

Protein existence of sperm proteome of *Apis mellifera iberiensis* drones

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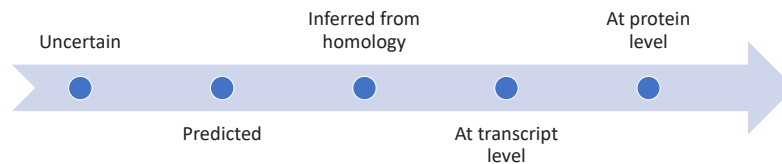
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INTRODUCTION

- The identification and characterization of sperm proteins is essential for a better understanding of their physiological function.
- One way to assess the confidence of the proteins that make up spermatozoa from species that are not widely studied, as honeybees, is to examine the evidence type that supports the protein existence in UniprotKB.

OBJECTIVE

To study the evidence types of protein existence of UniprotKB in sperm of *Apis mellifera iberiensis* adult drones



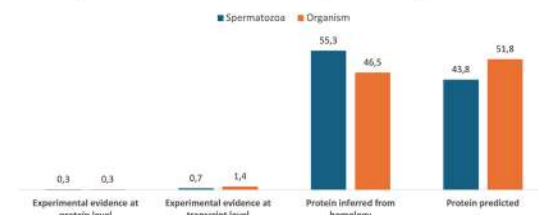
MATERIAL AND METHODS

- 38 ejaculates were obtained from *Apis mellifera iberiensis* adult drones using manual procedures and were pooled in 4 samples of 8-10 drones.
- Semen samples were washed four times in PBS discarding the supernatant and storing the wet pellet.
- Proteome were analyzed in the Proteomics Unit (University of Valencia). The LC-MS/MS was performed in a tims TOF fleX mass spectrometer and data were acquired using the DIA-PASEF mode.
- Results were generated with the unique genes and protein groups quantified with $FDR \leq 1\%$. Protein existence data from the proteome (gene count) of the organism and spermatozoa were obtained from the Uniprot database (UP000005203; 06/02/2025).

RESULTS

- A total of 1,425 proteins (unique genes) were detected, of which 733 were common to all 4 pooled sperm samples, representing 3.8% of the total proteins in the proteome of the organism (19,054).

Percentage of evidence types of protein existence in *Apis mellifera*



CONCLUSIONS

- The low protein evidence rate at protein or transcript level could make functional analysis difficult.
- We have validated, at protein level, a significant number of predicted proteins that will contribute to consolidate the honeybee proteome and facilitate its annotation.

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The identification and characterization of sperm proteins is essential for a better understanding of their physiological function. UniprotKB is one of the most widely used databases for studying of cellular proteome. One way to assess the confidence of the proteins that make up spermatozoa from species that are not widely studied in this database is to examine the evidence type that supports the protein existence. UniprotKB classified five evidence types of protein existence; at protein level, at transcript level, inferred from homology, predicted or uncertain. Thirty-eight ejaculates were obtained from *Apis mellifera iberiensis* adult drones using manual procedures and were pooled in 4 samples of 8-10 drones. Semen samples were washed four times in PBS discarding the supernatant and storing the wet pellet. Proteome were analyzed in the Proteomics Unit (University of Valencia). The LC-MS/MS was performed in a Tims TOF fleX mass spectrometer and data were acquired using the DIA-PASEF mode. Results were generated with the unique genes and protein groups quantified with $FDR \leq 1\%$. Protein existence data from the proteome (gene count) of the organism and spermatozoa were obtained from the Uniprot database (UP000005203; 06/02/2025). A total of 1,425 proteins (unique genes) were detected, of which 733 were common to all 4 pooled sperm samples, representing 3.8% of the total proteins in the proteome of the organism (19,504). Less than 1% of the sperm proteins were classified with experimental evidence at protein (0.3%) or transcript level (0.7%), slightly less than the organism proteome (1.7%). This rate is lower than that observed in studies of other livestock species such as pigs or cattle (> 30% proteins classified for these protein existence classes). The majority of sperm proteins of *A. mellifera* database were inferred from homology (55.3%) or predicted (43.8%). The low protein evidence rate at protein or transcript level could make functional analysis difficult. We have validated, at protein level, a significant number of predicted proteins that will contribute to consolidate the honeybee proteome and facilitate its annotation. This work was supported by the Spanish AEI-MICINN (grant PID2023-148475OB-I00), DGA-FSE (grant A07_23R), Generalitat Valenciana CIAICO/2022/174, PROMETEO/2022/062, AGROALNEXT/2022/063 and NextGenerationEU (PRTR-C17.I1).