Notes from the Lab: The Latest Bee Science Distilled

by Scott McArt

Varroa *and* Tropilaelaps *feed primarily on honey bee hemolymph when they reproduce*

Until 2019, everyone knew varroa was a vampire that fed on honey bee hemolymph. But then Sammy Ramsey published his paradigm-altering study (Ramsey et al., 2019; highlighted in the April 2019 *Notes from the Lab*: 159(4):443-445), showing varroa was actually more like a werewolf since it fed primarily on the fat bodies of adult honey bees.

But this paradigm shift didn't sit quite right with some researchers. Ramsey's study was excellent, but some people felt the full feeding situation across all mite and bee life stages might be a bit more complex. Reproduction (i.e., making new mites) takes a lot of protein, which is present in hemolymph, while dispersal (i.e., finding new hosts) takes a lot of energy, which is present in fat. Because of this, a smart mite would probably feed on hemolymph *and* fat bodies, depending on whether it's trying to reproduce or disperse.

So, is this the case? Does varroa feed primarily on fat bodies when it's trying to disperse, but hemolymph when it's trying to reproduce? And what about that other parasitic mite that still hasn't arrived in the USA but every beekeeper is concerned about? What does *Tropilaelaps mercedesae* primarily feed on? These are the topics for the seventy-fourth *Notes from the Lab*, where I summarize ***"Life-history stage determines the diet of ectoparasitic mites on their honey bee hosts,"*** written by Bin Han and colleagues and published in the journal ***Nature Communications* [2024].**

For their study, Han and colleagues expanded on Sammy Ramsey's groundbreaking 2019 study. To get us up to speed, let's briefly review what Ramsey and colleagues found. The Ramsey study observed varroa feeding on adult bees, finding multiple lines of evidence that varroa feeds on fat bodies during this dispersal stage. In addition, they conducted an elegant bioassay with reproductive-stage mites, finding that they survived longer and were more likely to produce offspring when fed a greater proportion of honey bee fat body compared to hemolymph.

Han and colleagues have built on Ramsey and colleagues' study in several ways. First, they observed varroa feeding on honey bee pupae (i.e., the reproductive stage of varroa) by introducing mites to 1,285 worker pupae and recording the specific area where feeding occurred. They used the slick fluorescent labeling methods pioneered by Ramsey and colleagues to differentiate whether varroa fed on hemolymph, which absorbs green Uranine stain, or the fat body, which absorbs Nile red stain. Both stains were fed to bees during their development, transferred into mites via feeding, and monitored via fluorescent imaging.

Next, Han and colleagues conducted a detailed proteomic and metabolomic analysis of reproductive- vs. dispersal-stage varroa. In

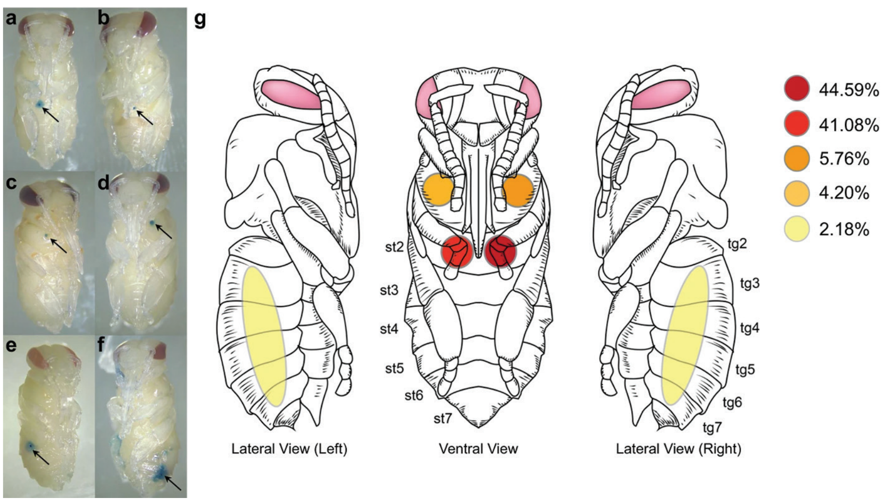

Fig. 1 Varroa destructor *shows a strong preference for feeding on the 2nd abdominal segment of honey bee pupae.* **a–f:** *Representative photos of different* V. destructor *feeding sites, detected by vital staining of integumental wounds in different parts of the pupa. Each arrow indicates the location of a perforation. A total of 1,285 pupae were inspected in eight trials.* **g:** *Diagram showing the frequency of integumental wounds found in each location on 1,285 pupae (st, sternite; tg, tergite).*

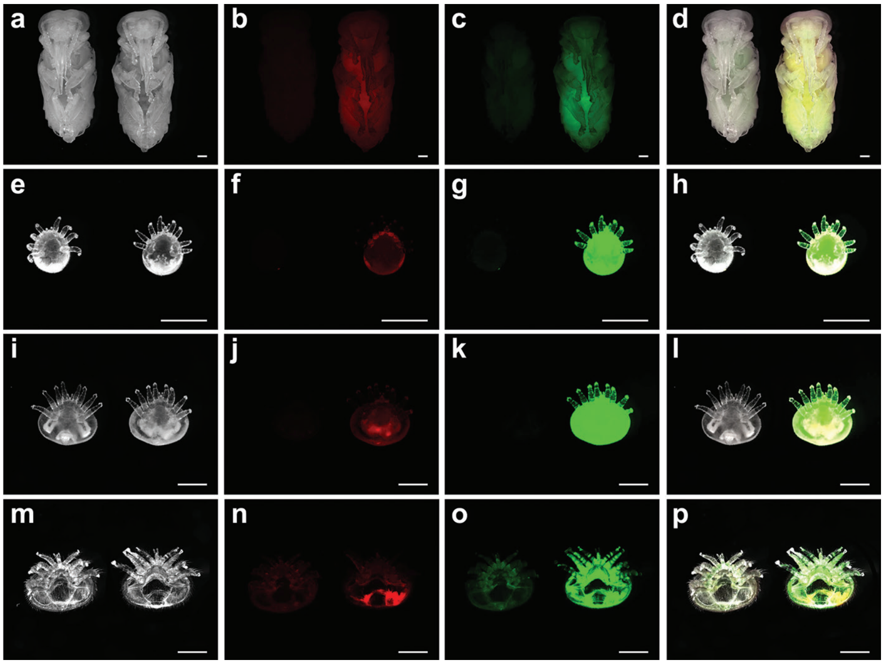

Fig. 2 Varroa destructor *ingests large amounts of honey bee pupae hemolymph during its reproductive stage. Photos show honey bee pupae that fed as larvae on biostains and* V. destructor *mites fed on these biostained pupae in brightfield (far left), fluorescence from these samples associated with lipophilic Nile red (staining predominantly fat body, center left) and hydrophilic Uranine (staining predominantly hemolymph, center right), and all three images merged together (far right). Within each representative photo, the specimen on the left is an unlabeled control and the one on the right depicts a representative labeled sample. All scale bars represent 1mm. Ventral views of honey bee pupae (***a–d***),* V. destructor *protonymphs (***e–h***), deutonymphs (***i–l***), and foundresses (***m–p***). Each row of photos is representative of at least 60 individuals from six independent experiments.*

other words, they extracted proteins and metabolites from mites that were either in the dispersal stage (mites obtained from adult worker bees) or reproductive stage (mites obtained from pupae), separated all the individual proteins and metabolites via chromatography, and determined their identity via mass spectrometry. The specific types of proteins and metabolites found in dispersal-stage mites were compared to those found in reproductive-stage mites.

Finally, the authors observed another parasitic mite, *Tropilaelaps*, feeding on honey bee pupae and conducted fluorescent staining experiments identical to those conducted with varroa.

So, what did they find? Does varroa feed primarily on hemolymph when it parasitizes honey bee pupae? Yes. As can be seen in Figure 1, reproductive-stage varroa feed primarily on the second tergite of the abdomen of honey bee pupae. Directly under this section of the abdomen is a large space containing hemolymph.

Confirming that hemolymph is indeed the primary dietary source for reproductive-stage varroa, foundresses and nymphs glowed brightly green with Uranine stain (see Figure 2, panels g, k and o) while much less Nile red stain showed up in foundresses and nymphs (see Figure 2, panels f, j and n). Again, the green Uranine stain is hydrophilic and easily permeates hemolymph, while the Nile red stain is hydrophobic and permeates fat.

Were different proteins and metabolites consumed by reproductive-stage varroa compared to dispersal-stage varroa? Yes. Reproductive-stage varroa consumed a protein-rich diet comprised of at least 272 proteins, while dispersal-stage varroa consumed 182 proteins. Of the 105 proteins that were unique to reproductive-stage varroa, 78 of them (74%) are known to come from honey bee hemolymph. In contrast, only 15 proteins were unique to dispersal-stage varroa and five (33%) are known to come from honey bee hemolymph. These results support the notion that reproductive-stage varroa obtain a protein-rich diet by consuming primarily honey bee hemolymph.

Do reproductive-stage mites produce more offspring when they feed on hemolymph compared to fat bodies? We don't know. This is a logical question to ask, especially since Ramsey and colleagues found reproductive-stage mites benefited from consuming a fat body-rich diet. Unfortunately, Han and colleagues weren't able to directly test this question due to logistical difficulties. But we do know that mites feeding on pupal hemolymph survive twice as long as mites that feed on larval or adult hemolymph (Piou et al., 2023). Clearly there's something very nutritious in pupal hemolymph, which is what reproductive-stage mites consume.

What about *Tropilaelaps*? Do they feed on hemolymph? Yes. As can be seen in Figure 3, hemolymph is the primary dietary source of foundresses and nymphs. All life stages glowed brightly green with Uranine stain (see Figure 3, panels c, g and k) while less Nile red stain was observed (see Figure 3, panels b, f and j).

Interestingly, the difference in green vs. red stain isn't quite as pronounced for *Tropilaelaps* as it is for varroa (compare the intensity of green vs. red stain in Figure 2 to Figure 3). One possibility for why this might be true is that *Tropilaelaps* exclusively parasitizes brood and has a very brief dispersal stage. So, unlike varroa which obtains fats and nutrients from feeding primarily on fat bodies during its dispersal stage, *Tropilaelaps* needs to get its fats and nutrients during its reproductive stage.

What's the take-home message? One take-home message is that scientific knowledge is not static. Scientists and beekeepers originally thought varroa fed on hemolymph. That changed in 2019, when we learned at least one stage of varroa fed primarily on fat bodies. With this new study by Han and colleagues, we've learned that both statements are true. Dispersal-stage varroa feed primarily on fat bodies of honey bee adults, while reproductive-stage varroa feed primarily on hemolymph of honey bee pupae. Cool!

Another take-home message is that this new knowledge can be applied. Because varroa and *Tropilaelaps* are pests of honey bees, there are many treatments that seek to control them. Some of the most interesting treatments currently being envisioned and tested are therapeutics that are delivered orally, selectively targeting mites as they feed on honey bee

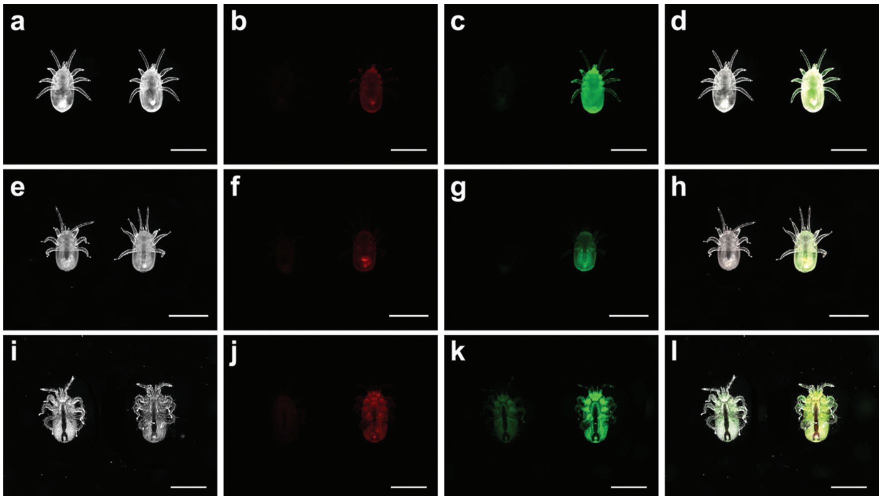

Fig. 3 Tropilaelaps mercedesae *ingests large amounts of honey bee pupal hemolymph. Photos show* T. mercedesae *mites fed on biostained pupae in brightfield, fluorescence from these samples associated with Uranine and Nile red, and all three images merged together. In each photo, the specimen on the left is the control and the one on the right is the treatment. All scale bars represent 1 mm. Ventral views of protonymphs (***a–d***), deutonymphs (***e–h***), and foundresses (***i–l***) of* T. mercedesae *mites. Each row of photos is representative of at least 60 individuals from six independent experiments.*

tissues (similar therapeutics have improved cancer treatment for humans in recent years). Importantly, these therapeutics for mites were originally designed to be hydrophilic so they'd be expressed in hemolymph. Since 2019, they've been designed to be lipophilic so they're expressed in fat bodies. Now, we know that both tissues are appropriate for delivering therapeutics, depending on the life stage that's most important and effective to target. Here's to hoping we see some major advances on this topic in the next decade.

Until next time, bee well and do good work.
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References:

Han, B., J. Wu, Q. Wei, F. Liu, L. Cui, O. Rueppell and S. Xu. 2024. Life-history stage determines the diet of ectoparasitic mites on their honey bee hosts. *Nature Communications* 15:725. **https://doi.org/10.1038/s41467-024-44915-x**

Piou, V., C. Vilarem, S. Blanchard, J.-M. Strub, F. Bertile, M. Bocquet, K. Arafah, P. Bulet and A. Vétillard. 2023. Honey bee larval hemolymph as a source of key nutrients and proteins offers a promising medium for *Varroa destructor* artificial rearing. *International Journal of Molecular Sciences*. 24:12443. **https://doi.org/10.3390/ijms241512443**

Ramsey, S.D., R. Ochoa, G. Bauchman, C. Gulbronson, J.D. Mowery, A. Cohen, D. Lim, J. Joklik, J.M. Cicero, J.E. Ellis, D. Hawthorne and D. vanEngelsdorp. 2019. *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. *Proceedings of the National Academy of Sciences of the United States* 116:1792-1801. **www.pnas.org/cgi/doi/10.1073/pnas.1818371116**

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