Exploring RNAi as a method for controlling *Varroa destructor*

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Summary

The varroa mite, *Varroa destructor,* is the worst pest of the Western honey bee (*Apis mellifera*) and responsible for declines in honey bee populations worldwide. In this study we used RNA interference (RNAi) technology to disrupt its life cycle by either causing death immediately or causing a reduction in reproduction. We developed a method to inject double stranded RNA (dsRNA) into *varroa* mites with a high survival rate. We then tested three candidate genes and determined how they affected mite survival or reproduction. RNA interference for the gene Rpl8 caused a reduction in reproduction, while RNA interference for Pros26.4 and Rps13 caused a reduction in survival.

Introduction

Varroa destructor is the worst pest of the Western honey bee (*Apis mellifera*) and responsible for declines in honey bee populations worldwide. The acaricide resistance and residues are of pressing concern to the U.S. beekeepers, because varroa mite has become resistant to the two most effective acaricides registered in the U.S.: Apistan (active ingredient fluvalinate), and Checkmite+ (active ingredient coumaphos). The colony collaspse disorder (CCD) of honey bees during the last few years may also be partly caused by varroa mite (Huang, 2009).

In this study, we attempted to determine the effect of injecting the double stranded RNA of a few selected genes on varroa mite survival and reproduction. Once this step is successful, we will explore ways that RNAi can be introduced to mites via the honey bee larvae.

Materials and Methods

1. RNA interference assays:

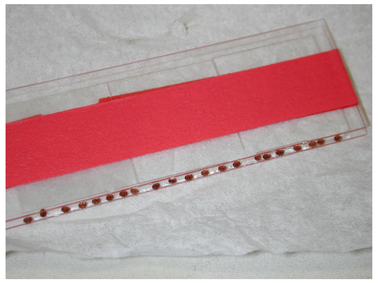
RNA interference (RNAi) was performed using a modification of the standard procedure used for mosquitoes (Xi et al., 2008). ds-RNA was prepared using a T7 MEGAscript kit (Ambion, Austin, TX). dsRNA was synthesized by Integrated DNA Technologies (Skokie, IL, USA) and verified to be correct by PCR. Varroa mites were glued with their ventral side up using a thin layer of honey on two pieces of glass slides (off-set such that mites were not able to slide away during injection). Approximately 20 nl dsRNA (at a concentration of 4 µg/µl) was injected into the idiosoma between the dorsal and ventral plates just behind the capitulum using a Picospritzer II Injector (General Valve Corporation). Injection was performed under a microscope at x40 magnification with a moving stage (VWR Scientific products). The injection time was set to 95 MSec and the injection pressure was set to 16 PSi. Injected mites were then cleaned off the honey using a moistened brush and enclosed inside a small petri dish, with about 20 mites per dish and 3-4 honey bee worker pupae(white eye stage) as food. We injected mites dsRNA of the **green florenscent** protein (GFP) gene, which does not exist in mites, **as a control for non-specific effects of dsRNA**. The injected mites were incubated at 27°C and RH 75%.

2. Assessment of varroa mite reproduction:

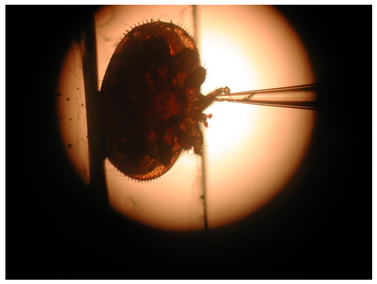
We first assessed the survival of mites during the 2 days post injection (p.i.). If dsRNA injected mites did not show significant reduction in survival, then we assessed whether mite reproduction was affected (Xie and Huang, 2008). We selected recently sealed (within 6 hours) brood cells as transfer hosts. This is done by mapping the brood that was nearly capped at one time and then remapping it again 6 hours later. We transferred dsRNA injected mites into brood cells with a paint brush after each cell was opened with a small pin. The opening was immediately sealed with melted beeswax after mite introduction. The brood frames were incubated at 35°C (50% RH) for 9 days after which each cell is opened and mite progenies scored.

3. List of candidate genes:

We searched for the following gene orthologs in the newly established varroa mite genome (http://www.ncbi.nlm.nih.gov/nuccore/ADDG00000000.1?ordinalpos=1&itool=EntrezSystem2.PEntrez.Sequence.Sequence_ResultsPanel.Sequence_RVDocSum). We tested the genes for ribosome protein L8(Rpl8), proteasome 26S subunit (Pros26.4) and ribosome protein S13(Rps13), all of which have shown to play roles in survival or reproduction in other tick species. Ticks are more closely related to mites than other insect species are related to mites.



Varroa mites glued ventral side up



A varroa mite being injected

Varroa mite injection by Xianbing Xie.

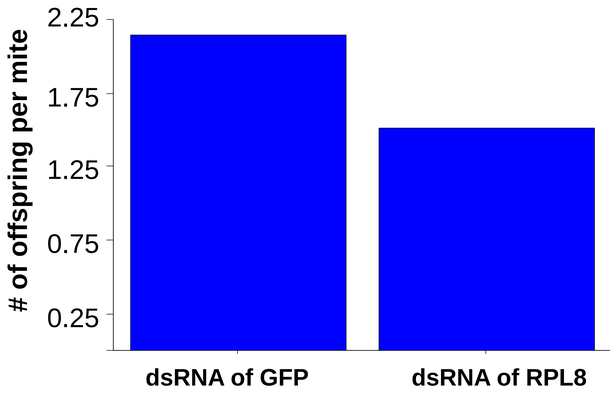


Fig. 1. RPL8 ds-RNA injected mites show siginificantly reduced reproduction.

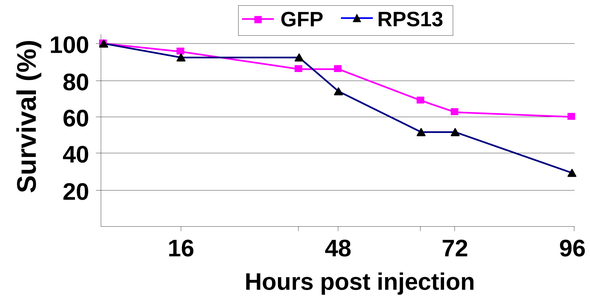


Fig. 2. RPS13 ds-RNA injected mites show siginificantly lower rate of survival.

Results and Discussion

We have shown previously that using our microinjection protocol, mite survival 48 h p.i. was 78.8% (N = 41 mites), and a 77% efficincy gene knock-down efficiency was achieved. The mean (± SD) fecundities of mites which were injected with dsRNA of RPL8 gene and of GPF (control) were 1.510 ± 1.405 and 2.146 ± 1.442, respectively (Fig. 1, T-test, P = 0.035). RPL8 therefore seems to be affecting reproduction in *Varroa destructor*. The other two genes Pts26.4 and RPS13 affected mite survival significantly and we did not assess their effect on mite reproduction. Ds-Pts26.4 gene injection caused a 65.6% reduction in rate of survival compared to the GFP control (P<0.05, data not shown). Ds-RPS13 injection showed a significantly reduced survival, with a 96 h post-injection survival of 29.6%, compared to 60% of the GFP (Fig. 2, G-test, P<0.05).

Conclusions

Our results here show that

1). That we can obtain high survival rates in mites injected with ds-GFP gene or those that does not affect mite survival, and

2). Varroa mites show differences in survival or reproduction, after injected with ds RNA of candidate genes, compared to the control mites which were injected with GFP dsRNA.

We will continue to screen for more genes next summer and then test the same ds-RNA on honey bees to make sure that the selected dsRNAs affect mite survival or reproduction but do not adversely affect honey bees.

Acknowledgment

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