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ORIGINAL ARTICLE

Genes important for survival or reproduction in *Varroa destructor* identified by RNAi

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Abstract The *Varroa* mite, (*Varroa destructor*), is the worst threat to honey bee health worldwide. To explore the possibility of using RNA interference to control this pest, we determined the effects of knocking down various genes on *Varroa* mite survival and reproduction. Double-stranded RNA (dsRNA) of six candidate genes (*Da*, *Pros26S*, *RpL8*, *RpL11*, *RpP0* and *RpS13*) were synthesized and each injected into *Varroa* mites, then mite survival and reproduction were assessed. Injection of dsRNA for *Da* (*Daughterless*) and *Pros26S* (Gene for proteasome 26S subunit adenosine triphosphatase) caused a significant reduction in mite survival, with 3.57% ± 1.94% and 30.03% ± 11.43% mites surviving at 72 h post-injection (hpi), respectively. Control mites injected with green fluorescent protein (GFP)-dsRNA showed survival rates of 81.95% ± 5.03% and 82.36 ± 2.81%, respectively. Injections of dsRNA for four other genes (*RpL8*, *RpL11*, *RpP0* and *RpS13*) did not affect survival significantly, enabling us to assess their effect on *Varroa* mite reproduction. The number of female offspring per mite was significantly reduced for mites injected with dsRNA of each of these four genes compared to their GFP-dsRNA controls. Knockdown of the target genes was verified by real-time polymerase chain reaction for two genes important for reproduction (*RpL8*, *RpL11*) and one gene important for survival (*Pros26S*). In conclusion, through RNA interference, we have discovered two genes important for mite survival and four genes important for mite reproduction. These genes could be explored as possible targets for the control of *Varroa destructor* in the future.

Key words *Apis mellifera*; reproduction; RNAi; survival; *Varroa destructor*

Introduction

The honey bee (*Apis mellifera*) is generally regarded as the most important insect to humans due to their pollination service (Calderone, 2012), hive products (Boukraa, 2015) and as a research model (Giurfa & Menzel, 2003; Ihle *et al.*, 2015). However, managed honey bee colonies are currently decreasing worldwide because of many biotic and abiotic factors (Neumann & Carreck, 2010). Among these factors, the acarine ecto-parasite *Varroa* mite (*Varroa destructor*, Anderson & Trueman, 2000) is suspected to play a major role in the recent honey bee colony collapse disorder (CCD). This is not only because of their direct deleterious effect, but also due to their vectoring of several honey bee viruses (Shen *et al.*, 2005; Gisder *et al.*, 2009; Di Prisco *et al.*, 2011; Wilfert *et al.*, 2016). The *Varroa* mite has developed resistance to several synthetic acaricides (Milani, 1999), and acaricide residues have appeared in honey and other bee products (Wallner, 1999). These residues may be another factor contributing to the recent bee decline (Huang, 2009). Therefore, it is urgent that we learn more about *Varroa* mite biology and use this knowledge for developing improved control methods.

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RNA interference (RNAi) is a widely used technology which introduces double-stranded RNA (dsRNA) into organisms to reduce transcription of a specific gene, and which is a post-transcriptional gene silencing mechanism, also called "gene knockdown". RNAi was successfully used to reduce mating success and number of eggs in cattle ticks (de la Fuente *et al.*, 2006). In *Varroa destructor*, Campbell *et al.* (2010) first tested the feasibility of RNAi by using the mu-class glutathione *S*-transferase gene (*Vd*GST-mu1). However, their injection method caused high mortality in mites, with only 50% surviving at 48 hpi (hours post-injection), so they chose to immerse mites in dsRNA solution to deliver dsRNA. In a more recent study, Campbell *et al.* (2016) showed that two neural genes (an allatostatin gene and a crustacean hyperglycaemic hormone (CHH)-like gene) can be targeted by dsRNA for potential mite control. They again used immersion to introduce dsRNA but avoid injection trauma. Garbian *et al.* (2012) showed that dsRNAs ingested by honey bees are transferred to *Varroa* mites and vice versa. However, in that study a cocktail of dsRNAs from 14 genes was used at the colony level, so we do not know which genes are responsible, nor do we know the mechanisms for the observed reduction in mite population. In this study, we developed an injection method that results in high mite survival and then determined the effects of introducing dsRNA for several genes on *Varroa* mites. Our objective was to identify *Varroa* genes and dsRNA sequences that reduce target gene expression via RNAi-mediated gene knockdown in order to control mite infestations.

Materials and methods

Honey bees and mites

Experiments were conducted during the summer of 2012 in East Lansing, Michigan, USA (42°40′44″ N, 84°28′38″ W). Honey bees were a mixture of subspecies typical of North America and managed according to standard beekeeping practices.

Varroa mites were harvested from workers on "open brood" (combs with larvae) by using powdered sugar (Macedo & Ellis, 2000). After being cleaned off the sugar with a wet brush, they were provided with white-eyed drone pupae in a Petri dish and stored in an incubator at 28°C and 75% relative humidity (RH).

RNA isolation and cDNA synthesis

RNA was extracted from 10 mites by RNeasy Mini Kit (Qiagen, Germantown, MD, USA) according to the manufacturer's instructions. The concentration and quality of isolated RNA was measured by a Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

First strand cDNA synthesis was performed by QuantiTect Reverse Transcription Kit (QIAGEN, USA) in accordance with the manufacturer's instructions. The cDNA was kept at −20°C until use.

Target fragment amplification and recovery

When a gene that was related with survival or reproduction in either cattle tick (Gong *et al.*, 2008), mosquito (Nene *et al.*, 2007) or fruit fly (Kurscheid *et al.*, 2009) was found to have 50% or more identity (in amino acid sequence) with a gene in the *Varroa* mite genome (http://www.ncbi.nlm.nih.gov/nuccore/ADDG00000000.1?ordinalpos=1&itool=EntrezSystem2.PEntrez.Sequence.Sequence_ResultsPanel.Sequence_RVDocSum), we chose it as a candidate gene and amplified the fragment from the mite cDNA by polymerase chain reaction (PCR). Each PCR was done with a 50 μL reaction system consisting of 45 μL Supermix (Invitrogen, Carlsbad, CA, USA), 0.4 μL each of F and R Primers (sequences shown in Table 1), 1 μL cDNA, and 3.2 μL ribonuclease (RNase)-free water. The PCR program was run as 95°C for 5 min, followed by 40 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 45 s, and then 72°C for 7 min. The primers for each gene were designed by SnapDragon (http://www.flyrnai.org/cgi-bin/RNAi_find_primers.pl), which encompasses a targeted stretch of RNA with 500–550 bp. Each primer pairs (about 20 bp) were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA, USA). We were able to PCR amplify genes for *Daughterless* (*Da*), Proteasome 26S subunit adenosine triphosphatase (*Pros26S*), Ribosomal protein L8 (*RpL8*), Ribosomal protein L11 (*RpL11*), Ribosomal protein P0 (*RpP0*), Ribosomal protein S13 (*RpS13*), but attempts to amplify genes Fruitless (*Fru*), Intersex (*Ix*), Transformer-2 (*Tra-2*) and Ubiquitin-63E (*Ubi*) were unsuccessful.

Electrophoresis was conducted to make sure the PCR products were the target fragments (500–550 bp) for each gene with a 2% agarose gel. Target fragment was recovered from the 2% agarose gel after being electrophoresed with QIAquick® Gel Extraction Kit (Qiagen) according to the manufacturer's instructions and sequenced to verify that the correct gene was obtained.

dsRNA preparation and Varroa mite injection

dsRNA synthesis was performed *in vitro* by following the procedure of Kurscheid *et al.*, (2009) with

Table 1 Gene-specific primers used in double-stranded RNA (dsRNA) synthesis and quantitative real-time polymerase chain reaction (qPCR). Lower-case bases were T7 promoters.

Gene	Primer	Primer sequence	Reference
Da	dsRNA-F	5′taatacgactcactatagggCTTTAAGCAGGCCACTTTCG3′	Nene *et al.*, 2007
	dsRNA-R	5′taatacgactcactatagggTGAGGTAAAGGACGAATCGG3′	
	qPCR-F	5′CGCGGGACTTGAAGTCTTTG3′	
	qPRC-R	5′GTTATGGTCAGGCGGGAAGTCC3′	
Pros26S	dsRNA-F	5′taatacgactcactatagggCCCACTAGAGCACAACCCTC3′	Kurscheid *et al.*, 2009
	dsRNA-R	5′taatacgactcactatagggTACCCGATCAACCTTTCGTC3′	
	qPCR-F	5′CAGCAATCAAATCTATTATAATAAC3′	
	qPRC-R	5′CGTTCAAACCAATAAGGGGAGTGGC3′	
RpL8	dsRNA-F	5′taatacgactcactatagggAGCCAATGAGGTGTTATCGG3′	Kurscheid *et al.*, 2009
	dsRNA-R	5′taatacgactcactatagggTAGAAGCCAAGGCTGAAGGAC3′	
	qPCR-F	5′TTCTTCGACCGACATGTTTGCCAGTC3′	
	qPRC-R	5′CGTCCCTTGATCGGTGACGTGGGCTG3′	
RpL11	dsRNA-F	5′taatacgactcactatagggCAGGTGAGTGTTTGCTGAGG3′	Kurscheid *et al.*, 2009
	dsRNA-R	5′taatacgactcactatagggGGCGGGATGAACTTAAAACAC3′	
	qPCR-F	5′CACTTATGTGCGGAACTCGTTATAG3′	
	qPRC-R	5′TCTAACCAATAAGTGCGCTATGAAC3′	
RpP0	dsRNA-F	5′taatacgactcactatagggACATCGAGAACAATCCGACC3′	Gong *et al.*, 2008
	dsRNA-R	5′taatacgactcactatagggGAGTGGGTGCTCAGAGAAGG3′	
	qPCR-F	5′GCTTCGGTTCAACAGAATAG3′	
	qPRC-R	5′GTACAATAAGAAATGAGGTG3′	
RpS13	dsRNA-F	5′taatacgactcactatagggTCCCTGCAAATCGTTTTCTC3′	Kurscheid *et al.*, 2009
	dsRNA-R	5′taatacgactcactatagggACCGAGCGTGACGACTAAAG3′	
	qPCR-F	5′GAATAAAAAGTACTGGTGTGC3′	
	qPRC-R	5′TGACAAAAAGTAGGTGAAAAC3′	
GFP	dsRNA-F	5′taatacgactcactatagggAGAAGAACTTTTCACTGG3′	Li *et al.*, 2015
	dsRNA-R	5′taatacgactcactatagggCTTCTACCTAGGCAAGTT3′	
Actin	ActinF	5′CATCACCATTGGTAACGAG3′	Campbell *et al.*, 2010
	ActinR	5′CGATCCAGACGGAATACTT3′	

modifications. Briefly, the 500–550 bp fragment from each gene, or the green fluorescent protein (*GFP*) gene (pMW1650), were generated to be complementary to the cDNA sequences of the gene and pMW1650 plasmid, respectively, with PCR, using each gene-specific primer pair with T7 promoter sequences (5′ - TAATACGACTCACTATAGGG- 3′) appended at the 5′ end of each PCR primer (Table 1). dsRNA was synthesized from the PCR template with the opposing T7 promoter sequences by *in vitro* transcription using the MEGAscript T7 High Yield Transcription kit (ThermoFisher, Waltham, MA, USA), according to the manufacturer's instructions. The dsRNAs were then purified by phenol/chloroform extraction followed by ethanol precipitation. Appropriate RNAase-free water (~40 μL) was used to dissolve the dsRNA and the concentration was adjusted to 4 μg/μL with a centrifuge vacuum (Eppendorf, Hamburg, Germany) after being quantified with the Nanodrop 2000.

Mites were carefully glued to a glass slide upside down (with ventral side up) by using a fine brush (size 10/0, 511337 Golden Taklon Series: 2000 Round, Hobby Lobby, USA). We used regular honey (predried in a Petri dish for 24 h) as a gluing agent because it can be easily diluted with water to release the mites. Two microscope slides (Ted Pella, Redding, CA, USA) were taped together but offset for 3 mm. Mites were glued to the lower piece of glass so that they would not be pushed away during injection because the edge of the top glass provided a barrier for further movement (Fig. 1A). Approximately 0.8 ng dsRNA of target gene or GFP (at a concentration of 4 μg/μL in 0.2 nL) was injected into the idiosoma of mites between the dorsal and ventral plates just behind the capitulum (Fig. 1B). Injection was performed

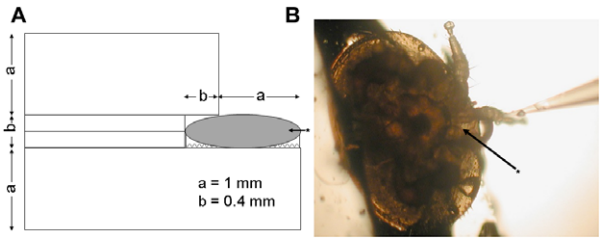

Fig. 1 Diagram showing the apparatus for fixing *Varroa* mites (A) and actual photo showing site of injection into a mite (B). The mite-fixing apparatus is composed of a sandwich design with two pieces of microscope glass (1 mm thick) encompassing two pieces of cover glass (total thickness = 0.4 mm). The top glass slide was offset by 1 mm to provide room for mites. The recess distance of the two cover glasses in the middle was 0.4 mm which allowed each mite to slip in between and not spinning around while being injected. The two arrows after *indicate the site of injection for both A and B.

using a Picospritzer II (Parker, Cleveland, OH, USA) under a compound microscope at 40 times magnification, using a micromanipulator (World Precision Instruments M3301, Sarasota, FL, USA). The injection time was set at 195 msec and the pressure set at 6.8 kg per square inch (psi) provided by a nitrogen tank. Injected mites were then cleaned of honey using a moistened brush and transferred to a small Petri dish (60 × 15 mm, D × H), with about 20 mites per dish and 3–4 white-eyed drone pupae as food. The injected mites were incubated in total darkness at 27°C and a RH of 75%. Injection of dsRNA of each candidate gene was accompanied by injection of dsRNA of the *GFP* gene as a control. We replicated each gene for three different trials with 120 mites (60 for genes and 60 for GFP) injected per trial. Because only one gene can be studied per day, we elected to use new *GFP* controls for each trial in case mites were slightly different due to colony origin or differences in their physiological status.

Gene expression analysis

Total RNA was extracted with three microinjected mites at 24 or 72 hpi and cDNA was synthesized and quantified by quantitative real-time PCR (qPCR). We then replicated with three independent cDNA samples (total nine mites) for each time point and for each target gene or GFP control. The quantity of cDNA was measured using Nanodrop 2000 prior to qPCR. qPCR was then performed with the SYBR Green master mix kit (Qiagen) and ABI 7900 Real Time PCR system (Applied Biosystems, Foster City, CA, USA). Gene-specific primers were used in each well on a 96-well plate, in which every qPCR reaction contained 7.5 μL SYBRmix, 0.15 μL each of F and R primers (Table 1), 1 μL cDNA and 6.2 μL RNase-free water (15 μL total volume). All samples were measured in triplicates and the reaction cycle consisted of a melting step of 95°C for 15 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

Varroa mite survival and reproduction assessment

We assessed the survival of mites at 48 or 72 hpi. If dsRNA injected mites did not show significant reduction in survival, then we assessed whether mite reproduction was affected using an established method (Le Conte *et al.*, 2015). Briefly, recently capped (within 6 h) brood cells from low-mite source colonies were selected as transfer hosts. This was done by mapping the larval cells that were being capped at one time and then remapping them again 6 h later. Any cells being capped at the first time, but completely capped at the second time were deemed to be capped within 6 h. Injected mites were each transferred into a cell with a paint brush after each cell was opened with an insect pin (size 1, Fine Science Tools, Foster City, CA, USA). The opening was immediately sealed with molten beeswax after mite introduction. The brood frames were incubated at 34°C (50% RH) for 9 days after which each cell was opened and mite progenies scored following the method in Le Conte *et al.* (2015).

Sequence similarity comparison

Because using a particular dsRNA for mite control would require that it does not affect honey bees, we compared the identity of the six candidate genes between *Varroa destructor* and *Apis mellifera*. We searched the similarity in the genome of honey bee for the six candidate genes by DNAMAN software (Lynnon Biosoft, San Ramon, CA, USA).

Data analyses

All data were presented as mean ± SEM (standard error of the mean). For gene knockdown efficiency, we used the expression levels for each gene compared to a control gene (actin), relative to the expression levels of the same gene in the GFP dsRNA injected mites, as in

$$\left\{1-\frac{2\hat{}[\mathrm{CT(actin)}-\mathrm{CT(TAR)}]}{2\hat{}[\mathrm{CT(actin)}-\mathrm{CT(GFP)}]}\right\}*100\%,$$

where CT is the threshold cycle value of each gene, TAR is the target gene studied and the denominator is the average of three biological samples, each containing RNA of three mites injected with dsRNA of GFP. This is a

derivation of relative expression ratio R by Pfaffl (2001). By definition, mites injected with GFP dsRNA would have average knockdown efficiency of 0%.

Differences in survival were compared by Kaplan–Meier survival analysis using Log-Rank tests, differences in fecundity and gene expression were compared using Student *t*-tests. All statistical analyses were performed with StatView (v 5.01, SAS Institute Inc., Gary, NC, USA) with significance levels set at $P < 0.05$.

Results

Varroa mite injection method development

Due to not using double-sided stickytape and lower injection volume, survival for dsGFP injected mites were higher than 85% after 48 hpi. The apparatus for fixing mites and injection location into the mites are shown in Figure 1.

dsRNA preparation

Six genes (*Da*, *Pros26S*, *RpL8*, *RpL11*, *RpP0* and *RpS13*) were amplified successfully and their dsRNA synthesized.

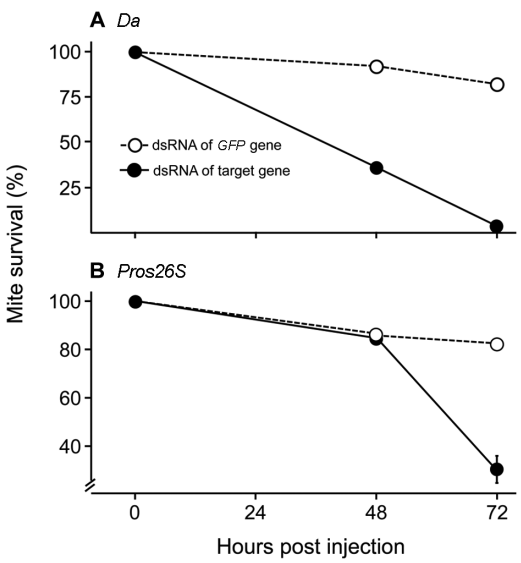

Fig. 2 Survival of *Varroa destructor* after being injected with double-stranded RNA (dsRNA) of *Daughterless* (*Da*, A) and ribosome protein *PROS26S* (B) genes, compared to mites injected with dsRNA of green fluorescent protein (GFP) gene. Injected mites were kept at 27°C and 75% relative humidity with drone pupae as food. Data based on three batches of mites ($n = 60$ per batch).

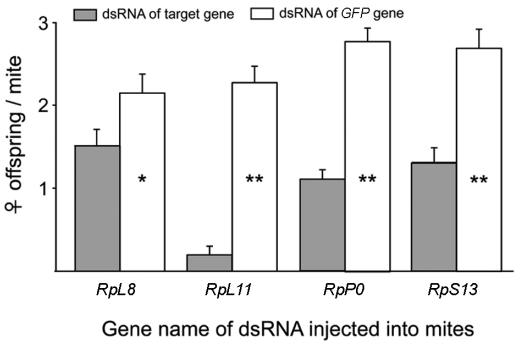

Fig. 3 Mean female offspring per mother mite (±SEM) for *Varroa* that were injected with with double-stranded RNA (dsRNA) of four different genes and their respective dsGFP (green fluorescent protein) control. Because normally only one gene was injected per day, we had to have dsGFP control for each day because mites might be slightly different due to colony conditions and/or handling. Stars indicate $P < 0.05$ (*) or $P < 0.01$ (**) for differences between the two groups of each gene by Student's *t*-tests. Data based on three batches of mites ($n = 30$ mites per batch).

Mite survival

Two genes caused significant reduction in mite survival. Mites injected with *Da* dsRNA showed a significant reduction in survival at 48 hpi. At 72 hpi, almost all mites (96.5% ± 1.94%) were dead (Fig. 2A). Mites injected with *Pros26S* dsRNA showed a significant reduction in survival, but only at 72 hpi (Fig. 2B). For the other genes, *RpL8*, *RpL11*, *RpP0* and *RpS13*, the survival of mites injected with dsRNA was not significantly different from the GFP control at 72 hpi (data not shown), so we estimated their effects on mite reproduction.

Mite fecundity

Since over 85% of mites were still alive at 72 hpi after being injected with dsRNA of *RpL8*, *RpL11*, *RpP0* and *RpS13*, we assessed whether their reproduction was affected by RNAi. Mites injected with dsRNA of *RpL8*, *RpL11*, *RpP0* and *RpS13* showed significant reductions compared to their own dsRNA GFP control (*t*-test, $P < 0.05$ for each gene) in the number of female offspring per mother (Fig. 3). *RpL11* showed the highest suppression of reproduction, followed by *RpP0*, *PpS13* with *RPL8* having the lowest effect.

Gene expression analysis

Mites injected with one survival-related gene, *Pros26S*, and two reproduction-related genes, *RpL8* and *RpL11*

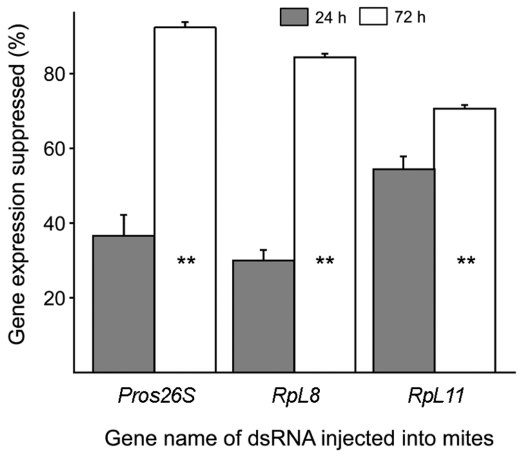

Fig. 4 Percent gene knockdown (±SEM) after with double-stranded RNA (dsRNA) injection of three different genes compared to mites injected with dsRNA of green fluorescent protein (*GFP*). Each sample was also normalized to their actin levels. All gene knockdown efficiencies were significantly higher than 0 (no knockdown, as represented by those injected with *GFP* dsRNA) even at 24 h post-injection, but the **here indicates significant differences ($P < 0.01$) between knockdown efficiencies at 24 and 72 h post-injection. Data based on three biological samples, each with three injected mites.

were chosen to verify gene knockdown by qPCR at 24 and 72 hpi. All mites injected with dsRNA candidate genes showed significant gene knockdown compared to their GFP control at 24 hpi. In addition, all mites showed higher knockdown efficiency at 72 hpi compared to those at 24 hpi (*t*-tests, $P < 0.01$, Fig. 4).

Gene similarity analysis

Sequence alignment and comparison between each *Varroa* gene and the honey bee genome showed that only *RpP0* of *Varroa* showed high identity (98%) to the sequence of the honey bee *RpP0*, but other genes showed much less (<30%) identity (Table 2).

Discussion

The main finding of this study is that knocking down of two genes, *Da* and *Pros26S*, caused high mortality in *Varroa* mites, suggesting that they play critical roles in the mites; that knocking down of six genes, *RpL8*, *RpL11*, *RpP0* and *RpS*, reduced *Varroa* offspring, suggests that these genes regulate *Varroa* reproduction.

We tested 10 candidate genes that were related to survival or reproduction in other organisms, but four genes (*Fru*, *Ix*, *Tra-2* and *Ubi-P63E*) failed to be amplified after we tried at least three pairs of primers for each. Even though we verified significant knockdown in only three out of six genes, we speculate that knockdown worked in all six genes because mites showed phenotypical changes after dsRNA injection, in either survival (2 genes) or reproduction (4 genes).

It was noteworthy that *Da* was shown to cause reduced reproduction in *Drosophila* and *Aedes aegypti* (Gong *et al.*, 2008), but in the current study, the same gene affected mite survival, because only 36.34% ± 2.63% and 3.57% ± 1.94% of dsRNA injected mites were alive at 48 and 72 hpi, respectively.

Mite survival in our study was high even after microinjection. For example, mites injected with GFP dsRNA survived at 81.95% ± 5.03% (Fig. 2A) or 82.36% ± 2.81% (Fig. 2B) and averaged 85.51% (±1.98) at 72 hpi for mites injected with the four genes that affected reproduction (*RpL8*, *RpL11*, *RpP0* and *RpS13*). In contrast, mite survival was 50% at 48 hpi for Campbell *et al.* (2010) and below 70% at 72 hpi for Campbell *et al.* (2016), even after they excluded damaged mites at 1 hpi. This difference might be due to the different mite-restraining methods; Campbell *et al.* (2010, 2016) placed the mites ventral side up on double-sided stickytape. But we used

Table 2 Percent identities of DNA sequences between *Varroa destructor* and *Apis mellifera* for the six genes that affected mite survival or reproduction (by DNAMAN software). Only RpP0 showed too high a similarity between the two species, with all others with identities below 34%.

Gene	Messenger RNA length	Sequence ID of the *Varroa* mite	Range	Identities (%)
Da	1748 bp	ADDG02019158.1	31336 to 33084	29.3
Pros26S	2682 bp	ADDG02019401.1	47271 to 49953	19.9
RpL8	1856 bp	ADDG02019401.1	54073 to 55929	13.1
RpL11	1590 bp	ADDG02002429.1	43348 to 44938	33.6
RpP0	1938 bp	ADDG02020171.1	165520 to 167458	89.9
RpS13	1671 bp	ADDG02019401.1	56559 to 58230	28.7

honey as a glue for mites, because honey can be diluted with water to easily release mites. Furthermore, our injection used a much smaller amount (20 pL at 4 μg/μL) of dsRNA, compared to their 20 nL at 2.5 μg/μL (Campbell *et al.*, 2010, 2016). Their immersion method required even higher amounts with 20 μL at 2.5 μg/μL. Garbian *et al.* (2012) fed 30 μg dsRNA in 200 μL sucrose syrup for 8 days in a treatment which had even higher amounts of dsRNA than Campbell *et al.* (2010, 2016). For laboratory studies, microinjection should be preferred because feeding and immersion methods require much higher doses, significantly increasing experimental costs.

Gene knockdown at 72 h was significantly higher compared to those at 24 h for all the genes (Fig. 4). These results are consistent with previous *Varroa* studies showing that gene knockdown efficiency was 62%–84% (Campbell *et al.*, 2010) or 55%–85% (Campbell *et al.*, 2016), and a *Macrobrachium* study (Zhang *et al.*, 2013) showing that the efficiency of RNAi could be sustained for more than 7 days.

In conclusion, we have discovered two genes important for *Varroa* survival and four genes important for *Varroa* reproduction. *RpP0* is too similar between the honey bee and *Varroa*, so it is not suitable as a candidate gene for mite control. But the other five genes can be used for controlling *Varroa* mites. A previous study (Garbian *et al.*, 2012) has already shown that a cocktail of many dsRNAs can be fed to honey bees at the colony level and they will be picked up by *Varroa* mites and suppress *Varroa* population. It remains to be seen whether a single gene, such as one of the five genes found here, can be used to the same effect at a colony setting.

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Disclosure

The authors declare no competing or financial interests.

Author contributions

Zachary Y. Huang and Zhiyong Xi conceived and planned the study and participated in elements of data collection, analysis and prepared the manuscript. Experiments were performed by Xianbing Xie and Guowu Bian. Zhiyong Xi contributed materials to the study, and all authors revised the manuscript.

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