



## Short communication

# Reduced genetic diversity in Bearded Vultures *Gypaetus barbatus* in Southern Africa

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The Bearded Vulture *Gypaetus barbatus* occurs throughout its range in small and dwindling population fragments with limited genetic differentiation between populations, suggesting that the species might be managed as a single entity. The numbers of East and Southern African Bearded Vultures included in previous studies were small, so we determine the genetic variation within, evolutionary placement of and connectivity among sub-Saharan African populations. Mitochondrial DNA fragment analyses detected little or no differentiation between populations in Ethiopia and Southern Africa, with reduced haplotype diversity in Southern Africa compared with populations in the Northern Hemisphere. The results inform conservation management of this species globally and locally, and offer guidelines for translocations should populations continue to decline.

**Keywords:** Bearded Vulture, conservation management, genetic diversity, population connectivity.

Many natural populations are becoming increasingly fragmented as a result of anthropogenic influences. Fragmentation negatively affects population viability through loss of genetic diversity, inbreeding, and ultimately population growth and adaptability (e.g. Lowe *et al.* 2005 and references therein). A case in point is the Bearded Vulture *Gypaetus barbatus*, a large scavenging raptor inhabiting mountainous areas across an extensive range. In the past, the species was widely distributed throughout much of

Asia, Europe and Africa but its current distribution is severely fragmented. Although the species never occurred in high numbers, trends of recent decline throughout much of its range have been documented (Margalida *et al.* 2008, Birdlife International 2014, Krüger *et al.* 2014).

Two subspecies have been recognized based on plumage characteristics (Hiraldo *et al.* 1984, Mundy *et al.* 1992), *G. b. barbatus* north of the Tropic of Cancer and *G. b. meridionalis* south of the Tropic of Cancer. Southern birds have similar phenotypes in that they lack a black 'ear' tuft and breast collar and are smaller in size than their northern counterparts. A genetic study (Godoy *et al.* 2004) confirmed the presence of two lineages, but these had little geographical correspondence to the described subspecies. The first lineage occurs mainly in Western Europe, the second predominantly in Africa, Eastern Europe and Central Asia. These findings led to the suggestion by Godoy and co-workers that the species should be managed as a single population. Only a few sub-Saharan African, and specifically Southern African, samples were included in the Godoy *et al.* (2004) study. For this reason, the evolutionary position of the Southern (and to a lesser extent Eastern) African populations, and the level of connectivity (migrant exchange) between them, remains unclear.

African Bearded Vulture populations are in decline or presumed to be declining. Ash and Atkins (2009) suspected that the Ethiopian population was much smaller than the crude estimate by Mundy *et al.* (1992) of 1430 pairs. Populations in Kenya, Tanzania and Uganda are estimated at only 10 pairs (S. Thomsett pers. comm.) whereas the Southern African population is estimated at 109 pairs (Krüger *et al.* 2014). The latter also suffered a significant range contraction (Boshoff *et al.* 1983, Krüger *et al.* 2014).

For the long-term persistence of the Bearded Vulture in Southern Africa it is essential to maintain an adequate population size and high levels of genetic diversity. If there is some exchange of individuals between sub-Saharan populations, genetic diversity could benefit. We therefore aim to extend the study of Godoy *et al.* (2004) by increasing the Southern and East African (specifically Ethiopian) sample sizes. Knowledge regarding the exact evolutionary placement of Southern and East African populations, and suggestions regarding connectivity among populations, will contribute information towards the management of Bearded Vultures locally as well as globally.

## METHODS

### Samples

Blood, tissue or feather samples for DNA analyses were collected from 40 individuals between 2000 and 2012 (details provided in Supporting Information Table S1).

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Only one feather was collected at each site to avoid possible duplication of samples from a single specimen.

### DNA isolation, polymerase chain reaction (PCR) and sequencing

DNA was extracted using a commercial DNA extraction kit (DNeasy Blood & Tissue kit; Qiagen, Hilden, Germany). As our study follows on from the work of Godoy and co-workers, we followed their laboratory protocols, including PCR parameters, to ensure comparable data. Amplification was performed using the control region primers QHD-2F and Fbox-2R. Eleven individuals (Table S1), including representatives of each haplotype identified by the control region data, were subsequently sequenced for cytochrome b using primers L14841 (Kocher *et al.* 1989) and H15915 (Irwin *et al.* 1991). Sequencing was performed with BigDye Terminator 3.1 mix (Applied Biosystems, Warrington, UK). Purified sequencing products were run on an Applied Biosystems 3130 *xl* Genetic Analyzer. Sequences were edited using GENIOUS R6 (Biomatters, available from [www.geneious.com](http://www.geneious.com)).

### Data analysis

Data were aligned with those published by Godoy *et al.* (2004) using CLUSTALW (Larkin *et al.* 2007) within MEGA 6 (Tamura *et al.* 2013). Analyses were based on 40 samples comprising 216 bp. The Oriental White-backed Vulture *Gyps bengalensis* was included as an outgroup (GenBank accession number EU752279; Johnson *et al.* 2008).

As is appropriate for closely related haplotypes, we constructed a haplotype network in TCS (Clement *et al.* 2000). We had to introduce a 2-bp indel to ensure optimal alignment, and followed Joly *et al.* (2007) in handling missing data. To support the network analyses, we constructed phylogenetic trees in MEGA 6 under a maximum likelihood approach. The evolutionary model that best fitted our data (the Kimura two-parameter model, gamma distributed with invariant sites) was determined on the basis of the Bayesian information criterion (BIC) using MEGA 6. Nodal support was determined through 10 000 bootstrap replicates. To determine whether genetic diversity in the Southern African/Ethiopian samples is geographically structured, an analysis of molecular variance (AMOVA) was performed in ARLEQUIN 3.5 (Excoffier *et al.* 2005). For this, we performed two analyses: one in which we specified all populations as a single group and a second in which we considered the Ethiopian samples separate from the Southern African samples.

To assess genetic variation within the Southern African region, we selected samples from Godoy *et al.*

(2004) as a reference group. We specifically looked for an area with similar geographical coverage and positioning (edge of the range) and high sampling density. The Iberian Peninsula ( $n = 61$  specimens) matched these selection criteria. We then sub-sampled Godoy *et al.*'s (2004) Iberian haplotypes through 100 000 bootstrap iterations to compare with the number of haplotypes recovered from the 37 Southern African samples.

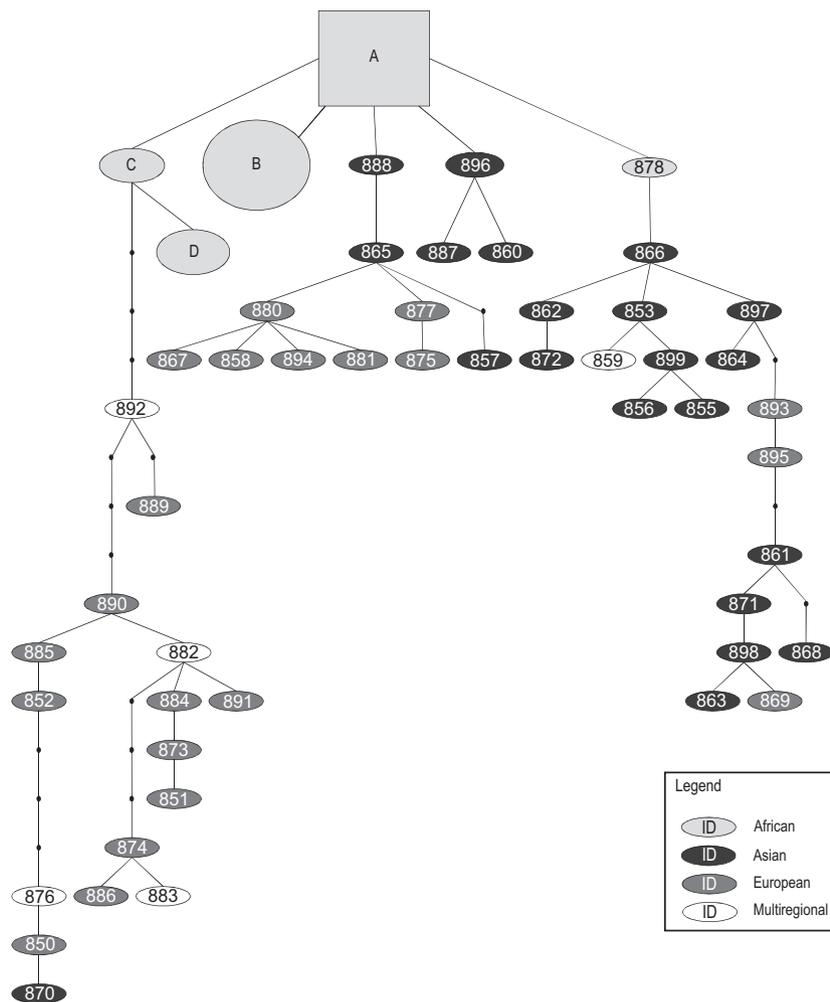
### RESULTS

Three polymorphic sites were identified in the Southern African birds, which defined four haplotypes (A–D: GenBank accession numbers KM203404–KM203407; Table S1) based on the control region sequences. One of these, haplotype A, was identical to a published sequence for birds collected from North Africa, Ethiopia and South Africa (haplotype 30, GenBank accession number AJ566879; Godoy *et al.* 2004). The three Ethiopian Bearded Vultures all had a single haplotype (A). Cytochrome b sequences (813 bp) generated for 11 specimens representative of the four control region haplotypes had an identical haplotype (GenBank accession number KM203403) and therefore no further analyses were conducted on the cytochrome b data.

AMOVA indicated no significant partitioning of genetic variation when the populations from sub-Saharan Africa were considered as a single group, with 89% of the variation accounted for by the within-population component ( $\Phi_{ST} = 0.11$ ,  $P = 0.11$ ). When the Ethiopian samples were grouped separately from the Southern African samples, the largest proportion of variation remained within population (84%), with no significant variation between Ethiopia and Southern Africa ( $\Phi_{CT} = 0.07$ ,  $P = 0.28$ ).

For the bootstrap analysis, sampling 37 individuals from Southern Africa should have recovered an average of 7.86 different haplotypes if diversity in Southern Africa was the same as on the Iberian Peninsula. Our finding of only four control region haplotypes (A–D) suggests a significant deficit in haplotype diversity in Southern Africa ( $P = 0.0025$ ).

The haplotype network is presented in Figure 1. Our four haplotypes were closely related, with three mutational steps separating the two most divergent haplotypes (D and B). The haplotypes retrieved in the current study group closely with haplotypes from birds sampled in North Africa, Southern Africa and Ethiopia (AJ566879, haplotype 30, Godoy *et al.* 2004, our haplotype A) and Ethiopia (AJ566878, haplotype 29, Godoy *et al.* 2004). The haplotype network corresponded well with the maximum likelihood topology shown in Supporting Information Figure S1.



**Figure 1.** Haplotype network demonstrating the relationships between haplotypes generated by Godoy *et al.* (2004) and the present study. Haplotypes generated in the present study are indicated using grey shading. Haplotypes generated by Godoy *et al.* (2004) are shaded by sampling location and all have the prefix 'AJ566'. For details on haplotypes A–D, see Table S1.

## DISCUSSION

The evidence presented here suggests that apart from the known environmental threats to Bearded Vultures in Southern Africa (Krüger *et al.* 2006), genetic diversity is already considerably reduced. Uncertainties also exist regarding the number of subspecies or ESUs (evolutionarily significant units) that should be recognized and the connectivity among regional populations, an important consideration in the management of the species.

Our samples from Ethiopia and Southern Africa group with samples from Africa, Europe, Asia and the Middle East. This clustering resolves the uncertainties of the Godoy *et al.* (2004) study, and strengthens their suggestion that Bearded Vultures should be managed as a single panmictic population due to the absence of

significant differences in life history traits, morphology, habitat, behaviour or genetics across geographical regions. Although it may be difficult to conceive of high levels of connectivity between populations in Africa south of the Sahara Desert and Europe/Asia, these results, based on a mitochondrial marker, are unambiguous. The haplotype network suggests that the African haplotypes, particularly type A, are major links within the network and may well link the two clades identified by Godoy *et al.* (2004). This diversity pattern hints at Africa being the ancestral population, with the possibility that the movement of birds took place across the Horn of Africa (Somali Peninsula) rather than directly across the desert.

The Southern African population is subject to a range of threats that are resulting in a continued decline

(Krüger *et al.* 2006, 2014). The low diversity at the mitochondrial level suggests these threats have had a real impact on the genetics of this critically endangered population. Similar levels of low genetic variability (haplotype diversity) have been found in other endangered raptors such as Bonelli's Eagle *Hieraaetus fasciatus* (Cadahía *et al.* 2007) and Spanish Imperial Eagle *Aquila adalberti* (Martínez-Cruz *et al.* 2004), and more specifically in other vultures: Eurasian Black Vultures *Aegypius monachus* (Poulakakis *et al.* 2008), Egyptian Vulture *Neophron percnopterus* (Kretzmann *et al.* 2003) and other Old World vultures (Lerner & Mindell 2005). These low levels of diversity have been ascribed to recent demographic declines mainly as a result of human pressure or population fragmentation. Other factors that may explain the low diversity in a population include drastic population size fluctuations over time, limited time since it diverged from its closest relative for genetic variation to appear, repeated bottlenecks and a selective sweep (Roques & Negro 2005, Fuchs *et al.* 2014). The most likely explanation for the low diversity in the Southern African population is the drastic range reduction experienced by the population over the past three centuries, more recently attributed to anthropogenic factors resulting in a low effective population size. As such, significant concerns exist regarding the long-term viability and management of this isolated population.

Although translocations are not currently part of the management of this population, the possibility of augmenting the Southern African population has been considered. From previous translocations in Europe, it is clear that this could be an effective management strategy (Hirzel *et al.* 2004, Schaub *et al.* 2009). Given that the Ethiopian population appears to be large (Mundy *et al.* 1992) and shares an otherwise unique haplotype with Southern Africa, individuals from Ethiopia may be used to supplement the Southern African population in areas where the habitat is suitable.

Major changes to the management strategy of a species threatened with extinction should be carefully deliberated based on several lines of evidence. Although the mitochondrial DNA shows that birds from Southern Africa and Ethiopia share haplotypes, it is imperative to add nuclear markers which will further inform questions such as whether connectivity between East and Southern Africa is ongoing (gene flow), whether populations are inbred as well as whether nuclear diversity is similarly low.

The information presented here is useful in guiding management decisions on the future of Bearded Vultures in Southern Africa, and the consideration of exchange of individuals with other countries to ensure that sufficient genetic variability is maintained. Further management options of increasing population size by maximizing the reproductive rate through improving the environment and protecting the population from

environmental changes, disease and persecution must also be considered.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Maximum likelihood topology generated under the evolutionary model that best fitted the data.

Table S1. Summary list of samples used in this study.