









RESEARCH ARTICLE

A queen's tale: Assessing the hidden potential of beeswax specimens in Natural History Museum collections

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Abstract

Background

Natural history museum specimens of historical honeybees have been successfully used to explore the species' genomic past, indicating fast and rapid changes between historical and modern specimens, possibly as a response to current challenges. In our study we explore a potential new untapped archive from natural history collections - specimens of historical beeswax. We examine an intact and closed *Apis mellifera mellifera* queen cell specimen from the 19th century.

Methods

In our study, we examine the queen cell by X-ray Computed Tomography (CT). Subsequently, a micro-destructive approach was used to explore the possibility of protein extraction from the cell for a palaeoproteomic analysis.

Results

Our results to reveal a perfectly preserved queen bee inside her cell. We were successful in extracting proteins from the residual material inside the queen cell, and were able to identify the material as

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containing several bee-related proteins, including major royal jelly proteins (MJRPs).

Conclusions

Our study show that studies on specimens such as the queen cell provide valuable information about the past rearing of queens, their diet, and their development, which is relevant for understanding current honeybees and their challenges.

Plain language summary

This study investigated a 19th-century queen bee cell from a natural history museum to reveal a perfectly preserved queen bee and successfully extracted proteins from the inside of the cell with information about the development and diet of the bee. These findings offer valuable insights into historical bee rearing practices, which can help us better understand and address current challenges faced by honeybees.

Keywords

Apis mellifera, honeybees, queen bee, beeswax, MRJPs, X-ray Computed Tomography, palaeoproteomics, natural history museum collections



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Introduction

Today, natural history museums play a crucial role in advancing scientific knowledge, offering invaluable resources for research into biodiversity, evolution, and climate change. They serve as educational hubs, fostering public understanding and appreciation of the natural world through exhibits and outreach programs. Additionally, these museums are vital for conservation efforts, providing historical baselines to track environmental changes and inform protection strategies for endangered species and habitats.

The western honeybee, *Apis mellifera*, is a eusocial insect vital for its irreparable role in ecology, agriculture and economy (Crane, 1999; Lamei, 2018). Each bee has an important role in the colony, yet there is only one bee without which the entire hive will not survive, the queen, who is also the mother of all bees in the colony (Woodward, 2007). Through her pheromones, the colony maintains its order and functions that reflect the requirements of the colony, e.g. the need for foraging (Crane, 1999; Woodward, 2007).

Natural history museum collections, with their preserved specimens and historical data, can offer critical insights into the evolution, health, and behaviours of honey bee populations over time. By studying these collections, researchers can identify genetic diversity, disease patterns, and environmental impacts, aiding in the development of effective conservation strategies to protect and sustain honey bee populations, which are essential for pollination and ecosystem health (Kasso *et al.*, 2023). There have been studies successful in extracting DNA from museum specimens of bees (Lozier & Cameron, 2009; Mikheyev *et al.*, 2015; Parejo *et al.*, 2020; Strange *et al.*, 2009), which is especially important for observing the decrease in genetic diversity that has occurred in honeybees over time (Espregueira Themudo *et al.*, 2020). As an alternative to ancient DNA, palaeoproteomics explores ancient proteins that could be used to explore questions relating to paleogenomics and paleomicrobiology (Hendy *et al.*, 2018), while also being more likely to preserve than DNA.

Human led queen rearing (i.e. raising and replacing) has been a prominent practice in beekeeping ever since the 19th century, as the quality of the queen significantly affects the whole hive, including its reproductivity and resistance to disease (Crane, 1999; Woodward, 2007). In Denmark, the bees currently used in beekeeping are a developed hybrid species from 3–4 subspecies of *Apis mellifera*, which has replaced the native bee to the area, *Apis mellifera mellifera*, the European Dark bee (i.e. *den brune bi*), that has almost disappeared from Denmark (Nielsdatter *et al.*, 2021).

In this study, we investigated a queen cell specimen from the Natural History Museum of Denmark collections, which holds several historical honeycomb specimens. This specimen (Figure 1) is formed of two queen cells, with one cell still capped (Figure 2), therefore with the possibility of having an undisclosed queen inside. Therefore, the specimen was studied

with X-ray Computed Tomography (CT) to non-destructively image the interior of the cell and micro-destructive palaeoproteomics was applied to study the material inside the cell. If the queen was present, proteomics would aid in gaining information about the queen and the material surrounding her.

An experimental study to extract proteins directly from historical beeswax was also conducted; due to limited results it is available as Extended Data (ED) (See Data Availability, ED 1).

Methods

The queen cell

The specimen used in this study was made accessible to us thanks to Lars Vilhelmsen from the Natural History Museum of Denmark. The specimen is estimated to be from the late 19th century, based on the hand-written label (Figure 1b) of the sample, which reads “*Apis mellifica* L 2 Dronningcell Ulvedal 7.88 Borries M 8.4.89”. “8.4.89” could be interpreted as the date in 1889 when the specimen was collected or recorded by the museum, or it could simply be a catalogue number. Ulvedal could be Ulvedalen in Denmark and Borries the name of the beekeeper - yet no historical archival information was found to support this, so we must leave this to speculation. *Apis mellifica* L indicates European dark bees, which today as a honeybee subspecies is rare in Denmark due to its displacement by other subspecies and hybrids, such as the Italian *Apis mellifera ligustica* which was introduced to Denmark ca. 1860 (Nielsdatter *et al.*, 2021).

X-ray computed tomography

The CT-scanning was performed at the 3D Imaging Centre at DTU using a ZEISS Xradia 410 Versa micro CT, which allows a resolution of a few micrometres and typical sample sizes from millimetres to a few centimetres. The maximum power of the instrument is 10 W, and the energy of the employed X-rays can be varied in the range 40–150 keV to optimise contrast that allows for the separation and configuration of different materials, such as the chitinous insect from the lipidic beeswax. The sample was scanned without a filter using 40 kV and 6.6 W, the large field of view objective and a 2x2 detector binning. 3201 projections were acquired with an exposure time of 2 s per projection, leading to a full scanning time of 3 hours. The reconstructed voxel size was 40.6 µm which results in an approximate resolution of 100 µm. During the scanning, only the 2D images of the X-rays travelling through the full sample, which is rotated 360 degrees, are visible for the different angles, but it was not possible to see the queen. However, the reconstruction of the 3D volume and the subsequent visualisation using the ThermoFisher Avizo software (<https://www.thermofisher.com/dk/en/home/electron-microscopy/products/software-em-3d-vis/avizo-software.html>) allowed the bee to be seen and to segment different components of the image in different colours. Similar analysis and visualisation can be performed with the software tool ITK-SNAP (www.itksnap.org) (Yushkevich *et al.*, 2006).

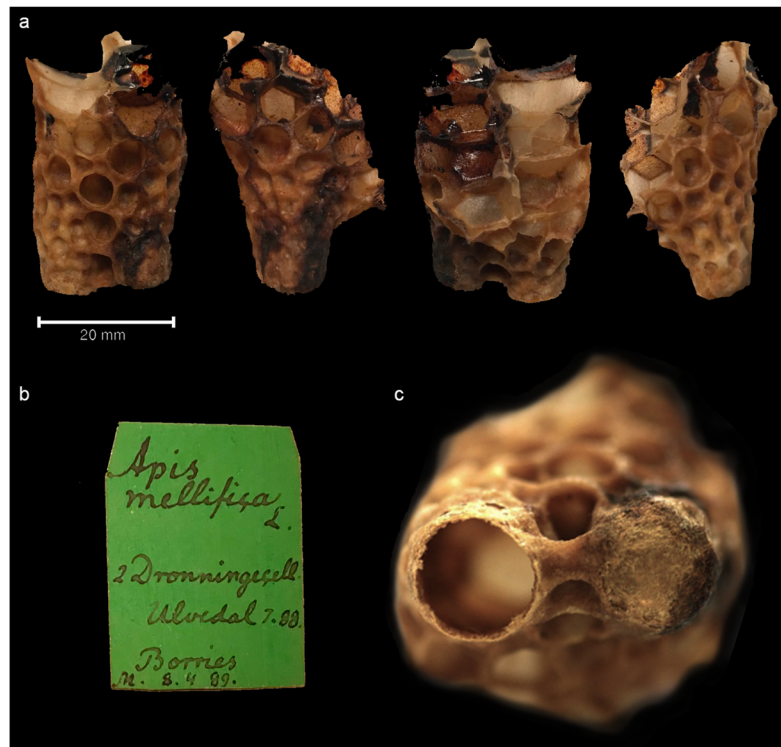


Figure 1. The queen bee cell specimen from all sides. Figure 1a. Piece of honeycomb with queen cell specimen shown from all sides. Figure 1b. Museum label of the specimen. Figure 1c. Close-up of top of the cell (far right): the left one is open and possibly unused, the right one is capped.

Palaeoproteomics

From the reconstructed 3D image obtained from X-ray CT, a layer of material can be seen beneath the queen bee (see Figure 2B). We assumed that this substance was likely royal jelly and/or faecal or other deposited material from the bee: the meconium i.e. the gut contents. It was decided to experiment with extracting this material for ancient proteins to learn about this specimen, as it was considered unethical to sample the queen itself. Accessing the queen would have required opening the cell and this would risk damaging or destroying the specimen irreplaceably, posing limitations for future research to study the morphology of the bee and its genetic material.

For the micro-destructive extraction of proteins from the queen cell, 100µl extraction buffer injected through the wall of the capped cell near the unknown material, using a syringe with a 1mm diameter needle. The extraction buffer was prepared (75:5:4:8:8) with 8M guanidine hydrochloride (Sigma-Aldrich cat. no. G7294), 1M Tris (Invitrogen™ cat. no. 10055704), 0.5M tris(2-carboxyethyl)phosphine) (Sigma-Aldrich cat. no. 646547), 0.5M chloroacetamide (Sigma-Aldrich cat. no. 22790), and ultra-pure H₂O (AccuGENE™ Molecular Biology Water cat. no. 7732-18-5). It was delicately partially aspirated and dispensed in and out of the cell two times. After this careful resuspension, the extraction buffer was fully

aspirated and placed into a 1.5 mL Protein LoBind tube (Eppendorf cat. no. 022431081). An immediate observation was a colour change of the buffer from transparent to translucent dark brown, indicating that the extraction was likely to be successful in retrieving sample material from inside the cell. The following analysis was performed according to best practice for palaeoproteomic samples to limit modern contamination, including the use of a dedicated laboratory space and nitrile gloves (Hendy *et al.*, 2018). A blank extraction was also performed to control for laboratory contamination. The sample and blank were first incubated at 80°C for 1h. Protein quantification was made using bicinchoninic acid (BCA) assay Pierce™ BCA Protein Assay Kits cat. no. 23225. This confirmed that a sufficient quantity of protein was extracted and available for downstream processing. The sample was then digested first with 1µl (0.4µg/µL) of Lys-C (Promega cat. no. V1671) at 37°C for 1h, followed by digestion with 1µl (0.4µg/µL) trypsin (Promega cat. no. V5111) overnight at 37°C. The purification of peptides was subsequently performed via StageTips (Rappsilber *et al.*, 2007). The peptides were eluted in 30 µL of 50% acetonitrile (ACN, Thermo Fisher Scientific/Pierce cat. no. 51101) 0.1% trifluoroacetic acid (TFA, Sigma-Aldrich cat. no. T6508).

The samples were analysed using liquid chromatography tandem mass spectrometry (LC-MS/MS) using an EASY nLC

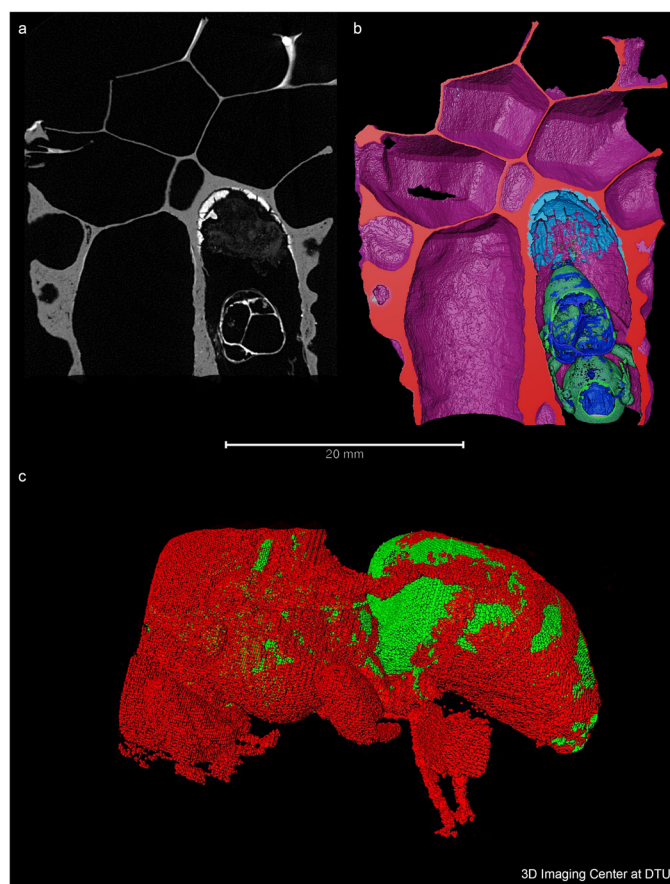


Figure 2. A 2D, 3D volumetric and 3D representation of the bee. Figure 2a. A 2D slice showing one plane of the 3D volume obtained by X-ray CT. The bee is located in the bottom right cell and the residual layer appears bright on the top of this cell, meaning that it has a higher density or is composed of materials with higher atomic number than the material in the darker areas. This plane is used to virtually cut the volume which enables the view shown in Figure 2b. Figure 2b. A 3D volumetric representation, colour coded according to the identity of the different materials. Note the residual layer (illustrated in light blue) at the bottom of the cell. Figure 2c. 3D representation of the bee isolated from the wax.

1200 (Proxeon/Thermo Fisher Scientific) coupled to an Exploris 480 mass spectrometer (Thermo Fisher Scientific), based on methods already published for historical samples (Mackie *et al.*, 2018), outlined as follows. The elutions were vacuum centrifuged at 45°C until approximately 5 μ L of sample remained. Samples were then resuspended in 8 μ L 5% ACN 0.1% TFA in water. 2 μ L of each sample was injected for measurement. The samples were separated on an in-house laser-pulled 15 cm column (75 μ m inner diameter, Polymicro Technologies cat. no. TSP075375) and packed with 1.9 μ m C18 beads (ReproSil Pur 120 C18-AQ, Dr. Maischcat. no. r119.aq) over a 77 min gradient with increasing ACN in 0.1% formic acid (FA, Merck/Supelco cat. no. 5330020050). In short, the MS parameters were as follows: MS1- scan range of 350–1400 m/z, 120k resolution, maximum injection time (IT) of 25 ms, and an AGC target of 300% in Top 10 mode. MS2- 60k resolution, maximum IT of 118 ms, minimum intensity 2e5, AGC target 200%, normalised collision energy of 30%, a dynamic exclusion of 20 s, and an isolation window of 1.2 m/z. To

hinder cross-contamination, a wash-blank method using 0.1% TFA, and 5% ACN was run in between each sample.

The raw MS/MS data was analysed in two steps. Firstly, a primary screening was made using MaxQuant (v.1.6.3.4, RRID:SCR_014485) (Cox & Mann, 2008), with a tryptic search of the Swiss-Prot database (downloaded 10/02/22, RRID:SCR_021164) to determine the possible sources of protein in the sample. Search parameters were the defaults for an Orbitrap mass spectrometer. Modifications were as follows: fixed carbamidomethylation of cysteine; variable oxidation of methionine, deamidation of asparagine and glutamine, and pyroglutamic acid of glutamine and glutamic acid. Error tolerances were 10 ppm for the precursor and 0.02 Da for the fragment ions, and the false discovery rate (FDR) was set to 1%. Minimum score cut-off was 60. Identifications from this search led to further searches of the Uniprot (RRID:SCR_002380) honey bee proteome (unspecific digestion search), and a search each for the known Uniprot proteins from *Aspergillus*

and *Penicillium* species (trypsin specific), followed by more specific species proteomes with an unspecific digestion search.

All hits from these search paths were then combined into a final database and searched with the same modifications as above but with semi-specific trypsin specificity (max peptide length of 8–25 amino acids), based on the results of the previous searches. The discovered proteins and peptides were then authenticated and identified: proteins with only one peptide detected in the entire dataset were discarded, as well as contaminants clearly deriving from the laboratory process, such as those present in the extraction blank, and from handling, such as keratins. Peptide species specificity was determined by using pBLAST (Altschul *et al.*, 1990) (RRID:SCR_004870). Deamidation was assessed using publicly available code (<https://github.com/dblyon/deamidation>) (Mackie *et al.*, 2018).

In order to examine other post-translational modifications (PTMs), another search was made with this database using PEAKS (version 7.5, RRID:SCR_022841), to utilise the PEAKS PTM module (Han *et al.*, 2011) to find unspecified modifications. The results of this search identified N-glycosylation sites, specifically N-Acetylhexosamine, which were confirmed

with another MaxQuant search with this as a variable modification.

Results and discussion

X-ray CT

The CT investigation successfully resulted in locating the queen and 3D images were generated of the bee that looks well developed (Figure 2). The formation stages of the queen bee from a *larvae* to a queen are well known (Woodward, 2007), and the ready queen usually emerges from the cell around the 16th day. As both the development and hatching of the bee needs a temperature of 35°C (Woodward, 2007) maintained by the worker bees, removing this cell from the beehive and keeping it at a lower temperature likely terminated the development of the queen. However, any indications of present pathogens that may also have caused disease and impacted the queen, was considered during the proteomic data analysis.

Palaeoproteomics

We successfully identified 120 proteins from the encapsulated cell sample (ED 2), with the most relevant groups (Figure 3) being bee-related proteins, including major royal jelly proteins (MRJPs) and silk fibroin proteins, as well as proteins from

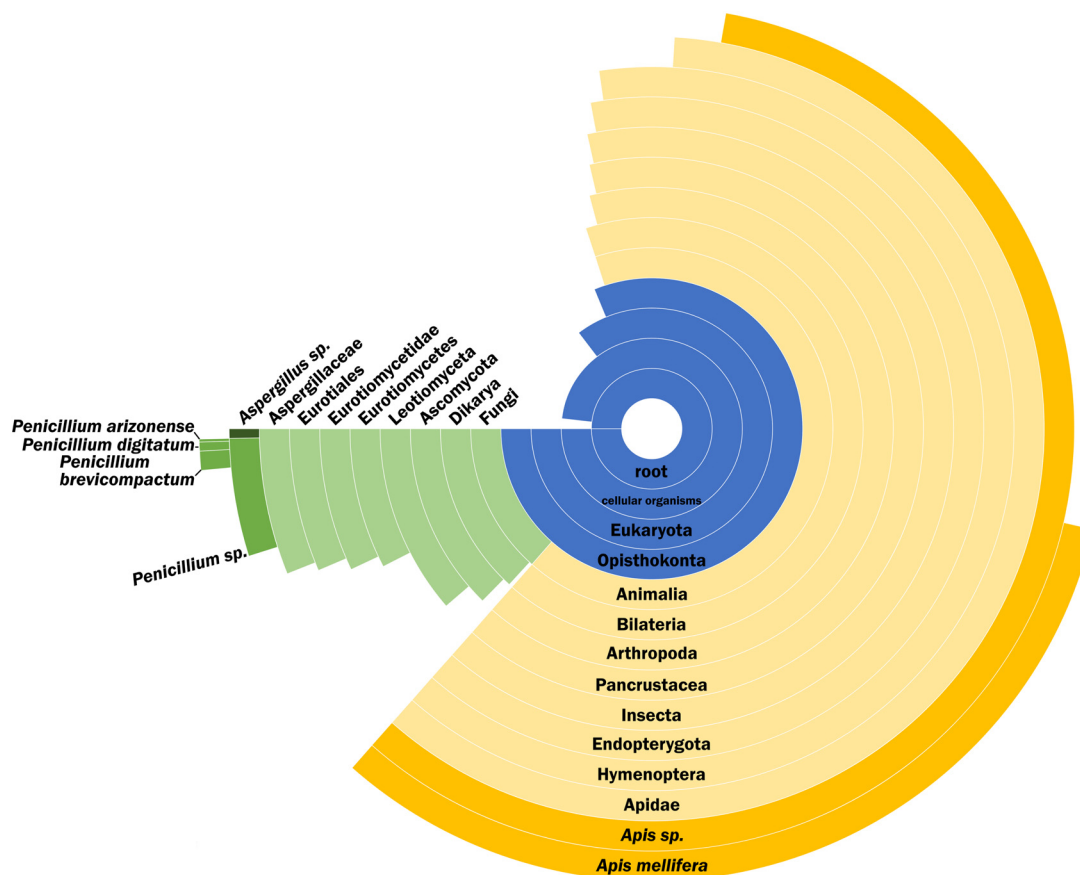


Figure 3. Peptide Species Specificity distribution of the sample. Peptide Species Specificity distribution of the sample after removal of lab reagent contamination and proteins reported with only one peptide. Percentage of the circle of each group indicates the percentage of peptides specific to that group.

Penicillium and *Aspergillus* species. During the development of the bee, the larvae makes a silk cocoon for itself, covering the cells (Hepburn *et al.*, 2013; Micas *et al.*, 2016). There are four different types of bee silk proteins (Micas *et al.*, 2016), and we identified all of them in our data.

There is no inherent difference between the eggs which develop into workers and queens. The only difference is the nutrition provided, with the prospective queens fed royal jelly (Buttstedt *et al.*, 2014; Woodward, 2007), which is more nutritious than the standard worker fare. Royal jelly (or 'bee milk') is high in both protein and carbohydrates and triggers the queen's development; its quality is critical for this event, despite the actual biological mechanism behind it not being completely understood (Buttstedt *et al.*, 2014; Crane, 1999; Woodward, 2007). MRJPs form up to 15% of the royal jelly that is fed to the queen during its development and its life (Buttstedt *et al.*, 2014; Woodward, 2007). We were able to identify five of the nine MRJPs known (MRJP 1, 2, 3, 5 and 7), and these are also the most abundant ones in royal jelly (Buttstedt *et al.*, 2014), explaining the better coverage of them in our sample.

In addition to its nutritional value, royal jelly is considered to be both antifungal and antimicrobial (Shen *et al.*, 2012). Some *Apis* specific enzymes found were Defensin-1 and glucose oxidase. Defensin-1 is found in royal jelly (Bucekova *et al.*, 2017), and in low concentrations it acts as a mechanism against gram-positive bacteria, e.g. *Paenibacillus larvae*, the cause of American Foulbrood disease (Ryba *et al.*, 2009; Shen *et al.*, 2012). Related to antimicrobial properties, we were also able to detect N-Acetylhexosamine glycosylation at several sites in MRJPs 1 and 2 (ED 3) three of which have been predicted to occur from sequence analysis (see UniProt Knowledgebase, <https://www.uniprot.org>, for these proteins). In addition, there was spectral evidence for a previously unrecorded hexose modification site on MJRP3. Some other sites were detected with varying confidence (ED 3), and more research needs to be done to confirm their presence. Their presence is important here because this modification has shown to give these proteins antimicrobial effects (Bíliková *et al.*, 2009; Mureşan *et al.*, 2022). In MRJPs, it is also related to metabolic activities important for the high metabolic fuel demands of an egg-laying queen (Zhang *et al.*, 2014). This modification also may increase the stability of the proteins (Lis & Sharon, 1993), which may explain why it has been found in modern and ancient bones, as well as ancient eggshell (Cleland, 2018; Demarchi *et al.*, 2016; Schroeter, 2024).

The microbial environment of the hive is a delicate balance that protects the bees against pathogens, including fungi. The fungi *Penicillium* and *Aspergillus* are present in the natural environment, therefore also present in the hive as well. *Penicillium* and *Aspergillus* species also thrive indoors, and are associated with dust and/or biodeterioration of museum pieces,

therefore, they could originate from the sample being from a (natural) museum environment. In fact, some of the specific species detected have been detected in indoor Danish dust (Andersen *et al.*, 2021; Frisvad & Gravesen, 1994), although it is obviously not specific to Denmark. However, dust contamination is somewhat unlikely as the cell itself was closed, and the area outside cleaned prior to perforating the wall of the specimen for extraction. It is possible that the fungi proteins are endogenous to the sample, as honeybees also collect fungal spores for nutrition (Parish *et al.*, 2020). Therefore, another advantage of using closed cells in future biomolecular studies of historical bees is the isolated environment that may reflect a pristine proxy for the original developmental environment of the queen bee.

In addition, the deamidation rate tells us about the preservation and the relative age of the proteins from the samples. This modification occurs to the amino acids asparagine (N) and glutamine (Q) over time. Older, and therefore more likely to be endogenous proteins, should have higher deamidation. The contamination present (mostly human keratins) has much lower rates of deamidation than those relating to the actual sample, supporting that the proteins from the actual sample are endogenous to the queen cell (ED 4). It is also of note that the fungi and bee samples seem equally damaged, despite the bee samples appearing to have more unspecific hydrolytic cleavage (ED 4). This cleavage also occurs over time with the breakdown of proteins due to hydrolysis, and is associated with older samples (Cappellini *et al.*, 2018; Schroeter & Cleland, 2016). The similar deamidation levels could indicate that the fungal and bee proteins are relatively the same age, therefore the fungi proteins could be from an earlier source than dust contamination. However, it really only shows that they are similarly damaged, as deamidation cannot really be used as an age indicator (Ramsøe *et al.*, 2021; Schroeter & Cleland, 2016).

Conclusions

With the help of X-ray CT, a bee and the residue of its developmental environment were identified in a closed cell. This allowed us to carefully identify and place the optimum sampling location for maximum information with as little intervention to the bee as possible through micro-destructive sampling. We were successful in extracting proteins from the residual material inside the queen cell, and palaeoproteomics was able to identify the material as containing several bee related proteins, including major royal jelly proteins (MJRPs). The MJRPs and defensive enzymes are interesting from the perspective of the development and nutrition of the queen, in addition to the protective microbiome of the hive against pathogens. The detection of glycosylation sites informs on antimicrobial properties of MRJPs in the past. The source of the fungi *Penicillium* and *Aspergillus* is unknown due to its multiple possible sources of origin, either as dust contamination or collected by the bees themselves.

The aim of the micro-destructive sampling strategy was to maximise the information retrieved from this sample without damaging the queen to preserve the specimen for future studies. Discovering and imaging the fully preserved queen in conjunction with the proteomics data will allow comparisons with modern samples. These studies could inform on changes in queen development over time, such as possible changes and developments in major royal jelly proteins.

This experimental study of a queen cell from the Natural History Museum of Denmark brings forward the profound potential these collections hold. This small study not only illuminates aspects of the queen bee's diet and hive conditions but also demonstrates how such preserved specimens can serve as time capsules, providing invaluable data for future scientific research. In contrast to historical beeswax specimens, which we attempted unsatisfactorily (ED 1), closed cells are an excellent source of proteins and potentially other biomolecules. Their preservation and isolation from the environment render these samples the most promising for future studies on historical bees, and could be a future direction or application for this type of targeted sampling.

Ethics and consent

Ethical approval and consent were not required.

Data availability

Underlying data

EMBL-EBI :PRIDE database: Assessing the hidden potential of beeswax specimens in Natural History Museum collections.

The underlying data has been deposited in the ProteomeXchange Consortium via the PRIDE partner repository, accession number PXD034106: <https://www.ebi.ac.uk/pride/archive/projects/PXD034106> (Mackie, 2024).

Data are available under the terms of the Creative Commons Public Domain license (CC0).

Extended data

EMBL-EBI :PRIDE database: Assessing the hidden potential of beeswax specimens in Natural History Museum collections. <https://www.ebi.ac.uk/pride/archive/projects/PXD034106> (Mackie, 2024).

This project contains the following extended data:

- Extended_Data_2_Queen_Cell_protein_tables.xlsx
- Extended_Data_1-3-4.pdf

Data are available under the terms of the Creative Commons Public Domain license (CC0).

References

- Altschul SF, Gish W, Miller W, et al.: **Basic local alignment search tool.** *J Mol Biol.* 1990; **215**(3): 403–410.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Andersen B, Frisvad JC, Dunn RR, et al.: **A pilot study on baseline fungi and moisture indicator fungi in Danish homes.** *J Fungi (Basel).* 2021; **7**(2): 71.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bíliková K, Mirgorodskaya E, Bukovská G, et al.: **Towards functional proteomics of minority component of honeybee Royal Jelly: the effect of post-translational modifications on the antimicrobial activity of apalbumin2.** *Proteomics.* 2009; **9**(8): 2131–2138.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Bucekova M, Sojka M, Valachova I, et al.: **Bee-derived antibacterial peptide, defensin-1, promotes wound re-epithelialisation *in vitro* and *in vivo*.** *Sci Rep.* 2017; **7**(1): 7340.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Buttstedt A, Moritz RFA, Eler S: **Origin and function of the Major Royal Jelly Proteins of the honeybee (*Apis mellifera*) as members of the yellow gene family.** *Biol Rev Camb Philos Soc.* 2014; **89**(2): 255–69.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Cappellini E, Prohaska A, Racimo F, et al.: **Ancient biomolecules and evolutionary inference.** *Annu Rev Biochem.* 2018; **87**(1): 1029–1060.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Cleland TP: **Solid digestion of demineralized bone as a method to access potentially insoluble proteins and post-translational modifications.** *J Proteome Res.* 2018; **17**(1): 536–542.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Crane E: **The world history of beekeeping and honey hunting.** Routledge, Inc. Abingdon, Oxford, 1999.
[Reference Source](#)
- Cox J, Mann M: **MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification.** *Nat Biotechnol.* 2008; **26**(12): 1367–1372.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Demarchi B, Hall S, Roncal-Herrero T, et al.: **Protein sequences bound to mineral surfaces persist into deep time.** *eLife.* 2016; **5**: e17092.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Espregueira Themudo G, Rey-Iglesia A, Tascón LR, et al.: **Declining genetic diversity of European honeybees along the twentieth century.** *Sci Rep.* 2020; **10**(1): 10520.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Frisvad JC, Gravesen S: ***Penicillium* and *Aspergillus* from Danish homes and working places with indoor air problems. Identification and mycotoxin determination.** In: *Health Implications of Fungi in Indoor Environments: Air Quality Monographs.* Pergamon Press, London, England, 1994; **2**: 281–290.
[Reference Source](#)
- Han X, He L, Xin L, et al.: **PeaksPTM: Mass spectrometry-based identification of peptides with unspecified modifications.** *J Proteome Res.* 2011; **10**(7): 2930–2936.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hendy J, Welker F, Demarchi B, et al.: **A guide to ancient protein studies.** *Nat Ecol Evol.* 2018; **2**(5): 791–799.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hepburn HR, Duangphakdee O, Pirk CWW: **Physical properties of honeybee silk: a review.** *Apidologie.* 2013; **44**: 600–610.
[Publisher Full Text](#)
- Kasso T, Bruun Jensen A, Johns A, et al.: **Beescapes – extracting pollen from historical Danish beeswax to explore honeybee foraging.** *Palynology.* 2023; **48**(2): 2288220.
[Publisher Full Text](#)
- Lamei S: **The effect of honeybee-specific Lactic Acid Bacteria on American foulbrood disease of honeybees.** Doctoral dissertation. Uppsala: Sveriges Lantbruksuniversitet. Acta Universitatis Agriculturae Suecicae. 1652-6880; 2018; **23**.
[Reference Source](#)
- Lis H, Sharon N: **Protein glycosylation. Structural and functional aspects.** *Eur J Biochem.* 1993; **218**(1): 1–27.
[PubMed Abstract](#) | [Publisher Full Text](#)

- Lozier JD, Cameron SA: **Comparative genetic analyses of historical and contemporary collections highlight contrasting demographic histories for the bumble bees *Bombus pennsylvanicus* and *B. impatiens* in Illinois.** *Mol Ecol.* 2009; **18**(9): 1875–1886.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Mackie M: **Assessing the hidden potential of beeswax specimens in natural history museum collections.** *PRoteomics IDentifications Database.* 2024.
[Reference Source](#)
- Mackie M, R  ther P, Samodova D, et al.: **Palaeoproteomic profiling of conservation layers on a 14th century Italian wall painting.** *Angew Chem Int Ed Engl.* 2018; **57**(25): 7369–7374.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Micas AFD, Ferreira GA, Laure HJ, et al.: **Proteins of the integumentary system of the honeybee, *Apis mellifera*.** *Arch Insect Biochem Physiol.* 2016; **93**(1): 3–24.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Mikheyev AS, Tin MMY, Arora J, et al.: **Museum samples reveal rapid evolution by wild honey bees exposed to a novel parasite.** *Nat Commun.* 2015; **6**: 7991.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Mureşan CI, Dezmirean DS, Marc BD, et al.: **Biological properties and activities of Major Royal Jelly Proteins and their derived peptides.** *J Funct Foods.* 2022; **98**: 105286.
[Publisher Full Text](#)
- Nielsdatter MG, Larsen M, Godthaab Nielsen L, et al.: **History of the displacement of the European dark bee (*Apis mellifera mellifera*) in Denmark.** *J Apic Res.* 2021; **60**(1): 13–18.
[Publisher Full Text](#)
- Parish JB, Scott ES, Hogendoorn K: **Nutritional benefit of fungal spores for honey bee workers.** *Sci Rep.* 2020; **10**(1): 15671.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Parejo M, Wragg D, Henriques D, et al.: **Digging into the genomic past of swiss honey bees by whole-genome sequencing museum specimens.** *Genome Biol Evol.* 2020; **12**(12): 2535–2551.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Rams  e A, Crispin M, Mackie M, et al.: **Assessing the degradation of ancient milk proteins through site-specific deamidation patterns.** *Sci Rep.* 2021; **11**(1): 7795.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Rappsilber J, Mann M, Ishihama Y: **Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using StageTips.** *Nat Protoc.* 2007; **2**(8): 1896–906.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ryba S, Titera D, Haklova S, et al.: **A PCR method of detecting American Foulbrood (*Paenibacillus* larvae) in winter beehive wax debris.** *Vet Microbiol.* 2009; **139**(1–2): 193–196.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Schroeter ER, Cleland TP: **Glutamine deamidation: an indicator of antiquity, or preservational quality?** *Rapid Commun Mass Spectrom.* 2016; **30**(2): 251–255.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Schroeter ER: **Characterization of diagenetiforms in an expanded proteome of the extinct moa (dinornithidae): identifying biological, diagenetic, experimental artifact, and mislabeled modifications in degraded tissues.** *Minerals.* 2024; **14**(2): 137.
[Publisher Full Text](#)
- Shen L, Liu D, Li M, et al.: **Mechanism of action of recombinant acc-royalisin from Royal Jelly of asian honeybee against gram-positive bacteria.** *PLoS One.* 2012; **7**(10): e47194.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Strange JP, Knoblett J, Griswold T: **DNA amplification from pin-mounted bumble bees (*Bombus*) in a museum collection: effects of fragment size and specimen age on successful PCR.** *Apidologie.* 2009; **40**: 134–139.
[Publisher Full Text](#)
- Woodward DR: **Queen bee: biology, rearing and breeding.** Balclutha, New Zealand, 2007.
[Reference Source](#)
- Yushkevich PA, Piven J, Cody Hazlett H, et al.: **User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability.** *Neuroimage.* 2006; **31**(3): 1116–28.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Zhang L, Han B, Li R, et al.: **Comprehensive identification of novel proteins and N-glycosylation sites in Royal Jelly.** *BMC Genomics.* 2014; **15**(1): 135.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

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Nelson Enrique Arenas

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The study presented in the natural history of *A. mellifera* specimens from the Natural History Museum collection is quite relevant to understanding the biology of pollinators through ancient times.

Abstract: This section must be strengthened since the findings are interesting, but its abstract is the weakest. Methods and results are not described in detail.

Introduction: Authors should connect paragraphs to present the background of the study sequentially. For example, one paragraph describes honeybees, and the next Natural History Museum collections are without any context. Perhaps check how to order the whole story more connected and add the study aims at the end of this section. Authors should describe better if there is any other study in the field of palaeoproteomics with a similar approach. The fifth paragraph must be moved to the "Results and Discussion" section including Figures 1 and 2.

Methods: In the section "Palaeoproteomics" separate the data analysis or bioinformatic methods from the experimental settings. I also recommend to define a little longer subtitle for each section.

Results and discussion: The CT's results appeared in the introduction section and should be moved here. I suggest including a list of the proteins identified as supplementary material despite that no differences were found. Authors must include a major information regarding the fold change of MRJPs and perhaps comparing with other honeybees' species or consider to review if abundances have any explanation through ancient times. Since the *A. mellifera*'s proteome has thousands of proteins, authors must explain why this low number of proteins, if any technical problems at obtaining clean protein fractions which is difficult due to exoskeleton or complex polysaccharides, wax or lipid content, or some specific inhibitors. The last part of the technical issues in sample processing was not clear.

Conclusions: The authors must summarize this section.

The manuscript must be proofread by a native speaker.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and does the work have academic merit?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Biochemistry, molecular biology, bioinformatics.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
