GENETIC DIVERSITY IN TURKISH HONEY BEES

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Abstract: *Apis mellifera* shows geographic variation in morphology and in genetic characteristics. Allozyme polymoprhisms and mitochondrial DNA variation are particularly useful tools for the study of genetic and geographic variation in honey bees. Mitochondrial DNA (mtDNA) variation reveals four lineages of bees: West European, East European, African and a newly recognized Middle Eastern lineage. In Turkey, mtDNA study shows that *A. m. anatoliaca* and *A. m. caucasica* belong to the East European group. Approximately 86% of sample colonies of *A. m. anatoliaca* in Thrace share genetic traits with *A. m. carnica*. *A. m. caucasica* mtDNA was found in 77% of colonies examined near the Georgian border, 29% of sample colonies in Erzurum, and 25% of sample colonies from Muş, Bitlis and Van. Samples from Hatay showed that 57% had mtDNA belonging to the Middle Eastern group. These are believed to represent *A. m. syriaca*. Allozyme variation also exists among Turkish populations, but a high frequency of Mdh1^{100} is characteristic of all populations examined. There is still much to learn about genetic variation among Turkish honey bees, but much variation may be lost due to migratory beekeeping, large scale queen production from limited stocks and distributing them to all over the country.

Keywords: *Apis mellifera subspecies*,Turkish honeybees, Allozyme, mtDNA

TÜRKİYE BALARILARINDA GENETİK FARKLILIKLAR

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Özet: *Apis mellifera* morfoloji ve genetik karakterler bakımından farklılık göstermektedir. Enzim ve mitokondri DNA (mtDNA) farklılıklarının incelenmesi balarılarında genetik ve coğrafik farklılıkları göstermede oldukça yararlı metodlardır. MtDNA farklılıkları 4 arı soyunu açığa çıkarmaktadır. Batı Avrupa, Doğu Avrupa, Afrika ve yeni açıklanan Ortadoğu soy kütüğüdür. Türkiye'de mtDNA çalışmaları Anadolu ve Kafkas arısının Doğu Avrupa'ya ait olduğunu göstermektedir. Trakya bölgesinde toplanan Anadolu ırkı numulerinin yaklaşık %86'sı Karniyol arısının genetik karakterlerini paylaşmaktadır. Erzurum'da toplanan arı numulerinin % 29, Muş, Bitlis, ve Van da %25 Kafkas ırkını temsil etmektedir. Hatay'dan alınan örneklerde %57 sinin Suriye arısıma ait olduğuna inanılmaktadır. Enzim farklılıkları Türkiye'de arılarda genetik farklılıklar açısından öğrenilecek çok şey olmasına rağmen bu farklılıklar değişik nedenlerle gezginci arıcılık, az sayıda, aynı kökenden gelen kraliçe arılar kullanılarak her bölgenin kendine has arı ırkı veya ekotipinin kaybedilmesi gibi ciddi bir tehlike söz konusudur.

Türkiye coğrafik olarak Avrupa, Asya, ve Orta Doğunun birleşme noktasıdır ve sınırları içerisinde çok geniş bir iklim ve bölge yelpazesine sahiptir. Bu durum bio-coğrafik çalışmalar için ideal bir konum oluşturur. Bu yüzden tahmin edileceği gibi Türkiye`deki balarıları dış görünüş, davranış, ekoloji, ve hastalıklara direnç açısından önemli farklılıklar gösterir. Enzim ve mtDNA analizleri morfolojik sonuçlarla benzerlik göstermenin yanında yeni bilgiler de açığa çıkarmıştır. Ruttner (1988) Türkiye'de 4 arı ırkı önermiştir; Anadolu, Kafkas, İran ve Suriye arısı. Kafkas arısı Kuzeydoğu Anadolu, Doğu Karadeniz kıyıları, İran arısı Güneydoğu, Suriye arısı Güneydoğu ve Suriye ile sınır kısımlarında ve Anadolu arısının Trakya dahil Türkiye`nin geri kalan kısımlarında bulunduğunu belirtmiştir. Ruttner

Kafkas, Anadolu, Ermeni ve Girit arılarını Oryantal grup içine almıştır. Fakat genetik çalışmalar Anadolu ve Kafkas arısının Doğu Avrupa grubu içinde Karniyol ve İtalyan arıları ile C grubunda olması gerektiğini göstermiştir. Trakya bölgesindeki Anadolu arısı, Anadolu`daki Anadolu arısından bazı farklılıklar, Karniyol arısına ise benzerlik göstermiştir. Bu genetik farklılıklar arıcılıkta sorunları çözmenin, Ör. hastalıklara dirençli arı hatları geliştirmenin hammaddeleridir. Bu farklı ırkların kaybedilmesi durumunda işlenecek hammadde olmayacaktır.

Türkiye'deki bal arılarının genetik farklılıkları daha yeni çalışılmaya başlanmıştır ve DNA çalışmaları daha ayrıntılı bilgilere ulaşmamızı sağlayacaktır. Bu genetik farklılıklar ekonomik yönden oldukça önemlidir. Çünkü her bölgenin kendi arısı o bölgenin ekolojik koşullarına adapte olmuş, ve bölgesinde nasıl hayatta kalabileceğini bilen arılardır. Yeni ulaşım vasıtaları ve gezginci arıcılık Ör. Varroa gibi hastalıkların çok hızlı bulaşmasına neden olmuş ve farklı bölgelerin arısı yeni bölgedeki iklime uyum sağlamasının zorluğu yanında yeni bölgedeki hastalık ve arı zararlıları ile mücadelesi oldukça zordur. Bugün Türkiye arılarının genetik farklılıkların belirlenmeden kaybedilmesi riski bulunmaktadır.

Anahtar kelimeler: *Apis mellifera* alt-türleri,Türkiye balarıları, Allozyme, mtDNA

Overview of Variation in *Apis mellifera*

The natural range of *Apis mellifera* includes most of Africa, Europe and parts of the Middle East. Every beekeeper and honey bee biologist is aware that bees from different locations may differ in size, color, the relative proportions of body, limbs and wings, wing venation, disease resistance, tendency to swarm, colony defensiveness, etc. The bees of Turkey are particularly exciting for studies of honey bee biogeography. Turkey is the geographic crossroad between Europe, Asia, and the Middle East, and it encompasses a wide range of climates and habitats within its borders. Not surprisingly, the honey bees of Turkey are also very diverse, as shown by morphometrics (e.g., Guler 1996, Guler et al 1999), behavior, ecology, and disease resistance (e.g., Adam 1954, 1964, 1977, 1983, Ruttner 1988, 1992). Variation among honeybee populations may be attributed to two factors: adaptation of honey bee populations to their diverse local environments, and history.

At various times during the Pleistocene, glaciers and cold temperatures made much of Europe uninhabitable for honey bees, and probably led to isolation of small populations of honeybees in relatively sheltered locations in southern Europe, such as the Iberian, Italian, and Balkan peninsulas (Ruttner 1988, 1992, Hewitt 1996). When Europe was experiencing glaciers, much of Africa was experiencing arid conditions, and when Europe experienced warm periods between glaciers, Africa experienced moist conditions (van Zinderen Bakker 1976, Hamilton 1982). This caused expansion and contraction of different types of habitat throughout Africa, especially during the past one million years (Potts and Behrensmeyer 1992). These conditions created small, isolated populations of honey bees.

Small isolated populations can easily become different from one another. New genetic mutations constantly arise in any population. In small populations, new mutations can spread rapidly by chance alone (called "genetic drift"). New mutations may also spread if they produce some benefit, such as disease resistance (the process of natural selection). Because populations are isolated from each other, mutations that appear in one population cannot spread to the others, and over time each population accumulates its own set of genetic characteristics.

While honey bee populations were isolated from one another in European refuges and habitat patches in Africa they could accumulate genetic differences through mutation, genetic drift and natural selection. After the Pleistocene glaciers receded formerly isolated populations could expand their ranges and come into contact again. Thus, these two factors---historical patterns of population isolation and adaptation to current environment---contribute to the geographic variation in morphology, behavior, population biology and other characters observed among honey bee populations today (Adam 1983, Ruttner 1988, 1992).

At present, 26 subspecies of *Apis mellifera* are recognized, primarily on the basis of morphometric characters (Buttel-Reepen 1906, Alpatov 1929, Skorikov 1929, DuPraw 1965, Ruttner et al. 1978, Cornuet et al. 1988, Ruttner 1988, 1992, Cornuet and Fresnaye 1989, Crewe et al. 1994, Sheppard et al. 1997, Engel 1999). Ruttner (1988, 1992) suggested that these subspecies could be grouped into four subspecies groups or lineages: (1) subspecies from northern and western Europe and northern Africa; (2) subspecies of the northern Mediterranean region and Eastern Europe; (3) subspecies of sub-Saharan Africa; and (4) subspecies of the eastern Mediterranean region and Iran.

More recently, genetic tools, particularly DNA sequence analysis and allozyme electrophoresis, have been applied to the study of honey bee diversity. The results of these studies are broadly similar to results of morphometric studies, but also reveal new information.

Mitochondrial DNA studies: Mitochondria are the tiny organelles that provide energy to a cell. Insect flight requires a tremendous amount of energy, so it is no surprise that the flight muscle of honey bees is packed with mitochondria. Each mitochondrion contains its own small, circular chromosomes. Unlike the genetic traits coded in the chromosomes of the cell nucleus, which an individual can inherit from both the mother and father, mitochondria are inherited only from the mother --- thus all the workers and drones in a colony will share the same mitochondrial DNA (mtDNA for short) as the queen.

Variation in the mtDNA of honey bees provides tremendous insight into the biogeography of honey bees, and, like morphometric data, mtDNA variation reveals four main lineages of *Apis mellifera* (Smith and Brown 1988, 1990, Smith et al. 1989, Cornuet and Garnery 1991, Hall and Smith 1991, Smith 1991 a and b, Garnery et al. 1992, Arias and Sheppard 1996 Palmer et al. 2000). The mtDNA groups are (1) Western (or "W"): western and northern European populations, mainly *A. m. mellifera* and some *A. m. iberiensis*; (2) Eastern (or "C"): southeastern European plus northern and eastern Mediterranean populations, including *A. m. carnica*, *A. m. ligustica*, Turkish and Georgian *A. m. caucasica*, and Turkish *A. m. anatoliaca*; (3) African (or "A"): African populations north and south of the Sahara, including *A. m. capensis*, *A. m. intermissa*, *A. m. litorea*, *A. m. monticola*, *A. m. sahariensis* and *A. m. scutellata*; and (4) a recently discovered Middle Eastern group (M) including *A. m. syriaca*, and perhaps others. Variation is also detected among some of the subspecies within each lineage (Moritz et al. 1994, Sheppard et al 1996).

Allozymes: Allozymes are different forms of an enzyme produced by different alleles at a single gene locus. Honey bees have few variable (or "polymorphic") enzymes compared to other insects, but the few enzymes that are variable are very useful tools for the study of honey bee populations. The most widely used enzyme in honey bee studies is malate dehydrogenase (MDH1, Enzyme Commission number 1.1.1.37; Contel et al.1977). Several authors have suggested that different forms of the malate dehydrogenase enzyme --- which is part of the system that provides energy to the muscles and other tissues --- may enable bees to perform optimally in habitats with different temperature regimes (e.g., Coelho and Mitton 1988, Cornuet et al. 1995; Harrison et al. 1996, Nielsen et al. 1994, Hepburn et al. 1999).

MDH1 shows substantial allele frequency differences among the four honey bee lineages. Bees in the western European or “W” group of have high frequencies of the *Mdh1*^{80} or "medium" allele, ranging from 85% to 100% (Cornuet 1982; Nunamaker et al. 1984, Sheppard and Berlocher 1984). African bees exhibit high frequencies of the *Mdh1*^{100} or "fast" allele, ranging from 95% to 100% (Nunamaker and Wilson 1981, Nunamaker et al. 1984, Ndiritu et al. 1986, Meixner et al. 1994, Sheppard and Huettel 1988). Bees from southeastern European plus northern and eastern Mediterranean populations (“C” group) show regional variation in allele frequencies (e.g., Dedej et al. 1996), but many subspecies have moderate to high frequency of the *Mdh1*^{65} (or "slow") allele, which is rare in the W and C groups. Allozymes have not been thoroughly investigated in the Middle Eastern group.

Genetic variation in Turkish *A. mellifera*

Ruttner (1988) suggested four subspecies occur in Turkey: *A. m. anatoliaca, A. m. caucasica*, *A. m. meda* and *A. m. syriaca*. According to Ruttner, *A. m. caucasica* occurs in the extreme northeast of Anatolia, with bees resembling *A. m. caucasica* occurring along the eastern Black Sea coast as far as Samsun. *A. m. meda* is found in the southeast, and *A. m. syriaca* in the extreme south, near the border with Syria. *A. m. anatoliaca* occurs throughout the rest of Turkey, including Thrace.

Mitochondrial DNA: Study of the mtDNA of Turkish honey bees shows that *A. m. anatoliaca* and *A. m. caucasica* belong to the Eastern or "C" mitochondrial lineage (Smith et al. 1997, Palmer et al. 2000) as do *A. m. carnica* and *A. m. Ligustica* **(Figure 1)**. This differs from Ruttner's placement of *A. m. caucasica* in a separate Oriental group of subspecies along with *A. m. armeniaca*, *A. m. anatoliaca* and *A. m. adami* (Ruttner 1992).

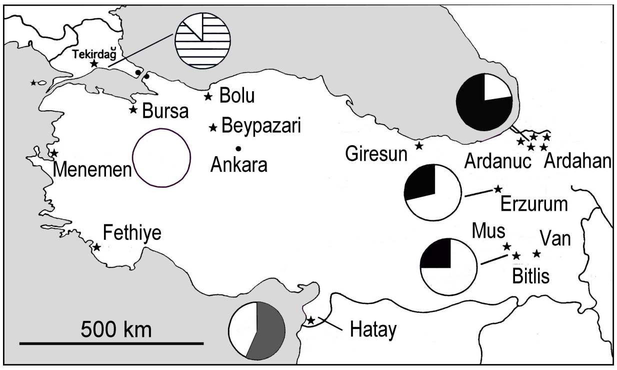

Figure 1. Distribution of honey bee mitochondrial DNA types in Turkey. Stars indicate collection sites. Pie diagrams show frequencies of mtDNA types in 6 regions. White indicates an East European type of mtDNA found in Anatolian *A. m. anatolia*. White with stripes indicates an East European type of mtDNA found in Thracian *A. m. anatoliaca* and *A. m. carnica*. Black indicates an East European type of mtDNA found in *A. m. caucasica*. Grey indicates a Middle Eastern mtDNA type found in *A. m. syriaca*.

Within the Eastern group, *A. m. anatoliaca* from Thrace differed slightly from the *A. m. anatoliaca* from Anatolia. Some of the bees of Thrace share mtDNA types with *A. m. carnica* from Austria, Slovenia and Croatia (Smith and Brown 1990, Meixner et al. 1993). This suggests some interbreeding among the bees of Thrace, the Balkans and southern Austria. It is possible that the bees of Thrace form a bridge between the Balkan and Anatolian bees. The fact that the Thrace mtDNA variation was not found in any bees from Anatolia suggests there may be a barrier to breeding between these two regions; more sampling in northwest Anatolia is needed to test this.

The "homeland" of *A. m. caucasica* is in the Caucasus Mountains, southern valleys of the Caucasus, and the higher reaches of the Little Caucasus mountains (Ruttner 1988), primarily in

Georgia and neighboring republics. There is some debate over the range of this bee in Turkey. Ruttner states that bees resembling *A. m. caucasica* were found along the Black Sea coast of Anatolia as far as Samsun (Ruttner 1988; 178, 192-198). A detailed look at the mtDNA of *A. m. anatoliaca* and *A. m. caucasica* showed DNA sequence differences between the two. As expected, colonies sampled near the border with Georgia showed a high frequency (77%) of *A. m. caucasica* mtDNA. However, A. *m. caucasica* mtDNA also occurred far to the south of the proposed range of *A. m. caucasica*. Twenty-nine percent of colonies sampled near Erzurum and 25% of colonies sampled around Lake Van had *A. m. caucasica* mtDNA. On the other hand, we have no evidence of the Caucasian mtDNA along the Turkish Black Sea coast, though we had very few samples from this region and may have missed it.

If the mtDNA sequence that our lab and others (Garnery et al. 1992) found in *A. m. caucasica* is truly characteristic of all *A. m. caucasica*, then our results indicate a wide zone of overlap between *A. m. caucasica* and *A. m. anatoliaca*, at least from Lake Van to the Georgian border. This could be due to natural gene flow and dispersal, or to transportation of *A. m. caucasica* by humans. More extensive collections from the north-eastern area of Turkey and the heart of the *A. m. caucasica* range in Georgia would enable us to determine if the DNA sequence we call "Caucasian" is actually characteristic of all or most *A. m. caucasica*, and help us to determine the true range of *A. m. caucasica* in Turkey. Nothing in our mtDNA data suggested that the bees from the Lake Van region, supposedly in the range of *A. m. meda*, were in any way distinct from other Anatolian populations.

In southeast Anatolia, 57% of colonies sampled from Hatay were found to have a type of mtDNA that, at that time, had not been found in any other bees. We called this the Middle Eastern, or "M" lineage (Palmer et al. 2000). The bees from Hatay appear to be *A. m. syriaca*, whose range is reported to include the eastern coast of the Mediterranean north of the Negev desert, including parts of Israel, Jordan, Syria and Lebanon. The M type of mtDNA has subsequently been found in Israel and Jordan (D. Smith and S. Shafir, unpublished data). Little genetic work has been carried out on the native honey bees of the Middle East, principally because imported *A. m. ligustica*, *A. m. carnica* and other races have largely replaced the native honey bee where modern apiculture is practiced (Y. Lensky, pers. com.).

Allozymes: A high frequency of the $Mdh1^{100}$ (or fast) allele is sometimes considered typical of African honey bees (e.g., Nunamaker et al. 1984). However, this allele is also present in high frequency in many Eastern subspecies, including the bees of Turkey. The $Mdh1^{100}$ allele frequencies reported in the Eastern group of honey bee subspecies range from a low of approximately 23% in north Italian *A. m. ligustica* (Badino et al. 1985, Sheppard and Berlocher 1985) to nearly 100% in *A. m. caucasica* and *A. m. anatoliaca* in Turkey (Asal et al. 1995, Kandemir and Kence 1995, Palmer et al. 2000). We have no information yet on allozyme frequencies in Middle Eastern bees such as *A. m. syriaca*.

Preserving the diversity of Turkish honey bees

The study of genetic diversity of Turkish honey bees has only just begun, and work remains to be done. In the future, techniques such as the study of microsatellites and DNA fingerprinting (e.g., Estoup et al. 1995) may show a more detailed picture of the diversity that exists among Turkish honey bees. Some of this variation may prove to be of economic importance. Local populations of honey bees are generally adapted to the ecological conditions found in their native habitat. For exampleAdam (1983) noted that the Anatolian honey bee, *A. m. anatoliaca*, shows superb resistance to the cold winters and hot dry summers of central Anatolia.

In addition, different races or subspecies of bees may differ in resistance to diseases, predators and parasites. With modern transportation, new parasites and diseases can spread quickly around the world. One has only to look at the spread of the *Varroa* mite to see that this is true. Who can predict which bee races will be most resistant to new the next new bee disease? Genetic variation is like a store of ammunition to use against invading pests. New genetic techniques for mapping the chromosomes of animals (Hunt and Page 1995) may soon enable apicultural scientists to locate and identify genes involved with particular traits, such as resistance to a particular pathogen. However, much of the genetic diversity of Turkish bee populations is in danger of being lost before it can be identified and studied.

Some common aspects of modern bee-keeping --- such as requeening colonies from one or few genetic stocks --- may have immediate, short term economic benefits for the bee-keeper. However, they may also lead to problems in the future. These problems include introduction of bees that are not suited to local climates, introduction of new diseases, parasites and predators, and loss of native genetic variation. Genetic studies of Turkish honey bee populations are important for detecting, maintaining and utilizing the genetic diversity of Turkey's bees.

ACKNOWLEDGEMENTS

I wish to thank Professor O. Kaftanoğlu, Drs. A. Güler, Dr. F. Genç, I. Cakmak and many Turkish bee-keepers for their help in collecting samples for the studies summarized here. The studies were partly supported by grants from the University of Kansas General Research Fund, the U. S. National Science Foundation, and the U. S. Department of Agriculture NRICGP 51.2.

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