Journal of Archaeological Science: Reports 41 (2022) 103262

Contents lists available at ScienceDirect

Journal of Archaeological Science: Reports

journal homepage: www.elsevier.com/locate/jasrep

Determination of different predictors affecting DNA concentration isolated from historical hairs of the Finnhorse

Tuija Kirkinen [a,*], Johanna Honka [b], Daniela Salazar [b], Laura Kvist [b], Markku Saastamoinen [c], Karin Hemmann [a]

[a] Dept. of Cultures, Archaeology, P.O. Box 59, 00014 University of Helsinki, Finland
[b] Dept. of Ecology and Genetics, P.O. Box 3000, Linnamaa, 90014 University of Oulu, Finland
[c] Natural Resources Institute Finland Luke, Production Systems, Tietotie 2, 31600 Jokioinen, Finland

ARTICLE INFO

Keywords:
Mammalian hair
Taphonomy
Microscopy
Historical DNA
Horses
Equus caballus

ABSTRACT

Everyday objects manufactured from raw materials of animal origin, such as skin, hair and bone, are innumerable in cultural historical museums and private collections. Besides their value as memoirs of past techniques, livelihoods and communities, they are a unique source for studying past animal populations by means of molecular analysis.

Here, we deal with horse mane and tail hair, a type of predecessor of modern synthetic material utilized, for example, for brushes, strings, tennis rackets, ropes, textiles, dolls' hair, rocking horses, and filling. By investigating the presence and quality of DNA in horsehair, we have studied the origins of the Finnhorse, the only native horse breed in Finland. Degradation of DNA in old samples is an issue that needs to be considered when selecting material for DNA analysis. For assessing the usability of historical artefacts for DNA-based studies, we study how DNA is preserved in horsehair and how well DNA can be isolated from 50 to 150-year-old artefacts, raw material bundles and archaeological finds. We investigate how the properties of hair and sample storage conditions affect the concentration of DNA extracts and success in Polymerase Chain Reaction (PCR).

Our analysis showed that historical hair shafts, stored in various environments and used for multiple purposes, are of sufficient quantity and quality for amplification by PCR. Therefore, their value for the research of past animal populations should be noticed when curating cultural historical collections. We also provide advice for the storage conditions for hair samples.

1. Introduction

Within cultural heritage research, artefacts manufactured from materials originating from plants and animals have allowed new insights about the past by using advanced scientific analyses, such as lipid analysis, Fourier-transform infrared spectroscopy (FTIR), and Zoo mass spectrometry (ZooMS). As a result, new information about the diets, health, environments and livelihood of ancient people has been gained (e.g. Buckley, 2017; Copley et al., 2005; Monnier et al., 2017). Similarly, past animal populations have been studied by utilizing natural historical collections (e.g. Martínková and Searle, 2006; Besnard et al., 2016; Castañeda-Rico et al. 2020).

During the last decades, DNA has become a significant source of increased knowledge of past events, extending from DNA analyses of recent specimens (a few hundred years of age, historical DNA, hDNA) to archaeological specimens and fossils which are thousands of years old (ancient DNA, aDNA; Billerman and Walsh, 2019). DNA can be isolated from most naturally occurring biological tissues, but, its quality and quantity vary substantially due to several factors relating to biological role and biogenesis of the tissues (Bengtsson et al., 2012). For example, a tissue's cell density may have an effect on the total amount of extractable DNA per unit mass sampled. It follows that bone, hair and nails contain less cells than blood, for example. Cell densities also vary between individuals within a species, and even within single individuals (Bengtsson et al., 2012).

Mammalian hairs have been a source of ancient DNA for the study of past animal populations (e.g. in analysing processes of domestication and extinctions of breeds and species) in only a limited number of

* Corresponding author.
E-mail addresses: tuija.kirkinen@helsinki.fi (T. Kirkinen), johanna.honka@oulu.fi (J. Honka), danielalisasalazar@gmail.com (D. Salazar), laura.kvist@oulu.fi (L. Kvist), markku.saastamoinen@luke.fi (M. Saastamoinen), karin.hemmann@helsinki.f (K. Hemmann).

https://doi.org/10.1016/j.jasrep.2021.103262
Received 12 January 2021; Received in revised form 23 August 2021; Accepted 19 November 2021
Available online 4 December 2021

studies (Bonnichsen et al., 2001; Amory et al., 2007; Gilbert et al., 2004; Gilbert et al. 2007; Brandt et al., 2011; Rasmussen et al., 2010Clack et al., 2012). This is because keratinous tissue is generally considered an inferior source of ancient and historical DNA, compared to other tissue types, such as bone or teeth, due to scarcity of hair in archaeological and historical material and also due to fragmented DNA in hair shafts (Campos and Gilbert, 2019). However, keratinous tissue offers some advantages over other tissue types, as it is easy to decontaminate. For example, hair samples have shown very little contamination, hypothetically because exogenous DNA contaminations are easy to remove, the hairs are less permeable to contaminant DNA, or both (Gilbert et al., 2004; Gilbert et al., 2006). Gilbert et al. (2006) have suggested that the hydrophobic and impermeable keratin structures of hair protect hair shafts from exogenous DNA. As cells undergo dehydration and catabolic breakdown of nucleic acids and organelles during keratinization (Forslind and Swanbeck, 1966), the DNA present in hair is not just at a low amount but also heavily fragmented (Higuchi et al., 1988; Linch and Prahlow, 2001). Most of the DNA in hair is located in the root and surrounding sheath cells (Hukkelhoven et al., 1981) whereas hair shafts/shed hairs may contain less than 10 ng (Higuchi et al., 1988). When Brandhagen et al. (2018) studied both fresh and approximately 50-year-old human hair samples, they found that nuclear DNA is surprisingly abundant in hair shafts compared to mitochondrial DNA (mtDNA), although it is highly fragmented. Interestingly, while the mtDNA seemed to consistently become more fragmented along the length of the hair shaft, there appeared to be no clear pattern of fragmentation for the nuclear DNA. In Brandhagen et al. (2018), the authors obtained sequencing data for historical hairs (cut and collected in 1958–1965, preserved in room temperature), and found that the average size of the mtDNA reads were between 55 and 87 bp. For historical genomic DNA, the average size of the nuclear DNA reads varied between 49 and 88 bp. Data for modern hair material showed that the average mtDNA size decreased from 168 bp at the proximal end to 91 bp at the distal end.

In cultural historical museums and private collections, items produced from hair are numerous. This includes not only textiles manufactured from sheep, goat, rabbit, and camel hair but countless objects, such as ropes, nets, sieves, toys, bags, brushes, strings, tennis rackets, and furniture and mattress filling, manufactured from horsehair. Therefore, we argue that the value of hair as a source of genetic data of past animal populations and the history of domestication should be recognized and taken into account in curating museum collections.

2. Research aims

In our research project *Interdisciplinary research strategies of biological cultural heritage – surveying, archiving, analysing and sharing historical DNA from Finnhorses* (2019–2021) (Interdisciplinary Research Strategies of Biological Cultural Heritage, 2020; Suomenhevosen varhaisvaiheiden tutkimushanke, 2020), we study the beginning of modern horse breeding in Finland by analysing historical DNA from samples collected from cultural historical museums and private persons and by excavating old horse burials. We concentrate on the time period of 1850–1950, which is elemental for the creation of the Finnhorse, the only native horse breed in Finland (Fig. 1).

To assess the usability of historical artefacts for PCR-based DNA-studies, we here study how DNA is preserved in the horse hairs, and how well DNA can be isolated from historical artefacts and raw material bundles. We investigate how properties of the hairs and changes in sample storage conditions affect the concentration and quality of DNA extracts and how this affects success in PCR, by amplifying mitochondrial DNA.

3. Materials and methods

3.1. Horsehair morphology and properties

Horse offers several types of fibres (i.e. body, tail and mane hair). Here, we refer to tail and mane hair by the term ‘horsehair’ and to the pelage by ‘body hair’. Body hair is 60–100 μm in diameter and oval or round in cross-section. The scale structure is regular mosaic, and the medulla is continuous tubular, containing flat, small-structured gas spaces (Rast-Eicher, 2016, 215). For the structure of hair, see Fig. 2. The colour of hair varies from white to black with different shades of red,

Fig. 1. Ploughing with stallions Leksi and Nurja in the 1930s. Photo: Southwest Finland Horse Breeding Association.

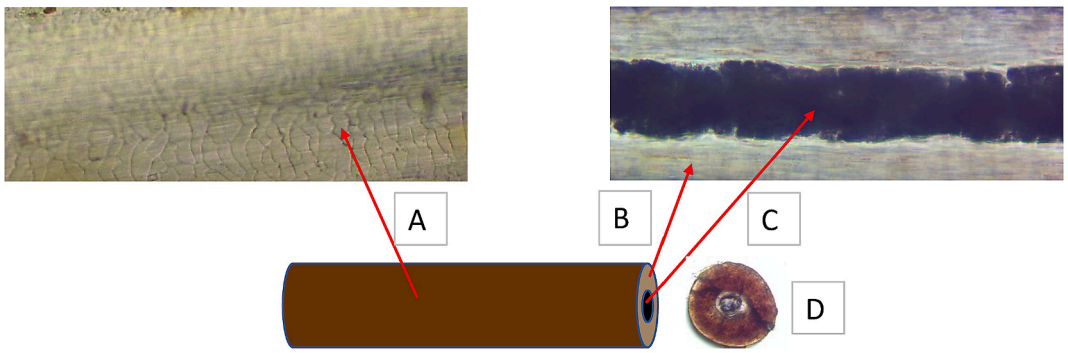

Fig. 2. The structure of modern horse tail hair: shaft A) cuticular scales (i.e. the outermost protective layer of the hair), B) cortex, which is made primarily of hydrophobic fibrous proteins, and C) medulla, the cells of which are mostly empty (air) or contain lipids and exogenous substances (see Bertrand et al., 2014, 487). D) Cross-section. Drawing: T. Kirkinen.

yellow and brown. Variation in hair colour is a product of the melanin type and level. Eumelanin contributes mostly to browns and blacks, and phaeomelanin to reds and yellows.

Tail hairs are 60–80 cm long, with the average diameter ranging from 75 to 280 μm (Von Bergen, 1961), up to 400 μm (Kalayci et al., 2019). The average yearly growth rate of domestic horse tail hair is 46 cm (Sharp et al., 2003, 1714), so shortening of the tail produced raw material for multiple purposes. The mane hairs are shorter and finer with a diameter between 50 and 150 μm (Von Bergen, 1961), up to 200 μm (Kalayci et al., 2019). The scale structure is waved with rippled scale margins, and in the mane hair the distance between the scale margins is wider than in tail hair (Rast-Eicher, 2016, 215). The medulla varies in width, being tubular or multicellular in structure.

In particular, tail hair is a strong fibre, a kind of a predecessor of

Fig. 3. Examples of origin of artefacts and materials for horsehair samples: A) a doll with hair made from horsehair, private collection; B) a stool, the seat of which is filled with horsehair, private collection; C) a brush made in 1888, private collection; D) horse tail hair bundles saved as raw material for brush-making, private collection; E) fabrics woven from horse tail hair, City Museum of Helsinki; F) a rocking horse, private collection; G) horse hide, private collection; H) a paintbrush, private collection. Photos: R. Sjöström, S. Ahola, K. Mantua-Kommonen, M. Elsinen, T. Kirkinen, T. Peltosaari, K. Helminen, and M. Hänninen, respectively.

modern synthetic usurpers (Robson and Ekarius, 2011, 2011, 398–399). It remains solid in wet conditions, which makes it usable, for example, for fishing lines (Rast-Eicher, 2016, 215). Horsehair is also resistant to wear, and its good ventilation properties make it a superb filling material, for mattresses, saddles, furniture, and such (Kalayci et al., 2019). Horsehair has been exploited for brushes, strings, ropes, textiles, dolls' hair, rocking horses, and filling (Fig. 3). Horse body hairs originate mostly from pelts, which have been used for covering in sledges but also as hangings on the walls. Loose hair has been used for filling in the same way as tail and mane hair.

3.2. Samples

The samples used here consist of 147 tail, mane and body hairs from Finnish horses born between the 1850s and 1960s. The number also includes single reference samples from horses up to the 2010s. The material was collected in 2017–2019 from cultural historical museums, private persons and excavations (see graphical abstract). The samples are listed in the Appendix.

We received 24 samples from the museums (e.g. from a taxidermic head, lab equipment cleaning material, and toys). These samples represent only a minor part of the total number of horse-related artefacts archived in Finnish museums. Private persons donated 121 samples, mostly originating from raw material bundles saved for brush-making, sewing and filling, which were stored in stables and unheated outbuildings (Fig. 4). Additionally, horsehair, tails, pelts and even legs were stored as biomemories of late animal companions.

Finally, two samples were collected from excavations of two famous trotters of their time, Rymy-Murto and Valokas (1932–1953) in 2019 (see graphical abstract).

3.3. Methods

3.3.1. Morphology of hairs

Subsamples of 5–15 hairs were separated from the material and washed by stroking them gently with a soft brush. Hairs were placed in parallel on a microscope slide and mounted with water. The material was studied with a transmitted light microscope, using a Leica DM 2000

Fig. 4. Horsehair bundles stored in an outbuilding. Photo K. Ojala.

LED microscope with 100–400x magnification, and documented with a Leica ICC50 E camera. Part of the material was analysed with an Amscope 40X–1600X Advanced Professional Biological Research Kohler Compound Microscope and documented with a 10MP USB 3.0 camera.

The colour and diameter of the hair, scale and medulla structures, medulla width, and medullar index (medulla width equalling hair diameter) are presented in the Appendix. The morphology of medulla and cuticular scales were classified after Tóth (2017) and Rast-Eicher (2016). Horse mane hairs were sorted from tail hairs according to the diameter of hair (<200 μm [Kalayci et al., 2019]) and the wider distance between scale margins (Rast-Eicher, 2016, 215).

In statistical analyses, hair colours were coded as follows: 1 = white and yellow hairs mixed, 2 = light, consisting of cream and very light brown hairs, 3 = light brown and light red hairs, 4 = medium brown and medium red hairs, 5 = dark brown hairs, 6 = black hairs. Black hairs were not considered in morphological analyses, because their morphology could not be verified due to invisibility of the structures.

3.3.2. Hair degradation

In historical horsehair samples, the mechanisms that degrade keratin are manifold. Therefore, the changes in hair structure caused by fungi, bacteria and insects, as well as heat, light and mechanical stress, were examined visually, microscopically and by a simple testing of tensile strength. The degradation of hair was classified after Tridico et al. (2014a; 2014b, 71) and Wilson et al. (2010). The resulting 15 variables were grouped according to the types of damage and impurities located primarily 1) on the surface of the fibre, 2) on cuticular scales, 3) in the cortex, 4) in the medulla, and 5) in two or more layers.

The first group indicates the presence of dirt (Fig. 5A), fungal hyphae (Fig. 5B), and bacteria pits on the surface of the fibre. Dirt might indicate the presence of adherent contaminants that can cause problems in PCR, such as PCR inhibitors present in soil, plant-based material or bacterial cells (Wilson, 1997; Schrader et al., 2012). In the second group, major damages detected on the outermost cuticular scale layer – for example, the loosening and/or removal of scales (Fig. 5C) – were recorded. The third group consists of a number of microbial structures, which operate in the cortex by penetrating into it through the scales (Fig. 5G and H) or medulla (Fig. 5I). In the fourth group, microbiological activity hollowing or destroying the medulla was detected (Fig. 5J and K). In the fifth group, agents which affect large areas/several layers of hair were recorded. This group includes the brittleness of the hair (tensile strength), colour changes (possible photodegradation), insect damage (Fig. 5D), bacterial and fungal infusion (Fig. 5L), and the effects of heat and mechanical processing (Fig. 5E and F).

In addition to examining different variables, hairs were scored from 0 to 5, with 0 indicating little or no damage, and 5 indicating the poorest preservation (see Wilson et al., 2010, 471). These scores were determined by totalling the damaged structures per group (see above classification) per hair.

Although the degradation of hair (e.g. by fungi) can begin while the animal is still living (Lewin et al., 1981; Tridico et al., 2014a), most damage is caused by time due to storage and handling of the material. Therefore, the storage place (e.g. museum, barn, stable) and use of the hair (e.g. biomemory, filling) were documented in the Appendix. On the basis of this information, the hairs were classified in three classes based on exposure to sunlight (1 = no exposure, 2 = some exposure, 3 = exposed to sunlight), humidity (1 = dry [room preservation], 2 = some humidity [outbuilding preservation], 3 = very humid [archaeological samples]) and temperature variation (1 = no variation [room temperature, about 20–25 °C], 2 = some variation [archaeological samples], 3 = lot of variation [outbuilding temperature, about −35 to +35 °C]).

3.3.3. DNA extraction and PCR

DNA was extracted from 140 hair samples by cutting hairs into ~1 cm long pieces. Approximately 40–50 pieces were included per sample, if available. If the follicle ends were identifiable, they were included. However, most samples were cut, not plucked, and included no root sections; some samples included only a couple of short hairs. Altogether 12 samples (see Appendix, DNA values in the brackets) were re-extracted after an extra step of washing the sample with sterile H_2O prior to extraction. The samples were put into 200 μl of QuickExtract DNA extraction solution (Lucigen) and extracted following the protocol of the manufacturer. Handling of the hairs was performed in a clean laboratory room dedicated to DNA work with low-quality samples. The room and fume hood in it were treated with UV light for at least two hours before and after working, and all equipment was cleaned with sterile water and alcohol between the samples. Normal protective clothing (gloves, lab coat) were worn when handling the samples. In addition, no PCR products were handled in this room. DNA concentrations and purities (absorbance ratio of A_{260}/A_{280}) were measured with the Nanodrop spectrophotometer (ThermoFisher) using 1 μl of the DNA extract.

We chose short horse-specific fragments to be amplified by PCR based on previous experience from horsehair samples (Kvist et al., 2019). PCR was performed by amplifying a part of the horse mitochondrial control region using either primers L305 (5′-GTCCCAATCCTCGCTCCGGGCCCAT-3′) and H532 (5′-GACTGCGTC-GAGGCCTTTGACGGCC-3′, producing ~250 bp fragments) or L450 (5′-CAGCCCATGCTCACACATAACTGT-3′) and H690 (5′-TTGTTTCTTAT GTCCCGCTACC-3′, producing ~240 bp fragments). We chose the length of the PCR product to be relatively short, but still long enough to likely result in lower PCR success in older samples if fragmentation of DNA is causing problems in the time frame of our samples. These two primer sets were applied to obtain a longer region of mtDNA for further studies of these samples. PCR reactions were performed in 10 μl reaction volumes, including 0.5 μl of both primers (10 μM), 1 μl of dNTPs (10 mM), 0.8 μl of $MgCl_2$ (50 mM), 1 μl of 10 × reaction buffer (Biotools), 0.2 μl of Biotools DNA Polymerase (5 U/μl, Biotools) and 10–50 ng of template DNA. 10 ng was used first as template, and in case of no PCR products, the amount of DNA was increased up to 50 ng. A PCR touchdown profile was used for primer pair L305 and H532 as follows: 94 °C for 5 min followed by 2 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 45 s, then 2 cycles of 94 °C for 30 s, 58 °C for 30 s and 72 °C for 45 s, 56 °C for 30 s and 72 °C for 45 s, 54 °C for 30 s and 72 °C for 45 s, 52 °C for 30 s and 72 °C for 45 s and 30 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 45 s, finishing with 72 °C for 7 min. The PCR profile for the primers L450 and H690 was 98 °C for 4 min followed by 30 cycles of 98 °C for 30 s, 53 °C for 30 s and 72 °C for 40 s, finishing with 72 °C for 10 min. Success of the PCR reaction was checked on an agarose gel. Samples that failed to amplify were attempted to be amplified at least for a second time.

3.3.4. Statistical tests

Statistical tests were chosen based on whether the studied variables were nominal or scale variables and whether they were normally distributed or not. A Bonferroni correction was used when multiple testing.

3.3.4.1. Hair morphology and damage vs. DNA concentration and purity and effect of time. DNA concentration and purity differences between non-washed and washed samples were tested using the Related-Samples Wilcoxon Signed Rank test. To examine how damage of the hairs affected DNA concentration and purity, the damage score described above was used and a Spearman's correlation test was performed with DNA concentration and purity. In addition, we checked for correlation between DNA concentration and purity (unwashed samples) and looked for the effect of hair diameter and medulla width on DNA concentration and purity using Pearson correlation. Furthermore, we examined if hair damage and other hair parameters (medulla width and hair diameter) were correlated by using Spearman's correlation test. We looked for the effect of hair colour on DNA concentration and purity and the effect of

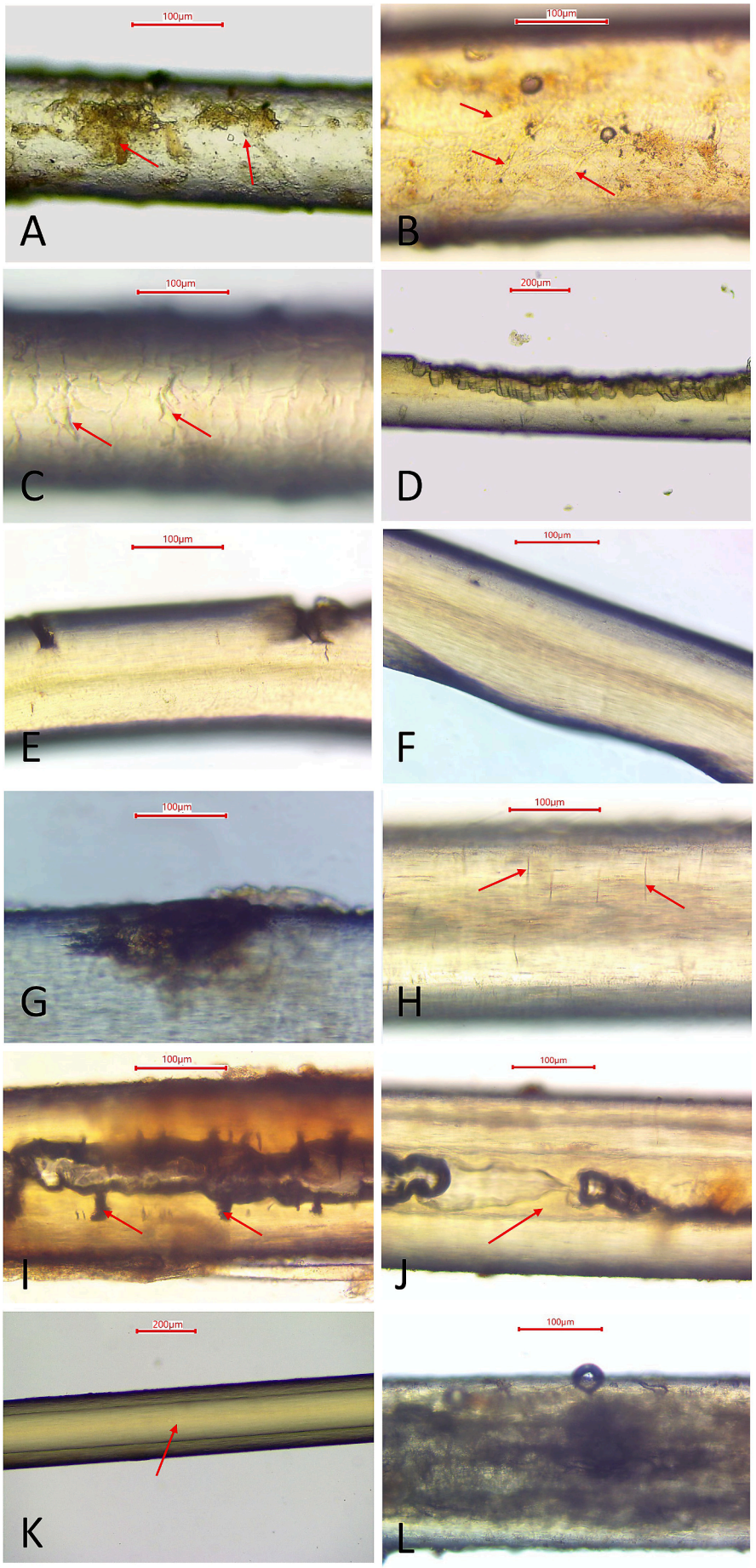

Fig. 5. Impurities and damages in hair shafts: A) dirt; B) fungal threads on the surface of the hair; C) loosened scales; D) insect damage; E–F) mattress filling material that was prepared among other things by twisting (F) the hair with a special machine and by cutting them into pieces (see cut marks in E); G) fungal borer-type damage; H) thin hyphae invading the hair; I) fungal finger-like stellate; J) hollowing out of the medullary canal; K) empty medulla; and L) mass of hyphae. Photos: T. Kirkinen.

hair damage and storage conditions by using Kruskall-Wallis test. Next, we tested the effect of sample age on hair diameter, medulla width, hair condition, DNA concentration and purity by using Spearman's correlation. Further, we performed a generalized linear analysis with log link function for log transformed DNA concentration using hair damage and medulla width as factors to see if there is any interaction between the two.

3.3.4.2. PCR success. We then examined if DNA concentration, DNA purity, hair damage, morphology or age had an effect on success in PCR amplification. This was done by classifying hair samples into two groups based on whether the PCR failed (=0) or PCR amplification was detected (=1), that is, either one or both of the primer pairs produced a band of correct size in the agarose gel. These groups were then compared with ANOVA analyses for DNA purity, hair diameter and medulla width and with a Mann-Whitney *U* test for DNA concentration, sample age and hair colour, damage and storage conditions. In addition, we performed a generalized linear model analysis for the PCR success, using medulla width, hair damage and colour as factors and another similar analysis using storage conditions; temperature variation and humidity and age as factors to study the effects and interactions of these terms.

All statistical tests were performed in IBM SPSS Statistics v. 26.0.0.1.

4. Results

4.1. DNA extraction and concentration

DNA concentrations after extraction varied from 34.57 ng/μl to 955.41 ng/μl and absorbance ratio A_{260}/A_{280} from 1.18 to 1.67. When samples were re-extracted after inclusion of an extra washing step, DNA concentrations dropped from a mean of 241.50 ng/μl (SD = 205.34) to 107.20 ng/μl (SD = 97.59) (Fig. 6a). This change was significant (z = 4.000, P = 0.006, N = 12). Difference in DNA purity before (A_{260}/A_{280} = 1.396, SD = 0.075) and after (A_{260}/A_{280} = 1.373, SD = 0.043) the washing step was non-significant (z = 33.000, P = 0.637, N = 12; Fig. 6b). DNA concentration and purity were significantly negatively correlated (r = −0.361, P = 0.000, N = 140; Fig. 6c).

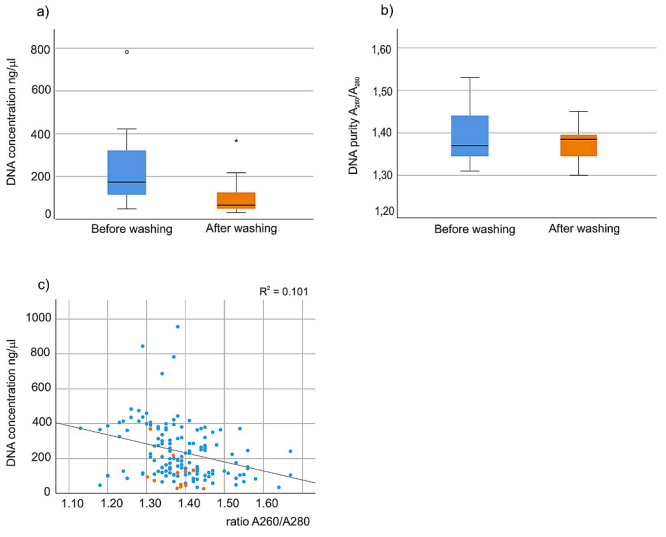

Fig. 6. A) Differences in DNA concentration and B) differences in DNA purity before and after adding an extra washing step in DNA extraction. C) Correlation between DNA concentration and DNA purity. Washed samples are included here in orange although not included into the calculations for correlation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.2. Morphology and degradation

The analysed hair samples were visually well-preserved with a few exceptions in which the fragile nature of the fibre was evident even with the naked eye. However, microscopic examination revealed a number of forms in which the hairs were damaged. As a result, only 8% of the hairs were scored as to 0–1 (i.e. to best preserved fibres) according to the number of degraded structures of the hair. Most of the hairs (65%) were scored to 2–4 and a quarter of the hairs (27%) to the most damaged groups scored to 5–6. As expected, the best-preserved hairs were biomemories or artefacts such as toy horses, and none of them was stored in cold outbuildings.

4.3. Hair morphology and degradation vs. DNA concentration and purity and effect of time

There was a significant positive correlation between DNA concentration and hair damage scores (r_s = 0.216, P = 0.020, N = 116; Fig. 7a), but this did not remain significant after Bonferroni correction. DNA purity was also positively correlated with hair damage, although not significantly (r_s = 0.083, P = 0.375, N = 116). Hair diameter, cortex width and medulla width were significantly positively correlated with DNA concentration (r = 0.309, P = 0.001, N = 116; Fig. 7b); r_s = 0.247, P = 0.016 (NS after Bonferroni correction), N = 94; r = 0.230, P = 0.025 (NS after Bonferroni correction), N = 94, respectively), but these did not affect DNA purity (r = 0.013, P = 0.887, N = 116; r = −0.072, P = 0.489, N = 94). Furthermore, medulla width and hair damage scores

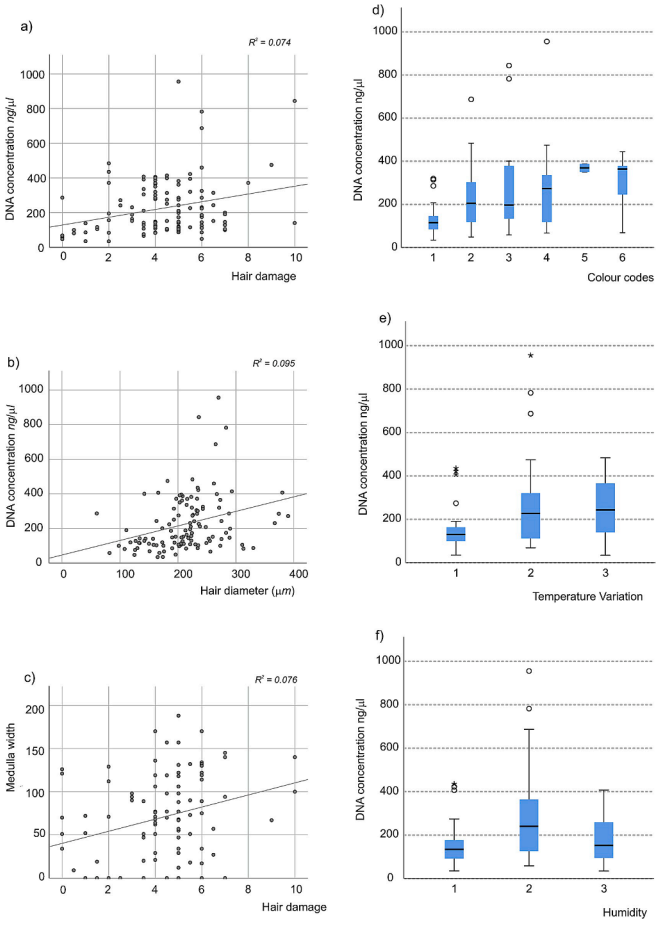

Fig. 7. Correlations between A) hair damage and DNA concentration, B) hair diameter and DNA concentration, C) hair damage and medulla width and DNA concentrations classified on the basis of D) colours, E) temperature variation and F) humidity (see codes in Materials and methods).

were significantly positively correlated (Fig. 7c), whereas hair diameter and damage were not correlated ($r_s = 0.316$, $P = 0.002$, $N = 98$; $r_s = 0.110$, $P = 0.233$, $N = 120$, respectively). Different hair colours resulted in different DNA concentrations (Kruskall-Wallis $H = 24.952$, $df = 5$, $P = 0.000$, $N = 138$; Fig. 7d, group 1 showing significant differences ($P < 0.05$) from groups 2, 4 and 6 in pairwise comparisons); in general, the darker hairs resulted in higher DNA concentrations, whereas no effect of colour could be seen on DNA purity or hair damage ($H = 9.142$, $df = 5$, $P = 0.104$, $N = 138$; $H = 2.612$, $df = 4$, $P = 0.625$, $N = 119$). None of the correlations of age with the other studied parameters were significant (DNA concentration: $r_s = 0.000$, $P = 0.996$, $N = 110$, purity: $r_s = -0.045$, $P = 0.639$, $N = 110$; hair diameter: $r_s = 0.049$, $P = 0.634$, $N = 96$; medulla width $r_s = -0.124$, $P = 0.290$, $N = 75$; hair damage $r_s = -0.133$, $P = 0.199$, $N = 95$). Generalized linear model did not reveal any effect of medulla width ($p = 0.835$) or hair damage ($p = 0.396$) either, although showed some, although non-significant, interaction between these terms ($p = 0.077$). Temperature variation and exposure to humidity seemed to affect DNA concentration (temperature variation: $H = 8.395$, $df = 2$, $P = 0.015$, $N = 133$; Fig. 7e; humidity: $H = 7.971$, $df = 2$, $P = 0.019$, $N = 137$; Fig. 7f); however, significance of humidity disappeared after Bonferroni correction. Exposure to sunlight did not affect DNA concentration ($H = 3.550$, $P = 0.169$, $df = 2$, $N = 138$). There was no effect of temperature variation, humidity or exposure to sunlight on DNA purity (temperature variation: $H = 0.040$, $df = 2$, $P = 0.980$, $N = 133$; humidity: $H = 2.363$, $df = 2$, $P = 0.307$, $N = 137$; exposure to sunlight: $H = 1.458$, $df = 2$, $P = 0.483$, $N = 138$).

4.4. PCR success

ANOVA analyses showed no difference in any of the variables in PCR success (hair diameter: $F = 0.334$, $P = 0.565$, $N = 98$; medulla width: $F = 0.263$, $P = 0.610$, $N = 80$; DNA purity: $F = 1.574$, $P = 0.213$, $N = 97$). In Mann-Whitney U tests, only DNA concentration turned out to be significant for PCR success, but the significance disappeared after Bonferroni correction (DNA concentration: $U = 1416$, $P = 0.036$, $N = 121$; age: $U = 1360.5$, $P = 0.096$, $N = 96$; hair damage: $U = 1036$, $P = 0.185$, $N = 99$; hair colour: $U = 1862$, $P = 0.838$, $N = 121$; exposure to sunlight: $U = 1758$, $P = 0.688$, $N = 121$; humidity: $U = 1705$, $P = 0.524$, $N = 120$; temperature variation: $U = 1758$, $P = 0.993$, $N = 119$). The general linear model showed significant effect of the hair damage ($p = 0.001$) but no effect of medulla width ($p = 0.875$) or colour ($p = 0.100$). Interaction between hair damage and medulla width ($p = 0.003$) and between hair damage and colour ($p = 0.000$) were significant. No effects were observed of temperature variation ($p = 0.570$), humidity ($p = 0.117$) or age ($p = 0.575$) to PCR success and there were no interactions between these factors (all p-values > 0.115). Samples with very high concentrations did not amplify at all, whereas samples with concentrations around 100 ng/μl were the most likely to succeed in PCR (Fig. 8).

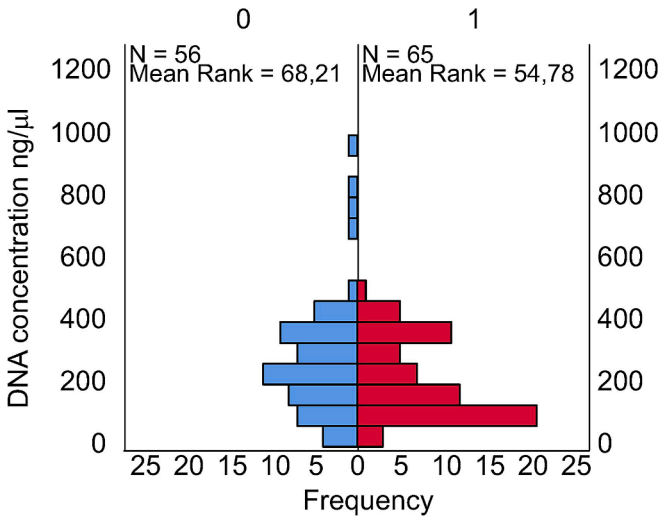

Fig. 8. Mann-Whitney test results of PCR success explained by DNA concentration. The best PCR amplification results were from samples with DNA concentration of around 100 ng/μl. 0 = no PCR product, 1 = PCR succeeded.

5. Discussion and conclusions

Pre-washing of hairs prior to DNA extraction decreased DNA concentration but did not affect the purity of the DNA (Fig. 6). Samples which have been stored for several decades by hanging on the walls of stables and other cold outbuildings can be assumed to have organic dirt, such as bacteria, yeast or mould, on the surface. DNA in this organic dirt increases the total amount of DNA and can be removed by washing the sample. However, pre-washing of the samples did not seem to affect PCR success. This might be due to the specificity of the PCR primers used for horse DNA; therefore, the non-targeted DNA did not interfere much. McNevin et al. (2005) have suggested that human hair samples should not be washed prior to DNA extraction, because washing removes nucleated epithelial cells adhering to the outer surface of the shaft that might contain more DNA than the shaft itself. Contrary to results observed by McNevin et al. (2005), Amory et al. (2007) found no differences in DNA concentrations or STR genotyping success between washed and unwashed hairs. According to Amory et al. (2007), variation in the final DNA concentration between these two extraction protocols results more likely from stochastic variation than to a clear pattern. Our samples with really high DNA concentrations (>600 ng/μl) did not amplify at all, suggesting that high DNA concentrations are likely due to the presence of exogenous DNA rather than the target horse DNA. Thus, the washing step likely needs to be optimized for each specific sample material. Based on our results, we recommend rinsing horse hair samples that have visible dirt on the surface.

Since the absorbance ratio A_{260}/A_{280} was less than 1.8, which is the ratio of pure DNA, it can be concluded that the extracted DNA samples contain some proteins, and as the ratio did not change during washing, this step did not affect the relative ratio of DNA and proteins. However, during the extraction procedure, proteins are fragmented into small pieces and proteins and enzymes that might be inhibitory in PCR (such as melanins and eumelanins) are likely broken down. The decrease in the A_{260}/A_{280} ratio with increasing DNA concentration suggests that the amount of proteins tends to increase at a higher rate than the amount of DNA with increasing DNA concentrations. Thus, it is advisable to optimise the amount of sample material for suitable DNA concentrations in DNA extracts that yield the best PCR amplifications. For our horse material and PCR primers, the best PCR success was achieved with samples which had a concentration of around 100 ng/μl after extraction.

DNA concentration increased with greater hair diameter. The reason for this might be simply that thick hairs have a larger volume, meaning that there are more cells and thus more DNA remaining. The role of medulla width in this equation is complicated. Although TEM observations by de Cássia Comis Wagner et al. (2007) have shown that medulla fibril material resembles cortical cells, probably indicating that the medulla is a shapeless cortex, the medulla is still mostly filled with air, lipids and exogenous substances (Bertrand et al., 2014, 487). Therefore, it is reasonable to assume that the cortex volume is critical for DNA concentration. Our research supports this hypothesis, as the correlation between DNA concentration and cortex width was positive, although significance disappeared after Bonferroni correction.

White hairs contain no melanins, which act as inhibitors to PCR (Wilson and Budowie, 1993); thus, we would have expected the most lightly coloured hairs to perform best in PCR amplification. Contrary to this hypothesis, however, the colour of the hair did not have an effect on PCR amplification. Furthermore, we found that, in general, darker hair had higher DNA concentration. Melanins protect DNA from damage induced by UV light (Kobayashi et al., 1993) and there is evidence that

melanins also protect DNA from reactive oxygen species, eumelanin being superior to pheomelanin in this. Thus, it is possible that there is more DNA in dark hairs because it is better preserved in dark eumelanin-containing cells (Swope and Abdel-Malek, 2018). Wilson et al. (2007, 453–455) found that there is a difference in degradation of melanin and keratin structures in the hair shaft. Melanin granules could resist microbial degradation, whereas keratinaceous structures degraded easily. This might increase even more the probability of good DNA preservation in dark hairs. Experiments done on feathers, which are also keratin derivatives, have shown that melanised feathers resist microbial degradation better (Goldstein et al., 2004; Gunderson et al., 2008). We are not aware of similar experiments on hairs, but if melanised hairs are also more resistant to degradation caused by microorganisms, this could explain their higher DNA concentration.

We also found that DNA concentration increased with hair damage. It can be assumed that the most damaged hairs also contained the largest amounts of contaminating DNA from the environment. The biodegradation of hair is dependent on the activity of keratinolytic microorganisms, such as keratinous fungi, bacteria and insects, which colonise and exploit different structures of hair as a nutrient source (Wilson et al., 2007; Bertrand et al., 2014; Tridico et al., 2014a); thus, the amount of DNA from these organisms likely increases with the damage seen in the hairs. The rate of degradation depends primarily on moisture, temperature, sun exposure, and bacterial and fungal activity (Chang et al., 2005; Wilson et al., 2007; Bertrand et al., 2014, 488). For example, in biological museum collections, airborne microorganisms are reported to attack hair fibres especially if the relative humidity level is high (Hawk and Rowe 1988). Most interestingly, keratinolytic fungi prefer sites frequented by animals (e.g. stables and zoological gardens). At these sites, destruction of hair can begin already during the lifetime of an animal (Tridico et al., 2014a, 5).

In our research material, temperature variation and humidity were found to have a negative effect on DNA concentration, but the significance of humidity disappeared after Bonferroni correction. Surprisingly, the samples stored in conditions with high temperature variation also contained the highest amounts of DNA. Most of the samples had been stored for decades in outbuildings and in unheated attics. In Finland, seasonal temperature fluctuations are great from −35 to +35 °C. Despite this, it was possible to obtain successful PCR products of mtDNA from many of these samples, because these environments are in general quite cool and temperatures are near or below zero for long periods each year. Humidity and light conditions vary reasonably little in these outbuildings and attics. The samples were not exposed to direct sunlight; if there was any sunlight, it was filtered through (dirty and/or small) windows. This may explain why the DNA was preserved quite well and no significant differences were observed between different classes of humidity and light.

We found no effect of the age of the samples on DNA concentration, PCR success or hair damage. DNA is known to become more and more fragmented with time (Pääbo et al., 2004), and obtaining DNA with good quantity and quality is often problematic, even in the time scale of historical samples and samples stored in museums, due to preservatives used for storing the sample, not the DNA in it (e.g. Staats et al., 2013; McCormack et al., 2016). As an example, McGaughran (2020) studied samples of pinned moth specimens (*Helicoverpa armigera*) ranging in age from 4 to 116 years and found that older samples resulted in lower DNA concentrations and produced a lower number of sequenced and mapped reads in NGS (Next-generation sequencing). McGaughran (2020) concluded that sample age has significant, measurable impacts on the quality of NGS data. The pinned moth samples had likely been treated with a chemical prior to storage and stored under stable conditions. Our samples were stored with no preservatives that we were aware of, and we found no effect of time on DNA preservation, likely because the effect was masked by other factors, such as damage due to varying environmental conditions, the amount of exogenous DNA, and protection of DNA by melanins. In addition, as we tested only for amplification of mitochondrial DNA, the larger copy number of mitochondrial DNA compared to nuclear DNA was likely advantageous, as there are likely more mitochondrial DNA-molecules spanning the entire length of the hair for PCR than there would have been for nuclear PCR.

To conclude, it is possible to isolate mtDNA of sufficient quality and concentration suitable for at least PCR-based DNA- studies from old horsehair samples stored in different places and for different lengths of time (environmental exposure). However, it is advisable to rinse very dirty horsehair samples before DNA isolation so that contaminating DNA does not interfere with PCR amplification. The obtained samples should be properly stored in museums (i.e. placed indoors where there are standardized conditions, and constant temperature is especially important). Because samples can be eaten by pests, they must be kept out of reach of microorganisms in museums and freezing for microorganisms must not be continuous.

The material which we sampled from museum collections and private persons was from everyday objects, such as brushes, toys and mattress filling (i.e. artefacts which have been in use in almost every household). Importantly, already Wandeler et al. (2007) concluded that cultural historical and ethnographic museums can offer older samples for the study of population genetics than most natural historical museums. Our research provides important information about the use of animal hair in the past and about the Finnish horses, which are of special value for private persons who have stored these items as memories of past ways of living and past animal companions. For our research project, samples opened the way to study early 20th-century animals and the effects of breeding on local animal populations.

Acknowledgements

We are thankful to Taru Peltosaari and all those who donated photos for this paper. We also thank Soile Alatatalo and Hannele Parkkinen for their help with laboratory work, and Mária Tóth for her kind help with hair structures. This work was supported by the Alfred Kordelin Foundation in Finland. We also thank the two anonymous reviewers for their helpful comments on improving this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jasrep.2021.103262.

References

Amory, S., Keyser, C., Crubézy, E.B., Ludes, B., 2007. STR typing of ancient DNA extracted from hair shafts of Siberian mummies. Forensic Sci. Int. 166 (2–3), 218–229. https://doi.org/10.1016/j.forsciint.2006.05.042.

Bengtsson, C.F., Olsen, M.E., Brandt, L.Ø., Bertelsen, M.F., Willerslev, E., Tobin, D.J., Wilson, A.S., Gilbert, T., 2012. DNA from keratinous tissue. Part 1: Hair and Nail. Ann. Anat. 194 (1), 17–25. https://doi.org/10.1016/j.aanat.2011.03.013.

Bertrand, L., Vichi, A., Doucet, J., Walter, P., Blanchard, P., 2014. The fate of archaeological keratin fibres in a temperate burial context: microtaphonomy study of hairs from Marie de Bretagne (15th c., Orléans, France). J. Archaeol. Sci. 42, 487–499. https://doi.org/10.1016/j.jas.2013.11.028.

Besnard, G., Bertrand, J.A.M., Delahaie, B., Bourgeois, Y.X.C., Lhuillier, E., Thebaud, C., 2016. Valuing museum specimens: high-throughput DNA sequencing on historical collections of New Guinea crowned pigeons (*Goura*). Biol. J. Linn. Soc. 117, 71–82. https://doi.org/10.1111/bij.12494.

Billerman, S.M., Walsh, J., 2019. Historical DNA as a tool to address key questions in avian biology and evolution: a review of methods, challenges, applications, and future directions. Mol. Ecol. Resour. 19, 1115–1130. https://doi.org/10.1111/1755-0998.13066.

Bonnichsen, R., Hodges, L., Ream, W., Field, K., Kirner, D.L., Selsor, K., Taylor, R.E., 2001. Methods for the study of ancient hair: radiocarbon dates and gene sequences from individual hairs. J. Archeol. Sci. 28, 775–785. https://doi.org/10.1006/jasc.2000.0624.

Brandt, L.Ø., Tranekjer, L.D., Mannering, U., Ringgaard, M., Frei, K.M., Willerslev, E., Gleba, M., Gilbert, M.T.P., 2011. Characterising the potential of sheep wool for ancient DNA analyses. Archaeol Anthropol Sci. 3, 209–221. https://doi.org/10.1007/s12520-011-0055-2.

Brandhagen, M.D., Loreille, O., Irwin, J.A., 2018. Fragmented nuclear DNA is the predominant genetic material in human hair shafts. Genes 9, 640. https://doi.org/10.3390/genes9120640.

Buckley, M., 2017. Zooarchaeology by Mass Spectrometry (ZooMS) Collagen Fingerprinting for the Species Identification of Archaeological Bone Fragments. In: Giovas, C.M., LeFebvre, M.J. (Eds.), Zooarchaeology in Practice. Springer, Cham, pp. 227–247.

Campos, P.F., Gilbert, M.T.P., 2019. DNA extraction from keratin and chitin. In: Barlow, A., Heintzman, P.D., Hofreiter, M., Paijmans, J.L.A., Soares, A.E.R., Shapiro, B. (Eds.), Ancient DNA. Humana Press, New York, NY, pp. 57–63.

Castañeda-Rico, S., León-Paniagua, L., Edwards, C.W., Maldonado, J.E., 2020. Ancient DNA from museum specimens and next generation sequencing help resolve the controversial evolutionary history of the critically endangered puebla deer mouse. Front. Ecol. Evol. 8 (April) https://doi.org/10.3389/fevo.2020.00094.

Chang, B.S., Hong, W.S., Lee, E., Yeo, S.M., Bang, I.S., Chung, Y.H., Lim, D.S., Mun, G.H., Kim, J., Park, S.O., Shin, D.H., 2005. Ultramicroscopic observations on morphological changes in hair during 25 years of weathering. Forensic Sci. Int. 151 (2–3), 193–200. https://doi.org/10.1016/j.forsciint.2004.12.037.

Copley, M.S., Berstan, R., Mukherjee, A.J., Dudd, S.N., Straker, V., Payne, S., Evershed, R.P., 2005. Dairying in antiquity. III. Evidence from absorbed lipid residues dating to the British Neolithic. J. Archaeol. Sci. 32 (4), 523–546. https://doi.org/10.1016/j.jas.2004.08.006.

Clack, A.A., MacPhee, R.D., Poinar, H.N., 2012. *Mylodon darwinii* DNA sequences from ancient fecal hair shafts. Ann. Anatomy-Anatomischer Anzeiger 194 (1), 26–30. https://doi.org/10.1016/j.aanat.2011.05.001.

De Cássia Comis Wagner, Kiyohara, R.K.P., Silveira, M., Joekes, I., 2007. Electron microscopic observations of human hair medulla. J. Microsc. 226, 54–63. https://doi.org/10.1111/j.1365-2818.2007.01747.x.

Forslind, B., Swanbeck, G., 1966. Keratin formation in the hair follicle. Exp. Cell Res. 43, 191–209.

Gilbert, M.T.P., Wilson, A.S., Bunce, M., Hansen, A.J., Willerslev, E., Shapiro, B., Higham, T.F.G., Richards, M.P., O'Connell, T.C., Tobin, D.J., Janaway, R.C., Cooper, A., 2004. Ancient mitochondrial DNA from hair. Curr. Biol. 14 (12), R463–R464, 10.106fjcub.204.06.008.

Gilbert, M.T., Lynn, P., Tomsho, L.P., Rendulic, S., Packard, M., Drautz, D.I., Sher, A., Tikhonov, A., Dalén, L., Kuznetsova, T., Kosintsev, P., Campos, P.F., Higham, T., Collins, M.J., Wilson, A.S., Shidlovskiy, F., Buigues, B., Ericson, P.G.P., Germonpré, M., Götherström, A., Iacumin, P., Nikolaev, V., Nowak-Kemp, M., Willerslev, E., Knight, J.R., Irzyk, G.P., Perbost, C.S., Fredrikson, K.M., Harkins, T.T., Sheridan, S., Miller, W., Schuster, S.C., 2007. Whole-genome shotgun sequencing of mitochondria from ancient hair shafts. Science 317 (5846), 1927–1930. https://doi.org/10.1126/science.1146971.

Gilbert, M.T.P., Menez, L., Janaway, R.C., Tobin, D.J., Cooper, A., Wilson, A.S., 2006. Resistance of degraded hair shafts to contaminant DNA. Forensic Sci. Int. 156 (2–3), 208–212. https://doi.org/10.1016/j.forsciint.2005.02.021.

Goldstein, G., Flory, K.R., Browne, B.A., Majid, S., Ichida, J.M., Burtt Jr., E.H., 2004. Bacterial degradation of black and white feathers. Auk 121, 656–659. https://doi.org/10.1093/auk/121.3.656.

Gunderson, A.R., Frame, A.M., Swaddle, J.P., Forsyth, M.H., 2008. Resistance of melanized feathers to bacterial degradation: is it really so black and white? J. Avian Biol. 39, 539–545. https://doi.org/10.1111/j.2008.0908-8857.04413.x.

Hawk, C.A., Rowe, W.F., 1988. Deterioration of hair by airborne microorganisms: implications for museum biological collections. Biodeterioration 7, 461–465. https://doi.org/10.1007/978-94-009-1363-9_62.

Higuchi, R., von Beroldingen, C.H., Sensabaugh, G.F., Erlich, H.A., 1988. DNA typing from single hairs. Nature 332, 543–545.

Hukkelhoven, M.W., Vromans, E., Markslag, A.M., Vermorken, A.J.M., 1981. A simple fluorimetric microassay for DNA in hair follicles or fractions of hair follicles. Anticancer Res. 1 (6), 341–344.

Interdisciplinary Research Strategies of Biological Cultural Heritage, 2020. Project webpages. University of Helsinki (available 16.12.2020).

Kalayci, E., Avinc, O., Yavas, A., 2019. Usage of horse hair as a textile fiber and evaluation of color properties. Ann. Univ. Oradea: Fascicle Text., Leatherwork XX, 57–62.

Kobayashi, N., Muramatsu, T., Yamashina, Y., Shirai, T., Ohnishi, T., Mori, T., 1993. Melanin reduces ultraviolet-induced DNA damage formation and killing rate in cultured human melanoma cells. J, Invest. Dermatol. 101 (5), 685–689. https://doi.org/10.1111/1523-1747.ep12371676. PMID: 8228329.

Kvist, L., Niskanen, M., Mannermaa, K., Wutke, S., Aspi, J., 2019. Genetic variability and history of a native Finnish horse breed. Genet. Sel. Evol. 51, 35. https://doi.org/10.1186/s12711-019-0480-8.

Lewin, R.A., Farnsworth, P.A., Yamanaka, G., 1981. The algae of green polar bears. Phycologia 20 (3), 303–314. https://doi.org/10.2216/i0031-8884-20-3-303.1.

Linch, C.A., Prahlow, J.A., 2001. Postmortem microscopic changes observed at the human head hair proximal end. J. Forensic Sci. 46, 15–20. https://doi.org/10.1520/JFS14906J.

Martínková, N., Searle, J.B., 2006. Amplification success rate of DNA from museum skin collections: a case study of stoats from 18 museums. Mol. Ecol. Notes 6, 1014–1017. https://doi.org/10.1111/j.1471-8286.2006.01482.x.

McCormack, J.E., Tsai, W.L., Faircloth, B.C., 2016. Sequence capture of ultraconserved elements from bird museum specimens. Mol. Ecol. Resour. 16 (5), 1189–1203. https://doi.org/10.1111/1755-0998.12466.

McGaughran, A., 2020. Effects of sample age on data quality from targeted sequencing of museum specimens: what are we capturing in time? BMC Genomics 21 (1), 188. https://doi.org/10.1186/s12864-020-6594-0.

McNevin, D., Wilson-Wilde, L., Robertson, J., Kyd, J., Lennard, C., 2005. Short tandem repeat (STR) genotyping of keratinised hair. Part 2. An optimised genomic DNA extraction procedure reveals donor dependence of STR profiles. Forensic Sci. Int. 153 (2–3), 247–259. https://doi.org/10.1016/j.forsciint.2005.05.005.

Monnier, G., Frahm, E., Luo, B., Missal, K., 2017. Developing FTIR microspectroscopy for analysis of plant residues on stone tools. J. Archaeol. Sci. 78, 158–178. https://doi.org/10.1016/j.jas.2016.12.004.

Pääbo, S., Poinar, H., Serre, D., Jaenicke-Després, V., Hebler, J., Rohland, N., Kuch, M., Krause, J., Vigilant, L., Hofreiter, M., 2004. Genetic analyses from ancient DNA. Annu. Rev. Genet. 38, 645–679. https://doi.org/10.1146/annurev.genet.37.110801.143214.

Rasmussen, M., Li, Y., Lindgreen, S., Pedersen, J.S., Albrechtsen, A., Moltke, I., et al., 2010. Ancient human genome sequence of an extinct Palaeo-Eskimo. Nature 463, 757–762. https://doi.org/10.1038/nature08835.

Rast-Eicher, A., 2016. Fibres. Microscopy of archaeological textiles and furs. Archaeolingua, Budapest.

Robson, D., Ekarius, C., 2011. The fleece and fiber sourcebook. More than 200 fibers from animal to spun yarn. Storey Publishing, North Adams, MA.

Schrader, C., Schielke, A., Ellerbroek, L., Johne, R., 2012. PCR inhibitors – occurrence, properties and removal. J. Appl. Microbiol. 113 (5), 1014–1026. https://doi.org/10.1111/j.1365-2672.2012.05384.x.

Sharp, Z.D., Atudorei, V., Panarello, H.O., Fernández, J., Douthitt, C., 2003. Hydrogen isotope systematics of hair: archeological and forensic applications. J. Archaeol. Sci. 30, 1709–1716. https://doi.org/10.1016/S0305-4403(03)00071-2.

Staats, M., Erkens, R.H.J., van de Vossenberg, B., Wieringa, J.J., Kraaijeveld, K., Stielow, B., Geml, J., Richardson, J.E., Bakker, F.T., Caramelli, D., 2013. Genomic treasure troves: complete genome sequencing of herbarium and insect museum specimens. e69189 PLoS ONE 8 (7). https://doi.org/10.1371/journal.pone.0069189.

Suomenhevosen varhaisvaiheiden tutkimushanke 2020. Facebook group. <https://www.facebook.com/groups/1739756833002506>. (available 16.12.2020).

Swope, V.B., Abdel-Malek, Z.A., 2018. MC1R: front and center in the bright Side of Dark Eumelanin and DNA repair. Int. J. Mol. Sci. 19, 2667. https://doi.org/10.3390/ijms19092667.

Teerink, B.J., 1991. Hair of West-European Mammals: Atlas and Identification Key. Cambridge University Press, Cambridge.

Tóth, M., 2017. Hair and Fur Atlas of Central European Mammals. Pars Ltd, Hungary.

Tridico, S.R., Koch, S., Michaud, A., Thomson, G., Kirkbride, K.P., Bunce, M., 2014a. Interpreting biological degradative processes acting on mammalian hair in the living and the dead: which ones are taphonomic? Proc. R. Soc. B 281 (1796), 20141755. https://doi.org/10.1098/rspb.2014.1755.

Tridico, S.R., Rigby, P., Kirkbride, K.P., Haile, J., Bunce, M., 2014b. Megafaunal split ends: microscopical characterisation of hair structure and function in extinct woolly mammoth and woolly rhino. Quat. Sci. Rev. 83, 68–75. https://doi.org/10.1016/j.quascirev.2013.10.032.

Von Bergen, W., 1961. Wool Handbook I. Textile Book Publishers Inc, New York.

Wilson, I.G., 1997. Inhibition and facilitation of nucleic acid amplification. Appl. Environ. Microbiol. 63, 3741–3751.

Wilson, A.S., Dodson, H.I., Janaway, R.C., Pollard, A.M., Tobin, D.J., 2007. Selective biodegradation in hair shafts derived from archaeological, forensic and experimental contexts. Br. J. Dermatol. 157 (3), 450–457. https://doi.org/10.1111/j.1365-2133.2007.07973.x.

Wilson, A.S., Dodson, H.I., Janaway, R.C., Pollard, A.M., Tobin, D.J., 2010. Evaluating histological methods for assessing hair fibre degradation. Archaeometry 52 (3), 467–481. https://doi.org/10.1111/j.1475-4754.2010.00524.x.

Wilson, M.R., Budowie, B., 1993. Inhibition of Taq polymerase by melanin: possible solutions for forensic applications. 45th Annual Meeting of the American Academy of Forensic Sciences. Williams Printing, Colorado Springs, Boston, MA.