Whole mitochondrial genome capture from low-quality fecal samples and museum-preserved specimens

Tom van der Valk^{1,*}, Frida Lona Durazo^{1,#}, Love Dalén², Katerina Guschanski^{1,*}

¹ Animal Ecology, Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, 752 36, Uppsala, Sweden

² Department of Bioinformatics and Genetics, Swedish Museum of Natural History, SE-10405 Stockholm, Sweden

[#]Current address: Department of Anthropology, University of Toronto Mississauga, 3359 Mississauga Road, Mississauga, ON, L5L 1C6, Canada

Correspondence: *Tom van der Valk, email: tom.vandervalk@ebc.uu.se

*Katerina Guschanski, email: katerina.guschanski@ebc.uu.se

Key word: Target enrichment, mtDNA, non-invasive samples, natural history collections, great apes

Running title: MtDNA capture from fecal and museum samples

Abstract

Population-scale molecular studies of endangered, threatened, and cryptic species are often limited by access to high-quality samples. Non-invasively collected samples, such as feces, are more readily available. In addition, museum-preserved specimens represent a valuable resource that reduces the pressure on natural populations. However, endogenous DNA content in such samples is low, making shotgun sequencing a financially prohibitive approach. Here, we apply a widely used target enrichment method to retrieve complete mitochondrial genomes from 56 non-invasively collected fecal samples and 65 museum specimens of two great ape species, Grauer's gorilla and eastern chimpanzee. We show that the applied method is suitable for a wide range of sample types that differ in endogenous DNA content, increasing the proportion of target reads to

over 300-fold. By systematically evaluating possible biases that can be introduced during target enrichment, we show that capture is less efficient for fragments that are shorter or longer than the baits, that the proportion of human contaminating reads increases post-capture although capture efficiency is lower for human compared to gorilla fragments with a gorilla-generated bait, and that the rate of jumping PCR is considerable, but can be controlled for with a double-barcoding approach. We succeed in capturing mitochondrial genomes from gorilla and chimpanzee fecal samples, but observe reduced capture efficiency as sequence divergence increases between the bait and target species. This study establishes mitochondrial genome capture from field-collected fecal samples and museum specimens as a robust, easy-to-implement, and reliable approach, opening doors to population-wide studies of non-model organisms.

Introduction

A limiting factor for the genetic study of endangered, threatened and cryptic species is access to high-quality samples, as these are rarely collected from wild animal populations for ethical and/or logistical reasons. In contrast, non-invasive samples, such as feces, hair, and food remains are relatively readily available and can be obtained at a population-wide scale. However, DNA in such samples is generally of poor quality, characterized by low endogenous content, short fragment size and presence of inhibitors (Gagneux et al. 1997; Taberlet et al. 1999; Snyder-Mackler et al. 2016). These traits are shared with museum-preserved specimens (Burrell et al. 2015), another sample source that allows studies of wild animal populations without subjecting them to dangers and stress of high-quality sample collection. The use of natural history collections for genomic analyses has become increasingly popular in recent years (e.g. Bi et al. 2013; Guschanski et al. 2013; Good et al. 2015; Weiß et al. 2016; McCormack et al. 2016). This offers the chance to study species and populations that are difficult or impossible to sample in the wild, because they occupy remote, difficult-to-access ranges, or are extinct in certain locations. Furthermore, historical samples provide a snapshot of the past, allowing the investigation of temporal population dynamics, which is frequently of interest, particularly for endangered species (Wandeler et al. 2007; Xenikoudakis et al. 2015). In addition to the classical hallmarks of low-quality DNA shared with feces, historical samples also show DNA damage, typically concentrated at the ends of the molecules (Nyström et al. 2006).

Modern molecular and analytical approaches effectively open doors to studies based on low-quality samples. They include extraction methods that retain short DNA fragments (Rohland et al. 2007; Dabney et al. 2013), account for or remove DNA damage (Skoglund et al. 2014; Rohland et al. 2015), and effectively neutralize PCR inhibitors (Vallet et al. 2008; McDevit et al.

2009; Zhang et al. 2010). Although next generation sequencing (NGS) is well suited for highly degraded samples, the low endogenous DNA content of fecal and historical samples, frequently below 1% (Perry et al. 2010; Särkinen et al. 2012; Carpenter et al. 2013; Damgaard et al. 2015; Snyder-Mackler et al. 2016), makes shotgun sequencing a financially costly approach. Therefore, methods that increase the proportion of endogenous DNA in the sample or enrich the sample for a particular region of interest are usually preferred. Multiple enrichment approaches have been developed (Briggs et al. 2009; Gnirke et al. 2009; Burbano et al. 2010). In hybridization-based capture, “baits” that cover the genomic region of interest are used to “catch” fragments from the complex mixture of DNA molecules contained in the sample extract. However, typically, bait construction relies on the availability of a reference genome or prior sequence knowledge, requires considerable bioinformatics expertise, and the synthesis costs of capture probe sets are rather high. All this makes the application of the approach prohibitive for studies of non-model organisms. Recently, alternative methods, in which a high quality DNA sample from the species of interest or a close relative is used as a template for bait construction, have been developed and successfully applied to non-model organisms (e.g. Bi et al. 2013; Carpenter et al. 2013; Mariac et al. 2014; Peñalba et al. 2014; Good et al. 2015; Gasc et al. 2016; Snyder-Mackler et al. 2016). In the widely used capture method described in Maricic et al. (2010), baits are constructed by PCR-amplifying genome regions of interest from a high quality sample. These PCR products are biotinylated, immobilized on magnetic beads, and used for in-solution target capture. The hybridization reaction allows target enrichment across large phylogenetic distances even from degraded low-quality samples (Guschanski et al. 2013; Peñalba et al. 2014), making it highly applicable for studies of non-model organisms from non-invasive samples. This technically relatively simple and cost-effective method can be carried out with standard lab equipment. Even

though this approach has been extensively applied to high quality modern (Delfin et al. 2014; Lippold et al. 2014; M. Li et al. 2015; C. Li et al. 2015), ancient (Dabney et al. 2013; Meyer et al. 2013; Thalmann et al. 2013), and museum-preserved historical samples (Guschanski et al. 2013; Liedigk et al. 2015; Hawkins et al. 2016), a number of possible biases that can arise from target enrichment have not been systematically evaluated. These include preferential retention of fragments of certain size, reduced capture efficiency of chemically damaged molecules, and capture performance in the face of contaminating sequences from closely related species. In addition, no study so far has evaluated the suitability of this capture protocol for the use with non-invasive samples, such as feces.

Here we apply the PCR-based target enrichment method of Maricic et al. (2010) to capture complete mitochondrial genomes from museum-preserved specimens and field-collected fecal samples of two great ape species, the critically endangered Grauer's gorillas (*Gorilla beringei graueri*) and the endangered eastern chimpanzees (*Pan troglodytes schweinfurthii*) (IUCN 2016). We focus on mitochondria, because the higher copy numbers of mitochondrial compared to nuclear genomes means that the former are more easily sequenced from low-quality samples and they continue to be widely used in population genetics and systematics (Desalle et al. 2017). It should also be noted that PCR-based mtDNA studies of great apes, and specifically gorillas, have been hampered by the high prevalence of numts (Thalmann et al. 2004). Target capture and subsequent NGS effectively overcome this problem, due to the great number of true mtDNA fragments compared to nuclear genome-encoded numts (Li et al. 2012; Guschanski et al. 2013).

To maximize the number of complete mitochondrial genomes that can be captured and sequenced simultaneously, we used a highly multiplexed setup, in which short incomplete and individually barcoded Illumina sequencing adapters are ligated to DNA extracts prior to capture (Rohland et al. 2015). This significantly reduces reagent and sequencing costs and increases capture efficiency, since non-hybridizing adapter overhangs remain short during capture. Adapters are double-indexed and completed post-capture, allowing us to pool and sequence 270 mtDNA enriched libraries on a single Illumina HiSeq2500 lane using only 18 index primers and 15 additional barcodes, retaining high certainty of sample identity after sequencing. The aim of our study was two-fold: i) to evaluate how target enrichment affects endogenous content, levels of human contamination, read length distribution, prevalence of DNA damage, and frequency of jumping PCR by comparing shotgun-sequenced and target-enriched data from historical samples and ii) to investigate the possibility to use mtDNA genome capture from fecal samples, evaluating its performance compared to museum specimens and the ability to capture mtDNA genomes from closely related species.

Methods

Sample collection

Historical samples (N = 69) were collected at the Royal Belgian Institute of Natural Sciences (RBINS) in Brussels and the Royal Museum for Central Africa (RMCA) in Tervuren, Belgium. From the historical specimens, we collected teeth or soft tissue samples (ear cartilage, fingertips or parts of hides, targeting the hardest tissue available). Fecal samples (N = 64) were collected in 2014 in the Democratic Republic of Congo using 2-step method (Nsubuga et al. 2004) from great ape night nests a few hours after defecation in two different locations: the high altitude sector of the Kahuzi-Biega National Park (KBNP) and the low altitude region of Nkuba-Biruwe, located between Kahuzi-Biega and Maiko national parks. Three social groups of great apes were targeted: one habituated gorilla group from KBNP and one unhabituated group each of gorillas and eastern chimpanzees from Nkuba-Biruwe region.

Sample extraction and individual identification

Historical samples were extracted in a dedicated ancient DNA laboratory. To remove surface contamination from teeth, we exposed the roots to UV light (245nm) for 10 minutes, abraded the surface with an engraving cutter, using a Dremel 8100 drill, and again subjected the cleaned surfaces to UV light for 10 minutes. We collected tooth powder from either dentin or cementum fraction with an engraving cutter. Whole root, ear cartilage, fingertip or hide samples were pulverized with mortar and pestle. The samples (50-90 mg/sample) were extracted using an ancient DNA protocol that is optimized for retention of short fragments (Dabney et al. 2013). We

added 0.0065 % v/v of TritonX-100 to the extraction buffer for tissue samples. Four dentin samples were extracted a second time with Vivapsin filters (Brace et al. 2012).

All fecal samples were extracted in a dedicated fecal extraction laboratory with the QIAamp DNA Stool mini kit following the manufacture's protocol with slight modifications (Nsubuga et al. 2004). Extraction success was tested via a standard PCR reaction with primers targeting a 100 bp region of the amelogenin locus (Bradley et al. 2001). Presence of PCR and library preparation inhibitors was detected in one third of the samples (n=23). Consequently, these samples were re-extracted using the 2-CTAB/PCI method (Vallet et al. 2008) with few adjustments. Specifically, we did not perform the RNA digestion step and second lysis was shortened to one hour. Approximate individual age was determined by dung bolus size (Schaller et al. 1963) and we excluded juvenile samples from further analyses (n=8). A total of 56 samples were subjected to our capture protocol. Since fecal samples were collected from night nests of unknown individuals, we inferred individual identity by genotyping the samples at 13 autosomal microsatellite loci as in Guschanski et al. (2009). Individual identification analysis was performed in CERVUS 3.0.7 and we found that 15 individuals in our dataset were sampled repeatedly (2-4 times). These samples served as biological replicates.

Fecal and museum samples were extracted in batches of 12 or 20, and for every batch two to four extractions blanks were included to check for contamination via the amelogenin PCR test (Bradley et al. 2001). No contamination was detected during extraction, but we nonetheless propagated multiple blanks throughout capture and sequencing.

Shotgun sequencing

Low coverage shotgun sequencing data was generated for 40 historical samples and two extraction blanks (Table S1). Each sample was labeled with two internal barcodes that were directly adjacent to the insert, and two indices (Fig. S1, Kircher et al. 2012, Rohland et al. 2015). Libraries were constructed as in Rohland et al. (2015), amplified during indexing for 12 cycles and pooled in equimolar amounts in two pools of 22 and 18 samples, respectively. Blanks were treated like the samples and we added 2.5 μ L of each blank to the pool of 22 samples, matching their volume to that of an average sample. The pool of 18 samples was combined with another sequencing library unrelated to this project and both pools were subjected to 125 bp paired-end shotgun sequencing on the Illumina HiSeq2500 platform (High Output Mode).

Capture

We followed the capture protocol as described in Maricic et al. (2010) with slight adjustments. For bait construction, we amplified the entire mitochondrial genome from a high quality western lowland gorilla (*Gorilla gorilla gorilla*) blood sample in two overlapping PCR fragments of 8 kb and 10 kb, using primate-specific mitochondrial primers as described in Finstermeier et al. (2013) and Guschanski et al. (2013). The PCR products were sonicated on the Covaris E220 to an average size of 200 bp (10% duty cycle, 175 PIP, 250 cycles per burst, for the duration of 350 sec. at 6°C). Based on Bioanalyzer results, we concluded that DNA fragment length for fecal samples was normally distributed around 1000 bp. We therefore sonicated the fecal samples before capture to the average size of 250 bp (conditions identical to above with 160 sec. duration). The average fragment size of museum samples was ~100 bp as assessed by Bioanalyzer and therefore shearing was not needed. Extraction blanks from different extraction

190 batches (n=13), museum and fecal samples (n=65 and n=56, respectively) were blunt-ended and
191 ligated to Illumina-adapters, each containing a 7 bp barcode (Rohland et al. 2015). After adapter
192 ligation, samples were quantified by qPCR with PreHyb-F and PreHyb-R primers (Rohland et al.
193 2015, fig. S1) and subsequently amplified with the same primers to ca. 10^{12} fragments/ μ L (18
194 cycles on average). Blanks were amplified for 18 cycles to correspond to an average sample.
195 Amplified libraries were quantified with qPCR and samples pooled, aiming for equimolar
196 proportions (ca. 5×10^{12} fragments/sample), in nine different capture pools, each containing 15
197 samples. Three pools contained only historical samples, three pools only fecal samples, and three
198 pools contained both sample types (table S2). Fecal and historical extraction blanks were either
199 contained within the mixed pools or with the pool corresponding to the sample type. Following
200 the first capture round, we divided the eluate of every capture pool into two equal volume
201 fractions. The first fraction was turned into a sequencing library by completing the adapters with
202 a double-indexing PCR, following Kircher et al. (2012), for 12 to 14 cycles depending on the
203 eluate concentration (table S2). The second fraction was subjected to a second round of capture
204 under the exact same conditions as before, without additional amplification. The eluate of the
205 second capture was subsequently turned into sequencing libraries as above, but because DNA
206 concentration was slightly lower, 16 cycles were used. Every capture pool eluate was amplified
207 in a separate indexing reaction with a unique combination of indexing primers, each containing a
208 7 bp index (fig. S1). By combining barcoded adapters with the double-indexing library
209 preparation approach, we were able to pool and sequence 270 different DNA libraries on a single
210 HiSeq2500 lane (High Output Mode, 125 bp paired-end) with just 15 barcodes and 18 indices.

213 **Sequence data analysis**

214 Sequences were demultiplexed by pool using the unique combination of indices and allowing for
215 a single sequencing error per index using an in-house python script (Supporting information). All
216 indices were designed with a minimum distance of 3 bp (Kircher et al. 2012), therefore
217 conversion of one index into another through a single base misincorporation or sequencing error
218 was not possible. Within each pool, samples were demultiplexed based on unique barcodes, using
219 an in-house python script with the same criteria as above. We estimated the prevalence of
220 jumping PCR per sample as the fraction of reads containing wrong barcode pairs after PCR
221 amplification (see Results) and these reads are discarded before downstream analysis. After
222 demultiplexing, barcodes were removed using an in-house python script, reads overlapping by a
223 minimum of 11 bp were merged and all reads shorter than 15 bp discarded (AdapterRemovalV2,
224 --collapse). Merged and unmerged reads were mapped separately to either the gorilla reference
225 genome (GorGor4) or chimpanzee reference genome (Pan_tro 3.0), depending on the species
226 identity of the sample. We used BWA-MEM with standard parameters and the resulting two
227 BAM files were combined after mapping with samtools merge. PCR duplicates were removed
228 with Picard MarkDuplicates. Mpileup files were generated for the complete mitochondrial
229 genomes with samtools mpileup. To call the consensus sequence, we required that each position
230 is covered by at least three independent sequencing reads and >90% of nucleotides at this
231 position are identical. All other positions were set as unidentified, encoded by the character “N”.
232 Since the mitochondrial D-loop region in great apes is typically problematic to sequence and
233 assemble (Prado-Martinez et al. 2013), we removed this region before subsequent analysis.
234 Human contamination in gorilla samples was estimated by mapping all sequenced reads
235 simultaneously against a panel of 21 human mtDNA genomes (same panel as used in Prado-

Martinez et al. 2013), the reference western lowland gorilla mtDNA (GCA_000151905.3) and one Grauer's gorilla mtDNA genome (KF914213.1). For chimpanzee samples, the human panel and the reference chimpanzee mtDNA genome (GCA_000001515.5) were used. The fraction of reads uniquely mapping to the human sequences was taken as an estimate of human contamination. Mitochondrial DNA damage was assessed by calculating the frequency of C-to-T and G-to-A substitutions per read position across all mtDNA reads using mapDamage2.0 (Jónsson et al. 2013). All statistical tests were performed in R and adjusted for multiple testing with Bonferroni correction (Rice 1989).

Results

Evaluating capture efficiency and assessing possible capture biases

If biases are introduced during capture of low-quality samples, downstream analyses can be significantly impacted. We therefore systematically assessed capture efficiency and presence of biases in the Maricic et al. (2010) method, by comparing sequence data from the same set of samples pre and post capture.

Forty historical samples were shotgun-sequenced, without any capture, to an average coverage of $0.24 \times$ (95% CI: 0.01 - $0.9 \times$), producing $4.5 \cdot 10^6$ unique sequencing reads per sample on average (table S1). Two extraction blanks yielded $0.0023 \cdot 10^6$ unique sequencing reads on average, a factor of 2000 less than the samples, suggesting that contamination during the experimental procedure was low. Endogenous DNA content varied greatly, ranging from less than 1% to over 99% (mean = $42.9 \pm 6.37\%$, Fig. S2A). Samples derived from soft tissues (fingertips and ear cartilage) had significantly higher endogenous DNA content than tooth samples (T-test, $P = 5.18 \times 10^{-7}$, Fig. S2A). The proportion of mitochondrial reads ranged from 0.003% to 0.95% (mean = $0.15 \pm 0.03\%$) and did not differ significantly across different sample types (pairwise T-test, all $P > 0.4$, Fig. S2B).

Target enrichment after capture

Thirty of the 40 shotgun-sequenced samples were subjected to mtDNA capture procedure (see methods, table S1). Capture significantly increased the proportion of target sequences from 0.13

± 0.04% mtDNA reads before capture to $29.15 \pm 3.23\%$ ($P = 7.4 \times 10^{-10}$) and $40.5 \pm 3.78\%$ ($P = 9.7 \times 10^{-13}$) after first and second capture round, respectively (Fig. 1), a total increase of over 310-fold. We found no significant correlation between the proportions of mitochondrial reads pre- and post-capture ($r = 0.16$, $P = 0.39$ and $r = 0.33$, $P = 0.067$ for pre-capture vs. one and two capture rounds, respectively, Fig. S3). However, the proportional increase of mtDNA was higher for samples with low endogenous mtDNA content ($r = -0.50$, $P = 0.024$, Fig S5b). Genome coverage was relatively equal across the entire length of the mitochondrial genome, suggesting no pronounced regional capture biases (Fig. S4).

Fig 1, Capture performance of shotgun sequenced and captured museum samples. Proportion of mtDNA reads out of all sequenced reads significantly increased following one and two capture rounds (* P -value = <0.05 , *** P -value $< 10^{-10}$).

Human contamination

To investigate if capture leads to increased proportion of human contamination, we estimated the percentage of human reads before and after capture. Gorillas and humans are closely related and therefore cross-hybridization of human reads with the gorilla-generated bait is possible. In addition, the frequent handling of museum-preserved specimens makes the presence of human contamination likely. Average human contamination, measured as the proportion of human mtDNA reads out of all mtDNA reads mapped to the reference panel (see Materials and Methods), was low before capture ($0.83 \pm 0.15\%$), and significantly increased after one capture round ($2.67 \pm 0.23\%$, $P = 1.23 \times 10^{-8}$, Fig. S5a). Human contamination decreased following the second capture round ($1.5 \pm 0.26\%$, $P = 0.0012$, Fig. S4). Nevertheless, gorilla reads were captured 84 times more efficiently than human reads (Fig. S5b/c).

Read length and DNA damage

We found no significant difference in average read length before and after capture (t-test, $P = 0.71$). However, the proportion of reads below 75 bp and above 175 bp was significant lower following capture (t-test, pre-capture versus one capture round, $P_{\text{reads} < 75\text{bp}} = 0.020$, $P_{\text{reads} > 175\text{bp}} < 0.00078$, Fig 2).

Fig 2, Read length distribution following shotgun sequencing, one and two capture rounds. Averages and SE are shown. Short (<75 bp) and long (>175 bp) fragments are captured less efficiently.

DNA damage in our historical samples was low, a factor of 10 lower compared to a 500-600 year old horse sample (Sawyer et al. 2012), which can be explained by the young age of our samples (on average 65.4 years, ranging from 29 to 105 years of age). We did not find any difference in the number of C-to-T and G-to-A transition before and after capture (T-test: $P_{C-to-T} = 0.14$, $P_{G-to-A} = 0.07$ and $P_{C-to-T} = 0.16$, $P_{G-to-A} = 0.09$ for one and two capture rounds, respectively, Fig. 3), suggesting that low levels of DNA damage do not lead to capture biases.

Fig. 3, Frequency of G-to-A and C-to-T transitions averaged over all mtDNA reads for the first 25 bp. Vertical lines depict 95% CI.

Chimeric reads and jumping PCR

We assessed how the proportion of reads with wrong barcode pairs changed following capture. Before capture, 0.034% of reads contained a wrong barcode pair, with all barcode represented in equal proportions among these chimeric reads. After capture, the proportion of chimeric reads increased significantly to 0.55% and 0.64% after one and two capture rounds, respectively (T-test, $P_{\text{pre-capture} - 1 \text{ capture round}} = 5.1 \times 10^{-4}$ $P_{1\text{-capture round} - 2 \text{ capture rounds}} = 4.4 \times 10^{-4}$). We evaluated the effect of the number of amplification cycles and the amount of starting material on the proportion of jumping PCR. No interactions between number of PCR cycles and starting material were observed (ANCOVA, $F=0.65$, $P = 0.58$). More chimeric reads were found in samples that went through a higher number of PCR amplification cycles (ANCOVA, $F=4.31$, $P = 0.0057$, Fig S6).

Although no significant correlation between input DNA and jumping-PCR was seen ($F=1.92$, $P = 0.17$), samples with low amount of input material tended to show more chimeric reads. This is especially pronounced in blanks, which contained on average three times more chimeric reads than samples (Fig S6). Following capture, a single barcode (#8) was significantly more often found to be paired with another barcode (T-test $P = 1.14 \times 10^{-11}$), suggesting that cross-contamination happened before capture with this specific barcode.

mtDNA sequence reconstruction

Finally, to evaluate if identified biases can result in erroneous mitochondrial sequences, we compared the mitochondrial genome sequences reconstructed from shotgun and capture data for the same samples ($N = 30$). No differences were found in consensus sequences obtained from data before and after one and two capture rounds with our standard base-calling criteria (coverage threshold of 3x, see Methods).

Capture efficiency by sample type in museum specimens

We did not observe a significant difference in endogenous DNA content between dentin and cementum tooth fractions, in contrast to previous findings (Damgaard et al. 2015). The proportions of on-target reads were similar among different historical sample types, although they differed markedly in the endogenous DNA content pre-capture (Fig. S2). Following the second capture round, the proportion of on-target reads increased for almost all museum samples compared to first capture (Fig. S7). However, this increase was significant only for the soft tissue samples (ear cartilage, fingertip, and hide combined, T-test $P = 0.046$, Fig. 4).

Capturing fecal samples

Whole mtDNA capture is an established procedure for historical and archaeological samples (Guschanski et al. 2013; Briggs et al. 2009; Maricic et al. 2010). However, this approach has not yet been applied to non-invasive fecal samples. After correcting for multiple testing, the proportion of on-target reads did not differ between fecal and museum samples after one and two capture rounds, respectively (Fig. 4). Similar to the historical samples, the proportion of on-target reads increased for fecal samples following the second capture round, but this increase was not significant. Overall, our results suggest that the used capture approach works as efficiently for fecal samples as for museum specimens.

Fig. 4, On-target reads per sample type after one and two capture rounds. No significant differences were observed within each capture round across sample types. However, percentage of on-target reads was significantly higher in soft tissue samples following the second capture round ($P < 0.05$).*

Effect of evolutionary distance on capture efficiency

Grauer's gorilla and eastern chimpanzee fecal samples were captured with baits constructed from a western lowland gorilla mtDNA genome. If capture efficiency decreases with genetic distance between bait and sample, we expect to find lower percentage of on-target reads in chimpanzee fecal samples compared to gorilla. We also expect to find lower coverage in the regions of mitochondrial genome that show greater divergence between bait and target. In concordance with our expectations, chimpanzee samples yielded significantly fewer on-target reads than gorilla fecal samples (T-test: $P < 0.013$, Fig. 5a). We also observed a significant negative correlation between local genome divergence and coverage (Pearson's $R = -0.15$, and $R = -0.31$, for gorillas and chimpanzees, respectively, both P values $< 2.2 \times 10^{-16}$, Figs. 5B, 5C), suggesting that mitochondrial genome regions with relatively higher divergence to the western lowland gorilla were captured less efficient in both great ape species. Nevertheless, in chimpanzee samples, capture increased the proportion of endogenous mtDNA reads to 6.5% and 10.3% on average after one and two capture rounds, respectively, despite more than eight million years divergence between chimpanzees and gorillas (Langergraber et al. 2012). This shows that although capture is more efficient if the bait is constructed from a closely related species, capture and reconstruction of complete mitochondrial genomes from fecal samples is still possible across moderate evolutionary distances.

In general, we observe a negative correlation between local coverage and GC-content ($R = -0.17$, P -value $< 2.2 \times 10^{-16}$). Upon closer examination, we found reduced coverage in both high and low GC regions (Fig. S10), which can be explained by the properties of the used polymerase during library amplification (see Discussion).

Fig. 5 A) Percentage of on-target reads for gorilla and chimpanzee fecal samples after one and two capture rounds ($*** P < 0.0001$). B) Relative coverage of chimpanzee samples, calculated as the average coverage of the bin divided by the average coverage of the whole mtDNA, for sliding window bins of 30 bp against sequence divergence to western lowland gorilla, used for bait construction. Dotted line shows fitted linear regression ($r = -0.31$, $P\text{-value} < 0.0001$) C) Same as b, but for all gorilla samples ($r = -0.15$, $P < 0.0001$).

Human contamination in fecal samples

We found the presence of human mtDNA reads in 98.5% of fecal samples and in 90.0% of museum specimens. Estimated human contamination in gorilla fecal samples was significantly lower than in museum specimens (t-test, $P = 5.5 \times 10^{-9}$). In contrast to our observation in museum specimens, it did not change significantly between one and two capture rounds in fecal samples (t-test, $P = 0.71$, mean = $0.88 \pm 0.14\%$ and $0.82 \pm 0.08\%$, respectively, Fig. S8). In chimpanzees, the proportion of human reads among all mitochondrial reads was significantly higher than in gorilla samples (mean = $4.2 \pm 0.61\%$, $P = 1.32 \times 10^{-8}$ and $4.6 \pm 0.73\%$, $P = 1.592.2 \times 10^{-13}$ after one and two capture rounds, respectively, Fig. S8).

Sequence confirmation with replicates

With our multiplexed capture approach we were able to recover over 90% complete mitochondrial genomes for 95% of museum samples, 80% of gorilla fecal samples, and 40% of chimpanzee fecal samples (Fig. S9). Accurate reconstruction of mitochondrial genomes are frequently needed for phylogenetic or population genetic analyses. We therefore included 48 replicates, 36 of which were from individuals represented by multiple fecal samples and 12 from museum specimens that were extracted twice with different extraction protocols (Methods). At 3x coverage threshold, all 48 replicates produced identical mtDNA genomes with the exception of a single chimpanzee sample that showed five differences within a 100 bp region. After manual inspection, these discrepancies could be attributed to the presence of three human contaminant reads mapped to this region.

Discussion

The ability to generate genomic data for natural populations from low-quality, non-invasive samples is the next essential step for many questions in ecology, evolution, and conservation. Here we apply an easy-to-implement, flexible enrichment method to effectively capture complete mitochondrial genomes from a wide range of sample types, including historical specimens (soft and hard tissues, ranging in age from 29 to over 100 years) and field-collected fecal samples from two different tropical locations. Our study thus uses sample types frequently encountered by researchers working with natural history collections, and fecal material obtained from difficult environmental conditions, known to induce rapid degradation and chemical modification of DNA (Allentoft et al. 2012).

Capture biases

A major concern when applying capture methods is the introduction of biases that could influence downstream analyses. In low-quantity, degraded, contaminated, and damaged samples, the sources of possible biases are manifold, specifically because rare initial errors will be amplified during PCR reactions that are part of capture and library preparation protocols. We extensively evaluated how the applied capture procedure affects coverage across the mitogenome, read length distribution, prevalence of jumping PCR, and the proportion of damaged and contaminating reads.

Previous studies utilizing target enrichment reported reduced coverage in regions with low GC content (Briggs et al. 2009; Guschanski et al. 2013). In contrast, we find a mild bias against both high- and low-GC regions (Fig. S10), which reflects known GC biases of different polymerases

used during library preparation (Dabney et al. 2012). Specifically, Goldtaq polymerase used in previous studies (e.g. Briggs et al. 2009; Guschanski et al. 2013) shows low efficiency in low-GC regions, whereas Pfu TurboCx polymerase used in this study performs best at intermediate GC content (Dabney et al. 2012). Because we find the same GC-bias in pre-and post-capture data, it is best explained by the use of the polymerase enzyme during library indexing and not by the capture procedure.

Target capture has been reported to result in preferential retention of longer fragments (Cruz-Dávalos et al. 2016; Enk et al. 2013). In contrast to these findings, we observed no change in median read length post capture, but instead detect reduced retention of both short and long fragments (Fig 3). Because our museum-preserved samples contain long fragments that are rarely found in ancient specimens, we could detect this additional bias against long reads. Unhybridized overhangs of long fragments that exceeds the length of the bait can re-associate with the complementary strand and thereby reduce overall hybridization efficiency, an effect known to occur in PCR capture experiments based on oligonucleotide microarrays (Suzuki et al. 2007; Tomlinson et al. 2014). Therefore, the average fragment size of the bait should ideally be identical to the average fragment size of the extract.

DNA damage is known to occur in ancient samples and has been demonstrated in museum specimens (Nyström et al. 2006; Sawyer et al. 2012). Specifically, deamination of cytosine to uracil at the single-stranded fragment overhangs leads to C-to-T and G-to-A transition in the resulting sequence data (Hofreiter et al. 2001; Gilbert et al. 2003; Briggs et al. 2007). Damaged fragments could be captured less efficiently, since they are more divergent to the bait compared

to non-damaged fragments. Consistent with previous studies (Sawyer 2012), DNA damage in our museum samples was low and accordingly we found no change in proportion of damaged reads post-capture.

Since our multiplexed capture approach relies on bulk amplification of pooled samples during indexing, cross-contamination and jumping PCR, leading to false read assignment during demultiplexing, can occur (Gilbert et al. 2003, Kircher, et al. 2012; Wright et al. 2016). The resulting chimeric sequences can have a pronounced influence on biological inferences and therefore their detection and removal is essential. We detected chimeric reads containing wrong barcode pairs in both pre- and post-capture sequences. The significant increase in chimeric reads following capture suggests that they arise from jumping PCR during bulk amplification. The proportion of chimeric reads increased with the number of amplification cycles, suggesting that these should be kept to the minimum, as previously suggested (Kim et al. 2015). Our finding further demonstrates the importance of double-barcoding design when applying a multiplexed capture strategy. Detecting and removing chimeric reads is particularly important in studies that use low-coverage genomes, as the presence of few read hybrids can significantly impact the results (e.g. Snyder-Mackler et al. 2016, Wall et al. 2016 Mol Ecol).

Because museum specimens are extensively handled by humans, human contamination will likely be present, even if great care is taken during sample collection and laboratory work. When studying close human relatives, such as primates, even low-level human contamination can lead to erroneous results, particularly if it increases in proportion during capture. We captured Grauer's gorilla historical samples with western lowland gorilla bait, so that the genetic distance

between bait and target species was lower than the genetic distance between bait and contaminating human fragments. Previous studies reported successful capture of sequences that are divergent from the bait (Guschanski et al. 2013; Peñalba et al. 2014) and it is therefore important to evaluate if this “promiscuity” of the capture method can negatively affect the results in the presence of closely related contaminants. As expected, we detected human mitochondrial fragments in almost all museum samples despite employing a rigorous decontamination procedure (Methods). Although capture is considerably less efficient for human fragments, as demonstrated by lower fold-increase in human compared to gorilla reads when their initial concentrations are similar (Fig. S5c), the proportion of human mitochondrial reads increased following capture. This is because there are considerably fewer human reads present in the sample and the proportional increase after capture is greater for fragments with low starting abundance (Fig. S5b). The proportion of human reads in historical samples significantly decreased following the second capture round, possibly due to the increased competition with gorilla mitochondrial reads, but the overall slightly higher percentage of human reads post-capture shows that human contamination remains a challenge when working with low-quality samples.

Performance of different museum sample types

Exploration of natural history collections is gaining in popularity, as they represent an invaluable resource for genomic studies (Burrell et al. 2014). As the pressure on museum collections intensifies, the selection of sampling material becomes increasingly important. We show that sample types differ significantly in their endogenous DNA content, with soft tissue samples consistently displaying very high proportions of endogenous DNA (>80%, Table S1). In contrast

to previous studies (Damgaard et al. 2015), we do not observe significant differences in endogenous content among different tooth fractions (cementum and dentin). We also find differences in average fragment length in different sample types, with soft tissue samples showing considerably shorter fragments than hard tissues (data not shown). This difference is likely the effect of harsh chemical treatment of hides for preservation (tanning) and possible of older sample age (tooth samples ~ 55 years, soft tissues ~95 years). Importantly, after capture, the difference in the proportion of on-target reads among sample types disappears. Our study thus suggests that target capture can be performed from any sample. However, if shotgun sequencing from museum specimens is considered, soft tissues may be more useful and should be preferentially targeted.

Capture from fecal samples

Only few studies have attempted target capture from fecal samples (Perry et al. 2010; de Manuel et al. 2016; Snyder-Mackler et al. 2016) and none has evaluated capture performance for mitochondrial genomes. Fecal samples share many characteristics with historical and ancient samples and in addition, we encountered considerable influence of PCR inhibitors, which also affected library preparation. Here, we successfully overcome the problem of inhibition by using a modified 2CTAB/PCI extraction protocol (Vallet et al. 2008), which allowed us to make every studied fecal sample amenable for target capture.

Capture efficiency for fecal samples was comparable to museum specimens, showing that non-invasively collected samples with low endogenous content (Perry et al. 2010; Snyder-Mackler et al. 2016) can be efficiently used for population-scale studies in ecology and evolution. In contrast

to museum-preserved specimens, the proportion of on-target reads did not increase following a second capture round in fecal samples. Our data thus suggests that a single capture may be sufficient for fecal samples.

Because species identification based on fecal morphology can be difficult, we evaluated capture performance for two closely related species: Grauer's gorillas and eastern chimpanzee. Gorillas and chimpanzees occur sympatrically over large parts of their distribution ranges and their fecal samples can be confused even by experienced field workers. Similar situations are well known for carnivores and ungulates (Davison et al. 2002.; Yamashiro et al. 2010). For some research questions, it is desirable to be able to characterize a complex sympatric species community in a single experiment, without *a priori* knowledge of species identity. Our analyses showed that although chimpanzee samples could be captured with gorilla-derived baits, capture efficiency was significantly lower for these samples, resulting in less complete mitochondrial genomes and overall lower coverage compared to gorilla samples. This behavior may have been exacerbated in our experiment that combined chimpanzee and gorilla samples in the same capture pools and therefore "competition" during hybridization may have favored less divergent reads. More generally, we find that regions with high divergence between bait and sample show significantly lower coverage post-capture compared to less diverged regions, consistent with reduction of capture efficiency with sequence divergence.

Even though we employed strict measures against contamination during sample collection and laboratory work, we found low amounts of human contamination in almost all gorilla fecal samples. In chimpanzee samples, human contamination was much more prevalent, ranging from 4.5% to 8.1% post-capture. We argue that the high levels of human contamination in chimpanzee

samples are unlikely to be due to high pre-capture contamination, but are rather explained by equal capture efficiency for human and chimpanzee fragments using the western lowland gorilla baits. This observation strongly argues against applying capture approaches in situations, where the study species and the likely contaminating species are evolutionary equidistant from the bait.

Conclusions

The multiplexed capture and sequencing approach, as used in this study, allowed us to generate mitochondrial sequence data from all samples and reconstruct high quality complete mitochondrial genomes for over 80% of gorilla fecal and museum samples. The presented approach produces reliable genomic sequences that can be effectively used to study natural population. Although mitochondrial genomes provide a wealth of information about important evolutionary processes (Desalle et al. 2017), nuclear sequence data is needed to answer questions about demography, population history, levels of inbreeding, genetic diversity, relatedness, etc. Several studies have successfully used non-invasively collected fecal samples to targeted specific chromosomes (Perry et al. 2010; de Manuel et al. 2016), characterized thousands of SNPs from across the genome (Hernández et al., personal communication) or captured low coverage nuclear genomes (Snyder-Mackler et al. 2016). Some of these studies extended the capture method applied here to the entire genome (Carpenter et al. 2013; Snyder-Mackler et al. 2016). However, the strength of PCR-based capture approaches, such as the Maricic et al. (2010) method, lies mainly in the target capture of a modest number of loci across potentially large phylogenetic distances without the need of a reference genome (Jones and Good, 2015). We show here that this method can be used as an effective, easy-to-implement approach for population-scale studies

of cryptic, rare and elusive non-model species from degraded, contaminated, low-quality non-invasive samples and museum specimens.

Acknowledgments:

We would like to thank the Dian Fossey Gorilla Fund International, Institut Congolais pour la Conservation de la Nature and especially Radar Nishuli for permission and logistical support of research in the Democratic Republic of Congo; Damien Caillaud, Urbain Ngobobo, Escobar Binyinyi for providing Grauer's gorilla fecal samples; Gontran Sonet, Emmanuel Gilissen, Patrick Semal, and Zoltan T. Nagy for their support during the study of natural history collections; Nadin Rohland for help with laboratory protocols; Pauline Baas for providing genotyping data from fecal samples. The authors acknowledge support from Science for Life Laboratory, the Knut and Alice Wallenberg Foundation, the National Genomics Infrastructure funded by the Swedish Research Council, and Uppsala Multidisciplinary Center for Advanced Computational Science for assistance with massively parallel sequencing and access to the UPPMAX computational infrastructure. This research was supported by the following grants: Human Frontier Science Program Postdoctoral Fellowship to KG (LT000800/2011-L), the Jan Löfqvist and the Nilsson-Ehle Endowments of the Royal Physiographic Society of Lund to KG, and FORMAS (project 2015-676) to LD.

References

- Allentoft M, Morten E, Collins M et al. (2012). The Half-Life of DNA in Bone: Measuring Decay Kinetics in 158 Dated Fossils. *Proceedings of the Royal Society of London B: Biological Sciences* **279**, 1748.
- Bi K, Linderöth T, Vanderpool D et al. (2013). Unlocking the Vault: Next-Generation Museum Population Genomics. *Molecular Ecology*, **22**, 6018–6032.
- Brace S, Palkopoulou E, Dalén L et al. (2012). Serial Population Extinctions in a Small Mammal Indicate Late Pleistocene Ecosystem Instability. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 20532–20536.
- Bradley B, Brenda J, Chambers K and Vigilant L. (2001). Accurate DNA-Based Sex Identification of Apes Using Non-Invasive Samples. *Conservation Genetics*, **2**, 179–81.
- Briggs A, Good WJM, Green RE et al. (2009). Targeted Retrieval and Analysis of Five Neandertal mtDNA Genomes. *Science* **325**, 318–21.
- Briggs A, Stenzel U, Johnson PLF et al. (2007). Patterns of Damage in Genomic DNA Sequences from a Neandertal. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 14616–14621.
- Burbano HA, Hodges E, Green RE et al. (2010). Targeted Investigation of the Neandertal Genome by Array-Based Sequence Capture. *Science* **328**, 723–725.
- Burrell AS, Disotell TR and Christina M. Bergey. (2015). The Use of Museum Specimens with High-Throughput DNA Sequencers. *Journal of Human Evolution* **79**: 35–44.
- Carpenter ML, Buenrostro JD, Valdiosera C et al. (2013). Pulling out the 1%: Whole-Genome Capture for the Targeted Enrichment of Ancient DNA Sequencing Libraries. *The American Journal of Human Genetics* **93**, 852–64.

- 626 Cruz-Dávalos DI, Llamas B, Gaunitz C et al. (2016). Experimental Conditions Improving in-
627 Solution Target Enrichment for Ancient DNA. *Molecular Ecology Resources*
- 628 Cyrielle G, Peyretailade E, and Peyret P. (2016). Sequence Capture by Hybridization to Explore
629 Modern and Ancient Genomic Diversity in Model and Nonmodel Organisms. *Nucleic Acids*
630 *Research*, **44**, 4504–4518.
- 631 Dabney J, Knapp M, Glocke I et al. (2013). Complete Mitochondrial Genome Sequence of a
632 Middle Pleistocene Cave Bear Reconstructed from Ultrashort DNA Fragments. *Proceedings*
633 *of the National Academy of Sciences of the United States of America*, **110**, 15758–15763.
- 634 Dabney J, and Matthias Meyer. (2012). Length and GC-Biases during Sequencing Library
635 Amplification: A Comparison of Various Polymerase-Buffer Systems with Ancient and
636 Modern DNA Sequencing Libraries. *BioTechniques* **52**, 87–94.
- 637 Damgaard PB, Margaryan A, Schroeder H et al. (2015). Improving Access to Endogenous DNA
638 in Ancient Bones and Teeth. *Scientific Reports*, **5**, 11184
- 639 Davison A, Birks JDS, Brookes RC et al. (2002). On the Origin of Faeces: Morphological versus
640 Molecular Methods for Surveying Rare Carnivores from Their Scats. *Journal of Zoology*,
641 **257**, 141–143.
- 642 de Manuel M, Kuhlwilm M, Frandsen P et al. (2016). Chimpanzee Genomic Diversity Reveals
643 Ancient Admixture with Bonobos. *Science*, **354**, 6311.
- 644 Delfin F, Min-Shan Ko A, Mingkun L et al. (2014). Complete mtDNA Genomes of Filipino
645 Ethnolinguistic Groups: A Melting Pot of Recent and Ancient Lineages in the Asia-Pacific
646 Region. *European Journal of Human Genetics* **22**, 228–237.
- 647

- 648 Desalle R, Schierwater B, and Heike Hadrys. (2017). MtDNA: The Small Workhorse of
649 Evolutionary Studies. *Frontiers in Bioscience*, **22**, 873–87.
- 650 Enk J, Rouillard JM, and Poinar H. (2013). Quantitative PCR as a Predictor of Aligned Ancient
651 DNA Read Counts Following Targeted Enrichment. *BioTechniques*, **55**, 300–309.
- 652 Finstermeier K, Zinner D, Brameier M et al. (2013). A Mitogenomic Phylogeny of Living
653 Primates. *PLoS ONE*, **8**, e69504.
- 654 Gagneux P, Boesch C, and Woodruff DS. (1997). Microsatellite Scoring Errors Associated with
655 Noninvasive Genotyping Based on Nuclear DNA Amplified from Shed Hair. *Molecular*
656 *Ecology*, **6**, 861–68.
- 657 Gilbert TMP, Willerslev E, Hansen AJ et al. (2003). Distribution Patterns of Postmortem
658 Damage in Human Mitochondrial DNA. *Am. J. Hum. Genet*, **72**, 32–47.
- 659 Gnirke A, Melnikov A, Maguire J et al. (2009). Solution Hybrid Selection with Ultra-Long
660 Oligonucleotides for Massively Parallel Targeted Sequencing. *Nature Biotechnology*, **27**,
661 182–189.
- 662 Good JM, Vanderpool D, Keeble D et al. (2015). Negligible Nuclear Introgression despite
663 Complete Mitochondrial Capture between Two Species of Chipmunks. *Evolution*, **69**, 1961–
664 1972.
- 665 Guschanski K, Krause J, Sawyer S, et al. (2013). Next-Generation Museomics Disentangles One
666 of the Largest Primate Radiations. *Systematic Biology* **62**, 539–554.
- 667 Guschanski K, Vigilant L, McNeillage A et al. (2009). Counting Elusive Animals: Comparing
668 Field and Genetic Census of the Entire Mountain Gorilla Population of Bwindi Impenetrable
669 National Park, Uganda. *Biological Conservation*, **142**, 290–300.
- 670 Hawkins MTR, Leonard JA, Helgen KM et al. (2016). Evolutionary History of Endemic

- 671 Sulawesi Squirrels Constructed from UCEs and Mitogenomes Sequenced from Museum
672 Specimens. *BMC Evolutionary Biology*, **16**, 80.
- 673 Hofreiter M, Jaenicke V, Serre D et al. (2001). DNA Sequences from Multiple Amplifications
674 Reveal Artifacts Induced by Cytosine Deamination in Ancient DNA. *Nucleic Acids
675 Research*, **29**, 4793–99.
- 676 IUCN. (2016). *The IUCN Red List of Threatened Species. Version (2016)-3*.
677 <http://www.iucnredlist.org>.
- 678 Jónsson H, Ginolhac A, Schubert M et al. (2013). mapDamage2.0: Fast Approximate Bayesian
679 Estimates of Ancient DNA Damage Parameters. *Bioinformatics*, **29**, 1682–84.
- 680 Kim H, Erlich HA and Calloway CD. (2015). Analysis of Mixtures Using next Generation
681 Sequencing of Mitochondrial DNA Hypervariable Regions. *Croatian Medical Journal*, **56**,
682 208–217.
- 683 Kircher M, Sawyer S, and Matthias Meyer. (2012). Double Indexing Overcomes Inaccuracies in
684 Multiplex Sequencing on the Illumina Platform. *Nucleic Acids Research*, **40**, e3.
- 685 Langergraber KR, Prüfer K, Rowney C, Boesch C, Crockford C, Fawcett K, Inoue E et al.
686 (2012). Generation Times in Wild Chimpanzees and Gorillas Suggest Earlier Divergence
687 Times in Great Ape and Human Evolution. *Proceedings of the National Academy of
688 Sciences of the United States of America*, **109**, 15716–15721.
- 689 Li C, Corrigan S, Yang L et al. (2015). DNA Capture Reveals Transoceanic Gene Flow in
690 Endangered River Sharks. *Proceedings of the National Academy of Sciences of the United
691 States of America*, **112**, 13302–13307.
- 692 Li M, Schröder R, Ni S et al. (2015). Extensive Tissue-Related and Allele-Related mtDNA
693 Heteroplasmy Suggests Positive Selection for Somatic Mutations. *Proceedings of the*

- 694 *National Academy of Sciences of the United States of America* **112**, 2491–2496.
- 695 Li M, Schroeder R, Ko A et al. (2012). Fidelity of Capture-Enrichment for mtDNA Genome
696 Sequencing: Influence of NUMTs. *Nucleic Acids Research* **40**, e137.
- 697 Liedigk R, Kolleck J, Böker K et al. (2015). Mitogenomic Phylogeny of the Common Long-
698 Tailed Macaque (*Macaca Fascicularis Fascicularis*). *BMC Genomics*, **16**, 222.
- 699 Lippold S, Xu H, Ko A et al. (2014). Human Paternal and Maternal Demographic Histories:
700 Insights from High-Resolution Y Chromosome and mtDNA Sequences. *Investigative*
701 *Genetics*, **5**, 13.
- 702 Mariac C, Scarcelli N, Pouzadou J et al. (2014). Cost-Effective Enrichment Hybridization
703 Capture of Chloroplast Genomes at Deep Multiplexing Levels for Population Genetics and
704 Phylogeography Studies. *Molecular Ecology Resources*, **14**, 1103–1113.
- 705 Maricic T, Whitten M, and Pääbo S. (2010). Multiplexed DNA Sequence Capture of
706 Mitochondrial Genomes Using PCR Products. *PLoS ONE*, **5**, 11.
- 707 Mason VC, Li G, Helgen KM et al. (2011). Efficient Cross-Species Capture Hybridization and
708 next-Generation Sequencing of Mitochondrial Genomes from Noninvasively Sampled
709 Museum Specimens. *Genome Research*, **21**, 1695–1704.
- 710 McCormack JE, Tsai WLE, and Faircloth BC. (2016). Sequence Capture of Ultraconserved
711 Elements from Bird Museum Specimens. *Molecular Ecology Resources*, **16**, 189–1203.
- 712 McDevit DC, and Saunders GW. (2009). On the Utility of DNA Barcoding for Species
713 Differentiation among Brown Macroalgae (Phaeophyceae) Including a Novel Extraction
714 Protocol. *Phycological Research* **57**, 131–141.
- 715 Meyer M, Fu Q, Aximu-Petri A et al. (2013). A Mitochondrial Genome Sequence of a Hominin
716 from Sima de Los Huesos. *Nature*, **505**, 403–406.

- 717 Nsubuga AM, Robbins MM, Roeder AD et al. (2004). Factors Affecting the Amount of Genomic
718 DNA Extracted from Ape Faeces and the Identification of an Improved Sample Storage
719 Method. *Molecular Ecology*, **13**, 2089–2094.
- 720 Nyström V, Angerbjörn A and Dalén L. (2006). Genetic Consequences of a Demographic
721 Bottleneck in the Scandinavian Arctic Fox. *Oikos*, **114**, 84–94.
- 722 Peñalba J, Joshua V, Smith LL et al. (2014). Sequence Capture Using PCR-Generated Probes: A
723 Cost-Effective Method of Targeted High-Throughput Sequencing for Nonmodel Organisms.
724 *Molecular Ecology Resources*, **14**.
- 725 Perry GH, Marioni JC, Melsted P et al. (2010). Genomic-Scale Capture and Sequencing of
726 Endogenous DNA from Feces. *Molecular Ecology*, **19**, 5332–5344.
- 727 Prado-Martinez J, Sudmant PH, Kidd JM et al. (2013). Great Ape Genetic Diversity and
728 Population History. *Nature*, **499**, 471–475.
- 729 Rice WR. (1989). Analyzing Tables of Statistical Tests. *Evolution*, **43**, 223.
- 730 Rohland N, Harney E, Mallick S et al. (2015). Partial Uracil-DNA-Glycosylase Treatment for
731 Screening of Ancient DNA. *Philosophical Transactions of the Royal Society of London.*
732 *Series B, Biological Sciences*, **370**, 20130624.
- 733 Rohland N and Michael Hofreiter. (2007). Ancient DNA Extraction from Bones and Teeth.
734 *Nature Protocols*, **2**, 1756–1762.
- 735 Särkinen T, Staats M, Richardson JE et al. (2012). How to Open the Treasure Chest? Optimising
736 DNA Extraction from Herbarium Specimens. *PLoS ONE*, **7**, e43808.
- 737 Sawyer S, Krause J, Guschanski K et al. (2012). Temporal Patterns of Nucleotide
738 Misincorporations and DNA Fragmentation in Ancient DNA. *PLoS ONE*, **7**, e34131.
- 739 Schaller GB. (1963). The Mountain Gorilla: Ecology and Behavior. *American Anthropologist*,

- 740 **65**, 1231–1431.
- 741 Skoglund P, Northoff BH, Shunkov MV et al. (2014). Separating Endogenous Ancient DNA
742 from Modern Day Contamination in a Siberian Neandertal. *Proceedings of the National*
743 *Academy of Sciences of the United States of America*, **111**, 2229–2234.
- 744 Snyder-Mackler N, Majoros WH, Yuan ML et al. (2016). Efficient Genome-Wide Sequencing
745 and Low-Coverage Pedigree Analysis from Noninvasively Collected Samples. *Genetics*,
746 **203**, 699–714.
- 747 Suzuki S, Ono N, Furusawa C et al. (2007). Experimental Optimization of Probe Length to
748 Increase the Sequence Specificity of High-Density Oligonucleotide Microarrays. *BMC*
749 *Genomics*, **8**, 373.
- 750 Taberlet P, Waits LP and Luikart G. (1999). Noninvasive Genetic Sampling: Look before You
751 Leap. *Trends in Ecology & Evolution*, **14**, 323–327.
- 752 Thalmann O, Shapiro B, Cui P et al. (2013). Complete Mitochondrial Genomes of Ancient
753 Canids Suggest a European Origin of Domestic Dogs. *Science*, **342**, 6160.
- 754 Thalmann O, Hebler J, Poinar HN et al. (2004). Unreliable mtDNA Data due to Nuclear
755 Insertions: A Cautionary Tale from Analysis of Humans and Other Great Apes. *Molecular*
756 *Ecology*, **13**, 321–335.
- 757 Tomlinson J, Harrison C, Boonham N et al. (2014). Influence of the Length of Target DNA
758 Overhang Proximal to the Array Surface on Discrimination of Single-Base Mismatches on a
759 25-Mer Oligonucleotide Array. *BMC Research Notes*, **7**, 251.
- 760 Vallet D, Petit EJ, Gatti S et al. (2008). A New 2CTAB/PCI Method Improves DNA
761 Amplification Success from Faeces of Mediterranean (Barbary Macaques) and Tropical
762 (Lowland Gorillas) Primates. *Conservation Genetics*, **9**, 677–680.

- Wandeler P, Hoeck PE and Keller LF. (2007). Back to the Future: Museum Specimens in Population Genetics. *Trends in Ecology & Evolution*, **22**, 634–642.
- Weiß CL, Schuenemann VJ, Devos J et al. (2016). Temporal Patterns of Damage and Decay Kinetics of DNA Retrieved from Plant Herbarium Specimens. *Royal Society Open Science*, **3**, 160239.
- Wright ES, Vetsigian KH, Martiny JBH et al. (2016). Inhibitory Interactions Promote Frequent Bistability among Competing Bacteria. *Nature Communications*, **7**, 11274.
- Wright ES and Vetsigian KH. (2016). Quality Filtering of Illumina Index Reads Mitigates Sample Cross-Talk. *BMC Genomics*, **17**, 876.
- Xenikoudakis G, Ersmark E, Tison JL et al. (2015). Consequences of a Demographic Bottleneck on Genetic Structure and Variation in the Scandinavian Brown Bear. *Molecular Ecology*, **24**, 3441–3454.
- Yamashiro A, Yamashiro T, Baba M et al. (2010). Species Identification Based on the Faecal DNA Samples of the Japanese Serow (*Capricornis Crispus*). *Conservation Genetics Resources*, **2**, 409–414.
- Zhang Z, Kermekchiev MB and Barnes WM. (2010). Direct DNA Amplification from Crude Clinical Samples Using a PCR Enhancer Cocktail and Novel Mutants of Taq. *The Journal of Molecular Diagnostics* **12**, 152–161.

Data Accessibility

Read alignment BAM files are available for download at the European Nucleotide Archive (these will become available upon paper acceptance).

786 **Author contribution**

787 TvdV and KG designed the study. TvdV, FL and KG extracted the samples. FL and TvdV
788 performed laboratory experiments. TvdV wrote in-house python scripts. TvdV and
789 KG performed data analyses. LD provided essential technical support and critical feedback
790 throughout the study. TvdV and KG wrote the article, with input from all co-authors.

For Review Only