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Cover image - Patch reef near the wall off Grotto Beach (photo by Lee Florea).

The natural history of *Craspeduchus pulchellus* (Hemiptera, Lygaeidae) on San Salvador Island, The Bahamas

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1. Abstract

On San Salvador Island, *Craspeduchus pulchellus* have been observed frequently on the fruit of the shrub *Corchorus hirsutus*. These bugs are known seed predators; they pierce the fruit wall to extract nutrients from the developing seeds. In Florida, the bug is reported to feed exclusively on *Corchorus siliquosus*. Both plants are native to The Bahamas and have been reported on San Salvador Island, but *C. siliquosus* is less common. Here we describe morphological and behavioral feeding characteristics of individuals feeding on *C. hirsutus*, and compare the traits to those reported for populations feeding on *C. siliquosus*. Observations were recorded during three time periods: June 2014, March 2015 and June 2015. We observed five immature instars as well as adults, some in the process of mating. The bugs were usually on the fruits, but were also found on the leaves and stems, particularly at the distal ends of the branches. Regardless of age, all bugs exhibited feeding behaviors on the fruits. We estimated the population density by recording the number of individuals observed on tagged branches on 25 *C. hirsutus* plants, and estimated movement between plants and distance traveled by marking adult bugs and releasing them onto five target plants, then recording their position over time. On average, we found 1.072 bugs ± 0.125 SE per 0.5 m of branch. Less than half of the marked bugs were found on the original target plants, although they did leave and return over three days. Beak (mouth-parts) length and diameter were measured and compared with the width of the fruit wall and the diameter of holes observed in seeds. Beak diameter was consistent in older instars and adults (0.1 mm ± 0.000 SE), and matched holes that were drilled in the seeds. Beak length was 11-22% greater than the lengths reported for instars and adults feeding on *C. siliquosus*. *Corchorus hirsutus* fruit walls are thicker than those of *C. siliquosus*, and this difference in fruit morphology could act as a selective filter influencing beak length in the bugs.

2. Introduction

The Woolly Corchorus shrub (*Corchorus hirsutus* L.; Malvaceae) is a common plant in coastal communities throughout The Bahamas (Correll and Correll 1982). We noticed that these shrubs frequently act as host plants for *Craspeduchus pulchellus* (Fabr.) (Hemiptera, Lygaeidae) which are known to act as seed predators on the closely related plant, *Corchorus siliquosus* L. in south Florida populations (Baranowski and Slater 1975). *Craspeduchus pulchellus* is a widespread neotropical species that is thought to have been introduced to Florida within the last 60 years (Slater and Baranowski 1990), possibly due to hurricane dispersal (Baranowski and Slater 1975). When feeding, *C. pulchellus* drills a hole through the fruit wall and sucks the tissues from the developing seeds. Nothing is known about the rate of herbivory on the seeds of either plant, but studies of related insects have found significant reductions (60-90%) in the germination rate of seeds that have been fed upon by seed bugs (Cervantes and Carranza 2008).

There have not been many studies of *C. pulchellus*. Baranowski and Slater (1975) observed adults and five instars in Key Largo, Florida, exclusively on *C. siliquosus* shrubs, which are native to South Florida. They also noted that *C. pulchellus* eggs were laid in clusters on the ground, and both the nymphs and adults were observed feeding on the long thin seed pods of *C. siliquosus*. Wolcott (1936) reported *C. pulchellus* under a previous name (*Lygaeus pulchellus*) on *C. hirsutus* in Puerto Rico, but Baranowski and Slater (1975) suggested that Wolcott's plant identification required verification because he indicated that the plant identification was presumed. The Key Largo study did not indicate whether *C. hirsutus* was present in the plant community (Baranowski and Slater 1975). *Corchorus hirsutus* is not native to south Florida, but vouchers from a single specimen were collected in nearby Miami-Dade County in 2000 and 2001 (Wunderlin et al. 2016). *Craspeduchus pulchellus* was also included in one field guide under another previous name, *Ochrostomus bilimeki* (Elliott et al. 2009). In this paper, we are using nomenclature from the most recent published works on the classification of this group (Ashlock 1975; Ashlock and Slater 1988).

Here we describe the morphology of the beak (piercing and sucking mouth-parts) and the feeding behaviors of *C. pulchellus* on *C. hirsutus* in one coastal plant community on San Salvador Island, The Bahamas. *Corchorus siliquosus* is also found on San Salvador (Smith 1993), but it is absent from coastal plant communities (Smith 1993; Landry et al. 2013). We discuss whether differences in the fruit morphology of the two *Corchorus* species could lead to differences in insect beak morphology due to natural selection.

3. Methods

This study was conducted on Sandy Hook, San Salvador Island, The Bahamas (Figure 1) during June 2014, March 2015, and June 2015. Reproductive, feeding, and other behaviors were recorded throughout the study. Demographic information was collected on the *C. pulchellus* bugs found on *C. hirsutus* plants. The leaf-covered segments of two branches (21-25 cm long) on each of 25 plants were randomly chosen along two transects, flagged, and the presence of immature fruits, mature fruits, flowers, and/or flowers buds was recorded. The numbers of adult and juvenile bugs initially observed on the branches were recorded; the branches were surveyed four more times, 6-12 days after flagging. To calculate the descriptive statistics (average and standard error), the number of bugs found on the two branches of each plant were pooled for each collection day because branches on the same plant are connected and therefore, are not true replicates. A two-way ANOVA was used to test for differences between plants and between morning versus afternoon observation periods. Correlations between the number of immature fruits and the number of bugs were investigated using linear regression analysis. Pearson's correlation coefficient was calculated to compare the number of mature bugs with the number of immature bugs.

To determine whether insects moved between host plants and if so, how far they traveled, five sets of five adult insects were randomly selected, placed in a small ice-filled container and cooled for five minutes to slow their movements. The bugs were then marked with a small dot of Testors™ enamel paint on the dorsal anterior portion of the thorax; care was taken to avoid painting the head or wings. Each set of five insects was painted a different color, and insects painted the same color were placed together on one of five targeted *C. hirsutus* plants. The plants were monitored for 3 days to observe the movements of the bugs. In addition to checking the target plants each day, all plants within 10 m of the target plant were inspected for marked bugs, and the distance from the target plant was recorded for each marked insect observed. The plants were inspected between the hours of 9 am and 1 pm. The average number of marked bugs found on each target plant and within 10 m of the target plant were calculated for each observation day. A one-way ANOVA was used to test for differences between target plants.

Figure 1. San Salvador Island, The Bahamas, with Sandy Hook (inlay); star indicates the specific site location.

Insect vouchers were collected by picking the bugs off the plants by hand. The specimens were preserved in alcohol, and a subset were exported to Ohio where the beaks were measured. The average beak length and maximum beak width was estimated for two adult insects, one second instar nymph, and three nymphs of the third through fifth instars. To estimate the total length of the beak of each specimen, we used a dissecting microscope and a stage micrometer to measure the length of each segment of the beak, and then summed the lengths. The beak length was compared to the width of *C. hirsutus* fruits. The maximum beak diameter was measured at the widest point and compared to the diameter of holes drilled into *C. hirsutus* seeds. Plant and insect vouchers were deposited at the Gerace Research Centre's (GRC) Natural History Repository on San Salvador Island, The Bahamas. Most insects that were exported to Ohio for study will be returned to the GRC Repository or submitted to the Bahamian National Entomological Collection in Nassau, but a series including representatives of all immature stages as well as adults will be submitted to the Museum of Biological Diversity at The Ohio State University, Columbus, Ohio.

4. Results

We observed insects representing five instars on reproductively mature *C. hirsutus* plants; no insects were observed on reproductively immature *C. hirsutus* individuals or on any other plant species in the community. The *C. pulchellus* instars not only differ in size, but each instar possesses a distinctive color pattern on the dorsal side of the thorax and abdomen (Figure 2). The bugs were typically found on the *C. hirsutus* fruits but also on stems and the upper and lower surfaces of leaves near fruits, usually at the distal ends of the branches. The late instar and mature bugs were frequently observed standing on the sides of green fruits while feeding, with their beaks inserted into the fruit (Figure 3). The much smaller early instars burrowed head first into the woolly fruits, with their abdomens and legs sticking out as if they were standing on their heads. The smallest instar individuals almost "disappeared" into the layer of wool that covers the fruit. We also observed bugs moving in and out of brown fruits that had dried and split open while still attached to the plant.

On average, 1.072 bugs ± 0.125 SE were found per 50 cm of leafy branch. There were significant differences between observation dates (Figure 4; ANOVA, df = 4, F-ratio = 2.968, p = 0.022) and between plants (ANOVA, df = 24, F-ratio = 3.737, p < 0.001) but not between observations performed in the morning versus the afternoon (ANOVA, df = 1, F-ratio = 0.070, p = 0.792). There was a significant positive relationship between the number of fruits present and the number of bugs on the branches (Figure 5) for green fruits (OLS Regression, t = 4.180, p < 0.001) and brown fruits (OLS Regression, t = 4.981, p < 0.001), and the effect was significantly greater for green fruits (ANOVA, df = 2, F-ratio = 17.474, p < 0.001).

Figure 2. A series of *Craspeduchus pulchellus* specimens with scale (mm), viewed at 40x magnification. The series includes, from left to right: Top) 1st through 3rd instar specimens, and Bottom) 4th and 5th instar and adult specimens.

Figure 3. Immature *Craspeduchus pulchellus* individual (5th stage instar) feeding on immature *Corchorus hirsutus* fruit.

There are no obvious morphological differences between male and female individuals, but we know both genders were present on the plants because we frequently observed mating pairs. Fertilization is internal; males and females face in opposite directions during copulation (Figure 6), and we observed mating pairs that remained attached to one another for up to 30 minutes. Less than half of the marked bugs were found on, or within 10 m of, the target plants after they were marked (Figure 7). The number of bugs observed on or near individual target plants differed significantly (ANOVA, df = 4, F-ratio = 5.85, p-value = 0.011).

The average beak length and beak diameter of bugs increased with the age of the bugs (Table 1). Insect beaks reached the maximum diameter (0.100 mm) by the 4th instar, while the length increased throughout development.

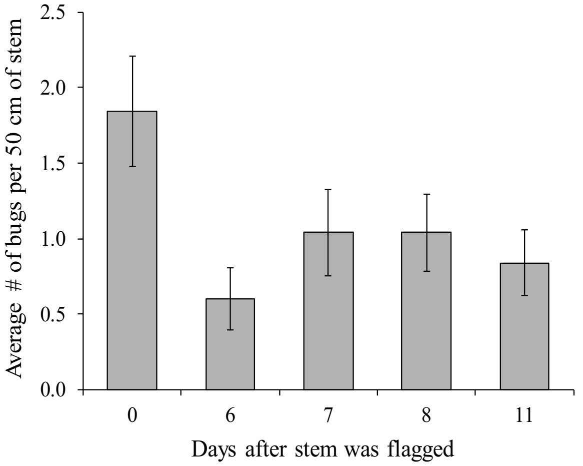

Figure 4. Average number of bugs present on each of five observation days, with SE bars. For each observation day. N = 25 plants with flagged stems, each with two flagged stems.

5. Discussion

The demographic data demonstrate that *C. pulchellus* bugs were more likely to move away from the target plant, and less likely to return, when the target plants were isolated from other *C. hirsutus* shrubs. The bugs moved away and returned to target plants when other *C. hirsutus* shrubs were nearby, which suggests that the bugs were more likely to stay in a small area when many plants with fruits were available, and did not forage over longer distances if they could avoid doing so. Alternatively, the bugs may have been responding to hot and dry conditions. When the study was conducted in 2015, San Salvador had been experiencing very little rain. It is possible the bugs chose to forage in patches of *C. hirsutus* versus solitary plants because the overlapping branches of nearby plants provided more shade.

Holes drilled into developing seeds matched the maximum beak diameter of the bugs, so bugs exhibiting feeding behaviors on immature fruits were feeding on the seeds. It is not clear what the bugs were doing inside the brown dehiscent fruits. It is possible that they were feeding on mature seeds, but this seems unlikely because when the seed is mature, the seed coat is very hard. The bugs could have been using the fruits as a hiding place from predators, although we did not observe bird or insect predators nearby when the behavior was exhibited. Alternatively, they may have been using open fruits in thermoregulation because the fruit interior would provide shade – a microhabitat with lower temperatures than

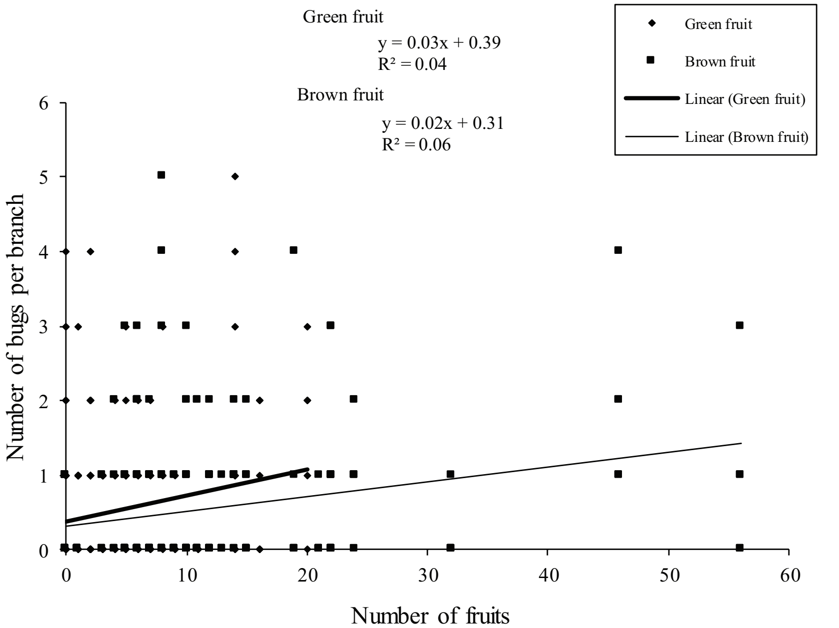

Figure 5. Number of bugs present versus number of immature (green) and mature (brown) fruits on flagged branches, with trend lines. N = 25 plants, each with two flagged stems.

Figure 6. Mating pair of *Craspeduchus pulchellus* bugs on *Corchorus hirsutus* fruit.

the plant surfaces exposed to direct sunlight. We also observed fewer bugs at midday versus the morning (data not shown), which could be attributed to thermoregulatory behaviors. Baranowski and Slater (1975) reported that *C. pulchellus* individuals were found in the leaf litter under plants, which would also be cooler than the temperature in full sun. We did not survey leaf litter in our study.

All instars of *C. pulchellus* appeared to be specialized on *C. hirsutus* in the coastal community that we studied, as was also reported for populations in Puerto Rico (Wolcott 1936), but contrasting with Baranowski and Slater's (1975) report that the bug was specialized on *C. siliquosus* in south Florida. The two plant species produce fruits that are very different in morphology. *Corchorus hirsutus* fruits are cylindrical, approximately 6 mm in diameter including a thick layer of woolly hairs, while the fruits of *C. siliquosus* are flattened, approximately 3 mm wide on the broad axis of the cross-section, and are either smooth or covered with fine hairs (Correll and Correll 1982). Theoretically, bugs with longer beaks would be more successful when feeding on *C. hirsutus*, while bugs with shorter beaks would be more successful when feeding on *C. siliquosus*; therefore, we would expect to see divergence in beak length between bug populations feeding on the different plants. Selection on beak length has been reported for another hemipteran seed predator in Australia (Andres et al. 2013). In the Australian study, bugs feeding on the large fruits of an introduced plant species had longer beaks than those feeding on native plants in nearby communities that lacked the non-native plant.

Our preliminary evidence indicates that the mouth-parts of 2nd to 5th instar nymphs and

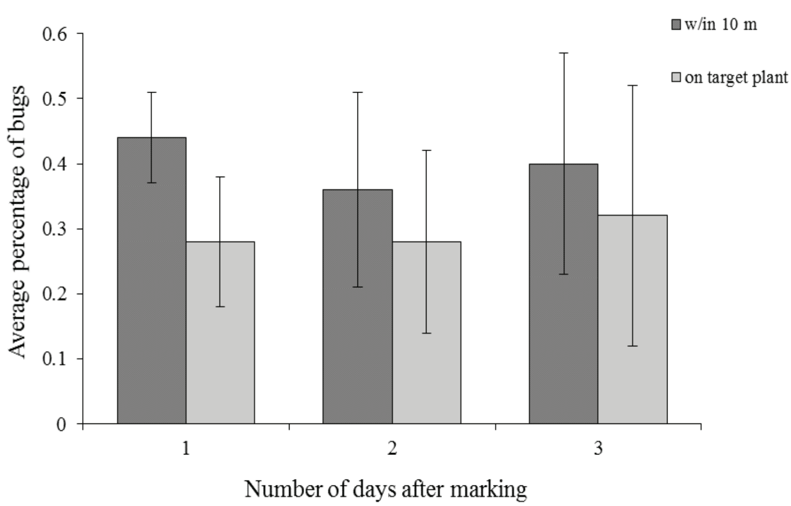

Figure 7. Average proportion of marked *Craspeduchus pulchellus* bugs observed on target plants and within 10 m of target plants (including target plants), with SE bars. For each observation day, N = 5 target plants, n = 5 marked bugs per target plant.

Table 1. Average beak length and maximum beak diameter for *Craspeduchus pulchellus* bugs at five different life history stages, collected on *Corchorus hirsutus*.

Life History Stage	Average Beak Length ± SE (mm)	Average Maximum Beak Diameter ± SE (mm)	Sample Size
Mature adult	3.25 ± 0.00	0.100 ± 0.000	2
5^{th} instar	2.92 ± 0.08	0.100 ± 0.000	3
4^{th} instar	2.50 ± 0.14	0.100 ± 0.000	3
3^{rd} instar	1.83 ± 0.08	0.092 ± 0.008	3
2^{nd} instar	1.50	0.050	1

adults feeding on C. *hirsutus* were 11-22% longer than those reported for bugs feeding on *C. siliquosus* in south Florida (Baranowski and Slater 1975), with the percent difference increasing as the insects age. *Craspeduchus pulchellus* individuals do not forage over large areas, so those feeding on *C. hirsutus* in coastal plant communities would not encounter bugs feeding on *C. siliquosus* because the two plant species are found in different communities on San Salvador. If insect populations feeding on *C. hirsutus* are reproductively isolated from those feeding on *C. siliquosus*, then we predict that the difference in fruit morphology would lead to differences in insect beak length due to natural selection. Additional work in multiple populations is necessary to rigorously test this hypothesis.

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