Taylor & Francis
Taylor & Francis Group

Journal of Apicultural Research

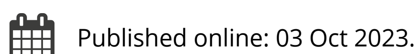
ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tjar20

Genomic DNA extraction from honey bee (*Apis mellifera*) queen spermathecal content

Carlos A. Yadró, Ana R. Lopes, Dora Henriques, Eduard Musin, Jakob Wegener & M. Alice Pinto

To cite this article: Carlos A. Yadró, Ana R. Lopes, Dora Henriques, Eduard Musin, Jakob Wegener & M. Alice Pinto (03 Oct 2023): Genomic DNA extraction from honey bee (*Apis mellifera*) queen spermathecal content, Journal of Apicultural Research, DOI: 10.1080/00218839.2023.2262871

To link to this article: https://doi.org/10.1080/00218839.2023.2262871



Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=tjar20

JOURNAL OF APICULTURAL RESEARCH
https://doi.org/10.1080/00218839.2023.2262871

IBRA INTERNATIONAL BEE RESEARCH ASSOCIATION
Taylor & Francis
Taylor & Francis Group

NOTES & COMMENTS

Check for updates

Genomic DNA extraction from honey bee (*Apis mellifera*) queen spermathecal content

Carlos A. Yadró[a,b], Ana R. Lopes[a,b], Dora Henriques[a,b], Eduard Musin[c], Jakob Wegener[c] and M. Alice Pinto[a,b]

[a]Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal; [b]Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Bragança, Portugal; [c]Institute for Bee Research Hohen Neuendorf, Hohen Neuendorf, Germany

ABSTRACT
Genetic analysis of the honey bee spermathecal content can be particularly useful to provide an estimate of the genetic diversity and purity of the surrounding populations. Here we compared the concentration and quality of DNA extracted from queen spermatheca using four commercial kits to determine the best method to obtain DNA suitable for single nucleotide polymorphism genotyping by next-generation sequencing. The four kits were tested with different adjustments in the lysis incubation time, use of RNA-carrier, elution conditions and number of re-elutions. Only the use of QIAamp DNA Microkit with 3 h of lysis incubation, the addition of RNA-carrier and multiple re-elutions produced a DNA concentration over the required threshold.

ARTICLE HISTORY
Received 8 September 2022
Accepted 31 May 2023

KEYWORDS
Honey bee; spermatheca; genomic DNA isolation; DNA extraction

In honey bee (*Apis mellifera* L.) conservation and breeding programs, isolated mating stations are established to avoid unwanted crosses, although these are not always effective as matings with unwanted drones are commonly reported (Gregorc et al., 2008; Jensen et al., 2006). An interesting method to monitor the degree of isolation could be the genetic analysis of the queen's spermathecal content. While this method implies that queens selected for monitoring are sacrificed, it can be a powerful way of assessing the effectiveness of mating stations because it would allow easy detection of unwanted alleles. However, obtaining enough quantities of drone DNA from the queen's spermatheca for genetic analysis can be challenging for two main reasons. First, only 3-5% of the drones' ejaculates are stored in the spermatheca (Koeniger & Koeniger, 1991), which corresponds to 3.66 to roughly 7 million spermatozoa (Bieńkowska et al., 2011; Güler et al., 1999; Güler & Alpay, 2005). Second, high chromatin condensation, *via* a protamination-like process, has been described for several insect species (Jayaramaiah Raja & Renkawitz-Pohl, 2005), which may also affect the drone's sperm. While such a process has not been described for the honey bee, a subset of sperm-specific proteins is thought to mediate a similar process with specific adaptations to long-term sperm storage in the queen's spermatheca (Zareie et al., 2013).

Here, we assessed the performance of four commercial kits, with modifications, for DNA extraction of the queen spermathecal content. The ultimate goal was to identify the best extraction protocol for single nucleotide polymorphism (SNP) genotyping by next-generation sequencing (NGS), which requires a higher DNA input for NGS library construction than PCR-based applications (e.g. microsatellite genotyping, mtDNA haplotyping). To the best of our knowledge, this is the first study reporting on DNA extraction from queens' spermathecal content for such downstream applications.

Spermathecae were removed from 43 mated queens using a pair of thin and pointed forceps. Great care was taken to avoid injuring the poison gland or sac. The spermathecal fluid was collected from each spermatheca by piercing and squeezing out the spermatheca in a droplet (20 µL) of K^+ (Wegener et al., 2014). The droplet was taken up into 280 µL of K^+, briefly vortexed, and stored at −70 °C. Later, the droplet was thawed and centrifuged at 5,000 × g for 5 min. The supernatant was

CONTACT M. Alice Pinto apinto@ipb.pt

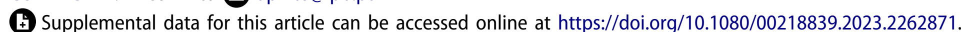

Supplemental data for this article can be accessed online at https://doi.org/10.1080/00218839.2023.2262871.
Spermathecae collection and preparation were performed by Jakob Wegener and Eduard Musin. M. Alice Pinto, Dora Henriques, Ana R. Lopes, and Carlos A. Yadró contributed to the study conception and design. Ana R. Lopes, Carlos A. Yadró, Dora Henriques performed the molecular work. The first draft of the manuscript was written by Carlos A. Yadró and M. Alice Pinto. Dora Henriques and Ana R. Lopes commented in previous versions of the manuscript. All authors read and approved the final manuscript.
© 2023 International Bee Research Association

Table 1. Commercial DNA extraction kits, protocols and adjustments assayed. MP: manufacturers' protocol.

Kit	Protocol	Adjustments
NucleoSpin® Tissue (Macherey-Nagel™, Düren, DE)	**Kit 1 A**. Support protocol for the isolation of genomic DNA from semen	• Final elution in 100 µL of DEPC water (MP, Step 11)
Monarch Genomic DNA Purification (New England Biolabs® Inc., Massachusetts, US)	**Kit 2 A**. Protocol for extraction and purification of genomic DNA from tissues	• Lysis incubation for 1 h (MP, Part 1, Step 3) • Final elution in 40 µL of DEPC water (MP, Part 2, Step 6)
QIAamp DNA Mini Kit (Qiagen®, Hilden, DE)	**Kit 3 A**. DNA purification from tissues	• Lysis incubation for 2 or 4 h (MP, Step 3) • Final elution in 50 µL of DEPC water, with RT incubation for 5 min before centrifugation (MP, Step 11)
	Kit 3B. User-developed protocol for isolation of genomic DNA from sperm	• Final elution in 50 µL of DEPC water, with RT incubation for 10 min before centrifugation (MP, Step 11)
QIAamp DNA Micro Kit (Qiagen® Hilde, DE)	**Kit 4 A**. Isolation of genomic DNA from small volumes of blood with RNA-carrier	• Addition of 1 µL of RNA-carrier (1 µg/µL) (MP, Step 4) • Lysis incubation for 1 or 3 h (MP, Step 5) • Elution in 25 µL of DEPC water with RT incubation for 10 min before centrifugation (MP, Step 13)
	Kit 4B. Isolation of genomic DNA from tissues	• Lysis incubation for 6 h or overnight (MP, Step 4) • Elution in 25 µL of DEPC water with RT incubation for 10 min before centrifugation (MP, Step 12)
	Kit 4 C. Isolation of genomic DNA from tissues with RNA-carrier	• Lysis incubation for 1 h, 3 h, 6 h, or overnight (MP, Step 4) • Addition of 1 µL of RNA-carrier (1 µg/µL) (MP, Step 5) • Elution in 25 µL of DEPC water with RT incubation for 10 min before centrifugation (MP, Step 12)
	Kit 4D. Isolation of genomic DNA from tissues with RNA-carrier and multiple elutions	• Lysis incubation for 3 h (MP, Step 4) • Addition of 1 µL of RNA-carrier (1 µg/µL) (MP, Step 5) • Elution in 25 µL of DEPC water with RT incubation for 10 min before centrifugation (MP, Step 12) • Re-use of the first eluate in 2, 3 or 4 elution steps

removed, and the semen pellet was stored in 400 µL of 98% ethanol.

Four different DNA extraction kits were tested on 43 semen samples. DNA extractions were performed according to the manufacturers' protocols (MPs), with slight adjustments described below (Table 1). Before DNA extraction, the semen pellets were centrifuged at 5,000 × g for 2 min, and the ethanol was carefully removed. The pellets were dried for 10 min at room temperature (RT) to evaporate as much ethanol as possible.

Total nucleic acid yield and purity (A_{260}/A_{280} ratio) were determined by spectrophotometry with the SpectroStar®Nano LVis Plate (BMG Labtech, Ortenberg, DE). Additionally, the dsDNA was quantified by fluorometric methods using the Quantus™ Fluorometer apparatus (Promega, Wisconsin, US) with the QuantiFluor® dsDNA System. Since NGS library construction with the NEBNext Direct® Genotyping Solution (New England Biolabs® Inc., Massachusetts, US) requires a volume of 6 µL containing a minimum of 10 ng of DNA (New England Biolabs, 2019), we established a concentration threshold of 1.7 ng/µL to assess the DNA extracts.

The success of the DNA extractions was assessed by PCR-amplification of the nuclear gene L-5S1ter (Suazo & Hall, 2002) in 34 DNA extracts. The reactions were carried out in a 10 µL final volume containing 5 µL of Q5® High-Fidelity 2X Master Mix (New England Biolabs® Inc., Massachusetts, US), 0.5 µM of each primer, 2 µL of DEPC water, and 2 µL of the DNA extract. The PCR-thermal conditions followed the Q5 manufacturer's instructions with the annealing temperature set at 65 °C. The amplicons were analysed in the LabChip GX Touch Nucleic Acid Analyzer using the DNA High Sensitivity Kit (Perkin-Elmer, Massachusetts, US).

According to spectrophotometric quantitation of total nucleic acid, all the protocols produced DNA extracts with a median concentration of 27.04 ng/µL, well-above the established threshold (Figure 1a and Supplementary Material 1). However, when analysing the A_{260}/A_{280} ratio, 82.9% of these samples showed values $\geq$ 2.0, indicating elevated proportions of RNA (Figure 1b). Hence, dsDNA quantitation was chosen to assess the performance of the different extraction protocols. Only the tissue protocol of QIAamp DNA Microkit, with the addition of RNA-carrier and 3 or 6 h of lysis incubation (method Kit 4 C), produced dsDNA concentrations exceeding the 1.7 ng/µL threshold (Figure 1c). Moreover, repeating the elution step two and three times greatly increased the yields, when compared to the single elution (69% increase for double re-elution and 154% for triple re-

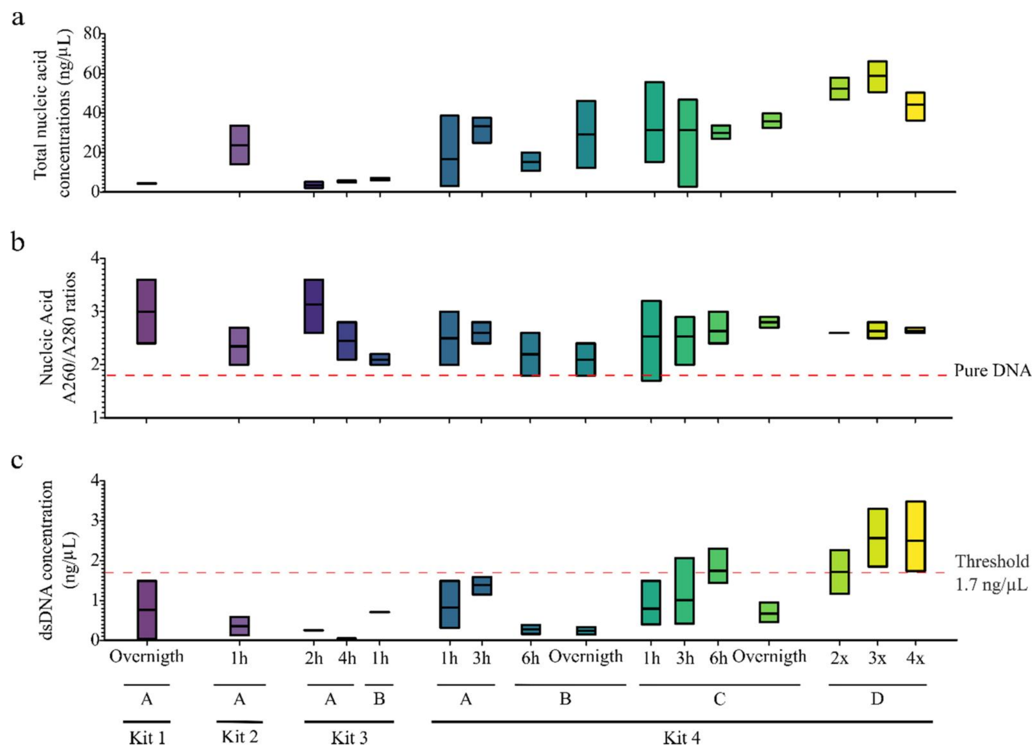


Figure 1. Comparative results of the extraction methods, expressed as median and Q2-Q3 ranges. (a) Total nucleic acid concentrations estimated by SpectroStar®Nano Lvis Plate. (b) DNA purity estimated by the A_{260}/A_{280} absorbance ratio. The red dashed line marks the 1.8 ratio, expected for pure DNA. (c) dsDNA concentrations estimated with Quantus ™ Fluorometer. The red dashed line represents the threshold of 1.7 ng/µL.

elution). A fourth re-elution did not increase the DNA yield much further.

The DNA extracts were further assessed by PCR. Of the 34 analysed samples, 28 (82.4%) were successfully amplified (Supplementary Material 2 and 3), producing the expected 500-bp fragments (Suazo & Hall, 2002). Amplification success was not related to dsDNA concentration values. For example, samples extracted using the protocols of Kit 3 A and B showed strong amplification bands, despite having dsDNA concentrations as low as 0.71 and 0.02 ng/µL (Figure 1a and Supplementary Material 2). This finding indicates that while most of the extraction protocols generated DNA concentrations below the threshold required for library construction with the NEBNext Direct® Genotyping Solution, they can still be used for PCR applications.

Our results showed that the QIAamp DNA Microkit, with slight modifications (Kit 4D), provides the highest DNA concentrations. We recommend lysis incubation for 3 h, and the addition of RNA-carrier to enhance DNA recovery. Furthermore, the use of multiple re-elution steps helps to obtain the minimum DNA yields required for genotyping by NGS. This method was later used to extract DNA from sperm obtained from 126 spermathecae, which were successfully genotyped by NGS despite the high amount of RNA in all the samples.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

Contributions of Jakob Wegener and Eduard Musin were supported by funds of the Federal Ministry of Food and Agriculture based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food, grant number 2818BM040. Dora Henriques was supported by BEEHAPPY (POCI-01-0145-FEDER-029871; FCT and COMPETE/QREN/EU). Ana R. Lopes and Carlos A. Yadró were supported by Fundação para a Ciência e a Tecnologia (FCT) grants SFRH/BD/143627/2019 and 2021.06948.BD, respectively. FCT provided financial support by national funds (FCT/MCTES) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021).

References

Bieńkowska, M., Panasiuk, B., Węgrzynowicz, P., & Gerula, D. (2011). The effect of different thermal conditions on drone semen quality and number of spermatozoa entering the spermatheca of queen bee. *Journal of Apicultural Science*, ***55***(2), 161–168.

Gregorc, Ǎ., Lokar, V., & Škerl, M. I. S. (2008). Testing of the isolation of the Rog-Ponikve mating station for Carniolan (*Apis mellifera carnica*) honey bee queens. *Journal of Apicultural Research*, ***47***(2), 137–140. https://doi.org/10.1080/00218839.2008.11101440

Güler, A., & Alpay, H. (2005). Reproductive characteristics of some honeybee (*Apis mellifera* L.) genotypes. *Journal of Animal Veterinary Advances*, ***4***(10), 864–870.

Güler, A., Korkmaz, A., & Kaftanoğlu, O. (1999). Reproductive characteristics of Turkish honeybee (*Apis mellifera* L.) genotypes. *Hayvansal Üretim*, ***40***(1), 113–119.

Jayaramaiah Raja, S., & Renkawitz-Pohl, R. (2005). Replacement by *Drosophila melanogaster* protamines and Mst77F of histones during chromatin condensation in late spermatids and role of sesame in the removal of these proteins from the male pronucleus. *Molecular and Cellular Biology*, ***25***(14), 6165–6177. https://doi.org/10.1128/MCB.25.14.6165-6177.2005

Jensen, A. B., Palmer, K. A., Chaline, N., Raine, N. E., Tofilski, A., Martin, S. J., Pedersen, B. V., Boomsma, J. J., & Ratnieks, F. L. (2006). Quantifying honey bee mating range and isolation in semi-isolated valleys by DNA microsatellite paternity analysis. *Conservation Genetics*, ***6***(4), 527–537. https://doi.org/10.1007/s10592-005-9007-7

Koeniger, N., & Koeniger, G. (1991). An evolutionary approach to mating behaviour and drone copulatory organs in *Apis*. *Apidologie*, ***22***(6), 581–590. https://doi.org/10.1051/apido:19910602

New England Biolabs. (2019). *NEBNext Direct® Genotyping Solution. INSTRUCTION MANUAL New England Biolabs*. https://www.neb.com/-/media/nebus/files/manuals/manuale9500_e9530.pdf?rev=8b4e2a66937941b3a87bedd1f89214d6

Suazo, A., & Hall, H. G. (2002). Nuclear DNA PCR-RFLPs that distinguish African and European honey bee groups of subspecies. II: Conversion of long PCR markers to standard PCR. *Biochemical Genetics*, ***40***(7–8), 241–261. https://doi.org/10.1023/a:1019882817120

Wegener, J., May, T., Kamp, G., & Bienefeld, K. (2014). New methods and media for the centrifugation of honey bee (Hymenoptera: Apidae) drone semen. *Journal of Economic Entomology*, ***107***(1), 47–53. https://doi.org/10.1603/ec13159

Zareie, R., Eubel, H., Millar, A. H., & Baer, B. (2013). Long-term survival of high quality sperm: Insights into the sperm proteome of the honeybee *Apis mellifera*. *Journal of Proteome Research*, ***12***(11), 5180–5188. https://doi.org/10.1021/pr4004773