Conservation Genetics Resources (2024) 16:293–305
https://doi.org/10.1007/s12686-024-01370-6

METHODS AND RESOURCES

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

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[5] · Haitham Al-Amri[3] · Bisnu B. Tiwari[3] · Said Nasser Al-Harsusi[3] · Abdullah Ghasab Al-Harsusi[3] · Qais Al-Rawahi[6] · Kara L. Dicks[2]

Received: 30 September 2022 / Accepted: 2 October 2024 / Published online: 9 October 2024
© The Author(s) 2024

Abstract
Conservation of elusive species affected by habitat degradation, population fragmentation and poaching is challenging. The remaining wild populations of a desert-adapted ungulate, Nubian ibex (*Capra nubiana*), within Oman are small and fragmented. 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 threatened species, it is essential to assess the genetic relationships between the wild and captive populations, and to investigate hybridisation with domestic goats (*Capra hircus*). We identified 5,775 high-quality SNPs using double digest restriction-site associated DNA (ddRAD), to assess genetic structure, gene flow and divergence between Oman's wild populations of Nubian ibex and in captivity, which are likely of North African provenance. We detected hybridisation with goats in captivity and recommend that genetic assessments of captive individuals are routinely used to evaluate their suitability for conservation programs. Building on previous mitochondrial evidence, substantial nuclear divergence (F_{ST} = 0.540) was found between wild Oman and captive populations, providing further evidence that Nubian ibex may be composed of multiple species and urgently needs a taxonomic review. Additionally, an appropriate insurance population should be established for Oman's threatened wild population. The data provided here will be invaluable for developing marker systems to assess wild populations using low-quality DNA from non-invasive sampling. Consequently, it will support further research into Nubian ibex throughout their range and highlights the need to integrate genetic information for effective conservation management of Nubian ibex.

Keywords ddRAD · SNP markers · *Capra* · Ex-situ conservation · Ungulate genetics · Oman · Hydridisation

Introduction

The conservation of wild species in their native habitat is a priority for any wildlife management program. However, many species are currently facing multiple threats imposed by anthropogenic activities, causing declines in wild populations (Nyhus 2016). In some cases, it may be appropriate to establish insurance populations to preserve a representative portion of the wild population for use as sources for reintroductions and reinforcements, known as ex-situ conservation (Price 1989; Beck et al. 1994; Leus 2011). If an ex-situ conservation strategy is to be effective, it is vital to determine precise taxonomies of the target populations to resolve any relationships between closely related species or populations, so the efforts and resources can be correctly targeted (Frankham et al. 2004). Setting conservation

✉ Mataab K. Al-Ghafri
alghafrimksa@yahoo.com

[1] School of Applied Sciences, Edinburgh Napier University, Edinburgh, UK

[2] RZSS WildGenes, Royal Zoological Society of Scotland, Edinburgh, UK

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

[4] Centre for Conservation & Restoration Science, Edinburgh Napier University, Edinburgh, UK

[5] Department of Biology, College of Science, Sultan Qaboos University, Muscat, Oman

[6] Department of Veterinary Medicine, A'Sharqiyah University, Ibra, Oman

strategies for understudied species is particularly challenging due to a lack of available data about their population size, dispersal, geographic range, threats, genetic diversity and taxonomy, and this can limit our understanding of the appropriate course of action for species recovery (Primack 2014).

Investigating the degree of distinction between species and populations assists with accurately identifying the level of vulnerability to extinction and, in turn, assists with prioritising appropriate management, to plan reintroduction or reinforcement strategies, and to avoid mixing between species or distantly related populations (Allendorf et al. 2012). Knowledge of the genetic diversity amongst populations of threatened species can be used for effective management planning, including developing plans for genetic rescue by translocating individuals and planning appropriate insurance populations suitable for future population reinforcement. Fundamentally, it is essential to preserve and protect multiple populations of endangered wild species in their current habitats to guarantee wide levels of genetic diversity which will ensure that the species retains the evolutionary resilience to cope with future environmental changes (Allendorf et al. 2012).

Nubian ibex are classified as Vulnerable by the IUCN Red List (Ross et al. 2020a) and are threatened by several human-induced changes, including hunting and habitat loss, that have exacerbated population fragmentation seen today (Giangaspero and Al-Ghafri 2014). In Oman, Nubian ibex were known to have a wide distribution through the southern and central regions of the country, while the rapid expansion of urbanisation has created small isolated populations, many of which have become locally extinct (Alkon et al. 2008). In the central region, the Al-Wusta Wildlife Reserve (WWR) population is thought to be small and isolated. Such populations have reduced gene flow, leading to inbreeding, accumulation of deleterious alleles and loss of genetic diversity (Frankham et al. 2019). Unchecked, these factors in the long-term can exacerbate population decline and increasingly smaller population sizes, which then might be subjected to detrimental stochastic events and increased risk of extinction (Reed 2004). Therefore, to conserve the Nubian ibex in Oman, it is vital to implement conservation management including enhanced protection, translocation and a captive-breeding program to act as an insurance population.

There is an existing captive-breeding program for Nubian ibex in Oman, but a recent study (Al-Ghafri et al. 2021) has raised concerns about its appropriateness and the possibility of taxonomic inaccuracy. Using mitochondrial DNA markers, Al-Ghafri et al. (2021) revealed significant differentiation between the wild population of the Nubian ibex in Oman and captive populations in both Oman and the United Arab Emirates (F_{ST}: D-loop 0.725, cytochrome b 0.968). In the same study, population structure was found within central and southern populations in Oman, and initial evidence that the southern population is more genetically diverse than the central one.

Whilst mitochondrial DNA analysis is a powerful tool for conservation, enabling rapid, low-cost genetic data generation from even the low-quality samples that are often available for threatened species (e.g. faecal samples), it is not recommended for use as a sole genetic marker (Hurst and Jiggins 2005). It has limited power to detect fine-scale genetic structure, especially within and among populations of the same species, or hybridisation because it is maternally inherited and thus represents only the female evolutionary history (Patwardhan et al. 2014). Multiple markers representative of the entire genome, such as microsatellites, single nucleotide polymorphisms (SNPs) and whole genome sequencing (WGS), can provide estimations of many biological parameters of vital importance to conservation biology, such as population connectivity, hybridisation, accurate estimations of genetic diversity, inbreeding, population structure and effective population size (Carroll et al. 2018).

The development of genetic resources for non-model species can be expensive and time-consuming, and requires high-quality DNA samples or reference genomes (Andrews et al. 2016). Reduced representation techniques to congruently identify and genotype SNPs, including double-digest restriction site-associated DNA sequencing (ddRAD; Peterson et al. 2012) have revolutionised their utility in conservation (Hohenlohe et al. 2021). ddRAD sequences DNA which has been fragmented by two restriction enzymes to genotype thousands of SNPs across the genome in a relatively inexpensive way without the need for existing species-specific genomic resources. However, this approach needs relatively high-quality DNA that retains intact restriction enzyme sites to minimise bias in genome representation between sample types and produce sequences with sufficiently high read depth to yield accurate SNP genotypes. Ascertainment bias during SNP identification can skew conclusions (Lachance and Tishkoff 2013), particularly where SNPs are called using a non-specific reference genome. This bias could influence certain applications, such as studies of rare variants or specific genomic regions not well-represented in the ascertainment process (Geibel et al. 2021). It can result in the underestimation of heterozygosity, overestimation of inbreeding coefficients and can result in biased patterns of population structure and distort the signal of selective processes.

The use of ddRAD for SNP genotyping has proven informative and accurate in assisting practitioners to develop conservation plans. For example, SNP panels generated by

ddRAD have been used to assess relatedness and inbreeding (sturgeon (*Acipenser sturio*) - Roques et al. 2019), produce conservation and sustainable management plans (e.g. beavers (*Castor fiber*) - Senn et al. 2014; elephants (*Loxodonta cyclotis*) - Bourgeois et al. 2018; and Siamese rosewood (*Dalbergia cochinchinensis*) - Hartvig et al. 2020), delineate taxonomy, divergence and gene flow (black-footed albatrosses (*Phoebastria nigripes*) - Dierickx et al. 2015; manta rays (Mobulidae) - Hosegood et al. 2020; canids - Krofel et al. 2022), investigate population structure, genetic diversity and speciation (ticks (*Dermacentor variabilis*) - Lado et al. 2019; Arabian tahr (*Arabitragus jayakari*) - Ross et al. 2020b), identify microsatellites (goldsinny wrasse (*Ctenolabrus rupestris*) -Jansson et al. 2016) and develop DNA capture probes for scat samples (snow leopards (*Panthera uncia*) - Janjua et al. 2020). It has also been used for monitoring hybridization and introgression between populations (Senn et al. 2019; Mirzaei and Wesselingh 2021), and delineating the evolutionary history of species (Muniz et al. 2018).

Here, we employ a ddRAD approach to characterize and genotype nuclear SNPs in wild and captive Nubian ibex. This will create a valuable genetic toolkit for the species which will allow us to: (i) evaluate the population genetic structure and assess levels of genetic diversity; (ii) confirm nuclear genetic differentiation between the wild and captive population; (iii) assess any possible hybridisation or introgression between domestic goats and Nubian ibex; (iv) aid in developing a set of nuclear baits probes used to capture SNPs from low quality DNA. The overall aim of using this is to use the information obtained as a monitoring tool that will assist future sustainable management and conservation of this threatened species in the wild.

Materials and methods

The total sample set consisted of 47 samples representing wild Nubian ibex, captive-bred Nubian ibex and domestic goats. The wild ibex samples consist of twelve Nubian ibex samples (11 tissue samples and one blood sample) collected from wild animals at Al-Wusta Wildlife Reserve ($n=2$), Shalim ($n=9$) and one tissue sample was obtained from the Natural History Museum of Oman from a whole frozen animal originated from WWR (stored at -20 C, died 1988). The nine tissue samples were obtained from nine confiscated animals across two separate incidents, and a tissue sample taken during the post-mortem of a wild-caught animal which died prior to its return to the wild. A whole blood sample (EDTA) was taken from a wild, live-caught animal during the fitting of radio collaring for future monitoring.

The samples from captive animals consist of 30 blood samples collected from captive centres in Oman (one institution) and United Arab Emirates (UAE, two institutions). These whole blood (EDTA) samples were taken after approval obtained from the centres' authorities and during routine veterinary examination.

Lastly, five blood samples from goats were collected from Al-Wusta Wildlife Reserve (WWR), approved by both the reserve administration and the owner, and collected during veterinary treatments. All samples described above were collected between 2013 and 2018 and are detailed in supplementary material (S1).

DNA extraction

DNA from tissue and blood samples were extracted using DNeasy Blood and Tissue kit (QIAGEN®, Germany) according to the manufacturer's instructions. A detailed methodology is included in the supplementary materials of Al-Ghafri et al. (2021).

Construction of ddRAD libraries

SNP discovery was conducted via ddRAD sequencing using a modified Peterson et al. (2012) protocol, described in Brown et al. (2016) and Manousaki et al. (2016). Detailed methods and minor additional modifications are included in supplementary material (S2) and are described briefly here. DNA was normalised to 7ng/µl prior to digestion with *SbfI* (restriction site; CCTGCA^GG) and *SphI* (restriction site; GCATG^C). Combinatorial 5–7 bp inline barcodes and Illumina primer sequences were then ligated to DNA fragments, after which the samples were pooled and size-selected to (400 –700 bp). The purified DNA was amplified by PCR to incorporate Illumina adapter sequences, and the resultant, cleaned library was sequenced on a single lane of an Illumina HiSeq 2500/4000/X. ddRAD and sequencing was performed across two separate libraries, with positive controls within each library and across libraries to enable data quality checking (see the supplementary material (S1) for a list of the repeated samples).

Bioinformatics

FastQC (Andrews 2010) was used to check for the quality of reads for each sample. The raw reads were trimmed to 135 bp and demultiplexed using the *process_radtags* module in STACK v2.52 (Rochette et al. 2019) with default parameters to remove low quality reads and those with uncalled bases.

SNP genotyping was performed as described in Dicks et al. (2023) by first aligning reads against the domestic goat

reference genome (*Capra hircus*) (GCA_001704415.1) using the Burrows-Wheeler Aligner v0.7.17 (Burrows and Wheeler 1994). Closely related reference genomes, including congeneric species, have been shown to be highly effective for SNP identification where conspecific references are unavailable, with limited bias on downstream analyses (Galla et al. 2019; Samaha et al. 2021). Unmapped reads were excluded using Samtools v1.10 (Li et al. 2009) and SNPs were called using *gstacks* in STACKS with the marukilow model, retaining only the first SNP on a RAD tag. A custom Snakemake pipeline (Koster and Rahmann 2012) was used for mapping and SNP calling (Dicks et al. 2023).

Genotype filtering

SNP filtering was conducted for all samples (30 captives, 11 wilds and 5 goats) using PLINK 1.9 (Chang et al. 2015). Loci were discarded if more than 10% of the genotypes were missing and if the minor allele frequency (MAF) fell below 5%. Individuals were excluded if more than 10% of the genotypes were missing.

Linkage disequilibrium was calculated for each of the three putative populations independently (captive ibex, wild ibex, and goats) after excluding two putative hybrids (NUB021 and NUB039 – see below for classification criteria). For any pair of SNPs with r^2 of 0.5 or greater in any single population, we retained the SNP with the highest global genotyping rate (Fig. S1). Additionally, we assessed SNPs for deviations from Hardy-Weinberg Equilibrium (HWE) for each population using PLINK, where SNPs deviating from HWE in multiple populations were assumed to be technical artifacts. No SNPs were found to deviate from HWE in multiple populations after correcting for multiple testing using the Benjamini and Yekutieli (2001) false discovery rate.

Kinship was estimated to determine repeatability across positive controls and assess levels of relatedness amongst samples. The KING-robust method (Manichaikul et al. 2010), an allele-frequency independent method, was selected due to the expectation of admixture and variation in allele-frequency spectrums amongst populations/species. KING-robust estimates were calculated within NgsRelate v2 (Hanghøj et al. 2019; Nøhr et al. 2021) and relationships were supported by comparison with R0 and R1 estimates following Waples et al. (2019). We pruned the sample set to retain a single genotyping attempt for all samples identified as identical, known hereafter as the "full sample set". We then further pruned the sample set to retain only those individuals considered third-degree relatives or more distantly related by applying a KING-robust threshold of 0.0884 within the PLINK 2.0 command `--king-cutoff`, known hereafter as the "unrelated sample set". All subsequent analyses were carried out using the "unrelated sample set" unless specified.

Many population genetic analyses assume independence of loci (e.g. STRUCUTRE). Linkage between loci increases as inbreeding increases within populations, and therefore identifying independent loci becomes increasingly challenging in such populations. Therefore, the effect of increasingly stringent linkage disequilibrium (LD) thresholds was assessed: $r^2 < 0.5$, $r^2 < 0.2$ and $r^2 < 0.05$, estimated using PLINK 1.9 (Chang et al. 2015) using the "unrelated sample set" after excluding repeated samples.

Population structure

The Bayesian clustering method STRUCTURE v2.3.4 (Pritchard et al. 2000) was used to assess the demographic history of the population, parallelised using ParallelStructure v1.0 (Besnier and Glover 2013) and each number of clusters (K 1 to 8) was repeated 10 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. POPHELPER (Francis 2017) and CLUMPP (Jakobsson and Rosenberg 2007) were used to combine the outputs from multiple runs and align clusters for visualisation. The Evanno method (Evanno et al. 2005) was used to assess the most probably number of clusters and visualised using POPHELPER (Francis 2017). STRUCTURE was carried out using the "full sample set" and the "unrelated sample set".

With all three populations included (goats, wild ibex and captive ibex), individuals were classified as putative hybrids when an individual had at least 25% assignment to an alternative population cluster K = 3. Additionally, STRUCTURE was carried out for wild and captive ibex within the "unrelated sample set" only, both including (4,449 variable SNPs and 22 samples) and excluding (2,297 variable SNPs and 21 samples) the putative hybrid.

To further investigate population structure, we used the ADEGENET R package (Jombart 2012) to run Principal Component Analysis (PCA). Pairwise F_{ST} was calculated in HIERFSTAT (Goudet 2005; de Meeûs and Goudet 2007) after excluding putative hybrids, and significance was calculated using 999 bootstraps with 95% confidence intervals.

Population genetic diversity analysis

Prior to estimates of genetic diversity, putative hybrid individuals (NUB021 and NUB039) were excluded. Observed (H_O) and expected heterozygosity (H_E) were estimated using HIERFSTAT version 0.5.10 in R (Goudet 2005; de Meeûs and Goudet 2007). Allelic richness (AR) and private allelic richness (PA) between the wild and captive

individuals were calculated using the rarefaction method in ADZE-1.0 (Szpiech et al. 2008) based on the smallest sample size (wild samples; $n = 7$). Unless specified, all analyses were carried out in R version 4.1.3 (R Core Team 2022). PGDspider v.2.0.5.0 (Lischer and Excoffier 2012) was used to convert file types.

Results

SNP discovery and genotype filtering

A total of 32,461 SNPs were identified and genotyped in this study. After SNP filtering, 5,775 SNPs were retained for subsequent analysis (Fig. S2). These included 5,500 SNPs mapped to 29 autosomal chromosomes on the domestic goat genome, 155 SNPs located on the sex chromosomes, and a further 120 SNPs located on unassembled scaffolds. There were 1,271 variable SNPs in wild Nubian ibex, 1,405 variable SNPs in captive Nubian ibex, and 973 variable SNPs in goats. After removing low quality and repeated samples, 46 individual samples were retained (11 wild Nubian ibex, 30 captive Nubian ibex and 5 goats). An initial assessment of population structure identified two captive ibex individuals (NUB021 and NUB039) as putative hybrids with goats (Fig. S3).

Kinship analyses identified all positive control samples as identical (wild ibex NUB003 and NUB153, and goat HIR001), demonstrating high repeatability of genotyping between and within libraries. Additionally, two wild ibex individuals were identified as being sampled repeatedly (individual 1 - NUB002, NUB005, NUB007 and NUB008; individual 2 – NUB003, NUB004; see Table S1). A typical kinship distribution was found across the captive ibex, except for the two individuals detected as hybrids which showed depressed R1 estimates (Fig. S4). Pruning related individuals removed a further 15 captive ibex, resulting in the "unrelated sample set" containing seven wild ibex, 15 captive ibex (including one of the two putative hybrids) and five goats (Table S2).

The impact of LD thresholds was tested by increasing stringency from r^2 of 0.5, testing r^2 0.2 and r^2 0.05, resulting in the total number of SNPs decreasing from 5775 to 2184 and 1509, respectively. Within these SNP sets, the number of variable SNPs dropped to a minimum of 135 within the wild population (Fig. S5a). Population structure analyses using PCA and STRUCTURE showed minimal impact of LD threshold on the major patterns between the three primary populations (captive and wild ibex, and goats) (Fig. S5B-D). Additionally, at both r^2 0.2 and 0.05, putative hybrid individuals (discussed subsequently in more detail) could not be detected by PCA, and admixture detected by STRUCTURE was less pronounced than at r^2 0.5. F_{ST} estimates at more stringent LD thresholds also show the same patterns of divergence (Table S3), where pairwise F_{ST} increases as the LD threshold becomes more stringent. The LD threshold of $r^2 = 0.5$ was therefore selected due to the enhanced information provided by greater number of SNPs, given the minimal impact of allowing some non-independence of loci on downstream analyses and acknowledging the inherent challenges of assessing linkage across putatively divergent taxa in the context of raised identity-by-descent.

Population structure

Both STRUCTURE and PCA analyses reveal three primary groupings: wild Nubian ibex, captive Nubian ibex and goats (Fig. 1A and B). The most likely number of clusters (K) in STRUCTURE was found to be between three and four using the Evanno method (Fig. S6). For all values of K, goats were identified as clustering independently from both the wild and captive ibex (Fig. 1A), suggesting reduced divergence between the wild and captive ibex than between both ibex groups and goats. However, separation of the wild and captive ibex was apparent from K = 3 (averaged Q scores shown in Table S4). This pattern was reflected in the PCA analysis where PC1, which captured 69.1% of the variation, separates the ibex from goats (Fig. 1B). From K = 3 onwards in STRUCTURE, additional population structure was identified between wild and captive Nubian ibex (Fig. 1A). PC2 also detected this divergence between the two Nubian ibex groups (Fig. 1B).

One captive Nubian ibex (NUB021) showed high levels of admixture between goats and captive Nubian ibex in STRUCTURE (Fig. 1A) and divergence from the primary population clusters in the PCA (Fig. 1B). During an initial STRUCTURE analysis prior to kinship filtering, a second individual (NUB039) was found to be related to NUB021 and showed similar admixture (Fig. S2A). These two individuals were therefore considered putative ibex: goat hybrids.

Population structure between wild and captive Nubian ibex was further investigated by repeating these analyses for ibex only, excluding both goats and putative hybrid individuals (Fig. 1C and D). The Evanno method identified the most likely value of K to be either two or three (Fig. S5). Wild ibex are first separated from captive ibex in both STRUCTURE K = 2 (Fig. 1C) and PCA (Fig. 1D), where PC1 reflects this divergence, capturing 49.9% of the variation. Additional substructure is subsequently identified within the captive ibex population. In STRUCTURE, six captive ibex showed assignment to both the captive ibex (cluster 1 in Fig. 1C) and an alternative cluster (cluster 2 or 3 in Fig. 1C), however only for three individuals was

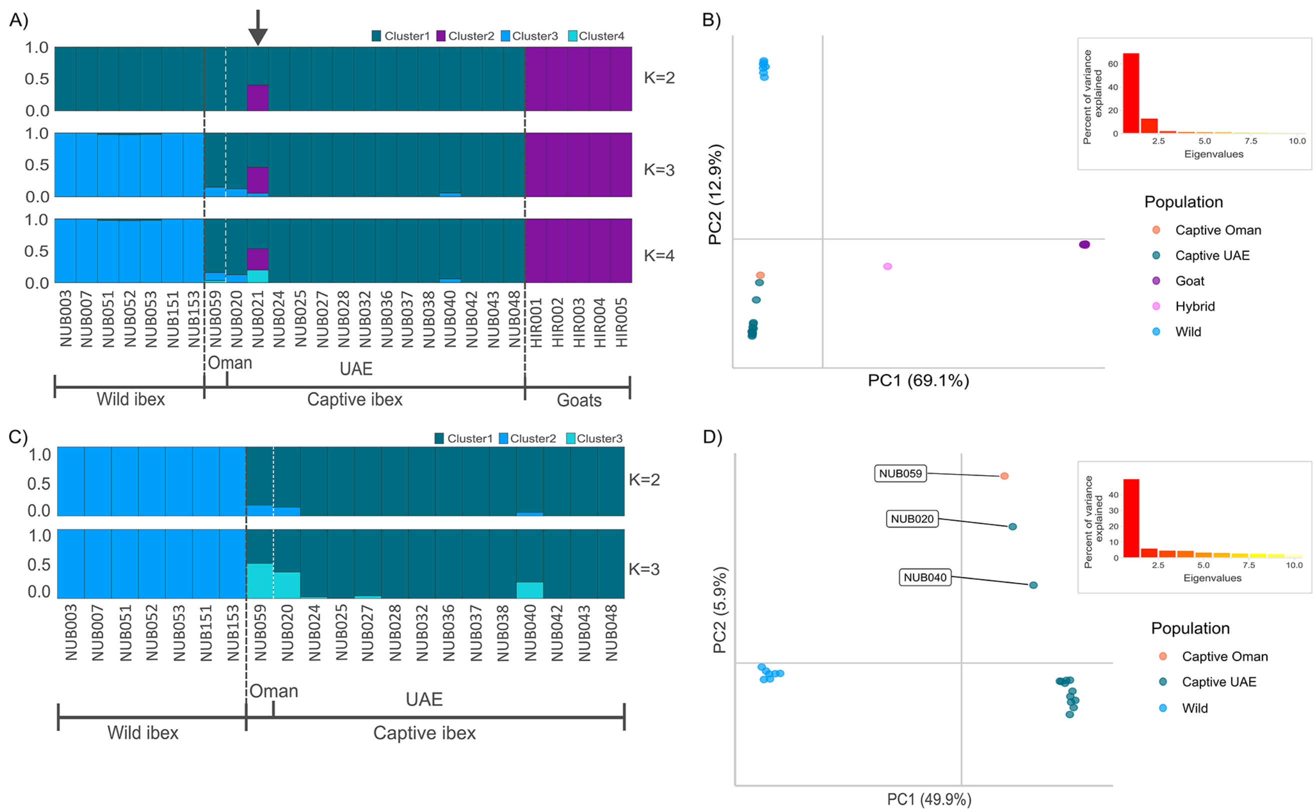

Fig. 1 Population *structure of unrelated samples of wild Nubian ibex, captive Nubian ibex and goats (5775 variable SNPs)* (**A**) and (**B**), and of wild and captive ibex only (2297 variable SNPs) (**C**) and (**D**). Inferences from STRUCTURE are visualised in (**A**) and (**C**). Each column represents 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. The arrow indicates the individual identified as a putative hybrid. PCA principal components (PC) 1 and 2 are visualised in (**B**) and (**D**), where each point represents an individual and colours indicate the population it was sampled from (legend shown on right). The proportion of variance captured by each PC is shown on axis titles in brackets. Inserts show the ten highest eigenvalues for each PCA

Table 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

	Wild	Captive	Goats
Wild		0.498-0.529	0.874-0.885
Captive	0.514		0.876-0.888
Goats	0.880	0.882	

the assignment greater than 0.05 (averaged Q scores shown in Table S5). At K = 2, the secondary cluster was the same as wild ibex. At K = 3, a unique third cluster was identified as the source of the admixture for those three individuals (NUB020 – $Q_{Cluster3}$=0.382, NUB040 – $Q_{Cluster3}$=0.236, and NUB059 – $Q_{Cluster3}$=0.506) (Fig. 1C). Within the PCA, PC2 (5.9% of variation) captures structure within the captive ibex population and the same three high-proportion admixed individuals were identified as deviating from the main cluster (Fig. 1D). These three individuals did not deviate towards the wild ibex cluster, supporting a possible alternative source of admixture.

Pairwise F_{ST} between wild and captive ibex was 0.514 (Table 1). Higher levels of fixation were observed for comparisons between wild ibex and goats (F_{ST} = 0.880) and between the captive ibex population and goats (F_{ST} = 0.882).

Population genetic diversity

Table 2 shows the population genetic diversity estimates for the three populations. H_O and H_E estimates were overlapping within and between wild (Ho = 0.080 and H_E = 0.089), but captive Nubian ibex had reduced observed heterozygosity compared with expectations (Ho = 0.072 and H_E = 0.082). Estimates for goats were marginally lower (Ho = 0.072 and H_E = 0.073) but overlapped with estimates for captive ibex. F_{IS} was raised in both the wild and captive populations (wild F_{IS} = 0.081; captive F_{IS}= 0.112), with 95% CI not overlapping zero, indicating a deviation from Hardy-Weinberg Equilibrium in this population.

A subtle difference was found in private alleles between wild (PA = 0.178) and captive populations (PA = 0.170). The

Table 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}), private allelic richness (PA) and allelic richness (AR). 95% confidence limits (CL) are shown in brackets for each measure. NA indicates this measure was not calculated

Population	Sample size	Number of variable SNPs	H_O (95% CL)	H_E (95% CL)	F_{IS} (95% CL)	AR (95% CL) ($n=7$)	PA (95% CL) ($n=7$)
Wild	**7**	1,263	0.080 (0.075–0.084)	0.089 (0.084–0.094)	0.081 (0.059–0.104)	1.1957 (1.1956–1.1958)	0.1779 (0.1777–0.1780)
Captive	14[a]	1,397	0.073 (0.069–0.077)	0.082 (0.078–0.087)	0.112 (0.096–0.129)	1.1872 (1.1871–1.1873)	0.1693 (0.1692–0.1695)
Goats	5	973	0.072 (0.067–0.077)	0.073 (0.069–0.078)	-0.006 (-0.034–0.022)	NA	NA

[a] note that one captive individual identified as a putative hybrid was excluded from these analyses

allelic richness was found to be slightly higher in the wild population compared to the captive (AR = 1.20 and 1.19 for wild and captive respectively). Detailed results with confidence intervals of the estimation of the allelic richness and private alleles between populations are shown in Table S6.

Discussion

The availability of genomic resources for organisms of high conservation importance is advantageous to support conservation management decisions by enabling targeted approaches for degraded DNA (Primmer 2009), and by generating reliable and precise estimations of population genetic statistics and identify relationships between populations and species. Here, we developed this resource for Nubian ibex in the form of > 32,000 RAD-tags which can be used for future development a genotyping system for degraded DNA samples, such as target enrichment probes. Using this resource here, we were able to carry out a first genomic assessment of Nubian ibex using 5,775 high-quality SNPs to carry out a preliminary assessment of the wild ibex in Oman and determine the utility of the current captive insurance populations for reintroductions and reinforcement in Oman.

Captive Nubian ibex are divergent from wild Omani Nubian ibex, and not just because of recent genetic drift induced by small population size in captivity.

We identified clear evidence of divergence between the wild and captive Nubian ibex populations using both Structure and PCA. This was supported by a high level of differentiation using pairwise F_{ST} (F_{ST} = 0.514). This level of divergence is in line with whole genome sequencing SNP estimates for giraffe subspecies (F_{ST} 0.51–0.62) (Bertola et al. 2024) for which taxonomic revision to species level has been recommended (Fennessy et al. 2016). These results support those obtained by the mitochondrial study, which did not detect any shared haplotypes between the same wild and captive Nubian ibex populations, and where the F_{ST} estimated for D-loop was 0.725 and for cytochrome *b* was 0.968 (Al-Ghafri et al. 2021). Although signals of population strucure can arise rapidly in captive populations because they often experince high levels of drift due to small founder base, the differntiation observed here is extreme. The current results support the possibility that these might be different taxa.

The native range of Nubian ibex is naturally fragmented throughout the Arabian peninsula, the Levant and North Africa (Ross et al. 2020a). Anecdotal evidence suggests that the founder base of the captive population originated in North Africa (Al-Ghafri 2023). Without additional samples from across the native range of Nubian ibex, it is therefore not possible to fully evaluate the potential combined influences of isolation by distance, evolutionary divergence due to speciation, and genetic drift or selection within captivity on the results presented here. However, they do highlight an urgent need for further study to understand the population structure and taxonomy of Nubian ibex using both genetic and traditional taxonomic methods. The levels of divergence seen suggest very low levels of gene flow between these two populations for an extended period and that the risk of outbreeding depression is not insignificant if these populations are mixed (Frankham et al. 2011).

Captive populations should not be used for reinforcements or reintroductions in Oman, and possibly elsewhere in the southern Arabian Peninsula. Our results suggest that there are no known appropriate insurance populations for the populations of Nubian ibex from Oman, at least within the captive instsitutions assessed.

Hybridisation within captive nubian ibex

Two samples from the captive population were identified as putative hybrids using STRUCTURE. We sequenced two mitochondrial markers (D-loop and cytochrome *b*) following Al-Ghafri et al. (2021) (data not shown here), and both individuals had haplotypes matching domestic goats. This evidence is strongly indicative of a recent cross with domestic goats.

In addition to introgression from goats, evidence of introgression into the captive population from a secondary source was detected (Fig. 1). Recent introgression from the Omani population of wild ibex into the captive population is possible (Fig. 1A); however, when considering ibex only, both STRUCTURE K = 3 (Fig. 1C) and PCA (Fig. 1D) suggest an alternative source. This alternative source was not detected when goats were included in the analyses (Fig. 1A and B), which is unsurprising when source populations are unsampled "ghost populations" (Lawson et al. 2018), and may be exacerbated by a bias towards alleles shared with goats resulting from SNP calling using the goat reference genome. In an additional analysis (Fig. S3B), the presence of a single ibex: goat hybrid within the ibex-only analysis was, however, sufficient to separate goat ancestry from the "ghost" source population (K = 4 onwards). It is therefore likely that the captive ibex have experienced admixture from either (i) other unsampled geographic localities of Nubian ibex or (ii) another species which is more closely related to Nubian ibex than goats.

Hybridization between non-goat *Capra spp* and the domestic goat *C. hircus* is known to occur and can produce fertile offspring (Iacolina et al. 2019). For example introgression was found between Alpine ibex (*C. ibex*) and domestic goats in the Swiss Alps (Grossen et al. 2014), and between Alpine ibex, Nubian ibex and bezoar (*C. aegagrus*) (Turcek and Hickey 1951). Introgression is increasingly possible where pastoralism and wild populations overlap (Harrison and Larson 2014), and occurs accidently or deliberately during captive breeding (Stüwe and Nievergelt 1991).

Introgression from domestic goats into the captive Nubian ibex population presents potential threats to the captive population, such as genetic swamping and outbreeding depression (Adavoudi and Pilot 2021). In addition, hybrid individuals may face enhanced challenges to survival upon release due to a combination of adaptation to captivity and a loss of genetic adaptations to the extreme environments that Nubian ibex inhabit (Muñoz-Fuentes et al. 2010; Torres et al. 2017). However, the impact of hybridisation can range from negligible to significant effects, which either put species survival in jeopardy or can serve as a potential pathway to evolution (Hirashiki et al. 2021). Both putative ibex: goat individuals are likely first or second generation crosses (NUB021 $Q_{Alternative}$=0.474/0.62, NUB039 $Q_{Alternative}$=0.345/0.481, where $Q_{Alternative}$ is the sum of assignments to alternative clusters at K = 3/K = 4), so it remains unclear if these they would be fully fertile. Using hybridised individuals within reintroductions could lead to introgression of non-ibex alleles into the wild population, with unknown consequences. Therefore, detecting hybrids is of high importance in captive breeding centers to ensure appropriate insurance populations exist and is vital when selecting individuals for reintroduction efforts.

The existence of introgression from goats into captive Nubian ibex raises concern about possibility of this happening in wild populations, especially with increasing encroachment of the land for pastoralism. These concerns need to be addressed in detail in future studies through wider sampling and regular monitoring of the wild Nubian ibex population to ascertain if there is any evidence of possible hybridisation and assess the likelihood of this risk. The power of the SNP panel developed in this study to detect hybridisation is evident and it will therefore be valuable for future monitoring of hybridisation in wild populations.

Genetic diversity within nubian ibex

Although the primary purpose of these analyses was to develop a genetic toolkit for Nubian ibex, we were able to take a preliminary look at the nuclear genetic diversity within the captive population and Oman's wild Nubian ibex. The captive populations were previously found to have low mitochondrial diversity (Al-Ghafri et al. 2021), and nuclear data continues to indicate low levels of genetic diversity. The fixation index was raised for the captive ibex (F_{IS} = 0.113 ± 0.016 after excluding the putative hybrid), which is indicative of raised inbreeding levels. It is possible this could be a Wahlund effect, whereby observed heterozygosity is reduced compared to expectations due to the presence of multiple populations, given the evidence of low levels of introgression into the captive population (Fig. 1). Captive populations frequently have low genetic diversity due to genetic drift and small founder bases (Jiang et al. 2005; Reiners et al. 2014; Chen et al. 2019), although this can be mitigated by appropriate management (Lacy et al. 2012).

Despite our limited sample set from a single subpopulation (Shalim) in Oman, we found that genetic diversity shows indications of being reduced (F_{IS} = 0.081 ± 0.023). The small sample size of the wild ibex population used in this study may lead to ascertainment bias, particularly reduced identification of rare alleles (Malomane et al. 2018), and an underestimation of its genetic diversity (Kalinowski 2005). Shalim was previously found to have the highest haplotype diversity, but intermediate nucleotide diversity at the mitochondrial D-loop, compared to other populations in Oman (Al-Ghafri et al. 2021). Therefore, caution must be taken in extrapolating these results across Oman. A larger study is required to assess nuclear diversity throughout the wild population and careful conservation management may be required to prevent further loss of genetic diversity.

Development of a genetic resource to inform conservation

Conservation management decisions are greatly enhanced by integrating genetic information (Pierson et al. 2016; Hoban et al. 2022), and reduced genome representation methods have proven valuable for generating accurate information for selecting individuals for captive breeding and for release into the wild (Chattopadhyay et al. 2019; Ogden et al. 2020). The thousands of nuclear loci developed in this study have already identified substantial divergence between wild and captive ibex, identifying a need for taxonomic review and a lack of appropriate source population for reintroduction into Oman.

The preliminary population genetic data presented here for the wild Oman population hint at reduced diversity and a need for further assessment to define appropriate management action for this metapopulation. Nubian ibex are elusive and inhabit challenging terrain, and faecal samples are frequently the only available additional material for genetic analysis (Al-Ghafri et al. 2021). The > 32,000 RAD-tags generated here will enable the development of advanced molecular techniques, such as target capture (Jones and Good 2016) will be needed to overcome the challenges presented by high levels of non-target DNA and degraded target DNA. Once developed, such a method would also enable on-going monitoring of wild populations with directly comparable genetic data.

Conclusion

The evaluation of population structure of Nubian ibex in Oman will play an imperative role in directing conservation efforts towards the successful genetic management of this species. Achieving this goal has thus far been greatly hampered by a lack of nuclear genetic resources. The thousands of SNPs discovered in this study for this threatened and understudied species in the far east of the Arabian Peninsula will provide an important conservation toolbox to aid in its protection and provide a vital foundation for future conservation planning.

This research has revealed an urgent need to review taxonomy of Nubian ibex throughout its range to understand whether or not Nubian ibex in Oman should be considered to be a different subspecies or species and how this relates to (as yet unsampled) populations across the wider Arabian Peninsula. Should taxonomic review separate these groups, the southern Arabian Nubian ibex will be at a much greater extinction risk than current assessments conclude (Ross et al. 2020a). Additionally, these data provide immediately relevant conservation information. Firstly, they have provided clarity that an appropriate insurance population for Oman is lacking, and that reintroduction efforts should carefully consider the genetic provenance of source populations, both in terms of appropriateness of the founder base as well as introgression from domestic goat. Finally, they reveal that the inbreeding levels of the wild population are uncertain and further genetic assessments are needed. The genomic tools generated throughout this study will greatly facilitate empirical evidence on which conservation strategies can be fine-tuned.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12686-024-01370-6.

Acknowledgements We would like to express our sincere thanks to the Diwan of the Royal Court, Oman, for the grant and support to this project. We would like to thank Mr Yasser Al Salami, The Director General of Office for Conservation of Environment for his continuous support to the project. Also, a special thanks to Al-Wusta Wildlife Reserve administration for providing help and logistical support for samples collection. 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 and Mark Craig, Al Ain Zoo, UAE for the sharing of captive samples. Thanks to Muhammad Ghazali and Jennifer Kaden from RZSS WildGenes for assistance and guidance in the lab.

Author 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 bioinformatic analyses, supervised SNP filtering and population genetic analyses, and participated in editing and reviewing the manuscript. All authors gave final approval for publication.

Funding This project is funded and supported by the Diwan of the Royal Court and Office for Conservation of Environment, Sultanate of Oman. However, the funders did not have any additional role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability The raw, demultiplexed ddRAD data for this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB54683. SNP calling is available on GitHub (https://github.com/karadicks/Nubian_ibex_SNPs). The link will lead to three files. The first one is a VCF file containing the 32461 unfiltered SNPs. The two remaining files are PLINK files containing the filtered 5775 SNPs).

Declarations

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

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

Al-Ghafri MK (2023) Genetic assessment and development of genomic resources for Nubian ibex conservation management in Oman [Unpublished doctoral dissertation]. Edinburgh Napier University. https://doi.org/10.17869/enu.2024.3789498

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. https://doi.org/10.1098/rsos.210125

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. https://doi.org/10.2305/IUCN.UK.2008.RLTS.T3796A10084254.EN

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

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

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

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

Benjamini Y, Yekutieli D (2001) The control of the false discovery rate in multiple testing under dependency. The Annals of Statistics 29. https://projecteuclid.org/journals/annals-of-statistics/volume-29/issue-4/The-control-of-the-false-discovery-rate-in-multiple-testing/10.1214/aos/1013699998.full

Bertola LD et al (2024) Giraffe lineages are shaped by major ancient admixture events. Current Biology. https://linkinghub.elsevier.com/retrieve/pii/S0960982224002343

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. https://dx.plos.org/https://doi.org/10.1371/journal.pone.0070651

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. http://www.jstor.org/stable/2387021%0Ahttp://about.jstor.org/terms

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. http://bmcgenomics.biomedcentral.com/articles/https://doi.org/10.1186/s12864-016-2773-4

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

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. https://onlinelibrary.wiley.com/doi/https://doi.org/10.1111/eva.12600

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. https://academic.oup.com/gigascience/article-lookup/doi/https://doi.org/10.1186/s13742-015-0047-8

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. https://onlinelibrary.wiley.com/doi/https://doi.org/10.1111/mec.15023

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. https://peerj.com/articles/6643

de Meeûs T, Goudet J (2007) A step-by-step tutorial to use HierFstat to analyse populations hierarchically structured at multiple levels. Infect Genet Evol 7:731–735

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

Dierickx EG, Shultz AJ, Sato F, Hiraoka T, Edwards SV (2015) Morphological and genomic comparisons of hawaiian and Japanese black-footed albatrosses (Phoebastria nigripes) using double digest RADseq: implications for conservation. Evol Appl 8:662–678. https://doi.org/10.1111/eva.12274

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. https://onlinelibrary.wiley.com/doi/https://doi.org/10.1111/j.1365-294X.2005.02553.x

Fennessy J, Bidon T, Reuss F, Kumar V, Elkan P, Nilsson MA, Vamberger M, Fritz U, Janke A (2016) Multi-locus Analyses Reveal Four Giraffe Species Instead of One. Current Biology 26:2543–2549. https://linkinghub.elsevier.com/retrieve/pii/S0960982216307874

Francis RM (2017) pophelper: an R package and web app to analyse and visualize population structure. Molecular Ecology Resources 17:27–32. https://onlinelibrary.wiley.com/doi/https://doi.org/10.1111/1755-0998.12509

Frankham R, Ballou JD, Briscoe DA, McInnes KH (2004) A Primer of Conservation Genetics. Cambridge University Press. 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. https://onlinelibrary.wiley.com/doi/https://doi.org/10.1111/j.1523-1739.2011.01662.x

Frankham R, Ballou JD, Ralls K, Eldridge M, Dudash MR, Fenster CB, Lacy RC, Sunnucks P (2019) A practical guide for genetic management of Fragmented Animal and Plant populations. A practical guide for genetic management of Fragmented Animal and Plant populations

Galla SJ, Forsdick NJ, Brown L, Hoeppner MP, Knapp M, Maloney RF, Moraga R, Santure AW, Steeves TE (2019) Reference genomes from distantly related species can be used for discovery

of single nucleotide polymorphisms to inform conservation management. Genes 10. https://doi.org/10.3390/genes10010009
Geibel J, Reimer C, Weigend S, Weigend A, Pook T, Simianer H (2021) How array design creates SNP ascertainment bias. PLoS ONE 16:1–23. https://doi.org/10.1371/journal.pone.0245178
Giangaspero M, Al-Ghafri MK (2014) Poaching: A Hreat for Vulnerable Wild Animal Species in Oman. Tropical Medicine & Surgery 2. https://www.walshmedicalmedia.com/open-access/poaching-a-threat-for-vulnerable-wild-animal-species-in-oman-2329-9088.1000e121.pdf
Goudet J (2005) hierfstat, a package for r to compute and test hierarchical F-statistics. Molecular Ecology Notes 5:184–186. http://doi.wiley.com/https://doi.org/10.1111/j.1471-8286.2004.00828.x
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. https://dx.plos.org/https://doi.org/10.1371/journal.pgen.1004438
Hanghøj K, Moltke I, Andersen PA, Manica A, Korneliussen TS (2019) Fast and accurate relatedness estimation from high-throughput sequencing data in the presence of inbreeding. GigaScience 8. https://academic.oup.com/gigascience/article/doi/https://doi.org/10.1093/gigascience/giz034/5481763
Harrison RG, Larson EL (2014) Hybridization, Introgression, and the Nature of Species Boundaries. Journal of Heredity 105:795–809. https://academic.oup.com/jhered/jhered/article/2961884/Hybridization
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. https://link.springer.com/https://doi.org/10.1007/s10592-020-01279-1
Hirashiki C, Kareiva P, Marvier M (2021) Concern over hybridization risks should not preclude conservation interventions. Conservation Science and Practice 3. https://onlinelibrary.wiley.com/doi/10.1111/csp2.424
Hoban S et al (2022) Global genetic diversity status and trends: towards a suite of Essential Biodiversity Variables for genetic composition. Biological Reviews 97:1511–1538. https://onlinelibrary.wiley.com/doi/https://doi.org/10.1111/brv.12852
Hohenlohe PA, Funk WC, Rajora OP (2021) Population genomics for wildlife conservation and management. Molecular Ecology 30:62–82. https://onlinelibrary.wiley.com/doi/https://doi.org/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. https://onlinelibrary.wiley.com/doi/https://doi.org/10.1111/mec.15683
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. https://doi.org/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. https://onlinelibrary.wiley.com/doi/https://doi.org/10.1111/mam.12140
IUCN/SSC (2013) Guidelines for Reintroductions and Other Conservation Translocations.Version 1.0. Gland, Switzerland: Page IUCN Species Survival Commission. https://www.iucn.org/content/guidelines-reintroductions-and-other-conservation-translocations
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. https://academic.oup.com/bioinformatics/article-lookup/doi/https://doi.org/10.1093/bioinformatics/btm233
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. https://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. http://link.springer.com/https://doi.org/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. 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 MR, Good JM (2016) Targeted capture in evolutionary and ecological genomics. Molecular Ecology 25:185–202. https://onlinelibrary.wiley.com/doi/10.1111/mec.13304
Kalinowski ST (2005) Do polymorphic loci require large sample sizes to estimate genetic distances? Heredity 94:33–36. http://www.nature.com/articles/6800548
Koster J, Rahmann S (2012) Snakemake–a scalable bioinformatics workflow engine. Bioinformatics 28:2520–2522. https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/bts480
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. https://onlinelibrary.wiley.com/doi/https://doi.org/10.1111/jzo.12946
Lachance J, Tishkoff SA (2013) SNP ascertainment bias in population genetic analyses: Why it is important, and how to correct it. BioEssays 35:780–786. https://onlinelibrary.wiley.com/doi/https://doi.org/10.1002/bies.201300014
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. https://onlinelibrary.wiley.com/doi/https://doi.org/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. Ann Entomol Soc Am 112:433–442. https://doi.org/10.1093/aesa/saz025
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. http://www.nature.com/articles/s41467-018-05257-7
Leus K (2011) Captive breeding and conservation. Zoology in the Middle East 54:151–158. http://www.tandfonline.com/doi/full/10.1080/09397140.2011.10648906
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. https://academic.oup.com/bioinformatics/article-lookup/doi/https://doi.org/10.1093/bioinformatics/btp352
Lischer HEL, Excoffier L (2012) PGDSpider: an automated data conversion tool for connecting population genetics and genomics programs. Bioinformatics 28:298–299
Malomane DK, Reimer C, Weigend S, Weigend A, Sharifi AR, Simianer H (2018) Efficiency of different strategies to mitigate ascertainment bias when using SNP panels in diversity studies. BMC Genomics 19:22. https://bmcgenomics.biomedcentral.com/articles/https://doi.org/10.1186/s12864-017-4416-9

Manichaikul A, Mychaleckyj JC, Rich SS, Daly K, Sale M, Chen W-M (2010) Robust relationship inference in genome-wide association studies. Bioinformatics 26:2867–2873. https://academic.oup.com/bioinformatics/article-lookup/doi/https://doi.org/10.1093/bioinformatics/btq559

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 https://doi.org/10.1534/g3.115.023432

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. http://link.springer.com/https://doi.org/10.1007/s12686-020-01181-5

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. https://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. http://link.springer.com/https://doi.org/10.1007/s10592-009-9974-1

Nøhr AK, Hanghøj K, Garcia-Erill G, Li Z, Moltke I, Albrechtsen A (2021) NGSremix: a software tool for estimating pairwise relatedness between admixed individuals from next-generation sequencing data. G3 Genes|Genomes|Genetics 11. https://academic.oup.com/g3journal/article/doi/https://doi.org/10.1093/g3journal/jkab174/6279082

Nyhus PJ (2016) Human–Wildlife Conflict and Coexistence. Annual Review of Environment and Resources 41:143–171. https://www.annualreviews.org/doi/10.1146/annurev-environ-110615-085634

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. https://doi.org/10.1016/j.biocon.2019.108244

Patwardhan A, Amit R, Samit R (2014) Molecular markers in phylogenetic Studies-A review. Journal of Phylogenetics & Evolutionary Biology, 2.

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. https://doi.org/10.1371/journal.pone.0037135

Pierson JC, Coates DJ, Oostermeijer JGB, Beissinger SR, Bragg JG, Sunnucks P, Schumaker NH, Young AG (2016) Genetic factors in threatened species recovery plans on three continents. Frontiers in Ecology and the Environment 14:433–440. https://esajournals.onlinelibrary.wiley.com/doi/https://doi.org/10.1002/fee.1323

Price MR (1989) Animal reintroductions: the arabian Oryx in Oman. Cambridge University Press, Available from www.cambridge.org

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. https://onlinelibrary.wiley.com/doi/https://doi.org/10.1111/j.1749-6632.2009.04444.x

Pritchard JK, Stephens M, Donnelly P (2000) Inference of Population Structure Using Multilocus Genotype Data. Genetics 155:945–959. https://academic.oup.com/genetics/article/155/2/945/6048111

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. https://onlinelibrary.wiley.com/doi/https://doi.org/10.1111/1755-0998.12512

R Core Team (2022) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org

Reed DH (2004) Extinction risk in fragmented habitats. Animal Conservation 7:181–191. http://doi.wiley.com/10.1017/S1367943004001313

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. 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. https://onlinelibrary.wiley.com/doi/https://doi.org/10.1111/mec.15253

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. Ecol Evol 9:7017–7029. https://doi.org/10.1002/ece3.5268

Ross S, Alqamy H, El AT (2020a) Capra nubiana, Nubian Ibex. The IUCN Red List of Threatened Species 2020. https://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. https://doi.org/10.1007/s42991-020-00072-4

Samaha G, Wade CM, Mazrier H, Grueber CE, Haase B (2021) Exploiting genomic synteny in Felidae: cross-species genome alignments and SNV discovery can aid conservation management. BMC Genomics 22:601. https://bmcgenomics.biomedcentral.com/articles/https://doi.org/10.1186/s12864-021-07899-2

Senn H, O'Donoghue P, McEwing R, Ogden R (2014) Hundreds of SNPs for the Endangered pygmy hippopotamus (Choeropsis liberiensis). Conservation Genetics Resources 6:535–538. http://link.springer.com/https://doi.org/10.1007/s12686-014-0178-8

Senn HV, 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. https://onlinelibrary.wiley.com/doi/https://doi.org/10.1111/eva.12720

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. https://linkinghub.elsevier.com/retrieve/pii/016815919190262V

Szpiech ZA, Jakobsson M, Rosenberg NA (2008) ADZE: a rarefaction approach for counting alleles private to combinations of populations. Bioinformatics 24:2498–2504. https://academic.oup.com/bioinformatics/article-lookup/doi/https://doi.org/10.1093/bioinformatics/btn478

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. https://linkinghub.elsevier.com/retrieve/pii/S161650471630221X

Turcek FJ, Hickey JJ (1951) Effect of Introductions on Two Game Populations in Czechoslovakia. The Journal of Wildlife Management 15:113. https://www.jstor.org/stable/3796784?origin=crossref

Waples RK, Albrechtsen A, Moltke I (2019) Allele frequency-free inference of close familial relationships from genotypes or low-depth sequencing data. Molecular Ecology 28:35–48. https://doi.org/10.1111/mec.14954

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.