Selectivity of prey capture based on prey size in the Venus fly trap
(Dionaea muscipula Ellis)
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Abstract

Venus fly traps (*Dionaea muscipula* Ellis) are carnivorous plants that live in nutrient poor soils and must digest insects to supplement their diets. When a Venus fly trap captures an insect, the plant's traps do not fully close for several minutes, which may allow small prey to escape. It would be beneficial for the plant to consume a large, nutrient-rich prey item as opposed to a small prey item. We tested the hypothesis that Venus fly traps select larger prey by offering plants small and large crickets. A Kruskal-Wallis test and Wilcoxon signed rank test were used to test significance of the results. These tests showed that the plants did not select prey based on size and instead captured and digested prey opportunistically.

Introduction

Charles Darwin studied carnivorous plants to a great extent calling them the "most wonderful plants in the world" (Ellison and Gotelli, 2009). Carnivorous Venus fly traps (*Dionaea muscipula* Ellis) possess snap traps that evolved only once among the carnivorous plant lineage (Cameron et al., 2002). Traps are formed from modified leaves, and plants vary in the number of traps they possess. Plants are able to sense mechanical stimuli through mechanosensitive channels found in their cells (Volkov et al., 2008b). Inside each trap there are hairs that have to be triggered twice to cause an action potential that closes the leaves around the prey (Pavlovic et al., 2010). The action potential causes cells within the leaves to experience a change in their volume, shape, and curvature. These cellular changes allow for fast closure of the traps, which occurs in about 0.3 seconds (Volkov et al., 2008a).

Venus fly traps are found in the Southeastern Coastal Plain of North Carolina and South Carolina (Luken, 2005). Lewis Ocean Bay Heritage Preserve is one area in South Carolina where these carnivorous plants can be found. The reason that the plant grows only in the Southeastern Coastal Plain is because it is a habitat specialist. Venus fly traps are found mostly between ecotones of wet evergreen-shrub bogs and dry sandy regions containing Long-leaf Pine.

Burnings of the surrounding area are considered a necessity for the plants to flourish. These habitual burnings allow for the decomposition of organic matter in the soil where nutrients are often low, allowing the plants to benefit from the added nutrients (Roberts and Oosting, 1958). The burnings decrease the insect availability for carnivorous plants in the area, but increase the ability for absorbance of light by decreasing the surrounding vegetation (Luken, 2007). Luken (2007) did an experiment with Venus fly traps in their normal habitat. He had control quadrats and quadrats where he trimmed back the surrounding vegetation allowing for

greater light availability. His results showed that the plants with the surrounding vegetation trimmed did not affect the growth of the plants. This demonstrates that the trimming only affects the light exposure, and the burnings may have a more complex environmental impact (Luken, 2007). After burnings the plants gain up to 75% of the nitrogen they need from insects (Schulze et al., 2001). The plants gain additional nutrients from prey to compensate for the lack of nutrients in the soil whether or not burning occurs (Adamec, 2002). Plants are able to digest the soft parts of insects using digestive enzymes.

Trap closure happens in a series of five stages: open state, closed state, locked state, constriction and digestion, and semi-open state back to open state. The trap is triggered causing a change in shape from convex to concave in 100 ms bringing the two modified leaves of the trap together (Volkov et al., 2011). Shahinpoor and Thompson (1995) proposed a theoretical model for the bending change in epidermal cells in an electric field which predicts this bending of the modified leaves due to the action potential. Volkov, Coopwood, and Markin (2008) performed an experiment in which the stages of trap closure were photographed in order to better understand the kinetics. The cilia come together to form a wall. The trap will stay in this position for a few hours allowing for the beginning of digestion. If prey is not large enough to digest or escapes, the trap will reopen. The next step includes the trap closing completely in order to seal the edges together allowing for the digestive juices to be concentrated inside the trap. One week later the trap will start to re-open with digestion complete (Volkov et al., 2011).

Venus fly traps have evolved various ways to attract prey. The plants contain a red pigment called anthocynanin that is thought to attract insects (Volkov et al., 2008a). An experiment was performed using Kodak Pan-X films with normal cameras that contained a filter to produce pictures of ultraviolet patterns on carnivorous plants. *Dionaea muscipula* had a

specific ultraviolet pattern that was clearly visible when the trap was open. The authors speculated that this pattern may attract certain insects to the trap (Joel et al., 1985). Trap size is an aspect of carnivorous plants that may affect prey capture as well. Plants with larger trap areas are able to catch a greater amount of prey (Bhattarai and Horner, 2009). This was further confirmed by Green and Horner (2007) in their experiment on pitcher plants. The pitcher plants which had a significantly smaller pitcher size captured significantly less total mass.

Dr. John J. Hutchens and Dr. James O. Luken, professors at Coastal Carolina University, did an experiment to test the selectivity of prey by Venus fly traps. Their experiment consisted of plants found at Lewis Ocean Bay Preserve that were observed over nine months. They found that the plants did not select prey based on size and instead ate opportunistically (Hutchens and Luken, 2009). However, there is little additional information on prey selectivity by Venus fly traps. I repeated aspects of the experiment that Hutchens and Luken (2009) performed to see if I could replicate their results. Carnivory in plants evolved so that plants could gain additional nutrients (Adamec, 2002); so, it is likely that Venus fly traps select larger prey to gain greater amounts of nutrients.

On the edge of each of the leaves are hair-like projections called cilia. When the leaves contract the trap stays partially open for a few minutes before becoming fully closed (Volkov et al., 2008a). Once the traps close, these cilia form an impenetrable wall which only small prey would be able to escape from (Volkov et al., 2011). This could allow for selectivity of prey based on size; if the traps stay partially open, allowing small prey to escape, the plants could be selecting for larger prey.

My experiment tested the hypothesis that Venus fly traps select large prey. Venus fly traps are considered to have active traps because the traps snap shut, and active traps have a high

energy requirement (Ellison and Gotelli, 2009). It takes a total of five to seven days for the traps to reopen once shut and the plants cannot obtain nutrients from prey again until after this time. During this time the Venus fly traps digest the prey they have captured (Volkov et al., 2008a). The plant would gain more nutritional value from larger prey than small prey. Due to this it would be advantageous for the plants to capture larger insects, which would give them the most energy in return.

A variable that could have an effect on the plants' selectively of prey size is the behavior of the differently sized crickets. When the crickets were placed through the hole in the plastic cups, the larger crickets seemed to be more active. The smaller crickets preferred to hide in the cracks in the soil. If the large crickets were found to be more active then it is possible that this would cause more large crickets to be captured in the plants. However, this could mean that they are able to move quicker to escape the traps before they are able to close on the crickets.

A nonparametric Kruskal-Wallis test was used to test significant difference between the three groups in the trials. An ANOVA test has a few assumptions that must be met. In this experiment the assumption of normal distribution and equal variance is not met. Kruskal-Wallis test is nonparametric and therefore does not have these same assumptions. It tests the data in order of rank like most nonparametric tests do. In the case of this experiment the ranks were given based on the number of prey that were captured, zero, one, or two. A Wilcoxon signed rank test was performed if there was a significant difference found by the Kruskal Wallis to identify what groups were significantly different. This test is the nonparametric version of the paired t-test (McDonald, 2009).

Materials and Methods

Thirty Venus fly traps (*Dionaea muscipula*) were purchased from a farm in North Carolina (www.flytrapfarm.com). Each plant was given a unique label and put on a Petri dish under greenhouse lights. The Petri dishes were filled with distilled water twice per week.

The number of traps per plant and the length (cm) of the largest and smallest traps of each plant were calculated. Before the experiment, each plant with more than eight traps was trimmed so that each plant possessed eight traps. The trimming was done in order to keep a variety of small and large sized traps. A clear plastic cup was inverted over the top of each plant to prevent prey from escaping. A hole was cut in each cup and covered by tape to allow crickets, purchased from Pet Smart, to be provided to the plants through the hole but not subsequently escape. Crickets were sorted into groups of small ($\leq$1.0 cm) and large ($\geq$1.5 cm) crickets.

The plants were assigned randomly to three groups of eight by using a random number table (www.random.org). Each plant received one of three treatments: two small crickets, one small and one large cricket, and two large crickets. Live crickets were placed in each cup using tweezers. The plants were then watched to observe immediate encounters between crickets and traps. Two hours later observations were made again and the size of trapped crickets was recorded. Observations of prey capture were taken over the next three days, providing a total of four days of observations. On the fourth day, the crickets that were not captured were removed from the cups. The size of the traps that captured the crickets was recorded using a caliper.

A non-parametric Kruskal-Wallis test was used to test the significance of the results. A Wilcoxon signed rank test was used to show which groups were significantly different if the Kruskal-Wallis test showed that there was a significant difference. The experiment was run twice more for a total of three trials.

Results

In trial one each plant trapped at least one cricket of the two provided except for one plant in group three which did not capture any. Six plants in group one captured both small crickets; the remaining two plants captured only one. Five plants in group two captured both small crickets; the remaining three plants captured only one. One plant in group two captured only the large cricket, and the other two plants that captured only one caught the small cricket. In group three, four plants captured both small crickets, three plants captured only one small cricket, and one plant did not catch any of the small crickets. Of the crickets trapped, trial one had an average small cricket size of 7.3 millimeters and an average large cricket size of 15.6 millimeters.

In trial two, all eight plants in group one captured both of the small crickets. In group two, three plants captured both of the crickets, four plants captured only one cricket, and one plant did not capture either of the crickets. The smaller cricket was the one caught in the plants that captured only one cricket. In group three, three plants captured both of the crickets, three plants captured only one large cricket, and two did not capture either of the two crickets. In trial two, the average size of small crickets trapped was about eight millimeters, and the average size of large crickets captured was 16.5 millimeters.

In trial three, six plants in group one captured both of the small crickets. The two other plants did not catch either of the crickets. In group two, three plants captured both the small and large cricket, four plants captured only the small cricket, and one plant captured only the large cricket. In group three, five plants captured only one of the large crickets, and three plants did not capture either of the large crickets. The average size of small crickets trapped in trial three was 7.5 millimeters, and the average size of large crickets trapped was 16.4 millimeters.

The nonparametric Kruskal-Wallis test for trial one demonstrated that Venus fly traps do not select their prey based on differences in size. There was no significant difference between the three groups in trial one ($H = 1.325$; $df = 2$; p-value = 0.516; $\alpha = 0.05$). The Kruskal-Wallis test performed on the data from trial two showed that there was a significant difference between the three groups ($H = 7.771$; $df = 2$; p-value = 0.021; $\alpha = 0.05$). A Wilcoxon signed rank test on trial two data showed that there was a significant difference between group one and group two (p-value = 0.034; $\alpha = 0.05$), and between group one and group three (p-value = 0.038; $\alpha = 0.05$). The Kruskal-Wallis test done on trial three demonstrated that there was a significant difference between the three groups ($H = 6.671$; $df = 2$; p-value = 0.036; $\alpha = 0.05$). The Wilcoxon signed rank test for trial three showed a significant difference between group two and group three (p-value = 0.014; $\alpha=0.05$) and between group one and group three (p-value = 0.020; $\alpha = 0.05$).

Discussion

The nonparametric Kruskal-Wallis test for trial one demonstrated that Venus fly traps do not select their prey based on differences in size. During observations, the traps were seen to not differentiate between small and large crickets. If a cricket travelled into the trap the trap closed on it. However, in trial two and three the Kruskal-Wallis test demonstrated a significant difference among the groups. Overall, from the data obtained during trial two and three, more small crickets were captured than large crickets. If this is the case then my thoughts that more large crickets would be captured for their higher nutrient content are false.

Through observing the behavior of the crickets, I believe that the significant difference between the amounts captured of small versus large prey can be explained. The larger crickets were in some cases as large as the traps that were trying to capture them. Due to this and their ability to move quicker, they were able to escape the trap before it closed. Many of the larger crickets that were captured were only caught in the trap by their head or tail-end, with the other end protruding out of the trap.

As Volkov et al. (2008) describes, the traps do not close completely until a few minutes after prey capture. This allows a short window for the smallest prey to escape. However, Volkov et al. (2011) also stated that the cilia form an impenetrable wall. Every time a trap closed on prey, they did not escape, whether they were small or large. If the cilia were removed and could not form the wall, it is possible that the insects would then be able to escape. It is probably for this reason that the Venus fly trap evolved the cilia.

Using the observations made and the data collected, it can only be concluded that the plants do not select prey based on size but trap opportunistically. This further proves the results

found by Hutchens and Luken (2009). Venus fly traps close once triggered whether the prey is of better nutritional value or not.

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