The Relationship Between *Wyeomyia smithii* and *Metriocnemus knabi* Larvae and the Insectivorous Plant, *Sarracenia purpurea*

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Abstract: Species coexistence may lead to specialized interactions. The abundance or limitation of resources may be an integral factor in determining which adaptations species evolve. The relationship between the pitcher plant, *Sarracenia purpurea*, and the larvae,*Wyeomyia smithii* and *Metriocnemus knabi*, that inhabit its water-filled pitcher, was investigated. Because the environment *S. purpurea* inhabits is nitrogen poor, nitrogen was assumed to be an indicator of plant fitness. The relationship between the larvae and the plant was determined by examining the correlation between larval biomass, detrital biomass, the total amount of solid nitrogen in the detritus, and the total amount of soluble nitrogen in pitcher water. There was no significant correlation among these variables. Thus, the relationship between *S. purpurea* and the two insect species was found to be neither mutualistic nor parasitic but commensal, and to have no direct correlation with plant fitness as measured by available nitrogen.

Introduction

The coexistence of plants and animals may lead to specialized plant-animal interactions. Such interactions may be restricted by both resource dynamics, i.e. changes in resource abundance and availability, and population dynamics, e.g. fluctuations in population size arising from seasonal and microclimatic variations. In an area where resources are limited, special adaptations allow organisms to overcome or to compensate for the limitation. Two species in close contact may undergo stepwise coevolution, thereby adapting to both their environment and one another. The relationship, be it mutualistic, parasitic, or commensal, may affect the fitness of one or both species.

Most of the vegetation in nutrient-poor areas have adapted mechanisms to overcome nitrogen limitation. *Sarracenia purpurea* , an insectivorous plant that inhabits glacial peat bogs and similarly nutrient-poor environments, is one such example (Gates 1942). The plant attracts insects and other invertebrates with the reddish coloration of its leaves, the nectar coating on its outer lip, and the pheromones it releases (Cresswell 1991). Once lured to the plant, prey fall into its water-filled pitcher. The pitcher's downward-facing hairs and waxy inner lip prevent the prey's escape (Harper 1918, Hepburn 1920, Jones

1921). Bacterial coenzymes and proteolytic digestive enzymes secreted by the plant aid in beginning the decomposition of its prey (Hepburn, St. John and Jones 1920, Jones 1921, Plummer and Jackson 1963). The decomposing prey help compensate for the nitrogen deficiency of the soil.

Species coexistence may be the result of resource partitioning; therefore, interactions among species may be determined by resource availability. For the mosquito, *Wyeomyia smithii* , and the midge, *Metriocnemus knabi, S. purpurea* provides a protected environment in which females lay their eggs. The mosquito and midge offspring remain in the pitcher throughout the egg, larval, and pupal stages, thereby avoiding predators and increasing their chance of surviving to adulthood (Istock 1975). The prey-larvae-plant interactions have been described as a processing chain commensalism in which *W. smithii* and *M. knabi* consume prey at different stages of decay (Heard 1994). Both species feed on prey carcasses, but competition between the species is eliminated because they feed at different stages of decay. *M. knabi* larvae feed on the solid prey carcass while *W. smithii* larvae filter feed on suspended organic particulates (Fish and Hall 1978). The breakdown of prey carcasses may contribute to the amount of available soluble nitrogen for *S. purpurea* thereby increasing overall plant fitness. While the larval relationship is commensal rather than competitive, the relationship, be it parasitic or mutualistic, between the larvae and the plant has yet to be determined.

Because *S. purpurea* grows in environments that are nitrogen-poor, its fitness may be directly affected by the amount of nitrogen it receives. If plant fitness is indeed nitrogen limited, and if the larvae increase nitrogen availability, then those pitchers with the greatest larval biomass should have the highest fitness and the relationship can be considered mutualistic. If there is a negative correlation, then those pitchers with the greatest biomass should have lower fitness and the relationship can be considered parasitic. If there is no correlation between the amount of nitrogen and larval biomass, but a linear relationship between the detrital biomass and the amount of soluble nitrogen, then digestive

enzymes and bacterial coenzymes may be responsible for the breakdown of prey and subsequent increase of soluble nitrogen in the pitcher water. If the only direct relationship found is that between the biomass of larvae and the biomass of detritus, then oviposition preference and the subsequent larval biomass may depend upon a pitcher's capture rate and resultant prey, i.e. detrital, biomass.

How the availability of soluble nitrogen varies with larval biomass, a factor of its adaptive landscape, can be evaluated by examining the relationships among soluble nitrogen and relative larval and detrital biomasses.

The following hypotheses will be investigated: 1) There is no correlation between the biomass of dead prey and the amount of soluble nitrogen in the *S. purpurea* water. 2) There is no correlation between the biomass of larvae and the biomass of dead prey. 3) There is no correlation between the biomass of larvae and the amount of soluble nitrogen in the *S. purpurea* water.

Materials and Methods

Water samples from twenty-five *S. purpurea* were taken from both Mud Lake bog and Grass Bay in Cheboygan County, Michigan. Mud Lake bog is an encroaching sphagnum mat lined with tamarack and Ericacaeous plants. Protected by the Nature Conservancy, Grass Bay is an inundated swail on the shore of Lake Huron. Both the rosettes and the pitchers sampled were randomly chosen.

The plant water, including the organic material suspended within the pitcher, was removed by suction with aquatic tubing attached to either a large syringe or turkey baster. The turkey baster was used only to initially remove large volumes from bigger pitchers. Thereafter, the syringe was used to suction the remaining fluid and detritus from each pitcher. This process was repeated until no water remained in the pitcher. Each water sample was placed in a 15 mL glass or plastic centrifuge tube and covered with Parafilm.

Each pitcher was then repeatedly flushed with deionized water to remove residual organic matter. This solution was removed by syringe and placed into a Boston jar. Finally, the pitcher was partially filled with deionized water and the base of the stem was pierced with a syringe and twice injected with air to dislodge any remaining organic matter. This solution was removed by syringe and placed into a Boston jar. Any organic matter remaining in the aquatic tubing was flushed into the jar with deionized water. The tubes and jars were labeled and refrigerated. (Note: refrigerator controls were improperly calibrated. As a result, all samples froze).

The centrifuge tubes, containing the original pitcher water, the larvae, and the prey were centrifuged at 1500 rpm for 25 minutes. The supernatant (SN) was decanted into a 100 mL volumetric flask . The pellet was resuspended in deionized water and centrifuged at 1500 rpm for 25 minutes. The SN was decanted into the volumetric flask. This process was repeated twice. (Note: a small number of the centrifuge tubes cracked when the samples froze. Those tubes were covered with a jacket of Parafilm to minimize leakage and the centrifuge sleeves were cleansed to minimize sample contamination should leakage occur. Leakage was minimal and deemed insignificant).

The liquid in the volumetric flasks was brought to 100 mL with deionized water. Samples were prepared for nitrogen analysis by the total nitrogen persulfate method. A one to seven dilution of the sample water was made. Three mL of 1.5M $K_2S_2O_8$ and 0.25 mL of 1.5M NaOH were added to each dilution. All samples were autoclaved for 30 minutes on slow exhaust to oxidize all organic and inorganic nitrogen into nitrates. Standards, controls and blanks were run according to the basic persulfate of nitrogen analysis procedure (E.I. du Pont de Nemours and Company, Inc., Wilmington, Delaware) and were used as procedural checks.

Contents of the Boston jars were suction filtered and examined under a dissecting microscope to remove *W. smithii* and *M. knabi* larvae. Pellets of detritus from the centrifuge were similarly examined. Larvae from each pellet and corresponding jar were

combined into a single container and dried for 24 hours at 50°C to determine total dry weight of larvae.

Remaining pellet detritus was combined with the detritus from the jar, dried for 24 hours at 50°C, weighed, and ground into a fine powder using liquid nitrogen. Powder samples were dried for 12 hours at 50°C and analyzed by a CHN analyzer to determine total solid nitrogen content.

Regression analyses were performed to determine if there were correlations between the larval biomass and the total amount of soluble nitrogen, detritus biomass and the total amount of soluble nitrogen, and larval biomass and detritus biomass.

Results

Data from Mud Lake bog and Grass Bay were analyzed independently and the results were compared. Results were not significantly different; therefore, the data from both sites were analyzed together. There was no correlation between the biomass of detritus and the amount of soluble nitrogen in the pitcher water ($p= 0.314$, $r^2= 0.022$). The detrital biomass ranged from 2.9 mg to 519.4 mg. The nitrogen content of the pitcher water ranged from 0.011 mg to 1.750 mg. There was no correlation between the biomass of larvae and the biomass of detritus ($p=0.167$, $r^2 =0.041$). The larval biomass ranged from 0.1 mg to 108.8 mg. There was no correlation between the biomass of larvae and the amount of soluble nitrogen in the pitcher water ($p=0.905$, $r^2 =0.000$). There was no correlation between the amount of soluble nitrogen in the water and the amount of nitrogen in the detritus ($p= 0.428$, $r^2 = 0.014$). The nitrogen content of the detritus ranged from 0.0550 mg to 36.825 mg. There was no correlation between the biomass of larvae and the amount of solid nitrogen in the detritus ($p= 0.372$, $r^2 = 0.017$).

Discussion

As our results indicated, there was no correlation between total larval biomass and total amount of soluble nitrogen in the pitcher water and therefore no direct relationship between the larvae and plant fitness. Because nitrogen was a limiting factor at each site, we assumed it was a limiting factor of overall plant fitness. Our data show no correlation between the total amount of soluble nitrogen in the pitcher, the total amount of nitrogen in the detritus, the detrital biomass, and the larval biomass. Because our data indicated that there was no linear relationship between larval biomass and the total amount of soluble nitrogen in pitcher water, we can assume that the fitness of *S. purpurea* is not directly affected by the presence of larvae. Thus, it is conceivable that the fitness of *S. purpurea* is instead affected by fluctuations in its adaptive landscape rather than the presence or abundance of larvae in its pitchers.

No correlation was found between larval biomass and detrital biomass. Bradshaw (1983), Bradshaw and Holzapfel (1983) and Fish and Hall (1978) found that the majority of mosquito oviposition occurs before the pitcher captures its prey. They found no substantial amount of prey present in the pitcher at the time of oviposition; therefore, mosquito adult females are not likely to show plant preference based on prey biomass. Choice of oviposition site may be influenced by the volume of water in the pitcher because desiccation rate, which threatens larval survival, decreases as volume increases (Kingsolver 1981). Oviposition preference and resultant larval biomass, then, may depend on other variables such as microclimate or relative size of the pitcher.

Detrital biomass was not correlated with the amount of soluble nitrogen in the pitcher water. Thus, plant fitness is not affected by detrital biomass.

There was no correlation between larval biomass and the amount of soluble nitrogen in the pitcher water. Similarly, no correlation was found between the larval biomass and the nitrogen content of detritus. A future line of inquiry might involve further examination of differences in larval abundance among pitchers. Kingsolver (1978) found

that desiccation frequency (determined by pitcher volume and location) may account for significant differences in larval developmental rates, voltinism, and mortality. Larval biomass may also be a function of leaf age because as the pitcher ages, pH of pitcher water becomes increasingly acidic (Fish and Hall 1978). Thus, larval success may be correlated with conditions within the pitcher that were not measured in this study.

Larval biomass is not related to plant nitrogen or detrital nitrogen. Instead, larval biomass may change seasonally with the growth and development of the larvae. As larvae progress through instars, a greater biomass is expected. Later in the year, as larvae reach their last instar, they are bigger and require more nitrogen. Therefore, nitrogen becomes a more limited resource. Thus, we may see no correlation in the early spring because the larvae are so small that despite their abundance, nitrogen is not yet a limiting resource.

Conclusion

We found that soluble nitrogen content of pitchers was not correlated to larval biomass, detrital biomass, or total amount of solid nitrogen in detritus. We concluded that the relationship between the larvae *W. smithii* and *M. knabi* and the pitcher plant , *S. purpurea* , is neither mutualistic nor parasitic; instead, it is commensal. The larvae benefit from the plant in the protection and resources it provides, while the plant remains unaffected by the larvaes' presence. Plant fitness is not affected by the larvae in its pitchers. Further interactions within *S. purpurea* may be addressed to explain variation in nitrogen content. For example, the presence and abundance of bacteria within the pitcher may affect the decomposition of prey and therefore nitrogen availability. Other factors such as resource availability, stochastic events, or temporal changes in environment may play important roles in nitrogen availability and plant fitness.

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