EXPLORATION FOR FACULTATIVE ENDOSYMBIONTS OF SHARPSHOOTERS

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ABSTRACT
Glassy-winged sharpshooters (GWSS) were collected in California and several states in the southeastern US in 2002 and 2003 to search for pathogenic or beneficial bacteria of GWSS. Various tissues were examined for the presence of bacteria by PCR: hemolymph, eggs, and bacteriomes. Hemolymph could contain primary and secondary symbionts, either resident in hemolymph or in transit to other tissues. Eggs were expected to contain any transovarially transmitted microorganisms, described or otherwise, and bacteriomes were expected to contain at least the primary symbionts. A small subset of hemolymph and egg samples were cloned and sequenced based on unique digest patterns of their extracted 16s DNA. Cloned sequences were identified as belonging to the primary symbiont *Baumannia* (in eggs and hemolymph) and *Acinetobacter* and *Stenotrophomonas* (in hemolymph). *Wolbachia* was also cloned from hemolymph, and subsequently found in 62% (24/39) of egg, 8% (5/66) of hemolymph, and 76% (31/41) of bacteriome samples by PCR.

INTRODUCTION
Most Homoptera so far examined, including the leafhoppers (Cicadellidae), are hosts to endosymbiotic bacteria that live within the body cavity of the host insect (Buchner 1965; Douglas 1994; Moran and Baumann 2000). Some endosymbionts ("primary" symbionts), have a mutually obligate association with their host: they are housed in specialized host cells, bacteriocytes (= mycetocytes) within a bacteriome (collection of bacteriocytes); are transovarially (i.e., vertically) transmitted from mother to offspring, and cannot be cultivated outside of the host. In aphids, the primary symbionts (*Buchnera*) have been shown to provide the host with essential nutrients and are necessary for the proper development of the host (e.g., Douglas 1998). Little is known about the specific role of leafhopper primary symbionts. Suggestions include roles in osmoregulation, respiration, circadian rhythms (Schwemmler 1980) and nutrition (Douglas 1988). Recent work on the bacteriome-associated symbionts of GWSS described two bacteria, one of which has been partially sequenced and placed in the γ-Proteobacteria, (named *Baumannia cicadellinicola)*, the second being a Flavobacteria (Moran et al. 2003).

Primary symbionts are of great interest in terms of their effects on their hosts. However, of interest for this work are bacterial associates that are facultative (also referred to as "secondary"), i.e., that occur in some individuals or populations but not others, and that could be introduced into, or be augmented in, pest populations. Facultative bacterial associates have been described in a variety of Homoptera such as mealybugs (Fukatsu and Nikoh 2000), psyllids (Thao et al. 2000), aphids (Buchner 1965, Chen et al. 1996, Fukatsu et al. 2000, 2001, Sandstrom et al. 2001) and leafhoppers (Swezy and Severin 1930, Schwemmler 1974, McCoy et al. 1978, Purcell et al. 1986). The potential importance of these long-unrecognized microbial associates has become increasing clear from recent studies with aphids, where the effects of facultative symbionts varied from positive to negative, and affected host plant suitability (Chen et al. 2000, Leonardo and Muiru 2003), susceptibility to high temperatures (Chen et al. 2000, Montllor et al. 2002) and to parasitism (Oliver et al. 2003), often at dramatic levels.

We assume that similar bacterial symbionts and associated bacteria in leafhoppers such as sharpshooters are likely to be as common as in aphids and also to have large and important effects on the population biology of their hosts. Molecular tools have recently made their detection and characterization more feasible (Moran et al. 2003). A cultivable, facultative symbiont (called BEV) that occurs in the leafhopper *Euscelidius variegatus* was transmitted transovarially from mother to offspring (Purcell et al. 1986). BEV reduced fecundity by 80%, doubled development time, and increased mortality of its host (Purcell et al. 1986, Purcell and Suslow 1987). In addition to being transovarially transmitted, BEV was transmitted efficiently among *E. variegatus* leafhoppers (i.e., "horizontally") via feeding within plant tissues (Purcell et al. 1994).

Facultative symbionts could be convenient agents for introducing genetic materials into leafhopper populations. Advances in molecular biology have made the prospects of identifying, isolating, and manipulating even non-cultivable symbionts realistic. Essentially, symbionts that have a high rate of maternal transmission represent a type of cytoplasmically-inherited genome that may be more amenable to molecular manipulations than leafhopper chromosomal genes. Therefore it is of major importance to better understand these potentially useful bacteria in GWSS and related leafhoppers.

OBJECTIVES

1. Survey glassy-winged sharpshooter and other sharpshooters in California and the southeastern U.S. for facultative bacterial endosymbionts.
2. Determine by DNA sequencing the identity of any bacteria discovered.
3. Depending on type of microorganism and relative frequency in surveyed insects, select candidate symbionts to (a) attempt to culture, (b) determine whether they can be transmitted by injection of hemolymph from infected to uninfected GWSS or to other sharpshooter species, (c) determine whether they are transovarially transmitted, (d) determine whether they can be horizontally transmitted through plants and (e) determine whether any are beneficial or pathogenic to GWSS in terms of life history traits (growth, fecundity, longevity, parasitism).

RESULTS AND CONCLUSIONS

GWSS hemolymph samples from California and the southeast collected in 2002 and 2003 were extracted and examined by PCR for eubacterial 16s ribosomal DNA (Table 1).

Table 1. ***GWSS tissues sampled for bacterial DNA—collection location and date.***

LOCALITY	DATE	TISSUES[1] (NO. OF SAMPLES)	TOTAL NO. EXTRACTS
Bakersfield CA	Apr 2002 Aug 2002 June 2003	H (2) , B (3) H (25), B (14), E (10) H (18)	72
Riverside CA	Apr 2002 Oct 2002	H (3), B (5) H (9), B (2)	19
Baton Rouge LA	May 2002 Sept 2002	H (39), B (20), E (14) H (5), B (4)	82
Pearl River LA	June 2003	H (15), B (5),E (5)	25
Quincy FL	May 2002 Aug 2002	H (25), B (9), E (7) H (18)	59
Crestview FL	June 2003	H (24), B (8),E (19)	51
Tallahassee FL	June 2003	H (9), E (3)	12
Dothan AL	June 2003	H (9), E (3)	12
State Line AL	June 2003	H (6)	6
Martinville MS	June 2003	H (8), E (1)	9
McComb MS	June 2003	H (6), E (3)	9

[1]H=hemolymph, B=bacteriocytes, E=eggs

Forty-three percent of hemolymph samples from all localities tested positive for eubacterial 16sDNA by PCR. Twenty-six individuals of another four species of sharpshooters from California were also tested for bacteria in hemolymph, of which five (19%) were positive by PCR. DNA from a total of 12 GWSS tissue samples was chosen for cloning, and four produced multiple transformed *E. coli* colonies with 16s rDNA inserts. DNA from 11 of these colonies was chosen for sequencing. The most common sequence was identical to that of *Baumannia*, a recently described bacteriome-associated symbiont of the GWSS (Moran et al. 2003), which was cloned from hemolymph and eggs of GWSS from Louisiana and Florida (Table 2). Like other bacteriome inhabitants, *Baumannia* is presumably transovarially transmitted from mother to offspring via hemolymph (Buchner 1965). *Wolbachia*, a commonly found facultative symbiont of many insects, including GWSS (Moran et al. 2003), was also cloned from hemolymph of a California GWSS.

Table 2. ***Cloned bacterial DNA from GWSS tissue samples.***

COLLECTION LOCATION (SAMPLE / NO. CLONES SEQUENCED)	GWSS TISSUE	16S RDNA SEQUENCE IDENTITY OF INSERTS
Bakersfield (UC2/ 2)	hemolymph	*Wolbachia, Acinetobacter*
Louisiana State Univ (L20-8/ 5)	hemolymph	*Baumannia, Stenotrophomonas*
Crestview FL (CF7/ 2)	eggs	*Baumannia*
Pearl River LA (PRE/ 2)	eggs	*Baumannia*

DNAs from two additional bacteria not previously described from GWSS were also cloned from our samples (Table 2): *Acinetobacter* and *Stenotrophomonas* are aerobic γ-Proteobacteria, and not uncommon as environmental contaminants and nosocomial pathogens (e.g., Towner et al. 1991, Ribbeck et al. 2003). However, both have also been isolated from ticks and fleas (Murrell et al. 2003); and *Stenotrophomonas*, among other bacteria, was isolated from the guts of ants, where it was presumed to provide nutrients and to be passed to offspring (Jaffe et al. 2001). *Stenotrophomonas* was also described as an endosymbiont of a fly (Otitidae), which failed to develop properly without its complement of bacteria (Wozniak and Hinz 1995).

After isolating *Wolbachia* from GWSS, we surveyed 146 of our extracts by PCR and detected this bacterium in 62% (24/39) of egg, 8% (5/66) of hemolymph, and 76% (31/41) of bacteriome samples by PCR. We will assess the frequency of the other bacteria we have detected in GWSS samples to date, and continue to look for additional facultative symbionts in extracted material and new collections. Finding a facultative symbiont of GWSS could impact the biological control of GWSS if such symbionts could be manipulated or eliminated from populations of GWSS.

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