Conservation management strategy impacts inbreeding and genetic load in scimitar-horned oryx

Emily Humble[1], Martin A Stoffel[2], Kara Dicks[3], Alex D Ball[3], Rebecca M Gooley[4,5], Justin Chuven[6,7], Ricardo Pusey[6], Mohammed al Remeithi[6], Klaus-Peter Koepfli[4,5], Budhan Pukazhenthi[5], Helen Senn[3], Rob Ogden[1]

[1]Royal (Dick) School of Veterinary Studies and the Roslin Institute, University of Edinburgh, EH25 9RG, Edinburgh, UK

[2]Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, EH9 3FL, UK

[3]RZSS WildGenes, Conservation Department, Royal Zoological Society of Scotland, Edinburgh, EH12 6TS, UK

[4]Smithsonian-Mason School of Conservation, George Mason University, Front Royal, VA 22630 USA

[5]Smithsonian's National Zoo and Conservation Biology Institute, Center for Species Survival, Front Royal, Virginia 22630 and Washington, D.C. 20008 USA

[6]Terrestrial & Marine Biodiversity Sector, Environment Agency – Abu Dhabi, United Arab Emirates

[7]US Fish and Wildlife Service, Colorado, USA

Corresponding Author:

Emily Humble, Royal (Dick) School of Veterinary Studies and the Roslin Institute University of Edinburgh, EH25 9RG, UK

Phone: 0131 506 210, Email: emily.humble@ed.ac.uk

Keywords:

Ex-situ populations, runs of homozygosity (ROH), deleterious mutations, reintroduction, effective population size, antelope conservation

Abstract

In an age of habitat loss and overexploitation, small populations, both captive and wild, are increasingly facing the effects of isolation and inbreeding. Genetic management has therefore become a vital tool for ensuring population viability. However, little is known about how the type and intensity of intervention shape the genomic landscape of inbreeding and genetic load. We address this using whole genome sequence data of scimitar-horned oryx (*Oryx dammah*), an iconic antelope that has been subject to contrasting management strategies since it was declared extinct in the wild. We show that unmanaged populations are enriched for long runs of homozygosity (ROH) and have significantly higher inbreeding coefficients than managed populations. These patterns were associated with a partial deficit of highly deleterious mutations but a considerable excess of weakly deleterious mutations. These findings emphasise the risks associated with multiple generations of inbreeding and highlight the complex dynamics of mutation accumulation and purging in captivity. As wildlife management strategies continue to diversify, our study reinforces the importance of maintaining genome-wide variation in vulnerable populations and has direct implications for one of the largest reintroduction attempts in the world.

Significance statement

The preservation of genetic variation has long been recognised as a critical component of conservation management. However, recent observations in small and isolated populations have led some to challenge this paradigm. We investigate the impact of contrasting management strategies on the genomic landscape of inbreeding and genetic load in captive populations of scimitar-horned oryx. We reveal how several decades of management have prevented the formation of long runs of homozygosity and buffered the impacts of deleterious mutations. Our findings validate consensus thinking on the importance of genome-wide variation for population viability and have direct implications for future management of threatened species.

Main text

Introduction

Captive populations have become an essential insurance against extinctions in the wild (1). However, due to inbreeding and drift, they are intrinsically vulnerable to reduced genetic variation and the expression of partially recessive deleterious mutations (2–6). It is therefore of paramount importance that appropriate plans are in place to safeguard their potential as source populations. *Ex-situ* management strategies fall along a continuum from high-intensity pedigree-based breeding (7), to low-intensity pedigree-free group management (8, 9), to a complete absence of breeding intervention whatsoever. Empirical evidence on how these approaches influence the combined landscape of inbreeding and deleterious variation is limited (10, 11). As wildlife management strategies begin to diversify (12–15), there is a pressing need to leverage current genomic techniques to validate consensus thinking on maximising genetic diversity and minimising inbreeding in captivity and beyond (16–18).

Alongside this, recent debate on the significance of neutral genetic variation in conservation biology has raised practical considerations for sourcing populations for restorations (19–23). For example, an increasing number of studies are uncovering genomic evidence for purging in the wild (24–29), some of which have used this to challenge the small population paradigm (25, 26). Furthermore, simulation-based studies on the interaction between effective population size, genetic variation and extinction risk have called for more emphasis on functional genomic variation in genetic rescue attempts (21, 22). These observations go against decades of empirical and theoretical work in favour of maximising genetic variation to enhance population viability (30–33) including recent studies highlighting the complex dynamics of deleterious mutation frequencies in small populations (34–38). Founder selection for translocations rests on a complex set of considerations, with genetics making up only one component (39). In most cases, conservation practitioners will favour a unifying strategy to minimise risk and maximise return (40–42). In light of this, empirical data on the patterns of inbreeding and deleterious mutations in species undergoing active conservation management is urgently required.

Ex-situ populations of scimitar-horned oryx provide an excellent opportunity to evaluate the genomic consequences of management in the context of a global reintroduction. This iconic antelope was once widespread across North Africa, yet during the 20th century, hunting and land-use competition led to their rapid population decline and eventual extinction from the wild (43). Prior to disappearing, captive populations had already been established from what is

thought to be less than 100 animals originating from Chad in the 1960s (43). In the following years, the *ex-situ* population has grown to reach approximately 15,000 individuals (44). Around 1,000 of these are held within coordinated breeding programmes, but the vast majority are held in collections in places like Texas and the Arabian Peninsula where little to no genetic management takes place. Crucially, the scimitar-horned oryx is now being reintroduced back into its former range and *ex-situ* populations with varying management strategies have been used to source individuals for release. Here, we use runs of homozygosity (ROH) and predicted deleterious mutations to evaluate the impacts of captive-breeding practices on inbreeding and genetic load in scimitar-horned oryx, and discuss the implications for its ongoing management.

Results

We generated whole-genome sequence data for 49 scimitar-horned oryx from four *ex-situ* populations. Two of these, the EAZA *Ex situ* Programmes (EEP, n = 8) and the USA (n = 17), represent captive populations where genetic management practices are in place. The USA population comprised individuals from both privately owned ranches and institutions within the AZA Species Survival Plan ® (SSP). The remaining populations from the Environment Agency – Abu Dhabi originate from two genetically unmanaged collections in the United Arab Emirates (EAD A: n = 9 and EAD B: n = 15). Census sizes for the EEP and SSP population are approximately 619 and 223 respectively while those for EAD A and EAD B are approximately 3,000 and 70. For further details on population origins, management strategies and sampling approach, please refer to the Supplementary Material.

High coverage sequencing (~15X) was performed for 20 of the individuals and the remaining 29 were sequenced at a lower depth (6–8X, Table S1). Sequencing reads were mapped to the scimitar-horned oryx reference genome (45) and to account for coverage biases, SNPs and genotype likelihoods were called after downsampling high coverage individuals (see Methods for details). Analysis of population structure using NGSadmix and PCAngsd detected differentiation between the four sampling groups (Figures S2–4). Individual admixture proportions highlighted two major ancestral source populations (Figures S2A), with further hierarchical structure being resolved up to values of K=4 (Figures S2B and S3), corresponding to the four *ex-situ* groups. PCA distinguished EEP and USA populations as discrete clusters along PC2 and PC3, while EAD A and EAD B clustered separately along PC1 (Figure S4).

Levels of inbreeding across management strategies

To investigate how genomic patterns of inbreeding vary with management strategy, we examined the ROH landscape across individuals (Figure 1). The average number and total length of ROH was 247 (min = 65, max = 638) and 2.0 Mb (0.5–22.0 Mb) respectively, which on average spanned 20% of the autosomal genome (min = 0.03, max = 0.55, Figure 1A and Figure S6). Oryx from managed populations had significantly lower inbreeding coefficients (F_{ROH}) than oryx from unmanaged populations (ß = -0.19, 95% CI = -0.24–-0.14, $P = 6.43^{e-9}$, Figure 1A). This pattern was driven by both the number and length of ROH, the former being almost three times higher in the most inbred population than in the least inbred population (Figure 1B and Figure S7).

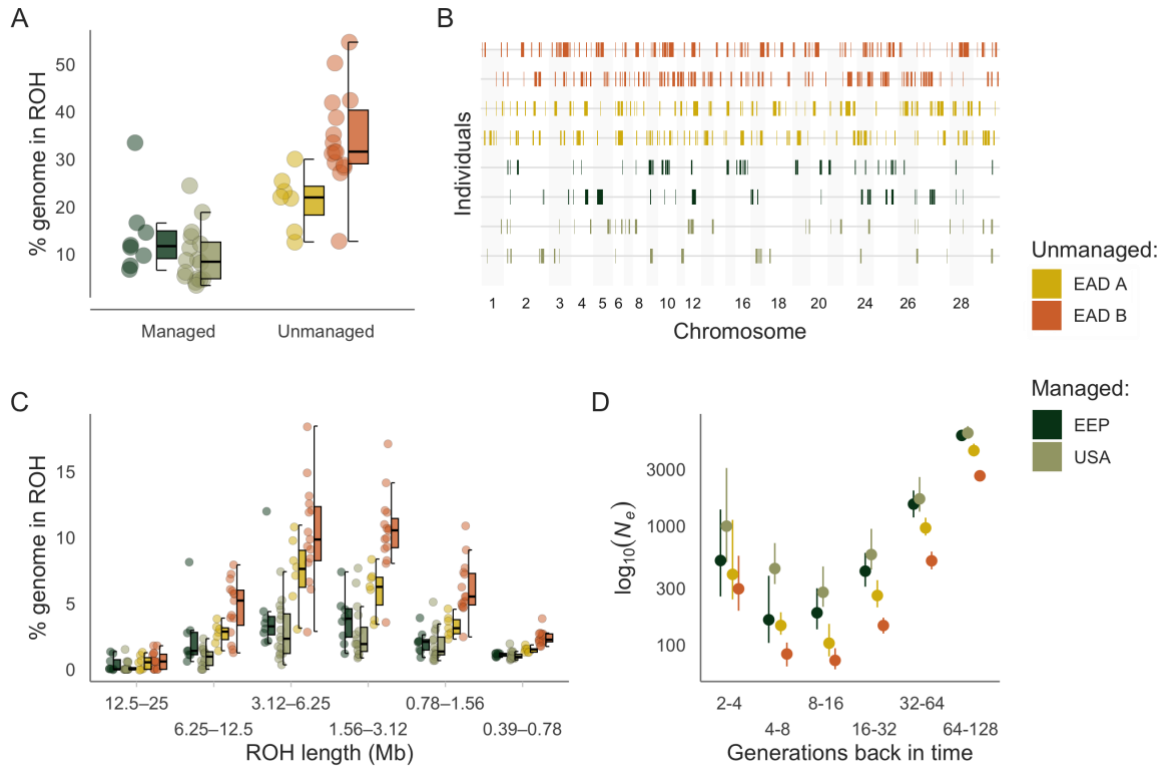

Figure 1. Runs of homozygosity (ROH) landscape across contrasting management strategies of scimitar-horned oryx. (A) Distribution of F_{ROH} among scimitar-horned oryx management strategies. Values were multiplied by 100 to reflect the percentage of the autosomal genome in ROH. Centre lines of boxplots reflect the median, bounds of the boxes reflect the 25th and 75th percentile and upper and lower whiskers reflect the largest and smallest values. **(B)** ROH in the two individuals with intermediate inbreeding coefficients F_{ROH} from each population. **(C)** Distribution of ROH within different length classes. Data points represent the percentage of ROH of a given length within an individual's autosomal genome. **(D)** Effective population size estimates inferred from the mean F_{ROH} in a population for a given time-period (see Methods for details). Error bars represent 95% bootstrap confidence intervals.

ROH length distribution and recent demography

We also observed variation in the abundance of ROH across different length classes. There was a steep decrease in frequency of ROH above lengths of around 6.25 Mb (Figure 1C). ROH longer than this made up a relatively small fraction of the genome, reaching a minimum average frequency of 0.4% between 12.5–25 Mb. ROH between 3.12–6.25 Mb had the highest frequency, making up on average 6.2% of an individual's genome. This pattern of abundance was observed in each population however there was variation in absolute proportions across individuals. For example, the most abundant length class 3.12–6.25 Mb made up on average only 3% of the genome in the least inbred population, USA, while it comprised on average 10% in the most inbred population, EAD B (Figure 1C). Interestingly,

long ROH >12.5 Mb which are likely the result of recent inbreeding, were identified in less than 30% of individuals from managed populations, yet were present in over 60% of individuals from unmanaged populations.

As ROH lengths decrease, their underlying haplotypes are expected to have originated from ancestors further back in time (Thompson 2013). The abundance of ROH in different length classes can therefore provide insights into past changes in effective population size (N_e) (46, 47). In line with this, our estimates of N_e based on individual inbreeding coefficients were proportional to patterns of ROH abundance (Figure 1D). N_e declines to reach a minimum of around 150 individuals between 8–16 generations ago, after which it shows a steady increase towards the present day. Interestingly, despite small census sizes, managed populations had higher N_e estimates across all time periods than unmanaged populations (mean N_e: USA = 1,672, EEP = 1,429 versus EAD A = 1,028, EAD B = 625). These patterns were reflected in estimates of mean pairwise nucleotide diversity which were also higher in managed (USA = 0.46×10^5, EEP = 0.44×10^5) than unmanaged populations (EAD A = 0.42×10^5 , EAD B = 0.27×10^5).

Mutation load landscape across management strategies

We next investigated how genetic load varies across management strategies using two approaches, both based on putative deleterious variants identified using annotation-based methods. As the overall patterns were qualitatively similar across two variant effect prediction programs (Figure S8), results using annotations from SnpEff are presented here. We first explored the present and potential impacts of putatively deleterious mutations by estimating two components of genetic load; inbreeding and drift load (Bertorelle *et al.* 2022, see Methods for details). Inbreeding load corresponds to the potential reduction in population fitness due to the burden of recessive deleterious mutations that may become homozygous through inbreeding. It was approximated as the absolute number of missense and loss of function heterozygotes per individual. Drift load corresponds to a reduction in fitness of a population due to the increased frequency and fixation of deleterious mutations. It was approximated as the absolute number of alternative missense and loss of function homozygotes per individual.

Inbreeding load for both missense and loss of function mutations was consistently higher in managed than unmanaged populations (Missense: ß = 1079, 95% CI = 769–1390, $P = 1.12^{e-8}$, LoF: ß = 21.7, 95% CI = 12.7–30.6, $P = 1.51^{e-5}$, Figure 2A–B). As expected, this pattern inversely tracked overall inbreeding levels, where individuals with lower inbreeding coefficients had a larger number of heterozygotes at missense and loss of function sites (Figure S9). In direct contrast, the drift load for weakly deleterious missense mutations was lower in managed

populations than in unmanaged groups (ß = -267, 95% CI = -398–-137, $P = 1.65^{e-4}$, Figure 2C). Interestingly, the drift load for highly deleterious loss of function mutations displayed no difference across management strategies, with similar numbers of alternative homozygotes present among individuals (ß = 0.89, 95% CI = -3.07–4.86, $P = 6.5^{e-1}$, Figure 2D).

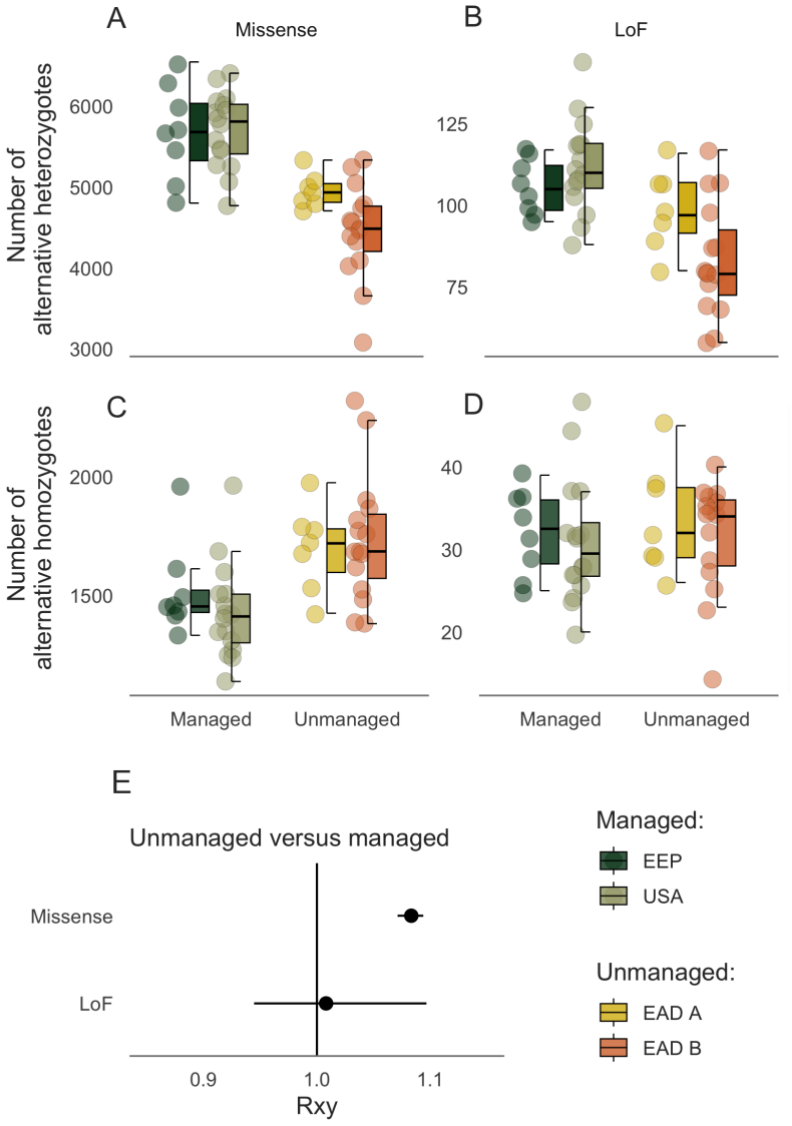

Figure 2. Deleterious load landscape across contrasting management strategies of scimitar-horned oryx based on SNPeff annotations. Distribution of the number of heterozygotes per individual (inbreeding load) for missense **(A)** and loss of function mutations **(B)** across management strategies. Distribution of the number of alternative homozygotes per individual (drift load) for missense **(C)** and loss of function **(D)** mutations across management strategies. **(E)** Relative number (*Rxy*) of alternative alleles at missense and loss of function sites. *Rxy* > 1 indicates a relative frequency excess of a given category of sites in unmanaged versus managed populations. Error bars represent 95% bootstrap confidence intervals.

We next used the measure *Rxy* to determine whether there was an excess of putative deleterious mutations in one management strategy over another. *Rxy* compares the relative frequency of derived alleles within a given impact category and is standardised over a set of intergenic SNPs, making it robust to population-specific biases. Overall, unmanaged populations displayed a significant excess of missense mutations compared to the managed populations (Figure 2E), indicating an accumulation of weakly deleterious mutations in the unmanaged groups. In contrast, no difference in the frequency of highly deleterious loss of function mutations could be detected between the management groups (Figure 2E).

Discussion

The scimitar-horned oryx was declared Extinct in the Wild in 2000, yet the species has persisted *ex-situ*. Understanding how management shapes the genomic landscape of inbreeding and genetic load is essential for improving species viability. We used whole genome resequencing data to characterise runs of homozygosity and deleterious mutations in scimitar-horned oryx populations undergoing contrasting management strategies. Our study highlights the complex dynamics between inbreeding, genetic load and population size and has broad-reaching implications for practical conservation management.

We first demonstrated how signatures of recent population history can be identified in the genomes of present-day animals. Across *ex-situ* oryx populations, both managed and unmanaged, we observed a peak in ROH abundance between 3.12–6.25 Mb. Although it is not possible to precisely estimate the time to the most recent common ancestor (MRCA) when ROH are inferred using physical positions (49), ROH of this size are expected to originate from haplotypes approximately 8–16 generations ago (50). This shift in abundance indicates a smaller population size around this time-period which we could reconstruct with our measures of N_e. Interestingly, assuming a generation time of around seven years (44), this directly corresponds to the mid 20th century when oryx were close to extinction in the wild and when *ex-situ* populations were founded (15, 43). These findings highlight the power of ROH for inferring the strength and timing of recent bottlenecks and for placing contemporary patterns of nucleotide diversity into a historical context.

The overall pattern of ROH abundance was qualitatively similar across populations yet the absolute proportion of the genome in ROH was considerably lower in managed than unmanaged populations for all length classes. Long ROH are indicative of recent inbreeding because recombination has had little opportunity to break up haplotypes (51–54). The relative absence of long ROH therefore strongly indicates that close inbreeding is uncommon in

managed populations, which work to mitigate this process. Furthermore, the smaller proportion of short ROH suggest these populations also have lower levels of background relatedness (53, 55). Although historic data on the origins of the unmanaged populations are lacking (15), it is not unreasonable to expect a higher level of relatedness among founder individuals compared to those of breeding programmes. Overall, these findings reveal the genomic effects of multiple generations of inbreeding, while on the other hand demonstrate how 30–40 years of *ex-situ* management has been successful at maximising the genetic diversity of captive populations.

We next shed light on the relationship between inbreeding, diversity and deleterious mutations by exploring how proxies for genetic load compare across management strategies. At an individual level, we show that animals from collections employing genetic management practices have a higher inbreeding load for both missense and loss of function mutations than animals from unmanaged populations. Theory and simulations (23, 56, 57) predict that in large populations, higher frequencies of segregating deleterious mutations will lead to higher inbreeding load. This is in part due to being masked from the effects of purifying selection in populations with larger N_e, but also by genetic drift driving deleterious mutations to fixation in small populations. In line with this, we show that despite their small census sizes, managed populations of oryx have higher nucleotide diversity and effective population sizes than unmanaged collections.

The presence of segregating deleterious variation within insurance populations may be considered a concern for conservation management. Indeed, there has been recent debate surrounding the risks associated with sourcing individuals for restoration from large, genetically diverse populations, given the higher expected levels of masked load (21, 22). However, these concerns are unlikely to be relevant for restoration and reintroduction programs that follow established recommendations (39). Notably, as advised by IUCN/SSC guidelines, sourcing individuals from genetically differentiated populations, releasing large numbers of animals over extended time frames and maximising initial population growth rate all serve to increase genetic variation and prevent the inbreeding load from being expressed (19, 58–60). The scimitar-horned oryx reintroduction plan has followed these best practice guidelines having so far released over 250 animals over a five-year time-period, and in eight separate release batches. Consequently, the released population has now reached close to 400 individuals, with over 150 calves born in the wild. Follow-up monitoring of the release herds will provide a rare opportunity to validate these efforts within the context of a large-scale reintroduction effort.

In addition to the inbreeding load, we also considered how drift load varies across populations. Several recent studies have demonstrated significant reductions in the relative number of highly deleterious mutations in small versus large (24–26, 29, 37) and in modern versus historical populations (27, 28), and attributed these differences to the effects of purifying selection. While we did not observe a complete reversal in the patterns of drift load for loss of function mutations, we did see a reduction in frequency relative to missense mutations in unmanaged collections. In small inbred populations, partially recessive deleterious mutations will be expressed as homozygotes thereby exposing them to the effects of purifying selection (62–64). However, as selection strength declines with decreasing N_e, fewer deleterious mutations are expected to be removed through purging (65, 66). In line with this, we also show that unmanaged collections have accumulated a considerable burden of weakly deleterious mutations compared to managed populations.

Recent studies distinguishing weakly and strongly deleterious mutations and different genetic load components have uncovered equivalent patterns in wild populations (34, 36, 38). Taken together, our results provide further support for the notion that the presence of purging of large-effect mutations does not imply the absence of inbreeding depression (19, 23). This is consistent with recent studies on a small population of Soay sheep (*Ovis aries)*, where long-term fitness and genomic data revealed strong inbreeding depression caused largely by many weakly deleterious mutations (35, 67). Consequently, despite some evidence for purging, unmanaged populations of oryx are likely to carry a higher fitness cost associated with inbreeding. With regard to their long-term genetic management, this would imply a need for reciprocal transfer of individuals between *ex-situ* collections. Not only would this serve to reduce inbreeding, but would produce populations with enhanced genetic diversity for enabling adaptation to changing environmental conditions and for release back into the wild. As part of the World Herd approach (68), mixing of animals from multiple collections is now a key part of the scimitar-horned oryx reintroduction management strategy.

Ex-situ breeding and species reintroduction planning are ultimately exercises in risk management, with genetics making up only one component of a multifaceted set of considerations (39). Overall, our study provides empirical support for the value of genetic management of *ex-situ* populations and reinforces the risks associated with multiple generations of inbreeding. These findings advocate for a strategy in line with conventional wisdom to maintain genetic variation and maximise differentiation in captive populations and restoration programmes (7, 60, 69–72). While such actions will be possible using largely traditional measures of genetic variation, our study demonstrates how the application of whole genome sequencing in the context of *ex-situ* management has the power to resolve previously

unknown aspects of variation. We recognise that it is impractical to consider comprehensive genomic approaches for the genetic management of every species (73). Rather, we suggest the application of studies such as this to guide conservation breeding strategies across diverse taxa. When combined with best-practice guidelines, this approach will help lead to healthy populations, with the greatest chance of survival.

Materials and methods

Sampling and sequencing

Blood (in EDTA) and tissue (in 100% ethanol) samples were collected for whole genome resequencing from 49 scimitar-horned oryx representing four *ex-situ* populations: the EEP (n = 8), USA (n = 17), EAD A (n = 9) and EAD B (n = 15). The EEP and USA are captive collections undergoing genetic management practices, while EAD A and EAD B represent collections in the United Arab Emirates with no genetic management in place (Supplementary Methods). Total genomic DNA was extracted between one and five times per sample using the DNeasy Blood and Tissue Kit (Qiagen, Cat. No. 69504). Elutions were pooled and concentrated in an Eppendorf Concentrator Plus at 45°C and 1400 rpm until roughly 50 µl remained. Library construction was carried out using the Illumina TruSeq Nano High Throughput library preparation kit (Illumina CA, UKA). Twenty samples from across all four populations were 150 bp paired-end sequenced on an Illumina HiSeq X Ten platform at a target depth of coverage of 15X. The remaining 29 samples from three of the populations were 150 bp paired-end sequenced on an Illumina NovaSeq 6000 instrument at a target depth of coverage of 7X (Table S1).

Read processing and alignment

Sequence reads were assessed for quality using FastQC v0.11.7 (74) and trimmed for adaptor content using cutadapt v1.16 (75). Reads were then mapped to the scimitar-horned oryx reference genome assembly (*Oryx dammah* assembly version 1.1, Genbank accession number GCF_014754425.2) using BWA MEM v0.7.17 (76) with default parameters. Unmapped reads were removed from the alignment files using SAMtools v1.9 (77). Alignments were then sorted, read groups added and duplicates removed using Picard Tools v2.18.16. This resulted in a set of 49 filtered alignment files, one for each of the resequenced individuals. To account for coverage variation in our data (78), we used SAMtools to downsample our 20 high coverage alignment files to approximately 6X, which was the average depth of coverage of our low coverage samples. All subsequent analyses were carried out on the set of alignments with comparable coverage.

Variant calling and filtering

Haplotype Caller and GenotypeGVCFs in GATK v3.8 (79) were used for joint genotyping across all samples. The resulting SNP data were filtered for biallelic sites using BCFtools v1.9 (80). To obtain a high-quality set of variants we then used VCFtools (81) to remove loci with a quality score less than 30, a mean depth of coverage less than 5 or greater than 53, a genotyping rate less than 95% and a minor allele count less than 1. We removed SNPs originating from the X chromosome or any of the unplaced scaffolds within the assembly. One individual with a high relatedness score was dropped from subsequent analysis (Figure S1, see Supplementary Methods for details). The resulting SNP dataset contained over 10 million polymorphic sites with a genotyping rate of 98%.

Population structure

We characterised population structure using genotype likelihood based approaches in NGSadmix (82) and PCAngsd (83). Genotype likelihoods were first estimated from bam files in ANGSD (84) using the GATK model (-GL 2), inferring major and minor alleles (-doMajorMinor 1) and outputting only polymorphic sites (-SNP_pval 1e-6) with data in at least 60% of individuals (-minInd 30). We restricted this analysis to the 28 chromosome-length autosomes and included only regions with Phred quality and mapping scores over 30. Admixture proportions for the individuals in our dataset were calculated using NGSadmix. We performed admixture runs for ancestry clusters ranging from K=1–6, with ten runs for each K. The runs with the highest likelihood were plotted. The optimal K was identified based on the maximum value of the mean estimated *ln* probability of the data (85) and the Delta K method (86). Two individuals with intermediate admixture proportions between EAD A and EAD B were dropped from further analysis (Figure S3, see Supplementary Methods for details). We next performed a principal components analysis (PCA) using PCAngsd with the default parameters. Eigenvectors were computed from the covariance matrix using R.

ROH calling and individual inbreeding coefficients

We used the filtered SNP genotypes to estimate inbreeding as the proportion of the genome in runs of homozygosoty (F_{ROH}). ROH were called with a minimum length of 500 kb and a minimum of 50 SNPs using the --homozyg function in PLINK v1.9 (87) and the following parameters: --homozyg-window-snp 50 --homozyg-snp 50 --homozyg-kb 500 --homozyg-gap 1000 --homozyg-density 50 --homozyg-window-missing 5 --homozyg-window-het 3. We then calculated individual inbreeding coefficients F_{ROH} as the sum of the detected ROH lengths for each individual over the total autosomal assembly length (2.44 Gb). To explore the effect of management on inbreeding coefficients we ran linear models with F_{ROH} as the response variables and management strategy as the predictor variable. We also calculated F_{ROH} based

on ROH inferred using bcftools roh and the following parameters: --AF-dflt 0.16 (average minor allele frequency), -G 30 and -M 1.2 (cattle recombination rate, Mouresan *et al.* 2019). We observed a near-perfect correlation (r = 0.99) with our PLINK-based estimates (Figure S5).

ROH length distribution and recent demography

To assess recent changes in oryx population size, we characterised the abundance of ROH in seven different length classes (≥25, 12.5–25, 6.25–12.5, 3.12–6.25, 1.56–3.12, 0.78–1.56 and 0.39–0.78 Mb). Categories were calculated using the formula 100/(2g) (50), and reflect the expected lengths of ROH when the underlying haplotypes have most recent common ancestors <2, 2–4, 4–8, 8–16, 16–32, 32–64 and 64–128 generations ago respectively. These generations were chosen to capture the time-period during which the wild population of oryx went extinct and captive populations were established. As there is no linkage map for the oryx, physical map lengths as opposed to genetic map lengths were used. For each length class, F_{ROH} was calculated as the sum of the detected ROH lengths for each individual over the total autosomal assembly length (2.44 Gb). We then used individual measures of F_{ROH} to infer recent changes in effective population size across each population. For each time-period described above (*t)*, N_e was estimated given the following expression where $F_{ROH,t}$ corresponds to the individual inbreeding coefficient at time *t*:

$$F_{ROH,t} = 1 - (1 - \frac{1}{2N_e})^t$$

To calculate 95% confidence intervals around our estimates, we randomly resampled 50% of individuals within each population without replacement 100 times, and recalculated N_e.

Nucleotide diversity

Nucleotide diversity estimates were calculated for each population using ANGSD. We first estimated the unfolded site-frequency spectrum (SFS) using the -doSaf and -realSFS commands while restricting the analysis to the 28 chromosome-length autosomes and regions with Phred quality and mapping scores over 30. Per-site pairwise nucleotide diversity estimates were then calculated using the -thetaStat command.

Identification of deleterious mutations

As most deleterious mutations are likely to be derived alleles, we first polarised our SNP genotypes as ancestral or derived using the blue wildebeest (*Connochaetes taurinus*), topi (*Damaliscus lunatus*) and hartebeest (*Alcelaphus buselaphus*) as outgroup species. Short read sequencing data from one wildebeest (SRR6902709), one topi (SRR6913384) and one

hartebeest (SRR6922939 and SRR6922940) were downloaded from NCBI and mapped to the scimitar-horned oryx reference genome using BWA MEM with the default parameters. The alignments were then merged using SAMtools. A consensus was generated by selecting the most common base from the alignment using the doFasta 2 and doCounts 1 options in ANGSD. We then used PLINK v2.0 to polarise the oryx SNPs in our VCF based on the alleles in the consensus. First, we removed SNPs from our VCF whose positions were not present in the consensus sequence. Second, we removed SNPs where the ancestral allele in the consensus matched neither allele in the VCF file. Finally, we rotated alleles so that the reference allele in our VCF matched the ancestral allele in the consensus.

To identify deleterious mutations, we predicted the functional effects of the polarised SNP variants using both SnpEff v5.0 (89) and the Variant Effect Predictor v99.2 (90). These methods compare a set of variants to an annotation database and predict the consequence of the alternative alleles on genes, transcripts and proteins. Both were run using the NCBI RefSeq scimitar-horned oryx genome annotation downloaded from: https://ftp.ncbi.nlm.nih.gov/genomes/all/annotation_releases/59534/100/GCF_014754425.2_SCBI_Odam_1.1/. For each approach, sites with warnings were removed from the VCF file and SNPs were then categorised as loss of function or missense according to the classifications provided in Table S2. For each dataset, we also extracted a random subset of 100,000 intergenic SNPs for use in the Rxy analysis below. For each set of SNPs, genotypes were extracted for all individuals using a combination of VCFtools and PLINK.

Genetic load landscape across management strategies

To assess how the genetic load varies across populations we used two approaches. First, we approximated two components of genetic load; inbreeding and drift load (48). Inbreeding load was measured as the total number of heterozygotes per individual for both loss of function and missense variants. Drift load was measured as the total number of alternative homozygotes per individual for both loss of function and missense variants. Second, we used the Rxy statistic to estimate the relative frequency of loss of function and missense mutations in one population over another (91). Alternative allele frequencies were calculated based on individuals from managed and unmanaged populations separately. A random subset of 100,000 intergenic SNPs was used to standardise our estimates and account for population-specific biases. To calculate 95% confidence intervals around our estimates, we randomly resampled 70% of SNPs within each impact category without replacement and recalculated Rxy. This was repeated 100 times. To explore the effect of management on genetic load, we ran linear models with inbreeding or drift load as the response variable and management strategy as the predictor variable.

Data availability

EEP samples are archived at the EAZA Biobank https://www.eaza.net/conservation/research/eaza-biobank. Whole genome resequencing data will be deposited to the European Nucleotide Archive. Analysis code will be available on Zenodo and GitHub.

Author contributions

RO, HS, AB, KD and EH conceived and designed the study. JC, RP and MaR contributed materials and funding. BP provided samples from SSP populations. EH analysed the data with input from MAS and RG. EH wrote the manuscript. All authors commented on and helped improve the final manuscript.

Acknowledgements

We thank the EAD, EAZA and AZA institutions that provided samples for this study. We thank Jennifer Kaden for initial processing of samples and DNA extraction, and Edinburgh Genomics for carrying out the whole genome sequencing. SaharaConservation provided materials and wider project support. We also acknowledge Katerina Guschanski for helpful discussions and Tania Gilbert at Marwell Wildlife for information on the international studbook of scimitar-horned oryx.

References

1. P. J. K. McGowan, K. Traylor-Holzer, K. Leus, IUCN guidelines for determining when and how ex situ management should be used in species conservation. *Conservation Letters* **10**, 361–366 (2017).

2. M. Kimura, T. Maruyama, J. F. Crow, The mutation load in small populations. *Genetics* **48**, 1303–1312 (1963).

3. R. C. Lacy, Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. *Conservation Biology* **1**, 143–158 (1987).

4. R. Lande, Risk of population extinction from fixation of new deleterious mutations. *Evolution* **48**, 1460–1469 (1994).

5. M. Lynch, J. Conery, R. Burger, Mutation accumulation and the extinction of small populations. *The American Naturalist* **146**, 489–518 (1995).

6. D. Spielman, B. W. Brook, R. Frankham, Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 15261–15264 (2004).

7. J. Ballou, R. Lacy, “Identifying genetically important individuals for management of genetic diversity in pedigreed populations” in *Analytical Methods and Strategies in Small Population Conservation*, (1995).

8. R. M. Gooley, C. J. Hogg, K. Belov, C. E. Grueber, The effects of group versus intensive housing on the retention of genetic diversity in insurance populations. *BMC Zool* **3**, 2 (2018).

9. D. Wildt, *et al.*, Breeding centers, private ranches, and genomics for creating sustainable wildlife populations. *BioScience* **69**, 928–943 (2019).

10. R. M. Gooley, *et al.*, Comparison of genomic diversity and structure of sable antelope (*Hippotragus niger*) in zoos, conservation centers, and private ranches in North America. *Evolutionary Applications* **13**, 2143–2154 (2020).

11. M. J. Lott, B. R. Wright, L. F. Kemp, R. N. Johnson, C. J. Hogg, Genetic management of captive and reintroduced bilby populations. *The Journal of Wildlife Management* **84**, 20–32 (2020).

12. D. J. Pritchard, J. E. Fa, S. Oldfield, S. R. Harrop, Bring the captive closer to the wild: redefining the role of *ex situ* conservation. *Oryx* **46**, 18–23 (2012).

13. K. H. Redford, D. B. Jensen, J. J. Breheny, Integrating the captive and the wild. *Science* **338**, 1157–1158 (2012).

14. K. R. Schwartz, E. C. M. Parsons, L. Rockwood, T. C. Wood, Integrating in-situ and ex-situ data management processes for biodiversity conservation. *Frontiers in Ecology and Evolution* **5** (2017).

15. R. Ogden, *et al.*, Benefits and pitfalls of captive conservation genetic management: Evaluating diversity in scimitar-horned oryx to support reintroduction planning. *Biological Conservation* **241**, 108244 (2020).

16. J. Fernández, M. A. Toro, A. Caballero, Managing individuals’ contributions to maximize the allelic diversity maintained in small, conserved populations. *Conservation Biology* **18**, 1358–1367 (2004).

17. J. Ballou, *et al.*, “Demographic and genetic management of captive populations” in *Wild Mammals in Captivity: Principles and Techniques for Zoo Management, Second Edition*, (University of Chicago Press, 2010).

18. J. Ivy, R. Lacy, “Using molecular methods to improve the genetic management of captive breeding programs for threatened species” in *Molecular Approaches in Natural Resource Conservation and Management*, (2010).

19. K. Ralls, P. Sunnucks, R. C. Lacy, R. Frankham, 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 (2020).

20. J. A. DeWoody, A. M. Harder, S. Mathur, J. R. Willoughby, The long-standing significance of genetic diversity in conservation. *Molecular Ecology* **30**, 4147–4154 (2021).

21. C. C. Kyriazis, R. K. Wayne, K. E. Lohmueller, Strongly deleterious mutations are a primary determinant of extinction risk due to inbreeding depression. *Evolution Letters* **5**, 33–47 (2021).

22. J. C. Teixeira, C. D. Huber, The inflated significance of neutral genetic diversity in conservation genetics. *Proceedings of the National Academy of Sciences* **118**, e2015096118 (2021).

23. M. Kardos, *et al.*, The crucial role of genome-wide genetic variation in conservation. *Proceedings of the National Academy of Sciences* **118**, e2104642118 (2021).

24. Y. Xue, *et al.*, Mountain gorilla genomes reveal the impact of long-term population decline and inbreeding. *Science* **348**, 242–245 (2015).

25. J. A. Robinson, C. Brown, B. Y. Kim, K. E. Lohmueller, R. K. Wayne, Purging of strongly deleterious mutations explains long-term persistence and absence of inbreeding depression in island foxes. *Curr Biol* **28**, 3487–3494 (2018).

26. J. A. Robinson, *et al.*, Genomic signatures of extensive inbreeding in Isle Royale wolves, a population on the threshold of extinction. *Science Advances* **5**, eaau0757 (2019).

27. T. van der Valk, D. Díez-del-Molino, T. Marques-Bonet, K. Guschanski, L. Dalén, Historical genomes reveal the genomic consequences of recent population decline in eastern gorillas. *Current Biology* **29**, 165-170.e6 (2019).

28. J. von Seth, *et al.*, Genomic insights into the conservation status of the world's last remaining Sumatran rhinoceros populations. *Nat Commun* **12**, 2393 (2021).

29. N. Dussex, *et al.*, Population genomics of the critically endangered kākāpō. *Cell Genomics* **1**, 100002 (2021).

30. R. Lande, S. Shannon, The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution* **50**, 434–437 (1996).

31. I. Saccheri, *et al.*, Inbreeding and extinction in a butterfly metapopulation. *Nature* **392**, 491–494 (1998).

32. D. H. Reed, R. Frankham, Correlation between fitness and genetic diversity. *Conservation Biology* **17**, 230–237 (2003).

33. C. Bozzuto, I. Biebach, S. Muff, A. R. Ives, L. F. Keller, Inbreeding reduces long-term growth of Alpine ibex populations. *Nat Ecol Evol* **3**, 1359–1364 (2019).

34. C. Grossen, F. Guillaume, L. F. Keller, D. Croll, Purging of highly deleterious mutations through severe bottlenecks in Alpine ibex. *Nature Communications* **11**, 1–12 (2020).

35. M. A. Stoffel, S. E. Johnston, J. G. Pilkington, J. M. Pemberton, Mutation load decreases with haplotype age in wild Soay sheep. *Evolution Letters* **5**, 187–195 (2021).

36. S. Mathur, J. A. DeWoody, Genetic load has potential in large populations but is realized in small inbred populations. *Evol Appl* **14**, 1540–1557 (2021).

37. A. Khan, *et al.*, Genomic evidence for inbreeding depression and purging of deleterious genetic variation in Indian tigers. *Proceedings of the National Academy of Sciences* **118**, e2023018118 (2021).

38. D. Kleinman-Ruiz, *et al.*, Purging of deleterious burden in the endangered Iberian lynx. *Proceedings of the National Academy of Sciences* **119**, e2110614119 (2022).

39. IUCN/SSC, "Guidelines for reintroductions and other conservation translocations. Version 1.0" (2013).

543 40. S. Edmands, Between a rock and a hard place: evaluating the relative risks of inbreeding
544 and outbreeding for conservation and management. *Molecular Ecology* **16**, 463–475
545 (2007).

546 41. A. R. Weeks, *et al.*, Assessing the benefits and risks of translocations in changing
547 environments: a genetic perspective. *Evolutionary Applications* **4**, 709–725 (2011).

548 42. C. J. Hogg, *et al.*, Preserving the demographic and genetic integrity of a single source
549 population during multiple translocations. *Biological Conservation* **241**, 108318 (2020).

550 43. T. Woodfine, T. Gilbert, "The Fall and Rise of the Scimitar-Horned Oryx" in *Antelope*
551 *Conservation*, (John Wiley & Sons, Ltd, 2016), pp. 280–296.

552 44. T. Gilbert, *International studbook for the scimitar-horned oryx* Oryx dammah, Seventeenth
553 edition (Marwell Wildlife, 2022).

554 45. E. Humble, *et al.*, Chromosomal-level genome assembly of the scimitar-horned oryx:
555 Insights into diversity and demography of a species extinct in the wild. *Molecular Ecology*
556 *Resources* **20**, 1668–1681 (2020).

557 46. I. M. MacLeod, D. M. Larkin, H. A. Lewin, B. J. Hayes, M. E. Goddard, Inferring
558 demography from runs of homozygosity in whole-genome sequence, with correction for
559 sequence errors. *Molecular Biology and Evolution* **30**, 2209–2223 (2013).

560 47. S. R. Browning, B. L. Browning, Accurate non-parametric estimation of recent effective
561 population size from segments of identity by descent. *The American Journal of Human*
562 *Genetics* **97**, 404–418 (2015).

563 48. G. Bertorelle, *et al.*, Genetic load: genomic estimates and applications in non-model
564 animals. *Nat Rev Genet*, 1–12 (2022).

565 49. M. Kardos, A. Qvarnström, H. Ellegren, Inferring individual inbreeding and demographic
566 history from segments of identity by descent in *Ficedula* flycatcher genome sequences.
567 *Genetics* **205**, 1319–1334 (2017).

568 50. E. A. Thompson, Identity by descent: variation in meiosis, across genomes, and in
569 populations. *Genetics* **194**, 301–326 (2013).

570 51. R. McQuillan, *et al.*, Runs of homozygosity in European populations. *The American Journal*
571 *of Human Genetics* **83**, 359–372 (2008).

572 52. M. Kirin, *et al.*, Genomic runs of homozygosity record population history and consanguinity.
573 *PLoS ONE* **5**, e13996 (2010).

574 53. T. J. Pemberton, *et al.*, Genomic patterns of homozygosity in worldwide human
575 populations. *The American Journal of Human Genetics* **91**, 275–292 (2012).

576 54. M. Kardos, G. Luikart, F. W. Allendorf, Measuring individual inbreeding in the age of
577 genomics: marker-based measures are better than pedigrees. *Heredity* **115**, 63–72 (2015).

578 55. H. Ringbauer, J. Novembre, M. Steinrücken, Parental relatedness through time revealed by
579 runs of homozygosity in ancient DNA. *Nat Commun* **12**, 5425 (2021).

580 56. D. Charlesworth, B. Charlesworth, Inbreeding depression and its evolutionary
581 consequences. *Annual Review of Ecology & Systematics* **18**, 237–268 (1987).

582 57. M. C. Whitlock, Selection, load and inbreeding depression in a large metapopulation.
583 *Genetics* **160**, 1191–1202 (2002).

584 58. L. N. Tracy, G. P. Wallis, M. G. Efford, I. G. Jamieson, Preserving genetic diversity in
585 threatened species reintroductions: how many individuals should be released? *Animal*
586 *Conservation* **14**, 439–446 (2011).

587 59. A. R. Whiteley, S. W. Fitzpatrick, W. C. Funk, D. A. Tallmon, Genetic rescue to the rescue.
588 *Trends in Ecology & Evolution* **30**, 42–49 (2015).

589 60. R. Frankham, Genetic rescue of small inbred populations: meta-analysis reveals large and
590 consistent benefits of gene flow. *Molecular Ecology* **24**, 2610–2618 (2015).

591 61. R. Frankham, Genetic rescue benefits persist to at least the F3 generation, based on a
592 meta-analysis. *Biological Conservation* **195**, 33–36 (2016).

593 62. J. Wang, W. G. Hill, Effect of selection against deleterious mutations on the decline in
594 heterozygosity at neutral loci in closely inbreeding populations. *Genetics* **153**, 1475–1489
595 (1999).

596 63. A. García-Dorado, Understanding and predicting the fitness decline of shrunk populations:
597 inbreeding, purging, mutation, and standard selection. *Genetics* **190**, 1461–1476 (2012).

598 64. B. Charlesworth, Mutational load, inbreeding depression and heterosis in subdivided
599 populations. *Molecular Ecology* **27**, 4991–5003 (2018).

600 65. R. C. Lacy, J. D. Ballou, Effectiveness of selection in reducing the genetic load in
601 populations of *Peromyscus polionotus* during generations of inbreeding. *Evolution* **52**, 900–
602 909 (1998).

603 66. R. Frankham, D. M. Gilligan, D. Morris, D. A. Briscoe, Inbreeding and extinction: Effects of
604 purging. *Conservation Genetics* **2**, 279–284 (2001).

605 67. M. A. Stoffel, S. E. Johnston, J. G. Pilkington, J. M. Pemberton, Genetic architecture and
606 lifetime dynamics of inbreeding depression in a wild mammal. *Nat Commun* **12**, 2972
607 (2021).

608 68. K. Mertes, *et al.*, Management background and release conditions structure post-release
609 movements in reintroduced ungulates. *Front. Ecol. Evol.* **7** (2019).

610 69. M. E. Montgomery, *et al.*, Minimizing kinship in captive breeding programs. *Zoo Biology* **16**,
611 377–389 (1997).

612 70. M. Pickup, D. L. Field, D. M. Rowell, A. G. Young, Source population characteristics affect
613 heterosis following genetic rescue of fragmented plant populations. *Proceedings of the*
614 *Royal Society B: Biological Sciences* **280**, 20122058 (2013).

615 71. R. Frankham, *et al.*, *Genetic management of fragmented animal and plant populations*
616 (Oxford University Press, 2017) https:/doi.org/10.1093/oso/9780198783398.001.0001
617 (March 21, 2022).

618 72. M. Ørsted, A. A. Hoffmann, E. Sverrisdóttir, K. L. Nielsen, T. N. Kristensen, Genomic
619 variation predicts adaptive evolutionary responses better than population bottleneck history.
620 *PLOS Genetics* **15**, e1008205 (2019).

621 73. M. Kardos, A. B. A. Shafer, The peril of gene-targeted conservation. *Trends in Ecology &*
622 *Evolution* **33**, 827–839 (2018).

623 74. S. Andrews, *FastQC: A quality control tool for high throughput sequence data. Available*
624 *online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/* (2010).

625 75. M. Martin, Cutadapt removes adapter sequences from high-throughput sequencing reads.
626 *EMBnet.journal* **17**, 10–12 (2011).

627 76. H. Li, Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM.
628 *arXiv* (2013).

629 77. H. Li, *et al.*, The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**, 2078–
630 2079 (2009).

631 78. R. N. Lou, N. O. Therkildsen, Batch effects in population genomic studies with low-
632 coverage whole genome sequencing data: Causes, detection and mitigation. *Molecular*
633 *Ecology Resources* **22**, 1678–1692 (2022).

634 79. G. A. Van der Auwera, *et al.*, From FastQ data to high confidence variant calls: the
635 Genome Analysis Toolkit best practices pipeline. *Curr Protoc Bioinformatics* **43**, 11.10.1-
636 11.10.33 (2013).

637 80. H. Li, A statistical framework for SNP calling, mutation discovery, association mapping and
638 population genetical parameter estimation from sequencing data. *Bioinformatics* **27**, 2987–
639 2993 (2011).

640 81. P. Danecek, *et al.*, The variant call format and VCFtools. *Bioinformatics* **27**, 2156–2158
641 (2011).

642 82. L. Skotte, T. S. Korneliussen, A. Albrechtsen, Estimating individual admixture proportions
643 from next generation sequencing data. *Genetics* **195**, 693–702 (2013).

644 83. J. Meisner, A. Albrechtsen, Inferring population structure and admixture proportions in low-
645 depth NGS data. *Genetics* **210**, 719–731 (2018).

646 84. T. S. Korneliussen, A. Albrechtsen, R. Nielsen, ANGSD: Analysis of Next Generation
647 Sequencing Data. *BMC Bioinformatics* **15**, 356 (2014).

648 85. J. K. Pritchard, M. Stephens, P. Donnelly, Inference of population structure using multilocus
649 genotype data. *Genetics* **155**, 945–959 (2000).

650 86. G. Evanno, S. Regnaut, J. Goudet, Detecting the number of clusters of individuals using the
651 software structure: a simulation study. *Molecular Ecology* **14**, 2611–2620 (2005).

652 87. S. Purcell, *et al.*, PLINK: a tool set for whole-genome association and population-based
653 linkage analyses. *The American Journal of Human Genetics* **81**, 559–575 (2007).

654 88. E. F. Mouresan, *et al.*, Mapping recombination rate on the autosomal chromosomes based
655 on the persistency of linkage disequilibrium phase among autochthonous beef cattle
656 populations in spain. *Frontiers in Genetics* **10** (2019).

657 89. P. Cingolani, *et al.*, A program for annotating and predicting the effects of single nucleotide
658 polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118;
659 iso-2; iso-3. *Fly (Austin)* **6**, 80–92 (2012).

660 90. W. McLaren, *et al.*, The Ensembl Variant Effect Predictor. *Genome Biology* **17**, 122 (2016).

661 91. R. Do, *et al.*, No evidence that selection has been less effective at removing deleterious
662 mutations in Europeans than in Africans. *Nat Genet* **47**, 126–131 (2015).

663