LEHTI SAAG

The prehistory of Estonia from a genetic
perspective: new insights from ancient DNA

TARTU ÜLIKOOL · UNIVERSITAS TARTUENSIS · 1632

LEHTI SAAG

The prehistory of Estonia from a genetic perspective: new insights from ancient DNA

Institute of Molecular and Cell Biology, University of Tartu, Estonia

Dissertation was accepted for the commencement of the degree of Doctor of Philosophy in Gene Technology on 12th of June 2019 by the Council of the Institute of Molecular and Cell Biology, Faculty of Science and Technology, University of Tartu.

Supervisors: Mait Metspalu, PhD; Director and Senior Research Fellow of Modern Population Genetics, Institute of Genomics, University of Tartu, Estonia

Kristiina Tambets, PhD; Vice Director and Senior Research Fellow of Population Genetics, Institute of Genomics, University of Tartu, Estonia

Toomas Kivisild, PhD; Professor, Department of Human Genetics, KU Leuven, Belgium; Senior Research Fellow of Population Genetics, Institute of Genomics, University of Tartu, Estonia

Opponent: Daniel Bradley, PhD; Professor and Head of School, Smurfit Institute of Genetics, Trinity College Dublin, Ireland

Commencement: Room No 105, 23B Riia St, Tartu, on 12th of September 2019, at 10:15.

Publication of this thesis is granted by the Institute of Molecular and Cell Biology, University of Tartu.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

Tables:

Figures:

LIST OF ORIGINAL PUBLICATIONS

I
Allentoft ME, Sikora M, Sjögren KG, Rasmussen S, Rasmussen M, Stenderup J, Damgaard PB, Schroeder H, Ahlström T, Vinner L, Malaspinas AS, Margaryan A, Higham T, Chivall D, Lynnerup N, Harvig L, Baron J, Della Casa P, Dąbrowski P, Duffy PR, Ebel AV, Epimakhov A, Frei K, Furmanek M, Gralak T, Gromov A, Gronkiewicz S, Grupe G, Hajdu T, Jarysz R, Khartanovich V, Khokhlov A, Kiss V, Kolář J, Kriiska A, Lasak I, Longhi C, McGlynn G, Merkevicius A, Merkyte I, Metspalu M, Mkrtchyan R, Moiseyev V, Paja L, Pálfi G, Pokutta D, Pospieszny Ł, Price TD, **Saag L**, Sablin M, Shishlina N, Smrčka V, Soenov VI, Szeverényi V, Tóth G, Trifanova SV, Varul L, Vicze M, Yepiskoposyan L, Zhitenev V, Orlando L, Sicheritz-Pontén T, Brunak S, Nielsen R, Kristiansen K, Willerslev E. 2015.
Population genomics of Bronze Age Eurasia. *Nature* 522(7555):167-72.

II
Saag L, Varul L, Scheib CL, Stenderup J, Allentoft ME, Saag L, Pagani L, Reidla M, Tambets K, Metspalu E, Kriiska A, Willerslev E, Kivisild T, Metspalu M. 2017.
Extensive Farming in Estonia Started through a Sex-Biased Migration from the Steppe. *Current Biology* 27(14):2185-2193.

III
Saag L, Laneman M, Varul L, Malve M, Valk H, Razzak MA, Shirobokov IG, Khartanovich VI, Mikhaylova ER, Kushniarevich A, Scheib CL, Solnik A, Reisberg T, Parik J, Saag L, Metspalu E, Rootsi S, Remm M, Mägi R, D'Atanasio E, Crema ER, Díez-del-Molino D, Thomas MG, Kriiska A, Kivisild T, Villems R, Lang V, Metspalu M, Tambets K. 2019.
The Arrival of Siberian Ancestry Connecting the Eastern Baltic to Uralic Speakers Further East. *Current Biology* 29(10):1701-1711.

IV
Tambets K, Yunusbayev B, Hudjashov G, Ilumäe AM, Rootsi S, Honkola T, Vesakoski O, Atkinson Q, Skoglund P, Kushniarevich A, Litvinov S, Reidla M, Metspalu E, **Saag L**, Rantanen T, Karmin M, Parik J, Zhadanov SI, Gubina M, Damba LD, Bermisheva M, Reisberg T, Dibirova K, Evseeva I, Nelis M, Klovins J, Metspalu A, Esko T, Balanovsky O, Balanovska E, Khusnutdinova EK, Osipova LP, Voevoda M, Villems R, Kivisild T, Metspalu M. 2018.
Genes reveal traces of common recent demographic history for most of the Uralic-speaking populations. *Genome Biology* 19(1):139.

Author's contributions to the listed articles are as follows:

REF I: I performed DNA laboratory work of the Estonian sample and provided input for writing the paper.

REF II: I was part of conceiving the study, performed laboratory work, participated in all data analyses, interpreted results and co-wrote the manuscript.

REF III: I was part of conceiving the study, performed laboratory work, participated in all data analyses except estimating kinship and phenotypes, interpreted results and co-wrote the manuscript.

REF IV: I compiled the dataset for ancient DNA comparisons and calculated f3 statistics, interpreted results and provided input for writing the paper.

ABBREVIATIONS

aDNA	ancient DNA (deoxyribonucleic acid)
AMH	anatomically modern human
BCE	Before the Common Era
CE	Common Era
chrX	X chromosome
chrY	Y chromosome
dsDNA	double-stranded DNA
EDTA	ethylenediaminetetraacetic acid
EHG	Eastern hunter-gatherers
hg	haplogroup
IBD	identity-by-descent
LGM	Last Glacial Maximum
LGP	Last Glacial Period
LBK	Linearbandkeramik
mtDNA	mitochondrial DNA
NGS	next generation sequencing
PCA	principal component analysis
PCR	polymerase chain reaction
SHG	Scandinavian hunter-gatherers
SNP	single nucleotide polymorphism
ssDNA	single-stranded DNA
WHG	Western hunter-gatherers

1. INTRODUCTION

The genetic changes that have happened in the past and the processes that have shaped the demographic histories of populations through time have been studied for decades using modern genetic data. After the development of next generation sequencing (NGS) technologies almost 15 years ago (Margulies et al., 2005), ancient DNA (aDNA) research has started to contribute significantly to this field of interest. Modern genomes contain a lot of information about the past and the large datasets available now provide a good representation of existing genetic diversity, enabling us to model how and when different processes affected populations in the past. Studying aDNA, however, allows us to confirm the more exact time when these processes were going on and adds a geographic component since each ancient genome is connected to a specific time and place in the past. Furthermore, aDNA can shed light on the social structure in the past and reveal genetic lineages that have not managed to contribute to the genomes of modern populations. To this end, aDNA research has already shown higher than expected levels of mobility and admixture (most unexpectedly between humans and other hominins).

The main restrictions of aDNA research come from the available sample size often being quite small, geographic limitations due to differential preservation depending on environmental conditions and the high cost – chemical processes causing different types of damage lead to highly degraded DNA which calls for the use of dedicated clean-room facilities and abundant funds for sequencing, often coupled with expensive laboratory methods to improve sequencing efficiency. Due to this, the researchers in this field are constantly trying to find ways to improve their laboratory and bioinformatic methods to generate more data and to be able to answer more questions.

The field of studying the human past is evolving and benefitting more and more from transdisciplinary research in recent times – combining the data and knowledge of genetics, archaeology, linguistics etc is crucial for being able to understand demographic processes and to get as clear of a picture of the past as possible.

The main topic of this thesis is the demographic history of Europe and more specifically Estonia. Anatomically modern humans (AMHs) reached Europe by around 45,000 years ago (Benazzi et al., 2011). These hunter-gatherers persisted in refugia during the Last Glacial Maximum (LGM), re-expanded later and formed genetically, geographically and, in some cases (Jones et al., 2015; Mittnik et al., 2018), temporally distinguishable groups. Around 8,000 years ago (Pinhasi et al., 2005), farming was brought into Europe by people with ancestry from Anatolia and Levant (Lazaridis et al., 2016), nowadays most frequent in modern Sardinians.

Archaeological data suggests that the demographic history of Estonia is somewhat different from more central areas of Europe – the first evidence of human settlement is from after the Last Glacial Period (LGP) around 11,000

years ago (Veski et al., 2005; Kriiska and Lõugas, 2009) and farming arrived only with the Late Neolithic Corded Ware culture (CWC) people. The most apparent thing that sets modern Estonians, Finns and some smaller populations apart from the rest of Europe is that they speak Uralic languages. The origin of this language family is probably in the Volga-Kama region from where the precursors of modern Uralic languages spread in several directions to be spoken today in areas of Europe and Siberia, far from each other.

This thesis looks into the Neolithic in Estonia in the context of migrations affecting much larger areas in Eurasia (REF I), the more specific processes and changes in Estonia through time (REF II, III), and the genetic makeup of modern Estonians, particularly connections with other Uralic speakers (REF IV).

2. LITERATURE OVERVIEW

The literature overview first gives a general background of the different aspects of using aDNA for studying the past. The later chapters explain the genetic, archaeological and linguistic background of the more specific questions addressed in the thesis.

2.1. Ancient DNA (aDNA)

Using aDNA to learn about the past is a relatively new development in the field of genetics. There are some characteristics of aDNA that make it more difficult to use in research compared to modern DNA, thus also impacting the history of aDNA studies and influencing the way that this research is conducted now.

2.1.1. The history of aDNA studies

The field of aDNA research started almost 35 years ago when Higuchi and colleagues (Higuchi et al., 1984) managed to sequence 229 bp of mitochondrial DNA (mtDNA) from a museum specimen of a quagga (*Equus quagga*), a mammal similar to extant zebras that went extinct in 1883. Already the next year, Svante Pääbo reported having cloned DNA from an Egyptian mummy (Pääbo, 1985). During the following years, many more articles were published, claiming to have successfully produced sequences from organisms who lived as long as millions of years ago (called "antediluvian"), including insects and plants stuck in amber (DeSalle et al., 1992; Cano et al., 1993; Poinar et al., 1993) and a Cretaceous dinosaur (Woodward et al., 1994).

The first enthusiastic phase of aDNA studies was followed by a period of questioning the results of those first studies and the limits of aDNA studies in general. The supposed dinosaur sequence was already classified as contamination a year after it was published when cytochrome b sequences of the so-called Cretaceous bone fragments were compared with those of extant tetrapods and shown to cluster with humans (Figure 1) (Hedges and Schweitzer, 1995; Zischler et al., 1995). The same conclusion was reached for many of the early aDNA results in the following years, often by the same researchers who had participated in the false discoveries (Austin et al., 1997; Cooper and Poinar, 2000; Pääbo et al., 2004). However, this also led to the establishment of the first laboratory protocols for avoiding contamination (Handt et al., 1996; Ward and Stringer, 1997; Cooper and Poinar, 2000), which have been built upon in later years (Knapp et al., 2012).

Human
91
Cretaceous bone
40
Bat
4
Dog
10
Rabbit
21
Rhinoceros
8
Dugong
17
Rodent
48
15
Whale
Cow
Sicklebill
Shoebill
92
Domestic fowl
70
Cuckoo
62
Frog

0.1
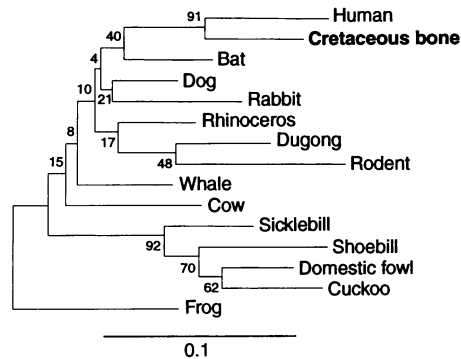
Figure 1. Phylogenetic tree of partial cytochrome b DNA sequences in representatives of extant tetrapod groups and putative dinosaur DNA sequence (majority rule consensus) derived from Cretaceous bone fragments (Woodward et al., 1994). Numbers on nodes are bootstrap confidence probabilities. A frog was included to root the tree. Tree shown is neighbor-joining with transversion distance. Figure reprinted with permission from Figure 1 (Hedges and Schweitzer, 1995), The American Association for the Advancement of Science.

A major breakthrough in the field of aDNA research came with the development of the NGS technology in 2005 (Margulies et al., 2005). In 2006, Poinar and colleagues published 0.5% (13 million bp) of the genome sequence of the extinct woolly mammoth (Poinar et al., 2006) and in 2008 Miller and others presented sequences covering 70% of the genome (Miller et al., 2008), making this the first ancient mammalian genome sequenced on a large scale. This was followed by the first human and Denisovan full genomes and a draft Neanderthal genome in 2010 (Rasmussen et al., 2010; Reich et al., 2010; Green et al., 2010), and the first high coverage Neanderthal genome in 2014 (Prüfer et al., 2014). The oldest aDNA genome sequenced so far is from a horse that lived 735,000 years ago (Orlando et al., 2013), the oldest genetically studied hominins lived in Spain 430,000 years ago (Meyer et al., 2014, 2016). The as of yet oldest genome sequences of AMHs from the Old and New World date to 45,000 and 12,600 years ago, respectively (Fu et al., 2014; Rasmussen et al., 2014).

2.1.2. Main characteristics of aDNA

2.1.2.1. Preservation influenced by the environment

It was already shown during the 1990's that cool and stable environmental conditions contribute to the preservation of DNA in remains (Höss et al., 1996a, 1996b; Hadly et al., 1998; Noro et al., 1998; Wayne et al., 1999). This is also supported by the fact that most of the first or oldest genomes mentioned in the previous chapter came either from permafrost conditions or caves and none of them from (sub)tropical conditions.

The negative impact of hot climate on aDNA preservation is exemplified by studies on Egyptian mummies. One of the first aDNA studies was conducted on mummies (Pääbo, 1985), but was later classified as contamination (Pääbo et al., 2004). The feasibility of aDNA studies of ancient Egyptian material has been debated in a series of publications (Gilbert et al., 2005; Marota et al., 2002; Zink and Nerlich, 2003). The first reliable results from Egyptian mummies were published only in 2017 and even then the success rate for obtaining mtDNA haplogroups (hgs) was 90/151 and for whole-genome data 3/151 (Schuenemann et al., 2017). The first aDNA results from Oceania (Skoglund et al., 2016; Lipson et al., 2018; Posth et al., 2018) and sub-Saharan Africa (Skoglund et al., 2017) were also published only recently. The dependency of DNA preservation on temperature has also been shown computationally (Allentoft et al., 2012).

So far aDNA extraction has been unsuccessful from burned bones due to extensive heat-damage (Hansen et al., 2017) and also from remains found in bogs (so-called bog-bodies) (Lynnerup, 2015), most likely due to DNA damage caused by the highly acidic environment (Allentoft et al., 2012).

2.1.2.2. Presence of exogenous DNA

An important aspect that has to be taken into account when conducting aDNA research is that ancient samples always contain DNA that is not endogenous (belonging to the specimen from which the sample was taken), i.e. is exogenous (Pääbo, 1989 and all later publications). The proportion of exogenous DNA varies significantly between individual samples (Figure 2) (Der Sarkissian et al., 2015) but it is not uncommon that a published sample contains only around 1% endogenous DNA (Green et al., 2010; Miller et al., 2012; Orlando et al., 2013).

Low endogenous DNA content makes sequencing aDNA (much) more expensive than modern DNA which is why a lot of effort goes into finding the best materials for extracting aDNA and into developing methods to maximize the endogenous DNA yield (discussed in more detail below).

Most of the exogenous DNA belongs to environmental organisms which have ended up inside the sample after the death of the organism the sample comes from (Der Sarkissian et al., 2014). However, in some cases, some of the DNA sequences belong to pathogens that were present in the blood of the organism at the time of their death (Spyrou et al., 2019 and references therein). The possibility of extracting DNA from pathogens infecting sampled individuals was first shown using PCR technology (Spigelman and Lemma, 1993) and the first full pathogen genome was that of plague (*Yersinia pestis*) from Black Death victims (Bos et al., 2011). Ancient plague sequences have also already been published from two Estonian CWC individuals (Rasmussen et al., 2015; Andrades Valtueña et al., 2017).
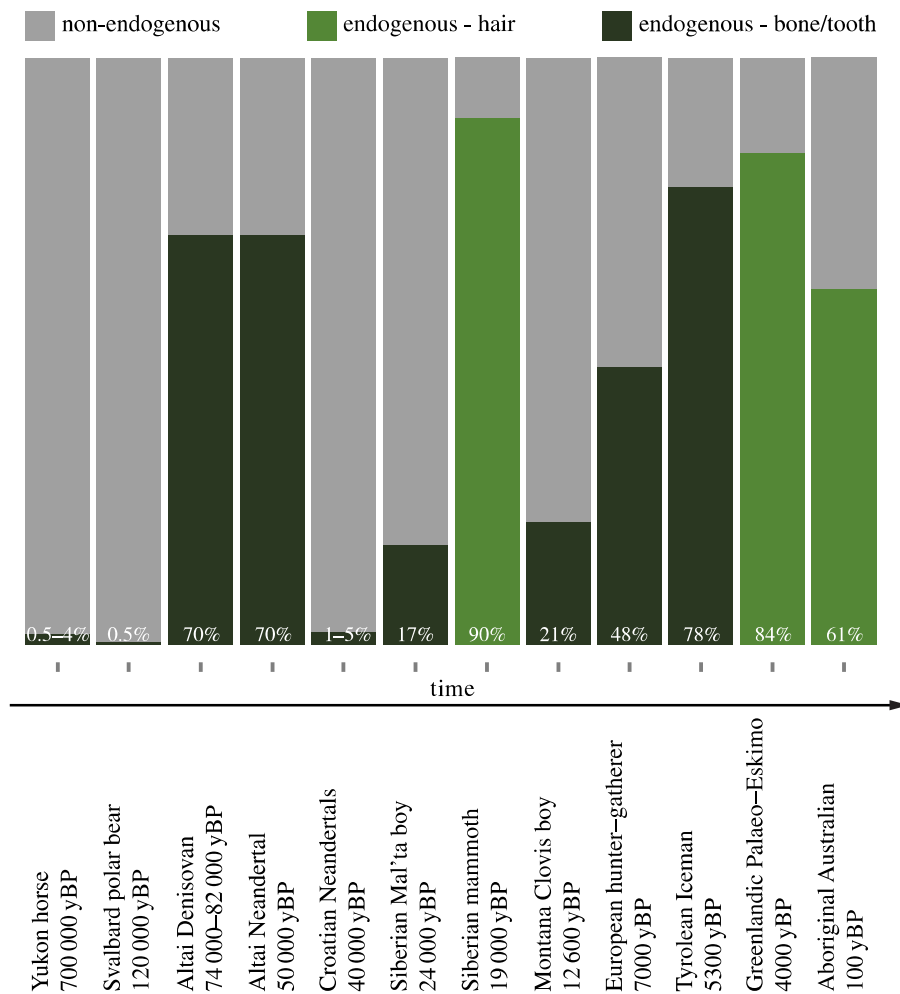
Figure 2. Endogenous content of ancient genomic extracts. Datasets are ordered from the most ancient sample to the most recent. 'yBP', years before present. Figure adapted from Figure 2 (Der Sarkissian et al., 2015), licenced under the CC BY Licence.

2.1.2.3. Degradation into short fragments

After the death of an organism, its DNA starts decaying, leading to short fragment length being characteristic of aDNA (Pääbo, 1989). One of the reasons for this is nuclease activity within the cell (Darzynkiewicz et al., 1997). Once the organism starts decomposing, DNA is also digested by microorganisms (Eglinton Geoffrey et al., 1991; Lindahl, 1993). Furthermore, DNA is not very chemically stable, which leads to strand breaks also in living cells, there repaired by enzymatic repair systems (Lindahl, 1993) that no longer function after the death of the organism. Depurination (the loss of purine residues) is an example of the chemical decay – purine residues are removed from deoxyriboses through

hydrolysis, resulting in abasic sites where DNA strands break (Lindahl and Nyberg, 1972; Lindahl and Andersson, 1972). As a consequence of the depurination strand breaks, adenosine (A) and guanosine (G) are relatively over-represented in reference sequences next to the ends of aDNA sequence reads (Briggs et al., 2007). Interestingly, the purine/pyrimidine ratio is higher in younger samples compared to older ones (Sawyer et al., 2012).

Since the fragmentation is random (Deagle et al., 2006; Schwarz et al., 2009), the relationship between the number of fragments and their length is exponential – the more fragments there are, the shorter they are. The relationship between DNA decay (relative copy number) and time is also exponential, hence DNA decay has a constant rate (given that conditions are also constant) meaning that DNA has a predictable half-life (Allentoft et al., 2012). The predicted time until DNA reaches an average length of 1 bp is 22,000 years in 25 °C and 6.83 million years in –5 °C (Allentoft et al., 2012) which means that even in ideal conditions there is a time constraint for aDNA studies, limiting research to relatively recent times in evolutionary terms.

The short fragment length characteristic of aDNA is used as a measure to authenticate a sample as ancient or to find signs of modern contamination (Ginolhac et al., 2011; Jónsson et al., 2013).

2.1.2.4. Presence of chemical modifications leading to nucleotide misincorporations in sequences

Chemical damage in aDNA was first studied with gas chromatography/mass spectrometry and the amount of pyrimidine oxidation products was found to negatively correlate with the DNA amplification success (Höss et al., 1996a). The chemical modifications result mostly in cytosine (C) to thymine (T) and guanine (G) to adenine (A) substitutions in sequences (Hofreiter et al., 2001; Stiller et al., 2006; Gilbert et al., 2007). The C to T substitutions are caused by cytosine deamination – the amino group of the nitrogenous base is removed by hydrolysis, turning it into a uracil (U) (Lindahl, 1996), which is not available in DNA amplification/sequencing reactions and hence shows up in sequences as a thymine (T) (Hofreiter et al., 2001). C to T substitutions are most frequent in 5' and G to A substitutions in 3' ends of fragments (Briggs et al., 2007). Thus G to A substitutions seen in sequences likely appear during sample preparation for sequencing when the complementary strand is synthesized to a fragment containing a deaminated cytosine (Briggs et al., 2007).

The proportion of C to T substitutions in the 5' ends of sequence reads, as the sole cause of deamination damage (Brotherton et al., 2007), and the apparent excess of G to A substitutions in the 3' ends is positively correlated with the age of the sample (Sawyer et al., 2012). On this basis, this characteristic, in addition to fragment length, is used to authenticate ancient samples (Ginolhac et al., 2011; Jónsson et al., 2013; Sawyer et al., 2012).

2.1.3. Main materials and laboratory methods in aDNA studies

Due to the characteristics described in chapter 2.1.2 complicating research, a lot of effort is put into finding the best materials for extracting aDNA and into developing laboratory methods.

2.1.3.1. Materials used for extracting aDNA

Teeth are relatively well protected from environmental conditions (Alvarez García et al., 1996; Schwarz et al., 2009) and have been shown to be a better source of DNA than most bones (Alonso et al., 2001; Ricaut et al., 2005). More detailed studies comparing different parts of teeth revealed that root cementum yields more DNA than dentine (Adler et al., 2011; Damgaard et al., 2015). It has also been shown that visual inspection is a useful (although not absolute) tool for predicting endogenous DNA preservation (Hansen et al., 2017).

In recent years, the petrous part of the temporal bone (*pars petrosa*) has been identified as a premium source of aDNA (Rasmussen et al., 2014; Gamba et al., 2014; Pinhasi et al., 2015; Hansen et al., 2017). More specifically, the dense inner ear bone yields more endogenous DNA when compared to other parts of the temporal bone (Pinhasi et al., 2015) and the highest yields are obtained from the cochlea (Pinhasi et al., 2019). Using the petrous bone is especially advantageous if the teeth from the same individual have low endogenous DNA content (<10%) while in case of higher proportions of endogenous DNA in teeth, using petrous bones often does not increase the DNA yield (Hansen et al., 2017). Obtaining DNA from cremated remains has so far not been successful even when using petrous bones (Hansen et al., 2017). Although the high endogenous content of petrous bones is highly beneficial for population genetic studies, this material is not well suited for metagenomic studies since the microbial diversity in petrous samples is much lower compared to teeth, inhibiting research involving pathogens or the oral microbiome (Margaryan et al., 2018).

2.1.3.2. Methods used for extracting aDNA

In 2007 Rohland and Hofreiter compared and analyzed previous DNA extraction methods (Rohland and Hofreiter, 2007a) and developed a protocol based on the results and further modifications and testing (Rohland and Hofreiter, 2007b). The protocol includes extracting DNA using a buffer containing ethylenediaminetetraacetic acid (EDTA) for demineralizing the bone/tooth and proteinase K for degrading proteins, binding to silica suspended in solution, washing with an ethanol-based buffer, and eluting in Tris-EDTA (Rohland and Hofreiter, 2007b).

Dabney and colleagues developed a modified method in 2013, altering the binding buffer, using larger volumes of binding buffer relative to DNA extract,

and as the biggest technological change switching from in-solution binding to silica spin-columns (Dabney et al., 2013).

Further modifications to these protocols have been suggested in later years, for example retaining shorter fragments (Allentoft et al., 2015; Glocke and Meyer, 2017). To decrease the proportion of contaminant DNA in extracts, pre-digestion (Damgaard et al., 2015), using a phosphate buffer to release surface-bound DNA (Korlević et al., 2015), and treatment with sodium hypochlorite (Korlević et al., 2015; Hajdinjak et al., 2018; Scheib et al., 2018) have been proposed.

2.1.3.3. Methods used for generating aDNA data

The first highly parallel or so-called NGS technology was developed in 2005 (Margulies et al., 2005). Since that, data output amounts have increased and sequencing costs decreased on a logarithmic scale (Muir et al., 2016). The most commonly used NGS technologies require that the pool of DNA fragments is converted into a so-called sequencing library where the ends of all fragments are made identical by adding specific adapters, and in order to pool multiple libraries together in one sequencing run (multiplex), index sequences that are different for each library are added to all fragments (https://emea.illumina.com/science/technology/next-generation-sequencing.html).

Most commonly, aDNA sequencing libraries are prepared using double-stranded DNA (dsDNA) fragments based on a protocol developed by Meyer and Kircher (Meyer and Kircher, 2010) with modifications for aDNA (Orlando et al., 2013; Malaspinas et al., 2014). The main steps of this protocol are: 1) end-repair –3' single-stranded overhangs are digested and 5' overhangs filled in; 2) adapter ligation – double-stranded sequencing adapters are added to the ends of fragments; 3) adapter fill-in – the shorter strands of adapters are filled in; 4) indexing PCR – multiplexing indexes are added and libraries are amplified (Figure 3) (Meyer and Kircher, 2010). Originally, the solution is purified between steps (Meyer and Kircher, 2010; Orlando et al., 2013; Malaspinas et al., 2014) but recently a single-tube protocol without purification steps was developed (Carøe et al., 2018).

Another method for preparing aDNA for sequencing is by following the single-stranded DNA (ssDNA) protocol by Gansauge and Meyer (Gansauge and Meyer, 2013). The main steps of the protocol are: 1) removing phosphate groups and deoxyuracils from the ends of fragments while denaturing frag-ments; 2) ligating biotinylated adapters hybridized with primers to 3' ends of; 3) binding biotinylated adapters to streptavidin-coated beads and synthesizing the complementary strand; 4) removing excess primers by washing at elevated temperature and removing 3' overhangs; 5) ligating adapters to the 3' ends of the synthesized strands; 6) washing away excess primers and denaturing frag-ments; adding indexes and amplifying the libraries with PCR (Gansauge and Meyer, 2013). This method retains information from dsDNA fragments with

single-stranded breaks and avoids DNA loss during purifications but is more expensive and more time-consuming than the dsDNA protocols (Gansauge and Meyer, 2013). To reduce costs, the protocol has been updated to include hybridization with the 6 random nucleotides of a splinter oligonucleotide otherwise complementary to the adapter and nick closure using a less expensive ligase (Gansauge et al., 2017).
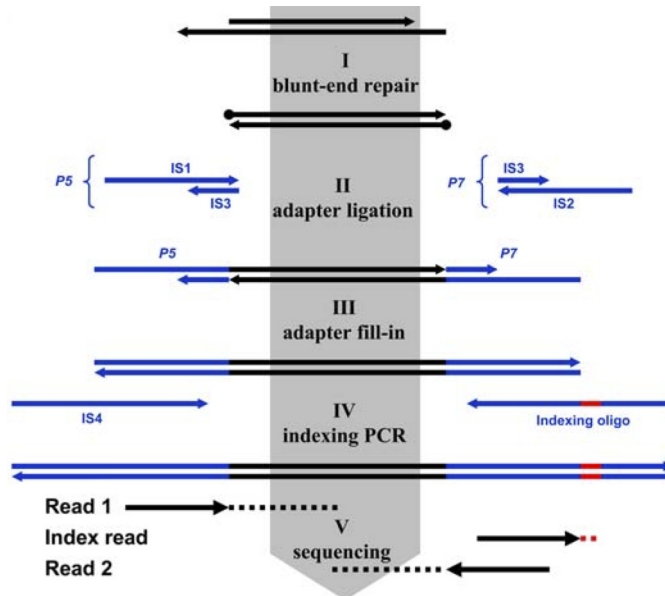
Figure 3. Main steps of the dsDNA library preparation protocol. Figure reprinted with permission from Figure 1A (Meyer and Kircher, 2010), Cold Spring Harbor Laboratory Press.

The libraries prepared using the dsDNA or ssDNA protocols are often sequenced as they are (shotgun sequencing) but due to low amounts of endogenous DNA, methods for enriching libraries for specific sequences have also been developed (Briggs et al., 2009; Burbano et al., 2010; Maricic et al., 2010; Fu et al., 2013a; Carpenter et al., 2013). The first of these methods captured limited parts of the genome, for example mtDNA (Briggs et al., 2009; Maricic et al., 2010), specific protein-coding positions (Burbano et al., 2010) or one chromosome (Fu et al., 2013a). However, in 2013 a whole-genome enrichment method was developed (Carpenter et al., 2013), which involves transcribing a biotinylated RNA bait library from a genomic DNA library with T7 RNA polymerase promoters, hybridization between the RNA bait library and the aDNA library, capturing the hybridized fragments to streptavidin-coated magnetic beads, and sequencing only the captured fragments (Carpenter et al., 2013). A large proportion of aDNA data is generated using the so-called 1240k capture first described in 2015, which uses the same principle described above but with baits synthesized only for fragments of the human genome surrounding

1,237,207 single nucleotide polymorphisms (SNPs) chosen for being infor-
mative in population genetic or functional analyses (Mathieson et al., 2015).

2.1.4. Perspectives in studying the human past using ancient biomolecules

Since the development of NGS almost 15 years ago, the main objective of
research using ancient materials has been extracting DNA from skeletal remains
to produce genomic data to infer the ancestry of the studied population.
However, in recent years, the scope of the field has begun to widen. Good
examples of this are studies where DNA is extracted from sediments instead of
skeletal remains, where the whole metagenome is studied not only the endo-
genous DNA or even pathogens, where proteins instead of DNA are used, or
where DNA is extracted from a whole group of nearby graves to find out about
the social structure of the population not only about the ancestry components
present.

2.1.4.1. Sediment DNA

The first study using DNA extracted from sediments was conducted already
more than 15 years ago (Willerslev et al., 2003) and since then, taxonomically
informative fragments of sediment DNA (sedaDNA) have been amplified by
polymerase chain reaction (PCR) and analyzed to gain insight into changes in
floral and faunal communities through time (Haile et al., 2009; Anderson-
Carpenter et al., 2011; Giguet-Covex et al., 2014; Willerslev et al., 2014;
Kisand et al., 2018).

Recently, however, shotgun sequencing has been employed on DNA
extracted from parasite eggs from an Iron Age settlement (Tams et al., 2018)
and from mammalian and also more specifically hominin mtDNA from
Pleistocene cave sediments (Slon et al., 2017). Articles presenting nuclear
hominin DNA are to be expected soon as shown in conference presentations.

2.1.4.2. Metagenomics and proteomics of dental calculus

Dental calculus has been recognized as a source of information about diet in the
past already for some time and studied using microscopy (Henry et al., 2011;
Hardy et al., 2009). More recently, researchers have also started to use calculus
from archaeological remains to extract both DNA and proteins (Adler et al.,
2013; Warinner et al., 2014a; Weyrich et al., 2017). Since calculus builds up in
layers over time, it contains information not only about the time close to an
individual's death but about a longer period during their life (Jin and Yip, 2002).

The metagenomic approach has already been used to characterize ancient
oral microbiomes, for example looking at changes in microbial communities in

connection to major cultural changes (Adler et al., 2013), the presence of pathogens, the diet and behaviour (Warinner et al., 2014a; Weyrich et al., 2017). One caveat of metagenomic research is currently the incompleteness and untrustworthiness of sequence databases but fortunately this will probably become less of a problem in the future and additional information can then be gained from already generated data (Breitwieser et al., 2017).

Ancient protein sequences are a valuable addition to aDNA data, confirming the results gained from metagenomic analyses (Warinner et al., 2014a) and providing information about periods beyond the reach of DNA preservation, up to millions of years ago (Cappellini et al., 2014; Demarchi et al., 2016). Studies analyzing proteins in dental calculus have already started to confirm or provide new information about the pathogens (Warinner et al., 2014a) and diet (Warinner et al., 2014b, 2014a; Hendy et al., 2018) of past human populations.

2.1.4.3. Studying social structure in the past

The social structure of past societies can be studied by looking into the ancestry and kinship of individuals from close burials in combination with archaeological data. This has been done already for years using autosomal short tandem repeats (STRs) in case of sufficient preservation and Y chromosomal (chrY) or mtDNA hgs to see if paternal or maternal relatedness is excluded or not (Keyser-Tracqui et al., 2003; Dissing et al., 2007; Haak et al., 2008; Vanek et al., 2009).

Recently, methods for estimating the relatedness of ancient individuals using low-coverage genome-wide data have been developed (Monroy Kuhn et al., 2018; Waples et al., 2019). These methods have been used, for example, to study the social structure in Migration Period Longobard cemeteries in Hungary and Italy (Figure 4) (Amorim et al., 2018), in an Early Middle Age Allemanic cemetery in Germany (O'Sullivan et al., 2018) and in a Late Neolithic Globular Amphora culture mass grave in Poland (Schroeder et al., 2019).
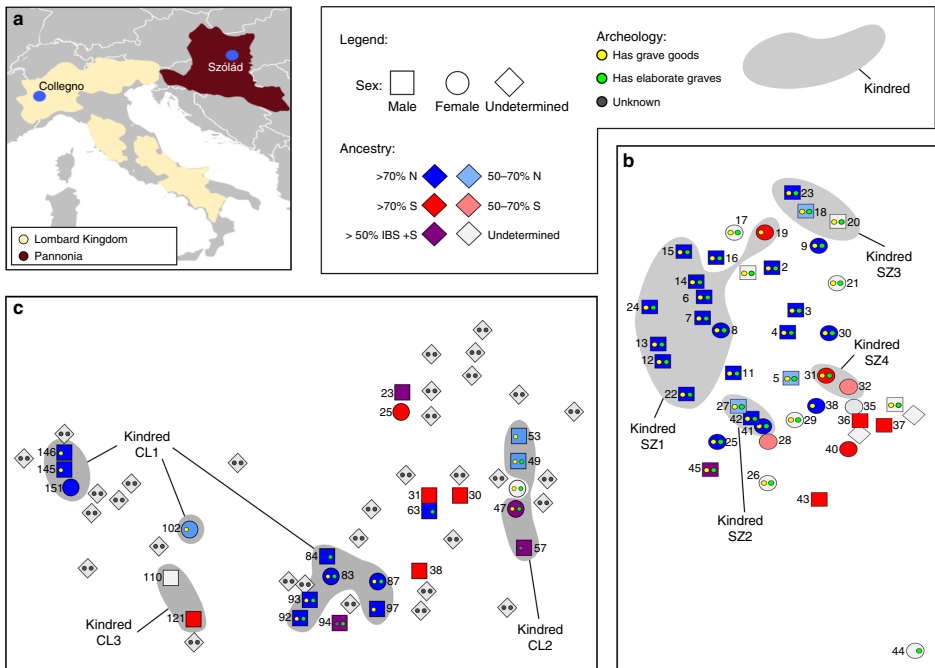
Figure 4. Archeological and genetic characterization of Szólád and Collegno. A. Map of Europe showing the location (blue dots) of the two cemeteries and regional context is included (the Roman province of Pannonia in burgundy and the Longobard Kingdom in beige). B, C. Spatial distribution of graves in Szólád and Collegno (first period burials only) with indication of sex (different shapes), genetic ancestry (different colors) and summary of archeology (yellow dots for presence/absence of grave furnishings and green dots for the presence of wooden elements in grave structure). Kindreds (in the biological sense) are indicated by gray shading in B and C. N = FIN + GBR + CEU, S = TSI. Figure reprinted from Figure 1 (Amorim et al., 2018), licenced under CC BY Licence.

2.2. The demographic history of Europe before the Late Neolithic from an aDNA perspective

Anatomically modern humans (AMHs) reached Europe by around 45,000 years ago (Benazzi et al., 2011). The hunter-gatherer lifestyle of these people persisted for tens of thousands of years before farming spread to Europe around 7,500 years ago bringing along drastic changes in social structure (Ammerman and Cavalli-Sforza, 1984; Gronenborn, 1999; Price, 2000). The genetic diversity and changes during these periods are discussed below.

2.2.1. European hunter-gatherers

2.2.1.1. Paleolithic hunter-gatherers

Although the oldest known AMH remains in Europe are around 45,000 years old (Benazzi et al., 2011), the oldest remains that have yielded nuclear DNA so far are those of a 37,000–42,000-year-old individual from Romania called Oase 1 (Fu et al., 2015) and of an individual from Russia called Kostenki 14, dating to 36,000–39,000 years ago (Krause et al., 2010; Seguin-Orlando et al., 2014). Oase 1 shared more alleles with early Europeans and modern East Asians than modern Europeans, likely belonging to a population that did not contribute significantly to the latter (Fu et al., 2015). Kostenki 14, on the other hand, showed affinity to both ancient and modern Siberians and Europeans over East Asians, which means that the divergence between East Asians and other Eurasians happened before this individual was born (Seguin-Orlando et al., 2014). The same applies for later Paleolithic individuals and until 14,000 years ago they all seem to descend from a single ancestral population (Fu et al., 2016). At the end of the Paleolithic, European hunter-gatherers started to show increasingly more affinity to present-day Near Eastern populations, pointing to a migration of the ancestors of the latter into Europe (Fu et al., 2016).

The mtDNA diversity during the first 20,000 years of AMHs in Europe before the LGM was quite high with individuals belonging to different sub-hgs of U, hg R and even hg M (https://www.phylotree.org/tree/index.htm) no longer present in Europe later (Figure 5) (Benazzi et al., 2011; Seguin-Orlando et al., 2014; Fu et al., 2013b; Posth et al., 2016). After the LGM, mostly hg U2'3'4'7'8'9 lineages expanded from refugia (Posth et al., 2016). However, during the LGP, hg U5 was most frequent (Figure 5) (Fu et al., 2013b; Jones et al., 2015; Posth et al., 2016), potentially indicating a population replacement from another pre-LGM refugium (Posth et al., 2016).

The chrY hgs present in Paleolithic males in Europe were C (Seguin-Orlando et al., 2014; Fu et al., 2016), F (Fu et al., 2016), I (Fu et al., 2016), NO (Fu et al., 2015) and R (Fu et al., 2016) (https://www.yfull.com/tree/).

Sequencing of the oldest AMH genomes from Eurasia, Ust'Ishim (Fu et al., 2014) and Kostenki 14, has revealed that these individuals, living closer to the time of Neanderthal admixture, shared much longer genomic tracts with Neanderthals (Seguin-Orlando et al., 2014) than present-day genomes, consistent with an archaic introgression date 55,000 years ago. A linear decline in Neanderthal ancestry estimated for European ancient genomes through time (Fu et al., 2016) has recently been shown to be a technical artifact caused by gene flow among modern populations (Petr et al. 2019). However, some individuals did differ significantly in the length distribution of the Neanderthal fragments. Oase 1, in particular, had unusually long Neanderthal fragments and up to 10% of Neanderthal ancestry and the length of Neanderthal tracts in his genome indicates a Neanderthal ancestor as recently as 4–6 generations ago (Fu et al., 2015, 2016). Furthermore, the Neanderthal ancestry was not distributed randomly across the

genome – the frequency of Neanderthal alleles was significantly decreased in coding sequences and other conserved regions of the genome due to negative selection (Sankararaman et al., 2014; Fu et al., 2016; Petr et al., 2019).

Figure 5. Late Pleistocene and Early Holocene Archeological Sites and Hunter-Gatherer Mitochondrial DNA (mtDNA) Haplogroups. A. Pre-Last Glacial Maximum dispersal of non-African populations, carrying both M and N lineages (haplogroups R, U, U5, and U2'3'4'7'8'9 belong to the N clade, distinct from the M clade). B. Post-LGM re-expansion in Europe while ice sheets retracted. C. Late Glacial shift in mtDNA haplogroup (hg) frequency. D. Holocene hunter-gatherer mtDNA, mainly belonging to hg U5. Figure reprinted with permission from Figure 1 (Posth et al., 2016), Cell Press.

2.2.1.2. Mesolithic hunter-gatherers

Mesolithic hunter-gatherers in Europe could be divided into three groups based on ancestry. The first of these was the Western hunter-gatherer (WHG) group defined by the La Braña individual from Spain (Olalde et al., 2014) and the Loschbour individual from Luxembourg (Lazaridis et al., 2014). This ancestry has later been shown to have been present all the way from Iberia (Olalde et al., 2019) to the Balkans (Mathieson et al., 2018) and the Eastern Baltic (Jones et al., 2015; Mittnik et al., 2018). The second group was Eastern hunter-gatherers (EHG), defined by two individuals from Karelia and Samara, Russia (Haak et al., 2015). Three more individuals from Russia have been added later (Mathieson et al., 2015; Mittnik et al., 2018). The third group was Scandinavian hunter-

gatherers (SHG) with mixed WHG and EHG ancestry, consisting of individuals from Sweden (Lazaridis et al., 2014; Haak et al., 2015; Mittnik et al., 2018; Günther et al., 2018). Individuals from Ukraine and Latvia had intermediate EHG-SHG and WHG-SHG ancestry, respectively (Jones et al., 2017; Mathieson et al., 2018).

Interestingly, while most Mesolithic hunter-gatherers had ancestry associated with less than 14,000 years old Paleolithic individuals (Villabruna cluster), Iberian individuals also showed ancestry related to earlier genetically distinguishable individuals (El Mirón cluster) (Posth et al., 2016; Villalba-Mouco et al., 2019).

Mesolithic individuals mostly belonged to mtDNA hg U5 (Figure 5) (Bramanti et al., 2009; Sánchez-Quinto et al., 2012; Fu et al., 2013b; Bollongino et al., 2013; Haak et al., 2015; Posth et al., 2016; Jones et al., 2017; Mittnik et al., 2018; Mathieson et al., 2018), similarly to the previous Late Glacial individuals described above. However, some individuals, mostly with EHG or SHG ancestry, belonged to hgs U4 and U2 (Bramanti et al., 2009; Haak et al., 2015; Jones et al., 2017; Mathieson et al., 2018; Mittnik et al., 2018).

The chrY hgs present in Mesolithic Europe were C (Olalde et al., 2014), I (Haak et al., 2015; Jones et al., 2015), J (Mathieson et al., 2015) and R (Haak et al., 2015; Jones et al., 2017; Mittnik et al., 2018) (importantly not R1b-M269 or R1a-M417 that became very frequent in the Late Neolithic (Kivisild, 2017)).

2.2.1.3. Neolithic hunter-gatherers

In peripheral areas of Europe, for example Scandinavia (Malmström et al., 2009; Skoglund et al., 2012, 2014) and the Eastern Baltic (Jones et al., 2017; Mittnik et al., 2018), the hunter-gatherer way of life persisted also during the Neolithic, although the use of pottery was already common. In Scandinavia, these late hunter-gatherers of the Pitted Ware culture were similar to Mesolithic SHGs and very different from the contemporaneous Neolithic farmers of the same region (Skoglund et al., 2012, 2014), while in the Eastern Baltic the Comb Ceramic culture individuals were most similar to Mesolithic EHG individuals (Jones et al., 2017; Mittnik et al., 2018).

2.2.2. Early farmers

Farming originated multiple times independently in the world and the earliest evidence of farming-based economies in West-Eurasia came from the Fertile Crescent (modern-day western Iran, Iraq, southeastern Turkey, Syria, Lebanon, Jordan, Israel, Palestinian territories and Egypt) in the Near East around 12,000 years ago (Harris, 1996; Pinhasi et al., 2005; Balaresque et al., 2010). Farming reached the Balkan Peninsula around 8,000 and Central Europe around 7,000 years ago (Pinhasi et al., 2005; Balaresque et al., 2010; Fort, 2015). It was

debated for decades whether this was mainly mediated by cultural transmission or demic diffusion (Ammerman and Cavalli-Sforza, 1984; Whittle, 1996; Renfrew and Boyle, 2000) before aDNA studies set out to answer this question.

The ancestry of the Neolithic farmers of Europe was first studied using mtDNA and it was seen that their genetic diversity was much higher than that of preceding hunter-gatherers – they belonged to hgs H, HV, J, K, N1a, U, V, W and X (Haak et al., 2005; Bramanti et al., 2009; Malmström et al., 2009; Haak et al., 2010; Lacan et al., 2011; Hervella et al., 2012; Brotherton et al., 2013; Brandt et al., 2013; Hervella et al., 2015; Szécsényi-Nagy et al., 2015; Rivollat et al., 2016; Szécsényi-Nagy et al., 2017).

The chrY hgs present in the European first farmers were C (Gamba et al., 2014; Mathieson et al., 2015), F (Haak et al., 2010; Szécsényi-Nagy et al., 2015), G (Haak et al., 2010; Lacan et al., 2011; Keller et al., 2012; Szécsényi-Nagy et al., 2015; Mathieson et al., 2015; Hofmanová et al., 2016), H (Günther et al., 2015), I (Gamba et al., 2014; Szécsényi-Nagy et al., 2015; Mathieson et al., 2015) and R (Mathieson et al., 2015) (importantly not R1b-M269 or R1a-M417 that became very frequent in the Late Neolithic (Kivisild, 2017)).

The first Neolithic farmer studied on a genome-wide level was the Tyrolean Iceman Ötzi (Keller et al., 2012; Sikora et al., 2014). Numerous other individuals have been studied since and it is evident that they were all genetically most similar to modern Southern Europeans, more specifically Sardinians, regardless of being from the Balkan Peninsula (Hofmanová et al., 2016; Mathieson et al., 2018), Central Europe (Lazaridis et al., 2014; Gamba et al., 2014; Haak et al., 2015; Mathieson et al., 2015; Lipson et al., 2017; Fernandes et al., 2018; Mathieson et al., 2018), Iberia (Haak et al., 2015; Mathieson et al., 2015; Olalde et al., 2015; Lipson et al., 2017; Olalde et al., 2019), the British Isles (Brace et al., 2019) or Scandinavia (Skoglund et al., 2012, 2014). It is worth mentioning that farming reached Britain, Scandinavia and other peripheral areas of Europe more than a thousand years later than Central Europe (Collard et al., 2010; Malmer, 2002; Fort, 2015).

In contrast to the demic diffusion model, which implied admixture between local hunter-gatherers and expanding farmers at the 'front' of the admixture wave, aDNA evidence shows that after reaching Europe, the early farmers did not start to admix with local hunter-gatherers immediately. Instead, the admixture was delayed in some regions for thousands of years, followed by the small resurgence of hunter-gatherer ancestry in the later stages of the Neolithic (Brandt et al., 2013; Skoglund et al., 2014; Haak et al., 2015; Mathieson et al., 2015; Lipson et al., 2017; Mathieson et al., 2018; Olalde et al., 2019). An exception to this trend is Britain where no such resurgence can be seen (Brace et al., 2019).

The Near Eastern origin of farming has also been confirmed with aDNA – Anatolian Neolithic farmers were very similar to those from Europe, having only slightly more affinity to modern Near Easterners than the latter (Figure 6) (Mathieson et al., 2015; Hofmanová et al., 2016; Omrak et al., 2016; Lazaridis et al., 2016). This ancestry extended to the Levant (modern-day Jordan and

Israel) in the western Fertile Crescent in space and to around 14,000-year-old Natufians in time (Figure 6) (Lazaridis et al., 2016). It did not, however, extend to the Neolithic farmers of the eastern Fertile Crescent (modern-day Iran) (Broushaki et al., 2016; Lazaridis et al., 2016) who shared ancestry with hunter-gatherers from the Caucasus (Jones et al., 2015) (Figure 6).
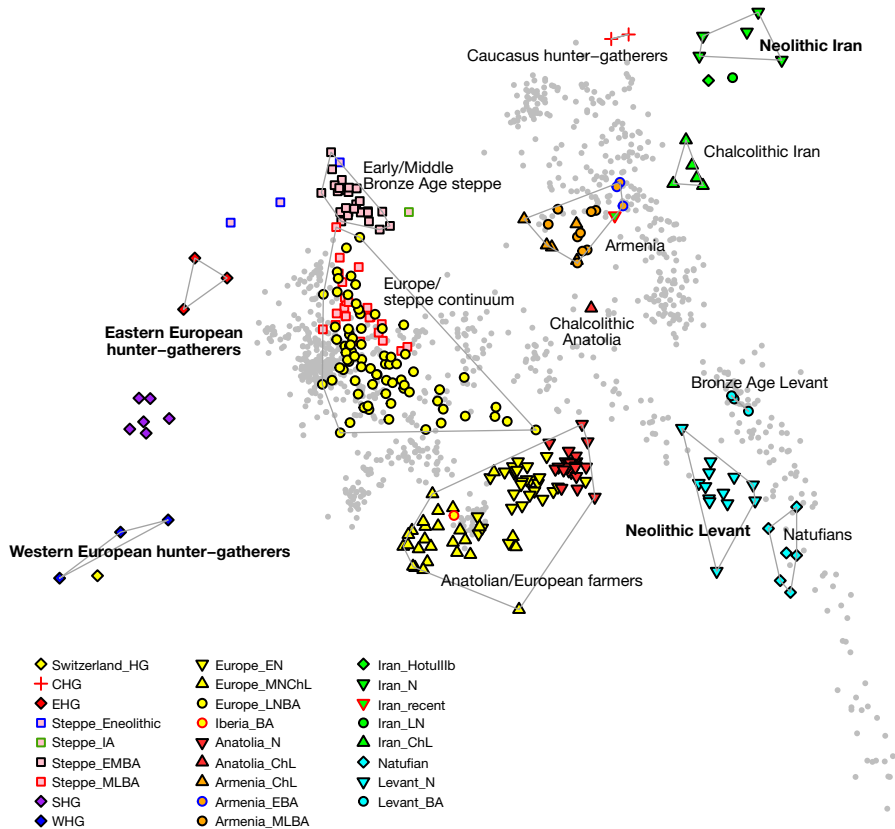
Figure 6. Genetic structure of ancient West Eurasia. Principal component analysis of 991 present-day West Eurasians (grey points) with 278 projected ancient samples. Figure reprinted with permission from Figure 1B (Lazaridis et al., 2016), Springer Nature.

2.3. The demographic history of Estonia from an archaeological perspective

Since time periods have different time spans and related cultural layers in different parts of the world and also of Europe, information about the archaeological background is crucial for understanding the demographic history of Estonia.

Table 1. Chronology of periods and cultures in Estonia. BCE – Before the Common Era; CE – Common Era. After Kriiska and Lang (Lang, 2007; Kriiska, 2009; Kriiska et al., 2017).

Period	Sub-period	Stage/culture	
Mesolithic (9,000–3,900 BCE)	Early Mesolithic (9,000–7,000 BCE)	Pulli stage (9,000–8,500 BCE)	
		Kunda stage (8,500–7,000 BCE)	
	Late Mesolithic (7,000–3,900 BCE)	Sindi-Lodja stage (7,000–5,200 BCE)	
		Narva stage (5,200–3,900 BCE)	
Neolithic (3,900–1,800 BCE)	Early Neolithic (3,900–2,800 BCE)	Comb Ceramic culture (3,900–1,800 BCE)	
	Late Neolithic (2,800–1,800 BC)		Corded Ware culture (2,800–1,800 BCE)
Bronze Age (1,800–500 BCE)	Early Bronze Age (1,800–1,100 BCE)		
	Late Bronze Age (1,100–500 BCE)		
Iron Age (500 BCE–1200 CE)	Early Iron Age (500 BCE–450 CE)	Pre-Roman Iron Age (500 BCE–50 CE)	
		Roman Iron Age (50–450 CE)	
	Middle Iron Age (450–800 CE)	Migration Period (450–550 CE)	
		Pre-Viking Era (550–800 CE)	
	Late Iron Age (800–1,200 CE)	Viking Age (800–1,050 CE)	
		Final Iron Age (1,050–1,200 CE)	
Middle Ages (1,200–1,600 CE)			

2.3.1. The Mesolithic

During the LGM Estonia was fully covered by the Scandinavian Ice Sheet that started to retreat after ~15,000 years ago (Mangerud et al., 2004). The first known human settlement in Estonia is Pulli in Southwestern Estonia dated to around 11,000 years ago to the Mesolithic (Veski et al., 2005; Kriiska and Lõugas, 2009). The Mesolithic in Estonia can be divided into the Early (9,000–7,000 Before the Common Era (BCE)) and the Late (7,000–3,900 BCE) periods which in turn can be divided into four cultural stages: Pulli (9,000–8,500 BCE), Kunda (8,500–7,000 BCE), Sindi-Lodja (7,000–5,200 BCE) and Narva (5,200–3,900 BCE) (Table 1). The Mesolithic people in Estonia lived near rivers and lakes or by the sea and lived off of hunting-and-gathering (Kriiska, 2003).

The oldest human remains have been found from the Late Mesolithic from around 8,500 years ago (Tõrv, 2016). So far there is knowledge of four Mesolithic burial grounds – Kivisaare, Veibri, Kõnnu and Narva Joaorg – and of some human bones from settlements (Tõrv, 2016).

2.3.2. The Neolithic

The Neolithic in Estonia started around 5,900 years ago (Table 1) when Comb Ceramic culture (CCC), a culture that gets it's name from clay pots that were decorated with a comb-like stamp, reached the area (Jaanits et al., 1982). The CCC people were hunter-gatherers, confirmed by stable isotope analyses of human remains and by animal bones found at settlements (Kriiska, 2001; Tõrv, 2016). The CCC lasted until the end of the Neolithic in Estonia (Lang and Kriiska, 2001).

The Corded Ware culture (CWC) arose around 4,800 years ago (Włodarczak, 2009) and quickly spread around Europe (Krainov, 1972; Kruk and Milisauskas, 1999; Nordqvist, 2016), including to Estonia in the Late Neolithic (Kriiska, 2009) (Table 1). The culture is named after clay vessels with cord impressions as decorations in English and after boat-shaped stone axes in Estonian (Jaanits et al., 1982). The CWC people were farmers, as evidenced by *Cerealia* pollen in bog and lake sediments, barley seed and a seed imprint on a pot shard, sheep/goat, pig and cattle bones and bone artefacts at burial sites, and ^{13}C and ^{15}N stable isotope analyses of human bones (Kriiska, 2003; Lõugas et al., 2007; Rasmussen et al., 2015).

There are four certain CCC burial grounds in Estonia – Kõljala, Naakamäe, Valma and Tamula – and some human remains from settlements (Tõrv, 2016; Kriiska, 1996). A few dozen CWC burial grounds are known in Estonia (mostly from Northern Estonia and Saaremaa (Kriiska, 2000)), but most of the remains have been unearthed during ploughing etc. and have been reburied or lost and only very limited research has been possible at Sope, Narva-Jõesuu, Ardu and Kunila sites (Moora, 1932; Indreko, 1935, 1938; Jaanits, 1985).

2.3.3. The Bronze Age

The Bronze Age in Estonia started around 3,800 years ago and can be divided into Early (1,800–1,100 BCE) and Late Bronze Age (1,100–500 BCE) (Table 1). The first bronze artefacts from Estonia are from the Early Bronze Age (Lang and Kriiska, 2001) but very few settlements can be linked to that period (Lang, 2007). During the Late Bronze Age, people lived in two types of settlements – open (mostly single farms) and enclosed (fortified or hilltop) settlements (Lang, 2007; Kriiska et al., 2017).

While little is known about the burial practices during the Early Bronze Age, the Late Bronze Age is characterized by distinct circular stone structures with a cist inside called stone-cist graves in coastal areas (Kriiska et al., 2017). The grave type and some grave goods point to connections to the west while other grave goods provide a link to the Eastern European Forest Belt (Lang, 2015, 2018). However, stone-cist graves were probably only used by a small part of the society and the burial places of most of the Bronze Age people are unknown (Lang, 2011).

2.3.4. The Iron Age

Settlement types during the Early Iron Age (Table 1) were similar to those of the Late Bronze Age – mostly single households and some fortified settlements (Lang, 2007). There was a sharp decline in the population in the Middle Bronze Age (Table 1) followed by recovering and the establishment of strongholds and villages with active contacts with neighbouring areas, especially during the Viking Age (Table 1) (Kriiska et al., 2017).

The burial practices of the Early Iron Age are somewhat different from those of the Late Bronze Age – circular stone-cist graves are replaced by *tarand* cemeteries with several quadrangular stone enclosures connected to each other (Lang, 2007). These structures have been proposed to mimic the so-called houses of the dead of the Uralic-speaking groups in the Volga-Kama region (Patrushev, 2000). The well-visible stone structures being a burial place only for some sort of an elite is also true during the Early Iron Age (Lang, 2011). Burial practices changed considerably after the Early Iron Age with cremation burials becoming the norm in the Middle and Late Iron Age (Kriiska et al., 2017).

2.3.5. The Middle Ages

The Middle Ages in Estonia started as late as the beginning of the 12th century Common Era (CE) (Table 1) when crusaders conquered the area after which Northern Estonia belonged to Denmark and Southern Estonia became part of Livonia under the rule of the Teutonic Order (Kala et al., 2012). Northern Estonia was also sold to the Order in 1346 and the medieval period in Estonia ended at the end of the 15th century CE (Table 1) with the Russian-Livonian war (Kala et al., 2012).

Probably due to the violent fashion of Christianization, many pre-Christian traditions remained in medieval Estonia, for example most rural people were still buried in cemeteries near villages not in churchyards (Muižnieks, 2015; Valk, 2001). Since the mostly German nobility was buried in churchyards, the village cemeteries represent the local population (Muižnieks, 2015; Valk, 2001).

2.4. The Uralic languages

Estonians together with Hungarians, Finns and other smaller peoples differentiate from most European populations by their languages – the majority of Europeans speak Indo-European languages while these peoples speak languages belonging to the Uralic family.

The Uralic language family is made up of around 40 languages (Abondolo, 1998; Salminen, 2007; https://www.ethnologue.com/subgroups/uralic) and has been proposed to derive from a protolanguage which split into the Samoyed and the Finno-Ugric branch (Figure 7) 4,000–6,000 years ago (Kallio, 2006; Honkola et al., 2013). Most researchers support the hypothesis that Uralic languages originated in the Volga river basin (Koivulehto, 2001; Häkkinen, 2009).
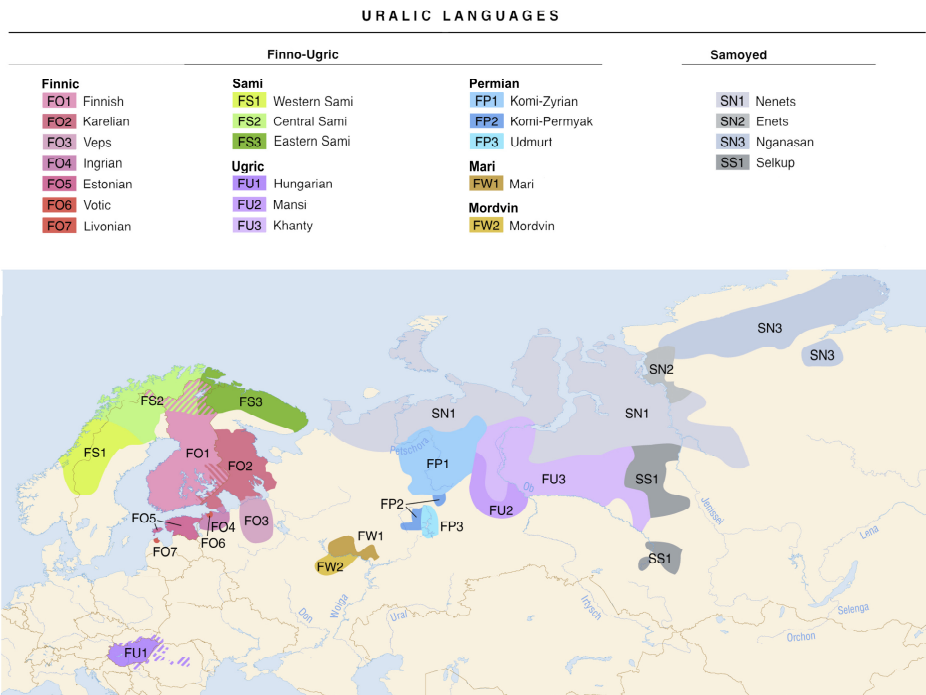
Figure 7. Map of the geographic spread of Uralic languages. Figure adapted with permission from Wikimedia Commons (author: Nug, derived from Chumwa (Maximilian Dörrbecker)), licenced under CC BY-SA Licence.

2.4.1. Uralic languages in Europe

The Uralic languages spoken today in Europe belong to the Finno-Ugric branch of the language family (except the Nenets language from the Samoyed branch spoken both in Europe and in Siberia) (Abondolo, 1998). The classification of the languages of the Uralic linguistic family and the localization of the original core areas of different sub-groups in space are not solved uniformly, but the major subdivisions of the Finno-Ugric branch could be described as follows: Ugric containing Hungarian and the Ob-Ugric sub-group spread in Siberia, Permian containing Komi and Udmurt, Mari containing Mari, Mordvin containing Erzya and Moksha, Saami containing Eastern, Central and Western Saami, and Finnic containing, for example, Estonian, Finnish, Karelian and Veps (Korhonen, 1981) (Figure 7). Many of these languages can be subdivided further (https://www.ethnologue.com/subgroups/uralic). The precursors of the Mordvin and Mari groups and of the Udmurt language of the Permian group remained in the Volga river basin while Proto-Permian (precursor of Komi) spread north (Abondolo, 1998), Proto-Saami northwest (Frog and Saarikivi, 2015) and Proto-Finnic west (Honkola et al., 2013; Lang, 2015). Proto-Hungarian spread southwest during the first millennium CE (Abondolo, 1998).

2.4.2. Uralic languages in Siberia

The Uralic languages spoken in Siberia belong to the Samoyed branch and the Ugric group of the Finno-Ugric branch (Abondolo, 1998). The Samoyed branch consists of Nganasan, Selkup, Nenets and Enets languages, and the Siberian Ob-Ugric sub-group consists of Khanty and Mansi (Korhonen, 1981) (Figure 7). The precursors of Samoyed and Ugric languages were most likely spread near the Ural Mountains (Häkkinen, 2009; Janhunen, 2009). The Samoyed languages later spread east and reached the Taymyr peninsula as recently as in the 16th century CE (Popov, 1966; Carpelan, 2006).

3. AIMS OF THE STUDY

The aim of this thesis was to study the demographic history of Estonia and more broadly, Europe, and to look for genetic similarities between Uralic-speaking populations. The introduction of NGS has allowed for the rapid development of the aDNA field during the last decade. Taking advantage of this new possibility of studying the past using samples from the periods of interest, the more specific aims were as follows:

First (REF I), to study the genetic background of the major cultural changes in Eurasia during the Late Neolithic/Bronze Age.
- To extract DNA from the first ancient individual studied from Estonia and to see how the data fits into the wider context of European Late Neolithic/Bronze Age.

Second (REF II), to find out if the shift from hunting-gathering to farming was accompanied by genetic changes in Estonia.
- To extract DNA from Mesolithic and Neolithic individuals from Estonia.
- To characterize the composition of the autosomal as well as mtDNA, X chromosome (chrX) and chrY ancestries of these individuals in the context of modern and other ancient populations.

Third (REF III), to try to pinpoint the arrival of the ancestry that connects Estonians to Uralic speakers in Siberia.
- To extract DNA from Bronze, Iron and Middle Age individuals from Estonia and Ingria, Russia.
- To characterize the composition of the autosomal as well as mtDNA, chrX and chrY ancestries of these individuals in the context of modern and other ancient populations.

Fourth (REF IV), to characterize the genetic affinities of Uralic-speaking populations from east to west, including Estonians.
- To compare the autosomal affinities of both European and Siberian Uralic speakers to other modern and ancient populations, taking into account geographic and linguistic origins.
- To contrast the autosomal and chrX affinities of a population of interest to other modern populations, taking into account geographic origins and the probability of a man from the population of interest and a man from the comparison population sharing chrY hg N3-M178.

4. MATERIALS AND METHODS

The origin and archaeological context of the ancient samples of this study is described in detail in the supporting materials of the research articles. The aDNA samples were obtained through a collaboration with the archaeologists of the University of Tartu. The samples were collected following the Code of Ethics of Estonian archaeologists (www.arheo.ut.ee/eesti-arheoloogide-eetikakoodeks).

The modern DNA samples were obtained from unrelated volunteers after receiving informed consent in accordance with the guidelines of the ethical committees of the institutions involved.

The experimental and bioinformatic methods of the present study are described in the respective publications and their supplementary materials.

5. RESULTS AND DISCUSSION

This section is a summary of the four research articles that present the novel results of this study. The aim of the section is to provide an overview of the main results of the articles; more detailed information about the findings can be found in the publications themselves and their supplemental materials.

5.1. The cultural changes in Late Neolithic/Bronze Age Eurasia are a result of large-scale human migrations (REF I)

In this study, we assembled a set of 101 ancient genomes from Eurasia from the Late Neolithic to the Iron Age – the largest aDNA dataset published at the time (Figure 8). This was possible due to improving aDNA extraction methods in the course of the study (Methods, REF I). One example of this, which I also did with the Estonian individual, is sampling both the root cementum and the inner dentine of a tooth and seeing that the cementum produces more endogenous DNA.

5.1.1. Genetic changes in Bronze Age Europe

We compared our newly sequenced genome-wide data to previously published modern and ancient samples (Lazaridis et al., 2014; Fu et al., 2014; Seguin-Orlando et al., 2014; Gamba et al., 2014; Skoglund et al., 2014; The 1000 Genomes Project Consortium, 2012; Raghavan et al., 2014; Olalde et al., 2014; Keller et al., 2012) and found that the Late Neolithic/Bronze Age individuals of Europe (Scandinavia, Eastern Baltic and Central Europe) showed an influx of genetic material compared to the Early Neolithic farmers of Central Europe and Scandinavia. Principal component analysis (PCA) and ADMIXTURE analysis (Alexander et al., 2009) point to the Caucasus as the origin of this ancestry (Figure 2, REF I). However, the Caucasian ancestry reached Europe through the steppe since CWC individuals from Central Europe and Estonia were closer to Yamnaya culture individuals from the Pontic-Caspian steppe than to the individuals of Bronze Age Armenia using D statistics (Extended data table 1). CWC having been the result of admixture between Early Neolithic farmers and Yamnaya culture people is further confirmed by negative admixture f3 values (Extended data table 2, Supplementary table 12, REF I).

The genetic change was also clear in the male lineages with most of the individuals sampled from the context of the CWC belonging to chrY hg R1a (Extended data figure 6, REF I) not found among the hunter-gatherers and early farmers of Europe (Kivisild, 2017). Interestingly though, most of the Yamnaya individuals of the study belonged to chrY hg R1b and none to R1a (Extended data figure 6, REF I).

Our autosomal, as well as mtDNA and chrY results show that the Late Neolithic/Bronze Age genetic structure was much more similar to that of modern Europe than it had been during the previous time layers. However, further changes during later periods and the more specific demographic processes in various parts of Europe remain to be addressed in following studies.
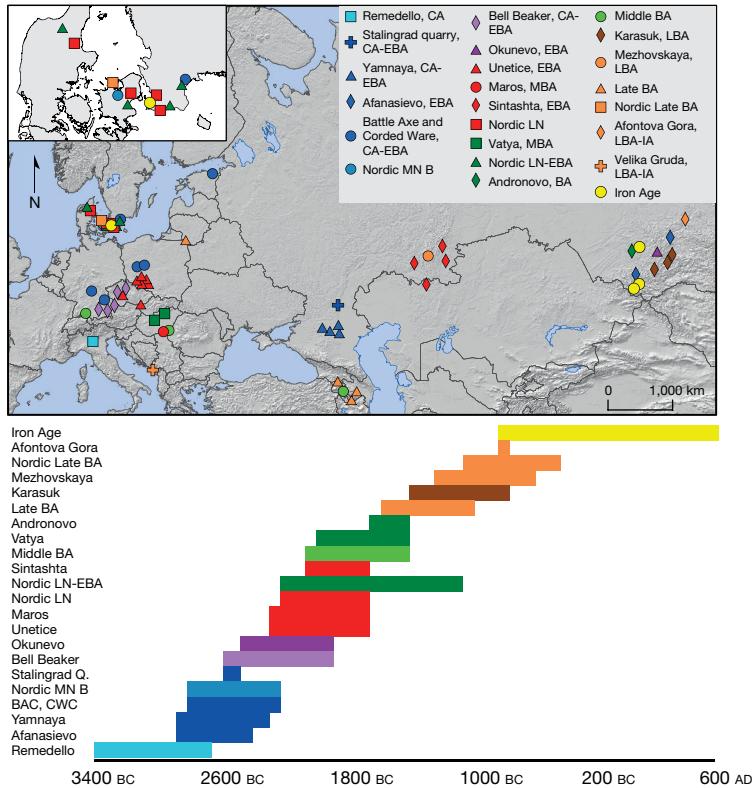
Figure 8. Sites, cultural context and approximate timeline of the samples of this study. CA, Copper Age; MN, Middle Neolithic; LN, Late Neolithic; EBA, Early Bronze Age; MBA, Middle Bronze Age; LBA, Late Bronze Age; IA, Iron Age; BAC, Battle Axe culture; CWC, Corded Ware culture. Figure reprinted with permission from Figure 1 left (REF I), Springer Nature.

5.1.2. Genetic changes in Bronze Age Asia

We also made comparisons between individuals of Bronze Age Asia and previously published modern and ancient data (Lazaridis et al., 2014; Fu et al., 2014; Seguin-Orlando et al., 2014; Gamba et al., 2014; Skoglund et al., 2014; The 1000 Genomes Project Consortium, 2012; Raghavan et al., 2014; Olalde et al., 2014; Keller et al., 2012), which showed that human migrations played an equally important role there. The Afanasievo culture people of the Altai-Sayan

region were extremely similar to Yamnaya culture people, meaning that there was no admixture involved in the emergence of this culture unlike for CWC in Europe (Figure 3b, Extended data figure 2b, Extended data table 1, REF I). These results also point to the migration from the Pontic-Caspian steppe being multidirectional, expanding both towards the west and the east.

The Sintashta culture people, who lived on the border of Europe and Asia, and the Andronovo culture people of Central Asia, on the other hand, were most similar to the CWC individuals of Central Europe and Estonia (Extended data figure 2ac, REF I). This is indicative of these cultures being the result of back migration from west to east.

What is more, the Okunevo people of the Sayano-Altai region were similar to modern Native Americans (Extended data figure 2d, REF I). This means that they might have been a remnant of the Upper Paleolithic population that contributed to the gene pool of Native Americans (represented by the Mal'ta boy (Raghavan et al., 2014)).

5.1.3. Temporal dynamics of phenotypically informative variants

We found an increase in the frequency of two alleles associated with light skin pigmentation (rs1426654, rs16891982) between the Mesolithic and the Bronze Age in Europe (Figure 4a, Supplementary table 13, REF I). On the other hand, there was no considerable increase in the frequency of the lactase persistence allele (rs4988235), pointing to the onset of the positive selection on this allele starting later than the start of dairying in Europe (Figure 4, Supplementary table 13, REF I). The allele associated with blue eyes (rs12913832) was at intermediate frequency in Bronze Age Europeans but absent in the Pontic-Caspian steppe populations (Figure 4, Supplementary table 13, REF I).

5.2. A sex-biased migration is connected to farming reaching Estonia (REF II)

To find out if migration of people (rather than ideas) had a significant role in the spread of farming to Estonia, we extracted DNA from 10 individuals from Mesolithic and Neolithic Estonia radiocarbon dated to between around 6300 to 4500 years ago. One of the individuals was a Mesolithic hunter-gatherer of Narva culture, 4 were Neolithic hunter-gatherers of CCC and 5 were Neolithic farmers of CWC (Figure 1, Table 1, Methods, REF II).

5.2.1. Maternal and paternal lineage diversity in Mesolithic and Neolithic Estonia

All of the successfully haplogrouped hunter-gatherers of the study at hand – the Mesolithic Narva culture individual as well as 3 Neolithic CCC individuals – belonged to mtDNA hg U (Table 1, Table S2, REF II) as expected from previous studies (Skoglund et al., 2012, 2014; Sánchez-Quinto et al., 2012; Fu et al., 2013b; Jones et al., 2015; Fu et al., 2016; Posth et al., 2016). The Neolithic farmers of CWC, on the other hand, displayed a much larger variety of mtDNA lineages, belonging to hgs T, J and H in addition to U (Table 1, Table S2, REF II). This is also in accordance with previous studies focusing both on the Early Neolithic and the CWC farmers of Europe (Skoglund et al., 2012, 2014; Haak et al., 2005, 2010; Bollongino et al., 2013; Brandt et al., 2013, 2015; Szécsényi-Nagy et al., 2015; Mathieson et al., 2015; Haak et al., 2015; REF I).

Due to low coverage, the chrY hg of Mesolithic Narva individual could not be determined, but the only male CCC hunter-gatherer belonged to hg R1a5 (Table 1, Table S3, REF II). That is an early lineage of R1a, and similar lineages have also been found in EHG (Haak et al., 2015). All four CWC males, however, belonged to hg R1a-Z645 (Table 1, Table S3, REF II), a lineage most frequent in the carriers of this culture also in Central Europe (REF I; Haak et al., 2015; Mathieson et al., 2015). These results suggest that admixture between CCC hunter-gatherers and CWC farmers was limited, at least in the male lineages.

5.2.2. Genome-wide ancestries of Neolithic Estonia

We compared our newly generated data to previously published modern and ancient genomes (Lazaridis et al., 2016; Jones et al., 2017) using PCA and ADMIXTURE analysis (Alexander et al., 2009) as well as f3 and D statistics. We found that the last hunter-gatherers of Estonia were most similar to another CCC hunter-gatherer from Latvia and to EHGs (Figure 9; Figure 2B, Figure 3AB, Figure S2, Data S1, REF II). Other studies, however, have shown that Mesolithic hunter-gatherers from the Eastern Baltic were most similar to WHGs (Jones et al., 2017; Mittnik et al., 2018). This points to a genetic influx from the east being involved in the arrival of CCC in Estonia and in the Eastern Baltic.

Furthermore, when using the same analyses to make comparisons involving the CWC samples of this study, we found that these individuals were similar to Late Neolithic/Bronze Age populations in other parts of Europe and clearly different from the previous CCC people (Figure 9; Figure 2B, Figure 3AB, Figure S2, Data S1, REF II). This means that farming in Estonia started as a result of human migration. However, it is important to note that the first farmers of Estonia were not the descendants of the first wave of farmers into Europe from Anatolia (Mathieson et al., 2015 and others, see chapter 2.2.2) but the next big migration wave into Europe from the Pontic-Caspian steppe (REF I; Haak et al., 2015).
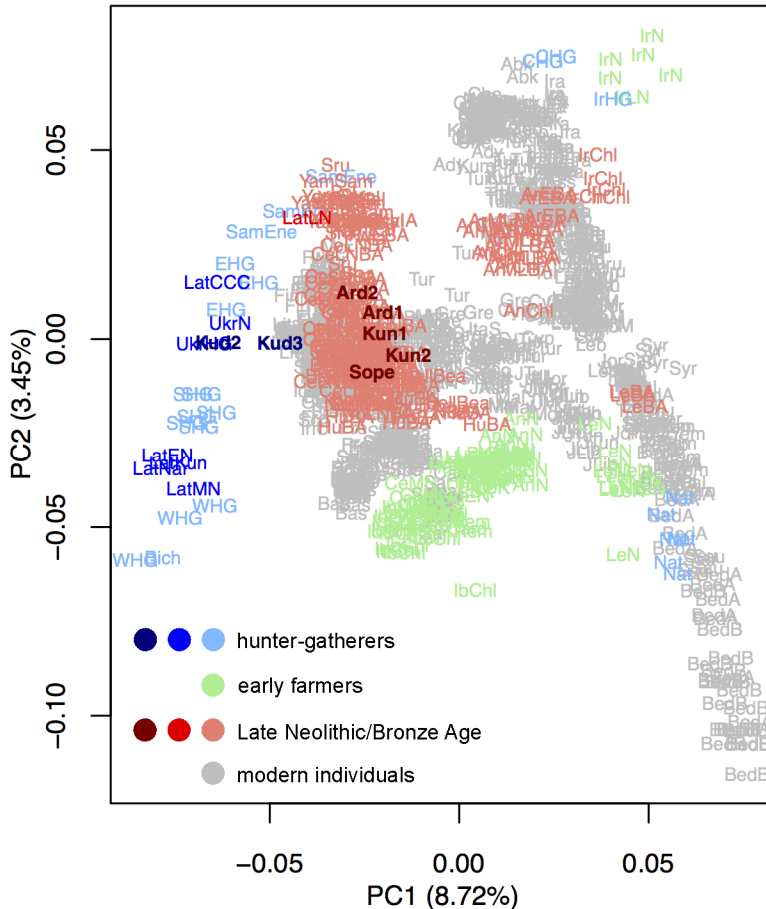
Figure 9. Principal component analysis results. Principal component analysis results of modern West Eurasians with ancient individuals (including seven ancient Estonians) projected onto the first two components (PC1 and PC2). In the legend, the following are shown from left to right: this study, Jones et al. 2017, and Lazaridis et al. 2016. Kud2, 3 – Kudruküla; Ard1, 2 – Ardu; Kun1, 2 – Kunila. Figure reprinted with permission from Figure 2A (REF II), Elsevier.

We studied the demographic processes involved in farming reaching Estonia further by comparing the autosomal and chrX ancestries of the Estonian CWC individuals. Both men and women contribute one of each autosomal chromosome to all children, but men only pass on their chrX to their daughters while boys always get their chrX from their mother. This means that in this comparison, autosomal data gives more information about the male and X chromosomal data about the female lineage. We found that the Anatolian early farmer ancestry that reached Estonia through CWC people was more prominent in the chrX (Figure 3D, Data S1, REF II), suggesting that the admixture involved was biased towards Yamnaya ancestry in the male and early farmer ancestry in the female lineages.

5.3. Bronze and Iron Age genomes reveal genetic influxes in Estonia from multiple directions (REF III)

We newly sequenced 56 ancient genomes to study the demographic history of Estonia from the Bronze Age onward and to find out, when chrY hg N3a (nomeclature as in Karmin et al., 2015) reached the Eastern Baltic. This hg is frequent in modern Estonians, but also in other, mostly Uralic-speaking, European populations and many populations in West Siberia and the Far East (Ilumäe et al., 2016; Tambets et al., 2004; Pliss et al., 2006; Rootsi et al., 2007; REF IV). What is more, the time of the transition from Bronze to Iron Age matches the hypothesized period of the diversification of western Uralic languages (Honkola et al., 2013) and of the Proto-Finnic language reaching the Eastern Baltic from the east (Kallio, 2006; Häkkinen, 2009). Our sample consists of 23 individuals mostly from stone-cists graves from Late Bronze Age Estonia (1200–400 BC), 14 mostly from *tarand* cemeteries from Pre-Roman Iron Age Estonia (800/500 BC–50 AD), 12 from Pre-Roman to Roman Iron Age Ingria, Russia (500 BC–450 AD) and 7 from Middle Age Estonia (1200–1600 AD) (Figure 1A, Table 1, Table S1, REF III).

5.3.1. Maternal and paternal lineage diversity in Bronze to Middle Age Estonia

The samples of the given study belonged to various different hgs – multiple lineages of hgs U, T, J, H, K, W, HV and I (Table 1, Table S1, REF III). A comparison to modern Estonian whole mtDNA sequences (unpublished) revealed that all of these lineages are also present in the modern-day Estonian population and not restricted to a specific area.

The chrY diversity was low in the Bronze Age with all 16 successfully haplogrouped males belonging to hg R1a and none to N3a (Table 1, Table S1, Table S2, REF III). This means that no change could be seen from the previous CWC in the Eastern Baltic (REF II; Jones et al., 2017; Mittnik et al., 2018; Mathieson et al., 2018). Admittedly, the sample size is small, but even so, the frequency of N3a in the population should not exceed 17% with 95% confidence. We did, however, detect hg N3a in the Iron and Middle Age groups (3/6 in both) (Table 1, Table S1, Table S2, REF III). From these results, we can conclude that chrY hg N3a most likely reached the Eastern Baltic during the transition from Bronze to Iron Age.

5.3.2. Genome-wide ancestry in Estonia from the Bronze Age onward

We also tested if autosomal data reveals similar patterns by using PCA and ADMIXTURE analysis (Alexander et al., 2009), f3 and D statistics, the program qpAdm (Patterson et al., 2012) and the CromoPainter-NNLS pipeline (Lawson et al., 2012; Hofmanová et al., 2016; Leslie et al., 2015; Montinaro et al., 2015). Unlike uniparental lineages, autosomal data revealed a detectable shift in ancestry between the CWC and the Bronze Age individuals. The Bronze Age samples displayed an increase in ancestry related to WHGs (Figure 1BC, Figure 2A, Figure S1, Figure S2AB, Data S1, REF III).

Furthermore, the change in paternal lineage diversity between Bronze and Iron Age was not as clearly mirrored in autosomal data. Individuals from Bronze, Iron and Middle Ages, and modern Estonians seemed quite similar in some of the analyses (Figure 1BC, Figure 2A, Figure S1, Data S1, REF III), but more detailed studies revealed the presence of around 3% Siberian (Nganasan used as proxy) ancestry starting from the Iron Age (Figure 10; Data S1, REF III). A small increase in early farmer ancestry was also detected between Bronze and Iron Age individuals and modern Estonians (Data S1, REF III) while the sex-bias seen in this ancestry in the CWC individuals (REF II) decreased in time (Figure S2C–F, Data S1, REF III).
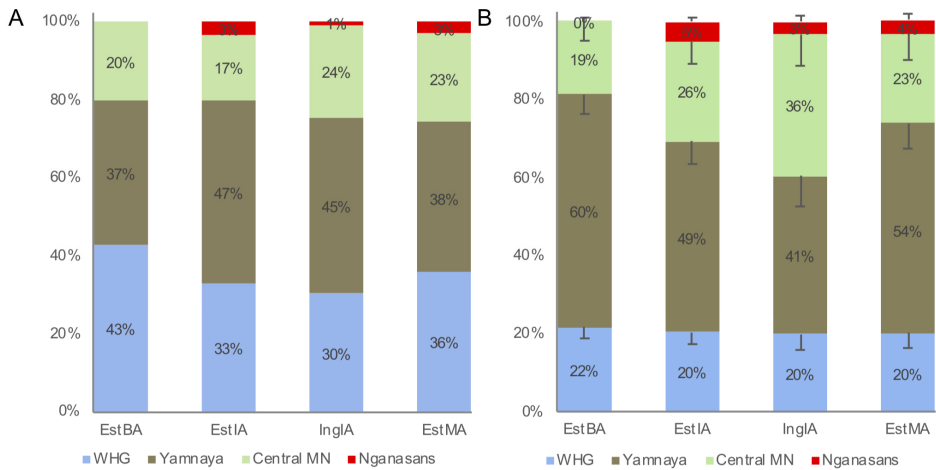

Figure 10. ChromoPainter-NNLS and qpAdm results. EstBA – Estonian Bronze Age; EstIA – Estonian Iron Age; IngIA – Ingrian Iron Age; EstMA – Estonian Middle Ages; WHG – Western hunter-gatherers; Central MN – Central European Middle Neolithic. A. ChromoPainter unlinked mode summarized results. B. qpAdm results. Figure reprinted with permission from Figure 3 (REF III), Cell Press.

5.3.3. Relatives in Bronze Age sample and phenotypic changes
in the Eastern Baltic through time

We used READ (Monroy Kuhn et al., 2018) and found a pair of second-degree relatives in our Bronze Age sample – most likely an uncle and his sister's son (Figure S3A–D, REF III). Interestingly, these individuals were not buried in the same grave group, but 13 km apart. These results point to the stone-cist graves being used for a limited circle of people.

We further imputed the genotypes of phenotype informative positions (Methods, REF III) and inferred that the frequency of the lactase persistence allele was low in Estonia in the Late Neolithic, as elsewhere in Europe (REF I), but increased considerably (to over 50%) in the Bronze Age (Data S2, REF III). We also inferred a change in the frequencies of alleles connected with skin pigmentation, hair and eye colour from mostly dark for the hunter-gatherers and Neolithic farmers of Latvia and Estonia to lighter starting from the Bronze Age in Estonia (Data S2, REF III).

5.4. Traces of shared demographic history can be found
in the genomes of most of modern Uralic speakers (REF IV)

In this study, we set out find out whether genetic connections can be found between modern Uralic speakers from Europe and Siberia not only in their paternal lineages but also in the rest of the genome. For that, we put together a genotyping dataset of individuals from 15 populations (Table S1, REF IV), covering the main groups of the Uralic language family (Table S2, REF IV).

5.4.1. Female and male demographic histories of Uralic speakers

The mtDNA lineages of most of the studied Uralic speakers are typical to West Eurasia (Figure 1B, Table S3, Table S4, REF IV). Nganasans and West Siberian Nenets are the exception with mostly East Eurasian lineages (Figure 1B, Table S3, Table S4, REF IV).

However, when looking at chrY, East Eurasian lineages, more specifically hg N lineages, are much more common – especially in Siberian, but also in European Uralic speakers (Figure 1B, Table S5, REF IV). Here the exceptions are Hungarians with almost no prevalence of this hg, and Selkups with frequencies much lower than in other Samoyed speakers (Table S5, REF IV).

We further tested the differences in the male and female demographic histories of Uralic speakers by comparing their autosomal and chrX ancestries using outgroup f3 analysis (Figure 11). We grouped the populations based on the probability of a randomly chosen pair of men – one from a given comparison population and the other from the study population – to share chrY hg N3-M178.

We found that population pairs for which this probability is over 10% compared to under 5% have a significant although small excess of autosomal rather than chrX affinity to each other (Figure 11).
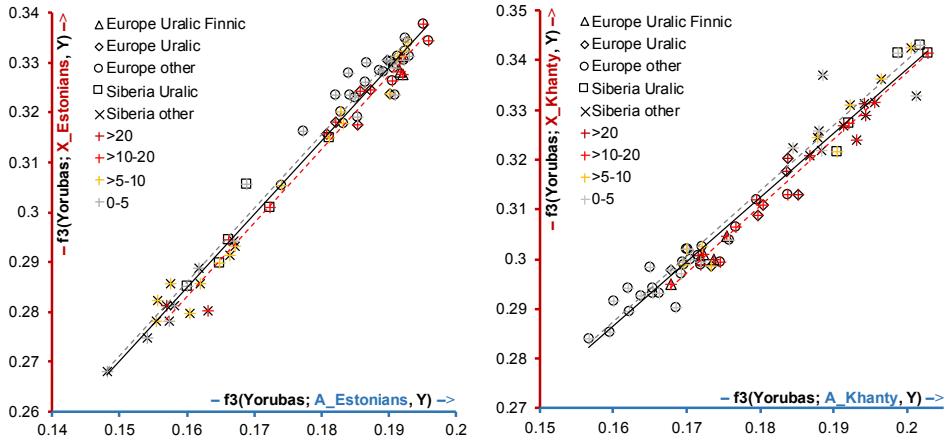
Figure 11. Comparison of autosomal (x-axis) and X chromosome (y-axis) outgroup f3-statistics for Estonians and Khanty. Four classes of probability values (%) for a pair of men from two populations − Estonians (left) or Khanty (right) vs others − to share Y chromosome hg N3-M178 are shown with crosses of different colours. The overall trendline (black) and trendlines for lower (<5%, grey) and higher (>10%, red) probability classes are shown. Differences of slopes of the grey and red trendlines were non-significant (Estonians p=0.96, Khanty p=0.73). Differences of the interception points of the grey and red trendlines were significant (Estonians p=0.01532, Khanty p=0.00542). Figure adapted with permission from Figure S13 (REF IV), Springer Nature.

5.4.2. The population structure and genetic connections of Uralic speakers

PCA, F_{ST} distances and lower K's of ADMIXTURE analysis (Alexander et al., 2009) mirror the geographic rather than the linguistic relationships of the populations (Figure 2, Figure 3A, Figure S1, Figure S2, Figure S3, REF IV). Higher K's of ADMIXTURE, however, reveal a genetic component that is shared by most of Uralic speakers and some of their neighbouring populations (Figure 3, Figure S3, REF IV). This component shows the highest frequencies in Ob-Ugric and Samoyed speakers, and also neighbouring Kets (Figure 3, Figure S3, REF IV). Both Uralic- and Turkic-speaking populations of the Volga-Ural region also have high frequencies of this component and it is further present in most of Northeast European Uralic speakers (in especially high frequency in Saami from Sweden) and interestingly also North Russians (Figure 3, Figure S3, REF

IV). The westernmost Uralic speakers Estonians and Hungarians, however, have very little or none of the component (Figure 3, Figure S3, REF IV).

D statistics suggested that most of Western Uralic speakers (Saami, Finns and Estonians), compared to their Indo-European neighbours share more genetic ancestry with Siberian populations while other European populations share more with their proximate geographic neighbours (Figure S4, REF IV). Notably, Hungarians behaved like non-Uralic speakers in this analysis (Figure S4, REF IV).

Next, we tested identity-by-descent (IBD) (Browning and Browning, 2011) segment sharing and found that Uralic speakers compared to non-Uralic speakers from Siberia and the Volga-Ural region (except Mordovians) share more with most of the other Uralic-speaking populations from these two regions and also with some Northeast European Uralic speakers (Figure 4, Table S8, Table S9, REF IV). What is more, Northeast European Uralic speakers rather than their non-Uralic-speaking neighbours mostly share more with other Uralic speakers from their own region, the Volga-Ural region and Siberia, and in some cases even with distant non-Uralic speakers (Figure 4, Table S7, REF IV). The only populations that do not show a higher genetic affinity to Uralic speakers from other regions are Estonians and Hungarians (Figure 4, Table S7, Table S8, REF IV).

The haplotype-based regional Globetrotter analysis (Hellenthal et al., 2014) (Figure 5B, Table S10, Table S11, REF IV), which reveals admixture events between sample clusters (identified using fineSTRUCTURE (Lawson et al., 2012) (Figure S5, Figure S6, REF IV)) not belonging to the same geographic region as the target, points to the connections between Uralic speakers in Europe and Siberia being unidirectional from east to west (Figure 5B, Table S10, Table S11, REF IV). The Siberian clusters mostly show admixture events within the region or also involving the Volga-Ural region (Figure 5B, Table S10, Table S11, REF IV). Samoyed-speaking clusters further have admixture from East Asia/South Siberia (Figure 5B, Table S10, Table S11, REF IV). Interestingly, as estimated using ALDER, the admixture from East Eurasia into Europe took place 800–900 CE or earlier but from West Eurasia into Siberia later than 1500 CE (Figure S7, Table S12, REF IV).

We also found that lexical and genetic distances of Uralic speakers are significantly positively correlated when using autosomal genetic data in the form of a fineSTRUCTURE (Lawson et al., 2012) matrix based on coancestry determined by shared chunk counts, or an F_{ST} distance matrix (Table S14, Table S15, REF IV). However, geographic distances significantly predict genetic distances when lexical distance is kept constant, meaning that both the geographic and lexical distances contribute to the genetic variation of Uralic speakers (Table S15, REF IV).

5.4.3. Affinities between Uralic speakers and ancient Eurasians

Lastly, we used the program qpGraph (Patterson et al., 2012) to see, if and in which proportions modern Uralic-speaking populations can be modeled as a

mixture of five ancestral populations: WHG, EHG, Linearbandkeramik (LBK; early farmers), CWC (steppe ancestry) and Nganasan (Siberian proxy) (Figure S10, REF IV). We found that the Siberian ancestry accounts for most of the genome of Uralic speakers in West Siberia, up to one third in the Volga-Ural region and in Saami, and less than 10% in Northeast Europe (5% in Estonians) (Figure 6, REF IV). Hungarians are the only Uralic-speaking population that could not be modeled using these ancestral populations due to them not having the Siberian component. What is more, the non-Uralic-speaking neighbours of Estonians (Latvians and Lithuanians) have almost no Siberian ancestry in their autosomes (Figure 6, REF IV).

6. CONCLUSIONS

- A genetic influx from the east was involved in the arrival of the hunting-gathering Comb Ceramic culture in Estonia during the Early Neolithic.
- The major cultural changes in Eurasia during the Late Neolithic/Bronze Age were connected to the expansion of the Yamnaya culture from the Pontic-Caspian steppe. In Europe (including Estonia), the Yamnaya expansion and admixture with Neolithic early farmer populations resulted in the Corded Ware culture. In Asia, this expansion gave rise to the Afanasievo culture, without significant admixture with local populations. However, the Sintashta and Andronovo cultures in Asia were likely the result of back migration of Corded Ware culture people from Europe.
- The Corded Ware culture people with Yamnaya ancestry were the first farmers of Estonia, meaning that the Anatolian early farmers who brought farming into most parts of Europe did not reach Estonia. What is more, the limited amount of early farmer ancestry that reaches Estonia with the Corded Ware culture people was more prominent in the female lineages, pointing to sex-biased admixture.
- An increase in Western hunter-gatherer ancestry can be seen in the autosomal data of Bronze Age Estonians. Interestingly, this change was not detected in their mtDNA and chrY lineages. Furthermore, light hair, skin and eyes, and lactose tolerance become frequent in Estonia during this period.
- A genetic component most similar to modern Siberians had been added to the genomes of Iron Age Estonians, very clearly visible in the composition of their chrY lineages (hg N3a) and less apparent in their autosomal data. ChrY hg N3a is present in high frequencies in modern Estonians, in other Uralic speakers in northeastern Europe and in populations of West Siberia and the Far East. The time of the arrival of the Siberian ancestry in Estonia coincided with the proposed arrival of Proto-Finnic language in the Eastern Baltic region.
- On a genome-wide scale, modern Uralic speakers are most similar to their geographic neighbours but, importantly, most of them share an ancestry component of possibly Siberian origin and share more IBD segments with each other over long distances than with other equidistant populations.

SUMMARY IN ESTONIAN

Eesti esiajalugu geneetika vaatevinklist:
uus teave vana DNA uuringutest

Juba aastakümneid on uuritud populatsioonide demograafilist ajalugu vorminud minevikus aset leidnud geneetilisi muutusi ja protsesse, kasutades selleks tänapäeval elavate inimeste geneetilisi andmeid. Pärast teise põlvkonna sekveneerimistehnoloogiate kasutuselevõttu peaaegu 15 aastat tagasi on sellesse valdkonda hakanud oluliselt panustama ka vana DNA uurimisel põhinev teadustöö. Tänapäevaseid genoome analüüsides on võimalik ennustada, millised protsessid ja millal populatsioone mõjutanud on. Vana DNA uurimine võimaldab neid ennustusi kontrollida ja lisab neile geograafilise mõõtme, kuna iga ammune genoom on seotud mingi kindla aja ja kohaga. Lisaks on vana DNA abil võimalik leida populatsioone, millel tänapäeva genoomidesse panustada ei õnnestunud.

Vana DNA uuringute peamised piirangud on seotud saadavaloleva bioloogilise materjali piiratud hulga, keskkonnatingimustest sõltuvast DNA säilimisest tulenevate geograafiliste piirangute ja sedalaadi uurimistöö kulukusega. Viimase põhjuseks on see, et vana DNA on mitmesuguste keemiliste protsesside tagajärjel tugevalt kahjustunud, mistõttu on selle uurimiseks vaja kasutada spetsiaalset puhaslaborit ning spetsiifilisi meetodeid. Nende piirangute mõju vähendamiseks arendatakse pidevalt laboritöö ja analüüside metoodikaid, et oleks võimalik suurema hulga andmete abil vastata rohkematele küsimustele.

Selle doktoritöö peamiseks uurimisteemaks on Euroopa ja täpsemalt Eesti demograafiline ajalugu. Nüüdisinimesed jõudsid Euroopasse umbes 45 000 aastat tagasi. Need küttimisest ja korilusest elatuvad inimesed elasid refuugiumides üle ka viimase jääaja maksimumi ning asustasid hiljem taas kogu Euroopa ja moodustasid geneetiliselt, geograafiliselt ja mõnel juhul ka ajaliselt eristuvad grupid. Umbes 8000 aastat tagasi jõudis Euroopasse põlluharimine koos Anatoolia ja Levanti päritolu inimestega, kes tänapäeva populatsioonidest olid geneetiliselt kõige sarnasemad sardiinlastega.

Arheoloogilistele andmetele toetudes erineb Eesti demograafiline ajalugu mõneti Euroopa kesksemate alade omast – esimesed inimasustuse jäljed pärinevad jääajajärgsest perioodist umbes 11 000 aastat tagasi ning põlluharimine sai alguse alles hilisneoliitikumis nöörkeraamika kultuuri saabumisega. Tänapäeva eestlasi, soomlasi ja mitmeid väiksemaid Ida-Euroopa ja Lääne-Siberi rahvaid eristab ülejäänud eurooplastest kõige selgemini meie keel. Uurali keelkond pärineb tõenäoliselt Volga-Kama piirkonnast, kust tänaste uurali keelte eelkäijad levisid mitmes suunas, nii et praegused nende keelte kõnelejad elavad üksteisest kaugel Euroopa eri piirkondades ja Siberis.

Selle doktoritöö raames uuriti tänasel Eesti territooriumil nooremal kiviajal ehk neoliitikumis elanud inimeste geenipärandit suuri Euraasia piirkondi mõjutanud migratsioonide kontekstis, Eesti alal erinevatel ajaperioodidel toimunud

spetsiifilisemaid muutusi ja protsesse ning tänapäeva eestlaste geneetilist pärit-olu ja eriti seoseid teiste uurali keelte kõnelejatega.

Töö peamised tulemused ja järeldused on järgmised:
- Küttimise-korilusega seostatud varaneoliitilise kammkeraamika kultuuri Eestisse jõudmisega kaasnes inimeste lisandumine idast.
- Hilisneoliitikumis ja pronksiajal aset leidnud suured kultuurilised muutused olid seotud aukhaudade kultuuri inimeste rändamisega Musta ja Kaspia meredega piirnevalt stepialalt nii Euroopa kui Aasia suunas. Euroopas (sh Eestis) tekkis varajaste põlluharijatega segunemise järel nöörkeraamika kultuur ning Aasias ilma erilise segunemiseta Afanasjevo kultuur ja nöör-keraamika kultuuri inimeste tagasirände tulemusena Sintashta ja Andronovo kultuurid.
- Eesti esimesed põlluharijad olid stepirahvastest pärinevad nöörkeraamika kultuuri esindajad, mis tähendab, et Anatoolia päritoluga varased põlluhari-jad Eestisse ei jõudnud. Nöörkeraamika kultuuri inimestega meie alale jõud-nud vähene Anatoolia päritolu oli sagedasem emaliinis, mis viitab soospet-siifilisele segunemisele – stepipäritolu seostub eelkõige meeste ja Lähis-Ida mõjutused naistega.
- Pronksiaegsete kivikirstkalmetesse maetute genoomides oli varasemaga võrreldes suurenenud Lääne-Euroopa küttide-korilastega seotud geneetiline komponent, mis samas ei kajastunud huvitaval kombel nende mitokondriaal-ses DNA-s ega Y kromosoomis. Just pronksiajal muutusid sagedaseks ka tänapäeva eestlastele (ja põhjaeurooplastele) omased heledad juuksed, nahk ja silmad ning laktoositaluvus.
- Rauaajal tarandkalmetesse maetute genoomidesse oli lisandunud geneetiline komponent, mis on sage tänapäevastes Siberi populatsioonides. See idapoolne mõju oli sage Y kromosoomis (haplogrupp N3a) ja väiksemamahuline üle-jäänud genoomis. Haplogrupp N3a on sage ka tänapäeva eestlaste ja teiste Kirde-Euroopa uurali keelte kõnelejate ning Lääne-Siberi ja Kaug-Ida popu-latsioonide meesliinides. Ühtlasi kattus Siberi päritolu Eestisse jõudmise aeg keeleteadlaste poolt välja pakutud läänemeresoome keelte saabumisajaga Läänemere idakaldale.
- Tänapäevased uurali keelte kõnelejad on ülegenoomsete andmete alusel kõige sarnasemad enda geograafilistele naabritele, kuid sellegipoolest jagab enamik neist veidi rohkem hiljutist ühise päritoluga geneetilist materjali teiste sama keelkonna esindajatega kaugemal, kui nende teisi keeli kõnelevad naabrid.

REFERENCES

Abondolo, D. (1998). The Uralic Languages (London: Routledge).

Adler, C.J., Haak, W., Donlon, D., and Cooper, A. (2011). Survival and recovery of DNA from ancient teeth and bones. J. Archaeol. Sci. *38*, 956–964.

Adler, C.J., Dobney, K., Weyrich, L.S., Kaidonis, J., Walker, A.W., Haak, W., Bradshaw, C.J.A., Townsend, G., Sołtysiak, A., Alt, K.W., et al. (2013). Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. Nat. Genet. *45*, 450–455, 455e1.

Alexander, D.H., Novembre, J., and Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. Genome Res. *19*, 1655–1664.

Allentoft, M.E., Collins, M., Harker, D., Haile, J., Oskam, C.L., Hale, M.L., Campos, P.F., Samaniego, J.A., Gilbert, M.T.P., Willerslev, E., et al. (2012). The half-life of DNA in bone: measuring decay kinetics in 158 dated fossils. Proc. Biol. Sci. *279*, 4724–4733.

Allentoft, M.E., Sikora, M., Sjögren, K.-G., Rasmussen, S., Rasmussen, M., Stenderup, J., Damgaard, P.B., Schroeder, H., Ahlström, T., Vinner, L., et al. (2015). Population genomics of Bronze Age Eurasia. Nature *522*, 167–172.

Alonso, A., Andelinović, S., Martín, P., Sutlović, D., Erceg, I., Huffine, E., de Simón, L.F., Albarrán, C., Definis-Gojanović, M., Fernández-Rodriguez, A., et al. (2001). DNA typing from skeletal remains: evaluation of multiplex and megaplex STR systems on DNA isolated from bone and teeth samples. Croat. Med. J. *42*, 260–266.

Alvarez García, A., Muñoz, I., Pestoni, C., Lareu, M.V., Rodríguez-Calvo, M.S., and Carracedo, A. (1996). Effect of environmental factors on PCR-DNA analysis from dental pulp. Int. J. Legal Med. *109*, 125–129.

Ammerman, A.J., and Cavalli-Sforza, L.L. (1984). The Neolithic Transition and the Genetics of Populations in Europe (Princeton: Princeton University Press).

Amorim, C.E.G., Vai, S., Posth, C., Modi, A., Koncz, I., Hakenbeck, S., La Rocca, M.C., Mende, B., Bobo, D., Pohl, W., et al. (2018). Understanding 6th-century barbarian social organization and migration through paleogenomics. Nat. Commun. *9*, 3547.

Anderson-Carpenter, L.L., McLachlan, J.S., Jackson, S.T., Kuch, M., Lumibao, C.Y., and Poinar, H.N. (2011). Ancient DNA from lake sediments: bridging the gap between paleoecology and genetics. BMC Evol. Biol. *11*, 30.

Andrades Valtueña, A., Mittnik, A., Key, F.M., Haak, W., Allmäe, R., Belinskij, A., Daubaras, M., Feldman, M., Jankauskas, R., Janković, I., et al. (2017). The Stone Age Plague and Its Persistence in Eurasia. Curr. Biol. *27*, 3683-3691.e8.

Austin, J.J., Ross, A.J., Smith, A.B., Fortey, R.A., and Thomas, R.H. (1997). Problems of reproducibility – does geologically ancient DNA survive in amber–preserved insects? Proc. R. Soc. Lond. B Biol. Sci. *264*, 467–474.

Balaresque, P., Bowden, G.R., Adams, S.M., Leung, H.-Y., King, T.E., Rosser, Z.H., Goodwin, J., Moisan, J.-P., Richard, C., Millward, A., et al. (2010). A Predominantly Neolithic Origin for European Paternal Lineages. PLOS Biol. *8*, e1000285.

Benazzi, S., Douka, K., Fornai, C., Bauer, C.C., Kullmer, O., Svoboda, J., Pap, I., Mallegni, F., Bayle, P., Coquerelle, M., et al. (2011). Early dispersal of modern humans in Europe and implications for Neanderthal behaviour. Nature *479*, 525–528.

Bollongino, R., Nehlich, O., Richards, M.P., Orschiedt, J., Thomas, M.G., Sell, C., Fajkosová, Z., Powell, A., and Burger, J. (2013). 2000 years of parallel societies in Stone Age Central Europe. Science *342*, 479–481.

Bos, K.I., Schuenemann, V.J., Golding, G.B., Burbano, H.A., Waglechner, N., Coombes, B.K., McPhee, J.B., DeWitte, S.N., Meyer, M., Schmedes, S., et al. (2011). A draft genome of *Yersinia pestis* from victims of the Black Death. Nature *478*, 506–510.

Brace, S., Diekmann, Y., Booth, T.J., Dorp, L. van, Faltyskova, Z., Rohland, N., Mallick, S., Olalde, I., Ferry, M., Michel, M., et al. (2019). Ancient genomes indicate population replacement in Early Neolithic Britain. Nat. Ecol. Evol. *3*, 765.

Bramanti, B., Thomas, M.G., Haak, W., Unterlaender, M., Jores, P., Tambets, K., Antanaitis-Jacobs, I., Haidle, M.N., Jankauskas, R., Kind, C.-J., et al. (2009). Genetic discontinuity between local hunter-gatherers and central Europe's first farmers. Science *326*, 137–140.

Brandt, G., Haak, W., Adler, C.J., Roth, C., Szécsényi-Nagy, A., Karimnia, S., Möller-Rieker, S., Meller, H., Ganslmeier, R., Friederich, S., et al. (2013). Ancient DNA reveals key stages in the formation of central European mitochondrial genetic diversity. Science *342*, 257–261.

Brandt, G., Szécsényi-Nagy, A., Roth, C., Alt, K.W., and Haak, W. (2015). Human paleogenetics of Europe--the known knowns and the known unknowns. J. Hum. Evol. *79*, 73–92.

Breitwieser, F.P., Lu, J., and Salzberg, S.L. (2017). A review of methods and databases for metagenomic classification and assembly. Brief. Bioinform. *120*.

Briggs, A.W., Stenzel, U., Johnson, P.L.F., Green, R.E., Kelso, J., Prüfer, K., Meyer, M., Krause, J., Ronan, M.T., Lachmann, M., et al. (2007). Patterns of damage in genomic DNA sequences from a Neandertal. Proc. Natl. Acad. Sci. U. S. A. *104*, 14616–14621.

Briggs, A.W., Good, J.M., Green, R.E., Krause, J., Maricic, T., Stenzel, U., Lalueza-Fox, C., Rudan, P., Brajkovic, D., Kucan, Z., et al. (2009). Targeted retrieval and analysis of five Neandertal mtDNA genomes. Science *325*, 318–321.

Brotherton, P., Endicott, P., Sanchez, J.J., Beaumont, M., Barnett, R., Austin, J., and Cooper, A. (2007). Novel high-resolution characterization of ancient DNA reveals C > U-type base modification events as the sole cause of post mortem miscoding lesions. Nucleic Acids Res. *35*, 5717–5728.

Brotherton, P., Haak, W., Templeton, J., Brandt, G., Soubrier, J., Jane Adler, C., Richards, S.M., Sarkissian, C.D., Ganslmeier, R., Friederich, S., et al. (2013). Neolithic mitochondrial haplogroup H genomes and the genetic origins of Europeans. Nat. Commun. *4*, 1764.

Broushaki, F., Thomas, M.G., Link, V., López, S., van Dorp, L., Kirsanow, K., Hofmanová, Z., Diekmann, Y., Cassidy, L.M., Díez-del-Molino, D., et al. (2016). Early Neolithic genomes from the eastern Fertile Crescent. Science *353*, 499–503.

Browning, S.R., and Browning, B.L. (2011). Haplotype phasing: existing methods and new developments. Nat. Rev. Genet. *12*, 703–714.

Burbano, H.A., Hodges, E., Green, R.E., Briggs, A.W., Krause, J., Meyer, M., Good, J.M., Maricic, T., Johnson, P.L.F., Xuan, Z., et al. (2010). Targeted investigation of the Neandertal genome by array-based sequence capture. Science *328*, 723–725.

Cano, R.J., Poinar, H.N., Pieniazek, N.J., Acra, A., and Poinar, G.O. (1993). Amplification and sequencing of DNA from a 120-135-million-year-old weevil. Nature *363*, 536–538.

Cappellini, E., Collins, M.J., and Gilbert, M.T.P. (2014). Unlocking Ancient Protein Palimpsests. Science *343*, 1320–1322.

Carøe, C., Gopalakrishnan, S., Vinner, L., Mak, S.S.T., Sinding, M.H.S., Samaniego, J.A., Wales, N., Sicheritz Pontén, T., and Gilbert, M.T.P. (2018). Single-tube library preparation for degraded DNA. Methods Ecol. Evol. *9*, 410–419.

Carpelan, C. (2006). On Archaeological Aspects of Uralic, Finno-Ugric and Finnic Societies before AD 800. In The Slavicization of the Russian North. Mechanisms and Chronology, J. Nuorluoto, Ed., (Helsinki: Slaciva Helsingiensia), pp. 78–92.

Carpenter, M.L., Buenrostro, J.D., Valdiosera, C., Schroeder, H., Allentoft, M.E., Sikora, M., Rasmussen, M., Gravel, S., Guillén, S., Nekhrizov, G., et al. (2013). Pulling out the 1%: Whole-Genome Capture for the Targeted Enrichment of Ancient DNA Sequencing Libraries. Am. J. Hum. Genet. *93*, 852–864.

Collard, M., Edinborough, K., Shennan, S., and Thomas, M. (2010). Radiocarbon evidence indicates that migrants introduced farming to Britain. J. Archaeol. Sci. *37*, 866–870.

Cooper, A., and Poinar, H.N. (2000). Ancient DNA: Do It Right or Not at All. Science *289*, 1139–1139.

Dabney, J., Knapp, M., Glocke, I., Gansauge, M.-T., Weihmann, A., Nickel, B., Valdiosera, C., García, N., Pääbo, S., Arsuaga, J.-L., et al. (2013). Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. Proc. Natl. Acad. Sci. *110*, 15758–15763.

Damgaard, P.B., Margaryan, A., Schroeder, H., Orlando, L., Willerslev, E., and Allentoft, M.E. (2015). Improving access to endogenous DNA in ancient bones and teeth. Sci. Rep. *5*, 11184.

Darzynkiewicz, Z., Juan, G., Li, X., Gorczyca, W., Murakami, T., and Traganos, F. (1997). Cytometry in cell necrobiology: Analysis of apoptosis and accidental cell death (necrosis). Cytometry *27*, 1–20.

Deagle, B.E., Eveson, J.P., and Jarman, S.N. (2006). Quantification of damage in DNA recovered from highly degraded samples – a case study on DNA in faeces. Front. Zool. *3*, 11.

Demarchi, B., Hall, S., Roncal-Herrero, T., Freeman, C.L., Woolley, J., Crisp, M.K., Wilson, J., Fotakis, A., Fischer, R., Kessler, B.M., et al. (2016). Protein sequences bound to mineral surfaces persist into deep time. ELife *5*, e17092.

Der Sarkissian, C., Ermini, L., Jónsson, H., Alekseev, A.N., Crubezy, E., Shapiro, B., and Orlando, L. (2014). Shotgun microbial profiling of fossil remains. Mol. Ecol. *23*, 1780–1798.

Der Sarkissian, C., Allentoft, M.E., Ávila-Arcos, M.C., Barnett, R., Campos, P.F., Cappellini, E., Ermini, L., Fernández, R., da Fonseca, R., Ginolhac, A., et al. (2015). Ancient genomics. Philos. Trans. R. Soc. B Biol. Sci. *370*.

DeSalle, R., Gatesy, J., Wheeler, W., and Grimaldi, D. (1992). DNA sequences from a fossil termite in Oligo-Miocene amber and their phylogenetic implications. Science *257*, 1933–1936.

Dissing, J., Binladen, J., Hansen, A., Sejrsen, B., Willerslev, E., and Lynnerup, N. (2007). The last Viking King: a royal maternity case solved by ancient DNA analysis. Forensic Sci. Int. *166*, 21–27.

Eglinton Geoffrey, Logan Graham A., Ambler R. P., Boon J. J., Perizonius W. R. K., Eglinton Geoffrey, and Curry G. B. (1991). Molecular preservation. Philos. Trans. R. Soc. Lond. B. Biol. Sci. *333*, 315–328.

Fernandes, D.M., Strapagiel, D., Borówka, P., Marciniak, B., Żądzińska, E., Sirak, K., Siska, V., Grygiel, R., Carlsson, J., Manica, A., et al. (2018). A genomic Neolithic time transect of hunter-farmer admixture in central Poland. Sci. Rep. *8*, 14879.

Fort, J. (2015). Demic and cultural diffusion propagated the Neolithic transition across different regions of Europe. J. R. Soc. Interface *12*, 20150166.

Frog, M., and Saarikivi, J. (2015). De situ linguarum fennicarum aetatis ferreae. RMN Newsl. *9*, 64–115.

Fu, Q., Meyer, M., Gao, X., Stenzel, U., Burbano, H.A., Kelso, J., and Pääbo, S. (2013a). DNA analysis of an early modern human from Tianyuan Cave, China. Proc. Natl. Acad. Sci. U. S. A. *110*, 2223–2227.

Fu, Q., Mittnik, A., Johnson, P.L.F., Bos, K., Lari, M., Bollongino, R., Sun, C., Giemsch, L., Schmitz, R., Burger, J., et al. (2013b). A revised timescale for human evolution based on ancient mitochondrial genomes. Curr. Biol. CB *23*, 553–559.

Fu, Q., Li, H., Moorjani, P., Jay, F., Slepchenko, S.M., Bondarev, A.A., Johnson, P.L.F., Petri, A.A., Prüfer, K., de Filippo, C., et al. (2014). The genome sequence of a 45,000-year-old modern human from western Siberia. Nature *514*, 445–449.

Fu, Q., Hajdinjak, M., Moldovan, O.T., Constantin, S., Mallick, S., Skoglund, P., Patterson, N., Rohland, N., Lazaridis, I., Nickel, B., et al. (2015). An early modern human from Romania with a recent Neanderthal ancestor. Nature *524*, 216–219.

Fu, Q., Posth, C., Hajdinjak, M., Petr, M., Mallick, S., Fernandes, D., Furtwängler, A., Haak, W., Meyer, M., Mittnik, A., et al. (2016). The genetic history of Ice Age Europe. Nature *534*, 200–205.

Gamba, C., Jones, E.R., Teasdale, M.D., McLaughlin, R.L., Gonzalez-Fortes, G., Mattiangeli, V., Domboróczki, L., Kővári, I., Pap, I., Anders, A., et al. (2014). Genome flux and stasis in a five millennium transect of European prehistory. Nat. Commun. *5*, 5257.

Gansauge, M.-T., and Meyer, M. (2013). Single-stranded DNA library preparation for the sequencing of ancient or damaged DNA. Nat. Protoc. *8*, 737–748.

Gansauge, M.-T., Gerber, T., Glocke, I., Korlevic, P., Lippik, L., Nagel, S., Riehl, L.M., Schmidt, A., and Meyer, M. (2017). Single-stranded DNA library preparation from highly degraded DNA using T4 DNA ligase. Nucleic Acids Res. *45*, e79.

Giguet-Covex, C., Pansu, J., Arnaud, F., Rey, P.-J., Griggo, C., Gielly, L., Domaizon, I., Coissac, E., David, F., Choler, P., et al. (2014). Long livestock farming history and human landscape shaping revealed by lake sediment DNA. Nat. Commun. *5*, 3211.

Gilbert, M.T.P., Barnes, I., Collins, M.J., Smith, C., Eklund, J., Goudsmit, J., Poinar, H., and Cooper, A. (2005). Long-term survival of ancient DNA in Egypt: Response to Zink and Nerlich (2003). Am. J. Phys. Anthropol. *128*, 110–114.

Gilbert, M.T.P., Binladen, J., Miller, W., Wiuf, C., Willerslev, E., Poinar, H., Carlson, J.E., Leebens-Mack, J.H., and Schuster, S.C. (2007). Recharacterization of ancient DNA miscoding lesions: insights in the era of sequencing-by-synthesis. Nucleic Acids Res. *35*, 1–10.

Ginolhac, A., Rasmussen, M., Gilbert, M.T.P., Willerslev, E., and Orlando, L. (2011). mapDamage: testing for damage patterns in ancient DNA sequences. Bioinforma. Oxf. Engl. *27*, 2153–2155.

Glocke, I., and Meyer, M. (2017). Extending the spectrum of DNA sequences retrieved from ancient bones and teeth. Genome Res. *27*, 1230–1237.

Green, R.E., Krause, J., Briggs, A.W., Maricic, T., Stenzel, U., Kircher, M., Patterson, N., Li, H., Zhai, W., Fritz, M.H.-Y., et al. (2010). A Draft Sequence of the Neandertal Genome. Science *328*, 710–722.

Gronenborn, D. (1999). A Variation on a Basic Theme: The Transition to Farming in Southern Central Europe. J. World Prehistory *13*, 123–210.

Günther, T., Valdiosera, C., Malmström, H., Ureña, I., Rodriguez-Varela, R., Sverris-dóttir, Ó.O., Daskalaki, E.A., Skoglund, P., Naidoo, T., Svensson, E.M., et al. (2015). Ancient genomes link early farmers from Atapuerca in Spain to modern-day Basques. Proc. Natl. Acad. Sci. *112*, 11917–11922.

Günther, T., Malmström, H., Svensson, E.M., Omrak, A., Sánchez-Quinto, F., Kılınç, G.M., Krzewińska, M., Eriksson, G., Fraser, M., Edlund, H., et al. (2018). Population genomics of Mesolithic Scandinavia: Investigating early postglacial migration routes and high-latitude adaptation. PLoS Biol. *16*, e2003703.

Haak, W., Forster, P., Bramanti, B., Matsumura, S., Brandt, G., Tänzer, M., Villems, R., Renfrew, C., Gronenborn, D., Alt, K.W., et al. (2005). Ancient DNA from the first European farmers in 7500-year-old Neolithic sites. Science *310*, 1016–1018.

Haak, W., Brandt, G., de Jong, H.N., Meyer, C., Ganslmeier, R., Heyd, V., Hawkesworth, C., Pike, A.W.G., Meller, H., and Alt, K.W. (2008). Ancient DNA, Strontium isotopes, and osteological analyses shed light on social and kinship organization of the Later Stone Age. Proc. Natl. Acad. Sci. U. S. A. *105*, 18226–18231.

Haak, W., Balanovsky, O., Sanchez, J.J., Koshel, S., Zaporozhchenko, V., Adler, C.J., Der Sarkissian, C.S.I., Brandt, G., Schwarz, C., Nicklisch, N., et al. (2010). Ancient DNA from European early neolithic farmers reveals their near eastern affinities. PLoS Biol. *8*, e1000536.

Haak, W., Lazaridis, I., Patterson, N., Rohland, N., Mallick, S., Llamas, B., Brandt, G., Nordenfelt, S., Harney, E., Stewardson, K., et al. (2015). Massive migration from the steppe was a source for Indo-European languages in Europe. Nature *522*, 207–211.

Hadly, E.A., Kohn, M.H., Leonard, J.A., and Wayne, R.K. (1998). A genetic record of population isolation in pocket gophers during Holocene climatic change. Proc. Natl. Acad. Sci. U. S. A. *95*, 6893–6896.

Haile, J., Froese, D.G., Macphee, R.D.E., Roberts, R.G., Arnold, L.J., Reyes, A.V., Rasmussen, M., Nielsen, R., Brook, B.W., Robinson, S., et al. (2009). Ancient DNA reveals late survival of mammoth and horse in interior Alaska. Proc. Natl. Acad. Sci. U. S. A. *106*, 22352–22357.

Hajdinjak, M., Fu, Q., Hübner, A., Petr, M., Mafessoni, F., Grote, S., Skoglund, P., Narasimham, V., Rougier, H., Crevecoeur, I., et al. (2018). Reconstructing the genetic history of late Neanderthals. Nature *555*, 652–656.

Häkkinen, J. (2009). Kantauralin ajoitus ja paikannus: perustelut puntarissa. Suom.-Ugr. Seuran Aikakauskirja *92*, 9–56.

Handt, O., Krings, M., Ward, R.H., and Pääbo, S. (1996). The retrieval of ancient human DNA sequences. Am. J. Hum. Genet. *59*, 368–376.

Hansen, H.B., Damgaard, P.B., Margaryan, A., Stenderup, J., Lynnerup, N., Willerslev, E., and Allentoft, M.E. (2017). Comparing ancient DNA preservation in petrous bone and tooth cementum. PloS One *12*, e0170940.

Hardy, K., Blakeney, T., Copeland, L., Kirkham, J., Wrangham, R., and Collins, M. (2009). Starch granules, dental calculus and new perspectives on ancient diet. J. Archaeol. Sci. *36*, 248–255.

Harris, D.R. (1996). The origins and spread of agriculture and pastoralism in Eurasia (Washington, D.C.: Smithsonian Institution Press).

Hedges, S.B., and Schweitzer, M.H. (1995). Detecting dinosaur DNA. Science *268*, 1191–1192.

Hellenthal, G., Busby, G.B.J., Band, G., Wilson, J.F., Capelli, C., Falush, D., and Myers, S. (2014). A genetic atlas of human admixture history. Science *343*, 747–751.

Hendy, J., Warinner, C., Bouwman, A., Collins, M.J., Fiddyment, S., Fischer, R., Hagan, R., Hofman, C.A., Holst, M., Chaves, E., et al. (2018). Proteomic evidence of dietary sources in ancient dental calculus. Proc. Biol. Sci. *285*.

Henry, A.G., Brooks, A.S., and Piperno, D.R. (2011). Microfossils in calculus demonstrate consumption of plants and cooked foods in Neanderthal diets (Shanidar III, Iraq; Spy I and II, Belgium). Proc. Natl. Acad. Sci. *108*, 486–491.

Hervella, M., Izagirre, N., Alonso, S., Fregel, R., Alonso, A., Cabrera, V.M., and Rúa, C. de la (2012). Ancient DNA from Hunter-Gatherer and Farmer Groups from Northern Spain Supports a Random Dispersion Model for the Neolithic Expansion into Europe. PLOS ONE *7*, e34417.

Hervella, M., Rotea, M., Izagirre, N., Constantinescu, M., Alonso, S., Ioana, M., Lazăr, C., Ridiche, F., Soficaru, A.D., Netea, M.G., et al. (2015). Ancient DNA from South-East Europe Reveals Different Events during Early and Middle Neolithic Influencing the European Genetic Heritage. PLOS ONE *10*, e0128810.

Higuchi, R., Bowman, B., Freiberger, M., Ryder, O.A., and Wilson, A.C. (1984). DNA sequences from the quagga, an extinct member of the horse family. Nature *312*, 282.

Hofmanová, Z., Kreutzer, S., Hellenthal, G., Sell, C., Diekmann, Y., Díez-Del-Molino, D., van Dorp, L., López, S., Kousathanas, A., Link, V., et al. (2016). Early farmers from across Europe directly descended from Neolithic Aegeans. Proc. Natl. Acad. Sci. U. S. A. *113*, 6886–6891.

Hofreiter, M., Jaenicke, V., Serre, D., von Haeseler, A., and Pääbo, S. (2001). DNA sequences from multiple amplifications reveal artifacts induced by cytosine deamination in ancient DNA. Nucleic Acids Res. *29*, 4793–4799.

Honkola, T., Vesakoski, O., Korhonen, K., Lehtinen, J., Syrjänen, K., and Wahlberg, N. (2013). Cultural and climatic changes shape the evolutionary history of the Uralic languages. J. Evol. Biol. *26*, 1244–1253.

Höss, M., Jaruga, P., Zastawny, T.H., Dizdaroglu, M., and Pääbo, S. (1996a). DNA damage and DNA sequence retrieval from ancient tissues. Nucleic Acids Res. *24*, 1304–1307.

Höss, M., Dilling, A., Currant, A., and Pääbo, S. (1996b). Molecular phylogeny of the extinct ground sloth Mylodon darwinii. Proc. Natl. Acad. Sci. U. S. A. *93*, 181–185.

Ilumäe, A.-M., Reidla, M., Chukhryaeva, M., Järve, M., Post, H., Karmin, M., Saag, L., Agdzhoyan, A., Kushniarevich, A., Litvinov, S., et al. (2016). Human Y chromosome haplogroup N: a non-trivial time-resolved phylogeography that cuts across language families. Am. J. Hum. Genet. *99*, 163–173.

Indreko, R. (1935). Sépultures néolithiques en Estonie. In Õpetatud Eesti Seltsi Aastaraamat 1933., (Tartu), pp. 202–223.

Indreko, R. (1938). Ein Hockergrab in Ardu, Ksp. Kose. In Õpetatud Eesti Seltsi Toimetised 30., (Tartu), pp. 185–200.

Jaanits, L. (1985). Могильник культуры ладьевидных топоров в Кунила. (Corded Ware Culture burial site in Kunila). In Изыскания По Мезолиту СССР. (Mesolithic Research USSR)., (Leningrad), pp. 84–90. (In Russian).

Jaanits, L., Laul, S., Lõugas, V., and Tõnisson, E. (1982). Eesti esiajalugu. (Tallinn: ENSV Teaduste Akadeemia Ajaloo Instituut; Eesti Raamat).

Janhunen, J. (2009). Proto-Uralic — what, where, and when? In The Quasquicentennial of the Finno-Ugrian Society, J. Ylikoski, Ed., (Helsinki: Tiedekirja), pp. 57–78.

Jin, Y., and Yip, H.-K. (2002). Supragingival calculus: formation and control. Crit. Rev. Oral Biol. Med. Off. Publ. Am. Assoc. Oral Biol. *13*, 426–441.

Jones, E.R., Gonzalez-Fortes, G., Connell, S., Siska, V., Eriksson, A., Martiniano, R., McLaughlin, R.L., Llorente, M.G., Cassidy, L.M., Gamba, C., et al. (2015). Upper Palaeolithic genomes reveal deep roots of modern Eurasians. Nat. Commun. *6*, 8912.

Jones, E.R., Zarina, G., Moiseyev, V., Lightfoot, E., Nigst, P.R., Manica, A., Pinhasi, R., and Bradley, D.G. (2017). The Neolithic transition in the Baltic was not driven by admixture with early European farmers. Curr. Biol. CB *27*, 576–582.

Jónsson, H., Ginolhac, A., Schubert, M., Johnson, P.L.F., and Orlando, L. (2013). mapDamage2.0: fast approximate Bayesian estimates of ancient DNA damage parameters. Bioinforma. Oxf. Engl. *29*, 1682–1684.

Kala, T., Kaljundi, L., Kreem, J., Leimus, I., Markus, K., Mänd, A., Põltsam-Jürjo, I., Russow, E., Selart, A., Tamm, M., et al. (2012). Eesti ajalugu II. Eesti Keskaeg. (Tartu: Ilmamaa).

Kallio, P. (2006). Suomen kantakielten absoluuttista kronologiaa. Virittäjä *1*, 2–25.

Karmin, M., Saag, L., Vicente, M., Wilson Sayres, M.A., Järve, M., Talas, U.G., Rootsi, S., Ilumäe, A.-M., Mägi, R., Mitt, M., et al. (2015). A recent bottleneck of Y chromosome diversity coincides with a global change in culture. Genome Res. *25*, 459–466.

Keller, A., Graefen, A., Ball, M., Matzas, M., Boisguerin, V., Maixner, F., Leidinger, P., Backes, C., Khairat, R., Forster, M., et al. (2012). New insights into the Tyrolean Iceman's origin and phenotype as inferred by whole-genome sequencing. Nat. Commun. *3*, 698.

Keyser-Tracqui, C., Crubézy, E., and Ludes, B. (2003). Nuclear and Mitochondrial DNA Analysis of a 2,000-Year-Old Necropolis in the Egyin Gol Valley of Mongolia. Am. J. Hum. Genet. *73*, 247–260.

Kisand, V., Talas, L., Kisand, A., Stivrins, N., Reitalu, T., Alliksaar, T., Vassiljev, J., Liiv, M., Heinsalu, A., Seppä, H., et al. (2018). From microbial eukaryotes to metazoan vertebrates: Wide spectrum paleo-diversity in sedimentary ancient DNA over the last ~14,500 years. Geobiology *16*, 628–639.

Kivisild, T. (2017). The study of human Y chromosome variation through ancient DNA. Hum. Genet. *136*, 529–546.

Knapp, M., Clarke, A.C., Horsburgh, K.A., and Matisoo-Smith, E.A. (2012). Setting the stage - building and working in an ancient DNA laboratory. Ann. Anat. *194*, 3–6.

Koivulehto, J. (2001). The earliest contacts between Indo-European and Uralic speakers in the light of lexical loans. In Early Contacts between Uralic and Indo-European: Linguistic and Archaeological Considerations, C. Carpelan, Ed., (Helsinki: Suomalais-Ugrilaisen Seuran Toimituksia), pp. 235–264.

Korhonen, M. (1981). Johdatus lapin kielen historiaan (Helsinki: Suomalaisen Kirjallisuuden Seura).

Korlević, P., Gerber, T., Gansauge, M.-T., Hajdinjak, M., Nagel, S., Aximu-Petri, A., and Meyer, M. (2015). Reducing microbial and human contamination in DNA extractions from ancient bones and teeth. BioTechniques *59*, 87–93.

Krainov, D.A. (1972). Древнейшая история Волго-Окского междуречья. Фатья-новская культура. (Prehistory of the Volga-Oka interfluve. The Fatyanovo culture). (Moscow). (In Russian).

Krause, J., Briggs, A.W., Kircher, M., Maricic, T., Zwyns, N., Derevianko, A., and Pääbo, S. (2010). A complete mtDNA genome of an early modern human from Kostenki, Russia. Curr. Biol. CB *20*, 231–236.

Kriiska, A. (1996). Stone Age settlements in the lower reaches of the Narva river, north-eastern Estonia. In Coastal Estonia: Recent Advances in Environmental and Cultural History. PACT 51., (Strassbourg: European Council), pp. 359–372.

Kriiska, A. (2000). Corded ware culture sites in North-Eastern Estonia. Temporibus Antiq. Ad Honor. Lembit Jaanits Muinasaja Tead. *8*, 59–79.

Kriiska, A. (2001). Stone Age settlement and economic processes in the Estonian coastal area and islands. (Helsinki: Helsingin yliopisto, kulttuurien tutkimuksen laitos).

Kriiska, A. (2003). From hunter-gatherer to farmer. Changes in the Neolithic economy and settlement on Estonian territory. Archaeol. Litu. *4*, 11−26.

Kriiska, A. (2009). The beginning of farming in the Eastern Baltic area. In East European Plain on the Eve of Agriculture. British Archaeological Reports International., (Oxford Archaeopress), p. 159−179.

Kriiska, A., and Lõugas, L. (2009). Stone Age settlement sites on an environmentally sensitive coastal area along the lower reaches of the River Pärnu (south-western Estonia), as indicators of changing settlement patterns, technologies and economies. In Mesolithic Horizons, S. McCartan, R. Schulting, G. Warren, P. Woodman, Ed., (Oxford-Oakville: Oxbow Books), p. 167−175.

Kriiska, A., Mäesalu, A., Selart, A., Põltsam-Jürjo, I., Piirimäe, P., Seppel, M., Andresen, A., Pajur, A., and Tannberg, T. (2017). Eesti ajalugu. (Tallinn: Avita).

Kruk, J., and Milisauskas, S. (1999). Rozkwit i upadek społeczeństw rolniczych Neolitu. (Krakow: Instytut Archeologii i Etnologii Polskiej Akademii Nauk).

Lacan, M., Keyser, C., Ricaut, F.-X., Brucato, N., Tarrús, J., Bosch, A., Guilaine, J., Crubézy, E., and Ludes, B. (2011). Ancient DNA suggests the leading role played by men in the Neolithic dissemination. Proc. Natl. Acad. Sci. *108*, 18255–18259.

Lang, V. (2007). The Bronze and Early Iron Ages in Estonia. Estonian Archaeology, 3. (Tartu: University of Tartu Press).

Lang, V. (2011). Traceless death. Missing burials in Bronze and Iron Age Estonia. Est. J. Archaeol. *15: 2*, 109–129.

Lang, V. (2015). Formation of Proto-Finnic – an archaeological scenario from the Bronze Age/Early Iron Age. In Congressus Duodecimus Internationalis Fenno-Ugristarum, Oulu 2015. Plenary Papers, H. Mantila, K. Leinonen, S. Brunni, S. Palviainen, J. Sivonen, Ed., (Oulu: University of Oulu), pp. 63–84.

Lang, V. (2018). Läänemeresoome tulemised. (Tartu: University of Tartu Press).

Lang, V., and Kriiska, A. (2001). Eesti esiajaloo periodiseering ja kronoloogia. J. Est. Archaeol. *5*, 83–109.

Lawson, D.J., Hellenthal, G., Myers, S., and Falush, D. (2012). Inference of population structure using dense haplotype data. PLoS Genet. *8*, e1002453.

Lazaridis, I., Patterson, N., Mittnik, A., Renaud, G., Mallick, S., Kirsanow, K., Sudmant, P.H., Schraiber, J.G., Castellano, S., Lipson, M., et al. (2014). Ancient human genomes suggest three ancestral populations for present-day Europeans. Nature *513*, 409–413.

Lazaridis, I., Nadel, D., Rollefson, G., Merrett, D.C., Rohland, N., Mallick, S., Fernandes, D., Novak, M., Gamarra, B., Sirak, K., et al. (2016). Genomic insights into the origin of farming in the ancient Near East. Nature *536*, 419–424.

Leslie, S., Winney, B., Hellenthal, G., Davison, D., Boumertit, A., Day, T., Hutnik, K., Royrvik, E.C., Cunliffe, B., Wellcome Trust Case Control Consortium 2, et al. (2015). The fine-scale genetic structure of the British population. Nature *519*, 309–314.

Lindahl, T. (1993). Instability and decay of the primary structure of DNA. Nature *362*, 709.

Lindahl, T. (1996). The Croonian Lecture, 1996: endogenous damage to DNA. Philos. Trans. R. Soc. Lond. B. Biol. Sci. *351*, 1529–1538.

Lindahl, T., and Andersson, A. (1972). Rate of chain breakage at apurinic sites in double-stranded deoxyribonucleic acid. Biochemistry *11*, 3618–3623.

Lindahl, T., and Nyberg, B. (1972). Rate of depurination of native deoxyribonucleic acid. Biochemistry *11*, 3610–3618.

Lipson, M., Szécsényi-Nagy, A., Mallick, S., Pósa, A., Stégmár, B., Keerl, V., Rohland, N., Stewardson, K., Ferry, M., Michel, M., et al. (2017). Parallel palaeogenomic transects reveal complex genetic history of early European farmers. Nature *551*, 368–372.

Lipson, M., Skoglund, P., Spriggs, M., Valentin, F., Bedford, S., Shing, R., Buckley, H., Phillip, I., Ward, G.K., Mallick, S., et al. (2018). Population Turnover in Remote Oceania Shortly after Initial Settlement. Curr. Biol. CB *28*, 1157-1165.e7.

Lõugas, L., Kriiska, A., and Maldre, L. (2007). New dates for the Late Neolithic Corded Ware Culture burials and early husbandry in the East Baltic region. Archaeofauna *16*, 21–31.

Lynnerup, N. (2015). Bog Bodies. Anat. Rec. *298*, 1007–1012.

Malaspinas, A.-S., Lao, O., Schroeder, H., Rasmussen, M., Raghavan, M., Moltke, I., Campos, P.F., Sagredo, F.S., Rasmussen, S., Gonçalves, V.F., et al. (2014). Two ancient human genomes reveal Polynesian ancestry among the indigenous Botocudos of Brazil. Curr. Biol. CB *24*, R1035-1037.

Malmer, M.P. (2002). The Neolithic of south Sweden: TRB, GRK, and STR (Stockholm: Royal Swedish Academy of Letters, History, and Antiquities : Distributed by Almquist & Wiksell International).

Malmström, H., Gilbert, M.T.P., Thomas, M.G., Brandström, M., Storå, J., Molnar, P., Andersen, P.K., Bendixen, C., Holmlund, G., Götherström, A., et al. (2009). Ancient DNA reveals lack of continuity between neolithic hunter-gatherers and contemporary Scandinavians. Curr. Biol. CB *19*, 1758–1762.

Mangerud, J., Jakobsson, M., Alexanderson, H., Astakhov, V., Clarke, G.K.C., Henriksen, M., Hjort, C., Krinner, G., Lunkka, J.-P., Möller, P., et al. (2004). Ice-dammed lakes and rerouting of the drainage of northern Eurasia during the Last Glaciation. Quat. Sci. Rev. *23*, 1313–1332.

Margaryan, A., Hansen, H.B., Rasmussen, S., Sikora, M., Moiseyev, V., Khoklov, A., Epimakhov, A., Yepiskoposyan, L., Kriiska, A., Varul, L., et al. (2018). Ancient pathogen DNA in human teeth and petrous bones. Ecol. Evol. *8*, 3534–3542.

Margulies, M., Egholm, M., Altman, W.E., Attiya, S., Bader, J.S., Bemben, L.A., Berka, J., Braverman, M.S., Chen, Y.-J., Chen, Z., et al. (2005). Genome sequencing in microfabricated high-density picolitre reactors. Nature *437*, 376–380.

Maricic, T., Whitten, M., and Pääbo, S. (2010). Multiplexed DNA sequence capture of mitochondrial genomes using PCR products. PloS One *5*, e14004.

Marota, I., Basile, C., Ubaldi, M., and Rollo, F. (2002). DNA decay rate in papyri and human remains from Egyptian archaeological sites. Am. J. Phys. Anthropol. *117*, 310–318.

Mathieson, I., Lazaridis, I., Rohland, N., Mallick, S., Patterson, N., Roodenberg, S.A., Harney, E., Stewardson, K., Fernandes, D., Novak, M., et al. (2015). Genome-wide patterns of selection in 230 ancient Eurasians. Nature *528*, 499–503.

Mathieson, I., Alpaslan-Roodenberg, S., Posth, C., Szécsényi-Nagy, A., Rohland, N., Mallick, S., Olalde, I., Broomandkhoshbacht, N., Candilio, F., Cheronet, O., et al. (2018). The genomic history of southeastern Europe. Nature *555*, 197–203.

Meyer, M., and Kircher, M. (2010). Illumina sequencing library preparation for highly multiplexed target capture and sequencing. Cold Spring Harb. Protoc. *2010*, pdb.prot5448.

Meyer, M., Fu, Q., Aximu-Petri, A., Glocke, I., Nickel, B., Arsuaga, J.-L., Martínez, I., Gracia, A., de Castro, J.M.B., Carbonell, E., et al. (2014). A mitochondrial genome sequence of a hominin from Sima de los Huesos. Nature *505*, 403–406.

Meyer, M., Arsuaga, J.-L., de Filippo, C., Nagel, S., Aximu-Petri, A., Nickel, B., Martínez, I., Gracia, A., de Castro, J.M.B., Carbonell, E., et al. (2016). Nuclear DNA sequences from the Middle Pleistocene Sima de los Huesos hominins. Nature *531*, 504–507.

Miller, W., Drautz, D.I., Ratan, A., Pusey, B., Qi, J., Lesk, A.M., Tomsho, L.P., Packard, M.D., Zhao, F., Sher, A., et al. (2008). Sequencing the nuclear genome of the extinct woolly mammoth. Nature *456*, 387–390.

Miller, W., Schuster, S.C., Welch, A.J., Ratan, A., Bedoya-Reina, O.C., Zhao, F., Kim, H.L., Burhans, R.C., Drautz, D.I., Wittekindt, N.E., et al. (2012). Polar and brown bear genomes reveal ancient admixture and demographic footprints of past climate change. Proc. Natl. Acad. Sci. U. S. A. *109*, E2382-2390.

Mittnik, A., Wang, C.-C., Pfrengle, S., Daubaras, M., Zariņa, G., Hallgren, F., Allmäe, R., Khartanovich, V., Moiseyev, V., Tõrv, M., et al. (2018). The genetic prehistory of the Baltic Sea region. Nat. Commun. *9*, 442.

Monroy Kuhn, J.M., Jakobsson, M., and Günther, T. (2018). Estimating genetic kin relationships in prehistoric populations. PloS One *13*, e0195491.

Montinaro, F., Busby, G.B.J., Pascali, V.L., Myers, S., Hellenthal, G., and Capelli, C. (2015). Unravelling the hidden ancestry of American admixed populations. Nat. Commun. *6*, 6596.

Moora, H. (1932). Die Vorzeit Estlands. (Tartu).

Muir, P., Li, S., Lou, S., Wang, D., Spakowicz, D.J., Salichos, L., Zhang, J., Weinstock, G.M., Isaacs, F., Rozowsky, J., et al. (2016). The real cost of sequencing: scaling computation to keep pace with data generation. Genome Biol. *17*, 53.

Muižnieks, V. (2015). Bēru tradīcijas Latvijā pēc arheoloģiski pētīto 14.–18. gadsimta apbedīšanas vietu materiāla. Latvijas Nacionāla Vēstures Muzeja Raksti, 21. (Riga).

Nordqvist, K. (2016). From separation to interaction: Corded Ware in the Eastern Gulf of Finland. Acta Archaeol. *87*, 49–84.

Noro, M., Masuda, R., Dubrovo, I.A., Yoshida, M.C., and Kato, M. (1998). Molecular phylogenetic inference of the woolly mammoth Mammuthus primigenius, based on complete sequences of mitochondrial cytochrome b and 12S ribosomal RNA genes. J. Mol. Evol. *46*, 314–326.

Olalde, I., Allentoft, M.E., Sánchez-Quinto, F., Santpere, G., Chiang, C.W.K., DeGiorgio, M., Prado-Martinez, J., Rodríguez, J.A., Rasmussen, S., Quilez, J., et al. (2014). Derived immune and ancestral pigmentation alleles in a 7,000-year-old Mesolithic European. Nature *507*, 225–228.

Olalde, I., Schroeder, H., Sandoval-Velasco, M., Vinner, L., Lobón, I., Ramirez, O., Civit, S., García Borja, P., Salazar-García, D.C., Talamo, S., et al. (2015). A common genetic origin for Early Farmers from Mediterranean Cardial and Central European LBK cultures. Mol. Biol. Evol. *32*, 3132–3142.

Olalde, I., Mallick, S., Patterson, N., Rohland, N., Villalba-Mouco, V., Silva, M., Dulias, K., Edwards, C.J., Gandini, F., Pala, M., et al. (2019). The genomic history of the Iberian Peninsula over the past 8000 years. Science *363*, 1230–1234.

Omrak, A., Günther, T., Valdiosera, C., Svensson, E.M., Malmström, H., Kiesewetter, H., Aylward, W., Storå, J., Jakobsson, M., and Götherström, A. (2016). Genomic evidence establishes Anatolia as the source of the European Neolithic gene pool. Curr. Biol. CB *26*, 270–275.

Orlando, L., Ginolhac, A., Zhang, G., Froese, D., Albrechtsen, A., Stiller, M., Schubert, M., Cappellini, E., Petersen, B., Moltke, I., et al. (2013). Recalibrating Equus evolution using the genome sequence of an early Middle Pleistocene horse. Nature *499*, 74–78.

O'Sullivan, N., Posth, C., Coia, V., Schuenemann, V.J., Price, T.D., Wahl, J., Pinhasi, R., Zink, A., Krause, J., and Maixner, F. (2018). Ancient genome-wide analyses infer kinship structure in an Early Medieval Alemannic graveyard. Sci. Adv. *4*, eaao1262.

Pääbo, S. (1985). Molecular cloning of Ancient Egyptian mummy DNA. Nature *314*, 644.

Pääbo, S. (1989). Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification. Proc. Natl. Acad. Sci. U. S. A. *86*, 1939–1943.

Pääbo, S., Poinar, H., Serre, D., Jaenicke-Després, V., Hebler, J., Rohland, N., Kuch, M., Krause, J., Vigilant, L., and Hofreiter, M. (2004). Genetic Analyses from Ancient DNA. Annu. Rev. Genet. *38*, 645–679.

Patrushev, V. (2000). The Early History of the Finno-Ugric Peoples of European Russia. (Oulu: Societas Historiae Fenno-Ugricae).

Patterson, N., Moorjani, P., Luo, Y., Mallick, S., Rohland, N., Zhan, Y., Genschoreck, T., Webster, T., and Reich, D. (2012). Ancient admixture in human history. Genetics *192*, 1065–1093.

Petr, M., Pääbo, S., Kelso, J., and Vernot, B. (2019). Limits of long-term selection against Neandertal introgression. Proc. Natl. Acad. Sci. U. S. A. *116*, 1639–1644.

Pinhasi, R., Fort, J., and Ammerman, A.J. (2005). Tracing the Origin and Spread of Agriculture in Europe. PLOS Biol. *3*, e410.

Pinhasi, R., Fernandes, D., Sirak, K., Novak, M., Connell, S., Alpaslan-Roodenberg, S., Gerritsen, F., Moiseyev, V., Gromov, A., Raczky, P., et al. (2015). Optimal Ancient DNA Yields from the Inner Ear Part of the Human Petrous Bone. PLOS ONE *10*, e0129102.

Pinhasi, R., Fernandes, D.M., Sirak, K., and Cheronet, O. (2019). Isolating the human cochlea to generate bone powder for ancient DNA analysis. Nat. Protoc. *14*, 1194–1205.

Pliss, L., Tambets, K., Loogväli, E.-L., Pronina, N., Lazdins, M., Krumina, A., Baumanis, V., and Villems, R. (2006). Mitochondrial DNA portrait of Latvians: towards the understanding of the genetic structure of Baltic-speaking populations. Ann. Hum. Genet. *70*, 439–458.

Poinar, H.N., Cano, R.J., and Poinar, G.O. (1993). DNA from an extinct plant. Nature *363*, 677.

Poinar, H.N., Schwarz, C., Qi, J., Shapiro, B., Macphee, R.D.E., Buigues, B., Tikhonov, A., Huson, D.H., Tomsho, L.P., Auch, A., et al. (2006). Metagenomics to paleogenomics: large-scale sequencing of mammoth DNA. Science *311*, 392–394.

Popov, A.A. (1966). The Nganasan the material culture of the Tavgi Samoyeds (Bloomington: Indiana University).

Posth, C., Renaud, G., Mittnik, A., Drucker, D.G., Rougier, H., Cupillard, C., Valentin, F., Thevenet, C., Furtwängler, A., Wißing, C., et al. (2016). Pleistocene mitochondrial genomes suggest a single major dispersal of non-Africans and a Late Glacial population turnover in Europe. Curr. Biol. CB *26*, 827–833.

Posth, C., Nägele, K., Colleran, H., Valentin, F., Bedford, S., Kami, K.W., Shing, R., Buckley, H., Kinaston, R., Walworth, M., et al. (2018). Language continuity despite population replacement in Remote Oceania. Nat. Ecol. Evol. *2*, 731–740.

Price, T.D. (2000). Europe's First Farmers (Cambridge: Cambridge University Press).

Prüfer, K., Racimo, F., Patterson, N., Jay, F., Sankararaman, S., Sawyer, S., Heinze, A., Renaud, G., Sudmant, P.H., de Filippo, C., et al. (2014). The complete genome sequence of a Neanderthal from the Altai Mountains. Nature *505*, 43–49.

Raghavan, M., Skoglund, P., Graf, K.E., Metspalu, M., Albrechtsen, A., Moltke, I., Rasmussen, S., Stafford, T.W., Orlando, L., Metspalu, E., et al. (2014). Upper Palaeolithic Siberian genome reveals dual ancestry of Native Americans. Nature *505*, 87–91.

Rasmussen, M., Li, Y., Lindgreen, S., Pedersen, J.S., Albrechtsen, A., Moltke, I., Metspalu, M., Metspalu, E., Kivisild, T., Gupta, R., et al. (2010). Ancient human genome sequence of an extinct Palaeo-Eskimo. Nature *463*, 757–762.

Rasmussen, M., Anzick, S.L., Waters, M.R., Skoglund, P., DeGiorgio, M., Stafford Jr, T.W., Rasmussen, S., Moltke, I., Albrechtsen, A., Doyle, S.M., et al. (2014). The genome of a Late Pleistocene human from a Clovis burial site in western Montana. Nature *506*, 225–229.

Rasmussen, S., Allentoft, M.E., Nielsen, K., Orlando, L., Sikora, M., Sjögren, K.-G., Pedersen, A.G., Schubert, M., Van Dam, A., Kapel, C.M.O., et al. (2015). Early Divergent Strains of Yersinia pestis in Eurasia 5,000 Years Ago. Cell *163*, 571–582.

Reich, D., Green, R.E., Kircher, M., Krause, J., Patterson, N., Durand, E.Y., Viola, B., Briggs, A.W., Stenzel, U., Johnson, P.L.F., et al. (2010). Genetic history of an archaic hominin group from Denisova Cave in Siberia. Nature *468*, 1053–1060.

Renfrew, C., and Boyle, K.V. (2000). Archaeogenetics: DNA and the population pre-history of Europe (Cambridge: McDonald Institute for Archaeological Research).

Ricaut, F.-X., Keyser-Tracqui, C., Crubézy, E., and Ludes, B. (2005). STR-genotyping from human medieval tooth and bone samples. Forensic Sci. Int. *151*, 31–35.

Rivollat, M., Réveillas, H., Mendisco, F., Pemonge, M.-H., Justeau, P., Couture, C., Lefranc, P., Féliu, C., and Deguilloux, M.-F. (2016). Ancient mitochondrial DNA from the middle neolithic necropolis of Obernai extends the genetic influence of the LBK to west of the Rhine. Am. J. Phys. Anthropol. *161*, 522–529.

Rohland, N., and Hofreiter, M. (2007a). Comparison and optimization of ancient DNA extraction. BioTechniques *42*, 343–352.

Rohland, N., and Hofreiter, M. (2007b). Ancient DNA extraction from bones and teeth. Nat. Protoc. *2*, 1756–1762.

Rootsi, S., Zhivotovsky, L.A., Baldovic, M., Kayser, M., Kutuev, I.A., Khusainova, R., Bermisheva, M.A., Gubina, M., Fedorova, S.A., Ilumäe, A.-M., et al. (2007). A counter-clockwise northern route of the Y-chromosome haplogroup N from Southeast Asia towards Europe. Eur. J. Hum. Genet. EJHG *15*, 204–211.

Salminen, T. (2007). Europe and North Asia. In Encyclopeia of the World's Endan-gered Languages, C. Moseley, Ed., (New York: Routledge), pp. 211–280.

Sánchez-Quinto, F., Schroeder, H., Ramirez, O., Avila-Arcos, M.C., Pybus, M., Olalde, I., Velazquez, A.M.V., Marcos, M.E.P., Encinas, J.M.V., Bertranpetit, J., et al.

(2012). Genomic affinities of two 7,000-year-old Iberian hunter-gatherers. Curr. Biol. CB *22*, 1494–1499.

Sankararaman, S., Mallick, S., Dannemann, M., Prüfer, K., Kelso, J., Pääbo, S., Patterson, N., and Reich, D. (2014). The genomic landscape of Neanderthal ancestry in present-day humans. Nature *507*, 354–357.

Sawyer, S., Krause, J., Guschanski, K., Savolainen, V., and Pääbo, S. (2012). Temporal Patterns of Nucleotide Misincorporations and DNA Fragmentation in Ancient DNA. PLoS ONE *7*.

Scheib, C.L., Li, H., Desai, T., Link, V., Kendall, C., Dewar, G., Griffith, P.W., Mörseburg, A., Johnson, J.R., Potter, A., et al. (2018). Ancient human parallel lineages within North America contributed to a coastal expansion. Science *360*, 1024–1027.

Schroeder, H., Margaryan, A., Szmyt, M., Theulot, B., Włodarczak, P., Rasmussen, S., Gopalakrishnan, S., Szczepanek, A., Konopka, T., Jensen, T.Z.T., et al. (2019). Unraveling ancestry, kinship, and violence in a Late Neolithic mass grave. Proc. Natl. Acad. Sci. 201820210.

Schuenemann, V.J., Peltzer, A., Welte, B., van Pelt, W.P., Molak, M., Wang, C.-C., Furtwängler, A., Urban, C., Reiter, E., Nieselt, K., et al. (2017). Ancient Egyptian mummy genomes suggest an increase of Sub-Saharan African ancestry in post-Roman periods. Nat. Commun. *8*, 15694.

Schwarz, C., Debruyne, R., Kuch, M., McNally, E., Schwarcz, H., Aubrey, A.D., Bada, J., and Poinar, H. (2009). New insights from old bones: DNA preservation and degradation in permafrost preserved mammoth remains. Nucleic Acids Res. *37*, 3215–3229.

Seguin-Orlando, A., Korneliussen, T.S., Sikora, M., Malaspinas, A.-S., Manica, A., Moltke, I., Albrechtsen, A., Ko, A., Margaryan, A., Moiseyev, V., et al. (2014). Paleogenomics. Genomic structure in Europeans dating back at least 36,200 years. Science *346*, 1113–1118.

Sikora, M., Carpenter, M.L., Moreno-Estrada, A., Henn, B.M., Underhill, P.A., Sánchez-Quinto, F., Zara, I., Pitzalis, M., Sidore, C., Busonero, F., et al. (2014). Population genomic analysis of ancient and modern genomes yields new insights into the genetic ancestry of the Tyrolean Iceman and the genetic structure of Europe. PLoS Genet. *10*, e1004353.

Skoglund, P., Malmström, H., Raghavan, M., Storå, J., Hall, P., Willerslev, E., Gilbert, M.T.P., Götherström, A., and Jakobsson, M. (2012). Origins and genetic legacy of Neolithic farmers and hunter-gatherers in Europe. Science *336*, 466–469.

Skoglund, P., Malmström, H., Omrak, A., Raghavan, M., Valdiosera, C., Günther, T., Hall, P., Tambets, K., Parik, J., Sjögren, K.-G., et al. (2014). Genomic diversity and admixture differs for Stone-Age Scandinavian foragers and farmers. Science *344*, 747–750.

Skoglund, P., Posth, C., Sirak, K., Spriggs, M., Valentin, F., Bedford, S., Clark, G.R., Reepmeyer, C., Petchey, F., Fernandes, D., et al. (2016). Genomic insights into the peopling of the Southwest Pacific. Nature *538*, 510–513.

Skoglund, P., Thompson, J.C., Prendergast, M.E., Mittnik, A., Sirak, K., Hajdinjak, M., Salie, T., Rohland, N., Mallick, S., Peltzer, A., et al. (2017). Reconstructing Prehistoric African Population Structure. Cell *171*, 59-71.e21.

Slon, V., Hopfe, C., Weiß, C.L., Mafessoni, F., de la Rasilla, M., Lalueza-Fox, C., Rosas, A., Soressi, M., Knul, M.V., Miller, R., et al. (2017). Neandertal and Denisovan DNA from Pleistocene sediments. Science *356*, 605–608.

Spigelman, M., and Lemma, E. (1993). The use of the polymerase chain reaction (PCR) to detect Mycobacterium tuberculosis in ancient skeletons. Int. J. Osteoarchaeol. *3*, 137–143.

Spyrou, M.A., Bos, K.I., Herbig, A., and Krause, J. (2019). Ancient pathogen genomics as an emerging tool for infectious disease research. Nat. Rev. Genet. *20*, 323–340.

Stiller, M., Green, R.E., Ronan, M., Simons, J.F., Du, L., He, W., Egholm, M., Rothberg, J.M., Keates, S.G., Keats, S.G., et al. (2006). Patterns of nucleotide misincorporations during enzymatic amplification and direct large-scale sequencing of ancient DNA. Proc. Natl. Acad. Sci. U. S. A. *103*, 13578–13584.

Szécsényi-Nagy, A., Brandt, G., Haak, W., Keerl, V., Jakucs, J., Möller-Rieker, S., Köhler, K., Mende, B.G., Oross, K., Marton, T., et al. (2015). Tracing the genetic origin of Europe's first farmers reveals insights into their social organization. Proc. Biol. Sci. *282*.

Szécsényi-Nagy, A., Roth, C., Brandt, G., Rihuete-Herrada, C., Tejedor-Rodríguez, C., Held, P., García-Martínez-de-Lagrán, Í., Magallón, H.A., Zesch, S., Knipper, C., et al. (2017). The maternal genetic make-up of the Iberian Peninsula between the Neolithic and the Early Bronze Age. Sci. Rep. *7*, 15644.

Tambets, K., Rootsi, S., Kivisild, T., Help, H., Serk, P., Loogväli, E.-L., Tolk, H.-V., Reidla, M., Metspalu, E., Pliss, L., et al. (2004). The western and eastern roots of the Saami − the story of genetic "outliers" told by mitochondrial DNA and Y chromosomes. Am. J. Hum. Genet. *74*, 661–682.

Tams, K.W., Jensen Søe, M., Merkyte, I., Valeur Seersholm, F., Henriksen, P.S., Klingenberg, S., Willerslev, E., Kjær, K.H., Hansen, A.J., and Kapel, C.M.O. (2018). Parasitic infections and resource economy of Danish Iron Age settlement through ancient DNA sequencing. PloS One *13*, e0197399.

The 1000 Genomes Project Consortium (2012). An integrated map of genetic variation from 1,092 human genomes. Nature *491*, 56–65.

Tõrv, M. (2016). Persistent Practices. A Multi-Disciplinary Study of Hunter-Gatherer Mortuary Remains from c. 6500–2600 cal. BC, Estonia. Dissertationes archaeologiae Universitatis Tartuensis 5. (Tartu: University of Tartu).

Valk, H. (2001). Rural Cemeteries of Southern Estonia 1225–1800 AD. CCC Papers, 3. (Visby–Tartu: Gotland University College, Centre for Baltic Studies, University of Tartu Archaeology Centre).

Vanek, D., Saskova, L., and Koch, H. (2009). Kinship and Y-Chromosome Analysis of 7th Century Human Remains: Novel DNA Extraction and Typing Procedure for Ancient Material. Croat. Med. J. *50,* 286–295.

Veski, S., Heinsalu, A., Klassen, V., Kriiska, A., Lõugas, L., Poska, A., and Saluäär, U. (2005). Early Holocene coastal settlements and palaeoenvironment on the shore of the Baltic Sea at Pärnu, southwestern Estonia. Quat. Int. *130*, 75–85.

Villalba-Mouco, V., van de Loosdrecht, M.S., Posth, C., Mora, R., Martínez-Moreno, J., Rojo-Guerra, M., Salazar-García, D.C., Royo-Guillén, J.I., Kunst, M., Rougier, H., et al. (2019). Survival of Late Pleistocene Hunter-Gatherer Ancestry in the Iberian Peninsula. Curr. Biol. *29*, 1169-1177.e7.

Waples, R.K., Albrechtsen, A., and Moltke, I. (2019). Allele frequency-free inference of close familial relationships from genotypes or low-depth sequencing data. Mol. Ecol. *28*, 35–48.

Ward, R., and Stringer, C. (1997). A molecular handle on the Neanderthals. Nature *388*, 225.

Warinner, C., Rodrigues, J.F.M., Vyas, R., Trachsel, C., Shved, N., Grossmann, J., Radini, A., Hancock, Y., Tito, R.Y., Fiddyment, S., et al. (2014a). Pathogens and host immunity in the ancient human oral cavity. Nat. Genet. *46*, 336–344.

Warinner, C., Hendy, J., Speller, C., Cappellini, E., Fischer, R., Trachsel, C., Arneborg, J., Lynnerup, N., Craig, O.E., Swallow, D.M., et al. (2014b). Direct evidence of milk consumption from ancient human dental calculus. Sci. Rep. *4*, 7104.

Wayne, R.K., Leonard, J.A., and Cooper, A. (1999). Full of Sound and Fury: History of Ancient DNA. Annu. Rev. Ecol. Syst. *30*, 457–477.

Weyrich, L.S., Duchene, S., Soubrier, J., Arriola, L., Llamas, B., Breen, J., Morris, A.G., Alt, K.W., Caramelli, D., Dresely, V., et al. (2017). Neanderthal behaviour, diet, and disease inferred from ancient DNA in dental calculus. Nature *544*, 357–361.

Whittle, A. (1996). Europe in the Neolithic: The creation of new worlds (Cambridge: Cambridge University Press).

Willerslev, E., Hansen, A.J., Binladen, J., Brand, T.B., Gilbert, M.T.P., Shapiro, B., Bunce, M., Wiuf, C., Gilichinsky, D.A., and Cooper, A. (2003). Diverse plant and animal genetic records from Holocene and Pleistocene sediments. Science *300*, 791–795.

Willerslev, E., Davison, J., Moora, M., Zobel, M., Coissac, E., Edwards, M.E., Lorenzen, E.D., Vestergård, M., Gussarova, G., Haile, J., et al. (2014). Fifty thousand years of Arctic vegetation and megafaunal diet. Nature *506*, 47–51.

Włodarczak, P. (2009). Radiocarbon and dendrochronological dates of the Corded Ware culture. Radiocarbon *51*, 737–749.

Woodward, S.R., Weyand, N.J., and Bunnell, M. (1994). DNA sequence from Cretaceous period bone fragments. Science *266*, 1229–1232.

Zink, A., and Nerlich, A.G. (2003). Molecular analyses of the "Pharaos:" Feasibility of molecular studies in ancient Egyptian material. Am. J. Phys. Anthropol. *121*, 109–111.

Zischler, H., Höss, M., Handt, O., von Haeseler, A., van der Kuyl, A.C., and Goudsmit, J. (1995). Detecting dinosaur DNA. Science *268*, 1192–1193; author reply 1194.

Web resources

Introduction to NGS
    https://emea.illumina.com/science/technology/next-generation-sequencing.html
PhyloTree
    https://www.phylotree.org/tree/index.htm
YFull
    https://www.yfull.com/tree/
Ethnologue: Languages of the World – Uralic language family
    https://www.ethnologue.com/subgroups/uralic
Code of Ethics of Estonian archaeologists
    http://www.arheo.ut.ee/eesti-arheoloogide-eetikakoodeks

ACKNOWLEDGEMENTS

PUBLICATIONS

CURRICULUM VITAE

Name: Lehti Saag
Date of Birth: April 30, 1991
Contacts: University of Tartu, Institute of Genomics, Riia 23b, 51010 Tartu, Estonia, +372 737 5053
E-mail: lehti.saag@ut.ee

Education:

2015–2019 Doctoral studies, University of Tartu, Faculty of Science and Technology, Institute of Molecular and Cell Biology, Chair of Evolutionary Biology

2013–2015 *MSc, cum laude*, University of Tartu, Faculty of Science and Technology, Institute of Molecular and Cell Biology, Chair of Evolutionary Biology, supervisors Mait Metspalu, Ene Metspalu, "The Neolithic transition in Estonia – sequencing and mito-chondrial DNA analysis of the representatives of Comb Ceramic and Corded Ware culture"

2010–2013 *BSc,* University of Tartu, Faculty of Science and Technology, Institute of Molecular and Cell Biology, Chair of Evolutionary Biology, supervisor Ene Metspalu, "The distribution of the populations of the Caucasus based on the subhaplogroups H1, H2, H5, H6, H10, H11 and H13 of haplogroup H and its corre-lation with their geographic and linguistic distribution"

Distinctions and Awards:

2016 Estonian Academy of Sciences competition "Science in 3 Minutes" finalist

2012 Rotalia Foundation scholarship grantee

Administrative work:

2017–… Member of the council of the Collegium for Transdisciplinary Studies in Archaeology, Genetics and Linguistics of the University of Tartu

Creative work and additional career information:

2019 Participation in the popular science show "Falling apple" at Raadio Kuku

2019 Popular science article in the journal Tutulus

2018–… Supervisor on the master's level in the University of Tartu

2018–2019 Collaboration for the population history section of the exhibition "Geneius" in Tallinn TV Tower

2018 Participation in the popular science show "Worldmix" at Raadio Elmar

2018	Popular science article in the journal Horisont
2017	Participation in the multidisciplinary project of the University of Tartu Art Museum studying Egyptian mummies
2017	Participation in the popular science show "Centre of Excellence of Curiosity" of the national television station
2017	Participation in the Science city of the Tartu Hanseatic Days
2017	Organizing a "Crazy scientist's experiment morning" in University of Tartu Museum
2017	Collaboration for the aDNA section of the exhibition "Glory of the Cathedral" in University of Tartu Museum
2016–…	Lecturer, instructor of practical courses and supervisor on the bachelor level in the University of Tartu

Publications:

Järve M, **Saag L**, Scheib CL, Pathak AK, Montinaro F, Pagani1 L, Flores R, Guellil M, Saag L, Tambets K, Kushniarevich A, Solnik A, Varul L, Zadnikov S, Petrauskas O, Avramenko M, Magomedov B, Didenko S, Toshev G, Bruyako I, Grechko D, Okatenko V, Gorbenko K, Smyrnov O, Heiko A, Reida R, Sapiehin S, Sirotin S, Tairov A, Beisenov A, Starodubtsev M, Vasilev V, Nechvaloda A, Atabiev B, Litvinov S, Ekomasova N, Dzhaubermezov M, Voroniatov S, Utevska O, Shramko I, Khusnutdinova E, Metspalu M, Savelev N, Kriiska A, Kivisild T, Villems R. 2019. **Shifts in the genetic landscape of the western Eurasian Steppe associated with the beginning and end of the Scythian dominance.** *Current Biology* accepted

Saag L, Laneman M, Varul L, Malve M, Valk H, Razzak MA, Shirobokov IG, Khartanovich VI, Mikhaylova ER, Kushniarevich A, Scheib CL, Solnik A, Reisberg T, Parik J, Saag L, Metspalu E, Rootsi S, Remm M, Mägi R, D'Atanasio E, Crema ER, Díez-del-Molino D, Thomas MG, Kriiska A, Kivisild T, Villems R, Lang V, Metspalu M, Tambets K. 2019. **The Arrival of Siberian Ancestry Connecting the Eastern Baltic to Uralic Speakers Further East.** *Current Biology* 29(10):1701-1711

Tambets K, Yunusbayev B, Hudjashov G, Ilumäe AM, Rootsi S, Honkola T, Vesakoski O, Atkinson Q, Skoglund P, Kushniarevich A, Litvinov S, Reidla M, Metspalu E, **Saag L**, Rantanen T, Karmin M, Parik J, Zhadanov SI, Gubina M, Damba LD, Bermisheva M, Reisberg T, Dibirova K, Evseeva I, Nelis M, Klovins J, Metspalu A, Esko T, Balanovsky O, Balanovska E, Khusnutdinova EK, Osipova LP, Voevoda M, Villems R, Kivisild T, Metspalu M. 2018. **Genes reveal traces of common recent demographic history for most of the Uralic-speaking populations.** *Genome Biology* 19(1):139.

Margaryan A, Hansen HB, Rasmussen S, Sikora M, Moiseyev V, Khoklov A, Epimakhov A, Yepiskoposyan L, Kriiska A, Varul L, **Saag L**, Lynnerup N, Willerslev E, Allentoft ME. 2018. **Ancient pathogen DNA in human teeth and petrous bones.** *Ecology and Evolution* 8(6):3534-3542.

Saag L, Varul L, Scheib CL, Stenderup J, Allentoft ME, Saag L, Pagani L, Reidla M, Tambets K, Metspalu E, Kriiska A, Willerslev E, Kivisild T, Metspalu M. 2017. **Extensive Farming in Estonia Started through a Sex-Biased Migration from the Steppe.** *Current Biology* 27(14):2185-2193.

Pagani L, Lawson DJ, Jagoda E, Mörseburg A, Eriksson A, Mitt M, Clemente F, Hudjashov G, DeGiorgio M, Saag L, Wall JD, Cardona A, Mägi R, Wilson Sayres MA, Kaewert S, Inchley C, Scheib CL, Järve M, Karmin M, Jacobs GS, Antao T, Iliescu FM, Kushniarevich A, Ayub Q, Tyler-Smith C, Xue Y, Yunusbayev B, Tambets K, Mallick CB, **Saag L,** Pocheshkhova E, Andriadze G, Muller C, Westaway MC, Lambert DM, Zoraqi G, Turdikulova S, Dalimova D, Sabitov Z, Sultana GNN, Lachance J, Tishkoff S, Momynaliev K, Isakova J, Damba LD, Gubina M, Nymadawa P, Evseeva I, Atramentova L, Utevska O, Ricaut FX, Brucato N, Sudoyo H, Letellier T, Cox MP, Barashkov NA, Skaro V, Mulahasanovic L, Primorac D, Sahakyan H, Mormina M, Eichstaedt CA, Lichman DV, Abdullah S, Chaubey G, Wee JTS, Mihailov E, Karunas A, Litvinov S, Khusainova R, Ekomasova N, Akhmetova V, Khidiyatova I, Marjanović D, Yepiskoposyan L, Behar DM, Balanovska E, Metspalu A, Derenko M, Malyarchuk B, Voevoda M, Fedorova SA, Osipova LP, Lahr MM, Gerbault P, Leavesley M, Migliano AB, Petraglia M, Balanovsky O, Khusnutdinova EK, Metspalu E, Thomas MG, Manica A, Nielsen R, Villems R, Willerslev E, Kivisild T, Metspalu M. 2016. **Genomic analyses inform on migration events during the peopling of Eurasia.** *Nature* 538(7624):238-242.

Rasmussen S, Allentoft ME, Nielsen K, Orlando L, Sikora M, Sjögren KG, Pedersen AG, Schubert M, Van Dam A, Kapel CM, Nielsen HB, Brunak S, Avetisyan P, Epimakhov A, Khalyapin MV, Gnuni A, Kriiska A, Lasak I, Metspalu M, Moiseyev V, Gromov A, Pokutta D, **Saag L**, Varul L, Yepiskoposyan L, Sicheritz-Pontén T, Foley RA, Lahr MM, Nielsen R, Kristiansen K, Willerslev E. 2015. **Early divergent strains of Yersinia pestis in Eurasia 5,000 years ago.** *Cell* 163(3):571-82.

Allentoft ME, Sikora M, Sjögren KG, Rasmussen S, Rasmussen M, Stenderup J, Damgaard PB, Schroeder H, Ahlström T, Vinner L, Malaspinas AS, Margaryan A, Higham T, Chivall D, Lynnerup N, Harvig L, Baron J, Della Casa P, Dąbrowski P, Duffy PR, Ebel AV, Epimakhov A, Frei K, Furmanek M, Gralak T, Gromov A, Gronkiewicz S, Grupe G, Hajdu T, Jarysz R, Khartanovich V, Khokhlov A, Kiss V, Kolář J, Kriiska A, Lasak I, Longhi C, McGlynn G, Merkevicius A, Merkyte I, Metspalu M, Mkrtchyan R, Moiseyev V, Paja L, Pálfi G, Pokutta D, Pospieszny Ł, Price TD, **Saag L**, Sablin M, Shishlina N, Smrčka V, Soenov VI, Szeverényi V, Tóth G, Trifanova SV, Varul L, Vicze M, Yepiskoposyan L, Zhitenev V, Orlando L, Sicheritz-Pontén T, Brunak S, Nielsen R, Kristiansen K, Willerslev E. 2015. **Population genomics of Bronze Age Eurasia.** *Nature* 522(7555):167–72.

ELULOOKIRJELDUS

Nimi: Lehti Saag
Sünniaeg: 30. aprill 1991
Kontakt: Tartu Ülikool, Genoomika Instituut, Riia 23, Tartu 51010,
               +372 737 5053
E-post   lehti.saag@ut.ee

Haridus:
2015–2019 Doktoriõpinguid, Tartu Ülikool, Loodus- ja täppisteaduste valdkond, Tartu Ülikooli Molekulaar- ja rakubioloogia instituut, Evolutsioonilise bioloogia õppetool

2013–2015 *MSc*, *cum* laude, Tartu Ülikool, Loodus- ja tehnoloogiateaduskond, Molekulaar- ja rakubioloogia instituut, Evolutsioonilise bioloogia õppetool, (juh) Mait Metspalu, Ene Metspalu, "Neoliitiline transitsioon Eestis – kamm- ja nöörkeraamika kultuuride esindajate DNA sekveneerimine ning mitokondriaalse DNA analüüs"

2010–2013 *BSc*, Tartu Ülikool, Loodus- ja tehnoloogiateaduskond, Molekulaar- ja rakubioloogia instituut, Evolutsioonilise bioloogia õppetool, (juh) Ene Metspalu, "Kaukaasia populatsioonide jaotus mitokondriaalse DNA haplogrupp H alamhaplogruppide H1, H2, H5, H6, H10, H11 ja H13 alusel ning selle vastavus geograafilisele ja keelelisele jaotusele"

Tunnustused ja auhinnad:
2016 Eesti Teaduste Akadeemia konkursi "Teadus 3 minutiga" finalist
2012 *Rotalia Foundation*'i stipendiaat

Administratiivtöö:
2017–… Tartu Ülikooli Arheoloogia, geneetika ja lingvistika sidusuuringute kolleegiumi nõukogu liige

Teenistuskäigu lisainfo:
2019   Osalemine Raadio Kuku populaarteaduslikus saates "Kukkuv õun"
2019   Populaarteaduslik artikkel ajakirjas Tutulus
2018–… Magistritaseme üliõpilase juhendaja Tartu Ülikoolis
2018–19 Kaastöö näitusele "Geeniaalne" Tallinna Teletornis populatsiooniajaloo osas
2018   Osalemine Raadio Elmari populaarteaduslikus saates "Maailmamix"
2018   Populaarteaduslik artikkel ajakirjas Horisont
2017   Osalemine Tartu Ülikooli Kunstimuuseumi multidistsiplinaarses Egiptuse muumiate uurimise projektis

2017	Osalemine ETV populaarteaduslikus saates "Uudishimu tipp-keskus"
2017	Osalemine Tartu Hansapäevade Teaduslinnas
2017	Tartu Ülikooli Muuseumi "Hullu teadlase eksperimendihommiku" korraldamine
2017	Kaastöö Tartu Ülikooli Muuseumi näituse "Toomkiriku hiilgus" vana DNA osas
2016–…	Loengupidaja, praktikumijuhendaja ja tudengi juhendaja bakalaureuse tasemel Tartu Ülikoolis

Teaduspublikatsioonid:
loetletud ingliskeelse CV rubriigis *Publications*

DISSERTATIONES BIOLOGICAE
UNIVERSITATIS TARTUENSIS

1. **Toivo Maimets**. Studies of human oncoprotein p53. Tartu, 1991, 96 p.
2. **Enn K. Seppet**. Thyroid state control over energy metabolism, ion transport and contractile functions in rat heart. Tartu, 1991, 135 p.
3. **Kristjan Zobel**. Epifüütsete makrosamblike väärtus õhu saastuse indikaatoritena Hamar-Dobani boreaalsetes mägimetsades. Tartu, 1992, 131 lk.
4. **Andres Mäe**. Conjugal mobilization of catabolic plasmids by transposable elements in helper plasmids. Tartu, 1992, 91 p.
5. **Maia Kivisaar**. Studies on phenol degradation genes of *Pseudomonas* sp. strain EST 1001. Tartu, 1992, 61 p.
6. **Allan Nurk**. Nucleotide sequences of phenol degradative genes from *Pseudomonas sp.* strain EST 1001 and their transcriptional activation in *Pseudomonas putida*. Tartu, 1992, 72 p.
7. **Ülo Tamm**. The genus *Populus* L. in Estonia: variation of the species biology and introduction. Tartu, 1993, 91 p.
8. **Jaanus Remme**. Studies on the peptidyltransferase centre of the *E.coli* ribosome. Tartu, 1993, 68 p.
9. **Ülo Langel**. Galanin and galanin antagonists. Tartu, 1993, 97 p.
10. **Arvo Käärd**. The development of an automatic online dynamic fluorescense-based pH-dependent fiber optic penicillin flowthrought biosensor for the control of the benzylpenicillin hydrolysis. Tartu, 1993, 117 p.
11. **Lilian Järvekülg**. Antigenic analysis and development of sensitive immunoassay for potato viruses. Tartu, 1993, 147 p.
12. **Jaak Palumets**. Analysis of phytomass partition in Norway spruce. Tartu, 1993, 47 p.
13. **Arne Sellin**. Variation in hydraulic architecture of *Picea abies* (L.) Karst. trees grown under different enviromental conditions. Tartu, 1994, 119 p.
13. **Mati Reeben**. Regulation of light neurofilament gene expression. Tartu, 1994, 108 p.
14. **Urmas Tartes**. Respiration rhytms in insects. Tartu, 1995, 109 p.
15. **Ülo Puurand**. The complete nucleotide sequence and infections *in vitro* transcripts from cloned cDNA of a potato A potyvirus. Tartu, 1995, 96 p.
16. **Peeter Hõrak**. Pathways of selection in avian reproduction: a functional framework and its application in the population study of the great tit (*Parus major*). Tartu, 1995, 118 p.
17. **Erkki Truve**. Studies on specific and broad spectrum virus resistance in transgenic plants. Tartu, 1996, 158 p.
18. **Illar Pata**. Cloning and characterization of human and mouse ribosomal protein S6-encoding genes. Tartu, 1996, 60 p.
19. **Ülo Niinemets**. Importance of structural features of leaves and canopy in determining species shade-tolerance in temperature deciduous woody taxa. Tartu, 1996, 150 p.

20. **Ants Kurg**. Bovine leukemia virus: molecular studies on the packaging region and DNA diagnostics in cattle. Tartu, 1996, 104 p.
21. **Ene Ustav**. E2 as the modulator of the BPV1 DNA replication. Tartu, 1996, 100 p.
22. **Aksel Soosaar**. Role of helix-loop-helix and nuclear hormone receptor transcription factors in neurogenesis. Tartu, 1996, 109 p.
23. **Maido Remm**. Human papillomavirus type 18: replication, transformation and gene expression. Tartu, 1997, 117 p.
24. **Tiiu Kull**. Population dynamics in *Cypripedium calceolus* L. Tartu, 1997, 124 p.
25. **Kalle Olli**. Evolutionary life-strategies of autotrophic planktonic microorganisms in the Baltic Sea. Tartu, 1997, 180 p.
26. **Meelis Pärtel**. Species diversity and community dynamics in calcareous grassland communities in Western Estonia. Tartu, 1997, 124 p.
27. **Malle Leht**. The Genus *Potentilla* L. in Estonia, Latvia and Lithuania: distribution, morphology and taxonomy. Tartu, 1997, 186 p.
28. **Tanel Tenson**. Ribosomes, peptides and antibiotic resistance. Tartu, 1997, 80 p.
29. **Arvo Tuvikene**. Assessment of inland water pollution using biomarker responses in fish *in vivo* and *in vitro.* Tartu, 1997, 160 p.
30. **Urmas Saarma**. Tuning ribosomal elongation cycle by mutagenesis of 23S rRNA. Tartu, 1997, 134 p.
31. **Henn Ojaveer**. Composition and dynamics of fish stocks in the gulf of Riga ecosystem. Tartu, 1997, 138 p.
32. **Lembi Lõugas**. Post-glacial development of vertebrate fauna in Estonian water bodies. Tartu, 1997, 138 p.
33. **Margus Pooga**. Cell penetrating peptide, transportan, and its predecessors, galanin-based chimeric peptides. Tartu, 1998, 110 p.
34. **Andres Saag**. Evolutionary relationships in some cetrarioid genera (Lichenized Ascomycota). Tartu, 1998, 196 p.
35. **Aivar Liiv**. Ribosomal large subunit assembly *in vivo*. Tartu, 1998, 158 p.
36. **Tatjana Oja**. Isoenzyme diversity and phylogenetic affinities among the eurasian annual bromes (*Bromus* L., Poaceae). Tartu, 1998, 92 p.
37. **Mari Moora**. The influence of arbuscular mycorrhizal (AM) symbiosis on the competition and coexistence of calcareous grassland plant species. Tartu, 1998, 78 p.
38. **Olavi Kurina**. Fungus gnats in Estonia (*Diptera: Bolitophilidae, Keroplatidae, Macroceridae, Ditomyiidae, Diadocidiidae, Mycetophilidae*). Tartu, 1998, 200 p.
39. **Andrus Tasa**. Biological leaching of shales: black shale and oil shale. Tartu, 1998, 98 p.
40. **Arnold Kristjuhan**. Studies on transcriptional activator properties of tumor suppressor protein p53. Tartu, 1998, 86 p.
41. **Sulev Ingerpuu**. Characterization of some human myeloid cell surface and nuclear differentiation antigens. Tartu, 1998, 163 p.

42. **Veljo Kisand**. Responses of planktonic bacteria to the abiotic and biotic factors in the shallow lake Võrtsjärv. Tartu, 1998, 118 p.

43. **Kadri Põldmaa**. Studies in the systematics of hypomyces and allied genera (Hypocreales, Ascomycota). Tartu, 1998, 178 p.

44. **Markus Vetemaa**. Reproduction parameters of fish as indicators in environmental monitoring. Tartu, 1998, 117 p.

45. **Heli Talvik**. Prepatent periods and species composition of different *Oesophagostomum* spp. populations in Estonia and Denmark. Tartu, 1998, 104 p.

46. **Katrin Heinsoo**. Cuticular and stomatal antechamber conductance to water vapour diffusion in *Picea abies* (L.) karst. Tartu, 1999, 133 p.

47. **Tarmo Annilo**. Studies on mammalian ribosomal protein S7. Tartu, 1998, 77 p.

48. **Indrek Ots**. Health state indicies of reproducing great tits (*Parus major*): sources of variation and connections with life-history traits. Tartu, 1999, 117 p.

49. **Juan Jose Cantero**. Plant community diversity and habitat relationships in central Argentina grasslands. Tartu, 1999, 161 p.

50. **Rein Kalamees**. Seed bank, seed rain and community regeneration in Estonian calcareous grasslands. Tartu, 1999, 107 p.

51. **Sulev Kõks**. Cholecystokinin (CCK) – induced anxiety in rats: influence of environmental stimuli and involvement of endopioid mechanisms and serotonin. Tartu, 1999, 123 p.

52. **Ebe Sild**. Impact of increasing concentrations of O_3 and CO_2 on wheat, clover and pasture. Tartu, 1999, 123 p.

53. **Ljudmilla Timofejeva**. Electron microscopical analysis of the synaptonemal complex formation in cereals. Tartu, 1999, 99 p.

54. **Andres Valkna**. Interactions of galanin receptor with ligands and G-proteins: studies with synthetic peptides. Tartu, 1999, 103 p.

55. **Taavi Virro**. Life cycles of planktonic rotifers in lake Peipsi. Tartu, 1999, 101 p.

56. **Ana Rebane**. Mammalian ribosomal protein S3a genes and intron-encoded small nucleolar RNAs U73 and U82. Tartu, 1999, 85 p.

57. **Tiina Tamm**. Cocksfoot mottle virus: the genome organisation and translational strategies. Tartu, 2000, 101 p.

58. **Reet Kurg**. Structure-function relationship of the bovine papilloma virus E2 protein. Tartu, 2000, 89 p.

59. **Toomas Kivisild**. The origins of Southern and Western Eurasian populations: an mtDNA study. Tartu, 2000, 121 p.

60. **Niilo Kaldalu**. Studies of the TOL plasmid transcription factor XylS. Tartu, 2000, 88 p.

61. **Dina Lepik**. Modulation of viral DNA replication by tumor suppressor protein p53. Tartu, 2000, 106 p.

62. **Kai Vellak**. Influence of different factors on the diversity of the bryophyte vegetation in forest and wooded meadow communities. Tartu, 2000, 122 p.

63. **Jonne Kotta**. Impact of eutrophication and biological invasionas on the structure and functions of benthic macrofauna. Tartu, 2000, 160 p.

64. **Georg Martin**. Phytobenthic communities of the Gulf of Riga and the inner sea the West-Estonian archipelago. Tartu, 2000, 139 p.

65. **Silvia Sepp**. Morphological and genetical variation of *Alchemilla L.* in Estonia. Tartu, 2000. 124 p.

66. **Jaan Liira**. On the determinants of structure and diversity in herbaceous plant communities. Tartu, 2000, 96 p.

67. **Priit Zingel**. The role of planktonic ciliates in lake ecosystems. Tartu, 2001, 111 p.

68. **Tiit Teder**. Direct and indirect effects in Host-parasitoid interactions: ecological and evolutionary consequences. Tartu, 2001, 122 p.

69. **Hannes Kollist**. Leaf apoplastic ascorbate as ozone scavenger and its transport across the plasma membrane. Tartu, 2001, 80 p.

70. **Reet Marits**. Role of two-component regulator system PehR-PehS and extracellular protease PrtW in virulence of *Erwinia Carotovora* subsp. *Carotovora*. Tartu, 2001, 112 p.

71. **Vallo Tilgar**. Effect of calcium supplementation on reproductive performance of the pied flycatcher *Ficedula hypoleuca* and the great tit *Parus major,* breeding in Nothern temperate forests. Tartu, 2002, 126 p.

72. **Rita Hõrak**. Regulation of transposition of transposon Tn*4652* in *Pseudomonas putida*. Tartu, 2002, 108 p.

73. **Liina Eek-Piirsoo**. The effect of fertilization, mowing and additional illumination on the structure of a species-rich grassland community. Tartu, 2002, 74 p.

74. **Krõõt Aasamaa**. Shoot hydraulic conductance and stomatal conductance of six temperate deciduous tree species. Tartu, 2002, 110 p.

75. **Nele Ingerpuu**. Bryophyte diversity and vascular plants. Tartu, 2002, 112 p.

76. **Neeme Tõnisson**. Mutation detection by primer extension on oligonucleotide microarrays. Tartu, 2002, 124 p.

77. **Margus Pensa**. Variation in needle retention of Scots pine in relation to leaf morphology, nitrogen conservation and tree age. Tartu, 2003, 110 p.

78. **Asko Lõhmus**. Habitat preferences and quality for birds of prey: from principles to applications. Tartu, 2003, 168 p.

79. **Viljar Jaks**. p53 – a switch in cellular circuit. Tartu, 2003, 160 p.

80. **Jaana Männik**. Characterization and genetic studies of four ATP-binding cassette (ABC) transporters. Tartu, 2003, 140 p.

81. **Marek Sammul**. Competition and coexistence of clonal plants in relation to productivity. Tartu, 2003, 159 p

82. **Ivar Ilves**. Virus-cell interactions in the replication cycle of bovine papillomavirus type 1. Tartu, 2003, 89 p.

83. **Andres Männik**. Design and characterization of a novel vector system based on the stable replicator of bovine papillomavirus type 1. Tartu, 2003, 109 p.

84. **Ivika Ostonen**. Fine root structure, dynamics and proportion in net primary production of Norway spruce forest ecosystem in relation to site conditions. Tartu, 2003, 158 p.

85. **Gudrun Veldre**. Somatic status of 12–15-year-old Tartu schoolchildren. Tartu, 2003, 199 p.

86. **Ülo Väli**. The greater spotted eagle *Aquila clanga* and the lesser spotted eagle *A. pomarina*: taxonomy, phylogeography and ecology. Tartu, 2004, 159 p.

87. **Aare Abroi**. The determinants for the native activities of the bovine papillomavirus type 1 E2 protein are separable. Tartu, 2004, 135 p.

88. **Tiina Kahre**. Cystic fibrosis in Estonia. Tartu, 2004, 116 p.

89. **Helen Orav-Kotta**. Habitat choice and feeding activity of benthic suspension feeders and mesograzers in the northern Baltic Sea. Tartu, 2004, 117 p.

90. **Maarja Öpik**. Diversity of arbuscular mycorrhizal fungi in the roots of perennial plants and their effect on plant performance. Tartu, 2004, 175 p.

91. **Kadri Tali**. Species structure of *Neotinea ustulata*. Tartu, 2004, 109 p.

92. **Kristiina Tambets**. Towards the understanding of post-glacial spread of human mitochondrial DNA haplogroups in Europe and beyond: a phylogeographic approach. Tartu, 2004, 163 p.

93. **Arvi Jõers**. Regulation of p53-dependent transcription. Tartu, 2004, 103 p.

94. **Lilian Kadaja**. Studies on modulation of the activity of tumor suppressor protein p53. Tartu, 2004, 103 p.

95. **Jaak Truu**. Oil shale industry wastewater: impact on river microbial community and possibilities for bioremediation. Tartu, 2004, 128 p.

96. **Maire Peters**. Natural horizontal transfer of the *pheBA* operon. Tartu, 2004, 105 p.

97. **Ülo Maiväli**. Studies on the structure-function relationship of the bacterial ribosome. Tartu, 2004, 130 p.

98. **Merit Otsus**. Plant community regeneration and species diversity in dry calcareous grasslands. Tartu, 2004, 103 p.

99. **Mikk Heidemaa**. Systematic studies on sawflies of the genera *Dolerus, Empria,* and *Caliroa* (Hymenoptera: Tenthredinidae). Tartu, 2004, 167 p.

100. **Ilmar Tõnno**. The impact of nitrogen and phosphorus concentration and N/P ratio on cyanobacterial dominance and N_2 fixation in some Estonian lakes. Tartu, 2004, 111 p.

101. **Lauri Saks**. Immune function, parasites, and carotenoid-based ornaments in greenfinches. Tartu, 2004, 144 p.

102. **Siiri Rootsi**. Human Y-chromosomal variation in European populations. Tartu, 2004, 142 p.

103. **Eve Vedler**. Structure of the 2,4-dichloro-phenoxyacetic acid-degradative plasmid pEST4011. Tartu, 2005. 106 p.

104. **Andres Tover**. Regulation of transcription of the phenol degradation *pheBA* operon in *Pseudomonas putida*. Tartu, 2005, 126 p.

105. **Helen Udras**. Hexose kinases and glucose transport in the yeast *Hansenula polymorpha*. Tartu, 2005, 100 p.

106. **Ave Suija**. Lichens and lichenicolous fungi in Estonia: diversity, distribution patterns, taxonomy. Tartu, 2005, 162 p.

107. **Piret Lõhmus**. Forest lichens and their substrata in Estonia. Tartu, 2005, 162 p.

108. **Inga Lips**. Abiotic factors controlling the cyanobacterial bloom occurrence in the Gulf of Finland. Tartu, 2005, 156 p.

109. **Kaasik, Krista**. Circadian clock genes in mammalian clockwork, metabolism and behaviour. Tartu, 2005, 121 p.

110. **Juhan Javoiš**. The effects of experience on host acceptance in ovipositing moths. Tartu, 2005, 112 p.

111. **Tiina Sedman**. Characterization of the yeast *Saccharomyces cerevisiae* mitochondrial DNA helicase Hmi1. Tartu, 2005, 103 p.

112. **Ruth Aguraiuja**. Hawaiian endemic fern lineage *Diellia* (Aspleniaceae): distribution, population structure and ecology. Tartu, 2005, 112 p.

113. **Riho Teras**. Regulation of transcription from the fusion promoters generated by transposition of Tn*4652* into the upstream region of *pheBA* operon in *Pseudomonas putida*. Tartu, 2005, 106 p.

114. **Mait Metspalu**. Through the course of prehistory in India: tracing the mtDNA trail. Tartu, 2005, 138 p.

115. **Elin Lõhmussaar**. The comparative patterns of linkage disequilibrium in European populations and its implication for genetic association studies. Tartu, 2006, 124 p.

116. **Priit Kupper**. Hydraulic and environmental limitations to leaf water relations in trees with respect to canopy position. Tartu, 2006, 126 p.

117. **Heili Ilves**. Stress-induced transposition of Tn*4652* in *Pseudomonas Putida*. Tartu, 2006, 120 p.

118. **Silja Kuusk**. Biochemical properties of Hmi1p, a DNA helicase from *Saccharomyces cerevisiae* mitochondria. Tartu, 2006, 126 p.

119. **Kersti Püssa**. Forest edges on medium resolution landsat thematic mapper satellite images. Tartu, 2006, 90 p.

120. **Lea Tummeleht**. Physiological condition and immune function in great tits (*Parus major* l.): Sources of variation and trade-offs in relation to growth. Tartu, 2006, 94 p.

121. **Toomas Esperk**. Larval instar as a key element of insect growth schedules. Tartu, 2006, 186 p.

122. **Harri Valdmann**. Lynx (*Lynx lynx*) and wolf (*Canis lupus*) in the Baltic region: Diets, helminth parasites and genetic variation. Tartu, 2006. 102 p.

123. **Priit Jõers**. Studies of the mitochondrial helicase Hmi1p in *Candida albicans* and *Saccharomyces cerevisia*. Tartu, 2006. 113 p.

124. **Kersti Lilleväli**. Gata3 and Gata2 in inner ear development. Tartu, 2007, 123 p.

125. **Kai Rünk**. Comparative ecology of three fern species: *Dryopteris carthusiana* (Vill.) H.P. Fuchs, *D. expansa* (C. Presl) Fraser-Jenkins & Jermy and *D. dilatata* (Hoffm.) A. Gray (Dryopteridaceae). Tartu, 2007, 143 p.

126. **Aveliina Helm**. Formation and persistence of dry grassland diversity: role of human history and landscape structure. Tartu, 2007, 89 p.

127. **Leho Tedersoo**. Ectomycorrhizal fungi: diversity and community structure in Estonia, Seychelles and Australia. Tartu, 2007, 233 p.

128. **Marko Mägi**. The habitat-related variation of reproductive performance of great tits in a deciduous-coniferous forest mosaic: looking for causes and consequences. Tartu, 2007, 135 p.

129. **Valeria Lulla**. Replication strategies and applications of Semliki Forest virus. Tartu, 2007, 109 p.

130. **Ülle Reier**. Estonian threatened vascular plant species: causes of rarity and conservation. Tartu, 2007, 79 p.

131. **Inga Jüriado**. Diversity of lichen species in Estonia: influence of regional and local factors. Tartu, 2007, 171 p.

132. **Tatjana Krama**. Mobbing behaviour in birds: costs and reciprocity based cooperation. Tartu, 2007, 112 p.

133. **Signe Saumaa**. The role of DNA mismatch repair and oxidative DNA damage defense systems in avoidance of stationary phase mutations in *Pseudomonas putida*. Tartu, 2007, 172 p.

134. **Reedik Mägi**. The linkage disequilibrium and the selection of genetic markers for association studies in european populations. Tartu, 2007, 96 p.

135. **Priit Kilgas**. Blood parameters as indicators of physiological condition and skeletal development in great tits (*Parus major*): natural variation and application in the reproductive ecology of birds. Tartu, 2007, 129 p.

136. **Anu Albert**. The role of water salinity in structuring eastern Baltic coastal fish communities. Tartu, 2007, 95 p.

137. **Kärt Padari**. Protein transduction mechanisms of transportans. Tartu, 2008, 128 p.

138. **Siiri-Lii Sandre**. Selective forces on larval colouration in a moth. Tartu, 2008, 125 p.

139. **Ülle Jõgar**. Conservation and restoration of semi-natural floodplain meadows and their rare plant species. Tartu, 2008, 99 p.

140. **Lauri Laanisto**. Macroecological approach in vegetation science: generality of ecological relationships at the global scale. Tartu, 2008, 133 p.

141. **Reidar Andreson**. Methods and software for predicting PCR failure rate in large genomes. Tartu, 2008, 105 p.

142. **Birgot Paavel**. Bio-optical properties of turbid lakes. Tartu, 2008, 175 p.

143. **Kaire Torn**. Distribution and ecology of charophytes in the Baltic Sea. Tartu, 2008, 98 p.

144. **Vladimir Vimberg**. Peptide mediated macrolide resistance. Tartu, 2008, 190 p.

145. **Daima Örd**. Studies on the stress-inducible pseudokinase TRB3, a novel inhibitor of transcription factor ATF4. Tartu, 2008, 108 p.

146. **Lauri Saag**. Taxonomic and ecologic problems in the genus *Lepraria* (*Stereocaulaceae*, lichenised *Ascomycota*). Tartu, 2008, 175 p.
147. **Ulvi Karu**. Antioxidant protection, carotenoids and coccidians in green-finches – assessment of the costs of immune activation and mechanisms of parasite resistance in a passerine with carotenoid-based ornaments. Tartu, 2008, 124 p.
148. **Jaanus Remm**. Tree-cavities in forests: density, characteristics and occupancy by animals. Tartu, 2008, 128 p.
149. **Epp Moks**. Tapeworm parasites *Echinococcus multilocularis* and *E. granulosus* in Estonia: phylogenetic relationships and occurrence in wild carnivores and ungulates. Tartu, 2008, 82 p.
150. **Eve Eensalu**. Acclimation of stomatal structure and function in tree canopy: effect of light and CO_2 concentration. Tartu, 2008, 108 p.
151. **Janne Pullat**. Design, functionlization and application of an *in situ* synthesized oligonucleotide microarray. Tartu, 2008, 108 p.
152. **Marta Putrinš**. Responses of *Pseudomonas putida* to phenol-induced metabolic and stress signals. Tartu, 2008, 142 p.
153. **Marina Semtšenko**. Plant root behaviour: responses to neighbours and physical obstructions. Tartu, 2008, 106 p.
154. **Marge Starast**. Influence of cultivation techniques on productivity and fruit quality of some *Vaccinium* and *Rubus* taxa. Tartu, 2008, 154 p.
155. **Age Tats**. Sequence motifs influencing the efficiency of translation. Tartu, 2009, 104 p.
156. **Radi Tegova**. The role of specialized DNA polymerases in mutagenesis in *Pseudomonas putida*. Tartu, 2009, 124 p.
157. **Tsipe Aavik**. Plant species richness, composition and functional trait pattern in agricultural landscapes – the role of land use intensity and landscape structure. Tartu, 2009, 112 p.
158. **Kaja Kiiver**. Semliki forest virus based vectors and cell lines for studying the replication and interactions of alphaviruses and hepaciviruses. Tartu, 2009, 104 p.
159. **Meelis Kadaja**. Papillomavirus Replication Machinery Induces Genomic Instability in its Host Cell. Tartu, 2009, 126 p.
160. **Pille Hallast**. Human and chimpanzee Luteinizing hormone/Chorionic Gonadotropin beta (*LHB/CGB*) gene clusters: diversity and divergence of young duplicated genes. Tartu, 2009, 168 p.
161. **Ain Vellak**. Spatial and temporal aspects of plant species conservation. Tartu, 2009, 86 p.
162. **Triinu Remmel**. Body size evolution in insects with different colouration strategies: the role of predation risk. Tartu, 2009, 168 p.
163. **Jaana Salujõe**. Zooplankton as the indicator of ecological quality and fish predation in lake ecosystems. Tartu, 2009, 129 p.
164. **Ele Vahtmäe**. Mapping benthic habitat with remote sensing in optically complex coastal environments. Tartu, 2009, 109 p.

165. **Liisa Metsamaa**. Model-based assessment to improve the use of remote sensing in recognition and quantitative mapping of cyanobacteria. Tartu, 2009, 114 p.

166. **Pille Säälik**. The role of endocytosis in the protein transduction by cell-penetrating peptides. Tartu, 2009, 155 p.

167. **Lauri Peil**. Ribosome assembly factors in *Escherichia coli.* Tartu, 2009, 147 p.

168. **Lea Hallik**. Generality and specificity in light harvesting, carbon gain capacity and shade tolerance among plant functional groups. Tartu, 2009, 99 p.

169. **Mariliis Tark**. Mutagenic potential of DNA damage repair and tolerance mechanisms under starvation stress. Tartu, 2009, 191 p.

170. **Riinu Rannap**. Impacts of habitat loss and restoration on amphibian populations. Tartu, 2009, 117 p.

171. **Maarja Adojaan**. Molecular variation of HIV-1 and the use of this knowledge in vaccine development. Tartu, 2009, 95 p.

172. **Signe Altmäe**. Genomics and transcriptomics of human induced ovarian folliculogenesis. Tartu, 2010, 179 p.

173. **Triin Suvi**. Mycorrhizal fungi of native and introduced trees in the Seychelles Islands. Tartu, 2010, 107 p.

174. **Velda Lauringson**. Role of suspension feeding in a brackish-water coastal sea. Tartu, 2010, 123 p.

175. **Eero Talts**. Photosynthetic cyclic electron transport – measurement and variably proton-coupled mechanism. Tartu, 2010, 121 p.

176. **Mari Nelis**. Genetic structure of the Estonian population and genetic distance from other populations of European descent. Tartu, 2010, 97 p.

177. **Kaarel Krjutškov**. Arrayed Primer Extension-2 as a multiplex PCR-based method for nucleic acid variation analysis: method and applications. Tartu, 2010, 129 p.

178. **Egle Köster**. Morphological and genetical variation within species complexes: *Anthyllis vulneraria* s. l. and *Alchemilla vulgaris* (coll.). Tartu, 2010, 101 p.

179. **Erki Õunap**. Systematic studies on the subfamily Sterrhinae (Lepidoptera: Geometridae). Tartu, 2010, 111 p.

180. **Merike Jõesaar**. Diversity of key catabolic genes at degradation of phenol and *p*-cresol in pseudomonads. Tartu, 2010, 125 p.

181. **Kristjan Herkül**. Effects of physical disturbance and habitat-modifying species on sediment properties and benthic communities in the northern Baltic Sea. Tartu, 2010, 123 p.

182. **Arto Pulk**. Studies on bacterial ribosomes by chemical modification approaches. Tartu, 2010, 161 p.

183. **Maria Põllupüü**. Ecological relations of cladocerans in a brackish-water ecosystem. Tartu, 2010, 126 p.

184. **Toomas Silla**. Study of the segregation mechanism of the Bovine Papillomavirus Type 1. Tartu, 2010, 188 p.

185. **Gyaneshwer Chaubey**. The demographic history of India: A perspective based on genetic evidence. Tartu, 2010, 184 p.
186. **Katrin Kepp**. Genes involved in cardiovascular traits: detection of genetic variation in Estonian and Czech populations. Tartu, 2010, 164 p.
187. **Virve Sõber**. The role of biotic interactions in plant reproductive performance. Tartu, 2010, 92 p.
188. **Kersti Kangro**. The response of phytoplankton community to the changes in nutrient loading. Tartu, 2010, 144 p.
189. **Joachim M. Gerhold**. Replication and Recombination of mitochondrial DNA in Yeast. Tartu, 2010, 120 p.
190. **Helen Tammert**. Ecological role of physiological and phylogenetic diversity in aquatic bacterial communities. Tartu, 2010, 140 p.
191. **Elle Rajandu**. Factors determining plant and lichen species diversity and composition in Estonian *Calamagrostis* and *Hepatica* site type forests. Tartu, 2010, 123 p.
192. **Paula Ann Kivistik**. ColR-ColS signalling system and transposition of Tn*4652* in the adaptation of *Pseudomonas putida.* Tartu, 2010, 118 p.
193. **Siim Sõber**. Blood pressure genetics: from candidate genes to genome-wide association studies. Tartu, 2011, 120 p.
194. **Kalle Kipper**. Studies on the role of helix 69 of 23S rRNA in the factor-dependent stages of translation initiation, elongation, and termination. Tartu, 2011, 178 p.
195. **Triinu Siibak**. Effect of antibiotics on ribosome assembly is indirect. Tartu, 2011, 134 p.
196. **Tambet Tõnissoo**. Identification and molecular analysis of the role of guanine nucleotide exchange factor RIC-8 in mouse development and neural function. Tartu, 2011, 110 p.
197. **Helin Räägel**. Multiple faces of cell-penetrating peptides – their intracellular trafficking, stability and endosomal escape during protein transduction. Tartu, 2011, 161 p.
198. **Andres Jaanus**. Phytoplankton in Estonian coastal waters – variability, trends and response to environmental pressures. Tartu, 2011, 157 p.
199. **Tiit Nikopensius**. Genetic predisposition to nonsyndromic orofacial clefts. Tartu, 2011, 152 p.
200. **Signe Värv**. Studies on the mechanisms of RNA polymerase II-dependent transcription elongation. Tartu, 2011, 108 p.
201. **Kristjan Välk**. Gene expression profiling and genome-wide association studies of non-small cell lung cancer. Tartu, 2011, 98 p.
202. **Arno Põllumäe**. Spatio-temporal patterns of native and invasive zooplankton species under changing climate and eutrophication conditions. Tartu, 2011, 153 p.
203. **Egle Tammeleht**. Brown bear (*Ursus arctos*) population structure, demographic processes and variations in diet in northern Eurasia. Tartu, 2011, 143 p.

205. **Teele Jairus**. Species composition and host preference among ectomy-corrhizal fungi in Australian and African ecosystems. Tartu, 2011, 106 p.
206. **Kessy Abarenkov**. PlutoF – cloud database and computing services supporting biological research. Tartu, 2011, 125 p.
207. **Marina Grigorova**. Fine-scale genetic variation of follicle-stimulating hormone beta-subunit coding gene (*FSHB*) and its association with reproductive health. Tartu, 2011, 184 p.
208. **Anu Tiitsaar**. The effects of predation risk and habitat history on butterfly communities. Tartu, 2011, 97 p.
209. **Elin Sild**. Oxidative defences in immunoecological context: validation and application of assays for nitric oxide production and oxidative burst in a wild passerine. Tartu, 2011, 105 p.
210. **Irja Saar**. The taxonomy and phylogeny of the genera *Cystoderma* and *Cystodermella* (Agaricales, Fungi). Tartu, 2012, 167 p.
211. **Pauli Saag**. Natural variation in plumage bacterial assemblages in two wild breeding passerines. Tartu, 2012, 113 p.
212. **Aleksei Lulla**. Alphaviral nonstructural protease and its polyprotein substrate: arrangements for the perfect marriage. Tartu, 2012, 143 p.
213. **Mari Järve**. Different genetic perspectives on human history in Europe and the Caucasus: the stories told by uniparental and autosomal markers. Tartu, 2012, 119 p.
214. **Ott Scheler**. The application of tmRNA as a marker molecule in bacterial diagnostics using microarray and biosensor technology. Tartu, 2012, 93 p.
215. **Anna Balikova**. Studies on the functions of tumor-associated mucin-like leukosialin (CD43) in human cancer cells. Tartu, 2012, 129 p.
216. **Triinu Kõressaar**. Improvement of PCR primer design for detection of prokaryotic species. Tartu, 2012, 83 p.
217. **Tuul Sepp**. Hematological health state indices of greenfinches: sources of individual variation and responses to immune system manipulation. Tartu, 2012, 117 p.
218. **Rya Ero**. Modifier view of the bacterial ribosome. Tartu, 2012, 146 p.
219. **Mohammad Bahram**. Biogeography of ectomycorrhizal fungi across different spatial scales. Tartu, 2012, 165 p.
220. **Annely Lorents**. Overcoming the plasma membrane barrier: uptake of amphipathic cell-penetrating peptides induces influx of calcium ions and downstream responses. Tartu, 2012, 113 p.
221. **Katrin Männik**. Exploring the genomics of cognitive impairment: whole-genome SNP genotyping experience in Estonian patients and general population. Tartu, 2012, 171 p.
222. **Marko Prous**. Taxonomy and phylogeny of the sawfly genus *Empria* (Hymenoptera, Tenthredinidae). Tartu, 2012, 192 p.
223. **Triinu Visnapuu**. Levansucrases encoded in the genome of *Pseudomonas syringae* pv. tomato DC3000: heterologous expression, biochemical characterization, mutational analysis and spectrum of polymerization products. Tartu, 2012, 160 p.

224. **Nele Tamberg**. Studies on Semliki Forest virus replication and pathogenesis. Tartu, 2012, 109 p.

225. **Tõnu Esko**. Novel applications of SNP array data in the analysis of the genetic structure of Europeans and in genetic association studies. Tartu, 2012, 149 p.

226. **Timo Arula**. Ecology of early life-history stages of herring *Clupea harengus membras* in the northeastern Baltic Sea. Tartu, 2012, 143 p.

227. **Inga Hiiesalu**. Belowground plant diversity and coexistence patterns in grassland ecosystems. Tartu, 2012, 130 p.

228. **Kadri Koorem**. The influence of abiotic and biotic factors on small-scale plant community patterns and regeneration in boreonemoral forest. Tartu, 2012, 114 p.

229. **Liis Andresen**. Regulation of virulence in plant-pathogenic pectobacteria. Tartu, 2012, 122 p.

230. **Kaupo Kohv**. The direct and indirect effects of management on boreal forest structure and field layer vegetation. Tartu, 2012, 124 p.

231. **Mart Jüssi**. Living on an edge: landlocked seals in changing climate. Tartu, 2012, 114 p.

232. **Riina Klais**. Phytoplankton trends in the Baltic Sea. Tartu, 2012, 136 p.

233. **Rauno Veeroja**. Effects of winter weather, population density and timing of reproduction on life-history traits and population dynamics of moose (*Alces alces*) in Estonia. Tartu, 2012, 92 p.

234. **Marju Keis**. Brown bear (*Ursus arctos*) phylogeography in northern Eurasia. Tartu, 2013, 142 p.

235. **Sergei Põlme**. Biogeography and ecology of *alnus*- associated ectomycorrhizal fungi – from regional to global scale. Tartu, 2013, 90 p.

236. **Liis Uusküla**. Placental gene expression in normal and complicated pregnancy. Tartu, 2013, 173 p.

237. **Marko Lõoke**. Studies on DNA replication initiation in *Saccharomyces cerevisiae.* Tartu, 2013, 112 p.

238. **Anne Aan**. Light- and nitrogen-use and biomass allocation along productivity gradients in multilayer plant communities. Tartu, 2013, 127 p.

239. **Heidi Tamm**. Comprehending phylogenetic diversity – case studies in three groups of ascomycetes. Tartu, 2013, 136 p.

240. **Liina Kangur**. High-Pressure Spectroscopy Study of Chromophore-Binding Hydrogen Bonds in Light-Harvesting Complexes of Photosynthetic Bacteria. Tartu, 2013, 150 p.

241. **Margus Leppik**. Substrate specificity of the multisite specific pseudouridine synthase RluD. Tartu, 2013, 111 p.

242. **Lauris Kaplinski**. The application of oligonucleotide hybridization model for PCR and microarray optimization. Tartu, 2013, 103 p.

243. **Merli Pärnoja**. Patterns of macrophyte distribution and productivity in coastal ecosystems: effect of abiotic and biotic forcing. Tartu, 2013, 155 p.

244. **Tõnu Margus**. Distribution and phylogeny of the bacterial translational GTPases and the Mqsr/YgiT regulatory system. Tartu, 2013, 126 p.

245. **Pille Mänd**. Light use capacity and carbon and nitrogen budget of plants: remote assessment and physiological determinants. Tartu, 2013, 128 p.

246. **Mario Plaas**. Animal model of Wolfram Syndrome in mice: behavioural, biochemical and psychopharmacological characterization. Tartu, 2013, 144 p.

247. **Georgi Hudjašov**. Maps of mitochondrial DNA, Y-chromosome and tyrosinase variation in Eurasian and Oceanian populations. Tartu, 2013, 115 p.

248. **Mari Lepik**. Plasticity to light in herbaceous plants and its importance for community structure and diversity. Tartu, 2013, 102 p.

249. **Ede Leppik**. Diversity of lichens in semi-natural habitats of Estonia. Tartu, 2013, 151 p.

250. **Ülle Saks**. Arbuscular mycorrhizal fungal diversity patterns in boreonemoral forest ecosystems. Tartu, 2013, 151 p.

251. **Eneli Oitmaa**. Development of arrayed primer extension microarray assays for molecular diagnostic applications. Tartu, 2013, 147 p.

252. **Jekaterina Jutkina**. The horizontal gene pool for aromatics degradation: bacterial catabolic plasmids of the Baltic Sea aquatic system. Tartu, 2013, 121 p.

253. **Helen Vellau**. Reaction norms for size and age at maturity in insects: rules and exceptions. Tartu, 2014, 132 p.

254. **Randel Kreitsberg**. Using biomarkers in assessment of environmental contamination in fish – new perspectives. Tartu, 2014, 107 p.

255. **Krista Takkis**. Changes in plant species richness and population performance in response to habitat loss and fragmentation.Tartu, 2014, 141 p.

256. **Liina Nagirnaja**. Global and fine-scale genetic determinants of recurrent pregnancy loss. Tartu, 2014, 211 p.

257. **Triin Triisberg**. Factors influencing the re-vegetation of abandoned extracted peatlands in Estonia. Tartu, 2014, 133 p.

258. **Villu Soon**. A phylogenetic revision of the *Chrysis ignita* species group (Hymenoptera: Chrysididae) with emphasis on the northern European fauna. Tartu, 2014, 211 p.

259. **Andrei Nikonov**. RNA-Dependent RNA Polymerase Activity as a Basis for the Detection of Positive-Strand RNA Viruses by Vertebrate Host Cells. Tartu, 2014, 207 p.

260. **Eele Õunapuu-Pikas**. Spatio-temporal variability of leaf hydraulic conductance in woody plants: ecophysiological consequences. Tartu, 2014, 135 p.

261. **Marju Männiste**. Physiological ecology of greenfinches: information content of feathers in relation to immune function and behavior. Tartu, 2014, 121 p.

262. **Katre Kets**. Effects of elevated concentrations of CO_2 and O_3 on leaf photosynthetic parameters in *Populus tremuloides*: diurnal, seasonal and interannual patterns. Tartu, 2014, 115 p.

263. **Külli Lokko**. Seasonal and spatial variability of zoopsammon communities in relation to environmental parameters. Tartu, 2014, 129 p.

264. **Olga Žilina**. Chromosomal microarray analysis as diagnostic tool: Estonian experience. Tartu, 2014, 152 p.

265. **Kertu Lõhmus**. Colonisation ecology of forest-dwelling vascular plants and the conservation value of rural manor parks. Tartu, 2014, 111 p.

266. **Anu Aun**. Mitochondria as integral modulators of cellular signaling. Tartu, 2014, 167 p.

267. **Chandana Basu Mallick**. Genetics of adaptive traits and gender-specific demographic processes in South Asian populations. Tartu, 2014, 160 p.

268. **Riin Tamme**. The relationship between small-scale environmental heterogeneity and plant species diversity. Tartu, 2014, 130 p.

269. **Liina Remm**. Impacts of forest drainage on biodiversity and habitat quality: implications for sustainable management and conservation. Tartu, 2015, 126 p.

270. **Tiina Talve**. Genetic diversity and taxonomy within the genus *Rhinanthus*. Tartu, 2015, 106 p.

271. **Mehis Rohtla**. Otolith sclerochronological studies on migrations, spawning habitat preferences and age of freshwater fishes inhabiting the Baltic Sea. Tartu, 2015, 137 p.

272. **Alexey Reshchikov**. The world fauna of the genus *Lathrolestes* (Hymenoptera, Ichneumonidae). Tartu, 2015, 247 p.

273. **Martin Pook**. Studies on artificial and extracellular matrix protein-rich surfaces as regulators of cell growth and differentiation. Tartu, 2015, 142 p.

274. **Mai Kukumägi**. Factors affecting soil respiration and its components in silver birch and Norway spruce stands. Tartu, 2015, 155 p.

275. **Helen Karu**. Development of ecosystems under human activity in the North-East Estonian industrial region: forests on post-mining sites and bogs. Tartu, 2015, 152 p.

276. **Hedi Peterson**. Exploiting high-throughput data for establishing relationships between genes. Tartu, 2015, 186 p.

277. **Priit Adler**. Analysis and visualisation of large scale microarray data, Tartu, 2015, 126 p.

278. **Aigar Niglas**. Effects of environmental factors on gas exchange in deciduous trees: focus on photosynthetic water-use efficiency. Tartu, 2015, 152 p.

279. **Silja Laht**. Classification and identification of conopeptides using profile hidden Markov models and position-specific scoring matrices. Tartu, 2015, 100 p.

280. **Martin Kesler**. Biological characteristics and restoration of Atlantic salmon *Salmo salar* populations in the Rivers of Northern Estonia. Tartu, 2015, 97 p.

281. **Pratyush Kumar Das**. Biochemical perspective on alphaviral nonstructural protein 2: a tale from multiple domains to enzymatic profiling. Tartu, 2015, 205 p

282. **Priit Palta**. Computational methods for DNA copy number detection. Tartu, 2015, 130 p.
283. **Julia Sidorenko**. Combating DNA damage and maintenance of genome integrity in pseudomonads. Tartu, 2015, 174 p.
284. **Anastasiia Kovtun-Kante**. Charophytes of Estonian inland and coastal waters: distribution and environmental preferences. Tartu, 2015, 97 p.
285. **Ly Lindman**. The ecology of protected butterfly species in Estonia. Tartu, 2015, 171 p.
286. **Jaanis Lodjak**. Association of Insulin-like Growth Factor I and Corticosterone with Nestling Growth and Fledging Success in Wild Passerines. Tartu, 2016, 113 p.
287. **Ann Kraut**. Conservation of Wood-Inhabiting Biodiversity – Semi-Natural Forests as an Opportunity. Tartu, 2016, 141 p.
288. **Tiit Örd**. Functions and regulation of the mammalian pseudokinase TRIB3. Tartu, 2016, 182. p.
289. **Kairi Käiro**. Biological Quality According to Macroinvertebrates in Streams of Estonia (Baltic Ecoregion of Europe): Effects of Human-induced Hydromorphological Changes. Tartu, 2016, 126 p.
290. **Leidi Laurimaa**. *Echinococcus multilocularis* and other zoonotic parasites in Estonian canids. Tartu, 2016, 144 p.
291. **Helerin Margus**. Characterization of cell-penetrating peptide/nucleic acid nanocomplexes and their cell-entry mechanisms. Tartu, 2016, 173 p.
292. **Kadri Runnel**. Fungal targets and tools for forest conservation. Tartu, 2016, 157 p.
293. **Urmo Võsa**. MicroRNAs in disease and health: aberrant regulation in lung cancer and association with genomic variation. Tartu, 2016, 163 p.
294. **Kristina Mäemets-Allas**. Studies on cell growth promoting AKT signaling pathway – a promising anti-cancer drug target. Tartu, 2016, 146 p.
295. **Janeli Viil**. Studies on cellular and molecular mechanisms that drive normal and regenerative processes in the liver and pathological processes in Dupuytren's contracture. Tartu, 2016, 175 p.
296. **Ene Kook**. Genetic diversity and evolution of *Pulmonaria angustifolia* L. and *Myosotis laxa sensu lato* (Boraginaceae). Tartu, 2016, 106 p.
297. **Kadri Peil**. RNA polymerase II-dependent transcription elongation in *Saccharomyces cerevisiae*. Tartu, 2016, 113 p.
298. **Katrin Ruisu**. The role of RIC8A in mouse development and its function in cell-matrix adhesion and actin cytoskeletal organisation. Tartu, 2016, 129 p.
299. **Janely Pae**. Translocation of cell-penetrating peptides across biological membranes and interactions with plasma membrane constituents. Tartu, 2016, 126 p.
300. **Argo Ronk**. Plant diversity patterns across Europe: observed and dark diversity. Tartu, 2016, 153 p.

301. **Kristiina Mark**. Diversification and species delimitation of lichenized fungi in selected groups of the family Parmeliaceae (Ascomycota). Tartu, 2016, 181 p.

302. **Jaak-Albert Metsoja**. Vegetation dynamics in floodplain meadows: influence of mowing and sediment application. Tartu, 2016, 140 p.

303. **Hedvig Tamman**. The GraTA toxin-antitoxin system of *Pseudomonas putida*: regulation and role in stress tolerance. Tartu, 2016, 154 p.

304. **Kadri Pärtel**. Application of ultrastructural and molecular data in the taxonomy of helotialean fungi. Tartu, 2016, 183 p.

305. **Maris Hindrikson**. Grey wolf (*Canis lupus*) populations in Estonia and Europe: genetic diversity, population structure and -processes, and hybridization between wolves and dogs. Tartu, 2016, 121 p.

306. **Polina Degtjarenko**. Impacts of alkaline dust pollution on biodiversity of plants and lichens: from communities to genetic diversity. Tartu, 2016, 126 p.

307. **Liina Pajusalu**. The effect of CO_2 enrichment on net photosynthesis of macrophytes in a brackish water environment. Tartu, 2016, 126 p.

308. **Stoyan Tankov**. Random walks in the stringent response. Tartu, 2016, 94 p.

309. **Liis Leitsalu**. Communicating genomic research results to population-based biobank participants. Tartu, 2016, 158 p.

310. **Richard Meitern**. Redox physiology of wild birds: validation and application of techniques for detecting oxidative stress. Tartu, 2016, 134 p.

311. **Kaie Lokk**. Comparative genome-wide DNA methylation studies of healthy human tissues and non-small cell lung cancer tissue. Tartu, 2016, 127 p.

312. **Mihhail Kurašin**. Processivity of cellulases and chitinases. Tartu, 2017, 132 p.

313. **Carmen Tali**. Scavenger receptors as a target for nucleic acid delivery with peptide vectors. Tartu, 2017, 155 p.

314. **Katarina Oganjan**. Distribution, feeding and habitat of benthic suspension feeders in a shallow coastal sea. Tartu, 2017, 132 p.

315. **Taavi Paal**. Immigration limitation of forest plants into wooded landscape corridors. Tartu, 2017, 145 p.

316. **Kadri Õunap**. The Williams-Beuren syndrome chromosome region protein WBSCR22 is a ribosome biogenesis factor. Tartu, 2017, 135 p.

317. **Riin Tamm**. In-depth analysis of factors affecting variability in thiopurine methyltransferase activity. Tartu, 2017, 170 p.

318. **Keiu Kask**. The role of RIC8A in the development and regulation of mouse nervous system. Tartu, 2017, 184 p.

319. **Tiia Möller**. Mapping and modelling of the spatial distribution of benthic macrovegetation in the NE Baltic Sea with a special focus on the eelgrass *Zostera marina* Linnaeus, 1753. Tartu, 2017, 162 p.

320. **Silva Kasela**. Genetic regulation of gene expression: detection of tissue- and cell type-specific effects. Tartu, 2017, 150 p.

321. **Karmen Süld**. Food habits, parasites and space use of the raccoon dog *Nyctereutes procyonoides*: the role of an alien species as a predator and vector of zoonotic diseases in Estonia. Tartu, 2017, p.

322. **Ragne Oja**. Consequences of supplementary feeding of wild boar – concern for ground-nesting birds and endoparasite infection. Tartu, 2017, 141 p.

323. **Riin Kont**. The acquisition of cellulose chain by a processive cellobio-hydrolase. Tartu, 2017, 117 p.

324. **Liis Kasari**. Plant diversity of semi-natural grasslands: drivers, current status and conservation challenges. Tartu, 2017, 141 p.

325. **Sirgi Saar**. Belowground interactions: the roles of plant genetic related-ness, root exudation and soil legacies. Tartu, 2017, 113 p.

326. **Sten Anslan**. Molecular identification of Collembola and their fungal associates. Tartu, 2017, 125 p.

327. **Imre Taal**. Causes of variation in littoral fish communities of the Eastern Baltic Sea: from community structure to individual life histories. Tartu, 2017, 118 p.

328. **Jürgen Jalak**. Dissecting the Mechanism of Enzymatic Degradation of Cellulose Using Low Molecular Weight Model Substrates. Tartu, 2017, 137 p.

329. **Kairi Kiik**. Reproduction and behaviour of the endangered European mink (*Mustela lutreola*) in captivity. Tartu, 2018, 112 p.

330. **Ivan Kuprijanov**. Habitat use and trophic interactions of native and invasive predatory macroinvertebrates in the northern Baltic Sea. Tartu, 2018, 117 p.

331. **Hendrik Meister**. Evolutionary ecology of insect growth: from geo-graphic patterns to biochemical trade-offs. Tartu, 2018, 147 p.

332. **Ilja Gaidutšik.** Irc3 is a mitochondrial branch migration enzyme in *Saccharomyces cerevisiae.* Tartu, 2018, 161 p.

333. **Lena Neuenkamp**. The dynamics of plant and arbuscular mycorrhizal fungal communities in grasslands under changing land use. Tartu, 2018, 241 p.

334. **Laura Kasak.** Genome structural variation modulating the placenta and pregnancy maintenance. Tartu, 2018, 181 p.

335. **Kersti Riibak.** Importance of dispersal limitation in determining dark diversity of plants across spatial scales. Tartu, 2018, 133 p.

336. **Liina Saar.** Dynamics of grassland plant diversity in changing landscapes. Tartu, 2018, 206 p.

337. **Hanna Ainelo.** Fis regulates *Pseudomonas putida* biofilm formation by controlling the expression of *lapA*. Tartu, 2018, 143 p.

338. **Natalia Pervjakova.** Genomic imprinting in complex traits. Tartu, 2018, 176 p.

339. **Andrio Lahesaare.** The role of global regulator Fis in regulating the expression of *lapF* and the hydrophobicity of soil bacterium *Pseudomonas putida*. Tartu, 2018, 124 p.

340. **Märt Roosaare.** *K*-mer based methods for the identification of bacteria and plasmids. Tartu, 2018, 117 p.
341. **Maria Abakumova.** The relationship between competitive behaviour and the frequency and identity of neighbours in temperate grassland plants. Tartu, 2018, 104 p.
342. **Margus Vilbas.** Biotic interactions affecting habitat use of myrmecophilous butterflies in Northern Europe. Tartu, 2018, 142 p.
343. **Liina Kinkar**. Global patterns of genetic diversity and phylogeography of *Echinococcus granulosus* sensu stricto – a tapeworm species of significant public health concern. Tartu, 2018, 147 p.
344. **Teivi Laurimäe.** Taxonomy and genetic diversity of zoonotic tapeworms in the species complex of *Echinococcus granulosus* sensu lato. Tartu, 2018, 143 p.
345. **Tatjana Jatsenko.** Role of translesion DNA polymerases in mutagenesis and DNA damage tolerance in Pseudomonads. Tartu, 2018, 216 p.
346. **Katrin Viigand.** Utilization of α-glucosidic sugars by *Ogataea* (*Hansenula*) *polymorpha.* Tartu, 2018, 148 p.
347. **Andres Ainelo.** Physiological effects of the *Pseudomonas putida* toxin grat. Tartu, 2018, 146 p.
348. **Killu Timm**. Effects of two genes (DRD4 and SERT) on great tit (*Parus major*) behaviour and reproductive traits. Tartu, 2018, 117 p.
349. **Petr Kohout**. Ecology of ericoid mycorrhizal fungi. Tartu, 2018, 184 p.
350. **Gristin Rohula-Okunev**. Effects of endogenous and environmental factors on night-time water flux in deciduous woody tree species. Tartu, 2018, 184 p.
351. **Jane Oja.** Temporal and spatial patterns of orchid mycorrhizal fungi in forest and grassland ecosystems. Tartu, 2018, 102 p.
352. **Janek Urvik**. Multidimensionality of aging in a long-lived seabird. Tartu, 2018, 135 p.
353. **Lisanna Schmidt**. Phenotypic and genetic differentiation in the hybridizing species pair *Carex flava* and *C. viridula* in geographically different regions. Tartu, 2018, 133 p.
354. **Monika Karmin.** Perspectives from human Y chromosome – phylogeny, population dynamics and founder events. Tartu, 2018, 168 p.
355. **Maris Alver.** Value of genomics for atherosclerotic cardiovascular disease risk prediction. Tartu, 2019, 148 p.