OP-219 [Beekeeping Technology and Quality]
The Effect of Honeybee Genotype on the Quality and Protein Content of Bee Venom Collected From Different Geographical Regions of Türkiye

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Honeybee venom is a valuable bee product that has a wide range of uses in health, pharmacology, cosmetics and Apitherapy due to its unique biological properties. It has been reported that there are various factors affecting the quality of bee venom and one of them is the species of bees. Turkey has an important place in the world in terms of beekeeping and the production of bee products due to its phytogeographic location, plant and honeybee biodiversity. In this study, bee venom samples were collected by electroshock method from 57 apiaries representing seven different regions of Turkey to examine phenotypic and genotypic effects on bee venom quality and worker bee samples were collected to determine the species. In order to determine the relationship between bee venom and genotype, the amount of bee venom and total protein contents were analyzed, and honeybee species and ecotypes were compared using geometric morphometry method. According to the results of the study, when the quality of honey bee venom was evaluated in terms of both geographical differences and honey bee species, it was found that the most prominent venoms in terms of quantity and quality were those collected from local and protected species and ecotypes located in isolated areas with rich and diverse pollen-nectar resources, away from environmental pollution, and collected under appropriate seasonal and temperature conditions. Our results show that genotype is an important parameter in bee venom production, although it cannot be evaluated alone. Environmental factors should also be taken into consideration in high quality bee venom production.

THE EFFECT OF HONEYBEE GENOTYPE ON THE QUALITY AND PROTEIN CONTENT OF BEE VENOM COLLECTED FROM DIFFERENT GEOGRAPHICAL REGIONS OF TURKIYE

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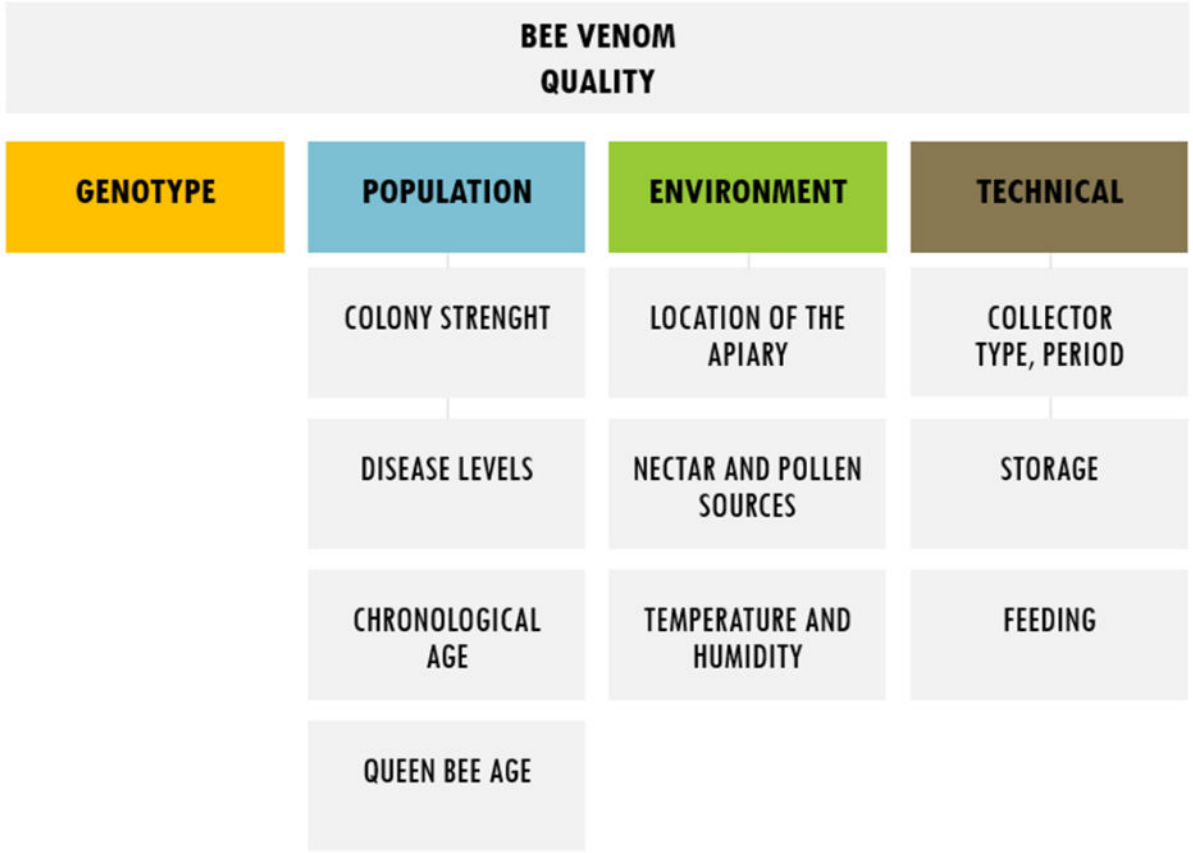
Introduction

Bee venom has been used in apitherapy, cosmetics, and pharmacology since ancient times. The use of bee venom in apitherapy dates back to Ancient Egypt. Written sources indicate that bee venom therapy has been practiced since the time of Hippocrates and Galen (370-460 AD).

It is known that the quality of bee venom can vary depending on various factors. Identifying these factors is critical for producing high-quality venom.



Aim of the Study

Determination of the biochemical characteristics of honeybee (*Apis mellifera*) breeds and ecotypes in different geographical regions of Turkey, which is rich in honeybee and plant biodiversity.

Identification of the diversity and regional variations of bee venoms across these regions.

Examination of genotype as a major factor influencing bee venom quality and composition.

Investigation of the production and utilization potential of bee venom and contribution to its sustainable development.

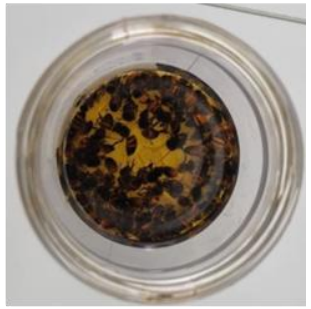
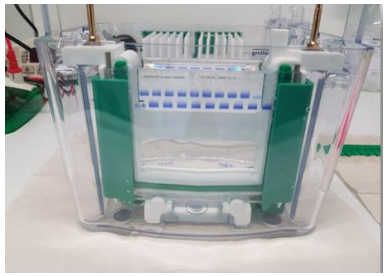
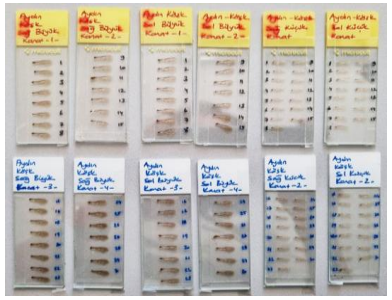
Provision of nationally based scientific data to enhance bee venom production capacity in Turkey and establish national standards.

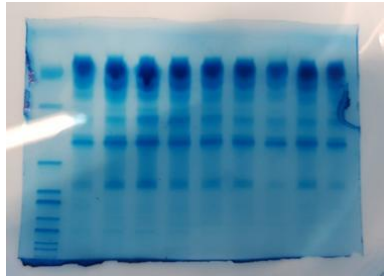
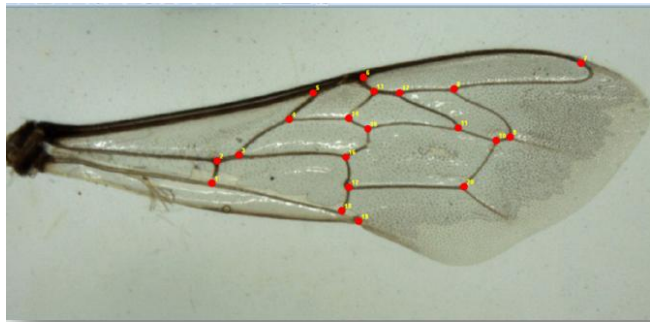
Materials and Methods

Fig.1. Schematic representation of venom collection points

<u>Field study</u> • 48 city • 57 apiary • 64 bv sample • 2565 bee sample	<u>Recordings:</u> Locations of apiaries, Feeding conditions, Temperature-humidity values, Collecting date and time, Weather conditions, Beekeeping system, Nectar flow status, Water source , Queen bee information, Frame counts	Each sample was collected by the same researcher, using the same equipment, at the same time of day and in the same season, by visiting the apiary in person. This minimized any researcher-related errors that might have occurred.

Materials and Methods

BV collection Elektroshock method

Lyophilisation

Determination of Total protein BCA assay kit

SDS Page Protein profile

Geometric morphometrics

tpsdig2, tpsUtil 64, MorphoJ, NTSYS-PC (2.2)

ANOVA, PCA, CVA, DFA, UPGMA analysis

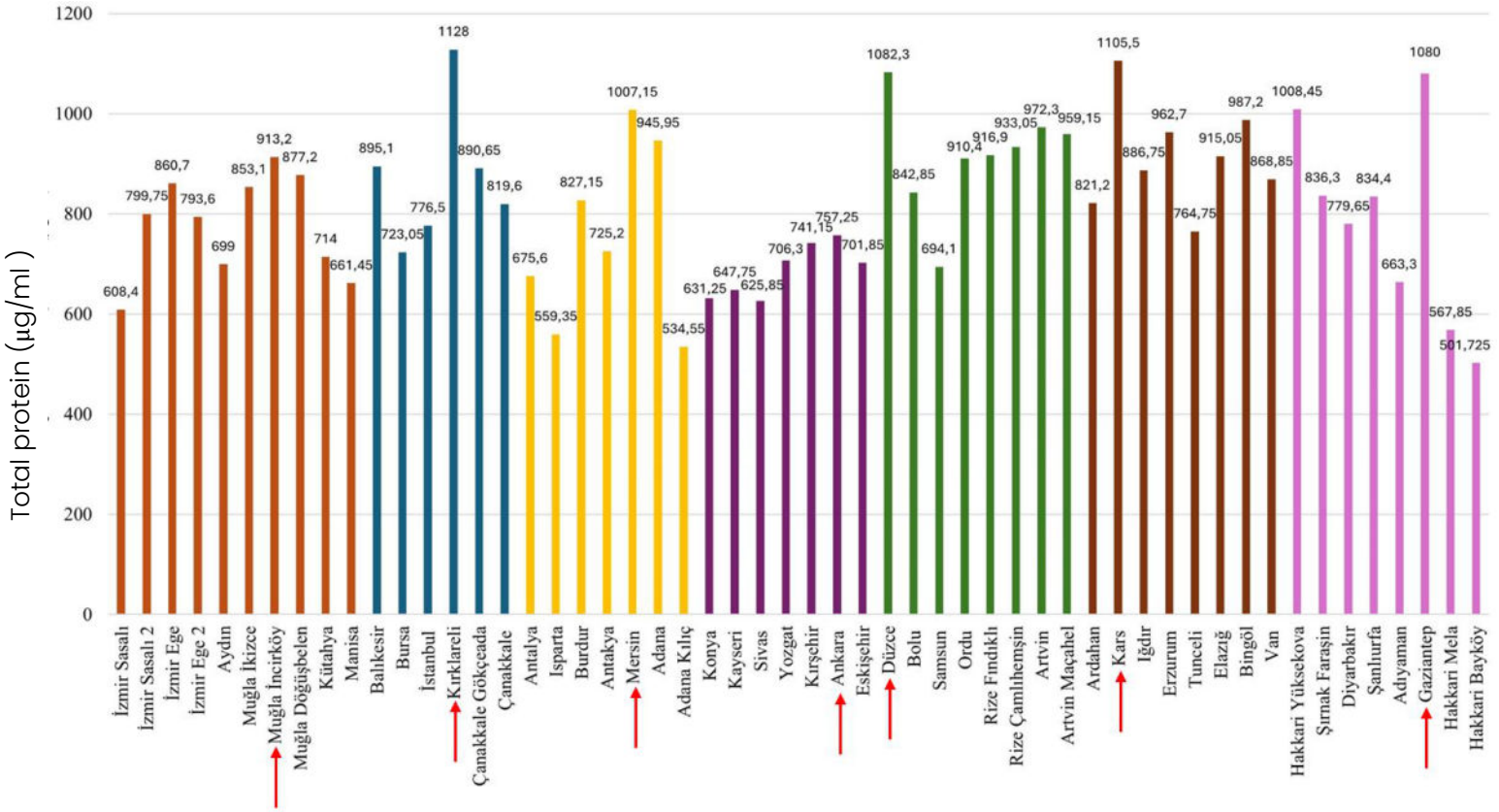
In vitro cytotoxicity

Cell culture

Proteomics

Genomics

RESULTS – Protein content



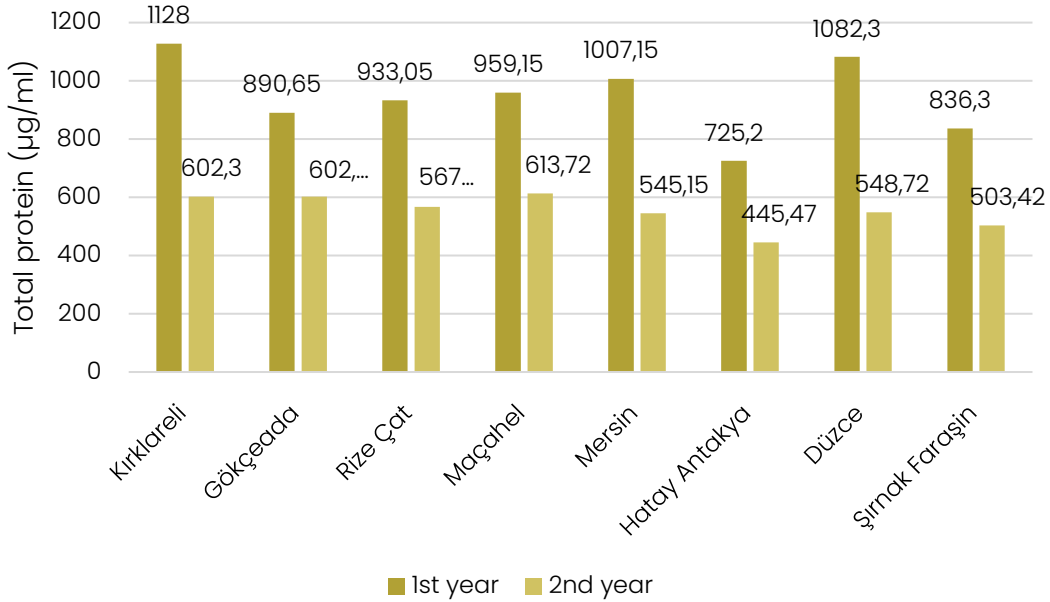
The amounts of crude venom collected throughout Turkey ranged from **9.30 mg to 303.30 mg** after the lyophilization process.

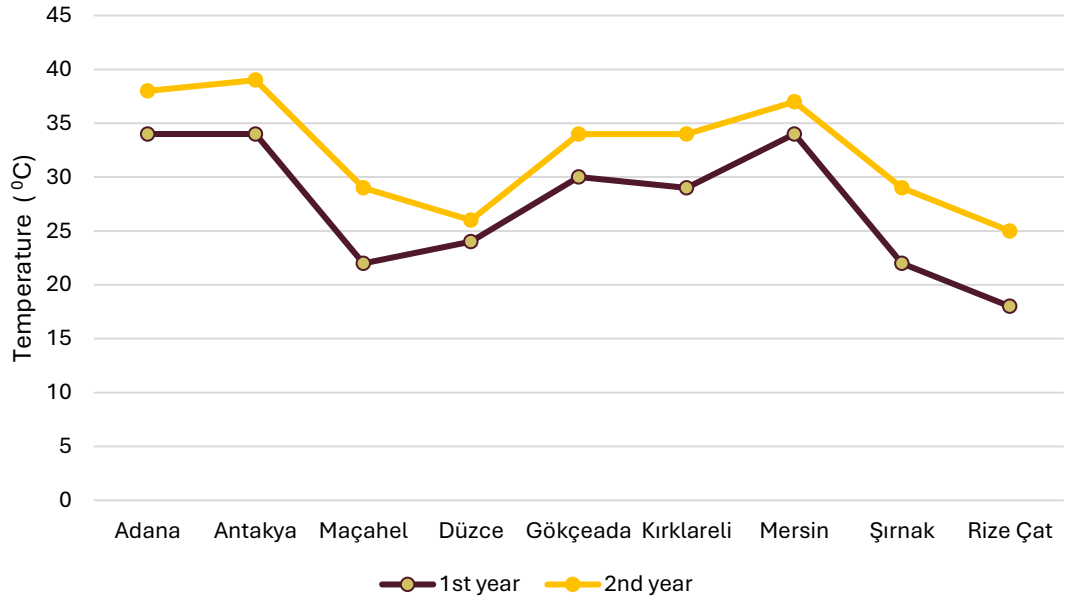
The protein concentration of lyophilized crude *Apis mellifera* venom (1 mg/ml), determined by the Smith test, ranged from **559.35 µg/ml to 1128.0 µg/ml** for all honeybee venom samples.

Bee venom collected from the Eastern Anatolia, Black Sea, and Southeastern Anatolia regions has been found to have a higher protein content.

RESULTS – Protein content

Following the initial fieldwork conducted in first year, sampling was repeated in next year, particularly in provinces located in isolated areas that are important in terms of genotype and have high protein content, away from industrial and residential areas (Adana, Hatay, Artvin Maçahel, Düzce, Gökçeada, Kırklareli, Mersin, Şırnak Faraşin, and Rize Çat).





The venom contents collected from the same apiaries and hives at similar times and in similar seasons, one year apart, were compared. It was determined that the total protein content in all venoms was on average 50.69% to 67.65% lower than the previous year.

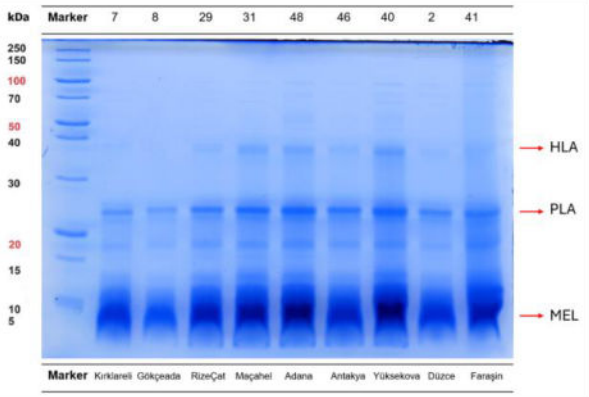
RESULTS – Protein content

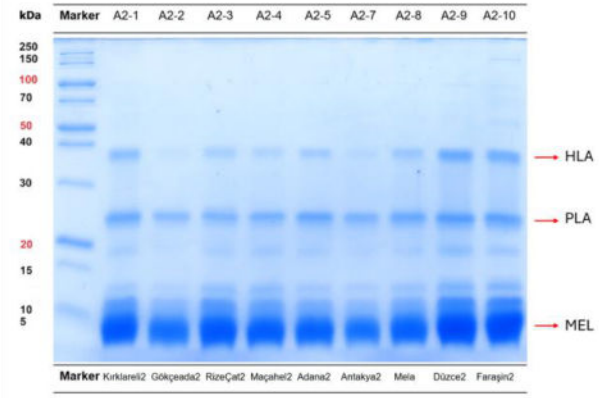
The difference between crude venom samples can be attributed to many factors, such as the honeybee genotype, temperature conditions, and the variety and quantity of nectar and pollen sources.

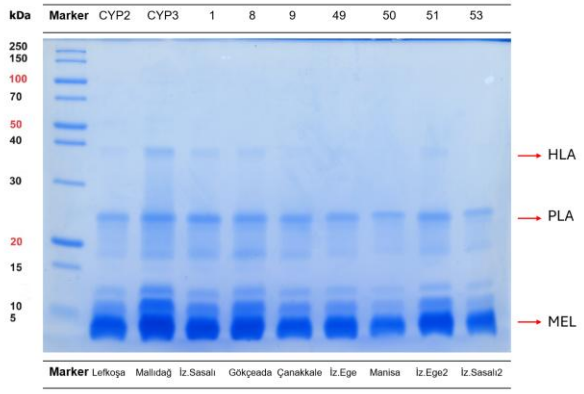
The highest protein content in the study was detected in the Kırklareli sample (1128.0 µg/ml), which is associated with the abundant phacelia (*Phacelia tanacetifolia*) and oak (*Quercus* spp.) pollen and nectar sources in the region. The high nutritional value of phacelia and oak pollen meets the protein and amino acid requirements of honeybees, supporting brood development (Owayss et al., 2020; Giovanetti et al., 2022; Ghosh and Jung, 2017).

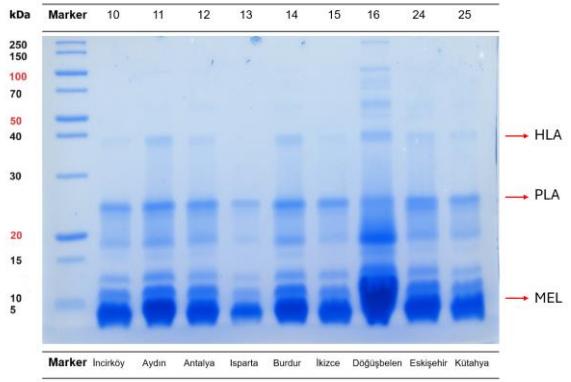
Our study determined that high temperatures in the second year, along with changes in nectar-pollen sources, caused a decrease in protein content in honeybee venom. Furthermore, it was observed that excessive heat caused stress in bees, prioritizing thermoregulation and reducing venom efficiency, consistent with previous studies.

RESULTS – SDS Page







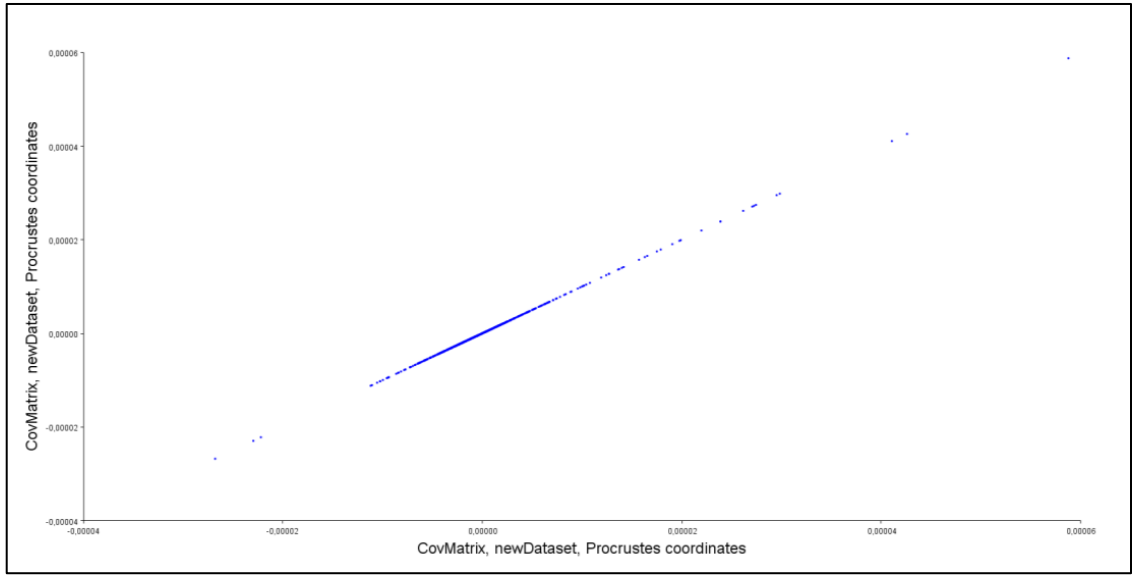


Protein bands with different molecular weights (MW) were obtained by SDS-PAGE. Protein bands ranging from 5 to 14 in number were obtained on gels separated by region.

The highest number of protein bands in SDS-PAGE analysis was observed in samples from Kırşehir (14), Yozgat (13), and Sivas (12), and it was determined that the thyme (*Thymus* spp.) and astragallus (*Astragallus* spp.) plants predominantly found in these provinces constitute a rich food source for bees. It is thought that these plants, with their medicinal and aromatic properties, may affect the quality and quantity of honey by supporting bee health.

RESULTS – Geometric Morphometrics

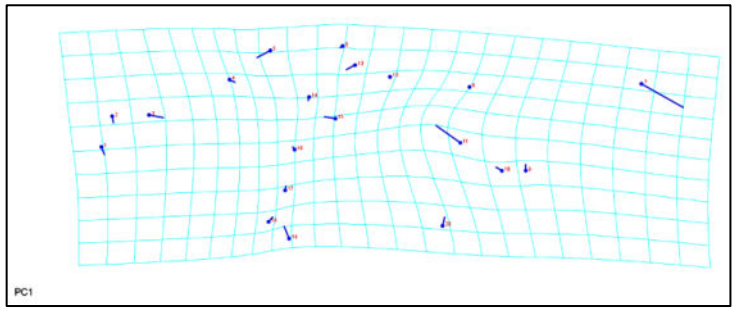
Centroid size							
Effect	SS	MS	df	F	P (param.)		
Individual	282275	5040	56	22,42	<0.0001		
Residual	371619	224	1653				
Shape, Procrustes ANOVA							
Effect	SS	MS	df	F	P (param.)	Pillai trace	P (param.)
Individual	0,32802	0,000162	2016	10,88	<0.0001	6,17	<0.0001
Residual	0,8898	0,0000149	59508				

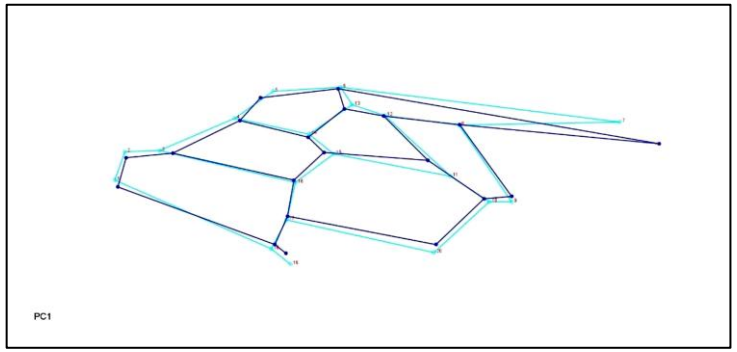


1,710 right wing samples from 57 different honeybee species were analyzed using the MorphoJ program.

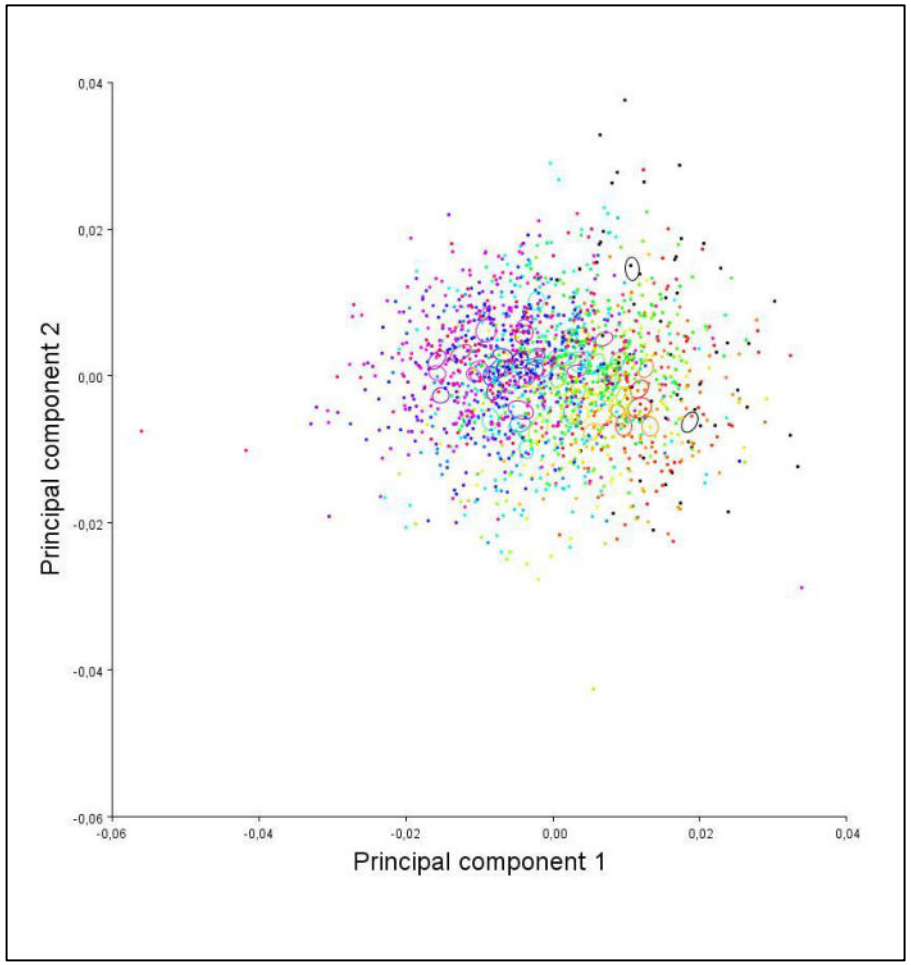
When individual samples were evaluated using the Procrustes ANOVA test, significant differences were found between locations in terms of both centroid size and wing shape ($P < 0.0001$).

RESULTS – Geometric Morphometrics PCA



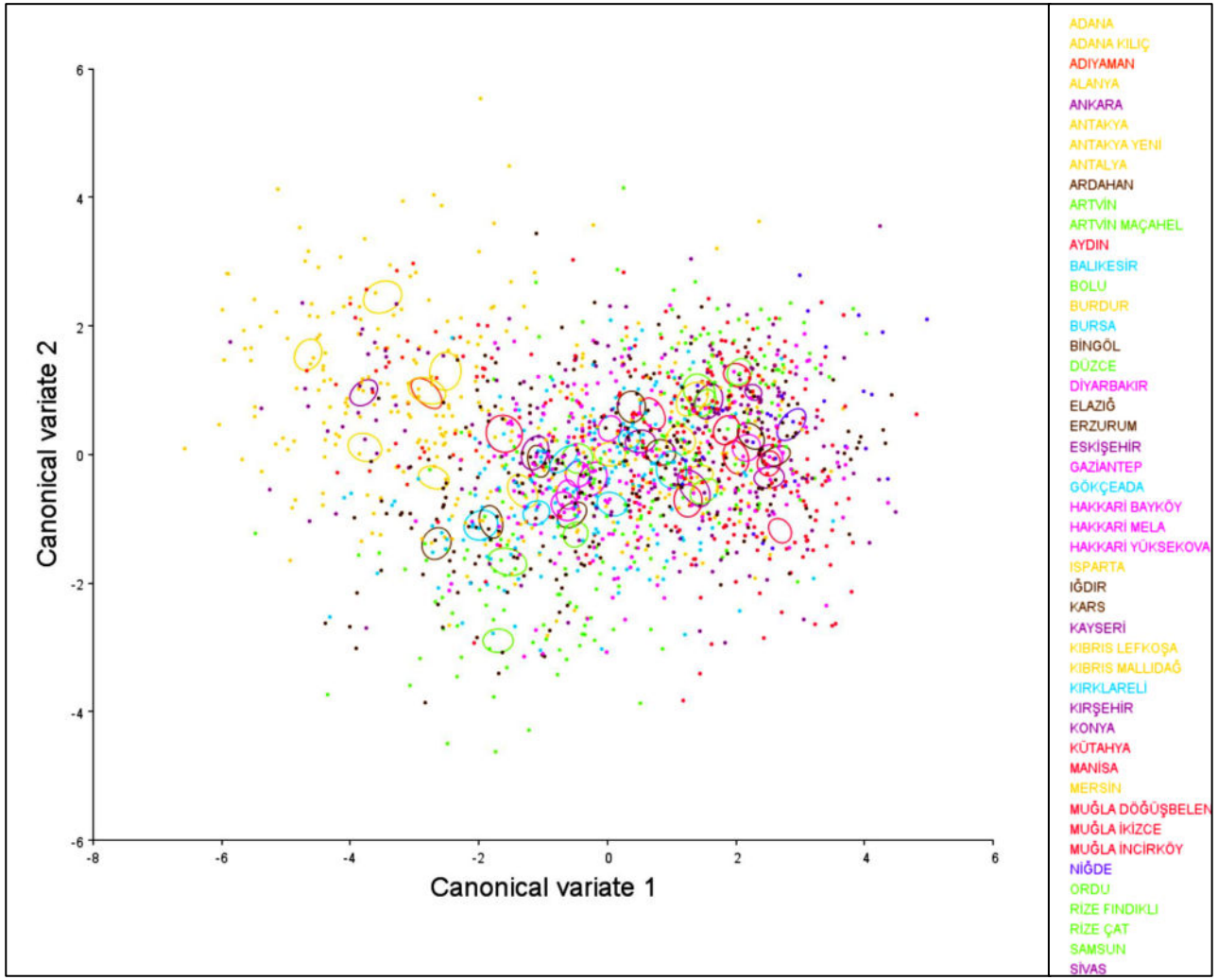


Landmarks 7, 11, 5, and 3 showed the highest variation in PCA.



Populations from Gökçeada, Antakya Yeni, and Artvin Maçahel formed distinct clusters. Other provinces showed overlapping populations.

RESULTS – Geometric Morphometrics CVA



The **first canonical axis** explained **34.28%** of the total variation (the most discriminative axis).

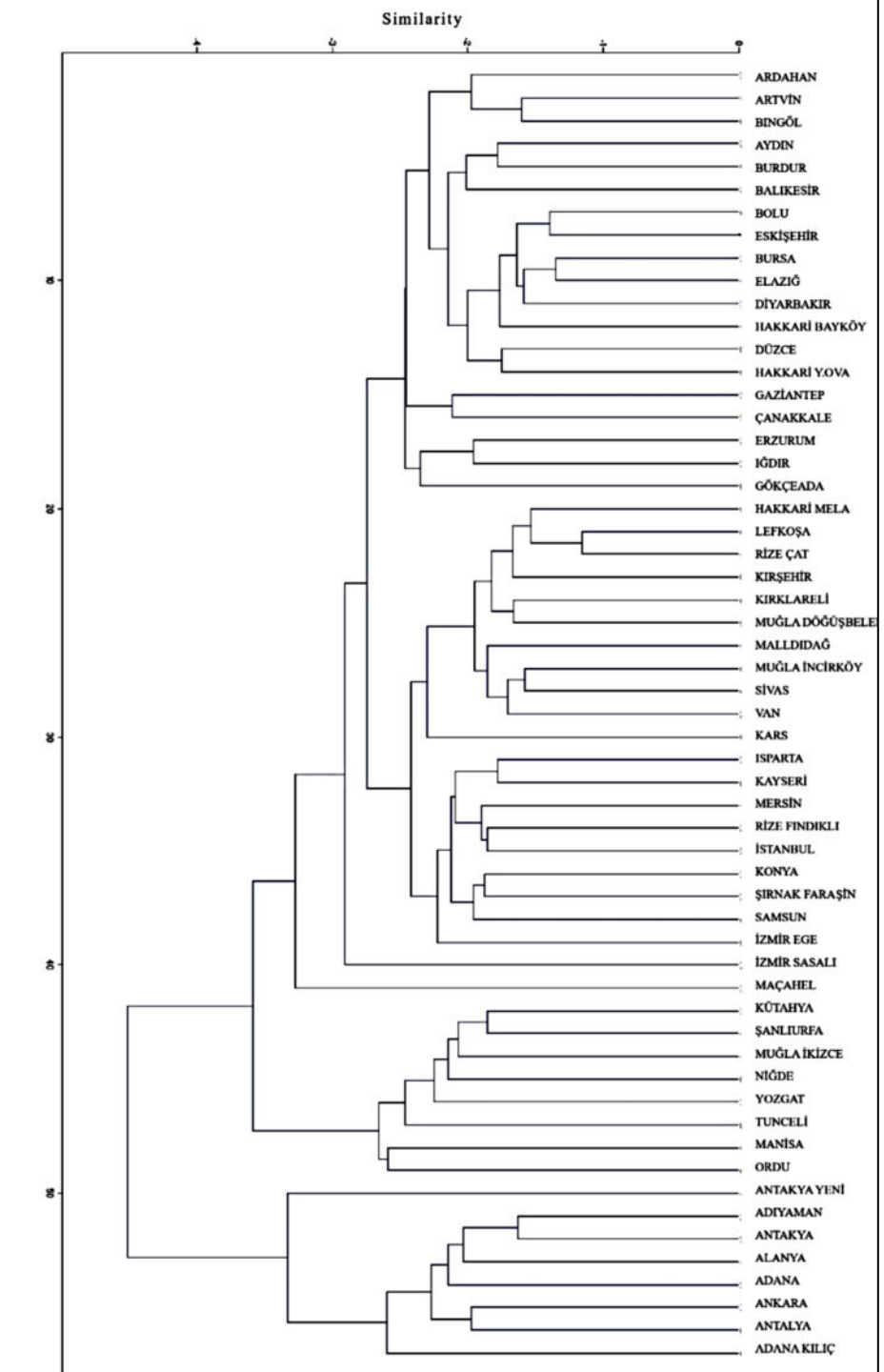
Populations such as **Adana Kılıç, Antakya, Antalya, Artvin Maçahel, and Gökçeada** formed **separate clusters**.

RESULTS – Geometric Morphometrics DFA

Comparison	Procrustes distance	Procrustes distance (P)	T-square (P)	Mahalanobis distance	T-square (value)	P- value (parametric)
Adana - Adana Kılıç	$1.05*10^{-2}$	0.0030	0.0030	4.22	267	0.0040
Adana - Adıyaman	$1.10*10^{-2}$	0.0020	0.0440	3.47	180	0.0427

Similarity groups identified:
Muğla Döğüşbelen ↔ Muğla İkizce
Artvin ↔ Artvin Maçahel
Çanakkale ↔ Düzce, Elazığ, Lefkoşa
Van ↔ Tunceli, Mersin, Niğde, Muğla İncirköy, Rize Çat

Conservation regions as **Muğla Döğüşbelen–İkizce** (Apis mellifera anatoliaca Muğla ecotype) and **Artvin–Maçahel** (Apis mellifera caucasia) showed significant similarities with each other ($P > 0.0001$).



RESULTS – Geometric Morphometrics UPGMA

UPGMA dendrogram shows two main clusters with several sub-clusters, indicating regional differentiation among honey bee populations.

PCA, CVA and UPGMA analyses revealed distinct clusters for **Maçahel, Gökçeada, Muğla and Antakya**.
Maçahel confirmed preservation of pure *Apis mellifera caucasica*.
Antakya appeared as a unique, locally adapted and aggressive population.

Most other populations overlapped, reflecting hybridization caused by migratory beekeeping and queen trade.

Results emphasize the urgent need for conservation strategies to protect Turkiye's honeybee biodiversity.

RESULTS – EFFECT OF GENOTYPE ON BEE VENOM COMPOSITION

High protein content & conservation regions

Kırklareli (Trakya bee conservation area, nectar & pollen rich flora) showed the **highest venom protein content**.

Muğla İncirköy & Muğla Döğüşbelen (Muğla bee), Düzce (Yığılca bee), Artvin–Maçahel (Caucasian bee), Bingöl (Bingöl bee) all correspond to **protected/local ecotypes** with higher protein levels.

Antakya Yeni, Adana, Şırnak Faraşin: **isolated ecotypes**, genetically distinct and venom-rich.

Environmental & floral sources

High protein samples (e.g., Kars, Gaziantep, Hakkari, Mersin, İzmir Sasalı, Balıkesir, Burdur, Kırşehir, Yozgat, Erzurum) often came from **nectar/pollen-rich habitats** (thyme, astragalus, eucalyptus, oak, phacelia, etc.).

SDS-PAGE bands were highest in regions with **medicinal plant dominance** (Kırşehir, Yozgat, Sivas).

İzmir Sasalı & Mersin Tarsus (Eucalyptus forest): **synergistic effect of bee species + floral source**.

Year-to-year variation

Despite sampling from the **same colonies & apiaries** in 2 years, **protein contents decreased by ~50–67% in 2nd year**, highlighting that beyond genetics, **floral availability & environmental stress** critically shape venom composition.

CONCLUSION

Turkiye has a high potential for bee venom production due to its **unique phytogeographic** diversity.

The chemical and protein composition of bee venom varies significantly depending on **species, ecotype, geography, climate, and floral sources**.

Local honeybee species and ecotypes showed **richer protein profiles**, offering advantages for commercial production.

Genotype is important but not the sole determinant; **environmental factors and food sources are also critical**.

Geometric morphometric results highlight the need for **conservation of honeybee biodiversity**.

With proper methods, high-quality venom can be harvested without colony stress.

SUGGESTIONS

1. Protect local honeybee races and ecotypes; develop selective breeding programs.
2. Limit migratory beekeeping and prevent uncontrolled queen trade.
3. Develop region-specific production strategies.
4. Create models tailored for apitherapy, pharmacology, and cosmetics.
5. Establish bee venom production centers and promote contract-based production.
6. Investigate the influence of flora and climate on venom quality.
7. Use proteomic and genomic methods to determine venom composition differences.
8. Purify bioactive molecules and ensure industrial diversification of venom components.
9. Develop national quality standards and apply strict control at all production stages.

Ege University Office of Scientific Research Projects

The Turkish Beekeepers Association (TAB)

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EUROPEAN COOPERATION
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APiMAK

THANK YOU