

Original Research Article

Morphological, Genetic and Chemical Composition Variations of Honey from the Honeybee (Hymenoptera; Apidae) *Apis mellifera* in Northern Iraq

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Abstract: *Objective:* The molecular genetic and phenotypic relationship between four populations of Iraqi worker honeybees (*Apis mellifera*) was studied. These four populations are from northern Iraq, specifically from four districts in the Sulaymaniyah Governorate. *Materials and Methods:* Fifty worker honeybees were collected from the Sulaymaniyah Governorate for the purpose of studying and determining their genetic and phenotypic structure. The phenotypic characteristics of the honeybees included in this study are: forewing length, forewing width, wing venation, number of cells per wing, presence of wing reticulum, dorsal abdominal color, ventral abdominal color, eye color, head length and width, and the extent of genetic similarity and difference between honeybee strains by determining the DNA sequence of the mitochondrial gene region (COX2). Gas chromatography (GC-Mass) was also used to analyze honeydew tissue from the honeybees. *Results:* The analysis showed that the molecular weight of the COX2 DNA amplification product in worker honeybees (*A. mellifera*) was 715 bp base pairs in 12 samples from the population. The results indicated slight genetic diversity in the form of point mutations of the deletion and substitution types. A total of 27 mutations were found in the COX2 gene, distributed across the districts of Sulaymaniyah Governorate. These mutations were translocations and substitutions. Regarding the phenotypic study, the results showed that head length was highest in Sulaymaniyah Governorate, reaching 5.1 mm. The genetic distance values revealed that the highest genetic distance was 0.021 between the districts of Juwarta in Sulaymaniyah Governorate, while the lowest was 0.002 between the districts of Sulaymaniyah City Center and Chamchamal in Sulaymaniyah Governorate. The remaining values varied. As for the honey material, the results of the gas chromatography (GC-Mass) showed that (19) organic materials were separated and identified in all the samples. The samples varied in their content of some of these materials or not, as well as in their percentages in the samples. The carbohydrate materials (sugars) mostly constitute a percentage exceeding 80%, meaning that the percentages in the table represent the percentage in the remaining 20% and not within the total percentage of materials in the honey.

Keywords: Honeybee, *Apis Mellifera*, Molecular Evaluation, Insect Extract.

INTRODUCTION

Honeybees have been known since ancient times as one of the most important social insects. Honeybee colonies are characterized by cooperation and division of labor, similar to other social insects such as ants and some wasps. The most important of these tasks is searching for food, providing it, and raising brood [1]. In Surah An-Nahl (The Bees), God Almighty accurately describes the characteristics of bees in terms of food and shelter, as well as the medical and economic benefits of their products, which researchers later discovered. He then summarizes this in verse 69, which encourages the mind to reflect on the lives of bees, their collective work, shelter, food, nectar collection, and caring for their nests, no matter how long or complicated the path. Thus, the two verses contain the essence of what researchers and studies have reached on the behavior of bees over the past years [2].

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The honey bee, *Apis mellifera* L., is an insect of economic importance in the production process of many agricultural crops, vegetable crops, and fruit trees, in addition to what bees produce of honey, pollen, royal jelly, wax, and propolis, which have medicinal and industrial importance, as well as providing job opportunities for a large group of workers [3]. Honey bees contribute significantly to increasing the rates of flower pollination, and thus the prevailing concept about the importance of the bee as an insect that produces honey and other materials has changed to an insect of great and wide importance in improving agricultural production, both quantitatively and qualitatively [4]. The development of molecular markers has provided methods that are accurate, fast, and save time and effort for better selection through early identification of the best performers. These markers utilize DNA and RNA in the body of a living organism, enabling researchers to overcome all the obstacles faced by previous methods.

MATERIAL AND METHOD

1- Collect Insect Samples:

Honeybee workers were collected from the apiaries belonging to the collection areas. They were collected from the northern region, represented by Sulaymaniyah Governorate, which included the districts of Jamjamal, Jawarta, the city center, and the Jabal Birmakron area. District. Fifty samples were collected from each study area during the period from September 1, 2025, to December 1, 2026. The samples were then sent to the Research Center and the Natural History Museum at the University of Baghdad for identification. The identification confirmed that the studied samples were the western honeybee, *A. mellifera*, which is the species required for the current study.

2-Sample Diagnosis:

The insects under study were diagnosed at the Research Center and Museum of Natural History, University of Baghdad, according to book numbered No. 56, dated 9/18/2025, where the diagnosis proved that the studied samples are the western honeybee *A. mellifera*, which is required for the current study.

3- DNA Extraction:

Body parts of the samples were used for DNA extraction. Genomic DNA was extracted because a large amount of body parts was required for the thoracoabdominal junction, which made it impossible to collect some data. In this case, the samples were cut into pieces, and both body and leg parts were used, then homogenized. The samples were first washed with distilled water and then stored in 90% alcohol for half an hour to ensure sterility. To store the ant material in the freezer for half an hour, the ant material was first ground with liquid nitrogen using a mortar and pestle, then boiled. After adding the proteinase K solution, homogenization continued in a water bath for 3 hours, alternating between half an hour at 60°C and half an hour without heat [5].

4- DNA Concentration and Purity Measurement:

Nano droplet device was used to determine the concentration and purity of DNA. A single drop of extracted DNA was placed in the sample compartment of the device. The device was then switched on and the computer began measuring the DNA after the device was set to Elution Buffer (Tris-HCL, pH 8.5). The device displayed the desired measurement on the computer screen, using Nano grams/ml as the unit of measurement. The sample was then diluted to a concentration of 50 ng/ml at -80°C until it was used for molecular profiling studies. The mitochondrial COI gene was amplified, and a nucleotide sequence for this gene was generated and completed. It was then matched with the global gene bank to determine the evolutionary lineage of the ants in this research. The nucleotide sequences were designed by [6].

5- PCR Amplification:

Amplification by the polymerase chain reaction (PCR) is fundamental to genetic analysis of the ants sampled in this study. A suite of PCR protocols are outlined, with specific primers provided Table 1. These will target two loci, the mitochondrial cytochrome oxidase 1 (COI) gene, which is used as a standard locus in insect systematics, using a PCR device to obtain the COI gene product according to the following reaction as shown in the Table 2 and Table 3:

Table 1: Shows the materials involved and their concentrations in the PCR reaction

Component	volume	Final concentration
Nuclease-free water	21 µl	-----
Gotaq® Green Master Mix, 2X	25µl	1X
Forward primer, 10µM	1µl	0.2 µM
Reverse primer, 10µM	1µl	0.2 µM
DNA-template	2µl	< 250 ng

Table 2: Shows the primer used in the study and its sequences

Primer	Sequence (5'-----3')	Company/Origin
COX2	F ATATGGCAGAATAAGTGCATTGAACT	Macrogen South Korea
	R TGTAATTAAACGTAGTGGGATTTTATTGGA	

Table 3: Shows the components of the main reaction mixture

Step	Temperature (°C)	Time	No. of Cycles
Initial denaturation	95	2 minutes	-
Denaturation	95	30 second	40
Annealing	55	30 second	
Extension	72	45 second	
Final extension	72	5 minutes	-

6-Data Analysis:

The molecular weight (MW) was calculated using computer software, which detects the images of the bands produced by the PCR reaction and compares them with the known size of the DNA ladder indicator bands, which includes 36 bands from 100 to 2000 bp.

7-DNA Sequencing:

The purification process of the PCR reaction product containing the gene to be studied is carried out according to the purification kit (Gel/PCR Fragment Extraction Kit Protocol) prepared by Bionner. At this stage, we obtain pure DNA for the studied genes, COX2. After completing the purification process of the polymerase chain reaction product containing the gene for which we want to find the nitrogenous base sequence, the DNA samples are sent to the Bioneer company in North Korea to perform the sequence of the nitrogenous bases for the studied gene, COX2.

8- Phenotypic Study of the Studied Specimens:

After collecting samples from the studied sites, we take 24 samples for phenotypic study directly to the laboratory for dissection, following the steps outlined by researchers [7], (with some modifications as follows:).

9-Dissection of Specimens:

1. The insect was placed under a dissecting scale to carefully separate the wings using forceps, taking care to prevent the specimen from drying out by adding a few drops of distilled water. The wings were then placed in 71% ethanol in numbered bottles (forewings, hindwings, right, and left) until the dissection was complete.
2. Separate the insect's head from its thorax using forceps and a scalpel, and do the same for the antennae and mouthparts. Place them in a square container with 25 compartments and a portable container. Sodium hydroxide (NaOH) at a concentration of 11%.
3. The thorax was separated from the abdomen, then the hind and middle legs were separated from the thorax, and then they were placed in a Petri dish.

10-Gas Chromatography:

As the name suggests, gas chromatography uses an inert gas to carry the sample during the separation process. This gas acts as the mobile phase, transporting the sample molecules through the chromatograph ideally without reacting with the molecules or damaging the apparatus.

The sample enters the chromatograph via injection or is transferred from a sampling device that allows for the collection of chemical components from solid or liquid sample arrays. The sample is then injected through the apparatus inlet via a membrane that injects the sample without allowing any loss or leakage of the gas component of the mobile phase. The loading column, a long (ranging from 11 m to 151 m) narrow tube (with an internal diameter of 1.1 mm to 1.53 mm) made of Smica or metal, is then connected to the inlet.

The stationary phase is saturated with a very high internal ionization charge. The loading column is then heated using heaters in the furnace during the loading process, initiating the separation of components. Materials are separated from least volatile to most volatile.

RESULTS AND DISCUSSION**1. Results of Amplification of the COX2 Gene**

Figure (1) shows the amplification of the COX1 DNA gene from the mitochondria of honeybee workers *A. mellifera*, which were collected from different regions of northern Iraq (Sulaymaniyah), central Iraq. The analysis results showed that the molecular weight of the bands resulting from the electrophoresis of the amplified COX1 DNA gene from

honeybee workers *A. mellifera* is 715bp base pairs. This result is consistent with a number of international studies conducted in the same field, as well as the same mitochondrial DNA gene from honeybee workers *A. mellifera*, for example, the study conducted by [7]. He studied three DNA sites, where the COX1 gene was one of the genes he studied, and he also used the polymerase chain reaction technique. The results they reached in studying this gene of the honeybee insect go back to the evolutionary line C, which represents the type of the northern Mediterranean region. Other studies are those conducted by [8]. In Saudi Arabia, where he used the polymerase chain reaction technique and also conducted the sequencing of the COX1-COX11 region of mtDNA, the results of the sequence analysis showed that all the various copies belong to the evolutionary line O, as well as other studies similar to those he conducted [9], where he studied three genes of mitochondrial DNA, namely 16SrDNA, COX1 and ND5, where he studied 15 different communities of honeybee workers in Turkey using the RFLP technique and used 17 types of restriction enzymes. After conducting the PCR amplification process for the aforementioned gene, as well as conducting the sequencing and also comparing the resulting sequences with the sequences of the gene bank, where he found 6 types of various copies. Finally, the study he conducted [10], on the genetic region (COX1-OX11) of mitochondrial DNA, where it shows a very high accuracy of genetic diversity for samples taken from the southern and central regions of the United States, where he used 469 samples from 14 countries to find out the DNA sequence, where Four mitotypes were found from the Middle East, i.e. in the (O) region.

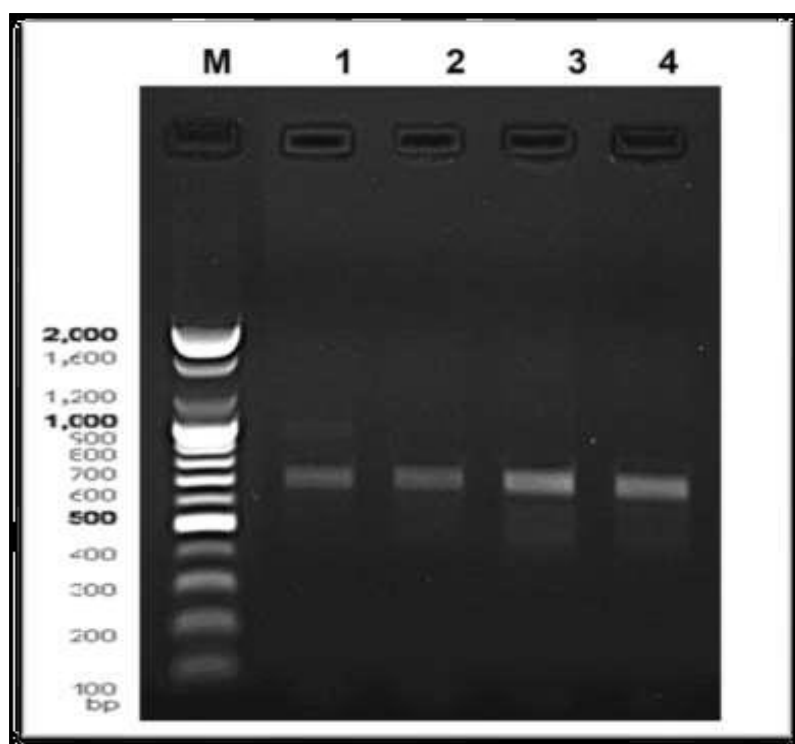


Figure 1: The amplification result of the cox2 gene in the mitochondrial DNA of honey bee workers *Apis mellifera* in Sulaymaniyah Governorate

2-Studying of Nitrogen Bases Sequencing:

A sequential nitrogen sequencing study was conducted on samples from honeybee workers collected from the studied areas in Sulaymaniyah Governorate. The extended method was used to study the nitrogenous base sequence of the three genes COX1 and ND5 found in mitochondrial DNA. Although this method is expensive, it gives us very accurate results. In addition, it is characterized by the ability to detect any sequence of nitrogenous bases in which a change or mutation occurred, even if that change occurred in only one nitrogenous base, without relying on obtaining ready results. After reviewing what was published of nitrogenous base sequences in global studies, research, and on the website of the global gene bank located at the American National Center for Biotechnology Information (NCBI) on the Internet, in order to carry out the process of comparison or matching between the results of the study of the studied samples and similar samples of the same studied species, so that there are no differences between them at the species level and on these matching samples that are found in the data bank, the sequence of the bases was extracted Nitrogenous and using the ready-made program to analyze the results of the sequential sequence of nitrogenous bases of genes called Bioedit, the study of the sequences of nitrogenous bases by the device may not be clear enough, so a part of the nucleotide sequence is left for the ends of the studied genetic region, where all the distinct and very clear sequences are read and published only to avoid any error, even if it was simple, so that we obtain results that are as clear and accurate as possible.

3-Comparison between *A. Mellifera* Honeybee Populations in Terms of the Sequence of the COX2 Gene

The sequence of the COX2 gene region in mitochondrial DNA was compared, and the group from northern Iraq in Sulaymaniyah Governorate, represented by Jamjamal district (sy1), Jawarta district (sy2), Sulaymaniyah center (sy3), and Jabal Birmekron (sy4), were compared. In order to compare the sequences of the samples of the population communities of the selected sites during the study, and also to conduct the process of Matching with global samples in the data bank, Table (1) represents a comparison of the sequencing results between the samples of the studied regions in the nitrogenous base sequences of a part of the COX2 gene, where some of the nucleotide sequences were left at its left end because they were not clear enough, so the distinct and clear sequences were taken starting from site 1 to site 439 to maintain the accuracy of the results and moreover to avoid falling into any error, where the sequencing results of the samples from the north from some regions or districts of Sulaymaniyah Governorate were clarified in it, as it was shown that the number of mutations for all samples is 27 mutations, All of them are replacement and transfer units, specifically in the northern region, represented by the Sulaymaniyah Governorate.

A similar study was conducted on the COX1-COX2 gene region of mitochondrial DNA, which was conducted by [12]. The aim of this study was to know the genetic and quantitative diversity of the honey bee population in southern Iran. Honey bee workers were collected from 6 different regions and the PCR-RFLP technique was used in the presence of Dra I restriction enzymes. It was found that the honey bee in the southern region of Iran belongs to the evolutionary line C. Another result of this same study, which was obtained, is the absence of genetic diversity among the samples collected from southern Iran.

4- Results of Genetic Variation Based on COX2 Results for Gene DNA-Sequences

A- Sample sequence (1) from *A. Mellifera* Sulaimaniyah:

Sample (1), isolated from *A. mellifera*, was analyzed using DNA sequencing techniques. The results from the Jumjamal (Sulaimaniyah) sample revealed three mutations, all of which were translocations. At position 3638, thymine (NCBI ID:OM) was translocated to the cytosine position, compared to the control sample 203338.1. At position 3773, cytosine was translocated to the thymine position, and at position 3813, thymine was translocated to the cytosine position. The homology ratio was 99%.

Table 4: Nitrogen base variations in sample (1) in Sulaymaniyah Governorate

nitrogenous base	the site	Type of variation (mutation)
T\C	3638	transmission
C\T	3773	transmission
T\C	3803	transmission

B- Sample Sequence (2) from *A. Mellifera* Sulaimaniyah:

Based on the results of DNA-sequencing technology for the *A. mellifera* species, sample [2], isolated from the Juwarta district (Sulaymaniyah), this sample obtained 6 mutations, all of which are transition mutations compared to the standard sample NCBI ID:OM203338.1. At position 3632, adenine was transposed to the guanine site, at position 3638, thymine was transposed to the cytosine site, at position 3655, adenine was transposed to the guanine site, at position 3773, cytosine was transposed to the thymine site, at position 3793, adenine was transposed to the guanine site, and at position 3801, adenine was transposed to the guanine site, with a similarity rate of 99%.

Table 5: Nitrogen base variations in sample (2) in Sulaymaniyah Governorate

nitrogenous base	the site	Type of variation (mutation)
A\G	3632	transmission
T\C	3638	transmission
A\G	3655	transmission
C\T	3773	transmission
A\G	3793	transmission
A\G	3801	transmission

C- Sample Sequence (3) from *A. Mellifera* Sulaimaniyah:

Based on the results of DNA-sequencing technology for the *A. mellifera* type, sample (3) isolated from the Al-Markaz district (Sulaymaniyah), this sample obtained two mutations, both of which are transfer mutations compared to the standard sample NCBI ID:OM203338.1. At position 3638, thymine was transferred to the cytosine site, and at position 3773, cytosine was transferred to the thymine site, with a similarity rate of 99%.

Table 6: Nitrogenous base variations in sample (3) in Sulaymaniyah Governorate

nitrogenous base	the site	Type of variation (mutation)
T\C	3638	transmission
C\T	3773	transmission

D- Sample Sequence (4) from *A. Mellifera*_ Sulaimaniyah:

Based on the results of DNA-sequencing technology for the *A. mellifera* species, sample [4], isolated from the district of Jabal Birmakron (Sulaymaniyah), this sample obtained 4 mutations, all of which are transfer mutations compared to the standard sample NCBI ID:OM203338.1. At position 3638, thymine was transferred to the cytosine site, at position 3773, cytosine was transferred to the thymine site, at position 4010, cytosine was transferred to the thymine site, and at position 4023, cytosine was transferred to the thymine site, where the similarity rate was 99%.

Table 7: Nitrogen base variations in sample (4) in Sulaymaniyah Governorate

nitrogenous base	the site	Type of variation (mutation)
T\C	3638	transmission
C\T	3773	transmission
C\T	4010	transmission
C\T	4023	transmission

5- Results of Honey Analysis in the Studied Samples Using Gc-Ms Technology:

Nineteen (19) organic substances were isolated and identified in all samples. The samples varied in the presence or absence of some of these substances, as well as in their percentages. Table shows these substances, their retention times, and their percentages in each sample. Figures show the chromatograms of analysis for each sample included in the study, using the Gc-Ms technique. From Table we observe the identification of 19 substances. For example, substances in sequences [7-14], were present in most samples, except for one sample, (Sulimanya-bermicron). It should be noted that the percentages specified in the table represent the percentages in other honey components, excluding carbohydrates (sugars), which generally constitute more than 80%. Therefore, the percentages in the table represent the percentage in the remaining 20%, not the total percentage of substances in the honey.

6- Results of Honey Analysis for the Samples Studied in Sulaymaniyah Governorate Using the Gc-Ms Technique:

The results showed that 19 honey components were identified in the studied samples from Sulaymaniyah Governorate, as detailed in the table. These 19 components were distributed across four districts within the governorate. The longest retention time was observed for Octadecanoic acid derivatives, ranging from 18 to 23 minutes, while the shortest retention time was for oxalic acid cyclobutyl nonyl ester, at 3.05 minutes. This retention time was consistent across all samples studied in all governorates included in the study.

In the Juwarta district of Sulaymaniyah Governorate, the highest percentage of this component was 39.14%, with a retention time of 13.2 minutes. The lowest percentage was for Carbamic acid (3,4,4-trimethyl-1,2-dioxetan) at 0.04%, with a retention time of 3.58 minutes. The remaining components varied between these two percentages.

**Figure 2: Chromatogram analysis of the study of its neighbors in the Sulaymaniyah Governorate, which is included in the study, using the Gc-Ms technique.**

As for the Chamchamal district of Sulaymaniyah Governorate, the highest percentage was for 2,6-dihexadecaoate (34.03%) with a retention time of 12.40 minutes, while the lowest percentage was for 2-methyl octacosane (0.72%) with a retention time of 8.30 minutes. The remaining substances varied between these two percentages.

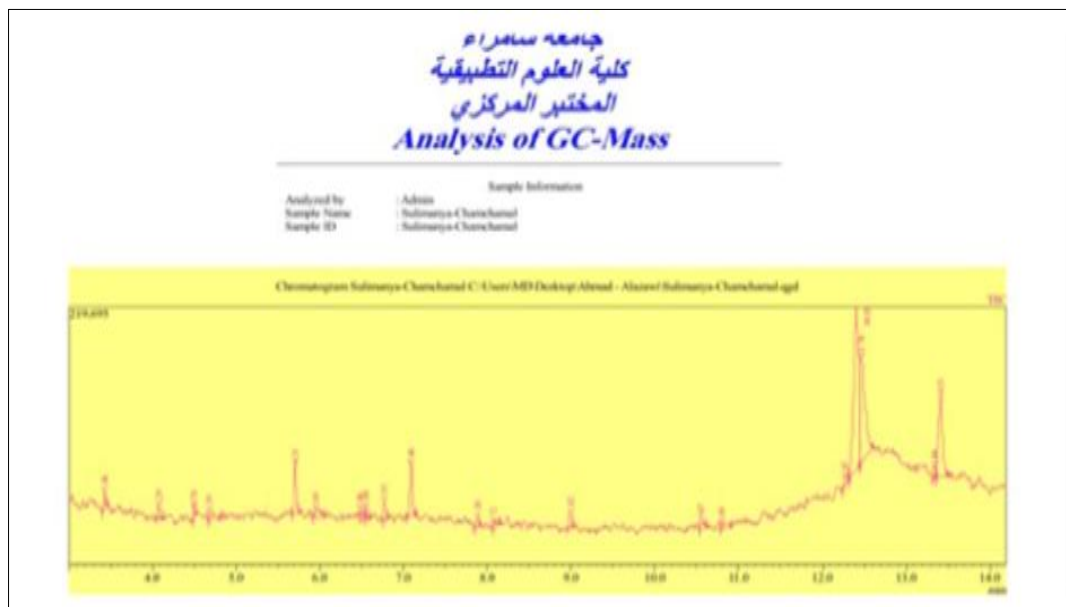


Figure 3: Chromatogram analysis of Chamchamal district in Sulaymaniyah Governorate included in the study by Gc-Ms technique

As for the Sulaymaniyah Central District of Sulaymaniyah Governorate, the highest percentage was for Tetradecane at 11.32% with a retention time of 7.10 minutes, while the lowest percentages were for Oxalic acid, bis(6-ethyloct-3-yl) ester and Pentadecane, 2,6,10,14-tetramethyl at 2.66% with retention times of 6.60 minutes and 6.77 minutes, respectively. The remaining substances varied between these two percentages.

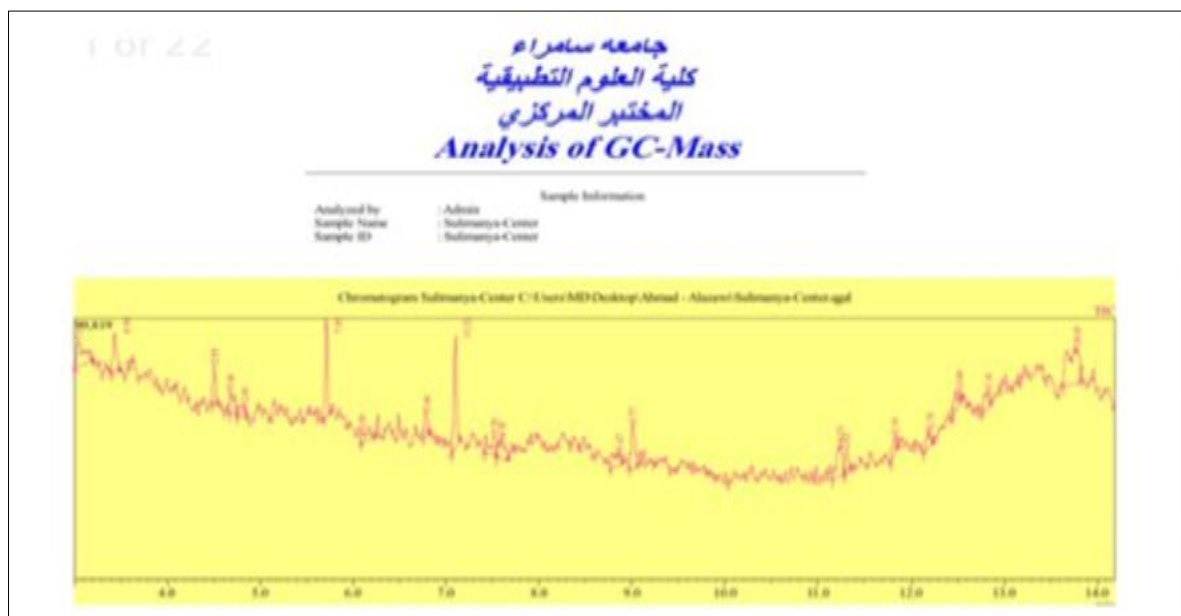


Figure 4: Chromatogram analysis of the central district in Sulaymaniyah Governorate included in the study by Gc-Ms technology

As for the district of (Mountain) Pirmakron, which belongs to the Sulaymaniyah Governorate, all substances showed a percentage of 0% except for the substance Octadecanoic acid- Derivatives 2.10%, with a retention time of 18.23 minutes.

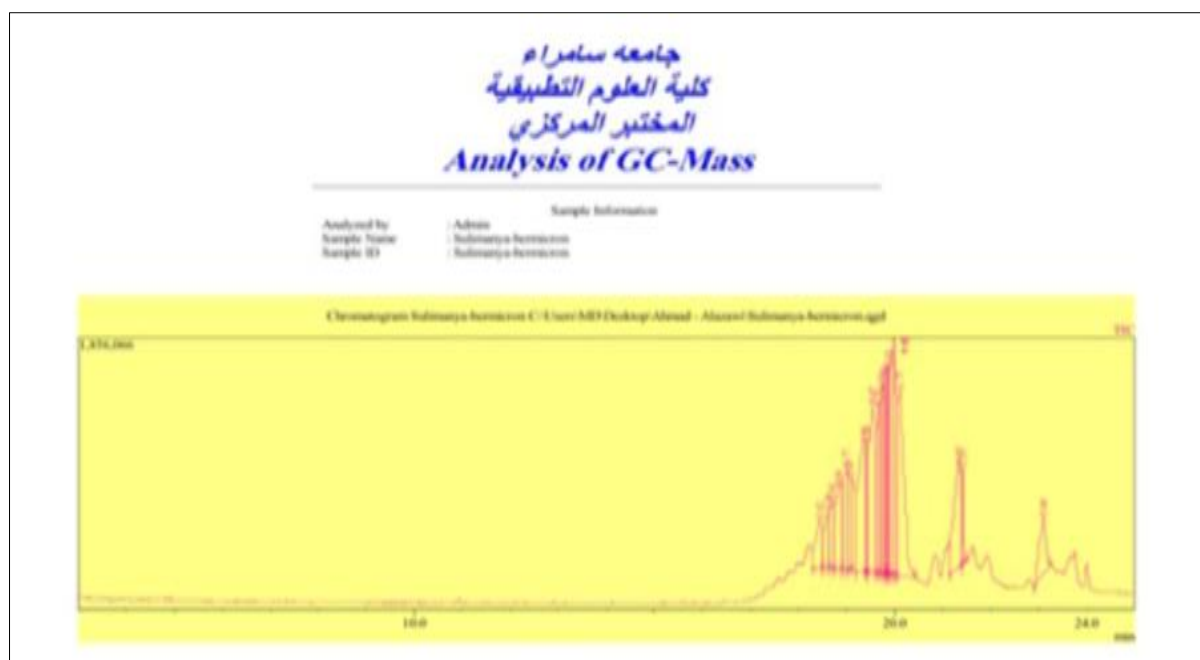


Figure 5: Chromatogram analysis of the district (mountain) of Pirmakron in the Sulaymaniyah Governorate included in the study by Gc-Ms technique.

7- Results of the Morphological Characteristics of Honeybee Samples from the Study Areas:

Several morphological characteristics of the honeybee were studied according to the regions included in the study, which include the northern region, represented by the Sulaymaniyah Governorate, which includes four districts. These morphological characteristics are the length of the forewing, the width of the forewing, the veining of the wings, the number of cells in the wing, the presence of the wing meshing apparatus, the color of the dorsal abdomen, the color of the ventral abdomen, the presence of hairs in the forehead region between the compound eyes and on the back, the color of the eyes, the length and width of the head. These characteristics include all samples from the regions included in this study.

8- Results of Phenotypic Characteristics in Sulaymaniyah Governorate

The above-mentioned phenotypic characteristics were studied in the Sulaymaniyah Governorate, which included four districts: Chamchamal District, (Jabal) Pirmakron District, Sulaymaniyah Center District, and Juwarta District. The results for samples from Sulaymaniyah Governorate showed that forewing length was highest in Chamchamal District at 10.1 mm, while it was lowest in Sulaymaniyah City at 9.8 mm. The remaining districts showed a range between these two sizes. In the districts of Jabal Birmakrun and its surrounding areas, forewing length was 10 mm, indicating a close correlation between the results. Forewing width was highest in Sulaymaniyah City at 4.2 mm, while it was lowest in the samples from Chamchamal and its surrounding areas at 3.8 mm, and 4 mm in Jabal Birmakrun. These results do not align with the study by [14]. Which found a forewing length of 8.85 mm and a forewing width of 3.15 mm for worker honeybees of the species *Apis mellifera ligustica*. This finding is consistent with, or close to, the study by [15], which found that the average forewing length and wing width in honeybees of the species *Apis mellifera carnica* were 8.41–9.37 mm, 4.74–6.46 mm, and 3.05–3.31 mm, respectively. This result is also consistent with the study by [16], which found the highest average forewing length to be 10.700 mm in the strain *Apis mellifera florea*.

As for wing venation, all were identical in terms of longitudinal venation. The highest number of cells in the forewing was found in the Sulaymaniyah district center, reaching 6 cells, while the number of cells was equal in the rest of the districts, at only 5 cells each. As for the wing netting device, it was identical in all the district samples. It was of the hook type, where the number of hooks was identical between the districts.

As for the color of the abdomen from the dorsal side, in the district of Chamchamal, the color of the abdomen from the dorsal side was black to gray with a dark brown band on the second and third abdominal rings and a gray or lead band on the fourth and fifth abdominal rings. In the district of (Jabal) Pirmakron, the color of the abdomen from the dorsal side was black gray with a reddish-brown (orange) band on the second, third and fourth abdominal rings and a light gray or lead band on the fifth and sixth abdominal rings. In the district of Sulaymaniyah Center, the color of the abdomen from the dorsal side was black gray with a reddish-brown band on the second, third and fourth abdominal rings and a light lead band on the fifth, sixth and seventh abdominal rings. In the district of Juwarah, the color of the abdomen from the dorsal side was blackish-gray with a reddish-brown (orange) band on the second, third and fourth abdominal rings and a blackish-gray band on the fifth and sixth abdominal rings.

As for the color of the abdomen from the ventral side, in the district of Chamchamal, the color of the abdomen from the ventral side was dark gray with a reddish-brown band on the second, third and fourth ventral rings with a dark gray or black band on the fifth, sixth and seventh ventral rings. In the district of (Jabal) Pirmakron, the color of the abdomen from the ventral side was dark gray with a reddish-brown band on the fifth, sixth and seventh ventral rings. In the district of Sulaymaniyah Center, the color of the abdomen from the ventral side was dark gray with a reddish-brown band on the third, fourth, fifth, sixth and seventh ventral rings. In the district of Juwarah, the color of the abdomen from the ventral side was dark gray with a reddish-brown band on the fifth, sixth and seventh ventral rings.

Regarding head characteristics (head length and width), head length was highest in the districts of Chamchamal and Sulaymaniyah city, reaching 5 mm, while it was lowest in the district of (Jabal) Pirmakrun at 4.5 mm, and in the district of Juwarta at 4.8 mm. Head width was also highest in the districts of Chamchamal and Sulaymaniyah city, reaching 4.5 mm, while it was lowest in the districts of (Jabal) Pirmakrun and Juwarta at 4 mm. Thus, all phenotypic characteristics were similar across the four districts of Sulaymaniyah Governorate, indicating their proximity and similar environmental conditions. These results are consistent with those of [16], whose findings showed the highest mean length and width for the forewings to be 5.57 and 4.55, respectively.

The results showed slight differences between the morphological characteristics of the body and wings of the *A. mellifera* honeybee subspecies in the studied areas of Sulaymaniyah Governorate. The highest values for wing length, wing width, head length, and head width were recorded in Chamchamal District, which gives the bee colonies of this district a greater ability to collect nectar, pollen, and wax secretion, while the rest of the districts recorded the lowest values for these, which gives the bee colonies of these districts a lesser ability to collect nectar, pollen, and wax secretion. This indicates the small size of the bees in these districts compared to other areas (Chamchamal District) in terms of external morphological characteristics.

The methods of raising honeybees vary from one region to another, and this variation results in phenotypic changes. One of these methods is the introduction of geographical patterns (subspecies) in an unstudied manner, which leads to hybridization between colonies and a difference in phenotypic traits [17]. demonstrated in a study of the phenotypic traits of the honeybee strain *A. m. intermissa* that all samples were similar in the traits of forewing length, forewing width, proboscis length, antenna length, number of hooks, and other traits. Furthermore, the body size and proboscis length traits of honeybees are positively correlated with the phenotypic traits of flowers, reflecting their vital role in foraging, honey production, and pollen collection [18]. These traits vary from region to region [19], noted variations in head length, width, and proboscis length in regions of India, reaching 3.19 mm, 3.78 mm, and 6.37 mm respectively [20], studied the phenotypic variation of the Iranian honeybee *A. m. meda* in 20 provinces of Iran reported a large variation in the studied phenotypic traits (proboscis length, first sternal plate index, shield color, third dorsal plate color, hind leg length, third and fourth dorsal plate length, forewing length and width, hindwing length and width, forewing index, and angles A4, D7, and G18) in all studied regions. There were no 5% significant differences in abdomen color, reflecting the great diversity of Iranian bees.

As for the wing venation characteristics in the studied areas of Sulaymaniyah Governorate, the highest measurement in the width of the forewing was recorded in the Sulaymaniyah Central District and the lowest measurement in the districts of Chamchamal and its surroundings. Wing characteristics give a wider range of flight distances and these measurements are important in classifying subspecies whose size affects the ability of honeybees to fly [21]. The difference in wing venation characteristics may be due to several factors. One of these factors is the presence of different lineages among the bee populations, as well as other factors such as spatial differences that affect the morphological measurements of honeybees [22].

CONCLUSIONS

There is clear genetic variation among the studied samples depending on the environment in which the honeybees live. Scotch-synthesis is one of the best techniques for detecting genetic variation, even at the level of a single nitrogenous base. The phenotype of bees varies according to the environmental conditions in which they live. The chemical composition of honey varies according to diet, environment, and genetics.

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