TINA SAUPE

The genetic history of the Mediterranean
before the common era:
a focus on the Italian Peninsula

TINA SAUPE

The genetic history of the Mediterranean before the common era: a focus on the Italian Peninsula

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

European Union
European Regional
Development Fund

Investing
in your future

"Nur wer die Vergangenheit kennt,
kann die Gegenwart verstehen
und die Zukunft gestalten."

"Only those who know the past,
can understand the present
and shape the future."

August Bebel (1840-1913)

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

LIST OF ORIGINAL PUBLICATIONS

I   Bortolini E, Pagani L, Oxilia G, Posth C, Fontana F, Badino F, **Saupe T**, Montinaro F, Margaritora D, Romandini M, Lugli F, Papini A, Boggioni M, Perrini N, Oxilia A, Cigliano RA, Barcelona R, Visentin D, Fasser N, Arrighi S, Figus C, Marciani G, Silvestrini S, Bernardini F, Menghi Sartorio JC, Fiorenza L, Cecchi JM, Tuniz C, Kivisild T, Gianfrancesco F, Peresani M, Scheib CL, Talamo S, D'Esposito M, Benazzi S. 2021. **Early Alpine occupation backdates westward human migration in Late Glacial Europe.** *Curr Biol.* 2021 Jun 7;31(11):2484–2493.e7.

II   **Saupe T***, Montinaro F*, Scaggion C, Carrara N, Kivisild T, D'Atanasio E, Hui R, Solnik A, Lebrasseur O, Larson G, Alessandri L, Arienzo I, De Angelis F, Rolfo MF, Skeates R, Silvestri L, Beckett J, Talamo S, Dolfini A, Miari M, Metspalu M, Benazzi S, Capelli C, Pagani L, Scheib CL. 2021. **Ancient genomes reveal structural shifts after the arrival of Steppe-related ancestry in the Italian Peninsula.** *Curr Biol*. 2021 Jun 21;31(12):2576–2591.e12.

III   Aneli S*, Saupe T*, Montinaro F, Solnik A, Molinaro L, Scaggion C, Carrara N, Raveane A, Kivisild T, Metspalu M, Scheib CL, Pagani L. 2022. **The Genetic Origin of Daunians and the Pan-Mediterranean Southern Italian Iron Age Context**. *Molecular Biology and Evolution*, Volume 39, Issue 2, February 2022, msac014, https://doi.org/10.1093/molbev/msac014.

    * Authors contributed equally to the study.

The author's contributions to the listed articles are as follows:

Reference (REF) I: I performed the laboratory work and first raw data analysis including mapping, estimating the average human coverage, and sex determination, provided the information to the first authors and reviewed the manuscript.

REF II: I was part of conceiving the study, performed the laboratory work, participated in data analysis focusing on raw data analysis and population genomic studies, interpreted the results, and co-wrote the manuscript.

REF III: I was part of conceiving the study, performed the laboratory work, participated in the data analysis focusing on raw data analysis, kinship estimation, mtDNA and Y chromosome haplogroup estimation, interpreted the results, and co-wrote the manuscript.

ABBREVIATIONS

1240k	124,000 SNPs overlapping the Human Origins SNP Array
A	adenine
aDNA	ancient DNA (deoxyribonucleic acid)
AN	Anatolian Neolithic
ANE	Ancient North Eurasians
BA	Bronze Age
BCE	Before the common era
bp	base pair
C	cytosine
CA	Chalcolithic/Copper Age
CE	Common era
CHG	Caucasus hunter-gatherers
EBA	Early Bronze Age
EEF	Early European farmers
EHG	Eastern hunter-gatherers
EN	European Neolithic
G	guanine
IA	Iron Age
IN	Iranian Neolithic
k	thousand
LGM	Last Glacial Maximum
MDS	Multidimensional scaling
mtDNA	mitochondrial DNA
N	Neolithic
NGS	Next-generation sequencing
NNLS	non-negative least square (algorithm)
PCA	Principal component analysis
PCR	Polymerase chain reaction
P	Palaeolithic
READ	Relationship estimation from ancient DNA
REF	Reference
sedaDNA	sedimentary DNA
SHG	Scandinavian hunter-gatherers
SNPs	single nuclear polymorphisms
T	thymine
WHG	Western hunter-gatherers
ya	years ago
Y chr	Y chromosome

1. INTRODUCTION

Numerous and varied studies have given a new insight into our understanding of the human past. The history of human habitation in Europe includes multiple stages starting with hunter-gatherers living in Western Eurasia during the Palaeolithic, the introduction of agriculture and domestication to Europe through the first contacts with non-local people incoming from the Levant and Anatolia (West Asia), and the arrival of 'Steppe' people from the Pontic-Caspian Steppe. Most of the studies have focused on the wider spectrum of genetic changes in Eurasia and the connection with archaeological evidence and historical events. Previously, researchers focussed on one culture, one geographical area, and/or one country. However, some areas of Eurasia are still understudied and their investigation and exploration might add more knowledge to the open gaps in the human migration history.

In particular, the Mediterranean Basin, a geographic landscape around the Mediterranean Sea located between the three continents – Africa in the South, Asia in the East, and Europe in the North – has played a central role in the history of human civilization. The climate around the Mediterranean Sea is mostly characterised by its mild winters and hot, dry summers, which makes the region a suitable place for human habitation. The Italian Peninsula, however, has long been understudied in the field of genetic history, despite its geographic location in the centre of the Mediterranean Basin and its socio-cultural connections to the surrounding populations. In addition, we know more about the historical events related to the Roman Empire and thereafter than about the migration events before the Iron Age and its genetic impact on the gene pool of present-day Italians.

Due to the development of next-generation sequencing (NGS) and successful extraction methods for ancient deoxyribonucleic acid (aDNA) from human remains, it is possible to combine the knowledge of history, archaeology, anthropology, and genetics to shed new insights into human history and present a new understanding of demographic changes. Nevertheless, the usage of aDNA is limited due to the inconsistent preservation of DNA molecules in human remains from areas with different environmental conditions, the limited number of available samples from geographical locations of interest, the degradation of DNA over time, and the high costs of analyses. Because of these factors, researchers have been improving laboratory workflows and bioinformatic methods to generate more data and provide the public with the results.

This thesis focuses on the genetic and social structure-related changes in the Italian Peninsula between the end of the last glacial maximum (LGM, 19,000-17,000 years ago (ya)) and ~2k ya parallel with the beginning of the Roman Republic. Genome-wide data was generated from human remains excavated from several archaeological sites in the northern, central, and southeastern regions of the Italian Peninsula.

This data was analysed in the context of previously published data to shed light on the genetic composition (and changes therein) of the ancient populations over time. The final dataset consisted of individuals dated between the Palaeolithic (43,000-5,000 BCE) and Iron Age (1,100-700 BCE), thus allowing us to study the three major demographic movements in conjunction with archaeological evidence in the Palaeolithic, the Neolithic, and during the Chalcolithic/Bronze Age transition (4,500-2,500 BCE).

2. LITERATURE OVERVIEW

The following chapter will highlight innovations in the field of aDNA and will include an overview of historical events related to the genetic changes in the human genome of Eurasians and ancient Italians.

2.1. Overview of ancient DNA studies

2.1.1. Insights into the aDNA history

Once upon a time, almost 40 years ago, the first era of aDNA studies (the 1980s-1990s) began with the first attempts to extract DNA from soft tissues, hair, and bones. The first partial DNA was extracted from a small piece of the dried muscle of an extinct animal called the Quagga (*Equus quagga*), which was stored at the Museum of Natural History in Mainz, Germany (Higuchi *et al.*, 1984). Here, the researchers were able to obtain 229 base pair (bp) long fragments of mitochondrial DNA (mtDNA), inserted them into bacterial clones to multiply the DNA fragments, and compared them to the mtDNA of present-day mountain zebra *E. zebra*.

Through the development of the polymerase chain reaction (PCR) method, which enabled the rapid amplification of finite copies of specific DNA fragments using thermal cycling methods and the two main reagents – primers and a thermostable DNA polymerase (Kleppe *et al.*, 1971; Mullis, 1990; *The Nobel Prize in chemistry 1993*, 1990), the previous method of bacterial cloning was replaced (Pääbo, 1984, 1985; Shizuya *et al.*, 1992; Shizuya and Kouros-Mehr, 2001); and researchers were able to amplify DNA fragments from ancient human brains, maize remains, archaeological human skeletal remains, and dry skins of the extinct marsupial wolf and kangaroo rats as well as from New Zealand moas (Pääbo, Gifford and Wilson, 1988; Rollo *et al.*, 1988; Hagelberg, Sykes and Hedges, 1989; Thomas *et al.*, 1989, 1990; Cooper *et al.*, 1992). One early example of an attempt to isolate human DNA was published in 1984 by Svante Pääbo (Pääbo, 1984, 1985). In his publication, he described the successful extraction of DNA from a 2,400-year-old mummified child and the analysis of the DNA fragments, which seemed to contain little or no signs of postmortem changes; this, however, may be due to the lack of reference studies. However, with the improvement of DNA extraction protocols and the development of dedicated aDNA laboratories, he clarified that he unfortunately extracted modern human DNA from the mummy (Pääbo, 2008, 2014). Nevertheless, Pääbo's work and the introduction of stringent measures against modern DNA contamination opened the door for the second era of aDNA studies from the 1990s to 2010. The second era was marked by the development of new technologies and methods to extract and analyse the DNA of various skeletal remains (Cooper *et al.*, 1992; Höss, Dilling, *et al.*, 1996; Yang, Golenberg and Shoshani, 1996; Poinar *et al.*, 1998; Leonard, Wayne and Cooper, 2000;

Thomas *et al.*, 2000; Greenwood *et al.*, 2001), but it also led to intense debate by archaeologists and anthropologists around the trustworthiness of aDNA research. In the late 1990s, Pääbo and his team extracted and sequenced DNA from a Neanderthal-type specimen, thereby introducing a series of laboratory standards that are still used today (Krings *et al.*, 1997; Ward and Stringer, 1997; Poinar and Cooper, 2000). Later, the aDNA field changed rapidly following the development of sequencing technology from shotgun sequencing to next-generation sequencing (NGS). In 2005, researchers were able to recover partial DNA genomes (Noonan *et al.*, 2005) and later, the complete mtDNA genome of a mammoth (Enk *et al.*, 2011). The first genome of a 4,000 old Palaeo-Eskimo (Rasmussen *et al.*, 2010) was obtained from a human specimen stored at the museum. Finally, the first draft sequence of a Neanderthal genome was published (Green *et al.*, 2010).

Since 2010, the field has made significant advances in the demographic history of ancient human populations (Olalde *et al.*, 2018; Racimo *et al.*, 2020; Patterson *et al.*, 2022), and in the history of pathogens (Bos *et al.*, 2015; Harkins *et al.*, 2015; Warinner, Speller and Collins, 2015; Keller, 2019), and the relationship(s) between animals and humans (Allaby *et al.*, 2015; Evin *et al.*, 2015; Ludwig *et al.*, 2015). Some of the latest studies have demonstrated a connectedness between particular lifestyles and their geographical spread such as the introduction of agriculture and domestication (Haak *et al.*, 2015; Hof-manová *et al.*, 2016; Olalde *et al.*, 2018).

2.1.2. From Bone to Sequencing

The preservation of aDNA depends on various factors, such as climate and geographical location, the presence of exogenous DNA from the environmental organisms, its degradation into short fragments and the damage of the DNA fragments over time (Green *et al.*, 2010; Miller *et al.*, 2012; Orlando *et al.*, 2013; Der Sarkissian *et al.*, 2015). Researchers from the 1990s to the present day have discussed the potential for DNA preservation in cool and stable environmental conditions such as permafrost or caves (Höss, Dilling, *et al.*, 1996; Höss, Jaruga, *et al.*, 1996; Hadly *et al.*, 1998; Noro *et al.*, 1998; van der Valk *et al.*, 2021) and the negative impact of hot climate on DNA preservation as shown in studies from Egyptian mummies (Pääbo, 1984, 1985; Marota *et al.*, 2002; Zink and Nerlich, 2003, 2005) and ancient sub-Saharan African individuals (Skoglund *et al.*, 2017). The first successful genome-wide study of three Egyptian mummies was published in 2017 (Schuenemann *et al.*, 2017). However, some of the distinct biogeographical units stay understudied due to environmental conditions or the burial practices of various cultures following the failure to extract DNA (Pusch, Broghammer and Scholz, 2000; Joshua Levine, 2017; Djinis, 2021).

Moreover, an organism or human body starts to decompose after death under different (biological and chemical) processes leading the DNA to decay into shorter fragments (Pääbo, 1989; Lindahl, 1993; Deagle, Paige Eveson and

Jarman, 2006). Examples of the processes are the nuclease activity within the cell and that DNA is not chemically stable leading to breaks in the DNA double-strand, which cannot be repaired after the death of an organism (Nishino and Morikawa, 2002). An example of a chemical process is depurination (Lindahl, 1993). Here, the purine residues are removed from the deoxyribose through hydrolysis, which may eventually lead to a break in the DNA filament. As a result, the fragmentation, as well as the length, are random and the fragment length distribution can be taken as an indicator for aDNA studies (Poinar *et al.*, 2006; Allentoft *et al.*, 2012). Another significant characteristic of aDNA is the chemical modifications of the amino acid cytosine (C) to thymine (T) and guanine (G) to adenine (A) in the sequences. In particular, the C to T substitutions are caused by cytosine deamination turning cytosine to uracil (U) leading to sequenced thymine. Following this, a G to A substitution is most likely visible in sequences during the library preparation and the synthesis process (Fogg, Pearl and Connolly, 2002). Based on that, the number of short fragments and the proportions of C to T substitutions can be used as a measure to authenticate the sequenced library of an individual as ancient or to find the presence of modern contamination (Briggs *et al.*, 2007; Ginolhac *et al.*, 2011; Jónsson *et al.*, 2013).

All the characteristics mentioned above are instrumental in the development of new methods and technology (Meyer and Kircher, 2010; Hofreiter *et al.*, 2015). Today, researchers can keep the present-day (human) contamination limited and increase the availability of targeted aDNA fragments using a laboratory workflow for DNA extraction with minimal damage to the bone (Rohland and Hofreiter, 2007; Pinhasi *et al.*, 2015; Margaryan *et al.*, 2018; Rohland *et al.*, 2018), prepare libraries for highly degraded DNA fragments for high throughput sequencing (Meyer and Kircher, 2010; Orlando *et al.*, 2013), and use target enrichment via in-solution hybridisation or array to enrich the targeted DNA fragments (Gnirke *et al.*, 2009; Meyer and Kircher, 2010; Carpenter *et al.*, 2013; Bos *et al.*, 2015; Haak *et al.*, 2015; Rohland *et al.*, 2022). For the sample extraction, researchers prefer petrous bone portions, in particular, a core sample of the inner ear, to increase the endogenous human DNA content (Pinhasi *et al.*, 2015; Hansen *et al.*, 2017), and teeth roots or dentin for sampling pathogen DNA (Margaryan *et al.*, 2018). In recent years, researchers have tested different parts of the human skeletal remains (Parker *et al.*, 2020) and found that the three auditory ossicles (malleus, incus, and stapes) are additional sources for well preserved human DNA (Sirak *et al.*, 2020). Unfortunately, the availability of these ossicles is limited due to their small size and loss during archaeological excavations. Another additional source to study the human history is the dental calculus allowing the reconstruction of the diet profile of an individual by comparing the results with other individuals to get additional insights into the socio-cultural context of an ancient human population (de La Fuente, Flores and Moraga, 2013; Weyrich, Dobney and Cooper, 2015; Arriola, Cooper and Weyrich, 2020; Sawafuji *et al.*, 2020; Ottoni *et al.*, 2021).

Besides the DNA extraction from skeletal remains, researchers have started to extract DNA from sediments to study the whole metagenomic profile of individuals found in a cave or from a mass burial (Epp, Zimmermann and Stoof-Leichsenring, 2019; Voldstad *et al.*, 2020; Linderholm, 2021; Sarhan *et al.*, 2021; Murchie *et al.*, 2022). Interestingly, sedimentary aDNA (sedaDNA) allows researchers to recover and analyse ecologically informative environmental DNA which is rapidly metabolised by bacteria or degraded, hence, can be bound to sedimentary materials (Murchie, Kuch, *et al.*, 2021; Murchie, Monteath, *et al.*, 2021). The results can be used to investigate organism diversity in understudied geographical regions and build a fine-scale taxonomic resolution of plant groups and mammals (Murchie *et al.*, 2022).

2.1.3. Most common strategies and statistical analyses in aDNA population genetics

Different approaches have been used to investigate the ancestral components and genetic relationships of ancient as well as present-day individuals (Racimo *et al.*, 2020). The most common approaches to studying the ancestral compo–nents are principal component analysis (PCA) (Patterson, Price and Reich, 2006; Novembre *et al.*, 2008), ADMIXTURE for unrelated individuals (Alexander, Novembre and Lange, 2009), and F-statistics (Peter, 2016). The latter is especially used as a base to reconstruct admixture events using model-based approaches like qpAdm/qpWave (Haak *et al.*, 2015; Harney *et al.*, 2021). Researchers perform PCA and ADMIXTURE to study population structure by fitting ancient individuals into structural clusters, which can then be used to undertake F-statistics as well as model-based analyses. Here, the archaeological records of the studied site(s) as well as relative and absolute dates (radiocarbon dating using the C14 method) is considered to name the clusters and place the individuals into the context of previous studies. For the analyses, the genome-wide data of ancient individuals are used to calculate the "distance" between the newly generated individuals and available published ancient as well as present-day individuals. The PCA for genome-wide analysis is based on a covariance matrix and the visualised results of the principal components, known as eigenvectors, represent the highest individual variance (Patterson, Price and Reich, 2006; Novembre *et al.*, 2008). Complementary to the PCA, different cluster-based methods using admixture algorithms are available to fit the ancient individuals into separate clusters based on their ancestral components. The first programs using these algorithms were STRUCTURE, which used a Bayesian framework (Pritchard, Stephens and Donnelly, 2000), FRAPPE (Tang *et al.*, 2005), and ADMIXTURE (Alexander, Novembre and Lange, 2009).
The latter expanded the capacities of STRUCTURE using maximum likelihood approaches. To perform the ADMIXTURE analysis, the researchers give several *K*-s to predefine the different clusters to which individuals can be assigned. The individual's ancestry can thus be represented between one or more predefined *K*-s. However, the results of the ADMIXTURE and other

programs such as CHROMOPAINTER (Lawson *et al.*, 2012) are limited by the number of available SNPs for ancient individuals and should be taken into account in the interpretation. Another program called DyStruct is a model-based algorithm using stochastic variational inference to estimate shared ancestry from temporally genotype data (Joseph and Pe'er, 2018, 2019). DyStruct includes the dating of an individual to model the respective ancestries observed for populations whose allele frequencies drift over time.

Beyond the study of the ancestral components, researchers have also increasingly focused on the reconstructing ancient social structures such as kinship and descent through the integrated analysis of archaeological, osteological and stable isotopes data (Haak *et al.*, 2008; Mittnik *et al.*, 2019; Sjögren *et al.*, 2020; Ingman *et al.*, 2021). To investigate social organisation from a genetic point of view, one program, Relationship Estimation from Ancient DNA or READ, is commonly used to estimate the genetic relatedness of individuals within a burial site or excavated area (Monroy Kuhn, Jakobsson and Günther, 2018; Hugo Reyes-Centeno, 2021). This method is specially developed to estimate the relatedness between ancient individuals with low coverage and a low number of available SNPs, but it is limited to the number of available individuals and the high number of possible closely-related individuals in a dataset. The final results of the program and the uniparental markers are used to build the family tree(s) of the individuals. Uniparental markers are classified as mtDNA haplogroups, which are inherited from the mother to the child (both daughter and son) through the mitochondrion, and the Y chromosome (Y chr) haplotype, which is inherited from the father to the son as male individuals through the Y chromosome. Additionally, the diversity of uniparental markers has previously been studied extensively before the access of genome-wide data, and therefore gives additional insights into population structures (see section 2.2.).

2.2. A summary of the demographic history of Eurasia

2.2.0. The ancestral components

The genetic compositions of present-day populations from Eurasian countries have been extensively studied. Novembre et al. (2008) analysed the genetic variation of 3,000 modern European individuals and found a close correspondence between genetic and geographic distances (Novembre *et al.*, 2008). Here, the PCA presented a reproduction of the European map with recognizable geographic features such as the Iberian Peninsula, Italian Peninsula, and Southeastern Europe.

One cluster contains French-, German-, and Italian-speaking people groups within Switzerland, suggesting shared genetic compositions. Hence, this map is the first indicator of the genetic differentiation of European populations based on geography.

Previous studies have proposed **three main** groups of ancestral populations as possible sources for the gene pool of present-day Eurasians: (I) Eurasian Hunter-Gatherers including Caucasus/Iranian HG (CHG), Eastern Hunter-Gatherers (EHG), and Western Hunter-Gatherers (WHG) representing the geographical region of published ancient individuals; (II) Early European Farmers (EEF) through migration events (=Neolithic revolution) from West Asia associated with the Neolithic farmers from Anatolia; and (III) 'Steppe-related' ancestry from the Pontic-Caspian Steppe associated with the culture in archaeology known as Yamnaya (Lazaridis *et al.*, 2014; Haak *et al.*, 2015) (Figure 1). However, those components did not arrive all at once in Eurasia but rather were admixed populations of known, as well as unknown, migration events (Jones *et al.*, 2015; Lazaridis *et al.*, 2016; Günther *et al.*, 2018). In specific, the CHG/Iranian HG component has been shown to be a distinct clade that split from WHG and Neolithic farmers representing a possible source of ancestry to the Yamnaya people (Jones *et al.*, 2015; Fu *et al.*, 2016; Narasimhan *et al.*, 2019). In reference to earlier studies, additional components namely Ancient North Eurasians (ANE) (Patterson *et al.*, 2012; Lipson *et al.*, 2013), and Iranian Neolithic farmers (IN) (Lazaridis *et al.*, 2016; Fernandes *et al.*, 2020), have contributed to the gene pool of ancient and present-day populations. Also a small proportion of Neanderthal DNA has been found within ancient and present-day populations (Prüfer *et al.*, 2014, 2021; Hajdinjak *et al.*, 2021). Hence, the arrival of these components can be pinpointed in time through the combination of archaeological, historical, and genetic data. The section below will present an overview of what we know so far.

Approximately 45,000 years ago, modern humans arrived in Europe (Benazzi *et al.*, 2011; Fu *et al.*, 2014; Hublin *et al.*, 2020). During the Last Glacial Maximum (25,000-19,000 years ago, LGM), the northern part of Europe was still covered with ice and human settlement was restricted to certain refugia areas (Gamble *et al.*, 2004; Mangerud *et al.*, 2004). These first humans lived a hunter-gatherer lifestyle before farming spread to Europe around 7,500 years ago along with changes in the social structure (see section 2.2.3.). Genetic studies on ancient individuals dated to the Upper Palaeolithic (50,000-12,000 ya) and Mesolithic (15,000-5,000 ya) periods indicate a population replacement with evidence of a continuous hunter-gatherer lifestyle during the Neolithic revolution in northern Europe (see section 2.2.3.). With the arrival of people from the Pontic-Caspian Steppe, there was a movement toward the usage of tools made out of bronze, copper and tin alloy (Dolfini, 2020).
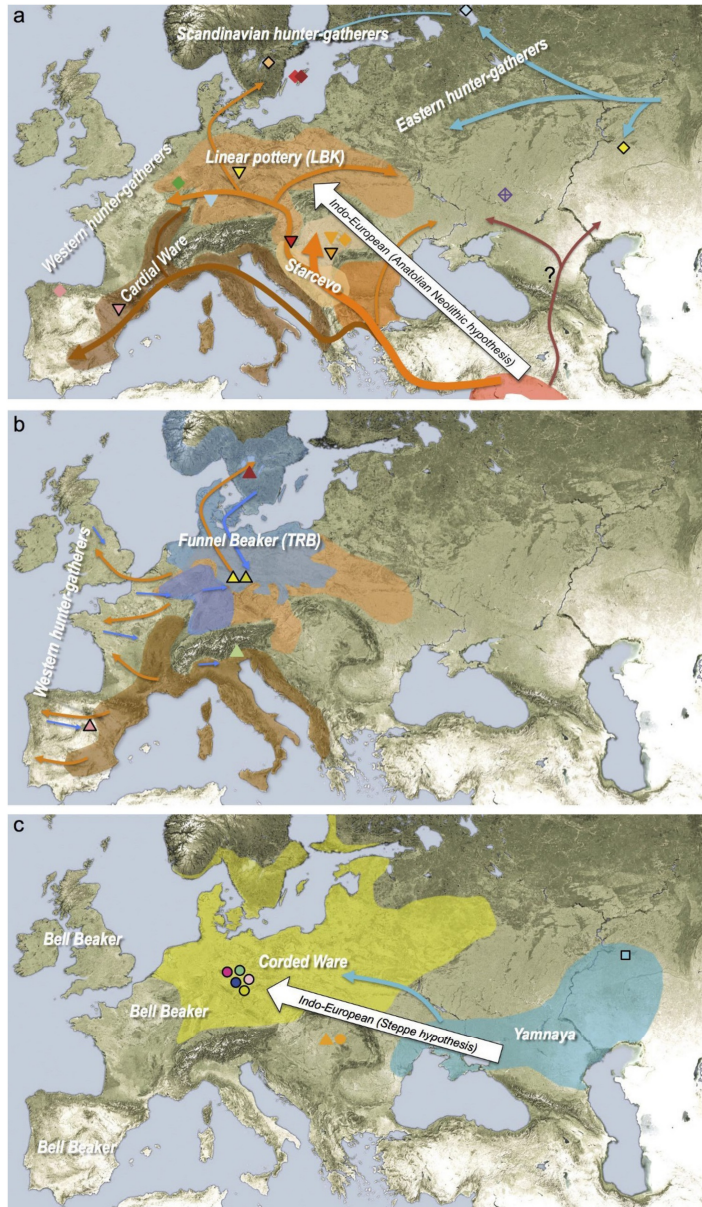
Figure 1. Geographic distribution of archaeological cultures and graphic illustration of proposed population movements/turnovers discussed in the main text.
a, Proposed routes of migration by Early farmers into Europe, 9,000-27000 years ago. b, Resurgence of hunter-gatherer-related ancestry during the Middle Neolithic, 7,000-25,000 years ago. c, Arrival of Steppe-related ancestry in central Europe during the Late Neolithic, 4,500 years ago. White arrows indicate the two possible scenarios of the arrival of Indo-European language groups. Figure reprinted with permission from Extended data figure 4 (Haak *et al.*, 2015), Springer Nature.

2.2.1. Palaeolithic Hunter-Gatherers

Despite the limited number of human remains from ancient individuals dated to the Palaeolithic in western Eurasia, some human remains yielded enough nuclear DNA to study the genetic diversity of Palaeolithic hunter-gatherers in the Pre-LGM (45,000-25,000 ya), Post-LGM (19,500-14,500 ya) and in the transition to the Holocene (~17,500 ya-present). The oldest human remains at present are from Bulgaria (Bacho Kiro, 43-47 ka), Czech (Zlatý kůň, ≥45,000 years), Romania (Oase I, 41,640-37,580 cal BP), and Russia (Ust'-Ishim, 47,480-42,560 cal BP) (Krause *et al.*, 2010; Fu *et al.*, 2014, 2015, 2016; Seguin-Orlando *et al.*, 2014; Posth *et al.*, 2016; Hublin *et al.*, 2020; Prüfer *et al.*, 2021).

The majority of the published ancient individuals carry the Y chr haplogroups C, F, I, J, K, and R whereby the Y chr haplogroups C with sub-haplogroups C1 and CT are mostly found in ancient individuals from the Gravettian period (33,000-21,000 cal BP) and/or during the Pre-LGM period (Fu *et al.*, 2014, 2016; Seguin-Orlando *et al.*, 2014). The Y chr haplogroups C, I, and J are found more abundantly in ancient individuals from the Magdalenian (17,000-12,000 cal BP) and Epigravettian (~21,000-10,000 cal BP) periods. *Fu et al. 2016* report one individual from Villabruna (14,180-13,780 cal BP) carrying the Y chr haplogroup R1b*, which later became common among ancient individuals arriving from the Pontic-Caspian Steppe during the Bronze Age migration events (Haak *et al.*, 2015) (see section 2.2.4.). This indicates an early link between Europe and the western fringe of the Steppe Belt of Eurasia.

The study of the mtDNA haplogroups revealed a higher diversity during the Pre-LGM period in ancient individuals carrying mtDNA haplogroup U with various sub-haplogroups, as well as haplogroups M and R. the haplogroup M is primarily found in present-day Asian populations and is absent in modern European populations. It was, however, assigned to one individual (Ostuni1, 27,810-27,430 cal BP) in South Italy, indicating a possible timing for the dispersal of modern humans into Eurasia (Kivisild, 2015; Posth *et al.*, 2016). In the Post-LGM period, mtDNA haplogroups U2'3'4'7'8'9 lineages are present in individuals from western Europe, but not in individuals dated to the Late Glacial period (14,500–11,500 ya) or during the transition to the Holocene (Fu *et al.*, 2016; Posth *et al.*, 2016; Mathieson *et al.*, 2018; Catalano *et al.*, 2019). Here, the most frequent mtDNA haplogroup is U5b*, suggesting a population shift after the end of the Late Glacial period (Fu *et al.*, 2016; Posth *et al.*, 2016; Modi *et al.*, 2021).

Besides the mtDNA and Y chr haplogroup diversity, researchers were able to study the genetic history of Upper Palaeolithic European individuals and identified at least 5 genetic clusters ('El Mirón', 'Mal'ta', 'Satsurblia', 'Věstonice', and 'Villabruna') sharing a major amount of genetic drift (Fu *et al.*, 2016; Villalba-Mouco *et al.*, 2019). As a result, each cluster was named after the oldest individuals within, rather than after, the archaeological cultural complexes.

The latter cluster 'Villabruna' is dated to around 14,000 ya and contains ancient individuals dated to Azilian, Epigravettian, and Mesolithic (see section 2.2.2.) sharing more ancestral components with non-European populations, e.g. from West Asia, reflecting migrations or population shifts within Europe with a possible population expansion from southeastern European or West Asian refugia correlating with the Bølling-Allerød interstadial (14,690-12,890 BP) and the cultural transitions within the Epigravettian and Magdalenian-to-Azilian. On the other side, ancient individuals, especially Kostenki 14, dated to the Pre-LGM and before the end of the LGM period showed more genetic affinities to modern Europeans than to East Asians and perhaps contributed genetic components to ancient and modern Siberians, indicating a divergence from a single ancestral population in Europe, without evidence of substantial genetic influx from elsewhere (Seguin-Orlando *et al.*, 2014; Fu *et al.*, 2016). External genetic influences were instead detected in another ancient European genome, Goyet-Q116, whose genome can be described as predominantly similar to the Kostenki14 one with detectable contributions from an East Asian source.

2.2.2. Mesolithic Hunter-Gatherers

During the Mesolithic in Europe (15,000-5,000 BP), the hunter-gatherers could be divided into three major groups based on their ancestral components and geographical locations. The first group, identified in 2014, was the Western European hunter-gatherers (WHG), which includes ancient individuals from Loschburg (8,160-7,940 cal BP) and La Braña 1 (7,940-7,690 cal BP). This ancestry component still contributes to present-day Europeans (Lazaridis *et al.*, 2014; Olalde *et al.*, 2014; Antonio *et al.*, 2019). Mostly, the WHG individuals belong to the 'Villabruna' cluster sharing the major mtDNA haplogroup U5 (Lazaridis *et al.*, 2014; Olalde *et al.*, 2014; Fu *et al.*, 2016; Posth *et al.*, 2016).

The second group – Eastern European hunter-gatherers (EHG) – was firstly defined by two individuals (Karelia (5,500-5000 BCE) and Samara (5,650-5,555 cal BCE)) from Russia carrying a significant amount of ANE and suggesting the possible source of this ancestry in present-day Europeans (Lazaridis *et al.*, 2014; Haak *et al.*, 2015). Different studies have linked the genetic composition of the third group called Scandinavian hunter-gatherers (SHG) as an admixed group between WHG and EHG (Lazaridis *et al.*, 2014; Haak *et al.*, 2015; Lazaridis *et al.*, 2016). The group was named after the first individuals (e.g. Motala 12 (7,670-7,580 cal BCE)) with their geographical origin in Sweden (Lazaridis *et al.*, 2014). Another study from 2018 suggests two different early postglacial migrations into Scandinavia from the South and Northeastern part of Eurasia. Here, researchers analysed genome-wide data of SHGs and found significant differences in the amount of WHG ancestry in the individuals (Günther *et al.*, 2018).

Further hunter-gatherer individuals from Southeastern Europe and the Baltic region were analysed and showed a similar pattern of admixture between WHG and EHG based on their geographical location (Mathieson *et al.*, 2018; Mittnik

et al., 2018). Mathieson et al. 2018 highlighted the predominance of WHG ancestry during the Mesolithic and its decrease in the transition to the Neolithic.

2.2.3. Neolithic Hunter-Gatherer and Farmers

In the transition from the hunter-gatherer lifestyle to agriculture (~8,000-10,000 ya), the hunter-gatherer-related ancestry component slowly decreased with the arrival of populations from the Eastern Mediterranean Basin, later known as Anatolian Neolithic Farmers (Lazaridis *et al.*, 2014), following its resurgence during the Middle Neolithic (Günther *et al.*, 2015; Haak *et al.*, 2015; Martiniano *et al.*, 2017). However, evidence of regional variability suggested that these populations admixed with local hunter-gatherers who adopted the farming lifestyle (Gamba *et al.*, 2014; Allentoft *et al.*, 2015; Günther *et al.*, 2015; Cassidy *et al.*, 2016; Hofmanová *et al.*, 2016; González-Fortes *et al.*, 2017; Lazaridis *et al.*, 2017; Lipson *et al.*, 2017; Tassi *et al.*, 2017; Fernandes *et al.*, 2018; Valdiosera *et al.*, 2018), with some exceptions in the Baltic region (Jones *et al.*, 2017; Saag *et al.*, 2017; Mathieson *et al.*, 2018; Mittnik *et al.*, 2018) and Scandinavia (Malmström *et al.*, 2009; Skoglund *et al.*, 2012, 2014). Here, the regions seemed to be more affected by the adoption of the farming lifestyle than the excess of Anatolian Farmer-related ancestry. Interestingly, in Britain, the story is quite different. Researchers found indications that the ancestral components were almost completely replaced during the Early Neolithic period with the later arrival of the Neolithic transition in the British Isles (Collard *et al.*, 2010; Brace *et al.*, 2019; Patterson *et al.*, 2022).

Before the availability of ancient genome-wide data, the dispersal routes (the Mediterranean vs. Balkan route), as well as the very demic or cultural nature of the Neolithic transition were extensively discussed in the field of archaeology and history (Ammerman, 1973; Ammerman and Cavalli-Sforza, 2014; Fort, 2015; Shennan, 2017). The origin of farming has been attributed to the Fertile Crescent in West Asia around 12,000 years ago. The farming lifestyle reached the Balkan region around 8,000 and Central Europe around 7,000 years ago (Porčić *et al.*, 2020, 2021).

Initial studies focused on the mtDNA haplogroup diversity from various geographical locations in Eurasia and found a higher genetic diversity mainly in the haplogroup lineages H, HV, J, K, N1a, U, V, W, and X (Haak *et al.*, 2005; Sampietro *et al.*, 2007; Bramanti *et al.*, 2009; Malmström *et al.*, 2009; Lacan, Keyser, Ricaut, Brucato, Duranthon, *et al.*, 2011; Hervella *et al.*, 2012; Brandt *et al.*, 2013; Brotherton *et al.*, 2013; Hervella *et al.*, 2015; Szécsényi-Nagy *et al.*, 2015, 2017; Rivollat *et al.*, 2017). Interestingly, based on the first mtDNA haplogroup studies, the admixture events between local hunter-gatherers and the Early European farmers were rejected because of the high differences between the assigned haplogroups to the local hunter-gatherers and the local farmers (Bramanti *et al.*, 2009). However, mtDNA is a small part of the human genome inherited by the maternal line and lacks recombination, which restricts the generalisation to a population scale.

The most frequent Y chr haplogroups found in ancient Neolithic farmers are C, F, G, H, I, and R (Rootsi *et al.*, 2004; Battaglia *et al.*, 2009; Keyser *et al.*, 2009; Haak *et al.*, 2010; Lacan, Keyser, Ricaut, Brucato, Tarrús, *et al.*, 2011); however, the latter is not associated with R1b (Kivisild, 2017). Among these, haplogroup G2a~ was most common in Early European farmers suggesting homogeneity of paternal lineages among early farmers but is rarely presented in present-day Europeans (Lacan, Keyser, Ricaut, Brucato, Tarrús, *et al.*, 2011; Szécsényi-Nagy *et al.*, 2015).

From a genetic perspective, the growing availability of genome-wide data from ancient individuals of any European country dated to the Neolithic allows researchers to build a detailed picture of the transition to farming and later to the Bronze Age. Early publications connected the first European farmers with their genetic origin in Anatolia and their spread over Europe through the Balkan routes (Lazaridis *et al.*, 2016; Feldman *et al.*, 2019). Various studies have presented evidence that the spread of farming reached Europe around 9,000 years ago and spread northwards along the Danube river and westwards across the Mediterranean coast (Brandt *et al.*, 2013; Skoglund *et al.*, 2014; Haak *et al.*, 2015; Mathieson *et al.*, 2015, 2018; Olalde *et al.*, 2015, 2019; Lipson *et al.*, 2017; Rivollat *et al.*, 2020). Researchers, however, did not find evidence that the big migration event (diffusion model) followed a direct admixture between the local hunter-gatherers and expanding farmers. Rather, it has been shown that the admixture was delayed in some regions of Eurasia for thousands of years seen in the presence of a recurring hunter-gatherer-related ancestry in the later stages of the Neolithic (Porčić *et al.*, 2020, 2021; Papac *et al.*, 2021). Interestingly, through the increase of genome-wide data from various geographical regions in Eurasia, the spread of farming and the imbalance of the WHG ancestry component can be seen in the cluster-based analysis of all individuals (Olalde *et al.*, 2015; Marcus *et al.*, 2020; Aneli *et al.*, 2021).

In addition to the genetic ancestry composition of ancient cultural groups and their connectedness to the spread of farming from Anatolia, recently the kinship of individuals has been a major research focus and revealed more insight into the social organisation of these groups. Other studies have reconstructed the social relationship between individuals in a high-resolution analysis connecting genetic relationships and archaeological records and found evidence of paternal lineages in Neolithic tombs and cemeteries suggesting patrilocality (Goldberg *et al.*, 2017; Amorim *et al.*, 2018; Schroeder *et al.*, 2019; Cassidy *et al.*, 2020; Sjögren *et al.*, 2020; Fowler *et al.*, 2022).

2.2.4. The arrival of ancestral components during the Bronze Age period

The Bronze Age period in Europe (~3,200-600 BCE) is occupied by the rapid arrival of the Western Steppe herders including Early Bronze Age Yamnaya culture, among others, from the Pontic-Caspian Steppe around 3,000 BCE, and major cultural changes, perhaps associated with a different perception of economics and social belonging of an individual (Allentoft *et al.*, 2015; Haak *et al.*, 2015). In recent years, the spread and impact of the Yamnaya culture have been studied extensively in Western Eurasia and the associated genetic ancestry is commonly referred to as Steppe-related ancestry (Lazaridis *et al.*, 2014). It has been suggested that the Steppe-related ancestry component is a mix of mostly EHG-related ancestry, CHG-related ancestry, an unknown source from Armenia, and Iranian-Neolithic-related ancestry (Haak *et al.*, 2015; Jones *et al.*, 2015; Lazaridis *et al.*, 2016).

Various studies have highlighted the divergence of the Steppe-related ancestry component in ancient individuals dated to the sub-periods of the Bronze Age and its impact in the context of different ancient cultures and its geographical expansion (Lazaridis *et al.*, 2017; Martiniano *et al.*, 2017; Saag *et al.*, 2017, 2019; Olalde *et al.*, 2018, 2019; Papac *et al.*, 2021; Villalba-Mouco *et al.*, 2021; Patterson *et al.*, 2022). At the beginning of the Early Bronze Age period, the Steppe-related ancestry component is absent in most of the ancient individuals in Western and Southern Europe and it increases during the Middle Bronze Age and stabilises at the end of the Bronze Age (Allentoft *et al.*, 2015; Olalde *et al.*, 2019; Fernandes *et al.*, 2020; Patterson *et al.*, 2022). The delay in the expansion of the Steppe-related ancestry component is visible in genome-wide data available from the islands in the western Mediterranean Sea suggesting genetic isolation from Bronze Age populations on the mainland (Fernandes *et al.*, 2020; Marcus *et al.*, 2020).

On the other side, studies focusing on Southern European human history have found an extent of the so-called Iranian-Neolithic-related ancestry component suggesting an arrival of this component during the Bronze Age period (Lazaridis *et al.*, 2017) and a presence of a Northern African component in some of the ancient individuals confirming the beginning of the trade network around the Mediterranean Basin (Antonio *et al.*, 2019; Fernandes *et al.*, 2020; Marcus *et al.*, 2020).

Aside from the arrival of the Steppe-related ancestry component in Western Eurasia, researchers have found an almost total replacement of the most common Y chr haplogroups from the Neolithic period by the Y chr haplogroup lineages R1b* and R1a*, the latter of which had been unknown in Western Eurasia until the Early Bronze Age period (Haak *et al.*, 2015; Underhill *et al.*, 2015). The Y chr haplogroup R1b* was found in individuals dated to the Palaeolithic and Mesolithic (see sections 2.2.1. and 2.2.2.) (Fu *et al.*, 2016; Mathieson *et al.*, 2018). The turnover of the Y chr haplogroups was thoroughly studied in individuals dated to the Chalcolithic/Bronze Age transition from the

British Isles and the Iberian Peninsula (Olalde *et al.*, 2018, 2019; Villalba-Mouco *et al.*, 2021) and archaeological sites located closely together (Papac *et al.*, 2021). The results suggested a higher genetic contribution of non-local male individuals than non-local female individuals, which also has been confirmed by the divergence of the ancestral components between the individuals within a burial site (Olalde *et al.*, 2019; Papac *et al.*, 2021). Additionally, other studies have emphasized strong sex bias during the migration event indicated by the Y chr turnover (Goldberg *et al.*, 2017; Martiniano *et al.*, 2017; Mittnik *et al.*, 2019) and differences in ancestry profiles associated with the Corded Ware culture (Saag *et al.*, 2017; Papac *et al.*, 2021).

In addition, recently researchers have started to focus more on the social structures in the context of different ancient cultures, e.g. Bell Beaker (~2,750-1,800 BCE), Corded ware (~2,900-2,300 BCE), and Globular Amphora culture (dated to Late Neolithic period), and found little changes in the paternal lineage during the Bronze Age, but various studies have implied female exogamy through the higher mtDNA diversity and the absent of closely related female individuals in the studied archaeological sites (Knipper *et al.*, 2017; Olalde *et al.*, 2018; Mittnik *et al.*, 2019; Freilich *et al.*, 2021; Papac *et al.*, 2021; Villalba-Mouco *et al.*, 2021; Žegarac *et al.*, 2021).

2.3. The demographic history of the Italian Peninsula from an archaeological and aDNA perspective

The following section gives an overview of the demographic history of published ancient and present-day individuals from the Italian Peninsula. Since time periods and cultural expansion varied worldwide, and especially in Eurasia, the following periods are based on archaeological records in the Italian Peninsula (Figure 2).
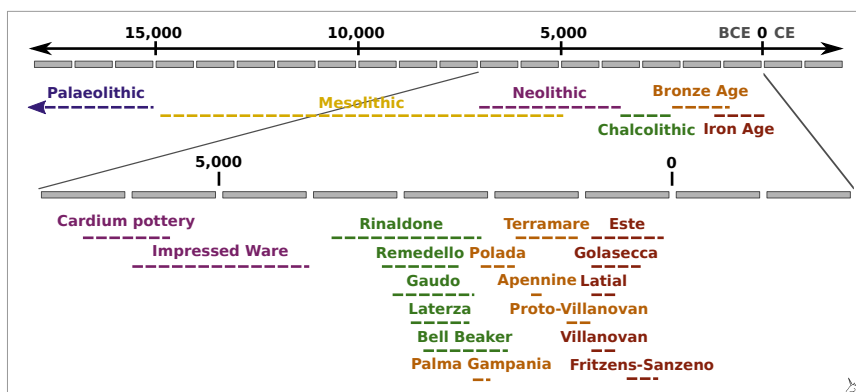

Figure 2. Chronology of periods and examples of cultures in the Italian Peninsula before the common era.
(All dates are approximal, more details can be found in the respective sections below.)

2.3.0. Gaps of knowledge based on Italian modern DNA

In the study by Raveane et. al, the genetic distribution of present-day Italians and other European populations was studied extensively to investigate their ancestral composition as a result of migration and admixture (Raveane *et al.*, 2019). To evaluate this, genome-wide data of more than 1600 individuals from 20 Italian administrative regions and over 140 worldwide reference populations including genome-wide data of published ancient individuals were used. As a result, the genetic differentiation of present-day Italians can be separated into three main groups/clusters scattering the geographical locations: Sardinia, Northern (North/Central-North Italy), and Southern Italy (South/Central-South Italy and Sicily). For the investigation of the ancestral compositions of the modern clusters, different combinations of ancient putative sources were tested, using Chromopainter and non-negative least squares (NNLS) algorithms. Comparing all the analyses, the so-called Italian clusters were characterised by high amounts of Anatolian Neolithic (AN) contribution with a closer affinity to AN in present-day Italians from Northern Italy and Sardinia. The other two components mostly represented were WHG and CHG with higher amounts in present-day Italians from the South. Besides these components, the EHG-related component with higher frequency in North Italy and the IN component with a higher concentration in the south were found and suggested as secondary sources of genetic ancestry in the region.

Besides these primary contributions, the researchers evaluated the North-South differences as a source of the presence and absence of the Anatolia Bronze Age and Steppe Bronze Age ancestries. For a better understanding of these ancestries and the high genetic differences between the North and South of Italy, the genetic affinities of genome-wide data of ancient published individuals from the Italian Peninsula were explored and confirmed in previous studies (Keller *et al.*, 2012; Allentoft *et al.*, 2015; Olalde *et al.*, 2018). Here, the authors interpreted/suggested that parts of the differentiation in ancient ancestry composition in the different areas of Italy are partly due to the migration events during the Bronze Age period. However, given the limited number of genome-wide sequences from published ancient individuals e.g. dated to the Neolithic and Iron Age, the obtained results can only be seen as a suggestion of different migration events.

2.3.1. The Palaeolithic and Mesolithic

During the Upper Palaeolithic (~50,000-12,000 BP) and at the end of the Ice Age/end of the Pleistocene, the Italian Peninsula was connected with the islands of Elba and Sicily allowing the first *Homo sapiens* to inhabit the Italian Peninsula and the island of Sicily (Benazzi *et al.*, 2011; Douka *et al.*, 2012; Sineo *et al.*, 2015). One of the oldest human remains was discovered at the archaeological site Riparo Mochi (Italian Peninsula) dated 48,000 years ago (Douka *et al.*, 2012).

Genetic studies initially focused on the mtDNA diversity of hunter-gatherers dated to the Gravettian (33,000-21,000 BP) and Epigravettian (~21,000-10,000 cal BCE) and found that the individuals dated pre-LGM carry the mtDNA haplogroups M, U2*, and U8 (Posth *et al.*, 2016; Modi *et al.*, 2021). After the LGM period, the individuals carry the mtDNA haplogroup U5* which has predominantly been found in ancient individuals dated to Epigravettian and Mesolithic, suggesting a replacement of the mtDNA haplogroups through the arrival of populations from West Asia (Mathieson *et al.*, 2015; Fu *et al.*, 2016; Posth *et al.*, 2016; Modi *et al.*, 2021).

The replacement has also been seen in the conducted genome-wide analyses, given that ancient individuals (Ostuni1, Ostuni2, Paglicci108, and, Paglicci133) dated to the pre-LGM belong to the so-called 'Věstonice' cluster sharing more genetic shifts with ancient and present-day Siberians, while ancient individuals dated to the Epigravettian (Continenza and Villabruna) belong to the so-called 'Villabruna' cluster, in which most of the WHG individuals fall (see section 2.2.1.) (Seguin-Orlando *et al.*, 2014; Fu *et al.*, 2016; Antonio *et al.*, 2019; Catalano *et al.*, 2019; Villalba-Mouco *et al.*, 2019).

2.3.2. The Neolithic

The Neolithic (7,000-3,500 BCE) in the Italian Peninsula was dominated by two cultures – Cardium pottery (6,400-5,500 BCE) and Impressed Ware (6,000-4,000 BCE) – connecting the Italian Peninsula with other regions along the Adriatic Sea in the East and the Ligurian Sea in the West (Broodbank, 2006; Zilhão, 2014; Martins *et al.*, 2015; Capelli *et al.*, 2017). Both cultures showed similarly decorative styles of pottery with little differences in the use of the materials to imprint the patterns.

From a genetic perspective, studies focusing on the Italian Peninsula, including the biggest Italian islands Sardinia and Sicily, have shown similar genetic ancestries as in other parts of Europe during that period (Antonio *et al.*, 2019; Fernandes *et al.*, 2020; Marcus *et al.*, 2020). However, the cluster-based analysis of the Early Neolithic individuals has shown that the individuals fall separately within the so-called European Neolithic cluster suggesting an imbalance of the WHG ancestry component and the difference in connectedness within the Mediterranean Basin (Olalde *et al.*, 2015; Marcus *et al.*, 2020; Rivollat *et al.*, 2020; Aneli *et al.*, 2021). Particularly ancient individuals from the island of Sardinia showed a stronger genetic affinity toward western Mediterranean populations than to the Italian Peninsula (Fernandes *et al.*, 2020; Marcus *et al.*, 2020).

2.3.4. The Chalcolithic and Bronze Age

From an archaeological point of view, the Chalcolithic (3,500-2,200 BCE) and Bronze Age (2,200-900 BCE) periods are significant for the appearance of metallic techniques and the establishments of the first economic stratification represented in different cultures. Thus, the Remedello, Rinaldone, and Gaudo cultures can be found in the North of the Italian Peninsula while the Laterza and Abealzu-Filigosa cultures are found in the South of the Italian Peninsula and Sardinia. The Chalcolithic/Bronze Age transition is marked by the spread of the Bell Beaker culture into the Italian Peninsula around 2,200 BCE. The end and start of one of the cultures are uncertain because of the integration of new "foreign elements" into the existing cultures.

The genetic changes during the Chalcolithic/Bronze Age transition and the arrival of the Western Steppe herders have been extensively studied in recent years (Allentoft *et al.*, 2015; Haak *et al.*, 2015; Jones *et al.*, 2015; Mathieson *et al.*, 2015, 2018; Olalde *et al.*, 2018; Villalba-Mouco *et al.*, 2021). However, the genetic data was limited to six ancient individuals from the Italian Peninsula dated to this period of change (Allentoft *et al.*, 2015; Olalde *et al.*, 2018). All six individuals were collected from the Northern part of the Peninsula and radiocarbon dated to the Chalcolithic and Early Bronze Ages. These individuals are associated with the Remedello and Bell Beaker culture, respectively, and especially the latter culture was widely spread over West Europe reaching Great Britain (Olalde *et al.*, 2018) and additionally presenting a wide spread of the Steppe-related ancestry component in the regions. In the Italian Peninsula, the Steppe-related ancestry component is significantly presented in one (I2478: 2195–1940 cal BCE) out of the three individuals associated with the Bell Beaker culture dated to the Early Bronze Age.

On the other hand, the Steppe-related ancestry component was not detectable in one female individual (RISE486: 2134-1773 cal BCE) from the Remedello culture dated to the Early Bronze Age, indicating that the region might not be affected by the expansion of the Yamnaya-related group from the Pontic-Caspian Steppe or due to the limitation of three ancient individuals from the geographical location. The two other individuals from the Remedello culture were dated to the Chalcolithic and fall within the genetic cluster with other ancient individuals from the Neolithic and Chalcolithic (Allentoft *et al.*, 2015) as seen in other publications focusing on the Italian Peninsula and the islands Sardinia and Sicily (Antonio *et al.*, 2019; Fernandes *et al.*, 2020; Marcus *et al.*, 2020). The Steppe-related ancestry component was found, however, in individuals dated to the Iron Age (Antonio *et al.*, 2019).

In addition, the presence of the Y chr haplotype R1b associated with the spread of the Steppe-related ancestry component was present in the individuals dated to the Iron Age in the central Italian Peninsula, but not present in individuals related to the Remedello and Bell Beaker culture (Allentoft *et al.*, 2015; Olalde *et al.*, 2018; Antonio *et al.*, 2019).

2.3.5. The Iron Age

The Iron Age in the Italian Peninsula was characterised by the mass presence of iron artefacts in archaeological sites and the growth of the political stratification as well as the trading network through the Mediterranean Sea routes and across the Alps to Northern Europe (Hodos, 2020). Furthermore, a lot of cultural groups were present in the Italian Peninsula during this period showing a high diversity of the material culture (Figure 2). From a genetic point of view, the generated genome-wide data is limited to individuals from the central Italian Peninsula and Venose (Southern Italian Peninsula) showing a high genetic heterogeneity and confirming the mobility of people during the time period (Antonio *et al.*, 2019; Posth *et al.*, 2021). In detail, the individuals presented significant compositions of the Steppe-related ancestry and/or Iranian-Neolithic-related ancestry components shifting from Central to the Southern Italian Peninsula confirming the trading network with the eastern part of the Mediterranean Basin. However, the exact influx of the Iranian-Neolithic-related ancestry component in the Southern Italian Peninsula is uncertain. In addition, a Northern African-related ancestry component was found in individuals from the central Italian Peninsula with an increase during the Roman Republic indicating additionally the mobility of people (Antonio *et al.*, 2019; Fernandes *et al.*, 2020; Marcus *et al.*, 2020).

3. AIMS OF THE STUDY

This thesis aims to investigate the demographic history of ancient individuals from the Italian Peninsula, the broad connection to ancient Eurasians and Africans, and the resulting genetic influence on present-day Italians. Using NGS, new genome-wide data of ancient individuals from archaeological sites in Northern, Central, and Southeastern Italy was generated at the Ancient DNA Laboratory of the Institute of Genomics, the University of Tartu in Estonia and assembled with published data to explore the more specific aims compiled in the three references:

The first study (REF I) examines the re-population of Southern Europe and subsequently Eurasia after the end of the LGM around 19k-17k ya and the potential relation to the Villabruna cluster, which is dated to the Bølling-Allerød around 14,000 years ago. For this reason, genome-wide data of one ancient individual from Northern Italy will be generated and analysed with published individuals dated between ~45,000 and 7,000 ya to characterise the genetic affinities with individuals associated with the introduced genetic cluster.

The second study (REF II) aims to investigate the genetic and social structure-related changes during the transition from the Chalcolithic to the Bronze Age period in the Italian Peninsula. To conduct the study, a genome-wide dataset of ancient individuals from Northeastern and Central Italy will be generated. To investigate the genetic changes and structural shifts during the transition in a more detailed study/framework, published genome-wide data of ancient individuals from the Italian Peninsula, Sardinia, and Sicily dated from the Neolithic to the Iron Age will be added to the dataset.

The third study (REF III) explores the external gene flow in Southeastern Italy during the Iron Age and its consequences on the genetic distribution of present-day Italians. New genome-wide data of ancient individuals from Southeastern Italy will be generated and placed into context with other ancient individuals from the Italian Peninsula. To characterise the genetic origin of an Iron Age group – the Daunians – whose cultural place of origin is under debate from an archaeological and historical perspective; specifically, the genetic affinities of published individuals from the Balkan and Aegean will be investigated. Besides the study of the external gene flow, the possible foundation of present-day Italians in South Italy will be explored.

4. MATERIALS AND METHODS

For the three listed publications, a dataset of newly generated genome-wide data at the Ancient DNA Laboratory at the Institute of Genomics, the University of Tartu in Estonia and published data from ancient and present-day individuals was compiled. The list of published ancient and present-day individuals can be found in the supplemental materials of the REF II and REF III. The following figure 3 represents A) the geographical location of the archaeological sites featuring the periods, B) and the absolute or relative radiocarbon dates of the newly generated ancient individuals (included in the final dataset for autosomal analysis) from the Italian Peninsula (Figure 3). I here provide, for each publication, a paragraph summarising the materials and methods used in each publication. Additional information about the experimental and computational methods can be found in the original publications and/or their supplemental materials.

Figure 3. Overview of the newly generated data from the Italian Peninsula used in these studies.
(A) The geographical location of the archaeological sites. (B) Absolute or relative radiocarbon dates of the individuals included in the genome-wide analyses.

4.1. Reference I

In this study, new genome-wide data was obtained from one individual uncovered at the archaeological site of Riparo Tagliente (Stallavena di Grezzana, Verona, Italy) in the Northeastern part of the Italian Peninsula and is currently stored at the Natural History Museum of Verona, Italy. The individual was directly radiocarbon dated to 16,980-16,510 cal BP (95.4% probability) and associated with Late Epigravettian cultural artefacts.

To explore the genetic ancestry and relationship to contemporary hunter-gatherers, different quantitative and statistical approaches, e.g. multidimensional scaling (MDS), Outgroup $f3$ and $f4$ statistics as well as qpGraph using Admixtools, were performed (Patterson *et al.*, 2012). The evolutionary history of the mtDNA was inferred by using the Maximum Likelihood method and General Time Reversible models (Nei and Kumar, 2000) (see STAR methods, REF I).

4.2. Reference II

In this study, DNA was extracted from human remains from four archaeological sites geographically located in the Northeastern and Central Italian Peninsula. After screening and additional sequencing, the program READ (Monroy Kuhn, Jakobsson and Günther, 2018) was used to identify genetically identical individuals and calculate the potential minimum number of individuals due to the commingled remains (Adams, 2014; Dolfini, 2020) in three out of the four archaeological sites. To evaluate the significance of the genetic changes during the Chalcolithic/Bronze Age transition in the Italian Peninsula, Sardinia, and Sicily, different genomic analyses including PCA, Admixture/DyStruct, Chromopainter/NNLS and Sourcefinder, and qpAdm were performed (see STAR methods, REF II). In addition to the validation of the ancestry patterns, the genetic relatedness of the ancient individuals dated to between the Neolithic and Iron Age were estimated to investigate the social pattern, e.g. related to the burial practices. Kinship analyses were performed in combination with the uniparental markers and obtained archaeological information. Furthermore, the phenotype features of the newly generated individuals were studied to answer open questions related to skin pigmentation, metabolism and digestion, and the role of pathogens.

4.3. Reference III

In this study, DNA was extracted from human remains from three closely located archaeological sites in Southeastern Italy. After quality control, the final dataset was merged with ancient and present-day Eurasian individuals, especially present-day individuals from Apulia to study the origin of the Daunians

and put the ancient individuals into the Pan-Mediterranean genetic context. Besides the genomic analysis focusing on model-based approaches such as the qpAdm/qpWave framework, the relatedness of the individuals was tested using READ and the comparison of uniparental markers.

5. RESULTS AND DISCUSSION

The following section provides an overview of the three scientific publications compressing the main results and discussions. Figure 4 presents the average human coverage of the ancient individuals included in the final genome-wide analyses (Figure 4). More detailed information can be found in the publications and their respective supplemental information.
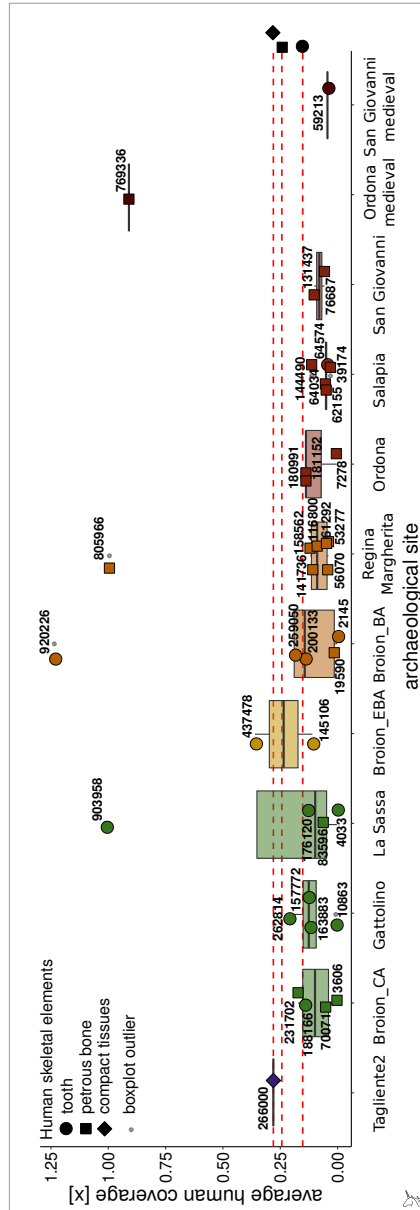
Figure 4. The final genome-wide average human coverage of the newly published ancient individuals was used in these studies.
Here the individuals are ordered by the periods (blue=Palaeolithic, green=Chalcolithic, yellow=Early Bronze Age, orange=Bronze Age, light red=Iron Age, dark red=Mediaeval period) and the shapes highlight the representative sample location of each individual (rhombus=compact tissue (mean=0.28×), cycle=tooth (mean=0.152×), square=petrous bone (mean=0.243×)). The red lines show the mean coverage of the sample location. The numbers represent the number of SNPs overlapping with the 1240K Human Origin SNP Array.

5.1. A new study of a Palaeolithic individual backdates the westward human migration in Southern Europe (REF I)

For this study, DNA was extracted from five human skeletal elements of one individual (Tagliente2) with focal cemento-osseous dysplasia (compact tissue) discovered at the archaeological site Riparo Tagliente (Delai *et al.*, 2015). Here, the library of the compact tissue had the highest DNA concentration compared to the tooth root and dentin powder of the individual proposing another potential source for DNA preservation in addition to the regular sources, e.g. petrous bone core or the other alternatives such as auditory ossicles and talus (Parker *et al.*, 2020; Sirak *et al.*, 2020). After quality control and additional sequencing of the selected library, the genome-wide coverage was 0.28×-fold yielding 266k SNPs overlapping with the 1240k Human Origin SNP Array.

5.1.1. mtDNA and Y chr haplogroup diversity

First, the genetic sex of the individuals was determined to be male using the sex/autosomal chromosome ratio allowing the estimation of the Y chr haplogroup. The Y chr haplogroup was estimated to be I2, which has been found in the majority of ancient individuals in Europe ~14,000 years ago (Figure 3B, Supplemental data S1C and S1E, REF I). The estimated mtDNA haplogroup of Tagliente2 is U2'3'4'7'8'9, which he shares with several other ancient individuals from West Europe during the Post-LGM, including two other ancient individuals (Paglicci 108 (28,430-27,070 cal BP), San Teodoro 2 (15,232-14,126 cal BP)) from the Italian Peninsula and one individual (Oriente C (layer: 12,250-11,850 BCE) from Sicily (Fu *et al.*, 2016; Posth *et al.*, 2016; Mathieson *et al.*, 2018; Catalano *et al.*, 2019; Modi *et al.*, 2021) (Figure 3A, Supplemental figure S4, Supplemental Data Table S1C and S1D, REF I).

5.1.2. Population perspective

Results of the MDS analysis revealed that Tagliente2 falls into a genetic group with WHG individuals mostly assigned to the 'Villabruna' cluster and dated to the Post-LGM period (Figure 4A, REF I) (Fu *et al.*, 2016). Because the genetic affinities of those individuals within the 'Villabruna' cluster indicated a large replacement of the previous European hunter-gatherer populations after ~14,000 ya and showed a higher affinity with a West Asian genetic component, statistical tests (*f*3 and *f*4 statistics) of Tagliente2 were performed (Figure 4B, Supplemental figure S2, S3B–S3B, REF I). The results expressed the shared genetic features of the individuals within the 'Villabruna' cluster with Tagliente2 and confirmed the affinity with West Asian genetic components.

These results proposed evidence that backdates the presence of a population associated with the 'Villabruna' cluster by ~3,000 years in Northern Italy and supports two different scenarios of migration: i) an involvement from a board network of a Southern Europe-Eastern Europe refugia during and immediately

after the LGM, or ii) a more-abrupt genetic turnover with the distribution of both genetic and cultural similarities. Throughout the scenarios described in detail in REF I cannot be tested with the available archaeological evidence, Tagliente2 provides new insights into the major migration events during the Upper Palaeolithic affecting the genetic background of all Europeans and its presence already in Southern Europe during the cold phase following the LGM peak. However, for a better understanding of the migration events, additional genome-wide data of ancient individuals from Southern Europe would be needed to investigate the genetic changes in more detailed times and connect the network between Europe, the Balkan, and Eastern Europe/Western Asia.

While writing this thesis, a preprint manuscript has presented newly generated genome-wide data of two individuals from Sicily (San Teodoro 3 (not dated) and San Teodoro 5 (15,322-14,432 cal BP)) associated with the Late Epigravettian period showing a yet unknown genetic Italian lineage within the 'Villabruna' cluster (Scorrano *et al.*, 2021). Both individuals share mtDNA and Y chr haplogroups with other European Palaeolithic individuals and confirm the conclusions based on the uniparental markers stated above. On the other hand, the authors found a difference in the shared genetic drifts between the Sicilian individuals and ancient HG from the Iberian and the Italian Peninsula suggesting a gene flow involving ancestry related to Pre-LGM hunter-gatherers in Southern Italy as previously observed in the Iberian Peninsula and a different ancestry proportion in ancient individuals within the 'Villabruna' cluster.

5.2. Changes in the (social) structure after the arrival of Steppe-related ancestry (REF II)

For this study, DNA was extracted from 51 human skeletal elements including tooth roots and petrous bones from 3 commingled cave burial sites (Broion, La Sassa, and Regina Margherita) and one single grave burial site (Gattolino) following the standard protocols for aDNA (Meyer and Kircher, 2010). After validation of each sequenced library (one sample per individual except 4 individuals), the relatedness between the samples was estimated using READ (Monroy Kuhn, Jakobsson and Günther, 2018) (Supplemental data S4, REF II). The results were compared with the estimated mtDNA and Y chr haplogroups to identify genetically identical samples and to calculate the minimum number of unique individuals. The identical samples were merged together leaving 22 unique individuals (Broion=11, Gattolino = 4, La Sassa = 4, Regina Margherita = 4) (Figure 4; Figure 1A and 1B, Table 1, Supplemental data S1A and S1B). For the study, a subset of ancient and present-day individuals in Eurasia was selected and merged with the newly generated individuals (Keller *et al.*, 2012; Gamba *et al.*, 2014; Lazaridis *et al.*, 2014; Olalde *et al.*, 2014; 1000 Genomes Project Consortium *et al.*, 2015; Allentoft *et al.*, 2015; Haak *et al.*, 2015; Jones *et al.*, 2015; Mathieson *et al.*, 2015; Lazaridis *et al.*, 2016; Broushaki *et al.*, 2016; Fu *et al.*, 2016; Hofmanová *et al.*, 2016; Lazaridis *et al.*,

2017; Lipson *et al.*, 2017; Martiniano *et al.*, 2017; Saag *et al.*, 2017; van den Brink *et al.*, 2017; Olalde *et al.*, 2018; Mathieson *et al.*, 2018; Damgaard *et al.*, 2018; Fregel *et al.*, 2018; Günther *et al.*, 2018; Harney *et al.*, 2018; Mittnik *et al.*, 2018; Valdiosera *et al.*, 2018; Antonio *et al.*, 2019).

5.2.1. Arrival of Steppe-related ancestry component

To explore the arrival of the Steppe-related ancestral component in the Italian Peninsula, the genome-wide data of the newly generated individuals were analysed with selected published ancient and present-day individuals (STAR methods, REF II). The results confirmed the diversity of ancestry components during the Chalcolithic and pinpointed the arrival of the Steppe-related ancestry component in ancient individuals in the central Italian Peninsula as early as 1,600 BCE, with an increase through time.

We applied different statistical, model-based clustering, and orthogonal methods (STAR method section, REF II) to estimate the ancestral components of the newly generated and published ancient individuals from the Italian Peninsula dated between the Neolithic and Iron Age (Figure 1A and 1B, Table 1, Supplemental data S1A and S1B, REF II). We found that the newly generated individuals and published individuals dated to the Chalcolithic are within the so-called European Neolithic (EN) cluster featuring Eastern European Neolithic individuals on the right side in the PCA (Figure 5; Figure 2A, REF II). This pattern has already been reported by (Marcus *et al.*, 2020) highlighting that ancient individuals from Sardinia dated to the Neolithic are genetically closer to Western European Neolithic individuals than to Eastern European Neolithic individuals including Neolithic individuals from the Italian Peninsula. The individuals dated to the Early Bronze Age (EBA) fall within the Post-Neolithic cluster overlapping with present-day Eurasian and published ancient individuals dated to the Bronze Age period (Figure 2A, REF II). We further investigated the genetic affinities of the Chalcolithic individuals to WHG individuals using $f3$ and $f4$ outgroup statistics (Supplemental data S2H, REF II). The results of the $f4$ statistics in form $f4$(Mbuti.DG, Italy_Mesolithic.SG; Italy_Sardinia_N, X) indicated that Italy_Sardinia_N shares more with Italy_Central_Mesolithic than with the Chalcolithic individuals from the Italian Peninsula explaining the overlap of ancient Sardinian individuals dated to the Chalcolithic in the right side of the EN cluster (Fernandes *et al.*, 2020; Marcus *et al.*, 2020).

To estimate the genetic contribution of the Steppe-related ancestry component in the ancient individuals dated between the Neolithic and Iron Age, we performed qpWave/qpAdm, DyStruct, Admixture, and $f4$ statistics in form $f4$(Mbuti.DG, Yamnaya Kalmykia; X, Anatolia_N) (modified figure 6; Figure 2B+C, Supplemental figure S2–S4, Supplemental data S3, REF II). The results of these allele frequency-based tests were all consistent and indicated significant enrichment of the Steppe-related ancestry component in ancient individuals dated to the EBA and Bronze Age, (represented by two published ancient individuals related to the Bell Beaker culture in the Northern Italian Peninsula

(Olalde *et al.*, 2018)), all newly generated individuals dated to the EBA and BA and published ancient individuals from the central Italian Peninsula dated to Pre-Roman Imperial time (Antonio *et al.*, 2019) (Figure 2C, Supplemental figure S4, REF II). We further tested the presence of the Iran-Neolithic related ancestry component in Pre-Bronze Age individuals as indicated by (Antonio *et al.*, 2019; Fernandes *et al.*, 2020), but did not find any significant results. Our study does not reject the hypothesis of the Iran-Neolithic related ancestry component in the Pre-Bronze Age time period nor the presence of the Steppe-related ancestry component in the southern Italian Peninsula, however, more genome-wide data from different geographical locations in the Italian Peninsula would be needed to define the presence of those genetic components and assess the dynamics of the spread of the Steppe-related ancestry component.
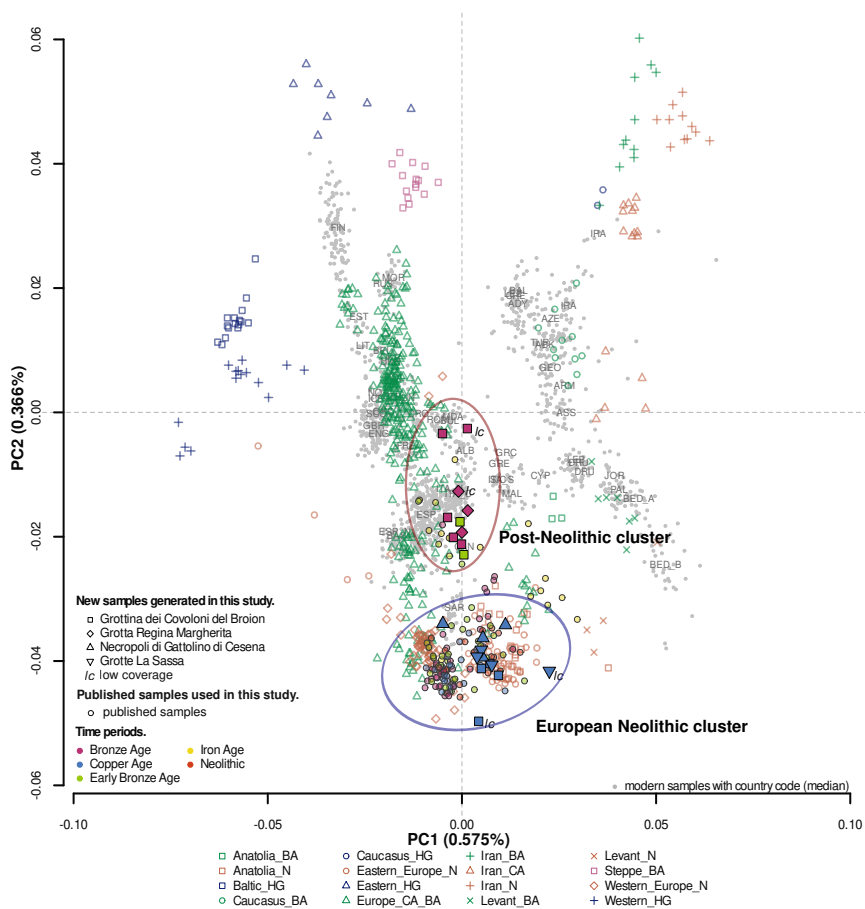

Figure 5. Principal component analysis of newly generated individuals with previously published data projected onto the variation from present-day populations. The two highlighted clusters (European Neolithic cluster and Post-Neolithic cluster) present the genetic affinities of the newly generated ancient individuals. Figure reprinted with permission from figure 2A (REF II), Elsevier.

5.2.2. Shift in uniparental markers

The uniparental markers of the ancient individuals were estimated and compared to published ancient individuals from Eurasia to investigate a structure among the ancient groups. Mostly, the individuals carried subclades of the mtDNA haplogroups H, J, K, and N1 (Table 1, Supplemental data S1B and S1E, REF II), equivalent to the mtDNA haplogroup diversity of published ancient individuals dated to the Late Neolithic and Bronze Age (Lazaridis *et al.*, 2014; Haak *et al.*, 2015). We found a change in the Y chr haplotypes with the enrichment of the Steppe-related ancestry component during the EBA in the newly generated individuals in comparison to the Y chromosome haplotypes of published ancient individuals from previous studies (modified figure 6; Figure 2C, REF II) (Keller *et al.*, 2012; Allentoft *et al.*, 2015; Olalde *et al.*, 2018; Antonio *et al.*, 2019; Fernandes *et al.*, 2020; Marcus *et al.*, 2020). The results are consistent with previous studies focusing on the spread of the Steppe-related ancestry component and the Y chr haplotype R1 (Allentoft *et al.*, 2015; Haak *et al.*, 2015; Olalde *et al.*, 2019; Papac *et al.*, 2021; Villalba-Mouco *et al.*, 2021).
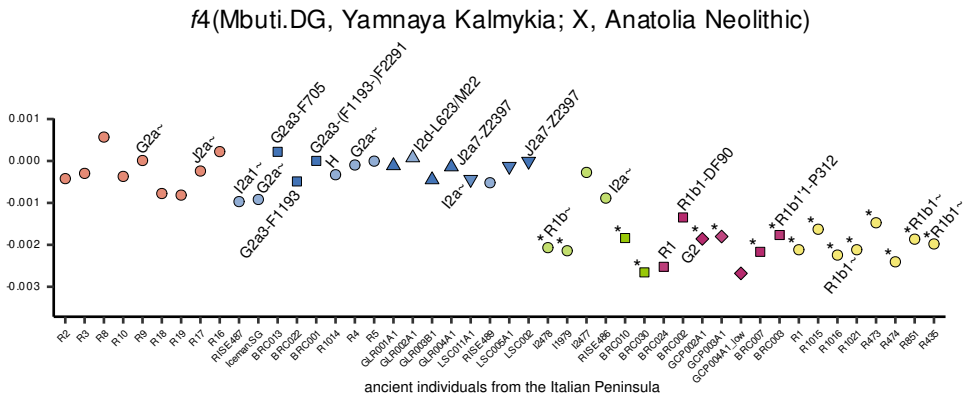

Figure 6. Result of the f4 statistics to estimate the enrichment of the Steppe-related ancestry component in ancient individuals from the Italian Peninsula and the respective Y chr haplotypes of male individuals.
In detail, the Figure presents the analysis of the Steppe-related ancestry component in selected published (transparent) and newly generated ancient individuals (X) from the Italian Peninsula using *f*4 statistics in form *f*4(Mbuti.DG, Yamnaya Kalmykia; X, Anatolia Neolithic) (samples with * have a Z score less than or equal to 3). Tests with less than 5,000 SNPs were not included. The colours present the periods (red=Neolithic, blue=Chalcolithic, green=Early Bronze Age, purple=Bronze Age, yellow=Iron Age) included in this study. The Y chr haplotypes of male individuals are added to the figure to give a better understanding of the shift in uniparental markers during the transition. The modified figure is reprinted with permission from figure 2A (REF II), Elsevier.

5.2.3. Social structural-related shifts

Besides the evaluation of the Steppe-related ancestry component, we explored the relatedness of the ancient individuals from the commingled cave burials during the transition, especially from the archaeological site Broion covering the periods Chalcolithic and Bronze Age, and tested whether the shift of burial practices occurred with the shift of genetic ancestry as featured in another recent study (Villalba-Mouco *et al.*, 2021).

In general, the number of ancient individuals in our study is approximately equal between the Chalcolithic and Bronze Age periods (n=12 and 10, respectively). However, the number of male individuals within the Chalcolithic cave burials differs from the expected value (binomial; p-value = 0.038) hinting at a bias in the cave burial toward males. On the other hand, we tested this phenomenon using $f3$ outgroup statistics and confirmed the absence of a male-sex bias associated with the arrival of the Steppe-related ancestry component as reported by other studies (Saag *et al.*, 2017) (Supplemental figure S5, REF II). However, these results are by the limited number of ancient individuals from the Italian biassed Peninsula.

We did not find genetic relationships among the individuals within the Bronze Age cave burials including available published ancient individuals. Within the Chalcolithic cave burials, we estimated five potential 1st- to 3rd-degree relationships between six individuals sharing uniparental markers, where all the relationships were detected between males (More details explained in the manuscript of REF II; Table 2, REF II). Interestingly, all the male individuals from the archaeological site Broion share the Y chr marker G2a-P15 and, despite the low average Y chr coverage, share the same haplotype G2a (modified figure 6; Figure 2C, Supplemental data S1F, REF II).

In addition, we examined the runs of homozygosity (ROH) by calculating the segment number and length of four length categories and estimating the size and homogeneity of populations dated from Mesolithic to Bronze Age and present-day Italians (STAR methods, Figure 4, Supplemental figure S7, Supplemental data S4, REF II). The ROH>1.6 cM showed a change in the population structure after the Mesolithic and more similar values to present-day Italians. We showed a significant difference of ROH>1.6 cM segments when comparing ancient individuals from the Neolithic and the Bronze Age indicating two possible scenarios: i) larger effective population size during the Bronze Age period in the Italian Peninsula or ii) increased diversity after an admixture event with local individuals during the Chalcolithic period.

5.2.4. Phenotypic features of ancient Italians

For this part of the study, in total 332 ancient individuals from the Italian Peninsula were selected and imputed with genetic markers related to 115 phenotypes associated with metabolism, immunity, and pigmentation (Allentoft *et al.*, 2015; Lazaridis *et al.*, 2016; Mathieson *et al.*, 2018; Antonio *et al.*, 2019; Fernandes *et al.*, 2020; Marcus *et al.*, 2020) (STAR methods, Supplemental data S6, REF II). In total, 15 variants were significant but were interpreted carefully due to the limited number of individuals. We estimated significant differences in the variants during and after the Roman Imperial period than during the Chalcolithic/Bronze Age transition (more details are explained in the manuscript of REF II). One interesting finding here is the decrease in alleles associated with the protection against Hansen's disease (leprosy) and the fundamental increase of the disease in bioarchaeological findings and historical records in Europe from 3,000 BCE and its decline ~1,000 CE (Donoghue *et al.*, 2005; Köhler *et al.*, 2017) (Table 3, Data S6C, REF II).

In the review by Aneli et al. 2021, the newly generated individuals were added to explore the five variants – lactose metabolism, fatty acid metabolism, immune system, skin pigmentation, and eye colour, which have been reported to be selected with the genetic changes during the major movements (Mathieson *et al.*, 2015; Aneli *et al.*, 2021). Here, the results presented a similar observation as reported in Mathieson et al. 2015. However, the selection for the lactase persistence is absent in individuals from the Italian Peninsula, Sardinia, and Sicily dated to the Bronze Age and first, it is presented in individuals from the Italian Peninsula dated to the Antiquity confirming our results stated above.

5.3. Genetic distribution of present-day Southern Italians during the Iron Age (REF III)

For this study, DNA was extracted from human skeletal remains from three necropoleis geographically located in modern Apulia, Southeastern Italy. The archaeological sites are dated to the Iron Age and Mediaeval periods referring to archaeological findings and historical records. After raw data processing, the final dataset contained 16 ancient individuals (Ordona = 8, Salapia = 5, San Giovanni Rotondo = 3), which were merged with selected ancient and present-day individuals from i) AADR (https://reich.hms.harvard.edu/allen-ancient-dna-resource-aadr-downloadable-genotypes-present-day-and-ancient-dna-data, version 44.3; more details in the supplemental materials, REF III), ii) Chalcolithic/Bronze Age individuals (Saupe *et al.*, 2021), ii) Tagliente2 individual (Bortolini *et al.*, 2021), iii) genome-wide data of present-day Italian individuals (Raveane *et al.*, 2019), iv) present-day Apulian individuals (Sallustio *et al.*, 2015), v) haploid genomes representing the Eurasian component of modern Ethiopians (Molinaro *et al.*, 2019).

5.3.1. Genetic heterogeneity of Iron Age Apulia

We investigated the genetic heterogeneity of the ancient individuals from Apulia to understand the connectedness of the geographical area with locals from the Italian Peninsula, Western Europeans, the Balkan; especially Croatians, the Aegean; especially Cretan, and the Levantines. The results of the PCA showed that the ancient individuals dated to the Iron Age period cluster largely between ancient and present-day populations stretching along the PC2 and overlapping with other Iron Age individuals from the Mediterranean Basin (Figure 1C, REF III). We investigated these genetic affinities further using the qpWave/qpAdm framework and modelled the individuals using different combinations of ancestral components (WHG, Anatolian Neolithic, Steppe-related, and CHG or Iranian-Neolithic- related) reported across Western Europe at that time (Figure 7; Method section, Figure 2B and 2C, Supplemental figure S7A , Supplemental data S5, REF III). The results showed that the Iron Age Apulians, mainly, could be modelled as a two-way admixture between Anatolian Neolithic and Steppe-related ancestry; however, an alternative model compiled by Anatolian Neolithic and CHG/Iranian-Neolithic-related ancestry could also be a fit for a subset of the ancient individuals. At the same time, the ancient individuals shifting towards present-day populations from West Asia could be modelled as a three-way admixture between Anatolian Neolithic, Steppe-related, and CHG/Iranian-Neolithic-related ancestry explaining the shift within the PCA (Figure 1C, in detail Figure 2B-C, Supplemental figure S5A, REF III).

Remarkably, alternative models, including Minoans, as a possible source from the Aegean and Amhara_NAF (linked with Sea People), required an additional source for the model to be a better fit. The additional required sources are mostly related to Iranian-Neolithic/CHG, Steppe, or WHG individuals. In addition, we modelled and compared the genetic composition of ancient individuals dated to the Roman Republic and re-confirmed the major presence of the Steppe-related ancestry component as reported in (Antonio *et al.*, 2019). Based on the results and the close geographical location of the newly introduced archaeological sites, we were able to conclude a high genetic heterogeneity during the Iron Age in the southeastern Italian Peninsula, which was impacted by the local ancestry with previous Bronze Age sources such as Minoans and Sea People.
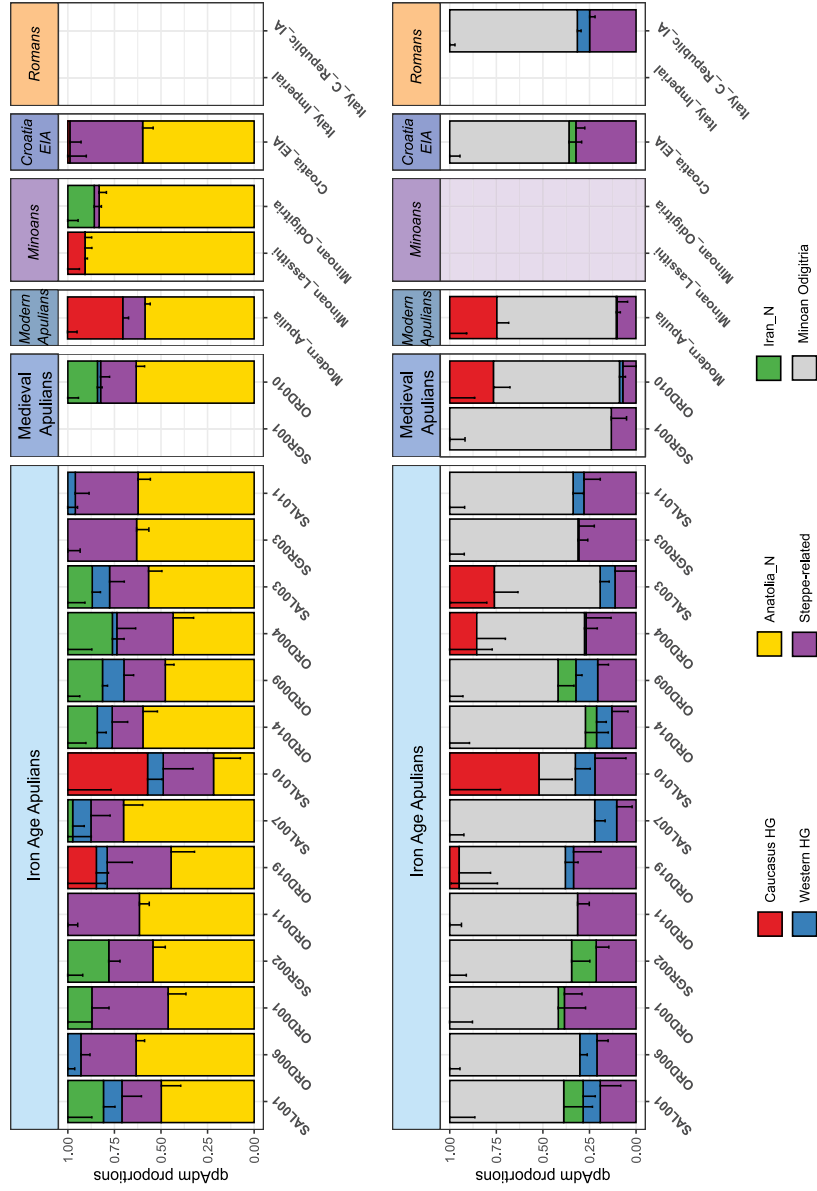
Figure 7. Overview of the best-fitted models to present the genetic contributions in the newly generated ancient individuals from Apulia, Southeastern Italy.
Figure reprinted with permission from figure 2B+C (REF III), Oxford University Press.

5.3.2. Ancient heterogeneity as a base for the gene pool of present-day Italians in Southeastern Italy

To examine the contributions of local Iron Age and Neolithic populations to the present-day southeastern Italians, we tested the genetic affinities of the ancient individuals using the results of the PCA visualisation and performed $f4$ statistics in form $f4$(Modern Apulian, Iron Age Apulian (IAA); Neolithic/Chalcolithic populations, Mbuti) (Figure 1C and 2A, REF III). We found that the ancient individuals primarily scatter between present-day Italians (including Sardinians), whereas the picture is different for other European populations dated to the Iron Age (Schiffels *et al.*, 2016; Saag *et al.*, 2019). On the other side, we found that two ancient individuals dated to the Mediaeval period show a shift towards present-day West Asian and Caucasus populations indicating the potential source of the split between northern-central and southern present-day Italians. This shift was already seen in central Italian individuals dated to the Republican-Imperial Roman period, but it is unclear whether southeastern Apulian individuals would have shown a similar shift towards the present-day Italian gene pool (Antonio *et al.*, 2019).

5.3.3. The origin of the Daunians

We further investigate evidence of the genetic origin of the Daunians using $f3$ statistics, qpWave/qpAdm framework, and $f4$ statistics. The results of the $f3$ statistics, using Minoans, Iron Age Croatians, and the local Roman Republicans as potential genetic sources respectively, declined a genetic origin from the Aegean (Figure 3A, REF III). The majority of the ancient individuals from the southeastern Italian Peninsula showed a higher genetic affinity with Roman Republicans suggesting a local Iron Age Italian ancestry.

Moreover, four individuals clustering closer with present-day Italians in the PCA (Figure 1C, REF III), presented a higher affinity towards Iron Age Croatian individuals making the Cretan or Arkadian origin less likely.

5.3.4. Relatedness of Iron Age individuals from Southeastern Italy and genetic heterogeneity

We estimated the genetic relatedness of the ancient individuals from the archaeological sites using READ (Monroy Kuhn, Jakobsson and Günther, 2018) and found a 1st-degree relationship between two female individuals from the archaeological site Ordona and two 2nd-degree relationships between one individual from Ordona and two individuals from Salapia. The latest relationship was tested combining the sites differently and declined based on the comparison of the uniparental markers plus the obtained radiocarbon dates (more details in the Material and Method section of REF III).

Notably, the genetic compositions differ between the two female individuals from Ordona, who shared the 1st-degree relationship and the identical mtDNA haplogroup H5c.

Both individuals pointed to different genetic contributions in the PCA with one individual having a closer genetic affinity towards West Asia and Caucasus present-day populations, although their radiocarbon dates overlapped (Figure 1C, Supplemental figure S4+S7, REF III). We further investigated the genetic affinities using $f4$ statistics using ancient individuals dated from the Neolithic to the Roman Republic as a potential genetic source and confirmed the affinities towards published ancient individuals with significant genetic contributions from CHG (Supplemental figure S4, REF III). Based on the results, we were able to find additional evidence for the genetic heterogeneity during the Iron Age period in the southeastern Italian Peninsula. However, more genome-wide data from other archaeological sites along the Eastern coastline would be needed to fully understand the genetic contributions of groups from the Balkans.

6. CONCLUSIONS

The results presented in this thesis showed that the Italian Peninsula has been shaped by continuous migration events reflected in the gene pool of present-day Italians and leaving their marks on the cultures. The main conclusions are the following:

REF I:
- The newly generated genome-wide data of one individual – Tagliente2 – dated 17,000 years ago showed genetic affinities with ancient individuals associated with the Villabruna cluster suggesting at least 3,000 years earlier human migration events in Southern Europe.
- The genetic analyses showed that the major migrations in Southern Europe occurred during the cold phase following the LGM peak and that Southern Europe, the Balkans, and Eastern Europe/Western Asia were already connected through the same network of potential LGM refugia sharing both genes and cultural information.

REF II:
- The first genome-wide data of ancient individuals dated to the (Early) Bronze Age from the northeastern and central Italian Peninsula indicated the genetic changes during the Chalcolithic/Bronze Age transition, connected with the appearance of the Steppe-related ancestry component in Bronze Age individuals and its continuous presence during the Iron Age.
- Two commingled cave burial sites suggested a shift in the social structure-related pattern during the Chalcolithic/Bronze Age transition. Five 1st- to 3rd-degree relationships between male individuals were only found in the sites dated to the Chalcolithic suggesting a close patrilineal kinship pattern. However, no close relationships were found between individuals dated to the Bronze Age.
- The study of phenotypic-related alleles in both the newly generated and published ancient individuals from Neolithic to Modern times suggested that noticeable changes occur during or after the Roman Imperial period as exemplified in the decrease of the alleles associated with the protection against leprosy.

REF III:
- Newly generated genome-wide data from ancient individuals geographically located in the Southeastern Italian Peninsula suggested a high genetic heterogeneity during the Iron Age period, comparable with central Italian samples from the Republican and Imperial Roman periods.

- Our data depict a pan-Mediterranean genetic landscape for the southeastern Italian Peninsula and its connectedness to the Eastern Mediterranean Basin with Mediaeval period individuals being genetically shifted toward West Asians.
- Despite the sites being geographically close and sharing a material culture, we estimated the 1st-degree relationship for two female individuals characterized by different ancestral components, additionally indicating high mobility between the Mediterranean regions.

SUMMARY IN ESTONIAN

Vahemere piirkonna geneetiline kujunemine enne meie ajaarvamist: fookusega Apenniini poolsaarele

Rohkearvulised ja erisugused uuringud on panustanud meie arusaamisesse inimajaloost. Inimasustuse ajalugu Euroopas sisaldab mitmeid etappe, seal-hulgas küttide-korilaste Lääne-Euraasias elamine paleoliitikumis, põlluharimise ja karjakasvatuse kasutuselevõtt läbi kontaktide Levantist ja Anatooliast (Lääne-Aasia) Euroopasse rännanud inimestega ja Ponto-Kaspia stepirahvaste migrat-sioon. Enamik senistest uuringutest on keskendunud üldistele geneetilistele muutustele Euroopas ja nende seostele arheoloogiliste tõendite ja ajalooliste sündmustega. Teadlased on varasemalt keskendunud ka kindlale kultuurile, geograafilisele piirkonnale ja/või riigile. Sellegi poolest on mõned Euroopa piirkonnad jätkuvalt väheuuritud ja neile keskendumine võib aidata täita lünki inimeste rändeajaloos.

Üks selline ala on Vahemere piirkond, mis jääb kolme maailmajao – Aafrika lõunas, Aasia idas ja Euroopa põhjas – vahele ning on mänginud kesket rolli inimtsivilisatsioonide ajaloos. Vahemere ümbruse kliimat iseloomustavad pea-miselt pehmed talved ja kuumad, kuivad suved, mis teevad selle piirkonna inimasustusele sobivaks. Apenniini poolsaare geneetiline ajalugu on pikalt olnud väheuuritud, vaatamata poolsaare geograafilisele paiknemisele Vahemere piirkonna keskmes ning sotsiaal-kultuurilistele sidemetele ümbritsevate popu-latsioonidega. Lisaks teame me rohkem ajaloolistest sündmustest, mis on seotud Rooma impeeriumi ja sellele järgnenud perioodidega, kui migratsioonidest enne rauaaega ja nende mõjust tänapäevaste itaallaste genoomidele.

Tänu teise põlvkonna sekveneerimistehnoloogia (NGS) kasutuselevõtule ja ammuse desoksüribonukleiinhappe (vana DNA) inimsäilmetest eraldamise meetodite väljatöötamisele on võimalik kombineerida teadmised ajaloost, arheoloogiast, antropoloogiast ja geneetikast, et heita valgust inimajaloole ja demograafilistele muutustele. Siiski piiravad vana DNA uurimist DNA mole-kulide ebaühtlane säilimine erinevate keskkonnatingimustega piirkondades, kättesaadavate proovide piiratud arv huvipakkuvatest paikadest, DNA lagune-mine ajas ja uuringute kulukus. Nende faktorite tõttu arendavad teadlased laboris kasutatavaid töövooge ning bioinformaatilisi meetodeid, et genereerida rohkem andmeid ja produtseerida tulemusi, mida avalikkusele tutvustada.

See doktoritöö keskendub geneetilise ja sotsiaalse struktuuriga seotud muu-tustele Apenniini poolsaarel viimase jääaja maksimumi lõpust (19–17 tuhat aastat tagasi) Rooma vabariigi loomiseni (umbes 2000 aastat tagasi). Genereeriti ülegenoomsed andmed inimsäilmetest, mis pärinesid Itaalia põhja-, kesk- ja kaguosast. Neid andmeid analüüsiti varem avaldatud andmete kontekstis, et uurida ammuste populatsioonide geneetilist ülesehitust (ja selle muutusi) läbi aja. Lõplik andmestik koosnes indiviididest, kes pärinevad paleoliitikumist (43–5 tuhat aastat enne meie aega) rauaajani (1100–700 aastat enne meie aega), või-

maldades arheoloogiliste andmete kontekstis uurida kolme peamist rahvasti-kuliikumist paleoliitikumis, neoliitikumis ja eneoliitikumi-pronksiaja üleminekul (4500–2500 aastat enne meie aega).

Käesoleva doktoritöö tulemused näitasid, et Apenniini poolsaart on mõjutanud järjestikused migratsioonid, mis peegelduvad tänapäeva itaallaste geenitiigis ja on jätnud jälje kultuuri. Peamised järeldused on järgmised:

REF I:
- Ühe 17 000 aasta vanuse indiviidi, Tagliente2 vastgenereeritud ülegenoomsed andmed näitasid geneetilist lähedust Villabruna grupiga seostatud ammuste indiviididega, viidates varem teada olnust vähemalt 3000 aastat varasematele migratsioonidele Lõuna-Euroopas.
- Geneetilised analüüsid näitasid, et Lõuna-Euroopa suured ränded toimusid viimase jääaja maksimumile järgnenud külmal perioodil ning et Lõuna-Euroopa, Balkan ja Ida-Euroopa/Lääne-Aasia olid omavahel seotud juba potentsiaalsete jääaja maksimumi refuugiumite kaudu, jagades nii geene kui kultuurilist infot.

REF II:
- Esimesed ülegenoomsed andmed Apenniini poolsaare kirde- ja keskosas (vanemal) pronksiajal elanud inimestelt viitasid eneoliitikumi-pronksiaja üleminekul toimunud geneetilistele muutustele, mis olid seotud stepiga seostatud päritolu ilmumisega pronksiajal ja selle jätkuva esinemisega rauaajal.
- Kaks segatud materjaliga matmispaika koobastes vihjasid muutustele sotsiaalse struktuuriga seotud mustrites eneoliitikumi-pronksiaja üleminekul. Eneoliitikumi indiviidide seast leiti 5 esimese kuni kolmanda astme meessugulaste paari, mis viitab patrilineaarsetele ehk isa kaudu seotud sugulusmustritele. Pronksiaja indiviidide hulgast sealjuures lähisugulasi ei leitud.
- Nii uue kui varem avaldatud neoliitikumist kuni uusajani pärinevate indiviidide fenotüübiga seotud alleelide uurimine viitas märgatavatele muutustele Rooma impeeriumi ajal või pärast seda, mida väljendab näiteks leepravastase kaitse pakkumisega seostatud alleelide sageduse vähenemine.

REF III:
- Uued ülegenoomsed andmed Apenniini poolsaare kaguosa ammustelt indiviididelt paljastavad suure geneetilise varieeruvuse rauaajal, mis on võrreldav Kesk-Itaalia Rooma vabariigi ja impeeriumi perioodidega.
- Meie andmed näitavad, et Apenniini poolsaare kaguosa geneetiline maastik oli Vahemere piirkonna ülene ning sellel alal oli ühendusi ka Vahemere piirkonna idaosaga, mida väljendab keskaegsete indiviidide suurenenud geneetiline sarnasus Lääne-Aasiaga.
- Kuigi kaasatud matmispaigad paiknesid üksteisele lähedal ja jagasid materiaalset kultuuri, esines nende seas vaid üks paar esimese astme sugulasi, kes seejuures olid erineva geneetilise päritoluga naised, mis viitab suurele liikuvusele Vahemere erinevate piirkondade vahel.

REFERENCES

1000 Genomes Project Consortium *et al.* (2015) "A global reference for human genetic variation," *Nature*, 526(7571), pp. 68–74.

Adams, B. (2014) "Commingled Remains: Field Recovery and Laboratory Analysis," in Smith, C. (ed.) *Encyclopedia of Global Archaeology*. New York, NY: Springer New York, pp. 1575–1580.

Alexander, D. H., Novembre, J. and Lange, K. (2009) "Fast model-based estimation of ancestry in unrelated individuals," *Genome research*, 19(9), pp. 1655–1664.

Allaby, R. G. *et al.* (2015) "Using archaeogenomic and computational approaches to unravel the history of local adaptation in crops," *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 370(1660), p. 20130377.

Allentoft, M. E. *et al.* (2012) "The half-life of DNA in bone: measuring decay kinetics in 158 dated fossils," *Proceedings. Biological sciences / The Royal Society*, 279(1748), pp. 4724–4733.

Allentoft, M. E. *et al.* (2015) "Population genomics of Bronze Age Eurasia," *Nature*, 522(7555), pp. 167–172.

Ammerman, A. J. (1973) "A population model for the diffusion of early farming in Europe," *The Explanation of Culture Change*. Duckworth, pp. 343–357.

Ammerman, A. J. and Cavalli-Sforza, L. L. (2014) *The Neolithic Transition and the Genetics of Populations in Europe*. Princeton University Press.

Amorim, C. E. G. *et al.* (2018) "Understanding 6th-century barbarian social organization and migration through paleogenomics," *Nature communications*, 9(1), p. 3547.

Aneli, S. *et al.* (2021) "Through 40,000 years of human presence in Southern Europe: the Italian case study," *Human genetics*, 140(10), pp. 1417–1431.

Antonio, M. L. *et al.* (2019) "Ancient Rome: A genetic crossroads of Europe and the Mediterranean," *Science*. American Association for the Advancement of Science, 366(6466), pp. 708–714.

Arriola, L. A., Cooper, A. and Weyrich, L. S. (2020) "Palaeomicrobiology: Application of ancient DNA sequencing to better understand bacterial genome evolution and adaptation," *Frontiers in ecology and evolution*. Frontiers Media SA, 8. doi: 10.3389/fevo.2020.00040.

Battaglia, V. *et al.* (2009) "Y-chromosomal evidence of the cultural diffusion of agriculture in Southeast Europe," *European journal of human genetics: EJHG*, 17(6), pp. 820–830.

Benazzi, S. *et al.* (2011) "Early dispersal of modern humans in Europe and implications for Neanderthal behaviour," *Nature*, 479(7374), pp. 525–528.

Bortolini, E. *et al.* (2021) "Early Alpine occupation backdates westward human migration in Late Glacial Europe," *Current biology: CB*. doi: 10.1016/j.cub.2021.03.078.

Bos, K. I. *et al.* (2015) "Parallel detection of ancient pathogens via array-based DNA capture," *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 370(1660), p. 20130375.

Brace, S. *et al.* (2019) "Ancient genomes indicate population replacement in Early Neolithic Britain," *Nature ecology & evolution*, 3(5), pp. 765–771.

Bramanti, B. *et al.* (2009) "Genetic discontinuity between local hunter-gatherers and central Europe's first farmers," *Science*, 326(5949), pp. 137–140.

Brandt, G. *et al.* (2013) "Ancient DNA reveals key stages in the formation of central European mitochondrial genetic diversity," *Science*, 342(6155), pp. 257–261.

Briggs, A. W. *et al.* (2007) "Patterns of damage in genomic DNA sequences from a Neandertal," *Proceedings of the National Academy of Sciences of the United States of America*, 104(37), pp. 14616–14621.

van den Brink, E. C. M. *et al.* (2017) "A Late Bronze Age II clay coffin from Tel Shaddud in the Central Jezreel Valley, Israel: context and historical implications," *Levantina*. Routledge, 49(2), pp. 105–135.

Broodbank, C. (2006) "The Origins and Early Development of Mediterranean Maritime Activity," *Journal of Mediterranean Archaeology*. Equinox Publishing Group, 19(2), pp. 199–230.

Brotherton, P. *et al.* (2013) "Neolithic mitochondrial haplogroup H genomes and the genetic origins of Europeans," *Nature communications*, 4, p. 1764.

Broushaki, F. *et al.* (2016) "Early Neolithic genomes from the eastern Fertile Crescent," *Science*, 353(6298), pp. 499–503.

Capelli, C. *et al.* (2017) "The circulation of Early Neolithic pottery in the Mediterranean: A synthesis of new archaeometric data from the Impressed Ware culture of Liguria (north-west Italy)," *Journal of Archaeological Science: Reports*, 16, pp. 532–541.

Carpenter, M. L. *et al.* (2013) "Pulling out the 1%: whole-genome capture for the targeted enrichment of ancient DNA sequencing libraries," *American journal of human genetics*, 93(5), pp. 852–864.

Cassidy, L. M. *et al.* (2016) "Neolithic and Bronze Age migration to Ireland and establishment of the insular Atlantic genome," *Proceedings of the National Academy of Sciences of the United States of America*, 113(2), pp. 368–373.

Cassidy, L. M. *et al.* (2020) "A dynastic elite in monumental Neolithic society," *Nature*, 582(7812), pp. 384–388.

Catalano, G. *et al.* (2019) "Late Upper Palaeolithic hunter-gatherers in the Central Mediterranean: new archaeological and genetic data from the Late Epigravettian burial Oriente C (Favignana, Sicily)," *bioRxiv*. bioRxiv. doi: 10.1101/692871.

Collard, M. *et al.* (2010) "Radiocarbon evidence indicates that migrants introduced farming to Britain," *Journal of archaeological science*, 37(4), pp. 866–870.

Cooper, A. *et al.* (1992) "Independent origins of New Zealand moas and kiwis," *Proceedings of the National Academy of Sciences of the United States of America*, 89(18), pp. 8741–8744.

Damgaard, P. de B. *et al.* (2018) "137 ancient human genomes from across the Eurasian steppes," *Nature*, 557(7705), pp. 369–374.

Deagle, B. E., Paige Eveson, J. and Jarman, S. N. (2006) "Quantification of damage in DNA recovered from highly degraded samples – a case study on DNA in faeces," *Frontiers in Zoology*. doi: 10.1186/1742-9994-3-11.

Delai, D. *et al.* (2015) "Florid Cemento-osseous Dysplasia: A Case of Misdiagnosis," *Journal of endodontia*, 41(11), pp. 1923–1926.

Der Sarkissian, C. *et al.* (2015) "Ancient genomics," *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 370(1660), p. 20130387.

Djinis, E. (2021) "Last meal of sacrificial bog body was surprisingly unsurprising, researchers say," *National Geographic*, 20 July. Available at: https://www. nationalgeographic.com/history/article/tollund-mans-last-meal (Accessed: April 20, 2022).

Dolfini, A. (2020) "From the Neolithic to the Bronze Age in Central Italy: Settlement, Burial, and Social Change at the Dawn of Metal Production," *Journal of Archaeological Research*, 28(4), pp. 503–556.

Donoghue, H. D. *et al.* (2005) "Co-infection of Mycobacterium tuberculosis and Myco-bacterium leprae in human archaeological samples: a possible explanation for the historical decline of leprosy," *Proceedings. Biological sciences / The Royal Society*, 272(1561), pp. 389–394.

Douka, K. *et al.* (2012) "A new chronostratigraphic framework for the Upper Palaeo-lithic of Riparo Mochi (Italy)," *Journal of human evolution*, 62(2), pp. 286–299.

Enk, J. *et al.* (2011) "Complete Columbian mammoth mitogenome suggests inter-breeding with woolly mammoths," *Genome biology*, 12(5), p. R51.

Epp, L. S., Zimmermann, H. H. and Stoof-Leichsenring, K. R. (2019) "Sampling and Extraction of Ancient DNA from Sediments," in Shapiro, B. et al. (eds.) *Ancient DNA: Methods and Protocols*. New York, NY: Springer New York, pp. 31–44.

Evin, A. *et al.* (2015) "Unravelling the complexity of domestication: a case study using morphometrics and ancient DNA analyses of archaeological pigs from Romania," *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 370(1660), p. 20130616.

Feldman, M. *et al.* (2019) "Late Pleistocene human genome suggests a local origin for the first farmers of central Anatolia," *Nature communications*, 10(1), p. 1218.

Fernandes, D. M. *et al.* (2018) "A genomic Neolithic time transect of hunter-farmer admixture in central Poland," *Scientific reports*, 8(1), p. 14879.

Fernandes, D. M. *et al.* (2020) "The spread of steppe and Iranian-related ancestry in the islands of the western Mediterranean," *Nature ecology & evolution*. doi: 10.1038/s41559-020-1102-0.

Fogg, M. J., Pearl, L. H. and Connolly, B. A. (2002) "Structural basis for uracil recogni-tion by archaeal family B DNA polymerases," *Nature structural biology*, 9(12), pp. 922–927.

Fort, J. (2015) "Demic and cultural diffusion propagated the Neolithic transition across different regions of Europe," *Journal of the Royal Society, Interface / the Royal Society*, 12(106). doi: 10.1098/rsif.2015.0166.

Fowler, C. *et al.* (2022) "A high-resolution picture of kinship practices in an Early Neolithic tomb," *Nature*, 601(7894), pp. 584–587.

Fregel, R. *et al.* (2018) "Ancient genomes from North Africa evidence prehistoric migrations to the Maghreb from both the Levant and Europe," *Proceedings of the National Academy of Sciences of the United States of America*, 115(26), pp. 6774–6779.

Freilich, S. *et al.* (2021) "Reconstructing genetic histories and social organisation in Neolithic and Bronze Age Croatia," *Scientific reports*, 11(1), p. 16729.

Fu, Q. *et al.* (2014) "Genome sequence of a 45,000-year-old modern human from western Siberia," *Nature*, 514(7523), pp. 445–449.

Fu, Q. *et al.* (2015) "An early modern human from Romania with a recent Neanderthal ancestor," *Nature*, 524(7564), pp. 216–219.

Fu, Q. *et al.* (2016) "The genetic history of Ice Age Europe," *Nature*, 534(7606), pp. 200–205.

Gamba, C. *et al.* (2014) "Genome flux and stasis in a five millennium transect of European prehistory," *Nature communications*, 5, p. 5257.

Gamble, C. *et al.* (2004) "Climate change and evolving human diversity in Europe during the last glacial," *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 359(1442), pp. 243–53; discussion 253-4.

Ginolhac, A. *et al.* (2011) "mapDamage: testing for damage patterns in ancient DNA sequences," *Bioinformatics* , 27(15), pp. 2153–2155.

Gnirke, A. *et al.* (2009) "Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing," *Nature biotechnology*, 27(2), pp. 182–189.

Goldberg, A. *et al.* (2017) "Ancient X chromosomes reveal contrasting sex bias in Neolithic and Bronze Age Eurasian migrations," *Proceedings of the National Academy of Sciences of the United States of America*, 114(10), pp. 2657–2662.

González-Fortes, G. *et al.* (2017) "Paleogenomic Evidence for Multi-generational Mixing between Neolithic Farmers and Mesolithic Hunter-Gatherers in the Lower Danube Basin," *Current biology: CB*, 27(12), pp. 1801-1810.e10.

Green, R. E. *et al.* (2010) "A draft sequence of the Neandertal genome," *Science*, 328(5979), pp. 710–722.

Greenwood, A. D. *et al.* (2001) "A molecular phylogeny of two extinct sloths," *Molecular phylogenetics and evolution*, 18(1), pp. 94–103.

Günther, T. *et al.* (2015) "Ancient genomes link early farmers from Atapuerca in Spain to modern-day Basques," *Proceedings of the National Academy of Sciences of the United States of America*, 112(38), pp. 11917–11922.

Günther, T. *et al.* (2018) "Population genomics of Mesolithic Scandinavia: Investigating early postglacial migration routes and high-latitude adaptation," *PLoS biology*, 16(1), p. e2003703.

Haak, W. *et al.* (2005) "Ancient DNA from the first European farmers in 7500-year-old Neolithic sites," *Science*, 310(5750), pp. 1016–1018.

Haak, W. *et al.* (2008) "Ancient DNA, Strontium isotopes, and osteological analyses shed light on social and kinship organization of the Later Stone Age," *Proceedings of the National Academy of Sciences of the United States of America*, 105(47), pp. 18226–18231.

Haak, W. *et al.* (2010) "Ancient DNA from European early neolithic farmers reveals their near eastern affinities," *PLoS biology*, 8(11), p. e1000536.

Haak, W. *et al.* (2015) "Massive migration from the steppe was a source for Indo-European languages in Europe," *Nature*, 522(7555), pp. 207–211.

Hadly, E. A. *et al.* (1998) "A genetic record of population isolation in pocket gophers during Holocene climatic change," *Proceedings of the National Academy of Sciences of the United States of America*, 95(12), pp. 6893–6896.

Hagelberg, E., Sykes, B. and Hedges, R. (1989) "Ancient bone DNA amplified," *Nature*, 342(6249), p. 485.

Hajdinjak, M. *et al.* (2021) "Initial Upper Palaeolithic humans in Europe had recent Neanderthal ancestry," *Nature*, 592(7853), pp. 253–257.

Hansen, H. B. *et al.* (2017) "Comparing Ancient DNA Preservation in Petrous Bone and Tooth Cementum," *PLoS one*, 12(1), p. e0170940.

Harkins, K. M. *et al.* (2015) "Screening ancient tuberculosis with qPCR: challenges and opportunities," *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 370(1660), p. 20130622.

Harney, É. *et al.* (2018) "Ancient DNA from Chalcolithic Israel reveals the role of population mixture in cultural transformation," *Nature communications*, 9(1), p. 3336.

Harney, É. *et al.* (2021) "Assessing the performance of qpAdm: a statistical tool for studying population admixture," *Genetics*, 217(4). doi: 10.1093/genetics/iyaa045.

Hervella, M. *et al.* (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(4), p. e34417.

Hervella, M. *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(6), p. e0128810.

Higuchi, R. *et al.* (1984) "DNA sequences from the quagga, an extinct member of the horse family," *Nature*, 312(5991), pp. 282–284.

Hodos, T. (2020) *The Archaeology of the Mediterranean Iron Age: A Globalising World c.1100–600 BCE*. Cambridge University Press.

Hofmanová, Z. *et al.* (2016) "Early farmers from across Europe directly descended from Neolithic Aegeans," *Proceedings of the National Academy of Sciences of the United States of America*, 113(25), pp. 6886–6891.

Hofreiter, M. *et al.* (2015) "The future of ancient DNA: Technical advances and conceptual shifts," *BioEssays: news and reviews in molecular, cellular and developmental biology*, 37(3), pp. 284–293.

Höss, M., Jaruga, P., *et al.* (1996) "DNA damage and DNA sequence retrieval from ancient tissues," *Nucleic acids research*, 24(7), pp. 1304–1307.

Höss, M., Dilling, A., *et al.* (1996) "Molecular phylogeny of the extinct ground sloth Mylodon darwinii," *Proceedings of the National Academy of Sciences of the United States of America*, 93(1), pp. 181–185.

Hublin, J.-J. *et al.* (2020) "Initial Upper Palaeolithic Homo sapiens from Bacho Kiro Cave, Bulgaria," *Nature*, 581(7808), pp. 299–302.

Hugo Reyes-Centeno, K. H. (2021) *Ancient Connections in Eurasia*, *Kerns Verlag Tübingen*. Available at: https://kernsverlag.com/en/book/ancient-connections-in-eurasia/ (Accessed: April 19, 2022).

Ingman, T. *et al.* (2021) "Human mobility at Tell Atchana (Alalakh), Hatay, Turkey during the 2nd millennium BC: Integration of isotopic and genomic evidence," *PloS one*, 16(6), p. e0241883.

Jones, E. R. *et al.* (2015) "Upper Palaeolithic genomes reveal deep roots of modern Eurasians," *Nature communications*, 6, p. 8912.

Jones, E. R. *et al.* (2017) "The Neolithic Transition in the Baltic Was Not Driven by Admixture with Early European Farmers," *Current biology: CB*, 27(4), pp. 576–582.

Jónsson, H. *et al.* (2013) "mapDamage2.0: fast approximate Bayesian estimates of ancient DNA damage parameters," *Bioinformatics* , 29(13), pp. 1682–1684.

Joseph, T. A. and Pe'er, I. (2018) "Inference of Population Structure from Ancient DNA," in *Research in Computational Molecular Biology*. Springer International Publishing, pp. 90–104.

Joseph, T. A. and Pe'er, I. (2019) "Inference of Population Structure from Time-Series Genotype Data," *American journal of human genetics*, 105(2), pp. 317–333.

Joshua Levine, P. by C. A. (2017) *Europe's famed bog bodies are starting to reveal their secrets*, *Smithsonian Magazine*. Available at: https://www.smithsonianmag.com/science-nature/europe-bog-bodies-reveal-secrets-180962770/ (Accessed: April 20, 2022).

Keller, A. *et al.* (2012) "New insights into the Tyrolean Iceman's origin and phenotype as inferred by whole-genome sequencing," *Nature communications*, 3, p. 698.

Keller, M. (2019) *Ancient Yersinia pestis genomes from across Western Europe reveal early diversification during the First Pandemic (541–750)*, *PNAS*. Available at: https://www.pnas.org/doi/full/10.1073/pnas.1820447116 (Accessed: April 19, 2022).

Keyser, C. *et al.* (2009) "Ancient DNA provides new insights into the history of south Siberian Kurgan people," *Human genetics*, 126(3), pp. 395–410.

Kivisild, T. (2015) "Maternal ancestry and population history from whole mitochondrial genomes," *Investigative genetics*, 6, p. 3.

Kivisild, T. (2017) "The study of human Y chromosome variation through ancient DNA," *Human genetics*, 136(5), pp. 529–546.

Kleppe, K. *et al.* (1971) "Studies on polynucleotides: XCVI. Repair replication of short synthetic DNA's as catalyzed by DNA polymerases," *Journal of molecular biology*, 56(2), pp. 341–361.

Knipper, C. *et al.* (2017) "Female exogamy and gene pool diversification at the transition from the Final Neolithic to the Early Bronze Age in central Europe," *Proceedings of the National Academy of Sciences of the United States of America*, 114(38), pp. 10083–10088.

Köhler, K. *et al.* (2017) "Possible cases of leprosy from the Late Copper Age (3780-3650 cal BC) in Hungary," *PloS one*, 12(10), p. e0185966.

Krause, J. *et al.* (2010) "The complete mitochondrial DNA genome of an unknown hominin from southern Siberia," *Nature*, 464(7290), pp. 894–897.

Krings, M. *et al.* (1997) "Neandertal DNA sequences and the origin of modern humans," *Cell*, 90(1), pp. 19–30.

de La Fuente, C., Flores, S. and Moraga, M. (2013) "DNA FROM HUMAN ANCIENT BACTERIA: A NOVEL SOURCE OF GENETIC EVIDENCE FROM ARCHAEOLOGICAL DENTAL CALCULUS," *Archaeometry*. Wiley, 55(4), pp. 767–778.

Lacan, M., Keyser, C., Ricaut, F.-X., Brucato, N., Duranthon, F., *et al.* (2011) "Ancient DNA reveals male diffusion through the Neolithic Mediterranean route," *Proceedings of the National Academy of Sciences of the United States of America*, 108(24), pp. 9788–9791.

Lacan, M., Keyser, C., Ricaut, F.-X., Brucato, N., Tarrús, J., *et al.* (2011) "Ancient DNA suggests the leading role played by men in the Neolithic dissemination," *Proceedings of the National Academy of Sciences of the United States of America*, 108(45), pp. 18255–18259.

Lawson, D. J. *et al.* (2012) "Inference of population structure using dense haplotype data," *PLoS genetics*, 8(1), p. e1002453.

Lazaridis, I. *et al.* (2014) "Ancient human genomes suggest three ancestral populations for present-day Europeans," *Nature*, 513(7518), pp. 409–413.

Lazaridis, I. *et al.* (2016) "Genomic insights into the origin of farming in the ancient Near East," *Nature*, 536(7617), pp. 419–424.

Lazaridis, I. *et al.* (2017) "Genetic origins of the Minoans and Mycenaeans," *Nature*, 548(7666), pp. 214–218.

Leonard, J. A., Wayne, R. K. and Cooper, A. (2000) "Population genetics of ice age brown bears," *Proceedings of the National Academy of Sciences of the United States of America*, 97(4), pp. 1651–1654.

Lindahl, T. (1993) "Instability and decay of the primary structure of DNA," *Nature*, 362(6422), pp. 709–715.

Linderholm, A. (2021) "Palaeogenetics: Dirt, what is it good for? Everything," *Current biology: CB*, pp. R993–R995.

Lipson, M. *et al.* (2013) "Efficient moment-based inference of admixture parameters and sources of gene flow," *Molecular biology and evolution*, 30(8), pp. 1788–1802.

Lipson, M. *et al.* (2017) "Parallel palaeogenomic transects reveal complex genetic history of early European farmers," *Nature*, 551(7680), pp. 368–372.

Ludwig, A. *et al.* (2015) "Twenty-five thousand years of fluctuating selection on leopard complex spotting and congenital night blindness in horses," *Philosophical*

transactions of the Royal Society of London. Series B, Biological sciences, 370(1660), p. 20130386.

Malmström, H. *et al.* (2009) "Ancient DNA reveals lack of continuity between neolithic hunter-gatherers and contemporary Scandinavians," *Current biology: CB*, 19(20), pp. 1758–1762.

Mangerud, J. *et al.* (2004) "Ice-dammed lakes and rerouting of the drainage of northern Eurasia during the Last Glaciation," *Quaternary science reviews*, 23(11), pp. 1313–1332.

Marcus, J. H. *et al.* (2020) "Genetic history from the Middle Neolithic to present on the Mediterranean island of Sardinia," *Nature communications*, 11(1), p. 939.

Margaryan, A. *et al.* (2018) "Ancient pathogen DNA in human teeth and petrous bones," *Ecology and evolution*, 8(6), pp. 3534–3542.

Marota, I. *et al.* (2002) "DNA decay rate in papyri and human remains from Egyptian archaeological sites," *American journal of physical anthropology*, 117(4), pp. 310–318.

Martiniano, R. *et al.* (2017) "The population genomics of archaeological transition in west Iberia: Investigation of ancient substructure using imputation and haplotype-based methods," *PLoS genetics*, 13(7), p. e1006852.

Martins, H. *et al.* (2015) "Radiocarbon dating the beginning of the Neolithic in Iberia," *Journal of Mediterranean archaeology*. Equinox Publishing, 28(1), pp. 105–131.

Mathieson, I. *et al.* (2015) "Genome-wide patterns of selection in 230 ancient Eurasians," *Nature*, 528(7583), pp. 499–503.

Mathieson, I. *et al.* (2018) "The genomic history of southeastern Europe," *Nature*, 555(7695), pp. 197–203.

Meyer, M. and Kircher, M. (2010) "Illumina Sequencing Library Preparation for Highly Multiplexed Target Capture and Sequencing," *Cold Spring Harbor Protocols*, p. db.prot5448-pdb.prot5448. doi: 10.1101/pdb.prot5448.

Miller, W. *et al.* (2012) "Polar and brown bear genomes reveal ancient admixture and demographic footprints of past climate change," *Proceedings of the National Academy of Sciences of the United States of America*, 109(36), pp. E2382-90.

Mittnik, A. *et al.* (2018) "The genetic prehistory of the Baltic Sea region," *Nature communications*, 9(1), p. 442.

Mittnik, A. *et al.* (2019) "Kinship-based social inequality in Bronze Age Europe," *Science*, 366(6466), pp. 731–734.

Modi, A. *et al.* (2021) "More data on ancient human mitogenome variability in Italy: new mitochondrial genome sequences from three Upper Palaeolithic burials," *Annals of human biology*, 48(3), pp. 213–222.

Molinaro, L. *et al.* (2019) "West Asian sources of the Eurasian component in Ethiopians: a reassessment," *Scientific reports*, 9(1), p. 18811.

Monroy Kuhn, J. M., Jakobsson, M. and Günther, T. (2018) "Estimating genetic kin relationships in prehistoric populations," *PLoS one*, 13(4), p. e0195491.

Mullis, K. B. (1990) "The Unusual Origin of the Polymerase Chain Reaction," *Scientific American*, 4 January. doi: 10.1038/scientificamerican0490-56.

Murchie, T. J., Monteath, A. J., *et al.* (2021) "Collapse of the mammoth-steppe in central Yukon as revealed by ancient environmental DNA," *Nature communications*, 12(1), p. 7120.

Murchie, T. J., Kuch, M., *et al.* (2021) "Optimizing extraction and targeted capture of ancient environmental DNA for reconstructing past environments using the

PalaeoChip Arctic-1.0 bait-set," *Quaternary Research*. Cambridge University Press, 99, pp. 305–328.

Murchie, T. J. *et al.* (2022) "Pleistocene mitogenomes reconstructed from the environmental DNA of permafrost sediments," *Current biology: CB*, 32(4), pp. 851-860.e7.

Narasimhan, V. M. *et al.* (2019) "The formation of human populations in South and Central Asia," *Science*, 365(6457). doi: 10.1126/science.aat7487.

Nei, M. and Kumar, S. (2000) *Molecular Evolution and Phylogenetics*. Oxford University Press.

Nishino, T. and Morikawa, K. (2002) "Structure and function of nucleases in DNA repair: shape, grip and blade of the DNA scissors," *Oncogene*, 21(58), pp. 9022–9032.

Noonan, J. P. *et al.* (2005) "Genomic sequencing of Pleistocene cave bears," *Science*, 309(5734), pp. 597–599.

Noro, M. *et al.* (1998) "Molecular phylogenetic inference of the woolly mammoth Mammuthus primigenius, based on complete sequences of mitochondrial cytochrome b and 12S ribosomal RNA genes," *Journal of molecular evolution*, 46(3), pp. 314–326.

Novembre, J. *et al.* (2008) "Genes mirror geography within Europe," *Nature*, 456(7218), pp. 98–101.

Olalde, I. *et al.* (2014) "Derived immune and ancestral pigmentation alleles in a 7,000-year-old Mesolithic European," *Nature*, 507(7491), pp. 225–228.

Olalde, I. *et al.* (2015) "A Common Genetic Origin for Early Farmers from Mediterranean Cardial and Central European LBK Cultures," *Molecular biology and evolution*, 32(12), pp. 3132–3142.

Olalde, I. *et al.* (2018) "The Beaker phenomenon and the genomic transformation of northwest Europe," *Nature*, 555(7695), pp. 190–196.

Olalde, I. *et al.* (2019) "The genomic history of the Iberian Peninsula over the past 8000 years," *Science*, 363(6432), pp. 1230–1234.

Orlando, L. *et al.* (2013) "Recalibrating Equus evolution using the genome sequence of an early Middle Pleistocene horse," *Nature*, 499(7456), pp. 74–78.

Ottoni, C. *et al.* (2021) "Tracking the transition to agriculture in Southern Europe through ancient DNA analysis of dental calculus," *Proceedings of the National Academy of Sciences of the United States of America*, 118(32). doi: 10.1073/pnas.2102116118.

Pääbo, S. (1984) "Über den Nachweis von DNA in altägyptischen Mumien," *Das Altertum*, 30, pp. 213–218.

Pääbo, S. (1985) "Preservation of DNA in ancient Egyptian mummies," *Journal of archaeological science*, 12(6), pp. 411–417.

Pääbo, S. (1989) "Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification," *Proceedings of the National Academy of Sciences of the United States of America*, 86(6), pp. 1939–1943.

Pääbo, S. (2008) "Imagine: An interview with Svante Pääbo. Interview by Jane gitschier," *PLoS genetics*. Public Library of Science (PLoS), 4(3), p. e1000035.

Pääbo, S. (2014) *Neanderthal Man: In Search of Lost Genomes*. Hachette UK.

Pääbo, S., Gifford, J. A. and Wilson, A. C. (1988) "Mitochondrial DNA sequences from a 7000-year old brain," *Nucleic acids research*, 16(20), pp. 9775–9787.

Papac, L. *et al.* (2021) "Dynamic changes in genomic and social structures in third millennium BCE central Europe," *Science advances*, 7(35). doi: 10.1126/sciadv.abi6941.

Parker, C. *et al.* (2020) "A systematic investigation of human DNA preservation in medieval skeletons," *Scientific reports*, 10(1), p. 18225.

Patterson, N. *et al.* (2012) "Ancient admixture in human history," *Genetics*, 192(3), pp. 1065–1093.

Patterson, N. *et al.* (2022) "Large-scale migration into Britain during the Middle to Late Bronze Age," *Nature*, 601(7894), pp. 588–594.

Patterson, N., Price, A. L. and Reich, D. (2006) "Population structure and eigenanalysis," *PLoS genetics*, 2(12), p. e190.

Peter, B. M. (2016) "Admixture, Population Structure, and F-Statistics," *Genetics*, 202(4), pp. 1485–1501.

Pinhasi, R. *et al.* (2015) "Optimal Ancient DNA Yields from the Inner Ear Part of the Human Petrous Bone," *PloS one*, 10(6), p. e0129102.

Poinar, H. N. *et al.* (1998) "Molecular coproscopy: dung and diet of the extinct ground sloth Nothrotheriops shastensis," *Science*, 281(5375), pp. 402–406.

Poinar, H. N. *et al.* (2006) "Metagenomics to paleogenomics: large-scale sequencing of mammoth DNA," *Science*, 311(5759), pp. 392–394.

Poinar, H. N. and Cooper, A. (2000) "Ancient DNA: do it right or not at all," *Science*, 5482(1139), p. 416.

Porčić, M. *et al.* (2020) "The timing and tempo of the Neolithic expansion across the Central Balkans in the light of the new radiocarbon evidence," *Journal of Archaeological Science: Reports*, 33, p. 102528.

Porčić, M. *et al.* (2021) "The Neolithic Demographic Transition in the Central Balkans: population dynamics reconstruction based on new radiocarbon evidence," *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 376(1816), p. 20190712.

Posth, C. *et al.* (2016) "Pleistocene Mitochondrial Genomes Suggest a Single Major Dispersal of Non-Africans and a Late Glacial Population Turnover in Europe," *Current biology: CB*, 26(6), pp. 827–833.

Posth, C. *et al.* (2021) "The origin and legacy of the Etruscans through a 2000-year archeogenomic time transect," *Science advances*, 7(39), p. eabi7673.

Pritchard, J. K., Stephens, M. and Donnelly, P. (2000) "Inference of population structure using multilocus genotype data," *Genetics*, 155(2), pp. 945–959.

Prüfer, K. *et al.* (2014) "The complete genome sequence of a Neanderthal from the Altai Mountains," *Nature*, 505(7481), pp. 43–49.

Prüfer, K. *et al.* (2021) "A genome sequence from a modern human skull over 45,000 years old from Zlatý kůň in Czechia," *Nature ecology & evolution*, 5(6), pp. 820–825.

Pusch, C. M., Broghammer, M. and Scholz, M. (2000) "Cremation practices and the survival of ancient DNA: burnt bone analyses via RAPD-mediated PCR," *Anthropologischer Anzeiger; Bericht uber die biologisch-anthropologische Literatur*, 58(3), pp. 237–251.

Racimo, F. *et al.* (2020) "Beyond broad strokes: sociocultural insights from the study of ancient genomes," *Nature reviews. Genetics*, 21(6), pp. 355–366.

Rasmussen, M. *et al.* (2010) "Ancient human genome sequence of an extinct Palaeo-Eskimo," *Nature*, 463(7282), pp. 757–762.

Raveane, A. *et al.* (2019) "Population structure of modern-day Italians reveals patterns of ancient and archaic ancestries in Southern Europe," *Science advances*, 5(9), p. eaaw3492.

Rivollat, M. *et al.* (2017) "Investigating mitochondrial DNA relationships in Neolithic Western Europe through serial coalescent simulations," *European journal of human genetics: EJHG*, 25(3), pp. 388–392.

Rivollat, M. *et al.* (2020) "Ancient genome-wide DNA from France highlights the complexity of interactions between Mesolithic hunter-gatherers and Neolithic farmers," *Science advances*, 6(22), p. eaaz5344.

Rohland, N. *et al.* (2018) "Extraction of highly degraded DNA from ancient bones, teeth and sediments for high-throughput sequencing," *Nature protocols*, 13(11), pp. 2447–2461.

Rohland, N. *et al.* (2022) "Three Reagents for in-Solution Enrichment of Ancient Human DNA at More than a Million SNPs," *bioRxiv*. doi: 10.1101/2022.01.13.476259.

Rohland, N. and Hofreiter, M. (2007) "Ancient DNA extraction from bones and teeth," *Nature protocols*, 2(7), pp. 1756–1762.

Rollo, F. *et al.* (1988) "Short but faithful pieces of ancient DNA," *Nature*, 335(6193), p. 774.

Rootsi, S. *et al.* (2004) "Phylogeography of Y-chromosome haplogroup I reveals distinct domains of prehistoric gene flow in europe," *American journal of human genetics*, 75(1), pp. 128–137.

Saag, Lehti *et al.* (2017) "Extensive Farming in Estonia Started through a Sex-Biased Migration from the Steppe," *Current biology: CB*, 27(14), pp. 2185-2193.e6.

Saag, Lehti *et al.* (2019) "The Arrival of Siberian Ancestry Connecting the Eastern Baltic to Uralic Speakers further East," *Current biology: CB*, 29(10), pp. 1701-1711.e16.

Sallustio, F. *et al.* (2015) "Genome-wide scan identifies a copy number variable region at 3p21.1 that influences the TLR9 expression levels in IgA nephropathy patients," *European journal of human genetics: EJHG*, 23(7), pp. 940–948.

Sampietro, M. L. *et al.* (2007) "Palaeogenetic evidence supports a dual model of Neolithic spreading into Europe," *Proceedings. Biological sciences / The Royal Society*, 274(1622), pp. 2161–2167.

Sarhan, M. S. *et al.* (2021) "Ancient DNA diffuses from human bones to cave stones," *iScience*, 24(12), p. 103397.

Saupe, T. *et al.* (2021) "Ancient genomes reveal structural shifts after the arrival of Steppe-related ancestry in the Italian Peninsula," *Current biology: CB*. doi: 10.1016/j.cub.2021.04.022.

Sawafuji, R. *et al.* (2020) "Ancient DNA analysis of food remains in human dental calculus from the Edo period, Japan," *PloS one*, 15(3), p. e0226654.

Schiffels, S. *et al.* (2016) "Iron Age and Anglo-Saxon genomes from East England reveal British migration history," *Nature communications*, 7, p. 10408.

Schroeder, H. *et al.* (2019) "Unraveling ancestry, kinship, and violence in a Late Neolithic mass grave," *Proceedings of the National Academy of Sciences of the United States of America*, 116(22), pp. 10705–10710.

Schuenemann, V. J. *et al.* (2017) "Ancient Egyptian mummy genomes suggest an increase of Sub-Saharan African ancestry in post-Roman periods," *Nature communications*, 8, p. 15694.

Scorrano, G. *et al.* (2021) "Genomic ancestry, diet and microbiomes of Upper Palaeolithic hunter-gatherers from San Teodoro cave (Sicily, Italy)," *bioRxiv*. doi: 10.1101/2021.12.08.471745.

Seguin-Orlando, A. *et al.* (2014) "Paleogenomics. Genomic structure in Europeans dating back at least 36,200 years," *Science*, 346(6213), pp. 1113–1118.

Shennan, S. (2017) "The Revolution in Studies of the Neolithic Transition in the West Mediterranean," in García-Puchol, O. and Salazar-García, D. C. (eds.) *Times of Neolithic Transition along the Western Mediterranean*. Cham: Springer International Publishing, pp. 399–408.

Shizuya, H. *et al.* (1992) "Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in Escherichia coli using an F-factor-based vector," *Proceedings of the National Academy of Sciences of the United States of America*, 89(18), pp. 8794–8797.

Shizuya, H. and Kouros-Mehr, H. (2001) "The development and applications of the bacterial artificial chromosome cloning system," *The Keio journal of medicine*, 50(1), pp. 26–30.

Sineo, L. *et al.* (2015) "Human peopling of Sicily during quaternary." iris.unipa.it. Available at: https://iris.unipa.it/handle/10447/147632.

Sirak, K. *et al.* (2020) "Corrigendum: Human auditory ossicles as an alternative optimal source of ancient DNA," *Genome research*, 30(6), p. 938_1-938_1.

Sjögren, K.-G. *et al.* (2020) "Kinship and social organization in Copper Age Europe. A cross-disciplinary analysis of archaeology, DNA, isotopes, and anthropology from two Bell Beaker cemeteries," *PloS one*, 15(11), p. e0241278.

Skoglund, P. *et al.* (2012) "Origins and genetic legacy of Neolithic farmers and hunter-gatherers in Europe," *Science*, 336(6080), pp. 466–469.

Skoglund, P. *et al.* (2014) "Genomic diversity and admixture differs for Stone-Age Scandinavian foragers and farmers," *Science*, 344(6185), pp. 747–750.

Skoglund, P. *et al.* (2017) "Reconstructing Prehistoric African Population Structure," *Cell*, 171(1), pp. 59-71.e21.

Szécsényi-Nagy, A. *et al.* (2015) "Tracing the genetic origin of Europe's first farmers reveals insights into their social organization," *Proceedings. Biological sciences / The Royal Society*, 282(1805). doi: 10.1098/rspb.2015.0339.

Szécsényi-Nagy, A. *et al.* (2017) "The maternal genetic make-up of the Iberian Peninsula between the Neolithic and the Early Bronze Age," *Scientific reports*, 7(1), p. 15644.

Tang, H. *et al.* (2005) "Estimation of individual admixture: analytical and study design considerations," *Genetic epidemiology*, 28(4), pp. 289–301.

Tassi, F. *et al.* (2017) "Genome diversity in the Neolithic Globular Amphorae culture and the spread of Indo-European languages," *Proceedings. Biological sciences / The Royal Society*, 284(1867). doi: 10.1098/rspb.2017.1540.

The Nobel Prize in chemistry 1993 (1990) *Nobelprize.org*. Available at: https://www.nobelprize.org/prizes/chemistry/1993/press-release/ (Accessed: April 18, 2022).

Thomas, M. G. *et al.* (2000) "Molecular and morphological evidence on the phylogeny of the Elephantidae," *Proceedings. Biological sciences / The Royal Society*, 267(1461), pp. 2493–2500.

Thomas, R. H. *et al.* (1989) "DNA phylogeny of the extinct marsupial wolf," *Nature*, 340(6233), pp. 465–467.

Thomas, W. K. *et al.* (1990) "Spatial and temporal continuity of kangaroo rat populations shown by sequencing mitochondrial DNA from museum specimens," *Journal of molecular evolution*, 31(2), pp. 101–112.

Underhill, P. A. *et al.* (2015) "The phylogenetic and geographic structure of Y-chromosome haplogroup R1a," *European journal of human genetics: EJHG*, 23(1), pp. 124–131.

Valdiosera, C. *et al.* (2018) "Four millennia of Iberian biomolecular prehistory illustrate the impact of prehistoric migrations at the far end of Eurasia," *Proceedings of the National Academy of Sciences of the United States of America*, 115(13), pp. 3428–3433.

van der Valk, T. *et al.* (2021) "Million-year-old DNA sheds light on the genomic history of mammoths," *Nature*, 591(7849), pp. 265–269.

Villalba-Mouco, V. *et al.* (2019) "Survival of Late Pleistocene Hunter-Gatherer Ancestry in the Iberian Peninsula," *Current biology: CB*, 29(7), pp. 1169-1177.e7.

Villalba-Mouco, V. *et al.* (2021) "Genomic transformation and social organization during the Copper Age-Bronze Age transition in southern Iberia," *Science advances*, 7(47), p. eabi7038.

Voldstad, L. H. *et al.* (2020) "A complete Holocene lake sediment ancient DNA record reveals long-standing high Arctic plant diversity hotspot in northern Svalbard," *Quaternary science reviews*, 234, p. 106207.

Ward, R. and Stringer, C. (1997) "A molecular handle on the Neanderthals," *Nature*, 388(6639), pp. 225–226.

Warinner, C., Speller, C. and Collins, M. J. (2015) "A new era in palaeomicrobiology: prospects for ancient dental calculus as a long-term record of the human oral microbiome," *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 370(1660), p. 20130376.

Weyrich, L. S., Dobney, K. and Cooper, A. (2015) "Ancient DNA analysis of dental calculus," *Journal of human evolution*, 79, pp. 119–124.

Yang, H., Golenberg, E. M. and Shoshani, J. (1996) "Phylogenetic resolution within the Elephantidae using fossil DNA sequence from the American mastodon (Mammut americanum) as an outgroup," *Proceedings of the National Academy of Sciences of the United States of America*, 93(3), pp. 1190–1194.

Žegarac, A. *et al.* (2021) "Ancient genomes provide insights into family structure and the heredity of social status in the early Bronze Age of southeastern Europe," *Scientific reports*, 11(1), p. 10072.

Zilhão, J. (2014) "Early prehistoric navigation in the Western Mediterranean: Implications for the Neolithic transition in Iberia and the Maghreb," *Eurasian Prehistory. Island Archaeology and the Origins of Seafaring in the Eastern Mediterranean*, 11(1–2), pp. 185–200.

Zink, A. and Nerlich, A. G. (2003) "News and Comments: Molecular Analyses of the" Pharaos:" Feasibility of Molecular Studies in Ancient Egyptian Material," *American journal of physical anthropology*. JOHN WILEY & SONS LTD, 121(2), pp. 109–111.

Zink, A. R. and Nerlich, A. G. (2005) "Long-term survival of ancient DNA in Egypt: Reply to Gilbert et al," *American journal of physical anthropology*. Wiley, 128(1), pp. 115–118.

ACKNOWLEDGEMENTS

Thanks to Andrew for sending me pictures of his cats and the country which stole my heart forever – Scotland. You always knew when to send me the pictures!

The last thanks and the most countable thanks of all goes to my parents and it has to be in German: Ich danke euch für immer und ewig. Ohne euch wäre ich niemals in der Lage gewesen diesen Schritt zu gehen und dabei noch so erfolgreich zu sein. Ohne euch hätte ich niemals die Welt mit anderen Augen gesehen. Ich danke euch für die guten Urlaube in den Alpen und das ihr die Launen meiner Natur akzeptiert habt. Ich danke euch für eure Hilfe und euer Wissen und eure Liebe. Ich bin so stolz euch meine Eltern zu nennen. Wir sind wie Gilmore Girls!

<div align="right">Unicorns don't judge!</div>

PUBLICATIONS

CURRICULUM VITAE

Name: Tina Saupe
Date of Birth: June 5, 1991
Place of Birth: Leipzig, Germany
Contacts: University of Tartu, Institute of Genomics,
Riia 23b, 51010 Tartu, Estonia
E-mail: tina.saupe@ut.ee

Education:

2018–2022 Doctoral studies Gene Technology, University of Tartu, Faculty of Science and Technology, Institute of Molecular and Cell Biology, Chair of Evolutionary Biology

2016–2018 *MSc*, Johannes Gutenberg University of Mainz, Faculty of Biology, supervisors Joachim Burger and Valentina Coia (EURAC Research, Institute of Mummy Studies, Bolzano, Italy), "Genetic analyses of human remains from valleys in South Tyrol dated to Early Middle Age"

2012–2016 *BSc*, University of Applied Sciences Mittweida, Faculty of Applied Computer Science and Biology, Department of Biology, supervisors Röbbe Wünschiers and Stephan Schiffels (Max Planck Institute for Evolutionary Anthropology, Department of Archaeogenetics, Leipzig, Germany), "Genetic sex determination from low coverage sequencing of ancient human DNA"

2014–2015 *BSc*, University of the West of Scotland, School of Health and Life Sciences, Lanarkshire, UK

Professional employment:

2018– University of Tartu, Faculty of Science and Technology, Institute of Genomics, Junior Researcher

Membership and administrative work:

2021– Member of the European Association of Archaeologists

2021–2022 Member of the Student Parliament at the University of Tartu, University of Tartu, Estonia

2021–2022 Member of the Faculty Council of the Faculty of Science and Technology, University of Tartu, Estonia

2020–2021 Member of the Institute Council of the Institute of Molecular and Cell Biology, University of Tartu, Estonia

Publications:

Aneli S, **Saupe T**, Montinaro F, Solnik A, Molinaro L, Scaggion C, Carrara N, Raveane A, Kivisild T, Metspalu M, Scheib CL, Pagani L, **The genetic origin of Daunians and the Pan-Mediterranean southern Italian Iron Age context**, *Molecular Biology and Evolution*, 2022, msac014, https://doi.org/10.1093/molbev/msac014.

Aneli, S., Caldon, M., **Saupe, T.** et al. **Through 40,000 years of human presence in Southern Europe: the Italian case study**. *Hum Genet* 140, 1417–1431 (2021). https://doi.org/10.1007/s00439-021-02328-6.

Saupe T, Montinaro F, Scaggion C, Carrara N, Kivisild T, D'Atanasio E, Hui R, Solnik A, Lebrasseur O, Larson G, Alessandri L, Arienzo I, De Angelis F, Rolfo MF, Skeates R, Silvestri L, Beckett J, Talamo S, Dolfini A, Miari M, Metspalu M, Benazzi S, Capelli C, Pagani L, Scheib CL. 2021. **Ancient genomes reveal structural shifts after the arrival of Steppe-related ancestry in the Italian Peninsula.** *Curr Biol.* 2021 Jun 21;31(12):2576–2591.e12.

Bortolini E, Pagani L, Oxilia G, Posth C, Fontana F, Badino F, **Saupe T**, Montinaro F, Margaritora D, Romandini M, Lugli F, Papini A, Boggioni M, Perrini N, Oxilia A, Cigliano RA, Barcelona R, Visentin D, Fasser N, Arrighi S, Figus C, Marciani G, Silvestrini S, Bernardini F, Menghi Sartorio JC, Fiorenza L, Cecchi JM, Tuniz C, Kivisild T, Gianfrancesco F, Peresani M, Scheib CL, Talamo S, D'Esposito M, Benazzi S. 2021. **Early Alpine occupation backdates westward human migration in Late Glacial Europe.** *Curr Biol.* 2021 Jun 7;31(11):2484–2493.e7.

Haber M, Nassar J, Almarri MA, **Saupe T**, Saag L, Griffith SJ, Doumet-Serhal C, Chanteau J, Saghieh-Beydoun M, Xue Y, Scheib CL, Tyler-Smith C. 2020. **A Genetic History of the Near East from an aDNA Time Course Sampling Eight Points in the Past 4,000 Years**. *Am J Hum Genet.* 2020 Jul 2;107(1):149–157.

ELULOOKIRJELDUS

Nimi: Tina Saupe
Sünniaeg: 5. juuni 1991
Sünnikoht: Leipzig, Saksamaa
Kontakt: Tartu Ülikool, genoomika instituut,
Riia 23b, 51010 Tartu, Eesti
E-post: tina.saupe@ut.ee

Hariduskäik:

2018–2022 Doktorantuur, Tartu Ülikool, loodus- ja täppisteaduste valdkond, molekulaar- ja rakubioloogia instituut (geenitehnoloogia)

2016–2018 Magistrikraad, Mainzi Johannes Gutenbergi Ülikool, bioloogia valdkond, juhendajad Joachim Burger ja Valentina Coia (EURAC Teadusuuringud, Muumia Uuringute instituut, Bolzano, Itaalia), "Genetic analyses of human remains from valleys in South Tyrol dated to Early Middle Age"

2012–2016 Bakalaureusekraad, Mittweida rakenduskõrgkool, rakendusliku arvutiteaduse ja bioloogia teaduskond, bioloogia osakond, juhendajad Röbbe Wünschiers ja Stephan Schiffels (Max Plancki Evolutsioonilise Antropoloogia Instituut, arheogeneetika osakond, Leipzig, Saksamaa), "Genetic sex determination from low coverage sequencing of ancient human DNA"

2014–2015 Bakalaureusekraad, Lääne-Šotimaa Ülikool, Tervise- ja bioteaduste kool, Lanarkshire, Suurbritannia

Töökogemus:

2018– Tartu ülikool, genoomika instituut, vana DNA nooremteadur

Teadusorganisatsiooniline ja -administratiivne tegevus

2021– Euroopa Arheoloogide Assotsiatsiooni liige
2021–2022 Tartu ülikooli üliõpilasesinduse nõukogu liige
2021–2022 Tartu ülikooli Loodus- ja täppisteaduste valdkond nõukogu liige
2020–2021 Tartu Ülikooli molekulaar- ja rakubioloogia instituut nõukogu liige

Teaduspublikatsioonid:

Loetletud inglisekeelse 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 micro-organisms 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: 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. **Krista Kaasik**. 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 intra-cellular trafficking, stability and endosomal escape during protein trans-duction. 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 zoo-plankton species under changing climate and eutrophication conditions. Tartu, 2011, 153 p.

203. **Egle Tammeleht**. Brown bear (*Ursus arctos*) population structure, demo-graphic 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 pseudo-uridine 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 boreo-nemoral 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 cellobiohydrolase. 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 relatedness, 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 geographic 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.

356. **Lehti Saag**. The prehistory of Estonia from a genetic perspective: new insights from ancient DNA. Tartu, 2019, 171 p.

357. **Mari-Liis Viljur**. Local and landscape effects on butterfly assemblages in managed forests. Tartu, 2019, 115 p.

358. **Ivan Kisly**. The pleiotropic functions of ribosomal proteins eL19 and eL24 in the budding yeast ribosome. Tartu, 2019, 170 p.

359. **Mikk Puustusmaa**. On the origin of papillomavirus proteins. Tartu, 2019, 152 p.

360. **Anneliis Peterson**. Benthic biodiversity in the north-eastern Baltic Sea: mapping methods, spatial patterns, and relations to environmental gradients. Tartu, 2019, 159 p.

361. **Erwan Pennarun**. Meandering along the mtDNA phylogeny; causerie and digression about what it can tell us about human migrations. Tartu, 2019, 162 p.

362. **Karin Ernits**. Levansucrase Lsc3 and endo-levanase BT1760: characterization and application for the synthesis of novel prebiotics. Tartu, 2019, 217 p.

363. **Sille Holm**. Comparative ecology of geometrid moths: in search of contrasts between a temperate and a tropical forest. Tartu, 2019, 135 p.

364. **Anne-Mai Ilumäe**. Genetic history of the Uralic-speaking peoples as seen through the paternal haplogroup N and autosomal variation of northern Eurasians. Tartu, 2019, 172 p.

365. **Anu Lepik**. Plant competitive behaviour: relationships with functional traits and soil processes. Tartu, 2019, 152 p.

366. **Kunter Tätte**. Towards an integrated view of escape decisions in birds under variable levels of predation risk. Tartu, 2020, 172 p.

367. **Kaarin Parts**. The impact of climate change on fine roots and root-associated microbial communities in birch and spruce forests. Tartu, 2020, 143 p.

368. **Viktorija Kukuškina**. Understanding the mechanisms of endometrial receptivity through integration of 'omics' data layers. Tartu, 2020, 169 p.

369. **Martti Vasar**. Developing a bioinformatics pipeline gDAT to analyse arbuscular mycorrhizal fungal communities using sequence data from different marker regions. Tartu, 2020, 193 p.

370. **Ott Kangur**. Nocturnal water relations and predawn water potential disequilibrium in temperate deciduous tree species. Tartu, 2020, 126 p.

371. **Helen Post**. Overview of the phylogeny and phylogeography of the Y-chromosomal haplogroup N in northern Eurasia and case studies of two linguistically exceptional populations of Europe – Hungarians and Kalmyks. Tartu, 2020, 143 p.

372. **Kristi Krebs**. Exploring the genetics of adverse events in pharmacotherapy using Biobanks and Electronic Health Records. Tartu, 2020, 151 p.

373. **Kärt Ukkivi**. Mutagenic effect of transcription and transcription-coupled repair factors in *Pseudomonas putida.* Tartu, 2020, 154 p.

374. **Elin Soomets**. Focal species in wetland restoration. Tartu, 2020, 137 p.

375. **Kadi Tilk**. Signals and responses of ColRS two-component system in *Pseudomonas putida.* Tartu, 2020, 133 p.

376. **Indrek Teino**. Studies on aryl hydrocarbon receptor in the mouse granulosa cell model. Tartu, 2020, 139 p.

377. **Maarja Vaikre**. The impact of forest drainage on macroinvertebrates and amphibians in small waterbodies and opportunities for cost-effective mitigation. Tartu, 2020, 132 p.

378. **Siim-Kaarel Sepp**. Soil eukaryotic community responses to land use and host identity. Tartu, 2020, 222 p.

379. **Eveli Otsing**. Tree species effects on fungal richness and community structure. Tartu, 2020, 152 p.

380. **Mari Pent**. Bacterial communities associated with fungal fruitbodies. Tartu, 2020, 144 p.

381. **Einar Kärgenberg**. Movement patterns of lithophilous migratory fish in free-flowing and fragmented rivers. Tartu, 2020, 167 p.

382. **Antti Matvere**. The studies on aryl hydrocarbon receptor in murine granulosa cells and human embryonic stem cells. Tartu, 2021, 163 p.

383. **Jhonny Capichoni Massante**. Phylogenetic structure of plant communities along environmental gradients: a macroecological and evolutionary approach. Tartu, 2021, 144 p.

384. **Ajai Kumar Pathak.** Delineating genetic ancestries of people of the Indus Valley, Parsis, Indian Jews and Tharu tribe. Tartu, 2021, 197 p.

385. **Tanel Vahter.** Arbuscular mycorrhizal fungal biodiversity for sustainable agroecosystems. Tartu, 2021, 191 p.

386. **Burak Yelmen.** Characterization of ancient Eurasian influences within modern human genomes. Tartu, 2021, 134 p.

387. **Linda Ongaro.** A genomic portrait of American populations. Tartu, 2021, 182 p.

388. **Kairi Raime.** The identification of plant DNA in metagenomic samples. Tartu, 2021, 108 p.

389. **Heli Einberg.** Non-linear and non-stationary relationships in the pelagic ecosystem of the Gulf of Riga (Baltic Sea). Tartu, 2021, 119 p.

390. **Mickaël Mathieu Pihain.** The evolutionary effect of phylogenetic neighbourhoods of trees on their resistance to herbivores and climatic stress. Tartu, 2022, 145 p.

391. **Annika Joy Meitern.** Impact of potassium ion content of xylem sap and of light conditions on the hydraulic properties of trees. Tartu, 2022, 132 p.

392. **Elise Joonas.** Evaluation of metal contaminant hazard on microalgae with environmentally relevant testing strategies. Tartu, 2022, 118 p.

393. **Kreete Lüll.** Investigating the relationships between human microbiome, host factors and female health. Tartu, 2022, 141 p.

394. **Triin Kaasiku.** A wader perspective to Boreal Baltic coastal grasslands: from habitat availability to breeding site selection and nest survival. Tartu, 2022, 141 p.

395. **Meeli Alber.** Impact of elevated atmospheric humidity on the structure of the water transport pathway in deciduous trees. Tartu, 2022, 170 p.

396. **Ludovica Molinaro.** Ancestry deconvolution of Estonian, European and Worldwide genomic layers: a human population genomics excavation. Tartu, 2022, 138 p.