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RESEARCH

Isolation and Characterization of Nine Microsatellite Loci From *Bemisia tabaci* (Hemiptera: Aleyrodidae) Biotype B

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ABSTRACT. Nine microsatellites were isolated from *Bemisia tabaci* (Gennadius) biotype B and screened across 60 individuals from two populations (biotype B) to examine polymorphism. Two to 12 alleles were observed per locus. Observed and expected heterozygosities ranged from 0.033 to 0.967 and 0.033 to 0.854, respectively. There was no significant deviation from Hardy–Weinberg equilibrium and no significant linkage disequilibrium between loci. One locus showed evidence for null alleles. These loci will be useful in future studies of the genetic structure of worldwide biotypes and gene flow analyses between and within biotypes of *B. tabaci*.

Key Words: invasive, biotype, microsatellite

The whitefly *Bemisia tabaci* (Gennadius) is a taxonomically diverse group, consisting of more than 20 cryptic members worldwide with enormous variation in ecological and biological traits. At least two of these cryptic members have spread from their native range and have become invasive in many areas globally (Brown et al. 1995, Perring 2001, Brown 2010, Gill and Brown 2010). These variants, referred to as biotypes, have been studied extensively using allozymes, restriction fragment length polymorphisms, amplified fragment length polymorphisms, random amplified polymorphic DNA, and, more recently, mitochondrial DNA sequences and microsatellites (see Hadjistylli et al. 2010, Gill and Brown 2010 for a review). Despite these efforts to characterize biotypes genetically and to resolve the systematics of this species, the only comprehensive studies done at the global-level scale have used the mitochondrial cytochrome oxidase I (mtCOI) gene (Frohlich et al. 1999, Boykin et al. 2007, Brown 2010, Dinsdale et al. 2010, Gill and Brown 2010), whereas studies utilizing microsatellite markers have focused to date at more local and regional levels, including populations or single biotypes (De Barro et al. 2005, 2008; Delatte et al. 2006; Tsagkarakou et al. 2007; Dalmon et al. 2008). Because there is insufficient evidence to elevate these cryptic members to species-level taxa (see Brown 2010 for a review, but see De Barro et al. 2011), we consider microsatellites to be particularly informative in describing genetic polymorphism within this group.

To undertake population genetics analyses of worldwide populations, including locally restricted and widespread or invasive biotypes, and characterize their genetic variation, we isolated and characterized nine microsatellite loci from biotype B, the most widespread and invasive biotype of this sibling species group. Although 50 loci for *B. tabaci* exist in the literature (De Barro et al. 2003, Tsagkarakou and Roditakis 2003, Delatte et al. 2006, Tsagkarakou et al. 2007, Dalmon et al. 2008, Gauthier et al. 2008) and were all screened across our multiple populations, they were not sufficient to provide a working set of loci for our studies because they were isolated mostly from laboratory strains reared from locally collected samples (Australia, Indonesia, Vietnam, biotype Q from Greece and Israel, and biotype B from Australia, Israel, and the island of La Réunion). Therefore, it was necessary to isolate additional loci that cross-amplified in other biotypes and were able to capture variation across globally collected samples (at least 50 populations) spanning nine clades of the global mtCOI phylogeny (Brown 2010).

Materials and Methods

We used the methodology described by Glenn and Schable (2005) to construct a microsatellite-enriched library. The extraction of genomic DNA was performed using the Qiagen (www.qiagen.com) DNeasy Blood & Tissue kit using approximately 25 mg of whole male and female biotype B whiteflies from a laboratory-reared population sampled from Arizona. We followed with an optional ribonuclease treatment. We then separately digested the DNA with the restriction enzymes *Rsa*I and *Bst*UI (New England BioLabs, www.neb.com). Digested fragments were ligated to the SuperSnx24 linker oligos and amplified with polymerase chain reaction (PCR). Following evidence of successful ligation, the linker-ligated DNA from both digestions was pooled before enrichment. Enrichment was performed using both the ligated DNA and a PCR of the ligation. We enriched for microsatellite sequences using streptavidin-coated beads (Dynalbead) and the following mix of 3′-biotinylated probes: $(ACTG)_4$, $(ACCT)_4$, $(AAAG)_4$, $(AATC)_4$, $(AATG)_4$, $(AAAC)_4$, $(ACAG)_4$, and $(ACTC)_4$. Those probes were selected to isolate tetranucleotides to minimize scoring errors and polymerase slippage. Also, di- and trinucleotides were already available in the literature, and most did not cross-amplify successfully in other biotypes. A PCR of the enrichment using the SuperSnx primer was performed to recover sequences containing repeats. This PCR product was enriched again using the same mix of probes as above and again recovered with PCR. PCR products were inserted into a vector and transformed into *Escherichia coli* using the Topo TA cloning kit (Invitrogen, www.invitrogen.com). Four cloning reactions were performed, and colonies were grown overnight. In total, 384 positive clones were then picked, cultured overnight, and PCR amplified using the M13 forward and reverse primers. A set of 194 clones seemed to have inserts of the appropriate size (300–1,200 bp). PCR products from these clones were purified using ExoSAP-IT (GE Healthcare, www.gehealthcare.com) and sequenced in the forward and reverse directions with the M13 primers and BigDye version 3.1 (Applied Biosystems, ABI, www.appliedbiosystems.com) on an ABI 3730 DNA Analyzer. Of the clones sequenced successfully, 79 appeared to contain

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Table 1. Properties of nine polymorphic tetra- and pentanucleotide microsatellite loci cloned from *Bemisia tabaci* biotype B

Microsatellite locus name	Primer sequence (5′–3′ direction)	Repeat motif	Fluorescent dye color (Applied Biosystems)	$MgCl_2$ (mM)	Accession numbers	Biotypes (other than B) and haplotypes successfully cross-amplified
WF1B11	F: GCATTGAACATTTTTCTGCATGCGCG R: GCACACAGCTCTCCAAAAGAAAGGTC	$(CCTGA)_{12}$imp	NED	1	JQ436838	A, H, K, L, M, Ms, Q, S, T, African, Asian, Australian, and New World haplotypes
WF2C01	F: ATGATACCGCACGAAAAAGAGGACG R: CTTGAATTACATCAAACGCAGCAGC	$(GTTT)_{11}$imp	NED	1	JQ436842	L, M, Q, African, Asian, and New World haplotypes
WF2H06	F: TATTCGCCAATCGATTCCTT R: CGGCGGAAATTTCGATAAA	$(TTTG)_{11}$	NED	0.75	JQ436844	A, L, M, Ms, Q, African, New World, and Yemen haplotypes
WF1B06	F: TCTCATAGAAAATTTTGGAGAAAGAAA R: TGCGGAATCTCAGGGTCATA	$(ACTC)_8$	PET	0.75	JQ436837	L, Ms, Q (only Spain Q or Q1), African, New World, and Yemen haplotypes
WF2E11	F: TCTCCAACCATAATTTTTAATCTCG R: GTCTGGGCAGGAAAACGAT	$(GATT)_{27}$imp	PET	1	JQ436843	L, M, Ms, Q, African, New World, and Yemen haplotypes
WF1D04	F: GTTGTTAGGTTACAGGGTTTGTC R: GTCTTTACTTCTCTTTTCCTCCG	$(CAAA)_{16}$	6-FAM	1	JQ436839	A, S, T, African, and New World haplotypes
WF1G03	F: CTCCAAAATGGGACTTGAAC R: GTAGAAGCCACACATACTAGCAC	$(GTTT)_8$	NED	1.125	JQ436840	H, K, M, Ms, Q, T, African, Asian, Australian, and New World haplotypes
WF2A05	F: ATTGATCATTTTCGGCTACCTTATC R: CGACCCTTCTGTAAATGATAGACT	$(CAAA)_{12}$imp	NED	1.125	JQ436841	
WF2A02	F: GAAGTGCATAAAAACATCGTCGCCG R: CAATTGAAAGGCTAGACTCCCTGGC	$(CTTT)_{10}$imp	NED	1	JX853746	

Imp, imperfect.

Locus name, primer sequences, repeat motif, fluorescent dye color used in fragment analysis, $MgCl_2$ concentration, GenBank accession numbers, and successful cross-amplification in non-B biotypes and haplotypes.

Table 2. Population-specific characteristics of the nine microsatellite loci tested in two populations from biotype B

Population tested	Cyprus biotype B				Egypt biotype B			
Locus name	Allele size range (bp) (N_A)	*f* null alleles	*n*	H_o/H_e	Allele size range (bp) (N_A)	*f* null alleles	*n*	H_o/H_e
WF1B11	106–193 (11)	0.0137	30	0.800/0.825	106–173 (8)	0.0596	30	0.700/0.808
WF2C01	150–187 (10)	**0.1478**	30	0.567/0.838	150–187 (9)	**0.1662**	30	0.500/0.799
WF2H06	172–196 (7)	−0.0235	30	0.867/0.824	172–204 (8)	−0.0432	30	0.867/0.789
WF1B06	158–162 (2)	−0.0005	30	0.033/0.033	146–162 (3)	0.0305	30	0.200/0.238
WF2E11	220–260 (9)	0.0459	29	0.655/0.735	199–260 (12)	0.0509	30	0.667/0.756
WF1D04	124–172 (12)	−0.0688	30	0.967/0.840	124–164 (10)	0.0114	30	0.833/0.854
WF1G03	139–162 (5)	−0.0077	30	0.167/0.158	158–162 (2)	−0.0005	30	0.033/0.033
WF2A05	141–158 (4)	−0.0134	23	0.217/0.201	154–158 (2)	0.0724	22	0.045/0.127
WF2A02	155−159 (2)	−0.0028	26	0.077/0.074	155–159 (2)	−0.001	22	0.045/0.044

Loci with evidence for null alleles in this population after analysis in MICROCHECKER (Van Oosterhout et al. 2004) are in bold.

Allele size range, N_A: number of observed alleles and *n*: number of individuals scored, estimated null allele frequency (Van Oosterhout et al. 2004), and observed (H_o) and expected (H_e) heterozygosity.

microsatellite sequences. Primers were designed manually for the best 18 candidates (see Supp Material [online only]) and analyzed using the Integrated DNA Technologies (Integrated DNA Technology 2007. www.idtdna.com) OligoAnalyzer tool, resulting in 18 primer pairs that were synthesized by IDTDNA.

The primer pairs were tested over a range of annealing temperatures and $MgCl_2$ concentrations, and eight pairs that failed to amplify the expected loci were discarded and were not tested further. The remaining 10 loci were tested for variation by screening across two populations belonging to biotype B. For fragment analysis, microsatellite forward primers were synthesized and labeled with a fluorescent dye by Applied Biosystems (Table 1). The PCR conditions were optimized to work across all 10 loci using a touchdown protocol. Reactions were done in 10 µl volumes using 1 µl of genomic DNA, 0.75 mM dNTPs mix, 0.5 µM of each primer, 1 µl of 0.1 mg/ml bovine serum albumin (BSA, New England BioLabs, www.neb.com), 1.25 µl of 10× PCR Buffer from Applied Biosystems (12.5 mM Tris-HCl, pH 8.3, 62.5 mM KCl), 0.75–1.125 mM $MgCl_2$ (ABI) (Table 1), and 0.5 U of AmpliTaq Gold DNA Polymerase (ABI). Amplification of all loci was done using touchdown PCR with the following thermocycling conditions: 1 cycle of 95°C for 10 min followed by 2 cycles of 94°C for 1 min, 60°C for 1 min, 70°C for 35 s followed by 18 cycles of 45 s at 93°C, 45 s at 59°C (lowering 0.5°C each cycle), and 45 s at 70°C. The final amplification step consisted of 20 cycles of 30 s at 92°C, 30 s at 50°C, and 1 min at 70°C followed by a final extension step at 70°C for 5 min. Final PCR products were mixed with a cocktail of 48:1 Hi-Di formamide (ABI)/LIZ500 size standard (ABI) (0.5 µl of PCR product, 0.2 µl of LIZ, and 9.3 µl of formamide) and were denatured at 95°C for 5 min. Fragments were run on an ABI 3730 DNA sequencer, and genotypic data were visualized and scored manually using the software GeneMapper version 4.0 (ABI).

Screening was done using 30 females from each population belonging to biotype B (from Cyprus and Egypt). Observed and expected heterozygosities, and allelic richness were calculated using GenAlEx 6.1 (Peakall and Smouse 2006). Deviation from Hardy–Weinberg equilibrium and gametic linkage disequilibrium were tested using GENEPOP (Raymond and Rousset 1995). An analysis for the presence of null

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alleles was carried out using the software MICROCHECKER (Van Oosterhout et al. 2004).

Results and Discussion

Among the 10 loci that were examined, one was difficult to score, showing ambiguous peaks. We selected the remaining nine loci to report and use in subsequent analyses, which showed good amplification results in populations from both biotypes and were easily scored, with clear, reproducible peaks.

The nine loci had 2–12 alleles in the tested populations, with observed heterozygosity ranging from 0.033 to 0.967 and expected heterozygosity ranging from 0.033 to 0.854 (Table 2). There was no significant deviation from Hardy–Weinberg equilibrium after correcting for multiple comparisons using the sequential Bonferroni correction (Rice 1989; $P < 0.05$), and no significant linkage disequilibrium was detected between loci ($P < 0.05$). However, one locus (WF2CO1) showed evidence for null alleles in the two populations (Table 2).

Of the loci reported here, seven were successfully amplified in non-B biotypes and haplotypes from worldwide mtCOI clades of *B. tabaci* as indicated in Table 1. This successful cross-biotype amplification may be attributed to the fact that tetranucleotides have a lower mutation rate compared with dinucleotides (Chakraborty et al. 1997, Lee et al. 1999), and thus may be more useful in studies of sibling species—species that are more closely related than any other pair of species but more genetically divergent compared with populations within a single species—as seems to be the case of *B. tabaci.* This is the first study that reports the isolation and characterization of a number of tetranucleotides from *B. tabaci* and may prove particularly useful in subsequent studies of multiple biotypes. The loci reported here are being used to study the population genetic structure of worldwide biotypes within the *B. tabaci* species complex, to examine gene flow between Mediterranean populations belonging to invasive biotypes B and Q, and to study the invasion history of biotype Q in the United States.

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