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METHODS AND RESOURCES

Historical genomic variation of Eurasian otters (*Lutra lutra*) in Britain, from hunting trophies

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Abstract
Genomic data from historical specimens can provide insights into populations and processes no longer evident from modern samples. Identifying viable sources of historical DNA is key to this. Here we aimed to explore the viability of trophy hunting paws as sources of historical DNA, and to use this data for preliminary analyses to evaluate changes in population structuring over time. We aimed to search for the presence of genomic signals of the putative recent introduction of Eurasian otters (*Lutra lutra*) from Asia to the UK previously identified using modern data, by analysis of two trophy paws from 1929 to 1952. Substantial proportions of endogenous DNA (77–85%) were identified in both samples for which genomic DNA extraction and sequencing was attempted, illustrating the potential of such specimens for historical DNA research. Autosomal variation suggests that the historical specimens cluster closest to modern specimens from Wales, and not with the variation identified in the East of England, putatively associated with the recent introduction of Eurasian otters from Asia. Similarly, the divergent mitochondrial lineage found in modern otters from the East of England and Asia, was not found in the historical British specimens. Although limited inferences can be made from a sample size of two, this preliminary dataset indicates the huge potential of historical trophy paw samples to assess past population genetic dynamics, calling for further research in this area.

Keywords Historical DNA · *Lutra* · Population genomics · Trophy hunting

Introduction

Following their colonisation of the British Isles, Eurasian otters (*Lutra lutra*) became isolated from their mainland counterparts around 6–12 thousand years ago, as ice sheets retreated and sea levels rose, flooding the land bridge previously connecting modern day southeast England to the Netherlands, Germany and Denmark (Ward et al. 2006). From medieval times through to the mid-1900s, otters were hunted in Britain for fur, food, sport, and pest control (Allen 2010; Raye 2023). The anthropogenic pressures on the species culminated in the 1950–70s when the bioaccumulation of chemical pollution led to a severe and rapid population crash (Chanin and Jefferies 1978), followed by a remarkable population expansion in Britain, after the banning of many chemical pollutants (Crawford and Scholey 2010; Kean and Chadwick 2021). Specifically, Eurasian otters in Britain show population structuring which reflects the stronghold regions that survived the bottleneck, as identified using

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microsatellites (Hobbs et al. 2011; Stanton et al. 2014; Thomas et al. 2022, 2025).

Recent genomic analyses highlighted the severity of the bottleneck in some stronghold populations (in the east and southwest of England), in contrast to a less extreme bottleneck in Wales, and a gradual, but long term, decline in the north of England and Scotland (du Plessis et al. 2023a). It is likely that this resulted in the genetic population structuring and diversity observed in modern populations, for example through isolation and drift. This process of isolation and drift may have overwritten the genetic structuring present from before these anthropogenic pressures, as in brown bears, *Ursus arctos* (Xenikoudakis et al. 2015). However, if this is not the case, and modern populations show historic, adaptive population structuring, it is important to conserve the results of these evolutionary processes (Leonard 2008).

Alongside characterising the population bottlenecks across Britain, recent genomic analyses have also suggested the likely introduction of Eurasian otters from Asia to the east of England during the population bottleneck (du Plessis et al. 2023a). For example, two mitochondrial lineages were identified in Britain, which diverged at least 80 thousand years ago, including a lineage that has otherwise only been found in Asia (du Plessis et al. 2023b), henceforth referred to as Lineage 1. If Lineage 1 was to be identified in Britain before the population bottleneck and hypothesised Asian introductions, this would necessitate other explanations for the presence of this mitochondrial lineage in Britain.

One potential source of historical DNA from British otters are hunting trophies. As otter hunting shifted from pest control to sport in the 1900s, the collection of trophies became increasingly common. Otter hunting in recent times has been reasonably well documented (Allen 2010; Williams 2010), and therefore hunt success was one of the first measures used to identify the population declines in the 1950s (Chanin and Jefferies 1978). There is little literature on the collection of trophies from these hunts, however taxidermy paws, tails, heads and whole skins can be found online and in museum collections, which date from the early 1900s. Interestingly, these specimens are also often inscribed with both location and date of the hunt, providing an opportunity to assess genetic variation across time and space on a fine scale. Little is known about the treatment of these specimens during the taxidermy process, and therefore whether DNA is likely to have been damaged. Although dried otter muscle and skin from museum specimens have been used to successfully genotype mitochondrial variation in Eurasian otters (Margaryan et al. 2021; Mohd Salleh et al. 2017; Waku et al. 2016), autosomal variation has not yet been assessed using historical specimens for the species.

Since both the prevalence of sport hunting in recent history and the preservation of trophy paws is widespread across Britain, it is worth investigating the viability of these specimens for historical DNA analyses (Atkulwar et al. 2020; Gubili et al. 2015; Polanc et al. 2012). Here, we assessed the use of historical trophy paws for genomic analyses of Eurasian otters based on two specimens, one from 1929 and the other from 1952. Specifically, we aimed to:

1. Explore the viability of trophy hunting paws as sources of endogenous DNA.
2. Investigate if the historical trophy paws align or differ with modern day population structuring based on their geographic origin.
3. Search for signals associated with a potential introduction of Asian otters observed in modern populations, such as the divergent mitochondrial Lineage 1.

Methods

Sample details

Two historical trophy paws in total were sampled for this study, solely based on their availability for destructive sampling: LUT01 and LUT02 (Fig. 1). Endogenous DNA content prior to extraction was unknown for both samples. Sample LUT01 was a taxidermy right otter paw, mounted on a wooden plaque. This sample was donated to the Essex Wildlife Trust from a member of the public who was given the paw as a trophy in 1952 following an otter hunt at the Great Holland Pits, Essex, UK (Fig. 1). Sample LUT02 was also a taxidermy right otter paw, mounted as a brooch, donated to the Cardiff University Otter Project. Engraved on the metal casing, is 'B.O.H.' for Bucks Otter Hunt (the name of the hunting organisation), the date 'MAY 20TH 1929' and 'HALFORD', as the location (a village in Warwickshire, Central England, Fig. 1). Typical of most available trophy paws, there is no information of how either specimen was prepared for taxidermy.

Data generation

Both samples were processed under strict clean conditions in a dedicated ancient DNA laboratory at the Globe Institute, University of Copenhagen in Denmark. Specifically, DNA extraction and library preparation were conducted in clean rooms to minimise contamination of modern DNA, following the best practice guidelines, which included the use of full body suits, positive pressure ventilated rooms and positive and negative controls throughout. Destructive sampling was used for both specimens. Specifically, for LUT01, two sub-samples of skin from the digit pad of the 4th toe were taken using a sterile drill. For LUT02, one sub-sample was taken of the nail on the 5th toe, and another sub-sample was

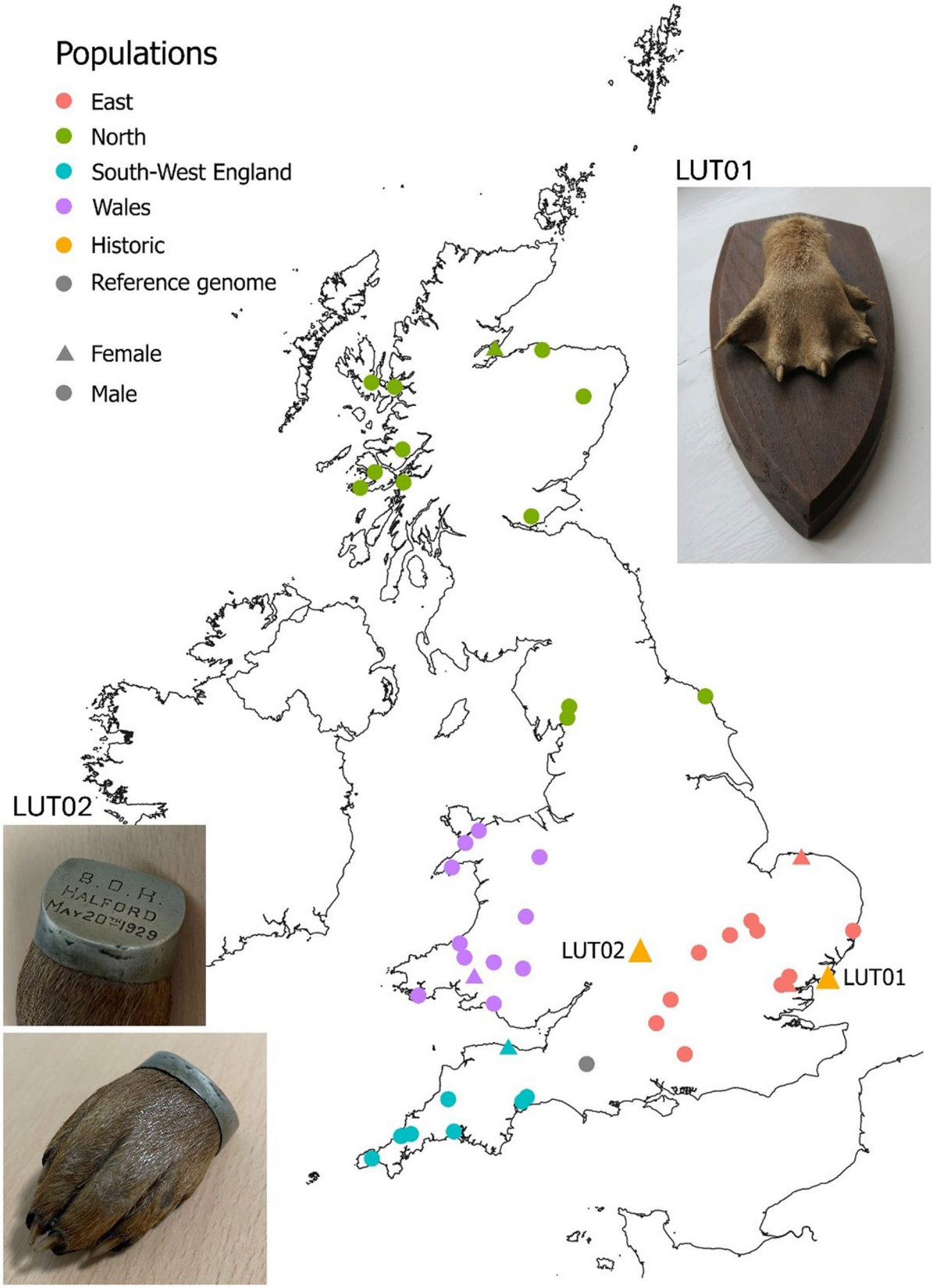


Fig. 1 Map of modern (du Plessis et al. 2023a) and historical sample locations, including approximate locations for historical samples LUT01 (Great Holland Pits, Essex, from 1952) in the east, and LUT02 (Halford, Warwickshire, from 1929) in central England

taken of the end of the toe and base of the nail of the 5th toe. Genomic DNA extraction was conducted for all samples using a phenol-chloroform protocol described in Campos and Gilbert (2012). DNA concentrations were checked on a Qubit Fluorometer in ng/µl, ranging from 1.82 to 70 ng/µl. Illumina libraries were built using the Santa Cruz Reaction (SCR) Protocol (Kapp et al. 2021). Libraries were amplified using Q5U Hot Start High-Fidelity DNA Polymerase (New England Biolabs Inc) with the following PCR cycle: 98 °C for 30 s, followed by 10 cycles of 98 °C for 10 s, 65 °C for 30 s, 72 °C for 60 s, and a final extension of 72 °C for 5 min, before being held at 4 °C. Amplified libraries were then purified using HighPrep PCR beads (MagBio Genomics) to remove adapters. An Agilent 2100 Bioanalyzer was used to assess DNA concentrations and the fragment size of the libraries, with concentrations ranging from 0.72 to 1.91 ng/µl. Initially, low coverage screening was conducted, identifying endogenous (target species) DNA, and followed by further sequencing to generate 120 million read pairs per specimen. All samples were sequenced as 150 bp paired-end reads on an Illumina NovaSeq 6000 instrument (Novogene, UK).

Data processing and bioinformatic analyses

Short reads were filtered, trimmed, and mapped to the Eurasian otter reference genome (mLutLut1.2, (Mead et al. 2020)) using PALEOMIX v1.3.7 (Schubert et al. 2014), AdapterRemoval (Lindgreen 2012), bwa v0.7.17 (Li 2013; Li and Durbin 2009) and Picard v2.22.2 MarkDuplicates (see SM for details). Postmortem DNA damage was assessed using mapDamage v2.0 (Jónsson et al. 2013; see Figures SM1-4 and Table SM1). Summary outputs include average read length, depth, coverage, number of endogenous reads and number of PCR duplicates (Table SM1). Samtools v1.17 (Danecek et al. 2021) was used to estimate breadth of coverage.

Due to the sequencing depth of 1.1-2.1x and breadth of 62–84% for the historical samples, no new variation was genotyped based on these reads, and instead, locations of variants in modern data were interrogated in the historical data. Specifically, the previously generated high coverage dataset of 45 modern samples (Fig. 1) (du Plessis et al. 2023a) was used to contextualise the nuclear genomes of the low coverage trophy paws. Briefly, these were Eurasian otters from across Britain from 2016 to 2020, which were sequenced at 16-40x coverage. Random reads were sampled from the historical data to determine the allele at each site genotyped in the modern, high coverage data, using BCFtools v1.14 (Danecek et al. 2021) and ANGSD v0.933 (Korneliussen et al. 2014; see SM for details).

Where sites consisted of only two possible alleles across all samples included, they were considered biallelic. From these biallelic sites, the number of transitions and transversions were calculated, and for subsets of only historical, and only modern samples, using VCFtools v0.1.16 (Danecek et al. 2011).

The number of private SNPs (i.e. variants found only in one population), was calculated as a measure of unique genomic variation among populations. Specifically, private SNPs from each stronghold population (East, North, Southwest England and Wales) were identified using only the modern data (du Plessis et al. 2023a). If SNPs that are private to the East in modern populations are also private to the East in historical populations, this suggests long-term population structuring maintained through the bottleneck. If not found, this suggests changes to population structuring over time and potentially the introduction of novel variation. To assess this, from the previously identified modern, private SNPs, the number identified in either or both historical samples was recorded (see SM for details).

To assess the genetic ancestry of the trophy paw samples, and to place them within the context of modern population structure of Eurasian otters in Britain, ADMIXTURE v1.3.0 (Alexander and Lange 2011) was run from K = 1 to 8. To minimise the potential biases introduced by using small sample sizes of low coverage data (particularly in allele frequency estimation), historical samples were projected against high coverage, large sample size modern data. Specifically, ADMIXTURE was run on the modern data only, and these allele frequencies were used to project the lower coverage trophy paws using the flag '-P', with both modern data and projected trophy paws combined for plotting. Within EIGENSOFT v7.2.1 (Price et al. 2006) the script 'convertf' was used to convert the data files from PLINK to EIGENSTRAT format. Then SMARTPCA (Patterson et al. 2006) was used to run a Principal Component Analysis (PCA) on the modern data only, and project the lower coverage, historical samples onto the modern principal components. Principal components 1 and 2 primarily explain variation of samples within the East, and therefore the resolution of variation within the remaining populations and samples is unclear. Therefore, to investigate the fine-scale positions of the historical samples, a subsequent PCA was conducted on all modern samples excluding those from the East.

To contextualise the historical samples against divergent mitochondrial variation identified in modern British sequences, whole mitochondrial genomes were assembled for both historical samples. Only reads mapping to the reference Eurasian otter mitochondrial scaffold (LR822067.1) in the PALEOMIX output were selected and converted to fastq format using Samtools. MITObim v1.9.1 (Hahn et

al. 2013) was used to assemble both whole mitochondrial genomes using the reference mitochondrial sequence as the seed, with both samples converging to a circular sequence after two iterations. These sequences were aligned with 44 modern, British, whole mitochondrial genome sequences (generated in (du Plessis et al. 2023a)) using MAFFT v7.481 (Katoh et al. 2002). Fasta sequences were aligned using the '—auto' setting, and the FFT-NS-2 (fast, progressive method) alignment strategy. Geneious Prime v2023.1.2 (https://www.geneious.com) was used to remove the low -quality region surrounding the tandem repeat within the control region (positions 16,050−16,202 on the reference scaffold) and overhanging sequences, leaving a total alignment length of 16,408. PopArt (Clements et al. 2019) was used to produce a TCS statistical parsimony network and identify the number of mutation steps between haplotypes. Summary statistics, including haplotype richness, haplotype diversity and nucleotide diversity (π), were calculated using the R packages pegas (Paradis 2010) and ape (Paradis and Schliep 2019).

Data handling and visualisation was conducted in R version 4.3.0 (R Core Team 2023) using RStudio version 2023.06.0 (RStudio Team 2023) and the tidyverse packages (Wickham et al. 2019), unless stated otherwise.

Results

It was possible to extract and sequence DNA from both specimens used in this study. Endogenous DNA made up 77% of reads for LUT01 and 85% for LUT02, and depth of coverage was 1.09x for LUT01 and 2.11x for LUT02, and breadth of coverage was 62.4% for LUT01 and 84.5% for LUT02 (Table SM1). Both samples were inferred to be female based on differences in X, Y and autosomal chromosome sequencing depth (Table SM3). Of the 8,931,760 sites interrogated, in the historical samples, reads mapped to 7,086,562 sites. Of these, 3,453,148 sites were biallelic across the pool of both historical and modern datasets. The Ts/Tv ratio was higher in historical samples (2.26) than modern samples (1.96), aligning with the evidence of DNA damaged observed using mapDamage (Figures SM1-4), and supporting the use of only transversions (n=1,207,020) in all analyses.

Table 1 Geographic matching of alleles in historical samples to private variation within modern British otter populations. Counts of transversion SNPs private to the four modern British populations, and their identification (count and %) in either one or both historical samples.

Population	Modern, private SNPs	LUT01 (%)	LUT02 (%)
East	269,359	2,374 (0.88)	1,906 (0.71)
North	86,627	2,255 (2.60)	2,013 (2.32)
SWEng	62,734	1,308 (2.08)	1,091 (1.74)
Wales	69,826	1,779 (2.55)	2,439 (3.49)

Autosomal variation

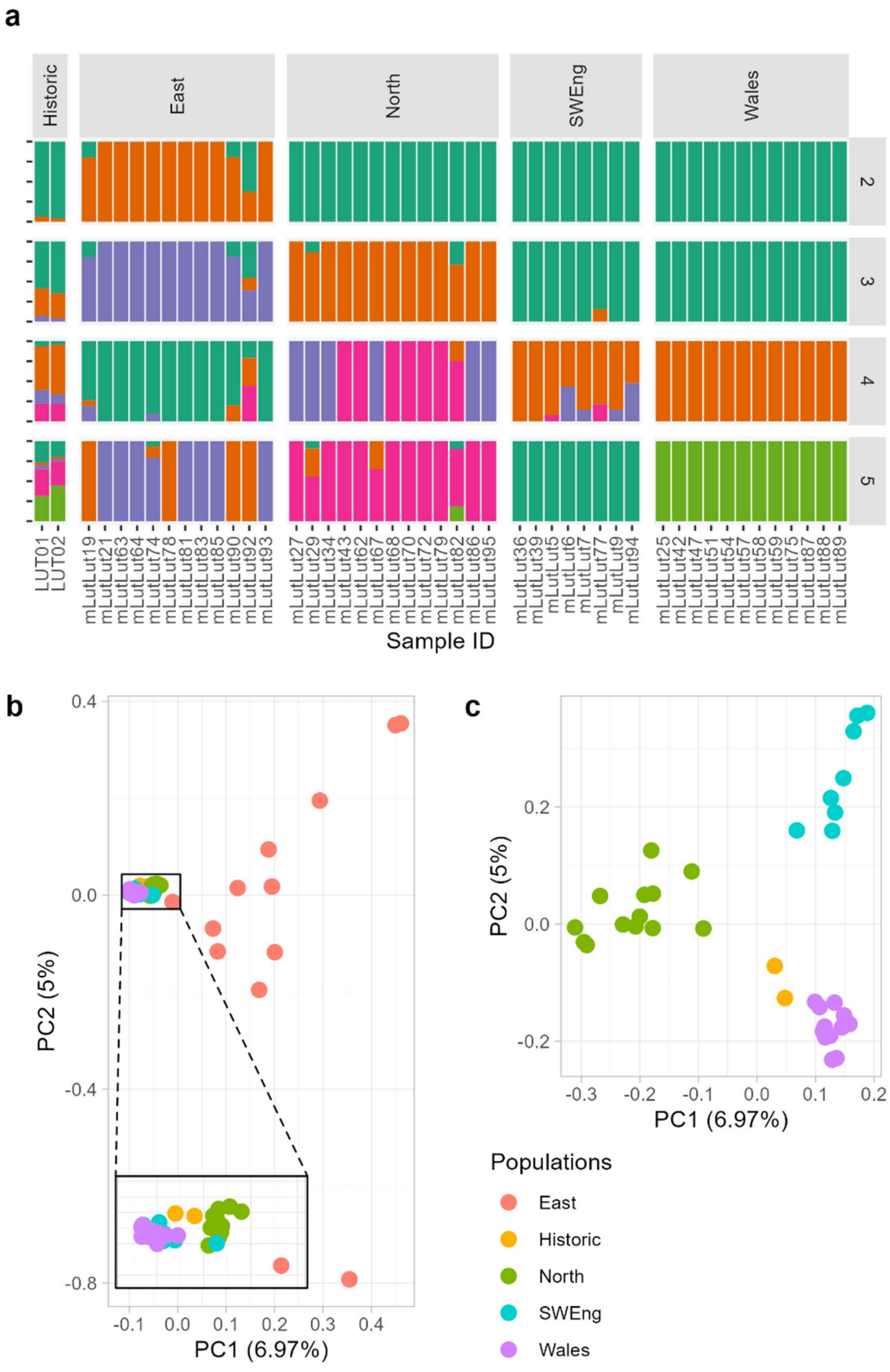
Of the 1,207,020 transversion SNPs included in these analyses, a proportion were private alleles in one of the four modern populations, and of these private SNPs, a small proportion (0.71–3.49%) were identified in the historical samples (Table 1).

Cross-validation (CV) errors of ADMIXTURE runs of the modern samples only for K=1 to 8 showed an increase up to K=7, with the lowest CV-error for K=1, indicating the best-fitting number of populations to be 1 (Figure SM5). Given the established stronghold population structuring, and the interest in fine-scale structuring within Britain, K=2 to 5 are included in the results. ADMIXTURE was used to project the historical samples onto the population structure observed in the modern data, using the allele frequencies learned from the modern data, from K=2 to 5 (Fig. 2a). Across all values of K, both historical samples are dominated by clusters found in North, Southwest England and Wales, and with minimal evidence of the cluster (or clusters) primarily identified in the East. This indicates that they likely share ancestry with the modern samples from North, Southwest England and Wales, rather than the East of England.

When PCA was conducted on all samples, both historical samples clustered within variation observed in the North, Southwest England and Wales, with no overlap with samples from the East (Fig. 2b). A PCA was also conducted on all modern samples excluding those from the East (Fig. 2c) to clarify the population structure among the remaining three populations. As expected, the modern populations show some structure, with the two historical samples projected closest to modern samples from Wales and furthest from modern samples from Southwest England; however, there is considerable variation among individuals from all populations.

Whole mitochondrial genomes

Whole mitochondrial genomes were successfully assembled from both historical samples, with a depth of coverage of 305x for LUT01 and 155x for LUT02. The TCS network of historical and modern sequences indicated that both historical sequences were one mutational step from modern haplotypes from the East and Wales, respectively (Fig. 3). Both historical samples were placed within lineage 3, which is primarily found in Britain, and not within lineage 1, primarily found in Asia and in modern samples from the east of England. Two different haplotypes were identified from


a
Historic
East
North
SWEng
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2
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LUT01
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Sample ID
b
0.4
0.0
-0.4
-0.8
PC2 (5%)
-0.1
0.0
0.1
0.2
0.3
0.4
PC1 (6.97%)
c
0.2
0.0
-0.2
PC2 (5%)
-0.3
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PC1 (6.97%)
Populations
East
Historic
North
SWEng
Wales

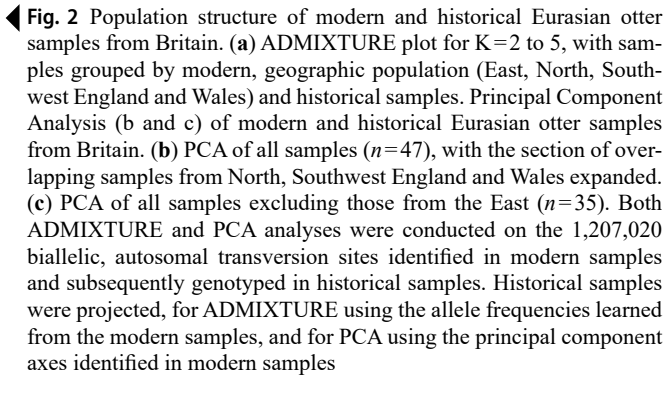

Fig. 2 Population structure of modern and historical Eurasian otter samples from Britain. (**a**) ADMIXTURE plot for K = 2 to 5, with samples grouped by modern, geographic population (East, North, Southwest England and Wales) and historical samples. Principal Component Analysis (b and c) of modern and historical Eurasian otter samples from Britain. (**b**) PCA of all samples ($n = 47$), with the section of overlapping samples from North, Southwest England and Wales expanded. (**c**) PCA of all samples excluding those from the East ($n = 35$). Both ADMIXTURE and PCA analyses were conducted on the 1,207,020 biallelic, autosomal transversion sites identified in modern samples and subsequently genotyped in historical samples. Historical samples were projected, for ADMIXTURE using the allele frequencies learned from the modern samples, and for PCA using the principal component axes identified in modern samples

the two historical samples, and the number of segregating sites and nucleotide diversity were lower in the historical samples than the modern samples (with the exception of southwest England, Table 2).

Discussion

DNA preservation in museum specimens is difficult to predict and depends on a range of factors, including age, and preservation method (Bi et al. 2013; Cassidy et al. 2017; Martínková and Searle 2006; McDonough et al. 2018; Wandeler et al. 2007). Nonetheless, despite the unknown handling and treatment of the analysed trophy paws during taxidermy and up to 91 years of storage, we were able to characterise both autosomal and mitochondrial variation in both specimens included in this study. Our results provide the first insights into genomic variation of Eurasian otters in Britain from before the anthropogenic population bottleneck in the mid-1900s. Although low-coverage sequencing was conducted in this study, the high endogenous DNA content indicates that trophy paws can be excellent sources of DNA for high coverage sequencing, enabling genotype likelihoods (not employed in this study) to be generated and novel variation characterised. Given the extent of otter hunting, there are likely to be significant numbers of trophy paw samples in private and museum collections, although a full assessment of the availability of such samples should be conducted. Sequencing of such specimens, in addition to osteocrusts and skins obtained from traditional museum specimens (McDonough et al. 2018), would allow a thorough assessment of population variation and genetic load over time, most notably from before and after the population bottleneck, while also providing insights into the genetic consequences of hunting itself (Allendorf and Hard 2009; Chiyo et al. 2015; Coltman et al. 2003; Knell and Martínez-Ruiz 2017).

Given the small sample size included in this study, conclusions should be considered preliminary, however the two historical samples genotyped in this study suggest that historical structuring is not consistent with modern structuring of British Eurasian otters. Instead, the results suggest that modern population structuring may have arisen through the isolation and drift of stronghold populations during and after the population bottleneck. Given the geographic proximity of the analysed historical samples to modern samples from the east of England, if we assume historical structuring was maintained through the bottleneck, we would mainly expect SNPs private to the modern East stronghold to be found in the historical samples. In contrast, SNPs private to all modern populations were identified in the two historical samples, with the smallest proportion of modern, private SNPs from the East of England. This aligns with the admixture proportions we inferred, which suggested the trophy paws cluster with samples from Wales, Southwest England and the North, and minimal admixture proportions shared with samples from the East. Similarly, in the PCA, the two trophy paw samples fall centrally between Wales, Southwest England and the North. Cumulatively, these results indicate that the trophy paws sampled in this study, despite their geographic origin in the east of England, look genetically more closely related to modern otters from Wales, Southwest England and the North. A significant limitation to this conclusion is the sample size and restricted geographical distribution of the analysed historical samples. Sample number and range would need to be substantially expanded before changes in population structure can be fully elucidated.

Interestingly, the ADMIXTURE results for the modern samples differ slightly to the ADMIXTURE analyses previously conducted for the same data (du Plessis et al. 2023a). Specifically, at K = 4, there is sub structuring in the North in this study, and sub-structuring in the East in prior analyses. The notable difference in methods is that for this study, only 1.2 million haploid, transversion SNPs were assessed, rather than previous analyses of 8.9 million diploid SNPs (both transitions and transversions). Broad patterns in clustering remain across analyses, indicating that this may be an effect of random sub-sampling of variants, however since transitions occur naturally at higher rates than transversions (Ts/Tv ratio of 2), and the removal of both DNA damage and true transitions in this study is associated with loss of patterns of sub-structuring in the East of England, it is possible that this signal is from a recent process, and characterised by differences in transitions, rather than an older signal of sub-structuring in the North, characterised by differences in transversions (see supplementary material 1.6). Although, this is a minor methodological difference, it could be an important consideration when interpreting studies of historical specimens, for which thousands rather than millions of SNPs could be characterised.

Modern Eurasian otter genomes from Britain were shown to contain a divergent genetic lineage in the east of England

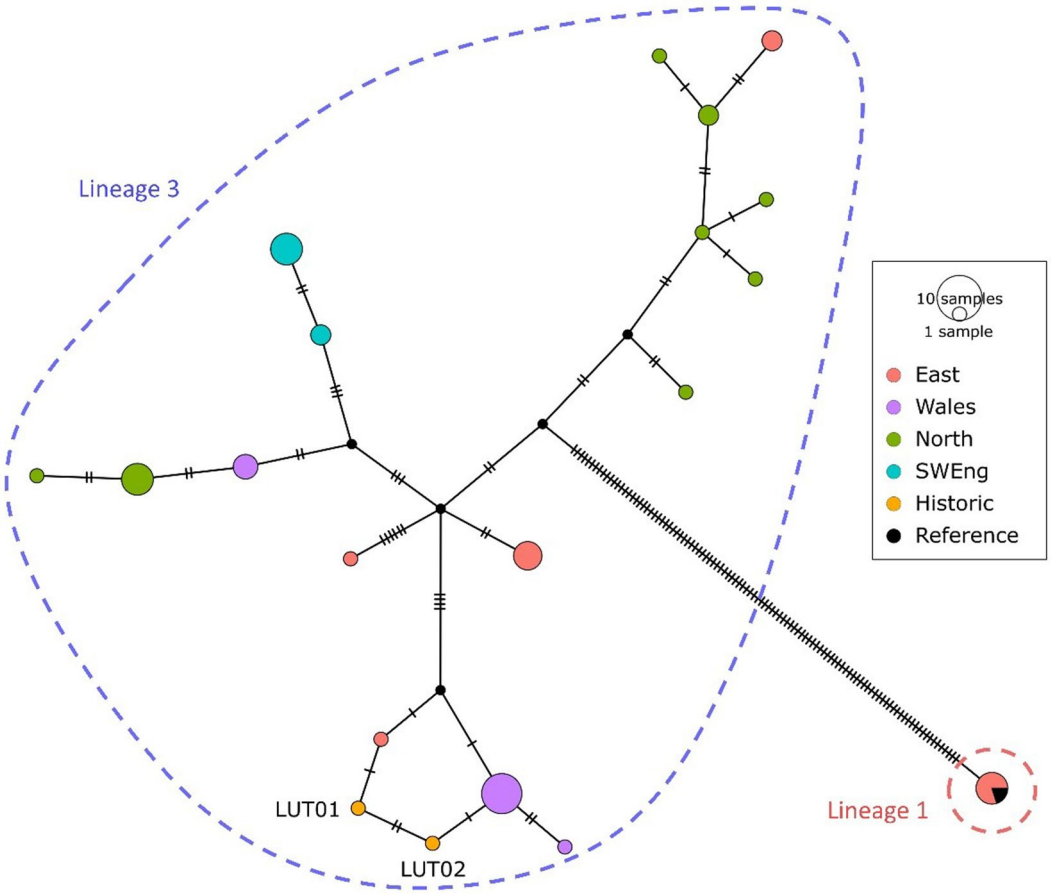

Fig. 3 TCS network of whole mitochondrial genomes (16,408 bp) of historical and modern samples of Eurasian otters from across Britain. Modern samples are colour coded based on the geographic population; East, Wales, North, Southwest England, the reference genome sequence is in black (Lineage 1), and the two historical samples are in orange, and labelled with the sample name. Sequences from Lineages 1 and 3 (as identified (du Plessis et al. 2023a) are circled

Table 2 Summary statistics of whole mitochondrial genome diversity of modern and historical Eurasian otters

	N	nh	s	h	π
Samples grouped by geographic population:					
East	12	5	132	0.80	0.0035
North	13	8	24	0.86	0.0005
Southwest England	7	2	2	0.48	0.0000
Wales	12	3	12	0.53	0.0003
Historical	2	2	2	1.00	0.0001
All British samples:	46	20	157	0.94	0.0016
Samples grouped by mitochondrial lineage:					
Lineage 1	4	1	-	-	-
Lineage 3	42	19	51	0.93	0.0006
All samples:	46	20	157	0.94	0.0016

Samples are grouped by modern, geographic population or as historical samples, and separately grouped by mitochondrial lineage. Sample size (N), number of haplotypes (nh), segregating sites (s), haplotype diversity (h) and nucleotide diversity (π)

(du Plessis et al. 2023a; Plessis et al. 2023b). We hypothesised that this divergent variation was derived from Eurasian otters from Asia, introduced to the east of England around the population bottleneck (1950–70s), and intensified as this genetic variation increased in frequency as the population expanded. The specimens sampled in this study, from before the proposed introduction, support this hypothesis in two ways. First, the divergent mitochondrial lineage (Lineage 1) was not identified in either of the two historical samples, but instead they clustered within Lineage 3, the more common lineage found in Britain and across most of the northern Eurasian part of the range (du Plessis et al. 2023b). Second, there is little evidence of autosomal genomic ancestry from the current East population in the two historical samples, as assessed through private variation, ADMIXTURE and PCA analyses. Hence, although limited by sample size, this work finds that the two analysed trophy paws show no evidence of the divergent, putative Asian lineage prior to the start of the population bottleneck and therefore support the hypothesis of introduced Eurasian otters from Asia.

To conclude, we have demonstrated the viability of extracting, sequencing and genotyping both autosomal and mitochondrial variation from trophy hunting paws and used this data to evaluate changes in population structuring of Eurasian otters in Britain. Although limited inferences can be made based on this dataset, the viability of these methods for historical specimens of this species highlight that increased sampling and sequencing depth can be used to comprehensively assess the historical population structuring.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12686-025-01383-9.

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Author contributions SJdP, FH and MTPG designed the research, SJdP and EAC sourced the samples, MTPG, NFM, SSTM and SJdP planned the laboratory work, and NFM conducted the laboratory work with SJdP, SJdP conducted bioinformatic analyses following guidance from XS and DWGS, SJdP wrote the paper with guidance from FH, K-PK, EAC. All co-authors provided feedback on the paper.

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Data availability Newly generated raw reads were deposited on SRA (BioProject PRJNA1081818, BioSamples SAMN40188547-8, SRA SRR28150401-2), and mitochondrial haplotypes on GenBank (PP409971-2). Previously published (du Plessis et al. 2023a) raw data were available through the DToL Data Portal (https://portal.darwintreeoflife.org/data/root/details/Lutra%20lutra) and haplotype data from NCBI Nucleotide Database (OR633269-86 and BK064833-5). Bioinformatic code used to conduct all analyses available on GitHub (https://github.com/sduplessis1/EurasianOtter_TrophyPaws) and in electronic supplementary material.

Declarations

Competing interests The authors declare no competing interests.

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