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Intraspecific genetic diversity of the oak gall wasp *Andricus lucidus* (Hymenoptera: Cynipidae) populations in Anatolia

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Abstract: Intraspecific genetic diversity and phylogenetic relationships of *Andricus lucidus* haplotypes from Turkey were studied by PCR-RFLP analysis. A total of 26 haplotypes were detected among 144 individuals collected from 9 populations. The estimated average haplotype and nucleotide diversity within populations were 0.8089 and 0.115542, respectively. Nucleotide divergence estimates among the analyzed oak gall wasp populations ranged between 0.012% and 7.3%. Dendrograms indicated that there was a relationship between the geographical distribution of haplotypes and their clustering. AMOVA analysis for estimation of the partitioning of genetic differentiation at all different hierarchical levels was statistically significant. Analysis of variance revealed that the highest genetic variance (61.21%) was present within populations and a significant partitioning of variance (26.85%) was found among groups. Overall, the present study indicates that the oak gall wasp haplotypes found in different populations from Turkey have a significant amount of genetic diversity and form geographically significant groupings.

Key words: *Andricus lucidus*, gall wasp, mitochondrial DNA, PCR-RFLP, phylogeography, Turkey

Meşe mazı arısı *Andricus lucidus* (Hymenoptera: Cynipidae) populasyonlarının Anadolu'daki türiçi genetik çeşitliliği

Özet: Ülkemizde bulunan *Andricus lucidus*'un tür içi genetik çeşitliliği ve filogenisi PCR-RFLP yöntemi kullanılarak çalışılmıştır. Toplam 9 populasyondan 144 bireyde 26 haplotip belirlenmiştir. Çalışılan populasyonlarda haplotip ve nükleotid çeşitliliği 0,8089 ve 0,11542 olarak hesaplanmıştır. Mazı arısı populasyonlarındaki nükleotid farklılaşması ise % 0,012 ile % 7,3 arasında değişmektedir. Elde edilen dendrogramlar haplotiplerin coğrafik dağılımı ile bunların gruplanmaları arasında anlamlı bir ilişkinin olduğunu göstermiştir. AMOVA kullanılarak elde edilen sonuçlar genetik çeşitliliğin dağılımının istatistiksel olarak önemli olduğunu göstermektedir. Bu analizler genetik varyansın en fazla populasyonlar içinde (% 61,21) ve sonra da gruplar arasında (% 26,85) dağıldığını ortaya koymuştur. Bu çalışma ülkemizde bulunan farklı populasyonlardaki meşe mazı arısı haplotiplerinin oldukça fazla bir genetik çeşitliliğe sahip olduğunu ve anlamlı coğrafik gruplar oluşturduğunu ortaya koymuştur.

Anahtar sözcükler: *Andricus lucidus*, mazı arısı, mitokondriyal DNA, PCR-RFLP, filocoğrafya, Türkiye

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Introduction

Recent advances in molecular biology and mitochondrial phylogeography have provided important insights into different fields such as population genetics, taxonomy, phylogeny, and evolution (Avise, 1994). Genetic data obtained using molecular techniques allow us to infer geographic structure by revealing relationships among genotypes from different populations relative to their geographic location (Avise, 2000). Apart from this, mitochondrial DNA (mtDNA) has proven to be a powerful tool for the assessment of phylogeographic patterns due to a lack of recombination and a relatively rapid rate of evolution compared to that of nuclear genomes. Its mode of inheritance provides information for identification of maternal lineages and allows inferences about time, since its divergence is from a common ancestor (Harrison, 1989). Thus, it has been extensively used for investigating population structure and assessing the influence of historical events on the divergence and distribution of current populations of insects (Chapco et al., 1992). The application of mtDNA variation across a species range has also been used to reconstruct phylogenies and to deduce the evolutionary origins and history of populations of oak gall wasps (Rokas et al., 2003; Stone et al., 2007).

Andricus lucidus (Hartig, 1843) is a gall wasp species with the ability to induce multichambered galls on the shoots of oaks (Atkinson et al., 2002). Parthenogenetic generation of *A. lucidus* can be found most commonly on *Quercus infectoria* Oliv. Other oak species are also preferred, including *Q. petraea* (Matt.) Liebl., *Q. pubescens* Willd., *Q. robur* L., and *Quercus infectoria*, which are found in almost all woodlands in Turkey; however, they grow most commonly in southeastern Anatolia (Eroğlu, 2000). As an obligate parasite of the white oak species and despite its large range in the eastern and southeastern parts of Turkey, very little is known about the genetic population structure of *A. lucidus* at a molecular level in Turkey.

Turkey is known to play a significant role as a corridor to allow the dispersal of many animal taxa from eastern to western and from northern to southern parts of the region (Kosswig, 1955). Anatolia is accepted as a nonhomogenous refugium allowing many species to use it as a shelter area to escape from the analogous effects of both glacial and interglacial cycles of the Quaternary periods (Çıplak, 2008). The presence of a diverse range of micro- and macrohabitats in Anatolia associated with varied topography makes Turkey a remarkable area for many endemic species and a hotspot for speciation, especially for insects (Çıplak et al., 1996). Furthermore, Turkey and more eastern parts, important for the Irano-Turanian center of endemism, are considered to be the center of diversification of oak gall wasp species (Stone et al., 2009).

In the present study, PCR-RFLP analysis of 2 mtDNA regions of *A. lucidus* collected from southeastern and eastern Turkey, which is the most mountainous part of Anatolia, was employed as a tool for assessing the levels of genetic diversity of the species across the sampled populations, and some implications for the current population structure and the effects of the varied topography of Anatolia in shaping the current genetic structuring on the studied species are discussed.

Materials and methods

Sampling and laboratory protocols

In total, 144 individuals from 9 populations of *A. lucidus* were collected between 2006 and 2008, covering the known distribution range of the species from the eastern and southeastern parts of Turkey. The locations of collection sites and sample size are shown in Figure 1 and Tables 1 and 2, respectively. After rearing the specimens in jars in the laboratory, they were stored at –80 °C until DNA isolation. Total genomic DNA was extracted from a single individual from each gall with the DNeasy Tissue Kit (QIAGEN). Extracted DNA was checked using 0.8% agarose gel electrophoresis, then diluted to the appropriate concentration for PCR amplification.

For PCR-RFLP analysis, 2 mtDNA regions were used: a 2540-base pair (bp) mitochondrial DNA region including ND4, ND6, and Cyt B genes, and a second 1800-bp fragment comprising the ATPases (6, 8) and COIII. Primer sequences for both mtDNA fragments were as follows: CBN 5'-ATTACACCTCCTAATTTATTAGGAAT-3', ND4 5'-GGAGCTTCAACATGAGCCT-3' (Simon et al., 1994)

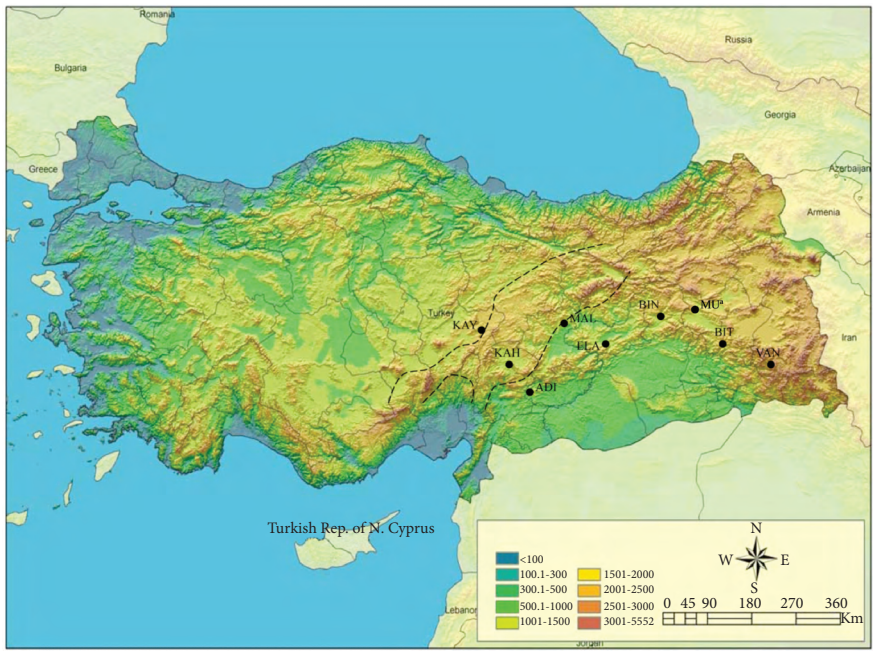

Figure 1. Location of sampling sites for *Andricus lucidus*. Abbreviations of the locations are given in Table 1.

Table 1. Localities of sampled populations of *Andricus lucidus* with their abbreviations and coordinates.

Population	**Locality**	**Coordinates of Localities**
1. Adıyaman (ADI)	Gölbaşı	37°34.568′N, 37°29.213′E
2. Bingöl (BIN)	Muş Road	38°57.407′N, 40°11.432′E
3. Bitlis (BIT)	Baykan	38°21.019′N, 42°02.412′E
4. Elazığ (ELA)	Poyraz Village	38°42.720′N, 39°55.515′E
5. Kayseri (KAY)	Pınarbaşı	38°40.512′N, 36°19.973′E
6. Kahramanmaraş (KAH)	Göksun	37°43.514′N, 36°40.038′E
7. Malatya (MAL)	Hekimhan	38°42.144′N, 38°06.992′E
8. Muş (MUŞ)	Günkırılı Village	38°36.793′N, 41°56.529′E
9.Van (VAN)	Çatak	37°55.015′N, 42°57.828′E

Table 2. Composite haplotypes and their frequencies among 9 *A. lucidus* populations (see Table 1 and Figure 1). Composite haplotypes based on digestion pattern of the used restriction enzymes shown by capital letters are given in the following order: ND4, ND6, Cyt B: HinfI, ClaI, HindII, MboII; ATPases, COIII: HinfI, EcoRI, HindII, HaeIII.

Haplotype	Composite Haplotype	Adıyaman	Bingöl	Bitlis	Elazığ	Kayseri	K.maraş	Malatya	Muş	Van	Total
Type 1	**AAAAAAAA**	5	4	3	4		4	2	3	2	**27**
Type 2	**AABAAABA**	4					2				**6**
Type 3	**BABAAAAA**	7					1				**8**
Type 4	**AAAAAABA**		6						3		**9**
Type 5	**AABAAAAA**		5	2	2				3		**12**
Type 6	**BAAAAABA**		3								**3**
Type 7	**AAAADAAA**			3							**3**
Type 8	**AABADAAA**			3							**3**
Type 9	**AACADAAA**			2							**2**
Type 10	**AABABAAA**				3						**3**
Type 11	**BAAACAAA**		4		3						**7**
Type 12	**AACAAAAA**				4						**4**
Type 13	**AAAABAAA**					12		2			**14**
Type 14	**AADCBAAA**					8					**8**
Type 15	**BACBBAAA**					2					**2**
Type 16	**BABBBAAA**					4					**4**
Type 17	**BACCAAAA**						1				**1**
Type 18	**BABBAAAA**						2				**2**
Type 19	**BABCAAAA**						3				**3**
Type 20	**BAABBAAA**	2						3			**5**
Type 21	**AADBFAAA**							2			**2**
Type 22	**BACBAAAA**								1		**1**
Type 23	**AAAAEAAA**									6	**6**
Type 24	**AACAEAAA**			2						3	**5**
Type 25	**AADBEAAA**									3	**3**
Type 26	**BADCEAAA**								1		**1**
Sample Size		**18**	**22**	**15**	**16**	**26**	**13**	**9**	**11**	**14**	**144**

and mt19 5'-GAAATTTGTGGAGCAAATCATAG-3', mt22 5'-TCAACAAAGTGTCAGTATCA-3' (Moretto and Arias, 2005). Amplification reactions were carried out in 25 µL volumes containing 0.5 µL of the total DNA extraction, 2.5 µL of 10× PCR buffer (Sigma), 2.0 µL of $MgCl_2$ (25 mM), 1.0 µL of dNTPs (2 mM each), 0.75 µL of each primer (20 µM), and 1.25 U of Taq DNA polymerase (Promega). Amplifications of both regions were performed under the following conditions: 5 min at 94 °C, 35 cycles of 1 min at 94 °C, 80 s at 44 °C, 2 min at 64 °C, and a final extension of 10 min at 64 °C. PCR products were visualized on 1% agarose gel buffered with Tris-Boric acid-EDTA (TBE), stained with ethidium bromide, and visualized under UV light.

The amplified ND4, ND6, and Cyt B fragments were digested with the restriction endonucleases HinfI, ClaI, HindII, and MboII (MBI Fermentas and TAKARA), and ATPases (6, 8) and COIII segments were digested with HinfI, EcoRI, HindII, and HaeIII (TAKARA), respectively. The restriction enzymes employed for RFLP analysis in this study

were previously used with other insects (Moretto and Arias, 2005). Restriction digestion was performed overnight under conditions specified by enzyme manufacturers. The digested fragments were separated by electrophoresis in 1% agarose gel containing ethidium bromide. The sizes of the DNA fragments were compared to the PCR marker run on the same gel.

Data analysis

For both mtDNA fragments, variable restriction patterns for each enzyme were alphabetically designated as they were encountered. The presence or absence of restriction sites were inferred for each enzyme from completely additive fragment patterns and each individual insect was designated a composite haplotype based on the observed RFLP patterns. The composite haplotype data and the restriction site matrix were used for a number of analyses, using the REAP software package (McElroy et al., 1991). Haplotype and nucleotide diversity within each population and divergence among populations were estimated according to the methods of Nei and Tajima (1981) using the "DA" programs contained in the REAP program. From the basic matrix of presence-absence of restriction sites for each haplotype generated by the program REAP GENERATE, the data were bootstrapped with 1000 replicates using the PHYLIP SEQBOOT program (Felsenstein, 1992). Dollo parsimony trees were constructed using the PHYLIP DOLLOP program. The CONSENSE program in the same program package was employed to summarize all of the most parsimonious topologies in a majority-rule consensus tree. The average number of nucleotide substitutions per site between haplotypes was used to obtain a neighbor-joining tree using the NEIGHBOR program present under PHYLIP. The degree of geographic heterogeneity of mtDNA haplotype distributions was assessed using χ^2 statistics (Roff and Bentzen, 1989). The significance level was obtained by 10,000 Monte Carlo randomizations using the Monte routine from the REAP package.

Genetic differentiation among populations was quantified by analysis of molecular variance (AMOVA), implemented by the program ARLEQUIN 3.1 (Excoffier et al., 2005). AMOVA is an analysis of variance procedure that partitions molecular variance according to sampling design, and it calculates genetic distances based on pair-wise F_{ST} indices between all pairs of populations (Excoffier et al., 1992). The significance of the F_{ST} statistics was tested for significance using 1000 permutations. Variance components were analyzed at 3 levels based on the geographic locations of the populations and tested between groups, among populations within groups, and within populations (Excoffier et al., 2005).

Results

mtDNA variation within and among populations

Polymorphisms within the ND4/ND6/CytB and ATPases (6, 8)/COIII gene regions were revealed using 6 different restriction enzymes, and a total of 26 composite haplotypes were identified. The size of the amplified fragments of the ND4/ND6/CytB and ATPases (6, 8)/COIII of mtDNA were 2.5 kb and 1.8 kb in length, respectively. The mtDNA regions from 144 individuals had a total of 37 cut sites for the enzymes employed in this study, representing a total of an estimated 210 nucleotides. The composite haplotypes and their frequencies in each population are given in Table 2. The most common haplotype (Type 1) was present in 8 populations among the 9 studied populations. Based on frequency of haplotypes, Type 13 was detected in 14 individuals from 2 populations; however, haplotype 5 was found in 12 individuals from 4 populations. Among the 26 detected haplotypes, 3 were private (Type 17, 22, and 26), that is to say, observed in only 1 individual. Based on the haplotype numbers observed in the 9 examined populations, Kahramanmaraş and Bitlis had 6 haplotypes; Bingöl, Elazığ, and Muş had 5 haplotypes; and only 4 haplotypes were detected in each remaining population.

The haplotype and nucleotide diversity of each *A. lucidus* population, calculated using the REAP program, are given in Table 3. The average haplotype diversity for the studied populations was 0.8089. Among the 9 analyzed populations, the highest haplotype diversity (0.8590) was estimated for the Kahramanmaraş and Bitlis populations, followed by the Bingöl population (0.8514). On the other hand, the lowest haplotype diversity was calculated for

Table 3. Mean ± SE for haplotype and nucleotide diversity for the *A. lucidus* populations.

Population	Haplotype Diversity	Nucleotide Diversity
1. Adıyaman	0.7516 ± 0.05574	0.078927
2. Bingöl	0.8514 ± 0.03094	0.162035
3. Bitlis	0.8590 ± 0.04961	0.079268
4. Elazığ	0.8417 ± 0.04276	0.087912
5. Kayseri	0.6892 ± 0.05757	0.066764
6. Kahramanmaraş	0.8590 ± 0.06326	0.152323
7. Malatya	0.8333 ± 0.08001	0.109356
8. Muş	0.8364 ± 0.07016	0.154532
9. Van	0.7582 ± 0.07691	0.147947
Average	0.8089 ± 0.00041	0.115542 ± 0.0001656

the Kayseri population (0.6892). Overall, haplotype diversity was significantly high for the studied populations. The average nucleotide diversity for the analyzed populations was 0.115542. Within-population nucleotide diversity was lower than the interpopulation nucleotide diversity (0.145371). Among the 9 examined *A. lucidus* populations, the highest nucleotide diversity (0.162035) was observed in the Bingöl population, followed by the Muş (0.154532) and Kahramanmaraş (0.152323) populations, respectively. The lowest nucleotide diversity (0.066764) was found in the Kayseri population.

Phylogenetic relationships among haplotypes

The nucleotide divergence between the populations calculated using the DA program of REAP ranged from 0.012% to 7.3% (Table 4). Based on pair-wise comparisons of nucleotide divergence of *A. lucidus* populations, the Kayseri and Bitlis

Table 4. Pair-wise nucleotide diversity (above diagonal) and net nucleotide divergence (below diagonal) among the populations of *A. lucidus*.

	Adıyaman	Bingöl	Bitlis	Elazığ	Kayseri	K.maraş	Malatya	Muş	Van
Adıyaman		0.150379	0.119311	0.133513	0.116818	0.122211	0.092601	0.158332	0.169423
Bingöl	0.029898		0.135746	0.141164	0.137392	0.163253	0.152323	0.154078	0.164467
Bitlis	0.040213	0.015095		0.119725	0.146156	0.155661	0.154876	0.136733	0.172488
Elazığ	0.050094	0.016191	0.036135		0.099004	0.161685	0.162482	0.157053	0.157507
Kayseri	0.043973	0.022993	0.073141	0.021665		0.128952	0.129989	0.151749	0.160087
K.maraş	0.006586	0.006074	0.039864	0.041567	0.019408		0.11863	0.166637	0.159389
Malatya	0.001541	0.016628	0.060565	0.063848	0.041929	0.001221		0.155628	0.159832
Muş	0.041603	0.004206	0.019833	0.035831	0.041101	0.013209	0.023684		0.168087
Van	0.055986	0.009476	0.058881	0.039578	0.052731	0.009254	0.031181	0.016847	

populations are the most diverged populations (7.3%), while the Malatya and Kahramanmaraş populations are the least diverged (0.012%). A neighbor-joining tree resulting from the distance matrix among all mtDNA haplotypes is presented in Figure 2 with associated bootstrap values (>50 are shown on the branches). As shown, haplotypes form 2 main clades. The first clade is composed of 2 clusters, of which the small cluster is formed by the haplotypes 2, 18, and 19, representing the Adıyaman and Kahramanmaraş populations. The second, larger cluster includes 2 groupings: the first contains the most widely distributed haplotype (Type 1), found in 8 populations among all the studied populations at the basal position of the group, including haplotypes from the Bingöl, Bitlis, Elazığ, Muş, Adıyaman, and Malatya populations. Haplotypes from Adıyaman, Kahramanmaraş, Kayseri, and Malatya are placed in the next group. The second main clade in the dendrogram is composed of haplotypes 4, 6, 7, 8, 9, 11, 22, 23, 24, 25, and 26 from the Van, Bingöl, Bitlis, Muş, and Elazığ populations; the absence of Adıyaman, Kahramanmaraş, Kayseri, and Malatya is striking and implies a geographic grouping of some haplotypes in the haplotype tree.

A haplotype data matrix based on percent sequence divergence was used to reconstruct a UPGMA dendrogram of the 9 *A. lucidus* populations (Figure 3). In the dendrogram, 2 main clusters are formed by the haplotypes observed in the studied populations. Haplotypes found in the Adıyaman and Kahramanmaraş localities are placed at the basal position of the first cluster. The most common haplotype (Type 1), shared among 8 populations, the second most common haplotype (Type 5), shared among the Bingöl, Bitlis, Elazığ, and Muş populations, and other haplotypes from Adıyaman and Malatya are placed in this cluster. On the other hand, 2 haplotypes found in Elazığ formed a subcluster of the larger cluster. Moreover, a distinct grouping was formed by the haplotypes found in the Kahramanmaraş, Kayseri, and Malatya populations in this cluster. The

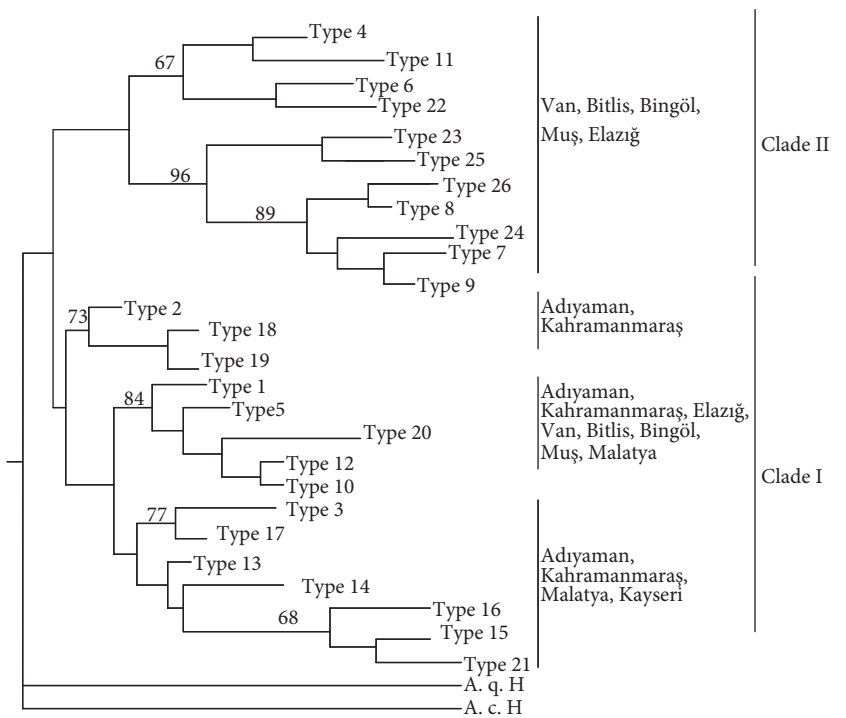

Figure 2. Dendrogram based on neighbor-joining cluster analysis of 26 detected mtDNA haplotypes. Corresponding population names are also shown (see Table 2). A. q. H and A. c. H are the out group haplotypes of *A. quercustozae* and *A. caputmedusae* gall wasp species. Numbers above branches represent the bootstrap values obtained from 1000 replicates of the restriction fragment data between the haplotypes. Support values <50% are not given.

second main cluster in the UPGMA dendrogram was composed of haplotypes only from Bitlis, Muş, Van, Bitlis, and Elazığ localities.

An unrooted Dollo parsimony majority-rule consensus tree of mtDNA haplotypes with supporting bootstrap values of >50 out of 1000 replicates is represented in Figure 4. As shown, haplotypes from the Adıyaman and Kahramanmaraş populations in Cluster I form a distinct grouping. Haplotypes representing the Adıyaman, Kahramanmaraş, Muş, Van, Bingöl, Bitlis, Elazığ, and Malatya populations are grouped in Cluster II. A distinct cluster formed by the haplotypes from Kahramanmaraş, Kayseri, and Malatya comprise Cluster III. The Bitlis, Muş, and Van haplotypes are grouped under Cluster IV. Finally, the last cluster is composed of the haplotypes representing the Bingöl, Muş, and Elazığ populations. Similar groupings are also seen in Figures 2 and 3.

For estimating the extent of genetic differentiation at different hierarchical levels, an AMOVA analysis was performed with 5 groupings obtained with an unrooted Dollo majority-rule consensus tree (Figure 4). For all grouping options, analysis of variance using AMOVA showed that all components of variance partitioning (among groups, among populations within groups, and within populations) were statistically significant. Analysis of variance using AMOVA revealed highly significant amounts of genetic variation (61.21%, $P < 0.001$) within populations, indicating that each population has high levels of genetic variation (Table 5). It also revealed that a significant partitioning of variance (26.85%) was found among groups and that the least amount of variance was among populations within groups (11.94%). When several different grouping schemes were analyzed using the ARLEQUIN program, AMOVA analysis produced similar results, in which within-population variation was highest compared to variations among groups and among populations within groups. Due to shared common haplotypes among populations, AMOVA results did not show the highest value of difference among groups. Current data indicate that each population developed its own genetic variation over time in addition to having an obvious differentiation among groups.

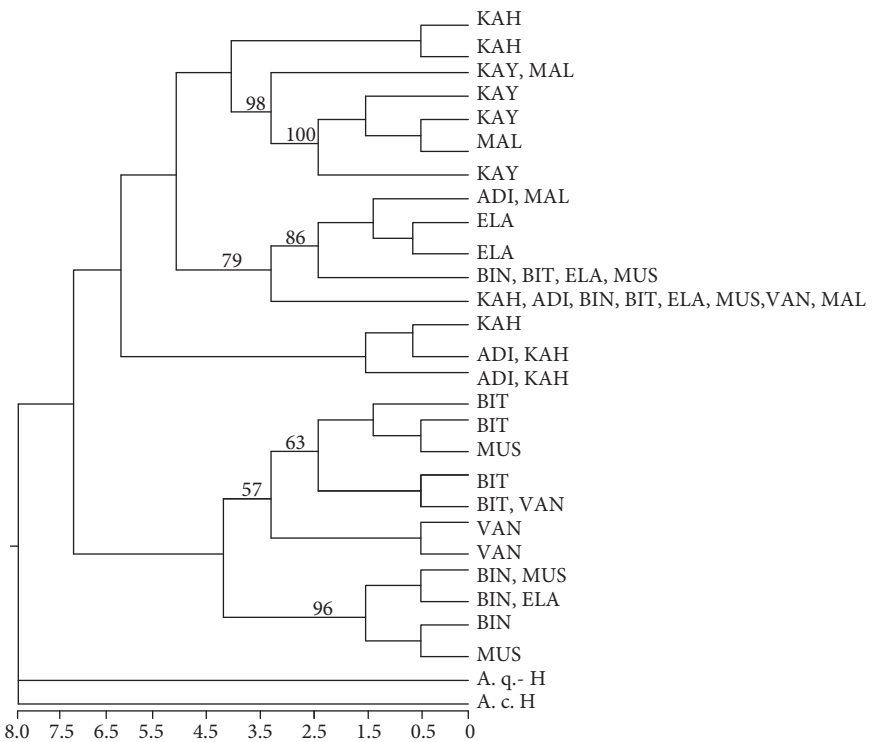

Figure 3. UPGMA dendrogram of 9 oak gall wasp populations (see Figure 1 and Table 1) based on pair-wise estimates of percent sequence divergence.

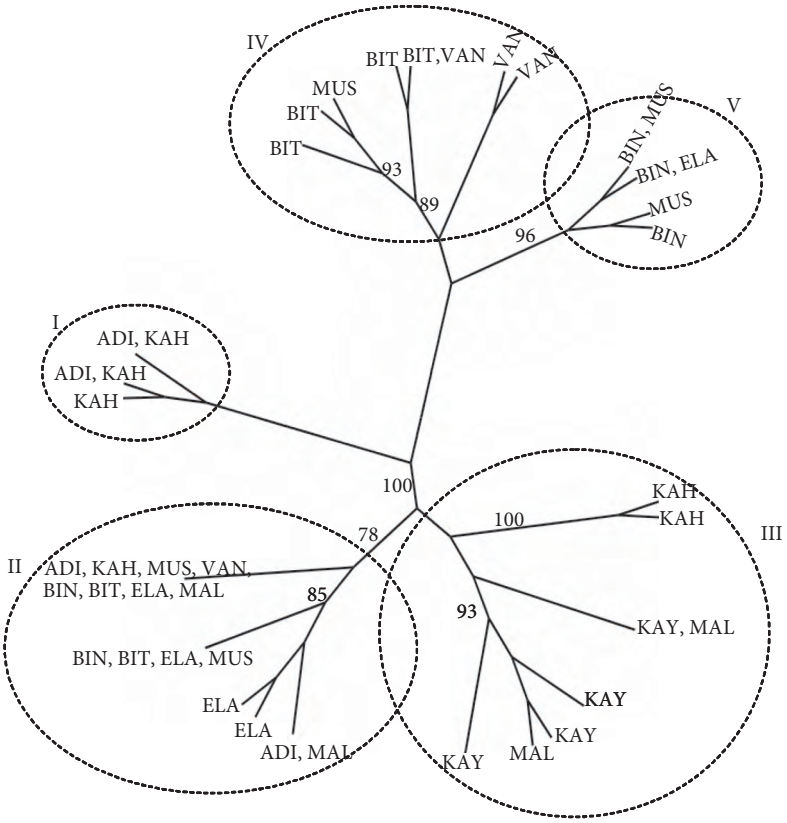

Figure 4. Unrooted Dollo parsimony majority-rule consensus tree of mtDNA haplotypes. Numbers at nodes indicates bootstrap values. Support values <50% are not shown.

Table 5. Analysis of molecular variance (AMOVA) among the studied oak gall wasp populations grouped into 5 groupings obtained with the unrooted Dollo parsimony majority-rule consensus tree of mtDNA haplotypes. *P < 0.001 after 1000 permutations. Va, Vb, and Vc are the associated covariance components.

Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation	Fixation Indices
Among groups	4	212.766	1.45609 Va	26.85*	F_{CT} = 0.26847
Within groups	4	49.787	0.64762 Vb	11.94*	F_{ST} = 0.38787
Within populations	135	448.203	3.32002 Vc	61.21*	F_{SC} = 0.16323

Discussion

mtDNA variation within and among populations of the Turkish oak gall wasp species

The present analysis of PCR-RFLP site variation at the ND4, ND6, Cyt B, ATPases (6, 8), and COIII regions of the mtDNA of Turkish oak gall wasps was motivated by evidence that these regions were variable in *A. lucidus* and thus could reveal genetic differences among the examined populations. Although using mitochondrial data alone may not always give high resolution and may be responsible for misleading results in phylogeography, it has been shown that mitochondrial DNA data and nuclear genetic data have been strongly concordant (Rokas et al., 2001;

Rokas et al., 2003; Stone et al., 2007). The existence of variation in the analyzed segments in the gall wasp species *A. lucidus* from Turkey is in accordance with other Hymenopteran sites, with some additional cut sites present (Francisco et al., 2001; Moretto and Arias, 2005). Thus, the results presented here reveal the presence of several variable restriction sites in these segments and allowed identification of 26 haplotypes in the Turkish oak gall wasp species.

In another Turkish oak gall wasp species, *A. caputmedusae*, 32 haplotypes were detected covering a sampling area in Anatolia. The average haplotype and nucleotide diversity within the populations were 0.4643 and 0.1021, respectively (Mutun, 2010). With this study, higher haplotype diversity was observed, with an average of 0.8089 for the *A. lucidus* populations. Among the 9 analyzed *A. lucidus* populations, the highest haplotype diversity was estimated for the Kahramanmaraş and Bitlis populations (0.8590). A high level of haplotype diversity is also reported in other species from Turkey (Rokas et al., 2003; Korkmaz et al., in press). On the other hand, the average nucleotide diversity for the *A. lucidus* populations (0.1155) was higher than that observed for the *A. caputmedusae* species in Turkey. In the oak gall wasp species *A. quercustozae*, studied in the Palearctic region, revealed that the European gall wasp populations possibly derived from Turkey. The Turkish populations overall showed not only greater genetic variations, but also represented the center of genetic diversity for the species. Moreover, phylogenetic and population structure suggested that the oak gall wasp species, now widespread in Europe, diverged in Anatolia and expanded its range westward prior to the Pleistocene (Rokas et. al., 2003). Studies on *A. coriarius* revealed the presence of higher genetic diversity with a westward expansion of about 1.6 million years before present, which might have possibly taken place with a distinct origin from Turkey and Iran (Challis et al., 2007). Likewise, similar results showing a signature of westward colonization with greater genetic variation were reported for the oak gall wasp species *A. kollari* (Stone et al., 2007). High genetic diversity and pre-Pleistocene separation of genetic lineages were also reported for other animal species from Turkey (Gündüz et al., 2005; Bardakçı et al., 2006).

Phylogeography of *Andricus lucidus*

The southeastern and eastern Anatolia region from which the *A. lucidus* specimens were collected for this study is one of the most mountainous areas of Turkey, fragmented by many valleys. Due to the highly heterogeneous topography, the studied area is an important topographic district for defining historical biogeography, contemporary species richness, and genetic patterns in Anatolia (Çıplak, 2008). The considerable nucleotide divergence between the lineages of oak gall wasps within its distribution range in Turkey is striking. The estimated pair-wise nucleotide divergence range among *A. lucidus* populations varied between 0.012% and 7.3%. It is often assumed that mtDNA evolves at a constant rate. The general application of the estimated mutation rate for insect mtDNA is a 2.3% sequence divergence per million years (Brower, 1994). Pair-wise distance comparisons between the Turkish oak gall wasp populations showed that the most ancient split between the lineages from Kayseri and Bitlis (7.3%) corresponds to a divergence time from a common ancestor of about 3.17 million years BP (using the conventional mutation calibration rate for insect mtDNA), suggesting a pre-Pleistocene separation. Likewise, the next split is 2.7 million years BP between the lineages from Malatya and Elazığ, as well as a split between Malatya and Bitlis dating back to 2.6 million years BP (see Table 4). Current findings on divergence estimates predate Pleistocene cycles and also seem to be closely correlated with the geographic barriers in the study area. The deep genetic divergence among some mtDNA lineages of the Turkish oak gall wasp species most probably shaped the population structuring currently observed, along with the geological history of Turkey, showing signs of pre-Pleistocene orogenic events. It is apparent that more current divergences also took place between some *A. lucidus* mtDNA lineages. The presence of a low divergence estimate among the Malatya, Adıyaman, and Kahramanmaraş populations (see Table 4) may imply a recent gene flow and resulting low level of isolation.

It is generally accepted that the existence of a population or lineage in a particular geographic area is a result of a number of historical events occurring over a long period of time, including previous historical

events and more current geologic and environmental factors (Cox and Moore, 1980). In the present study, there is a relationship among haplotypes and their geographical distribution (Figures 2 and 3, Table 2). Results obtained for *A. lucidus* from Turkey appear to involve 2 main clades, each composed of a number of subgroupings. The distribution of the 2 major clades identified by the analyses stands out in a number of aspects. Clade I, as a distinct lineage, is composed of haplotype 2 with a restricted distribution in the Adıyaman and Kahramanmaraş populations, and 2 additional haplotypes (Types 18 and 19) fixed to the Kahramanmaraş population. The second clade contains 2 internal lineages having the most common haplotype (Type 1), which is distributed in most parts of the sampling area, including the Adıyaman and Kahramanmaraş populations. The basal placement of the most common haplotype may imply that it is more of an ancestral haplotype compared to other haplotypes in this clade. For many species, it has been shown that the phylogeny of mtDNA haplotypes corresponds well to the geographical distribution of populations (Avise, 2000), and under a commonly accepted phylogeographic approach, more commonly distributed haplotypes are ancestral haplotypes when compared to the derived haplotypes that show more restriction in their distribution (Neigel and Avise, 1993).

The Amanos Mountains are located in the southern part of the sampling area of *A. lucidus*, and the southeastern Taurus Mountains extend from the connecting section of the southern, southeastern, and eastern Taurus (the Maraş Triangle) in the eastern part of the sampling area. In both the Kahramanmaraş and Bitlis populations, the observed high level of haplotype diversity may imply several points; the area where the Kahramanmaraş population was sampled is located at the Maraş Triangle, which is known for its high species/lineage diversity (Çıplak, 2008). Likewise, high levels of genetic variation were shown for *A. caputmedusae* for the Kahramanmaraş population, which coincides with the Maraş Triangle (Mutun, 2010). Moreover, geographically closely located to Kahramanmaraş, the Adıyaman population also shows a high haplotype diversity, with both sharing 3 haplotypes (Types 1, 2, and 3, respectively; see Table 2). On the other hand, haplotypes found in both locations are placed at the more basal position of 3 distinct subclusters of Clade I in the dendrogram shown in Figure 2. These findings may suggest that haplotypes found in the Adıyaman and Kahramanmaraş populations are more ancient in their origin and that at least one dispersal event might have taken place from this area to the other close populations. It is assumed that the more derived haplotypes found in more restricted numbers of populations originated from the more commonly found lineages (Neigel and Avise, 1993). Derived lineages of *A. lucidus* have increased over time.

Overall, this study presented information on the genetic structure of the Turkish oak gall wasp species with some distinct groupings within the range of *A. lucidus* in Turkey. Moreover, molecular data on the studied species provided new insights into the significance of varied topography playing a significant role in shaping the current population genetic structure of the Turkish oak gall wasp species. However, clarifying the center of origin and expansion of the species from Iran to Anatolia requires detailed sampling from the area. Recently, explorations have begun to obtain the necessary data for resolving the underlining scenario.

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References

Atkinson, R.J., McVean G.A.T. and Stone, G.N. 2002. Use of population genetic data to infer oviposition behavior: species-specific patterns in four oak gall wasps species (Hymenoptera: Cynipidae). P. Roy. Soc. B. 269: 383-390.

Avise, J.C. 1994. Molecular Markers, Natural History and Evolution, Chapman and Hall, NY, USA.

Avise, J.C. 2000. Phylogeography: The History and Formation of Species, Harvard University Press, Cambridge. MA.

Bardakçı, F., Değerli, N., Özdemir, O. and Başıbüyük, H.H. 2006. Phylogeography of the Turkish brown trout *Salmo trutta* L.: mitochondrial DNA PCR-RFLP variation. J. Fish Biol. 68: 36-55.

Brower, A.V.Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. P. Ntl. Acad. Sci.-Biol. 91: 6491-6495.

Challis, R.J., Mutun, S., Nieves-Aldrey, J.L., Preuss, S., Rokas, A., Aebi, A., Sadeghi, E., Tavakoli, M. and Stone, G.N. 2007. Longitudinal range expansion and cryptic eastern species in the western Palaearctic oak gall wasp, *Andricus coriarius*. Mol. Ecol. 16: 2103-2114.

Chapco, W., Kelln, R.A. and McFadyen, D.A. 1992. Intraspecific mitochondrial DNA variation in the migratory grasshopper, *Melanoplus sanguinipes*. Heredity 69: 547-557.

Cox, C.B. and Moore, P.D. 1980. Biogeography: An Ecological and Evolutionary Approach, Wiley and Sons, NY, USA.

Çıplak, B. 2008. The analogy between interglacial and global warming for the glacial relicts in a refugium: A biogeographic perspective for conservation of Anatolian Orthoptera. In: Insect Ecology and Conservation (ed. S Fattorini), India, pp. 135-163.

Çıplak, B., Demirsoy, A. and Bozcuk, A.N. 1996. Malatya (Türkiye) Ensifera (Orthoptera, Insecta) faunası. Turk. J. Zool. 20: 247-254.

Eroğlu, M. 2000. Gall production strategy that is inferring with life cycles of two important gall-maker species, *Andricus gallaetinctoriae* (Oliv.) and *Andricus kollari* Htg. (Hymenoptera: Cynipidae) in Turkey. Seminar on 'Harvesting on Non-wood Forest Products,' Menemen-İzmir (Turkey), Joint FAO/ECE/ILO Committee on Forest Technology, Management and Training, p. 10.

Excoffier, L., Laval, G. and Schneider, S. 2005. Arlequin Version 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1: 47-50.

Excoffier, L., Smouse, P.E. and Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479-491.

Felsenstein, J. 1993. PHYLIP (Phylogenetic Inference Package) Version 3.5c. Dept. of Genetics, University of Washington, Seattle.

Francisco, F.O., Silvestre, D. and Arias, M.C. 2001. Mitochondrial DNA characterization of five species of *Plebeia* (Apidae: Meliponini): RFLP and restriction maps. Apidologie 32: 323-332.

Gündüz, İ., Rambau, R.V., Tez, C. and Searle, J.B. 2005. Mitochondrial DNA variation in the western house mouse (*Mus musculus domesticus*) close to its site of origin: studies in Turkey. UCLA Sym. Biol. 84: 473-485.

Harrison, R.G. 1989. Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. Trends Ecol. Evol. 4: 6-11.

Korkmaz, M., Sarı, M. and Başıbüyük, H.H. (in press). Genetic structure of *Chorthippus parallellus* (Orthoptera: Acrididae: Gomphocerinae) populations in Anatolia: a stable rear edge population. Ann. Entomol. Soc. Am.

Kosswig, C. 1955. Zoogeography of the Near East. Syst. Zool. 4: 49-73.

McElroy, D., Moran, P., Bermingham E. and Kornfield, J. 1991. REAP: The Restriction Enzyme Analysis Package, version 4.0. Dept. of Zoology, University of Maine, Orono.

Moretto, G. and Arias M.C. 2005. Detection of mitochondrial DNA restriction site differences between the subspecies of *Melipona quadrifascinata* Lepeletier (Hymenoptera: Apidae: Meliponini. Neotrop. Entomol. 34(3): 381-385.

Mutun, S. 2010. Intraspecific genetic variation and phylogeography of the oak gall wasp *Andricus caputmedusae* (Hymenoptera: Cynipidae): effects of the Anatolian diagonal. Acta Zool. Hung. 56(2): 153-172.

Nei, M. and, Tajima, F. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics 97: 145-163.

Neigel, J.E. and Avise, J.C. 1993. Application of a random walk model to geographic distributions of animal mitochondrial DNA variation. Genetics 135: 1209-1220.

Roff, D.A. and Bentzen, P. 1989. The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. Mol. Biol. Evol. 6: 539-545.

Rokas, A., Atkinson, R.J., Brown, G., West, S.A. and Stone, G.N. 2001. Understanding patterns of genetic diversity in the oak gall wasp *Biorhiza pallida*: demographic history or a Wolbachia selective sweep? Heredity 87: 294-305.

Rokas, A., Atkinson, R.J., Webster, L.M.I., György, C. and Stone, G.N. 2003. Out of Anatolia: longitudinal gradients in genetic diversity support an eastern origin for a circum-Mediterranean oak gall wasp *Andricus quercustozae*. Mol. Ecol. 12: 2153-2174.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. and Flook, P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87(6): 651-701.

Stone, G.N., Challis, R.J., Atkinson, R.J., Csöka, G., Hayward, A., Melika, G., Mutun, S., Preuss, S., Rokas, A., Sadeghi, E. and Schröngrogge, K. 2007. The phylogeographical clade trade: tracing the impact of human-mediated dispersal on the colonization of northern Europe by the oak gall wasp *Andricus kollari*. Mol. Ecol. 16: 2768-2781.

Stone, G.N., Hernandez-Lopez, A., Nicholls, J.A., Di Pierro, E., Pujade-Villar, J., Melika, G. and Cook, J.M. 2009. Extreme host plant conservatism during at least 20 million years of host plant pursuit by oak gall wasps. Evolution 63: 854-869.