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Short Communication

Examination of host genome for the presence of integrated fragments of *Solenopsis invicta* virus 1

Steven M. Valles [a,*], Blake Bextine [b]

[a] USDA-ARS, Imported Fire Ant and Household Insects Research Unit, 1600 SW 23rd Drive, Gainesville, FL 32608, USA
[b] University of Texas at Tyler, 3900 University Blvd., Tyler, TX 75799, USA

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ABSTRACT

A series of oligonucleotide primer pairs covering the entire genome of *Solenopsis invicta* virus 1 (SINV-1) were used to probe the genome of its host, *S. invicta*, for integrated fragments of the viral genome. All of the oligonucleotide primer sets yielded amplicons of anticipated size from cDNA created from an RNA template from SINV-1. However, no corresponding amplification was observed when genomic DNA (from 32 colonies of *S. invicta*) was used as template for the PCR amplifications. Host DNA integrity was verified by amplification of an ant-specific gene, *SiGSTS1*. The representation of fire ant colonies included both social forms, monogyne and polygyne, and those infected and uninfected with SINV-1. Furthermore, no amplification was observed from genomic DNA from ant samples collected from Argentina or the US. Thus, it appears that SINV-1 genome integration, or a portion therein, has not likely occurred within the *S. invicta* host genome.

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1. Introduction

Solenopsis invicta virus 1 (SINV-1) is a dicistrovirus that infects the red imported fire ant, *S. invicta* (Valles et al., 2004). SINV-1 is the first virus reported to infect any ant (Formicidae) species (Oi and Valles, 2009). Since its discovery, additional characterization studies have been conducted including, host specificity (Valles et al., 2007), molecular detection and quantitation (Hashimoto et al., 2007), tissue tropism (Hashimoto and Valles, 2007), structural protein elucidation (Valles and Hashimoto, 2008), genotyping (Tufts et al., 2010; Valles and Strong, 2005), genome mutation rate (Allen et al., 2010), cap-independent translation (Nakashima and Shibuya, 2006; Nakashima and Uchiumi, 2008), and phylogenetic relationships (Bonning, 2009; Bonning and Miller, 2010). Thus, the biology of this virus is gradually emerging.

SINV-1 infection of fire ants resembles similar RNA virus infections in honeybees. Specifically, the virus occurs as a chronic, asymptomatic infection with no overt effects. However, when the infected host experiences external stress, the virus rapidly replicates often resulting in characteristic symptoms and/or death (Chen and Siede, 2007; Valles et al., 2004). Recently, portions of some positive-strand RNA viruses (including a dicistrovirus) have been reported to be integrated into their host genomes. This result is highly unexpected because no DNA stage is synthesized during the life cycle of positive-strand RNA viruses. Furthermore, and most interestingly, the integration event apparently afforded protection to the host from infection by the corresponding virus (Crochu et al., 2004; Maori et al., 2007; Tanne and Sela, 2005). Indeed, shrimp purposely transfected with, and expressing an anti-sense *Taura syndrome virus* (TSV) coat protein, had a nearly 2-fold increased survival rate compared with wild-type shrimp against TSV challenge (Lu and Sun, 2005). Although SINV-1 appears to cause a persistent, asymptomatic infection in *S. invicta*, its effect on the host population is not understood. It has been suggested that SINV-1 may be exploited as a microbial control agent of *S. invicta*. Thus, integration of a portion of the SINV-1 genome into the host, *S. invicta*, may influence its effectiveness as a microbial control agent. With this possibility in mind, experiments were conducted to determine whether a portion of the SINV-1 genome had been integrated into the fire ant host genome.

2. Materials and methods

S. invicta ants used for the study included colony samples of workers from Florida, Texas, and Argentina (Table 1). In all cases, RNA and DNA were extracted from the same colonies and each examined by molecular analyses to determine the social form of the colony, the SINV-1 virus infection status, and whether integration of a portion of the SINV-1 genome occurred based on production of an amplicon by PCR with genomic DNA as template.

Total RNA was extracted from 10 field-collected fire ant worker ants by the Trizol method according to the manufacturer's instructions (Invitrogen, CA). The RNA was evaluated for SINV-1 infection by the multiplex RT-PCR method described previously (Valles et al., 2009). DNA was extracted from a pooled group of 10 worker ants

* Corresponding author. Fax: +1 352 374 5818.
E-mail address: steven.valles@ars.usda.gov (S.M. Valles).

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Table 1
Summary data for *Solenopsis invicta* colonies examined for SINV-1 genome integration.

Colony sample	Collection		SINV-1 infection status	Social form	Integration result
	Location	Date			
37/11A/42/6	Newberry, FL	Oct-2010	Negative	Monogyne	Negative
39/11A/27/1	Windsor, FL	Oct-2010	Negative	Monogyne	Negative
43/11A/44/4	Otter creek, FL	Nov-2010	Negative	Monogyne	Negative
43/11A/44/3	Otter creek, FL	Nov-2010	Negative	Monogyne	Negative
55/103/145/197	Kenedy, TX	May-2010	Negative	Monogyne	Negative
57/103/135/1101	Formosa, Argentina	Oct-2008	Negative	Monogyne	Negative
57/103/135/986	Formosa, Argentina	Oct-2008	Negative	Monogyne	Negative
57/103/135/990	Formosa, Argentina	Oct-2008	Negative	Monogyne	Negative
37/11A/39/2	Paines Prairie, FL	Oct-2010	Positive	Monogyne	Negative
39/11A/27/3	Windsor, FL	Oct-2010	Positive	Monogyne	Negative
42/11A/22/1	Micanopy, FL	Nov-2010	Positive	Monogyne	Negative
42/11A/22/2	Micanopy, FL	Sept-2010	Positive	Monogyne	Negative
43/11A/33/2	Gainesville, FL	Oct-2010	Positive	Monogyne	Negative
57/103/135/962	Formosa, Argentina	Oct-2008	Positive	Monogyne	Negative
57/103/135/1000	Formosa, Argentina	Oct-2008	Positive	Monogyne	Negative
55/103/145/208	Kenedy, TX	Oct-2006	Negative	Monogyne	Negative
55/103/145/217	Kenedy, TX	Oct-2006	Negative	Monogyne	Negative
55/103/145/245	Kenedy, TX	May-2010	Positive	Monogyne	Negative
37/11A/44/6	Otter Creek, FL	Nov-2010	Negative	Polygyne	Negative
55/103/145/196	Kenedy, TX	Oct-2006	Negative	Polygyne	Negative
57/103/135/985	Formosa, Argentina	Oct-2008	Negative	Polygyne	Negative
57/103/135/988	Formosa, Argentina	Oct-2008	Negative	Polygyne	Negative
57/103/135/978	Formosa, Argentina	Oct-2008	Negative	Polygyne	Negative
37/11A/44/3	Otter Creek, FL	Nov-2010	Positive	Polygyne	Negative
39/11A/27/2	Windsor, FL	Oct-2010	Positive	Polygyne	Negative
39/11A/27/4	Windsor, FL	Oct-2010	Positive	Polygyne	Negative
42/11A/22/5	Micanopy, FL	Sept-2010	Positive	Polygyne	Negative
42/11A/22/6	Micanopy, FL	Sept-2010	Positive	Polygyne	Negative
43/11A/24/2	Otter Creek, FL	Nov-2010	Positive	Polygyne	Negative
55/103/145/257	Kenedy, TX	Sept-2010	Positive	Polygyne	Negative
55/103/145/341	Kenedy, TX	Sept-2010	Positive	Polygyne	Negative
55/103/145/342	Kenedy, TX	Sept-2010	Positive	Polygyne	Negative

from each corresponding colony (Valles et al., 2002). Nucleic acid concentration was determined spectrophotometrically and adjusted to 50 ng/μl by further dilution with diethyl-pyrocarbonate-treated water (RNA) or TE (10 mM Tris, pH 8.0, 1 mM EDTA) buffer (DNA). Colony social form was determined by conducting allele-specific PCR at the *Gp-9* locus using a DNA template (Valles and Porter, 2003). Homozygous ($Gp\text{-}9^{BB}$) samples were considered monogyne and heterozygous ($Gp\text{-}9^{Bb}$) samples polygyne (Krieger and Ross, 2002).

Oligonucleotide primers were designed and synthesized to provide complete coverage of the SINV-1 genome (Genbank accession number AY634314) in approximately 150 nucleotide sections (Supplementary Table). Once the oligonucleotide primers were synthesized, they were evaluated for their ability to amplify the corresponding section of the SINV-1 genome by PCR with cDNA synthesized from SINV-1-infected *S. invicta* (i.e., SINV-1 RNA). The reverse oligonucleotide primer from each pair was used in the cDNA synthesis reaction. Two-step RT-PCR was employed to amplify each SINV-1 region. First, 0.5 μl (25 ng) of the total extracted RNA was mixed with 10 mM dNTPs, 1 μM reverse oligonucleotide primer, heated to 65 °C for 5 min, and then placed on ice for at least 1 min. First strand buffer and Superscript reverse transcriptase (RT, Invitrogen) were then added and the reaction mixture incubated at 55 °C for 1 h before inactivating the RT at 70 °C for 15 min.

PCR was subsequently conducted with the cDNA and DNA templates. The reaction was conducted in a 25 μl volume containing 2 mM $MgCl_2$, 200 μM dNTP mix, 0.5 units of Platinum *Taq* DNA polymerase (Invitrogen), 0.2 μM of each primer, and 5 μl of the cDNA preparation. PCR products were separated on a 1% agarose gel and visualized by SYBR-safe (Invitrogen) staining. The integrity of each of the DNA sources was verified by amplification (with an identical quantity of DNA used in the integration assays) of a long template for the *S. invicta* glutathione *S*-transferase gene, *SiGSTS1* (Valles et al., 2003). Negative controls (non-template) were run alongside treatments. Amplification was completed in a thermal cycler under the following temperature regime: one cycle at 94 °C for 2 min, 35 cycles of 94 °C for 15 s, 55 °C for 15 s, 68 °C for 2 min, followed by a final elongation step of 68 °C for 5 min. Several oligonucleotide primer sets required lower annealing temperatures as indicated in the Supplementary Table.

3. Results and discussion

All oligonucleotide primer sets yielded amplicons of anticipated size from cDNA created from an RNA template from SINV-1-infected ants indicating that the oligonucleotide primers were specific for SINV-1 and functioning properly (Fig. 1). However, no corresponding amplification was observed when genomic DNA (from 32 colonies of *S. invicta*) was used as template for the PCR amplifications (Fig. 1). Furthermore, no extraneous bands were observed in PCR amplifications from DNA templates. However, artifacts were observed for a few reactions. These diffuse bands were significantly smaller than the corresponding amplicon from cDNA templates and considered dimerized primers. Integrity of each of the DNA templates was verified by successful amplification of a portion of the *SiGSTS1* gene from these DNA sources (Fig. 1). The representation of fire ant colonies included both social forms, monogyne and polygyne, and those infected and uninfected with SINV-1 (Table 1).

The possibility that a bottleneck could have occurred during the founding event of *S. invicta* into the US in which a SINV-1-containing integrated "allele" (i.e., integrant) could have been lost was considered. Consistent with this possibility is the fact that SINV-1 prevalence tends to be slightly higher among US *S. invicta*

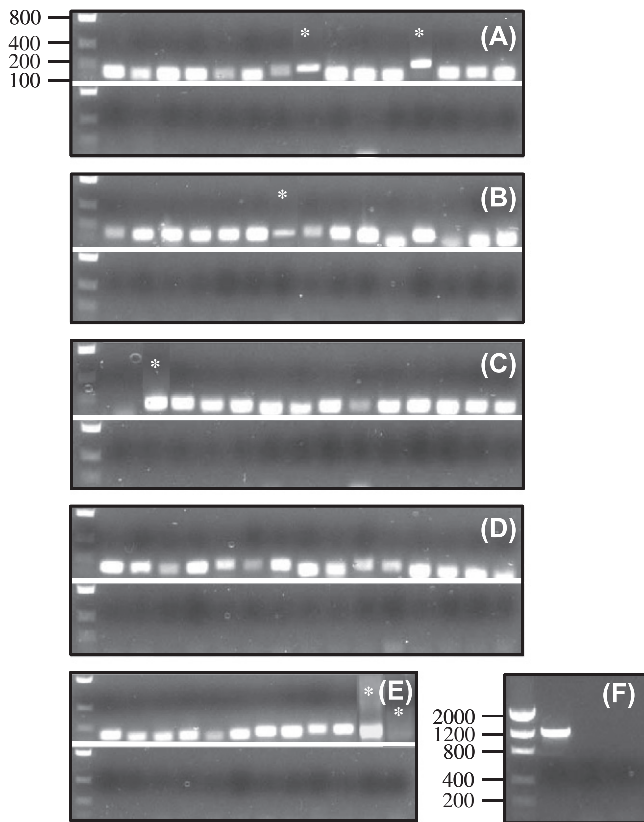

Fig. 1. Representative summary of PCR amplification results with cDNA (upper panel of each block) prepared from RNA extracted from SINV-1-infected *S. invicta* and DNA (lower panel in each block) from *S. invicta* with oligonucleotide primer pairs covering the genome of SINV-1 (colony designation 37/11A/44/3 from Table 1). Molecular marker positions are identified in the first gel of panel A and are consistent for all gels. Panel A includes the genome region covered by oligonucleotide primer sets 1 through 15 (see Supplementary Table for key); B includes 16 through 30; C includes 31 through 44; D includes 45 through 59; E includes 60 through 71. Panel F represents an evaluation of the DNA integrity by amplification of the fire ant gene, *SiGSTS1*. Lane assignments for panel F include: (1) molecular marker (2) *SiGSTS1* amplicon (3) non-template control. Reactions conducted at a lower annealing temperature (see Supplemental Table) are indicated with an asterisk.

populations compared with Argentinean populations (Valles et al., 2007). However, no region of the SINV-1 genome was amplified from genomic DNA purified from Argentinean or US sample colonies. Thus, it appears that SINV-1 genome integration, or a portion therein, has not likely occurred within the *S. invicta* host genome. Obviously, limitations in the methodology used and the possibility of rare integration events having occurred must be considered. Although the oligonucleotide primer sets completely covered the entire SINV-1 genome (mean amplicon size yielded by each oligonucleotide primer set was 133 ± 24 nucleotides), a smaller fragment or fragment across the boundaries of the oligonucleotide primer margins could be present which would have resulted in a false negative, or non-detection. However, among the integration events reported to date, virus genome fragments detected in host genomes range in size from 428 to 1500 nucleotides, a sizable proportion of those viral genomes (Crochu et al., 2004; Geuking et al., 2009; Katzourakis and Gifford, 2010; Maori et al., 2007; Tanne and Sela, 2005). Despite these precedents, a smaller fragment may still be integrated in the *S. invicta* genome – a possibility that must remain a consideration.

The frequency of integration must also be considered. Maori et al. (2007) reported that 30% of tested honey bee (*Apis mellifera*) individuals from 19 colonies carried a segment of the dicistrovirus, Israeli acute paralysis virus (IAPV), which apparently conferred host resistance to this virus. Indeed, if a portion of the SINV-1 genome was integrated into the *S. invicta* genome and it provided a competitive advantage against viral infection, it would be expected to be present in a moderate portion of the population. However, among the 32 colony samples examined from the US and Argentina (each comprised of 10 ant workers), the introduced and native ranges of *S. invicta*, respectively, the ant genome used as template for PCR failed to amplify any region of the SINV-1 genome. Hypothetically, in cases where an integrant was contributed from a male fire ant during mating, all members of the resulting colony would possess the integrant because males are haploid and all of their genetic material is passed on to their progeny (assuming a single mating event). A similar result would occur in situations where the integrant was contributed by a homozygous fire ant queen (diploid). If an integrant was contributed from a heterozygous (for the integrant) fire ant queen, 50% of her progeny would possess the integrant. In this case, the binomial probability of identifying a single integrant from 10 workers sampled randomly from a colony would be expected to be greater than 99.9%. Thus, although the possibility of SINV-1 genome integration exists, the lack of amplification from genomic ant (host) DNA, and the fact that positive, single-strand RNA viral integration has been reported rarely, support our results for SINV-1. However, it is impossible to fully support a negative result and, as such, integration of a portion of the SINV-1 genome into the fire ant host genome must remain a possibility.

Although SINV-1 appears to cause a persistent, asymptomatic infection of *S. invicta*, its effect on the host population is not understood. SINV-1 could be exploited as a microbial control agent or even as a vector to shuttle deleterious genes or toxins into *S. invicta*. In these situations, SINV-1 genome (or genome fragment) integration may influence the efficacy of such uses. In addition, knowing whether integration of SINV-1 into the *S. invicta* genome occurs, advances the understanding of the biology of this virus.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jip.2011.04.005.

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