ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

FOREWING DEFORMATIONS IN TURKISH HONEY BEE POPULATIONS

Türkiye Bal Arısı Populasyonlarında Ön Kanat Deformasyonları

(Genişletilmiş Türkçe Özet makalenin sonunda verilmiştir)

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ABSTRACT

Honey bees (*Apis mellifera*) have two pairs of membraneous wings that show a general pattern of vein formation almost identical in all individual bees. The wing vein formation as well as the shapes of cells enclosed by veins has been used as morphometric character for honey bee taxonomic studies. However, in addition to this general vein structure, extra-vein formations, vein connections or a specific loss of piece in the existing veins can be seen for unknown reasons, in some individuals. In this study, our aim is to investigate the irregular vein anomalies in forewings of honey bee populations in different regions of Turkey. A total of 6681 honey bee samples from 23 provinces in Turkey were used in the study. In order to visualize the anomalies, structural differences in the veins on the left forewing of individual worker bees were photographed. Different types of formations on the wing veins were observed in some honey bee samples; as formations of new veins, junctions or missing veins. A total of 13 different vein formations were identified. The two most common formations were on the outer edge of the marginal cell and the posterior quarter of the outer surface of the 2rs-m cross vein (previously described as adventitious distal abscissa, "aRs_2") respectively. The vein formation 1 located at the posterior quarter of the outer surface of the 2rs-m cross vein has the highest proportion in the honey bee populations of Black Sea region. The most common adventitious vein (formation 2) has the highest proportion in the honey bee populations of Aegean region. Other deformations on the wing venations were found to occur in low percentages.

Key words: Honey bees, forewing, wing venation, wing cells, anomalies

ÖZ

Bal arıları, (*Apis mellifera*) hemen hemen tüm bireylerde aynı damar oluşumunun genel bir modelini gösteren iki çift zararlı kanada sahiptir. Kanat hücrelerinin şekilleri ile birlikte kanat damarı oluşumu, bal arısı taksonomisi çalışmaları için morfometrik karakter olarak kullanılmıştır. Bununla birlikte, bu genel damar yapısına ek olarak, bilinmeyen nedenlerle bazı bireylerde, ekstra damar oluşumları, damar bağlantıları veya mevcut damarlarda belirli bir parça kaybı görülebilir. Bu çalışmada amacımız, Türkiye'nin farklı bölgelerindeki bal arısı populasyonlarının kanatlarında düzensiz damar anomalilerini araştırmaktır. Çalışmada Türkiye'de 23 ilden toplam 6681 işçi bal arısı kullanılmıştır. Anomalileri görselleştirmek için, işçi arı bireylerinin sol kanatları üzerindeki damarlarda yapısal farklılıklar fotoğraflanmıştır. Bal arısı örneklerinde, kanat damarlarında farklı tipte oluşumlar gözlenmiştir; Yeni damar oluşumları bağlantı noktaları veya eksik damarlar gibi. Toplam 13 farklı damar anomalisi tespit edilmiştir. En yaygın iki oluşum, sırasıyla marjinal hücrenin dış kenarında

(oluşum 2) ve 2rs-m çapraz damarının dış yüzeyinin arka çeyreğinde (oluşum 1) meydana gelmiştir. 2rs-m çapraz damarının dış yüzeyinin arka çeyreğinde meydana gelen damar (oluşum 1) en yüksek Karadeniz Bölgesi bal arısı populasyonlarında görülmüştür. Ek olarak oluşan en yaygın damar (oluşum 2) ise Ege Bölgesi bal arısı populasyonlarında en yüksek oranda kaydedilmiştir. Kanat damarlanmasındaki diğer bozuklukların düşük oranda olduğu tespit edilmiştir.

Anahtar kelimeler: Bal arıları, ön kanat, kanat damarlanması, kanat hücreleri, anomaliler

INTRODUCTION

Honey bees (*Apis mellifera*) have two pairs of membraneous wings stabilized by a complex pattern of wing veins. Especially, the forewings are rich source for genetic and taxonomic analyses in honey bee species or subspecies. The general shapes of the wing vein formations are more or less the same in all individual worker honey bees. Based on this general vein formation, vein geometry and angles between veins have been used for discriminating different honey bee populations, classifying honey bee species and subspecies (Alpatov, 1929; Goetze, 1964; Ruttner et al., 1978; Ruttner, 1988; Bouga et al. 2011) and also discriminating evolutionary honey bee lineages (Kauhausen and Keller, 1994). Within honey bees (Apidae: *Apis)* especially *A. mellifera*, geometric morphometric analysis of wing formed by vein system has been extensively studied to recognize the honey bee groups, subspecies and species (Francoy et al., 2006, 2008; Tofilski, 2008; Kandemir et al., 2009; Rattanawannee et al., 2010; Kandemir et al., 2011; Koca, 2012). Although vein formations and cell shapes on forewing are generally the same in all individual bees, taxonomically unimportant extra-vein formations, irregular vein connections and junctions or a specific loss of piece in the existing veins and asymmetries between right and left wing (Smith et al., 1997) have been found in some individuals. Many researchers have pointed out deformations (changes in wing venation pattern) in the workers, queens or drones in their studies (Akahira and Sakagami, 1959; Baehrman, 1963; Goetze, 1964; Tan et al., 2008; Wegrzynowicz, 2010; Mazeed, 2011; Porporato et al., 2014). Among these studies, the two most common extra-vein formations take place on the outer edge of the marginal cell and the posterior quarter of the outer surface of the 2rs-m cross vein (respectively described as adventitious distal abscissa, “aRs_2”, previously) (Akahira and Sakagami, 1959; Tan et al., 2008; Mazeed, 2011). Goetze (1959) stated that forewing deformations are not inherited and may arise from unsuitable environmental factors acting on the development of the honey bees. On the contrary, Baehrman (1963) indicated that honey bee wing deformations can only be caused by genetic factors. In this study, our aim is to investigate the irregular vein anomalies in forewings of honey bee populations in different regions of Turkey.

MATERIALS AND METHOD

A total of 6681 forewings of individual worker bees (from 686 colonies) were sampled from 23 provinces belong to different geographic regions in Turkey **(Table 1, Figure 1)**. The left forewings of individual worker bees were photographed with Leica MZ16 microscope system and each wing image was saved as a .jpg file **(Figure 2)**. About 5-10 forewings were sampled from each colony and examined for the presence of wing anomalies. Observed deformations (extra-vein formations, vein connections or a loss of piece in the veins) were divided into groups, registered and also, recorded as presence in each individual forewing. The percentage of deformations according to provinces in the regions were calculated **(Table 2)**. Overall data from 23 provinces were assigned to different regions in Turkey (Marmara-Thrace, East, Aegean, South, Black Sea and Central) and also to different subspecies according to the distribution given in Ruttner (1988) as *A. m. anatoliaca*, Western Anatolian honeybees, *A. m. caucasica*, *A. m. meda* and the *A. m. carnica* like bees in Thrace. The similarity of percentages of the groups was tested based on correlation by using ANOSIM implemented in PAST (Hammer et al., 2001).

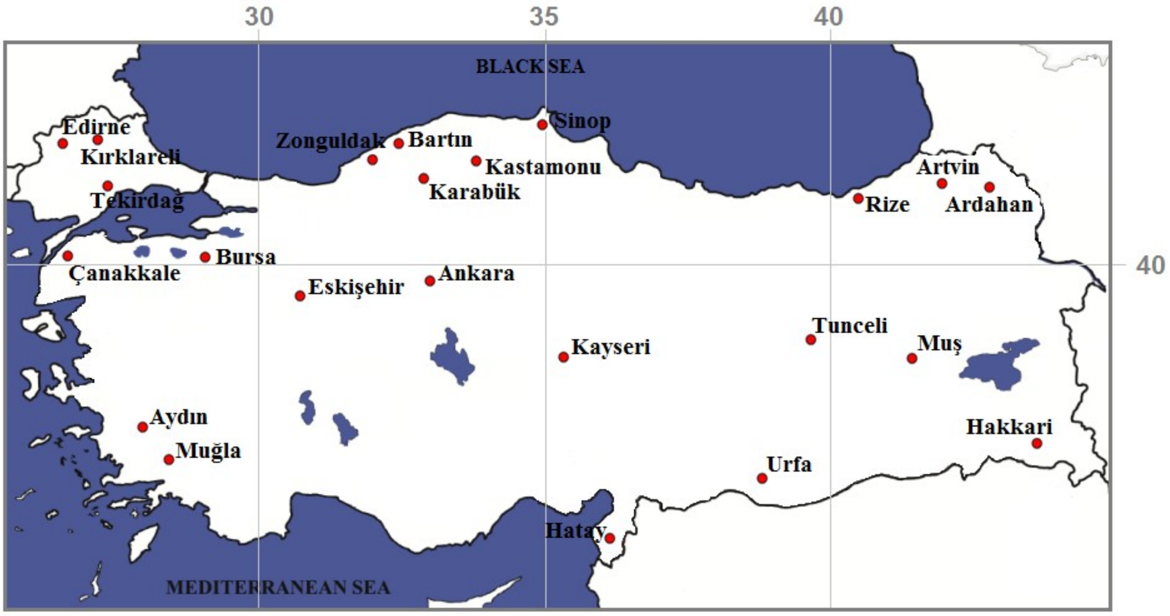

Figure 1. Map indicating sampling provinces in Turkey.

Table 1. Sampling locations from different regions in Turkey.

Region	Province	# of Hive	# of Individual
Thrace and Marmara	Bursa	23	230
	Çanakkale	45	426
	Tekirdağ	19	186
	Edirne	19	190
	Kırklareli	28	279
Aegean	Aydın	15	150
	Muğla	99	990
Central Anatolia	Eskişehir	37	368
	Ankara	43	428
	Kayseri	20	181
Black Sea	Zonguldak	14	136
	Bartın	21	204
	Karabük	18	178
	Kastamonu	9	83
	Sinop	15	145
Northeast Anatolia	Rize	29	285
	Artvin	42	404
	Ardahan	89	863
East and Southeast Anatolia	Muş	20	200
	Tunceli	15	124
	Hakkari	29	263
	Urfa	8	78
	Hatay	29	290

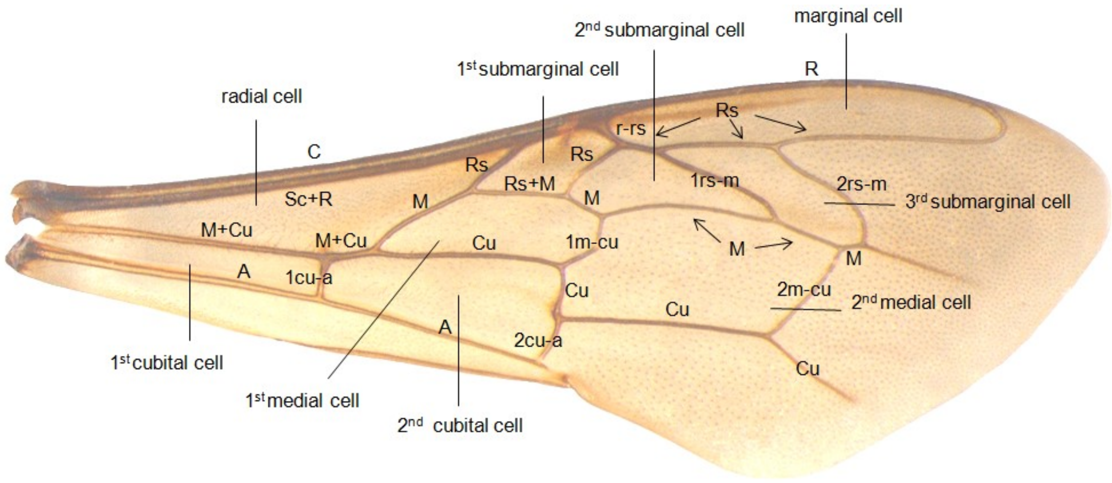

Figure 2. Honey bee forewing structure, name of the wing cells and the main veins on the forewing. *A*: anal vein; *C*: costal vein; *Cu*: cubital vein; *M*: medial (basal) vein; *R*: radial vein; *Rs*: radial sector; *Sc*: subcostal vein.

RESULTS

Different types (extra-vein formations, vein connections or loss of piece in veins) of deformations on the forewing veins were observed in honey bee samples belonging to different honey bee populations in Turkey **(Figure 3)**. Although, worker bees generally have standard venation pattern, some individuals have forewings with both additional vein extensions and lost veins, and in some cases there is a single type of deformation in the forewings of worker honey bee individuals. A total of 13 different irregular vein deformations, most of them indicated in previous studies, were recorded **(Figure 4)**. Most of these deformations were observed as the formation of irreguler new vein extension excluding vein formations 11 and 12. The formation of irregular new veins, numbering 1, 2, 4, 5, 6, 7, 8 and 10, occurred with different lengths, while the new vein extension, numbering 3, did not appear on a different length. Deformations 11 and 12 occurred in the form of a specific loss on apical part of 1rs-m vein. The formation of irregular new vein extensions, with the exception formations 1 and 2, was more likely to occur in the interior of the wing cells. The vein formations, numbering 1 and 2, are the most common extra-veins and extend out from the cells to the wing surrounding surface. In some individuals, a new additional vein developed on both veins (on 1rs-m and M veins), thus creating an open or even closed new small marginal cell. The average rate of vein deformation varies between 0.3% and 17.7% **(Figure 4)**. As shown in **Figure 4**, and with respect to wing venation pattern of the forewing, the highest percentage (17.7%) of vein formation (identified as the formation of new extra-vein, appearing with different lengthes in previous study) was registered on the end of the outer edge of the marginal cell (formation 2) and this rate was followed by formation 1, located at the posterior quarter of the outer surface of the 2rs-m cross, with a percentage of 16.3%. Conversely, the vein deformation, numbered 11 (average 0.3%) were seen the lowest percentage in the honey bee populations **(Figure 4).**

The most common vein (formation 2), located at the outer edge of the marginal cell, was seen the highest percentage (average 46%) in the honey bee populations of Aegean region and the lowest percentage (average 9%) in the populations of Central Anatolia region. The highest percentage of worker bees having this extra-vein was registered in Hakkari and Aydın provinces at a percentage of 75.7% and 62%, respectively and it was registered less than 50% in other provinces. And also, second most common vein (formation 1), located on the posterior quarter of the outer surface of the 2rs-m cross vein, was seen the highest percentage (average 21%) in the honey bee populations of Black Sea region and the lowest percentage (average 14%) in the populations of Thrace and Marmara region.

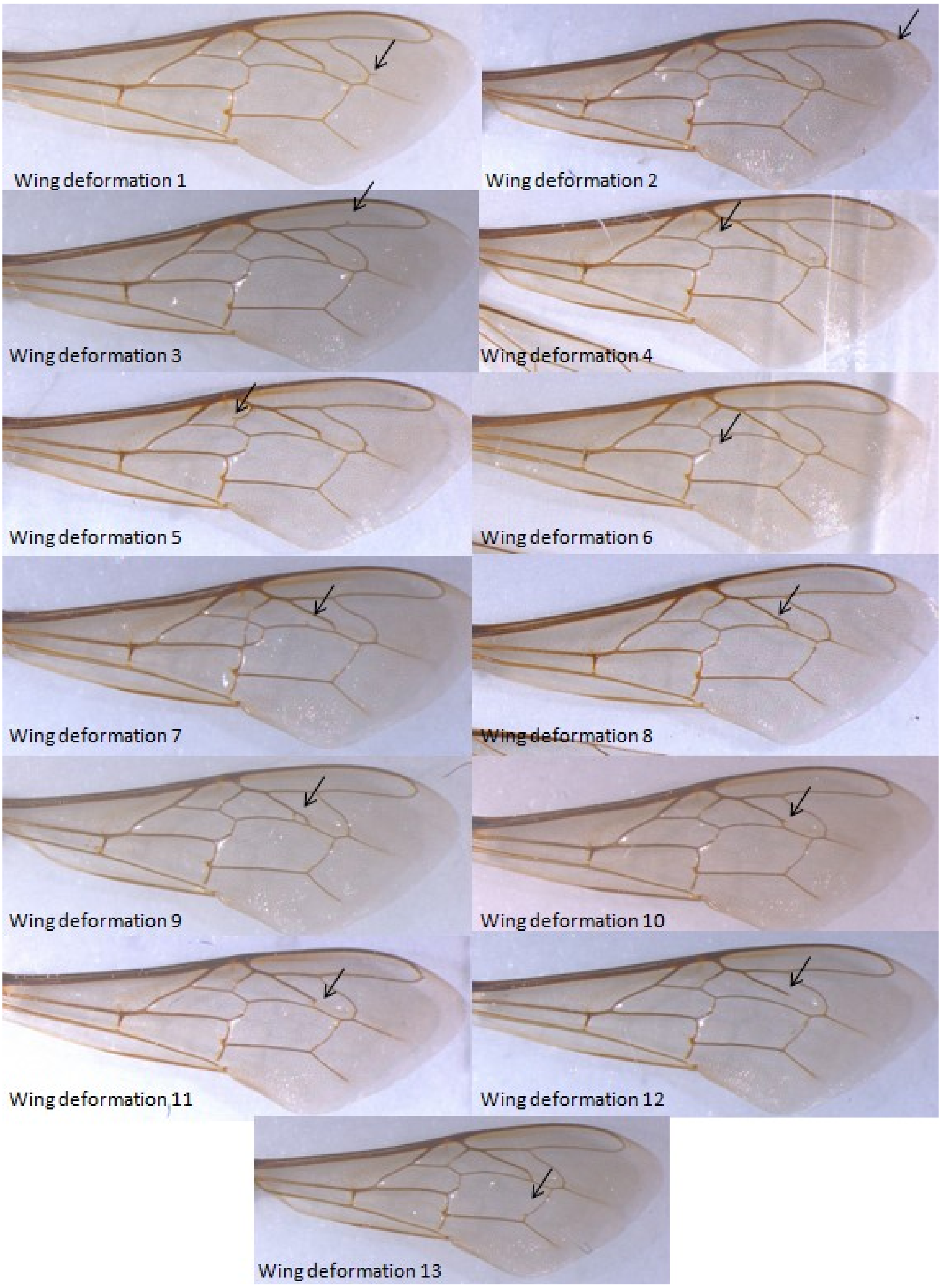

Figure 3. All honey bee forewing deformations observed in the study.

Table 2. Distribution of deformation percentages with respect to provinces.

Province	# of Hive	# of Individual	Deformation %												
			1	2	3	4	5	6	7	8	9	10	11	12	13
Bursa	23	230	17.4	13.9	2.6	0.4	8.7	5.7	6.5	0.9	1.7	7.4	0.0	2.6	0.4
Çanakkale	45	426	21.1	8.2	0.0	0.1	9.3	7.0	4.4	0.0	2.3	9.1	0.0	3.0	0.7
Tekirdağ	19	186	9.1	16.7	0.0	0.0	11.8	5.9	4.8	0.0	4.8	10.2	0.0	2.2	0.5
Edirne	19	190	11.1	8.9	0.0	1.6	4.7	5.8	4.2	1.1	1.1	4.2	0.0	1.1	0.0
Kırklareli	28	279	12.5	12.2	1.1	0.4	9.7	3.2	5.4	0.7	1.1	4.3	0.4	0.7	1.4
Aydın	15	150	18.0	62.0	4.0	1.3	24.7	9.3	2.7	0.0	4.7	16.0	0.7	1.3	0.7
Muğla	99	990	16.0	30.0	1.4	1.0	13.0	4.9	3.7	2.0	3.0	12.0	1.4	3.0	0.1
Eskişehir	37	368	12.7	8.9	2.4	0.3	12.2	4.1	4.1	0.8	1.9	5.7	0.5	1.4	0.3
Ankara	43	428	14.0	7.7	0.9	0.5	6.3	4.7	7.2	1.4	2.6	7.7	0.0	1.9	0.5
Kayseri	20	181	18.1	10.4	0.0	2.2	13.7	7.1	8.8	1.1	1.6	6.6	0.0	0.0	0.0
Zonguldak	14	136	24.3	49.3	2.2	4.4	28.7	36.0	2.9	1.5	2.9	2.2	0.7	2.2	0.0
Bartın	21	204	14.7	9.4	0.3	0.3	5.3	4.2	3.1	0.6	0.6	4.4	0.0	0.8	0.0
Karabük	18	178	28.1	4.5	2.2	3.9	14.6	5.6	5.6	0.0	2.8	11.8	0.6	2.2	0.6
Kastamonu	9	83	3.1	2.5	0.0	0.3	1.9	0.6	1.4	0.0	0.6	0.6	0.0	0.3	0.0
Sinop	15	145	33.1	14.5	0.7	1.4	8.3	12.4	5.5	0.7	3.4	7.6	0.0	1.4	0.7
Rize	29	285	20.4	13.3	0.7	0.7	4.9	9.1	4.2	0.7	0.7	2.5	0.0	0.7	0.4
Artvin	42	404	12.1	14.9	0.2	0.7	6.7	8.9	6.2	0.2	1.0	2.5	0.2	0.2	1.0
Ardahan	89	863	11.0	7.5	1.3	0.9	12.4	5.4	4.4	0.0	0.3	1.0	0.1	0.1	1.4
Muş	20	200	15.0	10.0	2.5	4.0	9.5	7.0	7.5	1.5	2.5	6.5	0.5	1.5	0.5
Tunceli	15	124	21.8	10.5	0.0	1.6	7.3	5.6	8.9	1.6	0.0	5.6	0.0	0.0	1.6
Hakkari	29	263	27.0	75.7	1.1	1.9	27.8	24.7	12.5	3.0	1.5	4.2	0.0	0.4	0.8
Urfa	8	78	3.8	6.4	0.0	2.6	3.8	5.1	3.8	0.0	1.3	3.8	0.0	2.6	0.0
Hatay	29	290	16.8	14.7	0.5	1.5	7.5	5.0	0.8	5.9	3.1	7.2	0.0	0.2	0.0

The highest percentage of worker bees, having this extra-vein, was registered in Sinop provinces at a percentage of 33.1% and it was registered less than 30% in other provinces. The other deformations were found to be more rare in each of the honey bee populations. The vein deformation (deformation 11), determined as a specific losses at the inferior edge of the 1rs-m vein occured at a very low percentage (less than 0.3%) in honey bee populations of all regions **(Table 2).**

Similarity of percentages of the groups (both regional and subspecies) showed that only Western Anatolian honeybees and the Aegean region is significantly different than the other subspecies and the regions with a P value 0.0215 and 0.045 respectively. The other regional groups and the subspecies group did not differ significantly based on the wing deformation percentages.

DISCUSSION

In the present detailed study, we found that there are 13 wing deformations in the form of extra and missing veins in the forewings in honey bee populations of Turkey. The great majority of deformations that are observed have been reported in previous studies on the forewings of honey bee populations. Most of these vein deformations are in the form of short extensions that extend into the cell. Two vein deformations commonly found in our study have been encountered in all of the previous studies (Akahira and Sakagami, 1959; Tan et al., 2008; Wegrzynowicz, 2010; Mazeed, 2011; Porporato et al., 2014). The short vein extension on the Rs5 vein close to the intersection with the 2rs-m vein (formation 3) was first found in this study **(Figure 4)**. Akahira and Sakagami (1959) reported that the new veins in the form of extension are found in both forewing of worker honey bees and drones. In our study, only worker bees were used for the existence of vein formations.

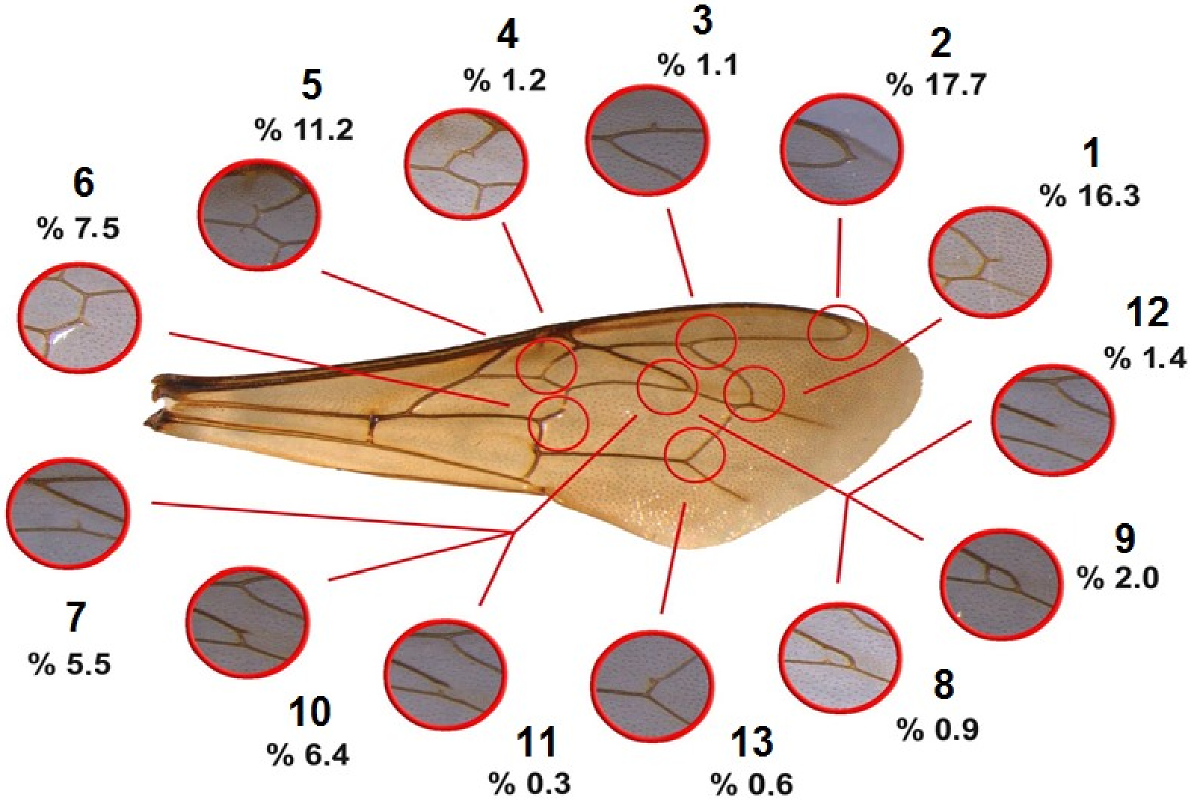

Figure 4. Forewing venation pattern showing the different wing cells with different kinds of vein deformations (each circle shows a different deformation).

Both marginal and 3rd submarginal cells in the forewing have the most common number of adventitious veins, especially the vein formations 1, located at the outer edge of the marginal cell and formations 2, described as adventitious distal abscissa, "aRs_2", previously. Mazeed (2011) has emphasized the same result in his research on Carniolan and Egyptian bee populations in Egypt. Porporato et al. (2014) studied on *A. mellifera mellifera*, *A. m. ligustica*, *A. m. carnica*, and hybrid colonies and also they described these two anomalies as the most widespread vein formations. Tan et al. (2008) have shed light on their ancestral status by testing whether these two formations are in *Apis* species and *Apis mellifera* subspecies. They also indicated that the highest frequency of *aRs*2 in *A. mellifera* populations, was found in the "Oriental" lineage and the honey bee populations that we have studied are also the same lineage. Since both cells, in which the two most common vein formations are encountered, are used to distinguish honey bee populations, it can be said that apical forewing cells may be more susceptible to selective pressures than other forewing cells, thus showing higher variation due to subspecies differentiation (Goetze, 1964; Francoy, 2006; Mazeed, 2011). Our findings suggested that the bees found on the southern locations were prone to more deformation percentages than the central and northern locations when the percentage data considered. However the statistical test showed only part of the southern locations (Muğla and Aydın) were significantly different than the other locations when grouped into regions. Similarly when data grouped into different subspecies based on Ruttner (1988) only Western honeybees (distributed only in Aydın and Muğla provinces) were significantly different than the other subspecies distributed in the rest of the country. Thus based on the results, it is hard to come to a conclusion about the formation of changes in the forewings that are caused by a genetic, developmental and environmental conditions. But it is seen that there are differences other than those mentioned but this difference among groups are not statistically significant.

The wing venation of bees was revised and terminology was standardized by Engel (2001). The wing venation pattern is widely used for the discrimination of honey bee subspecies based on standard morphometric and geometric morphometric approach. Except for general pattern of wing veins, the formations and/or losses that may occur due to genetic, developmental and environmental conditions in the veins were determined in different studies. The formations observed in the wings of the worker bees are ignored in morphometric studies; first there is no clear explanations of the reason and second it is random and there is no point of usage of such deformations in subspecies delimitations or assignment as mostly morphometrics used for. With the effect of selective pressures, presence of parasites in the hive and diseases of bees are frequently mentioned to cause these anomalies. And to explain the cause of such deformation a better experimental design should be designed considering to include genetical, and environmental factors. The results of such carefully designed study may indicate the reason of reacquisition of ancestral traits and thus may provide an insight into honey bee evolutionary history (Tan et al., 2008).

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GENİŞLETİLMİŞ ÖZET

GİRİŞ

Bal arıları (*Apis mellifera*) Hymenoptera takımında yer almakta ve zarlı bir yapı ile çevrili iki çift kanada sahip bulunmaktadır. Evrimsel açıdan damar oluşumlarının genel şekilleri, bütün arı bireylerinde aynıdır. Damar oluşumları ve damarlar içindeki ve damarlar arasındaki hücre şekilleri farklı taksonomik düzeylerde bal arılarını ayırt etmek için morfometrik karakterler olarak yaygın bir şekilde kullanılmaktadır. Genel damar yapısının dışında, bilinmeyen nedenlerle bazı bireylerde ekstra damar oluşumları, damar bağlantıları veya mevcut damarlarda belirli bir parça kaybı görülebilir. Bu oluşumların çevresel ve genetik faktörlerden kaynaklanmış olduklarına dair öngörüler ortaya konulmuştur. Bu çalışmada ülkemizde yayılış gösteren farklı bal arısı populasyonlarında toplanan bal arısı örneklerinin kanatları üzerindeki deformasyonlar ya da anomalilerin incelenmesi amaçlanmıştır.

MATERYAL ve METOT: Bu çalışmada amacımız doğrultusunda farklı projeler çerçevesinde (TBAG 107T154 and 110T518) farklı illerden çok sayıda bal arısı örneği toplanmıştır. Sunulan araştırmada farklı bölgelerde yer alan toplam 23 il ziyaret edilmiş ve 686 kovandan 6681 işçi bal arısı elde edilmiştir (**Tablo 1, Şekil 1**). Yukarıda adı geçen projeler çerçevesinde bu kolonilere ait örneklerin sol kanatları mikroskop lamları arasına sabitlenmiştir (**Şekil 2**). Kanatlar MZ-16 Leica mikroskobu ile fotoğraflanmıştır ve tüm kanatlarda anomaliler gözle tek tek taranmış ve var olan anomaliler not edilmiştir (**Şekil 3**).

SONUÇLAR

Çalışmamızda kullandığımız bal arısı örneklerinde, sol kanat damarlarında farklı tipte oluşumlar (deformasyonlar-anomaliler) gözlenmiştir; Yeni damar oluşumları, buna bağlı yeni hücre oluşumları gibi. Buna ek olarak bazı bireylerde oluşumlar bağlantı noktaları veya eksik damarlar olarak gözlenmiştir. Toplam olarak çalışmamızda 13 farklı damar anomalisi tespit edilmiştir (**Şekil 3**). En yaygın iki oluşum, sırasıyla marjinal hücrenin dış kenarında ve 2rs-m çapraz damarının dış yüzeyinin arka çeyreğinde meydana gelmiştir. 2rs-m çapraz damarının dış yüzeyinin arka çeyreğinde meydana gelen damar (oluşum 1) en yüksek Karadeniz Bölgesi bal arısı populasyonlarında görülmüştür. Ek olarak oluşan en yaygın damar (oluşum 2) ise Ege Bölgesi bal arısı populasyonlarında en yüksek oranda kaydedilmiştir. Diğer deformasyonların yüzdesi düşük bulunmuştur (**Tablo 2, Şekil 4**).

TARTIŞMA

Genel olarak tüm anomalilerin dağılımları göz önüne alındığında, güneydeki lokasyonlarda bulunan arıların, Orta Anadolu ve kuzeydeki lokasyonlarda bulunanlardan daha fazla deformasyon yüzdelerine sahip olduğu görülmektedir. Bunun da en önemli nedenleri arasında kanat gelişimi üzerinde farklı baskılara maruz kaldığı düşünülebilir. Bundan dolayı kanat damar oluşumlarının gelişim sürecindeki farklı çevresel koşullar tarafından şekillendirildiği düşünülebilir. Ayrıca daha önce yapılan çalışmalarda genetik faktörlerin de bu anomalilere neden olabileceği bildirilmiştir. Çok zamandan beri kanat üzerindeki bu damar oluşumlarının morfometrik çalışmalarda dışlandığı görülmektedir. Bunun da en önemli nedeni bu tür anomalilerin neden kaynaklandığının açık bir şekilde açıklanamamasıdır. Ancak son yıllarda yapılan çalışmalar bu tür anomalilere neden olarak farklı seçici baskısının, kovanda yer alan parazitler ve hastalıkların bal arısı kanadında bu tür anomalilerin oluşmasına neden olabileceğini göstermektedir. Ek damarların meydana gelmesi belki de atasal karakterlerin yeniden kazanımına bir gösterge olabilir. Bu da bal arısı evrimsel tarihçesine bir ışık tutmada yararlı olacaktır.