GENETIC STRUCTURE AND PHYLOGENETIC RELATIONS OF CINEREOUS VULTURE (*AEGYPIUS MONACHUS*) POPULATIONS IN TURKEY

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ABSTRACT

GENETIC STRUCTURE AND PHYLOGENETIC RELATIONS OF CINEREOUS VULTURE (*AEGYPIUS MONACHUS*) POPULATIONS IN TURKEY

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Throughout the world, vulture populations have suffered threatening declines in numbers in recent years. The Cinereous Vulture (*Aegypius monachus*) is a highly philopatric scavenger that ranges across southern Europe and the central Asian plateau. Turkey holds the second most numerous population in the Western Palearctic but there has been no research on the genetic structure of this particular population. In the present study, we describe the current genetic status of the Turkish population using both mitochondrial cytochrome b sequences and nuclear microsatellite markers. Although, mitochondrial (mt) DNA revealed extremely low genetic variability (haplotype diversity), nuclear diversity based on allelic richness and expected heterozygosity demonstrated moderate levels of genetic variation. Phylogenetic analyses indicated that the Turkish lineage, together with the Caucasus population, occupies an intermediate position between European (Balkan and Iberian) and Central Asian (Mongolian) lineages. We could not detect any significant differentiation between the four sub-populations (Çatacık, Tandır, Türkmenbaba and Köroğlu), which suggests that the sampled 81 individuals originated from a single large, panmictic population. Additionally, we observed evidence of a recent bottleneck in the Turkish populations and a low effective population size of 112 (95% CI 74–201). A population viability analysis using VORTEX revealed predicted population dynamics. These findings suggest that conservation strategies should be developed for treating Turkish populations as a single management unit.

Keywords: Cinereous Vulture, Microsatellites, Mitochondrial DNA, PVA, Turkey

ÖZ

TÜRKİYE'DEKİ KARA AKBABA (*AEGYPIUS MONACHUS*) POPÜLASYONLARININ GENETİK YAPISI VE FİLOGENETİK İLİŞKİLERİ

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Son yıllarda, tüm dünyada akbaba populasyon sayılarında tehdit eden düşüşler gözlenmiştir. Kara Akbaba (*Aegypius monachus*) Orta Asya platosu ve Güney Avrupa'da yayılış gösteren filopatrik bir leşçildir. Türkiye, Batı Palearktik'te ikinci en çok nüfusa sahip olan ülke olmakla birlikte bu populasyonun genetik yapısı hakkında hiç bir çalışma bulunmamaktadır. Bu çalışmada, hem mitokondriyal (mt) DNA sitokrom b dizileri, hem de nükleer mikrosatellit belirteçleri kullanılarak Türkiye populasyonunun mevcut genetik durumunu tanımladık. Mitokondrial DNA'da genetik değişkenlik (haplotip çeşitliliği) oldukça düşük olmasına rağmen, allelik zenginliğe ve beklenen heterozigotluğa dayalı nükleer çeşitlilik orta düzeyde bir genetik varyasyon sergiledi. Filogenetik analizler, Türkiye soyunun - Kafkasya ile birlikte -Avrupa (Balkan ve İber) ve Orta Asya (Moğolistan) soyları arasında bir konumda olduğunu gösterdi. Çalışılan dört alt populasyon (Çatacık, Tandır, Türkmenbaba ve Köroğlu) arasında anlamlı bir farklılık tespit edilememiştir. Bu bulgu çalışılan 81 bireyin tek bir panmiktik populasyondan köken aldıklarını göstermektedir. Buna ek olarak, Türkiye populasyonun bir darboğazdan geçmiş olabileceğinin kanıtını ve etkili nüfus büyüklüğünün 112 (%95 CI 74-201) gibi düşük bir düzeyde olduğunu gözlemledik. VORTEX kullanarak yapılan populasyon yaşayabilirlik analizi tahmin edilen populasyon dinamiklerini ortaya çıkarmıştır. Bu bulgular, koruma stratejileri geliştirilirken Türkiye populasyonlarının tek bir yönetim birimi olarak muamele edebilmesi gerektiğini önermektedir.

Anahtar Kelimeler: Kara akbaba, Mikrosatelit, Mitokondriyal DNA, PVA, Türkiye

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LIST OF ABBREVIATIONS

°C: Degrees Celsius
°N: Degrees North
°E: Degrees East
µl: Microliter
µM: Micromole
2D: Two Dimensional
bp: Base Pair
cm: Centimeter
CR: Control Region
Cy b: Cytochrome b
DNA: Deoxyribonucleic Acid
EV: Environmental Stochasticity
F: Female
h: Haplotype Diversity
ha: Hectare
H_E: Expected Heterozygosity
H_O: Observed Heterozygosity
HS: Half-Sibling
IUCN: The International Union for Conservation of Nature
LD: Linkage Disequilibrium
M: Male
m: Meter
MCMC: Markov Chain Monte Carlo
min: Minute
ML: Maximum Likelihood
mtDNA: Mitochondrial DNA

N: Population Size

n: Sample Size

Ne: Effective Population Size

ng: Nanogram

NT: Near Threatened

PCR: Polymerase Chain Reaction

PIC: Polymorphic Information Center

PVA: Population Viability Analysis

r: Relatedness

RFLP: Restriction Fragment Length Polymorphism

RHD: Reduce Human Disturbance

rRNA: Ribosomal Ribonucleic Acid

SD: Standard Deviation

sec: Second

SNP: Single Nucleotide Polymorphism

SSRs: Simple Sequence Repeats

tRNA: Transfer Ribonucleic Acid

U: Unrelated

UV: Ultraviolet

VFF: Vulture Friendly Forestry

VNTRs: Variable Number of Tandem Repeats

Vu: Vulnerable

π: Nucleotide Diversity

PART I

GENERAL INTRODUCTION

In this thesis, Cinereous Vulture (*Aegypius monachus* Linnaeus, 1766) populations from Turkey were examined by means of different genetic markers in order to elucidate their population genetics and to understand conservation needs better.

I.1 Biology of the Cinereous Vulture

Cinereous Vulture or Eurasian Black Vulture (*Aegypius monachus*) is one of the largest birds of prey with a length of 98 to 107 cm and a 2.5-3.1 m. wingspan (Birdlife-International 2017). It belongs to the order Accipitriformes (Birdlife-International 2017).

It is a globally 'Near Threatened' (NT) species with a Eurasian distribution (Birdlife-International 2017), and 'Vulnerable' (VU) at the European level (Tucker and Heath 1994). Its global population is estimated to be between 7,200 and 10,000 pairs, with 1,700–1,900 pairs in Europe (including Turkey, Birdlife-International 2017) and 5,500–8,000 pairs in Asia (Anonymous 2004). The Spanish population (~ 2000 pairs) constitutes 87% of the European population and between 18-25% of the world population (De la Puente 2007). Turkey has the second largest population (50-200 pairs) after Spain in Europe (Heredia et al. 1997; Yamaç and Günyel 2010). In Europe, Spanish, French (reintroduced population) and Portuguese populations demonstrate increasing population trends, whereas Greek and Macedonian populations are stable. Population is Turkey, Armenia, Azerbaijan, Georgia, Russia and Ukraine are reported to be declining (Barov and Derhé 2011). There is not sufficient information about the large Asian populations (Birdlife-International 2017). Additionally, very little is known about population situation in its wintering territories (Gavashelishvili and McGrady 2006).

Adult Cinereous vultures are resident in the western half of their range (Meyburg and Meyburg 1983). In Spain, Greece and Turkey, most juveniles may disperse to limited distances (Vasilakis et al. 2008; Yamaç and Bilgin 2012). However, in eastern populations from Central Asian Republics, Mongolia and China, migration of both juveniles and adults were reported between breeding and wintering areas (Reading et al. 2010).

The species nest in wooded areas in mountains at 300-1,400 m above sea level and forage for carrion in diverse habitats (Cramp and Simmons 1980). They breed in loose colonies of highly variable density (Birdlife-International 2017). Generally, the nest is built on large trees, such as oak (*Quercus*) and pine (*Pinus*) (Guerrero-Casado et al. 2013). Eggs are laid in February–March, and incubation is by both adults and last 50–54 days (Cramp and Simmons 1980; Heredia 1996). A single, semi-altricial chick remains in the nest for 95–110 days (Hiraldo 1983). Diet can vary based on animals living their region (Cramp and Simons 1980). Traditionally, wild rabbit *Oryctolagus cuniculus* carcasses was the main food supply for Cinereous vultures in Spain. Declining rabbit populations caused a switch to domestic ruminants, and in some areas, to wild ungulates (Costillo et al. 2007).

Direct mortality caused by humans and food scarcity are the two main threats (Birdlife-International 2017). Reduced numbers of domestic livestock, poisoning, and changes in nesting habitat may lead to population declines (Donázar 1993; Donázar et al. 2002). Declines in food resources such as Saiga antelope (*Saiga tartartica*) in Central Asia and sheep numbers in the Caucasus have had a negative effect on the populations (Birdlife-International 2017). Cutting of nest trees and other direct disturbances in Georgia (Gavashelishvili et al. 2006), and killing for feathers in China (GRIN 2010) are local threats.

I.2 Cinereous Vulture Populations in Turkey

Turkey has the second largest population (50-200 pairs) of *Aegypius monachus* in Europe after Spain (Barov and Derhé 2011; Heredia et al. 1997; Yamaç 2004). In Turkey, population size was reported as stable or declining based on expert opinion, although Yamaç (2004) observed variable but moderate breeding success (Table I.1). Cinereous vultures prefer to nest on mature Austrian Pine (*Pinus nigra*) trees in Turkey (Yamaç 2004; Yamaç 2007) (Figure I.1). The main threats for this species were detected to be forestry activities, fires and recreational activities (like picnics) in Turkey (Kirazlı and Yamaç 2013; Yamaç 2004). The only study on prey items showed 76.6% of all pellets including sheep followed by Wild Boar (44.1%) and chicken (22.5%) (Yamaç and Günyel 2010).

Figure I.1 Active nest at Sündiken.

Table I.1 Breeding populations and their sizes in Turkey (*: sites with verified nests).

Sites	Size	References
Akdağ-Çivril	4 breeding pairs	(Birdlife-International 2017; KAD 2004; Yarar and Magnin 1997)
Ardahan Forest	3-5 breeding pairs	(Birdlife-International 2017; KAD 2004; Yarar and Magnin 1997)
Eastern Black Sea Mountains	10-13 breeding pairs	(Birdlife-International 2017; Heredia 1996; KAD 2004; Yarar and Magnin 1997)
Iğdır Plain	15-20 individuals	(Birdlife-International 2017)
Kızılcahamam*	7-10 breeding pairs	(Birdlife-International 2017; Erdoğan 1998; Heredia 1996; KAD 2004; Yarar and Magnin 1997)
Köroğlu Mountains*	5 breeding pairs	(Birdlife-International 2017)
Murat Mountain	2-4 breeding pairs	(Birdlife-International 2017; KAD 2004; Yarar and Magnin 1997)
Sarıkamış Forest	10 breeding pairs	(Birdlife-International 2017)
Şemdinli Valley	10 breeding pairs	(Birdlife-International 2017)
Ilgaz Mountains	12 breeding pairs	(Heredia 1996)
Hamam Mountain*	2-5 breeding pairs	(KAD 2004; Yarar and Magnin 1997)
Kavaklı Mountain*	1-5 breeding pairs	(KAD 2004; Yarar and Magnin 1997)
Kazankaya	0-2 breeding pairs	(KAD 2004)
Dikmen Mountain	0-2 breeding pairs	(KAD 2004)
Uludağ	1 breeding pair	(Erdoğan 1998; Heredia 1996; KAD 2004)
Kızıltaş	1 breeding pair	(Erdoğan 1998)
Sündiken Mountains*	46 breeding pairs	(Kirazlı and Yamaç 2013)
Türkmenbaba Mountain*	26 breeding pairs	(Yamaç 2004)

I.3 Molecular Markers in Population Genetic Studies

A genetic marker is “a gene or DNA sequence with a known location on a chromosome and associated with a particular gene or trait” (Gu and Wang 2012). A wide range of genetic markers are available. It may be a short DNA segment, such as a single nucleotide polymorphism (SNP), a restriction fragment length polymorphism (RFLP), or a simple sequence repeats (SSRs) like microsatellites (Gu and Wang 2012).

However, it is crucial to remember that no universal ‘best’ technique exists. As Allendorf and Luikart (2007) state “the best technique to examine genetic variation depends upon the question being asked”.

Molecular markers are very useful tools for investigating the genetic variation and biodiversity with high levels of accuracy and reproducibility (Arif and Khan 2009). These markers are mainly classified into two types; mitochondrial and nuclear markers.

I.3.1 Mitochondrial DNA (mtDNA) as Marker

Animal mitochondrial DNA (mtDNA) has been often used to detect genetic variation in natural populations. It is a small circular molecule of approximately 16,000 base pairs (bp) that is relatively easy to isolate from genomic DNA and occurs in thousands of copies per cell (Allendorf and Luikart 2007). It contains 37 genes; two for rRNAs, 13 for proteins and 22 for tRNAs, all of which are essential for normal mitochondrial function (Boore 1999).

Animal mtDNA has for a long time played an important role as a genetic marker in population and evolutionary biology (Harrison 1989). Mitochondrial DNA has many special features such as (i) it is haploid and maternally inherited in most species (Lansman et al. 1983), (ii) it does not undergo recombination, which makes mtDNA molecules valuable for reconstructing phylogenetic trees (Allendorf and Luikart 2007), and (iii) mtDNA’s evolving rate is nearly ten times higher than its nuclear counterpart, with certain regions evolving even faster (control region, CR) (Wilson et al. 1985).

All mtDNA genes provide similar information (per base pair sequenced) for very closely related taxa (Sorenson 2003). However, for birds, the nucleotide sequence of the mitochondrial cytochrome b (Cyb) is more useful to resolve phylogenetic events (Wink 1995).

I.3.2 Microsatellites as Marker

Microsatellites are being widely used as a DNA marker in population genetic studies since 2000s. They are also called variable number of tandem repeats (VNTRs) or simple sequence repeats (SSRs). They form tandem repeats of a short sequence motif of one to six nucleotides (e.g., cgtcgtcgtcgtcgt, which can be represented by (cgt)n where n = 5), with PCR products usually between 75 and 300 bp long (Allendorf and Luikart 2007). Applications of microsatellites are in studies of hybridization, population history, and phylogeography; in detecting population bottlenecks and inbreeding; and assessing the social structure and dispersal effects on the genetic structure of endangered populations (Goldstein and Schlötterer 1999).

The major advantages of microsatellite markers are as follows: (i) They are generally highly polymorphic, even in small populations and in endangered species due to high mutation rates (10^{-3}-10^{-4} per generation); (ii) they are inherited codominantly, heterozygotes can be distinguished from homozygotes, and their variation is mostly neutral (Schlötterer 2000); (iii) in many species they are relatively easy to obtain, either directly through the isolation of species-specific markers, which involves constructing a genomic DNA library (Hammond et al. 1998), or by the application of markers originally isolated from related species (Rico et al. 1996); (iv) different loci can be used according to their level of variation; (v) as genetic systems, they are comparatively easy to automate, with multiplex amplification of up to eight loci possible in a single PCR reaction; and (vi) they are amplified by PCR and easy to study with non-invasively sampled material, which increases the possibility of tracking the demography, movements and social structure of populations without needing to come into direct contact with the animals themselves (Morin and Voodruff 1996).

Disadvantages of microsatellites include (i) obtaining microsatellites from certain group of organisms can be very difficult (undocumented anecdotal), (ii) different PCR problems (substitutions, insertions or deletions within the priming sites) cause non-amplification of certain alleles and lead to null alleles (Pemberton et al. 1995), and (iii) each locus is in just one copy per cell (mtDNA with multiple copies) so amplification problems (allelic dropout, non-specific amplifications) can arise (Taberlet et al. 1999).

I.4 Genetic Studies on Cinereous Vulture Populations

Except for population size, breeding biology and threats (Kirazlı and Yamaç 2013; Yamaç 2004), little is known about the Cinereous vulture populations in Turkey. A previous genetic study (Poulakakis et al. 2008) included species from Spain, Germany, the Caucasus and Mongolia but excluded samples from Turkey. Poulakakis et al. (2008) aimed to study current genetic diversity across the whole distributional range and compare the Balkan and Iberian populations with those from Asia (Mongolia), where the species is common.

They sequenced the mitochondrial cytochrome b gene and genotyped eight nuclear microsatellites in three European as well as several Asian populations to obtain information about which past or present processes can be linked to the observed genetic diversity in the species, and to help decide on proper management action. Despite a lower mitochondrial diversity in both European populations than in Mongolia, there was no genetic erosion according to microsatellite data. Additionally, they detected seven haplotypes, which were located within four geographically distinct lineages: A-Balkans (Greek population), B-Iberia (Spain population), C-Caucasus (Armenia, Georgia, Kazakhstan populations), and D-Mongolia (Mongolian population) (Poulakakis et al. 2008).

THE STRUCTURE OF THIS THESIS

This thesis has three hypotheses:

1. High dispersal rates, geographic proximity of the current subpopulations, and observed cases of population connectivity lead to a panmictic population.
2. Phylogenetic position of Turkish lineages is intermediate between Europe and central Asia and the Caucasus, mirroring its geographic location.
3. Despite published assessments of a declining population, the extinction risk of Turkish Cinereous vulture population remains low.

Part I includes general introduction part about the biology of the species and specific markers for population genetic analysis.

Part II tests if the study populations consist of a single panmictic population or if there is evidence of subpopulation structure, based on 15 polymorphic microsatellites. It reveals the genetic diversity of samples from four breeding sites in Turkey (Çatacık, Tandır, Türkmenbaba and Köroğlu), as well as degree of inbreeding and relatedness between those four sites. Finally, effective population size is estimated, and it is tested whether there is evidence of any recent bottleneck or population expansion.

Part III describes phylogeographic position of Turkish Cinereous vulture populations compared to other breeding populations within Europe, Caucasus and Mongolia by estimating its genetic differentiation, and detects current genetic diversity based on mitochondrial DNA cytochrome b sequences, and tests whether any population expansion exists.

Part IV evaluates the extinction probability of this near-threatened vulture species in Turkey by using the software VORTEX. A population viability analysis is carried out to model population dynamics and to better understand the threats the species is currently trying to cope with or may face in the future. The impact of various management implications to the outcome are also tested.

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PART II

GENETIC DIVERSITY IS RETAINED IN A BOTTLENECKED CINEREOUS VULTURE POPULATION IN TURKEY

(*with Ç. Akın Pekşen, C. Kirazlı, E. Yamaç, S. Bensch, and C.C. Bilgin, under review in IBIS*)

II.1 Abstract

Throughout the world, vulture populations have suffered threatening declines in numbers in recent years. The Cinereous vulture (*Aegypius monachus*) is a highly philopatric scavenger that ranges across southern Europe and the central Asian plateau. Turkey holds the second most numerous population in the Western Palearctic but there has been no research on the genetic structure of this particular population. In this study, we used 15 polymorphic microsatellite loci to investigate levels of nuclear diversity, relatedness of individuals, and signatures of a recent bottleneck as well as estimate the effective population size. We genotyped 81 individuals from four locations: Çatacık, Tandır, Türkmenbaba, and Köroğlu. Nuclear diversity based on allelic richness and expected heterozygosity demonstrated moderate levels of genetic diversity. We could not detect any significant differentiation between the four sub-populations, which suggest that the sampled individuals originated from a single large panmictic population. Additionally, we observed evidence of a recent bottleneck in the Turkish populations and a low effective population size 112 (95% CI 74–201). Besides, we could not detect any sign of population expansion. These findings suggest that it is urgent to implement conservation strategies for the Turkish populations and treat these as a single management unit.

Key words: Cinereous Vulture, Microsatellites, Genetic Diversity, Population structure, Recent Bottleneck, Turkey

II.2 Introduction

Despite their essential ecological roles like reducing spread of infectious diseases (Margalida et al. 2012; Şekercioğlu et al. 2004), and dispose of carrion (Ogada et al. 2012), nearly 69% of vulture species are considered near-threatened, threatened or endangered across many parts of the world (Birdlife-International 2017). This is mainly due to human disturbances such as growing threats of poisoning (Janss and Ferrer 2001), shooting (Ogada et al. 2012), food scarcity due to overhunting of livestock (Houston 1987), habitat alteration (>98% in West Africa) (Heredia 1996; Thiollay 2006) and wind farm collisions (Vasilakis et al. 2016). The major threat to Asian (>95% abundance of *Gyps* species) and African vultures was diclofenac contamination of livestock carcasses between 1990-2006 (Oaks et al. 2004; Ogada et al. 2016; Swan et al. 2006). The conservation status of most vulture species may reach critical levels in the near future (Ogada et al. 2016), because they are large scavengers with relatively low population density, large home range-requirements and slowing breeding rates (Carrete and Donázar 2005; Cramp and Simmons 1980).

The Cinereous Vulture (known as also the Eurasian Black Vulture) (*Aegypius monachus* Linnaeus, 1766) is one of the largest bird of prey. This highly philopatric scavenger is distributed from the Iberian Peninsula across southern Europe and through the central Asian plateau to Mongolia and China (Birdlife-International 2017). The species is classified globally as Near Threatened (Birdlife-International 2017) since it has experienced a considerable decline during the last centuries. However, the European population is now recognized as 'Least Concern' due to increasing population sizes in particular on the Iberian Peninsula (Spain constitutes 87% of the European population with ~2000 breeding pairs) (Moreno-Opo et al. 2010). Despite this increase, many breeding areas across the European continent were lost due to anthropogenic effects (poisoning, shooting, and nest destruction) (Carrete and Donázar 2005).

Turkey has the second largest Cinereous vulture population in Europe with 50-200 pairs (Heredia et al. 1997; Yamaç and Günyel 2010), and local populations show variable breeding success (Kirazlı and Yamaç 2013; Yamaç 2004). The species like most other vultures is monogamous (Nam and Lee 2009). The main threats for the species were detected to be forestry activities, fires and recreational disturbance in Turkey (Kirazlı and Yamaç 2013; Yamaç 2004).

The maintenance of genetic diversity is essential for the adaptation and fitness of a species (Frankham et al. 2002). Small populations are vulnerable to the effects of genetic drift and may lose valuable genetic variation, which might eventually lead to local extinction due to inbreeding depression and decreased adaptation (Mills 2012). For the successful management and conservation of any species in decline, investigating the reasons and impact of sudden population drops is critical (Xenikoudakis et al. 2015). In this study, based on 15 microsatellites, we will specifically test if the study populations (Çatacık, Tandır, Türkmenbaba and Köroğlu) consist of a single panmictic metapopulation or if there is evidence of subpopulation structure, attempt to reveal the genetic diversity of samples from four breeding sites in Central Anatolia, as well as detect inbreeding and relatedness, and estimate effective population size, and whether or not there is evidence of any recent bottleneck or population expansions.

II.3 Methods

II.3.1 Study area and sample collection

The samples were collected from Türkmenbaba Mountain (covers 17,500 ha area) located between Eskişehir and Kütahya (39.50°N, 30.33°E), from Sündiken Mountains (covers 218,068 ha area) in the Middle Sakarya Region (39.93 °N, 31.18 °E), both in northwestern Central Anatolia, and from Köroğlu Mountain located between Bolu and Ankara (covers 146,330 ha) in the western Black Sea Region (40.43 °N 31.98 °E) (Kirazlı 2013; Kirazlı and Yamaç 2013; Yamaç 2004) (Figure II.1). The

Sündiken population is made up of two separate sub-populations, namely Tandır and Çatacık.

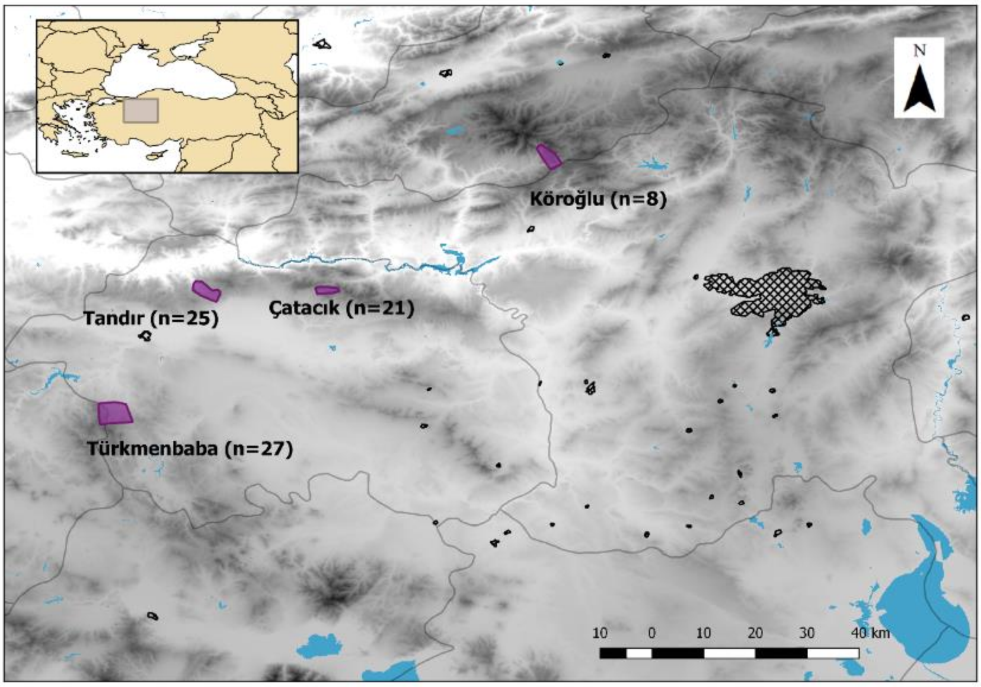

Figure II.1 A map of the location of the study populations and sample sizes

A total of 291 samples (molted feathers (n = 270, 93%), plucked feathers (n=16, 5%) and blood from four chicks and one adult in different nests (n = 5, 2%) were collected from the wild between the years 2009-2012, and 2015-2016. For each active nest, we included either feathers from one female and one male, or blood samples from a juvenile in those 5 cases when these were available. The closest distance between the two active nests was 51 m and 140 m in Sündiken populations (central Çatacık population and west Tandır population) and Türkmenbaba population, respectively (Kirazlı and Yamaç 2013; Yamaç 2004). On this basis, only feathers found at or within

150 m of an active nest-site were used for genotyping purposes. The two exceptions to this rule was when feathers were collected directly from one juvenile bird in Tandır population, or if samples collected from the same nest site but in different years were determined to belong to different individuals. All feathers were firstly sexed, and if possible, one male and one female sample were selected from each nest site within a single year for microsatellite analysis. Because of this procedure, to obtain feathers from one male and female per nest site, we isolated almost all samples (n=262), except samples of down feathers (n=24). Finally, we restricted the microsatellite analyses to 146 samples containing a DNA concentration >19 ng/ µl.

II.3.2 DNA extraction, molecular marker selection and PCR

The DNA from molted feathers were extracted by utilizing the blood spot within the superior umbilicus of the feather (Horváth et al. 2005). Qiagen DNeasy Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany) was used to extract both feather and blood samples. Isolated DNA was quantified using NanoDrop 2000 UV-Visible spectrophotometer. Molecular sexing was done by using the primers CHD1F/CHD1R as previously described (Lee et al. 2010), according to procedure in Çakmak et al. (2017).

We first screened primers for 31 microsatellite loci originally developed for Bearded Vulture *Gypaetus barbatus* (Gautschi et al. 2000), Eurasian Griffon Vulture *Gyps fulvus* (Mira et al. 2002), the Asian Vultures *Gyps bengalensis*, *Gyps tenuirostris*, and *Sarcogyps calvus* (Kapetanakos et al. 2014), and Egyptian Vulture *Neophron percnopterus* (Agudo et al. 2008). For the amplification, locus-specific fluorescently labelled (6-FAM, HEX, ROX, and TAMRA, Applied Biosystems) forward primers were used in eight different multiplex sets. Template DNA was amplified using FIRE POL Master mix (Solis Biodyne) in a total reaction volume of 25 µl, containing 1 µl of genomic DNA, 0.1-0.3 µM of each primer pair and 1x Master Mix (Solis Biodyne). Polymerase chain reaction (PCR) were conducted using touch down PCR under the following conditions: 15 min 95 ^{o}C heat activation followed by 35 cycles of 20 sec at

95 ^{o}C, 1 min at TA ^{o}C (optimum annealing temperature (TA) for each multiplex set, Table II.1), 1 min at 72 ^{o}C, and a final extension of 10 min at 72 ^{o}C. 2% agarose gel electrophoresis was used to qualify PCR amplifications. In the case of low peak signal, genotyping was repeated three times per sample. Allele sizes were determined in an ABI-PRISM 3100 sequencer (Applied Biosystems). Raw data from the sequencer were treated with the PEAK SCANNER 2.0 (Applied Biosystems) software for the identification of peaks and fragment sizing by using 500 LIZTM size standard. Genotypes (n=146) were firstly scored by eye; fifty samples were found to be duplicates of identical genotypes leaving 96 samples that differed at least for one allele in one locus.

Table II.1 Fifteen nuclear loci used in this study and their characteristics.

Locus	**Sequences (5’- 3’)**	**Repeat motif**	**TA (°C)**	**Expected allele size**	**Observed allele size**	**Label**	**References**
BV11	TGTTTGCAAGCTGGAGACC*	(CA)22	61	164-180	161-181	HEX	(Gautschi et al. 2000)
	AAAAGCCTTGGGGTAAGCAC						
GB4-4G	CGGTGAGCGGCCTCATTATC*	(GTTT)8	58	164-176	156-160	TAMRA	(Kapetanakos et al. 2014)
	GCTCAACTTTCAGTTCCACTTC						
GB3-2C	ATGAATCCAGGCTCAGTCAGAAC*	(AGA)50	58	404-461	389-401	HEX	(Kapetanakos et al. 2014)
	AGACATGGTAAGGAGTCAGCAGC						
Gf3F3	GATCTTTCCCCTTCTGTG*	(CT)10	56	180	173-175	ROX	(Mira et al. 2002)
	TTCGTGCAGTGATGCTGGTG						
Gt3-35	CCCCTTGTATGACAATGGTACAGTAT*	(ATT)10(ACT)13	56	216-264	255-282	ROX	(Kapetanakos et al. 2014)
	GTTTCTGTATTCAAAAGACATGACATCCAC						
GT4-20	GTGAGCCCTCCCATTGAGTCAT*	(GAAA)12(GA)8	56	298-334	272-280	HEX	(Kapetanakos et al. 2014)
	CTCAAGTGCATGCCCGCTG						
BV6	AATCTGCATCCCAGTTCTGC*	(CA)11	56	127-161	123-165	HEX	(Gautschi et al. 2000)
	CCGGAGACTCTCAGAACTTAAC						
NP141	GGAAGCCAATGAAAGCTCAG*	(CA)18	54	290-300	300-302	FAM	(Agudo et al. 2008)
	ACACATTTGCTGTGGTCTGG						
NP163	ACCATTCCTTAAGATTGAGAACAC*	(GT)6TT(GT)8	54	226-236	223-229	FAM	(Agudo et al. 2008)
	CATGCAGGACAGGAAAACAAATAG						
NP166	TGCAGTCAAACAGAGTAAAAGG*	(CA)11	54	190-200	170-172	TAMRA	(Agudo et al. 2008)
	CTAGCTCCACACTGAGACACAA						
NP229	AGGCACCTCACTGACACGTA*	(CTAT)5CTAC(CTAT)4	54	171-187	165-177	ROX	(Agudo et al. 2008)
	AACAAAAATCCCGTATCACGA						
BV17	TGATGTGCAGATGCGTGAC*	(CA)11	52	185-187	187-189	ROX	(Gautschi et al. 2000)
	GGACTCTGATGAAGCCAAGC						
NP39	TATCCCTCTGTCCCCCTTTT*	(CA)17	52	276-310	282-296	FAM	(Agudo et al. 2008)
	AGAATGGGAAGGTGCTCTTG						
BV20	GAACAGCACTGAACGTGAGC*	(CA)13	52	136-140	140-148	ROX	(Gautschi et al. 2000)
	GTTTCTCCTGACAGTGAAATAACTC						
BV13	AAAACAGAGTTTTCACATTTTCATAAG*	(CA)16	50	174-176	176-190	TAMRA	(Gautschi et al. 2000)
	TTCAGGAAACAGAAGCATGAAC						

II.3.3 Microsatellite analysis

II.3.3.1 Reliability of the data

Nineteen of these 31 loci proved polymorphic in the Cinereous vulture, but four (BV16, GB2-4B, Gf9-C1, and NP155) had high null allele frequency ($r>0.2$) tested using FREENA (Chapuis and Estoup 2006) with 10 000 replicates, and were removed from further analysis. For all populations the possible presence of linkage disequilibrium for the remaining 15 loci were calculated by FSTAT version 2.9.3 (Goudet 2001). . Genotyping errors, allelic dropout, and scoring of stutter peaks, were assessed statistically using MICROCHECKER version 2.2.3 (Van Oosterhout et al. 2004). The software GIMLET version 1.3.3 (Valière 2002) was used to estimate the probability of two individuals carrying identical multilocus genotypes, which demonstrates discriminative power of the microsatellites to distinguish between individuals. Histograms illustrating the pairwise similarity of individuals were made in R.

II.3.3.2 Population genetic structure

The polymorphic information content (PIC) value that demonstrates possible utility of the markers in identifying individuals was estimated by software CERVUS version 3.0.7 (Kalinowski et al. 2007; Marshall et al. 1998) where $PIC>0.5$ is highly informative, $0.5>PIC>0.25$ is reasonably informative, and $PIC<0.25$ is slightly informative (Botstein et al. 1980). F_{ST} (Weir and Cockerham 1984) values between pairs of populations was assessed by Arlequin v3.5 (Excoffier and Lischer 2010) where the statistical significance was tested by using 10000 permutations. Presence of spatial structure among populations were tested for up to five clusters (K) by using STRUCTURE version 2.3.4 (Pritchard et al. 2000). The burn-in period was 100000 and 500000 Markov Chain Monte Carlo (MCMC) iterations for each run. Due to close geographic proximity of these populations and the likelihood that they are relatively closely related, we allowed admixture and correlated allele frequencies because there is no reason to consider each population as completely discrete (Porras-Hurtado et al.

2013). A LOCPRIOR model was chosen to incorporate the location of individuals in sampling groups as a priori because LOCPRIOR model has been perform better in the case of weak population structure (Mims et al. 2016; Pritchard et al. 2000). STRUCTURE HARVESTER version 0.6.94 (Earl 2012; Earl and Vonholdt 2012) was used for estimating the most probable *K* value which determined by Evanno's method (Evanno et al. 2005) where delta-*K* value cannot be calculated for $K = 1$. Because the most probable *K* is assessed by the second-order rate of change in the log-likelihood. So, if $K = 1$ has the greatest loglikelihood, $K = 1$ is accepted most likely for runs (Spear et al. 2012). The CLUMPP software (Jakobsson and Rosenberg 2007) was used to permute the membership coefficients of individuals. Results were visualized by using DISTRUCT version 1.1 (Rosenberg 2004). Additionally, GENETIX version 4.05 (Belkhir et al. 1996; Belkhir et al. 2004) was used for visualization of the differentiation of individuals by factorial correspondence analysis (FCA) in two-dimensional space (2D).

II.3.3.3 Relatedness and relationships

The ML-RELATE software (Kalinowski et al. 2006) was conducted to estimate relatedness and relationship between pairs of individuals. This method calculates the maximum likelihood estimates of relatedness (*r*) by using the downhill simplex routine that is started from 11 sets of points, one of which is unrelated, full sibs, and parent-offspring (PO). The other ten are the random value. The software also assigns pairs of samples into four common pedigree relationships: unrelated (U), half-siblings (HS), full-siblings (FS), and parent-offspring (PO). Although the log-likelihood of four types of relationships were typically non-significant, we used these classifications to ask whether related birds were more common within than between sub-populations.

II.3.3.4 Genetic diversity and bottleneck

Basic statistics like number of alleles and allelic richness were calculated by FSTAT version 2.9.3 (Goudet 2001). Inbreeding coefficients for each locus and population were calculated using GENEPOP version 3.4 (Raymond and Rousset 1995), where the significance was evaluated by Fisher exact test P-values, applying the Markov chain method (10000 dememorization). Deviations from Hardy-Weinberg equilibrium (HWE) were carried out using exact tests implemented in Arlequin v3.5 (Excoffier and Lischer 2010). BOTTLENECK version 1.2.02 (Cornuet and Luikart 1996; Piry et al. 1999) was run to detect any recent bottleneck in the population. Statistical significance of the heterozygosity excess was performed by Wilcoxon sign rank test under two phase mutation model (TPM). The Mode-shift, a graphical method, was also used to estimate an allele frequency distortion after a bottleneck (Luikart et al. 1998). In bottlenecked populations, alleles with intermediate frequencies (e.g., 0.1-0.2) are expected to be more common than alleles with low frequencies (<0.1) (Ganapathi et al. 2012). Effective population size of the population was calculated in NeESTIMATOR version 2.01 (Do et al. 2014) by using linkage disequilibrium (LD) method. Finally, to estimate any population expansion KGTESTS (Bilgin 2007) was used.

II.4 Results

II.4.1 Reliability of the data

All possible comparisons between pairs of loci in each population yielded no significant ($p = 0.000119$ for 5% nominal level) linkage disequilibrium after Bonferroni correction, so they could be treated as independent. Analyses using FREENA and CERVUS showed no evidence of presence of null alleles among the 15 loci in the four populations, but MICROCHECKER detected the presence of null alleles at one locus (BV6) in the Türkmenbaba population. However, because this finding was not consistent among subpopulations, we kept the locus for further analysis. There was no evidence of genotyping errors due to stuttering and allele dropout at the 15 loci in the study populations. Since the samples consisted of shed feathers, it is possible that some individuals were included in more than one sample. As this may introduce a bias in estimating of population structure and effective population size changes, we first used GIMLET to construct a matrix of the pairwise comparisons of these 96 genotypes. A histogram of the number of identical loci between samples clearly showed a bimodal distribution (Fig II.2, gray bars). We manually inspected the pairs of outliers that had >13 loci with identical alleles and concluded that the differences between the obtained genotypes could be explained by allelic dropouts, and hence, likely representing the same individual. We extracted one genotype from each of these pairs by keeping the heterozygote genotype for these mismatching loci. After these removals, we obtained a final data set of genotypes corresponding to 81 unique individuals (Figure II.2, black bars). Thus, this reduced dataset should be reliable for investigating the genetic population diversity and structure of Turkish Cinereous vultures.

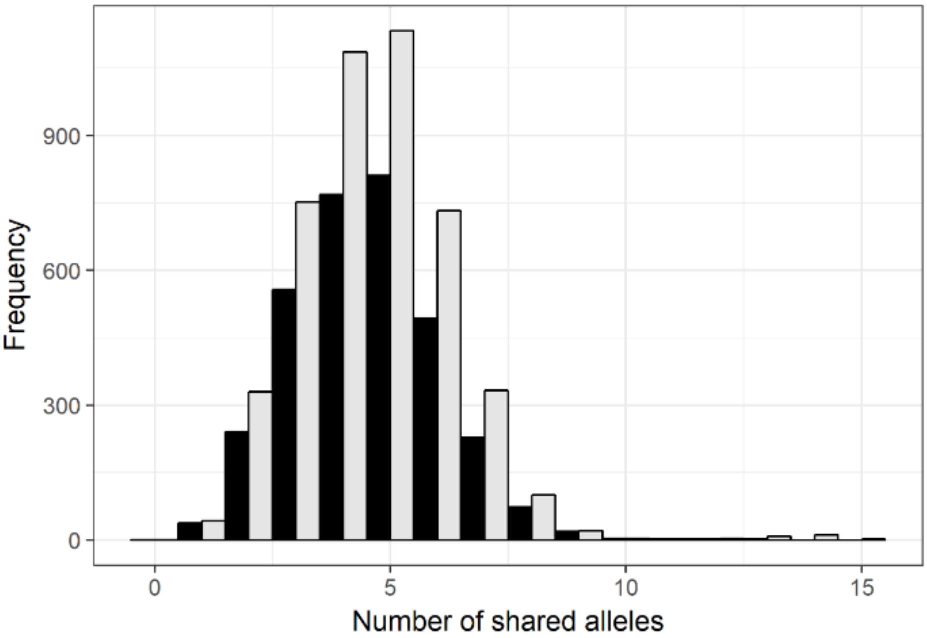

Figure II.2 Histogram of pairwise comparison of genotypes. Gray bars demonstrate the numbers of shared alleles with all samples, and black bars show the numbers of shared alleles with unique genotypes.

II.4.2 Population genetic structure and relatedness

Overall, there was no evidence of population structure between the four populations. F_{ST} analyses between population pairs were calculated and found to be non-significant ($p>0.05$), ranging from 0.000 to 0.011 (Table II.2). According to results of STRUCTURE from 20 replicates, the mean likelihood score (Ln(*K*)) was highest for *K*=1 (Figure II.3a) although the Evanno method suggested the number of clusters to be two ($\Delta K=2$) (Figure II.3b, c). Similar to the F_{ST} and STRUCTURE results, Factorial Correspondence Analysis (FCA) also failed to differentiate these four populations (Figure II.4).

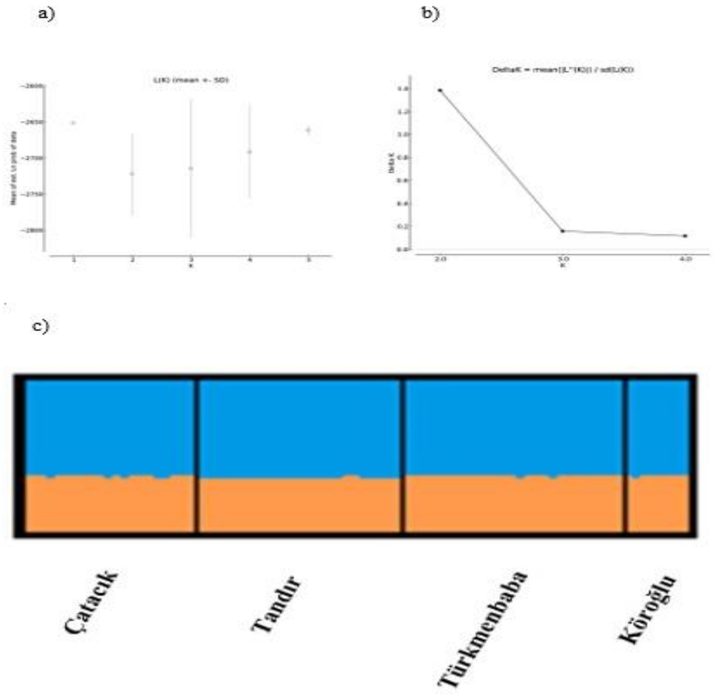

Figure II.3 Clustering analysis of genotypes at fifteen microsatellite loci performed using the STRUCTURE software a) The mean likelihood of each cluster for *K*=1 to 5, where the error bar demonstrates standard deviations b) Delta *K*=2 c) The visualization of outputs, *K*=2.

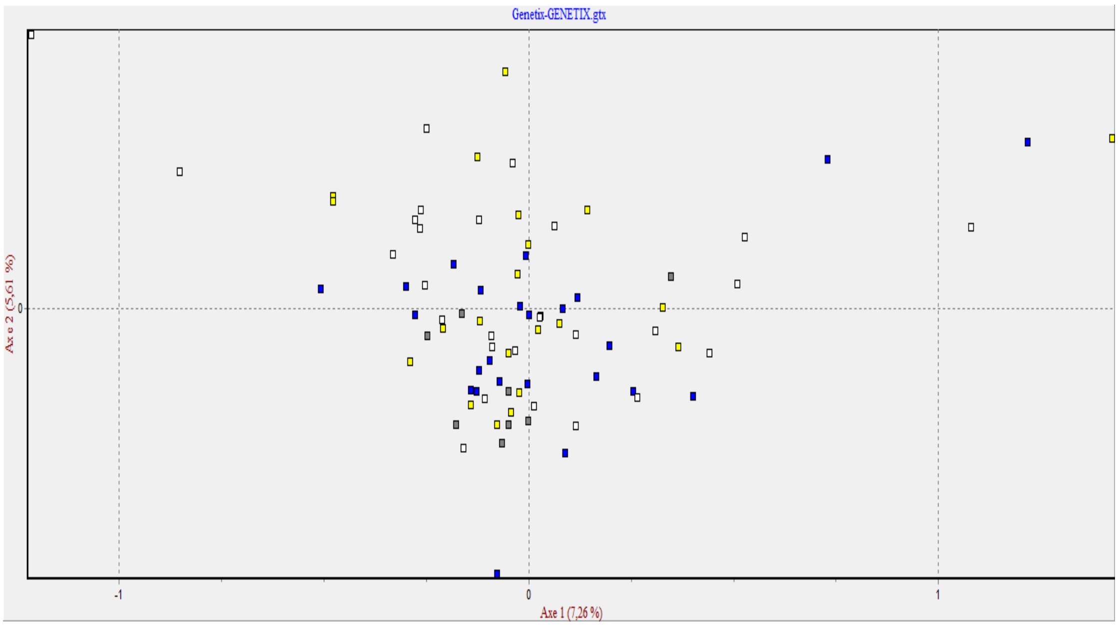

Figure II.4 Factorial Correspondence Analysis of overall data (Axe 1: 7.26%, Axe 2: 5.61%). Yellow color: Çatacık pop., Blue color: Tandır pop., White color: Türkmenbaba pop., and Gray color: Bolu pop.

Of 3240 possible pairwise combinations among the 81 individuals, ML-RELATE estimated 564 family relations. These were classified as 55 full siblings, 480 half siblings and 29 parent–offspring relationships. None of the individual pairs of samples that were assigned to the three relatedness classes were significant on their own (and may thus contain misclassifications), but taken together, allowed us to examine whether related birds were more likely to be found within than between colonies. When comparing the relationships between each pair of individuals from the four study sub-populations, 173 of the 564 family relations (PO, FS, or HS) were from within the study sub-populations and whereas 391 involved individuals sampled in different sub-populations (Table II.2 and Figure II.5) (chi-square test, p= 0.35). Additionally, eleven of the fifteen nesting pairs were classified as unrelated (73%).

Table II.2 Pairwise F_{ST} values between four population samples of *Aegypius monachus* based on 15 nuclear microsatellite loci above the diagonal and the proportion of pairs of individuals in the complete data set identified as being related (parent-offspring, sibs or half sibs) within (diagonal) and between study populations below the diagonal.

	Çatacık	Tandır	Türkmenbaba	Köroğlu
Çatacık	0.200	0.006	0.002	0.000
Tandır	0.175	0.203	0.005	0.001
Türkmenbaba	0.162	0.181	0.179	0.011
Köroğlu	0.196	0.130	0.120	0.250

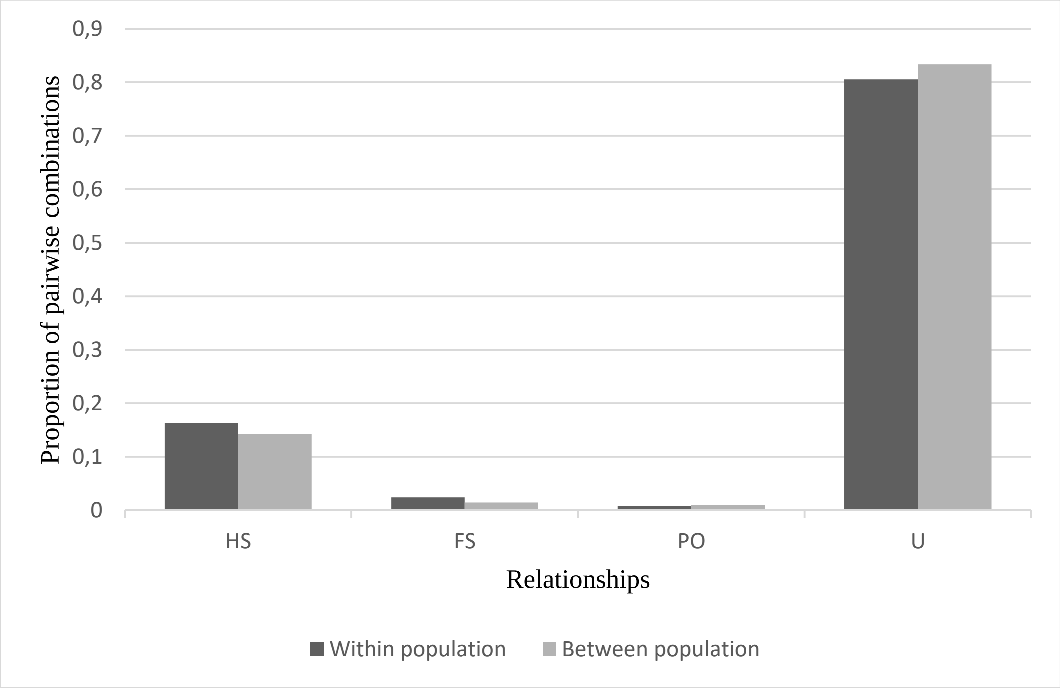

Figure II.5 Comparison of the proportion of pairwise combinations and relationships within and between populations. HS: Half-siblings, FS: Full-siblings, PO-Parent-offspring, U-Unrelated.

II.4.3 Genetic diversity and bottleneck

The fifteen nuclear loci were successfully genotyped in 81 individuals from the four locations; Çatacık (n=21), Tandır (n=25), Türkmenbaba (n=27), and Köroğlu (n=8) (Table II.3 and II.4). A total of 67 alleles were detected in all loci throughout all populations, with an average number of four alleles per locus and varying between two to 10 (BV11 and GT3-35) alleles. These fifteen loci demonstrated overall moderate level of genetic diversity, based on allelic richness (A_R, range 1.803-7.195) and expected heterozygosity (H_E, range 0.189-0.882), and were similar in four populations. The most informative eight loci (PIC>0.5) were BV11, GT3-35, BV6, NP163, NP229, NP39, BV20, and BV13 (Table II.5). All populations were in HWE, except for locus NP163 (p= 0.0008) in the Türkmenbaba population. Besides, none of the estimates of inbreeding coefficient (F_{IS}) differed significantly from zero, except again for NP163 in the Türkmenbaba population. No private allele was detected in those four populations.

Table II.3 Sample size (n), the mean number of alleles per locus (N_a), observed heterozygosity (H_O), and expected heterozygosity (H_E) for each population based on the fifteen microsatellite loci.

Population	**n**	**N_a**	**H_O**	**H_E**
Çatacık	21	4.0667	0.5206	0.5384
Tandır	25	4.2000	0.5361	0.5473
Türkmenbaba	27	4.2667	0.5132	0.5466
Köroğlu	8	3.4667	0.5214	0.5259
Mean		**4.0000**	**0.523**	**0.540**

Table II.4 A comparison of genetic diversity at fifteen microsatellite loci between *Aegypius monachus* populations.

Locus	**Çatacık population**							**Tandır population**							**Türkmenbaba population**							**Köroğlu population**						
	N	**Na**	A_R	H_O	H_E	**p**	F_{IS}	**N**	**Na**	A_R	H_O	H_E	**p**	F_{IS}	**N**	**Na**	A_R	H_O	H_E	**p**	F_{IS}	**N**	**Na**	A_R	H_O	H_E	**p**	F_{IS}
BV11	21	9	6.939	0.857	0.875	0.752	0.020	25	9	6.680	0.720	0.860	0.079	0.165	26	10	7.218	0.846	0.876	0.202	0.035	8	7	6.725	1	0.875	0.920	-0.154
GB4-4G	21	2	2	0.381	0.511	0.379	0.259	25	2	1.999	0.400	0.470	0.662	0.152	27	2	2	0.556	0.507	0.707	-0.099	8	2	2	0.500	0.500	1	0.000
GB3-2C	21	2	1.884	0.238	0.215	1	-0.111	25	2	1.993	0.360	0.393	0.641	0.085	27	3	2.452	0.444	0.475	0.688	0.066	8	3	2.867	0.125	0.342	0.067	0.650
Gf3F3	21	2	1.715	0.143	0.136	1	-0.053	24	2	1.952	0.167	0.284	0.090	0.418	27	2	1.895	0.185	0.230	0.357	0.198	8	2	1.875	0.125	0.125	1	0.000
Gt3-35	21	9	7.308	1	0.890	0.882	-0.128	24	8	6.360	0.875	0.865	0.379	-0.023	27	9	6.537	0.889	0.857	0.877	-0.038	8	6	5.867	0.625	0.867	0.080	0.293
GT4-20	21	3	2.262	0.238	0.292	0.452	0.187	25	3	2.471	0.458	0.377	0.049	-0,222	27	2	1.926	0.296	0.257	1	-0.156	8	2	2	0.250	0.400	0.384	0.391
BV6	21	5	4.461	0.571	0.712	0.023	0.201	25	5	3.852	0.400	0.519	0.149	0.233	27	4	3.597	0.370	0.549	0.018	0.329	8	5	4.850	0.750	0.675	0.650	-0.120
NP141	21	2	2	0.571	0.511	0.675	-0.122	25	2	2	0.560	0.509	0.699	-0.102	27	2	1.998	0.407	0.440	1	0.074	8	2	2	0.571	0.527	1	-0.091
NP163	21	4	3.954	0.619	0.758	0.085	0.188	25	4	3.699	0.720	0.680	0.071	-0.060	27	4	3.866	0.593	0.727	**0.0008***	**0.188**$^{+}$	8	4	3.992	0.875	0.767	0.547	-0.153
NP166	21	2	1.817	0.190	0.177	1	-0.081	25	2	1.822	0.200	0.184	1	-0.091	27	2	1.895	0.111	0.230	0.037	0.522	8	2	1.875	0.125	0.125	1	0.000

Table II.4 (continued).

Locus	**Çatacık population**							**Tandır population**							**Türkmenbaba population**							**Köroğlu population**						
	N	**Na**	A_R	H_O	H_E	**p**	F_{IS}	**N**	**Na**	A_R	H_O	H_E	**p**	F_{IS}	**N**	**Na**	A_R	H_O	H_E	**p**	F_{IS}	**N**	**Na**	A_R	H_O	H_E	**p**	F_{IS}
NP229	21	4	3.701	0.714	0.682	0.461	-0.049	25	4	3.255	0.680	0.667	0.461	-0.020	27	4	3.815	0.704	0.719	0.146	0.022	8	3	3	0.625	0.625	0.731	0.000
BV17	21	2	1.956	0.333	0.285	1	-0.177	25	2	1.962	0.280	0.301	1	0.072	27	2	1.926	0.222	0.257	0.455	0.138	8	2	2	0.375	0.458	1	0.192
NP39	21	5	4.609	0.714	0.770	0.844	0.074	25	8	5.766	0.800	0.817	0.680	0.021	27	8	5.573	0.704	0.787	0.009	0.108	8	5	4.875	0.875	0.817	0.704	-0.077
BV20	21	5	4.350	0.714	0.747	0.701	0.044	25	5	3.967	0.840	0.706	0.512	-0.194	27	5	4.595	0.777	0.776	0.700	-0.002	8	3	2.875	0.500	0.592	1	0.164
BV13	21	5	4.078	0.524	0.714	0.054	0.272	25	4	3.979	0.600	0.697	0.062	0.142	27	5	3.877	0.593	0.668	0.606	0.115	8	4	3.983	0.500	0.725	0.276	0.325

003, $^{+}p<0.05$

Table II.5 Summary statistics per loci. N_a, observed allele number; A_R, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; PIC, polymorphism information content.

Locus	N_a	A_R	H_O	H_E	PIC
BV11	10	7.015	0.825	0.871	0.852
GB4-4G	2	2.000	0.457	0.502	0.374
GB3-2C	3	2.214	0.333	0.376	0.316
Gf3-F3	2	1.843	0.163	0.211	0.187
Gt3-35	10	7.195	0.888	0.882	0.864
Gt4-20	3	2.336	0.325	0.313	0.273
BV6	5	3.992	0.469	0.595	0.558
NP141	2	2.000	0.513	0.497	0.372
NP163	4	3.870	0.667	0.728	0.674
NP166	2	1.803	0.160	0.189	0.170
NP229	4	3.604	0.691	0.680	0.616
BV17	2	1.945	0.284	0.296	0.251
NP39	8	5.433	0.753	0.795	0.762
BV20	5	4.223	0.753	0.728	0.677
BV13	5	3.884	0.568	0.684	0.623
Mean	**4.47**	**3.56**	**0.523**	**0.556**	**0.505**

For the accuracy of the bottleneck test and effective population size calculation, all four populations were assumed as a single panmictic population. According to Wilcoxon test, the observed proportion of heterozygotes departed significantly from the expectation under a mutation-drift equilibrium by using a two phase mutation model (TPM) in the population (one tail for H excess: $p = 0.00003$), which indicated that this population has suffered a recent bottleneck. The proportion of alleles in different allele frequency classes (0-0.1 low; 0.9-1 high allele frequency class), showed a mode shifted distribution in contrast to a normal L-shaped distribution as would be expected for non-bottlenecked populations (Figure II.6). This is further supporting that the population deviates from a mutation-drift equilibrium and that it has experienced a recent bottleneck. The effective population size estimate was 112 (95% CI 74–201).

We could not detect any signs of population expansion (number of loci with a negative k=7; k-test p value= 0.65).

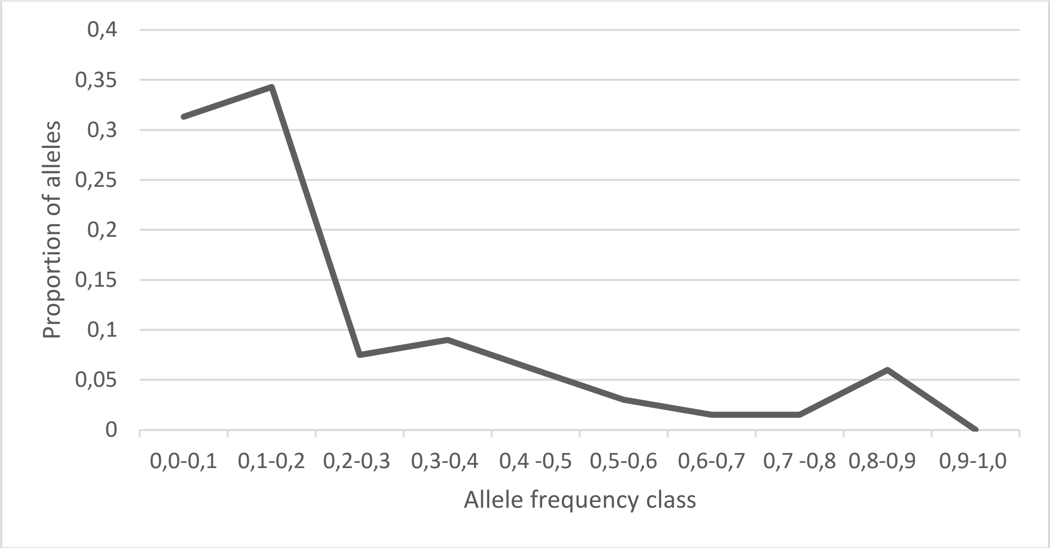

Figure II.6 Mode shift of the allele frequencies indicating the occurrence of a recent genetic bottleneck.

II.5 Discussion

We had hypothesized that these four sub-populations would constitute one panmictic population, and analyses of population genetic structure suggested that the sampled individuals originated from a single population. High dispersal rates and the relatively short distances between studied locations may explain our finding of an undifferentiated population structure (Craig et al. 2016; Jones et al. 2004; Le Gouar et al. 2005). The genetic structure in the population will disappear when there is significant gene flow, which is a homogenizing force that lead allele frequencies in subpopulations to converge (Wilson and Bossert 1971). Besides, Yamaç and Bilgin

(2012) tracked newly fledged birds from Türkmenbaba population by using GPS-GSM telemetry and observed one bird moving 80 km north to Çatacık immediately after fledging.

We also could not detect any significant inbreeding coefficient larger than zero, which may either be explained by limited sample size (Bourke et al. 2010; Ogden et al. 2015) or by birds choosing unrelated individuals as mates (Smith et al. 1997) like in this study (73%). Several studies demonstrated that choosing unrelated individuals as mates may be common in other species also rather than birds (Smith et al. 1997; Hoffman et al. 2007). However, a full understanding of the overall consequences of inbreeding in wild populations requires not only the detection of relatedness but also long term measurements of breeding success and survivorship (Brzeski et al. 2014; Pemberton 2004).

We detected a signature of a genetic bottleneck suggesting that the demographic decline in the 20th Century have had a critical effect on the Turkish population of Cinereous vultures. However, despite the bottleneck, the relatively high genetic diversity observed could be the result of long generation times of the vulture species, which is expected to buffer the effects of the experienced bottleneck (Hailer et al. 2006, Nadaeu 2012) because it produce shorter effective time of an experienced bottleneck (Hailer et al. 2006). In bottlenecked populations with population decline and reduced genetic diversity, the alleles in the population are lost faster compared to heterozygosity (Nadaeu 2012); thus, the lack of inbreeding may demonstrate that the bottleneck is too recent to detect the effects of inbreeding in the population (Canal et al. 2016). It is also considered that in the case of populations at small size for many generations, it may be less likely to show signatures of inbreeding due to the removal of recessive deleterious alleles in the demographic history of the species (Leberg and Firmin 2008). Thus, after a bottleneck, excess gene diversity could be observed for a short time (Ganapathi et al. 2012). It can be concluded that the demographic bottleneck has had, at least until now, little effect on the genetic diversity of the Cinereous vulture population.

The ML-RELATE method may describe many false-positive relatives (Allentoft et al. 2015) and it is likely that pairs of samples here classified as half-sibs may contain lower level relatives such as cousins and nieces. Given this uncertainty, the proportion of birds estimated to be related was not higher within populations than between populations, indicating that birds frequently move away from their natal colony for breeding, which is consistent with the F_{ST} analyses and again suggest that the study sites make up a single panmictic population.

The level of heterozygosity was consistent with other raptor populations (Craig et al. 2016; Godoy et al. 2004; Rudnick et al. 2005). Similar to the small relict population in Greece, our population also demonstrated higher observed heterozygosity than was found in the much larger Spanish Cinereous vulture population (Poulakakis et al. 2008). Although HWE could have been affected by small sample size (Györffy et al. 2004; Hui et al. 2017), Köroğlu population (n=8) showed no deviation from HWE.

The Turkish population was reported to be stable or declining, despite their variable breeding success (Kirazlı and Yamaç 2013; Yamaç 2004). The estimated countrywide population varied from 50 pairs up to 200 pairs, assuming the presence of undiscovered colonies, particularly in the east. We estimated the effective population size to be 112 individuals, which is lower than the total number of active nests (83) reported in Kirazlı and Yamaç (2013); and Yamaç (2004). Moreover, coverage of nesting colonies is rarely complete, and unsampled smaller colonies that are probably part of the studied meta-population exist in northern Central Anatolia (Kirwan et al. 2008; Yarar and Magnin 1997). Since strict monogamy is the rule among Cinereous vultures, N_e is expected to be close to the censused number of breeding adults. Similar results were also obtained in the study of Bourke et al. (2010). In their study, N_e was found as 437 individuals and census population size was estimated as 442 pairs in the monogamous British Golden eagle species. However, Frankham (1995) estimated N_e as much lower than census size. Similarly, Doyle et al. (2016) also estimated very low effective populatipon size (nearly 400) compared to census population size (nearly 32.000). The discrepancy observed can partly be explained by highly overlapping generations of breeders (Felsenstein 1971), and long-term pair bonds and spatial fidelity to particular localities (Hartl 2000).

II.5.1 Implications for conservation

Despite decades of speculating on the presence of undiscovered colonies elsewhere (Kirwan et al. 2008) and intensive searching in northeastern Turkey (DKM 2011; Turak et al. 2011), no new colonies have been detected. Therefore, the studied sub-populations likely represent the breeding core of this species in Turkey, for which there is no evidence of substructure. Our findings suggest that management actions should consider Cinereous vulture populations in Turkey as a whole (i.e. a single management unit), which might reduce costs and enable a more focused, comprehensive effort for their conservation.

Current moderate level heterozygosity suggested that there is limited need for intervention to increase the present genetic diversity. In the short-term, the aim should be to increase population size by providing continuous protection for individuals and habitat management.

The possible reasons for the inferred decline of Cinereous vultures leading to a bottleneck are many and complicated but they appear to include the increasing intensification of the livestock industry, and the widespread and indiscriminate use of poison by the rural populace during 1960-1995. On the other hand, the lack of any evidence of post-bottleneck expansion is a further cause of worry. Despite the much reduced use of poisons recently and the availability of new sources of food near poultry farms (Yamaç and Günyel 2010), the country population appears to have neither increased in size nor founded new colonies in the last two decades. These observations may possibly be explained by high rates of post-fledging mortality at the winter quarters (Yamaç and Bilgin 2012) and highly conservative behavior of adults in nest site selection (Dias et al. 2017; Yamaç unpubl.data), respectively, although data is not sufficient to prove or disprove either hypothesis. Continued monitoring of colonies both by conventional nest counts and through non-invasive genetic sampling is recommended.

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PART III

EXTREMELY LOW MITOCHONDRIAL DNA DIVERSITY IN A NEAR THREATENED SPECIES: CINEREOUS VULTURES IN TURKEY

(*with Ç. Akın Pekşen, C. Kirazlı, E. Yamaç, and C.C. Bilgin, to be submitted*)

III.1 Abstract

The Cinereous Vulture (*Aegypius monachus*) is a near threatened raptor species that occurs in isolated populations across its range. The only global genetic study so far excluded samples from Turkey in its analyses, although they form a significant part of the species' range. In this study, we aimed to obtain sequences (311 bp) of the mitochondrial cytochrome b gene from 58 Turkish individuals to assess genetic diversity, phylogeny and whether any signals for population expansion exists. Phylogenetic analyses indicate that the Turkish birds hold, along with those from the Caucasus, an intermediate position between European (Balkan and Iberian) and North Asian (Mongolian) lineages. Our findings also demonstrate extremely low mitochondrial diversity and suggest no population expansion. We propose that conservation plans should take into account the observed low genetic variation and investigate further the reasons for lack of population increase despite high productivity.

Keywords: mtDNA, Genetic Diversity, Turkey

III.2 Introduction

As a result of human disturbance, most species of Palearctic raptors have experienced demographic declines through the 20th century (Martinez-Cruz 2011). These population declines may cause the loss of diversity (Frankham et al. 2002), which even makes it difficult to predict future trends in population size. Additionally, population breakdowns lead to severe bottleneck which both increases extinction risk of the species and decreases evolutionary potential (Frankham et al. 2002). Generally, genetic differentiation among bird species is very low due to their high dispersal rates accompanied by gene flow (Barrowclough 1983). However, significant differentiation may be induced by behavioral characteristics like philopatry (Godoy et al. 2004), geographical barriers (Sonsthagen et al. 2012), or isolation on islands (Kretzmann et al. 2003). The understanding of the processes that affect the diversity of wild species and the genetic differentiation among them is critically important to develop suitable conservation and management plans (Allentoft and O’Brien 2010).

The Cinereous Vulture (*Aegypius monachus* Linnaeus, 1766) is a near threatened, large raptor species in the Palearctic region. The species experienced a severe decline in population size which causes the current patchy distribution throughout its range (Birdlife-International 2017). Many breeding areas were lost in Europe, and the species is regionally extinct in Cyprus, Italy, Moldova, Romania and Slovenia. Additionally, its current population trend is decreasing, despite Iberian Peninsula being a stronghold (Birdlife-International 2017).

The species is highly monogamous, with changing of partners rarely seen (Del Moral and De la Puente 2017). Cinereous vultures start breeding at the age of five or six and lay one egg which is incubated for 50-55 days from mid-February till May (Cramp and Simmons 1980; Heredia 1996). The period that hatchling plumage is observed lasts about 30-40 days and the juveniles usually fledge from the nest between mid-August and early September in Turkey (Yamaç 2004). The species breeds in loose colonies where nests are separated from each other by a few dozens to several hundred meters (Cramp and Simmons 1980).

A previous genetic study Poulakakis et al. (2008) included species from Spain, Germany, the Caucasus and Mongolia but excluded samples from Turkey, where a population with 50-200 breeding pairs deserves conservation interest at the European scale (Heredia et al. 1997). Here, we examine mitochondrial cytochrome b sequence diversity in this Turkish population in order to infer its phylogeographic position of with respect to populations elsewhere by estimating its genetic differentiation, and detect current genetic diversity and any signs of population expansion.

III.3 Methods

III.3.1 Sampling

Molted feathers (n = 55), and blood samples (n = 3) were collected from 58 wild individuals between the years 2009-2012, and 2015-2016 (details provided in Table III.1). All samples were firstly sexed, and if possible, one male and one female sample were selected from each nest site within a single year for mitochondrial DNA analysis.

III.3.2 DNA extraction, PCR and Sequencing

Genomic DNA was extracted both from feather and blood samples using Qiagen DNeasy Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany). The primer set CHD1F/CHD1R, as previously described (Lee et al. 2010), was used to sex individuals according to the procedure in Çakmak et al. (2017).

A partial sequence of cytochrome b gene (311 bp) was amplified using the universal primers L14841 and H15149 (Palumbi 1996). PCR amplification of this marker was conducted using FIRE POL Master mix (Solis Biodyne) in a total reaction volume of 25 μl, containing 1 μl of genomic DNA, 0.3 μM of each primer pair and 1x Master Mix (Solis Biodyne). An initial denaturation step of 5 min at 94 ^{o}C was followed by 35 cycles of at 94 ^{o}C for 30 s, at 47 ^{o}C for 30 s, and at 72 ^{o}C for 45 s; and PCR was terminated by a final extension step at 72 ^{o}C for 10 min. PCR products were sequenced in both directions to increase accuracy. Electrophoresis and detection of fluorescently labeled nucleotides were made with an automatic DNA sequencer (ABI 3730x1

Genetic Analyzer, Applied Biosystems). Mitochondrial DNA sequences representing distinct haplotypes were deposited in GenBank under the accession numbers given in Table III.1.

Table III.1 Summary list of samples used in this study.

Nest ID	Sample ID	Locality	Country	Year collected	Tissue	Haplotype
CB01	E65	Çatacık	Turkey	2011	Feather	TR1
CB02	E237	Çatacık	Turkey	2011	Feather	TR1
CB02	E238	Çatacık	Turkey	2011	Feather	TR1
CB03	E114	Çatacık	Turkey	2011	Feather	TR2
CB03	E262	Çatacık	Turkey	2011	Feather	TR1
CB03	E70	Çatacık	Turkey	2011	Feather	TR1
CB04	E72	Çatacık	Turkey	2011	Feather	TR1
CB05	E73	Çatacık	Turkey	2011	Feather	TR1
CB06	E241	Çatacık	Turkey	2011	Feather	TR1
CB06	E74	Çatacık	Turkey	2011	Feather	TR1
CB07	E121	Çatacık	Turkey	2011	Feather	TR1
CB09	E244	Çatacık	Turkey	2011	Feather	TR1
CB11	E223	Çatacık	Turkey	2011	Feather	TR1
CB11	E226	Çatacık	Turkey	2011	Feather	TR1
CB31	E131	Çatacık	Turkey	2011	Feather	TR1
CB71	E233	Çatacık	Turkey	2015	Feather	TR1
CB83	E260	Çatacık	Turkey	2015	Feather	TR1
CB13	E211	Tandır	Turkey	2011	Feather	TR1
CB16	E217	Tandır	Turkey	2011	Feather	TR1
CB17	E207	Tandır	Turkey	2011	Feather	TR1
CB18	E116	Tandır	Turkey	2011	Feather	TR1
CB19	E100	Tandır	Turkey	2011	Feather	TR1
CB20	E101	Tandır	Turkey	2011	Feather	TR1
CB22	E103	Tandır	Turkey	2011	Feather	TR1
CB23	E115	Tandır	Turkey	2011	Feather	TR1
CB27	E111	Tandır	Turkey	2011	Feather	TR1
CB29	E136	Tandır	Turkey	2011	Feather	TR1
CB29	E205	Tandır	Turkey	2011	Feather	TR1
CB29	E206	Tandır	Turkey	2011	Feather	TR1
CB30	E132	Tandır	Turkey	2011	Feather	TR1
CB61	E57	Tandır	Turkey	2012	Feather	TR1

Table III.1 (continued).

Nest ID	Sample ID	Locality	Country	Year collected	Tissue	Haplotype
CB62	E59	Tandır	Turkey	2012	Feather	TR1
CB33	E199	Türkmenbaba	Turkey	2011	Feather	TR1
CB35	E191	Türkmenbaba	Turkey	2011	Feather	TR1
CB40	E177	Türkmenbaba	Turkey	2011	Feather	TR1
CB40	E178	Türkmenbaba	Turkey	2011	Feather	TR1
CB41	E174	Türkmenbaba	Turkey	2011	Feather	TR1
CB42	E144	Türkmenbaba	Turkey	2011	Feather	TR1
CB46	E46	Türkmenbaba	Turkey	2009	Feather	TR1
CB47	E47	Türkmenbaba	Turkey	2009	Feather	TR1
CB48	E48	Türkmenbaba	Turkey	2009	Feather	TR1
CB50	E33	Türkmenbaba	Turkey	2010	Feather	TR1
CB54	E37	Türkmenbaba	Turkey	2009	Feather	TR1
CB55	E38	Türkmenbaba	Turkey	2009	Feather	TR1
CB66	E139	Türkmenbaba	Turkey	2015	Feather	TR1
CB78	E255	Türkmenbaba	Turkey	2016	Feather	TR1
CB79	E256	Türkmenbaba	Turkey	2016	Feather	TR1
CB80	E257	Türkmenbaba	Turkey	2016	Feather	TR1
CB81	E258	Türkmenbaba	Turkey	2016	Feather	TR1
CB82	E259	Türkmenbaba	Turkey	2016	Feather	TR1
CB67	E140	Köroğlu	Turkey	2015	Feather	TR1
CB70	E143	Köroğlu	Turkey	2015	Feather	TR1
CB72	E245	Köroğlu	Turkey	2016	Feather	TR1
CB74	E247	Köroğlu	Turkey	2016	Feather	TR1
CB75	E249	Köroğlu	Turkey	2016	Feather	TR1
CB76	E250	Köroğlu	Turkey	2016	Feather	TR1
CB76	E251	Köroğlu	Turkey	2016	Feather	TR1
CB77	E253	Köroğlu	Turkey	2016	Feather	TR1

III.3.3 Data analysis

The alignment of mtDNA sequences was conducted using the CLUSTAL W algorithm (Thompson et al. 1997) in MEGA 6.06 (Tamura et al. 2013). Alignments were then checked by eye, and corrected manually. Haplotype diversity (h) and nucleotide diversity (π) (Nei 1987) were calculated using DnaSP 5.0 (Librado and Rozas 2009).

Model of sequence evolution were selected on the basis of the Bayesian Information Criterion (BIC) (Schwarz 1978) as implemented in MEGA. The best-fitting model for the data set was Kimura two-parameter model (K2) (Kimura 1980), (lnL= -630.27; BIC= 3997.17). To reveal genetic distance among distinct western Palearctic populations, uncorrected p distance (Nei and Kumar 2000) were calculated in MEGA.

To reveal the phylogenetic position of Turkish Cinereous vulture populations within the Cinereous vulture of the western Palearctic, 40 mtDNA haplotypes from Spain, Greece, Armenia, Georgia and Kazakhstan and Mongolia were downloaded from GenBank and combined with 58 Turkish haplotypes. The Lappet-faced Vulture *Torgos tracheliotus* was included as an outgroup (GenBank accession number EU496463) (Arshad et al. 2009).

Phylogenetic relationships of mtDNA haplotypes were reconstructed with maximum likelihood as implemented in MEGA 6.06 using all sites for gaps/missing data treatment and Nearest-Neighbor-Interchange as the heuristic search method. The robustness of the tree was assessed using bootstrapping (Felsenstein 1985) with 1000 replications. In addition, evolutionary relationships and probable ancestral connections among haplotypes were evaluated by constructing a median-joining network in NETWORK 4.6.1.0 (Bandelt et al. 1999).

Population genetic analysis of Turkish populations based on 15 polymorphic microsatellite loci indicated that Turkish populations consist of one panmictic populations (Çakmak et al. unpublished data). Therefore, to make inferences about historical demography of the Turkish Cinereous vulture population, neutrality tests (Tajima's D and Fu's Fs) and mismatch analyses were performed in DnaSP 5.0 with all populations combined. Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) are used to test selective neutrality and obtain demographic information. Statistically significant negative values indicate a recent population expansion, while significantly positive values suggest bottlenecks (Guo et al. 2016; Rogers and Harpending 1992).

The mismatch analysis is based on the pairwise differences for all sequences. Demographic history of populations defines the shape of the distribution of these mismatches. A unimodal distribution is typical of a recent population expansion, while a ragged, multimodal distribution reflects populations at demographic equilibrium or constant over a longer period of time (Rogers and Harpending 1992). DnaSP calculates the observed mismatch distributions and expected values both in a stable population and in growing and declining populations (Rogers and Harpending 1992; Slatkin and Hudson 1991). Moreover, it computes the raggedness statistics by quantifying the smoothness of the observed mismatch distribution, so it can distinguish data from expanded and stationary populations (Harpending 1994).

III.4 Results

A 311 base pairs long section of the mitochondrial cytochrome b partial region was successfully sequenced in 58 samples. Among those sequences, only two distinct haplotypes were identified. TR1 was the dominant haplotype which was found to be fixed for 57 of our 58 samples. TR2 was present in only one individual and differed for only one nucleotide compared to Turkish and Caucasian populations. Haplotype diversity (h: 0.03) and nucleotide diversity (π: 0.0001) were found to be extremely low for the Turkish populations. The topologies of the ML tree (Figure III.1) and the median-joining network (Figure III.2) for the combined samples revealed four distinct lineages in Eurasia, namely Iberian (1) and Balkan (2) populations in Europe, a Mongolian (3) population in Central Asia, and Turkish and Caucasian (4) populations from western to Central Asia. This last group of populations, Turkish and Caucasian, are very closely related to each other with only one mutation difference and 0.6% of genetic divergence (Table III.2). These two populations hold an intermediate phylogenetic position between European and Mongolian population in both the tree and network (see Figure III.1 and Figure III.2). However, they are closer to Mongolian populations compared to Iberian and Balkan populations based on haplotype network analysis (Figure III.2).

Both the results of neutrality tests and mismatch analysis supported a constant population size for the Turkish population. Neither Tajima's D (-1.09, P > 0.10) nor Fu's Fs (-1.76, P > 0.10) were significantly different from zero. Similarly, the mismatch analysis was in accordance with a constant population size and the raggedness value was 0.87 (<0.04 is observed in expanded populations). There is no sign for population expansion.

Table III.2 Estimates of evolutionary divergence over sequence pairs between populations.

	Mongolia	**Greece**	**Spain**	**Caucasus**	**Turkey**
Mongolia					
Greece	0.031				
Spain	0.027	0.011			
Caucasus	0.011	0.027	0.023		
Turkey	0.012	0.021	0.017	0.006	

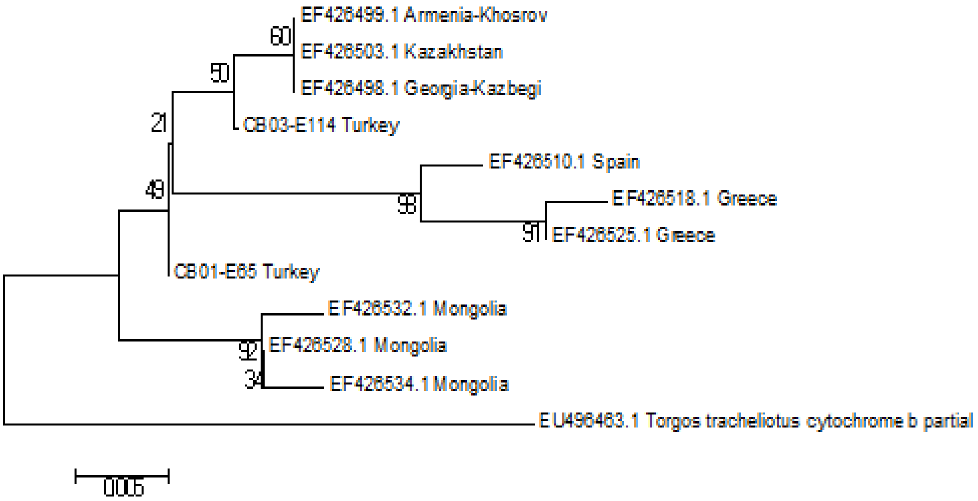

Figure III.1 Maximum likelihood topology generated under the evolutionary model that best fitted the data. Values at the nodes represent bootstrap value.

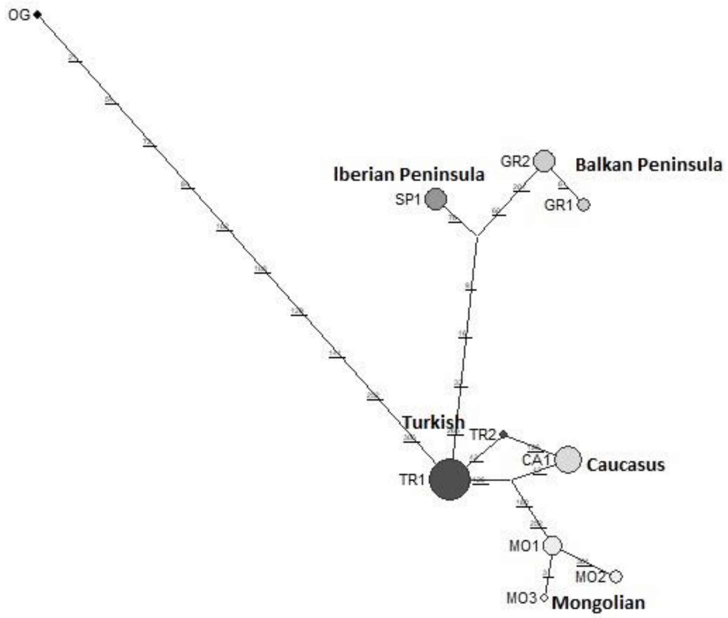

Figure III.2 Network of Cinereous Vulture mtDNA cytochrome b haplotypes. The size of the circle is positively correlated with the number of samples sequenced.

III.5 Discussion

Phylogenetic analysis of Turkish haplotypes with Iberian, Balkan, Caucasian and Mongolian haplotypes reveal that - together with Caucasian populations - Turkish populations are at an intermediate position between European and Asian populations. Therefore, our findings fill the “phylogenetic gap” between European and Asian populations. However, Turkish haplotypes are closer to Asian rather than European haplotypes. Interestingly, despite a minimum of 900 km distance between Turkish and Caucasian breeders, they are genetically identical, except for a single mutation. Regardless of this extreme genetic closeness, these two populations do not share any haplotypes. These findings support previous results by Poulakakis et al. (2008) that there is a strong within species phylogeographic pattern. This can be explained by the highly philopatric behaviour of this species which leads to limited gene flow among populations (Godoy et al. 2004; Poulakakis et al. 2008). Yet observations of single birds moving over the Bosporus or north-eastern Turkey exist (unpubl. data).

However, such movements are particularly rare for the Balkan population since it is resident (Birdlife-International 2017). On the other hand, first-year vultures from both Turkish and Caucasian populations move south during their first winter, where their ranges partly overlap (Gavashelishvili et al. 2012; Yamaç and Bilgin 2012). The potential for occasional switching of populations in the winter quarters and/or a common founding population at the end of the LGM might explain the genetic similarity between Turkish and Caucasian population.

Extremely low haplotype and nucleotide diversity of the Turkish population is again consistent with Poulakakis et al. (2008), and with data from other vulture species such as Egyptian Vulture *Neophron percnopterus* (Kretzmann et al. 2003) and other Old World vultures (Lerner and Mindell 2005). Neither the shape of mismatch distribution nor the results of neutrality tests show any sign of expansion for Turkish populations. These findings are in line with the results obtained in a separate genetic analysis using 15 polymorphic microsatellite loci, which indicated a recent bottleneck and nearly low effective population size for our vulture population (Çakmak et al. unpublished data).

Recent bottlenecks and a decline in population size may cause reduced genetic diversity (Roques and Negro 2005; Rutkowski et al. 2015). In the case of low mitochondrial DNA diversity compared to the moderate level nuclear diversity can be explained by male-mediated gene flow (Martinez-Cruz and Godoy 2007). Apart from these, different regions of mitochondrial DNA show different polymorphisms, which should be taken into account. For instance, fragments of hypervariable domains I and II of the non-coding mitochondrial control region show higher polymorphisms than other mitochondrial genes (Ruokonen and Kvist 2002).

A reduced mtDNA diversity can compromise the recovery of an endangered species by decreasing its viability and fitness (Tollefsrud et al. 2009). To increase adaptation and fitness of a species and to ensure the long-term persistence, genetic diversity should be maintained (Frankham et al. 2002). Additionally, if there is a need in the future, introductions of individuals particularly from Caucasus populations when looking at genetic results may be only way to prevent this genetic erosion in this population (Luikart et al. 1998).

III.6 Acknowledgement

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PART IV

VIABILITY ANALYSIS REVEALS POPULATION DYNAMICS, THREATS AND RELEVANT CONSERVATION PRACTICES FOR CINEREOUS VULTURE (*AEGYPIUS MONACHUS*)

(***with M. Kükrer, E. Yamaç, and C. C. Bilgin***)

IV.1 Abstract

VORTEX was used to run 37 simulations on the viability of the Cinereous Vulture *Aegypius monachus* in Turkey to better understand population dynamics. Here, we incorporate demographic parameters (adult, immature and juvenile mortality rates, longevity, age of first reproduction, number of lethal alleles, and dispersal rates) and anthropogenic impact (hunting, poisoning, wind farm development or habitat deterioration) in an individual-based model. Conservation scenarios were based on reasonable options of population management, including effects of supplementary feeding, vulture friendly forestry and human reduced disturbance as well as release of individuals via captive breeding program. In our baseline scenario, there was no extinction risk of Cinereous vulture in Turkey within 50 years. High juvenile mortality rates seemed to effect the viability in a way that was comparable to adult mortalities. Additionally, removing poisoning from the model alone resulted in a relatively rapid recovery of the population. The highest level of contribution to recovery was possible with ‘supplementary feeding’ which was shown to reduce immature mortalities. Besides, once prevention of fledgling mortalities related to lumber production around nesting sites as in the ‘vulture friendly forestry’ scenario was achieved, each subpopulation benefited from the situation with increased population sizes. The same holds for ‘reducing human disturbance’ related to human crowd, presence of roads, recreational and sportive activities around nesting sites. When both measures were applied synchronously, results comparable to supplementary feeding were achieved. Just these two simple applications might be enough to carry each of the subpopulations to positive growth rates. Consequences for this large, long-living raptor species can be applicable for other raptors especially those suffering from reduced reproductive rates due to human activities.

Key words: Human Disturbance, Juvenile Mortality, PVA, Vortex

IV.2 Introduction

Population viability analyses (PVAs) are very useful quantitative process in risk assessment studies to estimate the extinction probabilities of a particular population for a certain time interval (Beissinger and McCullough 2002). PVA can incorporate demographic parameters or life cycle components like age specific survival and reproduction rates into population models (Boyce et al. 2007). Additionally, it can estimate the extinction probability which is important in the conservation and management of rare and endangered species, and especially is useful for management options by means of sensitivity analysis (Akçakaya 2000; Akçakaya and Sjögren-Gulve 2000). It is widely used to address both deterministic and stochastic threats to natural populations of species of concern (Brook et al. 2002). Detection of the whether population size is increasing or decreasing, and which life stage is most contributing to the growth rate are the basic steps in PVA (Boyce 1992; Caswell 2001).

VORTEX simulation software (Lacy and Pollak 2014) is the most famous tool for PVA, and enable us to perform different modeling scenarios with increasingly complex population dynamics (Velevski et al. 2014). Indeed, this software is an individual based, stochastic simulation program, however, it incorporates deterministic forces besides demographic, environmental and genetic (like inbreeding) stochastic events on threatened populations used for the modeling procedure for a given time period (Miller and Lacy 2005). In the beginning, the software has been used to model birds and mammals, but now enlarged to other taxonomic groups (Miller and Lacy 2005). It has also been used for predicting viability of vulture populations (Buckley et al. 2016; Carrete et al. 2009; García-Ripollés and López-López 2011). Population modeling in threatened or near threatened species is important since models can be used for estimating population trajectories, determining the factors that affect the population most -so needed to be deeply studied- and decision making for different management options (Akçakaya and Sjögren-Gulve 2000).

The Cinereous Vulture (*Aegypius monachus*) is a large, long-living raptor species in the Palearctic region (height 250-295 cm, weight 7,5-12,5 kg) which inhabits mainly pine and mixed pine forests between 280 and 1880 m and predominantly feeds on hard tissues and external parts of the carcass of ungulates and lagomorphs (Donázar 1993; Yarar and Magnin 1997). The species is highly monogamous even if exchange of partners can rarely be seen (Del Moral and de la Puente 2010). It starts breeding at the age of five or six years and lays one egg which is incubated for 50-55 days from mid-February till May (Cramp and Simmons 1980; Heredia 1996; Tewes 1996). The period that hatchling plumage is observed lasts about 30-40 days and the juvenile bird seems to fledge from the nest after mid-August to September in Turkey (Yamaç 2004). The raptor species is also colonial that it breeds in loose colonies where nests are separated from each other by a few dozens to several hundred meters (Cramp and Simmons 1980; Hiraldo 1977).

The species was once very abundant in the middle zone (North-South) of Palearctic region with its distribution extending from Korea to Spain (Cramp and Simmons 1980). Today the distribution is much more limited mainly due to anthropogenic factors like habitat changes and forestry practices at breeding sites, poisoning, food scarcity, forest fires, exploitation and other types of human disturbance (Heredia 1996). The species is listed as near threatened status of IUCN Red List (Birdlife-International 2017). The Cinereous vulture populations are thought to be declining in Asia and keeps growing in Western Europe (Spain, Greece, and France) whereas populations in Caucasus region and Turkey seems fluctuating (Barov and Derhé 2011; Birdlife-International 2017). Populations in Spain recovered steadily thanks to conservation practices during the last 30 years, expanded to Portugal and new populations are successfully established in France through a reintroduction program (González 1990). This shows that, given suitable conditions, Cinereous vulture populations are resilient and have a great potential to meliorate. The fluctuating population sizes in Western Anatolia are needed to be studied in depth both for understanding various pressures on the species which limit population growth rates and for determining management implications.

In Turkey, the Cinereous vulture is well known to breed at Sündiken Mountains-Eskişehir (46 pairs) Türkmenbaba Mountain-Eskişehir (28 pairs), and Köroğlu Mountains-Kızılcahamam Forest-Bolu/Ankara (21 pairs) (Kirazlı and Yamaç 2013; Özcan and Yamaç 2015; Yamaç 2004). It is also stated that a few (between 1 and 10) pairs of the vulture species breed at each of the following sites: Ilgaz Mountain-Çankırı, Hamam Mountain-Eskişehir, Murat Mountain-Kütahya, Akdağ-Afyonkarahisar, Kazankaya-Yozgat, and Eastern Blacksea Mountains (Heredia 1996; Yarar and Magnin 1997). In Turkey, the most important threat to breeding success was detected as forestry activities due to the continuous existence of people and vehicles in the breeding area (Kirazlı and Yamaç 2013).

In this study, we have used VORTEX to evaluate the extinction probability of the near-threatened vulture species in Turkey. We applied population viability analysis to unravel population dynamics and to better understand threats the species is currently trying to cope with or potential hazards the population may face. Anthropogenic impact on survival and fecundity rates may play a prominent role in the stagnation observed and accordingly, by taking into consideration variable factors within sites, we also compare the effect of various management implications to pick out relevant conservation practices. The Cinereous vulture is usually treated as a flagship species in wildlife conservation and the consequences for this large, long-living raptor species can be applicable for other raptors which are on the verge of an extinction vortex (Donázar et al. 2016). Besides, the importance of the Turkish population comes from its relative size in comparison to world population as well as its potential role for gene flow between the Asian and European populations (Poulakakis et al. 2008).

IV.3 Materials and Methods

We used VORTEX 10.2.11.0 (Lacy and Pollak 2014) to model the viability of the Cinereous vulture population in western Turkey. The model we built was an age-structured, pre-breeding (censuses at the time of egg laying), stochastic one based on several demographic parameters including mortality rates, percent of breeding adults, initial population size, as well as initial age distribution and carrying capacity. Model parameters can be examined in Table IV.1. The order of events was assumed as follows to allow harvesting of juvenile individuals: breeding, harvesting, mortality, aging, dispersal, growth rate calculation, carrying capacity truncation, and census. Iteratively, the program creates an image of each individual and follows its fate through its life. Pseudorandom numbers which detect whether the bird lives or dies, disperse or not, etc., was used for every iteration (Miller and Lacy 2005). As a result, Vortex calculates a stochastic population growth rate (r) which is the ratio of the population size in the current year to the previous year for each scenario (Miller and Lacy 2005).

For each simulation, the model was run for 1000 times and for 50 years to minimize the standard error and to test the long-term viability of the populations, where population management for conservation practices can be carried out and monitored (Bretagnolle et al. 2004; Harris et al. 1987; Pavokovic and Susic 2005).

Among the several sites used by Cinereous vultures for breeding purposes, currently there are 5 breeding sites in Western Anatolia that are relatively well studied. Since some of those places are close enough to consider them as a single site, by joining ones closer than 50 km, we modeled the Western Anatolian population as if it was consisting of 3 subpopulations. These subpopulations were Sündiken (composed of Mihalgazi and Gökçekaya Dam breeding sites), Türkmenbaba and Köroğlu (by adjoining of Kıbrıscık and Kızılcahamam breeding sites). Number of breeding pairs in each of those sites were determined as 46, 28 and 21 respectively (Kirazlı and Yamaç 2013; Özcan and Yamaç 2015; Yamaç 2004; Yamaç 2006; Yamaç 2016). Modeling the population by dividing it in subpopulations allowed us to differentiate various factors between sites and hence, better understand dynamics.

Table IV.1 Parameters for the standard model.

Simulation Input	Parameters	Values	References/Comments
Scenario settings	Number of iterations	1000	(Harris et al. 1987)
	Number of years	50	(Bretagnolle et al. 2004; Pavokovic and Susic 2005)
	Extinction definition	Total N < critical size 1	Recolonizations are possible through dispersal.
	Number of populations	3 (Sündiken-Türkmenbaba-Köroğlu)	(Kirazlı and Yamaç 2013; Özcan and Yamaç 2015; Yamaç 2004; Yamaç 2006; Yamaç 2016)
Dispersal	Dispersal age	1-4	(Gouar et al. 2008)
	% Survival of dispersers	100	(Gavashelishvili et al. 2012; Yamaç and Bilgin 2012)
	% of dispersers	From Sündiken to Türkmenbaba: 8.7 From Türkmenbaba to Sündiken: 23.5 From Sündiken to Köroğlu: 3.5 From Köroğlu to Sündiken: 16.8 From Türkmenbaba to Köroğlu: 3.8 From Köroğlu to Türkmenbaba: 6.8	(Gavashelishvili et al. 2012; Gouar et al. 2008; Yamaç and Bilgin 2012) Dispersal rate was considered as 1000*N(recipient)/N(source)/distance in km.
Reproductive system	Reproductive system	Long-term monogamy	(Cramp and Simmons 1980; Del Moral and De la Puente 2017)
	Age of first offspring for female (F)/male (M)	5	(Cramp and Simmons 1980; Del Moral and De la Puente 2017)
	Maximum age of reproduction for f/m	20	(Ferrer and Calderón 1990; Flower 1937)
	Maximum lifespan	21	To let the birds breed at age 20.
	Max number of broods per year	1	(Del Moral and De la Puente 2017).
	Max number of progeny per brood	1	(Del Moral and De la Puente 2017)
	Sex ratio at birth – in % males	50	Assumed.

Table IV.1 (continued).

Simulation Input		Parameters	Values			References/Comments
Reproductive rates		% Adult female breeding =(100-((100-50)*((N/K)^B)))*(N/(A+N))	=(100-((100-50)*((N/K)^4)))*(N/(0+N))			(Fernández-Bellon et al. 2016; García-Ripollés and López-López 2011) N is the population size at each round of the simulation and K is the carrying capacity. A=0 and B=4.
		SD in % breeding due EV	Sündiken: 6.1 Türkmenbaba: 8.4 Köroğlu: 8.4			(Kirazlı and Yamaç 2013; Özcan and Yamaç 2015; Yamaç 2004; Yamaç 2006; Yamaç 2016)
		Distribution of broods per year	Sündiken: 69 Türkmenbaba: 85 Köroğlu: 69			(Kirazlı and Yamaç 2013; Özcan and Yamaç 2015; Yamaç 2004; Yamaç 2006; Yamaç 2016)
		Distribution of number of offspring per female per brood (exact distribution)	Always single offspring.			(Cramp and Simmons 1980; Del Moral and De la Puente 2017)
Mortality rates %	**F/M**		Sündiken	Türkmenbaba	Köroğlu	
		Mortality from age 0 to 1	50	50	50	(Gavashelishvili et al. 2012; Yamaç and Bilgin 2012)
		SD in 0 to 1 mortality due to EV	5	5	5	
		Mortality from age 1 to 2	29.4	29.4	29.4	(Mihoub et al. 2014; Oro et al. 2008)
		SD in 1 to 2 mortality due to EV	2.5	2.5	2.5	
		Mortality from age 2 to 3	29.4	29.4	29.4	(Mihoub et al. 2014)
		SD in 2 to 3 mortality due to EV	2.5	2.5	2.5	
		Mortality from age 3 to 4	2.2	2.2	2.2	(Mihoub et al. 2014)
		SD in 3 to 4 mortality due to EV	2.6	2.6	2.6	
		Mortality from age 4 to 5	2.2	2.2	2.2	(Mihoub et al. 2014)

Table IV.1 (continued).

Simulation Input	**Parameters**	**Values**			**References/Comments**
	SD in 4 to 5 mortality due to EV	1.8	1.8	1.8	
	Annual mortality after age 5	2.2	2.2	2.2	(Mihoub et al. 2014)
	SD in mortality after age 5	1.8	1.8	1.8	
Catastrophes	Poison	Frequency (%): 6.66			(Kirazlı and Yamaç 2013; Özcan and Yamaç 2015; Yamaç 2004)
		Severity: 0.9			
Mate	% Males in Breeding Pool	100			Assumed.
Initial population	Initial population size	Sündiken: 166			(Vasilakis et al. 2017)
		Türkmenbaba: 101			
		Köroğlu: 76			
Carrying Capacity	Carrying capacity (K) for each population	450			Assumed.
	SD in K in EV	45			Assumed.
Harvest	Population harvested	A female aged 0-1 in Sündiken/year			(Kirazlı and Yamaç 2013)
	First and last year of harvest	1-10			

Initial population sizes and age distributions were provided in Table IV.2. It was assumed that the proportion of juvenile, immature and adult individuals followed that of the population in Dadia National Park in Greece (Vasilakis et al. 2017) with a similar population trend with Western Anatolian populations. Equal number of individuals were allocated at first to each age within the age category in question and then a single individual was added until no individuals remained for that category. Ratio of females and males were assumed to be equal but when a single individual remained to be allocated it was considered as a male.

Table IV.2 Initial age distribution of subpopulations.

	Ages	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**Total**
Sündiken	**Females**	0	10	9	6	6	5	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0	82
	Males	0	11	10	6	6	5	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0	84
																								166
Türkmenbaba	**Females**	0	6	6	4	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	0	50
	Males	0	7	6	4	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	0	51
																								101
Köroğlu	**Females**	0	5	4	3	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	0	37
	Males	0	5	5	3	3	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	0	39
																								76

A population was considered extinct when no individuals were left during the simulations since recolonizations through dispersal were allowed. Adult birds show site fidelity like other territorial raptors (Grande 2006), and hence only subadult birds were assumed to disperse.

Since subpopulations are quite close that birds would daily travel from one site to another, we considered no dispersal penalty in terms of survival (Gavashelishvili et al. 2012; Gouar et al. 2008; Yamaç and Bilgin 2012). There are no tangible data on dispersal between subpopulations but it's known that dispersal in colonial birds are effected by the population size of source and recipient populations as well as the distance between them (Gouar et al. 2008). Telemetry data show that young birds tend to spend time in regions where conspecifics known to breed and one bird originating from Türkmenbaba visited the breeding site at Sündiken before initiating migration (Gavashelishvili et al. 2012; Yamaç and Bilgin 2012). Dispersal rates between subpopulations were taken as directly proportional with the size of the recipient population and inversely proportional to the size of the source population and the distance between populations.

A total of three age classes were used: juveniles (age <1), immatures (1< age <5) and adults (age >5). Only the adult birds were assumed to be reproducing even if breeding of immature birds were reported in many species of large raptors due to reduced intraspecific competition or immatures being able to attain breeding condition when environmental factors are favorable (Newton 2010). Age of first offspring for this long-term monogamous species was set as 5 years (Cramp and Simmons 1980; Del Moral and De la Puente 2017). VORTEX uses the average age of first offspring rather than the onset of maturity, so age of 5 was chosen. The species reproduces once per year by laying a single egg. And the sex ratio of hatchlings was assumed as 1:1. Reproduction and survival were assumed to be perfectly correlated since there is a long chick rearing period, from mid-February to late-August (Yamaç 2004).

The longevity of the species in nature was not determined, however, the longevity of Eurasian Griffon Vulture (*Gyps fulvus*) in wild was estimated as 22.41 to 32.86 years and a captive Cinereous vulture is known to live for 39 years (Flower 1937; Piper et al. 1999). Longevity of Spanish Imperial Eagle (*Aquila adalberti*) with similar demographic characteristics can reach 21-22 years in wild (Ferrer and Calderón 1990). So, the maximum age of reproduction was chosen as 20 years.

Under ideal conditions, all the adult female birds were assumed to attempt breeding but as the carrying capacity is approached, less females would initiate breeding (Fernández-Bellon et al. 2016). VORTEX models the % of breeding adult females with a function 100-((100-50)*((N/K)^B)))*(N/(A+N). Here, N is the population size at the specific round of the simulation and K is the carrying capacity. Allee effect was not reported and the movement capacity of the bird is quite high, also birds frequently gather at feeding sites. So, A was assumed to be 0. Percentage of breeding adult females dropped as low as 50 and steepness parameter B was estimated as 4 for the Griffon Vulture (García-Ripollés and López-López 2011). Determining standard deviation in the number of breeding birds was possible for each site but since data for Köroğlu was incomplete, value for Türkmenbaba that's more variable was used for that site too (Kirazlı and Yamaç 2013; Özcan and Yamaç 2015; Yamaç 2004; Yamaç 2006; Yamaç 2016). It was assumed that no mate monopolization occurred for males. It was assumed that carrying capacity for each site was about to harbor 125 adult pairs which corresponds to 450 individuals in total. Standard deviation was set as 10% of the parameter value set.

Breeding attempts were considered as successful if a chick was reared and it managed to fledge. Breeding success in Sündiken and Türkmenbaba is being monitored since as far as 16 years, but the data was again incomplete for Köroğlu. One of the breeding sites in Köroğlu lies within the Kızılcahamam National Park where recreational activities are frequent and the population densities as well as presence of roads around is high due to proximity to capital city of Ankara. This puts Köroğlu subpopulation in a situation that's more closely resembling Sündiken. So, breeding success as well as mortality rates were taken as the same for both subpopulations. Breeding success were determined as 69% and 85% for Sündiken and Türkmenbaba, respectively (Kirazlı and Yamaç 2013; Özcan and Yamaç 2015; Yamaç 2004; Yamaç 2006; Yamaç 2016).

Mortality rates for breeding sites in Western Anatolia were not known so the parameter values were estimated from other studies. 10 juvenile individuals from Anatolia and Caucasus were tracked by telemetry and only 4 of them were proved to reach the next age (Gavashelishvili et al. 2012; Yamaç and Bilgin 2012). This doesn't necessarily mean that the rest of the individuals died but telemetry signals would have ceased due other reasons so a similar value of 50% juvenile mortality was assumed. Immature and adult mortalities were estimated from Mihoub et al. (2014) after correction for the effect of supplementary feeding being implemented in those regions. 20% decrease in immature mortalities due to supplementary feeding was added back to the immature mortalities (Oro et al. 2008). This was not considered for immature ages (3 and 4 years) which were shown to reach adult survival rates before breeding age was reached (Mihoub et al. 2014).

Regarding the population in question, a single case of poisoning event was recorded during the 15 years of study (Kirazlı and Yamaç 2013; Özcan and Yamaç 2015; Yamaç 2004). The severity, frequency and the extent of poisoning events are not known widely. Most of the poisoning events occur from poisoned baits to kill carnivores and several vultures are spotted around a single carcass. It can be assumed that the effect would be locally wider than the individuals caught sick. We assumed poisoning events affected each site individually with a probability of once in 15 years (6,66 percent/year) with a 10% increase in mortalities and the same ratio of decrease in reproduction.

During the three-years survey in Sündiken hunting towards the species at the nests and feeding sites were recorded in first two years but didn't occur at other two sites (Kirazlı and Yamaç 2013). It was also seen that target practicing by the villagers and hunters was common in Sündiken which puts more the subpopulation at risk of hunting. We assumed that this harvesting of individuals in Sündiken would go on for another 10 years and that a juvenile female individual was harvested each year.

A number of scenarios were simulated in order to better understand population dynamics, reveal the impact of potential threats and test the usefulness of various conservation measures that can be taken. A summary of parameters changed within each scenario were given in Table IV.3. In the first set, we simulated 16 scenarios to better understand population dynamics. Demographic parameters that are adult, immature and juvenile mortality rates, longevity, age of first reproduction, number of lethal alleles, and dispersal rates were changed to higher or lower levels or completely omitted so that we were able to observe their relative contributions in the model.

In the second set, we considered 14 scenarios consisting of potential threats resulting from either hunting, poisoning, wind farm development or habitat deterioration were constructed to check for extent of their impacts. Higher, medium or lower values as substitutes for baseline parameter estimates were used as well as their absence. Killing of nestlings by shooting from ground and a possible avoidance behavior of experienced individuals from humans let us to limit the hunting age to juveniles only. Besides severity, the frequency of poisoning events was also simulated as more common or rarer occurrences too. Habitat deterioration due to loss of suitable nesting and/or foraging sites or due to reductions in quality would also cause serious problems; therefore, we incorporated them in our model as a certain amount of yearly decrease in carrying capacity.

Windfarm development can be a major problem for many aerial species since collisions with turbines during foraging or migration may inflict higher mortalities (Carrete et al. 2012). Installation of major windfarm facilities within or close to the breeding range of the Cinereous vulture population in Western Anatolia is already planned which could exert additional pressure on the population. Avoidance rate was estimated as 98% for White-tailed Eagles and it was proposed as 99-99,5% for the Cinereous vulture each resulting in particular mortality increases (Vasilakis et al. 2016). Three different mortality estimates based on those avoidance rates were used to construct relevant scenarios.

Table IV.3 Input parameters for the scenarios.

Category	Scenario	Parameter	Values
Population dynamics	**Adult_Mortality_H** **Adult_Mortality_L**	Adult mortality	3.3 1.1
	Immature_Mortality_H **Immature_Mortality_L**	Immature mortality (ages 2 and 3)	32.3 26.5
	Juvenile_Mortality_H **Juvenile_Mortality_L**	Juvenile mortality	55 45
	Longevity_H **Longevity_L**	Longevity	22 18
	First_Reproduction_H **First_Reproduction_L**	Age of first reproduction	6 4
	Inbreeding_H **Inbreeding_L** **Inbreeding_None**	Lethal alleles (6,29 in the standard model)	9.44 3.14 0
	Dispersal_H **Dispersal_L** **Dispersal_None**	Dispersal rates	baseline*1.5 baseline*0.5 no dispersal
Potential threats	**Harvest_H** **Harvest_M** **Harvest_None**	Harvest (hunting continues for 50 years)	1 female of ages 1-4 1 female of ages 1-2 no hunting
	Poison_H **Poison_L** **Poison_None** **Poison_C** **Poison_R**	Frequency/severity	6.66/0.81 6.66/0.99 no poisoning 9.99/0.90 3.33/0.90

Table IV.3. (continued)

Category	Scenario	Parameter	Values
	Wind_Farm_H **Wind_Farm_M** **Wind_Farm_L**	Mortalities	+10.8 +5.4 +2.7
	Habitat_Deterioration_H **Habitat_Deterioration_M** **Habitat_Deterioration_L**	Carrying capacity (yearly loss for 20 years)	-3.75 -2.5 -1.25
Conservation practices	**Supplementary_Feeding**	Immature mortality (ages 2 and 3)	15.2
	Vulture_Firendly_Forestry **Reduced_Human_Disturbance** **VFF_RHD**	Distribution of 1 broods per year (Sündiken/Türkmenbaba/Köroğlu)	75/91/75 85/85/85 91/91/91
	Release_L **Release_M** **Release_H**	Supplementation (from year 5 to 20 to each site)	1 female aged 4 2 females aged 4 3 females aged 4

Lastly, a third set of simulations included seven management scenarios for conservation. These scenarios which could be grouped under three different strategies of decreasing mortality rates, increasing reproductive rates and reintroductions are widely implemented for various species at risk. But the final outcome is usually found to be closely associated with the species or population under study.

“Supplementary Feeding” scenario was designed to simulate the effect of artificial feeding stations if they were to be used as a conservation management tool like in European populations for increasing immature survival, recolonization of abandoned areas and providing food safety (Gilbert et al. 2007; Mundy et al. 1992; Piper et al. 1999; Wilbur et al. 1974). The effect was limited to immature birds only, since they were the ones that used the so called “Vulture Restaurants” most (Oro et al. 2008). It was estimated that through supplementary feeding, immature survival rates would equal those in Western Europe.

Forestry and other types of human disturbance were shown to affect breeding density (Donázar et al. 2002; Poirazidis et al. 2004; Sánchez-Zapata and Calvo 1999). “Vulture Friendly Forestry” scenario made use of data obtained from Sündiken and Türkmenbaba subpopulations where the effect of anthropogenic impact on breeding success of the subpopulation related to forestry practices were recorded (Kirazlı and Yamaç 2013; Yamaç 2004). The breeding success was lowered to 6% in both of the sites.

“Reduced Human Disturbance” scenario was based on unsuccessful breeding attempts by death of nestlings that could be attributed to human activities other than lumbering (Kirazlı and Yamaç 2013). In Sündiken, recreational activities, sports hunting around, picnicking, economic activities and increased human and vehicle traffic resulting from them constituted a large share of reduced breeding success in the area (Kirazlı and Yamaç 2013). In their absence we assumed that the breeding success would equal to that of Türkmenbaba subpopulation. It was again assumed that reproductive rates in Köroğlu subpopulation more resembled Sündiken.

For both scenarios, loss of offspring in relationship to these factors were taken as reversed. A combination of both implications were simulated under the “VFF_RHD” scenario where breeding success reached more than 90%. Values higher than that were consistently observed in Türkmenbaba subpopulation and is not uncommon in Europe too where populations recovered quickly in the last 30 years.

A third strategy focusing on captive breeding and frequent restocking was also included. Immediate launch of a supplementing project within 5 years and ceasing the release of individuals at year 20 was simulated in order to check for sustainability of the method.

IV.4 Results

IV.4.1 Standard model

The standard model constructed as a baseline scenario predicted an almost stable population trend with considerable differences within subpopulations. Sündiken subpopulation that has the highest number of breeding pairs seems to sustain a healthy population with a growing trend at the beginning and a mild slope of decline in the later stages (stochastic growth rate at year 16 and 50 were r = 0.010 and r = -0.006) whereas Türkmenbaba and Köroğlu populations seem suffering from population declines (r = 0.003 and r = -0.008 for Türkmenbaba and r = -0.020 and r = -0.007 for Köroğlu at year 16 and 50). The case with Köroğlu was worse than the case with Türkmenbaba. Population trends can be tracked from Figure IV.1 and stochastic growth rates from Table IV.4.

Figure IV.1 Population and subpopulation trends in the standard model.

Table IV.4 Stochastic growth rates at year 16, 33, and 50.

Scenario/ Population	Year 16	Year 33	Year 50
Standard_Model Metapopulation	0,003	-0,005	-0,007
Standard_Model Sundiken	0,010	-0,004	-0,006
Standard_Model Turkmenbaba	0,003	-0,003	-0,008
Standard_Model Koroglu	-0,020	-0,012	-0,007
Longevity_H Metapopulation	0,009	0,003	-0,001
Longevity_L Metapopulation	-0,019	-0,018	-0,020
First_Reproduction_H Metapopulation	-0,001	-0,011	-0,013
First_Reproduction_L Metapopulation	0,008	0,000	0,000
Adult_Mortality_H Metapopulation	-0,004	-0,013	-0,015
Adult_Mortality_L Metapopulation	0,012	0,004	0,000
Immature_Mortality_H Metapopulation	-0,003	-0,009	-0,013
Immature_Mortality_L Metapopulation	0,010	0,000	-0,001
Juvenile_Mortality_H Metapopulation	-0,004	-0,011	-0,012
Juvenile_Mortality_L Metapopulation	0,012	0,003	0,001
Inbreeding_H Metapopulation	0,003	-0,005	-0,007
Inbreeding_L Metapopulation	0,005	-0,003	-0,003
Inbreeding_None Metapopulation	0,005	-0,002	-0,007
Dispersal_H Metapopulation	0,004	-0,002	-0,005
Dispersal_L Metapopulation	0,005	-0,004	-0,006
Dispersal_None Metapopulation	0,005	-0,002	-0,004
Poison_H Metapopulation	-0,002	-0,014	-0,014
Poison_L Metapopulation	0,011	0,001	0,001
Poison_None Metapopulation	0,011	0,001	-0,001
Poison_C Metapopulation	0,001	-0,004	-0,007
Poison_R Metapopulation	0,008	-0,001	-0,001
Supplementary_Feeding Metapopulation	0,031	0,014	0,012
Vulture_Firendly_Forestry Metapopulation	0,010	0,000	-0,001
Reduced_Human_Disturbance Metapopulation	0,014	0,003	0,004
VFF_RHD Metapopulation	0,019	0,006	0,003
VFF_RHD_NoH Metapopulation	0,020	0,008	0,004
VFF_RHD_NoH_NoP Metapopulation	0,010	0,002	0,000
Release_L Metapopulation	0,013	-0,007	-0,004
Release_M Metapopulation	0,019	-0,010	-0,006
Release_H Metapopulation	0,022	-0,013	-0,003
Harvest_H Metapopulation	-0,006	-0,015	-0,025
Harvest_M Metapopulation	0,004	-0,007	-0,006
Harvest_None Metapopulation	0,006	-0,005	-0,007
Habitat_Deterioration_H Metapopulation	-0,018	-0,037	-0,027
Habitat_Deterioration_M Metapopulation	-0,004	-0,018	-0,012
Habitat_Deterioration_L Metapopulation	0,002	-0,008	-0,007
Wind_Farm_L Metapopulation	-0,030	-0,038	-0,051
Wind_Farm_M Metapopulation	-0,065	-0,090	-0,132
Wind_Farm_H Metapopulation	-0,142	-0,171	-0,100

IV.4.2 Population dynamics

IV.4.2.1 Mortality rates

A 50% increase in adult mortality rates and just a 10% increase in juvenile mortality resulted in highly similar outputs. A similar 10% increase for mortality of immature birds (age 2-4) affected the model less than the other two (Figure IV.2). On the other hand, increased survival for adult and juvenile birds had distinct outputs. Despite still comparable, decreasing adult mortality by 50% had a larger impact than decreasing juvenile mortality by 10% in contrast to peer results obtained by increased mortalities. Decreased mortalities of immature birds again seemed to be less effective in the recovery that would be observed.

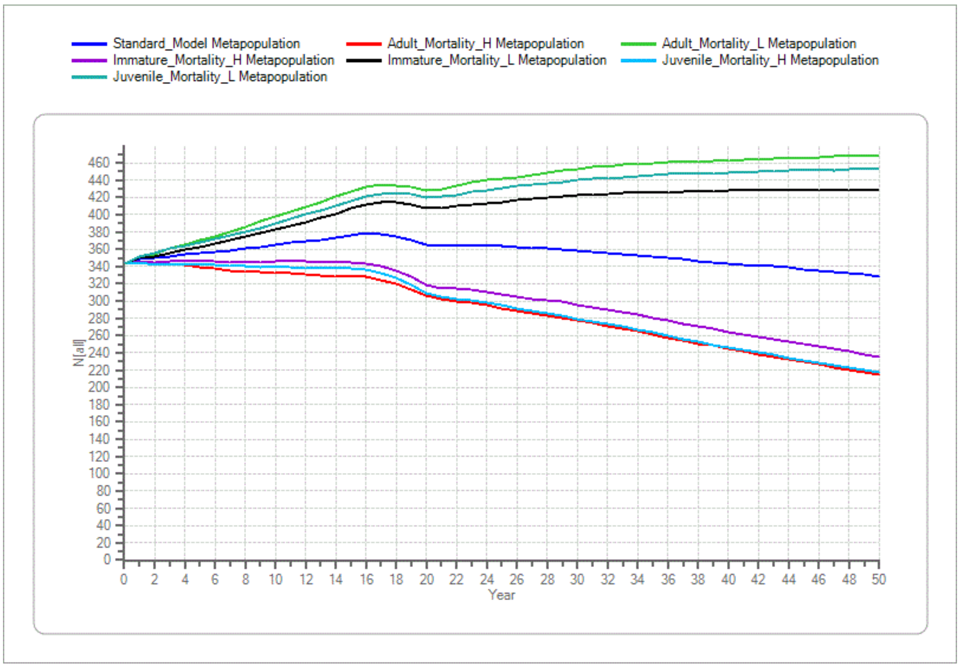

Figure IV.2 Differential impacts of increase and decrease of adult (4,6), immature (2,5) and juvenile (3,7) mortalities.

IV.4.2.2 Longevity and age of first reproduction

As can be traced in Figure IV.3, a 10% increase in longevity contributed more to population growth rates than a larger 20% reduction in the age of reproduction. The opposite also holds, decreased longevity had a larger impact than an augmented age of first reproduction.

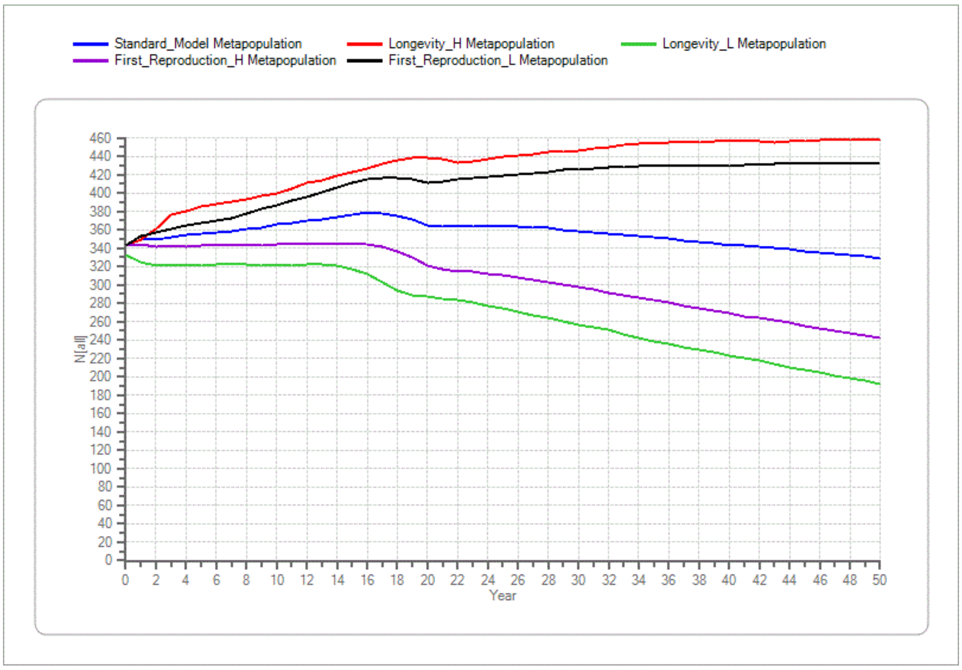

Figure IV.3 Relative roles of longevity and age of first reproduction.

IV.4.2.3 Inbreeding

Adjusting inbreeding levels had an obviously very low effect (Figure IV.4). Scenario in which the number of lethal alleles were set to 0, produced relatively higher values in terms of final population numbers of the whole population. The case was not distinguishable for particular inbreeding scenarios for Köroğlu subpopulation which had the smallest number of breeding pairs and thus might be considered more prone to inbreeding.

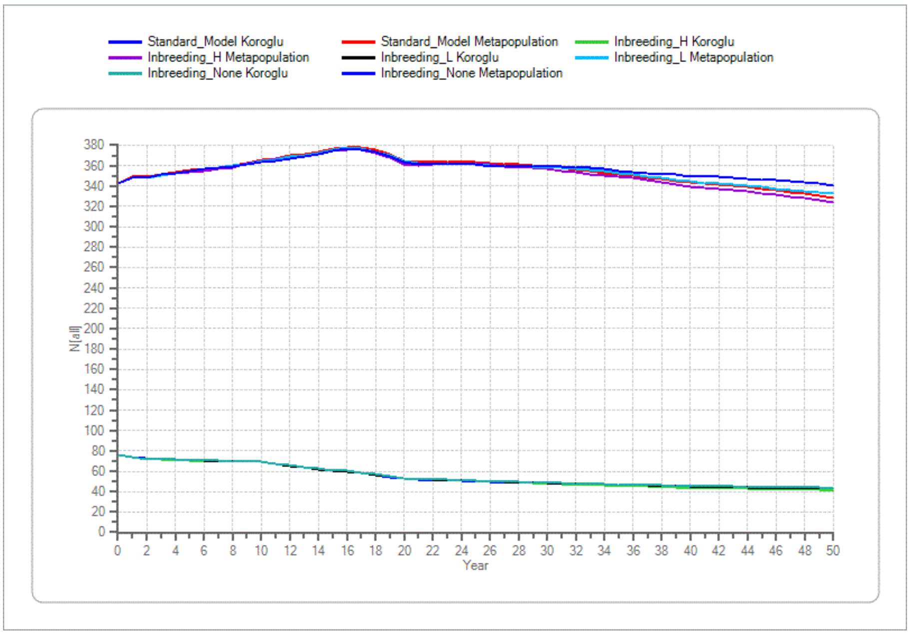

Figure IV.4 Effect of inbreeding on population numbers in the whole population and Köroğlu subpopulation.

IV.4.2.4 Dispersal

Despite there was no penalty set on survival for dispersal, limiting dispersal had still a positive effect on population numbers (Figure IV.5). The effect was still separable between populations (Figure IV.6). The highest output in terms of extant individuals at the end of simulations was in the high dispersal scenario for Sündiken subpopulation contrasted by the lowest outputs for Türkmenbaba and Köroğlu subpopulations as well as the whole population.

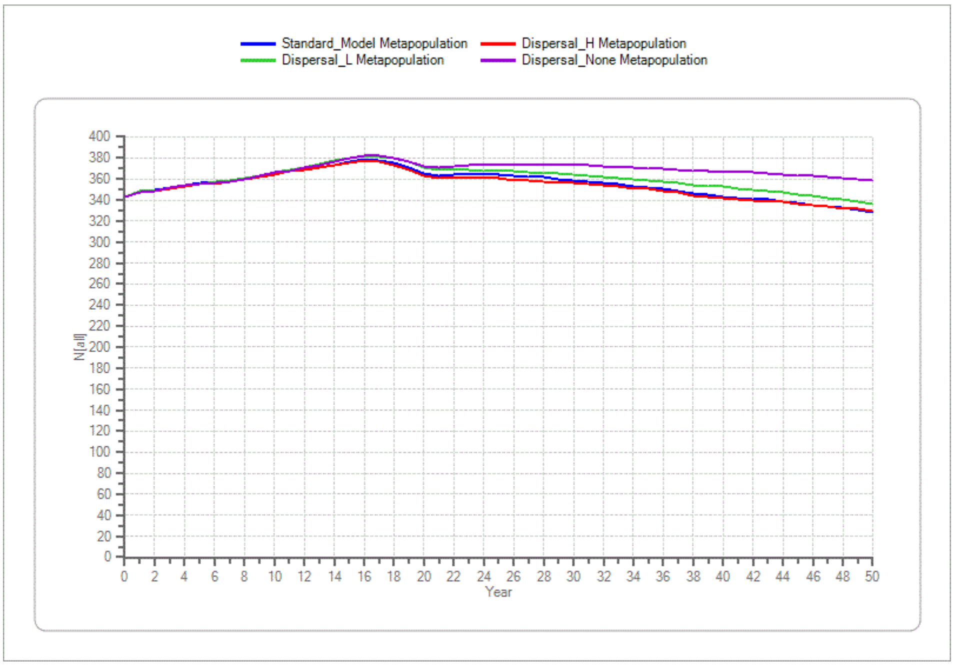

Figure IV.5 Effect of dispersal on population numbers.

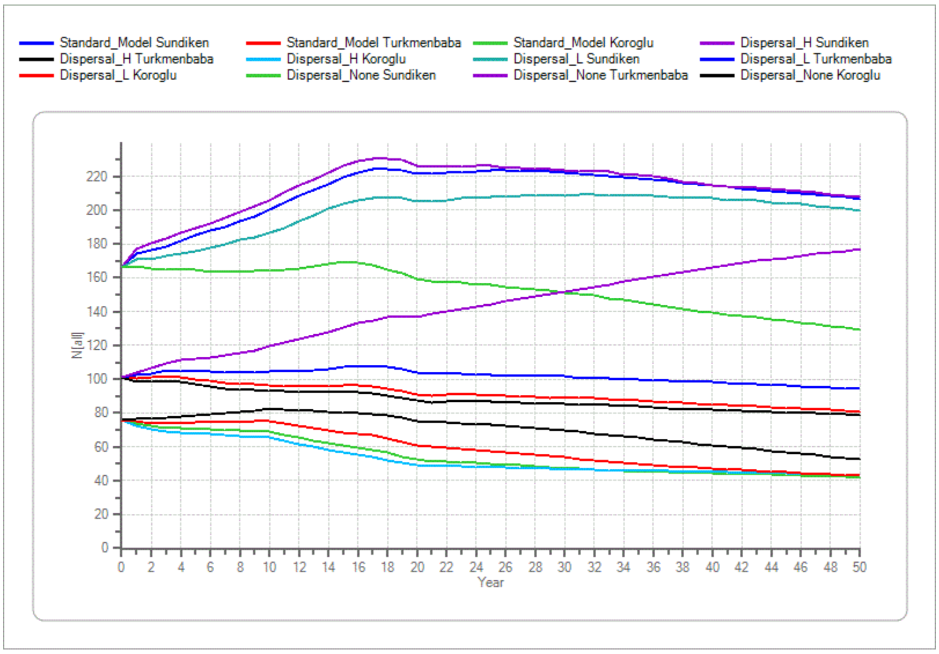

Figure IV.6 Differential effect of changing dispersal rates on subpopulations.

IV.4.3 Threats

IV.4.3.1 Hunting

A few cases of hunting were documented in Sündiken but the extent is not fully known (Kirazlı and Yamaç 2013). Except the extreme case simulated in the Harvest_H scenario of removing 1 female from each of the first 4 age classes for 50 years, the effect of hunting seems relatively low (Figure IV.7). Almost there was no difference between standard model and the no hunting scenario.

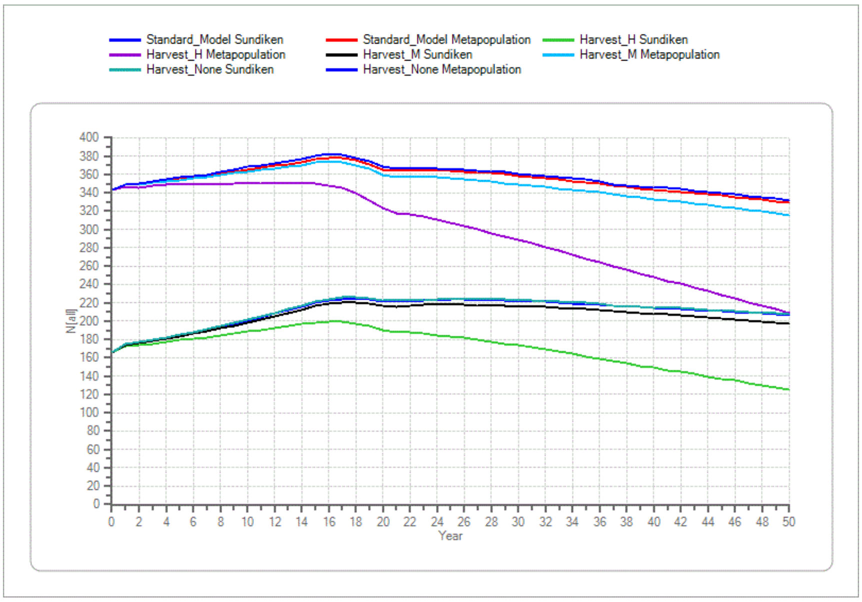

Figure IV.7 Impact of hunting on the model in Sündiken and in the case of whole population.

IV.4.3.2 Poisoning

The effect of poisoning seems to influence the model with a high level. The severity of poisoning on reproduction and survival rates seemed to affect more than the frequency, whether the incidences were more common or rarer (Figure IV.8).

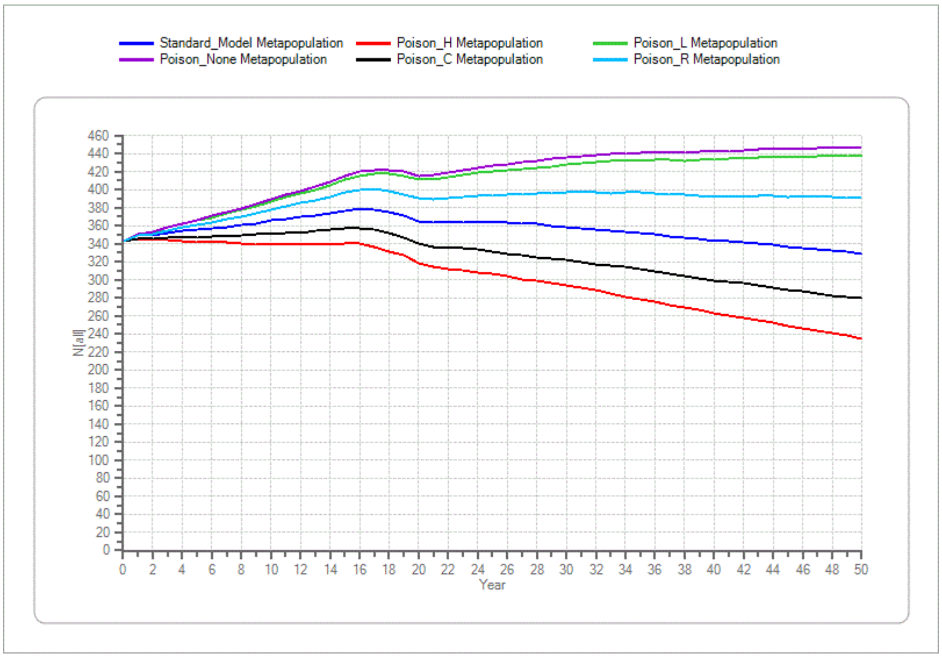

Figure IV.8 The effect of severity and frequency of poisoning events.

IV.4.3.3 Windfarm development

If not addressed properly by taking into account of foraging ranges and breeding sites, our models show that development of windfarms may have a devastating effect on the populations (Figure IV.9). Even cases of rapid and prolonged extinctions were forecast with minimal probabilities of persistence of populations in many cases (Figure IV.10).

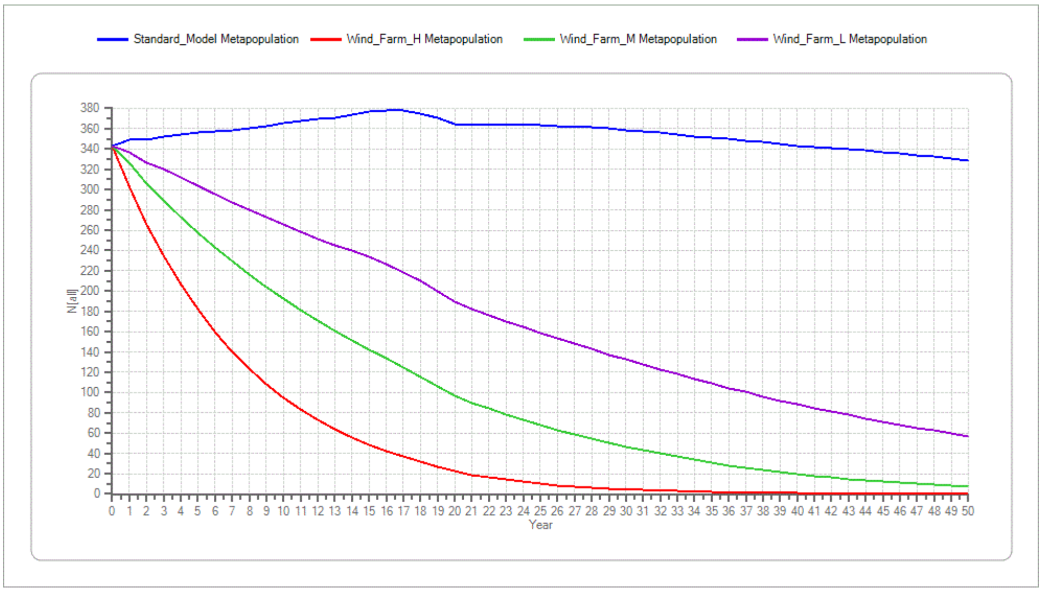

Figure IV.9 Effect of windfarm development.

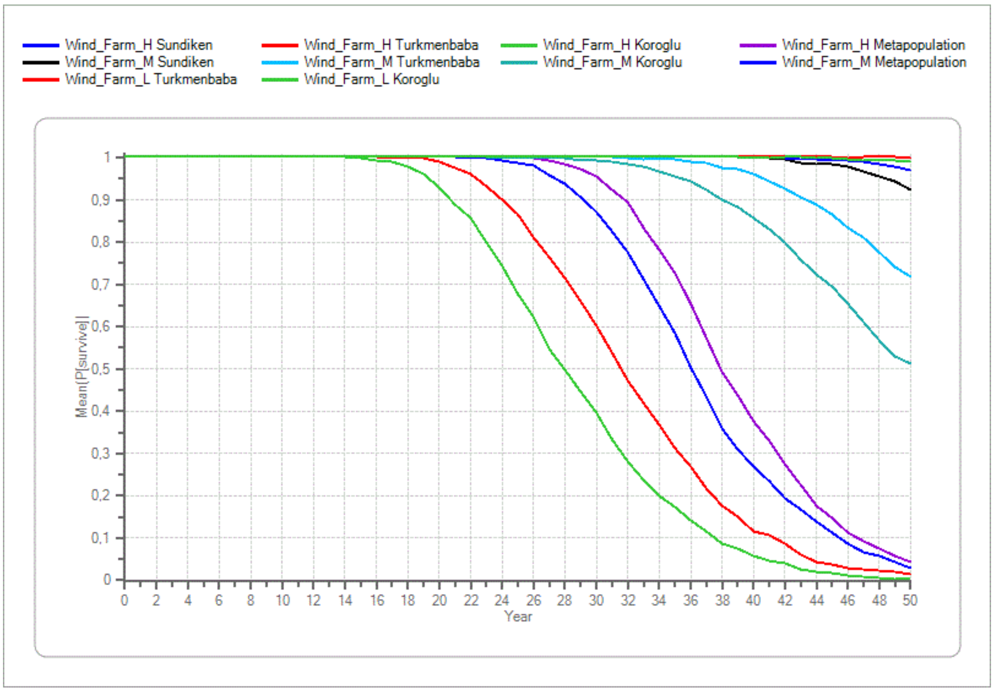

Figure IV.10 Extinction probabilities of subpopulations and the whole population under various avoidance rates from windfarms.

IV.4.3.4 Habitat deterioration

Human activities continuously narrow down the suitable habitat for many wildlife species. In the case of Cinereous vultures this corresponds to a reduction in the availability of nestable sites as well as foraging area in terms of both absolute loss of sites or lowering of quality. Although all the subpopulations expressed a high degree of sensitivity, Sündiken seemed to get effected more by habitat loss (Figure IV.11).

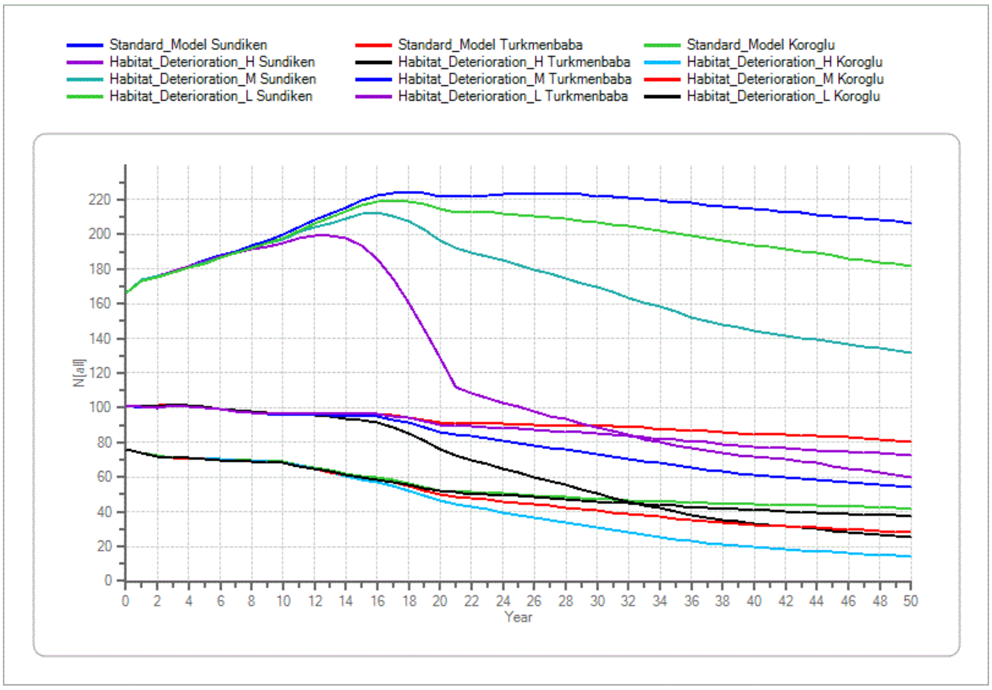

Figure IV.11 Effect of habitat deterioration on subpopulations.

IV.4.4. Conservation practices

IV.4.4.1 Reducing mortalities and invigorating breeding success

The highest level of contribution to recovery was possible with ‘supplementary feeding’ which was shown to reduce immature mortalities as expected (Figure IV.12). Increasing the survival rate of immature individuals to the levels realized in Western European populations quickly springs out the population size in each subpopulation, so in general.

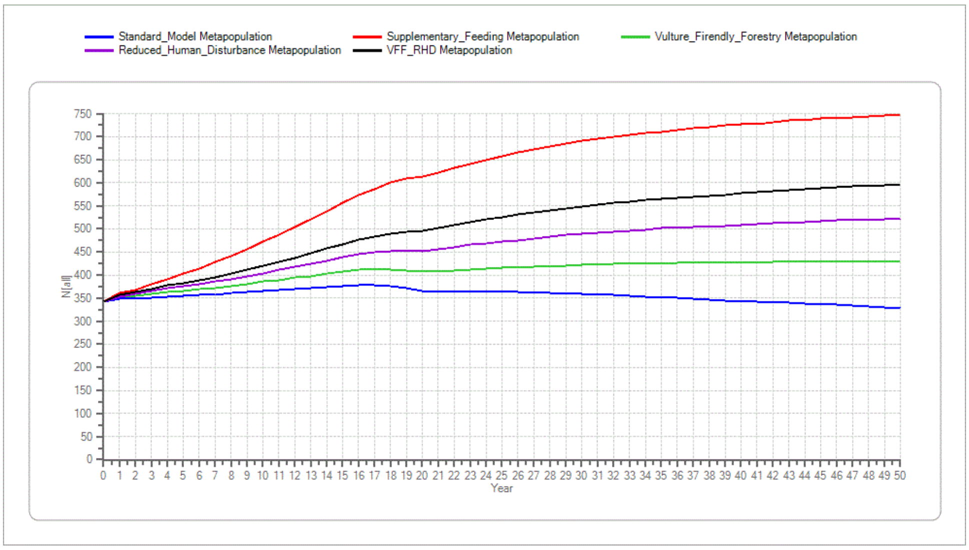

Figure IV.12 Relative impacts of various conservation practices on Cinereous Vulture population in Western Anatolia.

A second approach would focus on improving breeding success through limiting the proportion of breeding attempts lost due to human activities like forestry, recreation etc. (Figure IV.13, IV.14, IV.15). Once prevention of fledgling mortalities related to lumber production around nesting sites as in the ‘vulture friendly forestry’ scenario was achieved, each subpopulation benefited from the situation with increased population sizes. The same holds for ‘human reducing disturbance’ related to human crowd, presence of roads, recreational and sportive activities around nesting sites as well as advanced economic exploitation within the area. When both measures were applied synchronously, results comparable to supplementary feeding were achieved with population come backs without any need of improvements. Just these two simple applications might be enough to carry each of the subpopulations to positive growth rates.

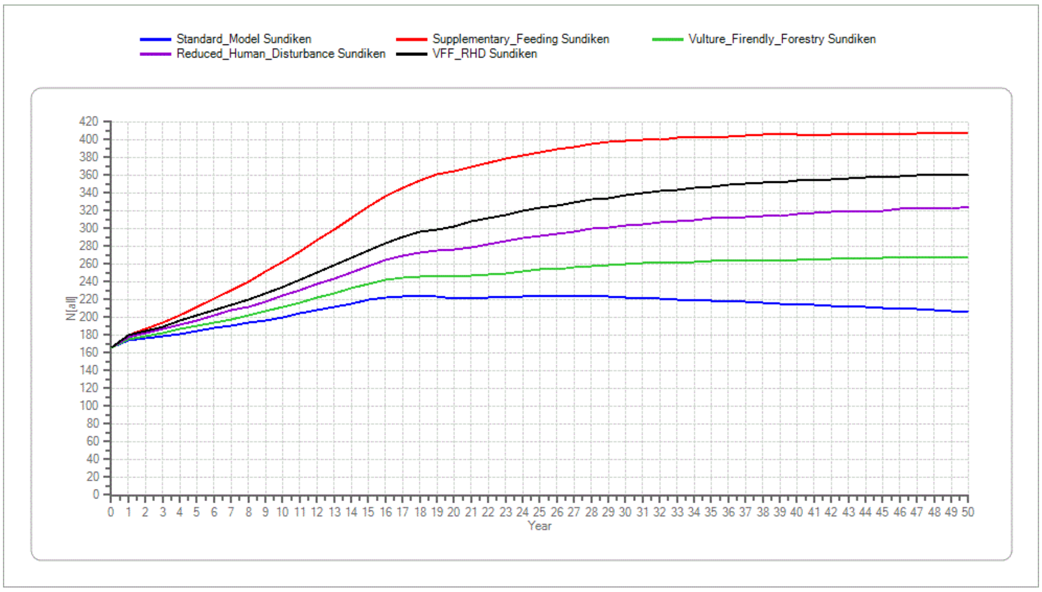

Figure IV.13 Relative impacts of various conservation practices on Sündiken subpopulation.

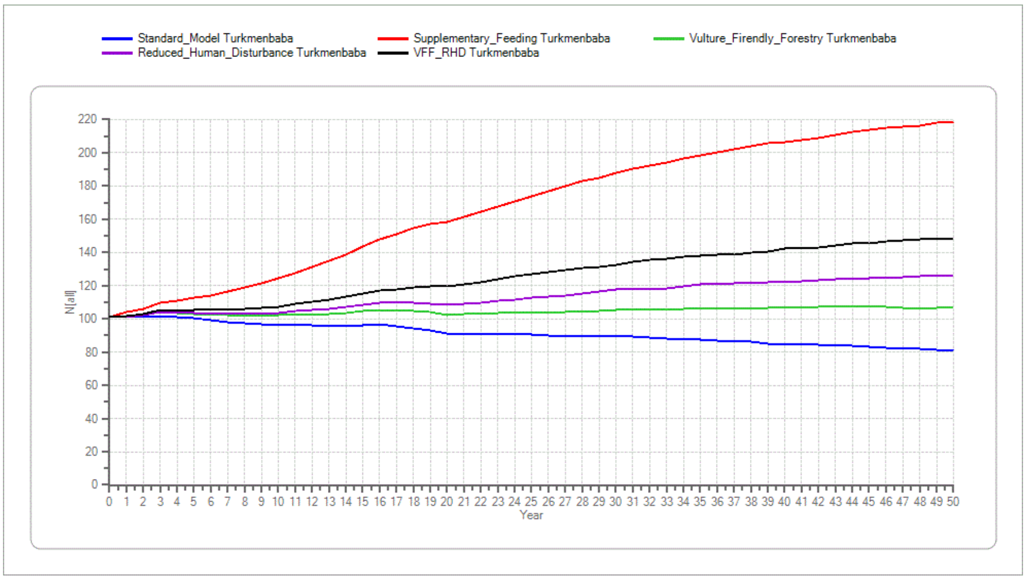

Figure IV.14 Relative impacts of various conservation practices on Türkmenbaba subpopulation.

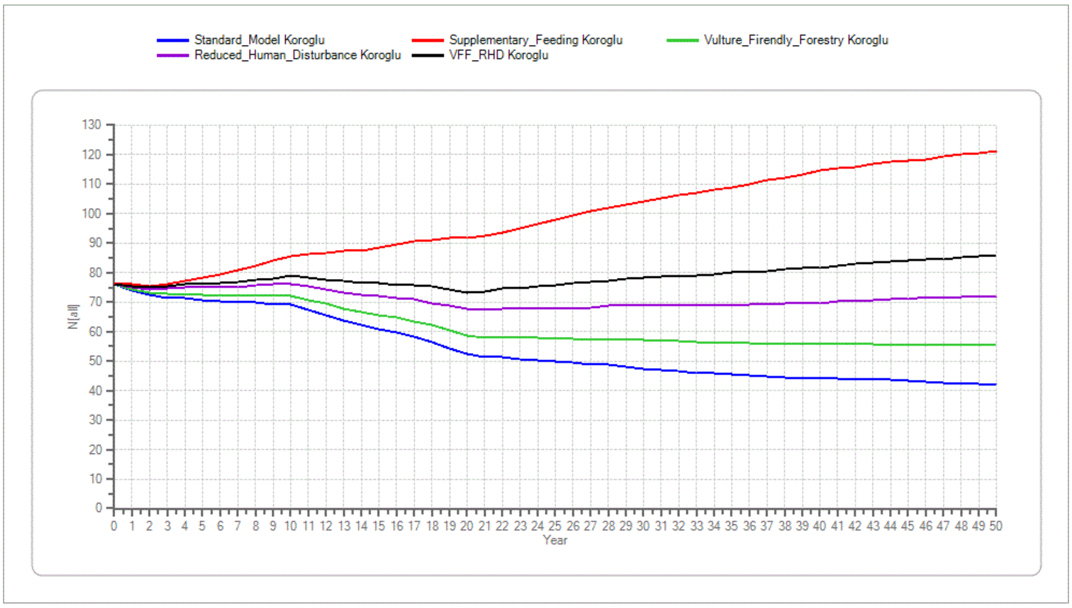

Figure IV.15 Relative impacts of various conservation practices on Köroğlu subpopulation.

IV.4.4.2 Captive breeding and reintroductions

Supplementing each subpopulation with a certain amount of individuals obviously helped building up population numbers as long as the reintroductions were kept on with a later decrease in the number of extant individuals after the program ended (Figure IV.16).

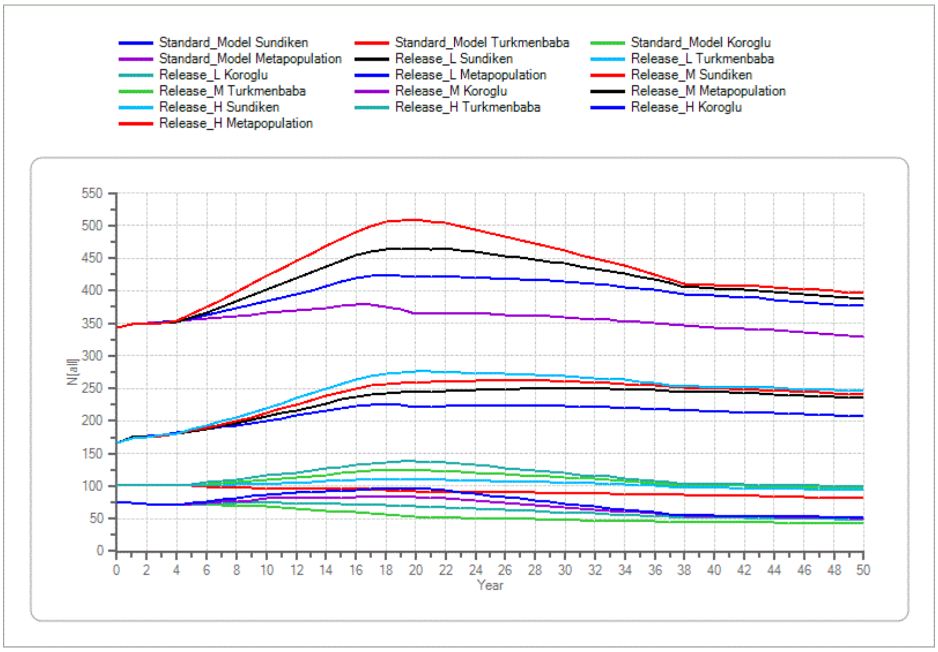

Figure IV.16 Effect of reintroduction of various number of individuals (1-3) on a yearly basis.

IV.4.5 Discussion

PVAs are very effective tool not only for risk assessment studies (Beissinger and McCullough 2002), but also for future management prescriptions (Soutullo et al. 2008). Small populations are known to be more prone to risks that may more easily end up in population declines, and as a result in extinction events (Boyce 1992). Situation in Türkmenbaba and Köroğlu in comparison to Sündiken might well be a reflection of such a circumstance. According to our results, Köroğlu is effected more than Türkmenbaba by human activities resulting in a higher pressure on population numbers despite both have similar number of breeding pairs. Even though a medium length (15 years) monitoring study in Türkmenbaba (Yamaç 2004) points to a stable population -even with signs of an increase in the number of breeding pairs- such a trend was not inconsistent with the model we constructed. For Türkmenbaba subpopulation in the first fifteen years, the slope of decline was mild with more pronounced effects after that time.

Usually adult mortality rates are considered as the main determining factor in population viability of long living raptors (Bennett and Ian 2002; Grande et al. 2009; Meretsky et al. 2000; Ortega et al. 2009); however, in that particular case high juvenile mortality rates seemed to effect the viability in a way that was comparable to adult mortalities. A smaller amount of (just 10%) increase in juvenile mortality would have an impact that is as much as 50% increase in adult mortality. The study of Manlik et al. (2016) showed also the importance of juvenile survival in the long term population recovery of two dolphin populations. The possible cause of the high mortality in inexperienced juvenile birds may be related with the migration routes or wintering areas (Penteriani et al. 2005; Thiollay 2006; Velevski et al. 2014). Although not directly comparable, an increase in the survival of juvenile birds instead of adults, which already have quite high survival rates would also be immensely beneficial for the population dynamics and parental fitness (Maness and Anderson 2013).

The bird species is known to form loose colonies at breeding sites and it was shown that such aggregations were facilitated further by dispersing young birds between breeding sites (Dunne 2017). The decision of subadults before establishing in a breeding colony was influenced by both the relative size and proximity of source and recipient sites (Brown 1978; Greenwood and Harvey 1982). Sündiken is located in the middle of two subpopulations and has the highest number of breeding pairs which may be result of benefiting more from dispersal. A juvenile from Türkmenbaba tracked by telemetry spent some time at the breeding sites in Sündiken before southward migration (Yamaç and Bilgin 2012), which would be ascribed to conspecific attraction seen in colonial species (Liminana et al. 2011; Sergio and Penteriani 2005). Despite higher number of breeding pairs, lower reproductive success rates observed in Sündiken subpopulation might be the reason of lowered number of extant individuals. By young adults establishing in Sündiken more and more, the site became even more attractive. In the simulations, with time the accumulated opportunity cost for population resulting from more individuals choosing to breed at the site with the lowest reproductive rates was overwhelming and an overall depression was seen except Sündiken. Since exact dispersal rates are not known, it could be speculated that the high number of breeding pairs in Sündiken might be supplied by outlying subpopulations. But more concrete studies on dispersal rates are needed, especially those make use of banded juveniles establishing in other breeding sites.

Severity, frequency, and the extent of poisoning events (decreasing fecundity and survival) are not very well studied. It had been possible to document a few cases (Gangoso et al. 2009; Hernández and Margalida 2009), but the broad effects, reasons and dynamics of poisoning remain to be investigated. Besides from high mortality rates of juvenile individuals, a covered but a prominent cause of steadiness of Western Anatolian population would be related with poisoning. Removing poisoning from the model alone resulted in a relatively rapid recovery of the population. In the study of García-Ripollés and López-López (2011), the effect of poisoning even in low frequency had also extremely negative effect on population trends. Although there weren't cases documented in Western Anatolia where the population suffered from mass poisoning events, the effect of poisoning seems to influence the model with a high level not only in our study, but also it is very effective threat to vultures worldwide (Green et al. 2004; Green et al. 2006; Virani et al. 2011).

Bird deaths arising from collisions with wind turbines (increasing mortality) in worldwide ranged from 0 to almost 40 deaths per turbine per year (Sovacool 2009).

Mortality caused by collision events are influenced by both site specific factors like landscape feature, flight paths and weather (Marques et al. 2014), in addition to the avoidance behavior of the birds (Sugimoto and Matsuda 2011). Scenarios we simulated here take into account different avoidance rates but since the end result was always additional mortality caused by collisions, the outcomes would be comparable for the case with decision of locations for wind turbines. The results associated with windfarm development does not necessarily say that the population will automatically suffer from population declines but instead points to the vitality of the site selection for installation of such premises (Marques et al. 2014). Collisions cannot be considered unique to Cinereous vultures, other species of birds also faced with negative effects (Barrios and Rodriguez 2004; Carrete et al. 2009; Drewitt and Langston 2006), so judgements and ecological cost evaluations should take into account the impact on other species as well.

Relative roles of survival and reproduction in long-living and slow-growing vertebrates were discussed both in earlier (Heppel et al. 2000) and recent (Manlik et al. 2016) studies. Thus in addition to try increasing survival rates, an alternative conservation strategy would focus on increasing reproductive rates. In contrast to role of survivals, such a clue was not easily obtained from our analysis of model parameters in the population dynamics part and from the analysis of potential threats. Instead, field data on breeding success in two distinct zones of Sündiken and Türkmenbaba and potential impact of anthropogenic factors on the values observed in distinct sites opened the way for developing these alternative strategies mainly focusing on breeding rates. Subpopulation in Sündiken enjoys the availability of highest number of breeding pairs on one hand and on the other it suffers most from the human disturbance. Mining and other types of economic activities as well as recreational areas around makes it more prone to habitat deterioration (Kirazli 2016; Kirazlı 2013; Kirazlı and Yamaç 2013).

Supplementary feeding through so-called ‘vulture restaurants’ would also serve conservation purposes by preventing poisoning and facilitating a lower level of dispersal of subadult birds thus further indirectly decreasing mortalities and increasing population sizes (García-Ripollés and López-López 2011; Parra and Tellería 2004). However, some existing vulture restaurants were closed to prevent the possible risk of transmission of neurodegenerative diseases (García-Ripollés and López-López 2011). Thus, demographic effect of supplementary feeding is still controversial (Margalida et al. 2010). Other negative effects of supplementary feeding include the reduction of productivity (Carrete et al. 2006) and buffering effect on dispersal by staying in the natal population aa a result of conspecific attraction (Oro et al. 2008). Nevertheless, if supplementary feeding involves individual pairs rather than large feeding places reduced the risk of mass poisoning (Velevski et al. 2014).

To reduce the probability of extinction, supplementation may also be employed as a management tool (Bustamante 1996). Generally, in raptors, hacking method (Dzialak et al. 2006) placing chicks in artificial nests and caring with them until they fledge-was used. In the aviary method, immatures were released after spending one year in the aviaries at the release site (Mihoub et al. 2014). In our results, releasing increased population size, but after program ended there was a decrease. Thus, after releases, success of reintroductions should be monitored and assessed (Mihoub et al. 2014; Sutherland et al. 2010).

Consequences for this large, long-living vulture species can be applicable for other raptors as well as other vertebrates including large mammals especially those suffering from reduced reproductive rates due to human activities.

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PART V

GENERAL CONCLUSION

First time in this study, black vulture samples from Turkey were studied genetically. In the present study, we describe the current genetic status of the Turkish population using both mitochondrial cytochrome b sequences and nuclear microsatellite markers.

Conclusions of the current study can be listed as follows:

- Analyses of population genetic structure suggested that the sampled individuals originated from a single panmictic population.

- The proportion of birds estimated to be related was not higher within populations than between populations.

- We could not detect any significant inbreeding coefficient larger than zero.

- We detected a signature of a genetic bottleneck with moderate level of heterozygosity and relatively low effective population size Analyses of population genetic structure suggested that the sampled individuals originated from a single panmictic population.

- Our findings suggest that management actions should consider Cinereous vulture populations in Turkey as a whole (i.e. a single management unit), which might reduce costs and enable a more focused, comprehensive effort for their conservation.

- Current moderate level heterozygosity suggested that there is limited need for intervention to increase the present genetic diversity. In the short-term, the aim should be to increase population size by providing continuous protection for individuals and habitat management.

- There is a strong within species phylogeographic pattern as a result of highly phylopatric behaviour of Cinereous vulture populations.

- The extremely low mitochondrial DNA diversity compared to the moderate level nuclear diversity can be explained by male-mediated gene flow.

- The standard model constructed as a baseline scenario predicted an almost stable population trend within 50 years.

- High juvenile mortality rates seemed to effect the viability in a way that was comparable to adult mortalities.

- Although there weren’t cases documented in Western Anatolia where the population suffered from mass poisoning events, the effect of poisoning seems to influence the model with a high level.

- The results associated with windfarm development does not necessarily say that the population will automatically suffer from population declines but instead points to the vitality of the site selection for installation of such premises.

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Peer Reviewed Articles:

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