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RESEARCH ARTICLE

Genetic structure of Mediterranean fruit fly (Diptera: Tephritidae) populations from Turkey revealed by mitochondrial DNA markers

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Abstract. *Ceratitis capitata* is one among the most destructive and economically important agricultural pests worldwide. Despite its economic significance, the population structures of this pest have remained relatively unexplored in the eastern Mediterranean basin. Using two mitochondrial markers, the present study aimed to examining the population genetic structure and diversity of *C. capitata* populations in Turkey, the region that covers a large part of the eastern Mediterranean area. Our results revealed that the Turkish Mediterranean fruit fly populations are characterized by low levels of genetic diversity and limited population differentiation. For comparison purposes, we merged the sequences identified in the present study with the previously reported sequences from across the world into the data matrix. The haplotype network showed that, unlike the African samples the Mediterranean samples and samples from the new world (America, Pacific region and Australia) did not show any clear pattern of geographical structuring, which indicates that the Mediterranean basin, particularly the eastern Mediterranean region populations, may have played a more important role in the colonization of *C. capitata* populations to the new world. The results also revealed a close genetic relationship between the Turkish and Iranian populations, suggesting that the Iranian *C. capitata* populations probably originated from Turkey.

Keywords. population structure; mitochondrial variation; colonizing species; medfly; Tephritidae; *Ceratitis capitata*.

Introduction

Tephritid fruit flies have been recognized as one of the most serious pests of agricultural crops worldwide (Meeyen *et al.* 2014). Among the members of Tephritidae, the genus *Ceratitis* MacLeay comprises several extremely polyphagous species that are of economic importance and known to be highly invasive or potentially invasive (Barr *et al.* 2012). Among these species, *Ceratitis capitata* (Wiedemann), the Mediterranean fruit fly or medfly is considered as one of the most destructive and economically important agricultural pests worldwide (Reyes and Ochando 1998a; Barr *et al.* 2012). In Turkey, *C. capitata* is particularly damaging fruits such as citrus, peach, apricot, fig and nectarines (Elekçioğlu 2009) and the damage is severe, estimated around 5–75% (Aydemir 2008).

Globally, the genetic structure and the possible colonization route of natural populations of *C. capitata* were analysed using both biochemical and molecular markers (Gasperi *et al.* 1991, 2002; Baruffi *et al.* 1995; Gasparich *et al.* 1997; Gomulski *et al.* 1998; Malacrida *et al.* 1998; Reyes and Ochando 1998a; Bonizzoni *et al.* 2004; Barr 2009). All the markers were consistent in showing that the amount of genetic variation was not distributed homogenously in the species range. Southeast African flies, in particular from Kenya, are the most polymorphic and have the highest level of genetic variability, which supports the assertion that this region is the area of origin of the species (Baruffi *et al.* 1995; Gasparich *et al.* 1997; Gomulski *et al.* 1998; Malacrida *et al.* 1998; Reyes and Ochando 2004). The Mediterranean fruit fly seems to have travelled with man from its home range to the primary introduction area in the northern Mediterranean basin,

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southern Spain followed by a spread to other coastal, northern and eastern Mediterranean locations (Malacrida *et al.* 1998, 2007; Gasperi *et al.* 2002; Reyes and Ochando 2004) in the beginning of the 19th century (Fimiani 1989). It was introduced into Australia from Europe around 1897 as a secondary colonization event (Hooper and Drew 1989; Bonizzoni *et al.* 2004). However, the expansion of the Mediterranean fruit fly into Latin America and the Pacific is reported to be the result of independent and repeated colonization events from different geographical regions (Africa and Mediterranean) or from regions with a high population substructure (Gasparich *et al.* 1997; Malacrida *et al.* 1998, 2007; Bonizzoni *et al.* 2004; Lanzavecchia *et al.* 2008). The observed variation can be explained by various evolutionary forces, singly or in concert, acting differently in the different geographic areas, including genetic drift, bottleneck effects, selection and gene flow (Gasperi *et al.* 1991, 2002; Gomulski *et al.* 1998).

The genetic variability data estimated in the species range are highly correlated with the dates on which this pest was first documented in different countries. *C. capitata* was first recorded in Spain early in 1842, later in Italy in 1863 and in Turkey in 1904 (Fimiani 1989). It was reported from Argentina in 1905 (Gallo *et al.* 1970), from Hawaii in 1910 (Headrick and Goeden 1996) and this pest had established itself in Central America on or before 1955 (Sheppard *et al.* 1992). *C. capitata* was first originally recorded from Iran in 1958 (Rajabiyan *et al.* 2015).

Owing to its maternal inheritance, simple structure and relatively fast rate of sequence divergence, the mitochondrial DNA has been proven to be one of the most useful markers in the determination of genetic diversity, population differentiation, phylogeny, origination, invasion and expansion patterns existing in many insect species (Zhang and Hewitt 1997; Barr 2009; Nardi *et al.* 2010; Dogac *et al.* 2013).

Despite the widespread presence and considerable economic importance of *C. capitata*, little or no information is available from populations in the eastern Mediterranean basin. Knowledge of the genetic structure of medfly populations from this region will increase our understanding of the contemporary genetic structure of worldwide medfly populations and help to trace the route of its colonization. Accordingly, this study aims, for the first time, to examine the overall genetic structure of *C. capitata* populations in Turkey, the region that covers a large part of the eastern Mediterranean area based on the genetic analysis of mitochondrial DNA sequences. Secondly, to further resolve the possible dispersal patterns of this fly, we also compared the Turkish haplotypes with those reported from other geographical regions that represent the current range of this insect population. Its ability to adopt to a wide range of environments, threatening important fruit production areas with subtropical or Mediterranean climates, and its reproductive potential make the medfly one of the most dangerous agricultural pests. The analyses of medfly genetic variability in the species range will provide us valuable information for the effective planning and success of any control and/or management programmes.

Materials and methods

Sample collection

Adults of *C. capitata* were collected in August–September 2016 from seven different geographical localities in Turkey (figure 1). All populations considered were sampled from localities that are known for intensive fruit production. Pheromone traps were used to capture the specimens in citrus farms. We also analysed samples from a laboratory strain maintained at the Directorate of Plant Protection Research Institute (Bornova Izmir, Turkey). This strain was initiated from a local field population and reared under standardized laboratory conditions. All samples were stored at –80°C for the molecular study.

Selection of polymorphic mtDNA regions

Briefly, our analyses centred on two highly variable sections of mtDNA that have been previously used to reveal the genetic structure and diversity of *C. capitata* populations (Gasparich *et al.* 1997; Reyes and Ochando 2004; Lanzavecchia *et al.* 2008; Barr 2009; Elfékih *et al.* 2010a, b; Rajabiyan *et al.* 2015). The first polymorphic region selected for amplification and sequencing in our survey, segment I, includes *cytochrome oxidase I* gene (30% coverage), while the second polymorphic region, segment II, includes the NADH dehydrogenase subunit 4 (*ND4*) (78% coverage), *tRNA-His* and *ND5* (15% coverage) genes. The total length of these sections (1727 bp) represents ~11% of the complete mitochondrial genome of the Mediterranean fruit fly (Reyes and Ochando 2004). Prior studies reported that the direct analysis of the mitochondrial DNA sequences was an efficient method for documenting the existence of genetic variation in populations of *C. capitata* (Elfékih *et al.* 2010a, b).

Molecular studies

Total DNA was extracted from each single fly following the Lifton DNA isolation protocol (Bender *et al.* 1983). A total of 228 flies representing eight populations from Turkey were sequenced for both segments with an average size of ≥12 flies per population.

A 457-bp portion of the fly's mitochondrial DNA, segment I, was amplified by the polymerase chain reaction (PCR) from each individual using the primer pairs TY-J-1460 (5′-TACAATTTATCGCCTAAACTTCAGCC) and

Figure 1. Map showing the sites of medfly sampling in this study.

C1-N-2191 (5′-CCCGGTAAAATTAAAATATAAACTTC) (Barr *et al.* 2012). PCR reactions were carried out in a final volume of 25 μL with 2 μL $MgCl_2$ (2.5 mM), 1.5 μL primers (0.1 μM, Thermo Scientific, Lithuania), 0.5 μL dNTPs (10 mM each), 2.5 μL 10× PCR buffer (Thermo Scientific), 0.5 U *Taq* DNA polymerase (Thermo Scientific), 1 μL of template DNA and 17.5 μL double distilled H_2O. The PCR amplification profile consists of an initial denaturation step of 5 min at 94°C (one cycle) followed by 39 repeating cycles of 30 s at 94°C, 25 s at 46°C and 1 min at 72°C followed by a final extension step of 8 min at 72°C.

The 1270-bp fragment of the mtDNA, segment II, was amplified by using the forward primer N5-J-7908 (5′-ACGATTAATATTGATATCTCC) (Barr 2009) and reverse primer N4-N-9243 (5′-TTAGTTTTAACATTTAGAAG) (Gasparich *et al.* 1995). The same reaction mix was used for segment I with a higher $MgCl_2$ concentration (3 μL). The following thermal profile was used: primary denaturation step of 5 min at 94°C followed by 39 cycles of denaturation at 94°C for 30 s, annealing at 43°C for 30 s, 1.5 min extension at 72°C and a final extension of 8 min at 72°C. Amplifications were achieved on an Eppendorf Mastercycler gradient thermal cycler (Eppendorf, Hamburg, Germany).

The PCR amplification products were confirmed by 1.3% agarose gel electrophoresis and purified via QIAquick Gel Extraction kit (Qiagen, Hannover, Germany). To increase accuracy, purified PCR products were sequenced in both directions by using the same primers as in the PCR, on an ABI 3130 automated sequencer (Applied Biosystems).

Data analysis

Sequences were aligned and edited using the software program CLUSTALW, as implemented in MEGA7.0 (Kumar *et al.* 2016). Three types of basic descriptive indices, namely the nucleotide diversity (π), haplotype diversity (*Hd*) and mean number of nucleotide differences (k), for each geographical population of *C. capitata* were estimated using DNASP v5.10.01 (Librado and Rozas 2009). The number and distribution of the mitochondrial haplotypes and polymorphic sites were also assessed using the same program.

To further characterize the population structure, the degree of genetic differentiation among populations was quantified by pairwise F_{ST} values. The analysis of molecular variance (AMOVA) method was used to determine the population genetic variability. Both pairwise F_{ST} and AMOVA analyses were conducted using Arlequin v3.1 (Excoffier *et al.* 2005).

We constructed a median joining (MJ) haplotype network to investigate the relationships between mitochondrial DNA haplotypes using Network 4.6.10 (Bandelt *et al.* 1999; Polzin and Daneschmand 2003). For comparison purposes, the sequences identified in the present study were added to additional mitochondrial DNA sequences deposited in the GenBank (see accession numbers in tables 1, 2 and 3 in electronic supplementary material at http://www.ias.ac.in/jgenet/) that constitute a reasonably complete coverage of the distributional range of this species worldwide. For segment I, in addition to 109 sequences identified from the present study, 120 sequences from 14

different countries were included in the haplotype network analysis. For segment II, further with the new data collected here, first we added 27 sequences (899 bp in length) for flies from Iran, Greece, Tunisia, Argentina, Guatemala and USA and compared it with the MJ network analysis. We also merged the sequences of this segment with the sequences with shorter (585 bp in length) but from wider geographical scales (105 sequences from 35 countries from previous publications).

A Mantel test (Mantel 1967) was performed to determine the relationship between genetic differentiation (F_{ST}) and geographical distance (km) to test an isolation-by-distance effect. Google Earth v 4.2 (http://earth.google.com/download-earth.html) was used to estimate the geographical distance between each pair of populations.

Results

Sequence data and haplotypes

Intraspecific genetic variation was investigated by analysis of DNA sequence-based mitochondrial haplotypes in individuals from seven natural populations plus a laboratory strain of the Mediterranean fruit fly *C. capitata* from Turkey. The results have revealed low levels of genetic diversity. For both segments, the AT-rich sequences (≥75%) were detected in nucleotide composition, similar to values found in other *C. capitata* populations (Gasparich *et al.* 1995). No insertions or deletions were observed for both segments.

For segment I, we detected a total of eight haplotypes, a variant sequence forms, among 109 individuals from the eight populations of the Mediterranean fruit fly from Turkey. Of the 457 characters, nine were polymorphic sites, composed of six parsimony informative and three singleton polymorphic sites. One of the haplotype, H1, was shared by all populations, three haplotypes were shared by at least two populations and four haplotypes were unique. The haplotype frequencies determined for each location are presented in table 1.

For segment II, of the 1270-bp nucleotide sites, 17 were variable including three singleton polymorphic sites and 14 parsimony informative sites. A total of 13 distinct haplotypes were identified from the 119 individuals of the eight *C. capitata* populations. With regard to the distribution of mitochondrial DNA haplotypes, haplotype H1 was found in all studied populations and considered as a common haplotype, 11 of the haplotypes were only present in single populations (table 1). Izmir was the most variable locality with four haplotypes for both segments. The complete sequence of each of the haplotype variants for both segments identified here were deposited in GenBank (accession numbers MG764535–MG764540).

Two and four different haplotypes were found in the analysed samples from the laboratory strain for segments I and II, respectively. Under laboratory conditions, the selection of specific mitochondrial DNA haplotypes has been reported previously (Reyes and Ochando 1997) and was also proposed as a possible process of geographical differentiation in field populations (Nigro and Prout 1990; Nigro 1994; Reyes and Ochando 1998b).

Table 1. Distribution and frequency of haplotypes by locality (segments I and II).

Haplotype/location	Yalova	İzmir	Aydın	Muğla	Antalya	Mersin	Adana	Laboratory strain
Segment I								
H1	11	14	15	14	9	12	2	1
H2								13
H3		1						
H4		2			1			
H5		2			5	2		
H6				1				
H7						2	1	
H8	1							
Segment II								
H1	11	11	14	19	22	6	4	1
H2	1					5		
H3						1		
H4				2				
H5				1				
H6			2					
H7			1					
H8		1						
H9		1						
H10		1						
H11								13
H12								1
H13								1

Population genetic structure

The haplotype diversity (*Hd*), nucleotide diversity (π) and average number of nucleotide difference (k) values of *C. capitata* based on two mitochondrial DNA segments for all considered eight populations are shown in table 2. Relatively low genetic diversity values were observed for both segments. For segment I, the mean haplotype and nucleotide diversity values were 0.32 and 0.001, respectively. For segment II, the average values of haplotype and nucleotide diversities were 0.264 and 0.0005, respectively. The values reported here are notably lower than those previously reported for other *C. capitata* populations from South Africa (Karsten *et al.* 2013), Morocco and Tunisia (Elfékih *et al.* 2010a, b) and Spain (Reyes and Ochando 2004). The level of polymorphism in the laboratory population was within the range of the Turkish field populations. It has been previously reported that the Mediterranean fruit fly goes through a narrow bottleneck when initially adapted to laboratory conditions (Haymer and McInnis 1994; Baruffi *et al.* 1995; Reyes and Ochando 1998b). But several generations later, these populations recover and the polymorphism levels can increase (Ochando *et al.* 2003).

Table 2. Results obtained from genetic diversity analysis among the eight populations of *C. capitata* in Turkey (segments I and II).

Population	*n*	*H*	*Hd*	π	*k*
Segment I					
Yalova	12	2	0.166	0.0004	0.167
İzmir	19	4	0.457	0.0011	0.503
Aydın	15	1	0.000	0.0000	0.000
Muğla	15	2	0.133	0.0006	0.267
Antalya	15	3	0.562	0.0013	0.61
Mersin	16	3	0.433	0.001	0.467
Adana	3	2	0.666	0.0015	0.667
Laboratory strain	14	2	0.143	0.0013	0.571
Mean	13.6	2.38	0.32	0.001	0.407
Segment II					
Yalova	12	2	0.168	0.0001	0.17
İzmir	14	4	0.396	0.001	0.98
Aydın	17	3	0.324	0.001	1.71
Muğla	22	3	0.256	0.0002	0.26
Antalya	22	1	0.000	0.0000	0.00
Mersin	12	3	0.621	0.001	1.21
Adana	4	1	0.000	0.000	0.00
Laboratory strain	16	4	0.35	0.001	1.43
Mean	14.9	2.63	0.264	0.0005	0.72

n, Sample size; *H*, number of haplotypes in each population; *Hd*, haplotype diversity; π, nucleotide diversity; *k*, average number of nucleotide differences per population.

For both segments, the population pairwise F_{ST} values indicated overall a low level of genetic structure, the majority of the values were not significant in natural *C. capitata* populations of Turkey, suggesting that the Mediterranean fruit flies can move freely across the geographic regions. However, the laboratory population was significantly isolated from the other natural populations analysed (table 3). Due to the low sample size from the Adana population, the values should be taken with some caution for this population.

Considering all field populations analysed here, the AMOVA results showed that variation among the populations contributed $\leq$11% to the overall genetic variance for both segments. However, if the laboratory strain was included in the calculations, this value increases to $\leq$67% for both segments, revealing the presence of significant genetic differences among populations ($P < 0.05$).

Table 3. Pairwise F_{ST} values among the eight *C. capitata* populations in Turkey (segments I and II).

Populations	Yalova	İzmir	Aydin	Muğla	Antalya	Mersin	Adana	Lab. strain
Segment I								
Yalova	–							
İzmir	0.01665	–						
Aydın	0.01932	0.02769	–					
Muğla	−0.00391	−0.01024	0.0000	–				
Antalya	0.18061*	0.04100	0.23810*	0.1039	–			
Mersin	0.03800	0.00381	0.06093	−0.00117	0.05116	–		
Adana	0.21649	0.06454	0.53125	0.15179	0.14352	−0.08401	–	
Laboratory strain	0.89876*	0.86279*	0.92574*	0.89242*	0.85414*	0.86991*	0.85491*	–
Segment II								
Yalova	–							
İzmir	0.00973	–						
Aydın	0.05985	0.03663	–					
Muğla	0.01041	0.02163	0.09863	–				
Antalya	0.05376	0.06046	0.11932*	0.03175	–			
Mersin	0.17273	0.14239*	0.15214	0.29134*	0.37589*	–		
Adana	−0.12821	−0.11474	−0.06164	−0.11485	0.0000	0.11111	–	
Laboratory strain	0.86282*	0.81164*	0.76821*	0.88148*	0.90472*	0.80613*	0.82202*	–

*Statistical significance at $P < 0.05$.

The Mantel test revealed a weak but significant correlation between geographic distance and pairwise F_{ST} for segment I ($r = 0.3883$; $P = 0.04$). However, for segment II, the analyses showed no significant correlations between these two variables ($r = -0.1552$; $P = 0.7070$), indicating the absence of isolation by distance (figure 1 in electronic supplementary material.

Genetic relationships between populations

A set of medfly mitochondrial DNA sequences from GenBank was used to compare the Turkish populations with the medfly populations from other geographically widespread regions.

For segment I, the MJ network was calculated from 229 sequences (109 sequences identified in this study and 120 sequences reported in the GenBank) of the Mediterranean fruit fly (figure 2a). The relationships between Turkish *C. capitata* and sequences from other geographic regions are as follows: haplotype H1 is the most common and widely distributed haplotype. It was found in samples from different geographical regions of the world (including the Mediterranean region, South America and Australia) and can be probably considered the ancestral haplotype for these regions. The other haplotypes from different parts of the new world were connected to this haplotype by one or two mutation steps. Most of the Iranian (11/13) samples also shared this haplotype and the other two haplotypes H44 and H45 were single mutations from Iran away from this haplotype. However, specific African haplotypes were grouped together. In general, this network is consistent with the medfly colonization history: the old-derived Mediterranean basin populations group together with the new adventive populations (from Hawaii, South America and Australia) and show some divergence from the ancestral African populations.

For segment II, the MJ mitochondrial haplotype network of 146 sequences, 899 bp in length, (119 sequences from the present study and 27 sequences previously published) showed no clear association between the haplotypes and geographical locality in *C. capitata* populations (figure 2b). Haplotype H2 has the highest frequency in Turkey and four samples from Iran also shared this haplotype. The other haplotypes from Iran, H3 and H5, were connected to this haplotype by one and three mutation steps, respectively. Our analysis also revealed that the haplotypes from Turkey, Tunisia, Argentina and USA did not associate with geographic origins, i.e., populations were mixed. There was one missing haplotype in the network.

With additional 15 missing intermediate haplotypes, the MJ mitochondrial haplotype network that included 585-bp sequences from five major geographical regions (Africa, Europe, Middle East – including Turkey, Iran and Israel –, Australia and America) revealed high degree of haplotype richness with no clear association between the haplotype phylogeny and geographic distribution in *C. capitata* populations, even though some of the African haplotypes were grouped together (figure 2c). Haplotype H48 is the most common and present in all Turkish populations and can be probably considered to be the ancestral haplotype in the eastern Mediterranean region. Four samples from Iran and one sample from Brazil also shared this haplotype. Further, our analysis has revealed that many haplotypes from different geographical regions evolved from the central haplotype H87 from the Mugla population of Turkey. Unique haplotypes from the eastern Mediterranean regions such as Israel (H74), Iran (H83), Greece (H84) and Turkey (85) connected to haplotype H48 by a one-mutation step which suggests that these haplotypes probably might have appeared after the colonization of this area. The presence of such unique haplotype variants from different geographical populations may provide valuable tools for understanding different aspects of the colonization process of this and other pest species. However, a larger 'scale study and higher number of samples per population are needed to verify the uniqueness of these haplotype variants.

Discussion

In this study, we have attempted to examine the genetic diversity and structure of the Mediterranean fruit fly populations and also provide some insight into the possible worldwide colonization pathways of this organism. To the best of our knowledge, this report is the first on the population genetic structure of this species in Turkey.

The analysis of mitochondrial DNA variation has revealed an overall low level of genetic variability with little or no genetic differentiation in the Turkish populations of *C. capitata*. This result is largely due to the following factors: (i) the 'relatively' recent colonization of this country by this pest. The presence of the Mediterranean fruit fly was reported for the first time in Turkey in the beginning of the 20th century (Fimiani 1989), then the species expanded rapidly over the next decades and became widespread in the region, (ii) the presence of significant gene flow (i.e., genetic exchange) among populations which could limit geographical differentiation when there is little or no selection. In the case of the studied natural populations, the high levels of gene flows were found ($Nm_{seg\ I} = 7.76$ and $Nm_{seg\ II} = 4.01$). The spaces between the sampling localities have been filled with host plants that effectively present a continuous habitat for the fly, with no significant physical barriers within the region; probably this could promote the gene flow among populations, (iii) the absence of selective pressures in relation with climatic conditions such as annual rainfall and minimum temperature in sampling areas as all these were already reported to be important factors in the development and survivorship of the flies (Reyes and Ochando 1998a) and (iv) intensive trading activities

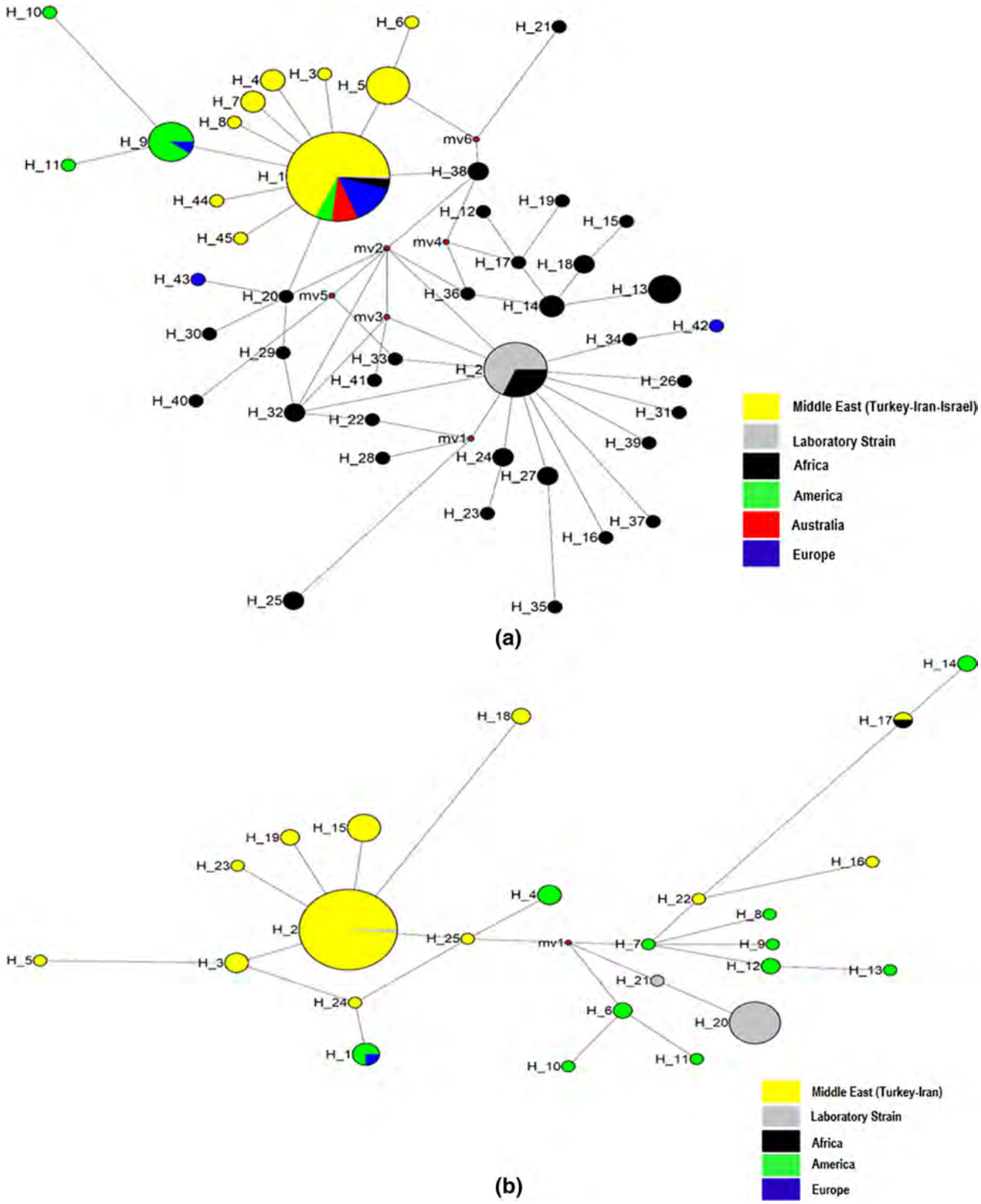

Figure 2. Continued

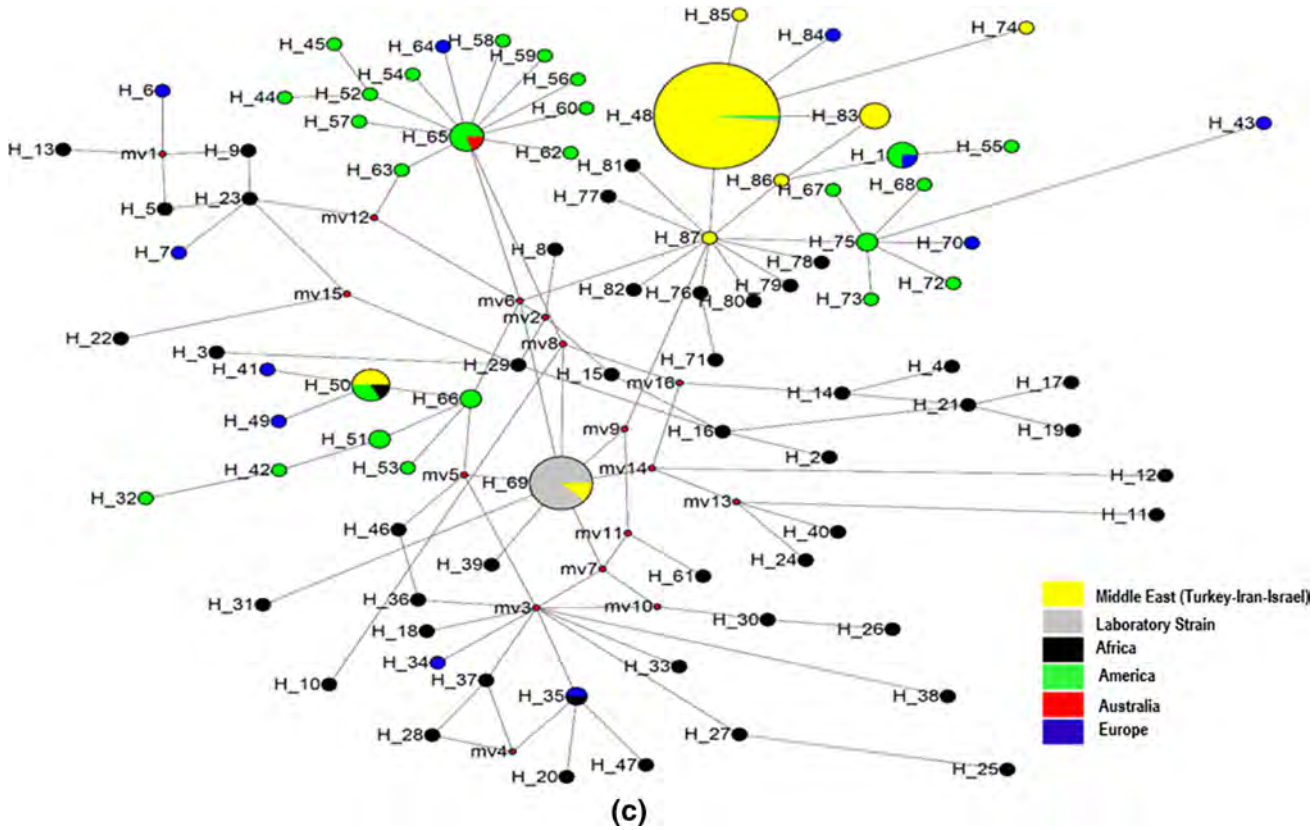

Figure 2. Mitochondrial haplotype networks for (a) segment I, (b) segment II and (c) 585-bp sequences of segment II of Mediterranean fruit fly populations. Each circle represents a haplotype and sizes are proportional to the number of individuals having that haplotype. The empty circles, named mv, correspond to intermediate haplotypes that were not sampled. Geographical locations are colour coded. Haplotype numbers and their distribution by region are listed in tables 1, 2 and 3 in electronic supplementary material, respectively. A full colour version of this figure is available online.

and human-mediated dispersal of the pest should also be considered between these regions. The results of our analysis are consistent with prior studies that also reported limited or no population genetic differentiation in other *C. capitata* populations from different parts of the world (Reyes and Ochando 1998b; Bonizzoni *et al.* 2004; Beroiz *et al.* 2012; Karsten *et al.* 2013).

Invasive/alien species, compared to their native ranges, typically have lower genetic diversity in their introduced ranges (Lockwood *et al.* 2005). With respect to the worldwide colonization pattern, Malacrida *et al.* (1998) suggested that medfly populations can be grouped into three main categories: ancestral populations from mainland Africa, ancient populations from the Mediterranean region and new populations from America and the Pacific area. The geographical dispersal from source area, mainland Africa to Greece is associated with a gradual loss of genetic variability content which is indicative of a colonization process probably through subsequent bottlenecks (Baruffi *et al.* 1995; Gomulski *et al.* 1998; Malacrida *et al.* 1998; Gasperi *et al.* 2002). The results of our analysis are compatible and complementary with previous studies. Here, we have shown that populations within Turkey have a lower level of genetic variability (in terms of *Hd*, π and *H*) compared to the results previously reported from natural populations of southern Africa (Karsten *et al.* 2013) and western Mediterranean countries such as Spain (Reyes and Ochando 2004), Tunisia and Morocco (Elfékih *et al.* 2010a, b). This confirms and supports the existence of a decreasing trend in the levels of genetic polymorphism from South Africa to eastern Mediterranean area and agrees with the framework for the colonization process of this pest species.

With the mitochondrial markers examined here, some generalizations for the colonization process of wild *C. capitata* can be made. The networks constructed based on both mitochondrial DNA segments revealed close genetic similarity among the populations of Turkey and Iran. Although its origin in Iran is unclear, most of the *C. capitata* samples from this country shared the same haplotypes with Turkish samples or are separated from the Turkish haplotypes by one or two mutational steps which probably suggest that Turkey could be the source of the colonizing populations in Iran.

Recently Rajabiyan *et al.* (2015) have reported a lower degree of genetic variability by using mtDNA markers for *C. capita* populations in Iran compared to the results obtained from Turkish populations. The authors also suggested that the colonization of the pest has occurred more recently in Iran and probably the pest has not had sufficient time for genetic divergence. This decreasing trend in genetic variability seems to indicate that the colonization process of this organism continues from Turkey to Iran. Humans have played an important role in introduction and altering the natural distribution of many fruit fly species (Malacrida *et al.* 2007). Considering geographic, human demographic and historical ties between these two countries, it is possible that the medfly could have been migrated from Turkey to Iran via growing fruit and vegetable trade (including transportation of nursery material and ornamental plants) human travel and/or active dispersal of the species across the climate zones. However, this hypothesis should be taken with caution as only limited number of samples and locations (only Mazandaran province) have been analysed from Iran. Such examples are available for other pest species; a recent study (Eti *et al.* 2018) suggested that *Bactrocera oleae*, another Tephritid fruit fly, was probably introduced to Iran from the western parts of Turkey. However, we cannot exclude the possibility that other European and/or eastern Mediterranean basin countries could be possible sources of Iranian medflies, especially considering the extensive global trade networks, opening new trade routes and increasing human mobility. More extensive medfly sampling throughout the species range and using high-resolution genetic markers will help us to gain broad information about the population structure and the possible colonization route of this economically important pest species. Deeper knowledge gained from the Mediterranean fruit fly will also provide us a model to approach and understand the colonization process of other Tephritid fruit fly species such as the genera *Bactrocera* and *Anastrepha,* which represent a potential threat in the globalized world. However, there is no doubt that as global trade increases, biological invasions will be a potential threat for natural biodiversity, human well-being and food security.

The genetic diversity and population structure of insects can be strongly influenced by the invasion pathways that led to their initial establishment (Gallo-Franco *et al.* 2017). In the present study, the observed distribution of haplotypes, for both segments, also suggests that unlike the African samples, the Mediterranean samples and the samples from the new world (including America, Pacific region and Australia) did not show any clear pattern of geographical structuring, which indicates that in the Mediterranean basin, particularly the eastern Mediterranean region, populations may have played a more important role than previously thought (Reyes and Ochando 2004) in the colonization of *C. capitata* populations to the new world. Greater resolution of geographic origin, historical factors and subsequent dispersal patterns of this species may be achieved by further molecular analysis including other parts of the genome such as nuclear gene markers in populations from different regions of the world.

The exception to the overall genetic homogeneity is the significant differentiation of the laboratory population which has been kept under standardized laboratory conditions for more than 30 years. All pairwise comparisons revealed significant F_{ST} values. This is due to the presence of highly divergent haplotypes found in this strain. Similar to the results presented here, considerable degree of genetic differentiation has also been reported between field and laboratory populations for different insect species (Arias *et al.* 2005; Lainhart *et al.* 2015). It seems laboratory conditions could alter the population genetic structure of *C. capitata.*

In addition to the history of colonization, selection is another process that cannot be ignored with respect to geographical variation in mitochondrial DNA. The presence of different selective pressures in newly colonized areas may favour and predominate different haplotypes (Nigro 1994). The role of selection in *C. capitata* populations has also been shown at the nuclear level by using microsatellites (Bonizzoni *et al.* 2004), isozymes and RAPD-PCR (Baruffi *et al.* 1995; Beroiz *et al.* 2012).

In conclusion, we present a comprehensive survey of *C. capitata* mitochondrial DNA variation in natural populations representing the current range of this insect in Turkey. The results revealed that this species is genetically homogenous and there is a low level of genetic structure associated with different geographical regions. Our study has made important progress in understanding the genetic structure of medfly populations in the eastern Mediterranean basin. In future studies, it will be important to concentrate efforts on sampling Iranian populations to further confirm the population genetic structures and invasion history of *C. capitata*. It has also been previously reported that information regarding population genetic diversity, genetic structure and gene flow are crucial for the development and success of resistance-management programmes (Roderick and Navajas 2003). Thus, the present study will assist in designing appropriate pest control and management strategies, especially in the Mediterranean region.

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References

Arias L., Bejarano E. E., Marquez E., Moncada J., Velez I. and Uribe S. 2005 Mitochondrial DNA divergence between wild and laboratory populations of *Anopheles albiminus* Weidmann (Diptera: Culicidae). *Neotrop. Entomol.* **34**, 499–506.

Aydemir M. (ed.) 2008 *Zirai mücadele teknik talimatları cilt V.* Agricultural combat technical instructions, volume V, pp. 57–60. Gıda Tarım ve Hayvancılık Bakanlığı, Ankara, Turkey (in Turkish).

Bandelt H. J., Forster P. and Rohl A. 1999 Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* **16**, 37–48.

Barr N. B. 2009 Pathway analysis of *Ceratitis capitata* (Diptera: Tephritidae) using mitochondrial DNA. *J. Econ. Entomol.* **102**, 401–411.

Barr N. B, Islam M. S., De Meyer M. and Mcpheron B. A. 2012 Molecular identification of *Ceratitis capitata* (Diptera: Tephritidae) using DNA sequences of the COI barcode region. *Ann. Entomol. Soc. Am.* **105**, 339–350.

Baruffi L., Damiani G., Guglielmino C. R., Bandi C., Malacrida A. R. and Gasperi G. 1995 Polymorphism within and between populations of *Ceratitis capitata*: comparison between RAPD and multilocus enzyme electrophoresis data. *Heredity* **74**, 425–437.

Bender W., Spierer P. and Hogness D. S. 1983 Chromosomal walking and jumping to isolate DNA from the Ace and Rosy loci and bithorax complex in *D. melanogaster*. *J. Mol. Biol.* **168**, 17–33.

Beroiz B., Ortego F., Callejas C., Hernandez-Crespo P., Castañera P. and Ochando M. D. 2012 Genetic structure of Spanish populations of *Ceratitis capitate* revealed by RAPD and ISSR markers: implications for resistance management. *Span. J. Agric. Res.* **10**, 815–825.

Bonizzoni M., Guglielmino C. R., Smallridge C. J., Gomulski M., Malacrida A. R. and Gasperi G. 2004 On the origins of medfly invasion and expansion in Australia. *Mol. Ecol.* **13**, 3845–3855.

Dogac E., Kandemir I. and Taskin V. 2013 The genetic polymorphisms and colonization process of olive fly populations in Turkey. *PLoS One* **8**, e56067.

Elekçioğlu N. Z. 2009 Akdeniz Meyve sineği. *Biyoloji Bilimleri Araştırma Dergisi* **2**, 61–65.

Elfékih S., Makni M. and Haymer D. S. 2010a Detection of novel mitochondrial haplotype variants in populations of the Mediterranean fruit fly, *Ceratitis capitata*, from Tunisia, Israel and Morocco. *J. Appl. Entomol.* **134**, 647–651.

Elfékih S., Makni M. and Haymer D. S. 2010b Genetic diversity of ND5 mitochondrial patterns in *Ceratitis capitata* (Diptera: Tephritidae) populations from Tunisia. *Ann. Soc. Entomol. Fr.* **46**, 464–470.

Eti C. N., Dogac E., Taskin B. G., Gokdere G. and Taskin V. 2018 Population structure and patterns of geographic differentiation of olive fly *Bactrocera oleae* (Diptera: Tephritidae) in Eastern Mediterranean Basin. *Mitochondrial DNA Part A* **29**, 1051–1062.

Excoffier L., Laval G. and Schneider S. 2005 ARLEQIN (ver. 3.0): an integrated software package for population genetics data analysis. *Evol. Bioinform. Online* **1**, 47–50.

Fimiani P. 1989 Mediterranean region. In *Fruit flies: their biology, natural enemies and control* (ed. A. S. Robinson and G. H. Hooper), vol. 3A, pp. 39–50. Elsevier, Amsterdam.

Gallo D. N. O., Wiendel F. M., Silvera Neto S. and Ricardo P. L. C. 1970 *Manual de entomologia agronomica*. Ceres, Sao Paulo.

Gallo-Franco J. G., Velasco-Cuervo S. M., Aguirre-Ramirez E., Obando R. G., Carrejo N. S. and Toro-Perea N. 2017 Genetic diversity and population structure of *Anastrepha striata* (Diptera: Tephritidae) in three natural regions of southwestern Colombia using mitochondrial sequences. *Genetica* **145**, 79–89.

Gasparich G. E., Sheppard W. S., Han H. Y., McPheron B. A. and Steck G. J. 1995 Analysis of mitochondrial DNA and development of PCR-based diagnostic molecular markers for the Mediterranean fruit fly (*Ceratitis capitata*) populations. *Insect Mol. Biol.* **4**, 61–67.

Gasparich G. E., Silva J. G., Han H. Y., Mcpheron B. A., Steck G. J. and Sheppard W. S. 1997 Population genetic structure of Mediterranean fruit fly (Diptera: Tephritidae) and implications for worldwide colonization patterns. *Ann. Entomol. Soc. Am.* **90**, 790–797.

Gasperi G., Guglielmino C. R., Malacrida A. R. and Milani R. 1991 Genetic variability and gene flow in geographical populations of *Ceratitis capitata* (Wied.) (medfly). *Heredity* **67**, 347–356.

Gasperi G., Bonizzoni M., Gomulski L. M., Murelli V., Torti C., Malacrida A. R. *et al.* 2002 Genetic differentiation, gene flow and the origin of infestations of the medfly, *Ceratitis capitata*. *Genetica* **116**, 125–135.

Gomulski L. M., Bourtzis K., Brogna S., Morandi P. A., Bonvicini C., Sebastiani F. *et al.* 1998 Intron size polymorphism of the Adh1 gene parallels the worldwide colonization history of the Mediterranean fruit fly, *Ceratitis capitata*. *Mol. Ecol.* **7**, 1729–1741.

Haymer D. S. and McInnis D. O. 1994 Resolution of populations of the Mediterranean fruit fly at the DNA level using random primers for the polymerase chain reaction. *Genome* **37**, 244–248.

Headrick D. H. and Goeden R. D. 1996 Issues concerning the eradication or establishment and biological control of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in California. *Biol. Control* **6**, 412–421.

Hooper G. H. S. and Drew R. A. I. 1989 Australia and South Pacific islands. In *Fruit flies: their biology, natural enemies and control* (ed. A. S. Robinson and G. H. Hooper) vol. 3A, pp. 67–72. Elsevier, Amsterdam.

Karsten M., van Vuuren B. J., Barnaud A. and Terblanche J. S. 2013 Population genetics of *Ceratitis capitata* in South Africa: implications for dispersal and pest management. *PLoS One* **8**, e54281.

Kumar S., Stecher G. and Tamura K. 2016 MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **33**, 1870–1874.

Lainhart W., Bickersmith S. A., Moreno M., Rios C. T., Vinetz J. M. and Conn J. E. 2015 Changes in genetic diversity from field to laboratory during colonization of *Anopheles darlingi* (Diptera: Culicidae). *Am. J. Trop. Med. Hyg.* **93**, 998–1001.

Lanzavecchia S. B., Cladera J. L., Faccio P., Marty N. P., Vilardi J. C. and Zandomeni R. O. 2008 Origin and distribution of *Ceratitis capitata* mitochondrial DNA haplotypes in Argentina. *Ann. Entomol. Soc. Am.* **101**, 627–638.

Librado P. and Rozas J. 2009 DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451–1452.

Lockwood J. L., Cassey P. and Blackburn T. 2005 The role of propagule pressure in explaining species invasions. *Trends Ecol. Evol.* **20**, 223–228.

Malacrida A. R., Marinoni F., Torti C., Gomulski L. M., Sebastiani F., Bonvicini C. *et al.* 1998 Genetic aspects of the worldwide colonization process of *Ceratitis capitata*. *Heredity* **89**, 501–507.

Malacrida A. R., Gomulski L. M., Bonizzoni M., Bertin S., Gasperi G. and Guglielmino C. R. 2007 Globalization and fruit fly invasion and expansion: the medfly paradigm. *Genetica* **131**, 1–9.

Mantel N. 1967 The detection of disease clustering and a generalized regression approach. *Cancer Res.* **27**, 209–220.

Meeyen K., Sopaladawan N. P. and Pramual P. 2014 Population structure, population history and DNA barcoding of fruit fly *Bactrocera latifrons* (Hendel) (Diptera: Tephritidae). *Entomol. Sci.* **17**, 219–230.

Nardi F., Carapelli A., Boore J. L., Roderick G. K., Dallai R. and Frati F. 2010 Domestication of olive fly through a multi-regional host shift to cultivated olives: comparative dating using complete mitochondrial genomes. *Mol. Phylogenet. Evol.* **57**, 678–686.

Nigro L. 1994 Nuclear background affects frequency dynamics of mitochondrial DNA variants in *Drosophila simulans*. *Heredity* **72**, 582–586.

Nigro L. and Prout T. 1990 Is there selection on RFLP differences in mtDNA? *Genetics* **125**, 551–555.

Ochando M. D., Reyes A., Callejas C., Segura D. and Fernández P. 2003 Molecular genetic methodologies applied to the study of fly pests. *Trends Entomol.* **3**, 73–85.

Polzin T. and Daneschmand S. V. 2003 On Steiner trees and minimum spanning trees in hypergraphs. *Oper. Res. Lett.* **31**, 12–20.

Rajabiyan M., Shayanmehr M. and Sharif M. M. 2015 The Mediterranean fruit fly (*Ceratitis capitata*) in Iran: genetic diversity and comparison with other countries. *J. Entomol. Acarol. Res.* **47**, 4055.

Reyes A. and Ochando M. D. 1997 Fitness of mitochondrial DNA haplotypes in *Ceratitis capitata*. *IOBC/wprs Bull.* **20**, 175–185.

Reyes A. and Ochando M. D. 1998a Genetic differentiation in Spanish populations of *Ceratitis capitata* as revealed by abundant soluble protein analysis. *Genetica* **104**, 59–66.

Reyes A. and Ochando M. D. 1998b Use of molecular markers for detecting the geographical origin of *Ceratitis capitata* (Diptera: Tephritidae) populations. *Ann. Entomol. Soc. Am.* **91**, 222–227.

Reyes A. and Ochando M. D. 2004 Mitochondrial DNA variation in Spanish populations of *Ceratitis capitata* (Wiedemann) (Tephritidae) and the colonization process. *J. Appl. Entomol.* **128**, 358–364.

Roderick G. K. and Navajas M. 2003 Genes in new environments: genetics and evolution in biological control. *Nat. Rev. Genet.* **4**, 889–899.

Sheppard W. S., Steck G. J. and Mcpheron B. A. 1992 Geographic populations of the medfly may be differentiated by mitochondrial DNA variation. *Experientia* **48**, 1010–1013.

Zhang D. X. and Hewitt G. M. 1997 Insect mitochondrial control region: a review of its structure, evolution and usefulness in evolutionary studies. *Biochem. Syst. Ecol.* **25**, 99–120.

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