

Accepted Preprint
voltage of 175 V. The instrument state mass range was set to 1700 m/z with 4 GHz digitalization rate, which leads to a mass resolution of 40 000 (measured at m/z 1521). Mass spectra were acquired in profile mode over a m/z range of 100 - 1700 by 1 Hz in scan and 3 Hz in MS/MS mode. The auto-MS/MS experiment was selected using the 20 most abundant precursor ions per cycle with activated precursor abundance-based scan speed. The quadrupole precursor ion isolation width was set to medium (~ 4 m/z) with a dynamic collision energy based as a function of the m/z value (slop = 3, offset = 2). Active exclusion of a precursor mass was one repeat and release after 15 seconds. The system was controlled with MassHunter Data Acquisition B.08.01 service pack 1 (Agilent Technologies, USA).

Protein identification

The raw data files were processed with Mascot Distiller 2.7 (Matrix Science, UK) to generate a peak list. A database search to identify proteins was conducted using an in-house Mascot Server 2.6 (Matrix Science, UK) against the *Apis mellifera* sequence database (15323 protein sequences of *Apis mellifera*, downloaded in November 2018 from Uniprot.org) and sequences for common protein contaminants (cRAP, downloaded from thegpm.org). The search parameters were: enzyme trypsin, maximum missed cleavages 2, fixed modification carbamidomethylation of cysteine and variable modification oxidation of methionine. Peptide mass tolerance window was set to 7 ppm and fragment ion mass tolerance to 0.70 Da. The received search results were sent to the Scaffold software 4.7.3 (Proteome Software, USA). The loaded results were searched against a second search engine X! Tandem (thegpm.org, version Cyclon 2010.12.01.1) to increase confidence. The Local FDR algorithm with 95 % probability was used for validating peptide identifications. The Protein Prophet algorithm with 99.0 % probability was used for protein identification, and contained at least 2 identified peptides (Nesvizhskii et al. 2003).

Vitellogenin identification by Western Blot

Immunoblots were performed according to the Licor protocols (https://www.licor.com/bio/applications/quantitative_western_blot/protocol.html) with slight modifications. For immunoblots, size-separated proteins were electrophoretically transferred to nitrocellulose membrane (Amersham™ Protran® Western blotting membranes, 0.45 µm: GE10600002 or 0.2 µm: GE10600004) using either Bjerrum Schafer-Nielsen Buffer with SDS (48 mM Tris, 39 mM glycine, 20% methanol (pH 9.2), 0.1% SDS) with constant current of 140 mA for 1h 30 min (OWL Device) or Pierce 1-Step Transfer Buffer with constant high current of 1.3-2.5 A for 12 min (Pierce™ Power Blotter). To control the transfer efficiency, the membrane was briefly rinsed with water after transfer, and incubated for 5 min in Revert Total Protein Stain, rinsed twice for 30 sec in washing solution (Licor: 926-11015) before acquiring the Revert total stain signal in the 700 nm channel using the Licor Odyssey® CLx Imaging System. The membranes were then incubated in Licor Blocking buffer (927-40100) for 1h at room temperature or overnight at 4°C with agitation. After addition of 0.2% Tween-20, the membranes were incubated with the polyclonal antibody against vitellogenin at a 1:7000 dilution (Davids Biotechnologie Regensburg) for 1 h at room temperature or overnight at 4°C with agitation. After 3 washes with PBS-T (0.2% Tween 20) the membranes were incubated for 45 min at room temperature in secondary antibody solution (IRDye® 800CW Goat anti-Rabbit IgG (H + L), 1:40.000 in Licor blocking buffer diluted 1:1 with PBS-T). After 3 washes with PBS-T (0.2% Tween 20) the vitellogenin specific signals were acquired in the 800 nm channel. The detected fluorescence signals were quantified using NIH software Image J (Christen et al. 2007). The intensity of vitellogenin bands was normalized against the intensity of the uncharacterized *Apis mellifera* protein, which is also detected by the antibody. Although not specified, this protein was well suited as a loading control, due to the fact that this protein is stably expressed in all analysed tissues. Moreover, there was a lack of available commercial antibodies, which are specific for honey bees and antibodies against proteins of other species may lack cross-reactivity.

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