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# INTRASPECIFIC GENETIC VARIATION AND PHYLOGEOGRAPHY OF THE OAK GALLWASP *ANDRICUS CAPUTMEDUSAE* (HYMENOPTERA: CYNIPIDAE): EFFECTS OF THE ANATOLIAN DIAGONAL

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Physical barriers and major climatic oscillations in the Pleistocene are of enormous importance for the distribution and current population genetic structure of many animal taxa. Anatolia was one of the main corridors for postglacial colonization of Europe and it is characterized by rich biodiversity. In the present study, mitochondrial DNA (mtDNA) RFLPs were used to assess i) the phylogeographic relationships among 26 populations of an oak gallwasp, *Andricus caputmedusae*, and ii) the impact of the heterogenous topography on the geographic structure of populations. PCR was used to amplify a *ca*. 2540 base pair mtDNA region spanning the genes ND4, ND4L, $tRNA^{Thr}$, $tRNA^{Pro}$, ND6 and part of cytochrome b. Digestion of this region with eight restriction enzymes yielded a total of 31 haplotypes that divided sampled populations into three phylogenetic assemblages reflecting their geographic location. The average haplotype and nucleotide diversities within populations were 0.4631 and 0.101214, respectively. AMOVA analysis attributed high levels of genetic variation to variation within populations (31.26%), variation within groups (24.85%), and variation among groups (43.89%). Estimation of the age of divergence between mitochondrial lineages with reference to the geological history of Anatolia suggests that the current population structure of *A. caputmedusae* was shaped by both the Pleistocene climatic fluctuations and the heterogenous topography of Anatolia.

Key words: Anatolian diagonal, *Andricus caputmedusae*, mtDNA, oak gallwasp, phylogeography

## INTRODUCTION

Recent advances in molecular biology and phylogeography continue to improve our understanding of the impact of historical events on the divergence and distribution of current populations (AVISE 1994). Many phylogeographic analyses have assessed the significance of Pleistocene climatic fluctuations and dispersal routes for the colonization history of the European biota and Anatolia has been suggested as an important refuge and source for recolonization of Europe both by animals and plants (HEWITT 1999, SEDDON *et al*. 2002). Recent phylogenetic studies have inferred populations of many western Palearctic species to originate from more eastern parts of their distribution range including Turkey, and those easterly

located populations represent significant centers of genetic diversity (ROKAS *et al*. 2003, GÜNDÜZ *et al*. 2005, 2007, CHALLIS *et al*. 2007, STONE *et al*. 2007).

Located in the Alpine–Himalayan Mountain belt between Eurasia, Africa and Arabia, Turkey has a complex geological history which is a result of the collision of the Arabian and African plates with the European plate, promoting the closure of the Tethys Sea (RÖGL 1998). The most important consequences of this event for Anatolia were the upfolding of the Caucasus and the Taurus mountains and the uplift of the Central Anatolian highlands (BOZKURT 2001). Anatolia acted as a corridor for the dispersal of African animals during the Early Miocene, and finally acted as refuge area during the climatic fluctuations of the Quaternary period (ÇIPLAK *et al*. 1996, ÇIPLAK 2003). Furthermore, the highland divide across Anatolia known as the Anatolian Diagonal has been proposed as a significant geographic barrier shaping current species composition of various species across Turkey and dividing species/lineage distribution into east and west (DAVIS 1971, ÇIPLAK *et al*. 1993, ROKAS *et al*. 2003, ÇIPLAK 2004*a*). The Anatolian Diagonal is a line of mountain ranges that run from the south of Gümüşhane – Bayburt in the north southwest across Turkey to the Taurus Mountains (DAVIS 1971, EKIM & GÜNER 1986). Several previous studies of species distribution and regional composition have suggested that, together with the Tertiary history of Turkey, the Anatolian Diagonal might be responsible for breaks in distributions at both specific and generic levels (ÇIPLAK *et al*. 1993, ÇIPLAK 2003). In addition to the Diagonal, several other altitudinal belts in Anatolia have been proposed either to fragment species/lineage distributions or provide limits for east-west or north-south distributions (ÇIPLAK 2008). Thus, defining range distributions of lineages or genetic structuring of individual species has particular importance in understanding the biogeography of Anatolia.

A range of molecular markers are available for studying population structure, phylogeography and phylogenetic relationships at various taxonomic levels (AVISE 2000). Mitochondrial DNA (mtDNA) has been extensively used for phylogeographic studies because of its small size and maternal inheritance, a fast evolutionary rate relative to coding regions of nuclear DNA, and lack of recombination (BERMINGHAM & MORITZ 1998, HARRISON 1989). A growing number of phylogeographic studies on animals have used mtDNA as a marker, including studies of oak gallwasps (LILJEBLAD & RONQUIST 1998, ROKAS *et al*. 2003, CHALLIS *et al*. 2007, STONE *et al*. 2007). As obligate parasites, oak gallwasps induce species- and generation-specific galls on different parts of oak trees. The gallwasp *Andricus caputmedusae* (HARTIG, 1843) is distributed from the Iberian Peninsula in the west to Iran in the east. Its parthenogenetic generation induces unilocular galls at the edge of acorn cups of white oak (*Quercus* section *Quercus sensu stricto)* species includ-

ing *Q. infectoria*, *Quercus petraea*, *Q. robur* and *Q. pubescens* (OĞURLU & AVCI 1998). The surface of the galls is covered by 4 cm long spines that are glutinous and sticky when young and hard in later stages. The galls are initially pink, turning pale yellow as they mature. To date, no detailed study has examined the possible effects of the Anatolian Diagonal in creating a genetic break between populations of a species distributed to both east and west. Because *A. caputmedusae* is widely distributed in Turkey and spans the Diagonal it should be a good model species i) to explore the phylogenetic structure of *A. caputmedusae* in Anatolia, ii) to reveal the possible influences of the major historical events on the distribution patterns of genetic variation of oak gallwasp species across Turkey, and iii) to test whether the Anatolian Diagonal and other high altitude regions have in fact acted as barriers to dispersal shaping phylogeographic structure, as suggested by many previous studies on distribution patterns of plant and animal species (DAVIS 1971, EKIM & GÜNER 1986, ÇIPLAK *et al*. 1993). To address these questions, I analyzed spatial variation in mitochondrial restriction fragment length variation using PCR-RFLP in populations of *A. caputmedusae* sampled across Anatolia.

## MATERIALS AND METHODS

### *Sampling and molecular techniques*

A total of 180 individuals from 26 populations of *A. caputmedusae* were collected in the summer of 2006–2008 from sites spanning most of the distribution range of this species in Turkey. Sampled populations were specifically chosen based on their location relative to the Anatolian Diagonal shown in Fig. 1. All specimens were stored at –80°C until DNA isolation. Total genomic DNA was extracted from single individuals with the DNeasy Tissue Kit (QIAGEN). A *c.* 2.5 kb mitochondrial fragment spanning the ND4, ND4L, $tRNA^{Thr}$, $tRNA^{Pro}$, ND6 regions and a part of the cytochrome b gene was amplified using primers ND4 and CBN (SIMON *et al*. 1994). PCR was carried out in 25 µl volumes containing 0.5 µl of the total DNA extraction, 2.5 µl 10× PCR buffer (Promega), 2.0 µl $MgCl_2$ (25 mM), 1.0 µl dNTPs (2mM each), 0.75 µl of each (20 µM) and 1.25 U of Taq DNA Polymerase (Promega). PCRs were carried out in a thermal cycler (Techne, UK) using the following program: 5 min at 94°C, 35 cycles of 1 min at 94 °C, 1 min 20 s at 44 °C, 2 min at 64 °C, and a final extension step of 10 min at 64 °C. The amplified mtDNA region was digested with eight restriction enzymes (HinfI, ClaI, HindII, MboII, VspI, ApaI, SspI and PstI: MBI Fermentas and TAKARA) also used in previous work on other insects (e.g. FRANSISCO *et al*. 2001, MORETTO & ARIAS 2005). Restriction fragments were separated by electrophoresis in 1% agarose gels containing ethidium bromide with 1× TBE running buffer (0.089 M Tris, 0,089 M Boric acid, 0.001 M disodium EDTA), visualized under UV light and photographed.

## *Data analysis*

Different restriction patterns for each restriction enzyme were assigned a letter as they were observed. The presence or absence of restriction sites was inferred for each enzyme from completely additive fragment patterns and each individual insect were designated a composite haplotype based on the observed RFLP patterns. Haplotype and nucleotide diversity within each population and divergence among populations were estimated according to NEI and TAJIMA (1981) using the DA programs contained in the software REAP (MCELROY *et al*. 1991). From the basic presence–absence matrix 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). Unrooted DOLLO parsimony trees were constructed using the PHYLIP DOLLOP program. From these trees, the consensus unrooted phylogenetic tree for haplotypes was obtained using the PHYLIP CONSENSE program. The average number of nucleotide substitutions per site between haplotypes was used to obtain a neighbour joining tree using the PHYLIP NEIGHBOR program. The degree of geographic heterogeneity of mtDNA haplotype distributions was assessed using $\chi^2$ statistics (ROFF & BENTZEN 1989). The significance level was obtained by 10,000 Monte Carlo randomizations using the Monte routine from the REAP package.

The partitioning of molecular variation was revealed by analysis of molecular variance (AMOVA) implemented in the program ARLEQUIN 3.1 (EXCOFFIER *et al*. 2005). AMOVA is an analysis of variance procedure that partitions molecular variance according to sampling design.

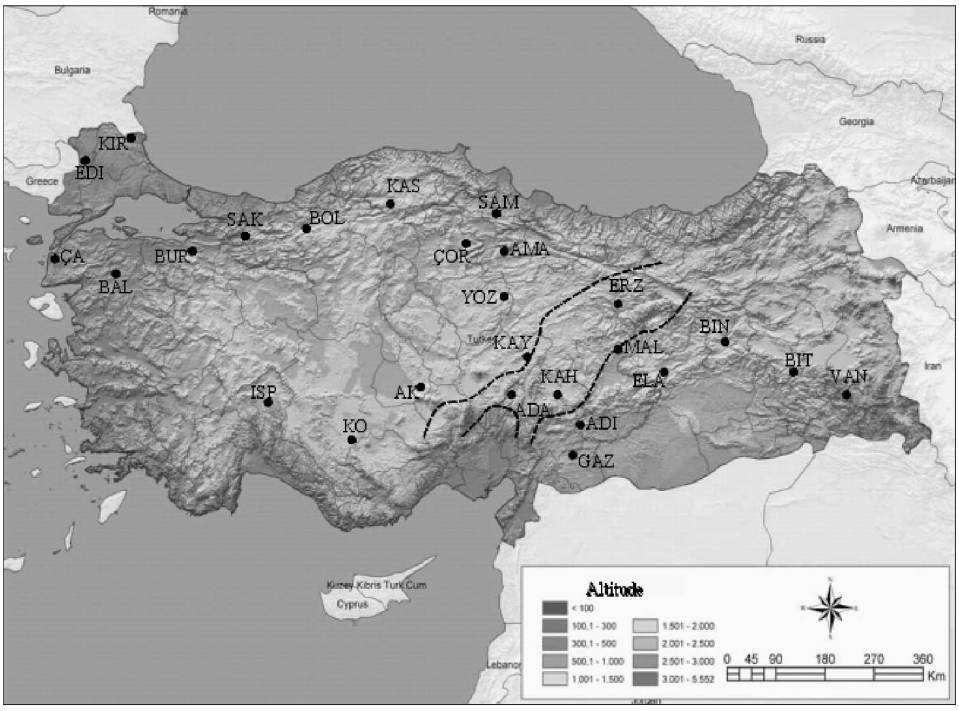


**Fig. 1.** Geographic distribution of the twenty six *Andricus caputmedusae* populations used in the present study and the location of the Anatolian Diagonal (indicated by dashed line) shown in a topographic map of Turkey

AMOVA 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 10000 permutations. Related $F_{ST}$ statistics are defined as follows: $F_{CT}$ is the correlation of random haplotypes within a population group, relative to that of random pairs of haplotypes from the entire data set; $F_{SC}$ is the correlation of molecular diversity of random haplotypes within populations, relative to that of random pairs of haplotypes from within the region; $F_{ST}$ is the correlation of random haplotypes within populations, relative to that of random pairs of haplotypes from the entire data set. AMOVA conducted on the whole data set was performed using different grouping options based on the geographic locations of the populations including three groupings obtained by unrooted Dollo parsimony majority rule and neighbor-joining trees as follows: (i) east/in the Diagonal, (ii) east/near west of the Diagonal and (iii) west of the Anatolian Diagonal. The partitioning of the variation was tested for the i) among groups, ii) among populations within groups, and iii) within populations (EXCOFFIER *et al*. 2005).

**Table 1.** Localities of sampled populations of *A. caputmedusae*.

| Population | Abbreviation | Locality | Coordinates |
|---|---|---|---|
| 1.Van | VAN | Çatak | N 37°55.015' E 42°57.828' |
| 2. Bitlis | BIT | Baykan | N 38°21.019' E 42°02.412' |
| 3. Bingöl | BIN | Near the Elazığ Road | N 38°57.407' E 40°11.432' |
| 4. Elazığ | ELA | near the Hazar Lake | N 38°29.886' E 39°22.599' |
| 5. Adıyaman | ADI | Besni | N 37°45.869' E 37°43.136' |
| 6. Gaziantep | GAZ | Araban | N 37°22.975' E 37°33.292' |
| 7. Erzincan | ERZ | Kemaliye | N 39°18.807' E 38°30.273' |
| 8. Malatya | MAL | Hekimhan | N 38°42.144' E 38°06.992' |
| 9. Kahramanmaraş | KAH | Göksun | N 37°43.514' E 36°40.038' |
| 10. Adana | ADA | Saimbey | N 38°09.720' E 36°06.555' |
| 11. Samsun | SAM | Ladik | N 40°57.086' E 35°48.006' |
| 12. Amasya | AMA | Near the Tokat Road | N 40°33.404' E 36°08.295' |
| 13. Çorum | ÇOR | Near the Amasya Road | N 40°36.487' E 35°04.802' |
| 14. Yozgat | YOZ | Çamlık | N 39°40.452' E 35°47.627' |
| 15. Kayseri | KAY | Pınarbaşı | N 38°40.512' E 36°19.973' |
| 16. Aksaray | AK | Hasan Mountain | N 38°09.931' E 34°11.463' |
| 17. Kastamonu | KAS | Daday | N 41°28.877' E 33°34.995' |
| 18. Bolu | BOL | Gölköy | N 40°40.380' E 31°25.991' |
| 19. Sakarya | SAK | Taraklı | N 40°29.257' E 30°20.749' |
| 20. Bursa | BUR | Uludağ | N 40°09.556' E 29°00.802' |
| 21. Balıkesir | BAL | Edremit | N 39°35.623' E 27°04.082' |
| 22. Çanakkale | ÇA | Üvecik | N 39°53.213' E 26°11.747' |
| 23. Edirne | EDI | Havsa | N 41°21.260' E 26°46.425' |
| 24. Kırklareli | KIR | İğneada | N 41°56.657' E 27°40.765' |
| 25. Isparta | ISP | Eğirdir | N 37°51.986' E 30°49.219' |
| 26. Konya | KO | Hadim | N 36°56.671' E 32°29.996' |

## RESULTS

In total, 180 individuals collected from 26 populations of *A.caputmedusae* representing the entire distribution range of the species across Turkey were used in this study. For the sample collections the location of the Anatolian Diagonal was considered as the populations from Van, Bitlis, Bingöl, Elazığ, Adıyaman and Gaziantep are located in the eastern part of the Anatolian Diagonal, the populations from Erzincan, Malatya, Kahramanmaraş and Adana are situated in the Diagonal and remaining populations are found in the western part of the Diagonal (Fig. 1, Table 1). RFLP analysis of the 2.5 kb mtDNA fragment revealed no restriction sites for the enzymes VspI, ApaI, SspI, PstI. The four remaining enzymes identified a total of 57 digestion sites, representing an estimated total of 264 nucleotides. Seven distinct digestion patterns (A-G) were detected with the restriction enzymes HinfI, HindII and MboII, and 3 distinct patterns (A-C) were produced with the enzyme ClaI. A total of 31 composite haplotypes were detected in the sampled populations. The composite haplotypes and their frequencies in each population are given in Table 2. Among the 31 composite haplotypes, Type 1 was the most widely distributed, found in 45 individuals from 9 populations. Type 24 was the second most abundant haplotype, present in 21 individuals from 5 populations. Some rare haplotypes were nevertheless relatively widely distributed: for example, Type 2 was found only in 15 individuals but was present in 6 different localities. Of the 31 composite haplotypes observed, 8 were private haplotypes observed only in one individual in one population. Among these private haplotypes, Type 7 was found only in the Malatya population, Types 11–13 only in the Aksaray population, Types 19–20 only in the Kırklareli population, and Types 22 and 25 only in the Bolu population.

### *Phylogenetic relationships among mtDNA haplotypes*

Haplotype and nucleotide diversity of the *A. caputmedusae* populations calculated using the REAP program is given in Table 3. Of the 26 analyzed populations eighteen had more than one haplotype. The average haplotype diversity for the studied populations was 0.4631. Within population nucleotide diversity was 0.101214, lower than the inter-population nucleotide diversity (0.222603). Among the 26 analyzed populations the highest haplotype diversity (0.9524) was estimated for the Gaziantep population followed by Adıyaman (0.9048) and Aksaray (0.8571). The highest nucleotide diversity (0.229664) was observed in the Balıkesir population followed by Edirne (0.210000), Adıyaman (0.209957) and Aksaray (0.202223).

**Table 2.** Composite haplotypes and their frequencies among 26 populations of *A. caputmedusae* based on the digestion pattern of eight restriction enzymes. Composite haplotypes shown by capital letters are given in the following order: VspI, ApaI, SspI, PstI, HinfI, ClaI, HindII, and MboII. HT = Haplotype, CH = composite haplotype

| HT | CH | Population | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | VAN | BIT | BIN | ELA | ADI | GAZ | ERZ | MAL | KAH | ADA | SAM | AMA | COR | YOZ | KAY | AK | KAS | BOL | SAK | BUR | BAL | CA | EDI | KIR | ISP | KO | Σ |
| 1 | AAAAAAAA | 4 | 8 | 8 | 4 | 2 | 2 | 7 | | | 6 | | | | | 4 | | | | | | | | | | | | 45 |
| 2 | AAAAAABA | 3 | | | 3 | | 1 | 2 | 4 | | | | | | | 2 | | | | | | | | | | | | 15 |
| 3 | AAAAAABB | | | | 1 | 2 | 1 | | | 8 | 2 | | | | | | | | | | | | | | | | | 14 |
| 4 | AAAAAAAB | | | | | 1 | 1 | | | | | | | | | | | | | | | | | | | | | 2 |
| 5 | AAAAAABC | | | | | 1 | 1 | | | | | | | | | | | | | | | | | | | | | 2 |
| 6 | AAAAAACA | | | | | 1 | 1 | | | | | | | | | | | | | | | | | | | | | 2 |
| 7 | AAAAAADA | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | 1 |
| 8 | AAAAAAAC | | | | | | | | 3 | | | | | | | | | | | | | | | | | | | 3 |
| 9 | AAAAAAAD | | | | | | | | | | | | | | | | 3 | | | | | | | | | | | 3 |
| 10 | AAAAAABD | | | | | | | | | | | | | | | | 2 | | | | | | | | | | | 2 |
| 11 | AAAAAAED | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | 1 |
| 12 | AAAAAAEA | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | 1 |
| 13 | AAAAABAA | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | 1 |
| 14 | AAAAAAGD | | | | | | | | | | | | | | | | | | | | | 2 | | | | | | 2 |
| 15 | AAAAAABG | | | | | | | | | | | | | | | | | | 3 | | | 2 | | | | | | 5 |
| 16 | AAAAAAFF | | | | | | | | | | | | | | | | | | | | | | | 3 | | | | 3 |
| 17 | AAAAABDA | | | | | | | | | | | | | | | | | | | | | | | 2 | | | | 2 |
| 18 | AAAAAABE | | | | | | | | | | | | | | | | | | | | | | | | 3 | | | 3 |
| 19 | AAAAAACG | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | 1 |
| 20 | AAAAABCG | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | 1 |
| 21 | AAAAACAG | | | | | | | | | | | | | | | | | | | | | | | | 2 | | | 2 |

**Table 2** (continued)

| HT | CH | Population | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | VAN | BIT | BIN | ELA | ADI | GAZ | ERZ | MAL | KAH | ADA | SAM | AMA | COR | YOZ | KAY | AK | KAS | BOL | SAK | BUR | BAL | CA | EDI | KIR | ISP | KO | Σ |
| 22 | AAAABBBA | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | 1 |
| 23 | AAAABBCB | | | | | | | | | | | 3 | | 2 | | | | 2 | | | | | | | | | | | 7 |
| 24 | AAAACABA | | | | | | | | | | | 2 | 6 | 4 | 7 | | | 2 | | | | | | | | | | | 21 |
| 25 | AAAACAAA | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | 1 |
| 26 | AAAACAAG | | | | | | | | | | | | | | | | | | | 6 | 6 | 1 | 6 | | | | | | 19 |
| 27 | AAAADABA | | | | | | | | | | | | | | | | | | | | | | | | | | 3 | | 3 |
| 28 | AAAADABC | | | | | | | | | | | | | | | | | | | | | | | | | | 4 | | 4 |
| 29 | AAAAEAAA | | | | | | | | | | | | | | | | | | | | | | | | | | 3 | | 3 |
| 30 | AAAAFAAA | | | | | | | | | | | | | | | | | | | | | | | | | | | 5 | 5 |
| 31 | AAAAGABA | | | | | | | | | | | | | | | | | | | | | | | | | | | 5 | 5 |
| Sample size | | 7 | 8 | 8 | 8 | 7 | 7 | 9 | 8 | 8 | 8 | 5 | 6 | 6 | 7 | 6 | 8 | 4 | 5 | 6 | 6 | 5 | 6 | 5 | 7 | 10 | 10 | 180 |

Pair wise sequence divergence estimates among the 31 haplotypes varied from 0.0–3.5 % (Table 4). The most divergent haplotypes were found between several populations including Adıyaman and Gaziantep being diverged from the Aksaray population and with the same estimated value compromising haplotypes from Bitlis-Bingöl and Sakarya, Bursa and Çanakkale populations. Moreover, the Çanakkale population is diverged from the Erzincan population with an estimated value of 0.03263. The average nucleotide divergence of all the populations is 0.03204±0.00003. Calculated Monte Carlo $\chi^2$ values with 1000 randomizations indicated statistically significant variation in haplotype distributions ($\chi^2$= 1845.60, $p < 0.01$). The nucleotide divergence between haplotypes is shown in the form of a UPGMA dendrogram in Fig. 2. Haplotype 4, shared between Gaziantep and Adıyaman populations, is located most basally in the dendrogram. Haplotype 13 from the Aksaray population was placed in the next most basal position in the dendrogram. Except for this haplotype, all other following haplotypes are observed in the populations from Malatya, Adana, Kayseri, Bingöl, Elazığ,

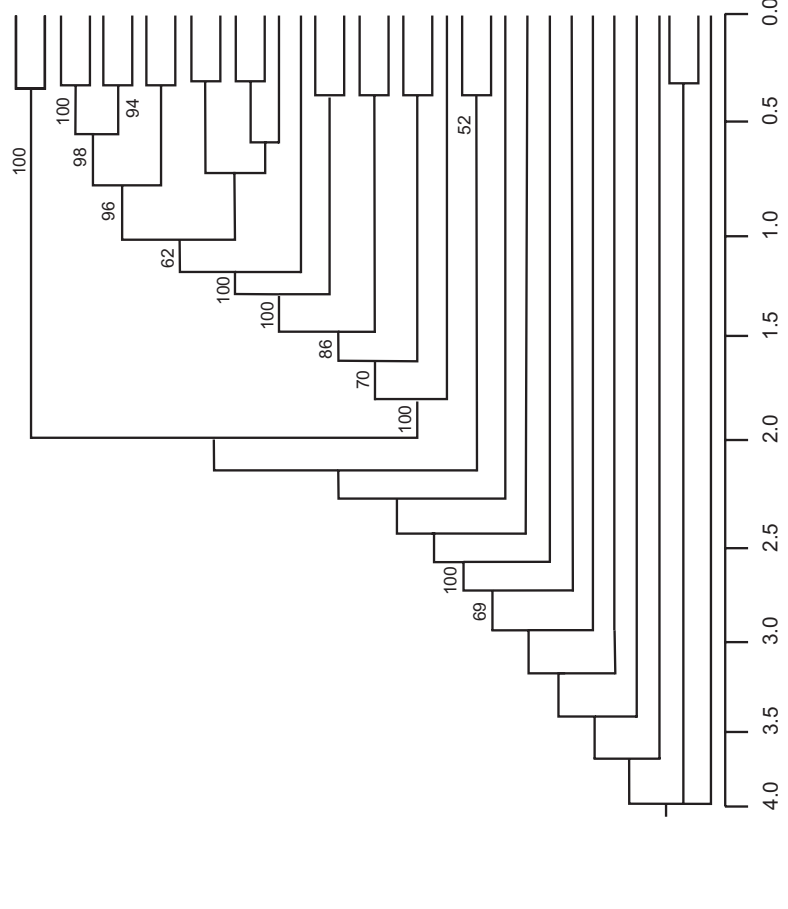


**Fig. 2.** UPGMA dendrogram of *A. caputmedusae* populations (see Fig. 1 and Table 1) based on pair wise estimates of percentage sequence divergence. A. q. H-1, A. q. H-2 are the haplotypes of *A. quercustozae* and A. l. H is the haplotype of *A. lucidus* gall wasp species used as outgroups. Numbers above branches represent the bootstrap values obtained from 1000 replicates of the restriction fragment data between the haplotypes. Support values < 50% are not given

**Table 3.** Mean±S.E. haplotype and nucleotide diversity for the *A. caputmedusae* populations.

| Population | Haplotype diversity | Nucleotide diversity |
|---|---|---|
| 1. Van | 0.5714±0.11950 | 0.069565 |
| 2. Bitlis | 0.0000±0.00000 | 0.000000 |
| 3. Bingöl | 0.0000±0.00000 | 0.000000 |
| 4. Elazığ | 0.6786±0.12204 | 0.120319 |
| 5. Adıyaman | 0.9048±0.10330 | 0.209957 |
| 6. Gaziantep | 0.9524±0.09552 | 0.191023 |
| 7. Erzincan | 0.3889±0.16440 | 0.047343 |
| 8. Malatya | 0.6786±0.12204 | 0.091024 |
| 9. Kahramanmaraş | 0.0000±0.00000 | 0.000000 |
| 10. Adana | 0.4286±0.16870 | 0.150000 |
| 11. Samsun | 0.6000±0.17527 | 0.171594 |
| 12. Amasya | 0.0000±0.00000 | 0.000000 |
| 13. Çorum | 0.5333±0.17213 | 0.152528 |
| 14. Yozgat | 0.0000±0.00000 | 0.000000 |
| 15. Kayseri | 0.5333±0.17213 | 0.064928 |
| 16. Aksaray | 0.8571±0.10825 | 0.202223 |
| 17. Kastamonu | 0.6667±0.20412 | 0.190660 |
| 18. Bolu | 0.7000±0.21836 | 0.200963 |
| 19. Sakarya | 0.0000±0.00000 | 0.000000 |
| 20. Bursa | 0.0000±0.00000 | 0.000000 |
| 21. Balıkesir | 0.8000±0.16395 | 0.229664 |
| 22. Çanakkale | 0.0000±0.00000 | 0.000000 |
| 23. Edirne | 0.6000±0.17527 | 0.210000 |
| 24. Kırklareli | 0.8095±0.12984 | 0.152124 |
| 25. Isparta | 0.7818±0.07494 | 0.084576 |
| 26. Konya | 0.5556±0.07454 | 0.093074 |
| Average | 0.4631±0.00447 | 0.101214±0.000272 |

Adıyaman, Gaziantep, Erzincan Kahramanmaraş, Van and Bitlis, most of which are located to the east of the Anatolian Diagonal. The remaining haplotypes from populations west of the Anatolian Diagonal together with one haplotype (Type 6) shared between the Adıyaman and Gaziantep populations form two clusters. One small grouping comprises Haplotypes 29 and 31 from the Isparta and Konya populations, respectively. The second larger cluster comprises haplotypes from the populations of Aksaray, Edirne, Balıkesir, Kırklareli, Bolu, Bursa, Sakarya, Çanakkale, Samsun, Çorum, Kastamonu, Amasya and Yozgat, all of which are geographically located west of the Anatolian Diagonal.

The basic matrix of presence/absence of restriction sites for each haplotype was also used to reconstruct a neighbor-joining dendrogram, shown with bootstrap values >50% in Fig. 3. The dendrogram comprises two clusters. The first cluster comprises haplotypes from sites to the East of the Diagonal (Gaziantep, Van, Erzincan, Kayseri, Malatya, Bitlis, Elazığ, Adana, Kahramanmaraş, and Adıyaman populations. The second cluster comprises a haplotype (Type 6) common in the Adıyaman and Gaziantep populations, Haplotype 8 from the Malatya population, haplotypes found in the Aksaray population, and a subcluster of haplotypes from populations to the west of the Anatolian Diagonal (Kırklareli, Edirne, Sam-

**Table 4.** Pair wise nucleotide divergence among the populations of *A. caputmedusae*.

| | VAN | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| VAN | | BİT | | | | | | | |
| BİT | 0.001739 | | BİN | | | | | | |
| BİN | 0.001739 | 0.000000 | | ELA | | | | | |
| ELA | 0.000869 | 0.002924 | 0.002924 | | ADI | | | | |
| ADI | 0.002700 | 0.007980 | 0.007980 | 0.000270 | | GAZ | | | |
| GAZ | 0.000747 | 0.005666 | 0.005666 | 0.001017 | 0.002518 | | ERZ | | |
| ERZ | 0.000241 | 0.000338 | 0.000338 | 0.000393 | 0.004679 | 0.002552 | | MAL | |
| MAL | 0.000757 | 0.003057 | 0.003057 | 0.000958 | 0.001770 | 0.000018 | 0.000515 | | KAH |
| KAH | 0.002377 | 0.003500 | 0.003500 | 0.017834 | 0.007038 | 0.010404 | 0.028618 | 0.021416 | |
| ADA | 0.000250 | 0.001250 | 0.001250 | 0.000848 | 0.000244 | 0.000649 | 0.000091 | 0.000147 | 0.018750 |
| SAM | 0.011314 | 0.018485 | 0.018485 | 0.007991 | 0.002430 | 0.003570 | 0.014203 | 0.009276 | 0.008512 |
| AMA | 0.007217 | 0.015162 | 0.015162 | 0.005899 | 0.006804 | 0.005505 | 0.010479 | 0.005914 | 0.020454 |
| ÇOR | 0.006633 | 0.014148 | 0.014148 | 0.004201 | 0.001514 | 0.001570 | 0.009688 | 0.004922 | 0.010960 |
| YOZ | 0.007217 | 0.015162 | 0.015165 | 0.005899 | 0.006804 | 0.005505 | 0.010479 | 0.005914 | 0.020454 |
| KAY | 0.000927 | 0.000811 | 0.000811 | 0.000567 | 0.003333 | 0.001299 | 0.000653 | 0.000450 | 0.025732 |
| AK | 0.014026 | 0.018684 | 0.018684 | 0.011649 | 0.035000 | 0.035000 | 0.015705 | 0.012914 | 0.018790 |
| KAS | 0.008248 | 0.015548 | 0.015548 | 0.005259 | 0.000776 | 0.001510 | 0.011199 | 0.006332 | 0.008119 |
| BOL | 0.012129 | 0.019527 | 0.019527 | 0.008844 | 0.004098 | 0.005152 | 0.015127 | 0.010623 | 0.009630 |
| SAK | 0.031521 | 0.035000 | 0.035000 | 0.028266 | 0.021822 | 0.023589 | 0.032632 | 0.030448 | 0.029257 |
| BUR | 0.031521 | 0.035000 | 0.035000 | 0.028266 | 0.021822 | 0.023589 | 0.032632 | 0.030448 | 0.029257 |
| BAL | 0.017545 | 0.023516 | 0.023516 | 0.014067 | 0.008047 | 0.009593 | 0.019856 | 0.016056 | 0.013504 |
| ÇA | 0.031521 | 0.035000 | 0.035000 | 0.028266 | 0.021822 | 0.023589 | 0.032632 | 0.030448 | 0.029257 |
| EDİ | 0.015021 | 0.017937 | 0.017937 | 0.013234 | 0.010124 | 0.010320 | 0.015862 | 0.013506 | 0.024500 |
| KIR | 0.019583 | 0.027393 | 0.027393 | 0.015779 | 0.009294 | 0.010864 | 0.022780 | 0.017493 | 0.012924 |
| ISP | 0.003414 | 0.009165 | 0.009165 | 0.002579 | 0.003911 | 0.002478 | 0.005619 | 0.002422 | 0.020516 |
| KO | 0.001404 | 0.005159 | 0.005159 | 0.001140 | 0.003579 | 0.001874 | 0.002648 | 0.001113 | 0.023073 |

**Table 4** (continued)

| | ADA | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ADA | | SAM | | | | | | | |
| SAM | 0.008492 | | AMA | | | | | | |
| AMA | 0.008985 | 0.008579 | | ÇOR | | | | | |
| ÇOR | 0.005851 | 0.000953 | 0.001906 | | YOZ | | | | |
| YOZ | 0.008985 | 0.008579 | 0.000000 | 0.001906 | | KAY | | | |
| KAY | 0.000458 | 0.012366 | 0.008441 | 0.007762 | 0.008441 | | AK | | |
| AK | 0.011210 | 0.010280 | 0.016970 | 0.010393 | 0.016970 | 0.014520 | | KAS | |
| KAS | 0.006191 | 0.003813 | 0.004766 | 0.002859 | 0.004766 | 0.009329 | 0.009012 | | BOL |
| BOL | 0.009553 | 0.006589 | 0.010211 | 0.005339 | 0.010211 | 0.013232 | 0.009489 | 0.004809 | |
| SAK | 0.026064 | 0.019762 | 0.021257 | 0.017567 | 0.021257 | 0.031753 | 0.020854 | 0.017628 | 0.003205 |
| BUR | 0.026064 | 0.019762 | 0.021257 | 0.017567 | 0.021257 | 0.031753 | 0.020854 | 0.017628 | 0.003205 |
| BAL | 0.013513 | 0.009169 | 0.015898 | 0.009300 | 0.015898 | 0.018331 | 0.002147 | 0.007907 | 0.000342 |
| ÇAN | 0.026064 | 0.019762 | 0.021257 | 0.017567 | 0.021257 | 0.031753 | 0.008013 | 0.017628 | 0.003205 |
| EDİ | 0.012078 | 0.009710 | 0.019404 | 0.011159 | 0.019404 | 0.015128 | 0.013338 | 0.008943 | 0.014218 |
| KIR | 0.016276 | 0.008517 | 0.019152 | 0.010384 | 0.019152 | 0.020778 | 0.013321 | 0.007906 | 0.000230 |
| ISP | 0.004503 | 0.006882 | 0.007624 | 0.004352 | 0.007624 | 0.004151 | 0.011321 | 0.004622 | 0.011163 |
| KON | 0.002137 | 0.009194 | 0.008243 | 0.005911 | 0.008243 | 0.016975 | 0.014321 | 0.006652 | 0.011969 |
| | SAK | | | | | | | | |
| SAK | | BUR | | | | | | | |
| BUR | 0.000000 | | BAL | | | | | | |
| BAL | 0.006449 | 0.006449 | | ÇA | | | | | |
| ÇA | 0.000000 | 0.000000 | 0.006449 | | EDİ | | | | |
| EDİ | 0.024500 | 0.024500 | 0.006500 | 0.024500 | | KIR | | | |
| KIR | 0.010545 | 0.010545 | 0.000967 | 0.010545 | 0.011140 | | ISP | | |
| ISP | 0.030771 | 0.030771 | 0.015914 | 0.030771 | 0.014095 | 0.016840 | | KO | |
| KO | 0.030346 | 0.030346 | 0.016428 | 0.030346 | 0.014090 | 0.018619 | 0.000084 | | |

Average Nucleotide Divergence: 0.0320437 +/–0.0000293

sun, Çorum, Yozgat, Kastamonu, Amasya, Bolu, Konya, Balıkesir, Çanakkale, Bursa, Sakarya and Isparta).

An unroted Dollo parsimony majority-rule consensus tree of mtDNA haplotypes is shown in Fig. 4. Results revealed three main clusters of gallwasps from Turkey reflecting a geographical grouping. Cluster A includes haplotypes primarily from sites to the East of the Diagonal (Gaziantep, Adıyaman, Adana, Elazığ, Kahramanmaraş, Kayseri, Bingöl, Erzincan, Van, Malatya and Bitlis); exceptions are haplotypes from Kayseri and Malatya. Cluster B includes one haplotype shared between the Adıyaman and Gaziantep populations, and haplotypes from the Aksaray population. Cluster C comprises haplotypes from sites to the west of the Diago-

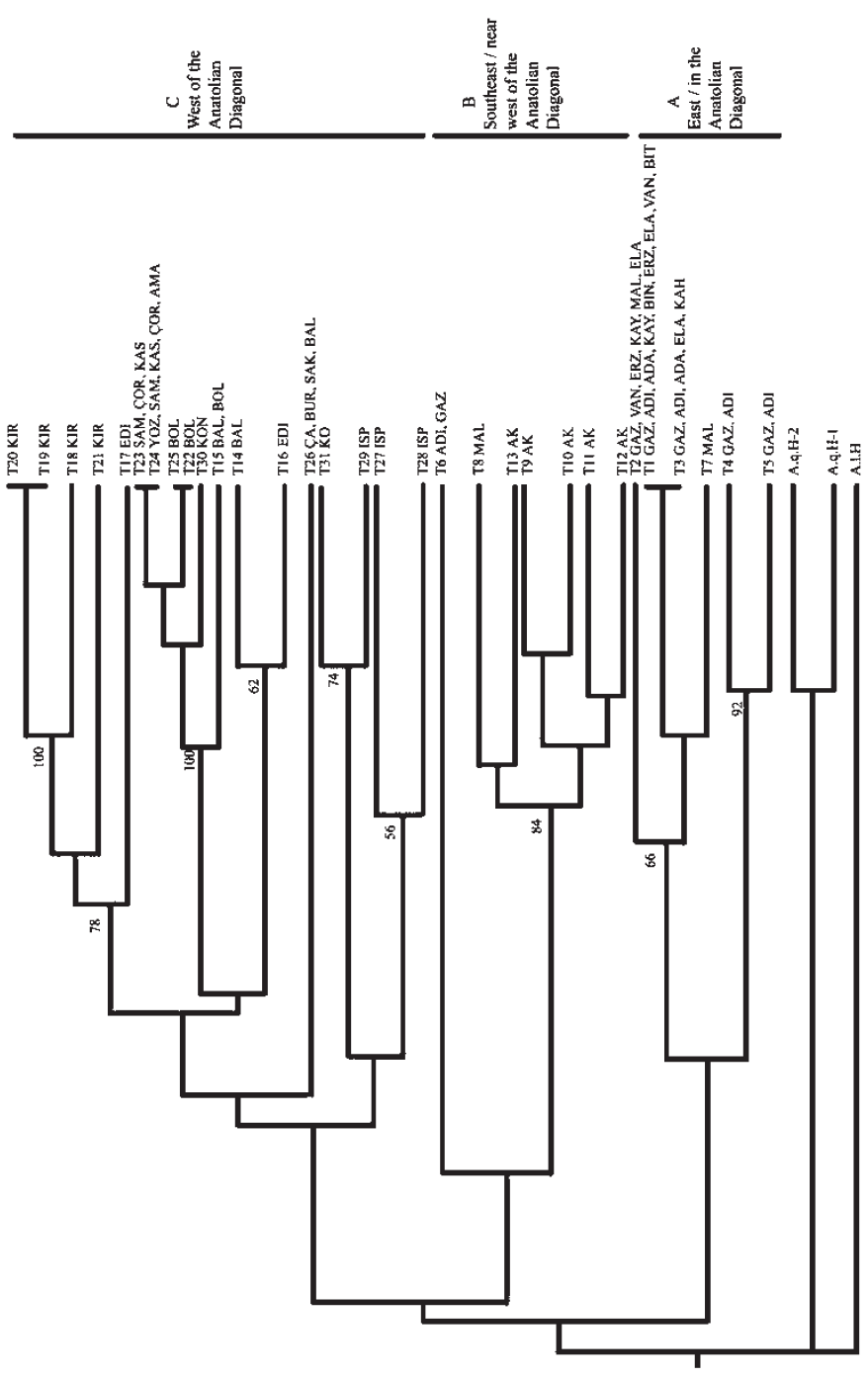


**Fig. 3.** Neighbor-joining dendrogram of the 31 haplotypes of *A. caputmedusae*. Numbers above the branches represent the bootstrap values 1000 replicates of the restriction fragment data between the haplotypes. Support values < 50% are not represented

**Table 5.** Analysis of molecular variance (AMOVA) among the studied oak gallwasp populations grouped into three groupings with respect to their locations: east/in the Diagonal, southeast/near west of the Diagonal and west of the Diagonal. * $P < 0.05$ after 10,000 permutations. Va, Vb, and Vc are the associate covariance components.

| Source of variation | d.f. | Sum of squares | Variance components | Percentage of variation | Fixation indices |
|---|---|---|---|---|---|
| Among groups | 2 | 117.831 | 1.73922 Va | 43.89* | $F_{CT} = 0.43887$ |
| Within groups | 23 | 184.763 | 0.98487 Vb | 24.85* | $F_{ST} = 0.68740$ |
| Within populations | 154 | 190.778 | 1.23882 Vc | 31.26* | $F_{SC} = 0.44290$ |

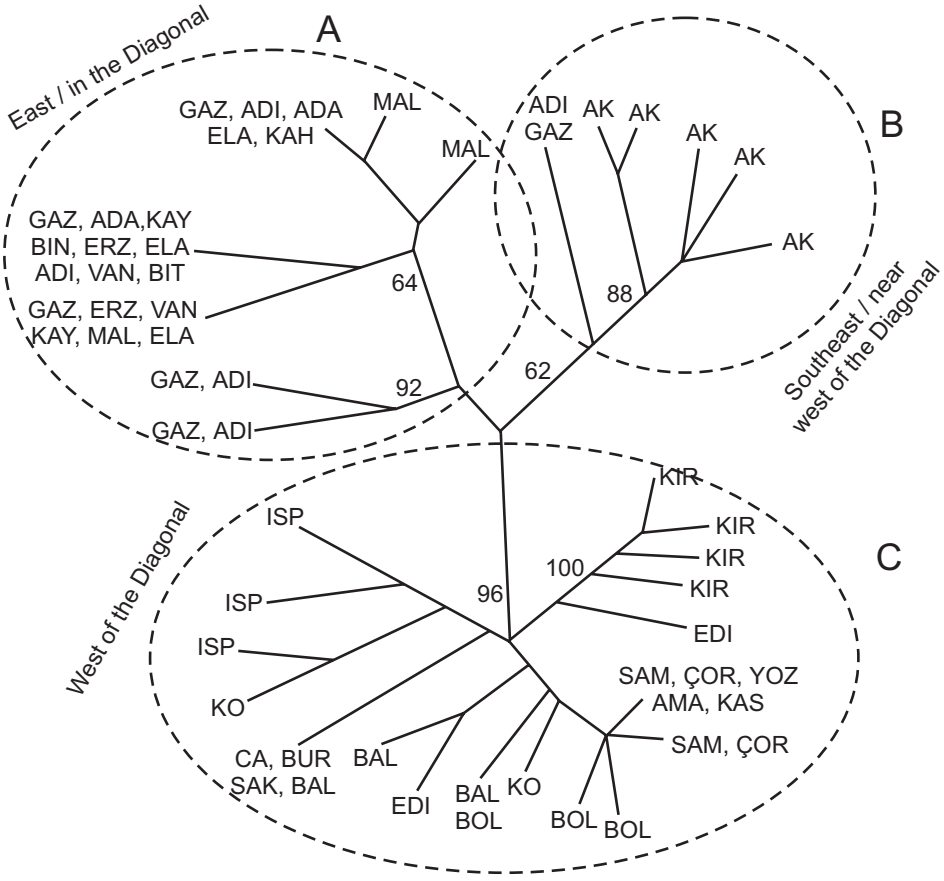


**Fig. 4.** Unrooted Dollo parsimony majority-rule consensus tree of mtDNA haplotypes. Numbers at nodes indicates bootstrap values. Support values < 50% are not represented

nal (Isparta, Konya, Çanakkale, Bursa, Sakarya, Balıkesir, Edirne, Kırklareli, Bolu, Samsun, Çorum, Yozgat, Amasya, Kastamonu). Cluster C further includes a smaller sub-cluster composed of one haplotype from the Edirne population being basal to the haplotypes from the Kırklareli population.

AMOVA analysis revealed that 43.89% of variance was distributed among groups, 24.85% among populations within groups, and 31.26% reflected variance within populations (Table 5). A significant ($P < 0.005$) partitioning of among-group variance indicated that when populations were grouped into three clusters (Clusters A, B and C as seen in Figs 3 and 4) a significant partitioning of molecular variation is obvious and overall AMOVA statistically supports high genetic diversification among clusters A, B and C. Moreover, significant genetic variation is also found within populations of *A. caputmedusae*.

## DISCUSSION

Phylogeographic analyses explore the relationships between gene genealogies and geography that underlie genetic population structure within species. In many species the phylogeny of mtDNA types corresponds well to the geographical distribution of population, and in many instances geographical barriers have been found to shape the current distribution of the lineages (AVISE 2000). Because its complicated geological history (GÖRÜR & TÜYSÜZ 2001) is likely to have been associated with both local and larger scale isolation between faunal elements, the Anatolian Diagonal has been accepted as an active physical barrier dividing species distributions into eastern and western components (ÇIPLAK *et al.* 1993, ÇIPLAK 2004*b*). The present study has revealed an obvious correlation between the genetic relationships among mtDNA haplotypes and their geographical distribution relative to the Anatolian Diagonal in all analyses (Figs 3 and 4). Most of the common haplotypes are shared and present only in eastern populations. Under a phylogeographic approach, it is assumed that more common haplotypes are ancestral haplotypes compared to the derived haplotypes that have geographically restricted distributions (SLATKIN 1991, CRANDAL & TEMPLETON 1993, NEIGEL & AVISE 1993). This suggests that eastern haplotypes are ancestral to those further west.

The oak gallwasp mtDNA haplotypes clustered into three major lineages including internal groupings (Fig. 3). Cluster A comprises haplotypes from populations located east of the Anatolian Diagonal. The second main cluster is divided into two groupings including cluster B, which includes haplotypes found to the southeastern and near west of the Anatolian Diagonal. Cluster C comprises haplo-

types found only to the west of the Diagonal. A very similar pattern is apparent in the unrooted Dollo parsimony majority-rule tree (Fig. 4). The presence of a common haplotype (Type 6) shared between the Adıyaman and Gaziantep populations grouped in cluster B, and haplotypes from the Kayseri and Malatya populations in cluster A may indicate a historical dispersal event in the past from the populations located to the east of more western populations. Furthermore, AMOVA analysis of the mtDNA data supported the presence of high level of genetic structuring (43.89%) among groups when all the populations were grouped into three groupings as east/in the Diagonal (cluster A), south east/near west of the Diagonal (cluster B) and west of the Anatolian Diagonal (cluster C).

Among the studied populations the highest observed level of pair wise sequence divergence among *A. caputmedusae* haplotypes was 3.5%, between the Sakarya, Bursa and Çanakkale populations and those from Bitlis and Bingöl. Adıyaman and Gaziantep populations are similarly diverged from the Aksaray population (Table 4). Over all populations, nucleotide divergence is 3.2%. Mitochondrial DNA divergence can be used to calculate the divergence time of the analyzed mtDNA lineages. Using the general approximation of 2.3% pair wise divergence per million years for insect mtDNA (BROWER 1994), divergence between the most divergent haplotypes and the rest of the lineages dated back to 1.5 MYA, during the Pleistocene climatic fluctuations. The lesser divergence seen among other *A. caputmedusae* populations may indicate higher levels of gene flow or more recent genetic divergence.

The current genetic structures of populations have been greatly influenced both by Pleistocene ice ages and climatic oscillations during the Quaternary periods (AVISE 2000, HEWITT 2000). Although only the highlands of Anatolia were covered by ice sheets during the Pleistocene, the climatic and environmental fluctuations seem to have played a major role in the diversification of *A. caputmedusae* populations in Turkey. The divergent groups are nested, respectively, within one clade containing the eastern haplotypes sampled from the populations east of the Diagonal and another clade containing haplotypes only from southeastern / western populations. Such a separation clearly indicates a genetic barrier between populations based on their location relative to the Anatolian Diagonal, suggesting a causal role for this feature in concert with Pleistocene climatic oscillations in structuring genetic variation in *A. caputmedusae*. All phylogenetic reconstructions have well-supported and congruent topologies, with eastern haplotypes always found as the basal group, and western haplotypes as a single cluster. A region-wide study on a closely-related oak gallwasp species, *Andricus quercustozae* (ROKAS *et al.* 2003), found that Anatolia is not only genetically distinct with refuge specific haplotypes, but also that this region is the center of genetic diversity for this species, with the

Turkish lineages being sources to more western European populations. The greatest nucleotide diversity was observed in Turkey (0.2–4.2%) followed by the lower diversity and divergence estimates in the Balkans (0.2–1.4%), Italy (0.2–0.7%) and Iberia (0.2–1.0%). Furthermore, as in this study a major genetic divide was observed between northeastern and southwestern lineages of *A. quercustozae* spanning the Anatolian Diagonal (ROKAS *et al*. 2003). Although the genetic diversity that can be revealed through PCR-RFLP has less power compared with analysis of haplotype sequences, both haplotype and nucleotide diversities observed in the current study are strikingly high and underline the significance of Anatolia. Similar patterns of higher nucleotide diversity in Turkish than European populations, and genetic subdivision between southern and central-eastern Turkish lineages has also been reported for other species including *Mus musculu*s (GÜNDÜZ *et al*. 2005), and ground squirrels (GÜNDÜZ *et al*. 2007).

The influence of Pleistocene climatic changes in shaping genetic structure has been recognized for many taxa in Europe and in North America. Repeated cycles of restriction to refugia during glacial periods and outward expansion during interglacials have left distinctive marks on the genome of many plants and animal species (HEWITT 1996). During periods of glacial expansion in the Pleistocene, many high-latitude organisms were confined to refugia. During interglacial periods, populations are thought to have expanded from refugia with lineages previously isolated in separate refugia often coming into contact to form geographic zones of genetic discontinuity. Therefore, genetic discontinuities are closely associated with refugia and many of these zones occur in deglaciated regions (HEWITT 1996). Anatolia is accepted as a large non-homogenous refuge area comprising smaller and distinct areas for different taxa to escape from the analogous effects of both glacial and interglacial cycles of the Quaternary period, from which a range of corridors were exploited to disperse into neighboring suitable areas (HEWITT 1999, 2000, ÇIPLAK 2008). In the present study, the observed haplotype number in the Gaziantep population is 6 haplotype, and 5 haplotypes in the Adıyaman population for the oak gallwasp species from Turkey. In addition to the haplotype numbers haplotype and nucleotide diversity in these populations are conspicuously high. Haplotype and nucleotide diversity are calculated as 0.9524 and 0.191023 for the Gaziantep population, 0.9048 and 0.209957 for the Adıyaman population, respectively. The area which extends from the adjoining regions of the Southern, Southeastern and Eastern Taurus Mountains is known as the Maraş triangle, and it is rich in species diversity (ÇIPLAK 2008). The presence of high genetic diversity for *A. caputmedusae* populations adjoining this same region (Gaziantep and Adıyaman populations) indicates that the Triangle is also a centre of intraspecific genetic diversity.

According to the refugia model, genetic diversity is conspicuously higher in or near the refuge area (CHAPCO 1997). Current data may indicate the presence of a possible refuge near the Aksaray population, and Aksaray–Hasan Mountain can thus be considered as a distinct hotspot area. Furthermore, the current results show both Balıkesir and Kırklareli populations to have high haplotype and nucleotide diversity, with 3 haplotypes in 5 analyzed individuals from the Balıkesir population giving with 0.80 haplotype and 0.23 nucleotide diversity values. Likewise, in the Kırklareli population haplotype diversity was 0.81 and nucleotide diversity was 0.15 (Table 3). The current finding of high genetic variation in both populations suggests that these localities may be hotspot areas for diversity and requires further attention. If true, an obvious prediction to test is that similarly distributed taxa, including other oak gallwasps, will show similar and parallel patterns in within-species genetic diversity. This hypothesis is currently under test.

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