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Detection of Genetic Relationship and Genetic Variation of the Four Species of Real Flies Using Molecular Markers

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ABSTRACT

Objective: To uncover the genetic relationship and the genetic variation of four types of true flies, which are as follows: *Musca domestica, Chrisomya megacephala, Sarcophaga africa Tabanus* regulars, using RAPD-PCR markers.

Methods of work: Samples were collected from Tarmiyah district in Baghdad governorate using traps. The species of flies were diagnosed at the Research Center and Museum of Natural History at the University of Baghdad. The species were preserved using 70% ethanol alcohol, the DNA extraction process was carried out, the purity and concentration of the DNA was measured and adjusted using a nano drop device, then the RAPD- PCR reactions were performed using (17) primers and the results were migrated to the acarose gel and the packages were photographed using a digital camera, after which the statistical analysis was performed. Her and the results are saved.

Results: The results of the RAPD markers showed different types of bands, and the total loci identified by the primers on the samples genome were (151) loci, of which (11) are general loci and (140) are differentiated loci. A locus, while the two primers produced (P5, P7), the lowest number of loci reached (6) loci. The total number of produced bands was (276) (a band of which (231) polymorphic bands and (44) monomorphic bands. as for the general variation ratio of the produced primers, it was 84%. The study showed the presence of distinctive bands, the total of the distinctive bands was (85), and of which (67) were unique bands and (18) were absent. (12) bands As for the absent bands, the house fly was distinguished by the lowest number of absent bands (3) band, while the horse fly got the highest number of absent band, reaching (6) bands. From the results of the interactions of the genetic distance, the values of the genetic distance ranged between (0.652 - 0.975), where the least genetic distance was between the meat fly and the horse fly, as it reached (0.652). This indicates that these two types are the most similar among the studied species, and it was the highest genetic distance between It is between the metal fly and the horse fly with a genetic distance (0.975), which indicates that they are more different within the studied species.

Conclusion: There is a close degree of affinity between the four types of flies, even though they belong to families, so molecular markers can be used in classifying insects, especially flies, accurately and determining their genetic fingerprint instead of adopting the phenotypic features in the classification because molecular markers are fixed traits and do not change with changing conditions.

Keywords- Genetic Relationship, Genetic Variation, Real Flies, Molecular Markers.

I. INTRODUCTION

The flies belong to the two-winged order, Diptera, which is considered one of the major insect orders, as it includes about 120,000 species and was placed in 177 families. The classification is primarily based on the study of the phenotypic traits and their comparison with other species (Schuman, 1992).

The classification at the phenotypic level was not sufficient to distinguish between the types of flies. Therefore, in the past few years, researchers have resorted to using DNA markers in diagnosing the species as well as estimating its genetic distance (Benecke and Wells, 2001) and among these markers are the RAPD markers (Random amplified Polymorphic DNA) and the markers of DNA are one of the most used markers in the field of biotechnology and are widely used in the study of variation and heterogeneity in the nucleotide sequence of the genetic material of living organisms. Mutation that occurs in chromosomes in general and genes in particular (Mondini, *et al*., 2009).

Molecular markers can identify specific and genetic loci by using the primer or probe, which depend on the presence or absence of complementary loci (Williams *et al*., 1995). Researchers have sought to develop these markers and normalize them to work since their discovery until now (Williams *et al*., 1990). Many of these markers, especially RAPD, have been widely used in studying the genetic variation between flies and determining the genetic signature of flies (Singh *et al*., 2016: Sunitha *et al*., 2015: Malviya *et al*., 2010). The aim of the research is to uncover the genetic relationship and the genetic variation of four types of real flies using molecular markers.

II. MATERIALS AND METHODS OF WORK

Collecting and diagnosing samples

Samples were collected from the Tarmiyah district of Baghdad Governorate, where they were

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collected from livestock farms, as well as near vegetable markets and butchers' shops. The types of flies were diagnosed in the Research Center and the Museum of Natural History / University of Baghdad, in 7/12/2020. The species were preserved using 70% ethanol alcohol and the samples were placed in special marked plastic bottles until the DNA extraction process was performed. They are four types of whole flies, as shown in Table (1):

Table 1: Types of flies used in the study

N	The scientific name	Local name
1	*Musca domestica*.L. (1758.)	House fly
2	*Chrisomya megacephala* (Fabricius,1794)	The metal fly
3	Sarcophaga africa wiedeman(1824)	Meat fly
4	*Tabanus regulars Jeannine* (1866)	Horse fly

DNA Extraction

DNA was extracted from insects using a modified method of the first two methods mentioned by (Boyce, 1989), and the second method is (Al-Sugmiany, 2017) from (Haung, 2013).

DNA Purification

The process of measuring the concentration and purity of DNA was done using a (nano drop) device, and then the sample was diluted to a concentration of 50ng / ml and preserved by freezing until use**.**

Gel Electrophoresis

The necessary solutions, materials, and gels are prepared and samples loaded in the electrophoresis process according to the method mentioned by (Sambrook et al. 1989: Al-Sugmiany, 2017).

RAPD - PCR Reactions

RAPD markers were performed based on (Williams *et al*., 1990) for four types of flies using (17) primers shown in Table (2), and the components of the reaction shown in Table (3).

Table 2: The primers used in the study

No.	Primer	Sequence 5´→→→ 3´	No.	Primer	Sequence 5´→→→ 3´
1.	OP A-01	CAGGCCCTTC	10.	OP C-16	CACACTCCAG
2.	OP A-06	GGTCCCTGAC	11.	OP C-10	TGTCTGGGTG
3.	OP B-04	GGACTGGAGT	12.	OP D-03	GTCGCCGTCA
4.	OP B-12	CCTTGACGCA	13.	OP D10	GGTCTACACC
5.	OP B-14	TCCGCTCTGG	14.	OP D-18	GAGAGCAAC
6.	OP C-08	TGGACCGGTG	15.	OP G-02	GGCACTGAGG
7.	OP H-16	TCTCAGCTGG	16.	OP G-08	TCACGTCCAC
8.	OP J-04	CGGAACACGG	17.	OP G-14	GGATGAGACC
9.	OP B-20	GGACCCTTAC	18.	OP Y-04	GGCTGCAATG

Table 3: Solutions used in the RAPD markers

C	Components	Volume
1	Green Master mix	12.5 µ l
2	Primer	2 µ l
3	Nuclease free water	8.5µ l
4	DNA template	2µ l
5	Total Volume	25µ l

The reaction program was applied with the pre denaturation temperature (94) for a period of (4) minutes for one cycle only, after which (40) cycle consisted of denaturation heat (93) for (45) seconds and the annealing temperature (36) for (45) seconds and the extension heat (72). For (1) minute and a final heat of extension (72) for (7) minutes, one cycle. The end of the reaction time, the tubes were removed from the thermoplastic device and kept in the freezing, and (5) microliters were withdrawn from the tubes and the mixture was loaded onto the

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prepared acarose gel at a concentration of 1.5% and stained with the Red say dye with the Marker volumetric guide. Then the samples were removed on the acarose gel. Then the jelly was photographed with a high-resolution digital camera and the images were saved in a computer.

Statistical Analysis

The results of the multiplication operations of the primers used in the RAPD markers were taken and converted into tables, depending on the presence or absence of the DNA bands and comparing it between the different samples, where the presence of the bands is symbolized by the number (1) and the absence of the bands by the number (0), the genetic similarity coefficient was calculated As well as the genetic distance between the studied samples using Nei's factor 72 (Nei and Li. 197) The similarities and differences in the genetic material (DNA) that can be obtained from the application of RAPD-PCR markers can be adopted to determine the genetic distance, which will convert the results obtained. Which appears in the gel to characterization tables by setting (1) when the beam is present and (0) when the bands is absent.

III. RESULTS AND DISCUSSION

Results of RAPD Markers

In this research, the genetic variation of four types of flies referred to in Table (1) was studied using the RAPD markers. (17) primers were used as shown in Table (2). Monomorphic bands and polymorphic bands were detected on acarose gel in the presence of a DNA marker (DNA ladder100bp). (11) general loci for all samples and (140) different loci . The primer (P1) was distinguished by the highest number of loci produced, as it reached (14) loci, while the primers (P5, P7) produced the lowest number of loci, as it reached (6) loci. The total number of total bands produced from those loci was (276) a band of which (232) polymorphic bands and (44) a main bands. (P8) the lowest number of bands as it reached (8) bands, while the genetic variation ratio of the produced primers was 84% (Singh and Achint, 2017: Bajpai and Tewari., 2010).

Table 4: Results of the primers used in RAPD reactions for samples

C	Primer Number	Loci number	Monomorphic loci	Polymorphic loci number	Bands number	Monomorphic bands number	Polymorphic band number	Unique bands	Absent bands	Variation ratio %
1	P1	14	-	14	21	-	21	9	2	100
2	P2	9	-	9	11	-	11	7	-	100
3	P3	10	-	10	13	-	13	8	1	100
4	P4	10	-	10	15	-	15	6	1	100
5	P5	6	-	6	8	-	8	4	-	100
6	P6	9	-	9	13	-	13	5	-	100
7	P7	6	-	6	10	-	10	2	-	100
8	P8	8	1	7	15	4	11	3	-	87
9	P9	9	1	8	19	4	15	3	2	88
10	P10	10	1	9	19	4	15	3	-	90
11	P11	8	1	7	19	4	15	1	2	87
12	P12	10	5	5	34	20	14	-	3	50
13	P14	8	-	8	14	-	14	3	1	100
14	P15	11	1	10	22	4	18	4	2	91
15	P16	7	-	7	11	-	11	3	-	100
16	P17	7	1	6	16	4	12	2	2	85
17	P18	9	-	9	16	-	16	4	2	100
Total		151	11	140	276	44	232	67	18	84

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The molecular sizes of the resulting bands varied, ranging between (100-2000bp) where the lowest molecular size was (100bp) in the primer (P6) and the highest molecular size in each of the primer (P1)), where the molecular size was (2000 bp). As for the proficiency of the primers used in the study, it varied between the studied samples, so the highest efficiency was for the primer (P15) as it reached (8)) and the lowest efficiency was for the primer (P5) where it scored (2.9). As for the ability of discriminatory primer, the primer (P1) was distinguished by the highest discriminatory ability, reaching (8.7), while the least discriminatory ability of the primer was (P5), reaching (3.4) Singh and Achint, 2017) (Sultan and Qadir, 2015).

The study also showed the presence of distinct bands (Absent bands, Unique bands) as shown in Table (6). The total of distinct bands resulting from the primers was (85) bands of which (67) were unique bands and (18) bands were absent. , The metal fly was characterized by the highest percentage of unique bands, reaching (24) bands, while the horse fly got the lowest percentage of unique bands, reaching (12) bands. As for the absent bands, the house fly got the least number of absent bands, it was (3) A bands Whereas the horse fly got the highest number of absent bands as it reached (6) bands, and these bands are used as a diagnostic and distinguishing characteristic for those types, so the appearance of those bands in one type indicates a mutation in a specific loci that led to the identification of the primers of this loci and the emergence of the unique bands, as well Absent bands, as a mutation occurs in the recognition site of the initiator only in one species without the other types, which leads to the cancellation of that recognition and the bands disappears and this is consistent with the results of most researchers (Sharma *et al*., 2015a: Brito *et a.*, 2008: malviya *et al*. , 2011,2012,2015).

Table 5: Distinctive bands the efficiency of the primers, and the discriminatory ability

N	primer name	Molecular waght	Distinctive bands in flies								The efficiency	Discriminatory ability
			A–Musca domestica		B–*Chrisomya megacephala*		C– *sarcophagae africa*		D– *Tabanus regulars*			
			unique	Absent	unique	Absent	unique	Absent	unique	Absent		
1	P1	–300 2000bp	–	–	5	2	3	–	1	–	7.3	8.7
2	P2	–400 1600 bp	3	–	1	–	3	–	–	–	4	4.8
3	P3	–200 1500	1	–	4	1	3	–	–	–	4.7	5.6
4	P4	–200 1600	–	–	1	–	2	–	3	1	5.4	6.5
5	P5	–300 1500	1	–	1	–	–	–	2	–	2.9	3.4
6	P6	–100 1250	2	–	1	–	2	–	–	–	4.7	5.4
7	P7	–300 1000	1	–	–	–	1	–	–	–	3.6	4.3
8	P8	–250 1500	1	–	1	–	–	–	1	–	5.4	4.8
9	P9	–200 1500	–	1	2	1	–	–	1	–	6.5	6.1
10	P10	–200 1500	–	–	1	–	–	–	2	–	6.9	6.5
11	P11	–250 1000	–	1	1	–	–	–	–	1	6.9	6.5
12	P12	–350 1500	–	1	–	–	–	3	–	–	12	5.6
13	P14	–250 1200	1	–	1	–	–	1	1	–	5.1	6.1

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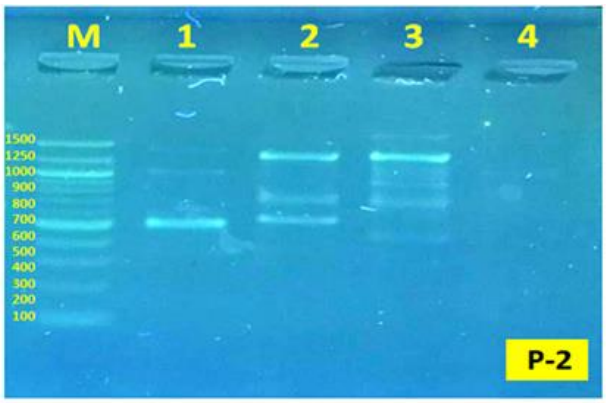
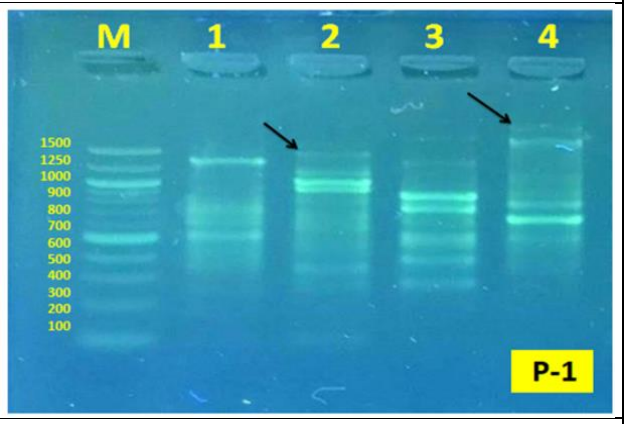
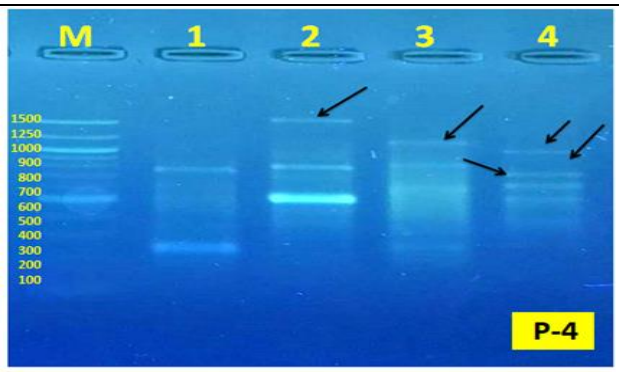
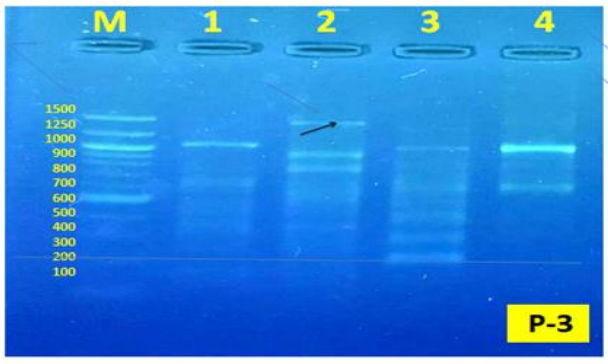
14	P15	<–300 1500	2	–	1	–	1	–	–	2	8	7.8
15	P16	–300 1500	1	–	1	–	–	–	1	–	4	4.8
16	P17	–300 1300	–	–	2	1	–	–	–	1	5.8	5.2
17	P18	–200 1500	1	–	1	–	2	1	–	1	5.8	6.9
Total		Llm	14	3	24	5	17	4	12	6		
			17		29		20		18			
			85									



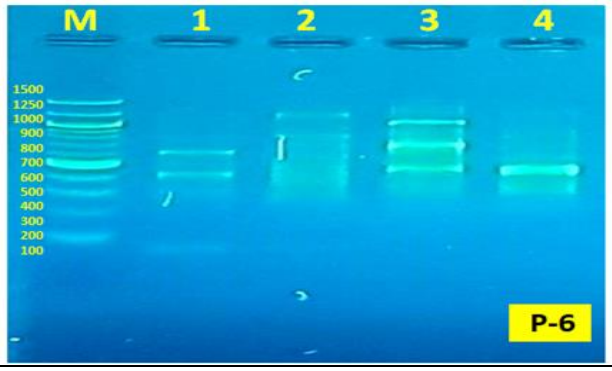


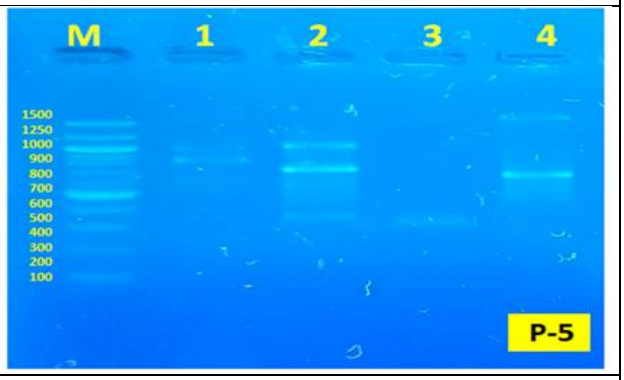
Picture (1,2) The products of the primers P1, P2 of the four types of stage flies





Picture (3,4) The products of the primers P3, P4 of the four types of stage flies





Picture (5,6) The products of the primers P5, P6 of the four types of stage flies

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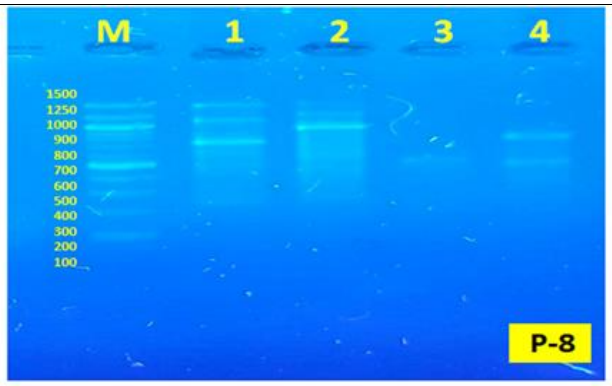
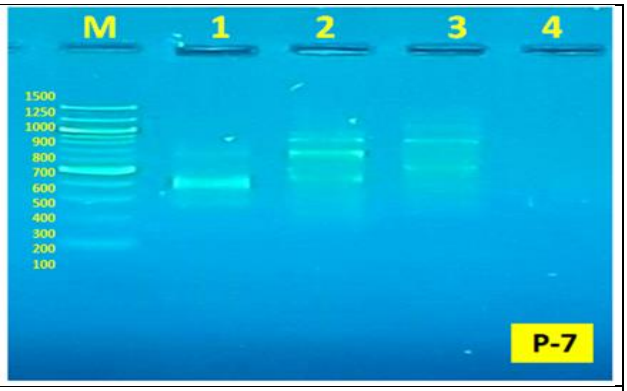
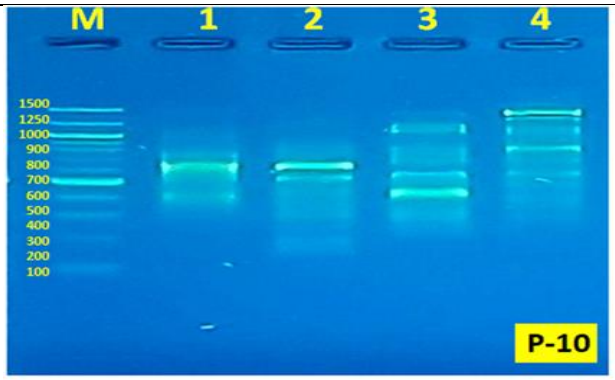
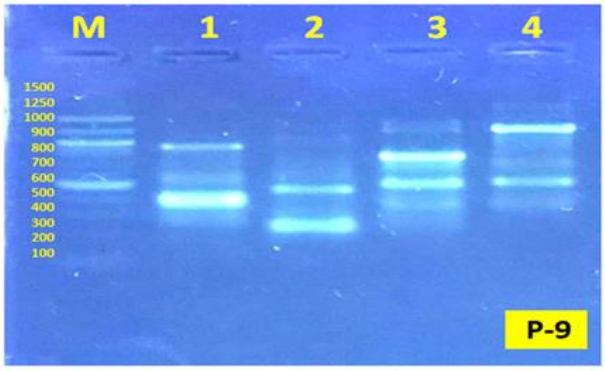


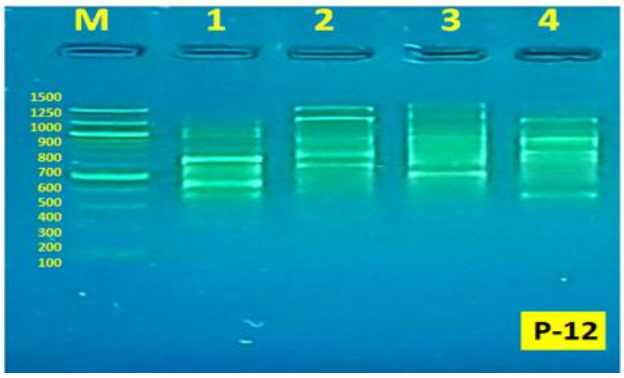


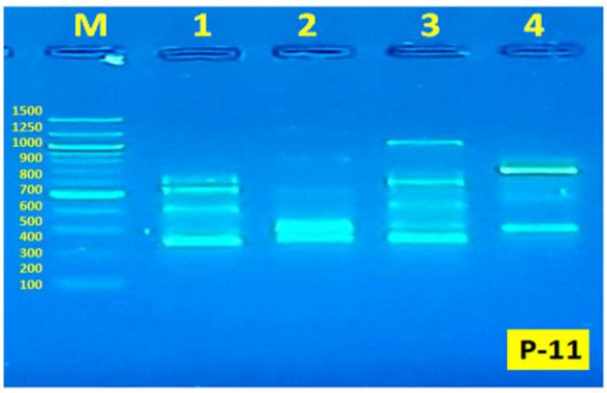
Picture (8,7) The products of the primers P8, P7 of the four types of stage flies



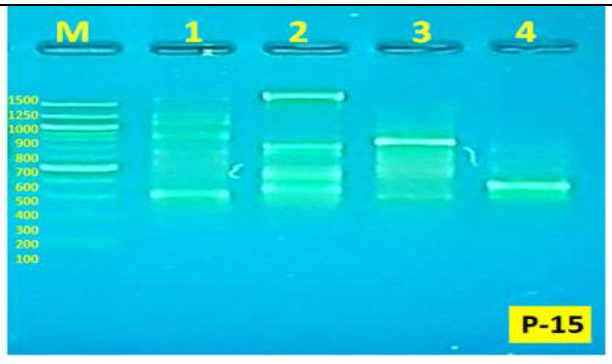


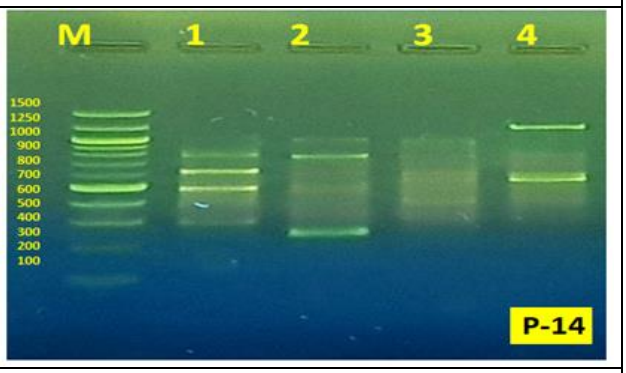
Picture (9,10) The products of the primers P9, P10 of the four types of stage flies





Picture (11,12) The products of the primers P11, P12 of the four types of stage flies





Picture (14,13) The products of the primers P14, P15 of the four types of stage flies

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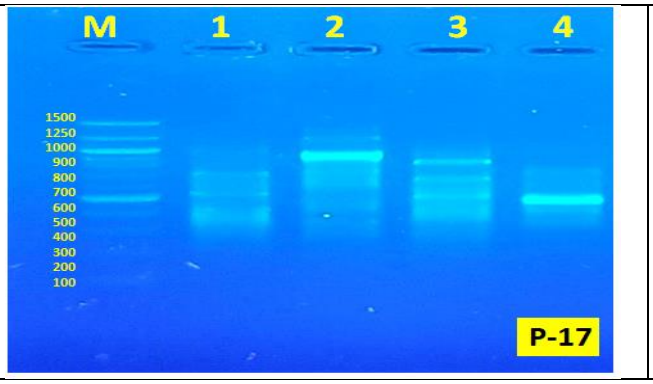
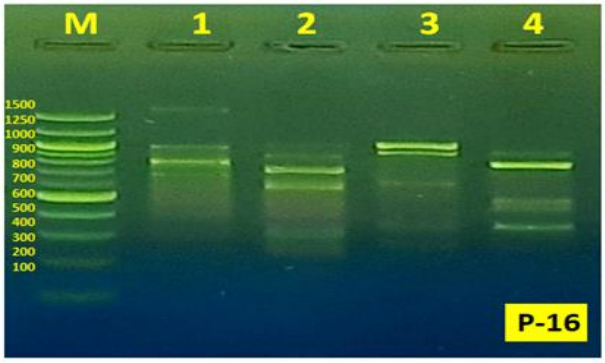
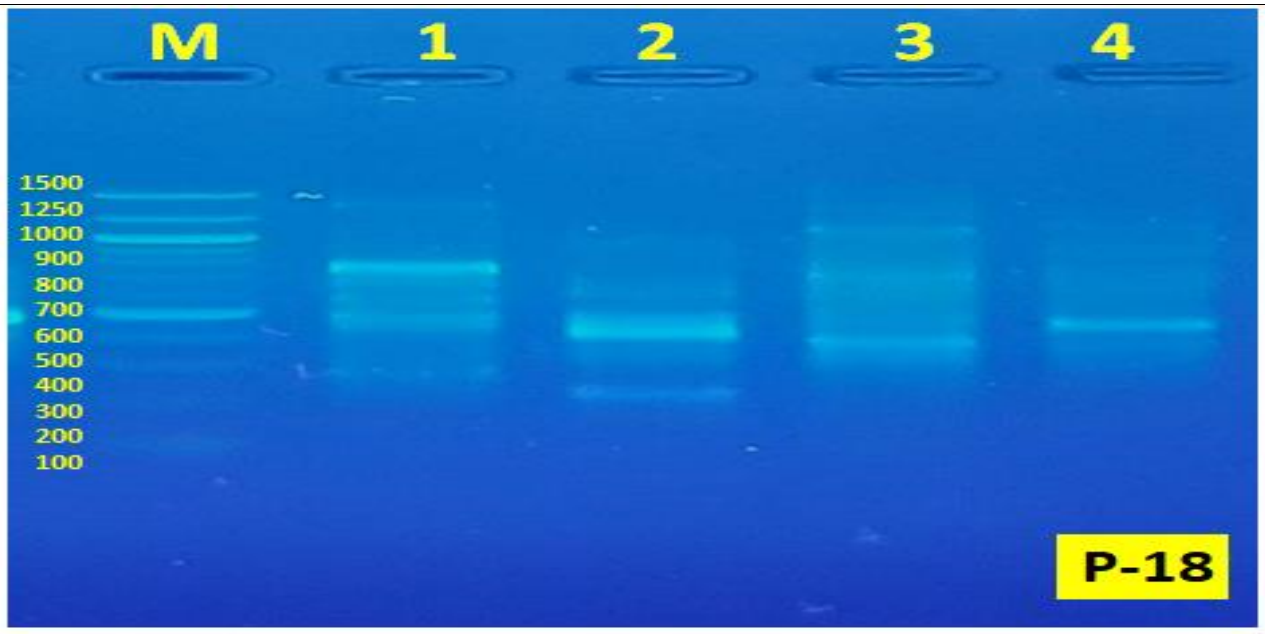




Picture (15,16) The products of the primers P16, P17 of the four types of stage flies



Picture (17) The products of the primers P18 of the four types of stage flies

Estimating the Genetic Distance

The estimation of the genetic distance was performed from the results of the RAPD markers between the four types of flies using the genetic program (NTSYS-PC.version 2.10) which depends in its analyzes on the equation (Nei and Li, 1979). Table (7) shows the values of the genetic distance of the four types of flies. The study was studied using (17) random primers from the RAPD primers, it was found through the results of statistical analysis that the values of the genetic dimension ranged between (0.652 - 0.975) where the lesser genetic distance is between the two species (meat fly and horse fly) as it reached (0.652). However, there is a large similarity ratio between the two types within the studied species, and the highest genetic dimension was between the metal fly and the horse fly, as it reached (0.975), and this is the lowest similarity ratio between the two species within the studied species, and this applies with what he mentioned (malviya, 2011-2012-2015: Sultan and Qadir, 2015).

Cluster Analysis

Based on the values of the genetic distance of the studied species obtained from the results of the RAPD, the cluster analysis group shown in chart (1) was created, where the genetic relationship through chart (2) showed that it was divided into two main groups, group (A and B), which included Group (A) the metal fly only, and this indicates that the metal fly is more different from the rest of the species, and therefore it has the highest genetic distance within the studied species, while group (B) included the other three species, and group (B) was divided into two subgroups, which are group B1 and B2, where group (B1) included the house fly only, while group B2 was divided into two subgroups (B2a, B2b) and the group (B2a) included the meat fly, while group (B2b) included the fourth type, which is the horse fly, and this explains However, group (B2), which includes both the horse fly and the meat fly, has the least genetic distance and is therefore the most similar among the species that have been studied molecularly and this applies to what

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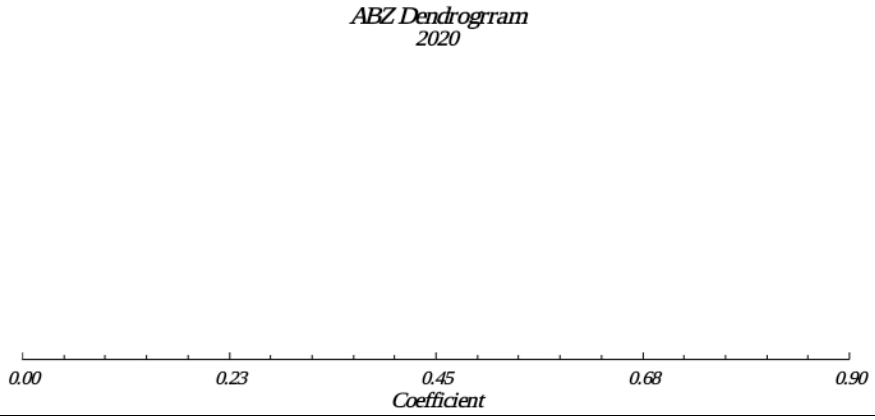
was mentioned by (Sultan and Qadir., 2015) and (malviya, 2011,2012,2015).) On the genetic distance.

IV. CONCLUSIONS

We conclude from the above results the degree of close affinity between the four types of flies, although they belong to different families, but they all belong to one order, which is the Diptera order, and this indicates that the molecular markers, including the RAPD, can be used in the classification of insects, especially flies, in a precise classification and identification The genetic imprint has instead of adopting the phenotypic traits in the classification because the phenotypic traits are unstable and change with the change of environmental conditions in addition to the great similarity between the types of flies, especially the species, including the genus, which makes the phenotypic classification process difficult and imprecise.

Table 6: The values of the genetic dimension of Molecular Data

0.000 **0.898 0.000** **0.736 0.704 0.000** **0.846 0.975 0.652 0.000**
Diagram (1) shows the genetic relationship of the four types of flies depending on the values of the genetic dimension


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