

## Sex-Specific Interactions of Microbial Symbioses on Cricket Dietary Selection

RYAN B. SCHMID,<sup>1,2</sup> R. MICHAEL LEHMAN,<sup>2</sup> AND JONATHAN G. LUNDGREN<sup>2,3</sup>

Environ. Entomol. 43(4): 896–902 (2014); DOI: <http://dx.doi.org/10.1603/EN13311>

**ABSTRACT** The nutrients found in prey and nonprey foods, and relative digestibility of these foods, has a major influence on diet selection by omnivorous insects. Many insects have developed symbiotic relationships with gut bacteria to help with extracting nutrition from nonprey diets. *Gryllus pennsylvanicus* (Burmeister) (Orthoptera: Gryllidae) was assigned to one of two treatment groups, antibiotic-treated and nonantibiotic-treated, and consumption of seeds (nonprey) and eggs (prey) were measured. Male crickets administered antibiotics consumed more seeds and greater seed weight, while antibiotic-fed female crickets consumed fewer seeds and less seed weight, relative to the untreated male and female crickets, respectively. Both male and female antibiotic-treated crickets consumed similar weight of eggs as nonantibiotic-treated male and female crickets, respectively. These results provide evidence that gut symbionts influence diet selection of male and female *G. pennsylvanicus* differently. This sex-specific dietary selection may be because of the fact that male and female crickets have different nutritional requirements.

**KEY WORDS** *Gryllus pennsylvanicus*, *Chenopodium album*, *Ephestia kuehniella*, nutrition, bacteria

Many insects are classified as omnivores and consume nonprey foods as well as prey (Slansky and Rodriguez 1987, Coll and Guershon 2002, Lundgren 2009). It is often assumed that most prey consumed by predators provide the insects with the required nutrients to survive, and it is true that nutrients received from prey diets promote many physiological functions such as maturation, fecundity, development, and reproduction (Reznik and Vaghina 2013, Rodrigues et al. 2013). However, nonprey foods can be equally critical to a predatory insect's physiology and behavior, affecting many life history parameters such as reproduction, dispersal, diapause, and demographics (Lundgren 2009). Nonprey foods can be important food sources not only when prey is limited but also when prey is plentiful. These nonprey food sources are important to an omnivore because nutrients found in nonprey foods such as seeds can often meet or exceed nutrient levels found in insect prey (Lundgren 2009). Extracting nutrients from these nonprey foods poses a unique set of challenges (relative to prey diets) that must be overcome by insects for these nonprey foods to be effectively used as a source of nutrients. Symbionts are one mechanism by which insects can exploit the nutrition from nonprey foods.

Microbial symbionts expand the dietary breadth of a host using numerous mechanisms, from the aiding in breakdown of food which the host cannot to synthe-

sizing essential amino acids or vitamins that are not found in the host's diet (Kaufman and Klug 1991, Feldhaar et al. 2007, Pais et al. 2008, Hosokawa et al. 2010). The production of enzymes to digest otherwise indigestible food is a common way that symbionts aid in extracting nutrients from nonprey diets (Kukor and Martin 1983, Sinsabaugh et al. 1985, Kukor et al. 1988, Shi et al. 2013, Takasuka et al. 2013). Gut symbionts provide a plethora of nutritional benefits for insects, which include production of vitamins and sterols, processing of foods, and synthesis of essential nutrients (Douglas 2009). All of these aforementioned forms of nutritional upgrading provided by microbial symbionts allow a variety of hosts, from animals to plants, to gather and consume an expanded diet (Belanger et al. 2013, Mortimer et al. 2013, Zindel et al. 2013).

Differences in the physiology and behavior of different sexes has frequently led to sex-specific animal diets. For example, female insects require a source of proteins and lipids to realize their reproductive potential. *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) females consumed 10 times more pollen under field conditions than male beetles (Lundgren et al. 2005b). Female parasitoid wasps frequently host feed as a way to support egg production, whereas male wasps are exclusively glucophagous (Heimpel and Collier 1996). Similarly, male mosquitoes are glucophagous, and females consume blood to produce eggs (Clements 1992). In butterflies, males more frequently display "puddling" behavior, consuming salts that can be provided to females as a nuptial gift or possibly because of being excluded from floral nectar by females (Boggs and Jackson 1991). Although com-

<sup>1</sup> Department of Biology and Microbiology, South Dakota State University, Brookings, SD 57007.

<sup>2</sup> USDA-ARS, North Central Agricultural Research Laboratory, Brookings, SD, 57006.

<sup>3</sup> Corresponding author, e-mail: [Jonathan.Lundgren@ars.usda.gov](mailto:Jonathan.Lundgren@ars.usda.gov).

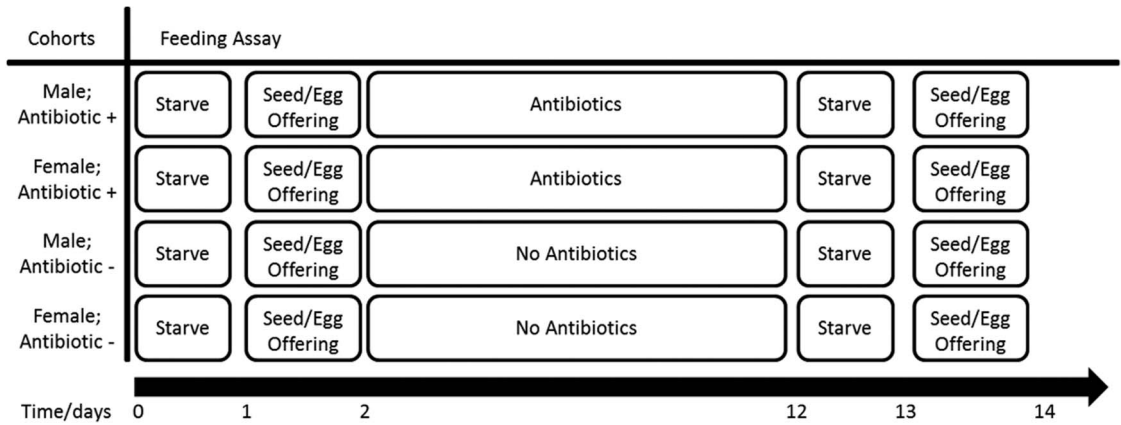


Fig. 1. Diagram outlining the treatment structure of the feeding assay.

monly recognized, the role of symbionts in the sex-specific dietary selection by animals has not been previously examined.

*Gryllus pennsylvanicus* (Burmeister) (Orthoptera: Gryllidae) is an omnivorous field cricket which consumes both prey and nonprey diets (O'Rourke et al. 2006, Lundgren and Harwood 2012). *G. pennsylvanicus* and other crickets have been shown to be hosts of several bacteria, including a diverse gut microbial community (Kaufman and Klug 1991, Giordano et al. 1997, Santo Domingo et al. 1998). The relationship developed between a host insect and a microbial symbiont has helped to expand the host diet in many cases. It has been shown that elimination of certain gut symbionts affects the amount of a specific food that is consumed by the host but typically only one diet is offered during these feeding assays (Lundgren and Lehman 2010, Schmid et al. 2014). We hypothesized that antibiotics would 1) reduce the gut bacterial community of *G. pennsylvanicus*, and 2) that this reduction would affect seed consumption rates. In observing the resulting data, it was quickly realized that male and female insects had a dramatically different feeding response when their bacterial symbionts were reduced that will have important implications for future research on symbiont-host interactions.

### Materials and Methods

**Crickets.** Crickets (F1 generation of a laboratory-reared colony collected locally, Brookings, SD) were kept at 27°C and a photoperiod of 16:8 (L:D) h. They were reared in 28 by 30 cm cages with aluminum wire mesh sides (Charcoal Aluminum Screening Vista Weave Standard Mesh Phifer Wire Products Inc., Tuscaloosa, AL), *n* = 50 crickets per cage. *G. pennsylvanicus* were fed a diverse diet throughout the rearing process (Appendix 1). *G. pennsylvanicus* selected for the assay lacked any visible signs of external physical damage.

**Feeding Assay.** As seen in Fig. 1, *G. pennsylvanicus* (*n* = 60 male and 60 female) were placed individually into clean plastic petri dishes containing only a water-

saturated cotton wick. The crickets were starved for 24 h at 27°C, after which they were weighed to the nearest 0.01 mg and placed into clean petri dishes containing lambsquarter (*Chenopodium album* L., Caryophyllales: Amaranthaceae) seeds and *Ephesthia kuehniella* (Zeller) (Lepidoptera, Phycitidae) eggs. The seeds and eggs were simultaneously presented affixed (with double-sided tape) on separate sticky note papers that were affixed to the bottom of a petri dish; this allowed the paper and food to be removed to weigh the seeds and eggs independently. Nonsterile silicon sand covered the exposed areas of tape to prevent the insects from becoming entrapped. Seed dishes each contained 50 undamaged lambsquarter seeds, ≈0.042 g of uninspected lambsquarter seeds in addition to the 50 undamaged seeds, and 0.062 g of *E. kuehniella* eggs; these quantities were found to represent more food than the crickets would typically eat in a 24-h period. *G. pennsylvanicus* were exposed to the seeds and eggs simultaneously for 24 h inside the petri dish, after which the insects were weighed and placed into petri dishes containing a water-saturated cotton wick and diet (Lundgren et al. 2005a). One cohort (*n* = 30 males and 30 females each) received a diet containing the antibiotics erythromycin, tetracycline, and rifampicin, and the fungicide sorbic acid for 10 d, while the other cohort (*n* = 30 males and 30 females) were given the same diet without antibiotics for 10 d. During this 10-d period, water wicks were refilled and diet was replaced daily. The crickets were placed in new sterile petri dishes every 3 d. *G. pennsylvanicus* were starved for 24 h again, weighed, and were placed into sterile petri dishes containing lambsquarter seeds and *E. kuehniella* eggs as previously described. After 24 h, the crickets were weighed. The seeds and eggs were weighed again to determine the amount eaten by each cricket. Pretreatment (before crickets were separated into antibiotic- or nonantibiotic-fed cohorts) number of seeds consumed, weight of seeds consumed, and weight of eggs consumed were equivalent between cohorts fed antibiotics or not, as determined using a two-way analysis of variance (ANOVA) to demonstrate equality between sex and

antibiotic treatments. Males consumed significantly less seed and egg weight than females (seed weight:  $F_{1, 116} = 7.43$ ,  $P = 0.01$ ; egg weight:  $F_{1, 116} = 16.75$ ,  $P < 0.001$ ).

**Examination of *G. pennsylvanicus* Gut Microbiota.** Ten crickets from each of the treatment groups of both the male and female crickets had their guts aseptically dissected and frozen at  $-20^{\circ}\text{C}$  in  $1 \times \text{PBS}$  immediately after completion of the feeding assay. DNA was extracted from cricket guts using DNeasy Blood and Tissue kit (Catalog No. 69506, Qiagen Sciences, Germantown, MD) as per manufacturer's instructions for gram-positive bacteria. DNA extractions were screened on 0.7% agarose gel (100V for 25 min). The 16S rRNA genes were qPCR-amplified in triplicate using the eubacterial universal primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 518R (5'-ATTACCGCGTCTGCTGG-3'). The reaction, 25  $\mu\text{l}$  total volume, consisted of 12.5  $\mu\text{l}$  SYBR Green qPCR Master Mix (Stratagene, Agilent Technologies Inc., Santa Clara, CA), 8.0  $\mu\text{l}$  of molecular-grade water, 1.5  $\mu\text{l}$  of each 5  $\mu\text{M}$  primer, 0.5  $\mu\text{l}$  of bovine serum albumin, and 1.0  $\mu\text{l}$  of template DNA. The extraction samples were amplified using a Mx3000P qPCR system (Stratagene, Agilent Technologies Inc., Santa Clara, CA) under the following conditions:  $95^{\circ}\text{C}$  for 15 min, followed by 40 cycles of  $95^{\circ}\text{C}$  for 1 min,  $53^{\circ}\text{C}$  for 30 s, and  $72^{\circ}\text{C}$  for 1 min. Fluorescence was recorded at 492 nm during the annealing step of each cycle. A dissociation curve was produced for each reaction by heating the samples to  $95^{\circ}\text{C}$  for 1 min, then cooling to  $55^{\circ}\text{C}$  and then heating up at  $0.2^{\circ}\text{C}/\text{s}$  up to  $95^{\circ}\text{C}$ , and monitoring fluorescence continuously. Threshold cycle ( $C_T$ ) values resulting from qPCR were determined for each cricket sampled and an average of the thrice replicated qPCR  $C_T$  values of each sample was calculated.

**Data Analysis.** Analyses were conducted using two-way ANOVA to reveal potential relationships between cricket sex and antibiotic treatment on seed and egg weight consumed. Upon revealing a significant interaction between these two variables, we resorted to the more conservative Kruskal-Wallis nonparametric ANOVA to compare within-sex patterns in seed consumption. This test has the added benefit of being applicable to nonnormal data distributions and inequality of variances associated with the assumptions of ANOVA. Data from crickets that died during the seed or egg dish feeding were excluded. Weight of seeds consumed, weight of eggs consumed, and number of seeds consumed was adjusted for cricket body mass. Two-way ANOVA (antibiotic status and sex of the crickets were factors in the analysis) were used to compare the effect of antibiotics on the abundance of gut microbiota (as determined using the  $C_T$ ). Statistical significance for  $P$  value was set at  $\alpha = 0.05$ , and a marginally significant  $P$  value was set at  $\alpha = 0.10$ .

## Results

**Effects of Antibiotics on Gut Microbiota.** Antibiotic treatment was effective at lowering the abundance of

gut bacteria in crickets. The antibiotic-treated crickets had higher  $C_T$  values ( $14.24 \pm 0.24$ ) than the nonantibiotic-treated crickets ( $12.81 \pm 0.28$ ; sex:  $F_{1, 36} = 0.05$ ,  $P = 0.82$ ; antibiotic:  $F_{1, 36} = 16.24$ ,  $P < 0.001$ ; sex  $\times$  antibiotic:  $F_{1, 36} = 4.53$ ,  $P = 0.04$ ). The significant interaction is a result of the antibiotic-treated male crickets having a higher  $C_T$  value ( $14.66 \pm 0.32$ ) than the antibiotic-treated female crickets ( $13.83 \pm 0.32$ ). This interaction has no consequence on the interpretations of the results because male and female crickets' seeds and eggs consumption are never compared directly with each other. DNA quantity is inversely related to the  $C_T$ , so crickets not fed antibiotics had more bacteria in their guts than the antibiotic-treated crickets.

**Effects of Antibiotics on Seed Consumption.** Males and females displayed sex-specific reactions to the antibiotic treatment in their consumption rates of seeds and eggs. Male crickets fed antibiotics had an increase in seed consumption, but feeding female crickets antibiotics resulted in decrease in seed consumption. A significant interaction between sex and antibiotic treatment was discovered for the number of seeds consumed (sex:  $F_{1, 112} = 0.21$ ,  $P = 0.65$ ; antibiotic:  $F_{1, 112} = 0.49$ ,  $P = 0.49$ ; sex  $\times$  antibiotic:  $F_{1, 112} = 12.90$ ,  $P < 0.001$ ). A significant interaction between sex and antibiotic treatment was also seen for the weight of seeds consumed (sex:  $F_{1, 112} = 26.90$ ,  $P < 0.001$ ; antibiotic:  $F_{1, 112} = 0.93$ ,  $P = 0.34$ ; sex  $\times$  antibiotic:  $F_{1, 112} = 8.17$ ,  $P = 0.01$ ). The significant interactions indicated that the two sexes reacted differently to the antibiotic treatments in the number and weight of seeds consumed. The effect was a result of the antibiotic-treated males consuming a marginally significant greater weight of seeds ( $0.053 \pm 0.006$ ;  $\chi^2_1 = 3.37$ ;  $P = 0.07$ ) and significantly greater number of seeds ( $32.10 \pm 5.43$ ;  $\chi^2_1 = 10.68$ ;  $P = 0.001$ ) than males not treated with antibiotics (weight of seeds:  $0.036 \pm 0.003$ ; number of seeds:  $10.39 \pm 2.77$ ; Fig. 2). The effect was also a result of the antibiotic-treated females consuming a marginally significant less weight of seeds ( $0.017 \pm 0.003$ ;  $\chi^2_1 = 3.64$ ;  $P = 0.06$ ) and significantly fewer number of seeds ( $11.61 \pm 3.81$ ;  $\chi^2_1 = 5.48$ ;  $P = 0.02$ ) than females not treated with antibiotics (weight of seeds:  $0.025 \pm 0.005$ ; number of seeds:  $26.27 \pm 7.24$ ; Fig. 2).

A significant effect of sex and antibiotic treatment was also discovered for the ratio of weight of seeds to weight of eggs consumed (sex:  $F_{1, 112} = 15.57$ ,  $P < 0.001$ ; antibiotic:  $F_{1, 112} = 0.94$ ,  $P = 0.33$ ; sex  $\times$  antibiotic:  $F_{1, 112} = 3.91$ ,  $P = 0.05$ ). The significant interaction reinforces the idea that the two sexes reacted differently to the antibiotic treatments. A significant effect resulting from antibiotic treatment was not observed in the male crickets ( $\chi^2_1 = 0.47$ ;  $P = 0.50$ ; antibiotic+:  $0.61 \pm 0.08$ ; antibiotic-:  $0.55 \pm 0.07$ ), but a significant effect was seen in the female crickets ( $\chi^2_1 = 7.95$ ;  $P = 0.005$ ). The significant effect observed for the female crickets was caused by the antibiotic-fed treatment having a smaller seed to egg weight consumption ratio ( $0.23 \pm 0.04$ ) than female crickets not fed antibiotics ( $0.42 \pm 0.06$ ) (Fig. 3). No signifi-

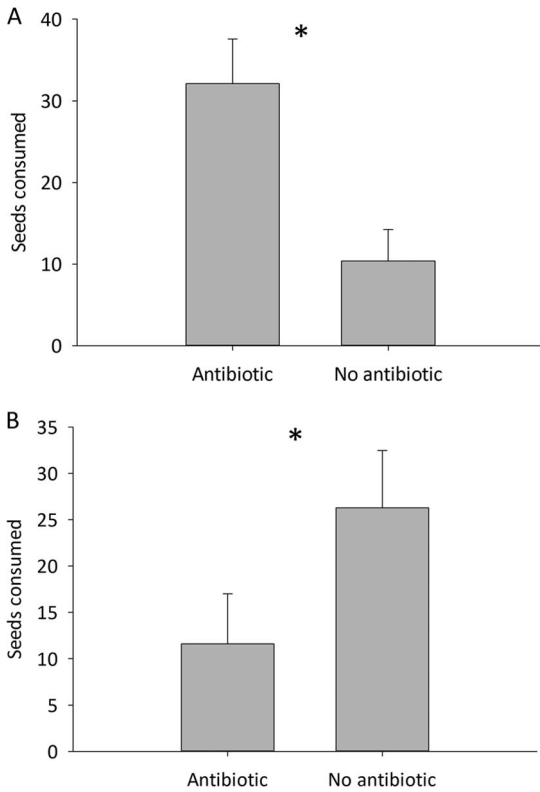


Fig. 2. The effects of antibiotic treatment on consumption by (A) male (antibiotic  $n = 30$ ; no antibiotic  $n = 29$ ) and (B) female (antibiotic  $n = 29$ ; no antibiotic  $n = 28$ ) *G. pennsylvanicus* on number of seeds consumed per individual (male:  $\chi^2_1 = 10.68$ ,  $P = 0.001$ ; female:  $\chi^2_1 = 5.48$ ,  $P = 0.02$ ). The asterisk denotes significant differences within the antibiotic or no antibiotic treatments ( $\alpha = 0.05$ ).

cant effect of sex and antibiotic treatment was observed for weight of eggs consumed (sex:  $F_{1, 112} = 1.72$ ,  $P = 0.19$ ; antibiotic:  $F_{1, 112} = 1.89$ ,  $P = 0.17$ ; sex  $\times$  antibiotic:  $F_{1, 112} = 0.07$ ,  $P = 0.79$ ).

Discussion

The effects of antibiotics lead to sex-specific diet selection by crickets. The results of this feeding assay showed that antibiotic-treated males consumed a greater number and weight of seeds than males not treated with antibiotics. Contrary to males, females treated with antibiotics consumed fewer seeds and less seed weight than females not treated with antibiotics. While antibiotic treatment affected seed consumption, it had no effect on egg consumption in either sex.

Often male and female animals have different nutritional requirements, which lead to sex-specific dietary selection. The varying nutrients found in different foods help to support the sex-specific physiological needs such as egg production, physical development, and metabolic needs of each sex (Katsuki et al. 2012, Raya Rey et al. 2012, Owen et al. 2013). Environmental stresses are another factor that can lead to diet partitioning (Azorit et al. 2012). These environmental stresses can vary greatly according to the species, ranging from lack of food, lack of water, lack of suitable shelter, restraint, and weaning (Tannock and Savage 1974, Bailey and Coe 1999, Buynitsky and Mostofsky 2009). Deer are an excellent example of how environmental stressors can cause selection of sex-specific diets. During late summer, drought often restricts food availability or quality and male deer feed preferentially on lower quality forage, leaving the high quality forage for the

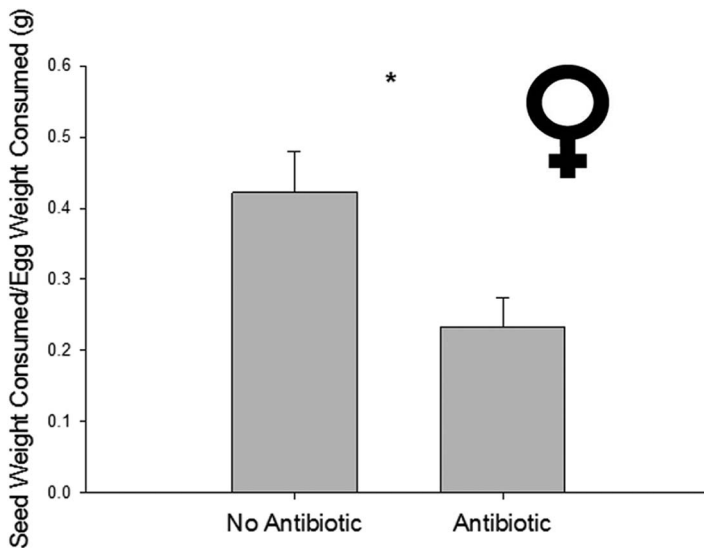


Fig. 3. The effects of antibiotic treatment on weight of seeds consumed per weight of eggs consumed by female *G. pennsylvanicus* per individual. The asterisk denotes significant differences within the antibiotic or no antibiotic treatments ( $\alpha = 0.05$ ). Antibiotic  $n = 29$ ; no antibiotic  $n = 28$ .

females (Miranda et al. 2012). It is interesting to note that the crickets displayed a sex-specific diet selection as a result of antibiotic treatment. Much like the sex-specific reaction in deer caused by environmental stress, antibiotic treatment caused the male crickets to consume a larger proportion of the lower quality seed diet, while the treatment caused females to consume a smaller proportion of the lower quality seed diet (Fig. 3). The question then becomes why did the antibiotic treatment cause sex-specific reactions in the crickets?

Antibiotics reduced the gut microbiota in the treated crickets, and the gut microbial community apparently was related to male and female dietary choices. It should be noted that antibiotics can cause other direct effects on the host including allergic reactions and toxic effects resulting from high dosage concentration, age, or natural susceptibility of the host (Nester et al. 2009, Slonczewski and Foster 2009). These other direct effects from antibiotics are less consistent and more dependent on other factors, which would result in more variability and less effective outcomes. If antibiotics were having a direct physiological effect on the host, then we might have expected general feeding behavior on prey to be reduced, which was observed neither in this study system nor a similar study system involving carabid beetles (Lehman and Lundgren 2010). Antibiotics affecting gut microbial communities related to diet is a much more substantiated claim (Newton et al. 2013). In other animals, gut microbial communities can cause physical distress to their host, as is commonly seen in the cases where gut microbes are related to insulin resistance, obesity, and gastrointestinal tract disorders (Cani et al. 2007, Nester et al. 2009, Slonczewski and Foster 2009, Fei and Zhao 2013). Recent evidence even shows that gut microbiota can affect stress response, anxiety, behavior, and even the central nervous system of an animal (Lyte et al. 1998, Goehler et al. 2008, Rao et al. 2009, Neufeld et al. 2011). These examples in other animals lead credence to the interpretation that disruption of the gut microbial community may have stressed the crickets. This stress may cause diet partitioning that is optimal for the survival of the species (i.e., females consume a smaller proportion of the less nutritious seeds, while the males consume a larger proportion of the less nutritious seeds thus leaving more eggs for the females). As previously stated, this type of behavior has already been documented in other animals; it is interesting to note that similar behavior may be happening in crickets.

This type of behavior, which was likely caused by the gut microbiota, is important to keep in mind when considering where crickets and other insects fit within food webs, and the ecological services that these organisms can provide. As demonstrated by this experiment, gut microbiota is an important factor when considering insect diet selection. By harnessing the effects of insect gut microbiota, many benefits could be gained. For example, insects with certain gut bacteria consume more seeds than insects without those

beneficial bacteria (Lundgren and Lehman 2010, Schmid et al. 2014). These beneficial gut bacteria have the potential to transform insects into a cost-effective natural control agent of weed seeds in agricultural fields. Because of the sex-specific reaction of the crickets to the different diets, which was influenced by the manipulation of the gut microbiota, the sex of the insect is an important factor to remember when analyzing the benefits reaped from insects. The results of this experiment add another layer of understanding to the effects that gut microbiota has on insect behavior or diet selection.

### Acknowledgments

Janet Fergen, Mallory and Megan Wentzel, Mike Bredeson, and Beth Choate helped with data collection and assay procedures. Mention of proprietary products does not constitute endorsement by the U.S. Department of Agriculture.

### References Cited

- Azorit, C., S. Tellado, A. Oya, and J. Moro. 2012. Seasonal and specific diet variations in sympatric red and fallow deer of southern Spain: A preliminary approach to feeding behaviour. *Anim. Prod. Sci.* 52: 720–727.
- Bailey, M. T., and C. L. Coe. 1999. Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys. *Dev. Psychobiol.* 35: 146–155.
- Belanger, P. A., J. P. Bellenger, and S. Roy. 2013. Strong modulation of nutrient distribution in *Alnus glutinosa* as a function of the actinorhizal symbiosis. *Botany-Botanique* 91: 218–224.
- Boggs, C. L., and L. A. Jackson. 1991. Mud puddling by butterflies is not a simple matter. *Ecol. Entomol.* 16: 123–127.
- Buynitsky, T., and D. I. Mostofsky. 2009. Restraint stress in biobehavioral research: Recent developments. *Neurosci. Biobehav. Rev.* 33: 1089–1098.
- Cani, P. D., J. Amar, M. A. Iglesias, M. Poggi, C. Knauf, D. Bastelica, A. M. Neyrinck, F. Fava, K. M. Tuohy, C. Chabo, et al. 2007. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 56: 1761–1772.
- Clements, A. N. 1992. The biology of mosquitoes: Volume 1: Development, nutrition and reproduction. CABI Publishing, Oxon, United Kingdom. p. 532.
- Coll, M., and M. Guershon. 2002. Omnivory in terrestrial arthropods: Mixing plant and prey diets. *Annu. Rev. Entomol.* 47: 267–297.
- Douglas, A. E. 2009. The microbial dimension in insect nutritional ecology. *Funct. Ecol.* 23: 38–47.
- Fei, N., and L. P. Zhao. 2013. An opportunistic pathogen isolated from the gut of an obese human causes obesity in germfree mice. *ISME J.* 7: 880–884.
- Feldhaar, H., J. Straka, M. Krischke, K. Berthold, S. Stoll, M. J. Mueller, and R. Gross. 2007. Nutritional upgrading for omnivorous carpenter ants by the endosymbiont *Blochmannia*. *BMC Biol.* 5: 48.
- Giordano, R., J. J. Jackson, and H. M. Robertson. 1997. The role of *Wolbachia* bacteria in reproductive incompatibilities and hybrid zones of *Diabrotica* beetles and *Gryllus* crickets. *Proc. Natl. Acad. Sci. U.S.A.* 94: 11439–11444.
- Goehler, L. E., S. M. Park, N. Opitz, M. Lyte, and R.P.A. Gaykema. 2008. *Campylobacter jejuni* infection in-

- creases anxiety-like behavior in the holeboard: Possible anatomical substrates for viscerosensory modulation of exploratory behavior. *Brain Behav. Immun.* 22: 354–366.
- Heimpel, G. E., and T. R. Collier. 1996. The evolution of host-feeding behaviour in insect parasitoids. *Biol. Rev.* 71: 373–400.
- Hosokawa, T., R. Koga, Y. Kikuchi, X.-Y. Meng, and T. Fukatsu. 2010. *Wolbachia* as a bacteriocyte-associated nutritional mutualist. *Proc. Natl. Acad. Sci. U.S.A.* 107: 769–774.
- Katsuki, M., Y. Okada, and K. Okada. 2012. Impacts of diet quality on life-history and reproductive traits in male and female armed beetle, *Gnatocerus cornutus*. *Ecol. Entomol.* 37: 463–470.
- Kaufman, M. G., and M. J. Klug. 1991. The contribution of hindgut bacteria to dietary carbohydrate utilization by crickets Orthoptera Gryllidae. *Comp. Biochem. Physiol.* A 98: 117–124.
- Kukor, J. J., and M. M. Martin. 1983. Acquisition of digestive enzymes by Siricid woodwasps from their fungal symbiont. *Science* 220: 1161–1163.
- Kukor, J. J., D. P. Cowan, and M. M. Martin. 1988. The role of ingested fungal enzymes in cellulose digestion in the larvae of Cerambycid beetles. *Physiol. Zool.* 61: 364–371.
- Lehman, R. M., and J. G. Lundgren. 2010. Manipulation of the gut bacterial community in a ground beetle, *Harpalus pensylvanicus*, influences its feeding behavior. *In*, American Society for Microbiology, May 26, 2010, San Diego, CA.
- Lundgren, J. G. 2009. Relationships of natural enemies and non-prey foods, 7 ed. Springer, New York, NY.
- Lundgren, J. G., and M. Lehman. 2010. Bacterial gut symbionts contribute to seed digestion in an omnivorous beetle. *PLoS ONE* 5: e10831.
- Lundgren, J. G., and J. D. Harwood. 2012. Functional responses to food diversity: The effect of seed availability on the feeding behavior of facultative granivores. *J. Entomol. Sci.* 47: 160–176.
- Lundgren, J. G., J. J. Duan, M. S. Paradise, and R. N. Wiedenmann. 2005a. Rearing protocol and life history traits for *Poecilus chalcites* (Coleoptera : Carabidae) in the laboratory. *J. Entomol. Sci.* 40: 126–135.
- Lundgren, J. G., A. Huber, and R. N. Wiedenmann. 2005b. Quantification of consumption of corn pollen by the predator *Coleomegilla maculata* (Coleoptera: Coccinellidae) during anthesis in an Illinois cornfield. *Agric. For. Entomol.* 7: 53–60.
- Lyte, M., J. J. Varcoe, and M. T. Bailey. 1998. Anxiogenic effect of subclinical bacterial infection in mice in the absence of overt immune activation. *Physiol. Behav.* 65: 63–68.
- Miranda, M., M. Sicilia, J. Bartolome, E. Molina-Alcaide, L. Galvez-Bravo, and J. Cassinello. 2012. Foraging sexual segregation in a Mediterranean environment: Summer drought modulates sex-specific resource selection. *J. Arid Environ.* 85: 97–104.
- Mortimer, P. E., M. R. Le Roux, M. A. Perez-Fernandez, V. A. Benedetto, A. Kleinert, J. C. Xu, and A. J. Valentine. 2013. The dual symbiosis between arbuscular mycorrhiza and nitrogen fixing bacteria benefits the growth and nutrition of the woody invasive legume *Acacia cyclops* under nutrient limiting conditions. *Plant Soil* 366: 229–241.
- Nester, E. W., D. G. Anderson, C. E. Roberts Jr., and M. T. Nester. 2009. Digestive system infections, pp. 581–617. *In* Microbiology: A human perspective, 6 ed. McGraw-Hill, New York, NY.
- Neufeld, K.-A.M., N. Kang, J. Bienenstock, and J. A. Foster. 2011. Effects of intestinal microbiota on anxiety-like behavior. *Commun. Integr. Biol.* 4: 492–494.
- Newton, D. F., S. Macfarlane, and G. T. Macfarlane. 2013. Effects of antibiotics on bacterial species composition and metabolic activities in chemostats containing defined populations of human gut microorganisms. *Antimicrob. Agents Chemother.* 57: 2016–2025.
- O'Rourke, M. E., A. H. Heggenstaller, M. Liebman, and M. E. Rice. 2006. Post-dispersal weed seed predation by invertebrates in conventional and low-external-input crop rotation systems. *Agric. Ecosyst. Environ.* 116: 280–288.
- Owen, E., F. Daunt, C. Moffat, D. A. Elston, S. Wanless, and P. Thompson. 2013. Analysis of fatty acids and fatty alcohols reveals seasonal and sex-specific changes in the diets of seabirds. *Mar. Biol.* 160: 987–999.
- Pais, R., C. Lohs, Y. Wu, J. Wang, and S. Aksoy. 2008. The obligate mutualist *Wigglesworthia glossinidia* influences reproduction, digestion, and immunity processes of its host, the Tsetse fly. *Appl. Environ. Microbiol.* 74: 5965–5974.
- Rao, S., R. Srinivasjois, and S. Patole. 2009. Prebiotic supplementation in full-term neonates - A systematic review of randomized controlled trials. *Arch. Pediatr. Adolesc. Med.* 163: 755–764.
- Raya Rey, A., M. Polito, D. Archuby, and N. Coria. 2012. Stable isotopes identify age- and sex-specific dietary partitioning and foraging habitat segregation in southern giant petrels breeding in Antarctica and southern Patagonia. *Mar. Biol.* 159: 1317–1326.
- Reznik, S. Y., and N. P. Vaghina. 2013. Effects of photoperiod and diet on diapause tendency, maturation and fecundity in *Harmonia axyridis* (Coleoptera: Coccinellidae). *J. Appl. Entomol.* 137: 452–461.
- Rodrigues, A.R.D., A. F. Spindola, J.E.D. Oliveira, and J. B. Torres. 2013. Dietary effects upon biological performance and lambda-dacyhalothrin susceptibility in the multicolored Asian lady beetle, *Harmonia axyridis*. *Phytoparasitica* 41: 285–294.
- Santo Domingo, J. M., M. G. Kaufman, M. J. Klug, J. M. Tiedje. 1998. Characterization of the cricket hindgut microbiota with fluorescently labeled rRNA-targeted oligonucleotide probes. *Appl. Environ. Microbiol.* 64: 752–755.
- Schmid, R. B., R. M. Lehman, V. S. Brözel, & J. G. Lundgren. 2014. An indigenous gut bacterium, *Enterococcus faecalis* (Lactobacillales: Enterococaceae), increases seed consumption by *Harpalus pensylvanicus* (Coleoptera: Carabidae). *Fla. Entomol.* 97: 575–584.
- Shi, W. B., S. X. Xie, X. Y. Chen, S. Sun, X. Zhou, L. T. Liu, P. Gao, N. C. Kyrpides, E. G. No, and J. S. Yuan. 2013. Comparative genomic analysis of the endosymbionts of herbivorous insects reveals eco-environmental adaptations: Biotechnology applications. *PLoS Genet.* 9: e1003131.
- Sinsabaugh, R. L., A. E. Linkins, and E. F. Benfield. 1985. Cellulose digestion and assimilation by three leaf-shredding aquatic insects. *Ecology* 66: 1464–1471.
- Slansky, F., and J. G. Rodriguez. 1987. Nutritional ecology of insects, mites, spiders, and related invertebrates, Wiley, New York, NY.
- Slonczewski, J. L., and J. W. Foster. 2009. Microbial pathogenesis, pp. 937–978, *Microbiology: An evolving science*, 1 ed. W. W. Norton and Company, Inc., New York, NY.
- Takasuka, T. E., A. J. Book, G. R. Lewin, C. R. Currie, and B. G. Fox. 2013. Aerobic deconstruction of cellulose biomass by an insect-associated *Streptomyces*. *Cell. Rep.* 3: 1030.

- Tannock, G. W., and D. C. Savage. 1974. Influences of dietary and environmental stress on microbial populations in the murine gastrointestinal tract. *Infect. Immun.* 9: 591–598.
- Zindel, R., M. Ofek, D. Minz, E. Palevsky, E. Zchori-Fein, and A. Aebi. 2013. The role of the bacterial community in the nutritional ecology of the bulb mite *Rhizoglyphus robini* (Acari: Astigmata: Acaridae). *FASEB J.* 27: 1488–1497.

*Received 1 November 2013; accepted 29 April 2014.*

---

**Appendix 1.** The diet fed to *G. pennsylvanicus* during rearing

---

Ground Oatmeal: 1 part (by wt)  
Ground Cat Food: 1 part  
Dry Milk: 1 part  
In a separate mixture:  
Ground Cat Food: 2 parts (by wt)  
Ground Cichlid Food: 2 parts  
Giant Foxtail Seed: 1 part  
Pigweed Seed: 1 part  
Crabgrass Seed: 1 part  
Lambsquarter Seed: 1 part  
Green Foxtail Seed: 1 part

---

Mixed together until ingredients were evenly distributed amongst each other.