Open Access Insect Physiology

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Transcription profiling of guanine nucleotide binding proteins during developmental regulation and pesticide response in *Solenopsis invicta* (Hymenoptera: Formicidae)

This article was published in the following Dove Press journal:
Open Access Insect Physiology
5 October 2012
Number of times this article has been viewed

Liming Zhao
Jian Chen
Walker A Jones

Biological Control of Pests Research Unit, National Biological Control Laboratory, Mid South Area, Agricultural Research Service, United States Department of Agriculture, Stoneville, MS, USA

Abstract: Guanine nucleotide binding proteins (GNBP), glycoproteins anchored on the cytoplasmic cell membrane, are mediators for many cellular processes. Complete cDNA of the GNBP gene β-subunit (*SiGNBP*) was cloned and sequenced from *Solenopsis invicta* Buren (Hymenoptera: Formicidae) worker ants. To understand whether *SiGNBP* is developmentally regulated in *S. invicta*, its expression levels in different developmental stages were examined using quantitative real-time polymerase chain reaction. *SiGNBP* was expressed in each developmental stage, and was especially highly expressed in the late larval and early pupal stages, as well among the dealate females (queens) ~10 days post nuptial flight. Quantitative real-time polymerase chain reaction also showed that mRNA transcription levels of *SiGNBP* in *S. invicta* workers were regulated during the duration of the study in response to heat shock, ultraviolet light, and boric acid treatment. These results suggest that the *SiGNBP* gene may play an important role in the development of *S. invicta*, and this has potential for use as a target in new insecticides for the control of fire ants.

Keywords: *Solenopsis invicta*, guanine nucleotide binding proteins, development, boric acid, mRNA transcription

Introduction

Transcriptional profiling is a powerful method for investigating the biological effects of chemicals and other stressors. It also offers a significant approach to identifying the relationship of gene expression to the development of the organisms. Information generated in such an approach is useful in identifying biomarkers for environmental and chemical stresses; this information can also be used in assessing new targets for drug development, such as in new insecticides for pest insect control. Guanine nucleotide binding proteins (GNBP), known as GTP-binding proteins and GTPases, are glycoproteins anchored on the cytoplasmic cell membrane, and are mediators for many cellular processes including signal transduction, protein transport, growth regulation, and polypeptide chain elongation. G-β subunits from heterotrimeric GNBPs modulate a wide array of signaling cascades by binding directly to diverse proteins, including effectors and regulators. GNBPs have been found in a variety of animals, plants, fungi, and insects.[1–6] The evolutionary aspects of GNBP-coupled receptors and their signaling pathways have revealed certain insect-specific features,[7] indicating that the GNBP may be a good target for developing new specific insecticides.

Correspondence: Liming Zhao
BCPRU-MSA-ARS USDA, Insect Physiology, 59 Lee Road, Stoneville, MS 38776 USA
Tel +66 268 630 67
Fax +66 268 659 22
Email liming.zhao@ars.usda.gov

The red imported fire ant is a widespread pest ant with significant medical and economic importance.[8,9] As in all organisms, the development of ants requires multigene regulation, which is affected by environmental conditions such as temperature, radiation, and challenges from pesticides and parasites.[10–22] However, the role of GNBPs in *Solenopsis invicta* Buren development has never been examined. In this study, the complete cDNA of the guanine nucleotide-binding protein gene β-subunit (*SiGNBP*) was cloned and sequenced from *S. invicta* workers, and its transcriptional expression related to the developmental stages and pesticide response was examined.

Materials and methods

Fire ants

Colonies of *S. invicta* were collected in Washington County, Mississippi in 2010 and 2011. All developmental stages of *S. invicta,* (ie, eggs, larvae, pupae, and adults – including workers, male alates, female alates, and queens) were collected at numerous time points within each stage in the laboratory. Larval stages were morphologically identified by microscopy according to the criteria of Petralia and Vinson.[23] For the egg stage, 50 μg of three samples were collected. For the larval stage, 50–100 larvae per sample were collected for RNA extraction. For pupae and adults, each sample contained 5–20 pupae and adults. Small workers and large workers were separated according to their sizes. An average of 20 large ants weighed 74.6 ± 1.5 mg, while an average of 20 small ants weighed 15.6 ± 2.19 mg. Three different colonies from Washington County, Mississippi in 2010 were used. Reproductive larvae were separated from the colony and were maintained with workers together in growth chambers in order to collect the time course samples for developmental study. Samples were frozen at −80°C in a freezer, and RNA extractions were processed when the collection stage was completed. Newly dealated queens were collected in Leland, Mississippi and were used for the time course studies of queens. Three queens were collected in 1, 5, 10, 16, 21, and 32 days, individually. The queens were provided with 10% sugar as food in a 50 mL glass test tube with cottons plugs. After about 8–10 days, the queens produced their first set of eggs.

RNA extraction

Total RNAs were extracted using TRIzol reagent according to the manufacturer's instructions (Invitrogen, Life Technologies Corporation, Carlsbad, CA). Poly $(A)^+$ RNA was isolated by applying Oligotex-dT suspension (QIAGEN, Valencia, CA). mRNA samples were quantified by SmartSpec™ Plus Spectrophotometry (Bio-Rad Laboratories, Inc, Hercules, CA).

GeneRacer cloning

The GeneRacer™ kit was used to amplify the full-length gene of 5′ and 3′ cDNA ends by slightly modifying the manufacturer's instruction (Invitrogen). Polymerase chain reaction (PCR) products were cloned using the TOPO TA Cloning® kit for sequencing (Invitrogen). Transformed plasmids were inserted into One Shot® TOP10 Competent Cells (Invitrogen) and grown overnight on Luria-Bertani plates containing ampicillin and X-Gal (5-bromo-4-chloro-3-indolyl- beta-D-galactopyranoside). Clones were isolated and grown overnight in LB-ampicillin broth at 37°C, and shaken at 230 RPM in the Thermo Scientific Analog MaxQ 4000 Shaker (Thermo Fisher Scientific Inc, Waltham, MA).

Gene sequencing of GeneRacer library

Clones of the GeneRacer library were purified with a QIAprep Miniprep (QIAGEN). The plasmid DNAs (0.5 μg) were then digested using the EcoRI enzyme (2.5 U) for 1.5 hours, and were run on a 1% agarose gel to confirm the DNA insert. Selected clones were then sent to the DNA Sequencing Core at the Interdisciplinary Center for Biotechnology Research at the University of Florida (Gainesville, FL) to be sequenced and analyzed using the National Center for Biotechnology Information BLASTN program to identify sequence homologies. The sequences were submitted into the National Center for Biotechnology Information GenBank; the accession number was HM130685.

cDNA synthesis for qPCR

To ensure that genomic DNA did not contaminate the sample, the Oligotex mRNA mini Kit (QIAGEN) was used to purify total RNA. A 250 ng aliquot of purified mRNA was reverse transcribed in a 20 μL reaction volume using the Clone AMV first-Strand Synthesis Kit, while also using the Oligo (dT)20 primer for cDNA synthesis according to the manufacturer's instructions (Invitrogen). The reaction was terminated by heat inactivation at 95°C for 5 min. The cDNA samples used for the quantitative real-time PCR (qPCR) analysis were taken from ants in their developmental stages undergoing boric acid treatment; controls were diluted by adding 80 μL ddH_2O (~450 ± 50 ng/μL) and stored at −20°C.

To design gene-specific primers, detailed analyses of the nucleotide sequence of genes found in the library (Genbank accession number: HM130685) were performed using the PRIMER3-Design Primer Pairs and Probes program from Biology Workbench (Department of Bioengineering, University of California, San Diego, CA). The primers for the *S. invicta* actin gene (Genbank accession number: HM130684) were also designed for internal control and comparison purposes.

qPCR amplification

The qPCR assay for the *SiGNBP* gene in *S. invicta* was performed using Platinum® SYBR® Green qPCR SuperMix-UDG with ROX (Invitrogen) in a volume of 15 µL on an Applied Biosystems 7300 Fast Real-Time PCR System (Life Technologies Corporation, Foster City, CA). The PCR mixture consisted of 1 µL of diluted cDNA (~450 ± 50 ng/µL), 0.5 µM primers, and 1X master mix. In every qPCR run, actin was used as an internal control to normalize for variation in the amount of the cDNA template. The PCR primers for the actin gene were *SiActin*-783-F 5′-CCTCTTCCAACCTTCCTTCC-3′, *SiActin*-948R 5′-CTTTTGCATACGATCAGCGA-3′, *SiGNBP*-13F 5′-TTACAGCTGAGAGGGACGCT-3′, and *SiGNBP*-267R 5′-AAGACGCAATGTTTTGTCCC-3′. The PCR thermal cycling parameters were the same as described previously.[22,24] Relative expression levels were calculated as follows. First, *SiGNBP* transcript levels relative to a standard (*SiActin*) were calculated using the following formula:

$$\Delta CT = CT\ (SiGNBP) - CT\ (SiActin) \quad (1)$$

Second, an average ΔCT value for each sample was calculated. Third, relative expression levels were calculated using the equation:

$$100 \times 2^{[-\text{average } \Delta CT]} \quad (2)$$

Boric acid experiments

Boric acid was delivered in a 10% sugar solution using feeding stations at room temperature (27°C). Two concentrations were used: 0.85 mg/mL (low dose, LD) and 8.5 mg/mL (high dose, HD). To trace the RNA expression levels of differentially transcribed genes for each dose, workers (200 mg) were sampled from each of the three different colonies at 4, 8, 12, and 14 days. Control workers were simultaneously collected from colonies that were supplied with boric acid-free sugar water. This time course study was replicated three times. mRNA was extracted and a cDNA library was created as previously described. qPCR was performed using primers designed for the genes *SiGNBP* and *SiActin*.

Sequence data processing

Multiple sequence alignments of GNBP and orthologues from other insects were performed with the Molecular Evolutionary Genetics Analysis 5.05 program (MEGA; http://www.megasoftware.net). A phylogenetic tree was constructed using the neighbor-joining method with the MEGA 5.05 program.[25] The neighbor-joining method is based on the minimum-evolution criterion, and is also a bottom-up clustering method for the creation of phenetic trees.[26]

Statistical analysis

Comparisons of means were analyzed using the paired *t*-test, and *t*-values and *P*-values were reported when normality and equal variance tests were passed. Significant differences between the data were determined using SigmaPlot software (SigmaPlot®11.2; Systat Software, Inc, San Jose, CA).

Results

Identification of *Solenopsis invicta* guanine nucleotide-binding protein subunit beta-like protein gene

The complete cDNA sequence of the *SiGNBP* gene of *S. invicta* was deposited in the GenBank (accession number: HM130685). *SiGNBP* is 951 base pairs in length, and encodes a protein of 317 amino acids with a molecular mass of 35.7 kDa. A phylogenetic tree for *SiGNBP* nucleic acid sequences of guanine nucleotide binding proteins from other insect orthologues was constructed using the neighbor-joining method with the MEGA 5.05 program (Figure 1). The phylogenetic analysis showed that *SiGNBP* was closely related to guanine nucleotide binding proteins from *Apis mellifera* L. and *Bombus terrestris* L.

SiGNBP RNA profile in different developmental stages of *Solenopsis invicta*

To understand how *SiGNBP* is regulated during the development of *S. invicta*, qPCR was performed to examine the relative transcription levels of *SiGNBP* in eggs, larvae, pupae, workers, male alates, female alates, and queens (Table 1A and B). In the egg stage, the relative RNA transcription level of *SiGNBP* ranged from 13.217 ± 1.665 to 58.919 ± 0.935.

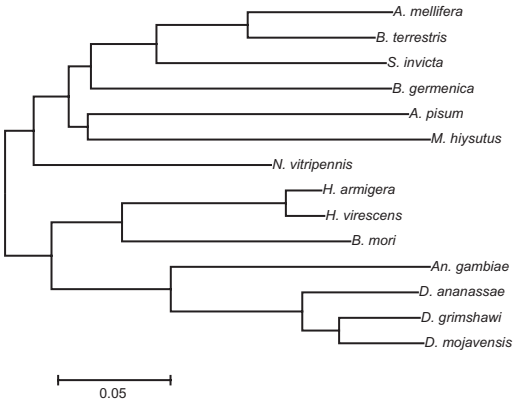

Figure 1 Construct/test neighbor-joining GNBP tree.
Notes: A phylogenetic tree was constructed using the neighbor-joining method for *SiGNBP* nucleic acid sequences of GNBPs from other insect orthologues using the MEGA 5.05 program. The scale bar indicates the number of changes inferred as having occurred along each branch. The accession numbers of reference nucleotide sequences used in this analysis are: HM130685, XM_003394751, XM_392962, XM_001600102, DQ885470.1, EF070464.1, JF417987.1, AF368031.1, AF368031.1, AY737531.1, HM449904.1, DQ073455.1, AY588074.1, XM_001988797.1, XM_968486.2, XM_001948034.2, HQ638203.1, XM_002065121.1, EU259815.1, BX063203, and XM_002003720.1.
Abbreviations: GNBP, guanine nucleotide binding proteins; *SiGNBP*, guanine nucleotide binding proteins gene β-subunit.

During the early worker larval stages, *SiGNBP* RNA expression decreased from first instar (37.288 ± 1.300) to third instar (4.264 ± 0.037), but these levels significantly increased during the fourth instar (111.71 ± 1.417) as compared to the third instar (Table 1A, and supplementary Table S1).

For reproductive larvae, *SiGNBP* RNA expression increased significantly in the fourth instar as compared to all the early instars, reaching (35.525 ± 0.425) (Table 1B and supplementary Table S1). There was a high *SiGNBP* RNA expression in early reproductive pupae (144.053 ± 1.997); however, expression significantly decreased in the late reproductive pupae. The *SiGNBP* RNA expression level was similar in the younger small workers, larger workers, and female alates (Table 1A and B).

In the male alates and queens, the relative *SiGNBP* RNA levels were differentially expressed from teneral male alates (8.249 ± 0.395) to the mature male alates (95.703 ± 4.111) (Table 1B and supplementary Table S1). The relative *SiGNBP* RNA levels were differentially expressed in queens' postnuptial flight. About 10 days postnuptial flight, the relative *SiGNBP* RNA expression reached its highest expression level (991.56 ± 15.46), which is significantly increased compared with day 1 (4.855 ± 1.842) and day 5 (57.784 ± 0.821) (Table 1B and supplementary Table S1). However, the relative *SiGNBP* RNA expression level was significantly reduced among the queens at 16 days postnuptial flight, as compared with the queens at day 10 postnuptial flight (Table 1B and supplementary Table S1).

Effects of boric acid on *SiGNBP* gene expression

To determine whether the transcription of *SiGNBP* in *S. invicta* was affected by boric acid treatment, workers were treated with different concentrations of boric acid in a 10% sugar solution. The qPCR time courses of *SiGNBP* expressed in workers were different between the two concentrations of the boric acid (HD and LD). In workers, relative *SiGNBP* expression levels increased two-fold for LD at 4 days of boric acid treatment, and then slightly increased for LD at 8-day and 12-day boric acid treatments compared with controls (Table 2A and B, Figure 2, and supplementary Table S2). After 14 days, *SiGNBP* expression was slightly decreased for LD boric acid when treatment compared with the control treatment of 10% sugar alone. However, boric acid HD-treated workers showed a decrease in *SiGNBP* RNA expression at 4, 8, 12, and 14-day exposures. Boric acid HD-treated workers showed a decrease in *SiGNBP* RNA expression (Figure 2, Table 2A and B, and supplementary Table S2).

Discussion

SiGNBP gene expression during *Solenopsis invicta* development

We examined and analyzed changes in *SiGNBP* RNA expression in *S. invicta* eggs, larvae, pupae, and adults. The *SiGNBP* gene was expressed in varying quantities during the egg and early instar stages. Previous studies showed that different forms of GNBP-coupled sensory transduction may mediate developmental interactions during both early and late stages of *Drosophila* embryogenesis.[6] Guanine nucleotide-binding proteins mediate signals between serotonin receptors and adenylate cyclase in *Schistosoma mansoni*, which indicates that the expression of this gene is developmentally mediated.[10]

There were significant differences in the expression of the *SiGNBP* gene between early (first to third instars) and late larval stages of worker ants (fourth instar). In addition, the expression of the *SiGNBP* gene in the early pupal stage was significantly higher than that in the late pupal stages of both worker and reproductive ants. These changes may indicate that the expression of the *SiGNBP* gene is important for late larval and pupal development in *S. invicta*. The fourth instar larvae play a critical role in the survival of the colony; they digest solid foods and provide protein and other nutrients to the other members in the colony.[15] An ant colony may be sensitive toward any disruption to the fourth instar larvae.[15] *SiGNBP* was highly expressed in fourth instar larvae, which may suggest that *SiGNBP* is important to fourth instar larvae

Table 1A Expression of *SiGNBP* in different developmental stages of *Solenopsis invicta*

Sample stage	Sample name	Mean Ct ± SD		Relative *SiGNBP* expression level			
		SiActin	*SiGNBP*	ΔCt-1	ΔCt-2	ΔCt-3	$100 \times 2^{-\Delta Ct} \pm SD$
Egg	Egg 1	21.325 ± 0.157	24.245 ± 0.024	2.738	3.101	2.920	13.217 ± 1.665
	Egg 2	16.488 ± 0.004	17.251 ± 0.026	0.786	0.740	0.763	58.919 ± 0.935
	Egg 3	18.191 ± 0.086	18.978 ± 0.040	0.833	0.741	0.787	57.951 ± 1.848
	Egg 4	19.839 ± 0.308	21.549 ± 0.058	1.651	1.767	1.709	30.582 ± 1.233
Larvae	L1	18.733 ± 0.010	20.157 ± 0.041	1.474	1.373	1.423	37.288 ± 1.300
	L2	18.518 ± 0.002	21.172 ± 0.015	2.666	2.641	2.654	15.893 ± 0.140
	L3	18.040 ± 0.029	22.592 ± 0.016	4.563	4.540	4.552	4.264 ± 0.037
	L4	21.171 ± 0.007	21.012 ± 0.017	–0.178	–0.141	–0.160	111.71 ± 1.417
Pupae	P1	19.687 ± 0.045	20.860 ± 0.071	1.148	1.198	1.173	44.353 ± 0.781
	P2	17.163 ± 0.035	22.447 ± 0.015	5.333	5.235	5.284	2.567 ± 0.088
Small workers	SW	18.215 ± 0.193	19.448 ± 0.021	1.060	1.405	1.233	42.558 ± 5.098
	SW	17.278 ± 0.088	18.999 ± 0.029	1.604	1.838	1.721	30.335 ± 2.462
	SW	17.391 ± 0.152	19.374 ± 0.010	1.831	2.134	1.983	25.305 ± 2.656
	SW	18.688 ± 0.008	22.266 ± 0.034	3.621	3.536	3.579	8.370 ± 0.246
	SW	18.397 ± 0.025	22.064 ± 0.061	3.631	3.704	3.667	7.871 ± 0.198
	SW	18.028 ± 0.003	21.143 ± 0.012	5.022	4.911	4.966	3.199 ± 0.124
Larger workers	LW	16.076 ± 0.024	17.710 ± 0.010	1.640	1.613	1.627	32.386 ± 0.299
	LW	16.672 ± 0.061	18.878 ± 0.024	2.243	2.170	2.206	21.673 ± 0.549
	LW	18.029 ± 0.023	20.991 ± 0.002	2.965	2.982	2.974	12.730 ± 0.281
	LW	18.028 ± 0.013	21.143 ± 0.012	3.124	3.107	3.116	11.538 ± 0.070
	LW	17.862 ± 0.036	21.620 ± 0.025	3.748	3.770	3.759	7.388 ± 0.056
	LW	18.122 ± 0.016	23.018 ± 0.076	4.803	4.896	4.988	3.360 ± 0.216

Abbreviations: *SiGNBP*, guanine nucleotide binding proteins gene β-subunit; Ct, cycle threshold; SD, standard deviation.

Table 1B Expression of *SiGNBP* in different developmental stages of *Solenopsis invicta*

Sample stage	Sample name	Mean Ct ± SD		Relative *SiGNBP* expression level			
		SiActin	*SiGNBP*	ΔCt-1	ΔCt-2	ΔCt-3	$100 \times 2^{-\Delta Ct} \pm SD$
Reproductive larvae	RL1	16.248 ± 0.007	19.364 ± 0.019	3.129	3.116	3.103	11.537 ± 0.102
	RL2	16.235 ± 0.004	20.139 ± 0.007	3.915	3.894	3.904	6.678 ± 0.050
	RL3	15.125 ± 0.011	18.148 ± 0.036	2.998	3.048	3.023	12.304 ± 0.263
	RL4	16.083 ± 0.022	17.560 ± 0.039	1.459	1.493	1.476	35.525 ± 0.425
Reproductive pupae	RP1	19.147 ± 0.007	18.621 ± 0.027	–0.507	–0.547	–0.527	144.05 ± 1.997
	RP2	19.623 ± 0.046	21.610 ± 0.075	1.959	2.015	1.987	25.225 ± 0.495
Female alates	FA	17.150 ± 0.022	18.354 ± 0.028	1.199	1.210	1.205	43.386 ± 0.162
	FA	19.219 ± 0.003	21.012 ± 0.039	1.738	1.847	1.793	28.860 ± 1.091
	FA	18.015 ± 0.003	21.136 ± 0.029	3.153	3.089	3.121	11.493 ± 0.253
Male alates	MA1[a]	18.714 ± 0.023	22.314 ± 0.046	3.531	3.669	3.599	8.249 ± 0.395
	MA4	16.753 ± 0.036	21.648 ± 0.113	4.971	4.817	4.894	3.362 ± 0.178
	MA8	19.733 ± 0.012	22.962 ± 0.005	3.212	3.246	3.229	10.666 ± 0.124
	MA12	18.478 ± 0.026	22.075 ± 0.171	3.452	3.742	3.597	8.265 ± 0.832
	MA14	24.174 ± 0.056	24.237 ± 0.118	0.001	0.125	0.063	95.703 ± 4.111
	MA18	21.121 ± 0.492	23.547 ± 0.121	2.205	2.426	2.630	18.817 ± 2.772
Queens	Q1[b]	18.193 ± 0.079	22.307 ± 0.071	4.105	4.123	4.114	5.775 ± 0.035
	Q5	20.659 ± 0.005	21.451 ± 0.003	0.789	0.793	0.791	57.784 ± 0.821
	Q10	26.346 ± 0.058	23.037 ± 0.036	–3.332	–3.287	–3.310	991.56 ± 15.46
	Q16	19.137 ± 0.040	22.762 ± 0.065	3.649	3.601	3.625	8.106 ± 0.136
	Q21	21.078 ± 0.030	22.177 ± 0.011	1.080	1.118	1.099	46.679 ± 0.607
	Q32	19.205 ± 0.038	20.969 ± 0.142	1.868	1.660	1.764	29.445 ± 2.123

Notes: [a]Male alates day post emerging from reproductive pupae; [b]dealate female (queens) day post nuptial flight.
Abbreviations: *SiGNBP*, guanine nucleotide binding proteins gene β-subunit; Ct, cycle threshold; SD, standard deviation.

Table 2A Expression of *SiGNBP* under boric acid feeding conditions in *Solenopsis invicta*

Time point	Ct ± SD		Relative *SiGNBP* expression level		
	SiActin	*SiGNBP*	ΔCt-1	ΔCt-2	ΔCt-3
Cont-4d'[a]	19.023 ± 0.121	21.842 ± 0.041	2.656	2.981	2.818
Cont-8d[a]	18.029 ± 0.023	20.991 ± 0.002	2.965	2.982	2.974
Cont-12d[a]	18.028 ± 0.013	21.143 ± 0.012	3.124	3.107	3.116
Cont-14[a]	17.770 ± 0.083	20.727 ± 0.052	2.988	2.927	2.957
BA-4d[b]	19.230 ± 0.025	22.114 ± 0.011	2.848	2.884	2.920
BF-8d[b]	18.156 ± 0.044	21.282 ± 0.037	3.133	3.120	3.126
BF-12d[b]	18.760 ± 0.116	22.282 ± 0.037	3.968	3.841	3.905
BF-14d[b]	19.266 ± 0.011	23.766 ± 0.123	4.365	4.634	4.499

Notes: [a]Nonboric acid, only 10% sugar feeding in *Solenopsis invicta* workers; [b]boric acid (0.85 mg/mL) within 10% sugar feeding in *Solenopsis invicta* workers.
Abbreviations: *SiGNBP,* guanine nucleotide binding proteins gene β-subunit; Ct, cycle threshold.

and thus is an excellent target for developing intervening methods, such as a new insecticide.

Numerous physiological changes occur during the development of ant adults. A previous study showed that arginine kinase, a primary enzyme in cell metabolism and adenosine 5′-triphosphate-consuming processes, was differentially expressed among larvae and adults, queens and workers, and among female alates and queens.[12] The results showed that the *SiGNBP* gene is not only differentially expressed among eggs, larvae, adults, workers, female alates, and queens, but it is also differentially expressed within the larval (ie, first instar, second instar, third instar, and fourth instar), pupal (early and late pupae), as well as the worker (early and late workers) stages. The relatively low levels of *SiGNBP* expression in older workers and female alates suggest that the *SiGNBP* gene may play a role in the attenuation of gene expression.

SiGNBP gene responses to pesticide

Many insecticides or repellents, including bifenthrin, chlorfenapyr, fipronil, and thiamethoxam, have been evaluated for activity against the red imported fire ant, *S. invicta*.[28–32] Molecular studies of insecticide resistance, including identification of the genes involved in target site and metabolic resistance mechanisms, have advanced rapidly over the past decade.[32–34] A previous study showed that boric acid significantly decreased the survivorship of workers in the target organs.[35]

SiGNBP expression levels of worker *S. invicta* were upregulated at 4, 8, and 12 days, and were downregulated at 14 days after a low dose of boric acid treatment. However, *SiGNBP* expression levels were downregulated at 4, 8, 12, and 14 days after a high dose of boric acid treatment.

In conclusion, expression of the *SiGNBP* gene in the life cycle of *S. invicta* was highly regulated developmentally and environmentally. *SiGNBP* RNA expression has, for the first time, been examined in detail for all developmental stages of *S. invicta*. The current study suggests that *SiGNBP* plays an important role in the development of *S. invicta*, and it may provide useful information for designing novel strategies for fire ant control. Using RNA interference technology to knock down the *SiGNBP* gene may provide additional targets that can be developed as new pesticides.

Table 2B Expression of *SiGNBP* under boric acid feeding conditions in *Solenopsis invicta*

Time point	Ct ± SD		Relative *SiGNBP* expression level		
	SiActin	*SiGNBP*	ΔCt-1	ΔCt-2	ΔCt-3
Cont-4d'[a]	19.023 ± 0.121	21.842 ± 0.041	2.656	2.981	2.818
Cont-8d[a]	18.029 ± 0.023	20.991 ± 0.002	2.965	2.982	2.974
Cont-12d[a]	18.028 ± 0.013	21.143 ± 0.012	3.124	3.107	3.116
Cont-14[a]	17.770 ± 0.083	20.727 ± 0.052	2.988	2.927	2.957
BA-4d[b]	19.230 ± 0.025	22.114 ± 0.011	2.848	2.884	2.920
BF-8d[b]	18.156 ± 0.044	21.282 ± 0.037	3.133	3.120	3.126
BF-12d[b]	18.760 ± 0.116	22.282 ± 0.037	3.968	3.841	3.905
BF-14d[b]	19.266 ± 0.011	23.766 ± 0.123	4.365	4.634	4.499

Notes: [a]Nonboric acid, only 10% sugar feeding in *Solenopsis invicta* workers; [b]boric acid (0.85 mg/mL) within 10% sugar feeding in *Solenopsis invicta* workers.
Abbreviations: *SiGNBP,* guanine nucleotide binding proteins gene β-subunit; Ct, cycle threshold.

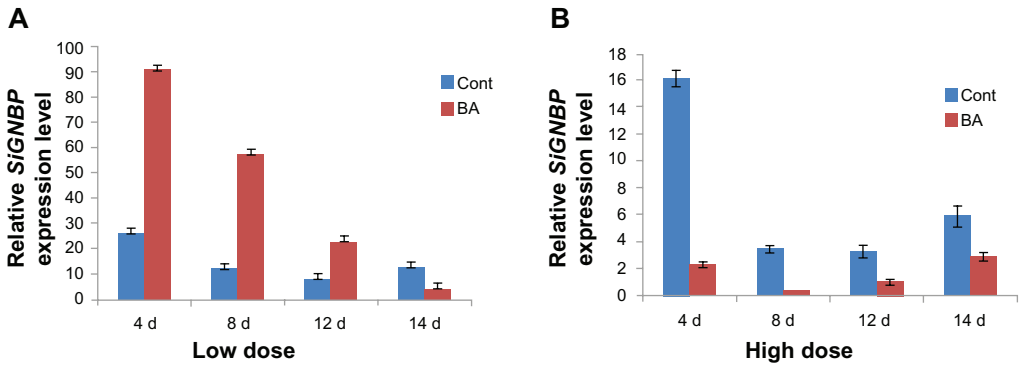

Figure 2 Boric acid treatment.
Notes: The *SiGNBP* transcription level in response to boric acid treatments, quantified by qPCR. *S. invicta* workers were exposed to low dose (0.85 mg/mL), and high dose (HD, 8.5 mg/mL) at 4, 8, 12, and 14 days.
Abbreviations: Cont, control; BA, boric acid; *SiGNBP*, guanine nucleotide binding proteins gene β-subunit; qPCR, quantitative real-time polymerase chain reaction.

Acknowledgments

We thank Drs. Zibiao Guo (University of Mississippi Medical Center) and Cesar Solorzano Torres (USDA-ARS) for critical reviews of the manuscript. We also thank Richard Evans and Sharla Burke (USDA-ARS) for technical support.

Disclosure

The authors report no conflict of interest in this work.

References

1. Kinoshita M. The septins. *Genome Biol*. 2003;4(11):236.
2. Friedman EJ, Temple BR, Hicks SN, Sondek J, Jones CD, Jones AM. Prediction of protein-protein interfaces on G-protein beta subunits reveals a novel phospholipase C beta2 binding domain. *J Mol Biol*. 2009;392(4):1044–1054.
3. Miura N, Atsumi S, Tabunoki H, Sato R. Expression and localization of three G protein alpha subunits, Go, Gq, and Gs, in adult antennae of the silkmoth (*Bombyx mori*). *J Comp Neurol*. 2005;485(2):143–152.
4. Mosser VA, Amana IJ, Schimerlik MI. Kinetic analysis of M2 muscarinic receptor activation of Gi in Sf9 insect cell membranes. *J Biol Chem*. 2002;277(2):922–931.
5. Quan F, Thomas L, Forte M. Drosophila stimulatory G protein alpha subunit activates mammalian adenylyl cyclase but interacts poorly with mammalian receptors: implications for receptor-G protein interaction. *Proc Natl Acad Sci U S A*. 1991;88(5):1898–1902.
6. Wolfgang WJ, Quan F, Thambi N, Forte M. Restricted spatial and temporal expression of G-protein alpha subunits during *Drosophila* embryogenesis. *Development*. 1991;113(2):527–538.
7. Broeck J. Insect G protein-coupled receptors and signal transduction. *Arch Insect Biochem Physiol*. 2001;48(1):1–12.
8. Ascunce MS, Yang CC, Oakey J, et al. Global invasion history of the fire ant Solenopsis invicta. *Science*. 2011;331(6020):1066–1068.
9. Vinson SB. Invasion of the red imported fire ant (Hymenoptera: Formicidae): spread, biology, and impact. *American Entomologist*. 1997;43:23–39.
10. Iltzsch MH, Bieber D, Vijayasarathy S, et al. Cloning and characterization of a cDNA coding for the alpha-subunit of a stimulatory G protein from *Schistosoma mansoni*. *J Biol Chem*. 1992;267(20):14504–14508.
11. Graber SG, Figler RA, Kalman-Maltese VK, Robishaw JD, Garrison JC. Expression of functional G protein beta gamma dimers of defined subunit composition using a baculovirus expression system. *J Biol Chem*. 1992;267(19):13123–13126.
12. Wang H, Zhang L, Lin Q, Liu N. Arginine kinase: differentiation of gene expression and protein activity in the red imported fire ant, *Solenopsis invicta*. *Gene*. 2009;430(1–2):38–43.
13. Liu N, Zhang L. CYP4AB1, CYP4AB2, and Gp-9 gene overexpression associated with workers of the red imported fire ant, *Solenopsis invicta* Buren. *Gene*. 2004;327(1):81–87.
14. Tracy JM, Demain JG, Quinn JM, Hoffman DR, Goetz DW, Freeman TM. The natural history of exposure to the imported fire ant (*Solenopsis invicta*). *J Allergy Clin Immunol*. 1995;95(4):824–828.
15. Whitworth ST, Blum MS, Travis J. Proteolytic enzymes from larvae of the fire ant, *Solenopsis invicta*. Isolation and characterization of four serine endopeptidases. *J Biol Chem*. 1998;273(23):14430–14434.
16. Shoemaker DD, Ross KG, Keller L, Vargo EL, Werren JH. Wolbachia infections in native and introduced populations of fire ants (Solenopsis spp.). *Insect Mol Biol*. 2000;9(6):661–673.
17. Haisheng T, Bradleigh VS, Coates CJ. Differential gene expression between alate and dealate queens in the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). *Insect Biochem Mol Biol*. 2004;34(9):937–949.
18. Valles SM, Strong CA, Dang PM, et al. A picorna-like virus from the red imported fire ant, *Solenopsis invicta*: initial discovery, genome sequence, and characterization. *Virology*. 2004;328(1):151–157.
19. Folgarait PJ, Patrock RJ, Gilbert LE. Development of Pseudacteon nocens (Diptera: Phoridae) on *Solenopsis invicta* and *Solenopsis richteri* fire ants (Hymenoptera: Formicidae). *J Econ Entomol*. 2006;99(2):295–307.
20. Park J, Kaufmann GF, Bowen JP, Arbiser JL, Janda KD. Solenopsin A, a venom alkaloid from the fire ant *Solenopsis invicta*, inhibits quorum-sensing signaling in *Pseudomonas aeruginosa*. *J Infect Dis*. 2008;198(8):1198–1201.
21. Tufts DM, Bextine B. Identification of bacterial species in the hemolymph of queen *Solenopsis invicta* (Hymenoptera: Formicidae). *Environ Entomol*. 2009;38(5):1360–1364.
22. Zhao L, Pridgeon JW, Becnel JJ, Clark GG, Linthicum KJ. Cytochrome c gene and protein expression: developmental regulation, environmental response, and pesticide sensitivity in *Aedes aegypti*. *J Med Entomol*. 2008;45(3):401–408.
23. Petralia RS, Vinson SB. Developmental morphology of larvae and eggs of the imported fire ant, *Solenopsis invicta*. *Annals of the Entomological Society of America*. 1979;72(4):472–484.

24. Zhao L, Pridgeon JW, Becnel JJ, Clark GG, Linthicum KJ. Identification of genes differentially expressed during heat shock treatment in Aedes aegypti. *J Med Entomol*. 2009;46(3):490–495.
25. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*. 2011;28(10):2731–2739.
26. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol*. 1987;4(4):406–425.
27. Wiltz BA, Suiter DR, Gardner WA. Activity of bifenthrin, chlorfenapyr, fipronil, and thiamethoxam against red imported fire ants (Hymenoptera: Formicidae). *J Econ Entomol*. 2010;103(3):754–761.
28. Chen J, Shang H, Jin X. Interspecific variation of Delta1,6-piperideines in imported fire ants. *Toxicon*. 2010;55(6):1181–1187.
29. Chen J. Repellency of an over-the-counter essential oil product in China against workers of red imported fire ants. *J Agric Food Chem*. 2009;57(2):618–622.
30. Oi DH, Oi FM. Speed of efficacy and delayed toxicity characteristics of fast-acting fire ant (Hymenoptera: Formicidae) baits. *J Econ Entomol*. 2006;99(5):1739–1748.
31. Appel AC, Gehret MJ, Tanley MJ. Repellency and toxicity of mint oil granules to red imported fire ants (Hymenoptera: Formicidae). *J Econ Entomol*. 2004;97(2):575–580.
32. Zhu F, Parthasarathy R, Bai H, et al. A brain-specific cytochrome P450 responsible for the majority of deltamethrin resistance in the QTC279 strain of Tribolium castaneum. *Proc Natl Acad Sci U S A*. 2010; 107(19):8557–8562.
33. Wu S, Li M, Tang PA, Felton GW, Wang JJ. Cloning and characterization of acetylcholinesterase 1 genes from insecticide-resistant field populations of Liposcelis paeta Pearman (Psocoptera: Liposcelididae). *Insect Biochem Mol Biol*. 2010;40(5):415–424.
34. Marcombe S, Poupardin R, Darriet F, et al. Exploring the molecular basis of insecticide resistance in the dengue vector Aedes aegypti: a case study in Martinique Island (French West Indies). *BMC Genomics*. 2009;10:494.
35. Sumida S, Silva-Zacarin EC, Decio P, Malaspina O, Bueno FC, Bueno OC. Toxicological and histopathological effects of boric acid on Atta sexdens rubropilosa (Hymenoptera: Formicidae) workers. *J Econ Entomol*. 2010;103(3):676–690.

Supplemental material

Table S1A Paired *t*-test data for comparison of relative *SiGNBP* gene transcription between developmental stages in *Solenopsis invicta*

Stage and ages	N	Df	t-value	*P*-value
L1 and L2	3	2	93.606	<0.001*
L2 and L3	3	2	38.995	<0.001*
L3 and L4	3	2	−55.610	<0.001*
L4 and P1	3	2	49.385	<0.001*
P1 and P2	3	2	85.455	<0.001*
SW(Y) and SW(O)	3	2	13.107	=0.006*
LW(Y) and LW(O)	3	2	591.843	<0.001*

Note: *Statistical significance ($P < 0.05$).
Abbreviations: *SiGNBP*, guanine nucleotide binding proteins gene β-subunit; SW, small worker; LW, large worker.

Table S1B Paired *t*-test data for comparison of relative *SiGNBP* gene transcription between developmental stages in *Solenopsis invicta*

Stage and ages	N	Df	t-value	*P*-value
RL1 and RL2	3	2	162.15	=0.001*
RL2 and RL3	3	2	144.81	<0.001*
RL3 and RL4	3	2	−100.45	<0.001*
RL4 and RP1	3	2	−77.33	<0.001*
RP1 and RP2	3	2	36.792	<0.001*
FA1 and FA2	3	2	27.166	=0.001*
FA2 and FA3	3	2	22.373	=0.002*
MA1 and MA4	3	2	14.762	=0.005*
MA4 and MA8	3	2	−41.739	<0.001*
MA8 and MA12	3	2	5.803	=0.028*
MA12 and MA14	3	2	−46.210	<0.001*
MA14 and MA18	3	2	38.975	<0.001*
Q1 and Q5	3	2	−1893.457	<0.001*
Q5 and Q10	3	2	−105.15	<0.001*
Q10 and Q16	3	2	111.133	<0.001*
Q16 and Q21	3	2	−89.963	<0.001*
Q21 and Q32	3	2	12.028	<0.007*

Note: *Statistical significance ($P < 0.05$).
Abbreviation: *SiGBNP*, guanine nucleotide binding proteins gene β-subunit.

Table S2 Paired *t*-test data for comparison of relative *SiGNBP* gene transcription between LD and HD boric acid treatments in *Solenopsis invicta* workers

Boric acid treatments	N	df	t-value	*P*-value
LD and Cont 4d	3	2	−38.448	<0.001*
LD and Cont 8d	3	2	−32.053	<0.001*
LD and Cont 12d	3	2	−5.023	=0.037*
LD and Cont 14d	3	2	104.372	<0.001*
HD and Cont 4d	3	2	49.802	<0.001*
HD and Cont 8d	3	2	157.478	<0.001*
HD and Cont 12d	3	2	73.056	<0.001*
HD and Cont 14d	3	2	14.788	=0.005*

Note: *Statistical significance ($P < 0.05$).
Abbreviations: *SiGNBP*, guanine nucleotide binding proteins gene β-subunit; LD, low dose; HD, high dose.