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Microsatellite based genetic diversity of Mediterranean fruit fly (*Ceratitis capitata*, Diptera: Tephritidae) populations from Southwest Turkey

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Abstract: Various pests cause economic losses in some agricultural products, especially in citrus fruits, which are grown in many countries and have a very important role in consumption and export. The Mediterranean fruit fly, *Ceratitis capitata* Wied. (Diptera: Tephritidae), which spreads throughout the Mediterranean basin, Europe, the Middle East, Central, and South America, and Australia, is considered the most destructive pest in the world. Turkey is an important part of the Eastern Mediterranean basin and, maybe plays a role in the colonization of this pest. This polyphagous species causes huge economic losses by negatively affecting yield and quality in many countries. In this study, population genetic structure and inter-population relationships of the Mediterranean fruit fly were studied by using eight microsatellite markers sampled from the Mediterranean and Aegean regions of Turkey. The results represent a high level of genetic diversity in the Mediterranean fruit fly populations in Turkey and there is no geographical clustering observed when the populations are evaluated in the geographical sense. As a result of the study for 7 provinces, the average number of alleles (n_a) was 6.95, the average number of effective alleles (n_e) was 3.58, the observed (H_o) and expected (H_e) heterozygosity values were 0.61 and 0.67, respectively. The gene flow (*Nem*) level among populations was found as *Nem* = 1.13. Higher genetic diversity values point out that Turkey may have an important role in the colonization of this organism.

Key words: *Ceratitis capitata*, Mediterranean basin, microsatellite, genetic diversity

1. Introduction

With the increasing world population, the need for food increases makes agriculture the most strategic sector of the century. As in many countries around the world, agricultural activities have great importance. Agricultural products are used not only for nutrition but also in many areas, particularly for industry. To be the winner in agriculture today, it is important to have biological, biotechnological, ecological, and bioinformatics knowledge, to be able to plan for the future, keep up with changing technologies, and manage the risks that may occur (Birişik, 2019). Agricultural pests are one of the most significant factors limiting agricultural production by causing major crop losses every year. The fact that the loss caused by these pests is very high makes it necessary to fight these pests. Various pests cause economic losses in many agricultural products grown in Turkey. Among these pests, the Mediterranean fruit fly (also known as medfly) (*Ceratitis capitata* Wiedemann 1824) is an important citrus pest causing huge economic losses by negatively affecting the yield and quality (Kahyaoğlu, 2011).

The Mediterranean fruit fly, which belongs to the Tephritidae family, is a polyphagous species and causes damage to plants such as apricot, apple, quince, pomegranate, peach, nectarine, and fig, especially citrus (Elekçioğlu, 2009). The Tephritidae family is known as fruit flies and is represented by approximately 4500 species in the world. Today, the Mediterranean fruit fly has been reported to cause damage to more than 260 plants in more than 70 countries around the world (Magana et al., 2007). This species is liable for economic bereavements in horticultural manufacturing and is the target of early detection, control, and elimination programs in every area (Beroiz et al., 2012). The origin of the Mediterranean fruit fly is in the African Sahara, and it is distributed in regions with tropical and subtropic climates such as North and South Africa, South and Central America, Europe, and Western Australia. It is also a common species in the Mediterranean countries such as Turkey, Israel, Lebanon, and Jordan (Thomas et al., 2004). Possible reasons for the widespread of the organism are its dispersal capabilities, human-mediated transport, the increase of trade routes

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in the world, the development of global trade policies, and the organism's ability to feed in many habitats and on many hosts. This pest was first reported in Turkey by Fimiani in 1904. *C. capitata* is the major and common pest in the Mediterranean and Aegean regions, but also the existence of this pest in the Eastern Black Sea region was reported by some researchers in recent years (Kaya et al., 2017). The colonization process of the Mediterranean fruit fly was revealed by Malacrida et al. (1998), and according to this study, it was reported that the probable source of these pests' spread to the Eastern Mediterranean basin was the Western Mediterranean basin (Malacrida et al., 1998).

The common tendency of decreasing grade of genetic variation from its adopted resource region towards those regions lately colonized is characteristically for medfly (Malacrida et al., 1992; Gomuliski et al., 1998; Malacrida et al., 1998). In many regions of the world, the probable colonization route and the genetic structure of natural populations of medfly were investigated both at the macro and micro geographical levels, by using biochemical and molecular markers (Gasperi et al., 1991; Malacrida et al., 1992; Baruffi et al., 1995; Gasparich et al., 1997; Gomuliski et al., 1998; Malacrida et al., 1998; Reyes and Ochando, 1998a; He and Haymer, 1999; Bonizzoni et al., 2000; Gasperi et al., 2002; Bonizzoni et al., 2004; Reyes and Ochando, 2004; Barr et al., 2009; Karsten et al., 2013; Karsten et al., 2015; Güler et al., 2019; Kurd et al., 2020; Nikolouli et al., 2020). The results of all studies show that the quantity of genetic variation is not dispersed homogeneously. The results, obtained in many studies indicating that the southeast African flies, especially from Kenya, have the most polymorphic and highest genetic diversity, support that this region is the origin of this organism (Baruffi et al., 1995; Gasparich et al., 1997; Gomulski et al., 1998; Malacrida et al., 1998; Reyes and Ochando, 2004; Malacrida et al., 2007).

Microsatellites are widely used to compare groups of organisms that are evolutionarily close, such as populations of a species. Also, microsatellites are one of the most used genetic markers in cases where a recent event (such as bottleneck effect, genetic drift, and inbreeding) is determined in populations. Microsatellites are a source of genetic markers suitable for use in population genetics and gene mapping studies in many organisms due to their high polymorphism (Holton, 2001; Bruford et al., 2003; Dogac et al., 2013).

Considering the extensive spread and important economic significance of medfly, there is limited knowledge about the populations in the Eastern Mediterranean basin. Understanding the genetic structure of Mediterranean fruit fly populations from this area will improve our comprehension of the advanced genetic structure of global medfly populations and assist to determine the colonization processes of this organism. This study aimed to better understand the genetic structure and colonization processes of the medfly populations by utilizing 8 polymorphic microsatellites markers obtained from distinct localities of the Mediterranean and Aegean regions of Turkey, which are of great importance for citrus cultivation. The medfly's ability to spread to a great number of regions, menacing significant fruit production fields with mild climates, and its reproduction capability make this species one of the worst hazardous horticultural pests. Illumination of *C. capitata* genetic structure will provide us helpful knowledge for the efficient and successful control and/or management programs.

2. Materials and methods

2.1. Sample collection

C. capitata adults were sampled from 7 provinces in Turkey (Figure. 1; Table 1). All populations sampled were collected from the areas that are recognized for intensive fruit production. All samples were caught by hanging "last fly" pheromone traps on trees at a certain distance from the citrus groves and then stored at –80 °C for further experimental studies.

2.2. DNA isolation and amplification of microsatellite loci

After morphological identification of the collected specimens (White and Elson-Harris, 1994), genomic DNA (gDNA) was extracted from every single fly according to a standard protocol by Bender et al. (1983). To determine the genetic diversity in the populations, the 8 most polymorphic microsatellite primers (Table 2) were implemented for this organism (Bonizzoni et al., 2000; Stratikopoulos et al., 2008). Microsatellite primers were labelled by using 3 different fluorescent dyes, 6-FAM, NED, and HEX. From each location, 20 adult individuals (40 from Muğla province) (a total of 160) were used for microsatellite analysis. Amplification of microsatellite loci was carried out as described by Bonizzoni et al. (2000; 2001; 2004), Stratikopoulos et al. (2008), and Karsten et al. (2013). Then, 1 µL of each reaction was run on 1.5 % agarose gel. PCR products were analysed on an ABI PRISM 3100 Genetic Analyser (Applied Biosystems). Electropherograms were checked with the Applied Biosystems Peak Scanner program[1] and scored.

2.3. Data analysis

Genetic diversity within populations was detected as the mean number of alleles per locus (n_a), the effective number of alleles (n_e), and expected (H_e) and observed heterozygosity (H_o) by using Popgene version 1.32[2]. The same software was used to compute the genetic distance values according to

[1] Applied Biosystems Peak Scanner. Website http://www.appliedbiosystems.com [accessed 10 April 2021].

[2] Yeh FC, Boyle T, Rongcai Y, Ye Z, Xiyan JM (1999). Popgene Ver. 1.32 Microsoft window-based Freeware for Population Genetic Analysis. Website http://www.ualberta.ca/_fyeh/ [accessed 18 June 2021]

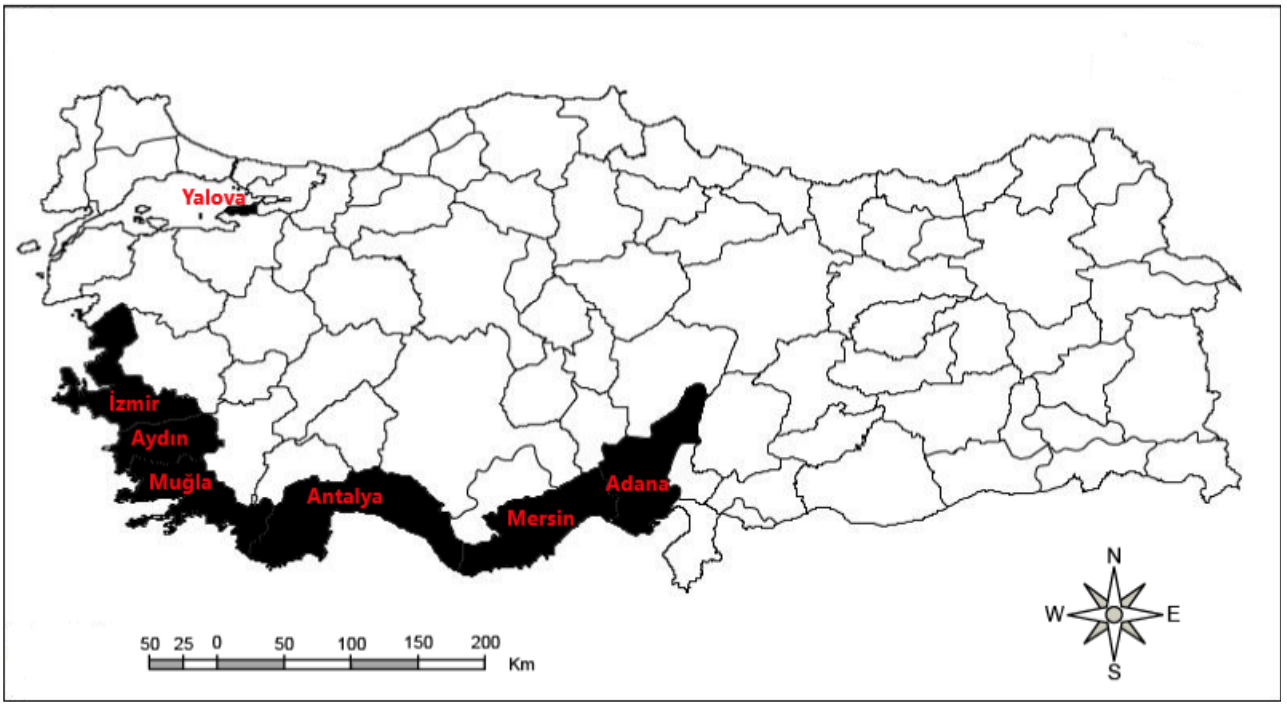

Figure 1. Map of Turkey with sampling sites.

Table 1. List of sampling locations of *C. capitata* populations sampled in Turkey.

Regions	Provinces	Number of Specimens	Latitude	Longitude
Aegean Region	Muğla	40	40°46.2'N	43°37.8'E
	Aydın	20	37°37.2'N	28°03.0'E
	İzmir	20	38°27.0'N	27°13.2'E
Mediterrenean Region	Mersin	20	36°49.2'N	34°46.2'E
	Adana	20	37°16.2'N	35°37.8'E
	Antalya	20	36°54.0'N	30°42.0'E
Marmara Region	Yalova	20	40°39.0'N	29°16.2'E

Nei (1972) and compliance of genotypic frequencies with Hardy-Weinberg equilibrium (HWE) in populations with chi-square (X^2) and likelihood ratio G^2; adjustments were done by implementing Bonferroni correction for multiple comparisons (Rice, 1989). Private alleles were detected with Genetix software version 4.05 [3]. The phylogenic relationships among populations adapted from genetic distances were visualized by a neighbor-joining tree, generated using Populations version 1.2.32 [4] by bootstrap method with 100 replicates. The Analysis of Molecular Variance (AMOVA) test was carried out with Arlequin version 3.5 (Excoffier et al., 2005). Genetic variation was divided into 3 levels; within individuals, among populations within individuals, and populations. Structure version 2.1 (Pritchard et al., 2000; Falush et al., 2003) was implemented to examine the number of probable genetic clusters of *C. capitata* populations in Turkey. This software supposes a model in which there are K populations, each of which is characterized by a set of allele frequencies at each locus. To define the most probable number of clusters (K)

[3] Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (1996–2004) GENETIX (ver. 4.02): logiciel sous Windows TM pour la ge´ne´tique des populations, Laboratoire Ge´nome, Populations, Interactions; CNRS UMR 5000; Universite´ Montpellier II, Montpellier (France). Website http://kimura. univ-montp2.fr/genetix/ [accessed 18 June 2021]

[4] Langella O (1999). Populations 1.2.31: a population genetic software. CNRS UPR9034. Website http://bioinformatics.org/~tryphon/populations [accessed 18 June 2021]

Table 2. Microsatellite loci.

Locus/5' label	Motif	Primer sequences (5'–3')	Allele size
Ccmic3 FAM 6 dye	(TG)11	F 5'- TGCACATGTATTCGTTCTTA R 5'- AATTACCTATAAACATGCATACTG	72-96
Ccmic6 NED dye	(TG)18	F 5'- AAGGTAGCCAGCAGTGTCTACG R 5'- ACGAATGGGAGTTATTCATACTGC	71-117
Ccmic8 FAM 6 dye	(TG)2GG(TG)5CG(TG)5	F 5'- GCGTTTCACTCTTCACTGGC R 5'- GAAACCATATTCCTCCGTCACAT	111-130
Ccmic9 HEX dye	(GA)9TA(GA)5TAGG(GA) 2TA(GA)6TAGATA(GA)13	F 5'- GAAGTGACTCATATTTTTAGGAACGA R 5'- TCTTTCTTTCATACTCACTCATTTCA	107-167
Ccmic14 FAM 6 dye	(CA)10CCAA(CA)2	F 5'- AATTCAGATACACGCTCACAAG R 5'- TCGTATTGCTATGCGCATAT	70-98
Ccmic15 HEX dye	(TG)4TA(TG)3A(GT)7	F 5'- GTTCGAAAGTGGGGTATGTACG R 5'- CACAAGAGCCAAAGACGCAT	85–109
Medflymic25 FAM 6 dye	(TA)9	F 5'- AGGCAAAGACAAGAAATTCG R 5'- GCAGCTTGAGGTGTAGTTTAAC	248-295
Medflymic96 NED dye	(TG)11(CAA)3	F 5'- GCCGCAACTATTTCTACACCC R 5'- ATGCTGCTGTTACTGTTCCTTC	159-237

in our *C. capitata* populations, we applied diverse values of K ranging from 1 to 7. Finally, the software Bottleneck version 1.2.02 (Cornuet and Luikart, 1997) was applied to conclude demographic expansion/contraction in all *C. capitata* populations.

3. Results

3.1. Microsatellite DNA variability

The analyses of 8 microsatellite loci in 160 flies, sampled from 7 localities, displayed high levels of variability (Table 3). According to this; the average number of alleles per locus was found at 17.25 ± 4.30, and this result indicates a high level of microsatellite diversity in the studied populations. The average number of alleles (n_a) per locus varied from 10 (Ccmic 14) to 24 (Ccmic 9). The mean number of effective alleles (n_e) detected per locus was 5.21 ± 1.71, while the highest number was observed at Ccmic 9 with 7.53; the lowest value was at Ccmic 8 with 3.33. According to the level of heterozygosity, the polymorphism ranges were also evaluated. Expected (H_e) and observed (H_o) heterozygosity values were 0.79 ± 0.07 and 0.61 ± 0.16, respectively, when averaged over loci. H_o values ranged from 0.22 (Medflymic 25) to 0.73 (Ccmic 3); H_e values ranged from 0.70 (Ccmic 8 and Ccmic 14) to 0.87 (Ccmic 9). Geographic dispersion of microsatellite alleles by population for every locus was also specified. The highest number of private alleles ranged from 1 (Ccmic 14) to 9 (Ccmic 9, Medfly 25, and Medfly 96) for all loci.

Table 3. Microsatellite variability of *C. capitata* in Turkey. N: number of flies used; n_a: number of actual alleles; n_e: number of effective alleles; H_o: observed heterozygosity; H_e: expected heterozygosity.

Locus	N	n_a	n_e	H_o	H_e
Ccmic 14	304	10.00	3.35	0.67	0.70
Ccmic 8	272	17.00	3.33	0.57	0.70
Ccmic 9	246	24.00	7.53	0.67	0.87
Ccmic 6	282	22.00	6.48	0.60	0.85
Ccmic 3	300	16.00	4.76	0.73	0.79
Medflymic 25	232	15.00	3.39	0.22	0.71
Ccmic 15	310	16.00	6.49	0.71	0.85
Medflymic 96	300	18.00	6.36	0.65	0.85
Mean	**281**	**17.25**	**5.21**	**0.61**	**0.79**
SD		**4.30**	**1.71**	**0.16**	**0.07**

After Bonferroni correction (Rice, 1989), all studied populations were verified to be in HWE at all microsatellite loci under X^2 and G^2 criteria (at P, 0.05). The mean value of genetic differentiation (F_{ST}) between populations was found to be 0.18, which, according to Wright (1978), indicates that there is low genetic differentiation between the studied populations. The average gene flow between populations, *Nem*, was found to be 1.13 [*Nem* = (1-Fst) / 4Fst)]. However, no statistically significant linkage-

disequilibrium was observed among genotypes at all loci and all were considered independent.

Total genetic diversity levels according to 8 loci for 7 analyzed *C. capitata* populations are presented in Table 4. The mean number of alleles (n_a) ranged from 4.50 (Antalya) to 8.25 (Aydın) and the mean number of effective alleles (n_e) from 2.70 (Antalya) to 4.55 (Aydın). The quantity of genetic diversity appeared to be homogeneously dispersed between distinct populations, taking into account average heterozygosity estimates of H_o and H_e. The observed and expected heterozygosity values varied from 0.50 (Antalya) to 0.69 (Mersin) and 0.56 (Antalya) to 0.75 (Aydın). While, Aydın population represented the highest level of genetic diversity consisting of the mean and effective number of alleles, the Mersin population represent the highest level of observed heterozygosity. In comparison to the other populations, lower variability amounts were observed in the Antalya population. In total, 49 new private alleles were determined, 21 of them in the Mediterranean region (Adana, Mersin, and Antalya). Although the number of private alleles is dependent on sample size (Slatkin 1985), all the studied populations had private alleles. The Adana population presented the highest number of private alleles with high frequency (≥0.094).

Genetic relationships between populations were evaluated by pairwise F_{ST} (Table 5). F_{ST} values ranged from 0.04429 (between İzmir-Aydın) to 0.38088 (between Muğla-Antalya). All F_{ST} values were low but significant among all sampled populations. This low differentiation can be elucidated by straight topography, continuous plant cultivation (also plant exchanges), and extensive citrus trade between provinces in these areas.

The Neighbor-Joining (NJ) tree of 7 *C. capitata* populations based on the genetic distance is represented in Figure 2. Although most of the branches have high bootstrap values, no regional grouping was observed in the obtained tree.

A homogeneity test between populations was carried out using AMOVA. In this context, the populations were grouped according to the geographical region. The results are outlined in Table 6. As a result of AMOVA, it was concluded that the principal contribution to genetic variation was based on the variation within individuals (83.11%). It was observed that the genetic variation among

Table 4. Genetic variability in field-collected of *C. capitata* from different geographical regions of Turkey. N: number of flies used; n_a: number of actual alleles; n_e: number of effective alleles; H_o: observed heterozygosity; H_e: expected heterozygosity.

Regions	**Locations**	**N**	n_a	n_e	***np***	***Ap***	H_o	H_e
Aegean Region	Muğla	40	7.00	3.28	9	0.065	0.51	0.67
	Aydın	20	8.25	4.55	7	0.051	0.65	0.75
	İzmir	20	6.63	3.30	3	0.022	0.66	0.69
Mediterrenean Region	Mersin	20	6.88	3.73	6	0.043	0.69	0.67
	Adana	20	7.63	3.61	13	0.094	0.63	0.68
	Antalya	20	4.50	2.70	2	0.015	0.50	0.56
Marmara Region	Yalova	20	7.75	3.92	9	0.065	0.66	0.67
	Mean		**6.95**	**3.58**	**7**	**0.051**	**0.61**	**0.67**

Table 5. The range of F_{ST} values among *C. capitata* populations and the significance of population differentiation estimated by F_{ST} values * $p < 0.05$.

Populations	**Muğla**	**Aydın**	**İzmir**	**Yalova**	**Antalya**	**Adana**	**Mersin**
Muğla	*******						
Aydın	0.06696*	*******					
İzmir	0.11436*	0.04429*	*******				
Yalova	0.05548*	0.06612*	0.11816*	*******			
Antalya	0.38088*	0.19808*	0.25178*	0.30580*	*******		
Adana	0.10646*	0.11647*	0.11289*	0.08959*	0.36944*	*******	
Mersin	0.19776*	0.10702*	0.12570*	0.12303*	0.29412*	0.18212*	*******

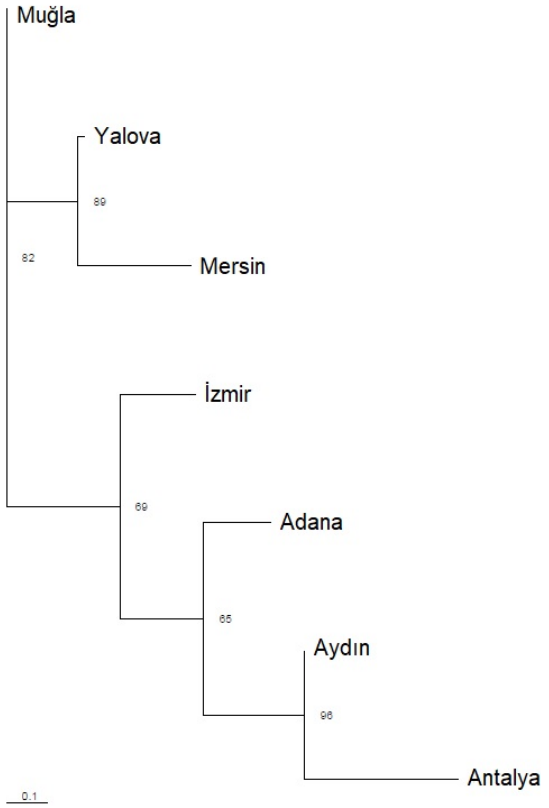

Figure 2. Unrooted Neighboor-Joinning (NJ) tree of 7 *C. capitata* populations using 8 polymorphic microsatellite markers.

populations/within groups was low (0.67% and 16.22%, respectively).

The genetic structure of the populations was also analyzed based upon microsatellites by using Structure version 2.1. Similar likelihood values were provided using diverse values of the length of the burn-in period (50.000) and Monte Carlo Markov Chain (MCMC) repetitions (100.000). For all *C. capitata* populations analyses, 6 values of K (K = 2 to K = 7) were applied. The highest ΔK was found at K = 4 (Figure 3 and Figure 4), and that demonstrated the possible number of clusters (Evanno et al., 2005). Table 7 represents the rate of individuals appointed into each of the 4 clusters depending upon the Q value obtained from the Structure analyses. The first cluster consisted of 92.1% of Adana and 69.9% of İzmir populations. The second cluster consisted of 88.6% of Muğla and 24.3% of Yalova populations. The third cluster was the most admixed one and this cluster contained 92.8% of Mersin, 60.7% of Yalova, 14% of Aydın, and 10.4% of İzmir. The fourth cluster was mainly formed by 97.2% of Antalya and 56.5% of Aydın mix. The hypothesis of a recent bottleneck based upon the TPM was also examined. The bottleneck test, with a mode shift in allele frequency classes, attributed an

Table 6. Analysis of molecular variance (AMOVA).

Source of variation	%Total variance	Fixation indices
Among groups	0.67	F_{CT} = 0.00673
Among populations/within groups	16.22	F_{SC} = 0.16334
Within individuals	83.11	F_{ST} = 0.1807

L-shaped distribution to all populations, in concordance with normal frequency class distribution ranges (P > 0.05).

4. Discussion

The quarantine pest Mediterranean fruit fly, which prevents the reach of products to international markets, is one of the most economically important fruit fly species because of its ability to stay alive in cooler climates more successfully than most other fly species. To better understand the damage and invasion risk of this pest in agricultural areas, we have to perform more studies about this organism's genetic structure and dispersal patterns. In this work, we investigated the genetic structure of the Mediterranean fruit fly by using microsatellites collected from different regions of Turkey. Our findings also provide some important implications about the colonization of the Eastern Mediterranean region where this pest is located.

The invasions of pest species, such as *C. capitata*, are generally defined by reduced genetic diversity. Invasive species, in proportion to their native areas, generally have a lesser genetic variation in their newly invaded areas (Lockwood et al., 2005; Karsten et al., 2015). The genetic structure and dispersal patterns of Mediterranean fruit fly populations have been analyzed at macrogeographic and microgeographic levels in previous studies. Gasperi et al. (2002) suggest that Mediterranean fruit fly populations are divided into three main groups at the macro geographical level. These groups are: (i) ancestral populations in sub-Saharan and Africa, (ii) ancient populations in the Mediterranean basin, and (iii) new populations from the Americas and Pacific region. At the micro geographical level, studies mainly include new populations from California, Florida, Argentina, and Australia (Meixner et al., 2002; Silva et al., 2003; Bonizzoni et al., 2004; Lazavecchia et al., 2008) and three Mediterranean countries (Turkey, Spain, and Greece *C. capitata* populations) (Ochando et al., 2003; Kourti, 2004; Güler et al., 2019; Kurd et al., 2020). Due to the agricultural activities and the geographical location of Turkey, it is suggested that Turkey may have participated in the invasion of the Mediterranean basin by Mediterranean fruit fly. However, there is a limited number of studies in Turkey on this organism, which is of great importance in illuminating this situation (Güler et al., 2019; Kurd et al.,

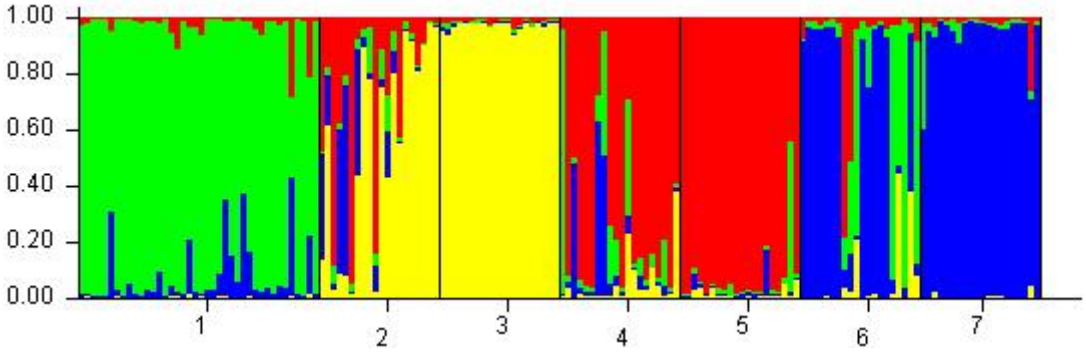

Figure 3. K = 4 clustering assignment depending on the Bayesian method under an admixture model obtained by Structure software. Individuals are represented by a vertical line and each color indicates a different cluster. 1: Muğla; 2: Aydın; 3: Antalya; 4: İzmir; 5: Adana; 6: Yalova; 7: Mersin.

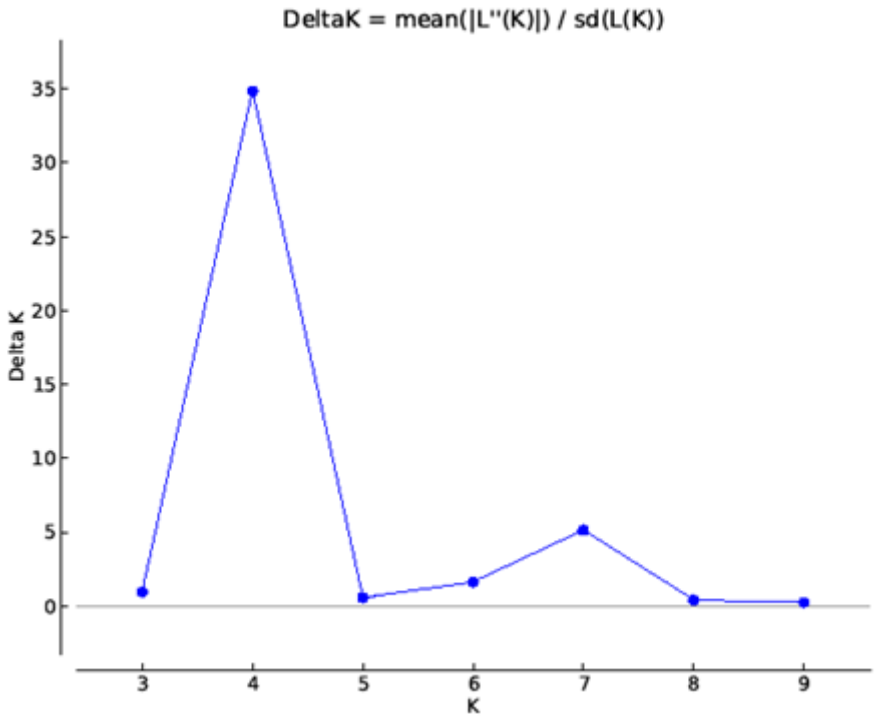

Figure 4. Δ*K* distribution along with different values of clusters (*K*) for 7 populations depending on Evanno's method (Evanno et al. 2005) using Structure Harvester application.

2020). Güler et al. (2019) showed that the colonization process of *C. capitata* continues from Turkey to Iran. Additionally, previous studies have shown that the genetic diversity values are very high in the Mediterranean fruit fly populations collected from the sub-Saharan Africa region of Central Africa and therefore the origin of the organism is Central Africa. It was stated that the colonization of the organism took place from Central Africa to the North African countries in the Mediterranean basin, then the Middle East, and finally to Southern Europe (Barr, 2009).

In this study, genetic diversity, population genetic structure, and inter-population relationships of the Mediterranean fruit fly were determined. Comparing all samples from 7 provinces, a high genetic similarity was observed in terms of both allele number and allele frequencies. In our study, microsatellite markers were used to elucidate the genetic structures of Mediterranean fruit fly populations. A high level of genetic variation was found in the Mediterranean fruit fly populations in Turkey, and when the populations were evaluated in the geographical sense, it has been observed that there is no significant degree of differentiation. Apart from the regions in this work, it is considered necessary to determine the genetic structures of the *C. capitata* populations in other regions of Turkey.

Table 7. The individuals' assignment into 4 clusters depending on the Q value at $K = 4$.

Clusters				
Populations	**1**	**2**	**3**	**4**
Muğla	0.030	0.886	0.077	0.007
Aydın	0.251	0.043	0.140	0.565
Antalya	0.012	0.009	0.007	0.972
İzmir	0.699	0.136	0.104	0.061
Adana	0.921	0.039	0.020	0.020
Yalova	0.085	0.243	0.607	0.065
Mersin	0.025	0.037	0.928	0.009

A high level of genetic diversity was observed among all examined populations by using 8 microsatellite markers. Our results are consistent with the previous studies in which a high level of genetic variation is reported on a regional geographic scale (Bonizzoni et al., 2001; Bonizzoni et al., 2002; Gasperi et al., 2002; Bonizzoni et al., 2004; Malacrida et al., 2007; Barr, 2009; Karsten et al., 2013; Karsten et al., 2015). In this study, the average number of alleles (n_a) for 7 provinces was 6.95, the average number of effective alleles (n_e) was 3.58, the observed (H_o) and expected (H_e) heterozygosity values were 0.61 and 0.67, respectively. Gasperi et al. (2002) reported the average number of alleles (n_a) 4.50, observed heterozygosity (H_o) 0.39 and expected heterozygosity (H_e) 0.46 for Italy, and the average number of alleles (n_a) 3.3, observed heterozygosity (H_o) 0.30 and expected heterozygosity (H_e) 0.34 for Greece, which are located in the Mediterranean basin. Bonizzoni et al. (2002) reported that the observed (H_o) and expected heterozygosity (H_e) values are 0.60 and 0.54, respectively, by using 4 different microsatellite loci, in a limited number of samples from the Mediterranean basin (Kos Island), which is the first invaded place outside Africa. Also, Bonizzoni et al. (2004) reported the average number of alleles (n_a) 4.4, the average number of effective alleles (n_e) 2.74, observed heterozygosity (H_o) 0.56, and expected heterozygosity (H_e) 0.55 for the Mediterranean basin (Spain, Kos-Greece, and Israel) by using 10 microsatellite loci. In this study, we found 49 new private alleles. Our results are consistent with the studies carried out in the Mediterranean basin (Bonizzoni et al., 2001; Gasperi et al., 2002; Bonizzoni et al., 2004; Kourti, 2004; Karsten et al., 2013; Karsten et al., 2015). These results indicate that Turkish populations have had enough time for private alleles to emerge after colonization. The long *C. capitata* existence in Turkey may have led to differentiation at microsatellite loci. As mentioned by Bonizzoni et al., (2001) the finding of private alleles in the Mediterranean indicates the ancient colonization of this area and, the detection of 49 new private alleles in this study is consistent with this result. Our results are parallel to the previous studies in this sense and higher genetic diversity values point out that Turkey may be an important colonization spot in the colonization of this organism. The possible explanations for this high genetic diversity are, (i) unlike resistance genes, microsatellite loci are not subject to selection pressure and (ii) the climate of Turkey is very suitable for this organism and it can produce quite a lot of annual offspring in many hosts.

The Neighbor-Joining (NJ) tree of the *C. capitata* populations in Turkey shows that there is no geographical clustering. These results are consistent with the previous studies (Güler et al., 2019; Kurd et al., 2020). Possible reasons for this situation are (i) the 'relatively' recent colonization of our country by this species (The existence of the *C. capitata* was reported for the first time in Turkey in 1904 (Fimiani, 1989), later *C. capitata* spread quickly and became common in this region), (ii) the existence of high migration (gene flow) among populations, (iii) the fact that this organism is a polyphagous species and has many hosts (the gaps between the sampling locations have been gorged with host plants that constitute a sustained habitat for this species), (iv) the lack of selective pressures with regard to climates like minimum temperature and yearly rainfall in sampling localities (all of these were previously declared to be significant factors for the growing and survival of this species (Reyes and Ochando, 1998a), (v) intensive trade of products between provinces and (vi) human-mediated activities. Previous studies indicated that commercial activities carried out by human influence, especially in fruit flies, play a critical role in the distribution of this organism in the world (Ramezani et al., 2015). The result of NJ tree was nearly consistent; two genetically distinct groups were obtained in Turkey. All examined populations apart from the population in Muğla were grouped. We can suggest that the genetic structure of *C. capitata* in Muğla is, likely, the consequence of complicated interactions between distinct forces that act at regional scopes (such as differences in pest eradication programs, agricultural implementations, climate and/or geographical structure). Muğla, one of the most important tourism centers in Turkey, is indeed suitable for host plants and *C. capitata*. There are also active trade routes connecting fruit distribution to other regions. The F_{ST} values showed that the Antalya population significantly differentiated from all other populations and comprehensive molecular analyses of this population will explain the difference that we observed.

The observed gene flow between populations (*Nem* = 1.13), seems to be sufficient to homogenize *C. capitata* populations in Turkey. In addition to high levels of genetic diversity, we observed limited genetic differentiation

between 7 *C. capitata* populations in Turkey. Our results are in accordance with previous studies that also stated restricted or no population genetic differentiation in other *C. capitata* populations from distinct regions of the world (Reyes and Ochando, 1998b; Bonizzoni et al., 2004; Beroiz et al., 2012; Karsten et al., 2013; Güler et al., 2019; Kurd et al., 2020).

The invasion of biological invaders can be described by some applications. Within this context, population genetics is one of the important methods for defining the genetic structure, colonization routes, and spread of pest species. Genetic characterization of 7 populations of Turkey showed higher genetic diversity. The high level of genetic diversity that we observed among all the studied populations supports the hypothesis that Australian flies are most probably derived from Mediterranean flies (Bonizzoni et al., 2004). As a result, it is clear that the analysis of Mediterranean fruit fly populations in Turkey from more locations, with more samples, will make a significant contribution to future studies in this field. In this sense, our work aims to shed light on future studies. Additionally, to better understand the colonization route of this organism in the Mediterranean basin and the possible genetic groups in this basin, North Africa, Eastern Mediterranean/America, and Western Mediterranean regions should be studied with further samples and molecular marker systems. Also, the geographical boundaries of the populations should be determined. The information to be obtained will also contribute to the development of more effective and efficient control and/or management programs against the *C. capitata*, which is the most important agricultural pest in the Mediterranean basin, where Turkey is located.

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