

2015

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### Recommended Citation

Faulkner, Zachary and Lampert, Evan (2015) "Variation in Culturable Gut Microorganisms of the Catalpa Sphinx Caterpillar (*Ceratomia catalpae*)," *Papers & Publications: Interdisciplinary Journal of Undergraduate Research*: Vol. 4 , Article 16.  
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# Variation in Culturable Gut Microorganisms of the Catalpa Sphinx Caterpillar (*Ceratomia catalpae*)

## **Acknowledgments**

This research was supported by the University of North Georgia Biology Department and a Roberta Williams Teaching Initiative grant. Bill Lott provided assistance locating caterpillars on catalpa trees, and Stephanie Brandys and Brittany Veloce provided technical assistance. Bea Chapman and Brandon Mangum helped pour plates. Dr. James Diggs, Dr. Davison Sangweme, Dr. Jeanelle Morgan, Holly Munro, and Melissa Parks provided feedback to improve earlier drafts of this manuscript.

## Introduction

Microorganisms exist in all ecosystems and are essential to the physiological, behavioral, and ecological interactions of essentially all living species. Insects, like other animals, rely heavily on the microorganisms living in them as mutualistic symbionts for a diverse variety of functions (reviewed in Engel and Moran 2013). For example, gut microorganisms can facilitate the ability of insect groups to acquire novel feeding styles (Russell et al. 2009), and can also provide resistance to infection by pathogens (Brownlie and Johnson 2009). Gut microorganisms may show some similarities among individuals within a species or guild of similar species (Yun et al. 2014), especially in regards to dominant microorganism taxa; however, the types of microorganisms can also be variable among individuals within a species because of factors such as diet, developmental stage, and social interactions (Broderick et al. 2004, Colman et al. 2012, Yun et al. 2014). Because gut microorganisms influence so many aspects of host insect biology, understanding the makeup of these communities has broad importance in such fields as medicine, ecology, and pest control.

Gut microorganisms potentially play an important role in the interactions between insect herbivores and their host plants. Most species of insect herbivores are plant specialists (Bernays and Graham 1988), with physiological systems, including potentially gut flora, that have adapted to feed mostly on a specific plant species or tissue. Gut microbe communities that are specialized based on an herbivore's diet may help herbivores acquire the most benefit from each plant's nutrients (Engel and Moran 2013). Gut microbes may also metabolize or modify the secondary metabolites that plants produce. These reactions may then affect the abilities of herbivores to metabolize these compounds and possibly feed on their host plants. Several species of herbivores sequester plant compounds, either modified or unmodified, to protect them from their own predators (Opitz and Müller 2009). Since plant compounds must cross the wall of the gut as part of their transport to the location where they are sequestered, it is likely that the microbes colonizing the gut play a role in the modification and transport of sequestered plant compounds.

The catalpa sphinx, *Ceratomia catalpae* (Lepidoptera: Sphingidae), is a specialist herbivore that feeds primarily on leaves of catalpa trees (*Catalpa speciosa* and *C. bignonioides*) (Bowers 2003). These caterpillars use two iridoid glycoside compounds produced by the trees, catalpol and its derivative catalposide, as phagostimulants (Nayar and Frankel 1963), and catalpol is sequestered in hemolymph at high concentrations (over 25% dry weight [Lampert et al. 2011, Lampert and Bowers 2015]). Catalpol sequestration can serve as a deterrent to a number of potential invertebrate predators of catalpa sphinx larvae (S. Brandys, unpublished data). Catalpol is excreted in frass (Bowers 2003), suggesting that catalpol that is not sequestered passes completely through the gut without being metabolized.

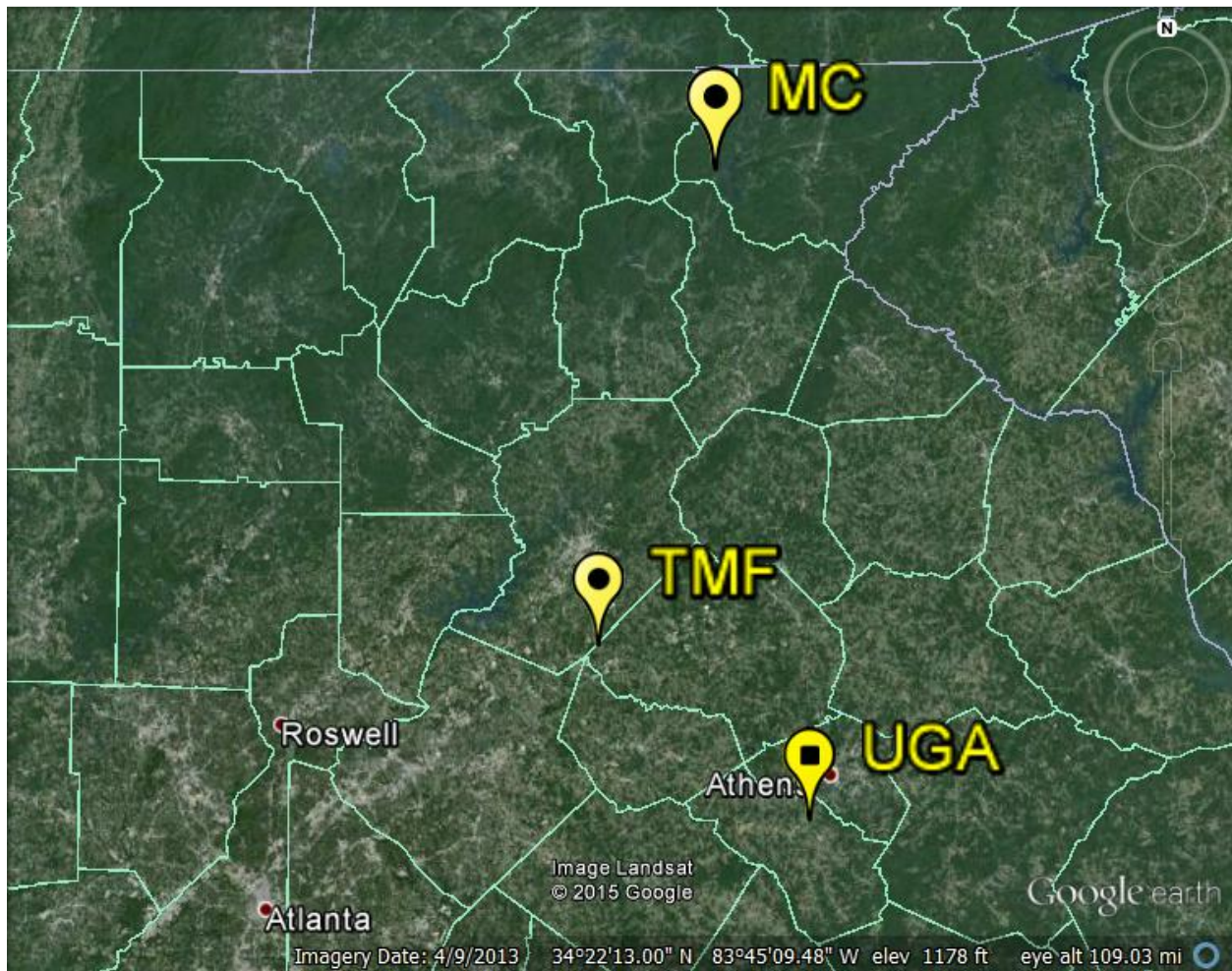
The gut microorganisms of catalpa sphinx larvae may play important roles in the midgut physiological interactions with iridoid glycosides and may contribute to the specialized diet and defensive chemistry of these herbivores. For instance, larvae seem to selectively catabolize catalposide into catalpol without hydrolyzing either into their aglycones (Bowers 2003). Microbes may protect iridoid glycosides from hydrolysis by interfering with glucosidase enzyme expression. Microbes might also influence the ability of catalpol to cross the gut into the hemocoel.

The objective of this research was to survey the culturable gut symbionts from catalpa sphinx larvae. Culturing gut symbionts provides important insights into gut microbe diversity (Broderick et al. 2004) and is a necessary first step in discovering their roles in physiology and

ecology of these caterpillars. We tested the hypothesis that gut microbe communities can vary among host populations or vary seasonally by collecting multiple samples of caterpillars from different populations that spanned two generations in late summer and early fall of 2014.

### Materials and Methods

*Catalpa* sphinx larvae were collected from three populations in three distinct areas in northeast Georgia in fall of 2014 (Figure 1; Table 1). Larvae were collected from Thompson Mills Forest on two separate dates; these larvae represented members of two different generations. Larvae from Moccasin Creek State Park were collected from several *Catalpa bignonioides* trees, while larvae from the other two locations were collected from a single *C. bignonioides* tree at each. All larvae were transported in coolers from the collection sites, then weighed immediately before being placed individually in sanitized plastic cups with leaves from the tree on which they fed in the field. After 24 h, frass was collected from 10 randomly selected larvae from each site to obtain samples of gut microbes.



**Figure 1.** Map of locations from which catalpa sphinx larvae were collected. Abbreviations are as follows: MC – Moccasin Creek State Park, UGA – University of Georgia Horticulture Farm, TMF 1 – Thompson Mills Forest.

**Table 1.** Dates of catalpa sphinx larva collections and number of larvae collected.

Collection Site	Insect Collection Date	Number of Larvae
Moccasin Creek State Park	16 September 2014	21
University of Georgia Horticulture Farm	19 September 2014	1
Thompson Mills Forest	18 September 2014	6
	2 October 2014	10

The collected frass from the individual caterpillars was suspended in 5 ml of sterilized 0.05% NaCl saline in sterile glass test tubes. The samples were vortexed for 30 s to separate and suspend the gut microbes. A sterilized cotton swab dipped into the frass suspension was used to spread the suspension onto nutrient agar Petri plates (BD Diagnostic Systems, catalog no. DF0001-17-0). The inoculated plates were left to incubate for 48 h at 27°C. After incubation, distinct colonies were identified using colony color, elevation, colony shape, and edge shape. Each phenotypically distinct colony was transferred to new nutrient agar plates with flame-sanitized metal loops using a quad streak-plate method to obtain pure colonies. The streaked plates were left to incubate for 48 h at 27°C.

Each colony that grew on these plates was Gram stained using a kit (Carolina Biological #821051). The slides were observed at 1000x magnification to determine cell shape and Gram stain results (Gram-positive cells appeared violet, while Gram-negative cells appeared pink).

## Results

Eighteen bacteria colonies were isolated to obtain pure cultures and Gram stained from the plates inoculated with catalpa sphinx larva frass suspensions. From these, five distinct types of bacteria colony morphologies, designated Bacteria 1-5 (Figure 2, Table 2), were identified. All five morphologies were cultured from larvae collected from Thompson Mills Forest, and two morphologies were cultured only from larvae collected from that location. Bacterium 5 was not cultured from any of the caterpillars collected in September. Both morphologies cultured from larvae collected at the University of Georgia horticulture farm were common to larvae from all three locations.

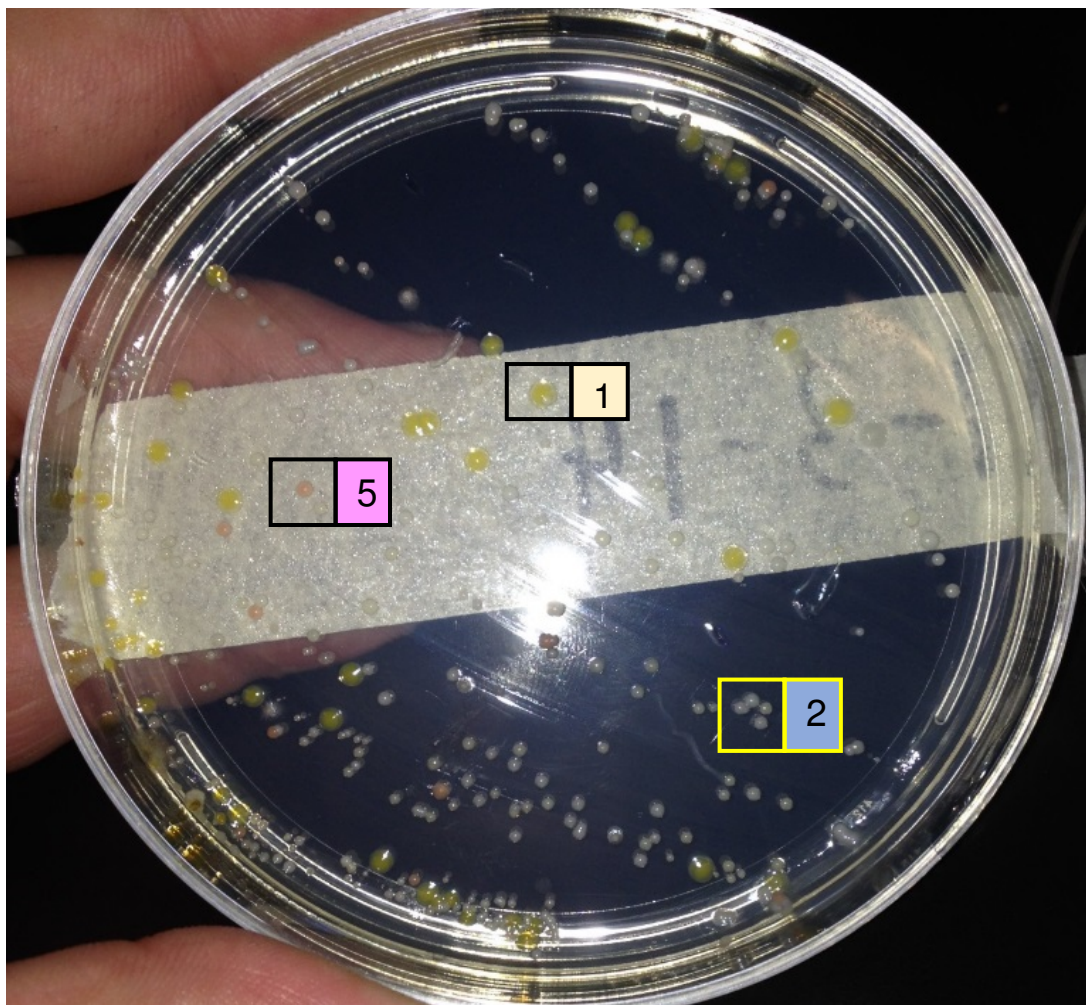
**Table 2.** Locations of the distinct types of bacterial colony morphologies cultured from catalpa sphinx larva frass. Collection site abbreviations are as follows: MC – Moccasin Creek State Park, UGA – University of Georgia Horticulture Farm, TMF 1 – Thompson Mills Forest, September, TMF 2 – Thompson Mills Forest, October.

Bacterium	Insect Collection Site
One	MC, UGA, TMF 1, 2
Two	MC, UGA, TMF 1
Three	TMF 1
Four	MC, TMF 1, TMF 2
Five	TMF 2

Colonies of Bacteria 2 and 3 were indistinguishable but differed in response to the Gram stain. All but one morphology were Gram-negative, and all were coccus-shaped (Table 3).

**Table 3.** Characteristics of the five distinct types of bacterial colony morphologies cultured from catalpa sphinx larva frass.

Bacterium	Colony Color	Colony Shape	Colony Appearance	Colony Elevation	Edge Shape	Gram Reaction	Cell Shape
One	yellow	round	glossy	raised	defined	negative	coccus
Two	white	round	glossy	flat	defined	positive	coccus
Three	white	round	glossy	flat	defined	negative	coccus
Four	eggshell	round	glossy	raised	undefined	negative	coccus
Five	pink	round	dry	raised	defined	negative	coccus



**Figure 2.** Nutrient agar plate showing three of the different bacterial colony morphologies cultured from the gut of a catalpa sphinx collected October 2, 2014 from Thompson Mills Forest. Bacteria are numbered as described in Tables 2 and 3.

## Discussion

This project was the first to examine the gut microorganisms of the catalpa sphinx, a common native caterpillar in the eastern US of interest because of both its harmful effects on ornamental trees (Baerg 1935) and its use as bait for fishing (personal observation). Several distinct bacteria colony morphologies were cultured from the guts of these caterpillars, including morphologies that were found in multiple populations and other morphologies that were specific to different populations and generations of caterpillars. These results are consistent with other studies that find variation in gut microorganisms among individuals within a single species (reviewed by Engel and Moran 2013).

Gut microorganisms have been examined previously in caterpillars, in particular, those of economic or ecological interest (Kingsley 1972, Broderick et al. 2004, Robinson et al. 2010, Hammer et al. 2013). Using both culture-dependent and culture-independent techniques, these studies have found that most caterpillar guts contain relatively few (6-15) different groups of bacteria. Certain groups such as Enterobacteriaceae and Proteobacteriaceae have been found in several different caterpillars. In this study, catalpa sphinx larvae contained comparable numbers of different bacteria types, although no more than three distinct bacterial types were cultured from any individual caterpillar. Bacterium Two showed some of the traits of enterococci; however, we lacked the instrumentation to confirm its identity. Most of the bacteria cultured in this study were Gram-negative, a trait shared by several dominant gut bacteria of caterpillars (Broderick et al. 2004). Further research may identify the colonies to higher taxonomic resolution and may also reveal if some of the colony morphologies represent multiple genetic species.

Bacterium Five was only present in larvae collected in October from Thompson Mills Forest. While catalpa sphinx larvae were collected from the same tree in September and October, the tree had replaced its leaves after being defoliated from the caterpillars present in September, and a subsequent generation of caterpillars had colonized the tree's new growth (personal observation). This bacterial morphology may represent one or more distinct symbionts of the lineage of caterpillars present at Thompson Mills Forest in October 2014, or may represent one or more bacterial taxa that changes in prevalence based on seasonality. Environmental changes such as changing seasons have been shown to influence microbial communities in insect guts (Jia et al. 2013). Future research may reveal if this bacterium can be found in caterpillars from other populations and if this bacterium's presence is influenced by phenological changes in catalpa leaves, the insects, or other factors.

Since many species of bacteria cannot be cultured (Ward et al. 1990), it is likely these larvae contained additional types of microorganisms. Culture-independent techniques such as 454 pyrosequencing or Illumina sequencing of 16S rRNA genes could be used in the future to reveal additional microorganisms.

In conclusion, this study adds to an important and rapidly growing literature base about the microbial communities of insect guts. Future research about the roles of these microorganisms, especially their role in plant-insect interactions, is recommended.

## References

- Baerg W. 1935. Three shade tree insects, II. Great elm leaf beetle, catalpa sphinx, and Eastern tent caterpillar. University of Arkansas Experimental Station Bulletin 317: 1-27.
- Bernays E and Graham M. 1988. On the evolution of host specificity in phytophagous arthropods. *Ecology* 69: 886-892.
- Bowers MD. 2003. Host plant suitability and defensive chemistry of the catalpa sphinx *Ceratomia catalpae* (Lepidoptera: Sphingidae). *Journal of Chemical Ecology* 29: 2359-2367.
- Broderick NA, Raffa KF, Goodman RM, and Handelsman J. 2004. Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture-independent methods. *Applied and Environmental Microbiology* 70: 293-300
- Brownlie JC and Johnson KN. 2009. Symbiont-mediated protection in insect hosts. *Trends in Microbiology* 17: 348-354.
- Colman DR, Toolson EC, and Takacs-Vesbach CD. 2012. Do diet and taxonomy influence insect gut bacterial communities? *Molecular Ecology* 21: 5124-5137.
- Engel P and Moran NA. 2013. The gut microbiota of insects—diversity in structure and function. *FEMS Microbiology Reviews* 37: 699-735.
- Hammer TJ, McMillan WO, and Fierer N. 2013. Metamorphosis of a butterfly-associated bacterial community. *PLoS ONE* 9: e86995.
- Jia S, Zhang X, Zhang G, Yin A, Zhang S, Li F, Wang L, et al. 2013. Seasonally variable intestinal metagenomes of the red palm weevil (*Rhynchophorus ferrugineus*). *Environmental Microbiology* 15: 3020-3029.
- Kingsley VV. 1972. Persistence of intestinal bacteria in the developmental stages of the monarch butterfly (*Danaus plexippus*). *Journal of Invertebrate Pathology* 20: 51-58.
- Lampert EC and Bowers MD. 2015. Incompatibility between plant-derived defensive chemistry and immune response of two sphingid herbivores. *Journal of Chemical Ecology* 41: 85-92.
- Lampert EC, Dyer LA, and Bowers MD. 2011. Chemical defense across three trophic levels: *Catalpa bignonioides*, the caterpillar *Ceratomia catalpae*, and its endoparasitoid *Cotesia congregata*. *Journal of Chemical Ecology* 37: 1063-1070.
- Nayar JK and Frankel G. 1963. The chemical basis of host plant selection in the catalpa sphinx, *Ceratomia catalpae* (Lepidoptera: Sphingidae). *Annals of the Entomological Society of America* 56: 119-122.
- Opitz SEW and Müller C. 2009. Plant chemistry and insect sequestration. *Chemoecology* 19: 117-154.
- Robinson CJ, Schloss P, Ramos Y, Raffa K, and Handelsman J. 2010. Robustness of the bacterial community in the cabbage white butterfly midgut. *Microbial Ecology* 59: 199-211.



- Russell JA, Moreau CS, Goldman-Huertas B, Fujiwara M, Lohman DJ, and Pierce NE. 2009. Bacterial gut symbionts are tightly linked with the evolution of herbivory in ants. *Proceedings of the National Academy of Sciences of the United States of America* 106: 21236-21241.
- Ward DM, Weller R, and Bateson MM. 1990. 16S rRNA reveal numerous uncultured microorganisms in a natural community. *Nature* 345: 63-65.
- Yun JH, Roh SW, Whon TW, Jung MJ, Kim MS, Park DS, Yoon C et al. 2014. Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. *Applied and Environmental Microbiology* 80: 5254-5264.