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NOTES AND COMMENTS

A standardized method to extract and store haemolymph from *Apis mellifera* and the ectoparasite *Varroa destructor* for protein analysis

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The ectoparasitic mite *Varroa destructor* is a serious problem for beekeeping. It sucks haemolymph from different stages of the bees causing significant damage (see review by Rosenkranz *et al*., 2010). Haemolymph collection from honey bees is required to perform immunological, physiological, nutritional and other assays. In the literature there are many dissimilar methodologies to extract and store haemolymph. The electrophoretic profile and protein contents of *V. destructor* have been little studied, but it is of great interest to evaluate the changes experienced by this tissue in response to different treatments in the host and its parasite. The aim of this study is therefore to propose a standard procedure for haemolymph collection and storage, extending previous studies and sharing our working experience.

In much previous published research, bee haemolymph extraction has been based on the dorsal puncture, between the second and third abdominal terga from the thorax. To avoid faecal contamination we prefer to make the extraction from the dorsal aorta. To perform our assays, honey bees and mites were obtained from an experimental apiary (38º 10′ 06″ S; 57º 38′ 10″ W), and were confined in wooden cages at the Arthropods Laboratory. To perform a protein analysis under laboratory conditions, it is necessary to provide a protein source to bees. According to our experience, feeding on pollen, candy and tap water separately (*ad libitum* from emergence) ensures prolonged honey bee survival. We observed a very low mortality under these feeding conditions when 100- 200 individuals were confined in medium size wooden cages (11 × 9 × 6 cm^3), allowing them to cluster, which possibly reduce the stress induced by long term confinement. The number of bees per cage is of great importance, particularly using this diet, as if more than 200 individuals are placed in this type of cage we commonly note death of individuals during the first week.

As mentioned above, honey bee haemolymph was collected by puncturing the dorsal aorta using a glass microcapillary. This collection is more abundant when bees remain hydrated, so it is important to spray the cage with water for at least 15 minutes before extraction. Because of the low volume obtained from each individual, haemolymph from 10 adults was pooled. The top of a plastic Pasteur pipette was employed to manipulate the adult bees to avoid stinging (Fig. 1.) and to make the extraction faster and more comfortable. The extraction process was carried out over ice to prevent any degradation of the proteins. The pooled haemolymph was placed in 1.5 ml plastic tubes containing 99.5 µl of frozen buffer developed by Mead *et al*. (1986) adapted to our biological material. This buffer prevents sample melanization, and also 0.5 µl of protease inhibitor was added (PMSF 200mM), then the sample was centrifuged to remove haemocytes at 5000 RPM at 4°C for 2 minutes. An aliquot of supernatant (1 µl) was used to determine the total protein concentration by the Bradford method (Bradford, 1976). It is essential to define *a priori* the age of bees, principally to avoid variation of protein content between samples.

To collect haemolymph from *V. destructor,* adult mites were held on sticky tape and manipulated under a stereomicroscope. Haemolymph was extracted with a glass microcapillary by puncturing within interescutal membranes (Tewarson, 1983). Due to the low volume obtained, a pool of 8 mites was made. Haemolymph was placed in 46 µl of frozen buffer (Mead *et al*., 1986) and 4 µl of PMSF 200mM. Then samples were centrifuged at 5000 RPM at 4°C for two minutes and their preservation was then as described for bee haemolymph. To SDS-PAGE, honey bee and mite samples, treated as above, were placed in Laemmli buffer 1X and boiled for five minutes and then stored at -20°C. It is not convenient to store untreated haemolymph

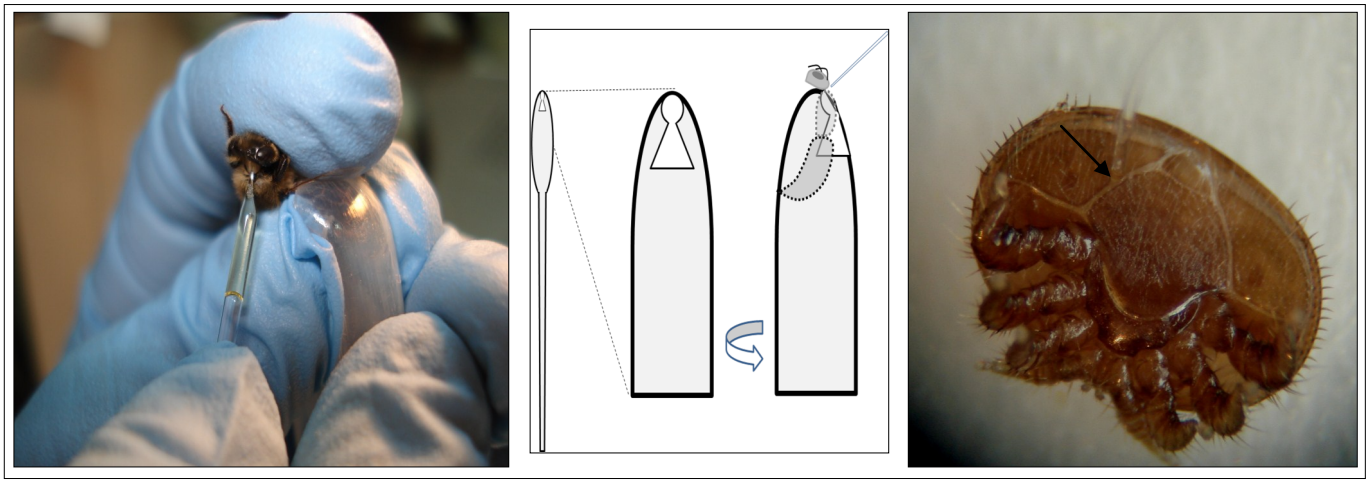

Fig. 1. a. Extraction of adult honey bee haemolymph from the dorsal aorta. ***b.*** To manipulate bees of any age we used a cut plastic Pasteur pipette. ***c.*** Varroa haemolymph extraction, the arrow indicates the microcapillary insertion.

at this temperature because we have observed protein degradation with time.

Extraction of haemolymph from mites by puncture has the disadvantage that it is tedious work, but it ensures the specificity of proteins from that particular tissue. In the case of bee haemolymph extraction, several protocols were tested, but this one was the most practical, with higher performance (it allows extraction up to 8 µl of haemolymph per bee), less contamination with surrounding tissues, the protein analysis was not interfered by buffer solution, moreover, individuals were easily manipulated.

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References

BRADFORD, M M (1976) A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Annual review of Biochemistry* 72: 248-254.

LAEMMLI, U K (1970) Cleavage of structural proteins during assembly of head of bacteriophage-T4. *Nature* 227: 680-685.

MEAD, G P; RATCLIFFE, N A; RENWRANTZ, L R (1986) The separation of insect haemocyte types on Percoll gradients; methodology and problems. *Journal of Insect Physiology* 25: 795-803.

ROSENKRANZ, P; AUMEIER, P; ZIEGELMANN, B (2010). Biology and control of *Varroa destructor*. Journal *of Invertebrate Pathology* 103: S96–S119. http://dx.doi.org/10.1016/J.JIP.2009.07.016

TEWARSON, N C (1983). Nutrition and reproduction in the ectoparasitic honey bee (*Apis* SP.) mite, *Varroa jacobsoni*. PhD thesis. Universität Tübingen; Germany.