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Genetic Diversity and Biological Activities of Nuclei from Swarming and Dividing of Honey Bee *Apis mellifera* L. Colonies.

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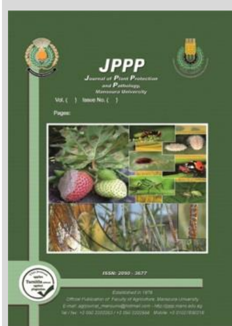

ABSTRACT

Honey bee swarming is a crucial behavior for the population reproduction of honey bee colonies in early spring and autumn. It is well known that the honey bee reproductive swarming always shows a genetic diversity among the swarmed individuals of specific genes. This type of genetic diversity always occurs for the individuals that naturally swarmed. Twelve honey bee nuclei were used; six from natural and the other six from artificial swarming. For studying the biological activity parameters including; honey, pollen, and brood. Moreover, the hygienic behavior test, queen weight, queen rearing, and ovarioles number were recorded for two years. Moreover, two different molecular techniques were used such as specific PCR and RAPD PCR. The molecular part helps in understanding the genetic diversity developed between swarm's individuals and those artificial ones. Concerning the biological activities; the results illustrated that the swarm nuclei had a highly significant difference compared with the artificial one in the first year, whenever, non-significant differences were observed between the two examined swarms in the second year. Furthermore, molecular differences were obtained assuming these differences back to the corresponding swarming related genes which mostly control some of the defense genes of hygienic and grooming behavior. These results revealed the presence of a tide-relationship between the bees' immune system and their genetic profiles. Thus, recommend leaving the colonies to prepare for a swarming to enhance their production ability and hygienic behavior after distributing the hunting hives in the apiaries to avoid missing colonies.

Keywords: *Apis mellifera*, swarm and dividing system, biological activity, hygienic behavior, RAPD PCR and DNA analysis.

INTRODUCTION

A natural phenomenon that is increases the number of honeybees is swarming. The remarkable process of colony reproduction arises from factors such as dense population density, scarce nectar, abundant pollen, and the genetic makeup of honeybee colonies. (Uzunov *et al*., 2014; Woyciechowski and Kuszewska, 2012; Taha and Al-Kahtani, 2013, 2019; Shawer et al., 2021). Growing colony size, brood nest congestion, worker age dispersion, and decreased transmission of pheromones produced by the queen all because swarming (Lensky and Slabezki, 1981; Winston., 1991). Seasonal and environmental factors, such as weather patterns and times when the colony's population is growing, can have an impact on swarming (Winston *et al*., 1991). Colony size includes several factors; number of sealed brood, number of eggs and unsealed brood, workers population, etc. However, the most important factor for swarming behaviour is the active population size, not the nest size. Swarming behaviour typically occurs in spring and early summer, and sometimes in other times of year, when local conditions allow, and starts during the warmer daylight hours (Landa, 1986; Seeley *et al*., 2006; Winston, 1980). There are many reasons for swarming, including the amount of food, the number of brood, the number of bees and the environment around the apiry as animies with thier sounds, also the temperature, and humidity in the hive system (Bencsik *et al*., 2011). Beekeepers need to take responsibility for managing swarming, which requires more manual control and relatively long periods of time (Ferrari *et al*., 2008; Zhu *et al*., 2019).

The "artificial swarming" division system is one of the most profitable products for beekeepers.They used these fission nuclei to increase the number of colonies in their apiary or to sell them to others to build new apiaries (Diao and Hou, 2018; Hatjina *et al*., 2014; Landa, 1986; Lewis and Schneider, 2008; Winston, 1980; Woyciechowski and Kuszewska, 2012). The production of packaged bees and nuclei requires a lot of experience and knowledge of the beekeeper to identify some key points related to the success of the division process.

The best seasons to divide, the best colonies to divide, types of queens, and how queens are introduced to new divisions have been studied by El-Hosainy *et al*. (2017); Sheikh (2019). Hygiene behavior testing for honey bees *Apis mellifera* L. includes inspecting, opening caps, and removing sick or dead bees from the colony. Removal (%) of artificially killed worker broods was considered an indicator of hygienic behavior. (Dietemann *et al.*, 2013; Uzunov *et al*., 2014; AlKahtani and Taha, 2022). Honey bee mitochondrial DNA (mtDNA) is considered a better genetic marker and has provided much data for studies on the phylogeny and biogeography of the honey bee (De La Rua *et al*., 2006; Kandemir *et al*., 2006; Bouga *et al*., 2007). As in most animals, mitochondria are inherited only from the mother, so all

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individuals within a colony have the same mitochondrial DNA (Crozier and Crozier, 1993). Mitochondrial DNA sequences are known to be useful in distinguishing honeybee lineages and variation between and within honeybee populations (Garnery *et al., 1992;* Moritz *et al.*, 1994). All honey bees have a non-coding sequence that appears to be unique to *Apis* sp. and is located between the mitochondrial COI and COII genes (Cornuet and Garnery, 1991). Analysis of DNA sequences can help provide phylogenetic context and allow conclusions to be drawn about relationships between populations and the history of population migration (Franck *et al.,* 2001). Variations in mtDNA are useful for constructing and identifying the phylogenetic composition of honeybees, as well as for analyzing subspecies and races (Moritz *et al.*, 1994).

Research into the unique evolution of the mitochondrial COI-COII intergenic region began in the early 1990s (Franck *et al.*, 1998). Since then, it has become a common tool for distinguishing evolutionary lineages in honey bee populations (Kozmus *et al.*, 2007). The COI region was chosen because it is favored among genes encoding mitochondrial proteins as an important molecular diagnostic system for identifying animal species, including honey bees (Hebert *et al*., 2003). The COI-COII region within the gene is often used to detect and track the gradual importation of honey bee queens (De La Rúa *et al.,* 2013), Phylogeographic studies in the region of origin (Franck *et al.,* 2001).

There are many tasks that every beekeeper must perform manually in their apiary to maintain the health of their bees (Masry *et al.*, 2015), five biological and morphometric analysis use for comparing between different colonies headed by artificially and naturally mated queens as number of sperm in queen spermatheca, genetic relatedness of offspring, number of eggs, sealed brood and pollen area measurment, (Crozier and Crozier, 1993, Hassona *et al* 2012).

Through this study different biological activity used to compare swarms and nuclei from division headed by queens unified the genetic origin and beekeeping process of both two types of studying nuclei and returning the biological activities, and hygienic behavior differentiation to the colony status whether it was a swarm or dividing colony. furthermore, DNA analysis; is the molecular tool, used in this study to differentiate between colonies from the natural and artificial swarming. For that reason, specific PCR (COI), RAPD-PCR, and DNA sequencing techniques were used. In addition, a DNA marker specific for the swarming was demonstrated in this investigation.

MATERIALS AND METHODS

Experimental procedure

A starting total of Twelve mother colonies were established and headed by mated queens obtained from Menzala, the previous region of isolated Carniolan bees in Egypt (Fathy *et al.*, 2019) transferred to the experimental apiary (Fig 1) of the City of Scientific Research and Technological Applications (SRTA-City), in the region of New Borg El-Arab (30° 50′ 56″ N, 29° 36′ 42″ E), Alexandria, Egypt at early spring (February) 2015 and ran until 31 May 2017.

Six swarms were caught from the mother colonies and housed separately, and six divisions were established and housed in a Langstroth hive simultaneously with the swarm mimicking approximately equal in stored honey, stored pollen area, combs covered with bees, brood combs, and queen age and whether mated or virgin.

Fig.1. The location of experimental farm of SRTA city.

Biological activity parameters:

The worker bees brood area (sealed and unsealed), stored pollen area, and honey area were recorded at 12 days intervals during the experiment period using a measuring standard Langstroth frame divided into square inches.

Hygienic behavior

One sealed brood comb was chosen from each three experimental colonies, then 10 x 5 cm were marked using a marker pen and counted as a total number 100 marked brood cells (X). A pin was used to kill the marked cells. Then, those treated combs were returned back to their colonies and after 12, 24, 48 hours sequentially the number of removed dead brood, from marked brood cells, by worker bees were counted and recorded as (Z). This observations were made in spring, summer, and autumn of the swarms and dividing nuclei in the second experimental year, the percentage of hygienic behavior (pin test) calculated by this formula HB%= Z/X*100 (Dietemann *et al.*, 2013; Hassona *et al.*, 2012; Uzunov *et al.,* 2014; Abou-shaara *et al.*, 2018).

Genotypic characterization of Honeybee (*Apis Mellifera,* L.) swarms

Sample collection

Sampling

Honeybee new-emerging workers were collected from all colonies monthly, separately, from the early spring of 2015 to the early summer of 2017 and maintained alive in ventilated cages, transported cold to the laboratory, where they were stored at -80°C until processing (Evans *et al.*, 2013; Scheiner *et al.*, 2013) At two time points for every year, a composite pooled sample of approximately 50 worker bees, equally derived from the same colony, was collected.

Mitochondrial DNA extraction

Total mitochondrial DNA was extracted from the medial leg and antennae of palling honeybee workers samples using a DNA preparation kit (Jena Bioscience, Germany) according to the manufacture procedure. The purity yield concentration of DNA was assessed by a Nano-drop spectrophotometer (BMG LABTECH, Germany). The obtained DNA was stored at -20°C until used.

DNA electrophoresis and visualization

The electrophoresis method used for mitochondrial DNA was carried out according to (Maniatis *et al.*, 1982) Agarose gel was prepared by melting 0.5 g agarose in 50 ml 0.5x Tris-base, 108 g Boric Acid, EDTA (TBE) buffer, and injected with 2 µl Ethidium Bromide (10 mg/ml). The DNA samples (5 µl) were mixed with 2 µl loading dye and by using DNA molecular marker, then the DNA was subjected to electrophoresis (mini-sub-DNA electrophoresis gel 170 - 4307 and a power supply 1000/500 from Bio-Rad, USA), At 80 volts for 30 min after that, the gel was visualized and

photographed using gel documentation system (Syngene, USA).

Polymerase chain reaction (PCR)

PCR using specific primers.

The PCR with specific primers (table, 1) reactions were performed in total volume 50 µl containing approximately 5 µl Taq polymerase buffer 10X (Promega, Germany) containing a final concentration of 2µl 25 mM $MgCl_2$, 2µl of 2.5 Mm d NTPs, 2µl of each primer (10 Pico moles/µl), 1µl (5 unit/ µl) Taq DNA polymerase (Promega Germany), and 2 µl DNA (50 ng).The PCR reaction conditions were; initial cycle with 94$^{\circ C}$ for 5 minutes and 35 cycles of denaturation at 94$^{\circ C}$ for 1 minute, annealing at 58 for 1 minute and Extension at 72 for 1 min. For final extension at 72$^{\circ C}$ was carried for 5 minutes. Amplified PCR products were checked in 1.5 % agarose gel as previously described.

Table 1. the primers used in amplification were selected from the published DNA sequences of the *Apis mellifera carnica* (Techer *et al.*, 2017).

Specific primer	Nucleotide sequence 5/ to3/	Amplicon length
Apis mellifera (carnica)	5\GGCAGAATAAGTGCATTG 3\ 5\CAATATCATTGATGACC 3\	550 – 850 bp
Apis mellifera (carnica west)	5\TTAAGATCCCCAGGATCATG3\ 5\GTTATCCACGTCATAAACGT 3\	1020 bp

RAPD-PCR

Three random primers each consists of 10 nucleotides were used in RAPD analysis (table, 2) to differentiate among the examined four samples (four group of *Apis mellifera)* under study. For RAPD-PCR analysis, the RAPD-PCR reaction was carried out in total volume 25µl; 12.5µl 2X power PCR mix (Biotech, Cairo, Egypt) contains 2µl of 25 mM $MgCl_2$, 1µl of 0.2 Mm d NTPs, 1µl of the primer (10.0 pmol/ µl), 1 µl 50 ng of honeybees mitochondrial DNA, 0.2µl (5 units/ µl) Taq DNA polymerase (Promega Germany) the volume completed to 25 µl by $_2H_2O$ water. DNA amplification was performed in a PTC-100 thermal cycler (M J Research, Inc.), and the reaction conditions; initial cycle with 95$^{\circ C}$ for 3 min, 35 cycles of denaturation 94$^{\circ C}$ for 1 min, annealing at 30$^{\circ C}$ for 1 min, and extension at 72$^{\circ C}$ for 1 min. Finally, an extra final extension step at 72$^{\circ C}$ for 5 minutes. Amplified RAPD-PCR products (12 samples) were checked in 1.5% agarose gel and electrophoresed in 0.5X (TBE) as a running buffer, for 30 min at 120 volts (Biometra, USA). PCR products (5 µl) were mixed with 2µl loading dye prior to loading of 10µl per gel pocket.

Table 2. primers and their nucleotide sequences employed in the RAPD-PCR analysis (Heikal *et al.*, 2008)

Number	Name	Nucleotide sequence 5\ to 3\
1	R3	AGC CAC CGA A
2	R7	ACC GCC GAA G
3	R10	GAG AGC CAA C

The RAPD-PCR band patterns and dendrogram construction

Analyzed data of the RAPD results were scored for computer analysis on the basis of the presence and absence of RAPD bands produced for each primer from the five primers. Products present in the samples were designated (1) and when absent it was designated (0) after excluding common bands. Based on the presence or absence of a unique and shared polymorphic product, was used to generate similarity coefficients (Dey *et al*., 2006).

Bands of honeybee DNA (RF) pattern were scored manually for all studied samples studied as 0.0 and 1.0 level of marker polymorphism according to the various molecular techniques. Similarity coefficients were used to construct a dendrogram for each sample produced by using software statistic(R) program version 5 (Gaspar *et al*., 2002).

Identification of the cytochrome C oxidase gene using universal primers

PCR amplification of the cytochrome C oxidase

The universal primers of the cytochrome C oxidase gene were used to amplify a specific conservative region of the cytochrome gene in the three selected honeybee colonies as usually used in eukaryotic identification (no one of the previous two sets of specific primers was used). The PCR reactions were performed in a total volume of 50 µl as previously mentioned. The PCR reaction conditions were; initial cycle with 94 $^{\circ C}$ for 2 minutes and 30 cycles of denaturation at 94$^{\circ C}$ for 1 minute, annealing at 48 for 1 minute, and extension at 72 for 1 minute. Then, the final extension was done at 72°C for 5 minutes. Amplified PCR products (4 samples) were checked in 1.5% agarose gel and electrophoresed at 0.5X (TBE) as a running buffer, for 30 minutes at 120 volts (Biometra, USA).

DNA Sequence of the cytochrome C PCR amplicon obtained by universal primers

QIA quick PCR purification kit (Qiagen. Germany) was used to purify the amplified products of PCR according to the manufacturing procedures. The sequence was performed at the center of Excellency, the City of Scientific Research and Technological Applications, New Borg El Arab City (16 capillary K B - 3130 xl – pop 7 – BDTV 3 mob genetic analyzers).

Sequence analysis and phylogenetic analysis

Pairwise and multiple DNA sequence alignments were carried out using Clustal W multiple sequence alignment program version 1.82 (http://www.ebi.ac.uk/clustalw) ,Bootstrap neighbor-joining tree was generated using MEGA version 5 (Kumar *et al.*, 2002) from the Clustal W alignment. Comparison with sequences in the Gene Bank database was achieved in BLASTN searches at the National Centre for Biotechnology Information site (http://ncbi.nlm.nih.gov) (Evans *et al*., 2013).

Statistical analysis

All statistical analysis was performed using the analysis of variance technique by means of the Graph Pad computer software package "One-way ANOVA followed by Dunnett's multiple comparisons test was performed using GraphPad Prism version 10.0.0 for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com ". The design of the experiment was split plot in time (split block). It was analyzed as a split-plot according to GraphPad Software, Inc. Accessed 5 November 2008 (Motulsky, 1999).

RESULTS AND DISCUSSION

Results

The biological activity of honey bee

Brood production, pollen collection, and honey in swarms and divided colonies.

The total mean areas by inch^2 of brood production, stored pollen, and stored honey for the swarming and dividing colonies through the four seasons of spring, summer, autumn, and winter in the two experimental years illustrated in (Fig.

2). Results Revealed that there were significant differences of production in the first year between swarming and dividing colonies, while there is no significant in the second year. For swarming colonies in the first year, were 251.95, 181.79, 346.15 of brood production, stored pollen, and stored honey $inch^2$/season respectively. Furthermore, in the second year, 239.55, 212.21, 339.42 of brood production, stored pollen, and stored honey $inch^2$/season respectively. Meanwhile, dividing colonies in the first year, were 149.50, 128.13, 232.59 of brood production, stored pollen, and stored honey $inch^2$/season respectively. Furthermore, in the second year, 168.31, 164.53, 286.59 of brood production, stored pollen, and stored honey $inch^2$/season respectively.

Moreover, there were significant differences of means of bee population (number of bees covered frames) between swarming and dividing colonies in both years, were 7, 8.92 of swarming colonies in 2015, and 2016 respectively. while there were 4.11, 5.21 of dividing colonies in 2015, and 2016 respectively. Furthermore, the highest covered frame was for swarming colonies in both autumn season 2015 and 2016.

Furthermore, there was significant difference of means of queen breeding (number cups and cells construction) between swarming and dividing colonies only in the 1st year of study 2015., were 3.35, 5.97 of swarming, and dividing colonies respectively. While there was no significant difference of means of means of queen cells construction between swarming and dividing colonies in the 2nd year, were 9.18, and 9.40 of swarming and dividing colonies respectively. The mean number of queen cups and cells building were higher in 2016 than 2015, start increasing in autumn after six months of swarms housing as compared with the results of the 2nd year 2016, was higher in spring and autumn.

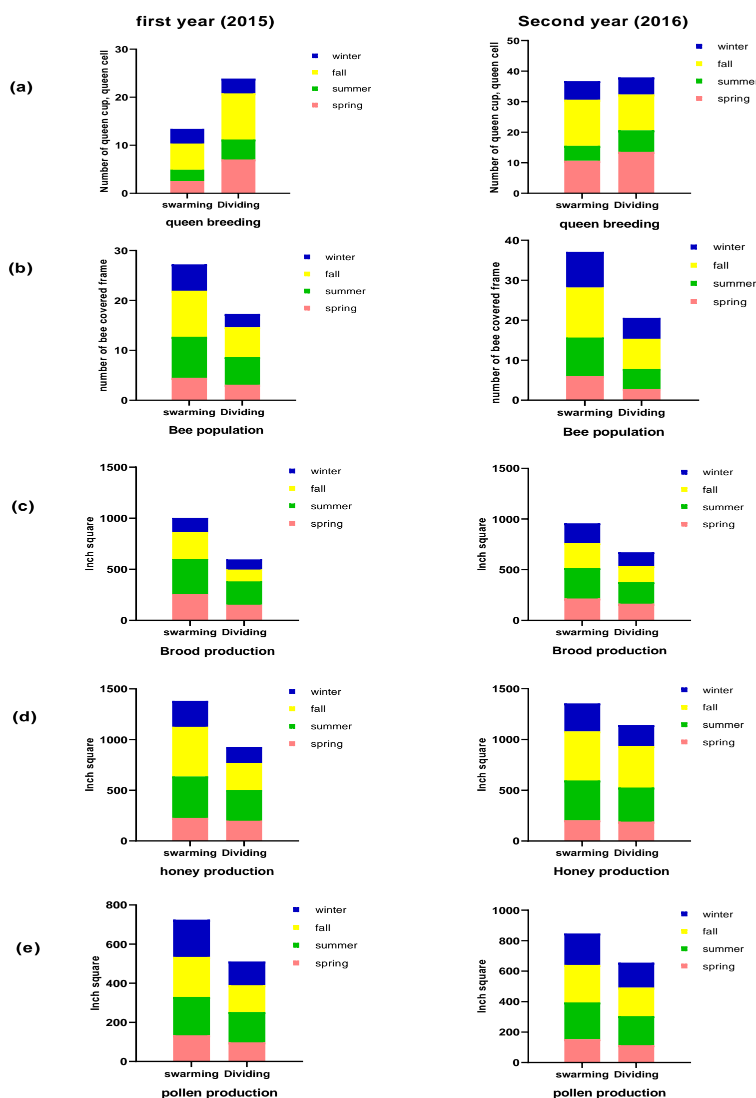

Fig. 2. Fluctuation of biological activity parameters for swarming and Dividing colonies during different seasons in two consecutive years 2015 (left), and 2016 (right) both of queen breeding based on cups and queen cells number (a), Bee population were estimated by counting the number of frames covered with bees every 21 days (b), furthermore production area measured by $inch^2$ all of Brood (c), stored honey (d), and stored pollen (e).

Hygienic behavior test:

Presented data in (Fig. 3) revealed that the mean of HB% In the swarming colonies was 30.67%, 88.33% and 86.33% after 12, 24, and 48 h respectively in spring. In addition, in summer the mean were 37.67%, 67.33% and 88.17% after 12.24 and 48 h respectively. Furthermore, it was 37.67%, 67.33% and 90.33% after 12, 24, and 48 h respectively in autumn.

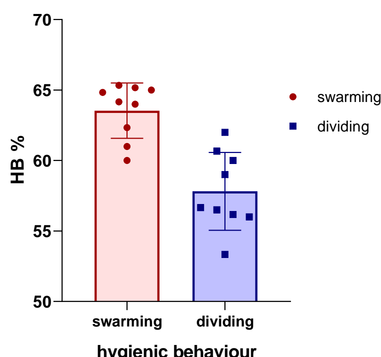

Fig. 3. hygienic behavior (HB%) of six queens both of natural swarm colonies and dividing system colonies carried out at the same time.

Meanwhile the mean of HB% In the dividing colonies was 25.83%, 58.83%, and 84.27% after 12, 24, and 48 h respectively in spring. Although, in summer were 30.33%, 66.83%, and 85.50% after 12, 24, and 48 h respectively. Furthermore, it was 22.33%, 60.50%, and 86.00% after 12, 24, and 48 h respectively in autumn.

The total mean for the 27-time test for swarming colonies was 63.54% and for divided colonies was 57.81% and T test was less than 0.05 and that means there was significant difference between swarming and dividing colonies in the hygienic behavior test.

Genotypic characterization of swarming and dividing honeybee colonies (*Apis Mellifera,* L.)

Genotypic characterization of Honeybee(*Apis Mellifera,* L.) swarms PCR specific primers for *Apis mellifera(carnica and west carnica)*

Data presented in (Fig. 4) showed that different amplicons were obtained by using the specific PCR of the Cytochrome gene applied by two specific primers (table, 1) and observed a presence of two amplicons with molecular sizes ranging between 550 – 850 bp. The 550 bp amplicon was obtained in the examined samples in lanes 2, 3, 4, 5, 8 and 10 whenever, the 650 bp amplicon was obtained with lane 9 and the 850 bp was obtained with lanes 1, 6, 7, 11 and 12.

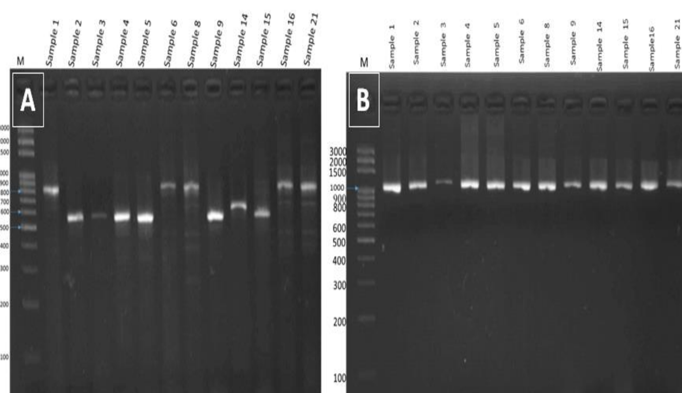

Fig. 4. PCR amplicons of the cytochrome C genes from different honey bee colonies using specific PCR. A: primers specific for Cytochrome C1 (E2, H2). B: Primers specific for Cytochrome C2 gene. Lanes; M: DNA marker 3 kbp. Lane1: colony1. Lane 2: colony 2, lane 3: colony 3, lane 4: colony 4, lane 5: colony 5, lane 6: colony 6, lane 7: colony 8, lane 8: colony 9, lane 9: colony14, lane 10: colony15, lane 11: colony 16, lane 12: colony 21.

The cytochrome C1 gene specific for Apis mellifera carnica and Cytochrome C2 specific for Apis mellifera carnica west were amplified using specific PCR. Cytochrome C1 primers succeeded to detect genetic variation between the 12 examined colonies and it was shown as; three different DNA band patterns with different molecular sizes of about; 550 bp of colonies 2, 3, 4, 5, 9, and 15 (artificial swarming) but a 650 of colony 14 and 850 bp of Colony 1, 6, 8, 16, and 21 (natural swarming) (Fig. 4 A). On the other hand, results obtained by using the specific primers Cytochrome C2 of A. m west carnica species; gave only one band with a molecular size of about 1020 bp was observed. The only difference was noticed in the band intensity (Fig. 4 B). According to Cytochrome C1 samples were divided into three groups; and from each group, one sample was chosen as (colony 4 from the first group (550 bp), colony 14 as the second group (650bp), and colony 1 (850bp) from the third group for RAPD – PCR examination.

RAPD – PCR examination

Fingerprinting for the selected three isolates based on the specific PCR analysis. The three selected isolate's DNA was subjected to RAPD –PCR examination using 3 different arbitrary primers (3, 7, and 10), the primer RAPD 3 grouped the three examined isolates into two groups. Group one contains samples 1 and 14. But the second group contains samples 4. In the case of primer RAPD 7, the three samples gave the same band pattern and no difference was observed. In addition, Primer RAPD 10, grouped the three samples into two main groups, group one contains sample 1 but the second group included the two other samples (Fig. 5).

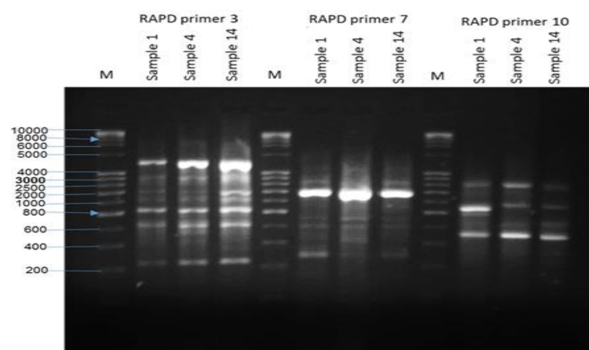

Fig. 5. DNA band patterns obtained by the RAPD primers when approached on the mitochondrial DNA isolated from different bee colonies. Lanes; M: DNA molecular marker 10kbp, Lane1: colon y1. Lane 2: colony 4, lane 3: colony 14, Lanes; 4: DNA molecular marker10 kbp, lane 5: colony 1, lane 6: colony 4, lane 7: colony 14, lane 8: DNA molecular marker10 kbp, lane 9: colony 1, lane 10: colony 4, lane 11: colony 14.

Results of dendrogram for the fingerprinted DNA in (Fig. 6) revealed that group one is a unique race that contains natural swarm colonies (1, 6, 8, 16, and 21) it was occurring separately in one cluster.

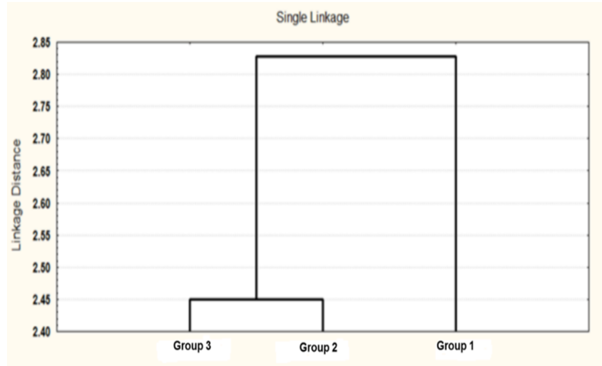

Fig. 6. Dendrogram for the fingerprinted DNA of three different honey bee colonies using three different arbitrary primers

But the other two groups have existed in one cluster together. This cluster was divided into two main groups; the first group contains artificial swarm colonies (2, 3, 4, 5, 9, and 15), and the second group included one natural swarm colony 14 only. In conclusion group, which included five natural swarm colonies (1, 6, 8, 16, and 21) and occurred in a separated cluster is a unique race. On the other hand, that unique race has similarities to the second cluster, which has two main groups [group 2 (artificial swarm colonies) and group 3 (natural swarm colony 14)], by a percentage of 82%. In addition, the two main groups [group 2 (artificial swarm colonies) and group 3 (natural swarm colony 14)] are closely related to each other by a percentage of more than 86%.

DNA sequencing

Based on the results obtained by the specific and RAPD – PCR in (Fig. 7) three samples were selected to be subjected to DNA sequencing. The PCR products (658 bp) of the three examined samples; 1, 4, 14 were purified using a PCR purification kit and sequenced at the Center Of Excellency, the City of Scientific Research and Technological Applications, New Borg El-Arab City (16 Capillary K B - 3130 x 1 - POP 7- BDTV3. Mob. Genetic - Analyzers)

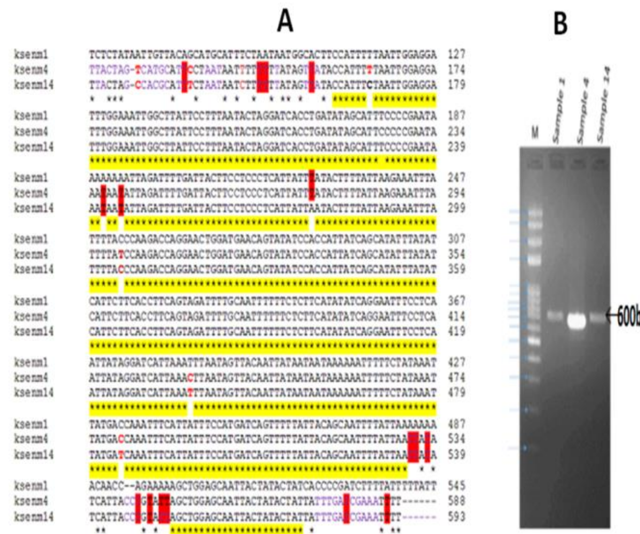

Fig. 7. PCR amplicons and the DNA sequence alignment of the Cytochrome C gene isolated from the three selected honey bee colonies using specific primers. A: sequence alignment of the obtained DNA nucleotide sequences and the conserved regions among these sequences. B: The PCR amplification of the three selected honey bee colonies, Lanes; M: DNA marker 3 k.bp. Lane1: colony1. Lane 2: colony 4, lane 3: colony 14.

In the phylogenetic analysis (Fig. 8) obtained by the three DNA nucleotide sequences of cytochrome genes, it was observed that colony1 considered an outer group because it was isolated in one cluster, whereas the other two colonies (4 and 14) are included in another cluster with a combined with other 21 cytochrome genes (listed in Gene Bank).

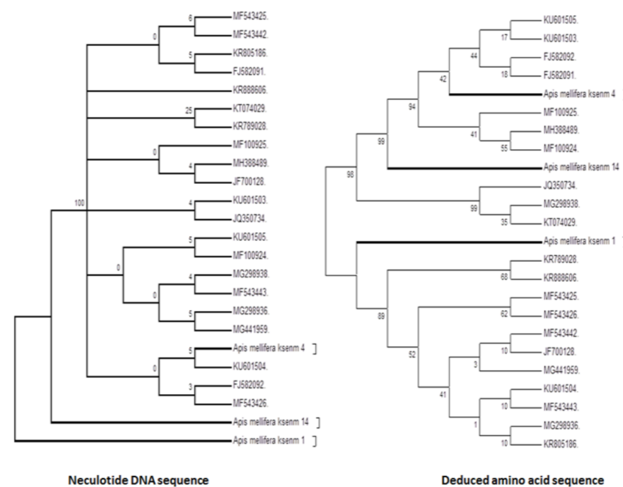

Fig. 8. Phylogenetic tree, of the obtained DNA sequence, of the cytochrome C1 gene for three honey bee colonies. The phylogenetic tree was constructed using the Mega 5 program. A: Phylogeny is constructed based on the DNA nucleotide sequences. B: Phylogeny constructed based on the deduced amino acid sequences. The three genes were compared with the other Cytochrome C genes listed on the Gene Bank.

The 23 cytochromes (cluster 2) are divided into two sub-clusters; sub-cluster one contains only sample 14. Whereas sub-cluster two was divided into 6 different groups, one of them is colony 4 which showed high similarity KU601504. On the other hand, the phylogenetic constructed based on the deduced amino acids of the three cytochrome genes compared with the other 21 cytochrome genes (Gene Bank), revealed that two clusters were observed, one of them containing colony1 and the other one cluster including the 4 and 14 colonies.

Discussion

The previous results indicated that in the first experimental year of 2015 the highest mean area of all brood for swarms was 342.82 $inch^2$/ colony in the summer season. In addition to dividing colonies, the highest mean area of all broods was 228.40 $inch^2$/colony in the summer season. In addition, in the second experimental year 2016 the highest mean of all brood for natural swarming colonies was 302.24 $inch^2$/ colony in the summer season and the same for dividing colonies the highest mean area of all brood was 212.47 $inch^2$/ colony in the summer season. That means that the highest brood areas were in summer in both types of colonies (natural swarm and artificial swarm) also in both of the study years (2015 and 2016), and that was normal for all colonies due to the summer season is the activation season for the honey bee colonies, and the rate of colonies individuals increased always in summer season as illustrated by (El-Hosainy *et al.*, 2017) that the highest rate of brood activity was in May and the highest number of bees was investigated in June and July. That was recorded by that the highest brood production was in summer followed by spring, autumn, and winter seasons. (Balhareth *et al.*, 2012) indicated that; there are significant differences between summer and other seasons.

This study noticed that through both years in natural swarmed colonies the highest mean area of all brood was in summer followed by autumn, spring, and winter seasons. On the other hand, in divided system colonies, the highest mean area of all broods was in summer followed by spring, autumn, and winter seasons. Here, natural swarmed colonies were collected and housed in spring 2015 and then started to be strong through the summer season also there is no significant difference between the two seasons (spring and autumn) in the mean brood areas for natural swarmed colonies. When comparing the two types of colonies noticed that the total mean of all brood for natural swarmed colonies in both years was higher than the total mean of all brood for divided system colonies according to (Richards, 2012) the swarm has younger workers which make the swarm strong and more active. Even if, the total mean of all brood for swarmed colonies in 2016 was lower than that in 2015 due to the old queens of the swarmed colonies did not change to another new queen in 2016 and they continued production until the end of 2016 that case the activation of queens may be decreased in the second study year of 2016 due a low number of spermatozoa in their spermatheca then decreased in laying eggs and brood production (Hassona *et al.*, 2006),also, may be due to the environmental condition (Koeniger and Koeniger, 2004; Rangel and Fisher, 2019). As a comparison between the two types of colonies in brood production, the swarmed colonies collected and housed was more activation and production in both years than divided colonies.

Through 2015 and 2016 results indicated that there was a significant difference between the mean pollen grains areas in different seasons in the two types of colonies. the results indicated that in swarmed colonies the highest mean of pollen grains areas was in Autumn 205.54 inch/colony in 2015 and 246.87 inch/colony in 2016 then followed by summer season 196.80 inch/colony in 2015 and 241.55 inch/colony in 2016. In addition, the mean of pollen grains areas in 2016 was bigger than that in 2015 due to the collected swarmed colonies in 2015 and increased their individuals in 2016 as a normal population. On the other hand, the dividing system colonies indicated that the highest mean area of pollen grains was 155.27inch/colony in summer 2015 and 191.37 inches/colony in summer 2016 then followed by autumn in both years, and in 2016 the mean of pollen area was the biggest due to the increased of the bee population. As a comparison between, all seasons in the two types of colonies in the two study years concluded that the mean areas of pollen grains that the workers bees collected in swarmed colonies were higher than in divided colonies in both years. In addition, in both years 2015 and 2016 the highest area of all brood was in the summer season which inverted in pollen grains production in the autumn season. Statistical analysis indicated that there is a correlation between brood and pollen grains by 48.6% with $LSD = 37.42$ and $P = 0.000$**. There was a direct relationship between pollen grains production and brood production in the Airland region (Masry and Abdelaal, 2016).

The bee honey production in the first experimental year of 2015 has the highest amount of honey in swarmed colonies 491.21 $inch^2$/colony in the autumn season followed by 408.57 $inch^2$/colony in the summer season. Compared with the divided colonies the highest amount was 304.83 $inch^2$/colony in the summer season. In addition, the total mean honey production in 2015 in swarmed colonies was higher than in divided colonies. In 2016 the total mean amount of honey was higher in swarmed colonies 339.42 $inch^2$/season than that in divided colonies 286.59 $inch^2$/season, the highest amount was 484.54 $inch^2$/colony in swarmed colonies in autumn followed by 391.83 $inch^2$/colony in summer compared with divided colonies autumn 409.13 $inch^2$/colony followed by summer 336.83 $inch^2$/colony. Previous results illustrated there were significantly different between bee honey productions stored through the two experimental years. Then noticed that; the highest amount was in the autumn season due to the activity of worker bees in autumn than in other seasons agreed with (Abolila, 2016) who indicated that the highest amount of honey production was in October and September than August. There is a correlation between sealed brood and stored honey 55% agreed with (Tahmasbi *et al.*, 2015) showed a significant correlation between bee population and honey production. In addition, (Masry and Abdelaal, 2016)showed that; there was a significant correlation between stored bee honey, pollen grains, brood, and the weather under the new Borg El-Arab region and the bee population increased from spring to winter then beekeepers can save their colonies in winter to manage the next season by easy way. (Bouga *et al* 2007) stated that honey yield affected the bee population size, and the colonies had many workers store a big amount of honey.

The ratio of HB% (Fig. 3) of divided colonies in the spring after 12h were 25.83%, while after 24h were 58.83%, while after 48h were 84.27%. in the summer after 12h were 30.33%, while after 24h were 66.83%, while after 48h were 85.50%. in the autumn after 12h were 22.23%, while after 24h were 60.50%, while after 48h were 86.00%. The previous results indicated that swarmed colonies have hygienic behavior better than divided colonies in different experimental seasons. The difference between hygienic colonies and non-hygienic colonies is that the hygienic colonies uncap dead brood more rapidly than non-hygienic colonies (Heikal *et al.*, 2008; Kozmus *et al.*, 2007). Hygienic is a defensive behavior for the colonies and has many complex environmental and genetic factors (Uzunov *et al.*, 2014) Here the swarmed colonies are more hygienic than divided colonies due to the swarmed colonies have stronger characters than other colonies. (Barrs *et al.*, 2021)indicated that there was a positive correlation between the removable larvae and hygienic brood behavior. Again (Batz *et al.*, 2022) indicated that the workers able to remove the brood have diseases and problems.

All previous results (Fig. 2) indicated that the swarmed colonies have a higher amount of brood, pollen grains, and bee honey than divided colonies. In addition, swarmed colonies have activation workers and a big bee population. On the other hand, these colonies have good defensive behavior against any disease that may attack them moreover, headed by stronger swarmed queens. That was mean the swarmed colonies, were not bad colonies and have no bad behavior at all, the beekeeper must follow their colonies and if any colony swarmed must collect this swarm and keep it in good condition then provide it with food and a place to save the genetic characters and mother queen have important genetic characters in this colony.

As a discussion result (Fig.2) indicated normality statues for all queens because any new queen in the beginning before mating has small size and lightweight, the queen mated and her physiology, especially her ovaries will be changing completely (Hassona and Mourad, 2016). On the other hand, during one year of heading the colony, her weight increased, and this was because the workers provided queens much feeding royal jelly to continue egg laying. Then, after the period of time (one or two or more years depending on the health and well mate of the queen) weight reduce and she stops laying eggs because she became older and, in this status, the colony changes her with another new queen (Sheikh *et al.*, 2019).

The three arbitrary RAPD-PCR genes belong bees also gave the same results which confirm that obtained the specific PCR. The results obtained by RAPD-PCR revealed that different band patterns were observed with polymorphic 82.7% and 17.2% monomorphic. The high percentage of polymorphic band patterns indicated that the genetic characteristics between the natural and artificial swarm colonies individuals are high. These results agreed with the results obtained by (Kumar Yogesh and Khan, 2014; Ali, 2011) when they used five different arbitrary primers (RAPD-PCR) for distinguishing between the honey bees genotypes collected from different places in India and they investigated that all the five used arbitrary primers succeeded to amplify; 38 (77.55%) were polymorphic and 11 (22.44%) were monomorphic bands varied.

Results assumed that this gap in genetic profiles between the examined honey bees' individuals due to 90% of natural swarm colonies, individuals mostly young and nurse workers (Garnery *et al.*, 1998). Moreover, (Richards, 2012) postulated that molecular and physiological characteristics of the depart individuals may differ from the rest of the colony. This assumption confirmed the results obtained in this investigation and showed that natural swarming colonies are isolated in group one (representative by colony 1) whenever; one of the natural swarmed colonies (14) is grouped with non-swarmed (divided system or artificial swarm) colonies (representative by colony 4) in the same cluster. This could explain that the similarity between the natural swarmed colony which is grouped with dividing colonies is 98%, but the similarity between cluster 2 (colony 14 and colony 4) and cluster 1 (colony 1) was 85%.

The results of specific PCR Cytochrome C specific gene for bees confirmed the obtained results by RAPD-PCR. This may ensure that swarming individuals have the tendency to segregate or are already segregated before their departure from the mother colony. Results of the two used molecular techniques (RAPD-PCR and the specific PCR for Cytochrome C) are mainly like what was speculated by (Kandemir et al., 2006),when used the mtDNA in the differentiation of the honey bee samples (*A. m. carnica*) have the same morphological appearance, but they collected from different regions. These results indicated that there are polymorphic and monomorphic patterns between the individuals collected from the three examined regions in Serbia. Whenever, amplified fragment with molecular size 658bp of cytochrome C oxidase 1 was used as a tool for species identification (Hebert *et al.*, 2003). Here it can conclude that; both genomic and mitochondrial DNA classified the examined natural and artificial swarmed colonies into three different groups. The differences between the three groups back to the mother queens' colonies and their swarm behavior colonies. The presence of three mtDNA genotypes in all honeybee samples may be evidence of the presence of more than one subspecies and this assumption was agreed with (Gaspar *et al.*, 2002) when obtaining only a unique mtDNA pattern they suggested that the examined three areas may contain two subspecies. On the contrary,(Kozmus *et al.*, 2007) postulated that honey bees of Serbia have only one native genetic pool founded in *A. m. carnica*.

Based on the DNA sequence alignment, only two ecotypes were observed, (4 -14) and (1) with an A-T transition of 67%. Whenever, there is a T-C transition within the ecotype (4 -14) with a percentage does not exceed 33%. Results obtained by (Kozmus *et al.*, 2007) agree with that obtained in this investigation, they found that T-C transition in 10% of the examined samples was associated with two polymorphic sites in the COI-COII region. (Kozmus *et al.*, 2007) discover a novel variable nucleotide position between two aligned sequences, but in this study the variable regions between the aligned sequences are many. Whenever, amplified fragment with molecular size 658 bp of cytochrome C oxidase 1 was used as a tool for species identification (Hebert *et al.*, 2003).

During the examining of the similarity percentage and the rooting of the genetic diversity among the examined colonies in this study, it was observed that the two constructed

phylogenetic trees (DNA and deduced amino acid sequence) revealed that, the colony1, from natural, swarmed colonies, was separated in one lineage or cluster. Whenever, colony 4, from artificially swarmed colonies, and natural swarmed colony 14 are separated into another cluster. Consequently, the second cluster is divided into two different groups, each group containing one colony. Previous results assumed that the genetic diversity among the individuals of colony 1 back to the number and types of honey bee drones that mated the queen and that was indicated before by using microsatellite loci (Hassona *et al.*, 2013). This assumption is confirmed by a number of T-A transitions in different loci along the amplified fragment of the cytochrome C gene. Whenever, a transition from T-C was only observed between colonies 4 and 14 which could be a determinant for the type of swarming, this type of transition was previously observed by obtained by *(*Kozmus *et al*., 2007). But the T-C transition is the first recorded in this study and the results assumed that this type of variation is linked with the type of swarm. This assumption needs more sequence for parts of the colony genomes, which will give a clear indication if the three colonies belong to one species or if they have differed especially colony number one.

CONCLUSION

As a conclusion the honey bee swarms have a very important genetics characters and need more studies to determine each of them and know in which gene exactly. For beekeepers, establishment of swarm traps in the apiary during the swarming season to attract swarms and protect swarms from losing, because the scout workers start to search for suitable housing among the rows of the apiary first before leaving. Delayed colony division, after leaving the colonies to simulate swarming or at least move towards it, allowing the bees to activate genes related to production and hygienic behavior. Leaving the swarm queen headed the colony, as it is good in terms of production and wintering for a full year from the swarming date.

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التنوع الوراثى والانشطة البيولوجية للأنوية الناتجة من التطريد والتقسيم فى طوائف نحل العسل

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الملخص

تطريد نحل العسل سلوكا حاسما للتكاثر العددى لطوائف نحل العسل فى اوائل الربيع والخريف ومن المعروف ان طرد تكاثر نحل العسل أظهر دائما تنوعا وراثيا بين أفراد التطريد من جينات معينة. هذا النوع من التنوع الجينى يحدث دائما للأفراد الذين يحشدون بشكل طبيعى. ومن ثم تم تقسيم أثنى عشر طائفة لنحل العسل الى ستة طوائف للتطريد وستة آخرى للتقسيم. ولدراسة عوامل النشاط البيولوجى بما فى ذلك مساحة العسل وحبوب اللقاح والحضنة المقاسة بالبوصة المربعة. علاوة على ذلك، تم تسجيل أختبار السلوك الصحى ووزن الملكات، وتربية الملكات ،وعدد المبايض لمدة عامين متتاليين. بالاضافة الى ذلك، تم استخدام جزيئين مختلفيين مثل PCR النوعى و RAPD PCR حيث ساعد الجزء الجزيئى فى فهم التنوع الجينى المتطور بين الافراد داخل التطريد الطبيعى وتلك الموجودة فى التقسييم الصناعى. فيما يتعلق بالأنشطة البيولوجية. وأوضحت النتائج أن الطوائف المطردة طبيعيا كان لديها أختلافات معنوية عالية مقارنة بالطوائف المقسمة صناعيا فى السنة الأولى، فى حين لوحظت أختلافات غير معنوية بين التطريد والتقسيم فى السنة الثانية. علاوة على ذلك، تم الحصول على الأختلافات الجزيئة بأفتراض أن هذة الأختلافات تعود الى الجينات المقابلة ذات الصلة بالكثرة العددية والتى تتحكم فى الغالب فى بعض الجينات الدفاعية للسلوك الصحى والعناية. كشف هذة النتائج عن وجود علاقة مد وجزر بين الجهاز المناعى للنحل وملامحة الوراثية. وبالتالى، نوصى بترك الطوائف للاستعداد لحالة التطريد لتعزيز قدرتها الإنتاجية وسلوكها الصحى بعد توزيع خلايا التطريد فى المناحل لتجنب فقدان الطوائف.

الكلمات الافتتاحية: نحل العسل ، التطريد والتقسيم للخلايا، القياسات البيولوجية، أختبار السلوك الصحى، تحليل DNAو PCR RAPD