MORPHOMETRIC AND GENETIC DIFFERENTIATION BETWEEN ANATOLIA AND CYPRUS *BOMBUS (BOMBUS) TERRESTRIS* (L. 1758) POPULATIONS

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I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

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ABSTRACT

MORPHOMETRIC AND GENETIC DIFFERENTIATION BETWEEN ANATOLIA AND CYPRUS *BOMBUS (BOMBUS) TERRESTRIS* (L. 1758) POPULATIONS

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Four microsatellite loci were used to investigate differentiation in *Bombus terrestris*, a bumblebee of interest for its high value crops pollination. Two bumblebee populations, one from Ankara (the capital of Turkey) and one from North Cyprus were analyzed. In these populations, the total number of alleles detected per polymorphic locus ranged from 7 to 12. F_{ST} genetic distance between Ankara and North Cyprus *B. terrestris* populations based on four microsatellite loci was calculated as 0,09351. This applies that there is significant (P<0,001) differentiation between Anatolian and Cypriot populations. Moreover, statistically significant differences between two populations were found in wing characters studied. According to the potential for local adaptation and individual fitness of bumblebees, microsatellite data calls for protection of *Bombus terrestris* populations against importation of bumblebees of foreign origin which are used as crop pollinator.

Keywords: *Bombus terrestris*, population genetics, microsatellite, morphometrics

ÖZ

ANADOLU VE KIBRIS *BOMBUS (BOMBUS) TERRESTRİS* (L. 1758) TOPLUMLARI ARASINDA MORFOMETRİK VE GENETİK FARKLILAŞMALAR

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Seralarda yetiştirilen yüksek ekonomik değere sahip tarım ürünlerinin birincil tozlaştırıcısı olan *Bombus terrestris* toplumları arasındaki genetik faklılıkları araştırmak için dört tane mikrosatelit lokusu kullanılmıştır. Biri Türkiye'nin başkenti olan Ankara ve diğeri de Kuzey Kıbrıs'tan olmak üzere iki farklı *Bombus terrestris* toplumu karşılaştırılmıştır. Bu toplumlardaki her mikrosatelit lokusuna ait alel sayısı 7 ile 12 arasında değişmektedir. Ankara ve Kuzey Kıbrıs *B. terrestris* toplumları arasındaki dört mikrosatelit lokusundan yararlanılarak hesaplanan F_{ST} genetik uzaklık değeri 0,09351 olarak hesaplanmıştır. Buna göre çalışılan iki toplum arasında istatistik bakımdan anlamlı ($P<0,001$) farklılıklar vardır. Ayrıca kanatlarda ölçülen morfometrik karakterler bakımından da toplumlar arasında anlamlı farklar bulunmuştur. Sonuç olarak, yüksek bölgesel adaptasyon ve bireysel yaşam başarısı özellikleri de göz önünde bulundurularak, yerel *Bombus terrestris* toplumlarının tozlaştırmada kullanılmak üzere ithal edilen yabancı menşeli türlere karşı korunması gerektiği sonucuna varılmaktadır.

Keywords: *Bombus terrestris*, populasyon genetiği, mikrosatelit, morfometri

To My Parents;

Tünay and Mustafa BETON

and
To My Sister;
Demet BETON

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CHAPTER 1

INTRODUCTION

Most of greenhouse crops, like greenhouse tomatoes, necessitate supplemental pollination for fruit set; therefore, they are usually pollinated by mechanical vibration (manual pollination), although it is labour intensive and expensive. To overcome these disadvantages of mechanical vibration, their natural pollinators started to be used as crop pollinators in the greenhouses as well as fields. More over, Dogterom *et. al.* indicated that using bumblebees as pollinators is better than the manual pollination technique (1998).

Bombus (Bombus) terrestris, a bumblebee species that is used for pollinating high-value crops (e.g., tomatoes and aubergines) in greenhouse industries, observed to be very successful. Accordingly, this species has received great attention. In Europe, laboratory reared colonies of *Bombus terrestris* have been in greenhouse industries since 1987 and started to replace manual pollination (van Ravenstjin & Nederpel, 1988; van Ravenstjin, 1989; van Heemert *et al.*, 1990). Improvement of rearing techniques has made mass production of colonies economically viable. After all, bumblebee rearing has been commercialised and some companies have specialized even in providing bees at appropriate stages of development.

After the use of *Bombus terrestris* in the greenhouses has been popular in Netherlands and Belgium, two companies from Netherlands with Turkish partners obtained a license from government agencies to breed *B. terrestris* in Turkey for export. However, instead of rearing, they have collected and transferred overwintered queens and nests abroad illegally for about 4 years (1989-1992) (Özbek, 1997). Yet,

in Turkey, *Bombus terrestris* rearing under legal conditions has started in 1996, and commercial growers has adopted them in a short time (Çağatay, Kence and Aytekin, 2000).

These commercially breeding activities sometimes involve Europe-wide transfer of colonies. For instance, Japan has been importing more than 40,000 *B. terrestris* colonies from Europe for pollination of tomato plants in greenhouses each year since 1991 (Ono, 1998; Goka *et. al*., 2001).

Obviously, such a large insect industry might have an ecological impact on natural fauna. This phenomenon mainly results from the great ecological flexibility of *Bombus terrestris* supporting it to flourish in various habitats (Dafni & Shmida, 1996). Introduction of *Bombus terrestris* to New Zealand represents good evidence to its adaptability, as was liberated in New Zealand in 1885 till 1906 from queens collected in England and have been very efficient in pollinating nearly 400 species of flowers (Macfarlane, 1995; Semmens, 1993).

There might be a couple of impacts of these commercially brought colonies. Since commercially bred bees originate from different stocks, they have different genetics from local populations and their escaping sexuals may introduce foreign alleles into the local population. In other words, they might change the gene pool of the local populations. Besides, this might be the case even when commercially bred bumblebees and native bumblebees are different species. It is shown that a Japanese native species, *B. hypocrita sapporoensis,* and *B. terrestris* can produce hybrids under laboratory conditions (Mitsuhata & Ono, 1996) and it is taught that they may even establish a colony. On the other hand, the competition for an ecological niche between introduced commercial species and native species. This might even cause extinction of the native species. Dafni & Shmida (1996) observed a reduction in numbers of various native bees correlated with an increase in *Bombus terrestris* numbers in the field in Israel. Then again, extinction due to carrying parasitic invaders might cause enormous losses of native species (Goka *et. al*., 2001). The varroa mite, *Varroa jacobsoni*, represents a really good example to this situation.

This species is native to eastern Asia, where it parasites the eastern honeybee, *Apis cerana*. At the same time, it is a virulent parasite of the European honeybee, *Apis mellifera* (Oudemans, 1904). After the varroa mite has been carried into the Western Hemisphere from Japan by the transportation of European honeybee colonies, it caused worldwide loss of European honeybee colonies (Goka *et. al*., 2001). Recently, Goka *et. al.* (2000) reported that an endoparasitic mite, *Locustacarus buchneri*, have been carried to Japan by the introduced *B. terrestris* colonies, however there is not any information or prediction about what would be its impacts. Therefore, it is especially urgent and important to control their continental transfer and its impacts.

Dogterom *et. al.* (1998) indicated that "In North America, Agriculture Canada and the United States Department of Agriculture restrict the importation of European bumblebee species, and restrict the movement of bumblebee species within North America to north-south movements within northern and western provinces or states".

1.1. General Information

1.1.1. General Information about *Bombus terrestris*

Family Apidae includes subfamilies such as, Bombinae and Psithyrinae. There are nearly 250 species of true bumblebees (Bombinae) and 45 species of cuckoo bumblebees (cleptoparasitic bumblebees) (Psithyrinae) in the world (Ito, 1985; Williams, 1985). *Bombus terrestris* belongs to the subfamily Bombinae, as shown above.

Class: Insecta

Order: Hymenoptera

Suborder: Apocrita

Superfamily: Apoidae

Family: Apidae

Subfamily: Bombinae

Syn. : *Bremus* Jurine, 1801

Bombus Latreille, 1802

Subfamily: Psithyrinae

Syn. : *Psithyrus* Lepeletier, 1832

Apathus Newman, 1834

Taxonomists have classified many subspecies of *Bombus terrestris* by the differences in their coat colour (Rasmont, 1983). However, these differences are so small, except for ssp. *xanthopus* from Corsica and ssp. *ferrugineus* from South-western Europe (Estoup *et. al*., 1996). So, it is quite difficult to distinguish them (Mayr, 1964).

The natural area of distribution of *B. terrestris* covers all of Continental Europe and includes the south of England and Scandinavia towards north, extends to northern Africa towards south and reaches east to the Caucasus and the Ural mountains. Moreover, *B. terrestris* is the only bumblebee species found almost on every major Mediterranean Island (Estoup *et. al*., 1996). Although it has got a great wide range, it is very hard to observe them higher than 1200m.

Different authors have recorded fifty five species of Bombinae in Turkey (Aytekin & Çağatay, 2002b). Rasmount, (1996) pointed out that distribution of bumblebees in Turkey seems mainly correlated to vegetation, altitude and main post-glacial dispersion centres. *Bombus terrestris* is one of the most widespread bumblebee species in Turkey, dispersed in the plain level particularly along the costal sites and in the areas which are not cultivated and protected (Rasmount, 1996; Özbek, 1997). *B. terrestris* is a primitive eusocial bee species that lives in colonies. In the early

spring, a post-hibernating queen emerges from its hibernation site in the ground in order to initiate the colony. This period coincides with the first flowering period of flowers most preferred by *B. terrestris*, such as *Trifolium pratense* and *Echium italicum* (Aytekin *et. al.*, 2002a; Davis, 1965; Kaya *et. al.*, 1999). First, she forages for about a week on flowers to develop her ovaries. Then she selects a nest site and collects pollen to lay her first eggs that will give rise to workers. The pollen stimulates the ovaries to produce eggs, which the queen lays on the ball of pollen. The first brood of workers hatches three to four weeks after the initiation of the colony and the larvae feed on their bed of pollen. Adult workers look after the nest, defend it and collect food for it. Once there are enough worker bees to do the foraging and housekeeping tasks, the queen concentrates on laying eggs. The colony peaks to a few hundred individuals. The newly emerged males begin to appear outside the nest about three to five weeks later than the first workers. After a couple of days, males leave the nest and usually do not return. About one week after the first appearance of the males new females emerge. New queens mate with the males which came from other nests around. Once sexuals are produced, the nest declines rapidly. The old queen, workers and males die. New mated queens leave the nest and seek out a suitable hibernation site where they will stay throughout winter (August-May) (Heinrich, 1979).

In Hymenoptera workers and queens are diploid (2n), whereas males are haploid (n). As a result, these animals are named as haplodiploid organisms.

1.1.2. Previous studies on *Bombinae*

The first use of numerical methods in bumblebee systematics was by Medler (1962), who determined the relationships between the castes and 35 different bumblebee species by examining the correlation between the radial cell of the front wing and mouthparts. Afterwards, Svensson (1973) identified two subspecies of *Bombus lapponicus* Fabricius by using morphological measurements of the seventh sternite and chemical features of cephalic secretions. The developments of the computerised

statistical methods have allowed bumblebee systematists to solve problems such as the morphometrical examination of geographic variations of certain species (Ito, 1987).

Protein electrophoresis provided a new approach that has been used alone or in combination with morphometry. Stephen & Cheldelin (1973) were the pioneers using these methods in Bombinae systematics. Afterwards, Pekkarinen (1979), Pekkarinen *et. al.* (1979), Pamilo *et. al.* (1987), Scholl *et. al.* (1990; 1992) and Pamilo *et. al.* (1996) have done studies on Bombinae by using at least one of these methods. In the short run, these methods have become a standard routine in molecular systematic works (Thorpe & Solé-cava, 1994). However, Owen *et. al.* (1985, 1992) indicated that *Bombus* species have considerably less allozyme variation than most other Hymenoptera.

Reinig (1967, 1971 and 1973) and Rasmount (1983) have done fauinstic studies on the bumblebees in Turkey. Özbek (1979, 1983 and 1990) has done researches on the male genitalia of some species found in East Anatolian Region. Besides, Aytekin & Çağatay, (2002b) included morphologic and colour variation data as well as male genitalia information to investigate the bumblebees in Ankara province. Afterwards, investigations have been improved by using protein electrophoresis and morphometric methods (Çağatay, Kence and Aytekin, 2000) and Aytekin and Çağatay (2002c) concluded that "*B. terrestris* exhibited inter-population variation but no intra-population variation for various enzymes studied". Scholl *et. al.* have also pointed out that allozyme studies have failed to show genetic differentiation among *B. terrestris* populations (1990).

Estoup *et. al.* have done the first studies on microsatellites in *Apis mellifera* and *Bombus terrestris* (1993) and found out that there is a high level of intrapopulational polymorphism with one tested honeybee microsatellite. This study and further

studies about microstaellites have shown that microsatellites tend to have many alleles and high heterozygosity even in social insects making them ideal markers for population genetics studies in Hymenoptera (Queller *et. al.,* 1993; Estoup *et. al.*, 1995).

1.1.3. General Information about Microsatellites

Microsatellites are tandem repeats of short sequence motifs, can be up to eight base long. These repeats can be up to 20 to 100 base pair long at each locus. These repeats have also been called as simple sequence repeats (SSR), short tandem repeat (STR) or variable tandem of repeat (VNTR). These tandem repeats are distributed throughout the genome (Tautz, 1993). There are three distinct parts within these sequences: i) initial flanking sequence, , ii) repeat region, and iii) the final flanking sequence. Initial and final flanking sequences are the parts from which the first and the second primer sequences are designed respectively. On the other hand, the number of repeats in the repeat region generates the polymorphism of that microsatellite locus (Goldstein & Schlötterer, 1999).

There are two extreme mutation models considered for microsatellite loci: "The Initial Alleles Model" (IAM, Kimura & Crow, 1964), which involves the loss or the gain of any number of tandem repeats, and "The Stepwise Mutations Model" (SMM, Kimura & Ohta, 1978),which represents the loss or the gain of a single tandem repeat. In 1994, Di Rienzo *et. al.* combined SMM and IAM and introduced a new model called "The Two Phase Mutation Model" (TPM). However, Estoup and Cornuet, implied in the book of Goldstein & Schlötterer (1999) that current knowledge of the mutation processes at microsatellite loci is insufficient to allow representation in any of the mutation models. Although no mutational model have been adopted for the microsatellites, their mutation process have been explained. Estoup and Cournet have stated that "The mutation process of microsatellites is composed of two processes, the generation of replication errors by slip-strand mispairing (SSM) and the correction of some of these errors by the exonucleolytic

proofreading or mismatches repair system. And they concluded that the rate and the type of these SSM errors and the success of the exonucleolytic proofreading and mismatch repair systems determine the mutation rate and the pattern of the microsatellite (Goldstein & Schlötterer, 1999)".

In 1989, 3 different research groups have indicated that microsatellites show high levels of polymorphism and they have reported how to exploit microsatellites in genome analysis (Litt & Luty, Weber & May and Tautz). As a result of their exceptionally high mutation rates, co-dominance, abundance throughout the genome and relative ease of scoring compared to minisatellites, microsatellites are considered as powerful markers for linkage analysis, identity testing, genetic mapping and population studies (Estoup *et. al.*, 1993; 1995).

As it was indicated before, allozyme studies were not informative in differentiating among *B. terrestris* populations; however microsatellites did (Aytekin & Çağatay, 2002c; Scholl *et. al.,* 1990; Estoup *et. al.*, 1993). Therefore, we have chosen to use microsatellite markers to study the population genetics of the Anatolian and Cypriot *Bombus terrestris* populations in combination with morphometry.

Estoup et. al. indicated that they could not find any significant difference among populations of the European continent, however there is significant difference among island populations and continental populations of *B. terrestris* (1996). In their study, they have used 10 microsatellite loci in concordance with a mitochondrial locus. Even though they have sampled from Cyprus in their study, they did not include any populations from Anatolia. With this study, they pointed out that the island populations of *B. terrestris* should be protected against the importation of bumblebees of foreign origin which are used as crop pollinators.

1.2. The Objective of the Study

Main objectives of this study are:

- To evaluate the amount of differentiation of different *Bombus terrestris* populations from Turkey and Cyprus by the use of morphological characters.

- To determine the extent of microsatellite variation in *B. terrestris* populations of Ankara and North Cyprus and estimate the amount of genetic differentiation.

- To find out the genetic relations between Ankara and other continent and island populations of *B. terrestris*.

- To produce data to be used in controlling continental transfer of *B. terrestris* and its impacts by characterizing their population genetic structure in Turkey and in Northern Cyprus.

CHAPTER 2

MATERIALS AND METHOD

2.1 Biological materials

Samples were all collected on plants, while they were foraging for nectar or pollen, by the help of a net and a vacuum jar. Coordinates, temperature and elevation of the particular sampling site as well as the flower that the sample has been collected have been recorded in order to have some ecological information about the species. In order to make sure that exact species had been collected, *B. terrestris* samples have been identified by Dr. Ahmet Murat Aytekin.

There are two main districts of the study, Turkey and North Cyprus. 116 samples (41 workers, 1 queen and 74 males) from various parts of North Cyprus, 74 samples (31 workers, 8 queens and 35 males) from various parts of Ankara, 6 worker samples from Adana, 8 samples (7 workers and 1 queen) from Antakya, 5 worker samples from Samsun and 5 worker samples from Bartın were collected (Table 1).

Table 1. Number of *B. terrestris* samples from each site belonging to each caste

***Bombus terrestris* Sample Castes/ Sites:**		**Worker (o-) (2n):**	**Queen (♀) (2n):**	**Male (♂) (n):**
SOUTHERN ANATOLİA	**ADANA**	6	-	-
	ANTAKYA	7	1	-
CENTRAL ANATOLİA:	**ANKARA**	28 (31)	8	45
NORTHERN ANATOLİA	**BARTIN**	5	-	-
	SAMSUN	5	-	-
CYPRUS		35 (41)	1	74
TOTAL		86 (95)	10	119

Just after they had been collected, samples have been put into pure or 70% ethanol to prevent any kind of deformation. Afterwards, bees were stored at 4 °C or at room temperature.

Only some of the diploid samples (workers and queens) have been used for molecular analysis as heterozygiosity can not be observed among haploid samples. On the other hand, only queens have been excluded from the morphometric studies because of their small sample size.

2.2. Classical Morphometric Procedure

Wings of the samples have been separated from the body by pliers. These parts have been glued on to the slides by entellan and all of the specimens have been labelled in order to classify each sample according to its' geographic site.

Only the right wings have been used for morphometric studies. All of the specimens' right wings have been photographed by a Leica MZ-7.5 stereoscopic zoom dissection microscope with a DC-300 digital camera system. Then a total of 23 characters were measured directly on the screen by the help of Image Tool and tpsUtil and tpsdig Programmes, which were chosen according to the study of Çağatay *et. al.* (2000). 12

of these characters were length and 11 of them were angle measurements from the front and the hind wings (Fig. 1, Fig. 2).

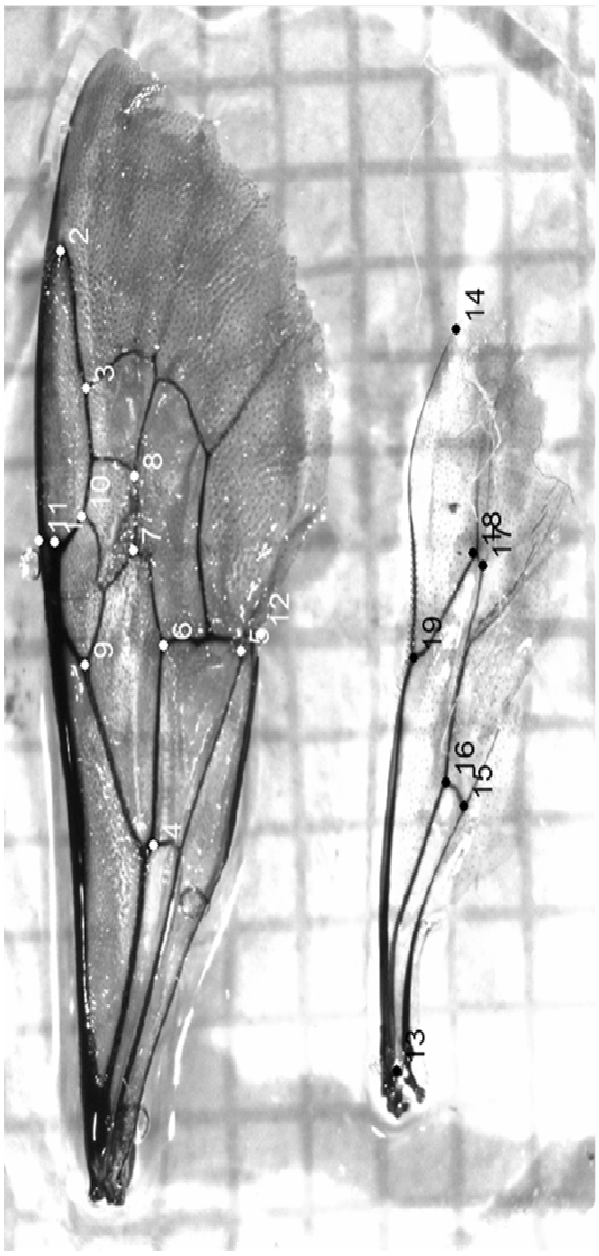

Figure 1. Totally 12 Size Variables (Length Characters (LC)) from The Front and The Hind Wings of *Bombus terrestris*; as: Fore-wing: LC-1: (1 to 2) Marginal Cell Length, LC-2: (2 to 3) Radial Sector Length, LC-3: (4 to 5) The length between the Opposite Corners of the 2nd Cubical Cell, LC-4: (6 to 7) The length between the First Medial Cell and the corner of the Second Submarginal Cell, LC-5: (7 to 8) 2nd Sub-marginal Cell Length, LC-6: (9 to 10) 1st Sub-marginal Cell Length, LC- 7: (11 to 12): Fore-wing Width; Hind-wing: LC-8: (13 to 14) Length of the Main Vein, LC-9: (15 to 16) Cubitus-Anal Vein Length, LC-10: (16 to 17) Media + Cubitus Length, LC-11: (17 to 18) + LC-12: (18 to 19) Radial Sector Length

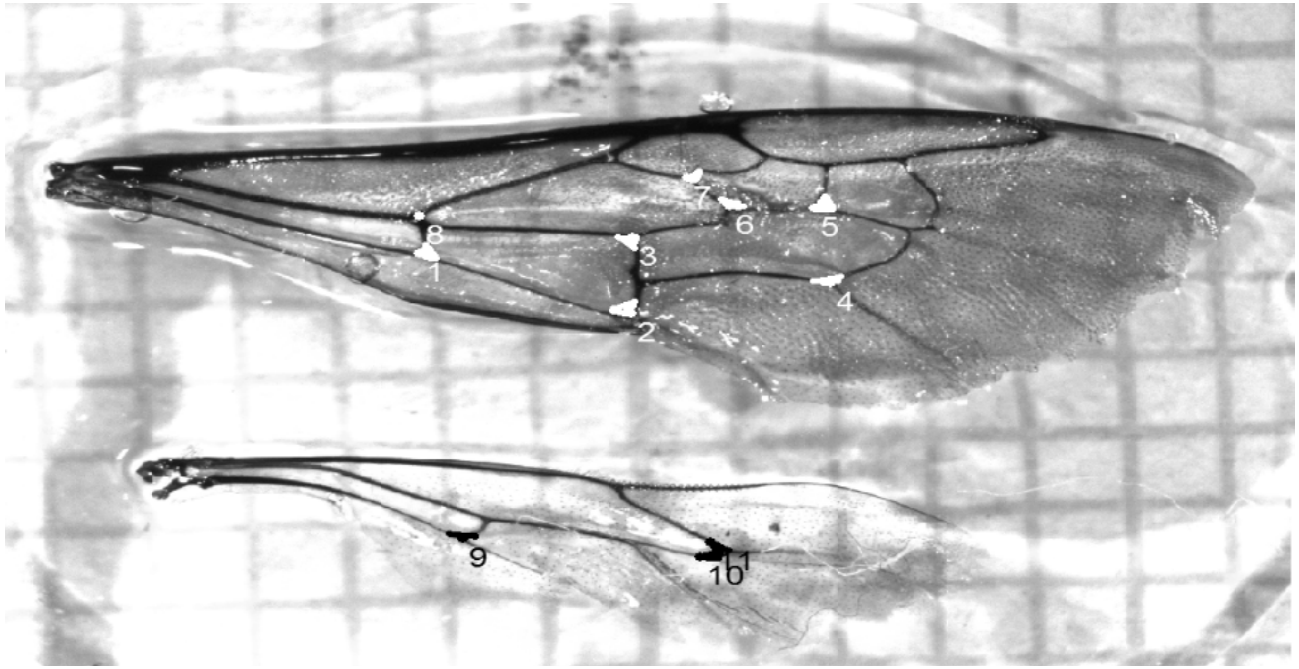

Figure 2. Totally 11 Shape Variables (Angle Charaters (AC)) from The Front and The Hind Wings of *Bombus terrestris*; as: Fore-wing: AC-13-14-15 (1, 2, 3): Cubital Cell Angles, AC-16 (4): Second Cubital Cells' Bottom Angle, AC-17 (5): The angle of the Third Submarginal Cell towards the Third Submarginal Cell, AC-18 (6): The angle of the Second Submarginal Cell between the First Medial and First Submarginal Cells, AC-19 (7): The angle of the Second Submarginal Cell between the First Medial and First Submarginal Cells, AC-20 (8): The angle of the First Medial Cell opposite to the Second Submariginal Cell; Hind-wing: AC-21 (9): The angle of the Medail and the Radial Sectors' corner, AC-22-23 (10, 11): Angles between the Anal and the Cubitoanal Vein

2.3. Genetic Procedure

2.3.1. DNA Isolation

Individual DNA extractions have been performed from the head or the thorax.

The head or the thorax of the bee has been removed after taking the bees out of the alcohol. They were put on a paper towel in order to get rid of the excess alcohol. Then each was put in a 1.5 ml sterile eppendorf tube and grinned by a sterile pestle. 750 µl of Wilson Buffer (Table 2) and 25 µl of 10 mg/ml Proteinase K were added into each tube. Tubes were mixed briefly before they had been incubated in a 50°C water bath for 2 hours. After a centrifugation step at 4°C at 10,000 rpm for 10 minutes, the supernatant (the upper phase of the solution) was pour into another sterile 1.5 ml tube. 750 µl of Phenol:Chloroform:Isoamylalcohol (25:24:1) mix was inverted gently into each tube for 5 minutes. And this homogenous mix had been centrifuged at 10,000 rpm for 20 minutes at 4°C. Then 600 µl of the supernatant was transferred to another sterile tube. 600 µl of Phenol:Chloroform:Isoamylalcohol (25:24:1) mix and after that 450 µl of Chloroform:Isoamylalcohol (24:1) mix was applied to the supernatant as it had been done by Phenol:Chloroform:Isoamylalcohol (25:24:1) mix. Then 300 µl of aqueous phase was recovered and transfer into new tubes for the last time. 30 µl NaOAc (3M) and 600 µl of cold alcohol were added to each tube and mixed. This mix was stored at –20°C for overnight. The day after, it had been centrifuged at 13,000 rpm for half an hour. Supernatant was discarded gently without dropping the pellet. 800-10,000 µl of 70% cold ethanol was added and centrifuged at 13,000 rpm again for 20 minutes at 4°C. After pouring the alcohol out, the tubes were replaced in the desiccator for 30 minutes to evaporate the rest of the alcohol. Then 50 µl sterile water was added and the tubes were left in the room temperature for 1 hour or overnight at 4°C.

Table 2. Preparation of Wilson Buffer

Wilson Buffer
Add the followings: • 10 ml from 1 M Tris.Cl pH: 8 stock solution. • 200 µl from 0.5 M EDTA stock solution. • 1 ml from 10% (NIV) Lauryl Sulfate (SDS) stock solution. • 0.771 gr of DTT. • 0.584 gr of Sodium Chloride (NaCl). Add distilled water to complete to 100 ml.

Afterwards, all of the tubes have been checked for the success of the application. 5 µl sample from each tube had been run on 1.6% agarose gel. Ethidium Bromide was applied to the gel in order to see the DNA under UV light.

After confirming the presence of DNA and its concentration for each tube, 250-350 µl water was added to each tube according to their DNA concentrations. At last the tubes could have been stored in –20°C for long time storage.

2.3.2. Microsatellite amplification by PCR

Four *Bombus terrestris* microsatellite loci, namely B126, B124, B11 and B100, were chosen out of 26 *Bombus terrestris* microsatellites available (Estoup *et al.* 1993). Their core regions and primer sequences are given (Table 3).

Table 3. Core sequences of the four microsatellites and their primers used for their amplification.

Locus	Core Sequence	Primers
B11	$(CT)_{5}$∘∘∘$(CT)_{10}(ATCT)_{6}$∘∘∘$(CT)_{3}$ $(ATCT)_{3}$	5′-GCAACGAAACTCGAAATCG-3′ 5′-GTTCATCCAAGTTTCATCCG-3′
B100	$(CT)_{12}GTC(CT)_{3}$	5′-CGTCCTCGTATCGGGCTAAC-3′ 5′-CGTGGAAACGTCGTGACG-3′
B124	$(CT)_{11}TCCTCTTCCAC(CT)_{14}$ $CCTC(GC)_{3}$∘∘∘$(GGCT)_{8}$	5′-GCAACAGGTCGGGTTAGAG-3′ 5′-CAGGATAGGGTAGGTAAGCAG-3′
B126	$(CT)_{12}GT(CT)_{10}$	5′-GCTTGCTGGTGAATTGTGC-3′ 5′-CGATTCTCTCGTGTACTCC-3′

Radioactive PCRs (Polymerase Chain Reactions) were carried out as described in Estoup *et al.* 1995. 10 µl of a mixture containing 5-10 ng of DNA template, 400 nM of each primer, 75 µM each 2′-deoxyguanidine 5′-triphosphate (dGTP), 2′-deoxycytidine 5′-triphosphate (dCTP), 2′-deoxythymidine 5′-triphosphate (dTTP), 6 µM 2′-deoxyadenosine 5′-triphosphate (dATP), 0.7 µCiα^{35}S-dATP, 1.0-1.5 mM $MgCl_2$ 20 µg/mL Bovine Serum Albumin (BSA), 1 x Promega Reaction Buffer and 0,4 unit of *Taq* polymerise. After a denaturing step aof 3 minutes at 94°C, samples were processed through 35 cycles consisting of 30 seconds at 94°C, 30 seconds at an optimal annealing temperature (T°m) and 30 seconds at 72°C. At last elongation step was lengthened to 10 minutes. The optimal annealing temperatures and $MgCl_2$ concentrations that were used for each microsatellite loci are given (Table 4).

Table 4. Optimal annealing temperatures and magnesium chloride concentrations for five microsatellite amplifications

Locus	Optimal Annealing Temperature (T°m) (°C)	Magnesium Chloride ($MgCl_2$) Concentration (mM)
B11	52	1.5
B100	58	1.0
B124	57	1.2
B126	57	1.2
B132	58	1.0

2.3.3. Sequencing polyacrylamide gel electrophoresis

Sequencing polyacrylamide gel apparatus has been used to separate alleles differing with one or a few nucleotides.

2.3.3.1. Cleaning the glass plates

Two glass plates of twenty five to forty five cm long were used in the preparation of each gel. Before pouring the gel, one side of each plate were cleaned first by deionised water and then by absolute ethanol in order to prevent anything on the surface to hamper the DNA fragments during electrophoresis. Then a silanizing (Sigmacote) solution was applied to the clean side of one of the plates. Once the gel has hardened, this solution would ease the separation of the plates by releasing gel from the side that it has been applied and residing it intact on the other plate.

2.3.3.2. Preparation of the gel

A 6% denaturing gel was used in the electrophoresis. Denaturing gel has been used in order to achieve discrimination in between single-stranded nucleic acid fragments. %6 acrylamide/urea solution (Table 5) contains 8M urea that is high enough to denature DNA. This mix was put into a light-tight bottle and kept at room temperature, ready to use. 650µl of 10% (v/v) ammoniumpersulfate (APS) and 30µl of N, N, N′, N′-tetramethylethylenediamine (TEMED) were added to 60µl of 6% polyacrylamide mix just before pouring the gel.

Table 5. Preparation of 6% Acrylamide/ Urea Solution

6% Acrylamide/ Urea Solution
Add the followings: • 75 ml from 40% acrylamide solution • 50 ml from 10xTBE • 240 g from urea Adjust the volume to 500 ml by adding distilled water

2.3.3.3. Pouring the gel

A gel caster, a shark's tooth comb and 0.4 mm plastic spacers were used. After the plates were cleaned, one of the plates was replaced on the gel caster. Before replacing the second plate on to the top of the first one, spacers were placed just to the sides of the first plate. The gel mix was applied immediately after the addition of TEMED. While applying the gel, plates were slid such that they would be on top of

each other. A shark's tooth comb was immediately squeezed in between the top side of the plates. Then metal clamps were applied to the both sides of the plates. And the gel was left to harden for 2 hours.

2.3.3.4. Loading and running the gel

10 µl of stop solution was added to each product of PCR (Table 6). Loading buffer for the denaturing polyacrylamide chain reaction contains Formamide that prevents the reaction to restart while it is heated again. Before loading, the tubes containing the mix were kept at 94°C for 2 minutes and denatured. 2.5 µl of each tube were loaded to each wheel of the shark's tooth comb separately. A marker set prepared by using α^{33}P-dATP was loaded to the gel as size marker to determine the size of the alleles (DNA fragments).

Table 6. Preparation of the Stop Solution (Loading buffer for the denaturing polyacryamide gel electrophoresis)

Stop Solution

Add the followings:

- 10 ml of Formamide
- 10 mg of Xylene cyanol FF
- 10 mg of Bromophenol blue
- 200 µl 0.5 EDTA (pH=8)

Upper and lower buffer reservoirs of the gel apparatus were filled with 1xTris-Borate-EDTA (TBE) buffer solution. Then it was run at 40 watt for 2.5-3 hours, depending on the size of the microsatellite loci and its' alleles.

2.3.4. Autoradiography

Once the electrophoresis was over, plates were separated. The gel that remained on one of the plates was taken onto a chromatography paper (Whatman 3MM). While the chromatography paper covered one side of the gel, the other side was covered by ordinary stretch film. Then it was dried on a vacuum dryer at 80°C for 25 minutes. Special autoradiography films (Kodak Biomax MR) were exposed to the dried gel in a dark room and placed in a light tight metal cassette for 2-7 days depending on the age of the radioactive material. The exposed films were developed in the medical centre of Middle East Technical University.

Some of these films and some of the alleles obtained for each loci are shown in figures 3, 4, 5 and 6.

Figure 3. Some of the alleles of loci B126; their lengths represented under each allele

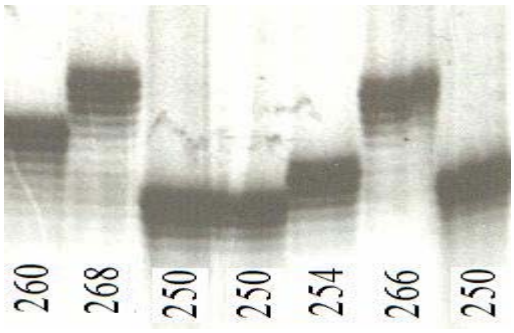

Figure 4. Some of the alleles of loci B124; their lengths represented under each allele

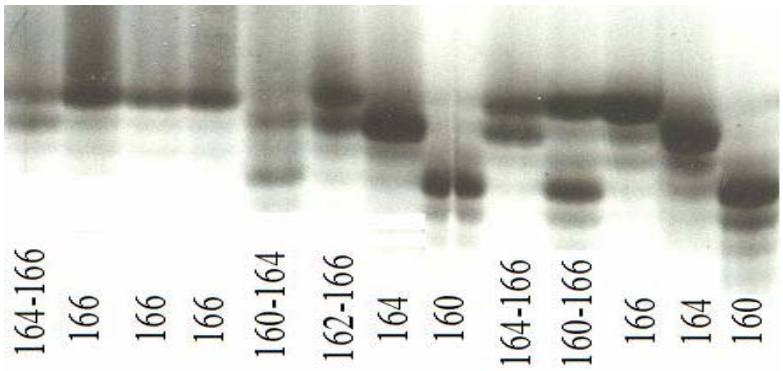

Figure 5. Some of the alleles of loci B11; their lengths represented under each allele

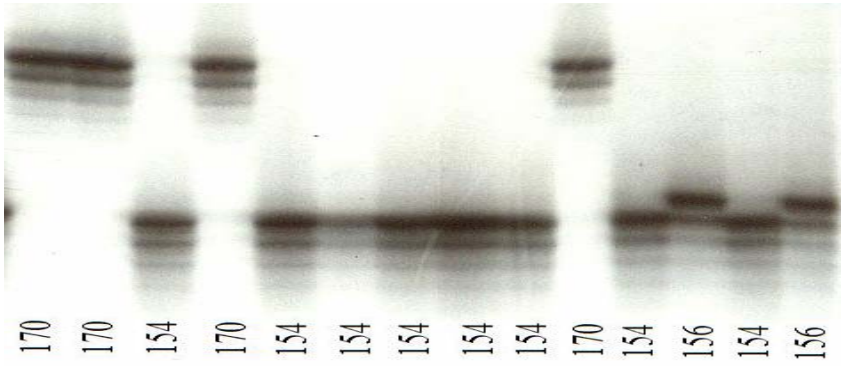

Figure 6. Some of the alleles of loci B100; their lengths represented under each allele

2.4. Statistical Analysis

2.4.1. Morphological Data Analysis

Principal Components Analyses (PCA) and Canonical Variates Analyses have been performed for workers and males separately, as different castes are proved to be morphologically different from each other by Çağatay *et. al.* (2000).

2.4.1.1. Principal Component Analysis (PCA)

Principal Component Analysis is an important method of ordination analysis. It is a way of identifying patterns in ungrouped data with continuous variables. In this study, it is used to see if there is a pattern in the distribution of the individuals in the principal components space. Before performing PCA, data has been standardized; as it consists of variables that are not all in the same units.

When PCA is performed, first the patterns of the individuals have been found. Then the standardized data has been compressed by constructing a new set of orthogonal coordinate axis so that the projection of the points onto them has the maximum variance. As a result, the first few principal component axes contain most of the variation in the data. Consequently, PCA is a powerful tool for summarizing the data, as it is hard to find patterns in the data of high dimensions, especially where graphical representation is not available.

Analyses have been performed with the help of computer program called NTSYS (Rolfh, 1993).

2.4.1.2. Canonical Variates Analysis

Canonical Variates Analysis is another method of ordination analysis. It is a way of finding the set of axes that allows for the greatest possible ability to discriminate between two or more groups. The analysis finds linearly uncorrelated axes by maximizing the distinction among groups and minimizing the variance within groups in the new canonical space. It determines how many CVA axes there are in the data by MANOVA test and computes the canonical variates scores of all the specimens entered in decreasing order. By plotting points in the first few canonical variates space, it gives the best pictures showing the separation of the groups. It permits the user to directly compare two or more data matrices, as in this study it is used to compare the amount of discrimination between different *B. terrestris* populations.

Analyses have been performed with the help of software program called SYNTAX (Podani, 1993).

The diagrams of both of the analysis are drawn in two dimensional space, as there are too many heterogeneously dispersed individuals obstructing a meaningful distribution view over a three dimensional diagram.

2.4.2. Population Genetic Data Analysis

2.4.2.1. Allele Frequencies and Heterozygosities

The statistical analysis of the molecular data started with the calculation of relative frequencies of each microsatellite allele in each of the studied populations. Allele frequencies were calculated as the proportion of the observed allele to the total number of alleles in the population, and this can be formulated as:

$$\text{Fq. of an Allele (p)} = \frac{(2\text{x Number of Homozygotes}) + (\text{Number of Heterozygotes})}{2\text{x Total Number of Individuals (N)}}$$

Observed and expected heterozygosities helps to measure the genetic variation in a population. Observed heterozigosity was calculated with the formula below:

$$\text{Observed Heterozigosity} = \frac{\text{Number of Heterozygotes}}{\text{Total Number of Indıviduals}}$$

Expected heterozygosity is computed from the allelic frequencies calculated from the population data meeting Hardy-Weinberg expectations.

Heterozygosities with Leven's correction and allelic frequencies have been calculated by using GENEPOP on the web software (Raymond and Rousset).

Nei have implied that average heterozygosity and, where variation results from the continued presence of different homozygotes, gene diversity seem to be the best parameters to measure genetic variation (Nei, 1975). Average heterozygosities and its sampling variances (Nei 1978) for both of the populations and gene diversity of each of the loci and all loci have been calculated by the help of DISPAN software (Ota, 1993). Average heterozygosities of each population is compared by *t* Test for paired comparisons.

2.4.2.2. Testing Hardy-Weinberg Equilibrium

The Hardy-Weinberg Principle allows a great simplification of the description of a population's genetic content by reducing the number of parameters that must be

considered, as it is based on the following assumptions: i) random mating; ii) allelic frequencies are conserved from generation to generation; ii) no significant migrations occur; and iv) mutation, genetic drift, selection and gene flow are negligible. By these assumptions it states that single-locus genotypic frequencies after one generation of random mating can be represented by a binomial (with two alleles) or multinomial (with multiple alleles) function of the allelic frequencies All of these assumptions were assumed to be true while calculating expected genotypic frequencies, like expected heteroygosity (Hedrick, 2000).

Chi-square (χ^2) test was employed to determine whether the observed numbers are consistent with Hardy-Weinberg predictions. The test has been carried out according to exact test of Haldane (1954), Weir (1990), Guo and Thompson (1992) and others; at the same time the Markov chain method have been used to estimate the P-value. Both of these calculations have been performed by using GENEPOP software on the web (Raymond and Rousset). Then the null hypothesis (H_0), stating that observed and expected heterozygosities are same, was evaluated.

2.4.2.3. Testing Linkage Disequilibrium

When there is random association between alleles within gametes, then the frequency of each gamete is a product of the frequencies of the alleles it contains. However, when there is non-random association of these alleles within gametes, gametic frequencies deviate from the expected frequencies under random associations. This is called linkage disequilibrium (Hedrick, 2000). It can have positive or negative values. LD is decreased by recombination. Thus, it decreases every generation of random mating unless some process opposing the approach to linkage 'equilibrium'. The main difference between the Hardy-Weinberg Equilibrium and linkage disequilibrium tests is that while HWE test look at two genes at the same locus on different gametes, linkage disequilibrium test looks at two genes on the same gamete

or on different gametes at different loci. Linkage disequilibrium tests have been performed by using GENEPOP on the web software (Raymond and Rousset).

2.4.2.4. Population Structure Analysis

2.4.2.4.1. Genic Differentiation

The null hypothesis of identical allelic distribution among populations was tested for each locus considering each population. Tests have been performed by using GENEPOP (Raymond and Rousset).

2.4.2.4.2. Genotypic Differentiation

The null hypothesis of identical genotypic distribution among populations was tested for each locus and for all loci by using GENEPOP (Raymond and Rousset).

2.4.2.4.3. Population Comparison and Structure Analysis by F Coefficients

Population subdivision would result in decreased heterozygosity relative to that expected heterozygosity. Wright developed three fixation indices to evaluate population subdivision: F_{IS} (interindividual), F_{IT} (total population), F_{ST} (subpopulations).

F_{IS} and F_{IT} are the measures of the deviation from Hardy-Weinberg proportions within subpopulations and in the total population respectively, where positive values indicate a deficiency of heterozygotes (inbreeding) and negative values indicate an excess of heterozygotes (outbreeding).

F_{ST} is a measure of the genetic differentiation over subpopulations, which is the reduction in heterozygosity in a subpopulation due to genetic drift. F_{ST} is always positive; it ranges between 0 = panmixis (no subdivision, random mating occurring, no genetic divergence within the population) and 1 = complete isolation (extreme subdivision) (Hedrick, 2000). Hartl and Clark have suggested qualitative guidelines for the interpretation of F_{ST}; as the range between 0-0.05 indicates little genetic differentiation, 0.05-0.15 indicates moderate genetic differentiation, 0.15-0.25 indicates great genetic differentiation and values above 0.25 indicates very great genetic differentiation. Single locus F-statistics are calculated according to Weir and Cockerham (1984) (Fwc), as the estimator does not make assumptions about numbers of population, sample size and heterozygote frequencies and are suited to small data sets. Calculations have been done with the help of GENEPOP software on the web (Raymond and Rousset).

2.4.2.4.4. Genetic Distance Calculations and Phenogram Construction

Phenograms are constructed according to the distance matrix calculated from allele frequencies of the microsatellite data. Nei's standard genetic distance (Nei, 1972) between the populations studied in Estoup *et. al*. (1996) (Gif-sur-Yvette (France), Tours (France), Banyuls-sur-mer (France), Madrid (Spain), Avellino (Italy), Nea-Moudania (Greece), Pleven (Bulgaria), Lublin (Poland), Samos (Island), Cyprus (Island), Elba (Island), Corsica (Island) and Sardinia (Island)) and the populations studied (Ankara (Anatolia) and Cyprus) is calculated by DISPAN software (Ota, 1993). Only B11 and B100 loci were common in both studies that the calculations were according to only these loci. Phenogram were constructed by the neighbor-joining method of Saitou and Nei (1987) from the Nei's standard genetic distance matrix with 1000 replications by using DISPAN software. Bootstraping percentage values give the proportion of the presence of that node that groups certain populations together, to all cases of 1000 bootstrapping resamplings. *Bombus lucorum* population has been used as outgroup in order to root the tree.

It should be noted that while using microsatellite data from other researcher's studies, there might be some calibration errors, as microsatellite data is so sensitive even to one base pair differences among alleles. In order to cope with this problem, the Cyprus data obtained by this study is changed in all probable ways and possible trees are constructed for each different data. Most appropriate tree, combining two Cyprus populations, one from the data obtained in this study and the other from the data obtained by Estoup *et. al.* (1996), with %100 bootstrap value is accepted as the true microsatellite data. The phenogram constructed by this data is accepted to be the right phenogram.

CHAPTER 3

RESULTS

3.1. Morphometric Analysis

Mean and Standard Error values of the variables in male and worker populations are given in Appendix A.

3.1.1. Male Morphometrics

Analysis of evaluation of the male morphometrics includes totally 119 individuals. These individuals have been arranged in such a way that the first group with 45 individuals are from Ankara population; where as all the rest are from various parts of North Cyprus, as Güzelyurt, Beşparmak Mountains and Girne with 6, 14 and 53 individuals respectively (Table 7).

Table 7. Groups of the males divided according to their provinces in order to be used in Principal Components Analysis and Canonical Variates Analysis

Group:	**1st Group: (Anatolia)**	**2nd Group: (North Cyprus)**	**3rd Group: (North Cyprus)**	**4th Group: (North Cyprus)**
Provinces:	Ankara	Güzelyurt	Beşparmak Mountains	Girne
Number of Individuals	45	6	14	53
Labels of Individuals:	1- 45	46-51	52-65	66- 118

3.1.1.1. Principal Components Analysis (PCA) of Males

Principal Components Analysis of males explained all of the variation by 23 components, as there are 23 variables. The first principal component explained %26.1810, while the second and the third explained % 8.1832 and %7.0471 of the overall variation respectively. So %42.1583 of the total variation is explained by the first three components (Table 8).

Table 8. Eigen Values of the first three principal components and total variation explained by each of them

Principal Components	**% Eigen Values**	**% of Total Variation**
1. Principal Component	26.1810	26.1810
2. Principal Component	8.1832	35.1112
3. Principal Component	7.0471	42.1583

Eigen vectors showed that all of the length character variables, except LC-5, LC-8 and LC-11, with AC-13, AC-19 and AC-23 give really high weights to the first component. The second component is constructed primarily by the variation on LC-3, LC-8, LC-11, AC 21 and AC-22. And, the third component is mainly created by the variation on LC-2, LC-5, LC-9, AC-13 and AC-20 (Table 9).

Table 9. Weights (Eigen Vectors) of 23 variables on the first 3 principal components

Variable	1st Principal C.	2nd Principal C.	3rd Principal C.
LC-1	7.8858018	1.0884606	2.8906535
LC-2	9.2485401	5.1327705	-9.322764
LC-3	7.4509144	6.1883229	-2.022772
LC-4	6.6550457	1.8199335	-4.538389
LC-5	4.3875327	-4.813510	-5.336879
LC-6	6.8165794	-1.044031	-2.835101
LC-7	8.6225236	5.0867002	3.1561694
LC-8	1.5405187	8.9317060	-3.599287
LC-9	6.0842178	2.6244447	7.8961741
LC-10	7.7183243	1.7418232	1.0408442
LC-11	4.1986698	6.3411379	-1.796905
LC-12	8.7542194	2.4048621	-3.550784
AC-13	7.9542194	1.2227118	6.0674283
AC-14	-3.769110	4.6386132	-4.074108
AC-15	-1.132958	-3.589923	3.1177352
AC-16	-1.516836	-1.948127	-3.253890
AC-17	-1.516836	1.0020174	-5.225270
AC-18	1.7815187	-2.432001	-1.391288
AC-19	8.3386660	-3.537252	-2.411185
AC-20	-2.074756	3.7366927	-6.278540
AC-21	-2.461325	5.8158182	1.0551066
AC-22	1.7234901	-7.520790	-2.874504
AC-23	8.8918446	-5.061970	2.1014523

Calculated correlation matrix of the variables represents the relative correlations of the variables according to the first and the second principal components (Appendix B). According to this matrix, except variables LC-5 and LC-6 to LC-11, the rest of

the size variables are positive correlated with each other. On the other hand, shape variables show both positive and negative correlations.

The scatter diagram of the male individuals, constructed by the first two principal components, has been drawn to show relative positions of the populations based on morphometric characters (Figure 7). According to the diagram above, these two populations does not show distinct clusters at all. Cyprus populations are grouped together, while Ankara population appears to be distributed more dispersed than Cyprus populations. However the individuals' distribution is rather heterogeneous on the right parts of the diagram, these two populations have an apparently different ordination according to the first principal component. It is obvious that the first axis has the most contribution to this difference in the ordination of the populations.

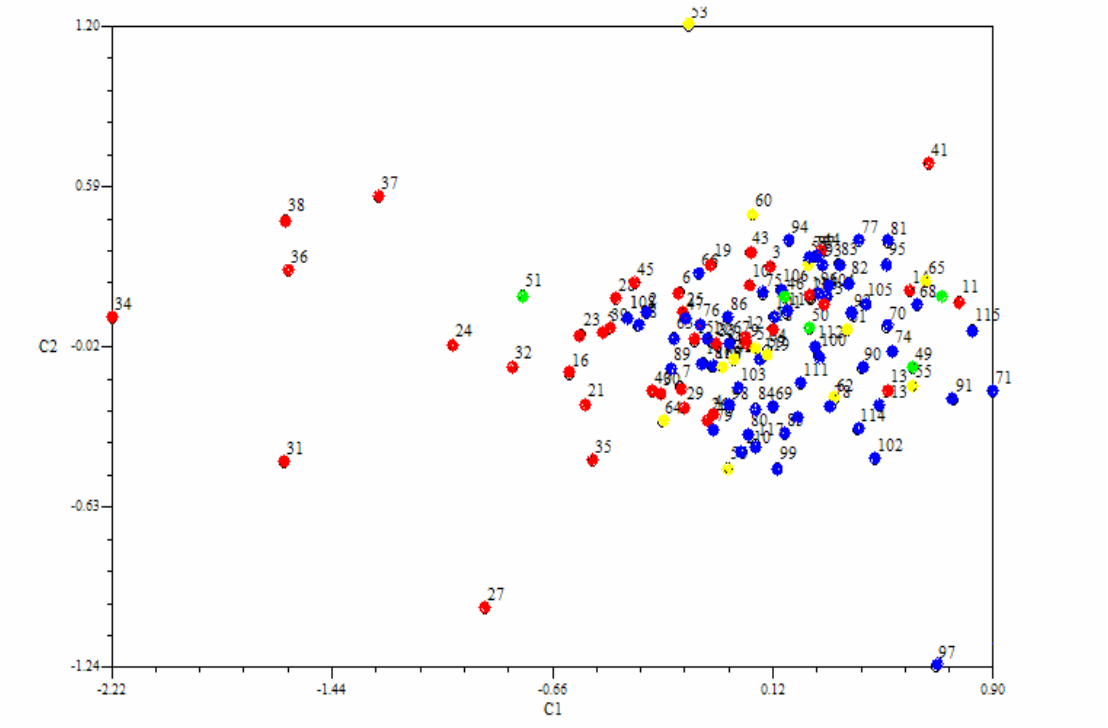

Figure 7. The Scatter Diagram of the Male Individuals, as a result of the first two principal components. Provinces have been represented in colours; as Ankara in red, Güzelyurt in green, Beşparmak M. in yellow and Girne in Blue.

3.1.1.2. Canonical Variate Analysis (CVA) of Males

Canonical Variates Analysis of males explained all of the variation only by 3 axes. The first canonical axis explained %56.61, while the second and the third (not shown) explained %29.38 and %14.01 of the overall variation respectively. Altogether these axes represent all of the variation (Table 10).

Table 10. Eigen Values for each of the canonical variates and total variation explained by each of them

Canonical Variates	% Eigen Values	% of Total Variation
1. Canonical Variate	56.61	56.61
2. Canonical Variate	29.38	85.99
3. Canonical Variate	14.01	100.00

Discriminant weights of the variables for each axis are the coefficients of the variables in the linear equations that define the canonical functions, as shown above (Table 11).

Table 11. Discriminant Weights of the variables for each canonical variate

Variable	1. Canonical V.	2. Canonical V.	3. Canonical V.
LC-1	-0.1143286	0.5127114	-0.2551850
LC-2	-0.8950803	-0.1914291	-0.2457349
LC-3	0.4171279	-0.2269086	0.2189912
LC-4	0.2161161	-0.4138465	0.5055472
LC-5	-0.8818007	0.5151891	0.2630184
LC-6	-0.1375485	-0.7739510	0.3276156
LC-7	0.6390064	-0.1276214	0.2751466
LC-8	-0.1585817	0.3893171	-0.7955138
LC-9	-0.2862758	-0.2909053	-0.4156979
LC-10	0.6999323	-0.6576500	0.6438922
LC-11	-0.1160137	-0.1120358	-0.1291966
LC-12	-0.8767216	0.3036503	0.9744187
AC-13	0.4895853	0.1218401	-0.5903208
AC-14	-0.5863513	-0.3048117	-0.1124379
AC-15	-0.9624732	0.5417433	-0.6203456
AC-16	0.2004018	0.1256238	-0.6877255
AC-17	0.1210628	-0.8204256	0.1681249
AC-18	0.3648660	-0.1224080	0.1241224
AC-19	0.2175132	-0.7155123	-0.4048375
AC-20	0.2245252	-0.1216739	-0.1794821
AC-21	-0.8688975	-0.7727473	-0.5829502
AC-22	-0.1086029	0.1556945	0.7170727
AC-23	0.1414146	-0.9475602	0.1529676

According to these weights, LC-2, LC-5 and LC-12 are the length and AC- 15 and AC-21 are the angle variables having the highest weights on the first axis, while LC-6, AC-17, AC-19, AC-21 and AC-23 have on the second. On the other hand, LC-8, LC-12 and AC-22 have been the most effective variables in constructing the third axis.

Figure 8 represents the scatter diagram of the individuals according to the first two canonical axes. As, the principal components scatter diagram, there is not distinct clusters of populations, however the scatter diagram above have been much more successful in grouping these populations. It should be noted that, while PCA scatter

diagram explained %35.11 of the total variation, %85.99 of the total variation have been explained by this diagram.

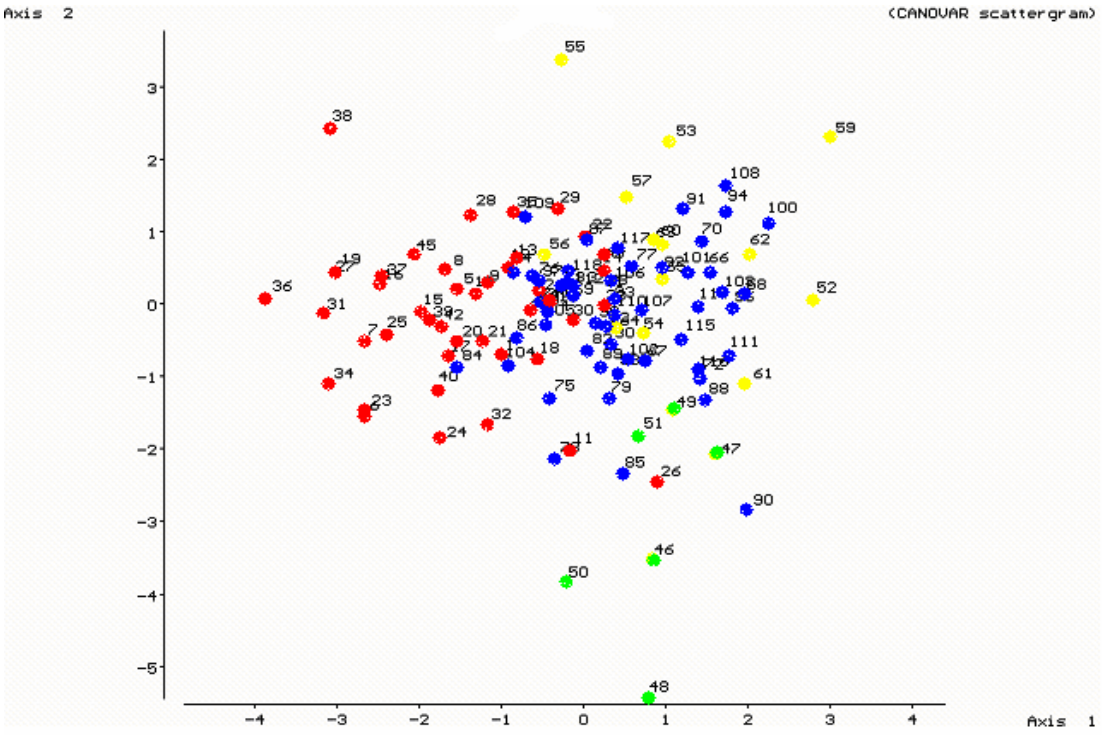

Figure 8. The Scatter Diagram of the Canonical Variates Analysis, obtained from the first two canonical variates. Provinces have been represented in colours; as Ankara in red, Güzelyurt in green, Beşparmak M. in yellow and Girne in Blue

When the MANOVA (Multivariate Analysis of Variance) statistic test has been performed; the analysis indicated that there is significant differentiation especially between Ankara and Cyprus populations (Figure 9). This discrimination between Ankara and Cyprus populations is due to the variation on the first canonical axis, and as indicated before this is the axis carrying more than half of the total variation.

Variables separating the clusters have been shown by the vectors on the diagram. The angles between the vectors indicate the correlations between the variables. It can be easily seen that LC-4 and LC-12 are %100 positively correlated to each other,

while the rest show positive and negative correlations (Figure 9). Also, analysis of variance has shown that all variables except AC-19 and AC-23 are significantly different among localities of the male populations (Table 12).

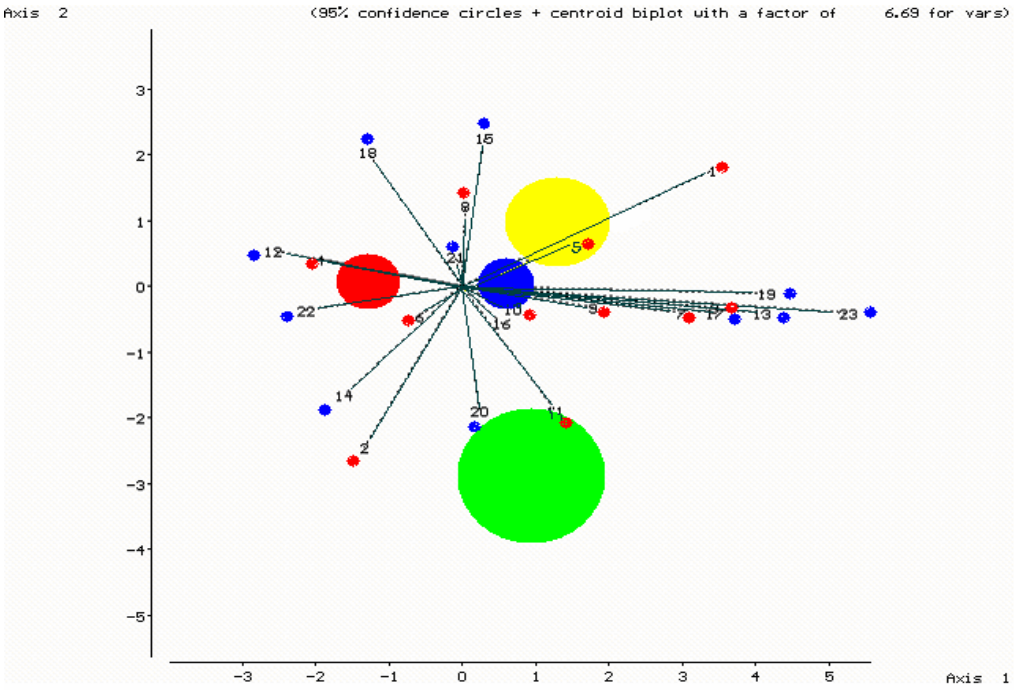

Figure 9. The Graph of the Separated Groups and Variables (with %95 confidence circles and centroid biplot with a factor of 6.69 for variance). Provinces have been represented in colours; as Ankara in red, Güzelyurt in green, Beşparmak M. in yellow and Girne in Blue

Table 12. F-Ratios calculated for each variable by the analysis of variance, with 3 and 114 degrees of freedom (* = P <0.05, ** = P<0.01, ns = not significant)

Var.	F-R.	Var.	F-R.	Var.	F-R.	Var.	F-R.
LC-1	7.246*	**LC-7**	4.650*	**AC-13**	8.479*	**AC-19**	15.768**ns**
LC-2	2.230*	**LC-8**	0.487*	**AC-14**	11.251**	**AC-20**	7.308*
LC-3	3.966*	**LC-9**	3.122*	**AC-15**	9.934**	**AC-21**	5.103*
LC-4	2.356*	**LC-10**	0.479*	**AC-16**	1.026*	**AC-22**	6.471*
LC-5	2.835*	**LC-11**	5.391*	**AC-17**	0.114*	**AC-23**	18.248**ns**
LC-6	0.765*	**LC-12**	7.240*	**AC-18**	8.709**		

3.1.2. Workers Morphometrics

Totally 86 individuals from 4 different populations of workers have been included in both of the analysis. These populations, their provinces and number of individuals are as shown in Table 7.

Table 13. Number of Individuals and their Labels in Different Populations of the *B. terrestris*

Groups:	**1st Group: Northern Anatolia**		**2nd Group: Southern Anatolia**		**3rd Group: Central Anatolia**	**4th Group: Northern Cyprus**
Provinces:	**Bartın**	**Samsun**	**Adana**	**Antakya**	**Ankara**	**Cyprus**
Number of Individuals	5	5	6	7	28	38
Labels of Individuals	1- 5	5- 10	11- 16	17- 23	24- 51	52- 89

3.1.2.1. Principal Components Analysis (PCA) of Workers

Principal Components Analysis of workers explained all of the variation by 23 components. The first, the second and the third principal components explained %40.6720, % 7.7396 and %6.3329 of the overall variation respectively. Totally %54.7446 of the overall variation is explained by the first three components (Table 14).

Table 14. Eigen Values of the first three principal components and total variation explained by each of them

Principal Components	% Eigen Values	% of Total Variation
1. Principal Component	40.6720	40.6720
2. Principal Component	7.7396	48.4116
3. Principal Component	6.3329	54.7446

Eigen vectors showed that all of the length character variables; except LC-8, and AC-18 have really high weights to the first component. The second component is constructed primarily by the variation on LC-2, LC-5, LC-6, LC-8, LC-10, AC-14, AC-16 and AC-20. And, the third component is mainly shaped by the variation on LC-3, LC-9, LC-10, LC-12, AC-20 and AC-22 (Table 15).

Table 15. Weights of 23 variables on the first 3 principal components

Variable	1st Principal C.	2nd Principal C.	3rd Principal C.
LC-1	9.7384619	3.0998316	2.2086356
LC-2	9.7193071	-6.708414	5.4324309
LC-3	9.0035733	-1.025724	-9.560283
LC-4	9.7436503	1.7346490	5.2614677
LC-5	9.7358157	5.4046774	4.0249219
LC-6	9.1142500	-7.679578	-4.494981
LC-7	9.5306332	-5.055121	2.1117977
LC-8	5.1434897	-9.434085	-4.838244
LC-9	8.2472944	-1.496870	-8.213797
LC-10	8.3051740	7.0881802	-9.256201
LC-11	5.7056167	-1.834094	1.6018765
LC-12	9.5155459	5.1016683	6.9454366
AC-13	1.4421608	5.2027490	-2.616428
AC-14	-1.157713	-6.318309	-1.965021
AC-15	1.5752802	1.2507347	-4.132326
AC-16	1.0088024	-9.427292	2.1909610
AC-17	-3.158419	2.1833923	-5.199367
AC-18	-8.449479	-3.237528	3.2499866
AC-19	-1.056928	-2.618115	-5.138426
AC-20	-2.137325	-7.499218	-6.843016
AC-21	1.2035620	-4.702784	-2.871329
AC-22	-1.609178	1.1345093	5.5166566
AC-23	-2.146527	-4.274192	2.0444766

The calculated correlation matrix of the variables according to the first and the second principal components shows that shape characters have both positive and negative correlations, yet size character variables are all positively correlated with each other (Appendix B). These results are in concordance with the results of analysis of males.

The scatter diagram of the workers, constructed by the first two principal components, has been drawn to show relative positions of the populations based on morphometric characters (Figure 10). According to the diagram, these two worker populations does not show exactly distinct clusters, yet it is obvious that they have been departed from each other much better than male populations did as a result of

the principal components analysis. Although there is a mix towards the middle part, Anatolia and Cyprus populations seem to have different ordination tendencies. In the graph, Cypriot and Anatolian individuals grouped towards opposite directions.

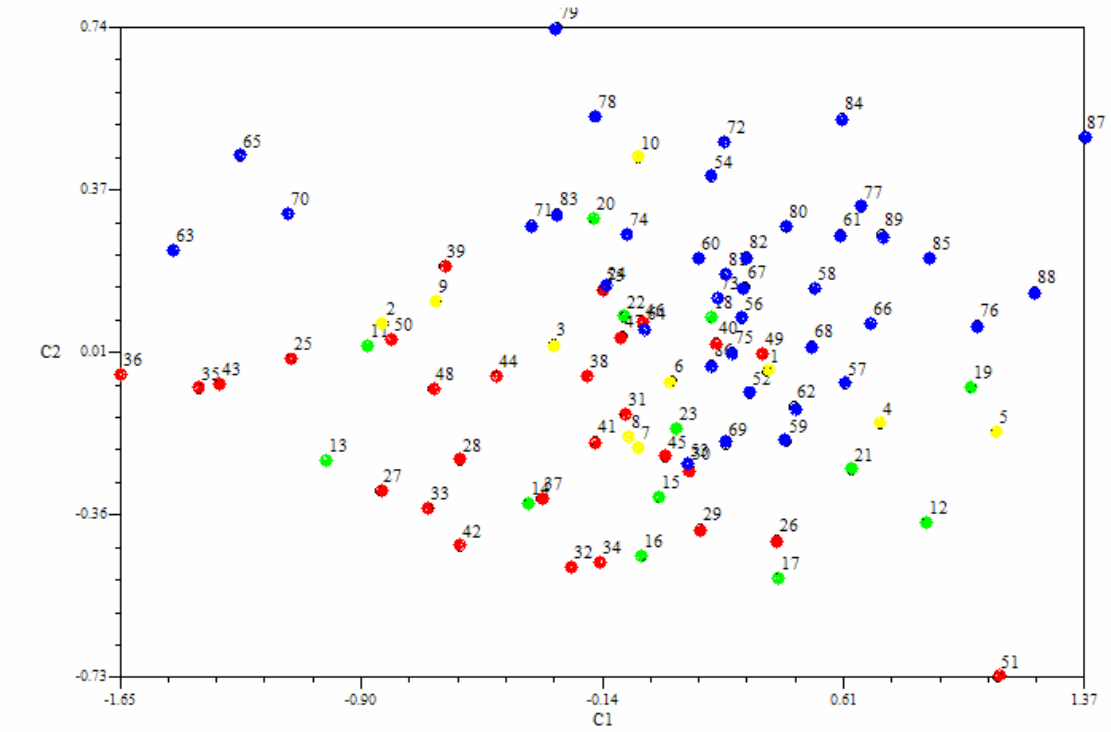

Figure 10. The Scatter Diagram of the Individuals, as a result of the first two principal components. Provinces have been represented in colours; as Northern Anatolia in Yellow, Southern Anatolia in green, Ankara in red, North Cyprus in Blue

3.1.2.2. Canonical Variates Analysis (CVA) of Workers

Canonical Variates Analysis of males explained all of the variation only by 3 axes. The first canonical axis explained %88.92, while the second and the third explained %6.39 and %4.69 of the overall variation respectively, such that altogether these axes represents all of the variation (Table 16).

Table 16. Eigen Values for each of the canonical variates and total variation explained by each of them

Canonical Variates	% Eigen Values	% of Total Variation
1. Canonical Variate	88.92	88.92
2. Canonical Variate	6.39	95.31
3. Canonical Variate	4.69	100.00

Discriminant weights of the variables for each axis are the coefficients of the variables in the linear equations that define the canonical functions, as shown above (Table 17).

Table 17. Discriminant Weights of the variables for each canonical variate

Variable	1. Canonical Variate	2. Canonical Variate	3. Canonical Variate
LC-1	-0.1219545	-0.4710316	-0.7501906
LC-2	-0.5847516	0.4863217	-0.6338460
LC-3	0.2002009	-0.1980107	0.1151273
LC-4	0.9297007	0.3786017	-0.1853132
LC-5	-0.1273224	-0.7462207	0.2141839
LC-6	0.1304706	0.2210823	0.2599081
LC-7	-0.1688829	0.7552596	0.2363881
LC-8	0.5070296	-0.2121810	0.2105833
LC-9	0.1124124	0.2391487	0.6703254
LC-10	0.2822021	0.6567471	0.1641982
LC-11	-0.4595760	-0.1163727	-0.1861461
LC-12	-0.3930961	0.5532438	-0.1497230
AC-13	-0.2992178	-0.4151263	-0.1909914
AC-14	0.1429853	-0.5622329	-0.4850703
AC-15	-0.6250984	0.3666155	-0.5131397
AC-16	-0.2320678	-0.3242370	-0.6521121
AC-17	-0.2431164	0.3664318	-0.3870671
AC-18	0.3404194	0.7982951	0.2199018
AC-19	0.1520439	0.1810922	-0.9520697
AC-20	0.1062346	-0.8714737	0.1788346
AC-21	0.4988214	0.6507369	-0.2555391
AC-22	0.5933916	-0.1642751	-0.2452545
AC-23	-0.1866836	0.4510109	0.9446889

As can be seen from the equations above, LC-4 has the highest weight on the first axis, while LC-5, LC-7, AC-18 and AC-20 are having on the second axis. AC-19 and AC-23 have been the most effective variables in constructing the third axis.

The scatter diagram of the individuals according to the first two canonical axes is as shown on the Figure 11. There are two distinct clusters on this diagram. This clustering indicates that Anatolian and Cypriot populations are apparently different from each other according to their wings' morphometric characteristics. The MANOVA statistic test supported this result, as can be seen on Figure 12. Also, analysis of variance has shown that all of the variables are significantly different

among localities of the worker populations (Table 18). The CVA of worker populations has shown that there is a significant differentiation between Ankara and Cyprus *B. terrestris* worker populations according to their wings' morphometric characteristics.

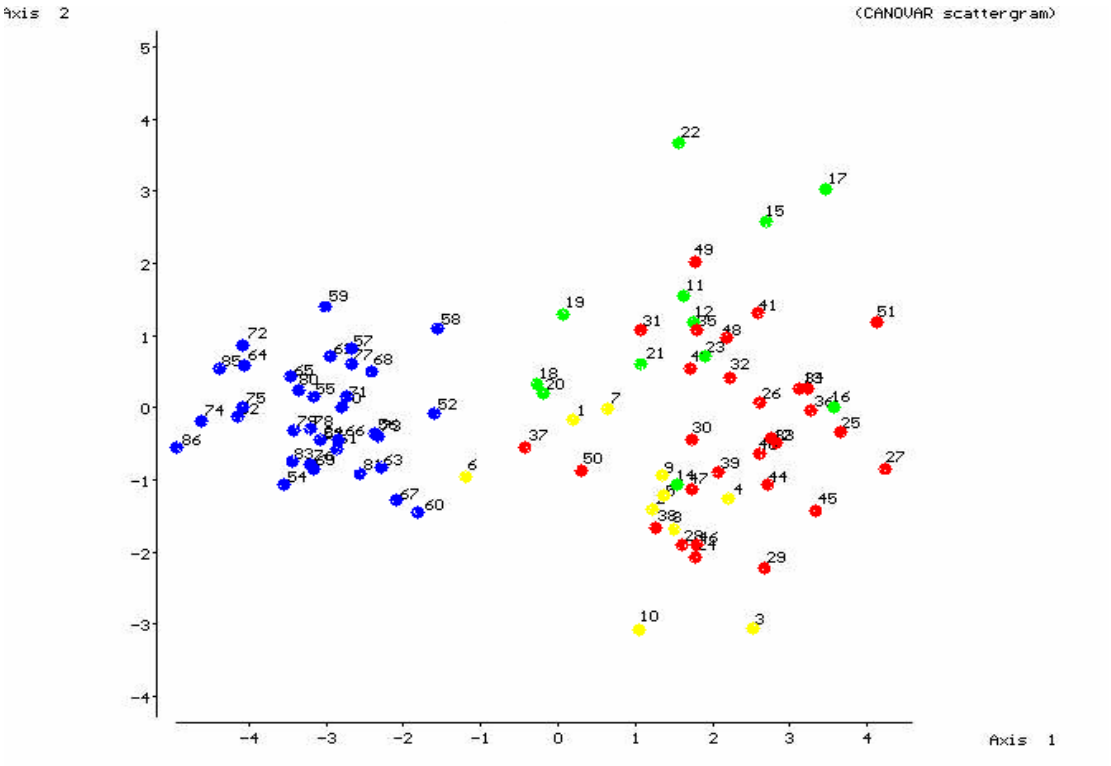

Figure 11. The Scatter Diagram of the Canonical Variates Analysis, obtained from the first two canonical variates. Provinces have been represented in colours; as Northern Anatolia in Yellow, Southern Anatolia in green, Ankara in red, North Cyprus in Blue

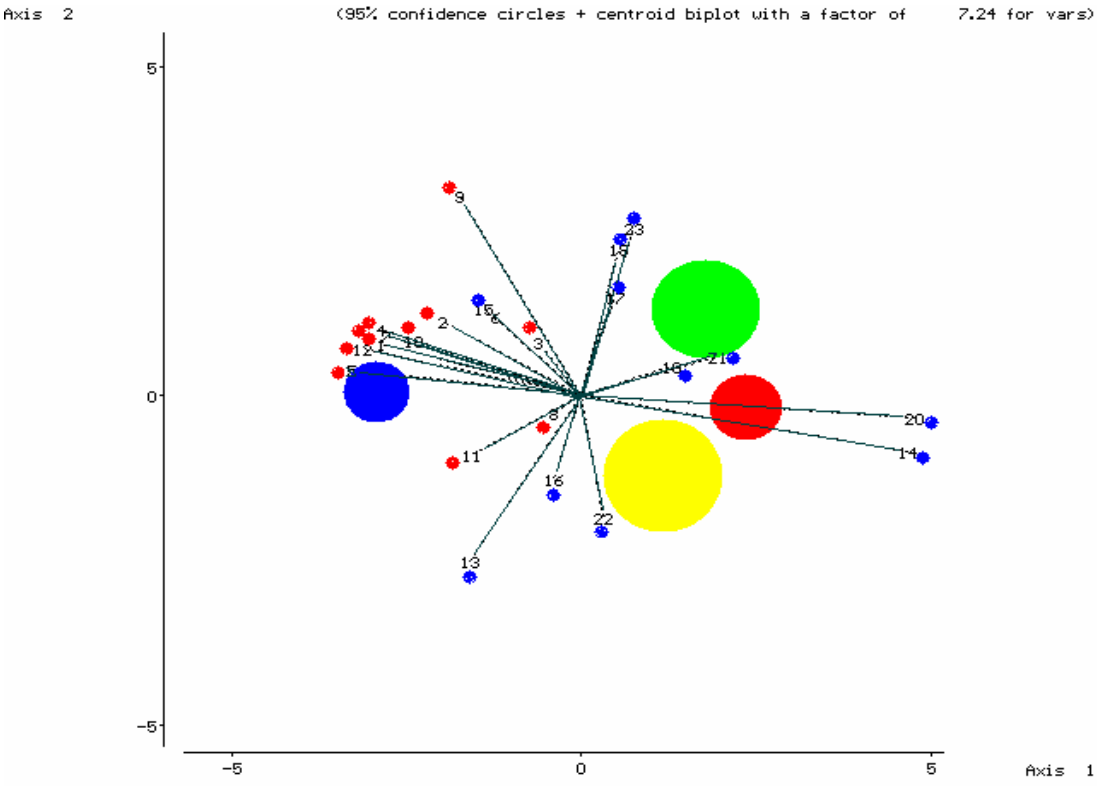

Figure 12. The Graph of the Separated Groups and Variables (with %95 confidence circles and centroid biplot with a factor of 7.24 for variance). Provinces have been represented in colours; as Northern Anatolia in Yellow, Southern Anatolia in green, Ankara in red, North Cyprus in Blue

Table 18. F-Ratios calculated by the analysis of variance; with 3 and 82 degrees of freedom (* = P <0.05, ** = P<0.01)

Var.	F-R.	Var.	F-R.	Var.	F-R.	Var.	F-R.
LC-1	5.276*	**LC-7**	5.668*	**AC-13**	3.060*	**AC-19**	0.806*
LC-2	3.208*	**LC-8**	0.293*	**AC-14**	15.616**	**AC-20**	16.456**
LC-3	2.322*	**LC-9**	3.799*	**AC-15**	1.230*	**AC-21**	1.903*
LC-4	5.612*	**LC-10**	3.341*	**AC-16**	0.340*	**AC-22**	0.625*
LC-5	7.473*	**LC-11**	1.309*	**AC-17**	0.803*	**AC-23**	1.433*
LC-6	2.841*	**LC-12**	5.952*	**AC-18**	0.947*		

3.2. Molecular Analysis

3.2.1. Allele Frequencies, Heterozygosities and Gene Diversities

Nine alleles for B126 locus, twelve alleles for B124 locus, eight alleles for B11 locus and seven alleles for B100 locus were found in Ankara and Cyprus populations. All four loci were polymorphic for both of the populations.

Ankara population found to have the highest number of population specific alleles (6 alleles) in locus B124 and Cyprus population found to have the highest number of population specific alleles (3 alleles) in locus B100. Each locus with all of the allele frequencies, observed and expected heterozygosities for both of the populations are given in table 19.

The mean number of alleles per locus of Cyprus equals to 5.25; while of Ankara is 7.25.

Ankara population has a significantly higher genetic variation than Cyprus population (P = 0.05), as the average heterozygosity of Ankara population is greater than it is in Cyprus population (Table 20).

Table 20. Average Heterozygosities of Each Population considering all of the Loci

Population	Average Heterozygosity	Standard Error
Cyprus	0.677065	0.034900
Ankara	0.848029	0.012802

Tablo 19. Allele frequencies, observed and expected heterozygosities for each locus (H_O: Observed Heterozygosity, H_E: Expected Heterozygosity, N: Number of Individuals) in each population

Locus B126			**Locus B124**			**Locus B11**			**Locus B100**		
Allele	**Cyp.**	**Ank.**	**Allele**	**Cyp.**	**Ank.**	**Allele**	**Cyp.**	**Ank.**	**Allele**	**Cyp.**	**Ank.**
176	0.00	0.19	**240**	0.06	0.00	**160**	0.03	0.06	**150**	0.00	0.20
178	0.38	0.14	**244**	0.00	0.07	**162**	0.00	0.22	**152**	0.00	0.30
180	0.04	0.24	**246**	0.00	0.02	**164**	0.16	0.03	**154**	0.64	0.30
182	0.00	0.19	**248**	0.00	0.12	**166**	0.41	0.22	**156**	0.07	0.00
184	0.08	0.05	**250**	0.06	0.10	**168**	0.31	0.22	**166**	0.14	0.00
186	0.35	0.12	**252**	0.56	0.12	**170**	0.06	0.08	**168**	0.00	0.20
188	0.15	0.00	**254**	0.00	0.10	**172**	0.00	0.17	**170**	0.14	0.00
190	0.00	0.02	**256**	0.22	0.22	**186**	0.03	0.00	**N**	7	5
196	0.00	0.05	**258**	0.00	0.02	**N**	16	18	**HO**	3	4
N	13	21	**260**	0.06	0.17	**HO**	10	15	**HE**	4,08	4,11
HO	5	14	**262**	0.00	0.02	**HE**	11,68	15,06			
HE	9,48	17,88	**266**	0.06	0.00						
			N	9	20						
			HO	5	18						
			HE	6,00	17,64						

3.2.2. Hardy Weinberg Equilibrium

Expected and observed frequencies of genotypes of each locus in each population, which are used in testing Hardy-Weinberg Equilibrium, are given in Appendix C. The P-values estimated by the Markov chain method for each locus in Cyprus and Ankara populations are as shown in tables 21 and 22 respectively. Results of the chi-square (χ^2) tests for each population and each locus with their degrees of freedoms are as indicated in table 23.

Table 21. P-values and their standard errors estimated by the Markov chain method for each locus in Cyprus population

Cyprus Population		
Locus	**P-value**	**Standard error**
B126	0.0070	0.0014
B124	0.3852	0.0184
B11	0.2297	0.0106
B100	0.0510	0.0041

Table 22. P-values and their standard errors estimated by the Markov chain method for each locus in Ankara population

Ankara Population		
Locus	**P-value**	**Standard error**
B126	0.0296	0.0051
B124	0.8931	0.0103
B11	0.1199	0.0087
B100	0.5325	0.0064

Table 23. Results of the chi-square (χ^2) tests for each population and each locus separately with their degrees of freedoms

Locus	Degrees of Freedom	χ2 Value	P- Value
B126	4	16.9628	0.0019
B124	4	2.1339	0.7114
B11	8	12.7706	0.1200
B100	16	33.4048	0.1250
Ankara	8	12.7706	0.1200
Cyprus	8	20.7243	0.0079

According to these calculations, except B126 locus, all of the loci are in Hardy-Weinberg Equilibrium in both of the populations. Overall tests of Hardy-Weinberg showed that, while Ankara population is in Hardy-Weinberg equilibrium, Cyprus population departs from the equilibrium. This departure of Cyprus population is due to B126 loci, as it shows a really strong deviation from Hardy-Weinberg equilibrium.

3.2.3. Linkage Disequilibrium Tests

The results of the linkage disequilibrium tests imply that only the B126-B11 locus pair in Cyprus population is in linkage disequilibrium, however it is in linkage equilibrium in Ankara population (Table 24). When the test is applied to each of the locus pair across both of the populations, only the B126-B11 locus is found to be in linkage disequilibrium (Table 25). The deviation in Cyprus population pulls the across populations values of this locus pair down, such that B126-B11 locus pair seem to be in linkage disequilibrium, although it is not in Ankara population.

Table 24. The Exact P values and their Standard Error Values of Linkage Equilibrium

Population	Locus # 1	Locus # 2	P- value	S.E.
Cyprus	B126	B124	0,55087	0,01032
	B126	B11	0,03202	0,00655
	B124	B11	0,63675	0,0139
	B126	B100	0,19859	0,00289
	B124	B100	0,33412	0,00188
	B11	B100	0,31408	0,00576
Ankara	B126	B124	0,21567	0,03697
	B126	B11	0,12187	0,023
	B124	B11	1	0
	B126	B100	1	0
	B124	B100	no information	no information
	B11	B100	0,30795	0,00761

Table 25. The χ^2 and exact P-values of Linkage Equilibrium for each locus pair across both of the populations

Locus # 1	Locus # 2	χ^2 Value	df	P-value
B126	B124	4.261	4	0.372
B126	B11	11.092	4	0.026
B124	B11	0.903	4	0.924
B126	B100	3.233	4	0.520
B124	B100	2.193	2	0.334
B11	B100	4.671	4	0.323

3.2.4. Population Differentiation Analyses

3.2.4.1. Genic Differentiation

Probability and standard error (S.E.) values calculated for each locus indicates a significant differentiation between Ankara and Cyprus populations (Table 26).

Table 26. Probability and standard error values of the test of Genic Differentiation between populations for each locus

Locus	Probability:	S.E.:
B126	0.00000	0.00000
B124	0.01694	0.00174
B11	0.00110	0.00025
B100	0.00450	0.00065

3.2.4.2. Genotypic Differentiation

As well as probability values and standard error (S.E.) values calculated for the genotypic differentiation of the Ankara and Cyprus populations by G-like test, genotypic differentiation of the Ankara and Cyprus populations across all loci calculated by Fisher's Method are given in table 27. There is a significant genotypic differentiation between these populations.

Table 27. Probability and standard error values of Genotypic Differentiation between Cyprus and Ankara Populations

Locus	Probability:	S.E:	
B126	0.0008	0.0004	
B124	0.0071	0.0010	
B11	0.0012	0.0003	
B100	0.0246	0.0018	
All	0.0001	$\chi^2 = 45.126$	df= 8

Results of the genotypic differentiation between these populations are in concordance with each other.

3.2.4.3. Population Differentiation (F Coefficients)

The positive values of F_{IS} and F_{IT} indicate a deviation from Hardy-Weinberg equilibrium in favour of homozygotes within subpopulations and in the total population respectively, which may be indicative of inbreeding (Table 28). Locus B126 shows the highest deficiency in heterozygotes, both within subpopulations and in the total population.

F_{ST} values are all higher than 0.05, which indicates differentiation of populations for each locus and all loci. All of the loci have a F_{ST} value between 0.05-0.15, pointing out moderate genetic differentiation between populations for these loci. Also, over all F_{ST} value is in concordance with these results and shows that these two populations are genetically different from each other.

Table 28. F Coefficients for Ankara and Cyprus Populations together for each and all loci

Locus	F_{IS}	F_{IT}	F_{ST}
B126	0.3125	0.3822	0.1014
B124	0.0482	0.1343	0.0905
B11	0.0736	0.1199	0.0500
B100	0.1645	0.2525	0.1053
All	0.1511	0.2213	0.0827

3.2.5. Genetic Distance and Phenogram

Nei's standard genetic distance for 15 *B. terrestris* populations and a *B. lucorum* population using two microsatellite loci (B11 and B100) were calculated and distance and its standard error matrices are given in tables 23 and 24. 13 of these *B. terrestris* populations (Gif-sur-Yvette (France), Tours (France), Banyuls-sur-Mer (France), Madrid (Spain), Avellino (Italy), Nea-Moudania (Greece), Pleven (Bulgaria), Lublin (Poland), Samos (Island), Cyprus (2) (Island), Elba (Island), Corsica (Island) and Sardinia (Island)) are studied by Estoup *et. al.* (1996), five of them are island populations from Mediterranean, including Cyprus (2) and the rest are Europe populations.

Neighbor-joining phenogram is constructed according to Saitou and Nei (1987) using the standard genetic distance values shown in tables 29 and 30 (Figure 13). The numbers at the end of the nodes are the bootstrap percentage values. According to this tree two Cyprus populations group together with a %100 bootstrap percentage value, as expected result from a true construction.

Table 29. Nei's standard genetic distance matrix of *B.terrestris* populations and a *B. lucorum* population from two microsatellite loc; B11 and B100

Population	Ank.	Gif/Y.	Tours	Bany.	Mad.	Avel.	Nea-m.	Plev.	Lublin	Sam.	Cyp. 1	Cyp. 2	Elba	Cors.	Sard.
Gif/Yvette	0.17														
Tours	0.35	0.01													
Banyuls	0.52	0.04	0.002												
Madrid	0.32	-0.02	0.05	0.06											
Avellino	0.24	-0.03	0.04	0.05	0.00										
Nea-m.	0.29	-0.001	0.01	0.04	0.03	0.02									
Pleven	0.22	0.02	0.08	0.06	0.03	-0.02	0.11								
Lublin	0.30	0.08	0.08	0.07	0.07	0.03	0.20	-0.023							
Samos	0.57	0.22	0.13	0.04	0.25	0.18	0.24	0.15	0.07						
Cyprus 1	0.28	0.06	0.19	0.27	0.07	0.02	0.13	0.12	0.12	0.34					
Cyprus 2	0.58	0.43	0.59	0.74	0.56	0.34	0.57	0.47	0.50	0.64	0.15				
Elba	1.15	0.53	0.46	0.21	0.59	0.34	0.62	0.28	0.30	0.28	1.03	1.59			
Corsica	2.07	1.03	0.75	0.40	1.13	0.78	1.10	0.48	0.54	0.44	2.18	4.62	0.03		
Sardinia	2.09	0.99	0.82	0.43	1.13	0.76	1.05	0.57	0.75	0.52	2.57	3.60	0.03	0.04	
B. lucorum	0.30	0.77	0.61	0.40	0.87	0.78	0.81	0.64	0.54	0.22	0.91	0.85	0.63	0.57	0.70

Table 30. Standard error of Nei's standard genetic distances

Population	Ank.	Gif/Y.	Tours	Bany.	Mad.	Avel.	Nea-m.	Plev.	Lublin	Sam.	Cyp. 1	Cyp. 2	Elba	Cors.	Sard.
Gif/Yvette	0.00														
Tours	0.22	0.06													
Banyuls	0.08	0.06	0.01												
Madrid	0.06	0.01	0.06	0.09											
Avellino	0.08	0.01	0.02	0.11	0.01										
Nea-m.	0.03	0.02	0.03	0.03	0.03	0.01									
Pleven	0.14	0.01	0.02	0.13	0.00	0.03	0.07								
Lublin	0.05	0.02	0.04	0.08	0.02	0.04	0.01	0.01							
Samos	0.02	0.09	0.16	0.10	0.10	0.08	0.16	0.02	0.03						
Cyprus 1	0.07	0.07	0.26	0.35	0.09	0.01	0.23	0.04	0.08	0.21					
Cyprus 2	0.02	0.24	0.48	0.72	0.42	0.12	0.54	0.21	0.27	0.53	0.03				
Elba	0.40	0.20	0.47	0.20	0.24	0.08	0.45	0.03	0.21	0.35	0.01	0.33			
Corsica	0.84	0.32	0.74	0.32	0.36	0.16	1.03	0.01	0.18	0.42	0.19	1.03	0.02		
Sardinia	0.84	0.36	0.85	0.38	0.38	0.11	0.91	0.00	0.40	0.54	0.08	1.00	0.00	0.03	
B. lucorum	0.11	0.34	0.38	0.25	0.20	0.32	0.48	0.05	0.04	0.07	0.02	0.05	0.43	0.29	0.52

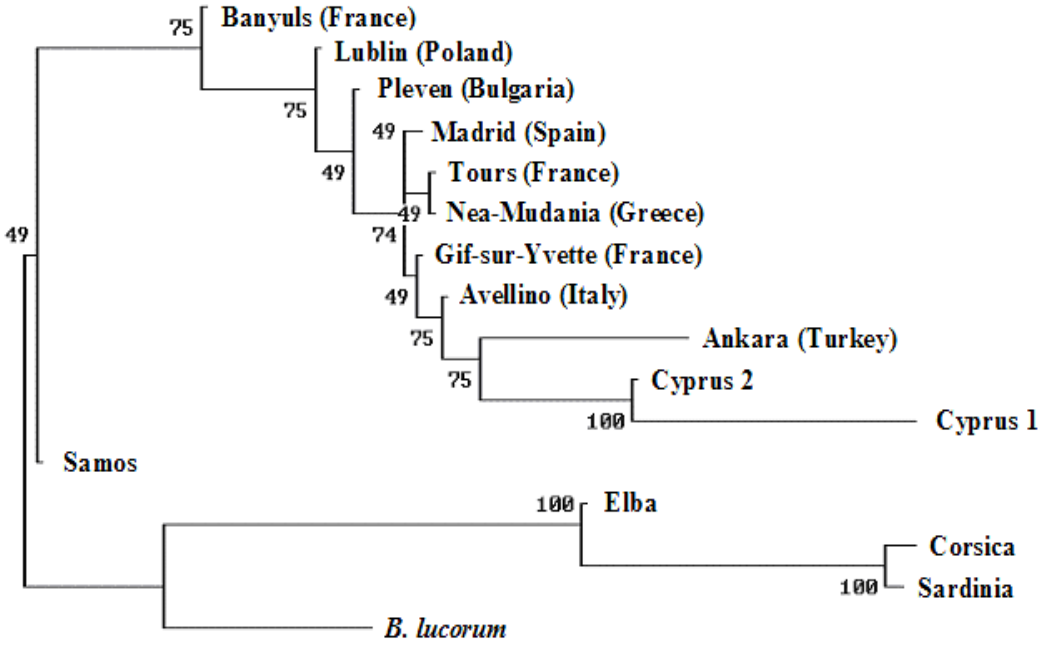

Figure 13. Neighbour-joining tree of the 15 B. terrestris populations and a B. lucorum population with bootstrap values

CHAPTER 4

DISCUSSION

4.1. Morphometrics of Bombus terrestris

One of the aims of this study is to evaluate the amount of differentiation of two *Bombus terrestris* populations from Turkey and Cyprus according to their morphometric character. In order to do so, 23 wing characters were analysed by principal components and canonical variates analysis for male and worker castes separately.

Results of principal components analysis of males indicate a slight difference in the ordination of Cyprus and Ankara populations on the scatter plot of individuals. Cyprus population is mainly grouped on the right, while Ankara population is more dispersed towards left side of the scatter plot. This difference in the ordination is due to the first principal axis. In this axis, 26.18% of the total variation has been explained mainly by the variation in most of the size variables as well as AC-13, AC-19 and AC-23 (cubital cell angles, the angle of the second submarginal cell between the first medial and first submarginal cells and angles between the anal and the cubitoanal vein). Moreover, it is known that in principal components analysis the first principal axis generally carries the overall variation in size (length characters). According to the ordination of the individuals, Ankara and Cyprus populations tend to have different wing sizes. And, when the mean values of the size variables of

males are compared, we can say that, male populations in Cyprus tend to have bigger wings than those in Ankara.

In the morphometric analyses of males, Cyprus population was divided into three subpopulations according to localities. These three subpopulations showed no difference in ordination on the scatter diagram of individuals of principal components analysis.

When principal components analysis is carried out with worker samples from Cyprus population and three different Anatolian populations, they showed different ordinations according to the second axis. While worker population in Cyprus disperse towards the top of the diagram, Anatolia population disperse towards the bottom of the diagram. The second axis was carrying 7.74% of the total variation and LC-2, LC-5, LC-6, LC-8, LC-10, AC-14, AC-16 and AC-20 (radial sector length, 2nd sub-marginal cell length, 1st sub-marginal cell length, length of the main vein, media + cubitus length, one of the cubital cell angles, second cubital cells' bottom angle and the angle of the first medial cell opposite to the second submariginal cell) characters have high loads on this axis. It should be noted that the size characters have less importance on the second axis compared to the first axis of the worker principal components analysis. Also, it is known that in principal components analysis, the second principal axis generally carries the overall variation in shape variables (angle characters). Consequently, according to the ordination of the individuals on this scatter diagram and the mean values of the shape variables, workers from Ankara tend to have different angle values than those from Cyprus.

In the analysis of worker populations, Anatolian populations have been divided into 3 subpopulations, as Northern Anatolia, Central Anatolia and Southern Anatolia. These populations exhibited no differentiation in ordinations on scatter plot of principal components analysis.

According to principal components analysis, most of the size characters are positively correlated with each other. Although when principal components analysis

is applied to males LC-11 (part of the radial sector length) showed a negative correlation with LC-5 (2^{nd} sub-marginal cell length) and LC-6 (1^{st} sub-marginal cell length), analysis of workers showed that all of the size variables have high positive correlations. On the other hand, shape characters are both positively and negatively correlated with each other.

When canonical variates analysis has been carried out for male individuals, Cyprus and Ankara populations are separated on the first canonical axis, carrying 56.61% of the total variation. This separation was due to the significant differences among populations of all of the variables except two angle characters; AC-19 (the angle of the second submarginal cell between the first medial and first submarginal cells) and AC-23 (one of the angles between the anal and the cubitoanal vein). LC-2, LC-5, LC-12, AC- 15 and AC-21 (Radial sector length, 2^{nd} sub-marginal cell length, part of radial sector length, one of cubital cell angles and the angle of the medail and the radial sectors' corner) are the variables having the highest weights on the first canonical axis.

A slight differentiation between three male populations of Cyprus is indicated by the canonical variates analysis; however it is only due to the second canonical axis carrying only 29.38% of the total variation. Girne and Beşparmak M. populations were shown to be less differentiated than Güzelyurt population. This pattern of the differentiation between these populations is positively correlated with the distance between these localities.

Canonical variates analysis of worker populations indicated a significant differentiation between the Anatolia and Cyprus populations. Although analysis of variance showed significant differences among populations for all of the characters, LC-4 (The length between the First Medial Cell and the corner of the Second Submarginal Cell) has the highest weight on the first canonical axis.

In the analysis of worker populations, Anatolia populations have been divided into three subpopulations, as Northern Anatolia, Southern Anatolia and Central Anatolia.

These populations revealed a slight dispersal from each other only on the second canonical axis. This axis is carrying only 6.39% of the total variation.

Although results of the canonical variates analyses are in concordance with each other, the differentiation of the Anatolian and Cyprus worker populations of *B. terrestris* according to their morphometric characteristics is shown to be much more apparent than it is for male populations of Ankara and Cyprus by the scatter diagrams of the canonical variates analysis.

Differentiation of morphological characteristics of wings within three populations of Anatolia populations and Cyprus populations might be due to different environmental conditions as well as genetic differentiation.

4.2. Microsatellite Characteristics of Bombus terrestris

Second aim of the study is to determine the extent of microsatellite variation in *B. terrestris* populations of Ankara and Cyprus and estimate the amount of genetic differentiation between these populations. For this purpose, four microsatellite loci, as B126, B124, B11 and B100, have been studied. All of the loci were polymorphic in both of the populations and these loci had 9, 12, 8 and 7 alleles respectively.

Cyprus population in our study was found to be more polymorphic in B11 locus; however it was less polymorphic in B100 compared to the results of Cyprus population in Estoup *et. al* (1996). This discrepancy in the B100 loci might be due to the smaller sample size used for B100 locus in this study; as 20 samples have been evaluated in their study, while 7 have been evaluated in this study. New alleles, as 166 of B100 loci and alleles 160, 162, 170 and 172 of B11 loci, have been found in Cyprus population. There are 4 alleles in locus B100 and one allele in locus B11 in Cyprus population that could not be found in this study, but have been recorded by Estoup *et. al.* (1996).

Alleles 176, 190 and 196 of locus B126, 244, 254, 258 and 262 of locus B124, 162 and 172 of locus B11, 150, 152 and 168 of locus B100 are found in Ankara population, not in Cyprus population. On the other hand, allele 188 of locus B126, 140 and 266 of locus B124, 186 of locus B11, 156 and 166 of locus B100 are found in Cyprus population, not in Ankara population. These alleles are the primary differentiating parameters of these populations.

When the mean number of alleles per locus of Cyprus (5.25) and of Ankara (7.25) is compared, Ankara shows a higher polymorphism than Cyprus population. The mean number of alleles per locus in island populations is less than in continental populations of *B. terrestris* has also been shown by Estoup *et. al.* (1996).

The difference in polymorphism of the populations is reflected by calculating the average heterozygosities of the populations as well. The *B. terrestris* populations from Ankara and Cyprus have high average heterozygosities of all loci, as 0.84 ± 0.01 and 0.68 ± 0.03 respectively. Difference between average heterozygozities emphasizes that Ankara population has a higher genetic variation than Cyprus population at a significance level of 0.05. Estoup *et. al*. (1996) showed that continental populations have higher average heterozygosities than island populations, which is also the case in this study.

Only locus B126 showed significant deviation from Hardy-Weinberg equilibrium in Ankara and Cyprus populations at a significance level of 0.05. Rest of the loci are in Hardy-Weinberg equilibrium. As a result of the deviation of B126 locus, Cyprus population is detected to be deviating from Hardy-Weinberg equilibrium, however Ankara did not. It should be noted that, this locus has not been used in population genetics studies of *B. terrestris* before. This deviation in both of the populations can not be due to some kind of a selective pressure on this locus, as microsatellite loci are neutral. On the other hand, this might mean there is a linkage between B126 locus and another locus under selective pressure.

Only B126 and B11 locus pair showed linkage disequilibrium. When this locus pair is evaluated separately in each population, it showed linkage disequilibrium in Cyprus population; however it did not in Ankara population, which supports the view that B126 locus is linked with a locus under selective pressure in Cyprus.

Genic differentiation tests applied for each locus have shown that these populations have significantly different ($P<0.05$) genic structure from each other. Similarly, all of the loci have shown significant ($P<0.05$) genotypic differentiation. Also, single-locus F_{ST} statistics of microsatellite loci indicated moderate genetic differentiation between these populations with high F_{ST} values. The best discrimination is provided by B126 and B100 loci, both of the F_{ST} values are as high as 0.1. On the other hand, the least discrimination is provided by B11 with a F_{ST} value of 0.05.

F_{IS} and F_{IT} values of all of the loci are positive, indicating the deficiency of heterozygotes in the populations and the variation in the allelic frequencies between populations. Locus B126 has the highest F_{IS} and F_{IT} values as it is the only locus that significantly deviates from Hardy-Weinberg equilibrium. These positive values of all of the loci are indicative of inbreeding. Hedrick (1999) pointed out that the genotypic changes due to inbreeding occur at all loci in the genome.

In their study Estoup *et. al* (1996) and Widmer *et. al* (1998) have found no significant differentiation among the continental samples and genetic difference between island and continental populations. Estoup *et. al* (1996) also pointed out that genetic differentiation gets higher as the distance between the island and the continent increases. Our results are in concordance with their findings.

In order to determine the genetic relatedness of Ankara and other continental and island populations of *B. terrestris*, standart genetic distance of populations from Ankara (Turkey), Gif-sur-Yvette (France), Tours (France), Banyuls-sur-Mer (France), Madrid (Spain), Avellino (Italy), Nea-Moudania (Greece), Pleven (Bulgaria), Lublin (Poland), Samos (Island), Cyprus (Island), Elba (Island), Corsica (Island) and Sardinia (Island) is calculated. Nei's standart genetic distance (1972) is

appropriate for microsatellite data as it is for long-term evolution when populations diverge because of drift and mutations. This distance assumes a neutral mutation rate for all loci and a new mutant is considered to be a novel allele not previously available in the population, so it is suitable for infinite allele model. Infinite allele model assumes that a mutation occurs within a microstellite locus with gain or loss of any number of tandem repeats. On the other hand, stepwise mutation model assumes the gain, or the loss of a single tandem repeat. In this study, analyses were done according to the infinite allele model, because not all of the alleles show a pattern as in stepwise mutation model; as it is in locus B11.

A neighbour-joining tree relating the island and continental populations was constructed based on microsatellite data using the Nei's standard genetic distance. According to this tree, Cyprus populations are closest to the Ankara population, while Ankara population is clustered together with other European populations. According to this tree, construction of all of the European populations are group in together and island populations are grouped close to each other, however Cyprus is closest to Ankara population. This closeness of Cyprus population with Ankara population might indicate that Cyprus population is diverged from Anatolia. Also, Estoup *et. al* (1996) indicated, island populations of *B. terrestris* probably diverged from continental populations probably by founder effect or genetic drift. On the other hand, the two populations in this study probably have diverged due to vicariance events. Yet, for any kind of inference about ancestral relations, further investigations including mtDNA with more samples should be carried out.

In addition to the study of Estoup *et. al* (1996), Widmer *et. al* (1998) have used microsatellite data to investigate the population genetic structure of *B. terrestris* populations in the islands at north-west of Africa. Their studies were not included in the comparisons of Anatolian and Cyprus populations with other continental and island populations as north-west Africa is far from Anatolia geographically. Moreover, the islands at north-west of Africa were monomorphic over all of the loci they have studied that they would not change construction of our tree.

4.3. Conclusion

To sum all, both morphometric and genetic variability in *Bombus terrestris* showed a significant differentiation between Ankara and Cyprus populations. This shows that, even though Cyprus is close to Anatolia, sea constitutes a geographical barrier for significant migration events and hence gene flow from continent to the island. Variety of factors including selection on dispersal rate, selective forces in a heterogenous environment and genetic drift lead to subdivision of these populations.

Microsatellites have been found to be much more polymorphic than alloenzyme data in Hymenoptera (Sholl *et. al*., 1990). However, because of its high mutation rate, microsatellite data sometimes overestimates genetic variation. Further investigations with a larger number of loci and a larger sample size would increase the resolution of the analysis by providing more data.

In their previous study, Estoup *et. al.* (1996) have shown that the practice of using *B. terrestris* for pollination and their transportation has not yet had noticeable impact on the current species structure. However, they have added that it should be considered in the future as an additional factor of genetic homogenization of populations. On the other hand, İstanbul Bosphorus is the only connection of Anatolia to Europe, which might be a geographic barrier to some extent for *Bombus terrestris*. Dispersal abilities of the queens should be investigated by mark and recapture methods than the amount of migration through this barrier would be estimated.

Genetic diversity in Cyprus population needs to be protected. This protection would be by controlling the importation of *B. terrestris* of foreign origin for crop pollination. Similarly, importation of Cyprus populations to continent should be avoided. On the other hand, further studies with more loci and different markers should be carried out, in order to do any reliable interpretations about the trading between Anatolian and European populations.

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APPENDIX A

Mean and Standard Error values of the variables in male and worker populations are given in tables above, respectively.

Males	**Ankara**		**Güzelyurt**		**Beşparmak M.**		**Girne**	
Var.	**Mean**	**StdEr**	**Mean**	**StdEr**	**Mean**	**StdEr**	**Mean**	**StdEr**
LC-1	269,57	17,87	272,30	43,86	288,77	8,75	281,81	10,70
LC-2	287,52	18,62	302,57	13,12	302,98	9,43	295,95	9,52
LC-3	141,21	10,08	143,49	10,64	147,22	6,62	142,65	6,17
LC-4	210,56	15,97	226,12	5,45	226,22	25,92	217,13	9,30
LC-5	277,47	19,95	294,46	8,83	291,57	9,39	289,61	44,41
LC-6	81,07	7,36	86,74	1,96	82,04	4,97	82,14	4,33
LC-7	138,10	10,42	146,77	3,68	146,29	6,15	144,99	4,19
LC-8	698,58	40,99	619,96	235,82	707,80	128,18	716,77	69,84
LC-9	37,06	3,89	40,73	2,50	40,92	2,68	39,14	3,33
LC-10	209,08	13,82	225,25	8,62	227,09	9,74	221,07	15,14
LC-11	12,01	2,42	11,31	0,76	11,84	1,43	11,91	3,82
LC-12	123,27	8,86	135,17	4,58	135,60	5,54	131,63	5,93
All LC	**207,13**	179,87	**208,74**	161,09	**217,36**	182,65	**214,57**	184,71
AC-13	84,16	7,86	86,03	5,68	86,21	8,94	86,36	9,80
AC-14	101,23	8,17	83,46	4,62	83,15	5,55	85,30	7,09
AC-15	123,42	6,48	125,52	7,08	124,29	5,67	124,73	7,29
AC-16	138,90	5,15	137,93	2,58	136,94	5,80	138,70	5,99
AC-17	79,88	7,56	75,33	11,39	78,60	8,95	78,02	7,49
AC-18	141,32	5,04	143,80	3,22	142,50	3,82	143,67	5,06
AC-19	141,32	11,67	78,15	7,35	78,01	7,62	79,86	11,61
AC-20	78,29	5,88	41,94	1,79	44,21	3,12	43,67	3,75
AC-21	79,23	7,33	80,21	5,96	76,49	7,09	76,67	7,95
AC-22	132,99	7,20	131,98	8,14	134,92	7,25	133,72	7,97
AC-23	118,70	8,20	114,16	7,52	116,72	7,95	119,97	8,04
All AC	**110,86**	26,79	**99,86**	32,48	**100,18**	32,16	**100,97**	32,48
All	**161,09**	137,55	**156,67**	128,64	**161,32**	143,99	**160,24**	144,59

Workers	Northern Anatolia		Southern Anatolia		Central Anatolia		Cyprus	
Var.	Mean	Std Er	Mean	Std Er	Mean	Std Er	Mean	Std Er
LC-1	151,03	142,97	150,02	120,87	231,25	20,54	252,09	20,89
LC-2	155,59	158,76	155,99	134,18	241,04	20,45	256,20	19,28
LC-3	76,13	74,87	75,98	63,28	118,95	9,49	122,79	9,60
LC-4	118,36	115,06	117,94	97,25	180,15	13,78	194,74	13,91
LC-5	156,38	149,00	155,46	125,96	232,64	19,19	256,12	19,31
LC-6	43,70	43,80	43,71	37,02	68,74	6,26	72,22	6,13
LC-7	75,34	77,42	75,60	65,44	117,86	10,18	128,31	9,81
LC-8	413,06	300,76	399,02	257,27	603,47	49,29	619,87	123,07
LC-9	21,55	20,51	21,42	17,34	31,04	4,28	33,95	3,92
LC-10	117,82	116,87	117,70	98,77	181,59	15,22	196,97	22,74
LC-11	6,84	5,40	6,66	4,60	10,61	2,10	11,41	2,03
LC-12	69,74	70,55	69,84	59,63	109,37	9,82	119,85	10,39
All LC	**117,13**	106,22	**115,78**	102,65	**177,23**	154,98	**188,71**	159,77
AC-13	47,17	42,78	46,62	36,19	80,02	7,66	84,89	10,73
AC-14	44,86	42,85	44,61	36,23	88,76	7,09	78,04	5,94
AC-15	65,76	64,73	65,63	54,71	121,11	5,88	124,15	6,86
AC-16	71,33	72,90	71,52	61,61	137,60	4,67	138,18	6,47
AC-17	43,30	37,31	42,55	31,60	78,14	8,57	78,10	7,46
AC-18	73,68	76,30	74,00	64,49	142,05	3,88	141,52	5,18
AC-19	43,77	38,27	43,08	32,40	79,46	9,15	76,29	7,80
AC-20	23,08	22,14	22,96	18,72	47,86	4,44	40,59	3,85
AC-21	42,39	38,80	41,95	32,82	81,59	10,31	77,51	6,45
AC-22	70,66	68,88	70,44	58,22	131,41	8,66	130,14	7,83
AC-23	62,39	59,79	62,07	50,54	118,40	8,65	117,41	7,75
All AC	**53,49**	16,16	**53,22**	16,35	**100,58**	30,71	**98,80**	32,78
All	**86,69**	82,56	**85,86**	80,07	**140,57**	118,19	**145,71**	123,94

APPENDİX B

Correlation Matrix of the variables as a result of the Male and Worker Principal Components Analysis.

Correlation Matrix of the variables as a result of the Male Principal Components Analysis.

	LC-1	LC-2	LC-3	LC-4	LC-5	LC-6	LC-7	LC-8	LC-9	LC-10	LC-11	LC-12
LC-1	1,00											
LC-2	6,73	1,00										
LC-3	5,13	8,22	1,00									
LC-4	4,97	6,11	4,67	1,00								
LC-5	2,87	3,13	2,02	2,29	1,00							
LC-6	4,45	7,28	5,57	3,94	2,66	1,00						
LC-7	6,76	7,98	6,04	5,22	2,79	5,79	1,00					
LC-8	7,47	1,35	1,14	9,99	7,60	7,25	1,01	1,00				
LC-9	5,44	4,92	3,49	3,81	2,07	2,59	5,25	2,02	1,00			
LC-10	5,59	6,44	4,67	4,36	3,49	4,45	6,36	9,73	4,06	1,00		
LC-11	9,48	8,21	3,03	6,87	-3,18	-5,31	6,09	3,60	1,53	5,99	1,00	
LC-12	6,46	7,63	5,74	5,25	3,87	5,56	7,21	1,48	5,31	7,17	6,87	1,00
LC-13	1,12	6,66	4,96	1,00	-5,57	-4,07	5,82	1,36	-4,29	7,29	-2,77	9,04
LC-14	-2,82	-2,64	-8,06	-2,59	-1,07	-8,29	-3,19	7,91	-2,15	-2,57	-6,36	-3,14
LC-15	-1,31	-1,22	-1,26	5,54	-1,92	-1,45	4,93	4,59	-5,26	-1,49	-6,67	-4,46
LC-16	-1,09	-9,92	-1,56	-1,08	1,32	-3,20	-1,73	8,77	-7,65	-1,43	-1,22	-1,18
LC-17	-1,63	-1,32	-7,31	-4,70	-1,69	4,30	-9,38	1,02	-1,00	-1,48	6,59	-6,70
LC-18	2,24	1,45	1,37	1,85	1,63	6,38	7,38	5,89	-4,72	2,25	-6,19	1,73
LC-19	1,82	1,64	5,75	1,02	3,92	1,35	3,73	-7,25	7,88	9,89	4,49	1,19
LC-20	-1,12	-1,38	-3,87	-7,57	-5,43	-5,02	-1,49	8,41	-1,00	-1,57	3,81	-2,65
LC-21	-1,51	1,52	-1,77	-4,43	-1,73	-1,13	-1,84	-6,67	-3,81	-1,87	2,43	-2,52
LC-22	6,71	1,75	4,34	3,61	2,63	1,21	4,39	-8,25	-1,12	9,01	-2,76	1,16

Correlation Matrix of the variables as a result of the Male Principal Components Analysis continues.

	LC-13	LC-14	LC-15	LC-16	LC-17	LC-18	LC-19	LC-20	LC-21	LC-22	LC-23
LC-1											
LC-2											
LC-3											
LC-4											
LC-5											
LC-6											
LC-7											
LC-8											
LC-9											
LC-10											
LC-11											
LC-12											
LC-13	1,00										
LC-14	-3,61	1,00									
LC-15	-5,45	9,71	1,00								
LC-16	9,23	1,14	1,84	1,00							
LC-17	-5,83	2,49	2,03	-1,66	1,00						
LC-18	-1,41	-1,06	1,02	7,69	1,54	1,00					
LC-19	2,20	2,87	-5,00	1,05	1,36	-1,08	1,00				
LC-20	5,76	4,31	-7,06	8,06	8,80	-3,34	7,23	1,00			
LC-21	1,06	5,00	-4,39	1,15	-6,93	-2,00	-6,72	2,96	1,00		
LC-22	4,74	-1,31	-3,47	1,27	-4,50	1,26	1,23	-1,08	-4,29	1,00	
LC-23	-9,38	-9,14	6,41	-8,46	-1,68	-1,95	2,26	-2,55	-1,87	3,46	1,00

Correlation Matrix of the Variables as a result of the Worker Principal Components Analysis.

	LC-1	**LC-2**	**LC-3**	**LC-4**	**LC-5**	**LC-6**	**LC-7**	**LC-8**	**LC-9**	**LC-10**	**LC-11**	**LC-12**
LC-1	1,00											
LC-2	9,58	1,00										
LC-3	8,64	9,19	1,00									
LC-4	9,57	9,61	8,73	1,00								
LC-5	9,58	9,49	8,61	9,78	1,00							
LC-6	8,82	9,24	8,86	8,85	8,61	1,00						
LC-7	9,34	9,24	8,49	9,40	9,42	8,64	1,00					
LC-8	4,67	4,39	4,43	4,56	4,53	3,50	5,05	1,00				
LC-9	7,96	7,70	6,92	7,83	7,56	7,22	7,09	4,26	1,00			
LC-10	7,83	7,93	7,04	7,98	8,07	7,03	7,42	3,80	6,34	1,00		
LC-11	5,38	4,86	4,03	4,83	4,98	4,79	4,72	2,88	5,12	4,42	1,00	
LC-12	9,28	9,13	8,13	9,22	9,44	8,37	8,80	4,45	7,51	8,19	5,88	1,00
LC-13	1,28	1,19	1,59	9,54	1,64	1,59	9,21	-2,17	-6,31	1,10	3,97	1,63
LC-14	-1,37	-4,89	2,90	-1,51	-1,13	-3,94	-5,55	-1,10	-1,52	-8,46	-4,30	-1,47
LC-15	1,12	8,11	1,14	1,48	1,48	1,12	1,48	1,27	1,61	1,90	7,80	1,10
LC-16	5,96	1,01	1,29	9,19	1,32	6,18	1,24	7,85	1,02	-1,82	5,02	7,08
LC-17	-2,75	-1,67	6,37	-6,10	-4,21	6,70	-2,54	-1,04	-6,38	-1,96	-1,19	-7,21
LC-18	-4,97	-1,45	-5,70	-4,09	-4,70	-4,01	-9,44	-1,10	-1,14	-8,44	-2,40	-5,65
LC-19	-8,35	-1,23	-6,14	-1,32	-1,23	-1,23	-1,34	4,63	-4,64	2,95	-7,91	-1,21
LC-20	-2,07	-1,62	-8,48	-2,18	-2,67	-9,62	-2,01	-1,34	-2,11	-1,96	-3,60	-2,39
LC-21	1,05	1,38	1,56	6,63	7,80	1,67	1,33	1,06	1,08	5,83	-1,95	8,17
LC-22	-3,95	-4,57	-4,63	-2,46	2,52	2,52	2,60	-9,54	-5,83	2,34	1,74	3,79

Correlation Matrix of the variables as a result of the Worker Principal Components Analysis continues.

	LC-13	LC-14	LC-15	LC-16	LC-17	LC-18	LC-19	LC-20	LC-21	LC-22	LC-23
LC-1											
LC-2											
LC-3											
LC-4											
LC-5											
LC-6											
LC-7											
LC-8											
LC-9											
LC-10											
LC-11											
LC-12											
LC-13	1,00										
LC-14	-1,21	1,00									
LC-15	-4,48	-5,98	1,00								
LC-16	9,62	1,07	-1,11	1,00							
LC-17	2,16	8,82	6,56	-4,52	1,00						
LC-18	-8,94	8,40	-1,04	9,27	-1,49	1,00					
LC-19	1,97	1,28	8,93	-5,24	3,82	-1,48	1,00				
LC-20	-3,58	4,01	-3,39	-6,13	-9,67	-2,77	1,17	1,00			
LC-21	-2,24	2,18	-3,40	-1,18	-1,68	-8,84	-1,65	1,29	1,00		
LC-22	-5,79	1,41	-6,21	-6,33	-1,18	-3,33	-1,45	2,27	-2,45	1,00	
LC-23	-5,91	9,65	-2,24	-7,04	-8,16	6,55	-8,66	1,65	8,59	-6,00	1,00

APPENDIX C

Expected and observed frequencies of genotypes for each loci in each population are given above.

Population: Cyprus		
Locus: B126		
Genotypes	**Observed Frequency**	**Expected Frequency**
178, 178	4	1,8
180, 178	0	0,4
180, 180	0	0
184, 178	1	0,8
184, 180	1	0,08
184, 184	0	0,04
186, 178	1	3,6
186, 180	0	0,36
186, 184	0	0,72
186, 186	3	1,44
188, 178	0	1,6
188, 180	0	0,16
188, 184	0	0,32
188, 186	2	1,44
188, 188	1	0,24

Population: Ankara		
Locus: B126		
Genotypes	**Observed Frequency**	**Expected Frequency**
176, 176	0	0,683
178, 176	1	1.171
178, 178	1	0,366
180, 176	2	1,951
180, 178	0	1,463
180, 180	3	1,098
182, 176	3	1,561
182, 178	2	1,171
182, 180	1	1,951
182, 182	1	0,683
184, 176	0	0,39
184, 178	1	0,293
184, 180	0	0,488
184, 182	0	0,39
184, 184	0	0,024
186, 176	1	0,976
186, 178	0	0,732
186, 180	0	1,22
186, 182	0	0,976
186, 184	0	0,244
186, 186	2	0,244
190, 176	0	0,195
190, 178	0	0,146
190, 180	0	0,244
190, 182	0	0,195
190, 184	1	0,049
190, 186	0	0,122
190, 190	0	0
196, 176	1	0,39
196, 178	0	0,293
196, 180	1	0,488
196, 182	0	0,39
196, 184	0	0,098
196, 186	0	0,244
196, 190	0	0,049
196, 196	0	0,024

Population: Cyprus		
Locus: B124		
Genotypes	**Observed Frequency**	**Expected Frequency**
240, 240	0	0.0
250, 240	0	0.059
250, 250	0	0.0
252, 240	1	0.588
252, 250	0	0.588
252, 252	3	2.647
256, 240	0	0.235
256, 250	0	0.235
256, 252	2	2.353
256, 256	1	0.353
260, 240	0	0.059
260, 250	0	0.059
260, 252	1	0.588
260, 256	0	0.235
260, 260	0	0.0
266, 240	0	0.059
266, 250	1	0.059
266, 252	0	0.588
266, 256	0	0.235
266, 260	0	0.059
266, 266	0	0.0

Population: Ankara		
Locus: B124		
Genotypes	**Observed Frequency**	**Expected Frequency**
244, 244	0	0,077
246, 244	0	0,077
246, 246	0	0
248, 244	0	0,385
248, 246	0	0,128
248, 248	0	0,256
250, 244	0	0,308
250, 246	0	0,103
250, 248	1	0,513
250, 250	0	0,154
252, 244	1	0,385
252, 246	0	0,128
252, 248	0	0,641
252, 250	1	0,513
252, 252	0	0,256
254, 244	0	0,308
254, 246	0	0,103
254, 248	2	0,513
254, 250	0	0,41
254, 252	2	0,513
254, 254	0	0,154
256, 244	2	0,692
256, 246	1	0,231
256, 248	1	1,154
256, 250	1	0,923
256, 252	1	1,154
256, 254	0	0,923
256, 256	1	0,923
258, 244	0	0,077
258, 246	0	0,026
258, 248	0	0,128
258, 250	0	0,103
258, 252	0	0,128
258, 254	0	0,103
258, 256	0	0,231
258, 258	0	0
260, 244	0	0,538
260, 246	0	0,179
260, 248	1	0,897
260, 250	1	0,718
260, 252	0	0,897

Expected and observed frequencies of genotypes for loci B124 in Ankara population are continuing above.

260, 254	0	0,718
260, 256	1	1,615
260, 258	1	0,179
260, 260	1	0,538
262, 244	0	0,077
262, 246	0	0,026
262, 248	0	0,128
262, 250	0	0,103
262, 252	0	0,128
262, 254	0	0,103
262, 256	0	0,231
262, 258	0	0,026
262, 260	1	0,179
262, 262	0	0

Population: Cyprus		
Locus: B11		
Genotypes	**Observed Frequency**	**Expected Frequency**
164, 160	1	0.161
164, 164	0	0.323
166, 160	0	0.419
166, 164	2	2.097
166, 166	4	2.516
168, 160	0	0.323
168, 164	2	1.613
168, 166	3	4.194
168, 168	2	1.452
170, 160	0	0.065
170, 164	0	0.323
170, 166	0	0.839
170, 168	1	0.645
170, 170	0	0.032
186, 160	0	0.032
186, 164	0	0.161
186, 166	0	0.419
186, 168	0	0.323
186, 170	1	0.065
186, 186	0	0

Population: Ankara		
Locus: B11		
Genotypes	**Observed Frequency**	**Expected Frequency**
160, 160	0	0,029
162, 160	0	0,457
162, 162	1	0,8
164, 160	0	0,057
164, 162	0	0,229
164, 164	0	0
166, 160	1	0,457
166, 162	3	1,829
166, 164	1	0,229
166, 166	1	0,8
168, 160	0	0,457
168, 162	3	1,829
168, 164	0	0,229
168, 166	0	1,829
168, 168	1	0,8
170, 160	0	0,171
170, 162	0	0,686
170, 164	0	0,086
170, 166	1	0,686
170, 168	0	0,686
170, 170	0	0,086
172, 160	1	0,343
172, 162	0	1,371
172, 164	0	0,171
172, 166	0	1,371
172, 168	3	0,514
172, 170	2	0,514
172, 172	0	0,429

Population: Cyprus		
Locus: B100		
Genotypes	**Observed Frequency**	**Expected Frequency**
3, 3	4	2,769
4, 3	1	0,692
4, 4	0	0
5, 3	0	1,385
5, 4	0	0,154
5, 5	0	0,077
7, 3	0	1,385
7, 4	0	0,154
7, 5	2	0,308
7, 7	0	0,077

Population: Ankara		
Locus: B11		
Genotypes	**Observed Frequency**	**Expected Frequency**
150, 150	0	0,111
152, 150	1	0,667
152, 152	0	0,333
154, 150	1	0,667
154, 152	0	1
154, 154	1	0,333
168, 150	0	0,444
168, 152	2	0,677
168, 154	0	0,667
154, 154	0	0,111