Effect of Food Restriction on Interaction with Opposite Sex in Male Milkweed Bugs,   
*Oncopeltus fasciatus*

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Abstract

Milkweed bugs have been used in research since the evo-devo revolution thanks to their short life span and manipulability. The current study questioned whether food restriction has an effect on interactions with the opposite sex. The research hypothesis was that food-restricted males would have a shorter duration of time interacting with females. Male milkweed bugs were divided into control and experimental conditions, with the food restriction enforced by removing food from Wednesday afternoon until the following Monday afternoon. To measure interactions with the opposite sex, a male and female were haphazardly selected from the appropriate conditions and placed in a 10cm diameter Tupperware container. For three minutes the duration of time in direct physical contact was recorded. Forty-five trials were conducted for each condition over a span of eight days. A nonparametric Wilcoxon Ranked Sums Test was run to determine if there was a difference in interaction duration between the control and food-restricted conditions. There was a highly significant difference in duration, indicating that males in the food-restricted condition spent a shorter duration of time interacting with the females. Further research is needed to determine the causes behind this behavioral difference.

Keywords: Milkweed, *Oncopeltus fasciatus*, feeding, food restriction, interaction, mating

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Native to temperate areas of North America, the milkweed bug (*Oncopeltus fasciatus*) has been studied to enhance entomological understandings in a number of areas since the evo-devo revolution began in the 20th century. It has been influential in understanding development in arthropods and used to consolidate evolutionary theories (Chipman, 2017). The current study seeks to prove its worth in relation to feeding and mating behaviors in an evolutionary context, by showing differentiated behaviors when resources and therefore energy are limited.

Adult milkweed bugs are being used in the current study due to their sexual maturity and developed markings. Six weeks elapse over which the insects pass through five instars before reaching adulthood, which lasts for an additional month (Missouri Botanical Garden, n.d.). Due to the fact that abdominal markings are present in adults, sex determination is more easily achieved. Females have a black stripe and two black dots, while males have two black stripes (Rea et al, 2003).

Young female milkweed bugs are sexually receptive on their third day, and males become sexually active on their fifth day (Loher et al. 1968). This activity occurs regardless of sexual maturity. Mature milkweed bugs copulate multiple times per day at varying durations. Isolation may increase male sexual activity if for one to two days (Loher et al, 1968). Mating in milkweed bugs has been shown to differ based on alteration of light and temperature. The maximum activity for both feeding and mating occurred eight hours after light was turned on (Caldwell & Dingle, 1967). This indicates that the present study should evaluate such behaviors in the afternoon hours.

Feir et al (1963) studied feeding behavior in milkweed bugs, defining the mechanisms and detection of food sources. Food detection is due to “sensory input from chemoreceptors in the stylus” (Feir et al, 1963, pp. 224). They stop feeding as a result of lowered salivary intake, and drink water after feeding. The present study provides a constant supply of water for ad libitum salivary intake. In studies of parental provisioning of food, food availability was limited and parental care was observed. The parents’ behavior of provisioning food was not affected by food availability but by the begging of the larvae (Smiseth and Moore, 2002). This indicates that food availability may not be as potent an indicator as social cues when it comes to fulfilling a biological need.

In relation to sharing a food resource, researchers at the University of Edinburgh found that partners will match behavior in consumption, with males compensating for size differences (Pilakouta et al., 2016). Water and its effects in copulation and fitness was also recently studied at Australia National University. It has been shown that water availability to females through male ejaculate does not significantly affect the fitness cost of the activity in seed beetles (Iglesias-Carrasco, et al 2018). Taken together, these previous studies confound the question of the costs and benefits of attaining biological needs.

The current study is important because the relationship between a manipulation of food availability and interactions between the opposite sex has been understudied. The energetic reactions to limited food may indicate priorities in energy reserve expenditure if the milkweed bugs spend more time in one activity as compared to another; if the animals spend the same amount of time copulating, this indicates it is a high priority activity. The results can also indicate how often the bugs need to be fed in a laboratory setting, if copulation were to be a measure of fitness in the animal. The primary research question was concerned with how food availability affects interactions with the opposite sex in *Oncopeltus fasciatus*. The null hypothesis stated that there would be no difference between the interactions of control and restricted milkweed bug males with the opposite sex. The alternative hypothesis predicted that there would be a difference between the interactions of control and experimental milkweed bugs with the opposite sex.

The current procedure involved two conditions, one of which included feeding ad libitum, with food in constant supply. The other condition involved food restriction with continued access to water. In this case, food was available for a limited time, followed by removal of the food for the remainder of the week. In these separate conditions, I hypothesized that those without a constant supply of food would conserve their energy more and have limited interaction with the opposite sex, as measured in duration of time in direct contact.

**Methods**

**Procedure**

One hundred milkweed bugs (*Oncopeltus fasciatus*) were obtained through Carolina Biological Supply Company. Two 7.5 gallon tanks and one 2.5 gallon tank were used as stock tanks for each condition. In each 7.5 gallon tank, a sponge soaked in water was placed inside along with a petri dish of sunflower seeds.  The sides of the tanks were labeled “constant male” and “constant female” with masking tape to indicate the food condition. These tanks were covered with a wire mesh top for airflow and paper was affixed to the underside to prevent the escape of baby milkweed bugs. The 2.5 gallon tank was labeled with “restricted males” and given cotton-soaked dental wicks and a petri dish of sunflower seeds during the feeding period.

The control condition contained a great number of milkweed bugs, in excess of twenty males and twenty females, who had food in constant supply and whose feeding was ad libitum. The experimental condition contained twenty male milkweed bugs that were fed from Monday afternoon at 2 p.m. until Wednesday afternoon at 2 p.m., amounting to forty-eight hours each week with food available. The milkweed bugs were tested on Monday, Wednesday, and Friday of one week after the initial food restriction began, as well as the following Monday.

The testing arena consisted of a white opaque Tupperware container that was 10cm in diameter. One male and one female from the appropriate conditions were haphazardly selected. This selection was used due to the great numbers in each condition as well as the death which made it improbable to account for the same milkweed bugs for each day of testing. Each was placed in the testing arena, after which a timer was set for three minutes. The duration of physical contact between the animals was timed using a stopwatch.

The procedure was repeated with each pair in the food restricted and control conditions, for a total of forty-five trials per condition.  The data was analyzed using a Wilcoxon Ranked Sums Test for the duration of time in direct contact with the opposite sex between male conditions. A pairwise comparison was then conducted to determine the significance of the day of testing on mean durations.

**Results**

The data was analyzed using JMP statistical software. A nonparametric Wilcoxon/Kruskal-Wallis Ranked Sums Test was conducted to determine whether there was a difference in duration of interactions with females between control and food-restricted males. The test indicated that control males interacted with females for a greater duration of time compared to food-restricted males (Q=11.91, p=0.0006), as shown in Figure 1. With this significance, I next ran a pair-wise test to see if there was a difference by day. The nonparametric comparisons resulted in a significant value, indicating that the interactions differed between days (Q=9.57, p=0.023). When viewed over the eight-day span of testing, the duration of interactions significantly differed between Wednesday and Friday (p=0.031) and between Friday and the following Monday (p=0.0093) as indicated in Figure 2.

**Discussion**

I hypothesized that when control and food-restricted male milkweed bugs were paired with females, food-restricted males would spend a shorter duration of time interacting (as measured by duration of direct physical contact). Over an eight-day span of testing (M/W/F/M), the results supported this hypothesis. Food-restricted males spent a significantly lower duration of time interacting with females (Figure 1). The data also indicated that there was a difference by day when data was collapsed across conditions (Figure 2).

Adult milkweed bugs have a lifespan of approximately 30 days to six weeks (SOURCE), so they may become less active or reduce the energy spent on activities not necessary for sustaining life, such as potential procreation by interacting with the opposite sex. In many invertebrate species, the male approaches the female to copulate SOURCE. During several interactions with the food-restricted males, however, the female ran laps around the inside of the Tupperware rim, avoiding the male entirely. This behavior was not replicated with control males, indicating a female preference for the control males.

One of the largest considerations for limitations to the study was the number of milkweed bugs in each condition. The rate of death was not equal across conditions, such that by the end of all trials there were fewer than five females, close to five control males and eighteen food restricted males. This limited number of females to be selected from for each trial meant that the same individuals were experimented with. This may have contributed to any resulting decrease in interaction.

Significant results in rejection of the null hypothesis lead to further questions as to the cause of these behavioral differences. While the original prediction posited that conservation of energy levels may motivate a male milkweed bug to spend less time interacting with females or engaging in activities nonessential to survival, the difference cannot be attributed to energy without further research. A manipulation of energy levels in further studies could support this prediction, such as by augmentation of physical activity.

Indications of a quality mate are evolutionarily adapted to promote genetic pairings for the survival of the species. These indications may come in coloration of chemical cues, known as pheromones, which attract mates. According to Nishida (2014), insects that feed on plants incorporate allelochemicals intended to ward off predation by herbivores into their own chemical signaling. The phytochemicals can be incorporated into sex pheromones and are used in conjunction with Juvenile Hormone (JH) for growth regulation in milkweed bugs (Nishida, 2014). The suppression of these chemicals by decreased availability through food sources may lead to limited output by the food-restricted males. This may serve as an indication to females that the food-restricted males are of lower quality either by reduced sex pheromone presence of by insufficient growth. Additional studies should measure variations in pheromone output depending on feeding (by the amount of food or how often males are fed) and see how this affects mating interactions.

The observation of females ignoring the food-restricted males by running laps around the rim of the container indicated that the females have an element of choice in mating interactions. While in most control male interactions the males initiated contact, this distinctive female behavior during several experimental trials alludes to an element of the female’s willingness or approval in mate choice to allow the interaction to take place. Further studies could explore this female preference for the quality of a male mate by manipulating feeding and other health factors. Female behavior in studies of mating interaction should be carefully recorded.

Food-restricted male milkweed bugs interacted with females for a shorter duration of time than did control males. Should this behavior be seen in related insects of Hemiptera, there may be ecological implications. When food resources are low, there is less food to be consumed and these indicators by way of potential pheromone changes may lead to lower levels of procreation and therefore smaller populations of the insects. When crops suffer, the bottom of the food chain will also be depleted, causing biological implications further up.

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Figures

Figure 1. The mean duration of interaction with females (in seconds) is displayed for each condition of milkweed bug males over forty-five trials of three minutes each. The difference in duration of time spent interacting with females by males in the two conditions was significant as indicated by a nonparametric Wilcoxon Ranked Sums Test (*Q*=11.91, *p*=0.0006).

Figure 2. Collapsed across conditions, the mean duration of time spent interacting with the opposite sex of milkweed bug males in three-minute trials is displayed by the day of testing. A comparison for each pair indicated significance using the nonparametric Wilcoxon method, *p*=0.023. The mean for Wednesday differed significantly from Friday (*p*=0.031) and the mean for Friday also differed significantly compared to the second Monday (*p*=0.0093).