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# Insights into the feeding behaviors and biomechanics of *Varroa destructor* mites on honey bee pupae using electropenetrography and histology

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ABSTRACT

Feeding behaviors and biomechanics of female *Varroa destructor* mites are revealed from AC-DC electropenetrography (EPG) recordings of mites feeding from *Apis mellifera* honey bee pupae and histology of mite internal ingestion apparatus. EPG signals characteristic of arthropod suction feeding (ingestion) were identified for mites that fed on pupae during overnight recordings. Ingestion by these mites was confirmed afterwards by observing internally fluorescent microbeads previously injected into their hosts. Micrographs of internal ingestion apparatus illustrate the connection between a gnathosomal tube and a pharyngeal lumen, which is surrounded by alternating dilator and constrictor muscles. Inspection of EPG signals showed the muscularized mite pharyngeal pump operates at a mean repetition rate of 4.5 cycles/s to ingest host fluids. Separate feeding events observed for mites numbered between 23 and 33 over approximately 16 h of recording, with each event lasting ~10 s. Feeding events were each separated by ~2 min. Consecutive feeding events separated by either locomotion or prolonged periods of quiescence were grouped into feeding bouts, which ranged in number from one to six. Statistical analyses of EPG data revealed that feeding events were prolonged for mites having lower pharyngeal pump frequencies, and mites having prolonged feeding events went unfed for significantly more time between feeding events. These results suggest that mites may adjust behaviors to meet limitations of their feeding apparatus to acquire similar amounts of food. Data reported here help to provide a more robust view of *Varroa* mite feeding than those previously reported and are both reminiscent of, as well as distinct from, some other acarines and fluid-feeding insects.

## 1. Introduction

The parasitiform mite *Varroa destructor,* hereafter *Varroa* mite, is an obligate ectoparasite of both *Apis cerana* Fabricius and *A. mellifera* Linnaeus honey bees. A native parasite of *A. cerana*, *Varroa* mites shifted to *A. mellifera* hosts within the last century (Beaurepaire et al., 2015). Adult female mites feed primarily on semi-digested host tissues (Ramsey et al., 2018, 2019) together with hemolymph (hereafter, fluids) of both honey bee adult and pupa hosts. Fluids are accessed through a hole made by mites in soft portions of the host's integument using paired, movable toothed digits of chelicerae, together with paired elongated corniculi having sharpened, serrated ventral edges, suitable for slicing though soft bee cuticle (De Lillo et al., 2001). The shapes of both the paired structures form internal concavities that, when pressed together, form a channel suitable for transferring host fluids internally (Ramsey et al., 2018). Feeding holes made by *Varroa* mites are relatively large, each having an average length of 100 µm (Kanbar and Engels, 2004; Ramsey et al., 2018), but can reach up to 300 µm in length (Kanbar and Engels, 2005). Female *Varroa* mites typically create a single feeding hole (Kanbar and Engels, 2004; Egekwu, et al., 2018; Calderón et al., 2009), which can remain open via suppression of host wound healing by anti-coagulant proteins found in the mites' saliva (Richards et al., 2011). Feeding holes are used repeatedly and communally by female *Varroa* mites and their progeny residing in the same wax-capped cells housing honey bee pupae (Kanbar and Engels, 2005). However, it remains unknown how intraspecific 'sharing' of feeding

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holes is facilitated, and whether the feeding behaviors of individual mites, and access to food, are thus impacted.

The feeding behaviors described above for *Varroa* mites may be adaptive, i.e., fewer wounds should minimize injury to the host from direct physical damage, and perhaps indirectly from water loss (Bowen-Walker and Gunn, 2001). Indeed, hygienic *Varroa*-sensitive nurse bees of their native host (*Apis cerana*), can detect highly parasitized pupae, which are discarded (Lin et al., 2016), thereby short-circuiting *Varroa* reproduction. Some colonies of the nascent host species (*Apis mellifera*) do exhibit signs of *Varroa*-sensitive hygiene (Mondet et al. 2016; Nazzi and Le Conte, 2016), which appears to have a genetic component (Hu et al., 2016). As a general response to injury and potential infection, individual honey bees can produce antimicrobial peptides that can recognize and dispatch foreign agents (Xu et al., 2009; Yang and Cox-Foster, 2005), and immunity-related enzymes (e.g., phenol oxidase, lysozymes) (Yang and Cox-foster, 2005) that promote wound healing. However, *Varroa* mites are able to suppress production of some antimicrobial peptides and immune-related enzymes that might have deterred or otherwise affected feeding behaviors (Yang and Cox-Foster, 2005). *A. cerana* has been reported to express antiparasitic proteins (Ji et al., 2015; Wang et al., 2019; Park et al., 2018). In contrast, workers of *A. mellifera*, a more recent host-parasite association for *Varroa* mites, do not respond to parasitism with anti-parasitic proteins, likely related to the immunosuppressive effects of mite saliva (Nazzi et al., 2012). These differences between host responses to *Varroa* parasitism may help explain the contrasting effects mites have on host colonies (Ji et al., 2015; Feng et al., 2014). It is not known how anti-feedant proteins produced by *A. cerana* honey bees might affect *Varroa* mite ingestion, or whether evolved responses in mite feeding behaviors are relaxed in *A. mellifera* honey bees.

Much remains unknown of whether and, if so, how intraspecific and interspecific interactions shape feeding behaviors of *Varroa* mites. Moreover, the feeding behaviors and biomechanics of fluid ingestion for individual *Varroa* mites are unclear, including how frequently, and for how long they feed, and the mechanism driving fluid intake.

A better understanding of individual *Varroa* mite feeding will provide a critical aid for deciphering both observed parasite-parasite and host-parasite interactions, which may be exploited by researchers for developing *Varroa* control methods. A recent study by Posada et al. (2018) showed that female *Varroa* mites deposit nearly 18 excretions per day. The authors suggested that the mites do not store ingested food but must feed frequently each day, consistent with their observations of frequent waste excretion. In the current study, AC-DC electropenetrography (EPG) (with emphasis on AC-branch signals), was used to investigate mechanisms of mite food ingestion. Recordings of EPG tracings (capacitor-coupled AC-generated signal), together with correlated visual observations of mite movements on honey bee pupa hosts, allowed characterization of feeding behaviors of individual female *Varroa* mites. Micrographs made of *Varroa* mite internal ingestion apparatus were examined for interpreting EPG signals of mite feeding, and for determining the mechanism driving fluid ingestion.

## 2. Materials and methods

### 2.1. Collections of Varroa mites and honey bee pupae

Queen-right (i.e., queen present) *A. mellifera* honey bee colonies having received no treatments against *Varroa* infestations served as sources of both mites and honey bee pupae. To collect mites, approximately 300 adult honey bees were collected by brushing them from brood frames removed from colony boxes into a large funnel atop a wide-mouth, pint Mason jar containing approximately two tablespoons of powdered sugar. Screened lids were affixed to jars, which were inverted and shaken over a pan of shallow water. Released mites were strained from the water and deposited onto moist paper towels lining the bottom of a 15 cm petri dish. The brood frames were then promptly transported to the laboratory. There, early developmental stage (white- to pink-eyed) pupae were carefully removed from their cells using wide-tipped featherweight forceps (BioQuip Products, Inc., Rancho Dominguez, CA) and immediately placed on a 32 °C warming plate. Mites selected for use in EPG recordings were picked up one at a time using a fine paintbrush and allowed to crawl onto a pupa host.

### 2.2. Electropenetrographic recordings

EPG recordings used the AC–DC 4-channel electropenetrograph (similar to Backus and Bennett, 1992; Backus et al., 2019), and was custom-built in 2010 by W.H. Bennett (EPG Instrument Co., Otterville, MO). Pilot EPG recording trials suggested that starved mites crawled more rapidly onto pupae than recently fed mites. Thereafter, only mites isolated from hosts 2–3 h in advance were used in overnight recordings. Mites that readily mounted a pupa when introduced had a 12.7 µm diameter fine gold wire (Sigmund Cohn Corp., Mt Vernon, NY) recording electrode affixed to their dorsal integument with conductive silver paint (Ladd Research Industries, Burlington VT; Supplemental Fig. 1A). The wire-tethered mite and its host pupa were placed together on a 32 °C warming plate under a stereoscope where a 76 µm diameter gold wire as reference electrode (A-M Systems, Carlsborg, WA) was shallowly inserted into the pupa to complete the electrical circuit. The recording electrode was attached to a head-stage preamplifier (gain = 100x, input impedance $< Ri > = 10^7$ Ohms) connected to the main amplifier control box/amplifier (gain = 1000×). A positive 40 mV DC was applied to the circuit as substrate voltage during recording. Both pre- and post-rectification signals were displayed so that native polarity of the signal could be determined. Positive offset voltage was added after the measuring point to invert any rectifier-induced inversions. This offset voltage explains the positive baseline shown in figures, in which only the post-rectified (cleaner) signal is displayed. Amplified mite activity signals were digitized through a DATAQ DI-720-UH analog-to-digital portable converter box before being recorded, at a sampling frequency of 100 Hz, using the WinDaq/Lite data acquisition software (DATAQ Instruments Inc., Akron, OH) on a Dell PC computer. Signals of mite activities were displayed in real time on the computer monitor. Each trial continued overnight, acquiring approximately 10–20 h of recording. The following morning, the surviving mites, confirmed by their active movement when prodded with a fine paintbrush, and their pupa hosts, were retained and subsequently examined microscopically for evidence of mite feeding. Samples of mites that did not survive overnight and their pupa hosts were also similarly examined for comparison.

EPG recordings were subsequently visualized using WinDaq Waveform Browser software (DATAQ Instruments Inc., Akron, OH), wherein EPG signals are plotted as changes in voltage amplitude over time. Signals were viewed at three levels of decreasing x-axis compression to identify the variability in signal voltage amplitudes and signal patterns that were suggestive of feeding activity. Initial EPG trials and observations of early periods of overnight trials provided *a priori* predictions of signal amplitudes and patterns of some *Varroa* mite behaviors. For example, signals having high voltage amplitudes correlated with locomotion of mite over host's integument, while other signals having small voltage amplitudes were correlated with mite activities, presumably fluid ingestion, while not walking on host's integument. EPG signals of *Varroa* ingestion were identified as rhythmic sinusoidal waveforms having short periods and relatively consistent peak-to-peak amplitudes with little or no interruption.

Durations of discrete feeding events identified from EPG recordings were measured, as well as the time between consecutive feeding events. For each feeding event, the number of voltage signal peaks were counted and divided by the event duration to calculate waveform repetition rate of active ingestion by *Varroa* mites. Additionally, the repetition rate of presumed pharyngeal pumping was determined for each mite by inspecting waveforms at a very low compression and counting

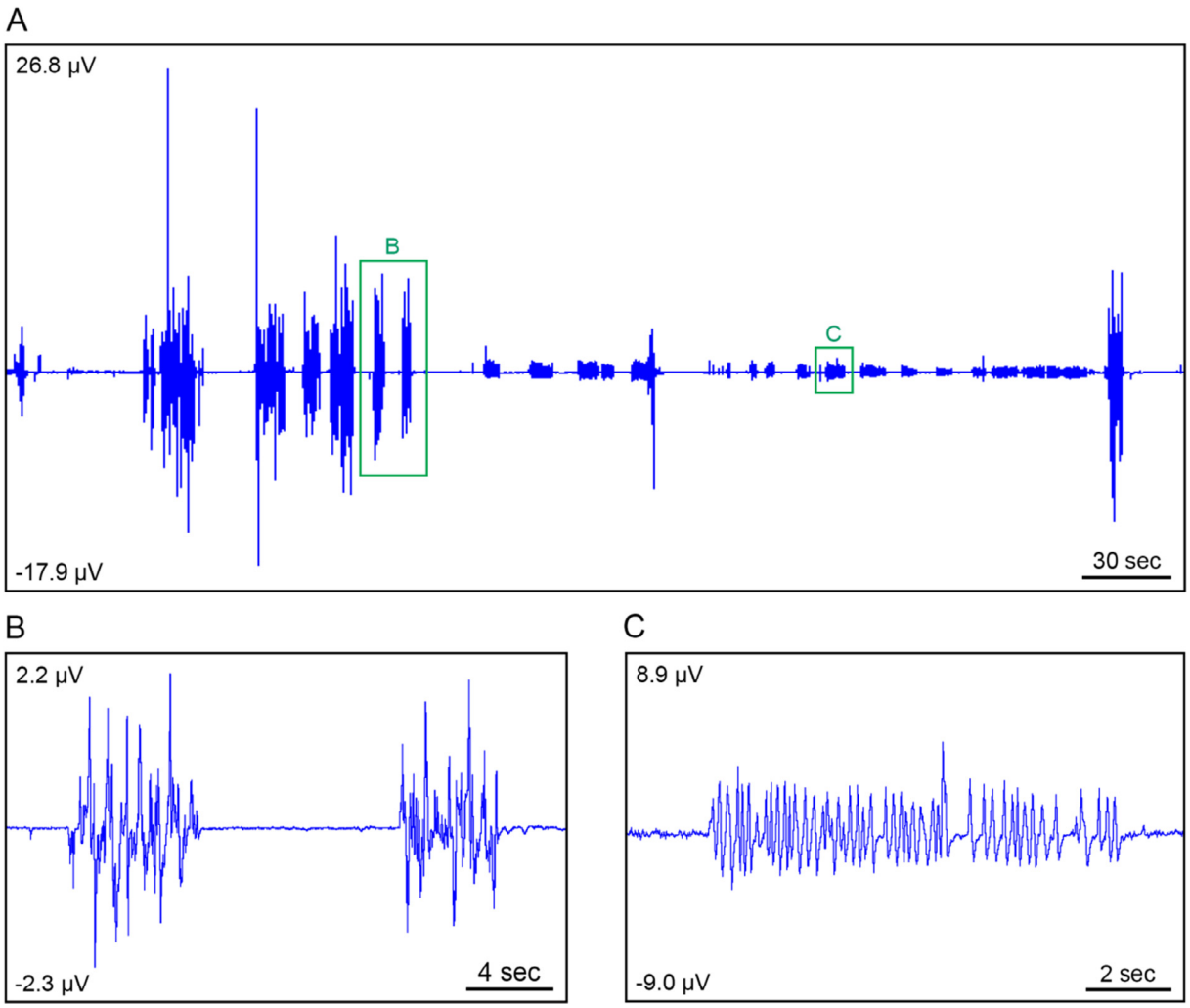


**Fig. 1.** Electropenetrographic tracings of *Varroa destructor* feeding activity on *Apis mellifera* pupa. (A) High compression view of EPG traces showing consecutive events of presumed *Varroa* mite feeding flanked on either side by EPG traces indicative of mite locomotion. (B) A low compression (spread-out) view of tandem EPG traces of *Varroa* mite locomotion showing irregularly spaced spikes having highly variable voltage amplitudes. (C) A low compression (spread-out) view of an EPG trace of presumed *Varroa* mite food ingestion showing regularly spaced spikes having relatively consistent voltage amplitudes. Green boxes in (A) represent locations of EPG traces shown in (B) and (C).

the number of peaks observed in exactly one second (E. Backus, pers. comm.). Acquired mite feeding data were further analyzed using JMP software version 10.0.0 (SAS, Cary, NC).

### 2.3. Confirmation of mite feeding

Confirming that mites had fed from their hosts during overnight EPG trials was accomplished by microscopically detecting fluorescein isothiocyanate (FITC)-labeled 1-µm polystyrene beads (Molecular Probes, Inc., Eugene, OR) (hereafter, microbeads), inside mite's bodies. Microbeads had previously been injected into host pupae. Prior to injection, microbeads were diluted to approximately $10^7$ beads/µl in PBS. Two microliters of this solution was injected between abdominal sternites of a pupa using a Micro4 microsyringe pump controller (World Precision Instruments, Sarasota, FL), equipped with a 10 µl Hamilton syringe and a 33-gauge needle (Reno, NV). Mites collected after the EPG trials were gently vortexed in 1 mL of 70% ethanol to remove any beads that may contaminate the body surface, blotted dry, then separately crushed and smeared onto a glass slide. Prepared specimens were examined under a Zeiss Model AX10 epifluorescence microscope (Thornwood, NY). As controls, three mites that did not show EPG patterns suggestive of food ingestion and had died during the overnight trials, as well as their pupa hosts, were both similarly examined.

To better visualize mite ingestion of microbeads, microbeads were imaged using fluorescent microscopy inside the bodies of both fed mites and their pupa hosts. To do so, individual honey bee pupae were injected with microbeads as above, and then placed in separate gelatin capsules together with a single *Varroa* mite. Gelatin capsules housing pupae and mites were kept in a dark incubator set at 32 °C and having a relative humidity of ~80% (Egekwu et al., 2018). As controls, four other pupae with and without injected microbeads were treated similarly. After 24 h, both injected and control pupae, as well as mites were inspected for presence of microbeads using a Zeiss AxioZoom stereozoom fluorescent microscope (Thornwood, NY) equipped with a 1× 0.25NA PlanNeoFluor objective lens. GFP filters and bandpass settings were used to obtain fluorescent images of pupae and mites. Camera exposure times were consistent between specimen of both injected and control pupae and mites. Two-dimensional images were produced from 80 to 120 z-stack images captured with 64-bit ZEN software (Zeiss, Thornwood, NY) using an extended depth of focus.

### 2.4. Histology of mite internal food ingestion apparatus

*Varroa* mite specimens for histology were first fixed in a 10%

neutral buffered formalin solution, and then embedded in a methyl methacrylate resin cured at low heat. Next, a Leica 2165 microtome formed 2 µm coronal sections from embedded specimen. Thin sections were mounted on glass slides and then stained with hematoxylin-1% eosin solution (Fisher Scientific, USA) (Ramsey et al., 2018, 2019). Slides were subsequently imaged on a Zeiss AxioZoom stereozoom microscope equipped with an Axio M2 camera (Thornwood, NY).

Additional mites were fixed for 2 h at room temperature in 2.5% glutaraldehyde, 0.05 M sodium cacodylate, 0.005 M calcium chloride (pH 7.0), then refrigerated at 4 °C overnight. Mites were rinsed 6 times with 0.05 M sodium cacodylate, 0.005 M calcium chloride buffer and post-fixed in 1% osmium tetroxide for 2 h at room temperature. Samples were then rinsed 6 times in the same buffer, dehydrated in a graded series of ethanol mixed with 1% Z-6040 silane primer followed by 3 exchanges of propylene oxide, infiltrated in a graded series of LX-112 resin/propylene oxide and polymerized in LX-112 resin at 65 °C for 24 h. Thin serial sections were cut at a thickness of 0.5 mm on a Reichert/American Optical Ultracut ultramicrotome (Vienna, Austria) with a Diatome diamond knife (Hatfield, PA) mounted on glass slides and stained with 1% Toluidine Blue O (Ted Pella, Inc., Redding, CA) (Ramsey et al., 2018, 2019). Images of specimen were captured with a Zeiss AxioZoom stereozoom microscope (Thornwood, NY) equipped with a 2.3× 0.57NA PlanNeoFluor objective lens.

## 3. Results

### 3.1. Electropenetrographic recordings

EPG patterns from recordings of mite locomotion on a host pupa was visually confirmed in real time for a representative mite. The EPG locomotion pattern is characterized by irregularly shaped and spaced spikes having large amplitudes; for mites for which EPG data were analyzed, these ranged from 2.53 − 14.2 µV (Fig. 1A, B; Fig. S1A, B and D, E). Visual confirmation of food intake during EPG recording was technically untenable, therefore the EPG ingestion patterns analyzed in this study can only be characterized as putative mite feeding. Nonetheless, in contrast to mite locomotion, the EPG patterns of presumed ingestion was characterized by uniformly shaped and spaced spikes having relatively small amplitudes ranging from 0.47 to 1.73 µV (Figs. 1A, C; S1A, C and D, F).

Visual inspection of prolonged EPG recordings of ten mites for patterns and amplitude variability in EPG signals suggested feeding activity was captured for three mites (Table 1). Evidence of mite locomotion or periods of quiescence greater or equal to 300 s were metrics used for grouping feeding events into separate feeding bouts. The latter metric was arbitrary but was also conservative; the value for the upper 95% confidence interval for data of mean time between feeding events (128.2 ± 15.7 s s.e.m) was 159.5 s. Using this grouping regime, mites ranged between a single and six separate feeding bouts over a total mean recording time of 16.8 ± 0.6 h. The duration of separate feeding bouts was highly variable, with a mean time of 19.4 ± 5.6 min. Feeding bouts were comprised of as few as a single feeding event to as many as 33 feeding events each. Among mites, the mean number of observed feeding events per mite (32 ± 1.0) was relatively consistent, compared to both their highly variable mean duration per event (9.9 ± 3.0 s) and time between feeding events (excluding time between feeding bouts) (104.6 ± 12.2 s). Using total number of peaks per event divided by the event duration gave for all mites a mean waveform repetition rate of presumed pharyngeal pumping of 4.5 ± 0.4 cycles/second. The waveform repetition rate of presumed feeding events calculated for one second for each mite is reported in Table 1, and the mean for all mites was equal to 4.6 ± 0.2 cycles.

**Table 2**
Results from separate one-way ANOVA testing differences between individual mites in variables acquired from visual analysis of EPG recordings. Asterisk indicates data were $\log_{10}$-transformed prior to running analysis.

| Variable | *F*-value | DF | *P*-value |
|---|---|---|---|
| Pump waveform repetition rate * | 48.05 | 2,90 | < 0.001 |
| Event duration* | 7.25 | 2,90 | 0.001 |
| Time between events* | 69.62 | 2,82 | < 0.001 |

Feeding event duration, time between events (excluding time between feeding bouts), and pump waveform repetition rate were all significantly different across the three examined mites (Table 2); two mites having higher pump frequencies, and both shorter feeding event durations and time between feeding events, consistently contrasted with the one other mite in Tukey-Kramer HSD *post hoc* tests (Supplemental Table 1). Including data for all mites, a significant negative correlation was observed between both $\log_{10}$-transformed pump waveform repetition rate and duration of feeding events ($r^2 = 0.30$; $F_{1,86} = 38.92$; $P < 0.0001$) (Fig. 2A). Additionally, a significant positive correlation was observed between both $\log_{10}$-transformed feeding event durations and the time elapsed between feeding events (excluding time between feeding bouts) ($r^2 = 0.14$; $F_{1,75} = 13.70$; $P = 0.0004$) (Fig. 2B).

### 3.2. Confirmation of mite feeding

Microbeads were detected in all mites for which putative feeding EPG patterns were recorded, and none were observed in the three control mites (data not shown). Microbeads were observed concentrated in the caeca of the digestive tract of mites housed in capsules with pupa injected with microbeads (Fig. 3). As seen in 3C, the autofluorescence of the mite was very low, while the fluorescent microbeads seen in 3D are highly fluorescent and were mainly concentrated in the 4 distinct lobes of the gastric caecum. Microbeads were observed in real time clearly flowing between the mouthparts, 4 lobes of the gastric caecum, rectum and legs, confirming that Varroa ingests microbeads from the bee pupae. No beads were detected inside mites that were housed with pupa not injected with microbeads. A single feeding hole was identified on honey bee pupa hosts of *Varroa* mites that survived overnight trials and for which putative food ingestion EPG patterns were analyzed (data not shown). For mites that died overnight (controls), no feeding holes were observed on hosts.

### 3.3. Histology of mite internal food ingestion apparatus

A sagittal section of the gnathosoma and adjacent podosomal region of a female mite show that the pharynx (collapsed) is a large sucking organ about 200 µm long, having at least seven constrictor muscles

**Table 1**
Data of feeding behaviors obtained from visual inspection of EPG recordings of *Varroa* mites on honey bee host pupae. Parenthetical values represent the standard error of means.

| Mite | Recording duration (hour) | # Bouts | Bout duration (min.) | # Events | Event duration (sec.) | #EPG signal peaks | Pump repetition rate |
|---|---|---|---|---|---|---|---|
| 1 | 15.8 | 1 | 13.6 | 33 | 6.2 (3.1) | 29.8 (14.1) | 5.3 (0.4) |
| 2 | 16.6 | 2 | 30.6 (29.2) | 30 | 7.6 (4.0) | 55.0 (47.2) | 3.9 (0.1) |
| 3 | 17.8 | 6 | 13.9 (10.27) | 30 | 15.8 (13.8) | 36.0 (16.3) | 4.5 (0.3) |

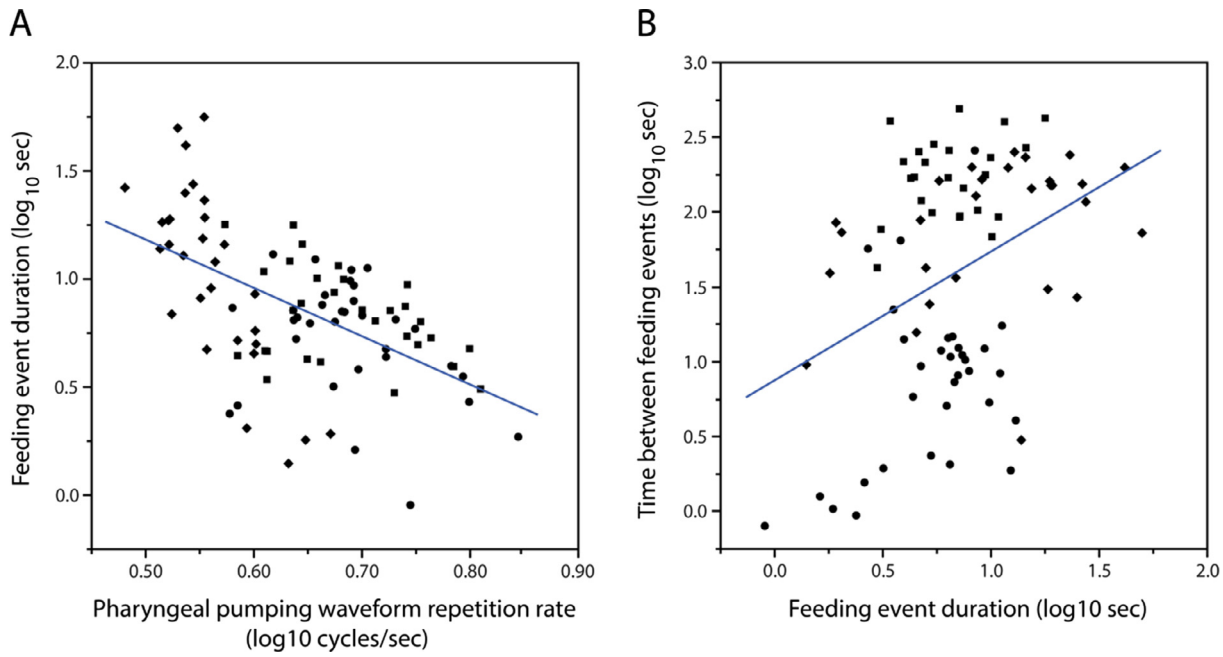


**Fig. 2.** Regression analyses of ($log_{10}$-transformed) data extracted from EPG recordings for presumed suction feeding (ingestion) of *Varroa* mites on honey bee pupae. (A) Plot of pharyngeal pump waveform repetition rate against duration of feeding events. (B) Plot of duration of feeding events against time between consecutive feeding events. In both A and B, circles = mite 1, diamonds = mite 2, squares = mite 3.

(Fig. 4A). A coronal section of the same region shows the pharynx with a fully expanded lumen, and with ~seven constrictor muscles (Fig. 4B, C). The number of pharyngeal dilator muscles is also at least seven, since the dilator muscles alternate in spaces between the constrictor muscles.

## 4. Discussion

In this study the feeding behaviors and biomechanics of fluid ingestion were described for *Varroa* mites on honey bee host pupae. Analyses of EPG recordings of behaviors together with histological examination of internal food ingestion apparatus reported here provide

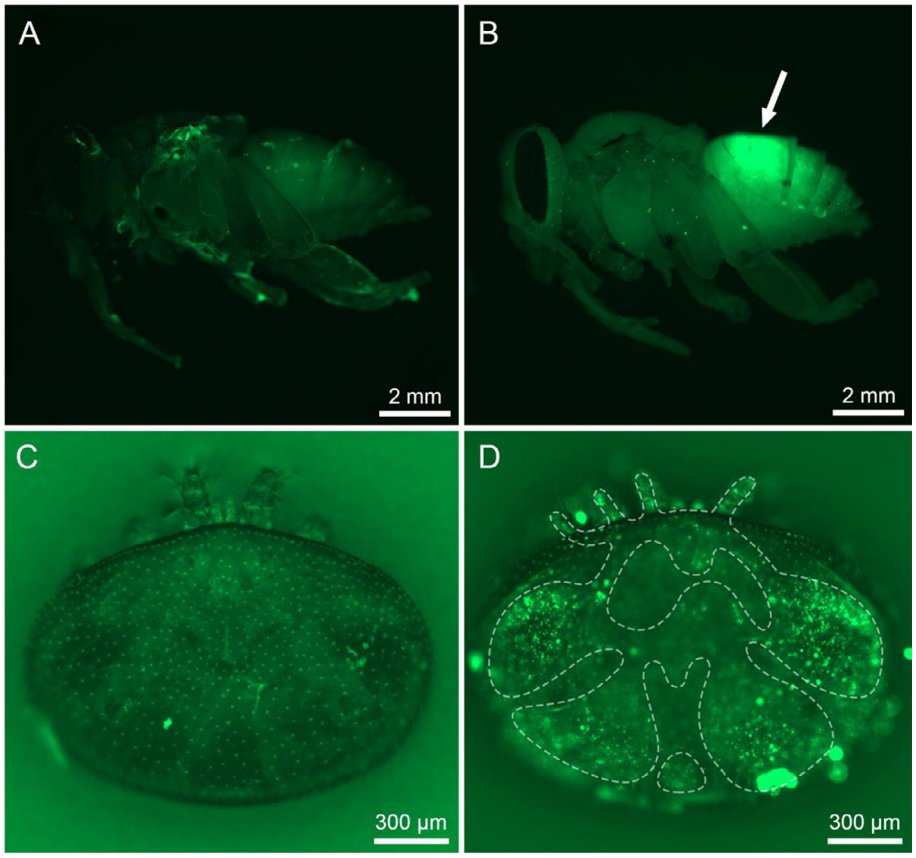


**Fig. 3.** Microscopic FITC-labeled fluorescent beads are transferred from pupae to *Varroa* confirming feeding by female *Varroa* mites. (A) Uninjected control pupa. (B) injected experimental pupa, with an arrow indicating the injection site in the abdomen containing a high concentration of fluorescent microbeads. (C) Mite that fed on uninjected control pupa, showing only faint background autofluorescence. (D) Mite that fed the pupa injected with fluorescent microscopic beads, showing aggregations of microbeads concentrated within the mite's caeca (dashed lines).

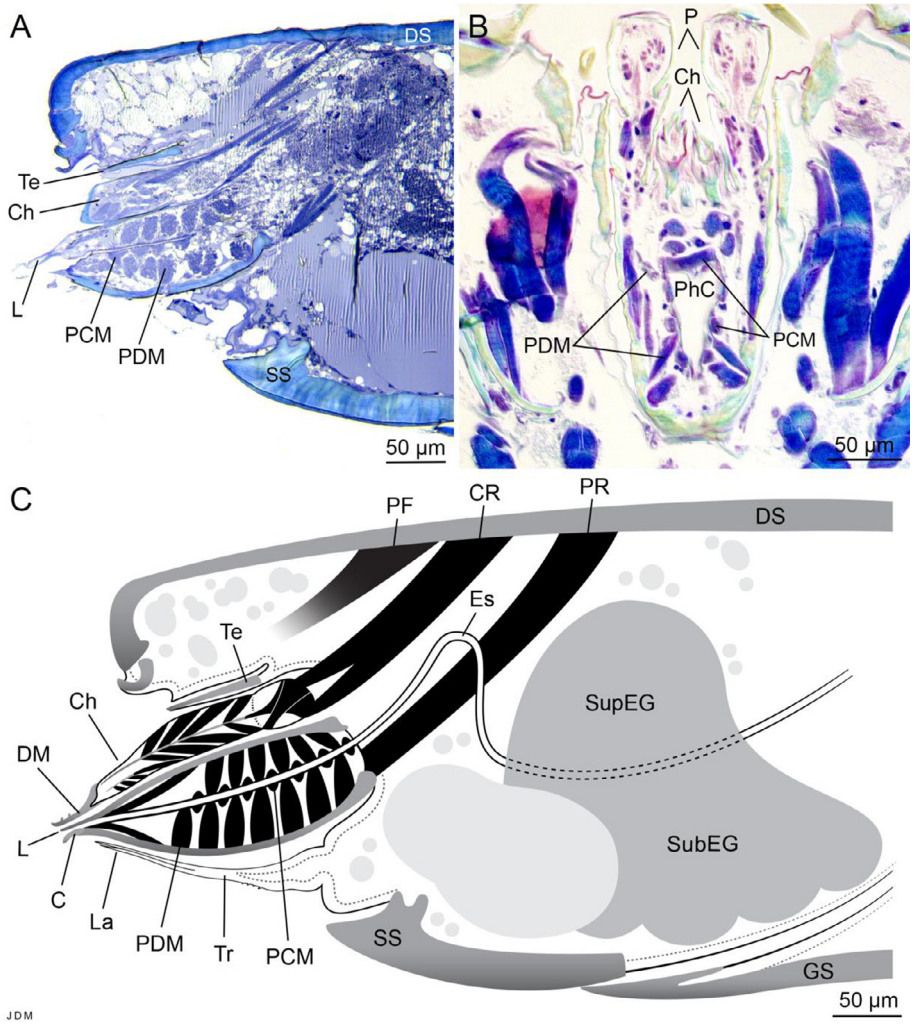


**Fig. 4.** Internal anatomy of the prosomal regions of female *Varroa destructor* mite showing the structure of the gnathosoma, pharynx and associated organs. (A) Micrograph of a sagittal view showing the alternating constrictor and dilator muscles surrounding the collapsed lumen. (B) Micrograph of a coronal view showing the dilated pharynx in the center of the gnathosomal base, with constrictor and dilator muscles. (C) Line drawing of the prosomal region showing the gnathosoma, pharynx, pharyngeal musculature, esophagus, synganglion and associated structures. C = corniculus; Ch = chelicera; CR = chelicera retractor muscle; Dm = movable digit (digitus mobilus); DS = dorsal shield; Es = esophagus; GS = genital sclerite; La = lacina; L = labrum; PCM = pharyngeal constrictor muscle; PDM = pharyngeal dilator muscle; PF = pedipalp flexor muscle; PR = pedipalp retractor muscle; SS = sternal sclerite; SubEG = subesophageal synganglion; SupEG = supraesophageal synganglion; Te = tectum; Tr = tritosternum.

a more robust understanding of *Varroa* mite feeding than previous studies. An early videographic study of *Varroa* mite feeding on honey bee pupae in the laboratory reported feeding events ranging in duration from 0.81 to 1.49 h (Donze and Guerin, 1994). A subsequent videographic study of *Varroa* mites with pupa hosts housed in artificial, transparent polystyrol brood cells located inside honey bee colonies reported feeding events lasting between 8.7 ± 8.4 (s.e.m) and 6.2 ± 4.0 min per hour, depending on developmental stage of host brood, giving a total feeding time between 200.5 ± 171.9 and 149.1 ± 96.0 min per day (Calderón et al., 2009). A preliminary analysis of *Varroa* mite feeding using an early version of the EPG system (Qin et al., 2006) reported a mean feeding duration per mite of 0.35 ± 0.12 h. The durations of feeding events from these studies correspond to feeding bout durations reported from our study; mean bout durations ranged between ~13 min to over 30 min (mean of ~10 min), and the longest feeding bouts continued for over 51 min. When separate feeding events were identified, they were reported to occur at a rate of between 0.3 ± 0.2 and 0.1 ± 0.1 events per hour, again depending on developmental stage of host brood (Calderón et al., 2009). The hourly occurrence of feeding events measured in our study ranged between 1.7 and 2.1 events, but the number of feeding bouts occurred at between 0.06 and 0.33 bouts per hour. Together, these data overlap with those reported in previous studies and demonstrate the variability in feeding behaviors exhibited by *Varroa* mites. What was missed in the above videographic studies, however, was the unseen movements of mites' mouthparts and activity of the sucking pump during food intake. From our study it was clear that although the mites often did not move away from the feeding hole, feeding was not continuous. From data collected using an early model of the EPG system, Qin et al. (2006) suggested the waveform repetition rate of the pumping structure was ~10 cycles/second, which was nearly double the rate as observed in our study.

The other feeding behaviors besides food ingestion have also been described from both observational and electronic monitoring studies of *Varroa* mites, other acarines, and also insects. For example, xylem-feeding leafhoppers, such as *Homalodisca vitripennis* (Hemiptera: Cicadelidae: Cicadellinae), is reported to have three feeding phases; pathway, Xwave and ingestion (Backus, 2016), during which salivation or sensing may occur (Dugravot et al., 2008). Similarly, ticks undergo sensory evaluation in multiple phases of feeding (Gregson, 1960). Feeding behaviors, such as probing, have also been suggested for *Varroa* mites (Qin et al., 2006). Careful observations of *Varroa* mites on honey bee host pupae reveal other consistent behaviors such as waving motions of their front legs possibly for detecting volatile chemical cues. In addition to EPG waveforms of mite walking on host pupae and putative pharyngeal pumping activities underlying fluid ingestion, we also recorded other mite EPG waveforms (data not shown) that could be associated with probing of the host's integument possibly with maxillary palps and piercing the cuticle with modified mouthparts for making a feeding hole. We also recorded mite EPG waveforms (Fig. S3) that are similar to tick salivation EPG waveforms (Li, unpublished data). In that study, individual ticks were topically treated with pilocarpine to induce salivation in a capillary feeding setup, tick salivation was visually observed, and corresponding EPG waveforms were recorded

simultaneously (Li, unpublished data). Because we were unable to visually confirm mite salivation through visual observation of behaviors, therefore we designate the EPG waveforms as "putative salivation waveforms" (Fig. S3). Moreover, we do not have clear histological preparations that show the salivary glands and their associated ducts. Future histological studies of *Varroa* mites should target elucidation of salivary gland structure and its relationship with other feeding anatomy, and other studies confirming EPG signal patterns for some of difficult to observe behaviors will increase the information extracted from both the current and previously published *Varroa* mite EPG data. However, a caveat to work using EPG to understand *Varroa* mite feeding behaviors is that trials are conducted outside of the mite and host's natural habitat. In the current study, care was taken to maintain temperature and lighting (dark) conditions within range of what would be naturally encountered by mites and their hosts.

Feeding behaviors of *Varroa* mites resemble those observed for some related acarines, but contrast with those of others (Stone et al., 1983; Zheng et al., 2015). For the former, suction feeding by the soft tick, *Ornithodoros turicata,* occurred at a repetition rate of 6.1–6.4 cycles per second, and feeding events extended for 19–22 min (Sonenshine and Anderson, 2014; Zheng et al., 2015). For a related Parasitiform mite, *Pergamasus longicornis*, feeding events were calculated to last ~10 min (Bowman, 2014). The comparable rates of suction feeding between *Varroa* mites and the soft tick is likely due to a similar arrangement of the constrictor and dilator muscles that operate their pharyngeal pumps (Sonenshine, 1991). In contrast, the hard ticks feed slowly and for much longer durations (Gregson, 1960; Sweatman and Gregson, 1970; Sonenshine and Anderson, 2014). Soft ticks have relatively large midguts and greatly expandable cuticles allowing for voluminous blood meals over relatively short periods of time, while hard ticks have a rigid cuticle, which must be synthesized *de novo* during feeding to accommodate the increasing blood volume (Sonenshine and Anderson, 2014). *Varroa* mites' bodies are rigid, and flattened dorso-ventrally; although some dorso-ventral expansion can be observed (e.g., when gravid), but the expansion is likely not due to synthesis of new cuticle. A flattened body form is necessary/ideal for inhabiting cramped spaces inside wax-capped honey bee brood cells during mites' reproductive stage, and for fitting their bodies under abdominal tergites/sternites of adult honey bee hosts during mites' phoretic stage (*sensu lato*). These constraints may have impacted the feeding behaviors, and possibly excretory behaviors of *Varroa* mites (Posada et al., 2018), and other Parasitiform mites (Bowman, 2014). For *Varroa* mites, constraints to feeding may be different depending on the life stage of both the mites and honey bees.

*Varroa* mite suction feeding is somewhat different compared to some other suction feeding insects, such as hemipterans. For example, during food ingestion by xylem-feeding *H. vitripennis*, EPG signals from the insect cibarial (suction) pump occurred at a waveform repetition rate of 1–2 cycles per second (Backus et al., 2005), a lower rate than observed for *Varroa* mites in our study. Moreover, voltage changes during leafhopper suction feeding varied from 0.35 mV to as high as 1.25 mV from G-level (Dugravot et al., 2008). The variation in amplitudes may be a result of different electrical signals produced from elastic and muscular forces driving the cibarial suction pump in hemipteran insects (Chapman, 1998). Whereas for mites and ticks, uniform signal amplitudes during suction feeding are likely a result of muscular forces that both contract and dilate the pharyngeal pump. Differences among fluid-ingesting arthropods in food ingestion rates may also be related to the physical properties of the foods they consume. For example, whether fluids are physically Newtonian or non-Newtonian in nature may impact how fluids move through often narrow tube-like food ingestion anatomy of liquid feeding arthropods (Cook, 2008). Honey bee host fluids consumed by *Varroa* mites contain some hemolymph with dissolved nutrients, but primarily semi-digested cellular components or entire cells (e.g., fat body tissues) (Ramsey et al., 2018, 2019), making the food more non-Newtonian in physical nature, similar to, e.g., mammal blood. Finer histological studies will likely be needed to fully examine the morphological features of *Varroa* internal feeding anatomy.

The feeding behaviors of individuals are mutable by interactions with other individuals. Feeding behaviors of *Varroa* mites may be influenced by both inter- and intraspecific interactions. For the former, it is unknown how differences between honey bee hosts in their responses to *Varroa* parasitism, and/or how *Varroa* mites counter these defenses, influence the mites' feeding behaviors. No published study is available for comparison that has described or quantified *Varroa* mite feeding behaviors on *Apis cerana* host honey bees. Moreover, the effects of proposed anti-feedant proteins produced by the mite's native host on *Varroa* mite feeding behaviors have not been studied. Intraspecific interactions may be facilitative or competitive; although communal use of a single feeding hole on host pupae by multiple mites is observed, and data suggest female mites may modify number and gender of offspring depending on mite density (Fuchs and Langenbach, 1989), it remains unclear whether these behaviors are altruistic. Correlations between feeding times and pump cycle frequencies observed in our study suggest that mites modify behaviors in order to acquire a given volume of food. This could mean that mites' feeding behaviors may be under some constraints, even in the absence of other mites. Both laboratory and field studies are required to more fully elucidate the altruistic or competitive interactions that may exist that influence *Varroa* mite feeding behaviors.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinsphys.2019.103950.

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