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Predation of Introduced Mosquito Larvae by the Midge *Metriocnemus knabi* in the Phytotelma of the Pitcher Plant *Sarracenia purpurea* and Colonization Following Dry Conditions

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Abstract - The leaves of the carnivorous *Sarracenia purpurea* (Purple Pitcher Plant) provide habitat for obligate insects. Within the pitchers of this plant, *Metriocnemus knabi* (Pitcher Plant Midge) larvae coexist with *Wyeomyia smithii* (Pitcher Plant Mosquito) larvae. No other mosquito species has been reported to utilize this habitat in the presence of the midge. We tested whether the midge larvae were responsible for the elimination of other mosquito species. We introduced 1 *Aedes triseriatus* (Eastern Treehole Mosquito) larva, into each of 90 different pitchers. After 45–75 minutes, we extracted the fluid from the Purple Pitcher Plant and counted mosquito and midge larvae. Although 98% of pitchers contained Pitcher Plant Mosquito larvae, we did not detect 61 of the Eastern Treehole Mosquito larvae. Of the 29 surviving introduced larvae, we found 13 (45%) in pitchers that had no midge larvae. Drier than normal conditions in 2012 provided the opportunity to investigate Purple Pitcher Plant leaves devoid of water and obligate insect larvae, and the potential for foreign mosquito larvae to colonize unoccupied pitchers. We found Pitcher Plant Midge or Pitcher Plant Mosquito larvae within 13 days following the addition of water. We observed no foreign mosquito larvae. The inquiline larvae did not develop when we added water to dry pitchers in the laboratory, suggesting that oviposition by Pitcher Plant Midge and Pither Plant Mosquito adults occurred after a rainfall event. During dry conditions, shaded Purple Pitcher Plants retained some fluids, and adults likely completed their life cycle in these plants. However, severe, prolonged drought may eliminate Purple Pitcher Plant inquilines and potentially make the pitchers available for exotic mosquito larvae.

Introduction

Ombrotrophic bogs are precipitation-filled wetlands (Small 1972) that contain uniquely adapted plant and animal communities. The Brighton Bog in upstate New York has a large population of the *Sarracenia purpurea purpurea* Wherry (Purple Pitcher Plant). This pitcher plant retains fluid, called phytotelma, in specialized leaves. Larvae of 3 obligate dipterans can occupy this liquid. *Wyeomyia smithii* Colquillett (Diptera: Culicidae, Pitcher Plant Mosquito) and *Metriocnemus knabi* Coquillett (Diptera: Chironomidae, Pitcher Plant Midge) are common, whereas

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Fletcherimyia fletcheri (Aldrich) (Diptera: Sarcophagidae, Pitcher Plant Flesh Fly) is occasionally found (Krawchuk and Taylor 2003, G.J. Torrisi, pers. observ.).

In the laboratory, Petersen et al. (2000) found that Pitcher Plant Midge eliminated introduced mosquito larvae from simulated Purple Pitcher Plant phytotelmata and suggested that the longer setae on Pitcher Plant Mosquito larvae are an adaptation to prevent predation by midge larvae. To our knowledge, these laboratory results have not been tested under natural conditions, which can often produce different results (e.g., Diamond 1986) and overestimate the effects of predation (Cooper et al. 1990). Therefore, our objective was to test predation by Pitcher Plant Midge in the field on larvae of *Aedes triseriatus* Say (Eastern Treehole Mosquito). Drier than normal conditions in the second year of our study also allowed us to investigate colonization of dry pitchers after water was artificially or naturally added.

Materials and Methods

Study site

We conducted this study at the Brighton Bog, in the town of Brighton, Franklin County, NY (44°26'50"N, 74°12'52"W), within the 2.43-million ha Adirondack Forest Preserve high-peaks region. The habitat investigated is an ombrotrophic wetland approximately 2.5 ha in size with a large open-water flark pool. The surrounding temperate forest is dominated by *Pinus strobus* L. (White Pine), *Tsuga canadensis* (L.) Carr. (Estern Hemlock), *Picea* spp. (spruces), *Larix laricina* (Du Roi) K. Koch (Tamarack), and *Quercus* spp. (oaks). The bog is covered in *Sphagnum* spp. (peat moss) and stunted *Picea mariana* (Mill.) Britton, Sterns & Poggenb. (Black Spruce). In addition, *Chamaedaphne calyculata* L. (Leather Leaf) and *Eriophorum* sp. (cotton-grass) grow around the perimeter ~20–30 m inward from the lagg. Johnson (1985) has characterized peatlands like Brighton Bog, which occurs at ~600 m elevation, as cold-climate habitats.

An abundance of Purple Pitcher Plants occurs within the shaded area of Brighton Bog. These plants contain an inquiline community dominated by the Pitcher Plant Midge, and the Pitcher Plant Mosquito. Our research in the bog began in 2010 and we conducted field experiments in 2011 and 2012. During these surveys, we found that Pitcher Plant Midge and Pitcher Plant Mosquito larvae occupied 98% of sampled Purple Pitcher Plant leaves with phytotelma (Torrisi 2013). In 2012, Brighton Bog received much less than average rainfall (Weather.org 2015). Some pitchers were either void of fluid or contained very little fluid, and in several instances, one or both obligate insects were absent from the leaves. By mid-July, many of the new pitcher plant leaves remained unopened, appeared stunted, and in some cases were senescing. The older red or purple leaves were also dying or dead. Approximately half of all the open pitchers in the bog were void of liquid.

Study organisms

We employed natural Pitcher Plant Mosquito and Pitcher Plant Midge communities for the field studies. We chose Eastern Treehole Mosquito as the foreign mosquito for our study because they proved to be the most abundant species colonizing our artificial containers. They were field collected and reared in containers.

Field introduction of larvae

In 2011, we collected Eastern Treehole Mosquito larvae from naturally colonized 2-L containers near Saratoga Springs, NY. We transported first-instar larvae to the bog, siphoned them from the transport container, and placed 1 larva into each of 40 large (15–20 cm tall) Purple Pitcher Plant pitchers. We marked selected pitchers by placing a color-coded wooden dowel next to the leaf. After a 45-min waiting period, we quickly removed the phytotelma of the pitchers using a modified culinary turkey baster fitted with clear, flexible, 7-mm diameter tubing. We squeezed the baster bulb, inserted the tubing to the bottom of the pitcher, and siphoned the fluid by allowing the bulb to expand. We examined the contents, removed any introduced larvae, discarded any surviving Eastern Treehole Mosquito larvae, and returned the fluid to the pitcher. We recorded the presence or absence of the foreign larva, predatory midge larvae, and Pitcher Plant Mosquito larvae. We repeated the experiment in 2012, introducing one Eastern Treehole Mosquito larva into leaves of 50 different Purple Pitcher Plants.

Dry-conditions investigation

In 2012, when near-drought conditions existed, we investigated 3 environmental conditions: pitcher leaves naturally retaining fluid, open pitcher leaves devoid of fluids, and dry pitcher leaves to which we added water. On 20 July 2012, we selected 50 pitchers for each treatment and marked each with a color-coded dowel. Purple Pitcher Plants were located in the shaded area of the bog perimeter, the edge boundary between shade and open bog, and the open, full sun-exposed center region of the bog. We selected 50 leaves that naturally contained fluid at the time of the investigation as controls, 50 leaves without fluid as dry controls, and 50 leaves of approximately the same size but without fluid for the treatment. We added 25 ml of untreated well water to each leaf in the latter group. On the fourth day of this study, a single rainstorm deposited 0.94 cm of precipitation which added water to all leaves.

On 3 August 2012, we examined each pitcher leaf for the presence or absence of insect larvae. We also recorded the size in cm above the moss bed, color scheme (either all green, all red, or mixed coloration), and venation patterns (slight, moderate, or heavily veined). Color and veination were qualified and not quantified descriptors of the pitcher leaves. We noted the number of damaged leaves, the larval stage of the insects found in the phytotelma, as well as leaves with reduced or increased amounts of phytotelma, which we measured (in ml) by removing the fluid and placing it into a graduated cylinder.

Results

Field introduction of larvae

Sampling from 40 pitcher leaves in 2011 revealed that all leaves contained Pitcher Plant Midge larvae and Pitcher Plant Mosquito Larvae. Approximately 45 min after we introduced the Eastern Treehole Mosquito larvae, they were no longer present in 31 of the 40 leaves (77.5%) (Table 1). We observed all 3 species in the remaining 9 leaves (22.5%).

In 2012, 30 of the 50 Eastern Treehole Mosquito larvae (60%) were absent from the pitchers during resampling. All samples with missing foreign mosquito larvae contained Pitcher Plant Midge larvae. In 7 of the leaves (14%), both Pitcher Plant Midge larvae and Eastern Treehole Mosquito larvae were present. The remaining 13 leaves (26%) contained the introduced Eastern Treehole Mosquito larva but no Pitcher Plant Midge larvae. During 1 resampling event, we observed a Pitcher Plant Midge larva clinging to the abdomen of the Eastern Treehole Mosquito larva but it released its hold upon transfer to the sampling container. We did not observe other interactions during the procedures. Across years, of the 90 total Purple Pitcher Plant leaves tested, 29 (32%) contained surviving Eastern Treehole Mosquito larvae, 88 (98%) had Pitcher Plant Mosquito larvae, and 77 (86%) of the leaves contained at least 1 Pitcher Plant Midge larva (Table 1). Elimination of foreign mosquito larva was significantly greater when the Pitcher Plant Midge was present than when it was absent (Mann-Whitney rank-sum test; $n = 90$, $t = 988$, $P < 0.001$).

Dry-conditions investigation

A total of 46 of the 50 leaves that contained pitcher phytotelma from the onset of our investigation maintained fluid throughout the investigation. Between visits, 3 pitchers were lost to leaf damage and we could not relocate 1 leaf during our second visit. We documented insect larvae in 43 of the 46 pitchers. Both midge and mosquito larvae were present in 26 of the leaves, Pitcher Plant Mosquito larvae alone were present in 13 leaves, and only Pitcher Plant Midge larvae were present in 4 leaves (Table 2).

Table 1. Recovery of the introduced larvae of *Aedes triseriatus* (Eastern Treehole Mosquito) and 1 or more *Metriocnemus knabi* (Pitcher Plant Midge) larvae from phytotelma of Purple Pitcher Plants at Brighton Bog, NY.

Year	Number of pitcher leaves	Foreign mosquito absent with midge present	Foreign mosquito present	
			With midge present	With midge absent
2011	40	31	9	0
2012	50	30	7	13
Totals	90	61	16	13

Table 2. Number of Purple Pitcher Plant leaves ($n = 50$)—categorized according to whether they had phytotelma, or were dry and had water added by investigators, or were dry and then filled only by a precipitation event—that contained inquiline larvae after 14 days at Brighton Bog, NY, during the summer of 2012.

Larval type	Fluid present	Water added	Precipitation filled
Midge only	4	7	4
Mosquito only	13	6	6
Both larvae present	26	12	6
No larvae present	6	25	34

Twenty-five of the leaves to which we added water contained insect larvae during our second visit and 8 additional leaves contained fluid but no larvae. We found Pitcher Plant Midge larvae in 7 leaves, Pitcher Plant Mosquito larvae in 6 leaves, and larvae of both insects in 12 of the leaves where water was added (Table 2). The remaining 17 leaves either dried out or were damaged during the investigation period.

Sampling of the dry leaves that did not receive additional water except from the rain event revealed 28 leaves that either remained dry or were damaged during our investigation. Of the remaining leaves that held fluid, 4 leaves contained only Pitcher Plant Midge larvae, 6 contained only Pitcher Plant Mosquito larvae, and 6 leaves contained both species. Only first-stage larvae were present in any of the leaves to which water had been added, either by the investigators or precipitation (Table 2). We did not detect Eastern Treehole Mosquito larvae in any of the pitchers with or without the midge.

We conducted an experiment to test if dry leaves were colonized or if desiccation-resistant eggs hatched when water was added. We brought 14 dry leaves back to the laboratory, added well water to each leaf, and checked the leaves every 2 days for the presence of insect larvae. No larvae were found after a 2-week period in the laboratory; thus, it appears that larvae found in the field were the result of oviposition by adults rather than by hatching from desiccation-resistant eggs.

Discussion

Purple Pitcher Plant leaves provide a microhabitat for a number of micro- and macro-invertebrates. Of these associates, the Pitcher Plant Mosquito and the Pitcher Plant Midge are obligate inhabitants (Fish and Hall 1978, Hamilton and Duffield 2002, Heard 1994, Miner and Taylor 2002, Nastase et al. 1995, Petersen et al. 2000). These insects live within the phytotelma and spend their egg, larval, and pupal periods in a single leaf.

This plant–insect ecosystem has served as the basis for much research extending back several decades (Bradshaw and Hozapfel 1983). Petersen et al. (2000) utilized a laboratory study to address the question of why no mosquito species other than Pitcher Plant Mosquito have been collected from the leaves of Purple Pitcher Plants. However, Milne et al. (2008) reported colonization of pitcher plant phytotelma by *Aedes albopictus* (Skuse) (Asian Tiger Mosquito), in the absence of Pitcher Plant Mosquito and Pitcher Plant Midge. Our results suggest that Pitcher Plant Midge larvae prey upon introduced *Aedes* (mosquito) larvae. Although we only directly observed one instance of this behavior, midge predation is the most likely explanation for our results. In 2012, foreign mosquitoes were always recovered from pitchers that did not contain Pitcher Plant Midge larvae (Table 1), which supports the conclusion that our use of the siphon was sufficient to capture introduced mosquito larvae. The disappearance of mosquito larvae was strongly correlated with the presence of the Pitcher Plant Midge with only 16 of 77 pitchers containing both the midge and the introduced larvae in 2012 (Table 1). These results support the conclusions of Petersen et al. (2000) that predation by the midge larva likely reduces interspecific interactions with other mosquito larvae for Pitcher Plant Mosquito larvae in pitcher phytotelma.

Successful colonization of an occupied niche by a new species requires displacement of residents, use of available resources, the availability of additional resources, or the absence of predators (Miller et al. 2002). In our investigation, introduced Eastern Treehole Mosquito larvae disappeared from the Purple Pitcher Plant leaves within 1 h in the presence of the Pitcher Plant Midge in ~80% of the tests. The midge appears to be an effective predator that is able to take advantage of a small habitat and is capable of capturing active prey (Petersen et al. 2000). These results suggest that the Pitcher Plant Midge may provide a form of guardianship for the Pitcher Plant Mosquito larvae by eliminating foreign mosquito larvae that hatch in the leaves of the pitcher plant and lack the long setae that seem to protect the former species from midge predation.

In our experiments, we allowed ~45 minutes for interactions and we always introduced a single first-instar Eastern Treehole Mosquito larva. It is likely that a longer time interval for interaction would have resulted in higher predation rates by the Pitcher Plant Midge larvae, though Petersen et al. (2000) reported that all introduced mosquito larvae placed in an observation chamber were killed by Pitcher Plant Midges within 30 minutes.

In 2012, when the bog experienced very dry conditions, many Purple Pitcher Plant leaves were either dry or nearly dry. Our observations of the bog during the predation experiments led us to question whether midge and mosquito larvae would colonize the pitcher plants if water was added to the leaves. Two questions arose: first, would the insects colonize the leaves late in the season and, second, would mosquitoes other than the Pitcher Plant Mosquito colonize the leaves in the absence of the predatory midge? In no more than 13 days after the addition of water, most leaves contained either the Pitcher Plant Mosquito, the Pitcher Plant Midge or both obligate species. On 24 July, 4 days after we experimentally added water to a set of pitchers, a rainstorm added additional water and provided water to the leaves that were dry. All insect larvae that we recovered from pitchers that had been dry were early instars. We found that pitchers containing water at the onset of the investigation contained both mosquito larval instars and some pupae; however, it seems that the dry conditions delayed the maturation of these larvae. We counted as many as 28 early-instar mosquito larvae from 3 pitchers that retained phytotelmata. Pitcher Plant Midge larvae were also in early stages but in much lower numbers, with 2–7 larvae observed.

After we added water to the 100 pitchers that were void of water, there was a mixed response among pitchers. Some had Pitcher Plant Midge larvae only, some had Pitcher Plant Mosquito larvae only, and in most cases, both species were present. At no time was a foreign mosquito larva found. It would seem that an unoccupied habitat, suddenly made available in a time when other oviposition sites were likely rare, would attract females of mosquito species other than Pitcher Plant Mosquito. Although treeholes hold more water than pitchers and are more protected from desiccation, the dry conditions were of such duration that even natural treeholes may have been dry or very nearly so, limiting available sites and likely reducing numbers of adult Eastern Treehole Mosquitoes. In a previous study, Eastern Treehole Mosquito females preferred larger and darker containers (Torrisi and Hoback 2013), and females that deposited eggs in pitchers may have differentiated among leaf ages. In the absence of the midge, it would seem possible that foreign mosquitoes could compete with Pitcher Plant Mosquito larvae. However, this assumption has not yet been tested. From our results, it appears that adult Pitcher Plant Mosquitos are able to persist in the environment and colonize newly available pitchers more readily than other mosquito species, which may survive dry conditions with aestivation of eggs.

The insect inquilines of the Purple Pitcher Plant require a phytotelma association in order to develop prior to adult emergence (Mouquet et al. 2008). It appears that the temporal delay in adult oviposition during drier conditions allows the adults to colonize newly grown or dried pitchers once precipitation fills the leaves. Climatic factors, primarily temperature and precipitation, strongly influence the development of these inquiline inhabitants (Tauber and Tauber 1976). Insect fauna within the leaves of the Purple Pitcher Plant are influenced by physical, chemical, and biological factors that modify their growth and affect their phenology (Juliano and Stoffregen 1994, Williams 1996). Our study emphasizes the importance of conducting field studies to confirm interactions observed in the laboratory and to determine the effects of variable or changing weather patterns on highly co-evolved systems.

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