Determining Demographic and Genetic Structure of Sedentary and Migratory Bears (*Ursus arctos*) within Eastern Anatolia Using Genome-Wide Genetic Markers

by

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Koç University

Graduate School of Sciences and Engineering

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To my beloved family

ABSTRACT

Determining Demographic and Genetic Structure of Sedentary and Migratory Bears (*Ursus arctos*) within Eastern Anatolia Using Genome-Wide Genetic Markers

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Large carnivores are known for altering their life-history strategies in response to environmental change. One such shift was recently discovered in brown bears (*Ursus arctos*) within Eastern Anatolia, where the availability of city dumps as a food source has led to the evolution of two distinct life strategies: sedentary bears that use city dumps as a primary food source and migratory bears that avoid the dump and migrate in search of food. Understanding the demographic and genetic processes that have led to the establishment of these life-history strategies is vital for predicting which life history strategy will dominate in the future and the overall impact of anthropogenic pressures on wild carnivores forced to live in human-dominated landscapes. Adaptive and genomic processes responsible for the two life-history strategies were determined using genotype data from 57 blood samples collected from captured bears, 31 of which were fitted with satellite collars and tracked almost for one year, providing information on bears' movement ecology and migratory behavior. We found that the Eastern Anatolian brown bear population is genetically highly differentiated and isolated from other world populations but contains high genetic diversity. Historical demography showed a recent dramatic increase in the effective population size of the bear population in Eastern Anatolia while relatedness analysis showed higher coefficient of kinship and sib-ship in sedentary bears utilizing the garbage dump. We also determined several genomic regions and distinct genotypes associated with sedentary and migratory behavior and strong signatures of positive selection at these loci. Outlier loci were associated with a number of transcript modifier genes, including the first exon of CCRL2, a gene that regulates immune response. Moreover, polygenic discrimination studies revealed 464 SNPs distributed throughout the genome actively contributing to difference in movement behaviorand could reliablt predict movement behavior in un-classified individuals. Gene ontology for these discriminatory SNPs revealed that the detection of sensory and chemical stimuli was the most important function that differentiated sedentary and migratory bears. Collectively our results indicate that adaptation to human-oriented landscapes in the East Anatolian brown bear population may have a strong genetic basis and emphasizes the importance of evolutionary genomics for understanding how species survive and adapt to human-mediated global change.

ÖZETÇE

Doğu Anadolu Bölgesindeki Yerleşik ve Göçmen Bozayıların (*Ursus arctos*) Demografik ve Genetik Yapısının Genom Çapındaki Genetik İşaretleyiciler Kullanılarak Belirlenmesi

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Büyük etoburların, çevresel değişime tepki olarak yaşam öyküsü stratejilerini değiştirdiği bilinmektedir. Böyle bir değişim, yakın zamanda Doğu Anadolu'daki boz ayılarda (*Ursus arctos*) keşfedildi ve buradaki şehir çöplüklerinin bir besin kaynağı olarak mevcudiyeti iki farklı yaşam stratejisinin gelişmesine yol açtı: şehir çöplerini birincil besin kaynağı olarak kullanan yerleşik ayılar ve çöplükten kaçan ve yiyecek aramak için göç eden göçmen ayılar. Bu yaşam öyküsü stratejilerinin oluşturulmasına yol açan demografik ve genetik süreçleri anlamak, gelecekte hangi yaşam öyküsü stratejisinin egemen olacağını ve antropojenik baskıların insan egemen bölgelerde yaşamaya zorlanan vahşi etoburlar üzerindeki genel etkisini tahmin etmek için hayati önem taşımaktadır. İki yaşam öyküsü stratejisi ile ilgili olan adaptif ve genomik süreçler, yakalanan ayılardan toplanan 57 kan örneğinden elde edilen genotip verileri kullanılarak belirlendi ve bu ayılardan uydu tasması takılan 31 tanesi neredeyse bir yıl boyunca takip edildi ve ayıların hareket ekolojisi ve göçmen davranışları hakkında bilgi verdi. Doğu Anadolu boz ayı popülasyonunun genetik olarak oldukça farklılaştığını ve diğer dünya popülasyonlarından izole olduğunu ancak yüksek genetik çeşitlilik içerdiğini bulduk. Tarihsel demografi, Doğu Anadolu'daki ayı popülasyonunun etkin nüfus büyüklüğünde yakın zamanda çarpıcı bir artış gösterirken, akrabalık analizi, çöplüğü kullanan yerleşik ayılarda akrabalık ve kardeşlik katsayısının daha yüksek olduğunu gösterdi. Ayrıca, yerleşik ve göçmen davranışla ilişkili birkaç genomik bölge ve farklı genotipler ve güçlü pozitif seçilim gösteren lokuslar belirledik. Aykırı lokuslar, bağışıklık tepkisini düzenleyen bir gen olan CCRL2'nin ilk ekzonu da dahil olmak üzere bir dizi transkript modifikasyon gen ile ilişkilendirildi. Ayrıca, poligenik ayrım çalışmaları, genom boyunca dağılmış 464 SNP'nin hareket davranışındaki farklılığa aktif olarak katkıda bulunduğunu ve sınıflandırılmamış bireylerde hareket davranışını güvenilir bir şekilde tahmin edebildiğini ortaya çıkardı. Bu ayrımcı SNP'ler için gen ontolojisi, duyusal ve kimyasal uyaranların tespitinin, yerleşik ve göçmen ayıları ayırt eden en önemli işlev olduğunu ortaya koydu. Sonuç olarak bu çalışma, Doğu Anadolu boz ayı popülasyonundaki insan kaynaklı etmenlere uyumun güçlü bir genetik temele sahip olabileceğini göstermekte ve türlerin nasıl hayatta kaldığını ve insan kaynaklı küresel değişime nasıl uyum sağladığını anlamak için evrimsel genomiğin önemini vurgulamaktadır.

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ABBREVIATIONS

GWAS	Genome-Wide Association Study
ANGSD	Analysis of Next Generation Sequencing
PCA	Principal Component Analysis
MAF	Minor Allele Frequency
DAF	Derived Allele Frequency
RF	Random Forest
SVM	Super Vector Machine
LDA	Linear Discriminant Analysis
GO	Gene Ontology
FDR	False Discovery Rate
IBD	Identical-By-Descent
EBSP	Extended Bayesian Skyline Plot
APN	Apennine brown bear population
EUR	European brown bear population
GEO	Georgian brown bear population
AK	Alaskan brown bear population
RUS	Russian brown bear population
SLO	Slovenian brown bear population
SLK	Slovakian brown bear population
GRE	Greek brown bear population
UM	Polar bear population
mya	Million years ago
kya	Thousands years ago

CHAPTER 1:
INTRODUCTION

Due to increasing human impacts, many mammal species have been forced to live in modified and fragmented environments (Goudie & Viles, 2013). Environmental changes can affect animal behavior, life history, migration patterns, and habitat choice (Nelson, 1998). Cognitively complex creatures may create plastic techniques to deal with novel and continuously changing environments, which can lead to the establishment of distinct alternative behaviors within the population (Flack et al., 2016).

A species that show remarkable adaptation to human-altered landscapes is the brown bear (*Ursus arctos*). They often opportunistically utilize the nutritional resources resulting from human activities (garbage dumps, campsites, and residual areas). Although food availability does not directly affect the home range of brown bears, they can reduce their home range size to easily access artificial food resources (Blanchard & Knight, 1991). However, rarely does a new life strategy based on anthropogenic food as the main food source and feeding area apart from opportunism becomes stable in natural populations. Such a change in life history was detected by our research team recently in the Eurasian brown bears (*Ursus arctos arctos*) living in Eastern Anatolia (Cozzi et al., 2016). The brown bear populations found in these regions with high anthropogenic impact show two basic life history strategies in terms of feeding behavior. The first strategy is 'sedentary', in which Eastern Anatolian brown bears use urban garbage dumps as their primary feeding area and rarely forage in natural habitats. The second type of strategy is 'migratory', in which Eastern Anatolian brown bears do not normally go to the dump but actively migrate to find food in natural habitats. Consequently, detailed studies of this population are essential, to understand how behavioral shifts towards anthropogenic nutritional sources develop and become stable in brown bear populations and to manage conflicts that may arise between bears and people as this new nutritional strategy becomes more widespread.

In this thesis, using genome-wide genetic markers, I investigate the genetic structure, diversity, relatedness, and demographic history of Eastern Anatolian brown

bears in relation to their conspecifics around the world and, using classical GWAS and polygenic discrimination analysis, evaluate the genetic basis of the observed life-history changes (i.e., movement behavior) in response to anthropogenic food sources.

Chapter 2 will present a description of the study area and our current knowledge regarding the ecology of the Eastern Anatolian brown bear population, as well as a literature review on studies focusing on the adaptation of wildlife to urban or human-dominated landscapes with a particular emphasis on genetic mechanisms. Detailed explanations of the study's purpose and rationale will be presented in Chapter 3, while Chapter 4 will provide all materials and experimental and computational methods used in this study. Results obtained throughout the study will be given in Chapter 5 and discussed in relation to current literature, conservation in Chapter 6. Finally, Chapter 7 will summarize all findings and present the study's main conclusions along with future directions.

CHAPTER 2:
LITERATURE REVIEW

2.1 Behavioral variation in adapting to human-dominated landscapes

The rapid expansion of urban areas has led to the large-scale destruction and degradation of natural habitats forcing many wildlife species to live in human-dominated landscapes and adapt to these modified environments (Chapron et al., 2014; Goudie & Viles, 2013; Hunter, 2007). Environmental variation caused by these new anthropogenic pressures can have an impact on an animal's behavior, life history, migration patterns, and choice of habitat (Nelson, 1998; Penteriani et al., 2018; Zarzo-Arias et al., 2018). For example, black-tailed gull *Larus crassirostris* is influenced by spatiotemporal variation in anthropogenic food resources in their foraging trips and selection of feeding grounds during their incubation and hatching period (Yoda et al., 2012). Similarly, the migrant white stork *Ciconia ciconia* has changed its life history from migratory to year-round residency to access additional food sources (Massemin-Challet et al., 2006). Major dietary shifts were observed in the spotted hyena (*Crocuta crocuta*) in northern Ethiopia due to the abundance of anthropogenic food sources (Yirga et al., 2012). Additionally, research on lions (Panthera leo) has shown how habitat selection differs between relocating and resident individuals (Elliot et al., 2014). The sensitivity of animals to human activity and how they have evolved to deal with the landscape changes can cause alterations in feeding habits, movement patterns, and habitat selection.

Many animal species now inhabit landscapes dominated by humans as a result of rising habitat loss and fragmentation (Hanski, 1999; Runge et al., 2014). They develop and employ cognitively complex mechanisms to deal with these novel and ever-changing environments (Goudie & Viles, 2013; Sol et al., 2008; Valeix et al., 2012), which can lead to the enhancement and fixation of different behavioral types within a population (Smith & Arcese, 1989). If left unaccounted for, these frequently observed behavioral changes can skew our knowledge of a species' environmental preferences (Elliot et al., 2014; Zeller et al., 2012). To execute conservation methods effectively, it is crucial to recognize and take into account appropriate behavioral patterns (Killeen et al., 2014). For

the implementation of successful conservation strategies and for the creation and upkeep of suitable and connected habitat areas, it is essential to comprehend the mechanisms that lead animals to make long-distance movements and to clarify the ecological and demographic effects of reduced mobility (Bowler & Benton, 2005; Clobert et al., 2001; Fischer & Lindenmayer, 2007; Runge et al., 2014).

Cognitively complex species are known to show remarkable behavioral plasticity in coping with novel and constantly changing environments, resulting in the establishment of distinct alternative behaviors within populations (Flack et al., 2016; Martin et al., 2010; Ordiz et al., 2013). One of the most prominent species in adapting to new environments formed as a result of human activities is the Eurasian brown bear (*Ursus arctos arctos*) (Cozzi et al., 2016; De Angelis et al., 2021; de Gabriel Hernando et al., 2020). The presence of anthropogenic food sources can have important consequences for the territorial behavior of brown bears which is often altered to accommodate these alternative food sources (Dahle & Swenson, 2003; Penteriani et al., 2021). Bears are highly opportunistic omnivores well known for using new environments and nutrient sources provided by human settlements such as house or city dumps, bee farms, poultry, and campsites (Cozzi et al., 2016; De Angelis et al., 2021; Kavčič et al., 2015; Krofel et al., 2017; Martin et al., 2010; Ordiz et al., 2014; Ordiz et al., 2017; Penteriani et al., 2021; Selva et al., 2017). Such tendencies increase human-bear interactions and have caused brown bears to be regarded as “nuisance” or even “problem” animals (Kavčič et al., 2015). The presence of anthropogenic food sources causes changes in the bears’ territory to benefit from these alternative food sources (Dahle & Swenson, 2003). Furthermore, this behavioral flexibility has been known to result in the emergence of new life history and nutrition strategies specific to human resources (Kaczensky et al., 2006; Martin et al., 2010; Ordiz et al., 2014). Therefore, the presence of anthropogenic food sources has the potential to permanently modify the territorial, roaming, and nutritional behavior of brown bears and lead to the establishment and spread of new life history strategies (Blanchard & Knight, 1991).

2.2 The Adaptation of Bears to Anthropogenic Food Resources

In the early 1900s, Yellowstone National Park, one of the biggest and most important protected areas in the United States, encouraged and occasionally allowed grizzly bears to feed from its garbage dumps. Between 1959 and 1968, there were few

management issues or grizzly bear issues in the Greater Yellowstone Ecosystem. However, as soon as all rubbish pits within the park and outside its borders were closed, human-bear interaction and conflict significantly rose (Craighead & Craighead Jr, 1971). As a result of this abrupt transition, grizzlies began to congregate around campgrounds and other areas frequented by tourists, dramatically raising the frequency of documented conflicts. Grizzly bear populations, fertility, and human-bear confrontations were tracked during that time (Cole, 1974; Craighead & Craighead Jr, 1971; Craighead et al., 1995). In the following years, first, the number of grizzly bears decreased until the 1980s, thus threatening the population in the Yellowstone National Park. A new grizzly bear management strategy was put into place in 1983 with a focus on the preservation of backcountry habitat. The objectives were to reduce bear-human interactions that would cause bears to become used to humans, avoid human-caused bear eviction from food sources, and reduce the danger of bear-caused human injury in bear-active areas. This program is still running today, and grizzly bear populations have grown, and bears now reoccupy historical ranges that are roughly seven times the size of Yellowstone National Park (Survey., 2016).

In urban Nevada, black bears (*Ursus americanus*) have been reported to have a highly enriched diet from the surrounding garbage, a consequent three-fold increase in population size as well as an increase in human-bear conflict as high as ten-fold (Beckmann & Lackey, 2008). They found that urban bears are less active than wildland ones because garbage is not a limited source. Although urban interface bears were not observed 10 to 15 years ago, black bears easily adapted to changing environments, and the increase in their body mass, population number, and reduction in their home ranges were observed (Beckmann, 2002; Beckmann & Berger, 2003a). Beckmann and Lackey (2008) found that the age-specific fecundity rates of urban female bears are higher even though they experience much higher age-specific mortality rates. There is a decline in the number of black bears in undeveloped areas and a rise in black bear populations around urban areas due to the inability of urban-adapted bears to recolonize their native habitats (rural/undisturbed) (Bateman & Fleming, 2012).

Perhaps one the most striking case study supporting the effect of anthropogenic food sources on brown bear ecology comes from Eastern Anatolia, where the availability of a large, open city dump, 3 kilometers west of Sarıkamış, Kars in Eastern Anatolia, has

led to the emergence of two distinct life history strategies: “Sedentary bears” that depend on the garbage dump regularly as a primary food source and “Migratory bears” that avoid the dump and migrate in search of food (Cozzi et al., 2016). At one time, over 30 bears have been observed feeding at the landfill at once, which they frequent at night (per. obs.). Furthermore, year-round monitoring around the garbage dump has shown that some females have up to four young instead of the typical two, and camera traps have photographed active bears in the middle of the winter. These data suggest that the year-round availability of high-calorie food sources at or near the garbage dump may be affecting the life history characteristics of brown bears such as movement, fertility, phenology, and hibernation patterns and could potentially be acting as a strong selective agent (Cozzi et al. 2016).

The emerging conflict between the bear population and people can determine whether this life history strategy can result in a more sedentary life for this bear population in Eastern Anatolia. It is essential to understand the ecology and population dynamics for the creation of conservation strategies and plans. To understand whether the change in complex characteristics such as life history strategy can be identified in a population, the two most important parameters must be known. One of them is the effect of this behavior on the fitness value of the individuals; in other words, understanding whether it positively affects the natural increase capacity or not, and the other parameter is the degree of inheritance of these behavioral characteristics to the next generations (Roff, 1997). As a response to the existence of city dumps, this resident life strategy observed in bears may increase the fitness of bears and the characters associated with this life strategy will be highly heritable, and this life strategy will be in danger due to rapidly spreading characters in the bear population in the studied region. Therefore, heritability values associated with this new life strategy seen in Eastern Anatolian brown bear populations is important.

2.3 The Evolutionary History of Bears

The evolution of Ursus species has been investigated using both mitochondrial and whole-genome sequence data (Barlow et al., 2018; Cahill et al., 2013; Leonard et al., 2000; Liu et al., 2014; Miller et al., 2012). It is estimated that the brown bear and the polar bear diverged approximately 300 thousand years ago, and this lineage diverged from black bears around 4-5 thousand years ago (Miller et al., 2012; Liu et al., 2014). The

evolutionary history of bears has been linked to significant climatic changes, and polar bears in particular have been shown to have undergone a sustained and sharp fall in effective population numbers over the past 500,000 years. The Alaskan brown bears are the closest relatives to polar bears (Miller et al., 2012). Even though they diverged fairly recently and sex-biased gene flow from brown bears to polar bears has been observed dating back to 150 kya, brown bears and polar bears are considered fully formed separate species (Hailer et al., 2012; Lindqvist et al., 2010; Liu et al., 2014; Wang et al., 2022).

The demography and ecology as well as genetic structure of European brown bears have been affected by climate and anthropogenic impacts. In modern Europe, although the brown bear is one of the most widespread large carnivores (Chapron et al., 2014), habitat destruction and human hunting have caused its distribution to decline (Servheen, 1990). Throughout most of the Holocene, the species range covered the entire continent, but it is now only found in the forested areas of northern and eastern Europe (Ersmark et al., 2019). After the last glacial maximum (LGM), mainly three clades of brown bears were observed to spread and expand from three separate refugia: the Iberian Peninsula, Italian-Balkan (Davison et al., 2011; Ersmark et al., 2019), and the Carpathian Mountains and Caucasus (Korsten et al., 2009; Saarma et al., 2007; Sommer & Benecke, 2005). The northern Eurasia brown bears (Finland, Estonia, and Western Russia) were founded probably in the Carpathian refugia after the LGM, and the population has expanded with several bottlenecks (Korsten et al., 2009; Saarma et al., 2007). More recent events on the population decline observed in Scandinavian brown bears, approximately 100 years ago, is because of intense hunting in the region (Xenikoudakis et al., 2015).

Calvignac et al. (2009) studied Middle East brown bears which are related to the Holarctic clade, which is today found in Eastern Europe, Türkiye, Japan, and North America. The closest relatives of Turkish and Syrian brown bears in Europe are Romanian brown bears (Calvignac et al., 2009). Turkish and Syrian samples demonstrate that Lebanese brown bears were recently separated from Western European brown bear populations by Eastern European populations (Calvignac et al., 2009). In Türkiye, three main clades can be found: the Western European clade, the Lebanese clade, and the Eastern clade also include Syrian brown bears.This demonstrates that European and Middle Eastern clades overlap in Türkiye and studies also show the presence of a novel divergent lineage with potential older antecedents (Çilingir et al., 2016).

2.4 The Genetic Structure of Bears

Given the significance of genetic diversity in preserving evolutionary potential and individual fitness, understanding the factors that affect the rate at which natural populations lose genetic diversity is a key component of conservation genetics. Large carnivores like brown bears, are usually dispersed at low densities and are extremely vulnerable to human-caused population fragmentation, raising concerns about the loss of genetic diversity (Paetkau et al., 1998). Therefore, understanding the genetic structure and genetic diversity of the species is highly important to determine its conservation strategies.

Studies have demonstrated that gene flow between brown bears and polar bears occurs in the direction of polar bears to brown bears due to brown bear admixture with male dominance (Barlow et al., 2018; Cahill et al., 2013; Liu et al., 2014; Wang et al., 2022). The genetic diversity of brown bears was historically higher at around 36 kya, especially in the Beringian brown bear populations, but it reached its current levels at around 15 kya (Leonard et al., 2000) and has remained relatively stable. These studies show that historical events like the LGM have had major effects on the diversity of and gene flow between polar and brown bears.

Ecuadorian Andean bears (*Tremarctos ornatus*) have very low genetic diversity, which is among the lowest ever recorded for a bear population, indicating that new conservation measures should be developed or existing ones should be revised (Cueva et al., 2018). Although Asiatic black bears (*Ursus thibetanus*) are widely distributed in mountainous areas and are regarded as vulnerable on a worldwide scale, they are not a high priority for conservation in Nepal, even though there are major threats from human-bear conflict, habitat fragmentation, and illegal hunting (Kadariya et al., 2018). Asiatic black bear populations indicated high genetic diversity; thus, the genetic analysis revealed no need for management actions to sustain genetic variety in these populations; nonetheless, human-bear conflict needs to be resolved if bears are to survive over the long run (Kadariya et al., 2018).

Another other study showed that although Himalayan brown bears (*Ursus arctos isabellinus*) living in Northern Pakistan have declined in population size during the last thousands of years due to human disturbance and glaciation, the Deosai population does

not indicate a reduction in genetic diversity and nor any inbreeding depression, probably because of gene flow between the surrounding populations (Bellemain et al., 2007).

Dinaric brown bears (*Ursus arctos*) in Croatia, one of the largest and most stable populations in Europe, exhibit higher nuclear genetic diversity than other current brown bear populations despite genetic evidence of a bottleneck brought on by past events (Kocijan et al., 2011). However, due to female philopatry and male-biased dispersal, mtDNA haplotype diversity was minimal in Dinaric brown bears, so the goal of management should be to keep the effective population size as large as possible while ensuring habitat connectivity with the surrounding populations (Kocijan et al., 2011). The Cantabrian brown bear population in Spain is under threat of very low genetic diversity, and the population is subdivided into two subpopulations with very small effective population sizes (Pérez et al., 2009).

Apennine brown bears comprise a small and isolated population living in the central Apennine mountains, Italy. Their population became completely isolated due to habitat fragmentation and farming activities (Benazzo et al., 2017). The accumulation of several deleterious mutations by genetic drift led to the loss of variation in this population; however, despite this loss of diversity several key genes associated with immune and olfactory receptors as well as changes in the tame/aggressive behavior and diet were found to be under positive selection (Benazzo et al., 2017; Lorenzini et al., 2004).

Brown bears used to live throughout most of the Middle East in historical times (McLellan, 2017). The populations from Egypt, Israel, Lebanon, and most recently, Syria, were driven to extinction (McLellan, 2017; Ridings, 2006). In Iraq, Iran, and Türkiye, where forest fragmentation and direct human persecution have led to population decreases over the past 50 years, brown bears manage to survive in small groups (Can & Togan, 2004). Despite these disturbances, the genetic diversity of Middle East brown bears remains high (Calvignac et al., 2009). Iranian brown bears are remarkably diverse and belong to a distinct group that is unrelated to any living bear species (Calvignac et al., 2009). Brown bears in the Caucasus belong to two genetically and geographically separate haplogroups that split apart from one another around the Last Glacial Maximum and contain bears from Eastern Europe and Asia (Murtskhvaladze et al., 2010). Genetic diversity analysis revealed that neither of the two brown bear subpopulations in Georgia had any signs of recent bottlenecks (Murtskhvaladze et al., 2010). Moreover, Çilingir et

al. (2016) indicated that in Türkiye there are three main clades originating from Europe, Iran, and Lebanon, resulting in high genetic diversity in Türkiye.

2.5 Polygenic Discrimination

The mechanisms that influence variance in wild populations can be improved by determining the genetic basis of phenotypic features and the individual genes mediating adaptation (Barson et al., 2015; Brieuc et al., 2015; Savolainen et al., 2013). With this knowledge, management and conservation efforts can be aided, and population responses to environmental change can be predicted (Bernatchez et al., 2017; Funk et al., 2012; Shafer et al., 2015). To understand the genetic architecture underlying polygenic traits, genome-wide association studies (GWAS) that look at polymorphisms throughout the genome are frequently performed. Comparing the genomes of several samples might reveal important details about various diseases (Yang et al., 2010), and GWAS can aid in identifying each individual's susceptibility to complicated diseases as well as their response to various medications based on genetic differences (Wang et al., 2005). High-throughput sequence data are now incredibly accessible and affordable thanks to the groundbreaking advances in next-generation sequencing technologies. The role of genome-wide association studies in clinical decision assistance for the diagnosis of complicated disorders is growing (Wang et al., 2005). GWAS studies often compare two groups of samples and typically read the single nucleotide polymorphisms (SNPs), which are markers showing the DNA sequence variation at a particular nucleotide site, rather than the full DNA sequence (Kruglyak & Nickerson, 2001). Thus, GWAS research aims to find complex relationships between various SNPs and environmental variables.

However, given the polygenic basis of the majority of phenotypic traits, genetic theory predicts that selection will lead to minor allelic changes among covarying loci rather than conspicuous changes at a few loci with substantial effects (Bourret et al., 2014; Le Corre & Kremer, 2012; McKay & Latta, 2002; Messer & Petrov, 2013; Pritchard et al., 2010). Therefore, when evaluating the genetics of complex traits such as life history and behavior we anticipate that evolution will result from the action of numerous genes spread across the genome, and for such traits, estimating polygenic effects should be given priority.

One of the most popular methods for identifying loci contributing to polygenic selection and discrimination is Random Forest (RF) (Breiman, 2001). It is a group of

trees that have been built from a training set of data and internally validated to produce a forecast of the response given the predictors for future observations. The application of RF to genomic investigations in order to identify the loci driving both discrete and quantitative features, especially when studying wild or non-model organisms has gained importance (Brieuc et al., 2018). In recent GWAS studies, SNP-SNP interaction discovery because of its inherent capacity to take many SNPs into account simultaneously in a nonlinear manner (Bao et al., 2005; Barenboim et al., 2008; Chen et al., 2007; Meng et al., 2009; Wang et al., 2005). It is possible to find significant SNP subsets linked to the outcome variables using the feature importance derived from RF.

Recent years have seen a rise in studies using RF for genetic analyses in evolutionary studies. As an illustration, RF was used to examine the relationships between the loci underlying Sitka Spruce's budset timing and cold resilience (Holliday et al., 2012). Similar to this, RF technology was used to identify loci responsible for white spruce trees' genetic adaptability to temperature and precipitation (Hornoy et al., 2015). During domestication, the brain and neural development genes have a polygenic basis (Carneiro et al., 2014). To study patterns of adaptive divergence of a crucial life history feature amongst populations of Chinook salmon, Brieuc et al. (2015) combined RF with FST outlier studies. Waters et al. (2018) investigated the effectiveness of gene flow in lowering domestication selection in a population improvement program utilizing trait-linked markers, discovered by RF, in the same species. In a panmictic species, repeated within-generation selection has been demonstrated to occur using markers associated to American eel ecotypes (Pavey et al., 2015), and loci that distinguish between eels in polluted and unpolluted settings have been found to covary with particular contaminants (Laporte et al., 2016). Additionally, RF has been used in related research to track invasion pathways (Fraimout et al., 2017) and select marker panels for population assignment (Sylvester et al., 2018).

Determining loci contributing to polygenic discrimination between traits can also be useful in genetic assignment methods where the aim is to assign unknown individuals into phenotypic or ecotypic groups based on genotype data (Remais et al., 2011). Genetic assignment of unknown individuals into phenotypic classes can be especially useful when working with species like wild carnivores which rely on non-invasive sampling

techniques and can be used to discriminate between local populations and individuals (Anderson, 2010; Pritchard et al., 2000; Wang, 2017; Waples & Gaggiotti, 2006).

Identification of the source populations from which immigrants originated and the capacity to distinguish between residents and immigrants can help with species management and conservation (Manel et al., 2005). The use of genetic markers has enhanced the understanding of population-level phenomena, such as life history variation (Gibbs et al., 1990; Koehler et al., 2008; Ross, 2001) and population dynamics (Hardesty et al., 2006; Hellberg et al., 2002). However, the methods currently in use for combining genetic data with information from other markers are insufficient and prevent us from fully understanding population structure and demography. The main idea behind them is to assign reference populations as the origins of individuals using multi-locus genotypes (Piry et al., 2004). One of the major limitations of the genetic assignment method is the inability to thoroughly assess a dataset's ability to Another one is the difficulty of effectively interpreting the resulting high-dimensional data and integrating diverse marker types (Bradbury et al., 2008; Chabot et al., 2012; Perrier et al., 2011; Smith & Campana, 2010). Large genomic datasets can be reduced in dimensionality using several techniques that use discriminant analysis of principal components (Jombart et al., 2010). However, these tools do not make it simple to combine genetic and non-genetic data or to separate PCs by data type (Jombart, 2008; Jombart & Ahmed, 2011). Numerous features are available in the assignPOP package in R (Chen et al., 2018) including a function to combine genetic and non-genetic data, principal component analysis (PCA) to reduce data dimensionality, resampling cross-validation to estimate assignment accuracy and membership probability, and a number of machine-learning classification algorithms to create adaptable predictive models (Chen et al., 2018) . All individuals from reference populations were separated randomly into training and test groups, and the assignment accuracies were calculated using Monte-Carlo cross-validation (Gajdárová et al., 2021). Therefore, genetic assignment analysis could be used to differentiate the sedentary and migratory individuals.

2.6 Kinship Relationships

Understanding behavioral changes and their evolution in wildlife species is extremely difficult because observing behavior across the course of an individual's lifetime and across generations is usually not possible. Despite this, such information can

have significant implications for management and conservation (Morehouse et al., 2016). Finding mates, choosing acceptable habitats, and finding adequate food are all essential for an animal to survive and reproduce. Such behaviors can be learned by social and asocial interactions, passed on by inheritance, or result from a combination of both (S. J. Arnold, 1981; Galef & Laland, 2005; Hopkins, 2013; Laland, 2004).

Learning has been linked to large brain size, developed memory, opportunism, curiosity, and behavioral plasticity in studies of both captive and wild animals (Giraldeau & Lefebvre, 1996; Mazur & Seher, 2008; Pokrovskaya, 2015). Bears are suited to social learning since they exhibit all these characteristics as well as a high level of mother investment in their offspring (Gilbert, 1999; Gittleman, 1986). Grizzly bears are rather asocial as adults, but because they normally remain with their mothers for 2-3 years (McLellan, 1994), cubs have the chance to learn social skills from their mothers. There is some evidence that a mother's behavior and the environments in which she raised her cubs have an impact on a cub's behavior (Hopkins, 2013; Mazur & Seher, 2008). Bears are opportunistic and adaptable foragers (Bojarska & Selva, 2012; Gilbert, 1999), so I might not anticipate strong evidence for social learning given the variety of options available to meet nutritional needs, especially in human-populated areas where anthropogenic resources are easily accessible (Beckmann & Berger, 2003a, 2003b; Breck et al., 2008). Therefore, the changes in the behavioral trait could be heritable and affect the fitness of the populations.

One way to understand the relative impact of learned vs inherited characteristics is to understand kinship patterns across behavioral types and to ascertain whether kinship is higher within different behavioral types. Traditionally calculating the degree of relatedness between individuals was based on pedigree information which can be extremely difficult to obtain from natural populations. Recently genomic data has enabled us to calculate the likelihood of identity-by-descent (IBD) of any genetic marker between two individuals, and when calculated across the genome, this matrix can enable researchers to calculate the coefficient of relatedness between any two individuals. Therefore genome-wide data is a powerful tool for understanding kinship relationships of individuals and populations in the wild and to tease apart learned vs inherited components of complex traits potentially.

Overall, this thesis aims to present the demographic and genetic structure, diversity, and adaptive variation in the Eastern Anatolian brown bear population and between migratory and sedentary bears and to understand the possible evolutionary spread of sedentary behavior as a response to anthropogenic food sources. The ability to predict which life history strategy will prevail in the future and the overall effects of anthropogenic pressures on wild carnivores compelled to live in human-dominated landscapes depends on an understanding of the demographic and genetic processes that have resulted in the establishment of these life-history strategies.

The results of the study will be also used to develop new biological conservation strategies that take into account the differences in habitat use of bears that have adopted two different life-history strategies and minimize bear-human conflicts in the region.

CHAPTER 3:
AIM AND RATIONALE OF THE THESIS

Bears often utilize nutrient resources (household or city dumps, beehives, domestic animals, farms, campsites, etc.) resulting from human activities. As a result of these opportunistic behaviors, bears are habituated to both anthropogenic food sources and to human habitats (Cozzi et al., 2016; De Angelis et al., 2021; de Gabriel Hernando et al., 2020). It has been frequently suggested in the literature that new life strategies towards a more "domestic" lifestyle in bears may emerge (Dahle & Swenson, 2003; Penteriani et al., 2021) as a result of this ongoing process. The existence of a natural brown bear population in Eastern Anatolia, in which some individuals are primarily established in and around a large open city dump and utilize it as predominant food source along with migratory bears that do not utilize the dump and actively forage in search of food presents a unique opportunity to study this phenomenon. Therefore, the outcomes of this thesis will be the first test of a hypothesis that has long been debated in the world literature but cannot be tested due to the lack of relevant natural populations or sufficient sampling of these populations.

This thesis aims to determine the genetic responses of brown bears in Eastern Türkiye to human dominated landscapes and to understand the genetic architecture of the observed shift in life histories as a result of the abundance of anthropogenic food sources. Using genome wide data from 31 bears tagged with radio collars and monitored for one year I determined genetic and adaptive variation within the East Anatolian population as well as that associated with the two life-history strategies. Our findings indicated that although the Eastern Anatolian brown bear population is genetically distinct from other world populations, it still has high genetic diversity and mixed ancestry. Historical demography showed a recent dramatic increase in the effective population size of the bear population in Eastern Anatolia while relatedness analysis showed higher coefficient of kinship and sib-ship in sedentary bears utilizing the garbage dump. Additionally, I identified a number of genomic regions, unique genotypes, and loci with signals of significant positive selection associated with sedentary and migratory

behavior. Furthermore, polygenic discrimination studies revealed 464 SNPs distributed throughout the genome actively contributing to foraging behavior. Gene ontology studies showed that these 464 SNPs were associated with 512 genes that predominantly regulate pathways associated with sensory perception in smell.

Collectively our results indicate that adaptation to human-dominated landscapes in the Eastern Anatolian brown bear population might have a strong genetic basis and emphasizes the importance of evolutionary genomics for understanding how species survive and adapt to human-mediated change. Moreover, the outputs of this thesis can help in designing new biological conservation strategies that take into account the evolutionary and adaptive dynamics of the species and reduce human-bear conflicts arising from bears that are forced to live in human-dominated habitats.

CHAPTER 4:
MATERIALS AND METHODS

4.1 Study Area

The study area covers the Kars and Ardahan regions at the intersection of the Caucasus and Iran-Anatolian global diversity hotspots in Northeast Anatolia, Türkiye (Figure 4.1). The area comprises 550 km^2 area centered around 40°20'N and 42°35'E. The city of Sarıkamış is located in the center of the study area. Forest cover consists mostly of Scots pine (*Pinus sylvestris*), while understory vegetation is scarce. Sarıkamış-Allahuekber Mountains National Park boundaries cover a total area of 225.1 km^2, but only include 49.69 km^2 of the forest, so only composed of 22.07% forest cover. There are 328.38 km^2 of total forest cover in the area, including a large forest area south of the national park (248.15 km^2).

The primary human activities in the forest are animal grazing, the collecting of forest products (such as fruits, pine cones, and mushrooms), and both legal and illegal timber extraction. These activities are extensive in both time and space and are only restricted by the bitter winter temperatures. From April to November, cattle (Bos taurus), sheep (Ovis aries), and goats (Capra hircus) can be found freely grazing on rangelands in the area (Capitani et al., 2016). 3 km west of Sarkamış city an unfenced municipal waste dump is a noticeable aspect of the terrain. Bears, wolves, and wild boar use the dump at night, making it a predictable supply of anthropogenic food. (personal observation).

The history of the Sarıkamış city dump is important for understanding the demographic history of the Sarıkamış bear population. Sarıkamış human population was around 35.000 in 1927, reaching a peak in 1955 with up to 80.000 people (Saraçoğlu, 2011). In the last census in 2021, the population of Sarıkamış was around 39.000 people. During 1940s, the population increased to the highest level due to the military base built in Sarıkamış during the Second World War (Saraçoğlu, 2011). After the end of World War II, the military population here was initially reduced and Sarıkamış human population decreased in the beginning of the 1950s. By 1955, the population of Sarıkamış

reached a peak, likely due to the military buildup of the Cold War, in which Kars was the easternmost outpost, the transition to the multi-party period and the effects of economic development initiatives in Turkey on Sarıkamış (Saraçoğlu, 2011). There is not much information about Sarıkamış region population size before the founding of the Turkish Republic in 1923.Moreover, even if I knew the population size in 1920s, I cannot be sure the location of the city dump at those years. However, I can estimate that the city dump is likely to have been established in its current location in 1940s, due to the population increase associated with World War II and the Cold War. Therefore, the Sarıkamış dump may have been a food resource for brown bears for 70 to 80 years.

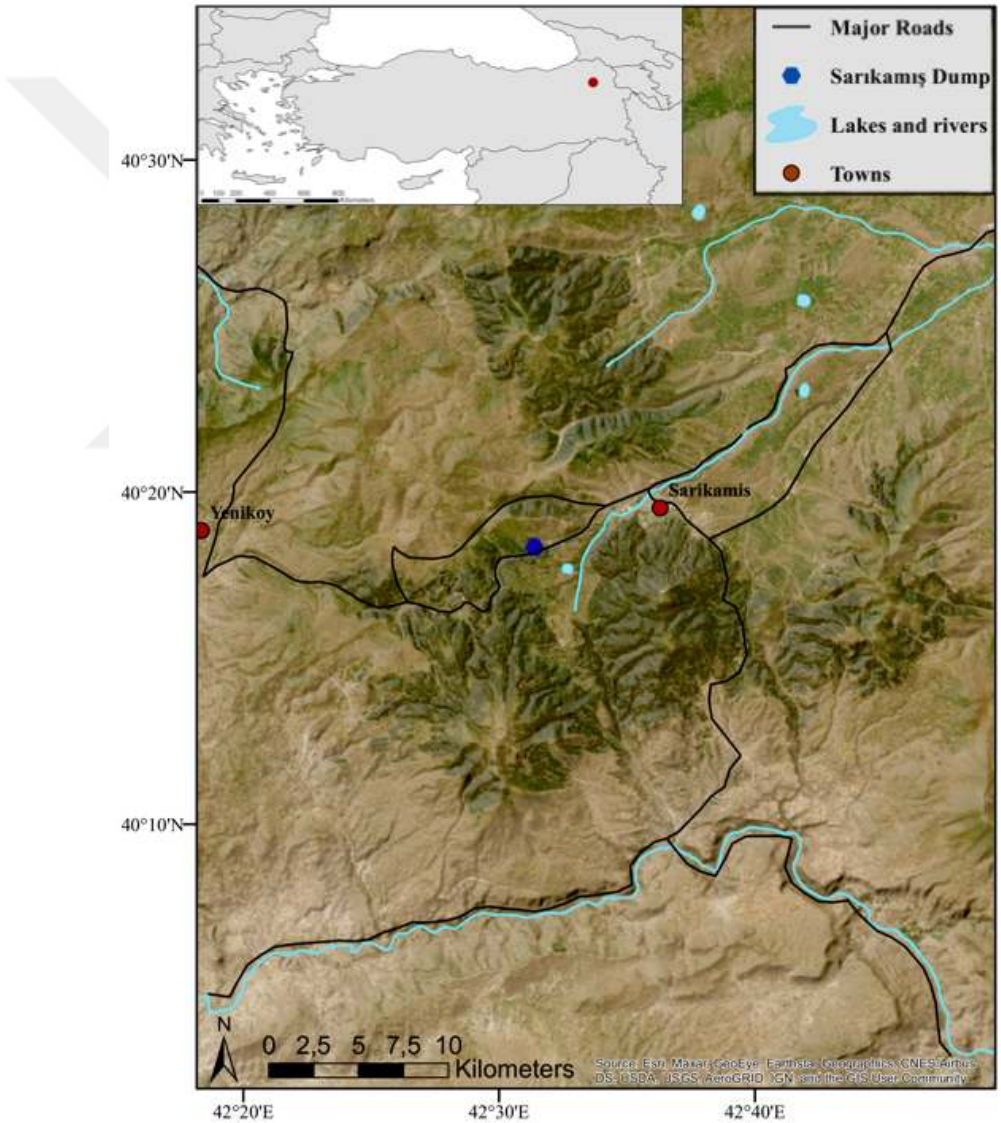

Figure 4.1 The study area in north-eastern Türkiye. Red dot is the center of Sarıkamış, Kars. Blue dot is the Sarıkamış dump.

4.2 Fieldwork and the collection of samples

Blood samples have been taken from captured animals. The Kuzey Doğa Association did the capturing and satellite-collaring as part of ongoing field surveys conducted in the region since 2012 (Table 4.1) (Akküçük & Şekercioğlu, 2016). Captured bears were immobilized and fitted with GPS/GSM or GPS/Iridium satellite-collars (GPS Plus; Vectronic Aerospace GmbH, Berlin, Germany) programmed to record one GPS location every hour. Before collaring, blood samples were taken from each bear, collected into EDTA tubes, and immediately frozen at -20°C prior to transport and DNA purification. Until 2019, 61 brown bears were captured and all information is given in Appendix A. I was able to obtain blood samples from 56 of the 61 captured animals and 31 were successfully tracked up to one year using GPS/GSM or GPS/Iridium radio-collars.

Table 4.1 Brown bears captured at Sarıkamış, Kars, Türkiye. ID, sex and capture dates are indicated.

Animal ID	Sex	Capture date	Animal ID	Sex	Capture date
BTR001	F	18.09.2012	BTR032	F	
BTR002	F	20.09.2012	BTR033	M	4.06.2017
BTR003	M	19.09.2012	BTR034	M	6.06.2017
BTR004	M	-	BTR035	M	-
BTR005	M	22.09.2012	BTR036	F	27.06.2017 / 29.06.2018
BTR006	M	25.09.2012	BTR037	F	28.06.2017
BTR007	M	26.09.2012	BTR038	M	29.06.2017
BTR008	M	29.09.2012	BTR039	M	30.06.2017
BTR009	F	1.10.2012	BTR040	F	4.07.2017
BTR010	F	4.10.2012	BTR041	F	20.07.2017
BTR011	M	5.10.2012	BTR042	M	16.06.2014
BTR012	M	11.06.2013 / 29.05.2013	BTR043	M	27.05.2018
BTR013	F	30.05.2013	BTR044	M	3.06.2018
BTR014	M	1.06.2013	BTR045	M	7.06.2018
BTR015	F	9.06.2013	BTR046	F	10.06.2018
BTR016	M	22.05.2014	BTR047	F	11.06.2018
BTR017	F	27.05.2014 / 25.06.2018	BTR048	M	13.06.2018
BTR018	M	28.05.2014	BTR049	M	30.06.2018
BTR019	M	30.05.2014	BTR050	F	11.07.2018
BTR020	M	30.05.2014	BTR051	M	01.06.2019
BTR021	F	2.06.2014	BTR052	F	07.06.2019

BTR022	M	5.06.2014	BTR053		09.06.2019
BTR023	F	6.06.2014	BTR054	M	10.06.2019
BTR024	F	6.10.2016	BTR055	M	13.06.2019
BTR025	M	16.10.2016	BTR056	M	13.06.2019
BTR026	M	16.10.2016	BTR057	M	15.06.2019
BTR027	F	15.10.2016	BTR058	F	2.07.2019
BTR028	M	26.05.2017 / 1.06.2017	BTR059	M	19.07.2019
BTR029	M	27.05.2017	BTR060	M	19.07.2019
BTR030	F	29.05.2017	BTR061	M	20.07.2019
BTR031	F	30.05.2017			

4.3 DNA Extraction

DNA was extracted from blood samples using the QIAamp DNA Blood Mini Kit following the manufacturer's protocol. The G10P primer pair was used to validate bear specific DNA in samples. The presence of bear DNA was determined by assessing the Cq values of each sample after qPCR amplification with the G10P primer pair (Paetkau & Strobeck, 1998).

4.4 Movement Behavior

For the 31 captured and satellite-collared bears, the average duration of monitoring was 370 days (range: 126–726 days). GPS locations with a position dilution of > 10 were removed from the dataset to avoid inaccurate position information (Elliot et al., 2014). In addition, I only used position data gathered pre- and post-individual hibernation (interquartile range: November 23rd–December 3rd to March 6th–April 1st) because GPS often fails to receive satellite data during the winter when bears hibernate in dens and caves. There is no bias between tracking days for sedentary and migratory bears (unpaired t-test, p-value=0.38). To calculate the maximum distance traveled by each individual, I determined the two most distant GPS locations in each day by taking the mean of the GPS coordinates for each individual and calculating the distance between these points. From this information, I calculated net squared displacement (NSD) (Bunnefeld et al., 2011) for each individual by measuring the square of the distance from the animal's starting point to successive daily locations. The NSD statistics are integrated with a nonlinear hierarchical modeling framework in this analytical method (Börger & Fryxell, 2012). Later NSD curves were used to classify each individual bear into migrant or sedentary categories using a model-based approach implemented in migrateR package

(Spitz et al., 2017) in R program version 3.6.2 (Team, 2019). The migrateR package fits each observed NSD curve into five a priori non-linear models, representing year-round residency, (2) nomad, (3) disperser, (4) migrant, and (5) mix-migrant movement behavior (Bunnefeld et al., 2011).

4.5 Sequencing, and mapping

To produce a high-quality genomic resource, I sampled the genome using RAD sequencing (Baird et al., 2008; Etter et al., 2012; Miller et al., 2007). Paired-end 150 bp sequence reads were generated for the 56 individuals using the Sfb1 restriction enzyme and the RAD protocol described in (Ali et al., 2016). Sequencing was carried out on the Illumina HiSeq 2500 platform, and resulting reads were sorted into individuals as matching forward and reverse fastq files using unique 8 bp barcodes.

Sequenced reads were aligned to the chromosome length assembly of the American black bear genome (Dudchenko et al., 2017; Dudchenko et al., 2018; Srivastava et al., 2019) using the BWA-MEM algorithm (Li & Durbin, 2009), which had the most complete assembly among available bear genomes. Aligned reads were outputted as bam files and indexed after sorting for proper pairs and removing PCR duplicates in samtools (v1.2) (Li et al., 2009). In addition, potential paralogous regions were marked using ngsParalog (Linderoth, 2018) and removed from further analysis.

4.6 Genotyping and SNP discovery

All analyses were conducted using the probabilistic framework in ANGSD (Korneliussen et al., 2014), which directly works with genotype likelihoods and does not require genotype calling, making it ideal for working with low depth data (Fumagalli et al., 2013; Korneliussen et al., 2014). Using ANGSD, I calculated genotype likelihoods (-GL 1) and probabilities (-doGlf 2), minor allele frequencies (-doMaf 1), and determined polymorphic sites along with the major and minor allele by screening for sites with a minor allele frequency (MAF) of over 0.05 and a significance threshold of $P<10^{-06}$ (-doMaf 1; -doMajorMinor 1; -SNP_pval 1e-06). Furthermore, I removed sites that did not meet a minimum quality score of 20 (-minQ 20) and mapping quality of 10 (-minMapQ 10) and filtered out any site that did not have an average per individual read depth of 6 and was not present in at least half of the individuals (-setMinDepth 220; -minInd 15).

4.7 Genetic Structure and Diversity

To determine the genetic structure of Eastern Anatolian brown bears in relation to worldwide brown bear populations, I performed a PCA analysis. Whole-genome sequences of brown bears from Georgia, Russia, Greece, Slovakia, Slovenia, Greece, Austria, and Alaska (Barlow et al., 2018; Benazzo et al., 2017; Cahill et al., 2013) downloaded from the Sequence Read Archive (SRA) at NCBI (Table 4.2) and aligned to the American black bear genome (Srivastava et al., 2019). Genotype likelihoods and polymorphic sites were calculated for overlapping regions within the whole data set as described above. PCA was conducted by first estimating a genetic covariance matrix between individuals based on genotype likelihoods in PCAngsd (Meisner & Albrechtsen, 2018) and later by classical eigenvalue decomposition of the derived matrix in R version 3.6.2 (Team, 2019).

To determine the genetic ancestry of Eastern Anatolian brown bears and shared ancestry between world populations, I performed admixture analysis for different numbers of clusters (K=2-8). Admixture proportions of individuals were estimated in NGSadmix (Skotte et al., 2013) based on genotype likelihoods calculated in the previous step. To avoid convergence to local optima, NgsAdmix was run 30 times per K value, and likelihoods from each run were used to calculate the most likely K value given Evanno's method (Evanno et al., 2005).

Table 4.2 Samples that their genome sequences downloaded from National Center for Biotechnology Information (NCBI). The references from the previously published studies and their SRA accession numbers are indicated.

Sample code	Species	Locality	Sex	Reference	Accession	Population in the study
APN1	*U. arctos*	Central Italy	F	Benazzo et al. (2017)	SRR5878350	APN
APN2	*U. arctos*	Central Italy	M	Benazzo et al. (2017)	SRR5878343	APN
APN3	*U. arctos*	Central Italy	F	Benazzo et al. (2017)	SRR5878342	APN
APN4	*U. arctos*	Central Italy	M	Benazzo et al. (2017)	SRR5878345	APN
APN5	*U. arctos*	Central Italy	F	Benazzo et al. (2017)	SRR5878344	APN
GRE1	*U. arctos*	Greece	M	Benazzo et al. (2017)	SRR5878355	EUR
GRE2	*U. arctos*	Greece	M	Benazzo et al. (2017)	SRR5878351	EUR
SLK	*U. arctos*	Slovakia	M	Benazzo et al. (2017)	SRR5878349	EUR

SLO	*U. arctos*	Hrusica, Slovenia	untested	Barlow et al. (2018)	ERR2678638	EUR
GEO	*U. arctos*	Georgia, Great Caucasus	untested	Barlow et al. (2018)	ERR2678639	GEO
RUS	*U. arctos*	Balakhtinsky District, Russia	untested	Barlow et al. (2018)	ERR2678640	RUS
AK1	*U. arctos*	Admiralty Island, Alaska	F	Cahill et al. (2013)	SRR830213	AK
AK2	*U. arctos*	Denali National Park, Alaska	F	Cahill et al. (2013)	SRR830337	AK
UM1	*U. maritimus*	Lancaster Sound	M	Cahill et al. (2013)	SRR827588	UM
UM2	*U. maritimus*	Wrangel Island	F	Cahill et al. (2013)	SRR827587	UM
UM3	*U. maritimus*	North Beaufort Sea	M	Cahill et al. (2013)	SRR827585	UM
UM4	*U. maritimus*	Chukchi Sea	M	Cahill et al. (2013)	SRR827584	UM
UM5	*U. maritimus*	North Beaufort Sea	M	Cahill et al. (2013)	SRR827600	UM
UM6	*U. maritimus*	West Hudson Bay	M	Cahill et al. (2013)	SRR827574	UM
UM7	*U. maritimus*	West Hudson Bay	F	Cahill et al. (2013)	SRR827537	UM

To summarize the genetic diversity of Eastern Anatolian brown bears compared to its conspecifics around the world, I calculated Watterson's theta (θw) estimated using the global site frequency spectrum (SFS). Watterson's theta is estimated by counting the expected number of polymorphic sites. The SFS of each population was estimated in ANGSD (-doSaf 1) and polarized as derived and ancestral based on the allelic states in the polar bear genome (Cahill et al., 2013). The estimated SFS was then used as a prior for calculating θw in 50-kb overlapping windows, with a step size of 25-kb using the thetaStat module in ANGSD (do_stat -win 50000 -step 25000). To reduce biased estimates, I eliminated any scaffold shorter than 25 MB and excluded windows with a number of sites less than 200. To eliminate the effect of sample size differences between the populations, I selected randomly five unrelated individuals from Eastern Anatolian brown bear population and calculated Watterson's theta by applying the same parameters forementioned.

4.8 Effective Population Size Estimation

In order to determine changes in population size over time, I used the extended Bayesian skyline plot (EBSP) (Heled & Drummond, 2008) approach in BEAST version 2 (Bouckaert et al., 2019). To this end, using custom scripts, I first discovered all RAD loci ranging between 300-800 bp, which are suitable for phylogenetic analysis. This resulted in a pool of 22,266 unique RAD loci. From this pool of RAD loci, I selected loci that had a high number of SNPs (around 6 per locus) to maximize the amount of information per marker and to maximize the accuracy and reliability of our inferences. From the resulting 338 RAD loci, I randomly selected 50 RAD loci to use in the analysis due to the computational limitations of the EBSP approach, which cannot deal reliably with high numbers of loci. I assigned a single mutation model (HKY) and strict clock to all loci but trees remained unlinked across loci. The mutation rate was set to $1.16*10^{-9}$ mutations per site per generation and generation time to 11.35 years (Benazzo et al., 2017; Liu et al., 2014). Default operator values were modified to increase mixing as suggested in Trucchi and Cristofari . I ran six independent MCMC analyses each with a chain length of 200 million generations, sampling every 20000 generations, and discarded the first 2 million generations as burn-in. Independent runs were conducted to ensure convergence and combined using Tracer (Rambaut et al., 2018) to increase ESS values to over 100 for all important parameters (Saarma et al., 2007).

4.9 Genome-Wide Association Study (GWAS)

I used the score statistics (-doAsso 4) implemented in ANGSD (Korneliussen et al., 2014) which has good statistical power even with lower coverage data, to detect the association between genotype and movement behavior (i.e., sedentary vs migratory). This test is based on genotype probabilities and uses a generalized linear framework to calculate likelihood scores for each SNP individually (Skotte et al., 2012). Association mapping was conducted by determining polymorphic sites (SNP_pval 1e-06), inferring major and minor alleles (doMajorMinor 1), calculating allele frequencies (doMaf 1), and retaining SNPs with a minor allele frequency over 5% (minMaf 0.05) by considering populations separately. I also filtered out any site that did not have at least ten numbers of homozygous major, heterozygous and homozygous minor genotypes (-minHigh 10). SNPs that passed all filters were assessed for significance using the likelihood ratio test (LRT) at a genome-wide significance threshold of $4.09x10^{-6}$ ($\alpha = 0.05$; 12,207 SNPs; LRT

> 21). I removed one individual (BTR36) from association mapping because of unambiguous movement behavior due to insufficient GPS data.

To determine whether any of the top associated SNPs showed extreme genetic differentiation either in sedentary or migratory bears, I compared population branch statistic (PBS) (Yi et al., 2010) scores at these sights against the mean PBS score calculated across the 37 scaffolds. PBS analyses were based on comparisons between migratory, sedentary, and European (Greece, Slovenia, and Slovakia) bears. I ran two separate PBS analyses treating migratory and sedentary bears as the focal population, in turn, allowing us to determine extreme differentiation unique to either behavioral type, which could be candidates for positive selection in these populations. PBS analyses were conducted in ANGSD (Korneliussen et al., 2014) by first estimating pairwise FST values between each population and then using these as input into the realSFS module to calculate PBS values across the 37 scaffolds in 20 bp overlapping windows with a step size of 5 bp.

Genotypes were called in ANGSD (-doGeno 4) using a population prior (-doPost 1) and a posterior probability cutoff of 90% (-postCutoff 0.9), and I used allelic states in the American black bear and polar bear genome (Cahill et al., 2013) to determine ancestral and derived states.

4.10 Polygenic Discrimination

To determine all SNPs contributing to the difference in behavior (i.e., polygenic discrimination), I performed a random forest (RF) analysis, which is a flexible method based on ensembled learning and decision trees (Breiman, 2001). Advantages of RF compared to other statistical classifier methods are very high classification accuracy, a novel method of determining variable importance, flexibility to perform several types of statistical data analysis and the ability to model complex interactions among predictor variables (Cutler et al., 2007). RF analysis was run using the R package randomForest (Liaw & Wiener, 2002) and was only applied to individuals with known behavior. Two thirds of the dataset was used to determine the associations between SNPs and behavior (i.e., sedentary vs. migratory), and then the remaining one third ("out-of-bag") was used to assess the models' predictive power to correctly assign individuals' behavioral types.

There are different main parameters of RF to be considered while creating the trees based on the data. The training data set (sampsize parameter), from which the tree is grown, is initially randomly sampled by the algorithm with replacement. Then, using the mtry parameter, RF randomly chooses a collection of predictors and looks for the predictor that best divides the response variable in the training data set. To give each candidate predictor adequate chances to be chosen, the number of trees in the forest should generally increase as the number of candidate predictors does (Boulesteix et al., 2012). Finding the predictor that minimizes the within-group variance (a categorical response variable) or error when regressed against the response is the foundation of partitioning (continuous response variable). The change in the tree's predictive power after each predictor has been randomly permuted across individuals is used to measure each predictor's significance. When a significant predictor is permuted, a tree's ability to predict the future will be lowered, but the accuracy of the tree won't be affected. In order to create a forest, many trees must be planted (ntree parameter); the significance values of the predictors are then averaged over the trees and examined in order to identify which ones best account for variation in the response variable (Brieuc et al., 2015; Brieuc et al., 2018).

Prior to analysis, I called genotypes in ANGSD (-doGeno 4) using a population prior (-doPost 1) and a posterior probability cutoff of 90% (-postCutoff 0.9). This resulted in 62,286 SNPs with high confidence genotype information to be used in random forest analysis. Then, I determined the best ntree parameter based on the least out-of-bag (OOB) error rates with different ntree parameters (500, 1000, 2000, 4000, 8000, 10000, 20000) based on 10 replicates. After the best ntree was determined, I searched for the best mtry values with 2,4,8,16,32, and 64 values. Using the best values of mtry and ntree, I ran randomForest function using R package randomForest (Liaw & Wiener, 2002) five times and recorded mean-decrease accuracy (MDA) values for each site. All SNPs with an MDA importance<0 were removed as non-discriminatory. After removal, I continued the same analysis with the remaining sites. I re-implemented RF run until OOB error rate reaches a minimum level (3.33% error). This resulted in 7 consecutive RF runs and gave us a final data set of 464 SNPs with high predictive and classification power.

For these selected 464 SNPs, derived allele frequencies (DAF) were calculated separately for migratory and sedentary individuals. The heatmap of the allele frequencies

for each behavioral type were generated with the 'heatmap.2' function of the 'gplots' R package (Warnes et al., 2009).

To determine the possible life history strategies of unknown individuals and to test the classification performance of the discovered SNPs, I performed a genetic assignment test, using the assignPOP package in R (Chen et al., 2018). All SNPs obtained from RF analysis were given as prior to the genetic assignment analysis. I ran and compared five different assignment methods all based on a Monte-Carlo framework: 1) linear discriminant analysis (LDA), 2) support vector machine (SVM), 3) naive Bayes, 4) decision tree, and 5) random forest. I set the maximum assignment accuracy at 97% and calculated assignment accuracy together with mean and standard deviation values for each model (Appendix C). Each model was tested using of the 464 SNPs and the 26 individuals with known behavior, and if the model correctly assigned individuals to their respective behavior class with 97% accuracy, I used the model to further classify individuals with unknown behavior to behavioral classes using the assign.X function in the assignPOP package in R (Chen et al., 2018).

4.11 Gene Ontology

To determine the associated genes and pathways for the 464 SNPs discriminating between sedentary and migratory bears, I conducted a gene ontology analysis. First, I scanned the gene annotation of the American black bear genome (Dudchenko et al., 2017; Dudchenko et al., 2018; Srivastava et al., 2019) to determine the SNPs that fell within a coding region or specific gene. After the genes were identified, I determined their human Uniprot IDs and using 'gprofiler2' package in R (Kolberg et al., 2020), I determined functional pathways and enrichment scores for the given gene list. I also used WebGestalt (Web-based Gene Set Analysis Toolkit) (Liao et al., 2019) to perform gene enrichment analysis with *Homo sapiens* as organism of interest, and geneontology and pathways (KEGG and Wikipathways) as functional database by setting FDR<0.05 as the cut-off criteria.

4.12 Relatedness

Relatedness of all 56 Eastern Anatolian brown bears was determined using NgsRelate (version 2) (Hanghøj et al., 2019). NgsRelate calculates the genetic kinship coefficient (θ) the likelihood that two randomly selected alleles from different individuals

are both identical-by-descent (IBD) between individuals using genotype probabilities and hence is suited for low-coverage genomic data. The kinship coefficient is based on the Cotternamn coefficients (K0, K1, and K2), which describe the probability that two individuals will share 0, 1, or 2 copies of an allele that are identical by descent at each site. Following this, the kinship coefficient can be calculated as $\theta = k1 / 4 + k2 / 2$. The relationship between two haploid genomes determines zygosity (i.e., whether an individual is heterozygous or homozygous) (Ackerman et al., 2017).

Using ANGSD (Korneliussen et al., 2014), I first calculated the genotype likelihoods (-GL 1) and probabilities (-doGlf 3), determined polymorphic sites along with the major and minor alleles by screening for sites with a minor allele frequency (MAF) of over 0.05 and a significance threshold of $P<10^{-06}$ (-doMaf 1; -doMajorMinor 1; -SNP_pval 1e-06) and I removed sites that did not meet a minimum quality score of 20 (-minQ 20) and mapping quality of 10 (-minMapQ 10) and filtered out any site that was not present in at least half of the individuals (-minInd 28) by considering the Eastern Anatolian population as one. The resulting genotype file contained 121252 variable sites, which were used as input for NgsRelate. For each pairwise comparison between individuals, I calculated the k0, k1, and k2, values, and from these calculated the θ statistic and zygosity, ρ. Table 4.3 shows the expected distribution of the Cotterman and kinship coefficients for 56 individual pairs and was used to classify relationships between our individuals. The Cotterman coefficients indicate the expected values from NgsRelate (Hanghøj et al., 2019), while kinship coefficients demonstrate the intervals related to Cotterman coefficients.

Table 4.3 Relatedness coefficient distributions for 56 samples were obtained from NgsRelate (Hanghøj et al., 2019).

Relationship	**K0**	**K1**	**K2**	**θ**
Mono-zygotic twin	0	0	1	0.45-0.5
Parent-Offspring	0	1	0	0.2-0.25
Full siblings	0.25	0.5	0.25	0.2-0.25
Half siblings	0.5	0.5	0	0.05-0.18
First cousins	0.75	0.25	0	0.05-0.1
Unrelated	1	0	0	0

CHAPTER 5:
RESULTS

5.1 Sample collection and DNA extraction

Between 2012 and 2019, I took blood samples from 57 out of 61 captured brown bears (Appendix A). The DNA from all of the blood samples was extracted successfully.

5.2 Movement Behavior

Based on NSD patterns, out of the 31 captured and satellite-collared bears, 15 were classified as resident, four as migrant, nine as mix-migrant, and two as nomadic. None of the individuals were classified as dispersers (Table 5.1). Following Cozzi et al. (2016), and to simplify the genetic analysis I grouped nomad and resident bears into a single category called "sedentary" and migrant and mix-migrants into a second category called "migratory" (Table 5.1). Out of the 16 individuals modeled for movement patterns in Cozzi et al. (2016), I converged on the same results for 12 of them (Table 5.1). Two individuals (BTR009 and BTR018) classified as migratory in Cozzi et al. (2016) were re-classified as sedentary as these bears showed no evidence of leaving the Sarıkamış forest. For the remaining two individuals (BTR016 and BTR019), GPS data were not complete in Cozzi et al. (2016) and thus were not classified previously. To conclude, our analysis of NDS patterns re-affirms the presence of two different life history strategies (sedentary vs. migratory) in Eastern Anatolian brown bears.

Table 5.1 Individuals that were captured and GPS-collared and tracked for the movement behaviors since 2012. The movement mode of the samples are indicated for this study, Cozzi et al., 2016.

Sample	Movement	Movement model in	Movement Mode (Cozzi et al.,
BTR002	Migratory	Migrant	Migratory
BTR007	Migratory	Migrant	Migratory
BTR012	Migratory	Migrant	Migratory
BTR013	Migratory	Mix-Migrant	Migratory
BTR016	Migratory	Mix-Migrant	Sedentary
BTR017	Migratory	Mix-Migrant	Migratory
BTR019	Migratory	Migrant	Nomadic

BTR029	Migratory	Mix-Migrant	-
BTR034	Migratory	Mix-Migrant	-
BTR049	Migratory	Mix-Migrant	-
BTR056	Migratory	Mix-Migrant	-
BTR058	Migratory	Mix-Migrant	-
BTR060	Migratory	Mix-Migrant	-
BTR003	Sedentary	Resident	Sedentary
BTR005	Sedentary	Resident	Sedentary
BTR009	Sedentary	Nomad	Migratory
BTR014	Sedentary	Resident	Sedentary
BTR015	Sedentary	Resident	Sedentary
BTR018	Sedentary	Resident	Migratory
BTR020	Sedentary	Resident	Sedentary
BTR022	Sedentary	Resident	Sedentary
BTR023	Sedentary	Resident	Sedentary
BTR031	Sedentary	Resident	-
BTR042	Sedentary	Resident	-
BTR043	Sedentary	Resident	-
BTR045	Sedentary	Resident	-
BTR048	Sedentary	Resident	-
BTR052	Sedentary	Resident	-
BTR054	Sedentary	Resident	-
BTR057	Sedentary	Nomad	-
BTR036	Undetermined	Resident	-

5.3 Sequencing

Average mapping success of RAD sequences obtained from the 56 samples to the American Black Bear genome was 99% and even after filtering out duplicate reads I still retained over 86% of the reads (Table 5.2) which indicated the mean of 1.7 million reads with 1.3 million read standard deviation for the number of aligned reads after filtered out all duplicates. I conclude that the mapped RAD contigs serve as a high-quality genome wide panel for SNP discovery and genotyping of Eastern Anatolian brown bears.

Table 5.2 Samples collected from the fieldwork at Sarıkamış, Kars, Türkiye. Indicated individuals were captured, GPS-collared and tracked for their movement behaviors since 2012.

Sample ID	Sex	Age	Capture Date	Tracking days	No of raw reads	No of raw aligned reads	No of duplicates removed aligned reads
BTR002	F	6-7	20.09.2012	186	6546068	6512174	5035992
BTR007	M	4	26.09.2012	387	1367904	1366086	598766
BTR012	M	10	29.05.2013	262	647512	646441	285751
BTR013	F	8-10	30.05.2013	378	2787038	2787058	1221008
BTR016	M	8	22.05.2014	297	2304848	2223257	981103
BTR017	F	6-10	27.05.2014-25.06.2018	652	3334394	3336143	1444531
BTR019	M	5	30.05.2014	290	4984962	4961392	3882910
BTR029	M	4	27.05.2017	511	10441852	10446312	4356768
BTR034	M	4	6.06.2017	168	1015230	1013681	448299
BTR049	M	4	30.06.2018	580	2021684	2022356	890344
BTR056	M	4	13.06.2019	581	2375174	2358160	1882694
BTR058	F	8	02.07.2019	326	2609184	2594049	2076601
BTR060	M	6	19.07.2019	484	3689308	3670024	2890088
BTR003	M	5	19.09.2012	410	5603392	5580074	4318888
BTR005	M	8-9	22.09.2012	161	9397768	9410257	3916377
BTR009	F	15-20	1.10.2012	213	3781342	3783550	1636292
BTR014	M	10-12	1.06.2013	173	1625824	1617602	1307130
BTR015	F	12-14	9.06.2013	343	1566146	1566864	694444
BTR018	M	6	28.05.2014	134	1383272	1382922	609612
BTR020	M	7	30.05.2014	417	713180	710590	312312
BTR022	M	9-10	5.06.2014	128	1371434	1372309	605133
BTR023	F	5.5	6.06.2014	715	4204632	4182197	1803209
BTR031	F	3	30.05.2017	423	3282440	3282981	1434689
BTR042	M		16.06.2014	173	4495340	4498475	1924439
BTR043	M	5	27.05.2018	622	2570562	2570195	1127519
BTR045	M	5	7.06.2018	726	1311324	1309994	578510
BTR048	M	15	14.06.2018	126	407200	406349	179679
BTR052	F	6	07.06.2019	415	2448418	2436148	1949320
BTR054	M	10	10.06.2019	227	4358548	4331379	3404147
BTR057	M	10	16.06.2019	279	2945814	2928307	2330933
BTR036	F	5-6	27.06.2017-29.06.2018	705	555774	554212	246464

5.4 Genetic Structure

Overall I captured high genetic structure between brown bear populations. Eastern Anatolian, Georgian, and Apennine brown bears were separated from other populations along PC1, while the Russian and Alaskan samples were further separated from East European populations along PC2 (Figure 5.1). Along PC1, Eastern Anatolian brown bears and the small isolated brown bear population from the Apennine mountains in Italy appeared on opposite ends of the axes, showing similar levels of differentiation from the rest of the brown bear populations. Although geographically close, the Georgian brown bear sample was still highly distinct from the Eastern Anatolian brown bear population positioned somewhat closer to the East European populations. A separate PCA using only the Eastern Anatolia samples revealed minimal structure within this population and showed no apparent difference between sedentary and migratory individuals (Figure 5.2).

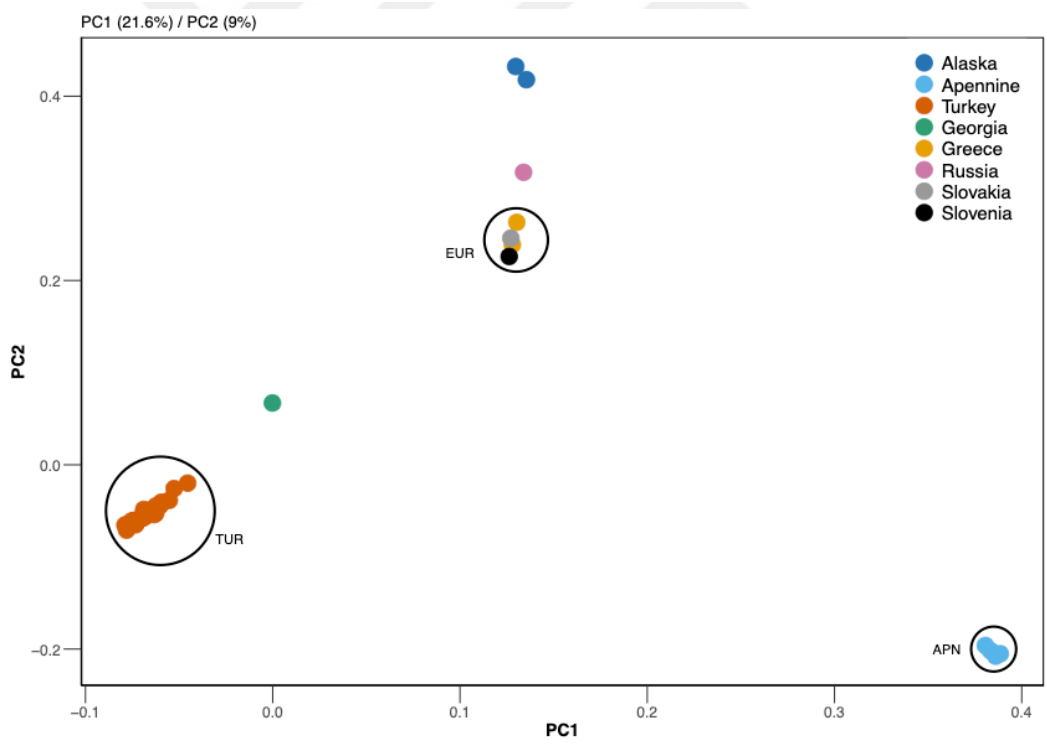

Figure 5.1 The PCA plot for all brown bear samples. Principal component analysis for European (n=4), Apennine (n=5) and Türkiye (n=31) populations. The dots outside the circles indicate Alaskan, Russian, and Georgian brown bears.

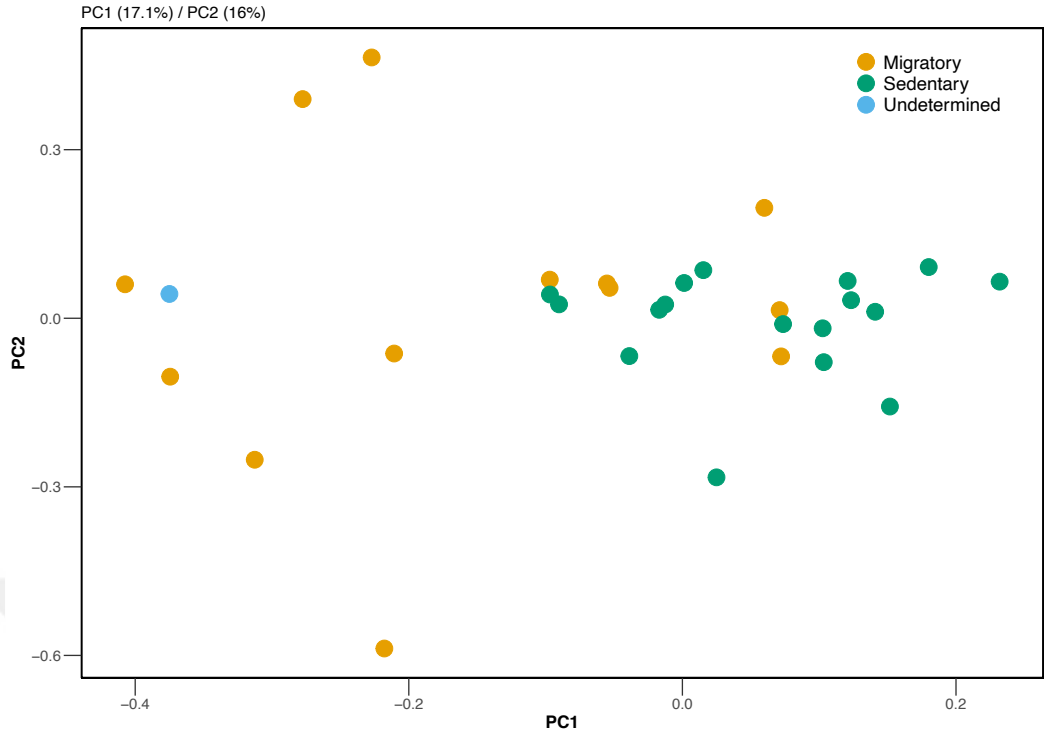

Figure 5.2 The PCA plot for Eastern Anatolian brown bear samples. Principal component analysis for the individual Eastern Anatolian brown bears. Green dots show sedentary bears (n=17), while orange dots show migratory bears (n=14).

Admixture analysis gave similar results to PCA and determined K=6 as the optimal number of clusters based on Evanno's method (Figure 5.3). Overall there was minimal shared ancestry between Apennine, European, and Eastern Anatolian brown bears, with the Georgian sample being the only individual showing shared ancestry between both Europe and Anatolia (Figure 5.4). In addition, both Eastern Anatolian brown bears and the Georgian sample showed a high amount of mixed ancestry, differentiating them further from European and Apennine brown bears, which showed minimal ancestral diversity even at higher K values (Figure 5.4). Finally, admixture analysis captured no difference in ancestry between sedentary and migratory bears (Figure 5.4).

To conclude, the genetic structure of Eastern Anatolian brown bears was highly distinct from other geographically close brown bear populations but I did not capture any genetic structure or distinct ancestry between behavioral types (sedentary vs. migratory) segregating in the Turkish population.

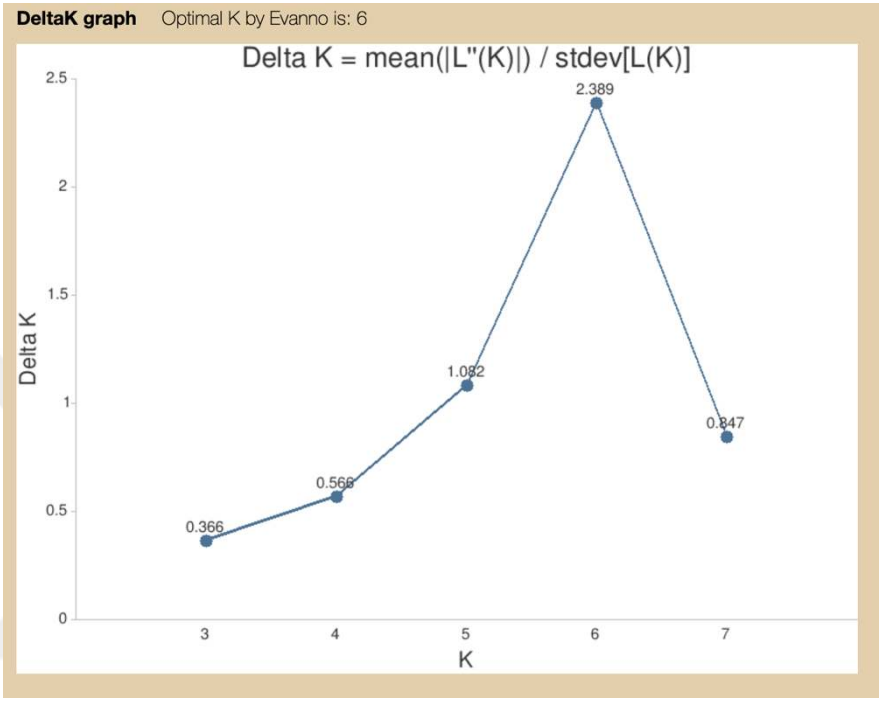

Figure 5.3 Delta K value for each K by Evanno's method (Kopelman et al., 2015).

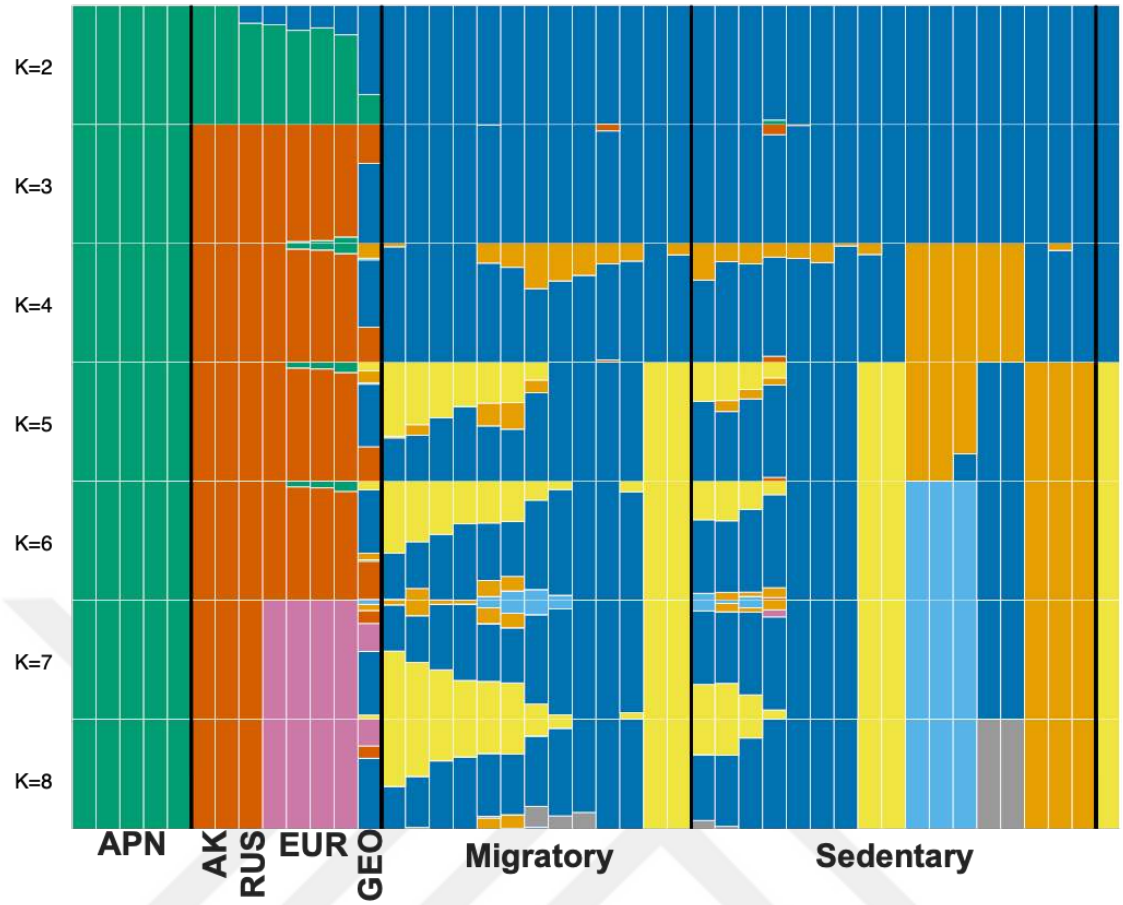

Figure 5.4 Admixture analysis for each population. Each color represents different ancestral state. The population and individuals' names are indicated.

5.5 Genetic Diversity

Excluding the Apennine bear population, genome-wide average nucleotide diversity (θw) was similar between most populations and ranged between 0.001 to 0.002 (Figure 5.5 and Appendix B). The highest diversity was observed in the East Anatolian population, while as expected, the Apennine bear population showed significantly lower diversity than all other populations. To understand how diversity is distributed throughout the genome across all populations, I calculated separate θw values across scaffolds over 25 MB. In all scaffolds, similar to genome-wide average values, the Eastern Anatolian population had the highest nucleotide diversity (Figure 5.5, Figure 5.6 and Appendix B). The results indicated that θw values did not affected from the sample size of the populations (Appendix B). In summary, although the Eastern Anatolian population is highly differentiated from other populations, it harbors relatively higher genetic diversity.

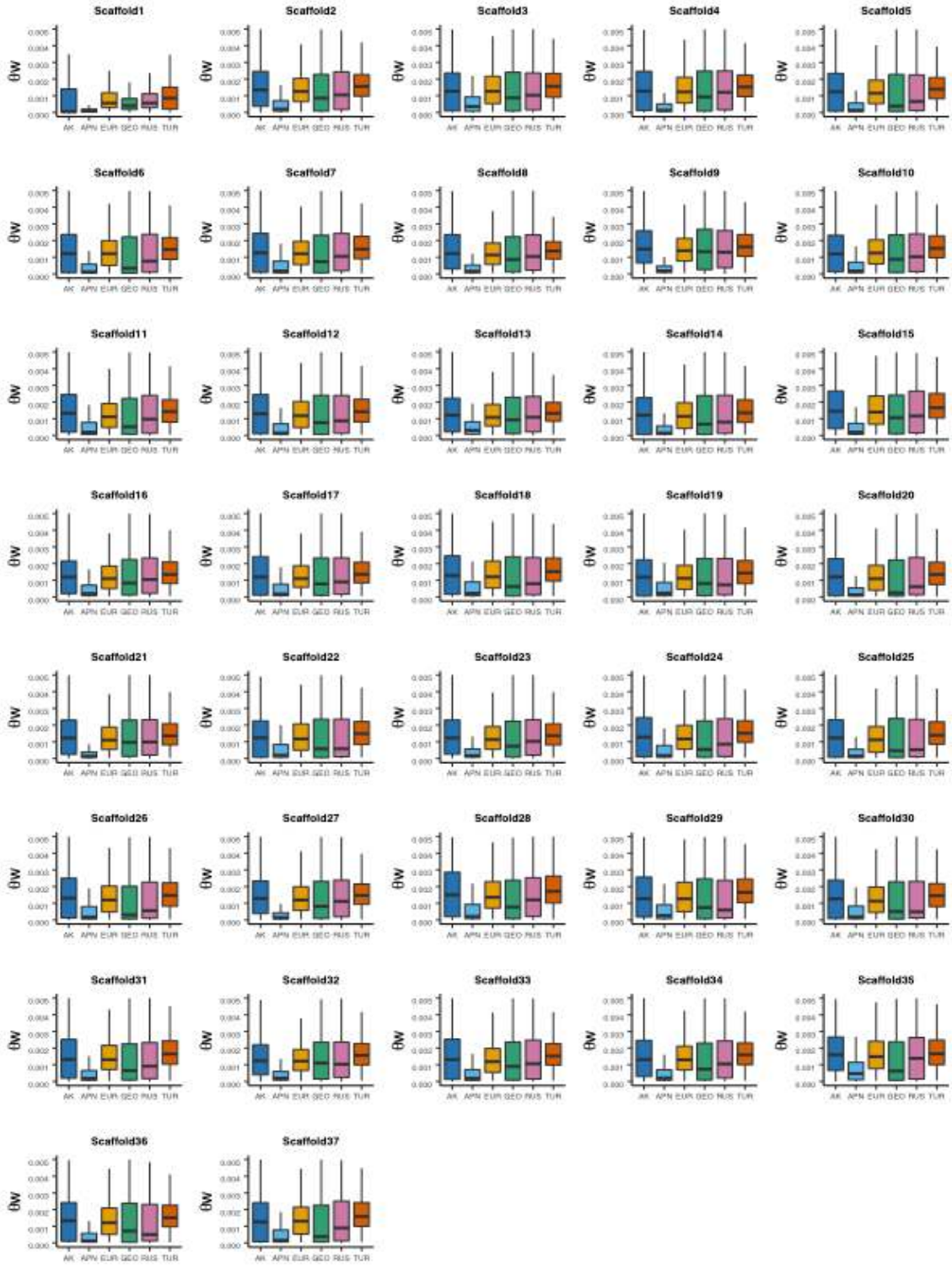

Figure 5.5 Box plots of Theta W (Watterson diversity) value in Türkiye, European, Apennine, Georgian, Alaskan and Russian populations indicated in red, orange, blue, green, dark blue, and pink colors, respectively. Each diversity plot is represents the selected top 37 scaffolds selected based on the length.

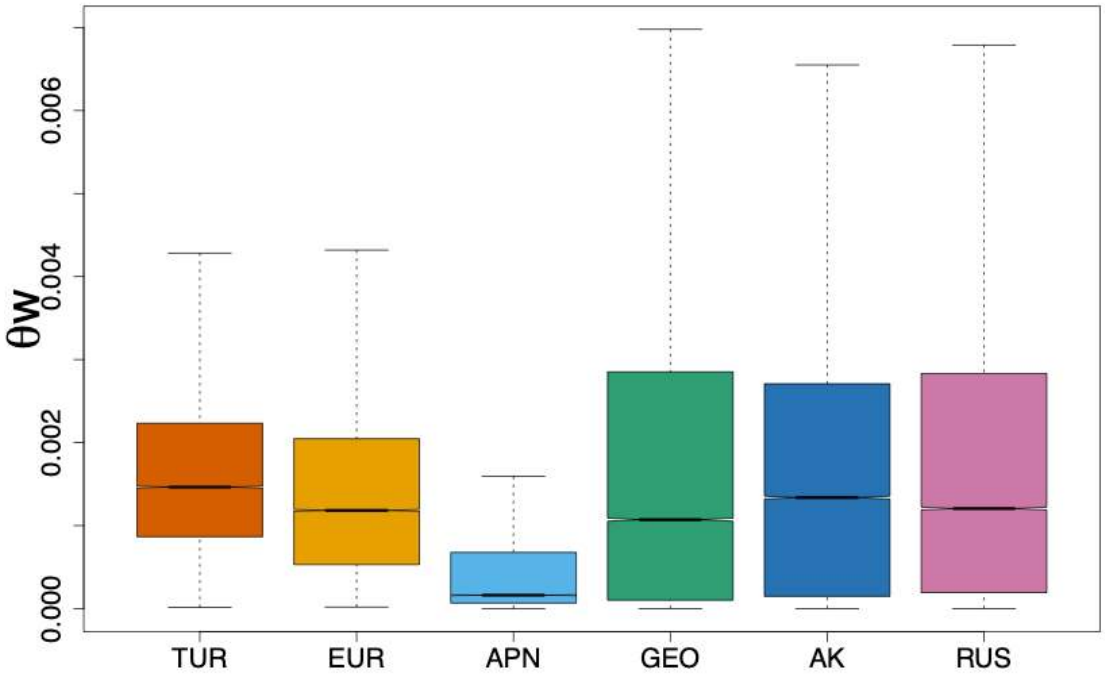

Figure 5.6 Box plots of Theta W (Watterson diversity) indices for Türkiye, European, Apennine, Georgian, Alaska, and Russian populations are indicated as red, orange, blue, green, dark blue, and pink colors, respectively.

5.6 Effective Population Size Estimation

The results of the EBSP analysis show a dramatic increase in effective population size of the Eastern Anatolian brown bear population starting around 20 kya and reaching present population sizes of around ca 250,000 individuals (Figure 5.7). The same analysis also shows that this relatively high effective populations size was historically not typical of the population which mostly stayed stable around 50,000 to 75,000 individuals with only a small reduction in size during the LGM (Figure 5.7).

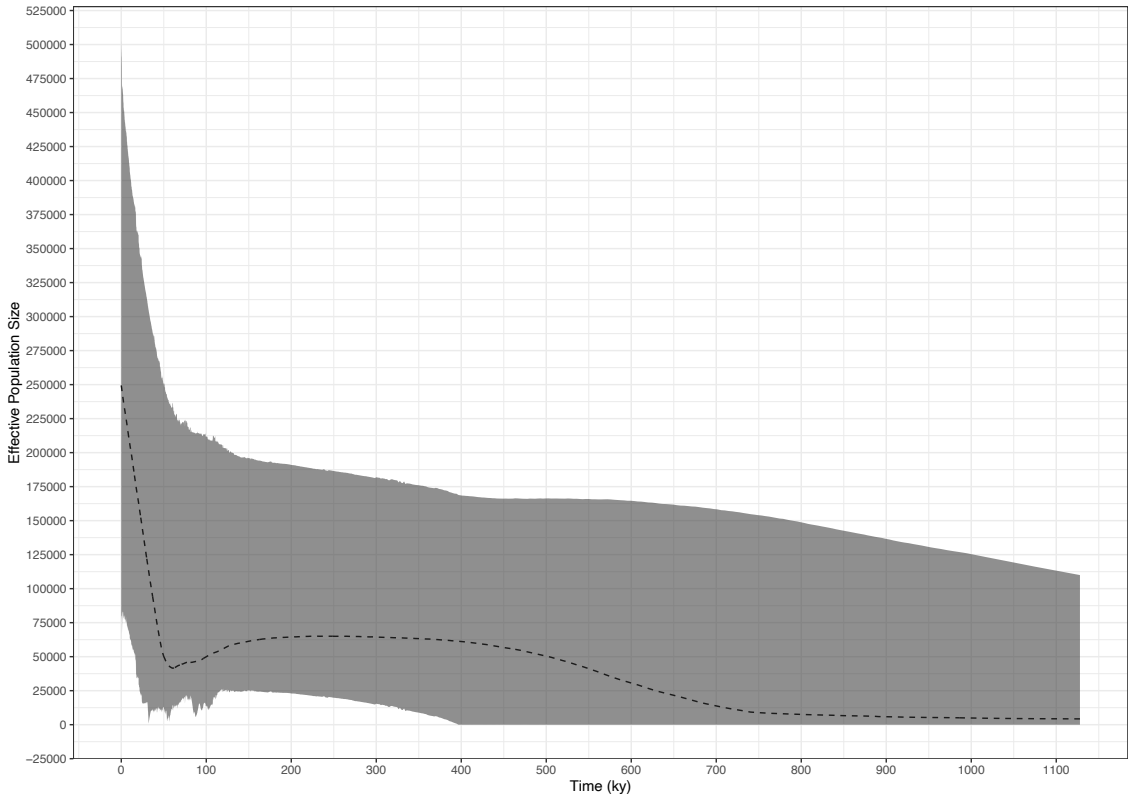

Figure 5.7 The EBSP plot. The effective population size of the Eastern Anatolia population with respect to time in thousands of years is shown. The median population size in the dashed line, and the 95% HPD in the lightly shaded grey.

5.7 Association and selection

Association mapping between sedentary and migratory bears returned no SNPs that reached genome-wide significance ($P < 4.09 \times 10^{-6}$, LRT > 21). To further investigate whether any of the top associated SNPs showed extreme genetic differentiation either in sedentary or migratory bears, I calculated population branch statistics (PBS) (Yi et al., 2010) for the top 20 SNPs (LRT > 13, $P < 3.00 \times 10^{-4}$) in our association panel and compared them against the mean PBS score calculated across the 37 scaffolds.

For PBS analysis, taking sedentary bears as the focal population the mean PBS value across the 37 scaffolds was 0.0205 with a standard deviation of 0.0330 whereas for analysis taking migratory bears as the focal population the mean PBS value was 0.0127 with a standard deviation of 0.0277. Among the 20 top associated SNPs, five fell over the 99.9 percentile range (PBS > 0.3547) in sedentary bears while another five fell over the

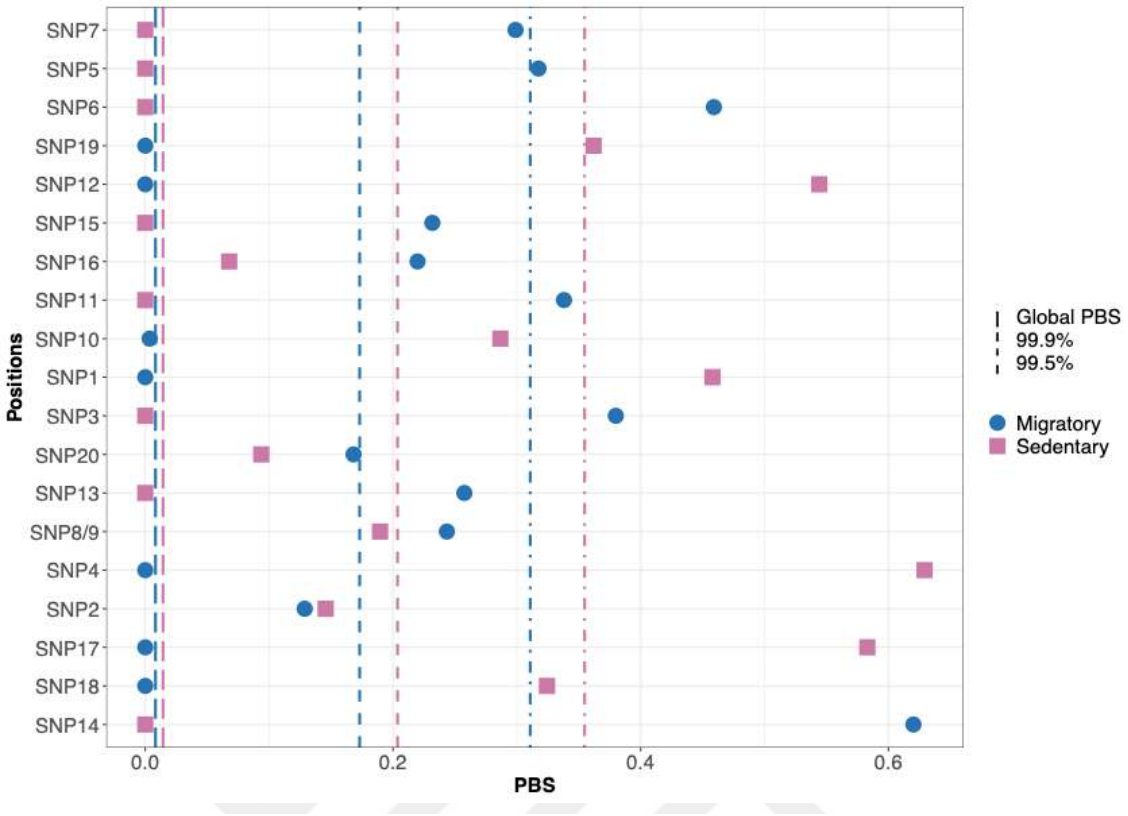

Figure 5.8 PBS values of each site found in the association analysis applied to European-Sedentary-Migratory and European-Migratory-Sedentary populations. Dark long-dashed lines represent the global PBS values for each case. Dot-dashed lines indicate 99.9% and dashed lines 99.5% significance thresholds for all PBS values with indicated colors.

99.9 percentile range (PBS > 0.3547) in migratory bears (Figure 5.8, Table 5.3). Moreover, all SNPs with PBS scores over the 99.9 percentile in either sedentary or migratory bears were at least 10 standard deviations above the mean (Table 5.4), providing good evidence for the outlier nature of these loci. Out of the 10 highly differentiated SNPs only one, SNP_TM3 (differentiated in migratory bears), fell within a protein-coding gene (upstream of the first exon of CCRL2). For the remaining highly differentiated SNPs I found 3 genes, Myo5b, RAN and TRDMT lying within 50 KB distance from SNP_TM4, SNP_TS3 and SNP_TS5 respectively (Table 5.4).

To further understand the genomic patterns of differentiation at the 10 SNPs showing extreme PBS values I genotyped each individual at these sites and categorized individuals as either heterozygous or homozygous for the ancestral or derived allele (Figure 5.9). Genotype calling at these sites revealed that two of the migratory SNPs showed ancestral state for migratory individuals while heterozygous for sedentary

individuals. All other SNPs were heterozygous for migratory individuals but indicated ancestral or derived states for sedentary individuals.

I conclude that even though statistically not significant at the genome-wide level, ten of the top 20 SNPs in our association panel showed extreme differentiation and distinct genotypes between sedentary and migratory bears, especially one of which was related to functional coding region, suggesting that migratory behavior might have a strong genetic basis.

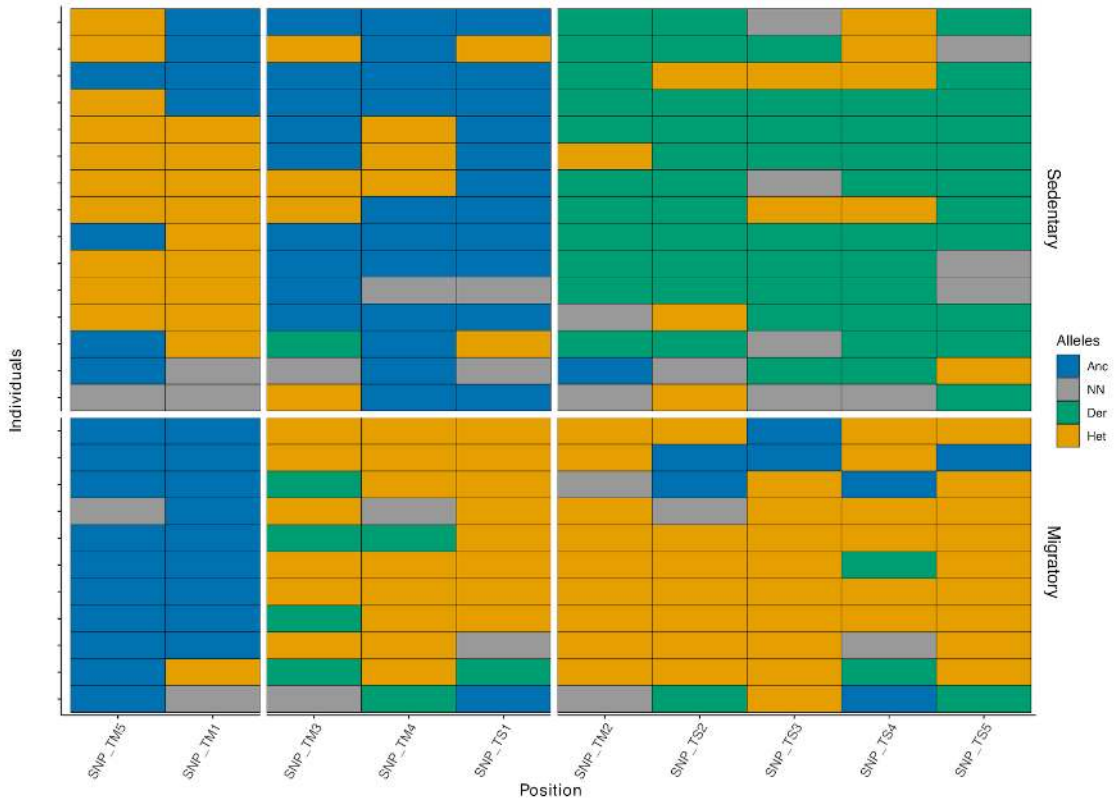

Figure 5.9 SNPs that have the highest log-likelihood ratios at each position for each individual separately for sedentary and migratory bears. The genotypes of the associated sites. Blue rectangles represent ancestral, yellow rectangles show heterozygous, and green ones represent derived alleles. Gray rectangles are the ones that the genotype is not known.

Table 5.3 Top 20 SNPs with allele frequencies, log-likelihood ratios, wildtype/heterozygous/homozygous individual ratios, reference and ancestral alleles, Major/minor alleles for migratory and sedentary individuals are indicated.

SNP Name	Position	Frequency	LRT	High WT/HE/HO	Reference Americanus Allele	Ancestral Polar Allele	Migratory Major/Minor	Sedentary Major/Minor
SNP1	HiC_scaffold_4-29070918	0.284268	18.883072	13/11/1	T	T	T/G	T/G
SNP2	HiC_scaffold_20-44992842	0.301007	16.867336	12/13/2	C	T	C/T	C/T
SNP3	HiC_scaffold_36-51993011	0.189913	16.184687	14/10/0	C	C	C/T	C/T
SNP4	HiC_scaffold_30-12480299	0.328073	15.697789	10/10/2	C	C	T/C	T/C
SNP5	HiC_scaffold_30-24131228	0.294255	15.358836	11/12/2	A	A	A/G	A/G
SNP6	HiC_scaffold_28-42755955	0.426213	15.27927	10/10/5	G	G	G/A	G/A
SNP7	HiC_scaffold_30-24131250	0.290398	15.261523	11/12/1	C	C	C/T	C/T
SNP8	HiC_scaffold_3-89691858	0.376305	15.198098	11/10/4	G	G	C/G	C/G
SNP9	HiC_scaffold_3-89691857	0.37706	15.165669	11/10/4	C	C	C/A	C/A
SNP10	HiC_scaffold_15-53316014	0.178035	15.113257	19/10/0	G	G	G/A	G/A
SNP11	HiC_scaffold_18-2297497	0.355604	14.75747	11/10/0	G	A	A/G	A/G
SNP12	HiC_scaffold_37-70763847	0.275576	14.479207	13/10/1	T	C	C/T	C/T
SNP13	HiC_scaffold_10-21737434	0.288273	13.10247	11/10/0	G	G	G/A	G/A

SNP14	HiC_scaffold_2-47325857	0.248919	12.982222	13/12/0	T	C	T/C	T/C
SNP15	HiC_scaffold_18-30542931	0.30257	12.824259	10/13/1	G	G	G/C	G/C
SNP16	HiC_scaffold_18-4714646	0.213437	12.59165	16/10/1	C	T	T/C	T/C
SNP17	HiC_scaffold_14-65416581	0.277475	12.537965	13/10/2	G	G	A/G	A/G
SNP18	HiC_scaffold_8183-263	0.249565	12.059821	11/13/0	T	C	C/T	C/T
SNP19	HiC_scaffold_34-17119378	0.300622	11.720096	13/10/2	T	A	A/T	A/T
SNP20	HiC_scaffold_16-20809862	0.267548	11.671161	15/13/1	A	A	A/G	A/G

Table 5.4 The SNPs that have the highest PBS values in the top 99.9% and labelled as TM if the migratory trait is the focal, and as TS if the sedentary trait is the focal in the PBS. Derived allele frequencies and mean, 99.9% and window PBS are indicated. Their BLAST results are shown in gene column.

SNP Name	Position	SNPs	DAF_Mig	DAF_Sed	DAF_Eur	MeanPBS	99.9 percentile PBS	SNPwindow Pbs	Distance to Nearest Gene	Gene
SNP_TM1	HiC_scaffold_2-47325857	SNP14	0.045271	0.393966	0.807156	0.01272476	0.310783	0.620202	-	LTR
SNP_TM2	HiC_scaffold_18-2297497	SNP11	0.394188	0.855803	0.770906	0.01272476	0.310783	0.338075	-	LTR
SNP_TM3	HiC_scaffold_28-42755955	SNP6	0.739289	0.195274	0.183508	0.01272476	0.310783	0.459069	Inside Exon	**CCRL2**
SNP_TM4	HiC_scaffold_30-24131228	SNP5	0.536806	0.125391	0	0.01272476	0.310783	0.317497	46501	Similar to Myo5b
SNP_TM5	HiC_scaffold_36-51993011	SNP3	0	0.333357	0.225375	0.01272476	0.310783	0.379928	4935	**ELOC**
SNP_TS1	HiC_scaffold_4-29070918	SNP1	0.540518	0.070973	0.511545	0.02049429	0.354704	0.457943	42113	RAN
SNP_TS2	HiC_scaffold_14-65416581	SNP17	0.495463	0.901103	0.192844	0.02049429	0.354704	0.582984	-	LTR
SNP_TS3	HiC_scaffold_30-12480299	SNP4	0.416979	0.8887	0.192721	0.02049429	0.354704	0.629213	-	LTR
SNP_TS4	HiC_scaffold_34-17119378	SNP19	0.455748	0.876957	0.469096	0.02049429	0.354704	0.361983	6926	**TRDMT**
SNP_TS5	HiC_scaffold_37-70763847	SNP12	0.503248	0.918521	0.372266	0.02049429	0.354704	0.544178	-	LTR

5.8 Polygenic discrimination

Random forest analysis identified 464 sites that were associated with movement behavior (sedentary versus migratory) and had an out-of-bag correct assignment rate of 97% out of the 62,286 SNPs considered initially. For the 464 discriminatory SNPs, I calculated the frequency of the derived allele in both sedentary and migratory bears (Figure 5.10). As expected for polygenic traits (Pavey et al., 2015), the difference in mean frequency of the derived allele at each of the discriminatory SNPs was moderate (ΔP). Nevertheless, there were notable SNP clusters in which sedentary individuals had lower DAF values (red) while migratory individuals had higher DAF values (white) and vice versa (Figure 5.10, black lines).

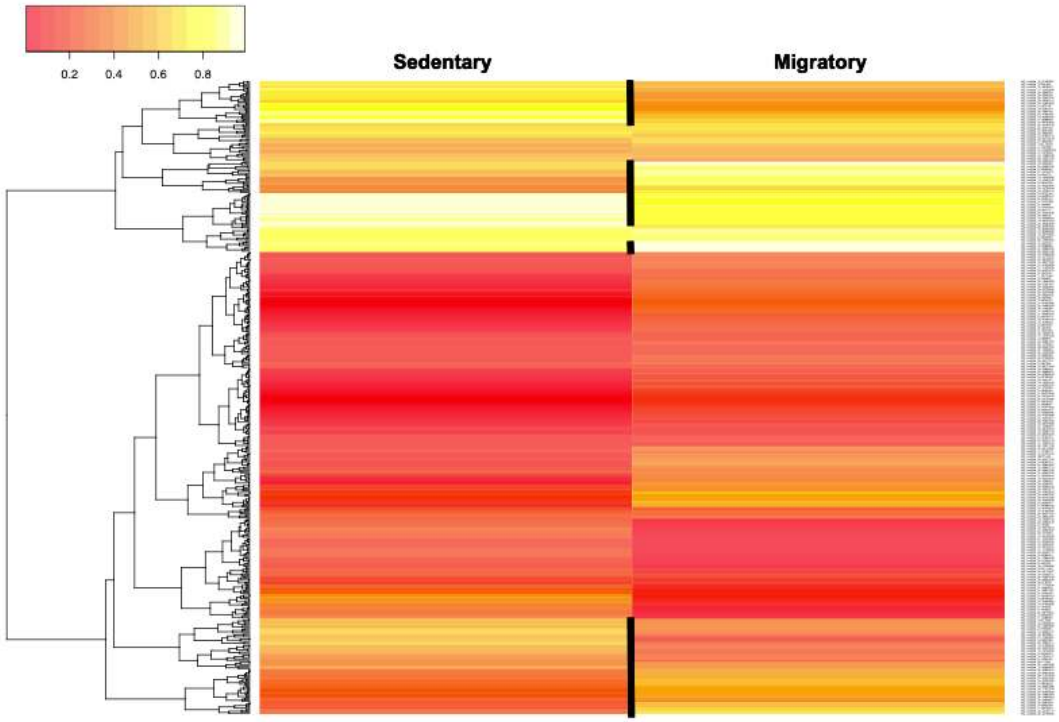

Figure 5.10 The heatmap of the derived allele frequency of 464 SNPs for sedentary and migratory individuals. Black lines indicate the DAF differences between columns.

To test the discriminatory and predictive power of these 464 SNPs I conducted a genetic assignment test using the assignPOP package in R (Chen et al., 2018) under 5 different models (SVM, naive Bayes, decision tree, RF, and LDA) on individuals with unknown behavioral states (Figure 5.11). All models reached 97% predictive accuracy based on the 464 sites and were able to assign unknown individuals as either migratory or sedentary with an average probability of 89% across all models (Table 5.5, Appendix

C). Predictions of different models for individuals were mostly in agreement except for BTR50 and 55 (Table 5.5). BTR50 was classified as sedentary in the naive Bayes model, whereas the remaining models classified this individual as migratory. BTR55 was assigned as migratory in the decision tree and RF models, while this individual was assigned as sedentary in the remaining models (Table 5.5).

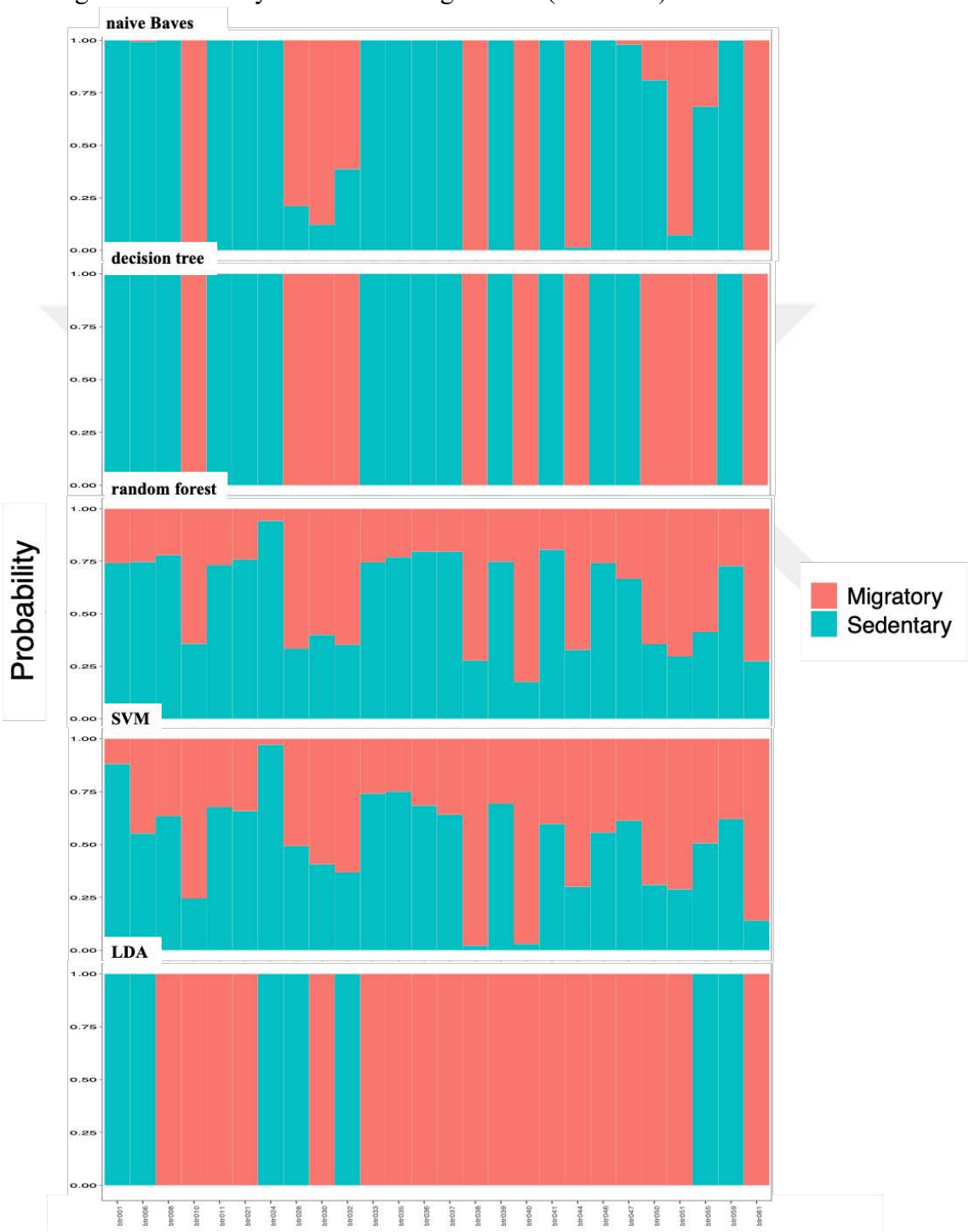

Figure 5.11 The genetic assignment scores of 26 individuals in each model (naive Bayes, decision tree, random forest, svm and lda).

Table 5.5 The behavioral trait for each 26 individuals from each genetic assignment model is indicated.

Animal ID	NaiveBayes	DecisionTree	RandomForest	SuperVectorMachine	Linear Discriminant Analysis
BTR001	Sedentary	Sedentary	Sedentary	Sedentary	Sedentary
BTR006	Sedentary	Sedentary	Sedentary	Sedentary	Sedentary
BTR008	Sedentary	Sedentary	Sedentary	Sedentary	Migratory
BTR010	Migratory	Migratory	Migratory	Migratory	Migratory
BTR011	Sedentary	Sedentary	Sedentary	Sedentary	Migratory
BTR021	Sedentary	Sedentary	Sedentary	Sedentary	Migratory
BTR024	Sedentary	Sedentary	Sedentary	Sedentary	Sedentary
BTR028	Migratory	Migratory	Migratory	Migratory	Sedentary
BTR030	Migratory	Migratory	Migratory	Migratory	Migratory
BTR032	Migratory	Migratory	Migratory	Migratory	Sedentary
BTR033	Sedentary	Sedentary	Sedentary	Sedentary	Migratory
BTR035	Sedentary	Sedentary	Sedentary	Sedentary	Migratory
BTR036	Sedentary	Sedentary	Sedentary	Sedentary	Migratory
BTR037	Sedentary	Sedentary	Sedentary	Sedentary	Migratory
BTR038	Migratory	Migratory	Migratory	Migratory	Migratory
BTR039	Sedentary	Sedentary	Sedentary	Sedentary	Migratory
BTR040	Migratory	Migratory	Migratory	Migratory	Migratory
BTR041	Sedentary	Sedentary	Sedentary	Sedentary	Migratory
BTR044	Migratory	Migratory	Migratory	Migratory	Migratory
BTR046	Sedentary	Sedentary	Sedentary	Sedentary	Migratory
BTR047	Sedentary	Sedentary	Sedentary	Sedentary	Migratory
BTR050	Sedentary	Migratory	Migratory	Migratory	Migratory
BTR051	Migratory	Migratory	Migratory	Migratory	Migratory
BTR055	Sedentary	Migratory	Migratory	Sedentary	Sedentary
BTR059	Sedentary	Sedentary	Sedentary	Sedentary	Sedentary
BTR061	Migratory	Migratory	Migratory	Migratory	Migratory

5.9 Gene Ontology

I ran a gene ontology analysis on the 464 discriminatory SNPs obtained from the RF analysis. The number of genes for each GO category; biological process, molecular function, and cellular component is given in Figure 5.12. In total I identified 514 unique genes (Appendix D), 423 of which were found to have *Homo sapiens* Uniprot IDs (Appendix D). These 423 genes were then used in an enrichment analysis which showed that most of the enriched genes ($p < 10^{-6}$) were transcription factors (TF) (Figure 5.13) and the most significant KEGG pathway was found to be olfactory transduction (Figure 5.13 and Figure 5.14B). GO terms within biological process demonstrated that detection of sensory and chemical stimuli (GO:0050906, GO:0050907, GO:0050911, GO:0007606, GO:0051606, GO:0007608, GO:0009593, and GO:0007600) were the most important functions that differentiated sedentary individuals from migratory individuals when corrected for FDR (Figure 5.13, Figure 5.14A). Other biological processes associated with the given genes were the G protein-coupled receptor signaling (GO:0007186) and nervous system pathways (GO:0050877) (Figure 5.13, Figure 5.14A).

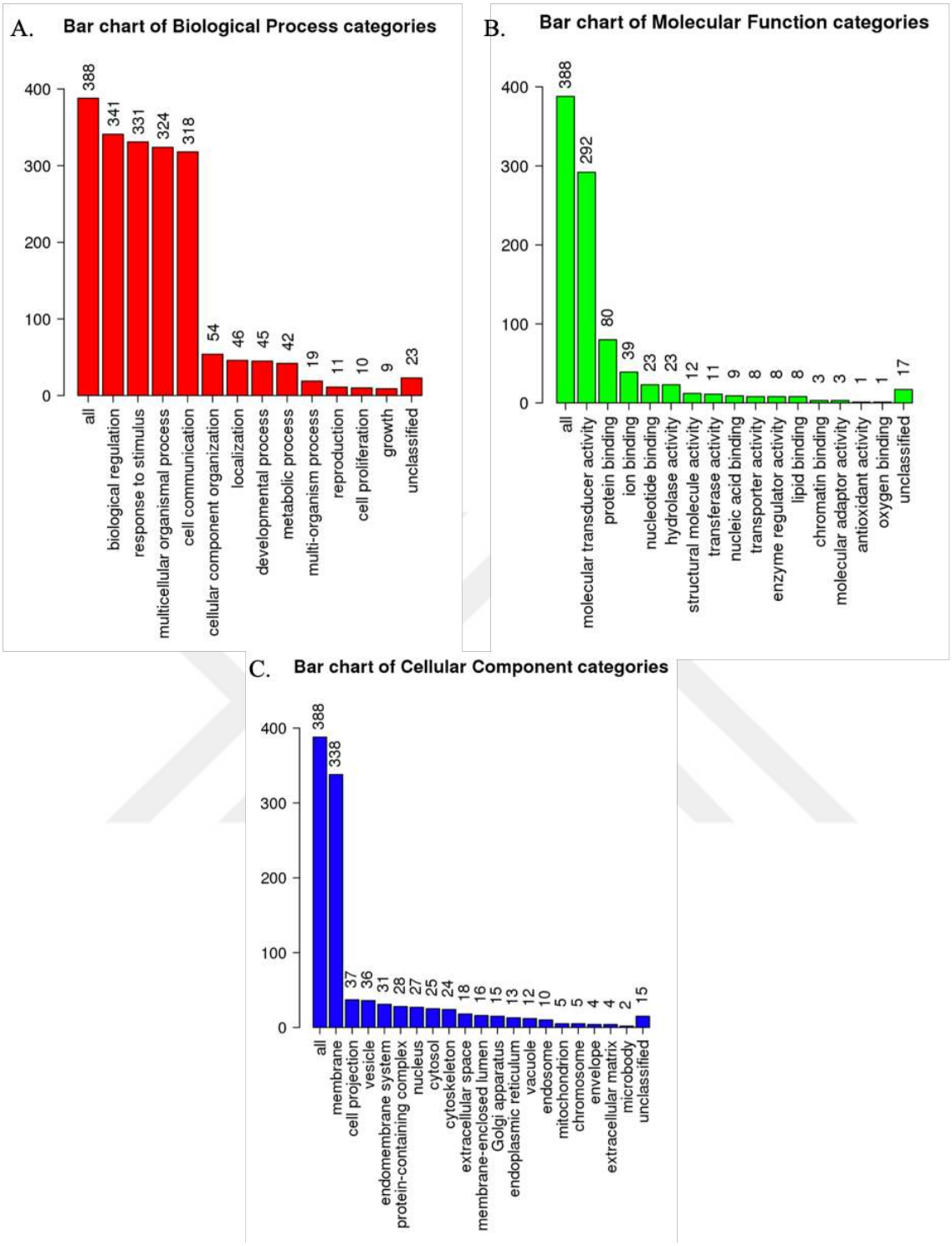

Figure 5.12 GO slim summary for the uploaded genes. Biological process (A), molecular function (B) and cellular component (C) represented as red, green or blue bar, respectively. The number of IDs in the category and user lists is indicated by the height of the bar.

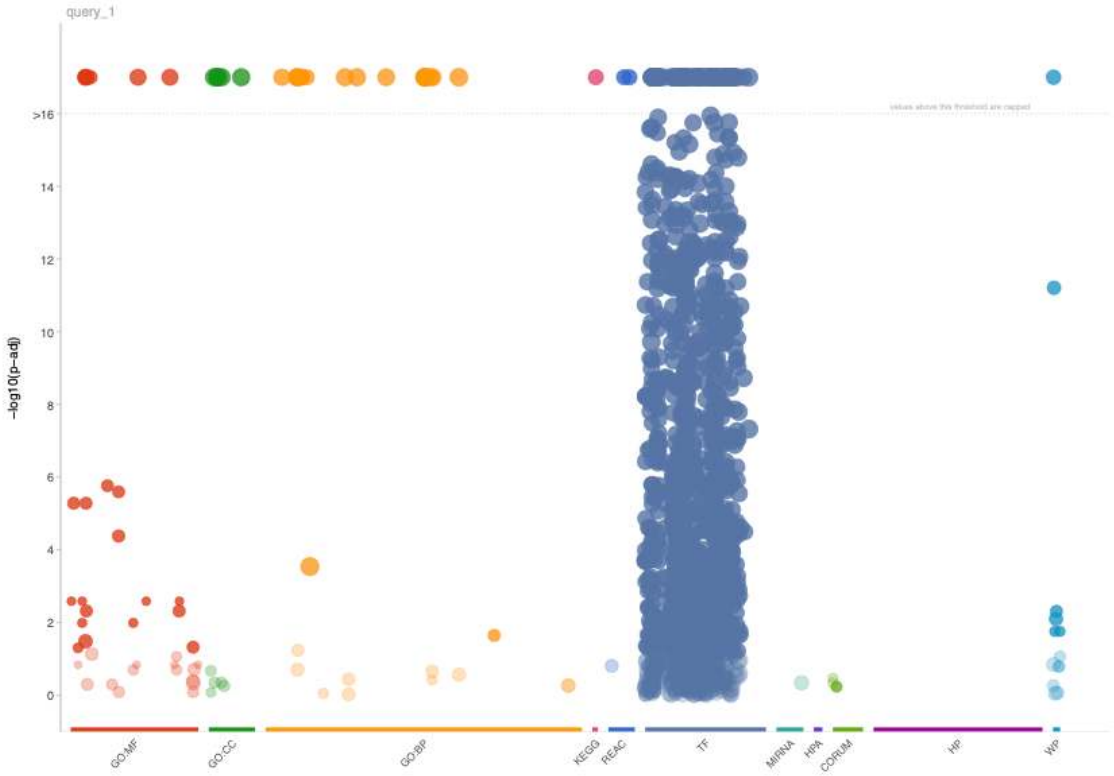

Figure 5.13 Manhattan plot of g:Profiler enrichment results for 423 SNPs obtained from gene annotation. The x-axis shows the terms and the y-axis shows the enrichment *p*-values on -the log10 scale.

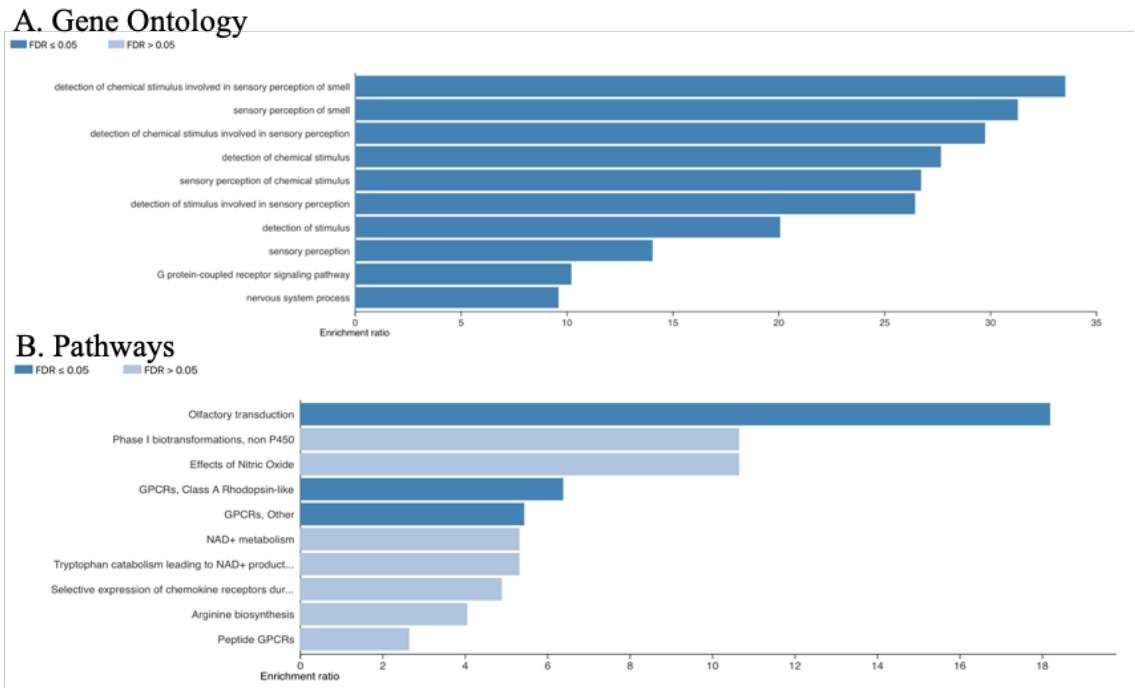

Figure 5.14 Gene enrichment results for GO analysis (A) and Pathways (B) including KEGG and Wikipathways. Dark blue colors indicate the FDR<=0.05, while blue colors indicate FDR>0.05. Taken from webgestalt.org (Liao et al., 2019).

5.10 Relatedness

Estimates of pairwise relatedness between the 56 samples revealed 3 mono-zygotic twins, 20 1st-degree kinships (parent-offspring pairs and full-sibs), and 50 2nd-degree kinships (half-sibs and first cousins), and the rest is unrelated (i.e., second-degree cousins and lower) individuals. When I only compare individuals with direct ancestry (theta > 0.05), I can observe higher kinship values within behavioral groups than across behavioral groups. Both sedentary-sedentary and migratory-migrator pairs had a much higher percentage of 1st-degree kinship (parent-offspring/full-sib) than migratory-sedentary comparisons (Figure 5.15). Moreover, when I compare the kinship and zygosity coefficients between groups I can observe that both are significantly higher within sedentary-sedentary pairs when compared to both migratory-migratory and sedentary-migratory pairs (Figure 5.16). In conclusion, I can say that although subtle, there seems to be a significant familiar structure within behavioral groups, especially among sedentary individuals.

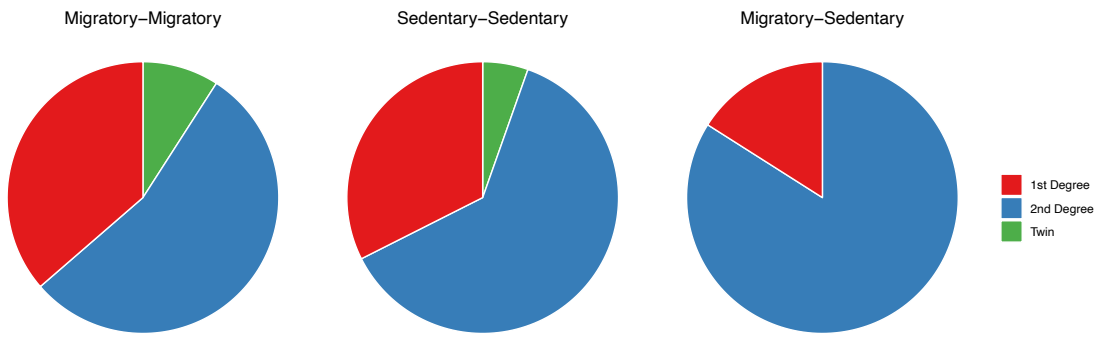

Figure 5.15 The pie charts for the pairwise relatedness for each pair of behavioral individuals based on the pair percentages.

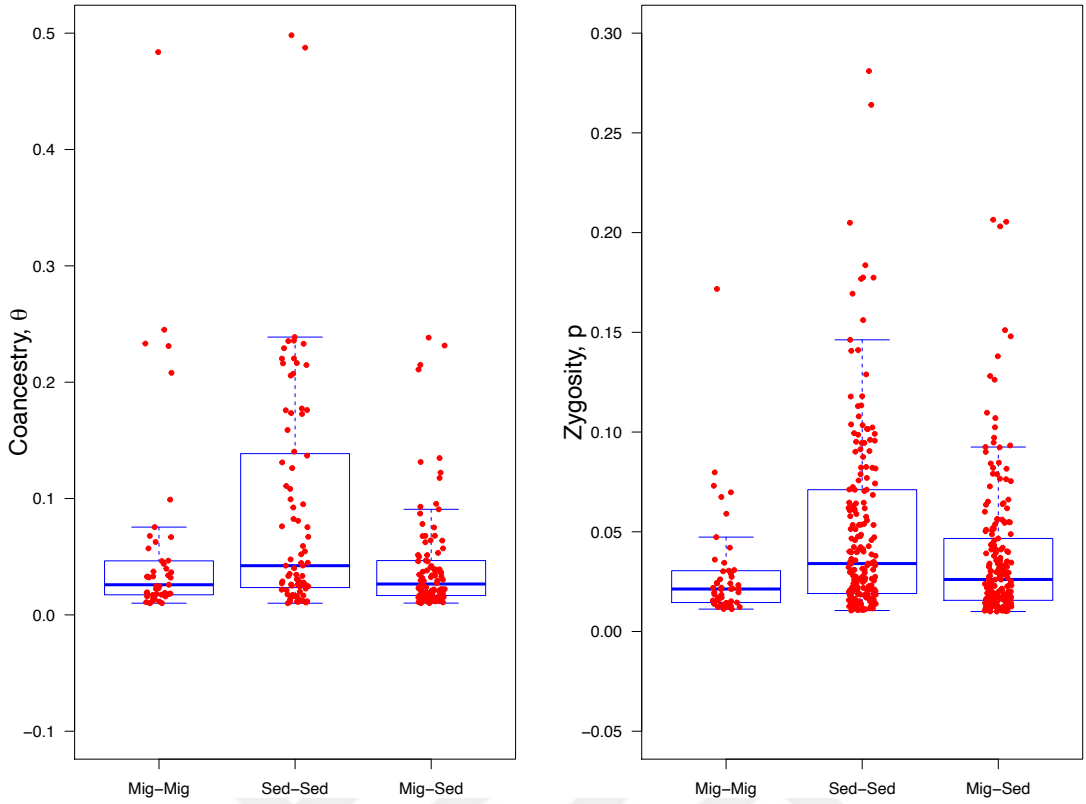

Figure 5.16 The co-ancestry and zygosity coefficients for each pair of behavioral differences, migratory-migratory, sedentary-sedentary and migratory-sedentary.

CHAPTER 6:
DISCUSSION

The rapid expansion of urban areas has resulted in the degradation and destruction of the natural habitats of many species. As opportunists, brown bears can adapt to new environments and new nutrient sources created by human activities such as city dumps, campsites, bee hives, etc. (Cozzi et al., 2016; De Angelis et al., 2021; de Gabriel Hernando et al., 2020). The adaptation of wildlife to novel and urban environments, as well as the development of new life strategies to cope with these new environments has been a topic of much interest (Dahle & Swenson, 2003; Penteriani et al., 2021). For example, Yellowstone National Park was home to grizzly bears that fed on garbage in the early 1900s. Later, park management closed this open dump, and suddenly human-bear conflicts in the area increased (Craighead & Craighead Jr, 1971), and Grizzly bear populations reportedly declined in the following years (Cole, 1974; Craighead & Craighead Jr, 1971; Craighead et al., 1995). Almost 40 years later, a similar situation was observed in the large open city dump near the city of Sarıkamış in Eastern Anatolia, where brown bears started feeding off the dump regularly, and Cozzi et al. (2016) recorded the presence of two types of life history based on movement and feeding behavior. Some individuals migrate in search of food and rarely go to the city dump (Migratory), and the others are generally feeding at the dump and staying at Sarıkamış throughout the year (Sedentary) (Cozzi et al., 2016). In this thesis, I studied the Eastern Anatolia brown bear population using genome-wide sequencing data to understand the demography and genetics of this life-history variation.

I showed that Eastern Anatolian bears are genetically distinct from other bear populations around the world but also harbored relatively high genetic diversity and mixed ancestry. I also identified recent population expansion and a higher degree of kinship within behavioral groups, especially among bears utilizing the garbage dump. Furthermore, I determined several genomic regions and distinct genotypes associated with sedentary and migratory behavior as well as an overall enrichment in genes associated with sensory perception in smell. All of these results imply that the observed life history diversity may have a substantial genetic basis. My findings suggest that the

Eastern Anatolian brown bear population may be subject to considerable selective pressures to adapt to anthropogenic resources.

The Eastern Anatolian brown bears have a genetic structure that is very different from other brown bear populations. Brown bears from Alaska, Russia, and Europe, including individuals from Slovenia, Slovakia, and Greece, clearly indicated a genetic structure that was geographically correlated and clustered together. Given Türkiye's neighboring status to Europe I would expect Türkiye to share some genetic similarities with the European populations but our analysis clearly shows that Turkish brown bears are as distinct from European brown bears as the highly isolated and diverged relict Apennine bear population in the Mountains of Italy. More interestingly even the Georgian sample was genetically distinct from the East Anatolian population even though Georgia is geographically very close and there is a good chance Eastern Türkiye and Georgian brown bears move in and out of one another's territory.

Admixture results gave more support to the genetic distinctiveness of the East Anatolian brown bear population as the Eastern Anatolia population did not share any ancestry with either the European, Russian or Alaskan brown bears. Moreover, the Eastern Anatolian brown bear population showed a high amount of mixed ancestry while other populations around the world showed mono-ancestry. The only exception was the Georgian brown bear which also had mixed ancestry and interestingly shared ancestry with both European and Eastern Anatolian brown bears. The findings obtained from admixture analysis coincided with the historical distributions of brown bears across Europe (Hewitt, 1999). Europe population has shared ancestry with Apennine populations and possible colonization of a clade from the Caucasus, including Georgia and Eastern Anatolia, through the north of Black Sea region (Hewitt, 1999). Moreover, the mtDNA analysis indicated that Eastern Anatolian brown bears have distinct haplotypes and the same clade as Georgian brown bears (Çilingir et al., 2016), which are similar to what this study suggested.

Comparisons between sedentary and migratory bears in East Anatolia revealed some structure between the two behavioral groups (PC1, Figure 5.3) although admixture analysis did not reveal any differences in ancestry. Therefore, even though there are observable behavioral differences within the Eastern Anatolian populations (sedentary vs migratory) and these behavioral groups show some genetic structure, these bears live in

a small geographic area and gene flow is still clearly widespread so the for the most part the system is still a mostly panmictic population.

The high amount of mixed ancestry in the East Anatolian brown bear population can also be taken as an indication of high genetic diversity. Indeed genetic diversity scans revealed that the East Anatolian brown bear population had the highest diversity along all of the 37 major scaffolds. As expected, the Apennine population has the least genetic diversity because they are completely isolated and have high inbreeding (Benazzo et al., 2017). It is worth noting that, genetic diversity in the East Anatolian population was comparable (actually slightly higher) than even the European population which includes individuals from a wider geographic area spanning several countries (i.e. Slovenia, Slovakia, and Greece). Interestingly, past studies based on mtDNA haplotype diversity captured similar trends - bears from Türkiye and the Middle East had relatively high haplotype diversity, while bears from Europe had relatively low haplotype diversity (Calvignac et al., 2009; Çilingir et al., 2016). Therefore this high diversity seems to be a property of eastern bear populations in general and together with mixed ancestry could indicate the presence of long running stable populations in this region with few historical founder effects or bottlenecks (Montgomery et al., 2000; Wright, 2001). The genetic variation of populations is an important parameter, especially in management of populations that are in threat of declining due to environmental change (Frankham, 2005; McNeely et al., 1990; Reed & Frankham, 2003). It is also crucial to understand how much genetic diversity is related to population size in populations that are of conservation concern, since this will provide insight into how accurate standard genetic predictions, which are based on the neutral theory of evolution, will be over time (Montgomery et al., 2000).

Indeed our estimates of the coalescent effective population size (Ne) through time (EBSP) of the East Anatolia brown bear population showed a relatively stable population with and Ne around 60K from 600 kya to 100 kya with only a slight drop to 30K between 100 - 50 kya. Interestingly after this short decline period the East Anatolia brown bear population grew exponentially in a short span of time reaching to an Ne of 250K in the present day. Therefore all of our findings point towards a brown bear population in Eastern Anatolia that has been able to maintain a high amount of genetic diversity through time and more recently is experiencing a phase of rapid demographic expansion. This

situation is not unique to Eastern Anatolian bears as there are other studies reporting demographic stability or expansion of brown bears around the world (Chapron et al., 2014; Coogan et al., 2018) and similar Ne estimates of around 250K were reported for brown bears (Liu et al., 2014; Wang et al., 2022).

Today, the long-term viability of large carnivores that require and occupy large territories depends heavily on their ability to adapt to human-altered and human-dominated environments (Sten et al., 2015). In this regard, brown bears have shown a remarkable capacity to adapt to human-dominated landscapes and are often considered a model species for studying interactions between big carnivores and humans (Ordiz et al., 2012; Ordiz et al., 2013). This situation is similarly observed in Eastern Türkiye where research and monitoring studies show an abundance of large carnivores relative to their natural prey base. These field observations when evaluated together with our findings of recent rapid demographic expansion, strongly suggest that such high population numbers of brown bears in the face of continued fragmentation, deforestation and urbanization of their natural habitats can be attributed to new foraging behaviors which enable easy access to high content food sources created by human activities.

High population numbers and high genetic diversity such as those observed in Eastern Anatolian brown bears would not only buffer such populations against environmental perturbations but also present ample fuel for genetic adaptation and phenotypic change due to the strong and consistent nature of human associated selective drivers. The great majority of recorded phenotypic changes in wild populations remain unclarified with respect to plasticity versus evolution (Merilä & Hendry, 2014; Pelletier & Coltman, 2018). Although numerous studies have found that trait changes do not correspond to adaptive expectations and even suggest evolutionary stasis, the abundance of examples of phenotypic and genetic changes for traits under selection (Bonnet et al., 2019) suggests that adaptive evolution commonly occurs in wild populations over contemporary timescales (Merilä et al., 2001; Pujol et al., 2018). Indeed more and more studies are showing that the most recent changes in wild animals can be explained by adaptive evolution (Bonnet et al., 2022). Adaptive evolutionary machinery frequently moves rapidly on generation-to-generation time periods, and the populations with additive genetic variance in fitness would likely have had significantly lower growth rates if ongoing adaptive genetic alterations had not occurred (Bonnet et al., 2022). Based on

this background the behavioral shifts observed in the Eastern Anatolian brown bear population in Sarıkamış as a result of the open city dump might be an example of rapid phenotypic evolution. Although I do not know the exact timing it is highly likely that the Sarıkamış dump was established in the 1940s after populations in the region started rising in the aftermath of World War II. Therefore, this site may have been serving as a food source for bears for around the past 80 years. When the average generation time of 11.35 years for bears is considered (Cronin et al., 2009; De Barba et al., 2010), a maximum of 80 years of adaptation to city dump covers approximately seven generations for the brown bear population. In this regard a shift from free foraging to a sedentary lifestyle observed in East Anatolian brown bears could also be the result of genetic changes in addition to behavioral plastic responses as the possible strong selective nature of a year-around available high-content food source could have caused rapid evolution.

This thesis is perhaps the first study to test this hypothesis and provides evidence that such behavioral shifts observed in many wildlife species could have a strong genetic basis. Even though GWAS scans could not determine any SNP associated with sedentary/migratory behavior that reached genome wide significance, 10 out of the 20 top-ranking SNPs in our association panel showed strong signatures of selection (Figure 5.7). 5 of these SNPs (SNP_TM1-5) showed extreme differentiation in migratory bears when allele frequencies at these sites were compared to sedentary and European bears whereas the remaining five (SNP_TS1-5) showed the reverse pattern (Table 5.4). Annotation of these SNPs revealed that 5 of them lay within 50kb distance of a gene with one SNP (SNP_TM3) being located in the first exon of the CCRL2 gene (Table 5.4). This gene belongs to the C-C chemokine receptor gene family (CCR1, CCR2, CCR3, CCR4, CCR5, CCR8, CC1L1, CCRL2, and CX3CR1), which are membrane proteins that bind to cytokines (Lira & Furtado, 2012). Chemokines are known to play a role in inflammation and autoimmunity, as well as homeostasis (Huber et al., 2008; Lira & Furtado, 2012) and CCRL2 has been shown to be integral to the immune response of mammals (Yoshimura & Oppenheim, 2011). C-C chemokines are also associated with a host of other functions in mammals for example CCR2 is linked with obesity and insulin resistance (Neels & Olefsky, 2006; Weisberg et al., 2006), CCR5 to olfactory recognition Kalkonde et al. (2011) and CCR4 with locomotor activity, anxiety and social exploratory behavior (Ambrée et al., 2016).The other genes (ELOC, RAN, TRDMT and Myo5b) within 50kb distance of SNPs that were under positive selection were all transcription

factors that play are role with a host of different cellular processes including RNA polymerase extension, DNA damage, DNA recombination, and mutation repair (Garrett & Garrett, 1994; Melchior et al., 1993; Ren et al., 1993; Subramaniam et al., 2014).

The genetics of behavioral traits is notoriously complex which are predominantly under the control of multiple genes with small effects (polygenic control) and very few genes of large effects. Therefore capturing genetic differences in behavioral traits using traditional GWAS is often difficult. To this end our scans for polygenic discrimination of migratory and sedentary behavior in contrast to classical GWAS resulted in a high number of signficant hits (464 SNPs) associated with 512 genes distributed throughout the bear genome. More interestingly our GO analysis showed that most of these genes played a role in the same metabolic pathway namely in control of olfactory and sensory perception of mammals (Figure 5.14). Positive selection on olfactory receptors have been a frequent observation in domestication of animals (Montague et al., 2014), and moreover it has been shown that a high-fat diet and the abundance of short-chain fatty acids can influence the function olfactory receptors (Pluznick et al., 2013; Primeaux et al., 2013).

The observed life history differences in the Eastern Anatolian brown bear population are driven by foraging and feeding behaviors that are most probably a response to the abundance of human-related resources. Large open garbage dumps like the one in Sarıkamış are a great source of high-content foods rich in fats and carbohydrates which seems to be the main difference between human-related resources and wild caught prey. That all of the associated genes and pathways I captured in relation to the observed behavioral differences are to do with factors related to high-fat diet, immune response and olfactory perception strengthens our initial hypothesis that the behavioral differences I am observing is a response to strong selection brought on by the abundance of human-resources. In addition, it has been shown that the adaptation of the Appenine brown bear to an highly isolated environment in the Appenine mountains of Italy was also associated with genes and pathways regulating the immune system, olfactory reception and diet (Benazzo et al., 2017). This might indicate the general importance of these pathways for adaptation of brown bears to novel environments.

Understanding the genetic architecture of behavioral traits in wild populations still remains a huge challenge especially since the capture of small genetic effects spread throughout hundreds of genes across the genome requires large sample sizes and high

resolution genetic mapping. In our case all our results were based on a very small sample size and on a very small portion of the overall genome (roughly 1-2%). This low resolution inevitably means that I have missed many more potential regions that could be contributing to the observed behavioral shifts and might be the main reasons why I did not capture any significant hits in our GWAS analysis. However, despite the low genomic resolution I was still able to capture a portion of the polygenic basis of genes and pathways controlling behavioral differences between brown bears in Easten Anatolia as well as signatures of selection in functionally important genes.

Sedentary bears stay in Sarıkamış region throughout the year and are mainly feeding from the city dump, while migratory bears migrate for the search of food during the summer and return back to Sarıkamış for hibernation. Even if two different life history traits are observed in the Eastern Anatolian brown bear population, all of the individuals hibernate and probably mate in the region. None of the tracked animals were found to be permanently nomadic or living in different habitats. Animal behaviors can be inherited genetically (S. Arnold, 1981), learned from the environment (Box et al., 1999; Galef Jr & Whiskin, 2001; Heyes, 1994), or they can be influenced by the interaction of both (Kandel et al., 2000). If behavioral differences in the population are genetic or under selection then I might expect higher kinship within behavioral classes and between behavioral classes. Thus, I looked at the kinship relationships of the individuals. I observed that the especially sedentary had a higher degree of kinship and overall, the level of 1st and 2nd degree relatedness was much higher within behavioral groups. Therefore, even though roaming and feeding behavior are undoubtedly learned traits (passed on from mother to cub) based on our analysis there seems to be some indication that these behavioral traits are also inherited to some extent and thus are under genetic control.

All of these results indicate that there might be a strong genetic basis for the observed phenotypic variation in this population. Furthermore, my results also show that the open garbage dump and easy access to human-related food sources could be acting as a strong selective agent that resulted in rapid evolutionary change in about seven generations.

CHAPTER 7:
CONCLUSION AND FUTURE DIRECTIONS

In this study, I examined two distinct life history strategies for the Eastern Anatolian brown bear population due to anthropogenic food resources; sedentary individuals adapted to feeding in a garbage dump and migratory individuals migrating to search for natural food. The Eastern Anatolian brown bear population is significantly differentiated and separated from the other world brown bear populations, but it has high genetic diversity. I also discovered a number of genomic areas and genotypes that are strongly linked to sedentary and migratory behavior, implying that the observed life history heterogeneity may have a substantial genetic basis. The one SNP discovered in the association study has a role in obesity and behavior, as well as the immune system, and the others are transcription factors with roles in cellular functions. The other genes found in polygenic selection analysis indicated the importance of olfactory and sensory perception in discriminating individuals as sedentary and migratory. Our findings suggest that considerable selective pressures for adaptation to human-dominated habitats may be acting on the Eastern Anatolian brown bear population.

Another future study would be perform whole-genome sequencing for the sedentary and migratory individuals to obtain significantly associated SNPs. That will enable the calculation of population genetic parameters such as comprehensive genetic diversity, effective population size and determining the possible genomic regions under selection by fully sequencing a large number of modern genomes from brown bears distributed in Eastern Anatolian and comparing them with other genomes available in databases, and the genetic differences between their conspecifics in the world and with reference to world populations. It is critical to examine the status of alleles and genotypes found to be associated with roaming behavior in other brown bear populations around the world and to determine the frequency and prevalence of behavior-related alleles worldwide.

Another future direction would be to examine the ancient samples from Türkiye with whole-genome sequencing and compare them with the other ancient samples around

the world and modern samples to determine the population demographic history. I can infer the impacts of human-related resources on the evolution and adaptation of brown bears.

Last but not least, it is critical to developing new biological conservation strategies that consider the differences in habitat use of these two different life strategies and minimize the region's human-bear conflict. Detection of which life history trait is predominant in the population using the absolute fitness values of sedentary-migratory bears and the inheritability of sedentary-migratory behavior will enable us to better protect Eastern Anatolian brown bears with their unique life history traits. The city dump could be moved more distance from the city center to decrease the human-bear conflict in the area. To protect the brown bears' natural habitat, the migratory individuals' route may be reforested, and wildlife overpasses should be constructed to decrease the deaths of the animals.

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APPENDICES

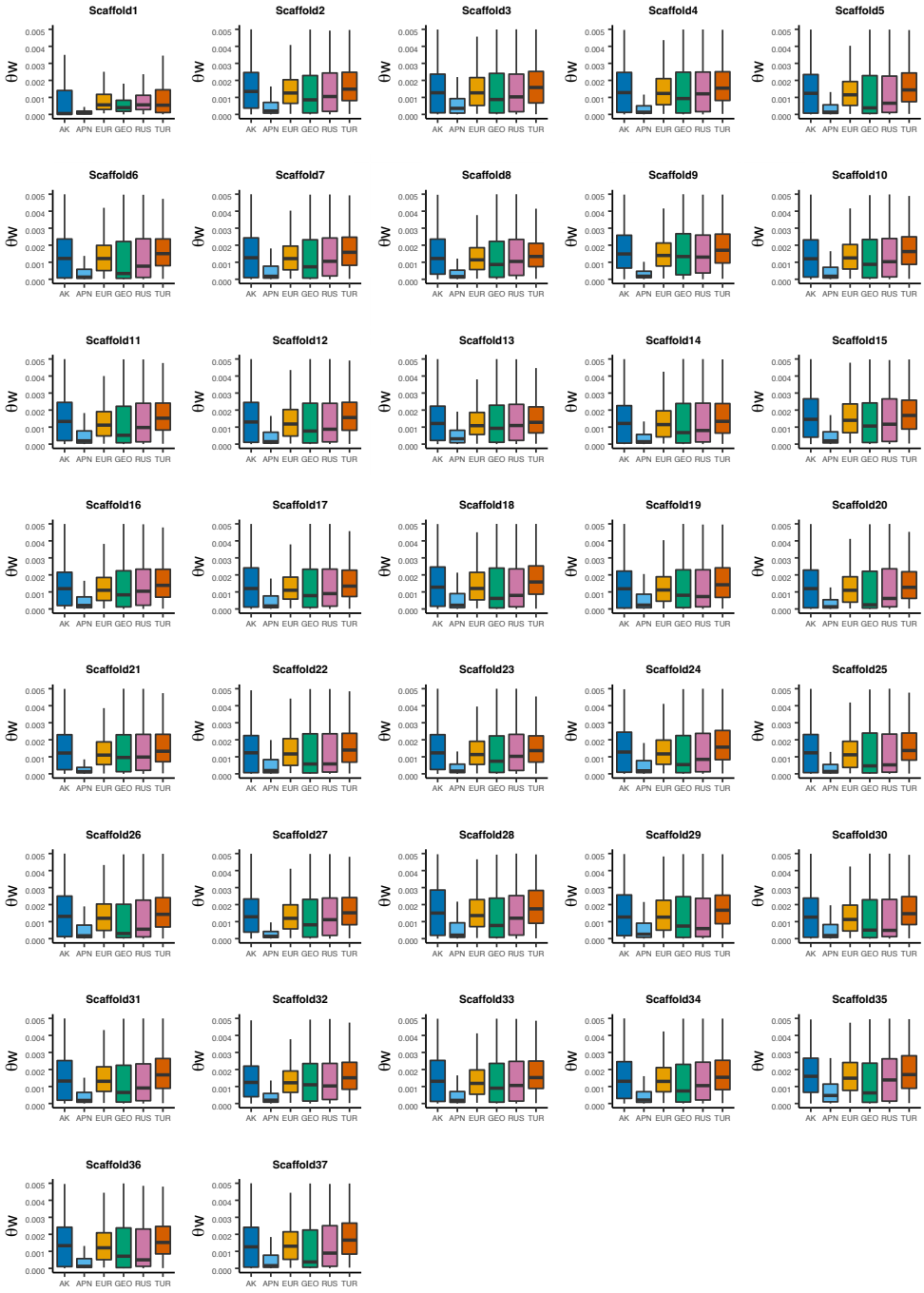
APPENDIX A: The information related to capture brown bears in the study area

AnimalID	AnimalName	Blood Availability	Sex	Age	Cq	DNA concentration (ng/ul)	Capture date
BTR001	Missed female	TRUE	F		20.7	33.2	18.09.2012
BTR002	Forest female	TRUE	F	6-7	19.8	32.6	20.09.2012
BTR003	Chest	TRUE	M	5	19.9	48.7	19.09.2012
BTR004	Cub001	FALSE	M				
BTR005	Bear 333	TRUE	M	8-9	20.8	42.8	22.09.2012
BTR006	Neck	TRUE	M		25.4	66.4	25.09.2012
BTR007	Four fingers	TRUE	M	4	21.8	22.6	26.09.2012
BTR008	Karadayi	TRUE	M		26.3	34.6	29.09.2012
BTR009	Old lady	TRUE	F	15-20	25.1	55.6	1.10.2012
BTR010	Yellow eye	TRUE	F		21.2	29	4.10.2012
BTR011	Hind leg	TRUE	M		20.7	30.1	5.10.2012
BTR012	Scarface	TRUE	M	10	23.6	56.4	11.06.2013 / 29.05.2013
BTR013	Old female (Skin)	TRUE	F	8-10	26.8	25.9	30.05.2013
BTR014	Lionheart	TRUE	M	10-12	21.1	5.1	1.06.2013
BTR015	Forest female 2	TRUE	F	12-14	19.5	76.8	9.06.2013
BTR016	Sabah	TRUE	M	8	21.4	15.3	22.05.2014
BTR017	Tiny	TRUE	F	6-10	20.1	29	27.05.2014 / 25.06.2018
BTR018	Digger	TRUE	M	6	18.5	58.4	28.05.2014
BTR019	Shy guy	TRUE	M	5	20.8	45.3	30.05.2014
BTR020	Blondy	TRUE	M	7	19.1	15.8	30.05.2014
BTR021	Teddy	TRUE	F		19.2	40.4	2.06.2014
BTR022	Sleepy	TRUE	M	9-10	21.1	47.6	5.06.2014
BTR023	Kung-fu	TRUE	F	5.5	21.7	87.6	6.06.2014
BTR024	Small face	TRUE	F	2	20	24	6.10.2016
BTR025	Collar guy	FALSE	M				16.10.2016
BTR026	Buddha	FALSE	M				16.10.2016

BTR027	Back side	FALSE	F				15.10.2016
BTR028	Faruk	TRUE	M		21.8	17.9	26.05.2017 / 1.06.2017
BTR029	Ekrem	TRUE	M	4	24.4	41.2	27.05.2017
BTR030	Cub002	TRUE	F		23	7.9	29.05.2017
BTR031	Tree hugging female	TRUE	F	3	20.3	30.2	30.05.2017
BTR032	Cub003	TRUE	F		25.2	12.7	
BTR033	Rocky	TRUE	M		20.4	26.2	4.06.2017
BTR034	Tarzan	TRUE	M	4	24.2	32.4	6.06.2017
BTR035	Cub004	TRUE	M		22.2	11	
BTR036	Ester	TRUE	F	5-6	22.3	5.8	27.06.2017 / 29.06.2018
BTR037	Nes	TRUE	F		24.4	26.2	28.06.2017
BTR038	Gokberk	TRUE	M		23.7	33	29.06.2017
BTR039	Karadogan	TRUE	M		25.5	7.5	30.06.2017
BTR040	Seha	TRUE	F		29.6	9.5	4.07.2017
BTR041	Mother bear	TRUE	F		18.1	12.8	20.07.2017
BTR042	Ben Ten	TRUE	M	-	24.9	33	16.06.2014
BTR043	Evening beer	TRUE	M	5	19.6	18.4	27.05.2018
BTR044	Bubba	TRUE	M		19	76.4	3.06.2018
BTR045	Kayahan	TRUE	M	5	19.1	27.4	7.06.2018
BTR046	Seha	TRUE	F		19.1	34.8	10.06.2018
BTR047	Princess (ear tag only)	TRUE	F		18.4	38.5	11.06.2018
BTR048	Short ear	TRUE	M	15	20.5	6.4	13.06.2018
BTR049	Hammer	TRUE	M	4	17.4	20.1	30.06.2018
BTR050	Haribo	TRUE	F		20.2	50.8	11.07.2018
BTR051	Two Toes	FALSE	M		21	15.7	01.06.2019
BTR052	Aramis	TRUE	F	6	20.5	23	07.06.2019
BTR053	Cub005	TRUE			22.1	2	09.06.2019
BTR054	Baloo	TRUE	M	10	20.1	26.2	10.06.2019
BTR055	Charger	TRUE	M		20	18	13.06.2019
BTR056	Lip	TRUE	M	4	20.1	11	13.06.2019
BTR057	Defender	TRUE	M	10	20.3	16.9	15.06.2019
BTR058	Eclipse	TRUE	F	8	21.7	10.5	2.07.2019
BTR059	Rocket	TRUE	M		19.29	26	19.07.2019
BTR060	Insomniac	TRUE	M	6	19.5	19	19.07.2019

BTR061	Apollo	TRUE	M		19.5	24.9	20.07.2019

APPENDIX B: Box plots of ThetaW value in Türkiye, European, Apennine, Georgian, Alaskan and Russian populations indicated in red, orange, blue, green, dark blue, and pink colors, respectively. Eastern Anatolian population consists of randomly selected five individuals. Each diversity plot is represents the selected top 37 scaffolds selected based on the length.



APPENDIX C: The genetic assignment scores for each model

Mean of SVM		
origin	Migratory	Sedentary
Migratory	0.94	0.06
Sedentary	0.03	0.97
Standard Deviation of SVM		
origin	Migratory	Sedentary
Migratory	0.15	0.15
Sedentary	0.09	0.09
Mean of LDA		
origin	Migratory	Sedentary
Migratory	0.9	0.1
Sedentary	0.08	0.92
Standard Deviation of LDA		
origin	Migratory	Sedentary
Migratory	0.26	0.26
Sedentary	0.15	0.15
Mean of naive Bayes		
origin	Migratory	Sedentary
Migratory	0.78	0.22
Sedentary	0.1	0.9
Standard Deviation of naive Bayes		
origin	Migratory	Sedentary
Migratory	0.31	0.31
Sedentary	0.2	0.2
Mean of decision tree		
origin	Migratory	Sedentary
Migratory	0.82	0.18
Sedentary	0.07	0.93

Standard Deviation of decision tree		
origin	Migratory	Sedentary
Migratory	0.26	0.26
Sedentary	0.15	0.15
Mean of Random Forest		
origin	Migratory	Sedentary
Migratory	0.8	0.2
Sedentary	0.04	0.96
Standard Deviation of Random Forest		
origin	Migratory	Sedentary
Migratory	0.28	0.28
Sedentary	0.1	0.1

APPENDIX D: The annotated genes obtained from Random Forest analysis

Uniprot_ID_human	Gene_Name	Uniprot_ID
Q75V66	ANO5_HUMAN	Q75V66
O00203	AP3B1_HUMAN	O00203
Q13367	AP3B2_HUMAN	Q13367
Q8N6T3	ARFG1_HUMAN	Q8N6T3
P98196	AT11A_MOUSE	P98196
Q9Y2G3	AT11B_HUMAN	Q9Y2G3
Q8NB49	AT11C_HUMAN	Q8NB49
O75110	ATP9A_MOUSE	O70228
O43861	ATP9B_RAT	D4ABB8
O75366	AVIL_HUMAN	O75366
Q9P1Z9	CC180_HUMAN	Q9P1Z9
P51676	CC1L1_MOUSE	P51676
P32246	CCR1_HUMAN	P32246
P41597	CCR2_HUMAN	P41597
P51677	CCR3_RAT	O54814
P51679	CCR4_MOUSE	P51680
P51681	CCR5_MOUSE	P51682
P51685	CCR8_MOUSE	P56484
O00421	CCRL2_HUMAN	O00421
P86790	CCZ1B_HUMAN	P86790
Q07092	COGA1_HUMAN	Q07092
Q07092	COGA1_MOUSE	Q8BLX7
P49238	CX3C1_BOVIN	A6QNL7
Q86SQ9	DHDDS_MOUSE	Q99KU1
Q7L190	DPPA4_HUMAN	Q7L190
O60469	DSCAM_RAT	Q8VHZ8
Q8TD84	DSCL1_MOUSE	Q4VA61
A6H8Z2	F221B_HUMAN	A6H8Z2
P08101	FCGR2_MOUSE	P08101
Q9C0D6	FHDC1_HUMAN	Q9C0D6

Q9NZU1	FLRT1_MOUSE	Q6RKD8
O43155	FLRT2_MOUSE	Q8BLU0
Q9NZU0	FLRT3_HUMAN	Q9NZU0
Q9HBA9	FOH1B_HUMAN	Q9HBA9
Q04609	FOLH1_MOUSE	O35409
O95684	FR1OP_HUMAN	O95684
O60318	GANP_HUMAN	O60318
O95166	GBRAP_RAT	P60517
Q9H0R8	GBRL1_RAT	Q0VGK0
P56159	GFRA1_MOUSE	P97785
Q92805	GOGA1_HUMAN	Q92805
Q96SL4	GPX7_HUMAN	Q96SL4
Q7LDG7	GRP2_HUMAN	Q7LDG7
Q8IV61	GRP3_HUMAN	Q8IV61
Q8TDF6	GRP4_HUMAN	Q8TDF6
P48507	GSH0_RAT	P48508
P35895	GU03_RAT	P35895
Q9NWT6	HIF1N_HUMAN	Q9NWT6
O43301	HS12A_HUMAN	O43301
Q96MM6	HS12B_HUMAN	Q96MM6
Q9UEF7	KLOT_RAT	Q9Z2Y9
Q86Z14	KLOTB_HUMAN	Q86Z14
Q14558	KPRA_MOUSE	Q9D0M1
O60256	KPRB_PONAB	Q5RBA8
A6NIV6	LRIQ4_HUMAN	A6NIV6
Q13021	MALL_HUMAN	Q13021
Q9UBB5	MBD2_MOUSE	Q9Z2E1
O95983	MBD3_HUMAN	O95983
Q96RN5	MED15_MOUSE	Q924H2
Q5VTT5	MYOM3_HUMAN	Q5VTT5
Q9Y3Q0	NALD2_HUMAN	Q9Y3Q0
O94856	NFASC_HUMAN	O94856
Q9HAN9	NMNA1_HUMAN	Q9HAN9
Q9BZQ4	NMNA2_MOUSE	Q8BNJ3
Q96T66	NMNA3_HUMAN	Q96T66

P29475	NOS1_RABIT	O19132
P35228	NOS2_HUMAN	P35228
P29474	NOS3_PIG	Q28969
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Q92823	NRCAM_HUMAN	Q92823
	O1002_MOUSE	Q8VFK2
	O1009_MOUSE	Q8VFK1
	O1013_MOUSE	Q7TR96
	O1019_MOUSE	Q8VGS3
	O1020_MOUSE	Q8VFK7
	O1030_MOUSE	Q8VFL5
	O1044_MOUSE	Q8VGR9
	O1052_MOUSE	Q8VGR8
	O1073_RAT	P35898
	O1078_RAT	P23265
	O1082_RAT	P23268
	O1086_MOUSE	Q8VFL9
	O1094_MOUSE	Q8VF13
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Q9H207	O10A5_HUMAN	Q9H207
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Q8NH08	O10AC_HUMAN	Q8NH08
Q8NGE0	O10AD_HUMAN	Q8NGE0
Q8NH19	O10AG_HUMAN	Q8NH19
Q96KK4	O10C1_HUMAN	Q96KK4
Q8NH80	O10D3_HUMAN	Q8NH80
Q8NGN7	O10D4_HUMAN	Q8NGN7
Q8NGC3	O10G2_HUMAN	Q8NGC3
Q8NGC4	O10G3_HUMAN	Q8NGC4
Q8NGN3	O10G4_HUMAN	Q8NGN3
Q8NH81	O10G6_HUMAN	Q8NH81
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Q8NGN5	O10G8_HUMAN	Q8NGN5

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O60403	O10H2_HUMAN	O60403
O60404	O10H3_HUMAN	O60404
Q8NGA5	O10H4_HUMAN	Q8NGA5
P30954	O10J1_HUMAN	P30954
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P0C629	O10J4_HUMAN	P0C629
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Q8NGX5	O10K1_HUMAN	Q8NGX5
Q6IF99	O10K2_HUMAN	Q6IF99
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Q8NGQ4	O10Q1_HUMAN	Q8NGQ4
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Q8NGN2	O10S1_HUMAN	Q8NGN2
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Q8NGI7	O10V1_HUMAN	Q8NGI7
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Q8NGY1	O10Z1_HUMAN	Q8NGY1
	O1102_MOUSE	Q8VES2
	O1174_RAT	P35896
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Q8NGC1	O11G2_HUMAN	Q8NGC1
Q8NH07	O11H2_HUMAN	Q8NH07
Q8NGC9	O11H4_HUMAN	Q8NGC9
Q8NGC7	O11H6_HUMAN	Q8NGC7
Q8NGC8	O11H7_HUMAN	Q8NGC8
Q8NGX0	O11L1_HUMAN	Q8NGX0
P0DN82	O12D1_HUMAN	P0DN82
P58182	O12D2_HUMAN	P58182
Q9UGF7	O12D3_HUMAN	Q9UGF7
	O1361_RAT	P23266
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Q8NGS9	O13C2_HUMAN	Q8NGS9

Q8NGS6	O13C3_HUMAN	Q8NGS6
Q8NGS5	O13C4_HUMAN	Q8NGS5
P0DN81	O13C7_HUMAN	P0DN81
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Q8NGT2	O13J1_HUMAN	Q8NGT2
	O1440_MOUSE	Q8VFV4
	O1444_MOUSE	Q8VFX2
	O1468_RAT	P23274
	O1493_RAT	P23271
	O1496_RAT	P23269
Q96R54	O14A2_HUMAN	Q96R54
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Q8NHC7	O14CZ_HUMAN	Q8NHC7
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Q8NHC6	O14L1_HUMAN	Q8NHC6
	O1500_RAT	P23273
	O1509_MOUSE	Q7TQQ0
	O1537_MOUSE	P34983
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Q96R47	O2A14_HUMAN	Q96R47
A4D2G3	O2A25_HUMAN	A4D2G3
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Q9H205	O2AG1_HUMAN	Q9H205
A6NM03	O2AG2_HUMAN	A6NM03
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Q8NG84	O2AK2_HUMAN	Q8NG84
Q8NGE2	O2AP1_HUMAN	Q8NGE2
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Q8NH01	O2T11_HUMAN	Q8NH01
Q8NH04	O2T27_HUMAN	Q8NH04
Q8NG76	O2T33_HUMAN	Q8NG76
Q8NGL6	O4A15_HUMAN	Q8NGL6
Q8NH70	O4A16_HUMAN	Q8NH70
Q6IF82	O4A47_HUMAN	Q6IF82
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A6NHA9	O4C46_HUMAN	A6NHA9
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O95013	O4F21_HUMAN	O95013
Q6IFG1	O52E8_HUMAN	Q6IFG1
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Q9NZP5	O5AC2_HUMAN	Q9NZP5
Q8NH90	O5AK2_HUMAN	Q8NH90
Q8NH89	O5AK3_HUMAN	Q8NH89
P0C617	O5AL1_HUMAN	P0C617
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Q8NGF4	O5AP2_HUMAN	Q8NGF4
Q8N127	O5AS1_HUMAN	Q8N127
Q8NGC0	O5AU1_HUMAN	Q8NGC0
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A6NDL8	O6C68_HUMAN	A6NDL8
A6NIJ9	O6C70_HUMAN	A6NIJ9
A6NCV1	O6C74_HUMAN	A6NCV1
A6NL08	O6C75_HUMAN	A6NL08
A6NM76	O6C76_HUMAN	A6NM76
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Q6IF36	O8G2P_HUMAN	Q6IF36
	OL139_MOUSE	Q60891
	OL140_MOUSE	Q60878
	OL141_MOUSE	Q60880
	OL142_MOUSE	Q60881
	OL143_MOUSE	P34985
	OL145_MOUSE	Q60882

	OL146_MOUSE	Q60884
	OL147_MOUSE	Q60886
	OL148_MOUSE	Q60887
	OL149_MOUSE	Q60888
	OL150_MOUSE	Q60895
	OL151_MOUSE	Q60893
	OL183_MOUSE	Q8VFB9
	OL186_MOUSE	Q8VEX5
	OL187_MOUSE	Q8VEX6
	OL226_RAT	P23270
	OL287_RAT	P23267
	OL469_MOUSE	Q8VF66
	OL473_MOUSE	Q8VG44
	OL474_MOUSE	Q8VFC9
	OL477_MOUSE	Q8VGI6
	OL478_MOUSE	Q8VG04
	OL480_MOUSE	Q8VEZ0
	OL481_MOUSE	Q8VGI5
	OL482_MOUSE	Q8VG03
	OL483_MOUSE	Q8VG05
	OL484_MOUSE	Q8VFD3
	OL486_MOUSE	Q8VFD0
	OL488_MOUSE	Q8VG02
	OL490_MOUSE	Q8VFD2
	OL491_MOUSE	Q8VG06
	OL492_MOUSE	Q8VFD1
	OL493_MOUSE	Q8VEW5
	OL494_MOUSE	Q8VG07
	OL498_MOUSE	Q8VEW2
	OL502_MOUSE	Q8VG09
	OL507_MOUSE	Q8VG13
	OL508_MOUSE	Q8VG42
	OL510_MOUSE	Q8VEW6
	OL867_MOUSE	Q7TRF3
	OL867_RAT	P34987

	OL958_MOUSE	Q8VEY3
	OL998_MOUSE	Q8VF76
	OLF1_CANLF	Q95154
	OLF1_MOUSE	Q8VGI1
	OLF10_MOUSE	Q60883
	OLF11_MOUSE	Q60890
	OLF12_MOUSE	Q60894
	OLF13_MOUSE	P34984
	OLF15_MOUSE	P23275
	OLF18_MOUSE	Q0VAX9
	OLF19_MOUSE	Q9JHB2
	OLF2_CANLF	Q95155
	OLF24_MOUSE	Q8VFM9
	OLF3_CANLF	Q95156
	OLF3_MOUSE	Q60879
	OLF4_CANLF	Q95157
	OLF49_MOUSE	Q9Z1V0
	OLF5_MOUSE	Q60889
	OLF50_MOUSE	Q8VGK5
	OLF56_MOUSE	Q8VGD6
	OLF6_MOUSE	P34986
	OLF63_MOUSE	Q8VBW9
	OLF8_MOUSE	Q60892
	OLF9_MOUSE	Q60885
	OLFD_CANLF	P30955
	OLFI9_RAT	P23272
Q9UHM6	OPN4_FELCA	Q5YKK9
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Q9Y585	OR1A2_HUMAN	Q9Y585
Q15619	OR1C1_HUMAN	Q15619
P34982	OR1D2_GORGO	Q9TU93
P58170	OR1D5_PANPA	Q9TU95
P47887	OR1E2_HUMAN	P47887
Q8WZA6	OR1E3_HUMAN	Q8WZA6

O43749	OR1F1_HUMAN	O43749
Q96R84	OR1F2_HUMAN	Q96R84
Q8NHA8	OR1FC_HUMAN	Q8NHA8
P47890	OR1G1_GORGO	Q9TU86
O60431	OR1I1_HUMAN	O60431
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Q8NGS2	OR1J2_HUMAN	Q8NGS2
Q8NGS1	OR1J4_HUMAN	Q8NGS1
Q8NGR3	OR1K1_HUMAN	Q8NGR3
Q8NH94	OR1L1_HUMAN	Q8NH94
Q8NH93	OR1L3_HUMAN	Q8NH93
Q8NGR2	OR1L6_HUMAN	Q8NGR2
Q8NGR8	OR1L8_HUMAN	Q8NGR8
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Q8NGR9	OR1N2_HUMAN	Q8NGR9
Q8NH06	OR1P1_HUMAN	Q8NH06
Q15612	OR1Q1_HUMAN	Q15612
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Q8NGT9	OR2A1_HUMAN	Q8NGT9
Q6IF42	OR2A2_HUMAN	Q6IF42
O95047	OR2A4_HUMAN	O95047
Q96R48	OR2A5_HUMAN	Q96R48
Q9GZK3	OR2B2_HUMAN	Q9GZK3
O76000	OR2B3_HUMAN	O76000
P58173	OR2B6_HUMAN	P58173
P59922	OR2B8_HUMAN	P59922
Q5JQS5	OR2BB_HUMAN	Q5JQS5
O95371	OR2C1_HUMAN	O95371
Q8N628	OR2C3_HUMAN	Q8N628
Q9H210	OR2D2_HUMAN	Q9H210
Q8NGH3	OR2D3_HUMAN	Q8NGH3
O95006	OR2F2_HUMAN	O95006
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Q5TZ20	OR2G6_HUMAN	Q5TZ20
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O95918	OR2H2_HUMAN	O95918
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O76001	OR2J3_HUMAN	O76001
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Q8NG85	OR2L3_HUMAN	Q8NG85
Q8N349	OR2LD_HUMAN	Q8N349
Q96R28	OR2M2_HUMAN	Q96R28
Q96R27	OR2M4_HUMAN	Q96R27
Q9NQN1	OR2S1_HUMAN	Q9NQN1
O43869	OR2T1_HUMAN	O43869
Q6IF00	OR2T2_HUMAN	Q6IF00
Q8NH00	OR2T4_HUMAN	Q8NH00
Q8NHC8	OR2T6_HUMAN	Q8NHC8
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Q96R30	OR2V2_HUMAN	Q96R30
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P47881	OR3A1_GORGO	Q9TU89
P47893	OR3A2_HUMAN	P47893
P47888	OR3A3_PANTR	Q9TUA0
P47883	OR3A4_HUMAN	P47883
Q8NH83	OR4A5_HUMAN	Q8NH83
P0C604	OR4A8_HUMAN	P0C604
Q8NGF8	OR4B1_HUMAN	Q8NGF8
Q8NH37	OR4C3_HUMAN	Q8NH37
Q8NGB2	OR4C5_HUMAN	Q8NGB2
Q8NH72	OR4C6_HUMAN	Q8NH72
Q6IEV9	OR4CB_HUMAN	Q6IEV9
Q96R67	OR4CC_HUMAN	Q96R67

Q8NGP0	OR4CD_HUMAN	Q8NGP0
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Q15615	OR4D1_HUMAN	Q15615
P58180	OR4D2_HUMAN	P58180
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Q8NGI6	OR4DA_HUMAN	Q8NGI6
Q8NGI4	OR4DB_HUMAN	Q8NGI4
P0C645	OR4E1_HUMAN	P0C645
Q8NGC2	OR4E2_HUMAN	Q8NGC2
Q8NH21	OR4F5_HUMAN	Q8NH21
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Q8NGD2	OR4K2_HUMAN	Q8NGD2
Q96R72	OR4K3_HUMAN	Q96R72
Q8NGD3	OR4K5_HUMAN	Q8NGD3
Q8NH42	OR4KD_HUMAN	Q8NH42
Q8NGD5	OR4KE_HUMAN	Q8NGD5
Q8NH41	OR4KF_HUMAN	Q8NH41
Q8NGC6	OR4KH_HUMAN	Q8NGC6
Q8NH43	OR4L1_HUMAN	Q8NH43
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Q8NGD1	OR4N2_HUMAN	Q8NGD1
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Q8IXE1	OR4N5_HUMAN	Q8IXE1
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Q8NH73	OR4S2_HUMAN	Q8NH73
Q8NH49	OR4X1_HUMAN	Q8NH49
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Q8NH48	OR5B3_HUMAN	Q8NH48
Q96R08	OR5BC_HUMAN	Q96R08
Q8NGF7	OR5BH_HUMAN	Q8NGF7
A6NL26	OR5BL_HUMAN	A6NL26
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Q8NGL4	OR5DD_HUMAN	Q8NGL4
Q8NGL3	OR5DE_HUMAN	Q8NGL3
Q8NGL1	OR5DI_HUMAN	Q8NGL1
O95221	OR5F1_HUMAN	O95221
P0C626	OR5G3_HUMAN	P0C626
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Q8NGV6	OR5H6_HUMAN	Q8NGV6
P0DN80	OR5H8_HUMAN	P0DN80
Q13606	OR5I1_HUMAN	Q13606
Q8NH18	OR5J2_HUMAN	Q8NH18
Q8NHB8	OR5K2_HUMAN	Q8NHB8
A6NET4	OR5K3_HUMAN	A6NET4
A6NMS3	OR5K4_HUMAN	A6NMS3
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Q8NGP6	OR5M8_HUMAN	Q8NGP6
Q8NGP3	OR5M9_HUMAN	Q8NGP3
Q6IEU7	OR5MA_HUMAN	Q6IEU7
Q96RB7	OR5MB_HUMAN	Q96RB7
Q8WZ92	OR5P2_HUMAN	Q8WZ92
Q8WZ94	OR5P3_HUMAN	Q8WZ94
Q8NH85	OR5R1_HUMAN	Q8NH85
Q8NG75	OR5T1_HUMAN	Q8NG75
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Q8NGG3	OR5T3_HUMAN	Q8NGG3
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Q8NH69	OR5W2_HUMAN	Q8NH69

O95222	OR6A2_HUMAN	O95222
O95007	OR6B1_HUMAN	O95007
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Q9NZP0	OR6C3_HUMAN	Q9NZP0
Q8NGE1	OR6C4_HUMAN	Q8NGE1
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Q8NGC5	OR6J1_HUMAN	Q8NGC5
Q8NGY2	OR6K2_HUMAN	Q8NGY2
Q8NGY3	OR6K3_HUMAN	Q8NGY3
Q8NGW6	OR6K6_HUMAN	Q8NGW6
Q8NGM8	OR6M1_HUMAN	Q8NGM8
Q8NGY5	OR6N1_HUMAN	Q8NGY5
Q8NGY6	OR6N2_HUMAN	Q8NGY6
Q8NGX9	OR6P1_HUMAN	Q8NGX9
Q8NGQ2	OR6Q1_HUMAN	Q8NGQ2
Q8NH40	OR6S1_HUMAN	Q8NH40
Q8NGN1	OR6T1_HUMAN	Q8NGN1
Q8N148	OR6V1_HUMAN	Q8N148
Q8NH79	OR6X1_HUMAN	Q8NH79
Q8NGX8	OR6Y1_HUMAN	Q8NGX8
Q8NGA2	OR7A2_HUMAN	Q8NGA2
Q15622	OR7A5_HUMAN	Q15622
O76100	OR7AA_HUMAN	O76100
O14581	OR7AH_HUMAN	O14581
O76099	OR7C1_HUMAN	O76099
O60412	OR7C2_HUMAN	O60412
Q96RA2	OR7D2_HUMAN	Q96RA2
Q8NG98	OR7D4_HUMAN	Q8NG98
Q8NGA0	OR7G1_HUMAN	Q8NGA0
Q8NG99	OR7G2_HUMAN	Q8NG99
Q8NG95	OR7G3_HUMAN	Q8NG95
P0DMU2	OR83P_HUMAN	P0DMU2

Q8NGG7	OR8A1_HUMAN	Q8NGG7
Q96RD0	OR8B2_HUMAN	Q96RD0
Q96RC9	OR8B4_HUMAN	Q96RC9
Q15620	OR8B8_HUMAN	Q15620
Q8NGG6	OR8BC_HUMAN	Q8NGG6
Q8WZ84	OR8D1_HUMAN	Q8WZ84
Q9GZM6	OR8D2_HUMAN	Q9GZM6
Q8NGM9	OR8D4_HUMAN	Q8NGM9
Q15617	OR8G1_HUMAN	Q15617
Q8NG78	OR8G5_HUMAN	Q8NG78
Q8NGG4	OR8H1_HUMAN	Q8NGG4
Q8N162	OR8H2_HUMAN	Q8N162
Q8N0Y5	OR8I2_HUMAN	Q8N0Y5
Q8NGP2	OR8J1_HUMAN	Q8NGP2
Q8NGG1	OR8J2_HUMAN	Q8NGG1
Q8NGG0	OR8J3_HUMAN	Q8NGG0
Q8NGG5	OR8K1_HUMAN	Q8NGG5
Q8NH51	OR8K3_HUMAN	Q8NH51
Q8NH50	OR8K5_HUMAN	Q8NH50
Q8NH09	OR8S1_HUMAN	Q8NH09
P0C7N1	OR8U8_HUMAN	P0C7N1
P0C7N5	OR8U9_HUMAN	P0C7N5
Q8NGU2	OR9A4_HUMAN	Q8NGU2
Q8NH87	OR9G1_HUMAN	Q8NH87
Q8NGQ1	OR9G4_HUMAN	Q8NGQ1
Q8NGQ6	OR9I1_HUMAN	Q8NGQ6
Q8NGE7	OR9K2_HUMAN	Q8NGE7
Q8NGQ5	OR9Q1_HUMAN	Q8NGQ5
Q8NGE9	OR9Q2_HUMAN	Q8NGE9
Q6UXH9	PAMR1_PONAB	Q5RDI1
O75051	PLXA2_HUMAN	O75051
P51805	PLXA3_RAT	D3ZPX4
Q9HCM2	PLXA4_HUMAN	Q9HCM2
P27169	PON1_HUMAN	P27169
Q15165	PON2_MOUSE	Q62086

Q15166	PON3_HUMAN	Q15166
Q9Y2K5	R3HD2_MOUSE	Q80TM6
Q68EM7	RHG17_HUMAN	Q68EM7
Q04912	RON_HUMAN	Q04912
Q8IYM1	SEP12_HUMAN	Q8IYM1
Q9UH03	SEPT3_RAT	Q9WU34
Q9UHD8	SEPT9_RAT	Q9QZR6
Q9H2V7	SPNS1_BOVIN	Q08DX7
Q6ZMD2	SPNS3_HUMAN	Q6ZMD2
A7MD48	SRRM4_HUMAN	A7MD48
Q5T011	SZT2_HUMAN	Q5T011
Q9H4B7	TBB1_MOUSE	A2AQ07
Q13509	TBB3_RAT	Q4QRB4
P68371	TBB4B_MESAU	P86221
Q3ZCM7	TBB8_PANTR	Q8WP14
Q9BT49	THAP7_HUMAN	Q9BT49
Q96FV9	THOC1_MOUSE	Q8R3N6
Q9NS62	THSD1_MOUSE	Q9JM61
P28289	TMOD1_HUMAN	P28289
Q9NZR1	TMOD2_HUMAN	Q9NZR1
Q9NZQ9	TMOD4_MOUSE	Q9JLH8
Q9Y5L0	TNPO3_MOUSE	Q6P2B1
Q8WVT3	TPC12_HUMAN	Q8WVT3
A0JNW5	UH1BL_HUMAN	A0JNW5
P09327	VILI_MOUSE	Q62468
O15195	VILL_HUMAN	O15195
Q6ZS81	WDFY4_HUMAN	Q6ZS81
A6NP61	ZAR1L_HUMAN	A6NP61
Q9UPR6	ZFR2_HUMAN	Q9UPR6