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Population structure and gene-flow among *Tetranychus urticae* populations collected from different geographic regions of Turkey

Türkiye'nin farklı coğrafi bölgelerinden toplanan *Tetranychus urticae* popülasyonlarındaki gen akışı ve popülasyon yapıları

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ABSTRACT

Tetranychus urticae Koch (Acari: Tetranychidae) is a devastating agricultural pest that can feed on more than 1000 host plants. This extremely polyphagous nature of this pest may allow random disperse of them. Although population movement and structure are of vital importance to design area-wide pest control programs, there is no such study focusing on this issue in Turkey. The present study showed that there was no genetic subdivision among *T. urticae* the populations collected from four geographic regions of Turkey (FST=0.090, p>0.05), based on *cytochrome c oxidase subunit I* (*COI*). In addition, the haplotype network supported these results since no clustering pattern was present. However, Black Sea populations had high genetic differentiation with other populations. This might be due to its isolated geography, different climate conditions, and limited sampling area. A high level of gene-flow between the Mediterranean and Aegean/Central Anatolian populations was determined. It is known that geography alone is not enough to explain population structure and genetic variation when excluding other ecological factors. Therefore, other factors such as current and historical climate data should be integrated to assess gene-flow in future studies.

INTRODUCTION

Turkey is geographically divided into seven regions, and each of them is of great importance for agricultural production. These zones normally limit the dispersal activity of agricultural pests due to their different climatic features, geographical structure, and plant diversity (Bebber et al. 2014, Mazzi and Dorn 2012). However, the knowledge is quite limited if this hypothesis is still valid for extremely polyphagous pests such as *Tetranychus urticae* Koch (Acari: Tetranychidae), considering the abundance of host plants.

T. urticae, the two-spotted spider mite, is a harmful agricultural pest that can feed on more than 1100 host plants that contribute to its worldwide dispersal (Migeon and Dorkeld 2021, Van Leeuwen et al. 2010). Although there are many studies about biological and chemical control of this pest (Attia et al. 2013, Van Leeuwen et al. 2009) as well as the potential of local entomopathogenic fungus isolates (Yucel 2021), there is limited information on its movement preference during dispersal and consequent gene-flow among populations in certain geographical areas.

T. urticae can spread via active and passive mechanisms (Hussey and Parr 1963, Kennedy and Smitley 1985). Active mechanisms such as moving often cause short-distance dispersal (Hussey and Parr 1963), on the other hand, passive dispersal by wind may result in the long-distance spread of spider mites (Osakabe et al. 2008). A better understanding of the dispersal ability of *T. urticae* populations across different geographic regions may contribute to pest control (Stinner et al. 1983).

The mitochondrial *cytochrome c oxidase subunit I* (*COI*) has been used in many studies to uncover relationships between and within spider mites including phylogeographic patterns (Ros and Breeuwer 2007). In this study, COI sequences belonging to *T. urticae* populations collected from four different geographic regions of Turkey were used to assess gene -flow and genetic differentiation between and within the regions. Additionally, COI haplotypes were determined and network analysis was performed to further elucidate the population structures.

MATERIALS AND METHODS

Tetranychus urticae populations

T. urticae populations were collected from three different geographic regions of Turkey: Black Sea (BS), Aegean (A), and Central Anatolia (CA) (Table 1). Mites were transferred into 70% and 90% alcohol for morphological and molecular identification, respectively. Prior to molecular analysis, morphological identification was performed using Hoyer's medium for permanent slides (Zhang 2003).

All the sequences obtained in this study were submitted to NCBI GenBank (accession numbers MZ824594-MZ824619). Besides the sequences herein obtained, some additional sequences, belonging to *T. urticae* populations in the Mediterranean (M) and CA region, from NCBI GenBank were used for further analyses (Table 1).

Genomic DNA extraction and gene amplification

Total DNA was extracted from pools of 10 adult female mites using Qiagen DNeasy Blood & Tissue Kit following the manufacturer's instructions. DNA extracts were stored at −20°C until further process. After the final washing step, genomic DNA was eluted with 100 µl of elution buffer per sample. The quality and purity of DNA were checked by agarose gel electrophoresis (1.5%) and UV spectrophotometer (Thermo Scientific NanoDrop 2000).

A partial fragment of COI gene was amplified by PCR using the following primers: 5'-TGATTTTTTGGTCACCCAGAAG-3'and 5'-TACAGCTCCTATAGATAAAAC-3' (Navajas et al. 1994). PCR conditions were as follows: 3 min at 95°C, 40 cycles of 30 s at 95°C, 30 s at 48°C and 60 s at 72°C, and a final extension of 7 min at 72 °C.

The PCR reaction was performed in a total volume of 30 µl, containing 5 µl of mite DNA, 0.5 µl of each primer, 18 µl of PCR grade water, and 6 µl of FIREPol Master Mix (Solis Biodyne, Estonia). Purification of PCR products was conducted using HighPrep PCR clean-up system (MagBio Genomics Inc.), according to the supplier's protocol and subsequent sequencing of amplicons was performed at Macrogen Inc. (Seoul, South Korea).

Population structure and gene-flow analyses

The number of haplotypes, haplotype diversity, nucleotide diversity was calculated using DnaSP v6.12.03 (Rozas et al. 2017) and PopArt v1.7 (Leigh and Bryant 2015) was used Analysis of Molecular Variance (AMOVA). Analyses of genetic differentiation and gene flow were assessed using DnaSP v6.12.03 (Rozas et al. 2017).

Haplotype network analysis

All sequences were aligned using MAFFT v.7 with default settings (Katoh et al. 2019). DnaSP v6.12.03 (Rozas et al. 2017) was used to generate haplotype data, and the haplotype network was constructed from 337 base pair (bp) long alignment using PopArt v1.7 (Leigh and Bryant 2015).

Table 1. Collection site and accession numbers of *Tetranychus urticae* populations

Region	City	Accession Numbers	Reference
Black Sea	Zonguldak	MZ824606-MZ824613	This study
Aegean	Aydin	MZ824614-MZ824619	This study
Central Anatolia	Ankara, Aksaray, Nevsehir, Konya, Karaman, Eskisehir	MZ824594-MZ824605; MW542505-MW542515	This study; İnak 2021
Mediterranean	Antalya, Mersin	MK508712-MK508722	İnak et al. 2019

Table 2. Number of samples, segregating sites, haplotypes, haplotype diversity, and nucleotide diversity among Tetranychus urticae populations from different geographic regions

Region	N	Segregating sites	Number of haplotypes (h)	Haplotype diversity	Nucleotide diversity (π)
Central Anatolia	22	31	7	0.74	0.032
Black Sea	8	4	4	0.75	0.004
Mediterranean	8	26	7	0.96	0.035
Aegean	6	15	5	0.93	0.025
Total	44	44	19	0.88	0.030

Table 3. F_{ST} and Nm values between *Tetranychus urticae* populations

CA*	-7.13	4,5	0,28	
BS	0,27	0,57		0,47
M	6,74		0,31	0,05
A		0,04	0,48	-0.03
	A	M	BS	CA

*CA: Central Anatolia, BS: Black Sea, M: Mediterranean, A: Aegean; FST values in the lower matrix. Nm values in the upper matrix.

RESULTS

Population structure

After alignment, a total of 337 bp COI fragment was used for further analyses. All obtained results related to haplotypes and nucleotides are presented in Table 2.

Overall, 293 out of 337 bp was conserved, and nucleotide diversity (π) was 0.030 (=3% genetic variation) among all sequences. Nucleotide diversity varied from 0.025 to 0.035 for various regions, except sequences belonging to the Black Sea region that has the lowest nucleotide diversity (0.004).

A total of nineteen different haplotypes were found, and the haplotypes' diversity values ranged from 0.74 to 0.96 based on a partial fragment of mitochondrial COI sequences. The diversity of haplotypes were the highest in Mediterranean populations; on the other hand, sequences belonging to Central Anatolia had the lowest haplotype diversity.

Genetic differentiation (F_{ST}) and gene-flow (Nm) between populations

The highest genetic differentiation was between the Black Sea and Aegean sequences (0.48), followed by Black Sea and Central Anatolia, in line with low Nm values between these regions that are 0.27 and 0.28, respectively (Table 3).

There was no genetic subdivision between the populations from Central Anatolia and Aegean regions, based on FST value. Mediterranean populations had also very low genetic differentiation with Aegean and Central Anatolia populations.

Haplotype network analysis and AMOVA

A total of 19 COI haplotypes were determined in *T. urticae* populations across Turkey. However, network analysis showed

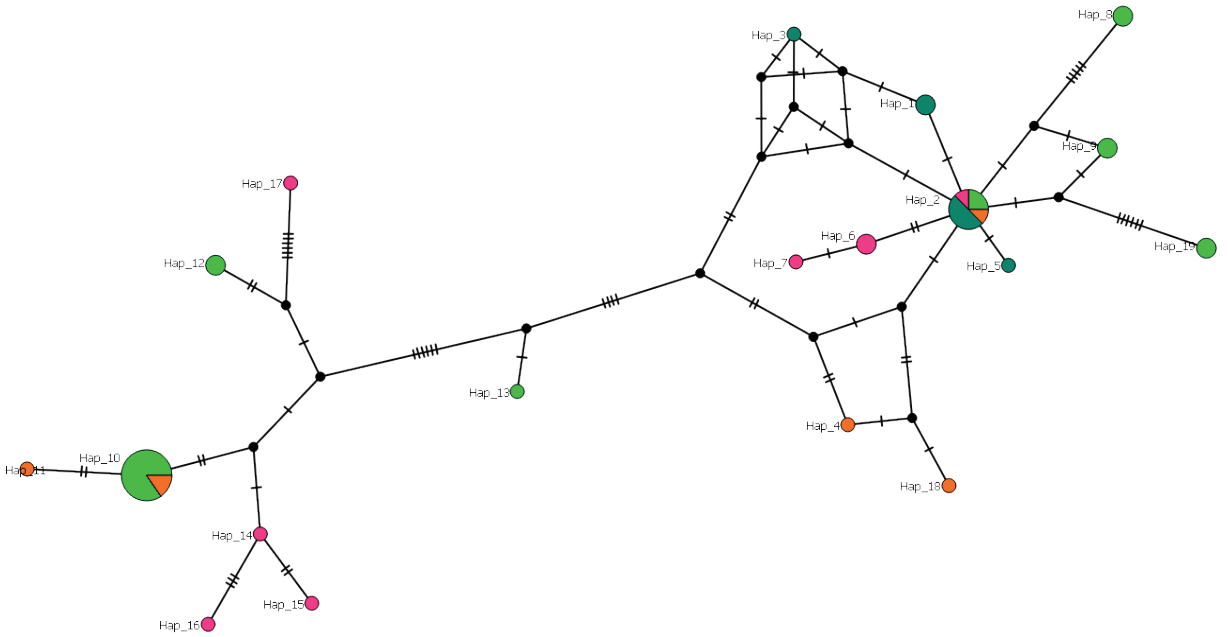

Figure 1. Haplotype network analysis of *Tetranychus urticae* populations from four geographic regions of Turkey. The sizes of the circles are proportional to haplotype frequency

that there is no clustering pattern for a certain region (Figure 1). Hap_10 and Hap_2 were the most frequent haplotypes that mostly included CA and A populations, respectively.

Results of AMOVA analysis among and within populations are given in Table 4. AMOVA analysis showed that the fixation index (FST) among all populations was 0.090. Molecular variance among populations was 9.09%, while 90.90% variation was determined within the population (Table 4).

Table 4. Analysis of molecular variance (AMOVA) and degrees of freedom (df) of among and within *Tetranychus urticae* populations

Variation	df	Sum of squares	Sigma2	%variation
Among populations	3	522.170	7.057	9.09174
Within populations	40	2822.375	70.559	90.90826
Total	43	3344.545	77.616	

DISCUSSION

Tetranychus urticae is an extremely polyphagous agricultural pest throughout the world (Van Leeuwen et al. 2010). Although population movement and structure are of vital importance to design area-wide pest control, there is no such study focusing on this issue in Turkey. To date, geographic distance and host plant adaptation has been associated with genetic differentiation and similarity, respectively, in *T. urticae* populations (Tsagkarakou et al. 1998, Tsagkarakou et al. 1999 Uesugi et al. 2009). In addition, since many parameters affecting geographic structure could be unique for the regions, studies should be performed with a special focus on target regions to investigate genetic differentiation and gene-flow among pest populations. In this study, genetic variation and movement of *T. urticae* populations collected from different geographic regions of Turkey were investigated.

Overall, 293 out of 337 nucleotides (86.9%) were conserved among all *COI* sequences and a total of 19 haplotypes were determined using 44 sequences, showing high haplotype diversity in Turkish T. urticae populations. In addition, AMOVA analysis showed 9% and 90.9% variation among and within populations, respectively. Low variation among populations showed the absence of subdivision among populations. The fixation index (F_{ST}) of all populations was not significant (p=0.065), indicating low genetic differentiation again. These results were also supported by haplotype network analysis which showed that no correlation existed between population diversity and geographic regions (Figure 1).

Estimation of FST value (Fixation index = Φ_{st}) that can range from 0 to 1, is the most frequent method to assess differentiation among populations since its first development in 1965 (Wright 1965). A higher degree of genetic differentiation among populations leads to a higher F_{ST} value (Meirmans 2006). On the contrary, a negative F_{ST} value has been considered equal to zero, indicating no population structure (Meirmans 2006). Wright (1978) suggested that FST values ranging from 0.05 and 0.15 indicate moderate genetic differentiation between pairs of populations. On the other hand, Nm (=number of migrants) estimates the gene-flow between populations (Whitlock and Mccauley 1999). A higher Nm value means higher gene migration, and when Nm>4, the presence of very high-level migration can be assumed (Beals et al. 2000).

The nucleotide diversity of the populations from various regions was close to each other, except the ones from the Black Sea region having very low diversity (0.004). This might be due to its isolated geography, different climate conditions, and limited sampling area. In line with this, a high level of genetic differentiation and low level of migration between BS and other regions have been determined based on F_{ST} and Nm values, In spite of having isolated geography, nucleotide and haplotype diversity were the highest in the Mediterranean region. It is known that geography alone is not enough to explain population structure and genetic variation when excluding other ecological factors (Jin et al. 2020). Therefore, other factors such as current and historical climate data should be integrated to assess gene-flow in future studies. In addition, human activities such as the importation of ornamental plants might cause the introduction of plant pests together with them and contribute to the increased genetic variation in the Mediterranean region.

Populations from the Aegean region showed low genetic differentiation with other populations (except BS). In addition, moderate differentiation between Mediterranean and Central Anatolian populations has been detected that might be explained by substantial differences in their climate regimes. However, since geographic regions do not match with the regions for climate regimes of Turkey (İyigün et al. 2013), more detailed studies considering climates regime zones, rather than geographic regions, are needed to get a better understanding of climate-migration relationships between *T. urticae* populations.

In conclusion, *T. urticae* populations collected from four different geographic regions showed low genetic differentiation and high gene-flow between each other, with the exception of Black Sea populations. However, more samplings from wider areas should be obtained in future studies. In addition, other ecological factors should be integrated to reveal gene-flow among T. urticae populations across the country.

ÖZET

Tetranychus urticae Koch (Acari: Tetranychidae) 1000'den fazla konukçu bitkiden beslenebilen tahrip edici bir tarımsal zararlıdır. Bu aşırı polifag doğası, bu zararlının rastgele dağılmasına olanak sağlayabilmektedir. Popülasyon hareketi ve yapısı geniş alanlarda zararlı kontrolü programları dizayn edilmesinde çok önemli olmasına rağmen, Türkiye'de bu konuda gerçekleştirilmiş bir çalışma bulunmamaktadır. Bu çalışmada, farklı coğrafik bölgelerden toplanan *T. urticae* popülasyonları arasında *sitokrom oksidaz c altünite I* (*COI*) genine dayanarak genetik alt bölünme olmadığını göstermektedir (F_{ST}=0.090, p>0.05). Ayrıca, haplotip network ağ analizinde kümelenme yapısı olmaması bu sonucu desteklemektedir. Ancak, Karadeniz popülasyonlarının diğer popülasyonlar ile yüksek genetik farklılığa sahip olduğu gösterilmiştir. Bu durum, bölgenin sahip olduğu izole coğrafyasından, farklı iklim koşullarından ve örnekleme yapılan alanın sınırlı olmasından dolayı olabilir. Akdeniz Bölgesi popülasyonları ile Ege ve İç Anadolu Bölgesi popülasyonları arasında yüksek gen akışı belirlenmiştir. Coğrafyanın tek başına popülasyon yapısı ve genetik varyasyonu açıklamada yeterli olmadığı bilinmektedir. Bu nedenle, güncel ve tarihsel iklim verileri gibi diğer faktörler ileri gen akışı çalışmalarında birleştirilmelidir.

Anahtar kelimeler: kırmızı örümcekler, genetik farklılaşma, *sitokrom oksidaz c altünite I*, haplotip ağ

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