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## Identification and Characterization of Single Nucleotide Polymorphisms (SNPs) in *Culex theileri* (Diptera: Culicidae)

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**ABSTRACT** *Culex theileri* Theobald (Diptera: Culicidae) is one of the most common mosquito species in northeastern Turkey and serves as a vector for various zoonotic diseases including West Nile virus. Although there have been some studies on the ecology of *Cx. theileri*, very little genetic data has been made available. We successfully sequenced 11 gene fragments from *Cx. theileri* specimens collected from the northeastern part of Turkey. On average, we found a single nucleotide polymorphism every 45 bp. Transitions outnumbered transversions, at a ratio of 2:1. This is the first report of genetic polymorphisms in *Cx. theileri* and single nucleotide polymorphism discovered from this study can be used to investigate population structure and gene-environmental interactions.

**KEY WORDS** *Culex theileri*, single nucleotide polymorphism, genetic polymorphism

*Culex theileri* Theobald, has a broad but discontinuous geographic distribution in Africa, the Middle East, Europe, and Asia (Becker et al. 2003) and it is one of the most common mosquito species in northeastern Turkey. Its distribution includes a broad range of ecological niches. Eggs hatch in Spring in flooded meadows, stagnant or slowly moving streams, ditches, rock pools, drains, swamps, and rice fields, but also frequently in artificial containers and strongly polluted water (Aitken 1954, Ramos et al. 1977). Larval habitats are usually in fresh or slightly saline water (2 g NaCl/liter), but they can tolerate salinity up to 16.6 g NaCl/liter (Jupp et al. 1966, Gutsevich et al. 1974, Pires et al. 1982). Females are zoophilic and also fairly ornithophilic, but sometimes feed on man and bite mainly in outdoors.

*Cx. theileri* has been shown to be naturally infected with West Nile virus, Rift Valley Fever virus, and Sindbis arboviruses in South Africa (Jupp et al. 1966, McIntosh 1967). In Iran, females were found to be naturally infected with the infective stage *Dirofilaria immitis* (dog heartworm) (Azari-Hamidian, 2009). Field and laboratory studies verified that the species is a natural vector of *D. immitis* on Maderia Island, Portugal (Santa-Ana et al. 2006). In Iraq *Cx. theileri* has been implicated in West Nile virus transmission (Abul-Hab, 1967).

First we hypothesized that body size of *Cx. theileri* may change with altitude and also that habitat differ-

ences associated with altitude may affect morphological characteristics. To test this we collected specimens of *Cx. theileri* from nine sites at different altitudes in northeastern Turkey (Fig. 1) and conducted geometric morphometrics analyses on wings to obtain shape and size differences between populations (Demirci et al. 2011). We focused our collection efforts on northeastern Turkey, because elevation changes dramatically over short distances in this region, providing a convenient surrogate for many variables, such as temperature and land cover, that affects mosquito bio-ecology. Geometric morphometrics is a powerful tool for capturing shape characteristics in several morphological aspects, particularly head, wings, and genitalia (Zelditch et al. 2004). This method involves examining the structures from which Cartesian coordinates can be taken. Wing size is an index of body size (Tantawy and Vetukhiv 1960, Cowley and Atchley 1990) and wings are very appropriate structures for biological shape studies because their two-dimensional flattened shape bears several useful landmarks (Groditsky 1999, Zelditch et al. 2004). The results for size and shape showed that there are some significant differences among populations from different altitudes (Demirci et al. 2012). Based on these analyses, we hypothesized that the result may be because of genetic variance in addition to environmental effects alone. However, despite its broad distribution and evidence indicating that *Cx. theileri* is a competent vector of human and domestic animal pathogens, very little genetic data has been collected for this species. One study successfully amplified the cytochrome oxidase I gene (Azari-Hamidian, 2009); however, there is no other genetic data available on the species.

We undertook this study to identify and characterize single nucleotide polymorphisms (SNPs) in the *Cx. theileri* genome with the aim of determining if

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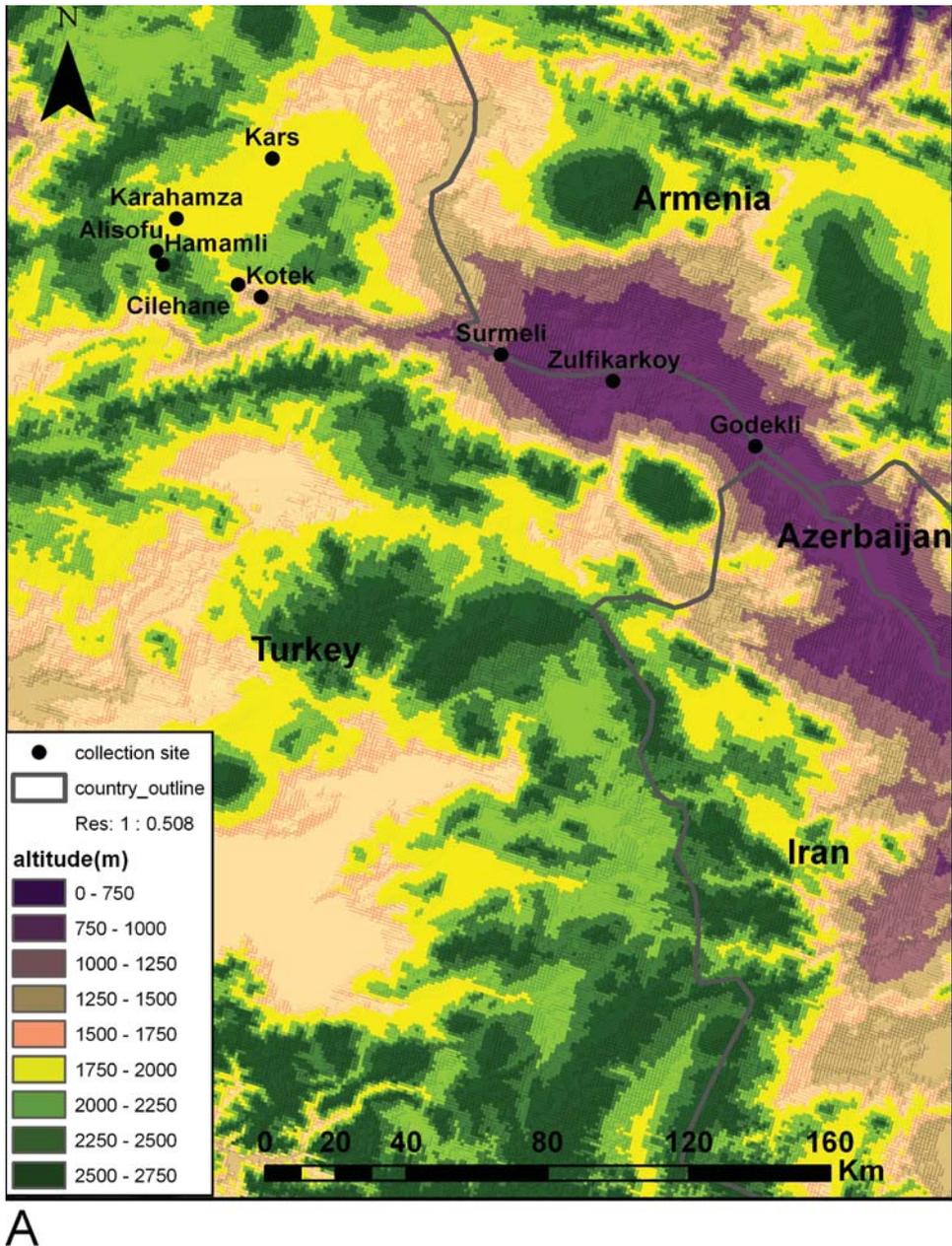


Fig. 1. Collection sites of *Cx. theileri* from the northeast part of Turkey. (A) Elevation map of collection sites. (B) Habitat type map of collection sites. (Online figure in color.)

there are genetic differences among *Cx. theileri* populations along habitat-climate-elevation gradients from northeast part of Turkey. SNPs represent the most widespread type of sequence variation in genomes. They are extremely abundant with an occurrence of about one SNP per kb in human (Wang et al. 1998) and about one SNP every 125 bp in *Anopheles gambiae* (Morlais and Severson 2003). SNPs located in noncoding regions of the genome and synonymous SNPs in coding regions, which have no impact on the

phenotype, may provide useful markers for population genetic studies. Nonsynonymous SNPs that alter the amino acid sequence and potentially the function of encoded proteins are useful markers for association studies to detect genetic variation linked with phenotypic traits (Wondji et al. 2007).

As part of our effort to create a genetic map for the *Cx. theileri* and to develop SNP markers for population genetics studies, we used specimens collected from sites at the lowest (Godekli, 808 m), intermediate

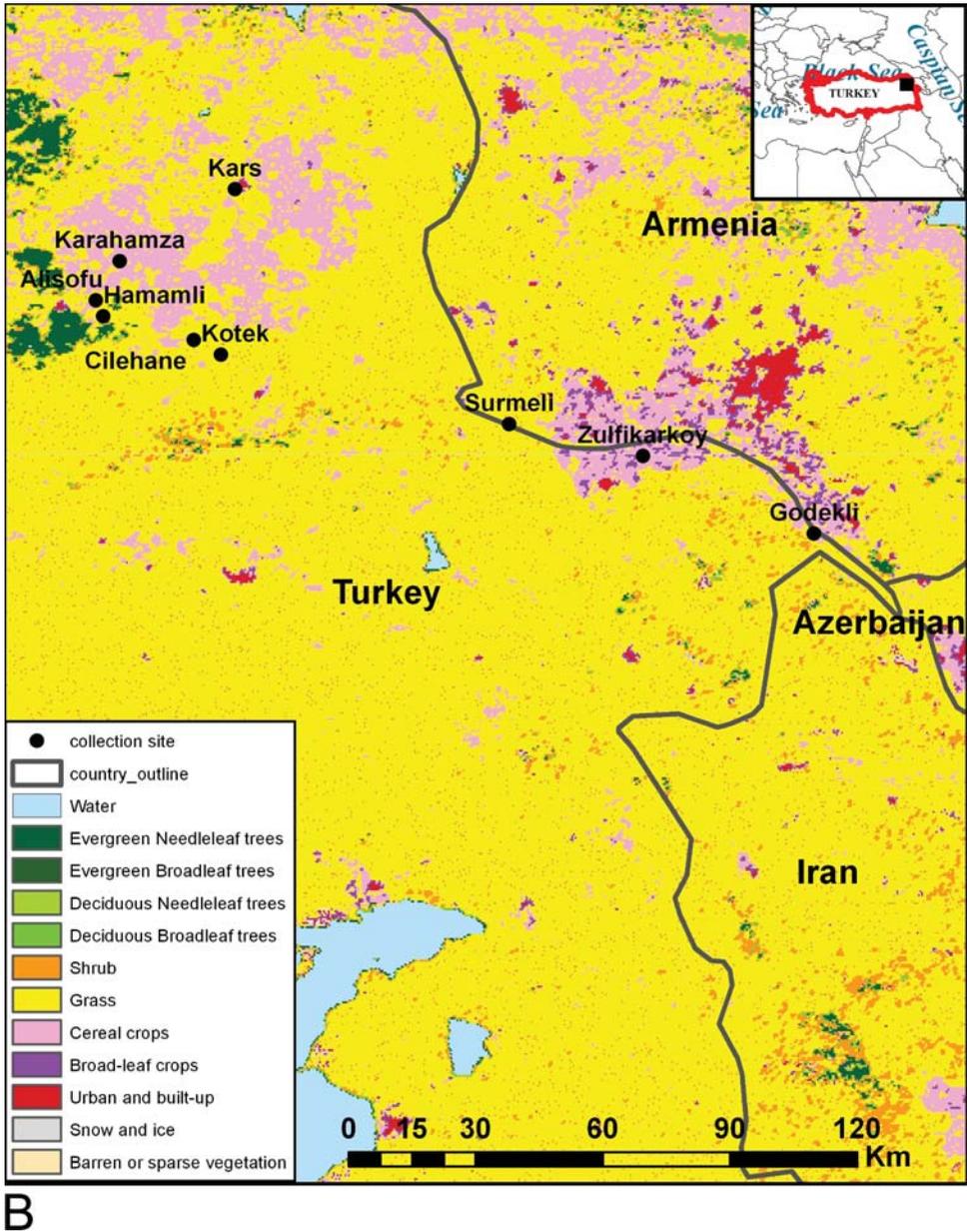


Fig. 1. (Continued)

(Kars, 1,876 m), and highest (Hamamli, 2130 m) altitudes (Table 1). DNA fragments from 11 genes were successfully amplified and sequenced from these specimens. In this article, we summarize the properties of SNPs recovered from these genes.

**Materials and Methods**

**Study Area.** Mosquito trapping was conducted from June to September 2009 in northeastern Turkey, along habitat-climate-elevation gradients ranging from plain habitats below 1,000 m to foothills low montane areas

(1,000–1,700 m), mid range montane areas (1,700–2,000 m) and high montane areas above 2,000 m. Sampling included sites located along the Aras River and in the Kars Plateau (Fig. 1). Aerial distance between the most remote locations (Godekli and Hamamli) is ≈ 200 km and the distance between the closest sites (Alisofu and Hamamli) is ≈ 5 km. Selected environmental site characteristics are given in Table 1.

The Kars plateau is located in the coldest region in Turkey and continental climate conditions prevail in the area. It is covered with snow over a 4–5 mo period each year and the thickness of the snow can reach 80

**Table 1. Geographic and ecological features of sampling stations**

City	Abbr.	Altitude	Latitude	Longitude	Habitat type
<b>Godekli</b>	<b>GDK</b>	808 m	39.820188°	44.596330°	The Iğdir Plain, abundant mosquito breeding habitat, intensive agricultural activities.
Zulfikarkoy	ZLK	848 m	39.994245°	44.145304°	
Surmeli	SRM	944 m	40.065487°	43.787455°	Transition zone between the Aras Valley and the Iğdir Plain, limited mosquito breeding habitats, intensive agricultural activities.
<b>Kotek</b>	<b>KTK</b>	1,350 m	40.253812°	42.943545°	
Cilehane	CLH	1,620 m	40.220713°	43.015578°	Kars Plateau, limited agricultural activities and limited mosquito breeding habitats.
<b>Kars</b>	<b>KRS</b>	1,768 m	40.592670°	43.077831°	
Karahamza	KRH	1,876 m	40.431349°	42.754236°	
Alisofu	ALS	2,070 m	40.342701°	42.682848°	
<b>Hamamli</b>	<b>HAM</b>	2,130 m	40.306989°	42.700422°	

The sampling stations from which samples were collected and used for SNP discovery are indicated in bold.

cm in some areas. According to the average climatic data of the 70 yr between 1935 and 2005 (Turkish State Meteorological Service [http://www.mgm.gov.tr/index.aspx]), the annual average temperature was 4.4°C; the highest temperature average was in July (17.3°C) and August (17.4°C) and the lowest temperature average was in January (-11.2°C). The annual rainfall is 489.5 mm.

The Aras River emerges in the vicinity of Erzurum Province in eastern Anatolia and flows into the Caspian Sea in Azerbaijan. The river forms part of the international boundaries of Turkey-Armenia, Iran-Nakhichevan (Azerbaijan), and Iran-Armenia. The Aras Valley is an important ecological corridor where desert fauna come into Anatolia. Suitable climate, poor drainage, high water table, and salinity combine to produce large populations of a number of mosquito species in the valley (Gündüz et al. 2009, Aldemir et al. 2010). Between 1990 and 2004, the annual average temperature, annual total rainfall, and annual average relative humidity in the area were 9.9°C, 250 mm, and 51.8%.

**Mosquito Collection and DNA Extraction.** Specimens were collected from cattle and sheep barns using mouth aspirators and New Jersey light traps (NJLTs) containing 40-watt light bulbs. On each trapping night, five to seven NJLTs were placed in each collection site. Traps were placed 1.5 m above ground and operated from 18:00–06:00. Specimens were collected and stored in individual microtubes and kept on dry ice. Specimens transported to the laboratory were identified using an existing taxonomic key (Schaffner et al. 2001). Whole mosquito tissue was homogenized using a Qiagen TissueLyser and genomic DNA extracted using a BioSprint96 DNA Blood Kit (Qiagen, CA).

**Sequencing.** Because, with the exception of the cytochrome oxidase one gene, there was no genetic data for *Cx. theileri*, we used polymerase chain reaction (PCR) primers developed for *Culex pipiens sp.* (L.) in the Vector Genetics Laboratory (PI: Dr. Anthony J. Cornel, University of California-Davis). In total, 35 primer pairs including 15 microsatellite markers were tested on *Cx. theileri* genomic DNA. Two primers (N1N-PDR and C3B-PDR) published in a previous study were used for amplifying the cytb gene (Esseghir et al. 1997).

Gene fragments successfully sequenced for this study include the putative genes; acetylcholine est-

terase 2 (*ACE-2*), *beta tubulin* (*BTUB*), cytochrome b (*cyt B*), esterase B1 (*ESTB-1*), fatty acid-binding protein (*FABP*), forkhead transcription factor (*FOXO*), heat shock protein 70 (*HSP70*), myosin light chain two (*MLC-2*), odorant receptor 10 (*ODR-10*), v-type ATP synthase B subunit (*VATPS-B*), and vitellogenin (*VIT*) (Table 2).

For amplification of the gene fragments (except cytb), each 50  $\mu$ l PCR reaction contained 0.5  $\mu$ M of each forward and reverse primer, 1 $\times$  PCR reaction buffer (Applied Biosystems, Foster City, CA), 1.5 mM MgCl<sub>2</sub>, 200  $\mu$ M dNTP mix, 1.25U AmpliTaq DNA polymerase, and 2  $\mu$ l of DNA template. Thermal cycling was performed using the following program: 5 min at 95°C; 35 cycles of 30 s at 95°C, 30 s at between 48 and 54°C (Table 1), 30 s at 72°C; followed by a final 5 min extension at 72°C. For each gene fragment, we optimized the PCR reaction by adjusting either the PCR mix and/or thermal cycling conditions for optimal amplification. Because the cytb gene fragment failed when using the same PCR mixture and cycling conditions used for the other gene fragments, we performed different conditions for this fragment. For amplification of the cytb gene fragment, the 50  $\mu$ l PCR reaction contained 0.4  $\mu$ M of each forward and reverse primer, 1 $\times$  PCR reaction buffer (Applied Biosystems), 2  $\mu$ M MgCl<sub>2</sub>, 200  $\mu$ M dNTP mix, 0.5 U AmpliTaq DNA polymerase and 3  $\mu$ l of DNA template. Thermal cycling was performed using the following program: 2 min at 94°C; 2 cycles for 30 s at 94°C, 30 s at 40°C, 1 min at 72°C; 30 cycles of 30 s at 94°C, 30 s at 45°C, 1 min at 72°C; followed by a final 3 min extension at 72°C. Amplicons were sequenced at the UC DNA Sequencing Facility (College of Biological Sciences, UC Davis) using an ABI 3730 Genetic Analyzer (Applied Biosystems, Carlsbad, CA).

**Sequence Analysis.** *ChromasLite* ver. 2.01 (Technelysium, Australia) was used to view chromatograms and convert chromatogram to text sequence. Sequences were aligned using BioEdit, version 7.0.5.3 (http://www.mbio.ncsu.edu/RNaseP/info/programs/BIOEDIT/bioedit.html). DnaSP version 5.10.01 was used for sequence analysis (Librado and Rozas 2009). Noncoding and coding regions were based on the gene annotation reported in the mosquito *Cx. pipiens quinquefasciatus* genome on Ensembl (http://metazoa.ensembl.org). The true reading frame in *Cx. theileri* may differ from these gene annotations. SNPs were first checked by visual inspection of alignments and

Table 2. Information on genes, PCR primers and conditions, Ensembl Gene ID and GenBank accession numbers used in this study

Gene	Abbr.	Primer sequences (5'-3')	Annealing temp	Super conlig	Coordinates	Ensembl gene ID	GenBank accession no.
Acetylcholine esterase 2 (exon 2)	ACE-2	F: TTGCACACTTCCAGGACGA R: GCCACGATTACCTTTCCAAAC	50°C	3.6	254683-258846	CPIJ000662	HQ893641-50
Beta tubulin (exon 1)	BTUB	F: CCGGACAACTTGTGTTCG R: TCTGGTACAGGGCTTCAATG	52°C	3.41	903520-907889	CPIJ003260	HQ893651-58 JN186294-99 HQ893625-40
Cytb (mitochondrial DNA)	CYTB	F: GAYYATTCAGGWGAATGATA R: GGTAYWTGCGTCGAWTTCGWTATGA	40°C	3.1381	14-4396	N/A	
Esterase BI precursor (exon 3-4)	ESTB-1	F: ACAGAAGGATGTGGTGTTCG R: CATGTGCTAGTGCACGGAAAC	50°C	3.512	34865-35143	CPIJ013918	HQ893659-68 JN186302-09
Fatty acid-binding protein (exon 1)	FABP	F: CGCATGACTACACTTCAAC R: AGCAAGCCAGCTACTGTC	54°C	3.1504	68695-72943	CPIJ018993	HQ893669-76 JN186310-19 HQ893677-92
Fork-head transcription factor (exon 1)	FOXO	F: CGGCTCAAGACCAACTTTC R: GAATACCGGAGTACATGTGG	54°C	3.2176	6094-10803	CPIJ020030	
Heat shock protein 70 (upstream + exon1)	HSP70	F: GCCTACCGACTGCAAGAA R: CCTAATCTCTGCAACGGTAGC	52°C	3.316	29607-34183	CPIJ011082	HQ893693-99 JN186320-25 HQ893713-22
Odorant receptor 10 (exon 2-4)	ODR-10	F: GATTTCTGCAACGGATCG R: AGATCTAGCTCACACGCCAGT	48°C	3.32	1489759-1494303	CPIJ002479	JN186338-45 HQ893703-12
Myosin light chain 2 (exon 2-3)	MLC-2	F: CAGAAGCATCGCCGAAT R: GTTGTCGAGGCGCTTGAAG	54°C	3.1999	32717-36881	CPIJ019398	JN186326-31 HQ893723-32
V type ATPase B subunit (exon 2)	VATPS-B	F: CGCTGGACAGAAATTCGCA R: CCTCAGCGTAAAGGAGCATG	54°C	3.585	129919-134286	CPIJ014700	JN186332-37 HQ893733-46
Vitellogenin (exon 1-3)	VIT	F: CTTCGATGCGCTTCTTGCAT R: GTCCATGCGCTTCTTGCAT	54°C	3.656	50494-54704	CPIJ015387	

chromatograms. Heterozygous positions were detected as multiple peaks at the same position in DNA sequence chromatograms.

Results

In total, 35 primer pairs including 15 microsatellite markers were tested on *Cx. theileri* genomic DNA and only 10 (28%) of these were successfully amplified. Combining all 11 fragments, we have sequenced 4,356 nucleotide base pair (bp) per individual *Cx. theileri* sample. The length of individual amplicons varied from 167 to 710 bp (Table 2). As amplification and/or sequencing of all 11 DNA fragments were not possible for all individuals studied, the number of individuals varied for different fragments (from a minimum number 14 to a maximum of 20; Table 3). We identified 96 SNPs, including 79 SNPs in the coding region (cSNPs) of the genes analyzed and 17 in noncoding regions. Our results yielded a SNP every 45 bp on average. We also observed that the abundance of SNPs varies considerably from one gene to another, ranging from none (BTUB) to 30 (ODR10). The distribution of SNPs among 11 genes are presented in Table 2.

The average GC content (the percentage of guanine and cytosine bases in an oligonucleotide sequence) observed in *Cx. theileri* is 52.48% and the GC content was significantly higher in coding regions (median = 54.30%) than noncoding regions (median = 31.17%, Mann-Whitney test,  $P = 3.119 \times 10^{-5}$ ; Table 2). We observed the lowest GC content in the mitochondrial cytb gene fragment (22.4%).

We estimated nucleotide diversity for both coding and noncoding regions of the genes studied (Table 3). The acetylcholinesterase two gene fragment contained no polymorphism and the beta tubulin gene fragment contained only one polymorphism. The odorant receptor 10 gene fragment was the most polymorphic ( $\pi = 0.01229$ ; Table 3).

For all SNPs observed, transition substitutions (C↔T and A↔G) were more common (67.94%) than transversions (31.25%; Fig. 2). Most common SNPs were C/T (40.62%) and A/G (28.13%). Transition substitutions are more prominent in coding regions where 51 were transitions (68.9%) and 23 transversions (32.06%).

Additionally, we found a higher frequency of SNPs occurring at the third codon position (49.36%) than at the first or second positions (Table 3), indicating that synonymous mutations were more common than non-synonymous mutations. Similar results have been observed for other mosquito species such as *Aedes aegypti* (L.) and *Anopheles funestus* Giles as well as three *Drosophila* species (Morlais and Severson 2003, Wondji et al., 2007, Moriyama and Powel, 1996). In a study of SNPs in *An. gambiae* immune signaling pathway genes it was found that synonymous SNPs that substitute common with rare codons are significantly associated with mosquito immunity against the malaria parasite, *Plasmodium falciparum* (Horton et al. 2010). We expect a subset of synonymous SNPs in *Cx. theileri*

Table 3. Summary of nucleotide polymorphism data

Gene	L	N	Coding region						Noncoding region						Nucleotide diversity				
			Transition			Transversion			Syn	Rep	Tr	Ts	Σ	%GCc	Eta	h	Hd	π	k
			First	Second	Third	First	Second	Third											
ACE-2	167	18	0	0	1	0	0	0	0	0	0	0	0	0	0.111	0.00067	0.11111		
BTUB	370	16	0	0	0	0	0	0	0	0	0	0	0	0.000	0.00000	0.00000			
CYTB	548	16	1	1	6	8	0	0	1	1	1	22.4%	9	0.950	0.00541	2.96670			
ESTB1	287	18	0	3	0	3	0	1	0	0	3	52.4%	7	0.562	0.00847	2.431			
FABP	249	20	2	0	2	0	1	0	1	1	59.8%	0	3	0.279	0.00156	0.38947			
FOXO	710	16	0	0	9	9	0	0	1	1	56.2%	10	0	0.858	0.00493	3.21667			
HSP70	577	16	7	2	0	9	9	2	0	0	53.6%	2	18	1.000	0.01116	6.442			
MLC-2	321	16	0	0	1	1	1	0	0	1	59.4%	1	3	0.775	0.00428	1.375			
ODR10	545	18	1	1	10	12	0	1	6	7	52.8%	16	3	0.974	0.01229	6.699			
VATPS	369	10	5	0	0	5	1	0	0	1	53.8%	0	6	0.892	0.00688	2.467			
VIT	213	14	0	1	3	4	0	0	1	1	54.3%	4	1	0.736	0.00729	1.47253			
Total	4,356		14	10	30	54	11	5	9	25	52.48%	44	35	0.649	0.005722	2.506407			
Average																			

L, amplicon length; N, no. of samples; Σ, total; Syn, synonymous substitutions; rep, replacement substitutions; Tr, transversions; Tv, transversions; %GCc, GC content in percent in coding regions; %GCnc, GC content percent in non-coding regions; Eta, total no. of mutations; h, no. of haplotypes; Hd, haplotype gene diversity; k, avg no. of nucleotide differences.

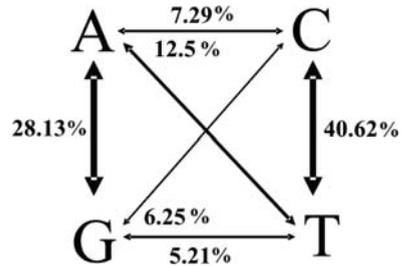


Fig. 2. Distribution of transitions and transversions among SNPs.

may result in functional changes in encoded proteins as well.

Polymorphisms in addition to SNPs were observed in this analysis. Five insertion/deletion polymorphisms (indels) were observed in four genes (FOXO, HSP70, VATPS, and VIT). Single base pair indels were observed in two gene fragments; insertion in the HSP70 (G/-) and deletion in VATPS (C/-) genes. A microsatellite of CT repeats and a 9 bp deletion (ACGATCTAC) were observed in the coding region of VIT. Some individuals were observed to have up to a 57 bp deletion in the FOXO gene.

Discussion

In this study, the average GC content observed in *Cx. theileri* is higher than the honey bee *Apis mellifera* with 32.7%, the mosquito species *Ae. aegypti* with 38.10%, *An. gambiae* with 44.30% and in the fruit fly *Drosophila melanogaster* with 42.30%, but lower than the congeneric mosquito species *Cx. pipiens* with 63.20%, suggesting that this mosquito genus is unusual in this regard (Samantha 2007). However, this difference could be incidental because our amplicons are disproportionately located in coding regions, which likely inflates the average GC content observed. The lowest GC content in the mitochondrial cytb gene fragment is consistent with the general pattern observed in previous studies where all mitochondrial genomes are GC poor, being lowest in insect and nematode genomes (range, 15–35%) (Saccone et al. 1999).

Because genes under strong selection are likely to show low levels of nucleotide polymorphism, low levels of nucleotide polymorphism that observed in the beta tubulin and acetylcholinesterase two fragments may indicate that these fragments are located in highly conserved domains (Moriyama and Powell 1996, Schmid and Tautz 1997, Morlais and Severson 2003, Wondji et al. 2007). In fact, beta tubulin sequence in *Cx. theileri* was 96–97% identical to that of *Cx. pipiens* complex (HQ881598-613) in California, and acetylcholinesterase two of *Cx. theileri* was 95% identical to that of *Cx. pipiens* complex (HQ881614-46) (Lee et al. 2012). Therefore, these two genes are fairly conserved within the genus *Culex*. Comparison of all the gene fragments sequenced in this study with the published *Cx. quinquefasciatus* genome revealed that BTUB,

ACE-2, FABP, FOXO, and VATPS gene fragments have >95% nucleotide identity, CYTB, VIT, ODR-10, HSP70, and MLC-2 have between 90–93% nucleotide identity and the ESTB1 has 83.85% nucleotide identity with *Cx. quinquefasciatus* (Say) ([http://metazoa.ensembl.org/Culex\\_quinquefasciatus/blastview](http://metazoa.ensembl.org/Culex_quinquefasciatus/blastview)).

The higher frequency of transition substitutions observed here is close to the frequency reported in the mosquito *An. funestus* (Wondji et al. 2007). The ratio of transitions/transversions observed ( $\approx 2:1$ ) is consistent with other organisms such as *Drosophila*, humans, and the mosquitoes *An. funestus* and *Ae. aegypti* (Moriyam and Powell 1996, Schmid and Tautz 1997, Morlais and Severson 2003, Wondji et al. 2007).

Similar results about a higher frequency of SNPs occurring at the third codon position than at the first or second positions have been observed for other mosquito species such as *Ae. aegypti* and *An. funestus* as well as three *Drosophila* species (Morlais and Severson 2003, Wondji et al., 2007, Moriyama and Powell, 1996). In a study of SNPs in *An. gambiae* immune signaling pathway genes it was found that synonymous SNPs that substitute common with rare codons are significantly associated with mosquito immunity against the malaria parasite, *Plasmodium falciparum* (Horton et al. 2010). We expect a subset of synonymous SNPs in *Cx. theileri* may result in functional changes in encoded proteins as well.

In this article we summarize the properties of SNPs recovered from the 11 genes with the aim of developing new molecular markers for *Cx. theileri*. We used these SNPs to determine if there are genetic differences between nine populations located along habitat-climate-elevation gradients in northeastern Turkey (Demirci et al. 2012). Genetic analyses showed that populations of *Cx. theileri* in this region are not genetically differentiated (Demirci et al. 2012). Failure to find genetic differentiation may to some extent relate to our sampling scheme, which was conservative with regard to our hypothesis. As mentioned above, we focused collection efforts on northeastern Turkey, because of the dramatic elevation changes over short distances in this region. Elevational changes provide a convenient surrogate for many variables, including temperature and land cover, which affect mosquito bioecology. Nevertheless, we considered collections from different altitudes as populations without considering other factors that can affect the degree of gene flow. Human transportation has been considered responsible for mosquito dispersal and genetic exchange (Pasteur et al. 1995, Lehmann et al. 1996, Failloux et al. 1997) and the intense human and animal transportation in the whole area (unpublished data) can be an important factor influencing gene flow in parallel with mosquito dispersal. In addition this failure may have been because of the short distances between populations and the absence of geographical barriers.

Although we did not find genetic differences among populations using these SNPs, we do believe that the development of an adequate genetic map of the *Cx. theileri* genome would allow researchers to choose

appropriate markers for a much needed future population genetics study of this species. According to molecular population genetic theory using SNP data, a minimum number of eight individuals per sample could capture most existing variation (Nei 1987). The distribution of genetics polymorphism within and among populations of *Cx. theileri* can provide insights into many ecological features including reproductive models and strategies, dispersal and population size, and structure (McCoy, 2008).

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