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Molecular evaluation of honey bees (Apis mellifera) in different regions of Iraq

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Abstract

The study aimed to find the genetic composition and the extent of genetic similarity and difference between honeybee strains from different regions of northern, central and southern Iraq by determining the DNA sequence of some mitochondrial gene regions (COX1 and ND5). The study was conducted in the laboratories of the College of Science - Department of Life Sciences at Tikrit University, where samples were collected from northern, central and southern Iraq. During the period from 8/13/2023 to 3/1/2024. Samples were collected and DNA was isolated according to the Genomic DNA Mini Kit Tissue Protocol. DNA concentration and purity were then determined, followed by electrophoresis to confirm DNA concentration and purity. Mitochondrial genes were engineered and polymerase chain reaction (PCR) was performed. The samples were sent to South Korea for nucleotide sequencing. The results showed that 22 mutations were detected for the COX1 gene, with the highest number of mutations appearing in northern Iraq, represented by Sulaymaniyah Governorate, where it was 10 mutations, while the lowest number appeared in southern Iraq, represented by Basra Governorate, where it was only 4 mutations, while in central Iraq, represented by Salah al-Din Governorate, it was 8 mutations. Large differences appeared between northern Iraq, which was 10 mutations, and southern Iraq, which was 4 mutations. The reasons for these differences are the different climatic conditions between the north and the south, as well as the great geographical distance between the north and the south.

Keywords: *Apis mellifera*, , Molecular evaluation, Insect extract.

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التقييم الجزيئي لنحل العسل (Apis mellifera) في مناطق مختلفة من العراق

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الملخص
هدفت الدراسة إلى معرفة التركيب الجيني ومدى التشابه والاختلاف الجيني بين سلالات نحل العسل من مناطق مختلفة من شمال ووسط وجنوب العراق، وذلك من خلال تحديد تسلسل الحمض النووي لبعض مناطق جينات الميتوكوندريا (COX1 و ND5). أُجريت الدراسة في مختبرات كلية العلوم - قسم علوم الحياة بجامعة تكريت، حيث جُمعت عينات من شمال ووسط وجنوب العراق. خلال الفترة من 2023/8/13 إلى 2024/3/1. تم جمع العينات وعزل الحمض النووي وفقًا لبروتوكول الأنسجة Genomic DNA Mini Kit. ثم تم تحديد تركيز الحمض النووي ونقاوته، تلا ذلك إجراء الرحلان الكهربائي لتأكيد تركيز الحمض النووي ونقاوته. تم هندسة جينات الميتوكوندريا وأجري تفاعل البوليميراز المتسلسل (PCR). تم إرسال العينات إلى كوريا الجنوبية لإجراء تسلسل النوكليوتيدات. أظهرت النتائج أنه تم الكشف عن 22 طفرة لجين COX1، مع ظهور أعلى عدد من الطفرات في شمال العراق، ممثلة بمحافظة السليمانية، حيث بلغ 10 طفرات، بينما ظهر أقل عدد في جنوب العراق، ممثلة بمحافظة البصرة، حيث بلغ 4 طفرات فقط، بينما في وسط العراق، ممثلة بمحافظة صلاح الدين، بلغ 8 طفرات. ظهرت اختلافات كبيرة بين شمال العراق، حيث بلغ 10 طفرات، وجنوب العراق، حيث بلغ 4 طفرات. أسباب هذه الاختلافات هي اختلاف الظروف المناخية بين الشمال والجنوب، وكذلك المسافة الجغرافية الكبيرة بين الشمال والجنوب.
الكلمات المفتاحية : Apis mellifera :، التقييم الجزيئي، مستخلص الحشرات.

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).

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

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. They were also collected from the central region, represented by Salah al-Din Governorate, which included the governorate center (Tikrit District), Al-Ishaqi Subdistrict, Balad District, and Al-Dujayl District. They were also collected from the southern region, represented by Basra Governorate, which included Al-Hartha District, Al-Qurna District, Shatt al-Arab District, and Abu Al-Khaseeb District. Fifty samples were collected from each study area during the period from September 1, 2022, to December 1, 2022. 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.

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 9/18/2024, where the diagnosis proved that the studied samples are the western honeybee A. mellifera, which is required for the current study.

Molecular Genetic Study

DNA Extraction

The extraction process was carried out according to the method Genomic DNA Mini Kit Tissue Protocol

Extraction method

First: Sample preparation and analysis:

1.Grind or crush 10–20 mg of the insect in liquid nitrogen.

2- Transfer the mixture to an Eppendorf tube.
3- Immediately add 800 µL of heated lysis buffer and then vortex the mixture to resuspend the sample.
4- Place the tube (mixture) in a water bath at 65°C for 20–30 minutes, mixing the sample by inverting the tube every 5 minutes.
5- Transfer the sample to a new Eppendorf tube, then add 600 µL of chloroform and vortex it for 30 seconds.
6- Place the tube in a centrifuge at 13,000 rpm for 5 minutes to precipitate insoluble particles.
7- Transfer 600 µL of the supernatant to a new Eppendorf tube

Second: DNA Binding

1- Add 900 µL of DNA binding solution and mix well by briefly stirring for 10 seconds.
2- Place the spin column and transfer 750 µL of the solution to the column.
3- Centrifuge for 1 minute at 11,000 rpm at room temperature. Discard the filter and place the spin column in the same 2 ml collection tube.
4- Add the remaining 750 µL of the mixture to the column and repeat the centrifugation process for 1 minute at 11,000 rpm.

Third: DNA Washing 1

1- Add 500 microliters of Wash Buffer 1 and centrifuge for 1 minute at 10,000 rpm.
2- Discard the filter and place the spin column in the same 2 ml collection tube.

Fourth: DNA Washing 2

1- Add 500 microliters of Wash Buffer 2 and centrifuge for 1 minute at 13,000 rpm.
2- Discard the filtered solution and place the spin column in the same 2ml collection tube.
3- Centrifuge for an additional 3 minutes at 13,000 rpm to remove any remaining ethanol.

Fifth: DNA extraction

1- Place the spin column in a new 1.5 ml tube.
2- Add 100-150 µL of preheated DNA elution solution (60°C) to the column and place it at room temperature for 5 minutes.
3- Centrifuge for 2 minutes at 11,000 rpm at room temperature.
4- Discard the column. It can be used immediately or stored at 4°C for later analysis. For long-strand DNA, store it at -20°C.

Determination of concenteration and purity for DNA:

DNA concentration was measured to estimate purity using a Nano Drop device. The absorbance of the UV spectrum was measured using a spectrophotometer at a wavelength of (260) nanometers. One (1) microliter of genomic DNA was taken and placed in the device. The device was then ordered to measure. This device provides concentrations in ng/ml. This device measures the concentration and purity of genomic DNA with the smallest amount of DNA. Purity is estimated by dividing the absorbance at a wavelength of (260) nanometers by the absorbance reading at a wavelength of (280) nanometers (5).

Gel Electrophoresis
Solutions used in the electrophoresis process:

1- Loading buffer (6x) :
Prepared by dissolving (0.25) g of bromophenol blue dye in (50%) glycerol, and adding (60) mM EDTA. The pH was then adjusted to (8.0) and the volume was completed with (100) ml of distilled water.
2- Sodium Borate Buffer (SB) solution (10x):
This solution was prepared at a 10x concentration by weighing (4) g of sodium hydroxide (NaOH) and dissolving it in (800) ml of distilled water. The pH was then adjusted to (8.5) using boric acid, and the volume was completed with distilled water to (1000) ml.

Method of preparing agarose gel and DNA electrophoresis:

To transfer the extracted genomic DNA, as well as detect it and estimate its molecular size, agarose gel was prepared at a concentration of (1%) and dissolved using a heat source, which is the microwave, for two minutes or until it boils once, and then it is left in the room atmosphere until it reaches a temperature of (50-60) degrees Celsius, then (3) microliters of Red Safe dye are added, and the method is as follows:
1- The gel solution was poured into a special tray in the electrophoresis device after the comb was installed to form wells at one end of the gel. Pouring was done gently to prevent bubbles from forming. If bubbles do form, they must be removed using a micropipette. The gel was then left to solidify.
2- The comb was then removed, and the tray was placed in the electrophoresis tank, which contained a suitable and sufficient amount of SBX1 solution to cover the loading wells.

3- Electrophoresis samples were prepared after mixing (5) microliters of the DNA sample with (3) microliters of loading buffer, using a micropipette for genomic DNA and also for the DNA volume indicator in a special well on one side of the agarose gel.

4_ The electrophoresis device is then operated using an electric current with a potential difference of (3) volts/cm, with the electrodes adjusted. The gel must be directed from the sample side toward the negative pole, and the sample migration direction must be toward the positive pole until the sample reaches the end. This process takes (30-35) minutes.

5- After completing the electrophoresis process, the gel is examined by exposing it to ultraviolet (UV) rays at a wavelength of (260) nanometers using a UV_Transilluminator device to visualize the DNA bands.

Specific polymerase chain reaction (PCR)

Specialized primers were used, namely:

(ND5 Gen, COX1 Gen) We arrange the nitrogenous bases in a customized and specific sequence which is made by Bioneer-Korea.

Table (1) shows the primers used in the study and their sequences

Primer	Sequence (5'----------3')		Company/Origin
COX1	**F**	TGGAGGATTTGGAAATTGGCTTATTC	**Macrogen South Korea**
	R	TCAACAGTAATAAGAATCTGGATAGTCTGA	
ND5	**F**	GGTTGAGATGGTTTAGGATTAATTTCTTATTG	
	R	CGAAATGAATAGGATACAGTAAAAATTGTACC	

How it works :

Table (2) shows the components of the main reaction mixture.

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 (5) shows the conditions for the Gene Specific – PCR COX1 reaction.

Step	**Temperature (°C)**	**Time**	**No. of Cycles**
Initial denaturation	**95**	**2 minutes**	-
Denaturation	**95**	**30 second**	**40**
Annealing	**60.5**	**30 second**	
Extension	**72**	**1 minute**	
Final extension	**72**	**5 minutes**	-

Table (4) shows the conditions for the Gene Specific – PCR ND5 reaction.

Step	**Temperature (°C)**	**Time**	**No. of Cycles**
Initial denaturation	**95**	**2 minutes**	-
Denaturation	**95**	**30 second**	**40**
Annealing	**53**	**30 second**	
Extension	**72**	**45 second**	
Final extension	**72**	**5 minutes**	-

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Gel Electrophoresis of the PCR reaction mixture

1- The gel was prepared by measuring 1 gram of acarose gel using a sensitive balance into 100 ml of TBE buffer at 1x strength. The gel was placed in a 500 ml graduated glass beaker. The beaker was then placed on a heating device, and a cellophane cover was placed over the opening of the beaker to prevent evaporation. The temperature reached 100°C until the mixture was completely dissolved, until bubbles and undissolved materials were removed. The mixture was then left to cool to 40°C–50°C.

2- Add 5 ml of Readsafe dye to the gel in the beaker, shaking the contents continuously for ten seconds.

3- Prepare the gel-pouring basin and place the comb in it before pouring the gel to create holes for the samples.

4- Pour the gel into the basin slowly and continuously to prevent bubbles. If bubbles occur, remove them using a fine pipette and allow it to solidify for a specified period of time.

5- Submerge the electrophoresis basin in a 1X TBE Buffer solution, prepared in advance by adding 50 ml of 10X TBE Buffer to 450 ml of distilled water.

6- Gently remove the comb from the gel after it has solidified in the casting mold. Then, carefully transfer the gel to the electrophoresis basin.

7- Place 7 ml of DNA ladders in the first and last wells of the gel for comparison purposes. Then, place 7 ml of each sample into the remaining wells, according to the sequence and numbering of each one. 8- Connect the electrodes of the electric current to the electrophoresis device, equipped with a power of 100 volts for 90 minutes. After the specified time for the electrophoresis process is completed, then gently lift the gel from the electrophoresis device and then place the gel in the Gel documentation device for the purpose of imaging and also to ensure the presence of the amplified gene bands in the PCR device.

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.

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.

How it works

1-Cut 300 mg of the PCR gel containing the bands for the CO1, COX2, and ND5 genes and place the gel in a 1.5 ml Eppendorf tube. -1

2- Add 500 ml of DF Buffer to the gel and place the tube in a Vortex shaker to dissolve the gel. -2

3- Place the tube in a water bath at 60°C for 15 minutes. -3

4- After incubation in the water bath is complete, transfer the contents of the tube to a DF Column tube, which contains a 2 ml filter included in the kit. -4

5- Place the tube in a centrifuge at 14,000 rpm for half a minute. 6- After completing the centrifugation process, remove the filter and place it in a new DF Column tube. Add 400 ml of W1 Buffer solution to it, then centrifuge at 14,000 rpm for half a minute. -5

7- Remove the filter from the DF Column tube and place it in a new DF Column tube. Add 600 ml of Wash Buffer solution. Leave the tube for one minute, then centrifuge at 14,000 rpm for three minutes. -6

8- Remove the filter from the DF Column tube and place it in an Eppendorf tube. Add 50 ml of Elution Buffer. Leave the tube for five minutes, then centrifuge at 14,000 rpm for two minutes. -7

At this stage, we obtain pure DNA for the studied genes, COX1 and ND5. 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 genes, COX1 and ND5.

Results and discussion:

DNA Isolation Results

Figure (1) shows the results of DNA isolation from honey bee workers A. mellifera, which were collected from different locations in northern, central, and southern Iraq, with relatively high purity. The purity of the DNA isolated from the samples of the studied areas in northern, central, and southern Iraq ranged from 1.1 to 1.94 nanometers. This purity is required for the polymerase chain reaction (PCR). This method of DNA extraction is similar to the method used by (6), who used molecular methods in his study to detect genetic diversity in the study of the screw fly Chrysomyia bezziana, as he isolated DNA from females and males of the insect using the same extraction kit.

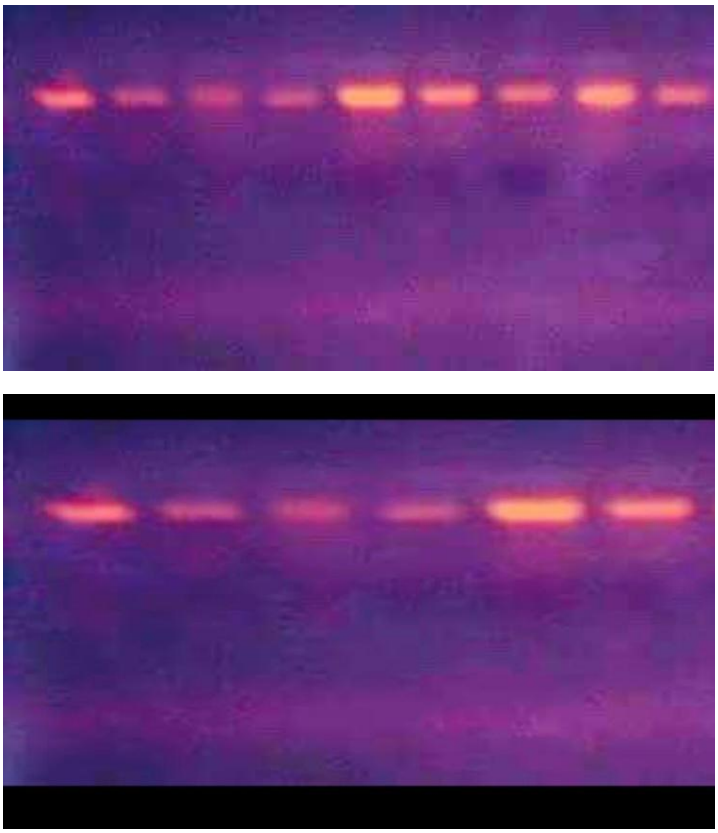

Figure (1) Results of genome migration on agarose gel

Results of amplification of the COX1 gene from mitochondrial DNA of honeybee workers A. mellifera

Figure (2,3,4) 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 (Salah al-Din), and southern Iraq (Basra). 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 1126 bp 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.

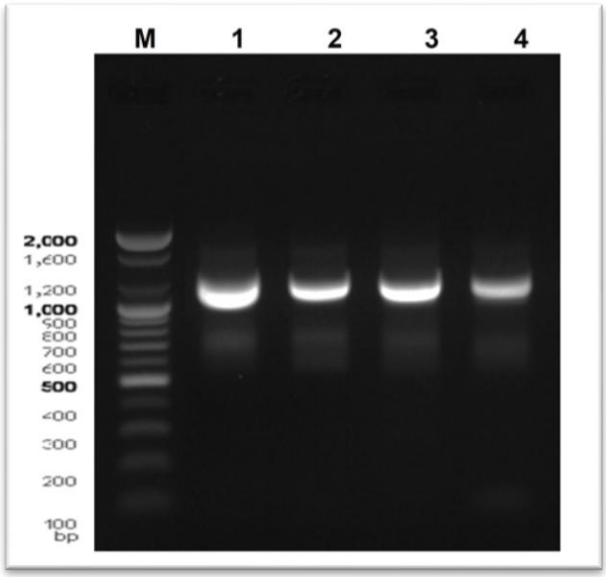

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Figure (2) The amplification result of the COI gene in the mitochondrial DNA of honey bee workers Apis mellifera in Salah al-Din Governorate.

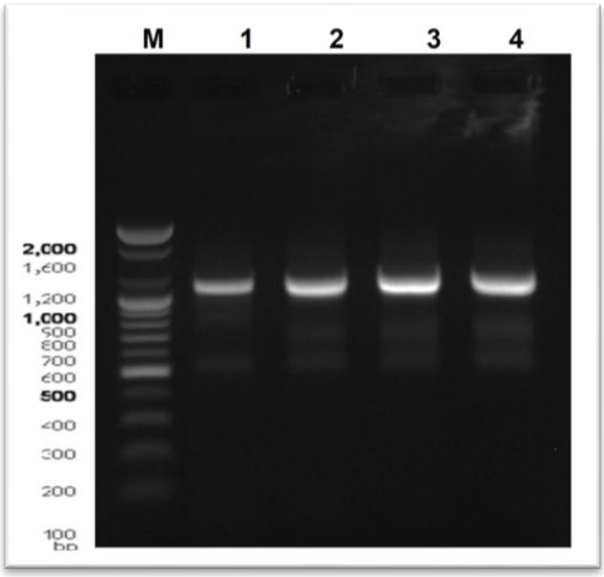

Figure (3) The amplification result of the COI gene in the mitochondrial DNA of honey bee workers Apis mellifera in Sulaymaniyah Governorate.

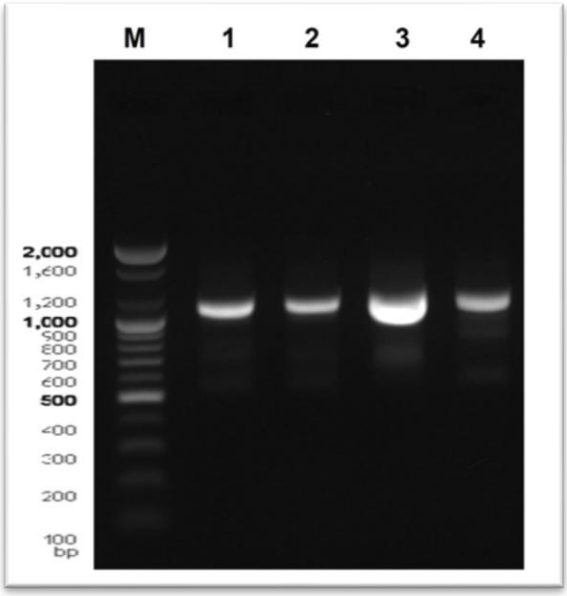

Figure (4) The amplification result of the COI gene in the mitochondrial DNA of honey bee workers Apis mellifera in Basra Governorate.

Results of ND5 gene amplification from mitochondrial DNA of honeybee workers A. mellifera.

Figure (3-5) shows the amplification of the ND5 gene from the mitochondrial DNA of honeybee workers A. mellifera, which were collected from some areas in northern, central and southern Iraq. The analysis results showed that the molecular weight of the bands resulting from the electrophoresis of the amplified

mitochondrial gene ND5 of honeybee workers A. mellifera is 821 bp when matched with the molecular weight on which the primer for this gene was designed using the molecular weight indicator 100-2000 bp Ladder DNA. The results of the current study are consistent with a number of international studies conducted in this field, as well as for the same gene, the mitochondrial DNA of honeybees, which is the ND5 gene. (9) studied the mitochondrial gene ND5, where 93 samples of honeybee workers from different regions of Anatolia were studied. Then, they performed a nucleotide sequence, where 6 variable sites were found, and the average genetic distance was 0.3%. The ND5 gene, which is the first study to identify the nucleotide sequence of the ND5 gene in honeybees in Turkey, was also studied in another study (11). Three mitochondrial DNA genes, 16S rDNA, CO1, and ND5, were studied to detect genetic variation in honeybee workers in Bulgaria. Nine different sites in Bulgaria were used, and four restriction enzymes (MDH, MEEST, and ALP) were used to complete the PCR-RFLP reaction.

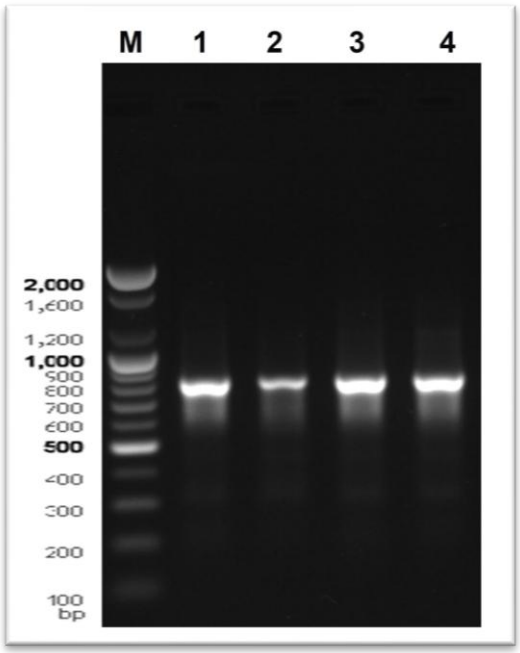

Figure (3-5) The amplification result of the ND5 gene in the mitochondrial DNA of honey bee workers Apis mellifera in Salah al-Din Governorate.

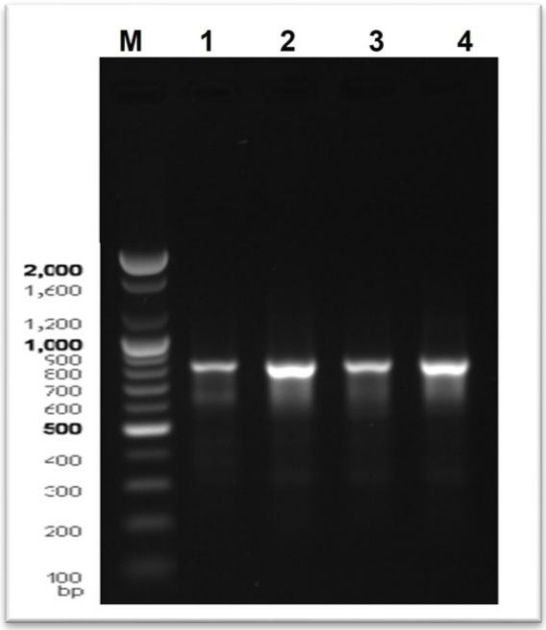

Figure (3-6) The amplification result of the ND5 gene in the mitochondrial DNA of honey bee workers Apis mellifera in Sulaymaniyah Governorate.

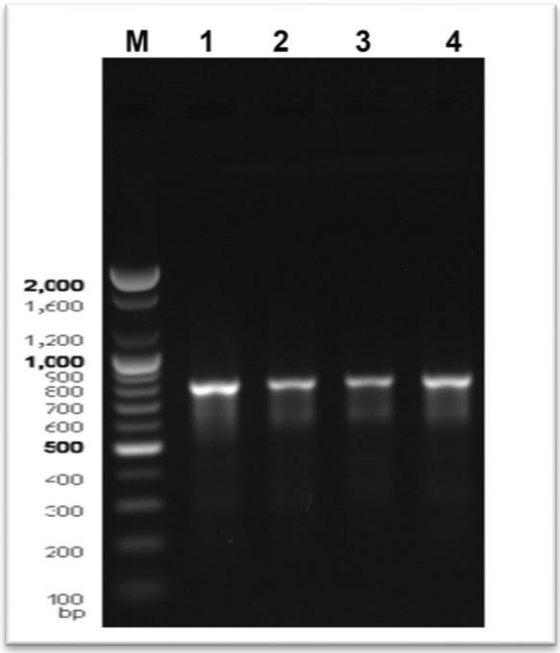

Figure (3-7) The amplification result of the ND5 gene in the mitochondrial DNA of honey bee workers Apis mellifera in Basra Governorate.

Studying of Nitrogen Bases Sequencing

A sequencing study was conducted for the nitrogenous bases of 12 samples of honey bee workers A. mellifera collected from the three governorates of Iraq. 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.

Comparison between A.mellifera honeybee populations in terms of the sequence of the COX1 gene

The sequence of the COX1 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), as well as the group from central Iraq in Salah al-Din Governorate, represented by Al-Dujayl district (sa1), Al-Ishaqi district (sa2), Balad district (sa3), and Tikrit district (sa4), as well as the group from southern Iraq in Basra Governorate, represented by Al-Hartha district (b1), Al-Qurna district (b2), Shatt al-Arab district (b3), and Abu Al-Khaseeb district (b4), 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 COX1 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 545 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 22 mutations, all of which are substitution mutations. In the northern region, represented by Sulaymaniyah Governorate, there are 10 mutations, where only one mutation was of the Transversion type and 9 mutations were of the Transition type. As for the samples of the central region, represented by Salah al-Din Governorate, there are 8 mutations, including two Transversion mutations and 6 Transition mutations. As for the samples of the southern region, represented by Basra Governorate, there are 4 mutations, including Two mutations of the Transversion type and two mutations of the Transition type. As a result of comparing the northern samples with the southern samples and through this number of mutations in all nucleotide sites, we note the presence of a divergence in this gene due to the increase in the number of nucleotide sites used for comparison between the samples, where it was found that the number of mutations in the northern samples is greater in terms of number, as the number of samples in the north reached 10 mutations, and these mutations are only substitution mutations, while the southern samples are only 4 mutations, and all of them are only substitution mutations. The reason for this may be due to the presence of a divergence between the northern samples and the southern samples in terms of the geographical distance between them, as well as the large difference in environmental factors, as well as climatic conditions represented by relative humidity and temperature, as well as the rainfall rate between the two regions. In addition to that, the reason may also be due to the studied samples not returning to a single geographical origin, meaning that they are hybrid strains and not pure, or the reason is due to the type. As for the substitution mutations, when comparing the number of mutations and their locations of occurrence between the central region and the southern region, the results were somewhat close, as the number The number of mutations for the middle samples is 8 mutations, while the number of mutations for the southern samples is only 4 mutations, as shown in Table (1), which shows a comparison of the percentage of occurrence of each type of mutation. The comparison between the samples of the three regions was conducted to clarify and understand the genetic variation between the samples of the studied regions and thus reach the reasons that led to the occurrence of this genetic variation, whether the reasons were natural or artificial, and by increasing the number of sites through which the samples of the study regions were compared, as the high number of substitution mutations in the northern samples is due to many reasons, the most important of which is the presence of a large number of natural barriers in the northern region. These barriers prevent the flow of genes between the three regions or population communities of honeybees compared to the central region and the southern region, in addition to the difference in environmental conditions between the three regions, such as low relative humidity and rainfall, as well as low temperatures. This data was taken from the General Authority of

The percentage of occurrence of each type of mutation %	Total sum of sequences	Number of mutations	Mutation type	Area name
1.83	545	10	replacing	Northern
1.46	545	8	replacing	Middle
0.73	545	4	replacing	Southern

Meteorology and Seismic Monitoring, where it was found that the direct reason for these differences between the three regions is the distance between them, as the distance between the south and the center is 450 km, while the distance between the northern region and the central region is 280 km.

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 l 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.

Also, the study conducted by (13), also for the COX1-COX2 gene region of mitochondrial DNA, where samples were taken from honey bee workers from four geographically different regions in Serbia, and after the process of extracting mitochondrial DNA and amplifying mitochondrial DNA using a PCR device, the results of the comparative analysis, which was conducted with samples from the gene bank, Table (5) shows a comparison of the percentage of occurrence of substitution mutations in the COX1 gene of the Iraqi honey bee A.mellifera between the northern, central and southern regions of Iraq.

showed a significant increase in the degree of similarity, reaching 99.4%.

4 Comparison between honeybee A.mellifera communities in terms of the sequence of the ND5 gene

The sequence of the ND5 gene region in mitochondrial DNA (mtDNA) was compared between three communities: the group from northern Iraq in Sulaymaniyah Governorate, represented by Jamjamal district (sy1), Jawarta district (sy2), Sulaymaniyah center (sy3), and Jabal Birmekron (sy4); the group from central Iraq in Salah al-Din Governorate, represented by Al-Dujayl district (sa1), Al-Ishaqi district (sa2), Balad district (sa3), and Tikrit district (sa4); and the group from southern Iraq in Basra Governorate, represented by Al-Hartha district (b1), Al-Qurna district (b2), Shatt al-Arab district (b3), and Abu Al-Khaseeb district (b4). In order to compare the sample sequences of the population communities of the selected sites during The study, and also the matching process with the global

samples present in the data bank, and Table (4) represents a comparison of the sequencing results between the samples of the studied regions in the sequences of the nitrogenous bases of a part of the ND5 gene, where some of the nucleotide sequences were left at its left end because they were not clear enough, as the distinct and clear sequences were taken, starting from site 1 to site 724 to maintain the accuracy of the results and, moreover, avoiding 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 42 mutations, all of which are substitution mutations. In the northern region, represented by Sulaymaniyah Governorate, there are 19 mutations, where 10 mutations were of the Transition type, and 9 mutations were of the Transition type. As for the samples of the central region, represented by Salah al-Din Governorate, there are 9 mutations, including 5 mutations of the Transition type and 4 mutations of the Transition type. As for the samples of the southern region, In Basra Governorate, there are 14 mutations, 10 of which were Transition mutations and 4 of which were Transversion mutations. As a result of comparing the northern samples with the central samples, and through this number of mutations in all nucleotide sites, we note the presence of genetic divergence in this gene due to the increase in the number of nucleotide sites used for comparison between the samples. It was found that the number of mutations in the northern samples is greater in terms of number, as the number of samples in the north reached 19 mutations, and these mutations are only substitution mutations, while the central samples have only 9 mutations, and all of them are only substitution mutations. The reason for this may be due to the presence of genetic divergence between the northern samples and the central samples in terms of the geographical distance between them, as well as the great difference in environmental factors, as well as climatic conditions represented by relative humidity and temperature, as well as the rate of rainfall between the two regions. In addition, the reason may also be due to the studied samples not returning to a single geographical origin, meaning that they are hybrid strains and not pure, or the reason is due to the type. As for substitution mutations, when comparing the number of mutations and sites Its occurrence between the central region and the southern region, the results were very close, as the number of mutations for the central samples is 9 mutations, while the number of mutations for the southern samples is only 14 mutations, as shown in Table (3), which shows a comparison of the percentage of occurrence of each type of mutation.

The comparison between the samples of the three regions was conducted to clarify and understand the genetic variation between the samples of the studied regions and thus reach the reasons that led to the occurrence of this genetic variation,

whether the reasons were natural or artificial, and by increasing the number of sites through which the samples of the study regions were compared, as the high number of substitution mutations in the northern samples is due to many reasons, the most important of which is the presence of a large number of natural barriers in the northern region. These barriers prevent the flow of genes between the three regions or population communities of honeybees compared to the central region and the southern region, in addition to the difference in environmental conditions between the three regions, such as low relative humidity and rainfall, as well as low temperatures. It was found that the direct reason for these differences between the three regions is the distance between them, as the distance between the south and the center is 450 km, while the distance between the northern region and the central region is 280 km

Similar studies were conducted on the ND5 gene region of mitochondrial DNA, where (14) and (15) studied the evolutionary relationships of five subspecies in Turkey, namely the Anatolian bee, the Caucasian bee, the Cosmic bee, the Syrian bee, and the Median bee. The aim of this study was to know the genetic structure and evolutionary relationships of five subspecies and ecological patterns of honey bees in Turkey through direct sequencing of the mitochondrial regions tRNAAleu-COX2 and ND5, with comparison to the reference mitochondrial genetic patterns as well as those published in previous studies.

Fragment lengths can be determined with certainty, but many mitochondrial genotypes cannot be identified by RFLP patterns alone. Genetic variation can be more sensitively determined by DNA sequence. For systematic and population biology, maternally inherited mtDNA without recombination is a pivotal tool. DNA sequence data can be analyzed within a simple evolutionary context, making mitochondrial data superior to morphological data. Occasionally, there may be differences between morphological and mitochondrial datasets. The ND5 gene region of mitochondrial DNA has been less studied at the population level, based on analysis of the mitochondrial ND5 subunit gene. The samples in this study belong to the same clade as the Apis mellifera ND5 type 3 gene (GU060468). In a study on the evolutionary relationships among subspecies based on mtDNA sequences, ND5 genotype 3 (GU060468) was observed in A. m. carnica (16). ND5 genotype 1 (JN410833) was captured in samples taken from the Konya-Cizme region of southeastern Turkey, where Sumatran bees are predominantly inhabited (9). The ND5 subunit 5 (ND5) gene fragments in the samples did not show any discrepancy with ND5 genotype 2 (JN410835) from Antalya and Konya, as well as several other genotypes. In that study, it was not possible to separate the races according to a cladogram based on ND5 sequences, as it belongs to the East European C lineage.

Conclusion

Molecular data on mitochondrial DNA (mtDNA) tRNAleu-COX2 and ND5 sequences concluded that all subtypes and ecotypes in this study were not genetically diverse.

The percentage of occurrence of each type of mutation %	Total sum of sequences	Number of mutations	Mutation type	Area name
2.56	742	19	replacing	Northern
1.21	742	9	replacing	Middle
1.88	742	14	replacing	Southern

Table (6) shows a comparison of the percentage of occurrence of substitution mutations in the ND5 gene of the Iraqi honey bee A.mellifera between the northern, central and southern regions of Iraq.

References

1- Gerges, Salem Jameel and Saeed, Mahdi Mohammed Saleh. (2010). The effect of temperature and colony strength on honey bee colonies (Apis mellifera L. (Apidae-Hymenoptera). Rafidain Journal of Agriculture. Volume (28), Issue (2).

2- Al-Khawli, Abdel Moneim Suleiman Ali and Issa, Ibrahim Suleiman. (1994). Honey bees: A study of behavior, production, and beehive care. Arab House for Publishing and Distribution.

3- Al-Jawrani, Redha Sakab; Khadir, Ghafouri Yas; Ibrahim, Ezz El-Din Hassan and Yassin, Abdul Aziz Ibrahim. 1990. Beneficial Insects. Dar Al-Hikma, Baghdad. -1

4-Al-Shadideh, Ahmed Nouri and Al-Abbadi, Amal Abdullah and Al-Atiyat, Mazen Ahmed (2012). Consumers' knowledge in the central region of Jordan about honeybee products and its relationship to some factors, Alexandria Journal.

5-Maniatis *et al* 2001 Both geometric morphometric and microsatellite data consistently support the differentiation of the *Apis mellifera M* evolutionary branch. – Apidologie 42: 150- 161 .

6- **Al-Tamimi, Hussein Mohammed Brisam. (2016).** A study of the molecular and quantitative genetic relationship between three populations of the Old World

screwworm fly, Master's thesis, College of Education for Pure Sciences, University of Diyala, Iraq.

7-Al-Baraki, Muhammad Ali, Karnoy, Lionel and Al-Baraki, Khalid, (2009). Genetic analysis of the Syrian honey bee Apis mellifera Syriaca and a study of its biodiversity using specialized molecular markers, the Sixth Conference of Arab Beekeepers, Saudi Arabia, 9-1.

8-Alattal , Y.; Alsharhl, M., Alghamdl, A., AlfaIfy ,S., Migdadi, H., Ansari, M., (2014). Characterization of the native honey bee subspecies in Saudi Arabia using the mtDNA COI-COII intergenic region and morphometric characteristics.- Bull of Insec, 67 (1): 31-37.

9-Ozdil,**F and ILhan,F**.(2012). Genetic Divergence of Turkish Apis mellifera Subspecies Based on Sequencing of ND5 Mitochondrial Segment. Sociobiolog y Vol. 59, No. 1, 2012.

10- Roxane, M., Magnus , Allen L., Szalanski ,.(2010):Mitochondrial DNA characterization of Africanized honey bee(*Apis mellifera L* .) populations from the USA. Social Insect Genetics Lab , Department of Entomology , University of Arkansas, Fayetteville , AR72701, USA Pages 177-185.

11- **Ivanova, Violeta, Marina Stefova, and Fabio Chinnici.(2010).** "Determination of polyphenol contents in Macedonian grapes and wines by standardized spectrophotometric methods." *Journal of the Serbian Chemical Society* 75.1 (2010): 45-59.

12- **Rahimi, A. (2015).** Study of the genetic diversity of Iranian honey bee (Apis mellifera meda Skorikow, 1829) populations using the mtDNA COI–COII intergenic region. *Biologija*, *61*(2).

13- **NedIc**,N.**; StanisavljevIc,L.; MladenovIc,M.;and StanIsavl jevIc, J** .(2009) . Molecular characterization of the honeybee Apis mellifera carnica in serbia , Arch. Biol. Sci., Belgrade, 61 (4), 587-598.

14-**Palmer, M. R., Smith, D. R., Kaftanoglu, O.** (2000): Turkish honey bees: genetic variation and evidence for a fourth lineage of *Apis mellifera* mtDNA. – J Hered 91: 42-46.

15- - **Henriques, D., Chávez-Galarza, J., Quaresma, A., Neves, C. J., Lopes, A. R., Costa, C., Costa, F. O., Rufino, J., Pinto, M. A. (2019)**: From the popular tRNAleu-COX2 intergenic region to the mitogenome: insights from diverse honey bee populations of Europe and North Africa. – Apidologie 50(2): 215-229. doi:10.1007/s13592-019-00632-9.

16- **Martimianakis, S., Klossa-Kilia, E., Bouga, M., Kilias, G.** (2011): Phylogenetic relationships of Greek *Apis mellifera* subspecies based on sequencing of mtDNA segments (COI and ND5). – J Apicult Res 50: 42-50.