Genetic changes influence behavioral shifts of brown bears in response to human-dominated landscapes in Eastern Türkiye

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Abstract

Large carnivores are known for altering their life-history strategies in response to environmental change. One such shift was recently discovered in Eurasian brown bears (*Ursus arctos arctos*) in Sarıkamış, Eastern Türkiye where an open city garbage dump has led to the emergence of two distinct life strategists: sedentary bears that use the city dump as a primary food source and migratory bears that avoid the dump and migrate in search of food. Understanding the genetic processes that have led to the establishment of these strategies is vital for predicting the overall impact of anthropogenic pressures on brown bears forced to live in human-dominated landscapes. To this end, we determined the amounts of genetic and adaptive variation associated with these two life-history strategies using genome-wide data obtained from 31 bears fitted with satellite collars and tracked for an average of one year. We found that the Eastern Türkiye brown bear population is genetically highly differentiated and isolated from other world populations but nevertheless contains high genetic diversity and mixed ancestry. We identified genomic regions and distinct genotypes associated with sedentary and migratory behavior and high differentiation between behavioral groups at these loci. Outlier loci were associated with several genes related to transcriptional modification, including a key SNP within the first exon of CCRL2 that regulates immune response. Collectively, our results present the first test of the genetic basis of behavioral shifts that may be playing an important role in the ongoing adaptation of Eastern Türkiye brown bears to human-dominated landscapes, emphasizing the importance of evolutionary genomics for understanding how species survive and adapt to global change.

Introduction

Rapid expansion of urban areas has resulted in large-scale reduction and destruction of natural habitats forcing many wildlife species to live in and adapt to human-dominated landscapes (Hunter, 2007; Goudie & Viles, 2013; Chapron et al., 2014). Environmental variation induced by anthropogenic pressures can have important consequences on the behavior, life history, movement patterns, and habitat selection of species (Nelson, 1998; Penteriani et al., 2018; Zarzo-Arias et al., 2018). For example, black-tailed gulls (*Larus crassirostris*) are influenced by spatiotemporal variation in anthropogenic food resources during foraging trips and selection of feeding grounds (Yoda et al., 2012). Similarly, some migrant white storks (*Ciconia ciconia*), have changed their life history from migratory to year-round residency to access additional food sources (Massemin-Challet et al., 2006). Significant dietary shifts were observed in the spotted hyenas (*Crocuta crocuta*) in northern Ethiopia due to the abundance of anthropogenic food sources (Yirga et al., 2012). Furthermore, vervet monkeys (*Chlorocebus pygerythrus*) became more aggressive when fed garbage and spent less time foraging and reproduced more (Lee et al., 1986).

Such behavioral or ecological responses of wildlife to human induced change will undoubtedly depend on both the sensitivity and adaptability of specific species to alterations in their landscape (Sih et al., 2011; Lowry et al., 2013; Wong & Candolin, 2015; Larson et al., 2016; Birnie-Gauvin et al., 2017; Tucker et al., 2018). In this regard, cognitively complex species show remarkable behavioral plasticity in coping with novel and constantly changing environments, resulting in distinct alternative behaviors within populations (Martin et al., 2010; Ordiz et al., 2013; Flack et al., 2016). Brown bears (*Ursus arctos*) are one of the most flexible species in adapting to new environments created by human activity (Cozzi et al., 2016; De Gabriel Hernando et al., 2020; De Angelis et al., 2021). The presence of anthropogenic food sources can have significant consequences on the space use and home range of brown bears, which often shift to accommodate these alternative food sources (Dahle & Swenson, 2003). Bears are highly opportunistic omnivores well known for using new environments and nutrient sources provided by humans, such as house or city dumps, orchards, beehives, poultry, and campsites (Martin et al., 2010; Ordiz et al., 2014; Kavčič et al., 2015; Cozzi et al., 2016; Krofel et al., 2017; Ordiz et al., 2017; Selva et al., 2017; De Angelis et al., 2021; Penteriani et al., 2021). Such tendencies increase human-bear interactions and have caused brown bears to be regarded as “nuisance” or “problem” animals (Kavčič et al., 2015). Furthermore, this behavioral flexibility has the potential to result in the emergence of new life histories and nutritional strategies specialized to exploit anthropogenic resources (Kaczensky et al., 2006; Martin et al., 2010; Ordiz et al., 2014).

Possibly the most striking observation supporting this prediction comes from Sarıkamış, Eastern Türkiye, where the availability of a large, open city dump has led to the emergence of two distinct life-history strategies: “sedentary bears” that use the city dump regularly as a primary food source and “migratory bears” that avoid the dump and migrate in search of food (Cozzi et al., 2016). This observation suggests that the year-round availability of a high-calorie food source may be an important factor in influencing the movement and feeding patterns of brown bears in Eastern Türkiye and could potentially be acting as a strong selective agent.

Here, we use genome-wide data to determine the genetic structure, diversity, and adaptive variation of brown bears in Eastern Türkiye and explore the genetic basis of sedentary and migratory behavior. Our results show that the Eastern Türkiye brown bear population is highly differentiated and isolated from other world populations but nevertheless contains high genetic diversity and mixed ancestry. We also determined high genetic differentiation at several genomic regions and distinct genotypes associated with sedentary and migratory behavior. We conclude that life history changes of brown bears in response to human-dominated landscapes in Eastern Türkiye may have a substantial genetic component.

Results

GPS tracking and movement modeling confirm the existence of two life history strategies.

To determine the movement behavior of brown bears in Eastern Türkiye we used GPS-collar data from 31 brown bears (10 female, 21 male) captured around the Sarıkamış, Kars province of Türkiye (Fig. 1) and tracked successfully for an average of 370 days (range: 126–726) (Table S1). From this data, we calculated the net squared displacement (NSD) of each individual by measuring the square of the distance from the animal's starting point to successive daily locations (Bunnefeld et al., 2011). The NSD data were then integrated into a nonlinear hierarchical modeling framework using the R package migrateR (Spitz et al., 2017) to classify individuals into five a priori non-linear models, representing (1) year-round residency, (2) nomad, (3) disperser, (4) migrant, and (5) mix-migrant movement behavior (Bunnefeld et al., 2011; Börger & Fryxell, 2012).

Out of the 31 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 S1, Movement Figures S1-S31). Following Cozzi et al. (2016), and to simplify genetic analyses we grouped nomadic and resident bears into a single category called “sedentary”, and migrant and mix-migrants into a second category called “migratory” (Table S1). Out of the 16 individuals modeled for movement patterns by Cozzi et al. (2016), we converged on the same results for 12 of them (Table S1). 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 (Table S1). For the remaining two individuals (BTR016 and BTR019), GPS data were not complete in Cozzi et al. (2016) and thus were not classified previously. Our analysis of NDS patterns confirms the presence of two different life-history strategies, sedentary vs. migratory, in Eastern Türkiye brown bears.

RADseq provides high quality genome-wide information for comparing Eastern Türkiye brown bears to their conspecifics.

To produce a high-quality genomic resource for the 31 GPS-tracked brown bears, we sampled the genome of each individual by paired end RAD sequencing (RADseq; Miller et al., 2007; Baird et al., 2008; Etter et al., 2012) using the Sfb1 restriction enzyme and the Illumina sequencing platform. Sequenced reads were aligned to the chromosome length assembly of the American black bear genome, which had the most complete assembly among available bear genomes (Dudchenko et al., 2017; Dudchenko et al., 2018; Srivastava et al., 2019).

Mapping success to the reference genome was high with over 99% of the reads mapped per individual (Table S1). Additionally, whole-genome sequences (WGS) of brown bears from Georgia, Russia, Greece, Slovakia, Slovenia, Austria, and Alaska (Cahill et al., 2013; Benazzo et al., 2017; Barlow et al., 2018) were downloaded from the Sequence Read Archive (SRA) at NCBI (Table S2) and aligned to the same reference genome.

In total we interrogated 21,745,475 sites that overlapped between the RADseq and WGS data, had an average per individual read depth of 6X and were present in at least 50% of the eastern Türkiye brown bears. Polymorphic sites (i.e., SNPs) were identified using the probabilistic framework in the program ANGSD (Korneliussen et al., 2014) and we discovered 108,777 SNPs with a minimum minor allele frequency (MAF) of 0.05 and a significance threshold of $P < 10 - 06$.

Eastern Türkiye brown bears are characterized by distinct genetic structure, mixed ancestry and high diversity.

To determine genetic structure and ancestry of Eastern Türkiye brown bears together with their conspecifics around the world, we conducted PCA, and calculated admixture proportions based on genotype likelihoods (Skotte et al., 2013; Meisner & Albrechtsen, 2018) using the genome-wide data described above. Eastern (Türkiye, Iran, and Georgia) and Apennine brown bears were separated from the remaining populations (Europe, Russia and Alaska) along PC1, while the Russian and Alaskan samples further separated from the European samples (Greece, Slovakia, Slovenia and Austria) along PC2 (Fig. 2A). Although clustered together relative to other populations and geographically close, the Iranian and Georgian brown bear samples were still highly distinct from the Eastern Türkiye brown bears (Fig. 2A). A separate PCA using only the Eastern Türkiye samples revealed minimal structure within this population and showed no apparent difference between sedentary and migratory individuals (Fig. 2B).

Admixture analysis gave similar results to those of the PCA and determined K = 5 as the optimal number of clusters based on Evanno’s method (Figure S1, Evanno et al., 2005). The three main groups along PC1 (Apennine; Europe + Russia + Alaska; and Eastern brown bears) separated into distinct ancestry classes with only the Georgian and Iranian bears showing slight levels of shared ancestry with European (Greece, Slovakia, Slovenia, and Austria) bears at K = 5 (Fig. 2C). Interestingly, Eastern brown bears (Eastern Türkiye, Iran, Georgia) showed higher amounts of mixed ancestry when compared to the rest of the world populations (Apennine, Europe, Russia, and Alaska) which showed mono ancestry even at higher K values (Fig. 2C). Finally, admixture analysis captured no difference between sedentary and migratory bears within Eastern Türkiye (Fig. 2C).

To summarize the genetic diversity of Eastern Türkiye brown bears in comparison to its conspecifics around the world, we calculated Watterson’s theta (θw) across the genome for the 37 scaffolds that were over 25Mb using the thetaStat module in ANGSD. 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 S2). Across the 37 scaffolds, the highest diversity was observed in the Eastern Türkiye population, followed by the Iran population (Fig. 3). As expected, the Apennine bear population had significantly lower diversity than all other populations (Fig. 3).

We conclude that Eastern Türkiye brown bears are characterized by distinct genetic structure, mixed ancestry, and higher levels of genetic diversity even when compared to geographically close populations. However, we did not capture any broad scale genetic differences between sedentary and migratory bears in Eastern Türkiye.

High genetic differentiation and distinct genotypes between sedentary and migratory bears at outlier loci.

To determine genomic regions associated with sedentary and migratory behavior we performed an association test using the score statistics (Skotte et al., 2012) implemented in ANGSD. In total we compared 12,207 SNPs between movement categories using a likelihood ratio test (LRT) with a genome-wide significance threshold of $4.09x10^{-6}$ (Bonferroni-corrected $\alpha = 0.05$; LRT > 21.2). The strongest associated SNP in our panel reached a significance level of $1.4x10^{-5}$ (LRT = 18.8) and we detected no SNP that reached genome-wide significance (Table S3).

To determine if any of the top 20 SNPs (LRT > 13, $P < 3.1x10^{-4}$) in our association panel could be candidate regions for behavioral differences between sedentary and migratory bears we performed an outlier test based on the population branch statistics (PBS; Yi et al., 2010) to test if any of these sites showed extreme genetic differentiation in either of the two groups. The mean PBS score across the genome was 0.0205 ± 0.0330 for sedentary bears and 0.0127 ± 0.0277 for migratory bears. 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 99.9 percentile range (PBS > 0.3106) in migratory bears (Fig. 4, Table S3). Moreover, all SNPs with PBS scores over the 99.9 percentile in either sedentary or migratory bears were at least 10 standard deviations above their respective means (Table 1), 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 (within the first exon of CCRL2). For the remaining highly differentiated SNPs, we found 3 genes (Myo5b, RAN, and TRDMT) lying within 1MB distance from SNP_TM4, SNP_TS3 and SNP_TS5, respectively (Table 1).

To further understand genomic patterns of differentiation at the 10 SNPs showing extreme PBS values, we genotyped each individual at these sites and categorized individuals as either heterozygous or homozygous for the ancestral or derived allele (Fig. 5). Genotype calling at these sites revealed three blocks with distinct genotype patterns between sedentary and migratory bears (Fig. 5). Block 1 and 2 constituted regions where the frequency of the derived allele was higher in sedentary bears, with most sedentary bears being heterozygous (block 1) or homozygous for the derived allele (block 2) in contrast to migratory bears which were mostly homozygous for the ancestral allele in block 1 and heterozygous in block 2 (Fig. 5). In contrast, Block 3 had the opposite pattern where the frequency of the derived allele was higher in migratory bears, with migratory bears being mostly heterozygous and sedentary bears being mostly homozygous for the ancestral allele (Fig. 5).

Even though not statistically significant at the genome-wide level, ten of the top 20 SNPs in our association panel showed extreme differentiation in either one of the two behavioral groups and were able to separate sedentary and migratory bears into distinct genotypes. This suggests that the differences in sedentary and migratory behavior might have an important genetic component.

Figure SEQ Figure * ARABIC 1 The study area in northeastern Türkiye. Blue dot indicated the Sarıkamış garbage dump.

Table 1
Derived allele frequencies of sedentary, migratory and European bears at the ten outlier loci. SNP_TM indicates outlier loci in migratory bears while SNP_TS indicates outlier loci in sedentary bears. The distance to the nearest genes within a 1 MB window of the SNPs are also indicated along with the mean, global, SNP window and 99.9 percentile PBS scores.

SNP Name	Position	SNPs	DAF_Mig	DAF_Sed	DAF_Eur	Mean PBS	Global PBS	99.9 percentile PBS	SNP window Pbs	Distance to Nearest Gene	Gene
SNP_TM1	HiC_scaffold_2-47325857	SNP14	0.0453	0.3940	0.8072	0.0127	0.0083	0.3108	0.6202	-	LTR
SNP_TM2	HiC_scaffold_18-2297497	SNP11	0.3942	0.8558	0.7709	0.0127	0.0083	0.3108	0.3381	-	LTR
SNP_TM3	HiC_scaffold_28-42755955	SNP6	0.7393	0.1953	0.1835	0.0127	0.0083	0.3108	0.4591	Inside Exon	**CCRL2**
SNP_TM4	HiC_scaffold_30-24131228	SNP5	0.5368	0.1254	0.0000	0.0127	0.0083	0.3108	0.3175	46,501 bp	**Similar to Myo5b**
SNP_TM5	HiC_scaffold_36-51993011	SNP3	0.0000	0.3334	0.2254	0.0127	0.0083	0.3108	0.3799	4,935 bp	**ELOC**
SNP_TS1	HiC_scaffold_4-29070918	SNP1	0.5405	0.0710	0.5115	0.0205	0.0143	0.3547	0.4579	42,113 bp	**RAN**
SNP_TS2	HiC_scaffold_14-65416581	SNP17	0.4955	0.9011	0.1928	0.0205	0.0143	0.3547	0.5830	-	LTR
SNP_TS3	HiC_scaffold_30-12480299	SNP4	0.4170	0.8887	0.1927	0.0205	0.0143	0.3547	0.6292	-	LTR
SNP_TS4	HiC_scaffold_34-17119378	SNP19	0.4557	0.8770	0.4691	0.0205	0.0143	0.3547	0.3620	6,926 bp	**TRDMT**
SNP_TS5	HiC_scaffold_37-70763847	SNP12	0.5032	0.9185	0.3723	0.0205	0.0143	0.3547	0.5442	-	LTR

Figure SEQ Figure * ARABIC 5 SNPs that have the highest log-likelihood ratios at each position for each individual sedentary and migratory bear, along with 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 where the genotype is not known.

Discussion

Using genome-wide sequencing data we examined genetic variation, structure, and differentiation among a brown bear population with a dimorphic life-history and living in a human-dominated environment. The availability of anthropogenic resources, like open garbage, can have significant effects on the feeding and movement behavior of brown bears and can even lead to animal habituation (Cozzi et al., 2016; De Gabriel Hernando et al., 2020; De Angelis et al., 2021). For example, the open garbage dumps in Yellowstone National Park in the early 1900s, became the principal feeding ground for the park's large bear population (Craighead & Craighead Jr, 1971). A century later, we observed similar behavior in the open city dump near Sarıkamış National Park, home to a large brown bear population, giving us the opportunity to study the genetic consequences of animal habituation.

Mixed ancestry and high relative diversity dictate the presence of a long-running stable bear population in Eastern Türkiye

Multiple studies have shown considerable structure among brown bear populations, even at regional scales (Montgomery et al., 2000; Wright, 2001; Benazzo et al., 2017). We also documented high amount of genetic structure and differentiation in this study, especially at the global scale. Excluding the highly isolated Apennine brown bear population, PCA clearly showed an east-west divide with bears from Alaska, Russia, and Europe (including individuals from Slovenia, Slovakia, and Greece) forming one group and bears from Georgia, Eastern Türkiye, and Iran forming another (Fig. 2A). Although there was a suggestion of this divide in a previous mtDNA study (Çilingir et al., 2016), the scale of this differentiation is surprising. Admixture results provided further support to the observed east-west divide. Eastern bears (Georgia, Türkiye, and Iran) were characterized by mixed ancestry, but they shared very little ancestry with western bears (Europe, Russian, and Alaska) which were mostly divided into distinct ancestral groups (Fig. 2C). It is also noteworthy that even though eastern brown bears clustered together in the PCA, eastern Türkiye brown bears were still highly distinct from bears in both Iran and Georgia. and were also the only ones not to share any ancestry with their European kin (Fig. 2C). Comparisons between sedentary and migratory bears in Eastern Türkiye revealed some loose genetic structure between the two behavioral groups (Fig. 2B), but admixture analysis did not reveal any differences in ancestry. Therefore, even though Eastern Türkiye brown bears show observable behavioral differences (sedentary vs migratory), they are still largely a panmictic population.

The high amount of mixed ancestry in eastern brown bears can also indicates high genetic diversity. Indeed, genetic diversity scans across the genome revealed that Eastern Türkiye brown bears had the highest diversity along all 37 major scaffolds. As expected, the Apennine population had the least genetic diversity because they are completely isolated and have a high degree of inbreeding (Benazzo et al., 2017). It is worth noting that, genetic diversity in Eastern Türkiye brown bears was comparable, and actually slightly higher than all European bears which included individuals from a wider geographic area spanning several countries (i.e., Slovenia, Slovakia, and Greece). Interestingly, a past study of mtDNA haplotype diversity also captured similar trends. Bears from Türkiye and the Middle East showed relatively high haplotype diversity, while bears from Europe showed 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 these regions with few historical founder effects or bottlenecks (Montgomery et al., 2000; Wright, 2001).

High allelic differentiation between migratory and sedentary bears at the protein coding gene CCRL2

Genetic variation plays a critical role in protecting populations against environmental perturbations (McNeely et al., 1990; Reed & Frankham, 2003; Frankham, 2005). Therefore, the high amount of diversity and mixed ancestry within Eastern Türkiye brown bears might not only buffer against population declines but can also provide ample genetic material for adaptation, especially in human-dominated landscapes with strong and consistent selective factors caused by anthropogenic factors. Although the effect of anthropogenic activities (especially the presence of human-related food sources) on bear feeding and roaming behavior is well known (Craighead & Craighead Jr, 1971; Beckmann, 2002; Beckmann & Lackey, 2008; Cozzi et al., 2016), until now there were no studies on the genetic consequences of such factors.

Here, by comparing sedentary and migratory bears within the same population, we present the first test of the genetic basis of behavioral shifts observed in brown bears as a response to feeding behavior and the usage of anthropogenic food sources. Even though GWAS scans could not determine any SNPs associated with sedentary/migratory behavior that reached genome-wide significance, 10 out of the 20 top-ranking SNPs in our association panel showed extreme differentiation between sedentary and migratory bears and were able to separate migratory and sedentary bears into distinct genotypes (Figs. 4 and 5). Four of these SNPs (SNP_TM5, SNP_TS1, SNP_TS4, and SNP_TM4) lay within 50 kb of multiple transcription-factor genes (ELOC, RAN, TRDMT and Myo5b, respectively) which influence a host of different cellular processes including RNA polymerase extension, DNA damage, DNA recombination, and mutation repair (Melchior et al., 1993; Ren et al., 1993; Garrett & Garrett, 1994; Subramaniam et al., 2014).

Most interestingly, SNP_TM3 showing extreme differentiation in migratory bears was in the first exon of the protein-coding gene CCRL2 (C-C Motif Chemokine Receptor Like 2) which plays an important role in the immune response of mammals including inflammation, autoimmunity, and homeostasis (Huber et al., 2008; Yoshimura & Oppenheim, 2011; Lira & Furtado, 2012). C-C chemokines (CCR1-5, CCR8, CC1L1-L2, and CX3CR1) 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 with olfactory recognition (Kalkonde et al., 2011) and CCR4 with locomotor activity, anxiety, and social exploratory behavior (Ambrée et al., 2016).

The frequent use of garbage dumps as primary food sources by brown bears increases the ingestion of toxins and foreign bodies, as well as the risk of pathogens (Coogan & Raubenheimer, 2016; Sato, 2017). Thus, our results are consistent with previous work showing that genes and pathways controlling immune function and diet in bears are involved in adaptation to novel environments (Benazzo et al., 2017).

Standing genetic variation could facilitate shifts in allele frequencies under strong selection

Genetic adaptation is a long process the speed of which depends on the amount of genetic variation (i.e., heritability of the target phenotype), the strength of selection and the persistence of the selective agent (Hartl et al., 1997; Pritchard & Di Rienzo, 2010). Based on the establishment of Sarıkamış as a major human settlement around the 1940s (Saraçoğlu, 2011) the large open garbage dump has likely been around for 70–80 years at most. Given an average generation time of 11.35 years for brown bears (Cronin et al., 2009; De Barba et al., 2010) this would mean that brown bears in the area have been interacting with this food source for around seven generations, too short a time for any novel genetic change to occur and get established in the population. However, humans and brown bears (or hominids and ursids in general) have been sharing the same environment and competing for resources since the mid-Pleistocene (Romandini et al., 2018). This long-shared history could potentially present brown bears with enough time to accumulate genetic variation for facilitating survival in human dominated landscapes. Strong selective pressures like the availability of a year-round high calorie food source (i.e., large open garbage dump at Sarıkamış) could then potentially sort this standing genetic variation into observable differences between phenotypes in a relatively short time (Matuszewski et al., 2015; Lai et al., 2019; Fuhrmann et al., 2023).

Long-term field observations conducted in the area since 2006 indicate that the large open garbage dump at Sarıkamış might have important fitness consequences for bears actively using the site. 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, including nine individuals simultaneously feeding in the garbage dump covered by a meter of snow (Şekercioğlu, pers. observation). These observations suggest that the year-round availability of a high-calorie food source could potentially be acting as a strong selective agent (Cozzi et al., 2016).

Conclusion

Brown bears are highly sensitive to human presence and activity (Zarzo-Arias et al., 2018; Morales-González et al., 2020) and like other carnivores and large mammals their long-term viability depends on their ability to adapt to human dominated landscapes (Ordiz et al., 2011; Støen et al., 2015). Yet we know very little about the genetic architecture of behavioral or life history traits that drives such adaptation in natural populations (Bubac et al., 2020). Here we conducted the first study to examine the genetic basis of phenotypic diversity in movement behavior of brown bears linked to the presence of a large open garbage dump in Sarıkamış. We were able to capture several genetic regions highly differentiated between the two behavioral groups that were linked to functionally important genes and could separate the two phenotypes into partially distinct genotypes.

Like most other complex traits, the genetic basis of life history variation in behavior is most likely quantitative and polygenic in nature (Santure et al., 2015; Bubac et al., 2020). Therefore, understanding the complete picture will require higher level genomic approaches that can capture small effect genes, epistatic interactions and quantitative trait loci spread across the genome. Despite these limitations, however, our results highlight that genetic changes could be playing an important role in the observed behavioral shifts in Eastern Türkiye brown bears, emphasizing the importance of evolutionary genomics for understanding how species survive and adapt to human-mediated global change. Thus, this system can serve as a model for understanding the genetic basis of rapid adaptation of large mammals to human-dominated landscapes.

Methods

Ethics Statement. This study was carried out in accordance with the ARRIVE guidelines (Percie du Sert et al., 2020) and methods were reported in detail following the suggested guidelines and regulations. Handling of bears including capture, collaring and blood sampling were conducted by specially trained scientists approved by the Republic of Türkiye Ministry of Agriculture and overseen by Boğaziçi and Kafkas University Animal Experiments Local Ethics Committees (BÜHADYEK and KAÜ-HADYEK respectively).

Study area. The study area covers the Kars, Erzurum and Ardahan provinces at the intersection of the Caucasus and Iran-Anatolian global diversity hotspots in northeastern Anatolia, Türkiye (Fig. 1), an understudied high biodiversity country experiencing a major conservation (Şekercioğlu et al., 2011 a,b). crisis Human activity in the forest is extensive in both time and space, limited only by harsh winter temperatures, consisting primarily of livestock grazing, legal and illegal timber extraction, and harvesting non-timber forest products (e.g., fruits, pinecones, mushrooms, Capitani et al., 2016; Chynoweth et al., 2016).

Fieldwork and sample collection. All fieldwork including capturing, collaring, GPS tracking and sample collection was conducted by KuzeyDoğa Society, a local conservation and ecological research non-profit organization, as part of a long-term large carnivore monitoring program conducted in

the region since 2011 (Akküçük & Şekercioğlu, 2016). Fieldwork was carried out under field permits No. 72784983-488.04-151690 and No. 72784983-488.04-114100 granted by the Republic Of Türkiye Ministry of Agriculture and Forestry, General Directorate of Nature.

Bears were captured using spring activated foot snares (Aldrich snares, USA) in the period between September 2012 and September 2019 following the protocols described in Cozzi et al. (2016) and Huber et al. (1996). Bears were caught both in the forest area more than 10 kilometers away from the garbage dump and within one kilometer of it. Our sampling procedure was not the cause of the observed dichotomy in genetic results, since some bears captured far from the dump visited it as well while some bears captured close to the dump, never visited it. The use of GSM alarms enabled response times to be within 20–30 minutes after capture. Captured bears were tranquilized using a mixture of 2.5 mg/kg tiletamine-zolazepam (Zoletil®, Vibrac) and 0.05 mg/kg medetomidine (*Domitor*®, Pfizer) injected via a dart fired from a CO_2-powered rifle (JM Special, Dan-Inject ApS, Denmark). Dosages were set following Kreeger & Arnemo (2009), and all treatments were supervised on-site by a wildlife veterinarian. All captured bears successfully recovered from anesthesia.

Captured bears were fitted with GPS/GSM or GPS/Iridium tracking collars (GPS Plus; and Vertex LITE collars from Vectronic Aerospace GmbH, Berlin, Germany) programmed to record one GPS location every hour. In addition blood samples were taken from each bear, collected into EDTA tubes, and immediately frozen at -20°C for transportation and DNA purification. In total we used data from 31 bears (10 adult female, 21 adult male) that were captured and collared and had tracking data for at least one year (Table S1).

Movement patterns. GPS locations with a position dilution of precision (PDOP) of > 10 were removed from the dataset to avoid inaccurate position information (Elliot et al., 2014). In addition, we only used position data gathered pre- and post-hibernation of individuals because when bears hibernate in caves or holes during the winter (hibernation interquartile range was November 23rd–December 3rd to March 6th–April 1st) the GPS system frequently failed to acquire satellites. (Cozzi et al., 2016). We 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 to obtain the daily maximum distance traveled. From this information, we 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.

Extraction, sequencing, and mapping. Genomic DNA was extracted from blood samples using QIAamp DNA Blood Mini Kit following the manufacturer's protocols and quantified using Qubit to determine the presence of total DNA. To produce a high-quality genomic resource, we sampled the genome using RAD sequencing (Miller et al., 2007; Baird et al., 2008; Etter et al., 2012). Paired end 150 bp sequence reads were generated for 31 individuals with known migratory/sedentary movement patterns using the Sfb1 restriction enzyme and the RAD protocol described in Ali et al. (2016). Library preparation and sequencing were carried out by the Micheal R. Miller laboratory at UC Davis using the Illumina platform.

Sequenced reads were sorted into individuals as matching forward and reverse fastq files using unique 8 bp barcodes. 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). Raw read counts, the number of aligned reads, and the number of alignments after removing PCR duplicates are given in Table S1. In addition, potential paralogous regions were marked using ngsParalog (Linderoth, 2018) and removed from further analysis.

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- to mid-depth data (Fumagalli et al., 2013; Korneliussen et al., 2014). Using ANGSD, we 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, we 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). Genotypes were called in ANGSD (-doGeno 4) using a population prior (-doPost 1) and a posterior probability cutoff of 90% (-postCutoff 0.9), and we used the American black bear and polar bear genomes (Cahill et al., 2013; Srivastava et al., 2019) to determine ancestral and derived states.

PCA and Admixture. Principal Component Analysis 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 (R Core Team, 2019). Admixture proportions of individuals were estimated in NGSadmix (Skotte et al., 2013) based on genotype likelihoods for K values of 2–8. 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).

Genetic diversity. Watterson's theta (θw) values were estimated based on the global site frequency spectrum (SFS). 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 genomes (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; Korneliussen et al., 2014). To reduce biased estimates, we eliminated any scaffold shorter than 25 MB and excluded windows having a number of sites less than 200. θπ values for each window were transformed into per-site values by dividing each statistic by the number of sites in the window and genome-wide values were obtained for each population by averaging across scaffolds.

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

Population branch statistics. Population branch statistics (PBS) analyses were based on the comparisons between migratory, sedentary, and eastern European (Greece, Slovenia, and Slovakia) bears. 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. We 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. Extreme genetic differentiation was determined by comparing the PBS score for a given site against the mean PBS score calculated across the 37 scaffolds and PBS values lying over the 99.9th percentile were regarded as significant hits (i.e., outliers).

Declarations

Data availability

The generated RAD sequencing data produced for this study are available in the NCBI Short Read Archive under study accession PRJNA1016121 BioProject ID. It will be publicly available after the publication.

Code availability

The pipelines and codes used in this study are available on the GitHub page. https://github.com/ciselkemahli/Brown-bear-genetics

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Author contributions

M.Ç.K.A., Ç.H.Ş., and İ.K.S. conceived, designed and planned the study. M.Ç.K.A., J.K., M.C., E.Ç., A.Ç., M.N. and Ç.H.Ş. collected samples. M.Ç.K.A. and İ.H. performed the lab work. M.Ç.K.A., and İ.K.S. prepared the data and performed data analyses. M.Ç.K.A., and İ.K.S. wrote the manuscript with input from Ç.H.Ş. and J.K. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

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Figures

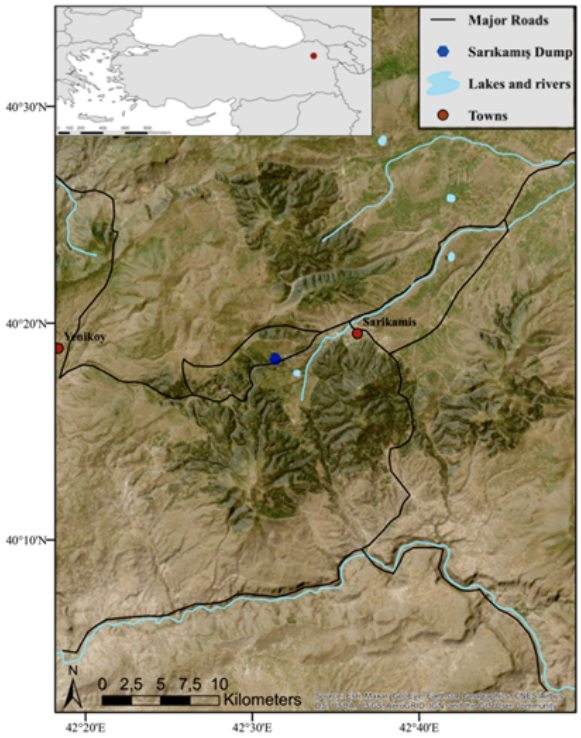

Figure 1

The study area in North Eastern Türkiye where bear sampling and tracking was conducted. The town of Sarıkamış is indicated in red and the open garbage dump in blue. The given area covers all locations visited by both sedentary and migratory bears (see supplementary movement Figures M1-M31 for detailed movement patterns of individual bears).

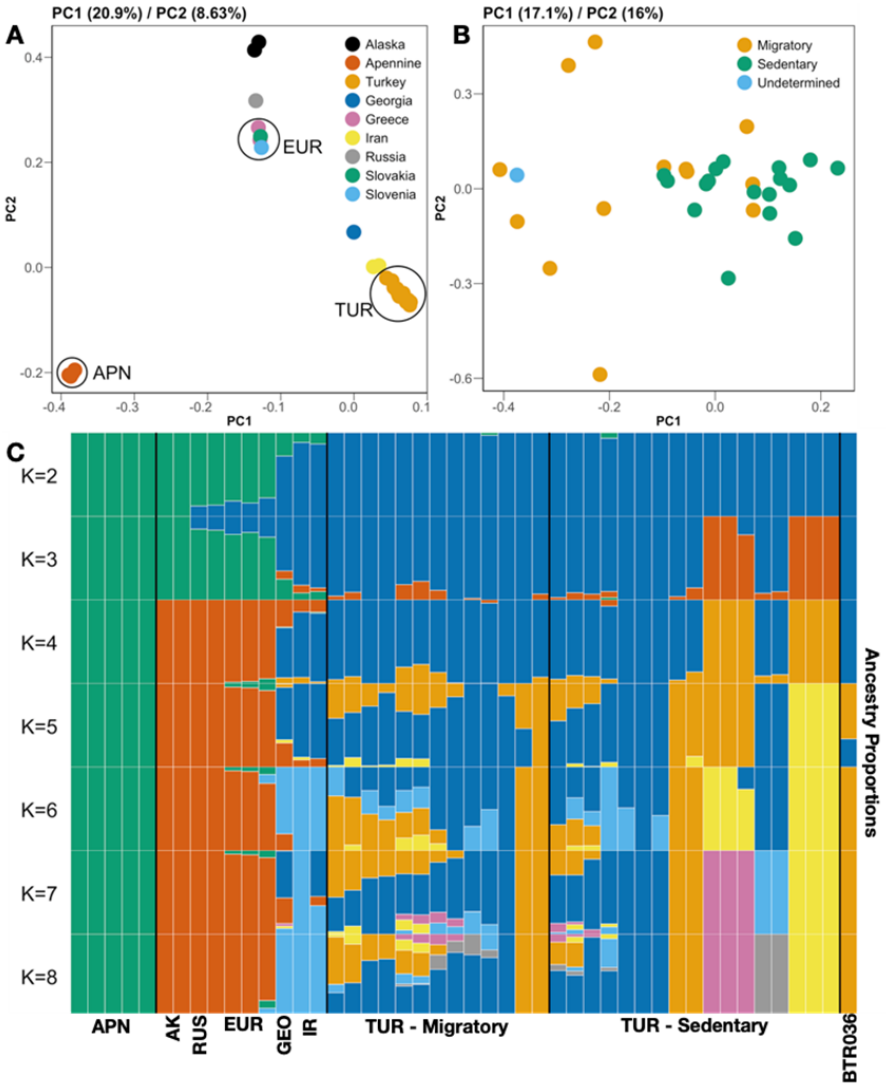

Figure 2

Genetic structure of brown bears from Eastern Türkiye and other world populations. (A) Principal component analysis (PCA) summarizing genetic variation between Apennine (n=5), European (Greece, Slovenia, and Slovakia, n=4), Alaskan (n=2), Russian (n=1), Georgian (n=1), Iranian (n=2) and Eastern Türkiye (n=31) brown bears. (B) Principal component analysis (PCA) summarizing genetic variation within Eastern Türkiye brown bears. (C) Admixture analysis depicting proportion of shared ancestry between bear samples for K=2-8 ancestral populations.

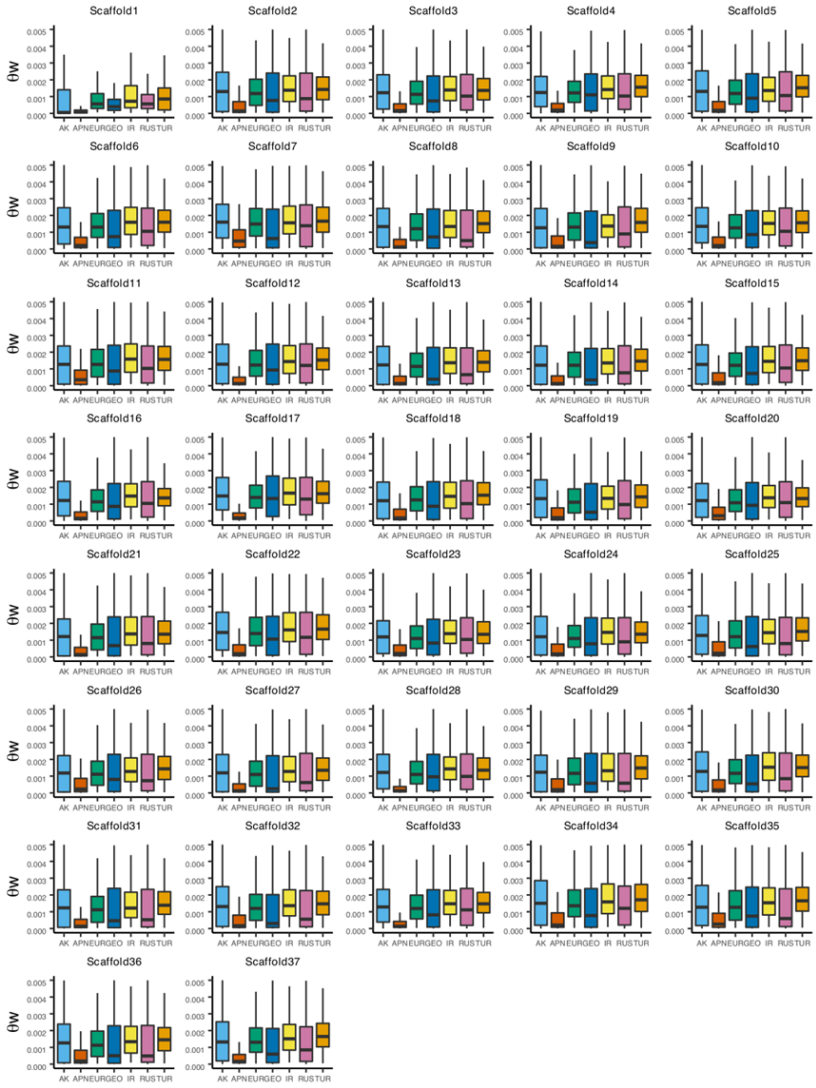

Figure 3

Average per-site nucleotide diversity (Watterson's theta,θw) across the 37 major scaffolds (> 25Mb) in brown bears from Eastern Türkiye and around the world. Total genome wide average values are given in supplementary Figure S2.

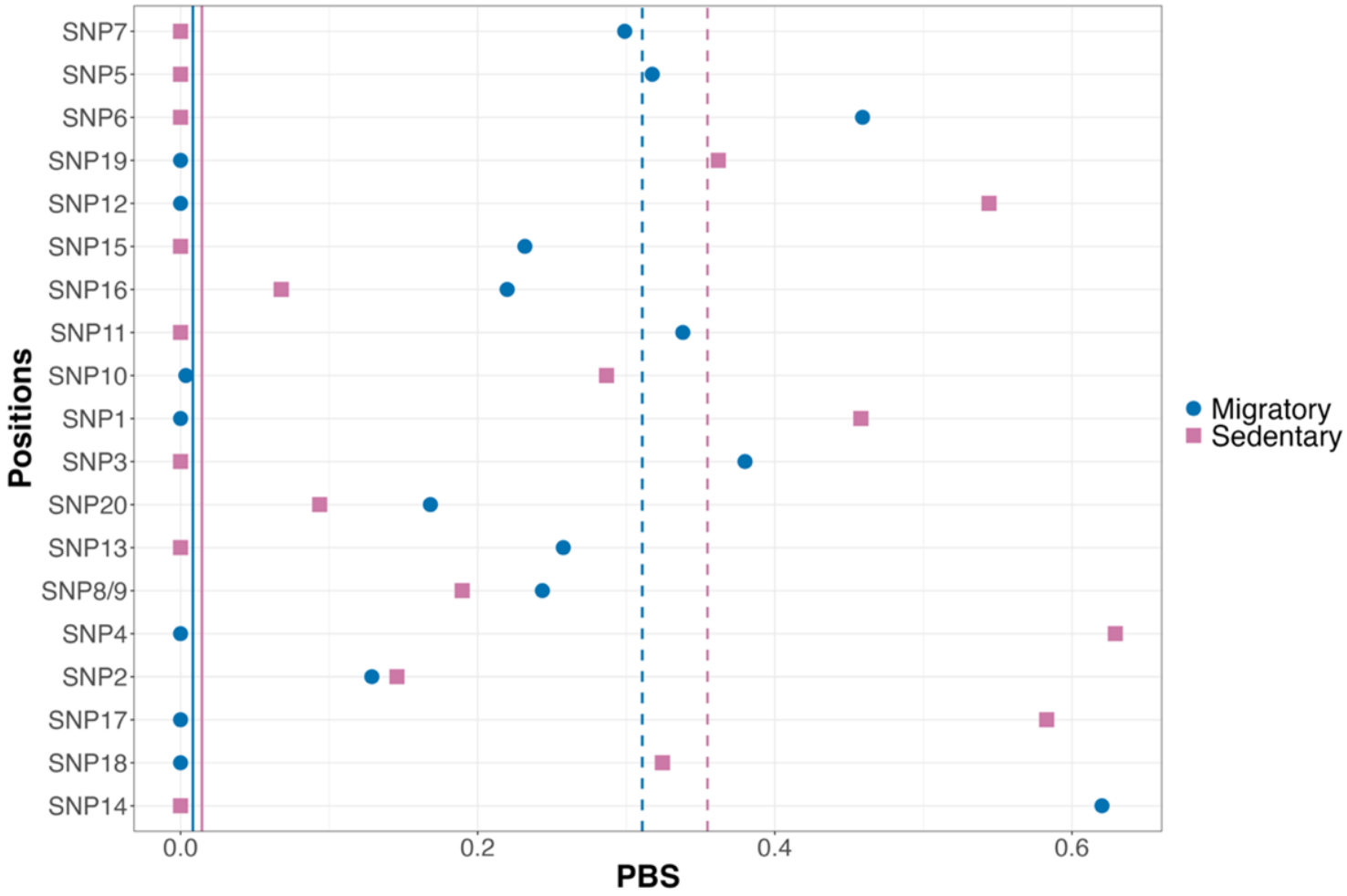

Figure 4

Population branch statistics (PBS values) of the top 20 SNPs (LRT > 13, P < 3.1x10-4) in our association panel for migratory (blue) and sedentary (pink) bears. Solid lines represent the global PBS values for each case across the 37 major scaffolds (> 25Mb) and dashed lines indicate the 99.9% percentile range.

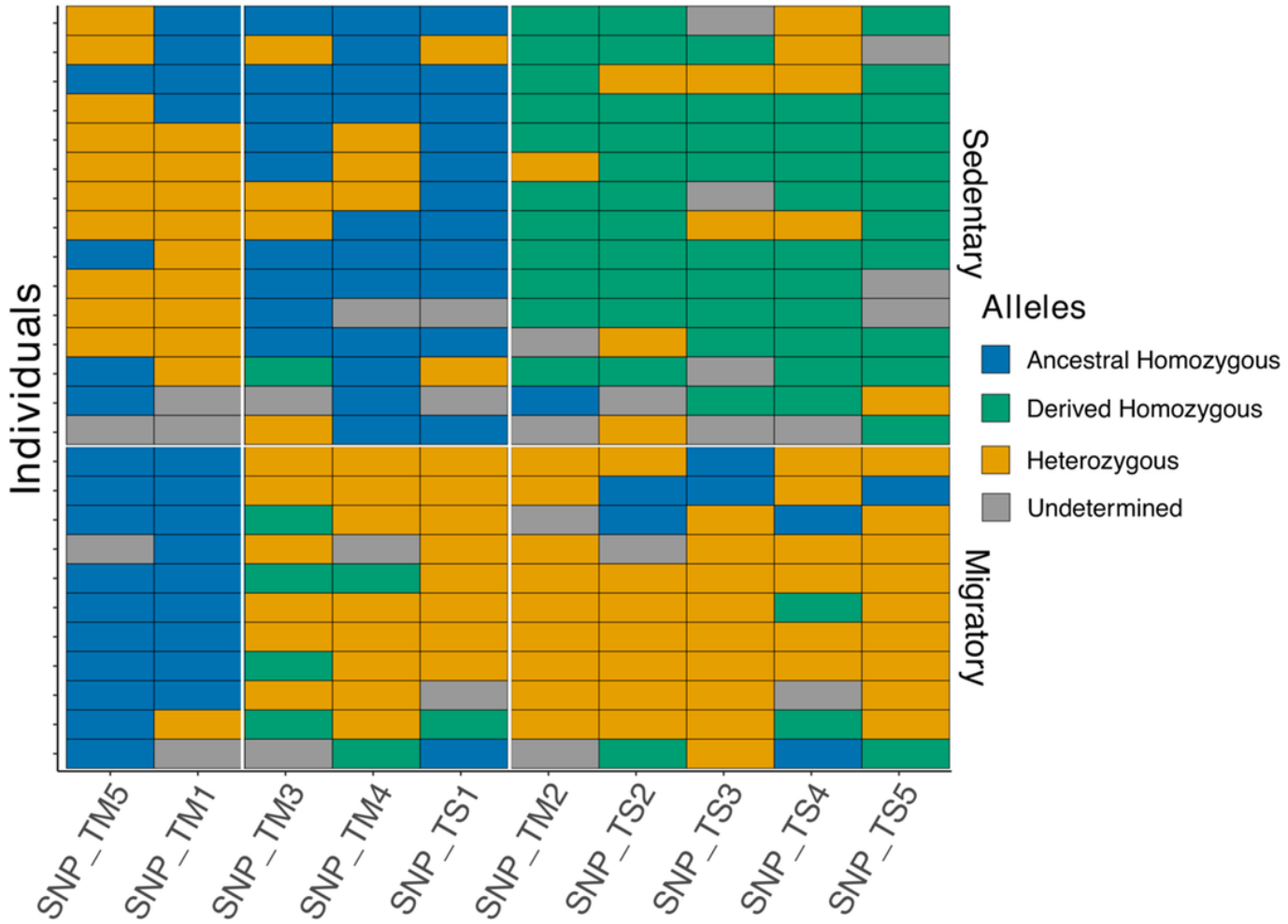

Figure 5

Genotype distribution of sedentary and migratory bears in Eastern Türkiye at the ten outlier SNPs as determined by PBS analysis. Blue boxes represent ancestral homozygous, green boxes represent derived homozygous, yellow boxes represent heterozygous genotypes and gray boxes represent missing genotype calls.

Supplementary Files

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