

Report from a BOU-funded project

Lack of mtDNA genetic diversity in the Black Harrier *Circus maurus*, a Southern African endemic

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The Black Harrier *Circus maurus* is one of the three rarest endemic avian species in South Africa (Curtis *et al.* 2004, Simmons *et al.* 2005), and certainly the rarest endemic raptor. Global populations of breeding Black Harriers are currently estimated at under 1000 mature individuals, mostly in the Northern and Western Cape (especially the south western corner: Curtis *et al.* 2004) provinces of South Africa, and sighted less frequently in the Eastern Cape and KwaZulu-Natal (Simmons *et al.* 2005). They nest primarily in protected areas and remnant patches of renosterveld in the Overberg, which has been highly fragmented by agriculture (Curtis *et al.* 2004), further reducing the suitable habitat for this species. Recent satellite-tagging studies have revealed that the birds in the Western Cape move freely to the east of the country and may spend the summer in Lesotho and the Free State (<http://www.blackharrierspace.blogspot.com/>). This suggests that the Eastern Cape and Free State population is not independent of the Western Cape core of the species' distribution and that the world population may be smaller than assumed (1000 adults). These factors have led to the species' recent reclassification as Endangered in South Africa's revised red data book (M. Taylor *in litt.* 2012). With a small population size, its increasingly degraded and fragmented habitat, and satellite data suggesting that the core Western Cape

population may be smaller than estimated, it has become imperative to determine the species' genetic structure, to help ensure that divergent lineages are protected. Here, we assess the mitochondrial DNA (mtDNA) genetic diversity for breeding adults sampled throughout the species' range and screened two nuclear introns for a subset of the individuals.

METHODS

Moulted Harrier feathers have been collected over the last decade from a number of nest-sites throughout the species breeding range (Fig. 1): Western Cape (32 nest-sites), Northern Cape (10 nest-sites) and the Eastern Cape (three nest-sites). Each recently moulted feather, mainly from the adult female, when collected was labelled with date, GPS location and a unique nest number. The likelihood that the same bird was repeat-sampled is low because unlike most other raptors, Black Harriers rarely return to the same nest-sites each year. For example, of 35 colour wing-tagged adult breeders ringed and followed over the last 10 years, only one male returned to the same nest in subsequent years and only two females returned to the same nesting area (not the same nest). Thus, the likelihood that unmarked birds were being re-sampled is very low.

Total genomic DNA was extracted from feather quills using a DNeasy Tissue Kit (Qiagen, Valencia, CA, USA) with the addition of 20 µL of dithiothreitol (DTT, 0.1 M). Prior to each extraction, the dedicated laboratory workstation and all equipment were cleaned using a bleach solution and UV light was applied to prevent contamination. The eluted final volume was 100 µL. We gathered sequence data from one of the fastest evolving mitochondrial protein coding genes (ATP6, 684 bp) and a contiguous portion of the CO3 (24 bp – the last bp of ATP6 and first bp of CO3 overlap), one autosomal intron (Vimentin intron-8) and one Z-linked intron (BRM intron 15), using primer pairs L9245–H9947 (Eberhard & Bermingham 2004), BRM15F–BRM15R (Goodwin 1997), and Vim.8 F–Vim.9R (Kimball *et al.* 2009), respectively. The thermocycling conditions were standard and included a hotstart at 94 °C, an initial denaturation at 94 °C for 3 min, followed by 35–40 cycles at 94 °C for 40 s, 52–60 °C for 30 s, and 72 °C for 30–60 s, and was completed with an extension step at 72 °C for 5 min. Purified PCR products were cycle-sequenced using the Big Dye terminator chemistry (ABI, Applied Biosystems, Foster, CA, USA) in both directions with the same primers as used for PCR-amplification.

Standard genetic diversity statistics (number of segregating sites *S*, haplotype diversity *H_d*, and nucleotide diversity *π*) were estimated with DNASP v. 5.0 (Librado & Rozas 2009).

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RESULTS

DNA extraction and sequencing were successful for 59 of the 84 feather samples. The complete mitochondrial ATP6 and partial CO3 sequence was obtained from 50 individuals collected in 13 sites. The sample size varied from one to 23 individuals per sampling locality. In addition, partial sequences (250 bp) of mtDNA were obtained from a further nine individuals. All individuals possessed an identical mtDNA haplotype (GenBank Accession: JX878406).

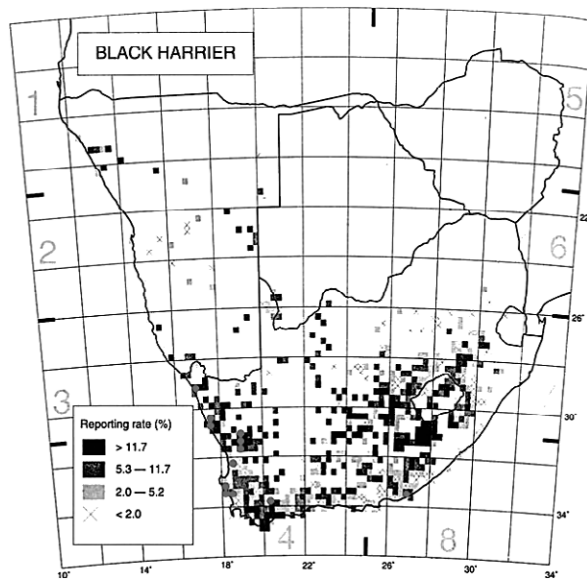


Figure 1. Global distribution of the Black Harrier (*Circus maurus*, squares indicate levels of occurrence) from the Bird Atlas of Southern Africa (Harrison *et al.* 1997) and sample localities for feathers (dots).

Screening of individuals for the autosomal VIM intron-8 (527 bp, 10 individuals from three sites; West Coast National Park, Jakkalsfontein and Bitterriver) and the Z-linked BRM intron-15 (350 bp, 10 individuals from four sites; West Coast National Park, Jakkalsfontein, Bitterriver, Bontebok NP) did not recover a single nucleotide polymorphism.

Hence, based on the mtDNA data and the screening of two introns from a subset of individuals, there appears to be no genetic variation in the Black Harrier across most of its breeding range.

DISCUSSION

Our screening for genetic diversity in both the mitochondrial and nuclear DNA genomes revealed the total absence of genetic variation in the three loci sampled for the Black Harrier, despite having sampled a significant portion of the species range, including from the Overberg (Western Cape) region where populations have probably remained the most stable over time. A comparison of the genetic diversity value obtained for Black Harriers in our study with that of other raptor species of conservation concern indicates that the total absence of variation is very uncommon but not unique (Table 1). Indeed, given the number of individuals sequenced to date, as well as the geographical range we sampled, only the Galapagos Hawk *Buteo galapagoensis*, where no variation was detected in 122 individuals over 1949 bp of the mtDNA Cyt-b, ND2 and the 5' region of CO1 (Bollmer *et al.* 2006), is likely to have as limited genetic variation as the Black Harrier. Several non-exclusive factors can explain this lack of variation, including (1) limited time since it diverged from its closest relative for genetic variation to appear, (2) use of an mtDNA locus with a substitution rate that is too slow for variation to yet be detected, (3) a selective sweep

Table 1. Comparison of the genetic diversity present in Accipitridae taxa that have been studied to date.

Taxon	<i>N</i>	<i>N</i> _{bp}	Gene region	<i>N</i> _h	π	Population estimate	Reference
<i>Aegypius monachus</i>	48	311	Cyt b	7	Not presented	14 000–20 000 mature	Poulakakis <i>et al.</i> (2008)
<i>Aquila adalberti</i>	60	345	CR	3	0.00098	300–400 mature	Martínez-Cruz <i>et al.</i> (2004)
<i>Aquila heliaca</i>	34	345	CR	7	0.00548	2500–9999 mature	Martínez-Cruz <i>et al.</i> (2004)
<i>Buteo galapagoensis</i>	122	2860	Cyt b, ND2, CO1, CR	7	Not presented	270–330 mature	Bollmer <i>et al.</i> (2006)
<i>Circus maurus</i>	50	707	ATP6, CO3	1	0	250–999 mature	This study
<i>Circus pygargus</i>	284	2777	ND2, CO1	51	0.000826	> 10 000	García <i>et al.</i> (2011)
<i>Gypaetus barbatus</i>	172	228	CR	50	0.0292	1300–6700 mature	Godoy <i>et al.</i> (2004)
<i>Haliaeetus albicilla</i>	228	500	CR	13	0.0068	13 000–26 000 mature	Hailer <i>et al.</i> (2007)
<i>Haliaeetus leucogaster</i>	128	553	CR	15	0.000806	670–6700 mature	Shephard <i>et al.</i> (2005)
<i>Harpia harpyja</i>	66	400–417	CR	22	0.00763	20 000–49 999	Lerner <i>et al.</i> (2009)
<i>Hieraetus fasciatus</i>	72	253	CR	4	0.00238	> 10 000	Cadahía <i>et al.</i> (2007)
<i>Milvus milvus</i>	105	357	CR	10	0.0032	13 000–41 000 mature	Roques & Negro (2005)

N, number of individuals sampled; *N*_{bp}, number of DNA base pairs; Gene region, mitochondrial gene analysed; *N*_h, number of haplotypes; π , nucleotide diversity. Population estimates are based on www.birdlife.org.

having acted upon the mtDNA, or (4) drastic population size fluctuations over time.

Based on a Cytochrome-b phylogeny, the closest relative of the Black Harrier is the Cinereous Harrier *C. cinereus* which is restricted to South America, the latter species clusters with the Pallid Harrier *C. macrourus* from the Eurasian steppes and then the Hen *C. cyaneus* Harriers (Simmons & Simmons 2000). The divergence time within the whole group (about 1.9 Myr, Simmons & Simmons 2000) is sufficient for mutational variation to have appeared in the Black Harrier. The mitochondrial locus we sequenced (ATP6 and partial CO3), although relatively short (707 bp), is among the fastest in the mitochondria, often as fast as some Control Region domains (Lerner *et al.* 2011, Pacheco *et al.* 2011). Hence, it is likely that if variation was present, at least some would have been detected with our sequencing effort.

The hypothesis of a strong selective sweep in the mtDNA (Ballard & Rand 2005) is plausible. However, based on the total lack of variation, such a selective sweep must have occurred recently. One way to explore this hypothesis further is to contrast the extent of genetic variation in the mitochondrial and nuclear genomes. If the nuclear diversity is much higher than the mitochondrial diversity, a selective sweep is likely. If both the mitochondrial and the nuclear genomes have low diversity, then it is more likely that the lack of diversity in the population is not due to selection but rather drift and fixation of alleles at small population sizes, as is expected to occur during a bottleneck. In our case, we did not detect a single nucleotide polymorphism in the nuclear data set, a result that suggests that mtDNA and nuclear DNA results are concordant and hence that a selective sweep on the mtDNA is unlikely to have occurred.

Our final hypothesis centres on demographic fluctuations and recovery from a substantial bottleneck. Indeed, because of their reliance on mice, numbers of which may fluctuate with rainfall, harrier populations are expected to fluctuate over time. This may explain the changes reported during the 20th century when the species was variously described as going extinct or increasingly numerous (van der Merwe 1981).

In conclusion, our current data favour the hypothesis of a lack of phylogeographical structure within this range-restricted species, although we can at present not conclusively exclude the hypothesis of a selective sweep having occurred on the mtDNA due to having sampled only two nuclear DNA loci, although from disparate chromosomes (Z and chromosome 2). Future efforts are needed to analyse more individuals from the eastern (Free State/Lesotho) part of the species' range as well as make use of microsatellite loci to assess the extant level of gene flow among different localities.

We are most grateful to the British Ornithologists' Union and Steve Dudley for helping fund this study via a BOU Research

Grant and to the NRF for long-term funding for field work to RES. We also thank Anna Sellas (CAS) and Joshua Penalba (MVZ) for help in the laboratory (MVZ) and to all volunteers who helped collect feathers especially: Anne Williams, Steyn Marais, Cat Simmons and Carets Doyle.

REFERENCES

- Ballard, J.W.O. & Rand, D.M. 2005. The population biology of mitochondrial DNA and its phylogenetic implications. *Annu. Rev. Ecol. Evol. Syst.* **36**: 621–642.
- Bollmer, J.L., Kimball, R.T., Whiteman, N.K., Sarasola, J.H. & Parker, P.G. 2006. Phylogeography of the Galápagos Hawk (*Buteo galapagoensis*): a recent arrival to the Galápagos Islands. *Mol. Phylogenet. Evol.* **39**: 237–247.
- Cadahía, L., Negro, L.L. & Urios, V. 2007. Low mitochondrial DNA diversity in the endangered Bonelli's Eagle (*Hieraetus fasciatus*) from SW Europe (Iberia) and NW Africa. *J. Ornithol.* **148**: 99–104.
- Curtis, O., Simmons, R.E. & Jenkins, A.R. 2004. Black Harrier *Circus maurus* of the Fynbos Biome, South Africa: a threatened specialist or an adaptable survivor? *Bird Cons. Int.* **14**: 233–245.
- Eberhard, J.R. & Bermingham, E. 2004. Phylogeny and biogeography of the *Amazona ochrocephala* (Aves: Psittacidae) complex. *Auk* **121**: 318–332.
- García, J.T., Alda, F., Terraube, J., Mougeot, F., Sternalski, A., Bretagnolle, V. & Arroyo, B. 2011. Demographic history, genetic structure and gene flow in a steppe-associated raptor species. *BMC Evol. Biol.* **11**: 333.
- Godoy, J.A., Negro, J.J., Hiraldo, F. & Donazar, J.A. 2004. Phylogeography, genetic structure and diversity in the endangered bearded vulture (*Gypaetus barbatus*, L.) as revealed by mitochondrial DNA. *Mol. Ecol.* **13**: 371–390.
- Goodwin, G.H. 1997. Isolation of cDNAs encoding chicken homologues of the yeast SNF2 and *Drosophila* Brahma proteins. *Gene* **184**: 27–32.
- Hailer, F., Helander, B., Folkestad, A.O., Ganusevich, S.A., Garstad, S., Hauff, P., Koren, C., Masterov, V.B., Nygård, T., Rudnick, J.A., Shiraki, S., Skarphedinsson, K., Volke, B., Wille, F. & Vila, C. 2007. Phylogeography of the white-tailed eagle, a generalist with large dispersal capacity. *J. Biogeogr.* **34**: 1193–1206.
- Harrison, J.A., Allan, D.G., Underhill, L.G., Herremans, M., Tree, A.J., Parker, V. & Brown, C.J. 1997. *The Atlas of Southern African Birds*, Vol 1. Cape Town: Birdlife South Africa and Avian Demography Unit.
- Kimball, R.T., Braun, E.L., Bowie, R.C.K., Braun, M.J., Chojnowski, J.L., Hackett, S.J., Han, K.-L., Harshman, J., Heimer-Torres, V., Huddleston, C.J., Marks, B.D., Miglia, K.J., Moore, W.S., Reddy, S., Sheldon, F.H., Smith, J.V., Witt, C.C. & Yuri, T. 2009. A well-tested set of resources to amplify nuclear regions across the avian genome. *Mol. Phylogenet. Evol.* **50**: 654–660.
- Lerner, H.R., Johnson, J.A., Lindsay, A.R., Kiff, L.F. & Mindell, D.P. 2009. It's not too late for the harpy eagle (*Harpia harpyja*): high levels of genetic diversity and differentiation can fuel conservation programs. *PLoS ONE* **4**: e7336.
- Lerner, H.R., Meyer, M., James, H.F., Hofreiter, M. & Fleischer, R.C. 2011. Multilocus resolution of phylogeny

- and timescale in the extant adaptive radiation of Hawaiian honeycreepers. *Curr. Biol.* **21**: 1–7.
- Librado, P. & Rozas, J.** 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**: 1451–1452.
- Martínez-Cruz, B., Godoy, J.A. & Negro, J.J.** 2004. Population genetics after fragmentation: the case of the endangered Spanish imperial eagle (*Aquila adalberti*). *Mol. Ecol.* **13**: 2243–2255.
- van der Merwe, F.** 1981. Review of the status and biology of the Black Harrier. *Ostrich* **52**: 193–207.
- Pacheco, M.A., Battistuzzi, F.U., Lentino, M., Aguilar, R., Kumar, S. & Escalante, A.A.** 2011. Evolution of modern birds revealed by mitogenomics: timing the radiation and origin of major orders. *Mol. Biol. Evol.* **28**: 1927–1942.
- Poulakakis, N., Antoniou, A., Mantziou, G., Parmakelis, A., Skartsi, T., Vasilakis, D., Elorriaga, J., de la Puente, J., Gavashelishvili, A., Ghasabyan, M., Katzner, T., McGrady, M., Batbayar, N., Fuller, M. & Natsagdorj, T.** 2008. Population structure, diversity, and phylogeography in the near-threatened Eurasian black vultures *Aegypius monachus* (Falconiformes; Accipitridae) in Europe: insights from microsatellite and mitochondrial DNA variation. *Biol. J. Linn. Soc.* **95**: 859–872.
- Roques, S. & Negro, J.J.** 2005. MtDNA genetic diversity and population history of a dwindling raptorial bird, the red kite (*Milvus milvus*). *Biol. Conserv.* **126**: 41–50.
- Shephard, J.M., Hughes, J.M., Catterall, C.P. & Olsen, P.D.** 2005. Conservation status of the White-Bellied Sea-Eagle *Haliaeetus leucogaster* in Australia determined using mtDNA control region sequence data. *Genetics* **6**: 413–429.
- Simmons, R.E. & Simmons, J.R.** 2000. *Harriers of the World: Their Behaviour and Ecology*. Oxford: Oxford University Press.
- Simmons, R.E., Curtis, O.E. & Jenkins, A.R.** 2005. Black Harrier. In Hockey, P.A.R., Dean, W.R.J. & Ryan, P. (eds) *Roberts' Birds of Southern Africa*: 502–503. Cape Town: John Voelcker Bird Book Fund, Black Eagle Publishing.