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Prey Species Influences Culturable Gut Symbionts of a Generalist Predator

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Introduction

Microbial symbionts, in particular those living in the guts of animals, play essential roles in animal ecology, physiology, and evolution. Communities of gut symbionts in insects can influence social interactions, interactions with entomopathogens, and the ability to successfully develop on host plants (reviewed in Engel and Moran 2013; Hansen and Moran 2014). Gut symbiont community structure can be highly variable within species, with variation found among ontogenetic stages (Hammer et al. 2013) and individuals feeding on different diets (Broderick et al. 2004; Hammer et al. 2013). Different midgut conditions as a result of the presence of different nutrients and allelochemicals in the gut is one potential explanation for the among-diets variation in gut microbe communities.

Predatory arthropods are vital members of terrestrial ecosystems and can provide a number of valuable ecosystem services (Kremen and Chaplin-Kramer 2007; Losey and Vaughan 2006; Symondson et al. 2002). Generalist predators can consume a variety of prey species, and therefore an individual may consume both high-quality and low-quality prey during its lifetime. Feeding on low-quality prey such as those that are toxic may cause deleterious effects in the predator such as delayed development and reduced longevity, body size, or fecundity (Ode 2006).

The different types of gut microbes in predatory insects may influence or be influenced by the predatory hosts' diets. Gut microbe communities may mediate the interactions between the insect and its diet, allowing it to digest specific food items. In contrast, the gut microbe communities may be affected by the predator's intrinsic physiological response to eating different prey species. While the differences in gut communities between herbivorous and carnivorous insects has been tested (e.g., Colman et al. 2012), there has been little research done on the effects of different prey species on gut microbes of predatory arthropods.

Podisus maculiventris (Hemiptera: Pentatomidae) is a predatory stinkbug that feeds on the bodily fluids of a variety of insects. Prior research with this species has shown that feeding on certain prey species can cause negative effects. For instance, consuming *Junonia coenia* caterpillars which sequester the iridoid glycosides aucubin and catalpol from their host plants has negative effects on *P. maculiventris* growth rate, body size, and feeding behavior compared to feeding on *Vanessa cardui* caterpillars (Strohmeyer et al. 1998).

Our objective in this study was to determine whether feeding on different prey species could affect gut microbes of a predatory arthropod. We hypothesized that different groups of microbes could be cultured from the guts of predators given two caterpillar prey species that differed in nutritional quality. In order to test this hypothesis, we provided *P. maculiventris* nymphs with two different caterpillar species, one which is known to be a prey species and another which is not.

Materials and Methods

Podisus maculiventris was obtained from a colony maintained for over five generations on a mixture of cabbage loopers (*Trichoplusia ni*; Lepidoptera: Noctuidae), and mealworms (*Tenebrio molitor*; Coleoptera: Tenebrionidae). The *T. ni* colony was reared on an artificial diet (Bio-Serv #9772) while *T. molitor* was reared on oatmeal. First instar *P. maculiventris* nymphs were given water-soaked cotton balls, replaced daily. The second and third instar nymphs were reared on third or fourth instar *T. ni* larvae, also replaced daily. The alternative prey, *Ceratomia*

catalpae, was collected as fourth instar larvae from a single *Catalpa bignonioides* tree in Jackson County, Georgia on 16 October 2014 and kept individually in 118 ml sanitized plastic cups until they voided their guts. *Ceratomia catalpae* consumes the iridoid glycosides catalpol and catalposide produced by its host plants but metabolizes catalposide into catalpol and sequesters catalpol only (Bowers 2003) in its hemolymph. Catalpol is sequestered at concentrations exceeding 25% dry weight (Lampert and Bowers 2015). *Trichoplusia ni* does not sequester catalpol.

For the experiment, ten *P. maculiventris* nymphs were kept on their original diet of *T. ni* larvae while another set of ten was provided *C. catalpae* larvae. Single nymphs were placed with individual caterpillar prey in sanitized 10 X 15 mm Petri dishes. All insects were handled with sanitized forceps, and prey were replaced daily for five days. After five days nymphs were immersed in 95% ethanol for five seconds to remove any microbes on the exterior of the body, then entire nymphs were macerated to obtain gut microbes. Each of the stinkbugs was placed into 5 ml of sterilized 0.5% NaCl saline and macerated with a flame-sterilized glass stir rod. Samples were vortexed to evenly suspend insect parts and microbes. The suspension samples were then streaked onto nutrient agar (BD Diagnostic Systems, catalog no. DF0001-17-0) plates with a sterile cotton swab. The plates were left to incubate for 48 h at 27°C. After incubation, each type of colony on the plates was separately transferred to new nutrient agar plates using a quad-streak method to isolate pure colonies of each. These plates were also incubated for 48 h at 27°C.

After incubation, a pure colony from each different type of microbe was added to a 0.1 ml of distilled water on a glass slide and Gram-stained using Carolina Biological kits (catalog no. 821051). Stains were observed at 1000X magnification. Bacteria were differentiated into morphological types by colony color, cell shape, and Gram stain reaction (positive or negative), but were not identified further. The diversity and types of gut microbes cultured were compared between nymphs reared on the two different prey items.

Results

Three distinguishable morphologies of bacteria colonies, tentatively designated as Bacteria 1-3, were cultured from the *P. maculiventris* nymphs (Table 1). Bacterium 1 was cultured from all 17 nymphs (three nymphs reared on *C. catalpae* were not used because they died during the experiment), and it was the only bacterium cultured from nymphs given *T. ni*. Bacteria 2 and 3 were found only in nymphs given *C. catalpae*, and Bacterium 3 was found only in a single nymph (Table 1).

Table 1. Different microbes cultured from the guts of fourth instar *Podisus maculiventris nymphs* reared on either *Trichoplusia ni* or *Ceratomia catalpae*.

Microbe	Number of nymphs reared on *Trichoplusia ni*	Number of nymphs reared on *Ceratomia catalpae*	Colony color	Cell shape	Gram reaction
Bacterium One	10/10	7/7	White	Coccus	Negative
Bacterium Two	0/10	5/7	Yellow	Coccus	Negative
Bacterium Three	0/10	1/7	Beige	Bacillus	Positive

Feeding on *C. catalpae* also had noticeable physical effects on nymphs. While the nymphs that were given *T. ni* were walking around dishes continuously prior to maceration, all of the nymphs given *C. catalpae* were markedly lethargic. Those nymphs did not move at all unless touched, and then they made only minimal movements to respond.

Discussion

In this study, we found that predatory stinkbugs that fed upon different caterpillar species had different groups of culturable bacteria present in their guts. All nymphs had a dominant morphological type of culturable bacteria in their guts, and bacteria showing those traits were the only bacteria cultured from the guts of nymphs that consumed *T. ni*. Other bacteria could be cultured from the guts of those nymphs that consumed *C. catalpae*. Since all nymphs were kept in the same conditions until the fourth instar, it is likely that these other types of bacteria either colonized during the fourth instar or were suppressed until the nymphs consumed *C. catalpae* hemolymph.

The ability to culture different bacteria in the *P. maculiventris* nymphs that fed on *C. catalpae* may be explained by two potentially non-exclusive hypotheses. First, a substance in the gut that was acquired from feeding on *C. catalpae* may have directly suppressed the ability of either Bacterium 1 or another non-culturable microbe to prevent colonization by other culturable bacteria. For instance, the iridoid glycoside catalpol sequestered at high concentrations by *C. catalpae* (Bowers 2003) has antimicrobial properties against some bacteria (Baden and Dobler 2009). Second, the negative physiological effects of feeding on *C. catalpae* may have indirectly influenced the gut microbe community of the nymphs. The nymphs reared on *C. catalpae* were in a visibly weakened state, and in this state their gut secretions or immune response may have altered the conditions experienced by gut microbes. Further experiments may lend support to one or both of these hypotheses.

Gut microbes may have also exacerbated the negative effects of feeding on *C. catalpae* larvae. *Podisus maculiventris* feeds on hemolymph, and *C. catalpae* hemolymph can contain up to 50% dry weight catalpol (Bowers 2003). Like other iridoid glycosides, catalpol is most pharmacologically active after the glucose has been hydrolyzed by glucosidase enzymes to release the toxic aglycones (Dobler et al. 2011). Microbes in the gut may produce glucosidase enzymes that release aglycones that harm the host while they benefit by using the glucose portion of the compound. Future experiments using *Spilosoma congrua* which can be reared to sequester or not sequester catalpol (Lampert and Bowers 2010) as prey for *P. maculiventris* may show more clearly whether catalpol affects gut microbes.

To our knowledge, this study is among the first to suggest that feeding on two different prey species can influence the culturable gut microbes of predatory insects. Culturing can only capture a partial census of any microbial community (Pace 1997), including that of insect guts (Broderick et al. 2004). While the cultured bacteria did show a measurable response to the different prey species, it is possible that non-culturable microbes may respond in a similar or dissimilar way. Culture-independent censuses may provide further insights to how prey species affect a predator's gut microbes.

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