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Genetic diversity is retained in a bottlenecked Cinereous Vulture population in Turkey

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Vulture populations worldwide have suffered precipitous declines in recent decades. The Cinereous Vulture *Aegypius monachus*, a highly philopatric scavenger distributed across southern Europe and the central Asian plateau, is threatened in many parts of its range. Turkey holds the second largest population of this species in the Western Palaearctic, but there has been no research on its genetic structure and the possible implications of this structure for the future of the species. Here we report nuclear diversity and relatedness determined by short tandem repeat genotyping of 81 individuals from the four largest colonies. Our results demonstrated no significant genetic structuring, suggesting a single panmictic metapopulation connected by frequent dispersal. Furthermore, we show that the study population has retained moderate levels of genetic diversity, despite passing through a recent demographic bottleneck. We estimated the effective population size to be 112 individuals (95% confidence interval 74–201). Our results imply that the observed lack of increase in population size since the 1990s has not been caused by lowered fitness due to genetic inbreeding but rather by increased mortality via demographic processes. In the short term, we suggest that conservation efforts should treat the Turkish subpopulations as a single management unit and aim to increase population size through effective protection, especially during the breeding season.

Keywords: *Aegypius monachus*, microsatellites, population structure.

Despite their essential ecological role in reducing the spread of infectious diseases (Şekercioğlu *et al.* 2004, Margalida *et al.* 2012) or disposing 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). Their unfavourable status is mainly caused by human impact through poisoning (Janss & Ferrer 2001), shooting (Ogada *et al.* 2012), food scarcity due to overhunting of mammals (Houston 1987), habitat alteration (> 98% in West Africa; Heredia 1996, Thiollay 2006) and wind farm collisions (Vasilakis *et al.* 2016). Another major threat to Asian (> 95% abundance of *Gyps* species) and African vultures has been diclofenac contamination of livestock carcasses between 1990 and 2006 (Oaks *et al.* 2004, Swan *et al.* 2006, Ogada *et al.* 2016), after which veterinary use of diclofenac was banned. The conservation status of most vulture species is expected to reach a critical level in the near future (Ogada *et al.* 2016), as they are large scavengers with relatively low population densities, large home-range requirements and low breeding rates (Cramp & Simmons 1980, Carrete & Donázar 2005).

The Cinereous Vulture (known also as the Eurasian Black Vulture) *Aegypius monachus* Linnaeus, 1766 is one of the largest birds of prey. This

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highly philopatric scavenger is distributed from the Iberian Peninsula across southern Europe and through western and central Asia to Mongolia and China (BirdLife International 2017). The species is classified globally as Near Threatened (BirdLife International 2017), as it has experienced a considerable decline within the last centuries. However, the European population is now recognized as 'Least Concern' due to increasing numbers, particularly in the Iberian Peninsula, where Spain contains 87% of the European population with ~2000 breeding pairs (Moreno-Opo *et al.* 2010). Despite this increase, many former breeding colonies across the European continent have been lost due to anthropogenic effects (poisoning, shooting and nest destruction; Carrete & Donázar 2005).

Turkey has the second largest Cinereous Vulture population in Europe with 50–200 pairs (Heredia *et al.* 1997, Yamaç & Günyel 2010) and local populations show variable breeding success (Yamaç 2004, Kirazlı & Yamaç 2013). Similar to most other vultures, this species is monogamous (Nam & Lee 2009). Cinereous Vultures start breeding at the age of 5 or 6 years, and lay one egg from mid-February to May which is incubated for 50–55 days (Cramp & Simmons 1980, Heredia 1996). The period during which hatchling plumage is observed lasts about 30–40 days and, in Turkey, the juveniles usually fledge from the nest between mid-August and early September (Yamaç 2004). The species breeds in loose colonies where nests are separated from each other by a few dozen to several hundred metres (Cramp & Simmons 1980).

The maintenance of genetic diversity is essential for adaptation and fitness (Frankham *et al.* 2002). Small populations are vulnerable to the effects of genetic drift and are prone to lose valuable genetic variation, which might eventually lead to local extinction due to inbreeding depression and lowered fitness (Mills 2013). 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). A previous genetic study by Poulakakis *et al.* (2008) included Cinereous Vulture samples from Spain, Germany, the Caucasus and Mongolia but none from Turkey. In this study, to remedy this shortcoming, we have genotyped Cinereous Vultures breeding at four colonies in Central Anatolia (Çatacık, Tandır, Türkmenbaba and Köroğlu) using 15 microsatellite loci. Due to the high dispersal ability of the species and the relatively close geographical proximity between breeding sites, we expected the colonies to constitute a single panmictic metapopulation. We tested this hypothesis, estimated effective population sizes and examined whether there was evidence of any recent bottleneck or population expansion. We discuss the implications of our results, including estimates of genetic diversity and levels of inbreeding and relatedness, for the planning of conservation efforts for the species in Turkey.

METHODS

Study area and sample collection

The samples were collected from all the largest colonies in Turkey, including the Türkmenbaba Mountain (17 500 ha) located between Eskişehir and Kütahya (39.50°N, 30.33°E), the Sündiken Mountains (218 068 ha) in the Middle Sakarya Region (39.93°N, 31.18°E), both in northwestern Central Anatolia, and the Köroğlu Mountain located between Bolu and Ankara (146 330 ha) in the western Black Sea Region (40.43°N, 31.98°E; Yamaç 2004, Kirazlı 2013, Kirazlı & Yamaç 2013; Fig. 1). The Sündiken population is made up of two separate colonies, namely Tandır and Çatacık.

A total of 291 samples (moulted 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 either included feathers from one female and one male, or blood samples from a juvenile in five cases when these were available. The closest distance between any two active nests was 51 and 140 m in Sündiken populations and the Türkmenbaba population, respectively (Yamaç 2004, Kirazlı & Yamaç 2013). On this basis, only feathers found within 150 m of an active nest-site were used for genotyping purposes. The two exceptions to this rule were when feathers were collected directly from one juvenile bird in the 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 first sexed and, if possible, one male and one female sample were selected from each nest-site within a single year for microsatellite analysis. We extracted DNA from almost all samples (n = 262) except those of down feathers

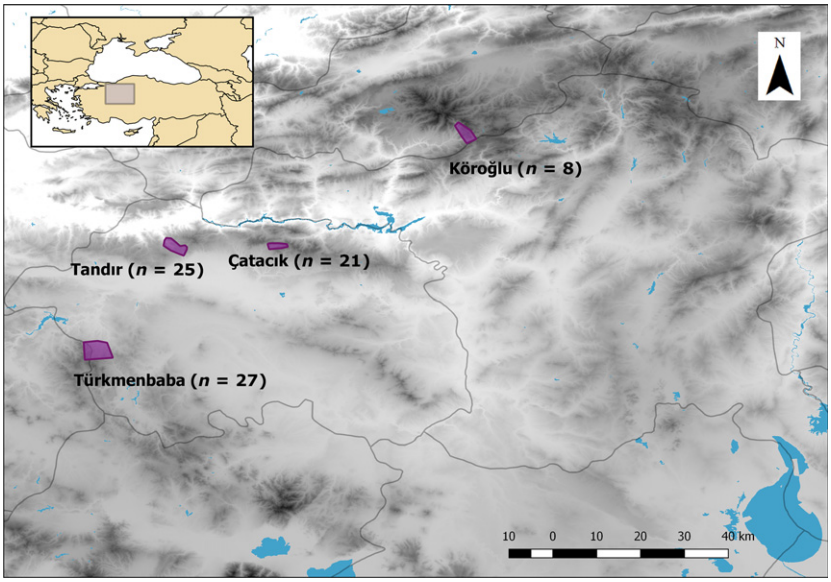

Figure 1. Map of the location of the study populations and sample sizes. Thin lines depict province boundaries. Shades of grey on the background represent altitude between 200 and 2200 m a.s.l.; darker shades are higher ground. [Colour figure can be viewed at wileyonlinelibrary.com]

(n = 24). Finally, we restricted the microsatellite analyses to 146 samples containing a DNA concentration > 19 ng/μL.

Molecular marker selection and laboratory procedures

DNA from moulted feathers was extracted by utilizing the blood spot within the superior umbilicus of the feather (Horváth *et al.* 2005). A Qiagen DNeasy Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany) was used to extract both feather and blood samples. Isolated DNA was quantified using a NanoDrop 2000 UV-Visible spectrophotometer (Thermo Fisher Scientific, Wilmington, USA). Molecular sexing was done using the primers CHD1F/CHD1R (Lee *et al.* 2010) according to the procedures given by 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) or Egyptian Vulture *Neophron percnopterus* (Agudo *et al.* 2008) on a subset of individuals. For the amplification, locus-specific fluorescently labelled forward primers (6-FAM, HEX, ROX and TAMRA, Applied Biosystems, Foster City, CA, USA) were used in eight different multiplex sets (Table S1). Template DNA was amplified using FIRE POL Master mix (Solis Biodyne, Estonia) in a total reaction volume of 25 μL, containing 1 μL of genomic DNA, 0.1–0.3 μM of each primer pair and 1× Master Mix (Solis Biodyne). Polymerase chain reaction (PCR) was conducted using touchdown PCR under the following conditions: 15 min of 95 °C heat activation followed by 35 cycles of 20 s at 95 °C, 1 min at TA °C (the optimum annealing temperature for each multiplex set, Table S1), 1 min at 72 °C, and a final extension of 10 min at 72 °C. To qualify PCR amplifications, 2% agarose gel electrophoresis was used. Allele sizes were determined on an ABI-PRISM 3100 sequencer (Applied Biosystems). Raw data from the sequencer were examined with the PEAK SCANNER 2.0 (Applied Biosystems) software for the identification of peaks and fragment sizing with the 500 LIZTM size standard. In the case of low peak signal, genotyping was repeated three times per sample. Genotypes (n = 146) were first scored by eye; 50 samples were found to be duplicates of identical genotypes, leaving 96 samples that differed in at least one allele in one locus.

Microsatellite data analysis

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 ($r > 0.2$), calculated using FREENA (Chapuis & Estoup 2006) with 10 000 replicates, and were removed from further analysis. Genotypic linkage disequilibrium between each pair of microsatellite loci was tested with FSTAT 2.9.3 (Goudet 2001). Genotyping errors, allelic dropout and scoring of stutter peaks were assessed statistically using MICROCHECKER 2.2.3 (Van Oosterhout *et al.* 2004). The software GIMLET 1.3.3 (Valière 2002) was used to estimate the probability of two individuals carrying identical multilocus genotypes, which gives information about the discriminative power of the microsatellites to distinguish between individuals. As the samples consisted of shed feathers, it is possible that some individuals were included in more than one sample. Because this may introduce a bias in estimates of population structure and effective population size changes, we first used GIMLET to construct a matrix of the pairwise comparisons of these 96 genotypes. Histograms illustrating the pairwise similarity of individuals were made in R v.3.3.2 (R Core Team 2016).

Population genetic structure and relatedness

Polymorphic information content (PIC), which demonstrates possible utility of markers in identifying individuals, was estimated by CERVUS 3.0.7 (Marshall *et al.* 1998, Kalinowski *et al.* 2007) 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 & Cockerham 1984) values between pairs of populations were calculated by ARLEQUIN v3.5 (Excoffier & Lischer 2010) where the statistical significance was tested using 10 000 permutations. The presence of spatial structure among populations was tested for up to five clusters (K) with 20 replicates using STRUCTURE 2.3.4 (Pritchard *et al.* 2000). The burn-in was set to 100 000 and the number of Markov chain Monte Carlo (MCMC) iterations to 500 000. Due to close geographical proximity of these populations and the likelihood that they are relatively closely related, we allowed admixture and correlated allele frequencies, as there is no reason to consider each population to be completely discrete (Porras-Hurtado *et al.* 2013). The LOCPRIOR model was chosen to incorporate the origin of individuals as prior information because this model performs better in the case of weak population structure (Pritchard *et al.* 2000, Mims *et al.* 2016). The software STRUCTURE HARVESTER 0.6.94 (Earl & Vonholdt 2012) was used to estimate the most probable K value determined by Evanno's method (Evanno *et al.* 2005) where the 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 $K = 1$ had the greatest log-likelihood, $K = 1$ was accepted as the most likely value (Spear *et al.* 2012). The CLUMPP software (Jakobsson & Rosenberg 2007) was used to average the estimated cluster membership coefficient matrices of multiple runs of the STRUCTURE clustering program. Output data of CLUMPP were used for the POPHELPER program (Francis 2016) graphically to display population structures. Additionally, GENETIX 4.05 (Belkhir *et al.* 1996, 2004) was used to visualize the differentiation of populations and individuals by factorial correspondence analysis (FCA) in two-dimensional space (2D).

The software ML-RELATE (Kalinowski *et al.* 2006) was used to estimate relatedness and relationships between pairs of individuals. This method calculates the maximum likelihood estimates of relatedness (r) using a downhill simplex routine. The software assigns pairs of samples into four common pedigree relationships: unrelated (U), half-siblings (HS), full-siblings (FS) and parent–offspring (PO). The Cinereous Vulture typically shows long-term monogamy, implying that true half-sibs should be rare in our data. However, there is no class for lower level relatedness (e.g. cousins and nieces) in these analyses. It is therefore reasonable to assume that these may be misclassified as HS, at least more frequently so than unrelated individuals. The results from these classifications were used to determine whether presumably related birds (classes HS, FS and PO) were more common within than between colonies.

Genetic diversity and bottleneck

Basic statistics such as number of alleles and allelic richness were calculated by FSTAT 2.9.3 (Goudet 2001). Inbreeding coefficients for each locus and population were calculated using GENEPOP 3.4 (Raymond & Rousset 1995), where the

significance was evaluated by Fisher's exact test *P*-values, applying the Markov chain method (10 000 dememorization). Deviations from Hardy–Weinberg equilibrium (HWE) were carried out using exact tests implemented in ARLEQUIN v3.5 (Excoffier & Lischer 2010). The software BOTTLENECK 1.2.02 (Cornuet & Luikart 1996, Piry *et al.* 1999) was run to detect any recent bottleneck in the population. The statistical significance of heterozygosity excess was determined using Wilcoxon's sign rank test under the two-phase mutation model (TPM). 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). The approximate date of possible demographic events was estimated by the Bayesian-based MSVAR method (Storz & Beaumont 2002). Analyses were run with 2 000 000 steps and 10 000 burn-ins. Generation time of the Cinereous Vulture is assumed to be 10 years (Chung *et al.* 2015) and μ was assumed to be 5×10^{-4} per nucleotide per generation (Agudo *et al.* 2010). Effective population size of the population was calculated in NEESTIMATOR 2.01 (Do *et al.* 2014) using the linkage disequilibrium (LD) method. Finally, to estimate any population expansion, KGTESTS (Bilgin 2007) was used.

RESULTS

Reliability of the data

None of the possible comparisons between pairs of loci in each population yielded any significant linkage disequilibrium (P critical = 0.000119 after Bonferroni correction), and therefore each was treated as independent. Analyses using FREENA and CERVUS showed no evidence for the presence of null alleles among the 15 loci in the four populations, but MICROCHECKER detected null alleles at one locus (BV6) in the Türkmenbaba population. However, because this finding was not consistent among colonies, we kept the locus for further analysis. There was also no evidence of genotyping errors due to stuttering or allele dropout at any of the 15 loci in the study populations based on MICROCHECKER. A histogram of the number of identical loci between samples (n = 96) clearly showed a bimodal distribution (Fig. S1, grey bars). We manually inspected the 15 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 from analyses of feathers obtained from the same individual. This is because the mismatching loci were genotypes that were homozygous vs. heterozygous, with the homozygous allele being shared with the heterozygous genotype. It is well known that allelic dropouts commonly affect this kind of marker, and the heterozygous genotype is more likely to correspond to the true genotype of the individual (Wang *et al.* 2012). We thus extracted one genotype from each of these pairs by keeping the heterozygous genotype for these mismatching loci. After these removals, we obtained a final dataset of genotypes corresponding to 81 unique individuals (Fig. S1, black bars; for our complete dataset see Table S2). Thus, this reduced dataset should be reliable for investigating the genetic population diversity and structure of Turkish Cinereous Vultures.

Population genetic structure and relatedness

There was no evidence of population structure. F_{ST} values between population pairs were calculated and found to be non-significant ($P > 0.05$), ranging from 0.000 to 0.011 (Table 1). According to results of STRUCTURE, the mean likelihood score (Ln(K)) was highest for $K = 1$ (Fig. S2a), although the Evanno method suggested the number of clusters to be two ($\Delta K = 2$; Figs S2b and 2). Similar to the F_{ST} and STRUCTURE results, FCA also failed to differentiate these four populations (Fig. S3).

Table 1. Pairwise F_{ST} values between four population samples of *Aegypius monachus* based on 15 nuclear microsatellite loci above the diagonal. Proportion of pairs of individuals in the complete dataset 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

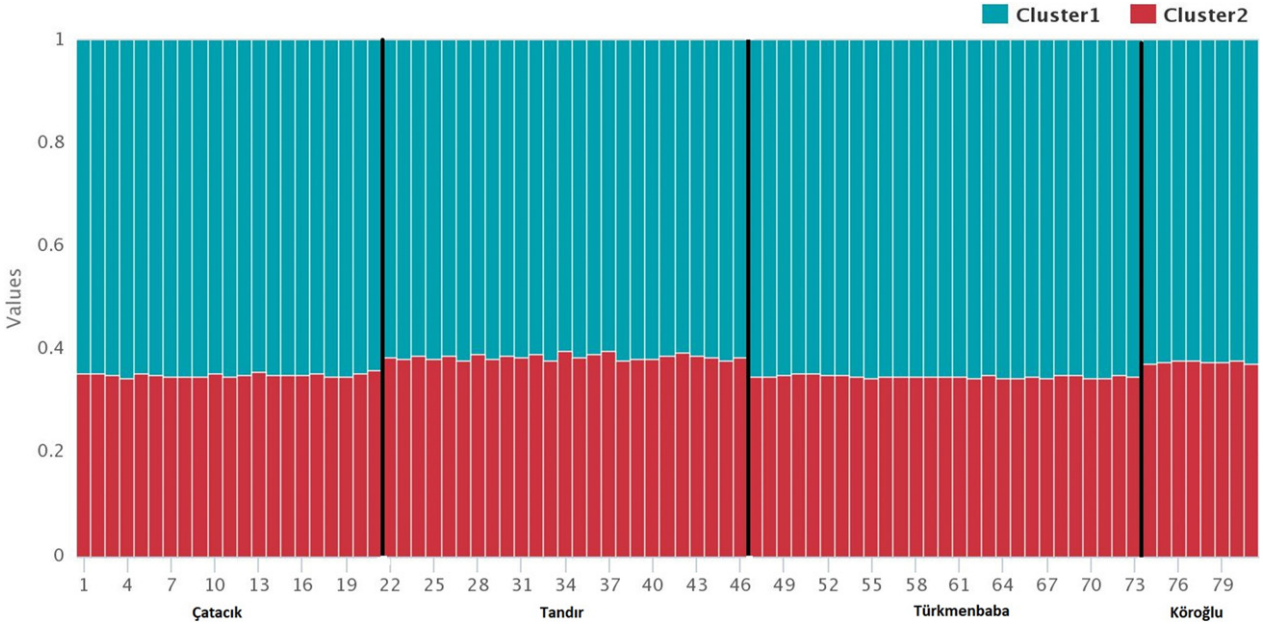

Figure 2. Clustering analysis of genotypes at 15 microsatellite loci performed using the STRUCTURE software. The visualization of outputs, $K = 2$. Each colour represents a different cluster and black segments separate the presumed populations. Blue and red colours represent clusters 1 and 2, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

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 was significant on its own. The results were used to examine whether related birds (classified as PO, FS or HS) were more likely to be found within than between colonies. When comparing the relationships between each pair of individuals from the four study colonies, 173 of the 564 family relationships (PO, FS or HS) were within the same study population, whereas 391 involved individuals sampled in different populations (Chi-square test $P = 0.35$; Table 1 and Fig. 3). Additionally, 11 of the 15 nests (73%) for which we had genotyped both the male and the female (using adult feathers) were classified as unrelated.

Genetic diversity and bottleneck

A total of 67 alleles were detected in all loci throughout all populations, with an average number of four alleles per locus, varying between 2 and 10 (BV11 and GT3-35) alleles (Tables S3 and S4). These 15 loci demonstrated overall a 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 for all four populations. The most informative eight loci (PIC > 0.5) were BV11, GT3-35, BV6, NP163, NP229, NP39, BV20 and BV13 (Table S5). All populations were in HWE, except for locus NP163 ($P = 0.0008$) in the Türkmenbaba population. None of the estimates of inbreeding coefficient (F_{IS}) differed significantly from zero, except again for NP163 in the Türkmenbaba population. No private alleles were detected in any of the four populations.

For the bottleneck analysis and effective population size calculation, all four populations were assumed to be a single panmictic population. According to the Wilcoxon test, the observed proportion of heterozygotes departed significantly from the expectation under mutation-drift equilibrium using a TPM in the population (one-tail for H excess: $P = 0.00003$), which indicated that this population had 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 the normal L-shaped distribution that would be

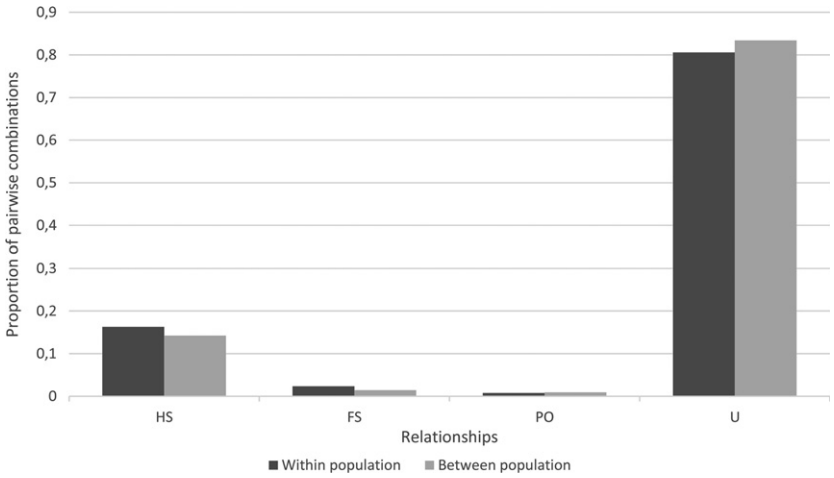

Figure 3. Comparison of the estimated proportions of pairwise combinations and relationships within and between populations. HS, Half-siblings (includes cousins and nieces); FS, full-siblings; PO, parent–offspring; U, unrelated.

expected for non-bottlenecked populations (Fig. 4). This further supports the notion that the population deviated from mutation-drift equilibrium and that it has experienced a recent bottleneck. The current N_e distribution for the Turkish population was estimated to be log 0.84. There was a dramatic population decline from the mean of the ancestral N_e distribution of log 4.96. Finally, the time since the population size started to decline was estimated as approximately 605 years ago (Fig. 5). The current effective population size estimate was 112 (95% confidence interval 74–201). We did not detect any signatures of population expansion (number of loci with a negative $k = 7$; k-test $P = 0.65$).

DISCUSSION

We had hypothesized that these four colonies would constitute a single genetic population connected via dispersal. This was supported by our analyses of population genetic structure, as we failed to find any evidence of differentiation between the colonies. High dispersal rates and the relatively short distances between studied locations may explain our finding of an undifferentiated population structure (Le Gouar *et al.* 2008, Craig *et al.* 2016). Yamaç and Bilgin (2012) tracked newly fledged birds from the Türkmenbaba population using GPS-GSM telemetry and observed one bird moving 80 km north to Çatacık immediately after fledging. 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 suggests that the study sites make up a single panmictic population.

Additionally, we did not detect any significant inbreeding coefficient larger than zero, which may be explained either by birds choosing unrelated individuals as mates (Smith *et al.* 1997) or the limited sample size of our study (Bourke *et al.* 2010, Ogden *et al.* 2015). Choosing mates among unrelated individuals is the dominant pattern in birds and mammals (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 (Pemberton 2004, Brzeski *et al.* 2014).

The level of heterozygosity observed was consistent with estimates from other raptor populations (Godoy *et al.* 2004, Rudnick *et al.* 2005, Craig *et al.* 2016). Similar to the small relict population of Cinereous Vultures in Greece, our population demonstrated higher observed heterozygosity than was found in the much larger Spanish Cinereous Vulture population (Poulakakis *et al.* 2008).

We detected a signature of a genetic bottleneck, suggesting that the demographic decline in the last few centuries has had an impact on the Turkish population of Cinereous Vultures. However,

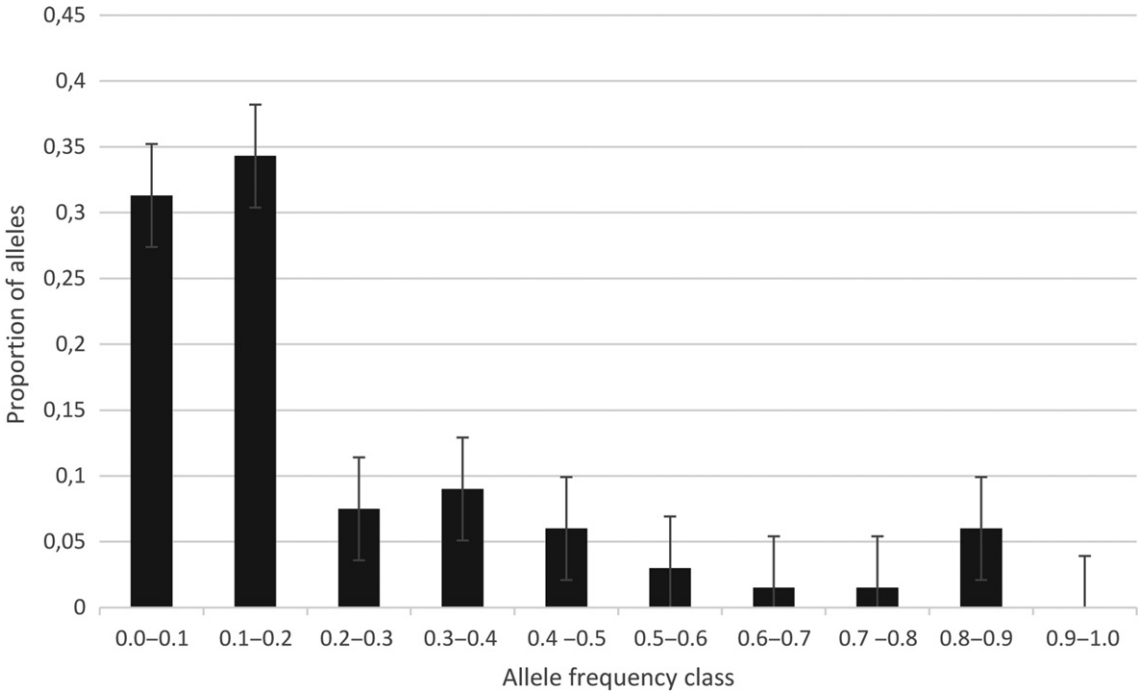

Figure 4. Mode shift of the allele frequencies indicating the occurrence of a recent genetic bottleneck.

despite the bottleneck, we observed relatively high genetic diversity. There are several reasons why this might be so. Long generation times, such as occurs in vulture species, can buffer against loss of genetic diversity during bottlenecks (Hailer *et al.* 2006). In small and declining populations, allelic diversity (loss of low-frequency alleles) decreases faster than heterozygosity (Nadeau 2012), so it might be that the demographic decline is too recent to detect signs of inbreeding in the population (Canal *et al.* 2017). Additionally, after a bottleneck, excess gene diversity can be observed for a short time (Ganapathi *et al.* 2012). Hence, it seems that the demographic bottleneck, at least until now, has had little effect on the genetic diversity of the Cinereous Vulture population in Turkey. The population size decline was estimated to have started approximately 605 years ago. However, there are few records previous to 1990 and these do not necessarily represent breeding birds. Therefore, it is impossible to comment on earlier population history or estimate a more reliable date for the bottleneck.

The current Turkish population has been reported to be stable or declining, despite their variable breeding success (Yamaç 2004, Kirazlı & Yamaç 2013). The estimated countrywide population size varied from 50 to 200 pairs, assuming the presence of undiscovered colonies, particularly in the east. As strict monogamy is the rule among Cinereous Vultures, N_e is expected to be close to the censused number of breeding adults. We estimated the effective population size to be 112 individuals, which would correspond to 56 nesting pairs. This is lower than the total number of active nests (83) reported by Yamaç (2004) and Kirazlı and Yamaç (2013). Moreover, surveys of nesting colonies are rarely complete, and unsampled smaller colonies that are probably part of the studied meta-population exist in northern Central Anatolia (Yarar & Magnin 1997, Kirwan *et al.* 2008), so the difference may be even greater. Similar results were also obtained by Bourke *et al.* (2010) on the monogamous British Golden Eagle *Aquila chrysaetos* where N_e was estimated to be 437 individuals although the census population size was 442 pairs. However, in another study of Golden Eagles, Doyle *et al.* (2016) estimated a very low effective population size (~400) compared with the census population size (~32 000). The discrepancies observed can be explained partly by the level of overlapping generations of breeders (Felsenstein 1971), and the length of pair bonds and spatial fidelity to particular localities

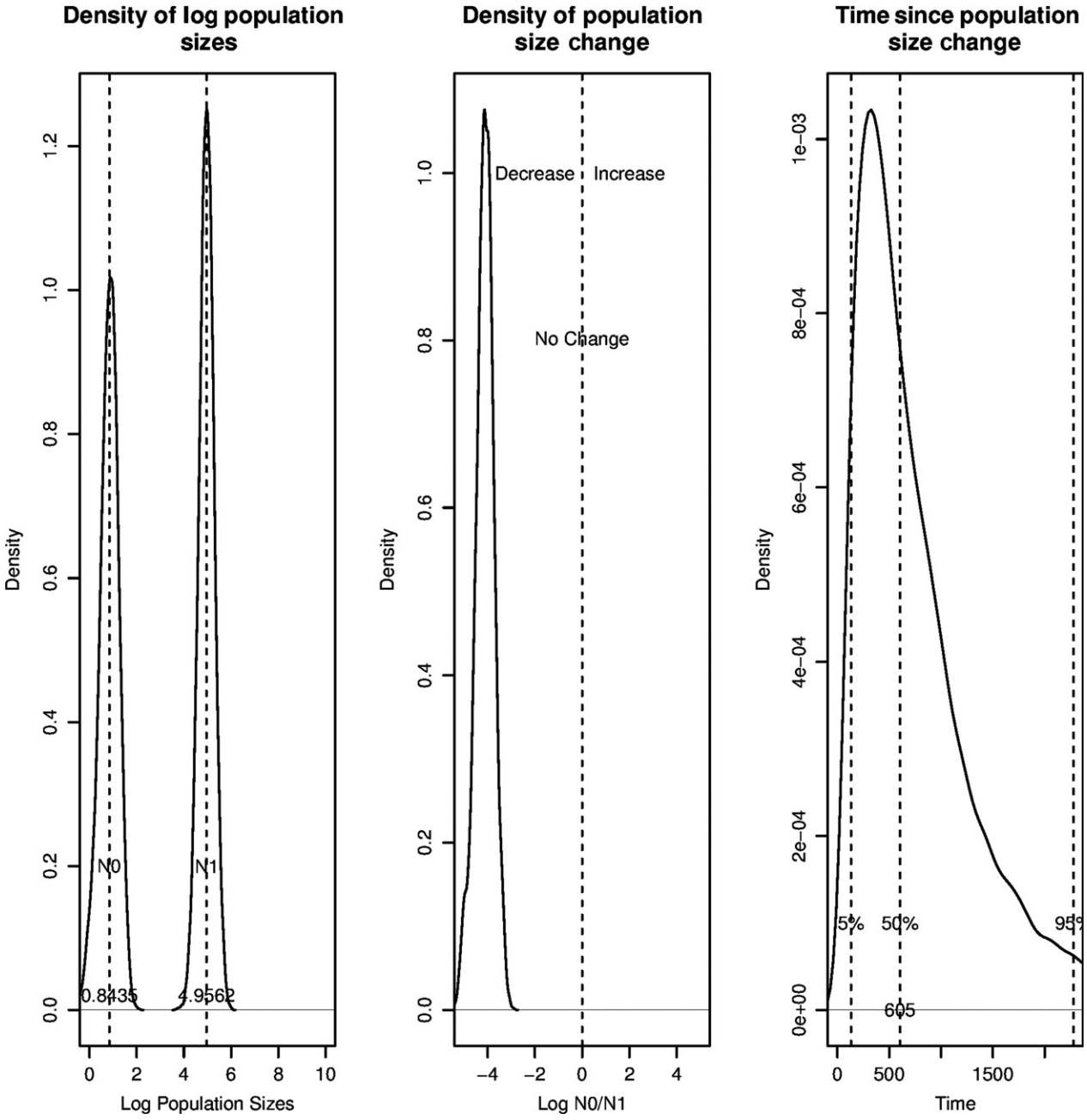

Figure 5. MSVAR results for the Turkish populations based on 15 microsatellite loci. Distribution of ancestral (N1) and current (N0) N_e on a logarithmic scale and time since N_e change.

(Hartl 2000). Vultures are long-lived with strongly overlapping generations, so the discrepancy observed in our population was most probably due to unequal reproductive success of the breeding pairs (Frey *et al.* 1995), as the population displayed an equal sex ratio.

The reasons behind the decline leading to the inferred bottleneck are probably many and complex but may include increasing intensification of the livestock industry, and the widespread and indiscriminate use of poison by the rural populace during 1960–95. 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 & Günyel 2010), the population of Cinereous Vultures in Turkey has 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 & Bilgin 2012) or by highly conservative behaviour of adults in nest-site selection (Dias *et al.* 2017, E. Yamaç unpubl. data), respectively. Continued monitoring of colonies both by conventional nest counts and through non-invasive genetic sampling is recommended.

Despite decades of speculating on the presence of undiscovered colonies elsewhere in Turkey (Kirwan *et al.* 2008) and intensive searches in the northeastern parts of the country (DKM 2011), no new colonies have been detected. Therefore, the studied colonies probably represent the 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 single management unit, which might reduce costs and enable more focused, comprehensive efforts for their conservation. The current moderate levels of heterozygosity suggest that there is a limited need for intervention to boost present genetic diversity. Therefore, in the short term, the aim should be to increase population size by providing effective protection for individuals and to encourage expansion through habitat management.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Histogram of pairwise comparison of genotypes. Grey bars demonstrate the numbers of shared alleles with all samples, and black bars show the numbers of shared alleles with unique genotypes.

Figure S2. (a) The mean likelihood of each cluster for K = 1–5, where the error bar demonstrates standard deviations. (b) Plot of delta K values from the STRUCTURE analyses.

Figure S3. Graphical representation of Factorial Correspondence Analysis (FCA) created using 2D module. Each colour represents different colonies; Çatacık—yellow; Tandır—blue; Türkmenbaba—white; Bolu—grey.

Table S1. Fifteen nuclear loci used in this study and their characteristics.

Table S2. A list of each individual with sex and genotypes for each locus.

Table S3. Sample size (n), mean number of alleles per locus (N_a), observed heterozygosity (H_O) and expected heterozygosity (H_E) for each population based on the 15 microsatellite loci.

Table S4. A comparison of genetic diversity at 15 microsatellite loci between *Aegypius monachus* populations.

Table S5. Summary statistics per loci. A_R, allelic richness; H_E, expected heterozygosity; H_O, observed heterozygosity; N_a, observed allele number; PIC, polymorphism information content.