

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

3 Emily Humble¹, Martin A Stoffel², Kara Dicks³, Alex D Ball³, Rebecca M Gooley^{4,5}, Justin
4 Chuyen^{6,7}, Ricardo Pusey⁶, Mohammed al Remeithi⁶, Klaus-Peter Koepfli^{4,5}, Budhan
5 Pukazhenth⁵, Helen Senn³, Rob Ogden¹

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

8 ²Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh,
9 Edinburgh, EH9 3FL, UK

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

12 ⁴Smithsonian-Mason School of Conservation, George Mason University, Front Royal, VA
13 22630 USA

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

16 ⁶Terrestrial & Marine Biodiversity Sector, Environment Agency – Abu Dhabi, United Arab
17 Emirates

18 ⁷US Fish and Wildlife Service, Colorado, USA

19 **Corresponding Author:**

20 Emily Humble, Royal (Dick) School of Veterinary Studies and the Roslin Institute

21 University of Edinburgh, EH25 9RG, UK

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

23 **Keywords:**

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

26 **Abstract**

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

42 **Significance statement**

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

52 Main text

53 Introduction

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

65 Alongside this, recent debate on the significance of neutral genetic variation in conservation
66 biology has raised practical considerations for sourcing populations for restorations (19–23).
67 For example, an increasing number of studies are uncovering genomic evidence for purging
68 in the wild (24–29), some of which have used this to challenge the small population paradigm
69 (25, 26). Furthermore, simulation-based studies on the interaction between effective
70 population size, genetic variation and extinction risk have called for more emphasis on
71 functional genomic variation in genetic rescue attempts (21, 22). These observations go
72 against decades of empirical and theoretical work in favour of maximising genetic variation to
73 enhance population viability (30–33) including recent studies highlighting the complex
74 dynamics of deleterious mutation frequencies in small populations (34–38). Founder selection
75 for translocations rests on a complex set of considerations, with genetics making up only one
76 component (39). In most cases, conservation practitioners will favour a unifying strategy to
77 minimise risk and maximise return (40–42). In light of this, empirical data on the patterns of
78 inbreeding and deleterious mutations in species undergoing active conservation management
79 is urgently required.

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

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

95 **Results**

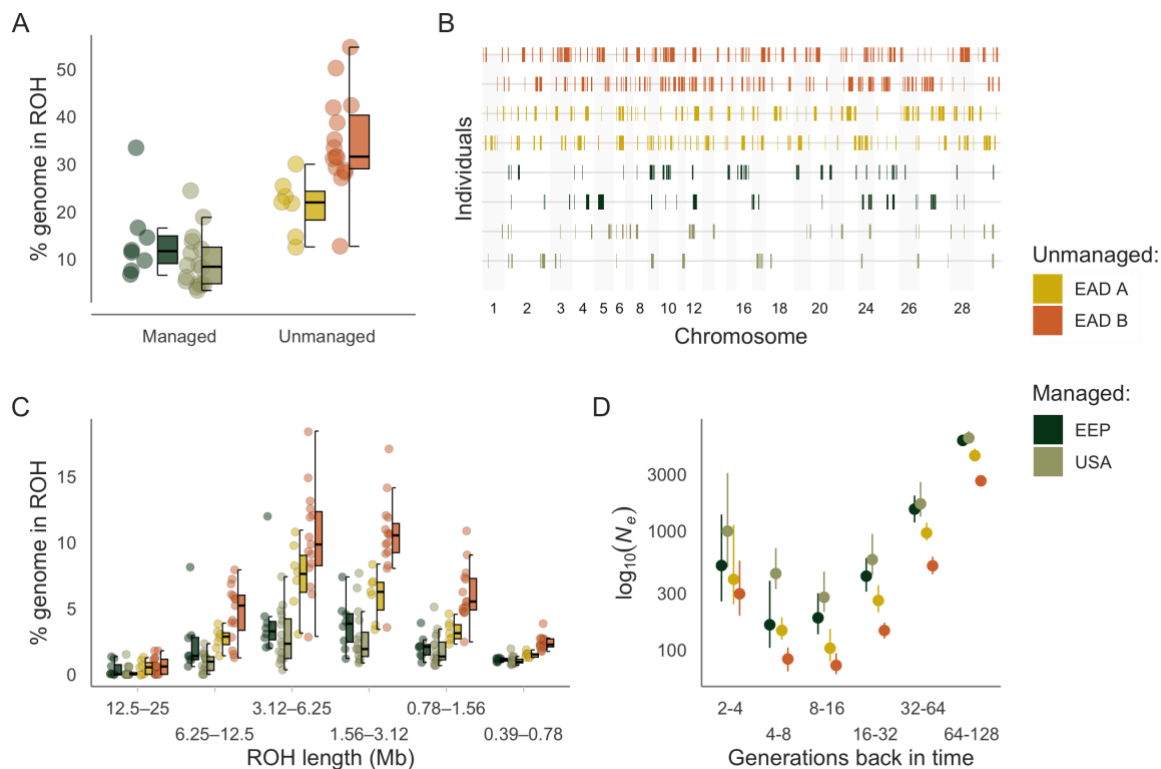
96 We generated whole-genome sequence data for 49 scimitar-horned oryx from four *ex-situ*
97 populations. Two of these, the EAZA *Ex situ* Programmes (EEP, $n = 8$) and the USA ($n = 17$),
98 represent captive populations where genetic management practices are in place. The USA
99 population comprised individuals from both privately owned ranches and institutions within the
100 AZA Species Survival Plan® (SSP). The remaining populations from the Environment Agency
101 – Abu Dhabi originate from two genetically unmanaged collections in the United Arab Emirates
102 (EAD A: $n = 9$ and EAD B: $n = 15$). Census sizes for the EEP and SSP population are
103 approximately 619 and 223 respectively while those for EAD A and EAD B are approximately
104 3,000 and 70. For further details on population origins, management strategies and sampling
105 approach, please refer to the Supplementary Material.

106 High coverage sequencing (~15X) was performed for 20 of the individuals and the remaining
107 29 were sequenced at a lower depth (6–8X, Table S1). Sequencing reads were mapped to
108 the scimitar-horned oryx reference genome (45) and to account for coverage biases, SNPs
109 and genotype likelihoods were called after downsampling high coverage individuals (see
110 Methods for details). Analysis of population structure using NGSadmix and PCAngsd detected
111 differentiation between the four sampling groups (Figures S2–4). Individual admixture
112 proportions highlighted two major ancestral source populations (Figures S2A), with further
113 hierarchical structure being resolved up to values of $K=4$ (Figures S2B and S3), corresponding
114 to the four *ex-situ* groups. PCA distinguished EEP and USA populations as discrete clusters
115 along PC2 and PC3, while EAD A and EAD B clustered separately along PC1 (Figure S4).

116 **Levels of inbreeding across management strategies**

117 To investigate how genomic patterns of inbreeding vary with management strategy, we
118 examined the ROH landscape across individuals (Figure 1). The average number and total
119 length of ROH was 247 (min = 65, max = 638) and 2.0 Mb (0.5–22.0 Mb) respectively, which
120 on average spanned 20% of the autosomal genome (min = 0.03, max = 0.55, Figure 1A and
121 Figure S6). Oryx from managed populations had significantly lower inbreeding coefficients
122 (F_{ROH}) than oryx from unmanaged populations ($\beta = -0.19$, 95% CI = -0.24–-0.14, $P = 6.43 \times 10^{-9}$,
123 Figure 1A). This pattern was driven by both the number and length of ROH, the former being
124 almost three times higher in the most inbred population than in the least inbred population
125 (Figure 1B and Figure S7).

126



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

136 ROH length distribution and recent demography

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

146 long ROH >12.5 Mb which are likely the result of recent inbreeding, were identified in less than
147 30% of individuals from managed populations, yet were present in over 60% of individuals
148 from unmanaged populations.

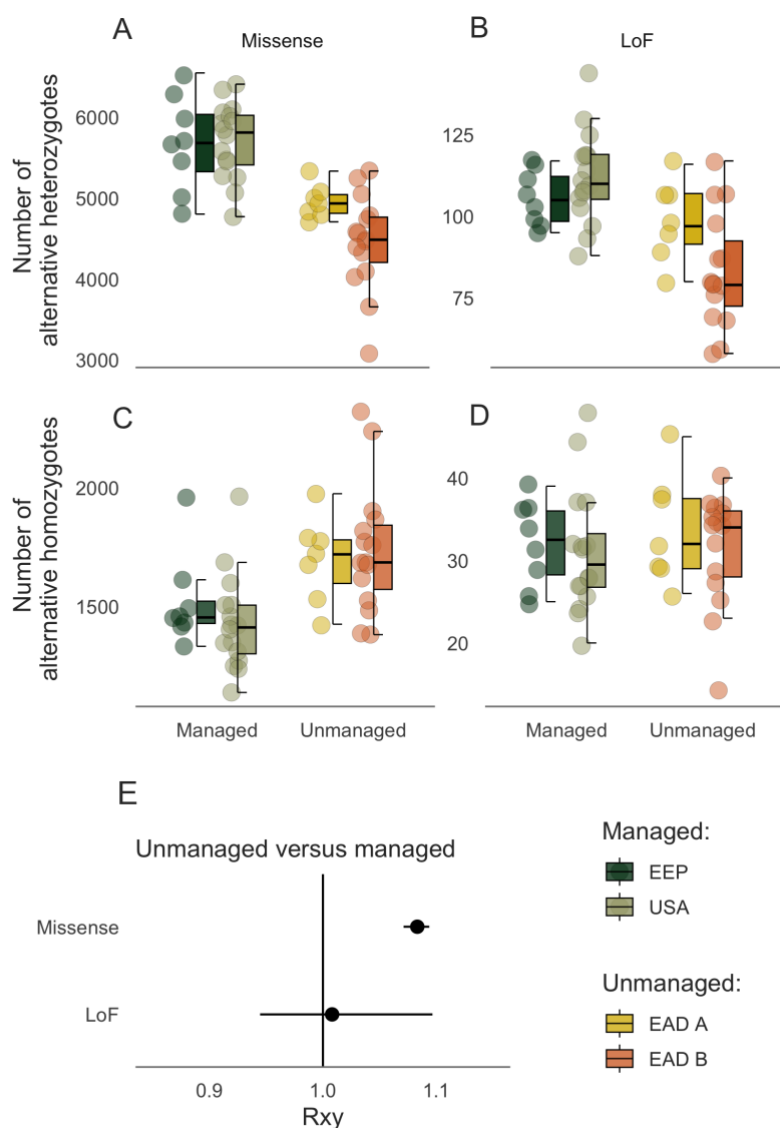
149 As ROH lengths decrease, their underlying haplotypes are expected to have originated from
150 ancestors further back in time (Thompson 2013). The abundance of ROH in different length
151 classes can therefore provide insights into past changes in effective population size (N_e) (46,
152 47). In line with this, our estimates of N_e based on individual inbreeding coefficients were
153 proportional to patterns of ROH abundance (Figure 1D). N_e declines to reach a minimum of
154 around 150 individuals between 8–16 generations ago, after which it shows a steady increase
155 towards the present day. Interestingly, despite small census sizes, managed populations had
156 higher N_e estimates across all time periods than unmanaged populations (mean N_e : USA =
157 1,672, EEP = 1,429 versus EAD A = 1,028, EAD B = 625). These patterns were reflected in
158 estimates of mean pairwise nucleotide diversity which were also higher in managed (USA =
159 0.46×10^5 , EEP = 0.44×10^5) than unmanaged populations (EAD A = 0.42×10^5 , EAD B =
160 0.27×10^5).

161 **Mutation load landscape across management strategies**

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

174 Inbreeding load for both missense and loss of function mutations was consistently higher in
175 managed than unmanaged populations (Missense: $\beta = 1079$, 95% CI = 769–1390, $P = 1.12 \times 10^{-8}$,
176 LoF: $\beta = 21.7$, 95% CI = 12.7–30.6, $P = 1.51 \times 10^{-5}$, Figure 2A–B). As expected, this pattern
177 inversely tracked overall inbreeding levels, where individuals with lower inbreeding coefficients
178 had a larger number of heterozygotes at missense and loss of function sites (Figure S9). In
179 direct contrast, the drift load for weakly deleterious missense mutations was lower in managed

180 populations than in unmanaged groups ($\beta = -267$, 95% CI = -398--137, $P = 1.65 \times 10^{-4}$, Figure
 181 2C). Interestingly, the drift load for highly deleterious loss of function mutations displayed no
 182 difference across management strategies, with similar numbers of alternative homozygotes
 183 present among individuals ($\beta = 0.89$, 95% CI = -3.07--4.86, $P = 6.5 \times 10^{-1}$, Figure 2D).



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

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

200 Discussion

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

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

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

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

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

244 The presence of segregating deleterious variation within insurance populations may be
245 considered a concern for conservation management. Indeed, there has been recent debate
246 surrounding the risks associated with sourcing individuals for restoration from large,
247 genetically diverse populations, given the higher expected levels of masked load (21, 22).
248 However, these concerns are unlikely to be relevant for restoration and reintroduction
249 programs that follow established recommendations (39). Notably, as advised by IUCN/SSC
250 guidelines, sourcing individuals from genetically differentiated populations, releasing large
251 numbers of animals over extended time frames and maximising initial population growth rate
252 all serve to increase genetic variation and prevent the inbreeding load from being expressed
253 (19, 58–60). The scimitar-horned oryx reintroduction plan has followed these best practice
254 guidelines having so far released over 250 animals over a five-year time-period, and in eight
255 separate release batches. Consequently, the released population has now reached close to
256 400 individuals, with over 150 calves born in the wild. Follow-up monitoring of the release
257 herds will provide a rare opportunity to validate these efforts within the context of a large-scale
258 reintroduction effort.

259 In addition to the inbreeding load, we also considered how drift load varies across populations.
260 Several recent studies have demonstrated significant reductions in the relative number of
261 highly deleterious mutations in small versus large (24–26, 29, 37) and in modern versus
262 historical populations (27, 28), and attributed these differences to the effects of purifying
263 selection. While we did not observe a complete reversal in the patterns of drift load for loss of
264 function mutations, we did see a reduction in frequency relative to missense mutations in
265 unmanaged collections. In small inbred populations, partially recessive deleterious mutations
266 will be expressed as homozygotes thereby exposing them to the effects of purifying selection
267 (62–64). However, as selection strength declines with decreasing N_e , fewer deleterious
268 mutations are expected to be removed through purging (65, 66). In line with this, we also show
269 that unmanaged collections have accumulated a considerable burden of weakly deleterious
270 mutations compared to managed populations.

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

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

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

299 **Materials and methods**

300 **Sampling and sequencing**

301 Blood (in EDTA) and tissue (in 100% ethanol) samples were collected for whole genome
302 resequencing from 49 scimitar-horned oryx representing four *ex-situ* populations: the EEP (n
303 = 8), USA ($n = 17$), EAD A ($n = 9$) and EAD B ($n = 15$). The EEP and USA are captive
304 collections undergoing genetic management practices, while EAD A and EAD B represent
305 collections in the United Arab Emirates with no genetic management in place (Supplementary
306 Methods). Total genomic DNA was extracted between one and five times per sample using
307 the DNeasy Blood and Tissue Kit (Qiagen, Cat. No. 69504). Elutions were pooled and
308 concentrated in an Eppendorf Concentrator Plus at 45°C and 1400 rpm until roughly 50 μ l
309 remained. Library construction was carried out using the Illumina TruSeq Nano High
310 Throughput library preparation kit (Illumina CA, UKA). Twenty samples from across all four
311 populations were 150 bp paired-end sequenced on an Illumina HiSeq X Ten platform at a
312 target depth of coverage of 15X. The remaining 29 samples from three of the populations were
313 150 bp paired-end sequenced on an Illumina NovaSeq 6000 instrument at a target depth of
314 coverage of 7X (Table S1).

315 **Read processing and alignment**

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

327 **Variant calling and filtering**

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

337 **Population structure**

338 We characterised population structure using genotype likelihood based approaches in
339 NGSadmix (82) and PCAngsd (83). Genotype likelihoods were first estimated from bam files
340 in ANGSD (84) using the GATK model (-GL 2), inferring major and minor alleles (-
341 doMajorMinor 1) and outputting only polymorphic sites (-SNP_pval 1e-6) with data in at least
342 60% of individuals (-minInd 30). We restricted this analysis to the 28 chromosome-length
343 autosomes and included only regions with Phred quality and mapping scores over 30.
344 Admixture proportions for the individuals in our dataset were calculated using NGSadmix. We
345 performed admixture runs for ancestry clusters ranging from $K=1-6$, with ten runs for each K .
346 The runs with the highest likelihood were plotted. The optimal K was identified based on the
347 maximum value of the mean estimated \ln probability of the data (85) and the Delta K method
348 (86). Two individuals with intermediate admixture proportions between EAD A and EAD B
349 were dropped from further analysis (Figure S3, see Supplementary Methods for details). We
350 next performed a principal components analysis (PCA) using PCAngsd with the default
351 parameters. Eigenvectors were computed from the covariance matrix using R.

352 **ROH calling and individual inbreeding coefficients**

353 We used the filtered SNP genotypes to estimate inbreeding as the proportion of the genome
354 in runs of homozygosity (F_{ROH}). ROH were called with a minimum length of 500 kb and a
355 minimum of 50 SNPs using the --homozyg function in PLINK v1.9 (87) and the following
356 parameters: --homozyg-window-snp 50 --homozyg-snp 50 --homozyg-kb 500 --homozyg-gap
357 1000 --homozyg-density 50 --homozyg-window-missing 5 --homozyg-window-het 3. We then
358 calculated individual inbreeding coefficients F_{ROH} as the sum of the detected ROH lengths for
359 each individual over the total autosomal assembly length (2.44 Gb). To explore the effect of
360 management on inbreeding coefficients we ran linear models with F_{ROH} as the response
361 variables and management strategy as the predictor variable. We also calculated F_{ROH} based

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

366 **ROH length distribution and recent demography**

367 To assess recent changes in oryx population size, we characterised the abundance of ROH
368 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
369 and 0.39–0.78 Mb). Categories were calculated using the formula $100/(2g)$ (50), and reflect
370 the expected lengths of ROH when the underlying haplotypes have most recent common
371 ancestors < 2 , 2–4, 4–8, 8–16, 16–32, 32–64 and 64–128 generations ago respectively. These
372 generations were chosen to capture the time-period during which the wild population of oryx
373 went extinct and captive populations were established. As there is no linkage map for the oryx,
374 physical map lengths as opposed to genetic map lengths were used. For each length class,
375 F_{ROH} was calculated as the sum of the detected ROH lengths for each individual over the total
376 autosomal assembly length (2.44 Gb). We then used individual measures of F_{ROH} to infer
377 recent changes in effective population size across each population. For each time-period
378 described above (t), N_e was estimated given the following expression where $F_{ROH,t}$
379 corresponds to the individual inbreeding coefficient at time t .

$$380 \quad F_{ROH,t} = 1 - \left(1 - \frac{1}{2N_e}\right)^t$$

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

383 **Nucleotide diversity**

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

389 **Identification of deleterious mutations**

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

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

403 To identify deleterious mutations, we predicted the functional effects of the polarised SNP
404 variants using both SnpEff v5.0 (89) and the Variant Effect Predictor v99.2 (90). These
405 methods compare a set of variants to an annotation database and predict the consequence
406 of the alternative alleles on genes, transcripts and proteins. Both were run using the NCBI
407 RefSeq scimitar-horned oryx genome annotation downloaded from:
408 https://ftp.ncbi.nlm.nih.gov/genomes/all/annotation_releases/59534/100/GCF_014754425.2
409 [SCBI Odam 1.1/](#). For each approach, sites with warnings were removed from the VCF file
410 and SNPs were then categorised as loss of function or missense according to the
411 classifications provided in Table S2. For each dataset, we also extracted a random subset of
412 100,000 intergenic SNPs for use in the Rxy analysis below. For each set of SNPs, genotypes
413 were extracted for all individuals using a combination of VCFtools and PLINK.

414 **Genetic load landscape across management strategies**

415 To assess how the genetic load varies across populations we used two approaches. First, we
416 approximated two components of genetic load; inbreeding and drift load (48). Inbreeding load
417 was measured as the total number of heterozygotes per individual for both loss of function
418 and missense variants. Drift load was measured as the total number of alternative
419 homozygotes per individual for both loss of function and missense variants. Second, we used
420 the Rxy statistic to estimate the relative frequency of loss of function and missense mutations
421 in one population over another (91). Alternative allele frequencies were calculated based on
422 individuals from managed and unmanaged populations separately. A random subset of
423 100,000 intergenic SNPs was used to standardise our estimates and account for population-
424 specific biases. To calculate 95% confidence intervals around our estimates, we randomly
425 resampled 70% of SNPs within each impact category without replacement and recalculated
426 Rxy. This was repeated 100 times. To explore the effect of management on genetic load, we
427 ran linear models with inbreeding or drift load as the response variable and management
428 strategy as the predictor variable.

429 **Data availability**

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

434 **Author contributions**

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

439 **Acknowledgements**

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

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