

THESIS

MICROEVOLUTION OF HUMAN TOLL-LIKE RECEPTORS IN CENTRAL ASIA DURING
BRONZE AND IRON AGES

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ABSTRACT

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The Toll-like receptors (TLRs) are an integral component of human immune defense because they act as first-responders to infection. Previous research has documented the presence of archaically-introgressed sequences of DNA into the TLR10-6-1 cluster (Deschamps *et al.*, 2016 & Dannemann *et al.*, 2016). This genetic region has also been suggested to be a hotspot for selection in both contemporary and ancient Eurasian populations (Barreiro *et al.*, 2009 & Mathieson *et al.*, 2015). Furthermore, ancient strains of Salmonella, Tuberculosis, and Plague were present on the Eurasian landmass, all of which would incite response from the TLRs (Andrades Valtueña *et al.*, 2022; Rasmussen *et al.*, 2015; Key *et al.*, 2020; Wu *et al.*, 2020; Hajam *et al.*, 2017; Caroff and Karibian *et al.*, 2003; Strahl and Errington 2017; Mukherjee *et al.*, 2014). Allen Ancient DNA Resource was used to access genotype data on five archaic TLR SNPs from 298 individuals who lived between 7922 and 1109 BP is used to examine their temporal and geographic distribution (Mallick and Reich, 2024 & Mallick *et al.*, 2024). Here, hypotheses are tested to determine whether lifestyle, time period, a combination of the two, or geography predict the presence of archaic TLR SNPs. The aim of this study was also to assess whether that distribution reflects microevolutionary change in the direction of drift or selection. A peripheral aim of this study was to answer whether that distribution is a result of adaptation to pathogens. It is concluded that the temporal and geographic distribution of these SNPs is both stochastic and does not reflect a signal of selection nor are lifestyle, time-period, a combination of the two, or geography predict the presence of archaic TLR SNPs.

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Microevolution of Human Toll-Like Receptors in Central Asia During Bronze and Iron Ages

Chapter I

Introduction

The continuation of any species is predicated on their capacity to adapt to changing conditions in their environment. Adaptation can be defined as a series of adjustments in the form of traits or behaviors that enhance an organism's ability to survive or reproduce in an environment (Darwin, 1859). This definition gives credence to the concept of adaptation as an ongoing process and an end-goal because the environment is constantly shifting (Rosalino et al., 2014 & Bijlsma and Loeschcke, 2005). Furthermore, if organisms adapt to their environment, then that fitness is measured in terms of reproductive success. Frequent iterations of reproductive success reflect adaptation to environmental stimuli which can be one or more variables an organism has to overcome. Anthropologists interested in adaptation can look to human-pathogen interactions because they have produced observable human adaptations that occupy many positions along the continuum from genetic to cultural interventions to pathogen exposure. Culture serves as an exosomatic means of offloading evolutionary problems from our biology (Tomassello, 2003). To that end, humans have a generative capacity to adapt to a multitude of shifting environments and can execute those adaptations within a lifetime. Cooking meat kills off a surface level population of bacteria that can make one sick (Doyle and Schoeni, 1984). Clothing shields the body from extreme temperatures (Gilligan, 2018). Both are cultural adaptations that further enable humans to propagate themselves into the next generation when faced with harsh conditions.

Humans are not limited to cultural adaptations, a combination of physiological, developmental, and genetic adaptations are often employed in response to pathogens (Harrison, 1998; Tomassello, 2003; Harris, 2013; Frisancho, 2009). For example, inflammation is a routine physiological adaptation to infection. Inflammation, which is the recruitment of immune cells, represents a physiological adaptation because it is a short-term chemical change in the body (Ahmed, 2011 & Harrison, 1998). Despite the personal inconvenience that comes with inflammation, it is the immune system's way of building an army in response to infection and natural selection would likely eliminate individuals without that ability. And while this is unfolding, the human immune system is making long-term adjustments to the presence of the pathogens in the form of adaptive, or acquired immunity whereby over time, exposure to certain pathogens will make an individual better suited to deal with similar ones (Müller *et al.*, 2018). This is a developmental process that can take place over the lifetime of an individual. The two operate on short timescales but have taken many generations of genetic modification to enable their success.

Similarly, biological anthropologists are keen to explore genetic adaptations because they reflect intense selective pressure. Genetic adaptations are a last-resort option for humans because on the short time scale we live on, natural selection prunes the population from unfit individuals and the time period for the correct adaptation to manifest in response to a pathogen may be prohibitively long (McDonald, 1983). For example, Klunk *et al.* (2022) characterized genetic variation around immune-related genes from 206 aDNA extracts recovered from skeletal remains discovered in London and across Denmark during the Black Death. They found that homozygosity of the C allele was associated with an increased ability to control intracellular *Yersinia pestis* in macrophages in a replicated sample of modern humans. These results indicate

that homozygous individuals with C allele of rs2549794 on the ERAP2 gene are selected for in response to *Y. pestis*. More importantly, they represent a genetic adaptation to a specific pathogen. In total, humans would not have possessed or evolved an adaptation like the one mentioned above if their shared history with pathogens was not deep and it represents a last-resort option for how humans have to manage their interactions with pathogens.

Partial human and pathogen genomes recovered from archeological sites demonstrate our shared history goes back millennia and reflects how humans have had difficulties in evolving genetic adaptations to overcome them. Studies such as HersHKovitz *et al.* (2008) and Sabin *et al.* (2019) have sequenced *Mycobacterium tuberculosis* genomes found in association with agro-pastoral and agricultural communities in northern Europe between 9250-8160 BP., and the Levant at 1679 CE, respectively. TB is currently a leading cause of death and these studies demonstrate that it has been a burden on our species for at least nine thousand years (World health organization, 2025). This fact alone supports how humans have been unable to evolve genetic adaptations to deal with it specifically. Similarly, ancient strains of plague and salmonella have been recovered from human remains throughout Eurasia dated between 6,500-2,920 BP. and their adaptation to humans can be inferred based on gene losses and additions (Rascovan *et al.*, 2019; Seersholm *et al.*, 2024; Andrades Valtueña *et al.*, 2022; Rasmussen *et al.*, 2015; Key *et al.*, 2020; Wu *et al.*, 2020).

There is a discrepancy between humans and pathogens' capacity to adapt to each other. Humans are static targets for infection because we evolve on a slower time scale compared to pathogens and one mechanism we have evolved to compensate for this discrepancy is through the innate immune system. By innate, what is meant is that its functions are genetically predetermined germline-encoded and the genes that code for them bestow a baseline

mechanism for dealing with pathogens (Turvey & Broide, 2010). In the event that pathogens breach constitutive barriers of the skin, lungs, mouth, and digestive tract, humans possess biological adaptations to ward against pathogens and one of them lies in the innate immune system (McDonald and Levy, 2019).

The innate immune system is a readily available defense system against pathogens. One function innate immunity works by employing a generalized arsenal of receptors to recognize pathogens. The Toll-Like receptors (TLR) are a family of innate immune receptors that are an essential component of host defense in response to pathogenic infection. The TLRs are a subset of pattern recognition receptors (PRRs) that are activated at the onset of innate immune reaction along with (Pal and Chakravarty, 2020). Their function is to detect pathogen-associated molecular patterns (PAMPs), detect damage-associated molecular patterns (DAMPs), and initiate the production of pro-inflammatory cytokines and type-I interferons following detection of PAMPs and DAMPs. They are also important for phagocytosis too. Macrophages/neutrophils engulf foreign pathogens via TLRs (Takeda and Akira, 2005; Kawai and Akira, 2006; Akira *et al.*, 2006). It can be hypothesized that repeated exposure to pathogens from birth would put pressure on a lineage to evolve a repertoire of defense mechanisms that increase their likelihood of survival or reproduction. Some authors have contemplated this (Buchmann, 2014; Brennan and Golmore, 2018), and it can be asserted that the innate immune system, and the toll-like receptors are both an evolutionarily conserved and ancient system. To that end, if pathogens were not selective agents then the immune system would not be encoded in human genomes. However, because humans have an innate immune system, that tells us that since its archaic development, pathogens have been a constant selective burden within our lineage.

The present study looks at microevolution in humans as a consequence of repeated interactions with pathogens by examining five archaic-like TLR SNPs in central Asians who lived between 7922 and 1109 BP. (Mallick and Reich, 2024 & Mallick *et al.*, 2024) for three reasons. First, central Asians, and Eurasians more broadly, represent a spatio-temporal and genetic continuity between modern humans and the Neanderthal lineage (Green *et al.*, 2010; Sankararaman *et al.*, 2014; Li *et al.*, 2024; Villanea and Schraiber, 2019; Wall *et al.*, 2013). The introgression events culminated in increased genetic variation, especially around the TLR10-1-6 region, within each deme and would have allowed for selection to operate more efficiently than drift on a microevolutionary scale (Simonti *et al.*, 2016; Barreiro *et al.*, 2009; Deschamps *et al.*, 2016; Dannemann *et al.*, 2016; Harris and Nielsen, 2016). Secondly, within the study area, researchers have documented the presence of several pathogens that would have elicited a response from the toll-like receptors (Andrades Valtueña *et al.*, 2022; Rasmussen *et al.*, 2015; Key *et al.*, 2020; Wu *et al.*, 2020; Hajam *et al.*, 2017; Caroff and Karibian *et al.*, 2003; Strahl and Errington 2017; Mukherjee *et al.*, 2014). More importantly, these pathogens are selective agents that present an opportunity for human populations they come in contact with to adapt or go extinct. In either case, individuals whose immune system is more fit for dealing with pathogens are more likely to reproduce. Third, Central Asians during the time period we are interested in exhibit a wide range of cultural lifestyles that would differentially put them in proximity to pathogens (Damgaard *et al.*, 2018; Narasimhan *et al.*, 2019; Gneccchi-Ruscione *et al.*, 2021). Because not all populations will equally interact with pathogens and not every population has an equal amount of Neanderthal TLR genes, selection will preferentially favor the individuals who are fit to their ecological conditions.

Relevance of this study

It has been a long-held assumption that the advent of agriculture accelerated an evolutionary arms race with pathogens as a consequence of humans residing in large numbers and in close proximity to animals for extended periods of time (Diamond, 1999). It is a fact that pathogens are able to propagate more efficiently when more hosts are available and several examples of zoonoses transmitting to humans can be traced to agricultural practices (Jones *et al.*, 2013 & Stephens *et al.*, 2021). Furthermore, it has been proposed that this process has simultaneously equipped agriculturalists with a more robust immune system, while also making them more prone to disease. Because pathogens can kill humans that makes them selective forces, but it is a matter of debate whether humans have adapted to them, and if so, then could it be observed on a microevolutionary scale? If researchers want to interrogate the biological implications of adopting agriculture and how it relates to infectious diseases, they need to consider microevolutionary processes that would have unfolded.

This investigation is based on a nexus that ties introgression with Neanderthals to the development of agriculture and modern human innate immune system variation. The study of ancient DNA has come with the revelation that a portion of human immune system variation can be explained by episodes of introgression with Neanderthals. Such a series of episodic intervals of admixture would have also represented an epidemiological transition between two previously diverged lineages (Houldcraft and Underdown, 2016). With genetic variation as a result of admixture, ancestral populations receive the phenotypic diversity of the two lineages (Martínez-Abadías *et al.*, 2006). When two previously diverged lineages admix with each other and introduce genetic variants related to immunological function, it shifts the genetic landscape and enables selection to operate more efficiently as a result of an increase in genetic variation

(Simonti *et al.*, 2016 & Harris and Nielsen, 2016). Furthermore, it is important to know that a disproportionate amount of modern human immune system genetic variation has archaic origin because their longevity would suggest that there is an adaptive benefit to being a carrier of those alleles. Deschamps *et al.* (2016) observed that protein coding genetic regions are generally depleted among Neanderthal alleles implying a selective sweep in those regions and genes conferring innate immunity are present at a higher rate in Neanderthal introgressed segments compared to the rest of the genome. This selective sweep can be thought of as a pruning effect because it is not Neanderthal ancestry that is specifically being selected for but instead genetic regions that are crucial for immunological function and in contemporary populations these genes are present in a disproportionate amount compared to other Neanderthal genes. Their work also presented evidence of Neanderthal ancestry being selected at the TLR10-1-6 cluster, however concluded the introgression of those genes is not a source of adaptive variation and that the variants do not present any signal of positive selection in modern populations. In other words, contemporary populations are not the recipients of any adaptive benefit. However, Dannemann *et al.* (2016) found the TLR10-1-6 cluster to be rich in regulatory elements that overlap many archaically introgressed sequences which makes them potential targets for selection.

Central Asia is an ideal place to study the relationship between humans and pathogens on a microevolutionary scale because of three reasons: First, they represent a spatio-temporal and genetic continuity between modern humans and the Neanderthal lineage (Green *et al.*, 2010; Sankararaman *et al.*, 2014; Li *et al.*, 2024; Villanea and Schraiber, 2019; Wall *et al.*, 2013). Additionally, we see evidence of selection operating on the TLRs in Eurasian populations. Barreiro *et al.* (2009) initially reported the TLR10-1-6 cluster exhibits clear signs of positive selection in Eurasians. Mathieson *et al.* (2015) later observed a higher frequency of selected

TLR10-6-1 SNPs in Steppe-derived populations compared to European Farmers. Such signals of positive selection do not come as the result of a stochastic process because these genes play a role in the immune system's defense against pathogens and without them the consequences are lethal. Secondly, various populations of central Asians have experienced repeated bouts of pathogenic exposure. Direct evidence of this comes in the form of archaic Plague strains that were found in Eastern Kazakhstan, Mongolia, and Russia (Andrades Valtueña *et al.*, 2022 & Rasmussen *et al.*, 2015) and ancient Salmonella strains recovered from Russia and the Xinjiang region (Key *et al.*, 2020 & Wu *et al.*, 2020). These bacteria have left traceable edifices that provide a spatial and temporal distribution that overlaps with the study population. From that, we know of selective agents which would have placed pressure on central Asia's populace to evolve genetic adaptations to manage infection more readily. Third, sedentary, semi-sedentary and mobile groups occupied Central Asia during the Bronze and Iron ages (Gnecchi-Ruscone *et al.*, 2021 & Damgaard *et al.*, 2018). The Inner-Asian Mountain Corridor [IAMC (Frachetti, 2012)] in particular served as a conduit through which many of these populations of differing lifestyles migrated through and admixed with each other during this time (Narasimhan *et al.*, 2019). The gene flow between them would allow microevolutionary processes by means of natural selection to operate more thoroughly in contrast to drift when pathogens are a consistent feature of their environment.

Theoretical framework:

Evolutionary theory hinges on the concept of adaptation – traits or behaviors that enhance an organism's ability to survive or reproduce in an environment. If organisms adapt to their environment then that fitness is measured in terms of reproductive success. Biological

anthropologists interested in adaptation look for evidence of fitness along a continuum of cultural and biological processes. Adaptation on longer and shorter timescales is contingent on macro- and microevolutionary processes. Macroevolution occurs above the species level and can lead to speciation over long stretches of time. Morphological differences between taxa can be accounted for under the lens of macroevolutionary explanation (Hautmann, 2020; Rolland *et al.*, 2023; Flegr, 2013). In such cases, the concept of adaptation is used to explain these traits. Contemporary examples of adaptation biological anthropologists regularly point to in the literature refer to macroevolutionary processes such as bipedalism, increased cranial capacity, narrowing of the ribcage (Thorpe *et al.*, 2007 Senut *et al.*, 2017; Henneburg, 1987; Bastir *et al.*, 2020). As a consequence of this fixation on macroevolutionary changes, paleoanthropologists have overlooked the significance of microevolutionary processes.

Microevolution is more relevant to this study than macroevolution because microevolution generally deals with shorter-time scales and is an arduous concept to apply to ancient hominin fossils. Microevolution is the change in allelic frequency within a population over a short period of time and results in the phenotypic diversification among conspecific populations or related species (Arnold *et al.*, 2001). Applying microevolutionary processes to the hominin fossil record has been laden with issues. The rates of anatomical change over a few generations can mimic evolutionary change over several thousands of years. Speciation events are often incited in observation of small but distinct morphological differences between taxa. Cranial capacity, body height, and cranial index are all phenotypes that can gradually shift; the microevolution of them is several times faster than those observed over longer time periods in hominid evolution (Henneburg, 2006). A more contemporary example of microevolutionary change in humans would be the increased prevalence of median artery of the forearms over the

last 150 years; its driving force is still unknown and remains to be said whether it is the result of changing selection pressures, or health insults to mother and offspring, or both (Lucas *et al.*, 2020). Together, these are anatomical shifts that reflect accelerated underlying genetic changes within the populations they were exhibited in.

Microevolution, however, needs a mechanism to explain its occurrence. Wright's fitness landscape (Wright, 1932) is a useful device to consider the evolutionary mechanisms that shape populations. The adaptive landscape can be imagined as a topographical map with fitness peaks and valleys with a third dimension representing population size. For large populations they will be relatively high on the z-axis. Here, the landscape appears highly detailed with many modest peaks and shallow valleys. These are navigable subtle fitness differences and are easily overcome by the strength of selection which is a function of large effective population size. Small populations will be relatively low on the z-axis, in contrast. If effective population size is too small for selection to minimize mutations then drift will take over. From their point of view, the landscape appears "blurred" or "smoothed out" and these populations can only respond to large fitness differences; smaller features disappear into an adaptive valley. They will have a greater challenge in responding to smaller fitness differences. There is a caveat to large effective population size expressed in the drift barrier hypothesis. The drift barrier hypothesis (Lynch, 2010) posits that selection generally acts to minimize mutation rates but is limited by the power of genetic drift; stochastic effects of drift impose a precision limit on selections power to maintain low mutation rate. This would effectively occur at an adaptive peak and in a large population it would inhibit them from leaving their adaptive peak.

With respect to the aforementioned, it is worth pointing out that adaptation is a rare occurrence. Several theoretical models have been proposed to describe the neutrality of many mutations and rarity of adaptively beneficial mutations. Kimura (1968; 1983) suggested the majority of existing polymorphisms and fixed differences between species are selectively neutral and functionally equivalent. Under this model, the frequency dynamics of neutral variants in the population is determined by their mutation rate and drift, highlighting a linear relationship between genetic variation and population size. Despite this theoretical prediction, this relationship is not reflected in the wide range of population sizes across species with similar genetic diversity. To explain this paradox, Smith and Haigh (1974) proposed genetic hitchhiking as an explanation. In this scenario neutral alleles that are in high linkage disequilibrium with favorable alleles will go to fixation which reduces linked genetic variation. Alternatively, Ohta's (1992) nearly neutral theory predicts that natural selection eliminates nearly neutral mutations in large populations but in smaller populations they behave as neutral and are randomly fixed.

At this point, it should be stated though that microevolution and genetic adaptation are not necessarily the same thing nor does microevolution imply genetic adaptation (Meyer and Harris, 2013). A population can evolve a genetic adaptation through microevolution but they can also drift and evolve a maladaptive genetic trait as well. Microevolution is an ongoing process, so is adaptation but genetic adaptation is contingent upon the mutation and propagation of beneficial alleles and at the molecular level it is rare.

Applying microevolutionary principles to the fossil record is more challenging for paleoanthropologists than it is for archeo-geneticists who see signatures of it in ancient DNA because modern and ancient genomes together provide a temporal and geographic ordering of genomic events to the archeological record. More importantly, they allow us to infer selective pressure and whether adaptation occurred. Waves of admixture, introgression, migration, diffusion, and drift establish geographic patterns of genetic variation and can be observed in time

series genetic data in which changes in allele frequencies occur along the human phylogeny (Hünemeier, 2024; Pollen *et al.*, 2023; Jay *et al.*, 2013; Green *et al.*, 2010). Human pathogen interactions, specifically provide high resolution insights into microevolutionary change in the human immune system. Klunk *et al.* (2022) serves as a reminder of this claim.

Research Questions and Goals

This study is interested in whether microevolutionary processes are driving populations in Central Asia up towards an adaptive peak in the span of approximately eight thousand years. Following previous research documenting the debate over a potential selective benefit of archaic sequence variants on the TLR10-6-1 cluster, this study focuses on Central Asia because it represents a spatio-temporal and genetic continuity with the first recipient of these variants through introgression (Barreiro *et al.* 2009; Green *et al.*, 2010; Deschamps *et al.*, 2016; Dannemann *et al.*, 2016). Also, this region was chosen because of the diversity of lifestyles people have lived for thousands of years (Damgaard *et al.*, 2018; Narasimhan *et al.*, 2019; Gneccchi-Ruscione *et al.*, 2021). These lifestyles, it is believed, represent a junction between

culture and biology because an individual's lifestyle refers to degrees of mobility and by proxy, pathogen exposure. The aims of this paper are threefold: First, to examine the temporal and geographic distribution of five archaic variants of TLR SNPs using genetic data from 298 individuals from central Asia who lived between 7922 and 1109 BP. (Mallick and Reich, 2024 & Mallick *et al.*, 2024). Secondly, this study aims to test the strength of selection using the selection coefficient (s). Researchers are better equipped to make inferences about potential selective agents through the use of s because it is a calculation of relative fitness, or rather, the likelihood a given allele will propagate into the next generation. The output ranges from 0 to 1 with 1 being total selection and no chance the allele survives whereas outputs closer to 0 reflect weak selection and are more likely influenced by drift.

Lastly, this study aims to test four interrelated hypotheses. Generally speaking, this study uses Type III Wald Chi-square to test if the probability that an individual carries an archaic allele for the TLRs is statistically different when comparing mobility patterns, time period, a combination of the two, and geography. Each of these variables are broken into their own hypothesis in redundant fashion as follows: First, H_0 : the mean presence of archaic TLR SNP variants are statistically equal between sedentary, semi-sedentary, and mobile groups. This is in competition with H_a : If the mean presence of archaic TLR SNP variants is not statistically equal between sedentary, semi-sedentary, and Mobile groups then the null hypothesis is rejected, and it is predicted that the mean archaic allele presence of semi-sedentary groups differs from sedentary and mobile groups. The competing null and alternative hypotheses are mutually exclusive because a single mobility group that would differ in the proportion of archaic TLR SNPs negates the possibility of them being immunologically unremarkable in comparison.

The second hypothesis tested is H_0 : The mean presence of archaic TLR SNP variants is statistically equal across the Bronze Age, Iron Age, and recent history. This hypothesis opposes H_a : if the mean presence of archaic TLR SNP variants is not statistically equal across the Bronze Age, Iron Age, and recent history then the null hypothesis is rejected, and it is predicted that the mean presence of archaic TLR SNP variants in the Bronze Age is statistically different compared to the Mesolithic, Neolithic, Iron Age, and recent history. These hypotheses are in competition with one another because either Central Asian populations experience a turn point in terms of their innate immunity or they did not. The two versions of history are thus incompatible.

Third, H_0 : the mean presence of archaic TLR SNP variants is statistically equal between Lifestyle categories and across time periods. This hypothesis is measured up against H_a : if the mean presence of archaic TLR SNP variants is not statistically equal between Lifestyle categories and across time periods then the null hypothesis is rejected, and it is predicted that the mean presence of archaic TLR SNP variants of semi-sedentary groups during the Bronze Age is statistically different compared to any alternative combination of lifestyle and time period. The null and alternative hypotheses are irreconcilable with each other because either a unique and genetic edifice was produced as the result of two convergent circumstances or, those factors operated independently.

Fourth, this study tests H_0 : the mean presence of archaic TLR SNP variants is statistically equal across Kazakhstan, China, Russia, Mongolia, and Kyrgyzstan. This hypothesis is in competition with H_a : if the mean presence of archaic TLR SNP variants is not statistically equal across Kazakhstan, China, Russia, Mongolia, and Kyrgyzstan then the null hypothesis is rejected, and it is predicted that the mean presence of archaic TLR SNP variants in Kazakhstan is statistically significant compared to the other political entities. The null and alternative

hypotheses present contradictory statements about the impact of geography on allele frequencies. this study frames them as opposing views because geography either leaves a detectable signature within the TLR10-1-6 cluster, or the presence of the archaic TLR SNPs are purely incidental.

Fifth and lastly, this study tests H_0 : the mean presence of archaic TLR SNP variants is statistically equal between the Central Asian study population, and the Kenyan pastoralists. This hypothesis stands in tension with H_a : if the mean presence of archaic TLR SNP variants is not statistically equal between the Central Asian study population, and the Kenyan pastoralists then the null hypothesis is rejected, and it is predicted that the mean presence of archaic TLR SNP variants in Central Asia is statistically significant compared to Kenya. The hypotheses are non-overlapping because they ask, "do two different microevolutionary histories and ecological conditions produce the same immunological outcome?"

This study is being done within the work of firstly, Smith and Haigh (1974)'s work that predicted neutral alleles in high linkage disequilibrium with favorable alleles will go to fixation. If the neutral alleles are in high LD with beneficial ones, then selection will be reflected in genetic distribution of the TLR SNPs between lifestyles, geographic region, and time period. Secondly, Ohta (1992)'s Nearly Neutral theory in which natural selection eliminates nearly neutral mutations in large populations but in smaller populations they behave as neutral and are randomly fixed. Random fixation would inform us whether selection or drift is a more plausible explanation for their frequency.

Synopses of Chapters

Chapter II provides background information on the molecular biology of the TLRs, the differences between the toll-like receptors and their signaling pathways and their primary

function and importance for the immune system are described here. This chapter also gives a general overview of central Asia and why this study used the genetic data from that region. The third chapter contains the materials and methods used for this study. This chapter will first provide an archaeological and population genetic background of each subsample in the form of a literature review. The purpose of this is to justify to the readers their inclusion into one of three mobility groups: Sedentary, Semi-sedentary, and Mobile. Our working logic here is that an individual's mobility would be reflected in pathogen exposure. The chapter will end with a detailed description of the statistical methods used to address the research goals previously laid out. In Chapter IV the results of this inquiry are reported. We start by examining the geographic distribution of the allele and how that distribution changes through time. Here, summary and descriptive statistics are dissected in conjunction with the results of the hypotheses tests. A majority of the figures and tables are presented here as a visual aid. In Chapter V, the results are discussed within the context of current research on aDNA, immunology, and microevolution in humans and fit the current study within our broader understanding of Central Asia as well as the agricultural revolution. Chapter VI concludes this work by summarizing the study and offers potential avenues of further research.

Chapter II

Background

Innate Immunity and the Toll-Like Receptors

Pathogens evolve more rapidly than humans due to their short generation times, large population sizes, and high mutation rates. Because of these factors, human genetic adaptations to the stresses they pose are costly because on the short time scale we live on; natural selection prunes the population from unfit individuals and the time period for the correct adaptation to manifest in response to a pathogen may be prohibitively long (McDonald, 1983). However, the human immune system has evolved mechanisms to compensate for this challenge through innate immunity and adaptive immunity.

The basis of innate immunity is to sustain a discriminatory boundary between self and the fungal, bacterial, and viral non-self. Innate immunity uses a limited number of generalized receptors to detect essential molecular components across a broad array of pathogens (Beutler, 2004; Akira *et al.*, 2006; Turvey and Broide, 2010; McDonald and Levy, 2019). Selective pressures have shaped host populations to optimize this detection capacity.(Beutler, 2004). In response, pathogens have acquired genetic components via natural selection that mimic the hosts through natural selection to evade their immune system.

Innate immunity differs from adaptive immunity in the sense that adaptive immunity exhibits a diverse repertoire of randomly generated receptors shaped by antigen exposure and clonal selection (Müller *et al.*, 2018). In contrast, innate immunity is germline encoded and does not require a developmental phase (Turvey and Broide, 2010). It should be noted however the two types of immunity are not siloed from each other and their cooperation is essential for

immunological function. This is evident based on their expression on T and B lymphocytes and on non-immune cells like epithelial and endothelial cells and fibroblasts (Delneste *et al.*, 2007).

As mentioned earlier, innate immunity works by employing a generalized arsenal of receptors to recognize pathogens. The toll-like receptors are a subset of pattern recognition receptors (PRRs) that are activated at the onset of innate immune reaction (Pal and Chakravarty, 2020). Their function is to detect pathogen-associated molecular patterns (PAMPs), detect damage-associated molecular patterns (DAMPs), and initiate the production of pro-inflammatory cytokines and type I interferons following detection of PAMPs and DAMPs. The downstream effects of PAMP-PRR interactions initially begin with the activation of Nuclear Factor- κ B (NF- κ B), a transcription factor that is responsible for the production of pro-inflammatory cytokines and type 1 interferons (Zhang and Liang *et al.*, 2016).

At present, 10 TLRs have been identified in humans (Takeda and Akira, 2005). While each has different responsibilities in pathogen detection, for simplification, they are subdivided based on which locality of the cell they are confined to. TLRs 1, 2, 4, 5, 6, and 10 reside on the plasma membrane and detect microbial membrane components or PAMPs such as lipoproteins, flagellin, and lipopolysaccharides (Gay *et al.*, 2014). TLRs 3, -7, -8, and 9 are found in the endosome and detect foreign nucleic acids. Furthermore, all TLRs have three primary structural domains. The first structure which is the outer domain and resides on the cell surface consists of a Leucine-rich repeat (LRR) which is a 20–30 amino acid structural motif that folds into a curved, horseshoe-shaped solenoid. The second structure is a single membrane-spanning α -helical segment that is responsible for transmitting a signal to the intracellular homology domain upon ligand binding. The third is a homology domain in the cytoplasm which consists of a ~150–160 amino acid chain responsible for initiating intracellular signaling (Jin and Lee, 2008;

Wang et al., 2024; Brennan and Gilmore, 2018). The LRRs that compose the first domain on the cell surface are especially important because they confer ligand specificity. This enables them to discern between various pathogens and damaged host cells but also to allocate responsibility between themselves (Chen et al., 2024). For example, TLR1 and 6 are functional as a heterodimer with TLR2. TLR6 however, can heterodimerize with both TLR 2 and 4 whereas TLR2 can operate independently of the two (Bezhadi et al., 2021). Similarly, there remains some controversy over the expression of intracellular TLRs 3, -7, and -9 when exposed to HIV and *Mycobacterium tuberculosis* (Nguyen et al., 2020). These examples bring to light the observation that no cell surface TLR is equally attentive to PAMPs because each ligand is tailored to detect a specific molecular pattern. The structural domains of TLRs — particularly the LRRs that govern ligand specificity — represent a phenotype under direct selection pressure. If variation in that structure translates directly into differences in pathogen recognition then the SNPs that impact TLR structure are unlikely to be selectively equivalent between populations that differ in their pathogen exposure.

The hypotheses laid out in this study are only plausible in the context of the biological mechanisms involved in the TLRs recruitment of immune cells. Figure 1 illustrates the steps involved in TLR signaling, amplification and eventual transcription of immune cells. Five different types of signaling adaptor proteins can be induced by the Toll/interleukin-1 receptor/resistance (TIR) domain in mammals which include: myeloid differentiation primary-response protein 88 (MyD88), TIR domain-containing adaptor protein (TIRAP or MAL), TIR domain-containing adaptor protein inducing IFN- β (TRIF), TRIF-related adaptor molecule (TRAM) which is a translocon-associated protein that helps insert proteins into the ER membrane or move them across it, and Sterile α - and armadillo-motif-containing protein

(SARM) (Akira and Takeda, 2004). TLRs recruit adapter proteins that act as a platform to send messages back into the genome to produce immune cells. These come in the form of downstream kinases and signaling intermediates that propagate and amplify the signal received from the TLRs. They include, IL-1R-associated protein kinases (IRAK) 1, 2, 4, and M which are signal transducers that trigger inflammatory and immune responses primarily recruited by MyD88, TGF- β -activated kinase 1-binding protein 2 (TAB2) which is an adapter protein crucial for mediating NF- κ B, Tumor necrosis factor receptor-associated factor 6 (TRAF6) which is another signal transducer that mediates activation of NF- κ B downstream of TLR. The recruitment of these adaptor proteins and signal transducers leads to nuclear translocation of a pro-inflammatory transcription factor, NF- κ B, upon detection of PAMPs or DAMPs, which is critical for an ambulatory engagement with pathogens (Li *et al.*, 2010). This is because a transcription factor like NF- κ B is responsible for the transcription of specific genes that encode different sets of proteins such as pro-inflammatory cytokines and type 1 interferon, chemokines (CXCL8 and CXCL10), and antimicrobial peptides (Moresco *et al.*, 2011).

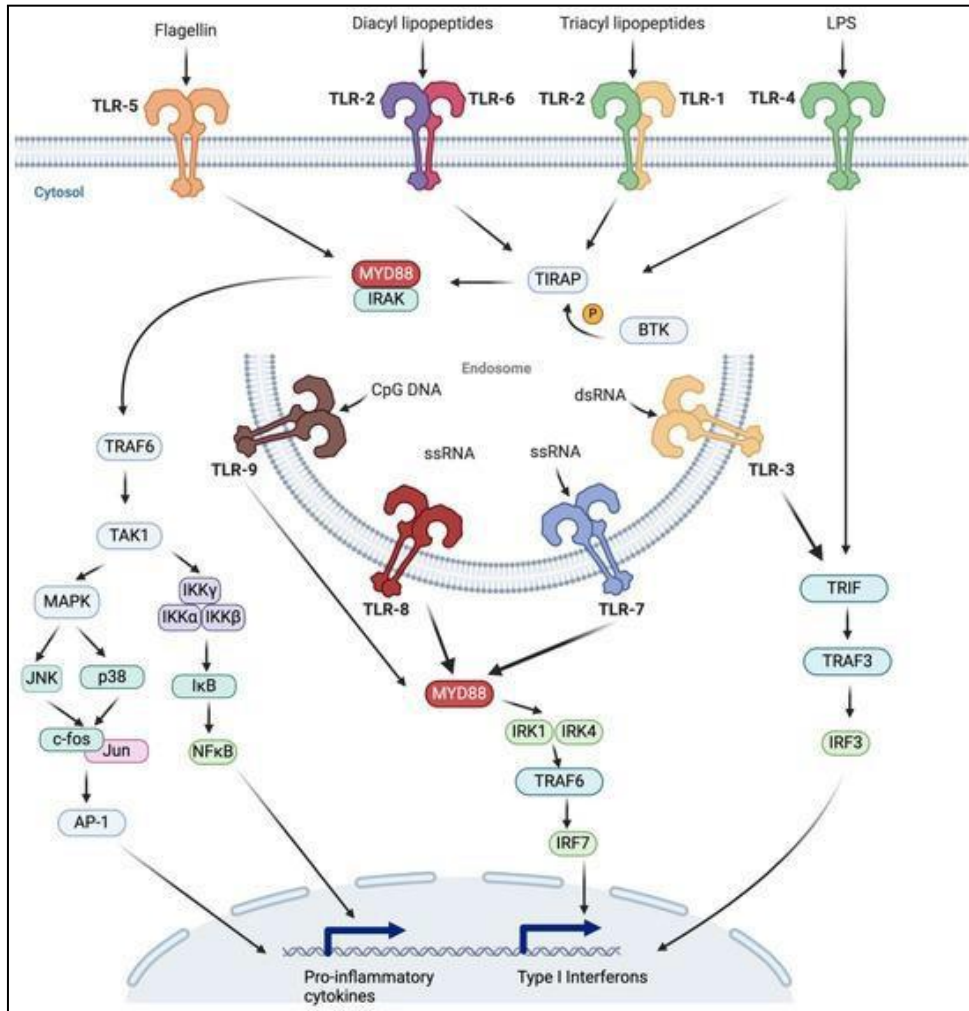


Figure 1. Image adapted from Chen *et al.* (2024) depicting TLR signaling pathway

This study is concerned with five SNPs in the TLR10-1-6 cluster on chromosome 4 (chr4: 38,723,860–38,908,438;). The tandem arrangement of this cluster, seen in Figure 2 appears to be the result of gene duplication as evidenced by a 56% sequence identity between TLR6 and 1 (Georgel *et al.*, 2009 & Kang *et al.*, 2009). This sequence identity also explains the heterodimeric relationship between TLRs 1 and 6 with TLR2 (Bezhadi *et al.*, 2021). Both TLR1 and 6 control ligand specificity of TLR 2, thus the function of TLR2 is dependent on the two (Kang *et al.*, 2009). Additional preliminary research also highlights a competitive role between the three TLRs. The TLR2-mediated response to phenol-soluble modulin, a factor secreted by

Staphylococcus epidermidis, was enhanced by TLR6 but inhibited by TLR1 (Hajjar *et al.*, 2001). TLR1 plays a role in dampening the cytokine response and preventing excessive immune-mediated tissue damage, whereas TLR6 exacerbates inflammatory response. TLR1 and 2 deficiencies increased intestinal inflammation and overgrowth of *Candida albicans* and *Escherichia coli* populations (Choteau *et al.*, 2017). The function of TLR10 remains elusive in contrast to TLR1 and 6. Mikacenic *et al.* (2013) report coding SNPs within TLR10, suggesting it may contribute to some mediation of disease susceptibility. Influenza virus infection has been shown to incite reaction from TLR10 yet its direct responsibility is not clear (Lee *et al.*, 2014). With respect to the aforementioned, this study aims to infer whether the five SNPs discussed in this study impact the function of the TLR10-1-6 cluster.

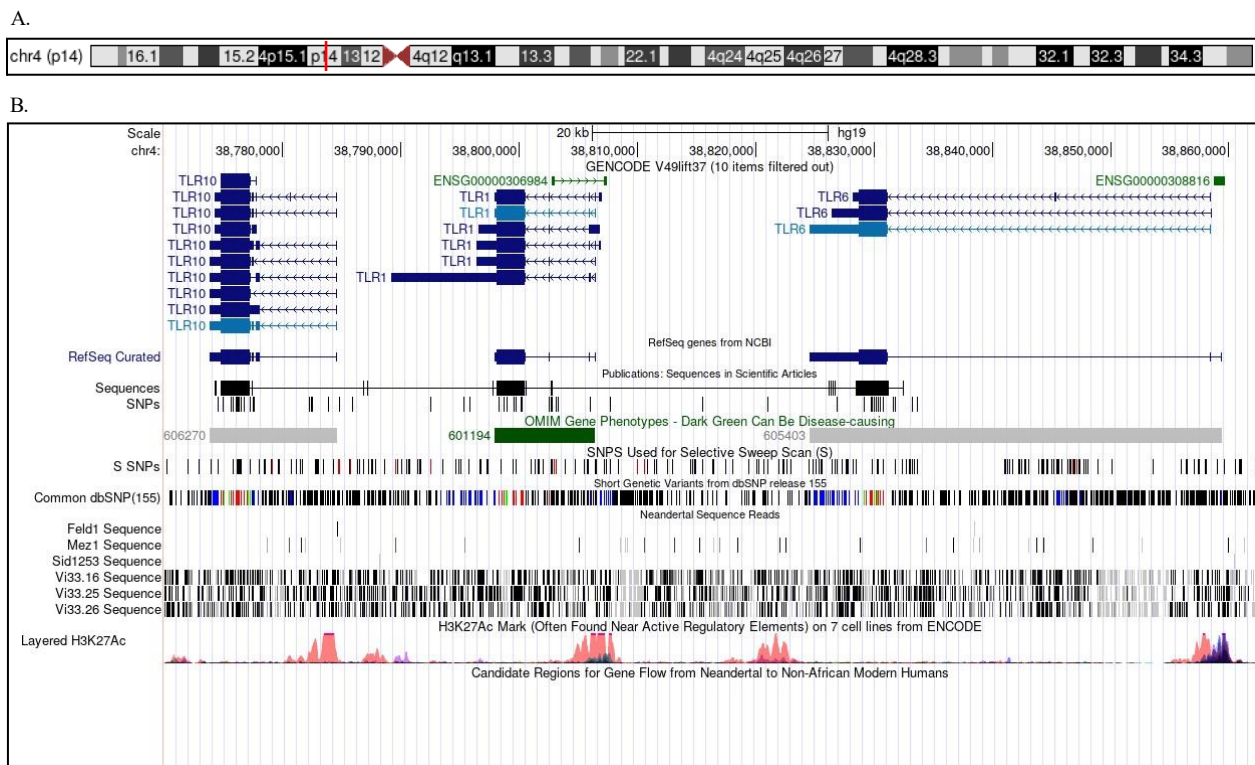


Figure 2. A, location of Toll-like Receptors 10-1-6 on chromosome 4. B, Alignment of TLRs 10-1-6 along hg19 and Neanderthal sequence taken from the UCSC Human Genome Browser, <https://genome.ucsc.edu> (Perez *et al.*, 2025)

TLRs are cellular components that are located across many cell lines in the human body. TLRs are abundantly expressed on all innate immune cells including macrophages, natural killer

(NK) cells, dendritic cells (DCs), monocytes, and neutrophils. TLRs 1, 6, and 10 specifically, are expressed most acutely in the hemolymphoid system (Figure 3.) which include blood cells, bone marrow cells, and in lymph nodes (Robinson-Rechavi *et al.*, 2020 & Bastian, 2021). They are not only distributed the peripheral blood leukocytes but are also expected to be distributed in tissues such as the heart, liver, pancreas, colon, small intestine, lung, kidney, ovary, placenta, testis, prostate, skeletal muscle, and brain (Zarembler and Godowski, 2002). This whole-body abundance reflects a concerted effort to ward off pathogens on multiple fronts. More importantly, the ubiquity of TLRs suggests that despite the evolutionary discrepancy between humans and pathogens, the former has evolved a suite of mechanisms in the form of adaptive and innate immunity to deal with entities it has not encountered.

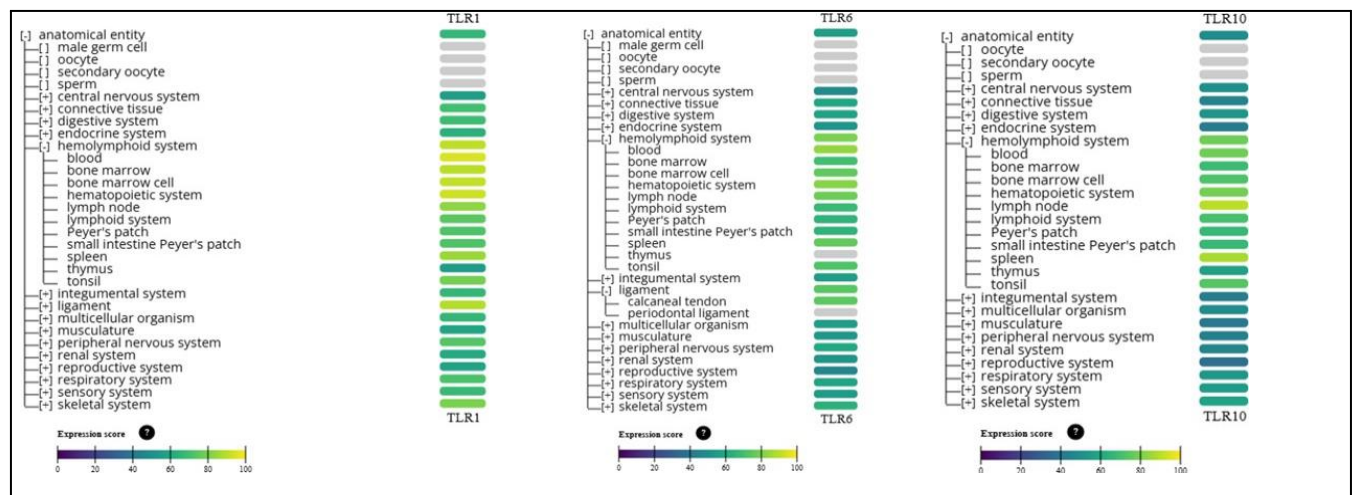


Figure 3. Expression profile of TLR 1, 6, and 10. Taken from the Bgee expression profile tool (Robinson-Rechavi *et al.*, 2020 & Bastian, 2021). In all three cases, expression is most prevalent in the hemolymphoid system, specifically, blood, bone marrow, bone marrow cells, and lymph nodes.

The TLR family is an ancient genetic structure indeed. TLR4 emerged as a distinct entity around 180 million years ago. Then about 150 million years ago, TLRs 3 and 5 diverged from a common ancestral gene. The divergence between TLR 3 and 5 is important because all TLRs

except TLR3 initiate the MyD88-dependent pathway to produce pro-inflammatory cytokines (Yamamoto et al., 2003). Furthermore, TLR3 appears to have co-opted the usage of NF- κ B, the evolutionary origin of which dates to one billion years ago among basal lineages of unicellular organisms on the edge of diversifying into multicellular organisms (Gilmore and Wolenski, 2012). More recently, TLRs 1 and 6 then diverged from a common mammalian ancestral gene ~95 million years ago (Roach et al., 2005). These studies suggest that even on the time scale of millions of years, the TLRs have remained staunchly conserved.

Inner Asian Mountain Corridor (IAMC)

This section provides an overview of the peoples who have historically called the IAMC home. A more detailed accounting than the one provided here is found in Chapter 3; the relevant archaeological literature is reviewed to justify the mobility pattern attributed to each of the subgroups used in this study. Information referring to their genetic background can be found in the Appendix.

It has been a long-held assumption that the transition from bands of hunter-gatherers to agricultural societies in recent history accelerated an evolutionary arms race with pathogens as a consequence of living in higher population densities and sharing more space with animals for extended periods of time. It has been proposed that this process has simultaneously equipped agriculturalists with a more robust immune system, while also making them more prone to disease. Central Asia serves as a case study for investigating allelic frequency changes related to this transition because the archeological remains that have been recovered span ages and belonged to hundreds of individuals who lived various lifestyles (Citations here).

Hunter-gatherer groups occupied the IAMC during 8000-6000 BP. Then between 6000-4000 BP, cultivation of barley, wheat, foxtail millet and broomcorn varied across the region and became part of the human diet, though these groups cannot be referred to as ‘agriculturalists’ (Yan *et al.*, 2025). For further clarification, populations in low-middle elevations consumed more C4 crops, mainly millets because they are drought resistant, than their higher elevation counterparts. In comparison C3 crops like wheat and barley are more typically found at higher elevations and can overcome colder climates (Wang *et al.*, 2020). Furthermore, high altitude valleys of the IAMC were conduits for the spread of broomcorn millet across Eurasia. C4 plants circulated among mountainous populations, likely as human and animal food during the third Millennium BC (Motuzaite Matuzeviciute *et al.*, 2022). Nevertheless, between 4000-3000 BP cattle, sheep and horse were main domestic animals, especially sheep extensive use of barley, wheat, foxtail millet and broomcorn, indicative of increasing population size. In 3000-2000 BP intensity of human activity spanned a broader range in the IAMC and horses became substantially more important during this period (Yan *et al.*, 2025).

Between migration, population expansion, and cultural advances such as adoption of agricultural practices, it has been proposed that central Asia served as a flourishing hotspot because of climatic factors. The Altai Holocene climate optimum took place during 6500-3600 BP. During its peak between 4700-4300 bp provided a favorable climate for prehistoric population expansion and cultural exchange (Xiang *et al.*, 2023). This is corroborated by a body of archeogenetic literature that suggests semi-sedentary and mobile groups of horse herders occupied Central Asia during the Bronze and Iron ages (Gnecchi-Ruscone *et al.*, 2021; Damgaard *et al.*, 2018; Narasimhan *et al.*, 2019). While the archaeology and population genetics of these groups are touched upon in Appendix A, at the moment it is imperative the following is

stated: the Inner-Asian Mountain Corridor served as a conduit for many populations of differing lifestyles to migrate through and admix with each other in ancient history.

This study focuses on lifestyle as a proxy for mobility because there are studies that suggest mobility differentially impacts exposure to novel pathogens and that novelty makes an individual more susceptible to negative impacts of the pathogens (Barnes *et al.*, 2017 & Harrison *et al.*, 2019). For example, Harrison *et al.* (2019) demonstrated that between Batwa rainforest HG and their agriculturalist Bakiga neighbors from Uganda, the viral burden was heavier among the Batwa HG cohort and their genomes contained more signatures of positive selection on innate immune response. A literature review by Barnes *et al.* (2017) outlined risk factors for zoonotic parasitic diseases typical among mobile and semi-sedentary pastoral peoples. In particular, interactions with dogs appear to be a unifying risk factor for the transmission of ZEP. Aside from dogs, herding animals across large ranges opens the door for multiple intermediate and definitive host species to proliferate pathogens between themselves. It may even be the case that the selective pressures imposed by pathogens onto humans may have a limiting factor related to population size. Slavin and Sebbane (2022) suggests that the domestication of horses and an increase of regional travel would have widened the variety of hosts available to *Y. pestis* but outbreaks would be limited by local communities and the route of transmission would have required constant contact.

Wherever humans go, pathogens tend to go with them. As mentioned above, this is evident based on the presence of archaic strains of *Yersinia pestis* found in Eastern Kazakhstan, Mongolia, and Russia and ancient *Salmonella enterica* strains recovered from Russia and the Xinjiang region (Andrades Valtueña *et al.*, 2022; Rasmussen *et al.*, 2015; Key *et al.*, 2020; Wu *et al.*, 2020). These are rare cases and Musralina *et al.* (2019) reported the highest percentage of

pathogens among ancient Central Asians were microorganisms associated with dental caries, gum diseases, and other soft tissue infections in the oral cavity. In contrast, *Yersinia pseudotuberculosis*, *Y. pestis*, *M. tuberculosis*, and *S. enterica* were among the lowest proportion of identified pathogens. The pathogenic burden is not entirely conclusive because we lack substantial data on the frequency of occurrence. Despite this shortcoming, these studies demonstrate that the history of pathogens and peoples in Central Asia is deep regardless of subsistence strategy and that the pathogenic burden is varied.

Chapter III

Materials and Methods

In the past decade and a half, genetic studies of ancient populations have used some amalgamation of various tools and exploratory methods that accentuate particular aspects of the data to provide useful summaries and visualizations to extract population history and reveal changes in allele frequencies over time (Peter, 2022). This study follows in a similar vein by leveraging data from the Allen Ancient DNA Resource (AADR): A curated compendium of ancient human genomes (Mallick & Reich, 2024). It is a publicly available dataset that includes over 16k individuals from across the globe, genotyped at approximately 1.23 million single nucleotide polymorphisms (SNPs) along hg19 (Mallick *et al.*, 2024). All data filtering, analyses, and visual productions were performed in Rstudio (v4.2.2).

Exclusion Criteria and Sample Construction

Because this paper focuses on Central Asia, samples in the AADR (Mallick & Reich, 2024 & Mallick *et al.*, 2024) were filtered by political entities and localities to include individuals found in Kazakhstan, Tajikistan, Mongolia, Kyrgyzstan, the Altai Mountains in Russian, and the Xinjiang region. After filtering by political entities and localities, the remaining number of individuals was 869 divided into 385 distinct archeological groups (here, archeological groups are used interchangeably with distinct genetic populations and populations more generally).

From there, it was necessary to establish group mobility as a variable in the data. The first step in this process was to set a 10-person minimum threshold and any group that did not meet this requirement was removed from the study. If groups spanned multiple sites or could be subsumed into larger cultural complexes then they were consolidated under such terms. In other

cases, a single site would be their designated group name for analysis. After the minimum threshold was met, archeological literature was used to infer mobility patterns. This was essential because when referring to subsistence strategy, there are degrees of mobility associated with them. The groups were given one of three labels of analysis: Semi-Sedentary, where a portion of its members are sedentary and others have extensive mobility or they inhabit multiple regions throughout the year in a cyclical manner; Sedentary – groups that inhabit their region on a year round basis and may build permanent structures; Mobile, the members of these groups do not set up permanent structures, nor do they set up temporary residence and return to it.

There were several groups of which exceeded the minimum threshold but were removed due to temporal inconsistencies. Time is an exclusionary criterion because groups that span millennia are unlikely to exhibit cultural continuity from their inception to their extinction which makes determining their mobility pattern a matter of subjectivity. Establishing group mobility is best done when the archeological group in question is localized to a specific time period. For example, Mongolia_Sukhbaatar_MLBAXiongnuLateMedieval (n = 11), was removed from further analyses due to their broad temporal range.

Mongolia_Khuvsgul_MLBALateMedieval (n = 11) was excluded due to temporal inconsistencies with the Xiongnu period. This culture lasted about 300 years from 200 BCE to 50 CE – the dating of these samples ranges from 282 BCE to 1376 CE (Brosseder and Miller, 2011). The Xiongnu cultural group whose burials have experienced episodes of intrusion at multiple sites along with severely high standard deviations in mean dates making their temporal placement an arduous task was removed from further analyses. Mongolia_LBA_CenterWest_4 (n = 10) draws from multiple sites whose persons cannot be unified under one cultural/ archeological group; therefore, they were excluded from further analysis. Individuals in

Mongolia_EIA_SlabGrave_1 previously reported in Wang *et al.*, (2021), were said to have lived in a singular region starting around the 10th - 8th century BCE until the 5th century BCE in their supplementary materials. The authors also mention the presence of burial goods dating to the 1st millennium BCE, ' due to a lack of descriptions regarding the archaeological context each of these sites were excavated in, they were omitted from further analyses.

Archaeological Sample

The data shrunk to 298 individuals grouped into 14 archeological groups from an original 869 after reviewing the relevant literature. Each archeological group's composition that lists the names used in the AADR can be found in Appendix A. Additionally, certain groups required a higher order of description and were left in Appendix A. For example, 13 individuals from the Chemurchek culture (originally reported in Damgaard *et al.*, 2018 & Jeong *et al.*, 2020) were given the label of "Mobile," although they were likely a part of a broader agro-pastoralist economy, given their association with the late Afanasievo culture (Wang *et al.*, 2021). At present, no settlements have been identified and are known only from their mortuary practices. In their burials, the remains of sheep, goat, and cattle were recovered and it has been demonstrated by Ventresca Miller *et al.* (2022) they consumed sheep (*Ovis*) and ruminant (*Caprinae*) milk. Furthermore, while they had exposure to domesticated animals, the periodicity of said contact is unknown. The genetic profile of the Chemurchek suggests that southern groups exhibit stronger ancestry of hunter-gatherers found in the Baikal region (80%), while the northern Chemurchek individuals are more closely related to a woman found at Botai, a hunter-herder site at ca. 3500 BCE (Damgaard *et al.*, 2018 & Jeong *et al.*, 2020).

This study used one control group in our study, "Kenya_PastoralN" (n=12), previously reported in Prendergast *et al.* (2019). Carbon and nitrogen isotope values indicate very high amounts of protein from C4 plants or C4 grazing-herbivores (Ambrose and DeNiro, 1986 & Ambrose, 1986), yet there is no indication the C4 in their diets came as the result of agricultural cultivation like in the case of the 13 individuals from the Ji'ierzankale (Kumar *et al.*, 2022). There is also no record of permanent habitation or temporary settlements pointing to seasonal migration. No grave goods were recovered but artifacts such as a stone bowl and chert flakes have pointed researchers to the conclusion they were pastoral. However, there are no faunal remains found in association with the individuals and Prendergast *et al.* (2019) reported only a single instance of lactose persistence in an individual not present in the sub-sample used here. In the view of the author, these are likely mobile individuals whose lives depended on hunting and gathering.

Chemurchek, Kenyan pastoralists and every other group's archeological background is summarized in Table 1 where the subsample name, n, the time period they lived in, political entity they were excavated from, mobility pattern, and the sources that were consulted during the process of this study. Additionally, these groups are depicted in Figure 4, which shows where in Central Asia they are found and the Time period they lived in, based on archaeological data.

Table 1. Archeological Sample

Subgroup	n	Time	Mobility	Habitation pattern	Mode of subsistence	Did they construct dwellings?	Source
Saka	38	2739 – 1129 BP	Semi-Sedentary	Intermittent	millet agriculture	Yes	Beisenov (2018); Jeong et al. (2019); Gneccchi-Ruscione et al. (2021)
Afanasievo	24	5061 – 4479 BP	Semi-Sedentary	seasonal migration	millet agriculture	Yes	Hermes et al. (2020); Honeychurch et al. (2021); Wang et al. (2021); Kumar et al. (2022); Narasimhan et al. (2019); Zhang et al. (2021)
Tian Shan Huns	24	1802 – 1134 BP	Sedentary	Permanent settlement	Unknown	No settlement has been found	Damgaard et al., (2018); Gneccchi-Ruscione et al. (2025)

Sarmatians	24	2400 – 1109 BP	Semi-Sedentary	Seasonal migration	cattle-breeding	No; some members adopted a totally sedentary lifestyle	Davis-Kimball et al. (1995); Damgaard et al. (2018); Gneccchi-Ruscione et al. (2021)
Tasmola	22	3318 – 2246 BP	Semi-Sedentary	Seasonal migration	Small-scale agriculture		Beisenov (2018).; Beisenov et al., (2016); Svyatko & Beisenov, (2017); Gneccchi-Ruscione et al. (2021)
Andronovo	21	3761 – 3100 BP	Semi-Sedentary	Seasonal migration	Consumption of domestic sheep, cattle and horse products; no evidence of agriculture	construction of fortified settlements	Outram et al. (2012); Jia et al. (2020); Grigoriev (2021); Damgaard et al. (2018); Narasimhan et al. (2019); Kılınç et al. (2021); Kumar et al. (2022); Blöcher et al. (2023)
Russian Angara HG	21	7992 BP – 4251 BP	Mobile	Frequent migration throughout the area	Hunting and gathering	No evidence of dwelling construction	Losey et al., (2016); Losey et al. (2017); Weber and Goriunova (2013); Yu et al. (2020); Kılınç et al. (2021)
Sagly	18	2326 – 2131 BP	Semi-Sedentary	intermittent		yes	Mary et al. (2019); Jeong et al. (2020); Wang et al. (2021);
Khövsgol	20	3389 – 2880 BP	Semi-Sedentary	Seasonal migration	Animal husbandry	Temporary settlement	Houle (2010); Frohlich et al. (2010); Jeong et al. (2018)
Zhagunluke	16	2415 – 1945 BP	Sedentary	Permanent habitation	horns and bones of sheep, goats, cattle, and horses along with horsewhips, wheat or barley products, harrow and millstone	Constructed permanent settlement	Yang et al. (2025); Want et al. (2025); Nobaru et al. (2015); Wang et al. (2003); Kumar et al. (2022)
Pazyryk	19	2300 – 1615 BP	Semi-Sedentary	Seasonal migration	agricultural cattle-breeding	Temporary log dwellings built	Tishkin and Dashkovsky (2019); Gneccchi-Ruscione et al. (2021); Polosmak (2023); Rubinson and Linduff (2024)
Chemurchek	13	4632 – 4038 BP	Mobile	Frequent migration	No evidence of agricultural practices	No settlement has been found	Damgaard et al. (2018a); Jeong et al. (2020); Wang et al. (2021); Ventresca Miller et al. (2022)
Ji'ierzankale	13	2515 – 2351 BP	Semi-Sedentary	Intermittent migration	Small scale agriculture indicated by consumption of C4 plants, likely millet	No settlement has been found	Wang et al. (2016); Xie et al. (2022); Kumar et al., (2022); Guo et al. (2020); Shen et al., 2015
Xiaohe	13	3959 – 3645 BP	Sedentary	No evidence of outward migration. DNA evidence suggests inward migration and settlement	Grain products and animal husbandry	No settlement has been found	Qu et al. (2018); Zhang et al. (2017); Li et al. (2015); Li et al. (2013); Bergman (1939); Yang et al. (2014); Zhang et al. (2021)
Kenyan Pastoralists	12	3241 – 1654 BP	Mobile	No evidence of permanent or temporary settlement	Likely hunting and gathering	No settlement has been found	Ambrose and DeNiro (1986); Ambrose (1986); Prendergast et al. (2019)

Semi-Sedentary, where a portion of its members are sedentary and others have extensive mobility or they inhabit multiple regions throughout the year in a cyclical manner; Sedentary groups inhabit their region on a year-round basis and may build permanent structures; Mobile, the members of these groups do not set up permanent structures, nor stay in one spot for extended periods of time. If groups spanned multiple sites then they were consolidated under identifiable cultures. In other cases, a single site would be their designated group name for analysis.

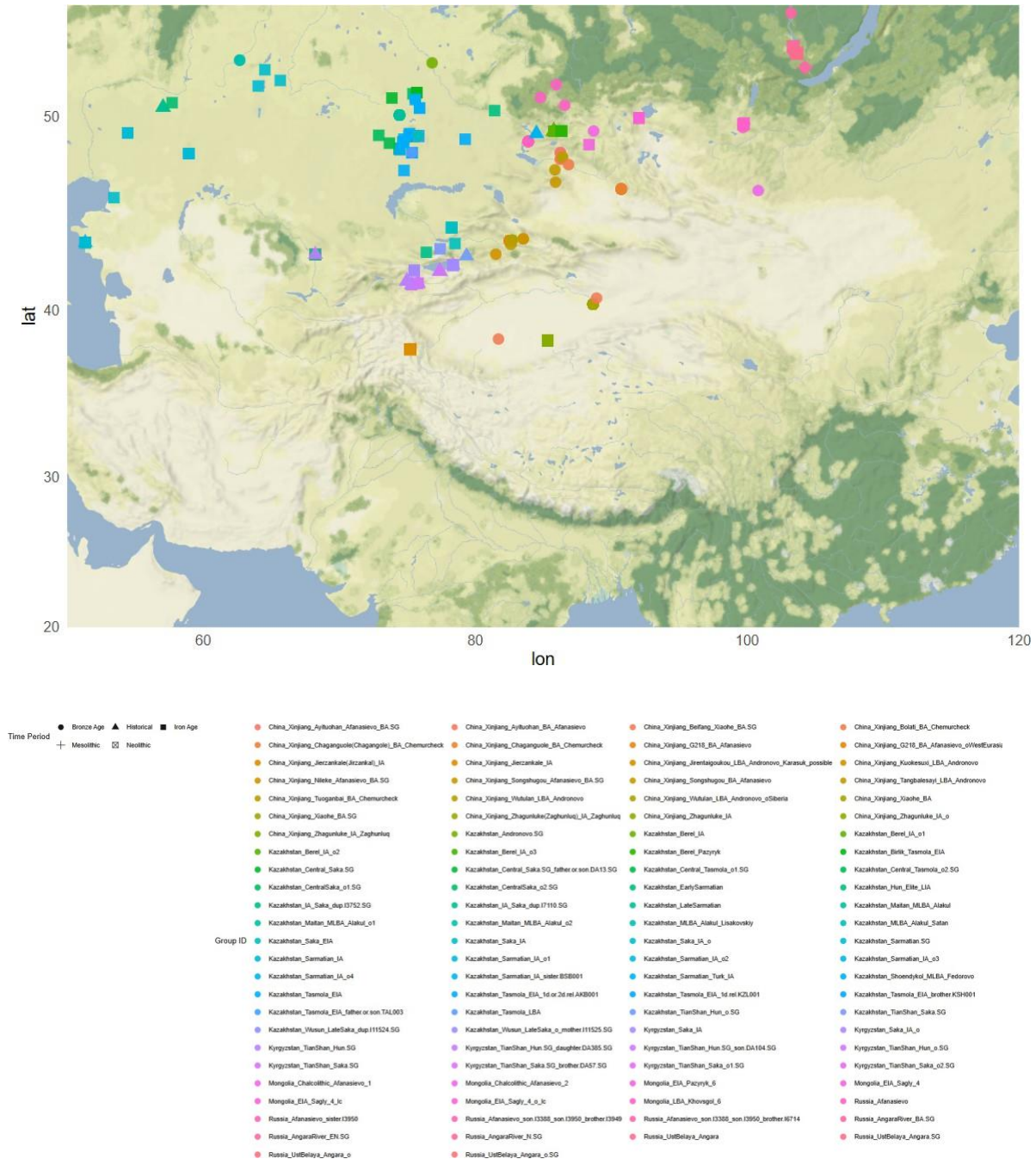


Figure 4. Archaeological Sample

After filtering through the data and determining the groups of interest mobility pattern through a literature review our sample was reduced to 298 individuals lumped into 9 semi-sedentary, 3 sedentary, and 3 mobile groups based on the archeological data reviewed above. Appendix B shows how each archeological group was lumped together.

Genetic Sample

Establishing a cadre of SNPs to examine was next. To do this, 30 archaic SNPs from the supplementary materials found in Table s10 in Danneman *et al.* (2016) were filtered for with the AADR. Out of the 30, only 5 were present – rs11466640, rs3924113, rs5743563, rs5743560, and rs11725779. Once a set of SNPs was established, Ensembl (release 155) [Dyer *et al.*, 2025]] was used to confirm their genetic components and find associated phenotypes and conditions. Table 2 provides each SNP, its allele, position on chromosome 4, the type of genetic component it is, how it is coded in the AADR, and phenotypes associated with those loci. Each SNP is described here: rs11466640 is an intron variant implicated in studies on risk for prostate cancer (Stark *et al.*, 2009; Tindall *et al.*, 2010; Kutikhin and Yuzhalin, 2012; Rogers *et al.*, 2013), moderate-to-severe asthma (Dong *et al.*, 2020), and *Helicobacter pylori* serologic status (Senturk and Ergoren, 2020). rs3924113 is an intergenic variant factored in circulating cytokine levels (Konieczny *et al.*, 2025). rs5743563 is a splice polypyrimidine tract variant implicated in studies on HIV-1 Infection (Rozman *et al.*, 2023), cryptococcal meningitis (Jiang *et al.*, 2018), Melioidosis (Chantratita *et al.*, 2014), bacterial vaginosis among HIV-1 infected adolescents (Royse *et al.*, 2012), and *Helicobacter pylori* serologic status (Senturk and Ergoren, 2020). rs5743560 is an intron variant associated with rheumatoid arthritis discussed in Jaen *et al.* (2009). rs11725779 is an intergenic variant with no known associations with ailments.

Table 2. SNPs available in AADR

SNP	Allele	Position on Chr 4	Type	Genotype	Associated Phenotypes
rs11466640	G	38,778,903	Intron variant	2	Risk for prostate cancer; moderate-to-severe asthma; <i>Helicobacter pylori</i> serologic status
rs3924113	G	38,796,255	Intergenic variant	2	Circulating cytokine levels
rs5743563	G	38,806,019	splice polypyrimidine tract variant	0	HIV-1 Infection; cryptococcal meningitis; Melioidosis; bacterial vaginosis among; HIV-1 infected adolescents; <i>Helicobacter pylori</i> serologic status
rs5743560	G	38,806,200	Intron variant	2	Rheumatoid arthritis
rs11725779	A	38,811,668	Intergenic variant	0	NA

Genotype matrices such as the one used here, often code SNPs as 1, 2, 0, or 9 (or NA) to reflect homozygosity for the alternative allele, heterozygosity, homozygosity for the reference allele, or missing data. But because genomic coverage of ancient samples is often low and subsequently produced as pseudohaploid data, zygosity is uninferable. To overcome this, it was decided that an absence/ presence system would be used for various purposes where 1 indicates a carrier of the archaic allele and 0 signifies the derived variant being present. NAs were left as they were. This counting system was used to determine the ratio of archaic-to-alternative alleles present in each archeological group. In Table 3, each subsample, their time period, mobility, sources, and ratio of archaic to alternative alleles, is depicted below.

Table 3. Genetic Sample

Subgroup	Political Entity	n	Time	Mobility	Source	rs11466640 Archaic : modern	rs3924113	rs5743563	rs5743560	rs11725779
Saka	Kazakhstan; Kyrgyzstan	38	2739 – 1129 BP	Semi-Sedentary	Beisenov (2018); Jeong et al. (2019); Gneecchi-Ruscione et al. (2021)	17:5	3:1	4:14	15:4	5:16
Afanasievo	China; Mongolia; Russia	24	5061 – 4479 BP	Semi-Sedentary	Hermes et al. (2020); Honeychurch et al. (2021); Wang et al. (2021); Kumar et al. (2022); Narasimhan et al. (2019); Zhang et al. (2021)	10:6	3:0	2:8	10:4	6:11
Tian Shan Huns	Kyrgyzstan; Kazakhstan	24	1802 – 1134 BP	Sedentary	Damgaard et al., (2018); Gneecchi-Ruscione et al. (2025)	13:2	2:0	3:6	9:5	6:11
Samatians	Kazakhstan	24	2400 – 1109 BP	Semi-Sedentary	Davis-Kimball et al. (1995); Damgaard et al. (2018); Gneecchi-Ruscione et al. (2021)	7:4	1:1	4:6	7:4	7:5
Tasmola	Kazakhstan	22	3318 – 2246 BP	Semi-Sedentary	Beisenov (2018).; Beisenov et al., (2016); Svyatko & Beisenov, (2017); Gneecchi-Ruscione et al. (2021)	16:2	3:0	2:11	16:2	5:16
Andronovo	China; Kazakhstan	21	3761 – 3100 BP	Semi-Sedentary	Outram et al. (2012); Jia et al. (2020); Grigoriev (2021); Damgaard et al. (2018); Narasimhan et al. (2019); Kilinç et al. (2021); Kumar et al. (2022); Blöcher et al. (2023)	10:2	1:0	2:10	12:1	0:12
Russian Angara HG	Russia	21	7992 BP – 4251 BP	Mobile	Losey et al., (2016); Losey et al. (2017); Weber and Goriunova (2013); Yu et al. (2020); Kilinç et al. (2021)	8:2	0:0	0:6	6:2	1:5
Sagly	Mongolia	18	2326 – 2131 BP	Semi-Sedentary	Mary et al. (2019); Jeong et al. (2020); Wang et al. (2021);	10:6	0:0	2:9	11:3	4:11
Mongolia_LBA_Khovsgol_6	Mongolia	20	3389 – 2880 BP	Semi-Sedentary	Houle (2010); Fröhlich et al. (2010); Jeong et al. (2018)	15:1	3:0	3:9	11:5	3:14
Zhagunluke	China	16	2415 – 1945 BP	Sedentary	Yang et al. (2025); Want et al. (2025); Nobaru et al. (2015); Wang et al. (2003); Kumar et al. (2022)	5:6	2:2	5:4	5:4	8:4
Pazyryk	Kazakhstan; Mongolia	19	2300 – 1615 BP	Semi-Sedentary	Tishkin and Dashkovsky (2019); Gneecchi-Ruscione et al. (2021); Polosmak (2023); Rubinson and Linduff (2024)	9:2	2:1	1:9	10:1	1:12
Chemurchek	China; Mongolia	13	4632 – 4038 BP	Mobile	Damgaard et al. (2018a); Jeong et al. (2020); Wang et al. (2021); Ventresca Miller et al. (2022)	9:2	1:1	2:5	8:2	3:9
China_Xinjiang_Jierzankale_IA	China	13	2515 – 2351 BP	Semi-Sedentary	Wang et al. (2016); Xie et al. (2022); Kumar et al., (2022); Guo et al. (2020)	4:5	0:2	2:3	1:4	5:5

Xiaohe	China	13	3959 – 3645 BP	Sedentary	Qu et al. (2018); Zhang et al. (2017); Li et al. (2015); Li et al. (2013); Bergman (1939); Yang et al. (2014); Zhang et al. (2021)	7:1	2:0	2:4	5:3	3:5
Kenya_PastoralN	Kenya	12	3241 – 1654 BP	Mobile	Ambrose and DeNiro (1986); Ambrose (1986); Prendergast et al. (2019)	4:2	1:0	2:3	3:2	2:4

Ratios of archaic to alternative alleles were based on a binary carrier status. These ratios do not add up to the total number of individuals (n) in each subsample due to missing data.

Several maps were created to assess when and where individuals in our data carry the archaic haplotypes for the five SNPs along with stacked bar charts and frequency graphs. This was essential because it permits inferences about the types of ecosystems that these alleles were subjected to. It was also important to be able to track how the frequency of these alleles changed over time in different regions and also among different mobility patterns.

Statistical Procedures

The first question this study aims to answer concerns the strength of selection acting on these alleles — specifically, the relative fitness difference conferred by each archaic allele, as quantified by the selection coefficient (s) (Haldane, 1926). To calculate s for SNPs rs11466640, rs3924113, rs5743563, rs5743560, and rs11725779, stepadna.R, a software described in Muktopavela *et al.* (2022), was utilized. This software utilizes pseudohaploid genotypes to estimate s and confidence intervals for both before and after 5000 BP – this demarcation is used to account for population dynamics and migration in deeper time. Because stepadna.R tracks $2s$ and some alleles were coded as 0, genotype coding was swapped for rs11466640, rs3924113, and rs5743560. Furthermore, a date of 45,000 ya was used as an anchor for the introduction of derived alleles via Neanderthal introgression into modern human populations, as admixture between the two lineages is assumed to have initiated selection in subsequent ancestors (Sankararaman *et al.*, 2012; Sümer *et al.*, 2024; Iasi *et al.*, 2024). Because stepadna.R requires modern data, a sample of 1,536 individuals from Kazakhstan, Tajikistan, Russia, China,

Mongolia, and Kyrgyzstan was merged with the ancient dataset for this analysis only. The outgroup (Kenya_PastoralN) was excluded from this analysis, because this study is focused on the strength of selection within Central Asia.

A principal components analysis (PCA) was then conducted to examine how the groups of interest cluster. PCA has been widely employed in archaeogenetic research since Reich et al. (2009); see also Patterson et al. (2006). The method leverages binary SNP data to create useful summaries of population genetic substructure. A common challenge when applying PCA to ancient genotype data is high amounts of missing data (Cipollaro *et al.*, 2005; Disotell, 2012). To overcome this, the mean value of in-group allele frequency was imputed to accommodate PCA. In rare instances, identical SNP vectors within Group IDs produced duplicate PCA coordinates. These were identified when multivariate outliers in PC1–PC2 space were detected using Mahalanobis distance with a $\chi^2(2, 0.975)$ threshold, which flagged multiple duplicate groups (Warren *et al.*, 2011). Although it is commonplace to project ancient samples onto contemporary ones, it was decided that since finding metadata pertaining to mobility patterns of contemporary populations would be an arduous task, this practice was neglected. This decision is also further justified by the fact that this study is not interested in lineage like most aDNA studies.

This study sought to determine the most significant predictor of allele presence for the five TLR-related SNPs. In short, the hypotheses under interrogation may be summarized as follows: factors such as mobility, time period, a combination of the two, and geography are not significant contributors to allele presence and each one of these variables may be further broken down into a null repudiating their contribution to allele presence as well. To test these hypotheses, a set of Generalized Linear Mixed Models (GLMM) were produced. GLMMs have recently been applied in ancient DNA research to detect signatures of selection (Akbari *et al.*,

2024; 2026). The present study follows suit with caveats: absence/ presence data like the one used here have been incorporated into ecological studies and GLMMs accommodate the non-normal distribution of repeated observations inherent to genotype data structured by time period and geography (Bolker *et al.*, 2009). GLMMs are also well-suited to non-orthogonal predictors, as time period and geography (and lifestyle) are not orthogonal and likely to be correlated in the archeological record therefore, archeological group membership must be modelled as a random effect.

Generally speaking, this study uses Type III Wald Chi-square to test if the probability that an individual carries an archaic allele for the TLRs is statistically different when comparing mobility patterns, time period, a combination of the two, and geography. Each of these variables are broken into their own hypothesis in redundant fashion as follows: First, H₀: the mean presence of archaic TLR SNP variants is statistically equal between sedentary, semi-sedentary, and Mobile groups. This is in competition with H_a: If the mean presence of archaic TLR SNP presence is not statistically equal between sedentary, semi-sedentary, and mobile groups. H_a predicts that semi-sedentary groups will differ from both sedentary and mobile groups. A multilevel logistic GLMM was used, with mobile groups as the reference category, because sedentary and semi-sedentary populations are more likely to experience pathogen pressures associated with sedentism.

Hypothesis 2 — H₀: Mean archaic TLR SNP presence is statistically equal across the Bronze Age, Iron Age, and recent history. H_a predicts that Bronze Age presence will differ from later periods. A GLMM with time period as an unordered factor with the Bronze age as the reference group was produced. The Mesolithic and Neolithic subsamples were excluded due to sparse representation (Mesolithic $n = 1$; Neolithic $n = 6$). Although population size contributes to allele frequency, our study is interested in whether an individual conceived during the Bronze

age or Iron age, for example, is more likely to inherit an archaic variant of the five TLR SNPs, compared to other time periods. In this analysis Kenyan pastoralists were excluded due to insufficient representation across time periods.

Hypothesis 3 — H_0 : Mean archaic TLR SNP presence is statistically equal across all combinations of lifestyle and time period. H_a predicts that semi-sedentary groups during the Bronze Age will differ from all other lifestyle–period combinations. A multifactorial GLMM was used to disentangle the potentially confounding effects of mobility and time period — for example, semi-sedentary Steppe populations may be disproportionately represented in the Bronze Age relative to sedentary farmers in the Iron Age. Mesolithic, Neolithic, and Historical subsamples were excluded due to sparse cells, and Kenyan pastoralists were also excluded because they perfectly confounded with Mobility for this test.

Hypothesis 4 — H_0 : the mean presence of archaic TLR SNP variants is statistically equal across Kazakhstan, China, Russia, Mongolia, and Kyrgyzstan. H_a predicts that Kazakhstan will differ significantly from the other regions. A GLMM with random effects was used to test if regional differences account for allele presence. The author thought this would be the appropriate test because biological variation is almost always correlated across SNPs, a random effect for SNP was included to control for baseline allele frequency differences would be needed to prevent inflated Type I error rates. Kenyan pastoralists were excluded from this comparison, as they perfectly confounded with the outgroup indicator, and were instead compared against the full Central Asian ingroup in a separate model.

Hypothesis 5 — H_0 : Mean archaic TLR SNP variants is statistically equal between the Central Asian study population and Kenyan pastoralists. H_a predicts that Central Asian ancestry is associated with significantly greater archaic TLR SNP presence. A GLMM with random effects was used to test whether Central Asian ancestry predicts the presence of archaic TLR

SNPs.

For all of the tests mentioned, statistical significance criteria were based on the returning p -values and Chi-square goodness of fit [χ^2 (Type III)]. Furthermore, there is a substantial imbalance in group sizes across all predictors. To deal with this, post hoc sensitivity analyses were performed using balanced subsamples — down-sampled to match the smallest group — to assess the robustness of results.

Chapter IV

Results

Chapter Summary

Chapter 4 compiles the results of this study. First, it includes the descriptive and summary statistics used for initial data analysis. Here, the mean allele presence was calculated along with allele trajectories for each SNP by lifestyle. Following this, readers can expect to see the results of a principal components analysis that was used to determine whether any clusters were formed based on allele presence, lifestyle, time period, and geography. Then the calculation of selection coefficients (s) for rs11466640, rs3924113, rs5743563, rs5743560, and rs11725779 are reported. Secondly, the chapter includes the results of this study's tested hypotheses. Each hypothesis was tested using a Generalized Linear Mixed Model (GLMM) to determine if lifestyle, time period, a combination of the two, or geography are significant predictors of archaic TLR allele presence.

Descriptive Statistics and Initial Analyses

Archaic variants are slightly more common than the alternative alleles across all Central Asian subsamples. Mean allele presence was highest among sedentary groups (0.562; $n = 53$), followed by mobile groups (0.531; $n = 34$) and semi-sedentary groups (0.518; $n = 211$) (Table X). The elevated presence among the sedentary groups runs counter to the initial assumption that semi-sedentary groups would have higher mean presence of these alleles as a result of selection pressure from both novel and endemic pathogens.

Table 4. Mean allele presence by lifestyle

Lifestyle	Mean archaic TLR presence
Sedentary	0.562
Mobile	0.531
Semi sedentary	0.518

This study sought to better understand the temporal and geographic distribution of five archaic TLR SNPs. Figure 6 depicts the distribution of each SNP by mobility pattern. Each map shows both allelic variants and individuals who are missing it. Most notably, throughout all time periods, carriers of archaic TLR SNPs cluster where the borders of Kazakhstan, Russia, Mongolia, and China meet in the Altai Mountain range. With the exception of rs5743563, the archaic haplotypes for the other four SNPs appear to be a consistent feature of Tian Shan populations.

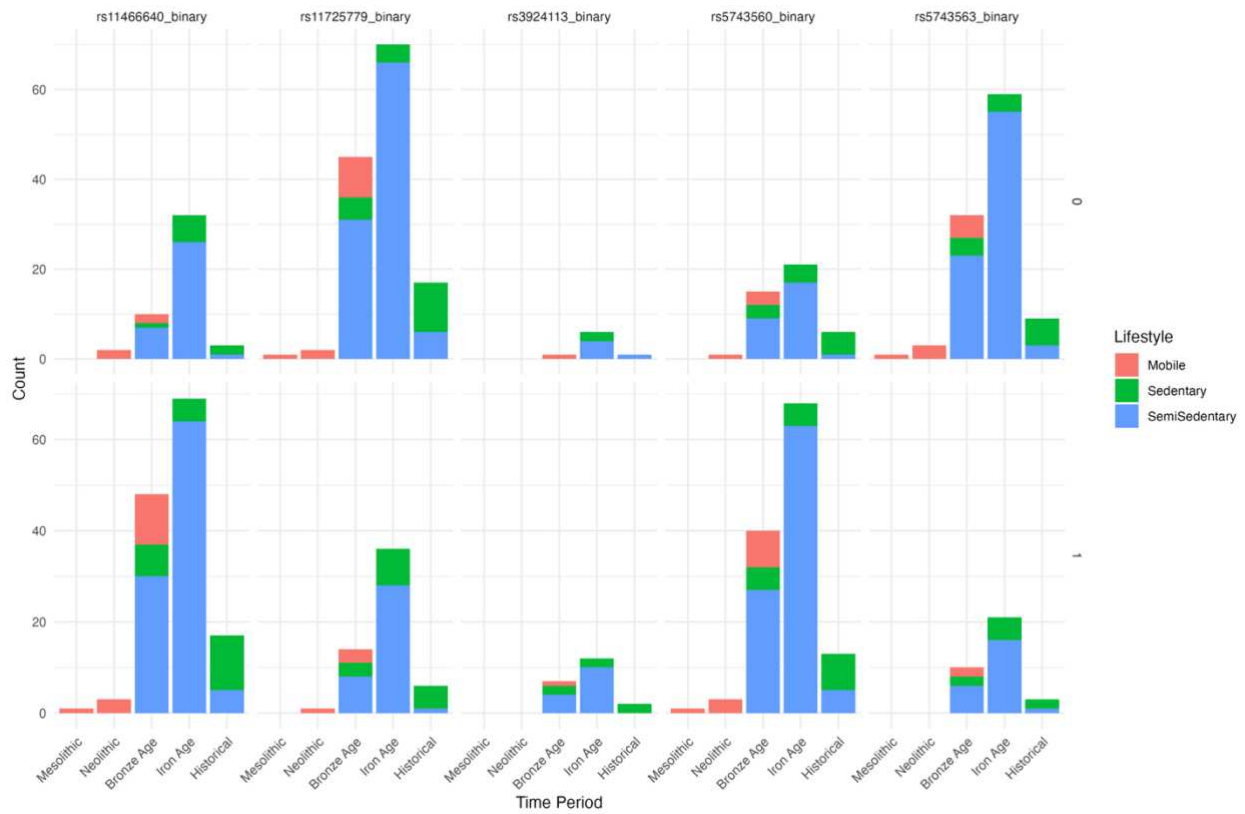


Figure 5. Archaic TLR SNP allele frequency across time periods by lifestyle. Each curve shows allelic frequency for rs11466640, rs3924113, rs5743563, rs5743560, and rs11725779 through time by mobility pattern, with the sample size for each time period labeled above the line. Orange: Mobile; Green: Sedentary; Blue: Semi-Sedentary.

Figures 6 and 7 show PC1 vs. PC2 and PC1 vs. PC3. No distinct clusters emerged along any axis for groupings by geography, lifestyle, or time period. Two multivariate outliers were identified in PC1–PC2 space: an Afanasievo individual from Xinjiang, China, and Iron Age individuals from Zaghunluq. It was found necessary to plot the first three principle components as they explain 44.8, 17.5, and 15.6% of the data.

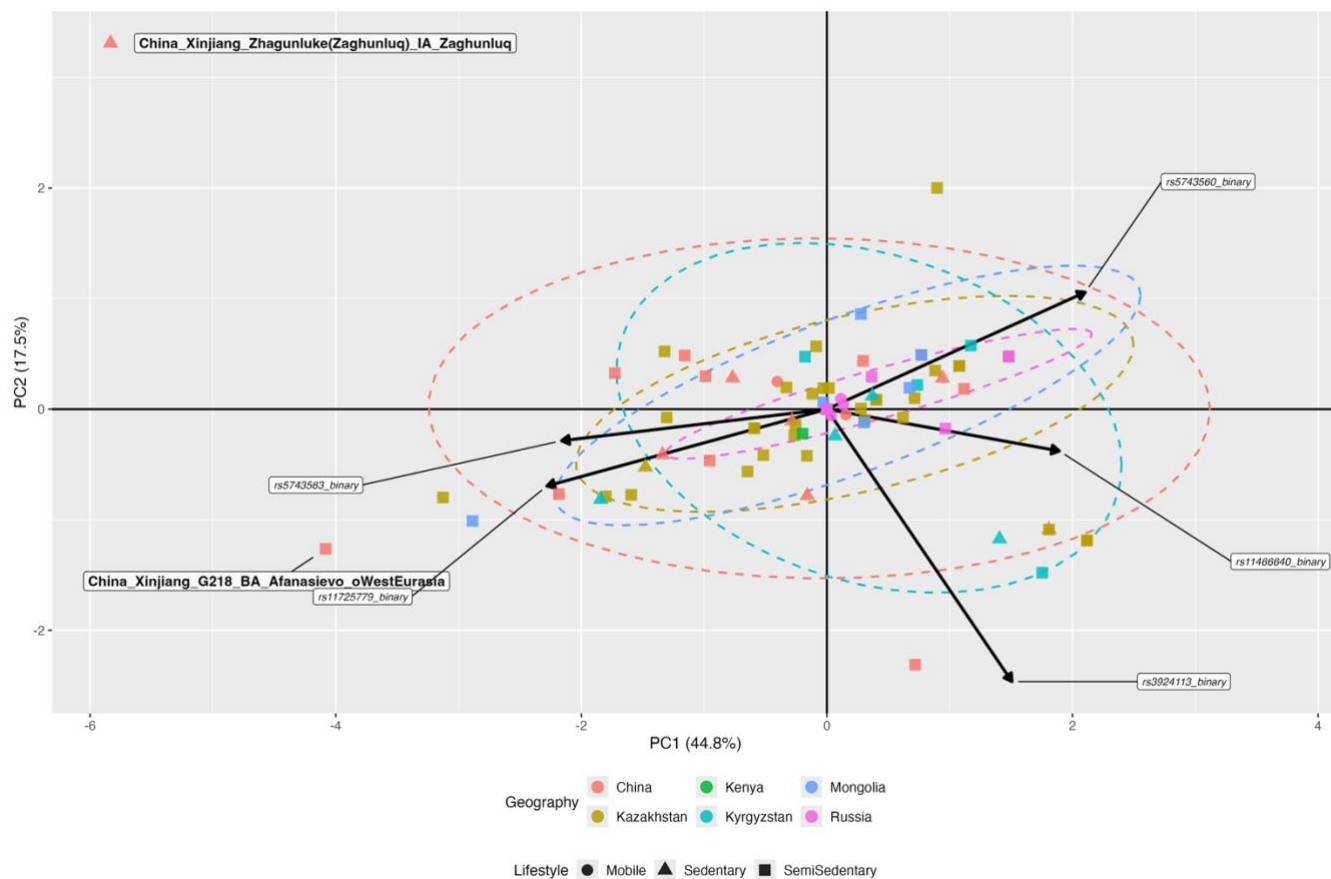


Figure 6. PC 1 vs PC 2 of each group by Time Period, Lifestyle and Geography, and archaic allele presence. Shapes reflect lifestyle, whereas color reflects geography. Here, both Xinjiang outliers were labeled.

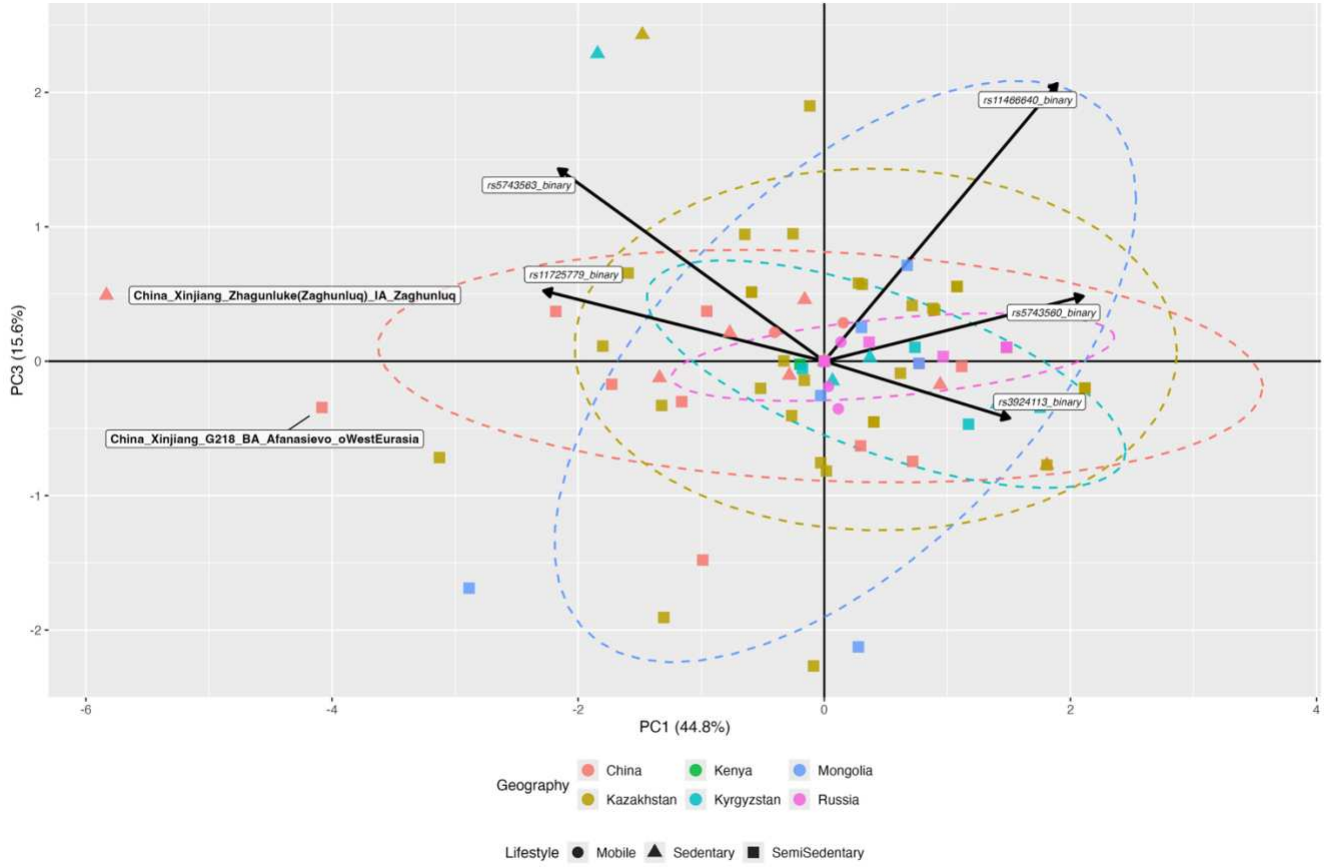


Figure 7. PC 1 vs PC 3 of each group by Time Period, Lifestyle and Geography, and archaic allele presence. Shapes reflect lifestyle, whereas color reflects geography.

Selection coefficients before and after 5000 BP are reported in Table 5. For every SNP, s was higher before the Bronze Age than after it, and post-5000 BP values approach neutrality. Before 5000 BP, all SNPs had $s < 0.02$ except rs5743560 ($s = 0.0223$ [-0.0024, 0.0471]); after 5000 BP, the largest coefficient was rs5743563 ($s = 0.0063$ [0.0032, 0.0095]), with the remainder at or near zero. Together these results indicate a marginal weakening of selection from before the Bronze Age into the current era and become nearly neutral. With the exception of rs5743560 prior to 5000 BP, all SNPs have an s of less than 0.02 prior to the bronze age and continued to decrease.

Table 5. Selection coefficients Before and After 5000 BP

SNP	<i>s</i> before 5000	CI low	CI high	<i>s</i> after 5000	CI low	CI high
rs11466640	0.0151	0.0085	0.0216	0.0003	-0.0122	0.0128
rs3924113	0.0171	0.0171	0.0171	0.0003	-0.001	0.0017
rs5743563	0.0162	0.0161	0.0162	0.0063	0.0032	0.0095
rs5743560	0.0223	-0.0024	0.0471	0.0009	0.0001	0.0017
rs11725779	0.017	0.0034	0.0307	0.0004	-0.0019	0.0027

Hypothesis testing

The present study had a goal of testing five hypotheses to determine if lifestyle, time period, a combination of the two, or geography predict the presence of archaic TLR SNPs. First, it was tested if Allele frequency was a function of mobility patterns as they are proxy for pathogen exposure using a generalized linear mixed-effects model (GLMM). Allele presence was modeled as a binomial response with Lifestyle as a fixed effect with the five SNPs included as a random intercept. In this scenario, mobility was not found to be a significant predictor of allele presence (Type III Wald $\chi^2 = 0.776$, $df = 2$, $p = 0.678$). Figure 8A depicts the predictive power of each lifestyle's effect on allele presence. These results indicate little differentiation in allele frequency between sedentary, semi-sedentary, and mobile groups.

Next, it was of interest whether allele presence is affected by time period. To answer this, a GLMM was used to model Time Period as a fixed effect and the five SNPs were included as a random intercept with polynomial contrasts to capture potential linear and nonlinear temporal trends. The results of this were nonsignificant (Type III Wald $\chi^2 = 0.010$, $df = 2$, $p = 0.995$; Figure 8B); the null hypothesis is not rejected. Because the sampled groups are confined to single time periods — so that their temporal, geographic, and cultural attributes cannot be fully disentangled — lifestyle and time period were also assessed jointly. Neither predictor was significant in the combined model (lifestyle: $\chi^2 = 0.719$, $df = 2$, $p = 0.698$; time

period: $\chi^2 = 0.000$, $df = 1$, $p = 0.990$; Figure 8C). Inclusion of both predictors did not improve model fit (AIC= 775.9) in comparison to models with single variable predictions. These results indicate that Lifestyle and Time Period have little combined effect on allele presence. Geography was assessed using two models. Comparing the ingroup subsamples, geography did not significantly predict allele presence (Type III Wald $\chi^2 = 1.262$, $df = 4$, $p = 0.868$; Figure 8D depicts the predictive power of each geographic region's effect on allele presence. The second model comparing the outgroup to the ingroups was found to be nonsignificant (Type III Wald $\chi^2 = 0.001$, $df = 1$, $p = 0.979$). With both of these results in mind, the author fails to reject the null hypothesis and will maintain the claim that there is no significant geographic differentiation of allele presence within the scope of SNPs examined here.

As mentioned earlier there is a substantial imbalance in group sizes across all predictors, post hoc sensitivity analyses on balanced subsamples confirmed that the results were consistent across imbalanced and balanced models. Two comparisons trended toward significance: in the balanced mobility model, sedentary versus semi-sedentary groups ($p = 0.068$), and in the balanced geography model ($p = 0.056$). Table 3 provides the results of the sensitivity analyses and results of the initial hypothesis testing. Each row provides the independent variable, comparative reference group, n for the sensitivity analyses, X^2 (Type III), p -value, notes that add context to the results.

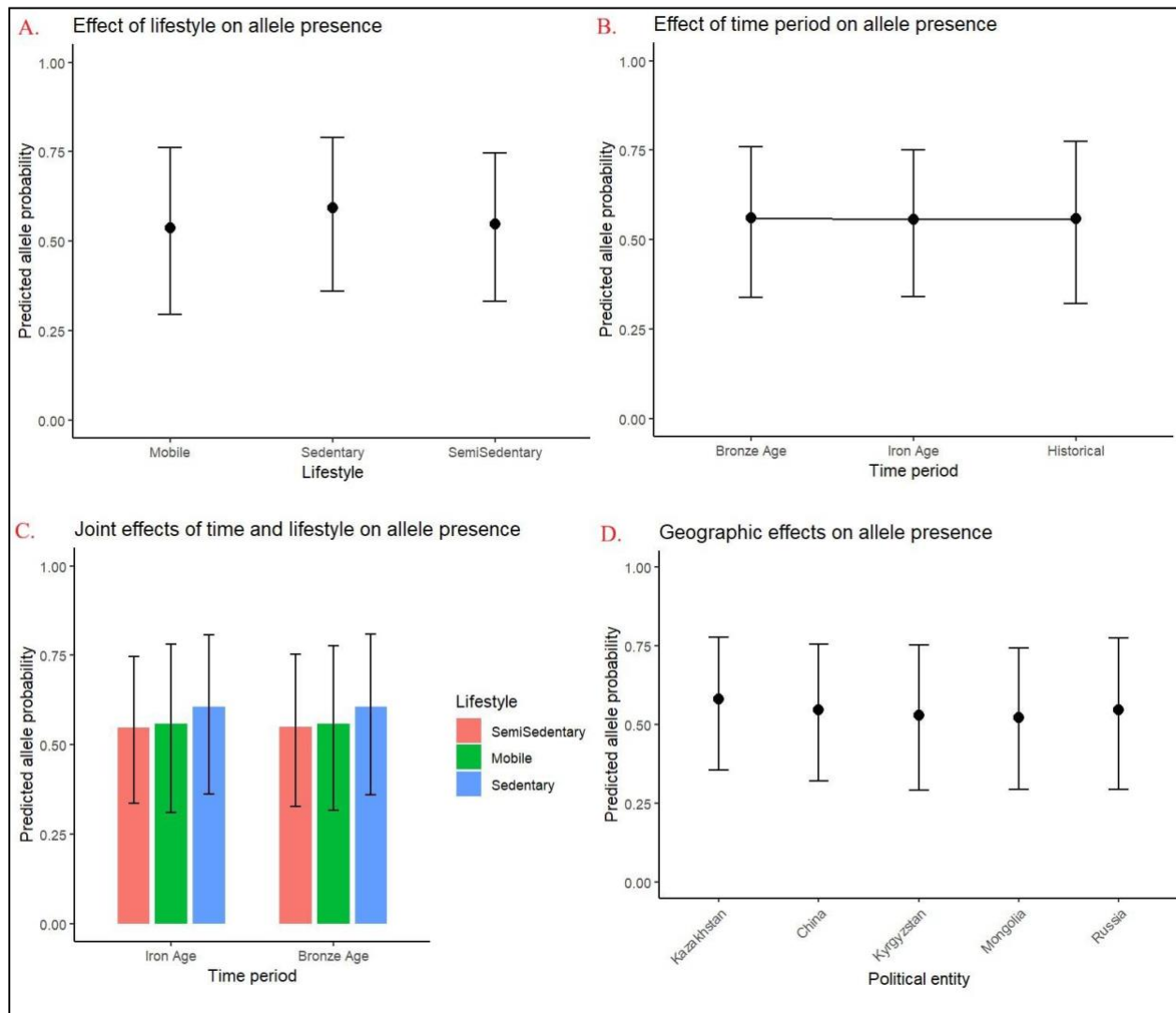


Figure 8. A. Effect of lifestyle on allele presence B. Effect of time period on allele presence C. Joint effect of time and lifestyle on allele presence D. Geographic Effects on Allele Presence

Table 6. Initial Hypothesis Testing and *Post Hoc* Sensitivity Results

Model	Predictor	Ref	<i>n</i>	df	X ² (Type III)	<i>p</i> -value	Notes
Lifestyle	Lifestyle	Semi-Sedentary		2	0.776	0.678	
Lifestyle (balanced)	Lifestyle		34	2	3.551	0.169	Sedentary trended <i>p</i> = 0.068 vs Semi-Sedentary
Time Period	Time Period	Bronze Age		2	0.010	0.995	Is Outgroup excluded — sparse across periods
Time Period (balanced)	Time Period		36	2	0.211	0.900	
Combined	Lifestyle	Semi-Sedentary / Iron Age		2	0.719	0.698	Historical excluded — sparse cells. IsOutgroup

							excluded — perfectly confounded with Lifestyle
	Time Period			1	0.000	0.990	
Ingroup countries	Political entity	Kazakhstan		4	1.262	0.868	Kenya excluded — perfectly confounded with IsOutgroup
Ingroup countries (balanced)	Political entity		32	4	3.882	0.422	China trended $p = 0.056$ vs Kazakhstan
Outgroup vs ingroup	IsOutgroup			1	0.001	0.979	

Chapter V Discussion and Conclusions

Discussion

The aim of this study was to investigate the temporal and geographic distribution of five archaic-like SNPs located in the Toll-like receptor 10-1-6 cluster and assess whether that distribution reflects microevolutionary change in the direction of drift or selection. More importantly, it was of interest to answer whether that distribution is a result of adaptation to pathogens. In doing so, this study was operating under a body of theory involving the mechanisms of adaptation through microevolution and is recapitulated as follows: Recall Wright's fitness landscape (1932) in which phenotypic diversity within species is governed by effective population size. Depending on effective population size, selection will push populations towards an adaptive peak, or drift will pull them into a valley. However, there is a precision limit on selection's ability to maintain low mutation rates imposed by drift which would effectively keep larger populations on an adaptive peak and inhibit them from leaving, expressed in the drift barrier hypothesis (Lynch, 2010).

Kimura neutral theory of evolution (1968; 1983) suggested the majority of existing polymorphisms and fixed differences between species are selectively neutral and functionally equivalent. However, the wide range of population sizes across species with similar genetic diversity does not conform to this relationship. Smith and Haigh (1974) proposed genetic hitchhiking as an explanation for this paradox. Under this model, neutral alleles that are in high linkage disequilibrium with favorable alleles will go to fixation which reduces linked genetic variation. In addition, Ohta's (1992) nearly neutral theory posits that natural selection eliminates nearly neutral mutations in large populations but in smaller populations they behave as neutral

and are randomly fixed. To that end, fitness is determined by a combination of alleles for a narrow set of genes.

Initial explorations of the data set out to describe the temporal and geographic distribution of rs11466640, rs3924113, rs5743563, rs5743560, and rs11725779. Figure 7 reveals these SNPs vary in frequency across time by mobility pattern; mobility groups are also varied in sample size which is the case when looking at the Mobile groups compared to semi sedentary and they're numerically different between time periods. One example of how this may raise concern is that during the Mesolithic, the frequency of rs5743560 is at 1.0, however, there is only 1 individual, when the sample increases and during the next time period, that frequency drops. Likewise, for several of the time periods, multiple SNPs exhibit a frequency of 0 and a subsample of 0 individuals who possess the archaic-like variants. But because the SNPs can be observed in contemporary populations, the most parsimonious explanation for this discrepancy is the taphonomic constraints on the preservation of DNA in archeological remains (Caruso *et al.*, 2020; Hedges, 2002; Leney, 2006). In other words, temporal gaps in allelic frequencies are the result of not having any data from that period. If an allele is observed at 10,000 BP, and it is observed in modern populations, then the temporal gap that would occur at 5,000 BP for example could be filled in by the recovery of a sample whose genome occupies that period.

At first this study used a principal components analysis on the data to gauge whether there were any observable clusters. Following this, 10 outliers were identified using a Mahalanobis distance. Figure 8 shows the data along PC1-PC2 and does not exhibit any distinct clusters attributable to allele presence based on lifestyle, time period, or geography. Additionally, the comparisons of the SNPs *s* before and after 5000 BP indicate a marginal decrease in the strength of selection before the Bronze Age into the current era and become nearly neutral. With

the exception of rs5743560 prior to 5000 BP, all SNPs have an s of less than 0.02 prior to the bronze age and continued to decrease. It remains to be said that they were not markers of a selectively advantageous innate immune system. The SNPs are intron variants or intergenic variants (Table 2)—relatively “selection free sequences” of DNA that are transcribed but sliced out before translation. While they can serve many functions, it is typically understood that they participate in the regulation of transcription and genomic architecture because their presence ensures proper 5’ to 3’ -end splicing (Chorev and Carmel, 2012). Nevertheless, the low selection coefficients suggest these SNPs have little responsibility in the performance of the TLRs. Instead, they likely serve as a mutational buffer to mitigate the consequences of deleterious mutations since introns reside on average ~40% of the total length of genes (Jo and Choi, 2015). In Danneman et al (2015) about 42 SNPs distinguish archaic-like core haplotypes from modern human core haplotypes. 46% of these are introns. The adaptive benefit of these loci may be instead of warding off pathogens more effectively, are actually meant to bolster the genetic integrity of the sequences they occupy.

It was thought that lifestyle patterns would contribute to allelic differences in immune system variation as a consequence of differential exposure to pathogens. However, the data do not indicate such a pattern. This study tested whether mobility was a significant predictor of allele presence and failed to reject the null hypothesis (Type III Wald $\chi^2 = 0.776$, $df = 2$, $p = 0.678$). This is in stark contrast to Harrison et al. (2019) who demonstrated that between Batwa rainforest HG and their agriculturalist Bakiga neighbors from Uganda, the viral burden and thus, signatures of positive selection on innate immune response, were more forceful in the HG cohort. One way to explain this is that if we assume that most pathogens of the type explained in the introduction, actually employ a sit-and-wait mode of transmission then groups that are more

mobile are more likely to come into contact with them as opposed to a sedentary group whose range is limited and the pathogens they've interacted with evolve towards benignness whereas the formers are more novel (Ewald, 1994). If this scenario were true, then we would expect the mobile groups to possess a higher proportion of archaically derived sequences under selection than the sedentary groups. However, the data does not follow this pattern and point in the opposite direction. The archaic variants are more common among the sedentary groups and have a mean allele presence of 0.56

This study also tested whether time was a significant predictor of allele presence and the results of our GLMM were not significant (Type III Wald $\chi^2 = 0.010$, $df = 2$, $p = 0.995$). Now, it must be stated that although allele frequencies are impacted by temporal constraints, here, we are interested if it is more likely one individual will have the archaic over the alternative allelic variant depending on the time period they are conceived in. In this case the differences in probability differ only marginally between the Bronze and Iron ages. This study specifically examines the differences between the two time periods and focuses on Central Asia. Along this temporal dimension the result is consistent with Deschamps *et al.* (2016) who suggested a majority of adaptations on innate immune genes occurred in the last 6,000-13,000 ya, predating a majority of the data used here.

On their own, Mobility and Time Period were found to not be significant predictors of allele presence and it was decided to analyze the two factors together; it is also observed that lifestyle has little effect on allele presence when accounting for time period (Type III Wald $\chi^2 = 0.719$, $df = 2$, $p = 0.698$), and the inverse (Type III Wald $\chi^2 = 0.000$, $df = 1$, $p = 0.990$) holds water as well, suggesting that across time periods and between lifestyle patterns there is no combination which would offer a stark preference for the archaic alleles. Central Asians were

used as the study population for four reasons. First, they represent a spatio-temporal and genetic continuity between modern humans and the Neanderthal lineage. Secondly, there is evidence of the Toll-Like Receptors being under selection. Thirdly, archaic strains of various pathogens have been recovered from archaeological remains in Central Asia. Finally, the Inner-Asian Mountain Corridor in particular served as a conduit through which many of these populations of differing lifestyles migrated through and admixed with each other during this time (Deschamps *et al.*, 2016; Dannemann *et al.*, 2016; Green *et al.*, 2010; Sankararaman *et al.*, 2014; Li *et al.*, 2024; Villanea and Schraiber, 2019; Wall *et al.*, 2013; Barreiro *et al.*, 2009; Mathieson *et al.*, 2015; Andrades Valtueña *et al.*, 2022; Rasmussen *et al.*, 2015; Key *et al.*, 2020; Wu *et al.*, 2020; Gneccchi-Ruscone *et al.*, 2021; Damgaard *et al.*, 2018; Narasimhan *et al.*, 2019). This digression aside, we may return to the observation that while there is an observable decrease in the frequency of archaic variants into the historical time period, note that this observation may be interpreted as an artefact of a dwindled sample size during this period. This is because there are now more archaic-like-alleles among contemporary populations than there were when the Neanderthals existed and when the study population was alive as well (Vernot and Akey 2014; Skov *et al.*, 2020; Witt *et al.*, 2022). Furthermore, a post hoc sensitivity analysis was performed and it reaffirmed the initial test (Table 3) – across time periods and between lifestyle patterns, selection has no preference over any combination of lifestyle and time period.

More recently, Simon and Coop (2024) observed the lack of a substantial contribution of linked selection, also referred to as hitchhiking which is the impact of correlated selection at neighboring loci. One expects this results in the compounding of positive covariances between allele frequency changes across generations but this effect is not observed here. They conclude the time periods under consideration (5425-0 B.P. and 5607-3037 B.P.) are not long enough for

strong covariances to build up as signals of linked selection reflect the dynamics of coalescent time scales of hundreds of thousands of years. With respect to the concept of "hitchhiking," Barreiro *et al.* (2009) and Deschamps *et al.* (2016) noted the TLR genes 10, 6, and 1 to be in strong LD with each other and the archaic SNPs within the cluster to be in strong LD with each other as well. Together, these results reflect an evolutionarily conserved structure within the TLR10-6-1 cluster which has experienced putative selection but not on the time scale observed here nor related to recent cultural adaptations to Central Asia.

It was found that geography is not a strong predictor of allele presence (Type III Wald $\chi^2 = 1.262$, $df = 4$, $p = 0.868$). Geographic distribution of the archaic alleles is rather uniform across political entities and not one locality appears to harbor a disproportionate amount of them. Ancestry by geography has little influence over which allelic variant one receives. It should be stated though, the probability does not exceed .60 across all political entities, suggesting a slight preference for the archaic variants – but this is a very minute edge over the alternative alleles – the immune system can juggle either variant of the TLR SNPs of interest here in grassy steppes, arid deserts, foothills, and glacial mountains. One way to explain the statistical insignificance is to take notice of the geographic distribution of each SNP; they tend to flip back-and-forth in terms of prevalence throughout the region. The alternative allele of rs11725779 clusters in Altai mountain range and Tian Shan during the Bronze age and in central Kazakhstan during and Iron age. The archaic variant A rare is across all time periods. The archaic rs5743560 G allele dominates central Kazakhstan, Altai mountain range, Tian Shan Mountains during the Bronze age and increases into the Iron age. The alternative rs5743563 allele is widely dispersed across all regions and the archaic allele is rare whereas the rs3924113 locus itself is rare across all time periods and regions. But the archaic rs11466640 G allele dominated China during the bronze

age. In the Iron age however, the allele clusters in Tian Shan, Tajikistan and central Kazakhstan. Also, during the Iron age, the alternative allele appears to span an East-to-West gradient across northern Kazakhstan, Altai mountains, and north Mongolia. All that to say, the archaic alleles do not exhibit a pattern one might expect if they were being selected for. This geographic hopping is likely an artefact of admixture events and eventual settlement because if they were selected for, there would be an observable discrepancy between the regions.

The present study differs from previous work that examines geographic patterns of allelic distribution in several ways. For example, Valente *et al.* (2015) identified CYP3A5, a locus involved in metabolic pathways, as the target of selective factors determined by proximity to the tropic of cancer. The present study does not specify geographic features nor looks at allelic distribution in relation to them. Here, national borders are used to sort groups and can overlook specific environmental conditions. Geographic clustering of populations based on thousands of loci have been revealed by PCA but in cases where geographic patterns regarding the distribution of particular genes that hold responsibility for phenotypes like lactase persistence (Ségurel and Bon, 2017), skin pigmentation (Sturm and Duffy, 2012), and sickle cell (Livingstone, 1958), researchers incorporate the selective stimuli that would incite those traits; that theoretical and subsequent methodological component is missing here and we do not have any pathogenic frequency of occurrence to measure up against the presence of the Toll-like receptor SNPs. Lastly, local conditions may provide very weak selective forces on the populations that inhabit them when underlying population substructure dominates genetic variation. Coop *et al.* (2009) indicates that while high *F_{st}* SNPs are rare in the genome, alleles that rise to fixation are rarely driven by selection; signals of selection can be mimicked by the stochastic nature of processes like drift and admixture because rapid adaptation to local environments do not occur at single

loci on the scale of a few thousand years. In the current study many of the populations have a genetic record of admixture which can also be traced to source populations; a scenario such as this could establish underlying population structure so that local adaptation is unobservable. Considering the alleles under interrogation here are introns, intergenic variants, or a splice polypyrimidine tract variant, and not exons, their exposure to local conditions is rather limited, thus reducing geography's predictive power.

Outgroup Discussion

It is at this point in the discussion, a section has been reserved to contemplate the outgroup used here. Because the Kenyan pastoralists occupy a phylogenetically distant branch compared to the central Asian groups, it was assumed that the data on them would act differently and this did not manifest. Kenyan Pastoralists were found to be an outlier in PCA space, likely because it carries the weight of being geographically isolated from the remaining subsamples. Furthermore, prior assumptions on their data would reflect a genetic architecture dissimilar to the Central Asian groups and unaffected by Neanderthal introgression (Sánchez-Quinto *et al.*, 2012; Llorente *et al.*, 2015; Haber *et al.*, 2016; Chen *et al.*, 2020). However, Kenyan Pastoralists were unaffected by Mobility ($p=0.678$) and in the model comparing ingroup and outgroup association between allele presence and geography, a p -value of 0.979 was produced. In summary, when the subsamples are compared to Kenyan pastoralists, neither mobility nor geography have little predictive power over the presence of rs11466640, rs3924113, rs5743563, rs5743560, and rs11725779.

Conclusions

Intellectuals often aspire to overturn hypotheses. This study is a divergence from that inclination and instead furnishes one more brick in the wall which is a canon on the Neanderthals contribution to modern human immune system variation. The presence of archaic alleles in central Asian populations during the Bronze and Iron ages are not predicted by mobility, time period, or any combination of the two, nor geography. Together, the results of this study reflect an evolutionarily conserved structure within the TLR10-6-1 cluster which has experienced putative selection but not on the scale observed here nor related to recent cultural adaptations to Central Asia. As population geneticist Sewall Wright suggested almost a century ago: selection doesn't operate on single alleles, it operates rather on how those alleles work in concert with each other (1931). With respect to Wright's comment, the archaic TLR SNPs likely serve as a mutational buffer to mitigate the consequences of deleterious mutations.

Nevertheless, the results of this study are most consistent with Ohta's nearly neutral theory. Initially they appear to be consistent with Kimura's neutral theory (1968; 1983) because the allelic distribution stays relatively constant throughout time and between lifestyles and comports with his idea that allelic frequency dynamics are directed by drift and mutation rate, not purely selection. When we consider the selection coefficients however, it becomes clear that these alleles are operating under very weak-to-moderate selection and are driven to a state of being nearly neutral, as Ohta would suggest. This is because the alleles behave differently depending on population context. As populations increase and expand into empires as they did during the bronze age, rs11466640, rs3924113, rs5743563, rs5743560, and rs11725779 collapsed under such complexity and behave as nearly neutral.

To determine the sample's mobility patterns a literature review was used – because the past is an inaccessible entity, the best one can do is make use of current archeological literature which may be wrong at times. Although this is a very weak problem, the lack of an overarching consensus on the mobility patterns of archeological groups opens the door for inconsistencies. The temporal range under investigation here and the geographic setting is heavily biased towards bronze and iron age nomadic cultures and the given populations under examination here are highly admixed and derive from similar source populations which may be a contributing factor to the lack of differentiation between groups. That is to say, this study suffers from previous observations in the sense that meaningful differences between them cannot be brought to light because of their similar genetic architecture – population history will govern much of the variation we see and override any environment effect having taken place on the scale of a few thousand years. Five SNPs is an extremely humble number of genetic markers to investigate and does not offer much room especially when looking at less than 300 individuals across several thousand years.

It is not unrealistic to think that selection on these innate immunity-related SNPs was not strong enough to exert drastic changes in allele frequency. Most of this is undergirded by population history that would require repetitive instances of admixture with newer populations to underdo. Furthermore, since rs11466640, rs3924113, rs5743563, rs5743560, and rs11725779 are not exons, the results of our GLMMs are consistent with evolutionary theory; protein coding genes, exons, are more consequential since they actually result in phenotypes. In comparison, introns and intergenic variants, because they remain in the nucleus, are less likely to be targets for selection and remain nearly neutral. To better understand the functional implications of these

alleles, comparative experiments may be necessary; selection operates on phenotypes – genes happen to direct them though – this may be easier under observation of model organisms.

The human innate immune system encompasses a set of mechanisms evolutionarily designed to alert the body of an infection. Without it the body would be less ambulatory in its response to healing itself and survival would be impeded. The genetic architecture that upholds those mechanisms is complex. As a part of that architecture is the presence of archaically introgressed sequences of DNA which have been a topic of debate for over a decade. While other research has placed emphasis on the source of these SNPs, the current study aimed to place them within an evolutionary framework. Dannemann *et al.* (2016) suggested they were not a source of adaptive introgression and the current study is in agreement with this assertion. The archaic TLR SNPs studied here exhibit a temporally and geographically stochastic distribution. In addition to this, lifestyle, time period, a combination of the two, and geography are not significantly predictive of the presence of them either. What this means for the innate immune system is that the genetic structure that undergirds the Toll-like receptors is resilient to microevolutionary change despite successive bouts of introgression from the Neanderthals and subsequent admixture

References Cited

- Ahmed, A. (2011). An overview of inflammation: Mechanism and consequences. *Frontiers in Biology*, 6. <https://doi.org/10.1007/s11515-011-1123-9>
- Akbari, A., Barton, A. R., Gazal, S., Li, Z., Kariminejad, M., Perry, A., Zeng, Y., Mittnik, A., Patterson, N., Mah, M., Zhou, X., Price, A. L., Lander, E. S., Pinhasi, R., Rohland, N., Mallick, S., & Reich, D. (2024). Pervasive findings of directional selection realize the promise of ancient DNA to elucidate human adaptation (p. 2024.09.14.613021). *bioRxiv*. <https://doi.org/10.1101/2024.09.14.613021>
- Akbari, A., Perry, A., Barton, A. R., Kariminejad, M., Gazal, S., Li, Z., Zeng, Y., Mittnik, A., Patterson, N., Mah, M., Zhou, X., Price, A. L., Lander, E. S., Pinhasi, R., Rohland, N., Mallick, S., & Reich, D. (2026). Ancient DNA reveals pervasive directional selection across West Eurasia. *Nature*, 1–10. <https://doi.org/10.1038/s41586-026-10358-1>
- Akira, S., & Takeda, K. (2004). Toll-like receptor signalling. *Nature Reviews Immunology*, 4(7), 499–511. <https://doi.org/10.1038/nri1391>
- Akira, S., Uematsu, S., & Takeuchi, O. (2006). Pathogen Recognition and Innate Immunity. *Cell*, 124(4), 783–801. <https://doi.org/10.1016/j.cell.2006.02.015>
- Allentoft, M. E., Sikora, M., Sjögren, K.-G., Rasmussen, S., Rasmussen, M., Stenderup, J., Damgaard, P. B., Schroeder, H., Ahlström, T., Vinner, L., Malaspinas, A.-S., Margaryan, A., Higham, T., Chivall, D., Lynnerup, N., Harvig, L., Baron, J., Casa, P. D., Dąbrowski, P., ... Willerslev, E. (2015). Population genomics of Bronze Age Eurasia. *Nature*, 522(7555), 167–172. <https://doi.org/10.1038/nature14507>
- Ambrose, S. H. (1986). Stable carbon and nitrogen isotope analysis of human and animal diet in Africa. *Journal of Human Evolution*, 15(8), 707–731. [https://doi.org/10.1016/S0047-2484\(86\)80006-9](https://doi.org/10.1016/S0047-2484(86)80006-9)
- Ambrose, S. H., & DeNiro, M. J. (1986). Reconstruction of African human diet using bone collagen carbon and nitrogen isotope ratios. *Nature*, 319(6051), 321–324. <https://doi.org/10.1038/319321a0>
- Andrades Valtueña, A., Neumann, G. U., Spyrou, M. A., Musralina, L., Aron, F., Beisenov, A., Belinskiy, A. B., Bos, K. I., Buzhilova, A., Conrad, M., Djansugurova, L. B., Dobeš, M., Ernée, M., Fernández-Eraso, J., Frohlich, B., Furmanek, M., Hałaszkó, A., Hansen, S., Harney, É., ... Herbig, A. (2022). Stone Age *Yersinia pestis* genomes shed light on the early evolution, diversity, and ecology of plague. *Proceedings of the National Academy of Sciences*, 119(17), e2116722119. <https://doi.org/10.1073/pnas.2116722119>
- Andreeva, T. V., Soshkina, A. D., Gusev, F. E., Malyarchuk, A. B., Dotsenko, G. S., Dudko, N. A., Plotnikova, M. Y., Kunizheva, S. S., Manakhov, A. D., Ustkachkintseva, T. V., Protasova, M. S., Volodin, S. A., Buzhilova, A. P., Razuvaev, Y. D., Berezina, N. Y., Berezin, Y. B., Kantorovich, A. R., Maslov, V. E., Barinov, D. G., ... Rogaev, E. I. (2025).

- Arnold, S. J., Pfrender, M. E., & Jones, A. G. (2001). The adaptive landscape as a conceptual bridge between micro- and macroevolution. In A. P. Hendry & M. T. Kinnison (Eds.), *Microevolution Rate, Pattern, Process* (Vol. 8, pp. 9–32). Springer Netherlands. https://doi.org/10.1007/978-94-010-0585-2_2
- Barnes, A. N., Davaasuren, A., Baasandagva, U., & Gray, G. C. (2017). A systematic review of zoonotic enteric parasitic diseases among nomadic and pastoral people. *PLOS ONE*, 12(11), e0188809. <https://doi.org/10.1371/journal.pone.0188809>
- Barreiro, L. B., Ben-Ali, M., Quach, H., Laval, G., Patin, E., Pickrell, J. K., Bouchier, C., Tichit, M., Neyrolles, O., Gicquel, B., Kidd, J. R., Kidd, K. K., Alcaïs, A., Ragimbeau, J., Pellegrini, S., Abel, L., Casanova, J.-L., & Quintana-Murci, L. (2009). Evolutionary Dynamics of Human Toll-Like Receptors and Their Different Contributions to Host Defense. *PLOS Genetics*, 5(7), e1000562. <https://doi.org/10.1371/journal.pgen.1000562>
- Bastian, F. B., Roux, J., Niknejad, A., Comte, A., Fonseca Costa, S. S., de Farias, T. M., Moretti, S., Parmentier, G., de Laval, V. R., Rosikiewicz, M., Wollbrett, J., Echchiki, A., Escoriza, A., Gharib, W. H., Gonzales-Porta, M., Jarosz, Y., Laurency, B., Moret, P., Person, E., ... Robinson-Rechavi, M. (2021). The Bgee suite: Integrated curated expression atlas and comparative transcriptomics in animals. *Nucleic Acids Research*, 49(D1), D831–D847. <https://doi.org/10.1093/nar/gkaa793>
- Bastir, M., García-Martínez, D., Torres-Tamayo, N., Palancar, C. A., Beyer, B., Barash, A., Villa, C., Sanchis-Gimeno, J. A., Riesco-López, A., Nalla, S., Torres-Sánchez, I., García-Río, F., Been, E., Gómez-Olivencia, A., Haeusler, M., Williams, S. A., & Spoor, F. (2020). Rib cage anatomy in *Homo erectus* suggests a recent evolutionary origin of modern human body shape. *Nature Ecology & Evolution*, 4(9), 1178–1187. <https://doi.org/10.1038/s41559-020-1240-4>
- Beisenov, A. Z., Bazarbaeva, G. A., & Duisenbay, D. B. (2017). Round dwellings of the saka epoch. *Samara Journal of Science*, 6(1), 89–94. <https://doi.org/10.17816/snv201761201>
- Beisenov, A. Z., Svyatko, S. V., Kassenalin, A. E., Zhambulatov, K. A., Duisenbai, D., & Reimer, P. J. (2016). First Radiocarbon Chronology for the Early Iron Age Sites of Central Kazakhstan (Tasmola Culture and Korgantas Period). *Radiocarbon*, 58(1), 179–191. <https://doi.org/10.1017/RDC.2015.18>
- Beisenov, A. (n.d.). ABOUT CULTURE OF THE POPULATION OF CENTRAL KAZAKHSTAN IN THE SAKA AGE.
- Beutler, B. (2004). Innate immunity: An overview. *Molecular Immunology, Innate Immunity*, 40(12), 845–859. <https://doi.org/10.1016/j.molimm.2003.10.005>
- Bijlsma, R., & Loeschcke, V. (2005). Environmental stress, adaptation and evolution: An overview. *Journal of Evolutionary Biology*, 18(4), 744–749. <https://doi.org/10.1111/j.1420-9101.2005.00962.x>

- Blöcher, J., Bami, M., Feinauer, I. S., Stolarczyk, E., Diekmann, Y., Vetterditz, L., Karapetian, M., Winkelbach, L., Kokot, V., Vallini, L., Stobbe, A., Haak, W., Papageorgopoulou, C., Krause, R., Sharapova, S., & Burger, J. (2023). Descent, marriage, and residence practices of a 3,800-year-old pastoral community in Central Eurasia. *Proceedings of the National Academy of Sciences*, 120(36), e2303574120. <https://doi.org/10.1073/pnas.2303574120>
- Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J.-S. S. (2009). Generalized linear mixed models: A practical guide for ecology and evolution. *Trends in Ecology & Evolution*, 24(3), 127–135. <https://doi.org/10.1016/j.tree.2008.10.008>
- Brennan, J. J., & Gilmore, T. D. (2018). Evolutionary Origins of Toll-like Receptor Signaling. *Molecular Biology and Evolution*, 35(7), 1576–1587. <https://doi.org/10.1093/molbev/msy050>
- Brosseder, U., & Miller, B. K. (2011). Xiongnu Archaeology. Multidisciplinary perspectives of the first steppe empire in Inner Asia. *Bonn Contributions to Asian Archaeology*, 5.
- Buchmann, K. (2014). Evolution of Innate Immunity: Clues from Invertebrates via Fish to Mammals. *Frontiers in Immunology*, 5. <https://doi.org/10.3389/fimmu.2014.00459>
- Caruso, V., Marinoni, N., Diella, V., Berna, F., Cantaluppi, M., Mancini, L., Trombino, L., Cattaneo, C., Pastero, L., & Pavese, A. (2020). Bone diagenesis in archaeological and contemporary human remains: An investigation of bone 3D microstructure and mineral-chemical assessment. *Archaeological and Anthropological Sciences*, 12(8), 162. <https://doi.org/10.1007/s12520-020-01090-6>
- Chen, L., Wolf, A. B., Fu, W., Li, L., & Akey, J. M. (2020). Identifying and Interpreting Apparent Neanderthal Ancestry in African Individuals. *Cell*, 180(4), 677–687.e16. <https://doi.org/10.1016/j.cell.2020.01.012>
- Chen, Y.-H., Wu, K.-H., & Wu, H.-P. (2024). Unraveling the Complexities of Toll-like Receptors: From Molecular Mechanisms to Clinical Applications. *International Journal of Molecular Sciences*, 25(9), Article 9. <https://doi.org/10.3390/ijms25095037>
- Chorev, M., & Carmel, L. (2012). The Function of Introns. *Frontiers in Genetics*, 3. <https://doi.org/10.3389/fgene.2012.00055>
- Choteau, L., Vancraeynest, H., Le Roy, D., Dubuquoy, L., Romani, L., Jouault, T., Poulain, D., Sendid, B., Calandra, T., Roger, T., & Jawhara, S. (2017). Role of TLR1, TLR2 and TLR6 in the modulation of intestinal inflammation and *Candida albicans* elimination. *Gut Pathogens*, 9(1), 9. <https://doi.org/10.1186/s13099-017-0158-0>
- Cipollaro, M., Galderisi, U., & Di Bernardo, G. (2005). Ancient DNA as a multidisciplinary experience. *Journal of Cellular Physiology*, 202(2), 315–322. <https://doi.org/10.1002/jcp.20116>
- Coop, G., Pickrell, J. K., Novembre, J., Kudaravalli, S., Li, J., Absher, D., Myers, R. M.,

- Cavalli-Sforza, L. L., Feldman, M. W., & Pritchard, J. K. (2009). The Role of Geography in Human Adaptation. *PLoS Genetics*, 5(6), e1000500. <https://doi.org/10.1371/journal.pgen.1000500>
- Damgaard, P. de B., Marchi, N., Rasmussen, S., Peyrot, M., Renaud, G., Korneliussen, T., Moreno-Mayar, J. V., Pedersen, M. W., Goldberg, A., Usmanova, E., Baimukhanov, N., Loman, V., Hedeager, L., Pedersen, A. G., Nielsen, K., Afanasiev, G., Akmatov, K., Aldashev, A., Alpaslan, A., ... Willerslev, E. (2018). 137 ancient human genomes from across the Eurasian steppes. *Nature*, 557(7705), 369–374. <https://doi.org/10.1038/s41586-018-0094-2>
- Damgaard, P., Martiniano, R., Kamm, J., Moreno-Mayar, J. V., Kroonen, G., Peyrot, M., Barjamovic, G., Rasmussen, S., Zacho, C., Baimukhanov, N., Zaibert, V., Merz, V., Biddanda, A., Merz, I., Loman, V., Evdokimov, V., Usmanova, E., Hemphill, B., Seguin-Orlando, A., ... Willerslev, E. (2018). The first horse herders and the impact of early Bronze Age steppe expansions into Asia. *Science*, 360(6396), eaar7711. <https://doi.org/10.1126/science.aar7711>
- Dannemann, M., Andrés, A. M., & Kelso, J. (2016). Introgression of Neandertal- and Denisovan-like Haplotypes Contributes to Adaptive Variation in Human Toll-like Receptors. *The American Journal of Human Genetics*, 98(1), 22–33. <https://doi.org/10.1016/j.ajhg.2015.11.015>
- Darwin, C. (1859). *On the origin of species by means of natural selection, or, The preservation of favoured races in the struggle for life*. London : John Murray, Albemarle Street. http://archive.org/details/onoriginofspecie00darw_0
- Davis-Kimball, J., Bashilov, V. A., & Yablonsky, L. T. (1995). *NOMADS OF THE EURASIAN STEPPES IN THE EARLY IRON AGE*.
- Delneste, Y., Beauvillain, C., & Jeannin, P. (2007). [Innate immunity: Structure and function of TLRs]. *Medecine sciences*, 23(1), 67–73. <https://doi.org/10.1051/medsci/200723167>
- Deschamps, M., Laval, G., Fagny, M., Itan, Y., Abel, L., Casanova, J.-L., Patin, E., & Quintana-Murci, L. (2016). Genomic Signatures of Selective Pressures and Introgression from Archaic Hominins at Human Innate Immunity Genes. *The American Journal of Human Genetics*, 98(1), 5–21. <https://doi.org/10.1016/j.ajhg.2015.11.014>
- Diamond, J. (1999). *Guns, Germs, and Steel: The Fates of Human Societies*. W. W. Norton & Company.
- Disotell, T. R. (2012). Archaic human genomics. *American Journal of Physical Anthropology*, 149(S55), 24–39. <https://doi.org/10.1002/ajpa.22159>
- Dong, Z., Ma, Y., Zhou, H., Shi, L., Ye, G., Yang, L., Liu, P., & Zhou, L. (2020). Integrated genomics analysis highlights important SNPs and genes implicated in moderate-to-severe asthma based on GWAS and eQTL datasets. *BMC Pulmonary Medicine*, 20(1), 270. <https://doi.org/10.1186/s12890-020-01303-7>

- Doyle, M. P., & Schoeni, J. L. (1984). Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. *Applied and Environmental Microbiology*, 48(4), 855–856. <https://doi.org/10.1128/aem.48.4.855-856.1984>
- Dyer, S. C., Austine-Orimoloye, O., Azov, A. G., Barba, M., Barnes, I., Barrera-Enriquez, V. P., Becker, A., Bennett, R., Beracochea, M., Berry, A., Bhai, J., Bhurji, S. K., Boddu, S., Branco Lins, P. R., Brooks, L., Ramaraju, S. B., Campbell, L. I., Martinez, M. C., Charkhchi, M., ... Yates, A. D. (2025). Ensembl 2025. *Nucleic Acids Research*, 53(D1), D948–D957. <https://doi.org/10.1093/nar/gkae1071>
- Flegontov, P., Altınışık, N. E., Changmai, P., Rohland, N., Mallick, S., Adamski, N., Bolnick, D. A., Broomandkhoshbacht, N., Candilio, F., Culleton, B. J., Flegontova, O., Friesen, T. M., Jeong, C., Harper, T. K., Keating, D., Kennett, D. J., Kim, A. M., Lamnidis, T. C., Lawson, A. M., ... Schiffels, S. (2019). Palaeo-Eskimo genetic ancestry and the peopling of Chukotka and North America. *Nature*, 570(7760), 236–240. <https://doi.org/10.1038/s41586-019-1251-y>
- Flegr, J. (2013). Microevolutionary, macroevolutionary, ecological and taxonomical implications of punctuational theories of adaptive evolution. *Biology Direct*, 8(1), 1. <https://doi.org/10.1186/1745-6150-8-1>
- Frachetti, M. D. (2012). Multiregional Emergence of Mobile Pastoralism and Nonuniform Institutional Complexity across Eurasia. *Current Anthropology*, 53(1), 2–38. <https://doi.org/10.1086/663692>
- Frisancho, A. R. (2009). Developmental adaptation: Where we go from here. *American Journal of Human Biology*, 21(5), 694–703. <https://doi.org/10.1002/ajhb.20891>
- Frohlich, B., Amgalantugs, T., Littleton, J., Erdene, B., Hunt, D., Nittler, E., Karstens, S., Frohlich, T., & Galdan, G. (2010). AN OVERVIEW OF THEORIES AND HYPOTHESIS PERTAINING TO MONGOLIAN BRONZE AGE KHIRGISUURS IN THE HOVSGOL AIMAG, MONGOLIA.
- Gay, N. J., Symmons, M. F., Gangloff, M., & Bryant, C. E. (2014). Assembly and localization of Toll-like receptor signalling complexes. *Nature Reviews Immunology*, 14(8), 546–558. <https://doi.org/10.1038/nri3713>
- Georgel, P., Macquin, C., & Bahram, S. (2009). The Heterogeneous Allelic Repertoire of Human Toll-Like Receptor (TLR) Genes. *PLoS ONE*, 4(11), e7803. <https://doi.org/10.1371/journal.pone.0007803>
- Gilligan, I. (2018). *Climate, Clothing, and Agriculture in Prehistory: Linking Evidence, Causes, and Effects*. Cambridge University Press. <https://doi.org/10.1017/9781108555883>
- Gilmore, T. D., & Wolenski, F. S. (2012). NF-κB: Where did it come from and why? *Immunological Reviews*, 246(1), 14–35. <https://doi.org/10.1111/j.1600-065X.2012.01096.x>
- Gnecchi-Ruscone, G. A., Khussainova, E., Kahbatkyzy, N., Musralina, L., Spyrou, M. A., Bianco,

- R. A., Radzeviciute, R., Martins, N. F. G., Freund, C., Iksan, O., Garshin, A., Zhaniyazov, Z., Bekmanov, B., Kitov, E., Samashev, Z., Beisenov, A., Berezina, N., Berezin, Y., Bíró, A. Z., ... Krause, J. (2021). Ancient genomic time transect from the Central Asian Steppe unravels the history of the Scythians. *Science Advances*, 7(13), eabe4414. <https://doi.org/10.1126/sciadv.abe4414>
- Gnecchi-Ruscone, G. A., Rácz, Z., Liccardo, S., Lee, J., Huang, Y., Traverso, L., Radzevičiūtė, R., Hajnal, Z., Szécsényi-Nagy, A., Gyuris, B., Mateovics-László, O., Bernert, Z., Szeniczey, T., Hajdu, T., Mészáros, B., Bálint, M., Mende, B. G., Miller, B., Samashev, Z., ... Hofmanová, Z. (2025). Ancient genomes reveal trans-Eurasian connections between the European Huns and the Xiongnu Empire. *Proceedings of the National Academy of Sciences*, 122(9), e2418485122. <https://doi.org/10.1073/pnas.2418485122>
- Grigoriev, S. (2021). Andronovo Problem: Studies of Cultural Genesis in the Eurasian Bronze Age. *Open Archaeology*, 7(1), 3–36. <https://doi.org/10.1515/opar-2020-0123>
- Guo, Y., Lou, J., Xie, S., Wu, X., & Fuller, B. T. (2020). Isotopic reconstruction of human diet in the Ji'erzankale site, Xinjiang Uyghur Autonomous Region, China. *International Journal of Osteoarchaeology*, 30(1), 65–72. <https://doi.org/10.1002/oa.2831>
- Haber, M., Mezzavilla, M., Bergström, A., Prado-Martinez, J., Hallast, P., Saif-Ali, R., Al-Habori, M., Dedoussis, G., Zeggini, E., Blue-Smith, J., Wells, R. S., Xue, Y., Zalloua, P. A., & Tyler-Smith, C. (2016). Chad Genetic Diversity Reveals an African History Marked by Multiple Holocene Eurasian Migrations. *American Journal of Human Genetics*, 99(6), 1316–1324. <https://doi.org/10.1016/j.ajhg.2016.10.012>
- Hajam, I. A., Dar, P. A., Shahnawaz, I., Jaume, J. C., & Lee, J. H. (2017). Bacterial flagellin—A potent immunomodulatory agent. *Experimental & Molecular Medicine*, 49(9), e373–e373. <https://doi.org/10.1038/emm.2017.172>
- Hajjar, A. M., O'Mahony, D. S., Ozinsky, A., Underhill, D. M., Aderem, A., Klebanoff, S. J., & Wilson, C. B. (2001). Cutting Edge: Functional Interactions Between Toll-Like Receptor (TLR) 2 and TLR1 or TLR6 in Response to Phenol-Soluble Modulin. *The Journal of Immunology*, 166(1), 15–19. <https://doi.org/10.4049/jimmunol.166.1.15>
- Haldane, J. B. S. (1926). A mathematical theory of natural and artificial selection. *Mathematical Proceedings of the Cambridge Philosophical Society*, 23(4), 363–372. <https://doi.org/10.1017/S0305004100015176>
- Harris, E. E. (2013). Gene Evolution and Human Adaptation. In *Encyclopedia of Life Sciences*. John Wiley & Sons, Ltd. <https://doi.org/10.1002/9780470015902.a0020773.pub2>
- Harris, K., & Nielsen, R. (2016). The Genetic Cost of Neanderthal Introgression. *Genetics*, 203(2), 881–891. <https://doi.org/10.1534/genetics.116.186890>
- Harrison, G. A. (1998). Physiological Adaptation. In *Human Adaptation*. Routledge.
- Harrison, G. F., Sanz, J., Boulais, J., Mina, M. J., Grenier, J.-C., Leng, Y., Dumaine, A., Yotova, V., Bergey, C. M., Nsoby, S. L., Elledge, S. J., Schurr, E., Quintana-Murci, L., Perry, G.

- H., & Barreiro, L. B. (2019). Natural selection contributed to immunological differences between hunter-gatherers and agriculturalists. *Nature Ecology & Evolution*, 3(8), 1253–1264. <https://doi.org/10.1038/s41559-019-0947-6>
- Hautmann, M. (2020). What is macroevolution? *Palaeontology*, 63(1), 1–11. <https://doi.org/10.1111/pala.12465>
- Hedges, R. E. M. (2002). Bone diagenesis: An overview of processes. *Archaeometry*, 44(3), 319–328. <https://doi.org/10.1111/1475-4754.00064>
- Henneberg, M. (1987). Hominid cranial capacity change through time: A darwinian process. *Human Evolution*, 2(3), 213–220. <https://doi.org/10.1007/BF03016107>
- Henneberg, M. (2006). The rate of human morphological microevolution. *Studies in Historical Anthropology*, 4, 49–59.
- Hermes, T. R., Tishkin, A. A., Kosintsev, P. A., Stepanova, N. F., Krause-Kyora, B., & Makarewicz, C. A. (2020). Mitochondrial DNA of domesticated sheep confirms pastoralist component of Afanasievo subsistence economy in the Altai Mountains (3300–2900 cal BC). *Archaeological Research in Asia*, 24, 100232. <https://doi.org/10.1016/j.ara.2020.100232>
- Hershkovitz, I., Donoghue, H. D., Minnikin, D. E., Besra, G. S., Lee, O. Y.-C., Gernaey, A. M., Galili, E., Eshed, V., Greenblatt, C. L., Lemma, E., Bar-Gal, G. K., & Spigelman, M. (2008). Detection and Molecular Characterization of 9000-Year-Old *Mycobacterium tuberculosis* from a Neolithic Settlement in the Eastern Mediterranean. *PLOS ONE*, 3(10), e3426. <https://doi.org/10.1371/journal.pone.0003426>
- Honeychurch, W., Rogers, L., Amartuvshin, C., Diimaajav, E., Erdene-Ochir, N.-O., Hall, M. E., & Hrivnyak, M. (2021). The earliest herders of East Asia: Examining Afanasievo entry to Central Mongolia. *Archaeological Research in Asia*, 26, 100264. <https://doi.org/10.1016/j.ara.2021.100264>
- Houle, J.-L. (2010). Emergent complexity on the Mongolian steppe: Mobility, territoriality, and the development of early nomadic polities [Ph.D., University of Pittsburgh]. <https://www.proquest.com/docview/746106437/abstract/AAD30D10C0A04C45PQ/1>
- Hünemeier, T. (2024). Biogeographic Perspectives on Human Genetic Diversification. *Molecular Biology and Evolution*, 41(3), msae029. <https://doi.org/10.1093/molbev/msae029>
- Iasi, L. N. M., Chintalapati, M., Skov, L., Mesa, A. B., Hajdinjak, M., Peter, B. M., & Moorjani, P. (2024). Neanderthal ancestry through time: Insights from genomes of ancient and present-day humans. *Science*, 386(6727), eadq3010. <https://doi.org/10.1126/science.adq3010>
- Jeong, C., Balanovsky, O., Lukianova, E., Kahbatkyzy, N., Flegontov, P., Zaporozhchenko, V., Immel, A., Wang, C.-C., Ixan, O., Khussainova, E., Bekmanov, B., Zaubert, V., Lavryashina, M., Pocheshkhova, E., Yusupov, Y., Agdzhoyan, A., Koshel, S., Bukin, A., Nymadawa, P., ... Krause, J. (2019). The genetic history of admixture across inner

- Eurasia. *Nature Ecology & Evolution*, 3(6), 966–976.
<https://doi.org/10.1038/s41559-019-0878-2>
- Jeong, C., Wang, K., Wilkin, S., Taylor, W. T. T., Miller, B. K., Bemmman, J. H., Stahl, R., Chiovelli, C., Knolle, F., Ulziibayar, S., Khatanbaatar, D., Erdenebaatar, D., Erdenebat, U., Ochir, A., Ankhsanaa, G., Vanchigdash, C., Ochir, B., Munkhbayar, C., Tumen, D., ... Warinner, C. (2020). A Dynamic 6,000-Year Genetic History of Eurasia's Eastern Steppe. *Cell*, 183(4), 890-904.e29. <https://doi.org/10.1016/j.cell.2020.10.015>
- Jeong, C., Wilkin, S., Amgalantugs, T., Bouwman, A. S., Taylor, W. T. T., Hagan, R. W., Bromage, S., Tsolmon, S., Trachsel, C., Grossmann, J., Littleton, J., Makarewicz, C. A., Krigbaum, J., Burri, M., Scott, A., Davaasambu, G., Wright, J., Irmer, F., Myagmar, E., ... Warinner, C. (2018). Bronze Age population dynamics and the rise of dairy pastoralism on the eastern Eurasian steppe. *Proceedings of the National Academy of Sciences of the United States of America*, 115(48), E11248–E11255.
<https://doi.org/10.1073/pnas.1813608115>
- Jia, P., Caspari, G., Betts, A., Mohamadi, B., Balz, T., Cong, D., Shen, H., & Meng, Q. (2020). Seasonal movements of Bronze Age transhumant pastoralists in western Xinjiang. *PLOS ONE*, 15(11), e0240739. <https://doi.org/10.1371/journal.pone.0240739>
- Jin, M. S., & Lee, J.-O. (2008). Structures of TLR–ligand complexes. *Current Opinion in Immunology*, 20(4), 414–419. <https://doi.org/10.1016/j.coi.2008.06.002>
- Jo, B.-S., & Choi, S. S. (2015). Introns: The Functional Benefits of Introns in Genomes. *Genomics & Informatics*, 13(4), 112–118. <https://doi.org/10.5808/GI.2015.13.4.112>
- Jones, B. A., Grace, D., Kock, R., Alonso, S., Rushton, J., Said, M. Y., McKeever, D., Mutua, F., Young, J., McDermott, J., & Pfeiffer, D. U. (2013). Zoonosis emergence linked to agricultural intensification and environmental change. *Proceedings of the National Academy of Sciences*, 110(21), 8399–8404. <https://doi.org/10.1073/pnas.1208059110>
- Kang, J. Y., Nan, X., Jin, M. S., Youn, S.-J., Ryu, Y. H., Mah, S., Han, S. H., Lee, H., Paik, S.-G., & Lee, J.-O. (2009). Recognition of Lipopeptide Patterns by Toll-like Receptor 2-Toll-like Receptor 6 Heterodimer. *Immunity*, 31(6), 873–884.
<https://doi.org/10.1016/j.immuni.2009.09.018>
- Kawai, T., & Akira, S. (2006). TLR signaling. *Cell Death & Differentiation*, 13(5), 816–825.
<https://doi.org/10.1038/sj.cdd.4401850>
- Key, F. M., Posth, C., Esquivel-Gomez, L. R., Hübner, R., Spyrou, M. A., Neumann, G. U., Furtwängler, A., Sabin, S., Burri, M., Wissgott, A., Lankapalli, A. K., Vågane, Å. J., Meyer, M., Nagel, S., Tukhbatova, R., Khokhlov, A., Chizhevsky, A., Hansen, S., Belinsky, A. B., ... Krause, J. (2020). Emergence of human-adapted *Salmonella enterica* is linked to the Neolithization process. *Nature Ecology & Evolution*, 4(3), 324–333.
<https://doi.org/10.1038/s41559-020-1106-9>
- Kılınç, G. M., Kashuba, N., Koptekin, D., Bergfeldt, N., Dönertaş, H. M., Rodríguez-Varela, R., Shergin, D., Ivanov, G., Kichigin, D., Pestereva, K., Volkov, D., Mandryka, P., Kharinskii,

- A., Tishkin, A., Ineshin, E., Kovychev, E., Stepanov, A., Dalén, L., Günther, T., ... Götherström, A. (2021). Human population dynamics and *Yersinia pestis* in ancient northeast Asia. *Science Advances*, 7(2), eabc4587. <https://doi.org/10.1126/sciadv.abc4587>
- Klunk, J., Vilgalys, T. P., Demeure, C. E., Cheng, X., Shiratori, M., Madej, J., Beau, R., Elli, D., Patino, M. I., Redfern, R., DeWitte, S. N., Gamble, J. A., Boldsen, J. L., Carmichael, A., Varlik, N., Eaton, K., Grenier, J.-C., Golding, G. B., Devault, A., ... Barreiro, L. B. (2022). Evolution of immune genes is associated with the Black Death. *Nature*, 611(7935), 312–319. <https://doi.org/10.1038/s41586-022-05349-x>
- Konieczny, M. J., Omarov, M., Zhang, L., Malik, R., Richardson, T. G., Baumeister, S.-E., Bernhagen, J., Dichgans, M., & Georgakis, M. K. (2025). The genomic architecture of circulating cytokine levels points to drug targets for immune-related diseases. *Communications Biology*, 8(1), 34. <https://doi.org/10.1038/s42003-025-07453-w>
- Kumar, V., Wang, W., Zhang, J., Wang, Y., Ruan, Q., Yu, J., Wu, X., Hu, X., Wu, X., Guo, W., Wang, B., Niyazi, A., Lv, E., Tang, Z., Cao, P., Liu, F., Dai, Q., Yang, R., Feng, X., ... Fu, Q. (2022). Bronze and Iron Age population movements underlie Xinjiang population history. *Science*, 376(6588), 62–69. <https://doi.org/10.1126/science.abk1534>
- Lee, S. M. Y., Kok, K.-H., Jaume, M., Cheung, T. K. W., Yip, T.-F., Lai, J. C. C., Guan, Y., Webster, R. G., Jin, D.-Y., & Peiris, J. S. M. (2014). Toll-like receptor 10 is involved in induction of innate immune responses to influenza virus infection. *Proceedings of the National Academy of Sciences*, 111(10), 3793–3798. <https://doi.org/10.1073/pnas.1324266111>
- Leney, M. D. (2006). Sampling Skeletal Remains for Ancient DNA (aDNA): A Measure of Success. *Historical Archaeology*, 40(3), 31–49. <https://doi.org/10.1007/BF03376731>
- Li, C., Ning, C., Hagelberg, E., Li, H., Zhao, Y., Li, W., Abuduresule, I., Zhu, H., & Zhou, H. (2015). Analysis of ancient human mitochondrial DNA from the Xiaohe cemetery: Insights into prehistoric population movements in the Tarim Basin, China. *BMC Genetics*, 16(1), 78. <https://doi.org/10.1186/s12863-015-0237-5>
- Li, J.-F., Abuduresule, I., Hueber, F. M., Li, W.-Y., Hu, X.-J., Li, Y.-Z., & Li, C.-S. (2013). Buried in Sands: Environmental Analysis at the Archaeological Site of Xiaohe Cemetery, Xinjiang, China. *PLOS ONE*, 8(7), e68957. <https://doi.org/10.1371/journal.pone.0068957>
- Li, X., Jiang, S., & Tapping, R. I. (2010). Toll-like receptor signaling in cell proliferation and survival. *Cytokine*, 49(1), 1–9. <https://doi.org/10.1016/j.cyto.2009.08.010>
- Li, Y. (2021). Agriculture and palaeoeconomy in prehistoric Xinjiang, China (3000–200 bc). *Vegetation History and Archaeobotany*, 30(2), 287–303. <https://doi.org/10.1007/s00334-020-00774-2>
- Livingstone, F. B. (1958). Anthropological Implications of Sickle Cell Gene Distribution in West Africa. *American Anthropologist*, 60(3), 533–562.

<https://doi.org/10.1525/aa.1958.60.3.02a00110>

- Llorente, M. G., Jones, E. R., Eriksson, A., Siska, V., Arthur, K. W., Arthur, J. W., Curtis, M. C., Stock, J. T., Coltorti, M., Pieruccini, P., Stretton, S., Brock, F., Higham, T., Park, Y., Hofreiter, M., Bradley, D. G., Bhak, J., Pinhasi, R., & Manica, A. (2015). Ancient Ethiopian genome reveals extensive Eurasian admixture in Eastern Africa. *Science*, 350(6262), 820–822. <https://doi.org/10.1126/science.aad2879>
- Losey, R. J., Nomokonova, T., & Savel'ev, N. A. (2016). Humans and animals at Bugul'deika II, a Trans-Holocene habitation site on the shore of Lake Baikal, Russia. *Quaternary International, Holocene Hunter-Gatherer Archaeology of Northern Eurasia* (Guest Editors: Peter Jordan and Andrzej Weber), 419, 62–73. <https://doi.org/10.1016/j.quaint.2014.08.021>
- Losey, R. J., Waters-Rist, A. L., Nomokonova, T., & Kharinskii, A. A. (2017). A Second Mortuary Hiatus on Lake Baikal in Siberia and the Arrival of Small-Scale Pastoralism. *Scientific Reports*, 7(1), 2319. <https://doi.org/10.1038/s41598-017-02636-w>
- Lucas, T., Kumaratilake, J., & Henneberg, M. (2020). Recently increased prevalence of the human median artery of the forearm: A microevolutionary change. *Journal of Anatomy*, 237(4), 623–631. <https://doi.org/10.1111/joa.13224>
- Lynch, M. (2010). Evolution of the mutation rate. *Trends in Genetics : TIG*, 26(8), 345–352. <https://doi.org/10.1016/j.tig.2010.05.003>
- Mallick, S., Micco, A., Mah, M., Ringbauer, H., Lazaridis, I., Patterson, N., & Reich, D. (2024). The Allen Ancient DNA Resource (AADR): A curated compendium of ancient human genomes.
- Mallick, S., & Reich, D. (2024). The Allen Ancient DNA Resource (AADR): A curated compendium of ancient human genomes (Version 9.1) [Dataset]. Harvard Dataverse. <https://doi.org/10.7910/DVN/FFIDCW>
- Martínez-Abadías, N., González-José, R., González-Martín, A., Van Der Molen, S., Talavera, A., Hernández, P., & Hernández, M. (2006). Phenotypic evolution of human craniofacial morphology after admixture: A geometric morphometrics approach. *American Journal of Physical Anthropology*, 129(3), 387–398. <https://doi.org/10.1002/ajpa.20291>
- Mary, L., Zvenigorosky, V., Kovalev, A., Gonzalez, A., Fausser, J.-L., Jagorel, F., Kilunovskaya, M., Semenov, V., Crubézy, E., Ludes, B., & Keyser, C. (2019). Genetic kinship and admixture in Iron Age Scytho-Siberians. *Human Genetics*, 138(4), 411–423. <https://doi.org/10.1007/s00439-019-02002-y>
- Mathieson, I., Lazaridis, I., Rohland, N., Mallick, S., Patterson, N., Roodenberg, S. A., Harney, E., Stewardson, K., Fernandes, D., Novak, M., Sirak, K., Gamba, C., Jones, E. R., Llamas, B., Dryomov, S., Pickrell, J., Arsuaga, J. L., de Castro, J. M. B., Carbonell, E., ... Reich, D. (2015). Genome-wide patterns of selection in 230 ancient Eurasians. *Nature*, 528(7583), 499–503. <https://doi.org/10.1038/nature16152>

- McDonald, D. R., & Levy, O. (2019). Innate Immunity. In *Clinical Immunology* (pp. 39-53.e1). Elsevier. <https://doi.org/10.1016/B978-0-7020-6896-6.00003-X>
- McDonald, J. F. (1983). The Molecular Basis of Adaptation: A Critical Review of Relevant Ideas and Observations. *Annual Review of Ecology and Systematics*, 14, 77–102.
- Meyer, D., & Harris, E. E. (2013). Nonadaptive Genetic Change in Human and Primate Evolution. In *Encyclopedia of Life Sciences*. John Wiley & Sons, Ltd. <https://doi.org/10.1002/9780470015902.a0024607>
- Mikacenic, C., Reiner, A. P., Holden, T. D., Nickerson, D. A., & Wurfel, M. M. (2013). Variation in the TLR10/TLR1/TLR6 locus is the major genetic determinant of interindividual difference in TLR1/2-mediated responses. *Genes & Immunity*, 14(1), 52–57. <https://doi.org/10.1038/gene.2012.53>
- Moresco, E. M. Y., LaVine, D., & Beutler, B. (2011). Toll-like receptors. *Current Biology*, 21(13), R488–R493. <https://doi.org/10.1016/j.cub.2011.05.039>
- Motuzaitė Matuzeviciute, G., Ananyevskaya, E., Sakalauskaite, J., Soltobaev, O., & Tabaldiev, K. (2022). The integration of millet into the diet of Central Asian populations in the third millennium BC. *Antiquity*, 96(387), 560–574. <https://doi.org/10.15184/aqy.2022.23>
- Mukherjee, S., Ganguli, D., & Majumder, P. P. (2014). Global Footprints of Purifying Selection on Toll-Like Receptor Genes Primarily Associated with Response to Bacterial Infections in Humans. *Genome Biology and Evolution*, 6(3), 551–558. <https://doi.org/10.1093/gbe/evu032>
- Muktupavela, R. A., Petr, M., Ségurel, L., Korneliussen, T., Novembre, J., & Racimo, F. (2022). Modeling the spatiotemporal spread of beneficial alleles using ancient genomes. *eLife*, 11, e73767. <https://doi.org/10.7554/eLife.73767>
- Müller, V., de Boer, R. J., Bonhoeffer, S., & Szathmáry, E. (2018). An evolutionary perspective on the systems of adaptive immunity. *Biological Reviews*, 93(1), 505–528. <https://doi.org/10.1111/brv.12355>
- Narasimhan, V. M., Patterson, N., Moorjani, P., Rohland, N., Bernardos, R., Mallick, S., Lazaridis, I., Nakatsuka, N., Olalde, I., Lipson, M., Kim, A. M., Olivieri, L. M., Coppa, A., Vidale, M., Mallory, J., Moiseyev, V., Kitov, E., Monge, J., Adamski, N., ... Reich, D. (2019). The formation of human populations in South and Central Asia. *Science*, 365(6457), eaat7487. <https://doi.org/10.1126/science.aat7487>
- Nguyen, H., Gazy, N., & Venketaraman, V. (2020). A Role of Intracellular Toll-Like Receptors (3, 7, and 9) in Response to *Mycobacterium tuberculosis* and Co-Infection with HIV. *International Journal of Molecular Sciences*, 21(17), 6148. <https://doi.org/10.3390/ijms21176148>
- Ohta, T. (1992). The Nearly Neutral Theory of Molecular Evolution. *Annual Review of Ecology and Systematics*, 23, 263–286.

- Outram, A. K., Kasparov, A., Stear, N. A., Varfolomeev, V., Usmanova, E., & Evershed, R. P. (2012). Patterns of pastoralism in later Bronze Age Kazakhstan: New evidence from faunal and lipid residue analyses. *Journal of Archaeological Science*, 39(7), 2424–2435. <https://doi.org/10.1016/j.jas.2012.02.009>
- Pal, A., & Chakravarty, A. K. (2020). Genes conferring immunity against viral infections. *Genetics and Breeding for Disease Resistance of Livestock*, 163–169. <https://doi.org/10.1016/B978-0-12-816406-8.00010-3>
- Patterson, N., Price, A. L., & Reich, D. (2006). Population Structure and Eigenanalysis. *PLOS Genetics*, 2(12), e190. <https://doi.org/10.1371/journal.pgen.0020190>
- Paul W. Ewald. (1994). *Evolution of infectious disease*. Oxford University Press.
- Perez, G., Barber, G. P., Benet-Pages, A., Casper, J., Clawson, H., Diekhans, M., Fischer, C., Gonzalez, J. N., Hinrichs, A. S., Lee, C. M., Nassar, L. R., Raney, B. J., Speir, M. L., van Baren, M. J., Vaske, C. J., Haussler, D., Kent, W. J., & Haeussler, M. (2025). The UCSC Genome Browser database: 2025 update. *Nucleic Acids Research*, 53(D1), D1243–D1249. <https://doi.org/10.1093/nar/gkae974>
- Peter, B. M. (2022). A geometric relationship of F2, F3 and F4-statistics with principal component analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 377(1852), 20200413. <https://doi.org/10.1098/rstb.2020.0413>
- Petr, M., Vernot, B., & Kelso, J. (2019). admixr—R package for reproducible analyses using ADMIXTOOLS. *Bioinformatics*, 35(17), 3194–3195. <https://doi.org/10.1093/bioinformatics/btz030>
- Pollen, A. A., Kilik, U., Lowe, C. B., & Camp, J. G. (2023). Human-specific genetics: New tools to explore the molecular and cellular basis of human evolution. *Nature Reviews Genetics*, 24(10), 687–711. <https://doi.org/10.1038/s41576-022-00568-4>
- Polosmak, N. V. (2023). The Pazyryk Dwelling. *Archaeology, Ethnology & Anthropology of Eurasia*, 51(1), Article 1.
- Prendergast, M. E., Lipson, M., Sawchuk, E. A., Olalde, I., Ogola, C. A., Rohland, N., Sirak, K. A., Adamski, N., Bernardos, R., Broomandkhoshbacht, N., Callan, K., Culleton, B. J., Eccles, L., Harper, T. K., Lawson, A. M., Mah, M., Oppenheimer, J., Stewardson, K., Zalzal, F., ... Reich, D. (2019). Ancient DNA reveals a multistep spread of the first herders into sub-Saharan Africa. *Science*, 365(6448), eaaw6275. <https://doi.org/10.1126/science.aaw6275>
- Qu, Y., Hu, X., Wang, T., & Yang, Y. (2020). Early interaction of agropastoralism in Eurasia: New evidence from millet-based food consumption of Afanasyevo humans in the southern Altai Mountains, Xinjiang, China. *Archaeological and Anthropological Sciences*, 12(8), 195. <https://doi.org/10.1007/s12520-020-01094-2>
- Qu, Y., Hu, Y., Rao, H., Abuduresule, I., Li, W., Hu, X., Jiang, H., Wang, C., & Yang, Y. (2018). Diverse lifestyles and populations in the Xiaohe culture of the Lop Nur region, Xinjiang,

- China. *Archaeological and Anthropological Sciences*, 10(8), 2005–2014.
<https://doi.org/10.1007/s12520-017-0520-7>
- Rascovan, N., Sjögren, K.-G., Kristiansen, K., Nielsen, R., Willerslev, E., Desnues, C., & Rasmussen, S. (2019). Emergence and Spread of Basal Lineages of *Yersinia pestis* during the Neolithic Decline. *Cell*, 176(1), 295–305.e10.
<https://doi.org/10.1016/j.cell.2018.11.005>
- Rasmussen, S., Allentoft, M. E., Nielsen, K., Orlando, L., Sikora, M., Sjögren, K.-G., Pedersen, A. G., Schubert, M., Van Dam, A., Kapel, C. M. O., Nielsen, H. B., Brunak, S., Avetisyan, P., Epimakhov, A., Khalyapin, M. V., Gnuni, A., Kriiska, A., Lasak, I., Metspalu, M., ... Willerslev, E. (2015). Early Divergent Strains of *Yersinia pestis* in Eurasia 5,000 Years Ago. *Cell*, 163(3), 571–582. <https://doi.org/10.1016/j.cell.2015.10.009>
- Reich, D., Thangaraj, K., Patterson, N., Price, A. L., & Singh, L. (2009). Reconstructing Indian population history. *Nature*, 461(7263), 489–494. <https://doi.org/10.1038/nature08365>
- Roach, J. C., Glusman, G., Rowen, L., Kaur, A., Purcell, M. K., Smith, K. D., Hood, L. E., & Aderem, A. (2005). The evolution of vertebrate Toll-like receptors. *Proceedings of the National Academy of Sciences*, 102(27), 9577–9582.
<https://doi.org/10.1073/pnas.0502272102>
- Robinson-Rechavi, M., Rech de Laval, V., Bastian, F. B., Wollbrett, J., & Bgee, T. (2020). The Expression Comparison Tool in Bgee. In C. Scornavacca, F. Delsuc, & N. Galtier (Eds.), *Phylogenetics in the Genomic Era* (p. 4.3:1–4.3:4). No commercial publisher | Authors open access book. <https://hal.science/hal-02535720>
- Rogers, E. N., Jones, D. Z., Kidd, N. C., Yeyeodu, S., Brock, G., Ragin, C., Jackson, M., McFarlane-Anderson, N., Tulloch-Reid, M., Sean Kimbro, K., & Kidd, L. R. (2013). Toll-like receptor-associated sequence variants and prostate cancer risk among men of African descent. *Genes & Immunity*, 14(6), 347–355.
<https://doi.org/10.1038/gene.2013.22>
- Rolland, J., Henao-Diaz, L. F., Doebeli, M., Germain, R., Harmon, L. J., Knowles, L. L., Liow, L. H., Mank, J. E., Machac, A., Otto, S. P., Pennell, M., Salamin, N., Silvestro, D., Sugawara, M., Uyeda, J., Wagner, C. E., & Schluter, D. (2023). Conceptual and empirical bridges between micro and macroevolution. *Nature Ecology & Evolution*, 7(8), 1181–1193. <https://doi.org/10.1038/s41559-023-02116-7>
- Rosalino, L. M., Verdade, L. M., & Lyra-Jorge, M. C. (2014). Adaptation and Evolution in Changing Environments. In L. M. Verdade, M. C. Lyra-Jorge, & C. I. Piña (Eds.), *Applied Ecology and Human Dimensions in Biological Conservation* (pp. 53–71). Springer.
https://doi.org/10.1007/978-3-642-54751-5_4
- Rubinson, K. S., & Linduff, K. M. (2024). A Distinct Form of Socio-Political and Economic Organization in the Pazyryk Culture. *Arts*, 13(1), Article 1.
<https://doi.org/10.3390/arts13010036>
- Sabin, S., Herbig, A., Vågene, Å. J., Ahlström, T., Bozovic, G., Arcini, C., Kühnert, D., & Bos, K.

- I. (2020). A seventeenth-century *Mycobacterium tuberculosis* genome supports a Neolithic emergence of the *Mycobacterium tuberculosis* complex. *Genome Biology*, 21(1), 201. <https://doi.org/10.1186/s13059-020-02112-1>
- Sánchez-Quinto, F., Botigué, L. R., Civit, S., Arenas, C., Ávila-Arcos, M. C., Bustamante, C. D., Comas, D., & Lalueza-Fox, C. (2012). North African Populations Carry the Signature of Admixture with Neandertals. *PLOS ONE*, 7(10), e47765. <https://doi.org/10.1371/journal.pone.0047765>
- Sankararaman, S., Patterson, N., Li, H., Pääbo, S., & Reich, D. (2012). The Date of Interbreeding between Neandertals and Modern Humans. *PLOS Genetics*, 8(10), e1002947. <https://doi.org/10.1371/journal.pgen.1002947>
- Seersholm, F. V., Sjögren, K.-G., Koelman, J., Blank, M., Svensson, E. M., Staring, J., Fraser, M., Pinotti, T., McColl, H., Gaunitz, C., Ruiz-Bedoya, T., Granehall, L., Villegas-Ramirez, B., Fischer, A., Price, T. D., Allentoft, M. E., Iversen, A. K. N., Axelsson, T., Ahlström, T., ... Sikora, M. (2024). Repeated plague infections across six generations of Neolithic Farmers. *Nature*, 632(8023), 114–121. <https://doi.org/10.1038/s41586-024-07651-2>
- Ségurel, L., & Bon, C. (2017). On the Evolution of Lactase Persistence in Humans. *Annual Review of Genomics and Human Genetics*, 18(Volume 18, 2017), 297–319. <https://doi.org/10.1146/annurev-genom-091416-035340>
- Senturk, N., & Ergoren, M. C. (2020). Developing an Online Portal for Determining the Genomic Signature of Archaic DNA that are Associated to Modern Human Genetic Diseases: A Meta-Analysis Study. *The Eurasian Journal of Medicine*, 52(2), 153–160. <https://doi.org/10.5152/eurasianjmed.2019.18424>
- Senut, B., Pickford, M., Gommery, D., & Ségalen, L. (2017). Palaeoenvironments and the origin of hominid bipedalism. *Historical Biology*. <https://doi.org/10.1080/08912963.2017.1286337>
- Shen, H., Wu, X., Tang, Z., Zhou, X., Sun, N., & Li, X. (2015). Wood Usage and Fire Veneration in the Pamir, Xinjiang, 2500 yr BP. *PLOS ONE*, 10(8), e0134847. <https://doi.org/10.1371/journal.pone.0134847>
- Sikora, M., Pitulko, V. V., Sousa, V. C., Allentoft, M. E., Vinner, L., Rasmussen, S., Margaryan, A., de Barros Damgaard, P., de la Fuente, C., Renaud, G., Yang, M. A., Fu, Q., Dupanloup, I., Giampoudakis, K., Nogués-Bravo, D., Rahbek, C., Kroonen, G., Peyrot, M., McColl, H., ... Willerslev, E. (2019). The population history of northeastern Siberia since the Pleistocene. *Nature*, 570(7760), 182–188. <https://doi.org/10.1038/s41586-019-1279-z>
- Simon, A., & Coop, G. (2024). The contribution of gene flow, selection, and genetic drift to five thousand years of human allele frequency change. *Proceedings of the National Academy of Sciences of the United States of America*, 121(9), e2312377121. <https://doi.org/10.1073/pnas.2312377121>
- Simonti, C. N., Vernot, B., Bastarache, L., Bottinger, E., Carrell, D. S., Chisholm, R. L., Crosslin,

- D. R., Hebbring, S. J., Jarvik, G. P., Kullo, I. J., Li, R., Pathak, J., Ritchie, M. D., Roden, D. M., Verma, S. S., Tromp, G., Prato, J. D., Bush, W. S., Akey, J. M., ... Capra, J. A. (2016). The phenotypic legacy of admixture between modern humans and Neandertals. *Science*, 351(6274), 737–741. <https://doi.org/10.1126/science.aad2149>
- Slavin, P., & Sebbane, F. (2022). Emergence and spread of ancestral *Yersinia pestis* in Late-Neolithic and Bronze-Age Eurasia, ca. 5,000 to 2,500 y B.P. *Proceedings of the National Academy of Sciences*, 119(21), e2204044119. <https://doi.org/10.1073/pnas.2204044119>
- Smith, J. M., & Haigh, J. (1974). The hitch-hiking effect of a favourable gene. *Genetical Research*, 23(1), 23–35. <https://doi.org/10.1017/S0016672300014634>
- Stephens, P. R., Gottdenker, N., Schatz, A. M., Schmidt, J. P., & Drake, J. M. (2021). Characteristics of the 100 largest modern zoonotic disease outbreaks. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 376(1837), 20200535. <https://doi.org/10.1098/rstb.2020.0535>
- Strahl, H., & Errington, J. (2017). Bacterial Membranes: Structure, Domains, and Function. *Annual Review of Microbiology*, 71(Volume 71, 2017), 519–538. <https://doi.org/10.1146/annurev-micro-102215-095630>
- Sturm, R. A., & Duffy, D. L. (2012). Human pigmentation genes under environmental selection. *Genome Biology*, 13(9), 248. <https://doi.org/10.1186/gb-2012-13-9-248>
- Sümer, A. P., Rougier, H., Villalba-Mouco, V., Huang, Y., Iasi, L. N. M., Essel, E., Bossoms Mesa, A., Furtwaengler, A., Peyrégne, S., de Filippo, C., Rohrlach, A. B., Pierini, F., Mafessoni, F., Fewlass, H., Zavala, E. I., Mylopotamitaki, D., Bianco, R. A., Schmidt, A., Zorn, J., ... Krause, J. (2025). Earliest modern human genomes constrain timing of Neanderthal admixture. *Nature*, 638(8051), 711–717. <https://doi.org/10.1038/s41586-024-08420-x>
- Svyatko, S. V., Владимировна, С. С., Beisenov, A. Z., & Зияденович, Б. А. (2017). First palaeodietary isotopic data for the Tasmola culture population. *Samara Journal of Science*, 6(3), 223–227. <https://doi.org/10.17816/snv201763223>
- Takeda, K., & Akira, S. (2005). Toll-like receptors in innate immunity. *International Immunology*, 17(1), 1–14. <https://doi.org/10.1093/intimm/dxh186>
- Thorpe, S. K. S., Holder, R. L., & Crompton, R. H. (2007). Origin of Human Bipedalism As an Adaptation for Locomotion on Flexible Branches. *Science*, 316(5829), 1328–1331. <https://doi.org/10.1126/science.1140799>
- Tishkin, A. A., & Dashkovsky, P. K. (2019). Social Complexity of Pazyrik Culture in Altai, 550–100 BC. *Social Evolution & History*, 18(2). <https://doi.org/10.30884/seh/2019.02.04>
- Tishkin, A., Hermes, T., & S., G. (2021). Nizhnyaya Sooru Settlement in Central Altai: Some Outcomes of the Study and Prospects for Further Research. *Teoriya i Praktika Arkheologicheskikh Issledovaniy*, 33(3), 125–141.

[https://doi.org/10.14258/tpai\(2021\)33\(3\).-08](https://doi.org/10.14258/tpai(2021)33(3).-08)

Tomassello, M. (2003). The Human Adaptation for Culture. In *Handbook of Evolution* (pp. 1–23). John Wiley & Sons, Ltd. <https://doi.org/10.1002/9783527619702.ch1>

Turvey, S. E., & Broide, D. H. (2010). Innate immunity. *Journal of Allergy and Clinical Immunology*, 2010 Primer on Allergic and Immunologic Diseases, 125(2, Supplement 2), S24–S32. <https://doi.org/10.1016/j.jaci.2009.07.016>

Valente, C., Alvarez, L., Marks, S. J., Lopez-Parra, A. M., Parson, W., Oosthuizen, O., Oosthuizen, E., Amorim, A., Capelli, C., Arroyo-Pardo, E., Gusmão, L., & Prata, M. J. (2015). Exploring the relationship between lifestyles, diets and genetic adaptations in humans. *BMC Genetics*, 16, 55. <https://doi.org/10.1186/s12863-015-0212-1>

Valtueña, A. A., Mitnik, A., Key, F. M., Haak, W., Allmäe, R., Belinskij, A., Daubaras, M., Feldman, M., Jankauskas, R., Janković, I., Massy, K., Novak, M., Pfrengle, S., Reinhold, S., Šlaus, M., Spyrou, M. A., Szécsényi-Nagy, A., Törv, M., Hansen, S., ... Krause, J. (2017). The Stone Age Plague and Its Persistence in Eurasia. *Current Biology*, 27(23), 3683–3691.e8. <https://doi.org/10.1016/j.cub.2017.10.025>

Ventresca Miller, A. R., Johnson, J., Makhortykh, S., Gerling, C., Litvinova, L., Andrukh, S., Toshev, G., Zech, J., Le Roux, P., Makarewicz, C., Boivin, N., & Roberts, P. (2021). Re-evaluating Scythian lifeways: Isotopic analysis of diet and mobility in Iron Age Ukraine. *PLOS ONE*, 16(3), e0245996. <https://doi.org/10.1371/journal.pone.0245996>

Ventresca Miller, A. R., Wilkin, S., Hendy, J., Turbat, T., Batsukh, D., Bayarkhuu, N., Giscard, P.-H., Bemmman, J., Bayarsaikhan, J., Miller, B. K., Clark, J., Roberts, P., & Boivin, N. (2022). The spread of herds and horses into the Altai: How livestock and dairying drove social complexity in Mongolia. *PLOS ONE*, 17(5), e0265775. <https://doi.org/10.1371/journal.pone.0265775>

Villanea, F. A., & Schraiber, J. G. (2019). Multiple episodes of interbreeding between Neanderthal and modern humans. *Nature Ecology & Evolution*, 3(1), Article 1. <https://doi.org/10.1038/s41559-018-0735-8>

Wall, J. D., Yang, M. A., Jay, F., Kim, S. K., Durand, E. Y., Stevison, L. S., Gignoux, C., Woerner, A., Hammer, M. F., & Slatkin, M. (2013). Higher Levels of Neanderthal Ancestry in East Asians than in Europeans. *Genetics*, 194(1), 199–209. <https://doi.org/10.1534/genetics.112.148213>

Wang, C.-C., Yeh, H.-Y., Popov, A. N., Zhang, H.-Q., Matsumura, H., Sirak, K., Cheronet, O., Kovalev, A., Rohland, N., Kim, A. M., Mallick, S., Bernardos, R., Tumen, D., Zhao, J., Liu, Y.-C., Liu, J.-Y., Mah, M., Wang, K., Zhang, Z., ... Reich, D. (2021). Genomic insights into the formation of human populations in East Asia. *Nature*, 591(7850), 413–419. <https://doi.org/10.1038/s41586-021-03336-2>

Wang, K., Goldstein, S., Bleasdale, M., Clist, B., Bostoen, K., Bakwa-Lufu, P., Buck, L. T., Crowther, A., Dème, A., McIntosh, R. J., Mercader, J., Ogola, C., Power, R. C., Sawchuk, E., Robertshaw, P., Wilmsen, E. N., Petraglia, M., Ndiema, E., Manthi, F. K., ...

- Schiffels, S. (2020). Ancient genomes reveal complex patterns of population movement, interaction, and replacement in sub-Saharan Africa. *Science Advances*, 6(24), eaaz0183. <https://doi.org/10.1126/sciadv.aaz0183>
- Wang, K., Huang, H., Zhan, Q., Ding, H., & Li, Y. (2024). Toll-like receptors in health and disease. *MedComm*, 5(5), e549. <https://doi.org/10.1002/mco2.549>
- Wang, L., Wang, Y., Li, W., Spate, M., Rehemani, K., Sun, Q., Wang, B., Xu, H., Zhang, Z., Zhang, G., & Jiang, H. (2021). Inner Asian agro-pastoralism as optimal adaptation strategy of Wupu inhabitants (3000–2400 cal BP) in Xinjiang, China. *The Holocene*, 31(2), 203–216. <https://doi.org/10.1177/0959683620941139>
- Wang, W., Liu, Y., Duan, F., Zhang, J., Liu, X., Reid, R. E. B., Zhang, M., Dong, W., Wang, Y., Ruan, Q., Li, W., & An, C.-B. (2020). A comprehensive investigation of Bronze Age human dietary strategies from different altitudinal environments in the Inner Asian Mountain Corridor. *Journal of Archaeological Science*, 121, 105201. <https://doi.org/10.1016/j.jas.2020.105201>
- Wang, X., Tang, Z., Li, Y., Zhang, G., Lu, L., Wang, B., Wang, W., Lü, H., & Roberts, P. (2025). Illuminating interaction networks along the Silk Roads: A multi-isotopic analysis of the Zaghunluq Cemetery, southern Xinjiang, China. *Antiquity*, 1–18. <https://doi.org/10.15184/aqy.2025.45>
- Wang, X., Tang, Z., Wu, J., Wu, X., Wu, Y., & Zhou, X. (2016). Strontium isotope evidence for a highly mobile population on the Pamir Plateau 2500 years ago. *Scientific Reports*, 6(1), 35162. <https://doi.org/10.1038/srep35162>
- Wang, Z., Guo, T., Xu, L., Liu, J., Hou, Y., Jin, J., Zhang, Q., Jiang, T., Zhao, Z., & Xue, Y. (2024). Regional differences of Mycobacterium tuberculosis complex infection and multidrug resistance epidemic in Luoyang. *BMC Infectious Diseases*, 24, 578. <https://doi.org/10.1186/s12879-024-09395-w>
- Warren, R., Smith, R. F., & Cybenko, A. K. (2011). Use of Mahalanobis Distance for Detecting Outliers and Outlier Clusters in Markedly Non-Normal Data: A Vehicular Traffic Example (No. AFRLRHWPTR20110070). Article AFRLRHWPTR20110070. <https://apps.dtic.mil/sti/html/tr/ADA545834/>
- Weber, A. W., & Goriunova, O. I. (2013). Hunter–gatherer migrations, mobility and social relations: A case study from the Early Bronze Age Baikal region, Siberia. *Journal of Anthropological Archaeology*, HUMAN BIOARCHAEOLOGY: GROUP IDENTITY AND INDIVIDUAL LIFE HISTORIES, 32(3), 330–346. <https://doi.org/10.1016/j.jaa.2012.01.006>
- Wright, S. (1932). The roles of mutation, inbreeding, crossbreeding, and selection in evolution. <http://www.esp.org/books/6th-congress/facsimile/contents/6th-cong-p356-wright.pdf>
- Wu, X., Ning, C., Key, F. M., Valtueña, A. A., Lankapalli, A. K., Gao, S., Yang, X., Zhang, F., Liu, L., Nie, Z., Ma, J., Krause, J., Herbig, A., & Cui, Y. (2021). A 3,000-year-old, basal *S. enterica* lineage from Bronze Age Xinjiang suggests spread along the Proto-Silk Road.

PLOS Pathogens, 17(9), e1009886. <https://doi.org/10.1371/journal.ppat.1009886>

Xiang, L. (2023). Prehistoric population expansion in Central Asia promoted by the Altai Holocene Climatic Optimum.

Xie, S., Guo, Y., & Wu, X. (2022). Millet consumption in south Xinjiang during the 1st millennium BCE: Isotopic evidence from bone bioapatite at the Ji'erzankale site. *Quaternary International*, 634, 38–47. <https://doi.org/10.1016/j.quaint.2022.06.014>

Yablonsky, L. T. (1995). *The Archaeology of Eurasian Nomads*.

Yamamoto, M., Sato, S., Hemmi, H., Hoshino, K., Kaisho, T., Sanjo, H., Takeuchi, O., Sugiyama, M., Okabe, M., Takeda, K., & Akira, S. (2003). Role of Adaptor TRIF in the MyD88-Independent Toll-Like Receptor Signaling Pathway. *Science (New York, N.Y.)*, 301, 640–643. <https://doi.org/10.1126/science.1087262>

Yamamoto, M., Sato, S., Mori, K., Hoshino, K., Takeuchi, O., Takeda, K., & Akira, S. (2003). Cutting Edge: A Novel Toll/IL-1 Receptor Domain-Containing Adapter That Preferentially Activates the IFN- Promoter in the Toll-Like Receptor Signaling. *Journal of Immunology (Baltimore, Md. : 1950)*, 169, 6668–6672. <https://doi.org/10.4049/jimmunol.169.12.6668>

Yan, Z., Du, L., Alipujang, N., Alimu, A., Zhang, Z., Qiu, M., & Dong, G. (2025). Spatio-temporal changes of prehistoric human activities and subsistence in relation to trans-Eurasian exchange in the Inner Asian Mountain Corridor. *Journal of Geographical Sciences*, 35, 1667–1682. <https://doi.org/10.1007/s11442-025-2388-2>

Yang, R., Yang, Y., Li, W., Abuduresule, Y., Hu, X., Wang, C., & Jiang, H. (2014). Investigation of cereal remains at the Xiaohe Cemetery in Xinjiang, China. *Journal of Archaeological Science*, 49, 42–47. <https://doi.org/10.1016/j.jas.2014.04.020>

Yang, X., Li, H., Wang, B., Zhu, K., He, H., Ji, Z., Wang, B., Zhao, J., & Wang, C.-C. (2025). Population dynamics in Iron Age Xinjiang inferred from ancient genomes of the Zhagunluke site. *Archaeological and Anthropological Sciences*, 17(4), 76. <https://doi.org/10.1007/s12520-025-02186-7>

Yu, H., Spyrou, M. A., Karapetian, M., Shnaider, S., Radzevičiūtė, R., Nägele, K., Neumann, G. U., Penske, S., Zech, J., Lucas, M., LeRoux, P., Roberts, P., Pavlenok, G., Buzhilova, A., Posth, C., Jeong, C., & Krause, J. (2020). Paleolithic to Bronze Age Siberians Reveal Connections with First Americans and across Eurasia. *Cell*, 181(6), 1232–1245.e20. <https://doi.org/10.1016/j.cell.2020.04.037>

Zarembek, K. A., & Godowski, P. J. (2002). Tissue Expression of Human Toll-Like Receptors and Differential Regulation of Toll-Like Receptor mRNAs in Leukocytes in Response to Microbes, Their Products, and Cytokines. *The Journal of Immunology*, 168(2), 554–561. <https://doi.org/10.4049/jimmunol.168.2.554>

Zhang, F., Ning, C., Scott, A., Fu, Q., Bjørn, R., Li, W., Wei, D., Wang, W., Fan, L., Abuduresule, I., Hu, X., Ruan, Q., Niyazi, A., Dong, G., Cao, P., Liu, F., Dai, Q., Feng, X., Yang, R., ... Cui, Y. (2021). The genomic origins of the Bronze Age Tarim Basin mummies. *Nature*,

599(7884), 256–261. <https://doi.org/10.1038/s41586-021-04052-7>

Zhang, F., Ning, C., Scott, A., Fu, Q., Bjørn, R., Li, W., Wei, D., Wang, W., Linyuan, F., Abuduresule, I., Hu, X., Ruan, Q., Niyazi, A., Dong, G., Cao, P., Liu, F., Dai, Q., Feng, X., Yang, R., & Cui, Y. (2021). The genomic origins of the Bronze Age Tarim Basin mummies. *Nature*, 599, 1–6. <https://doi.org/10.1038/s41586-021-04052-7>

Zhang, Y., Mo, D., Hu, K., Bao, W., Li, W., Abuduresule, I., Storozum, M. J., & Kidder, T. R. (2017). Holocene environmental changes around Xiaohe Cemetery and its effects on human occupation, Xinjiang, China. *Journal of Geographical Sciences*, 27(6), 752–768. <https://doi.org/10.1007/s11442-017-1404-6>

Appendix A: Archaeological and Genetic background of subsamples

Saka

There are several groups that are associated with the Scythian tribes. Namely, Tasmola, Saka and their sub-tribe, the Pazyryk. Scythian ancestry can be modelled as a three-way mixture between Khövsgol (inner Mongolia), steppe_MLBA, and small contributions from populations inhabiting the southern regions of the Caucasus/ Iran or BMAC (Bactria–Margiana Archaeological Complex). That proportion of Caucas/Iranian ancestry increases through time with up to ~20% in the Tian Shan Saka groups (Gnecchi-Ruscone *et al.*, 2021). It should be noted though the genetic profiles of these groups are distinct from those in the western part of the Scytho-Siberian world whose genetic profile receives substantially less influence from the steppe (Andreeva *et al.*, 2025).

The Saka were part of the Scythian federation of local groups which also included Tasmola, Sagly-Uyuk, and Pazyryk (Yablonsky, 1995) whose genetic heterogeneity can be explained by geography. Jeong *et al.* (2019) suggests Tian Shan populations may have derived some of their ancestry from populations originating from West/South Asia during the middle of the first millennium BC and into Kazakhstan. Alternatively, Kazakhstan Saka may be modelled as Ngansan + Srubnaya or Ulchi + Srubnaya + AG3. This is in agreement with Gnecchi-Ruscone *et al.* (2021) who later suggested Nomadic IA populations of the Kazakh steppe formed through consistent pulses of admixture between preceding MLBA populations from the steppe and the neighboring regions. Eastern groups may have descended from a homogeneous gene pool associated with Sintashta, Srubnaya, and Andronovo and from a specific eastern Eurasian source already present in Mongolia during the late Bronze Age. However, they suggest the ancestry of the Saka groups in Kazakhstan and Tian Shan can be explained differently. Tian Shan Sakas can

be modelled as a mixture of BMAC, steppe_MLBA and Khövsgöl with higher ancestry yet less uniformly distributed contributions from the southern Caucasus between 400-100 BCE-CE (Gnecchi-Ruscone *et al.*, 2021b).

Even though the Saka were considered nomadic horse warriors, some members engaged in sedentism and constructed dwellings and used millet agriculture (Yablonsky, 1995; Beisenov *et al.*, 2017; Ventresca Miller *et al.*, 2021; Polosmak, 2023). Saka sample was given the “Semi-Sedentary” label because of this. Likewise, their sibling culture, Sagly-Uyuk is represented by 18 individuals (originally reported in Jeong *et al.*, 2020 & Wang *et al.*, 2021) was also given the “Semi-Sedentary” label for analysis.

Tasmola

This study incorporated 22 individuals belonging to the Tasmola culture previously reported in Gnecchi-Ruscone *et al.* (2021). Furthermore, the genetic structure of Early IA Tasmola culture appears to be of equal mixture between preceding Sintashta, Srubnaya, and Andronovo groups as well as BMAC, although miniscule amounts of gene flow from Iranian farmers is also required (Gnecchi-Ruscone *et al.*, 2021). Tasmola sites provide evidence that their members had agricultural practices, consumed millet, strategically brokered seasonal migration between summer and winter pastures, and contain equipment used for horse harnessing (Beisenov n.d.; Beisenov *et al.*, 2016; Svyatko and Beisenov, 2017). The archeological findings suggest that they were mobile in the sense that they migrated between seasonal pastures with the assistance of horses and during those seasons, differentially used agriculture to subsist while they settled in those areas. They were given the “Semi-Sedentary” label for those reasons.

Pazyryk

The genomes of 16 people who belonged to the Pazyryk culture, originally reported in Gneocchi-Ruscione et al. (2021), were used in this study. The genetic architecture of the Pazyryk is similar to that of other Scythian groups — BMAC influence is present in the later fourth to first century BCE-CE Pazyryk site of Berel in Kazakhstan yet received more ancestry from a Eurasian source when compared to Tian Shan Sakas (Gneocchi-Ruscione et al., 2021).

Archaeological literature suggests the Pazyryk people had a mixed economy of nomadism and sedentary agricultural cattle breeding which was aided by the construction of log dwellings built on summer and winter pastures (Polosmak 2023 & Robinson and Linduff 2024). As such, they were assigned to the “Semi-Sedentary” label of analysis because of the cyclical habitation and agricultural practice they engaged in.

Andronovo

Andronovo culture represents a temporal and geographical extension of Sintashta culture whose genetic origins can be pin pointed either by eastward migration of Corded Ware peoples or descent by an earlier steppe population. In both cases contributions from EHG and CHG are required with differing amounts of Anatolian Farmer related ancestry between Andronovo and Sintashta groups. Regardless, Andronovo culture was later replaced in Xinjiang by East Asian peoples that appear in the later cultures such as Mezhovskaya and Karasuk (Allentoft et al., 2015; Damgaard et al., 2018; Kumar et al., 2022). This study incorporates the data of 21 individuals from the “Andronovo cultural-historical commonality,” which also includes groups known as the Federovo and Alakul (originally reported in Damgaard et al., 2018; Narasimhan et al., 2019; Kılınç et al., 2021; Kumar et al., 2022). They were designated as being

“Semi-Sedentary.” The justification of the “Semi-Sedentary” label was based on the work of Outram et al. (2012), who showed that the meat and milk of sheep, cattle and horses were consumed at a number of Andronovo sites. While there is no indication of cereal consumption, a level of sedentism is further supported by the construction of fortified settlements (Grigoriev 2021). Similarly, the work of Jia et al. (2020) compares the settlement pattern of modern pastoralists to the Andronovo and concludes that the Andronovo settlement pattern indicates seasonal migration.

The semi-sedentary label has some support on the molecular front as well – an Alakul site revealed the practice of exogamous marriage. Blöcher et al. (2023) reported genomic data of 32 individuals dated to ~3800 years old belonging to the “Srubnaya-Alakul” culture in the Southern Urals. They received genetic contributions from a substrate of preceding Sintashta-like people, Eneolithic Shamanka hunter-gatherers from lake Baikal, and the Russian Eneolithic Steppe. The authors also investigated familial ties among the recovered individuals. A reconstruction pedigree of 23 individuals placed six brothers at the center. The genetic relationships between this family indicate an emphasis on patrilineal descent and organization around the male line. In contrast, female contributions were more diverse, and female children above the age of five were absent from the site, suggesting they originated from neighboring groups.

Afanasievo

Much debate around the emergence of the hunter-herding Afanasievo culture has been recently based on archaeogenetic research (Honeychurch et al. 2021; Wang et al., 2021; Kumar et al., 2022; Narasimhan et al., 2019; Zhang et al., 2021). Narasimhan et al., (2019) suggest the

genetic profile of Afanasievo people contained contributions from Eneolithic peoples on the steppe, an Iranian farmer population, and a European farmer population. While they do exhibit some genetic similarity to Yamnaya, they do not form a clade with Western_Steppe_EMBA. This can be explained by an eastward migration of individuals with Yamnaya-related ancestry onto the steppe. The people of the Afanasievo culture were given the “Semi-Sedentary” label for analysis (n= 24, originally reported in Wang et al., 2021; Kumar et al., 2022; Narasimhan et al., 2019; Zhang et al., 2021). Afanasievo individuals in this study were given the semi-sedentary label for the following reasons: first, one settlement confirmed the use of domestic sheep at the site based on the presence of mitochondrial cytochrome b gene (MT-CYB) in *Ovis aries* (Hermes et al., 2020). Secondly, tools such as saws, burins, and grinding tools, point to both wood-working capabilities and metal processing. Faunal remains of cattle, sheep and horse were recovered as well (Tishkin et al., 2021). Third, Qu et al. (2020) presented evidence of differential millet consumption across multiple Afanasievo sites.

Tian Shan Huns

This study uses the genetic data of 24 individuals, originally reported in Damgaard et al., (2018), described as Tian Shan Huns. They have no genetic nor archaeological association with European Huns yet they also are distinct from Xiongnu or other Tian Shan inhabitants (Gnecchi-Ruscone et al., 2025). Despite their elusive context, it appears as though these individuals were part of a sedentary family. This is based on the single site location, genetic relationships, and the fact that the dating of these individuals' burials are separated by a few years. This represents a relatively sedentary community whereby individuals also left offspring who were recovered from nearby sites; justifying the “Sedentary” label of analysis in this study.

As a side note: individual DA101 showed detectable traces of *Yersinia pestis* in Damgaard et al. (2018) with full bubonic potential and flea-transmission. This is of particular importance because it demonstrates the presence of plague being found along the silk road, which would have provided ample hosts for propagation.

Sarmatians

Archeo-genetic studies on the Sarmatians such as Gneccchi-Ruscone et al. (2021) suggest they maintained a high level of genetic homogeneity whose members receive contributions from Iranian farmers, Khövsgol and steppe_MLBA-related ancestry while covering a wide geographic range and for multiple centuries. The Sarmatians in this study (n= 24; originally reported in Damgaard et al., 2018; Narasimhan et al., 2019; Gneccchi-Ruscone et al., 2021) were given the “Semi-Sedentary” label for analysis to account for the observation that different portions of their cultural complex were quite mobile, while others were on the other extreme and sedentary.

Marina Moshkova writes in Davis-Kimball *et al.* (1995; pp. 184-188), the Sarmatians participated in an incredibly mixed economy, composed of cattle breeders seasonally mobilizing their livestock between summer and winter pastures. Pursuits such as metallurgy, pottery production, and leather dressing were likely done by sedentary groups within their territory at the behest of nomadic aristocrats and militias.

Khövsgöls

20 LBA Khövsgöls (originally reported in Jeong et al., 2018) were included into the current dataset for analysis and given the semi-sedentary label for four reasons: Jeong et al. (2018) found that the dairy-adaptive -13910*T (rs4988235) lactase persistence (LP) allele

was not present in this group despite 7 out of 9 tested individuals containing dairy proteins in their dental calculus. Secondly, Jeong also found Early Bronze Age hunter-gatherers from the Baikal region to be their largest genetic contributor. Third, despite the burials were made entirely of stone and no culturally identifiable artefacts were recovered (likely due to looting having occurred shortly after burial), and many remains showed signs of violent interactions (), their burials reflect an association with the Deer Stone-Khirigsuur Complex (DSKC) which provides the highest fidelity of evidence for animal pastoralism on the Steppe prior to ca. 1200 BCE (Houle, 2010 & Frohlich et al. 2010). Fourth, in Houle, their typical settlement pattern is restricted to floodplains and foothills which reflects a cycle of seasonal migration between summer and winter pastures. Based on the genetic literature, a majority of LBA Khövsgöls received ancestry from an ANE source during the Neolithic period, and a much smaller proportion of WSH ancestry in the early Bronze Age. Genetic outliers varied in ancestry proportions. one showed higher affinities with early Neolithic individuals from the Russian far east and in general with contemporary Han Chinese. Another exhibited affinity toward MBA Sintashta culture, and early Bronze age Shamanka (Jeong et al., 2018).

Baikal HG

The sub-sample of 21 individuals (originally reported in: Kılınç et al., 2021; Flegontov et al., 2019; Sikora et al., 2019) from Angara River HG and the HG within the broader Baikal region were given the “Mobile” label for analysis here. This is because the populations in the Baikal region generally engaged in a pattern of HG in which they consumed large amounts of game, fish, and seal up until 2900-3275 cal. BP (post dating the subsample used here) where we see a dietary shift with the arrival of domestic herd animals (Weber and Goriunova, 2013; Losey et al.,

2016; 2017). Furthermore, there is no evidence they constructed dwellings, or engaged in agriculture. Genetically speaking, Baikal populations reported in Yu et al. (2020) showed highest genetic affinity with previously published Early neolithic and LNBA Baikal Populations. Admixture timing estimates ranged between ~8,000 and 5,500 BP suggesting LNBA could have been formed through a prolonged process of admixture between local groups and northeast Asian related populations in southern or central Siberia. For example, ANE ancestry increased from 14.3% among Baikal_EN to 22.7% in the LNBA population. However, outlier individuals point to interaction between LNBA Baikal and multiple bronze age western steppe populations, most likely from nearby Afanasievo in the Altai-Sayan region. This was later elucidated by Kılınç et al. (2021) who revealed the shift occurred between ~6100 and ~3700 where an increase in Western Eurasian-related ancestry was observed along with comparably low amounts of Northeast Asian components (Kılınç et al., 2021).

We also regard this region to be of interest because of the presence of *Y. pestis* on multiple occasions – two individuals in Yu et al. (2020) at 4,556 and 4,430 BP showed evidence of *Y. pestis* and both lacked Yamnaya related steppe ancestry. The two reconstructed *Y. pestis* genomes fell on the LNBA lineage previously described by Andrades Valtueña et al. (2017). These findings are consistent with Kılınç et al. (2021) who also documented the presence of *Y. pestis* in the Cis-Baikal region of Yakutia between ~4400 and ~3800 years ago.

Xiaohe

13 individuals, dated to Cal. 4–3.5 ka BP from the Lop Nur region of Xinjiang at the Xiaohe cemetery (originally reported in Zhang et al., 2021) were selected for the current study (Li et al., 2013). Genetically speaking, EMBA individuals buried at Xiaohe form a tight cluster

along a PCA with pre-bronze age central steppe and Siberian inhabitants who carry high amounts of ANE ancestry. These individuals were given the “Sedentary” label for several reasons: first, Inhabitants were able to practice agriculture nearby along the rivers due to an Oasis formation during 4.8--3.5 ka BP despite being mostly desert today (Zhang et al., 2017). Secondly, the discovery of common millet (*Panicum miliaceum*), caryopses of bread wheat (*Triticum aestivum*) and love grass (*Eragrostis sp.*) remains within tombs at the cemetery suggests agriculture was a common practice (Yang et al., 2014). Third, the commonality of *Populus euphratica* suggests it was not exclusively used for constructing coffins but also dwellings as well (Li et al., 2013 & Bergman 1939). Fourth, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of M13 from human remains suggest a high intake of animal protein (Qu et al., 2018). Fifth, mtDNA haplotype diversification suggests continued migration and settlement into the area and subsequent admixture (Li et al., 2015).

Zhagunluke

In this study, the genomic data of 16 individuals (originally reported in Kumar et al., 2022) belonging to the Zhagunluke culture were incorporated and treated as a sedentary group. The excavation of the Zhagunluke site revealed horns and bones of sheep, goats, cattle, and horses along with horsewhips, wheat or barley products, harrow and millstone (Wang et al., 2003). Furthermore, Nobaru et al. (2015) revealed a nearby settlement though no date of its construction has been ascertained. Despite this shortcoming, Isotopic analysis ($^{87}\text{Sr}/^{86}\text{Sr}$, $\delta^{18}\text{O}$, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of human dental remains and nearby plant remains from the sixth century BC to the first century AD done by Wang et al. (2025) would suggest the inhabitants of Zhagunluke were sedentary and were not integrated within a network of mountain pastoralists in the IAMC

model provided by Frachetti (2012). Genomic data generated from two individuals dated to 2800-1900 BP in (Yang et al., 2025) have some overlap with the 16 analyzed in the present study (2415 – 1945 BP), a majority of which date to 2190 BP. Their analysis reveals the continuation of Late Bronze age and Early Iron age Western Steppe pastoralist DNA at Zhagunluke with limited contribution from Scythian-Saka tribes and a notable contribution of genes from Yellow River farmers suggesting a migration and eventual settlement into the area.

Chemurchek

13 individuals from the Chemurchek culture (originally reported in Damgaard et al., 2018 & Jeong et al., 2020) were given the label of “Mobile,” although they were likely a part of a broader agro-pastoralist economy, given their association with the late Afanasievo culture (Wang et al., 2021). At present, no settlements have been identified and are known only from their mortuary practices. In their burials, the remains of sheep, goat, and cattle were recovered and it has been demonstrated by Ventresca Miller et al. (2022) they consumed sheep (*Ovis*) and ruminant (*Caprinae*) milk. Furthermore, while they had exposure to domesticated animals, the periodicity of said contact is unknown. The genetic profile of the Chemurchek suggests that southern groups exhibit stronger ancestry of hunter-gatherers found in the Baikal region (80%), while the northern Chemurchek individuals are more closely related to a woman found at Botai, a hunter-herder site at ca. 3500 BCE (Damgaard et al., 2018 & Jeong et al., 2020).

Ji'ezankale

The genomes of 13 individuals from the Ji'ezankale site previously reported in Kumar et al., (2022) were used. Their genetic background is little understood but they cluster with East

Asian hunter-gatherers and Iranian farmers. That being said, they were given the semi-sedentary label for this study. The choice to designate them as semi-sedentary in this study came as a result of the following reasons: Wang et al. (2016) suggests 10 people at ~2500 BP were migrants who permanently settled in the area following a previously mobile lifestyle whereas the remaining 24 were born and raised there, based on the ratios of strontium isotopes in dental enamel, and were found in association with several types of wood used to construct food vessels. Results of $\delta^{15}\text{N}$ from excavated sheep show a wide range of variation (+6.1 to +11.5‰ with a mean $\pm$ SD of +8.3 $\pm$ 1.5‰) suggesting they might have consumed a wide variety of plants in different environments (Guo et al., 2020). Human bioapatite-collagen $\delta^{13}\text{C}$ spacing indicates that there was a minor input of C4 plants in the diet, likely millet (Xie et al., 2022). This would suggest some level of sedentism because they either stayed at the site and maintained the crop for cultivation or it was planted and they returned to cultivate it. but no permanent settlements have been identified. In the dataset of the present study, one individual is dated to 2351 BP; the following 12 are dated to 2515 BP (Kumar et al., 2022) which may be indicative of frequent usage throughout the year.

Appendix B: Supplemental Tables and Figures

Table S1. Terms and acronyms

Adaptation	A series of adjustments in the form of traits or behaviors that enhance an organism's ability to survive or reproduce in an environment. Such examples come in the form of culture, physiological, developmental, and genetic.
Admixture	The exchange of genes between populations
Microevolution	shifting allelic frequency within a population over a short period of time and results in the phenotypic diversification among conspecific populations or related species
Macroevolution	Above species-level change that can be observed in terms of morphological differences
TLR	Toll-Like Receptor
SNP	Single Nucleotide Polymorphism
BP	Before Present
ZEP	Zoonotic enteric parasite
BMAC	Bactria–Margiana Archaeological Complex
IAMC	Inner Asian Mountain Corridor
LNBA	Late-Neolithic Bronze Age
MLBA	Middle-to-Late Bronze Age
Pseudohaploid	Genetic data that represent the individual by a randomly sampled sequence at each analyzed position
LBA	Late Bronze Age
IA	Iron Age
Innate Immunity	The baseline genetically predetermined and germline-encoded system for combatting pathogens
Introgression	Transfer of genetic material between distally related populations
EMBA	Early-to-Middle Bronze Age
ANE	Ancient North Eurasian

HG	Hunter-Gather
CHG	Caucasus Hunter-Gatherer
EHG	Eastern hunter-gatherer
LP	Lactase persistence
LD	Linkage disequilibrium
Intron	non-coding DNA sequence within genes that are transcribed into pre-mRNA but removed during translation
Exon	Coding DNA sequence that goes through the process of transcription and translation
Innate Immunity	The body's immediate front-line defense against pathogens
Adaptive Immunity	A specialized and learned immune defense mechanism tailored to defend against re-infection
Clonal selection	Generation of specific antibodies against pathogens
PAMPs	Pathogen-Associated Molecular Patterns
DAMPs	Damage-Associated Molecular Patterns
PRRs	Pattern Recognition Receptors
TIR domain	Toll/Interleukin-1 Receptor proteins; a 135–160 amino acid signaling module that forms the homology domain of TLRs
MyD88	Myeloid Differentiation Primary-Response Protein 88; an adapter protein that activates nuclear factor-kappa-B (NF- κ B)
TIRAP/MAL	Toll-interleukin-1 receptor domain-containing adapter protein or MyD88-adapter-like protein that binds MyD88 to TLR2 and TLR4
TRIF	TIR-domain-containing adapter-inducing interferon- β ; a protein that mediates TLR3 and TLR4 signaling to produce type I interferons and cytokines
TRAM	Translocating chain-associating membrane protein; a translocon-associated protein that helps insert proteins into the ER membrane or move them across it.
SARM	Sterile alpha and HEAT/Armadillo motif-containing

	protein; an adaptor protein that regulates pro-inflammatory cytokine expression
IRAK	Interleukin receptor-associated kinase; a signal transducer that triggers inflammatory and immune responses primarily recruited by MyD88
TRAF6	Tumor necrosis factor receptor-associated factor 6; an E3 ubiquitin ligase and signal transducer that mediates activation of NF- κ B downstream of TLR
TAB2	TGF- β -activated kinase 1-binding protein 2; an adapter protein crucial for mediating NF- κ B
NF- κ B	Nuclear Factor- κ B; a transcription factor that activates or deactivates genes responsible for the production of immune cells such as pro-inflammatory cytokines and Chemokines
Leucine-Rich Repeat	20–30 amino acid structural motifs that fold into curved, horseshoe-shaped solenoid domains
Heterodimer	a molecular complex composed of two distinct, non-identical polypeptide chains or monomers that are closely joined
Pro-inflammatory cytokines	signaling proteins produced by macrophages and Th1 cells that initiate, amplify, and regulate inflammation
Type I interferons	a family of cytokine signaling proteins produced by infected cells to induce an antiviral state, activate immune cells, and modulate adaptive immunity
TNF- α	Tumor Necrosis Factor Alpha; a pro-inflammatory cytokine primarily produced by activated macrophages, T-lymphocytes, and NK cells
IL-1 β , IL-6 (Interleukins)	interconnected pro-inflammatory cytokines
IFN- α , β (Interferons)	Type I cytokines crucial for innate immunity, primarily induced by virally infected cells to inhibit viral replication and modulate immune responses
Chemokines	a family of small, signaling proteins (cytokines) that guide the movement of white blood cells to tissues
CXCL8, CXCL10	proinflammatory chemokines that recruit leukocytes to inflammation sites
Antimicrobial peptides	small, naturally occurring cationic and amphiphilic molecules acting as a first-line innate immune defense

	against bacteria, viruses, fungi, and parasites
Macrophages	specialized white blood cells of the innate immune system that engulf and digest pathogens
Natural Killer cells	specialized lymphocytes that move throughout the body and target infected and diseased cells
Dendritic cells	bone marrow-derived immune cells that communicate between the innate and adaptive immune systems
Monocytes	white blood cells that fight infections, remove dead cells, and repair tissues by transforming into macrophages or dendritic cells
Neutrophils	The most common white blood cell that fights infection
T lymphocytes	A type of white blood cell originating from the bone marrow that migrates to the thymus to undergo a rigorous selection process, whereby they are screened to ensure they can recognize protein-based antigens bound to receptors on other cells without attacking the body's own tissues
B lymphocytes	white blood cells that mature in the bone marrow that produce antibodies
Hemolymphoid system	Bodily system that comprises bone marrow, thymus, spleen, and lymph nodes, functioning to produce blood cells and immune cells (lymphocytes, monocytes)
Lipoproteins	a membrane-associated protein that has a lipid molecule covalently attached to it. They are important for the nutrient uptake, transportation, and virality
Flagellin	the whip-like appendages bacteria use for movement
Lipopolysaccharides	a protective layer of gram-negative bacterial membranes
Phenol-soluble modulin	a family of small, amphipathic peptides that allow bacteria to attach and remove themselves from new sites, killing immune cells
Sepsis	An overwhelming and whole-body immune response in response to toxin-producing bacteria

Table S2. Sample breakdown.

Subgroup	Group ID in the AADR	Total n
Saka	Kazakhstan_Central_Saka.SG 5 Kazakhstan_Central_Saka.SG_father.or.son.DA13.SG 1 Kazakhstan_CentralSaka_o1.SG 1 Kazakhstan_CentralSaka_o2.SG 2 Kazakhstan_IA_Saka_dup.I3752.SG 1 Kazakhstan_IA_Saka_dup.I7110.SG 1 Kazakhstan_Saka_EIA 1 Kazakhstan_Saka_IA 3 Kazakhstan_Saka_IA_o 1 Kazakhstan_TianShan_Saka.SG 1 Kazakhstan_Wusun_LateSaka_dup.I11524.SG 1 Kazakhstan_Wusun_LateSaka_o_mother.I11525.SG 1 Kyrgyzstan_Saka_IA 7 Kyrgyzstan_Saka_IA_o 1 Kyrgyzstan_TianShan_Saka.SG 8 Kyrgyzstan_TianShan_Saka.SG_brother.DA57.SG 1 Kyrgyzstan_TianShan_Saka_o1.SG 1 Kyrgyzstan_TianShan_Saka_o2.SG 1	38
Afanasievo	China_Xinjiang_Ayituohan_Afanasievo_BA.SG 2 China_Xinjiang_Ayituohan_BA_Afanasievo 2 China_Xinjiang_G218_BA_Afanasievo 2 China_Xinjiang_G218_BA_Afanasievo_oWestEurasia 1	24

	China_Xinjiang_Nileke_Afanasievo_BA.SG 2 China_Xinjiang_Songshugou_Afanasievo_BA.SG 1 China_Xinjiang_Songshugou_BA_Afanasievo 1 Mongolia_Chalcolithic_Afanasievo_1 1 Mongolia_Chalcolithic_Afanasievo_2 1 Russia_Afanasievo 8 Russia_Afanasievo_sister.I3950 1 Russia_Afanasievo_son.I3388_son.I3950_brother.I394 9 1 Russia_Afanasievo_son.I3388_son.I3950_brother.I671 4 1	
Tian Shan Huns	Kazakhstan_Hun_Elite_LIA 1 Kazakhstan_TianShan_Hun_o.SG 1 Kyrgyzstan_TianShan_Hun.SG 16 Kyrgyzstan_TianShan_Hun.SG_daughter.DA385.SG 1 Kyrgyzstan_TianShan_Hun.SG_son.DA104.SG 1 Kyrgyzstan_TianShan_Hun_o.SG 4	24
Sarmatians	Kazakhstan_EarlySarmatian 1 Kazakhstan_LateSarmatian 1 Kazakhstan_Sarmatian.SG 3 Kazakhstan_Sarmatian_IA 12 Kazakhstan_Sarmatian_IA_o1 1 Kazakhstan_Sarmatian_IA_o2 1 Kazakhstan_Sarmatian_IA_o3 1	24

	Kazakhstan_Sarmatian_IA_o4 1 Kazakhstan_Sarmatian_IA_sister.BSB001 1 Kazakhstan_Sarmatian_Turk_IA 2	
Tasmola	Kazakhstan_Birlik_Tasmola_EIA 2 Kazakhstan_Central_Tasmola_o1.SG 1 36 Kazakhstan_Central_Tasmola_o2.SG 1 Kazakhstan_Tasmola_EIA 13 Kazakhstan_Tasmola_EIA_1d.or.2d.rel.AKB001 1 Kazakhstan_Tasmola_EIA_1d.rel.KZL001 1 Kazakhstan_Tasmola_EIA_brother.KSH001 1 Kazakhstan_Tasmola_EIA_father.or.son.TAL003 1 Kazakhstan_Tasmola_LBA 1	22
Andronovo	China_Xinjiang_Jirentaigoukou_LBA_Andronovo_Ka rasuk_possible 1 China_Xinjiang_Kuokesuxi_LBA_Andronovo 1 China_Xinjiang_Tangbalesayi_LBA_Andronovo 1 China_Xinjiang_Wutulan_LBA_Andronovo 1 China_Xinjiang_Wutulan_LBA_Andronovo_oSiberia 1 Kazakhstan_Andronovo.SG 1 Kazakhstan_Shoendykol_MLBA_Fedorovo 3 Kazakhstan_Maitan_MLBA_Alakul 7 Kazakhstan_Maitan_MLBA_Alakul_o1 1 Kazakhstan_Maitan_MLBA_Alakul_o2 1	21

	Kazakhstan_MLBA_Alakul_Lisakovskiy 2 Kazakhstan_MLBA_Alakul_Satan 1	
Russian Angara HG	Russia_AngaraRiver_BA.SG 5 Russia_AngaraRiver_EN.SG 1 Russia_AngaraRiver_N.SG 3 Russia_UstBelaya_Angara 9 Russia_UstBelaya_Angara.SG 1 Russia_UstBelaya_Angara_o 1 Russia_UstBelaya_Angara_o.SG 1	21
Sagly	Mongolia_EIA_Sagly_4 16 Mongolia_EIA_Sagly_4_lc 1 Mongolia_EIA_Sagly_4_o_lc 1	18
Khövsgol	Mongolia_LBA_Khovsgol_6	20
Zhagunluke	China_Xinjiang_Zhagunluke(Zaghunluq)_IA_Zaghunluq 1 China_Xinjiang_Zhagunluke_IA 7 China_Xinjiang_Zhagunluke_IA_o 1 China_Xinjiang_Zhagunluke_IA_Zaghunluq 7	16
Pazyryk	Kazakhstan_Berel_Pazyryk 4 Mongolia_EIA_Pazyryk_6 1 Kazakhstan_Berel_IA 9 Kazakhstan_Berel_IA_o1 1 Kazakhstan_Berel_IA_o2 1 Kazakhstan_Berel_IA_o3	19

	3	
Chemurcheck	China_Xinjiang_Bolati_BA_Chemurcheck 1 China_Xinjiang_Chaganguole(Chagangole)_BA_Chemurcheck 1 China_Xinjiang_Chaganguole_BA_Chemurcheck 9 China_Xinjiang_Tuoganbai_BA_Chemurcheck 2	13
Ji'ierzankale	China_Xinjiang_Jierzankale(Jirzankal)_IA 1 China_Xinjiang_Jierzankale_IA 12	13
Xiaohe	China_Xinjiang_Beifang_Xiaohe_BA.SG 2 China_Xinjiang_Xiaohe_BA 7 China_Xinjiang_Xiaohe_BA.SG 4	13
Kenyan Pastoralists	Kenya_PastoralN	12