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Article

The heart of *Varroa destructor*: description, function and inhibition following acaricide application

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Abstract

This study examines the heart and its activity in the mite *Varroa destructor*. Through light microscopy, the duration and frequency of the beat of the mite's heart, which is located over the synganglion, was recorded and analysed. The heart measured 165 µm by 60 µm, and consisted of two lateral trunks, inducing pulses with an average duration of 10 sec and an average frequency of 0.13 Hz. The use of cardiac activity as a new bioassay for accurate assessment of the effect of varroacide compounds was also examined.

Keywords: *Varroa destructor*, heart, cardiac activity, acaricide assessment

Introduction

The ectoparasitic mite, *Varroa destructor* (Anderson and Trueman, 2000) is undoubtedly the most serious worldwide pest of the western honeybee *Apis mellifera* (Spivak 1999, Rosenkranz *et al.* 2010). It can induce both direct and indirect damage to honey bees and honey bee colonies. Directly, these mites can parasitize and feed on honey bee brood, reducing both body weight and lifespan of emerged honey bees (De Jong *et al.* 1982; Amdam *et al.* 2004). Indirectly, they are excellent vehicles for the transmission of honey bee viruses which can lead to the weakening or collapse of entire colonies (Genersch *et al.* 2010; Hayes *et al.* 2008). Since *V. destructor* has transferred from the Asian honey bee (*Apis cerana*) and infested colonies of *A. mellifera*, research has focused mainly on the control of the parasite. A huge number of experimental results has been published about the "battle" against apiculture's number one threat using chemical products, "green products", natural substances, biotechnical manipulations, etc (Rosenkranz *et al.* 2010). Compared to the extended research on varroa control, other scientific fields, such as the anatomy and the physiology of the mite, have been relatively neglected. Research has mainly focused on the genital system and sensory organs of the mite (Ifantidis 1983; De Ruijter & Kaas 1983; Alberti & Hänel 1986; Dillier *et al.* 2006).

One of the less-examined anatomical systems of varroa is the circulatory system. To our knowledge, there is no bibliographic reference on the presence or absence of a heart in varroa. Research on the circulatory system of the Acari is rather limited. The small body size of most Acari makes them unsuitable for anatomical and physiological investigations (Obenchain & Oliver 1976). However, the relatively larger size of ticks (Ixodioidea) enables easier monitoring of the heart and has resulted in detailed descriptions of its morphology, anatomy and function (Obenchain & Oliver 1976; Binnington 1981; Šimo *et al.* 2009; Grubhoffer *et al.* 2014).

In the present study, we searched for the heart of varroa under the dorsal cuticle in the area close to the synganglion (brain) of this species. We monitored only live specimens in an effort to find and record the presence of a heart as well as to examine its function. We also examined the effect of amitraz application on cardiac activity as a preliminary test of a potential new bioassay for the assessment of varroa control compounds.

Material and Methods

Varroa destructor mites

Female varroa mites were picked from the bodies of adult honey bees in a colony of *A. mellifera macedonica* naturally infested by the parasitic mites. Mites in brood cells were not sampled in order to avoid age variation and physiological differentiations. The colony was located at the department of Biology of the Aristotle University of Thessaloniki (40º38'00.61"N, 22º57'27.31"E) and had been untreated for varroa for one year. The proximity of the colony to the laboratory meant that mites could be collected and used in experiments within a few minutes.

Observation and recording of the heart of *Varroa destructor*

The mites were placed on a microscope slide, ventral side down, and were fixed in place through a thin, transparent, nylon membrane cover. The specific procedure enabled immobilization of the mite without injury to or malfunction of its physiology. Monitoring was conducted through a Zeiss Axiostar Plus light microscope at magnifications of between 40-100x. Video recordings were obtained through a Canon A640 digital camera. The duration and the frequency of the cardiac beats were calculated through video analysis of the recordings using Sony Vegas Pro V13 software.

In addition, efforts were made to isolate and photograph the heart through a Confocal Laser Scanning Microscope (CLSM). To do this, the dorsal cuticles of dead varroa mites were removed to reveal the internal organs. The specimens were left to dry for more than 12 hours and were fixed in formaldehyde solution of 5% for 12 hours in 4ºC and dehydrated in alcohol series in WFI (50%, 70%, 90%, 100% for 10 minutes), infiltrated in methyl salicylate for 30 minutes and hydrated in alcohol series (100%, 90%, 70%, 50%) for 10 minutes. Then, the specimens were left to dry overnight and were sealed in Entellan. Finally, the heart was removed through section using fine razor blades and scissors. Photographs of the preparations were taken with a Zeiss AxioObserver equipped with Zeiss LSM 780 CLSM. The scanning was conducted with an argon laser at 488nm and images were obtained with ZEN 2011 software.

Amitraz application

Amitraz (Sigma-Aldrich, Germany), a formamidine insecticide frequently used against varroa, was applied according to a previous protocol described by Papachristoforou *et al.* (2011). The active ingredient was dissolved in dimethyl sulfoxide (DMSO) (Panreac, Spain), and freshly made stocks containing 0.02% amitraz (w/v) were prepared. Amitraz solutions were applied topically to varroa in droplets of approximately 5μL, using a micropipette. Varroa mites were fixed on a microscope slide, ventral side down with a transparent film, allowing the recording of the heart rate before and after the topical application of Amitraz. A puncture was made in the film to facilitate the fitting of the micropipette without displacing the mite.

Statistical analysis

Statistical analyses were conducted through the GraphPad Instat V3.10 software. Both duration and frequency of heartbeat were compared between all the individual mites monitored. For the comparison of means, nonparametric Kruskal-Wallis tests were applied. Evaluation of duration and frequency was performed on video recording of 5 mites under normal conditions, lasting from 4 to 20 minutes. For the evaluation of the duration and frequency of heartbeat following Amitraz application, 5 extra mites were used.

Results and discussion

Morphological description of the heart

The morphology of the varroa's heart is similar to other Arachnida and ticks previously described by Grubhoffer *et al.* (2104). The function of the varroa's heart is visible in Video 1 (https://youtu.be/t3ZvhwFllfk). The heart is approximately 165 µm long and 60 µm wide. Though the heart of varroa appears to be smaller when compared with previously described hearts of ticks, it is relatively large in relation to its body size. For example, the heart of a female *Amblyomma tuberculatum* is approximately 270 µm long and 200 µm wide (Obenchain & Oliver 1976) but the body size of *A. tuberculatum* is much larger than the body size of *V. destructor* (7.7 mm long and 5.5 mm wide, 1.2 mm long and 1.7 mm wide, respectively). Grubhoffer *et al.* (2104) presented images of the circulatory system of male *Ixodes ricinus* where the heart of the tick is approximately 110 µm long and 90 µm wide and its body size is approximately 2.6 mm long and 1.4 mm wide. Regarding heart's morphology, a sinus, called the pericardial septum, surrounds the heart (Figure 1A) and the haemolymph flows inside the myocardium heart region from the ostia. Arterial vessels (pedal arteries) direct the flow of haemolymph outside the heart cavity. The flow can be easily observed by the movement of hemocytes (Video 2: https://youtu.be/NYySIqWEz1s). Contrary to hearts of previously described mites, the heart of varroa appears to consist of two distinct ventricula (Figure 1B and Figure 2). Synchronized contractions of both parts lead haemolymph into the heart while expansions direct haemolymph to the body parts of the Acari. Another difference between the heart of varroa and other mites is the presence of 6 rather than 4 ostia (Figure 1B).

All the morphological information provided in the present preliminary study is a result of optical and CLS microscopy. Future research is required through scanning electron microscopy and microsections to obtain a detailed description of the morphology and the anatomy of varroa's heart.

Function of the heart and application of acaricide

The heartbeat of varroa presented an average duration of 7.39 sec (min 4.33, max 23.06). The average frequency of the heart rate was 0.13 Hz (min 0.04, max 0.22). The comparison between all mites showed that there were significant differences in both duration and frequency of heartbeat ($p<0.001$). Such variation amongst different specimens of the same mite species is not unusual. As noted by Obenchain & Oliver (1976), heart rate appears extremely variable in all species of ticks. They recorded heart rates varying between 12 and 180 beats per minute in *Dermacentor variabilis*. However, variation of heart rate within each individual varroa mite was very low (SEM = 0.11–2.87 for duration and 0.002–0.007 for frequency), suggesting a relatively constant pattern of heart function. This stability means that heartbeat has potential to be used for the assessment of the impact of an acaricide.

Amitraz application induced a rapid excitation of the varroa heartbeat a few seconds after contact (Video 3: https://youtu.be/vG1ovEnnrZ8). The duration of the beats evaluated on a single mite was 0.33 sec while frequency reached 1.35 Hz for about one minute and then gradually decreased until it completely ceased. Heart activity of 5 tested mites stopped in an average of 5.36

min (min 3, max 7.21min, SEM = 0.96). Using heart function for the assessment of acaricides' efficacy is a promising novel methodology. It can be applied easily, it is not time-consuming and requires no complex material. Future research will focus on the accurate assessment of a variety of acaricides, investigating different doses inducing both lethal and sublethal effects on *V. destructor*.

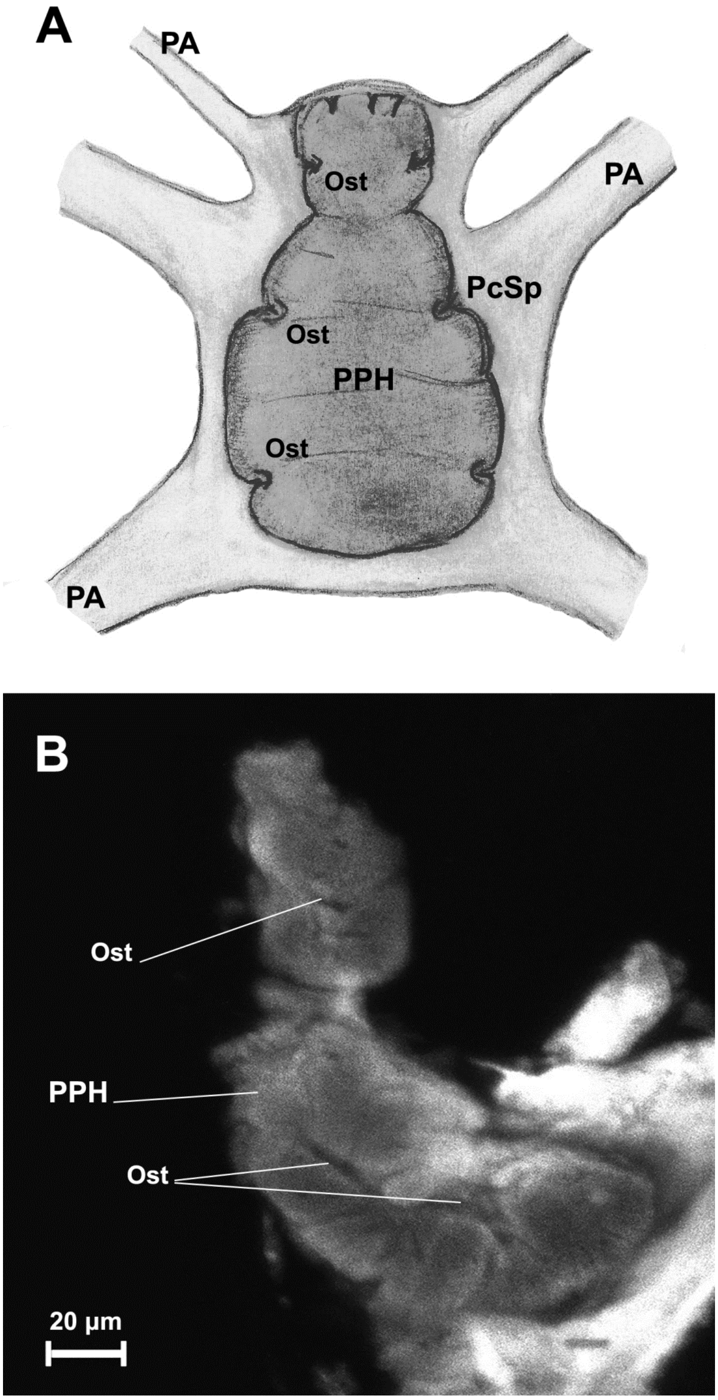

FIGURE 1. The heart of *Varroa destructor*. A: Graphical representation. B: Confocal laser scanning microscope image of the heart (costal view). PcSp: Pericardial septum PPH: Pulsative portion of heart, PA: Pedal arteries, Ost: Ostia.

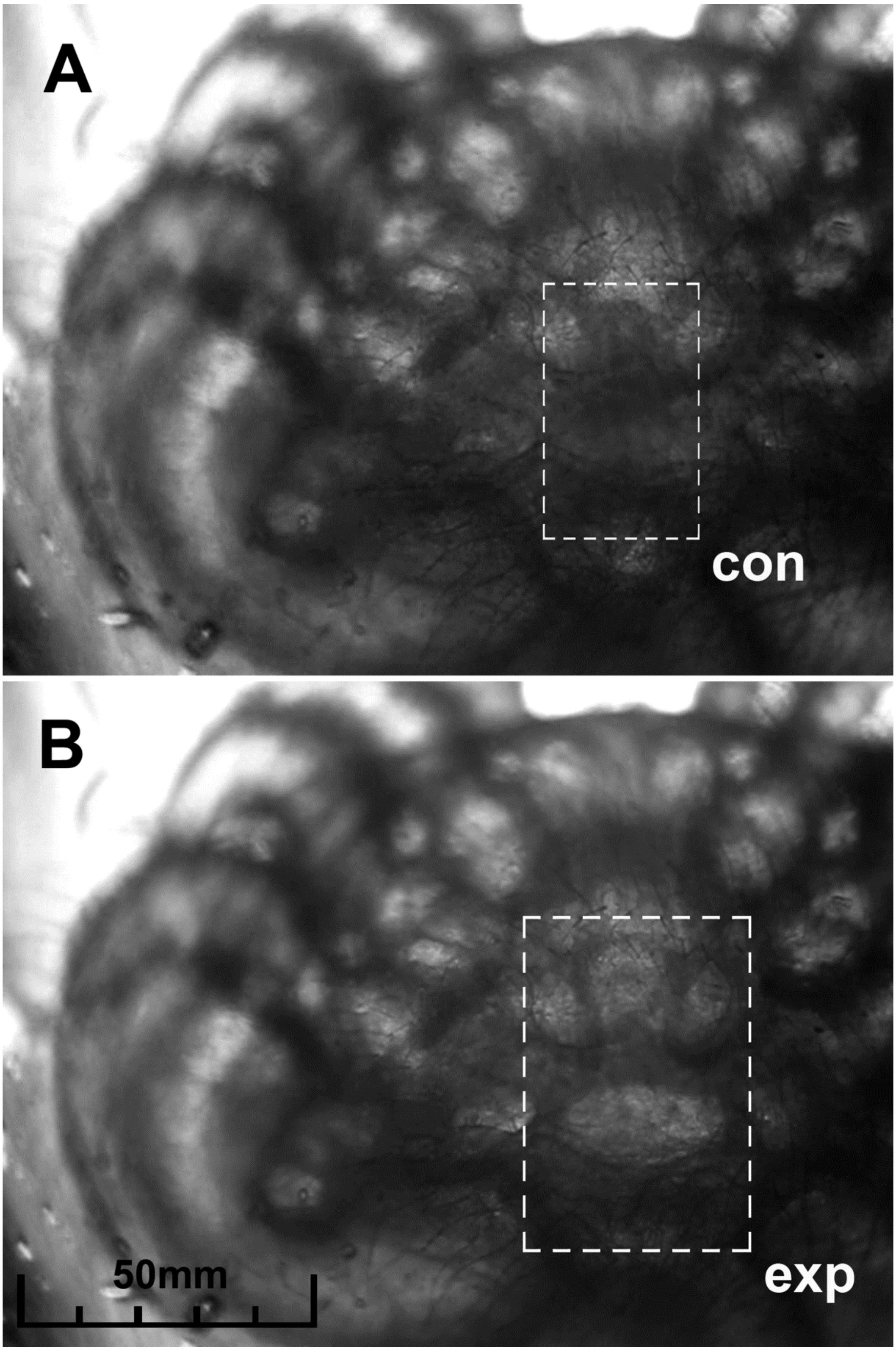

FIGURE 2. The heart of *Varroa destructor* (snapshot from live recording). A: Systolic phase. B: Diastolic phase. con: contraction, exp: expansion.

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Appendix

VIDEO 1. https://youtu.be/t3ZvhwFllfk
VIDEO 2. https://youtu.be/NYySIqWEz1s
VIDEO 3. https://youtu.be/vG1ovEnnrZ8