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Studying the genetic relationship among three populations for *Musca domestica* L. (Diptera : Muscidae) in Iraq by using RAPD-PCR Technique

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Abstract
Randomly Amplified Polymorphic DNA Polymerase Chain Reaction RAPD-PCR Technique was used in this study to investigate genetic relationship among three populations of housefly ***Musca domestica*** (L.) from Northern, Middle and Southern Iraq. Environmental conditions such as temperature, relative humidity and the amount of annual rainfall to be different in the northern, Middle and Southern Iraq , In addition to the northern of Iraq away from the southern a distance of 1,000 km and away from the middle distance of approximately 500 km or less, as well as there are some mountains and rivers separating northern Iraq from the middle and south , these environmental conditions act as geographical barriers to gen flow among the three population. We have used in this study nine primers and Tools For Population Genetic Analysis (TFPGA) software was used to calculate percentage efficiency of each primer and percentage of discriminatory ability of each primer , In addition to Nei's genetic identity. The results showed that the primer No. 3 gave the highest of percentage efficiency and percentage of discriminatory ability which is 17.24 and 17.07 respectively ., While primer No. 6 gave The lowest of percentage efficiency and percentage of discriminatory ability which is 5.74 and 6.09 respectively ., The genetic identity among three populations ranges from 1.383 to 1.586, which reveals that there is a low level of genetic identity reflecting the fact that the environmental conditions appear to act as a barrier to gene flow among these populations.
Keywords: *Musca domestica* , RAPD-PCR , Northern , Middle , Southern , Iraq

Introduction
The house fly, ***Musca domestica*** L., is a synanthropic fly of cosmopolitan distribution, It is the most common species found on hog and poultry farms, horse stables and ranches, These flies are also known to transport disease-causing organisms It has been established that housefly is a carrier of several pathogens causing serious and prevalent diseases such as salmonellosis, poliomyelitis, typhoid fever, cholera, infantile diarrhea and amoebic dysentery (Scott , *et al*., 2009 and Greenberg , 1970) . Recently, house flies were shown to spread a deadly strain of ***Escherichia coli*** in Japan (Sasaki , *et al*., 2000) . In recent years, Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction RAPD-PCR Technique has been extensively used for population genetic studies in several dipterans (Balbino , *et al*., 2006 and Sharma , *et al*., 2009) . Randomly Amplified Polymorphic DNA is a dominant expression marker for which no prior information about genomic organization is required (Welsh and McClelland , 1990) . The method is cost effective and uses ten base primers of arbitrary sequence which amplify numerous regions of the genome. The amplicons thus generated reveal abundant genetic information by comparing the genomic fingerprints and can be used successfully for identification and assessing genetic relationships in dipterans of medical, veterinary and economic importance (Infante-Malachias , *et al*., 1999 and Malviya , *et al*., 2011) . Randomly Amplified Polymorphic DNA, a polymerase chain reaction RAPD-PCR Technique, is of immense value for work on anonymous genomes, where only limited quantities of DNA are available. Its cost effectiveness enables one to resolve inter- and intra-specific relationships among insects as these organisms have a relatively large genome size, which increases the probability of finding polymorphism (Hadrys, *et al*., 1992 ; Williams , *et al*., 1990) . In this study , genetic relationship among three natural populations of housefly ***Musca domestica*** from Northern, Middle and Southern locations of Iraq has been analyzed on the basis of RAPD-PCR.

Materials and Methods
This research was performed in the Laboratory of Molecular genetic analysis, Department of Biology, Faculty of Education for pure Science, University of Diyala, Diyala. Iraq. Flies of the ***M.domestica*** were collected from three different locations from Northern, Middle and Southern Iraq , Collected 30 fly , 10 flies from each location . Specimens of the ***M. domestica*** collected were stored separately in 70% ethanol in a small labeled container according to location .

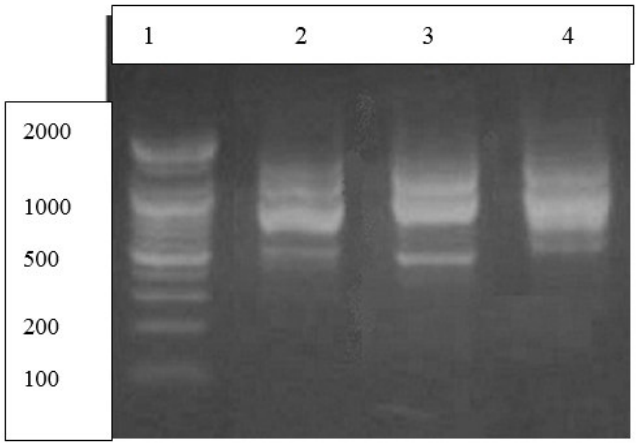
DNA was extracted by using Genomic DNA Mini Kit (Tissue) Protocol, and processed from the Bioneer company / Korea . The purity of DNA was determined by analyzing the ratio of the optical densities at 260 nm and 280 nm. The quality of DNA was assessed by running 20µl of the extracted DNA sample on 1% agarose gel. Extracted DNA was stored at -20°C for further use. The sequences of the primers (procured form

Bioneer company) used in this study have been presented in table (1). The reaction mixture was prepared at 4C° and 20µl of which comprised 5 µl from AccuPower® PCR PreMix which consists of (Top DNA polymerase 1U , d NTP 250µM , Tris-HCl 10m M , KCl 30m M , $MgCl_2$ 1.5m M) , 4 µl of primer (Containing 10 picomoles of primer) , 5 µl DNA and 6 µl Deionized water . Amplifications were performed in MULTIGENE OPTIMAX thermal cycler. The amplification profiles of all the primers are presented in table (1) . In order to detect any DNA contamination a negative control reaction was carried out for each primer sans the template DNA. For each Specimen the amplification reactions were repeated twice for each primer to determine consistency and reproducibility of the bands. The amplicons were loaded parallel with the low range DNA ladder (100 bp - 2000 bp) (Bioneer company) and electrophoresed on 1% agarose gel (Sambrook , *et al*., 1989) . Gels were photographed under Biovis Gel Documentation System, band detection and their molecular weight interpretation was determined using Biovis V4 1D gel analysis software taking low range DNA ruler as molecular weight marker. On the basis of presence or absence of bands in 1 Specimen of each location, a data matrix was prepared. Smears were not taken into consideration. Since RAPD is dominant expression marker hence a dominant allele at a particular locus is denoted by the presence of a band whereas the absence of a band is a manifestation of a homozygous recessive allele at that locus. Tools for Population Genetic Analysis (TFPGA) Software (Miller , 1997) , were used to calculate of Nei's (1972) genetic identity (Nei , 1972) average heterozygosity and clustering of the specimens . The percentage efficiency of each primer was calculation by the following equation : Percentage Efficiency of Primer = The total number of bands for primer / The total number of bands for all primers X 100 , While Percentage of Discriminatory Ability of Primer was calculation by the following equation = The number of polymorphic bands for primer / The total number of polymorphic bands for all primers X 100 (Grudmann , *et al*., 1995) .

Table 1. Random primer sequences used in this study and their amplification profiles

Primer number	Nucleotide sequence (5'-3')	Amplification profile	Reference
1	CCAGCCGAAC	Initial denaturation at 94C° for 5 min (1 cycle), 45 cycles of denaturation at 94C° for 1 min, annealing at 36C° for 1 min, extension at 72C° for 2 min and a final extension at 72C° for 7 min (1 cycle).	Infante-Malachias *et al* (1999)
2	GACTAGGTGG		
3	GGGACGTTGG		
4	AGGGTCGTTC		
5	TGCGTGCTTG		
6	GTCCCGACGA		
7	TGATCCCTGG		
8	CAGGCCCTTC		
9	TGCCGAGCTG		

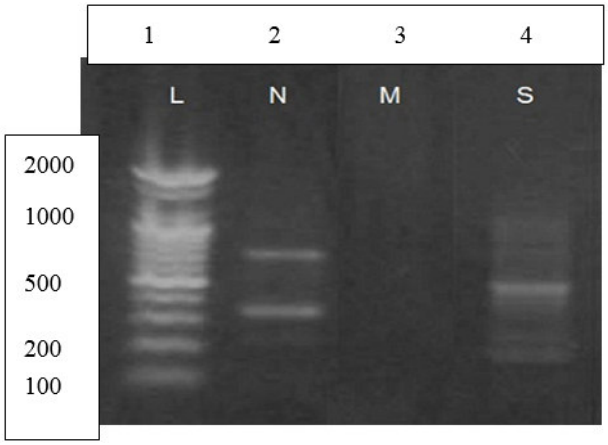
Results



L.	M.W	N	M	S
1	485	0	1	0
2	515	1	0	0
3	586	0	0	1
4	800	1	0	0
5	827	0	0	1
6	878	0	1	0
7	949	0	0	1
8	974	1	0	0
9	1070	0	1	0
10	1155	1	0	1

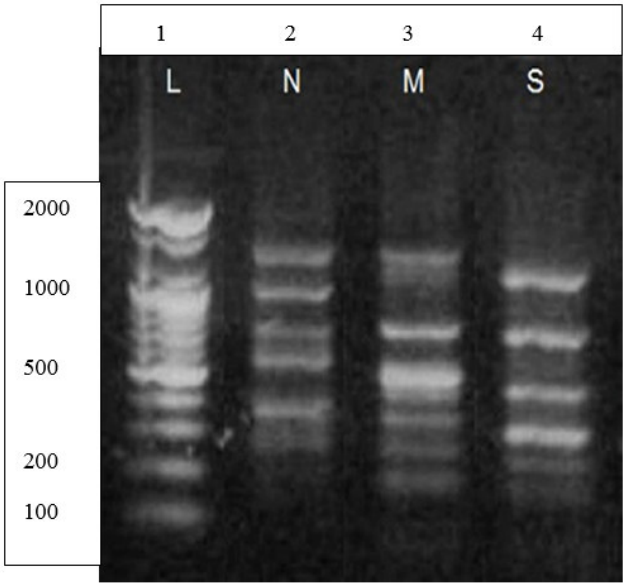
Fig. 1. RAPD-PCR banding patterns derived from primer 1(5" CCAGCCGAAC 3") Lane 1 : Ladder DNA (100 – 2000 bp) , Lane 2 : ***Musca* domestica** from North Iraq , Lane 3 : ***Musca domestica*** from Middle Iraq , Lane 4 :

Musca domestica from South Iraq



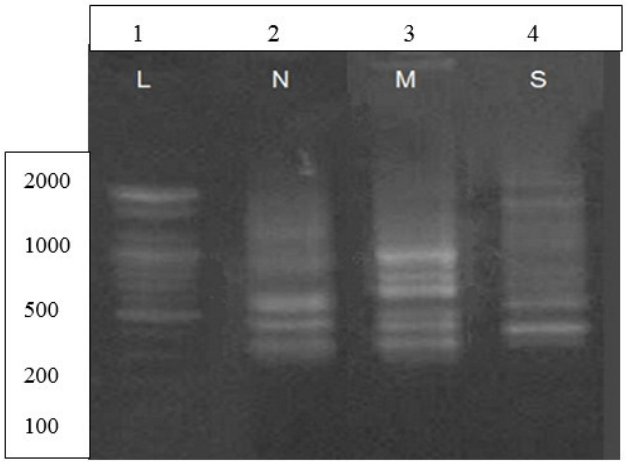
L.	M.W	N	M	S
1	163	0	0	1
2	230	0	0	1
3	321	1	0	0
4	344	0	0	1
5	465	0	0	1
6	700	1	0	0
7	736	0	0	1
8	959	0	0	1

Fig. 2. RAPD-PCR banding patterns derived from primer 2(5" GACTAGGTGG 3") Lane 1 : Ladder DNA (100 – 2000 bp) , Lane 2 : ***Musca domestica*** from North Iraq , Lane 3 : ***Musca domestica*** from Middle Iraq , Lane 4 : ***Musca domestica*** from South Iraq



L.	M.W	N	M	S
1	167	0	1	0
2	195	0	0	1
3	243	1	1	1
4	337	0	1	0
5	368	1	0	0
6	436	0	1	1
7	492	0	1	0
8	553	1	0	0
9	662	0	0	1
10	700	1	0	0
11	748	0	1	0
12	933	1	0	0
13	1011	0	0	1
14	1130	1	0	0
15	1147	0	1	0

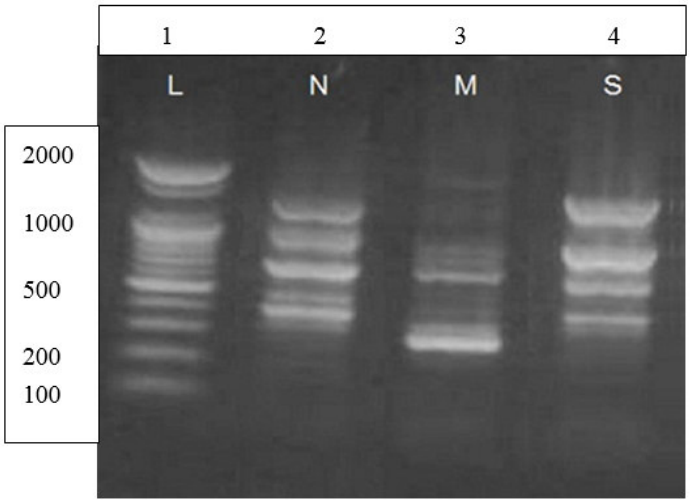
Fig. 3. RAPD-PCR banding patterns derived from primer 3(5" GGGACGTTGG 3") Lane 1 : Ladder DNA (100 – 2000 bp) , Lane 2 : Musca domestica from North Iraq , Lane 3 : Musca domestica from Middle Iraq , Lane 4 : Musca domestica from South Iraq



L.	M.W	N	M	S
1	140	1	0	0
2	162	0	1	0
3	237	1	1	1
4	370	1	0	1
5	482	0	1	0
6	591	0	1	0
7	646	1	0	0
8	700	0	1	0
9	816	1	0	0
10	933	0	0	1

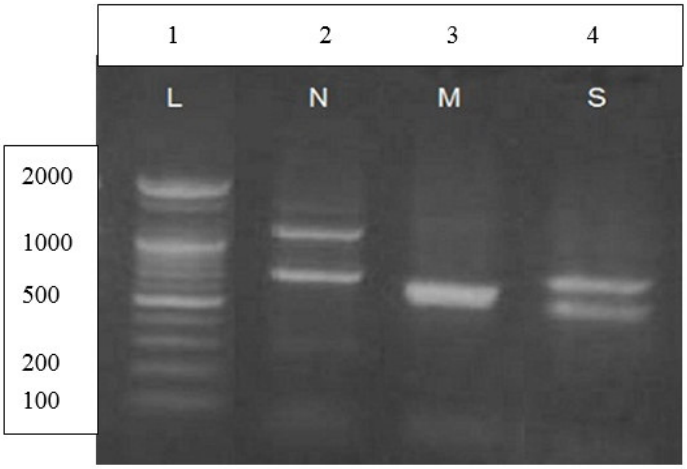
Fig. 4. RAPD-PCR banding patterns derived from primer 4(5" AGGGTCGTTC 3") Lane 1 : Ladder DNA (100

– 2000 bp) , Lane 2 : Musca domestica from North Iraq , Lane 3 : Musca domestica from Middle Iraq , Lane 4 : Musca domestica from South Iraq



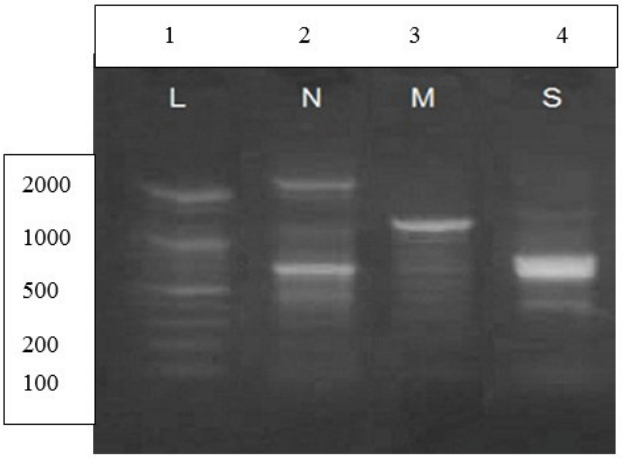
L.	M.W	N	M	S
1	239	0	1	0
2	278	0	1	0
3	308	0	0	1
4	336	1	0	0
5	433	1	0	0
6	478	0	0	1
7	562	0	1	0
8	600	1	0	0
9	647	0	0	1
10	700	0	1	0
11	826	1	0	0
12	1026	1	0	1

Fig. 5. RAPD-PCR banding patterns derived from primer 5(5" TGCGTGCTTG 3") Lane 1 : Ladder DNA (100 – 2000 bp) , Lane 2 : Musca domestica from North Iraq , Lane 3 : Musca domestica from Middle Iraq , Lane 4 : Musca domestica from South Iraq



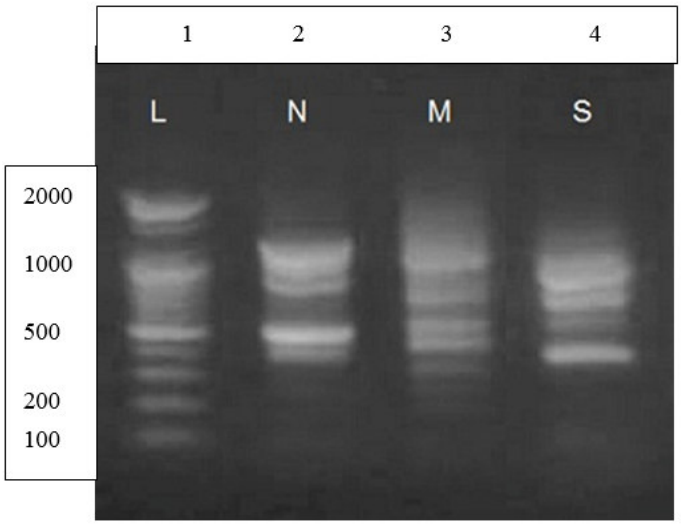
L.	M.W	N	M	S
1	446	0	0	1
2	540	0	1	0
3	600	0	0	1
4	683	1	0	0
5	834	1	0	0

Fig. 6. RAPD-PCR banding patterns derived from primer 6(5" GTCCCGACGA 3") Lane 1 : Ladder DNA (100 – 2000 bp) , Lane 2 : Musca domestica from North Iraq , Lane 3 : Musca domestica from Middle Iraq , Lane 4 : Musca domestica from South Iraq



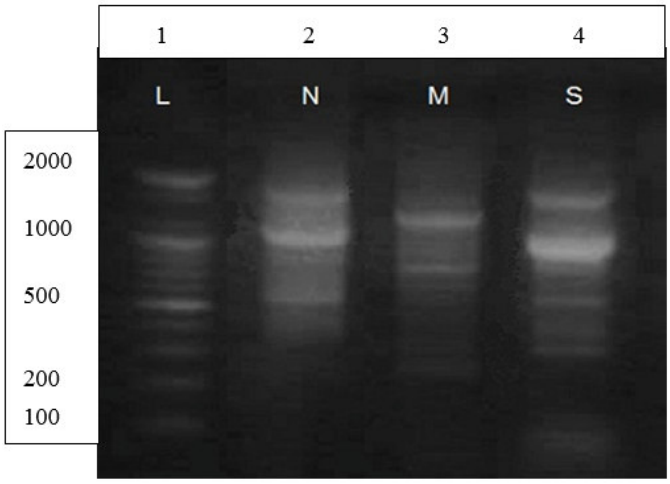
L.	M.W	N	M	S
1	434	0	0	1
2	456	1	0	0
3	571	1	1	1
4	604	0	1	0
5	638	0	1	0
6	867	1	0	0

Fig. 7. RAPD-PCR banding patterns derived from primer 7(5" TGATCCCTGG 3") Lane 1 : Ladder DNA (100 – 2000 bp) , Lane 2 : Musca domestica from North Iraq , Lane 3 : Musca domestica from Middle Iraq , Lane 4 : Musca domestica from South Iraq



L.	M.W	N	M	S
1	316	0	1	0
2	381	1	0	1
3	436	0	1	0
4	461	1	0	0
5	532	0	1	0
6	600	0	0	1
7	733	0	0	1
8	750	0	1	0
9	830	1	0	0
10	873	0	0	1
11	939	1	0	0
12	960	1	1	1

Fig. 8. RAPD-PCR banding patterns derived from primer 8(5" CAGGCCCTTC 3") Lane 1 : Ladder DNA (100 – 2000 bp) , Lane 2 : Musca domestica from North Iraq , Lane 3 : Musca domestica from Middle Iraq , Lane 4 : Musca domestica from South Iraq



L.	M.W	N	M	S
1	307	0	0	1
2	391	0	0	1
3	529	0	0	1
4	559	1	0	0
5	746	0	1	0
6	866	1	0	1
7	900	1	0	0
8	942	0	1	0
9	996	1	1	1

Fig. 9. RAPD-PCR banding patterns derived from primer 9(5" TGCCGAGCTG 3") Lane 1 : Ladder DNA (100 – 2000 bp) , Lane 2 : Musca domestica from North Iraq , Lane 3 : Musca domestica from Middle Iraq , Lane 4 : Musca domestica from South Iraq

Table 2 . The primers used total number of amplified fragments , range of amplified fragments , number of polymorphic fragments , percentage efficiency of primer and percentage of discriminatory of ability of primer among three population of *Musca domestica* in Iraq

Primer no.	Sequence 5"-------3"	Total number of amplified fragments	Range of amplified fragments in (bp)	Number of polymorphic fragments	Percentage efficiency of primer	Percentage of discriminatory ability of primer
1	CCAGCCGAAC	10	485-1155	10	11.49	12.19
2	GACTAGGTGG	8	163-959	8	9.19	9.75
3	GGGACGTTGG	15	167-1147	14	17.24	17.07
4	AGGGTCGTTC	10	140-944	9	11.49	10.97
5	TGCGTGCTTG	12	239-1026	12	13.79	14.63
6	GTCCCGACGA	5	446-834	5	5.74	6.09
7	TGATCCCTGG	6	434-864	5	6.89	6.09
8	CAGGCCCTTC	12	316-960	11	13.79	13.41
9	TGCCGAGCTG	9	307-996	8	10.34	9.75

Table 3. Neis(1972) Genetic identity based on comparison of RAPD patterns among *Musca domestica* populations from Northern , Middle and Southern of Iraq.

	M.domestica (S)	***M.domestica (M)***	***M.domestica (N)***
M.domestica (S)	0.0		
M.domestica (M)	1.507	0.0	
M.domestica (N)	1.586	1.383	0.0

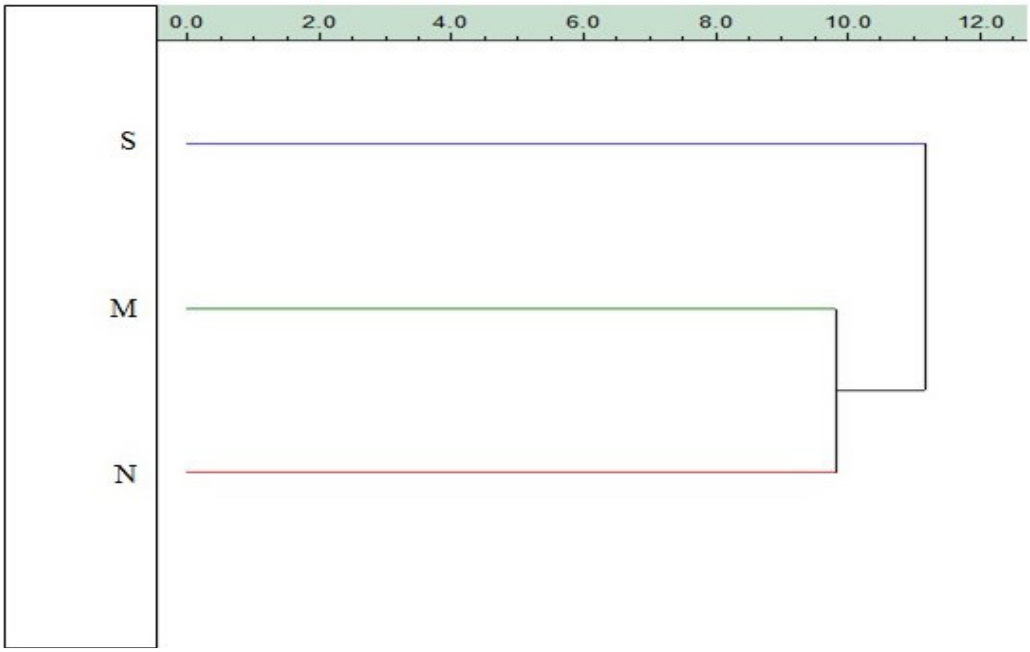


Fig. 10 . UPGMA dendogram as revealed by RAPD-PCR , S : Southern , M : Middle and N: Northern

Discussion

RAPD-PCR technique is an efficient method for analyzing genetic polymorphisms among diverse group of organisms (Skoda , *et al*., 2013 ; Bajpai and Tewari , 2010; Malviya , *et al*., 2011 and Chandra, *et al*., 2010) . The technique utilizes random decamer sequences as a single primer that anneals and primes at multiple locations throughout the genome of an organism. The patterns of amplicons produced are characteristic of the template DNA (Williams, *et al*., 1990). The presence and absence of a specific PCR product is diagnostic for the primer binding sites on genomic DNA, therefore, can serve as useful molecular markers for genetic characterization as well as assessment of genetic relationships (Williams and Bartel , 1995). The advantages of RAPD-PCR include its cost effectiveness, small DNA sample requirements, no requirement of prior knowledge of the sequence of DNA and the ability to identify hundreds of new markers in a short time (Hadrys , *et al*., 1992). Results shown in Table 2 that the primer No. 3 gave the highest of amplified fragment which amounted to 15 bands and it gave the highest of the polymorphic fragments which amounted to 14 bands and it gave the highest of the percentage efficiency and percentage of discriminatory ability which amounted to 17.24 , 17.07 respectively. While the primer No. 6 gave the lowest of amplified fragment which amounted to 5 bands and it gave the lowest of the polymorphic fragments which amounted to 5 bands and it gave the lowest of the percentage efficiency and percentage of discriminatory ability which amounted to 5.74 and 6.09 respectively . When the comparison among the three populations of ***M.domestica*** through genetic identity, as is evident in Table 3 was observed that ***M.domestica*** from northern Iraq are the nearest to ***M.domestica*** from Middle Iraq and valued at 1.383 while ***M.domestica*** from southern Iraq be beyond the ***M.domestica*** from northern and Middle of Iraq and valued at 1.507 and 1.586 respectively, This shows clearly in the picture No. 10 . The cause of heterogeneity among the three populations ***M.domestica*** may be ascribed to the fact that RAPD loci have higher mutation rates, hence are more polymorphic than that of other markers (Santos , *et a*l., 2011). Different regions in the genome evolve at different rates and single-copy genes have relatively low substitution rates because some alteration in a coding sequence may cause a severe effect on the coded protein product. However, changes in repetitive DNA apparently do not result in these consequences and thus the polymorphism in these regions is greater than coding regions (Li and Graur , 1991). The genetic identity values among the three populations of ***M.domestica*** were low, this could be ascribed to the fact that the three populations surveyed in this study are separated due to physical barriers i.e. rivers which prevents gene flow among them (Srivastava , 2009) . The genetic variation depends on colonization, host and reproductive pressures such that any species distributed over

a great variety of environmental conditions would be genetically more heterozygous as compared to the species of restricted distribution (Narang , 1980 ; Santos , *et al*., 2005 and Scarpassa and Hamada , 2003). Presence of variability in housefly is essential for their ability to survive and successfully respond to environmental stresses (Sharma , *et al*., 2009).

Conclusion

The RAPD-PCR technique is the generates diagnostic markers constant or polymorphic fragments diagnostic for a genus as well as between species within the genus. The data may also be used for elucidating systematic and genetic relationships. In this study RAPD-PCR analysis of the three populations for *M.domestica* from Northern , Middle and Southern Iraq reveals that the *M.domestica* from Northern Iraq close to *M.domestica* from Middle Iraq, While *M.domestica* from Southern Iraq Far away from the *M.domestica* of Northern and Middle Iraq. This is clear from the genetic identity of the three population.

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