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Matti Heino

CHALLENGING DNA SAMPLES ARE VALUABLE SOURCES FOR GENETIC INFORMATION OF POPULATIONS AND INDIVIDUALS

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CHALLENGING DNA SAMPLES ARE VALUABLE SOURCES FOR GENETIC INFORMATION OF POPULATIONS AND INDIVIDUALS

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Abstract

In my PhD thesis, I have focused on studying challenging DNA samples. In common to all the sub-projects is that the target DNA is badly preserved and the contamination risk from modern samples is high. All the samples under study have been in varying environmental conditions for varying amounts of time. In my research I therefore employed ancient DNA methods that have been developed to extract and amplify poor quality DNA. In all the sub-projects, we were able to gain information that would not have been possible to obtain by studying more conventional biological samples.

In article I, we studied whether non-invasively collected placentas of the Saimaa ringed seal (*Pusa hispida saimensis*) could be utilized in individual identification and population monitoring. The umbilical cord proved to give a reliable genotype of the pup and therefore placentas can be used in genetic monitoring of the population. In article II, I investigated the geographical origin of poorly documented tiger samples from the Finnish museum of natural history. All the samples under investigation could be identified to subspecies levels, and among them I observed for example a Javan tiger (*Panthera tigris sondaica*), which is extinct. In article III, I studied the domestication history of goose (*Anser anser*) using bones collected from archaeological sites in Russia. The majority of the studied samples belonged to genetic lines that are typical for domestic goose, but I also observed lines that have not been observed among domestic geese. In article IV, I studied what kind of role reindeer (*Rangifer tarandus*) has had in the contacts of the Sámi and the Swedes in the Middle Ages, by studying DNA from samples originating from archaeological sites. The genetic results suggest that the samples under investigation are more likely to originate from wild forest reindeer than domestic reindeer. In article V, I investigated whether the reindeer population that lived in the forest region in Tatarstan 4000 years ago had gone extinct or whether there is genetic continuation from this population among modern populations. I observed genetic continuity between the historical reindeer from Tatarstan and the wild reindeer from the taiga zone of northeastern part of European Russia.

Keywords: ancient DNA, mitochondrial DNA, population history

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Tiivistelmä

Olen väitöskirjatutkimuksessani keskittynyt haastavien DNA-näytteiden tutkimiseen. Yhteistä kaikille osatöilleni on se, että tutkimuksen kohteena olevien näytteiden DNA on huonosti säilynyttä, ja kontaminaatioriski moderneista näytteistä niihin on suuri. Kaikki tutkimuksen kohteena olevat näytteet ovat olleet vaihtelevissa ympäristöolosuhteissa pidempiä tai lyhyempiä aikoja. Tutkimuksissani sovellettiinkin huonolaatuisen DNA:n käyttöön kehitettyjä muinais-DNA:n eristys- ja monistusmenetelmiä. Kaikissa osatutkimuksissa onnistuttiin hankkimaan tutkimustietoa, jota ei olisi saatu tutkimalla tavanomaisempia biologisia näytteitä.

Artikkelissa I selvitettiin luonnosta kerättyjen saimaannorpan (*Pusa hispida saimensis*) istukoiden soveltuvuutta yksilöntunnistukseen ja populaation seurantaan. Istukan napanuoran kohdalta otetun näytteen todettiin soveltuvan luotettavasti poikasen genotyypin määrittämiseen ja täten istukkanäytteet soveltuvat hyvin populaation geneettiseen seurantaan. Artikkelissa II selvitin Suomen luonnontieteellisen keskusmuseon kokoelmissa puutteellisilla löytöpaikkatiedoilla olevien tiikerinäytteiden alkuperää. Kaikki tutkimuksen kohteena olevat näytteet onnistuttiin määrittämään alalajilleen, ja joukossa todettiin olevan mm. jo sukupuuttoon kuollut jaavantiikeri (*Panthera tigris sondaica*). Artikkelissa III tutkittiin hanhen (*Anser anser*) domestikaation historiaa venäläisiltä arkeologisilta kohteilta peräisin olevista luista. Suurin osa tutkituista näytteistä kuului geneettisiin linjoihin, jotka ovat tyypillisiä kesyhanhella, mutta näytteistä löytyi myös linjoja, joita ei kesyhanhella ole tavattu. Artikkelissa IV tutkittiin peuran (*Rangifer tarandus*) roolia saamelaisten ja ruotsalaisten välisissä kontakteissa keskiajalla mm. tutkimalla DNA:ta arkeologisilta kohteilta peräisin olevista peuran luista. Geneettiset tulokset viittaavat, että tutkimuksen kohteena olevat näytteet ovat todennäköisemmin peräisin villistä metsäpeurasta kuin kesytetystä porosta. Artikkelissa V selvitettiin ovatko Tatarstanin metsäalueella 4000 vuotta sitten esiintynyt peurapopulaatio kuollut sukupuuttoon, vai löytyykö tästä populaatiosta jatkumoa jossain nykyisessä populaatiossa. Tatarstanin historiallisten peurojen ja Venäjän puolisen Koillis-Euroopan taigavyöhykkeen villien metsäpeurojen välillä havaittiin geneettistä jatkumoa.

Asiasanat: populaatiohistoria, mitokondriaalinen DNA, muinais-DNA

Dedicated to Minna Ruokonen

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29.8.2020 Matti Heino

Abbreviations

AD	*anno Domini*
aDNA	ancient DNA
BP	Before Present
bp	base pairs
cal	calibrated years
COI	cytochrome c oxidase I gene
CytB	mitochondrially encoded cytochrome B gene
FS	foetal side of a placenta
mtDNA	mitochondrial DNA
MS	maternal side of a placenta
ND2	mitochondrially encoded NADH dehydrogenase 2 gene
ND6	mitochondrially encoded NADH dehydrogenase 6 gene
Numt	nuclear mitochondrial DNA
OP	orange particles found on the maternal side of a placenta
SNP	single nucleotide polymorphism
UC	umbilical cord

Original publications

This thesis is based on the following publications, which are referred to throughout the text by their Roman numerals:

I Valtonen, M., Heino, M., Aspi, J., Buuri, H., Kokkonen, T., Kunnasranta, M., Palo, J. U. & Nyman, T. (2015). Genetic monitoring of a critically-endangered seal population based on field-collected placentas. *Annales Zoologici Fennici, 52*(1-2), 51-65. doi:10.5735/086.052.0205

II Heino, M., Granroth, J., Aspi, J., & Pihlström, H. (2019). A previously undescribed Javan tiger *Panthera tigris sondaica* specimen, and other old and rare tiger specimens in the Finnish museum of natural history. *Mammal Study, 44*(1). https://doi.org/10.3106/ms2018-0036

III Honka, J., Heino, M., Kvist, L., Askeyev, I., Shaymuratova, D., Askeyev, O. V., Askeyev, A. O., Heikkinen, M. E., Searle, J. B. & Aspi, J. (2018). Over a thousand years of evolutionary history of domestic geese from russian archaeological sites, analysed using ancient DNA. *Genes, 9*(7), 367. doi:10.3390/genes9070367

IV Salmi, A., & Heino, M. (2019). Tangled worlds: The Swedish, the Sámi, and the reindeer. *International Journal of Historical Archaeology,* 1-23. doi:10.1007/s10761-018-0465-2

V Heino, M., Askeyev, I., Shaymuratova (Galimova), D., Askeyev, O., Askeyev, A., van der Valk, T., Pečnerová, P., Dalén, L., Aspi, J. (2019). 4000-year-old reindeer mitogenomes from the Volga-Kama region reveal continuity among the forest reindeer in northeastern part of European Russia. *Arheologiâ evrazijskih stepej, 4(179-190).*

Author contributions

Article	I	II	III	IV	V
Original idea	MK	HP, JG	IA, DS, OA, AA, JA, LK, MTH, JH	AKS	IA, MTH, DS, OA, AA, JA
Sample collection	TK, MK, MV	HP, JG	IA, DS, OA, AA	AKS	IA, DS, OA, AA
Laboratory work	MTH, MV, HB	MTH, HP, JG	JH, MTH	AKS, MTH	MTH, PP
Data analyses	MV, MTH, TN	MTH, HP, JG	JH	AKS, MTH	MTH, TvdV
Manuscript preparation	MV, TN, JP, MTH, JA, MK, TK	HP, MTH, JA, JG	JH, MTH, LK, IA, DS, OA, AA, MEH, JS, JA	AKS, MTH	MTH, IA, DS, OA, AA, TvdV, PP, LD, JA

Matti T. Heino (MTH), Jouni Aspi (JA), Igor Askeyev (IA), Dilyara Shaymuratova (DS), Oleg Askeyev (OA), Arthur Askeyev (AA), Henry Pihlstöm (HP), Janne Granroth (JG), Anna-Kaisa Salmi (AKS), Mia Valtonen (MV), Johanna Honka (JH), Mervi Kunnasranta (MK), Tommi Nyman (TN), Laura Kvist (LK), Tom van der Valk (TvdV), Patrícia Pečnerová (PP), Tuomo Kokkonen (TK), Hanna Buuri (HB), Jukka Palo (JP), Marja E. Heikkinen (MEH), Jeremy Searle (JS), Love Dalén (LD)

Contents

1 Introduction

DNA studies have traditionally targeted samples that originate from modern individuals. This approach has many caveats. Studies on modern individuals provides only a very limited and possibly wrong picture of evolution. This is because certain assumptions (e.g. about the mutation rate) are needed to infer the past processes, and these assumptions often contain uncertainty. Additionally, more recent evolutionary processes may mask those that have happened earlier. Targeting DNA from historical and ancient samples therefore offers a better alternative to study the past, because by comparing long-dead individuals to modern and other ancient individuals, one can make direct observations of evolution and demographic processes. Ancient DNA has proven to be especially helpful for studies on domestication (see e.g. Gaunitz et al., 2018; Skoglund, Ersmark, Palkopoulou, & Dalén, 2015), phylogeography (Doan et al., 2017), human migration (see e.g. Haak et al., 2015; Saag et al., 2019), bioarchaeology (see e.g. Schroeder et al., 2019), past environmental communities (see e.g. Willerslev et al., 2014), disease epidemics (see e.g. Rascovan et al., 2019) and historical trade routes (see e.g. Star et al., 2017).

In addition to showing a potentially distorted picture of the past, studying samples originating from living organisms may be problematic also because acquiring the sample is often harmful for the study individuals. Especially, research on endangered species and populations should preferably use methods that avoid interfering with the study individuals. It is sometimes possible to obtain DNA samples from living individuals without harming them, utilizing so-called non-invasive samples that originate from DNA which the individual has left behind in the environment. Such samples can be, for example, hair samples from hair traps (Rovang et al., 2015), shed feathers (Horváth et al., 2005), fecal samples (Ramon-Laca et al., 2015) or snow tracks (Dalén et al., 2007). More and more genetic monitoring is done using environmental DNA samples, for example by extracting DNA from water samples or soil (Thomsen & Willerslev, 2015, Taberlet et al., 2018). Even though environmental DNA is commonly used to investigate which taxa are present or absent in the environment, in some cases even population-level information of the study species can be obtained (Sigsgaard et al., 2016).

At the beginning of my PhD, the intention was to study purely ancient DNA of reindeer and seals. Confessedly, none of the projects that were initially in my PhD plan ended up in this thesis. Some of these original projects grew considerably larger than originally planned and therefore they are still work in progress. Some

where superseded by interesting side projects that developed during the PhD. The studies on ancient Russian reindeer and geese started when Igor and Oleg Askeyev and Dilyara Shaymuratova from the Institute of Problems in Ecology and Mineral Wealth of the Tatarstan Academy of Sciences contacted us regarding opportunities for collaboration. The study on historical tiger specimens on the other hand developed after Henry Pihlstöm and Janne Granroth from the Finnish museum of natural history re-discovered poorly documented tiger specimens from their museum collections. Henry and Janne asked if we could use DNA analyses to try to gain information on the likely geographic origin of these specimens. Despite these and other changes in my original plan, the major theme in my research remained as studies on degraded DNA, and the already previously done study on the Saimaa seal placentas fitted within this theme.

1.1 Ancient DNA

Ancient DNA can be loosely described as any DNA that is extracted from biological material that has not been preserved specifically for DNA research. The term is however more commonly used regarding DNA from ancient or historical samples. The samples in question usually derive from bones, but also hair, sediments, old wood, eggshells etc. can be used as sources. Due to exposure to environmental factors such as humidity, radiation, and acidity, ancient DNA is commonly highly fragmented and affected by post-mortem changes (e.g. Thomas & Gilbert, 2006).
The research field of ancient DNA is relatively young but has provided some ground-breaking insights on evolution and population history of several species. First ancient DNA studies were conducted in mid 1980s, when Svante Pääbo reported results of a 2,400 year old mummy from Egypt (Pääbo, 1985) and Higushi and coworkers presented results from an extinct quagga (Higushi et al., 1984). The early ancient DNA studies commonly targeted mitochondrial DNA sequences. The reason for this was that there are multiple copies of mitochondria per cell, making its recovery therefore more likely than recovering nuclear sequences from old, usually highly degraded samples. Even though mitochondrial DNA remains a valuable marker documenting maternal lineages, recent advances in sequencing technology and data interpretation (Hofreiter et al., 2015) have made it easier to target nuclear DNA as well. The field of ancient DNA has especially benefited from newly developed sequencing (and statistical) methods. Especially the technique called shotgun sequencing has had a large impact on ancient DNA studies, because

the technique can utilize fragmented DNA, which is typical for ancient DNA samples.

In addition to fragmentation, ancient DNA is also characterized by postmortem changes such as cross-links between DNA strands and deamination. Cross-links may hinder amplification of ancient DNA, a step usually required in ancient DNA analysis. Deamination on the other hand causes misincorporation of bases during the amplification step. Even though these modifications and fragmentation complicate the analysis of ancient DNA, they can also be used to distinguish authentic ancient DNA from contaminating modern DNA (Skoglund et al., 2014). Contamination from modern DNA or cross-contamination from other ancient samples are common problems when working with ancient DNA. Depending on study species, modern DNA is often more prevalent in the environment and may shadow the authentic ancient DNA of a sample. This may cause modern DNA to be interpreted as ancient, and lead to wrong conclusions. In order to minimize the possibility of contamination, sample collection should preferably be done in a sterile manner. After bringing the samples to the laboratory, certain procedures should be applied, as exemplified already by Cooper and Poinar (2000). These include performing the contamination prone steps of DNA extraction in laboratory facilities that are dedicated to work on ancient DNA. These need to be separated from laboratories that handle modern DNA and/or amplified DNA samples. Even if strict measures to prevent contamination are followed throughout the sample collection and genetic data production, it is still possible that the data will suffer from contamination. The level of contamination can be estimated using statistical methods, and the sequences likely resulting from contamination can be removed before data analysis. Finally, it is important to use common sense and evaluate if the data and results make sense in the light of what is already known about the study system.

1.2 Modern DNA

As the word implies, modern DNA derives from modern or relatively recent biological material. Taken that the samples have not degraded due to unfavourable storage conditions, these samples commonly contain more high-quality DNA than ancient samples, i.e. DNA is not heavily fragmented and is more abundant and therefore easier to study. However, this is not always the case. Researchers may also have to use biological material that has been exposed to elements that are unfavourable for DNA survival. Such material may come from e.g. long-dead

carcasses (predated or naturally died animals, road-kills) or samples that have been noninvasively collected as explained above.

Samples may also include inhibitors, which may prohibit extraction and/or amplification of DNA (a step usually required for downstream genetic analyses). Inhibition is caused by substances that interfere with chemicals used in DNA amplification and may derive from the tissue in itself (for example keratin in hair) or from the environment (soil humus). Many DNA extraction protocols take into account the possible presence on inhibiting substances by incorporating chemicals and steps that remove as much as possible of these substances (e.g. Schrader et al., 2012).

1.3 Domesticated study species

Domestication of animal and plant species has been one of the greatest advancements in the human history, enabling the development of large human communities and civilizations. Domestication is the process where animal or plant population is adapting to human control. This commonly happens through artificial selection where humans choose which individuals get to reproduce based on their favourable characteristics.

Biological traits of species dictates much of the domestication process. For example, generation interval affects how quickly species responds to artificial selection. The shorter the interval, more quickly the species may respond genetically to selection. For this reason, domestication of mammal species happens usually slowly and gradually, while for example in the case of certain plant species, domestication may happen quickly. Different domestic species are in different stages of domestication. For some species, such as for reindeer, the difference between domestic and wild populations is small, while for some species, the domestic and wild populations are clearly diverged.

Domestication may lead to genetic bottlenecks, first when only a certain portion of the wild population is subjected for domestication, and second, when this population is then subjected for increasing artificial selection through time (Zeder et al., 2006). On the other hand, sometimes human selection may increase genetic variation on certain loci. For example, many domestic mammal species such as horse have more coat colour variation than their wild relatives.

Ancient DNA can be used to obtain important knowledge about domestication. It can for example be used to pinpoint geographical locations where the domestication process of certain species has started (see e.g. Røed et al., 2008),

inform about the timing of domestication (see e.g. Skoglund et al., 2015) and evolution of the domesticated species through time (see e.g. Fages et al., 2019).

1.3.1 Goose

The European domestic goose (*Anser anser*) has been domesticated from the wild greylag goose (Shi, Wang, Zeng, & Qiu, 2006; Wang et al., 2010). Its domestication process has been proposed to have followed the prey-pathway, according to which it was hunted for meat before being domesticated (Larson & Fuller, 2014). However, the domestication history of the European domestic goose is still largely unknown. Historical records indicate that geese were used already by the ancient Egyptians, Romans and Mesopotamians (Zeuner, 1963) and it has been proposed that geese were domesticated around 3000 BCE either in south-eastern Europe (Crawford, 1984) or in Egypt (Zeuner, 1963). Domesticated geese were used for certain in Egypt and in Europe around 1550–1150 BCE (Zeuner, 1963). By the 1st century BCE, the Romans had already several different breeds of geese (Albarella, 2005) and in the Medieval Period, peasants commonly kept large flocks of geese (Albarella, 2005). The European domestic goose was probably introduced into Scandinavia during the Early Iron Age (400 BCE–550 CE), as indicated by archaeological evidence (Tyrberg, 2002).

Goose domestication has previously been studied using mitochondrial DNA analysis of exclusively modern geese demonstrating that modern domestic geese were derived from a limited genetic base (Heikkinen et al., 2015, Fig. 1). However, in that study, it was not possible to interpret if the observed low diversity of modern breeds was due to domestication or if it was due to foundation of the breeds some hundreds of years ago.

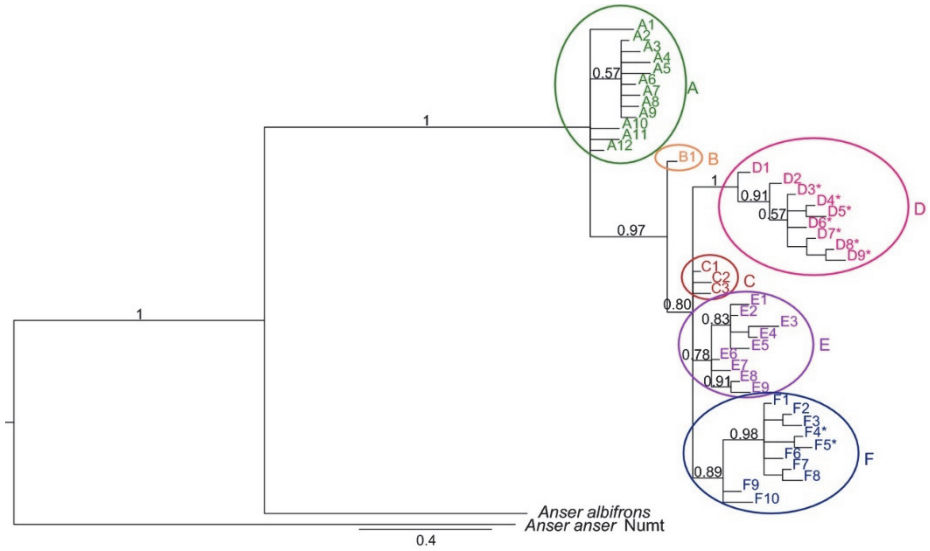

Fig. 1. Bayesian phylogeny showing the relationships of mitochondrial haplotypes of wild graylag (*Anser anser*) and European domestic goose. Asterisk indicates haplotypes that are shared between wild graylag and domestic goose. Posterior support values above 0.5 are shown. Six main haplogroups (A-F) are indicated. Outgroups: *Anser anser* Numt = graylag nuclear mitochondrial DNA insert, *Anser albifrons* = greater white-fronted goose. (Reprinted, with permission, from Heikkinen et al. 2015 ©John Wiley and Sons)

1.3.2 On reindeer domestication

Domestic reindeer differs from many other domesticated animal species in that the human control over the domestic herds is more relaxed than with many other domestic species, such as for example horses, pigs and chickens, which are kept in strong control over the whole lifespan of the individual. Domestic reindeer are therefore commonly referred as semi-domestic. Additionally, the semi-domestic reindeer herds commonly live in close proximity with wild herds leading to intermixing between the wild and semi-domestic herds. In Fennoscandia, semi-domestic reindeer populations have been mixing with wild Norwegian mountain reindeer populations, which are today restricted to south-central Scandinavian mountains, and with wild Finnish forest reindeer population in eastern Finland.

Not much is known about the early history of reindeer domestication. Some scholars have suggested that some form of reindeer herd control by humans has

already taken place during the Late Pleistocene (e.g. Patte, 1958). Other researchers have however refuted some of these views (e.g. Weinstock, 2000). The earliest definite descriptions of the use of semi-domestic reindeer come from written records from China from 499 AD in which a monk named Huei Shen described a people living possibly around the area of Baikal who, for example, milked reindeer and used them for pulling sledges (Laufer, 1917). The first written documents describing domestic reindeer in Fennoscandia come from an account of Norwegian chieftain Ohthere of Hålogaland (Ottar) who travelled to England and met King Alfred the Great in 890 AD. Ohthere said that he owned 600 reindeer of which six were used as decoys to lure and catch wild reindeer.

Røed et al. (2008) have shown that the reindeer has likely been domesticated at least twice by the people living in different regions of Eurasia, and that some wild populations, such as the Finnish forest reindeer, have not contributed much if any ancestry to the domestic herds. The mitochondrial lineages that at present are the dominating lineages among the Fennoscandian domestic reindeer appeared in southern (Røed, Flagstad, Bjørnstad, & Hufthammer, 2011; Røed et al., 2014) and northern Norway (Bjørnstad, Flagstad, Hufthammer, & Røed, 2012; Røed, Bjørklund, & Olsen, 2018) only about 500 years ago. This timing coincides with a change in reindeer pastoralism from people having small herds towards keeping larger herds. The origin of the two main Fennoscandian semi-domestic lineages remain elusive, but recently an origin east of Fennoscandia was tentatively suggested (Røed, Bjørklund, & Olsen, 2018).

1.4 On the tiger subspecies

Tigers have traditionally been divided into eight Recent subspecies: the Bengal or Indian tiger *P. t. tigris*, the Caspian tiger *P. t. virgata*, the Amur or Siberian tiger *P. t. altaica*, the South China tiger *P. t. amoyensis*, the Indochinese tiger *P. t. corbetti*, the Sumatran tiger *P. t. sumatrae*, the Javan tiger *P. t. sondaica*, and the Balinese tiger *P. t. balica* (Mazák, 1981; Mazák, 2013). Additionally, Luo et al. (2004, 2008, 2010) have recognized a ninth subspecies, the Malayan tiger *P. t. jacksoni*. Tiger taxonomy is however controversial subject, and other authors have suggested that only two or perhaps three subspecies should be recognized (Kitchener, 1999; Kitchener & Yamaguchi, 2010; Kitchener et al., 2017; Kitchener & Dugmore, 2000; Wentzel et al., 1999; Wilting et al., 2015).

Of the traditionally recognized subspecies, many have become extinct during the last 100 years. The Balinese tiger went extinct during the 1930's (Seidensticker,

1987). The Caspian and Javan tigers likely went extinct in the 1970's (Can, 2004; Seidensticker & Suyono, 1980; Seidensticker, 1987. The South China tiger went extinct in the wild in the early 1990's (Tilson, Defu, Muntifering, & Nyhus, 2004), but persists in captivity. The wild populations of the other remaining tiger subspecies are also threatened by extinction. Amur, Bengal, and Sumatran tigers are however relatively numerous in captivity (Luo et al., 2008).

1.5 Saimaa ringed seal

Lake Saimaa, which is the largest lake complex in Finland, harbours a ringed seal subspecies (*Pusa hispida saimensis*) (Fig. 2), which is thought to be derived from the Baltic Sea ringed seal (*Pusa hispida botnica*). The subspecies has been thought to have been landlocked in Saimaa since the postglacial land uplift separated part of the original population into the lake about 9000 years ago. Both nuclear and mitochondrial DNA of modern Saimaa ringed seal has been previously studied (Nyman et al., 2014; Valtonen et al., 2012). Based on these studies, Saimaa ringed seal in not however genetically particularly close to the modern Baltic ringed seal, suggesting that the histories of the ringed seal populations in the Baltic region may be more complex than generally thought.

Fig. 2. Saimaa ringed seal. Photo: Mia Valtonen.

The subspecies experienced a dramatic decline in population size from a possible 1000 individuals (Kokko et al., 1999) at the beginning of the 20th century due to hunting and fishing, and was on the verge of extinction in 1980s, with only about 120 individuals left. Since then, the population size has increased, with an estimate of 380-400 individuals in 2019 (http://www.metsa.fi/saimaannorppa/hyljekanta2019). Like other ringed seal subspecies, Saimaa ringed seal prefers giving birth in snow lairs that they dig from under the ice (Sipilä, 2003). Because the Saimaa ringed seal does not eat its placenta, after the breeding season, placentas can usually be located from the bottom of the lake from close proximity of the lair.

2 Genetic markers used in the study

Mitochondrial DNA was used as a genetic marker in all of the articles. Mitochondria are cell organelles, which produce most of the energy required by cells using aerobic respiration. Mitochondria derive from phagocytosised bacteria, which were absorbed early in the evolution of eukaryotes (Martin & Mentel, 2010). Mitochondria are usually transmitted maternally, (but see Kondo et al., 1990; Kvist, Martens, Nazarenko, & Orell, 2003; Luo et al., 2018; Skibinski, Gallagher, & Beynon, 1994), so their sequences are used to track the maternal history of individuals. Mitochondria have their own small circular genome that encodes components of the respiratory chain complexes (Fig. 3.) In animals, the size of the mitochondrial genome is around 16 kilo bases. Sequences of the mitochondria are commonly used in evolutionary studies to infer relationships of populations and individuals. Animal mitochondrial genomes commonly consists of 37 genes and other regions, including highly variable non-coding control region, which plays a role in replication and transcription of the mitochondrial DNA (Boore, 1999). Because of its high mutation rate, the control region is especially useful in studying relationships of populations within a species. The high mutation rate can also sometimes be problematic due to homoplasy where different lineages mutate to same state irrespective of their evolutionary relationship. Therefore, sequencing more slowly evolving regions/genes may sometimes be more desirable. Mitochondrial DNA is especially useful in aDNA research because it is found in up to thousands copies per cell, making it more likely for the mitochondrial DNA to survive over time compared to nuclear sequences, which are only found in two copies per each cell in diploid organisms.

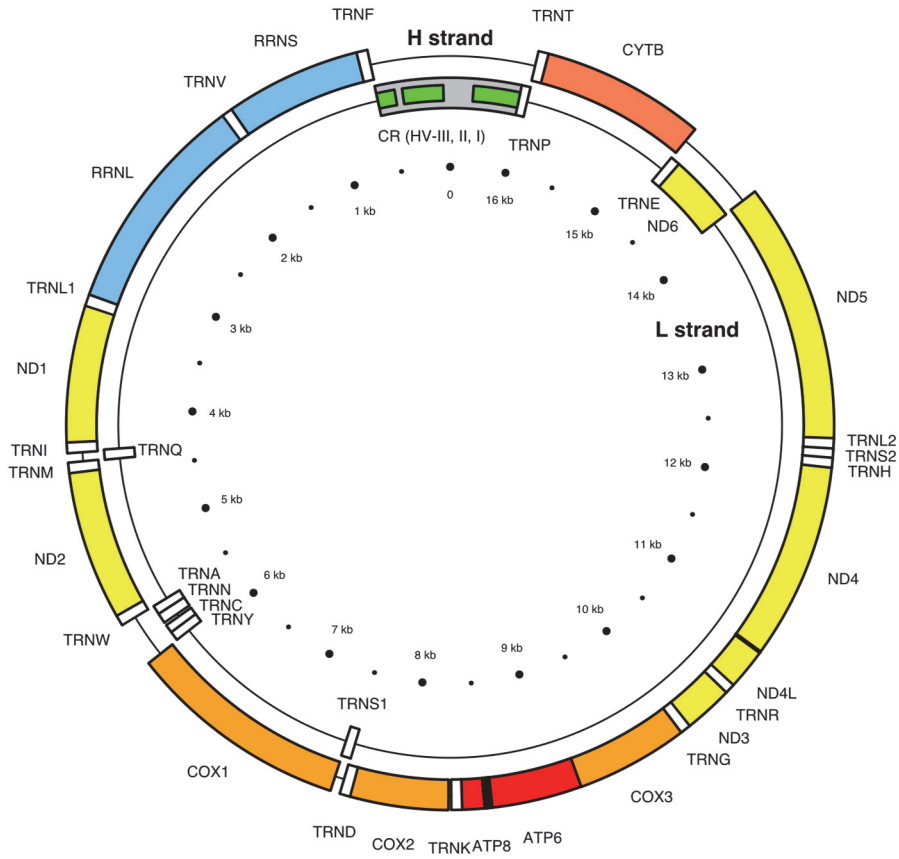

Fig. 3. Schematic picture of the human mitochondrial genome. Genes encoded on the heavy (H) and light (L) strand are shown. Species studied in this thesis (reindeer, tiger, ringed seal and domestic goose) have the same genes in the same order as human, with the exception that goose has ND6 and CytB genes in the opposite order. Lengths of the mitochondrial genomes of the studied species are: reindeer; 16362 bp, tiger; 16990 bp, ringed seal; 16754 bp, goose; 16738 bp. Picture: Emmanuel Douzery, CC BY-SA 4.0.

Because mtDNA alone has a very limited power to distinguish individuals from each other, in subproject I, we also studied nuclear microsatellite loci. Microsatellites are regions of repetitive DNA that are abundant in eukaryote genomes. A microsatellite may consist of tandem repeats of one to six nucleotides. The number of repeats in each loci is highly variable between individuals, which is why microsatellites are used in individual identification and forensics. The number

of repeats is commonly considered to evolve neutrally through insertions and deletions of single repeats at a time (Ellegren, 2004).

Mitochondria comprises only a part of the genetic material of an individual. Furthermore, as mitochondria is transmitted maternally, it can be used to study the maternal history of the study species. Therefore, results from studies that focus exclusively on mitochondria may give somewhat limited picture on the evolution and population history of the study species. Targeting nuclear loci would however have been difficult and out of the scope in many of the subprojects due to following reasons: 1) When these studies were conducted, genome-wide data was only available for one the study species, the tiger. Therefore, in order to make comparisons to modern populations, we would also have needed to generate data from modern samples. 2) There would have been available microsatellite data from modern reindeer populations (Røed et al., 2008). However, DNA in ancient samples is usually too degraded for microsatellite studies. 3) Therefore, the best option to generate nuclear data would have been to shotgun sequence the samples. In shotgun sequencing, sequence data is generated randomly across the genome. 4) Generating adequate amount of shotgun sequencing data would have been more costly.

2.1 Aims of the study

The aim in my research was to use challenging and unconventional samples to study the population history of the study species. The samples in question were either ancient (or historical), or non-invasively collected modern samples.

In subproject I, the utility of non-invasively collected Saimaa ringed seal placentas for genetic identification of individuals and measures of population level genetic parameters was evaluated. Because the Saimaa ringed seal is highly endangered and the genetic monitoring of the subspecies has so far relied on randomly found dead individuals, obtaining useful genetic data from placentas would be highly beneficial. Placentas are more numerous and more easily obtainable from the environment than any other types of tissue. Thus, the resolution of the monitoring would increase if they provided useful information. Because a placenta is composed of tissues that derive from both the mother and the pup (which would increase their utility), I attempted to genotype both the mother and the pup by sampling tissue from different spots on the placentas.

Natural history collections offer an enormous opportunity to study present and past biological diversity. Unfortunately, especially in the past, collected samples were often not carefully curated in the sense that their origin and other contextual

information was not included with the sample or may have been lost in time. The situation may have been exacerbated if the collections have been moved to different facilities, during which some records may have been lost. In article II I attempted to identify the subspecies and geographical origin of some tiger samples located at the Finnish Natural history museum for which no curated information was available. The aim therefore was to increase the scientific value of this tiger collection and present it to the wider public.

In article III we aimed to shed further light on the domestication of the goose by DNA sequencing goose remains from Russian archaeological sites. Special interest was to investigate if domestic goose haplotypes were present in the archaeological material, and to determine whether there had happened changes in the genetic diversity through time.

In article IV, my goal was to understand what kind of role reindeer had in the encounters of the Sámi and the Swedes during the Middle Ages and early modern times in northern Fennoscandia. Historically reindeer may have played an important role on these northern areas by comprising products for trading in addition to its use in transport. We utilized ancient DNA, stable isotopes and zooarchaeology on reindeer remains that originated from marketplaces, towns and agrarian settlements. Stable isotopes were used to study if the reindeer had been fed by humans, and aDNA analysis were used to establish whether the reindeer in question had been wild or domestic.

In article V I studied how 4000-year-old reindeer samples from the forest region of Tatarstan are genetically related to modern populations. As reindeer has gone extinct from Tatarstan, it was of interest to study if closely related lineages still prevail in some other regions.

3 Material and methods

3.1 Samples

3.1.1 Saimaa ringed seal placental samples

The studied placentas were collected by my collaborators. Most were collected from nest sites after the ice had melted in May (Auttila et al., 2014, Figs. 4, 5, 6), but some were collected already during the annual seal census counts. Population size of the Saimaa ringed seal is estimated annually by counting the number of found nests. Because the Saimaa ringed seal builds its nest in a snow pile close to the shore, after the ice has melted, placentas are commonly visible from the water surface and can be collected for example by using a long stick (Fig. 4). Between 2009 and 2011 a total of 59 placentas were collected, which is a considerable sample size taken the small population size on the Saimaa ringed seal. Most of the placentas were at least partly decomposed, evaluated by eye. Tissue samples were obtained from four different parts of each intact placenta: maternal (i.e., uterine) side (MS); foetal (i.e., membrane) side (FS); umbilical cord, or in absence of it, a vein (UC); and orange particles (OP), which are bilirubine-containing particles found on the maternal side of the placenta (van den Broeck, 1904) (Fig. 6). Additionally, blood samples were obtained from four placentas that looked exceptionally fresh. The samples were randomized and subjected to DNA extraction.

Fig. 4. Mia Valtonen picking up a Saimaa ringed seal placenta. Photo: Juha Taskinen.

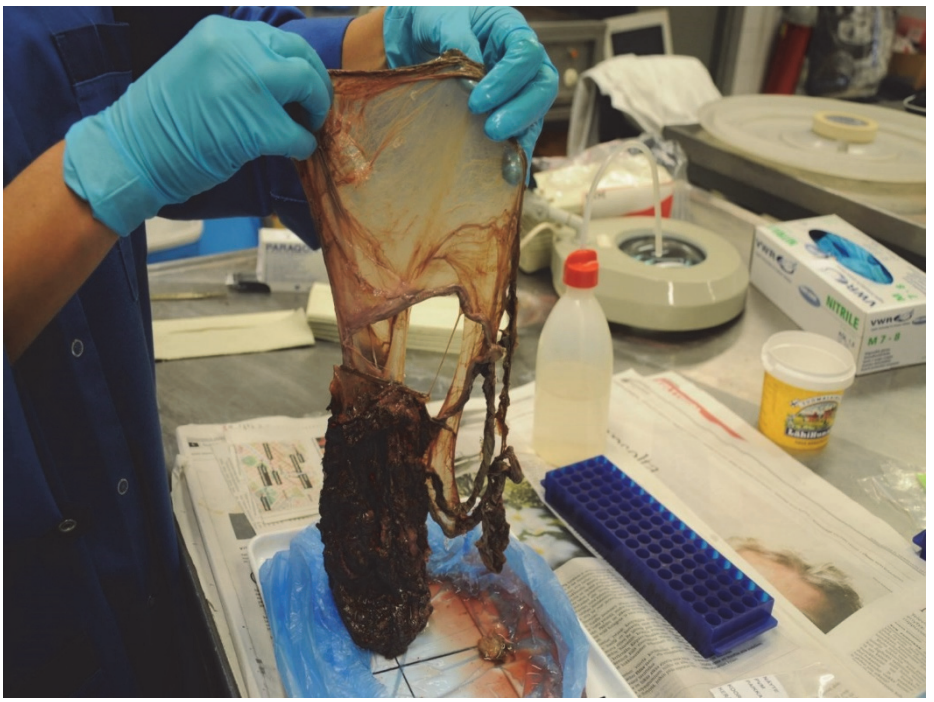

Fig. 5. Saimaa ringed seal placenta. Photo: Mia Valtonen.

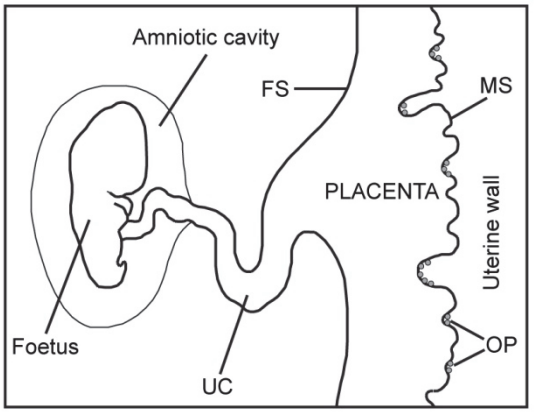

Fig. 6. Structure of the placenta, and different sampling spots used in this study: MS = maternal side, FS = foetal side, UC = umbilical cord, OP = orange particles. (Reprinted,

with permission, from Valtonen et al. 2015 ©Finnish Zoological and Botanical Publishing Board 2015)

Additional tissue samples were obtained from five dead pups, of which the natal site, and therefore the corresponding placenta were known. These included three that were found as stillborn and two that had accidentally been caught and died in fishing nets. The genotypes obtained from these pups could be compared to the genotypes that were obtained from different sampling spots of their placentas, and thus used to pinpoint the sampling spot on placenta that gives the most reliable pup genotype.

3.1.2 Tiger samples

Recently, during inventory of the collections of the Finnish Museum of Natural History, seven poorly documented tiger *(Panthera tigris*) samples were re-discovered. The samples originate from the 19th and 20th centuries and have very little background information about the subspecies or geographical origin. This is because during that time, specimens of taxa that were not native to Finland were commonly purchased from traders or obtained from foreign scientists. In order to gain information on the likely sub-species status and geographical origin of these samples, and therefore to increase the scientific value of these specimens, two skeletons and five pelts were sampled for teeth, bone, skin, hair or footpads (Fig. 7).

Fig. 7. Skull of UN 2485, one of the studied tiger specimens. Photo: Janne Granroth.

3.1.3 Archaeological geese samples

We sampled a total of 67 goose bones from Russian archaeological sites spanning the time between the 4th and 18th centuries. The bones were classified as belonging to domestic geese based on morphology. The study sites are located west from the Ural mountains in Tatarstan, Saratov, Chivash, Nizhny Novgorod, Leningrad and Pskov regions (Fig. 8). Most of the samples originate from Tatarstan (N = 51).

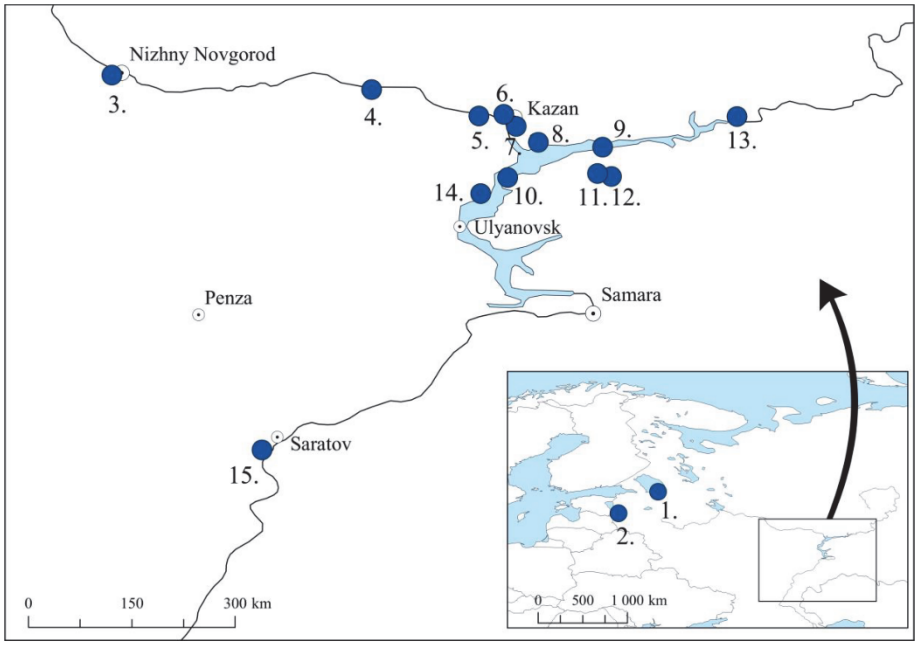

Fig. 8. Archaeological sites for the studied domestic geese from 4th–18th century: 1. Staraya Ladoga (Leningrad Region), 2. Pskov city (Pskov Region), 3. Nizhny Novgorod Kremlin (Nizhny Novgorod Region), 4. Chebosakry city (Chuvash Republic), 5. Sviyazhsk (Tatarstan Republic), 6. Kazan Kremlin (Tatarstan Republic), 7. Kazan State University, Kazan city (Tatarstan Republic), 8. Imenkov hillfort (Tatarstan Republic), 9. Ostolopovskoe settlement (Tatarstan Republic), 10. Bulgar (Tatarstan Republic), 11. Toretskoe settlement (Tatarstan Republic), 12. Bilyarsk (Tatarstan Republic), 13. Elabuga hillfort (Tatarstan Republic), 14. Tetyushkoe II hillfort (Tatarstan Republic), and 15. Bagaevskoe settlement (Saratov Region). (Reprinted from Honka et al., 2018, CC BY 4.0)

3.1.4 Archaeological reindeer samples from Finland

The samples for DNA analysis of reindeer originated from two archaeological sites, Oravaisensaari (N =2), and Ylikylä (N = 2), located in the southern part of Finnish Lapland (Article IV, p. 264, Fig. 1). Samples have been directly radiocarbon dated to between 1401-1797 calibrated years (cal) AD. It was not known whether the bones presented wild or domestic reindeer.

3.1.5 Ancient reindeer samples from Tatarstan

We subjected six reindeer samples for DNA analysis from the archaeological site Pestrechinskaya II. The site is located in present day Tatarstan and dated to around 4000 cal BP.

3.2 Laboratory methods

Mitochondrial DNA was targeted in all sub-projects. In article I, we studied also nuclear microsatellite markers. The targeted regions of each specific work are listed in table 1. Because it was expected that the DNA in the samples would be highly fragmented, we amplified short DNA fragments between 100-150 base pairs in each article II-V.

Table 1. Overview of taxons and targeted regions studied in each article.

Taxon	Markers	Article
Saimaa ringed seal	11 autosomal microsatelllite loci, mtDNA control region	I
Tiger	mtDNA regions ND2, COI, ND6, and CytB	II
Goose	mtDNA control region	III
Reindeer	mtDNA control region	IV
Reindeer	Whole mitochondrial genome	V

DNA work on historical and ancient bone samples, prior to amplification, was performed in ancient DNA laboratories either at the Centre for Material Analysis, University of Oulu, Finland or at the Swedish Museum of Natural History, Stockholm, Sweden. Procedures that minimize contamination and maximize the possibility of obtaining genetic data from the samples were followed (Fig. 9). First, the outer layer of the sampling location on each bone was polished off to get rid of as much of surface contaminants as possible. Then approximately 50 mg of bone powder was obtained by drilling inside the bone to provide bone powder from which the DNA was then extracted, using a modified version of the silica-column protocol first described by Yang, Eng, Waye, Dudar, & Saunders (1998) and later modified by Gamba et al. (2014; 2016) or alternatively a protocol outlined in Ersmark et al. (2015) which is a modified version of the protocol C in Yang et al. (1998).

Fig. 9. Working in the aDNA laboratory at the Centre for Material Analysis, University of Oulu. Photo: Pekka Moilanen.

Preparation of amplification mixtures for articles II, III, IV and V was also performed in the clean room to prevent contamination. All other steps of the protocols after that were performed in a regular molecular genetic lab. For article I, all laboratory work was performed at the molecular biology laboratory of the Ecology and Genetics Research Unit of the University of Oulu using standard protocols. The targeted DNA regions were amplified by PCR to provide enough template for subsequent sequencing reactions and microsatellite fragment length detection. PCR profiles for the amplifications can be found from the original papers (sub-projects I-V). Each reaction was replicated at least once to identify possible post-mortem changes and other sequencing artefacts.

The success of each amplification reaction was assessed using agarose gel electrophoresis, after which the successful reactions were cleaned using Exonuclease I - Alkaline Phosphatase-method, and sequenced using the BigDye® Terminator v1.1 Cycle Sequencing Kit (Thermo Fisher Scientific). The sequencing reactions were run on 3130xl Genetic Analyzer (Applied Biosystems).

3.2.1 Specific laboratory methods used in article V

DNA extracts were turned into Illumina sequencing libraries. The specific protocol can be found from the subproject V. Libraries were then sequenced together with other ancient reindeer and moose libraries on one Illumina MiSeq and HiSeq lane.

3.3 Data analyses

3.3.1 Initial processing of raw data

In article I, the chromatograms from the fragment length analysis were turned into genotypes using GENEMAPPER ver. 4.0 (Applied Biosystems). In articles II, III, IV and V, the mtDNA reads were inspected, edited and assembled into consensus sequences using CodonCode Aligner (Version 4.0.4, CodonCode Corporation).

3.3.2 Data validation

MICRO-CHECKER ver. 2.2.3 (Van Oosterhout et al., 2004), FreeNA (Chapuis & Estoup, 2007) and MICRODROP ver. 1.01 (Wang et al., 2012) were used to identify possible genotyping errors (i.e., stuttering, allelic dropout and null alleles) in the microsatellite data in article I.

3.3.3 Reference data

Reference data were incorporated in the analyses in all of the subproject. In subproject I, Saimaa ringed seal mtDNA data from Valtonen et al. (2012) and microsatellite data from Valtonen et al. (2014) were used. In subproject II, concerning the genetic identification of tiger specimens, I compared the genotypes of the study individuals with the genotypes obtained from Buddhakosai et al. (2016), Driscoll et al. (2009), Kitpipit, Tobe, & Linacre (2012), Luo et al. (2004), Sun et al. (2015), Wilting et al. (2015) and Xue et al. (2015). In subproject III where we studied ancient goose samples from Russia, data from Heikkinen et al. (2015), Honka et al. (2017), Lomakina et al. (2015), Ruokonen et al. (2000), Ruokonen et al. (2008) and Wang et al. (2010) were used. In subproject IV on the archaeological reindeer from Finland, I used data from Bjørnstad & Røed (2010), Bjørnstad et al. (2012), Røed et al. (2011) and Røed et al. (2008). Finally, in subproject V where I studied the genetic relatedness of ancient reindeer from Tatarstan to modern

populations, data from Røed et al. (2008), Kholodova et al. (2011), Baranova et al. (2012), Kvie et al. (2016a and 2016b), Korolev et al. (2017) and Ju et al. (2016) was used.

3.3.4 Genetic diversity, structure and isolation-by-distance

For the microsatellite data in article I, ARLEQUIN ver. 3.5.1.2 (Excoffier & Lischer, 2010) was used to calculate genetic diversity indices, GENEPOP ver. 4.1.3 (Rousset, 2008) was used to test departures from Hardy-Weinberg equilibrium, and SPAGEDI ver. 1.3 (Hardy & Vekemans, 2009) was used to test for the presence of isolation by distance.

Genetic diversity indices for the mtDNA sequence data in article I were calculated using ARLEQUIN and in article III using DnaSP v.5 (Librado & Rozas, 2009). ARLEQUIN was further used to calculate analysis of molecular variance (AMOVA) (Excoffier et al., 1992) and genetic distances between temporal groups in article III.

3.3.5 Phylogenetic analyses

MrBayes (Version 3.2, Ronquist et al., 2012) was used to build a Bayesian phylogenetic tree in article II and VI. TempNet (Prost & Anderson, 2011) was used to draw a temporal statistical parsimony network in article III, and PopART (Version 1.7, http://popart.otago.ac.nz) was used to build a Median-Joining network in articles III, IV and V.

3.3.6 Individual identification and kinship analyses

In article I, GENALEX ver. 6.41 (Peakall & Smouse, 2006, 2012) was used to calculate the probability if identity (PI) and the probability of exclusion (PE). PI is a likelihood that two randomly chosen individuals have the same genotype across all studied loci. In other words, it measures how reliably individuals can be differentiated from other individuals. PE measures how reliably any given individual can be ruled out as a parent of any other individual. It therefore estimates the power and utility of the microsatellite data in parentage analysis. In this article, COLONY ver. 2.0.4.1 (Jones & Wang, 2010) was further used to investigate the adequacy of microsatellite panels for inferring sibship and parentage.

Sequences of ancient reindeer from Tatarstan obtained in article V were BLAST searched against the GenBank database to infer the closest species matches.

3.3.7 Other statistical methods used in article I

χ^2-test for homogeneity was used to test whether the four main placental sampling spots differed with respect to overall genotyping success. ANOVA in SPSS Statistics 19 (IBM) was used to test the effect of the quality of the placenta on amplification success of UC samples, which were observed to yield the pups' genotypes. To see if placentas can be used to get population-level genetic parameters, the diversity and differentiation estimates obtained from the placentas were compared with the reference data. This was done by calculating an exact G-test in GENEPOP to test differences in allele frequencies between placentas and the reference data, and by calculating the Spearman's rankorder correlation in SPSS to test correlation between mtDNA haplotype frequencies in the two datasets.

4 Results and discussion

4.1 Saimaa ringed seal placentas as tools for population monitoring

It was clear from the microsatellite chromatograms that many samples contained DNA from more than one individual: More than two alleles at one locus, indicating a mixture of the mother's and pup's DNA, were detected most often in MS (maternal side) samples and least frequently in UC samples (34.6% and 3.6%, respectively). This was expected because placentas are chimeras containing tissue from both the mother and the pup. This however complicated the handling, analysis and interpretation of the results. For forensic researchers it is common to obtain DNA samples from crime scene that are mixtures of more than one individual. Usually however, the investigators have knowledge about the genotypes of victim/s and suspect/s of the crime to which the results from the DNA mixtures can be compared to. Using this analogy for the study on the Saimaa seal placentas, we didn't have either knowledge about the "victims" nor the "suspects" genotype before we obtained genotypes directly from some of the pups (from which we also had placentas) and could compare these genotypes with those obtained from different spots on the placenta. When comparing the genotypes of the five reference pups to those of their corresponding placentas, UC samples were the only ones that produced completely matching multi-locus genotypes. After this discovery, genotypes from the UC samples were taken to represent the genotypes of the pups, and all the following analysis on genetic diversity, individual identification relatedness etc. used these genotypes.

Microsatellite diversity was very low but observed (HO) and expected (HE) heterozygosities corresponded closely with estimates obtained previously using conventional samples. MtDNA haplotype frequencies obtained from placentas correlated strongly with haplotype frequencies of reference dataset. Due to low genetic variability in the studied markers, reliable estimates for relatedness of individuals were not obtained.

I was not able to reliably solve genotypes of the mothers. I tried to assess which spot on the placenta had most genetic differences compared to the genotype obtained from the umbilical cord of the same placenta. As the umbilical cord gave the pups genotype, reasoning was that the genotype most dissimilar to the pup's genotype would be closest to mother's genotype. This exercise however did not

provide consistent results and is not reported in the published article. Further insights could possibly be attained, if one would obtain tissue samples from some of the mothers and compare the genotypes to those obtained from the placentas of the same individuals.

Overall, when using the genotypes obtained from the umbilical cord, the work showed that the Saimaa ringed seal placentas collected at nest sites are useful samples for population monitoring and can be used to identify individuals. This is important because the placentas that remain after nesting are so called non-invasive samples and collecting them does not harm the individuals. Furthermore, genetic research so far on this elusive species has used samples obtained mainly from stillborn, by-caught or stranded seals. These samples present individuals which no longer belong to the population, whereas placental samples present individuals that still remain and possibly breed in the population. This is a clear advantage regarding population monitoring. Additionally, placentas are more abundantly available than individuals found dead, increasing the number of available samples and therefore the resolution of population monitoring. Because other seal species also leave their placentas after giving birth, this method could be extended also for these species.

Genotyping more nuclear loci than was done in this study could possibly result in data that could be used to obtain reliable estimates of relatedness between samples. This could be done either by shotgun sequencing the samples or typing them with a SNP panel. The upcoming *de-novo* genome of the Saimaa ringed seal will facilitate both of these approaches (https://www.saimaaringedseal.org/index.html). Furthermore, studying tissue samples from some of the mother's as an addition to their placenta's might help to figure out to extract the mother's genotype from the placenta.

4.2 Origin and subspecies status of the tiger samples in the Finnish Museum of Natural History

Five of the tiger samples could be identified by subspecies with reasonable confidence (Table 2). The genotyping results strongly suggested that the suspected Javan tiger UN 2485 is indeed an actual a Javan tiger (Fig. 10). Sample UN 365, which according to documentation originates from "India ost" (i.e., the East Indies, meaning present-day Southeast Asia), had a Sunda Island specific haplotype. It was however not possible to discern from which of the specific islands this individual originates from, because it had a haplotype that has been observed on all of the

main Sunda Islands. One would need to identify more SNPs that differentiate between tigers originating from these islands to better assess from where UN 365 originates.

Table 2. Inferred subspecies of the study samples.

Study sample	Inferred subspecies of the study sample
UN 2166	South China tiger
UN 365	Sumatran / Javan / Balinese tiger
UN 378	Bengal tiger
UN 2137	Amur tiger
UN 2485	Javan tiger
UN 2390	South China tiger
UN 2484	Malayan tiger

Specimen UN 378, and an individual without locality data but originating from the nineteenth century, had a haplotype which is specific to the Bengal tiger, therefore strongly suggesting that this individual is a Bengal tiger. The haplotypes of the two tigers known to originate from China were consistent with their locality: UN 2137 was identified as an Amur tiger, and UN 2166 as a South China tiger. The haplotype of the specimen UN 2390 was unique, but closest to one South China tiger haplotype. This individual therefore might represent South China tiger genetic diversity which has disappeared from the current population, or which previous studies had not captured. Sample UN 2484 also had a haplotype that so far has only been observed among Malayan tiger. UN 2484 is therefore most probably a Malayan tiger.

Overall, the research revealed that the samples harbor a surprisingly high subspecies diversity and include rare specimens such as the two Sunda Island tigers, of which one could be further identified as an extinct Javan tiger. This newly obtained data significantly increased the scientific value of the tiger collection of the Finnish Museum of Natural History and presented to the wider scientific community previously unknown rare tiger specimens.

Fig. 10. Specimen UN 365 that was determined to have originated from the Sunda Islands. The resolution of the genetic analysis was not enough to determine whether the individual is Sumatran, Javan or Balinese tiger, but the stripe pattern is *sondaica*-like suggesting that the individual may be a Javan tiger. Photo: Henry Pihlström. (Reprinted, with permission, from Heino et al., 2019 ©The Mammal Society of Japan)

4.3 History of domestic goose in Russia

46 out of the 67 studied samples produced reliable results. Samples belonged to eight haplotypes, of which three have not been observed before. The haplotypes fell into three different lineages: haplogroup D, haplogroup F, and the lineage constituting the taiga bean goose (*Anser fabalis fabalis*). The majority of known domestic goose haplotypes belong to haplogroup D and the rest to the F-haplogroup together with wild graylag goose haplotypes. The temporal statistical parsimony network together with the haplotype assignments of the studied samples is shown in Fig 11.

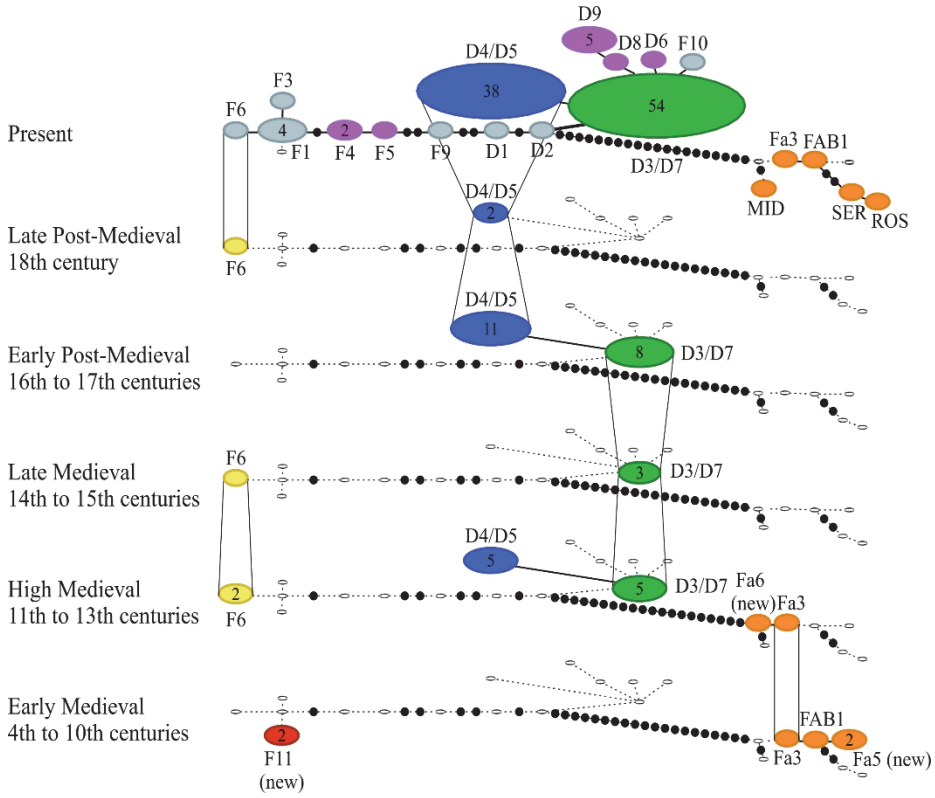

Fig. 11. Temporal statistical parsimony network of the historical goose samples from Russia and modern greylag goose (*Anser anser*) haplogroups D and F. Domestic haplotypes D3 and D7 are in green, D4–D5 in blue, and D6, D8–D9, and F4–F5 in purple. Wild greylag goose is in grey. Selected modern bean goose (*Anser fabalis*) haplotypes are in orange. MID, SER, and ROS indicate subspecies *Anser fabalis middendorffii*, *Anser fabalis serrirostris*, and *Anser fabalis rossicus*, respectively. FAB and Fa indicate *Answer fabalis fabalis*. Sizes of the ellipses are proportional to haplotype frequencies. Numbers inside ellipses indicate the number of samples having the haplotype (for haplotypes with more than one sample). White ellipses indicate missing haplotypes and black dots mutations. (Reprinted from Honka et al., 2018, CC BY 4.0)

The network shows that the typical domestic goose haplogroup D appears in the material in the studied sites between the 11th to 13th centuries and it's presence continues to the present day. Interestingly, haplotypes typical for the taiga bean goose were observed in the oldest studied material. It remains unclear whether these individuals present misidentified wild taiga bean geese or possibly domesticated taiga bean geese.

According to the nucleotide and haplotype diversity estimates, genetic diversity was highest during the High and Late Medieval Periods. This pattern however needs to be taken with caution, as the sample sizes varied between time periods.

4.4 Archaeological reindeer of northern Finland

The archaeological reindeer samples dated between 1400-1800 cal AD from northern Finland were not closely related to present-day domestic reindeer from Fennoscandia nor to the domestic reindeer from northern Fennoscandia originating from the twentieth century (Fig. 12). Instead, they were most closely related to present-day Finnish forest reindeer and ancient reindeer from Finnmark, mostly from the time period between ca. 3400 and 500 BCE. Based on size of the reindeer remains, on vegetation history and on genetic factors, Bjørnstad et al. (2012) have argued that the reindeer from Finnmark from the time period between ca. 3400 and 500 BCE may have been forest reindeer. Based on the above, it's likely that individuals studied in this work have been forest reindeer. This interpretation is in line also with what is known on the historical distribution of forest reindeer in Finland (Luukko 1954:111; Virrankoski 1973:271–272; Lundmark 1982:161). Because forest reindeer have not been shown to have contributed significant genetic ancestry to the present-day domestic reindeer in Fennoscandia (Røed et al., 2008), it is most parsimonious to conclude that the study samples most likely originated from wild individuals.

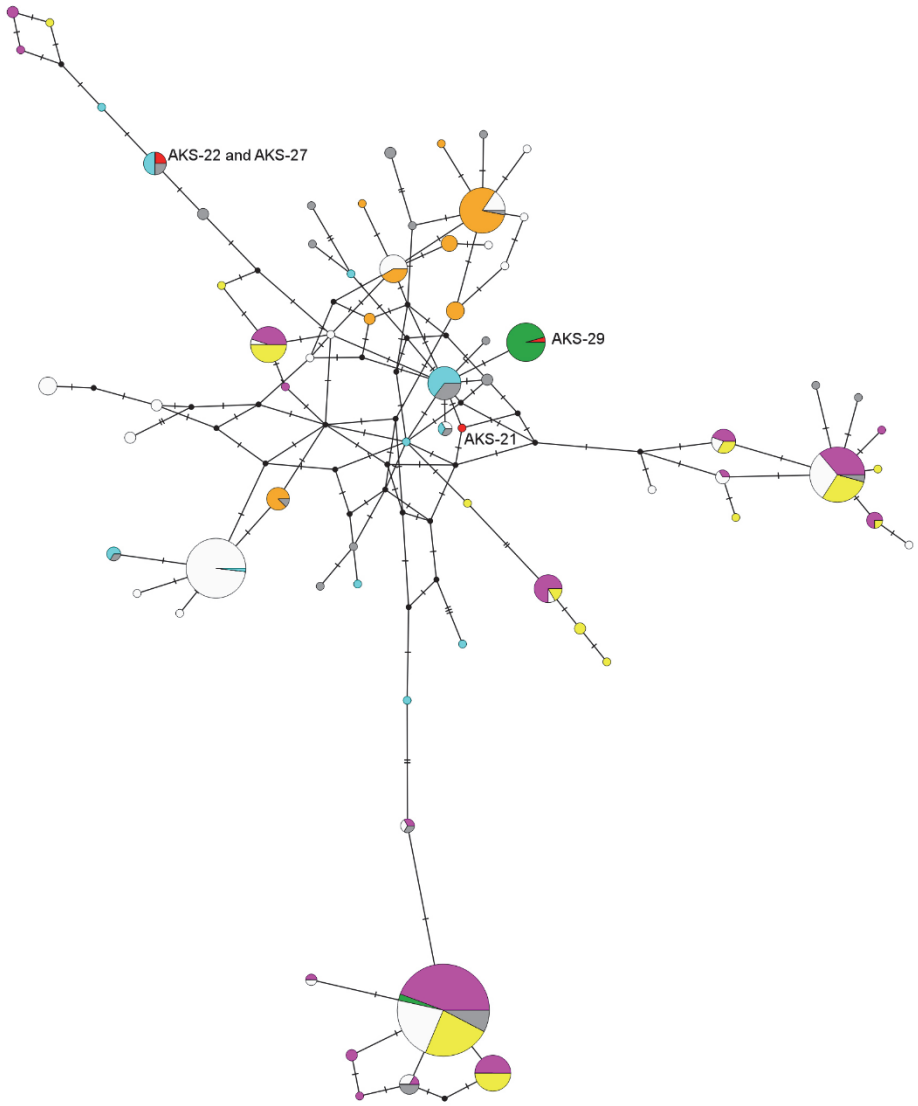

Fig. 12. Haplotype network of the studied samples and historical and modern reference data. Study samples are in red, modern domestic reindeer from Fennoscandia in pink, domestic reindeer from the early twentieth century from northern Fennoscandia in yellow, modern wild Norwegian mountain reindeer in white, modern Finnish forest reindeer in green, reindeer from Hardangervidda from the time period between ca. 1210–1310 AD in orange, reindeer from Finnmark from the time period between ca. 3400–500 BC in turquoise, and reindeer from Finnmark from the time period between ca. 100–1750

AD in grey. Sizes of the circles correspond to observed haplotype frequencies. Hatch marks indicate mutations, and black circles indicate median vectors. (Reprinted, with permission, from Salmi & Heino 2019 ©Springer Nature)

Unfortunately, there is no old reindeer bones for which it would be known that they present domestic or wild reindeer. We are therefore relying on modern data in the inferences of which genetic lineages are domestic and which are wild. This is problematic because modern populations do not necessarily reflect all the diversity that has been present in historical populations. For example, it is possible that the historical domestic populations included lineages that have since been lost from the domestic populations (for example as in the case of Prezwalski horse), so our interpretations are not on such solid ground. Rather, it is possible that before large-scale reindeer pastoralism spread over Fennoscandia, there were other local domestic lineages. It would be crucial therefore to study how much wild and domestic reindeer differ by their nuclear DNA and then investigate historical material for these possible differences. *De novo* genome and some re-sequenced genomes have recently been published (Weldenegodguad et al., 2020), but there isn't yet exact studies on the possible genome-wide genetic differences between wild and domestic individuals. Conducting this kind of study first on modern samples would facilitate the research also on ancient samples.

4.5 Genetic relatedness of the 4000-year-old reindeer from Tatarstan with modern populations

For all other samples except P13, 98-99% of the mitochondrial genome sequence was resolved at 3X coverage. Because at the time of the writing the article, only one mitogenome sequence of a modern individual was available in GenBank, most of the comparisons were based on mtDNA control region data, since a lot more reference data was available regarding this marker. Based on this marker, genetic relatedness between the historical reindeer of Tatarstan and the present-day wild populations of the northeastern part of European Russia was observed. The 4000-year-old reindeer from Tatarstan shared haplotypes especially with modern reindeer from the taiga zone of the northeastern part of European Russia, which implies that there is genetic continuity between these populations. However, as this study was conducted only on mitochondrial DNA, it provides only a very limited picture on the genetic relationships of the reindeer.

The phylogenetic tree that shows the haplogroup affiliations of the study samples is shown in Fig.5 (subproject V, page 186). The most interesting

observation from the tree is that four of the study samples are situated in relatively basal positions of haplogroup II. This haplogroup, together with haplogroup Ib, is the dominating haplogroup of Fennoscandian domestic reindeer (Flagstad and Røed, 2003; Røed et al., 2008; Kvie et al., 2016b). It was originally thought to have originated in Europe during the Late Pleistocene, south of the Fennoscandian ice sheet, but these results suggest that it may have originated east of Fennoscandia. The Fennoscandian domestic reindeer, however, likely has not directly originated from the population presented by the Pestrechinskaya II as the haplotypes observed in this material are not particularly close to the Fennoscandian haplotypes.

5 Conclusions

My research shows that valuable individual and population level genetic information can be obtained from unconventional biological samples. Each sub-project was successful in the sense that new relevant data and conclusions could be obtained.

In subproject I, we investigated the usability of non-invasively collected placentas of Saimaa ringed seal for individual identification. We were able to obtain pups genotypes from the placentas, and these genotypes could be used to calculate population level genetic parameters. Reliable estimates of relatedness of individuals were however not obtained, as the genetic variability in the studied microsatellite markers was too low in the population. Increasing the number of markers for example by typing single nucleotide polymorphism (SNPs) across the nuclear genome might provide enough resolution to obtain reliable estimates of relatedness.

In subproject II, I determined to which likely subspecies the historical tiger specimens belonged to, and from which likely geographical area they originate. Five of the samples could be reliably identified by subspecies. Subspecies diversity was surprisingly high and included rare specimens such as two Sunda Island tigers, of which one could be further identified as an extinct Javan tiger. As relatively few known Javan tiger specimens exist in the natural history collections in the world, the specimen identified in this study may in the future provide important additional information on this species. Additional genetic data obtained from this and other Javan tiger specimens could for example be used to study population level genetic changes in species that is going towards extinction.

Subproject III investigated the history of the domestic goose in Russia by studying specimens from archaeological sites. Analyses showed that the typical domestic goose haplotypes were present in the material from the 11th century onwards. Surprisingly, also bean goose haplotypes were observed among the studied material. The domestic/wild status of these individuals remains uncertain.

In subproject IV, I studied mtDNA from reindeer material originating from archaeological sites in Finland. As the studied specimens were genetically related to the present-day Finnish forest reindeer, a tentative conclusion is that they present wild reindeer. However, caution is needed, as the modern-day diversity of Fennoscandian domestic reindeer may not accurately present the historical diversity. I am at the moment studying archaeological reindeer samples from

additional sites in Fennoscandia in order to determine when large-scale reindeer pastoralism started and from which region did it originate.

In subproject V, the studied 4000-year-old reindeer samples from Tatarstan shared haplotypes especially with modern reindeer from the taiga zone of the northeastern part of European Russia, implying genetic continuity between these populations. Interestingly also a connection to the major Fennoscandian domestic haplogroup was observed, but the importance of this observation needs to be validated in future studies. The data generated in this study will be used in a large-scale investigation on the phylogeography of mitochondrial lineages of reindeer through the last Ice Age, which we are conducting at the moment together with my collaborators.

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