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Genetic characterization of field populations of *Culex pipiens* Linnaeus, 1758 (Diptera: Culicidae) sampled from the Aegean region of Turkey

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Abstract: Mosquitoes are one of the organisms subjected to frequent insecticide application due to their status as vectors that carry a wide range of life-threatening diseases. Turkey has climatic and other ecological features required for the breeding and living of 50 species from 8 genera. The members of the *Culex pipiens* complex are the dominant mosquitoes among them. In order to design more sustainable insecticide resistance management strategies, it is important to investigate the genetic structure of mosquito populations using molecular techniques. The aim of this study was the genetic characterization of field populations of *C. pipiens* sampled from 25 different sublocations belonging to 6 provinces in the Aegean region of Turkey by using random amplified polymorphic DNA (RAPD) markers. Eighty 10-mer RAPD primers were screened on a subset of DNAs. Among them, 20 reproducible and clear band-producing polymorphic primers were selected and applied to all study material. A low level of genetic differentiation and a high level of gene flow were detected between the populations.

Key words: *Culex pipiens*, genetic diversity, RAPD markers, Turkey

1. Introduction

Mosquitoes are medically important arthropods. They are the vectors of numerous arboviral and parasitic diseases adversely affecting millions of people in each year worldwide (WHO, 2009; Khan et al., 2010; Idrees and Ashfaq, 2012). Of the currently known approximately 500 arboviruses, more than 200 are known or suspected to be mosquito-borne. Due to its appropriate climatic and other ecological features required for breeding and living of mosquitoes, Turkey hosts 50 mosquito species from 8 genera (Ramsdale et al., 2001; Çağlar et al., 2008). Studies based on molecular methods confirmed the widespread presence of *Culex pipiens* complex members in different parts of Turkey (Ergunay et al., 2014; Gunay et al., 2015; Morcicek et al., 2018). Because of their global distribution, mosquitoes in the *C. pipiens* complex have a special place in terms of their medical and veterinary importance as vector species. *C. pipiens* L. and *C. quinquefasciatus* Say are the two widespread species of the complex. *C. pipiens* L. is known as the northern house mosquito species, mostly present in the temperate climatic zone, and *C. quinquefasciatus* Say is known as the southern house mosquito, which predominantly exists throughout subtropical and tropical areas (Farajollahi et al., 2011). *C. australicus* and *C. globocoxitus* are the other members of the complex. They are restricted to Australia and are poorly known (Dumas et al., 2013).

Detection of *C. quinquefasciatus* in Turkey is highly important in terms of arboviral disease transmission. This species feeds on both birds and mammals and has been widely charged as being an extremely effective vector of West Nile virus (WNV) across its distribution area. In other parts of its distribution area, *C. quinquefasciatus* also acts as a major vector of Japanese encephalitis virus in SE Asia (Kumari et al., 2013) and St Louis encephalitis virus in the USA (Rios et al., 2006), and it is capable of experimental transmission of *Plasmodium relictum* and *Wuchereria bancrofti* (Calherios et al., 1998; LaPointe et al., 2005).

Members of the *C. pipiens* complex are evolutionarily closely related species that often hybridize. In both morphological and genetic analyses, hybridization between *C. pipiens* and *C. quinquefasciatus* in North America and Asia was shown (Cornel et al., 2003; Fonseca et al., 2004, 2009). More recently, hybrids between *C. pipiens* and *C. quinquefasciatus* were reported on the Greek island of Kos

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(Shaikevich and Vinogradova, 2014). Northerly records of *C. quinquefasciatus* in central areas of Iran in sympatry with *C. pipiens* were reported by Dehghan et al. (2013). Many reports, in general, indicate a northerly expansion of the range of *C. quinquefasciatus* in the Palearctic region. This hybridization phenomenon is important because it was shown that hybridization has a significant effect on WNV infection, dissemination, and, particularly, transmission in *C. pipiens* L. complex mosquitoes, and enhanced transmission of WNV was found in all hybrid populations compared to one or both parental stains (Ciota et al., 2013).

Worldwide, to control the vectors and thus to limit the effect of vector-borne diseases, the main tactic is the use of insecticides. Unfortunately, the widespread use of insecticides for mosquito control and the use of such chemicals for agricultural purposes have resulted in the development and dispersal of resistance. The resistance phenomenon has presented an obstacle and ever-increasing problem in the endeavors to control important mosquito vectors (N'Guessan et. al., 2007). Therefore, to guarantee the sustainability of vector-control programs, monitoring of insecticide resistance in field mosquito populations is very crucial. In a number of recent studies the molecular basis of insecticide resistance for *C. pipiens* populations was documented from different parts of the world (Wondjii et al., 2008; Alout et al., 2009; Zhou et al., 2009; Tantaley et al., 2010; Osta et al., 2012; Wang et al., 2012; Kioulos et al., 2014), and more recently, as a first effort, the overall distribution and dynamics of the insecticide resistance-associated mutations in field populations of *C. pipiens* in Turkey were analyzed by Taskin Gocmen et al. (2016). In addition to the use of insecticides, climatic changes cause appreciable effects on the genetic structures of mosquito populations.

It has become necessary to understand the population structure of mosquito species on a genetic basis using molecular techniques in order to develop and implement successful control programs in specific areas, which is in turn important for evaluating the roles of mosquitoes in disease transmission and developing more effective management strategies for mosquito-borne diseases. Thus, the current study was designed to genetically analyze field populations of the *C. pipiens* complex sampled from 25 different sublocations belonging to 6 provinces in the Aegean region of Turkey to demonstrate the genetic variations and gene flow using random amplified polymorphic DNA (RAPD) markers. Occupying the western part of Turkey, the Aegean region has a total population of more than 10 million. The region is characterized by densely populated residential, commercial, and industrial areas as well as rural areas comprising agricultural areas and arable lands. The areas in the region lie along the routes of migratory birds constituting a considerable risk for WNV. In the region there are large international airports, which are potential ports for incoming and outgoing vector species. High insecticide usage for public health, personal protection, and the agroindustry is common throughout the region. Given the fact that the species in this complex are important vectors of human and animal diseases, it is very necessary to characterize the populations genetically in this critical region of Turkey to assess resistance gene dispersal and to determine effective insecticide management strategies, which is indispensable for combating mosquito-borne diseases. The RAPD marker system that determines nucleotide sequence polymorphisms using single primers of arbitrary nucleotide sequences has been used to investigate gene flow and genetic structure in different insect species (Jain et al., 2010). This marker system is generally faster and less expensive than other markers, no previous DNA sequence information is required, very low quantities of DNA are sufficient, whole genomes can be screened, and it can generate essentially unlimited numbers of loci for use in genetic analysis (Williams et al., 1990). Reliability and repeatability are the main problems of RAPD markers, but these problems can easily be minimized by optimizing experimental conditions.

2. Materials and methods

2.1. Specimens

Samples of *C. pipiens* complex mosquitoes were collected as egg rafts or as the first and second instar larvae from the same natural breeding sites after the final application of insecticides in October 2012 from 6 provinces of the Aegean region in Turkey. Among these provinces Muğla, Aydın, İzmir, Balıkesir, and Çanakkale have coastal sites on the Aegean Sea and have Mediterranean climatic conditions, while Denizli Province has a relatively mild continental climate due to its location. From each province 4 sublocations were sampled (only for İzmir Province 5 sublocations were sampled), 2 of them being under intense agricultural activities and 2 of them being under intense tourism activities. The sublocations and provinces from which the samples were collected are shown in Figure 1 and listed in Table 1. In the region the average winter and summer temperatures are 9.8 °C and 25.2 °C, respectively, and the annual rainfall is 631 mm. Egg rafts and larvae from breeding sites sampled in a given sublocality were pooled, transported to the laboratory, and reared to adults under standard conditions of 25–27 °C and 70 ± 5% RH with a 14:10 h light:dark photoperiod. Upon emergence, *Culex* species were identified based on morphological characters using the standard identification keys (Dubose and Curtin, 1965; Darsie and Samanidou-Voyadjoglou, 1997) and immediately used in genomic DNA extraction.

Figure 1. Map of collection localities for mosquitoes.

From each sublocation 15 individual mosquitoes (in total 375 mosquitoes) were used in RAPD analysis.

2.2. Genomic DNA extraction

Genomic DNA from individual mosquitoes was isolated following the modified protocol of the Lifton method of Bender et al. (1983). According to this modified protocol, an individual mosquito was homogenized in liquid nitrogen, and after addition of 400 µL of Lifton buffer solution (0.1 M Tris-HCl, 0.05 M EDTA, pH 9.1) and 100 µL of 0.5% SDS solution the homogenate was incubated at 65 °C for 30 min. Following the addition of 1 µL of proteinase K, it was incubated at 37 °C for 30 min. Then 200 µL of 0.6 M sodium acetate was added and incubated in ice for 1 h. The sample was centrifuged at 14,000 rpm for 10 min and the supernatant was removed to a new tube. Then 500 µL of phenol and a drop of chloroform:isoamyl alcohol (24:1) were added prior to centrifugation at 14,000 rpm for 5 min. To the supernatant removed to a new tube 250 µL of phenol and 250 µL of chloroform:isoamyl alcohol (24:1) were added and centrifugation was performed at 14,000 rpm for 5 min. Once more the supernatant was removed to a new tube and 500 µL of chloroform:isoamyl alcohol (24:1) was added before centrifugation at 14,000 rpm for 5 min. To the supernatant taken to a new tube 1 µL of RNase was added and the mixture was incubated at 37 °C for 30 min. After incubation, 96% ethanol was added and the mixture was centrifuged at 14,000 rpm for 15 min. Then 80% ethanol was added to the pellet and this was centrifuged at 14,000 rpm for 15 min. Finally, the ethanol was removed and the pellet was dried, resuspended in 30 µL of sterile dH_2O, and incubated at 4 °C overnight. Estimation of DNA concentration was made by measuring optical density (OD) at 260 nm and DNA quality was checked through 1% agarose gel electrophoresis.

2.3. DNA amplification conditions

In this study, 80 ten-base oligonucleotide primers from Operon Technologies (Alameda, California, USA) including kits from A (OPA) to R (OPR) were used (Table 2). Each PCR reaction was carried out in a final volume of 25 µL, containing 10 ng of template DNA, 5.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 1.0 U of Taq DNA polymerase, 10 pmol of primer, 2.5 µL of PCR buffer (from 10X), and

Table 1. Mosquito collection sites and map coordinates.

Provinces	Sublocations	Coordinates	Elevations (m)
Çanakkale	Gökçeada	40°08′15.87″N, 25°44′12.88″E	75.89
	Gelibolu	40°25′24.08″N, 26°40′05.44″E	37.79
	Ezine	39°47′05.18″N, 26°19′41.40″E	63.09
	Ayvacık	39°34′00.74″N, 26°10′04.71″E	20.11
Balıkesir	Erdek	40°07′02.15″N, 27°59′11.66″E	22.86
	Merkez	39°54′57.62″N, 28°09′51.03″E	42.06
	Ayvalık	39°16′25.80″N, 26°38′19.40″E	15.54
	Burhaniye	39°22′53.57″N, 26°50′06.60″E	14.93
İzmir	Seferihisar	38°11′03.19″N, 26°48′13.18″E	11.58
	Bergama	39°06′16.91″N, 27°10′06.33″E	44.19
	Menemen	39°01′05.52″N, 27°04′24.17″E	17.37
	Çiğli	38°29′34.04″N, 26°57′22.01″E	30.48
	Bornova	38°27′26.32″N, 27°14′01.35″E	39.01
Aydın	Çine	37°41′10.83″N, 27°59′59.04″E	59.13
	Söke	37°40′21.87″N, 27°21′46.15″E	07.92
	Karpuzlu	37°35′55.58″N, 27°50′05.06″E	112.77
	Kuşadası	37°21′04.64″N, 27°16′26.35″E	12.49
Muğla	Gökova	37°01′58.59″N, 28°20′21.22″E	03.04
	Datça	36°47′16.99″N, 28°01′53.41″E	03.96
	Fethiye	36°38′16.41″N, 29°05′40.91″E	51.20
	Köyceğiz	36°57′15.30″N, 28°42′01.72″E	04.87
Denizli	Çivril	38°19′20.75″N, 29°51′01.28″E	834.84
	Merkez	37°47′36.00″N, 29°04′46.08″E	365.15
	Beyağaç	37°14′14.51″N, 28°53′48.10″E	701.34
	Pamukkale	37°54′55.25″N, 29°06′53.78″E	245.36

0.3 µL of Tween-80. The PCR program comprised 45 cycles with initial denaturing of DNA at 94 °C for 4 min, denaturation at 94 °C for 1 min, primer annealing at 36 °C for 1 min, extension at 72 °C for 2 min, and final extension at 72 °C for 5 min in an Eppendorf Mastercycler gradient thermal cycler (Hamburg, Germany). The PCR products were run on 1% agarose gel at 70 V for 180 min.

2.4. Strategy for identification of RAPDs and data collection

Initially, 80 RAPD primers were screened against DNAs from a subset of 4 individuals randomly selected among the 375 individual mosquitoes from the 25 subpopulations of *C. pipiens***.** Among these, 20 primers were selected based on the reproducibility and number of polymorphic bands (Table 2). The primers were then screened with the DNAs of all individuals of the studied populations.

2.5. Data analysis

The RAPD band patterns of each individual mosquito sampled from studied populations were examined on an ultraviolet transilluminator and photographed using the Vilber Lourmat Infinity-115 Gel Documentation System. The sizes of amplified bands or loci were compared against the DNA marker. All the fragments were scored as present (1) or absent (0) for each sample. Ambiguous bands were not scored. The bands were counted by starting from top to the bottom in all lanes. In cases where no bands were detected throughout a lane, the locus was scored as missing data. The RAPD markers were analyzed using the following assumptions: (1) the RAPD alleles follow the Mendelian pattern of segregation, (2) monomorphic fragments are homologous (comigrate), (3) loci are independent from each other, and (4) mosquito populations are in Hardy–Weinberg equilibrium as described by Ayres et al. (2003).

In order to evaluate *C. pipiens* populations for intra- and interpopulation genetic diversity, several statistics were used. Polymorphism (*P%*), the proportion of polymorphic loci detected (criterion of 99% was used), mean number of observed (*Na*) and effective (*Ne*) alleles per locus (Kimura and Crow, 1964), Nei's gene diversity (*h*) as a measure of heterozygosity (Nei, 1973), and Shannon's information index (*I*) (Lewontin, 1972) were calculated. Total genetic variation (H_T), within-population genetic variation (H_S),

Table 2. Total number of bands with their size ranges and number of polymorphic bands produced by the screened 80 random 10-mer OPERON primers.

	Primer code	Sequence of the primer (5′ to 3′)	Total # of bands produced	Range of bands produced (bp)	Total # of polymorphic bands produced
1	OPA-11	CAATCGCCGT	6	500–3000	5
2	OPA-15	TTCCGAACCC	0	-	-
3	OPA-17	GAC CGCTTGT	0	-	-
4	OPA-18*	AGGTGACCGT	6	500–3000	5
5	OPA-20*	GTTGCGATCC	4	500–3000	3
6	OPB-13	TTCCCCCGCT	0	-	-
7	OPB-14	TCCGCTCTGG	6	500–3000	5
8	OPB-16	TTTGCCCGGA	11	500–3000	9
9	OPB-19	ACCCCCGAAG	6	500–3000	5
10	OPB-20*	GGACCCTTAC	8	500–3000	7
11	OPC-12	TGTCATCCCC	0	-	-
12	OPC-14	TGCGTGCTTG	0	-	-
13	OPC-17	TTCCCCCCAG	0	-	-
14	OPC-19*	GTTGCCAGCC	8	500–3000	6
15	OPD-11*	AGCGCCATTG	12	500–3000	9
16	OPD-14	CTTCCCCAAG	0	-	-
17	OPD-15	CATCCGTGCT	0	-	-
18	OPD-17	TTTCCCACGG	0	-	-
19	OPE-13	CCCGATTCGG	0	-	-
20	OPE-14*	TGCGGCTGAG	11	500–3000	8
21	OPE-16	GGTGACTGTG	4	1000–2000	3
22	OPE-17	CTACTGCCGT	8	500–3000	6
23	OPE-19	ACGGCGTATG	6	500–3000	5
24	OPF-11	TTGGTACCCC	0	-	-
25	OPF-14	TGCTGCAGGT	0	-	-
26	OPF-15	CCAGTACTCC	0	-	-
27	OPF-18	TTCCCGGGTT	0	-	-
28	OPF-19	CCTCTAGACC	0	-	-
29	OPG-11*	TGCCCGTCGT	8	500–3000	6
30	OPG-12	CAGCTCACGA	0	-	-
31	OPG-16	AGCGTCCTCC	0	-	-
32	OPG-18	GGCTCATGTG	0	-	-
33	OPG-20	TCTCCCTCAG	0	-	-
34	OPH-11	CTTCCGCAGT	0	-	-
35	OPH-12*	ACGCGCATGT	6	500–3000	5
36	OPH-17	CACTCTCCTC	0	-	-
37	OPH-19	CTGACCAGCC	0	-	-
38	OPI-11*	ACATGCCGTG	11	500–3000	8
39	OPI-13*	CTGGGGCTGA	9	500–3000	6

Table 2. (Continued).

40	OPI-15	TCATCCGAGG	0	-	-
41	OPI-16	TCTCCGCCCT	7	500–3000	7
42	OPI-18	TGCCCAGCCT	0	-	-
43	OPJ-12	GTCCCGTGGT	0	-	-
44	OPJ-16	CTGCTTAGGG	0	-	-
45	OPJ-17	ACGCCAGTTC	0	-	-
46	OPJ-19	GGACACCACT	0	-	-
47	OPK-14	CCCGCTACAC	0	-	-
48	OPK-15	CTCCTGCCAA	9	500–3000	4
49	OPK-17*	CCCAGCTGTG	10	500–3000	9
50	OPK-18	CCTAGTCGAG	0	-	-
51	OPL-13	ACCGCCTGCT	0	-	-
52	OPL-14	GTGACAGGCT	6	500–3000	5
53	OPL-17*	AGCCTGAGCC	13	500–3000	9
54	OPL-18	ACCACCCACC	0	-	-
55	OPM-11	GTCCACTGTG	0	-	-
56	OPM-12*	GGGACGTTGG	12	500–3000	9
57	OPM-14	AGGGTCGTTC	0	-	-
58	OPM-18	CACCATCCGT	0	-	-
59	OPM-20	AGGTCTTGGG	0	-	-
60	OPN-13*	AGCGTCACTC	10	500–3000	8
61	OPN-14*	TCGTGCGGGT	14	500–3000	6
62	OPN-15	CAGCGACTGT	12	500–3000	8
63	OPN-20*	GGTGCTCCGT	12	500–3000	9
64	OPO-12	CAGTGCTGTG	6	500–3000	5
65	OPO-15*	TGGCGTCCTT	10	500–3000	8
66	OPO-16*	TCGGCGGTTC	9	500–3000	8
67	OPO-18	CTCGCTATCC	0	-	-
68	OPP-13	GGAGTGCCTC	0	-	-
69	OPP-16*	CCAAGCTGCC	9	500–3000	8
70	OPP-17	TGACCCGCCT	5	500–3000	4
71	OPP-18	GGCTTGGCCT	0	-	-
72	OPQ-12	AGTAGGGCAC	0	-	-
73	OPQ-14	GGACGCTTCA	0	-	-
74	OPQ-19	CCCCCTATCA	0	-	-
75	OPQ-20	TCGCCCAGTC	0	-	-
76	OPR-11	GTAGCCGTCT	11	500–3000	9
77	OPR-12	ACAGGTGCGT	6	500–3000	4
78	OPR-14	CAGGATTCCC	0	-	-
79	OPR-16*	CTCTGCGCGT	5	500–3000	4
80	OPR-19	CCTCCTCATC	0	-	-

*Primers screened with the DNAs of all individuals.

and Nei's (1973) genetic differentiation coefficient (G_{ST}) were determined. Gene flow (*Nm*) was estimated from G_{ST} values using the relationship $Nm = 0.5 (1 - G_{ST}) / G_{ST}$, in which *N* is the effective population size and *m* is the proportion of the population that are migrants. Subsequently, genetic distance (D_N) coefficients among all possible population pairs were calculated (Nei, 1972) and a dendrogram was constructed using the UPGMA (unweighted pair-group method with arithmetic average) method. All calculations were performed with POPGENE (version 1.32) software (Yeh et al., 1999). Pearson's correlation coefficients (r_p) were computed to relate the genetic diversity measures (*P%*, *Na*, *Ne*, *h*, and *I*) of the populations to the elevations of the locations by using SPSS 11 for Windows according to Steel and Torrie (1980).

3. Results

Of the 80 RAPD primers that were initially screened against DNA from a subset of 4 individuals randomly selected among the 375 total individuals from 6 *C. pipiens* populations, 45 did not yield any RAPD bands and the remaining 35 revealed at least 3 polymorphic loci. Most of these 35 primers (21 primers) produced 6 to 10 bands, while 10 primers produced 11 to 14 bands and only 4 primers produced 1 to 5 bands. Sizes of these RAPD fragments were within the range of 500 and 3000 bp. Six to 9 polymorphic loci were detected by 21 primers and 3 to 5 polymorphic loci were detected by the other 14 primers (Table 2).

Screening the DNA from all individuals of the studied populations with 20 polymorphic RAPD primers resulted in a total of 252 loci. Only one of these loci was monomorphic, so the polymorphic loci ratio was 99.60%. Genetic parameters for intra- and interpopulation variability are given in Table 3. The percentage of polymorphic loci (*P%*) in populations varied between 64.29% (Çanakkale population) and 94.84% (Muğla population) with an overall mean of 82.41%. The overall mean number of observed alleles per locus (*Na*) was 1.82 ± 0.35, while the overall mean number of effective alleles per locus (*Ne*) was 1.44 ± 0.34. The Çanakkale population had the lowest value for *Na* (1.64 ± 0.48), and the Muğla population had the highest value (1.95 ± 0.22). *Ne* values of the populations varied between 1.34 ± 0.34 (Çanakkale population) and 1.48 ± 0.34 (Aydın population). The overall means of Nei's gene diversity (*h*) and Shannon's information index (*I*) were 0.27 ± 0.18 and 0.40 ± 0.24, respectively. *h* values ranged from 0.21 ± 0.19 in the Çanakkale population to 0.29 ± 0.17 in the Aydın population, and *I* values ranged from 0.31 ± 0.27 to 0.44 ± 0.22 in the same populations. Total genetic variation (H_T) was 0.29 ± 0.03. A substantial proportion of this variation, 0.27 ± 0.02 (93.10%), was due to within-population genetic variation (H_S). The genetic differentiation coefficient (G_{ST}) was 0.07 and mean gene flow (*Nm*) within a generation among the 6 populations was 6.61. Genetic distance coefficients (D_N) ranged from 0.0185 to 0.0560 among population pairs. The minimum distance was detected between the İzmir and Aydın and the İzmir and Balıkesir populations, while the maximum distance was detected between Muğla and Çanakkale populations (Table 4). A UPGMA dendrogram is presented in Figure 2. Correlation analysis was not significant between elevations of the populations and their diversity measures (*P%*, *Na*, *Ne*, *h*, and *I*) obtained in the study (data not shown).

4. Discussion

In relation with the global warming phenomenon, mosquito-borne diseases have been rapidly spreading during the last decade, threatening thousands of people in many parts of the world. Dispersal due to transportation, indiscriminate use of insecticide, and the elimination of natural and artificial breeding places had a significant effect on migration, genetic exchange, and the genetic structure of mosquitoes (Paupy et al., 2000; Lerdthusnee and Chareonviriyaphap, 2002).

It is very crucial to understand the population structures of mosquito species on a genetic basis using

Table 3. Genetic parameters for intra- and interpopulation variability in *C. pipiens* populations.

Population	*P%*	*Na*	*Ne*	*h*	*I*
Muğla	94.84	1.95 ± 0.22	1.46 ± 0.33	0.28 ± 0.16	0.43 ± 0.21
Aydın	92.46	1.92 ± 0.26	1.48 ± 0.34	0.29 ± 0.17	0.44± 0.22
İzmir	86.90	1.87 ± 0.34	1.47 ± 0.35	0.28 ± 0.18	0.42 ± 0.24
Balıkesir	77.38	1.77 ± 0.42	1.46 ± 0.36	0.27 ± 0.19	0.41 ± 0.26
Denizli	78.57	1.79 ± 0.41	1.45 ± 0.34	0.27 ± 0.18	0.41 ± 0.25
Çanakkale	64.29	1.64 ± 0.48	1.34 ± 0.34	0.21 ± 0.19	0.31 ± 0.27
Overall mean	82.41	1.82 ± 0.35	1.44 ± 0.34	0.27 ± 0.18	0.40 ± 0.24

Table 4. Estimates of Nei's (1972) genetic distance (D_N) coefficients among the *C. pipiens* populations.

Population	Muğla	Aydın	İzmir	Balıkesir	Denizli	Çanakkale
Muğla	*****					
Aydın	0.0236	*****				
İzmir	0.0294	0.0185	*****			
Balıkesir	0.0417	0.0307	0.0185	*****		
Denizli	0.0412	0.0275	0.0222	0.0187	*****	
Çanakkale	0.0560	0.0474	0.0407	0.0393	0.0375	*****

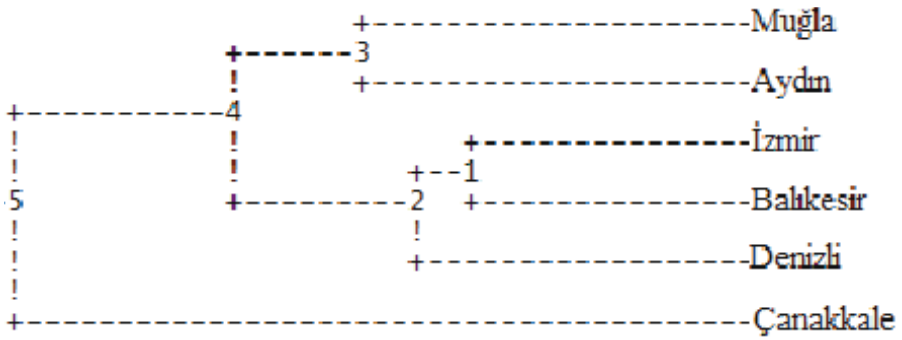

Figure 2. A UPGMA dendrogram based on Nei's (1972) genetic distances among *C. pipiens* populations.

molecular techniques to develop successful mosquito control programs in specific areas and to be successful in management strategies of mosquito-borne diseases. The genetic diversity in mosquito populations had been widely reported through various molecular techniques (Franco et al., 2002; Paduan et al., 2006). Particularly the polymerase chain reaction (PCR)-based DNA techniques are significantly increasing our knowledge of population structures and dynamics (Beroiz et al., 2012). Over the past two decades interest in mosquito population genetics has risen dramatically and many studies carried out on different geographical scales have been reported determining the genetic structure of mosquito populations. Although the earlier studies in mosquito population genetics focused on resolving taxonomic issues, especially in distinguishing and defining the geographic distributions of cryptic taxa, recently studies have shifted to vector control as a means of controlling mosquito-borne diseases (Lanzaro et al., 2006). These studies are promising for the understanding of vector genetic structure, vector transmission, and disease epidemiology and disease control (Failloux and Rodhain, 1999). However, to the best of our knowledge little or no information related to the population genetics of mosquito species has been available from Turkey, which covers a large part of the eastern Mediterranean basin. Therefore, this study, in which 25 *C. pipiens* complex populations from 6 provinces of a critical and relatively large region of Turkey were genetically characterized, is important.

Our results showed that the studied *C. pipiens* complex populations were genetically less differentiated (G_{ST} = 0.07), having a high level of gene flow (*Nm* = 6.61) among them. These low G_{ST} and high *Nm* values detected in the present study are consistent with the findings of many studies reported for different mosquito species from different parts of the world (Lehmann et al., 1996; Besansky et al., 1997; Humeres et al., 1998; Cui et al., 2007; Santos et al., 2011; Bibi et al., 2015; Preet and Gupta, 2017). In their study, Cui et al. (2007) compared the previous population genetic surveys of *C. pipiens* in different geographic areas around the world and reached a conclusion that this species needs considerable distances, at least between 500 and 1000 km, to show isolation by distance, irrespective of the subspecies (*C. p. pipiens, C. p. quinquefasciatus*, and *C. p. pallens*) or the geographic location. On the other hand, in some studies high G_{ST} and low *Nm* values were reported for mosquito species. For example, Souza et al. (2001) from Argentina (G_{ST} = 0.249, *Nm* = 0.75), Ayres et al. (2003) from Brazil (G_{ST} = 0.317, *Nm* = 0.54), and Ashraf et al. (2016) from Pakistan (G_{ST} = 0.341, *Nm* = 0.966) reported such G_{ST} and *Nm* values for *Aedes aegypti* populations. It has been widely accepted that genetic variability occurs in different insects both at intra- and interspecies level as a

result of differences in environmental processes, genetics, and various demographic factors. In contrast, genetic homogeneity in insect species occurs due to free movement, lack of barriers (Batool, 2012; Ashraf, 2013; De Lourdes et al., 2013), and migration as well as transportation through various means (Franco et al., 2002; Souza et al., 2001; Ayres et al., 2003). Our study also demonstrated that the majority of genetic variation was within populations (93.10%) while the remaining small portion (6.90%) was among populations. This low level of among-population variation in relation to within-population variation supports our findings where the values of G_{ST} and *Nm* depict a low level of genetic differentiation, indicating high rate of gene flow between populations. These findings suggest that *C. pipiens* mosquitoes are freely moving between the locations throughout the Aegean region in diverse habitats, establishing stable populations with high gene flow. Caprio (1990) reported that a gene flow rate between 6.3 and 10.6 does not prevent significant allelic variation between populations, while it is sufficient to move adaptively significant alleles relatively rapidly throughout populations. Considering insecticide resistance and vector capacity as the two important selected traits of a vector species, our findings are critical in terms of the control of *C. pipiens* complex populations to fight against the diseases caused by them. In this respect, we should emphasize the importance of well-designed region-wide insecticide management strategies covering the whole Aegean region of Turkey, rather than local management strategies. When developing such strategies, of course, the evaluation of the knowledge of the potential dynamics of insecticide resistance among populations of the *C. pipiens* complex together with the population genetics information will be critical. In order to get country-wide and world-wide success in the control of mosquito populations and the fight against the mosquito-borne diseases, we should be aware of the necessity of well-designed internationally coordinated studies on the evolution and dynamics of insecticide resistance as well as the population genetics of mosquitoes.

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