RZSS
THE WILDLIFE CONSERVATION CHARITY

Edinburgh Napier
UNIVERSITY

Genetic Assessment and Development of Genomic Resources for Nubian ibex Conservation Management in Oman

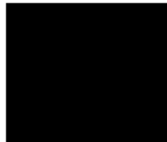
Mataab Khalaf Al-Ghafri

A thesis submitted in partial fulfilment of the requirements of Edinburgh Napier University, for the award of Doctor of Philosophy

March 2023

Declaration

I hereby declare that all contents of this thesis are my own independent work and all results presented here were undertaken and written by me. The parts that are the result of collaboration are clearly indicated, and my contribution to those parts is explicitly stated. I also declare that no part of this work has been submitted for any other degree or professional qualification.

Mataab Khalaf Al-Ghafri  date 08/03/2023

Abstract

The Nubian ibex (*Capra nubiana*) is listed as Vulnerable according to IUCN Red List. It is patchily distributed across parts of Africa and Arabia, including Oman where it is present in isolated populations in central and southern region of the country. It is further threatened by habitat degradation, human expansion, poaching, and population fragmentation.

In order to study the impact of these factors and inform future management plans, I investigated the genetic diversity, gene flow and divergence between wild Nubian ibex from Oman, in captivity and from museum samples, using mitochondrial DNA and nuclear DNA using ddRAD. In addition, a hybrid capture technique was performed to analyse nuclear DNA of low-quality samples (faecal and bones) and further investigate the population genetics of ibex in Oman, Yemen and Sudan. Lastly, population viability analysis (PVA) was carried out to assess the effects of different environmental and anthropogenic factors on the isolated ibex population in the central region.

Mitochondrial results showed that the population in the southern region of Oman was more diverse compared to the central region with restricted gene flow. There was a significant divergence between wild and captive populations (F_{ST}=0.725). Moreover, a significant differentiation was found between wild ibex from Oman and ibex samples from Sudan (F_{ST}=0.971), while results showed no differences between Omani and Yemeni ibex. These results were supported by 5,775 high quality SNP loci across Nubian ibex genome which showed significant genetic differentiation between wild and captive individuals ($F_{ST\ (5,775)}$ = 0.540).

Furthermore, the baits designed from the ddRAD reads generated in this study were successful in targeting DNA of low-quality samples of Nubia ibex populations in Oman, Yemen and Sudan. It generated 1,054 SNPs, which helped in assessment of genetic variation among these different populations.

My results showed a significant divergence between Oman and Sudan populations ($F_{ST\ (1,054)} = 0.513$). As well as a significant divergence between the Omani population and the captive individuals ($F_{ST\ (1,054)} = 0.433$), while no significant differences were found between Omani and Yemeni populations.

The PVA results indicate that wild ibex in central Oman under the current circumstances are subject to an increased risk of extinction over the next 100 years. High mortality was found to have the strongest influence on the dynamics of the population. Moreover, the analysis showed that the proportion of females played a vital role in population viability, thus they need to be in the central focus of any conservation program.

Synthesising genetic and PVA results, I recommended establishment of a captive breeding population formed from more genetically diverse animals selected from central and southern Omani populations, whilst protecting current populations. This will guarantee sufficient genetic diversity to retain evolutionary resilience to cope with future environmental changes.

My results indicate that captive Nubian ibex did not originate from wild populations in Oman. I advise that captive ibex should not be used for any future re-introduction or reinforcement programs. Nubian ibex in Oman should be treated as a distinct taxonomic unit, potentially at the species level.

Acknowledgements

First of all I would like to thank my supervisors Dr Helen Senn at the Royal Zoological Society of Scotland, Dr Patrick White and Prof. Rob Briers at Edinburgh Napier University for their teaching, advice and invaluable input and support during the numerous challenges I faced throughout my project. They played a vital role towards which without this work would not be complete.

I would like to thank WildGenes team in the Royal Zoological Society of Scotland for hosting me throughout my study period and provided me with all kind of support and help. Thanks to the lab team Elizabeth Heap, Muhammad Ghazali and Jennifer Kaden for assistance. They gave up their time to help and guide me through the lab work. Dr Alex Ball who helped me in data analysis and provide valuable comments that improve my writing and provided encouragement and friendship along the way. Dr Kara Dicks for discussions that expanded the horizons and honed my scientific thinking. She gives up her spare time without a second thought and provided me with guidance and valuable comments.

I would like to express my sincere thanks to the Diwan of the Royal Court, Oman, for the grant and support to this project. Particular thanks go to Mr Yasser Al-Salami, the director general of Office for Conservation of Environment (OCE) in Oman, for his great efforts in getting the required fund to this project and for his continuous support from the beginning to make the project successful. Thank you to Dr Mansoor Al-Jahdhami who made it possible for me to do this project and helped in securing the fund and support. His comments and suggestion were valuable. A special thanks to Dr Qais Al-Rawahi WWR field manager and Mr Salim Al-Rubaii field manager of OCE office in Dhofar for providing help and logistical support for samples collection. My thanks also go to all reserves rangers in both Al-Wusta Wildlife Reserve (WWR) and Dhofar for providing friendship, laughter and fun along the way with special thank you to

Said Nasser Al-Harsusi, Abdullah Ghasab Al-Harsusi, Salim Mubarak Al-Harsusi and Khalifa Mohammed Al-Harsusi for sharing their knowledge and experience about the central desert of Oman and for all the nights we spend in the wild. Special and heartfelt thank for my friend and colleague at OCE, Haitham Al-Amri who provided continuous support throughout the study. His passion and dedication for wildlife and conservation inspire me to do my best to contribute to the conservation of wildlife in Oman through this work. I want to thank Steven Ross, Taimur Al-Said Said Al- Mahroqi, Nasser Mabrook Al-Mahrooqi, Mudhafar Al-Umairi, Hani Al-Saadi , Ali Aka'ak, Ahmed Hardan, Nasser Zabanoot, Ibrahim Hassan and all the people who were kind, helpful and I was lucky enough to work with them at WWR and Dhofar.

I am extremely grateful to Balazs Buzas, Al Mayya Sanctuary and Mark Craig, Al Ain Zoo, from United Arab Emirates (UAE) and Dr Khalid Al-Rasbi from Oman for providing samples of captive Nubian ibex. I deeply thank Paul Vercammen, Operations Manager at Breeding Centre for Endangered Arabian Wildlife, Sharjah Desert Park, UAE, for his help and provision of samples of Nubian ibex from Yemen and for his valuable comments regarding the source of captive breed animal in Sharjah and Oman. In addition, we would like to extend special thanks to Ms Hanan Al-Nabhani for her help in collecting samples from the Natural History Museum of Oman. I also would like to thank the directorate general of meteorology for providing meteorological data for WWR and Dhofar.

I must also thank my friend David Hammond, who has provided unending support and advice throughout my study.

Thank you to my neighbour Anne Anstruther who showed untold kindness and friendship throughout my stay in Edinburgh. I enjoyed her home made cakes and jams.

Finally, I would like to thank my beloved family for their patience and support throughout my study. My mum who has always been proud of me and supported me at every step throughout my entire live and during this research

journey. To my sisters and brothers for the continuous support and assurances and their enduring confidence that I would successfully reach the end. The biggest thank you goes to my wife Yasara and my children; Abdullah, Leen, Haya, Yumna and Sura. They never complained for long stay in front of my PC, in contrary they provided me with a quite environment to work and complete this work. You are loving, caring and encouraging, and I am proud and very lucky of being together and share my life with you.

.

Table of Contents

DECLARATION II

ABSTRACT III

ACKNOWLEDGEMENTS V

LIST OF FIGURES X

LIST OF TABLES XV

CHAPTER 1 INTRODUCTION 1
- *1.1 Importance of biodiversity 1*
- *1.2 Population Genetic diversity 2*
- *1.3 Population size 3*
- *1.4 Genetic drift 4*
- *1.5 Inbreeding 4*
- *1.6 Effect of isolation 4*
- *1.7 Measuring population divergence 5*
- *1.8 Extinction risks 5*
- *1.9 Factors causing a decrease in the genetic diversity 6*
- *1.10 Antelope status in Oman 9*
- *1.11 Capra genus Taxonomy and distribution 11*
- *1.12 Nubian ibex (Capra nubiana) 12*
- *1.13 Study sites 19*
- *1.14 Challenges facing wildlife in Oman 25*
- *1.15 Conservation of Nubian ibex 30*
- *1.16 Potential for a captive program of Nubian ibex in Oman 30*
- *1.17 Thesis aims and outline 32*

CHAPTER 2 GENETIC DIVERSITY OF THE NUBIAN IBEX IN OMAN AS REVEALED BY MITOCHONDRIAL DNA ..35
- *2.1 Abstract 36*
- *2.2 Introduction 37*
- *2.3 Materials and Methods 42*
- *2.4 DNA extraction 46*
- *2.5 Mitochondrial DNA sequencing 46*
- *2.6 Sequence Analysis 48*
- *2.7 Phylogenetic analysis 49*
- *2.8 Genetic diversity statistics 49*
- *2.9 Results 50*
- *2.10 Discussion 61*
- *2.11 Conclusion 67*

CHAPTER 3 GENERATING ADDITIONAL GLOBAL MITOCHONDRIAL REFERENCE DATA FOR THE NUBIAN IBEX (*CAPRA NUBIANA*) FROM SUDAN AND YEMEN. 69
- *3.1 Abstract 69*
- *3.2 Introduction 70*
- *3.3 Materials and Methods 71*
- *3.4 Results 74*
- *3.5 Discussion 79*

CHAPTER 4 IMPLICATIONS OF NEWLY DEVELOPED SNPS FOR CONSERVATION PROGRAMMES FOR THE THREATENED NUBIAN IBEX (*CAPRA NUBIANA*) IN OMAN 81
4.1 *Abstract*....................82
4.2 *Introduction*....................83
4.3 *Materials and Methods*....................87
4.4 *Results*....................91
4.5 *Discussion*....................97
4.6 *Conclusion*....................102
CHAPTER 5 HYBRIDIZATION CAPTURE OF NUBIAN IBEX GENOME FROM NON-INVASIVE AND HISTORICAL SPECIMENS DNA....................105
5.1 *Abstract*....................105
5.2 *Introduction*....................106
5.3 *Materials and Method*....................111
5.4 *Results*....................120
5.5 *Discussion*....................134
5.6 *Conclusions*....................144
CHAPTER 6 POPULATION VIABILITY ANALYSIS FOR NUBIAN IBEX AT AL-WUSTA WILDLIFE RESERVE IN OMAN....................147
6.1 *Abstract*....................147
6.2 *Introduction*....................148
6.3 *Methods*....................156
6.4 *Estimating the effect of Drought and Hunting*....................160
6.5 *Establishment of a captive population*....................163
6.6 *Results*....................165
6.7 *Discussion*....................182
6.8 *Conclusion*....................192
CHAPTER 7 GENERAL DISCUSSION....................193
7.1 *Approaches used to investigate the genetic diversity of Nubian ibex in Oman*....................195
7.2 *Nubian ibex at WWR: PVA and genetics*....................198
7.3 *Conservation implications*....................199
7.4 *Establishment of bio-banking*....................201
7.5 *Limitations and obstacles*....................202
7.6 *Conclusions and recommendations*....................203
APPENDIXES....................205
REFERENCES....................244

List of Figures

Figure 1.1. *Map of Oman showing the location of central, WWR and southern region. The location of the two extinct Nubian ibex populations in Janaba hills and Ras Madrakah are indicated by black dots.* 16

Figure 1.2. *(A) The Haquf escarpment at WWR. (B) High salinity water pools at WWR. (C) The visual impact of vehicles on vegetation cover. Photos A and B by Mataab Al-Ghafri, photo C obtained from Google Earth Pro.* 17

Figure 1.3. *Annual average precipitation comparison between the centralregion (black bars) and southern region (gray bars) from 2010 to 2020. In 2018 a cyclone hit the cost of Dhofar hence the extreme value in that year. Data retrieved from Directorate General of Meteorology (www.met.gov.om).* 22

Figure 1.4. *Mean monthly temperature (TM; in °C) comparisons between central region (black solid line) and southern region (red dotted line). Error bars represent the standard deviation. The data are averaged across the years 2010 to 2020. Data retrieved from Directorate General of Meteorology (www.met.gov.om).* 23

Figure 1.5. *Schematic representation of hybridization between (A) Ardi goatand (B) Nubian ibex. The offspring differs in coat color and shape. It has the ear shape and long curved horns of the ibex (C). The rings in the horns are small and not clear compared to the wild ibex. Photos A and C courtesy to Obaid Al-Awni (Saudi Arabai), B to Steve Ross and Taimur Al-Said (office for conservation of nature, Oman).* 28

Figure 1.6. *Schematic representation of hybridization between (A) Tohamigoat and (B) Nubian ibex. The offspring has almost all Nubian ibex phenotypic traits (C). The rings in the horns of the produced animals are smaller and compacted and the leg coloration does not match exactly the wild ibex. Photos (A) courtesy to Obaid Al-Awni (Saudi Arabai), (C) from (https://twitter.com/khal4000/status/1556536873820033024/photo/1) and (B) to Steve Ross and Taimur Al-Said (office for conservation of nature, Oman).* 29

Figure 2.1. *A) Nubian ibex male captured by camera trap at Al-Wusta Wildlife Reserve (WWR) (Oman) (by Steven Ross & Taimur Al-Said). B) distribution range of C. nubiana in the Middle East and Africa (Ross et al. 2020a). C) Map of Oman showing the sampling locations represented by black circles. The samples were collected from three different places: Al Wusta Wildlife Reserve (WWR) and surrounds, Shalim and Dhofar.* 39

Figure 2.2. *The distribution of the mitochondrial DNA haplotypes within each location. The lower circle for each region represents cytochrome b, while the upper circle is D-loop. The size of the circle does not represent sample size.* 51

Figure 2.3. *Median-joining haplotype network for cytochrome b (487bp). The wild animals from Oman are in the right-hand blue box, while the captive animals are in the left-hand orange box. Each coloured circle represents a single haplotype, the size of which is proportional to the number of samples. The tick marks represent the mutational steps between haplotypes. Three reference sequences of C. nubiana from GenBank were used (Accession numbers given on the figure)*.. 52

Figure 2.4. *Median-joining haplotype network for D-loop (186bp).The wild animals from Oman are in the right-hand blue box while the captive animals are in the left-hand orange box. Each coloured circle represents a single haplotype, the size of which is proportional to the number of samples. The tick marks represent the mutational steps between haplotypes.* 53

Figure 2.5. *Haplotype accumulation curves for cytochrome b (left) and D-loop (right) within the wild Nubian ibex samples. For cytochrome b it is estimated that more than 98.5% of the haplotypes are represented, while for the D-loop this is 93.7%.The dashed lines show the number of haplotypes found corresponding with sampled individuals. The dotted lines represent the expected numbers of haplotypes which should be found in the given population*.. 54

Figure 2.6. *Bayesian phylogenetic tree of concatenated mtDNA sequences of cytochrome b (487bp) and D-loop (187bp) calculated by MrBayes. The blue box indicates Oman wild Nubian ibex (WildHAP1 to WildHAP13). The yellow box indicates the captive Nubian ibex (CaptiveHAP14 to CaptiveHAP18). GenBank sequences are indicated by their accession numbers adjacent to the species binomial. The numbers at the nodes represent the posterior probabilities.* 57

Figure 3.1. *Haplotype network for cytochrome b (487 bp). The wild samples from Oman and Yemen are in the right-hand blue box, while the captive samples (from the UAE and Oman) and museum samples from Sudan are in the left-hand orange box. Each coloured circle represents a single haplotype. The tick marks represent the mutational steps between haplotypes. Two reference sequences of C. nubiana from GenBank were used as representative samples from Israel (shown in green).* 75

Figure 3.2. *Bayesian phylogenetic tree for cytochrome b (487 bp) produced using MrBayes. The wild samples from Oman and Yemen are in the blue box, while the captive samples (from UAE and Oman) and museum samples from Sudan are in the orange box. GenBank sequences are indicated by their accession numbers. The numbers shown at the nodes represent the posterior probabilities.* 77

Figure 4.1. *Schematic illustration of SNP filtering process. Each right-handbox illustrates a step of the bioinformatics filtering process and the number of SNPs remaining after each step is given on the left. The last box indicates*

the final filtered SNPs (5775 SNPs) which were used for subsequent analysis. 92

Figure 4.2. *Genetic structures of Nubian ibex samples for wild Nubian ibex, captive Nubian ibex and goats inferred using STRUCTURE. Each column represents one sample divided into K colours, where K is the predefined number of clusters assumed, and the length of the coloured segment represents the individual's estimated proportion of membership to a particular cluster. Each panel represents a different value of K (shown on right). The y-axis represents the likelihood membership to each cluster. Arrows indicate which individuals were identified as potential hybrids.* 93

Figure 4.3. *Principal component analysis (PCA) plot of the genetic relationship matrix for 5,775 SNPs of the wild Nubian ibex (purple cross), captive Nubian ibex (red dot), goats (green triangle) and the two captive ibex hybrids as detected by STRUCTURE (blue square). The first principal component accounts for 86.8% of the underlying variation, while the second one describes 9.2% of the variation.* 94

Figure 5.1. *PCA plot for different criteria of missing genotypes: (A) Missing genotype = 0.25, (B) 0.30, (C) 0.40, (D) 0.50, (E) 0.60 and (F) 0.70. Each sample group is represented by a different colour. Note the colour key changes across plots.* 122

Figure 5.2. *Comparison between different filtering criteria of SNPs that are inLD. The stringent criteria removes SNPs that have R^2 > 0.5 and the relaxed criteria R^2 > 0.8: (A) Missing genotype = 0.25, (B) 0.30, (C) 0.40.* 123

Figure 5.3. *Genetic structures inferred by STRUCTURE of Nubian ibex samples for Goat, Captive, Oman (WWR, Shalim and Dhofar), Yemen and Sudan, based on 982 SNPs. Dashed white lines showed the boundaries of each sampling group. Hybrid and wild-goat individuals were indicated by dashed lines. The y-axis represents the likelihood of membership to each cluster. Each single column represents one sample divided into K colours, where K is the number of clusters assumed and the length of the coloured segment represents the individual's estimated proportion of membership to a particular cluster.* 126

Figure 5.4. *Principal component analysis (PCA) plot of the genetic relationship based on 982 SNPs of Oman Nubian ibex(WWR, Shalim and Dhofar), captive Nubian ibex , goats, Sudan and Yemen. The analysis showed two hybrids from captive individuals (green dots) and two samples from Oman (WWR-goat) clustering with goats (grey dots).* 129

Figure 5.5. *Principal component analysis (PCA) plot of the genetic relationship based on 589 SNPs of Oman Nubian ibex samples from WWR (blue dots), Shalim (green dots) and Dhofar (red dots) and Yemen (purple dot).* 130

Figure 5.6. *Genetic structures inferred by STRUCTURE of Nubian ibex samples for WWR, Shalim, Dhofar (all in Oman) and Yemen. Dashed white lines showed the boundaries of each cluster. This is based on 589 SNPs after excluding fixed SNPs and SNPs located on sex chromosomes. The y-axis represents the likelihood of membership to each cluster. Each single column represents one sample divided into K colours, where K is the number of clusters assumed and the length of the coloured segment represents the individual's estimated proportion of membership to a particular cluster.* 131

Figure 6.1. *Map showing the location of the Huqf escarpment (red dotted line) and WWR (black solid line). About 60 km of the escarpment is under the protection of the reserve rangers. The irregular section of the black line represents the weak points where the fence is either damaged or incomplete because the presence of a natural features such as rocky hills.* 152

Figure 6.2. *A group of seven Nubian ibex hunted from Shalim in 2018 consisting of one adult male, two sub-adult males, two adult females and two juveniles. (Courtesy of Office for conservation of Environment, Oman).* 163

Figure 6.3. *Trap used to capture wild Nubian ibex at WWR. Some hay is placed inside the trap and when animal hits the trigger the door closes. A transmission device sends a signal to alert the trapper(s) when the door is closed.* 164

Figure 6.4. *Probability of extinction (PE) and genetic diversity (GD) of different population sizes (from 5 individuals (Pop_5) to 250 individuals (Pop_250)). The dashed lines indicate initial populations of 70 and 100 individuals that had probability of persistence of 95.1% and 98.7% respectively. The 70 is the initial population that meets the PE<5% criteria and the 100 is that which also then meets the GE>=90% criteria.* 167

Figure 6.5. *Sensitivity analysis results of an initial population of 70 Nubian ibex individuals under different scenarios. Bars indicate the standard errors for population growth rate, probability of extinction, population size, expected heterozygosity. Base, baseline scenario (no-inbreeding); ID, inbreeding depression; HM, high mortality; LM, low mortality; HB-F, high breeding females; LB-F, Low breeding females; HB-M, high breeding males; LB-M, Low breeding males; SR45, females-biased sex ratio; SR55, males-biased sex ratio.* 170

Figure 6.6. *Sensitivity analysis results of an initial population of 100 Nubian ibex individuals under different scenarios. Bars indicate the standard errors for population growth rate, probability of extinction, population size, expected heterozygosity. Base, baseline scenario (no-inbreeding); ID, inbreeding depression; HM, high mortality; LM, low mortality; HB-F, high breeding females; LB-F, Low breeding females; HB-M high breeding males; LB-M*

Low breeding males; SR45 females-biased sex ratio; SR55 males-biased sex ratio.......... 173

Figure 6.7. *Drought scenarios at different frequencies (Freq10 = 1 in 10 years, Freq20 = 1 in 5 years, Freq33 = 1 in 3 years) and reproduction rates during drought years (relative to non-drought years) for a population of 100 individuals. The graphs show: (A) population growth rate; (B) probability of extinction; (C) population size; (D) expected heterozygosity.*.......... 175

Figure 6.8. *Drought scenarios at different frequencies (Freq10 = 1 in 10 years, Freq20 = 1 in 5 years, Freq33 = 1 in 3 years) and reproduction rates during drought years (relative to non-drought years) for a population of 250 individuals. The graphs showed (A) population growth rate; (B) probability of extinction; (C) population size; (D) expected heterozygosity.*.......... 176

Figure 6.9. *Sensitivity analysis results for population sizes of 100 and 250 individuals (Pop100 and Pop250 respectively) under different hunting intensities (15%, 20% and 25%) of adult females and males on population growth rate, probability of extinction, population size and expected heterozygosity.*.......... 179

Figure 6.10. *The effect on population viability of using different number of males and females to supplement a captive population of Nubian ibex that started with 10 individuals. In simulations, animals are added every 5 years, five times. The graphs show: (A) population growth rate; (B) probability of extinction; (C) population size; (D) expected heterozygosity.*.......... 181

Figure 6.11. *Water provision for the wildlife at WWR. (A) Arabian gazelle drinking from artificial water depression. (B) Fenced water place to prevent camels from using the water. A camera trap is fixed in one corner to monitor use of the site by wildlife.*.......... 187

List of Tables

Table 1.1. *Wild Antelope species in Oman and their population status.* 10

Table 1.2. *Population estimates of Nubian ibex by country.* 13

Table 2.1. *Published genetic studies investigating taxonomy of the Capra, highlighting their findings regarding the relationship between Nubian and Alpine ibex.* 41

Table 2.2. *Locations and types of samples collected for analysis.* 45

Table 2.3. *Primer sequences used to amplify targeted markers. All primers were designed for the purposes of this study.* 47

Table 2.4. *Molecular diversity measures for both cytochrome b and D-loop between wild and captive animals.* 57

Table 2.5. *Comparison of genetic diversity indices between Nubian ibex samples from the central and southern region* 58

Table 2.6. Comparison of genetic diversity indices for Nubian ibex from WWR, Shalim and Dhofar 59

Table 2.7. *Analysis of molecular variance for cytochrome b and D-loop among Nubian ibex in the wild (Oman) and in captivity (Oman and UAE).* 60

Table 3.1. *Genetic differentiation (F_{ST}) among populations based on cytochrome b of (487bp)* 78

Table 4.1. *Pairwise F_{ST} estimates for wild ibex, captive ibex and goats below diagonal. Above diagonal are the 95% confidence intervals. All pairwise comparisons were significant at $p \leq 0.001$. Hybrid individuals were excluded from this analysis.* 95

Table 4.2. *Genetic diversity estimates for the three populations based on 5,775 SNPs, showing observed heterozygosity (H_O), expected heterozygosity (H_E), fixation index (F_{IS}) and 95% confidence intervals (CI) for each measure.* 96

Table 5.1. *Samples used in ddRAD and hybrid capture from each population* 112

Table 5.2. *Effect of using different missing genotypes filtering criteria to filter Hybrid capture data and ddRAD SNPs by PLINK. Fixed criteria used are – individual genotyping rate of 10%, --MAF 0.05 and finally individual genotyping rate of 80% are the same in all filtering criteria.* 121

Table 5.3. *Number of SNPs that showed linkage disequilibrium distance more than 0.5 and 0.8.* 124

Table 5.4. *Number of variable variants found in each population based on 1,054 SNPs. In each population the MAF must be >0.* 125

Table 5.5. *Percentage of shared membership between Oman, captive, Sudan and goat population. These results based on 982 SNPs and obtained from STRUCTURE analysis at K=4. The dash sign means there is no shared ancestry*.. 127

Table 5.6. *Pairwise F_{ST} estimates for Oman ibex, captive ibex and goats, shown below the diagonal. Above the diagonal are the 95% confidence intervals. All pairwise comparisons were significant at $p \leq 0.001$. These results are based on 1,054 SNPs. Hybrid individuals and wild-goat were excluded from this analysis*.. 132

Table 5.7. *Pairwise F_{ST} estimates for Oman ibex samples in WWR (15 samples), Shalim (4 samples), Dhofar (10 samples) and Yemen (one sample).All pairwise comparisons were significant at $p \leq 0.001$. These results based on 589 SNPs after excluding fixed and sex SNPs. Wild-goat individuals were excluded from this analysis.*.. 133

Table 5.8. *Genetic diversity estimates for the three populations based on 589SNPs, showing observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F_{IS}) and 95% confidence intervals (CI) for each measure.*.. 134

Table 6.1. *Nubian ibex parameters for PVA simulations, including the reference where they were obtained and or reasons for the value used. "Default" refers to the default value provided in the VORTEX model.*..................... 156

Table 6.2. *Highest and lowest reproductive and mortality parameter values used for sensitivity analysis. The (*) means the value is same as the baseline. The Scenarios are: HM&LM (high and low mortality); HB-F&LB-F (high and low breeding females) HB-M&LB-M (high and low breeding males); SR45 (female-biased sex ratio); SR55 (male-biased sex ratio); INB (inbreeding factor).*.. 159

Table 6.3. *Parameters used to model different drought scenarios.*................ 161

Table 6.4. *The parameters used for simulating the effect of hunting on the Nubian ibex population at WWR. Several hunting intensities were tested on sub adult, and adult males and females.*.. 162

Table 6.5. *Parameters used for simulating the effect of supplementation to a proposed Nubian ibex captive population at WWR.*................................. 165

Table 6.6. *Simulation results of modelling different population sizes of Nubian ibex at WWR for a time period of 100 years. (stoch-r) mean rate of stochastic population growth, (PE) probability of extinction, (N-extant) population size, (GD) genetic diversity, (MeanTE) mean time to extinction and ($SD_{(x)}$) standard deviation of each parameter. Values of $PE < 0.05$ and $GD \geq 0.9$ are shown in bold, as these are the criteria used to assess minimum viable population. Simulations that met both these criteria are shown in italics.*.. 166

Table 6.7. *Results of mean rate of stochastic population growth (stoch-r), probability of extinction (PE), population size (N-extant) and genetic diversity (GD) of different scenario simulated by sensitivity test with an initial population of 70 individuals. SE = standard error. A negative value of stoch-r means population decline and a positive value means population growth. P-values are from t-tests between the two scenarios for each parameter, with those that were non-significant shown in red.* 169

Table 6.8. *Results of mean rate of stochastic population growth (stoch-r), probability of extinction (PE), population size (N-extant) and genetic diversity (GD) of different scenario simulated by sensitivity test with an initial population of 100 individuals population. SE standard Error. A negative value of stoch-r means population decline and a positive value means population growth. P-values are from t-tests between the two scenarios for each parameter, with those that were non-significant shown in red.* 172

Table 6.9. *Results of mean rate of stochastic population growth (stoch-r), probability of extinction (PE), population size (N-extant) and genetic diversity (GD) of different hunting scenario simulated by sensitivity test for 100 (pop100) and 250 (pop250) individuals in the initial population. SE = standard error.* 177

Chapter 1 Introduction

1.1 Importance of biodiversity

Biodiversity is comprised of the different types of complex ecosystems (i.e. marine, desert, tropical rainforest, fresh water etc.) that are inhabited or used by a wide variety of species, each of which harbours, in itself, a wide gene diversity (Primack 2014). From this, we can deduce that there are three components shaping biodiversity: ecosystem, species and gene diversity. These are the main components which the International Union for Conservation of Nature (IUCN) (Frankham et al. 2017) and Convention on Biological Diversity (https://www.cbd.int/) has recommended for conservation.

The degree of ecosystem diversity can simply be measured by the number of species living in that ecosystem, ranging from simple organisms like bacteria to the more complex, such as plants and animals (Malcolm & James 2006). On the other hand, the genetic diversity in an ecosystem is the amount of genes content in the species that allow them to adapt to changing environmental conditions (Cutter 2019). Unfortunately, the wide variety of life on earth is threatened and the rate of species extinction is increasing in a way that has never been recorded before (De Vos et al. 2015; Brondízio et al. 2019). There is a consensus that a sixth extinction is under way in a rapid manner caused mainly by human impact and an unsustainable exploitation of resources (Barnosky et al. 2011). Although extinction is a natural process, it is expected to be balanced by speciation, which requires a long time to occur.

The continuing change in climate factors, such as temperature and the annual precipitation, is affecting species habitats. This may change their characteristics and render them unsuitable for organisms to survive. Under these circumstances, the organisms do not have sufficient time to evolve and adapt to

such sudden changes, which may cause large numbers of organisms to perish. This will lead to the loss of genetic variation necessary for adaptation. Hence, genetic diversity is essential for acquiring new traits, which can then help organisms to cope with environmental changes (Pörtner et al. 2021).

1.2 Population Genetic diversity

There are many definitions of genetic diversity but no one accepted definition. The IUCN definition of genetic diversity is ''the range of genetic material found in the world's organisms'' (IUCN 1980). Another recent definition by Frankham et al. (2004) stated that "Genetic diversity is the variety of alleles and genotypes present in the group under study (population, species or group of species)".

The genetic constituency of a species plays a pivotal role in providing potential adaptation to changing conditions and to support speciation. It also helps conservation geneticists to distinguish between populations and species, and therefore identify threatened taxa from those causing less concern, and to focus conservation efforts accordingly. Wildlife biologists and conservation practitioners argue increasingly that genetic diversity is a linchpin for any evolutionary adaptation, which is fundamental for any species' long-term adaptation and survival (Schemske et al. 1994). Therefore, one key role of the wildlife conservation specialist is to maintain an adequate level of genetic diversity required for a species, especially any with small declining numbers, which are threatened with extinction, so that they can adapt to challenges in the future such as climatic changes, changes in environmental factors and emerging disease. In addition, they have to impede the accumulation of recessive deleterious alleles, which might cause a decrease in survival and fecundity rates coincidental with the small population. Furthermore, the study of genetic diversity can help to define the taxonomy of the species and evaluate if the species has any conservation significance (Ferson 2002). Small populations

face five genetic threats that increase the extinction risk. These are: loss of heterozygosity as a result of genetic drift, fixation of deleterious alleles, hybridization, inbreeding and out breeding (Dyke 2003). Some of these main factors are now known to affect Nubian ibex and will be discussed in the following sections.

1.3 Population size

The effective population size (N_e) is one of the measures used to estimate the rate of genetic diversity reduction. It represents the number of individuals that have the ability to pass their genetic materials to the next generation (Frankham 1995b). Therefore, N_e is always lower than the total population size where sub-adult animals, juvenile and old sterile individuals who are not able to mate and reproduce are not counted. There are several factors that cause reduction in N_e, for example, population bottlenecks and reproductive success variation between individuals (Frankham et al. 2010). Low N_e in an isolated population increases the loss rate of genetic variation because the effect of genetic drift. The rule of 50/500 was proposed by Soule and Franklin (Franklin 1980; Soule 1980) to aid conservation efforts of small populations, in which the minimum number of individuals that have the ability to pass their gametes to the next generation needed not to be less than 50 to avoid extinction due to inbreeding. This years at Cop15 preservation of the genetic diversity of wild species was recognised as important for the first time in the CBD targets. The indicator which has been selected is maintaining effective population size above 500 (www.cbd.int).

1.4 Genetic drift

The ideal populations where the assumptions of Hardy-Weinberg equilibrium are met should have stable or unchanged allele frequencies. The stochastic process that cause change in allele frequencies in a population through its generations due to random sampling is called genetic drift (Allendorf et al. 2012). Genetic drift causes loss of genetic variation especially in small, isolated populations, and can cause rapid changes in the genetic composition of small populations. Mutations and migration of individuals between populations can mitigate the effect of genetic drift (Frankham et al. 2004).

1.5 Inbreeding

Small populations are characterized by lower level of genetic variation due to effects of genetic drift and inbreeding. Inbreeding is defined as mating between closely related animals and it is more obvious in small populations because there are fewer choices of mating with unrelated animals. Consequently, this type of mating between related individuals will cause increase in homozygosity and accumulation and expression of recessive deleterious alleles which may cause inbreeding depression (Frankham et al. 2010), causing reduction in population fitness and increase the probability of extinction (Reed & Frankham 2003).

1.6 Effect of isolation

Isolated populations are those that do not have the ability to move and exchange individuals with other populations, especially with close proximity ones. This is because of natural barriers such as mountains, rivers and lakes or by human made constructions like roads, farms and industrial areas. As a result,

this prevents movement of genetic material between adjacent populations. Low individual numbers coupled with reduced genetic diversity of isolated populations, by means of genetic drift, increase populations' differentiation and exacerbate risk of extinction (Frankham et al. 2017). Therefore, it is important in conservation of isolated population that movement and exchange of individuals to be facilitated either by restoring corridors (Christie & Knowles 2015) or translocation of animals (Hedrick 1995).

1.7 Measuring population divergence

Populations that are isolated for long time will differentiate by developing different set of alleles compared to other populations. One measure of population differentiation is F statistics, with F_{ST} being the most used form (Wright 1943). It is estimated from heterozygosity or variance in gene frequencies among subpopulations by using genetic markers such as microsatellites, mtDNA and Single Nucleotide Polymorphisms (SNP). The Values of F_{ST} range from 0, indicating no differentiation, to 1, indicating complete differentiation (Frankham et al. 2010).

1.8 Extinction risks

Populations that are isolated, having low effective population size and low genetic variation are more likely for genetic drift and inbreeding to act on and increase the degree of extinction risk. Therefore, to counteract these factors or alleviate their effects, increase the levels of heterozygosity is necessary for adaptations and increase fitness and survival rate (Frankham et al. 2004). Other factors such as loss of biodiversity, habitat destruction, overexploitation, diseases and global climate change might contribute synergistically and increase the risk of extinction for a population (Brook et al. 2008). The rapid rate

of climate changes is one of the main concerning factor where wildlife species and especially the ones with low genetic variation will not have the ability to cope with (Thomas et al. 2004). Therefore, conservation management of isolated population need to act and think of ways to increase the gene exchange, reduce anthropogenic threats to guarantee the ability of population to respond to future changes.

1.9 Factors causing a decrease in the genetic diversity

1.9.1 Habitat alteration and fragmentation

The steady increase in the human population coupled with increasing rate of consumption per capita has caused a high demand for supplies of food, water and shelter. These necessities are provided by maximum exploitation of natural resources and, as a result, cause a decline in the overall species and ecosystem diversity. Human induced habitat alterations and disturbances will lead to fragmentation in habitat and isolation of wildlife populations in small patches (Fahrig et al. 2019). Unfortunately, even the protected areas are not safe from human encroachment and their domestic livestock competing with wildlife, consequently causing serious damage to protected ecosystems. This is clearly seen at Al-Wusta Wildlife Reserve in Oman where local people used the reserve as foraging area for their livestock and competing with wildlife for space and resources (personal observation).

A major threat that is causing reduction in biodiversity and thus reducing size and genetic diversity of wild population is fragmentation of wild habitats, which is mainly caused by anthropogenic activities (Newbold et al. 2015). One of the main effects of fragmentation is preventing any possibility of movement of individuals between different populations which, in turn, halts the gene flow that is important to maintain their genetic diversity (Frankham et al. 2019a).

Therefore, small populations that are highly fragmented will lose their genetic variability due to genetic drift. Roads and human settlements are also important factors causing habitat fragmentation and population isolations (Barrientos et al. 2021). Many wildlife species are suffering from isolation and human interferences in their habitats causing potentially irreversible damage. The genetic diversity of fragmented populations has been found to be lower than that of unfragmented or undisturbed populations (DiBattista 2008).

For example, the critically endangered north China leopard (*Panthera pardus japonensis*) was found to be affected dramatically by fragmentation and habitat degradation caused by the rapid expansion of human population and industrial development (Vitekere et al. 2020). It is currently distributed in several small isolated patches. Similarly, African rhinoceros species are currently extant in small isolated populations. Combined with illegal hunting and human induced habitat alterations such as, mining, logging and infrastructures constructions put these iconic species under the risk of extinction (Chanyandura et al. 2021). It has also been reported that that the major threat to the existence of these species is hunting for rhinoceros horns for international trade (Annecke & Masubelele 2016). The giant panda (*Ailuropoda melanoleuca*), another charismatic species which is affected by fragmentation and habitat loss, was previously known to be distributed from China and Myanmar to Vietnam. Currently it is only found in six isolated populations in the Tibetan mountains (Lu et al. 2001). Therefore, in the context of conservation, populations that are suffering from fragmentation should be given priority.

1.9.2 Illegal hunting

Other imminent threats to wildlife are clearly represented in hunting for sport and the illegal trade of critically endangered and endangered species. The

illegal trade of wildlife parts and species is estimated to be worth several billions of dollars annually. According to the United Nation Development Program, the global illegal wildlife trade is estimated to be worth 7-23 US billion dollars annually (UNDP 2019)

Illegal hunting is a significant cause of population size reduction in some species especially large-bodied and horned mammals that are persecuted by hunters for their meat or horns (Price & Gittleman 2007) and, therefore, thought to predispose the population into the effect of genetic drift and important alleles are likely to be lost. In addition, the reduction of population size caused by direct individual harvesting might cause inbreeding and consequently reduction in population fitness. Moreover specific targeting of individuals such as males for their horns might cause elimination of rare alleles (Coltman et al. 2003).

The loss or reduction in genetic variability of wildlife species places their existence in danger because this will reduce their reproduction fitness and their ability to adapt to changing environmental conditions and further reduce their ability to resist new diseases (Frankham 1995a). The global genetic diversity within species is thought to have declined by more than 6% (Leigh et al. 2019) which poses challenges to the ability of many species to adapt to climate changes.

To preserve the genetic diversity of species, wild populations needs to be protected by establishing reserves and national parks that safeguard threatened species. Establishing corridors for fragmented and isolated populations will allow gene flow and migration between populations (Frankham et al. 2017). Moreover, reintroduction and reinforcement of lost and small populations can also assist in raising the level of genetic variation in populations respectively (Allendorf et al. 2012). Altogether, the literature presented here highlights the importance of maintaining the genetic diversity of wildlife populations and endeavouring to mitigate the effect of factors that reduce it.

Estimation of genetic diversity and assessing its effect on natural populations can assist conservationists in identifying populations that need urgent conservation and protection. It can also indicate the factors that may have caused a reduction in genetic diversity and inform approaches designed to alleviate their effects on these populations. Moreover, understanding and identifying the historic and current factors that are impacting natural populations will help in setting protective plans in future (DiBattista 2008).

1.10 Antelope status in Oman

Oman is located in the southeast extremity of the Arabian Peninsula. It has a total area of 309,500 km^2 along with a coastline extending to more than 3,000 km. It is an arid country receiving on average less than 50 mm precipitation annually. However, it contains a wide array of diverse ecosystems including mountains, salt flats (called Sabkha), lagoons (called Khwars), gravel deserts and sand dunes. Oman has 18 official protected areas and more than 60 sites with conservation importance that are being considered for recognition later as protected areas. The officially protected areas represent 4.3% of the total area of Oman. Ninety-three mammal species have been identified in Oman of which 20 species are globally threatened (CBD 2014) (see Table A1.1 in appendixes for the list of species that have conservation importance in Oman). The Arabian leopard (*Panthera pardus nimr*) and the Arabian tahr (*Arabitragus jayakari*) are under Critically Endangered and Endangered classification respectively in Oman and IUCN (Spalton et al. 2006; Mallon & Budd 2011; Ross et al. 2019, 2020b).

There are five antelope species in Oman, some of which are only found in specific areas. For example, the Arabian tahr is only found in the northern part of Oman (Ross et al. 2020b) whereas, the Arabian Oryx (*Oryx leucoryx*) and the sand gazelle (*Gazella marica*) are found only in the central part. The Nubian

ibex (*Capra nubiana*) can be found in central and southern regions while the Arabian gazelle (*Gazella arabica*) is widespread across the Sultanate (Grobler 2002). Table 1.1 shows the five antelope species found in Oman and their estimated population size and population trend. Nubian ibex is one of three important antelope species in Oman that are still free roaming in the wild (alongside Arabian gazelle and Arabian Tahr). Its population is decreasing due to several threats, discussed later in this thesis. As the protection of wild species in their native habitat is a priority, a conservation action was initiated by the Office for Conservation of the Environment (OCE) to study this wild animal.

Table 1.1. *Wild Antelope species in Oman and their population status.*

Species	Population size	Population trend in Oman	IUCN status	References
Arabian Oryx	>800 in captivity	increasing	Vulnerable	OCE personal communication
Nubian Ibex	>1100 in wild	decreasing	Vulnerable	(Ross et al. 2020a)
	10 in captivity	stable		OCE personal communication
Sand Gazelle	>1000 in captivity	increasing	Vulnerable	OCE personal communication
Arabian Gazelle	<2000 in wild	decreasing	Vulnerable	(Al Hikmani et al. 2015; Al Jahdhami et al. 2017)
Arabian Tahr	>2000 in wild	decreasing	Endangered	(Ross et al. 2020b)

1.11 *Capra* genus Taxonomy and distribution

The *Capra* genus putatively consists of nine species (Pidancier et al. 2006). They are mountain specialists and adapted to live in higher altitudes and well suited to living within a rocky mountain environment. This genus is distributed widely in three different continents from the hot hyper-arid desert of the Arabian sands to the cooler areas of the Alpine mountains (Shackleton 1997). The central and western parts of the European continent are known to host two *Capra* species. They are Alpine ibex (*Capra ibex*) and Spanish ibex (*Capra pyrenaica*) (also known as Iberian wild goat) (Manceau et al. 1999a; Parrini et al. 2009). The majority of the other *Capra* species are only found in the Asian continent except Nubian ibex which are extant in both Africa and Asia, and Walie ibex (*Capra walie*) which are only found in Africa (specifically in Ethiopia) (see Table A1.2 in appendixes).

The reliance on the traditional method of taxonomy, such as horn morphology and coat pelage, to distinguish between different *Capra* species has created a dilemma, its consequences affecting the ability to delineate the number of species and subspecies of this genus (Schaller 1977). These phenotypic characteristics are subjected to environmental conditions. For example, fur colour may change between seasons. In addition, variation of a single trait within the same population is common. Moreover, hybridization has been observed between species of this genus (Couturier 1962; Hammer et al. 2008; Biebach & Keller 2010). Therefore, the taxonomy of the *Capra* genus is still under debate and there are no clear results as to their origin (Pidancier et al. 2006). However, different studies using mitochondrial and nuclear markers suggested that the ancestral origin of the *Capra* is thought to be from central Asia, where hybridization between the ancestor of the ibex type and the ancestor of Bezoar type (*Capra aegagrus aegagrus*) is thought to take place (Manceau et al. 1999b; Kazanskaya et al. 2005, 2007; Ropiquet & Hassanin

2005; Pidancier et al. 2006). The *Capra* species then started to distribute to the other ranges and habitats and this migration was estimated to be between one and six million years ago (Manceau et al. 1999b).

Due to several factors such as isolation and fragmentation of populations, and degradation of habitat (Shackleton 1997) populations of *Capra* have decreased with time. In some areas, it has become locally extinct in the wild, such as the Alpine ibex in Switzerland which was then reintroduced to the wild, and Nubian ibex which is extinct in Lebanon and Syria (Stüwe et al. 1992; Alkon et al. 2008).

1.12 Nubian ibex (*Capra nubiana*)

1.12.1 Distribution

The distribution of Nubian ibex currently extends from Africa (Egypt and Sudan) through the Middle East (Negev desert and Jordan) up to the Arabian Peninsula (Saudi Arabia, Yemen and Oman) (Ross et al. 2020a). Table 1.2 shows the population estimates of the Nubian ibex by country in the area of its distribution and it is clear that there is quite a shortage in data in some countries such as Sudan and Yemen.

Table 1.2. *Population estimates of Nubian ibex by country.*

Country	Population size	References
Eritrea	No Estimation	(Alkon et al. 2008)
Sudan	No Estimation	
Saudi Arabia	~ 400	
Egypt	200-250	(Ross et al. 2020a)
Israel	1200	
Oman	600-1100	
Yemen	No Estimation	
Jordan	~ 200	(Alkon et al. 2008)
Syria	extinct	(Alkon et al. 2008; Ross et al. 2020a)
Lebanon	extinct	

1.12.2 Adaptation

The Nubian ibex is found to be well adapted to harsh desert conditions, which are characterized by higher diurnal temperatures, scarce vegetation cover and limited water sources (Habibi 1994). One of its adaptive characteristics is possessing a shiny coat that protects it from intense sunlight and helps in minimizing the loss of water through its skin (Castelló et al. 2016). Conversely, the pelage tends to be thicker in the winter months which assists the animal to absorb more heat from the sun (Baharav & Meiboom 1982). It is also found to

prefer to settle in areas where there is minimum interaction either with humans or domestic livestock (Baharav & Meiboom 1981).

Nubian ibex possesses scimitar-shaped horns, which appear oval in cross section. The horns' surface is well defined and separated by prominent transverse ridges and they can reach a length of about 100 cm (Habibi 1994). In addition, they have the capability of growing throughout life and, by counting the annuli, can be used to estimate the age of an animal (Couturier 1962). However, horn growth may vary between locations because it is affected by food availability, environmental conditions and population density (Michallet et al. 1994; Giacometti et al. 2002), so caution is needed to avoid misleading counting due to false annuli. Nubian ibex is distinguishable from the Alpine ibex (*Capra ibex*) by having longer ears (100 to 120 mm notch to tip for Nubian ibex compared with 75 to 85 mm in Alpine ibex) as well as a longer beard in Nubian ibex males than Alpine ibex (Harrison 1991).

1.12.3 Nubian ibex in Arabia

Nubian ibex were known to people inhabiting the Arabian areas from ancient times. This can be seen from the drawing found in Paleolithic and Neolithic rocks in Jabal Tubaiq in Saudi Arabia depicting animals with curved horns chased by hunters (Carruthers 1935 cited in Harrison 1991). Also, Hoeck 1962 (cited in Harrison 1991) had pointed out that the symbol of Nubian ibex represented the Moon God in the days of Queen of Sheba who flourished between c.1000 BCE - c.901 BCE in Yemen (Britannica 2023). In addition, the same drawing representing Nubian ibex on rocks and some of them showing hunting groups of people flowing animal herds were discovered in the Negev desert (Avner et al. 2017).

1.12.4 Nubian ibex population in Oman

The Nubian ibex in Oman live in fragmented populations in central (specifically Al-Wusta Wildlife Reserve, WWR) and southern region (Figure 1.1) caused recently by anthropogenic activities. A lack of information available about this species creates more difficulties to conserve and protect it. This information includes population structure, population size, the range of the population, adaptation, ecology and genetic diversity. The main threats that face the Nubian ibex in Oman are poaching, competition with local livestock, habitat destruction and fragmentation (CBD 2014).

Nubian ibex inhabit the northeast parts of the WWR, which is called the Huqf escarpment (Figure 1.2A). It is characterized by steep terrain that extends about 150 km in length (Ross et al. 2020a) and about 4 km wide giving a total area of about 600 km^2.

Nubian ibex is known to have a wide distribution in the central part of Oman but because of urban sprawl, this wide population started to shrink into small, isolated populations. Several small populations of Nubian ibex were extirpated and were locally extinct in the central region such as populations in Janaba Hills and Ra's Madrakah which were probably diminished 50- 60 years ago (Figure 1.1) (Alkon et al. 2008). The last population in the central region is now located at WWR where part of this population is under a direct protection of the reserve rangers.

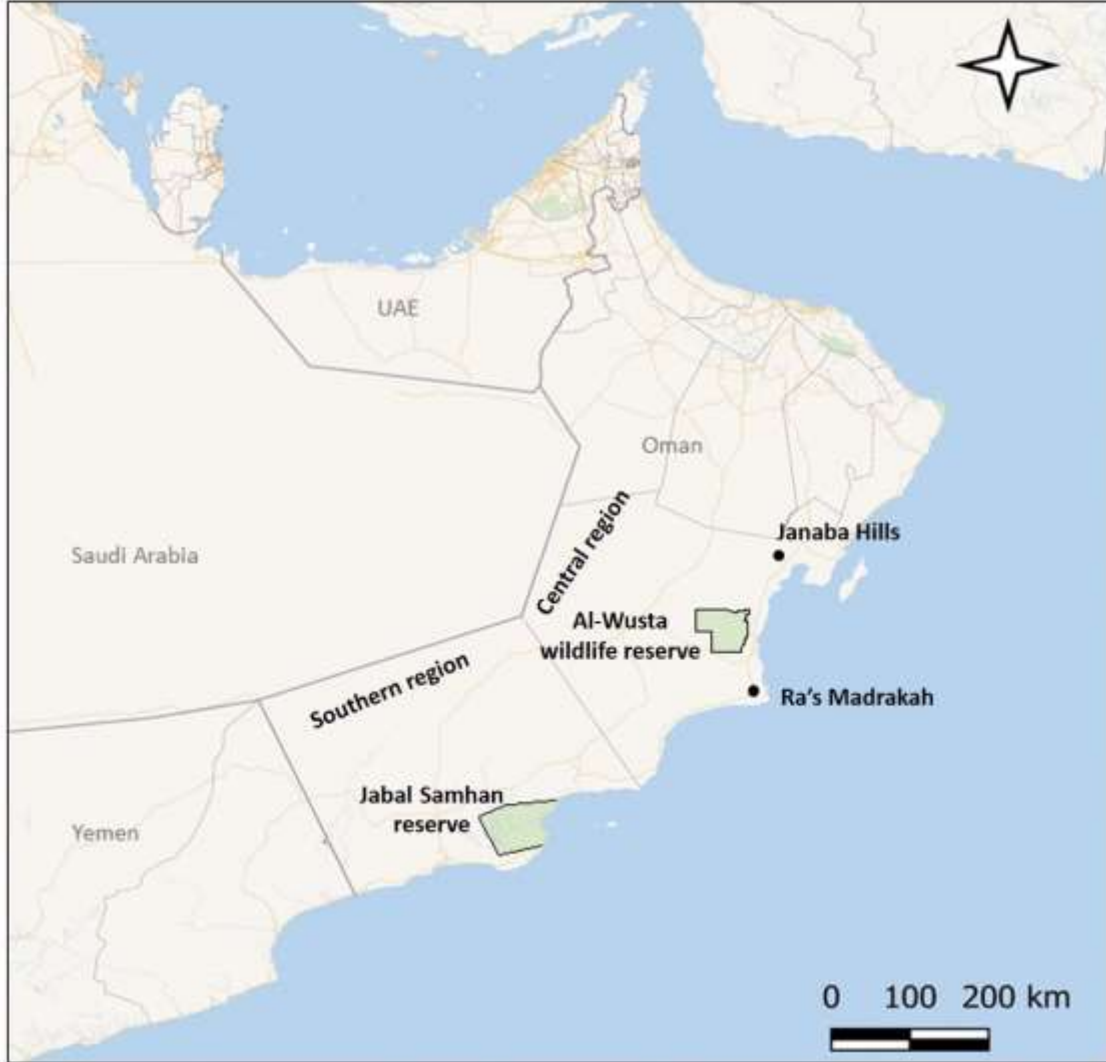


Figure 1.1. *Map of Oman showing the location of central, WWR and southern region. The location of the two extinct Nubian ibex populations in Janaba hills and Ras Madrakah are indicated by black dots.*

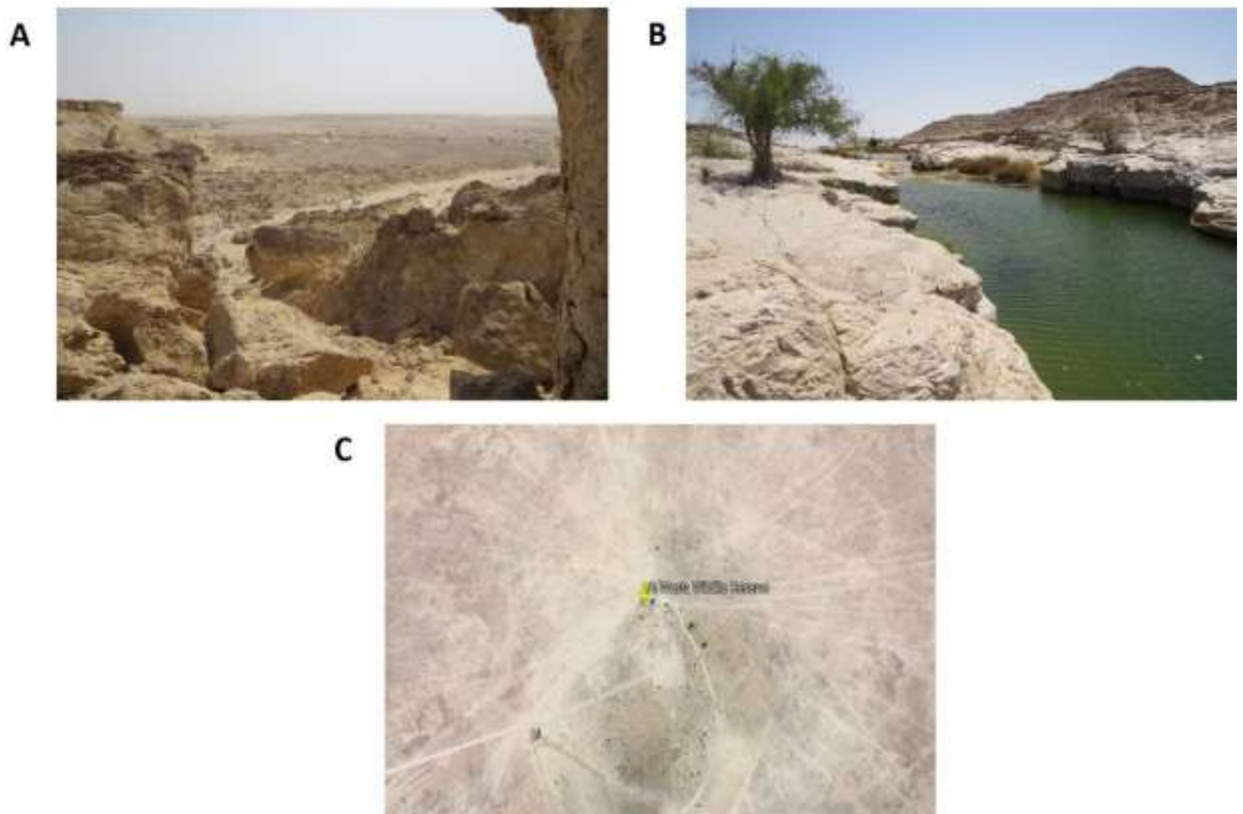


Figure 1.2. *(A) The Haquf escarpment at WWR. (B) High salinity water pools at WWR. (C) The visual impact of vehicles on vegetation cover. Photos A and B by Mataab Al-Ghafri, photo C obtained from Google Earth Pro.*

There are several anthropogenic made barriers that potentially prevent any possible exchange of individuals between central and southern region such as construction of roads network, oil exploration sites in the desert and construction of residential buildings. This problem if not solved will render the population in the WWR to be genetically isolated consequently will be in a high risk of local extinctions in the near future. If, however, individual exchange could be established (either naturally by corridors or artificially by translocation) there is likely to be a higher probability that these populations will persist for longer time.

1.12.5 Nubian ibex population size in Oman

The last population size estimate of Nubian ibex at WWR and the southern region was based on field survey and camera trapping and found that the size ranged from 100 to 250 individuals in WWR and from 600 to more than 1,000 in the southern region (Ross et al. 2020a). The WWR population is isolated and endangered by several threats such as hunting, habitat destruction and competition with domestic livestock (Giangaspero et al. 2014). On the other hand, the population in the southern region is larger and has a wider range despite the threats created human and their livestock. Therefore, it is important to reduce the impacts of these threats and avoid any further local extinctions of Nubian ibex populations in Oman. It will be fundamental to implement evidence-based conservation programs that will help in maintaining and preserving genetically and evolutionary viable population of this species for long term.

1.12.6 Nubian ibex hunting

Nubian ibex in Yemen (on Oman's south-west border) has been reported to range in the southeast part of the country and in the region of Hadramaut which is located in east-central Yemen, on the Gulf of Aden. It is facing heavy hunting pressure because it is a tradition in Yemen for local people to hang ibex horns as a trophy in front of their houses (Al-Jumaily 1998). Showler (1996) cited in Al-Jumaily (1998) reported that a single hunter was able to shoot about 70 ibex in one year. Nubian ibex horn specimens can be found in local markets in Yemen offered for sale, especially the male horns (Obadi 1993).

In Oman, many of the horns are used to produce musical instruments by creating some holes along the horns, while others are used to store gunpowder (personal observation).

1.12.7 Rutting season

The rut is defined as the condition of sexual excitement that is observed in certain mammals species such as goats, sheep, bison, and antelopes during the mating seasons (Powell & Evans 2019). The rutting behaviour is triggered by an increase in testosterone and oestrogen hormones levels in males and females respectively.

The beginning of the rutting season has been observed from October to November (Habibi 1997; Massolo et al. 2008). Some studies have reported that rutting starts earlier in the fall from September and extend to the beginning of December (Gross et al. 1995).

1.12.8 Nubian ibex diet

Nubian ibex prefer to feed on succulent plants that supply them with water during the hot months (Habibi 1994). After rainfall, feeding behaviour is switched to dependence on woody plants (Campbell 1997). The summer months in the Arabian Peninsula are characterized as dry and hot where almost no rainfall is combined with high temperatures (Price 1989). Therefore, during these months, the Nubian ibex is more active in the early morning and before the sunset when the temperature is lower compared with midday when they prefer to rest in the shade (Levy & Bernadsky 1991).

1.13 Study sites

Nubian ibex is patchily distributed in Oman. The populations are located in the central and southern regions of the country. In the central region the last

population is located at Al- Wusta Wildlife Reserve (WWR). In the southern region Nubian ibex can be found in several locations in Shalim and in the mountains of Dhofar. Therefore, the study will concentrate on the current distribution of the Nubian ibex population in these where samples will be collected from these two regions (i.e. the central and the southern region).

1.13.1 Central region (WWR)

Al-Wusta Wildlife Reserve (WWR) is a location formerly known as the Arabian Oryx Sanctuary with a total area of about 34,000km^2. It was proclaimed as a protected area by a Royal Decree (4/94) in 1994 and the same year it was inscribed as a UNESCO world heritage site (Al Jahdhami et al. 2011). In 2007, the Omani government decided to reduce its area by more than 90% to allow oil exploration activities to take place, consequently lead to delisting of the sanctuary from the UNESCO World Heritage Site list, becoming the first site ever to be deleted from this list (UNESCO 2007). Currently, WWR has a total area of about 2,824km^2 and it is protected by a fence covering almost the whole perimeter as well as more than 20 rangers patrolling the area day and night (personal communication).

It is a primarily flat landscape located in the central desert of Oman with a discrete limestone plateau consisting of different types of habitats such as sand dunes, wadi depressions and steep rocky terrain with patchily distributed vegetation.

1.13.2 The climate at WWR

The presence of water is a vital element in the survival of wild species and it sometimes plays a role in shaping behaviour, especially in the hyper-arid environment like the one at WWR. For example, Tear et al. (1997) observed a change in the mating and calf rearing behaviour of the Arabian Oryx after rainfall in WWR. The reserve receives a low amount of precipitation annually (< 50mm) and the groundwater is more than 150m below the surface except in some areas in the Huqf depression where water is a few meters deep but it has high salinity levels (Spalton et al. 1999) (Figure 1.3). Despite that, there are a few water seepages that can be used by wild animals. Salty water pools are also available in the east part of the reserve (Figure 1.2B). However, the reserve is generally characterized by daily fog in the summer months which contributes to the wide floral diversity (Price 1989). In the past, the local people used to condense the fog to gather sweat water. They used cotton and handmade fabric made of goat hair to collect water on the foggy nights which indicates how heavy the fog can be (personal communication with Hurasis tribesmen) also mentioned in (Dixon & Jone 1988). The temperature is high and can reach up to 45°C (Al Jahdhami 2010). The hottest months are June to August while the winter is between November and January (Figure 1.4)

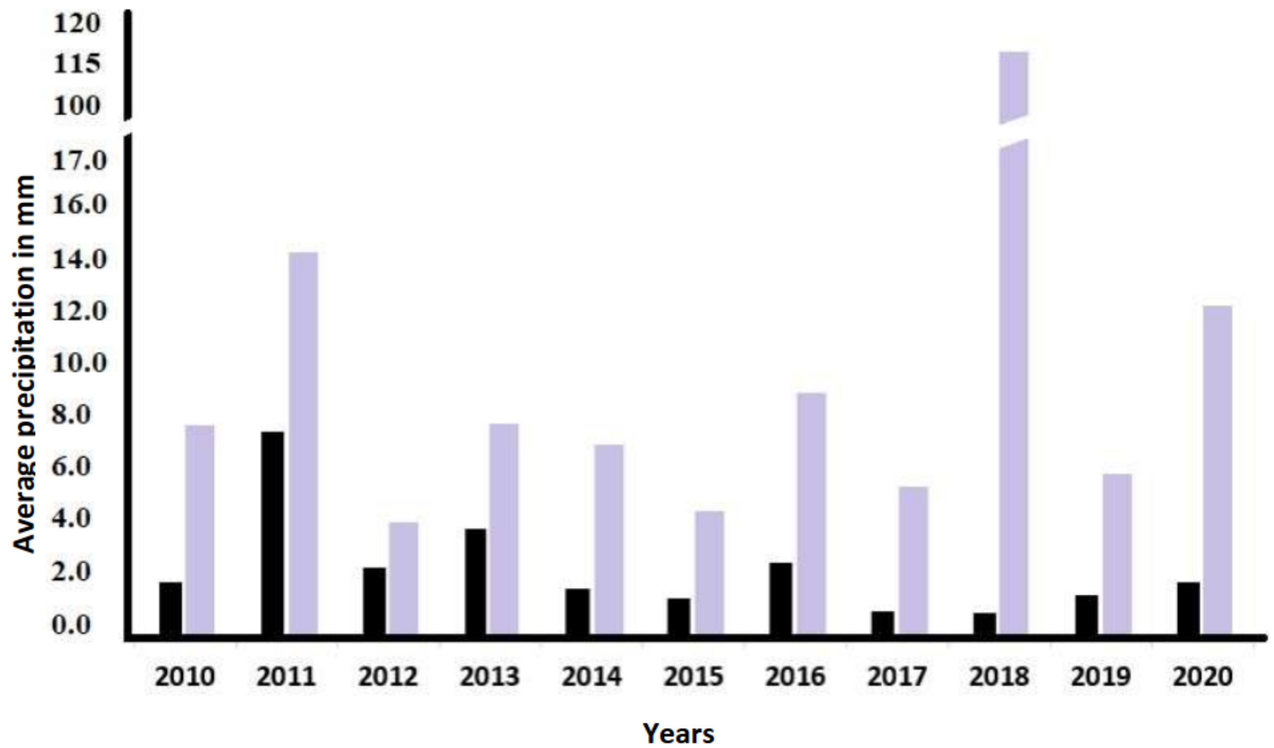


Figure 1.3. *Annual average precipitation comparison between the central region (black bars) and southern region (gray bars) from 2010 to 2020. In 2018 a cyclone hit the cost of Dhofar hence the extreme value in that year. Data retrieved from Directorate General of Meteorology (www.met.gov.om).*

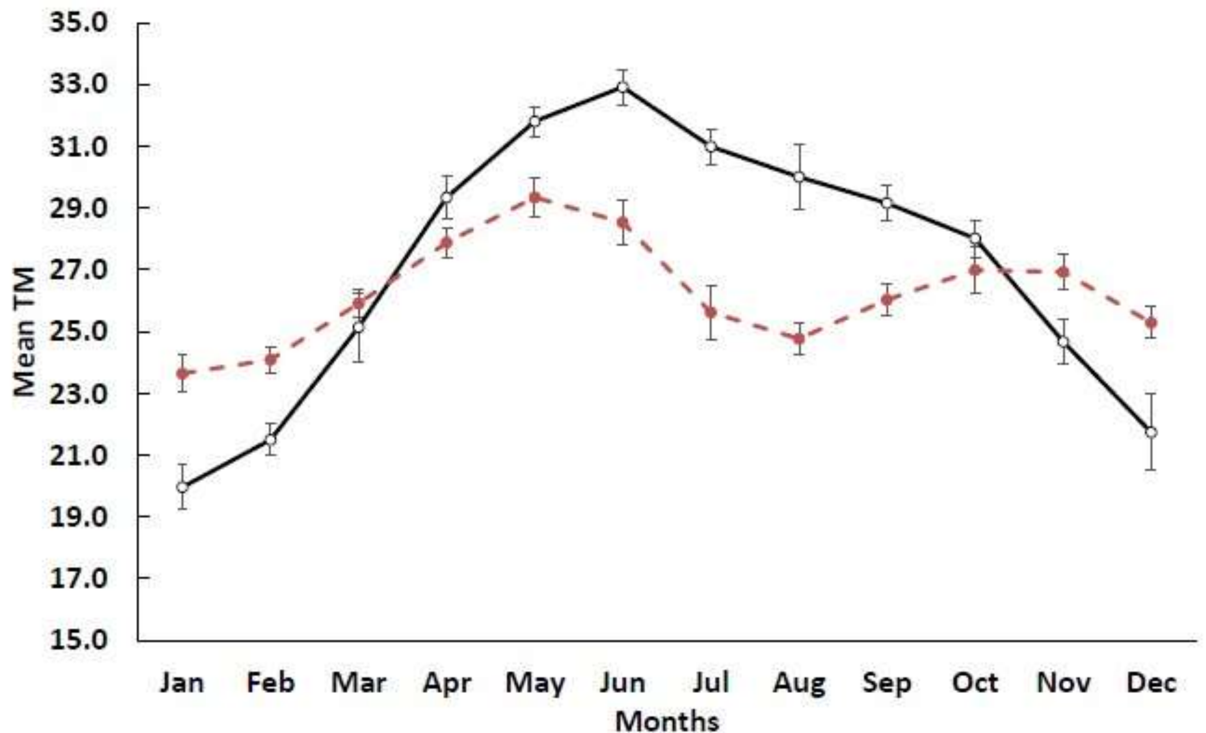


Figure 1.4. *Mean monthly temperature (TM; in °C) comparisons between central region (black solid line) and southern region (red dotted line). Error bars represent the standard deviation. The data are averaged across the years 2010 to 2020. Data retrieved from Directorate General of Meteorology (www.met.gov.om).*

There are no permanent water sources in WWR and so wildlife there depends on the water content in their food. The Arabian Oryx has been documented to withstand the absence of standing water for 11 months, depending mainly on the water content from the plant leaves and by licking water drops forming on leaves and rocks after foggy days (Tear 1992). The close proximity of the WWR to the Arabian Sea gives it an advantage of benefiting from the seasonal mist season (monsoon). The density of vegetation decreases as the distance from

the sea increases and the vegetation species vary from perennial to ephemeral, which just live for a short life cycle following a rainfall (Price 1989).

1.13.3 Flora & Fauna

The reserve supports diverse wildlife communities including: Arabian Oryx, mountain gazelle, sand gazelle (reintroduced in 2013), feral donkeys (*Equus asinus*), Cape hares (*Lepus capensis*), at least two species of foxes, different species of reptiles and rodents (Price 1989; Spalton 2002).

The main plant species found in WWR are *Acacia tortilis*, *Acacia ehrenbergiana* and *Prosopis cineraria* (Ghazanfar 2004). In addition there are different species of grasses which are important for Oryx, such as *Stipagrostis sokotrana* and *Dicanthium foveolatum* (Tear et al. 1997).

1.13.4 Southern region (Dhofar)

Dhofar is located in the southern part of Oman where it shares borders with Saudi Arabia in the north-west and Yemen in the south-west, while to the south-east it faces the Arabian Sea (Figure 1.1).

The mountains of Dhofar extend to a range of about 400 km in length and a maximum width of more than 23 km (Mazzolli 2009). The average elevation is about 2,100 m above sea level (asl). (Shackleton 1997). The well-known mountains in Dhofar are Jabel Samhan (2,030 m asl), Jabel Qara (1,050 m asl) and Jabel Qamr (1,460 m asl) (Arnold, E 1980). From mid-June to mid-September, a large area of these mountains receives annually a substantial amount of water during the monsoon (Ministry of Environment and Climate Affairs 2010). This supports a wide variety of vegetation and creates dense forests. These mountains create two habitats, depending on an area’s location in relation to the sea. Areas which faces the Arabian sea benefit from the dense

moisture carried by the monsoon (Rogers 1980), while the opposite side is dryer, yet still harbours scattered vegetation and it is a homeland for the economically important tree *Boswellia serrata* (Arnold, E 1980; Sale 1980; Galletti et al. 2016). Compared to WWR, Dhofar is considered a semi-arid land receiving annually 100–400 mm of rainfall (Kwarteng et al. 2009).

Unfortunately, there are still hunting incidences reported periodically despite the huge effort done by rangers to patrol the protected areas

The disappearance of mountain gazelles from parts of its former ranges in the Jabel Qamr and Jabal Al Qara mountains are considered a result of illegal hunting (Al Hikmani et al. 2015).

1.14 Challenges facing wildlife in Oman

1.14.1 Human activities

After the exploration of oil, and with a good health care system, the Omani population has increased exponentially (Didero et al. 2019). Along with that, the lifestyle has also changed dramatically, and cars have replaced the traditional modes of transport (i.e., camels, horses and donkeys), which created the necessity to construct roads to connect towns and villages. According to the Ministry of Transport, the road network in Oman is more than 30,000 km where 50% are paved roads (Ministry of Transport 2018). Roads traverse the area between the central region and southern region, potentially creating difficulties for wildlife to move between locations and reducing connectivity between populations and habitats. Roads also have been found to cause tremendous effects on the wildlife by creating barriers that prevent animals from movement between places, habitat fragmentation, and mortality of the wildlife because of road kills (Van Der Ree et al. 2011; Spooner 2015; Jackson 2000).

Similar results were obtained by investigating the effect of the oil exploration activities on wildlife behaviour. Mulondo and Mugiru (2011) found that mammal

species such as giraffe (*Giraffa camelopardalis*), oribi (*Ourebia ourebi*), Uganda kob (*Kobus kob thomasi*) and elephant (*Loxodonta africana*) are disturbed from the oil exploration sites. They found that animals avoided sites of construction and maintenance by a distance of more than 1,000 m. Furthermore, the network of roads built to connect different sites cause difficulties and add more restrictions to the wildlife movement. Moreover, disturbance can be caused by heavy traffic of big trucks and buses. The actual impact of road constructions and the oil exploration activities on the wildlife of Oman and how it affects the avoidance behaviour, movement and distribution maybe deserve to be further studied in the future.

There are no permanent settlements in the WWR. However, nomadic Bedouin use the reserve for their cattle foraging. Their daily movement by car between locations for herding and monitoring their animals causes an impact which results in the removal of vegetation cover (Figure 1.2C).

Anthropogenic activities can play a critical role in reducing wild populations. These activities include illegal hunting, habitat destruction and fragmentation (Spalton et al. 1999; Giangaspero et al. 2014). Nubian ibex face competition from feral donkey and livestock of the local people which share foraging areas with wildlife (Shackleton 1997).

The presence of human beings can cause disturbance and avoidance of wildlife to that specific area. Tadesse and Kotler (2012) have investigated the impact of the human presence on the Nubian ibex and found a significantly negative effect on their foraging behaviour. Female ibex are found to be more sensitive to the disturbance and this leads to less feeding time that subsequently may affect the rearing ability for new calves. Conversely, Nubian ibex spend more time in patches where there is no or minimal human presence (Kotler et al. 1994).

1.14.2 Nubian ibex hybridization

Hybridization between *Capra* species and domestic goats has been documented (Iacolina et al. 2019). Specifically in Saudi Arabia deliberate hybridization between Nubian ibex and goat is carried out by domestic livestock breeders for trade, ornamental and sport purposes (Obaid Al-Awni personal communication). Several goat breeds were used for hybridization with Nubian ibex and produce breeds that have some desired characteristics such as long curved horns, coat pelage, agility in climbing cliffs and have the ability to produce meat and milk. Some of the hybrid animals which resemble Nubian ibex are released into large, fenced farms (or into private reserves) for hunting.

The most common goat breeds used for hybridization with Nubian ibex are so-called Ardi and Tohami. These two breeds were found to harbour the highest genetic diversity and capability to tolerate high heat and low availability of food and water (Al-Atiyat & Aljumaah 2014; Al-Atiyat et al. 2015). Figure 1.5 showed an obvious example of hybridization between Ardi and Nubian ibex. The offspring have a black coat colour from the goat and long curved horns from the Nubian ibex. This type is not preferred by people especially the one who used them for sport, but rather for meat and milk production. The most sought-after type of hybrid is between Tohami goats and Nubian ibex (Figure 1.6). The offspring resemble the Nubian ibex in colour, pelage and horns, but the horns lack the rings. To my knowledge, there are no studies investigate this kind of hybridization and its possible impact on the wild Nubian ibex (Obaid Al-Awni personal communication). At the same time such practices is not regulated or monitored by governmental authorities or by conservation agencies. There is a real concern and fear that these kinds of hybrids will find their way into the wild and have the chance to mate with wild animals. Therefore, there is a pressing need to routinely investigate the possibility of putative hybridization between wild ibex and goats and the practice of hybridization need to be regulated and

monitored by officials. In this study I will incorporate samples of goats collected from WWR where these animals share overlapping areas with other wildlife species (Nubian ibex and Arabian gazelle) to test potential hybridization between the two species.

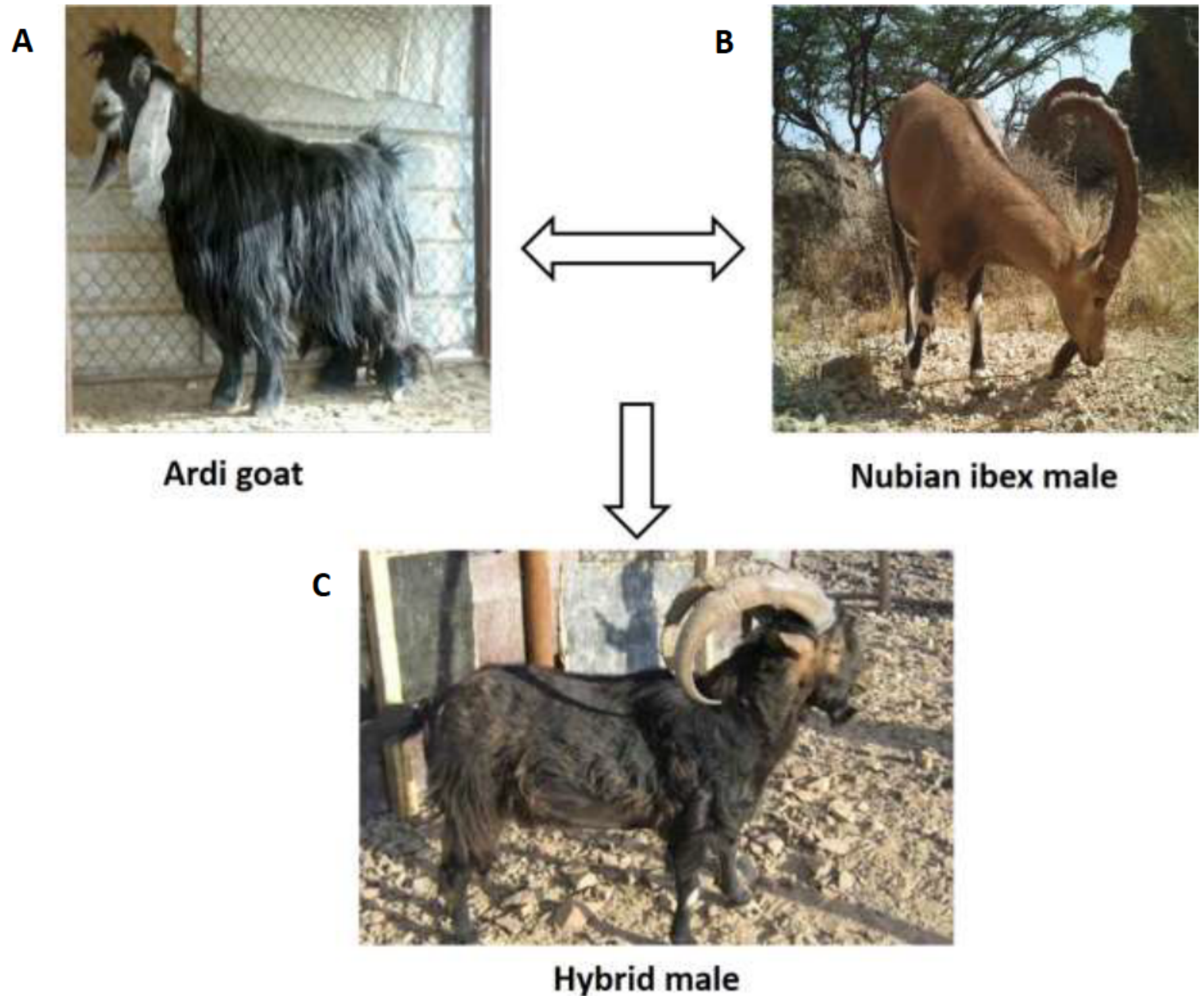


Figure 1.5. *Schematic representation of hybridization between (A) Ardi goatand (B) Nubian ibex. The offspring differs in coat color and shape. It has the ear shape and long curved horns of the ibex (C). The rings in the horns are small and not clear compared to the wild ibex. Photos A and C courtesy to Obaid Al-*

Awni (Saudi Arabai), B to Steve Ross and Taimur Al-Said (office for conservation of nature, Oman).

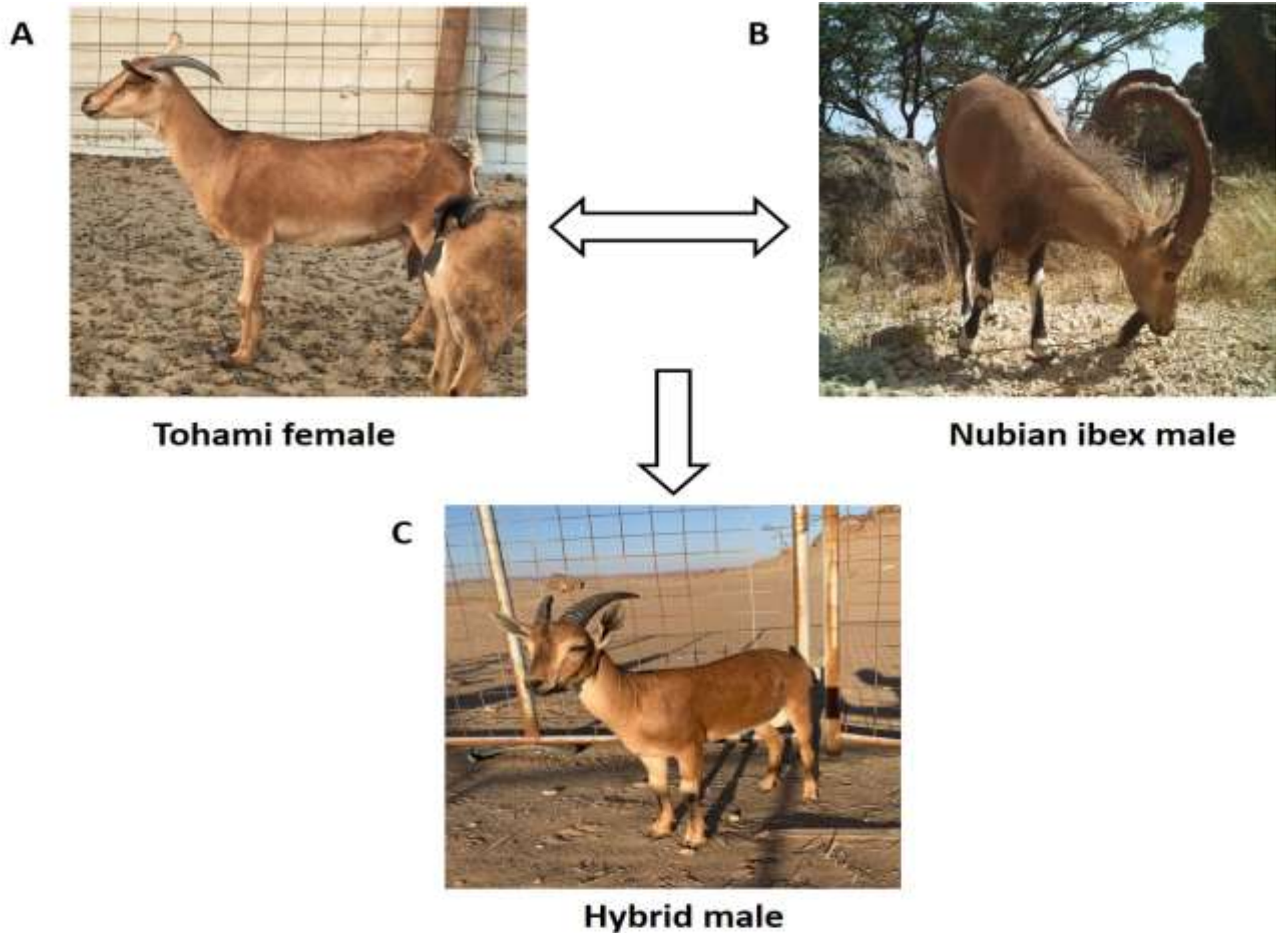


Figure 1.6. *Schematic representation of hybridization between (A) Tohamigoat and (B) Nubian ibex. The offspring has almost all Nubian ibex phenotypic traits (C). The rings in the horns of the produced animals are smaller and compacted and the leg coloration does not match exactly the wild ibex. Photos (A) courtesy to Obaid Al-Awni (Saudi Arabai), (C) from (https://twitter.com/khal4000/status/1556536873820033024/photo/1) and (B) to Steve Ross and Taimur Al-Said (office for conservation of nature, Oman).*

1.15 Conservation of Nubian ibex

The conservation of the wild Nubian ibex in Oman can be facilitated in two ways. The first is by protecting the extant populations in the central and southern region and ensure that they stay viable and diverse over time. The second is by establishing a captive population, which later can serve as source of individuals for future conservation plans. Nevertheless, for any such program to be successful, it needs to be informed by high quality data about Nubian ibex in the region, such as its approximate population size, demography, genetic variation and factors effecting its population growth rates and to what extant this population is viable.

To achieve these goals we need to (i) assess the genetic diversity of the wild Nubian ibex populations in Oman, and (ii) assess the genetic structure of the current captive animals in Oman to explore the possibility of using them for any future reintroduction or augmentation programs.

1.16 Potential for a captive program of Nubian ibex in Oman

Captive programs have played an imperative role in saving many endangered species from extinction such as addax (*Addax nasomaculatus*), scimitar-horned Oryx (*Oryx dammah*), Arabian Oryx and many others. These programs are designed to provide a safe haven for wildlife species which are no longer can survive without assistance due to several factors such as habitat destruction, over exploitation and population isolation. The main goals of captive programs are retaining genetic diversity, secure an insurance population of endangered species, educating the public on wildlife importance, assist in conservation

research and provide animal for reinforcement and reintroduction programs (Frankham et al. 2010).

It is more plausible to establish a captive program from wild individuals in Oman because there is already ongoing program and ready facilities along with experienced staff. To ensure effective *ex situ* management that would involve animals from WWR and the southern region this program would need to be carefully designed and many important factors must be taken into consideration. First, there are ecological differences between the WWR and southern populations, which might include specific adaptations to their respective habitat. Second, water seepages that are used by wildlife at WWR are characterized by high salinity levels, which may require a specific adaptation to tolerate it. In addition, adaptive traits related to immune response, visual development and tolerance to high solar radiation have already been identified in Nubian ibex (Chebii et al. 2020). Such information can play a key role in establishing captive programs that represent the wild animals which harbour these traits. Therefore, it is important to investigate any potential adaptations that might exist between the two regions before any translocation trials.

According to IUCN, there are two important factors that need to be met in order to establish a captive population. First, founder individuals should genetically represent the source population and second, these individuals should be able to live in the wild independently with minimal human interfere (IUCN/SSC 2013). Moreover, careful consideration needs to be concentrated towards environmental and demographic factors and the adaptive potential of the selected individuals for the captive breeding (Weeks et al. 2011). Animals sourced from different locations might have different adaptations (e.g. Nubian ibex in WWR might be adapted to tolerate high salinity water while the southern region population might lack this adaptation). Another consideration is that populations in captivity will develop genetic adaptations to captivity (Frankham 2008), which should be taken into consideration.

It is generally agreed that animals selected for captive programs need to be representative of > 95% of the level of genetic diversity of the source population (Miller et al. 2009; Frankham et al. 2010), which should enable the populations to persist into the future and adapt to future environmental change (Keller et al. 2012). Thus, for any such programme to work successfully, it will be important to assess the genetic diversity available in WWR and southern region in Oman for Nubian ibex using a variety of approaches including mitochondrial and cutting-edge nuclear DNA approaches. Ultimately, this will help in creating a captive population that represent the genetic diversity of the wild.

1.17 Thesis aims and outline

The overall aim of my research is to help inform future conservation programmes of Nubian ibex in Oman, with a particular focus on their genetic diversity and suitability for a future captive breeding programme.

The following chapters in this thesis will investigate the genetic diversity and population structure of Nubian ibex in Oman in comparison with captive individuals and samples from other populations (Yemen and Sudan). Special consideration was taken towards the population at WWR in the central region of Oman, due to its isolated nature and small size, where its viability was tested against several natural and anthropogenic factors.

In chapter two, I used mitochondrial markers to explore the genetic variation and population differentiation in and within Nubian ibex populations in Oman and compare it with that of captive individuals from UAE and Oman. I was able to design and test primers that can amplify cytochrome *b* and D-loop for Nubian ibex from samples quality ranging between low (faecal) to high (blood and tissue). The majority of the samples used were faecal as these are available and easy to collect especially when studying elusive and endangered species.

The tissue and blood samples were taking from animals that are either caught for study or killed by hunters. This chapter was published in Royal Society Open Science in 2021 (Al-Ghafri et al. 2021).

In chapter three, I used the same method developed in chapter two to amplify the same markers for museum samples from Yemen and Sudan. I was successful for cytochrome *b* which revealed the interspecific relationship between the populations. The results showed significant differentiation between Oman/Yemen populations and Sudan/captive samples.

In chapter four, nuclear DNA was examined by the Double-digest restriction-site associated DNA sequencing (ddRAD) method to conduct efficient and flexible SNP genotyping of the Nubian ibex genome. I was able to develop 5,775 high quality SNPs which confirmed the significant divergence between wild and captive Nubian ibex found in chapter two and three. In addition, these SNPs have the power to detected hybridization with goats within captive Nubian ibex and confirm the previous results revealed by mtDNA. This chapter has been submitted for publication to the Conservation Genetics Resources journal and is under review at time of writing.

In chapter five, I used the Hybridization capture approach that used baits (probes) designed from the previous results of ddRAD in chapter four, to target SNPs identified in chapter four, from faecal, bone and museum samples. I was successful in targeting DNA of low-quality samples. This technique provided valuable information on genetic composition of Nubian ibex populations in Oman, Yemen and Sudan, and will provide an essential tool or future genetic monitoring of ibex in Oman. The results showed a significant divergence between Oman and Sudan populations and confirmed the previously detected divergence between wild and captive ibex.

In chapter six, I carried out population viability analysis for the isolated WWR population. First, I used VORTEX10 software to estimate the minimum viable

population size of Nubian ibex at WWR, then I used sensitivity tests to test the effect of different parameters on the population viability (i.e. mortality rates, sex ratio and reproduction rates). To explore the effects of two key anthropogenic and environmental factors I tested scenarios where drought frequency and hunting intensity were varied. Finally, I proposed establishment of a captive population and I simulated a scenario of supplying this population with animals from the wild. This study indicated the importance of reducing the mortality rate especially in females, and the feasibility of establishing captive population starting with ten individuals that should be supplemented by wild individuals over time (i.e. each five years).

Finally, in chapter seven, I discussed the key findings of my thesis and relate them to the required efforts to conserve this species. I reviewed the limitations and obstacles encountered this study and I detailed the implications for conservation of Nubian ibex in Oman based on the study's findings.

Chapter 2 Genetic diversity of the Nubian ibex in Oman as revealed by mitochondrial DNA

An adapted version of this chapter was published in Royal Society Open Science journal as:

Mataab K. Al-Ghafri[1,2,3] , Patrick J. C. White[1], Robert A. Briers[1], Kara L. Dicks[2], Alex Ball[2], Muhammad Ghazali[2], Steven Ross[1], Taimur Al-Said[3], Haitham Al-Amri[3], Mudhafar Al-Umairi[3], Hani Al-Saadi[3] , Ali Aka'ak[3], Ahmed Hardan[3], Nasser Zabanoot[3], Mark Craig[4], Helen Senn[2]

1 Edinburgh Napier University, School of Applied Sciences, Edinburgh, United Kingdom

2 RZSS WildGenes, Royal Zoological Society of Scotland, Edinburgh, United Kingdom

3 Office for the Conservation of Environment, Diwan of Royal Court, Muscat, Oman

4 Al Ain Zoo, Life Sciences Department, Abu Dhabi, United Arab Emirates.

Authors' contributions. MKA participated in the design of the study, collected the field samples, carried out the molecular laboratory work, analysed the data and drafted the manuscript; PW and RAB participated in the design of the study and participated in reviewing the manuscript; KLD and A.B developed the genetic analysis protocols and participated in reviewing the manuscript; MG participated in developing the genetics protocol and helped perform the molecular laboratory work; ST and TA contributed to study deign and collected the field samples from the central region. MA and HA collected the field samples from the central region; HA, AA, AH and NZ collected the field samples from the southern region; MC

provided the samples from UAE captive centres; HS participated in the design of the study, developed the genetic analysis protocols and participated in reviewing the manuscript. All authors gave final approval for publication

2.1 Abstract

The Nubian ibex (*Capra nubiana*) is patchily distributed across parts of Africa and Arabia. In Oman, it is one of the few free-ranging wild mammals found in the central and southern regions. Its population is declining due to habitat degradation, human expansion, poaching, and fragmentation. Here we investigated the population's genetic diversity using mitochondrial DNA (D-loop 186bp and cytochrome *b* 487bp). We found that the Nubian ibex in the southern region of Oman was more diverse (D-loop HD; 0.838) compared to the central region (0.511) and gene flow between them was restricted. We compared the genetic profiles of wild Nubian ibex from Oman with captive ibex. A Bayesian phylogenetic tree showed that wild Nubian ibex form a distinct clade independent from captive animals. This divergence was supported by high mean distances (D-loop 0.126,cytochrome *b* 0.0528) and high F_{ST} statistics (D-loop 0.725,cytochrome *b* 0.968). These results indicate that captive ibex are highly unlikely to have originated from the wild population in Oman and the considerable divergence suggests that the wild population in Oman should be treated as a distinct taxonomic unit. Further nuclear genetic work will be required to fully elucidate the degree of global taxonomic divergence of Nubian ibex populations.

Keywords: Genetic diversity, Nubian ibex, cytochrome *b*, D-loop, Oman, conservation.

2.2 Introduction

The *Capra* (or goat) genus is distributed widely in the three continents of Europe, Africa and Asia, and its range extends from the cooler areas of the Alpine mountains to the hot hyper-arid desert of Arabia (Shackleton 1997). The number of species of *Capra* is debated and described as containing between six and nine species, but they are all rocky montane specialists (Schaller 1977; Shackleton 1997) . According to phenotypic characteristics, *Capra* species are divided into three groups: markhor, ibex and true goats (Groves & Grubb 2011). Heptner et al. (1961) divided the ibex into seven species: Spanish ibex (*C. pyrenaica*), Alpine ibex (*C. ibex*), Dagestan tur (*C. cylindricornis*), Caucasian ibex (*C. caucasica*), Siberian ibex (*C. sibirica*), Nubian ibex (*C. nubiana*) and Walie ibex (*C. walie*). The more widely accepted classification, which is used by the International Union for the Conservation of Nature (IUCN), is that the *Capra* consists of nine species: the seven ibex, a single species of markhor (*C. falconeri*) and wild goat (*C. aegagrus*) (see Table A1.2 in appendixes).

The Nubian ibex (*C. nubiana*) is the smallest *Capra* species (Groves & Grubb 2011). Males weigh between 55 and 65 kg and are distinguished by long curved horns, while females are much lighter, weighing in the region of 21 to 27 kg with smaller and thinner horns (Habibi 1997; Massolo et al. 2008) (Figure 2.1A). The distribution of the Nubian ibex extends from north east Africa through the Middle East and into the Arabian Peninsula (Alkon et al. 2008) (Figure 2.1B).

A

Figure 2.1. *A) Nubian ibex male captured by camera trap at Al-Wusta Wildlife Reserve (WWR) (Oman) (by Steven Ross & Taimur Al-Said). B) distribution range of C. nubiana in the Middle East and Africa (Ross et al. 2020a). C) Map of Oman showing the sampling locations represented by black circles. The samples were collected from three different places: Al Wusta Wildlife Reserve (WWR) and surrounds, Shalim and Dhofar.*

The taxonomic status of the Nubian ibex has been debated, with earlier studies tending to classify it as a subspecies of the Alpine ibex (*Capra ibex*) because of the close similarity in the morphology of the horns (Schaller 1977). Early comparisons used allozymes to compare the Nubian ibex with the Alpine ibex, but did not find enough supporting evidence to consider the Nubian ibex as a separate species (Granjon et al. 1990; Stüwe et al. 1992). On the other hand, a number of more recent studies have investigated the taxonomy of the genus *Capra* and classified the Nubian ibex as a separate species according to allozyme and mitochondrial DNA results (Hartl et al. 1990; Manceau et al. 1999b; Pidancier et al. 2006; Kazanskaya et al. 2007; Bibi et al. 2012), summarised in Table 2.1.

Groves and Grubb (2011) recommended further splitting of the Nubian ibex into two subspecies based on coat colour differences: the Sudanese Nubian ibex as *C. nubiana nubiana* (F. Cuvier, 1825), and those from the Dead Sea and Sinai as *C. nubiana sinaitica* (Ehrenberg, 1833). Groves and Grubb (2011) debated the merit of species or subspecies classification of the Nubian ibex, as well as postulating a third subspecies in the Arabian Peninsula. However, a lack of specimens from southern Arabia has hindered the resolution of this debate and prevented further understanding of population sub-structure.
Delimiting the boundaries between species is vital for informing management decisions in a conservation context. The IUCN, generally recognized as authoritative for the purposes of conservation, currently classifies the Nubian ibex as a single species with a Red List classification of Vulnerable (Ross et al. 2020a). Here, we reopen the debate by studying samples from the most south-easterly region of the Nubian ibex's range, Oman

Table 2.1. *Published genetic studies investigating taxonomy of the Capra, highlighting their findings regarding the relationship between Nubian and Alpine ibex.*

Reference	Study species	Marker used	Nubian ibex found to be a distinct species from Alpine ibex
Granjon et al. 1990	Alpine ibex; Nubian ibex	Allozymes	No
Hartl et al. 1990	Seven species of Caprini, and two species of *Rupicaprini*	Allozymes	Yes
Stüwe et al. 1992	Alpine ibex and Nubian ibex	Allozymes	No
Manceau et al. 1999b	Eight *Capra* species	Mitochondrial DNA (cytochrome *b* and D-loop)	Yes
Ropiquet & Hassanin 2006	17 Caprini species	Mitochondrial DNA (12S, CO2, Cytb, ND1) and nuclear genes (kCas, PRKC1, SPTBN1 and TG)	Yes
Pidancier et al. 2006	Eight *Capra* species	Mitochondrial DNA (cytochrome *b*) and nuclear Y-chromosome	Yes
Kazanskaya et al. 2007	Eight *Capra* species	Mitochondrial DNA (cytochrome *b* and D-loop)	Yes

The Nubian ibex is a flagship species for conservation efforts in Oman, alongside other important arid-land ungulates (Grobler 2002; CBD 2014). The species is located in fragmented populations from the central region down to the southern region of Oman (Grobler 2002; Alkon et al. 2008; Ross et al. 2020a) (Figure 2.1C). The Nubian ibex in the central region is restricted to the 100-150 km long Al Wusta wildlife Reserve (WWR) escarpment, a hyper-arid region (Massolo et al. 2008). The southern region, in contrast, is ecologically distinct from the central region, being higher and wetter, with the highest floral and faunal diversity of anywhere in Oman (Patzelt 2015). Throughout Oman, Nubian ibex populations are declining in response to poaching, human settlement expansion, feral livestock competition, habitat degradation, and population fragmentation (CBD 2014).
Perhaps surprisingly, there has so far been little genetic research on the Nubian ibex across its range, and this is especially lacking in the Arabian Peninsula. In Oman, there is a pressing need to design and inform a conservation management plan for the species. Therefore we aim to 1) investigate the genetic diversity of the Nubian ibex in Oman, and 2) address the question of whether animals from captive populations would be suitable for future re-introduction/reinforcement programmes.

2.3 Materials and Methods

2.3.1 The study area

The study areas consisted of three locations in Oman where the Nubian ibex is confirmed to exist: Al Wusta Wildlife Reserve (WWR), Shalim and Dhofar. WWR is a protected area in the hyper-arid central region (19.719960 N, 57.496767 E). The WWR sampling area (approximately 150km long) included the southern part of the reserve, where the Nubian ibex is free ranging, and extended beyond the protected area boundaries to cover the largest area of the Nubian ibex range within the central region (Figure 2.1C). The reserve contains no permanent human settlements, but is used frequently by nomadic pastoralists to herd camels and domestic goats. The

second location was Shalim (18.107289 N ,55.650218 E), which is approximately 350 km southwest of WWR in the southern region (Figure 2.1C). Finally, Dhofar is approximately 200 km south west of Shalim. Four locations throughout Dhofar were sampled because, it was expected to host a large population of Nubian ibex compared to WWR and Shalim.

2.3.2 Sampling

2.3.2.1 Wild samples

As a part of the Nubian ibex conservation programme launched by the Omani Office for Conservation of the Environment in 2014, 55 faecal samples were collected non-invasively during setting of camera traps in WWR. Additionally, 12 bone samples and two dried tissue samples were collected from skulls and horns of deceased animals. A single tissue sample was collected from each of six hunted animals, which were confiscated by Royal Oman Police (ROP) in January 2018 in the Shalim area (Oman 2018). Eight tissue samples from hunted ibex from the same area were also sent from Oman to the Royal Zoological Society of Scotland (RZSS) in 2015, but it was not recorded whether the number of samples represents eight individuals or fewer (i.e. multiple samples may have been taken from the same animal(s)).
From Dhofar 84 faecal samples were collected non-invasively and three bone/horn samples were collected from deceased animals. Additional samples were collected from the Natural History Museum of Oman, Muscat, which represent Nubian ibex specimens collected from the wild during survey studies. These included eight samples from WWR and two samples from Shalim.

2.3.2.2 Captive samples

Nine blood samples were collected from animals at Bait Al Barakah breeding centre (the Royal private collection in Muscat, Oman) as part of routine

veterinary examinations of captive-bred animals (Table 2.2). A further 56 blood samples of Nubian ibex from UAE captive populations were used with permission from Al Mayya Sanctuary and Al Ain Zoo in the United Arab Emirates. Faecal samples from the wild and blood samples from captive centres were collected between 2014 and 2018 (Table 2.2). A full list of samples is found in Table A2.1 in appendixes.

Table 2.2. *Locations and types of samples collected for analysis.*

Population	Sampling location/source	Collection Date	Faecal	Blood	Tissue	Bone / Horn	Total
Wild Oman (central region)	Al-Wusta Wildlife Reserve (WWR)	2014-2018	82	1+1*	3*	12	98
	WWR (Natural History Museum Oman)	2019	0	0	1	7	8
Wild Oman (southern region)	Shalim	2013-2018	0	0	14	0	14
	Shalim (Natural History Museum Oman)	2019	0	0	1	1	2
	Dhofar	2019	84	0	0	3	87
Oman Captive	Bait Al Barakah Breeding Centre (Muscat, Oman)	2017	0	9	0	0	9
UAE Captive	Al Mayya Sanctuary (Fujairah, United Arab Emirates) and Al Ain Zoo (Abu Dhabi, United Arab Emirates)	2015-2018	0	56	0	0	56

*indicates that one individual has two samples (one tissue and one blood)

2.3.3 DNA extraction

DNA from blood and tissue samples were extracted using the DNeasy Blood and Tissue kit (QIAGEN®, Germany) according to the manufacturer's instruction. For bone samples, DNA was extracted using QIAamp DNA Investigator Kit (QIAGEN®, Germany) according to the manufacturer's bones and teeth protocol. DNA was extracted from the faecal samples using the Isohelix Xtreme DNA Kit (XME-50), see the supplementary materials A2 for details.

2.3.4 Mitochondrial DNA sequencing

Primer pairs for both D-loop and cytochrome *b* were designed with Primer3Plus (Untergasser et al. 2007), using a GenBank reference sequence from each of the different *Capra* species in order to identify conserved regions (accession numbers included in Table A2.4 in appendixes). Because the D-loop is highly variable and the DNA from the faecal and bone samples was expected to be fragmented due to degradation over time (Lindahl 1993), one hyper-variable segment (242bp) was targeted and amplified using a single primer pair. For cytochrome *b,* three primer pairs were designed to amplify short, overlapping fragments. Table 2.3 shows the primer sequences used for the D-loop and cytochrome *b*.

Table 2.3. *Primer sequences used to amplify targeted markers. All primers were designed for the purposes of this study.*

Marker	Primer ID	Primer sequence (5'>3')	Estimated Fragment Length
D-loop	NUB_D-loop_RZSS_F1	ATGGCACTAATGCAACAAG	242bp
	NUB_D-loop_RZSS_R1	TGCTATGTACGGGTATGCAG	
Cytochrome *b*	NUB_CytB_RZSS_F1	GGACGAGGCCTATATTATGGA	244bp
	NUB_CytB_RZSS_R1	CGAAAAATCGGGTGAGAGTG	
	NUB_CytB_RZSS_F2	TTGGCACAAACCTAGTCGAA	282bp
	NUB_CytB_RZSS_R2	GCAGGTCGGGTGTGAATAGT	
	NUB_CytB_RZSS_F3	CTGCTCTTCCTCCACGAAAC	369bp
	NUB_CytB_RZSS_R3	TGGGCGGAATATTATGCTTC	

PCRs were carried out at a total volume of 10 uL and contained 1 μM of each primer, 1.4X DreamTaq Hot Start Master Mix (Thermo Fisher Scientific Inc., includes 0.28 mM of each dNTP and 2.8mM $MgCl_2$) and 1 μL of extracted DNA. For the faecal samples, 0.2 uL of Bovine Serum Albumin (BSA) were added to the master mix. The PCR program for both D-loop and cytochrome *b* was as follows. The initial denaturation step was at 95°C for 5 minutes. This was followed by 39 cycles of 95°C denaturation for 30 seconds, 55°C for 30 seconds to allow primer annealing and then 72°C for 60 seconds for elongation. A final 72°C extension for 10 minutes completed the programme. PCR products were confirmed by gel electrophoresis before being cleaned up with 0.5 μl of EXO1 enzyme and 0.5 μl FastAP. PCR products were Sanger sequenced in both directions using the Big Dye Terminator Kit v3.1 (Applied Biosystems) on an ABI 3730 DNA Analyzer (Applied Biosystems).

2.3.5 Sequence Analysis

Sanger sequences were trimmed and quality checked by eye using Geneious software (Version 11.1.5). The three overlapping cytochrome *b* sequences generated from the same sample were aligned and the consensus sequence generated. The good quality sequences were then aligned with reference sequences from GenBank (see Table A2.4 in appendixes) and trimmed to an equal size of 186bp (D-loop) and 486bp (cytochrome *b)* using MEGA X (Kumar et al. 2018).

To assess the genetic diversity of the Nubian ibex from Oman in relation to individuals from other sources, median-joining (Bandelt et al. 1999) and TCS haplotype networks were built using PopArt software v1.7 (Leigh & Bryant 2015) for both the D-loop and cytochrome *b*. To estimate the robustness of the sample sizes obtained by this study, we produced haplotype accumulation curves using the R (v.3.5.3) package HACSim (Phillips et al. 2015, 2019, 2020). This is used to estimate the total sample size, which is required to capture all the haplotypes in a specific population.

The accession numbers for other *Capra* species used to construct the phylogenetic trees can be found in Table A2.5 in appendixes. Concatenation of D-loop and cytochrome *b* to a total length of 673bp was performed using Geneious (Version 11.1.5). In addition, the cytochrome *b* sequences were translated into amino acids to check for evidence of incorrect amplification of nuclear insertions of mitochondrial sequences (NuMtS) (Hazkani-Covo et al. 2010)

2.3.6 Phylogenetic analysis

A phylogenetic tree was constructed for the concatenated sequences. Whole mitochondrial genome sequences for different *Capra* species along with other species sequences imported from GenBank were used and can be found in Table A2.4 in appendixes. Bos taurus (AY676870) was used as an outgroup. The evolutionary model used for conducting the analyses was HKY+Γ+I, as selected using jModelTest in the R package “phangorn” (Schliep 2010). The phylogenetic tree for the concatenated sequences was constructed by MrBayes (Huelsenbeck & Ronquist 2001) within Geneious (version 11.1.5). The parameters used were as follows: total chain length 1,000,000, subsample frequency 200, and a burn-in of 10% of the trees was applied.

2.3.7 Genetic diversity statistics

Mean genetic distances between the wild Nubian ibex samples from each location in Oman and the captive animals were calculated with MEGA X using the maximum composite likelihood (Tamura et al. 2004; Kumar et al. 2018). In addition, the genetic distance and differentiation between the wild Nubian ibex and the captive ibex were calculated using DnaSP v 6.12.03 (Rozas et al. 2017). Analysis of molecular variance (AMOVA) was carried out using Arlequin v3.5 (Excoffier & Lischer 2010). This was used to measure the population genetic structure within and between groups, using 159 and 131 sequences for D-loop and cytochrome *b,* respectively. The permutation was set to default (1,023) at a significance value of p=0.05.

2.4 Results

2.4.1 Haplotype networks

A total of 188 sequences were successfully amplified for the cytochrome *b*. The quality control check excluded 57 samples due to poor amplification. Therefore, the final sample set included 131 high quality sequences, which were used to create an alignment with total length of 487bp for the analysis (see Table A2.2 in appendixes). A total of seven cytochrome *b* haplotypes were found in this study (named, A to G). Wild Nubian ibex from Oman were found to have three haplotypes (A, B and C), while the captive populations were found to contain four haplotypes (D, E, F and G). There were no shared haplotypes between the wild and captive populations. Regarding the wild sampling locations in Oman, two haplotypes were identified in the WWR (A and B). Dhofar was found to share both these haplotypes while, Shalim only had one haplotype (A, shared with both WWR and Dhofar). Dhofar on the other hand had an additional unique haplotype (C). The geographical distribution of the haplotypes of the wild Nubian ibex in Oman is illustrated in Figure 2.2.

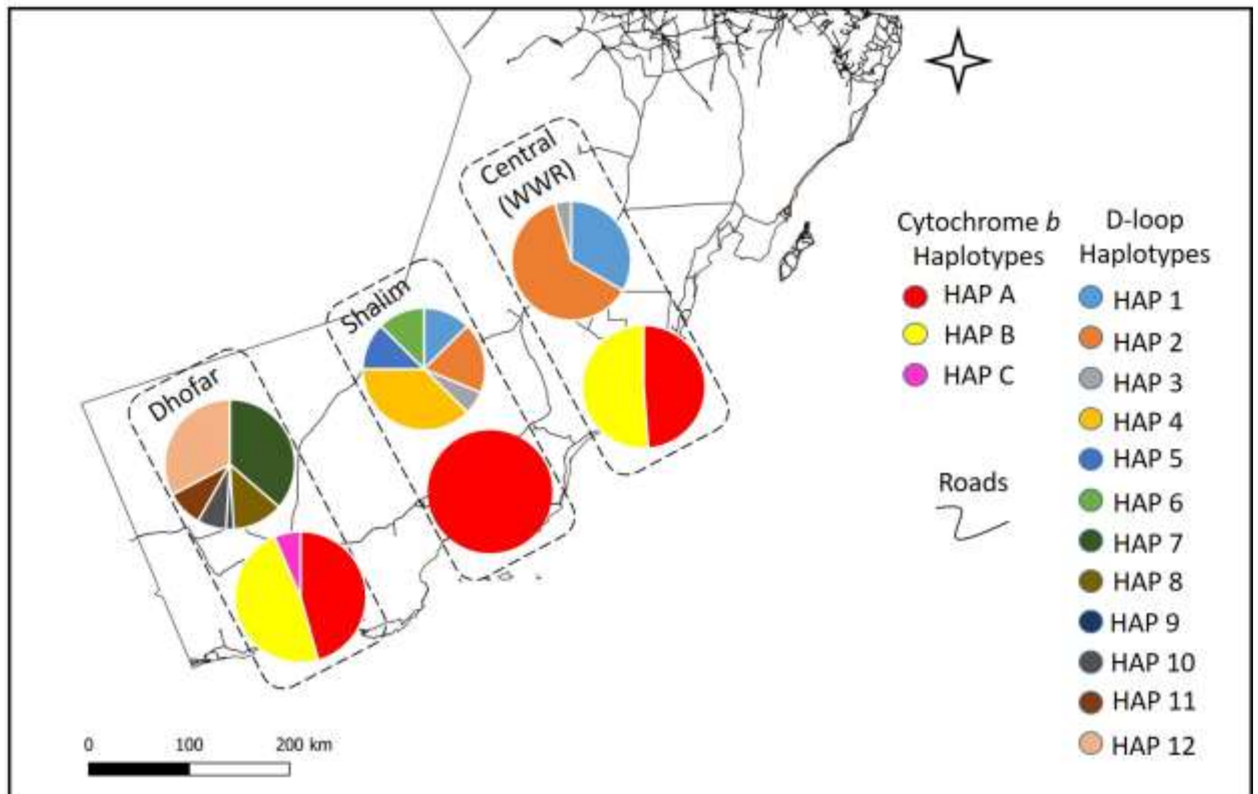


Figure 2.2. *The distribution of the mitochondrial DNA haplotypes within each location. The lower circle for each region represents cytochrome b, while the upper circle is D-loop. The size of the circle does not represent sample size.*

The Omani captive population had three cytochrome *b* haplotypes in total, including two unique haplotypes (D and G) and one haplotype shared with the UAE captive population (E). In addition, the UAE captive population had an additional haplotype (F; Figure 2.3) (Table A2.3 in appendixes). All haplotype sequences were submitted to NCBI GenBank under accession numbers (MW911255-MW911278).

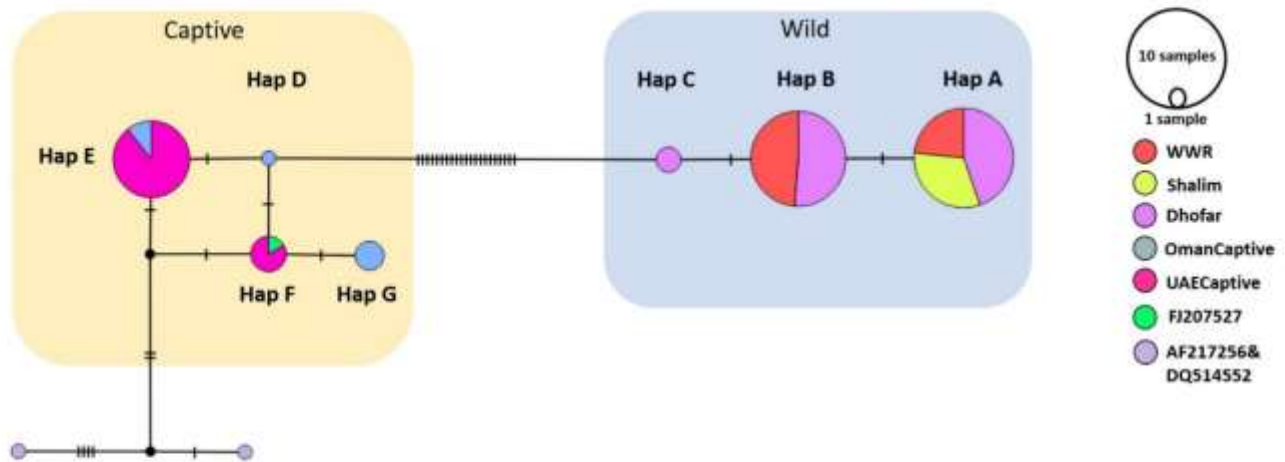


Figure 2.3. *Median-joining haplotype network for cytochrome b (487bp). The wild animals from Oman are in the right-hand blue box, while the captive animals are in the left-hand orange box. Each coloured circle represents a single haplotype, the size of which is proportional to the number of samples. The tick marks represent the mutational steps between haplotypes. Three reference sequences of C. nubiana from GenBank were used (Accession numbers given on the figure).*

A total of 159 sequences were successfully amplified for the D-loop and were used in the analysis. The total sequence alignment length was 186bp, revealing seventeen haplotypes (numbered 1 to 17). There were no haplotypes shared between the wild and captive Nubian ibex. The wild Nubian ibex exhibited 12 D-loop haplotypes (1 to 12) while, the captive ibex have five different haplotypes (13 to 17). WWR was found to have three D-loop haplotypes (1-3), all of which were shared with Shalim. Shalim had six D-loop haplotypes, three of which were unique (4, 5 and 8). Dhofar had six unique D-loop haplotypes (6, 7, 9, 10, 11

and 12), which were not shared with either Shalim or the WWR. On the other hand, the Omani captive animals were found to have three D-loop haplotypes, two of which were unique and one which was shared with the UAE captive animals. The UAE sample also had two unique D-loop haplotypes not shared with Omani captive animals (Figure 2.4).

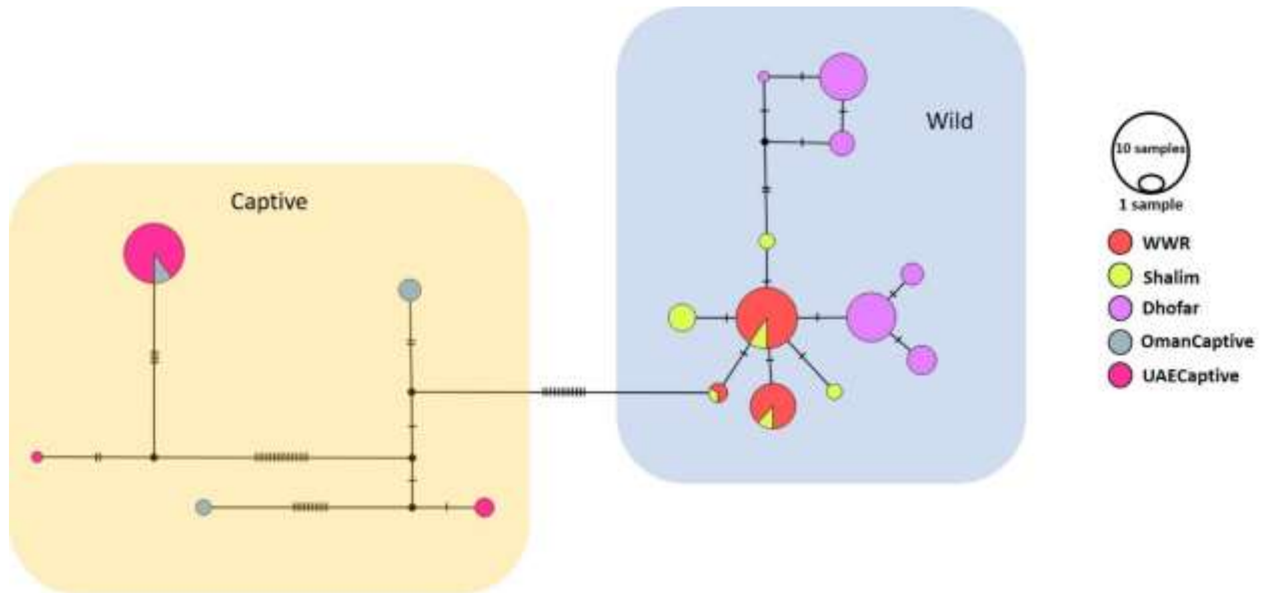


Figure 2.4. *Median-joining haplotype network for D-loop (186bp).The wild animals from Oman are in the right-hand blue box while the captive animals are in the left-hand orange box. Each coloured circle represents a single haplotype, the size of which is proportional to the number of samples. The tick marks represent the mutational steps between haplotypes.*

The median-joining networks showed a clustering pattern for the wild Nubian ibex in both the cytochrome *b* (Figure 2.3) and the D-loop (Figure 2.4). There are 21 mutations separating the wild from the captive ibex in cytochrome *b* and 12 mutations in D-loop.

For cytochrome *b,* the haplotype accumulation curve reached an asymptote (Figure 2.5) where 98.5% of the haplotypes have been sampled at p= 0.05 confidence. On the other hand, the haplotype accumulation curve for the D-loop was slightly below the asymptote, but the difference between the sampled and unsampled haplotypes is small (R= 93.7% of the haplotypes were sampled at a 95% confidence interval) (Figure 2.5). This indicates that the sampling process is likely representative of the Nubian ibex populations in Oman.

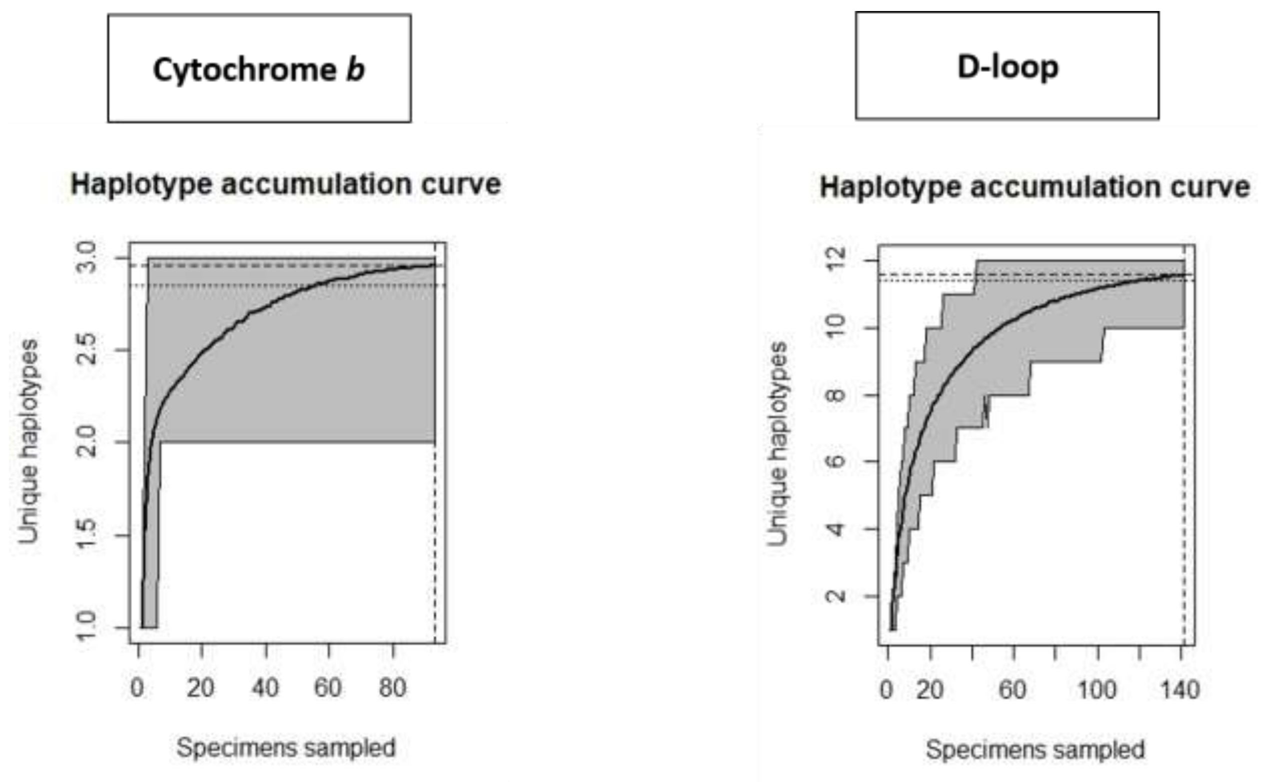


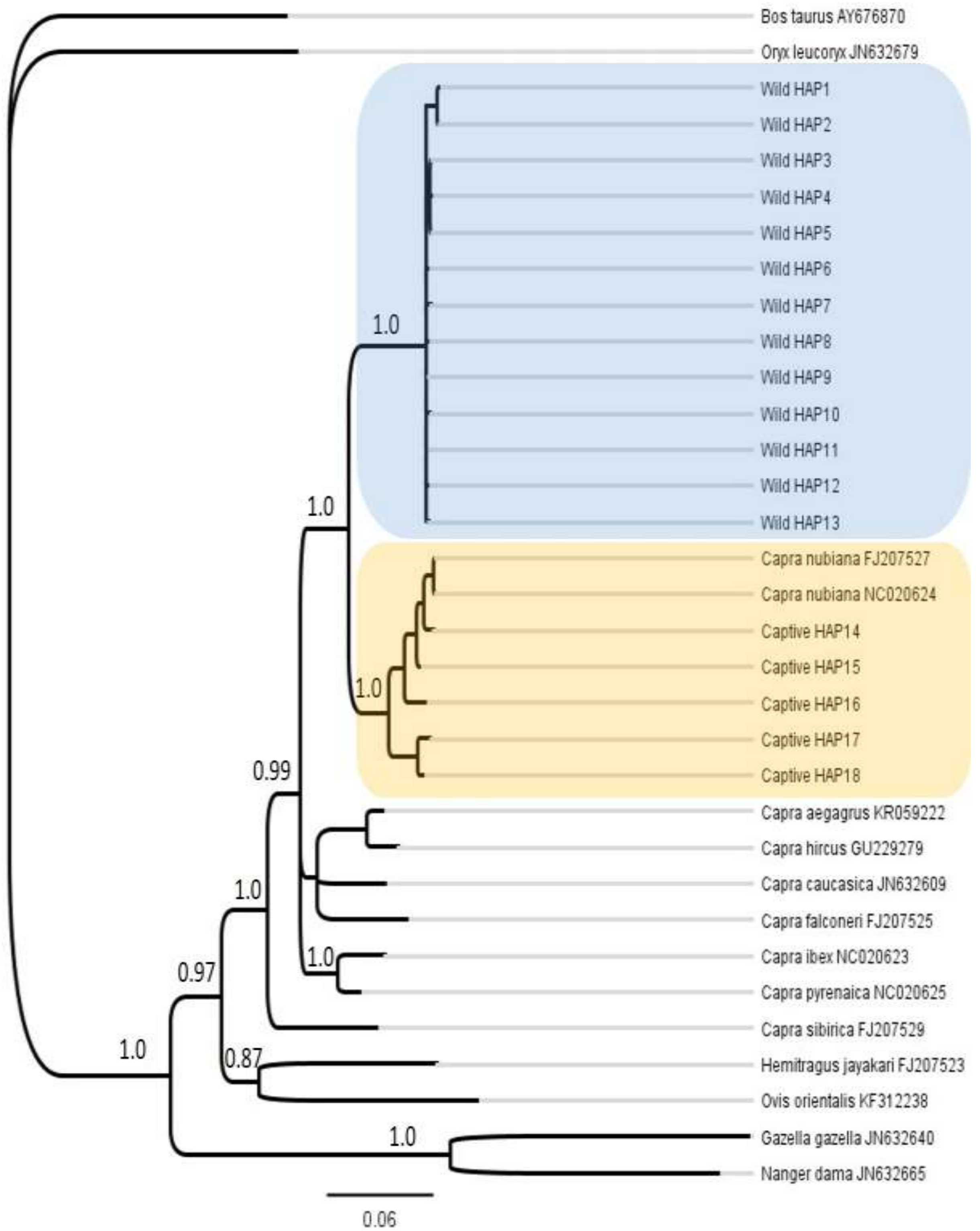
Figure 2.5. *Haplotype accumulation curves for cytochrome b (left) and D-loop (right) within the wild Nubian ibex samples. For cytochrome b it is estimated that more than 98.5% of the haplotypes are represented, while for the D-loop this is 93.7%.The dashed lines show the number of haplotypes found corresponding with sampled individuals. The dotted lines represent the expected numbers of haplotypes which should be found in the given population.*

2.4.2 Phylogenetic trees

The number of concatenated mitochondrial DNA (mtDNA) haplotypes (cytochrome *b* and D-loop) was 18. The wild Nubian ibex from Oman had 13 concatenated haplotypes (named WildHAP1 to WildHAP13). The captive animals had five concatenated haplotypes, named (CaptiveHAP14 to CaptiveHAP18).

The phylogenetic tree of the concatenated mtDNA sequences shows separation of the *C. nubiana* from the rest of the *Capra* species (Figure 2.6). This separation is supported with a 0.99 posterior probability. Our data indicate two well-supported clades within *C. nubiana*: the first one contained the wild samples from Oman, while the second one contains the samples from Oman and UAE captive animals. The divergence is supported by a posterior probability of 1.0 (Figure 2.6). The concatenated haplotypes from captive samples cluster with the reference sequences from GenBank (NC020624 and FJ207527) (Hassanin et al. 2009). This suggests that the original source of the captive animals in both Oman and the UAE is similar, but was not from the current distribution of the wild Omani population. Unfortunately, the original collection locations of the GenBank sequences (museum samples) were not detailed.

Additional trees, which were constructed using Bayesian analysis within StarBEAST 2 software (Bouckaert et al. 2019), retrieved the same tree topology ensuring the stability of the phylogenies (see Figures A2.1, A2.2 and A2.3 in appendixes). The Bayesian phylogeny shows a large evolutionary separation between the wild Nubian ibex in Oman and the captive Nubian ibex samples.



Bos taurus AY676870
Oryx leucoryx JN632679
Wild HAP1
Wild HAP2
Wild HAP3
Wild HAP4
Wild HAP5
Wild HAP6
Wild HAP7
Wild HAP8
Wild HAP9
Wild HAP10
Wild HAP11
Wild HAP12
Wild HAP13
Capra nubiana FJ207527
Capra nubiana NC020624
Captive HAP14
Captive HAP15
Captive HAP16
Captive HAP17
Captive HAP18
Capra aegagrus KR059222
Capra hircus GU229279
Capra caucasica JN632609
Capra falconeri FJ207525
Capra ibex NC020623
Capra pyrenaica NC020625
Capra sibirica FJ207529
Hemitragus jayakari FJ207523
Ovis orientalis KF312238
Gazella gazella JN632640
Nanger dama JN632665
1.0
1.0
1.0
0.99
1.0
1.0
0.97
1.0
0.87
1.0
0.06

Figure 2.6. *Bayesian phylogenetic tree of concatenated mtDNA sequences of cytochrome b (487bp) and D-loop (187bp) calculated by MrBayes. The blue box indicates Oman wild Nubian ibex (WildHAP1 to WildHAP13). The yellow box indicates the captive Nubian ibex (CaptiveHAP14 to CaptiveHAP18). GenBank sequences are indicated by their accession numbers adjacent to the species binomial. The numbers at the nodes represent the posterior probabilities.*

2.4.3 Nubian ibex genetic diversity

Both cytochrome *b* and D-loop had higher haplotype diversity in the wild Nubian ibex (0.54 and 0.85 respectively) compared with the captive ibex (0.44 and 0.47 respectively). However, in both regions, nucleotide diversity was higher in the captive ibex (0.002 and 0.046) compared with the wild Nubian ibex (0.001 and 0.014). Table 2.4 shows the molecular diversity measures of the wild and captive Nubian ibex.

Table 2.4. *Molecular diversity measures for both cytochrome b and D-loop between wild and captive animals.*

	Cytochrome *b*		**D-loop**	
Population	**Oman wild**	**Captive**	**Oman wild**	**Captive**
Number of haplotypes, h	3	4	12	5
Haplotype diversity, Hd	0.54	0.44	0.85	0.47
Segregating sites, S	2	3	12	32
Nucleotide diversity, Pi	0.001	0.002	0.014	0.046
Number of sequences	93	38	116	43
Sequence length	487bp		186bp	

Maximum- likelihood mean distances between wild Nubian ibex populations in Oman and the captive animals for cytochrome *b* and D-loop can be found in Table A2.6 in appendixes.

In terms of the wild Nubian ibex, it is clear that the haplotypes and nucleotide diversity is higher in the southern region, than in the central region (Table 2.5). In addition, the F_{ST} value between the central region and the southern region is 0.09 for cytochrome *b* and 0.32 for the D-loop.

Table 2.5. *Comparison of genetic diversity indices between Nubian ibex samples from the central and southern region*

	Central region		**Southern region**	
	Cytochrome *b*	D-loop	Cytochrome *b*	D-loop
Number of haplotypes, h	2	3	3	12
Haplotype diversity, Hd	0.465	0.511	0.587	0.838
Segregation Sites, S	1	2	2	12
Nucleotide diversity, Pi	0.001	0.002	0.0012	0.0177
Number of sequences	32	45	61	71

Furthermore, the genetic diversity of the Nubian ibex in terms of the samples location showed that haplotype diversity is higher in Dhofar (0.57) for cytochrome *b* compared with WWR (0.47) (Table 2.6). For the D-loop Shalim has the highest haplotype diversity (0.83), while Dhofar is intermediate (0.65), and WWR has the lowest diversity (0.51) compared with other locations (Table 2.6). Moreover, the F_{ST} for D-loop between Dhofar and Shalim was found to be 0.39, while the F_{ST} between Dhofar and WWR was 0.43 and between Shalim and WWR was 0.15. Therefore, the greatest similarity (and likely gene flow) is between Shalim and WWR.

Table 2.6. Comparison of genetic diversity indices for Nubian ibex from WWR, Shalim and Dhofar

	Cytochrome *b*			**D-loop**		
Population	WWR	Shalim	Dhofar	WWR	Shalim	Dhofar
Number of haplotypes, h	2	1	3	3	6	6
Haplotype diversity, Hd	0.47	0.0	0.57	0.51	0.83	0.65
Segregating sites, S	1	0	2	2	5	9
Nucleotide diversity, Pi	0.001	0.0	0.001	0.002	0.005	0.016
Number of sequences	32	15	46	45	16	55
Sequence length	487bp			186bp		

2.4.4 Genetic divergence between wild and captive Nubian ibex

Pairwise differences showed that there was 0.053 (5.3%) divergence at cytochrome *b* and 0.126 (12.6%) at the D-loop between the wild and captive Nubian ibex. In addition, the F_{ST} between the wild and captive populations calculated by DnaSP v6.0 was found to be 0.97 and 0.73 for cytochrome *b* and D-loop respectively, indicating a large genetic difference between the wild and the captive animals. AMOVA results showed high diversity in cytochrome *b* among the Nubian ibex populations in Oman and the captive animals (97.19%, $P<0.0001$; Table 2.7). The D-loop statistics support these results, with the highest diversity among populations compared to within populations (77.87%, $P<0.0001$). The significant diversity between wild Nubian ibex in Oman and the captive population is further supported by genetic distance values (F_{ST} 0.97, $p < 0.0001$ and F_{ST} 0.78, $p < 0.0001$) for cytochrome *b* and D-loop respectively (Table 2.7).

Table 2.7. *Analysis of molecular variance for cytochrome b and D-loop among Nubian ibex in the wild (Oman) and in captivity (Oman and UAE).*

D-loop among two populations (Omani Wild and Captive)						
Source of variation	df	Sum of square	Variance components	Percentage variation	Fixation Index (Fst)	P-value
Among populations	1	468.185	7.428 Va	77.87	0.779	<0.0001
Within populations	157	331.368	2.111 Vb	22.13		
Total	158	799.553	9.539			
Cytochrome *b* among two populations (Omani Wild and Captive)						
Among populations	1	635.652	11.775 Va	97.19	0.972	<0.0001
Within populations	129	43.966	0.341 Vb	2.81		
Total	130	679.618	12.11583			

The mean divergence distance between the wild Oman and reference samples from Egypt (AJ009879) and Israel (DQ514552 and AF217256) within cytochrome *b* was found to be 0.072 (7.2%) and 0.055 (5.5%) respectively. Additional comparison between wild Oman and other *Capra* species can be found in Table A2.7 in appendixes.

2.5 Discussion

Previous studies on the Nubian ibex in the Arabian Peninsula have mostly focused on ecology and population distribution (Habibi & Grainger 1990; Habibi 1997; Massolo et al. 2008). This is the first study to address the genetic diversity of the Nubian ibex in Oman. It provides the first insight into the population genetic structure of this species and produces vital baseline information for future management strategies.

Based on the results of mtDNA of 186bp D-loop and 487bp cytochrome *b* sequences, we found that the populations in the southern region (Shalim and Dhofar) are genetically more diverse than the central region (WWR). The haplotype accumulation curves indicate that the sampling process has provided a highly representative sample set that captures a good representation of haplotypes present in the Omani populations (Figure 2.5). The gene flow between these populations is restricted (F_{ST}=0.32 for the D-loop), although there may be greater gene flow between Shalim and WWR (evidenced by shared haplotypes and F_{ST} = 0.15 for D-loop). One cytochrome *b* haplotype is shared between the central and the southern region and three D-loop haplotypes are shared only between the central region and its closest population in the southern region, Shalim. This is likely to be attributed to a combination of a stepping-stone pattern (Frankham et al. 2017) between the three populations, the semi-isolation of the central region population, and the extremely limited connectivity between regions due to human activities such as road construction and oil exploration.

2.5.1 Population-level genetic substructure of the Nubian ibex in Oman

The results reveal genetic sub-structuring within Nubian ibex populations in Oman, clearly illustrated by the mtDNA haplotype distribution. Nubian ibex in Oman appear to display a stepping-stone pattern (Frankham et al. 2017), where

only neighbouring populations share haplotypes. The central region population (WWR) is in close proximity with the Shalim population in the southern region (F_{ST}= 0.15) which likely explains the shared D-loop haplotypes between them. The longer distance between the Dhofar population and both Shalim and the central population (WWR), resulting in very limited gene flow amongst the regions (Dhofar – Shalim $F_{ST=}$0.39 and Dhofar – WWR $F_{ST=}$0.43), likely explains the lack of shared haplotypes.

The genetic diversity of the Nubian ibex in WWR is remarkably lower than that of Dhofar. This low level of diversity may be attributed to the isolated nature of the population with restricted or even no gene flow, which could be due to human activities and, specifically, oil exploration or roads which bisect wildlife corridors. For example Ross et al. (2020b) found two genetic clusters in the Arabian tahr (*Arabitragus jayakari*) population in Oman, which were separated by human barriers (i.e. roads and highways). They speculated that these roadblocks will contribute to increasing the genetic divergence of these populations and will eventually cause an increase in inbreeding in the long term. The same factors found by Ross et al. (2020b) may be applied to the Nubian ibex population in the central and southern regions. These factors include resistance to movements between the populations, the long distances between them, and urbanisation and development. In addition, despite the substantial efforts taken by the authorities to limit and minimize hunting, wildlife populations (Arabian Oryx – *Oryx leucoryx*, Mountain gazelle- *Gazella gazella* and Nubian ibex) in the central region have suffered from high hunting pressure, especially between 1996 and 1999 (Spalton et al. 1999).

The relatively high genetic diversity found in the Dhofar population could be associated with a relatively large and highly diverse area of habitat. In addition the population estimated there is larger than that of WWR, which ranged between 600 and 1100 individuals (Ross et al. 2020a). Furthermore, the possible connectivity with the Yemeni population could play a vital role in exchange of immigrants between the populations. However, it is not clear

whether these populations are still connected given that many areas which could function as connections between the populations in Oman and Yemen are now occupied by human settlements or intersecting roads (CBD 2014). In addition, a security fence has been constructed between the borders of Oman and Yemen, which is likely to further limit any gene flow.

It should be noted that the analyses carried out here have used only mitochondrial DNA, which is only inherited maternally, and are therefore limited in their scope to elucidate population structure and gene flow in a species for which male-biased dispersal is typical. Further research using nuclear makers would provide valuable insight into whether gene-flow between populations in Oman remains limited after accounting for male-biased dispersal. Nevertheless further investigation is needed on anthropogenic effects (such as road building, hunting and oil exploration) on wildlife in Oman and how they affect avoidance behaviour, movement and distribution. Analyses involving nuclear markers may help to elucidate whether the extent of genetic sub-structuring of Nubian ibex populations in Oman is a result of historical or human-driven effects or both.

2.5.2 The genetic diversity of the Nubian Ibex in Oman

In general, the D-loop haplotype diversity estimate for the wild Nubian ibex in Oman (Hd=0.85) was found to be in the same range as those of other wildlife species, which indicates a considerable level of genetic diversity. On the other hand, the nucleotide diversity (Pi=0.014) was low compared with other wild ungulate species. For example haplotype and nucleotide diversity in Dama gazelles (*Nanger dama*) from the wild population in central Chad was found to be Hd=0.84 and Pi =0.031, while the captive population was found to be Hd=0.49 and Pi=0.013 (Senn et al. 2014a). This indicates that in general within a species wild populations tend to be more diverse compared with captive populations and the Nubian ibex population in Oman shows the same pattern.

Table A2.8 in appendixes shows additional comparisons of genetic diversity between the Nubian ibex and other species.

The haplotype networks for both the cytochrome *b* and D-loop, and the phylogenetic trees for the concatenated sequences illustrate a substantial differentiation between the Nubian ibex populations in the wild in Oman and those in captivity. The captive population haplotypes cluster with both the cytochrome *b* and D-loop haplotypes of the available NCBI reference sequences. This suggests that the source of these captive animals could be the Levant region or North Africa, as it is for the NCBI sequences with known geographical descriptors, given as Egypt (Manceau et al. 1999b) and the Dead Sea (Pidancier et al. 2006). The other NCBI sequences come from the Museum National d'Histoire Naturelle, France and are likely from the European zoo population which is thought to have originated in North Africa (Hassanin et al. 2009). It is unfortunate that more detailed descriptors of location are not available, and this illustrates the clear need for samples to be submitted to NCBI with more precise locality/origin descriptions.
There was no sharing of haplotypes between the wild Omani population and the captive animals at either mtDNA marker, which indicates that the Omani population is extremely distinctive from other Nubian ibex populations that have been studied and may deserve to be treated as a distinct taxonomic unit. This is supported by the AMOVA, results which showed a well-structured population with high variation among the populations (77.87% for D-loop and 97.19% for cytochrome *b*). The variance difference between the population is high (F_{ST} = 0.77 for D-loop and F_{ST} = 0.97 for cytochrome *b*). The distinctiveness of the Nubian ibex in Oman is also shown in the Bayesian phylogenetic trees of the concatenated sequences, which are supported by a high posterior probability of 1.0 (Figure 2.6).

In terms of genetic divergence, our results are in the same range as several other studies, which investigated the taxonomic status of ungulates. For examples Wronski et al. (2010) identified two different species of *Gazella*:

Gazella gazella in the Levant and *G. arabica* in the Arabian Peninsula. This study estimated the average distance between these two populations as 12.7% for D-loop which is a similar level of divergence to our results (D-loop = 12.6%). Manceau et al. (1999a) identified an evolutionary significant unit (ESU) for one Pyrenean ibex (*C. pyrenaica*) population where average distances between the Pyrenean and other Spanish populations were estimated to be D-loop=5.3% and cytochrome *b*=1.6%, and between the Pyrenean and the Alpine ibex to be D-loop=5.7% and cytochrome *b*=1.8%. Our results are higher than those of Manceau et al. (1999a) (D-loop =12.6%; cytochrome *b* =5.3%), which suggests strongly that the Nubian ibex in Oman may certainly deserve to be treated as a distinct taxonomic unit. Whether it would be appropriate for this to be at the species or subspecies level requires additional sampling and nuclear data. We recognise that the capability of mitochondrial markers to specifically differentiate between species is limited due to introgression or incomplete lineage sorting (Funk & Omland 2003).

In particular, the animals from captivity might not be reliable candidates for investigating the uniqueness of the Nubian ibex in Oman, because it has been documented that *Capra* species in captivity can hybridize with other species such as domestic goats (Hammer et al. 2008). Additionally, captive populations are susceptible to high levels of genetic drift due to founder effects. The founder history of the captive populations of the Nubian ibex is poorly known, however it is unlikely to have a large founder base. For example the breeding programme of the Association of Zoos and Aquariums in North America is thought to be founded from approximately 13 individuals (Putnam et al. 2020). Genetic drift could, therefore, exacerbate the divergence between the captive and wild populations.

Studies based on nuclear markers and samples from Saudi Arabia, Yemen, Jordan, Egypt and Sudan are now needed to ascertain the phylogeographical relationship between Nubian ibex populations across their range. However, this

study sheds light on the putative difference between the wild Nubian ibex in Oman and its counterpart in the Levant and North Africa.

2.5.3 Gene flow and genetic diversity

The results from this study reveal that the population in the central region may be isolated and has lower genetic diversity than the southern populations, especially the Dhofar population. Small, isolated populations with restricted gene flow are at risk of inbreeding depression and further loss of genetic diversity. Inbreeding may cause reduction in reproductive fitness and genetic diversity loss will reduce the ability of the population to adapt to changing environments (Frankham et al. 2004). Therefore, we recommend measures to limit the loss of genetic diversity. Wildlife corridors could be introduced and maintained between the Nubian ibex populations, or translocation of individuals between populations could replicate natural gene flow. The augmentation of an isolated, less genetically diverse population has been found to boost its genetic diversity and alleviate inbreeding depression (Frankham et al. 2017; Ralls et al. 2020). Protection of the Nubian ibex is particularly important for Oman, specifically as it is considered one of the only a few large wild mammals species with appreciable free-ranging populations (CBD 2014), and for the species as a whole given its vulnerability to extinction (Ross et al. 2020a), which may be greater than previously assessed given the taxonomic questions raised by this study. If corridors between subpopulations are to be maintained, then protections need to be put in place to prevent their destruction from human activities, such as oil exploration, roads, hunting and agriculture. This could take the form of mitigation measures that combat and limit levels of disturbance, especially where there are oil exploration activities. Such developments must implement measures to reduce fragmentation and destruction of wildlife habitat and to mitigate the level of disturbance caused by infrastructure during development.

2.6 Conclusion

This study has demonstrated that the Nubian ibex population in Oman is highly distinct and not closely related to any of the assessed captive Nubian ibex populations in Oman and UAE. The mtDNA analysis of both cytochrome *b* and D-loop showed a deep divergence between the wild and the captive animals. Therefore, we recommend that captive animals are not used for any future reintroduction programmes until more detailed genetic data become available. If deemed appropriate and necessary, a captive breeding programme in Oman should be initiated from wild individuals from southern and central regions, which should create a relatively diverse population that can be used for reienforcement to populations of low genetic diversity such as central region (IUCN/SSC 2014). With this in mind, we currently recommend treating the wild Nubian ibex populations in Oman as distinct from captive populations and that they are managed separately. Their habitat must be conserved and protected from further human destruction and monitoring strategies put in place that assess in relation to genetic diversity levels and population numbers through time.

Both translocation and captive-breeding strategies need to be carefully investigated prior to their inception to ensure that they do not harm existing wild populations, by following IUCN guidelines (IUCN/SSC 2013, 2014) and to ensure that they work in tandem with comprehensive *in-situ* management of threats (e.g. the OnePlan approach) (WAZA 2013).

Further nuclear data and global reference samples covering the extant range of the Nubian ibex are required to elucidate the taxonomic position of the Nubian ibex in Oman and whether it deserves to be treated as a distinct subspecies or species from Nubian ibex across the rest of Arabia and North Africa. This is not only important from an Oman conservation perspective, but is required to

spread further light on population structure and connectivity across the range of Nubian ibex.

Data accessibility. The datasets supporting this article have been uploaded as part of the supplementary material. The sequences generated in this study were deposited to NCBI GenBank under accession numbers MW911255-MW911278.

Ethics statement. Blood samples collected from captive centres in Oman and UAE were as part of routine veterinary examinations of captive bred animals. From Oman, the approval for collecting and using blood samples of the Nubian ibex was taken from Dr Khalid Al-Rasbi director general of Bait Al Barakah breeding centre (the Diwan of the Royal Court, Muscat, Oman). Samples from UAE were used after approval from Mark Craig (Al-Ain zoo).

The field work and samples collection were approved by the Office of Conservation for the Environment (Muscat, Oman) in 2014 as part of the Nubian ibex conservation program.

Funding. This project is funded and supported by the Diwan of the Royal Court and Office for Conservation of Environment, Sultanate of Oman.

Acknowledgements. We would like to express our sincere thanks to his excellency Sayyid Khalid bin Hilal Al-Busaidi the minister of the Diwan of the Royal Court, Oman, for his kind grant and support to this project. We would like to thank Mr Yasser Al-Salami, The Director General of Office for Conservation of Environment and Dr Mansoor Al-Jadhami for their continuous support to the project. Also a special thanks goes to Dr Qais Al-Rawahi and Salim Al-Rubaii for providing all the help and logistic support for collecting samples from central and southern regions respectively. In addition, we would like to extend special thanks to Ms Hanan Al-Nabhani for her help in collecting samples from the Natural History Museum of Oman. We are extremely grateful to Balazs Buzas, Al Mayya Sanctuary, Fujairah, UAE for the sharing of captive samples. Thanks also to David Mallon for comments on the draft.

Chapter 3 Generating additional global mitochondrial reference data for the Nubian ibex (*Capra nubiana*) from Sudan and Yemen.

3.1 Abstract

Populations of Nubian ibex in Oman are found in patchily distrubuted locations in the central and southern regions of the country. The isolated nature and discontinuous range of this species in Oman posed questions regarding its relationship with other Nubian ibex populations in other countries especially the neighbouring countries like Yemen. This is the first study to use museum samples from Sudan and Yemen to investigate the population structure and differentiation in Nubian ibex. A mitochondrial cytochrome *b* region (487bp) was used to assess the genetic structure of Nubian ibex populations in Oman, Yemen and Sudan. The results revealed a highly significant divergence between Nubian ibex in Oman and Sudan (F_{ST} = 0.971) and between Yemen and Sudan (F_{ST} = 0.979). In addition, the considerable divergence of the Nubian ibex in Oman from that of Sudan may suggest that it should be treated as a distinct taxonomic unit, perhaps even at the species level. Further sequencing of the nuclear genome will help elucidate this.

3.2 Introduction

Nubian ibex are one of the few remaining free-roaming ungulates in Oman. They are distributed between the central and southern desert of the country. Due to urbanisation and the building of complex road networks, people can penetrate the deserts with increasingly sophisticated hunting equipment which has had dramatic negative impacts on the numbers of large mammals (Price 1989; Spalton 1993; Didero et al. 2019). Nubian ibex is classified as Vulnerable according to IUCN (Alkon et al. 2008; Ross et al. 2020a) and it is distributed from north Africa through to the Arabian Peninsula .According to the Omani National Report 2014 (CBD 2014) and the Omani Strategic Plan, Nubian ibex is classified as near endangered in the country due to the population decline and habitat loss.

The estimated population of the wild Nubian ibex in Oman ranges from 150-200 individuals in the central region (WWR (Al-Wusta Wildlife Reserve) from now on) and 600-1000 individuals in the southern region (Dhofar from now on) (Ross et al. 2020a). The situation of the Nubian ibex at WWR is concerning because of low population size and low genetic diversity compared to the Dhofar region (Al-Ghafri et al. 2021; chapter 2). Small isolated populations are highly prone to the accumulation of deleterious alleles, and this has been evidenced in ibex species with larger population sizes than those of the Nubian ibex remaining in Oman (Grossen et al. 2020).

In chapter two I used mitochondrial DNA (MtDNA) to characterise the genetic diversity and population structure in the wild Nubian ibex in Oman (Al-Ghafri et al. 2021). I found that there is a significant population differentiation between the wild population and the captive ones. It is therefore important to investigate the genetic structure of the Nubian ibex from different localities in the wild as there is limited information on the origins of the captive populations (i.e., North Africa, Middle East and Arabian Peninsula).

The purpose of this study is first to assess the genetic differentiation between the Nubian ibex at the extremes of its current range, from Oman and Yemen in the Arabian peninsula, to Sudan in North Africa. This will be achieved using museum samples as a source for MtDNA and generating mitochondrial references data from wild Nubian ibex from Yemen and Sudan.

3.3 Materials and Methods

3.3.1 Museum samples

Four skin samples of deceased captive bred Nubian ibex and four wild samples from Yemen were obtained from the Breeding Centre for Endangered Arabian Wildlife (BCEAW) based in Sharjah, UAE. The wild samples originally collected from Yemen consisted of one single horn and three full skulls, some with attached skin and dry tissue. The collection dates of the samples were unknown but from the samples status it seems that they are not recent. Eleven Nubian ibex samples from Sudan were collected from the Powell-Cotton Museum in London. The samples were skin samples from animals collected in 1934 from different parts of Sudan. For more details see Table A4.1 in the appendixes. For the purposes of analysis, the samples were categorised into four groups. Samples collected from wild animals in Oman were referred to as “Oman wild”. “Yemen” and “Sudan” groups, represented samples collected from wild individuals in these two countries, and “captive” samples represented samples collected from captive breeding centres in Oman and UAE.

3.3.2 DNA extraction

The DNA from tissue and blood samples was extracted using DNeasy Blood and Tissue kit (QIAGEN®, Germany) according to the manufacturer’s instruction. The skin and museum samples were extracted by QIAamp DNA

Investigator Kit (QIAGEN®, Germany). Allowing a gap of several months, the extraction of the museum samples was carried out in a sterilised ducted fume cabinet to avoid any cross contamination and two separate DNA extractions were performed. Each extraction was sequenced separately to check the consistency and avoid contamination. Moreover, in each extraction process, two blank extractions with no tissue or bone were included as negative controls and were run in each PCR reaction to check for contamination. A detailed extraction protocol was previously documented see supplementary materials A2 in appendixes.

3.3.3 Cytochrome b sequencing and analysis

Three overlapping primers, forward and reverse were used to target the cytochrome *b* marker. The primers' sequences and the PCR protocol were detailed in chapter 2. Big Dye Terminator Kit v3.1(Applied Biosystems) was used to sequence the final PCR products on an ABI 3730 DNA Analyzer sequencer (Applied Biosystems).

Sequence qualities were checked using Geneious prime software (v. 2021.1.1) and the overlapping fragments were aligned to a reference from GenBank (FJ207527) to generate a consensus sequence. The final sequences were trimmed to 486bp, equal to Nubian ibex sequences already deposited into GenBank. To check if the sequence amplified is for the mitochondrial cytochrome *b,* the sequences were translated into amino acids in Geneious using genetic code as vertebrate mitochondrial and translation start from frame one.

3.3.4 Phylogenetic analysis

The Bayesian method was used to reconstruct the phylogenetic tree for the cytochrome *b* sequences. Oman wild and the captive sequences were obtained from the previous study (Al-Ghafri et al. 2021). *Bos taurus* (AY676670) was used as an outgroup along with other *Capra* and ungulates species imported from GenBank (accession numbers were shown in the phylogeny tree). The best fit evolutionary model for this data was HKY+Γ+I, which has been selected by using jModelTest in the R package "phangorn" (Schliep 2010). The phylogenetic tree was constructed using MrBayes (Huelsenbeck & Ronquist 2001) implemented in Geneious prime (v. 2021.1.1) using the following parameters: total chain length 1,000,000, subsample frequency 200, and 100,000 (10%) were discarded as burn-in.

3.3.5 Genetic diversity and population structure

A median joining (Bandelt et al. 1999) haplotypes network for cytochrome *b* was constructed using PopArt software (Leigh & Bryant 2015) to assess the genetic differentiation between Oman wild, Yemeni and Sudanese samples. Two sequences of cytochrome *b* from Israel (AF217256 and DQ514552) were imported from the GenBank and included in the haplotype network and genetic differentiation analysis. The genetic distances between the Nubian ibex populations were calculated by DnaSP v. 6.12.03 (Rozas et al. 2017) using a permutation test of 10000. To estimate the population genetic structure, analysis of molecular variance (AMOVA) was performed using Arlequin v. 3.5 (Excoffier & Lischer 2010) using haplotype sequences of cytochrome *b* found in each population. Numbers of permutations were set at (10000) at a significance value of $p = 0.05$.

3.4 Results

3.4.1 Mitochondrial Haplotypes

The sequences of the two independent extractions of the museum samples were consistent and no contamination was detected in the control samples. The translation of the cytochrome *b* resulted in 162 amino acids. Nine Sudanese samples and two samples from Yemen were successfully amplified for the cytochrome *b*. The success rate of amplification of the mitochondrial marker from museum and old samples was more than 70% (9 out of 11 for Sudan and 2 out of 4 for Yemen). There was one haplotype detected in the Sudanese samples (HAP F) which has been previously observed in a captive animal from UAE. Nubian ibex samples from Yemen were found to harbour two haplotypes. The first one is (HAP C) which has been observed in the wild Nubian ibex population from Dhofar in Oman. The second haplotype was unique to the Yemeni samples and was not detected before. This haplotype will be called (HAP H). The two successful samples from the captive bred animals from Sharjah were found to have two haplotypes (HAP E and HAP G) which had already been found in the captive animals from UAE and Oman (chapter two; Al-Ghafri et al. 2021). No shared haplotypes between the Sudanese and the Yemeni samples, nor between the Yemeni and the captive samples, were found.

A haplotype network built in PopArt including haplotypes found in the wild population in Oman previously and haplotypes of captive animals, along with the haplotypes found in this study, is illustrated in Figure 3.1. It shows two clear clusters: the first one contains wild Nubian ibex from Oman and Yemen and the second one contains the Sudanese and captive samples. The haplotype sequences will be submitted later to GenBank and for the meantime the sequences can be found in Supplementary materials A3 in appendixes.

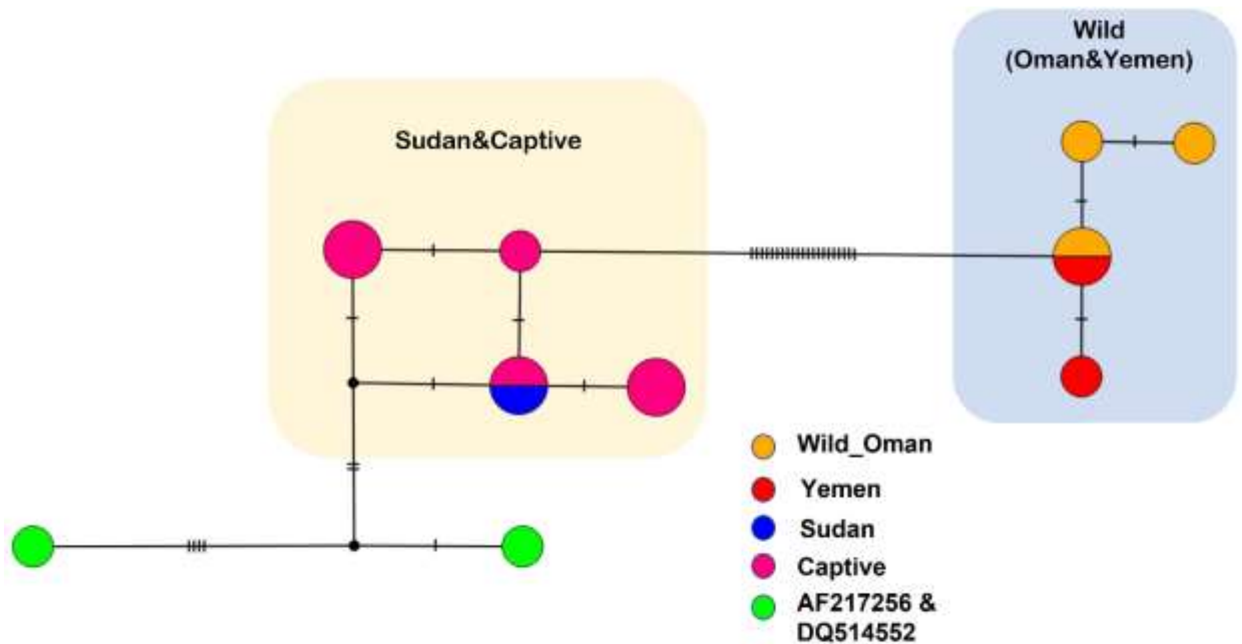


Figure 3.1. *Haplotype network for cytochrome b (487 bp). The wild samples from Oman and Yemen are in the right-hand blue box, while the captive samples (from the UAE and Oman) and museum samples from Sudan are in the left-hand orange box. Each coloured circle represents a single haplotype. The tick marks represent the mutational steps between haplotypes. Two reference sequences of C. nubiana from GenBank were used as representative samples from Israel (shown in green).*

3.4.2 Phylogenetic tree

A Bayesian phylogenetic tree was constructed for the cytochrome *b* sequences (486bp). The divergence between *Capra nubiana* and other *Capra* species was supported by over 0.96 posterior probability (Figure 3.2). In the *Capra nubiana* there are two highly supported clades: the first one represents samples from Yemen and Oman while the second one consists of samples from Sudan, Israel

and all the samples from captive individuals. These two clades are supported by 1.0 and 0.99 posterior probabilities respectively.

The clustering of Sudanese samples with the reference sequences from GenBank (NC020624) (Hassanin et al. 2009), (AF034740) (Hassanin et al. 1998a) and captive bred animals suggests that the origin of the captive animals is likely to be from North Africa, specifically Sudan. The origin of the samples from GenBank was not detailed but from the phylogeny tree it appears they could have been collected from North Africa.

The samples from Israel (AF217256) and (DQ514552) (Pidancier et al. 2006) form a sister clade from the rest of the Sudanese and captive samples, which is supported by a 0.85 posterior probability.

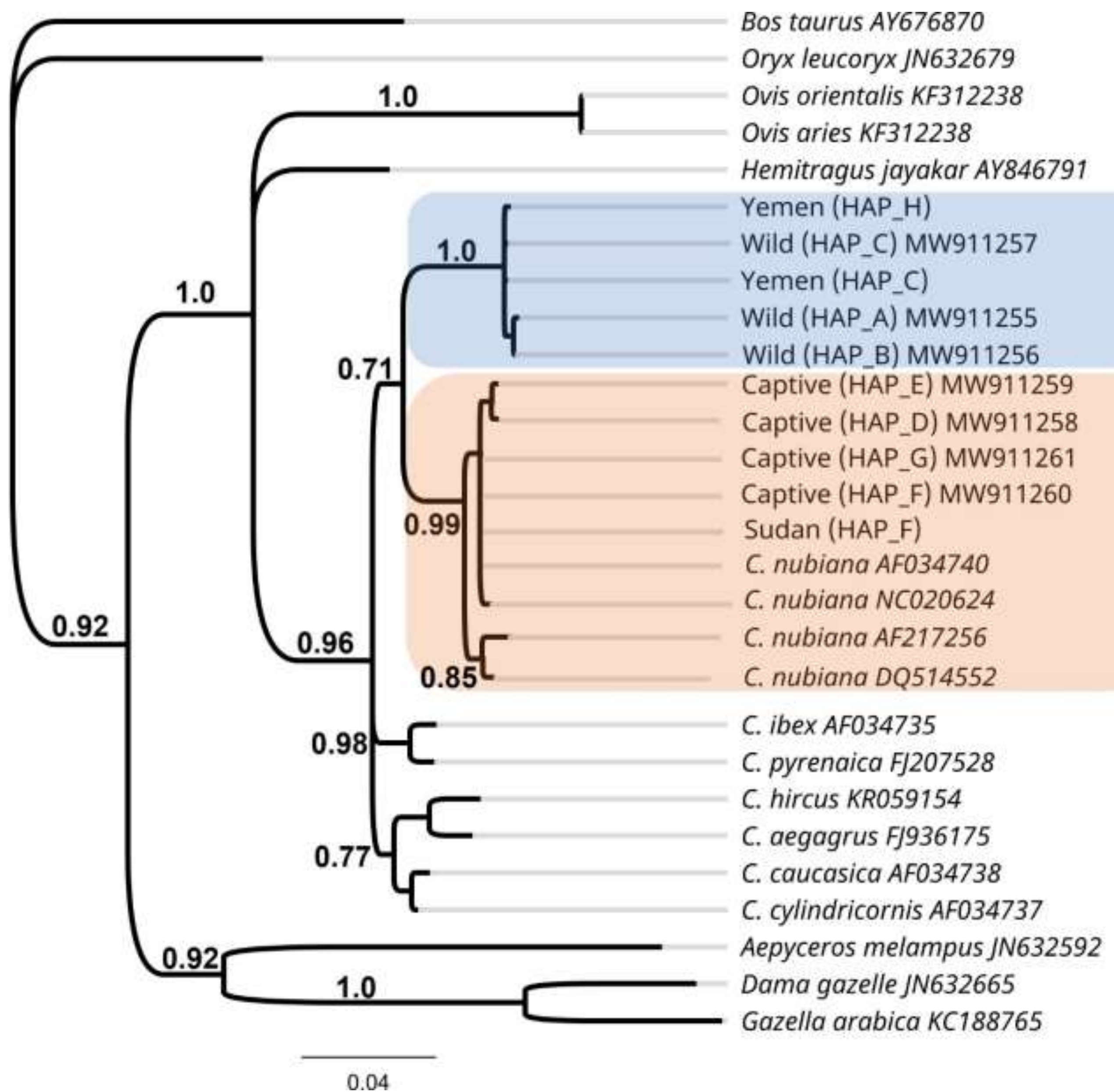


Figure 3.2. *Bayesian phylogenetic tree for cytochrome b (487 bp) produced using MrBayes. The wild samples from Oman and Yemen are in the blue box, while the captive samples (from UAE and Oman) and museum samples from Sudan are in the orange box. GenBank sequences are indicated by their accession numbers. The numbers shown at the nodes represent the posterior probabilities.*

3.4.3 Genetic divergence between Oman/Yemen and Sudan Nubian ibex

The fixation index (F_{ST}) between samples from different origin is represented in Table 3. The genetic differences between Oman/Sudan and Yemen/Sudan were found to be 0.971 and 0.979 respectively, while the genetic differences between captive/Sudan and Oman/Yemen was found to be 0.160 and 0.222 respectively.

Table 3.1. *Genetic differentiation (F_{ST}) among populations based on cytochrome b of (487bp)*

	Sudan	*Oman wild*	*Yemen*	*Captive*
Sudan				
Oman wild	0.971			
Yemen	0.979	0.222		
Captive	0.160	0.934	0.941	
Israel	0.545	0.872	0.880	0.444

The results of AMOVA showed high diversity in cytochrome *b* among the Nubian ibex populations in Oman and Sudan animals (98.8%, $p < 0.0001$). The same high diversity among populations (99.52%, $p < 0.05$) was observed between Sudan and Yemen. In contrast, there was no population structure observed between the Oman wild and Yemen samples. Here most of the diversity was observed within population (80.48%) and the population difference was not significant ($p > 0.05$). The same result was seen between Sudan and captive animals, where most of the diversity was found within population (75.4%, $p < 0.05$). The genetic distance values support significant divergence

between Oman wild/Sudan and Yemen/Sudan (F_{ST} 0.988, $p < 0.0001$ and F_{ST} 0.995, $p < 0.05$) respectively.

3.5 Discussion

For the first time the cytochrome *b* of museum samples of Nubian ibex from Sudan and Yemen were successfully amplified and analysed to assess the genetic relationship between these populations and the wild population in Oman. Mitochondrial cytochrome *b* results showed that there was a significant divergence between the Sudanese and the Oman wild and Yemen samples. The genetic distance analysis represented in the F_{ST} and AMOVA, along with the haplotypes and phylogenetic tree, showed the animals in the captive centres from Oman and UAE clustering in the same group with Sudanese samples, Furthermore, their genetic distance is not high, which suggest that these animals might have been brought from North Africa and specifically from Sudan.

The Nubian ibex population in Yemen and Oman were found to share just one haplotype (HAP C) and this haplotype was only found in the southern region, specifically in Dhofar, a region which shares a border with Yemen (Al-Ghafri et al. 2021). This suggests that there has been gene flow between the two populations. The unique haplotype in the Yemeni population (HAP H) was not observed in the Omani population despite the wide range of sampling from the Dhofar region, which may indicate that there is some resistance to movement between the populations which may have been exacerbated recently by human expansion and settlement and by road construction (Ross et al. 2020b). The current study suggests that there is no significant differentiation between populations in Oman and Yemen, but this result needs to be further investigated by collecting more wild samples from Yemen and assessing the genetic makeup of the population. Currently the status of the Yemeni population is not clear and its population size is not known (Ross et al. 2020a) and this population is

suffering from high hunting pressure related to the cultural practice of the Yemeni people who traditionally like to hang stuffed Nubian ibex skull in their homes as a sign of bravery and endurance (Al-Jumaily 1998).

The highly significant genetic differences observed between the Sudanese population and Oman/Yemen population are more likely a result of historical isolation and adaptation to different environments rather than a continuous gene flow influenced by geographic distance (isolation by distance).
(Wright 1943). This is strongly supported by the genetic distance between Sudanese population and Israel samples (F_{ST} = 0.545) which is just over half of that between Sudanese population and Omani wild and Yemeni populations (Table 1). According to the IUCN, the antelopes of North Africa are found to be completely distinct from the Arabian Peninsula species and they evolved separately (Species survival specialists & Antelope Specialist Group 2017). In a recent study on the sand cat (*Felis margarita)* across its range from north Africa up to Arabia, mitochondrial markers showed that there was degree of genetic differentiation between the two populations (Howard-McCombe et al. 2020). Therefore, my results indicate that Nubian ibex in captivity which have North African origin should not be mixed with wild animals in Oman, nor used as a source for any reinforcement or reintroduction program. The extent of divergence between the Sudanese population and the Oman/Yemen population has the potential to allow classification of the Nubian ibex in Oman and Yemen to be at subspecies or even species level. Further nuclear investigation is needed to confirm these results. The divergence time between the North African and Arabian (specifically Oman and Yemen) populations will help in understanding the evolutionary history of this species and assess in developing appropriate conservation strategies.

Chapter 4 Implications of newly developed SNPs for conservation programmes for the threatened Nubian ibex (*Capra nubiana*) in Oman

An adapted version of this chapter has been submitted for publication to Conservation Genetics Resources journal and is under initial review at the time of writing. The authors are:

Mataab K. Al-Ghafri[1,2,3] , Patrick J.C. White[1,4], Robert A Briers[1,4], Alex Ball[2], Helen Senn[2] , Mansoor H. Al-Jahdhami[3], Haitham Al-Amri[3], Bisnu B. Tiwari[3], Said Nasser Al-Harsusi[3], Abdullah Ghasab Al-Harsusi[3], Qais Al-Rawahi[3], Kara L Dicks[2]

1 School of Applied Sciences, Edinburgh Napier University, Edinburgh, United Kingdom

2 RZSS WildGenes, Royal Zoological Society of Scotland, Edinburgh, United Kingdom

3 Office for the Conservation of Environment, Environmental Authority, Muscat, Oman

4 Centre for Conservation & Restoration Science, Edinburgh Napier University, Edinburgh, United Kingdom.

Authors' contributions. M.K.A.-G. participated in the design of the study, collected the field samples, carried out the molecular laboratory work, carried out SNP filtering and population genetic analyses and drafted the manuscript; P.J.C.W. and R.A.B. participated in the design of the study and participated in reviewing the manuscript; A.B participated in laboratory study design and reviewing the manuscript; H.V.S. participated in the design of the study, and participated in reviewing the manuscript; M.H.A Study design and manuscript

revision; H.-A, B.B.T, S.N.-H, A.G.-H and Q.-R contributed to study deign and collected the field samples; K.L.D. participated in laboratory study design, designed and carried out bioinformatics analyses, supervised SNP filtering and population genetic analyses, and participated in editing and reviewing the manuscript. All authors gave final approval for publication.

4.1 Abstract

Conservation of elusive wildlife species affected by habitat degradation, population fragmentation and poaching is challenging. Nubian ibex (*Capra nubiana*) is a desert-adapted ungulate, and the remaining wild populations within Oman are small and fragmented, as are populations across the species' distribution range. The appropriateness of captive insurance populations for reinforcing existing, or establishing new, wild populations remains uncertain for Oman due to ambiguity regarding their genetic provenance. For effective management of this vulnerable species, it is essential to assess the genetic relationships between the wild and captive animals, and to investigate hybridisation with domestic goats (*Capra hircus*). To facilitate this, we used 5,775 high quality SNPs developed by double digest restriction-site associated DNA, to assess genetic structure, gene flow and divergence between wild and captive populations of Nubian ibex. We detected hybridisation with goats within captive Nubian ibex, and recommend that genetic assessment of captive individuals is routinely used to evaluate their suitability for reintroduction or reinforcement programs. Substantial population structure and significant genetic differentiation was found between wild and captive individuals (F_{ST} = 0.540), building on a previous mitochondrial study showing a divergence between the wild Nubian ibex in Oman and those in captivity. The data provided by this study will be invaluable for future development of marker systems to assess wild populations using low-quality DNA from non-invasive sampling. Consequently, it

Chapter 5 Hybridization capture of Nubian ibex genome from non-invasive and historical specimens DNA

5.1 Abstract

Hybridization (or hybrid) capture provides an unprecedented technique to address several conservation questions for non-model and under studied organisms that lack reference genomes by enabling the researcher to utilize low-quality samples such as faeces and historical museum specimens. Here we performed this method to recover SNPs from contemporary faecal and museum specimens of wild Nubian ibex from Oman, Yemen and Sudan. We combined the results of previous ddRAD study (chapter 4) with this study and we were able to capture 1,054 SNPs, which helped in assessment of genetic variation among these populations. Our results showed a significant divergence between Oman and Sudan populations (F_{ST}= 0.513). These results were congruent with mitochondrial diversity analysis results (chapter 3), which showed significant divergence with no shared haplotypes between the two populations. In contrast, the Yemeni samples were found to share one haplotype with Omani samples and there were no significant differences between them either by mitochondrial or nuclear markers. We detected a clear signature of admixture of some captive individuals within Oman and UAE to the Sudan samples which will need careful attention if these animals are intended to be used for reintroduction programs. Further sampling of other Nubian ibex populations in Saudi Arabia, Jordan, Israel and Egypt is needed to delimit the taxonomy and evolutionary history of this species.

5.2 Introduction

5.2.1 Wildlife monitoring

The rapid loss of biodiversity seen today is a result of several human-induced factors including habitat destruction, alteration and fragmentation, overexploitation and illegal haunting (Pimm et al. 2014; Fahrig et al. 2019). Since 1970, an average decrease of 68% in population sizes of mammals, birds, amphibians, reptiles and fish has been recorded (WWF 2020) and more than 28% of the assessed species are threatened with extinction (IUCN 2018). Monitoring the level of biodiversity loss and understanding its causes is imperative in de-escalating the rapid deterioration of ecosystem components. Species are core components of any ecosystem and thus it is crucial to preserve and protect them to maintain ecosystem functioning which can indirectly affect human well-being (Dirzo et al. 2014). Therefore, it is important to act swiftly and immediately before it is too late. Monitoring and assessing wildlife species in their natural habitat are often challenging, particularly where they are rare or elusive, and is potentially dangerous if trying to capture animals alive for taking samples. These challenges become more serious when dealing with threatened species.

5.2.2 Non-invasive samples

Non-invasive sampling provides an ideal choice for collecting samples of wildlife species without the need to harm or capture the animal and allows collection of larger sample sizes across the species' range (Waits & Paetkau 2005). Non-invasive samples such as faecal, hair, bone, urine, eggshell, saliva, and feathers can act as a source for DNA, which helps in assessing several parameters of wild species like genetic diversity, gene flow between populations, and estimation of population sizes (Zarzoso-Lacoste et al. 2018; Al-Ghafri et al. 2021).

There are many advantages of non-invasive samples, especially in the field of conservation of wildlife species. For example, they are frequently deposited into the environment by animals and are easy to collect without the need for special instruments or expert scientists or veterinarians. In addition, this type of sampling can minimize disturbance to the animals and their habitats. On the other hand, the main disadvantage which limits its use is that DNA is degraded quickly, for example by UV and endonucleases, causing a reduction in DNA quality and consequently resulting in low amplification success rates, and high genotyping errors (Hajkova et al. 2006).

Faecal samples are generally the most abundant non-invasive samples that can be easily identified and collected for terrestrial mammals. Beside the genotyping information retrieved from faecal samples, they can be used to assess diet, pathogens and reproductive status of the animal (Kohn & Wayne 1997). However, amplification of genetic markers using faecal samples is problematic due to the presence of PCR inhibitors such as proteins, fats and salts, having higher error rates and yielding low and fragmented amounts of DNA (Smith & Wang 2014). Furthermore, the use of faecal samples requires more processing efforts to obtain enough quality DNA for genotyping and avoid contamination during the extraction and amplification process (Frantzen et al. 1998).

5.2.3 Methods for utilizing low-quality DNA samples in conservation

Several protocols and techniques have been developed to overcome the limitation of using low-quality DNA resources (Nagai et al. 1998; Ball et al. 2007; Stenglein et al. 2010; de Flamingh et al. 2014; Ramón-Laca et al. 2015; Kubasiewicz et al. 2016; Bourgeois et al. 2019). The use of non-invasive DNA sources in conservation of wildlife has been reported for several species. For example, they have been used for differentiation between species and identification (e.g. Eurasian beaver *Castor fiber,* Iso-Touru et al. 2021); sloth

bear (*Melursus ursinus*, Thatte et al. 2018), disease monitoring (e.g. bovine tuberculosis infective agent, *Mycobacterium bovis*, Emami-Khoyi et al. 2021), species abundance (e.g. pine marten *Martes martes*, Sheehy et al. 2014), genetic diversity assessment for conservation (e.g. maned wolves *Chrysocyon brachyurus*, Mannise et al. 2018) and species monitoring (e.g. Cabrera vole *Microtus cabrerae*, Ferreira et al. 2018).

5.2.4 Next generation sequencing and the use of non-invasive samples

Previously the discovery of polymorphic markers that can be used to investigate population genetics for non-model species were time consuming and expensive and required high quality DNA samples (Thomson et al. 2010). However, advancement in sequencing techniques have provided a promising platform for using low-quality DNA samples in conservation research. Recent developments of molecular investigation techniques such as next-generation sequencing (NGS), genotyping-by-sequencing (GBS), single-nucleotide polymorphisms (SNPs) arrays and restriction site-associated DNA sequencing (RAD-Seq) has helped in development of high-density genotyping data without the need for prior knowledge of the species' genome (Davey et al. 2011; Ekblom & Galindo 2011; Andrews et al. 2016). However, one of the major challenges in getting maximum benefit from these platforms is the quality of the samples collected. Genetic research on the conservation of high-profile and/or endangered species relies mostly on the non-invasive sampling (Russello et al. 2015; Carroll et al. 2018).

Although the cost of whole genome sequencing is continually decreasing, applying this method for many samples for population studies is still not a cost-effective choice (McCormack et al. 2013). In addition, whole genome sequencing approaches generate excessive amounts of data, more than is needed to address questions such as phylogenetic, phylogeographic and population genetics. These questions can be answered by approaches based

on reduced representations of the genome that are able to produce large amounts of informative data of an unbiased subset of loci from many individuals pooled at the same time (Andrews & Luikart 2014).

5.2.5 Different approaches of next generation sequencing

There are several approaches for next generation sequencing based on the research interest, question being posed, availability of reference genome and quality of the DNA.
(Valencia et al. 2018). Two methods that can be used which are relatively cost effective and accurate and can be used for low-quality samples.
The first one is the use of restriction endonucleases to digest the genomic DNA into large amounts of varied-size fragments. This is known as Restriction site-Associated DNA sequencing (RAD-seq) (Baird et al. 2008) (see also chapter 4).
The second category is an enrichment approach which targets specific regions of interest in the genome. In the latter, the region of interest can be selected by DNA hybridization (or hybrid) capture or by direct targeted sequences such as targeting a specific exon, or non-coding region, that is highly conserved or of some vital evolutionary interest (Taron et al. 2021; Sevigny et al. 2021; Suchan et al. 2021). Hybrid capture techniques are now used to enrich loci of interest specifically in non-invasive samples (Perry et al. 2010).

5.2.6 Enrichment of SNPs from low-quality DNA

SNPs can be amplified from degraded DNA or from non-invasive samples such as faeces. This is because SNPs can be recovered by targeting short fragments of DNA (Morin & Mccarthy 2007). SNPs are widely used in conservation research due to several advantages. They are distributed evenly throughout the genome, can be identified by using next-generation sequencing technology, and large numbers of SNPs can be detected across the genome (Garvin et al. 2010; Helyar et al. 2011). In addition, they can be shared across laboratories

and they are good for non-invasive samples. They have already been used in the field of conservation biology. For example, Bourgeois et al (2018) produced high-quality candidate SNP markers from faecal samples of African forest elephant (*Loxodonta cyclotis*) for genetic monitoring. Nussberger et al (2018) monitored introgressive hybridization between European wildcats (*Felis silvestris*) and domestic cats (*Felis catus*) by using 70 nuclear SNPs and four mitochondrial SNPs. Eriksson et al (2020) used 26 SNPs for coyote (*Canis latrans*) identification and monitoring from non-invasive samples (e.g. hair) and compared it with results obtained from tissue samples. These studies indicate the capacity of non-invasive methods for monitoring and conserving elusive and endangered wildlife species. Materials such as hairs, faecal, saliva and bones are used to monitor population densities (Waits & Paetkau 2005; Kery et al. 2010; Wheat et al. 2016). In addition SNPs has been found to be more accurate than microsatellites due to huge number of SNPs that can be detected across the genome compared to few markers for microsatellites (Puckett 2017). Furthermore, SNPs were found to produce accurate estimation of the genotypes from highly fragmented DNA (Thaden von et al. 2017).

5.2.7 Hybridization Capture

Hybridization capture (also abbreviated as hybrid capture) is generally best used for poor quality or low concentration DNA where the majority of the DNA fragments are not long enough to comprise the restriction sites used in RAD seq or it is not possible to perform size selection. This approach can be scaled and replicated more easily and allow targeting of the same loci across different taxonomic groups (Harvey et al. 2016).

In this method, DNA libraries are prepared from randomly fragmented DNA templates derived from low-quality samples such as faecal samples. The prepared libraries are hybridized to sets of biotinylated synthetic oligonucleotide probes, known as “baits” (Gnirke et al. 2009). Baits are typically 60–120 bases

in length and are complementary to one of genomic fragments of interest in the sample solution. The targeted fragments are selected from already existed sequence data such as ddRAD data. Streptavidin-coated paramagnetic beads are used to separate a bait along with the hybridized (targeted) DNA, while non-hybridized DNA fragments are washed away. The captured DNA fragments are then released from the beads and sequenced.

In this study, we explore the potential of the hybrid capture method to decipher the genetic and geographic structure between Nubian ibex populations in Oman, Yemen and Sudan by using faeces and museum samples.
In addition, it will provide monitoring tool for Nubian ibex that can utilise low-quality samples and thus be able to collect larger sample sizes in a less invasive way.

5.3 Materials and Method

The total number of samples used in this study for hybrid capture was 63. Samples were selected based on the DNA quality of each sample assessed by 1% electrophoresis and the samples that were selected should appear on the gel as a bright solid band or as a bright smear. Fifty-five samples were from wild Nubian ibex in Oman (29 samples from WWR, two samples from Shalim and 24 samples from Dhofar). Eight museum samples from animals from different localities (two samples from Yemen and six samples from Sudan) were also included. Sudanese samples were collected from the Powell Cotton Museum (London).

For the purpose of analysis, the ddRAD results from chapter 4 (based on 30 samples from captive animals, eight wild samples from Oman and five samples from domestic goats) were used and combined with the MyBaits results from this chapter. Therefore, the total number of samples used was 107 (after

excluding the eight repeats). Table 5.1 shows the samples used in ddRAD and hybrid capture.

Captive samples that showed ancestral assignment to the goat population will be called “Hybrid” while samples collected from wild (Nubian ibex from Oman) that showed ancestral assignment to the goat population will be called “wild-goat”. Museum samples representing wild Nubian ibex from Oman, Sudan and Yemen will be referred to as “Oman”, “Sudan” and “Yemen” respectively. The samples from captive centres in Oman and UAE will be referred to as “captive”. Note that these captive samples have previously been shown to belong to a different clade of Nubian ibex more closely related to animals from Sudan (see chapters 2-4).

Table 5.1. *Samples used in ddRAD and hybrid capture from each population*

Population	Approximate date of sampling	Number of samples genotyped by ddRAD	Number of samples genotyped by Hybrid Capture
Captive			
UAE	2015-2018	27	-
Oman		1	-
Putative hybrids		2	-
Wild			-
Sudan	1935	-	6
Oman			
- Dhofar	2018	-	24
- Shalim	2013	4	2
- WWR	2016	4	29[a]
Yemen	No record	-	2
Goat samples	2018	5	-
Total		43	63

[a] During population structure analyses, two of these samples were determined to be goat samples.

5.3.1 DNA extraction

DNA from the faecal and bone samples were extracted by using Isohelix Xtreme DNA Kit (XME-50) and QIAamp DNA Investigator Kit (QIAGEN®, Germany) respectively. The method followed was according to the manufacturer's instructions. A detailed methodology of DNA extraction from faecal and bones can be found in the supplementary materials A3 in appendixes.

5.3.2 Identification of candidate SNPs

The ddRAD data generated in chapter 4 was used to identify candidate SNPs for bait design. The raw reads of ddRAD were demultiplexed and initially filtered by using the process_radtags module in STACK v2.52 (Rochette et al. 2019) as described in chapter 4. The ddRAD sequences were mapped against the goat reference genome (*Capra hircus*) (GCA_001704415.1_ARS1) using the default parameters of the Burrows-Wheeler Aligner v0.7.17 (Burrows & Wheeler 1994). Unmapped reads were excluded. Stacks v2.52 was used to identify SNPs in Nubian ibex samples (Oman and captive) only, as these are likely to produce the best balance between levels of variation across both populations and reduce bias to avoid over-representation of regions that were highly conserved across more distantly related taxa. SNPs were filtered to retain those that were sequenced within at least one sample within each population (i.e. one sample in Oman and one sample in captive). During this process no filters for linkage disequilibrium (LD), Hardy-Weinberg equilibrium (HWE), minor allele frequency (MAF) or individual sequencing rates were applied due to the small sample size of the wild population.

5.3.3 Bait design

Bait design was carried out by Arbor Biosciences (Ann Arbor, USA) using the candidate SNPs identified in previous step. The process was as follows. A total of 72,232 baits (80 bp in length) were produced for 21,591 of the rad loci. These baits were blasted against a masked version of the goat genome for filtering. Stringent criteria were used, in which baits were filtered out if they had a blast hit to a region of the genome that was greater than 25% soft-masked for repetitive elements, or fell outside of the GC% range of 25%-55% GC. Loci that had three baits that passed the filtering criteria were retained.

5.3.4 Library preparation for bait capture

The libraries were prepared for each sample by using NEBNext Ultra II FS DNA Library Prep Kits for Illumina according to the manufacturers protocol (New England BioLabs Inc) for sample inputs ≤ 100 ng without size selection. The total DNA concentration of each sample was normalised to 100 ng/26 µl. Then fragmentation was performed at 37 ℃ for 15 minutes followed by incubation at 65 ℃ for 30 minutes using NEBNext Ultra II FS Enzyme Mix (New England Biolabs Inc). Adaptors for Illumina sequencing (NEBNext Multiplex Oligos for Illumina; New England Biolabs Inc) were ligated to DNA fragments and incubated at 20 ℃ for 15 minutes. Clean-up of adaptor-ligated DNA was performed using AMPure XP magnetic beads (Beckman Coulter Inc). The magnetic beads should bind to adaptor-ligated DNA while unwanted fragments (the ones without adaptors) should be in the supernatant. Therefore, 0.8X of magnetic beads were added to each individual library which was then placed on a magnetic plate concentrator. When the solution became clear the supernatant was discarded leaving the beads with the wanted DNA fragments. Then the adaptor-ligated DNA was eluted from the beads by adding 0.1X TE buffer and

placed again on the magnetic plate concentrator. When the solution became clear 15 µl were used for PCR enrichment.

The retained DNA fragments were amplified for eight cycles using NEBNext Ultra II Q5 Master Mix and NEBNext index/universal primers (New England Biolabs Inc). The following PCR conditions were used: 1) initial denaturation at 98 ℃ for 30 seconds; 2) denaturation at 98 ℃ for 10 seconds; 3) annealing and extension at 65 ℃ for 75 seconds; and 4) final extension at 65 ℃ for 5 minutes. Libraries were quantified on the Qubit fluorometer 2.0 using high sensitivity dsDNA kit (Invitrogen)

5.3.5 Hybrid capture reaction

Each of the amplified libraries from the previous step were normalised to 250 ng and four libraries were pooled together into each capture reaction, giving a total of 1000 ng of DNA input. To minimise sequencing bias that might happen due to some of the samples having much high DNA concentrations than others, samples that had similar DNA concentrations were pooled together, assuming that total DNA concentration was correlated to target DNA concentration.

The hybrid capture reaction started with mixing libraries with adaptor blockers. To allow for hybridization between blockers and adaptors ligated to DNA fragments, the reaction is incubated at 65 ℃ for five minutes. Next, baits were added into the library mix and left at 65 ℃ for 24 hours to allow sufficient time for hybridisation between the baits and the libraries. Then, the reaction containing the hybridized DNA with baits were transferred to a streptavidin-coated magnetic bead solution and incubated at 65 ℃ for five minutes to allow the beads to bind to the hybridised baits. Then the beads were pelleted using a magnetic particle concentrator and the formed supernatants were discarded. Next the pelleted beads were washed four times using Wash Buffer X (Arbor

Biosciences). Bead-bound baits were resuspended in 30 ul of buffer E (Arbor Biosciences).

Fifteen ul of bead-bound baits libraries were PCR amplified for 14 cycles using P5 (AATGATACGGCGACCACCGA) and P7 (CAAGCAGAAGACGGCATACGA) primers and NEB Ultra II Q5 2X Master Mix, using the on-bead amplification protocol (New England BioLabs Inc). The PCR conditions were as follow: 1) initial denaturation at 98 ℃ for 2 minutes; 2) denaturation at 98 ℃ for 20 seconds, annealing at 60 ℃ for 30 seconds, extension at 72 ℃ for 45 seconds; and 3) final extension at 72 ℃ for 5 minutes. The amplified capture libraries were purified using 1.2x AMPure XP magnetic beads (Beckman Coulter Inc) and the final concentrations were measured using a Qubit dsDNA High Sensitivity Assay Kit (Thermofisher Scientific).
In total, 16 capture libraries (60 samples) were pooled in equimolar concentrations and 13 individual museum samples libraries were sequenced in a different lane of Illumina Hiseq 4000 using paired end sequencing. This is done because it has been found that the performance of fresh DNA outweighs that taken from museum specimens, due to the presence of highly degraded DNA and few long DNA fragments in historical samples (Linck et al. 2017). Therefore, in order to overcome this problem we perform hybridization reactions separately for each museum sample and the samples that had almost same DNA concentration were pooled together.

5.3.6 SNP calling

The ddRAD raw reads were prepared previously as described in chapter 4. MyBaits raw reads were processed using Trimmomatic v0.39 (Bolger et al. 2014) as follows. First, Illumina adapter sequences were removed and the reads bases from the beginning and from the end were trimmed if the base quality fell below Qphred ≤ 3. Next, a sliding window of four base pairs was used to trim and remove sequences that had a quality below Qphred < 15. Sequences that

fell below the length of 50 bp were removed. Then, both ddRAD & MyBaits data were mapped to goat genome using Burrows-Wheeler Aligner v0.7.17 (Burrows & Wheeler 1994). Samtools (v1.10) (Li et al. 2009) was used to exclude reads with low mapping quality (Qphred < 20) and reads which were not uniquely mapped (i.e. had secondary alignments or multiple maps) following suggestions by Lou et al. (2021) for low-coverage sequencing. Samples with < 200,000 mapped reads were excluded from further analysis. Picard tools (v2.18.7) (http://broadinstitute.github.io/picard/) was used to add read groups and remove PCR duplicates. The variants were called with GATK (v4.2.5.0) (https://gatk.broadinstitute.org) following the best-practice workflow for germline short variant discovery and filtered by retaining only biallelic SNPs which met quality and depth filters, while singletons were removed.

5.3.7 Assessment of putative hybrids

I used SNPs data along with the mitochondrial data (chapter 2 and 3) to test potential hybridization between Nubian ibex from WWR and goats. The results of mitochondrial markers were checked for the putative hybrid samples. I blasted search of the D-loop and cytochrome *b* sequences into the GenBank database. In addition, I preformed STRUCTURE analysis in order to estimate the percentage of hybridization between the two species. The sample was considered hybrid by estimating the proportion of alleles that were inherited from one of the two parental species (Buerkle, 2005). These two samples and the previously identified hybrid in the captive individuals were not included in genetic diversity analysis. Wild samples from Oman that showed 100% assignment to goats in STRUCTURE analysis will be called “wild-goat” as these samples are probably a result of errors during the sampling process whereby goat faeces were collected accidently and sampled as those of Nubian ibex.

5.3.8 SNP filtering

Different filtering criteria were used to exclude low-quality and high missing data samples and to check if the results are robust using PLINK 1.9 (Chang et al. 2015). First, a high stringency SNP set was produced, retaining SNPs that were genotyped in 75% of individuals. Secondly, a low stringency SNP set was generated, retaining SNPs genotyped in 30% of individuals. For both SNP sets, SNPs were subsequently excluded with MAF below 5% and individuals with fewer than 90% of the SNPs genotyped. Lastly, both SNP sets were filtered to exclude SNPs that were not genotyped in at least 80% of the remaining samples.

Filtering out the linkage disequilibrium (LD), for each population separately, was based on two criteria: the stringent SNP set where $R^2 < 0.5$ were retained and the relaxed one where SNPs with $R^2 <0.8$ will be retained. Finally, fixed SNPs and SNPs that were located on sex chromosomes were excluded.

5.3.9 Population structure

After excluding repeated samples, the Bayesian clustering method STRUCTURE v2.3.4 (Pritchard et al. 2000) was used to assess the demographic history of the populations and determine the number of genetic clusters (K) which best represent the data and assign individuals to clusters. Each number of clusters (K 2 to 8) was repeated five times to account for uneven sampling (Puechmaille 2016). The admixture model was run with 1,000,000 MCMC chains and a burn-in of 500,000. The most probable K value was estimated by the web-based Structure Harvester (Earl & vonHoldt 2012) and the Evanno method (Evanno et al. 2005) was used to assess the most probable number of clusters. The same criteria used in chapter 4 were applied here, in which individuals were classified as putative hybrids when an individual had at least 25% assignment to an alternative population (refers to different

putative taxon, not populations within Oman). For further confirmation of the population structure obtained by STRUCTURE, we used the ADEGENET R package (Jombart 2012) to run Principal Component Analysis (PCA).

Weir & Cockerham's (1984) pairwise F_{ST} was calculated in HIERFSTAT (Goudet 2005; de Meeûs & Goudet 2007) after excluding putative hybrids, repeated samples and Omani wild samples that clustered with goats (wild-goat). The significance of the results was calculated using 999 bootstraps with 95% confidence intervals. In addition, the genetic distance between localities within Oman (WWR, Shalim and Dhofar) were estimated using PCA and F_{ST} between the individuals in each location and we compared the results with the mitochondrial results in chapter 2. To check for data robustness, F_{ST} was calculated for high and low stringency SNP sets. T-tests were performed to check for any potential differences between the F_{ST} values between different SNP numbers. To account for multiple testing, a Bonferroni correction was applied.

5.3.10 Population genetic diversity analysis

To calculate genetic parameters between populations first I excluded repeated samples, putative hybrid individuals (NUB021 and NUB039) and wild-goat samples (NUB097 and NUB103). HIERFSTAT version 0.5.10 in R (Goudet 2005; de Meeûs & Goudet 2007) was used to estimate observed (H_o) and expected heterozygosity (H_e). The number of variants that were unique in each population was calculated by MAF in each population and identifying SNPs which had a MAF of 0 in all except one population. These steps were performed for the data that had less than 30% missing data (i.e. loci must present in more than 70% of the samples) and excluding SNPs that had LD > 0.5.

5.4 Results

Identification of candidate SNPs from the ddRAD data for baits design resulted in 22,707 SNPs. Table A5.1 in the appendixes shows the numbers of SNPs that were fixed or variable in the identified candidate SNPs from Oman and captive populations. Arbor Biosciences were able to design 27,750 baits each of 80bp in length for a total of 9,250 SNPs. Of these, 6,666 SNPs were randomly selected and thus the final bait panel included 19,998 baits where each SNP would be targeted by three baits.

The number of reads obtained by hybrid capture from faecal samples of Nubian ibex ranged from 0.2 to 17.7 million reads and the total sequence length ranged from 62 bp to 137 bp. The percentage of duplicate sequences ranged from 7% to 84.7%. This is quite a high proportion of duplicates with respect to the range of typical RAD-seq (Andrews et al. 2016). However, PCR duplicates have been found to not affect the estimation of population differentiation (Grossen et al. 2018). Museum samples were found to have more sequence duplication, as well as lower read numbers and read length than faecal samples (Table A5.2 in the appendixes). Two samples (NUB243_60 and NUB150_48) did not have sufficient raw or mapped reads and were not included in SNP calling.
The de-multiplex ddRAD reads obtained in chapter 4 were combined with the Hybrid capture reads, and a total of 4,682,399 SNPs were identified and genotyped in the combined 113 samples. Two samples of Oman Nubian ibex clustered with goats in the PCA. These samples are NUB097 and NUB103 (called wild-goat). The subsequent results are based on 107 samples, as six samples were removed due to being repeated samples.

5.4.1 SNPs filtering

In order to find an optimal filtering criteria that balances sample and SNP retention with minimising missing data across my samples. I used different

missing genotyping values and calculated the number of SNPs remaining, number of samples removed and genotyping rate in each stage (Table 5.2).

Table 5.2. *Effect of using different missing genotypes filtering criteria to filter Hybrid capture data and ddRAD SNPs by PLINK. Fixed criteria used are – individual genotyping rate of 10%, --MAF 0.05 and finally individual genotyping rate of 80% are the same in all filtering criteria.*

Missing genotype rate	% of samples loci must be present in	Samples removed	Samples remaining	SNPs remaining	Total SNP genotyping rate
--geno 0.25	75%	35	**78**	**397**	**0.989**
--geno 0.30	70%	35	**78**	**1,255**	**0.979**
--geno 0.40	60%	37	**76**	**2,489**	**0.937**
--geno 0.50	50%	61	**52**	**4,361**	**0.893**
--geno 0.60	40%	93	**20**	**9,438**	**0.843**
--geno 0.70	30%	86	**27**	**70,935**	**0.915**

PCA analyses for each missing genotype rate were plotted to assess the robustness of the results. All PCA plots showed the same pattern and distribution of the samples (Figure 5.1). Using higher missing genotype thresholds of 0.60 and 0.70 caused removal of samples from captive Nubian ibex, Yemen, Shalim and goats (Figure 5.1 E&F).

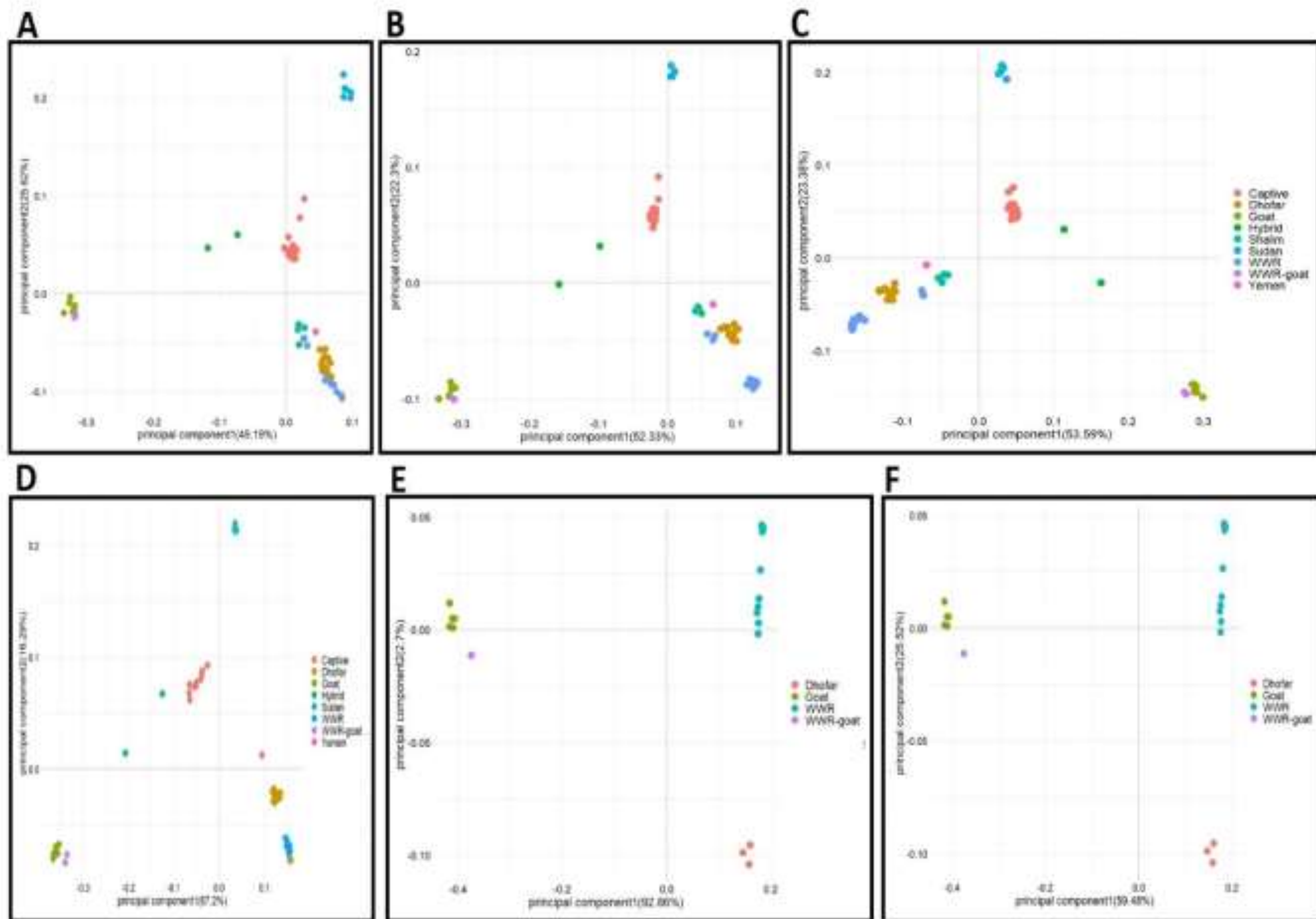


Figure 5.1. *PCA plot for different criteria of missing genotypes: (A) Missing genotype = 0.25, (B) 0.30, (C) 0.40, (D) 0.50, (E) 0.60 and (F) 0.70. Each sample group is represented by a different colour. Note the colour key changes across plots.*

Linkage disequilibrium (LD) was calculated for the first three values of the missing genotype (i.e. –geno 0.25, 0.30 and 0.40) to assess the robustness of the results. Two assessments were performed, stringent ($R^2 < 0.50$) and relaxed ($R^2 < 0.80$). The results showed no substantial differences in the results when using either criterion (Figure 5.2). All results showed the same clustering behaviour.

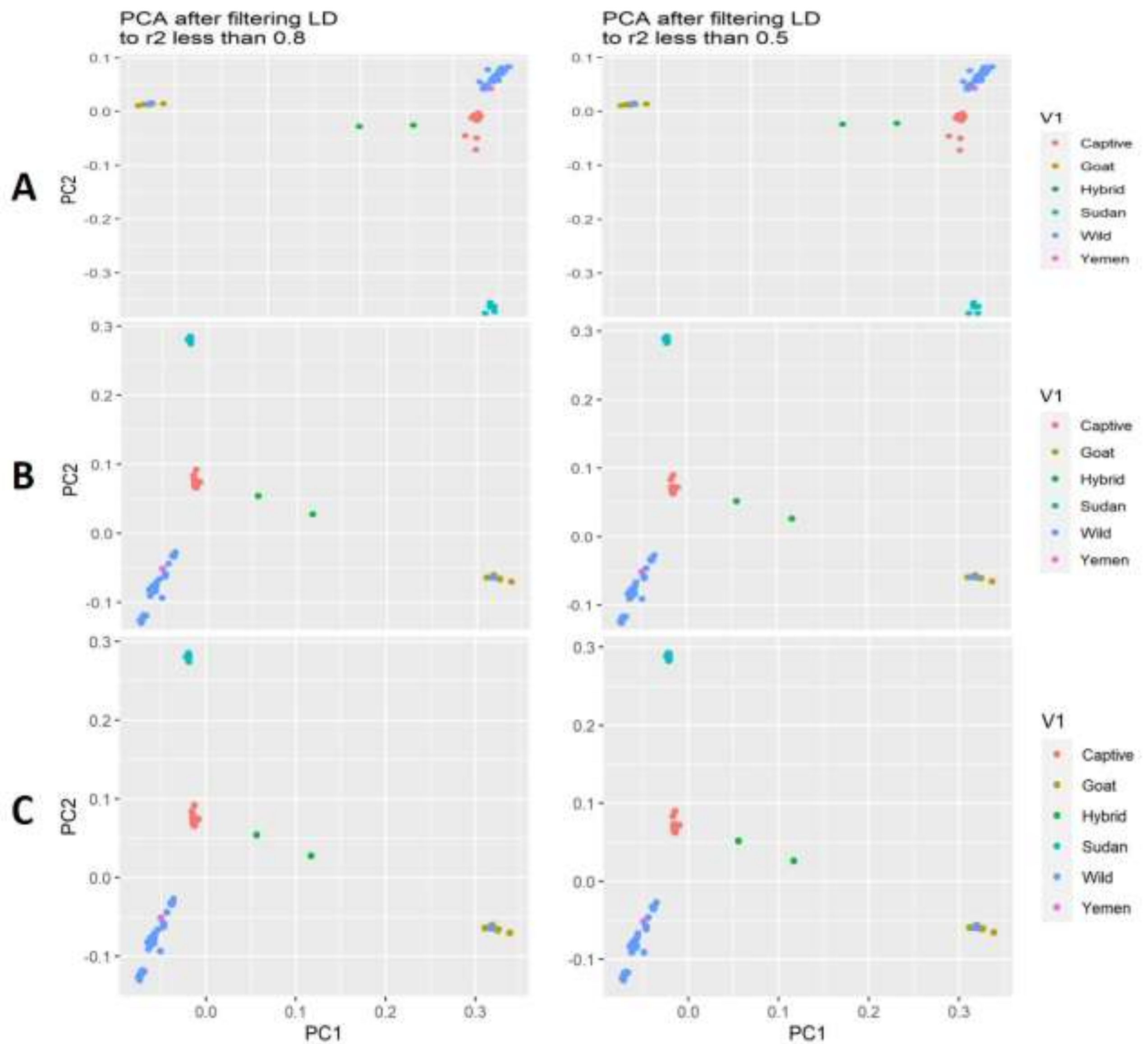


Figure 5.2. *Comparison between different filtering criteria of SNPs that are inLD. The stringent criteria removes SNPs that have R^2 > 0.5 and the relaxed criteria R^2 > 0.8: (A) Missing genotype = 0.25, (B) 0.30, (C) 0.40.*

Table 5.3 shows the comparison between number of SNPs when the R^2 value (i.e. the correlation between alleles at two loci) was greater than 0.8 and 0.5. The number of pairs of SNPs that have R^2 of more than 0.8 and 0.5 were 376

and 413 SNPs respectively. One SNP in each pair was randomly excluded leaving 1,076 and 1,054 SNPs.

The subsequent results are based on missing genotype criteria of less than 70% (-- geno0.30). These remaining 78 samples had an overall SNP genotyping rate of 0.96 across the 1,255 SNPs retained. This SNP number represents a balance: not so small that it might lack power to differentiate between different populations, and not so large to have a high amount of missing data. The final dataset includes 1,054 SNPs that have an R^2 less than 0.50. Further exclusion of fixed SNPs and SNPs that are located on sex chromosomes retained 982 SNPs.

Table 5.3. *Number of SNPs that showed linkage disequilibrium distance more than 0.5 and 0.8.*

	R^2 >0.8	**R^2 >0.5**
Number of SNPs that have high LD	376	413
Number of SNPs excluded	183	201
Remaining SNPs	1,076	1,054
Remaining Samples	78	78
Genotyping rate	0.979	0.979

Table 5.4 shows number of SNPs that were variable in each population estimated by MAF based on 1,054 SNPs and exclusion of R^2 > 0.5. There were 850 variable SNPs in the Oman Nubian ibex population, of which 489, 179 and 548 SNPs were variable in WWR, Shalim and Dhofar respectively.

Table 5.4. *Number of variants found in each population based on 1,054 SNPs. In each population the MAF must be >0.*

	Number of variable SNPs	Proportion of total SNPs
SNPs variables in **WWR, Oman**	489	***0.46***
SNPs variables in **Shalim, Oman**	179	***0.16***
SNPs variables in **Dhofar, Oman**	548	***0.52***
SNPs variables in **captive**	210	***0.20***
SNPs variables in **Sudan**	182	***0.17***
SNPs variables in **Yemen**	150	***0.14***
SNPs variables in **goats**	74	***0.07***

5.4.2 Population structure

STRUCTURE was run using 1,054 and 982 SNPs sets independently to check for the results robustness. The following results are based on 982 SNPs. The differentiation between groups was detected from K=2 to K=8 (Figure 5.3). At K=2, no differentiation was observed between populations of captive, Sudan and goats. The differentiation started to be clearer at K=3 which showed assignment of Sudan samples to captive and goats. From K=4 to K=8 there are clear differentiation between Oman, Sudan, captive and goats. Differentiation between Omani populations was observed at K=5, 7 and 8.

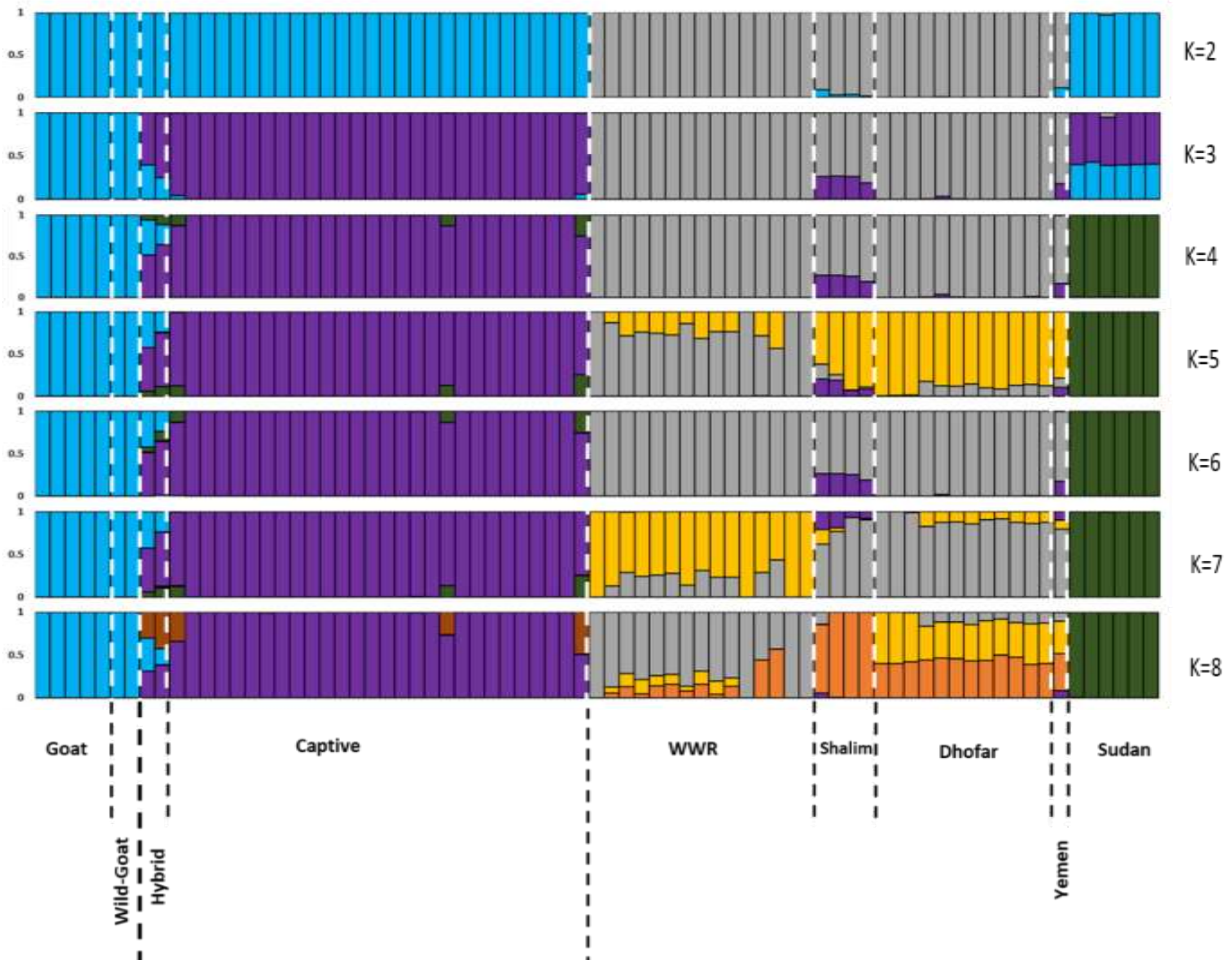


Figure 5.3. *Genetic structures inferred by STRUCTURE of Nubian ibex samples for Goat, Captive, Oman (WWR, Shalim and Dhofar), Yemen and Sudan, based on 982 SNPs. Dashed white lines showed the boundaries of each sampling group. Hybrid and wild-goat individuals were indicated by dashed lines. The y-axis represents the likelihood of membership to each cluster. Each single column represents one sample divided into K colours, where K is the number of clusters assumed and the length of the coloured segment represents the individual's estimated proportion of membership to a particular cluster.*

The two wild-goats samples showed 99% assignment to goats while the putative hybrids detected in the previous study (chapter 4) shared an ancestry with goats (0.25 and 0.43 for each sample), captive (0.52 and 0.62) and Sudan (0.05 and 1.3). There were five samples from Oman and Yemen that shared ancestral assignment with the captive individuals. These samples were detected in the previous study (chapter 4). Three captive samples also showed ancestral assignment with Sudan. The percentages of shared membership between the populations are shown in Table 5.5. From K=4 to K=8 the Sudan samples does not share any ancestral membership with any other group, which indicates that these group are unique. The results of using 1,054 SNPs, which produced the same results is showed in Figure A5.1 in the Appendixes.

Table 5.5. *Percentage of shared membership between Oman, captive, Sudan and goat population. These results based on 982 SNPs and obtained from STRUCTURE analysis at K=4. The dash sign means there is no shared ancestry.*

samples	The contribution of each group			
	goat	captive	Sudan	wild
Hybrid 1 (NUB021)	0.25	0.62	0.13	-
Hybrid 2 (NUB039)	0.43	0.52	0.05	-
Wild-goat (NUB097)	0.99	-	-	-
Wild-goat (NUB103)	0.99	-	-	-
Captive1(NUB020)	-	0.87	0.13	-
Captive 2 (NUB040)	-	0.88	0.12	-
Captive 3 (NUB059)	-	0.77	0.23	-
Oman 1 (NUB003)	-	0.26	-	0.74
Oman 2 (NUB052)	-	0.25	-	0.74

Oman 3 (NUB053)	-	0.17	-	0.83
Oman 4 (NUB140)	-	0.29	-	0.71
Oman 5 (NUB051)	-	0.26	-	0.74
Yemen (NUB121)	-	0.15	-	0.85

PCA results based on 982 SNPs showed differentiation of the data into four groups: Oman, Sudan, captive, goat (Figure 5.4). Two captive ibex samples, identified as putative hybrids in chapter 4, are shown here to be deviated from the main cluster toward the goat samples. In addition, two samples from Oman Nubian ibex (NUB097 and NUB103; 'wild-goat' in Figure 5.4) are clustered with goats. The Yemeni samples clustered with Oman Nubian ibex (PCA analysis based on 1054 SNPs for all population can be found in Figure A5.2 in appendixes. The PCA also showed that there is differentiation among the Omani populations, where some of the samples from WWR cluster together away from Dhofar. On the other hand, Shalim samples are clustered together away from WWR and Dhofar (Figure 5.4).

PCA analysis, based on 589 SNPs after excluding fixed and SNPs on sex chromosomes, was performed to assess the relationship between the samples within Oman and Yemen (Figure 5.5). It showed clustering of three groups almost fully distinct. WWR samples are grouped together away from Dhofar but some samples deviate towards Shalim. On the other hand, samples from Dhofar are grouped together but there is one sample with a slight deviation toward Shalim. These results correspond with the actual distribution of the Omani population on the ground, where WWR population is considered an isolated population to the north of Dhofar and Shalim. The Shalim population is located between Dhofar and WWR (see Figure 2.1C, chapter 2). For comparison, PCA was performed using 1.054 SNPs which showed almost the same pattern of samples distribution (Figure A5.3 in appendixes).

In addition, STRUCTURE analysis was performed for samples from the three locations in Oman and the Yemeni sample (Figure 5.6). The results are in agreement with the PCA analysis.

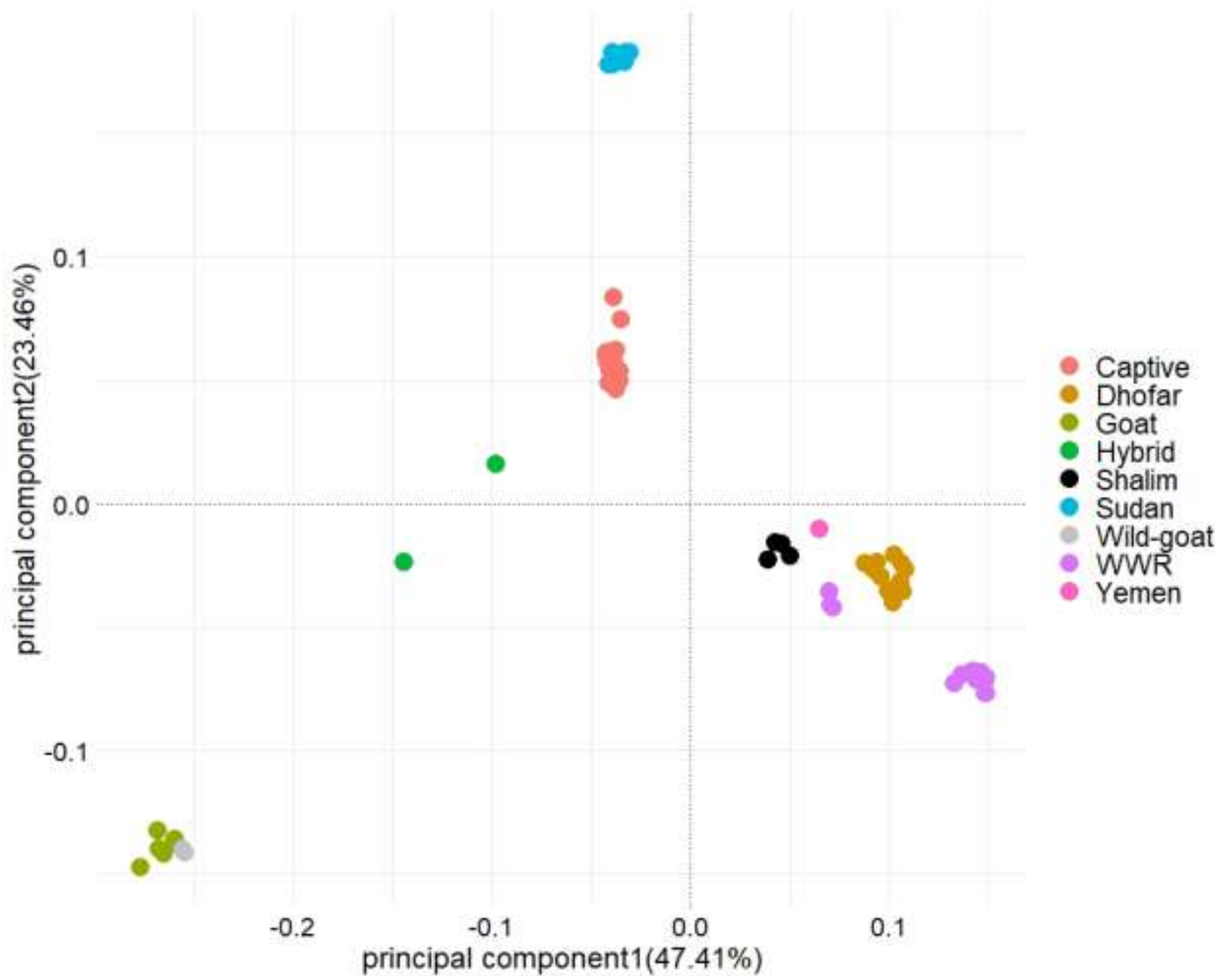


Figure 5.4. *Principal component analysis (PCA) plot of the genetic relationship based on 982 SNPs of Oman Nubian ibex(WWR, Shalim and Dhofar), captive Nubian ibex , goats, Sudan and Yemen. The analysis showed two hybrids from captive individuals (green dots) and two samples from Oman (WWR-goat) clustering with goats (grey dots).*

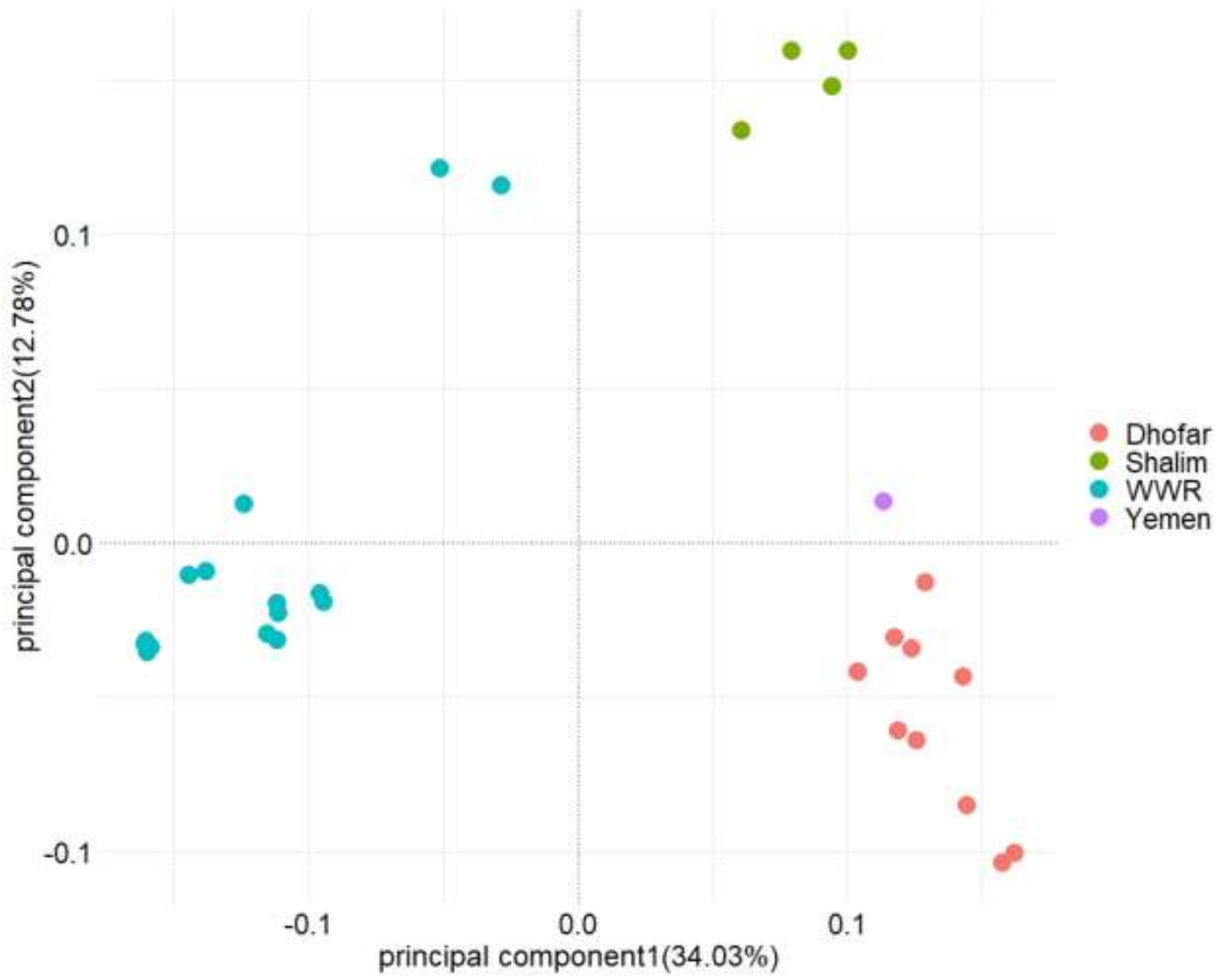


Figure 5.5. *Principal component analysis (PCA) plot of the genetic relationship based on 589 SNPs of Oman Nubian ibex samples from WWR (blue dots), Shalim (green dots) and Dhofar (red dots) and Yemen (purple dot).*

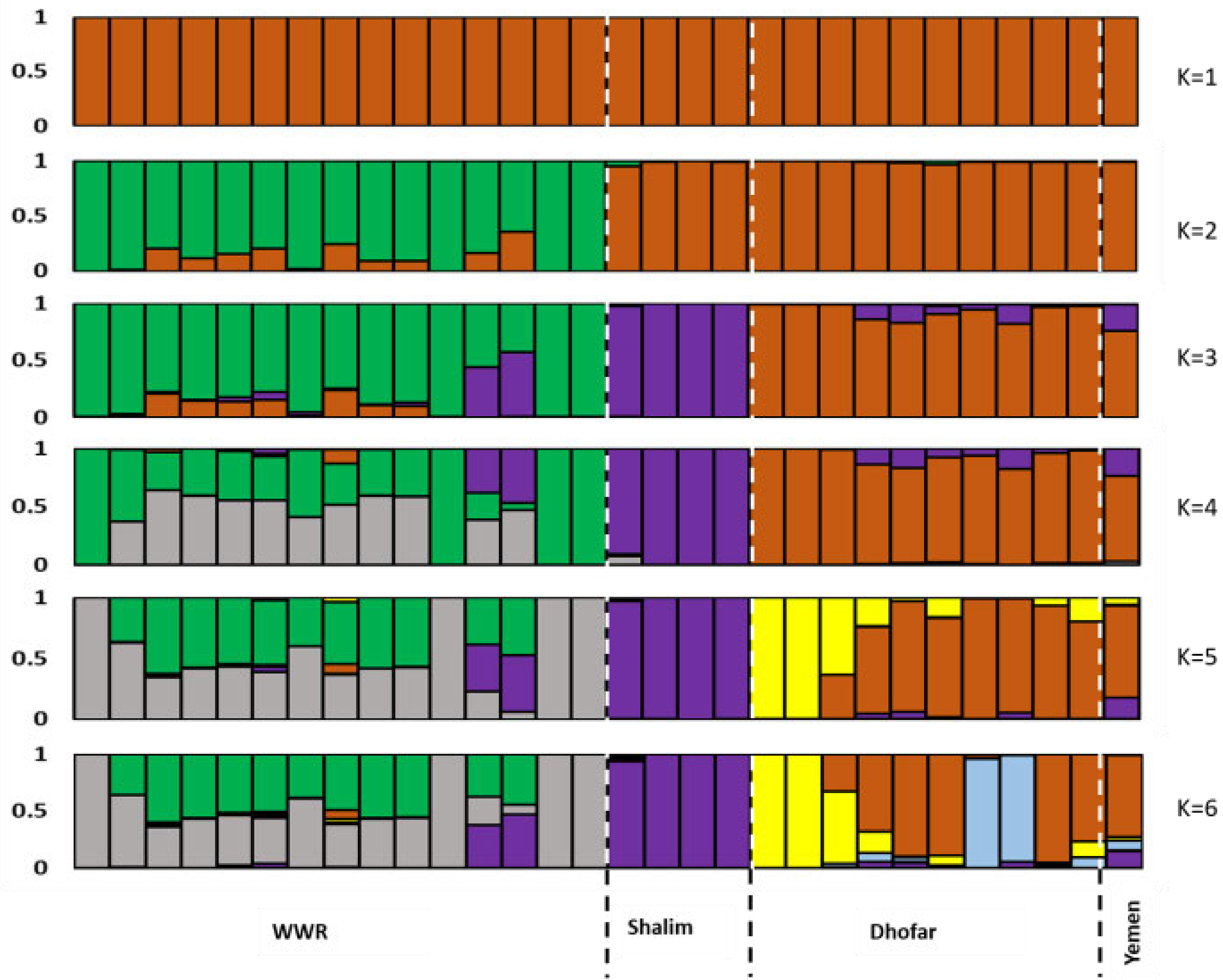


Figure 5.6. *Genetic structures inferred by STRUCTURE of Nubian ibex samples for WWR, Shalim, Dhofar (all in Oman) and Yemen. Dashed white lines showed the boundaries of each cluster. This is based on 589 SNPs after excluding fixed SNPs and SNPs located on sex chromosomes. The y-axis represents the likelihood of membership to each cluster. Each single column represents one sample divided into K colours, where K is the number of clusters assumed and the length of the coloured segment represents the individual's estimated proportion of membership to a particular cluster.*

Pairwise F_{ST} between Oman and captive ibex was 0.433 (Table 5.6), between Oman ibex and goats (F_{ST} = 0.620) and between Oman and Sudanese samples (F_{ST} = 0.513). On the other hand, very minimal differentiation was identified between Oman and Yemeni samples (F_{ST} = 0.062). Additional F_{ST} results between different SNP numbers (344, 1,054 and 1,927 SNPs) obtained by using different filtering criteria are showed in Table A5.3 in appendixes. T-tests showed no difference in F_{ST} values between the different SNP numbers, which suggest that all three filtering criteria give the same result. The assessment of genetic differentiation in the Nubian ibex population in Oman showed significant differentiation between WWR and Dhofar (F_{ST} = 0.231) and Shalim and WWR (F_{ST} = 0.291). Estimating MAF for samples from these three groups revealed that there are 59 SNPs unique only to Dhofar and there are no SNPs unique to WWR or Shalim (Table 5.7).

Table 5.6. *Pairwise F_{ST} estimates for Oman ibex, captive ibex and goats, shown below the diagonal. Above the diagonal are the 95% confidence intervals. All pairwise comparisons were significant at $p \le 0.001$. These results are based on 1,054 SNPs. Hybrid individuals and wild-goat were excluded from this analysis.*

	Oman	Captive	Sudan	Yemen	Goat
Oman		0.408- 0.459	0.482- 0.543	0.019- 0.097	0.592- 0.647
Captive	**0.433**		0.680-0.751	0.573-0.678	0.798-0.847
Sudan	**0.513**	**0.717**		0.701-0.768	0.858-0.889
Yemen	**0.062**	**0.626**	**0.737**		0.840-0.888
Goat	**0.620**	**0.823**	**0.874**	**0.865**	

Table 5.7. *Pairwise F_{ST} estimates for Oman ibex samples in WWR (15 samples), Shalim (4 samples), Dhofar (10 samples) and Yemen (one sample).All pairwise comparisons were significant at $p \leq 0.001$. These results based on 589 SNPs after excluding fixed and sex SNPs. Wild-goat individuals were excluded from this analysis.*

Population1	Population2	Lower bound CI limit	Upper bound CI limit	Fst
Shalim	**WWR**	0.259	0.323	**0.292**
Shalim	**Yemen**	0.136	0.335	**0.239**
Shalim	**Dhofar**	0.161	0.215	**0.189**
WWR	**Yemen**	0.233	0.321	**0.275**
WWR	**Dhofar**	0.207	0.258	**0.234**
Yemen	**Dhofar**	0.049	0.127	**0.088**

5.4.3 Population genetic diversity

The genetic diversity estimates between Omani populations based on 589 SNPs after excluding fixed SNPs and SNPs on sex chromosomes are shown in Table 5.8. H_o and H_e estimates were higher in Dhofar (H_o and H_e = 0.323) than in WWR (H_o = 0.273 and H_e = 0.281). Shalim showed the lowest genetic diversity and highest inbreeding coefficient (H_o= 0.016 and H_e= 0.170) (Table 5.8). This is expected as Shalim samples belong to six animals killed together by hunters and are thought to be one family, as shown in Figure 6.2 in chapter 6.

Table 5.8. *Genetic diversity estimates for the three populations based on 589SNPs, showing observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F_{IS}) and 95% confidence intervals (CI) for each measure.*

	Sample size	H_o (95% CI)	He (95% CI)	F_{IS} (95% CI)
Dhofar	10	0.323 (0.306 : 0.341)	0.323 (0.309:0.337)	-0.006 (-0.035:0.023)
Shalim	4	0.016 (0.010: 0.022)	0.170 (0.147:0.192)	0.864 (0.814:0.914)
WWR	15	0.273 (0.255: 0.292)	0.281 (0.266:0.297)	0.040 (0.010:0.070)
Yemen	1	0.265 (0.229: 0.300)	NA	NA

5.5 Discussion

Hybrid capture provides a cost effective technique to address genetic diversity, taxonomy, evolutionary and phylogeny questions for utilizing low-quality samples such as ancient DNA, historical museum specimens, chemically preserved samples and herbarium samples (Suchan et al. 2016; Schmid et al. 2017; O'Connell et al. 2022). In addition, it has been successfully used to reconstruct almost complete mitochondrial and chloroplast genomes from ancient specimens which has helped in addressing phylogeography questions (Mason et al. 2011; Schweizer et al. 2018; Seeber et al. 2019; Schulte et al. 2021).

This is the first study to assess the genetic structure between samples of Nubian ibex from Oman, Yemen and Sudan using the hybrid capture technique

with non-invasive and museum samples. In addition, previous ddRAD results were merged with the results of hybrid capture, which represent samples of captive Nubian ibex and goats to assess the genetic relationship between this range of different groups.

Here we were successful in capturing genomic sequencing from low-quality samples for different Nubian ibex populations. Despite the probe design being more biased towards the captive and Oman population, this technique has proven to be able to capture sequences in Yemen and Sudan populations. Several studies have shown the ability of this technique to capture homologous and orthologous sequences of even distantly related species (Hedtke et al. 2013; Ilves & López-Fernández 2014). For example, Li et al. (2013) have shown successful enrichment for species that have sequence divergence of up to 40%.

5.5.1 Robustness of results to filtering criteria

The use of different filtering criteria produced different numbers of SNPs. The number of SNPs produced according to the filtering criteria ranged from 397 SNPs when using stringent criteria to 70,935 SNPs when using relaxed criteria. The first two filtering criteria had almost the same number of remaining samples and the same genotyping rate but different SNPs numbers (Table 5.2). The PCA showed no substantial clustering differences between different filtering criteria. The only key difference was when SNPs were filtered in more than 60 and 70 percent of the samples, which caused the removal of captive, Sudan and Yemen samples (Figure 5.1). A total of 1,255 SNPs with a genotyping rate of 0.98 were used for estimating genetic parameters between populations.

The effects of two criteria used to filter LD were compared for this set of 1,255 SNPs, namely excluding any SNPs that have LD>0.8 and LD>0.5 respectively. The comparison showed no substantial differences when using either criteria on

the results (Figure 5.2), which indicates robustness of the data. After excluding the SNPs that had LD more than 0.5, the remaining SNPs were 1,054. The removal of the fixed SNPs and SNPs that were located on sex chromosomes resulted in a final number of 982 SNPs. All of the genetic analysis between the populations were based on this number of SNPs.

5.5.2 Population Structure

The molecular dataset results of this study yielded unprecedented resolution in the relationships between different Nubian ibex groups. Both, STRUCTURE and PCA analysis clearly indicate the divergence between Sudan, captive individuals and the southern Arabian populations in Oman and Yemen. The PCA analysis showed a clear separation of Sudan samples from other groups, while at the same time the captive individuals clustered together away from Sudan and Omani/Yemeni groups. As indicated before by ddRAD, there are two hybrid samples in captive individuals, which deviated toward the goat group and this result was supported by both mitochondrial and STRUCTURE results (see chapter4). In the current study we detected two samples of Oman Nubian ibex clustering with goat (see below for explanation).

The results based on the mtDNA investigation between Sudan, Yemen, Oman and captive individuals (see chapter 3) showed shared mtDNA haplotypes between Sudan and captive individuals. The current results based on SNPs reveal a significant divergence between Sudan and captive individuals (F_{ST} = 0.717, $p \leq 0.001$). The Sudanese samples were collected over 80 years before the contemporary samples in Oman, Yemen and captive populations. However, the effect of 80 years difference between sampling of the Sudan population and the captive animals on allele frequencies and loss of diversity might contribute to the high divergence seen here and possibly contribution from other populations not sampled here, e.g. Egypt, Jordan, Israel, KSA. Generally, these

results indicate the fine resolution of genomic approaches in delimitation between closely related species and taxon (Springer et al. 2001).

The STRUCTURE analyses identified five Omani samples with noticeably high level of assignment to the captive population, with Q-value (which represent the proportions of membership of each population to other population) ranging between 0.17 and 0.29. The observation was also found with the Yemeni samples where it had assignment to the captive with a Q-values of 0.15. The differentiation of the captive individuals from the Sudan samples might exclude the hypothesis that the source of the captive animals was North Africa, despite there being three animals in the captive population that had assignment to the Sudan with Q-values ranging between 0.05 and 0.23.

According to a personal communication from Paul Vercammen (Former manager of the Breeding Centre for Endangered Arabian Wildlife (BCEAW), Sharjah, United Arab Emirates), the BCEAW collection was built with animals received from South Africa and Bahrain, where 10 Nubian ibex individuals were imported by BCEAW from South Africa in 2000. The four Nubian ibex individuals imported from Bahrain were thought to originate from Saudi Arabia, but the exact location is not known. Another breeding centre in UAE, Al Bustan Wildlife Centre, had brought the same number of animals from Bahrain in the same importation trip. Although the animals in Bahrain are thought to come from Saudi Arabia, there is a possibility that they include animals acquired from Sudan as well. Subsequently at BCEAW, animals brought from Bahrain were mixed with descendants of South African animals. Out of this population, some animals were provided to the Oman Mammal Breeding Centre (the Centre I sampled in this study) and this was confirmed by the centre administration in Oman (Khalid Al Rasbi, personal communication). This likely explains the shared mtDNA haplotypes and clustering pattern of Omani captive individuals with captive individuals from UAE.

The South African animals mentioned above originated in the USA, but had originally been imported from Hai-Bar Nature Reserve in Israel. The Nubian ibex at the Wildlife Centre in Taif Saudi Arabia were also brought from USA in 1987 and 1988 (two animals from Cincinnati and 22 animals from San Diego Zoo; Paul Vercammen personal communication). A schematic representation of the sources of the captive animals in UAE and Oman is shown in Figure A5.4 in appendixes.

In addition, according to Paul Vercammen, Al Ain Zoo in UAE imported Nubian Ibex 50 years ago from either Sudan or Egypt. There is no documentation confirming the source of these animals nor showing if they were sent to any other collection in the Arabia. From these communications, we can say that there are some animals brought from North Africa and others sourced from the Levant. Whether animals originating from North Africa have been used for reintroductions or as founder for captive programs needs to be further investigated.

The results of this current study show that there are three genetic clusters of Nubian ibex: one represents the Sudan samples, one the captive animals from UAE, and one represents wild Nubian ibex from Oman and Yemen. STRUCTURE analysis was able to resolve population structure between these different groups and this was confirmed by repeating the analysis with different subsets of the data.

One possible explanation for the source of the captive animals is that they are either from the animals brought from South Africa to UAE or animals brought from Bahrain with Saudi Arabian origin. The assignment of three captive individuals to the Sudan group might be explained by possible non-deliberate mixing of animals from Sudan with captive animals. On the other hand, the assignment of some Omani and Yemeni animals to the captive population could

be explained by a possible recent gene flow between Omani/Yemeni population and populations un-sampled here, for example Saudi Arabia. This postulation could be true if one considers the captive population to include Saudi ancestry. However, this view needs further investigation by incorporating samples from wild animals from Saudi Arabia, Jordan and Egypt. The mitochondrial results (see chapters 2 and 3) showed that there are no shared haplotypes between captive animals and samples from Israel, however this result is based on just two samples retrieved from GenBank. Therefore, samples from across the range of the Nubian ibex distribution would be needed to assess the evolutionary and phylogeographic history of this population, which would help in establishment of conservation plans and avoid using species for reintroduction or captive programs not belonging to the area.

5.5.3 Divergence between Oman and Sudan Nubian ibex

In this study, for the first time, we detected a significant differentiation between Omani Nubian ibex and samples of Nubian ibex from Sudan (F_{ST} = 0.513). This is an important result which reveals part of the evolutionary history of this species. The mitochondrial results based on sequences of D-loop and cytochrome *b* markers in chapter 3 support this result, in that there were no shared haplotypes between populations in Oman and samples from Sudan. Moreover, pairwise genetic distance comparisons based on the mtDNA markers revealed significant differentiation between the two populations (see chapter 3). There were no incongruent results found between the mtDNA and nuclear based markers.

The Arabian peninsula has been considered a global biodiversity hotspot harbouring a rate of endemism for mammals of up to 9% (Mallon 2011). The differentiation between Arabian species and their African counterparts have been documented for several species. For example, Bray et al. (2013) found a significant divergence of Kuhl's pipistrelle (*Pipistrellus kuhlii*) between Arabian peninsula and North Africa populations of the same species. In addition

investigation of the lizard genus *Uromastyx* between the Arabian and African species has found that these two species diverged some 11–15 Mya (Amer & Kumazawa 2005). Furthermore, a species from the gecko genus *Stenodactylus* (*Stenodactylus cf. arabicus*) from Oman was found to be genetically distinct from similar species in North Africa (Metallinou et al. 2012). Finally, Fernandes (2011) has reported that white-tailed mongoose (*Ichneumon albicauda*) diverged from its counterparts in Africa from more than 32,500 years ago therefore advised against the use of individuals from Africa for introduction in Arabian Peninsula.

Generally, the distinctiveness and divergence found between Arabian and North African species has been attributed to several climatic conditions and geological evolution characterised by the break-up of the Arabian plate from Africa 30 Mya (Bosworth et al. 2005; Guiraud et al. 2005). In addition, the uplifting of the Yemen Plateau and extension and rifting of the Red Sea might be one of the possible explanations of the divergence between African and Arabian species (Geoffroy et al. 1998; Bosworth et al. 2005). Therefore, an estimation of the divergence time between the Africa Nubian ibex populations and Omani/Yemeni populations might support this notion and then could be correlated with the approximate time of separation of Arabian Peninsula from African continent. The sample size and the source of the Sudanese Nubian ibex specimens may hamper drawing an irrefutable conclusion about the evolutionary and taxonomic history between the two populations. However, this preliminary result from both nuclear and mitochondrial markers gives an indication about a possible differentiation between these two populations, which deserves to be further investigated in the future. More samples from wild Nubian ibex in Sudan are needed to confirm these results. The successful use of the hybrid capture technique in this study should galvanize the use of non-invasive samples from wild Nubian ibex in Sudan for the purpose of genetic studies.

5.5.4 Is it hybridization or contamination?

Surprisingly, there were two samples from WWR that clustered with goat group. The mitochondrial results for both of these samples showed that they have a D-loop marker consistent with *Capra nubiana*. The D-loop was amplified as a single fragment (one sequence with a total length of 242bp: see chapter 2) On the other hand, the cytochrome *b* markers were amplified in three overlapping fragments varying in size between 244 to 369 bp in length (see chapter 2). These three overlapping fragments should be aligned together and create a consensus sequence. Surprisingly, fragments numbered 1 and 3 for sample NUB103, when search blasted against the GenBank databases, aligned into the goat (*Capra hircus*) sequences, while the middle fragment (number 2) aligned into *Capra nubiana* sequence. This inconsistency of cytochrome *b* fragment alignment indicates that there is a likely contamination in this reaction. The PCR reaction for the D-loop and cytochrome *b* were repeated two times and negative control samples were used. However, the results of D-loop were the same and robust in that the sequence aligned completely with *Capra nubiana* reference sequence while it was inconsistent for cytochrome *b* therefore the sequences of cytochrome *b* were excluded (see chapter 2). To minimise possibility of contamination I did follow good practice were I used UV light on all instruments used for DNA extraction and PCR, cleaning working area, including negative controls and repeat PCR.

Although hybridization between *Capra nubiana* and *Capra hircus* was confirmed and documented as shown in our results in chapter 4 and elsewhere (Grossen et al. 2014; Iacolina et al. 2019), the hybridization between these two species based on these results at WWR is unlikely for several reasons. The first reason is the inconsistency of cytochrome *b* sequence alignment results, mentioned above. Second, the D-loop results showed these two samples as being *Capra nubiana*. In addition, it is known that there is no recombination in mitochondria, thus it is unlikely to have one marker from one species and the other marker

from another species. Third, there are other samples that showed the same discordant results between the D-loop and cytochrome *b* but, according to the results based on hybrid capture of nuclear DNA, they clustered as pure Nubian ibex. These samples include NUB084, 87, 91, 94, 98, 101, 115, 116 and 117. Fourth, the results of the STRUCTURE analysis revealed that these two samples were 100% assigned with goats, a result that is unlikely in terms of hybridization in the wild. For example, Senn & Pemberton (2009) identified putative hybrids in the wild as individuals with more than 5% and less than 95% proportion assigned to an alternative population. In addition, the hybrid individuals discovered in chapter 4 had admixture with goats of between 30 to 50% evidencing the capacity of the method to detect hybridisation. It is important to note that these hybrids were only detected in captive animals and have high chance of mating than the animal in the wild

One possible explanation of this incongruent result is that there was missampling or misidentification in which goat faecal samples were collected but though to be from Nubian ibex because the shape of faeces of goats and Nubian ibex are almost the same, and it is hard to discriminate between them. In addition, it is known that goats can use the same feeding areas as Nubian ibex. On the other hand, in the field I tried to collect samples from higher altitudes where goats were not expected to reach naturally or be herded to. A second possible explanation is that goat pellets were collected by mistake along with faecal sample of Nubian ibex and placed in the same container.

Despite the above possible alternative explanations, I cannot exclude the possibility of hybridization between Nubian ibex and goats in the wild, and the detection of hybrids in captivity demonstrates this potential. At WWR and Dhofar there are large numbers of domestic animals (both goats and sheep) pastured in the same foraging areas as the Nubian ibex. In addition, the low population size of wild Nubian ibex may produce restricted mate choice

(especially at WWR) (Ross et al. 2020a) and/or high population sizes of goats and sheep (especially in Dhofar), both of which may increase the risk of hybridization taking place between wild ibex and domestic animals. This type of hybridization has been documented in Scotland between exotic sika deer (*Cervus nippon*) and native red deer (*Cervus elaphus*) (Smith et al. 2018b; McFarlane et al. 2020). Therefore, this possibility of hybridization warrants investigation in the near future and action must be taken to prevent any further possible incidences. If introgression between wild ibex in Oman and goats is confirmed, this would mean that urgent action must be initiated to prevent range overlap between domestic goats and wild ibex. In addition, it will be important to consider establishing a captive breeding programme (see chapter 6) representing pure wild individuals from different populations in Oman.

5.5.5 Genetic diversity within Nubian ibex in Oman

The estimation of heterozygosity between Omani populations in this study showed that Dhofar population had the highest observed and expected heterozygosity (H_o and H_e = 0.323) while it was lower at WWR (H_o = 0.273 and H_e = 0.281). The Shalim population showed the lowest genetic diversity and highest inbreeding because the samples seem to belong to a family group of individuals. These samples were collected from six hunted animals consisting of two adult females, two calves, one sub-adult male and one adult male (Figure 6.2, chapter 6). This result suggests further study of the Nubian ibex population in Shalim is needed, as the population might consist of several isolated groups roaming together and there might be limitations or barriers which prevent the mixing of different groups with each other. Another explanation is that the population in Shalim is very low and as a result consists of few groups that forage in separate areas. This needs to be investigated further by collecting more samples from this area and conducting surveys to estimate the population size and study the geography of the area.

At the mitochondrial level, there were no shared D-loop haplotypes between WWR and Dhofar (chapter 2; Al-Ghafri et al. 2021), which, alongside the distinct nuclear signatures detected here, indicates both historical and contemporary geographical sub-structuring between these two locations. Additionally, a higher level of variation was found within Dhofar samples compared to WWR, as shown clearly by the haplotype (Hd) and nucleotide diversity (Pi) (chapter 2; Al-Ghafri et al. 2021). This pattern is supported by the nuclear results in this chapter based on 982 SNPs, which showed a significant F_{ST} value (0.231) estimated between the two regions.

The results of mitochondrial markers showed a shared haplotype between Yemen and Oman and, furthermore, the phylogenetic analysis support this result whereby the Yemeni samples aligned with samples from Oman. Although only one Yemeni sample was analysed, it showed clear clustering with Dhofar. This indicates the for potential for gene flow across the border between Oman and Yemen, which may provide a lifeline for a highly threatened population, yet more research into the Yemeni population and its connectivity to those in Oman is needed.

5.6 Conclusions

Here we were able to differentiate between three populations of Nubian ibex: Captive, Sudan and Oman/Yemen. Based on gathered verbal records we understand that the source of the captive individuals might be Saudi Arabia or the Levant, while genetic data suggest a closeness to wild populations sampled in Sudan (in 1935). More investigation is therefore needed to clarify the population origin for the captive animals. Given the genetic difference between North African and Arabian animals it would be a concern if some animals from North Africa have infiltrated into captive populations of Nubian ibex in the Arabian Peninsula, and could then be introduced to the wild. This concern is valid given the assignment of some captive individuals to the Sudan group.

Therefore, it is highly recommended that prior to any use of captive animals for Nubian ibex reintroduction programs, it is necessary to test the genetic makeup of these animals.

The sample from the Yemen population clustered with the Omani population, a result that would be expected due to the close proximity between the two populations. Furthermore, the assignment of some individuals from the wild population of Oman and Yemen to the captive population might indicate that at least some of the captive individuals might be from Saudi Arabia. This shared assignment might indicate a gene flow between these populations and the Saudi Arabia population, which would need further confirmation by sampling other Nubian populations in Saudi Arabia, Jordan, Israel and Egypt.

The results of mtDNA (chapter 2) which showed differentiation between WWR and Dhofar populations were confirmed here by nuclear data. Therefore, it will be important to facilitate individual exchange between the two regions. Surveying and establishment of possible ecological corridors used by wild animals, and prevention or mitigation of hunting pressure to permit migration between locations may aid in gene flow between these populations. Additionally, an *ex situ* conservation program should be considered in order to prevent any future loose of genetic diversity due to continued/further isolation or anthropogenic factors.

Finally, hybrid capture provided an exceptional method to investigate population genetic structure in Nubian ibex, by targeting degraded and fragmented DNA from low-quality samples. The successful use of this process will aid in future study and monitoring of not just Nubian ibex, but also other elusive wildlife species in Oman, such as carnivores or other antelope species. This is potentially further facilitated by the fact that an arid climate is relatively less damaging to DNA samples compared to humid conditions (Haile et al. 2009).

Although the design of probes for such a targeted capture technique depends on previous knowledge of loci sequences, where this knowledge is not available, it is still possible to perform hybrid capture by using the published genome of a related model organism (Förster et al. 2018).

Chapter 6 Population Viability Analysis for Nubian Ibex at Al-Wusta Wildlife Reserve in Oman

6.1 Abstract

Conservation of elusive species is challenging particularly if there is paucity in demographic, ecological and geographical information about them. Therefore, it is important to use whatever data are available about the species and deal with its uncertainty to make decisions for conservation management. In this chapter, I performed population viability analysis using VORTEX software to identify and estimate the threats and risks that might drive the small and genetically isolated Nubian ibex population at Al-Wusta Wildlife Reserve (WWR) in Oman to extinction. First, I estimated the minimum viable population of Nubian ibex at WWR and ran sensitivity analysis which modelled mortality rate, birth rate and sex ratio. Various drought and hunting scenarios were simulated to estimate the effects of these events on the survival of this population. In addition, captive populations with varying numbers of founder individuals and supplemental animals of different sexes were simulated in order to assess the feasibility of initiating such a program for Nubian ibex conservation in Oman.

A Nubian ibex population between 70 to 100 was found to be required to insure the persistence of WWR population for 100 years. The results of sensitivity analysis showed that wild Nubian ibex at WWR under the current circumstances are subjected to an elevated risk of extinction over the next 100 years. High individual mortality was found to have the strongest influence on the dynamics of the population, reducing the growth rate and elevating the probability of extinction. Moreover, the analysis showed that the proportion of females plays a

vital role in population viability, thus they need to be at central focus of any future conservation program such as translocation or captive breeding. The effect of drought and hunting scenarios depended on the initial population size. When these scenarios were simulated with a population size of 100 individuals, the results showed high impact on the viability of the population over 100 years. When simulation started with 250 individuals the effect was minimal and the population was found to have a highy probability of surviving for the next 100 years. The captive population simulations showed that starting with 10 individuals and supplementing the population every 5 years will increase its growth rate, size and genetic diversity. Supplementation with females was found to be more important than male supplementation.

6.2 Introduction

6.2.1 Population Viability Analysis

Population viability analysis (PVA) is a method that is widely used by conservation practitioners to assess threats, extinction risk and factors driving declines of a population, as well as to evaluate different management interventions and predict those that would best help in its recovery (Akçakaya & Sjögren-Gulve 2000).

PVA was developed by Shaffer (1981 and 1987). He defined four interrelated and dynamic types of stochasticity that have direct effect on populations. These are demographic, genetic, environmental and catastrophes. PVA uses the demographic statistics of a population to make predications on the magnitude effect of the threats on the population (Chaudhary & Oli 2020). It has been used in the field of conservation in order to: 1) predict population size; 2) estimate the probability of a population going extinct in future or after a specified period of time (e.g. after 100 years) (Coulson et al. 2001); 3) evaluate the population's conservation status; 4) predict the effect of stochastic events; 5) better

understand the causes which lead to population declines; and 6) assess the usefulness and feasibility of any management efforts and link these to any further need for action in order to protect and maintain the population in a viable state (Boyce 1992).

PVA has been used to predict the fate of different types of endangered and non-endangered species such as ungulates (Slotta-Bachmary et al. 2004; Alemayehu 2013), carnivores (Clark 2015; Tian et al. 2011) and primates (Smith et al. 2018; Lees et al. 2014) and has played pivotal role as a guide for research programs, design conservation plans, and help in decision making.

Chaudhary and Oli (2020) have specified the important points that a good PVA should contain. They have suggested that a good PVA should contain background information (i.e. approximate population size, sex ratio, mortality rate and growth rate) about the studied population. The study objectives need to be clear and well stated. Finally, a good PVA should describe the source of data, period taken to collect the data, and the method of data collection.

6.2.2 Minimum Viable Population

Population size plays an imperative role in determining the probability of persistence of wildlife species for a given period of time. Therefore, estimating minimum viable population size (MVP) is fundamental to plan for future conservation strategies. The main objective of MVP is to identify the minimum number of individuals required for any population to have a high probability of survival for a certain period of time (Shaffer 1981; Soule 1987).Therefore, any wild population smaller than the MVP criteria will be considered at a high risk of becoming extinct. PVA can be used to estimate the MVP using basic behavioural characteristics and demographical values such as type of mating (polygamy, monogamy), maximum number of offspring per year, age at which

males and females can start reproducing and the maximum lifespan of the animal.

6.2.3 Limitations and strengths of PVA

The usefulness and accuracy of PVA to predict the fate of a population and to set conservation policies has been, for long time, a controversial issue. For example, Coulson et al. (2001) discussed that a PVA cannot be accurate because high quality data regarding a population's vital rates need to be used and, in reality, these data are only found for rare cases. In addition, they argue that the distributions of vital rates are assumed to stay unchanged overtime, which is again impossible as populations are dynamic and affected by their dynamic ecosystems as well. Therefore they advise not to use PVA unless adequate data that represent the actual population parameters are available and the factors that influence the change in vital rates are estimated (Coulson et al. 2001).

Along the same lines, Ellner et al. (2002) argued that the precision in PVA estimation cannot be achieved when the data used are recent and do not therefore represent a detailed demographic history of the species. This, they argue, will cause difficulties in parameter estimation and will lead to inaccurate estimation of species extinction risk. Furthermore, they showed that risk estimates for longer time intervals (i.e. 100 years or more) produce imprecise results. Thus, they suggested using PVA as one tool among a range of other methods and indicators, such as studying habitat loss, population trends and examination of genetic factors (Ellner et al. 2002).

Some, on the other hand, appreciate the importance of PVA in conservation research but suggest some points to be taken into consideration if a PVA is to be used. For example, Reed et al. (2002) proposed that the data need to be of high quality, appropriate models need to be used, use of appropriate confidence

estimates is required, and results should be interpreted with great caution. They advocate for PVA to be used for making comparisons between different management interventions to enable assessment of which one is likely to have the better result (Reed et al. 2002). Akçakaya & Sjögren-Gulve (2000) also argue for interpreting the results in a relative way, and concentrating more on the causes of the species decline rather than on absolute extinction risks.

On the other hand, some go far in supporting PVA and considering it to be "accurate" in estimating extinction probability (Brook et al. 2000). Taking into consideration that the field of conservation itself is full of uncertainties and limited data about the majority of species, there is a need to act quickly with the best tools available which have the ability to provide information about certain problems even when the data used are uncertain (Brook et al. 2002). Other proponents of PVA have argued that it can help decision makers to understand the factors that are driving the populations to extinction, and indicate appropriate decisions and actions needed to restore habitat and recover species (Lindenmayer et al. 1993).

Regardless of its potential limitations and criticisms, PVA has been, and still is, used widely to predict the viability of wide range of species and it is a it is a commonly used tool of the IUCN-SSC Conservation Planning Specialist Group for conservation planning (https://www.cpsg.org/our-approach/science-based-tools/vortex). Perhaps, in summary, it should be understood that PVA is potentially a very useful tool available to design and prioritize management actions for conservation, but its results and conclusions should still be interpreted with caution (Elderd et al. 2003).

6.2.4 Al-Wusta Wildlife Reserve

Al-Wusta Wildlife Reserve (WWR) is located in the central part of Oman, about 500 km away from the capital Muscat. The reserve is fenced and its total area is

about 2,824 km^2. It contains the breeding centre of some flagship species of Oman wildlife including Arabian oryx (*Oryx leucoryx*), sand gazelle (*Gazella marica*) and Arabian gazelle (*Gazella Arabica*). Recently the Office for Conservation of the Environment (OCE) of Oman has released about 129 wild animals (consisting of Arabian oryx, Arabian gazelle and sand gazelle) (Oman Observer 2020). There are two free ranging wild large mammal species at WWR which have conservation importance: the Arabian gazelle and the Nubian ibex. The latter dwells the Huqf escarpment which is characterized by rough terrain (Figure 6.1).

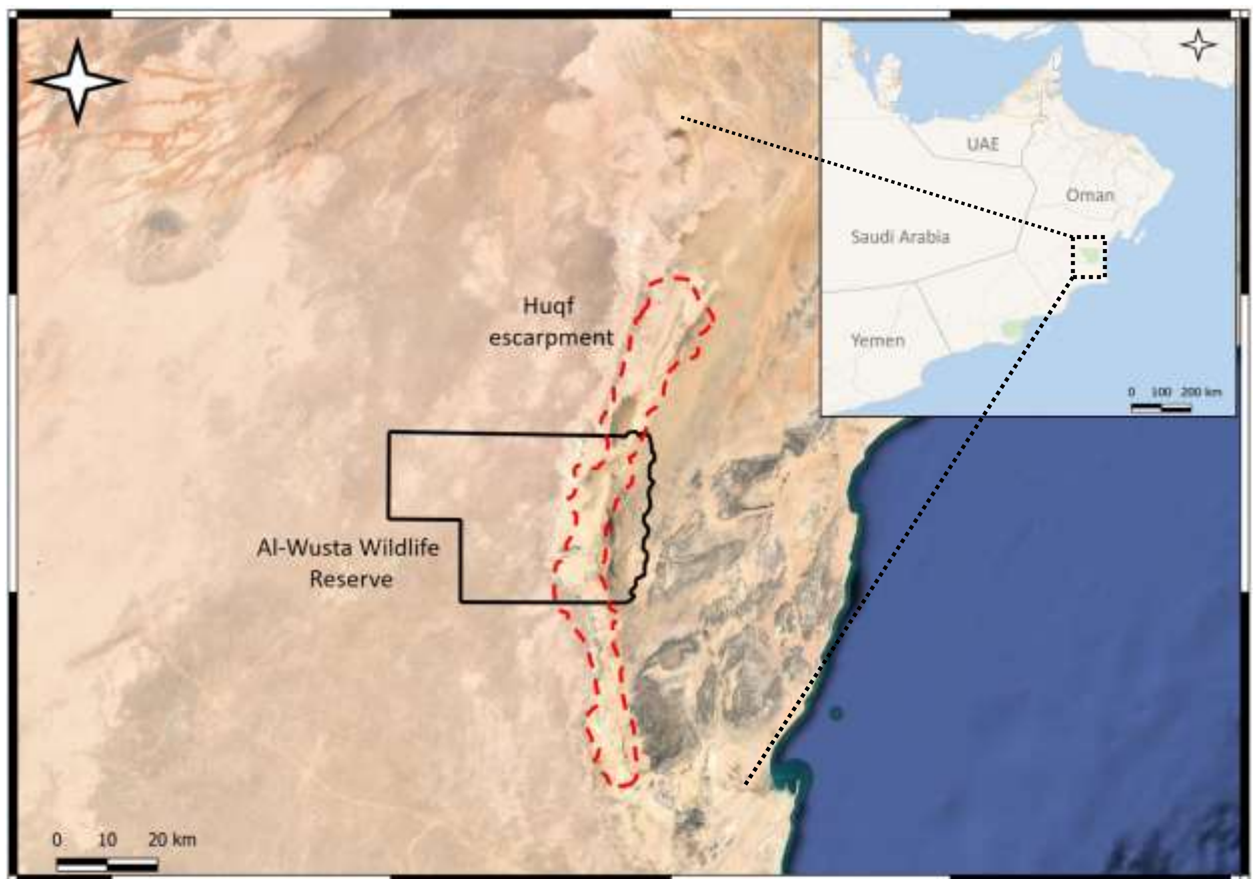


Figure 6.1. *Map showing the location of the Huqf escarpment (red dotted line) and WWR (black solid line). About 60 km of the escarpment is under the protection of the reserve rangers. The irregular section of the black line*

represents the weak points where the fence is either damaged or incomplete because the presence of a natural features such as rocky hills.

The population of the Nubian Ibex in WWR is estimated to be 100 to 250 individuals which is far less than the population in the Dhofar (southern part of Oman) which has been estimated as 600 to 1,100 individuals (Ross et al. 2020a). The density of the Nubian ibex in WWR has been estimated to be one individual /km^2 (Shackleton 1997). In addition, this population is restricted to a limited area of the escarpment that extends for about 150 km in length.

The mean group size estimated for the Nubian ibex population in WWR was 3.2 individuals and ranged from two to 15 animals (Massolo et al. 2008). A similar distribution was observed in Saudi Arabia where the mean group size was found to be 3.4 but with smaller numbers of individuals in each group, ranging from one to 8 animals (Habibi & Grainger 1990). A recent Nubian ibex herd in WWR observed on camera trap images from 2015 consisted of seven individuals made up of two sub-adult males, three adult females and two calves less than one year in age.. In addition, other camera trap recordings showed that a herd could consist of an adult female, a sub-adult male (2-3 years old) and a calf (0-1 year old). Camera traps in WWR have recorded individual males foraging alone as well as groups of females and calves together. Similar observations have also been made by other researchers (Gross et al. 1995; Habibi 1994; Massolo et al. 2008). Generally, herds WWR have been found to split into herds of smaller numbers and move for longer distances after vegetation cover starts to deteriorate (Tear & Ables 1999).

The population of Nubian ibex in WWR is small, so it is likely to be more vulnerable to genetic factors such as inbreeding depression and genetic drift. Genetic diversity is important factor affecting the viability of small populations (Frankham et al. 2017). In chapter two, we showed that the genetic flow between populations in the southern region and WWR is restricted. Because of

these factors, the population in WWR might be at an elevated risk of inbreeding depression and decline in its genetic diversity, which may require attention and monitoring. Other stochastic factors may be playing a role in the population dynamic of the Nubian ibex in WWR. These include drought periods which are caused by low or no rain for long periods of time, sometimes reaching up to 3 years in duration (Price 1989; Corp et al. 1998; Tear & Ables 1999)

6.2.5 Nubian ibex vital values

The gestation length of the Nubian ibex is between 147-180 days (Ronald.M 1983). Litter size is usually one; although twins may occur, this is excluded in the wild where harsh conditions are not in favour of having twins (Placentation 2005). However Massolo et al. (2008) suggested that the Nubian ibex might have a second breeding seasons at WWR, the first one starting between November and December with parturition in spring, while the second one is short and starts in March, the kids being born in summer.

The new-born weight is 1.0 – 1.9 kg, compared to 3.5 – 5 kg for the Alpine ibex (Placentation 2005). Tomson (2007) reports that the Nubian ibex can live up to 17 years and produce one litter per year. According to San Diego Zoo and the Animal Ageing and Longevity Database (AnAge 2017) the maximum known life span of the Nubian ibex is 22.4 years and its gestation lasts between 150 and 160 days. On the other hand, Ilani (1986) cited in Harrison (1991) stated that the Nubian ibex was recorded to live about 11 years (probably based on animals in captivity).

Females reach maturity at the age of two when they can produce their first calf. In contrast, males reach maturity at the age of 3-4 years, but the chances of mating at this age are very low because dominant males will exclude any males reaching maturity and starting to show interest in dominancy (Jones et al. 2009).

Typically, the main predators of the Nubian ibex are the Arabian wolf (*Canis lupus*), Striped hyaena (*Hyaena hyaena*) and leopards (*Panthera pardus*)

(Tomson 2007). However predation on the Nubian ibex in WWR is very rare and is likely to pose no real threat to population growth because there are so few predators recorded (Massolo et al. 2008). Predators are not completely absent, however. In 2013 the biology team at WWR found a striped hyaena cub which had fallen into a deep salty water hole, while in 2016 an Arabian wolf was killed on a road about 60 km away from WWR (personal observation). In addition, golden eagles (*Aquila chrysaetos*) are documented to inhabit the reserve and may prey on the newly born ibex or gazelle (Price 1989).

6.2.6 Objectives of this study

Here I will follow the suggestions and the good PVA practice guidelines outlined by Chaudhary and Oli (2020) in order to come up with an appropriate PVA model for Nubian Ibex to give us suitable predictions for the fate and management of the Nubian ibex population in WWR. Therefore, the main objective of this chapter is first to estimate the MVP that is needed for Nubian ibex population at WWR to have a high probability of survival for at least the coming 100 years. I will then apply drought and hunting scenarios of varying intensities on the best MVP to estimate the potential effect of these factors on the population. In addition, I will explore the viability of establishing a captive population of Nubian ibex at WWR to serve as an insurance population for future reintroduction or reinforcement programs. This will help to better understand the management options available in order to protect the population and keep it viable as long as possible.

Since comprehensive population data on wild Nubian ibex at WWR is not available, I created PVA models based on information available from different articles, books and scientific papers combined with data from field observations based on camera trapping at WWR.

6.3 Methods

In order to analyse the fate of the Nubian ibex population in WWR, I used the software VORTEX (version 10.0.7.9) (Lacy & Pollak 2020). This uses Monte Carlo simulation to predict the consequences of deterministic forces acting on the targeted population. The simulation also incorporates stochastic factors which can be demographic, environmental and genetic. VORTEX is also able to model the effects of catastrophic events on the population such as floods, drought, fires etc. The population dynamics are modelled as independent consecutive events that take place according to defined probabilities by the user. The population is simulated at a specific period of time by stepping through series of events of the individual life cycle (i.e. births, deaths and dispersal) which take place on annul basis (Marshall et al. 2008). These events include mating, mortality, dispersal, supplementation and harvest. The simulation runs for a number of iterations specified by the user. Outputs from the model include estimates of population growth rate, population extinction rate, and population extinction time (Lacy 2000).

Population parameters I used in the PVA process in this study are given in Table 6.1, along with their source.

Table 6.1. *Nubian ibex parameters for PVA simulations, including the reference where they were obtained and or reasons for the value used. "Default" refers to the default value provided in the VORTEX model.*

Parameters	Description/Value	Sensitivity Test	Reason or Reference
Mean group size	4.2±1.7		(Massolo et al. 2008)
Mean home range size at WWR	150 km		(Ross et al. 2020a)
Mating system	Polygamous		(Habibi 1994)

The gestation length	147-180		(Ronald.M 1983)
Lethal equivalents due to inbreeding depression	6.29		Default
Percent due to recessive lethal	50		Default
EV correlation between reproduction and survival rate	1		Default
Age of first offspring females	2		(Strauss 2006; Jones et al. 2009)
Maximum age of female reproduction	10		Estimated (AnAge 2017)
Age of first offspring males	4		(Jones et al. 2009)
Maximum age of male reproduction	10		Estimated (AnAge 2017)
Maximum life span	15		The mean maximum age
Maximum number of broods per year	1		(Massolo et al. 2008)
Maximum number of progeny per brood	1		Field camera traps
Sex ratio at birth, in % males	50		Default
% adult females breeding	50	See Table 6.2	estimated
SD in % breeding due to EV	10		Default
Mortality of females as %			See Sensitivity test
Juvenile females 0-1 years	50	See Table 6.2	
Adult females >2 years	10	See Table 6.2	
Mortality of males as %			See Sensitivity test
Juvenile males 0-1 years	50	See Table 6.2	
Adult males >4 years	10	See Table 6.2	
Catastrophe and frequency %	0		See Table 6.3 for catastrophes scenarios
Reproduction rate reduce to % due to catastrophe			
Survival rate reduce to % due to catastrophe			
% males in breeding pool	50%		Estimated
Initial population size	100		See Method
Carry capacity (K)	300		See Method

6.3.1 Estimating minimum viable population (MVP)

In order to estimate the MVP of the Nubian ibex at WWR we used the parameters in Table 6.1 to simulate population growth using different starting populations sizes (5 individuals, then 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150 and 250). These values were chosen and simulated in order to find the minimum population size that is estimated to have a high probability of survival (≥ 95%) for 100 years whilst sustaining genetic diversity of at least 90%. Therefore I ran simulations with as few as five individuals, and as many as 250 individuals, because the latter is the maximum population estimated at WWR by the IUCN (Ross et al. 2020a). Each population scenario was simulated for 500 iterations and inbreeding depression was not incorporated. These scenarios were modelled for 100 years, which I consider long enough for any long-term factors acting on the population to be observed and evaluated.

The following population parameters were estimated for each scenario: population stochastic growth rate (stoch-r), probability of population extinction (PE), mean population size (N-extant) and genetic diversity (GD). A population would be deemed non-viable if the probability of extinction was >5% and its genetic diversity was <90% after 100 years based on initial runs of data (Frankham et al. 2014).

6.3.2 Sensitivity analysis

Sensitivity tests were used to simulate the dynamics of the isolated Nubian ibex populations at WWR. This process should identify the parameters that will have a significant effect on the population and those that have less effect. The sensitivity of the model to certain population parameters (e.g. mortality, birth rate, sex ratio) may indicate that these parameters are important in order to maintain population viability. Thus any such parameters need to be carefully

monitored and management strategies need to be implemented to minimize any adverse impacts on them that might in turn negatively impact the population. Thus, sensitivity testing was used here to estimate the fate of the population under uncertainties of several parameters. The sensitivity test was used to estimate variation in the following parameters:

1. mortality rate
2. percentage of breeding females
3. percentage of breeding males
4. sex ratio

Sensitivity of the model to these parameters was examined by changing the value in the baseline scenario by ± 5% (Table 6.2). For each scenario, five hundred iterations were run for a period of 100 years and the carrying capacity was set to 300 because the maximum population size at WWR was estimated to be 250 individuals so from a conservative point I would consider that WWR cannot support more than 300 individuals. All the sensitivity scenarios were simulated without incorporating an inbreeding depression factor.

These sensitivity test analyses with above parameters were performed on the population that had been identified as the minimum viable population of Nubian ibex at WWR (see section above). Student's two-tailed t-tests were used to check for significance of the results between scenarios.

Table 6.2. *Highest and lowest reproductive and mortality parameter values used for sensitivity analysis. The (*) means the value is same as the baseline. The Scenarios are: HM&LM (high and low mortality); HB-F&LB-F (high and low breeding females) HB-M&LB-M (high and low breeding males); SR45 (female-biased sex ratio); SR55 (male-biased sex ratio); INB (inbreeding factor).*

			Scenario								
		Baseline	INB	HM	LM	HB-F	LB-F	HB-M	LB-M	SR45	SR55
Paramet	Inbreeding	No	Yes	*	*	*	*	*	*	*	*
	Mortality 0-1 years	50%	*	55%	45%	*	*	*	*	*	*
	Mortality	30%	*	35%	25%	*	*	*	*	*	*

	1-2 years										
	Mortality >2 years	10%	*	15%	5%	*	*	*	*	*	*
	Mortality 2-3 years Males	10%	*	15%	5%	*	*	*	*	*	*
	Mortality >4 years Males	10%	*	15%	5%	*	*	*	*	*	*
	% adult females breeding	50%	*	*	*	55%	45%	*	*	*	*
	% Males in breeding pool	50%	*	*	*	*	*	55%	45%	*	*
	Sex Ratio In %Males	50%	*	*	*	*	*	*	*	45%	55%
	Initial population	100	*	*	*	*	*	*	*	*	*
	Carrying capacity	300	*	*	*	*	*	*	*	*	*

6.3.3 Estimating the effect of Drought and Hunting

The effect of future potential drought and hunting scenarios, two factors that I considered may pose a future threat to Nubian ibex at WWR, were also simulated for population sizes of 100 and 250 individuals because this is the range of population sizes that has been estimated at WWR (Ross et al. 2020a).

6.3.3.1 Drought scenarios

Sensitivity testing was conducted to investigate the potential effects of different frequency and severity of future drought events. The frequency of droughts at WWR was simulated at 10% (the probability of a drought event happening once every 10 years), 20% (once every 5 years) and 33% (once every 3 years). In terms of drought severity, there are two factors that might be influenced, survival and reproduction. However, since animals would likely be able to survive such events, the survival rate was set at 100% for all sensitivity scenarios. In the reproduction section, several values were tested against the frequency of the event, starting from 0% reproduction up to 100% (i.e. 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%) (Table 6.3). For example, if the drought event took place every ten years, the first would assume that there were

no births during that year (0% reproduction), then 10% reproduction, 20% and so on up to a 100% reproduction rate, which means effectively that there is no effect of drought on reproduction. This process was repeated for each drought frequency in order to estimate the minimum reproduction rate that is needed in order for the population to survive and to estimate at what frequencies drought is predicted to have severe consequences for the population.

Table 6.3. *Parameters used to model different drought scenarios.*

Scenarios (Drought occurrence)	Frequency	Reproduction rate	Survival rate
Each three years	33%		
Each five years	20%	0% to 100%	100%
Each ten years	10%		

6.3.3.2 Hunting scenarios

The effect of hunting intensity on the 100 and 250 individuals was simulated independently using sensitivity testing. Several hunting intensity values were tested on sub adults (2-3 years) and adult individuals (> 3 years for females and > 4 years for males) in both sexes to see which intensity and sex targeted the model would be more sensitive to. Therefore, to evaluate the impact of hunting we set the sub adult (males and females) mortality at several intensities (50%, 55%, 60% and 65%) and that for adult males and females at 15%, 20% and 25% (Table 6.4).

The mortality rates I selected for hunting scenarios may be considered pessimistic, because the ibex hunting at WWR is less common when compared to the southern region, because of the nature of the terrain forces hunters to hunt on foot and wait for long periods, and this is less feasible for hunters in a protected area where they can be discovered by any stringent patrols. Nevertheless, this does not appear to exclude hunting attempts, which are

reported frequently. In addition, despite the fact that older animals are much preferred by trophy hunters, specifically the males, the whole family (group) can be terminated if they are encountered by hunters (see Figure 6.2).

Table 6.4. *The parameters used for simulating the effect of hunting on the Nubian ibex population at WWR. Several hunting intensities were tested on sub adult, and adult males and females*.

	Baseline	Hunting
Inbreeding	NO	NO
Female breeding %	50%	50%
Mortality rate		
Male & Female 2-3 years	50%	55%, 60% and 65%
Adult Female >2 years	10%	15%, 20%, 25%
Adult Male >4 years	10%	15%, 20%, 25%

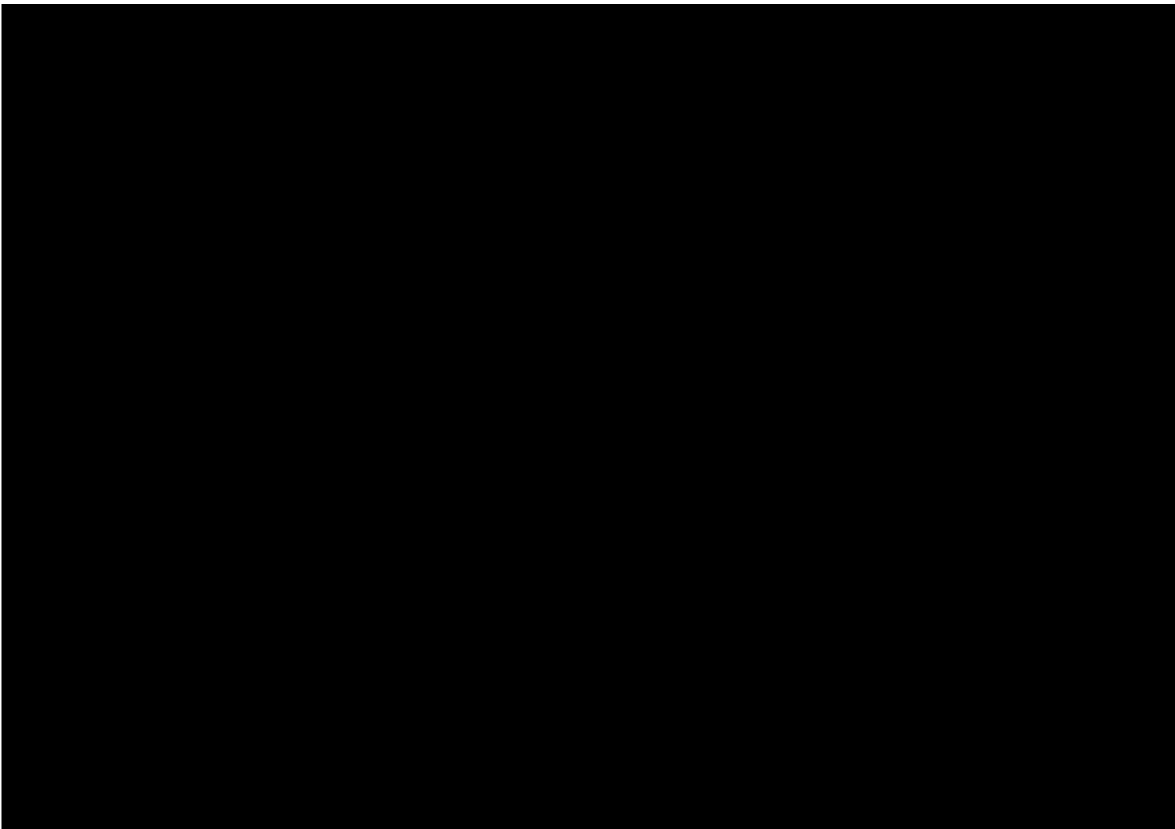


Figure 6.2. *A group of seven Nubian ibex hunted from Shalim in 2018 consisting of one adult male, two sub-adult males, two adult females and two juveniles. (Courtesy of Office for conservation of Environment, Oman).*

6.3.4 Establishment of a captive population

In this scenario, a captive population of Nubian ibex was proposed to be established at WWR. This simulated population will start with 10 individuals (five adult females > 2years and five adult males > 3years). The purpose of this captive population would be to create an insurance population under a scientific management to serve as a source for any future conservation programs such as introduction or translocation plans.

The same parameters used for the baseline scenario were used here, except for the initial population which was 10 individuals. The simulation was run for 500 iterations with inbreeding depression incorporated (Table 6.5). I consider starting with 10 individuals as realistic since capturing animals from the wild requires huge effort and logistical support. In addition, capturing just a few animals is unlikely to cause significant disturbance to the rest of the Nubian ibex population. Furthermore, animals from southern region, which was detected to be genetically more diverse (chapter 2 and 5), would need to be caught and transported to WWR, which could pose risk for animal survival. Therefore, ten animals seemed feasible as a starting point and, later on, these animals could be supplemented.

Supplementation scenarios were simulated to assess the minimum number of wild individuals needed to be added each year to the initial captive population in order to keep it viable for a longer time and more genetically diverse. The initial individuals would be collected from the wild population from WWR and the

southern region using a specialised trap that has proven efficient in capturing live wild animals without harming them (Figure 6.3).

Figure 6.3. *Trap used to capture wild Nubian ibex at WWR. Some hay is placed inside the trap and when animal hits the trigger the door closes. A transmission device sends a signal to alert the trapper(s) when the door is closed.*

The supplementation scenarios started from year two (after the first year of captive establishment) and lasted for 22 years. Wild animals would be captured from wild and supplied to the captive population every 5 years with total of five supplementations (Table 6.5).

Table 6.5. *Parameters used for simulating the effect of supplementation to a proposed Nubian ibex captive population at WWR.*

	Baseline	Supplementation
Inbreeding	NO	YES
Female breeding %	50%	50%
Mortality rate		
Male & Female 2-3 years	50%	50%
Adult Female >2 years	10%	10%
Adult Male >4 years	10%	10%
First year of supplement	-	2
Last year of supplement	-	22
Interval between supplement	-	5
Supplement female >2 years	-	Sensitivity test from 1[*] to 10 animals
Supplement male >4 years	-	

*This mean one individual from each sex; 1 female >2 years and 1 male >4 years

6.4 Results

6.4.1 Minimum viable population (MVP)

The MVP criteria were set as being a population that a have survival probability of 95% (PE ≤ 5%) and genetic diversity of more than 90% after 100 years. The results of simulating different initial population sizes showed that the larger population sizes (> 70 individuals) met the criteria of having a probability of extinction < 5% and genetic diversity ≥ 90% after 100 years (Table 6.6). In contrast, simulation with smaller initial populations (< 40 individuals) showed negative growth rate and higher probability of extinction. At an initial population size of 40 individuals, the growth rate started to increase but still there was a relatively high extinction probability (PE= 28.5%). The PE<5% criteria was met at an initial population number of 70 individuals, but at this population size only

88.8% of genetic diversity was retained. To meet both the PE<5 and GD>=90% criteria the initial population needed to be at least 100 (Figure 6.4).

Table 6.6. *Simulation results of modelling different population sizes of Nubian ibex at WWR for a time period of 100 years. (stoch-r) mean rate of stochastic population growth, (PE) probability of extinction, (N-extant) population size, (GD) genetic diversity, (MeanTE) mean time to extinction and ($SD_{(x)}$) standard deviation of each parameter. Values of PE < 0.05 and GD ≥ 0.9 are shown in bold, as these are the criteria used to assess minimum viable population. Simulations that met both these criteria are shown in italics.*

Population size (N)	stoch-r	SD(r)	PE	N-extant	SD(Next)	N-all	SD(Nall)	GD	SD(GD)	MeanTE
5	-0.035	0.262	0.999	33.00	0.000	0.03	1.040	0.606	0.000	14.4
10	-0.035	0.199	0.987	54.77	69.02	0.72	9.790	0.638	0.149	30.6
20	-0.020	0.162	0.806	98.78	97.39	19.21	57.950	0.697	0.178	51.0
30	-0.004	0.142	0.506	143.28	105.11	70.84	102.87	0.763	0.145	61.5
40	0.008	0.130	0.285	176.04	100.21	125.90	116.15	0.814	0.112	65.5
50	0.015	0.125	0.193	202.86	94.00	163.74	116.34	0.841	0.106	69.3
60	0.023	0.118	0.089	218.40	81.71	198.97	99.74	0.868	0.074	70.9
70	0.026	0.116	**0.049**	228.16	78.93	217.00	91.35	0.878	0.064	71.2
80	0.028	0.116	**0.039**	232.90	74.78	223.82	86.06	0.884	0.073	75.4
90	0.031	0.114	**0.017**	246.34	65.88	242.15	72.66	0.895	0.049	78.5
100	*0.033*	*0.114*	***0.013***	*244.60*	*64.30*	*241.42*	*69.64*	***0.900***	*0.046*	*77.8*
150	*0.037*	*0.113*	***0.000***	*257.36*	*52.73*	*257.36*	*52.73*	***0.915***	*0.026*	*0.00*
200	*0.039*	*0.112*	***0.000***	*261.35*	*47.76*	*261.35*	*47.76*	***0.920***	*0.020*	*0.00*
250	*0.040*	*0.113*	***0.000***	*260.98*	*47.72*	*260.98*	*47.72*	***0.922***	*0.018*	*0.00*

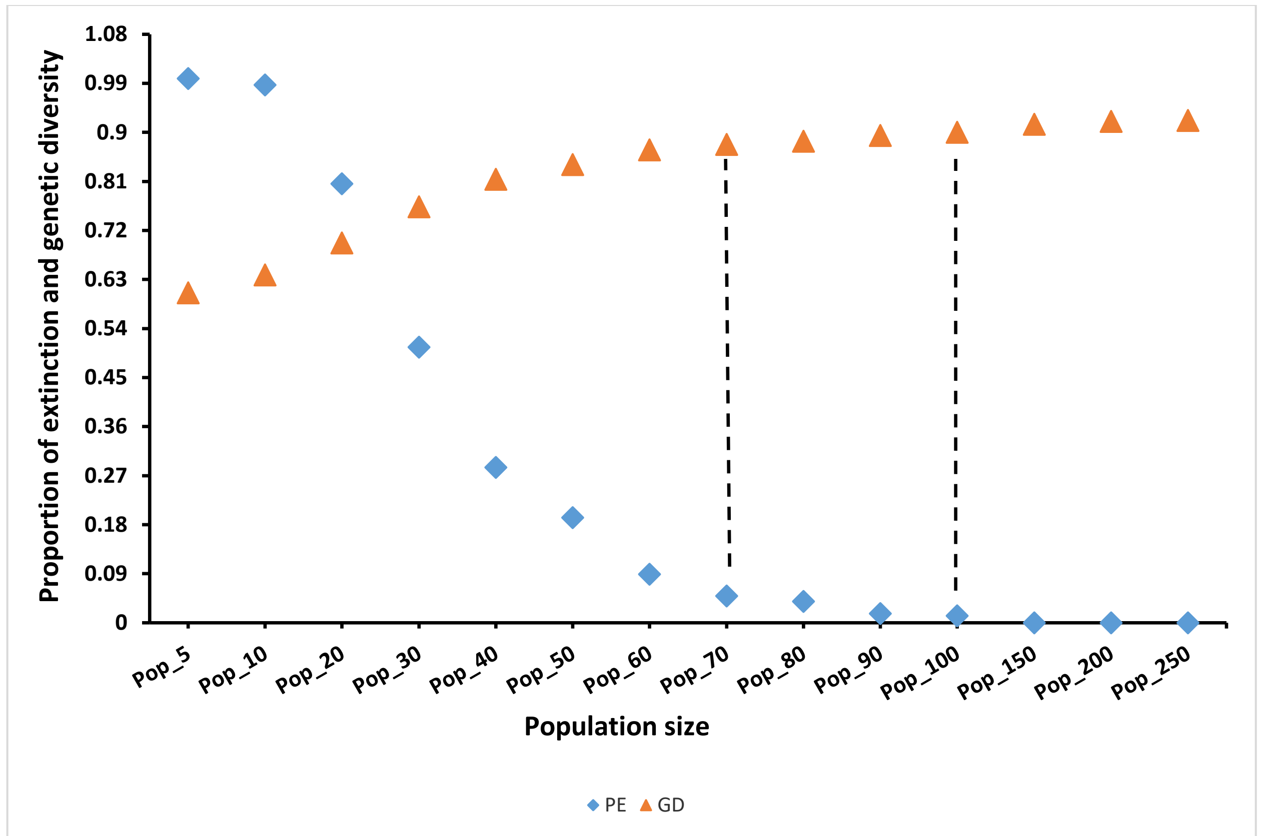


Figure 6.4. *Probability of extinction (PE) and genetic diversity (GD) of different population sizes (from 5 individuals (Pop_5) to 250 individuals (Pop_250)). The dashed lines indicate initial populations of 70 and 100 individuals that had probability of persistence of 95.1% and 98.7% respectively. The 70 is the initial population that meets the PE<5% criteria and the 100 is that which also then meets the GE>=90% criteria.*

6.4.2 Sensitivity test analysis of different parameters

From the MVP analysis, populations of 70 and 100 individuals were identified as the minimum populations that are needed in order for Nubian ibex population at WWR to persist for 100 years. A Population of 70 individuals was found to have 95.1% persistence probability over 100 years but genetic diversity of 88%, while population of 100 individual was found to have 98.7% persistence probability

and 90% genetic diversity. As such, I carried out sensitivity analyses on simulations with initial population sizes of 70 and 100 individuals respectively.

Simulations with an initial population of 70 individuals showed significant differences ($P<0.05$) between sensitivity scenarios. There were no significant differences between high and low breeding males scenario (Table 6.7).

This population size experienced a negative growth rate at high mortality scenario, while inbreeding depression, low breeding females and male-biased sex ratio resulted in lower growth rate compared with other scenarios (Table 6.7 and Figure 6.5). In contrast, scenarios with lower mortality rate, higher breeding females and female-biased sex ratio showed an increase in population growth rate.

At this population size, the probability of extinction was zero in the baseline scenario (i.e. no inbreeding depression), and in scenarios with low mortality, high and low breeding males and females and female-biased sex ratio (Table 6.7). On the other hand, the high mortality scenario showed the highest probability of extinction (PE= 54.4%) followed by the inbreeding depression scenario (PE= 6.6%). The male-biased sex ratio and low breeding females scenarios showed lower probability of extinction (PE= 1.6% and PE= 1.0%) respectively) (Figure 6.5).

High mortality caused the highest reduction in the population size (36.1±2.69 SE) followed by the inclusion inbreeding depression, while in other scenarios the population sizes exceeded 250 individuals (Figure 6.5). All scenarios maintained relatively high overall genetic diversity, ranging from 82% (in high breeding males) to 91% (in low mortality) except the high mortality scenario which reduced the genetic diversity to as low as 63% (Table 6.7).

Table 6.7. *Results of mean rate of stochastic population growth (stoch-r), probability of extinction (PE), population size (N-extant) and genetic diversity (GD) of different scenario simulated by sensitivity test with an initial population of 70 individuals. SE = standard error. A negative value of stoch-r means population decline and a positive value means population growth. P-values are from t-tests between the two scenarios for each parameter, with those that were non-significant shown in red.*

	stoch-r (SE)	P value	PE (SE)	P value	N-extant (SE)	P value	GD (SE)	P value
Base (no inbreeding)	0.057 (0.0005)	P<0.05	0 (0)	P<0.05	284.6 (1.26)	P<0.05	0.89 (0.0018)	P>0.05
Inbreeding (ID)	0.025 (0.0005)		0.066 (0.0111)		213.5 (4.2)		0.88 (0.0031)	
High Mortality (HM)	-0.025 (0.0012)	P<0.05	0.544 (0.0217)	P<0.05	36.1 (2.69)	P<0.05	0.63 (0.0179)	P<0.05
Low Mortality (LM)	0.124 (0.0005)		0 (0)		297.1 (0.51)		0.91 (0.0009)	
High breeding females (HB-F)	0.0769 (0.0006)	P<0.05	0 (0)	P>0.05	288.7 (0.94)	P<0.05	0.90 (0.001)	P<0.05
Low breeding females (LB-F)	0.036 (0.0006)		0.01 (0.0044)		264.1 (2.42)		0.87 (0.0026)	
High breeding males (HB-M)	0.057 (0.0005)	P>0.05	0 (0)	P>0.05	285.7 (1.05)	P>0.05	0.82 (0.0021)	P>0.05
Low breeding males (LB-M)	0.058 (0.0006)		0 (0)		284.9 (1.09)		0.89 (0.0030)	
Female-biased sex ratio (SR45)	0.078 (0.0005)	P<0.05	0 (0)	P<0.05	291.6 (0.86)	P<0.05	0.90 (0.0011)	P<0.05
Male-biased sex ration (SR55)	0.035 (0.0006)		0.016 (0.0055)		264.7 (2.65)		0.87 (0.0030)	

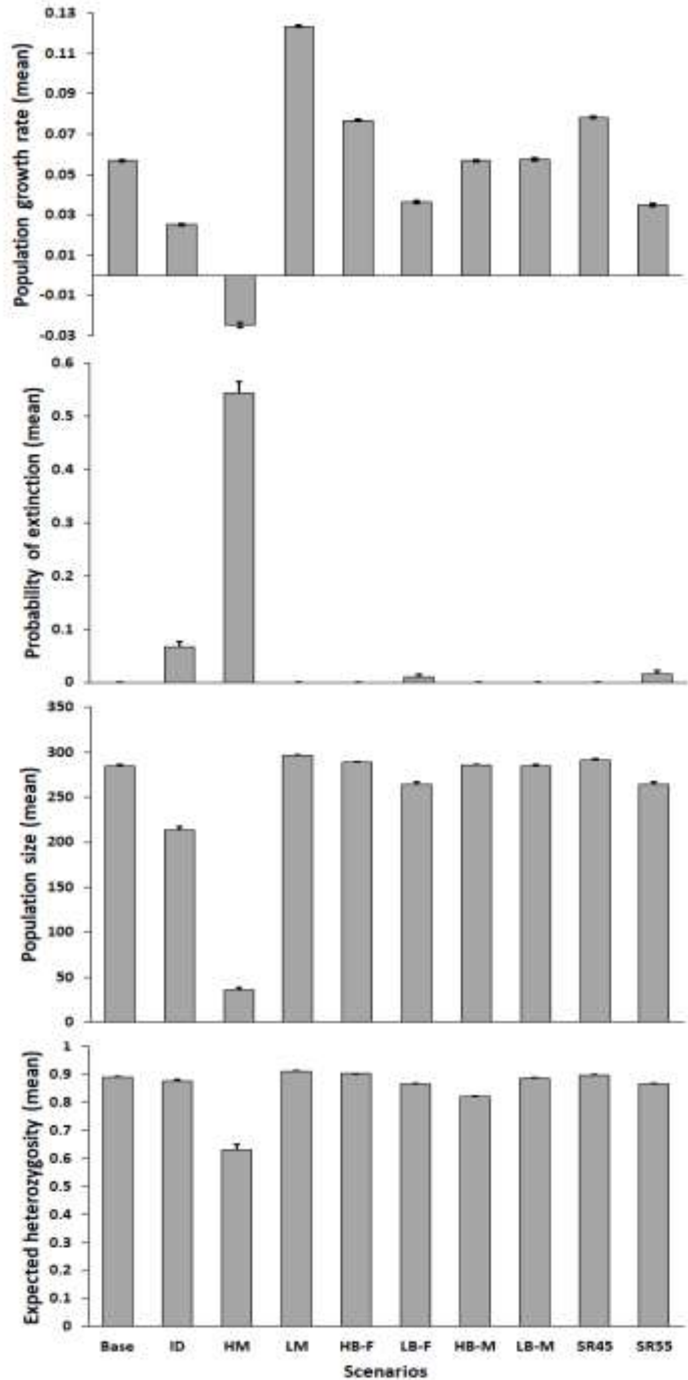


Figure 6.5. *Sensitivity analysis results of an initial population of 70 Nubian ibex individuals under different scenarios. Bars indicate the standard errors for population growth rate, probability of extinction, population size, expected heterozygosity. Base, baseline scenario (no-inbreeding); ID, inbreeding depression; HM, high mortality; LM, low mortality; HB-F, high breeding females;*

LB-F, Low breeding females; HB-M, high breeding males; LB-M, Low breeding males; SR45, females-biased sex ratio; SR55, males-biased sex ratio.

For scenarios with an initial population size of 100 individuals, probability of extinction was zero for all scenarios except for those which included inbreeding, high mortality, low breeding females and a male-biased sex ratio. High mortality showed the highest extinction probability (PE= 41%), while the low breeding females scenario showed the lowest (PE= 0.4%) (Table 6.8 and Figure 6.6).

The high mortality scenario had resulted in a negative growth rate while inbreeding depression, low breeding females and male-biased sex ratio resulted in a decrease in population growth (Figure 6.6). On the other hand scenarios with lower mortality rate, higher breeding females and female-biased sex ratio showed an increase in population growth rate.

Population size was reduced significantly in the high mortality scenario (48.6±2.98 SE). The inbreeding scenario has the second lowest population size of all scenarios, although this was above 240 individuals so represented a growth relative to the baseline (Table 6.8).

For the genetic diversity, all scenarios resulted in high gene variability except for high mortality, which reduced the overall genetic diversity to 70%.

Overall, the sensitivity analysis results suggest that the demographic parameters that the model is most sensitive to were mortality and change in percentage of females that breed in the population. Therefore, these factors are important and should be measured and monitored regularly regardless of population size.

Table 6.8. *Results of mean rate of stochastic population growth (stoch-r), probability of extinction (PE), population size (N-extant) and genetic diversity (GD) of different scenario simulated by sensitivity test with an initial population of 100 individuals population. SE standard Error. A negative value of stoch-r means population decline and a positive value means population growth. P-values are from t-tests between the two scenarios for each parameter, with those that were non-significant shown in red.*

	stoch-r (SE)	P value	PE (SE)	P value	N-extant (SE)	P value	GD (SE)	P value
Base (no inbreeding)	0.058 (0.0005)	P<0.05	0 (0)	P<0.05	284.64 (1.23)	P<0.05	0.91 (0.001)	P>0.05
Inbreeding (ID)	0.034 (0.0005)		0.018 (0.006)		244.37 (6.32)		0.91 (0.002)	
High Mortality (HM)	-0.020 (0.001)	P<0.05	0.408 (0.020)	P<0.05	48.632 (2.97)	P<0.05	0.70 (0.014)	P<0.05
Low Mortality (LM)	0.122 (0.0005)		0 (0)		297.71 (0.424)		0.92 (0.0007)	
High breeding females (HB-F)	0.078 (0.0005)	P<0.05	0 (0)	P>0.05	290.95 (0.850)	P<0.05	0.91 (0.0008)	P<0.05
Low breeding females (LB-F)	0.036 (0.0006)		0.004 (0.003)		266.48 (2.15)		0.89 (0.0018)	
High breeding males (HB-M)	0.058 (0.0007)	P>0.05	0 (0)	P>0.05	285.70 (1.21)	P>0.05	0.91 (0.0009)	P>0.05
Low breeding males (LB-M)	0.057 (0.0006)		0 (0)		284.26 (1.17)		0.90 (0.0013)	
Female-biased sex ratio (SR45)	0.078 (0.0006)	P<0.05	0 (0)	P<0.05	289.70 (0.868)	P<0.05	0.91 (0.0001)	P<0.05
Male-biased sex ration (SR55)	0.036 (0.0007)		0.016 (0.005)		265.12 (2.58)		0.89 (0.0021)	

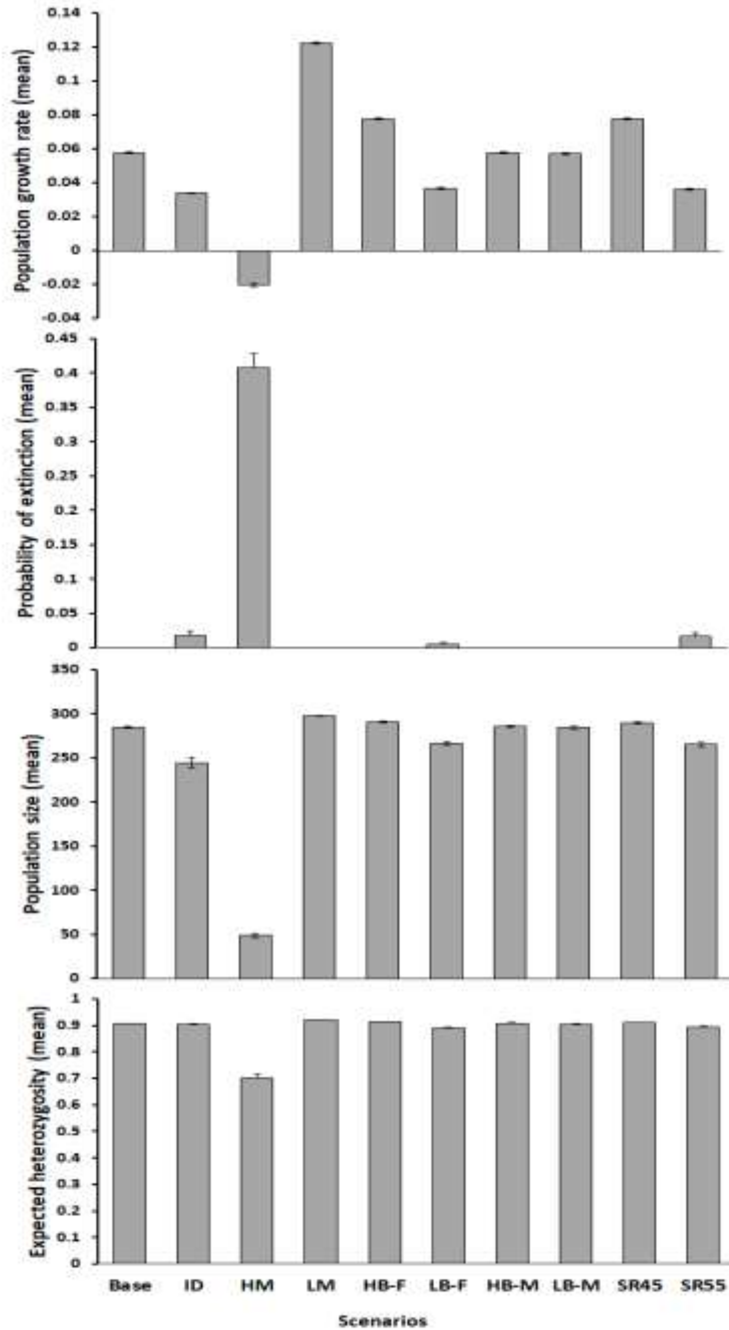


Figure 6.6. *Sensitivity analysis results of an initial population of 100 Nubian ibex individuals under different scenarios. Bars indicate the standard errors for population growth rate, probability of extinction, population size, expected heterozygosity. Base, baseline scenario (no-inbreeding); ID, inbreeding depression; HM, high mortality; LM, low mortality; HB-F, high breeding females;*

LB-F, Low breeding females; HB-M high breeding males; LB-M Low breeding males; SR45 females-biased sex ratio; SR55 males-biased sex ratio.

6.4.3 Drought scenarios

The effect of different drought scenarios was simulated first for population sizes of 100 then separately for population of 250 individuals because these are the range of population sizes of Nubian ibex estimated in 2020 at WWR. Sensitivity tests were used to simulate different reproduction rates during different drought frequency scenarios.

When drought frequency was set at 10% (occurring once each 10 years) the growth rate for population sizes of 100 and 250 individuals was positive but when the drought frequency increased to 20% or 33% (occurring once each 5 and 3 years respectively), the growth rate for both population sizes reached negative values (Figure 6.7 and 6.8). The probability of extinction for population size of 100 individuals was higher at the 0% reproduction rate (during the drought years) in the three frequency scenarios (PE=15%, 73% and 98% at frequency of 10%, 20% and 33% respectively) compared to the population size of 250 individuals (PE = 5%, 39% and 94%). The extant population size and the final genetic diversity (at 0% reproduction rate during drought years) decreased when the drought frequency increased for both population sizes (see Table A 6.1 and A 6.2 in appendixes).

A minimum reproduction rate of 80% during drought years was required for the population size of 100 individuals during different drought frequency events to attain more than 95% population survival after 100 years. The same population size would have to have more than a 90% reproduction rate during droughts in order to achieve genetic diversity of around 90% in the three drought frequency scenarios. On the other hand, only a 40% reproduction rate during drought years is needed for the population of 250 individuals to have a survival

probability of more than 95%, and a 60% reproduction rate to attain genetic diversity of around 90% for all three drought frequencies. Hence, the population of 100 individuals is more sensitive to higher frequency of drought than the larger population.

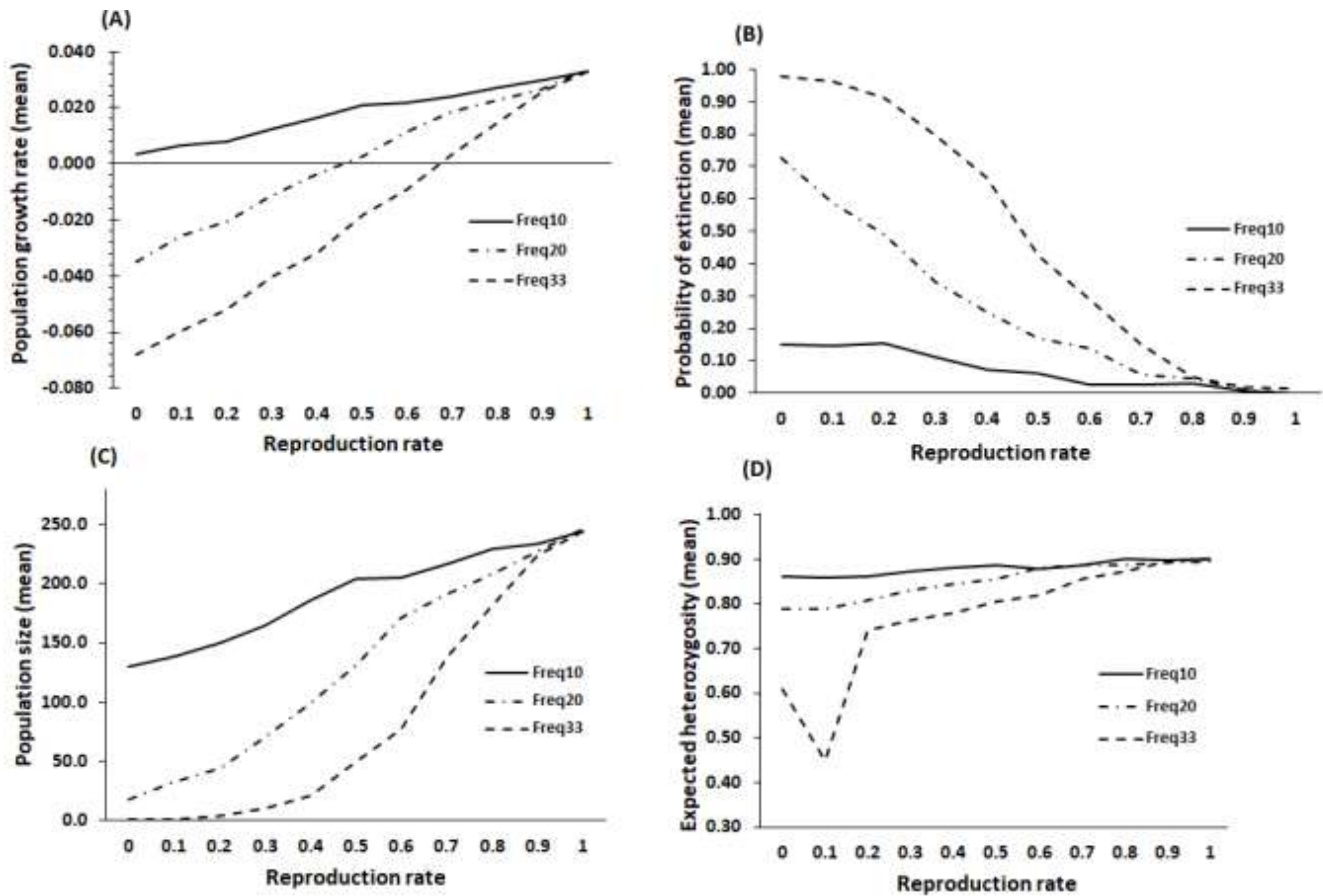


Figure 6.7. *Drought scenarios at different frequencies (Freq10 = 1 in 10 years, Freq20 = 1 in 5 years, Freq33 = 1 in 3 years) and reproduction rates during drought years (relative to non-drought years) for a population of 100 individuals.*

The graphs show: (A) population growth rate; (B) probability of extinction; (C) population size; (D) expected heterozygosity.

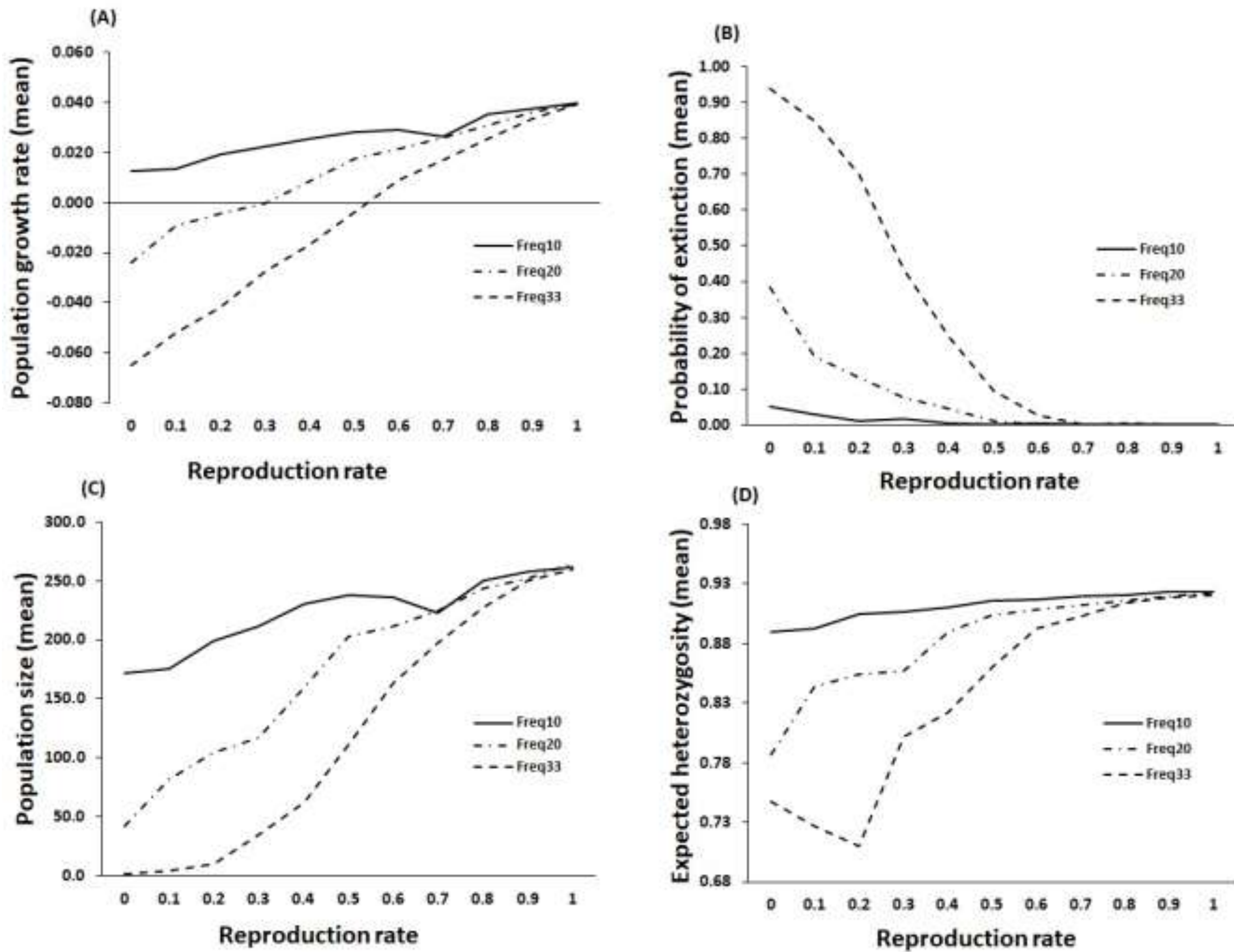


Figure 6.8. *Drought scenarios at different frequencies (Freq10 = 1 in 10 years, Freq20 = 1 in 5 years, Freq33 = 1 in 3 years) and reproduction rates during drought years (relative to non-drought years) for a population of 250 individuals.*

The graphs showed (A) population growth rate; (B) probability of extinction; (C) population size; (D) expected heterozygosity.

6.4.4 Hunting scenarios

In these scenarios, the age and the sex of individuals targeted by hunters were varied to identify which sex and age stages are likely to be more sensitive to hunting for population sizes of 100 and 250 Nubian ibex individuals.

Here only adult animals were simulated and tested for the effect of hunting, assuming that they are more likely to be targeted and easier to shoot from a distance. Hunting of adult males and females were tested under hunting intensities 15%, 20% and 25% each year for 100 years. The results showed that different simulated hunting intensities had no effect on growth rate, final population and genetic diversity when the hunting targeted adult males, regardless of the initial population size (Table 6.9). On the other hand, when the intensity was set at 20% and 25% hunting of adult females, it caused negative growth rate in both initial population sizes (100 and 250). The high intensity of hunting on adult females resulted in high probability of extinction and reduced genetic diversity in both population sizes (Figure 6.9). These results showed that Nubian ibex populations are more sensitive to hunting when adult females are targeted than when adult males are targeted.

Table 6.9. *Results of mean rate of stochastic population growth (stoch-r), probability of extinction (PE), population size (N-extant) and genetic diversity (GD) of different hunting scenario simulated by sensitivity test for 100 (pop100) and 250 (pop250) individuals in the initial population. SE = standard error.*

	Stoch-r (SE)	PE (SE)	N-extant (SE)	GD (SE)
Adult Females				
pop100				
15%hunting	0.023 (0.001)	0.007 (0.007)	238.967 (6.172)	0.861 (0.007)
20%hunting	-0.021 (0.002)	0.477 (0.040)	54.328 (7.319)	0.718 (0.024)
25%hunting	-0.060 (0.002)	0.933 (0.020)	0.927 (0.303)	0.155 (0.048)
pop250				
15%hunting	0.023 (0.001)	0 (0)	250.952 (5.042)	0.907 (0.003)
20%hunting	-0.015 (0.002)	0.187 (0.031)	84.626 (6.759)	0.805 (0.011)
25%hunting	-0.055 (0.001)	0.850 (0.021)	3.605 (0.801)	0.346 (0.048)
Adult Males				
pop100				
15%hunting	0.059 (0.001)	0 (0)	283.881 (2.128)	0.904 (0.002)
20%hunting	0.060 (0.001)	0 (0)	286.473 (1.991)	0.903 (0.002)
25%hunting	0.058 (0.001)	0 (0)	284.068 (2.153)	0.898 (0.002)
pop250				
15%hunting	0.058 (0.001)	0 (0)	283.663 (1.569)	0.919 (0.001)
20%hunting	0.057 (0.001)	0 (0)	282.772 (2.589)	0.918 (0.002)
25%hunting	0.058 (0.001)	0 (0)	278.707 (2.984)	0.912 (0.002)

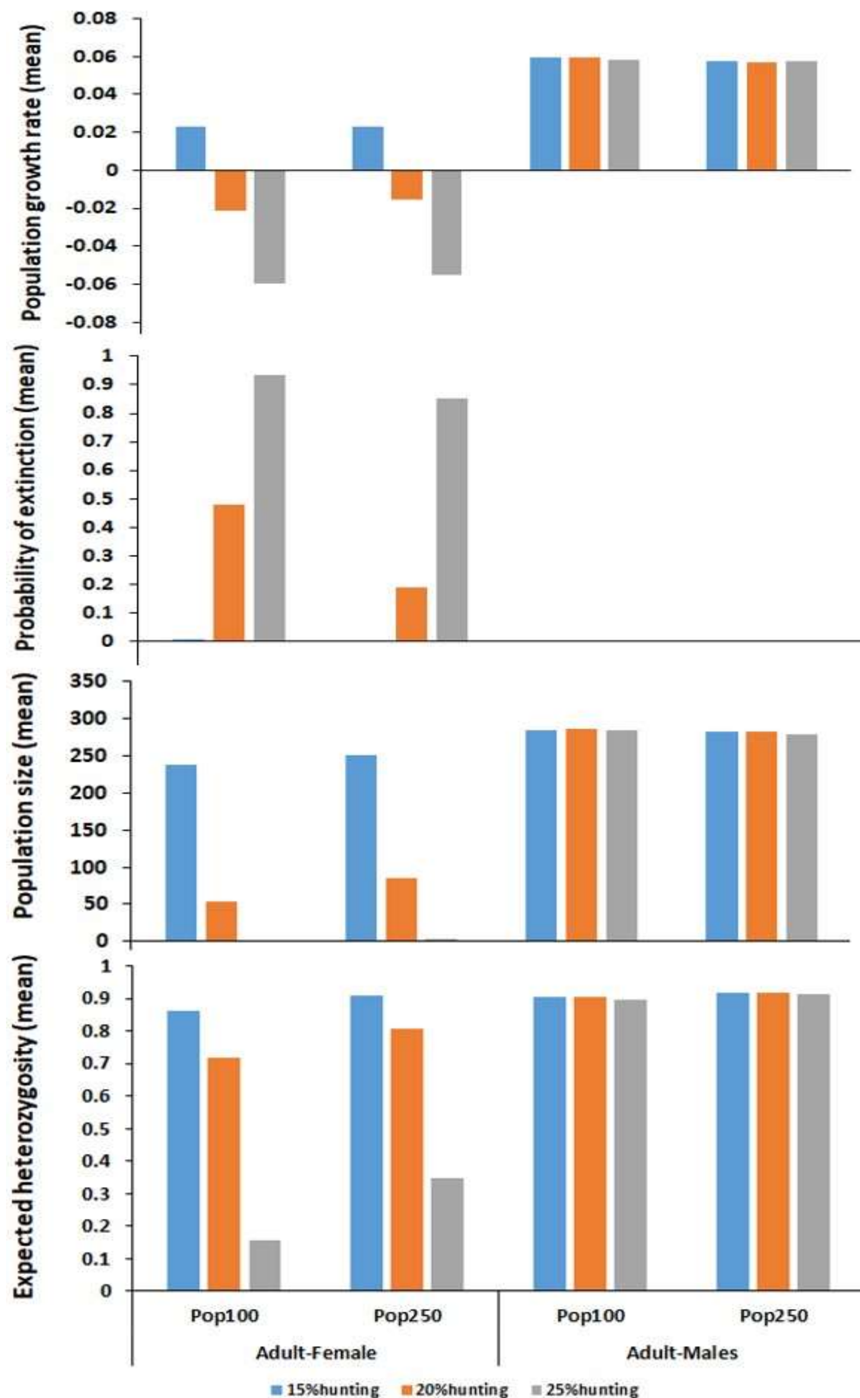


Figure 6.9. *Sensitivity analysis results for population sizes of 100 and 250 individuals (Pop100 and Pop250 respectively) under different hunting intensities (15%, 20% and 25%) of adult females and males on population growth rate, probability of extinction, population size and expected heterozygosity.*

6.4.5 Captive population and supplementation

A captive population starting with 10 individuals would need to be managed in a scientific way (e.g. using a studbook) to minimize mating between related animals. Therefore, such a population is deemed unviable if not managed properly. Simulation results for an unmanaged population (with no supplementation) predicted a probability of extinction of 95%. The sensitivity test of the number of males and females needed to keep this population viable and genetically diverse indicated that when the simulated number of animals used to supplement the captive population every 5 years increased, so did the growth rate, population size and genetic diversity (Figure 6.10). Moreover, supplementation with females seems to be critical for the simulated captive population, decreasing the probability of extinction and increased the other parameters more than supplementation with males (see Table A6.3 in appendixes).

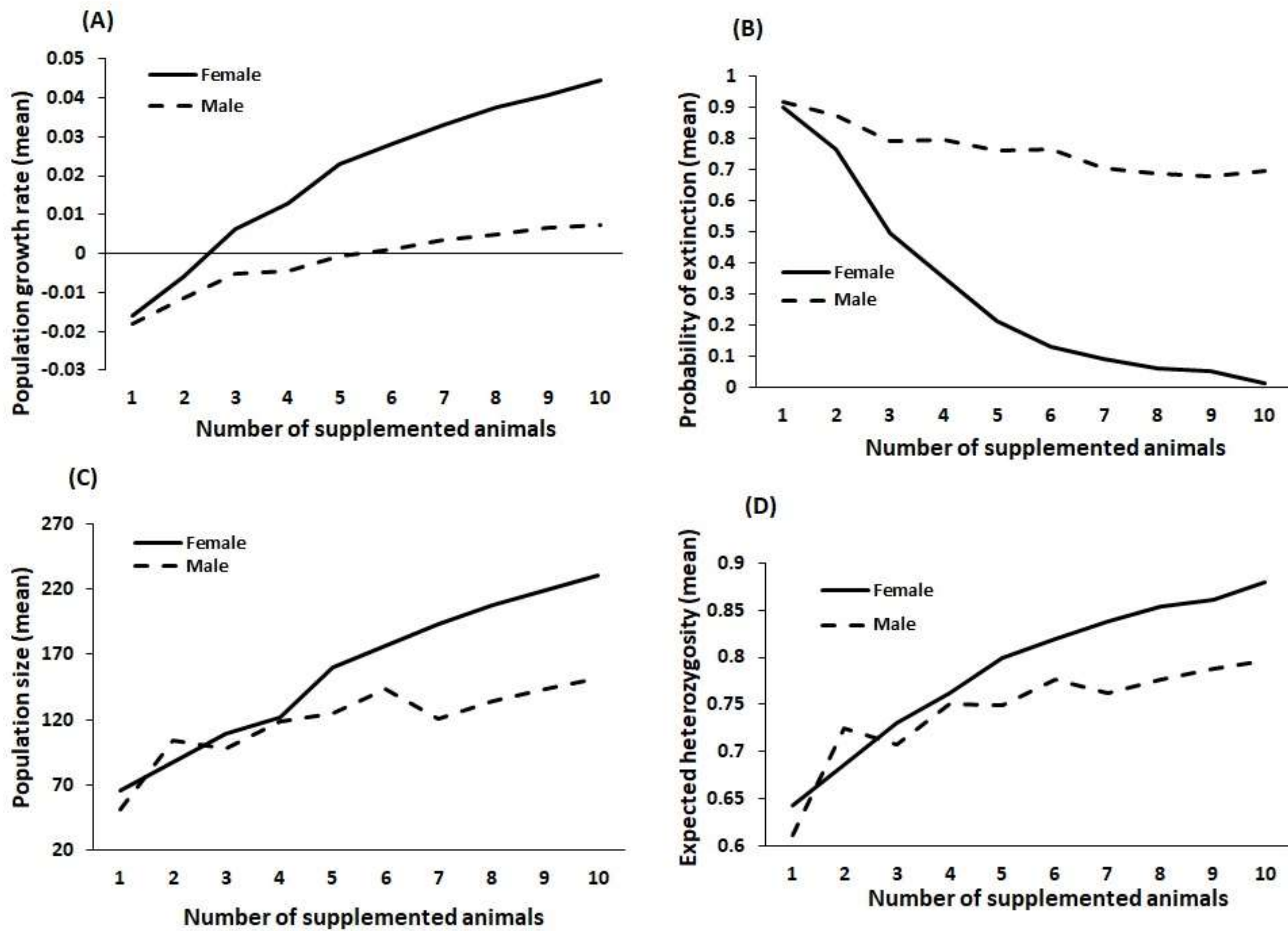


Figure 6.10. *The effect on population viability of using different number of males and females to supplement a captive population of Nubian ibex that started with 10 individuals. In simulations, animals are added every 5 years, five times. The graphs show: (A) population growth rate; (B) probability of extinction; (C) population size; (D) expected heterozygosity.*

6.5 Discussion

Nubian ibex were once known to be located in several isolated locations in the central desert of Oman but unfortunately the majority of these populations have become extinct in the past few decades (Alkon et al. 2008). WWR currently hosts the last Nubian ibex population in the central region of the country. This population is small and it is decreasing due to different factors such as habitat loss, fragmentation, and hunting. Therefore, it is important to minimize these threats and avoid any further local extinctions of wild populations by creation and implementation of conservation programs. Population viability analysis is one of the common tools used to assess the threats and estimate the factors that may affect the persistence of wild population.

Population size plays a vital role in the ability of any population to persist and adapt for changing environment, but the question to be asked is how large populations need to be (Reed et al. 2003). The minimum viable population analysis for Nubian ibex in this study used conservative parameters where thresholds of more than 95% survival rate and more than 90% genetic diversity were specified for a population to exist after 100 years.

Assuming that the model parameters and environmental conditions remain the same for the next 100 years, the results of simulating different initial population sizes showed that a population size of more than 100 individuals of Nubian ibex is likely to persist at WWR over the next 100 years. This population is estimated to have more than a 98% survival probability and 90% genetic variability remaining after 100 years. Thus, the estimated Nubian ibex population of between 100 and 250 at WWR (Ross et al. 2020a)should be large enough to be viable over the next 100 years assuming conditions remain the same. The simulated population of more than 200 individuals was found to be able to retain

more than 92% of genetic diversity which would be critical for adaptability and resilience to changing environmental conditions. On the other hand, the simulations also showed that small sized initial populations (70- 90 individuals) might have the ability to persist in the long term, but would likely suffer high loss of genetic diversity that may result in accumulation of deleterious alleles, therefore impacting the population over time (Frankham 1997). Consequently, conservation measures and monitoring techniques must be implemented to maintain genetic variation for the population at WWR.

6.5.1 Sensitivity analysis of uncertain parameters

High mortality, inbreeding depression, low numbers of breeding females and a male-biased sex ratio all showed significant effects on simulated Nubian ibex populations with initial population sizes between 70 and 100 individuals. High mortality had the strongest influence on the dynamics of the population, reducing the growth rate and elevating the probability of extinction. This means that any threats that reduce survival or reproductive rate, such as hunting or severe drought, have the potential to severely affect the population. The effect of mortality on wildlife population viability has been investigated widely for different mammal species. For example, the roan antelope (*Hippotragus equinus*) experienced population decline when mortality rate of adults and juveniles exceeded 40%, while when management action acted in reducing the mortality by 10% the population showed an increase (Kimanzi 2018). Similarly, the giant anteater (*Myrmecophaga tridactyla*) was found to experience a decrease in growth rate when female mortality reached 18% while the same mortality percentage for males did not affect the growth rate of the population (Desbiez et al. 2020).

In addition, my simulations showed that female breeding percentage had a significant effect on the growth rate of the Nubian ibex population. Similar results have been found for different mammals. For example, when the

percentage of breeding females of Asian elephants (*Elephas maximus*) was set at 12.5%, the growth rate decreased by around 21% while, when the percentage of breeding females increased to 25%, the growth rate was found to increase by 44% (Changhuan et al. 2020). Similarly, the percentage of breeding females for the giant anteater was simulated with low value (50%) and high value (70%) and the results showed that the growth rate increased from 0.03 to > 0.06 (Desbiez et al. 2020).

My results show that the Nubian ibex population at WWR is likely to be affected by the proportion of females in the population but it is insensitive to the proportion of males. This as aligns with the fact that in this species one adult male is known to mate with several females in the breeding season (Habibi 1997). Generally in *Capra* species it is known that the majority of males are not included in the breeding pool because of dominant males excluding them (Toïgo et al. 2007). Therefore for future conservation actions, females need to be a central focus of any program such as translocation or captive breeding.

For better preparation and management of the species, it is important to work on generating accurate values of Nubian ibex mortality rates and population demography in the wild. Accurate estimation of demographic data for wild Nubian ibex can be facilitated by using several methods including yearly field surveys or by using camera trapes (Attum et al. 2022).

6.5.2 Drought impacts and mitigation

Nubian ibex is known to be highly dependent on water and it needs to drink on a daily basis (Habibi 1994; Wakefield et al. 2008). Water availability plays a vital role in productivity in the species in the wild, and it has been found that the rutting season of the Nubian ibex occurs during the months of high rain probability (Massolo et al. 2008). Hence water availability is important in driving population growth.

In general, drought has been found to reduce reproductive success, decrease the productivity of ecosystems and increase mortality in species (Archaux & Wolters 2006). In low rainfall seasons the availability of food reduces, and with competition with other wild or domestic animals, food sources became scarcer. If, as a result, foraging does not satisfy individuals' calorific requirements, growth and reproduction can be delayed as these processes demand high energy (Bronson 2009).

The drought events which were simulated here, closely align to those previously documented at WWR, with different drought periods ranging from one to three years (Price 1989; Spalton 1999; Tear & Ables 1999). Stanley Price (1989) indicated that Arabian oryx has experienced periods of drought at WWR and a decision of supplementation with water and food during these drought periods was made to enable the population to cope with the harsh conditions. The oryx population was able to grow in size and reach more than 30 individuals during these periods. Another factor that might help oryx populations and other species is that the reserve's climate has been known to experience frequent dewfall and foggy days, which play a vital role in maintaining vegetation cover regardless of fewer rain events. Therefore, we can expect Nubian ibex to cope well with the harsh conditions and it is expected to have adapted very well to situations of drought and low food availability. These adaptations should be studied and explored in the future, especially the question as to whether Nubian ibex use high saline water sources that are available in several locations around the reserve.

The simulations performed here showed that the Nubian ibex population is likely to be able to tolerate low frequency drought seasons (once every ten years) even when the reproduction rate was set at zero in those years. In addition, the simulated population could tolerate high frequencies (once each three and five years) of drought periods if the reproduction rate was between 40% and 80% of its normal value in drought years, for initial populations of 250 and 100 respectively.

Accordingly, given the rarity of natural drinkable water sources in the desert, measures to help the wild species at WWR during drought seasons may be essential. It will be important to set up a plan for food and water provision when it is necessary during such severe drought seasons, which may help reduce impact on reproductive rate. This strategy has been found useful for mammal conservation (Arabian oryx (*Oryx leucoryx*) in Oman (Stanley Price 1989 and Spalton 1999), kangaroos and wallabies in Australia (James et al. 1999), striped hyenas (*Hyaena hyaena*) in Jordan (Attum et al. 2017) and African elephants (*Loxodonta africana*) in Zimbabwe (Tshipa et al. 2017)).

The management team of the WWR has already installed several permanent water spots targeted to areas containing the highest biodiversity of wildlife species. These water spots are connected with a water tank which is connected to a small reservoir that dispenses water mechanically when the water level in the hole decrease beyond the trigger point (Figure 6.11). A camera trap is fixed in one corner to capture animals that use the water. This idea has its drawbacks as well. For example, the fence surrounding the water source designed to prevent camels using the structure, still allows other domestic animals to enter (especially goats and sheep) which compete for water with wild animals, and this could be a source of disease transmission. Moreover, such features might encourage the local people with their livestock to stay in the area as long as the water is available, and hence cause disturbance to the wildlife. In addition, these water sources might act as hunting points where hunters can wait for the animals and kill several animals in one go (El Alqamy et al. 2010). These weak points need to be addressed and better solutions to be created to prevent such problems. For example, local people need to be educated about the importance of wildlife and how the existence of such animals can bring benefits to them. On the other hand, frequent patrolling of wildlife rangers at the water points might deter hunters, as they would be unlikely to stay for a long time if they perceived that the place is under constant protection.

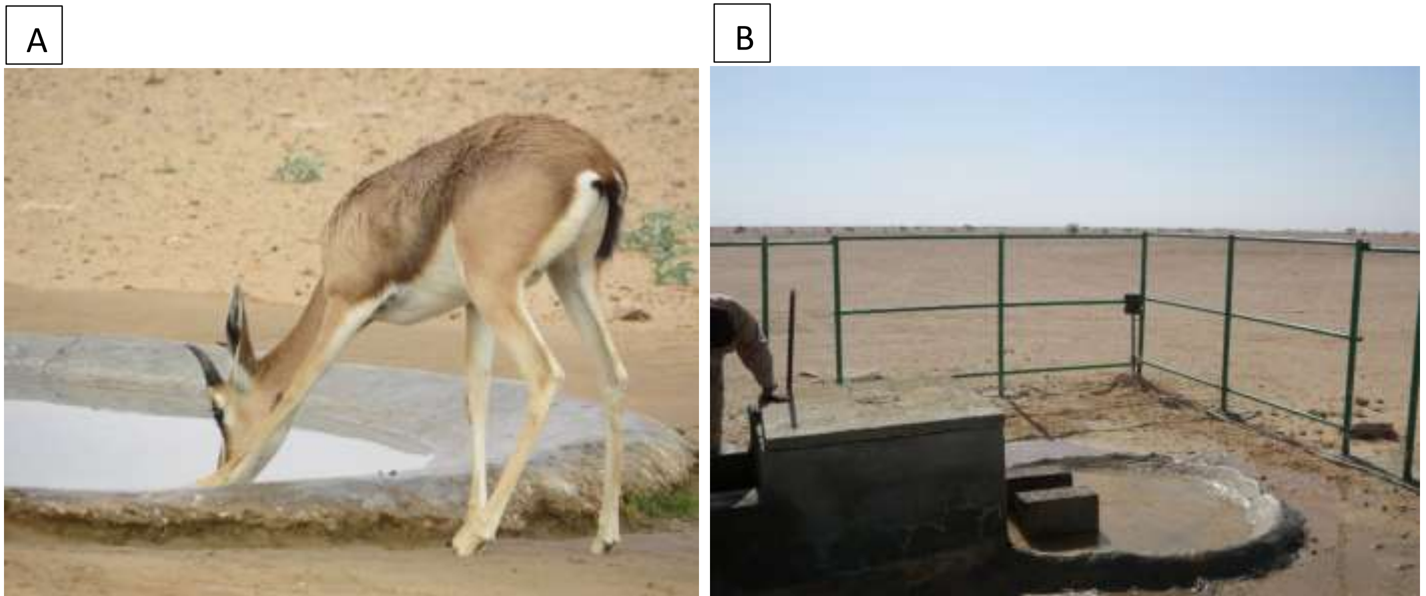


Figure 6.11. *Water provision for the wildlife at WWR. (A) Arabian gazelle drinking from artificial water depression. (B) Fenced water place to prevent camels from using the water. A camera trap is fixed in one corner to monitor use of the site by wildlife.*

6.5.3 Hunting impacts and mitigation

Hunting has been identified globally as one potential driver of species extinctions (Gross 2019). In the Arabian Peninsula, hunting has caused extinction of several wildlife species and put others in the brink of extinction (Barichievy et al. 2016). Economic and infrastructure development has been found to lead to more accessibility to wild populations to people, therefore increase hunting intensity (Davies et al. 2006). Nubian ibex is a large-bodied animal and lives in groups, which mean that they are easily seen and detected by hunters. They are hunted mainly as trophies for their distinct horns. In addition, their geographical range, particularly in WWR, is limited and this factor

has been associated with high extinction risk (Price & Gittleman 2007). As shown in the above scenarios, the simulated hunting events that caused a reduction in females numbers showed a more negative effect on the population while the events that effect the males have lower and sometimes negligible effects.

However, in this scenario the Nubian ibex population at WWR showed the ability to cope with a low level of female hunting (15%) where the population still can grow and maintain high genetic diversity, especially when the initial population was assumed to be 250 individuals. High hunting intensity caused a severe reduction in simulated populations. However, such a hunting scenario with high intensity is not likely to happen under current conditions at WWR because this area is under protection from rangers and Nubian ibex hunting is considered a challenging quarry species, needing highly skilled and experienced hunters (Attum 2007). However this does not eliminate hunting incidents, that are reported frequently (Giangaspero et al. 2014). At WWR, the population likely benefits from the protection enforced by the rangers who patrol the reserve day and night which minimizes the potential harm to the population caused by hunting.

According to my personal experience, several other effective measures have been implemented to reduce hunting, such as reinforcing the security fence around the reserve and concentrating monitoring at the weak points in the fence. In addition, camera traps were used to conduct surveys and spot the frequent and preferred locations visited or used by the Nubian ibex which would allow these areas to be protected and monitored regularly by rangers. Furthermore, wildlife aspects have been incorporated into education programs to communicate with locals about the importance of preserving wildlife species. Incorporating local people in protection and monitoring and reporting any hunting attempts to the reserve management will assist the protection efforts and help in preserving wild species. This can be done in several forms, such as

school visits, meeting with locals during social gatherings, and through social media, which the majority of people use nowadays.

6.5.4 Captive breeding

Captive populations or *ex situ* conservation has proven to be a useful strategy that have saved several endangered species from being extirpated (Conde et al. 2011; McGowan et al. 2017). Many species has been reintroduced to the wild by aid of captive breeding centers and zoos, such as California condors (*Gymnogyps californianus*), whooping cranes (*Grus americana*), Mauritius parakeets (*Psittacula eques*), black-footed ferrets (*Mustela nigripes*), Arabian oryx (*Oryx leucoryx*) and American bison (*Bison bison*) (Conway 2010). *Ex situ* conservation priority objectives are to increase the size and genetic diversity of the captive population, and it aims to create insurance population that can be used for reintroductions and supplementations to the wild, with the ultimate goal being to protect endangered species from extinction (Allendorf et al. 2022).

At WWR there are currently three species in captive management: Arabian oryx, Arabian gazelle and sand gazelle. The infrastructure for a captive program is thus already in place and it could be quite readily used for initiation of a captive program for the Nubian ibex with some modifications. These modifications should include wide enclosures that contain artificial hills and big boulders for climbing, and some caves for hiding that resemble the natural environmental habitat of this species. I have seen such modifications at the Breeding Centre for Endangered Arabian Wildlife (BCEAW) and at Sharjah Safari Park, UAE. This would ensure animal welfare and maintain natural behaviors. As per my simulations, I would propose starting with ten individuals in five pairs (5 females and 5 males), and each pair would be placed in one holding pen. Then there would be rotation of males among each holding pen. The offspring would be recorded in a studbook and mating between related individuals would be minimized as far as possible. The simulations showed that every five years a

minimum of at least 2-3 adult females and 1-2 adult males would be added to the captive population to maximize genetic variance and minimize genetic differentiation between captive and wild populations. Without supplementation and proper management the captive population would lose its genetic variability each generation by means of genetic drift (Frankham et al. 2019b). Supplementing the captive population with even a low number of individuals from the wild will assist in countering the effect of genetic drift and minimize the loss of genetic diversity (Weiser et al. 2013). This management option should create a population that is more genetically variable, less adapted to the captivity and has lower inbreeding (Margan et al. 1998).

Although captive programs have several advantages in term of assisting wildlife conservation, there are some problems and obstacles that need to be looked after and addressed in a scientific way:

1- The individuals taken from the wild represent only a subset of the original population. This subset might not capture all the genetic variation of the wild population. This could produce a bottleneck effect and founder effect and would cause reduction in genetic diversity in long term (Stanton et al. 2015).
2- Translocation of individuals from different habitats and ecosystems, where they already developed a local-adaption, to a new location with different physical and ecological characteristics might reduce their survival chance in the new habitat or in captivity, which would create a challenge (Weeks et al. 2011).
3- The nature of captive populations is that they are commonly made up of few individuals and this creates another challenge in which small populations are highly sensitive to genetic drift, inbreeding depression and the accumulation of deleterious mutations (Frankham 2008).
4- Reduction of genetic diversity of the captive population might take place during the release of a subset of animals into the wild (Sigg 2006).

Therefore, the animals that should be selected for release should represent a proportionate amount of genetic diversity found in the captive population so the release of these animals will not cause any such reduction of genetic diversity. In addition, continuous supplementation with animals from the wild to the captive population would maintain a good genetic diversity level in the latter. Thus screening of the population genetic parameters is required before any attempt of release, in order to choose the appropriate individuals.

5- If few founders are used, this might increase the probability of adaptation to captive conditions (Allendorf et al. 2022).

These effects need to be addressed carefully in managing captive populations. This can be done by choosing founder individuals with high genetic diversity (IUCN/SSC 2013). The scanning of the Nubian ibex range in Oman by the markers developed in this study (chapter 4 and 5) and by using non-invasive samples such as faeces, hair and bone, will give information about the magnitude of genetic diversity found in this species. This will enable selection of individuals for captive or translocation that have the highest level of gene diversity and mitochondrial haplotypes and it will assist in selecting individuals that are representative of the wild population (Seaborn et al. 2021).

6.6 Conclusion

In this chapter I used VORTEX software to test the effects of several future scenarios for the isolated Nubian ibex population at WWR, including different drought and hunting scenarios, as well as testing the effect of mortality in both sexes, percentage of breeding females and males, and the effect of sex ratio. In addition, I was able to assess the viability of a captive population of Nubian ibex, under different supplementation scenarios. Applying population viability analysis has helped to identify the factors that may affect Nubian ibex future population viability at WWR, with population viability being particularly linked to mortality rates. This knowledge will assist in conservation effort and in focussing future studies.

My simulations indicate that a captive breeding program seems feasible for the Nubian ibex, and could be implemented at WWR relatively easily since infrastructure for captive animals already exists. However, implementation of such plan will need careful consideration of the species' demography, ecology, behaviour and genetics as well as special modifications on captive holding pens as illustrated in discussion section. In terms of the wild population, results of my simulations under different drought and hunting scenarios shed important light on the essential factors that need to be managed or mitigated against for better conservation and habitat management of the Nubian ibex, both at WWR but potentially for other populations in Oman.

Chapter 7 General discussion

There are many threats facing the future of wildlife species which, if not prevented or at least mitigated, will cause extinction of many species (Woodruff 2001). Habitat loss and degradation, increasing pollution, climate change, over exploitation, isolation and fragmentation of wildlife populations are the main threats. Moreover, species are likely to face challenges in keeping pace with the factors causing rapid changes in their environment and habitat and their ability to adapt in response to such changes. Therefore, it is important to assess the status of wildlife species in order to evaluate the magnitude of these threats on their future persistence. Genomic approaches with no need for genomic references or any need for invasive sampling provide unprecedented ways of investigating the effect of abovementioned threats on wildlife populations.

This study provides the first extensive assessments of the genetic structure of one of the most important wild ungulates in Oman, the Nubian ibex. Here I showed several important results that can improve and assist the conservation efforts of this species. I assessed the relationship and the genetic structure of this species by starting with conventional methods in which I interrogated the mitochondrial markers in both wild and captive individuals of the species (chapters 2 and 3). Then I benefited from the advancement in genomic research, which has enabled investigation of genetic diversity to be applied in nearly any organism, including rare or elusive wildlife species. Moreover, the reduction in the cost of genomic approaches have made it feasible and widely used for any species. These approaches allow genotyping of thousands of SNPs for many individuals at the same time for less cost comparing to the use of microsatellites or other genetic approaches.

Genomic approaches allow several questions to be addressed which were hard to answer by simpler genetic approaches like microsatellites and mitochondria. Such questions include those that seek to identify adaptive loci and loci that are

associated with environmental variation, estimate effective population size of a species, estimate contemporary gene flow rates and identify ESUs (evolutionarily significant units) (Luikart et al. 2018).

Here first I applied the ddRAD approach by using high quality samples (blood and tissue) to explore the genetic structure between wild and captive Nubian ibex, as well as domestic goats (chapter 4). The results of this study formed the basis for developing probes (baits) to be used to capture DNA in low-quality samples (faecal and museum), and I then used hybrid capture approach for rapid genotyping of a large number of SNPs to investigate patterns of genetic variations between Nubian ibex populations (chapter 5).

The array of approaches used in this study revealed the uniqueness of Nubian ibex in Oman compared to other populations in the species' distribution range. Investigating nuclear and mitochondrial markers of different populations showed that the Nubian ibex population in Oman harboured distinguishing SNPs and haplotypes, which does not share with other populations except Yemeni population, which indicate their genetic isolation and distinctiveness which deserve to be conserved and protected.

Survey results and population estimation carried by OCE have shown that the Nubian ibex population at WWR is small, decreasing and isolated. In general, the reduction of population numbers for any wildlife species coupled with prevention of animal movement between populations might lead to genetic consequences such as inbreeding, accumulation of deleterious alleles and reduction in genetic diversity (Frankham et al. 2010) which in the long term (with other anthropogenic factors) will lead to population extinction which conservation practitioners try to avoid. Therefore, I carried out population viability analysis (chapter 6) to estimate the impact of several threats and natural conditions on the WWR Nubian ibex population. The population was found to be affected by high hunting intensity and prolonged periods of drought. The percentage of females in the population was found important to maintain

population growth and ensure its persistence over 100 years. A simulated captive program was assessed, which would help in creating an insurance population that can be used for reintroduction or reinforcement programs. The genetic results produced in this study helped in identifying the genetic structures between the populations and selecting populations that harbour more genetic diversity which can be used as source for individuals for such a captive program.

7.1 Approaches used to investigate the genetic diversity of Nubian ibex in Oman

7.1.1 Mitochondrial approach

Firstly, mitochondrial DNA markers were used to investigate the genetic diversity and the relationship between wild Nubian ibex and captive individuals. Goat samples were used to test potential hybridization in wild and captivity (chapter2). This analysis separated wild ibex from both ibex in captive populations, and domestic goats. A phylogenetic tree showed that wild ibex formed a separated clade from captive populations. Interestingly, two captive individuals showed introgression of goat mitochondria, which indicates possible hybridisation between the two species. Next, samples from Yemen and Sudan were incorporated in the analysis where only cytochrome *b* was amplified for both populations (chapter 3). The results based on this marker revealed that the Omani and Yemeni populations shared one mitochondrial haplotype, while all analyses showed that the Omani population is significantly different from that of Sudan. The limited number of samples from Yemen preclude our ability to further investigate this finding, and more samples might provide more information in the future about the population structure between these locations. Another limitation is that I was not able to amplify the D-loop marker for the

Yemeni and Sudanese samples, which can be ascribed to the primer compatibility and the hyper-variable nature of this marker.

7.1.2 Double digestion approach

By utilising the double digest restriction-association sequencing technique (ddRAD) I obtained a total of 5,775 high quality SNP loci, giving an overview of genomic variation in the wild and captive populations of the Nubian ibex (chapter4). Significant genetic differentiation was detected between wild and captive individuals. In addition, hybridisation in captivity between Nubian ibex and goats was identified which will necessitate careful attention by management authorities in future, to avoid any mixing of animals with unknown origin. Consequently, it is highly recommended that genetic assessment of captive individuals is performed if these animals are intended to be used for future conservation programs. These results concur with my previous study (chapter 2) using mitochondrial markers which showed a deep divergence between the wild and captive Nubian ibex in Oman. The SNP loci developed in this study were used as a basis for generating probe baits to be used in hybrid capture technique in chapter 5.

7.1.3 Hybrid capture approach

The probes (baits) that were designed from ddRAD results (chapter 4) were used to target sequences in low-quality samples. In this study, the technique has proven useful and successful in capturing SNPs from low DNA concentration libraries. The samples used in this study were faecal, bone and museum samples representing animals from Oman, Yemen and Sudan (chapter 5).

This study has genotyped 1,054 SNPs, which were used to investigate genetic variation among several Nubian ibex populations (Oman, Yemen, captive animals and Sudan). Confirming the mitochondrial results (chapters 2 & 3), a significant divergence between Oman and Sudan populations was disclosed (F_{ST}= 0.513). This result showed the effect of range expansion, where, in theory, the animals with close proximity should have more similar or closer genetic structure than those further apart. The isolated nature and the location of the Nubian ibex in the far east of the Arabian Peninsula made the current results unsurprising, especially with already documented divergence between a range of African and Arabian species (Derouiche et al. 2017). The location of the Yemeni population in close proximity with the Omani population explains the absence of genetic differentiation between the two, and would suggest there is or has recently been individual migration between the two populations. This potential movement between the two populations needs to be further explored, especially under the current situation in the borders between Oman and Yemen where Oman has built a security fence to prevent unauthorised crossing of the border. However, the Yemeni samples were found to share one mitochondrial haplotype with Omani samples and, based on the SNPs generated by hybrid capture, there were no significant differences detected between them.

Some captive individuals from Oman and UAE were clearly assigned to the Sudan group, which indicates introgression between individuals from the two populations in captivity. This would need careful attention when planning any future re-introduction or re-enforcement programmes, and such animals would need to be detected and separated from the rest of animals.

In order to be able to disentangle the whole taxonomy and evolutionary history of the Nubian ibex in the Arabian Peninsula, further sampling from its other populations in Saudi Arabia, Jordan, Israel and Egypt is needed.

7.2 Nubian ibex at WWR: PVA and genetics

Population viability analysis was carried out to explore the most significant factors that might potentially affect population growth of Nubian ibex population at WWR and predispose it to extinction (chapter 6). Mine and previous studies have shown that this population is small in size, isolated and genetically depauperate (Ross et al. 2020a; Al-Ghafri et al. 2021), making it a highly suitable candidate for population viability analysis (PVA). Several factors were tested and simulated using PVA in VORTEX (Lacy & Pollak 2020). The major and most significant factor that found to affect the viability of this population was an elevated rate of individual mortality. Mortality can be influenced by a range of factors such as disease, natural disaster, climate change or hunting. The prevalence of disease in the wild free-roaming animals has not been assessed at WWR, and warrants future research. In contrast, hunting incidence at WWR has been reported several times over the years, causing, in some cases, the death of several individuals (Giangaspero et al. 2014; Oman 2018). Simulation of high hunting intensity was found to cause a severe reduction in populations. Threats posed by hunting can be reduced by concentrating protection on areas that are known to encounter more hunting incidences.

Simulation of different drought scenarios showed that Nubian ibex population can tolerate low frequency drought seasons (once every ten years) but to overcome more frequent droughts the population would need to have a relatively high reproduction rate. As a mitigation step it was recommended to provide water in several spots of the reserve during summer and drought seasons.

Investigation of the minimum population size that can be viable for the next 100 years at WWR under prevailing conditions revealed that if the population falls below 100 individuals, the viability of the population will be severely impaired. The estimated population size of the Nubian ibex in this area has recently been estimated at between 150-250 individuals (Ross et al. 2020a). Routine survey

needs to be carried out annually at the reserve to estimate population size and trend and be prepared for action when needed.

This study provides recommendations to be carried out in order to face and be prepared in case of severe conditions. I proposed an establishment of captive breeding unit for Nubian ibex at WWR taking into account the genetic diversity and population structure discovered across Nubian ibex populations in Oman.

Further sub-structuring within wild Nubian ibex in Oman was indicated by PCA and estimation of the genetic distances (F_{ST}). This finding is congruent with the mitochondrial results where no D-loop haplotypes were shared between WWR and Dhofar. These correspond, on the ground, with the isolated nature of the WWR population. In light of these results, solutions need to be implemented in order to prevent the effect of isolation on the long-term viability and genetic diversity of the population (Frankham et al. 2017). Therefore, the possibility of reconnecting the populations by establishment natural corridors needs to be assessed.

7.3 Conservation implications

My findings can help inform conservation of Nubian ibex in several ways, including helping establish a monitoring programme for genetic diversity of the species in Oman, and informing a captive breeding programme.

7.3.1 Nubian ibex monitoring

My development of several thousand Nubian ibex SNPs using ddRAD can be used in the future to design a high resolution microfluidic arrays (Thaden von et al. 2017; Thaden et al. 2020). These could be used for fast genotyping of large number of Nubian ibex individuals at WWR and Dhofar using non-invasive samples, thus facilitating the monitoring of the population’s demographic and

genetic status and identifying individuals for translocation or captive programs. For example, SNPs has been used to monitor populations in the wild species such as North American river otters (*Lontra canadensis*), wolves (*Canis lupus*) and brown bears (*Ursus arctos*) (Norman et al. 2013; Kraus et al. 2015; Stetz et al. 2016).

7.3.2 Establishment of insurance population

The notion of the establishment of captive populations of wildlife species for protection and sustainability has been a point of contention between conservation biologists. Breeding wild animals in captivity can be prohibitively expensive and extremely challenging. Many programs have faced difficulties and even failure in reintroducing the captive animals to their natural habitat (Grey-Ross et al. 2009). However, regardless of caveats surrounding captive breeding programs, their benefits and advantages can outweigh their drawbacks.

A current trend in conservation is integrating *ex situ* and *in situ* management. That is, alongside ecosystem management and habitat rehabilitation, and protecting wildlife in their natural habitat, animals are also managed in captivity to act as an insurance population against any future events that might cause an unexpected dramatic reduction in the wild population (Pritchard et al. 2012; Schwartz et al. 2017; Mestanza-Ramón et al. 2020).

My results should help guide future establishment of *ex situ* populations for Nubian ibex based on adequate knowledge about the founders' genetic makeup and relationships between the individuals in captivity, which will promote proper management that considers all risks surrounding captive programs including genetic and behavioural risks. Genetic risks include founder effects, genetic drift, bottlenecks, accumulation of deleterious alleles and inbreeding (Witzenberger & Hochkirch 2011), while behavioural risks include adaptation to

captivity and to human presence (Christie et al. 2012; Schell et al. 2021). In addition, my results can guide to the selection of animals that as a group have a genetic diversity that is representative of that of the wild population (Dicks et al. 2023).

Using PVA (chapter 6) I assessed the feasibility of the establishment of such a captive breeding population of Nubian ibex that would be formed from individuals sourced from both WWR and the southern region of Oman. I proposed starting with 10 individuals (5 females and 5 males) and each five years supplementing the populations with at least 2-3 females and 1-2 adult males. Simulations showed that supplementation with females should be a central focus as they appear to be more important in decreasing the probability of extinction and increasing the growth rate and genetic diversity than supplementation with males. In addition, I proposed adding modifications to the holding pens to mimic the natural habitat of this species.

7.4 Establishment of bio-banking

There are other techniques that can be applied as a long-term insurance policy against the extinction of endangered wildlife species such as bio-banking of eggs and sperm using cryopreservation, artificial insemination (AI), and *in vitro* fertilization and embryo transfer (Comizzoli 2015). Such techniques have been used successfully to manage and recover endangered species such as giant panda in China (Wildt et al. 2006) and black-footed ferret in North America (Howard & Wildt 2009). Moreover, testicular tissue banking is used for several mammalian species to act as a stock of germ cells that can later on be propagated *in vitro* and then transferred to the host animal (Comizzoli & Wildt 2014).

Establishing bio-banking containing samples representing species of the Arabian Peninsula will be a significant advancement in wildlife conservation in

the area. Captive breeding centres would not need to use expensive and unsafe movement of animals in order to maintain the genetic diversity of the captive animals. All they would need would be a package with frozen semen that could be used with the AI technique. In addition, such techniques will support the conservation of critically endangered species in Oman such as Arabian leopard and Arabian Tahr. The use of such techniques in preserving and protecting wildlife in Arabia deserves to be looked at in order to try to explore the potential and opportunities.

7.5 Limitations and obstacles

7.5.1 Sample quality and availability

I tried to collect samples of Nubian ibex from different countries by contacting people and institutions. I was able to collect Sudan specimens from the Powell Cotton Museum (London, UK). These samples were collected by explorers in 1935. Additionally, BCEWA provided me with samples from Yemen and Saudi Arabia. In the analyses for chapter 3, I was only able to amplify cytochrome *b* of the Yemeni samples. The failure of amplification of the other samples could be a problem with the samples' condition, their preservation, the sampling process, the DNA condition or primer compatibility.

I faced problems with amplifying the D-loop due to the hyper-variable nature of this marker, therefore I designed primers that are located in a conserved region, by aligning several *Capra* species together. The region produced by these primers was comparatively small (186 bp after trimming). These primers failed to amplify the D-loop for the Sudan and Yemen samples, which indicates sequence differences due to the variability nature of this marker (Upholt & Dawid 1977). Thus I recommend designing primers that are specific to each species.

The baits I developed and reported in chapter 5 worked on the majority of the faecal and bone samples, although some of the samples failed to be amplified. Therefore, it is recommended that fresh and recent faecal samples to be collected and two or more replicates taken for each sampling location. In addition, two to three extracts could be performed to increase the DNA quantity (Goossens et al. 2000). Moreover, careful attention is needed while collecting samples from areas where two or more species from the same genus (e.g. Nubian ibex and goat) are present in order to avoid missampling and contamination

The absence in my study of samples from Saudi Arabia and the Levant limited the ability to draw more precise and conclusive picture of the population genetic structure of this species across its whole range. I posit that if we were able to get samples from these areas we would be able to assign the origin of the captive animals (from UAE and Oman) and resolve the mystery of presence of Sudan ancestry in the captive and the detection of similarities between the captive population and Oman/Yemen samples.

7.6 Conclusions and recommendations

For Nubian ibex, a species considered Vulnerable by the IUCN and thus of considerable conservation concern, this study should pave the way toward further genetic and genomic investigation of the species in its distribution range (i.e. Saudi Arabia, Jordan, Israel, Egypt and Sudan). The deposited mitochondrial sequences generated in this study for D-loop and cytochrome *b* representing animals from three different countries (Oman, Yemen and Sudan) should help and encourage other researchers to examine the genetic makeup of this species in their countries. This will help in understanding the species' evolutionary history and range expansion. In addition, the successful use of the hybrid capture technique for generating SNPs from faecal samples, provides a non-invasive tool to monitor and manage Nubian ibex genetic diversity in the

future, and may also promote the use of this technique to other researchers to study other elusive species, or dangerous species such as carnivores from which it is hard to get blood samples as capture methods are fraught with risks for the animals and humans alike.

More locally in Oman, I demonstrated that the southern population is or has recently been potentially connected with the Yemeni population, but that there has likely been genetic isolation between the southern population and that in the central region at WWR. This latter population is of particular concern due to its relatively small size and isolation. Because the origin of captive populations available may include animals from North Africa and/or possible hybrids with domestic goats, here I proposed, and assessed the viability of, the establishment of a new captive breeding program for the Nubian ibex based at WWR. The presence of existing captive breeding programs on site for several species, and experienced people and veterinary assistants at WWR make it the best choice for this new program. In addition, the purpose of such a program would be to preserve the Nubian ibex population in the central desert of Oman and establishment of the captive program at WWR would potentially help the captive population to acclimatise to the local climate and ease the release of animals to the wild at later stages.

Appendixes

Table A1.1. *Mammals of conservation importance in three main parts of Oman. The signs × indicates the species is not present, and √ means is present, while ? means no information is available.*

Species	Central region	Southern region	Northern region
Arabian leopard (*Panthera pardus nimr*)	×	√	×
Nubian ibex (*Capra nubiana*)	√	√	×
Arabian tahr (*Arabitragus jayakari*)	×	×	√
Arabian gazelle (*Gazella arabica*)	√	√	√
Arabian wolf (*Canis lupus arabs*)	√	√	√
Rock Hyrax (*Procavia capensis*)	×	√	×
Striped hyaena (*Hyaena hyaena*)	√	√	√
Caracal (*Caracal caracal*)	√	√	√
Sand gazelle (*Gazella marica*)	√	×	×
Cape hare (*Lepus capensis*)	√	√	√
Red fox (*Vulpes vulpes*)	√	√	√
Sand fox (*Vulpes rueppellii*)	√	?	?
Honey badger (*Mellivora capensis*)	√	√	?
Sand cat (*Felis margarita*)	?	×	√
Wildcat (*Felis silvestris*)	√	√	?
Porcupine (*Hystrix cristata*)	?	√	?
Hedgehog (*Hemiechinus auritus*)	√	√	√
Spiny mouse (*Acomys dimidiatus*)	?	√	?
Gerbil (*Gerbilus cheesmani*)	√	√	?

Table A1.1. *The nine generally agreed Capra species and their geographical distribution according to the IUCN.*

Species	Common name	Geographic distribution	Population trend	IUCN Species status	Reference
Capra aegagrus	Bezoar or Wild Goat	Russia, Turkey, Armenia, Afghanistan, Georgia, Iran, Azerbaijan	Decreasing	Near Threatened	(Weinberg & Ambarli 2020)
Capra ibex	Alpine ibex	Italy, Switzerland, Germany, French, Austria	Increasing	Least Concern	(Toïgo et al. 2020)
Capra sibirica	Siberian ibex	Russia, Pakistan, India, Mongolia, Afghanistan, Chain, Kazakhstan, Tajikistan, Kyrgyzstan	Unknown	Least Concern	(Reading et al. 2020)
Capra nubiana	Nubian ibex	Ethiopia, Sudan, Egypt, Syria, Jordan, Saudi Arabia, Yemen, Oman	Decreasing	Vulnerable	(Ross et al. 2020a)
Capra pyrenaica	Spanish ibex	Spain	Increasing	Least Concern	(Herrero & Luco 2020)
Capra falconeri	Markhor	Pakistan, India	Increasing	Near Threatened	(Michel & Rosen Michel 2015)
Capra caucasica	Western tur or Caucasian	Georgia, Russia	Decreasing	Endangered	(Weinberg 2020)
Capra cylindricornis	Eastern/Dagestan Tur	Georgia, Russia, Azerbaijan	Decreasing	Near Threatened	(Lortkipanidze & Weinberg 2020)
Capra walie	Walia ibex	Ethiopia	Increasing	Endangered	(Ejigu 2020)

Table A2.2. *Samples used in the analysis and explanation as to why some samples were excluded.*

Key:- L= Low-quality, F= Failed, S= Short sequence, Cyt b F1= cytochrome b fragment 1

UAE = United Arab Emirates, WWR= Al-Wusta Wildlife Reserve

Samples	Sample Type	Country	Collection date	D-loop 186bp	D-loop Excluded/Used Sequences	Reasons	Cyt b F1	Cyt b F2	Cyt b F3	Cyt b Excluded Sequences	Reasons
NUB001	Tissue	Oman/Shalim	2013		used					used	
NUB002	Tissue	Oman/Shalim	2013		used					used	
NUB003	Tissue	Oman/Shalim	2013		used					used	
NUB004	Tissue	Oman/Shalim	2013		used					used	
NUB005	Tissue	Oman/Shalim	2013		used					used	
NUB006	Tissue	Oman/Shalim	2013		used					used	
NUB007	Tissue	Oman/Shalim	2013		used					used	
NUB008	Tissue	Oman/Shalim	2013		used					used	
NUB009	Blood	UAE/Captive	2015-2018		used					used	
NUB010	Blood	UAE/Captive	2015-2018		used					used	
NUB011	Blood	UAE/Captive	2015-2018		used					used	
NUB012	Blood	UAE/Captive	2015-2018		used					Excluded	F
NUB013	Blood	UAE/Captive	2015-2018		used					used	
NUB014	Blood	UAE/Captive	2015-2018		used					used	
NUB015	Blood	UAE/Captive	2015-2018		used					used	
NUB016	Faecal	Oman/WWR	2016		used					used	
NUB017	Faecal	Oman/WWR	2016		used					Excluded	F
NUB018	Faecal	Oman/WWR	2016		used					Excluded	F
NUB019	Faecal	Oman/WWR	2016		used					Excluded	F
NUB020	Blood	UAE/Captive	2015-2018		used					used	
NUB021	Blood	UAE/Captive	2015-2018		Excluded	F				Excluded	L
NUB022	Blood	UAE/Captive	2015-2018		used					used	
NUB023	Blood	UAE/Captive	2015-2018		used					used	
NUB024	Blood	UAE/Captive	2015-2018		used					Excluded	F
NUB025	Blood	UAE/Captive	2015-2018		used					used	
NUB026	Blood	UAE/Captive	2015-2018		used					Excluded	F
NUB027	Blood	UAE/Captive	2015-2018		used					used	
NUB028	Blood	UAE/Captive	2015-2018		used					used	
NUB029	Blood	UAE/Captive	2015-2018		used					used	
NUB030	Blood	UAE/Captive	2015-2018		used					used	
NUB031	Blood	UAE/Captive	2015-2018		used					used	
NUB032	Blood	UAE/Captive	2015-2018		used					used	
NUB033	Blood	UAE/Captive	2015-2018		used					used	
NUB034	Blood	UAE/Captive	2015-2018		used					used	
NUB035	Blood	UAE/Captive	2015-2018		used					used	
NUB036	Blood	UAE/Captive	2015-2018		used					used	
NUB037	Blood	UAE/Captive	2015-2018		used					used	
NUB038	Blood	UAE/Captive	2015-2018		used					used	
NUB039	Blood	UAE/Captive	2015-2018		Excluded	L				Excluded	L
NUB040	Blood	UAE/Captive	2015-2018		used					used	
NUB041	Blood	UAE/Captive	2015-2018		used					used	
NUB042	Blood	UAE/Captive	2015-2018		used					used	
NUB043	Blood	UAE/Captive	2015-2018		used					used	
NUB044	Blood	UAE/Captive	2015-2018		used					used	
NUB045	Blood	UAE/Captive	2015-2018		used					Excluded	F
NUB046	Blood	UAE/Captive	2015-2018		used					used	

NUB047	Blood	UAE/Captive	2015-2018		used					used	
NUB048	Blood	UAE/Captive	2015-2018		used					used	
NUB049	Tissue	Oman/Shalim	2018		used					used	
NUB050	Tissue	Oman/Shalim	2018		used					used	
NUB051	Tissue	Oman/Shalim	2018		used					used	
NUB052	Tissue	Oman/Shalim	2018		used					used	
NUB053	Tissue	Oman/Shalim	2018		used					used	
NUB054	Tissue	Oman/Shalim	2018		used					used	
NUB055	Blood	Oman/Captive	2017		used					used	
NUB056	Blood	Oman/Captive	2017		used					used	
NUB057	Blood	Oman/Captive	2017		used					used	
NUB058	Blood	Oman/Captive	2017		used					Excluded	S
NUB059	Blood	Oman/Captive	2017		used					used	
NUB060	Blood	Oman/Captive	2017		used					used	
NUB061	Blood	Oman/Captive	2017		used					Excluded	L
NUB062	Blood	Oman/Captive	2017		used					used	
NUB063	Blood	Oman/Captive	2017		used					Excluded	S
NUB064	Bone	Oman/WWR	Old samples		used					used	
NUB065	Bone	Oman/WWR	Old samples		used					used	
NUB066	Bone	Oman/WWR	Old samples		used					used	
NUB067	Bone	Oman/WWR	Old samples		used					used	
NUB068	Bone	Oman/WWR	Old samples		used					used	
NUB069	Dry Tissue	Oman/WWR	Old samples		used					Excluded	S
NUB070	Bone	Oman/WWR	Old samples		Excluded	L				Excluded	L
NUB071	Bone	Oman/WWR	Old samples		used					used	
NUB072	Bone	Oman/WWR	Old samples		used					used	
NUB073	Bone	Oman/WWR	Old samples		used					Excluded	L
NUB074	Bone	Oman/WWR	Old samples		used					Excluded	L
NUB075	Bone	Oman/WWR	Old samples		used					used	
NUB076	Bone	Oman/WWR	Old samples		used					used	
NUB077	Dry Tissue	Oman/WWR	Old samples		used					used	
NUB078	Faecal	Oman/WWR	2017		used					used	
NUB079	Faecal	Oman/WWR	2017		used					Excluded	L
NUB080	Faecal	Oman/WWR	2017		used					Excluded	L
NUB081	Faecal	Oman/WWR	2017		used					Excluded	L
NUB082	Faecal	Oman/WWR	2017		used					Excluded	L
NUB083	Faecal	Oman/WWR	2017		used					used	
NUB084	Faecal	Oman/WWR	2017		used					Excluded	L
NUB085	Faecal	Oman/WWR	2017							Excluded	S
NUB086	Faecal	Oman/WWR	2017		used					Excluded	L
NUB087	Faecal	Oman/WWR	2017		used					Excluded	L
NUB088	Faecal	Oman/WWR	2017		used					Excluded	
NUB089	Faecal	Oman/WWR	2017		used					Excluded	S
NUB090	Faecal	Oman/WWR	2017		used					Excluded	S
NUB091	Faecal	Oman/WWR	2017		used					Excluded	S
NUB092	Faecal	Oman/WWR	2017		used					Excluded	L
NUB093	Faecal	Oman/WWR	2017		used					Excluded	S
NUB094	Faecal	Oman/WWR	2017		used					Excluded	L
NUB095	Faecal	Oman/WWR	2017		used					Excluded	L
NUB096	Faecal	Oman/WWR	2017		used					Excluded	L
NUB097	Faecal	Oman/WWR	2017		used					Excluded	L
NUB098	Faecal	Oman/WWR	2017		used					Excluded	L
NUB099	Faecal	Oman/WWR	2017		used					Excluded	L
NUB100	Faecal	Oman/WWR	2017		used					Excluded	L
NUB101	Faecal	Oman/WWR	2017		used					Excluded	L
NUB102	Faecal	Oman/WWR	2017		used					Excluded	L
NUB103	Faecal	Oman/WWR	2017		used					Excluded	L
NUB104	Faecal	Oman/WWR	2017		used					Excluded	S
NUB105	Faecal	Oman/WWR	2017		used					Excluded	L
NUB106	Faecal	Oman/WWR	2017		Excluded	S				Excluded	L
NUB107	Faecal	Oman/WWR	2017		Excluded	L				Excluded	L
NUB108	Faecal	Oman/WWR	2017		Excluded	F				Excluded	S
NUB109	Faecal	Oman/WWR	2017		used					used	
NUB110	Faecal	Oman/WWR	2017		used					Excluded	L
NUB111	Faecal	Oman/WWR	2017		used					Excluded	L
NUB112	Faecal	Oman/WWR	2017		used					Excluded	L
NUB113	Faecal	Oman/WWR	2017		Excluded	S				used	
NUB114	Faecal	Oman/WWR	2017		used					Excluded	L
NUB115	Faecal	Oman/WWR	2017		used					Excluded	L
NUB116	Faecal	Oman/WWR	2017		used					used	
NUB117	Faecal	Oman/WWR	2017		used					used	
NUB118	Faecal	Oman/WWR	2017		used					Excluded	S
NUB119	Faecal	Oman/WWR	2017		used					Excluded	S
NUB140	Dry Tissue	Oman/WWR	Museum		used					Excluded	S

NUB141	Bone	Oman/WWR	Museum		Excluded	S					F
NUB142	Bone	Oman/WWR	Museum		used						F
NUB143	Bone	Oman/Shalim	Museum		used						F
NUB144	Bone	Oman/WWR	Museum		Excluded	L					F
NUB145	Bone	Oman/WWR	Museum		used						F
NUB146	Bone	Oman/WWR	Museum		Excluded	L					F
NUB147	Bone	Oman/WWR	Museum		Excluded	L					F
NUB148	Bone	Oman/WWR	Museum		used						F
NUB149	Dry Tissue	Oman/Shalim	Museum		used					used	
NUB150	Tissue	Oman/WWR	2018		used					Excluded	L
NUB151	Tissue	Oman/WWR	2018		used					Excluded	L
NUB152	Tissue	Oman/WWR	2018		used					used	
NUB153	Tissue	Oman/WWR	2018		used					used	
NUB154	Tissue	Oman/WWR	2018		Excluded	F					F
NUB155	Faecal	Oman/WWR	2018		used						F
NUB156	Faecal	Oman/WWR	2018		Excluded	F					F
NUB157	Faecal	Oman/WWR	2018		Excluded	S					F
NUB158	Faecal	Oman/WWR	2018		Excluded	F					F
NUB159	Faecal	Oman/WWR	2018		used					used	
NUB160	Faecal	Oman/WWR	2018		used					Excluded	L
NUB161	Faecal	Oman/WWR	2018		used					used	
NUB162	Faecal	Oman/WWR	2018		used					used	
NUB163	Faecal	Oman/WWR	2018		used						F
NUB164	Faecal	Oman/WWR	2018		used					used	
NUB165	Faecal	Oman/WWR	2018		used					used	
NUB166	Faecal	Oman/WWR	2018		used					used	
NUB167	Faecal	Oman/Dhofar	2018		Excluded	F					F
NUB168	Faecal	Oman/Dhofar	2018		used					used	
NUB169	Faecal	Oman/Dhofar	2018		used					used	
NUB170	Faecal	Oman/Dhofar	2018		used					used	
NUB171	Faecal	Oman/Dhofar	2018		used					used	
NUB172	Faecal	Oman/Dhofar	2018		used					used	
NUB173	Faecal	Oman/Dhofar	2018		used					used	
NUB174	Faecal	Oman/Dhofar	2018		Excluded	L				used	
NUB175	Faecal	Oman/Dhofar	2018		Excluded	L				used	
NUB176	Faecal	Oman/Dhofar	2018		Excluded	L				used	
NUB177	Faecal	Oman/Dhofar	2018		used					used	
NUB178	Faecal	Oman/Dhofar	2018		used					Excluded	L
NUB179	Faecal	Oman/Dhofar	2018		used					used	
NUB180	Faecal	Oman/Dhofar	2018		used					Excluded	L
NUB181	Faecal	Oman/Dhofar	2018		used					Excluded	L
NUB182	Faecal	Oman/Dhofar	2018		used					used	
NUB183	Faecal	Oman/Dhofar	2018		used					Excluded	
NUB184	Faecal	Oman/Dhofar	2018		used					used	
NUB185	Faecal	Oman/Dhofar	2018		used					used	
NUB186	Faecal	Oman/Dhofar	2018		used					used	
NUB187	Faecal	Oman/Dhofar	2018		used					used	
NUB188	Faecal	Oman/Dhofar	2018		Excluded	L				used	
NUB189	Faecal	Oman/Dhofar	2018		used					Excluded	L
NUB190	Faecal	Oman/Dhofar	2018		used					used	
NUB191	Faecal	Oman/Dhofar	2018		used					used	
NUB192	Faecal	Oman/Dhofar	2018		Excluded	L				used	
NUB193	Faecal	Oman/Dhofar	2018		used					used	
NUB194	Faecal	Oman/Dhofar	2018		used					Excluded	L
NUB195	Faecal	Oman/Dhofar	2018		used					used	
NUB196	Faecal	Oman/Dhofar	2018		used					used	
NUB197	Faecal	Oman/Dhofar	2018		used					used	

NUB198	Faecal	Oman/Dhofar	2018		used					Excluded	L
NUB199	Faecal	Oman/Dhofar	2018		used					used	
NUB200	Faecal	Oman/Dhofar	2018		used					used	
NUB201	Faecal	Oman/Dhofar	2018		used					Excluded	L
NUB202	Faecal	Oman/Dhofar	2018		used					used	
NUB203	Faecal	Oman/Dhofar	2018		used					Excluded	F
NUB204	Faecal	Oman/Dhofar	2018		used					used	
NUB205	Faecal	Oman/Dhofar	2018		used					used	
NUB206	Faecal	Oman/Dhofar	2018		used					used	
NUB207	Faecal	Oman/Dhofar	2018		used					Excluded	F
NUB208	Faecal	Oman/Dhofar	2018		Excluded	L				used	
NUB209	Faecal	Oman/Dhofar	2018		Excluded	L					F
NUB210	Faecal	Oman/Dhofar	2018		used					used	
NUB211	Faecal	Oman/Dhofar	2018		Excluded	F				Excluded	F
NUB212	Faecal	Oman/Dhofar	2018		Excluded	F				Excluded	F
NUB213	Faecal	Oman/Dhofar	2018		Excluded	F				Excluded	F
NUB214	Faecal	Oman/Dhofar	2018		Excluded	F				Excluded	F
NUB215	Faecal	Oman/Dhofar	2018		Excluded	F				Excluded	F
NUB216	Faecal	Oman/Dhofar	2018		Excluded	F				Excluded	F
NUB217	Faecal	Oman/Dhofar	2018		Excluded	F				Excluded	F
NUB218	Faecal	Oman/Dhofar	2018		Excluded	F				Excluded	F
NUB219	Faecal	Oman/Dhofar	2018		Excluded	F				Excluded	F
NUB220	Faecal	Oman/Dhofar	2018		used					used	
NUB221	Faecal	Oman/Dhofar	2018		used					used	
NUB222	Faecal	Oman/Dhofar	2018		Excluded	L					F
NUB223	Faecal	Oman/Dhofar	2018		used					Excluded	L
NUB224	Faecal	Oman/Dhofar	2018		used					used	
NUB225	Faecal	Oman/Dhofar	2018		used					Excluded	L
NUB226	Faecal	Oman/Dhofar	2018		Excluded	S				used	
NUB227	Faecal	Oman/Dhofar	2018		used					used	
NUB228	Faecal	Oman/Dhofar	2018		used					used	
NUB229	Faecal	Oman/Dhofar	2018		used					used	
NUB230	Faecal	Oman/Dhofar	2018		used					used	
NUB231	Faecal	Oman/Dhofar	2018		used					used	
NUB232	Faecal	Oman/Dhofar	2018		used					used	
NUB233	Faecal	Oman/Dhofar	2018		Excluded	F					F
NUB234	Faecal	Oman/Dhofar	2018		Excluded	F					F
NUB235	Faecal	Oman/Dhofar	2018		Excluded	F					F
NUB236	Faecal	Oman/Dhofar	2018		used					used	
NUB237	Faecal	Oman/Dhofar	2018		used					used	
NUB238	Faecal	Oman/Dhofar	2018		Excluded	F					F
NUB239	Faecal	Oman/Dhofar	2018		used						F
NUB240	Faecal	Oman/Dhofar	2018		used					used	
NUB241	Faecal	Oman/Dhofar	2018		used						
NUB242	Faecal	Oman/Dhofar	2018		used					used	
NUB243	Faecal	Oman/Dhofar	2018		Excluded	L				used	
NUB244	Faecal	Oman/Dhofar	2018		Excluded	F					F
NUB245	Faecal	Oman/Dhofar	2018		Excluded	L					F
NUB246	Faecal	Oman/Dhofar	2018		used						F
NUB247	Faecal	Oman/Dhofar	2018		used						F

NUB248	Dry Tissue	Oman/Dhofar	2018		used					used	
NUB249	Horn	Oman/Dhofar	2018		Excluded	F					F
NUB250	Horn	Oman/Dhofar	2018		used						F

Table A2.3. *List of Cytochrome b haplotypes and the samples representing them.*

Cytb_HAP A	Cytb_HAP B	Cytb_HAP C	Cytb_HAP D	Cytb_HAP E	Cytb_HAP F	Cytb_HAP G
Shalim_001	Wusta_16	Dhofar_174	Oman_Captive_55	Oman_Captive_57	UAE_Captive_9	Oman_Captive_56
Shalim_002	Wusta_68	Dhofar_188		Oman_Captive_58	UAE_Captive_10	Oman_Captive_59
Shalim_005	Wusta_71	Dhofar_192		Oman_Captive_62	UAE_Captive_13	Oman_Captive_60
Shalim_006	Wusta_78			UAE_Captive_15	UAE_Captive_14	Oman_Captive_63
Shalim_007	Wusta_79			UAE_Captive_20	UAE_Captive_11	
Shalim_008	Wusta_80			UAE_Captive_22		
Shalim_003	Wusta_82			UAE_Captive_23		
Shalim_004	Wusta_83			UAE_Captive_25		
Shalim_0049	Wusta_84			UAE_Captive_27		
Shalim_0050	Wusta_109			UAE_Captive_28		
Shalim_0052	Wusta_113			UAE_Captive_29		
Shalim_0053	Wusta_116			UAE_Captive_30		
Shalim_0054	Wusta_117			UAE_Captive_31		
Shalim_0051	Wusta_152			UAE_Captive_32		
Wusta_64	Wusta_153			UAE_Captive_33		
Wusta_65	Wusta_159			UAE_Captive_34		
Wusta_66	Wusta_161			UAE_Captive_35		
Wusta_67	Wusta_162			UAE_Captive_36		
Wusta_69	Wusta_164			UAE_Captive_37		
Wusta_72	Wusta_165			UAE_Captive_38		
Wusta_73	Wusta_166			UAE_Captive_40		
Wusta_74	Dhofar_168			UAE_Captive_41		
Wusta_75	Dhofar_169			UAE_Captive_42		
Wusta_76	Dhofar_171			UAE_Captive_43		
Wusta_77	Dhofar_172			UAE_Captive_44		
Dhofar_170	Dhofar_177			UAE_Captive_46		
Dhofar_173	Dhofar_179			UAE_Captive_47		
Dhofar_175	Dhofar_182			UAE_Captive_48		

Dhofar_176	Dhofar_187					
Dhofar_184	Dhofar_190					
Dhofar_185	Dhofar_191					
Dhofar_186	Dhofar_195					
Dhofar_193	Dhofar_200					
Dhofar_196	Dhofar_220					
Dhofar_197	Dhofar_221					
Dhofar_199	Dhofar_224					
Dhofar_202	Dhofar_226					
Dhofar_204	Dhofar_227					
Dhofar_205	Dhofar_229					
Dhofar_206	Dhofar_230					
Dhofar_228	Dhofar_231					
Dhofar_232	Dhofar_236					
Dhofar_240	Dhofar_237					
Dhofar_242						
Dhofar_243						
Dhofar_248						
Shalim_149						

Table A2.4. *List of Dloop haplotypes and the samples representing them.*

Dloop_HAP1	Dloop_HAP2	Dloop_HAP3	Dloop_HAP4	Dloop_HAP5	Dloop_HAP6	Dloop_HAP7	Dloop_HAP8	Dloop_HAP9	Dloop_HAP 10
Shalim_049	Wusta_17	Wusta_81	Shalim_001	Shalim_143	Dhofar_169	Dhofar_185	Shalim_003	Dhofar_250	Dhofar_241
Shalim_050	Shalim_052	Wusta_89	Shalim_002	Shalim_149	Dhofar_170	Dhofar_186	Shalim_004		Dhofar_242
Wusta_64	Shalim_053	Shalim_051	Shalim_005		Dhofar_171	Dhofar_187			Dhofar_246
Wusta_65	Shalim_054		Shalim_006		Dhofar_173	Dhofar_189			Dhofar_248
Wusta_67	Wusta_68		Shalim_007		Dhofar_180	Dhofar_194			
Wusta_72	Wusta_69		Shalim_008		Dhofar_184	Dhofar_196			
Wusta_85	Wusta_71				Dhofar_193	Dhofar_197			
Wusta_86	Wusta_73				Dhofar_199				
Wusta_91	Wusta_74				Dhofar_201				
Wusta_93	Wusta_75				Dhofar_202				
Wusta_94	Wusta_76				Dhofar_203				
Wusta_98	Wusta_77				Dhofar_204				
Wusta_103	Wusta_87				Dhofar_205				
Wusta_110	Wusta_88				Dhofar_206				
Wusta_115	Wusta_95				Dhofar_207				

Wusta_118	Wusta_96				Dhofar_210				
Wusta_119	Wusta_97				Dhofar_228				
	Wusta_99				Dhofar_232				
	Wusta_100				Dhofar_239				
	Wusta_101				Dhofar_240				
	Wusta_102								
	Wusta_104								
	Wusta_105								
	Wusta_109								
	Wusta_111								
	Wusta_112								
	Wusta_142								
	Wusta_148								
	Wusta_155								
	Wusta_160								
	Wusta_163								

Continue of **Table A2.3**. *List of Dloop haplotypes and the samples representing them.*

Dloop_HAP11	Dloop_HAP12	Dloop_HAP13	Dloop_HAP14	Dloop_HAP15	Dloop_HAP16	Dloop_HAP17
Dhofar_198	Dhofar_168	UAE_Captive_15	UAE_Captive_20	UAE_Captive_13	Oman_Captive_56	Oman_Captive_61
Dhofar_223	Dhofar_172	UAE_Captive_22		UAE_Captive_14	Oman_Captive_59	Oman_Captive_55
Dhofar_225	Dhofar_177	UAE_Captive_23		UAE_Captive_9	Oman_Captive_60	
Dhofar_227	Dhofar_178	UAE_Captive_24			Oman_Captive_63	
Dhofar_231	Dhofar_179	UAE_Captive_25				
	Dhofar_182	UAE_Captive_26				
	Dhofar_183	UAE_Captive_27				
	Dhofar_190	UAE_Captive_28				
	Dhofar_191	UAE_Captive_29				
	Dhofar_195	UAE_Captive_30				
	Dhofar_200	UAE_Captive_31				
	Dhofar_220	UAE_Captive_32				
	Dhofar_221	UAE_Captive_33				
	Dhofar_224	UAE_Captive_34				
	Dhofar_229	UAE_Captive_35				
	Dhofar_230	UAE_Captive_36				
	Dhofar_236	UAE_Captive_37				
	Dhofar_237	UAE_Captive_38				

		UAE_Captive_40				
		UAE_Captive_41				
		UAE_Captive_42				
		UAE_Captive_43				
		UAE_Captive_44				
		UAE_Captive_45				
		UAE_Captive_46				
		UAE_Captive_47				
		UAE_Captive_48				
		Oman_Captive_57				
		Oman_Captive_58				
		Oman_Captive_62				

Table A2.5. *The reference sequences retrieved from NCBI for the Capra species used in phylogenetic analysis.*

Specie	Accession Number	Sample source	Sample Country	Reference
*Capra nubiana*1	FJ207527 NC020624	Jardin des Plantes, MNHN (FRANCE)	Not mentioned	(Hassanin et al. 2009)
*Capra nubiana*2	DQ514552	Israel	Israel	(Pidancier et al. 2006)
*Capra nubiana*3	AF217256	Israel	Israel	(Bar-Gal et al. 2002)
*Capra nubiana*4	AF034740	Bone 1938-1296 Laboratorie Mammiferes & Oiseaux, MNHN (FRANCE)	Not mentioned	(Hassanin et al. 1998a)
*Capra nubiana*5	AJ009879	Vienna Museum	Egypt	(Manceau et al. 1999b)
*Capra nubiana*6	AJ317871	Not mentioned	Not mentioned	(Luikart et al. 2001)
Capra caucasica	AF034738	Bone 1982-797 Laboratorie Mammiferes & Oiseaux, MNHN (FRANCE)	Not mentioned	(Hassanin et al. 1998b)
Capra cylindricornis	AF034737	Bone 1945-68 Laboratorie d Anatomie comparee, MNHN (FRANCE)	Not mentioned	(Hassanin et al. 1998b)
Capra falconer	FJ207525	Cyto 2001-214, MNHN	Not mentioned	(Hassanin et al. 2009)
Capra hircus	KR059154	Saudi Arabia	Saudi Arabia	(Colli et al. 2015)

Capra hircus	GU229279	China	China	Wu,Y.P. and Ma,Y.H.2009
Capra ibex	AJ010055 NC020623	Not mentioned	Not mentioned	(Manceau et al. 1999a)
Capra pyrenaica	FJ207528 NC020625	M149, Sierra Nevada, Spain	Spain	(Hassanin et al. 2009)
Capra sibirica	DQ246771	Altai Republic	Russia	(Kazanskaya et al. 2005)
Capra sibirica	FJ207529	Not mentioned	Not mentioned	(Hassanin et al. 2009)
Capra aegagrus	AF217255	Not mentioned	Not mentioned	(Bar-Gal & Greenblatt 1999)
Capra aegagrus	KR059222	Iran	Iran	(Colli et al. 2015)
Capra Falconeri	AF034736	Bone 1971-147 Laboratorie Mammiferes & Oiseaux ,MNHN (FRANCE)	Not mentioned	(Hassanin et al. 1998b)

Table A2.6. *The reference sequences retrieved from NCBI for other species used in phylogenetic analysis.*

Specie	Accession Number	Reference
Hemitragus_jemlahicus	AF034733	(Hassanin et al. 1998a)
Bos taurus	AY676870	Unpublished
Oryx leucoryx	JN632679	(Hassanin et al. 2012)
Hemitragus jayakari	FJ207523	(Hassanin et al. 2009)
Ovis orientalis	KF312238	(Sanna et al. 2015)
Gazella gazella	JN632640	(Hassanin et al. 2012)
Nanger dama	JN632665	

Table A2.7. *Maximum composite likelihood Between Groups mean distances for Cytochrome b (below diagonal) and D-loop (above diagonal).*

	WWR	*Shalim*	*Dhofar*	*Oman captive*	*UAE captive*
WWR		**0.00598**	**0.01775**	**0.11105**	**0.12704**
Shalim	0.00021		**0.01956**	**0.11322**	**0.12837**
Dhofar	0.00018	0.00019		**0.11749**	**0.13185**
Oman captive	0.00907	0.00907	0.00904		**0.08839**
UAE captive	0.00899	0.00917	0.00899	0.00051	

Table A2.8. *Cytochrome b Evolutionary distances between haplotypes calculated by maximum composite likelihood analysis with Mega.*

Wild Oman nubiana												
Captive nubiana	0.051											
*Capra nubiana**	0.050	0.002										
Dead Sea nubiana**	0.055	0.012	0.011									
Egypt nubiana***	0.072	0.039	0.039	0.045								
Capra aegagrus	0.076	0.060	0.058	0.059	0.074							
Capra caucasica	0.059	0.044	0.042	0.045	0.057	0.040						
Capra cylindricornis	0.054	0.039	0.038	0.041	0.057	0.036	0.008					
Capra falconeri	0.064	0.053	0.051	0.052	0.066	0.045	0.042	0.038				
Capra hircus	0.073	0.057	0.056	0.059	0.071	0.006	0.038	0.034	0.042			
Capra ibex	0.054	0.054	0.051	0.058	0.064	0.045	0.038	0.038	0.061	0.047		
Capra pyrenaica	0.052	0.047	0.045	0.051	0.062	0.047	0.036	0.031	0.054	0.045	0.018	
Capra sibirica	0.103	0.096	0.095	0.091	0.117	0.102	0.095	0.085	0.089	0.100	0.095	0.097

*France museum accession number FJ207527

**Two samples used here DQ514552 and AF217256

*** AJ009879

Table A2.9. *Comparison of mitochondrial DNA (D-loop) measures of genetic diversity between Nubian ibex from the wild and in captivity from this study, and other ungulate species.*

<table>
<tr><th></th><th>Wild/ Captive</th><th>Haplotype diversity</th><th>Nucleotide diversity</th><th>Population status</th><th>Population trend</th><th>Reference</th></tr>
<tr><td>Wild Nubian ibex in Oman</td><td>Wild</td><td>0.849</td><td>0.014</td><td rowspan="5">Vulnerable</td><td rowspan="4">Decreasing</td><td rowspan="2">This study</td></tr>
<tr><td>Captive Nubian ibex</td><td>Captive</td><td>0.466</td><td>0.046</td></tr>
<tr><td>Mainland Soemmerring’s gazelles</td><td>Wild</td><td>0.931</td><td>0.034</td><td rowspan="2">Ibrahim et al. 2020</td></tr>
<tr><td>Dahlak Soemmerring’s gazelles</td><td>Wild</td><td>0.778</td><td>0.005</td></tr>
<tr><td>Captive Arabian Oryx</td><td>Captive</td><td>0.789</td><td>0.009</td><td>Stable</td><td>Khan et al. 2011</td></tr>
<tr><td>Dama Gazelle population1</td><td>Wild</td><td>0.840</td><td>0.031</td><td rowspan="4">Critically endangered</td><td rowspan="6">Decreasing</td><td rowspan="4">Senn et al. 2014</td></tr>
<tr><td>Dama Gazelle population2</td><td>Wild</td><td>0.870</td><td>0.031</td></tr>
<tr><td>Dama Gazelle population3</td><td>Wild</td><td>0.510</td><td>0.006</td></tr>
<tr><td>Dama Gazelle population6</td><td>Captive</td><td>0.485</td><td>0.013</td></tr>
<tr><td>Dorcas gazella</td><td>Wild</td><td>0.692</td><td>0.002</td><td rowspan="4">Endangered</td><td>Godinho et al. 2012</td></tr>
<tr><td>Forest musk deer</td><td>Captive</td><td>0.836</td><td>0.056</td><td>Peng et al. 2008</td></tr>
<tr><td>Przewalski’s gazelle</td><td>Wild</td><td>0.950</td><td>0.015</td><td>Increasing</td><td>Lei et al. 2003</td></tr>
<tr><td>Dorcas gazelle</td><td>Wild</td><td>0.802</td><td>0.015</td><td>Decreasing</td><td>Hadas et al. 2015</td></tr>
</table>

Table A4.1. *Samples used for ddRAD.*

RZSS sample ID	species	Origen	collection date	sample type	remarks	Repeated samples
NUB002	Nubian ibex	wild/Oman/Shalim	2013	tissue	Hunted animal/stored -80C	
NUB003	Nubian ibex	wild/Oman/Shalim	2013	tissue	Hunted animal/stored -80C	Repeated
NUB004	Nubian ibex	wild/Oman/Shalim	2013	tissue	Hunted animal/stored -80C	
NUB005	Nubian ibex	wild/Oman/Shalim	2013	tissue	Hunted animal/stored -80C	
NUB007	Nubian ibex	wild/Oman/Shalim	2013	tissue	Hunted animal/stored -80C	
NUB008	Nubian ibex	wild/Oman/Shalim	2013	tissue	Hunted animal/stored -80C	
NUB020	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB021	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB022	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB023	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB024	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB025	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB026	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB027	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB028	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	

NUB029	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB030	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB031	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB032	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB033	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB034	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB035	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB036	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB037	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB038	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB039	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB040	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB041	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB042	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB043	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB044	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB045	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB046	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB047	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB048	Nubian ibex	captive/UAE	2015-2018	blood	stored -20C	
NUB051	Nubian ibex	wild/Oman/Shalim	2018	tissue	Hunted animal/stored -20C	
NUB052	Nubian ibex	wild/Oman/Shalim	2018	tissue	Hunted animal/stored -20C	
NUB053	Nubian ibex	wild/Oman/Shalim	2018	tissue	Hunted animal/stored -20C	
NUB059	Nubian ibex	captive/Oman	2017	blood	stored -20C	
NUB140	Nubian ibex	wild/Oman	1988	tissue		
NUB151	Nubian ibex	wild/Oman	2019	tissue	wild cought/died later/stored -20C	
NUB153	Nubian ibex	wild/Oman	2019	blood	wild cought/released/stored -20C	Repeated
HIR001	Goat	demostic/Oman	2018	blood	stored -20C	Repeated
HIR001	Goat	demostic/Oman	2018	blood	stored -20C	
HIR002	Goat	demostic/Oman	2018	blood	stored -20C	
HIR003	Goat	demostic/Oman	2018	blood	stored -20C	
HIR004	Goat	demostic/Oman	2018	blood	stored -20C	
HIR005	Goat	demostic/Oman	2018	blood	stored -20C	

Table A4.2. Genotyping errors were calculated per pair of repeated samples for SNPs identified from Wild nubian only or Wild & Captive. Genotyping errors were calculated only for SNPs genotyped in both samples and were classified as either allelic dropout

(one sample heterozygote, the other homozygote) or alternative homozygotes (both samples homozygous for alternative alleles).

SNP group	Total SNPs	No pairs samples	No SNPs geno/pair (range)	Proportion total errors	Mean Proportion errors	Proportion allelic dropout (range)	Mean proportion allelic dropout	Proportion alternative homozygote (range)	Mean proportio alternativ homozyg
Wild_Oman	13131	4	10293-12251	0.022-0.065	0.052	0.016-0.053	0.041	0.006-0.015	0.011
Captive	22707	4	17682-21487	0.007-0.024	0.018	0.005-0.018	0.014	0.002-0.005	0.004

Supplementary Materials S3

Table A4.3. *Analysis of Molecular Variance (AMOVA) by the poppr package in R using 5775 SNPs within and between populations.*

Captive VS Wild					
Source	Df	Sum.Sq	% Of Variance	Phi Φ*ST*	P-value
Within samples	39	16239.99	45.83262	0.541674	0.001
Between samples within populations	37	15318.92	-0.13126	-0.00287	0.504
Among populations	1	15998.12	54.29864	0.542986	0.001
Total	77	47557.04	100	NA	NA
Captive VS Goats					
Source	Df	Sum.Sq	% Of Variance	Phi Φ*ST*	P-value
Within samples	33	13459.72	11.34711	0.886529	0.001
Between samples within populations	31	12554.42	-0.04019	-0.00355	0.515
Among populations	1	54505.42	88.69308	0.886931	0.001
Total	65	80519.56	100	NA	NA
Wild VS Goats					
Source	Df	Sum.Sq	% Of Variance	Phi Φ*ST*	P-value
Within samples	16	5820.0	11.502	0.885	0.001

Between samples within populations	14	5139.82	0.053	0.005	0.474
Among populations	1	38825.81	88.444	0.884	0.001
Total	31	49785.63	100	NA	NA

Table A4.4. *Estimation of the Allelic richness and private alleles between wild and captive Nubian ibex populations using the rarefication method in ADZE-1.0.*

POP	Sample Size	SNP	Allelic richness Mean ± confidence interval
wild	2	5775	1.08493 ± (1.08498:1.08487)
wild	3	5775	1.12739 ± (1.12748:1.12730)
wild	4	5775	1.15228 ± (1.15238:1.15218)
wild	5	5775	1.16838 ± (1.16849:1.16827)
wild	6	5775	1.17951 ± (1.17963:1.17939)
wild	7	5775	1.18757 ± (1.18769:1.18745)
wild	8	5775	1.19362 ± (1.19375:1.19349)
wild	9	5775	1.19829 ± (1.19842:1.19816)
wild	10	5775	1.202 ± (1.20213:1.20187)
wild	11	5775	1.20501 ± (1.20514:1.20488)
captive	2	5775	1.07786 ± (1.07791:1.07781)
captive	3	5775	1.11678 ± (1.11686:1.11670)
captive	4	5775	1.14038 ± (1.14048:1.14028)
captive	5	5775	1.1563 ± (1.15641:1.15620)

captive	6	5775	1.16783 ± (1.16794:1.16772)
captive	7	5775	1.1766 ± (1.17672:1.17648)
captive	8	5775	1.18352 ± (1.1836:1.1834)
captive	9	5775	1.18915 ± (1.18927:1.18903)
captive	10	5775	1.19383 ± (1.19396:1.19371)
captive	11	5775	1.19779 ± (1.19791:1.19766)
POP	Sample size	SNP	**Private alleles** Mean ± confidence interval
wild	11	5775	0.114546 (0.1146-0.1144)
captive	11	5775	0.104912 (0.1049-0.1048)

Table A5.10. *Numbers of SNPs that are fixed or variable when selecting SNPs using just the Oman or both Oman and captive Nubian ibex.*

Variability	**Wild Captive NUB**
Both fixed	1462
Oman_(only)_variable	5345
UAE_(only)_variable	13658
Both variable	2242
Total SNPs	22707

Table A5.11. *SNP statistics comparison between faecal and old samples for Nubian ibex showing percentage duplicated, sequence length in base pairs, and total number of reads. Old samples consist of museum samples, bone and skin.*

	Duplicate %				read length (bp)				# of reads (millions (M))			
sample	> 50%	< 50%	High value	Low value	>100pb	< 100bp	High value	Low value	> 3 M	< 3 M	High value	Low value
Faecal	22%	78%	84%	7%	80%	20%	137	66	59%	41%	17.7	0.2

Old	38%	61%	84%	11%	4%	96%	102	62	13%	87%	16.4	0.8

Table A5.12. *Pairwise F_{ST} estimates for Oman ibex, captive ibex, goats, Sudan, Yemen and other groups. The 95% confidence intervals indicated by Lower bound CI limit and Upper bound CI limit. These results based on 344, 1,054 and 1,935 SNPs.*

Number of SNPs used	Population1	Population2	Lower bound CI limit	Upper bound CI limit	p-value	F_{ST}
344 SNPs	Goat	Oman	0.581	0.688	0.000	0.636
	Goat	Captive	0.770	0.859	0.000	0.820
	Goat	Hybrid	0.651	0.777	0.000	0.721
	Goat	WWR-Goat	-0.181	0.066	0.851	-0.061
	Goat	Yemen	0.791	0.886	0.000	0.843
	Goat	Sudan	0.831	0.888	0.000	0.861
	Oman	Captive	0.364	0.472	0.000	0.419
	Oman	Hybrid	0.283	0.424	0.000	0.354
	Oman	WWR-Goat	0.531	0.654	0.000	0.595
	Oman	Yemen	-0.036	0.125	0.157	0.044
	Oman	Sudan	0.523	0.629	0.000	0.578
	Captive	Hybrid	0.415	0.598	0.000	0.515
	Captive	WWR-Goat	0.750	0.851	0.000	0.805
	Captive	Yemen	0.452	0.656	0.000	0.563
	Captive	Sudan	0.683	0.789	0.000	0.739
	Hybrid	WWR-Goat	0.487	0.661	0.000	0.583
	Hybrid	Yemen	-0.005	0.296	0.031	0.150
	Hybrid	Sudan	0.595	0.716	0.000	0.662
	WWR-Goat	Yemen	0.778	0.868	0.000	0.828

	Population1	Population2	Lower bound CI limit	Upper bound CI limit	p-value	Fst
	WWR-Goat	Sudan	0.795	0.868	0.000	0.833
	Yemen	Sudan	0.649	0.765	0.000	0.711

	Population1	Population2	Lower bound CI limit	Upper bound CI limit	p-value	Fst
1,054 SNPs	Goat	Oman	0.592	0.645	0.000	0.620
	Goat	Captive	0.796	0.846	0.000	0.823
	Goat	Hybrid	0.689	0.767	0.000	0.731
	Goat	WWR-goat	-0.078	0.100	0.423	0.010
	Goat	Yemen	0.842	0.888	0.000	0.865
	Goat	Sudan	0.858	0.889	0.000	0.874
	Oman	Captive	0.407	0.458	0.000	0.433
	Oman	Hybrid	0.337	0.405	0.000	0.372
	Oman	WWR-goat	0.541	0.602	0.000	0.573
	Oman	Yemen	0.020	0.102	0.002	0.062
	Oman	Sudan	0.481	0.543	0.000	0.513
	Captive	Hybrid	0.483	0.593	0.000	0.539
	Captive	WWR-goat	0.774	0.833	0.000	0.806
	Captive	Yemen	0.570	0.676	0.000	0.626
	Captive	Sudan	0.684	0.750	0.000	0.717
	Hybrid	WWR-goat	0.551	0.649	0.000	0.605
	Hybrid	Yemen	0.263	0.427	0.000	0.348
	Hybrid	Sudan	0.658	0.722	0.000	0.692
	WWR-goat	Yemen	0.812	0.863	0.000	0.839
	WWR-goat	Sudan	0.827	0.867	0.000	0.848
	Yemen	Sudan	0.703	0.768	0.000	0.737

	Population1	Population2	Lower bound CI limit	Upper bound CI limit	p-value	Fst
1,935 SNPs	Goat	Oman	0.590	0.630	0.000	0.611
	Goat	Captive	0.769	0.810	0.000	0.791
	Goat	Hybrid	0.681	0.743	0.000	0.713
	Goat	WWR-goat	-0.009	0.148	0.044	0.068
	Goat	Yemen	0.843	0.880	0.000	0.863
	Goat	Sudan	0.856	0.880	0.000	0.869
	Oman	Captive	0.455	0.492	0.000	0.474
	Oman	Hybrid	0.372	0.420	0.000	0.395
	Oman	WWR-goat	0.526	0.572	0.000	0.550
	Oman	Yemen	0.081	0.141	0.000	0.112
	Oman	Sudan	0.512	0.555	0.000	0.535

	Captive	Hybrid	0.454	0.540	0.000	0.496
	Captive	WWR-goat	0.736	0.784	0.000	0.762
	Captive	Yemen	0.579	0.661	0.000	0.622
	Captive	Sudan	0.689	0.738	0.000	0.716
	Hybrid	WWR-goat	0.565	0.647	0.000	0.606
	Hybrid	Yemen	0.379	0.504	0.000	0.444
	Hybrid	Sudan	0.680	0.732	0.000	0.707
	WWR-goat	Yemen	0.805	0.849	0.000	0.829
	WWR-goat	Sudan	0.826	0.855	0.000	0.841
	Yemen	Sudan	0.732	0.779	0.000	0.756

Table A6.13. *Results of mean rate of stochastic population growth (stoch-r), probability of extinction (PE), final population size (N-extant) and genetic diversity (GD) of different drought scenarios simulated for a population of 100 individuals population. SD = standard deviation. and TE = the mean time to extinction.*

Drought frequency	Reproduction rate	stoch-r	SD(r)	PE	N-extant	SD(N-ext)	GD	SD(GD)	meanTE
	0	0.003	0.135	0.150	153.290	94.980	0.862	0.074	74.100
	0.1	0.007	0.131	0.147	161.843	93.583	0.859	0.090	71.867
	0.2	0.008	0.128	0.155	176.913	92.060	0.862	0.098	76.300
	0.3	0.012	0.124	0.110	185.683	90.987	0.873	0.082	77.367
	0.4	0.016	0.119	0.073	200.945	88.225	0.880	0.068	81.625
10%	0.5	0.021	0.117	0.060	216.688	80.455	0.885	0.071	84.925
	0.6	0.022	0.117	0.027	210.433	84.863	0.877	0.081	76.333
	0.7	0.024	0.114	0.025	222.940	80.080	0.887	0.060	42.500
	0.8	0.027	0.113	0.030	236.455	66.215	0.900	0.035	43.600
	0.9	0.030	0.113	0.005	234.975	67.700	0.898	0.036	39.000
	1	0.033	0.113	0.010	246.723	64.915	0.899	0.049	57.500
	0	-0.035	0.159	0.725	65.813	69.938	0.788	0.115	65.875
	0.1	-0.026	0.150	0.586	78.026	76.300	0.789	0.132	70.940
	0.2	-0.021	0.144	0.490	86.658	86.068	0.808	0.108	73.450
	0.3	-0.011	0.136	0.343	106.613	87.000	0.832	0.097	76.633
	0.4	-0.004	0.129	0.250	132.860	91.863	0.845	0.099	74.100
20%	0.5	0.003	0.124	0.170	157.094	93.294	0.855	0.091	74.580
	0.6	0.011	0.119	0.140	198.880	85.680	0.881	0.054	76.600
	0.7	0.018	0.116	0.058	203.648	83.068	0.886	0.053	78.925
	0.8	0.022	0.115	0.045	218.200	78.220	0.887	0.071	72.350
	0.9	0.027	0.115	0.020	231.780	75.480	0.891	0.062	31.250
	1	0.034	0.113	0.000	246.455	68.460	0.895	0.052	0.000
	0	-0.068	0.177	0.980	9.500	4.270	0.610	0.027	49.150
	0.1	-0.059	0.169	0.963	13.630	22.703	0.449	0.093	56.133
33%	0.2	-0.052	0.162	0.912	42.372	57.778	0.741	0.127	59.460
	0.3	-0.040	0.152	0.798	48.875	57.003	0.762	0.161	67.575
	0.4	-0.032	0.144	0.664	60.488	63.348	0.780	0.125	69.960

	0.5	-0.018	0.134	0.427	85.453	79.680	0.804	0.109	73.400
	0.6	-0.009	0.129	0.290	108.460	89.923	0.819	0.130	75.100
	0.7	0.004	0.120	0.152	163.237	94.843	0.856	0.088	76.083
	0.8	0.015	0.114	0.050	191.113	88.230	0.873	0.077	75.667
	0.9	0.026	0.114	0.010	226.650	74.160	0.895	0.039	55.000
	1	0.033	0.114	0.015	247.720	64.520	0.900	0.051	87.000

Table A6.14. *Results of mean rate of stochastic population growth (stoch-r), probability of extinction (PE), final population size (N-extant) and genetic diversity (GD) of different drought scenarios simulated for a population of 250 individuals population. SD = standard deviation and TE = the mean time to extinction.*

Drought frequency	Reproduction rate	stoch-r	SD(r)	PE	N-extant	SD(N-ext)	GD	SD(GD)	meanTE
10%	0	0.012	0.131	0.050	180.184	91.250	0.890	0.068	87.800
	0.1	0.013	0.126	0.030	181.380	91.160	0.893	0.082	83.300
	0.2	0.019	0.121	0.010	201.177	81.557	0.905	0.044	90.667
	0.3	0.022	0.119	0.017	215.173	77.077	0.907	0.050	88.667
	0.4	0.026	0.117	0.003	231.177	71.030	0.911	0.044	30.000
	0.5	0.028	0.115	0	237.840	64.810	0.916	0.027	0.000
	0.6	0.029	0.113	0.003	236.433	64.313	0.917	0.022	30.333
	0.8	0.035	0.112	0	250.347	55.330	0.919	0.020	0
	0.9	0.038	0.113	0	257.553	53.875	0.920	0.020	0
	1	0.040	0.112	0	261.390	48.295	0.923	0.019	0
20%	0	-0.024	0.151	0.385	67.370	73.420	0.786	0.147	77.550
	0.1	-0.010	0.143	0.190	101.370	86.850	0.844	0.101	79.700
	0.2	-0.004	0.132	0.133	120.560	87.610	0.854	0.093	85.300
	0.3	-0.001	0.127	0.077	125.923	89.780	0.857	0.112	87.500
	0.4	0.009	0.121	0.044	166.300	87.582	0.890	0.060	86.760
	0.5	0.017	0.116	0.010	204.495	82.083	0.903	0.040	38.075
	0.6	0.022	0.113	0.000	211.900	82.580	0.908	0.039	0
	0.7	0.026	0.114	0.005	226.060	76.145	0.912	0.035	50
	0.8	0.031	0.112	0	243.470	61.227	0.916	0.028	0
	0.9	0.036	0.112	0	252.343	56.453	0.920	0.019	0
	1	0.039	0.112	0	264.605	45.598	0.923	0.016	0
33%	0	-0.065	0.170	0.940	20.415	17.075	0.747	0.095	62.550
	0.1	-0.052	0.158	0.850	27.230	32.345	0.727	0.116	70.000
	0.2	-0.042	0.151	0.700	32.870	53.150	0.710	0.205	76.500
	0.3	-0.027	0.139	0.434	59.769	62.196	0.802	0.124	80.157
	0.4	-0.017	0.130	0.243	82.193	77.910	0.822	0.109	82.933
	0.5	-0.004	0.122	0.093	121.643	88.493	0.860	0.090	83.033

	0.6	0.009	0.116	0.028	168.010	84.338	0.893	0.052	89.800
	0.7	0.017	0.112	0.013	199.543	82.600	0.902	0.051	86.167
	0.8	0.025	0.111	0.003	228.407	70.732	0.914	0.030	33
	0.9	0.034	0.112	0	249.930	58.120	0.918	0.022	0
	1	0.039	0.113	0	259.400	55.590	0.921	0.020	0

Table A6.15. *Results of simulating different number of animals supplemented to a proposed captive population and comparison between male and female supplementation. The results showed population growth (stoch-r), probability of extinction (PE), final population size (N-extant) and genetic diversity (GD). SD = standard deviation.*

	stoch-r		**PE**		**N-extant**		**GD**	
#of animal supplied	**Female (SD)**	**Male (SD)**	**Female**	**Male**	**Female (SD)**	**Male (SD)**	**Female (SD)**	**Male (SD)**
1	-0.010 (0.176)	-0.018 (0.186)	0.821	0.918	78.719 (82.03)	51.014 (46.21)	0.663 (0.157)	0.611 (0.131)
2	-0.001 (0.164)	-0.011 (0.181)	0.640	0.875	97.170 (90.84)	104.189 (83.57)	0.736 (0.128)	0.725 (0.119)
3	0.010 (0.153)	-0.005 (0.175)	0.413	0.789	119.709 (95.15)	97.643 (94.31)	0.747 (0.140)	0.708 (0.156)
4	0.018 (0.147)	-0.004 (0.175)	0.276	0.798	149.037 (105.27)	119.184 (101.49)	0.778 (0.133)	0.751 (0.128)
5	0.026 (0.143)	-0.001 (0.175)	0.155	0.762	166.118 (98.38)	124.819 (101.76)	0.801 (0.121)	0.750 (0.139)
6	0.031 (0.141)	0.001 (0.178)	0.096	0.764	185.544 (95.26)	143.695 (108.07)	0.830 (0.093)	0.776 (0.123)
7	0.035 (0.140)	0.004 (0.182)	0.057	0.704	197.072 (92.66)	120.710 (93.73)	0.849 (0.076)	0.763 (0.125)
8	0.040 (0.140)	0.005 (0.183)	0.050	0.687	216.708 (80.72)	134.060 (111.35)	0.863 (0.049)	0.777 (0.132)
9	0.042 (0.141)	0.007 (0.182)	0.030	0.677	220.053 (81.95)	143.691 (103.16)	0.864 (0.067)	0.788 (0.125)
10	0.046 (0.141)	0.007 (0.187)	0.011	0.695	231.773 (72.44)	151.641 (100.68)	0.874 (0.059)	0.797 (0.110)

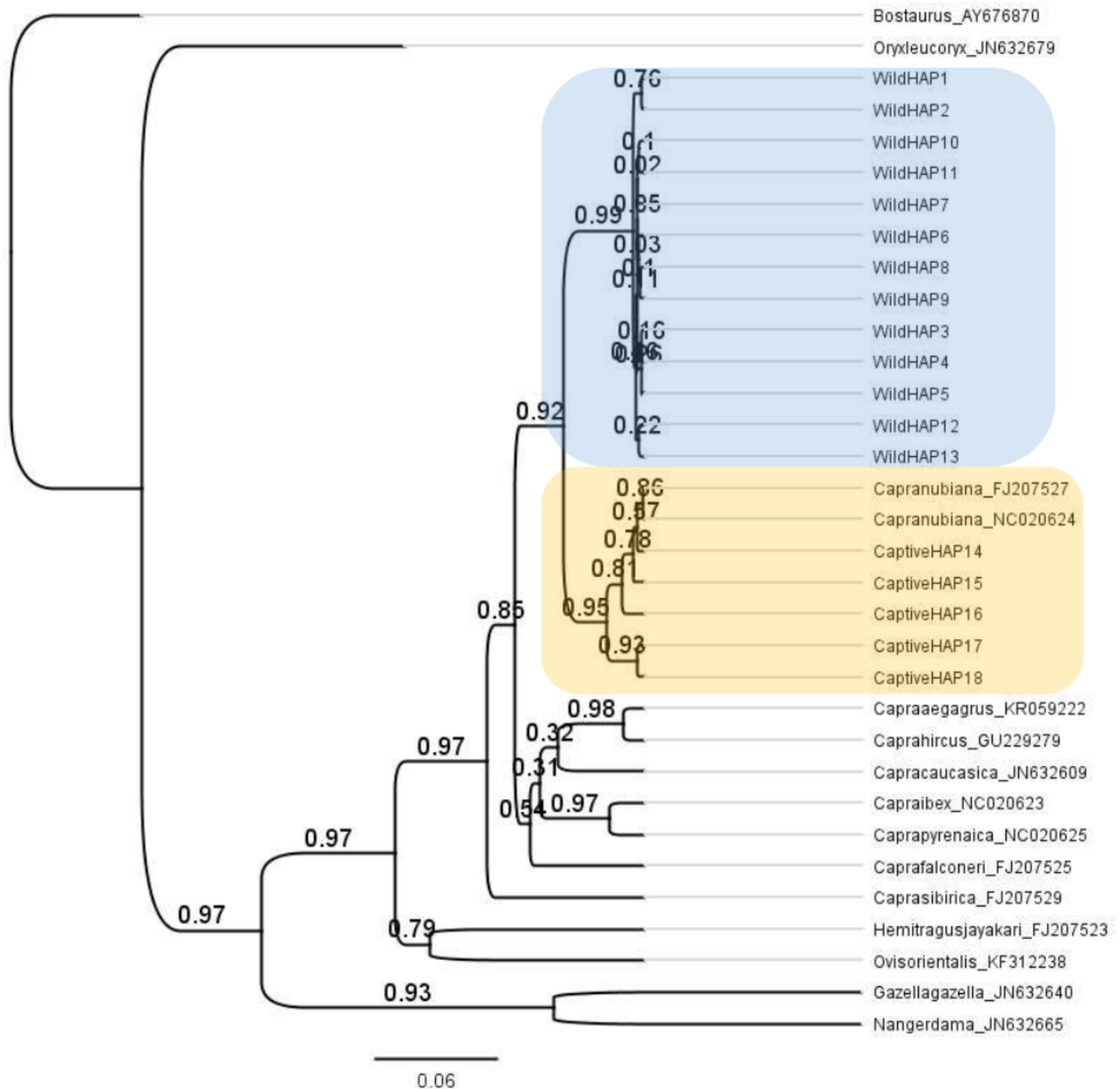


Figure A2.1. *Bayesian phylogenetic tree of concatenated mtDNA sequences of cytochrome b (487bp) and D-loop (187bp) calculated by StarBeast (Bouckaert et al. 2019). The blue shade represent Oman wild Nubian ibex (WildHAP1 to WildHAP13) which are forming a distinct clade supported by 99% bootstrap.*

The orange shade represent the captive Nubian ibex (CaptiveHAP14 to CaptiveHAP18) which cluster with other Nubian species from GenBank and this clade is supported by 95% bootstrap. The model used was HKY+Γ+I calculated by jModelTest in the R package “phangorn”(Schliep 2010). Discrete gamma distribution with four rate categories was used and a strict molecular clock was employed. The population function used was linear with constant root. The priors were set as default. The sample store was set every 5000 from a total of 5000,000 MCMC and 10% were discarded as burn-in

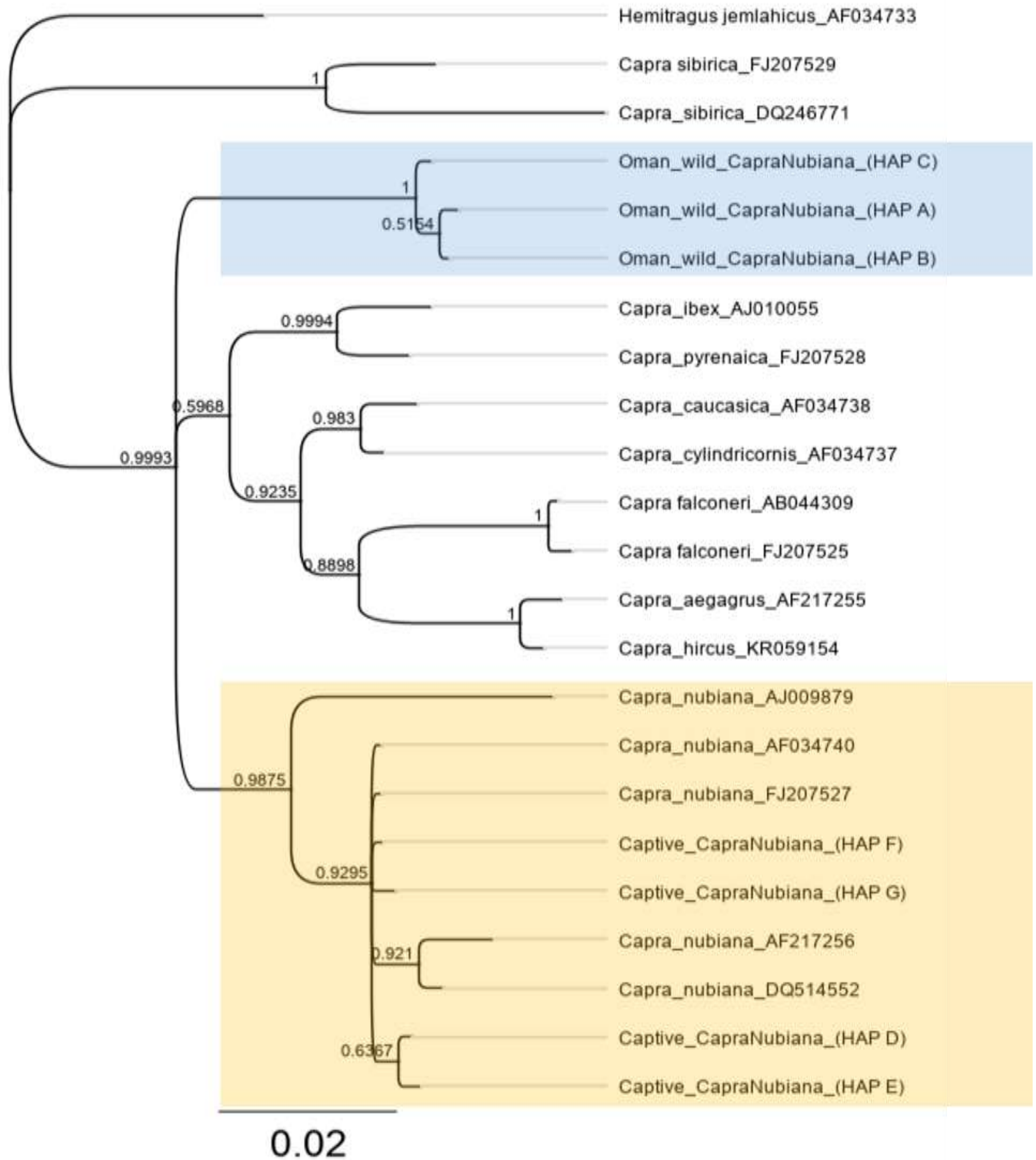


Figure A2.2. *Bayesian phylogeny based on Cytochrome b (487bp). The wild samples are highlighted with blue while the captive samples are highlighted with orange, the remaining samples are reference data obtains from GenBank (Table A2.4). The numbers at the nodes represent the posterior probability. In the tree,*

the wild Nubian ibex is referred as Oman_wild_CapraNubiana and captive ibex as Captive_CapraNubiana.

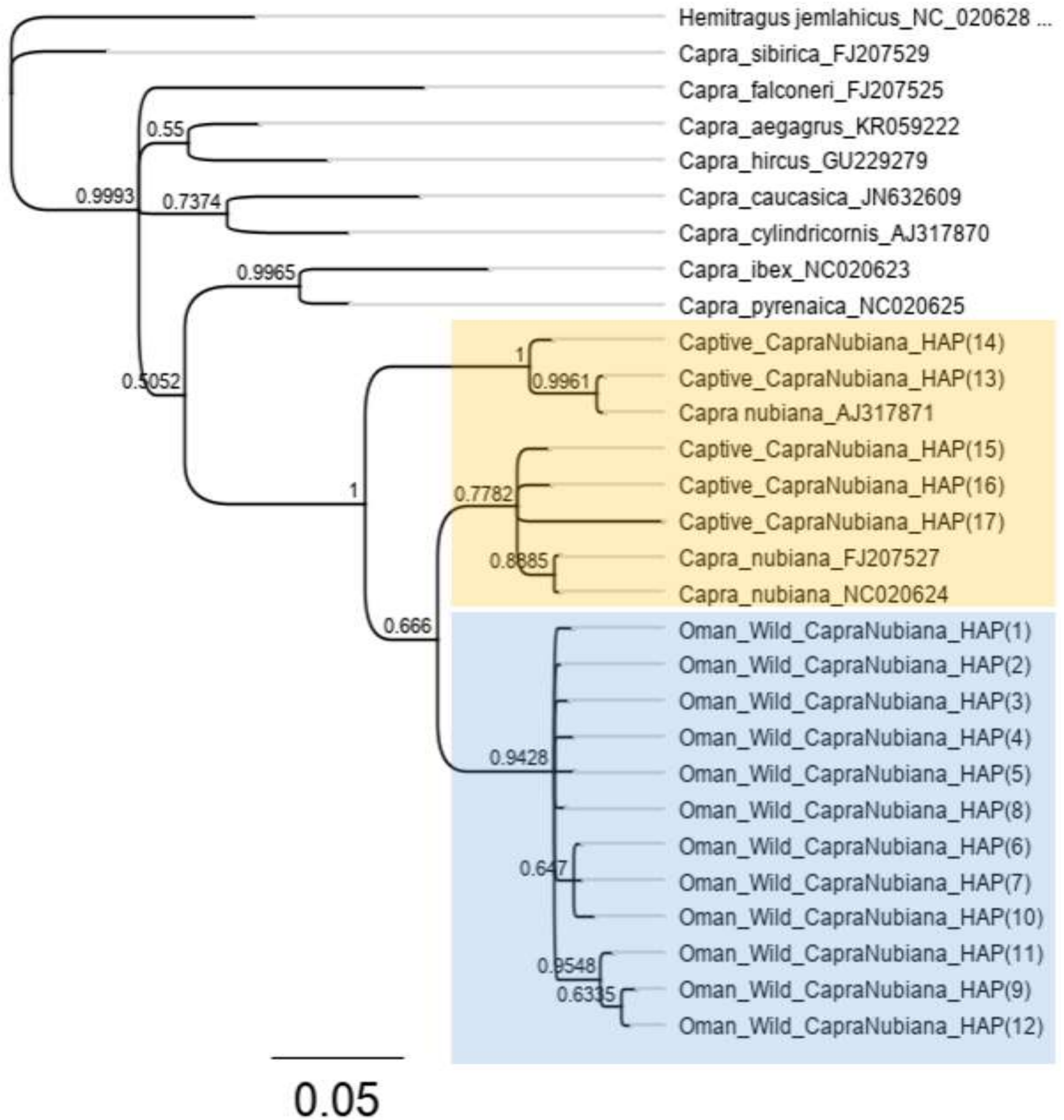


Figure A2.3. *Bayesian phylogeny based on D-loop (186bp). The wild samples are highlighted with blue while the captive samples are highlighted with orange,*

the remaining sequences are reference data from GenBank (Table 2.4). The numbers at the nodes represent the posterior probabilities. In the tree, the wild Nubian ibex is referred as Oman_wild_CapraNubian and captive ibex as Captive_CapraNubian.

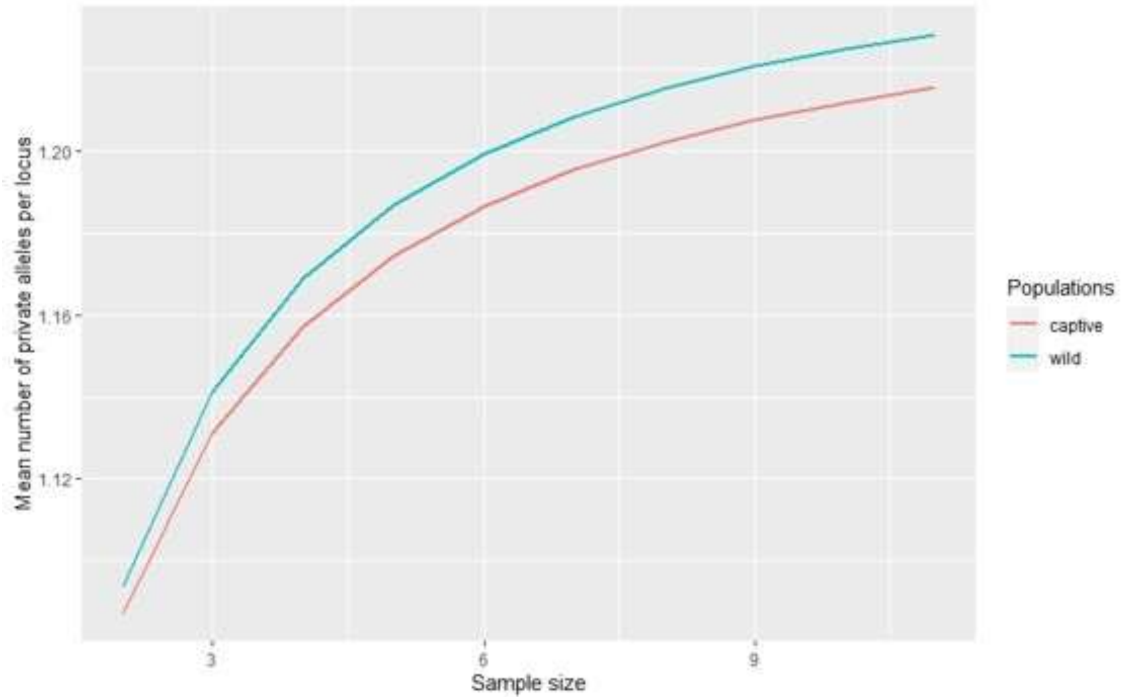


Figure A4.4. *Private allelic richness in wild and captive populations based on the rarefication method of up to 11 samples from each population.*

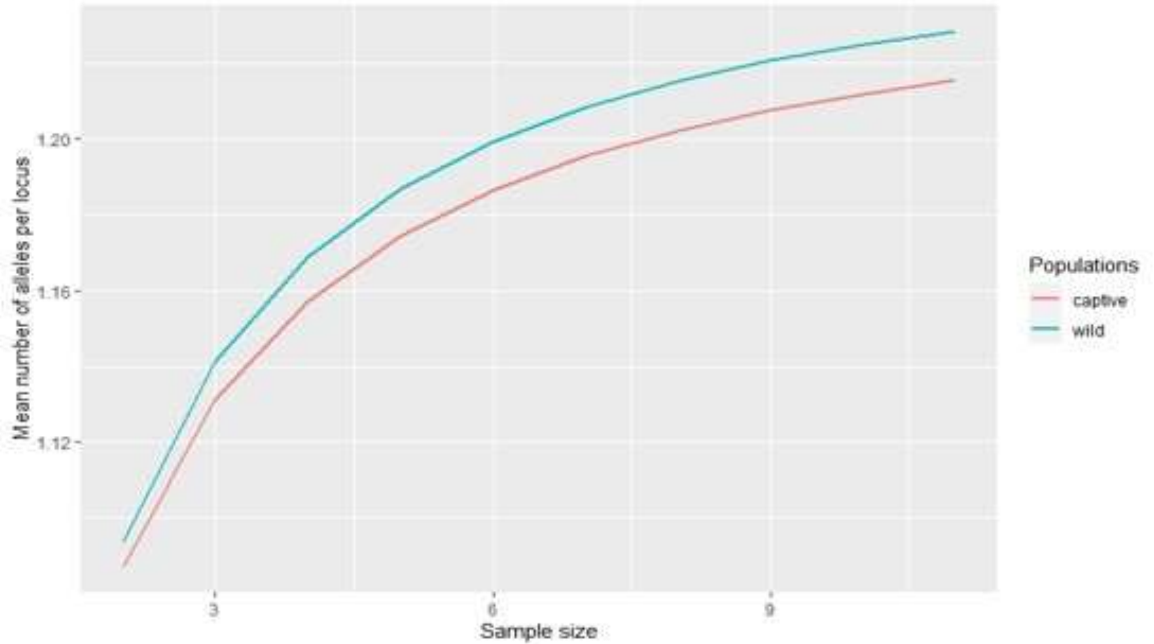


Figure A4.5. *Mean allelic richness in wild and captive populations based on the rarefication method of up to 11 samples from each population.*

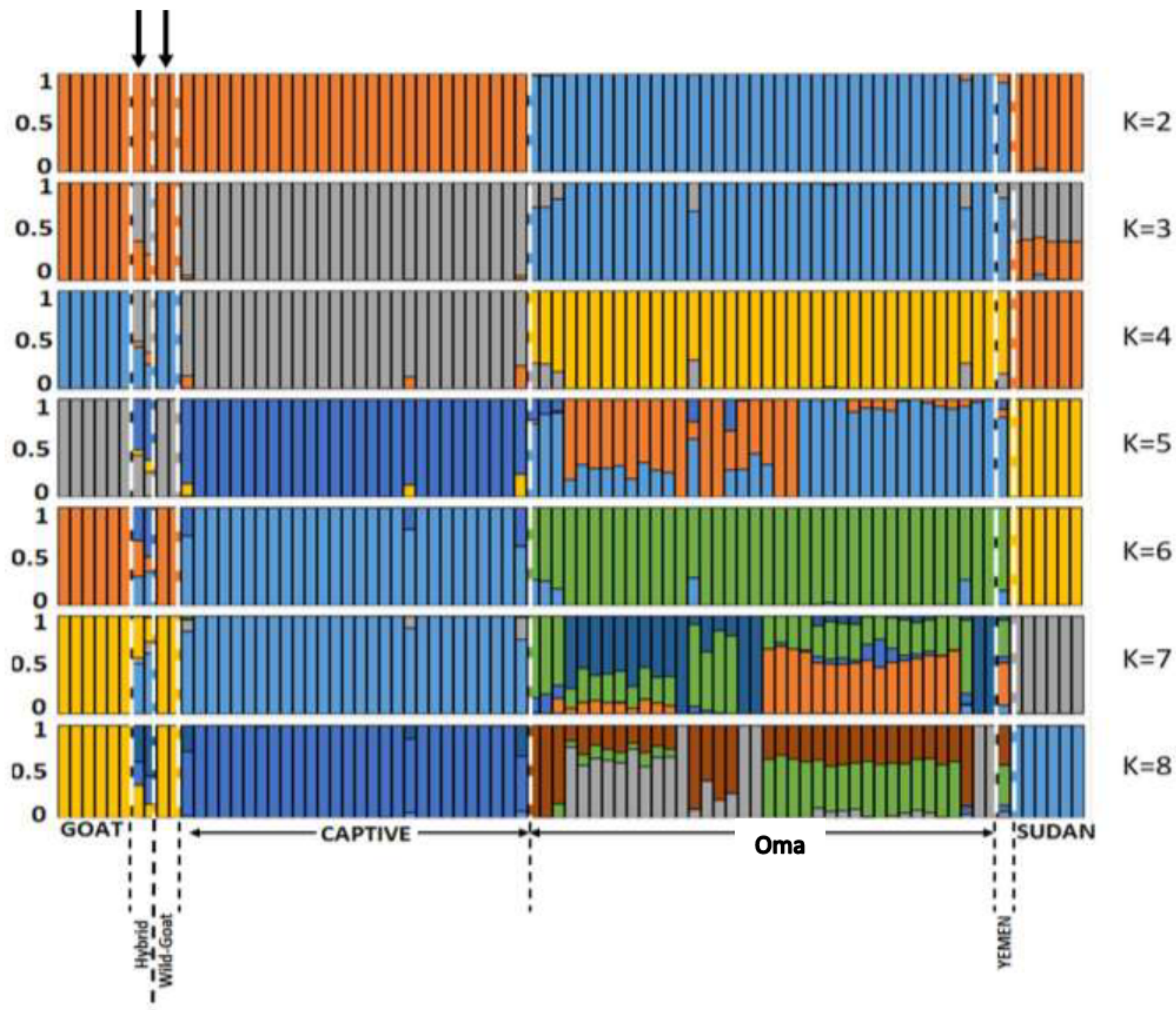


Figure A5.6. *Genetic structures inferred by STRUCTURE of Nubian ibex samples for Oman, captive, Yemen, Sudan and domestic goat based on 1,054 SNPs. Dashed white lines showed the boundaries of each cluster. Hybrid and wild-goat individuals are indicated by arrows. The y-axis represents the likelihood of membership to each cluster. Each single column represents one sample divided into K colours, where K is the number of clusters assumed and*

the length of the coloured segment represents the individual's estimated proportion of membership to a particular cluster.

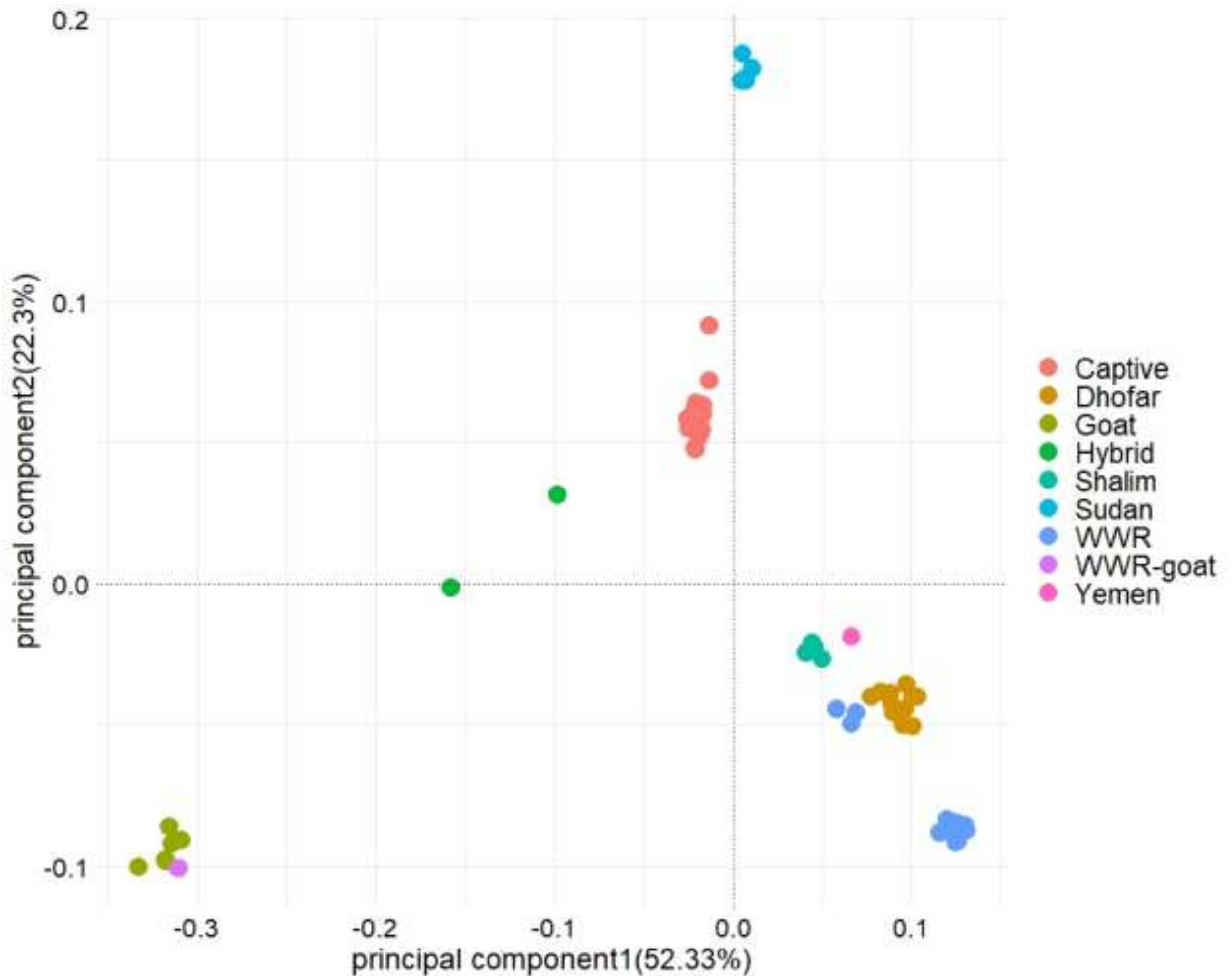


Figure A5.7. *Principal component analysis (PCA) plot of the genetic relationship based on 1,054 SNPs of Oman, captive, Sudan, Yemen and goats. The analysis showed two hybrids from captive individuals (green dots) and two samples from Oman (WWR-goat) clustering with goats (purple dots). The dashed circles encompass the samples within four populations: goats, southern Arabia, captive, Sudan.*

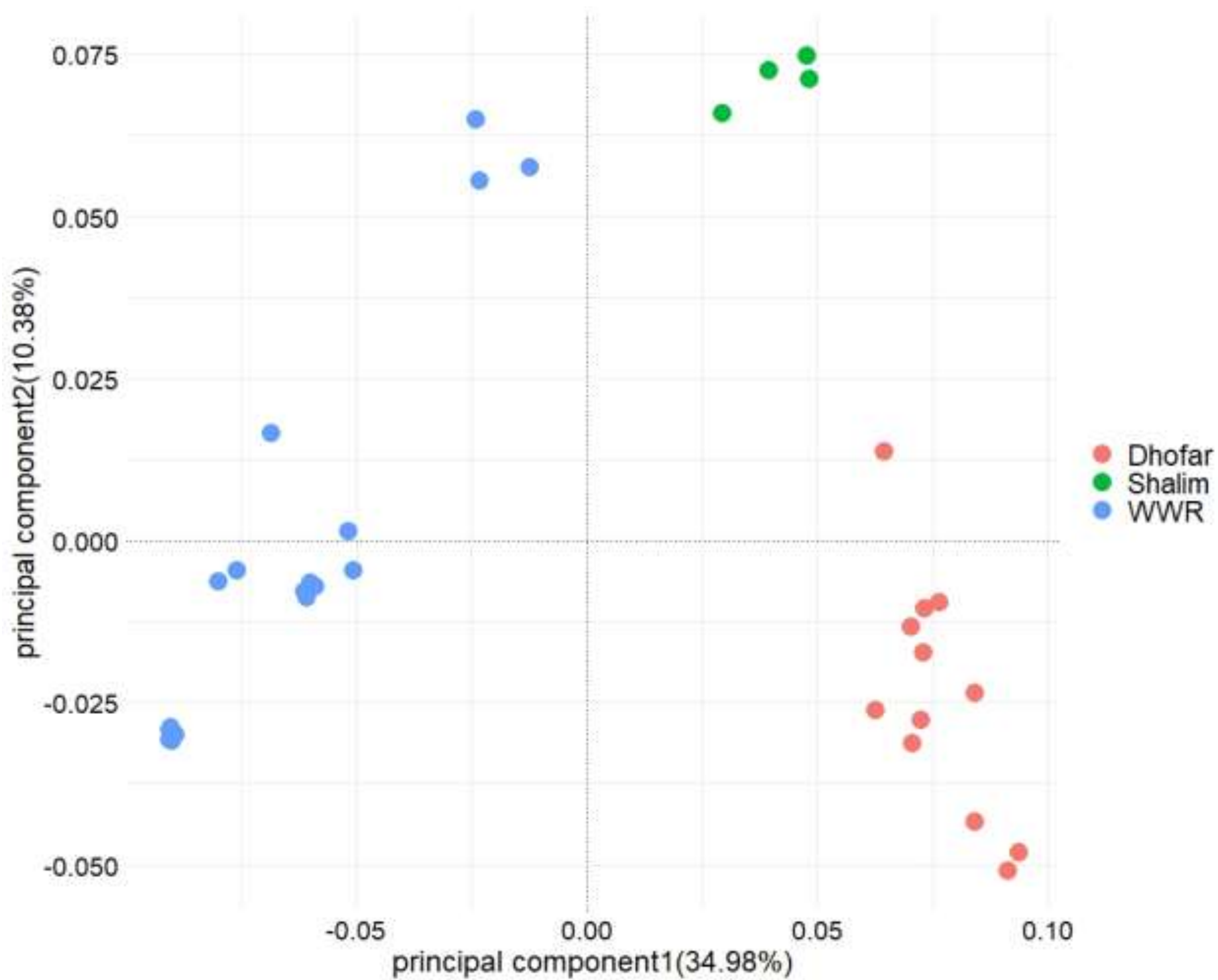


Figure A5.8. *Principal component analysis (PCA) plot of the genetic relationship based on 1,054 SNPs of Oman Nubian ibex samples from WWR (blue dots), Shalim (green dots) and Dhofar (red dots).*

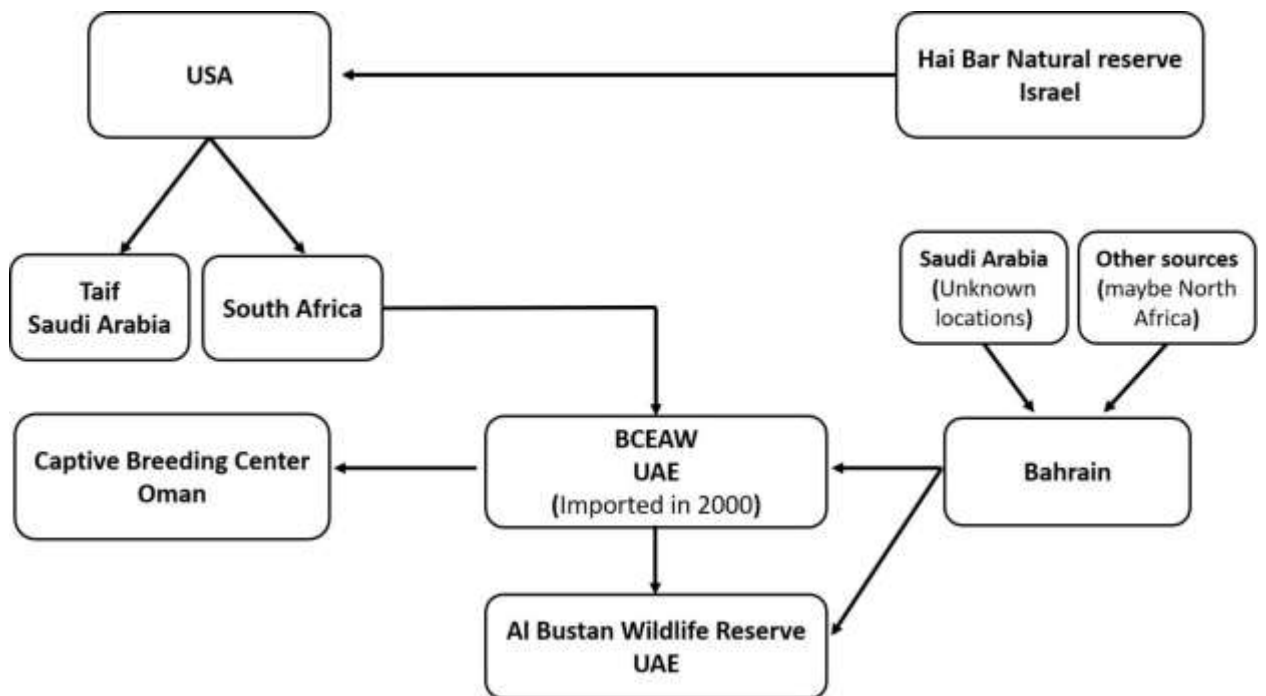


Figure A5.9. *Schematic representation showing the reported origin of the captive animals in UAE and Oman captive centres, based on email communications with Paul Vercammen in 2022, Operations Manager at Breeding Centre for Endangered Arabian Wildlife, Sharjah Desert Park, UAE. See discussion for details.*

Supplementary Materials A2

DNA extraction protocols

DNA extraction from blood

The DNA extraction from blood was carried out by using the QIAGEN-DNeasy Blood and Tissue kit (QIAGEN®, Germany). 100 μl of the blood from each sample were transferred into 1.5 ml tube. Then 20 μl of proteinase k was added to each tube in order to digest any protein in the solution. After that, 100 μl of PBS buffer was added to each blood tube, bringing the total volume up to 220

μl. Next, 200 μl of AL buffer was added. Each tube was vortexed and then spun for short time (5-10 seconds). All tubes were placed on a hot thermoblock at a temperature of 56 ˚C for 10 minutes. After that, 200 μl of absolute ethanol was added to each tube followed by vortexing and spinning for a short time. 600 μl of the blood solution was then transferred to the spin column (QIAGEN) and spun at 8000 rpm for 1 minute. Next, 500 μl of AW1 washing buffer was added and the tubes were spun at 8000 rpm for 1 minute followed by adding another 500 μl of AW2 washing buffer to each tube and spinning again at same power and time. In order to dry the membrane completely, the column tubes were spun for 2 minutes at full speed (14500 rpm) and then the membrane of each tube was transferred to a new 1.5 ml tube. A final step was to add 100 μl of AE buffer and spin at 8000 rpm for 1 minute in order to eluate the DNA from the membrane. The DNA quality was checked by using NanoDrop 1000 (Thermo Scientific).

Decalcification of bones

About 2 g of bone powder were obtained from each bone sample by drilling. The outer surface of the bone was avoided when possible to reduce contamination. Then 1 ml of 0.5M EDTA was added to each tube followed by vortexing to mix well. Next, all samples were placed on a thermoblock at 4˚C overnight. Next, the samples were centrifuged at 12000 rpm for 10 minutes and the supernatant was discarded. After that, 1 ml of EDTA was added and the solution was mixed by vortex. The samples were placed on a thermoblock for 24 h at 4˚C. Then, the samples were centrifuged at 12000 rpm for 10 minutes and the supernatant was discarded. One milliliter of EDTA was added to each samples and centrifuged at 12000 rpm for 10 minutes and the supernatant discarded again. Next, 1 ml of ddH2O was added and the samples were vortexed and they were left to settle down for 5 minutes before spinning at 12000 rpm for 10 minutes. This step was repeated three more times. The final

step was to discard the supernatant and keep the decalcified bone in the fridge to be used for DNA extraction.

DNA extraction from decalcified bones

To each sample tube 360 μl of ATL buffer and 40 μl of proteinase k were added. After mixing the tubes they were placed on a hot thermoblock at 56 ˚C applying shaking at 9000 rpm and kept digesting overnight. Next 400 μl of AL buffer were added and then vortexed and placed on the thermoblock at 70˚C for 10 minutes at 9000 rpm. After that the tubes were spun for 1 minute at full speed (14500 rpm) and the supernatant was transferred to 2 ml tube. An amount of absolute ethanol (400 μl) was added and mixed by vortex then spun for short time, the supernatant then transferred to 600 μl lysate QIAamp Min Elute column. The column was centrifuged at 9500 rpm for 1 minute. Then the elute column was placed on a clean tubes and 600 μl of AW1 was added to the elute column and then spin at 9500 rpm for 1 minute. The elute column was again placed in a new tube and 700 μl of AW2 were added followed by centrifugation at 9500 rpm for 1 minute. After that 700 μl of 100% ethanol was added to each elute column after placing them in a new clean tube. The elute columns were spun at 9500 rpm for 1 minute followed by 3 minutes to ensure that the membrane was completely dry of ethanol. Next, each elute column was placed in a new 1.5 ml and 50 μl of ATE buffer was added and spin at 14500 rpm for 1 minute. The quality of DNA was assessed by using NanoDrop 1000 (Thermo Scientific) and gel electrophoresis.

DNA extraction from tissue

The extraction was performed using the DNeasy Blood&Tissue kit (QIAGEN). Small pieces of tissue were used to perform DNA extraction from the tissue of the hunted ibex. They were placed in 1.5 ml tubes. 180 μl of AL buffer and 20 μl

of proteinase k were added to each tube. Then each tube was vortexed and spun for short time. Each tube was then placed on thermoblock at 56 ˚C until digested. Next 20 µl of AL buffer was added to each tube then vortexed and spun for short time. After that 200 µl of absolute ethanol was added to each tube followed by vortexing and spinning. Then the solution was transferred to a spin column and centrifuged at 8000 rpm for 1 min. In separate steps, 500 µl of AW1 buffer and AW2 buffer were added followed by spinning at 8000 rpm for 1 min. In order to make sure that the membrane in the column was dry it was spun at full speed for 2 min. Finally, the column was transferred to a 1.5 ml tube and 100 µl of AE buffer was added and left for 1 min before spinning at 8000 rpm for 1 min. The DNA quality was checked by using NanoDrop 1000 (Thermo Scientific).

DNA extraction from faecal samples

For DNA extraction from the faecal samples Isohelix Xtreme DNA Kit (XME-50) was used. The surface of the faecal pellet was scrubbed with a swab and then rinsed in the Isohelix buffer. This process was repeated 2-3 times. The 25 µl of proteinase k solution was added to the solution and then it was placed on a thermoblock at 60˚C and mixed for approximately one hour. 750 µl of CB buffer was added and then the tubes were vortexed at full speed to mix well followed by brief centrifuging to remove liquid from the lid. 1 ml of the solution was transferred into a clean 2ml tube then 1ml of ethanol was added and mixed gently. 700 µl of the sample was then pipetted into Xtreme DNA column and centrifuged for one minute at full speed. This step was repeated with new collection tubes until all the lysate was through the spin column. Each spin column was then placed into a clean collection tube and 500 µl of WB wash buffer was added, then spun for one minute at full speed. This step was repeated three times. After that each spin column was then transferred into a clean collection tube and centrifuged for 3 mins at full speed to remove all

ethanol traces. Then it was transferred to a labelled 1.5ml tube and 100 μl EB buffer was added to the membrane column to elute the DNA from the membrane. The buffer was preheated at 70˚C in a water bath prior to use in order to dissolve the participate which forms when it is stored for long time. The column was left to settle for one minute then spun for one minute at full speed, then the collected DNA was quantified by using the Nanodrop and stored at -20°C.

The quality of DNA in each sample was checked by NanoDrop 1000 (Thermo Scientific). The extracted DNA (especially from faecal samples) is expected to contain not only the targeted DNA of the animal, but also other organism's DNA, as well as proteins, plant tissues and impurities. These inhibitors will affect the PCR process by reducing its efficiency and the overall yield. In order to minimize their interference with our target DNA a 1:10 sample dilution was performed.

Supplementary materials A3

The haplotype sequences of Sudanese and Yemeni samples

>Sudan_cytochrome *b* .ab1. HAP F

TATGTCTTACCATGAGGACAGATATCATTCTGAGGGGCAACAGTCATTACT
AACCTTCTCTCAGCAATCCCATATATTGGCACAAACCTAGTCGAATGAATCT
GAGGAGGATTCTCAGTAGACAAAGCCACTCTCACCCGATTTTTCGCCTTCC
ACTTTATCCTCCCATTCATCATTGCAGCCCTCGCCATAGTCCACCTGCTCTT
CCTCCACGAAACAGGATCCAACAACCCCACAGGAATTCCATCAGACACAG
ACAAAATCCCATTTCACCCTTACTACACCATTAAAGACATCTTAGGCATCAT
GCTACTAATTCTTGTCCTAATATTACTAGTACTATTCACACCCGACCTGCTC
GGAGACCCAGACAACTACATCCCAGCAAACCCGCTCAATACACCCCCTCA
CATCAAACCTGAATGATACTTCCTATTTGCATACGCAATCCTACGATCAATT
CCCAACAAACTAGGAGGAGTTCTAG

>Yemen_cytochrome *b* .ab1. HAP C

TATGTCTTACCATGAGGGCAGATATCATTCTGAGGAGCAACAGTCATTACT
AACCTTCTCTCAGCAATCCCATATATTGGCACAAACCTAGTCGAATGGATCT
GAGGAGGATTCTCAGTAGACAAAGCCACTCTCACCCGATTCTTCGCTTTCC
ACTTTATCCTCCCATTCATCATTGCAGCCCTCGCCATAGTCCACCTGCTCTT
CCTCCACGAAACAGGATCCAACAACCCCACAGGAATTCCATCAGACATAGA
CAAAATTCCATTTCACCCCTACTACACCATTAAAGATATCTTAGGCGCCATA
CTACTAATTCTTACCCTAATATTACTAGTACTATTCATACCCGACCTGCTCG
GGGACCCAGACAACTATACTCCAGCAAACCCACTCAATACACCCCCTCACA
TCAAACCCGAATGATATTTCCTATTTGCATACGCAATCCTACGATCAATTCC
CAACAAACTAGGAGGAGTCCTAG

>Yemen_cytochrome *b* .ab1. HAP H

TATGTCTTACCATGAGGGCAGATATCATTCTGAGGAGCAACAGTCATTACT
AACCTTCTCTCAGCAGTCCCATATATTGGCACAAACCTAGTCGAATGGATC
TGAGGAGGATTCTCAGTAGACAAAGCCACTCTCACCCGATTCTTCGCTTTC
CACTTTATCCTCCCATTCATCATTGCAGCCCTCGCCATAGTCCACCTGCTC
TTCCTCCACGAAACAGGATCCAACAACCCCACAGGAATTCCATCAGACATA
GACAAAATTCCATTTCACCCCTACTACACCATTAAAGATATCTTAGGCGCCA
TACTACTAATTCTTACCCTAATATTACTAGTACTATTCATACCCGACCTGCTC
GGGGACCCAGACAACTATACTCCAGCAAACCCACTCAATACACCCCCTCA
CATCAAACCCGAATGATATTTCCTATTTGCATACGCAATCCTACGATCAATT
CCCAACAAACTAGGAGGAGTCCTAG

Supplementary materials A4

Construction of ddRADseq libraries

ddRAD libraries were constructed following modifications of the protocol described by Peterson et al. (Peterson et al. 2012), and described in Brown et al. (Brown et al. 2016) and Manousaki et al. (Manousaki et al. 2016). The full protocol, with minor modifications used in this study, are detailed here. Twenty-one ng of each DNA sample was digested with 0.42 U SbfI-HF (restriction site; CCTGCA^GG), 0.42 U SphI-HF (restriction site; GCATG^C), and 1X CutSmart buffer (all New England Biolabs), at a final volume of 6 µl. Digestions were incubated for overnight at 16°C. P1 (*Sbf*I-compatible) and P2 (*Sph*I-compatible) adapters, each containing 5 or 7 bp combinatorial inline barcodes and Illumina-compatible primer sequences (Brown et al. 2016) were added to digested DNA samples, to a final volume of 9 µL containing of 2 nM and 30 nM adapters, respectively, at a final ratio of 1:15. The adapters were incubated with the digested DNA at room temperature for 15 mins, before adding a ligation mixture up to a final volume of 12 µl containing 1X CutSmart buffer, 1 mM rATP) and 42 ceU T4Ligase. Each mixture was incubated at 22°C for 3 hours, after which 30ul of Qiagen PB buffer was added to inhibit ligase activity.

The entire volume of each sample was pooled to form a single library, which was then concentrated purified using the MinElute PCR purification kit (Qiagen, following the manufacturer's protocol) and eluting in 90 µl EB. The pooled library was then size selected from a 1% agarose gel (90 V for 95 minutes) to retain fragments of approximately 400 – 700bp (see Brown et al. (Brown et al. 2016) for further details), and purified using the MinElute gel extraction kit (Qiagen, following the manufacturer's protocol).

Illumina adapters were then bound to the fragments using PCR. To minimise PCR errors caused by high numbers of PCR cycles whilst generating sufficient

product for sequencing, amplifications were carried out in 32 independent 12.5µl PCR reactions, prior to re-pooling the amplified products. Each PCR reactions contained 1.5 µl of the pooled library, 1X Q5® Hot Start High-Fidelity *Taq* master mix (containing 2 mM Mg^{++}, New England Biolabs), and 0.32 µM of each primer. PCR cycling conditions were: initial denaturation at 95˚C for 30 seconds, 15 cycles of 98˚C for 10 seconds, 65˚C for 30 seconds and 72˚C for 30 seconds, with a final extension at 72˚C for 5 minutes. The PCR products were re-pooled and purified using the MinElute PCR purification kit (Qiagen), eluting into 20 µl EB. A final size selection was carried out using 1X AMPure XP magnetic beads (Beckman Coulter).

The samples (see methods for details) were run in two independent libraries and two samples were repeated in each library to enable data quality checking. Final ddRAD libraries were each sequenced on a single lane on Illumina HiSeq 4000 at Novogene (Hong Kong).

References

Adavoudi R, Pilot M. 2021. Consequences of Hybridization in Mammals: A Systematic Review. Genes **13**:50. Available from https://www.mdpi.com/2073-4425/13/1/50.

Akçakaya HR, Sjögren-Gulve P. 2000. Population Viability Analyses in Conservation Planning: An Overview. Ecological Bulletins:9–21. Oikos Editorial Office. Available from http://www.jstor.org/stable/20113245.

Al-Atiyat RM, Aljumaah RS. 2014. Genetic relatedness between Ardi, Black Bedouin and Damascus goat breeds. Genetics and Molecular Research **13**:4654–4665. Available from http://www.funpecrp.com.br/gmr/year2014/vol13-2/pdf/gmr3623.pdf.

Al-Atiyat RM, Alobre MM, Aljumaah RS, Alshaikh MA. 2015. Microsatellite based genetic diversity and population structure of three Saudi goat breeds. Small Ruminant Research **130**:90–94. Available from https://linkinghub.elsevier.com/retrieve/pii/S0921448815300328.

Al-Ghafri MK et al. 2021. Genetic diversity of the Nubian ibex in Oman as revealed by mitochondrial DNA. Royal Society Open Science **8**:210125. Available from https://royalsocietypublishing.org/doi/10.1098/rsos.210125.

Al-Jumaily MM. 1998. Review of the mammals of the Republic of Yemen. Fauna of Arabia **17**:477–499.

Al Hikmani H, Zabanoot S, Al Shahari T, Zabanoot N, Al Hikmani K, Spalton A. 2015. Status of the Arabian Gazelle, Gazella arabica (Mammalia: Bovidae), in Dhofar, Oman. Zoology in the Middle East **61**:295–299.

Al Jahdhami M, Al-Mahdhoury S, Al Amri H. 2011. The re-introduction of Arabian oryx to the Al Wusta Wildlife Reserve in Oman: 30 years on. Global Re-introduction Perspectives:194–198.

Al Jahdhami MH. 2010. Physiological monitoring of welfare for conservation of Arabian oryx, Oryx leucoryx. University of Exeter, UK. Available from http://hdl.handle.net/10036/3024.

Al Jahdhami MH et al. 2017. The status of Arabian Gazelles Gazella arabica (Mammalia: Cetartiodactyla: Bovidae) in Al Wusta Wildlife Reserve and Ras Ash Shajar Nature Reserve, Oman. Journal of Threatened Taxa **9**:10369–10373. Available from https://www.threatenedtaxa.org/index.php/JoTT/article/view/3398/4095 (accessed December 18, 2020).

Alemayehu K. 2013. Population viability analysis of Walia ibex (Capra walie) at Simien Mountains National Park (SMNP), Ethiopia. African Journal of Ecology **51**:280–287.

Alkon P., Harding L, Jdeidi T, Masseti M, Nader I, de Smet K, Cuzin, F. & Saltz D. 2008. Capra nubiana. The IUCN Red List of Threatened Species. The IUCN Red List of Threatened Species. Available from http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T3796A10084254.EN.

Allendorf FW, Funk WC, Aitken SN, Byrne M, Luikart G. 2022. Conservation Breeding and Restoration. Pages 487–511 Conservation and the Genomics of Populations. Oxford University Press. Available from https://academic.oup.com/book/41948/chapter/355152906.

Allendorf WF, Luikart GH, Aitken NS. 2012. Conservation and the Genetics of Populations, 2nd edition. Wiley-Blackwell.

Amer SAM, Kumazawa Y. 2005. Mitochondrial DNA sequences of the Afro Arabian spiny tailed lizards (genus Uromastyx, family Agamidae) phylogenetic analyses and evolution of gene arrangements. Biological Journal of the Linnean Society **85**:247–260. Available from https://academic.oup.com/biolinnean/article-lookup/doi/10.1111/j.1095-8312.2005.00485.x.

AnAge. 2017. “Capra nubiana” (On-line),Animal Ageing and longevity Database. Available from https://genomics.senescence.info/species/entry.php?species=Capra_nubiana (accessed November 26, 2020).

Andrews KR, Good JM, Miller MR, Luikart G, Hohenlohe PA. 2016. Harnessing the power of RADseq for ecological and evolutionary genomics.

Andrews KR, Luikart G. 2014. Recent novel approaches for population genomics data analysis.

Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. Babraham Institute, Cambridge, United Kingdom. Available from https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Annecke W, Masubelele M. 2016. A Review of the impact of militarisation: The case of rhino poaching in kruger national park, South Africa. Conservation and Society **14**:195. Available from http://www.conservationandsociety.org/text.asp?2016/14/3/195/191158.

Archaux F, Wolters V. 2006. Impact of summer drought on forest biodiversity: what do we know? Annals of Forest Science **63**:645–652. Available from http://www.edpsciences.org/10.1051/forest:2006041.

Arnold, E N. 1980. The Reptiles and Amphibians of Dhofar, Southern Arabia. The Journal of Oman Studies **2**:173–332. Available from https://gallotia.de/AF/Bibliografie/BIB_4508.pdf.

Attum O. 2007. Can landscape use be among the factors that potentially make some ungulates species more difficult to conserve? Journal of Arid

Environments **69**:410–419. Academic Press.

Attum O, Al Awaji M, Bender LC. 2022. The use of demographic data to monitor population trends of the Nubian Ibex, Capra nubiana in Jordan (Mammalia: Bovidae). Zoology in the Middle East **68**:1–11. Available from https://www.tandfonline.com/doi/full/10.1080/09397140.2021.2021654.

Attum O, Rosenbarger D, Al awaji M, Kramer A, Eid E. 2017. Population size and artificial waterhole use by striped hyenas in the Dana Biosphere Reserve, Jordan. Mammalia **81**. Available from https://www.degruyter.com/document/doi/10.1515/mammalia-2015-0155/html.

Avner U, Horwitz LK, Horowitz W. 2017. Symbolism of the ibex motif in Negev rock art. Journal of Arid Environments **143**:35–43. Elsevier Ltd. Available from http://dx.doi.org/10.1016/j.jaridenv.2016.11.009.

Baharav D, Meiboom U. 1981. The status of the Nubian ibex Capra ibex nubiana in the Sinai desert. Biological Conservation **20**:91–97. Available from http://www.sciencedirect.com/science/article/pii/0006320781900203.

Baharav D, Meiboom U. 1982. Winter thermoregulatory behaviour of the Nubian ibex Capra ibex nubiana in the Sinai Desert. Journal of Arid Environments **5**:295–298.

Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, Lewis ZA, Selker EU, Cresko WA, Johnson EA. 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS ONE **3**. Available from https://search-proquest-com.ezproxy.napier.ac.uk/docview/1312316079/fulltextPDF/3A6982FB49F44084PQ/1?accountid=16607 (accessed April 9, 2018).

Ball MC, Pither R, Manseau M, Clark J, Petersen SD, Kingston S, Morrill N, Wilson P. 2007. Characterization of target nuclear DNA from faeces reduces technical issues associated with the assumptions of low-quality and quantity template. Conservation Genetics **8**:577–586. Available from https://link.springer.com/10.1007/s10592-006-9193-y.

Bandelt HJ, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution **16**:37–48. Available from https://doi.org/10.1093/oxfordjournals.molbev.a026036.

Bar-Gal GK, Greenblatt C. 1999. The relationships between Capra species from the southern Levant to other Capra species based on mitochondrial cytochrome b sequence. Unpublished.

Bar-Gal GK, Smith P, Tchernov E, Greenblatt C, Ducos P, Gardeisen A, Horwitz LK. 2002. Genetic evidence for the origin of the agrimi goat (Capra aegagrus cretica). Journal of Zoology **256**:369–377. Cambridge University Press. Available from http://doi.wiley.com/10.1017/S0952836902000407

(accessed January 18, 2019).

Barichievy C, Sheldon R, Wacher T, Llewellyn O, Al-Mutairy M, Alagaili A. 2016. Conservation in Saudi Arabia; moving from strategy to practice. Saudi Journal of Biological Sciences:9–11. King Saud University. Available from https://doi.org/10.1016/j.sjbs.2017.03.009.

Barnosky AD et al. 2011. Has the Earth's sixth mass extinction already arrived? Nature **471**:51–57. Available from http://www.nature.com/articles/nature09678.

Barrientos R, Ascensão F, D'Amico M, Grilo C, Pereira HM. 2021. The lost road: Do transportation networks imperil wildlife population persistence? Perspectives in Ecology and Conservation **19**:411–416. Available from https://linkinghub.elsevier.com/retrieve/pii/S2530064421000699.

Beck BB, Rapaport LG, Price MRS, Wilson AC. 1994. Reintroduction of captive-born animals. Pages 265–286 Creative Conservation. Springer Netherlands, Dordrecht. Available from http://link.springer.com/10.1007/978-94-011-0721-1_13.

Besnier F, Glover KA. 2013. ParallelStructure: A R Package to Distribute Parallel Runs of the Population Genetics Program STRUCTURE on Multi-Core Computers. PLoS ONE **8**:e70651. Available from https://dx.plos.org/10.1371/journal.pone.0070651.

Bibi F, Vrba E, Fack F. 2012. A new african fossil caprin and a combined molecular and morphological bayesian phylogenetic analysis of caprini (Mammalia: Bovidae). Journal of Evolutionary Biology **25**:1843–1854. Available from http://doi.wiley.com/10.1111/j.1420-9101.2012.02572.x.

Biebach I, Keller LF. 2010. Inbreeding in reintroduced populations: The effects of early reintroduction history and contemporary processes. Conservation Genetics **11**:527–538.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics **30**:2114–2120. Available from https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/btu170.

Bosworth W, Huchon P, McClay K. 2005. The Red Sea and Gulf of Aden Basins. Journal of African Earth Sciences **43**:334–378. Available from https://linkinghub.elsevier.com/retrieve/pii/S1464343X0500124X.

Bouckaert R et al. 2019. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. PLOS Computational Biology **15**:e1006650. Available from https://dx.plos.org/10.1371/journal.pcbi.1006650.

Bourgeois S, Kaden J, Senn H, Bunnefeld N, Jeffery KJ, Akomo-Okoue EF,

Ogden R, McEwing R. 2019. Improving cost-efficiency of faecal genotyping: New tools for elephant species. PLOS ONE **14**:e0210811. Available from https://dx.plos.org/10.1371/journal.pone.0210811.

Bourgeois S, Senn H, Kaden J, Taggart JB, Ogden R, Jeffery KJ, Bunnefeld N, Abernethy K, McEwing R. 2018. Single-nucleotide polymorphism discovery and panel characterization in the African forest elephant. Ecology and Evolution **29**:150–152. Available from http://www.jstor.org/stable/2387021%0Ahttp://about.jstor.org/terms.

Boyce MS. 1992. Population viability analysis. Annual Review of Ecology and Systematics **23**:481–506.

Bray TC, Mohammed OB, Alagaili AN. 2013. Phylogenetic and Demographic Insights into Kuhl's Pipistrelle, Pipistrellus kuhlii, in the Middle East. PLoS ONE **8**:e57306. Available from https://dx.plos.org/10.1371/journal.pone.0057306.

Britannica. 2023. The Editors of Encyclopaedia. "Queen of Sheba." Available from https://www.britannica.com/biography/Queen-of-Sheba (accessed February 13, 2023).

Brondízio ES, Settele J, Díaz S, Ngo HT (eds). 2019. IPBES (2019), Global assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. Page Debating Nature's Value. Available from https://ipbes.net/global-assessment%0Ahttps://ipbes.net/global-assessment-report-biodiversity-ecosystem-services.

Bronson FH. 2009. Climate change and seasonal reproduction in mammals. Philosophical Transactions of the Royal Society B: Biological Sciences **364**:3331–3340. Available from https://royalsocietypublishing.org/doi/10.1098/rstb.2009.0140.

Brook B, Sodhi N, Bradshaw C. 2008. Synergies among extinction drivers under global change. Trends in Ecology & Evolution **23**:453–460. Available from https://linkinghub.elsevier.com/retrieve/pii/S016953470800195X.

Brook BW, Burgman MA, Akçakaya HR, O'Grady JJ, Frankham R. 2002. Critiques of PVA Ask the Wrong Questions: Throwing the Heuristic Baby Out with the Numerical Bath Water. Conservation Biology **16**:262–263. Available from https://onlinelibrary.wiley.com/doi/10.1046/j.1523-1739.2002.01426.x.

Brook BW, O'Grady JJ, Chapman AP, Burgman MA, Akçakaya HR, Frankham R. 2000. Predictive accuracy of population viability analysis in conservation biology. Nature **404**:385–387. Available from http://www.nature.com/articles/35006050.

Brown JK, Taggart JB, Bekaert M, Wehner S, Palaiokostas C, Setiawan AN,

Symonds JE, Penman DJ. 2016. Mapping the sex determination locus in the hāpuku (Polyprion oxygeneios) using ddRAD sequencing. BMC Genomics **17**:448. Available from http://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-016-2773-4.

Buerkle, CA. 2005. Maximum-likelihood estimation of a hybrid index based on molecular markers. Molecular Ecology Notes **5**:684–687. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.1471-8286.2005.01011.x.

Burrows M, Wheeler D. 1994. A Block-sorting Lossless Data Compression Algorithm. System Research Center.

Campbell P. 1997. A note on growing season food habits of mountain gazelles and Nubian ibex in Saudi Arabia. Journal of Arid Environments **36**:705–709.

Carroll EL, Bruford MW, DeWoody JA, Leroy G, Strand A, Waits L, Wang J. 2018. Genetic and genomic monitoring with minimally invasive sampling methods. Evolutionary Applications **11**:1094–1119. Available from https://onlinelibrary.wiley.com/doi/10.1111/eva.12600.

Castelló J., Huffman B, Groves C. 2016. Bovids of the World Antelopes, Gazelles, Cattle, Goats, Sheep, and Relatives. Princeton University Press, Princeton, NJ, USA.

CBD. 2014. 5th National Report to the Convention on Biological Diversity (CBD): Ministry of Environment and Climate Affairs. Available from https://www.cbd.int/reports/nr5/.

Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. GigaScience **4**:7. Available from https://academic.oup.com/gigascience/article-lookup/doi/10.1186/s13742-015-0047-8.

Changhuan HE, Jiaojiao DU, Zhu D, Zhang L. 2020. Population viability analysis of small population: a case study for Asian elephant in China. Integrative Zoology **15**:350–362.

Chanyandura A, Muposhi VK, Gandiwa E, Muboko N. 2021. An analysis of threats, strategies, and opportunities for African rhinoceros conservation. Ecology and Evolution **11**:5892–5910. Available from https://onlinelibrary.wiley.com/doi/10.1002/ece3.7536.

Chattopadhyay B, Garg KM, Soo YJ, Low GW, Frechette JL, Rheindt FE. 2019. Conservation genomics in the fight to help the recovery of the critically endangered Siamese crocodile Crocodylus siamensis. Molecular Ecology **28**:936–950. Available from https://onlinelibrary.wiley.com/doi/10.1111/mec.15023.

Chaudhary V, Oli MK. 2020. A critical appraisal of population viability analysis. Conservation Biology **34**:26–40.

Chebii VJ, Oyola SO, Kotze A, Domelevo Entfellner J-B, Musembi Mutuku J, Agaba M. 2020. Genome-Wide Analysis of Nubian Ibex Reveals Candidate Positively Selected Genes That Contribute to Its Adaptation to the Desert Environment. Animals **10**:2181. Available from https://www.mdpi.com/2076-2615/10/11/2181.

Chen G, Zheng C, Wan N, Liu D, Fu VWK, Yang X, Yu Y, Liu Y. 2019. Low genetic diversity in captive populations of the critically endangered Blue-crowned Laughingthrush (Garrulax courtoisi) revealed by a panel of novel microsatellites. PeerJ **7**:e6643. Available from https://peerj.com/articles/6643.

Christie MR, Knowles LL. 2015. Habitat corridors facilitate genetic resilience irrespective of species dispersal abilities or population sizes. Evolutionary Applications **8**:454–463. Available from https://onlinelibrary.wiley.com/doi/10.1111/eva.12255.

Christie MR, Marine ML, French RA, Blouin MS. 2012. Genetic adaptation to captivity can occur in a single generation. Proceedings of the National Academy of Sciences **109**:238–242. Available from https://pnas.org/doi/full/10.1073/pnas.1111073109.

Clark D. 2015. Assessment of Population Viability of Wolves in Oregon.

Colli L et al. 2015. Whole mitochondrial genomes unveil the impact of domestication on goat matrilineal variability. BMC Genomics **16**:1115. Available from https://doi.org/10.1186/s12864-015-2342-2.

Coltman DW, O'Donoghue P, Jorgenson JT, Hogg JT, Strobeck C, Festa-Bianchet M. 2003. Undesirable evolutionary consequences of trophy hunting. Nature **426**:655–658. Available from http://www.nature.com/articles/nature02177.

Comizzoli P. 2015. Biobanking efforts and new advances in male fertility preservation for rare and endangered species. Asian Journal of Andrology **17**:640. Available from http://www.ajandrology.com/text.asp?2015/17/4/640/153849.

Comizzoli P, Wildt DE. 2014. Mammalian fertility preservation through cryobiology: value of classical comparative studies and the need for new preservation options. Reproduction, Fertility and Development **26**:91. Available from http://www.publish.csiro.au/?paper=RD13259.

Conde DA, Flesness N, Colchero F, Jones OR, Scheuerlein A. 2011. An Emerging Role of Zoos to Conserve Biodiversity. Science **331**:1390–1391. Available from https://www.science.org/doi/10.1126/science.1200674.

Conway WG. 2010. Buying Time for wild animals With Zoos. Zoo Biology:n/a-n/a. Available from http://doi.wiley.com/10.1002/zoo.20352.

Corp N, Spalton A, Gorman ML. 1998. The influence of rainfall on range in a female desert ungulate: the Arabian oryx (Oryx leucoryx) in the Sultanate of Oman. Journal of Zoology **246**:369–377. Wiley. Available from http://doi.wiley.com/10.1111/j.1469-7998.1998.tb00169.x (accessed December 25, 2020).

Coulson T, Hudson E, Possingham H. 2001. The Use and Abuse of Population Viability Analysis. Trends in ecology & evolution **16**:219–221.

Couturier M. 1962. Le bouquetin des Alpes (Capra aegagrus ibex ibex L.) (Privately printed. Grenoble).

Cutter AD. 2019. A Primer of Molecular Population Genetics. Page A Primer of Molecular Population Genetics. Oxford University Press. Available from https://academic.oup.com/book/35101.

Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML. 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nature Reviews Genetics **12**:499–510. Nature Publishing Group. Available from http://dx.doi.org/10.1038/nrg3012.

Davies RG et al. 2006. Human impacts and the global distribution of extinction risk. Proceedings of the Royal Society B: Biological Sciences **273**:2127–2133. Available from https://royalsocietypublishing.org/doi/10.1098/rspb.2006.3551.

de Flamingh A, Sole CL, van Aarde RJ. 2014. Microsatellite repeat motif and amplicon length affect amplification success of degraded faecal DNA. Conservation Genetics Resources **6**:503–505. Available from http://link.springer.com/10.1007/s12686-014-0160-5.

de Meeûs T, Goudet J. 2007. A step-by-step tutorial to use HierFstat to analyse populations hierarchically structured at multiple levels. Infection, Genetics and Evolution **7**:731–735.

De Vos JM, Joppa LN, Gittleman JL, Stephens PR, Pimm SL. 2015. Estimating the normal background rate of species extinction. Conservation Biology **29**:452–462. Available from https://onlinelibrary.wiley.com/doi/10.1111/cobi.12380.

Derouiche L, Vercammen P, Bouhadad R, Fernandes C. 2017. Genetic evidence supporting the taxonomic separation of the Arabian and Northwest African subspecies of the desert hedgehog (Paraechinus aethiopicus). Gene **620**:54–65. Available from https://linkinghub.elsevier.com/retrieve/pii/S0378111917302524.

Desbiez ALJ, Bertassoni A, Traylor-Holzer K. 2020. Population viability analysis

as a tool for giant anteater conservation. Perspectives in Ecology and Conservation **18**:124–131. Associação Brasileira de Ciência Ecológica e Conservação. Available from https://doi.org/10.1016/j.pecon.2020.04.004.

DiBattista JD. 2008. Patterns of genetic variation in anthropogenically impacted populations. Conservation Genetics **9**:141–156. Available from http://link.springer.com/10.1007/s10592-007-9317-z.

Dicks KL et al. 2023. Genetic diversity in global populations of the critically endangered addax (Addax nasomaculatus) and its implications for conservation. Evolutionary Applications **16**:111–125. Available from https://onlinelibrary.wiley.com/doi/10.1111/eva.13515.

Didero M, Farooq A, Nebel S, Pfaffenbach C. 2019. Urban Oman: From Modern to Postmodern Mobility in Muscat? Middle East : Topics & Arguments **12**:87–98.

Dierickx EG, Shultz AJ, Sato F, Hiraoka T, Edwards S V. 2015. Morphological and genomic comparisons of Hawaiian and Japanese Black-footed Albatrosses (Phoebastria nigripes) using double digest RADseq: Implications for conservation. Evolutionary Applications **8**:662–678.

Dirzo R, Young HS, Galetti M, Ceballos G, Isaac NJB, Collen B. 2014. Defaunation in the Anthropocene. Science **345**:401–406. Available from https://www.science.org/doi/10.1126/science.1251817.

Dixon A, Jone D. 1988. Conservation and Biology of Desert Antelopes. Christopher Hem, London (GB).

Dyke F Van. 2003. Conservation biology : foundations, concepts, applications. McGraw-Hill, Boston, Mass.

Earl DA, vonHoldt BM. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources **4**:359–361.

Ejigu D. 2020. Capra walie (errata version published in 2020). The IUCN Red List of Threatened Species. Available from https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T3797A178652661.en%0ACopyright:

Ekblom R, Galindo J. 2011. Applications of next generation sequencing in molecular ecology of non-model organisms. Heredity **107**:1–15. Nature Publishing Group. Available from http://dx.doi.org/10.1038/hdy.2010.152.

Elderd BD, Shahani P, Doak DF. 2003. The Problems and Potential of Count-Based Population Viability Analyses. Pages 173–202. Available from http://link.springer.com/10.1007/978-3-662-09389-4_7.

Ellner SP, Fieberg J, Ludwig D, Wilcox C. 2002. Precision of Population Viability

Analysis. Conservation Biology **16**:258–261. Available from https://onlinelibrary.wiley.com/doi/10.1046/j.1523-1739.2002.00553.x.

Emami-Khoyi A et al. 2021. A New Non-invasive Method for Collecting DNA From Small Mammals in the Field, and Its Application in Simultaneous Vector and Disease Monitoring in Brushtail Possums. Frontiers in Environmental Science **9**. Available from https://www.frontiersin.org/articles/10.3389/fenvs.2021.701033/full.

Eriksson CE, Ruprecht J, Levi T. 2020. More affordable and effective noninvasive single nucleotide polymorphism genotyping using high-throughput amplicon sequencing. Molecular Ecology Resources **20**:1505–1516. Available from https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.13208.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Molecular Ecology **14**:2611–2620. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2005.02553.x.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources **10**:564–567. Available from http://doi.wiley.com/10.1111/j.1755-0998.2010.02847.x.

Fahrig L et al. 2019. Is habitat fragmentation bad for biodiversity? Biological Conservation **230**:179–186. Available from https://linkinghub.elsevier.com/retrieve/pii/S0006320718313375.

Fernandes CA. 2011. Colonization time of Arabia by the White-tailed Mongoose Ichneumia albicauda as inferred from mitochondrial DNA sequences. Zoology in the Middle East **54**:111–124. Available from http://www.tandfonline.com/doi/full/10.1080/09397140.2011.10648903.

Ferreira CM et al. 2018. Genetic non-invasive sampling (gNIS) as a cost-effective tool for monitoring elusive small mammals. European Journal of Wildlife Research **64**:46. Available from http://link.springer.com/10.1007/s10344-018-1188-8.

Ferson S. 2002. Quantitative Methods for Conservation Biology. Springer.

Förster DW et al. 2018. Targeted resequencing of coding DNA sequences for SNP discovery in nonmodel species. Molecular Ecology Resources **18**:1356–1373. Available from https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.12924.

Francis RM. 2017. pophelper : an R package and web app to analyse and visualize population structure. Molecular Ecology Resources **17**:27–32. Available from https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.12509.

Frankham R. 1995a. Inbreeding and Extinction: A Threshold Effect. Conservation Biology **9**:792–799. Available from http://doi.wiley.com/10.1046/j.1523-1739.1995.09040792.x.

Frankham R. 1995b. Effective population size/adult population size ratios in wildlife: a review. Genetical Research **66**:95–107. Available from https://www.cambridge.org/core/product/identifier/S0016672300034455/type/journal_article.

Frankham R. 1997. Do island populations have less genetic variation than mainland populations? Heredity **78**:311–327. Available from https://www.nature.com/articles/hdy199746.

Frankham R. 2008. Genetic adaptation to captivity in species conservation programs. Molecular Ecology **17**:325–333. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2007.03399.x.

Frankham R, Ballou JD, Briscoe DA. 2010. Introduction to Conservation Geneticssecond. Cambridge University Press.

Frankham R, Ballou JD, Briscoe DA, McInnes KH. 2004. A Primer of Conservation Genetics. Cambridge University Press. Available from http://ebooks.cambridge.org/ref/id/CBO9780511817359 (accessed January 27, 2019).

Frankham R, Ballou JD, Eldride MDB, Lacy RC, Ralls K, Dudash MR, Fenster CB. 2011. Predicting the Probability of Outbreeding Depression. Conservation Biology **25**:465–475. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.1523-1739.2011.01662.x.

Frankham R, Ballou JD, Ralls K, Eldridge M, Dubash MR, Fenster CB, Lacy RC, Sunnucks P. 2017. Genetic Management of Fragmented Animal and Plant Populations. Available from https://www.oup.com.au/books/higher-education/science/9780198783404-genetic-management-of-fragmented-animal-and-plant-populations (accessed January 22, 2018).

Frankham R, Ballou JD, Ralls K, Eldridge M, Dudash MR, Fenster CB, Lacy RC, Sunnucks P. 2019a. A Practical Guide for Genetic Management of Fragmented Animal and Plant Populations. A Practical Guide for Genetic Management of Fragmented Animal and Plant Populations.

Frankham R, Ballou JD, Ralls K, Eldridge M, Dudash MR, Fenster CB, Lacy RC, Sunnucks P. 2019b. A Practical Guide for Genetic Management of Fragmented Animal and Plant Populations. A Practical Guide for Genetic Management of Fragmented Animal and Plant Populations **2011**.

Frankham R, Bradshaw CJA, Brook BW. 2014. Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biological Conservation **170**:56–63. Available from

https://linkinghub.elsevier.com/retrieve/pii/S0006320713004576.

Franklin IR. 1980. Evolutionary change in small populations. Pages 135–149 Conservation Biology - An evolutionary-ecological perspective. Sinauer Associates, U.S.A, Sunderland, Massachusetts.

Frantzen MAJ, Silk JB, Ferguson JWH, Wayne RK, Kohn MH. 1998. Empirical evaluation of preservation methods for faecal DNA. Molecular Ecology **7**:1423–1428. Available from https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1365-294x.1998.00449.x.

Funk DJ, Omland KE. 2003. Species-Level Paraphyly and Polyphyly: Frequency, Causes, and Consequences, with Insights from Animal Mitochondrial DNA. Annual Review of Ecology, Evolution, and Systematics **34**:397–423. Available from http://www.annualreviews.org/doi/10.1146/annurev.ecolsys.34.011802.132421.

Galletti CS, Turner BL, Myint SW. 2016. Land changes and their drivers in the cloud forest and coastal zone of Dhofar, Oman, between 1988 and 2013. Regional Environmental Change **16**:2141–2153. Available from http://link.springer.com/10.1007/s10113-016-0942-2.

Garvin MR, Sattoh K, Gharrett AJ. 2010. Application of single nucleotide polymorphisms to non-model species: a technical review. Molecular Ecology Resources **10**:915–934. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.1755-0998.2010.02891.x.

Geoffroy L, Huchon P, Khanbari K. 1998. Did Yemeni Tertiary granites intrude neck zones of a stretched continental upper crust? Terra Nova **10**:196–200. Available from https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1365-3121.1998.00194.x.

Ghazanfar SA. 2004. Biology of the central desert of Oman. Turkish Journal of Botany **28**:65–71.

Giacometti M, Willing R, Defila C. 2002. Ambient Temperature in Spring Affects Horn Growth in Male Alpine Ibexes. Journal of Mammalogy **83**:245–251. Available from https://doi.org/10.1644/1545-1542(2002)083%3C0245:ATISAH%3E2.0.CO.

Giangaspero M, Al-Ghafri MK. 2014. Poaching: A Hreat for Vulnerable Wild Animal Species in Oman. Tropical Medicine & Surgery **2**. Available from https://www.walshmedicalmedia.com/open-access/poaching-a-threat-for-vulnerable-wild-animal-species-in-oman-2329-9088.1000e121.pdf.

Giangaspero M, Khalaf M, Al S. 2014. Poaching: A Threat for Vulnerable Wild Animal Species in Oman. Tropical Medicine & Surgery **2**:2–4.

Gnirke A et al. 2009. Solution hybrid selection with ultra-long oligonucleotides

for massively parallel targeted sequencing. Nature Biotechnology **27**:182–189. Available from http://www.nature.com/articles/nbt.1523.

Godinho R, Aba T, Lopes S, Essalhi A, Ouragh L, Cano M, Ferrand N. 2012. Conservation genetics of the endangered Dorcas gazelle (Gazella dorcas spp .) in Northwestern Africa:1003–1015.

Goossens B, Chikhi L, Utami SS, de Ruiter J, Bruford MW. 2000. A multi-samples, multi-extracts approach for microsatellite analysis of faecal samples in an arboreal ape. Conservation Genetics **1**:157–162. Available from https://doi.org/10.1023/A:1026535006318.

Goudet J. 2005. hierfstat, a package for r to compute and test hierarchical F-statistics. Molecular Ecology Notes **5**:184–186. Available from http://doi.wiley.com/10.1111/j.1471-8286.2004.00828.x.

Granjon L, Vassart M, Greth A. 1990. Genetic variability in nubian ibex. Mammalia **54**:665–667.

Grey-Ross R, Downs CT, Kirkman K. 2009. Reintroduction failure of captive-bred oribi (*Ourebia ourebi*) : research article. South African Journal of Wildlife Research - 24-month delayed open access **39**:34–38. Available from https://journals.co.za/doi/abs/10.10520/EJC117309.

Grobler M. 2002. A field guide to the larger mammals of Oman. Ministry of Regional Municipalities, Environment & Water Resources, Muscat. Available from www.mrmewr.gov.om.

Gross JE, Alkon PU, Demment MW. 1995. Grouping patterns and spatial segregation by Nubian ibex. Journal of Arid Environments **30**:423–439.

Gross M. 2019. Hunting wildlife to extinction. Current Biology **29**:R551–R554. Elsevier. Available from http://dx.doi.org/10.1016/j.cub.2019.05.063.

Grossen C, Biebach I, Angelone-Alasaad S, Keller LF, Croll D. 2018. Population genomics analyses of European ibex species show lower diversity and higher inbreeding in reintroduced populations. Evolutionary Applications **11**:123–139. Available from http://doi.wiley.com/10.1111/eva.12490.

Grossen C, Guillaume F, Keller LF, Croll D. 2020. Purging of highly deleterious mutations through severe bottlenecks in Alpine ibex. Nature Communications **11**:1001. Available from http://www.nature.com/articles/s41467-020-14803-1.

Grossen C, Keller L, Biebach I, Croll D. 2014. Introgression from Domestic Goat Generated Variation at the Major Histocompatibility Complex of Alpine Ibex. PLoS Genetics **10**:e1004438. Available from https://dx.plos.org/10.1371/journal.pgen.1004438.

Groves C, Grubb P. 2011. Ungulate Taxonomy. Johns Hopkins University

Press, Baltimore, Maryland.

Guiraud R, Bosworth W, Thierry J, Delplanque A. 2005. Phanerozoic geological evolution of Northern and Central Africa: An overview. Journal of African Earth Sciences **43**:83–143. Available from https://linkinghub.elsevier.com/retrieve/pii/S1464343X05001147.

Habibi K. 1994. The Desert Ibex; Life history, ecology and behaviour of the Nubian ibex in Saudi Arabiafirst. NCWCD Saudi Arabia.

Habibi K. 1997. Group dynamics of the Nubian ibex (Capra ibex nubiana) in the Tuwayiq Canyons, Saudi Arabia. Journal of Zoology **241**:791–801.

Habibi K, Grainger J. 1990. Distribution and status of Nubian ibex in Saudi Arabia. Oryx **24**:138–142. Available from https://www.cambridge.org/core/product/identifier/S0030605300033858/type/journal_article.

Hadas L, Hermon D, Boldo A, Arieli G, Gafny R, King R, Bar-Gal GK. 2015. Wild gazelles of the southern levant: Genetic profiling defines new conservation priorities. PLoS ONE **10**.

Haile J et al. 2009. Ancient DNA reveals late survival of mammoth and horse in interior Alaska. Proceedings of the National Academy of Sciences **106**:22352–22357. Available from https://pnas.org/doi/full/10.1073/pnas.0912510106.

Hajkova P, Zemanova B, Bryja J, Hajek B, Roche K, Tkadlec E, Zima J. 2006. Factors affecting success of PCR amplification of microsatellite loci from otter faeces. Molecular Ecology Notes **6**:559–562. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.1471-8286.2006.01269.x.

Hammer SE, Schwammer HM, Suchentrunk F. 2008. Evidence for Introgressive Hybridization of Captive Markhor (Capra falconeri) with Domestic Goat: Cautions for Reintroduction. Biochemical Genetics **46**:216–226. Available from http://link.springer.com/10.1007/s10528-008-9145-y.

Harrison DL. 1991. The Mammals of Arabia. Page (Bates PJ, editor), 2nd edition. Harrison Zoological Museum.

Harrison RG, Larson EL. 2014. Hybridization, Introgression, and the Nature of Species Boundaries. Journal of Heredity **105**:795–809. Available from https://academic.oup.com/jhered/jhered/article/2961884/Hybridization,.

Hartl GB, Burger H, Willing R, Suchentrunk F. 1990. On the biochemical systematics of the Caprini and the Rupricaprini. Biochemical Systematics and Ecology **18**:175–182.

Hartvig I et al. 2020. Conservation genetics of the critically endangered Siamese rosewood (Dalbergia cochinchinensis): recommendations for management

and sustainable use. Conservation Genetics **21**:677–692. Available from https://link.springer.com/10.1007/s10592-020-01279-1.

Harvey MG, Smith BT, Glenn TC, Faircloth BC, Brumfield RT. 2016. Sequence Capture versus Restriction Site Associated DNA Sequencing for Shallow Systematics.

Hassanin A et al. 2012. Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes. Comptes Rendus Biologies **335**:32–50. Available from http://www.sciencedirect.com/science/article/pii/S1631069111002800.

Hassanin A, Lecointre G, Tillier S. 1998a. The ‘evolutionary signal’ of homoplasy in proteincoding gene sequences and its consequences for a priori weighting in phylogeny. Comptes Rendus de l’Académie des Sciences - Series III - Sciences de la Vie **321**:611–620. Available from http://www.sciencedirect.com/science/article/pii/S0764446998804642.

Hassanin A, Pasquet E, Vigne J-D. 1998b. Molecular Systematics of the Subfamily Caprinae (Artiodactyla, Bovidae) as Determined from Cytochrome b Sequences. J. Mamm. Evol. **5**:217–236.

Hassanin A, Ropiquet A, Couloux A, Cruaud C. 2009. Evolution of the Mitochondrial Genome in Mammals Living at High Altitude: New Insights from a Study of the Tribe Caprini (Bovidae, Antilopinae). Journal of Molecular Evolution **68**:293–310. Available from http://link.springer.com/10.1007/s00239-009-9208-7.

Hazkani-Covo E, Zeller RM, Martin W. 2010. Molecular Poltergeists: Mitochondrial DNA Copies (numts) in Sequenced Nuclear Genomes. PLoS Genetics **6**:e1000834. Available from https://dx.plos.org/10.1371/journal.pgen.1000834.

Hedrick PW. 1995. Gene Flow and Genetic Restoration: The Florida Panther as a Case Study. Conservation Biology **9**:996–1007. Available from https://onlinelibrary.wiley.com/doi/10.1046/j.1523-1739.1995.9050988.x-i1.

Hedtke SM, Morgan MJ, Cannatella DC, Hillis DM. 2013. Targeted Enrichment: Maximizing Orthologous Gene Comparisons across Deep Evolutionary Time. PLoS ONE **8**:e67908. Available from https://dx.plos.org/10.1371/journal.pone.0067908.

Helyar SJ et al. 2011. Application of SNPs for population genetics of nonmodel organisms: new opportunities and challenges. Molecular Ecology Resources **11**:123–136. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.1755-0998.2010.02943.x.

Heptner VG, Nasimovich AA, Bannikov AG. 1961. Mlekopitayushchiye Sovetskogo Soyuza. Pamokopytnyye i Neparnokopytnyye [Mammals of the Soviet Union. Artiodactyls and perissodactyls]. Vyshaya Shkola Publishers

1:1–771.

Herrero A, Luco F De. 2020. Capra pyrenaica,. The IUCN Red List of Threatened Species. Available from https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T3798A170192604.en.

Hirashiki C, Kareiva P, Marvier M. 2021. Concern over hybridization risks should not preclude conservation interventions. Conservation Science and Practice **3**. Available from https://onlinelibrary.wiley.com/doi/10.1111/csp2.424.

Hohenlohe PA, Funk WC, Rajora OP. 2021. Population genomics for wildlife conservation and management. Molecular Ecology **30**:62–82. Available from https://onlinelibrary.wiley.com/doi/10.1111/mec.15720.

Hosegood J et al. 2020. Phylogenomics and species delimitation for effective conservation of manta and devil rays. Molecular Ecology **29**:4783–4796. Available from https://onlinelibrary.wiley.com/doi/10.1111/mec.15683.

Howard-McCombe J et al. 2020. A Mitochondrial Phylogeny of the Sand Cat (Felis margarita Loche, 1858). Journal of Mammalian Evolution **27**:525–534. Available from http://link.springer.com/10.1007/s10914-019-09473-w.

Howard JG, Wildt DE. 2009. Approaches and efficacy of artificial insemination in felids and mustelids. Theriogenology **71**:130–148. Available from https://linkinghub.elsevier.com/retrieve/pii/S0093691X08006766.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees . Bioinformatics **17**:754–755. Available from https://doi.org/10.1093/bioinformatics/17.8.754.

Hughes AR, Inouye BD, Johnson MTJ, Underwood N, Vellend M. 2008. Ecological consequences of genetic diversity. Ecology Letters **11**:609–623. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.1461-0248.2008.01179.x.

Hurst GD., Jiggins FM. 2005. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proceedings of the Royal Society B: Biological Sciences **272**:1525–1534. Available from https://royalsocietypublishing.org/doi/10.1098/rspb.2005.3056.

Iacolina L, Corlatti L, Buzan E, Safner T, Šprem N. 2019. Hybridisation in European ungulates: an overview of the current status, causes, and consequences. Mammal Review **49**:45–59. Available from https://onlinelibrary.wiley.com/doi/10.1111/mam.12140.

Ibrahim KM, Williams PC, Olson A, Torounsky R, Naser E, Ghebremariam FH, Masri MA. 2020. Genetic variation in morphologically divergent mainland and island populations of Soemmerring ’ s gazelles (Nanger soemmerringii):403–412. Mammal Research.

Ilves KL, López-Fernández H. 2014. A targeted next-generation sequencing toolkit for exon-based cichlid phylogenomics. Molecular Ecology Resources **14**:802–811. Available from https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.12222.

Iso-Touru T, Tabell J, Virta A, Kauhala K. 2021. A non-invasive, DNA-based method for beaver species identification in Finland. Wildlife Biology **2021**. Available from https://bioone.org/journals/wildlife-biology/volume-2021/issue-3/wlb.00808/A-non-invasive-DNA-based-method-for-beaver-species-identification/10.2981/wlb.00808.full.

IUCN/SSC. 2013. Guidelines for Reintroductions and Other Conservation Translocations.Version 1.0. Gland, Switzerland: Page IUCN Species Survival Commission. Available from https://www.iucn.org/content/guidelines-reintroductions-and-other-conservation-translocations.

IUCN/SSC. 2014. IUCN Species Survival Commission Guidelines on the Use of Ex Situ Management for Species Conservation. Version 2.:1–7. Available from https://portals.iucn.org/library/sites/library/files/documents/2014-064.pdf.

IUCN. 1980. WORLD CONSERVATION STRATEGY. Living Resource Conservation for Sustainable Development. IUCN,UNEP,WWF. Available from https://portals.iucn.org/library/efiles/documents/wcs-004.pdf.

IUCN. 2018. The IUCN Red List of Threatened Species. Available from https://www.iucnredlist.org/ (accessed September 20, 2022).

Jackson SD. 2000. Overview of transportation impacts on wildlife movement and populations. Wildlife and highways: seeking solutions to an ecological and socio-economic dilemma. The Wildlife Society:7–20. Available from http://www.umassextension.org/NREC/images/stories/linked_content/pdf_files/tws_overview_ms.pdf.

Jakobsson M, Rosenberg NA. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics **23**:1801–1806. Available from https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/btm233.

James CD, Landsberg J, Morton SR. 1999. Provision of watering points in the Australian arid zone: a review of effects on biota. Journal of Arid Environments **41**:87–121. Available from https://linkinghub.elsevier.com/retrieve/pii/S0140196398904670.

Janjua S, Peters JL, Weckworth B, Abbas FI, Bahn V, Johansson O, Rooney TP. 2020. Improving our conservation genetic toolkit: ddRAD-seq for SNPs in snow leopards. Conservation Genetics Resources **12**:257–261. Springer

Netherlands. Available from http://dx.doi.org/10.1007/s12686-019-01082-2.

Jansson E, Taggart JB, Wehner S, Dahle G, Quintela M, Mortensen S, Kvamme BO, Glover KA. 2016. Development of SNP and microsatellite markers for goldsinny wrasse (Ctenolabrus rupestris) from ddRAD sequencing data. Conservation Genetics Resources **8**:201–206. Available from http://link.springer.com/10.1007/s12686-016-0532-0.

Jiang P, Lang Q, Fang S, Ding P, Chen L. 2005. A genetic diversity comparison between captive individuals and wild individuals of Elliot's Pheasant (Syrmaticus ellioti) using mitochondrial DNA. Journal of Zhejiang University SCIENCE **6B**:413–417. Available from http://www.zju.edu.cn/jzus/article.php?doi=10.1631/jzus.2005.B0413.

Jombart T. 2012. A tutorial for Discriminant Analysis of Principal Components (DAPC) using adegenet 1.3-4:1–43.

Jones KE et al. 2009. PanTHERIA: a species-level database of life history, ecology, and geography of extant and recently extinct mammals. Ecology **90**:2648–2648.

Kamvar ZN, Tabima JF, Grünwald NJ. 2014. Poppr : an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ **2**:e281. Available from https://peerj.com/articles/281.

Kazanskaya E., Kuznetsova M., Danilkin A. 2005. Variability of consecution of gene cytb mDNA in genus Capra. Unpublished.

Kazanskaya EY, Kuznetsova M V., Danilkin AA. 2007. Phylogenetic reconstructions in the genus Capra (Bovidae, Artiodactyla) based on the mitochondrial DNA analysis. Russian Journal of Genetics **43**:181–189. Available from http://link.springer.com/10.1134/S1022795407020135.

Keller LF, Biebach I, Ewing SR, Hoeck PEA. 2012. The Genetics of Reintroductions: Inbreeding and Genetic Drift. Pages 360–394 Reintroduction Biology. Wiley. Available from https://onlinelibrary.wiley.com/doi/10.1002/9781444355833.ch11.

Kery M, Gardner B, Stoeckle T, Weber D, Royle JA. 2010. Use of Spatial Capture-Recapture Modeling and DNA Data to Estimate Densities of Elusive Animals. Conservation Biology:no-no. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.1523-1739.2010.01616.x.

Khan HA, Arif IA, Shobrak M, Homaidan AA Al, Farhan AH Al, Sadoon M Al. 2011. Application of mitochondrial genes sequences for measuring the genetic diversity of Arabian oryx:67–72.

Kimanzi JK. 2018. Population Viability Analysis of the Endangered Roan Antelope in Ruma National Park, Kenya, and Implications for Management. TheScientificWorldJournal **2018**:6015694. Hindawi.

Kohn MH, Wayne RK. 1997. Facts from feces revisited. Trends in Ecology & Evolution **12**:223–227. Available from https://linkinghub.elsevier.com/retrieve/pii/S0169534797010501.

Koster J, Rahmann S. 2012. Snakemake--a scalable bioinformatics workflow engine. Bioinformatics **28**:2520–2522. Available from https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/bts480.

Kotler B, Gross J, Mitchell W. 1994. Applying Patch Use to Assess Aspects of Foraging Behavior in Nubian Ibex Author. J Wildlife Manage **58**:299–307. Available from https://www.jstor.org/stable/3809395%0D.

Kraus RHS, VonHoldt B, Cocchiararo B, Harms V, Bayerl H, Kühn R, Förster DW, Fickel J, Roos C, Nowak C. 2015. A single-nucleotide polymorphism-based approach for rapid and cost-effective genetic wolf monitoring in Europe based on noninvasively collected samples. Molecular Ecology Resources **15**:295–305. Available from https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.12307.

Krofel M et al. 2022. Towards resolving taxonomic uncertainties in wolf, dog and jackal lineages of Africa, Eurasia and Australasia. Journal of Zoology **316**:155–168. Available from https://onlinelibrary.wiley.com/doi/10.1111/jzo.12946.

Kubasiewicz LM, Minderman J, Woodall LC, Quine CP, Coope R, Park KJ. 2016. Fur and faeces: an experimental assessment of non-invasive DNA sampling for the European pine marten. Mammal Research **61**:299–307. Available from http://link.springer.com/10.1007/s13364-016-0276-y.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular Biology and Evolution **35**:1547–1549. Available from https://academic.oup.com/mbe/article/35/6/1547/4990887.

Kwarteng AY, Dorvlo AS, Vijaya Kumar GT. 2009. Analysis of a 27-year rainfall data (1977-2003) in the Sultanate of Oman. International Journal of Climatology **29**:605–617. Available from https://onlinelibrary.wiley.com/doi/10.1002/joc.1727.

Lacy R., Pollak J. 2020. Vortex: A Stochastic Simulation of the Extinction Process. Version 10.4.0. Chicago Zoological Society, Brookfield, Illinois, USA.

Lacy RC. 2000. Structure of the VORTEX Simulation Model for Population Viability Analysis. Ecological Bulletins:191–203. Oikos Editorial Office. Available from http://www.jstor.org/stable/20113257.

Lacy RC, Ballou JD, Pollak JP. 2012. PMx: software package for demographic and genetic analysis and management of pedigreed populations. Methods

in Ecology and Evolution **3**:433–437. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.2041-210X.2011.00148.x.

Lado P, Cox C, Wideman K, Hernandez A, Klompen H. 2019. Population genetics of dermacentor variabilis say 1821 (Ixodida: Ixodidae) in the United States Inferred from ddRAD-seq SNP Markers. Annals of the Entomological Society of America **112**:433–442.

Lawson DJ, van Dorp L, Falush D. 2018. A tutorial on how not to over-interpret STRUCTURE and ADMIXTURE bar plots. Nature Communications **9**:3258. Available from http://www.nature.com/articles/s41467-018-05257-7.

Lees C, Rawson BM, Behie AM, Leonard N. 2014. Preliminary Population Viability Analysis of the Critically Endangered Cat Ba Langur (Trachypithecus poliocephalus):1–18. Available from http://www.cbsg.org/sites/cbsg.org/files/documents/Cat Ba Langur PVA_Final_EN.pdf.

Lei R, Hu Z, Jiang Z, Yang W. 2003. Phylogeography and genetic diversity of the critically endangered Przewalski’s gazelle. Animal Conservation **6**:361–367.

Leigh DM, Hendry AP, Vázquez-Domínguez E, Friesen VL. 2019. Estimated six per cent loss of genetic variation in wild populations since the industrial revolution. Evolutionary Applications **12**:1505–1512. Available from https://onlinelibrary.wiley.com/doi/10.1111/eva.12810.

Leigh JW, Bryant D. 2015. <scp>popart</scp> : full-feature software for haplotype network construction. Methods in Ecology and Evolution **6**:1110–1116. Available from http://popart.otago.ac.nz/index.shtml (accessed January 13, 2019).

Leus K. 2011. Captive breeding and conservation. Zoology in the Middle East **54**:151–158. Available from http://www.tandfonline.com/doi/full/10.1080/09397140.2011.10648906.

Levy N, Bernadsky G. 1991. Creche behavior of Nubian ibex Capra ibex nubiana in the Negev desert highland, Israel. Israel Journal of Zoology **37**:125–137. Taylor & Francis. Available from https://www.tandfonline.com/doi/abs/10.1080/00212210.1991.10688649.

Li C, Hofreiter M, Straube N, Corrigan S, Naylor GJP. 2013. Capturing protein-coding genes across highly divergent species. BioTechniques **54**:321–326. Available from https://www.future-science.com/doi/10.2144/000114039.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics **25**:2078–2079. Available from https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/btp352.

Linck EB, Hanna ZR, Sellas A, Dumbacher JP. 2017. Evaluating hybridization capture with RAD probes as a tool for museum genomics with historical bird specimens. Ecology and Evolution **7**:4755–4767.

Lindahl T. 1993. Instability and decay of the primary structure of DNA. Nature **362**:709–715. Available from http://www.nature.com/articles/362709a0.

Lindenmayer DB, Clark TW, Lacy RC, Thomas VC. 1993. Population viability analysis as a tool in wildlife conservation policy: With reference to Australia. Environmental Management **17**:745–758. Available from http://link.springer.com/10.1007/BF02393895.

Lischer HEL, Excoffier L. 2012. PGDSpider: An automated data conversion tool for connecting population genetics and genomics programs. Bioinformatics **28**:298–299.

Lortkipanidze B, Weinberg P. 2020. Capra cylindricornis , Eastern Tur. The IUCN Red List of Threatened Species. Available from https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T3795A91287260.en%0ACopyright:

Lou RN, Jacobs A, Wilder AP, Therkildsen NO. 2021. A beginner's guide to low-coverage whole genome sequencing for population genomics. Molecular Ecology **30**:5966–5993. Available from https://onlinelibrary.wiley.com/doi/10.1111/mec.16077.

Lu Z et al. 2001. Patterns of genetic diversity in remaining giant panda populations. Conservation Biology **15**:1596–1607.

Luikart G, Gielly L, Excoffier L, Vigne JD, Bouvet J, Taberlet P. 2001. Multiple maternal origins and weak phylogeographic structure in domestic goats. Proceedings of the National Academy of Sciences of the United States of America **98**:5927–5932.

Luikart G, Kardos M, Hand BK, Rajora OP, Aitken SN, Hohenlohe PA. 2018. Population Genomics: Advancing Understanding of Nature. Pages 3–79. Available from http://link.springer.com/10.1007/13836_2018_60.

Malcolm HJ, James G. 2006. Fundamentals of Conservation Biology, 3rd edition. Wiley-Blackwell. Available from https://www.wiley.com/en-gb/Fundamentals+of+Conservation+Biology%2C+3rd+Edition-p-9781405135450.

Mallon D, Budd K. 2011. Regional Red List Status of Carnivores in the Arabian Peninsula:1–52. Available from Mallon_&_Budd_2011_Red_List_Status_of_Carnivores_in_the_Arabian_Peninsula.pdf.

Mallon DP. 2011. Global hotspots in the Arabian Peninsula. Zoology in the Middle East **54**:13–20. Available from

http://www.tandfonline.com/doi/full/10.1080/09397140.2011.10648896.

Manceau V, Crampe J-P, Boursot P, Taberlet P. 1999a. Identification of evolutionary significant units in the Spanish wild goat, Capra pyrenaica (Mammalia, Artiodactyla). Animal Conservation **2**:S1367943099000335. Available from http://doi.wiley.com/10.1017/S1367943099000335.

Manceau V, Després L, Bouvet J, Taberlet P. 1999b. Systematics of the Genus Capra Inferred from Mitochondrial DNA Sequence Data. Molecular Phylogenetics and Evolution **13**:504–510. Available from https://linkinghub.elsevier.com/retrieve/pii/S1055790399906887.

Mannise N, Trovat RG, Duarte JMB, Maldonado JE, González S. 2018. Using non–invasive genetic techniques to assist in maned wolf conservation in a remnant fragment of the Brazilian Cerrado. Animal Biodiversity and Conservation **41**:315–319. Available from http://abc.museucienciesjournals.cat/volume-41-2-2018-abc/using-non-invasive-genetic-techniques-to-assist-in-maned-wolf-conservation-in-a-remnant-fragment-of-the-brazilian-cerrado/?lang=en.

Manousaki T et al. 2016. Exploring a nonmodel teleost genome through rad sequencing-linkage mapping in common pandora, Pagellus erythrinus and comparative genomic analysis. G3: Genes, Genomes, Genetics **6**:509–519. Genetics Society of America.

Margan SH, Nurthen RK, Montgomery ME, Woodworth LM, Lowe EH, Briscoe DA, Frankham R. 1998. Single large or several small? Population fragmentation in the captive management of endangered species. Zoo Biology **17**:467–480. Available from https://onlinelibrary.wiley.com/doi/10.1002/(SICI)1098-2361(1998)17:6%3C467::AID-ZOO1%3E3.0.CO;2-3.

Marshall AJ et al. 2008. Orangutan population biology, life history, and conservation. Pages 311–326 Orangutans. Oxford University Press. Available from https://oxford.universitypressscholarship.com/view/10.1093/acprof:oso/9780199213276.001.0001/acprof-9780199213276-chapter-22.

Mason VC, Li G, Helgen KM, Murphy WJ. 2011. Efficient cross-species capture hybridization and next-generation sequencing of mitochondrial genomes from noninvasively sampled museum specimens. Genome Research **21**:1695–1704. Available from http://genome.cshlp.org/lookup/doi/10.1101/gr.120196.111.

Massolo A, Spalton JA, Tear TH, Lawrence MW, Said al Harsusi L, Lovari S. 2008. Dynamic social system in Nubian ibex: can a second mating season develop in response to arid climate? Journal of Zoology **274**:216–225. Available from http://doi.wiley.com/10.1111/j.1469-7998.2007.00373.x.

Mazzolli M. 2009. Arabian leopard, panthera pardus nimr, status and habitat assessment in northwest dhofar, oman: (Mammalia: Felidae). Zoology in the Middle East **47**:3–11.

McCormack JE, Hird SM, Zellmer AJ, Carstens BC, Brumfield RT. 2013. Applications of next-generation sequencing to phylogeography and phylogenetics. Molecular Phylogenetics and Evolution **66**:526–538. Available from https://linkinghub.elsevier.com/retrieve/pii/S1055790311005203.

McFarlane SE, Hunter DC, Senn H V., Smith SL, Holland R, Huisman J, Pemberton JM. 2020. Increased genetic marker density reveals high levels of admixture between red deer and introduced Japanese sika in Kintyre, Scotland. Evolutionary Applications **13**:432–441. Available from https://onlinelibrary.wiley.com/doi/10.1111/eva.12880.

McGowan PJK, Traylor-Holzer K, Leus K. 2017. IUCN Guidelines for Determining When and How Ex Situ Management Should Be Used in Species Conservation. Conservation Letters **10**:361–366. Available from https://onlinelibrary.wiley.com/doi/10.1111/conl.12285.

Mestanza-Ramón C, Henkanaththegedara SM, Vásconez Duchicela P, Vargas Tierras Y, Sánchez Capa M, Constante Mejía D, Jimenez Gutierrez M, Charco Guamán M, Mestanza Ramón P. 2020. In-situ and ex-situ biodiversity conservation in ecuador: A review of policies, actions and challenges. Diversity **12**:315. MDPI.

Metallinou M, Arnold EN, Crochet P-A, Geniez P, Brito JC, Lymberakis P, Baha El Din S, Sindaco R, Robinson M, Carranza S. 2012. Conquering the Sahara and Arabian deserts: systematics and biogeography of Stenodactylus geckos (Reptilia: Gekkonidae). BMC Evolutionary Biology **12**:258. Available from https://bmcevolbiol.biomedcentral.com/articles/10.1186/1471-2148-12-258.

Michallet J, Loison A, Gaillard M, Gauthier D. 1994. Valeur de crite`res biome´triques externs pour la de´termination de l’aˆge du bouquetin des Alpes (Capra ibex ibex): role du sexe et de l’habitat. Gibier Faune Sauvage **11**:99–118.

Michel S, Rosen Michel T. 2015. Capra falconeri, Markhor. The IUCN red list of threatened species **8235**:e.T3787A97218336. Available from http://dx.doi.org/10.2305/IUCN.UK.2015- 4.RLTS.T3787A82028427.en.

Miller W, Wright SJ, Zhang Y, Schuster SC, Hayes VM. 2009. Optimization methods for selection founder individuals for captive breeding or reintroduction of endangered species. Pages 43–53 Biocomputing 2010. WORLD SCIENTIFIC. Available from http://www.worldscientific.com/doi/abs/10.1142/9789814295291_0006.

Ministry of Environment and Climate Affairs. 2010. Sultanate of Oman: Fourth National Report to the Convention on Biological Diversity:1–156. Available from https://www.cbd.int/doc/world/om/om-nr-04-en.pdf.

Ministry of Transport. 2018. Annual Report. Muscat. Available from https://www.transport.gov.om.

Mirzaei K, Wesselingh RA. 2021. Development of a large set of diagnostic SNP markers using ddRAD-seq to study hybridization in Rhinanthus major and R. minor (Orobanchaceae). Conservation Genetics Resources **13**:31–33. Available from http://link.springer.com/10.1007/s12686-020-01181-5.

Morin PA, Mccarthy M. 2007. Highly accurate SNP genotyping from historical and low-quality samples. Molecular Ecology Notes **7**:937–946. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.1471-8286.2007.01804.x.

Mulondo P, Mugiru G. 2011. Measuring Responses of Wildlife To Oil Operations in Murchison.

Muniz FL, Campos Z, Hernández Rangel SM, Martínez JG, Souza BC, De Thoisy B, Botero-Arias R, Hrbek T, Farias IP. 2018. Delimitation of evolutionary units in Cuvier's dwarf caiman, Paleosuchus palpebrosus (Cuvier, 1807): insights from conservation of a broadly distributed species. Conservation Genetics **19**:599–610. Springer Netherlands. Available from http://dx.doi.org/10.1007/s10592-017-1035-6.

Muñoz-Fuentes V, Darimont CT, Paquet PC, Leonard JA. 2010. The genetic legacy of extirpation and re-colonization in Vancouver Island wolves. Conservation Genetics **11**:547–556. Available from http://link.springer.com/10.1007/s10592-009-9974-1.

Nagai M, Yoshida A, Sato N. 1998. Additive effects of bovine serum albumin, dithiothreitol and glycerolon PCR. IUBMB Life **44**:157–163. Available from http://doi.wiley.com/10.1080/15216549800201172.

Newbold T et al. 2015. Global effects of land use on local terrestrial biodiversity. Nature **520**:45–50. Available from http://www.nature.com/articles/nature14324.

Norman AJ, Street NR, Spong G. 2013. De Novo SNP Discovery in the Scandinavian Brown Bear (Ursus arctos). PLoS ONE **8**:e81012. Available from https://dx.plos.org/10.1371/journal.pone.0081012.

Nussberger B, Currat M, Quilodran CS, Ponta N, Keller LF. 2018. Range expansion as an explanation for introgression in European wildcats. Biological Conservation **218**:49–56. Available from https://linkinghub.elsevier.com/retrieve/pii/S0006320717301507.

Nyhus PJ. 2016. Human–Wildlife Conflict and Coexistence. Annual Review of Environment and Resources **41**:143–171. Available from

https://www.annualreviews.org/doi/10.1146/annurev-environ-110615-085634.

O'Connell KA, Mulder KP, Wynn A, Queiroz K, Bell RC. 2022. Genomic library preparation and hybridization capture of formalin-fixed tissues and allozyme supernatant for population genomics and considerations for combining capture- and RADseq-based single nucleotide polymorphism data sets. Molecular Ecology Resources **22**:487–502. Available from https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.13481.

Obadi N. 1993. Animals of Yemen: Mammals. Obadi Piblication Center.

Ogden R, Chuven J, Gilbert T, Hosking C, Gharbi K, Craig M, Al Dhaheri SS, Senn H. 2020. Benefits and pitfalls of captive conservation genetic management: Evaluating diversity in scimitar-horned oryx to support reintroduction planning. Biological Conservation **241**:108244. Elsevier. Available from https://doi.org/10.1016/j.biocon.2019.108244.

Oman Observer. 2020, March. Project for settlement of wild animals launched. Online. Muscat. Available from www.omanobserver.com.

Oman T of. 2018, January 22. Three arrested for poaching in Oman - Times Of Oman. Muscat. Available from https://timesofoman.com/article/126561 (accessed January 28, 2019).

Parrini F, Cain JW, Krausman PR. 2009. Capra ibex (Artiodactyla: Bovidae). Mammalian Species **830**:1–12. Available from http://asmjournals.org/doi/abs/10.1644/830.1.

Patwardhan A, Amit R, Samit R. 2014. Molecular Markers in Phylogenetic Studies-A Review. Journal of Phylogenetics & Evolutionary Biology **02**.

Patzelt A. 2015. Synopsis of the Flora and Vegetation of Oman, with Special Emphasis on Patterns of Plant Endemism. Braunschweigische Wissenschaftliche Gesellschaft:282–317.

Peng H, Liu S, Zou F, Zeng B, Yue B. 2008. Genetic diversity of captive forest musk deer (Moschus berezovskii) inferred from the mitochondrial DNA control region:65–72.

Perry GH, Marioni JC, Melsted P, Gilad Y. 2010. Genomic-scale capture and sequencing of endogenous DNA from feces. Molecular Ecology **19**:5332–5344. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2010.04888.x.

Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE. 2012. Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS ONE **7**.

Phillips JD, French SH, Hanner RH, Gillis DJ. 2020. HACSim: an R package to

estimate intraspecific sample sizes for genetic diversity assessment using haplotype accumulation curves. PeerJ Computer Science **6**:e243. Available from https://peerj.com/articles/cs-243.

Phillips JD, Gillis DJ, Hanner RH. 2019. Incomplete estimates of genetic diversity within species: Implications for DNA barcoding. Ecology and Evolution **9**:2996–3010. Available from http://doi.wiley.com/10.1002/ece3.4757.

Phillips JD, Gwiazdowski RA, Ashlock D, Hanner R. 2015. An exploration of sufficient sampling effort to describe intraspecific DNA barcode haplotype diversity: examples from the ray-finned fishes (Chordata: Actinopterygii). DNA Barcodes **3**. Available from http://access.portico.org/stable?au=phx3c2h63ck.

Pidancier N, Jordan S, Luikart G, Taberlet P. 2006. Evolutionary history of the genus Capra (Mammalia, Artiodactyla): Discordance between mitochondrial DNA and Y-chromosome phylogenies. Molecular Phylogenetics and Evolution **40**:739–749. Available from https://linkinghub.elsevier.com/retrieve/pii/S1055790306001321.

Pimm SL, Jenkins CN, Abell R, Brooks TM, Gittleman JL, Joppa LN, Raven PH, Roberts CM, Sexton JO. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. Science **344**. Available from https://www.science.org/doi/10.1126/science.1246752.

Placentation. 2005. “Nubian Ibex Capra nubiana” (On-line),placentation. Available from http://placentation.ucsd.edu/ibex.html (accessed November 28, 2020).

Pörtner H-O et al. 2021. IPCC, 2022: Climate Change 2022: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Page Climate Change 2021: The Physical Science Basis.

Powell B, Evans KO. 2019. Rutting. Pages 1–3 Encyclopedia of Animal Cognition and Behavior. Springer International Publishing, Cham. Available from http://link.springer.com/10.1007/978-3-319-47829-6_1906-1.

Price MR. 1989. Animal Reintroductions: The Arabian Oryx in Oman. Cambridge University Press. Available from www.cambridge.org.

Price SA, Gittleman JL. 2007. Hunting to extinction: biology and regional economy influence extinction risk and the impact of hunting in artiodactyls. Proceedings of the Royal Society B: Biological Sciences **274**:1845–1851. Available from https://royalsocietypublishing.org/doi/10.1098/rspb.2007.0505.

Primack RB. 2014. Essentials of Conservation Biology, 6th edition. Sinauer Associates, Massachusetts USA.

Primmer CR. 2009. From Conservation Genetics to Conservation Genomics. Annals of the New York Academy of Sciences **1162**:357–368. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.1749-6632.2009.04444.x.

Pritchard DJ, Fa JE, Oldfield S, Harrop SR. 2012. Bring the captive closer to the wild: redefining the role of ex situ conservation. Oryx **46**:18–23. Available from https://www.cambridge.org/core/product/identifier/S0030605310001766/type/journal_article.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of Population Structure Using Multilocus Genotype Data. Genetics **155**:945–959. Available from https://academic.oup.com/genetics/article/155/2/945/6048111.

Puckett EE. 2017. Variability in total project and per sample genotyping costs under varying study designs including with microsatellites or SNPs to answer conservation genetic questions. Conservation Genetics Resources **9**:289–304. Available from http://link.springer.com/10.1007/s12686-016-0643-7.

Puechmaille SJ. 2016. The program does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. Molecular Ecology Resources **16**:608–627. Available from https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.12512.

Putnam AS, Nguyen TN, Mott A, Korody ML, Ryder OA. 2020. Assessing possible hybridization among managed Nubian ibex in North America. Zoo Biology **39**:121–128.

R Core Team. 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available from https://www.r-project.org.

Ralls K, Sunnucks P, Lacy RC, Frankham R. 2020. Genetic rescue: A critique of the evidence supports maximizing genetic diversity rather than minimizing the introduction of putatively harmful genetic variation. Biological Conservation **251**:108784. Available from http://www.sciencedirect.com/science/article/pii/S0006320720308429.

Ramón-Laca A, Soriano L, Gleeson D, Godoy JA. 2015. A simple and effective method for obtaining mammal DNA from faeces. Wildlife Biology **21**:195–203. Available from http://www.bioone.org/doi/10.2981/wlb.00096.

Reading R, Michel S, Suryawanshi K, Bhatnagar Y. 2020. Capra sibirica , Siberian Ibex. The IUCN Red List of Threatened Species. Available from https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T42398A22148720.en.

Reed DH. 2004. Extinction risk in fragmented habitats. Animal Conservation **7**:181–191. Available from

http://doi.wiley.com/10.1017/S1367943004001313.

Reed DH, Frankham R. 2003. Correlation between Fitness and Genetic Diversity. Conservation Biology **17**:230–237. Available from http://doi.wiley.com/10.1046/j.1523-1739.2003.01236.x.

Reed DH, O'Grady JJ, Brook BW, Ballou JD, Frankham R. 2003. Estimates of minimum viable population sizes for vertebrates and factors influencing those estimates. Biological Conservation **113**:23–34. Available from https://linkinghub.elsevier.com/retrieve/pii/S0006320702003464.

Reed JM, Mills LS, Dunning JB, Menges ES, McKelvey KS, Frye R, Beissinger SR, Anstett M, Miller P. 2002. Emerging Issues in Population Viability Analysis. Conservation Biology **16**:7–19. Available from https://onlinelibrary.wiley.com/doi/10.1046/j.1523-1739.2002.99419.x.

Reiners TE, Eidenschenk J, Neumann K, Nowak C. 2014. Preservation of genetic diversity in a wild and captive population of a rapidly declining mammal, the Common hamster of the French Alsace region. Mammalian Biology **79**:240–246. Available from https://linkinghub.elsevier.com/retrieve/pii/S1616504713002152.

Rochette NC, Rivera-Colón AG, Catchen JM. 2019. Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. Molecular Ecology **28**:4737–4754. Available from https://onlinelibrary.wiley.com/doi/10.1111/mec.15253.

Rogers TD. 1980. Meteorological records from the mountain region of Dhofar. The scientific results of the Oman flora and fauna survey 1977. Journal of Oman Studies **2**:55–58.

Ronald.M N. 1983. Walker's Mammals of the World, 4th edition. Johns Hopkins University Press. Available from https://www.press.jhu.edu/news/blog/walkers-mammals-world.

Ropiquet A, Hassanin A. 2005. Molecular phylogeny of caprines (Bovidae, Antilopinae): The question of their origin and diversification during the Miocene. Journal of Zoological Systematics and Evolutionary Research **43**:49–60.

Ropiquet A, Hassanin A. 2006. Hybrid origin of the Pliocene ancestor of wild goats. Molecular Phylogenetics and Evolution **41**:395–404. Available from https://linkinghub.elsevier.com/retrieve/pii/S1055790306002223.

Roques S, Chancerel E, Boury C, Pierre M, Acolas ML. 2019. From microsatellites to single nucleotide polymorphisms for the genetic monitoring of a critically endangered sturgeon. Ecology and Evolution **9**:7017–7029.

Ross S, Al-Rawahi H, Al-Jahdhami MH, Spalton JA, Mallon D, Al-Shukali AS,

Al-Rasbi A, Al-Fazari W, Chreiki M. 2019. Arabitragus jayakari. The IUCN Red List of Threatened Species 2019: e.T9918A128770408 **8235**. Available from http://dx.doi.org/10.2305/IUCN.UK.2019-1.RLTS.T9918A128770408.en.

Ross S, Alqamy H El, Alsaid T. 2020a. Capra nubiana, Nubian Ibex. The IUCN Red List of Threatened Species 2020. Available from https//dx.doi.org/10.2305/IUCN.UK.2020- 2.RLTS.T3796A22143385.en.

Ross S, Costanzi JM, Al Jahdhami M, Al Rawahi H, Ghazali M, Senn H. 2020b. First evaluation of the population structure, genetic diversity and landscape connectivity of the Endangered Arabian tahr. Mammalian Biology. Springer International Publishing. Available from https://doi.org/10.1007/s42991-020-00072-4.

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A. 2017. DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. Molecular Biology and Evolution **34**:3299–3302. Available from http://academic.oup.com/mbe/article/34/12/3299/4161815.

Russello MA, Waterhouse MD, Etter PD, Johnson EA. 2015. From promise to practice: pairing non-invasive sampling with genomics in conservation. PeerJ **3**:e1106. Available from https://peerj.com/articles/1106.

Sale JB. 1980. The environment of the mountain region of the Dhofar. The scientific results of the Oman flora and fauna survey 1977. Journal of Oman Studies **Special Re**:17–24.

Sanna D et al. 2015. The First Mitogenome of the Cyprus Mouflon (Ovis gmelini ophion): New Insights into the Phylogeny of the Genus Ovis. PLOS ONE **10**:e0144257. Available from https://dx.plos.org/10.1371/journal.pone.0144257.

Schaller GB. 1977. Mountain Monarchs. The University of Chicago Press, Chicago, London.

Schell CJ, Stanton LA, Young JK, Angeloni LM, Lambert JE, Breck SW, Murray MH. 2021. The evolutionary consequences of human–wildlife conflict in cities. Evolutionary Applications **14**:178–197. Available from https://onlinelibrary.wiley.com/doi/10.1111/eva.13131.

Schemske DW, Husband BC, Ruckelshaus M, Goodwillie C, Parker IM, Bishop JG. 1994. Evaluating approaches to the conservation of rare and endangered plants. Ecology **75**:584–606.

Schliep KP. 2010. phangorn: phylogenetic analysis in R. Bioinformatics **27**:592–593. Available from https://doi.org/10.1093/bioinformatics/btq706.

Schmid S, Genevest R, Gobet E, Suchan T, Sperisen C, Tinner W, Alvarez N.

2017. HyRAD-X, a versatile method combining exome capture and RAD sequencing to extract genomic information from ancient DNA. Methods in Ecology and Evolution **8**:1374–1388. Available from https://onlinelibrary.wiley.com/doi/10.1111/2041-210X.12785.

Schulte L, Bernhardt N, Stoof-Leichsenring K, Zimmermann HH, Pestryakova LA, Epp LS, Herzschuh U. 2021. Hybridization capture of larch (Larix Mill.) chloroplast genomes from sedimentary ancient DNA reveals past changes of Siberian forest. Molecular Ecology Resources **21**:801–815. Available from https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.13311.

Schwartz KR, Parsons ECM, Rockwood L, Wood TC. 2017. Integrating in-situ and ex-situ data management processes for biodiversity conservation. Frontiers in Ecology and Evolution **5**:120. Frontiers Media SA.

Schweizer RM et al. 2018. Natural Selection and Origin of a Melanistic Allele in North American Gray Wolves. Molecular Biology and Evolution **35**:1190–1209. Available from https://academic.oup.com/mbe/article/35/5/1190/4913657.

Seaborn T, Andrews KR, Applestein C V., Breech TM, Garrett MJ, Zaiats A, Caughlin TT. 2021. Integrating genomics in population models to forecast translocation success. Restoration Ecology **29**. Available from https://onlinelibrary.wiley.com/doi/10.1111/rec.13395.

Seeber PA, McEwen GK, Löber U, Förster DW, East ML, Melzheimer J, Greenwood AD. 2019. Terrestrial mammal surveillance using hybridization capture of environmental DNA from African waterholes. Molecular Ecology Resources **19**:1486–1496. Available from https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.13069.

Senn H et al. 2014a. Splitting or Lumping? A Conservation Dilemma Exemplified by the Critically Endangered Dama Gazelle (Nanger dama). PLoS ONE **9**:e98693. Available from https://dx.plos.org/10.1371/journal.pone.0098693.

Senn H, O'Donoghue P, McEwing R, Ogden R. 2014b. Hundreds of SNPs for the Endangered pygmy hippopotamus (Choeropsis liberiensis). Conservation Genetics Resources **6**:535–538. Available from http://link.springer.com/10.1007/s12686-014-0178-8.

Senn H V., Ghazali M, Kaden J, Barclay D, Harrower B, Campbell RD, Macdonald DW, Kitchener AC. 2019. Distinguishing the victim from the threat: SNP-based methods reveal the extent of introgressive hybridization between wildcats and domestic cats in Scotland and inform future in situ and ex situ management options for species restoration. Evolutionary Applications **12**:399–414. Available from https://onlinelibrary.wiley.com/doi/10.1111/eva.12720.

Senn H V., Pemberton JM. 2009. Variable extent of hybridization between invasive sika (Cervus nippon) and native red deer (C. elaphus) in a small geographical area. Molecular Ecology **18**:862–876. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2008.04051.x.

Sevigny J, Leasi F, Simpson S, Di Domenico M, Jörger KM, Norenburg JL, Thomas WK. 2021. Target enrichment of metazoan mitochondrial DNA with hybridization capture probes. Ecological Indicators **121**:106973. Available from https://linkinghub.elsevier.com/retrieve/pii/S1470160X20309122.

Shackleton D. 1997. Wild Sheep and Goats and their Relatives. Status Survey and Conservation Action Plan for Caprinae. Gland : IUCN, 1997. Available from https://www.iucn.org/content/wild-sheep-and-goats-and-their-relatives-status-survey-and-conservation-action-plan-caprinae.

Shaffer ML. 1981. Minimum Population Sizes for Species Conservation. BioScience **31**:131–134. Available from https://doi.org/10.2307/1308256.

Shaffer ML. 1987. Minimum viable populations: coping with uncertainty, In: Viable Populations for Conservation. Cambridge University Press.

Sheehy E, O’Meara DB, O’Reilly C, Smart A, Lawton C. 2014. A non-invasive approach to determining pine marten abundance and predation. European Journal of Wildlife Research **60**:223–236. Available from http://link.springer.com/10.1007/s10344-013-0771-2.

Sigg DP. 2006. Reduced genetic diversity and significant genetic differentiation after translocation: Comparison of the remnant and translocated populations of bridled nailtail wallabies (Onychogalea fraenata). Conservation Genetics **7**:577–589. Available from http://link.springer.com/10.1007/s10592-005-9096-3.

Slotta-Bachmary L, Boegel R, Kaczensky P, Stauffer C, Walzer C. 2004. Use of Population Viability Analysis To Identify Management Priorities and Success in Reintroducing Przewalski’S Horses To Southwestern Mongolia. Journal of Wildlife Management **68**:790–798.

Smith JH, King T, Campbell C, Cheyne SM, Nijman V. 2018a. Modelling Population Viability of Three Independent Javan Gibbon (Hylobates moloch) Populations on Java, Indonesia. Folia Primatologica **88**:507–522.

Smith O, Wang J. 2014. When can noninvasive samples provide sufficient information in conservation genetics studies? Molecular Ecology Resources **14**:n/a-n/a. Available from https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.12250.

Smith SL, Senn H V., Pérez-Espona S, Wyman MT, Heap E, Pemberton JM. 2018b. Introgression of exotic Cervus (nippon and canadensis) into red deer (Cervus elaphus) populations in Scotland and the English Lake District. Ecology and Evolution **8**:2122–2134. Available from

https://onlinelibrary.wiley.com/doi/10.1002/ece3.3767.

Soule M. 1987. Viable populations for conservation. Cambridge University Press, Cambridge, United Kingdom.

Soule M. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. Comservation biology: an evolutionary-ecological perspective:151–169. Sinauer Associates.

Spalton A. 2002. Canid News Canidae in the Sultanate of Oman. Control:1–4.

Spalton JA. 1993. A brief history of the reintroduction of the Arabian oryx Oryx leucoryx into Oman 1980–1992. International Zoo Yearbook **32**:81–90.

Spalton JA. 1999. The food supply of Arabian oryx (Oryx leucoryx) in the desert of Oman. Journal of Zoology **248**:433–441.

Spalton JA, Al Hikmani HM, Willis D, Said ASB. 2006. Critically Endangered Arabian leopards Panthera pardus nimr persist in the Jabal Samhan Nature Reserve, Oman. Oryx **40**:287–294.

Spalton JA, Lawerence MW, Brend SA. 1999. Arabian oryx reintroduction in Oman: successes and setbacks. Oryx **33**:168–175. Available from https://www.cambridge.org/core/product/identifier/S003060530003043X/type/journal_article.

Species survival specialists, Antelope Specialist Group. 2017. Antelopes of North Africa and the Arabian Peninsula.

Spooner PG. 2015. Minor rural road networks: Values, challenges, and opportunities for biodiversity conservation. Nature Conservation **11**:129–142.

Springer MS, DeBry RW, Douady C, Amrine HM, Madsen O, de Jong WW, Stanhope MJ. 2001. Mitochondrial Versus Nuclear Gene Sequences in Deep-Level Mammalian Phylogeny Reconstruction. Molecular Biology and Evolution **18**:132–143. Available from http://academic.oup.com/mbe/article/18/2/132/1079207.

Stanton DWG et al. 2015. Genetic structure of captive and free-ranging okapi (Okapia johnstoni) with implications for management. Conservation Genetics **16**:1115–1126. Available from http://link.springer.com/10.1007/s10592-015-0726-0.

Stenglein JL, De Barba M, Ausband DE, Waits LP. 2010. Impacts of sampling location within a faeces on DNA quality in two carnivore species. Molecular Ecology Resources **10**:109–114. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.1755-0998.2009.02670.x.

Stetz JB, Smith S, Sawaya MA, Ramsey AB, Amish SJ, Schwartz MK, Luikart G. 2016. Discovery of 20,000 RAD–SNPs and development of a 52-SNP

array for monitoring river otters. Conservation Genetics Resources **8**:299–302. Available from http://link.springer.com/10.1007/s12686-016-0558-3.

Strauss WM. 2006. Martin Struss Msc thesis PVA on Oryx. Bloomsbury, London (GB). Available from https://www.bloomsbury.com/us/mammals-of-africa-volume-vi-9781408122563/.

Stüwe M, Nievergelt B. 1991. Recovery of alpine ibex from near extinction: the result of effective protection, captive breeding, and reintroductions. Applied Animal Behaviour Science **29**:379–387. Available from https://linkinghub.elsevier.com/retrieve/pii/016815919190262V.

Stüwe M, Scribner KT, Alkon PU. 1992. A comparison of genetic diversity in Nubian ibex (Capra ibex nubiana) and Alpine ibex (Capra i. ibex). Z. für Sougetierkunde **57**:120–123.

Suchan T et al. 2021. Performance and automation of ancient DNA capture with RNA hyRAD probes. Molecular Ecology Resources. Available from https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.13518.

Suchan T, Pitteloud C, Gerasimova NS, Kostikova A, Schmid S, Arrigo N, Pajkovic M, Ronikier M, Alvarez N. 2016. Hybridization capture using RAD probes (hyRAD), a new tool for performing genomic analyses on collection specimens. PLoS ONE **11**.

Szpiech ZA, Jakobsson M, Rosenberg NA. 2008. ADZE: a rarefaction approach for counting alleles private to combinations of populations. Bioinformatics **24**:2498–2504. Available from https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/btn478.

Tadesse SA, Kotler BP. 2012. Impact of tourism on Nubian ibex (Capra nubiana) revealed through assessment of behavioral indicators. Behavioral Ecology **23**:1257–1262.

Tamura K, Nei M, Kumar S. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences **101**:11030–11035. Available from http://www.pnas.org/content/101/30/11030.abstract.

Taron UH, Paijmans JLA, Barlow A, Preick M, Iyengar A, Drăgușin V, Vasile Ștefan, Marciszak A, Roblíčková M, Hofreiter M. 2021. Ancient DNA from the Asiatic Wild Dog (Cuon alpinus) from Europe. Genes **12**:144. Available from https://www.mdpi.com/2073-4425/12/2/144.

Tear TH. 1992. Range use patterns and the develop- ment of a natural grazing system in reintroduced Arabian oryx (Oryx leucoryx) in the Sultanate of Oman. Idaho, Moscow.

Tear TH, Ables ED. 1999. Social system development and variability in a

reintroduced Arabian oryx population. Biological Conservation **89**:199–207.

Tear TH, Mosley JC, Ables ED. 1997. Landscape-Scale Foraging Decisions by Reintroduced Arabian Oryx. The Journal of Wildlife Management **61**:1142.

Thaden A et al. 2020. Applying genomic data in wildlife monitoring: Development guidelines for genotyping degraded samples with reduced single nucleotide polymorphism panels. Molecular Ecology Resources **20**:662–680. Available from https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.13136.

Thaden von A, Cocchiararo B, Jarausch A, Jüngling H, Karamanlidis AA, Tiesmeyer A, Nowak C, Muñoz-Fuentes V. 2017. Assessing SNP genotyping of noninvasively collected wildlife samples using microfluidic arrays. Scientific Reports **7**:10768. Available from http://www.nature.com/articles/s41598-017-10647-w.

Thatte P, Patel K, Ramakrishnan U. 2018. Rapid species identification of sloth bears from non-invasive samples: a PCR-based assay. Ursus **29**:67–70. Available from http://www.bioone.org/doi/10.2192/URSUS-D-17-00024.2.

Thomas CD et al. 2004. Extinction risk from climate change. Nature **427**:145–148. Available from http://www.nature.com/articles/nature02121.

Thomson RC, Wang IJ, Johnson JR. 2010. Genome-enabled development of DNA markers for ecology, evolution and conservation. Molecular Ecology **19**:2184–2195. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2010.04650.x.

Tian Y, Wu J, Smith AT, Wang T, Kou X, Ge J. 2011. Population viability of the Siberian Tiger in a changing landscape: Going, going and gone? Ecological Modelling **222**:3166–3180. Elsevier B.V. Available from http://dx.doi.org/10.1016/j.ecolmodel.2011.06.003.

Toïgo C, Brambilla A, Grignolio S, Pedrotti L. 2020. Capra ibex (Alpine Ibex). The IUCN Red List of Threatened Species 2020. Available from https://dx.doi.org/10.2305/IUCN.UK.2020- 2.RLTS.T42397A161916377.en.

Toïgo C, Gaillard JM, Festa-Bianchet M, Largo E, Michallet J, Maillard D. 2007. Sex- and age-specific survival of the highly dimorphic Alpine ibex: Evidence for a conservative life-history tactic. Journal of Animal Ecology **76**:679–686.

Tomson J. 2007. “Capra nubiana” (On-line), Animal Diversity Web. Available from https://animaldiversity.org/accounts/Capra_nubiana/ (accessed November 26, 2020).

Torres RT, Ferreira E, Rocha RG, Fonseca C. 2017. Hybridization between wolf and domestic dog: First evidence from an endangered population in central Portugal. Mammalian Biology **86**:70–74. Available from https://linkinghub.elsevier.com/retrieve/pii/S161650471630221X.

Tshipa A, Valls-Fox H, Fritz H, Collins K, Sebele L, Mundy P, Chamaillé-Jammes S. 2017. Partial migration links local surface-water management to large-scale elephant conservation in the world's largest transfrontier conservation area. Biological Conservation **215**:46–50. Available from https://linkinghub.elsevier.com/retrieve/pii/S0006320717309047.

Turcek FJ, Hickey JJ. 1951. Effect of Introductions on Two Game Populations in Czechoslovakia. The Journal of Wildlife Management **15**:113. Available from https://www.jstor.org/stable/3796784?origin=crossref.

UNDP. 2019. A RACE AGAINST TIME Combatting Illegal and Unsustainable Trade in Endangered Species in Indonesia. Available from https://undp-biodiversity.exposure.co/a-race-against-time (accessed September 16, 2021).

UNESCO. 2007. Oman's Arabian Oryx Sanctuary : first site ever to be deleted from UNESCO's World Heritage List. Available from https://whc.unesco.org/en/news/362.

Untergasser A, Nijveen H, Rao X, Bisseling T, Geurts R, Leunissen JAM. 2007. Primer3Plus, an enhanced web interface to Primer3. Nucleic acids research **35**:W71–W74. Oxford University Press. Available from https://pubmed.ncbi.nlm.nih.gov/17485472.

Upholt WB, Dawid IB. 1977. Mapping of mitochondrial DNA of individual sheep and goats: Rapid evolution in the D loop region. Cell **11**:571–583. Available from https://linkinghub.elsevier.com/retrieve/pii/0092867477900757.

Valencia LM, Martins A, Ortiz EM, Di Fiore A. 2018. A RAD-sequencing approach to genome-wide marker discovery, genotyping, and phylogenetic inference in a diverse radiation of primates. PLOS ONE **13**:e0201254. Available from https://dx.plos.org/10.1371/journal.pone.0201254.

Van Der Ree R, Jaeger JAG, Van Der Grift EA, Clevenger AP, Van Der Ree R, Jaeger JAG. 2011. Effects of Roads and Traffic on Wildlife Populations and Landscape Function Road Ecology is Moving toward Larger Scales Guest Editorial, part of a Special Feature on Effects of Roads and Traffic on Wildlife Populations and Landscape Function Effects of Ro. Ecology and Society **16**:48.

Vitekere K, Tulizo K, Zaman M, Karanja H, Hua Y, Jiang G. 2020. INSIGHTS ON THE NORTH CHINA LEOPARD (PANTHERA PARDUS JAPONENSIS GRAY, 1862): CHALLENGES IN DISTRIBUTION, POPULATION STATUS, THREATS, AND IMPLICATIONS FOR CONSERVATION. The Journal of Animal and Plant Sciences **31**. Available from http://www.thejaps.org.pk/Volume/2021/31-01/01.php.

Waits LP, Paetkau D. 2005. Noninvasive Genetic Sampling Tools for Wildlife Biologists: A Review of Applications and Recommendations for Accurate

Data Collection. The Journal of Wildlife Management **69**:1419–1433. [Wiley, Wildlife Society]. Available from http://www.jstor.org/stable/3803503.

Wakefield S, Attum O, Robinson ER, Sandoka MA. 2008. Seasonal use of a waterhole by Nubian ibex Capra nubiana (Artiodactyla: Bovidae). mammalia **72**. Available from https://www.degruyter.com/document/doi/10.1515/MAMM.2008.014/html.

WAZA. 2013. Towards Integrated Species Conservation. Towards Integrated Species Conservation **14**:2–4. Available from http://www.waza.org/files/webcontent/1.public_site/5.conservation/integrated_species_conservation/WAZA Magazine 14.pdf.

Weeks AR et al. 2011. Assessing the benefits and risks of translocations in changing environments: a genetic perspective. Evolutionary Applications **4**:709–725. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.1752-4571.2011.00192.x.

Weinberg P. 2020. Capra caucasica (Western Tur). The IUCN Red List of Threatened Species. Available from https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T3794A22143809.en.

Weinberg P, Ambarli H. 2020. Capra aegagrus , Wild Goat. The IUCN Red List of Threatened Species. Available from https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T3786A22145942.en.

Weiser EL, Grueber CE, Jamieson IG. 2013. Simulating retention of rare alleles in small populations to assess management options for species with different life histories. Conservation biology : the journal of the Society for Conservation Biology **27**:335–44. Available from http://www.ncbi.nlm.nih.gov/pubmed/23330669.

Wheat RE, Allen JM, Miller SDL, Wilmers CC, Levi T. 2016. Environmental DNA from Residual Saliva for Efficient Noninvasive Genetic Monitoring of Brown Bears (Ursus arctos). PLOS ONE **11**:e0165259. Available from https://dx.plos.org/10.1371/journal.pone.0165259.

Wildt DE, Zhang A, Zhang H, Janssen DL, Ellis S. 2006. Giant Pandas: biology, veterinary medicine and management. Cambridge University Press.

Witzenberger KA, Hochkirch A. 2011. Ex situ conservation genetics: a review of molecular studies on the genetic consequences of captive breeding programmes for endangered animal species. Biodiversity and Conservation **20**:1843–1861. Available from http://link.springer.com/10.1007/s10531-011-0074-4.

Woodruff DS. 2001. Declines of biomes and biotas and the future of evolution. Proceedings of the National Academy of Sciences **98**:5471–5476. Available from https://pnas.org/doi/full/10.1073/pnas.101093798.

Wright S. 1943. ISOLATION BY DISTANCE. Genetics **28**:114–138. Available from https://academic.oup.com/genetics/article/28/2/114/6033172.

Wronski T et al. 2010. Two reciprocally monophyletic mtDNA lineages elucidate the taxonomic status of Mountain gazelles (Gazella gazella). Systematics and Biodiversity **8**:119–129. Available from http://www.tandfonline.com/doi/abs/10.1080/14772001003613192.

WWF. 2020. Living Planet Report 2020 - Bending the curve of biodiversity loss. Page WWF. Switzerland. Available from https://livingplanet.panda.org/.

Zarzoso-Lacoste D, Jan P-L, Lehnen L, Girard T, Besnard A-L, Puechmaille SJ, Petit EJ. 2018. Combining noninvasive genetics and a new mammalian sex-linked marker provides new tools to investigate population size, structure and individual behaviour: An application to bats. Molecular Ecology Resources **18**:217–228. Available from https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.12727.