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BAKALÁŘSKÁ PRÁCE

Comparison of mitochondrial haplogroups in Neolithic and modern populations  
from central Europe

Porovnání variability mitochondriálních haploskupin v neolitických a  
současných populacích ve střední Evropě

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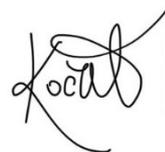
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Odevzdáním této bakalářské práce na téma Porovnání variability mitochondriálních haploskupin v neolitických a současných populacích ve střední Evropě potvrzuji, že jsem ji vypracovala pod vedením vedoucího práce samostatně za použití v práci uvedených pramenů a literatury. Dále potvrzuji, že tato práce nebyla využita k získání jiného nebo stejného titulu.



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## **ABSTRACT**

This work focuses on Neolithic period in connection to population genetics and Neolithic haplogroup frequencies in comparison to frequencies from contemporary populations. In order to gain needed archaeological and ancient cultural perspective, first part of this work focuses on Neolithic period itself. It firstly sets Neolithic period into chronological context and then describes its specificities. This work monitors the major changes occurring during Neolithic Revolution that significantly influenced later human development. The second part of this work focuses on mitochondria and mitochondrial DNA that plays significant role in population genetics. Thanks to two hypervariable regions in the non-coding section on mitochondrial genome, genetic mutation in mitochondria are formed in high frequency. Each specific type of mutation can be assigned to an existing group with similar mutations. These groups are called haplogroups. Because mitochondrial DNA – haplogroup is inherited only through maternal lineage, geneticists are able to track our genetic grandmothers back into the past several generations. Thanks to haplogroups, evolution of the entire populations can be monitored and studied. This work monitors haplogroup frequencies in Neolithic populations in central European region. Next, it monitors haplogroup frequencies in contemporary populations in central European region and at the end, the work compares these two frequencies and presents results, on how have the haplogroup frequencies changed through time from Neolithic up to the modern populations.

## **KEY WORDS**

Neolithic period, culture, mitochondrial DNA, haplogroups, central Europe

## **ABSTRAKT**

Táto práca se zaměřuje na období Neolitu ve spojení s populační genetikou a na frekvenci haploskupin v současných populacích a v populacích z období neolitu na území střední Evropy. Za účelem získání ucelené perspektivy na toto téma, první část práce se zaměřuje na samotné období Neolitu. Práce představuje Neolit v kontextu doby a upřesňuje jeho specifika. Dále práce opisuje podstatní období historie a to je Neolitická revoluce, která zásadně ovlivnila další lidský vývoj. Druhá část práce se zaměřuje na mitochondrie a na mitochondriální DNA, které hraje klíčovou roli v populační genetice. Díky dvěma hypervariabilním sekvencím na nekódující části mitochondriálního genomu, mitochondrie produkují vysoké množství mutací. Každá specifická mutace může být přiřazena do určité skupiny, která zahrnuje všechny mutace podobného typu. Tyto skupiny jsou nazývány haploskupiny. Díky tomu, že mitochondriální DNA – haploskupiny jsou děděny pouze po matce, nikoli po otci, odborníci jsou schopni vystopovat mateřské linie několik set generací do minulosti a sledovat tímto způsobem vývoj celých populací. Táto práca monitoruje frekvenci haploskupin v neolitických populacích na území střední Evropy. Dále práce monitoruje frekvenci haploskupin v současných populacích na území střední Evropy a nakonec práce porovnává frekvence haploskupin těchto dvou období. Tímto způsobem práce podává výsledky analýzy, která popisuje jak se haploskupiny měnili od období neolitu až po současnost.

## **KLÍČOVÁ SLOVA**

Neolit, kultura, mitochondriální DNA, haploskupina, střední Evropa

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## INTRODUCTION

The question of our own origin arises at some point in our life and keeps our curiosity wondering. Who am I as part of my community and where did I come from? Who were my ancestors? What kind of life they had and where are laying the deepest branches of my genetic roots?

The need to record and document accompanies people since early ages. Creativity, I believe, is in the core of human nature. The creative spirit captivated by everyday life drove man to create beautiful cave paintings, capture events, stories and legends onto the early canvases enriched by mesmerising decorative calligraphy. It is only to our benefit, that we now may look back on the historical remains and find answers to our questions.

Historical resources offer a great opportunity to explore human origin. But what if we want to dive deeper? I was driven by curiosity to gain a deeper understanding of my ancestor's life and how cultures and people evolved through time. In order to discover the answers to these questions, we need to turn inward. It is in ourselves that we carry the great story of our origin, written in miniscule sign of nucleic acid in each and every one of our body cell. Single part of our DNA can direct us to the right way leading to discover our personal ancestors. DNA in mitochondria tells even deeper and greater story of origin and inheritance. Thanks to the fact that mitochondrial DNA is inherited exclusively from mother to her descendants, we can trace our female grandmothers back thousands of years and track the movement and recombination of various populations. It can be done by retracting mitochondrial DNA from archaeological samples. When enough data is collected, extensive Phylogenetic maps can be created indicating origin of different cultures in different regions and their progress though time.

My work is divided into two main sections which are both contributing to produce an overall perspective on the main subject. The first section is focused on profiling Neolithic period in its cultural and historical context. The main focus of this section is to lay a foundation and summarise a current state of the knowledge, which will bring better perspective on ancient periods in connection to population genetics studied in following chapters. The second part of the work focuses on mitochondrial genome, its specifiers and types in relation to Neolithic cultures and Neolithic haplogroup distribution.

## EXPECTATIONS

Mitochondria are organelles inside human cells which play a significant role in population evolution studies. Due to that fact that mitochondrial DNA is inherited only in maternal lineage, geneticists are able to track the evolution and development of populations far back to the past. The frequency of a specific type of haplogroup among population in a given region gives us an information about the most frequent haplotypes present in people's genome. There is a significant time gap of several millennia between Neolithic period and the present and thus, mitochondrial haplogroups experienced different conditions and events that may have influenced their form.

The ability to mutate is the key characteristic of human DNA. It does not only bring the negative consequences in form of diseases but it holds the great advantage of evolution. Mitochondria genome has very high mutation rate that occurs in the non-coding hypervariable region of mitochondrial DNA. Kivisild (2015) states that the estimated mutation rate of mitochondrial genome is 10x higher than mutation rate of nuclear DNA. These mutations determine into which haplogroup certain genome will belong to. Kivisild explains, that this mutation phenomenon creates perfect conditions for studying population evolution. Due to the rapid genomic alterations, many generation can be studied in approximately short period of time. The mutation rate on hypervariable region determine how fast will each mutation change its form resulting in formation of new haplotypes. The mitochondrial mutation rate directly influences the haplogroup frequency in each population by creating new haplotypes and changing the form of existing ones.

Mutation rate is not the only attribute influencing haplogroup frequencies. Guha *et al.* (2013) links the haplogroup frequency in population with human migration. Authors state that due to the fact that each type of haplogroup is linked to specific geographical region, human migration represents an influential attribute that changes the genetic distribution in each region. Migration is one of the causes of genetic variability in each region because it enables the incorporation of new types of genes into younger generations.

As was stated above, mutation rate and human migration influence formation of haplogroups. Therefore, I expect the Neolithic haplogroup frequencies to be different from the haplogroup distribution in contemporary population of Europe.

Mentioned scientist and experts support the fact that mitochondrial genome undergoes constant evolutionary changes and so, haplogroups necessarily undergo constant developmental changes too.

In the practical part of this work I will try to answer the following question:

Research question: Is there a difference in mitochondrial haplogroup frequencies in the central European region from the Neolithic period in comparison to the present day?

H: Haplogroups frequencies in Neolithic populations are different than haplogroups frequencies among contemporary populations in central Europe.

## **MAIN GOALS**

Firstly, my goal for this work is to review the current state of knowledge. As science widened its field of expertise in the last few decades, massive amounts of data were collected from various fields. More data from anthropological samples were extracted, scientists deepened their understanding of the importance and uniqueness of mitochondrial DNA, and historians in scientific research were able to predict and describe possible layout of Neolithic populations and their progress.

Firstly, I will look closely on the Neolithic period. I will review occurring changes that happened during Neolithic Revolution such as domestication, migration of populations in the European region and changes in lifestyle of early Neolithic cultures, all of which influenced contemporary modern populations. I will characterise the cultures typical for Neolithic period in central European region.

Secondly, I will look closely on mitochondria itself. My goal will be to dive into understanding of the mitochondrial genome, its specificity and its role in population genetics. In order to gain better perspective on the main problematic it will be crucial to understand the principle behind haplotypes and haplogroups.

Thirdly, I will collect available data of mtDNA from published research articles from Neolithic period and the present day from central European region. Based on the collected data I will do haplogroup frequency comparison for both periods in order to find answer for my primary hypothesis. Next, I will compare the haplogroup frequencies from the two different time periods and find out, how did the frequencies changed through time. From the collected data I will be able to track, if and how population changed over the millennia by comparing the frequencies from these two periods.

# 1 NEOLITHIC PERIOD IN CENTRAL EUROPE

To gain an overall picture of the main issue of this work, we need to look closely on what preceded and what followed this time period and understand Neolithic period itself. It is important to understand the overall lifestyle and life challenges of our ancestors to better comprehend where certain types of haplogroups originated.

## 1.1 Late Mesolithic period

The very beginning of Neolithic transformation can be dated into the Late Mesolithic era, where cultural progress started to emerge. Crombé and Robinson (2014) link the beginning of a Late Mesolithic Era with continuous climate changes that positively influenced future population's evolution, when favourable conditions for agriculture started to form. According to these authors, Late Mesolithic can be roughly dated within Atlantic period between 9.000 and 6.000 years BC, when major part of Europe experienced higher temperatures and greater rainfalls than ever before.

This important change in environmental dynamics undoubtedly enabled the beginning process of major climate change, which resulted in vegetation and large bodied animal alternation. The increased rainfall played a significant role in softening and enriching the soil which was from the Late Palaeolithic period partially covered by glacier, and throughout the beginning of Mesolithic era still exposed to a cold temperature, which was, however, progressively increasing (Crombé & Robinson 2014). The major difference from a cultural point of view between Mesolithic era and the Neolithic is that humans progressed from hunting and gathering for subsistence to first forms of farming.

Svizzero and Tisdell (2014) offer a definition that is, in my opinion, very basic but accurate description of Mesolithic lifestyle:

*“Hunter-gatherers rely upon a mode of subsistence characterised by the absence of direct human control over the reproduction of exploited species, and little or no control over other aspects of population ecology such as the behaviour and distribution of food resources.”<sup>1</sup>*

As mentioned above, soil, climate and overall favourable environmental changes had significant impact on later human development. Hunter-gatherers (further on referred to as

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<sup>1</sup> SVIZZERO, S. & TISDELL, C. *Hunter-Gatherer societies: Their Diversity and Evolutionary Processes* [online] Work paper No. 197, Working papers on economics, ecology and the environment, Australia, The University of Queensland, 2014 [cited 30.12.2020 Bratislava]

HG) seized the surrounding environment mainly of fauna but also flora and used specialised tools, weapons and aids to hunt for animals and to process collected goods. This type of lifestyle required human migration which correlated with migration of large bodied animals that were seeking more suitable environmental habitat.

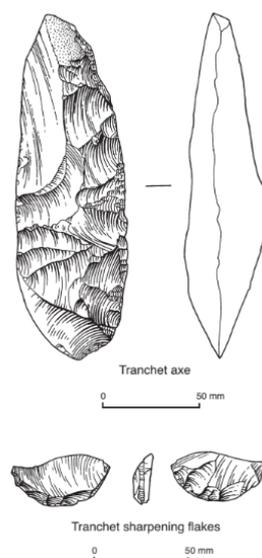
The method of obtaining food in Late Mesolithic differs according to location. Hunting for four legged wild animals with specially designed tools dominated in continental regions in addition to collecting greens harvested from the surroundings. Nevertheless, meat continued to be the main source of nourishment. As A. Whittle with his colleague V. Cummings mentioned at the conference at Cardiff University (2007), the landscape typology was known for post-glacial forests in which last groups of HG still coexisted with first farmers in European region. Post-glacial forests were moderately more spacious offering habitats where especially red deer and roe deer found perfect conditions for life, so naturally, they were also the most frequent pray of continental HG. Other additional elements of Late Mesolithic diet more in the Mediterranean zone were hazelnuts, pistachios, sloe, blackberry and additional mollusc collecting. Each Mesolithic group used different tools to obtain and process the collected food which differed according to a location. Naturally, different weapons were used in continental area and different ones in the coastal area.

The great importance of the sea in Mesolithic subsistence was confirmed in chemical analysis in carbon isotope content of human bone (Price 1987). Settlers in southern Europe living alongside coastlines were shown to consume at least 50% of marine food. Inhabitants in northern Europe (e.g. Great Britain) in later Mesolithic periods living also alongside coastlines were proven to consume at least 90% of marine diet. Price points out an interesting shift in human diet in later Mesolithic periods leading to Early Neolithic. What nicely represents the lifestyle change in Neolithic populations are results from mentioned Neolithic skeleton analysis showing a radical shift towards terrestrial recourses rather than to the marine ones. Similar changes pointing towards utilising fertile soils, trying to captivate animals and take care of them rather than kill them and use them instantly enabled people to start the process that we today determine as Neolithic Revolution, which was a definite step forward in a human evolution. Paul R. Preston (2008) states that the human response to these climate and environmental changes is reflected (besides diet) in changes in hunting technology and alterations in toolkits which are proven to exist during this transition period throughout archaeological evidence and palaeoenvironmentology.

Preston describes the change in shape of Mesolithic tools found in Great Britain. The habit in Early Mesolithic was to use a wider, broader and longer blades for processing any materials and goods. The usage of these specifically shaped tools was marked as “Long Blade Technology”. The example of tool transformation throughout Mesolithic can be shown on an example of approximately 12cm long and 5cm wide blade attached to a handle which served as an axe that can be seen in Fig 1, in comparison to the earlier weapon. The usage of wider tools in Mesolithic is also represented in Fig 1 as tranchet sharpening flakes with wider edge.

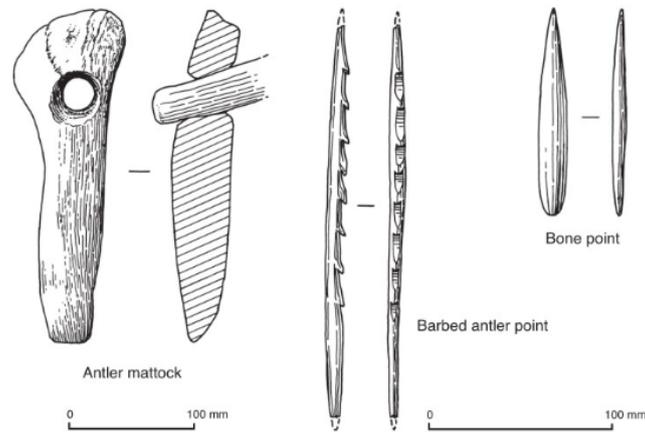
The diet in settlements, which were situated near coastlines and where the sea was the main source for subsistence, was naturally different from the diet in continental settlements. As a result, remains include more sea-related weapons and tools which suggest regular usage of hooks, nets and weaponry that were shaped to better process the marine animals. In contrast, HG in the continental regions used differently shaped and sharpened antlers and mattock made out of bones or stones to better process larger wild animals as can be seen in the Fig 2.

These instruments gradually transformed during Late Mesolithic-Early Neolithic from a wide, broad blade into narrow tools. The outburst of bone and antler work resulted in new styles of fish hooks, pins, awls and mattock heads as is shown in the Fig 2. All the modifications correlate with changes in the surrounding environment that required updated hunting techniques and tools.



*Fig. 1 the Long Blade Technology, Early Mesolithic, The Mesolithic Period, Preston, 2008*

*The Mesolithic Period*



*Fig. 2 Tools from Late Mesolithic Phase, The Mesolithic Period, Preston, 2008*

The significant lifestyle changes during Neolithic Revolution caused tools and weaponry alteration as HG changed their focus from collecting and hunting to mainly crop cultivation, farming and animal domestication. This transition enabled early farmers to take better control over their resources and became less dependent on migrating animals. As a result they could settle in certain locations for longer period of time. Domesticated animals later became a great source of power needed for maintaining soil and improving agriculture.

## 1.2 Neolithic Revolution

Different authors offer different theories on how and when the Neolithic period started, although the exact time cannot be precisely set. Even if the title “Neolithic Revolution” indicates rather abrupt turnover, the opposite is true. The transition from Mesolithic hunter-gathering into Neolithic farming happened gradually depending on location of various settlements and migration of tribes. During the process of neolithisation, different settlements reacted in their own specific way in their own time to social and technological adjustments.

Christian Violatti (2018) offers following explanation of the term “Neolithic Revolution” in the perspective of time:

*„The term Neolithic or New Stone Age is most frequently used in connection with agriculture, which is the time when cereal cultivation and animal domestication was introduced. Because agriculture developed at different times in different regions of the world, there is no single date for the beginning of the Neolithic. In the Near East, agriculture was developed around 9,000 BCE, in Southeast Europe around 7,000 BCE, and later in other regions. Even within a specific region, agriculture developed during different times.”<sup>2</sup>*

Since I’m focusing primarily on European region, I will focus on the main stream of neolithisation coming to Europe from Near East, Anatolia and Levant. Neolithic transition is up to this present day highly debated subject with numerous models and theories dealing with intracultural interactions, population genetic admixture, migration, adjustments and changes in standards of living. Neolithic revolution indeed started wave of transitions providing people with knowledge and skills for more independent lifestyle by practicing farming and agriculture, leaving the hunting and gathering to be mostly a matter of the past.

### 1.2.1 Fertile Crescent

The emerging agriculture and domestication made Fertile Crescent to be known as a Cradle of civilisation referring to its great importance in contribution to the human development. Civilisations thriving in the Fertile Crescent shaped and helped to form the character of many other cultures by providing the traits nowadays known from more sophisticated ancient communities. It is the region from where the Neolithic lifestyle spread further up north eventually reaching the region of central Europe. Fertile Crescent had a

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<sup>2</sup> VIOLATTI, C. *Neolithic Period Definition* [online] Ancient History Encyclopedia™, 2018, [cited 26.12.2020 Bratislava]

perfect location for both agriculture and animal domestication thanks to three great rivers Euphrates and Tigris and Nile River on the Egypt side that enriched the soil and provided bare necessities for life.

J. J. Mark (2018) describes Fertile Crescent as a territory that spreads throughout Middle-East passing through Persian Gulf, Syria, Iraq, Lebanon, Jordan, and Israel into the Northern Egypt which creates crescent-like shape through upper parts and around Saudi Arabia. An important part of the Fertile Crescent, which is well studied and from where many evidences were collected to confirm the transition from food gathering to farming, is a region called Levant, especially its sub region – southern Levant (Zeder 2011). (Levant is a part of ancient Fertile Crescent that spreads to the eastern Mediterranean covering today's Turkey, Syria and Lebanon (Van der Crabben 2011)). Another source of archaeological evidence originates in Anatolian region. (Anatolia - Portion of today's Turkey (Easton 2020)). This area confirms the link in development alongside the region of Fertile Crescent leaving behind evidence consisting of buildings and graves typical for Neolithic cultures - Aşıklı Höyük and Boncuklu Höyük (Düring 2013). Strategical geographical position of these two regions that connect Near East with Europe, enabled later Neolithic expansion to the north.

At the beginning of Neolithic period around the year 8.000 BC, Mesopotamia covered the majority of Fertile Crescent with Euphrates and Tigris being the dominant feature of the region. Some of the first complexed and sophisticated cultures emerged in this region. Around 9.500 BC people in Mesopotamia started to grow crops as an additional source of food but also as a way of attracting animals (Garland 2020). Dominant cultures of Mesopotamia such as Babylonians, Sumerians and later Assyrians, progressed rapidly in farming technologies, mastering the water management techniques, aerating and enriching the soil, crop harvesting and they also progressed in the cultural aspect gaining the deserved title as being the first complex communities (Janus 2017).

Looking more closely at the process of crop cultivation, it is problematic to provide an accurate data, but we can reliably predict the types of crops that played major role at the beginnings of agriculture. Crops such as lentil, pie, wheat, flax, barley belong to a group called “founder crops” that played key role in the cultivation process and were the first to be domesticated by our ancestors – these crops may have been domesticated separately in different regions in different time (Sadowski 2017).

Animal domestication followed the plant and crops domestication. M. A. Zeder (2011) believes that the time gap between the start of crop domestication and the start of animal

domestication wasn't that significant as was previously believed (approximately 1000 years), however, the crop domestication is believed to have started slightly sooner. It is only logical taking into consideration that cultivating crops is less challenging if population occupies fertile soils, which was the case of Mesopotamia, where people used the growing crops to attract animals. Another significant point was that many captivated animals needed the cultivated crops to enrich their diet because they were no longer able to migrate in searching for food. While crop and plant domestication took place in the southern Levant, according to Zeder's studies, animal domestication took place more into the north-east form Levant. Goats, sheep and gazelles were the most typical domesticated species in that region – goat preferring rocky and hilly environments, sheep preferably grazing in flatter and grassier valleys, while gazelles were not picky and fed on any easily-digested plants (Hole 1984).

Introduction of this particular lifestyle among Mesopotamian cultures is referred to as an agricultural sedentism (Garland 2020). Besides the practical conveniences of farming lifestyle that R. Garland describes, it was the sense of safety and security people felt after adapting to farming lifestyle that significantly changed their mind set. The constant migrating, worries concerning hunger, danger and instability in temporary settlements on top of climate fluctuations were no longer posing a threat. People were able to gain a sense of territoriality, stability and they were capable of managing their own food supplies, which brought them a great sense of comfort. They were able to shift their main focus from food obtaining to more noble activities that enriched the cultural life, such as art, music and literature but also science, mathematics and philosophy. It is no wonder that our ancestors symbolised the essence of this transition through replicas of heavily pregnant women symbolising fertility and newly gained wealth.

There lays the origin of the new Neolithic traits. It was only a matter of time, when these new lifestyle conveniences would be spread onward. The countries of Fertile Crescent and Levant were relatively close to the southern Europe and the existence of continental passage from northern Egypt into southern Europe was crucial. Because migration and colonisation is believed to be one of the key processes of neolithisation, the continental passages enabled early farmers to migrate into new and unknown territories.

### **1.2.2 From the Near East into the European region**

By further expansion of Neolithic traits, the new lifestyle made its way into the regions of today's Europe with Greece being the first country to be affected by neolithisation.

This process was accompanied by massive changes that took place around 7.000 BC, when first farmers settled in the homeland of HG (Perlès 2004).

I believe that this chapter is one of the most important one, because at this point we are discussing the first interaction between two communities that undertook entirely different development in distant environmental setting, whose habits, values and possibly religions were completely different. There are few models suggesting how did the Neolithic spread throughout the Near East and further into the central Europe. Some theories suppose that communities interacted, in which case the mixing of genomes is very probable, other theories believe that there was just form of theoretical diffusion of traits – without the personal interaction. I consider this topic very important for my further investigation of mtDNA and gene distribution. These models are nowadays considered out-dated, since newer representations have been introduced. Nevertheless, I will briefly introduce the essence of these models since they helped to shape an overall image of neolithisation in the academic community and they are currently applied as unified concept.

Experts discuss numerous possibilities which demonstrate the chronological and geographical background of the spread-out. Ron Pinhasi (2013) describes two opposing theoretical approaches that deal with Neolithic expansion.

First model predicts that process of neolithisation progressed further into the central Europe without migration of early farmers from Anatolia and there was hardly any interaction between the two different populations (cultural model). This model basically predicts that Mesolithic HG undertook some objects, goods and possibly domesticated animals from early farmers and they learned how to maintain them.

The second opposing model (demic model), that Pinhasi presents is process of neolithisation that was a result of an extend migration of early farmers from Anatolia into Greece and then further into the eastern and northern Europe. As early farmers were migrating they brought their domesticated pigs, cattle, sheep and goats along with cultivated plants and crops and tools that were new to Mesolithic HG and there was interaction between the two communities. Very interesting aspect is the linguistic side of this model, since the two different communities used different forms of communication, but this discussion is beyond the scope of this work.

To summarise these two models, migrationists believe that farmers migrated to a Mesolithic sites and they genetically completely replaced the traditional Mesolithic HG. In

contrast indigenists believe that there was no genetic replacement but rather admixture and they lay a great importance in people and ideas that caused the spread of Neolithic traits, but these two models cannot be applied separately (Galeta & Bruzek 2009).

Pinhasi provides unifying results of craniometrics studies clarifying the question of farmer's migration and confirms the personal interaction with HG.

*“An important source of information about past population movements is provided by statistical analysis of craniometric distances within and between late hunter-gatherer and early farming populations from the Near East, Anatolia, and Europe. For instance, there is a striking homogeneity in the skull morphology of Early Neolithic populations from central Anatolia, Greece, Bulgaria, Romania, Serbia, and Hungary. This homogeneity contrasts with the pronounced cranial heterogeneity of Pre-Pottery Neolithic groups in the Near East beyond Anatolia. The results imply continuous gene flow from central and western Anatolia into southeast and central Europe.”<sup>3</sup>*

As was mentioned, these two models are too homogenous and they strictly separate the two possible scenarios instead of unifying them. Vast majority of experts now views the process of neolithisation as a combination of both of these models while HG and farmers both worked actively on the process of farming transition and each region undertook its own specific model of adaptation involving signs of both demic and cultural model (Galeta & Bruzek 2014).

Neolithisation spread onward slowly and gradually. It altered various regions in Europe affecting existing communities and cultures and causing formation of new cultures as a result of migration and population growth. Neolithisation in Europe spread out in two main directions – upstream the Danube River (which I will focus on) crossing Romania, Serbia and Hungary up to the central Europe and the other way is alongside Mediterranean route covering the region of Italian, French and Spain's coastline up to Iberia (Rivollat *et al.* 2020).

These two main roads offered different life conditions in different environment, so cultures progressed and developed their lifestyle accordingly.

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<sup>3</sup> PINHASI, R. 2013, *20 Europe: Neolithic colonization* [online] Prehistory, Part II: Holocene Migrations The Encyclopedia of Global Human migration [cited 2.2.2021, Bratislava]

## 2 CULTURES IN NEOLITHIC PERIOD

I will focus on different cultures present in Early Neolithic that were the first ones practising the Neolithic lifestyle. In these cultures may still be present certain Mesolithic traits as a result of gradual transition. However, they are rather minor and they accompany and complete the big changes happening in Early Neolithic cultures.

European region is full of diversity and many cultures built their very first settlements alongside European rivers and began farming on the fertile lowlands and later on spread out further into the central Europe.

European region is rich in number of archaeological sites revealing variety of cultures. During the process of social development many new cultures and subcultures with various different branches were created leaving us little overwhelmed, because every region had its own pace of development with different outcomes. In order to fully describe the cultural representation of the whole Europe, I would need to dive deeply into too many cultures and their subcultures and that is not the main focus of my work, therefore I will focus strictly on the region of central Europe. It is important to know, where lays the origin of Neolithic cultures in central Europe, for that reason I will shortly mention the pre-developmental stage taking place in Balkan and later continuing up north.

## 2.1 Starčevo-Körös-Criș complex

Cultures from Near East including first farmers migrated gradually. In the span of several generations they slowly progressed further up north while settling down along the way. One of these cultures whose occupation region was in the middle between Near East and central Europe was Starčevo complex, that represents the cultures migrating from Near East that influenced the genetic background of today eastern Europe. Because these cultures represent important developmental stage of the Neolithic in central Europe and they influenced genetic haplogroup distribution in contemporary population, I will shortly characterise them.

The region of south and south-east Europe along Danube River and the region of Serbia play an important role in development of cultures migrating from Near East into the central Europe. These sites are best known for Starčevo-Körös-Criș complex.

*“This expansion reached the Carpathian Basin at the beginning of the 6th millennium BC by three branches: the Starčevo Culture in the southern part of Transdanubia on the right bank of the Danube, the Körös Culture in the Tisza valley in the Great Hungarian Plain, and the Criș Culture in Transylvania.”<sup>4</sup>*

Some historians like to consider these 3 cultures to be just 3 branches of one uniform culture (e.g. Astaloş *et al.* 2013). Since we can label the connection between these cultures as a complex, the regions of occupation for each culture may have intervened with one another.

### ***Starčevo culture***

Starčevo is a culture that has its origin in western Sarmia in Serbia, that later migrated and settled along the Drava River around 6000 BC (Botić 2016). Remains from one of the first Neolithic cultures that practised Neolithic lifestyle with few of the persisting HG lifestyle traits can be found in central Balkan as a part of Starčevo-Körös-Criș complex (Vitezović 2016). Selena Vitezović further characterises lifestyle of Starčevo culture as innovative because their main subsistence was provided from agriculture and farming. This culture nicely demonstrate the gradual transition towards Neolithic lifestyle because it well adapted to

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<sup>4</sup> MESTER, Z.& RÁCZ, B. 2010, *THE SPREAD OF THE KÖRÖS CULTURE AND THE RAW MATERIAL SOURCES IN THE NORTHEASTERN PART OF THE CARPATHIAN BASIN: A RESEARCH PROJECT* [online] Neolithization of the Carpathian Basin: Northernmost distribution of the Starčevo/Körös culture Kraków, the formation of Europe: prehistoric population dynamics and the roots of socio-cultural diversity, 2010, ISBN 978-83-7676-045-2 [cited 12.1.2021 Bratislava]

farming but still used some Mesolithic traits. With coming changes in lifestyle, settlements modified accordingly.

*„Settlements consisted of semi-dugout pit-dwellings and huts above ground made from wattle and daub. Their material culture brought in numerous innovations, the most important and most conspicuous being the introduction of clay technology: ceramics were used for daily and storage pots, everyday artefacts such as weights, but also figurines and other cult objects...“<sup>5</sup>*

In the northern parts of Starčevo region, the settlements reached the shores of Danube River. Starčevo culture expanded within Hungary more on the right side in Transdanubian region which is unusual choice for Early Neolithic farming settlement given the fact that this area is regularly being flooded, but after better geomorphological examination, this was the only place suitable for farming, because lower lands were covered with woods and unsuitable soil for cultivating crops (Bánffy 2010). Eszter Bánffy further connects the development of Starčevo culture with Körös culture, which occupied the Tisza valley in dense populated settlements.

The archaeological artefacts of Starčevo culture mainly consists of pottery, ceramics and fired clay. There is a great variety of types, colours, structure and composition of these samples. Some differ in colour from bright brown-yellowish colour all the way to darker grey, according to what type of material settlers used and the decoration or polish of the pottery also presents a great diversity from shiny coverage all the way to rough surfaces covered with grained sand (Regenye 2010).

György Szakmány and Elisabetta Starnini (2007) state, that pottery making was not necessary for livelihood of early Neolithic potters, but was rather household-scale industry that mainly adult women attended. On the other hand, Szakmány and Starnini claim that it is likely, that pottery was more created in dry season, right after the summer harvest and so can be associated and linked to agricultural events which indicates the main utilisation of pottery and ceramics.

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<sup>5</sup> VITEZOVIĆ, S. *Neolithisation of technology: innovation and tradition in the Starčevo culture osseous industry* [online] Documenta Praehistorica XLIII, vol 43, p. 123-138, 2016 [cited 26.2.2021 Bratislava]

Nevertheless, pottery relic are objects perfectly suitable for analysis. The decorations of pottery may be divided into groups according to a particular patterns occurring in different archaeological samples from different sites.

*„The Starčevo pottery assemblage appears typical. Sherds are organic-tempered, and red, light brown or greyish, with the signs of typical Early Neolithic low-temperature firing in their cross-sections.“<sup>6</sup>*

### ***Körös culture***

Körös culture can be dated to roughly almost the same time as the development of Starčevo culture with the years between 5.850 and 5.650 BC. The area of occupation was the basin of the Tisza River and the area alongside its shores, with especially significant region for early farmers - the Tokai region, which was rich in obsidian resources (Kaminská 2020).

Alasdair Whittle (2010) connects the development of Starčevo and Körös culture to early LBK cultures (Linear Pottery culture). Whittle describes the lifestyle of Körös culture in Transdanubian region, where they mainly lived in isolated and separated settlements and used the wide plains for cereal cultivating and sheep herding. This region was the source of many lithic materials such as mentioned obsidian, limnoquartzite and radiolite which was processed for creating pottery.

What was significant in Körös culture especially was the ceramics technology. Attila Kreiter (2010) was focusing in his research on petrographic analysis of Körös ceramic remains. He compares results from each examined Hungarian site and comes to a conclusion that Körös culture used mainly very fine and raw material (volcanic dust and sand) and organic temper for ceramic production. The fine texture of the fabric may be according to Kreiter a result of a volcanic activity in this region covering the ancient soil with fine volcanic dust. Since other specialist (e.g. Whittle 2010) state that this region was rich in obsidian, limnoquartzite and radiolite and other rocks and minerals, the composition of lithic component indicate the influence on the colour and structure of ceramics depending of region.

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<sup>6</sup> Oross, K. & Bánffy, E. & Osztás, A. *et al.* 2016, *The early days of Neolithic Alsónyék: The Starčevo occupation* [online] Bericht der Romisch-Germanischen Kommission, 94. 93-121, Henrich Editionen, ISBN 978-3-943407-70-9 [cited 1.3.2021 Bratislava]

## ***Criș culture***

Criș culture can be dated as the oldest branch of this complex with the years being as early as 6.300 BC. Since cultures migrated and set up settlements often near other cultures, we can now talk about complexes of cultures that inhabited the same region.

The location of Criș culture settlements is estimated in Transylvania region in Romania which later expanded further to the region in north-western Romania (e.g. Mester & Rácz 2010). In the north-eastern Carpathian basin of eastern Slovakia and Western Ukraine, remains were found from all three Körös, Criș and Starčevo culture which appoints to the regional cultural transmission (Kozłowski & Nowak 2010). This area is geomorphologically different from the region of Transdanubia, where the majority of Körös cultural remains were found. As an outcome, the settlement and farming technologies were different in hillier region.

Their pottery consisted of biconic vessels, bowls and jars with short necks. The typical Criș pottery is tempered with sand and silt which makes it rather soft pottery characteristic for Criș culture (Astaloş *et al.* 2013).

## 2.2. Early Neolithic period

As was already mentioned, exact date cannot be set to determine the beginning of early Neolithic period, but the beginning is generally estimated to the years around 10.000 BCE (Britannica, Neolithic, 2019). At this point in time, most of the cultures are well adapted to the new farming lifestyle.

### 2.2.1 Linear pottery culture - LBK

Linear pottery culture (LBK) is probably the first and biggest one to be practising farming lifestyle in continental central Europe in the early Neolithic. LBK culture is believed to have the starting point firstly as a one branch of Starčevo culture that later separated and created new subdivision (Oross & Bánffy2016). The period when LBK culture started to develop can be dated back to around the same time as Criş culture, back to 5600 BC. Oros and Bánffy also state that the first sites can be set into Hungarian Transdanubia, from where this culture spread through the entire central Europe also into the central Germany. Authors offer closer look at the transition of LBK settlements that changed their structure based on the changes of lifestyle. One major change was in choice of suitable habitat, when settlers built their houses on grassy plains to best care for the livestock and crops. Another change happened with the houses by themselves, when the walls and base of the house was constructed of wattle and daub creating timber framed structures. These houses were occupied by several generations.

The pottery technologies evolved and gain specific character with each culture. The ceramics were often decorated in order to give the clay a certain characteristics that made the pottery stronger and more efficient for storing goods. *“Even ancient potters knew that after their earthenware vessels had been fired, the clay walls remained slightly porous, allowing stored liquids to percolate slowly to the outside... A later technique for sealing earthen-ware involved the application of a glaze—a finely ground glass powder usually suspended in water. The glaze was painted, dipped, or sprayed on a fired pot, both inside and out. When the pot was fired a second time, the glaze would melt and form a fused, amorphous layer of glass, sealing the clay pores.”*<sup>7</sup>

The typical decorations on pottery were created by many technologies including burning and glazing. Choosing the right material which had specific characteristic when

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<sup>7</sup> ANDERSON, K.J. *Ceramic Glazes* [online] MRS Bulletin, volume 18, issue 12, p. 66, Cambridge University Press, 2013, [cited 3.3.2021 Bratislava]

burned and processed was also very important. Kevin J. Anderson (2013) explains the process of ancient pottery making and introduces applied techniques. One feature, and the most visually obvious one, is the colour of ceramics, which depends on the clay alone while being exposed to different conditions (e.g. the colour differs, when certain areas are covered in sand while burning – in that case, clay will turn out dark grey in contrast to a uncovered clay, which will turn out red). Other conditions that influenced the colour and properties of ancient pottery were ventilation, number of glaze layers, and properties of the wood which fuelled the fire.

The marks of cultural transition can be seen besides pottery also on gained habits and values which transformed the way of thinking of early farmers as can be seen in LBK cultures, mainly on the alteration of burial rituals, which changed as the LBK culture involved (Řídký *et al.* 2015). This change indicates also the shift in spirituality.

LBK culture expanded very rapidly from the central region of Hungary and Transdanubia to the west, up the Rhine River, but also to the north to central Poland and to the east into central Ukraine (Price *et al.* 2001). A research made focusing on Northern Germany site called Prenzlau revealed many unknown sites from LBK cultures that can be dated to a second half of 5<sup>th</sup> millennium, nearly one and a half millennium after the first expansion of Neolithic farmers migrating from the Near East (Benecke 2018). This research revealed that this site was well agriculturally developed with larger size cattle husbandry and goats, sheep and pigs as the second largest group of mammals providing subsistence.

## **2.3 Middle Neolithic in Europe**

The period around 4.580 - 3.950 BC can be classified as middle Neolithic. The most transformative changes from HG to farmers are a matter of past and middle Neolithic is a period of cultural enrichment, economic growth and social transformation and division in the sense of religion, social position and cultural specification. This middle period can be best enlightened through portrayal of authentic cultures.

### **2.3.1 Baden culture**

The first evidences of this culture in central Europe was discovered near Baden in eastern Austria to the south from Vienna (Wild *et al.* 2016). Baden culture developed in central Europe around 4.250-2.850 BC, which was the period when farmers already used wheeled vehicles to ease everyday life, which was an enormous step forward in agricultural, but also cultural development.

Baden culture has two main phases which further divide into smaller subgroups, the older one called Baden-Boleráz with typical pottery in all shades of brown with herringbone pattern. Later followed Baden-Classical period with typical ceramics being preferably dark coloured mugs with long and extended handles (Wild *et al.* 2016). The Baden cultural period can be divided according to typology of ceramics into subgroups, which can be demonstrated for instance on archaeological founding from Czech republic: a) the baden-boleráz period- the earliest stage –decorated pot with vertical groove decorations, with typical flat bottom; b) Baden-Classical period- the middle stage – ceramics with rounded and slightly pointed bottom, which can be further subdivided according to decorations (decorated surface– older stage; undecorated/modestly decorated surfaces – younger stage); c) Baden-Classical period – the youngest stage – ceramics with round and pointed bottom, with long and elevated handles above the top opening (Zápotocký 2008). This culture most probably emerged in central Europe (Lower Austria, Slovakia, Czech Republic, Moravia and western Hungary) and transmitted into the South-East (Horváth *et al.* 2008).

### **2.3.2 Funnelbeaker culture**

The dating of Funnelbeaker culture into the Neolithic chronology is dependent on each exact region, but it is generally estimated to thrive around the year 3300-2900 BC in Germany and further up north in Scandinavia (Kooijmans *et al.* 2018).

Similarly to other cultures, Funnelbeaker has its own unique type of pottery.

*“The main distinguishing trait for FBC is a pottery form called funnel beaker, a handle-less drinking vessel shaped like a funnel. These were hand-built from local clay and decorated with modeling, stamping, incising, and impressing.”*<sup>8</sup>

What is interesting about this culture is their burial rituals. They originally buried people in separate graves, but this habit changed and progressed towards communal burials – the habit was to burry one person, later reopen the grave and burry next person and so forth (Hirst 2019). Graves were supported by wooden poles which were later substituted by stones creating massive graves with pathways ending in chambers forming number of tombs that were discovered all around the region of funnel beaker culture.

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<sup>8</sup> HIRST, K.K. Funnel Beaker Culture: First Farmers of Scandinavia, Social Sciences, 2019 [cited 21.1.2021 Bratislava]

## 2.4 The Copper Age

Copper Age – also referred to as “Aeneolithic” or “Chalcolithic stage”- is a “pre-bronze” period, when copper was already manufactured and used, but bronze was not yet processed. The progression occurred during 3<sup>rd</sup> millennium BC and involved series of migration periods that led further into the central Europe and enabled further development of CWC and Beaker culture in central EU region (Sjögren *et al.* 2020). The link that Copper Age cultures share are the usage of similar materials and grave rituals that were very similar with similar artefacts left in graves during the burial ceremony. Agriculture and food production was greatly enriched by wheeled constructions easing the hard labour.

### 2.4.1 Corded Ware culture – CWC

In historical chronology Corded Ware culture is still dated in the late Neolithic but also overlaps with the next Bronze period, which can be dated between 3.000-2.500 BCE (the whole 3<sup>rd</sup> millennium according to some authors e.g. Linden 2007) where migration played significant role in redistribution of cultural traits (Kroon *et al.* 2019). The name “Corded Ware culture” is derived from the pottery decorations that were created by impressing cords in many ways leaving clay decorated with cord-like patterns.

Similarly to other cultures, CWC burying rituals had their significance. The principle used to bury people depended on the gender of dead person: “*In the Corded Ware complex, men and women are disposed according to a recurring pattern, men with their head to the west, lying on their right side and facing south and women with their head to the east, placed on their left side also facing south.*”<sup>9</sup> The author further explains that weapons and beakers were common objects found in men graves while women were equipped with various ornaments.

Graves were constructed for single-person burials, which naturally associate that person to a particular social group and express its individual importance rather than the social status he/she gained during life as a result of their achievements (Turek 2020). Turek states, that the distinct difference in the burial methods according to gender was probably the reflection of different social status of the person among family differing with women, men and children. That fact is supported by the objects found in graves alone that were often

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<sup>9</sup> LINDEN, M.V. *For Equalities Are Plural: Reassessing the Social in Europe during the Third Millennium BC* [online] *World Archaeology*, vol. 39, no. 2, pp. 177–193, 2007 [cited 23.1.2021 Bratislava]

typical for common responsibilities of each buried person depending on social role in family/community.

CWC is, as was mentioned above, known for its overlap to the Bronze Age, so bronze was naturally frequent material to be used for manufacturing weapons and other tools. Bronze daggers greatly influenced later development of weaponry in the beginning stages of Bronze Age (Salmon 2020), which I will discuss later.

### **2.4.2 Bell beaker culture**

Bell beaker culture developed during the second half of the 3<sup>rd</sup> millennium (2.750-2.000 BC (e.g. Allentoft 2015)) in central Europe and pose a transition stage between Neolithic and Bronze Age, and it seems to be greatly influenced by Corded Ware culture, (and possibly be the result of division from CWC), since some artefacts and traits from Corded Ware period persisted to the Bell beaker period (Besse 2004). Since Bell beaker culture developed in the second half of 3<sup>rd</sup> millennium and CWC is dated into the whole 3<sup>rd</sup> millennium, the chronological and geographical territories in central Europe have overlapped with these two cultures with bell beaker being greatly influenced by CWC, some experts however, still enforce the theory of separated development (Linden 2007). According to Marie Besser, Bell beaker cultural remains in central Europe can be found in Germany, Czech Republic – Moravia and Austria but also in Hungary and Slovakia.

Beaker culture gained its name after their bell-shaped beakers that were thoroughly decorated with great precision. People from this culture made a great effort to find and process copper, which is believed to have caused the spread of metallurgy across Europe and bronze became part of their everyday life from machinery to weaponry. (The Editors of Encyclopaedia 2011) Many researches were made in order to better understand division between many branches of Bell beaker culture and many experts now prefer the term “Bell beaker Phenomenon/Complex”, because the diversity of pottery (very often found as parts of remains from graves), weaponry and artefacts produced is too extent to call it a culture (Current Archaeology 2018).

There are certain doubts about how exactly Bell beaker culture spread so rapidly throughout Europe, whether it was a rapid migration or simply the result of interaction between cultures and passing on the traits. One study of ancient DNA (McNish 2018) confirms that Bell beaker cultural traits spread to central Europe from Iberia without

migration, which is supported by the fact that burial remains from Iberia are not genetically close to skeletons from central Europe.

## 2.5 The beginning of Early Bronze Age

The beginnings of Bronze Age differ with each location, but generally we can say that Aegean Bronze Age started approximately 3.200 B.C.E. with continental Europe slightly later around 2.200 BC and with the end around the years 800 BC (Rebay-Salisbury 2017).

The everyday lifestyle of Bronze Age people was very similar to late Neolithic populations with one bigger difference in technologies and preferable material being bronze for producing tools, artefacts and weapons, combined with other metals – for instance copper and tin. *“The majority of Bronze Age people lived in small-scale settlements and single farmsteads, practiced subsistence farming and animal husbandry, and crafted products like textiles and tools for use and exchange.”*<sup>10</sup>

Although this citation may paint the picture that Bronze Age did not included many life challenges, mentioned publication from Rebay-Salisbury describes the many difficulties that people needed to overcome, including wars and violence. This fact is supported by found artefacts (e.g. axes, swords, daggers etc.), that were objects first time in history created exclusively for combat. With the creation of combat weaponry, groups of specialised trained groups of men started forming. This is a very important turning point. Up to this point, there was no such thing as a combat weapon or any form of “army”, but with this progress, the whole social arrangement shifted. Suddenly, there is an individual/small group of individuals that are in charge of this combat group and thus have the power over other people that are not combat trained and so, they can press charges. The whole society experienced turnover in having leading groups of individuals – not in their own small family circle – but as a part of community and society. With that kind of leadership, the role of men and women in society changed so they served the authority. This historical period involves early forms of states with authority, social hierarchy and distinguished social roles according to sex (e.g. Furholt 2021).

Besides social alterations, which were accompanied by emerging of new religious ideologies with new mystical authorities, Bronze Age experienced very dramatic economic changes. After introducing agriculture, Bronze Age offer many technological transitions in means of new production practices with newly discovered materials (copper, bronze, etc.), which improved metallurgy and supported innovative ways of improving agriculture and food

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<sup>10</sup> REBAY-SALISBURY, K. *Bronze Age Beginnings: The Conceptualization of Motherhood in Prehistoric Europe* [online] Motherhood in Antiquity (chapter), p. 169-196, ISBN: 978-3-319-48901-8 [cited 2.3.2021 Bratislava]

production (wheeled vehicles) (Greenfield 2001). We can understand the social and economic changes in the Late Neolithic and Early Bronze Age as a way of social development towards what we know today as “medieval social hierarchy” with one authority, that had the power and support from some kind of aristocracy, army and often religion and ordinary people accepting the role of craftsman, farmer, soldier or worker if being a man, and role of mother, wife, maid or peasant if being a women.

### **3 MITOCHONDRIAL DNA**

The following chapters focus more on the genetics of us and our ancestors. Now, that I have characterised the ancient periods and how it affected the population evolution, it is necessary to understand the genetic heredity in order to gain understanding of how our ancestors influenced our own genomes and how cultures changed in the genetic aspects. In following chapters I will discuss mitochondrial DNA, haplotypes and haplogroups that play an important role in population genetics and that help us understand the ancient and modern population gene flow.

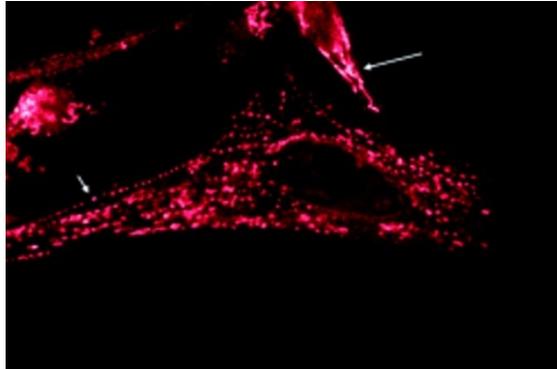
#### **3.1 Mitochondria**

Mitochondria are small organelles occupying cell's cytoplasm playing significant role in cell's metabolism and homeostasis. They are double membraned organelles of eukaryotic cell – outer membrane is smooth and penetrated by canals which are permeable especially for polar substances, in contrast to the inner larger membrane, not permeable for polar substances and is folded - creating mitochondria cristae. The inner space of mitochondria is filled with substance called matrix.

The number of mitochondria in one cell may differ according to a type of cell and location in organism, but generally there can be hundreds or even thousands of mitochondria in one cell when we study cells which demand a high energetic metabolism such as – muscle or kidney cell (Šmarda *et al.* 2013).

The main functions of mitochondria according to Šmarda, alongside with other metabolisms supporting homeostasis, are mainly to participate in breathing metabolism and energy cell metabolism and secondly to produce and store energy in form of ATP molecules produced by aerobic respiration.

*“Although some mitochondria probably do look like the traditional cigar shaped structures that appear in standard textbooks, it is more accurate to think of them as a budding and fusing network similar to the endoplasmic reticulum.”<sup>11</sup>*



*Fig. 3 Mitochondria, dyed mitochondria under microscope form chain like structures, Chinnery et al. (2003)*

*Human mitochondria. Scanning fluorescence confocal micrograph of a cultured human myoblasts stained with the Mitotracker, which is a fluorescent potentiometric dye taken up specifically by living mitochondria because of their membrane potential. Some mitochondria form discrete organelles (short arrow), but others form a reticulate network (long arrow).*

Mitochondria has its own DNA (mtDNA), which supports and suggest the theory, that mitochondria was originally an independent prokaryotic living organism which was later on absorbed by an eukaryotic cell during endocytosis. The fact that mitochondria may have subdued such process of endocytosis was originally suggested because of the existence of mtDNA (other organelles besides plastids do not possess their own DNA) and because of a double membrane that differ in structure and size. These facts suggest, that the outer membrane was created during endocytosis of the original prokaryotic cell and it is believed to have originated from cell's cytoplasmic membrane, which bended and completely bordered the larger inner membrane. That process caused the inner membrane to fold into cristae supporting the effectiveness of various metabolisms typical for eukaryotic cell. The benefits from mitochondrial presence in intracellular space must have been significant considering that host eukaryotic cell created a symbiotic bond with this organelle.

Mitochondria underwent its own process of development through time. Mitochondria as we know it today originally developed from pre-mitochondrial alphaproteobacterium going through few stages of development from independently living organism - to become an

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<sup>11</sup> CHINNERY P.F. & SCHON E.A. *Mitochondria* [online] Journal of Neurology, Neurosurgery & Psychiatry, 2003;74:1188-1199 [cited 24.2.2021 Bratislava]

endosymbiotic organelle of eukaryotic cell changing its form and increasing in diversity according to the type of cell that it eventually became part of (Roger *et al.* 2017). But why have eukaryotic cell created this symbiotic bond with mitochondria and what was the ultimate benefit of this form of relationship for the host cell? Mentioned paper from three scientists give answers to these questions:

*“Alternatively, the first endosymbiotic mitochondrial ancestor was suggested to have been a biochemically versatile facultatively aerobic photosynthetic bacterium. It would have been useful to its host by allowing it to either: move to aerobic niches, oxidize sulfide produced by host respiration, release hydrogen from fermentation, or by secreting organic photosynthate.”<sup>12</sup>*

The host cell got a significant advantages from this form of endosymbiotic relationship with mitochondria. But there are other theories that may have possibly happen, which suggest a completely different view on the relationship between mitochondria and eukaryotic cell.

The other possibility is that the pre-mitochondrial alphaproteobacterium was in fact a bacterial predator or parasite that invaded eukaryotic cell, which later resulted by many alteration in symbiotic state (Roger *et al.* 2017).

Besides the endocytic theories we can study the inheritance of mtDNA which can be used and analysed in order to gain more knowledge about ancient mixing of cultures. Because mitochondria is a living organelle it responds to the environment and other forces by changing and altering specific structural or functional attributes resulting in detectable alteration in its mtDNA. These changes can be detected and analysed providing genome profiles of certain area which can help to create a whole genetic overview of certain area, which will be mine main focus in practical part of this work.

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<sup>12</sup> ROGER, A. J. & MUÑOZ-GÓMEZ, S. A. & KAMIKAWA, R. *The Origin and Diversification of Mitochondria* [online] Current Biology, Volume 27, Issue 21, 2017 [cited 24.2.2021 Bratislava]

## 3.2 Mitochondrial genome

*“Human mtDNA is a circular double-stranded molecule with one strand (the heavy strand) relatively rich in G bases, and the other (the light strand) rich in C. tRNA genes are indicated by the three-letter name of the corresponding amino acid, remaining genes are protein coding, except for the two ribosomal RNA (rRNA) genes.”<sup>13</sup>*

Besides the coding regions of the genome there is a “control” region – also referred to as a D-loop - that is non-coding and it has very mutable nature allowing to create a great variability in particular mutation types (Sharma *et al* 2005). This region is crucial for mtDNA replication while it acts as a promotor for both the light and the heavy mtDNA strand and on top of that it carries essential elements for transcription and translation process (Sharma *et al.* 2005). Very important mentioned regions are the two hypervariable regions that are part of the D-loop. These sections are very prone to mutations. Based on which particular mutation will occur in these segments, we can link the specific mitochondrial genome with existing haplogroups, that each contain mtDNA that carry similar type of mutation in the hypervariable regions.

*“The 37 genes it (mitochondria, writer’s note) now carries all play roles in either the oxidative phosphorylation pathway, or mitochondrial protein synthesis. The other genes essential for mitochondrial function, including those encoding mitochondrial-specific DNA- and RNA polymerases and many structural and transport proteins, have been transferred to the nuclear genome.”<sup>14</sup>*

The two genomes that are responsible for the mitochondria synthesis are mtDNA itself alongside with nuclear DNA, whose proteins are translated and transported to the mitochondria by gene expression, they later combine with mitochondrial peptides which were originally created in mitochondrial matrix from mtDNA (Wei & Chinnery, 2020).

All of our somatic cells contain two copies of nuclear DNA coded in DNA double helix. mtDNA, however, is stored in mitochondria in thousands of copies (sometimes up to 100 000 copies – the number depends on type and function of the cell), and all of these copies are the exact same in a healthy individual (Chinnery & Schon, 2003). Authors further explain that this genetic supply is inherited always through the maternal path. Although sperm

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<sup>13</sup> JOBLING, M. *et al.* *Human evolutionary genetics*, 2<sup>nd</sup> edition, Garland Science, 2014, chapter 2, p. 36, Figure 2.21, ISBN: 978-0-8153-4148-2 [cited 18.3.2021 Bratislava]

<sup>14</sup> JOBLING, M. *et al.* *Human evolutionary genetics*, 2<sup>nd</sup> edition, Garland Science, 2014, chapter 2, p. 39, ISBN: 978-0-8153-4148-2 [cited 18.3.2021 Bratislava]

also contains mitochondria in the mid-piece of the sperm's "body" and so it necessarily contains also mtDNA (providing important energy for sperm movement), the mtDNA of the sperm does not enter the female ovum pass the zona pellucida, so only maternal mtDNA stays in the zygote and is passed onto the further generation.

Because mitochondria is responsible for many important processes and because there is not another set of genes from father to alternate any defected mitochondrial genes, the maternal inheritance must be done with the greatest caution and precision ensuing that only the healthy and strong mitochondria are passed on. The selective process of narrowing the selection of mitochondria must be very efficient. This process takes place before the process of fertilisation.

*"But before an oocyte is ready to pass on its mtDNA load to the offspring, it undergoes remarkable mitochondrial remodeling. The mtDNA bottleneck of the early female embryogenesis decreases the mtDNA copy number to <100 copies by an unknown mechanism, after which these mtDNAs are rapidly replicated to reach the hundreds of thousands of copies in the mature oocyte. The >1,000-fold increase of mtDNA copies means that a rare variant that survives the bottleneck can become the major mtDNA type in the mature egg and the offspring."*<sup>15</sup>

There are 3 important factors for which mitochondria is so important in studying population evolution (Jobling *et al.* 2014) with the first being that mitochondria, as was mentioned, has a high number of mtDNA. There may be even thousands of circular double stranded molecules within mitochondria, which gives us greater possibility to study and compare changes in different sections of genetic information. Secondly, mtDNA mutates very easily. Specialists suggest (e.g. Kivisild 2015) that the mutation rate in human mtDNA, especially in the control section of the circular DNA, is 10x higher than is normal in nuclear DNA, leaving great variability within a population. This factor is very beneficial for evolutionary geneticists, because the rapid changes can be detected and traced easily in relatively short period of time. The last fact is that mitochondrial DNA does not undergo the process of recombination during cell division, so we always inherit only the original maternal copy as it was, which is very easily detectable (Kivisild 2015).

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<sup>15</sup> SUOMALAINEN, A. *Mitochondrial DNA Inheritance in Humans: Mix, Match, and Survival of the Fittest*, *Cell Metabolism* [online] ScienceDirect, Volume 30, Issue 2, Pages 231-232, 2019 [cited 26.2.2021 Bratislava]

### 3.3 Mitochondrial haplotypes

Human genome is a collection of all nucleotide pairs in all DNA strands that make up the entire set of our chromosomes (e.g. Fridovich-Keil 2019). We are able to extract, study, identify and analyse the entire human genome, but what is more challenging is to track its evolution. For that purposes, we need to have a greater number of genome samples available. Studying genome of certain population and its development is a very complex and complicated research to do. In order to ease up the operation and to create a functioning system for determining certain branches of development, each group of genomes that have certain specific characteristics in their haplotypes are being put together in a sections called haplogroup.

*“In diploid organisms (such as humans) there are two (not completely identical) “copies” of each chromosome, and hence of each region of interest. A description of the data from a single copy is called a haplotype, while a description of the conflated (mixed) data on the two copies is called a genotype.”<sup>16</sup>*

When we look at a human genome, we will find two copies of DNA in our chromosomes, because humans are diploid organisms. Each strand of the chromosome carries the same genetic information. With a closer look on the nucleotides in double helix however, we can see some differences in the two strands at certain sections.

*“DNA genetic sequences vary considerably between individuals. These changes are collectively called DNA variants. Most DNA variants have little apparent functional significance, in which case they are known as DNA polymorphisms. By convention, a polymorphism is a difference in DNA sequence that occurs in  $\geq 1\%$  of the population.”<sup>17</sup>*

As was mentioned, if this change in DNA (on usually at least 2 alleles where occurred genetic mutation – base substitution (Jobling *et al.* 2014)) occurs among more than 1% in population, we talk about polymorphism. Certain changes can occur as polymorphism where we are usually dealing with alterations in more nucleotides at once. What is an important fact is that alteration can happen at the level of one single nucleotide. In this case we are not dealing with polymorphism, but SNPs - Single Nucleotide Polymorphisms. When we

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<sup>16</sup> GUSFIELD, D. *An Overview of Combinatorial Methods for Haplotype Inference* [online] Computational Methods for SNPs and Haplotype Inference, Springer, DIMACS/RECOMB Satellite Workshop Piscataway, Lecture Notes in Computer Science, vol. 2983, 2004, online ISBN: 978-3-540-24719-7 [cited 12.3.2021 Bratislava]

<sup>17</sup> TRENT, R. J. *Chapter 3 - DNA genetic testing* [online] Molecular Medicine (Fourth Edition), Academic press, p. 81 – 115, 2012, ISBN 9780123814517 [cited 17.3.2021 Bratislava]

compare the two strands of one DNA, some nucleotides at certain section on one strand are not equivalent to the nucleotides in the same section on the other strand although they carry the same genetic information. SNPs are differences that are affecting single position in DNA sequence and are very common and ferly easy to study. We talk about SNPs, if the certain type of variation is present in more than 1% of the population (Redfield 2015).

Professor Redfield describes haplotype as a “genotype of a short segment of a chromosome”, in other words – a collection of variations in certain position. If these variations (SNPs) occur in certain position among many people’s genome, we can call it a haplotype. Haplotypes are regionally-connected in general sense, in other words people from occupied area by our ancestor will have similar haplotype among each other and thus, their descendants are inheriting their mtDNA with certain haplotype. Tracing haplogroups enable us to discover the ancestors from different parts of world that at some point met, had offspring and passed the mtDNA onwards. We can analyse mitochondrial DNA and detect certain patterns in sequences of DNA and then look for similarities in the same sequences in mitochondrial genome of our ancestors. Because mtDNA is inherited only from mother side, geneticists are able to track our ancestral grandmothers and create so called Human phylogenetic tree describing the relationships between different cultures in context with time periods but also detecting the possible ancestral human migration which created a perfect potential for genome mixing.

### 3.4 Mitochondrial haplogroups

The lack of recombination in mitochondrial replication allowed geneticists to synchronise the data from mtDNA and divide them according to similarities in mutations into branches of Phylogenetic tree. The biggest and most common branches with data similarities represented in population got the alphabetical labels of capital letter, creating today known mitochondrial haplogroups.

*“The nomenclature of mtDNA haplogroups was introduced in the mid-1990s with A-G labels assigned to variation observed in Asian and American lineages, H-K to Europe whereas only a single letter, L, was assigned to describe the highest level of variation observed in Africa in a study using an Asian outgroup.”<sup>18</sup>*

In order to analyse mtDNA data and classify it into certain branches of Phylogenetic tree, scientists use high-resolution analysing method RFLP (Restriction Fragment Length Polymorphism) which analyse 14 enzymes, and based on the data, it will be assigned to certain haplogroup (Torrioni *et al.* 2000). Each group is marked by a letter or combination of letter and number.

My area of interest are the haplogroups (later on referred to as hg/hgs) occurring in central European region. The branches overlap each other and influence each other in some areas.

#### 3.4.1 Haplogroups in cultural context of Neolithic period

The concept of hg greatly helps in understanding the ancestral migrations and genetics, but the evolution of hg is far more complex. Further chapters will detect the hg frequency of distribution in each Neolithic culture and possible way of incorporation of particular hg into central European region. What have to be kept in mind is the mtDNA hg evolutionary background of this region. People underwent significant development also in the previous periods before Neolithic. This region had a certain starting hgs background of its own at the beginning of Neolithic era. The overlapping influence it had on the developing Neolithic hg distribution have to be taken into consideration.

Following hg characteristics are (unless stated otherwise) resourced from Eupedia online mtDNA database, which holds the data about lineage from the entire European region,

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<sup>18</sup> KIVISILD, T. *Maternal ancestry and population history from whole mitochondrial genomes* [online] Investigative genetics, vol. 6, 3, 2015 [cited 20.3.2021 Bratislava]

with the author being a specialist Maciamo Hay. This database is constantly evolving according to new discoveries, founding and updates in the academic community.

## Haplogroup H

- *First Farmers from Near East H5 hg;*
- *LBK culture - H16, H23 and H26 hgs;*
- *Bell Beaker Culture and Corded Ware Culture - H1, H3, H4, H6, H7, H11, H13 hg;*
- *Copper Age culture H1, H3;*

Hg H occurs in most of the present central European populations in high frequency. Research suggests (Brotherton *et al.* 2013) that hg H emerged in European region before Last Glacial Maximum surviving in south-western Europe and later in the post-glacial period it re-expanded and underwent dynamic modifications. Eupedia database presumes that sub-haplogroups present in the Late Mesolithic were mostly branches H4, H10, H17 and H45 which seems to be exclusively European.

Hg H has a great variability within itself with total of 11 subgroups (H1 – H11), each having its own sub-haplogroups (Achilli *et al.* 2004). Hg H is very common and dominates in population of what is today western Eurasia with multitude over 40% (Brotherton *et al.* 2013). This research article compares mitochondrial hg from Neolithic and Mesolithic with present day. After the period of great changes during Neolithic Revolution, many cultures especially from Fertile Crescent begun to migrate towards Greece and Anatolia. These first farmers potentially belonged to the hg H5, but they also carried hg J and K. Hg H was not at all that frequent in the Early Neolithic Period. Data from Eupedia database showed that Early Farmers distributed only about 7% of hg H in the central European region, which is very low number when compared to the frequency of hg H in central Europe, which is today 40-50%. This hg has today very wide geographical distribution in central Europe and is believed to have originated in Near East spreading with migration of early Neolithic farmers from Near East and in later periods expanding further (Achilli, A. *et al.* 2004).

The cultures of the later periods, however, played significant role in distribution of the hg H throughout the region, with the mutation rate being over 45% explaining the great diversity in hg H (Brotherton, P. *et al.* 2013). The sub-hg that persisted till this day in many populations are mainly e.g. H1, H3, H4, H6, H7, H11 and H13. Based on mentioned research Bell beaker culture and Corded Ware culture played significant role in development of this hg

whose mitochondrial hg are present in populations today. During late Neolithic and Copper Age the most represented hg were H1 and H3 which are both significant across Europe today.

## **Haplogroup HV**

- *First Neolithic Farmers and migrating HG from South in European region;*
- *Linear Pottery Culture;*
- *Bronze Age – Indo-Europeans;*

This hg was originally branch of hg H but it has later more defined and form entirely new hg HV. The frequency of HV hg across Europe ranges from 2 to 3%. The subdivisions of this branch range from HV1 up to HV17 expanding through the whole Europe and beyond. There is a probability that hg H, HV and V evolved from populations of hunter gatherers living in Mediterranean that later spread onwards during Neolithic period. HV hg was detected in low amount among Neolithic farmers in LBK culture and even less with Starčevo and Cardium Pottery cultures. Later in the Bronze Age HV hg thrived among Indo-Europeans. Overall, HV hg thrives today mostly among Near East populations where it is greatly extended among 10% of the population. The low percentage that occurs in central Europe is probably from the migration of Mediteranean hunter-gatherers up to the north.

## **Haplogroup HV0+V**

- *Sami culture – Last Hunter-gatherers in Europe*
- *Starčevo culture*
- *LBK culture*
- *Neolithic Rössen culture - Germany*

Hg V represents a large branch of hg HV0 that has separated. The mutation that defines hg HV0 is believed to have happened around the Last Glacial Maximum, while the mutation for hg V happened later around Late Glacial period.

The frequency of these hg in European region is around 5%. The highest frequency of these hg nowadays is in northern Scandinavia.

The geographical origin for these hgs is highly debated subject because it is not quite certain, but it is believed that these hgs did not originate in Near East but in Mesolithic Europe, because they were not present among populations from Near East. There are not enough accurate studies that would confirm the presence of these hgs before Neolithic period, but the samples that are well studied are only from Neolithic period and the later periods.

These hgs were extremely common (around 42%) among Sami culture, who were the last European hunter-gatherers. HV0+V were also found among LBK culture, Starčevo culture and in Rössen culture typical for the region of Germany.

## **Haplogroup J**

- *Linear Pottery Culture;*
- *Cardium Pottery Culture;*
- *Funnel Beaker culture;*
- *Indo-European invasion during Bronze Age J1b;*

This hg is quite evenly distributed with the frequency in the central Europe ranging from 5 to 9%. It is believed that the mutation that defined J hg happened around 45,000 years ago and mutated in the region of West Asia. Nowadays high frequencies of this hg are detected mostly in northern Germany and Denmark with the frequency around 5%. J hg subdivides into many smaller branches from J1 up to J2b2.

There are no reliable sources to confirm the fact, if hg J was not present among populations earlier than Neolithic period. There have been studies however, that confirmed the presence of J hg in several Neolithic cultures – LBK, Cardium Pottery culture and Funnel Beaker culture. They have been all tested and classified to hg J1 or J2. J1c is today the most common sub branch of all J sub-hg covering over 1/3 of all J hg carriers.

Sub-haplogroup J1b has never occurred in any samples before Bronze Age and based on that fact it is probable that J1b was brought to central Europe by invading Indo-Europeans.

## **Haplogroup U5**

- *Last Glacial Maximum – Gravettian culture;*
- *Early Hunter Gatherers in central Europe;*
- *Corded Ware Culture;*

Hg U5 is considered to be the most ancient one and occurred among populations of Upper Paleolithic (Malyarchuk *et al.* 2010). According to this article, U5 hg has two sub-haplogroups – U5a and U5b. Hg U5a seems to be older than hg U5b and is very common among populations of central and Eastern Europe. High frequency of this hg was detected in Mesolithic cultures to be around 65%. The frequency decreased rapidly, but it is still up to this day detectable in European population on average of 7% (Malyarchuk *et al.* 2010). The frequency in central Europe ranges from 7 to 10%.

During the Last Glacial Maximum that was around 23,000 years ago, populations of U5 hgs have been part of Gravettian culture. The lifestyle conditions were very harsh at that point and it took a great amount of energy from body metabolism to keep body in homeostasis, for that reason mitochondria as an energy producer was crucial organelle for survival. Why particularly mitochondria with genetic mutation belonging to U5 haplogroup best persisted during these difficult conditions is not certain, but there is a possible survival advantage that this branch holds. Analysis of mtDNA hg revealed that before Neolithic period the branch U5b was very frequent among people from the region of West Europe, but on the other hand U5a hg reached mostly up to the middle and eastern regions. U5 is very frequent among early HG in Europe. This shifted during Bronze Age, when majority of collected samples belonged to branch U5b among CWC.

## **Haplogroup K**

- *Early Neolithic Farmers;*

Hg K ranges in central Europe from 8% depending on specific region. Its origin is presumed to be in West Asia, where it separated from hg U8b around 30,000 years ago. Different sub branches have been present in European populations in different periods with K3 being present as far as Mesolithic period, on the other side, K1c, K2b and K2c were not present in population even in the Neolithic period. Hg K divides into many sub-haplogroups ranging from K1 up to K3. The highest frequency today can be found in Belgium (14%) Ireland (12%) and Netherlands (10%).

This hg seems to start developing with the early Neolithic. K1a, K1b and K2a sub branches have been found among early Neolithic farmers from Near East in western and central Europe. On the other hand sub branches K1c, K2b, K2c have never been found among Neolithic farmers. K1a is the most common sub branch today as it was among Neolithic farmers. K hg was detected in around 15% of the samples from Neolithic Farmers of central Europe.

## **Haplogroup T**

- *LBK;*
- *Cardium Pottery Culture;*
- *Corded Ware Culture;*

The main sub branches of this hg are T1 and T2. The highest frequencies of hg T1 in central Europe are among populations of Czech Republic and Austria. The incidence is around 4-5%. T2 sub-haplogroup is prevalent in the whole central Europe mainly in Slovakia, Hungary and Austria with occurrence around 7%.

The mutation probably happened around 29,000 years ago in eastern Mediterranean. The division of these two main hgs occurred about 21,000 years ago. T hg was found among late HG. The hypothesis is that both T1 and T2 entered the European region from Anatolia in the Late Glacial Period and the particular culture settled in southeast Europe. Later when first farming communities migrated around Europe, hg T geographically expanded (Pala *et al.* 2012). Cultures that had hg T in their genome played an important role in distribution agricultural traits across Europe. T hg was detected among LBK culture and Cardium Pottery culture. In a Bronze Age T1 and T2 were extracted from CWC samples.

### **Haplogroup N1a**

- *LBK culture;*
- *Starčevo culture;*
- *Funnel Beaker culture;*

This hg was very frequent among first farming communities in central Europe but in today's population it is quite rare. It is believed that this hg may have spread from Near East as well as from southern European region or Eastern Europe. N1a hg was not detected in samples from Palaeolithic nor Mesolithic and the first evidence of this hg existence is dated to early Neolithic. N1a had a significant distribution among Starčevo, LBK and Funnel Beaker culture reaching almost 12.5% in population.

By the time Corded Ware Culture spread throughout the European region N1a hg decreased significantly in the populations and it have never had as high frequency as it had in early Neolithic since.

In contemporary European population the frequency is only about 0.5%. This hg is nowadays very rare also in global scale with Yemen and Saudi Arabia having the highest distribution among population, around 2.5%.

## 4 MITOCHONDRIAL HAPLOGROUP FREQUENCY COMPARISON

In these following chapters I have made my own research in order to answer my research question and in order to find out if my primary hypothesis was correct or not.

### MATERIAL

#### *Neolithic samples:*

The data used for Neolithic hg overview were collected from online database gathering data from verified research articles: AmtDNA (<https://amtdb.org/>) database. This database was used in order to collect data from the central European region.

AmtDNA database generated the total number of 135 samples. All samples were from the region of central Europe, specifically from Germany, Austria, Czech Republic and Poland from 10 research articles in total:

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

**<https://doi.org/10.1038/nature14317>**

I have used the total number of 44 samples from this study. All of the samples were dated to Neolithic period from Germany. Sixteen samples belonged to LBK culture (I0019, I0020, I0021, I0022, I0023, I0024, I0025, I0026, I0027, I0054, I0056, I0101, I0102, I0796, I0795 and I0820). Seven samples (I0049, I0050, I0103, I0104, I0106, I0550 and I0114) belonged to CWC. One sample (I0059) belonged to BBC and one sample (I0172) belonged to BEC culture. Six samples (I0560, I0559, I0558, I0557, I0556 and I0807) belonged to Baalberge culture. Five samples (I0554, I0552, I0551, I0802 and I0800) belonged to Salzmünde culture and four samples (I0166, I0165, I0163 and I0162) belonged to Rössen culture. Two samples (I0799, I0798) belonged to Schöningen culture. The sample I0171 belonged to culture called Benzigerode Heimbürg, LN and sample I0118 belonged to LN.

**Lipson et al. 2017, *Parallel palaeogenomic transects reveal complex genetic history of early European farmers*, Nature, 551(7680), 368-372, available:**

**<https://doi.org/10.1038/nature24476>**

I have used the total number of 21 samples from this study. All of the samples were from Neolithic period, from Germany. All of the used 21 samples (I0046, I0048, I0057, I0100, I0659, I0821, I1550, I2008, I2014, I2029, I2026, I2022, I2021, I2020, I2017, I2016, I2038, I2037, I2036, I2032 and I2030) belonged to LBK culture.

**Mathieson et al. 2015, *Genome-wide patterns of selection in 230 ancient Eurasians*, Nature, 528(7583), pp.499-503, available: <https://doi.org/10.1038/nature16152>**

I have used the total of 19 samples from this study. Nine samples (I1532, I1534, I1536, I1544, I1542, I1541, I1540, I1539 and I1538) belonged to CWC from Germany. Eight samples (I5068, I5069, I5208, I5207, I5206, I5205, I5204 and I5070) belonged to LBK culture from Austria. One sample (I0797) belonged to LBK culture from Germany and one sample (I0551) belonged to Salzmünde culture from Germany.

**Tassi et al. 2017, *Genome diversity in the Neolithic Globular Amphorae culture and the spread of Indo-European languages*, Proceedings of the Royal Society B: Biological Sciences, 284(1867), 20171540, available: <https://doi.org/10.1098/rspb.2017.1540>**

I have used the total number of 3 samples from this study. All samples (I2301, I2803 and I2801) were from Neolithic period from Poland and they belonged to Globular Amphora culture.

**Olalde et al. 2018, *The Beaker phenomenon and the genomic transformation of northwest Europe*, Nature, 555(7695), 190-196, available: <https://doi.org/10.1038/nature25738>**

I have used 4 samples from this study, all of them were dated to Neolithic period. Sample I4893 belonged to culture Czech MN and the other three samples (I7272, I7280 and I7279) belonged to CWC from Czech Republic.

**Chyleński et al. 2017, *Late Danubian mitochondrial genomes shed light into the Neolithisation of central Europe in the 5th millennium BC*, BMC Evolutionary Biology, 17(1), available: <https://doi.org/10.1186/s12862-017-0924-0>**

I have used 5 Neolithic samples from this study. Four samples (KM1, KZ6, NHP1 and R18\_1) belonged to Lengyel culture in Poland and one sample (Sam1) belonged to LBK culture from Poland.

**Juras et al. 2016, *Investigating kinship of Neolithic post-LBK human remains from Krusza Zamkowa, Poland using ancient DNA*. Forensic Science International: Genetics, 26(?), 30-39, available: <https://doi.org/10.1016/j.fsigen.2016.10.008>**

I have used 15 samples from this study. Four samples from Poland (KZ1, KZ2, KZ3 and KZ4) belonged to Lengyel culture. Nine samples (poz323, poz287, poz286, poz282, poz281, poz280, poz279, poz235, poz234) from Poland belonged to CWC. Two samples (poz257, poz256) from Czech Republic belonged to CWC.

**Allentoft et al. 2015, *Population genomics of Bronze Age Eurasia*, Nature, 522(7555), 167-172, available: <https://doi.org/10.1038/nature14507>**

From this study I have used 8 samples. Two samples (RISE1 and RISE431) from Poland belonged to CWC. Five samples (RISE434, RISE471, RISE446, RISE436 and RISE435) from Germany belonged to CWC and one sample from Germany (RISE560) belonged to Bell Beaker culture.

**Schroeder et al. 2019, *Unraveling ancestry, kinship, and violence in a Late Neolithic mass grave*, Proceedings of the National Academy of Sciences, 116(22), 10705-10710, available: <https://doi.org/10.1073/pnas.1820210116>**

I have used 15 samples from this study (RISE1173, RISE1172, RISE1171, RISE1170, RISE1169, RISE1168, RISE1167, RISE1166, RISE1165, RISE1164, RISE1163, RISE1162, RISE1161, RISE1160 and RISE1159). They were all from Poland belonging to Blobular Amphora culture from Neolithic period.

**Lazaridis et al. 2014, *Ancient human genomes suggest three ancestral populations for present-day Europeans*, Nature, 513(7518), 409-413, available: <https://doi.org/10.1038/nature13673>**

I have used one sample (LBK380) from this study that was dated to Neolithic. It was from Germany and it belonged to LBK culture.

#### ***Samples from present day:***

For hg frequency overview from present day I have used several scientific studies. The total number of 1165 samples was collected. All samples were collected from 4 research articles from Germany, Czech Republic, Slovakia and Poland:

**Vanek et al. 2011, *Mitochondrial control region sequence of the Czech Republic population and comparison to other populations*, ScienceDirect, vol.3, issue 1, E415-E416, available: DOI:<https://doi.org/10.1016/j.fsigss.2011.09.069>**

I have used all 255 samples from this study that were collected from unrelated individuals from Czech Republic.

**Brandstätter et al. 2006, *Application of quasi-median network analysis for the visualization of character conflicts to a population sample of mitochondrial DNA control region sequences from southern Germany (Ulm)*, International Journal of Legal Medicine, 120:310–314, available: DOI [10.1007/s00414-006-0114-x](https://doi.org/10.1007/s00414-006-0114-x)**

From this study I have used all 100 samples collected from Southern Germany.

**Malyarchuk et al. 2002, *Mitochondrial DNA variability in Poles and Russians*, *Ann Hum Genet*, ul;66(Pt 4):261-83, available: doi: 10.1017/S0003480002001161**

I have used only 436 samples from this study that belonged only to Poles.

**Lehocký et al. 2008, *A database of mitochondrial DNA hypervariable regions I and II sequences of individuals from Slovakia*, *Forensic Sci. Int. Genet.* 2, e53–e59, available: <https://doi.org/10.1016/j.fsigen.2007.12.008>**

I have used all 374 samples from this study of randomly chosen individuals from Slovakia region.

## **METHODS**

I have collected all 135 samples from Neolithic period and calculated the frequency of each hg among populations in value of %. The results can be seen in Graph 1.

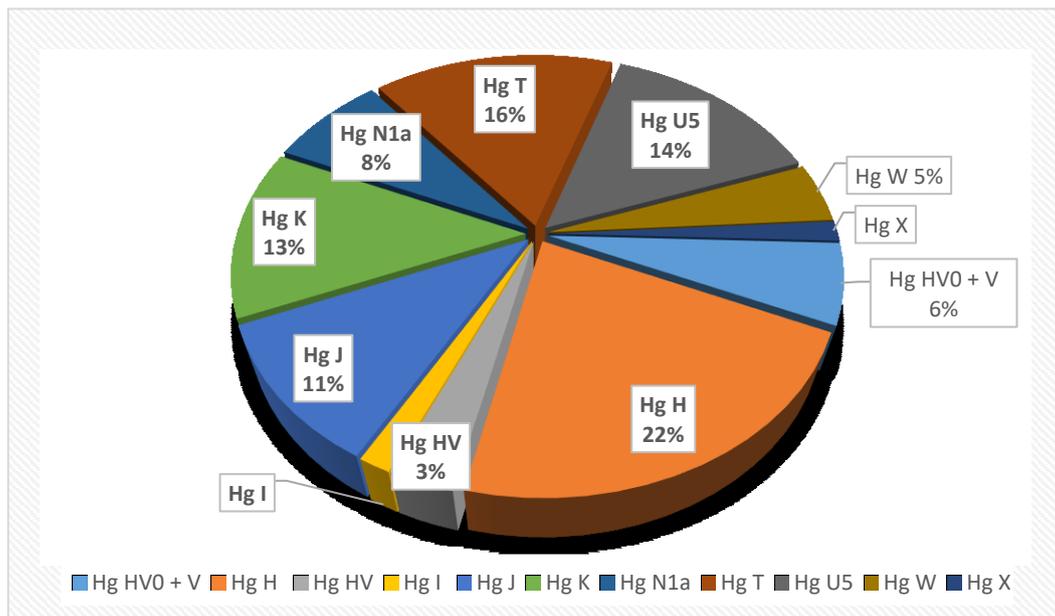
I have collected all 1165 samples from present day and I have calculated the frequency of each hg among contemporary populations in value of %. The results can be seen in Graph 2.

After the calculation of hg frequencies for each time period I have calculated the percentage change of each hg. The results can be seen in Table 1.

$$\text{Percentage change} = \frac{\text{new value} - \text{original value}}{\text{original value}} \times 100$$

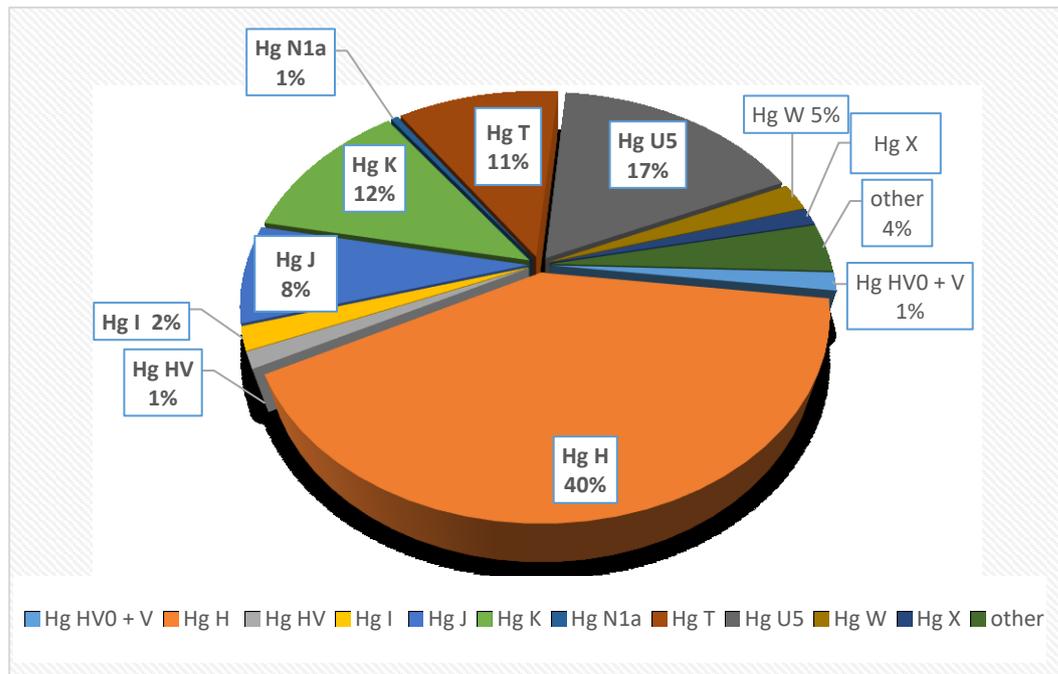
At the end I have created Graph 3 that shows the hg frequency from Neolithic period in comparison to present day.

## RESULTS



*Graph 1 Haplogroup frequency in Neolithic period from central European region*

Results from Neolithic period showed quite even distribution of all the major hgs typical for European region (marked with bold letters). Hg H showed the frequency of 22% which made it dominant. After hg H the second most frequent hg was hg T with frequency of 16% followed by hg U5 with 14%. Hg K showed to have had 13% frequency and hg J was represented by 11%. Hg N1a was represented by 8% followed by hg HV0+V with the frequency of 6%. Hgs that were represented by 5% or less were hg W with frequency of 5%, Hg HV with frequency of 3% and hg X and I had the smallest frequency of 1%.



*Graph 2 Haplogroup frequency in present central European region*

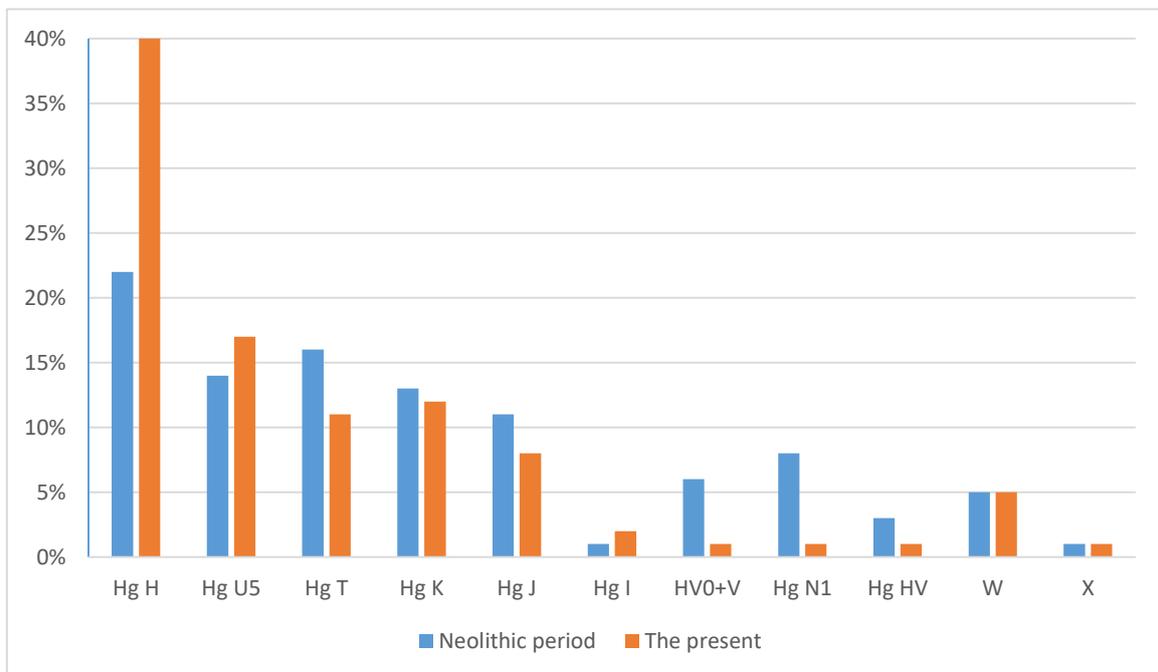
Results from the present show that hg H has the frequency of 40%, hg U5 had 17%, Hg K had the frequency of 12%, hg T had the frequency of 11% and Hg J had the frequency of 8%. Hgs that had the frequency 5% or less were hg W with 5%, followed by hg I with the frequency of 2%. Hgs HV, HV0+V, X and N1a had the smallest frequency of 1%.

Haplogroup	Neolithic period	The present	% change
H	22	40	81,8
U5	14	17	21,4
T	16	11	- 31,3
K	13	12	- 7,7
J	11	8	- 27,3
I	1	2	100
HV0+V	6	1	- 83,3
N1a	8	1	- 87,5

HV	3	1	- 66,7
W	5	5	0
X	1	1	0

*Table 1 Percentage change calculation results*

Percentage change for Hg H was 81.8%, for hg U5 it was 21.4%, for hg T -31.3%. Percentage change for hg K was -7.7%, for hg J it was -27.3% for hg I it was 100%, for hg HV0+V it was -83.3%, for N1a the percentage change was - 87.5% and for hg HV -66.7%. The percentage change for hg W and X was 0%.



*Graph 3 Haplogroup frequency comparison Neolithic vs the present*

Graph 3 shows the results of hg frequency comparison of Neolithic period with the present.

## DISCUSSION

The interpretation of results confirmed my primary hypothesis, that the hg representation is different in Neolithic period and in the present populations. Because my primary hypothesis was confirmed I have made additional calculations in order to find out how exactly each hg has changed.

Results from Neolithic period demonstrated on Graph 1 show quite even frequency of the main hg - H, U5, T, K, J and N1a. The results show that hg H was dominant in Neolithic period with 6% higher frequency among population than the other hgs. When we compare the hg H frequency from the Neolithic on Graph 1, which is 22%, with hg H frequency from the present day on Graph 2, which is 40%, we can see a significant increase in representation. Calculated percentage change for this hg was 81.8% as can be seen on Table 1. This major increase indicates that some other attribute, beside migration and mtDNA mutation, played a role in the development of this hg. Roostalu *et al.* (2006) confirmed that hg H was extended in European region and the Near East even before the Last Glacial Maximum period and on top of that hg H have reoccupied this region also after the Last Glacial Maximum. This study confirms a strong distribution of H hg even before the Neolithic period. This means that when the first hg during Neolithic period were brought to the region of central Europe, hg H was already present among population in higher frequency and thus it makes a strong hg foundation in many European populations. This fact might be the possible explanation for the strong hg H dominance in the present. Other possible reason might be the mentioned migration. While some other hg were being firstly introduced to the European region during Neolithic period by first farmers, hg H have already had a strong position among people. Due to the reason that hg H was highly represented also in Near East, first farmers migrating were bringing even more of the hg H (e.g. mentioned hg H5) into the European region which might have helped to cause such increased resulted in comparison to other hg.

The comparison of Graph 1 and Graph 2 showed that Hg T, which was represented in Neolithic by 16%, decreased in present populations to 11 %. Calculated percentage change showed results of -31.3%. The decrease in hg frequency is not dramatic and considering the constant migration influencing all hgs through millennia, besides other influencing attributes, the decrease it quite low. Due to that fact I consider the frequency of hg T to be quite stable in the populations of central European region.

Haplogroup U5 also showed to remain quite stable through the millennia in terms of frequency. Percentage change for hg U5 resulted in 21.4%. This slight increase may have

been the result of migration or simply population growth. Taking into consideration that I have compared two periods that are few millennia apart, this percentage change is low.

Another, even more stable hg, is hg J which had in the past the frequency of 11% and in contemporary population the frequency of 8%. The percentage change calculation resulted in -27.3%. This hg experienced just a slight decrease in frequency.

The percentage change in hg K was calculated to -7.7%. Due to the fact that the two compared groups from Neolithic and the present day consisted of quite high number of samples, result shows an accurate measuring. Due to that fact, I consider hg K to be very stable in terms of frequency. Isern *et al.* (2017) studied the hg K frequencies and the research confirmed that hg K is slowly but gradually decreasing from the period when First farmers entered the European region. Considering the high number of used samples during the comparison I assume that this hg is the most stable one.

Low frequency hg W and X in contemporary populations showed the percentage change of 0%. The frequency did not change but I assume that the results of this comparison would be more accurate with higher number of samples. Overall, the frequency comparison showed that the frequency of these hgs among ancient and contemporary population is the same.

Percentage change in the case of hg I showed 100%. The frequency in Neolithic was 1% and the frequency in contemporary Europe is about 2%. This high value is caused by the very low number of samples in both present population as well as the ancient ones, but it represents the change that occurred in populations. Even if the frequency is nowadays very low, it has doubled since the Neolithic period.

The hg frequency comparison generated quite interesting data with hg N1a. The N1a hg frequency in Neolithic period was 8% and in contemporary populations it is only 1%. The percentage change was calculated to -87.5% as is shown on Table 1. Hg N1a experienced the most significant decrease in frequency out of all hg. For that reason I assume there might be a possibility that hg N1a did not have a strong roots in the population of European regions as it was the case with hg H which dominated in genomes around European region and Near East. Other possibility is that it might have been carried away to other regions by migration. Fernandes *et al.* (2012) offer an insight into the regional development of hg N1a that is very deeply rooted in Eastern Africa and Arabian Peninsula. Authors assign this hg to Late Glacial Period when this hg entered Europe but because of migration (and other factors) this hg did

not stayed in European region and it did not create stronger roots within populations. The increase in Neolithic period among LBK culture might have been just regional re-expansion that did not persisted due to low representation among existing population.

Hg HV had the frequency of 3% in the Neolithic period that had decreased to 1% in contemporary populations. The percentage change was calculated to -66.7%. Hg HV0+V experienced even more significant decrease in frequency. The percentage change was calculated to -83.3%. Hg HV and HV0+V are nowadays very rare among contemporary populations.

Graph 3 shows the comparison of frequencies from Neolithic period and the present. Based on these results we can see which hg were the more stable ones in frequency through time and which hgs experienced major changes. I consider hg K to be the most stable one with percentage change only -7.7%. Hg W and X showed to be also stable with percentage change only 0%. In the case of these two hg I consider the results being not quite accurate due to the low number of samples that were compared. Another quite stable hgs that did not experiences major changes in frequency are hg U5, T and J. The hg that experienced the most significant increase in frequency are hg H and hg I. Hgs that experienced the most significant decrease in frequency are hg HV0+V, hg HV and hg N1a.

Based on these comparisons I have confirmed my primary hypothesis. Hg frequencies in Neolithic period differ from the hg frequencies of the present populations.

Attributes that had influenced the change were mutation rate and mutation change of hg themselves and population migration. There are attributes that contributed to the changes that I did not mentioned as a reason for the changes in my primary hypothesis. These attributes are genetic background of population that creates genetic foundation of some region (the case of hg H), local genetic development and genetic re-expansions (the case of hg N1a). In the case of hg H, the main reason for such extensive increase in frequency was the fact that hg H created a genetic foundation in ancient populations and its representation only increased over time. Another reason for changes in frequency was local genetic development (local genetic re-expansion). There are definitely other forces that influenced the development of each hg and each hg was probably influenced by some other forces that occurred based on time, geographical position, cultural interaction and many others.

### **Limitation of this study**

The main limitation of this study was the uneven number of samples from each period. The number of samples from Neolithic period is naturally lower, because archaeological findings with interpretable data are rarer than studies made in recent years among European populations in high numbers. It is probable that results of this study were influenced by this limitation.

Another limitation of this study is uneven distribution of archaeological samples. Most of used samples were from Germany, Austria, Poland and Slovakia which may have misrepresented the overall perspective on the hg profile of central European region.

Future research should be done with similar number of samples from each period. It could detect also the exact change in the low frequency hg such as W and X, of which results were inaccurate due to low number of samples. Next study could potentially explain the exact possible reason for the increase or decrease in frequency of each hg among contemporary population in comparison to the Neolithic ones. I have presented few possible reasons for these changes based on researches done in the past. The future research could aim on the reaching for exact reason that have caused the changes in that particular hg over time.

## **CONCLUSION**

In this work I have made a research comparing hg frequencies in Neolithic cultures in European region and compared it to the hg frequencies in contemporary European populations. My primary hypothesis was confirmed.

In the theoretical part of this work I have focused on the detailed description of the Neolithic period in order to set my research into context. I described the processes happening during Neolithic period that influenced Neolithic cultures and further human development. Then I have focused on mitochondria itself, on its purpose as a part of organisms and on its own mitochondrial DNA. I have looked closely on the haplotype and haplogroup phenomenon as a part of population genetics. In order to connect the archaeological and genetic point of view, I have presented historical hg overview of each culture, historical background of each hg and also contemporary representation in European population.

In the research part of this work I have focused on comparing hg frequencies in Neolithic period and in the present. After confirming my hypothesis I have calculated the percentage change for each hg.

One question still remains unanswered which I see as a potential research question for another study: What is the true reason for the change in representation of each haplogroup among population of central Europe?

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