Effect of Host Tree Species on Cellulase Activity and Bacterial Community Composition in the Gut of Larval Asian Longhorned Beetle

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ABSTRACT *Anoplophora glabripennis*, the Asian longhorned beetle, is a wood-boring insect that can develop in a wide range of healthy deciduous hosts and requires gut microbes to aid in wood degradation and digestion. Here we show that larval *A. glabripennis* harbor a diverse gut bacterial community, and this community can be extremely variable when reared in different host trees. *A. glabripennis* reared in a preferred host (*Acer saccharum*) had the highest gut bacterial diversity compared with larvae reared either in a secondary host (*Quercus palustris*), a resistant host (*Pyrus calleryana*), or on artificial diet. The gut microbial community of larval *A. glabripennis* collected from field populations on Brooklyn, NY, showed the highest degree of complexity among all samples in this study. Overall, when larvae fed on a preferred host, they harbored a broad diversity of gut bacteria spanning the α-, β-, γ-Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes. Cellulase activities (β-1,4-endoglucanase, β-1,4-exoglucanase, and β-1,4-glucosidase) in the guts of larvae fed in a preferred host (*A. saccharum*) or a secondary host (*Q. palustris*) were significantly higher than that of artificial diet fed larvae. Larvae that fed on wood from a resistant host (*P. calleryana*) showed suppressed total gut cellulase activity. Results show that the host tree can impact both gut microbial community complexity and cellulase activity in *A. glabripennis.*

KEY WORDS *Anoplophora glabripennis*, gut microbiota, host tree resistance, culture-independent community analysis

Gut microbiota often play important roles in the acquisition of nutrients for growth and development in insects that feed on nutrient-limiting sources (e.g., wood, blood, plant phloem fluids) (Dillon and Dillon 2004). Beneficial roles of gut microbiota include nitrogen fixation, lignocellulose degradation, amino acid biosynthesis, uric acid degradation, and fermentation (Dillon and Dillon 2004). These symbiotic interactions have evolved to permit insects to overcome nutritional, structural, and chemical barriers in their host.

The Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky), is a wood feeding insect in the family Cerambycidae. Exotic to the United States, *A. glabripennis* was first discovered infesting urban shade trees in 1996 in New York (Haack 1997). Native to China and Korea, the preferred hosts in Asia are trees in the poplar, maple, elm, and willow families. Unlike most cerambycids that feed on stressed, dying, or dead trees, *A. glabripennis* attacks apparently healthy, vigorous trees, in addition to weakened trees, (Hanks 1999, Lingafelter and Hoebeke 2002), making it a potential threat to urban and rural forests (Nowak et al. 2001). Although greenhouse studies show that the beetles' host range is likely to expand in the United States (Morewood et al. 2004a, 2005), maple (*Acer* spp.) is the primary domestic host, perhaps because of its relative abundance in infested areas (Haack 1997). Greenhouse studies have also shown tree species that are apparently resistant to *A. glabripennis,* including callery pear, *Pyrus calleryana;* the beetle cannot complete development in these species because of either constitutive defenses or induced responses to oviposition (Morewood et al. 2004b).

Few studies have examined microbial symbionts in cerambycids. Early work identified endosymbiotic yeasts houses in mycetocytes associated with some cerambycid species (Buchner 1965), thought to play a role in xylose degradation (Jones et al. 1999). Kukor and Martin studied the role of wood-rot fungi in cellulose digestion in cerambycid species that feed on

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dead and decaying wood (Kukor and Martin 1986a, Kukor et al. 1988). In these insects, ingested fungal enzymes played an important role in providing complete cellulase activity (Kukor and Martin 1986b). We are studying the role of fungal species in *A. glabripennis*, but in the data presented here, we limited our survey to bacterial species. In the beetle species examined to date that harbored gut bacteria, a broader diversity of microbes was associated with broader tree host range (Schloss et al. 2006). In *A. glabripennis*, a member of the derived cerambycid subfamily Laminae with a broad host range, a wide diversity of bacteria was found in the gut of insects collected in willow trees in China, whereas the linden borer (*Saperda vestita*), a cerambycid with a more restricted host range, contained only a small subset of these same bacteria (Schloss et al. 2006). Findings such as this raise questions about the role of an insect's gut microbial community in host range and host suitability, but this study was limited in that comparisons between host tree species/diet were not investigated, so it is difficult to identify key microbial species.

Cellulose digestion in insects occurs widely in different insect taxa (Martin 1983), and the proportion of cellulose digested can be extremely high (up to 99%) (Prins and Kreulen 1991). Cellulolytic enzymes may originate from gut symbionts, ingestion of enzymes produced by wood decay fungi, the insect itself, or some combination of these (Martin 1983, Breznak and Brune 1994, Watanabe and Tokuda 2001, Brune 2003, Suh et al. 2005). However, little is known about how cellulose digestion occurs in insects, outside of termites or other insects that feed on decaying wood. Enzymatic hydrolysis of cellulose to glucose proceeds by synergistic interaction of endo- and exoglucanases and β-glucosidases (Ljungdahl and Eriksson 1985). Many phytophagous insects can synthesize their own endo-glucanses and β-glucosidases but not exo-glucanases (Martin 1983, Watanabe and Tokuda 2001). Because insects are not known to encode genes for endogenous exo-glucanases, it is thought that gut symbionts are required to produce a complete cellulolytic enzyme complex (Breznak and Brune 1994, Ohkuma 2003, Suh et al. 2003, Delalibera et al. 2005). Several studies have investigated other cerambycids (Sugimura et al. 2003; Lee et al. 2004, 2005) from which endogenous endoglucanase genes were identified, but the interactions between cellulose digestion, host tree, and microbial community were not investigated.

The purpose of this study was to examine relationships between host tree species, gut microbial community composition, and cellulose digestion in *A. glabripennis.* Gut microbial communities and cellulase activity of larvae were compared among insects reared in a preferred host (sugar maple, *Acer saccharum*), an alternative host (pin oak, *Quercus palustris*) (Morewood et al. 2005), a third tree species that is highly resistant to *A. glabripennis* (callery pear, *Pyrus calleryana* cultivar Aristocrat) (Morewood et al. 2004b), and an artificial diet to elucidate relationships between host tree suitability and gut community complexity. Also, these data were compared with gut microbial communities of insects collected from a variety of host trees in an established population of *A. glabripennis* in New York City to understand variability in gut community composition and complexity between colony-derived and field populations.

Materials and Methods

Rearing Colony–Derived *A. glabripennis* on Different Host Trees

Gut microbial communities were studied using larvae reared in nursery lines of one of three tree species (sugar maple, pin oak, or callery pear). Trees were planted in 20-gal nursery containers filled with Fafard 52 pine bark medium (Fafard, Agawam, MA) and grown at an outdoor pot-in-pot nursery at the Pennsylvania State University, University Park campus, until they were 4–5 yr old. Several weeks before use in experiments, trees were moved into a quarantine greenhouse to allow for acclimation to greenhouse conditions. Three trees of each species were placed in large ($\approx$3 m high, 3 m long, 2 m wide) walk-in insect cages, each cage containing only one tree species, and maintained as described previously (Morewood et al. 2004a). Adult *A. glabripennis* were obtained from a quarantine research colony of mixed ancestry (Morewood et al. 2004b). The research colony is maintained on a cellulose-based artificial diet (Dubois et al. 2002) using Norway maple (*Acer platanoides*) for adult feeding and oviposition. For this experiment, three mated pairs of adults per tree species (n = 9 pairs) were maturation fed on twigs from either sugar maple or pin oak for 3–5 d. Callery pear was not used for maturation feeding because *A. glabripennis* do not survive or produce eggs when fed on twigs of this tree species (Morewood et al. 2004b). After maturation, mating pairs were released into a cage containing potted trees of the same tree species from which they maturation fed and were allowed to oviposit into these trees for 2 wk. At this point, adults were removed from the cages. The trees were held in the greenhouse for 90 d to permit larval establishment (Morewood et al. 2004a). After 90 d, each tree was dissected, and living larvae were collected for gut community analysis. Because *A. glabripennis* are recalcitrant to feed on and do not grow in callery pear (Morewood et al. 2004b), a portion of the larvae extracted from sugar maple were inserted into callery pear and allowed to feed for 2 wk (Ludwig et al. 2002) to examine the effect of this resistant host on the gut community. Callery pear trees were dissected 2 wk after larval insertion, and apparently healthy, feeding larvae were collected for gut community analysis. Larvae that fed on a cellulose-based artificial diet (Dubois et al. 2002) were also collected for gut community analysis from the quarantine laboratory colony of approximately the same age as the tree-reared larvae. Although the artificial diet does not contain any specific antibiotics, it does include components such as sodium propionate, sorbic acid, and p-hydroxybenzoic acid methyl ester, which have fungal and microbial inhibitory properties.

Collection of Larval *A. glabripennis* From Field Populations

To compare composition of the gut community harbored by colony-derived larvae with those of field-collected insects, *A. glabripennis* was field collected from populations located in Brooklyn, NY, in conjunction with eradication efforts by the USDA–APHIS–PPQ New York ALB Laboratory (Amityville, NY). Infested trees were identified based on exit holes and dieback of the trees and were located on homeowners' property in an urban setting. Four trees were cut for this study, two silver maples (*Acer saccharinum*), one sycamore maple (*Acer pseudoplatanus*), and one horse chestnut (*Aesculus hippocastanum*). Trees were cut into segments and dissected to remove larvae. Larvae were immediately frozen after removal from trees and stored at −80°C until use.

Culture-Independent Gut Community Analysis

Dissection and DNA Extraction. Larvae dissections were performed in a laminar flow hood to maintain sterility using sterile dissection tools. Larvae removed from trees were immediately chilled and dissected within 1 h of removal from trees. New York larvae were kept frozen until immediately before they were dissected and maintained on ice. Larvae were surface sterilized in 70% EtOH for 1 min and rinsed in sterile water before dissection. Whole guts were dissected by cutting the cuticle open laterally, ligating the gut at the anterior midgut and posterior hindgut, and carefully transferring the entire gut into a sterile microcentrifuge tube. Ten guts were pooled into a single tube for DNA extraction for each tree species to reduce individual variation within trees. Total DNA was extracted using the FastDNA SPIN for Soil Kit (MP Biomedicals, Solon, OH) and the FastPrep Instrument (BIO101, La Jolla, CA) for tissue homogenization following the manufacturer's protocol. This kit was used because of the complexity of the *A. glabripennis* gut contents (containing wood, bacteria, and fungi) to ensure complete DNA extraction from all organisms. A control DNA extraction was also performed by using the sterile water rinsate to confirm that no contaminating DNA was extracted. DNA concentration was determined by absorbance at 260 nm, and samples were stored at −20°C until use.

Polymerase Chain Reaction Amplification and Cloning. Bacterial 16S rRNA was amplified using polymerase chain reaction (PCR) from total gut DNA extractions from each treatment. Universal primers 530 F (5′-GTG CCA GCM GCC GCG G-3′) and 1392R (5′-ACG GGC GGT GTG TRC-3′) were used to amplify ≈860 bases of the 16S rRNA gene. PCR reactions were performed in 25-μl volumes with the following components: 5 μl of 5× GoTaq green reaction buffer, 1.25 U GoTaq DNA polymerase (Promega, Madison, WI), 1 μl 10 μM dNTP mix, 2 μl of 10 μM forward (530 F) and reverse primers (1392R), and 20 ng of template DNA. PCR conditions were 95°C denaturation for 3 min, 25 cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 1.5 min, with a final extension at 72°C for 5 min. Control DNA, extracted from sterile water rinsate, also underwent PCR to ensure that there was no contaminating DNA during extraction and positive and negative PCR controls. Agarose gel electrophoresis showed two bands amplified from the 16S rRNA PCR reaction. The larger band represented the 18S rRNA region, derived from eukaryotic organisms (insect), and the smaller band represented the 16S rRNA region from bacteria. To exclude the 18s fragments from the cloning reaction, the bands were resolved by gel electrophoresis in 0.75% low-melt agarose. The smaller band was excised under UV light using a clean razor blade, placed in a microcentrifuge tube, and stored at −20°C until used for cloning. Low-melt gel fragments were ligated into the pCR 2.1 TOPO vector (Invitrogen, Carlsbad, CA) following the manufacturer's protocol, with the exception that the low-melt fragments were first melted in a 65°C water bath and the ligation reaction was performed at 42°C. The vector was transformed into chemically competent *E. coli* cells (TOP10; Invitrogen) by heat shock, and 16S rRNA clone libraries were created.

Insert DNA from 16S rRNA clones was amplified using M13 primers using direct PCR. Twenty-five-microliter PCR reactions were set up in 96-well format with the following components: 5 μl of 5× GoTaq green reaction buffer, 1.25 U GoTaq DNA polymerase (1.25 U; Promega), 1 μl 10 μM dNTP mix, 2 μl of 10 μM forward primer (M13Universal), and 2 μl of 10 μM reverse primer (M13Rev). Individual colonies were picked from the clone library using a sterile pipette tip and immersed into the PCR mix to allow the bacteria cells to enter the PCR reaction. The PCR program had an initial 95°C denaturation step for 10 min to rupture bacteria cells, followed by 30 cycles of 95°C for 30 s, 55°C for 1:00 min, and 72°C for 1:30 min, with a final extension at 72°C for 5 min. Four microliters of the PCR product was cleaned up for sequencing by addition of 0.8 μl of ExoSAP-IT (USB, Cleveland, OH) following manufacturer's instructions. Two microliters of this reaction was used to sequence both DNA strands with M13 primers.

Culture-Dependent Gut Community Analysis

Dissection and Culturing of Aerobic and Anaerobic Gut Microbiota on General Media. Subsets of the colony-derived larvae from sugar maple trees and from the quarantine colony fed on an artificial diet were used for culture-dependent gut microbial community analysis under aerobic and anaerobic conditions. Larval dissections were performed under aseptic conditions as described above. Five guts were pooled into a single microcentrifuge tube containing 500 μl of sterile PBS solution (0.01 M, 0.138 M NaCl, 0.0027 M KCl, pH 7.4) for each tree, providing triplicate samples for both food sources (15 total guts/treatment). Gut material for each sample was weighed to standardize bacterial colony forming units (CFUs) between samples. A control tube containing only PBS was also set up to ensure there was no contamination

during dissection and plating. Tissue was homogenized using a disposable micropestle and vortexed at a medium speed for 30 s. Serial dilutions of each replicate were performed in PBS (1:10, 1:100, 1:1,000, and 1:10,000). One hundred microliters of each dilution was plated in triplicate onto LG agar plates (10 g tryptone, 5 g yeast extract, 5 g NaCl, 10 g glucose, 15 g agar for 1 liter, pH 7.0) for aerobic culturing and incubated at 28°C. For anaerobic culturing, 100 μl of each dilution was plated only onto LG agar plates to which 2 mg methylene blue was added as an oxygen indicator. The anaerobic samples were plated in an anaerobic glove box, transferred to an anaerobic jar system (BD GasPak system with GasPak Plus hydrogen + carbon dioxide generator) and incubated at room temperature. Colonies were grown for 2–4 d before being counted and used for 16S rRNA sequencing.

Gut Microbial Culturing on Restrictive Media. Gut samples were obtained as described in the previous section with three replicates from insects reared in sugar maple trees and artificial diet, with each replicate consisting of three insect guts. Serial gut dilutions were made (1:10, 1:100, 1:1,000), and 80 μl of each dilution was plated in triplicate onto CMC agar (Delalibera et al. 2005) (5 g carboxymethyl cellulose, 10 g tryptic soy broth, 0.03 g malt extract, and 12 g agar for 1 liter, pH 7.0). Plates were aerobically incubated at 28°C for 2–4 d, and colonies were counted and picked for sequencing. After picking colonies from plates, clearing zones, representing digestion of carboxymethyl cellulose, were visualized by staining plates with 0.1% Congo red for 10 min followed by destaining with repeated washes with 1 M NaCl. All colonies grown on these plates created clearing zones, showing the selectivity of this media for isolating cellulose-degrading bacteria.

Direct 16S rRNA PCR. After colony growth occurred from broad aerobic and anaerobic culturing and culturing on selective media, individual bacteria were identified by amplification of 16S rRNA gene and sequencing. We used only dilutions that had well-spaced colonies (between 50 and 150 CFUs/plate) for sequencing and for enumerating population numbers for each colony type to determine CFU/g of gut. Ninety-six bacterial colonies were amplified for each feeding treatment for both anaerobic and aerobic plates, with 32 colonies from each of the three replicates. Unique colony types from the selective media were also picked for amplification. Twenty-five-microliter PCR reactions were performed with 5 μl of 5× GoTaq green reaction buffer, 1.25 U GoTaq DNA polymerase (1.25 U; Promega), 1 μl 10 μM dNTP mix, 2 μl of 10 μM forward primer (530 F), and 2 μl of 10 μM reverse primer (1392R). Direct PCR from individual bacterial colonies were performed by addition of the colony to the PCR reaction as described above. Reaction conditions were 95°C denaturation for 10 min, followed by 30 cycles of 95°C for 30 s, 55°C for 1:00 min, and 72°C for 1:30 min, with a final extension at 72°C for 5 min. Four microliters of the PCR product was treated for sequencing by addition of ExoSAP-IT as described above, and 2 μl was used for sequencing of both strands.

16S rRNA Sequencing and Data Analysis

Sequencing using the BigDye Terminator method was performed at the Penn State Nucleic Acid Facility. Consensus 16S rRNA sequences were constructed for each clone analyzed. Alignment of forward and reverse sequences was performed using MEGA 4 (Tamura et al. 2007); the vector sequence was removed and consensus sequence created. Sequences for each library were aligned by performing a multiple alignment in ClustalX 2.0.3 (Higgins and Sharp 1988, Thompson et al. 1997). A Jukes-Cantor corrected distance matrix was created using the dnadist application of Phylip3.67 (Felsenstein 1989). From this, unique bacterial taxa were identified in each library, defined as operational taxonomic units (OTUs), using the DOTUR software package and based on 5% sequence variability (Schloss and Handelsman 2005). Initially each tree/diet library was analyzed separately to create rarefaction curves, perform Chao1 richness estimates (Chao 1984), and calculate a Simpson's diversity index (1-D) (Magurran 1988) for each library separately. All culture-independent sequences were built into a single alignment to assign shared OTUs between treatments. To assign a taxonomic name to the OTUs, 16S rRNA libraries were uploaded to the Ribosomal Database Project II (www.rdp.cme.msu.edu) and compared with the database using the Seqmatch tool (Cole et al. 2005, 2007). Seqmatch identifies the nearest neighbors to a query sequence, providing taxonomic identification along with a similarity score to sequences in the database. For the purposes of this experiment, OTUs were assigned to a genus only when the confidence to that genus was >90%; otherwise, family level assignment was given.

All DNA sequences were deposited into the GenBank database for both the culture-independent and culture-dependent sequencing. GenBank accession numbers for culture-independent gut bacteria 16S sequences from Penn State colony–derived insects are as follows: (1) EU879156–EU879238 for artificial diet-fed larvae, (2) EU879488–EU879610 for pin oak reared larvae, (3) EU879611–EU879736 for callery pear reared larvae, and (4) EU879737–EU879911 for sugar maple reared larvae. GenBank accession numbers for culture-independent gut bacteria 16S sequences from larvae removed from trees in New York City are as follows: (1) EU879239–EU879265 for the horse chestnut, (2) EU879266–EU879329 for silver maple 1, (3) EU879330–EU879412 for silver maple 2, and (4) EU879413–EU879487 for sycamore maple. GenBank accession numbers for representative samples from culture-dependent 16S rRNA sequences from sugar maple reared larvae are as follows: (1) EU918333–EU918341 for bacteria cultured on CMC agar, (2) EU918305–EU918314 and EU918342 for aerobic cultures on LB agar, and (3) EU918343 for anaerobic cultures on LB agar. GenBank accession num-

bers for representative samples from culture-dependent 16S rRNA sequences from artificial diet–reared larvae are as follows: (1) EU918320–EU918331 from aerobic cultures on CMC agar, (2) EU918315, EU918316, EU918318, and EU918319 for aerobic cultures on LB agar, and (3) EU918317 for anaerobic cultures on LB agar.

In Vitro Gut Cellulase Assays

Enzyme Extraction. Crude gut enzyme extracts were prepared in triplicate from pools of three larval guts from each tree species (sugar maple and pin oak) and from artificial diet–fed insects (Tokuda 2004, Tokuda et al. 2005). Gut dissections were performed as described above. Pooled guts were suspended in 500 μl of sodium citrate buffer (50 mM, pH 5.5) and homogenized with a micropestle on ice. Samples were centrifuged at 10,000*g* for 15 min at 4°C, and the supernatant was collected into a fresh centrifuge tube. Protein concentration of each samples was measured using Bradford chemistry (Bradford 1976, Bollag et al. 1996) with bovine serum albumin (BSA) as a protein standard (0–20 μg); samples were diluted to a working concentration of 60 μg/ml in sodium citrate buffer (50 mM, pH 5.5).

Reducing Sugar Assays. To determine the effect of host tree on gut cellulolytic activity, in vitro activities of β-1,4-glucosidase, β-1,4-endoglucanase, and β-1,4-exoglucanase were measured from crude gut extracts incubated with cellulose substrates based on release of reducing sugar measured by the dinitrosalicylic acid (DNS) assay (Bernfeld 1955, Miller 1959). Each enzyme extract was performed in duplicate, with three enzyme extracts prepared from each feeding treatment. Because of difficulty in obtaining larvae from callery pear, no enzyme assays could be performed from this treatment. Field-collected insects were not suitable for enzyme assays because of destructive sample collection techniques of field populations under quarantine restriction. For β-glucosidase activity, 500 μl of a 2% salicin solution (in 50 mM sodium citrate buffer, pH 5.5) was combined with 30 μg (500 μl of 60 μg/ml dilution) of crude gut extract. For β-endoglucanase activity 500 μl of a 2% carboxymethycellulose solution (Kukor et al. 1988) (in 50 mM sodium citrate buffer, pH 5.5) was combined with 500 μl of crude gut extract. For β-exoglucanase activity 500 μl of a 1% Avicel solution (Kukor et al. 1988) (in 50 mM sodium citrate buffer, pH 5.5) was combined with 500 μl of crude gut extract. Also, ground sugar maple and callery pear wood were incubated with maple gut enzyme extracts to study enzyme inhibition by compounds present in callery pear wood. Xylem tissue from these two trees was collected and ground finely through a 1-mm screen with a Wiley mill. Wood was lyophilized, and a 2% solution (in 50 mM sodium citrate buffer, pH 5.5) was combined with 500 μl of gut extract. For all assays, 100 μl of the reaction were pulled at time 0 to allow for subtraction of background sugar. Reactions were incubated at 37°C for 180 min, with 100-μl aliquots removed after 30, 90, and 180 min. For each aliquot, 100 μl DNS reagent was added to halt enzyme activity (Miller 1959). Samples were incubated in a boiling water bath for 8 min, and absorbance of a 150-μl aliquot was read in duplicate at 540 nm on a SpectraMax microplate reader (Molecular Devices, Sunnyvale, CA) along with glucose standards (standard curve, 20–1,000 μg). Statistical differences among treatments were determined at each time point by performing repeated-measures analysis of variance (ANOVA) followed by post hoc means comparisons with Tukey's least significant difference (LSD; SAS Institute, Cary, NC).

Results

Microbial Community of Larval Asian Longhorned Beetles. Study of the gut microbial community harbored by Asian longhorned beetle larvae using culture-independent 16S rRNA analysis and aerobic and anaerobic culturing allowed us to identify 63 unique bacterial types (OTUs) between both the Penn State colony–derived (PSU) and New York City field–derived (NYC) populations. 16S rRNA sequences from culture-independent analysis (n = 758 clones, across eight libraries) showed broad bacterial diversity, with most taxa being placed within γ-Proteobacteria (n = 391, 272 PSU/119 NYC), followed by Firmicutes (n = 169, 159 PSU/10 NYC), Actinobacteria (n = 87, 52 PSU/35 NYC), α-Proteobacteria (n = 80, 16 PSU/64 NYC), β-Proteobacteria (n = 19, 8 PSU/11 NYC), Bacteroidetes (n = 7, 3 PSU/4 NYC), Verrucomicrobia (n = 5, 0 PSU/5 NYC), Planctomycetes (n = 1, 0 PSU/1 NYC), and Cyanobacteria (n = 1, 1 PSU/0 NYC; Tables 1 and 3). Comparing results from research colony–derived insects (PSU) and field-collected insects (NYC) showed that the two groups harbored similar gut communities, both in terms of taxa and percentage species composition (Fig. 1). However, we also found some unique bacterial phyla in the NYC population (Verrucomicrobia and Planctomycetes) as well as the PSU population (Cyanobacteria), but these represented the least abundant bacterial taxa and may represent transient bacterial types (Tables 1 and 3).

Only a small portion of these bacteria could be identified by culture-dependent methods (aerobic and anaerobic; Table 2). Culturing on a nonrestrictive diet yielded five aerobic bacterial taxa and three anaerobic. Two types were identified using both aerobic and anaerobic culturing and thus are likely facultative anaerobes. Culturing on cellulose-based restrictive media yielded 11 bacterial types, 10 of which were different from the nonrestrictive cultures. All bacteria isolated through culturing were closely related to bacteria found using culture-independent techniques.

Rarefaction Analysis and Richness Estimates. Rarefaction analysis of distance matrices constructed for each clone library yielded rarefaction curves for relative species richness present in each sample. This allows for estimates of species richness to be made from each sample, using the subset of the clones sam-

Table 1. Bacterial taxa (OTUs) identified from culture-independent analysis of larval *A. glabripennis* gut contents from a quarantined research colony reared in three different host trees or artificial diet

Phylum	Class	Family	Genus	Sugar maple (177)	Pin oak (124)	Callery pear (126)	Artificial diet (84)
Actinobacteria	Actinobacteridae	Brevibacteriaceae	*Brevibacterium*	2	0	1	0
		Dermabacteraceae	*Brachybacterium*	1	1	0	0
		Microbacteriaceae	*Curtobacterium*	0	0	1	0
			Microbacterium	27	1	0	0
			Unassigned Microbacteriaceae 1	6	4	0	0
			Unassigned Microbacteriaceae 2	0	1	0	0
		Nocardiaceae	*Rhodococcus*	0	1	1	0
		Promicromonosporaceae	*Cellulosimicrobium*	0	2	0	0
		Streptomycetaceae	*Streptomyces*	3	0	0	0
Bacteroidetes	Sphingobacteria	Sphingobacteriaceae	*Sphingobacterium*	0	3	0	0
Cyanobacteria	Cyanobacteria	Unknown	*Unassigned Cyanobacteria*	1	0	0	0
Firmicutes	Bacilli	Enterococcaceae	*Enterococcus*	89	4	0	0
			Unassigned Enterococcaceae	0	0	0	2
		Lactobacillaceae	*Lactobacillus*	0	0	0	60
			Unassigned Lactobacillaceae	0	0	0	4
Proteobacteria	Alphaproteobacteria	Brucellaceae	*Ochrobactrum*	7	5	1	0
			Unassigned Brucellaceae	1	0	0	0
		Rhizobiaceae	*Rhizobium*	0	1	0	0
		Sphingomonadaceae	*Unassigned Sphingomonadaceae*	0	0	0	1
	Betaproteobacteria	Alcaligenaceae	*Achromobacter*	4	1	0	0
		Incertae sedis 5	*Methylibium*	0	0	0	1
		Oxalobacteraceae	*Unassigned Oxalobacteraceae*	0	0	0	2
	Gammaproteobacteria	Enterobacteriaceae	*Enterobacter 1*	1	0	116	0
			Enterobacter 2	29	92	1	0
			Raoultella	0	0	3	0
			Shigella	0	0	0	3
			Unassigned Enterobacteriaceae 1	0	0	2	0
			Unassigned Enterobacteriaceae 2	0	0	0	8
			Unassigned Enterobacteriaceae 3	0	0	0	2
			Unassigned Enterobacteriaceae 4	0	0	0	1
		Moraxellaceae	*Acinetobacter*	0	6	0	0
		Xanthomonadaceae	*Lysobacter*	1	0	0	0
			Stenotrophomonas	5	2	0	0
			Simpson's diversity index (1-D)	0.70	0.45	0.17	0.49

pled to extrapolate from. Because the number of clones analyzed for each library was different, terminal values of the curves cannot be directly compared. Instead, the shape of the curves was compared, with higher initial slopes of the curves representing greater richness. Also, curves that reached an asymptote indicated saturation of sampling, where additional sampling would be unlikely to yield a higher number of new OTUs. For the PSU-derived clone libraries, relative richness, based on curve shape, was higher in sugar maple and pin oak, followed by artificial diet and callery pear (Fig. 2). In NYC-derived clone libraries, relative richness was highest in the silver maple tree-2 clone library, followed by sycamore maple, horse chestnut, and finally silver maple tree-1 (Fig. 2). Of all libraries, only the curve for silver maple tree-1 reached a clear asymptote, suggesting that more sampling of the other libraries would yield further diversity.

Chao1 richness analysis estimated the probable number of OTUs in the sample (Fig. 3). Here, an asymptotic curve suggested that the estimate was unlikely to dramatically change with more sampling of clones. For the PSU colony–derived samples, richness estimates were highest for sugar maple and pin oak samples and lowest for callery pear and artificial diet. For the NYC-derived samples, Chao1 richness estimates were highest for silver maple tree-2, followed by sycamore maple, silver maple tree-1, and finally horse chestnut. The majority of the estimated curves reached a plateau, suggesting that these estimates are accurate given the number of clones sampled for each library (Fig. 3).

Effect of Host Tree Species on Gut Microbial Community Composition. Host tree species had a marked effect on diversity of the larval gut bacterial community. By calculating the Simpson diversity index (1-D) of the PSU colony–derived insects, reduced heterogeneity was observed in insects fed callery pear in comparison to the two preferred tree species: sugar maple and pin oak. The Simpson diversity index gives values from 0 to 1, with 0 representing no heterogeneity and 1 representing infinite complexity. Simpson diversity index values for gut bacterial communities in larvae fed sugar maple, pin oak, callery pear, and cellulose-based artificial diet were 0.70, 0.45, 0.17, and 0.49, respectively, showing a reduction in species diversity when feeding on the secondary host (pin oak) or on artificial diet and an even greater reduction in larvae fed on the resistant tree species (callery pear). Based on culture-independent data, the highest number of OTUs was found in the two preferred host trees: sugar maple and pin oak (14 OTUs each). Feeding in the resistant tree species callery pear and cellulose-

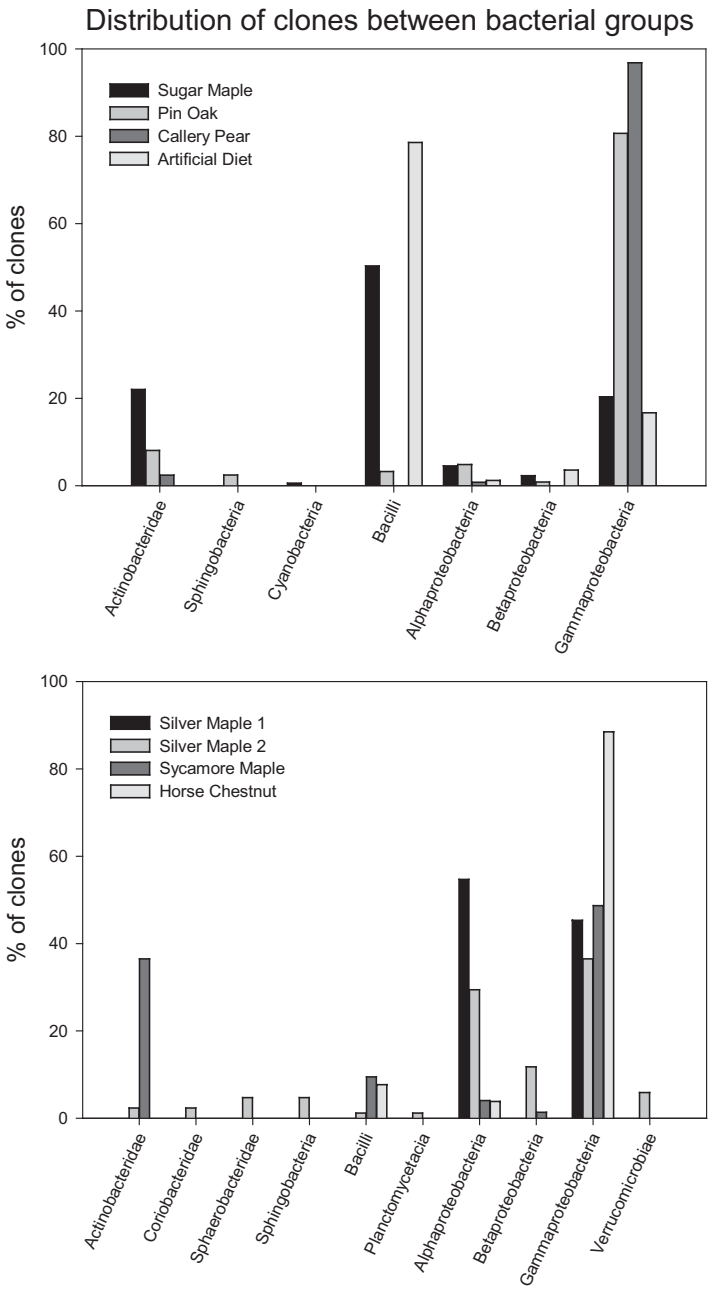

Fig. 1. Distribution of bacterial taxa between different host tree types from the Penn State quarantine research colony (PSU) versus field populations from Brooklyn, NY (NYC), clone libraries.

based artificial diets produced a reduction in species diversity with only 8 OTUs found in larval guts from callery pear and 10 from artificial diet. Also, although larvae from the two preferred hosts contained OTUs from six different bacterial classes, larvae reared on callery pear only produced OTUs placed in three different classes, with no taxa represented from the Cyanobacteria, Sphingobacteria, β-Proteobacteria, or Firmicutes (Fig. 1). The majority of the bacteria from larvae fed callery pear were from a single unclassified Enterobacteraceae (92% of clones). Similarly, larvae reared on the cellulose-based artificial diet only had OTUs placed in three different bacterial classes, with the majority of the sequences matching closely to *Lactobacillus* spp. (71% of clones), a taxonomic group we never found in larvae fed in trees (Table 1). Culture-dependent analysis of gut microbiota found in artificial diet–fed larvae and sugar maple–fed larvae

Table 2. Taxa of bacteria (OTUs) identified by culture-dependent analysis on broad and restrictive media of larval *A. glabripennis* gut contents from a quarantined research colony reared on sugar maple or artificial diet

Phylum	Class	Family	Genus (95% identity)[a]	Average no. of CFUs/g insect gut[b] cultured on:					
				Artificial diet			Sugar maple		
				Aerobic		Anaerobic	Aerobic		Anaerobic
				CMC	LG	LG	CMC	LG	LG
Actinobacteria	Actinobacteridae	Brevibacteriaceae	*Brevibacterium*	4.63×10^2	—	—	—	—	—
		Micrococcaceae	*Micrococcus*	5.21×10^2	—	—	—	—	—
		Streptomycetaceae	*Streptomyces*	3.47×10^2	—	—	—	—	—
Acidobacteria	Acidobacteriales		*Gp1*	2.59×10^2	—	—	—	—	—
Firmicutes	Bacilli	Bacillaceae	*Bacillus*	3.47×10^2	—	—	—	—	—
		Enterococcaceae	*Enterococcus*	—	—	—	—	—	2.54×10^5
		Lactobacillaceae	*Lactobacillus*	—	1.58×10^5	2.15×10^5	—	—	—
			Unassigned Lacobacillaceae	—	1.34×10^3	—	—	—	—
		Staphylococcaceae	*Staphylococcus*	1.56×10^2	—	—	—	—	—
Proteobacteria	Alphaproteobacteria	Brucellaceae	*Ochrobactrum*	—	—	—	1.50×10^4	—	—
		Rhizobiaceae	*Unassigned Rhizobiaceae*	—	—	—	8.46×10^3	—	—
	Betaproteobacteria	Burkholderiaceae	*Burkholderia*	1.34×10^2	—	—	—	—	—
	Gammaproteobacteria	Enterobacteriaceae	*Enterobacter*	—	—	—	3.91×10^2	4.30×10^6	2.54×10^6
			Unassigned Enterobacteriaceae	—	—	—	—	1.91×10^5	—
		Moraxellaceae	*Acinetobacter*	—	—	—	—	4.30×10^5	—
		Pseudomonadaceae	*Pseudomonas*	—	—	—	2.73×10^3	—	—

[a] OTU identity based on taxonomy of Ribosomal Database Project.
[b] CFUs/g gut presented are based on the average no. of colonies from three replicates gut pools plated in duplicate.
CMC, carboxymethyl cellulose agar; LG, glucose enriched Luria-Bertani (LB) agar.

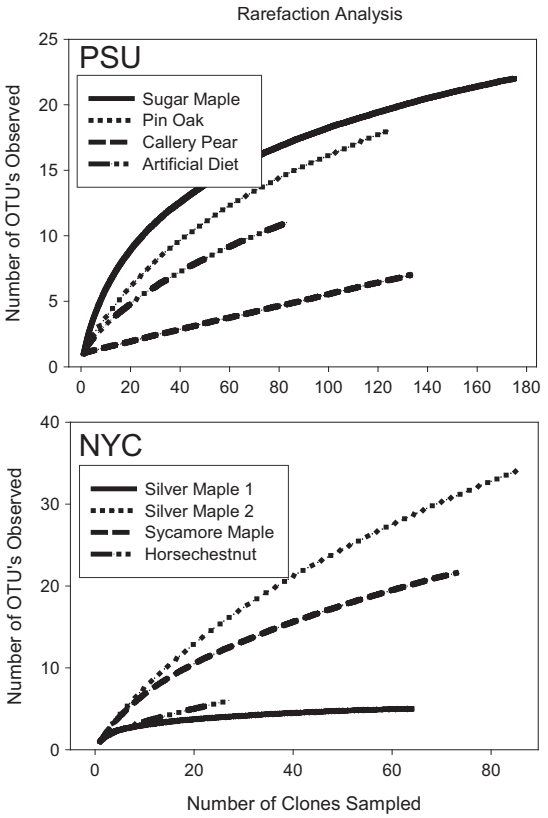

Fig. 2. Rarefaction analysis of clone libraries from the Penn State quarantine research colony (PSU) versus field populations from Brooklyn, NY (NYC), based on OTUs at 5% sequence difference.

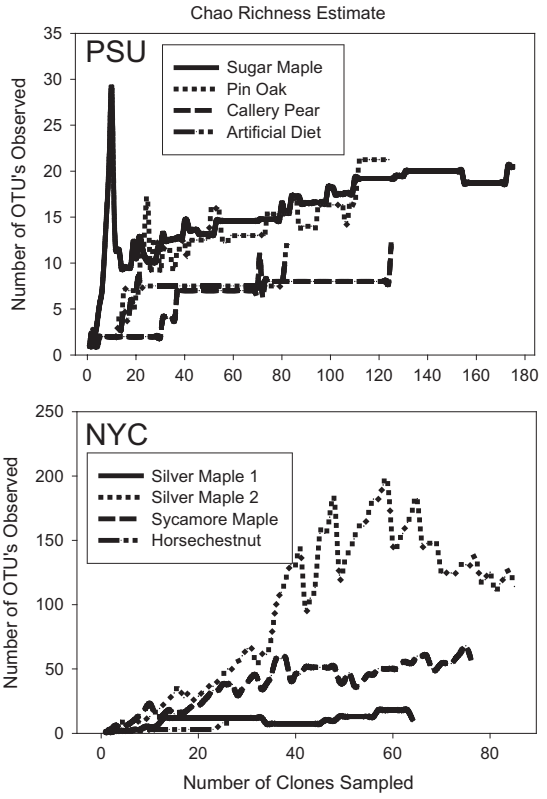

Fig. 3. Chao1 richness estimates. Curves of clone libraries collected from the Penn State quarantine research colony (PSU) versus field populations from Brooklyn, NY (NYC), based on OTUs at 5% sequence difference.

yielded strikingly different bacterial compositions. Aerobic and anaerobic, nonrestrictive culturing yielded almost entirely Enterobacteriaceae in sugar maple–fed larvae, whereas artificial diet–fed larvae yielded entirely Lactobacillaceae (Table 2). The number of CFUs was ≈10-fold higher in sugar maple–fed larvae. Restrictive culturing on CMC media also showed differences, with sugar maple–fed larvae producing only α- and γ-Proteobacteria, and artificial diet–fed larvae having a greater diversity across the Actinobacteria, Acidobacteria, Firmicutes, and β-Proteobacteria. Although the diversity using restrictive media was greater in artificial diet–fed larvae, there was an ~100-fold reduction in the total number of CFUs from these larvae (Table 2).

Within NYC field-collected samples, the host tree also had a marked impact on bacterial composition. The highest microbial diversity was seen in larvae collected from one of the silver maple trees (silver maple tree-2) and the sycamore maple, with Simpson's diversity indices (1-D) of 0.94 and 0.93, respectively. In contrast, diversity indices of 0.62 and 0.55 were calculated from larvae collected from horse chestnut and the second silver maple (silver maple tree-1) tree, respectively (Table 3). The sugar maple 1 larval clone library was composed almost entirely of two OTUs: a *Bradirhizobium* in the α-Proteobacteria (53% of clones) and an *Enterobacter* in the γ-Proteobacteria (42% of clones; Fig. 1). Although the horse chestnut–derived clone library did not have any *Bradirhizobium* present, it had the highest proportion of clones matching to *Enterobacter* (62% of clones). These less diverse samples lacked taxa from the Actinobacteria, Bacteriodetes, Plantomycetes, β-Proteobacteria, and Verrucomicrobia (Table 3). Clones from the diverse samples derived from sugar maple 2 and sycamore maple clone libraries were far more evenly distributed, with OTUs from eight and five bacterial phyla, respectively, and no single OTU representing >26% of the clone library (Fig. 1).

Gut Cellulolytic Activity. The enzyme activities of three different types of cellulases were compared among larvae reared on the different tree species or on artificial diet. Values for β-glucosidase activity, measured by the release of β-1,4-bonded glucose from salicin, endoglucanase activity as degradation of carboxymethyl cellulose, and exoglucanase activity, measured as degradation of crystalline cellulose (Avicel), were significantly higher in enzyme extracts from the guts of *A. glabripennis* fed on sugar maple and pin oak compared with those fed on artificial diet (Fig. 4; time,

Table 3. Taxonomic groups represented among bacteria (OTUs) identified by culture-independent analysis of larval *A. glabripennis* gut contents from different host trees in field populations located in Brooklyn, NY

Phylum	Class	Family	Genus	Silver maple 1 (64)	Silver maple 2 (83)	Sycamore maple (74)	Horse chestnut (26)
Actinobacteria	Actinobacteridae	Dermabacteraceae	*Brachybacterium*	0	0	2	0
		Microbacteriaceae	*Microbacterium*	0	0	9	0
			Unclassified Micrococcineae	0	2	2	0
			Unclassified Actinomycetales	0	0	2	0
			Unclassified Microbacteriaceae	0	0	3	0
		Corynebacteriaceae	*Corynebacterium*	0	0	8	0
		Gordoniaceae	*Gordonia*	0	0	1	0
	Coriobacteridae	Coriobacteriaceae	*Unassigned Coriobacteriaceae*	0	2	0	0
	Sphaerobacteridae	Sphaerobacteraceae	*Sphaerobacter*	0	2	0	0
Bacteroidetes	Sphingobacteria	Crenotrichaceae	*Chitinophaga*	0	2	0	0
		Flexibacteraceae	*Dyadobacter*	0	2	0	0
Firmicutes	Bacilli	Bacillaceae	*Geobacillus*	0	1	0	0
			Unassigned Lactobacillales	0	0	2	0
		Enterococcaceae	*Enterococcus*	0	0	5	1
		Leuconostocaceae	*Leuconostoc*	0	0	0	1
Planctomycetes	Planctomycetacia	Planctomycetaceae	*Planctomyces*	0	1	0	0
Proteobacteria	Alphaproteobacteria	Caulobacteraceae	*Asticcacaulis*	0	1	0	0
		Sphingomonadaceae	*Sphingomonas*	0	9	0	0
			Sphingoryxis	0	1	0	0
		Bradyrhizobiaceae	*Bradyrhizobium*	34	1	0	0
		Brucellaceae	*Ochrobactrum*	1	3	0	0
		Hyphomicrobiaceae	*Devosia*	0	2	0	0
		Phyllobacteriaceae	*Unassigned Phyllobacteriaceae*	0	2	0	0
		Rhizobiaceae	*Rhizobium*	0	0	0	1
		Rhodobacteraceae	*Paracoccus*	0	1	2	0
			Rhodobacter	0	5	1	0
	Betaproteobacteria	Alcaligenaceae	*Unclassified Alcaligenaceae*	0	0	1	0
		Comamonadaceae	*Unclassified Comamonadaceae*	0	1	0	0
		Incertae sedis 5	*Aquabacterium*	0	4	0	0
			Methylibium	0	2	0	0
		Methylophilaceae	*Unclassified Methylophilaceae*	0	1	0	0
		Rhodocyclaceae	*Shinella*	0	2	0	0
	Gammaproteobacteria	Enterobacteriaceae	*Leclercia*	1	1	4	2
			Brenneria	0	0	7	0
			Enterobacter	27	13	19	16
			Raoultella	1	10	0	2
			Unclassified Enterobacteriaceae	0	0	2	3
		Moraxellaceae	*Acinetobacter 1*	0	0	3	0
		Pseudomonadaceae	*Pseudomonas*	0	0	1	0
			Unclassified Pseudomonadaceae	0	1	0	0
		Xanthomonadaceae	*Pseudoxanthomonas*	0	2	0	0
			Luteimonas	0	2	0	0
			Lysobacter	0	2	0	0
Verrucomicrobia	Verrucomicrobiae	Opitutaceae	*Opitutus*	0	1	0	0
		Verrucomicrobiaceae	*Verrucomicrobium*	0	2	0	0
			Incertae Sedis	0	2	0	0
			Simpson's diversity index (1-D)	0.55	0.94	0.93	0.62

180 min; $P < 0.0001$ for salicin and CMC, $P < 0.05$ for Avicel). β-glucosidase activity was also significantly higher in pin oak gut extracts than sugar maple (Fig. 4; time, 180 min; $P < 0.0001$), but there were no significant differences between pin oak and sugar maple in endo- or exoglucanase activity.

Although enzyme assays could not be performed with callery pear–fed larvae because of difficulty in obtaining sufficient gut extracts, enzyme activities of gut extracts from sugar maple–fed larvae were tested on sugar maple and callery pear wood. Sugar release (degradation of carbohydrates) was significantly higher from sugar maple wood than from callery pear wood (Fig. 5; time, 180 min; $P = 0.0189$).

Discussion

The *A. glabripennis* larval gut harbors a rich diversity of bacteria, exemplified by marked variation in community complexity and composition as a function of host tree and geographic location. Chao1 richness estimates varied greatly, not only between larvae collected from different host tree species and different geographic locations, but also among insects grown in the same host tree species from the same geographic location. These estimates varied from 12 OTUs on artificial diet to 144 OTUs on the preferred host (silver maple). Despite this variability, it seems that certain classes of bacteria are required for survival and de-

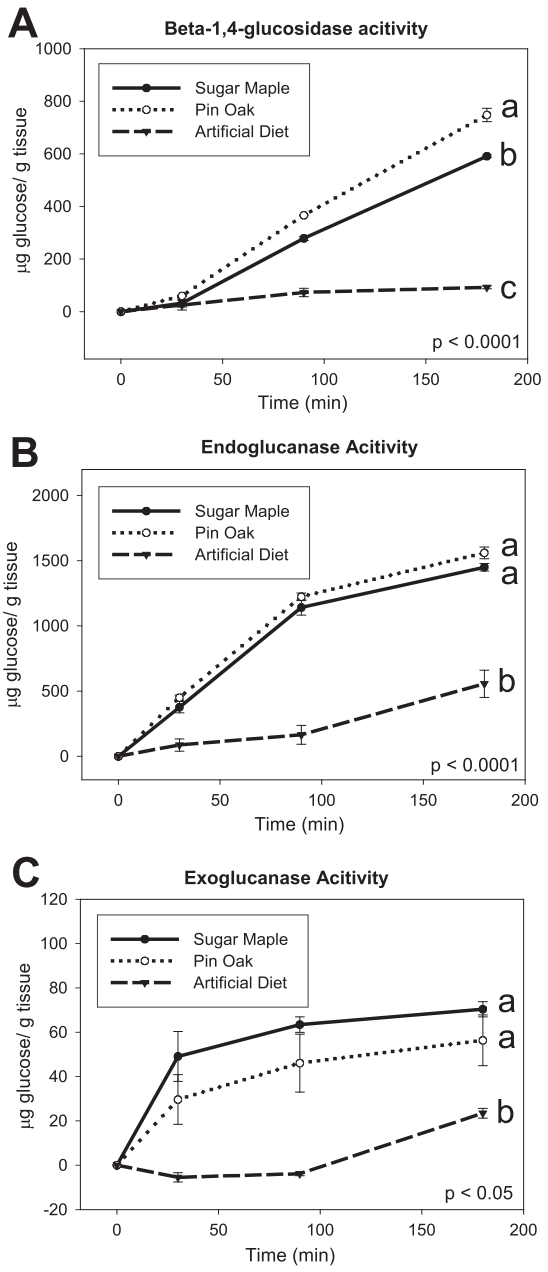

Fig. 4. Gut enzyme activity from larval *A. glabripennis* reared in sugar maple, pin oak, or artificial diet toward (A) salicin (β-1,4-glucosidase activity), (B) Avicel (exoglucanase activity), or (C) carboxymethyl cellulose (endoglucanase activity). Error bars represent ±SE, and significant differences at 180 s are denoted with different letters (ANOVA, $\alpha = 0.05$).

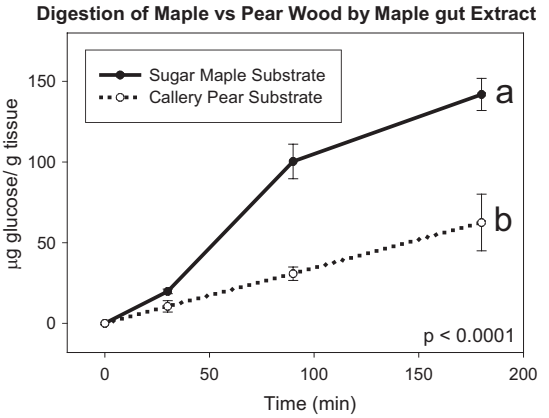

Fig. 5. Digestion of sugar maple and callery pear wood by gut extracts of sugar maple–reared *A. glabripennis*. Error bars represent ±SE, and significant differences at 180 s are denoted with different letters (ANOVA, $\alpha = 0.05$).

velopment because they were consistently present in insects fed in suitable host tree species. Our PSU colony has passed through multiple (at least 10) generations on artificial diet without adding field-collected insects to the mix. Also, these insects only encounter trees as adults in the colony, in the form of twigs for feeding. However, larvae reared only on artificial diet showed low diversity and species richness when analyzed by culture-independent methods, with the clones consisting largely of a single taxa of *Lactobacillus* that was not found in any of the other libraries. Thus, we suspect these bacteria represent an artifact of rearing on an artificial diet that contains a number of antimicrobial compounds (Table 1). They are likely not directly derived from the diet ingredients, because the ingredients are pasteurized during processing, and the diet is autoclaved and UV sterilized when made. However, casein is present in the diet, which may facilitate growth of lactobacillus species. Similar results were obtained by culture-dependent analysis on a nonrestrictive media, using both aerobic and anaerobic culture conditions (Table 2). Despite this, when bacteria were cultured on restrictive media containing cellulose as the only carbon source, low numbers of Actinobacteria were recovered that were not detectable by culture-independent analysis (Table 2).

When adults from our artificial diet–fed colony were allowed to lay eggs on preferred tree species (sugar maple and pin oak), the resulting larvae showed a broad diversity of bacteria as determined by culture-independent analysis, including a diversity of Actinobacteria, a single type of *Enterococcus*, and α-, β-, and γ-Proteobacteria (Table 1). It is not clear what the source of these bacteria are; a subset may be environmentally derived, whereas others may be vertically transmitted. Low numbers of Actinobacteria isolated from culturing guts of artificial diet–fed larvae suggest that these bacteria may be maintained in the colony at low levels through transmission from generation to generation, but vertical transmission of bacteria in this insect has not yet been studied. Interestingly, when larvae from sugar maple, with diverse gut communities, were inserted and allowed to feed on the resistant tree species (callery pear), the larval gut lacked diversity when analyzed using culture-inde-

pendent techniques, and the 16S rRNA clones consisted mostly of a single *Enterobacter* sequence (Table 1), raising the possibility of antimicrobial compounds being present in this tree species.

The NYC-derived larvae came from four trees located on Brooklyn, NY. Ten larvae from each of these trees were pooled to create a 16S rRNA clone library. The two silver maple–derived clone libraries, although they consisted of larvae from the same tree species, showed large differences in complexity and diversity of gut bacterial species. Silver maple tree-2 had by far the greatest diversity of any 16S library, having the highest number of OTUs (Table 1), classes of bacteria represented (Fig. 1), and Chao1 richness estimate (Fig. 3). In contrast, *A. glabripennis* larvae derived from silver maple tree-1 had the lowest diversity. The reason for this discrepancy is not clear; it could be because of differences in larval health or host tree quality. The trees that these larvae were collected from were urban street trees, and although it was thought that they were not treated with any insecticide that would disrupt the physiology of the insect, the complete history of the trees is not known and prior pesticide applications cannot be ruled out. At the same time, the role of insecticides on gut microbial communities has not received much attention.

Similarly, the 16S rRNA library created from larvae from a horse chestnut tree had low diversity, with many similarities to silver maple tree-1, and the same reasons can be speculated for the lack of diversity in this library as well. The clone library from sycamore maple–derived larvae showed the second highest diversity of any clone library sampled, with a high richness estimate of >50 OTUs. Previous characterization of *A. glabripennis* larval gut communities of insects collected from their native range in Hebei, China, showed similar community complexity, with bacteria across α-, β-, γ-Proteobacteria, Bacteriodtes, Actinobacteria, and Firmicutes (Schloss et al. 2006), but this study was also limited in the collection of larvae from China and the level of manipulation that could be done. Similar to what was seen between the NYC clone libraries, of the five clone libraries created from the Chinese-collected larvae, some had relatively high diversity. Samples from one field site were estimated to contain >65 OTUs based on Chao1 richness estimates, whereas libraries from another field site were estimated to contain only 10 OTUs. Plasticity of gut composition is present in both the native range and invasive range, as well as in colony-reared *A. glabripennis*, which may help explain the broad host range of this insect.

Comparisons between PSU and NYC clone libraries showed that, even after many generations on artificial diet, where the apparent "natural" bacterial community seems to be lost, shifting the insects to suitable host trees allows for reacquisition or rebalancing toward a "wild-type" bacterial community. The clone libraries from maple- and pin oak–reared PSU insects both have a broad diversity of OTUs, and, although the diversity is not as high as the most diverse clone libraries from the NYC insects, composition across bacterial classes was similar. Also, the PSU larvae were reared and developed on trees within a greenhouse, a more controlled environment, where there is less opportunity for bacteria from the environment to be present in the system.

Bacterial community composition and host tree both correlated well with cellulase activity using gut extracts from these larvae. Feeding on preferred host trees, both sugar maple and pin oak produced both high bacterial community diversity and cellulase enzyme activity. When fed artificial diet, both microbial community composition and enzyme activity were reduced. Although this does not provide direct evidence of bacterial species as the source of cellulase enzymes, it does show that larvae that have a high bacterial diversity have a greater ability to feed and develop on the woody tissue of their host tree. In addition, β-1,4-exoglucanses are not known to be produced by animals and thus are likely microbially derived in this system. The observed reduction in exoglucanase activity (Fig. 4C) correlated well with microbial community diversity, but in our analysis, we cannot determine which specific microbial species contributed to this enzyme activity. Even more insightful, when enzyme extracts from *A. glabripennis* larvae raised on sugar maple, which had high cellulase activity, were incubated with callery pear wood, the sugar released (cellulase activity) from this wood was approximately one third of the sugar released from sugar maple. The extract from sugar maple–reared larvae likely contained a high diversity of bacterial enzymes, but the ability of this extract to degrade callery pear wood was greatly reduced relative to activity on maple wood itself. This suggests that compounds could be present in callery pear that inhibit wood-degrading enzymes. It is known that callery pear contains high levels of calleryanin (Challice 1973), a phenolic glycoside, which may inhibit enzyme activity or microbial growth (Erasto et al. 2004, Das and Rosazza 2006). This may explain why *A. glabripennis* cannot develop in this tree, or it may simply be that the structure of the lignocellulose in callery pear is more difficult to degrade. This finding will be further studied to better understand host tree resistance to cerambycids.

Although we did not study the role of specific bacterial taxa in the gut community, we can postulate their role in *A. glabripennis* gut physiology based on their relationships to well-studied bacteria. For example, Actinobacteria, present in the most diverse samples and in low numbers in artificial diet–fed larvae, are likely candidates for wood degradation in *A. glabripennis*. There are many species of this group known to produce cellulases and xylanases (Lykidis et al. 2007, Park et al. 2007). Other species in this group are involved in aromatic ring degradation as per the monomeric subunits of lignin (Pasti and Belli 1985, Pasti et al. 1990). Nitrogen is limiting in wood and, interestingly, nitrogen-fixing bacteria were found in many of the 16S rRNA clone sequences. Some α-Proteobacteria including *Rhizobium*, *Ochrobactrum*, and *Rhodobacter*, as well as some γ-Proteobac-

teria including *Enterobacter*, are capable of fixing nitrogen (Potrikus and Breznak 1977, Ngom et al. 2004, Rasolomampianina et al. 2005), and may be playing this role in the *A. glabripennis* gut. Nitrogen fixation has been observed in termites, and nitrogen-fixing bacteria have been found in bark beetles and other cerambycids (Breznak et al. 1973, Bridges 1981). Overall, the *A. glabripennis* gut community is similar to that of other wood-feeding organisms and seems to be suitable for feeding and growing on lignocellulose (Breznak and Brune 1994, Cazemier et al. 1997, Dillon and Dillon 2004, Delalibera et al. 2005).

Anoplophora glabripennis is an invasive species, with a broad host range in both its native range, as well as in North America. Here, the plasticity of the larval gut community was shown in both field-collected and colony-derived insects, with a direct correlation between bacterial community diversity and gut cellulase activity. Further studies elucidating the roles of the bacterial community in the *A. glabripennis* gut may lead to identification of novel microbial species and the discovery of new enzymes involved in the degradation of hardwood lignocellulose by this wood-feeding beetle. Understanding the role of the gut community will provide new information on the gut physiology of *A. glabripennis*, which could lead to development of novel tactics for controlling wood boring insects by determining means to disrupt this community. These discoveries can also lead to the utilization of novel enzymes to increase the efficiency of conversion of cellulosic biomass, particularly wood, into ethanol, and may have other industrial applications as well.

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