Microsatellite Markers Reveal a Predominant Sugarcane Aphid (Homoptera: Aphididae) Clone is Found on Sorghum in Seven States and One Territory of the USA

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ABSTRACT

The sugarcane aphid (*Melanaphis sacchari*) has become a serious pest causing severe economic losses to sorghum [*Sorghum bicolor* (L.) Moench] grown in the southern United States. Since its original detection in four states in 2013, *M. sacchari* on sorghum has now, in 2016, spread to 19 states. The presence of one or multiple genotypes on sorghum in the United States has not yet been established. In this study, genome sequencing of *M. sacchari* was used to develop microsatellite markers. A total of 8,665,267 reads and 1.44 Gb of nucleotide sequences were generated, and 79.6% of the reads were from *M. sacchari*. *Melanaphis sacchari* DNA from 46 samples from 17 locations across seven states and one US territory was polymerase chain reaction (PCR) amplified using 38 newly created microsatellite markers, as well as 14 published microsatellite markers. Genotyping with the 52 microsatellite markers indicated that the samples of *M. sacchari* on sorghum were all one genotype, with the exception of a single sample collected from Sinton, TX, which had the predominant genotype as well as another genotype. Genotyping of the aphid samples with 12 microsatellite markers for *Buchnera aphidicola*, the obligate aphid symbiont, had nearly identical results. The invasive *M. sacchari* on sorghum appears to be spreading in the United States on sorghum as primarily one asexual clone.

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Abbreviations: GC, guanine-cytosine; MLL, multilocus lineages; PCR, polymerase chain reaction; SSR, simple sequence repeat; UPGMA, unweighted pair group method with arithmetic mean.

THE sugarcane aphid (*Melanaphis sacchari*) has worldwide geographical distribution being located in over 30 countries and has followed the cultivation of sorghum [*Sorghum bicolor* (L.) Moench] and sugarcane (*Saccharum* spp.) (Singh et al., 2004). Worldwide aphid samples collected in 2002 to 2009 revealed five

multilocus lineages (MLL) based on geography but not host plant (Nibouche et al., 2014). These five MLL were MLL-A from Africa, MLL-B from Australia, MLL-C from South America, the Caribbean, Reunion Island (Nibouche et al., 2015), and the Indian Ocean including East Africa, MLL-D from the United States, and MLL-E from China. Multilocus lineages A and C had a wide geographic distribution and matched the definition of a "superclone" (Nibouche et al., 2014). Interestingly, Nibouche et al. (2015) examined sugarcane aphid samples collected in Reunion Island and showed the existence of host plant specialization despite low genetic differentiation.

In the United States, prior to 2013, *M. sacchari* had a long-established association with sugarcane and was largely isolated to Florida, Hawaii, Louisiana, and Texas, although occasional reports of infestation on sorghum occurred as early as 1922 in Florida (Wilbrink, 1922; Denmark, 1988). In 2013, *M. sacchari* was found infesting grain sorghum in four US states (Texas, Louisiana, Oklahoma, and Mississippi) and northern Mexico (Villanueva et al., 2014). The aphid has rapidly spread in all directions and, as of 2016, has been found in 19 states. It is unknown if a new *M. sacchari* genotype(s) was introduced to the United States, or if the previous aphid genotype(s) mutated, or if sexual reproduction has occurred, allowing a host shift and thus causing the current outbreak of the aphid on sorghum.

An understanding of the number of *M. sacchari* genotypes currently present in the United States is important for identifying resistant sorghum plants and for insecticide use. Resistant plants are often only resistant to certain aphid biotypes such as greenbug (*Schizaphis graminum*) resistance in sorghum (Katsar et al., 2002), and the efficacy of insecticides can be biotype specific (Sloderbeck et al., 1991). Two products, Transform (Dow AgroSciences, 50% sulfoxaflor) and Sivanto Prime (Bayer Crop Science, 17.09% flupyradifurone) are available for use to control the sugarcane aphid in the United States (Bowling et al., 2016)

Simple sequence repeats (SSRs), also called microsatellites, are short elements consisting of tandem repeat units of one to six base pairs in length that are present in eukaryotic genomes and, to a lesser extent, in prokaryote genomes (Toth et al., 2000). Simple sequence repeat markers are polymerase chain reaction (PCR)-based markers that consist of primers that usually flank these tandemly repeated regions. They are frequently used for diversity studies due to their high information content, relative abundance, wide dispersal across the genome, codominant inheritance, high polymorphism, locus specificity, high rate of transferability across species of the same genera, and being easily scored (Tautz, 1989; Wang et al., 2009). Simple sequence repeat markers have been previously used to examine aphid diversity (Weng et al., 2007; Jun et al., 2012). Prior to this study, only 14 SSR markers have been created for *M. sacchari*

(Molecular Ecology Resources Primer Development Consortium et al., 2010). Previously, the generation of a high number of SSR markers had been technically challenging and costly (Csencsics et al., 2010). Recently, next-generation sequencing platforms such as Illumina's Genome Analyzer, Applied Biosystems's SOLiD, and Roche's 454 GS FLX have been used to generate large numbers of sequences that can be mined for the development of genetic markers (Davey et al., 2011; Jun et al., 2012).

Aphids harbor symbionts that are essential for aphid survival or can improve their fitness and thus enhance their distribution. The bacterium *Buchera aphidicola*, which is considered an essential endosymbiont, provides nearly all aphids with essential amino acids and vitamins that supplement their nutrient-poor diet of plant-phloem sap (van Ham et al., 2003). These essential bacteria are referred to as primary symbionts. Additionally, aphids can harbor additional maternally transmitted bacteria called secondary symbionts that are considered nonessential and are generally found at intermediate frequencies within the host population (Russell and Moran, 2006). Secondary symbionts, such as the bacteria *Hamiltonella defensa* and *Serratia symbiotica*, can protect the aphid from endoparasitoid wasps and thus reduce aphid mortality (Degnan et al., 2009). Because genetic sequences of *M. sacchari* cannot be obtained without including sequences from these associated organisms, these symbiont and pathogen sequences must be filtered out to obtain only those sequences that are representative of *M. sacchari*.

In this study, we used high-throughput sequencing of the sugarcane aphid DNA to develop additional microsatellite markers to determine the number of *M. sacchari* genotypes present on sorghum in 17 locations, which encompass seven states and one territory of the United States. We further confirmed these results by examining the genetic similarity of the endosymbiont *B. aphidicola* in all aphid samples.

MATERIALS AND METHODS
Aphid Sampling and DNA Isolation

Melanaphis sacchari samples used for sequencing were obtained from the lower leaves of *S. bicolor* hybrid K73-J6, which was grown at the Bellflower Farm (Tifton, GA). Aphids were pooled and placed into 2-mL collection tubes and stored at −80°C until DNA extraction. Four zinc-plated BBs (Daisy Outdoor Products) were added to each tube, and samples were then ground into a fine powder using a vortex mixer. Samples were kept frozen using liquid nitrogen during the grinding process by continually placing the samples back into the liquid nitrogen. The DNA was extracted using a GeneJET Plant Genomic DNA Purification Kit (Thermo Fisher Scientific) by following the manufacturer's recommendations, except aphids were used instead of plant tissue. The purified DNA was visualized on a 1% agarose gel to confirm quality and was quantified using a NanoDrop 2000c (Thermo Fisher Scientific).

Aphids were collected within the United States and its territories from August to November 2015 (Supplemental Table 1, Fig. 1). Each aphid collector removed multiple sorghum leaves infested with aphids from three areas from each field plot, if possible, and placed aphids collected in each area into separate Ziploc style bags containing wet paper towels. Aphids were shipped overnight to Tifton, GA, and all alive aphids from each Ziploc bag (sample) were immediately placed into a 2-mL collection tube using a paintbrush. The paintbrush and forceps used were bleached (6% sodium hypochlorite) and rinsed with water between each sample. Samples were then stored at −80°C. Aphid samples from Puerto Rico were shipped dead (placed in 2-mL tubes containing 95% ethanol and stored at −80°C until shipment). The DNA was extracted from each sample as described above. The DNA samples were aliquoted into strip tubes to form an array, and the aphid sample used for high-throughput sequencing (originating from Tifton, GA, sample labeled on the dendrogram as "Tifton_GA10", Supplemental Table 1) was included four times throughout the array to ensure PCR amplification reliability (i.e., an identical banding pattern was generated for each of the repeated samples). Additionally, five samples containing only water were present in the array.

Construction of the Genomic Library and Ion PGM Sequencing

To construct a DNA library (400 bp target reads) for *M. sacchari*, genomic DNA (1 µg) was digested for 4 min to obtain the desired fragment length using the Ion Xpress Plus gDNA Fragment Library Preparation kit (Life Technologies). After library preparation, the library was quantified using the Ion Library Quantitation Kit (Life Technologies). The quantified library was diluted to 20 pM for template preparation using the Ion PGM Template OT2 400 Kit (Life Technologies). The prepared library was loaded onto an Ion 318 Chip v2 (Life Technologies) and sequenced on the Ion PGM System (Life Technologies) using the Ion PGM Sequencing 400 Kit (Life Technologies).

De Novo Assemblies and Microsatellite Identification

Raw sequencing reads were trimmed using the default settings of the quality trimming option in CLC Genomics Workbench (Qiagen). Prior to de novo assembly, potential reads belonging to organisms with symbiotic relationships or pathogens of *M. sacchari* were removed by filtering using the map reads to reference option with the default settings in CLC Genomics Workbench (Qiagen). The following reference sequences were downloaded from the National Center for Biotechnology Information (NCBI) and used for filtering: *B. aphidicola* (NC_002528.1; Shigenobu et al., 2000), *H. defensa* (NC_012751.1; Degnan et al., 2009), *Regiella insecticola* (ACYF01; ACGA01; Degnan et al., 2010), *Rickettsia bellii* (NC_007940.1; NC_009883.1; Ogata et al., 2006), *S. symbiotica* (AENX01; Burke and Moran, 2011), *Spiroplasma mirum* (NZ_CP002082.1; unpublished), and *Verticillium* spp. (ABJE01; ABPE01; CVQH01; JPET01; Klosterman et al., 2011). All filtered reads were assembled de novo into contigs by first assembling reads into contigs and then mapping reads back to the contigs in the CLC Genomics Workbench (Qiagen). The default parameters were used for all assemblies, and the minimum contig size was 1000 bp for *M. sacchari* and 500 bp for the symbionts and pathogen. To ensure that the *M. sacchari* assembly did not contain *S. bicolor* sequencing reads, the *S. bicolor* reference genome version 181 (updated 23 Dec. 2010) was downloaded from the genome database (http://www.plantgdb.org/XGDB/phplib/download.php?GDB=Sb). The *S. bicolor* reference genome (Paterson et al., 2009) was used to check if contamination could arise from aphid feeding on the host. Assembled reads from all species were mapped to the *S. bicolor* genome using the default parameters in CLC Genomics Workbench. Only contigs with ≥10× coverage were searched for microsatellite motifs (di- to hexa-nucleotide) for *M. sacchari* and *B. aphidicola*. Microsatellites were identified and primer pairs were designed with BatchPrimer3 (You et al., 2008) using default settings except for the following primer parameters: minimum and optimum primer Tm of 55.0 and 60.0°C; maximum Tm difference between primer pairs of 5.0°C; optimum primer guanine–cytosine content (GC%) of 40%.
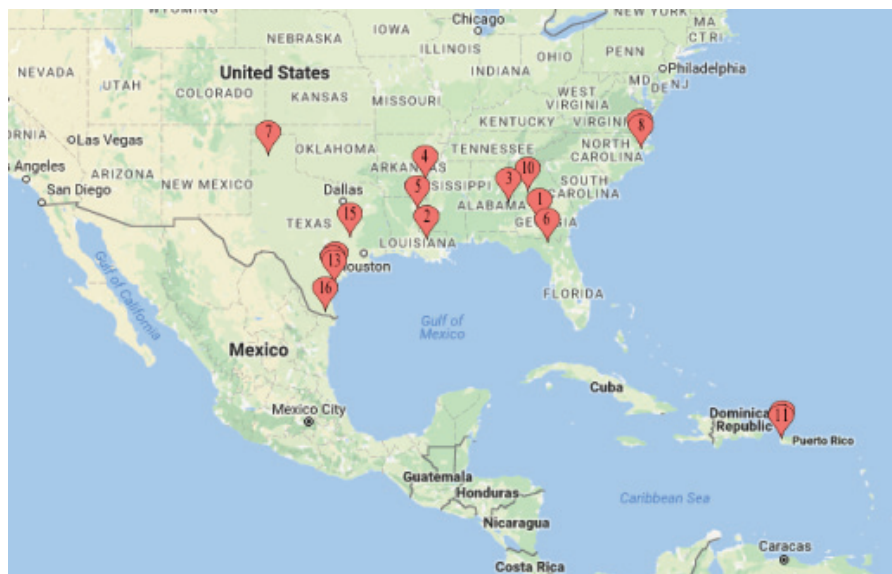

Fig. 1. The 17 locations where sugarcane aphids were collected from sorghum in the United States and its territories. The location numbers are (1) Tifton, GA, (2) Baton Rouge, LA, (3) Loachapoka, AL, (4) Watson, AR, (5) Winnsboro, LA, (6) Live Oak, FL, (7) Amarillo, TX, (8) Pantego, NC, (9) Plymouth, NC, (10) Griffin, GA, (11) Mayaguez, PR, (12) Isabela, PR, (13) Corpus Christi, TX, (14) Refugio, TX, (15) College Station, TX, (16) Weslaco, TX, and (17) Sinton, TX.

The de novo assembly sequence data for *M. sacchari* were deposited into the Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) of the NCBI under the accession number SRP072451. The BioProject ID is PRJNA316592.

Microsatellite Characterization and Data Analyses

Melanaphis sacchari and *B. aphidicola* primers were selected that amplify a microsatellite with a motif length that spans 15 to 27 bp and 12 to 18 bp, respectively. Furthermore, 14 microsatellite markers that had been previously identified for *M. sacchari* were amplified as well (Molecular Ecology Resources Primer Development Consortium et al., 2010). The microsatellite markers were amplified from the 46 *M. sacchari* aphid DNA samples, as well as single samples of *Rhopalosiphum maidis* (corn leaf aphid) and *Sipha flava* (yellow sugarcane aphid), which were used as outgroups (Supplemental Table 1).

Each microsatellite marker was amplified for each aphid sample using a 10-μL reaction volume that contained 2 μL of 5× Clear GoTaq reaction buffer (Promega), 1 μL of 25 mM $MgCl_2$, 0.8 μL of 2.5 mM deoxynucleotide mix, 0.5 μ of M13-tagged forward primer, 2.0 μL of reverse primer at 1 μM, 1.8 μL of 1 μM M13 primer (M13-TGTAAAACGACGGCCAGT) fluorescently labeled with the IRDye 800 CW fluorophore (Eurofins MWG Operon), 0.04 μL of GoTaq DNA polymerase (Promega), 0.86 μL of sterile water, and 1 μL of 2.5 ng μL^{-1} DNA. Thermocycler conditions were: an initial denaturation at 94°C for 3 min, 39 cycles of 94°C for 30 s, 50°C for 1 min (except for marker MS9 that had an annealing temperature of 55°C), 72°C for 1 min and 10 s, and a final elongation step at 72°C for 10 min. The thermocyclers used were a Gene Amp PCR System 9700 dual and single blocks (Applied Biosystems) and T100 Thermal Cyclers (Bio-Rad). The individual PCR products (2 μL) were combined with 5 μL of Blue Stop (LI-COR Biosciences), and 0.35 μL of this mixture was loaded on a 6.5% (v/v) acrylamide gel using a LI-COR Biosciences 4300 DNA Analyzer. Gel images were scored visually and coded as a "1" for the presence of a band, "0" for the absence of a band, or "9" for missing (failed reaction) for each accession for each fragment.

For the unweighted pair group method with arithmetic mean (UPGMA) cluster analysis, the binary marker data matrix was imported into NTSYSpc (Rohlf, 2008). Genetic similarity between each pair of samples was calculated using the SIMQUAL module using the DICE coefficient of similarity (Nei and Li, 1979). A dendrogram was generated from the similarity matrix by using the UPGMA procedure in the SAHN module of NTSYSpc. The cophenetic correlation was calculated by using the COPH and MXCOMP modules in NTSYSpc. The software program FreeTree (Hampl et al., 2001) was used to do the bootstrapping analysis, and the number of repetitions was 1000. Only bootstrap values ≥50% are shown (Fig. 2 and 3).

RESULTS
Ion Torrent PGM Sequencing Output and Assembly

After quality trimming, a total of 8,665,267 reads and 1.44 Gb of nucleotide sequences were generated from sequencing with two Ion 318 Chips (v2) on the Ion PGM System (Table 1). Because most aphid species have associations with microbes, we removed potential symbiont reads prior to de novo assembly of the *M. sacchari* reads. *Melanaphis sacchari* reads were filtered using the reference genome sequences of the symbiont or pathogen genomes of *B. aphidicola*, *H. defensa*, *R. insecticola*, *R. bellii*, *S. symbiotica*, *S. mirum*, and *Verticillium* spp. Additionally, to ensure that only *M. sacchari* reads were included in the assembly, we removed potential *S. bicolor* reads. This was performed because it was unknown if *S. bicolor* DNA was present inside of *M. sacchari* after feeding on the host. However, no *S. bicolor* reads were detected.

The summary of the de novo assembly for the symbionts and *M. sacchari* are reported in Table 1. After filtering of the symbiont reads, 79.6% of the reads were from *M. sacchari*. The largest percentage of the symbiont reads were from *Verticillium* spp. and *B. aphidicola* with 5.6 and 5.3%, respectively. The GC content of the reads was the lowest for *B. aphidicola* at 26.9% and the highest at 53.7% for *Verticillium* spp. The *M. sacchari* reads were assembled de novo into 49,129 contigs with an average length of 1663 bp and a N50 of 1636 bp, of which N50 is the weighted median statistic such that 50% of the entire assembly is contained in contigs or scaffolds equal to or larger than this value. In contrast, the de novo assemblies for the symbiont reads ranged from 5 (*S. symbiotica*) to 287 (*B. aphidicola*) contigs. Although *Verticillium* spp. comprised the greatest number of symbiont reads (489,229), only 11 contigs resulted from the assembly. This may be due to a lack of an available reference genome for *V. lecanii* or other *Verticillium* spp. The symbiont or pathogen DNA may have been digested for too long during genomic library construction, resulting in the high number of short reads (>95% of reads were 15–50 bp). We found similar results of short read lengths for all symbionts and the pathogen except for *B. aphidicola* (Supplemental Fig. 1).

Microsatellite Discovery

Contigs (≥10× coverage, *n* = 2424) from *M. sacchari* were screened for microsatellite motifs, and a total of 1455 were detected (Table 2). Tri- and tetra-nucleotide motifs were the most common microsatellite detected, with 70.6% of the motifs in these two classes (38.1 and 32.5%, respectively), followed by the penta-nucleotide and di-nucleotide motifs at 15 and 11.3%, respectively. Lastly, 3% of the microsatellites detected were hexa-nucleotide motifs. A total of 1179 primer pairs were designed for *M. sacchari*. Microsatellites were also detected for *B. aphidicola*, and tetra-nucleotide motifs were the most common in *B. aphidicola* (63.6%) (Table 2). A total of 30 primer pairs were designed for *B. aphidicola*.

For many plant species, as well as humans, a higher degree of polymorphism has been observed in Class I microsatellites (repeat length is ≥20 bp) than in Class II
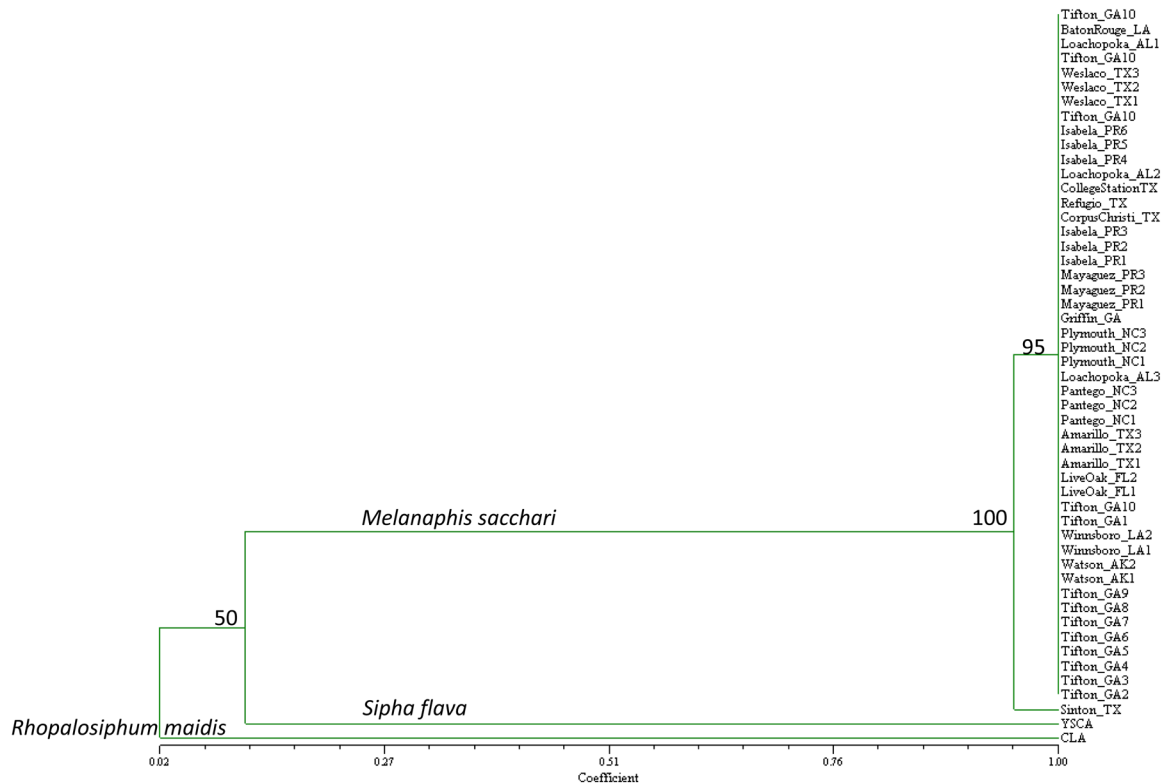
Fig. 2. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram of the 48 sugarcane aphid (*Melanaphis sacchari*), corn leaf aphid (CLA, *Rhopalosiphum maidis*), and yellow sugarcane aphid (YSCA, *Sipha flava*) samples analyzed with 52 microsatellite markers. The values for the *x*-axis are the DICE similarity coefficient values. Only bootstrap values (the numbers above the branches) of ≥50% are shown. Collection information for each sample is listed in Supplemental Table 1.
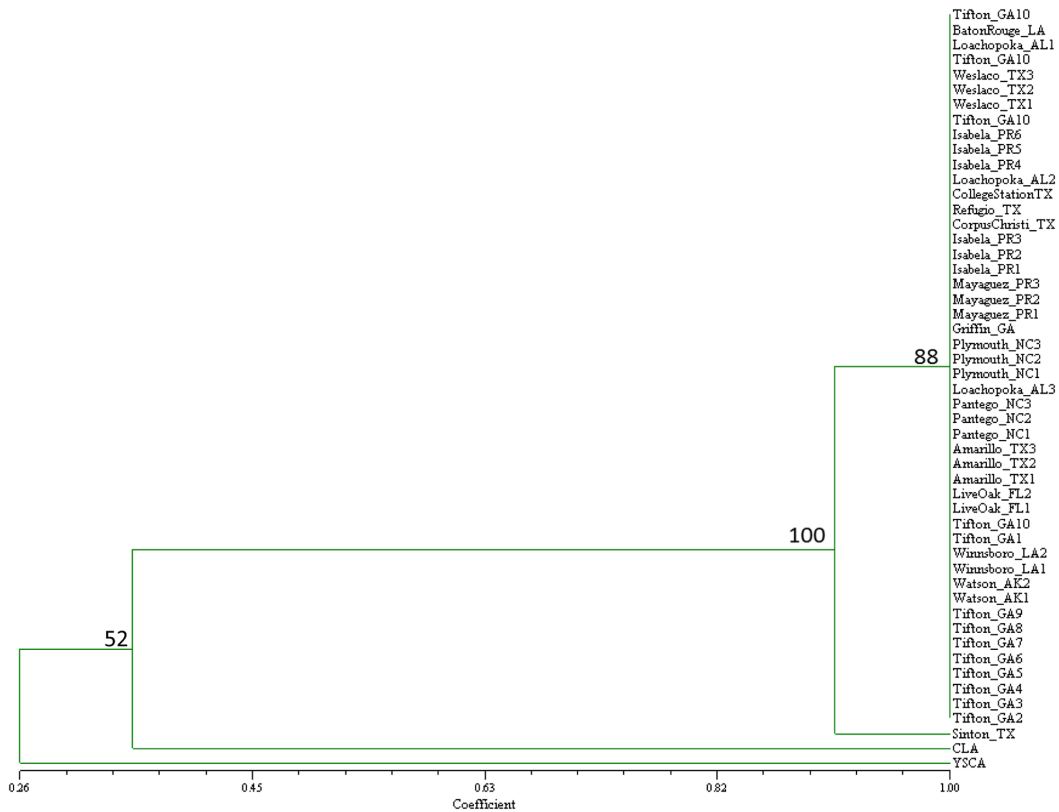

Fig. 3. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram of the 48 *Buchnera aphidicola* samples, an endosymbiont of the sugarcane aphid (*Melanaphis sacchari*), corn leaf aphid (CLA, *Rhopalosiphum maidis*), and the yellow sugarcane aphid (YSCA, *Sipha flava*) analyzed with 12 microsatellite markers. The values for the *x*-axis are the DICE similarity coefficient values. Only bootstrap values (the numbers above the branches) of ≥50% are shown. Collection information for each sample is listed in Supplemental Table 1.

Table 1. Assembly metrics from sequencing of *Melanaphis sacchari* and filtering of symbiont and pathogen genomes.

Organism	No. of reads	Reads	Total length of reads	GC%	No. of contigs	Total length of contigs	Average contig length	N50
		%	bp	%		————————— bp —————————		
Buchnera aphidicola	457,327	5.3	65,545,941	26.9	287	447,514	1,559	2,013
Hamiltonella defensa	179,123	2.1	11,030,746	34.6	34	31,612	930	825
Regiella insecticola	175,551	2.0	13,630,870	41.0	31	31,132	1,004	950
Rickettsia bellii	182,871	2.1	7,868,530	41.9	9	8,359	929	1,226
Serratia symbiotica	76,914	0.9	4,606,860	42.7	5	7,064	1,413	1,999
Spiroplasma mirum	204,364	2.4	8,412,639	44.8	6	6,196	1,033	1,323
Verticillium spp.	489,229	5.6	21,259,425	53.7	11	9,087	826	1,099
Melanaphis sacchari	6,899,888	79.6	1,306,363,947	30.6	49,129	81,700,192	1,663	1,636

Table 2. Microsatellite screening of contigs from the sugarcane aphid (*Melanaphis sacchari*) and associated symbiont *Buchnera aphidicola*.

Organism	No. of contigs	No. microsatellites (primer pairs designed)	Nucleotide repeat motif				
			Di	Tri	Tetra	Penta	Hexa
Melanaphis sacchari	2424	1455 (1179)	165	555	473	218	44
Buchnera aphidicola	287	33 (30)	2	7	21	2	1

microsatellites (repeat length is 12–19 bp) (Temnykh et al., 2001). Similarly, the Molecular Ecology Resources Primer Development Consortium et al. (2010) identified a greater number of alleles from the worldwide sugarcane aphid population from Class I microsatellites than Class II. Thus, 38 primer pairs were selected (Supplemental Table 2) for *M. sacchari* that amplified the microsatellites with the longest repeat lengths, ranging from 15 to 27 bp.

Genotyping of Collected Sugarcane Aphid Samples

The 38 primer pairs selected for microsatellite amplification, as well as 14 previously identified *M. sacchari* primer pairs (Molecular Ecology Resources Primer Development Consortium et al, 2010), were used to amplify DNA from all 46 *M. sacchari* samples that were collected from 17 locations representing seven states and one US territory (Supplemental Table 1). Two additional aphid species that are commonly found on sorghum, the corn leaf aphid (*R. maidis*) and the yellow sugarcane aphid (*S. flava*), were included as outgroups. Many of these primers amplified fragments in *R. maidis* and *S. flava* DNA samples (Supplemental Table 3) and may be useful for genetic studies for these aphids. The number of scored alleles (number of polymorphic alleles generated for all the aphid species) ranged from 1 to 8 (Supplemental Table 3). The number of alleles detected per *M. sacchari* sample for each marker ranged from one to three, with one and two alleles being the most frequent (42 and 20 markers, respectively), with the exception of the sample from Sinton, TX (Supplemental Table 3), which had as many as four alleles. One marker, MS37, generated three alleles and is likely amplifying multiple loci. For five markers (MS9, MS14, MS37, CIRMSB09, and CIRMSD02), the Sinton, TX, sample had the same alleles as the other *M. sacchari* samples, as well

as one to two additional alleles (Supplemental Table 3), suggesting that this sample is not clonal but rather a mixture of *M. sacchari* genotypes. Because most aphid samples had primarily one to two alleles per marker, this suggests that *M. sacchari* is likely a diploid, which is in agreement with previous aphid studies (Hales et al., 1997).

The UPGMA cluster analysis of the 127 scored fragments generated using *M. sacchari* markers from the 48 samples indicated that a single *M. sacchari* genotype is predominant on sorghum in the seven US states and Puerto Rico (Fig. 2). The matrix correlation was *r* = 0.99, indicating that the UPGMA clustering summarized the DICE similarity matrix very well.

The three aphid species we used in this study that are commonly found on sorghum, *M. sacchari*, *R. maidis*, and *S. flava*, had very few common alleles (Fig. 2). Using the *M. sacchari* SSR markers, *Sipha flava* and *M. sacchari*, *S. flava* and *R. maidis*, and *M. sacchari* and *R. maidis* were only 12, 7, and 2% genetically similar, respectively.

The genetic relationships among aphid samples containing *B. aphidicola*, an obligate aphid endosymbiont, were examined using 12 microsatellite markers (Supplemental Table 2, BA1- BA12) for all the aphid samples (Supplemental Table 1). Analysis of the 19 polymorphic fragments generated from the 12 microsatellite markers indicated that relationships among *B. aphidicola* samples were similar to those among samples of *M. sacchari* (Fig. 3). All *M. sacchari* samples had the same *B. aphidicola* genotype except the Sinton, TX, sample, which had an additional allele. The finding suggests that a mixture of aphids and thus a mixture of B. *aphidicola* genotypes exist for this sample. The genetic similarity between *B. aphidicola* obtained from the *M. sacchari* sample collected from Sinton, TX, and *B. aphidicola* obtained from aphid samples collected from other locations was 91%. This symbiont data supports

that a mixture of aphid genotypes exist for the Sinton, TX sample. The *R. maidis* sample (labeled as "CLA" on the dendrogram) and the *S. flava* sample (labeled as "YSCA" on the dendrogram) when compared with the predominant *M. sacchari* genotype had a *B. aphidicola* genetic similarity of 35 and 27%, respectively. From the *B. aphidicola* SSR markers, the *S. flava* aphid sample and *R. maidis* samples contained *B. aphidicola* that had a genetic similarity of 0%, suggesting these two species have symbiotic *B. aphidicola* bacteria that are extremely different.

DISCUSSION

A single predominant genotype, over a large geographic area, is consistent with an absence of sexual reproduction (Balloux et al., 2003) and suggests that parthenogenesis is the main reproductive mode for *M. sacchari* on sorghum in the United States. In agreement, Singh et al. (2004) noted that reproduction for the sugarcane aphid is predominantly asexual, with winged and nonwinged females bearing live young. Although sexual reproduction has been documented for the sugarcane aphid on sorghum (David and Sandu, 1976), it is rarely observed. Aphid populations are sometimes composed of a small number of dominant clones and many rare genotypes (Harrison and Mondor, 2011). "Superclones" are highly abundant clones distributed over a large geographical scale and persisting over time (Gilabert et al., 2015). The combination of asexual reproduction and high reproduction rates in aphids can lead to superclones (Piffaretti et al., 2013). Invasive aphid superclones have been reported previously in other aphid species. A single predominant clone of the tobacco aphid (*Myzus persicae nicotianae*) was observed in Chile and is also present in Argentina, Brazil, and the United States (Zepeda-Paulo et al., 2010). Similarly, a single clone was primarily identified in the oleander aphid (*Aphis nerii*) from populations collected in the southern United States (Harrison and Mondor, 2011). Furthermore widespread asexual lineages can be specialized on particular host plant species as seen for the cotton-melon aphid (*Aphis gossypii* Glover; Carletto et al., 2009). Although a lack of genetic diversity is usually considered to be harmful, *M. sacchari*, as well as many other aphid species, are quite successful in a range of environments with a predominant genotype. This may be due to a wide range of phenotypic plasticity, as aphids can produce winged or wingless forms in response to host plant quality, crowding, or daylength (Zepeda-Paulo et al., 2010). Alternatively, the success of an asexual lineage may be that they are not dependent on finding suitable mates or mating sites for their long-term survival (Piffaretti et al., 2013). Another hypothesis is that aphids that are superclones have the ability to tolerate allelochemicals, which allows high fitness across multiple hosts (Castañeda et al., 2010).

Interestingly, Nibouche et al. (2014) identified that *M. sacchari* samples collected from the Caribbean belonged to a different MLL than *M. sacchari* collected from the United States. The Caribbean MLL, MLL-C, identified from aphids collected from 2002 to 2009 was considered a superclone. In the current study, aphid samples collected in 2015 from Puerto Rico (an island in the Caribbean) and the continental United States were the same genotype. It is unknown if any of the MLL identified by Nibouche et al. (2014) correspond to the current predominant sugarcane aphid genotype on sorghum in the United States. Nibouche et al. (2014) identified MLL-A and MLL-C *M. sacchari* as superclones, and thus a genotype from MLL-A or MLL-C may be the predominant sugarcane aphid genotype on sorghum. Alternatively, the predominant aphid genotype on sorghum may not have been identified previously, perhaps arising from a rare sexual event or not sampled in his study. Furthermore, Nibouche et al. (2014) identified that sugarcane aphids collected in 2007 on sugarcane and Johnsongrass [*Sorghum halepense* (L.) Pers.] from Hawaii and Louisiana are MLL-D. It is unknown if the predominant aphid genotype on sorghum is also infesting sugarcane and Johnsongrass, although with Johnsongrass and sorghum sharing the same genus, it may be probable. Assuming that different aphid genotypes are found on Johnsongrass and sugarcane, we would expect a mixture of aphid genotypes on sorghum grown in close proximity to sugarcane or Johnsongrass. Genotyping is needed to determine what MLL(s) of *M. sacchari* is currently on sugarcane and Johnsongrass in comparison with the predominant aphid genotype on sorghum.

The *M. sacchari* sample from Sinton, TX, was unique in the current study and is likely a mixture of *M. sacchari* genotypes. Sinton, TX (28°2′11″ N, and 97°31′43″ W) is located in southern Texas but further north than where most sugarcane is grown (The Rio Grande Valley). It is possible that *M. sacchari* on sugarcane, Johnsongrass, or perhaps other sorghum containing a rare sugarcane aphid genotype (for sorghum) might have infested sorghum plants in Sinton, TX, by traveling on wind patterns and thus causing the mixture and colocalization of two different genotypes that we identified in the current study. Indeed many studies have reported that winged cereal aphids are capable of very long-distance movement with the aid of the jet stream, yet most alates tend to move short distances over their lifetime, from 20 to 100 m (Parry, 2013).

Melanaphis sacchari have at least three interacting genomes: their chromosome, the mitochondria, and the *B. aphidicola* chromosome and plasmids. Symbiont diversity can have major effects on aphid viability and adaptation potential (Swanevelder et al., 2010). A single point mutation in *B. aphidicola* allowed the pea aphid (*Acyrthosiphon pisum*) to survive at different temperatures (Dunbar et al., 2007). In this study, each of the different aphid species had a different *B. aphidicola* genotype, including the Sinton, TX, *M.*

sacchari sample that is likely a mixture of aphid genotypes. Similarly, in the Russian wheat aphid (*Diuraphis noxia*), *B. aphidicola* DNA sequence was found to be a high–resolution population genetic marker (Zhang et al., 2014).

In conclusion, *M. sacchari* collected on sorghum during 2015 from 17 locations across seven southern US states and one US territory were one clone, with the exception of one location where a mixture of clones on sorghum occurred. This mixture represented either a rare mixture of clones reproducing on sorghum or representing a mixture of a single clone reproducing on sorghum and wind–aided migrants coming from perhaps sugarcane or Johnsongrass. Examination of the endosymbiont *B. aphidicola* using microsatellite markers showed a similar pattern, as all the *M. sacchari* samples except one had the same *B. aphidicola* genotype. These data suggest that *M. sacchari* is reproducing asexually and spreading on sorghum in seven states and one territory of the United States, primarily as a single clone. Furthermore, a primarily single clonal aphid genotype where only two chemicals are used for control may lead to insecticide resistance by somatic mutation.

Conflict of Interest

The authors declare that there is no conflict of interest.

Supplemental Material Available

Supplemental material for this article is available online.

Acknowledgments

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