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Collective Prey Capture by Juvenile Venus Flytraps (*Dionaea muscipula*)

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Collective prey capture by juvenile Venus flytraps (*Dionaea muscipula*)

Steven Rosen

Abstract

The Venus flytrap (*Dionaea muscipula*), first described by Charles Darwin, is a unique carnivorous plant which acquires prey using leaf blades modified into snap-traps. A recent study showed that prey capture by flytraps was not selective. With this study we examined prey capture rates further by analyzing *D. muscipula* traps less than 1 cm in length to determine if juvenile flytraps differ from adults. Similar to the earlier findings, trap size appeared to play no role in prey capture success. This was likely due to the fact that 88% of all prey items recovered were smaller than the smallest collected trap, suggesting that lack of selection by traps could be due to limited prey type availability. Future research is needed to determine if flytraps in areas with greater variation in prey type instead favor selection.

Introduction

The Venus flytrap (*Dionaea muscipula*) is an insectivorous angiosperm (Darwin 1875) endemic to North and South Carolina in the United States. It inhabits wet, sandy soils that are generally nutrient-poor (Albert et al. 1992), such as along the borders of Carolina bays (Luken 2005b). While many plants would perish in such soil conditions, *D. muscipula* is well adapted to living in such due to its carnivorous lifestyle.

Although it relies somewhat on nutrients within the soil, the Venus flytrap gathers much of its needed nutrients from the prey it captures with its modified leaves (Darwin 1875; Schulze et al. 2001). These leaves have evolved into individual snap traps that works as follows: (1) two or more trigger hairs on the trap's lobe surfaces are stimulated within quick succession (Volkov et al. 2012); (2) the trap rapidly closes, interlocking the cilia extending from its edges to prevent escape; (3) as the prey continues to move around within the closed trap it repeatedly stimulates the flytraps trigger hairs, causing the trap to tighten; (4) digestive enzymes, including proteins, proteinase, phosphatase, DNase, and trace amounts of amylase (Scala et al. 1969; Volkov et al. 2008), are secreted within the now sealed trap to absorb the nutrients from the prey, usually leaving only the exoskeleton; (5) after digestion is complete, the trap reopens and awaits more prey.

D. muscipula also is a fire-dependent species (Roberts and Oosting 1958), relying on fire to clear out competition. Studies by Luken (2005a; 2007) showed that mowing of Carolina bay rims can serve to elicit similar responses in population growth in flytraps when compared to controlled burning and that reduced competition is necessary for *D. muscipula*'s survival. Also necessary for survival is consistent soil moisture (Luken 2012). Additionally, prey may be attracted to the general area of the flytraps by volatile organic compounds (Kreuzweiser et al. 2014) before being drawn to a specific trap by the attractive coloration.

The diet of *D. muscipula* is comprised mostly of crawling arthropods such as ants, spiders, and beetles, though it is not limited to such (Ellison and Gotelli 2009; Hutchens and Luken 2009). Nutrients absorbed from prey include high amounts of nitrogen, phosphorous, and potassium (Adamec 2002). Because it can only catch prey that literally walks into the trap, the Venus flytrap is classified as a passive filter feeder (Jeschke et al. 2004).

While the mechanics behind trap closing and prey capture are widely studied, this experiment will examine the variance in prey capture success rate between adult and juvenile flytraps, as well as any variation within prey type. A few studies have looked for similar information (Hatcher and Hart 2014; Hutchens and Luken 2009) but currently very little data exists emphasizing or focusing on juveniles. It is expected that adult flytraps will demonstrate higher rates of prey capture due to an increased capacity for prey size that encompasses what is available to juveniles as well as prey that would be too big for the smaller size of juvenile traps.

Field-site Description

The area used for this study was Lewis Ocean Bay Heritage Preserve (LOBHP), a 3640 ha protected area located in Horry County, South Carolina (lat 33°47'N, long 78°52'W) encompassing 22 Carolina bays. The vegetation in the area is comprised largely of pine savanna, with *D. muscipula* typically found on the ecotones between the bays and neighboring plant assemblages.

Materials and Methods

Nine populations of *D. muscipula* were used across the LOBHP site for the purpose of this study: 6 resident populations (naturally occurring) and 3 introduced populations (placed along mowed stretches). The resident sites were classified as Emily Plot (lat 33°48.37'N, long 78°53.332'W), Bearcub Bay (lat 33°47.757'N, long 78°51.088'W), Bearcub Central (lat 33°47.759'N, long 78°51.102'W), Open Longleaf, Luken Plot, and Near Ecotone. The introduced sites were classified as Introduced #1858, Introduced #1860, and Introduced #1865.

Population and Density Estimates

Over a five week span (August 25, 2015 through September 22, 2015) data were recorded across Emily Plot, Bearcub Bay, Bearcub Central, and the 3 introduced plots in regards to population sizes and densities. Three 0.25 m x 0.25 m quadrats of flytraps were sampled haphazardly within each population. The total number of *D. muscipula* with central roots within the quadrat were counted as well as the total number of traps per plant, the total number of closed traps per plant, and the petiole and trap lengths of each trap for each plant (using either standard or electronic calipers). A few plants did not have petiole length measured due to their burial within the substrate. Also, two populations (Bear Cub Bay and Introduced #1865) did not have three quadrats sampled due to small population size and a resultant inability to keep the sampling unbiased.

Flytrap Prey Collection

On September 29, 2015, the six sites used for population and density estimates were visited to collect traps for prey analysis in the lab. At each site, 25 closed traps of less than approximately 1 cm in length were collected at random for lab analysis. This was done by removing only the traps from their respective petioles and placing them into a labeled centrifuge tube for each population. Introduced site #1865 only had 5 closed traps less than 1 cm in length. The traps were preserved with 90% ethanol.

A second set of data was collected on October 22, 2015. We had aimed to collect 25 traps again from each of the first six populations used, but due to road closures we could only visit the Emily Plot. However, we were able to locate 3 additional populations (Open Longleaf,

Luken Plot, and Near Ecotone) for data collection. As before, 25 traps were collected from each population for lab analysis.

Prey Analysis

In the lab, the preserved traps were cleaned with deionized water before examination. After cleaning, each trap's length and width was measured using electronic calipers and then opened with forceps to determine whether an invertebrate was present in the closed trap. If present, the invertebrate was then identified to order, usually, and measured at its longest body length. After data were recorded, all traps and captured prey from the population were placed into a small glass vial filled with 70% ethanol for long term preservation.

Data Analysis

For determination of normality of trap lengths among resident and introduced populations, Shapiro-Wilk tests were performed. Linear regression was used to test for a relationship between petiole and trap lengths as well as between trap lengths and prey lengths for traps containing prey. Lastly, single-factor ANOVAs were performed for determination of significant differences between size classes (5-7 mm, 7-9 mm, and ≥ 9 mm; determined by averaging lengths of all collected traps) in prey type (i.e. whether different orders of prey were found in different size classes), prey length, and trap width.

Results

Size and Density

182 traps were measured for the resident populations and 241 were measured for the introduced populations for a total of 423 traps. A total of 24 plants were found and used in the

resident data set and 24 plants were used in the introduced data set, with resident plants averaging 11.8 traps/plant and introduced plants averaging 11.2 traps/plant. Additionally, 44% of all resident traps examined were closed compared to 43% of all introduced traps. Neither resident nor introduced populations showed Gaussian distributions of trap lengths after performance of Shapiro-Wilk tests (Figure 1).

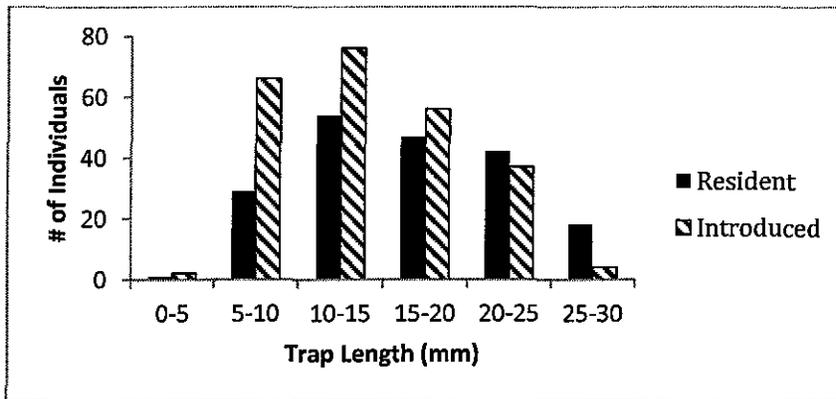


Figure 1. Size-frequency distributions of *D. muscipula* across 3 resident and 3 introduced populations. Both residents ($W=0.98$; $P=0.004$) and introduced ($W=0.97$; $P<0.001$) showed uneven distributions of trap lengths.

Petiole lengths were also measured for 369 of the 423 traps (191 resident traps and 178 introduced traps). Linear regression on traps that had both petiole and trap lengths measured showed a weak relationship for residents ($R^2=0.517$) and a slightly stronger relationship for introduced traps ($R^2=0.549$) (Figure 2).

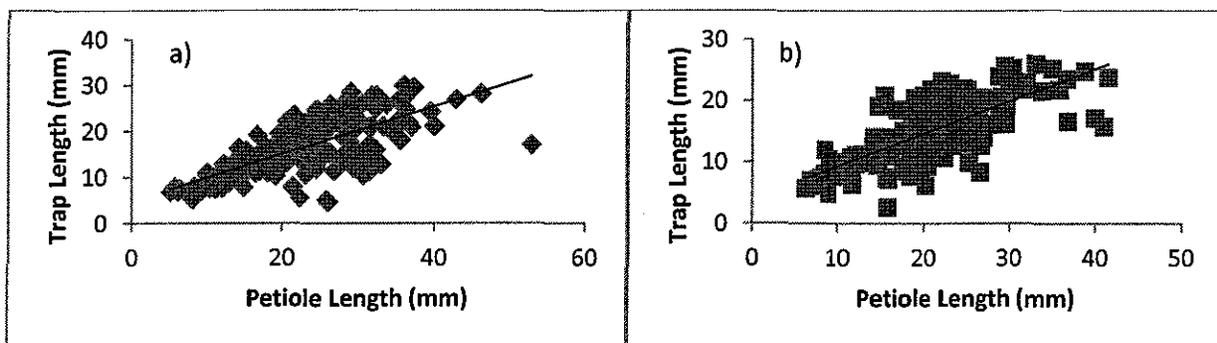


Figure 2. Relationships between trap lengths and petiole lengths in resident and introduced populations. Weak correlations were found in both a) 3 resident populations ($R^2 = 0.5172$) and b) 3 introduced populations ($R^2 = 0.5493$).

Collected Traps

	0-7mm	7-9mm	>9mm	P
Avg. Trap Length	5.62	7.93	9.86	<0.001*
Avg. Trap Width	3.37	4.70	5.64	<0.001*
Avg. Prey Length	2.27	2.58	3.35	0.420
Total # of closed traps	86	78	66	0.138*
Total # of closed traps with prey	32	34	17	0.009
% of closed traps with prey	37	44	26	0.082

Table 1. Mean values for variables of prey acquisition by plant size class.

*Concurrent with findings by Hutchens and Luken (2009)

Compared to a previous study by Hutchens and Luken (2009), similar descriptive statistics were found for the collected flytraps (Table 1). A total of 230 closed traps were collected across the ten populations. 83 of those contained prey across seven different orders (Table 2). Single-factor ANOVA tests were used to test for differences in prey capture between

Table 2. Prey breakdown across all collected traps

	Total	Formicidae	Chilopoda	Collembola	Dipteran (adult)	Dipteran (larvae)	Pseudoscorpiones	Araneae	Coleoptera
All	37%	75%	2%	10%	6%	1%	1%	7%	7%
Resident	34%	73%	0%	4%	3%	1%	1%	6%	9%
Introduced	44%	58%	8%	19%	12%	0%	0%	4%	0%
0-7mm	37%	63%	3%	19%	9%	0%	3%	3%	0%
7-9mm	44%	76%	3%	3%	3%	0%	0%	11%	5%
>9mm	26%	64%	0%	5%	5%	5%	0%	5%	18%

size classes. Although there was no significant difference in diet composition between size classes (df=83, P=0.177), there was a significant difference found across all collected traps

($P < 0.001$) (Figure 3). However, there was no significant difference found in prey length between size classes ($P = 0.420$). Linear regression was used to determine any relationship between trap length and prey length for both resident and introduced populations. Both residents ($R^2 = 0.145$) and introduced plants ($R^2 = 0.056$) showed no relationship between trap and prey lengths (Figure 4). For the three size classes, average trap widths were found to be 3.4 mm, 4.7 mm, and 5.6 mm (Table 1), respectively, and this was shown by a single-factor ANOVA test to be significantly different ($P < 0.001$).

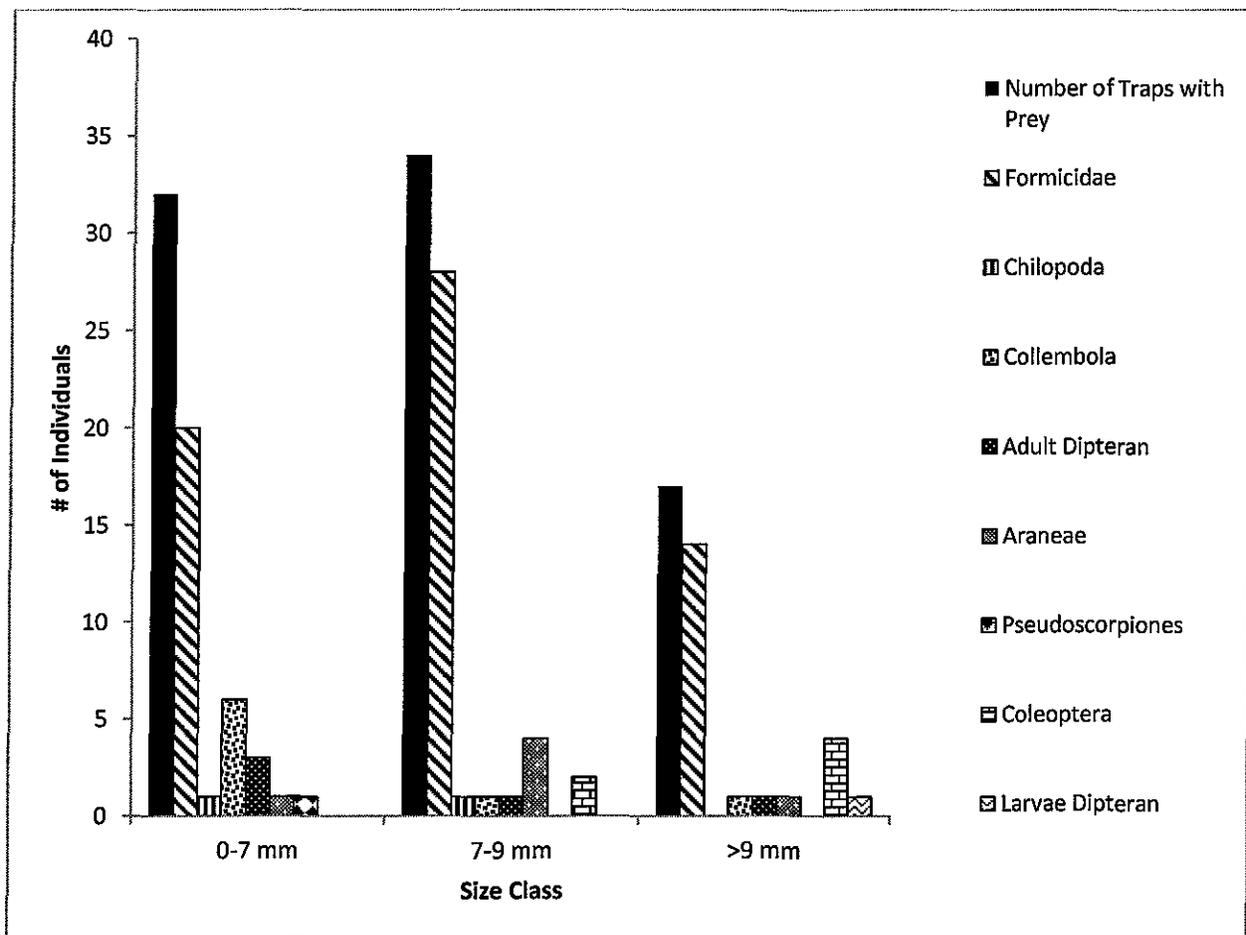


Figure 3. Distribution of prey types found across the three size classes. Formicidae was significantly more abundant ($p < 0.001$).

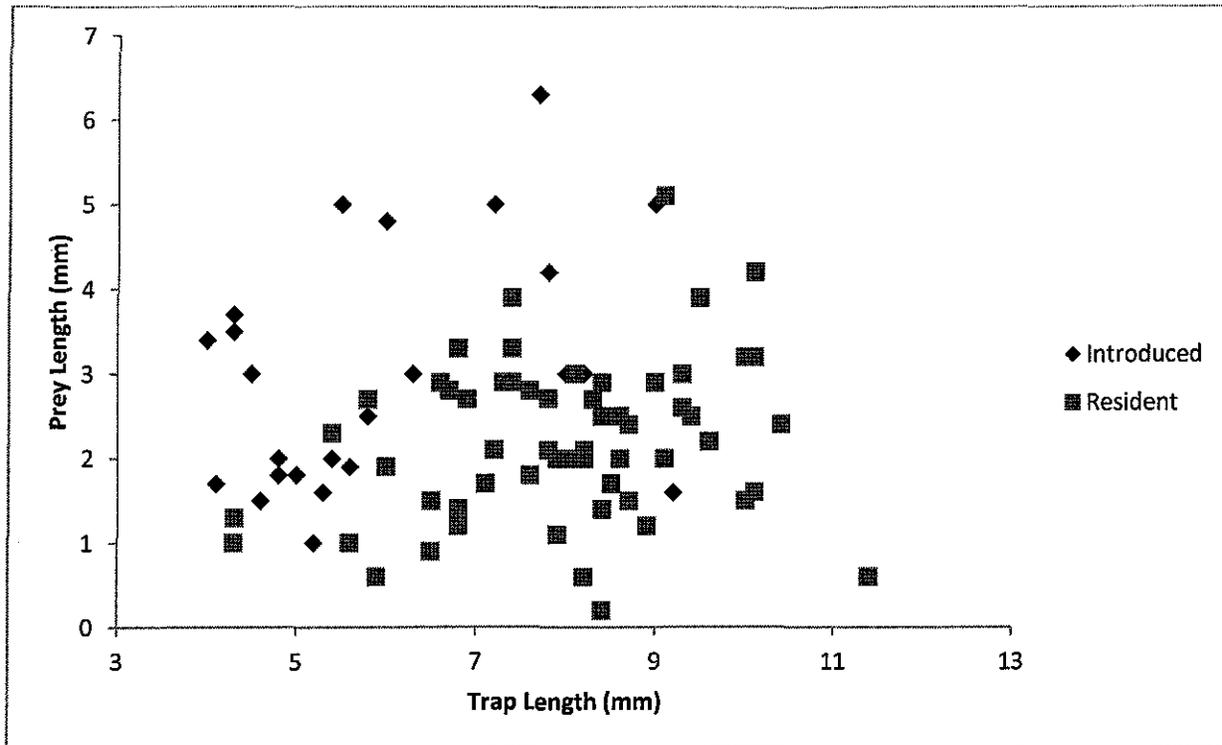


Figure 4. Relationship between trap length and prey length across the ten studied populations. No correlation was found for residents ($R^2 = 0.0563$) or introduced traps ($R^2 = 0.1451$).

Discussion

The results obtained here did not support the hypothesis that larger traps would have a higher prey capture success rate. This is in agreement with the findings of Hutchens and Luken (2009). We also found *D. muscipula*'s success at prey capture to be similar across the three trap size classes sampled. This could be due to the fact that all but 10 (88%) of the prey items recovered were smaller than the smallest trap collected (3.8 mm) so that even the biggest prey found could have fit into the smallest traps. In fact this was likely the case as there was no significant difference found in prey length between trap size classes. Likewise, there was no significant difference in prey type between size classes; Formicidae was the dominant prey found in all. This abundance of ants allowed us to determine that while prey type did not differ

between size classes, it did differ significantly across all traps concerning what was most eaten (the next most abundant were Collembolans, followed by Araneae and Coleopterans) according to a single-factor ANOVA test ($P < 0.001$) and a Tukey HSD test ($P < 0.01$). This leads to an alternative hypothesis that perhaps the lack of selection apparent in *D. muscipula* is due more in part to prey availability. In other words, perhaps there was no significant difference in trapping success between size classes because the most abundant prey type (ants) was small enough to fit in almost all traps collected for this study; had the distribution of prey across multiple taxonomic orders at the study site been more even then we may have obtained different results and correspondingly different conclusions.

The size and density estimates performed at the beginning of this study showed uneven distributions in trap length across both resident and introduced populations. Resident populations had only about 15% of traps <1 cm long while introduced populations showed about 27% <1 cm long. Introduced populations had prey capture success rates very similar to residents but saw slight differences in diet composition such as fewer Formicidae and Coleoptera but more Collembola and Diptera. This indicates that mowed patches for *D. muscipula* reestablishment are a successful method (Luken 2005a). Linear regression also showed weak correlations between petiole lengths and trap lengths in both residents and introduced populations. This could be due to traps encountering less competition as they grow their petioles and extend farther away from the other traps on the plant, allowing for increased prey acquisition and subsequent growth. However, the fact that these were weak correlations could be due in part to the fact that approximately 4 more traps were found per plant on average than usual (7), resulting in increased competition with petiole extension. Finally, trap width was shown to be significantly different across size classes, with longer traps displaying greater widths. This may be an adaptation to

increase the odds of capturing larger prey by increasing the area of the trap, thereby heightening the ratio of nutrient uptake to trap closure and ultimately maximizing energy gains while minimizing costs.

The findings here could just be an example of the often misguided assumption that natural selection is in control of every little detail of biological construction, commonly referred to as the Panglossian paradigm (Gould and Lewontin 1979). We have shown here that Venus flytraps (*D. muscipula*) will simply try to eat whatever comes their way with no regards to prey size and that trap size makes no difference in their diets. Further research on the subject may consider comparing diet composition across multiple populations at different field sites within their endemic range. Additionally, further efforts should be made towards locating and studying tinier traps (ideally less than 3 mm) to determine if these differ significantly in diet composition.

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