatial factors like season, humidity, surrounding flora/fauna, relative abundance of each arthropod group within regions, ecological disturbance, or temperature. Unfortunately, the recording of such factors was unstandardized across the compiled data with some studies reporting environmental factors as site data (i.e. “Wet+Dry+Humid”) or using a numerical system (i.e. “Site 1”) and omitting environmental factors altogether. Due to this, as well as a biased dataframe consisting of a majority of studies taking place from 1980-1990 with few at either end of the timeframe, inclusion of these factors within the analysis of this study would have been unreliable and heavily biased. Future analyses should focus on further investigation of these elements.

On a broader scope, the differences in prey capture reported by this study may reflect differing evolutionary pressures such as the opportunist availability of prey from ants present near or crawling into the pitcher versus chance prey like flying insects. It may even be possible that ants and mites, as well as flying insects in relation to sticky traps, may be attracted to that environment by the presence of a carnivorous plant (i.e. by providing the unsuspecting insect a haven to rest or hide in from the elements). Thus further elucidation of prey preferences may give a broader view of the role of carnivorous plants in their environment and what may happen should they disappear or if the environment changes.

Tables & Figures:

Table 1: Genera of carnivorous plants assigned to each predation group (n = 42)

Pitcher (n = 11)	**Active trapping** (n = 5)	**Sticky traps** (n = 26)
Nepenthes (n = 1) *Sarracenia* (n = 9) *Triphyophyllum* (n = 1)	*Utricularia* (n = 5)	*Pinguicula* (n = 10) *Drosera* (n = 16)

Table 2: Arthropod groups for which count data was grouped and used in analyses.

Arthropod Groups
Acarina
Araneae
Coleoptera
Collembola
Diptera
Formicidae
Hemiptera
Homoptera
Hymenoptera (not formicidae)
Lepidoptera

Figure 1: (A) Count data of major arthropod groups organized by carnivorous plant predation method. (B) Mean number (+/- SE) of insects captured per arthropod group organized by carnivorous plant predation method. Hymenoptera does not include formicidae, which were treated separately for analyses.

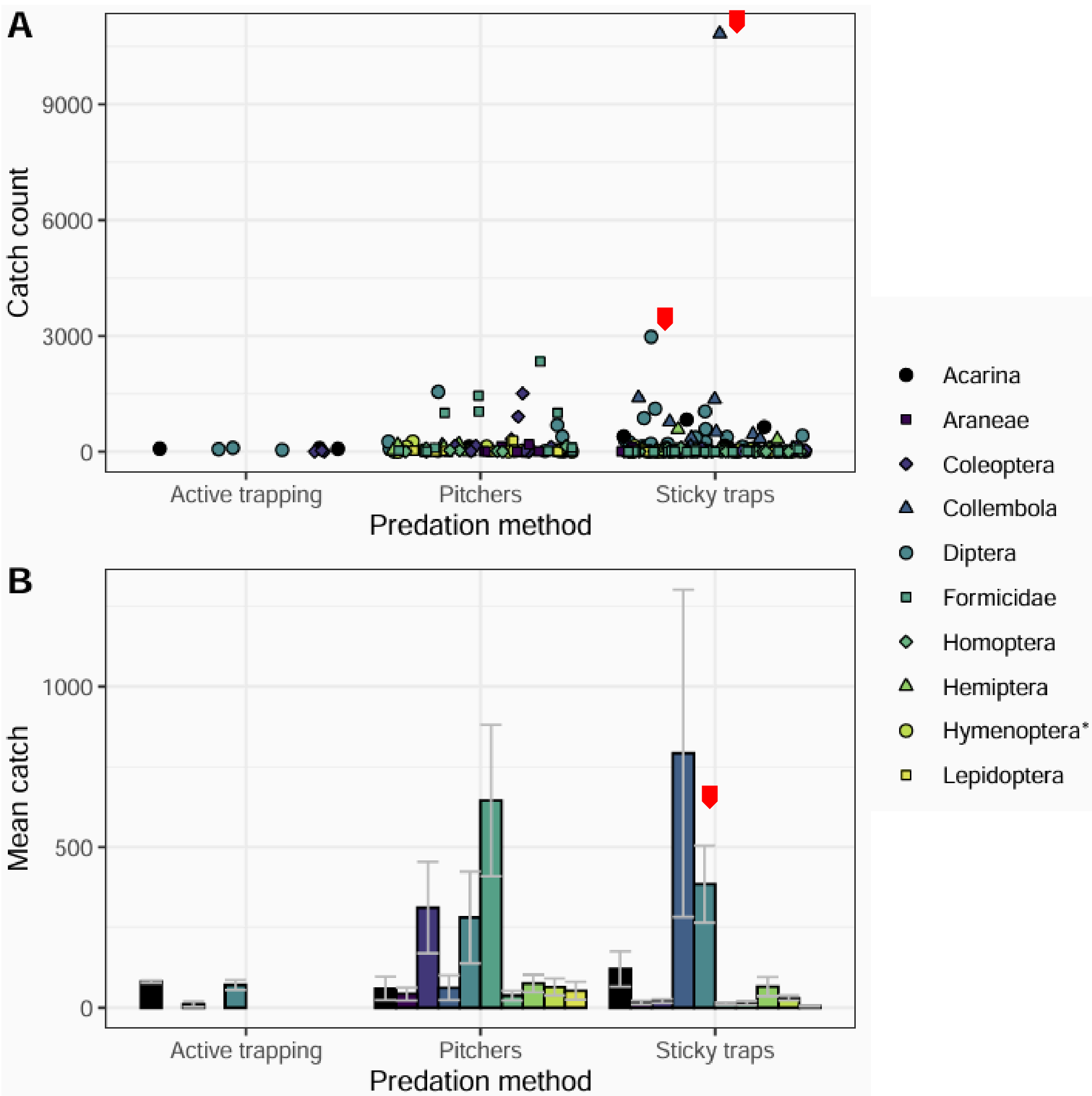
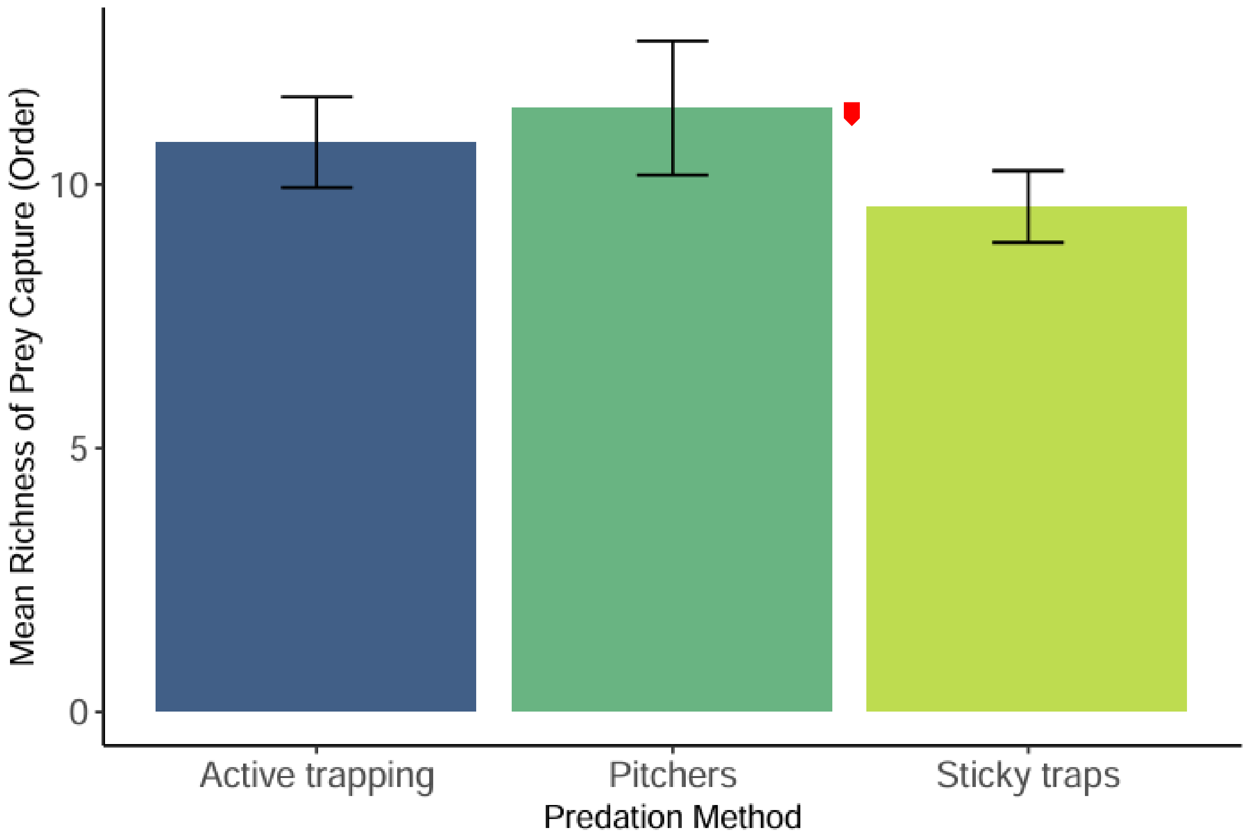


Figure 2: Mean richness of insects captured per predation type



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