

# **Update on Genetic Analyses of Arabian Sea Humpback Whales**

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## **Introduction**

Several lines of evidence have shown that Arabian Sea Humpback Whales (ASHW) form a discrete, small and non-migratory population restricted to the Northern Indian Ocean (e.g. Mikhalev, 1997; Minton et al., 2011; Minton et al., 2008; Pomilla et al., 2014; Reeves, Leatherwood, & Papastavrou, 1991). Analyses of genetic samples collected from humpback whales off the coast of Oman between 1998 and 2004 provided firm evidence that Arabian Sea Humpback Whales (ASHW) are genetically highly differentiated from all other humpback whale populations (Pomilla, Amaral et al. 2014). Low population abundance estimates coupled with a suite of threats raise concern for its survival (Baldwin, Willson, & Collins, 2015; Minton et al., 2008). The population can reasonably be described as being among the most threatened of large whale populations. The population has a current IUCN Redlist status of Endangered.

At IWC/SC/65 (2014) funds were provided to WCS by the IWC in order to complete analyses of archived ASHW tissue samples collected in the years subsequent to 2004. A series of priorities for genetic analyses were specified by the participants of the Dubai Workshop which represented the first regional meeting of the Arabian Sea Whale Network (Minton, Reeves, Collins, & Willson, 2015). The highest priority for immediate analyses was to provide sexing and genotypic identification for the complete dataset of ASHW samples from Oman (n = 92). Here we present some preliminary results on the levels of genetic diversity and genetic matching of individuals from two sampling periods: 1999-2004 and 2005-2015.

## **Material and Methods**

Tissue samples from 49 ASHW were collected between 2005 and 2015 along the coast of Oman. This included biopsies (n=40), sloughed skin (n=4) and tissues from stranded whales (n=5). Given known challenges associated with importing and exporting tissue samples and securing appropriate permits, a decision was made to await completion of fieldwork in November 2015 in order to send the entire Oman genetic archive in a single shipment, which occurred in late March 2016.

Genomic DNA was extracted using the DNAeasy tissue kit (Qiagen). Molecular sexing for all samples was conducted using the PCR primers and conditions described in (Berube & Palsboll, 1996). Ten microsatellite loci were genotyped as described in Pomilla et al. (2004): 199/200, 417/418 and 464/465 (Schlotterer, Amos, & Tautz, 1991), EV1Pm, EV37Mn, EV94Mn and EV96Mn (Valsecchi & Amos, 1996), and GATA028, GATA053 and GATA417 (Palsboll, Berube, Larsen, & Jorgensen, 1997). The program GeneMapper v.4.1 (Applied Biosystems, CA) was used for sizing of allele fragments. The program Micro-checker v.2.2.3 was used to check for the presence of genotyping errors such as scoring errors due to stuttering, large allele dropout or evidence for null alleles. Duplicate samples within this dataset were detected using the identity probability method as implemented in the program Cervus

v. 3.0.7. (Kalinowski et al. 2007); they were then eliminated from subsequent analyses of genetic diversity.

At this stage, we added 43 genotyped samples to the dataset that had been previously analysed in Pomilla et al. (2014; hereafter referred to as the ‘2004 dataset’) in order to compare individuals re-sampled since 2004 between the newly generated dataset (hereafter referred to as the ‘2015 dataset’).

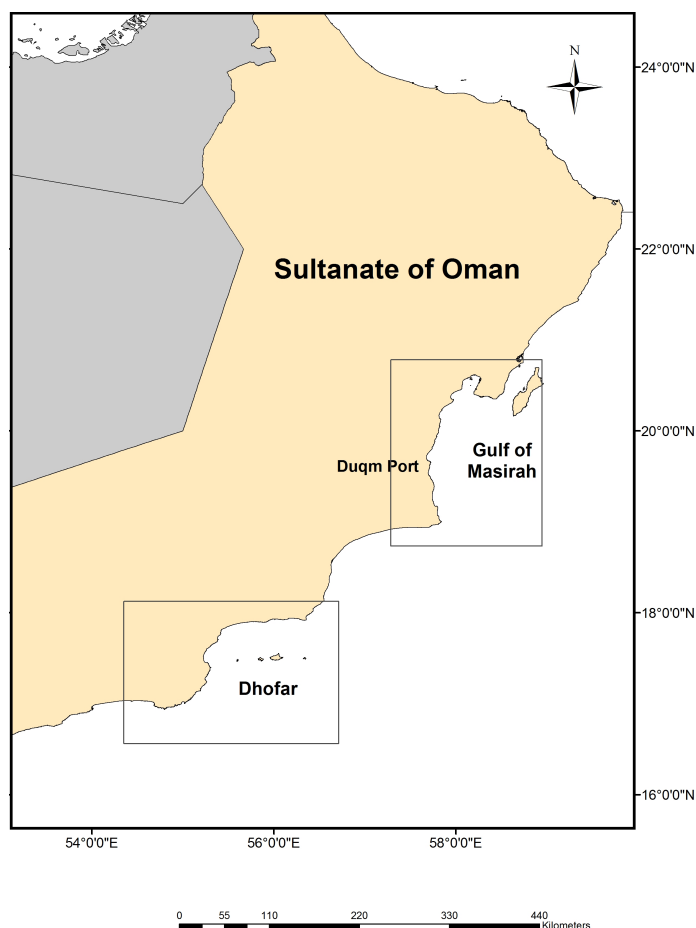
Genetic diversity measures such as the mean number of alleles per locus and observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosities were calculated in Arlequin v.3.5.1 (Excoffier & Lischer, 2010) and allelic richness ( $A_R$ ) and the inbreeding coefficient ( $F_{IS}$ ) calculated using FSTAT v.2.9.3 (Goudet, 1995).

## Results and Discussion

From the 49 new samples, 44 were successfully genotyped at 10 microsatellite loci. We identified 27 males and 17 females. Within this dataset, 7 duplicate samples were found. Five samples representing 3 individuals in the 2015 dataset registered as genotypic matches with animals sampled in the 2004 dataset. An additional 9 samples involving 6 individuals that represent potential matches between the 2015 and 2004 dataset are being reviewed to determine if they represent inter-year recaptures. As the data were generated very recently, further checks to clarify issues with some microsatellite loci will be completed. Six samples failed to amplify consistently for sexing markers and need to be revisited.

Analysis of the combined genotypic dataset will be completed by the end of June/early July 2016. The genotypic matches and sexing data will be incorporated into the Oman Photo-ID database, and used to generate robust updated photo-ID and genotypic mark-recapture estimates of population abundance. These data have also been used to determine or confirm the sex of the 9 satellite tagged whales off the coast of Oman (see SH28), and a significant number of other whales identified photographically off the coast of Oman. This will prove highly valuable if future satellite tagging efforts are undertaken in Oman, as previous tagging work has been significantly male-biased (See SH28), and future tagging should target known females.

Preliminary assessment of the new sexing data (although not yet fully complete) is providing valuable insight into possible difference in habitat use between the two primary study sites off the coast of Oman (see Figure 1). Minton et al. (2011) documented a heavy bias toward males in Dhofar and a ratio near parity in the Gulf of Masirah. These results were based on the total number of photographic captures of known sex individuals. The combination of new and old sexing information allows for a more nuanced examination of this phenomenon: the male-female ratio based on individual whales for which sex was determined in each study site (regardless of how frequently they were observed) is 2:1 in Dhofar ( $n=30$ ), whilst in the Gulf of Masirah this ratio is slightly biased toward females, with 45% of individuals of known sex observed in that study area being male, and 55% female ( $n=49$ ). More detailed analysis of these sexing data in relation to survey effort and photographic identification data will yield further important insights into habitat use, behaviour, and social interactions, critical for the conservation of this population.



**Fig. 1.** Study sites off the coast of Oman

**Table 1.** Genetic diversity statistics estimated for ASWH datasets.

Statistics	'2004 dataset' + '2015 dataset'	'2015 dataset'	'2004 dataset'
$H_E$	0.6866 (0.1826)	0.70003 (0.1785)	0.6737 (0.1935)
$H_O$	0.6716 (0.3556)	0.6973 (0.3699)	0.6408 (0.3472)
K	7.1 (3.143)	6.5 (2.877)	7.1 (2.998)
$A_R$	7.021	6.433	6.586
$F_{IS}$	-0.022	-0.033	-0.014

$H_E$  – expected heterozygosity;  $H_O$  – observed heterozygosity; K – mean number of alleles;  $A_R$  – allelic richness;  $F_{IS}$  – inbreeding coefficient.

### Considering Future Priorities for Genetic Work

Continued analyses of genetic material available from research projects in the Arabian Sea is crucial in order to further investigate population taxonomy, population structure and demography, habitat utilization, abundance and trends, and potentially whale health. Analyses of samples collected from strandings have proven problematic

and require more laboratory work. This is essential for determining if any of these dead animals were sampled as live animals during previous fieldwork.

A few samples are known to have been collected from strandings in other parts of the ASWH range (e.g. Pakistan,  $n=1$ ; 2006; India = unknown). The sample from Pakistan has been processed and mtDNA haplotype characteristics were compared with a dataset comprising all known haplotypes from the [Northern Indian Ocean], Eastern South Atlantic and Western Indian Ocean. The mtDNA sequence was a match to HBA019 – a haplotype found in all 9 of 12 sampling sites from these regions. The sampling site with the highest frequency of HBA019 is Oman at 30% (WCS unpublished data). More samples are needed before we can draw any conclusions about genetic relationships within the Arabian Sea, but this sample is worth noting.

Understanding the population status, identity and connectivity of humpback whales in the Arabian sea would be greatly enhanced if samples from the wider region are can be obtained in sufficient numbers (e.g., dedicated effort to collect stranding samples and/or live biopsy sampling or sloughed skin). Discussions with researchers in relevant range states are ongoing and it is hoped that permits will be issued for export of samples to allow them to be included in a regional study programme.

During the January 2015 Dubai workshop, participants reviewed an evaluation of priorities for further genetic work (see appendix 6.4 of Minton et al. 2015). These included: 1) clarification of the taxonomic status of the ASHW population; 2) confirmation of the stock of origin (i.e. founder population) and an improved estimate of the duration of isolation using additional, more informative nuclear markers; 3) better understanding of genetic variability (by inferring a family tree of pedigrees) and assessment of potential inbreeding depression; 4) assessment of population health status (by assessing genetic variability of immune genes, e.g. the major histocompatibility complex); 5) estimation of individual ages (using DNA methylation techniques to include in mark-recapture analyses); 6) mapping habitat use by sex and kin-group to inform spatial conservation measures; 7) incorporation of satellite tracking and small vessel survey data into spatial analyses of habitat use; and 8) consideration of the genetic potential for adaptation to climate change.

Based on the analyses we have conducted to date, and taking into consideration discussions subsequent to the Dubai workshop, we consider the following priorities for the ASHW over the next two years:

### ***1. Taxonomic status***

Aim: to determine if the ASHW population warrants new subspecies status. Our existing dataset we already supports the classification as subspecies, as levels of genetic differentiation (based on  $F_{ST}$ ) for the mtDNA control region and nuclear microsatellites between the ASHW population and other breeding stocks in the Southern Hemisphere fall within the range of differentiation reported for ocean-basin comparisons of the mtDNA control region and nuclear introns between the North Atlantic, North Pacific and Southern Ocean (Jackson et al. 2014). These levels of differentiation were deemed adequate to support sub-species designation of humpback whales in each of the three ocean basins (Jackson et al. 2014). Notwithstanding the mitochondrial and microsatellite support, additional nuclear markers would be desirable to confirm these results (e.g. population genomics using GBS). We also plan

to examine and extract DNA from a bone sample taken from the nominal species *M. indica* Gervais, 1883.

## **2. Relatedness, family trees and social structure, including inbreeding assessment**

Aim: study the genetic variability of the population in detail by inferring a family tree or pedigree and assess the degree of potential inbreeding, including considerations related to inbreeding depression, i.e. breeding of related individuals. In order to do this we would need a considerable number of highly variable genetic markers in order to obtain the genetic profile of each individual in the population (i.e. 20 or more microsatellites).

## **3. Health status/resiliency, genetics-based**

Aim: assess the health status of the population by assessing genetic variability of the MHC (major histocompatibility complex). For this some genes of this gene family would have to be sequenced.

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