Gene Expression Profiling in the Thiamethoxam Resistant and Susceptible B-biotype Sweetpotato Whitefly, Bemisia tabaci

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Gene expression profiling in the thiamethoxam resistant and susceptible B-biotype sweetpotato whitefly, *Bemisia tabaci*

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Abstract

Thiamethoxam has been used as a major insecticide to control the B-biotype sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). Due to its excessive use, a high level of resistance to thiamethoxam has developed worldwide over the past several years. To better understand the molecular mechanisms underlying this resistance in *B. tabaci*, gene profiles between the thiamethoxam-resistant and thiamethoxam-susceptible strains were investigated using the suppression subtractive hybridization (SSH) library approach. A total of 72 and 52 up- and down-regulated genes were obtained from the forward and reverse SSH libraries, respectively. These expressed *sequence* tags (ESTs) belong to several functional categories based on their gene ontology annotation. Some categories such as cell communication, response to abiotic stimulus, lipid particle, and nuclear envelope were identified only in the forward library of thiamethoxam-resistant strains. In contrast, categories such as behavior, cell proliferation, nutrient reservoir activity, sequence-specific DNA binding transcription factor activity, and signal transducer activity were identified solely in the reverse library.

To study the validity of the SSH method, 16 differentially expressed genes from both forward and reverse SSH libraries were selected randomly for further analyses using quantitative real-time PCR (qRT-PCR). The qRT-PCR results were fairly consistent with the SSH results; however, only 50% of the genes showed significantly different expression profiles between the thiamethoxam-resistant and thiamethoxam-susceptible whiteflies. Among these genes, a putative NAD-dependent methanol dehydrogenase was substantially over-expressed in the thiamethoxam-resistant adults compared to their susceptible counterparts. The distributed profiles show that it was highly expressed during the egg stage, and was most abundant in the abdomen of adult females.

Keywords: insecticide resistance, quantitative real-time PCR, NAD-dependent methanol dehydrogenase, sap-sucking insect, suppression subtractive hybridization

Abbreviations: EST, expressed sequence tag; **SSH**, suppression subtractive hybridization; **qRT-PCR**, quantitative real-time PCR

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Introduction

The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is one of the most widely distributed agricultural pests worldwide (Perring 2001), attacking agronomic, horticultural, and ornamental crops in subtropical and tropical agriculture, as well as in greenhouse production systems (Oliveira et al. 2001). It was first identified in China in the mid-1990s and then spread into more than 20 provinces within a very short time frame (Luo et al. 2002; Chu et al. 2005; Zhang et al. 2005; Chu et al. 2006). The phloem-feeding whitefly has caused severe crop losses through direct feeding, excretion of honeydew (which favors sooty mold development), and transmission of plant viruses (Jones 2003).

Due to its severe damages, *B. tabaci* has been controlled predominantly with chemical insecticides. However, as a result of extensive application of synthetic insecticides, *B. tabaci* has developed a high degree of resistance to a wide range of insecticides, including carbamates, organophosphates, pyrethroids, insect growth regulators (IGRs), and neonicotinoids (Horowitz et al. 1988; Prabhaker et al. 1988; Horowitz et al. 1999; Nauen et al. 2002; Ahmad et al. 2002; Kranthi et al. 2002; Ma et al. 2007; Erdogan 2008; Roditakis 2009; Wang et al. 2010). Neonicotinoid insecticides are generally considered systemic and have excellent efficacy, long-lasting residual activity, and favorable safety profiles. For example, thiamethoxam, discovered and developed by the Novartis Crop Protection (www.novartis.com), has played a crucial role in controlling *B. tabaci* and many other sap-sucking insect pests in China since its introduction in 2000. A high level of resistance to thiamethoxam (100- and 900-fold), however, has already been reported in B- and Q-biotype of *B. tabaci* strains from Israel and Spain, respectively (Rauch and Nauen 2003; Horowitz et al. 2004). In China, both biotypes have developed high levels of resistance to imidacloprid and thiamethoxam in the field (Wang et al. 2010).

In general, the safety and effectiveness of neonicotinoids have been attributed to their high affinity to nicotinic acetylcholine receptors (nAChRs). Consequently, resistance to neonicotinoids initially focused on the mutations in nAChRs (Liu et al. 2009). In addition, recent studies revealed that resistance of neonicotinoids in *B. tabaci* could be associated with an enhanced oxidative detoxification by cytochrome P450 monooxygenases (Karunker et al. 2008; Wang et al. 2009). Through biochemical characterization of B-biotype thiamethoxam-resistant strains, cytochrome P450 monooxygenase and carboxylesterase were found to be responsible for the thiamethoxam resistance in whiteflies (Feng et al. 2008, 2010).

Suppression subtractive hybridization (SSH) is a RNA-based method for identifying genes with unknown function, especially in species that lack primary genomic resources (Diatchenko et al. 1996; Lü and Wan 2008). This method has already been used to better understand the genetic basis of insecticide resistance, such as *Aedes aegypti* resistance to deltamethrin (Lertkiatmongkol et al. 2010) and *Nilaparvata lugens* resistance to triazophos (Bao et al. 2010). The SSH method has been applied to identify genes related to viral infection (Li et al. 2011) and heat-shock (Lü and Wang 2008) in *B. tabaci* as well. In this study, gene expression profiles between the thiamethoxam-resistant and

thiamethoxam-susceptible *B. tabaci* were investigated by both SSH and qRT-PCR analyses. Combined results give us a unique perspective in regards to the development of neonicotine resistance in the B-biotype *B. tabaci*.

Materials and Methods

Bemisia tabaci strains

The B-biotype *B. tabaci* susceptible strain (TH-S) and resistant strain (TH-R) were the same populations as described previously (Feng et al. 2008, 2010). Before sample collection, a leaf-dip bioassay (Feng et al. 2008) was conducted to confirm that the resistance factor (LC_{50} (TH-R)/LC_{50} (TH-S)) was at least 70-fold. About 3000 adult whiteflies from TH-R were treated with 2000 mg/L thiamethoxam (~LC_{80}) to eliminate the heterozygous individuals. Then, the survivors were collected after 48 hours and designated as the TH-2000. A total of 300 TH-S and TH-2000 adults, respectively, were collected, snap frozen in liquid nitrogen for three hours, and transferred to a −80 °C freezer for long-term storage. Different developmental stages, such as eggs, 3rd instar larvae, and two-day-old unmated adult females, and various tissues including head, thorax, abdomen, and wing of a two-day-old unmated adult female were collected to study the distribution profiles of genes of interest.

Total RNA isolation and reverse transcription

Total RNAs from both TH-2000 and TH-S adults were extracted using Trizol (Invitrogen, www.invitrogen.com) following manufacturer protocol. The resulting total RNA was re-suspended in nuclease-free water, and quantified by the Nanodrop 2000 (Thermo Scientific, www.thermoscientific.com). The first-strand cDNA and ds-cDNA were synthesized using SMARTer™ PCR cDNA Synthesis Kit (Clontech, www.clontech.com) and later, the ds-cDNA were purified with QIAquick PCR Purification Kit (QIAGEN, www.quiagen.com).

Construction of the SSH cDNA library

The SSH procedure was carried out using a PCR-Select™ cDNA Subtraction Kit (Clontech) following manufacturer protocol. A forward SSH library was constructed to isolate the up-regulated genes of the TH-2000 whitefly strain. The forward SSH library was used to identify clones in which the TH-2000 cDNA was used as the tester and the TH-S cDNA as the driver. In addition, a reverse SSH library was constructed to detect the down-regulated genes of the TH-2000 whitefly strain. The reverse SSH library was used to identify clones in which the TH-S cDNA was used as the tester and the cDNA from TH-2000 as the driver. After hybridization, the subtracted cDNA were ligated into the pGEM-T vector (Promega, www.promega.com) and transformed into *Escherichia coli* competent cells through electroporation.

DNA sequencing and EST analysis

Positive clones were selected by conventional blue-white screening. White clones were randomly selected from both forward and reverse libraries. The positive clones were further validated by colony PCR using nested PCR primers provided in the kit. The resulting products were subjected to the direct sequencing with M13 primers. The vector sequences were removed through a Perl script and checked through VecScreen (http://www.ncbi.nlm.nih.gov/VecScreen/VecScreen.html). Then, the remaining high-quality EST sequences were analyzed in the GenBank non-redundant (nr) database with BLASTX. A sequence was considered as

significantly matched when the E-value was < 10^{-5}. Functional annotation was carried out in the Swiss-Prot (http://expasy.org/people/swissprot.html). Gene Ontology (GO) terms were extracted and counted using map2slim and Perl scripts.

Quantitative real time PCR

Up to 150 (three biological replicates, n = 50) TH-S and TH-2000 adults, respectively, were collected for the qRT-PCR analysis. Approximately 0.5 μg of total RNA was used as a template to synthesize the first-strand cDNA using PrimerScript RT reagent Kit (Takara Bio Inc., www.takarabio.com) following manufacturer protocol. The resulting cDNA was diluted to a working concentration of 0.1μg/μL for the subsequent qRT-PCR analysis. To validate the differentially expressed genes detected by the SSH approach, 16 expressed ***sequence*** tags (ESTs) representing 11 putatively up-regulated genes and five down-regulated genes were randomly selected. The qRT-PCR primers were designed using Primer3 (http://frodo.wi.mit.edu/primer3) (Table 1). The cycling parameters were as follows: 95 °C for 30 sec, followed by 40 cycles of 95 °C for five sec and 62 °C for 34 sec, and ended with a melting curve analysis (65 °C to 95 °C in increments of 0.5 °C every five sec) to check for nonspecific product amplification. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. β-actin was used as the internal reference gene to eliminate sample-to-sample variations in the initial cDNA samples.

Results

Differential screening and EST sequencing

Based on the results of the differential screening, all 507 cDNA clones were randomly picked and sequenced from these two libraries. Specifically, 298 clones were from the forward library representing up-regulated genes, and 209 clones were from the reverse library representing down-regulated genes. After trimming, 127 and 63 high-quality ESTs from the forward and reverse library, respectively, were obtained.

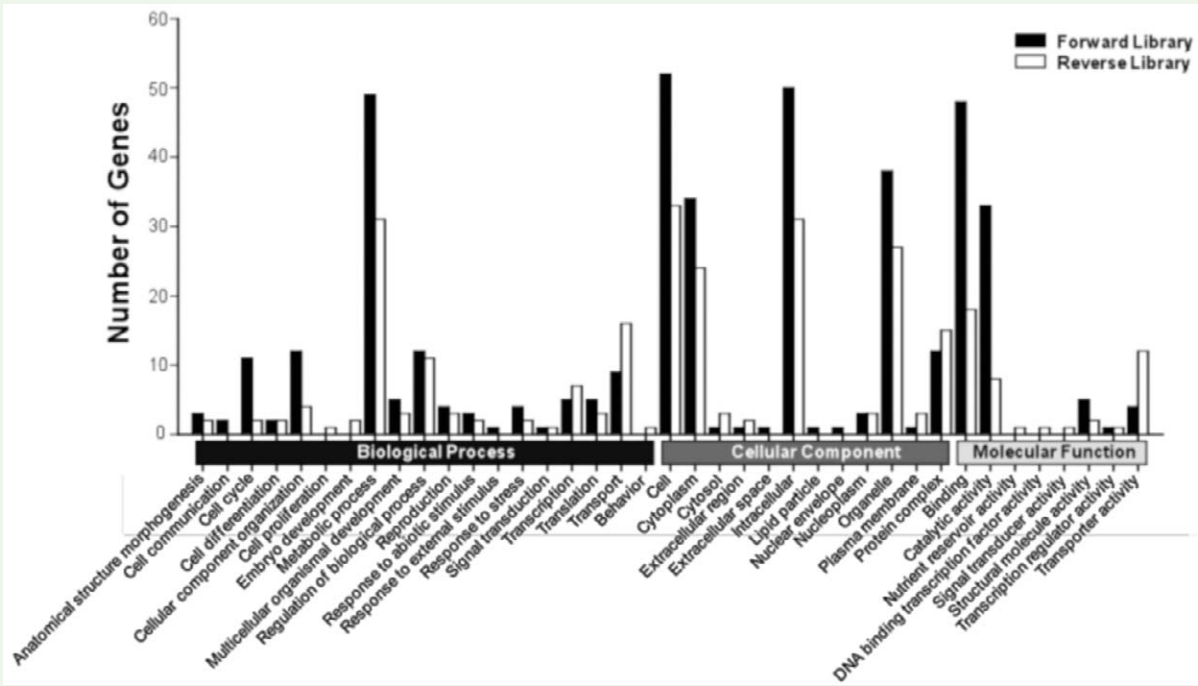

Figure 1. Gene Ontology (GO) classification of the differentially expressed expression ESTs in the forward and reverse SSH libraries. Based on the GO terms, the ESTs are categorized into putative functional groups. The black and white columns represent the up- and down-regulated genes from forward and reverse libraries, respectively. High quality figures are available online.

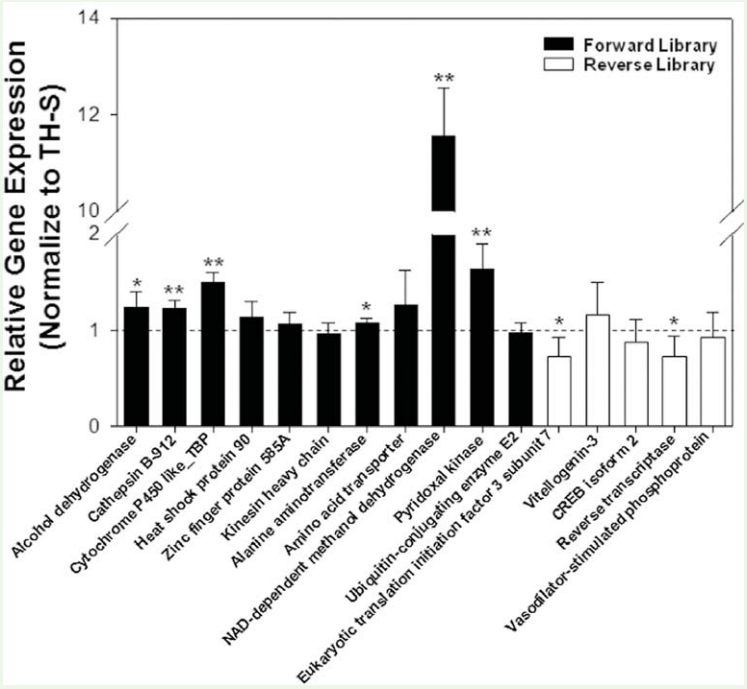

Figure 2. Quantitative real-time PCR validation. The gene expression level of 16 randomly selected ESTs, including 11 from SSH forward library (the black column) and five from SSH reverse library (the white column), was tested using qRT-PCR. The relative gene expression level in the resistant strains was normalized to the susceptible TH-S whiteflies. Data are presented as mean ± SE. Asterisks denote significant gene expression differences between the resistant and susceptible whiteflies, as determined by the pairwise *t*-tests (* $p < 0.05$, ** $p < 0.01$, LSD *t*-test). High quality figures are available online.

Functional annotation

The BLASTX results showed that among the 127 clones from the SSH forward library, 72 ESTs (56.7%) had significant matches (E-value < 10^{-5}) to known or predicted genes in GenBank, and these clones could be assembled into 61 distinct sequences (Supplementary Table 1). For the 63 clones from the SSH reverse library, 52 ESTs (83.8 %) had significant matches (E-value < 10^{-5}) with the database, among which 39 distinct sequences were identified (Table S2).

Based on the Gene Ontology (GO) terms, these distinct sequences were functionally annotated (Figure 1). Majority of the biological processes (such as cell cycle, metabolic process, response to external stimulus, response to stress, and transport), some cellular components (such as cell, plasma membrane, and protein complex), and some molecular functions (such as binding, catalytic activity, structural molecule activity, transcription regulator activity, and transporter activity) were presented in both libraries. Other GO terms, such as cell communication, response to abiotic stimulus, lipid particle, and nuclear envelope were identified only in the forward library. Vice versa, some GO terms such as behavior, cell proliferation, nutrient reservoir activity, sequence-specific DNA binding transcription factor activity, and signal transducer activity were identified only in the reverse library.

qRT-PCR validation

The qRT-PCR results from the randomly selected differentially expressed transcripts were, for the most part, consistent with the SSH results (Figure 2, Supplementary Tables 1 and 2). For the 11 up-regulated genes, 9 of them were over-expressed in the resistant *B. tabaci*, whereas 4 out of 5 down-regulated genes were under-expressed. However, only 50% of the genes exhibited significantly

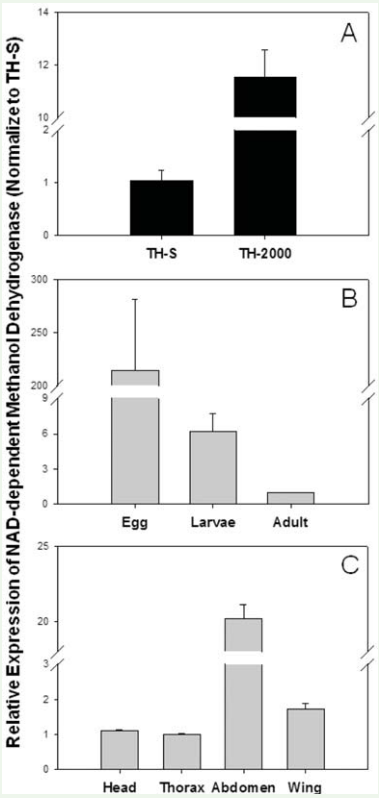

Figure 3. Gene expression profiles of a putative NAD-dependent methanol dehydrogenase in *Bemisia tabaci*. (A) Relative gene expression between resistant (TH-2000) and susceptible (TH-S) *Bemisia tabaci* adults. (B) Relative gene expression at different developmental stages including egg, 3rd instar larvae, and two-day-old unmated adult females. (C) Relative gene expression among different tissues including head, thorax, abdomen, and wing of two-day-old unmated adult females. High quality figures are available online.

different expression profiles between resistant and susceptible whiteflies (Figure 2). Most notably, F-TH_SS_58, a putative NAD-dependent methanol dehydrogenase EST, was over-expressed ~12-fold in the resistant TH-2000 whiteflies in comparison to the susceptible TH-S strains (Figure 2, Supplementary Table 1). To characterize this newly identified *B. tabaci* dehydrogenase gene, its expression profiles at different developmental stages and different tissues were examined (Figure 3). In general, the transcript level of this gene was much higher in the resistant whiteflies (Figure 3A), was most abundant in egg stage (Figure 3B), and was much higher in the abdomen of adult female than in any other tissues (Figure 3C).

Discussion

Thiamethoxam, a second-generation neonicotinoid insecticide (Maienfisch *et al.* 2001), has been used extensively for the sustainable management of *B. tabaci* in horticultural and other cropping systems (Nauen and Denholm 2005). However, like many other neonicotinoid insecticides, *B. tabaci* has developed a high degree of resistance to thiamethoxam under the laboratory selection (Feng *et al.* 2008, 2010), as well as in the field (Elbert and Nauen 2000; Horowitz *et al.* 2004; Wang *et al.* 2010) in the past decade. The molecular mechanism governing the thiamethoxam resistance in *B. tabaci*, however, has yet to be fully understood. In this study, the molecular basis of thiamethoxam resistance in *B. tabaci* was investigated using the SSH cDNA library approach. About 72 and 52 differentially expressed ESTs were obtained from forward and reverse libraries, respectively, representing up-regulated and down-regulated genes. The differentially expressed genes between the thiamethoxam-resistant and -susceptible *B. tabaci* include, but not limit to, cell communication, response to abiotic stimulus, response to stress, lipid particle, nuclear envelope, cell proliferation, and nutrient reservoir activity, etc. The accuracy of the SSH method was partially confirmed by the qRT-PCR analysis, with only 50% of the randomly selected ESTs showed significant differences. Similar to microarray analysis, RNA-based SSH method tends to generate false positives. Consequently, results from both analyses need to be validated by qRT-PCR.

Previous mechanistic studies suggested that neonicotinoid resistance could be associated with enhanced metabolic detoxification by

cytochrome P450 monooxygenases (Zhao *et al.* 2000; Rauch and Nauen 2003; Honda *et al.* 2006; Karunker *et al.* 2008; Wang *et al.* 2009). In this study, only one P450-like EST (F-TH_SS_19), that has the highest similarity with a tobacco cytochrome P-450-like gene (Accession No. BAA10929.1), was significantly over-expressed in the resistant whiteflies (1.50 ± 0.05, $p<0.01$, Table 2). Due to the limited resolution and coverage of this SSH method, it is not uncommon that some of the genes potentially involved in the thiamethoxam resistance in whiteflies were not included. It is worth noting, however, that a NAD-dependent methanol dehydrogenase-like EST from *B.tabaci* was substantially overexpressed in the resistant whiteflies (11.56 ± 0.57, $p<0.01$, Table 2).

Dehydrogenases including farnesol dehydrogenase, succinic semialdehyde dehydrogenase (SSADH), aldehyde dehydrogenase, glutamate dehydrogenase, and methanol dehydrogenase can oxidize a substrate by transferring one or more hydrides (H^-) to an acceptor, usually NAD^+/$NADP^+$ or a flavin coenzyme such as FAD or FMN. They are involved in various physiological and biochemical processes. In mammals, SSADH is thought to be responsible for the degradation of the inhibitory neurotransmitter GABA in the central nervous system (Blaner and Churchich 1979; Chambliss *et al.* 1995). SSADH homologues have been cloned and expressed in the parasitic insects *Lucilia cuprina* and *Ctenocephalides felis* (Rothacker *et al.* 2008). In addition, $NADP^+$-dependent farnesol dehydrogenase was found to be involved in the juvenile hormone synthesis in mosquito (Mayoral *et al.* 2009). The NAD-dependent methanol dehydrogenase found in this study shed new light on the molecular understanding of thiamethoxam resistance in whiteflies. Based on these results, future studies involving cloning and functional characterization of this NAD-dependent methanol dehydrogenase are warranted to elucidate its role in the whitefly thiamethoxam resistance.

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Table 1. Primers used for the quantitative real-time PCR analysis.

Putative Gene	Primers 5′-3′*	Temp (°C)**	Product (bp)
Alcohol dehydrogenase	F-CAATGCGAGCTGCTCTGGA	62	102
	R-GCTGGAATGGACGAGTGGAA		
Cathepsin B-912	F-CATCAGTAATCGCGGATCGC	62	101
	R-CCATCCGCACTTTTTGCAAC		
Cytochrome P450–like TBP	F-TCTGCCCAGTGCTCTGAATGT	62	101
	R-TGACGAGGCATTTGGCTACC		
Heat shock protein 90	F-CCTCCGGTTTTGCTCTTGAAG	62	104
	R-TTCAACCATGACTGGCTCGTC		
Zinc finger protein 585A	F-AGCCATCTGCCTGCTGATCTC	62	104
	R-CCTAGATGGATTCGCCTGTGG		
Kinesin heavy chain	F-TGATCGGCTCTTTGAGCAATG	62	181
	R-AATTCGTAGTCGCGGCGAG		
Alanine aminotransferase	F-ACACAGCCTCATTGGCTGAAT	62	84
	R-CAGGAATATCCAAGCCCCAGT		
Amino acid transporter	F- GGCATCACACTTCCGATTGC	62	101
	R-CCCAACATTGCTGAACGCA		
NAD-dependent methanol dehydrogenase	F-TCGAGTCCTTCACAGCATTGC	62	107
	R-GGCCCAAATATCGGAGATGG		
Pyridoxal kinase	F- TGAGACGGAAGTAGTGCCCAC	62	116
	R-TCCTTCCGTTTCATGCTGTTC		
Ubiquitin-conjugating enzyme E2	F-AGTCGGCGAACTTAACACGCT	62	102
	R-CGCCAGCCTTTCCTTTCTTC		
Eukaryotic translation initiation factor 3 subunit 7	F-AAGCCCCTGTCAAAAATCGTG	62	136
	R-CCACAGCAGGTAAGGTCTGCA		
Vitellogenin-3	F-AAAAATACGCCCGCAACGA	62	144
	R-GGATGGAAGGTCCGTTTTGG		
CREB isoform 2	F-CTGATTGCTGGTTGGCTTGAA	62	117
	R-GGATGGTGGAGGAAAACAGGA		
Reverse transcriptase	F-CGAGCCGACCAACTAAACCA	62	101
	R-ACACCTAGTGTTGCAGCCGG		
Vasodilator-stimulated phosphoprotein	F-GGATGGTGATGGTGTGTCAGG	62	129
	R-CCGCACCCAACAAGCTCTT		
β-actin	F- ACCGCAAGATTCCATACCC	60	129
	R-CGCTGCCTCCACCTCATT		

* F, forward primer; R, reverse primer; ** T, Annealing temperature.

Supplementary Table 1. Up-regulated genes identified in the SSH forward library.

Clone ID*	Length (bp)	Sequence Description	Accession No.	Hit Species	E-value**	Copy No.
F-TH SS 1	663	D-tyrosyl-tRNA(Tyr) deacylase 1	EFN74087.1	*Camponotus floridanus*	1.00E-46	1
F-TH SS 2	311	putative ribosomal protein L8e	ABM55545.1	*Maconellicoccus hirsutus*	8.00E-33	1
F-TH SS 3	350	non-ATPase regulatory subunit	EFN85092.1	*Harpegnathos saltator*	1.00E-07	1
F-TH SS 4	445	60S ribosomal protein L7	EFN75670.1	*Harpegnathos saltator*	8.00E-52	1
F-TH SS 5	477	60s ribosomal protein L9	ADD20176.1	*Glossina morsitans*	1.00E-33	1
F-TH SS 6	640	AGAP002569-PA	XP 312367.4	*Anopheles gambiae str. PEST*	6.00E-25	1
F-TH SS 7	817	AGAP004890-PB	XP 314288.4	*Anopheles gambiae str. PEST*	1.00E-138	1
F-TH SS 8	559	Alcohol dehydrogenase	XP 002429805.1	*Pediculus humanus corporis*	6.00E-71	1
F-TH SS 10	379	ATP synthase F0 subunit 6	YP 086805.1	*Bemisia tabaci*	3.00E-34	2
F-TH SS 11	670	cathepsin B-912	NP 001119612.2	*Acyrthosiphon pisum*	5.00E-30	1
F-TH SS 15	561		1KR1		8.00E-40	6
F-TH SS 18	306	class III chitinase	ACM45715.1	*Pyrus pyrifolia*	4.00E-27	1
F-TH SS 19	326	cytochrome P450 like TBP	BAA10929.1	*Nicotiana tabacum*	2.00E-23	1
F-TH SS 21	703	D-tyrosyl-tRNA(Tyr) deacylase 1	EFN74087.1	*Camponotus floridanus*	4.00E-47	1
F-TH SS 22	578	fbxl7	XP 002428011.1	*Pediculus humanus corporis*	6.00E-51	1
F-TH SS 23	478	formin	XP 002432855.1	*Pediculus humanus corporis*	5.00E-48	3
F-TH SS 26	972	GI13422	XP 002008326.1	*Drosophila mojavensis*	5.00E-15	1
F-TH SS 27	417	GK20166	XP 002060760.1	*Drosophila willistoni*	2.00E-30	1
F-TH SS 28	762	Glycerophosphodiester phosphodiesterase 1	EFN63344.1	*Camponotus floridanus*	6.00E-47	1
F-TH SS 29	871	GTP-binding ADP-ribosylation factor-like protein 1 protein	EFN82659.1	*Harpegnathos saltator*	7.00E-14	1
F-TH SS 30	461	heat shock protein 90	ACH85198.1	*Bemisia tabaci*	2.00E-57	1
F-TH SS 31	466	TcasGA2 TC008350	EFA02630.1	*Tribolium castaneum*	3.00E-10	1
F-TH SS 32	384	TcasGA2 TC009545	EFA06626.1	*Tribolium castaneum*	5.00E-47	1
F-TH SS 33	346	Importin subunit beta-1	EFN81834.1	*Harpegnathos saltator*	4.00E-30	1
F-TH SS 34	624	Integrator complex subunit 3	EFN89783.1	*Harpegnathos saltator*	1.00E-85	1
F-TH SS 35	396	Kinesin heavy chain	EFN71894.1	*Camponotus floridanus*	6.00E-37	1
F-TH SS 36	757	ribosomal protein, S11	XP 001650870.1	*Aedes aegypti*	7.00E-45	1
F-TH SS 37	716	Mitochondrial-processing peptidase subunit beta	EFN65875.1	*Camponotus floridanus*	7.00E-87	1
F-TH SS 38	514	multisynthetase complex auxiliary component p38	XP 002427467.1	*Pediculus humanus corporis*	5.00E-08	1
F-TH SS 39	663	Negative elongation factor A	XP 002429474.1	*Pediculus humanus corporis*	5.00E-24	1
F-TH SS 40	695	Phosphoacetylglucosamine mutase	EFN67555.1	*Camponotus floridanus*	7.00E-17	1
F-TH SS 41	385	acyl-Coenzyme A binding domain containing 4	XP 002719487.1	*Oryctolagus cuniculus*	7.00E-06	1
F-TH SS 42	767	ADP-ribosylation factor GTPase activating protein 2-like	XP 002740329.1	*Saccoglossus kowalevskii*	5.00E-09	1
F-TH SS 43	471	AGAP005404-PA	XP 971530.1	*Tribolium castaneum*	7.00E-13	2
F-TH SS 45	923	AGAP009657-PA	XP 001947057.1	*Acyrthosiphon pisum*	5.00E-20	1
F-TH SS 46	580	AGAP010331-PA	XP 975279.2	*Tribolium castaneum*	5.00E-22	1
F-TH SS 47	599	alanine aminotransferase	XP 001948711.1	*Acyrthosiphon pisum*	1.00E-83	1
F-TH SS 48	458	amino acid transporter	XP 001944922.1	*Acyrthosiphon pisum*	1.00E-37	1
F-TH SS 50	629	aminoacylase, putative	XP 969212.1	*Tribolium castaneum*	3.00E-43	2
F-TH SS 51	716	conserved hypothetical protein	XP 001601024.1	*Nasonia vitripennis*	2.00E-13	1
F-TH SS 52	298	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	XP 394723.2	*Apis mellifera*	2.00E-33	1
F-TH SS 53	750	DEAD box ATP-dependent RNA helicase	XP 001602897.1	*Nasonia vitripennis*	7.00E-98	1
F-TH SS 54	376	DEAD box ATP-dependent RNA helicase	XP 974045.1	*Tribolium castaneum*	5.00E-43	1
F-TH SS 55	618	GA15457-PA	XP 001602440.1	*Nasonia vitripennis*	6.00E-71	1
F-TH SS 56	520	GH10652p	XP 001602045.1	*Nasonia vitripennis*	1.00E-52	1
F-TH SS 57	489	MGC82386 protein	XP 001601848.1	*Nasonia vitripennis*	4.00E-14	1
F-TH SS 58	485	NAD-dependent methanol dehydrogenase	XP 001952147.1	*Acyrthosiphon pisum*	8.00E-60	1
F-TH SS 59	425	nucleolar KKE/D repeat protein	XP 001603746.1	*Nasonia vitripennis*	8.00E-44	1
F-TH SS 60	822	pugilist CG4067-PA	XP 623143.1	*Apis mellifera*	2.00E-80	1
F-TH SS 61	393	pyridoxal kinase	XP 001607107.1	*Nasonia vitripennis*	6.00E-10	1
F-TH SS 62	508	ring finger protein 141	XP 974067.1	*Tribolium castaneum*	9.00E-35	1
F-TH SS 63	599	Type III alcohol dehydrogenase CG3425-PA	XP 968236.1	*Tribolium castaneum*	6.00E-74	1
F-TH SS 64	544	ubiquitin-conjugating enzyme E2	XP 967918.1	*Tribolium castaneum*	1.00E-26	1
F-TH SS 65	856	Probable hydroxyacid-oxoacid transhydrogenase, mitochondrial	EFN79501.1	*Harpegnathos saltator*	4.00E-08	1
F-TH SS 66	395	putative enolase	ACT87785.1	*Meganola phylla*	3.00E-10	1
F-TH SS 67	230	REST corepressor	EFN80005.1	*Harpegnathos saltator*	1.00E-14	1
F-TH SS 68	173	ribosomal protein L8e	ABF60235.1	*Leptinotarsa decemlineata*	4.00E-18	1
F-TH SS 69	456	S-phase kinase-associated protein 1	ACO11412.1	*Caligus rogercresseyi*	4.00E-18	1
F-TH SS 70	548	transposase IS630	CAL47051.1	*Listonella anguillarum serovar O2*	9.00E-06	1
F-TH SS 71	104	transposase IS630	CAL47051.1	*Listonella anguillarum serovar O2*	1.00E-06	1
F-TH SS 72	449	Zinc finger protein 585A	EFN63352.1	*Camponotus floridanus*	2.00E-54	2

* Genes randomly selected for the qRT-PCR validation study are in the shade; ** transcripts were putatively identified by homology from BLASTX search using a cutoff E-value less than $1x10^{-5}$.

Supplementary Table 2. Down-regulated genes identified in the SSH reverse library.

Clone ID*	Length (bp)	Sequence Description	Accession No.	Hit Species	E-value**	Copy No.
R-SS_TH_1	570	60S ribosomal protein L27a	XP_001892460.1	*Brugia malayi*	7.00E-54	1
R-SS_TH_2	466	60S ribosomal protein L7	EFN75670.1	*Harpegnathos saltator*	5.00E-52	1
R-SS_TH_12	375	ATP synthase F0 subunit 6	YP_086805.1	*Bemisia tabaci*	3.00E-34	10
R-SS_TH_13	902	ATP-binding domain-containing protein 3	EFN65530.1	*Camponotus floridanus*	2.00E-81	1
R-SS_TH_14	494	ATP-dependent Clp protease	XP_002428757.1	*Pediculus humanus corporis*	2.00E-64	1
R-SS_TH_15	699	cathepsin B endopeptidase	XP_002404475.1	*Ixodes scapularis*	2.00E-33	1
R-SS_TH_16	186	conserved hypothetical protein	ABM53543.1	*uncultured beta proteobacterium CBNPD1 BAC clone 578*	1.00E-09	1
R-SS_TH_17	818	cyclic-AMP response element binding protein isoform 2	NP_001159940.1	*Nasonia vitripennis*	2.00E-50	1
R-SS_TH_18	418	DNA replication licensing factor MCM5	EFN70560.1	*Camponotus floridanus*	2.00E-44	1
R-SS_TH_19	705	DNA-directed RNA polymerases I, II, and III 17.1 kDa polypeptide	XP_002425189.1	*Pediculus humanus corporis*	1.00E-39	1
R-SS_TH_20	849	DnaJ-like protein subfamily C member 2	EFN63844.1	*Camponotus floridanus*	8.00E-51	1
R-SS_TH_21	844	Exostosin-3	EFN78322.1	*Harpegnathos saltator*	2.00E-57	1
R-SS_TH_22	584	GK21731	XP_002074462.1	*Drosophila willistoni*	2.00E-08	1
R-SS_TH_23	417	GL10889b	XP_002015686.1	*Drosophila pseudoobscura pseudoobscura*	2.00E-23	1
R-SS_TH_24	398	GroEL	ACC54436.1	*Candidatus Portiera aleyrodidarum*	2.00E-50	1
R-SS_TH_25	245	heat shock protein 90	ADG03469.1	*Bemisia tabaci*	6.00E-34	1
R-SS_TH_26	456	hypothetical protein AaeL_AAEL003385	XP_001656735.1	*Aedes aegypti*	3.00E-21	1
R-SS_TH_27	339	hypothetical protein AcavDRAFT_4806	ZP_06213060.1	*Acidovorax avenae subsp. avenae ATCC 19860*	2.00E-21	1
R-SS_TH_28	566	hypothetical protein LOC431729	NP_001002182.1	*Danio rerio*	1.00E-25	1
R-SS_TH_29	779	hypothetical protein TcasGA2_TC011939	EFA09797.1	*Tribolium castaneum*	2.00E-27	1
R-SS_TH_30	586	hypothetical protein TcasGA2_TC030638	EFA01434.1	*Tribolium castaneum*	1.00E-12	1
R-SS_TH_31	482	molecular chaperone DnaK	YP_742734.1	*Alkalilimnicola ehrlichii MLHE-1*	8.00E-06	1
R-SS_TH_32	811	propionyl-CoA carboxylase alpha chain, mitochondrial-like	XP_002937132.1	*Xenopus (Silurana) tropicalis*	1.00E-37	1
R-SS_TH_33	311	CG4364-PA	XP_001600892.1	*Nasonia vitripennis*	1.00E-33	1
R-SS_TH_34	585	ENSANGP00000013956	XP_001604161.1	*Nasonia vitripennis*	4.00E-63	1
R-SS_TH_35	435	erect wing CG3114-PF	XP_001947009.1	*Acyrthosiphon pisum*	6.00E-09	1
R-SS_TH_36	312	eukaryotic translation initiation factor 3 subunit 7	XP_001950255.1	*Acyrthosiphon pisum*	4.00E-22	1
R-SS_TH_37	368	mitochondrial ribosomal protein L30 CG7038-PA	XP_001942529.1	*Acyrthosiphon pisum*	1.00E-09	1
R-SS_TH_38	665	Protein enabled	XP_001943648.1	*Acyrthosiphon pisum*	2.00E-19	1
R-SS_TH_39	470	signal recognition particle receptor alpha subunit (sr-alpha)	XP_001807129.1	*Tribolium castaneum*	3.00E-23	1
R-SS_TH_40	162	SVH protein	XP_001947154.1	*Acyrthosiphon pisum*	6.00E-09	1
R-SS_TH_41	897	tomosyn CG17762-PC	XP_001952670.1	*Acyrthosiphon pisum*	2.00E-73	1
R-SS_TH_42	451	vacuolar ATPase subunit C	XP_001946227.1	*Acyrthosiphon pisum*	5.00E-59	1
R-SS_TH_43	696	vasodilator-stimulated phosphoprotein	XP_970236.1	*Tribolium castaneum*	1.00E-18	1
R-SS_TH_44	876	reverse transcriptase	BAC57914.1	*Anopheles gambiae*	3.00E-17	1
R-SS_TH_45	268	SWI/SNF-related matrix-associated actin-dependent regulator of	EFN84532.1	*Harpegnathos saltator*	2.00E-32	1
R-SS_TH_47	766	transposase IS630	CAL47051.1	*Listonella anguillarum serovar O2*	8.00E-07	5
R-SS_TH_51	1057	unnamed protein product	BAG64005.1	*Homo sapiens*	3.00E-71	1
R-SS_TH_52	669	vitellogenin-3	BAA88077.1	*Plautia stali*	2.00E-09	1
R-SS_TH_1	570	60S ribosomal protein L27a	XP_001892460.1	*Brugia malayi*	7.00E-54	1

* Genes randomly selected for the qRT-PCR validation study are in the shade; ** transcripts were putatively identified by homology from BLASTX search using a cutoff E-value less than 1×10^{-5}.