

Honeybee blood under the microscope: Characterizing hemocytes through surface protein profiling

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The global decline of honeybee populations is a pressing issue, yet their cellular immunity - specifically the blood cells, or hemocytes - remains significantly understudied. In contrast, the blood cells of other farm animals have been extensively characterized, leading to a deeper understanding of their immune systems. One of the key challenges in studying honeybee hemocytes is their morphological similarity, which makes it difficult to distinguish their functions. For instance, differentiating between plasmatocytes and prohemocytes, or oenocytoids and granulocytes, often requires advanced microscopy techniques such as interference or phase contrast microscopy. Additionally, researchers debate the existence of hemocyte subtypes like spherulocytes and coagulocytes. A standardized classification system is crucial to determine the hemocyte types present in honeybee blood and their roles in immunity.

In our study, we aim to characterize honeybee hemocytes based on the proteins present on the outer surface. This strategy, known as the Cluster of Differentiation (CD) system, has been widely applied to various cell types across different organisms. We enzymatically shaved the surface proteins from the hemocytes and identified them using mass spectrometry. Additionally, we extracted RNA to analyse gene expression, providing further insight on hemocyte function. Our combined approach revealed 1101 genes encoding surface proteins, indicating great diversity. We identified key immune pathways in insects - JAK/STAT, Imd, Toll, and JNK - and detected hemocyte markers from other invertebrates in honeybees. Notably, several of these genes encode viral receptors found in other insect species.

This knowledge opens the door for the development of monoclonal antibodies targeting potential hemocyte markers. By establishing a panel of antibodies that react with different hemocyte subtypes, researchers can investigate the impact of stress factors on honeybee blood cells. A better understanding of honeybee hemocytes could pave the way for new strategies to support colony health and resilience against environmental threats.

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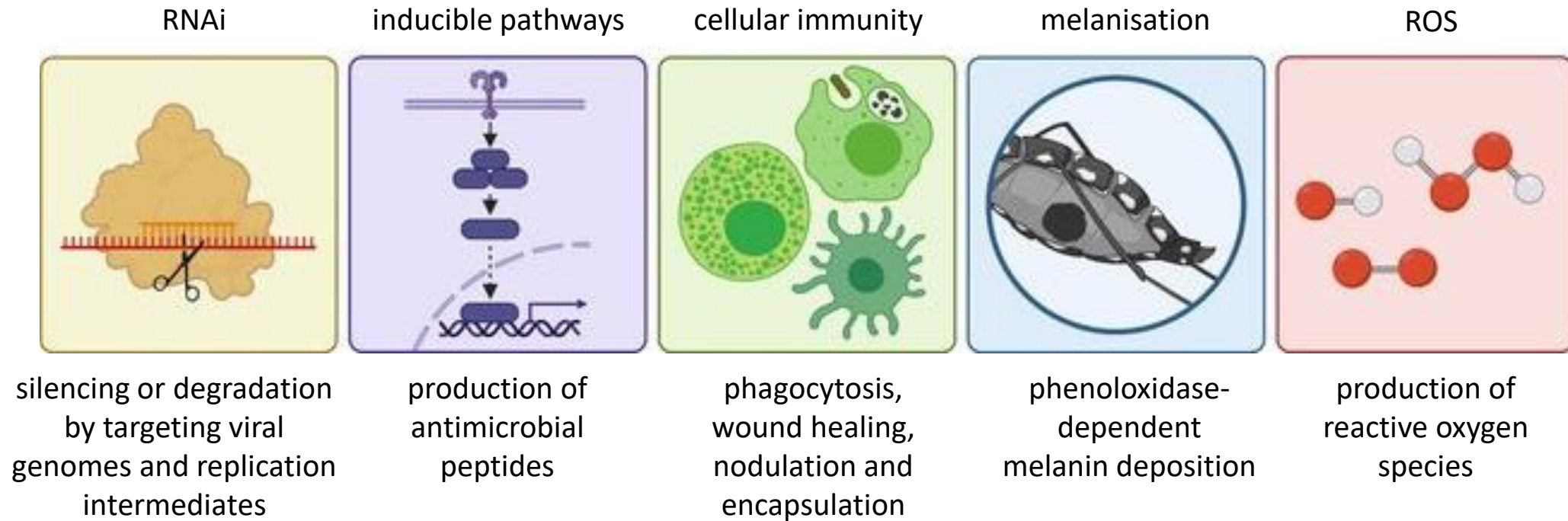


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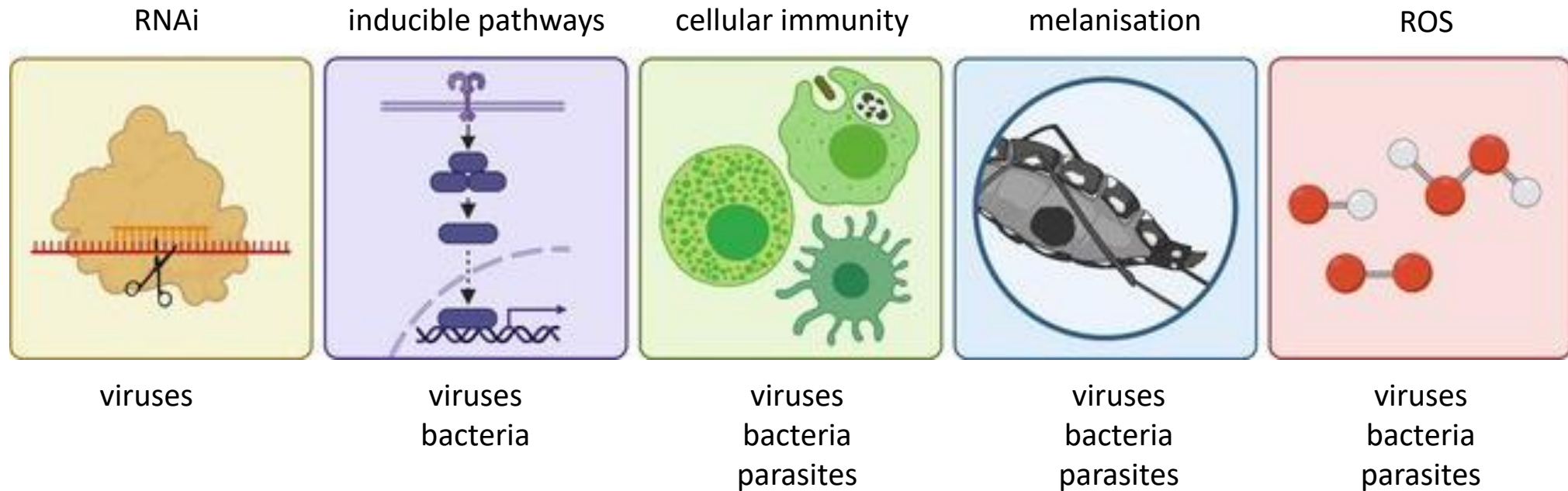
Introduction

Insect immune systems



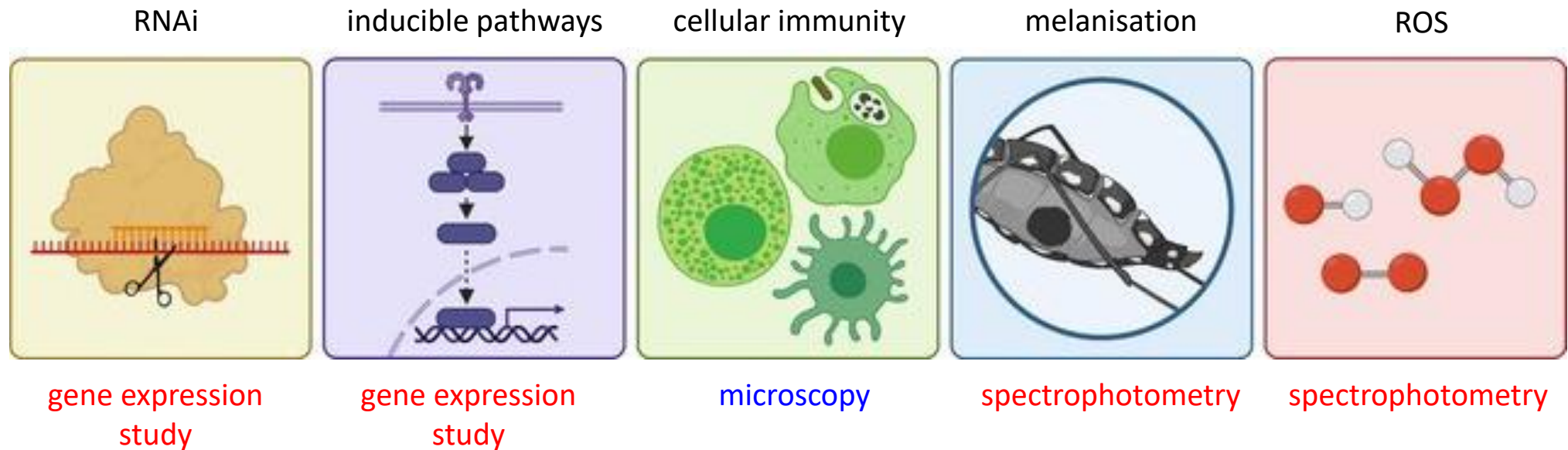
Introduction

Insect immune systems



Introduction

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Introduction

Honey bee immunity studies

122 results from Web of Science Core Collection for:

honeybee or honey bee (All Fields) and **hemocyte or haemocyte** (All Fields)



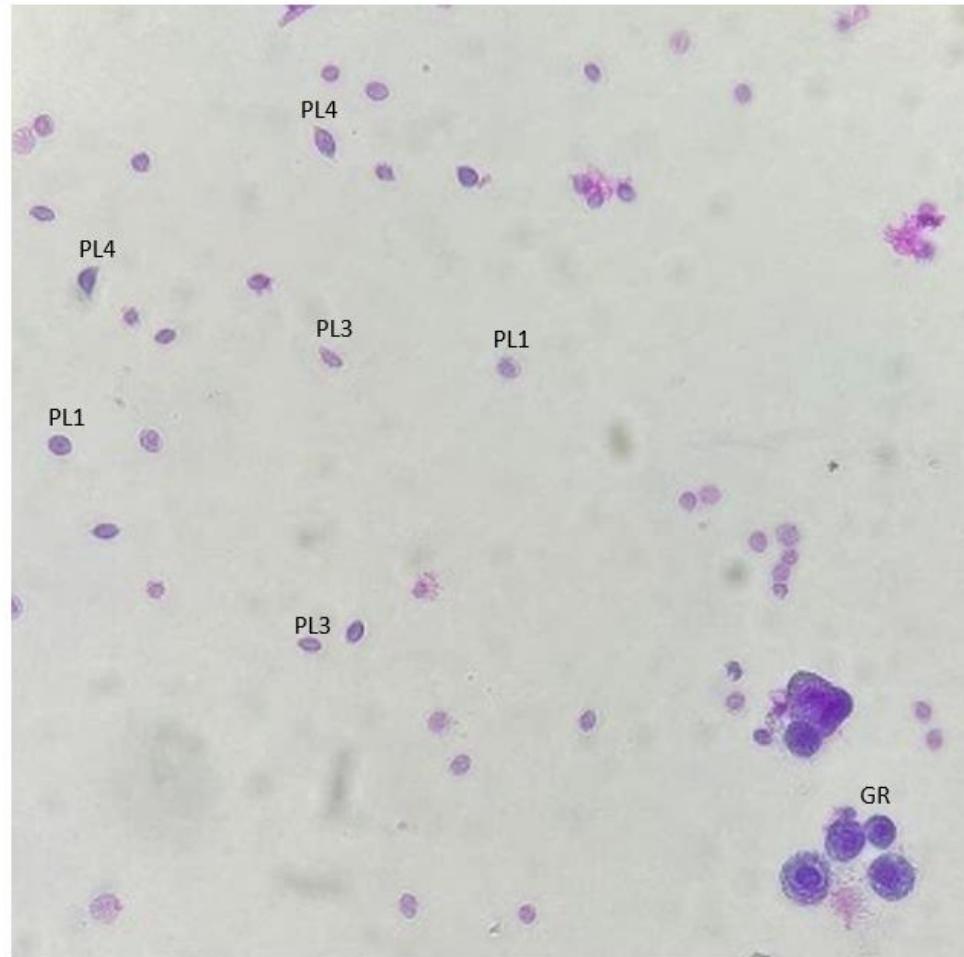
824 results from Web of Science Core Collection for:

honeybee or honey bee (All Fields) and **RNAi or antimicrobial peptide or melanization or ROS** (All Fields)



Introduction

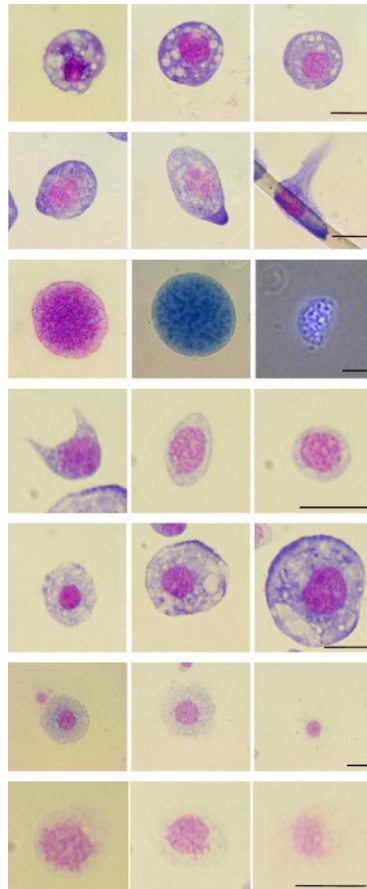
Microscopy of honey bee hemocytes



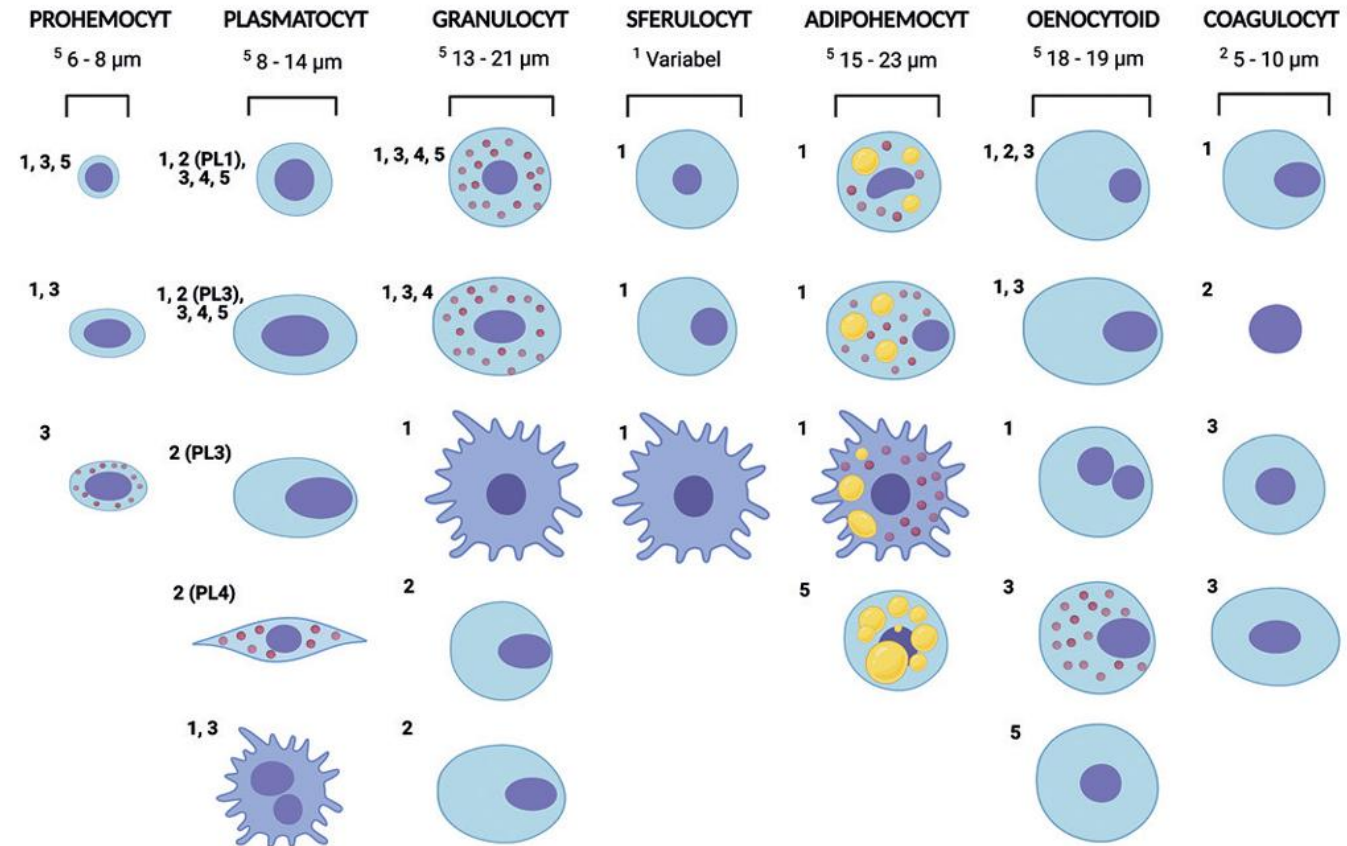
Haemocytes stained with May-Grünwald Giemsa on a blood smear

Introduction

Microscopy of honey bee hemocytes

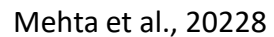


Richardson et al., 2018



Van Herzele et al., 2022

Hemocyte typology based on surface protein profiling

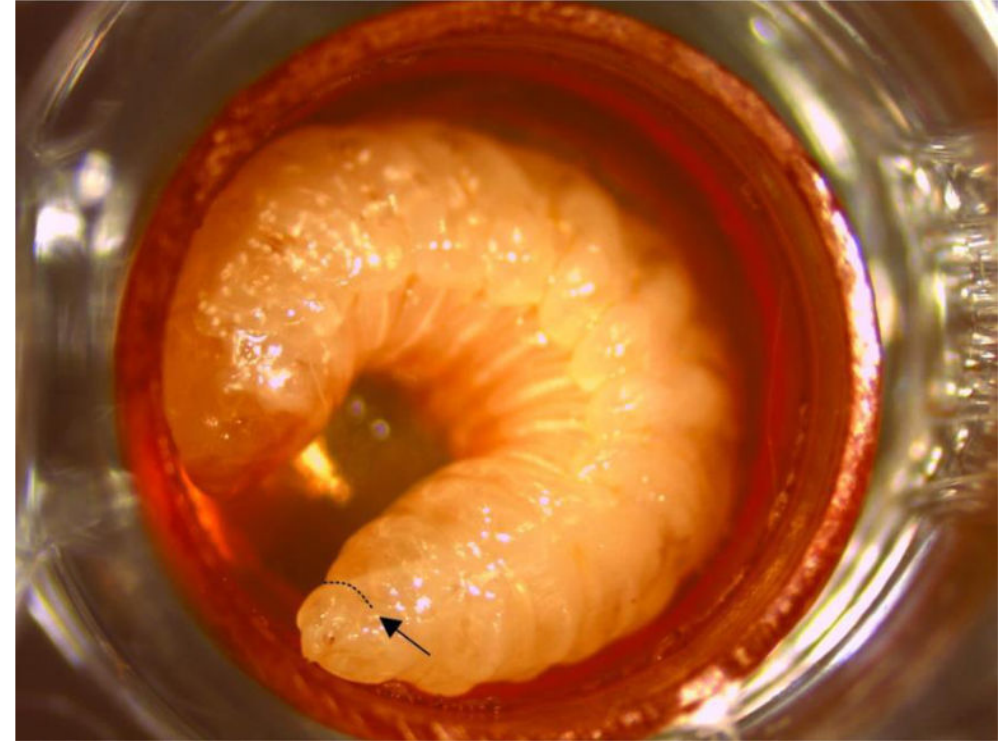


Materials and methods

Hemolymph bleeding



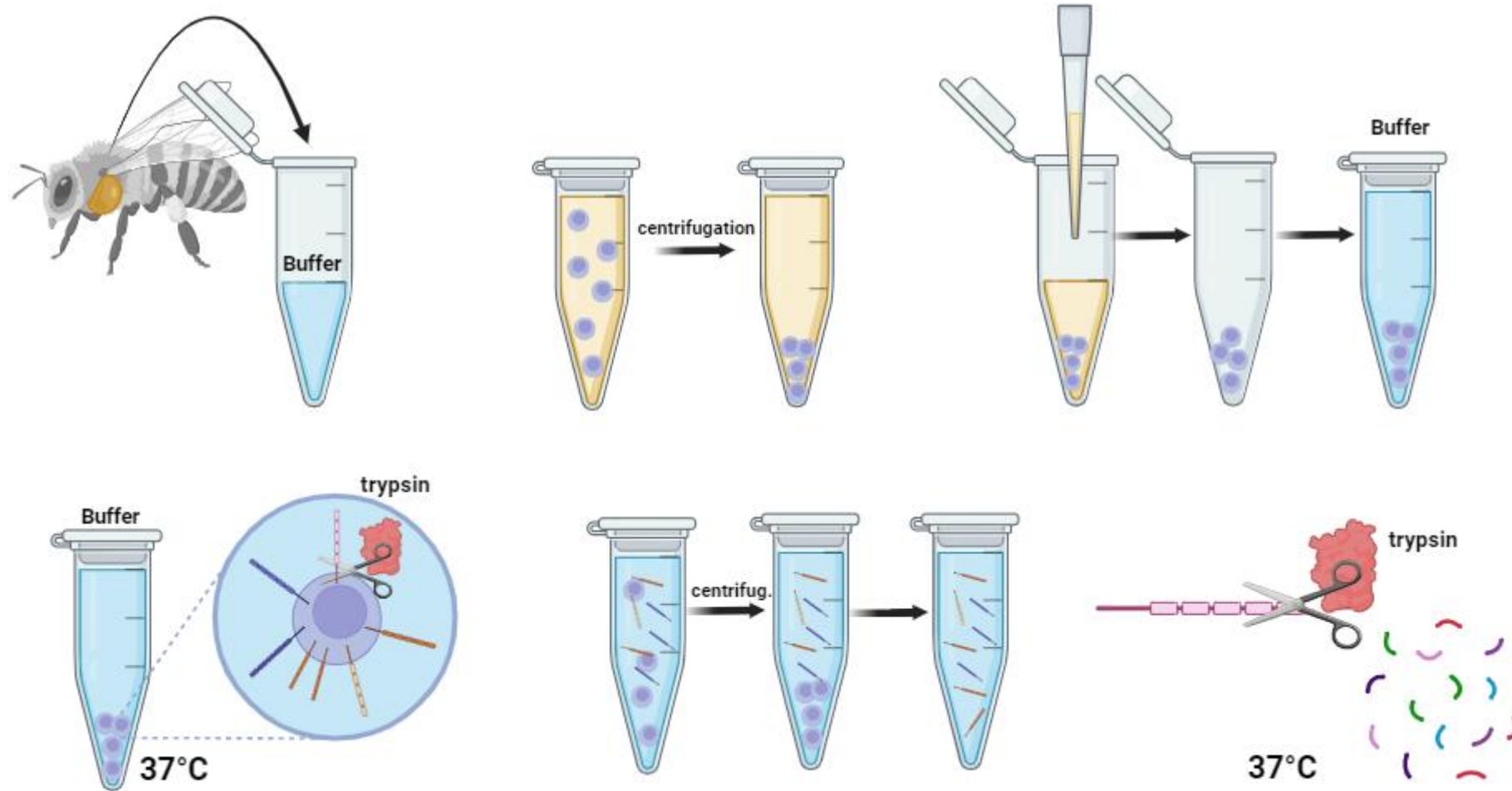
Haemolymph collection from workers bees by wing removal



Haemolymph collection from larvae by making an incision with scissors

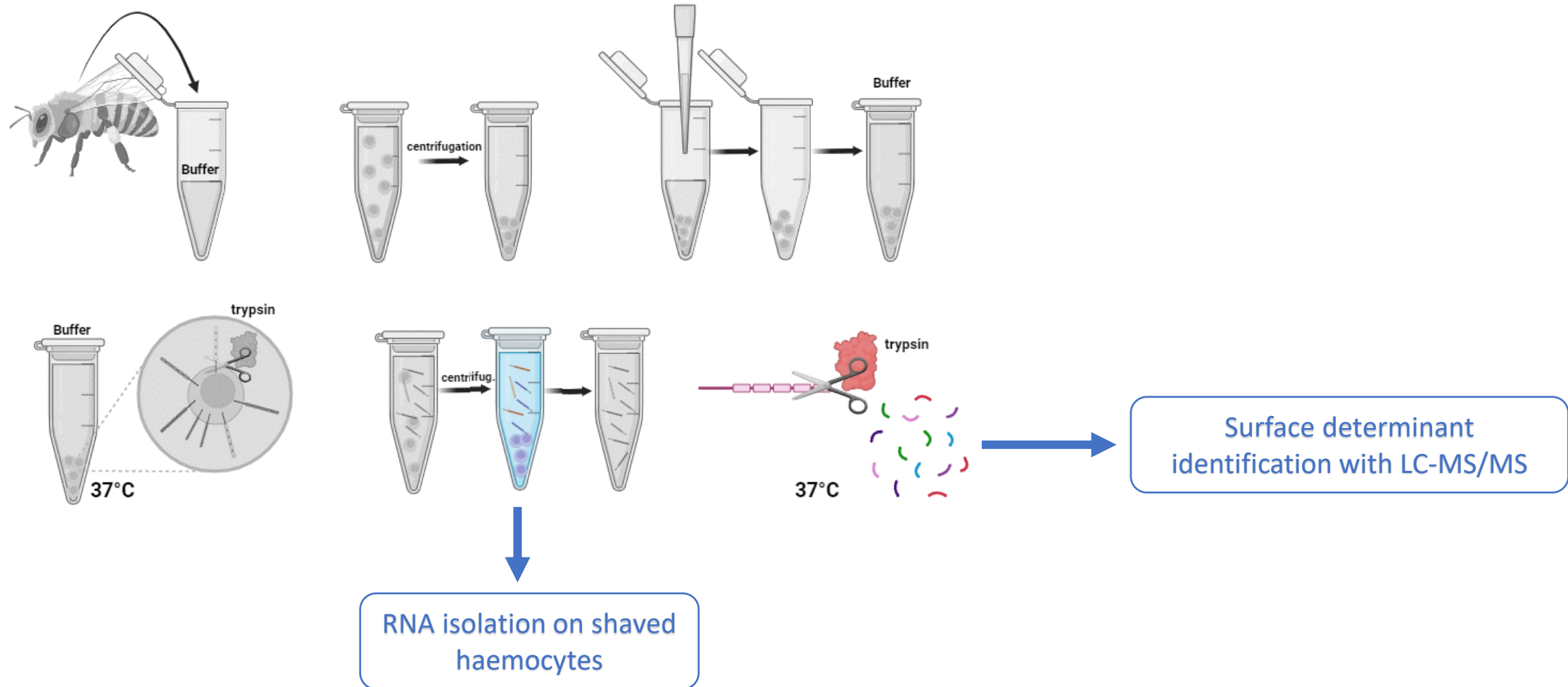
Materials and Methods

Proteomics and transcriptomics protocol



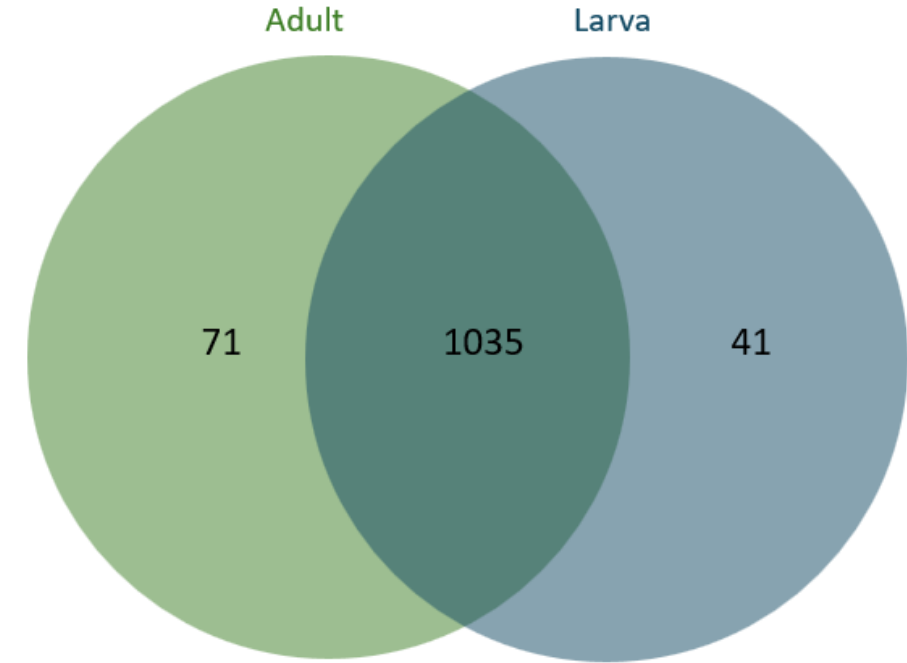
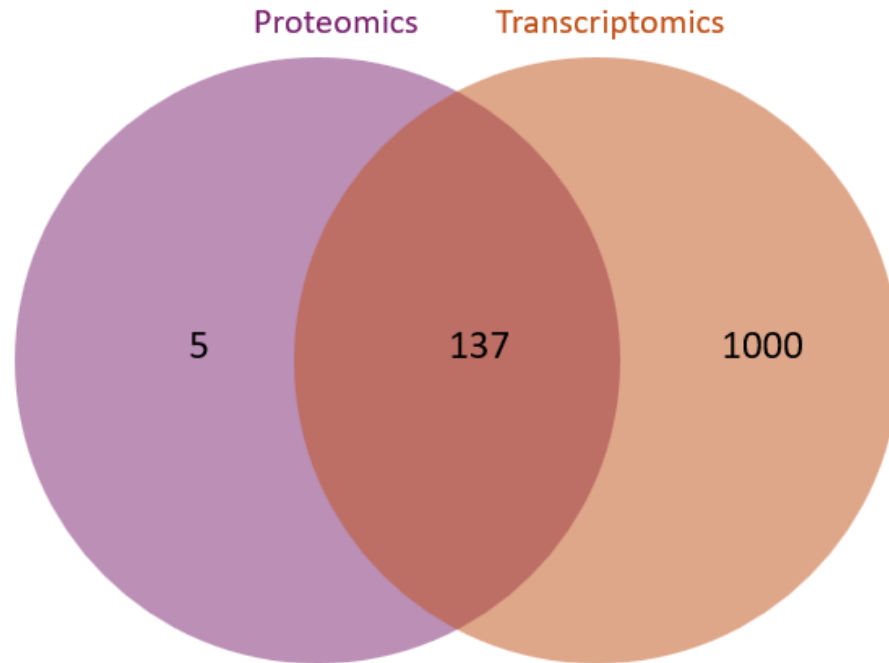
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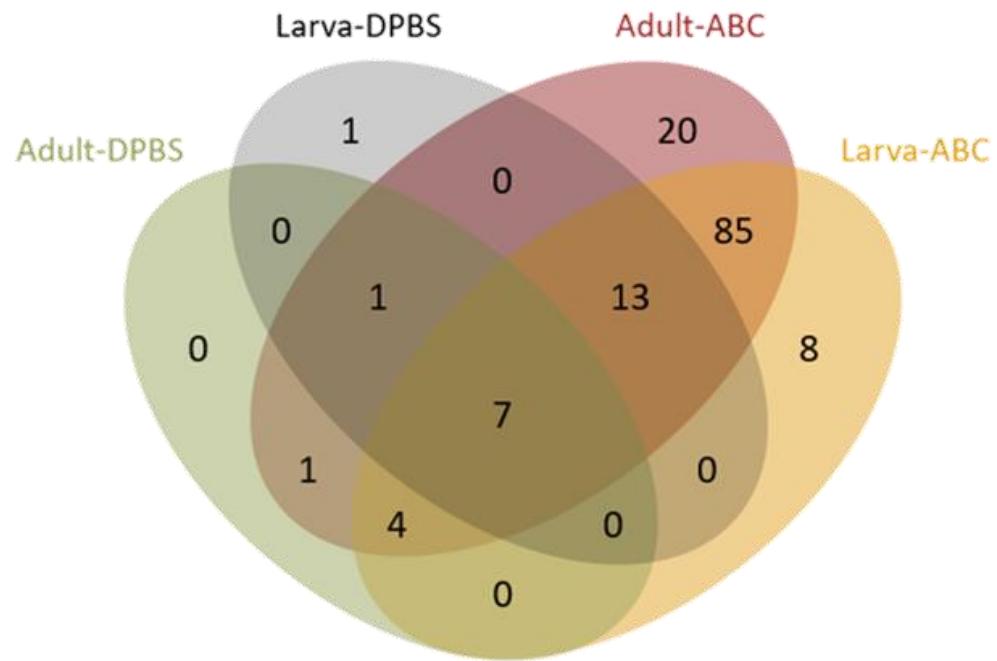
Results

Overall findings

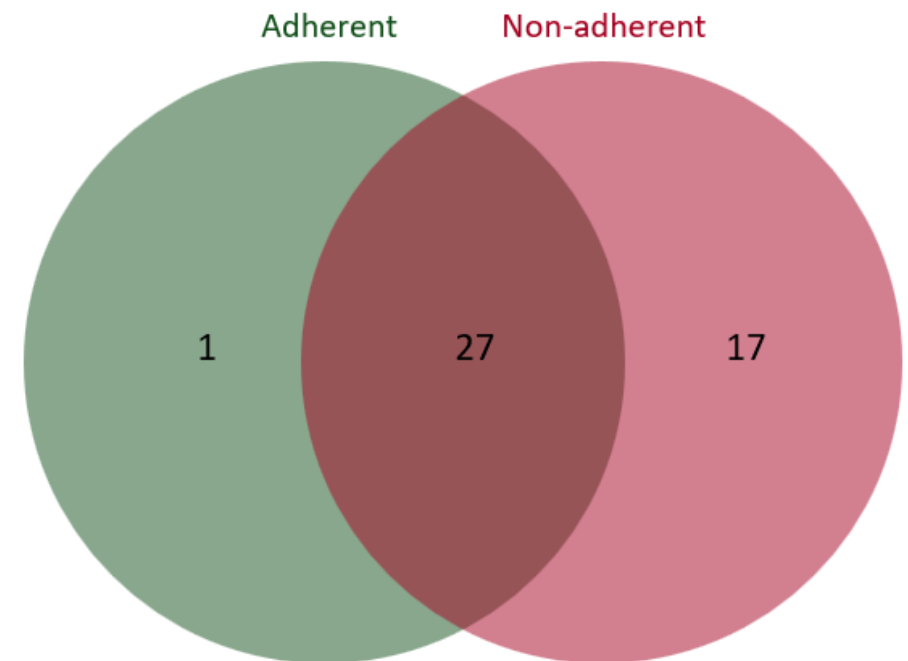


Results

Proteomic approach



Dulbecco's Phosphate-Buffered Saline (DBPS)
Ammonium Bicarbonate Buffer (ABC)



Results

Known surface markers (other invertebrates)

Gene name:	Hemocyte type:
CD9 antigen	Agranulocyte
ninjurin-2	Granulocyte
neprilysin-1	Granulocyte
lysosome-associated membrane glycoprotein 1	Granulocyte
CD151 antigen	Granulocyte
tyrosine-protein kinase transmembrane receptor Ror	Granulocyte-derived cell line
toll-like receptor 7	Granulocyte-derived cell line
dipeptidase 1	Phagocytic hemocytes
facilitated trehalose transporter Tret1	Plasmatocyte-derived cell line
scavenger receptor class B member 1	Plasmatocyte-derived cell line
sodium channel protein Nach	Plasmatocyte-derived cell line
neuroglian	Plasmatocyte
prominin-1-A	Plasmatocyte-derived cell line
integrin beta-PS	Plasmatocyte

Results

Genes encoding immunity-related surface proteins

Gene name:	Immune function:
TNF receptor superfamily member Wengen	JNK signaling
TNF superfamily member 12 eiger	JNK signaling
peptidoglycan-recognition protein LC	Imd pathway
toll-like receptor 4	Toll-pathway
toll-like receptor 7	Toll-pathway
domeless	JAK/STAT signaling
down syndrome cell adhesion molecule 1	Phagocytosis bacteria
croquemort (CD36)	Phagocytosis bacteria

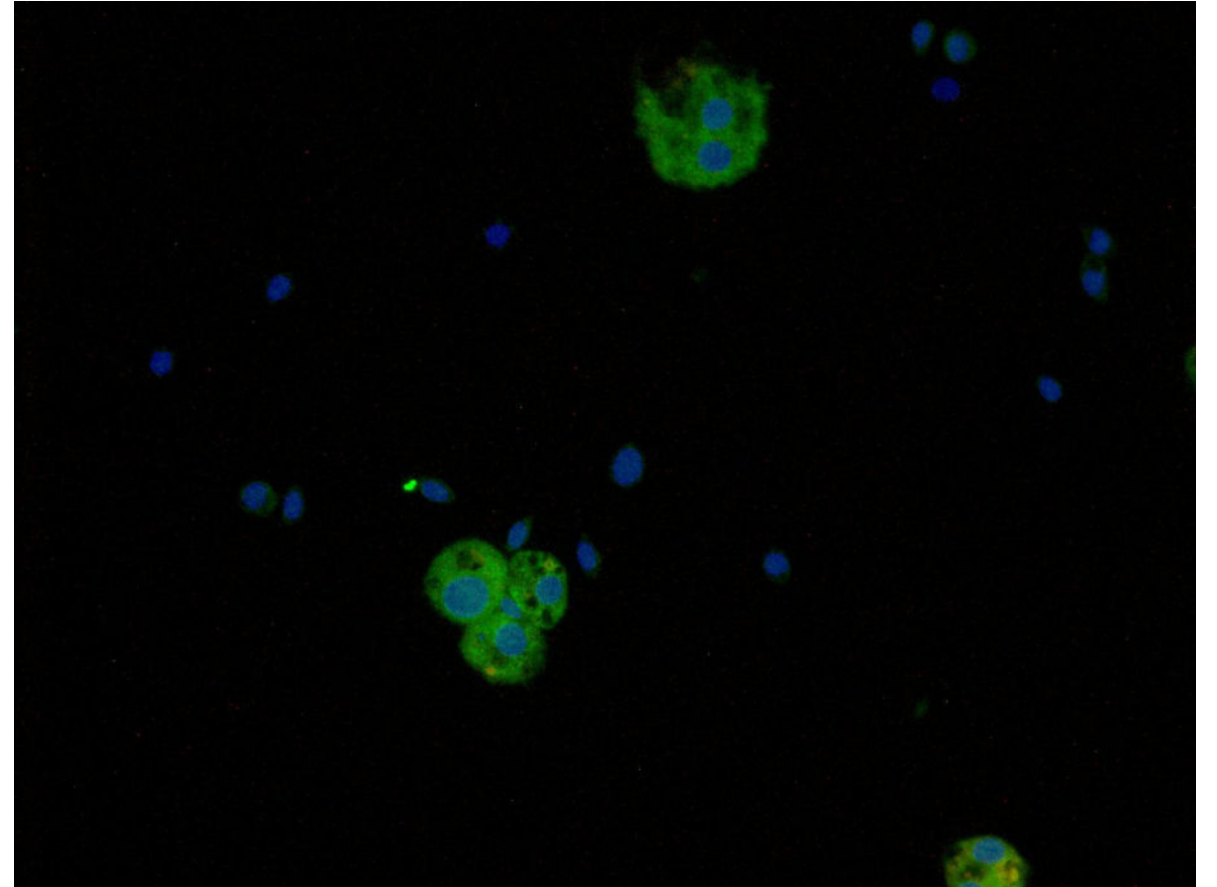
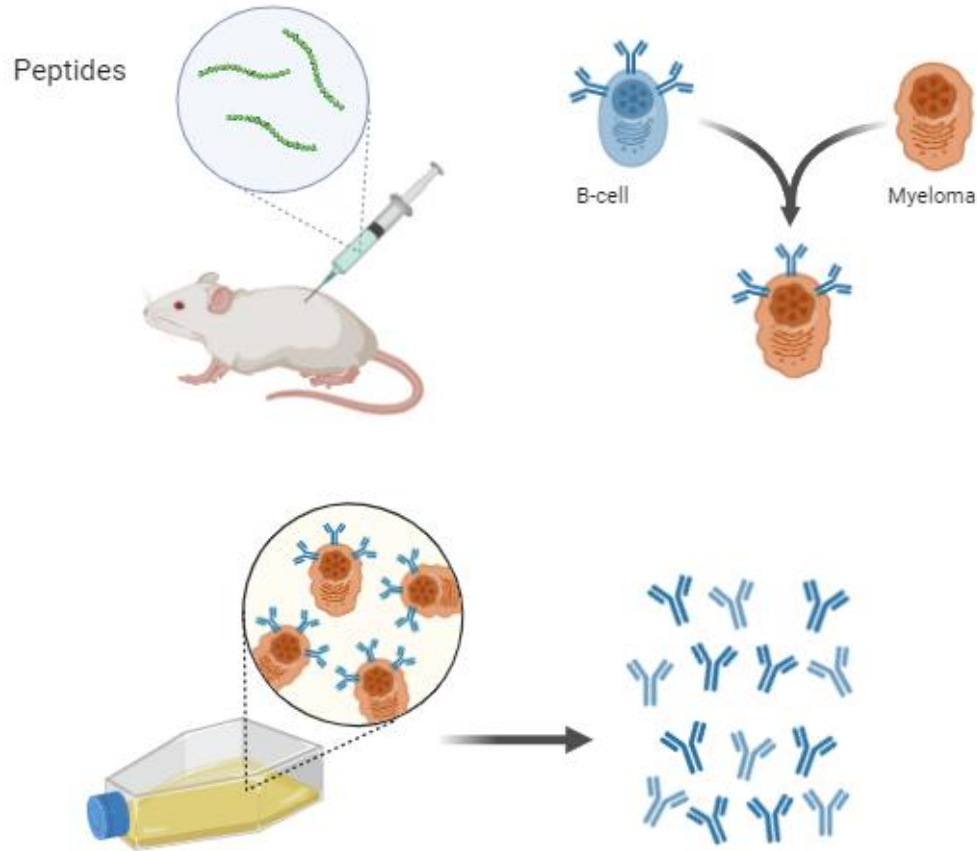
Results

Other interesting genes encoding surface proteins

Gene name:	Function:
anaplastic lymphoma kinase	Hemocyte proliferation
PDGF- and VEGF-receptor related protein	Hemocyte proliferation
innexin inx2	Immune response & apoptosis
tetraspanin-33	Innate immune response
CD63 antigen (552721)	Phagocytosis
sortilin-related receptor	Phagocytosis
transmembrane 9 superfamily member 4	Phagocytosis & cell adhesion
draper	Phagocytosis apoptotic cells, bacteria
plasmolipin	Viral receptor
dyslexia-associated protein	Viral receptor
ferritin light chain homologue	Soluble hemocyte marker
hemolectin	Soluble hemocyte marker

Next steps

Selection of monoclonal antibodies has already started



Conclusions

- First exploration of the honey bee hemocyte surfaceome revealed 1142 IDs using:
 - a proteomic approach by shaving surface determinants and mass-spectrometric identification
 - a transcriptomic approach by RNA sequencing targeting hemocyte mRNA
- We found:
 - markers already described in other invertebrate species
 - genes encoding immune-related surface proteins
 - other interesting genes, among which viral receptors
- This will be a solid foundation for a hemocyte typology using surface markers
- Paper on this has been accepted in *Insect Biochemistry and Molecular Biology*



Thank you for your attention!



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Better-B-project

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