

Polymorphism Among and Within Populations of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) in the Jordan Valley and Southern Syria

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ABSTRACT

A laboratory study was initiated to assess levels of genetic variations of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) using Random Amplified Polymorphic DNA (RAPD) method. 47 individuals of medfly were collected from six locations in the Jordan Valley and Southern Syria. Of 18 arbitrary primers screened, 6 were found to generate reproducible bands. Nei's coefficient of genetic distance ranged from 0.10 to 0.57 with an overall mean genetic distance of 0.39. This indicates that 39% of the tested medflies vary from the mean of their genetic constitution.

The lowest distance was recorded in Mazrahit Bait Jen population (0.10), while the highest distance was recorded in South Shuna (0.57). Results based on RAPD product data revealed that populations of *Ceratitis capitata* with common genomes tend to cluster together in the same group. Using 6 primers for RAPD analysis showed an average of 84% and 85.2% reproducibility for females and males, respectively. This indicates that RAPD method is a reliable approach for exploring genetic variation within and among medfly populations.

Keywords: Genetic variation, Medfly, RAPD, Genetic distance, Jordan, Syria.

INTRODUCTION

The Mediterranean fruit fly (Medfly) *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) is a serious fruit insect pest attacking ripening fruit (Liquido *et al.*, 1991; Malacrida *et al.*, 2006; Heath *et al.*, 2007). *C. capitata* has a broad host range, infesting more than 250 types of fruit grown commercially in the Mediterranean

area (Enkerlin *et al.*, 1989; Loquido *et al.*, 1990; Talhuok, 1969). Citrus, cherries, peach, mango, guava, locat, apple and Australian almond are considered hosts of medfly (Christenson and foot, 1960).

C. capitata which is apparently native to sub-Saharan Africa (White and Elson-Harris, 1992) has expanded its distribution in the past 200 years to include several countries in the Mediterranean region, the Hawaiian Islands, Central America and South America (Fimiani, 1989). In the second half of the previous century, medfly has also been sporadically detected in parts of North America, including California, Texas and Florida.

C. capitata is a well established insect pest in Jordan (Abdel- Jabbar, 1994) and Syria (Mofleh, 2000). It is a devastating pest for citrus fruits in the Jordan Valley and for stone fruits in the upper land of Jordan (Abdel- Jabbar, 1994). Infestation percentage ranged from 50-90% on

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fruits and vegetables and in some areas it may cause 100% losses in stone fruits (Abdel- Jabbar, 1994; Liquido *et al.*, 1990; Talhouk, 1969).

History of infestation and geographical spread of this species are well documented (Robinson and Hooper, 1989). However, genetic variation between and among its populations is still obscure.

Studying genomes of individuals of *C. capitata* in isolation from the surrounding environmental conditions reflects the genetic variation among individuals in DNA levels. This provides important data, such as; genetic structure of population and gene flow (Reyes and Ochando, 1998a; Haymer and McInnis, 1994). In addition, the possibility for allocating a gene responsible for insecticide-resistance may be found. This might be implicated for alleviating its disastrous status.

Several techniques have been used to study genetic variation in metapopulation of *C. capitata*. These include analysis of allozyme loci (Gasperi *et al.*, 1991; Malacrida, 1996, 1998, 2006; Reye and Ochando, 1998a) and abundant soluble protein (Reye and Ochando, 1998a) as well as fragment length polymorphism in mitochondrial DNA (Reye and Ochando, 1998b; Gasparich *et al.*, 1995; Reye and Ochando, 2004) and Microsatellites (Bonizzoni *et al.*, 2000, 2001).

Recently, techniques based on the polymerase chain reaction (PCR) using random primers have provided a powerful tool for producing a large amount of anonymous genetic markers called RAPD (random amplified polymorphic DNA (Williams *et al.*, 1990). RAPD has already been applied to *C. capitata* proving

that this approach rapidly generates a large number of markers for genome mapping and for population genetics (Haymer and McInnis, 1994; Haymer, 1994). Many studies revealed the occurrence of geographic differentiation in *C. capitata* by means of molecular approaches such as RAPD and Multilocus enzyme electrophoresis (MLEE) technique (Baruffi *et al.*, 1995; Haymer *et al.*, 1997) as well as restriction fragment length polymorphism in mitochondrial DNA (Reyes and Ochando, 1998b).

This study aims to determine the extent of genetic variation in some medfly populations at the DNA level.

MATERIALS AND METHODS

A laboratory experiment was conducted in the lab of molecular biotechnology at the National Center for Agricultural Research and Extension (NCARE), Jordan, in 2007. Specimens of *C. capitata* were collected from 4 locations from the Jordan Valley and 2 locations from Southern Syria. In addition, a sterile male laboratory strain (strain Vienna 8) was imported.

Adults of *C. capitata* were collected in 2005, 2006 and 2007 from citrus grooves using MacPhail traps in the Jordan Valley (North Shuna, Maa'de, Deir- Alla and South Shuna) and from peach and kaki trees in Jaramana and Mazrahit Bait Jen in Syria (Fig. 1). Laboratory sterile males (strain Vienna 8) previously imported from Biofly and released to control the medfly in Aqapa governorate were also analyzed before released. All fly specimens were stored in 70% ethanol until being used for RAPD analysis.

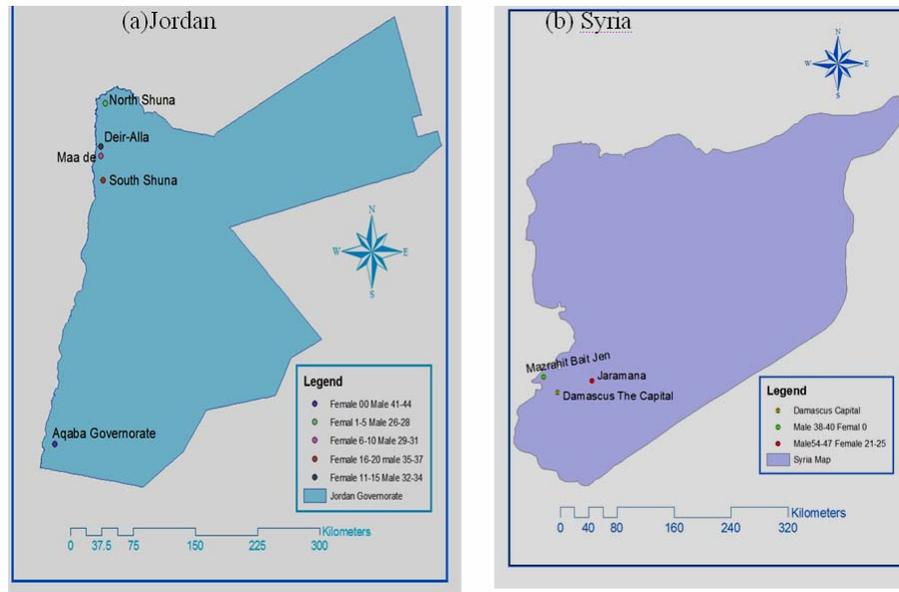


Figure 1. Jordan's (a) and Syria's (b) maps showing the distribution of medfly population *C. capitata* studied in the seventh geographical region in Jordan and Syria. (GIS Unit- NCARE, June, 2008).

DNA preparation from single adult fly was performed as described by Corsini *et al.* (1999) with some modifications for isolation of genomic DNA. Each individual conserved in 70% ethanol was dried under vacuum in a 1.5 ml Eppendorf tube and then ground at room temperature in 200 μ l of lysis buffer [50mM Tris- HCl (pH 8.0), 50 mM EDTA, 3% w/v SDS, 0.1 M 2- mercap-toethanol] with 25 μ l proteinase K (10 mg/ ml) until the solution turned to reddish color. Further 200 μ l of lysis buffer were added, and the mixture was incubated in a water bath (65 $^{\circ}$ C) for 15 min. 100 μ l of 5M potassium acetate were added, and the mixture was incubated on ice for 10 min. After the centrifugation of the mixture for 10 min at 12,000 rpm (Centrifuge 5415C), the supernatant was extracted with 1 volume of phenol: chloroform: isoamyl alcohol (25:24:1). The supernatant was again centrifuged for 15 minutes at 11,000 rpm. 0.6 volume of cold isopropanol was added to the supernatant and incubated overnight at -20 $^{\circ}$ C. The DNA was collected by centrifugation for 10 minutes at 11,000 rpm and the pellet of DNA was washed

with 70% ethanol, dried and then dissolved in 100 μ l TE buffer [10mM Tris-HCl, 1 mM EDTA (pH 8.0)]. DNA was incubated with 3 μ l R-NaseA (10mg/ ml) for each sample and kept at 37 $^{\circ}$ C for 30 min in a water bath to get rid of RNA (Doyle and Doyle, 1990). The DNA from five individual females and three males of each population sample was amplified, except for Mazrahit Bait Jen where only males were used, and from 4 males among the sterile males (strain Vienna 8) .

DNA was detected using gel documentation system (vilber Lourmat, IP, 010- SD, France) (Johanson, 1994). DNA quality and relative quantity were determined using agarose gel electrophoresis (0.7%) mixed with 5 μ l ethidium bromides, then placed in UV-Transilluminator (vilber lourmat, TCP- 20-M, France). DNA concentration was determined using a spectrophotometer (Bio Wave, S2100 Diode Array Spectrophotometer).

Random Amplified Polymorphic DNA (RAPD) Analysis

DNA from the 47 individuals was amplified using the

following oligonucleotide primers (Table 1):

Table 1. The primers and their oligonucleotides used in PCR amplification.

Primer's name	Nucleotide
OPB-01	5'- GTTTCGCTCC- 3'
OPB-02	5'-TGATCCCTGG- 3'
OPB-08	5'-GTCCACACGG- 3'
OPB-09	5'-TGGGGGACTC- 3'
OPB-10	5'-CTGCTGGGAC- 3'
OPB-13	5'-TTCCCCCGCT- 3'
OPB-14	5'-TCCGCTCTGG- 3'
OPB-15	5'-TGCGCCCTTC- 3'
OPB-17	5'-AGGGAACGAG- 3'
OPM-01	5'-GTTGGTGGCT- 3'
OPM-03	5'- GGGGGATGAG- 3'
OPM-08	5'-TCTGTTCCCC- 3'
OPM-09	5'-GTCTTGCGGA- 3'
OPM-10	5'-TCTGGCGCAC- 3'
OPM-13	5'-GGTGGTCAAG- 3'
OPM-16	5'-GTAACCAGCC- 3'
OPM-18	5'-CACCATCCGT- 3'
OPM-20	5'-AGGTCTTGGG- 3'

Amplification reactions were performed in a 25 µl volume, containing 10 ng of template DNA, 2.5 µl 10x Taq Rxn Buffer, 2.5 µl dNTP(0.02mM), 5Pmole from each primer, 1.5 µl MgSO₄ (1.2mM), 0.3 µl (1.5 unit) of Taq DNA polymerase (Promega Catalog # M3001). Double distilled and sterile water ddH₂O was added to make up the final volume of 25µl.

DNA amplification was carried out with the PTC-200 Peltier thermal cycler. The cycling parameters were similar to those described by Williams *et al.* (1990), except that we included an initial denaturation step of 5min at 95 °C (one cycle) and limited the total number of repeating cycles to 40 in order to cut down on spurious weak bands (Haymer and McInnis, 1997). The cycling profile consisted of: 1 min denaturation at 94 °C 1min annealing at 36 °C and

2 min for extension at 72 °C.

Seven nanograms taken from each previously amplified product were used as a template and amplified for 2 min, denaturated at 95 °C for 0.40 sec, annealed at 34 °C for 0.30 sec and extended at 72 °C. Amplified products were visualized using ethidium bromide staining of DNA after electrophoresis in 1.5% agarose gel. Ten µl DNA solution from each sample were mixed with 2 µl of a loading dye in 0.5 ml PCR tubes, 100 bp DNA ladder was used. The gel was allowed to run for 2 hours at 100Volts and DNA was detected using gel documentation system (Vilber Lourmat, IP, 010- SD, France).

Data Analysis

Markers generated from different primers were employed

to compute dissimilarity coefficient values. These bands were visually scored as present (1) or absent (0). All bands ranging in size from 50 to 1000 bp were taken into consideration.

Data analysis was first conducted using NTSys. 2.02 software package. Genetic distance analysis was run according to Nei (1972). The distances were then used to develop a dendrogram using UPGMA to develop a tree relationship between and within medfly populations.

RESULTS

The DNA concentration ranged from 1.7 to 2.2 mg/ μ l. 18 primers from Operon technology (Kit B and M) were randomly screened to test their ability to prime PCR amplification of medfly DNA (Table 1). Six primers (OPB-10, OPB-13, OPM-03, OPM-10, OPM-16 and OPM-20) were considered informative for the purpose of resolving genetic marker differences among and within medfly populations (Table 2).

Medfly populations collected from the six locations were genetically analyzed using the six primers mentioned above. A total of 626 for females and 648 for males of medfly reproducible and clearly scorable bands were produced from the six primers; 84% and 85.2% of them were polymorphic

for both sexes, respectively (Table 2). The six primers revealed a total of 47 and 58 polymorphic markers for females and males, respectively. The numbers of polymorphic markers ranged from 7 for primers OPB-10, OPM-10 and OPM-20 to 14 for OPB-13 and OPM-10. The most informative primer was OPB-13 which produced the largest polymorphic bands (182) (Table 2).

RAPD banding patterns are shown in Figures 2-5. The percentage of polymorphism ranged from 44% for primer OPM-03 to 100% for primers OPB-10, OPB-13 and OPM-16 (Table 2).

Genetic Distance between Medfly Populations

Nei's (1972) coefficient of genetic distance between 47 individuals of medfly populations collected from six locations in Jordan and Syria, in addition to the sterile males (Vienna 8) ranged from 0.10 to 0.57 with an overall mean genetic distance of 0.39, indicating that medfly populations had an average of 39% of their RAPD markers or fragments variable among medfly individuals from the [seven] locations. The lowest genetic distance (0.10) was recorded in Mazrahit Bait Jen group, while the highest one was recorded in South Shuna (0.57).

Table 2. Total number of bands, total number of markers, number of polymorphic markers and polymorphism (%) for medfly populations studied.

Primer marker	Primer name	Total no. of bands		Total no. of markers *		No. of polymorphic markers		Polymorphism %	
		Females	Males	Females	Males	Females	Males	Females	Males
1	OPB-10	42	64	7	7	7	7	100	100
2	OPB-13	134	182	14	14	14	14	100	100
3	OPM-03	165	156	9	9	4	4	44	44
4	OPM-10	69	68	8	14	7	14	88	100
5	OPM-16	80	59	8	11	8	11	100	100
6	OPM-20	136	119	10	12	7	8	70	67
Total		626	648	56	67	47	58	502	511
						7.5	9.5	84%	85.2%

* Bands across the samples.

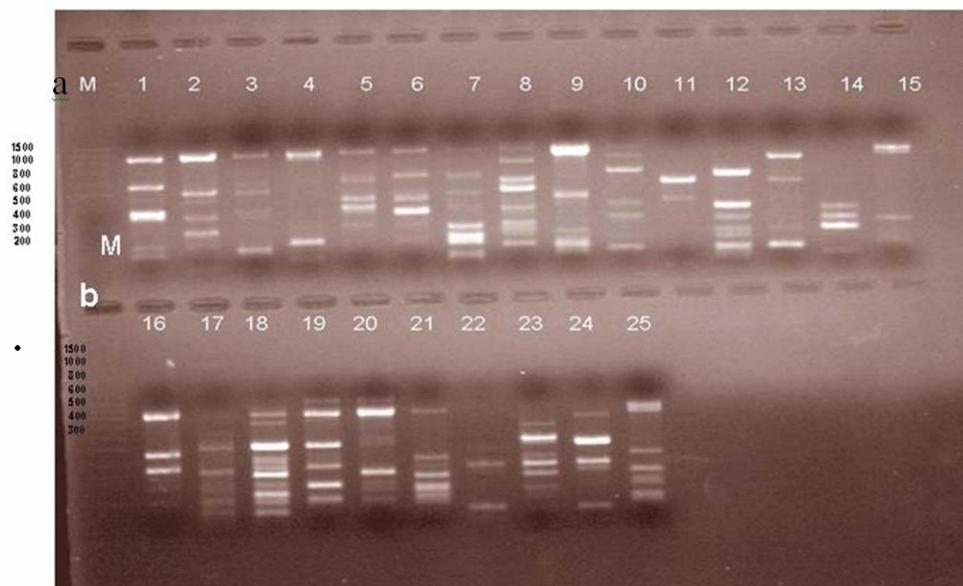


Figure 2. RAPD pattern using OPB-13 primer for females.

(a) M marker, Lanes 1-5 North Shuna; Lanes 6-10 Maa'de; Lanes 11-15 Deir-Alla.

(b): Lanes 16-20 South Shuna; Lanes 21-25 Jaramana.

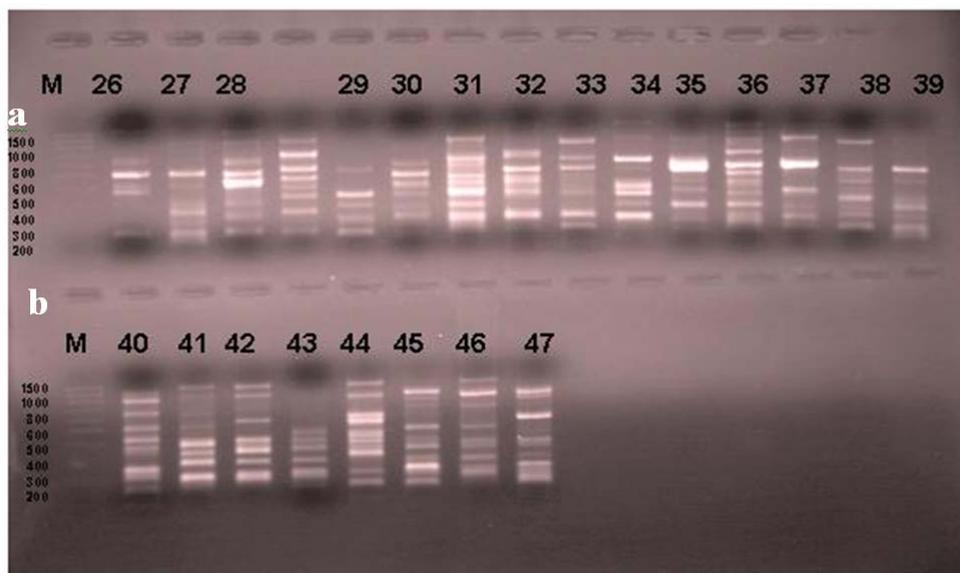


Figure 3. RAPD pattern using primer OPB-13 for males.

(a) M marker, Lanes 26-28 North Shuna; Lanes 29-31 Maa'de; Lanes 32-34 Deir - Alla. (b) Lanes 35-37 South Shuna; Lanes 38 - 40

Jaramana; Lanes 41-44 Vienna 8; Lanes 45- 47 Mazrahit Bait Jen.

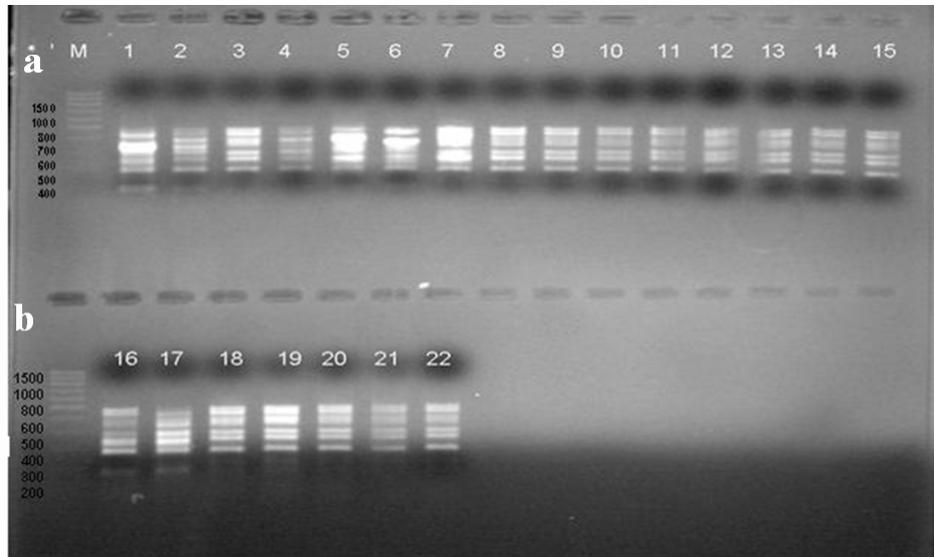


Figure 4. RAPD pattern using primer OPM-03 for females.
(a) M marker, Lanes 1-5 North Shuna; Lanes 6-10 Maa'de;
Lanes 11-15 Deir-Alla. (b): Lanes 16-20 South Shuna;
Lanes 21-22 Jaramana.

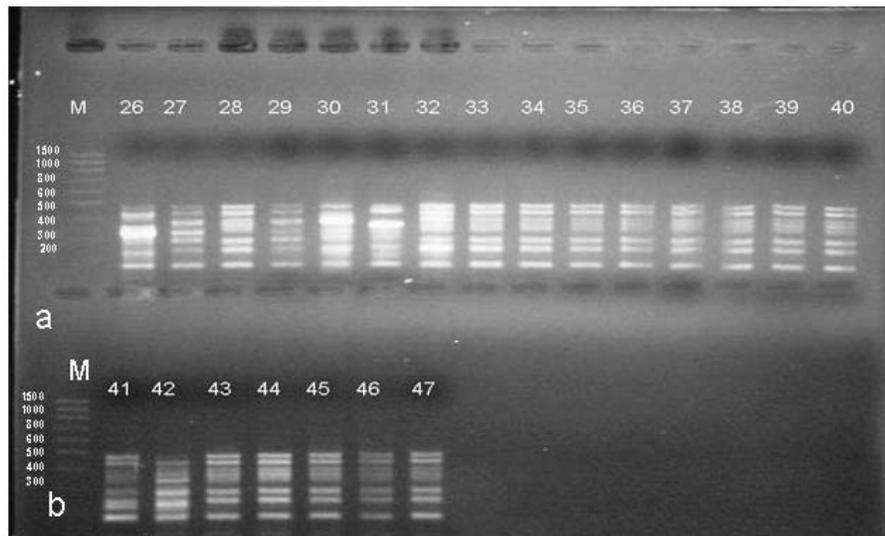


Figure 5. RAPD pattern using primer OPM-03 for males.
(a) M marker, Lanes 26-28 North Shuna; Lanes 29-31 Maa'de; Lanes 32
-34 Deir-Alla. (b) Lanes 35-37 South Shuna; Lanes 38-40
Jaramana; Lanes 41-44 Vienna 8; Lanes 45- 47 Mazrahit Bait Jen.

Genetic Relationships Among Medfly Populations
 The dendrogram constructed by the Unweighted Pair

Group Method with Arithmetic Average (UPGMA) method demonstrated that two main groups existed in the collection

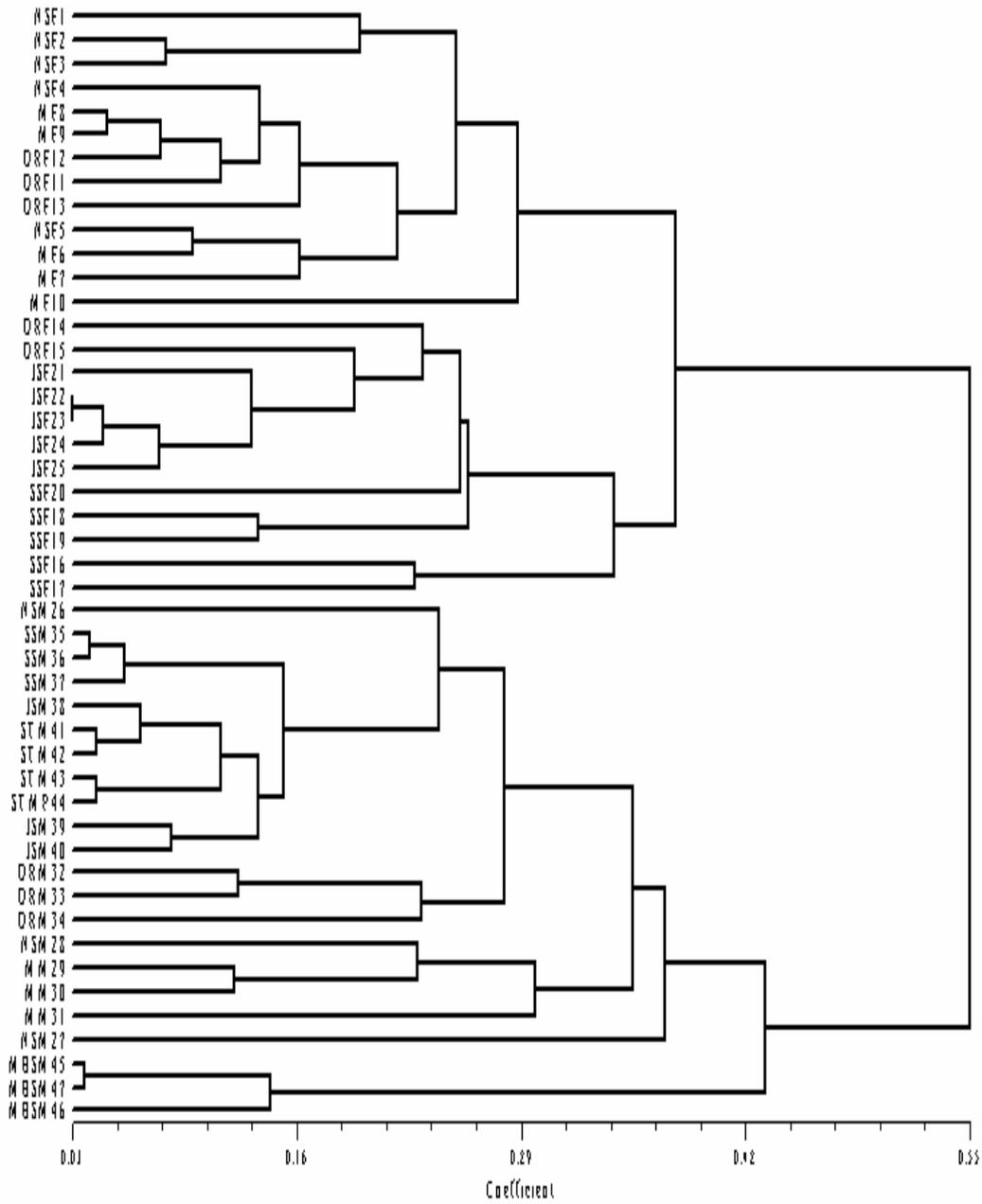


Figure 6. UPGMA dendrogram of 47 individuals of medfly populations collected from seven locations performed on the basis of genetic characters using Nei's (1972) coefficient based on unweighted pair groups.

(Figure 6). The dendrogram clearly separated males from females. The females' group was further subdivided into two main subgroups: I and II. The first subgroup (I) produced 2 clusters: one included individual 10 from Maa'de, which is completely separated from any of the Maa'de samples. The second cluster was divided into 3 sub-clusters. Samples representing metapopulations from the same region exhibit the closest relationships, such as populations 1,2 and 3 from North Shuna, 4,8,9, 11,12 and 13 from Maa'de and Deir-Alla and populations 5,6 and 7 from North Shuna and Maa'de. However, the Deir-Alla metapopulations do not cluster closely with each other. The genetic distance in sub-group I ranged from 0.10 to 0.14.

The second main sub-group (II) was divided into two clusters, the first one including individuals 16 and 17 with a distance of (0.23) and the second cluster divided into two sub-clusters, the first sub-cluster included individuals 18 and 19 from South Shuna with a genetic distance of 0.14. The second sub-cluster included individuals 14, 15, 20, 21, 22, 23, 24 and 25 from Deir-Alla and Jaramana/Syria.

The males' group was divided into two main subgroups: I and II. The first sub-group (I) included individuals 45, 46 and 47 from Mazrahit Bait Jen/Syria which formed a separate group with a distance of (0.04) among individuals. The second sub-group (II) was further divided into two clusters; the first one included individual 27 from North Shuna and the second cluster was divided into two sub-clusters. The first sub-cluster included individuals 28, 29, 30 and 31 from North Shuna and Maa'de. The second sub-cluster included the male individual 26 from North Shuna and the individuals 35, 36 and 37 from South Shuna and individuals 38 and 40 from Jaramana/ Syria and 41,42,43 and 44 from strain Vienna 8 and individuals 32,33 and 34 from Deir-Alla (Table 3).

DISCUSSION

By using DNA templates extracted from medfly individuals, the results showed that different RAPD primers identify regions of DNA that are variable among populations.

The various RAPD primers (OPB-10, OPB-13, OPM-10, OPM-16 and OPM-20) used in this study make it possible to resolve genetic variability among populations of the medfly. Some of the RAPD markers identified here are essentially monomorphic, like primer OPM-03 producing major bands that appeared (58%) monomorphic, Table (2). This primer appeared to amplify a region of the genome that is conserved (or less variable) among these individuals. Similar results were obtained by (Haymer, 1994). The lack of major amplification product for primer OPB-03 in some individuals' populations (Figs. 4 and 5) can also be explained by the lack of at least one primer binding site within the window of size ranges necessary for the RAPD procedure.

Other RAPD primers produce markers that are more variable among populations, identifying DNA polymorphism varying between flies originating from laboratory *versus* wild collections as well as from different geographic localities. Primer OPB-13 (Figs. 2 and 3) as an example clearly promotes the amplification of a more variable region of the genome of the medfly. Even the laboratory strain (Vienna 8) was a little bit monomorphic when we used primer OPB-03.

We used RAPDs to document population relationships in a number of ways. One method included the calculation of genetic dissimilarity index based on the degree of bands unshared between individuals. Using this method, one value (0.38-0.40) was obtained which was consistent, emphasizing the fact that individuals from the same population should represent a somewhat narrowly defined genetic pool or share similarities in their genetic make-up. The same index was also used to calculate genetic dissimilarity between populations. In

this case, the index values vary over a much boarder range. When we compared metapopulation or sub-groups sampled within broadly defined geographical areas (Jordan and Syria), results showed a dissimilarity of 39%. This indicates that these metapopulations are genetically very similar to each other, probably as a result of substantial amounts of gene flow occurring between these populations. Gasperi *et al.*(1991) previously used allozyme studies to show the effect of gene flow in minimizing genetic distances among medfly populations.

The neighbour-joining tree constructed (Fig. 6) for 47 individuals of medfly populations also showed that metapopulations from the same locations tend to cluster strongly together, again reflecting the minimal distances between these samples relative to each other, like the populations from Mazrahit Bait Jen. This is in agreement with the results found by (Haymer *et al.*, 1997) who reported that samples of *C. capitata* from some regions, such as Southern California and Guatemala , appear to be quite closely related to each other, whereas others, such as those from Greece and Argentina, show relatively little in common with any other sampled population. Female populations from Jaramana/ Syria appeared closely similar and clustered in one group. This is in agreement with the findings of Saitou and Nei (1987). Neighbour-joining trees are constructed by creating nodes that link the least distant taxa pairs. On

the other extreme, are male populations of Mazrahit Bait Jen/ Syria, which appeared to be an isolated gene pool, because they exhibit no similarity to any of the other populations sampled here. Here again, these relationships can be seen both in the dissimilarity indices (0.10) and in the neighbour-joining tree.

Our results indicated that genetic variability follows a clear geographic pattern of differentiation in *C. capitata*. Similar results were obtained by other researchers (Gasperi *et al.*, 1991; Reyes and Ochando, 1994; Haymer and McInnis, 1994; Barruffi *et al.*, 1995; Reyes and Ochando 1998b).

Selection is another process that can affect not only the amount of variability, loss of alleles and RAPD markers, but also their relative frequencies. Haymer and McInnis (1997), using six primers, have found the populations of *C. capitata* from the same geographical origin (natural and laboratory) present a similar RAPD pattern, suggesting that they could be used for determining geographical origins. Drift of gene flow in combination with natural selection may have played an important role in genetic differentiation in this species in Greece (Kourti, 2004).

Results obtained from the current study demonstrated that RAPD method is an efficient and good technique for documenting variation among and within populations of the Mediterranean fruit fly.

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Ceratitis capitata Wiedemann (Diptera: Tephritidae)

4 3 2 1

Ceratitis capitata Wiedemann

(Diptera Tephritidae)
(RAPD)

(18)

0.39 0.57 0.10 (Nei, 1972)
(0.10) .%39
/ (0.57) /

RAPD

85.2% %84

RAPD
RAPD

RAPD (Medfly)

(1
(2
(3
(4

.2010/4/28

2008/12/17