

## Determining the Relative Abundance of Honey Bee (*Apis mellifera* L.) Races in Kars Plateau and Evaluating Some of Their Characteristics

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Makale Kodu (Article Code): KVFD-2010-2081

### Summary

During June-July period 2009, 310 honeybee samples were collected from 32 different colonies in Kars Plateau. The majority of the samples (71.6%, n=222) were hybrid form of *Apis mellifera*. The ratio of *Apis mellifera caucasica* was 26.5% (n= 82) and only 1.9% (n=6) of samples were found to be *A. mellifera remipes*. Tarsi length, fore wing length, fore wing width, number of hamulus hind wing, femur length of hind leg, tibia length of hind leg, metatarsus length of hind length, metatarsus width of hind leg of *A. mellifera caucasica* and that of hybrid form of *A. mellifera* were measured and the measurements of those morphological characters of different subspecies were compared. In addition of the morphological traits, variation patterns of proteins bades on gel electrophoresis was measured from the two subspecies and their hybrids. Gel electro-phoresis patterns reveal that the total proteins of pure Caucasian race individuals from Ardahan and Kars were similar. On the other hand, the protein bands of pure Caucasian race individuals (66 kD, 45 kD, 29 kD and 18 kD) showed clear differences from that of hybrid individuals.

**Keywords:** *Apis mellifera caucasica*, *Apis mellifera remipes*, Hybridization, Kars Plateau

## Kars Platosu'ndaki Bal Arısı (*Apis mellifera* L.) Irklarının Nispi Yoğunlukları ve Bazı Özellikleri

### Özet

Haziran-Temmuz 2009 periyodunda Kars Platosu'ndaki 32 farklı koloniden 310 bal arısı toplandı. Toplanan örneklerin çoğunluğunun (%71.6, n=222) *Apis mellifera*'nın hibrit formu olduğu belirlendi. *Apis mellifera caucasica*'nın oranı %26.5 ti (n= 82). *A. mellifera remipes*'e ait yalnızca 6 birey (1.9%) tespit edildi. *A. mellifera caucasica* ve *A. mellifera* melez formlarının vücut uzunluğu, ön kanat uzunluğu, ön kanat genişliği, arka kanat çengel sayısı, arka bacak femur uzunluğu, arka bacak tibia uzunluğu, arka bacak metatarsus uzunluğu, arka bacak metatarsus genişliği morfolojik ölçümü yapıldı. Bu özelliklerden 3 tanesi (tarsi uzunluğu, çengel sayısı ve femur uzunluğu) saf kafkas ve melez formlar arasında istatistiksel olarak anlamlı farklılık gösterdi (P<0.05). Elektroforez çalışmalarıyla, Ardahan ve Kars'taki saf Kafkas arı ırklarının protein bantlaşmasının oldukça benzer olduğu belirlendi. Diğer taraftan melez formlar ile saf Kafkas ırkına ait protein bantlarında önemli farklılıklar (66 kD, 45 kD, 29 kD and 18 kD) tespit edildi.

**Anahtar sözcükler:** *Apis mellifera caucasica*, *Apis mellifera remipes*, Melezleşme, Kars Platosu

### INTRODUCTION

There has been limited investigation Apoidea in North East Anatolia <sup>1</sup>. Honeybees (*Apis mellifera* L.) from Anatolia express very high degrees of genetic diversity at subspecific level <sup>2,3</sup> and conventionally being cited as "the place which has no match in the world." Existence of important bee species in different geographical areas of Anatolia is well documented <sup>2</sup>. Presence of Caucasus race (*Apis mellifera caucasica*) which produces more

honey than other races in North East Anatolia, trans-Caucasian race (*Apis mellifera remipes*) and Armenian race (*Apis mellifera armeniaca*) in Eastern Anatolia <sup>3-7</sup>, the Anatolian race (*Apis mellifera anatoliaca*) in central Anatolia <sup>6,8-11</sup>, Karniyol race (*Apis mellifera carnica*) in Thrace region <sup>12</sup>, and Syrian race (*Apis mellifera syriaca*) and Iranian race (*Apis mellifera meda*) in Southeast Anatolia <sup>4,5,13</sup> were reported.

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Ruttner (1998) and Güler (1999) defined some ecotypes within each geographical region that serve as transition areas such as, Muğla (Aegean Region) and Borçka-Camili (Eastern and North Eastern Anatolia Region)<sup>5,6</sup>.

In Ardahan, Artvin, Kars and some neighboring provinces, presence of pure Caucasian bees were noted in first half of the last century, but after mobile apiculture began in 1950s the number of pure bee colonies in this region has gradually decreased. Questions are raised to the existence of any pure Caucasian race in these provinces nowadays<sup>14</sup>.

Motherland of Caucasian bees is Central Caucasia, and it has two types, namely *A. m. caucasica* (mountain type) and *A. m. remipes* (lowland type). *A. m. caucasica* is the grey-colored mountain type Caucasian bee which lives in the high valleys of the region; whereas *A. m. remipes* is the yellow-colored lowland type Caucasian bee that lives in the low areas of the region. In Turkey, mountain-type Caucasian bee (*A. m. caucasica*) can be found in pure and hybrid forms in the plateaus of Eastern Anatolia as well as in close proximity to the Caucasian region<sup>15</sup>. This race is also one of the economic honeybee races and widely apicultured in Turkey and around the globe<sup>16</sup>.

Firatlı and Budak<sup>17</sup> noted that the widespread mobile apiculture has altered the genetic structure of Anatolian bee populations leading a dramatic increase in admixed populations. It has been claimed that pure Caucasian races can solely be found in isolated areas<sup>18</sup> such as, Posof district of Ardahan Province and Camili basin in Borçka district of Artvin Province<sup>19</sup>. Increasing mobile beekeeping in Anatolia and commercial distribution of hybrid queen bees marked at pure Caucasian have collectively caused a high degree of hybridization among ecotypes adapted different ecogeographic regions leading extinction of unique genetic properties of the local landraces<sup>18</sup>.

The objectives of this study were (i) to determine the relative abundance of *Apis mellifera* races gathered from Kars Plateau, (ii) to conduct their morphometric analysis, and (iii) to analyze their proteins structures. We hope this study will contribute to conservation activities for *Apis mellifera caucasica* in the area.

## MATERIAL and METHODS

During June-July period 2009, 310 honeybee samples were taken from the entrance of hives from 32 different colonies in Kars Plateau (Table 1). In addition, 10 samples of *Apis mellifera caucasica* were taken from Ardahan province. All of the samples were transferred to the laboratory alive in special insect cages, were placed in eppendorf tubes, fixed at -20°C, and stored there until they were used. Carl Zeiss Jena brand stereo-microscope was used for morphological measurements and pictures.

In order to infer degree of admixture between races, pictures of big stains and striping in the first and second tergite of abdomen were taken. The identification of *A. mellifera* races was performed according to Güler et al.<sup>6</sup> and Güler<sup>14</sup>.

As a part of morphometric characterization of the collected samples, general body size, length of fore wing, width of fore wing, the number of hamulus in the hind wing, width of hind leg femur, metatarsus length of hind leg, and metatarsus width of hind leg were measured. Furthermore, dappling (brown spots) and striping (yellow-dark yellow) in first and second tergites of abdomen were also examined.

Means and standard deviations of all the morphological traits measured were computed for pure Caucasian race and hybrids separately from each location using SAS 9.2<sup>20</sup>. The difference of means for each morphological trait between *A. mellifera caucasica* and hybrid samples of *A. mellifera* were inferred via student t-test using SPSS<sup>21,22</sup>.

Abdomen, wing and leg parts of each sample was removed for electrophoretic analysis, and remaining head and thorax parts were homogenized in eppendorf tubes by adding 250 µl of pure water. Then, samples were centrifuged at 12.000 rpm for 15 min. at +4°C. After supernatant was obtained, it was transferred into tubes<sup>23</sup> and was kept at -20 °C until electrophoresis procedure. Total protein was determined according to biuret method<sup>24</sup>. SPS and PAGE procedures were performed according to Laemmli and O'Farrell method<sup>25,26</sup>. Protein molecule weights were calculated according to the method of Weber et al.<sup>27</sup>.

**Table 1.** Localities along with the elevations in Kars and its districts where bee samples were collected from

**Tablo 1.** Kars ve ilçelerinde arı örnekleri toplanan lokaliteler ve yükseklik değerleri

Localities	Kars	Akyaka	Arpaçay	Susuz	Dığor	Sarıkamış	Selim	Kağızman
The Number of Samples	30	50	50	50	20	40	30	40
Elevation	1750	1477	1675	1650	1450	2200	1830	1400

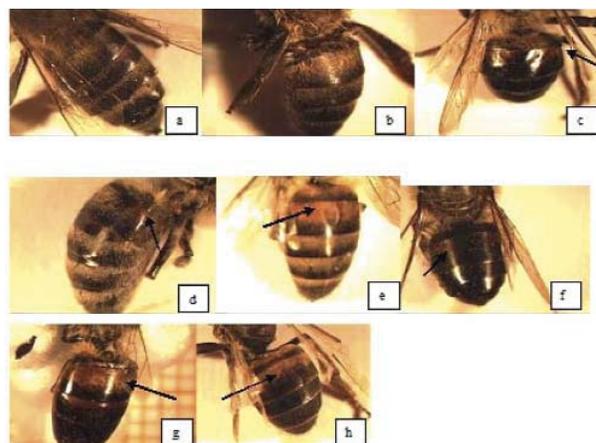
## RESULTS

A total of 310 specimens were collected during the study. The most dominant race was hybrid form of *A. mellifera* (71.6%, n=222) followed by *A. mellifera caucasica* (26.5%, n=82) and *A. mellifera remipes* (1.9%, n=6) (Table 2).

None of the samples collected from Ardahan, accepted as pure Caucasian race (*A. mellifera caucasica*), displayed bands on their tergites, but only a few had spots. The most of the samples taken from other colonies had dapples and in a few of them striping was observed. The six samples that showed striping were excluded from the statistical analyses due to the low sample size. The number of samples collected from each district along with the number of stained and non-stained samples were given in Table 2.

In addition to morphological analysis, pictures of brown dapples from mountain-type Caucasian race and other colonies were taken and shown in Fig. 1. Samples taken from Ardahan gene center and Queen production center had similar characteristics to the mountain-type Caucasian race bees (Fig. 1a, b). However, very weak brown stains were detected in 1<sup>st</sup> and 2<sup>nd</sup> tergites of some samples (Fig. 1c).

In the analysis of samples taken from Kars Plateau, most of the samples showed brown-yellow stains in their 1<sup>st</sup> and 2<sup>nd</sup> tergites (Fig. 1d, e and f) similar to the



**Fig 1.** Pictures of the first and the second tergite dappling and striping (a, b: tergite color of mountain-type Caucasian race; c, d and e: dappling in the 1<sup>st</sup> and 2<sup>nd</sup> tergites of bees in Kars Plateau; d, e and f: dappling in the 1<sup>st</sup> and 2<sup>nd</sup> tergites of bees in Kars Plateau; g, h: striping in the 1<sup>st</sup> and 2<sup>nd</sup> tergites of bees in Kars Plateau

**Şekil 1.** 1. ve 2. tergit beneklenme ve bantlaşma fotoğrafları (a, b: Kafkas ırkı dağ tipi tergit rengi; c, d, e: Kars Platosu'ndaki arıların 1 ve 2. tergit'lerindeki beneklenme; d, e, f: Kars Platosu'ndaki arıların 1 ve 2. tergit'lerindeki beneklenme; g, h: Kars Platosu'ndaki arıların 1 ve 2. tergit'lerindeki bantlaşma

mountain-type Caucasian race. In addition, it was found out that most of the samples had yellow stripes in their 1<sup>st</sup> and 2<sup>nd</sup> tergites (Fig. 1g, h).

The results of the morphological analysis of *A. mellifera caucasica* are given in Table 3 and that of hybrid form of *A. mellifera* in Table 4.

**Table 2.** The color of first tergite circle of all of the samples collected from Ardahan and Kars Plateau

**Tablo 2.** Ardahan ve Kars Platosu'ndan toplanan tüm örneklerin 1. tergit halkasının rengi

Localities	Ardahan	Kars	Akyaka	Arpaçay	Susuz	Digor	Sarıkamış	Selim	Kağızman
The number of samples	10	30	50	50	50	20	40	30	40
Non-stained	10	15	11	2	16	0	9	14	15
Stained	0	15	39	48	34	20	31	16	25

**Table 3.** Means (mm) and standard deviations of the tarsi length, fore wing length, fore wing width, number of hamulus hind wing, femur length of hind leg, tibia length of hind leg, metatarsus length of hind length, and metatarsus width of hind leg of samples belonging to pure Caucasian (*A. mellifera caucasica*) race collected from different district of Kars Province.

**Tablo 3.** Saf Kafkas (*A. mellifera caucasica*) bireylere ait örneklerin vücut uzunluğu, ön kanat uzunluğu, ön kanat genişliği, arka kanat çengel sayısı, arka bacak femur uzunluğu, arka bacak tibia uzunluğu, arka bacak metatarsus uzunluğu, arka bacak metatarsus genişliği değerleri ile bu değerlerin ortalaması (mm) ve standart sapma değerleri

Locality	Tarsi L.	No. of Hamulus	Wing L	Wing W	Metatarsus L	Metatarsus W	Femur L	Tibia L
Akyaka	1.12±0.07	21.91±1.81	0.92±0.03	0.30±0.01	0.20±0.01	0.13±0.02	0.30±0.01	0.31±0.01
Ardahan	1.25±0.05	21.20±1.48	0.98±0.07	0.33±0.02	0.20±0.00	0.14±0.01	0.30±0.00	0.30±0.00
Arpaçay	1.12±0.01	19.50±3.54	0.90±0.06	0.30±0.01	0.21±0.01	0.14±0.01	0.32±0.01	0.30±0.01
Kağızman	1.29±0.05	21.93±1.83	0.97±0.03	0.30±0.01	0.20±0.01	0.13±0.02	0.30±0.01	0.30±0.01
Merkez	1.23±0.04	21.07±1.03	0.95±0.02	0.30±0.01	0.21±0.01	0.13±0.01	0.30±0.01	0.31±0.01
Sarıkamış	1.19±0.02	21.44±1.24	0.95±0.04	0.32±0.01	0.20±0.01	0.12±0.01	0.30±0.01	0.31±0.01
Susuz	1.21±0.08	20.69±2.15	0.98±0.03	0.32±0.02	0.20±0.01	0.12±0.02	0.31±0.01	0.31±0.02
Selim	1.20±0.05	21.21±1.12	0.95±0.04	0.30±0.01	0.20±0.01	0.12±0.01	0.29±0.01	0.29±0.02
General	1.22±0.07	21.28±1.65	0.96±0.04	0.31±0.02	0.20±0.01	0.13±0.01	0.30±0.01	0.30±0.01

**Table 4.** Means (mm) and standard deviations of the tarsi length, fore wing length, fore wing width, number of hamulus hind wing, femur length of hind leg, tibia length of hind leg, metatarsus length of hind length, metatarsus width of hind leg of samples belonging to hybrid samples of *A. mellifera* collected from different district of Kars Province.

**Table 4.** Melez bireylere (*A. mellifera* melez formları) ait örneklerin vücut uzunluğu, ön kanat uzunluğu, ön kanat genişliği, arka kanat çengel sayısı, arka bacak femur uzunluğu, arka bacak tibia uzunluğu, arka bacak metatarsus uzunluğu, arka bacak metatarsus genişliği değerleri ile bu değerlerin ortalaması (mm) ve standart sapma değerleri

Locality	Tarsi L.	No. of Hamulus	Wing L	Wing W	Metatarsus L	Metatarsus W	Femur L	Tibia L
Akyaka	1.12±0.05	23.18±1.64	0.92±0.02	0.30±0.01	0.21±0.01	0.13±0.01	0.30±0.01	0.31±0.01
Ardahan	1.12±0.05	21.67±1.34	0.96±0.03	0.31±0.01	0.20±0.01	0.13±0.03	0.30±0.01	0.30±0.01
Arpaçay	1.21±0.05	21.60±1.35	1.00±0.02	0.31±0.02	0.20±0.01	0.13±0.02	0.30±0.01	0.30±0.01
Kağızman	1.30±0.04	22.52±1.50	0.97±0.02	0.30±0.01	0.21±0.01	0.13±0.01	0.30±0.01	0.30±0.01
Merkez	1.22±0.03	20.53±1.51	0.94±0.03	0.30±0.02	0.21±0.01	0.13±0.01	0.31±0.02	0.31±0.01
Sarıkamış	1.18±0.03	21.61±1.63	0.95±0.03	0.31±0.01	0.20±0.01	0.13±0.01	0.30±0.01	0.31±0.02
Susuz	1.21±0.07	21.21±1.43	0.97±0.03	0.32±0.02	0.20±0.01	0.13±0.02	0.32±0.01	0.32±0.02
Selim	1.20±0.04	21.50±1.41	0.95±0.05	0.30±0.01	0.20±0.01	0.12±0.02	0.29±0.02	0.28±0.01
General	1.18±0.08	21.85±1.64	0.95±0.03	0.31±0.02	0.20±0.01	0.13±0.02	0.30±0.01	0.31±0.02

All samples were either grouped as pure Caucasian or hybrid bees, and the means of each group for each trait along with p values of t-tests given in [Table 5](#). According to these results, when morphological characteristics of Caucasian samples and hybrid samples were compared, the only statistically significant differences were observed in tarsus length, number of hamulus, and femur length. Body of pure Caucasian samples were longer than hybrid ones, whereas they had fewer hamulus and shorter femurs.

So as to determine variation in total protein amount of mountain-type pure Caucasian race bees, the electrophoregrams were also conducted. Gel electrophoresis obtained as a result of electrophoregram is given in [Fig. 2](#).

Significant similarities were observed when comparing the electrophoregrams of pure Caucasian samples taken from Ardahan and from Kars province.

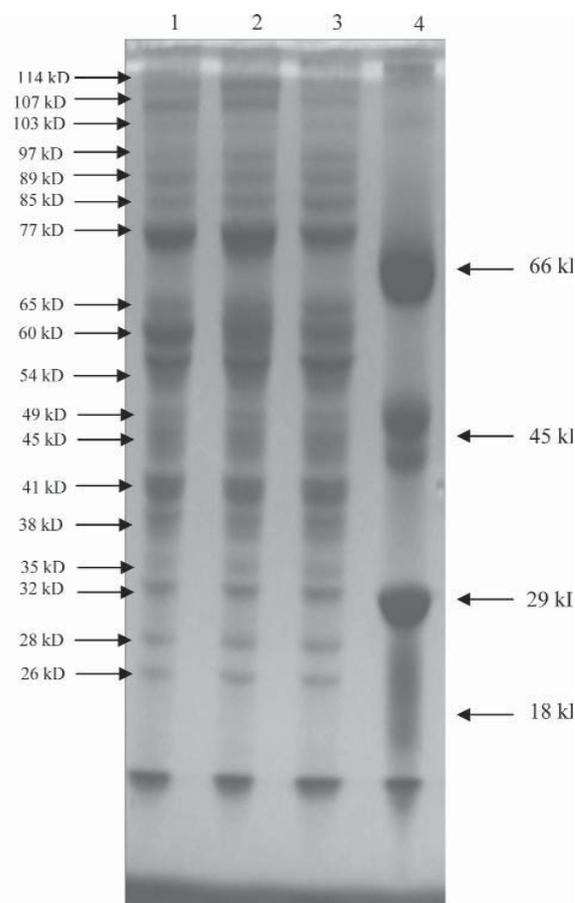
When we compared the electrophoregrams of the pure Caucasian race samples for Ardahan to the hybrids from Kars province, we observed that 77 kD, 114 kD, and 107 kD protein stripes of hybrid samples were

**Table 5.** Means (mm) of morphological traits measured from hybrid and pure Caucasian samples and P values of the difference between two groups as obtained with T-test

**Table 5.** Melez ve saf Kafkas bireylerinin ortalama (mm) ölçüleri ve her gurubun farklılığının T testi ile test edilmesine ait ( $P<0.05$ ) değerleri

Characters	Caucasian	Hybrid	P Value
Tarsi length	1.25	1.18	<0.001
Number of humulus	21.20	21.70	0.006
Wing length	0.98	0.95	0.758
Wing width	0.325	0.306	0.769
Metatarsus length	0.200	0.203	0.108
Metatarsus width	0.135	0.129	0.096
Femur length	0.300	0.303	0.043
Tibia length	0.300	0.305	0.510

thinner compare to pure Caucasian. However, hybrid samples produced thicker bands in 38 kD and 65 kD proteins.



**Fig 2.** The Protein electrophoregram of 1- Ardahan Caucasian sample, 2-Kars Caucasian sample, 3- Kars hybrid sample, and 4- Standard protein

**Şekil 2.** 1- Ardahan Kafkas Örneği, 2- Kars Kafkas örneği, 3- Kars Melez örneği, 4- Standart protein

## DISCUSSION

In the present study, morphological analysis of the samples taken from Ardahan gene center and Queen production center reveal that most of the samples showed similarities with the mountain-type Caucasian race bees (Fig. 1a, b). However, in the first and second tergite, weak brown spots were detected in some samples (Fig. 1c). The results are partially similar to İnci's data<sup>28</sup>.

The high abundance of hybrid samples (71.6% of the total catch) in our study is similar to Bodenheimer's finding that the research area is a sub-passage region. Identification of yellow-abdomen circled subjects, albeit a few, in the research makes us believe that, as Çakır reports, *A. m. remipes* living in Elazığ area hybridized with the bees in the region due to mobile apiculture activity<sup>29</sup>.

In the light of all these data, it can be asserted that colonies in and around Kars have been hybridized apparently and colonies in Ardahan gene center and Queen production station are gradually hybridized towards Caucasian race lowland-type or yellow-trans-Caucasian (*A. m. remipes*) race.

At the same time, it is a reality that although this region was announced as the center of origin, some hybridization has been observed due to mobile apiculture and lack of sufficient legal controlling mechanisms. Ardahan area was under protection with Artvin Province, but due to insufficient legal controlling mechanisms mobile apiculture and individual queen production stations were not controlled, which can make hybridization of the colonies in the region inevitable.

It has been found out that local apicultural colonies in the centre of Kars and its districts were not pure mountain-type Caucasian race, which was claimed before<sup>27</sup>; morphological analysis of samples showed that most of these bees were hybrids. The only reason for this phenomenon can be the existence of significant mobile apiculture in the districts (especially mobile apiculturists in Elazığ and Bingöl regions where *A. m. remipes* is dominant). Morphological analysis of the samples taken from Kars and its districts showed subjects with yellow stripes on 1<sup>st</sup> and 2<sup>nd</sup> tergites (albeit a little), and the existence of brown-yellow stains and dapples in 1<sup>st</sup> and 2<sup>nd</sup> tergites of most of the samples mean that hybridization still continues.

The conventional belief is that the mobile apiculture has negative effects on local bee races in Kars Plateau.

This study indicates the relative abundance of bee races of the region and has potential to contribute to the preservation efforts of local bee races of the Kars Plateau.

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