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A comparative evaluation of DNA yield, purity and integrity from different body segments of *Apis mellifera* using phenol–chloroform and commercial extraction

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Abstract

Efficient DNA extraction is essential for molecular studies in honey bees, yet extraction success may vary depending on methodology and tissue type. This study compared a classical phenol–chloroform protocol and a silica membrane–based commercial kit for genomic DNA isolation from head, thorax and abdomen tissues of the honey bee (*Apis mellifera*). DNA integrity, yield and spectrophotometric purity (A260/A280) were evaluated under standardized laboratory conditions. Agarose gel electrophoresis revealed that phenol–chloroform extraction produced high-molecular-weight DNA with minimal degradation across all body segments, whereas the commercial kit yielded weaker bands and partial smearing. Quantitative analyses showed that phenol–chloroform extraction generated significantly higher DNA concentrations than the commercial kit in all tissues. Two-way ANOVA confirmed a highly significant effect of extraction method on DNA yield ($p < 0.001$), while body segment alone did not significantly influence yield. However, a significant interaction indicated segment-dependent variation in extraction efficiency. DNA purity also differed significantly between methods ($p < 0.001$), with phenol–chloroform extraction producing more consistent A260/A280 ratios, particularly in thorax and abdomen samples. Overall, the extraction methodology was the primary determinant of DNA yield and quality. Although commercial kits offer advantages in speed and convenience, phenol–chloroform extraction remains a reliable method for obtaining high-quality genomic DNA suitable for molecular studies in *A. mellifera*.

Keywords: *Apis mellifera*, commercial kit, DNA extraction, DNA purity, DNA yield, phenol–chloroform

INTRODUCTION

Honey bees (*A. mellifera*) are essential pollinators that play a critical role in agricultural productivity and ecosystem stability. Their ecological and economic value is substantial, as they contribute to both crop yield and biodiversity through pollination services (Klein et al. 2007; Potts et al. 2010). In addition to their ecological significance, honey bees are also valuable subjects in scientific studies, including genetics, disease diagnostics, and population monitoring (Evans and Schwarz 2011; Harpur et al. 2012). Molecular tools that rely on DNA-based analyses, such as PCR and sequencing, are widely used for these purposes and require sufficient DNA quantity, purity, and integrity (Kek et al. 2018). High-quality genomic DNA is a fundamental prerequisite for a wide range of molecular analyses, including PCR-based diagnostics, population genetics, phylogenetic studies, and next-generation sequencing (Oppert et al. 2019, Psifidi et al. 2015, Soares et al. 2023). The success and reliability of these applications depend not only on DNA quantity but also on its purity and integrity, as contaminants and inhibitory compounds can severely compromise enzymatic reactions (Asghar et al. 2015, Boom et al. 1990). Consequently, selecting an appropriate DNA extraction method is a critical step in molecular research workflows. In insects, DNA extraction is frequently challenged by the presence of structural and biochemical components such as chitin, proteins, and lipids, which may co-extract with nucleic acids and negatively affect downstream enzymatic reactions (Boncristiani et al. 2011, Güler et al. 2018, Weis et al. 2021).

Comparative studies on insect tissues have demonstrated that these biological characteristics can substantially influence DNA quality, purity, and overall suitability for molecular applications, often resulting in inconsistent amplification success despite acceptable spectrophotometric measurements (Güler et al. 2018). These challenges highlight the limitations of relying solely on conventional purity indicators and emphasize the importance of method optimization for insect-derived samples. A wide range of DNA extraction protocols has therefore been developed for insect samples, with organic solvent–based methods and silica membrane–based commercial kits being the most commonly applied approaches (Firmansyah et al. 2023, Güler et al. 2018, Tatar et al. 2024). Among

organic methods, phenol–chloroform extraction has long been regarded as a reference technique due to its capacity to recover high-molecular-weight genomic DNA with relatively high yields (Boom et al. 1990, Green and Sambrook 2017, Modabber et al. 2016).

In insect-focused comparative studies, this method has frequently outperformed commercial kits in terms of DNA concentration, particularly when applied to structurally complex tissues or aged samples (Modabber et al. 2016, Tatar et al. 2024). However, despite these advantages, phenol–chloroform extraction is labor-intensive and time-consuming, and it involves the use of hazardous chemicals, which limits its routine applicability in laboratories processing large numbers of samples (Asghar et al. 2015, Modabber et al. 2016, Tatar et al. 2024). In contrast, commercial DNA extraction kits based on silica membrane technology offer rapid, standardized, and user-friendly alternatives that are well-suited for routine molecular analyses. These kits reduce operator-dependent variability and minimize exposure to toxic reagents, making them particularly attractive for high-throughput laboratory workflows. Nevertheless, several studies have reported that DNA yield and downstream amplification success obtained using commercial kits can vary considerably depending on sample type and biological matrix (Asghar et al. 2015, Issa et al 2013, Kek et al. 2018, Soares et al. 2023).

Importantly, accumulating evidence suggests that higher DNA concentrations do not necessarily translate into improved PCR performance, and that lower DNA yields obtained from kit-based extractions may be associated with superior amplification success due to more effective removal of inhibitory compounds (Weis et al. 2021). In honey bees (*A. mellifera*), DNA isolation challenges are further exacerbated by the complexity of specific biological matrices and the presence of PCR-inhibitory compounds in certain tissues (Boncristiani et al. 2011). Wax-associated materials and lipid-rich matrices such as beeswax have been reported to contain substances that may interfere with PCR amplification and downstream molecular analyses (Weis et al. 2021). In addition, reproductive tissues, such as the spermatheca, represent highly complex biological matrices, and obtaining sufficient, high-quality DNA for molecular applications remains technically challenging (Yadró et al. 2022). Studies focusing on beeswax and hive-associated samples have demonstrated that co-extracted lipids and other contaminants can severely inhibit PCR reactions, even when DNA concentrations appear sufficient according to spectrophotometric measurements (Weis et al. 2021).

Similarly, genomic DNA isolation from spermathecal contents has been reported to require carefully optimized protocols to obtain DNA of adequate quality and quantity for downstream genotyping applications (Yadró et al. 2022). Together, these findings clearly indicate that spectrophotometric purity ratios alone are insufficient indicators of DNA suitability in honey bee molecular studies. Despite the availability of numerous DNA extraction protocols, most comparative studies in insects have focused on a limited number of methods or specific sample types, making it difficult to disentangle methodological effects from biological variability (Asghar et al. 2015, Modabber et al. 2016). In honey bees, in particular, direct and systematic comparisons between organic extraction methods and commercial kits under standardized experimental conditions remain scarce. Therefore, the aim of the present study was to comparatively evaluate a phenol–chloroform–based protocol and a commercial silica column–based DNA extraction kit for genomic DNA isolation from honey bee samples, while also assessing the effects of different body segments (head, thorax, and abdomen) on DNA yield and purity.

MATERIALS AND METHODS

Sample Collection and Tissue Preparation

In this study, 40 healthy worker honey bees (*A. mellifera*) were randomly collected from the flora of the Akdeniz University campus. Honey bees actively foraging on flowers were guided into sterile Falcon tubes containing absolute ethanol without any physical contact, and individuals were rapidly immobilized upon contact with ethanol. All samples were transported to the laboratory under appropriate conditions. In the laboratory, each individual was dissected under sterile conditions and separated into three anatomical body segments: head, thorax, and abdomen. For each DNA extraction method, 20 samples per tissue type were prepared, yielding a total of 120 tissue samples in the study (phenol–chloroform method: 60; commercial kit method: 60).

DNA Extraction Procedures

Two different DNA extraction methods were applied in this study, both optimized according to standard protocols described in the literature to ensure suitability for honey bee tissues. Genomic DNA extraction using a commercial kit was performed with the BioBasic Spin Column Animal Genomic DNA Extraction Kit (Bio Basic Inc., Canada; Cat. No: [BS427/BS628], following the manufacturer's instructions. To enhance extraction efficiency, each tissue sample was mechanically homogenized in liquid nitrogen prior to extraction. Subsequently, the lysis, DNA binding, washing, and elution steps described in the kit protocol were carried out sequentially. Incubation times, centrifugation speeds, and buffer volumes were strictly standardized according to the manufacturer's guidelines. Using this method, genomic DNA was successfully isolated from 60 tissue samples. For classical DNA extraction, the phenol–chloroform protocol described by Freedman et al. (2008) was used as a reference and optimized for honey bee tissues. For phenol–chloroform extraction, the

protocol described by Freedman et al. (2008) was followed with modifications. Approximately 4–5 mg of tissue was homogenized under liquid nitrogen and transferred to 1.5 mL microcentrifuge tubes.

Each sample was incubated in 500 µL of lysis buffer containing 50 mM Tris-HCl (pH 8.0), 0.1 M EDTA, 1% SDS, and 40 mM DTT at 37°C for 1 hour. Subsequently, proteinase K (~11 µL; ~16 mg/mL) was added, and samples were incubated overnight at 37°C to ensure complete digestion. Following lysis, an equal volume of phenol (pH 8.0) was added, and samples were centrifuged at 15,400 × g for 20 minutes. The aqueous phase was transferred to a new tube and extracted twice with phenol:chloroform (1:1, v/v), with centrifugation at 15,400 × g for 5 minutes after each extraction. DNA was precipitated by adding cold 95% ethanol and incubating at −80°C for 1 hour. Samples were then centrifuged at 13,000 rpm for 30 minutes, and the DNA pellet was washed, air-dried, and resuspended in 80 µL nuclease-free water. Extracted DNA was stored at 4°C, and DNA concentration and purity were assessed spectrophotometrically.

Genomic DNA was isolated from 60 tissue samples using this method. All phase transfers, centrifugation conditions, and reagent volumes were standardized to ensure methodological consistency. To minimize technical variability, pipetting techniques, incubation temperatures and durations, mixing procedures (vortexing or gentle mixing), and centrifugation conditions were kept consistent for both extraction methods. All procedures were performed using single-use sterile consumables. To monitor potential contamination, negative control tubes were included in each extraction batch. Following DNA extraction, all samples were evaluated for the following parameters: DNA concentration (ng/µL), A260/A280 purity ratio, and DNA integrity. DNA concentration and purity ratios were measured using a NanoDrop spectrophotometer. DNA integrity was assessed by agarose gel electrophoresis, and gel images were examined for band integrity, potential degradation, and the presence of high-molecular-weight genomic DNA.

Statistical Analysis

Descriptive statistics were calculated for DNA yield (ng/µL) and DNA purity (A260/A280 ratio) for each extraction method × body segment combination and are presented as mean ± standard deviation. Because DNA yield data exhibited right-skewed distributions, values were $\log_{10}$-transformed prior to inferential analyses to improve normality and homogeneity of variance. The effects of the extraction method (phenol–chloroform vs. commercial kit), body segment (head, thorax, abdomen), and their interaction on DNA yield and DNA purity were evaluated using two-way analysis of variance (ANOVA) models fitted separately for each response variable. When a significant extraction method × body segment interaction was detected, segment-specific post-hoc comparisons were performed using Welch's t-tests with Holm correction to adjust for multiple comparisons. Effect sizes were calculated using Hedges' g to quantify the magnitude of differences between extraction methods. DNA purity was assessed by A260/A280 ratios; values between 1.8 and 2.0 were considered indicative of high-quality DNA. Graphical visualization included agarose gel electrophoresis images to qualitatively assess DNA integrity and boxplots to illustrate the distribution of DNA yield and purity across extraction methods and body segments.

All statistical tests were two-tailed, and statistical significance was set at $p < 0.05$. Statistical analyses and figure generation were performed using Python (SciPy, statsmodels, pandas, and matplotlib).

RESULTS

DNA Integrity Assessment by Agarose Gel Electrophoresis

The structural integrity of genomic DNA isolated from honey bee head, thorax, and abdomen tissues was evaluated using 1.5% agarose gel electrophoresis (Figure-1). DNA samples obtained using the phenol–chloroform method consistently showed high-molecular-weight bands with sharp, well-defined profiles and minimal smearing across all body segments. This pattern indicates effective preservation of DNA integrity by the classical extraction method. In contrast, DNA isolated using the commercial kit displayed noticeably weaker band intensities and, in several samples, pronounced low-molecular-weight smearing, suggesting partial DNA degradation or reduced recovery efficiency. These qualitative differences indicate that, in addition to lower yield, the commercial kit method may be less effective at maintaining DNA integrity than the phenol–chloroform protocol. Importantly, these visual patterns are consistent with the quantitative differences in DNA yield and purity described below.

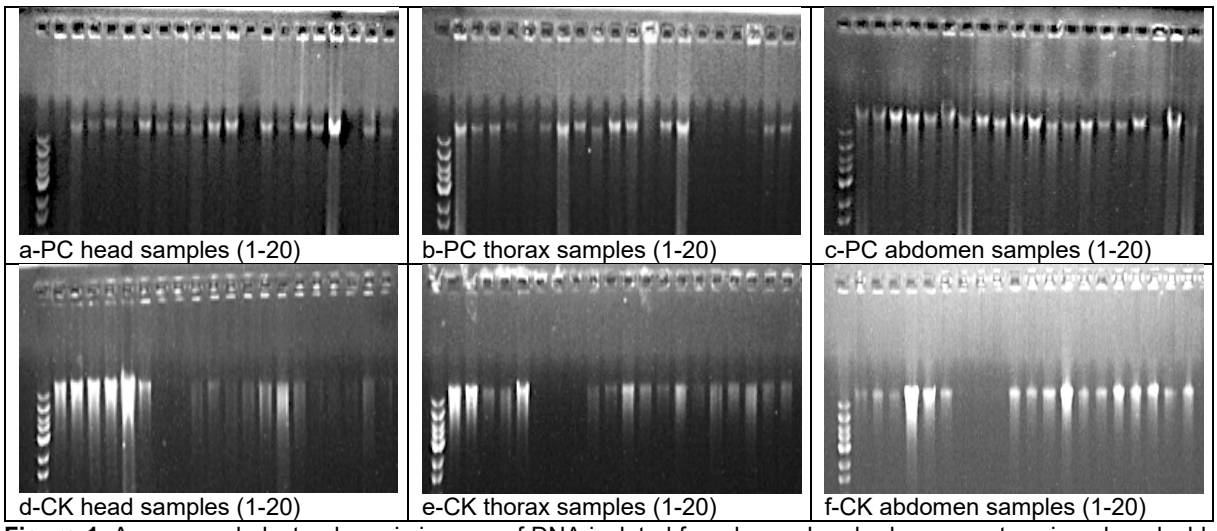


Figure-1. Agarose gel electrophoresis images of DNA isolated from honey bee body segments using phenol–chloroform and commercial kit methods. PC: Phenol–chloroform; CK: commercial kit. High-molecular-weight bands indicate intact genomic DNA.

Descriptive Statistics of DNA Yield and Purity

Descriptive statistics for DNA concentration and purity (A260/A280 ratio) obtained from each extraction method and body segment are summarized in Table-1. Across all tissues, the phenol–chloroform method produced substantially higher mean DNA concentrations than the commercial kit. When phenol–chloroform extraction was applied, abdomen and thorax samples generally yielded higher DNA concentrations than head samples. Regarding DNA purity, phenol–chloroform extraction resulted in A260/A280 ratios predominantly within the optimal range of 1.8–2.0, with relatively low variability. In contrast, the commercial kit exhibited lower mean A260/A280 values and greater dispersion, particularly in thorax and abdomen tissues.

Table-1. Summary statistics of DNA yield (ng/µL) and purity (A260/A280) obtained from honey bee head, thorax and abdomen, using phenol–chloroform and commercial kit extraction methods (mean ± SD).

Extraction method	n	Body segment	DNA yield (ng/µL) Mean ± SD	A260/A280 Mean ± SD
PC	20	Head	1120.15 ± 636.12	1.885 ± 0.158
	20	Thorax	1153.75 ± 659.06	2.010 ± 0.235
	20	Abdomen	864.60 ± 435.41	1.938 ± 0.104
CK	20	Head	77.20 ± 52.00	1.810 ± 0.189
	20	Thorax	39.30 ± 30.42	1.600 ± 0.182
	20	Abdomen	48.05 ± 19.99	1.647 ± 0.195

Values are presented as mean ± standard deviation (SD). A260/A280 ratios between 1.8 and 2.0 indicate high DNA purity. PC: Phenol–chloroform; CK: commercial kit.

Effects of Extraction Method and Body Segment on DNA Yield

Raw DNA yield values exhibited right-skewed distributions across all groups; therefore, $\log_{10}$-transformed values were used for inferential statistical analyses. Two-way ANOVA demonstrated a highly significant main effect of extraction method on DNA yield ($F(1,114) = 767.95$, $p < 0.001$), indicating that phenol–chloroform extraction consistently produced substantially higher DNA yields than the commercial kit (Table-2). The main effect of body segment was marginally non-significant ($F(2,114) = 2.99$, $p = 0.054$), suggesting that yield differences among segments were not uniform across extraction approaches. Importantly, a significant extraction method × body segment interaction was detected ($F(2,114) = 4.35$, $p = 0.015$), demonstrating that the relative performance of extraction methods differed among head, thorax, and abdomen samples.

The distribution of $\log_{10}$-transformed DNA yield values for each extraction method and body segment is illustrated in Figure-2. Across all body segments, phenol–chloroform extraction yielded markedly higher DNA than the commercial kit, with minimal overlap between interquartile ranges. This pattern was particularly evident in thorax and head samples, where the commercial kit consistently yielded lower DNA concentrations than phenol–chloroform extraction.

Table-2. Two-way ANOVA results for DNA yield and DNA purity

Response variable	Effect	df	F	p-value
Log10 DNA	Extraction method	1,114	767.95	<0.001
	Body segment	2,114	2.99	0.054
	Method × segment	2,114	4.35	0.015
DNA purity	Extraction method	1,114	60.73	<0.001
	Body segment	2,114	1.03	0.362
	Method × segment	2,114	8.71	<0.001

Two-way ANOVA models were fitted separately for $\log_{10}$-transformed DNA yield and DNA purity (A260/A280) to evaluate the effects of extraction method, body segment, and their interaction. Degrees of freedom (df), F-statistics, and p-values are reported. Statistical significance was set at $p < 0.05$.

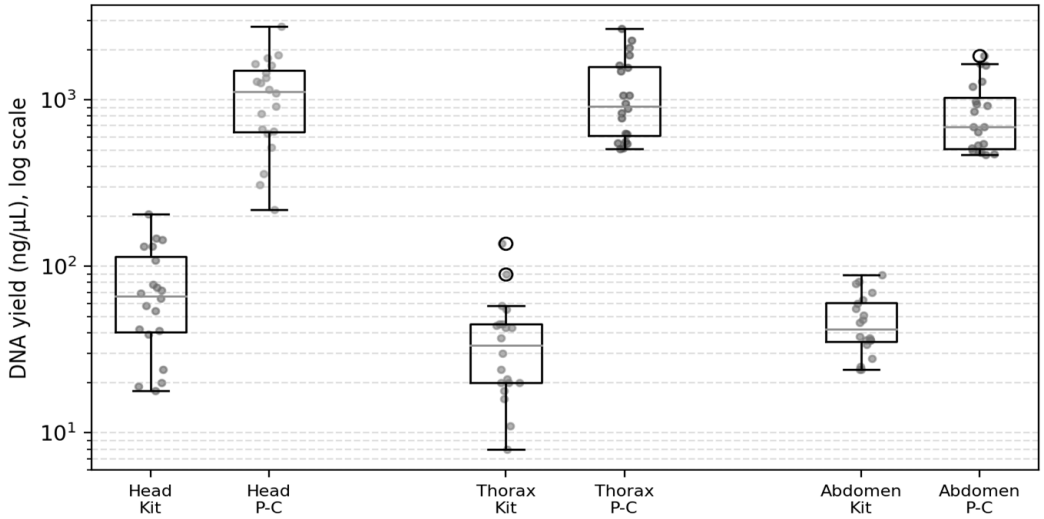


Figure-2. Distribution of DNA yields across extraction methods and body segments. Boxplots show DNA yield (ng/µL) measured in head, thorax, and abdomen samples isolated using phenol–chloroform and a commercial kit. Data are presented on a $\log_{10}$ scale. Boxes represent the interquartile range with median values indicated by horizontal lines; whiskers denote data dispersion, and individual points correspond to single samples.

Segment-specific post-hoc comparisons using Welch's t-tests with Holm correction confirmed that DNA yield was significantly higher for phenol–chloroform extraction in the head, thorax, and abdomen (all adjusted $p < 0.001$; Table-3). The magnitude of these differences was substantial, with effect size estimates indicating very large method-related effects across all segments (Hedges' g ranging from 3.83 to 6.41). These results indicate that phenol–chloroform extraction is superior to other methods for DNA yield, with robust, consistent results across anatomical regions. The interaction patterns underlying these effects are further illustrated in Figure 3, which shows non-parallel trends in extraction methods across body segments, highlighting segment-dependent differences in extraction efficiency.

Table-3. Segment-specific post-hoc comparisons between extraction methods

Response	Segment	t (Welch)	p (Holm)	Hedges' g
Log10 DNA yield	Abdomen	20.67	<0.001	6.41
	Head	12.37	<0.001	3.83
	Thorax	17.55	<0.001	5.44
DNA purity	Abdomen	5.88	<0.001	1.82
	Head	1.36	0.18	0.42
	Thorax	6.17	<0.001	1.91

Post-hoc comparisons were conducted using Welch's t-tests following significant extraction method × body segment interactions. Holm correction was applied to adjust for multiple comparisons. Effect sizes are reported as Hedges' g (phenol–chloroform minus commercial kit). For DNA yield, geometric mean ratios (phenol–chloroform / commercial kit) were calculated by back-transforming $\log_{10}$ differences. Significant comparisons ($p < 0.05$ after correction) are indicated.

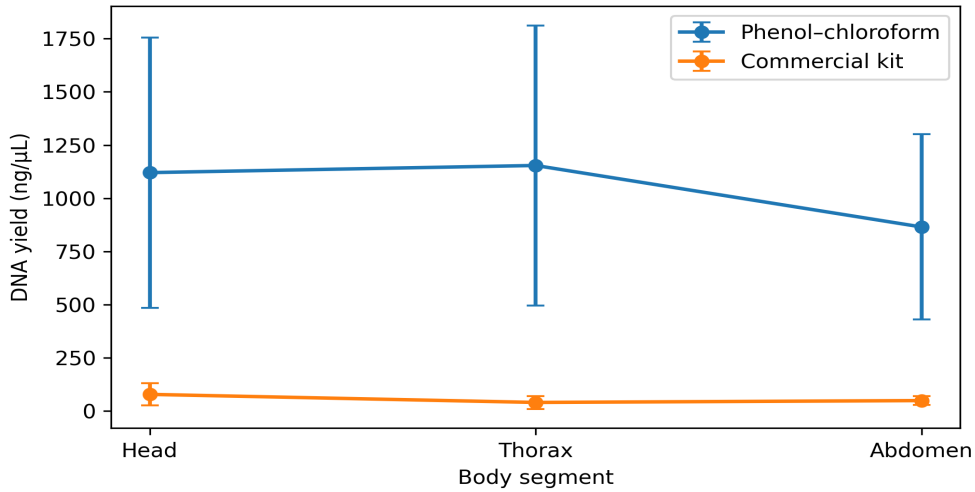


Figure-3. Interaction effects of extraction method and body segment on DNA yield. Interaction plot showing mean DNA yield (ng/µL) ± standard deviation for each extraction method across body segments. The non-parallel trends indicate a significant interaction between extraction method and body segment in the two-way ANOVA.

DNA Purity (A260/A280) Across Extraction Methods and Body Segments

DNA purity, assessed by A260/A280 ratios, also varied by extraction method and body segment (Table 1). Two-way ANOVA revealed a significant main effect of extraction method on DNA purity ($F(1,114) = 60.73$, $p < 0.001$), indicating overall differences in nucleic acid purity between phenol–chloroform extraction and the commercial kit (Table-2). In addition, a significant extraction method × body segment interaction was observed ($F(2,114) = 8.71$, $p < 0.001$), suggesting that method-related differences in DNA purity were not uniform across body segments. In contrast, the main effect of body segment alone was not statistically significant ($F(2,114) = 1.03$, $p = 0.362$). The distribution of DNA purity values across extraction methods and body segments is shown in Figure 4. Phenol–chloroform extraction generally yielded higher A260/A280 ratios and a narrower distribution of purity values than the commercial kit, particularly for thorax and abdomen samples. In these segments, phenol–chloroform extraction yielded DNA purity values that more frequently approached or fell within the commonly accepted optimal range.

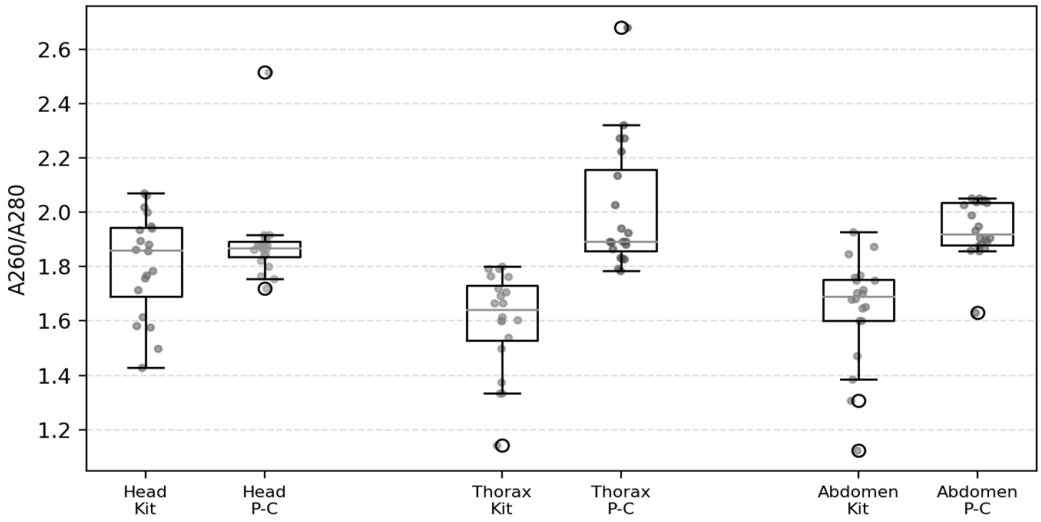


Figure-4. DNA purity (A260/A280) obtained from honey bee body segments using different extraction methods. Boxplots show A260/A280 ratios for DNA isolated from head, thorax, and abdomen samples using phenol–chloroform and a commercial kit. Boxes represent the interquartile range with median values indicated by horizontal lines; whiskers denote data dispersion, and individual points correspond to single samples. A260/A280 ratios between 1.8 and 2.0 indicate high DNA purity.

Post-hoc Welch's t-tests with Holm correction revealed that DNA purity was significantly higher for phenol–chloroform extraction compared with the commercial kit in the abdomen and thorax (both adjusted $p < 0.001$; Table-3). Correspondingly, large effect sizes were observed for these segments (Hedges' $g > 1.8$), indicating substantial method-related differences in DNA purity. Although the

difference was not statistically significant for the head segment (p = 0.18), the corresponding effect size (Hedges' g = 0.42) suggested only a modest difference between extraction methods. These segment-specific interaction effects are further visualized in Figure-5, which illustrates divergent trends in DNA purity between extraction methods across body segments.

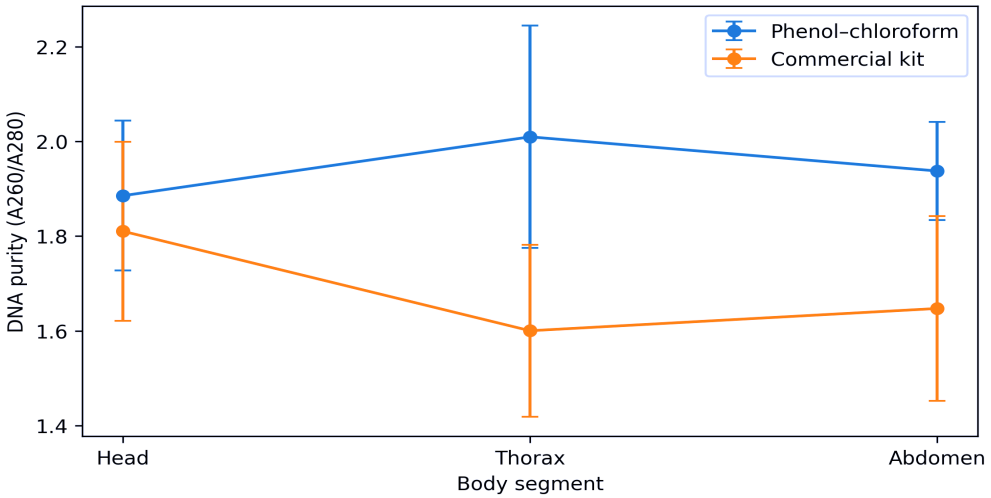


Figure-5. Interaction effects of extraction method and body segment on DNA purity. Interaction plot displaying mean DNA purity (A260/A280) ± standard deviation for phenol–chloroform and commercial kit methods across body segments. Divergent segment-specific patterns reflect the significant interaction between the extraction method and the body segment.

Proportion of High-Quality DNA Samples

When DNA purity was categorized according to widely accepted quality thresholds (A260/A280 = 1.8–2.0), clear differences emerged between extraction methods (Table 4). Across all body segments, phenol–chloroform extraction yielded a higher proportion of samples within the high-quality DNA range compared with the commercial kit. This difference was most pronounced in thorax samples, where high-quality DNA was infrequently obtained using the commercial kit but was commonly achieved following phenol–chloroform extraction. Similar, although less marked, trends were observed for abdomen and head samples.

Table-4. Proportion of samples with high-quality DNA purity (A260/A280 = 1.8–2.0)

Method	Body segment	High-quality DNA, n (%)
PC	Abdomen	12 (60%)
	Head	16 (80%)
	Thorax	11 (55%)
CK	Abdomen	3 (15%)
	Head	8 (40%)
	Thorax	1 (5%)

High-quality DNA was defined as samples with A260/A280 ratios between 1.8 and 2.0. Values are presented as the number of samples and corresponding percentages for each extraction method × body segment combination (n = 20 per group). Taken together, these results demonstrate that phenol–chloroform extraction consistently outperformed the commercial kit in both DNA yield and purity, with the magnitude of these differences varying across body segments.

DISCUSSION

The present study provides a comparative evaluation of two widely used DNA extraction approaches—phenol–chloroform extraction and a silica membrane–based commercial kit—for genomic DNA isolation from different honey bee (*A. mellifera*) body segments. By integrating DNA yield, spectrophotometric purity, and integrity assessments under a standardized experimental design, this study offers methodologically robust insights into DNA isolation performance in honey bees, a model organism characterized by complex tissue composition. Although a prior power analysis was not conducted, the large observed effect sizes indicate that the sample size was sufficient to detect substantial differences between extraction methods.

Extraction Method as the Dominant Factor Influencing DNA Yield

The most prominent outcome of this study is the overwhelming effect of extraction methods on DNA yield. Across all body segments, phenol–chloroform extraction produced substantially higher DNA concentrations than the commercial kit, indicating

pronounced method-dependent differences. Previous studies have similarly emphasized that DNA yield and quality are strongly influenced by extraction methodology in insect tissues (Modabber et al. 2016; Tatar et al. 2024). In particular, comparative analyses of insect samples have demonstrated that phenol–chloroform–based protocols often yield higher DNA recovery and greater integrity than alternative extraction methods (Tatar et al. 2024). The superior performance of the phenol–chloroform method can be attributed to its ability to achieve efficient cell lysis and extensive protein denaturation while minimizing DNA loss during purification. Extraction methodology has long been recognized as a critical determinant of DNA recovery and integrity in insect tissues, particularly in samples characterized by chitin-rich exoskeletons and dense protein matrices (Güler et al. 2018).

Comparative evaluations of insect DNA isolation protocols have similarly demonstrated that classical extraction approaches, including phenol–chloroform–based methods, frequently yield higher DNA quantities and improved integrity than several alternative procedures (Tatar et al. 2024). Although silica-based purification systems offer practical advantages in speed and handling, their performance may vary depending on sample complexity and tissue composition. The magnitude of yield differences observed in the present study—often exceeding an order of magnitude—indicates that extraction methodology is the primary determinant of DNA recovery in honey bee samples. Previous investigations have similarly emphasized that DNA yield and quality are highly dependent on the extraction protocol used, and that methodological optimization is essential for maximizing DNA recovery from insect tissues (Modabber et al. 2016). Comparative and review-based studies further highlight that no universal extraction method is suitable for all insect matrices, and that DNA recovery efficiency varies substantially depending on tissue composition and the selected protocol (Asghar et al. 2015). These observations support the conclusion that extraction strategy plays a decisive role in determining DNA yield in *A. mellifera* samples.

Influence of Body Segment on DNA Yield

Although body segment alone did not exert a statistically significant main effect on DNA yield, a significant interaction between extraction method and tissue type was detected. This suggests that while all body segments contain sufficient genomic material, the efficiency of DNA recovery depends largely on how effectively each method interacts with the biochemical composition of specific tissues. Previous studies have reported comparable patterns. Sudhagar et al. (2014) observed that differences in DNA yield among honey bee body parts were generally less pronounced than differences associated with extraction methodology, although thoracic tissues occasionally provided higher DNA concentrations due to their greater muscle content. Similarly, comparative studies in insect samples have emphasized that DNA yield and quality are strongly influenced by methodological factors, with extraction protocol often playing a more decisive role than tissue origin (Güler et al. 2018; Tatar et al. 2024). The slightly higher yields obtained from thorax and abdomen tissues in the present study may reflect increased cellular and mitochondrial content in these regions. However, the absence of a strong main effect of body segment indicates that, under optimized extraction conditions, all major body regions can serve as suitable sources of DNA for molecular analyses.

DNA Purity and Tissue-Specific Inhibitory Effects

In contrast to DNA yield, DNA purity showed a strong interaction between extraction method and body segment. Phenol–chloroform extraction consistently produced A260/A280 ratios within or close to the optimal range, whereas the commercial kit showed lower purity and greater variability, particularly in thorax and abdomen samples. These findings align with previous reports that have highlighted the presence of tissue-derived inhibitors in honey bee molecular studies. Boncristiani et al. (2011) demonstrated that certain honey bee tissues contain compounds that can interfere with PCR amplification, even when spectrophotometric purity values appear acceptable. Similarly, Weis et al. (2021) and Yadró et al. (2022) reported that lipid-rich and glandular tissues may introduce contaminants that are difficult to remove using rapid silica-based purification systems. The thorax and abdomen of honey bees are characterized by high protein content, lipid reserves, and glandular secretions, including wax-associated compounds. These components may co-purify with DNA during rapid kit-based extraction, leading to reduced purity and potential downstream inhibition. In contrast, the head contains relatively lower levels of such inhibitory substances, which likely explains the comparable purity values obtained between methods for head samples in this study. Comparable tissue-specific patterns were reported by Sudhagar et al. (2014), who found that DNA purity varied among body parts due to differences in biochemical composition.

DNA Integrity and Methodological Performance

Agarose gel electrophoresis revealed clear differences in DNA integrity between extraction methods. Phenol–chloroform extraction consistently produced high-molecular-weight DNA with minimal degradation, whereas commercial kit–derived samples often exhibited weaker bands and increased smearing. These results confirm that DNA integrity is closely linked to extraction chemistry and purification efficiency. Similar observations have been reported in previous comparative studies. Asghar et al. (2015) and Psifidi et al. (2015) emphasized that commercial kits, although rapid and

convenient, may result in partial DNA fragmentation or reduced recovery of high-molecular-weight DNA. Previous insect and food-derived DNA studies have further highlighted that DNA integrity and suitability for downstream applications are strongly influenced by extraction protocol and matrix complexity (Oppert et al. 2019; Soares et al. 2023). The present findings reinforce the concept that DNA quantity, purity, and structural integrity represent distinct but interrelated quality parameters. High DNA concentration alone does not guarantee suitability for downstream applications, as co-extracted inhibitors or fragmented DNA may compromise PCR amplification and sequencing performance (Asghar et al. 2015; Weis et al. 2021). A limitation of the present study is the absence of downstream validation (e.g., PCR amplification or sequencing), which would provide direct functional assessment of DNA suitability. Future studies should incorporate such analyses to further evaluate the practical performance of extraction methods.

Consistency with Previous DNA Extraction Comparisons

The overall superiority of phenol–chloroform extraction observed in this study is consistent with numerous comparative investigations across insect and animal systems. Modabber et al. (2016) and Issa et al. (2013) reported that extraction methodology plays a decisive role in determining DNA yield and purity, with conventional organic extraction protocols often providing reliable recovery of high-quality DNA from protein- and lipid-rich tissues. Likewise, comparative studies across different biological matrices have shown that although commercial kits offer advantages in speed, safety, and ease of use, conventional extraction methods may yield higher DNA yields and maintain structural integrity under certain conditions (Kek et al. 2018). These findings collectively support the conclusion that classical extraction methods remain highly relevant in molecular entomology, particularly when high-quality genomic DNA is required for demanding downstream applications such as sequencing, genotyping, or population genetic analyses.

Conclusion

In conclusion, this study demonstrates that DNA extraction method is the primary determinant of genomic DNA yield in honey bee (*A. mellifera*) samples, whereas DNA purity is shaped by method-dependent, tissue-specific effects. Across all body segments examined, phenol–chloroform extraction consistently produced higher DNA yields and stable purity values, confirming its robustness for isolating high-quality genomic DNA from structurally complex insect tissues. In contrast, body segment alone did not significantly influence DNA yield, indicating that head, thorax, and abdomen tissues provide comparable amounts of extractable DNA when effective extraction protocols are applied. Importantly, the significant method × body segment interaction observed for DNA purity highlights that the performance of commercial silica-based kits is more sensitive to the biological matrix of the sample. Reduced purity values in thorax and abdomen samples suggest incomplete removal of tissue-derived inhibitory compounds, whereas head tissues appeared less affected by method-dependent purification efficiency.

These findings emphasize that DNA concentration, purity, and integrity represent complementary but non-interchangeable metrics and should be jointly evaluated when assessing extraction success. From a practical perspective, the results indicate that phenol–chloroform extraction remains a reliable option for applications requiring high DNA yield and consistent quality, despite its labor-intensive nature. Commercial extraction kits offer advantages in speed and standardization but may require careful consideration of tissue type in honey bee molecular studies. Overall, this study provides methodologically sound guidance for selecting appropriate DNA extraction strategies in honey bees and underscores the importance of aligning extraction protocols with both the biological matrix and the intended downstream application.

AI Usage Statement: The authors declare that no artificial intelligence (AI)-assisted technologies were used in the design, analysis, or writing of this manuscript, except for minor language editing and reference organization.

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Conflict of Interest: The authors declare no conflicts of interest.

Data availability: All data generated or analyzed during this study are included in this published article.

Ethics Statement: This study did not involve human participants or vertebrate animals requiring ethical approval. Honey bee samples were collected under standard field and laboratory practices.

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