Mobility and Sedentism Across the Pre-Pottery Neolithic Fertile Crescent

an Integrative Study of Archaeological and Multi-Bioarchaeological Approaches

Inaugural-Dissertation
zur Erlangung des Doktorgrades der Philosophie der
Ludwig-Maximilians-Universität
München

vorgelegt von

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Zaozhuang, China

2023

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Tag der mündlichen Prüfung: den 4. Juli 2023

This is a dissertation submitted to Ludwig-Maximilians-Universität München.

Content

Deutsche Zusammenfassung

Die archäologische Forschung hat in den letzten Jahrzehnten verdeutlicht, dass es notwendig ist, die Neolithisierung als einen komplexen, nichtlinearen, vielfältigen und regional unterschiedlichen Prozess von einer Dauer von vielen Jahrhunderten zu verstehen. Die Untersuchung dieses Transformationsprozesses, insbesondere in Bezug auf die sich ändernden Arten mobiler bzw. sesshafter Lebensstile, erwies sich jedoch mit herkömmlichen archäologischen Ansätzen als schwierig. Das Ziel dieser Arbeit und der beiden zur Veröffentlichung anstehenden Manuskripte besteht deshalb darin, diesen Veränderungsvorgang nachzuvollziehen, insbesondere im Hinblick auf den Wechsel von mobiler zu sesshafter Lebensweise, das Tempo der Sesshaftwerdung im Verhältnis zur Entstehung und Entwicklung der Landwirtschaft sowie die Beziehungen zwischen diesen beiden Aspekten, die bislang kaum zu greifen waren. Dies ist nun erstmals durch naturwissenschaftliche Multiproxy-Ansätze möglich, die an bioarchäologischem Material, das bei Ausgrabungen geborgen wurde, nach direkten Beweisen suchen.

In der ersten Fallstudie der bioarchäologischen Forschung wurden isotopische und archäogenetische Analysen an menschlichen Individuen aus Nevalı Çori, einer bedeutenden Stätte des präkeramischen Neolithikums (PPN), durchgeführt. Dadurch konnten wir erstmals die Dynamik der sich ändernden Mobilität vs. Sesshaftigkeit innerhalb der Gemeinschaft aufzeigen, die die prächtigen und bekannten T-förmigen Pfeilergebäude von Göbekli Tepe und Nevalı Çori in Anatolien errichtet hat. Darüber hinaus haben wir archäogenetische Daten für Nevalı Çori und das südlevantinische Ba'ja generiert, die uns einen neuen Einblick in die genetische Vielfalt zu Beginn des Neolithikums im Fruchtbaren Halbmond geben.

Unser Ziel war es, einen interdisziplinären Ansatz zu verfolgen, der Osteoarchäologie, C14-Datierung, charakteristische Isotopen für Ernährung und Mobilität sowie archäogenetische Daten vom PPNB bis zur Eisenzeit mit genetischen Daten aus dem PPNB der südlichen Levante kombiniert. Da biomolekulare Daten menschlicher PPNB-

Überreste aufgrund schlechter Aufbewahrungsbedingungen knapp sind, ist jeder Datenpunkt wertvoll. Obwohl die dargestellte Stichprobe klein ist, deckt sie eine zeitliche und geografische Lücke in einem Zeitraum und Gebiet ab, die für das Verständnis der Entstehung der ersten landwirtschaftlichen Gesellschaften von entscheidender Bedeutung sind. Diese Studie präsentiert zudem neue isotopische, genetische und C14-Daten von menschlichen und tierischen Überresten aus dem PPNB in Südostanatolien.

Unsere Ergebnisse zeigen erstens einen Rückgang der menschlichen Mobilität nach der Anfangsphase des PPNB, während gleichzeitig eine zunehmende Abhängigkeit von domestizierten Ressourcen festgestellt wurde. Zweitens weisen die Daten auf eine hohe genetische Ähnlichkeit zwischen PPNB Nevalı Çori und anatolischen Jägern und Sammlern sowie Bauern hin, und es gibt auch Affinitäten zu Populationen im weiteren Fruchtbaren Halbmond. Zusätzlich berichten wir über neuartige humangenomweite Daten von der späten PPNB-Stätte Ba'ja in der südlichen Levante, die Einblicke in Beziehungen unter Blutsverwandten liefern. Schließlich präsentieren wir neuartige humangenomweite Daten aus dem postneolithischen Nevalı Çori, die eine zeitliche Perspektive auf die genetische Geschichte der Region bieten, die bisher fehlte.

In der zweiten Fallstudie der bioarchäologischen Forschung über Jericho haben wir isotopische und proteomische Analysen an menschlichen Individuen aus Jericho, ebenfalls ein bedeutender Fundort des PPN, durchgeführt. Hier können wir zum ersten Mal die Dynamik des Übergangs von Mobilität zu Sesshaftigkeit innerhalb der Gemeinschaft aufzeigen, die den ältesten monumentalen Turm und die ältesten Befestigungsmauern der Welt errichtet hat. Zusammen mit anderen kürzlich veröffentlichten Daten aus der nördlichen und südlichen Levante können wir damit neue Erkenntnisse über die Vielfalt und Dynamik der menschlichen Mobilität zu Beginn des Neolithikums im Fruchtbaren Halbmond vorlegen. Dies ist das erste Mal, dass Bioproben aus Jericho analysiert und die verfügbaren Datensätze aus der Levante für eine umfassendere Sr-Kartierung überprüft werden.

Unsere Ergebnisse legen einen überraschenden Fokus auf Lokalität nahe und zeigen das Fehlen von Fernmobilitätsmustern zu einer Zeit kurz nach der Sesshaftigkeit. Dies passt perfekt zu den Bemühungen der lokalen Gemeinschaft, einen dauerhaften Siedlungsraum zu schaffen, indem sie den weltweit ersten Turm und die erste Mauer um eine Siedlung herum errichtete. Darüber hinaus sind wir die ersten, die die proteomische Analyse von geschlechtsspezifischen Amelogeninpeptiden im Zahnschmelz zur Bestimmung des Geschlechts auf archäologischem Material aus der südlichen Levante anwenden und dadurch Einblick in geschlechtsbezogene Bestattungspraktiken geben können.

Zusätzlich führt diese Studie in den naturwissenschaftlichen Hintergrund und die Geschichte der Landwirtschaft im Fruchtbaren Halbmond ein und demonstriert die bioarchäologischen Methoden, die für diese Doktorarbeit angewendet wurden. Dies betrifft Isotopen- und aDNA-Analysen ebenso wie eine Überprüfung und Zusammenfassung relevanter Forschungsliteratur über Muster von Mobilität, Migration und Akkulturation sowie die Präsentation aktueller Fortschritte und Durchbrüche auf diesem Gebiet.

1. Introduction: Neolithization in Western Asia

1.1 The origins of agriculture, sedentism and the Fertile Crescent

Given the relevance of this thesis to understanding and interpreting the origins of agriculture at the start of the Holocene (around 12,000–8,000 BP) in the Near East, it is important to first present a series of key concepts underpinning these discussions. When we refer to "the origins of agriculture," we are not necessarily discussing an institutionalized, dominant economic system that operates maturely within a particular society, accompanied by corresponding political and cultural regimes. Instead, agriculture is here conceptualized focusing on the activities of plant cultivation and animal herding and domestication. From this perspective, the emergence of agriculture was not a single event or specific turning point but rather a long and complex process that may have started much earlier than observable evidence suggests, and shows varied, non-linear trajectories (Bar-Yosef and Meadow 1995; Peters, et al. 2013; Weisdorf 2005). As Flannery argued (Flannery 1973), it is inadequate to search for "the first domestic plant" or indeed for the first farmer.

Observing agriculture in the archaeological record is often focused on the emergence of biological traits associated with domestication of plants and animals that result from human interactions with these species (Cole 1959; Hole 1984; Putterman 2008; Watkins 2013; Weisdorf 2005). Writing in the early to mid-20th century, Gordon Childe first conceptualized this process as a 'Neolithic Revolution' to highlight the rupture between the preceding hunter-gatherer lifestyles with greater mobility to a sedentary way of life dependent on domesticated plants and animals (Childe, et al. 1951). This transition apparently first took place during the Pre-Pottery Neolithic B (PPNB) period (c. 10,500–8,400/200 BP, (Renfrew and Bahn 2014)) of the Near East region, though has been documented globally in areas including Western Asia, East Asia, Mesoamerica, and South America (Neumann 2003; Piperno 2011). Childe himself acknowledged that "Neolithic civilization" is a problematic term that applies to a wide range of cultural groups and different geographical zones. Indeed, it has now been clearly observed that each region displays very different historical processes in the context of the emergence of food production and varying reliance on sedentism and different domesticated resources (Barker 2006; Bellwood 2023; Flannery 2021; Jones and Liu 2009; Lillie 2000; Liu and Chen 2012; Shennan 2018). Nevertheless, it is also clear that the emergence of agriculture represents a major thresholds in humanity's relationship to the planet, altering global biodiversity, landscapes, and even the atmosphere (Zeder, 2017).

Since Childe, if not before, the Fertile Crescent has long been considered one of the key regions for exploring the origin of agriculture. It encompasses the upper reaches of the Tigris and Euphrates rivers in present-day southeastern Turkey and northern Syria. The flat alluvial plains of southern Mesopotamia, particularly the basins of the Euphrates and Tigris rivers, are commonly referred to as the cradles of civilization and urbanization, which developed later in Sumer during the Ubaid culture around the 5th millennium cal. BC (Campbell 2007; Carter and Philip 2010; Childe 1950; Sołtysiak 2006). However, in the context of the origins of agriculture, most attention has been centred on the so-called Fertile Crescent which generally refers to a region in Western Asia that includes present-day Iraq, Iran, Syria, Lebanon, Jordan, Israel, and Turkey. From a geographical perspective much attention in discussions of early cultivation has been focused on the lowlands of the Levantine region, especially the Southern Levant, where the Natufian pre-agricultural society thrived during the Epipaleolithic period (Bar-Yosef 1998). Meanwhile the hilly flanks of the Fertile Crescent rather than the flood plains in the lower reaches, such as the piedmont ranges of the Zagros and Taurus Mountains, also known as the Taurus/Zagros Arc in some cases have featured prominently in discussions of the plant and animal domestication (Braidwood 1960; Flannery 1969; Zeder 2017).

Earlier studies often discussed the concept of "core areas" within the Fertile Crescent in the context of agricultural origins, a concept that has faced both criticism and support (Abbo, et al. 2010; Fuller, et al. 2012; Fuller, et al. 2011b; Lev-Yadun, et al. 2000; Nesbitt 2004). The basis for this idea was that the wild ancestors of all seven Neolithic founder crops (einkorn wheat, emmer wheat, barley, lentil, pea, bitter vetch, and chickpea), as well as flax, are found together only in the southeastern Turkey at the north fringe of the Fertile Crescent. This conclusion was drawn from the findings at that time and empirical data, such as the discovery of the first domesticated rye and pulses at sites like Tel Abu Hureyra during the late Epipaleolithic (around 18,900–8600 cal. BC), Tell Mureybet I and II (around 9000–8000 cal. BC), Hallan Çemi, and Cayonü and Nevalı Çori (Hillman and Colledge 1998; Lev-Yadun, et al. 2000; Nesbitt and Samuel 1998; Pasternak 1998; Rosenberg, et al. 1995; Zohary and Hopf 1973). Zooarchaeological evidence also supports the limited presence of the wild ancestors of sheep (*Ovis orientalis*) and goats (*Capra aegagrus*) in the Taurus/Zagros Arc, as well as the initial domestication of pigs (Sus scrofa) and cattle (*Bos primigenius*) in the Fertile Crescent of Southwest Asia (Zeder 2017). New data from recent archaeological finds continue to accumulate. For example, more recent archaeobotanical studies have revealed the presence of abundant wild progenitor species of crops in Iranian sites before 10600 cal. BP, with very few wild progenitor species found at Körtik Tepe, dating back to 11700–11250 cal. BP (Riehl, et al. 2011).

Before discussing further theories and models of Neolithization, it is important to provide a brief overview of the early history of research on the origins of agriculture. In the 19th century, studies in this area were primarily rooted in anthropological and ethnological perspectives and, often colonially-embedded, socio-evolutionary ideas of ‘progress’ (Morgan 1964; Tylor 1871). Here, agriculture was seen as a necessary steppingstone on the way to civilization with all societies following a unilinear path towards greater complexity. Nevertheless, studies showing the ongoing resistance of hunting and gathering communities to agriculture (Marlowe 2002; Marlowe 2005; Sahlins 1998), the frequent return of past societies to mobile ways of living under conditions of stress (Köhler-Rollefson 1988), the failure of some agricultural strategies (Erickson 2006; Stevens and Fuller 2012), and the diverse compilation of lifeways encompassed under ‘food production’ (Cramp, et al. 2019; Gremillion, et al. 2014; Prendergast 2010; Smith 2001) have demonstrated such perspectives to be both simplistic and incorrect. Such work has, in turn, also highlighted interest in the external and internal factors explaining the emergence and spread of agriculture where and when it did take hold socially and economically.

From an external perspective, some have argued that environmental changes, particularly the climatic shifts accompanying the terminal Pleistocene-Holocene transition around 10,000 years ago, played a critical role in triggering the agricultural revolution (Bar-Yosef and Belfer-Cohen 2002; Moore and Hillman 1992). Gordon Childe supported the oasis hypothesis (Childe, et al. 1951), while Kathleen M. Kenyon identified the lowland oasis of Jericho as a primary site of the agricultural revolution (Kenyon 1954). However, the argument of environmental determinism, which emphasizes climatic change as the essential factor, is considered insufficient in explaining the origins of agriculture. Braidwood argued that the transition to food production occurred as a result of cultural differentiation and specialization *within* human communities (Braidwood 1960). Meanwhile, Hodder (Hodder 1990) criticized Braidwood's focus on psycho-cultural factors over material and economic factors. Additionally, new theories examining the cognitive aspects of this transition shed light on the symbolic ideas associated with the Neolithic period, emphasizing the intervention in the environment through agriculture as a human agency derived from the belief in personal divinities (Hodder 2001). The material dimensions of this transition can be studied through the works of Donald (Donald 1991) and Renfrew and Scarre (Renfrew and Scarre 1998), while the social dimension is explored by Bender (Bender 1978) and the symbolic dimension by Hodder (Hodder 1990).

Turning to Neolithization in the Fertile Crescent more specifically, Braidwood conducted excavations at the Sarab and Asiab sites near Kermanshah in the 1959-60s to study some of the earliest examples of domesticated resources. During this time, the west central Zagros area in Iran was identified as one of the first regions with great potential for studying the process of Neolithization in the Near East (Braidwood, et al. 1961). However, the relative scarcity of identified and excavated sites in the Zagros region since the 1990s prompted researchers to reassess its academic significance in studying the Near Eastern Neolithization process. Nevertheless, new materials continue to emerge from this region, providing updates to our understanding (Darabi 2012; Hole 1999; Hole, et al. 1969; Matthews, et al. 2020; Niknami and Nikzad 2012; Riehl, et al. 2011; Riehl, et al. 2013). Since the 1990s, research focus has shifted to the northern and western regions of the Fertile Crescent due to the larger accumulation of archaeological materials found there (Arbuckle 2014; Fuller, et al. 2012; Özdoğan 2015; Peters, et al. 2014; Peters, et al. 2013; Peters, et al. 2005; Vigne, et al. 2005; Willcox 2005). For example, Çilingiroğlu (Çilingiroğlu 2005) evaluated the meaning and applicability of the term "Neolithic package" by examining its appearance and regional chronologies in different parts of Anatolia.

Zeder's series of studies (Zeder 2011; Zeder 2017; Zeder 2008) have continued the work initiated by Bar-Yosef and Meadow in 1995 (Bar-Yosef and Meadow 1995), providing updates and characterizing significant developments in understanding the origins of agriculture within and beyond the Fertile Crescent. In the early 2000s, evidence pushed back the initial steps of plant and animal domestication in the Eastern Mediterranean to the 12th millennium cal. B.P. Different species seem to have been domesticated in different parts of the Fertile Crescent, and genetic analyses have revealed multiple domestic lineages for each species (Zeder 2008). Multi-linear models of agricultural emergence in the past were proposed as early as thirty years ago (Flannery 1973), and subsequent updates incorporating new findings and multiproxy evidence (e.g., (Larsen, et al. 2019; Munro, et al. 2018; Price and Bar-Yosef 2011; Sterelny and Watkins 2015; Willcox 2005) have further supported these arguments, including within the Near East. Advances in archaeological sciences, along with the application of technologies and methodologies such as isotopic and aDNA analysis, helped facilitate a more pluralistic and nuanced view of agriculture's origins. This perspective recognizes multiple centres and non-linear models influenced by both endogenous and exogenous factors. Current research emphasizes the need to examine the various intersections of culture, climate, and economy when approaching local processes of Neolithization. For example, Maeda et al. (2016) discuss how sickles worked to signal a new cultural identity of 'farmers' while also providing functional needs for crop processing.

The process of Neolithization has also commonly been associated with a transition to a more sedentary lifestyle. However, research within and beyond the Near East has highlighted the presence of sedentism without agriculture and vice versa. For example, early permanent settlements of the Natufian culture in the Southern Levant and the Gulf Coast of Florida lack evidence of domestic plants or only show the presence of "wild" plants in a phenotypic sense (Boyd 2006; Flannery 1973; Henry 1985; Valla 2018). It is crucial to untangle such commonly related notions and thoroughly investigate their relationship. As early as the late 19th century, Roth observed that while agriculture and domestication are often intertwined, they are not inherently connected. For example, people can domesticate animals without having the intention of using them for domestic purposes (Roth 1887).

Climate change, resource and population intensification, sedentism, and the growth of settlements are among the numerous factors that have been argued to have contributing contributed to the emergence of agriculture in the Near East, and they are deeply interconnected. In this thesis, I focus on key sites such as Nevalı Çori, Göbekli Tepe, Ba'ja, and Jericho that have played a major role in discussions of Neolithization in the Near East. I analyze and synthesize multiple lines of evidence with colleagues to comprehend the nuanced mechanisms governing the pace of transition between agriculture and sedentism. Additionally, I explore the long-term dynamics of admixture and mobility across the entire region to try and understand the origins and connections of some of the first ‘farmers’. While different zones within the Fertile Crescent may have experienced similar timelines for the development of agriculture, we demonstrate that the relationship between agriculture and sedentism in Southeastern Anatolia, the Southern Levant, the Zagros region, and the Caucasus should be considered as diverse, complex processes.

1.2 Mobility, migration, and admixture in prehistoric Western Asia

When thinking about sedentism and population interactions it is important to define what we even mean by ‘mobility’ and its expression across different spatial and temporal scales. When discussing the mobility of a group, it can be considered as a social or economic attribute of a society, as a sporadic behavior by specific individuals, as acquired routines certain populations followed, or a particular living strategy in response to historical changes or events as well. Kelly’s (Kelly 1992) comprehensive work provides an important review of the various conceptions of mobility in archaeological studies, from categorizing types (Beardsley, et al. 1955) to Binford's differentiation of mobility across different dimensions, with comparisons to ethnographic evidence (Binford 1980). In this thesis, I am particularly interested in the nature and cultural implications of mobility during the Pre-Pottery period in the Near East. This includes exploring whether group and individual mobility can be identified and the ways in which mobility might be tied to identity or the emergence of new economic practices. In the first study, I led a team examining people from different time periods at the same site, Nevalı Çori, which may represent a crucial transition in this community’ economic and cultural roles, shifting from being complete hunter-gatherers to becoming herder-farmers. This transition likely had a notable impact on the entire society, as reflected by varying degrees of mobility among the individuals.

The next challenge for archaeologists is to identify and detect mobility through material remains associated with the people involved, such as lithics, pottery, bone artifacts, grinding and cooking tools, and burials (Barzilai and Goring-Morris 2007; Li 2022; Mayer 2005; Verhoeven 2002; Yerkes, et al. 2003). Archaeological remains can provide indirect insights into mobility and sedentism. Understanding reduced mobility and identifying the presence of sedentism involves exploring significant changes in food storage, trade, territoriality, social and gender inequality, male/female work patterns, subsistence, and demography (Kelly 1992). Various studies related to human mobility in prehistoric and early historic Mesopotamia have been conducted which do not rely on isotope or aDNA analysis, such as on the interactions between Iranian and Mesopotamian peoples (Carter and Stolper 1984), on Mesopotamia during the Late Chalcolithic and Early Bronze Age (Sołtysiak 2006), on the relations between farmers and herders (Porter 2012), on population movements inferred from settlement patterns

(Altaweel and Palmisano 2015), and on human mobility during the formation of the first urban centers in the Late Chalcolithic (Algaze 2018; Ur, et al. 2011). Alternatively, researchers in the 1980s have studied zooarchaeological evidence for more settled ways of life, such as the frequencies of human commensals like house mice and house sparrows (Auffray, et al. 1988; Tchernov 1991).

Isotopic analysis has been used in archaeology to explore mobility since the 1980s (Beard and Johnson 2000; Bentley, et al. 2003b; Chenery, et al. 2010; Price, et al. 1994), and aDNA analysis has gained prominence particularly since the 2000s, providing direct evidence of mobility from targeted individuals. The former employs a multiproxy approach, often combining strontium and stable isotope proxies, which have proven effective in tracing an individual's mobility throughout their lifetime (Bentley, et al. 2003b; Eerkens, et al. 2014; Müldner, et al. 2009; Richards, et al. 2008). On the other hand, aDNA analysis utilizes the fundamental principles of evolutionary genetics, offering a new quantitative language to unravel human history (Furholt 2019; Preus, et al. 2011; Skourtanioti 2022; Vilà-Valls, et al. 2023; Villalba-Mouco, et al. 2023; Wang, et al. 2023a). Furthermore, advances in biomarker applications have opened new possibilities in archaeological research. For example, the identification of substantial faecal deposits at archaeological sites holds promise for gaining insights into human living arrangements and lifestyles (Shillito, et al. 2011).

The emergence, and particularly the expansion, of farming has often been linked to the movement of particular populations. For example, in 2003, Jared Diamond and Peter Bellwood described the correlated expansion of farming and the development of large language families through the demographic growth of farmers (Diamond and Bellwood 2003). The discovery that European farmers shared close affinities with ancient and present-day populations in the Near East also highlighted the ways in which demographic expansion may have carried the knowledge and domesticated resources underpinning a Neolithic 'package' (Brandt, et al. 2013; Lazaridis, et al. 2014b; Mathieson, et al. 2015b). Meanwhile, isotopic approaches to mobility in Neolithic Europe have highlighted the ways in which arriving farming communities and local hunter-gatherer populations might have interacted (Bentley et al., 2002).

Stable isotope analysis plays a crucial role in investigating the origins of agriculture, specifically in understanding the transition to a sedentary lifestyle and the reliance on crops as staple food. Strontium isotope analysis primarily aids in studying the former, while carbon and nitrogen stable isotope analyses are key for the latter (Ambrose and Norr 1993; Schwarcz 1991). Pearson et al., for example, applied strontium and stable oxygen isotope analysis to tooth enamel from 99 individuals spanning over 7,000 years at Pınarbaşı, Boncuklu, and Çatalhöyük. Their research suggested that prior to the emergence of farming, local kinship practices in the Fertile Crescent were influenced more by shared ideologies and associations involving fictive kinship (Pearson, et al. 2023). Stable carbon isotope analysis on the other hand can be used to explore human reliance on C_3 versus C_4 resources which can have agricultural implications. For example, this method has been extensively applied in East Asia and the Americas to investigate the origins of foxtail and broomcorn millet (*Setaria italica* and *Panicum miliaceum*) agriculture (Barton, et al. 2009; Hu, et al. 2006; Hu, et al. 2008; Liu, et al. 2012; Pechenkina, et al. 2005), as well as maize (*Zea mays*) (Hard, et al. 1996; Kennett, et al. 2020; Schoeninger 2009; Tykot 2016) agriculture that emerged in those regions. These case studies are powerful because C_4 crops are being introduced into predominantly C_3 wild environments. However, the resolution of this technique is limited in the Pre-Pottery period of the Near East where C_4 crops are not present. Another important study applying strontium and oxygen isotopes by Pearson et al. examined tooth enamel from 99 individuals spanning over 7,000 years at Pınarbaşı, Boncuklu, and Çatalhöyük. Their research suggested that prior to the emergence of farming, local kinship practices in the Fertile Crescent were influenced more by shared ideologies and associations involving fictive kinship (Pearson, et al. 2023).

Due to the limited preservation of DNA in hot, arid conditions and relatively limited bioarchaeological samples from relevant regions, many puzzles related to mobility patterns, population structure and genetic history of the first farmers in the Near East, and their connections to each other, have remained obscure. A pivotal investigation by

Lazaridis et al. in 2016 was one of the first to shed light on this topic using aDNA. They obtained late Pleistocene and early Holocene genomes from early farming sites in the Fertile Crescent, specifically the southern Levant, the Zagros Mountains in Iran, and Anatolia. These genomes exhibited significant genetic differentiation, suggesting long-term isolation between these regions. Furthermore, there was a high degree of genetic continuity between local Mesolithic hunter-gatherers and the early farmers in the Levant and Iranian Zagros (Lazaridis, et al. 2016). These findings align with the notion that the (pre-) Neolithic cultures were distinct from each other due to their distinct economic characteristics and self-sufficiency within each community (Childe, et al. 1951) as well as the multi-linear process of domestication (Fuller 2007; Fuller, et al. 2011a). This study paints a picture of substantial genetic continuity from the late Pleistocene to the early Holocene across the Fertile Crescent, and moreover, it reveals greater genetic diversity during earlier stages, including the Pre-Pottery Neolithic period and potentially even preceding it (Broushaki, et al. 2016; Feldman, et al. 2019; Kilinc, et al. 2016; Lazaridis, et al. 2016; Skourtanioti, et al. 2020; Wang, et al. 2023b).

Another study by Broushaki et al. revealed the adoption of farming by multiple genetically-differentiated hunter-gatherer populations in southwestern Iran, specifically the Zagros region of the eastern Fertile Crescent. They sequenced Early Neolithic genomes and identified a population that diverged from Early Neolithic farmers in Anatolia between 46,000 and 77,000 years ago, showing affinities with present-day Pakistani and Afghan populations (Broushaki, et al. 2016). Kılınç et al. (Kilinc, et al. 2016) conducted a study on human remains from two Neolithic settlements Boncuklu and Tepecik-Çiftlik in central Anatolia (approximately 8300 and 5800 cal. BC). Their research focused on understanding the demographic processes during the earliest (aceramic) phase of the Neolithic transition and the subsequent Pottery Neolithic period in Anatolia. Similarly, van de Loosdrecht et al. (van de Loosdrecht, et al. 2018) analyzed genomic data from seven 15,000-year-old individuals belonging to the Iberomaurusian culture. They found a genetic affinity between these individuals and early Holocene Near Eastern populations, specifically the Levantine Natufians. This suggests a preagricultural connection between Africa and the Near East dating back to the Pleistocene.

Additionally, Feldman et al. reported the first genome-wide data from a 15,000-year-old Anatolian hunter-gatherer and seven Anatolian and Levantine early farmers (Feldman, et al. 2019). Their findings revealed high genetic continuity between the hunter-gatherers and early farmers of Anatolia, as well as two distinct ancestral contributions: one from early Iranian/Caucasus-related populations and another from the ancient Levant. Skourtanioti et al. (Skourtanioti, et al. 2020) conducted genome-wide data analyses on 110 ancient individuals from Near East spanning the Late Neolithic to Late Bronze Age. They found that populations in North/Central Anatolia and the Southern Caucasus during the 6th millennium BC shared mixed ancestry, forming a genetic cline that developed during the Neolithic between Western Anatolia and regions in the Southern Caucasus/Zagros. Meanwhile, Yaka et al. (Yaka, et al. 2021) made progress in understanding genetic relatedness within Neolithic Anatolia. They analyzed 59 ancient genomes, including 22 new genomes from Asıklı Hoyuk and Çatalhöyük, and discovered frequent occurrences of sibling and parent-offspring relationships within domestic structures. This indicates close genetic ties among individuals buried together in these structures. However, they also found that genetic relatives were rare among subadults buried within and around houses during the 7th millennium, highlighting the likely diversity of kinship structures in Neolithic communities in the Fertile Crescent.

Lararidis et al. conducted a study presenting ancient DNA data from the Pre-Pottery Neolithic of Mesopotamia, Cyprus, Northwestern Zagros, and Neolithic Armenia. Their findings revealed that these populations and their neighboring groups were formed through admixture of pre-Neolithic sources related to Anatolian, Caucasus, and Levantine hunter-gatherers. This admixture resulted in a Neolithic continuum of ancestry that reflects the geographical distribution of West Asia. Furthermore, the Pre-Pottery populations of Anatolia originated from admixture between Mesopotamian-related and local Epipaleolithic-related sources, while Pottery Neolithic populations experienced additional gene flow from the Levant. These findings provide evidence for at least two migration events that have happened to the early farmers of Anatolia (Lazaridis, et al. 2022). Altınışık et al. (Altınışık, et al. 2022)) published the results of their study on 13 ancient genomes (approximately 8500 to 7500 cal. BC) from Pre-Pottery Neolithic Çayönü in the Tigris basin, which is closely related to the target site Nevalı Çori in their first case study. They discovered that Çayönü was a genetically diverse population with mixed ancestry from the western and eastern Fertile Crescent. The genetic profile of the population from the Neolithic site of PPNB Nevalı Çori, as concluded in their study, reflects a similar genetic structure.

The advancements in obtaining and analyzing genomic data from modern and ancient populations have greatly enhanced our understanding of human dispersals during the Holocene (Stoneking, et al. 2023). In light of genomic evidence, this overview provides a glimpse into Holocene dispersals that occurred in various regions worldwide, including Africa, Europe, Central and South Asia, East Asia and Mainland Southeast Asia, Island Southeast Asia (ISEA) and Oceania, and the Americas. These dispersals have a complex history, and further research on the sociocultural contexts of these dispersals would be valuable in unraveling their intricate narratives and modeling different social scenarios.

When examining human mobility associated with the Neolithic period, it is important to understand how different mobility strategies and ways of life (e.g. agro-pastoral, hunting and gathering) can intersect in different local contexts (Mathieson, et al. 2017). To gain a comprehensive understanding, it is necessary to explore multidisciplinary proxies and accumulate datasets from various dimensions. Genetic and isotopic studies have proven instrumental in providing evidence for migration and demographic connectivity at broad and local scales, respectively (Bentley, et al. 2003a). These approaches have been increasingly employed in recent research (e.g., (Krzewinska, et al. 2018; Manin, et al. 2018)) including our first case study described in Chapter 8 (MANUSCRIPT A). Strontium and oxygen isotopic analyses are powerful tools for tracing mobility history on the scale of the individual's lifetime. These methods can be used, for example, to determine whether a group, or some members of it, are first-generation immigrants (e.g., (Knipper, et al. 2017; Kroll, et al. 2022)). Moreover, they are especially useful for examining individuals buried with different material culture, in conjunction with specific archaeological and anthropological contexts (e.g., (Copeland, et al. 2011; Knudson, et al. 2016)). However, it is important to acknowledge the inherent limitations of isotopic methods. First, the homogenous geological context across a wide region, particularly in the Near East, leads to undistinguishable isotopic data, hindering the pinpointing of individual origins. Secondly, this method targets early life as it requires sampling on tooth enamel which develops during relatively early stage. Thus, movement afterwards cannot be excluded.

On the other hand, genetic studies have emerged as a separate approach for addressing numerous questions in evolutionary biology, anthropology, and archaeology (Orlando, et al. 2021) and have revolutionized our understanding of the past by providing another angle and scale of narratives of genetic admixture and demographic interaction (Haak, et al. 2015; Lazaridis, et al. 2014a; Moorjani, et al. 2016; Reich, et al. 2010; Skoglund, et al. 2012). However, a limitation of genetic studies lies in the lack of detailed information regarding individual mobility and migration patterns and it normally refers to large scales of time (the so-called 'big picture'). Meanwhile, multiple models and interpretations are needed to explore what kind of behaviour and events actually produced the studied results (Gamble, et al. 2005; Kristiansen 2022). For instance, Bentley et al. pointed out that geneticists should not over-interpret the continent-wide patterns of genetic data in terms of archaeological patterns observed at the local scale, until more localized genetic datasets available (Bentley, et al. 2003a). Indeed, genetic analysis can only provide insights into geographic demographic patterns to the extent that samples exist from known, localized archaeological contexts. To overcome this limitation, integrating genetic and isotopic analyses can yield more comprehensive insights into the complex processes that shaped the Neolithic revolution.

2. Objectives and highlights of the thesis

Chapter 1 has highlighted how in recent decades, archaeological research has documented the complexity of the Neolithization process and the need to understand it as a non-linear, manifold, and regionally diverse phenomenon spanning many centuries. However, tracing this transformative process, particularly in terms of the transition from mobile to sedentary lifestyles, has posed challenges using conventional archaeological methods. The goal of this thesis, which comprises two published manuscripts, is to trace this transformative process by investigating the changing modes of mobile and sedentary lifestyles, the pace of sedentism during the emergence and development of agriculture, and the relationships between these factors. These aspects have proven challenging to address using conventional archaeological approaches, necessitating the utilization of multiproxy scientific methods to seek direct evidence from bio-archaeological materials recovered from excavation sites. The following sections present the main issues and highlights of the specific case studies detailed in the two manuscripts.

Manuscript A

In MANUSCRIPT A, I conducted isotopic and archaeogenetic analyses on human individuals from the prominent Pre-pottery Neolithic site of Nevalı Çori with my collaborators. For the first time, this study reveals the dynamics of changing mobility and sedentism within the community responsible for constructing the T-shaped pillar buildings of Göbekli Tepe and Nevalı Çori in Anatolia. Additionally, we generated archaeogenetic data for Nevalı Çori and the Southern Levantine site of Baj'a, shedding new light on genetic diversity at the onset of the Neolithic period in the Fertile Crescent. Our multidisciplinary approach combines osteoarchaeology, radiocarbon dating, isotopic analysis of diet and mobility, and archaeogenetic data spanning from the Pre-pottery Neolithic B (PPNB) to the Iron Age, along with genetic data from the PPNB in the Southern Levant. Due to the poor preservation conditions, biomolecular data from PPNB human remains are scarce, making each data point valuable. Despite the small sample size, our study fills a temporal and geographical gap, providing crucial insights into the emergence of the first farming societies.

The primary questions addressed in this study are as follows:

1. Did the people of Nevalı Çori have connections with Göbekli Tepe and other T-shaped pillar sites during the PPNB?
2. Did the population of Nevalı Çori exhibit different lifestyles during different periods?
3. How can we interpret the group of T-shaped pillar sites beyond cultural or religious perspectives? What other hypotheses can explain the attribution or underlying mechanism of this phenomenon?
4. Is there evidence of special familial relationships, kin groups, intermarriage, or consanguinity during the PPNB in the Near East, and if so, how does it fit into the cultural and wider social context?

The highlights of the results from MANUSCRIPT A are as follows:

1. This study presents new isotopic, genetic, and radiocarbon data from human and animal remains from the Southeastern Anatolia PPNB.
2. The findings of this study indicate a decline in human mobility after the initial phase of the PPNB, coinciding with an increasing reliance on domesticated resources. We also observe a high genetic similarity between PPNB Nevalı Çori and Anatolian hunter-gatherers and farmers, as well as affinities with populations across the Fertile Crescent.
3. This study reports novel human genome-wide data from the late PPNB site of Ba'ja in the Southern Levant, providing insights into consanguineous unions.
4. Finally, this study presents novel human genome-wide data from post-Neolithic Nevalı Çori, offering a diachronic perspective on the genetic history of the region that was previously missing.

This study underscores the potential of an integrative archaeogenetic and isotopic approach in revealing human mobility and social organization during the Pre-Pottery Neolithic, aspects of life that often leave limited archaeological traces. We believe our work significantly advances knowledge of the Neolithization process in the ancient Near East, shedding new light on the transition from mobile to sedentary lifestyles, the relationship between Göbekli Tepe and Nevalı Çori, and the archaeogenetic diversity within the PPNB Fertile Crescent.

Manuscript B

In MANUSCRIPT B, I conducted isotopic and proteomic analyses on human individuals from the significant Pre-pottery Neolithic site of Jericho with my collaborators. This study represents the first demonstration of the dynamics involved in the transition from mobility to sedentism within the community responsible for constructing the oldest monumental tower and fortification walls in the world. By combining our new data with recently published findings from both the Northern and Southern Levant, it provides fresh insights into the diversity and patterns of human mobility at the beginning of the Neolithic era in the Fertile Crescent.

The main questions addressed in this study are as follows:

1. Considering that the earliest wall and tower in human history were discovered in Jericho, with intense debate existing regarding their function, we explore the mobility patterns of Jericho's inhabitants during the Pre-pottery Neolithic periods. Did these patterns undergo any changes? How does the detected mobility/migration pattern relate to the social organization of the settlement?

2. Since I was able to obtain rib and long bone samples from Jericho, and therefore infer the nutritional characteristics and trophic levels of the population, what insights can we gain about their potential dietary patterns?

3. Were the populations in Jericho genetically distinguishable from other contemporary groups in the Southern Levant? If so, which admixture models could explain these differences?

The aims of the results from MANUSCRIPT B are as follows:

1. This study presents an integrative isotopic dataset ($^{87}Sr/^{86}Sr$, $\delta^{18}O$, $\delta^{13}C$) from human individuals at the Pre-pottery Neolithic site of Jericho, contributing to our understanding of the mobility dynamics and social organization of the community responsible for building some of the earliest monumental structures in the Near East, indicating a focus on locality and an apparent lack of long-distance mobility at a time shortly after the turn to sedentism, which fits perfectly with the local community's effort to create a permanent settlement space.
2. It applied proteomics analysis to determine biological sex for the sampled individuals from PPN Jericho to undertake sex determination in the absence of both the complete skeletons for osteological and aDNA extraction for shotgun-genomic sex estimation. This is the first study to apply proteomic analysis of sex-specific amelogenin peptides in tooth enamel on archaeological material from the Southern Levant and provide thoughts about sex-related burial practices.
3. This study tried to shed light on the nutritional characteristics and trophic levels of the Jericho population based on rib and long bone samples, providing insights into their dietary patterns, and tried to explore the genetic distinctiveness of Jericho's population within the context of the Southern Levant for potential admixture models.

This study significantly enhances our knowledge of Jericho's role in the Pre-pottery Neolithic and its unique contributions to the cultural landscape of the Fertile Crescent. By addressing critical questions regarding mobility, social organization, nutrition, and genetic distinctiveness, we provide valuable insights into the multifaceted nature of this ancient settlement. These findings have broad implications for our understanding of the human societies in the region during the early Neolithic periods.

3. Methodologies and rationales of the applied bioarchaeological approaches

3.1 Isotope analyses of mobility in archaeology ($^{87}Sr/^{86}Sr$, $\delta^{18}O$ and $\delta^{13}C$)

Bio-archaeologists have been using isotopic analysis of human remains for over 30 years (Alexander Bentley 2006; Burton and Price 2013; Nehlich 2015; Slovak and Paytan 2012). Isotopic analysis of human remains has revolutionized the study of ancient mobility in prehistoric and historic human populations and paleoclimate for over 30 years. Bio-archaeologists have utilized radiogenic strontium to directly examine and analyze patterns of mobility in archaeological human and faunal skeletal samples (e.g., (Copeland, et al. 2011; Knipper, et al. 2017; Price, et al. 2008; Price, et al. 2015)). This approach provides insights into migratory patterns (e.g., (Harvig, et al. 2014; Montgomery 2010)), food resource catchment (e.g., (Hobson, et al. 2010; Montgomery, et al. 2013)), and relevant phenomena such as resource circulation/exchange, pastoralism/nomadism, and artifact provenance (e.g., (Erban Kochergina, et al. 2021; van Geldern, et al. 2006; Widerlund and Andersson 2006)). These analyses contribute to a better understanding of the attribution of specific prehistoric groups or societies, as well as the potential social complexity at a given time. Oxygen isotope analysis of enamel from humans, fauna, and other environmental materials is also used to study mobility by distinguishing geographical variations in annual temperature, precipitation, and seasonality of rainfall along longitudinal and altitudinal gradients (Buzon and Bowen 2010; Dansgaard 1964; Daux, et al. 2008; Gat 1996; Pederzani and Britton 2019). Finally, carbon isotopic analysis of plants, soils, and other surficial materials can provide insights into vegetation patterning and diets over a landscape (Ellsworth and Cousins 2016; Makarewicz 2017; Wallace, et al. 2013). This section aims to introduce the foundations and rationales of isotopic analyses in archaeology, including the properties of these proxies, as well as the related principles, materials, and methods.

Strontium (Sr) is an alkaline earth metal that is widely present in most igneous, metamorphic, and sedimentary rocks, as well as surface water, groundwater, seawater, and soil. During the weathering of rocks and soils, strontium is released into water, making it biologically available. This strontium is then absorbed by plants, animals, and humans through the food chain (Price, et al. 1986). Strontium has four naturally occurring stable isotopes (^{84}Sr, ^{86}Sr, ^{87}Sr, and ^{88}Sr), of which ^{87}Sr (7.040%) is radiogenic and results from the radioactive decay of ^{87}Rb (Rubidium) (Ericson 1985; Faure and Powell 1972). The amount of ^{87}Sr, expressed as the ^{87}Sr/^{86}Sr ratio, varies between geological units, typically ranging between 0.7000 and 0.7500, depending on the rubidium content and age of the rock (Faure and Powell 1972). Due to their similar ionic radii and chemical properties, strontium can substitute for calcium and be transferred through food chains without significant isotope fractionation (Graustein and Armstrong 1983; Montgomery 2010; Rokita, et al. 1993). The strontium isotope ratio in human or animal individuals reflects the bioavailable strontium taken up from the environment during tissue formation, typically occurring early in life for teeth. Therefore, different ratios of strontium isotopes in biomaterials reflect geographic variation in ^{87}Sr/^{86}Sr values based on their local geological context (Capo, et al. 1998; Montgomery 2010).

However, the application of strontium isotope analysis to reconstruct ancient migration and mobility patterns is limited in regions without bioavailable ^{87}Sr/^{86}Sr baselines. It is crucial to have datasets of bioavailable ^{87}Sr/^{86}Sr from a variety of modern and archaeological sources to estimate the local ^{87}Sr/^{86}Sr signature in a region due to differential processes of weathering, different ranges of animals, as well as potential changes in bioavailable through time (Grimstead, et al. 2017; Maurer, et al. 2012; Price, et al. 2002). When multiple baselines are combined across a region, the resulting maps can have widespread applications in ecology, food inspection, and forensic investigation (e.g., (Bataille, et al. 2021; Bataille, et al. 2018; Bataille, et al. 2020; Bentley and Knipper 2005; Wang, et al. 2023c)). Research on constructing ^{87}Sr/^{86}Sr maps, or what have become known as 'isoscapes' (though see (Holt, et al. 2021; Makarewicz and Sealy 2015)), began about two decades ago, with various empirically based and process-based models proposed. Some models involve direct sampling of average ^{87}Sr/^{86}Sr ratios from similar geological areas), while others use georeferencing models, such as Empirical

Bayesian Kriging (EBK), which predict $^{87}Sr/^{86}Sr$ ratios across landscapes based on known values (Tucker, et al. 2020). Recent studies are also integrating machine learning approaches into these models (Bataille, et al. 2018). Currently, the first predictions of bioavailable $^{87}Sr/^{86}Sr$ for East Africa have been made available (Janzen, et al. 2020) and forthcoming research will report a batch of large and comprehensive datasets for Western Africa (Wang et al., in press). These $^{87}Sr/^{86}Sr$ mapping efforts provide crucial resources for future studies on ancient and modern animal and human mobility in different regions.

Stable oxygen isotope analysis ($\delta^{18}O$) is commonly used as a supportive tool to identify the mobility and environmental contexts of humans and animals, providing insights into population movement, animal husbandry, and other management strategies (e.g., (Hartman, et al. 2016; Henton, et al. 2010; Laffoon, et al. 2017; Locosselli, et al. 2020)). This method relies on the spatial variation and gradients of oxygen isotopic composition ($^{18}O/^{16}O$ expressed as $\delta^{18}O$ in ‰ against V-SMOW) in meteoric water ($\delta^{18}O_{mw}$), which is imbibed by animals and humans through drinking water and food. $\delta^{18}O_{mw}$ values vary based on factors such as temperature, altitude, latitude, distance from the ocean, precipitation, and seasonality (Blumenthal, et al. 2014; Dansgaard 1964; Gat 1996; Gat and Dansgaard 1972; Laffoon, et al. 2017; Levinson, et al. 1987). In mammalian teeth and bones, oxygen is bound to the phosphate fraction ($\delta^{18}O_p$) or the structural carbonate ($\delta^{18}O_{ca}$) of the hydroxyapatite, which reflect the local environmental water (Dansgaard 1964; Longinelli 1984). The constant body temperature of mammals allows for the use of linear regression equations to estimate the oxygen isotopic values of consumed water based on bio-archaeological samples (Chenery, et al. 2012; Levinson, et al. 1987; Longinelli 1984; Luz, et al. 1984), albeit with documented errors (Pellegrini and Snoeck 2016). By comparing these values with existing datasets or converting $\delta^{18}O_{ca}$ or $\delta^{18}O_{dw}$ (drinking water) to $\delta^{18}O_{mw}$ values and comparing them with the oxygen isotopic composition of modern meteoric water ($\delta^{18}O_{prep}$), individuals with distinct values can be cautiously identified as potentially being non-local. $\delta^{18}O$ values can be used to reconstruct paleoclimate, paleoseasonality, human and animal origins, and related behaviors such as breastfeeding, animal husbandry, weaning, and culinary techniques (Makarewicz 2017; Roberts, et al. 2018; Ventresca Miller, et al. 2020).

$\delta^{13}C$ in bioapatite provides valuable additional information on mobility by linking environment, diet, and isotopic compositions, making it widely used in paleoecological and paleodietary studies to improve the interpretation of archaeological data (Hartman, et al. 2016; Makarewicz 2017; Roberts, et al. 2018; Ventresca Miller, et al. 2020). Stable carbon isotope ratios (expressed as $\delta^{13}C$) are useful for modeling mobility due to seasonal changes in $\delta^{13}C$ within plants and variations related to seasonality and altitude (Hartman and Danin 2010). Stable carbon isotope variation in terrestrial environments occurs as a result of different pathways of photosynthesis, with C_3 plants (Calvin cycle) discriminating more against the heavy isotope ^{13}C than C_4 plants (Hatch-Slack cycle) (Kohn and Cerling 2002). When measuring carbon in the bone and tooth enamel structural carbonate (bioapatite), it mainly reflects carbon from all dietary components, including fat, protein, and carbohydrates (Ambrose and Norr 1993). C_3 plants have an average isotope composition of around –26‰, while C_4 plants cluster around 14‰ higher at around –12‰. Herbivores that consume these plants inherit the isotope difference: C_4 consumers have $\delta^{13}C$ values approximately 14‰ higher than C_3 consumers (0 to +2‰ vs. around –12‰), and values falling between C_3 and C_4 diet endmembers likely reflect mixed feeding (Bender 1971; DeNiro and Epstein 1978; Smith and Epstein 1971). Additionally, $\delta^{13}C$ values in C_3 plants tend to be higher during the dry season due to decreased water availability (Farquhar, et al. 1989; Hartman and Danin 2010). The proportion of C_4 vegetation in mixed C_3/C_4 biomes varies seasonally and decreases with increasing altitude (Cavagnaro 1988; Li, et al. 2009; Rundel 1980; Tieszen, et al. 1979). $\delta^{13}C$ variation between C_3 and C_4 plants is also reflected in the values of different domesticated crops, which, in turn, influences the isotopic composition of the bones and tissues of their consumers. With the exception of a few species such as millet and maize, which were domesticated in the Old World and Americas respectively (Hunt, et al. 2008; Kennett, et al. 2020), the majority of staple foods today are C_3 crops including wheat, barley, rice, and others. In Southwest Asia, there is no evidence of C_4 crop domestication. The founder crops domesticated in this region, such as einkorn and emmer wheat, barley, and pulses, are all C_3 crops.

The materials used for isotopic analysis of humans, animals, and environmental samples to establish local $^{87}Sr/^{86}Sr$ baselines are important to discuss. In animals and humans, strontium is primarily incorporated into hydroxyapatite ($Ca_{10}(PO_4)_6OH_2$), the inorganic component of teeth and bones. Dental enamel, which is highly mineralized with low organic content, is particularly valuable for strontium analysis due to its resistance to diagenesis and post-mortem alteration, making it a reliable archive of isotopic values during early life (Alexander Bentley 2006). For the measurement of $^{87}Sr/^{86}Sr$ ratio, the most common sampling choices are the first, second, and third permanent molars, which form enamel at different stages: from pre-natal to around three years old, between three and seven years old, and between seven and fourteen years old, respectively (Hillson 1996). To avoid breast-feeding effects, the optimal choices for evaluating human $\delta^{13}C$ and $\delta^{18}O$ values are the second or third permanent molars (Wright and Schwarcz 1998).

It is important to note that interpreting isotopic data from bioarchaeological materials requires a strong understanding of paleogeological, geochemical, paleobiological, and pathological factors, including knowledge of dental development, nutrition, growth timeline, and properties of specific tissues in the studied species. These prerequisites contribute to a more precise interpretation of the isotopic data (Burton and Katzenberg 2018; Erban Kochergina, et al. 2021; Hedges, et al. 2006; Kohn 2004; Passey 2002; Schweissing and Grupe 2003; Sighinolfi, et al. 2018; Thomsen and Andreasen 2019; Van Ham-Meert, et al. 2020). Strontium isotope analysis is typically performed on the second or third permanent molars in humans, while high-crowned molars of cattle and sheep/goats are sampled near the apex and cervix to identify possible isotopic variation along the crown (e.g., (Kohn 2004; Lazzerini, et al. 2021; Zazzo, et al. 2012)).

For the analysis, enamel chips are separated from the crowns using a diamond-coated cutting disc attached to a dental drill. The chips are thoroughly cleaned to remove any surface contamination and dentine, and then ground into powder using an agate mortar. Generally, around 20 mg of enamel powder is used for strontium isotope measurement, and 10 mg for carbon and oxygen isotope measurements. The subsequent pretreatment, measurement, and calibration against international and internal standards follow similar protocols for each type of isotope, but specific details may vary between labs. These details should be provided in the Methods & Materials section of each Manuscript or in the Supplementary Information of the publication.

3.2 aDNA and population genetics

Over the past four decades, advances in genetics have enabled the study of ancient genomes from archaeological specimens, giving rise to a new field of research known as archaeogenetics or archeogenomics, also referred to as ancient DNA (aDNA). Molecular biology began providing quantitative insights into evolutionary history in the early 1960s (Cavalli-Sforza 1966; Wilson and Sarich 1969). In the 1980s, a significant breakthrough occurred when researchers traced the common ancestor of all 147 lineages to a woman who lived in Africa around 200,000 years ago. This landmark study utilized mitochondrial DNA (mtDNA), a non-recombining DNA marker inherited maternally (Cann, et al. 1987). Cavalli-Sforza (Cavalli-Sforza, et al. 1994) pioneered the use of principal component analysis (PCA) as a tool to summarize and visualize allele-frequency differences, which has since been widely employed in population genetics. However, this approach has also faced some criticism (Novembre and Stephens 2008). In the 2000s (Skourtanioti 2022), published archaeogenetic research primarily focused on mtDNA, Y-chromosome DNA, blood groups, protein polymorphisms, and microsatellite loci, creating an arena for studying human genotypes and their interactions in the past (e.g., (Fu, et al. 2016; Lazaridis, et al. 2014a; Mathieson, et al. 2015a; Olivieri, et al. 2006; Reich, et al. 2010; Skoglund, et al. 2012; Warinner, et al. 2014)). Following the initial applications of aDNA analysis in studying human history, the subsequent "aDNA revolution" greatly expanded the scope of relevant studies, and also enabled genetic variation to be documented at given points in the past, rather than relying on modern studies alone.

The International Human Genome Sequencing Consortium released the first draft assembly of the human genome, using Sanger sequencing (chain termination DNA sequencing) as the implemented method at that time. However, in 2005, the introduction of next-generation sequencing (NGS) technology revolutionized the field by enabling high-throughput production of biomolecular data and significantly reducing time and financial costs. In the 1980s, ancient DNA (aDNA) extraction initially relied on bacterial cloning to overcome low copy numbers (Higuchi, et al. 1984; Pääbo 1985), but it was soon replaced by the more powerful Polymerase Chain Reaction (PCR) approach (Pääbo, et al. 1988). With the advancement of NGS technology, standardized criteria and procedures for aDNA analysis, such as pre-amplification steps in clean rooms and contamination control for genomic library preparation, were established. The application of universal adaptors to aDNA fragments allowed for contamination estimation using statistical tools (Korneliussen, et al. 2014; Peyrégne and Peter 2020; Renaud, et al. 2015). Furthermore, metagenomic information and comprehensive aDNA preservation provided insights into the host organism, its microbiome, and pathogens (O'Sullivan, et al. 2018; Spyrou, et al. 2018). Improved protocols for sampling, preservation, and wet-lab operations, along with commercial toolkits, gained popularity among archaeologists and anthropologists. Recently, a lysate-to-indexed-library protocol integrated various wet-lab improvements, significantly reducing costs and time during the sampling process (Gansauge, et al. 2020; Rohland, et al. 2018). Another breakthrough was the capture of target sequences in the aDNA fraction using probes (Carpenter, et al. 2013; Fu, et al. 2013). The petrous portion of the inner ear on the temporal bone emerged as an exceptional source of well-preserved aDNA, allowing valuable information to be obtained from samples in severe environments (Gamba, et al. 2014; Pinhasi, et al. 2015).

In addition to wet-lab advancements, the development of computational tools for genome analysis, referred to as dry-lab research, has played a crucial role. These tools enable the study of population structure, admixture, demographic size, and other aspects of population genetics. Large-scale modern human genome projects, such as the HapMap Project (Gibbs, et al. 2003), 1000 Genomes Project (Sudmant, et al. 2015). Human Genome Diversity Project, and Simons Genome Diversity Project (Mallick, et al. 2016) have provided foundations and references for further computational tools. Principal Component Analysis (PCA), ADMIXTURE modeling of populations, and ADMIXTOOLS for investigating admixture and gene flow direction are among the frequently used methods in aDNA analysis using genome-wide data as input (Alexander, et al. 2009; Durand, et al. 2011; Patterson, et al. 2012). 2006 to 2010 represented a turning point when high-throughput sequencing (so-called 'next generation sequencing') began to replace the prior technique of polymerase chain reaction (PCR), allowing archaeogenetic studies to gain radically higher efficiency, providing new insights into evolution, ancient diseases, and human behavior (Hajdinjak, et al. 2021; Reich 2018; Slon, et al. 2018; Zeberg and Pääbo 2020). However, it is worth noting that the time lag between data production and publication can be longer than the time it takes to double the data in the field (Reich 2018).

This study follows a genome-scale approach and introduces specific steps for sampling and data production. The sampling primarily targets the petrous portions of the temporal bone and the teeth, as these skeletal elements have been statistically shown to better preserve DNA compared to others (Gamba, et al. 2014; Parker, et al. 2020; Pinhasi, et al. 2015). The sampling process follows standardized minimally invasive protocols, and detailed steps can be found in the archived protocols (https://www.protocols.io/workspaces/mpieva-archaeogenetics).

Bone powder lysates are converted into single-stranded libraries for Next Generation Sequencing (NGS) using an Illumina platform and a semi-automated liquid handling system (Gansauge, et al. 2017; Gansauge and Meyer 2013; Rohland, et al. 2018). Following targeted enrichment of approximately 1.2 million nuclear markers (SNPs) using the '1240K capture' method), libraries with very low amounts of genetic information are excluded from downstream analyses. The remaining libraries often contain a very low proportion of endogenous DNA after metagenomic shotgun screening (approximately 0.1% or more) and exhibit characteristic deamination patterns (Jónsson, et al. 2013). In some cases, multiple genomic libraries are prepared from the lysate of the sampled bone powder to increase the resolution of genetic data. The sequencing data from all enriched libraries are processed using the EAGER pipeline (Peltzer, et al. 2016), which involves clipping remaining adaptor sequences (AdapterRemoval [v2.2.0] (Schubert, et al. 2016)), mapping reads longer than 30bp to the human genome reference (hs37d5) using the Borrows-Wheeler Aligner (BWA) [v0.7.12] (Li and Durbin 2009), and removing duplicate reads using the embedded tool (dedup [v0.12.2]). Bam files are merged across libraries from the same sample, and genotypes are extracted with pileupCaller from the pileups generated with samtools (Morgan, et al. 2019) at the targeted 1240K SNPs covered by at least one sequencing read. For the three PPNB individuals, the mitochondrial genome and the Y-chromosome are enriched using in-solution hybridization protocols (Fu, et al. 2015; Rohrlach, et al. 2021).

Contamination rates are estimated using three methods with external modern human DNA sources, each harnessing different information: 1) schmutzi (Renaud, et al. 2015) estimates mitochondrial contamination based on divergence from haploidy in mitochondrial DNA and the presence of deamination at the ends of fragments, 2) Xcont from ANGSD (Korneliussen, et al. 2014) examines the divergence from haploidy in the X-chromosome in male individuals, and 3) AuthentiCT (Peyrégne and Peter 2020) leverages the non-random distribution of aDNA damage (C > T deaminations) across fragments.

Genetic sex determination in studied individuals was performed by calculating the ratio of coverage on the X-chromosome and Y-chromosome, normalized by the coverage on autosomal chromosomes (Skoglund, et al. 2014). To identify possible sample mix-ups and genetic relatives, the rate of mismatching genotypes between pairs of individuals was calculated (Jeong, et al. 2018; Kennett, et al. 2017). Uniparental haplogroups were assigned based on the sequencing data obtained after 1240K capture or mitochondrial and Y-chromosome enrichments. For the mitochondrial genome, consensus sequences were generated using Schmutzi (fasta format) and CircularMapper for mapping (Peltzer, et al. 2016), and haplogroups were assigned using HaploGrep (Weissensteiner, et al. 2016). Y-haplogroups were determined by generating pileups with Rsamtools (Morgan, et al. 2019), and calling Y-chromosome SNPs, followed by manual inspection for the presence of diagnostic SNPs for a specific Y-haplogroup.

To visualize the genetic structure, a principal component analysis (PCA) was performed using the *smartpca* program from the EIGENSOFT software (Patterson, et al. 2006; Price, et al. 2006). Typically, PCAs are computed from modern populations to account for missing data in ancient samples. Therefore, *smartpca* was run on present-day West Eurasian populations (approximately 1800 individuals), and all ancient data from the present study and relevant published datasets were projected using the *lsqproject* function within the software.

Visual observations from the PCA can be formally tested using f_4/D-statistics (Patterson, et al. 2012). These four-population tests calculate allele correlations between populations/individuals (e.g., f_4(A, B; C, D)) and assess whether C and D form a distinct clade with respect to A and B. If the tested topology is true and there has been no gene flow between A/B and C/D after their divergence, the statistic should be close to 0. However, if the tested topology is incorrect or gene flow has occurred, the statistic will deviate from 0, with ±3 SE commonly used as the significance threshold. When A is the outgroup to B, C, and D, the f_4/D-statistic measures excessive allele sharing between B and either C (negative value) or D (positive value). Multiple f_4-statistics can be combined in the *qpWave* framework to estimate independent ancestry streams between target and reference populations, while the *qpAdm* framework models a target population as a linear combination of specific source populations (Fig. S9) (Harney, et al. 2021). Valid results with *qpAdm* depend on the availability and choice of reference populations, and the power to resolve admixture scenarios relies on these factors. In *qpAdm*, a likelihood test compares the proposed admixture model (null model) to a full model where the target population varies freely. If the null model has a low p-value (typically ≤ 0.01 or ≤ 0.05), it is rejected, indicating the need for a more complex model to explain the data.

In contrast to the above methods, haplotype-based approaches utilize information from linked markers inherited from a single parent to the offspring. Homozygous stretches within an individual, known as Runs of Homozygosity (ROH), reveal information about demography (population sizes, bottlenecks) and/or inbreeding/consanguinity. The method *hapROH* (Ringbauer, et al. 2021) can effectively estimate ROH from genome-wide data, such as those generated from 1240K capture, using a reference panel of haplotypes from modern populations and it is recommended a coverage of at least 300,000 SNPs. The program provides embedded functions for plotting individual histograms of ROH with expected distributions under specific scenarios of parental relatedness.

4. The spatial distribution of the $^{87}Sr/^{86}Sr$ signatures in the Fertile Crescent

As mentioned in Chapter 3, the utilization of strontium isotope ($^{87}Sr/^{86}Sr$) analysis, either alone or in conjunction with other isotopic analyses (such as $\delta^{18}O$, $\delta^{13}C$, $\delta^{34}S$, $\delta^{66}Zn$, and Pb-208/Pb-206), has emerged as a powerful tool for investigating ancient population mobility. A comprehensive review of relevant research, particularly the spatial variation indicated by $^{87}Sr/^{86}Sr$ data, can enhance our understanding of the isotopic background and provide references for future case studies within the specified region. This can help trace locales as potential sources or key points during mobility or migration, characterized by distinct bio-isotopic features. Although strontium isotope analysis has been applied to studies on glass trade and provenance in the Near East and other locations (Degryse, et al. 2007; Henderson, et al. 2010; Ma, et al. 2016; Ma, et al. 2014), my focus here is primarily on studies that utilize strontium isotopes on bio-archaeological materials to explore migration and mobility patterns. The exploration of relevant sites across various modern countries and distinct regions within the Fertile Crescent has been unevenly conducted. The utilization of $^{87}Sr/^{86}Sr$ ratios on bio-archaeological materials is still relatively restricted, both in terms of the number of sites examined and the sample sizes measured, particularly in the Anatolian and Zagros regions. In contrast, more extensive datasets are available from the Levantine area.

Several comprehensive reviews have been conducted on this topic. Sheridan (Sheridan 2017) and Rose (Rose 2017) provide detailed assessments of bio-archaeological work in the ancient Near East. They present statistical lists of relevant publications categorized by chronology and specified journals, drawing from extensive literature. Sheridan defines the "Near East" more narrowly, focusing only on the southern Levant. The research covers various aspects, employing a holistic framework and applying the "Life course" theory to examine demographic, dietary, health, and mortality factors, as well as bio-distances. The studies also address regional challenges, including paradigm differences, issues related to curation and pretreatment of skeletal collections, religious and legal considerations, pressures to conform, and risks stemming from political conflicts such as the Syrian Civil War and the rise of the Islamic State of Iraq and the Levant (ISIL). In the seventh chapter of Prowse et al. (Prowse, et al. 2018), earlier isotope analyses in the ancient Mediterranean and Near East, mainly focused on the later periods, are summarized. This will be further expanded and explored later in our research. To provide a more comprehensive evaluation of the local bio-available strontium isotopic signatures, I here review and summarize the most recent work on strontium isotope analysis, primarily focusing on biological and environmental materials from distinct regions within the Fertile Crescent.

4.1 Anatolian region

In general, the Anatolian region exhibits tectonic complexity, encompassing various rock types. These include up-thrusted Mesozoic and Cenozoic limestones (with $^{87}Sr/^{86}Sr$ ratios ranging from 0.7075 to 0.7092), mafic volcanic rocks (characterized by low $^{87}Sr/^{86}Sr$ ratios from 0.703 to 0.706), as well as granites, other acidic igneous and volcanic rocks, and metamorphosed clastic sedimentary rocks with higher Rb contents and consequently higher $^{87}Sr/^{86}Sr$ ratios above 0.710 (Brinkmann 1976; Dufour, et al. 2007).

Regarding Southwestern Turkey, Dufour et al. (2007) conducted a study in which they analyzed archaeological carp remains from the coastal town site of Sagalassos in Anatolia, dating back to the Early Byzantine period (AD 450-650). They compared these remains to modern fish and water samples from rivers, lakes, and other water bodies in the larger western Anatolia region. Despite efforts to narrow down the geographic origin of the ancient carp by combining species identification, determining the precise location remains challenging. This method may have limitations in distinguishing between lakes with different bedrock settings of varying ages and geologies. Due to the complex geological situation, the dissolved strontium in lakes and rivers exhibited a wide range of $^{87}Sr/^{86}Sr$ ratios, ranging from 0.7038 to 0.7149. These variations were primarily influenced by the respective bedrock or lithology, indicating a diverse range of geochemical signals in surface waters. Most of the $^{87}Sr/^{86}Sr$ ratios of the water bodies and their corresponding modern samples were consistent, except for Eber Lake. This study provided a comprehensive overview of the local strontium isotopic signatures within the defined region, offering valuable insights for future analysis of aquatic materials.

When focusing on Southeastern Anatolia, Lang et al. (Lang, et al. 2013) conducted isotopic analyses, including $^{87}Sr/^{86}Sr$ and $\delta^{18}O$, on materials from the well-known prehistoric site of Göbekli Tepe. This site, known for its notable megalithic architecture represented by T-shaped pillars, held significant religious importance during the tenth to ninth millennia BCE in Southeastern Anatolia. Based on local rodents recovered from archaeological strata, the study reported a narrow range of local bio-available strontium isotopic ratios, specifically 0.70810–0.70827 (0.70817±0.000045, 2 SD). The results indicated that most gazelles found at Göbekli Tepe were from the local area, suggesting local hunting practices. Another significant piece of research conducted in Southeastern Anatolia is the study by Benz et al. (Benz, et al. 2016) on Körtik Tepe in the Upper Tigris region during the Pre-Pottery Neolithic A (PPNA) period. This research provided the only available human strontium isotope dataset for reference in Southeastern Anatolia. Based on modern plant samples and human tooth enamel, the study reported a range of $^{87}Sr/^{86}Sr$ ratios, approximately 0.7080–0.7088, for the modern water and plant samples collected from Hasankeyf, Bismil, and Diyarbakır (Karakadağ). The research indicated that the variations in stable isotope ratios were minimal, suggesting that the population of Körtik Tepe had limited mobility within a confined area.

In this context, our Manuscript A in Chapter six, which reports and analyzes multiple isotopic datasets from Nevalı Çori (Wang, et al. 2023b), plays a vital role in filling the research gap regarding the $^{87}Sr/^{86}Sr$ data source in the Euphrates Basin/Upper Mesopotamian region (also known as the Urfa region locally). In that study, I aim to explore past human mobility and migration during the Pre-Pottery Neolithic period in Southeastern Anatolia, in conjunction with the investigation of aDNA.

To enhance our understanding of the spatial distribution of $^{87}Sr/^{86}Sr$ in the South Central/Eastern Anatolia region, another noteworthy area besides the hilly flanks is the Amuq Valley, located further west of the Euphrates Basin, near the northeastern corner of the Mediterranean Sea. Among the many urban sites in the Near East during the Middle and Late Bronze Age, Tell Atchana, situated near the Orontes River in the Amuq Plain, served as the capital of a local Mukish kingdom dating from approximately 2200 to 1300 BC (Ingman, et al. 2020). The first strontium isotope study conducted by Meiggs in 2011 focused mostly on archaeological fauna, including teeth from ovicaprines and deer, as well as modern environmental samples including six snail shells and six plants from multiple locations around the valley (Meiggs 2011). The study provided a local baseline with a wide range of 0.707851–0.714678 (2 SD), which was not sufficiently narrow to serve as a useful reference. However, a subsequent study by Ingman et al. (Ingman, et al. 2020) built upon the materials from the same site. In addition to measuring five snail shells and two rodents from an archaeological context, they provided strontium isotope data for 53 individuals and oxygen isotope data for 77 individuals, establishing a local range for Alalakh and the Amuq Valley, respectively. The results showed no significant evidence for high levels of individual mobility. The newly reported dataset combined with new ancient DNA data from nine individuals in Ingman et al. (Ingman, et al. 2020), is currently unparalleled in quantity among the case studies in the Near East.

To the northeast of Alalakh, Lau et al. (Lau, et al. 2021) conducted strontium isotope data on faunal teeth retrieved from the Halaf site of Domuztepe (ca. 6000–5450 cal. BC). Halaf is a specific cultural horizon defined by a distinct suite of material culture the period of which is a special chronological sequence between the late Neolithic and the Chalcolithic present mostly in small scale settlements widespread throughout the northern Fertile Crescent, with the inhabitants either as settled agropastoralists or engaged in subsistence with great degree of mobility. The isotopic reference set samples exhibit a relatively narrow local isotopic reference range of 0.70782–0.70793 (2 SD). Aiming to explore the provisions of feasts and the meat supplies utilized by this site researchers applied isotope analyses to archaeologically-recovered samples, the results of which indicate that animals consumed at feasts were drawn from the same faunal population used as the inhabitants' everyday diet (Lau, et al. 2021).

Located northeast of Domuztepe, Meiggs (Meiggs 2009) studied caprine herding at Pre-Pottery Neolithic Gritille (ca. 8000–6800 cal. BC). Integrating data based on the fauna teeth of aurochs, equids, and cattle from Çayönü, Göbekli Tepe, and Titriş Höyük, that are all located in southeastern Turkey, this research yielded a baseline of ^{87}Sr/^{86}Sr for reference ranging 0.70798–0.70804 (unknown SD). The strontium isotope analyses of the samples studied were remarkably homogeneous, the majority of which indicated a geographic area restricted to the limestone plateaus surrounding the Euphrates in this region, extending southward to Syria, which highlighted equifinality across a wide region though. Moreover, this study mentioned that there are other regional archaeological data indicating potentially increasing economic autonomy of household through the Aceramic Neolithic (Pre-pottery Neolithic), which is consistent with the conclusive inference based on our case study of Nevalı Çori (Wang, et al. 2023b), see MANUSCRIPT A in Chapter 8.

In central Anatolia, Bogaard et al. (Bogaard, et al. 2014) summarized previously published stable oxygen isotope (^{18}O/^{16}O) analyses of sequential sheep tooth samples (Henton 2012) to test the model of long-distance sheep transhumance into the mountains beyond the Konya Plain. The newly reported strontium isotope ratios (^{87}Sr/^{86}Sr) in archaeological sheep tooth enamel and charred plant material revealed a north-south cline in Sr isotope values surrounding Çatalhöyük, with higher ratios observed in the alluvial plain and lower ratios in the limestone terraces (Bogaard, et al. 2014). The strontium results, in conjunction with previous oxygen isotope analyses, suggest that most of the sheep were herded within the plain. Another isotopic study in central Anatolia was conducted by Meiggs et al. (Meiggs, et al. 2017), who established a bioavailable ^{87}Sr/^{86}Sr range from regional background samples surrounding Köşk Höyük to trace grazing areas for the domestication of ovicaprines and exploitation of wild equids. The study demonstrated detailed changes in local land-use patterns and found consistency between shifts in production priorities and access rights to grazing spaces during the period from 6200 to 4900 cal. BC.

In Southcentral Anatolia, Yazıcıoğlu-Santamaria et al. (Yazıcıoğlu Santamaria 2015) conducted a study to investigate the local $^{87}Sr/^{86}Sr$ range of Kültepe (ancient Kanesh), a commercial center that emerged in the early 2nd millennium BCE. The local range of Kanesh was determined based on snail shells, which Maurer et al. (Maurer, et al. 2012) suggested might not be the optimal choice of material for strontium isotope analysis. The samples were collected from 12 locations surrounding Kültepe and 5 locations in the wider Kayseri plain and foothills, yielding a range of 0.70776–0.70781 (2 SD) for the former group and a range of 0.70772–0.70785 (2 SD) for the latter. Additionally, a regional project conducted in collaboration with Pınar Ertepınar and David Meiggs aimed to assess the $^{87}Sr/^{86}Sr$ ratios of agricultural soils in drainage basins of central Anatolia, providing a rough distribution of $^{87}Sr/^{86}Sr$ ratios for the defined region (Yazıcıoğlu Santamaria 2015). Some individuals sampled at the site of Kültepe were found to be non-local, and potential locations for their origin include southwestern Anatolia (i.e., the land of Purushaddum), the Amuq, upper and middle Euphrates, and Assyria.

A representative case study from Northern Anatolia is the isotopic analysis of a substantial collection of skeletal samples from the İkiztepe site (Welton 2014). İkiztepe is located in Bafra, Turkey, in the Kızılırmak delta near the Black Sea coast. The site presents a primary chronological sequence from the Late Chalcolithic to the Iron Age, with the majority of findings dating to the Early Bronze Age (Welton 2014). The predominant geological context consists of carbonates and island arc magmatics with relatively low strontium isotopic signatures. Eight animal samples were analyzed to establish a local baseline isotopic signature ranging from 0.70752 to 0.70781 (2 SD). By analyzing strontium and oxygen isotopes in the enamel of 72 individuals, the study revealed a high proportion of non-locals in the İkiztepe population. Northern Anatolia appears to have played a significant role in inter-regional interactions within the larger Pontic world during the 4th millennium BC.

The bioavailable baseline strontium isotope map in Southwestern Anatolia was systematically investigated by Megan Wong. In a case study conducted in 2018 (Wong, et al. 2018), she examined the human mobility of individuals from the Roman (1st to 7th century AD) and Byzantine (9th to 13th century AD) periods, recovered from the UNESCO World Heritage site of Hierapolis, Turkey. The results revealed that some individuals with strontium isotope values outside the local range were identified as migrants who could have originated from northern regions or even from somewhere in Europe, particularly France and Italy. However, the majority of the population exhibited strontium isotope values falling within the assumed local baseline. The geological context in southwestern Turkey is complex and varied (Brinkmann 1976) and Wong's more recent study (Wong, et al. 2021) presents a map of bioavailable strontium for this area based on measurements from 283 modern plants and land snail shells collected from 87 different locations. The strontium ratios were found to range from 0.703907 to 0.715162. The bioavailable strontium ratios generally grouped into two separate areas, with less radiogenic values in the northwest and more radiogenic values in the southeast, corresponding to variations in the underlying bedrock age. This comprehensive work significantly contributes to future studies on population mobility using strontium isotopes and provides a key reference for understanding the overall landscape of Southwestern Anatolia.

Another important study focused on available $^{87}Sr/^{86}Sr$ values for soil and plant samples in northern Mesopotamia based on environmental materials from several sites in northeastern Syria, including Tell Majnuna, Tell Barri, Tell Ashara, and Tell Masaikh in the Euphrates valley (Sołtysiak 2020). Additionally, two $^{86}Sr/^{87}Sr$ ratios of plants retrieved from Tell Brak are available (Henderson, et al. 2009). This study found no evidence suggesting mass migration to the city from places with detectably different strontium isotopic signatures. The increase in dispersal indicated higher resource heterogeneity and wider use of marginal areas to support the growing population in the mega-city at that time.

In summary, the bioavailable strontium baselines available for Anatolia are scattered across various regions. While there are relatively more informative studies conducted in Southwestern Anatolia and some case studies in Central and Southeastern Anatolia, which are key regions for better understanding the origins of agriculture and civilization, only one case study has been conducted in Northern Anatolia. Overall, the density of the dataset for the entire Anatolia region is still insufficient to establish a bioavailable strontium baseline map with a reasonable resolution. Although there is inevitable overlapping between the ranges from different regions of this area, but some interesting conclusions could still be drawn with archaeological context.

4.2 Zagros and Caucasus regions

Despite the increasing application of isotopic studies in archaeology, the strontium isotopic dataset and local bioavailable $^{87}Sr/^{86}Sr$ baselines in the Zagros and Caucasus regions are significantly fewer compared to the Anatolia region in the northern Fertile Crescent. There is also a lack of systematic study in any smaller regional zone within this range. While some relevant research has been published in local languages, particularly for the Zagros region (شعاعی and 2018 نیکنامی), which may limit accessibility for broader readers, this section aims to provide an overview of the current English strontium isotope studies in these regions other than those in Persian.

Most of the current research in this field has been conducted by M. B. Kasiri's team. Kasiri and Karimi (Kasiri and Karimi 2017) focused on studying human skeletal remains from the Iron Age site of the Blue Mosque in Tabriz, located in the northwest corner of Iran. The geological context of this area consists primarily of quaternary sediments, including alluvial and alluvial fans, fine-grained clay minerals, and river sediments. The results showed that all the samples, except for one female individual, could be considered local to the region. The researchers also examined the ratios of Sr/Ba and Ba/Ca in the bone samples and used the strontium isotope ratios of archaeological bone samples as the reference for local strontium baselines, which ranged from 0.7048 to 0.7064. This approach helped avoid the higher risk of contamination when measuring soil or plant samples from the cemetery located within the city.

Another case study was published in 2020 (B. Kasiri and Abedi 2020). However, as it has been published in Persian, it is challenging for an author like me to delve deeper into the details. The study analyzed strontium isotopes of teeth and bones from five individuals from Tepe Silveh, Piranshahr. This site is located in northwestern Iran and has a rich history of occupation, including the Early Chalcolithic Dalma (5000 BC) culture, Late Chalcolithic, Early Bronze Age culture of Hasan Ali or Nineveh V (3500–2700 BC), Iron Age, Parthian periods, and the Middle Islamic Periods (Seljuk era) through to the late Islamic Period. This study provided a local baseline with a range of 0.7692–0.8190 (Mean = 0.8076, ±2 SD) based on the strontium ratios of human bone samples. These bone samples were supposed to reflect the local strontium isotopic signature of the soil due to diagenesis. Among all of the tested samples, including five bone samples and five teeth samples from the five individuals, there were nine ratios above 0.8. These ratios are incredibly high compared to most common bioavailable strontium isotope ratios based on human tissue. However, in the same year, another paper published in English (Khojasteh, et al. 2020), seemingly based on the same batch of samples from Tepe Silveh and using the same methods, presented completely different data from the former study. In this paper, the reported local baseline ranged from 0.7290 to 0.7062 (Mean = 0.7176, ±2 SD). It is not reliable to accept the results proposed by the original study that all samples could be considered non-native based solely on the difference between the ratios of Ba/Ca and Sr/Ca in tooth enamel and bone. Based on the data from their results, there is one individual with a notably high enamel ratio, while the other four data points are not significantly outside the calculated local range. The confidence in these results might need to be improved with more data, both in terms of types and quantity, from environmental samples. However, this improvement is contingent upon the validity of all the datasets published in this paper, reflecting the authentic strontium isotope ratios of the samples *in vivo*. The section on pretreatment protocols did not mention the operations of using acetic acid (5 times) to eliminate diagenetic Sr contamination, nor dissolution with HNO_3 at higher temperature, nor Sr purification and separation steps. These omissions significantly diminish the reliability and confidence of the data generated by this study.

As for the Great Caucasus range and along the Black Sea coastline, there are few available cases to reference. A study by Trifonov et al. (Trifonov, et al. 2012) aimed to investigate the origin of people buried in the Kolikho dolmen, the first megalithic archaeological site in the Caucasus. However, the study calculated the local baseline for strontium isotope ratios using measured snail shells (*Helix pomatia*) collected near the dolmens. These ratios varied significantly from 0.7075 to 0.7087. It is worth noting that snail shell $^{87}Sr/^{86}Sr$ ratios are often biased towards values for soil carbonates, and thus, snail shells are not suitable for providing accurate estimates of biosphere $^{87}Sr/^{86}Sr$ (Maurer, et al. 2012). In conclusion, the study argued that human tooth samples were more homogeneous than bone samples and showed good agreement with the local 87Sr/86Sr baselines. The authors argued that half of the 70 sampled individuals spent the last decade of their lives far outside of the Kolikho Valley. However, bone samples should not be considered an optimal and reliable proxy for estimating individual mobility, as diagenesis can affect the data and provide information about burial soil or local biosphere strontium ratios (Price, et al. 1992; Sealy, et al. 1991). Therefore, the data and arguments presented in this research are not optimal for further reference.

In a joint study of strontium, oxygen, and carbon isotope analyses, Chazin and Chazin et al. (Chazin 2016; Chazin, et al. 2019) examined a large sample of herd animals (sheep, goats, and cattle) from Late Bronze Age sites (1500–1100 BC) in the Tsaghkahovit Plain, Armenia. The findings indicated that long-distance movements across geological zones were not common among the studied animals, but they were drinking from a range of water sources, providing insights into the relationships between pastoralist mobility and the development of new forms of political organization. The Tsaghkahovit Plain, located between the northern slope of Mount Aragats and the southwestern slopes of the Pambak range in central Armenia, exhibits significant geological diversity (Smith, et al. 2009). Due to the absence of a large-scale strontium isotope mapping project, the preliminary interpretation of the data in this study also used local baselines to determine bioavailable strontium. The local $^{87}Sr/^{86}Sr$ baseline range for bioavailable strontium in the Tsaghkahovit Plain was found to range from 0.7077 ± 0.0007 (2 SD). In summary, this study provides standard, meticulous, and informative research on strontium isotopes, shedding light on the topic of fauna herding in the central Caucasus. Additionally, it offers vital datasets for the bioavailable baseline range in this region.

4.3 The Levantine region

When it comes to the latest strontium isotopic data in the Levantine area, there are approximately 20 papers published between 2004 and 2021 that serve as references. I aim to integrate these papers to gain a comprehensive understanding of this field. By analyzing the existing data, we can explore the feasibility of constructing an iso-scape of ^{87}Sr/^{86}Sr ratios across the region and assess how this map could aid future relevant studies. To accomplish this, I will rely on geological maps (at a 1:50,000 scale) and other relevant publications on the Levantine area and beyond, encompassing regions such as present-day Jordan and Israel. These resources are crucial for referencing the distribution of lithologies and soils (Bender 1974; Bender 1975; Sneh, et al. 1998).

Shewan's 2004 study (Shewan 2004) examined 89 archaeological samples, including fauna bone samples, human cortical bone (n = 34), and enamel samples (n = 7) from six Natufian sites. Additionally, they provided several datasets based on modern fauna and plant samples from 18 locations. Unfortunately, only the metadata of grass samples were presented in the publication, which can be useful for integrating into the strontium isotopic map for the southern Levant region. Regrettably, the metadata of the archaeological samples lack explicit information regarding the corresponding site or coordinates. However, the researchers conducted test measurements on the archaeological bone material to determine the Sr/Ca and Ca/P ratios, ensuring that the ^{87}Sr/^{86}Sr ratios accurately represent the endogenous strontium *in vivo*.

Perry et al. (Perry, et al. 2008; Perry, et al. 2009; Perry, et al. 2017) and Shewan (Shewan 2004) conducted estimations on archaeological and modern fauna and flora samples to obtain multiple datasets for the local bio-available strontium isotopes in the Jordan Valley region, which encompasses parts of Israel, Jordan, and Syria. These datasets provide insights into the Sr isotopic zone within the defined area. The data revealed overlapping ranges among these regions, suggesting their applicability when utilized in practice. Notably, important burial sites from the Natufian to the late PPNB periods, such as Jericho, Kfar HaHoresh, Iraq ed Dubb, and Yiftah'el, were included in the study. Alt et al. (Alt, et al. 2013) reconstructed the ancient mobility of the Basta site by systematically analyzing tooth enamel samples, reporting a narrow range of local bio-available Sr values of 0.70821±0.00008 (2 SD) based on local animal bones (bulls and boar) and one soil sample. These values fall within the zone of the Western Highlands, both geographically and isotopically (Shewan 2004). Beherec et al. (Beherec, et al. 2016), which investigated the relationship and mobility patterns in the Iron Age at the Wadi Fidan 40 cemetery, also relied on the local $^{87}Sr/^{86}Sr$ signatures published in Shewan's study, using biological materials.

Another case study within this region focused on the bio-archaeological investigation of Byzantine St. Stephen's monastery in Jerusalem, where pilgrims from various parts of the Levant visited according to written and archaeological evidence (Sheridan and Gregoricka 2015), though with the potential issue that the local baseline based on faunal materials may be risky considering the animals could also from elsewhere throughout the Crusades and pilgrim networks. However, the study by Cooper et al. (Cooper, et al. 2007) has provided another dataset could offer a bolster, which exhibited a group of $^{87}Sr/^{86}Sr$ values from 0.707991 to 0.708522, with a local range of 0.7079–0.7086 (2 SD). Although this range is slightly wider than the Eastern Highlands zone to which the site belongs, the two ranges mentioned still provide mutual support.

Arnold et al. (Arnold, et al. 2016) reported an isotope study of the Early Bronze Age site of Tell es-Safi/Gath in Israel, where dental isotopes of archaeological animals ($n = 17$) indicated that the majority of animal resources were local. Sequential sampling provided evidence of animal trade from Egypt to ancient Canaan (the southern Levant). The study also tested modern plants ($n = 10$), yielding a mean value of 0.708732, consistent with the expected range based on the Calcareous sandstone/Sand zone (Hartman and Richards 2014) where the site is located. The latter study (Hartman and Richards 2014) also established a bioavailable $^{87}Sr/^{86}Sr$ mapping of Northern Israel and the Golan area. The investigation of strontium isotopic analysis on gazelle tooth enamel to reconstruct hunting ranges at Amud Cave (Hartman, et al. 2015), inhabited by Neanderthals northwest of the Sea of Galilee, found that it was more effective in pinpointing geographic locations and providing higher resolution insights into the environment in some cases.

The work conducted by Perry et al. (Perry, et al. 2017) in Southwest Jordan focused on the site of Aila, an ancient Byzantine port city located in modern Aqaba. They reported a local strontium isotope signature for Aila based on both rodent and sheep/goat fauna, which ranged from 0.7075779 to 0.7084145. Whereas there is uncertainty about the suitability of sheep/goat fauna as contextual material. Alternatively, using just the archaeological rodent dental enamel (n = 2) and modern groundwater samples (n = 2) to establish the local range for Aila, would yield a value of 0.7076473±0.0004 (2 SD) as a more narrowed range.

In Northern Jordan, the source of inhabitants at the site of Barsinia was identified, and contextual values of other sites were summarized in the study by Al-Shorman and El-Khouri (Al-Shorman and El-Khouri 2011). The study analyzed 12 third molars from excavated individuals and 12 rodent teeth samples, confirming that the sampled population was local. The mean strontium ratio of Barsinia's rodents was determined to be 0.708208 with a standard deviation of 0.000254, resulting in a range from 0.707700 to 0.708716 (±2 SD). Additionally, the average strontium ratios for the sites of Pella, Bediye, Ya'amun, and Dajaniya were found to be 0.707967, 0.708012, 0.708336, and 0.708035, respectively. Among these, the values for Bediye and Dajaniya sites were homogeneous and indistinguishable.

Spiro et al. (Spiro, et al. 2011) characterized the types of water sources in the catchment area and used the snail *Melannopsis sp.* as a tracer to investigate the paleo-hydrology of the Hula Lake during the corresponding period. They found a wide range of local Sr isotope composition, ranging from 0.7046 to 0.7079. These results highlight the importance of caution when using materials exposed to the environment during remote geological times. Therefore, it is crucial to select aquatic samples from well-mixed water sources of the archaeological period.

For more comprehensive information on the Sr baseline and other case studies in the Levant, a detailed review has been included in Manuscript B (the case study of Jericho) and its supplementary information in the Appendix (Chapter 10.2), which will be published. This region provides relatively abundant data for constructing a primary $^{87}Sr/^{86}Sr$ baseline map compared to the rest of the Fertile Crescent.

5. Outlook for future studies

As early as 1992, Renfrew argued that the availability of genetic data has great potential in providing valuable insights into questions of population mobility associated with linguistic and agricultural dispersal (Renfrew 1992). Meanwhile, while studies focusing on isotopic data from specific sites unveil a more nuanced understanding at the local level (Bentley, et al. 2003a), they also shed light on the complexities underlying the broader patterns observed in genetic investigations. Weighing the advantages and disadvantages of both methods, my two case studies have aimed to examine mobility patterns during the Pre-Pottery Neolithic (PPN) periods, which have long been argued to have marked a transitional stage towards a sedentary lifestyle and increased reliance on plant and animal domestication. From the perspective of regional archaeology, this dissertation significantly contributes to our understanding of this process in the Southeastern Anatolian region during the PPNB period, as well as the high degree of sedentism at the beginning of the PPNA period in the Southern Levant region. These findings highlight the regional diversity and varying pace of the trajectory towards sedentism in the western Fertile Crescent. These differences can be attributed to various factors, including distinct subsistence strategies, belief systems, and population structures.

My first case study (MANUSCRIPT A, Chapter 8) combined the isotopic data ($^{87}Sr/^{86}Sr$, $\delta^{18}O$, and $\delta^{13}C_{carb}$), new radiocarbon dating, and genome-wide data of the materials from the site of Nevalı Çori and Ba’ja, filling a research gap between prehistoric Anatolian and Levantine populations and revealing a broad connectedness within the Fertile Crescent during the earlier phases of Neolithization and evidence of consanguinity in the PPNB Levant. My second case study (MANUSCRIPT B, Chapter 9) detected no evidence for large-scale migration into the community or structural mobility, indicating a relatively high degree of consolidation of the social organization in the PPN Jericho. In addition to complementing previous studies on regional diversity during the Neolithization process in the western Fertile Crescent, this research also enriches the discussion on the complex and varied relationships between the development of agriculture and sedentism in different subregions of the Fertile Crescent. It becomes apparent that there is no unified model to determine whether sedentism preceded agriculture or *vice versa*, necessitating further examination of archaeological contexts on-site.

However, this does not imply abandoning efforts to develop a general model that articulates the overall mechanism of how agricultural origins and the development of sedentism interact in the process of Neolithization. Rather, it calls for a framework that reconciles the particularities observed through site-based studies with the broader regional perspective, highlighting the issue that both field and laboratory research should be closely incorporated in the future. Regarding the methodologies employed, this dissertation effectively combines isotope studies and ancient DNA analysis to comprehend the mobility and population structure in Southeastern Anatolia and the Southern Levant region on different scales. This integration enables a deeper understanding of how population migration and genetic communication were associated with the process of Neolithization, as manifested by the development of more settled communities and agricultural practices. Nevertheless, questions remain regarding the specific mechanisms, beyond population factors, that directly contributed to the microscale changes in mobility detected through strontium isotope analysis.

For example, in the second case study of Jericho (MANUSCRIPT B, Chapter 9), I integrated the local bioavailable strontium baseline based on newly measured data with the published data of surrounding areas in the existing literature relevant to develop a spatial mapping of $^{87}Sr/^{86}Sr$ ratios for the Southern Levant. However, the available baseline data show significant $^{87}Sr/^{86}Sr$ homogeneity across large areas of the region as well as overlapping values between regions which hampers resolution in terms of spatial mobility and the identification of immigration from adjacent regions. This limitation may mask the significance of short-distance migrations driven by trade, marriage, pilgrimage, or other reasons, as such migrations often occur gradually and on smaller scales. Archaeogenetic analysis can contribute to understanding some cultural underpinnings of the role of kinship networks in the region. For instance, one of the results in my first case study supports the evidence of consanguineous union in the PPNB Ba'ja and the Iron Age Nevalı Çori by analyzing the ROH distribution of the relevant individuals (MANUSCRIPT A, in Chapter 8). Additional fine-scale genetic studies focused on subregional and site-based contexts are required.

Beyond the specific scope of the region where our target sites are located, more challenges in current archaeological research remain across the whole Fertile Crescent. The first major challenge faced in this region are regional conflicts and political instability, which pose significant obstacles to conducting bioarchaeological research and limits access to skeletal remains or local funding for research. For example, the impact of the long-term civil war in Lebanon from 1975 to 1990 has resulted in a dearth of publications for this region, creating a significant gap in our understanding of the bioarchaeology of the southern Levant (Sheridan 2017). A similar situation can be observed in the territory of modern Iran. Initially, Braidwood chose sites in the western Zagros to conduct Neolithic research in order to better understand the origin of agriculture, as mentioned in Chapter 1.1. However, due to political issues, work in the area came to a halt in 1979, and the focus of research shifted towards the Levant and southern Turkey. Additionally, the northwest Iranian plateau is now, by some, referred to as Azerbaijan due to current political divisions (Khojasteh, et al. 2020). It is important for us to acknowledge these gaps when discussing general processes for the region and to focus on potential future work in poorly-understood regions where possible. Another problem hindering researchers, particularly laboratory analysts, is the inadequate management and storage of archived samples from early excavations. Sheridan provides an example of this issue using the case of Jericho, where the recovered skeletons are spread across multiple locations, making it challenging to determine their whereabouts (Sheridan 2017). However, for this study, I was fortunate enough to gain authorized access to a portion of the samples from Jericho. Unfortunately, the preservation of these samples is not sufficient to extract qualified collagen and DNA materials.

The lack of data for regions has become a common challenge that few archaeological scientists can escape. For studies involving strontium isotope analysis, it is, in particular, crucial to establish a reliable baseline from both the local site and the surrounding area. As Meiggs mentioned in the study of Tell Atchana, further work should aim to expand the reference sample, identify sources of variation within it, and increase the sample size of fauna and humans from different areas of the site (Meiggs 2011). It is highly beneficial if the researcher conducting the bioarchaeological analysis can personally visit the site and prepare a detailed sampling proposal in advance. Additionally, when interpreting the findings, it is essential to establish complete chains of evidence and to also accept the inherent limitations in knowledge of isotopic variability and its interpretation. Furthermore, it is important to have a deep understanding of the rationale and principles of the applied method (e.g., (Buonasera, et al. 2020; Castellano, et al. 2014; Harney, et al. 2020; Kohn 2004; Lugli 2019; Wathen, et al. 2022)). In doing so, we can avoid limiting ourselves to case studies within the current scope and actively pursue further foundational research.

The recent advances in integrative studies of genetics and archaeology hold promise for documenting more nuanced mechanisms of domestication. Zeder et al. (Zeder, et al. 2006) have highlighted several promising areas where the complementary perspectives of both disciplines could provide reciprocal illumination, including identification of wild progenitors of plant and animal domesticates and documenting the number and location of domestication events and the dispersal of domesticates. There are also scholars who have criticized a so-called ‘molecular chauvinism’ and pointed out that this leads to overreach in interpretation that can actually hamper archaeological progress (Horsburgh 2015). Similarly, Frieman and Hofmann (Frieman and Hofmann 2019) have argued that right wing and racist political activists make use of the results of archaeogenetic to bolster the ‘Fortress Europe’ idea against non-European immigrants and they note that “we are responsible for the co-created pasts we present to the world, and the impact of our research is more wide-ranging and consequential than its journal metrics or citation rate” (Frieman and Hofmann 2019, 539). Similar concerns have been raised also in other critical essays, e.g., (Hakenbeck 2019; Sykes, et al. 2019) and highlight the responsibility that scientists should take when presenting their work. On a more positive note, however, there are increasing efforts by archaeogeneticists to better contextualize ancestry profiles with ancient human behaviors, including, but not limited to: more complements and innovations in methodology (e.g., (Moorjani, et al. 2016)); more reviews, method primers and even popular science readings for wider readers who are not expertised in molecular genetics to have better understandings of this field (e.g., (Orlando, et al. 2021)); more systematic thinking, clarification and and promotion on key concepts and standardized terminology (e.g., (Eisenmann, et al. 2018)).

In any case, in this thesis, I would argue that aDNA and isotopic studies have offered currently unrivalled toolkits which can help archaeologists struggling to understand past mobility. Applying these techniques we can explore how cultural traits align, or do not align, with genetic ancestry. As Anthony et al. (Anthony and Brown 2017; Anthony 2023) have noted, race and genetic ancestry are quite different things and the mating network can be considered independently of other interaction spheres defined by shared cultural traits. Archaeolgists can determine the degree to which patterns of mobility correspond to economic or behavioural thresholds in individuals or societies and test the degree to which subsistence patterns, new socio-political hierarchies, and other novel social dynamics have demographic correlories. Utilizing DNA data in tandem with other proxies, including stable and radiogenic isotopes, macro- and micro-remains of fauna and flora, and paleoproteomic data, will greatly improve our knowledge about mobility across different temporal and spatial scales in the past and deepen the recognition of the complex and multi-generational processes that underpin a major period in human history that has left a trace on landscapes all around us today.

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شعاعی, فهیمه شیخ, and کمال الدین نیکنامی
آنالیز ایزوتوپ های پایدار استرانسیوم و تعیین افراد بومی یا غیربومی در گورستانهای باستانی، 2018
مطالعه موردی: گورستان عصر آهن محوطه باستانی گوهر تپه, 2018.

7. Acknowledgements

It all began with an email in response to my initial inquiry letter to Philipp W. Stockhammer, a German researcher who later became my PhD supervisor at the University of Munich. In 2018, we had our first meeting in Shanghai, where I also met his family—Christina, Sophie, and Julius. From the moment Philipp saw and spoke with me, he believed that I could accomplish our project goals and excel in my doctoral work. However, the truth is that without his initiative, encouragement, and unwavering faith in me, I would have never embarked on the study of the prehistory of Western Asia, nor would I have reached this moment of writing the most relaxing section of my PhD dissertation with a sense of pride and accomplishment. I cannot imagine a more ideal supervisor than Philipp. Our shared academic philosophy and interests have fostered a strong bond, and he has not only been a mentor but also an understanding friend, always there with his optimism and cheerfulness. Thank you, Philipp, for making all of this possible!

I am also immensely grateful to my second supervisor, Patrick Roberts. His writing and thinking have consistently illuminated my work from new and broader perspectives, helping me construct and organize my ideas with different angles. He has assisted me in expressing myself with greater precision in my writing, and his patience in responding to my questions, no matter how trivial, is greatly appreciated. I would also like to acknowledge Eirini Skourtanioti, a reliable and responsible collaborator in genetic analysis, whose professional skills and knowledge have bolstered our research efforts. Both Patrick and Eirini have consistently shown dedication and invested additional effort in our progress, always open to further communication. I continue to learn from them with each interaction.

My gratitude extends to the individuals who have provided warmth and support throughout my PhD journey, creating countless memorable moments together. To my dear friends and colleagues in Munich—Janine Fries-Knoblach, Maleen Leppek, Stefanie Eisenmann, and Fabian Heil—you have made me feel at home since my arrival in Germany. I fondly remember every helping hand you extended when I felt lost in the new environment, the time we spent together on campus, the shared meals and trips, and the recipes we exchanged. I will never forget the first time I encountered a "quince" which Janine brought from her garden. The view outside my workspace has always resembled an oil painting.

To my friends from China whom I met in Munich, who excel in their respective fields—Xiaodan Wang, Difei Han, Zhi Li, Wenkai Wu, Wei Liang, Wei Wang, Fangfang Liu, and Ling Cheng—some of you have been in Germany for decades, graciously offering me new perspectives, while others, like myself, were newcomers, exploring new foods and embarking on new adventures in this unfamiliar city.

I am immensely grateful to the team at the isotopic lab of the Max Planck Institute of Geoanthropology (GEA-MPI, formerly SHH-MPI): Jana Ilgner, Mary Lucas, Sara Marzo, and Erin Scott. I also extend my gratitude to the lab technicians from EVA-MPI—Raffaela Bianco, Cäcilia Freund, and Nuno Filipe—for their skilled training and invaluable assistance with the significant workload.

To the powerful support from the Max Planck Institute for Evolutionary Anthropology (EVA-MPI) and the members of the Max Planck Harvard Center for the Archaeoscience of the Ancient Mediterranean (MHAAM) group, I am particularly grateful to Johannes Krause, Angela Mötsch, Gunnar Neumann, Guido Gnecchi Ruscone, Anthi Tiliakou, Irene Högner, Stefanie Eisenmann, Nada Salem, and Megan Michel. Their enthusiasm for research and the friendly and vibrant environment they fostered inspired the second half of my PhD study.

I would like to extend my gratitude to the archaeologists with whom I have interacted to varying degrees: Marion Benz, Laura Dietrich, Michael Morsch, Tara Ingman, Eleni Asouti, Michael Schultz, Nadja Pöllath, and Joris Peters. Some of you have dedicated significant efforts to our collaboration, while my interactions with others may have been brief but meaningful. Your dedication to research and your enriched expertise always inspired me with new perspectives from different fields. I would also like to thank Petra Vaiglova for kindly inviting me as an external member to join their R-club session at WashU for a short but practical training. She was an excellent host!

To my dear friends and colleagues in Jena—Naihui Wang, Ke Wang, He Yu, Li Tang, Chao Ning, Xueye Wang, Yiming Wang, and Thomas Larsen—I am fortunate to know all of you, sharing common research interests and cultural backgrounds. We have spent so much time together, cooking during each home festival, and exploring the beautiful hills of this charming town. I look forward to establishing more connections with you when we return home for future endeavors. I would also like to express my gratitude to Xiaobao Li, Alina Naomi Hiß, and Hans Sell, who made my time in Jena easier and more enjoyable in their own unique ways.

To Xiaotong in Berlin, a childhood friend who has witnessed nearly all the significant turning points in my life, and to Jun, a friend I coincidentally met in Jena whose doctoral theme is Fichte, I am grateful for the memorable trip we took together before leaving Germany. I would also like to thank Richard from Newcastle in England, whom I met during my less-prepared hiking expedition to Mount Telde. My sincere thanks also go to the friends who supported me during the last year while I battled through the final rounds of manuscript revisions: Meng in Guangzhou, Shuhan and Jane in Shanghai, Xiang in Hefei, and Mingyue in Barcelona, who is currently still working on her doctoral thesis—keep up the good work! They have accompanied and supported me through challenging times.

Last but not least, I would like to thank my parents Yong and Rui who are always nourishing me with their loving cares and understandings, and my partner Yufeng who meanwhile has been a supportive comrade of mine along my life and academic paths.

The string of the affectionate names makes up the cherished memories of my PhD life in Germany. It has been a nice ride and I look forward to seeing and being part of the next play.

Ps. Many thanks to Patrick and Yufeng for patiently polishing the language and improving the content of the thesis, to Janine, whose timely help saved the language of the German abstract. I cannot achieve this without their crucial help.

8. Manuscript A (the case study of Nevalı Çori)

PNAS RESEARCH ARTICLE | ANTHROPOLOGY

Isotopic and DNA analyses reveal multiscale PPNB mobility and migration across Southeastern Anatolia and the Southern Levant

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Edited by Melinda Zeder, Smithsonian Institution, Frederick, MD; received June 20, 2022; accepted November 29, 2022

Growing reliance on animal and plant domestication in the Near East and beyond during the Pre-Pottery Neolithic B (PPNB) (the ninth to eighth millennium BC) has often been associated with a "revolutionary" social transformation from mobility toward more sedentary lifestyles. We are able to yield nuanced insights into the process of the Neolithization in the Near East based on a bioarchaeological approach integrating isotopic and archaeogenetic analyses on the bone remains recovered from Nevalı Çori, a site occupied from the early PPNB in Turkey where some of the earliest evidence of animal and plant domestication emerged, and from Ba'ja, a typical late PPNB site in Jordan. In addition, we present the archaeological sequence of Nevalı Çori together with newly generated radiocarbon dates. Our results are based on strontium ($^{87}Sr/^{86}Sr$), carbon, and oxygen ($\delta^{18}O$ and $\delta^{13}C_{carb}$) isotopic analyses conducted on 28 human and 29 animal individuals from the site of Nevalı Çori. $^{87}Sr/^{86}Sr$ results indicate mobility and connection with the contemporaneous surrounding sites during the earlier PPNB prior to an apparent decline in this mobility at a time of growing reliance on domesticates. Genome-wide data from six human individuals from Nevalı Çori and Ba'ja demonstrate a diverse gene pool at Nevalı Çori that supports connectedness within the Fertile Crescent during the earlier phases of Neolithization and evidence of consanguineous union in the PPNB Ba'ja and the Iron Age Nevalı Çori.

Neolithization | Near East | $\delta^{18}O$ and $\delta^{13}C$ isotopes | $^{87}Sr/^{86}Sr$ | ancient DNA

Significance

We present the integrative bioarchaeological study on the Pre-Pottery Neolithic B (PPNB) in the Southeastern Anatolia by combining isotopic data ($^{87}Sr/^{86}Sr$, $\delta^{18}O$, and $\delta^{13}C_{carb}$), new radiocarbon dates, and genome-wide data recovered from human skeletal remains from the site of Nevalı Çori. We also report human genome-wide data from post-Neolithic Nevalı Çori and the late PPNB site of Ba'ja in the Southern Levant. Our combined isotope and ancient DNA data fill a research gap between prehistoric Anatolian and Levantine populations. Our results indicate a decline in human mobility after the first phase of the PPNB in the Southeastern Anatolia accompanied by increasing reliance on domesticated resources and evidence of consanguinity in the PPNB Levant.

The classic model of "Neolithization" argues that there was a dramatic shift between mobile hunting and gathering to an increasingly sedentary herder/cultivator-based lifestyle as a result of growing economic reliance on animal and plant domestication during the Pre-Pottery Neolithic (PPN) in Southwestern Asia (1–4). Although the "Fertile Crescent" (FC) has often been seen as a vital region of early pathways to "agriculture," this process was heavily dependent on the distribution of the wild progenitors of domesticates, like wheat, barley, pulses, goat, sheep, pig, and cattle (5–12) in the FC (13), including the Levantine corridor, Southeastern (SE) Anatolia (which makes up a significant part of Upper Mesopotamia), and Zagros region, located at the western wing, the northeastern fringe, and the eastern wing of the FC, respectively. Nevertheless, despite decades of research into the "origins of agriculture," direct insights into the complex mechanisms underlying Neolithization, especially the pace, patterns, and the relationship between sedentism and the integration of hunting-gathering and agricultural lifeways, are still insufficiently understood (2, 14, 15). In particular, the correlation between sedentism and the adoption of agriculture has been hotly debated, especially in light of almost-sedentary Levantine Natufian hunter-gatherer communities (16–24).

Besides individual mobility during a lifetime, the importance of larger migrations during the process of Neolithization is also under debate. Early from the Pre-Pottery Neolithic A (PPNA) to the Pre-Pottery Neolithic B (PPNB) stages, it is marked by evidence for long-distance interactions throughout the wider region of the FC, where the presence of exotic objects, like obsidian, minerals, and lithic raw materials (25–27), shared ritual/cultural practices (28, 29) and feasting (30–33) increased. Ba'ja (ca. 7250 to 6800 cal. BC) in the late PPNB Southern Levant is a typical site of the "mega-site phenomenon," i.e., the sudden aggregation of population, enlargement of settlement size, and increased social differentiation, which has been explained under different models (34). One of these argues that domesticated species diffused as "packages" from their agricultural "homeland" in SE Anatolia (35, 36). However, there is also evidence indicating that the late PPNB Southern Levantine plant production was based on the cultivation of crops with a long history of local management (37). How these cultural similarities and the modes of exchange relate to human heritage and population connectedness between SE Anatolia

Author contributions: X.W. and P.W.S. designed research; X.W., E.S., J.I., M.L., P.L.R., and M.S. performed research; M.B., J.G., J.P., and N.P. contributed new reagents/analytic tools; X.W., E.S., M.M., H.R., J.K., P.R., and P.W.S. analyzed data; M.B., J.G., J.P., N.P., and M.S. contributed samples; and X.W., E.S., P.R., and P.W.S. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2210611120/-/DCSupplemental.

Published January 17, 2023.

and the Levant are still unclear (38–40) with indirect diffusion of ideas or direct interaction like larger migrations and individual mobility associated with trade, marriage, and other factors, all being proposed to explain the interplay between the different regions of the FC (3).

During the PPNA and PPNB, an enigmatic type of site with T-shaped pillars (TSP) emerged and flourished in SE Anatolia and has been seen as an iconic part of the early Neolithization process. The TSP themselves are believed to signify humans, with low reliefs representing head, arms, and clothing like belts and loincloth, and are often decorated with a variety of animal motifs, including snakes, scorpions, aurochs, and gazelles, and geometric patterns in low and high relief (41). Nevalı Çori is one of the key representative sites of the TSP society, the excavation of which not only documented the earliest occurrence to date of domesticated einkorn (42, 43) but also recovered a large amount of animal bones living in wooded habitats (*Bos, Sus, Cervus*, etc.) and open landscapes (*Ovis, Capra, Gazella*, etc.) (11, 44–46). Excavations at Nevalı Çori unearthed a series of five PPNB layers (47–51), i.e., phases I–V, with phase I being the oldest, based on its flint industry and architectural remains (47–51). In close proximity to Nevalı Çori (within a radius of ca. 60 km), the site of Göbekli Tepe is famously believed to have been the ritual center of this TSP society, many sites among which remain unexcavated or buried under the alluvium of the Harran Plain (52, 53). The younger layer (layer II) of the early and middle PPNB architectural phases at Göbekli Tepe is partially contemporaneous with the occupation of the earlier phase at Nevalı Çori for ca. 300 y, and both are characterized by smaller pillars (<2 m, compared with those of PPNA at Göbekli Tepe), rectangular stone buildings, and terrazzo floors (50, 54). The potential relatedness and dynamic interactions between these two sites, and the wider social landscape, require further investigation, however.

The social organization and subsistence strategies of the TSP society are key to better understanding the cultural transformations and the interplay of forager lifeways with the initial stages of agriculture. There has been so much work on subsistence in the FC given the focus on agricultural origins but much less on direct insights into mobility. Nevertheless, modes and degrees of human mobility strongly influence their cultural and social organization and have been central to arguments of agricultural dispersal across the regions (6, 55–58). However, the identification of human mobility on the basis of material culture has posed severe challenges (59). Multidisciplinary bioarchaeological approaches, especially archaeogenetic and isotopic analyses of ^{87}Sr/^{86}Sr and δ^{18}O, have increasingly served as powerful tools for investigating past mobility (60–65). The only available strontium isotope dataset for reference in SE Anatolia so far was generated for Körtik Tepe in the Upper Tigris region of the PPNA period (66), and bioarchaeological datasets aiming to investigate human mobility (Sr isotopic and archaeogenetic data) are missing for the TSP society in the Upper Euphrates. Furthermore, aDNA studies have documented the progressive reduction in genetic differentiation between populations from the Levant, Northwestern–Southcentral Anatolia, and as far as Zagros since the Neolithic, thereby shedding light on genetic admixture of a broad spatiotemporal scale within the FC (67–70). Geographically located among these regions, SE Anatolia is the critical missing link into further elucidating mobility patterns since the earliest phases of Neolithization.

In order to overcome this lack of data, we have conducted an integrative bioarchaeological analysis of human and animal remains from one of the key sites of the earliest Neolithic, i.e., Nevalı Çori. We report ^{87}Sr/^{86}Sr, δ^{18}O and $\delta^{13}C_{carb}$ data from 44 molar enamel samples belonging to 28 human individuals excavated from subphases PPNB I (ca. 8700 to 8300 BC), PPNB II (ca. 8300 to 7900 BC) and PPNB III (ca. 7900 to 7500 BC) and later periods. The site was reoccupied during the Halaf Culture (ca. 5500 to 5000 BC), Early Bronze Age I (EBA I, ca. 2900 to 2800 BC), the Iron Age (IA, ca. 1200 to 30 BC), and finally the Roman Imperial period (RI, ca. first to third century CE) (71) based on both archived and newly reported 14C data of bone and cereal materials recovered in situ (Dataset S4). Their distribution ranges from the PPNB to the RI occupation layers as follows: 23 samples (n = 13 individuals) dating to the early (I), middle (II), and late (III) layers of the PPNB, three samples (n = 2 individuals) from the period of Halaf Culture, nine samples (n = 6 individuals) from the EBA I, eight samples (n = 5 individuals) from the IA, and two samples (n = 2 individuals) from the RI. Additionally, we conducted strontium, carbon, and oxygen isotope measurements on 29 animal samples, most of which are gazelles, sheep/goats, and pigs recovered from PPNB Nevalı Çori (Dataset S3), aiming to identify a baseline for the local bioavailable strontium and to explore possible changes in subsistence strategies. We also developed human genome-wide data from PPNB Nevalı Çori and Ba'ja to explore evidence for transregional population mobility in the landscape of polycentric development of Near Eastern cultivation and domestication (27, 37). After sampling 35 individuals from Nevalı Çori and 24 from Ba'ja (archaeological background is provided in *SI Appendix*, Note S1 and Fig. S1), genome-wide data were successfully recovered from three PPNB individuals (NEV009, BAJ020, and BAJ022) due to the challenging preservation conditions. Supraregional comparisons of population genetics from these two sites with other early groups across the Near East have been conducted to achieve more insights into connections within the northwest wing of the FC and, more broadly, within the whole of Southwestern Asia. The detailed contextual and osteological information, as well as the shotgun metagenomic sequencing evaluation of all sampled individuals, are presented in Datasets S1 and S5.

Results

Strontium Isotope Analysis of Human Remains. In Figs. 1*B* and 2*A*, the range between the solid lines shows the bioavailable local strontium signature of Nevalı Çori [0.707818 to 0.708052 (mean ± 2 SD)] calculated based on the ^{87}Sr/^{86}Sr ratios of pigs (n = 6) and fox (n = 1) as archaeological fauna enamel expected to provide a more local range (Dataset S2). The resulting range is compatible with the geological background in the Urfa region, where limestone bedrock provides a significant contribution to strontium isotopic composition (*SI Appendix*, Note S2). We define any data falling outside the Nevalı Çori local baseline as "nonlocal" values. However, what this means in terms of cultural significance can vary. For instance, for people who are not completely sedentary, a nonlocal ^{87}Sr/^{86}Sr ratio could reflect use of a broader home range area across different geological contexts. During the subphase PPNB I, 6 out of 7 individuals showed nonlocal values, while only 1 out of 6 individuals is nonlocal during PPNB II–III, and none of the subsequent periods (from the Halaf Culture to the RI) provide nonlocal signatures. In order to contextualize the Nevalı Çori results within the wider region, a local range of bioavailable ^{87}Sr/^{86}Sr at Göbekli Tepe, drawn from the published values of Lang et al. (72) determined from gazelle bones and enamel of 0.708025 to 0.708255 (mean values ± SD), was used. As wild animals (73), the values of gazelles, which have never been domesticated, tend to be more dispersed, and we therefore used ±1 SD instead of ±2 SD, which has also been applied in other

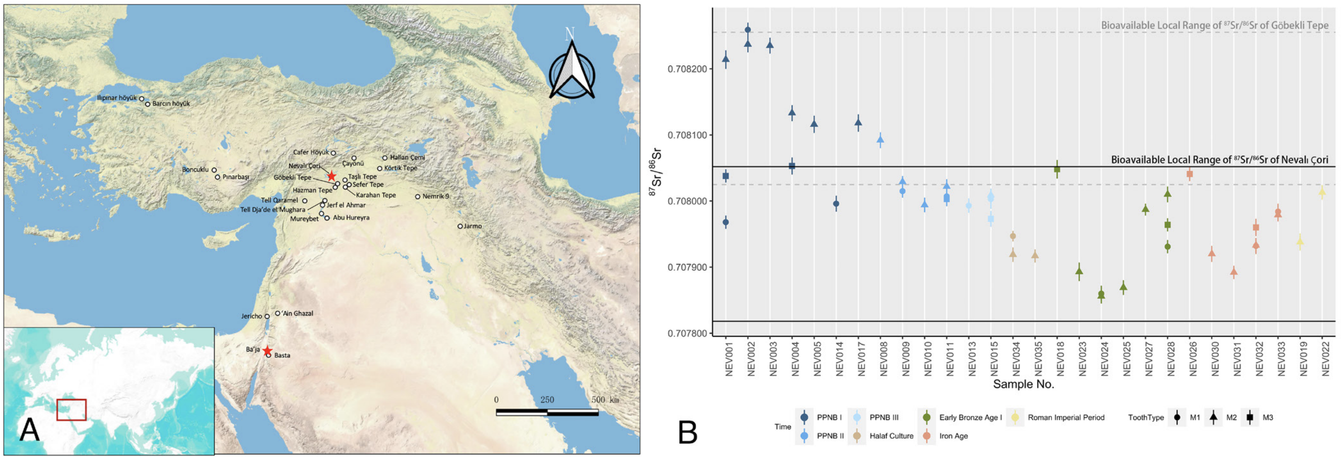

Fig. 1. *A*) Map of the prehistoric FC and Anatolia, with relevant sites in the text. *B*) Scatterplot of the $^{87}Sr/^{86}Sr$ ratios of the individuals from Nevalı Çori (Dataset S1); the range between the gray dashed lines shows the bioavailable $^{87}Sr/^{86}Sr$ interval based on the data from gazelles excavated at Göbekli Tepe; the range between the dark solid lines shows the bioavailable $^{87}Sr/^{86}Sr$ interval of Nevalı Çori based on the data of the archaeological animal remains measured in this study (Dataset S2).

studies, including the original publication of these gazelles (72), for a more conservative local range (64, 74).

Our $^{87}Sr/^{86}Sr$ ratios for human enamel samples from Nevalı Çori ranged from 0.707856 to 0.708259 (n = 44). An obvious decrease in mobility among the Nevalı Çori inhabitants occurred at the transition from PPNB I to II, as evident from the numbers of nonlocals in each period. Among the nonlocals from PPNB I, NEV001 shows the largest variation in the $^{87}Sr/^{86}Sr$ ratios, with the lowest value measured in the M1, which indicates that the individual's mother spent her pregnancy and breastfeeding period in a geological location compatible with Nevalı Çori. Meanwhile, this individual had a nonlocal value for the M2 and a local value for the M3 (Fig. 1*B* and Dataset S1). Similarly, NEV004 exhibits intraindividual variations in $^{87}Sr/^{86}Sr$ ratios also with M2 being nonlocal and M3 being local but M1 missing. Both NEV001 and NEV004 indicate a similar pattern of mobility: Both individuals seem to have been active around the Göbekli Tepe region during their later childhood (ca. 3 to 7 y old; *SI Appendix*, Note S5.2.2) and returned to Nevalı Çori later in their lifetimes (ca. 8 to 14 y old). Moreover, the $^{87}Sr/^{86}Sr$ ratios of the M1 and M2 of NEV002 are both highly consistent with the range of the Göbekli Tepe region, with the fact that NEV002 died between the ages of 12 to 15 y (Dataset S1), indicating that this individual was born outside Nevalı Çori and had lived beyond this area, although there is no indication from or differentiation in burial practices that would distinguish it from other contemporary individuals.

Stable Isotope Analyses of Human Remains. All tooth enamel samples measured for $^{87}Sr/^{86}Sr$ were also analyzed for $\delta^{13}C_{carb}$ and $\delta^{18}O_{carb}$. Forty-four matching $\delta^{13}C_{carb}$ and $\delta^{18}O_{carb}$ values were generated from all the 28 individuals ranging in periods from the PPNB to the RI. The $\delta^{13}C_{carb}$ values all fall into a range between −14‰ and −11‰ (Dataset S1 and *SI Appendix*, Fig. S2), which indicates an overall reliance on C_3 resources without significant diachronic variation. This fits well with archaeobotanical evidence for the dominant crops at the site being wheat (*Triticum* spp.) and pulses (e.g., *Lens* and *Pisum*) (42), as well as with the natural vegetation, highlighting that the graze and browse available for domesticated animals would have been made up primarily of C_3 plants, with the use of forested habitats perhaps also indicated by the relatively low values (75). Our results agree with the previously published $\delta^{13}C$ data of collagen material (76) that the people at Nevalı Çori lived on a heavily vegetarian diet based on C_3 plants. The newly published modeling research (77) quantified the plant biomass contribution as being as high as up to 90% in some individuals (average 87%). This overall reliance on plants may help to explain the close apparent linkage between growing reliance on domesticated resources and a reduction in mobility following the early PPNB phase.

The $\delta^{18}O_{carb}$ values of the human samples vary widely from −7.3‰ to −1.7‰, with values becoming higher over time. Twenty-one previously published $\delta^{18}O_{carb}$ values from Nevalı Çori (78) range from ca. −8.6‰ to −6.4‰, except for an extremely high outlier at the value of −5.55‰. The latter dataset therefore partially overlaps with the new data, agreeing with those from the PPNB I subphase in this study (−7.3‰ to −4.3‰), although they are, overall, lower. This was probably due to the limited temporal coverage and the dates of the previous sampling, which seem to have concentrated on the very early PPNB, although specific dating was not provided. For the $\delta^{18}O$ data presented in this study, we applied ANOVA and the Tukey Honestly Significant Difference (HSD) test, which indicated that both PPNB I and II values differ from those of the post-PPNB individuals, especially for PPNB I, of which the measured difference with EBA I is −3.9‰ (CI: −6.2, −1.7), with the Halaf Culture is −5‰ (CI: −8.2, −1.9), with the IA is −4.8‰ (CI: −7.1, −2.5), and with the RI is 4.6‰ (CI: 0.9, 8.3). However, no clear distinction is demonstrated between PPNB III individuals and their post-PPNB counterparts, and no isotopic meaningful difference can be inferred from individuals among the PPNB subphases (*SI Appendix*, Fig. S4 and Dataset S9). The change in $\delta^{18}O$ values over time could be related to different factors working together, like climate change or anthropogenic changes—e.g., mobility patterns or drinking water sources from different altitudes. However, the increase in the newly reported $\delta^{18}O$ values from the PPNB to later periods is consistent with Holocene $\delta^{18}O$ increases documented in paleoenvironmental records in the Eastern Mediterranean area, including the speleothem profiles from the Incesu Cave in Southcentral Anatolia, Turkey (79), and from the Soreq Cave in the Southern Levant (80), and of the stalagmites from the Sofular Cave in Northern Turkey (81, 82). The $\delta^{18}O$ ratios of the body water of large mammal and human enamel are systematically related to the isotope values of drinking water ($\delta^{18}O_{dw}$), with $\delta^{18}O_{precip}$ being calculated from $\delta^{18}O_{carb}$ (with an intermediate step of conversion to $\delta^{18}O_{phosphate}$ values using a series of equations) (83–85) (*SI Appendix*, Note S5.1.1). On a

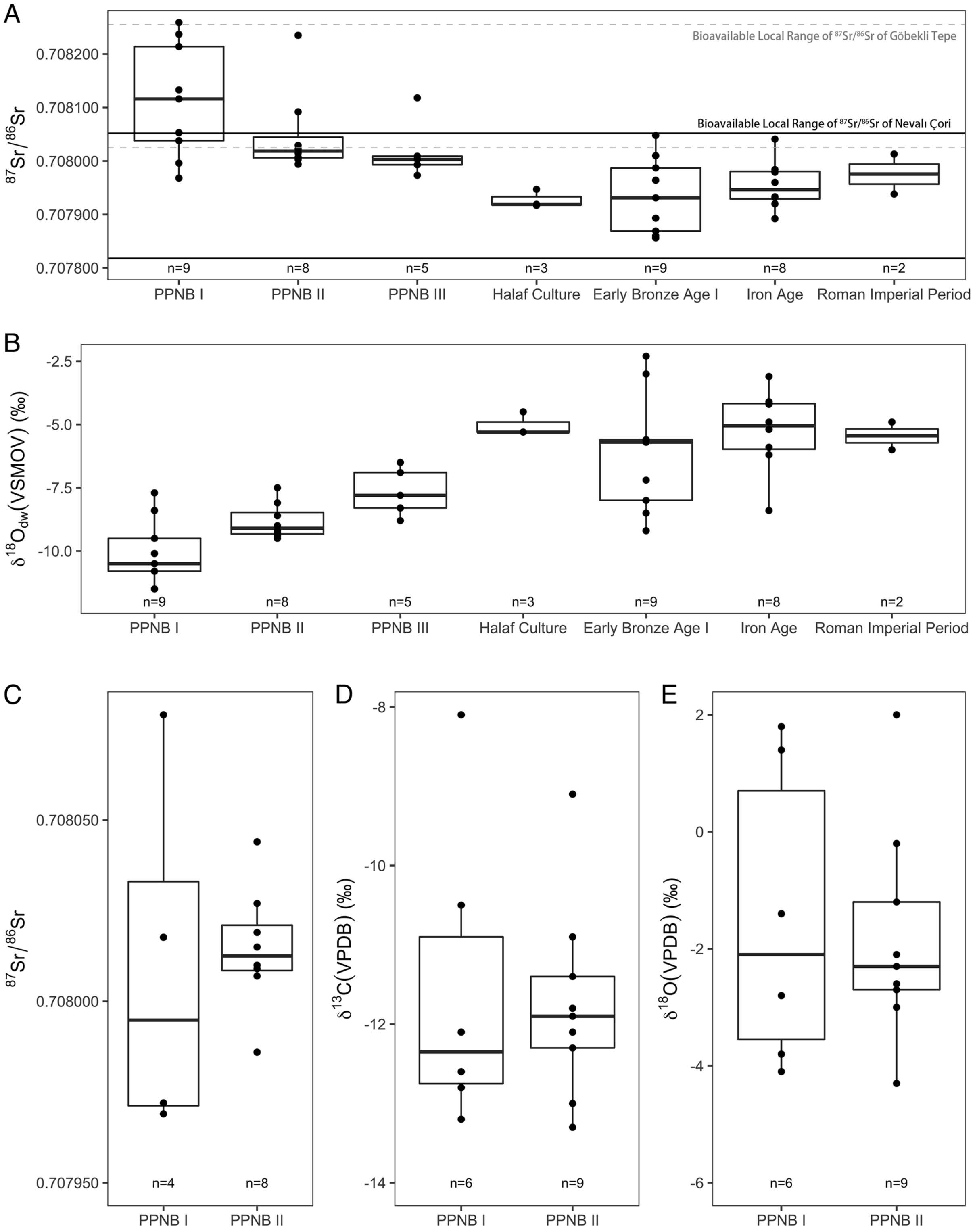

Fig. 2. *A*) Box plot of the $^{87}Sr/^{86}Sr$ ratios of the individuals from Nevalı Çori; the range between the gray dashed lines presents the bioavailable $^{87}Sr/^{86}Sr$ interval based on the data from gazelles excavated at Göbekli Tepe, and the range between the dark solid lines shows the bioavailable $^{87}Sr/^{86}Sr$ interval of Nevalı Çori. *B*) Box plot of $\delta^{18}O_{dw}$ (drinking water) values of the Nevalı Çori human samples grouped in different periods, spanning from the PPNB to the RI; VSMOW, Vienna standard mean ocean water. *C*) The $^{87}Sr/^{86}Sr$ ratios of the gazelles from Nevalı Çori grouped into PPNB I and II. *D*) $\delta^{13}C_{carb}$ values of the gazelles from Nevali Çori grouped into PPNB I and II. *E*) $\delta^{18}O_{carb}$ values of the gazelles from Nevalı Çori grouped into PPNB I and II.

larger temporal scale, there seems to be a warming trend in this region, which has gradually become closer to the modern environment, as represented by the annual mean precipitation $\delta^{18}O$ values ranging from −8‰ to −6‰ that generally agree with the $\delta^{18}O_{dw}$ values from post-PPNB (Fig. 2*B* and *SI Appendix*, Fig. S3) (86, 87).

Isotope Analyses of the PPNB Animals. First, as mentioned above, we primarily used pigs and fox to represent the local $^{87}Sr/^{86}Sr$ baselines in our study on the ecological understanding that these animals tend to be closely integrated with settlements at this time based on zooarchaeological identification (11, 12). Second, 26 matching $\delta^{18}O$ and $\delta^{13}C_{carb}$ data and 28 $^{87}Sr/^{86}Sr$ data were newly generated from 29 animal individuals recovered from the different PPNB subphases of Nevalı Çori (Dataset S3). In contrast to the above taxa (pig and fox), we use gazelle as a better marker of human use of the wider landscape and hunting. The scatterplot of the mammalian $\delta^{18}O$ and $\delta^{13}C_{carb}$ data (*SI Appendix*, Fig. S5 and Dataset S3) reveals that NC0289 and NC0518 (both gazelles) had C_4 food intake. Evidence for the widespread distribution of C_4 plants throughout temperate Eurasia is extremely low (75), and C_3 resources were dominant in the local vegetation (75). However, notable cover of C_4 plants in communities of sand dunes, salt marshes, and disturbed sandy ground of warm regions were found. Mediterranean, Irano-Turanian, and Hyrcanian ecosystems with high summer temperature, intensive light, nutrient-poor soils, and dry conditions are suitable habitats for C_4 plants (88–90). Therefore, there would have likely been C_4 food resources available for gazelles moving widely across the arid regions in Anatolia and the Levant.

The lack of a significant C_4 signal among the human individuals in this study is likely due to either the low frequency of C_4-consuming individuals among the whole gazelle population or a limited consumption of gazelles overall due to seasonal aspects of grazing and hunting. It is also possible that the hunted animals were only used for special feasts/occasions at specific times, especially in light of the fact that the inhabitants at Nevalı Çori depended largely on a plant-based diet as mentioned above (77, 78). It is worth noting that the box plot of the Nevalı Çori gazelles, clustered by stratigraphic level, shows that variation in PPNB II is lower than that in PPNB I for all isotopes (Dataset S3 and Fig. 2 *C*–*E*), which potentially indicates a shrinking of the meat resource catchment area from PPNB I to II. Additionally, $^{87}Sr/^{86}Sr$ data of the gazelles from Nevalı Çori are distinct from the PPNA and early–middle PPNB Göbekli Tepe (*SI Appendix*, Fig. S6), which show higher averages and, in general, greater variability. However, we should caution that variability cannot necessarily be directly compared between these two limestone-based sites due to local contingencies in geology. These two facts together possibly indicate declining mobility of hunters from the PPNA to PPNB along with a weakening of the dependence on a hunting-gathering economy over time.

Genetic Analysis of Human Remains. All the six genetically analyzed individuals passed quality control, including low contamination rates and ca. 0.25× coverage on 1,240K markers (≥300,000 SNPs) (SNP, Single Nucleotide Polymorphism), except one low-coverage sample BAJ020 (ca. 47,000 SNPs). A complete summary of quality assessment of the data, along with information on molecular sexing and uniparental haplogroups, is provided in Datasets S5 and S6.

To get an overview of the population genetic structure, we first computed a principal component analysis (PCA) using SNP array data (Human Origins, ca. 600,000 SNPs) from 85 modern western Eurasian populations. We then projected ancient individuals from this and relevant other studies onto the first two principal components (Fig. 3). Notably, the PPNB individuals from Ba'ja and Nevalı Çori (level II; NEV009) are separated along the PC2 and PC1. The former fall within the cluster formed by PPN individuals from the Southern Levant and one published sample from Ba'ja (BAJ001) (67), whereas NEV009 is placed along the PC1 between

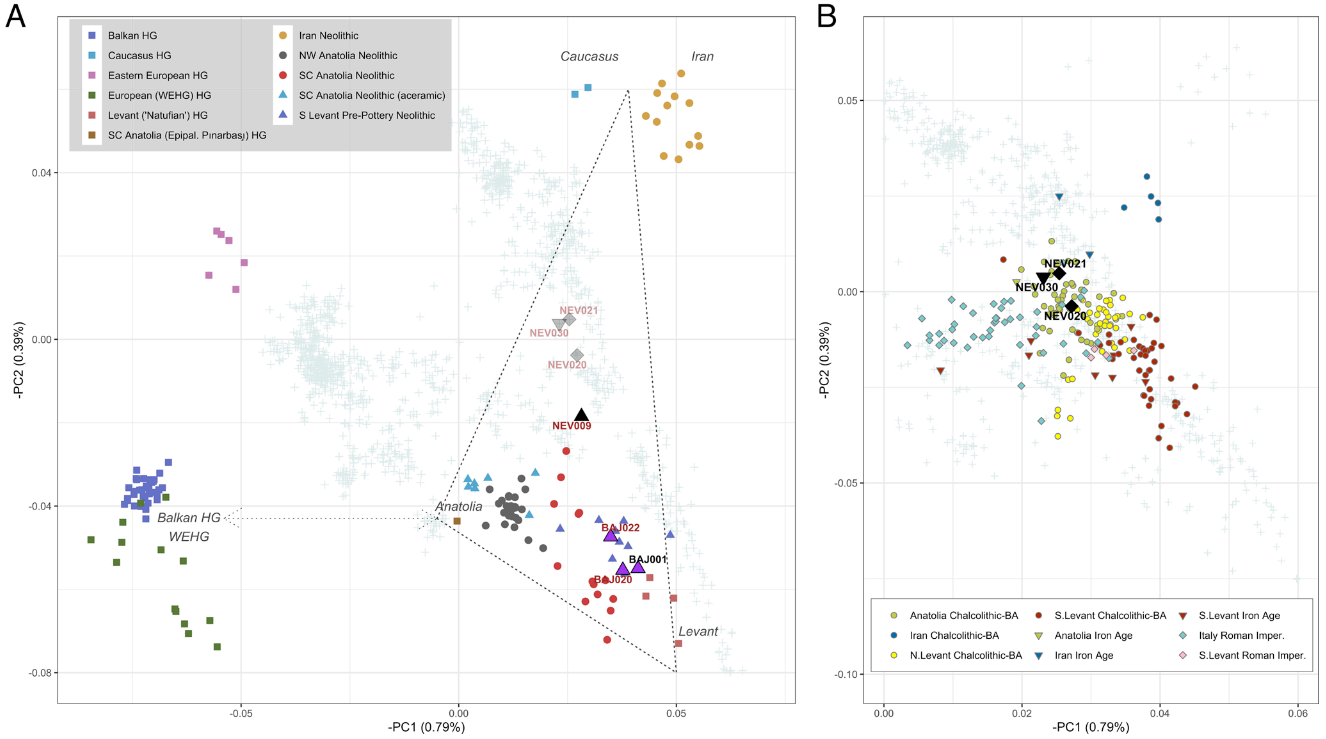

Fig. 3. Scatterplot of the PC1 and PC2 generated with *smartpca* on Human Origins (HO) data of 85 modern West Eurasian populations (light gray cross symbols). Ancient datasets (colorful points) were projected with the *lsqproject* option. *A*) Coordinates of the PPNB Nevalı Çori (NEV) and the Ba'ja (BAJ) individuals (black and purple upward triangles, respectively) with their IDs. The drawn polygon outlines the Early Holocene individuals from southwestern Asia, and the double arrow represents the more distal genetic link between the Anatolian HG and European HG. For reference, the IA and RI Nevalı Çori individuals are plotted in faded colors. *B*) The three later Nevalı Çori individuals are plotted along with post-Chalcolithic Southwest Asia and Rome from the Imperial Period. Abbreviations: HG = hunter-gatherers, NW = Northwestern, S = Southern, SC = Southcentral.

the cluster of Anatolian hunter-gatherers (HG) and PPN farmers and the Levantine cluster of PPN and Epipaleolithic (EP) individuals. However, NEV009 can be distinguished from these two groups along the PC2, being shifted toward the direction of the Early Holocene Iranian (Neolithic Iran; "Iran N") and Caucasian HG ("CHG") individuals. The intermediate coordinates between the Early Holocene Anatolia, the Southern Levant, and Iran/ Caucasus suggests varying genetic affinity with these populations. However, a higher genetic similarity can be proposed based on the distances between all the Neolithic individuals from within the Anatolia (including NEV009) and the Levant compared with Iran.

Almost eight millennia later, the three Nevalı Çori individuals dated to the IA and RI carry a distinct ancestry profile in contrast to the earlier inhabitants (68). Consistent with broad-scale post-Neolithic population mixing across the FC and the Caucasus (91, 92), all three individuals cluster with the Chalcolithic and Bronze Age Anatolian individuals. Interestingly, some other individuals from Rome during the RI overlap with the two Nevalı Çori RI. These new data indicate genetic continuity from the Bronze Age through the Iron Age and the Roman Imperial period and further corroborate the argument that people of the Anatolian origin contributed to the population of ancient Rome (93).

To gain further insights into ancestry patterns identified via our PCA, we tested several scenarios of asymmetric allele sharing. To delineate the evolutionary relationship between PPNB Nevalı Çori and Ba'ja and other Neolithic individuals, D-statistics of the form (outgroup, test; pop 3 and pop 4) were calculated (Fig. 4). As a four-population test, a D-statistic measures the excess of allele sharing between test and either pop 3 or pop 4, in which cases the statistic is expected to be negative or positive, respectively. For this analysis and the admixture modeling, the individuals were grouped and/or labeled by the site and period. Besides BAJ020 and BAJ022, the group "Ba'ja PPNB" comprised the previously published individual BAJ001. Collectively, Ba'ja PPNB exhibits evidence of gene flow from populations related to Anatolia when compared with the "Natufian" HG. This also applies to the PPN individuals from ʻAin Ghazal further to the north in Jordan. Furthermore, the genetic affinity with Anatolia might be stronger in ʻAin Ghazal than Ba'ja, although not significantly [Z = 1.3 for D (Mbuti, Anatolia Barcın–Menteşe N; ʻAin Ghazal PPN, Ba'ja PPNB)].

Nevalı Çori PPNB (NEV009) shares significantly more alleles with the Anatolian gene pool than the Levantine populations do, which indicates a common ancestry among the Anatolian populations. However, when we contrast NEV009 with the Anatolian populations, significant differences can be captured for test being western European HG (WEHG) and the Balkan HG (Z ≤ –6.5) (Fig. 4*A*). These test populations broadly represent a post–Last Glacial Maximum lineage that prevailed in Europe and had a higher affinity to the Anatolian hunter-gatherer from Pınarbaşı and present-day Near Easterners (67, 94). Consistently, NEV009 also shares less alleles with Pınarbaşı compared with the other Anatolian farmers, indicating a less direct genetic link to this ancestral Anatolian population. On the other direction, a trend for increased affinity of NEV009 with the Early Holocene Iranian/ Caucasian and Levantine can be drawn, especially when NEV009 is contrasted with Pınarbaşı—but stands below the significance threshold.

To address how the various signals from the D-statistics can be fitted under certain admixture models, we run qpAdm exploring

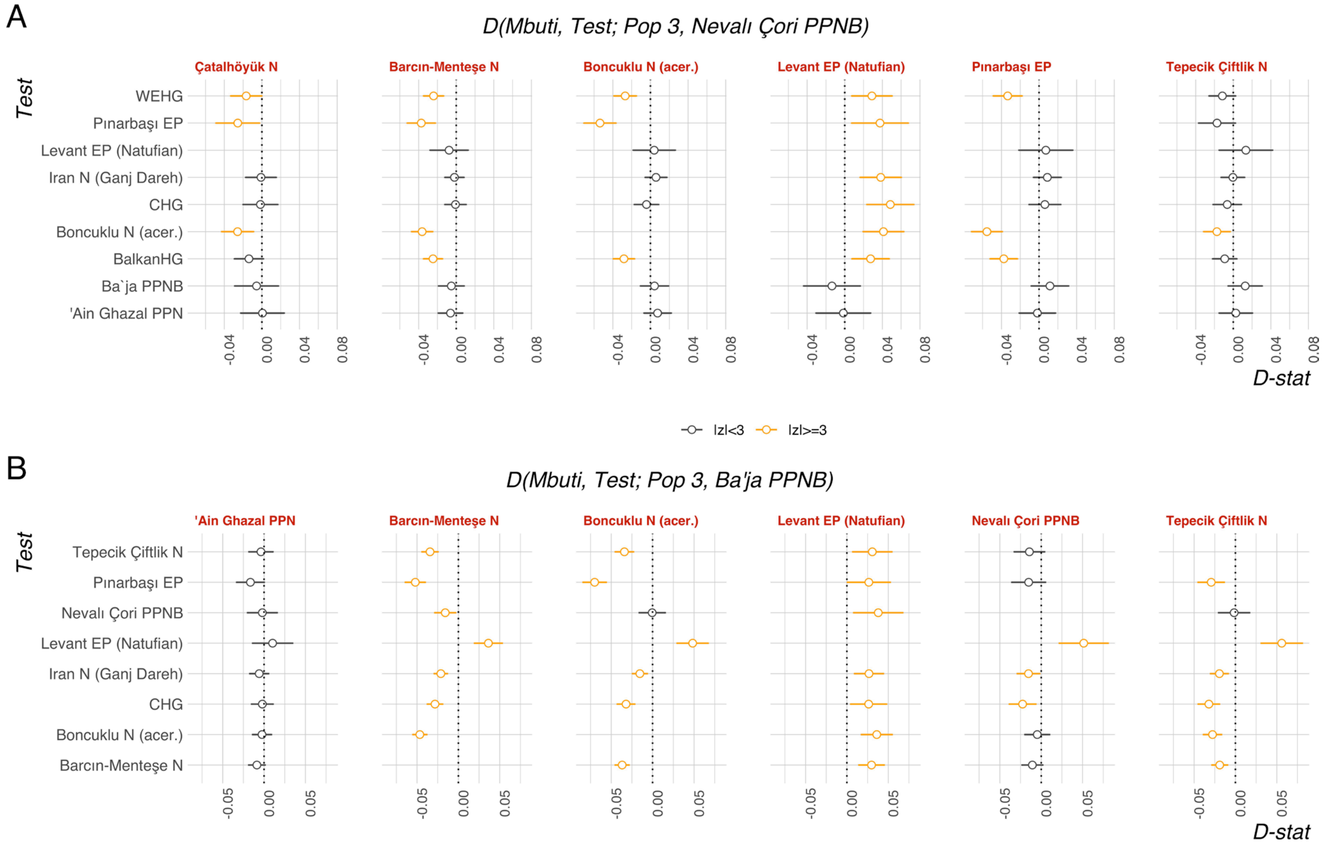

Fig. 4. D-statistics for allele-sharing differences between the Early Holocene Southwest Asian and European populations and *A*) Nevalı Çori PPNB (NEV009) or *B*) Ba'ja PPNB. Significant statistics (|Z| ≥ 3) are annotated in orange, and error bars are plotted as ±3 SE. For both groups, results are presented by pop 3 (columns). EP = Epipaleolithic, PPN = Pre-Pottery Neolithic, N = Neolithic, HG = hunter-gatherers, CHG = Caucasus HG, and WEHG = European (western) HG.

combinations from genetically and geographically distant sources within the FC (distal modeling) (*SI Appendix*, Fig. S7 and Dataset S7). To increase the resolution of the analysis, populations such as WEHG, CHG, and Levant EP ("Natufian") were added to the set of reference populations, which serve as a scaffold to relate the target with the source populations (*Materials and Methods*). In agreement with previous results, the later seventh millennium BC Anatolian farming communities require additional ancestry related to the 'Ain Ghazal Levant (i.e., Barcın–Menteşe) or Ganj Dareh Iran (i.e., Tepecik Çiftlik) compared with Pınarbaşı HG and the PPN farmers from Boncuklu. In stark contrast, Nevalı Çori cannot be modeled as a two-way combination from Pınarbaşı HG and either Iran N or Levant PPN. Instead, all three sources are necessary. Notably, this three-way admixture model becomes adequate only when CHG is removed from the reference populations, which can be explained by the real source of the Iranian-related source being linked to CHG-like populations as well, which agrees with the suggestion that CHG and Ganj Dareh Neolithic are interchangeable as sources of inland admixture for most cases (95). Furthermore, the high Levantine coefficient (45 ± 15%)—in spite of the lack of significant D-statistics for excessive affinity with the Levant—might result from a lower resolution owing to the low sample size of Nevalı Çori. Therefore, we take a qualitative rather than a strictly quantitative interpretation of the qpAdm model.

To gain insights into parental relatedness, we inferred runs of homozygosity (ROH) using the software hapROH (96). We analyzed five individuals from Ba'ja and Nevalı Çori spanning from the PPNB to the RI periods, with as low as ca. 25% coverage on 1,240K SNPs (Fig. 5). Populations having reduced effective population sizes exhibit a higher frequency of short ROH due to background relatedness (97). PPN famers from Boncuklu in Southcentral Anatolia and some early farmers from Iran have a higher proportion of short ROH equivalent to small mating pools in contrast to the seventh millennium BC farmers from -Northwestern Anatolia, a pattern already previously observed (96, 98). Similarly, high levels of short-range ROH [4 to 8 centimorgan (cM)] are observed within PPN Levant including the previously published individual from Ba'ja (BAJ001). On the contrary, the PPNB individual NEV009 exhibits lower levels of short ROH, which suggests larger population sizes on average, comparable with those later Anatolian farmers. Interestingly, in total, 3/5 individuals exhibit long-range ROH (≥20 cM), with the oldest being PPNB BAJ022 with an overall ROH distribution equivalent to offspring of a close-kin union (e.g., parents being first or second cousins). A similar conclusion can be drawn for the IA NEV030. However, the most striking evidence for consanguinity comes from the IA individual NEV020 whose ROH length distribution matches the parental scenario of full siblings (*SI Appendix*, Fig. S8).

Discussion

Foundation of the TSP Horizon in the Final PPNA. With the rapid amelioration of the climate after the Younger Dryas, an increase in population and settlements took place in the Tigris Basin and Upper Euphrates, also correlated with simultaneous changes in human behavior and productive subsistence strategies (87, 100, 101). Subsequently, wider settlement distributions evidently declined, while at the same time, occupation in the Urfa region increased as embodied by the appearance of the TSP sites (102). This change in settlement patterns has been interpreted as a "Late PPNA Hunter-Crisis" (102–105) corresponding to a rapid climate change interval indicated by a spike in the GISP2 potassium data (106, 107), which heralded the rise of the TSP communities. From the PPNA to early PPNB, Göbekli Tepe was a ritual center in the Urfa region characterized by its magnificent monolithic

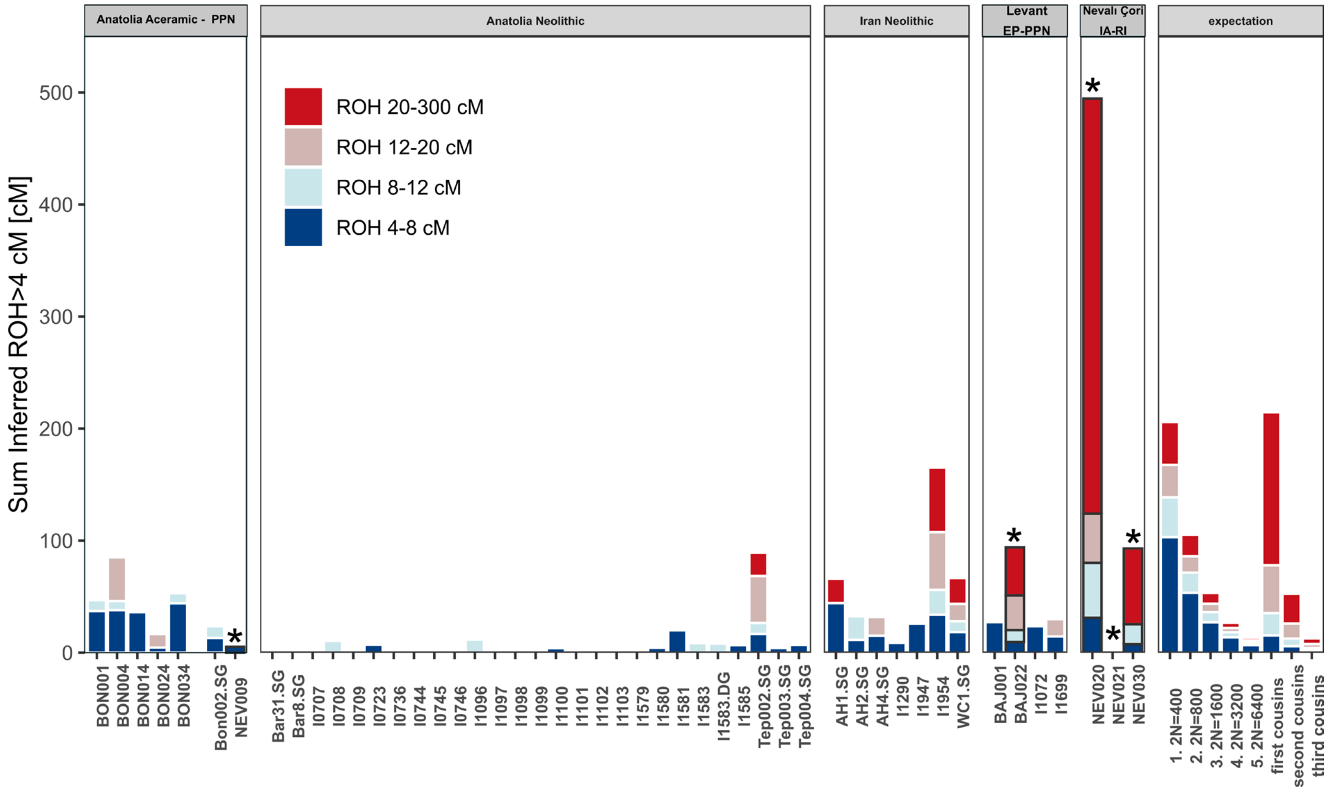

Fig. 5. Inference of Runs Of Homozygosity (ROH) from 1240K data using hapROH (99). The ROH distribution in the individuals of the present study (NEV009, NEV020, NEV021, NEV030, and BAJ022) and the published individuals from other Southwest Asian EP and Neolithic contexts.

structures. The hypothesized large events held in the TSP buildings might have been part of routine gathering and feasting events that the surrounding communities participated in (33). Göbekli Tepe is not the only place in the Urfa region where the geological context consists of a mixture of Eocene and Miocene limestones (*SI Appendix*, Note S2), the signature of which can be distinguished from that of Nevalı Çori. The other sites of the TSP are of similar geological context as Göbekli Tepe. Therefore, "Göbekli Tepe" here refers not only to the site itself but also to the TSP society as a whole, which covers the interactive sphere in the Urfa region from the PPNA to PPNB, including Karahan Tepe, Sefer Tepe, Harbetsuvan Tepesi, and so on (Fig. 1*A*) (102, 108). Taking the region represented by the bioavailable strontium signature of Göbekli Tepe into account, prior to this point, the interactive sphere of PPNB Nevalı Çori seems to have extended across a larger area. On top of that, the fact that the nonlocal ^{87}Sr/^{86}Sr ratios of Nevalı Çori humans during PPNB I match the ^{87}Sr/^{86}Sr signature of a broader area in the Urfa region where the other TSP sites located is very likely to be the consequence of mixed strontium intake across the whole area where TSP people were active by means of frequent interaction and networks. This supports the hypothesis that the establishment of the TSP ritual system was part of a demand for unifying and organizing the people in the Upper Euphrates to engage in concerted hunting events and other cultural practices (102, 109–112). In turn, these collective activities further consolidate the ritual system, and social stability was strengthened, with more connections being built among more stable communities in the area.

A trans-FC comparison of the populations at a genetic level is also now possible, given the credit that Nevalı Çori fills the missing connection within the northwest wing of the FC. Individual NEV009 was shown to not only descend from the Early Holocene populations like those from the Southcentral Anatolia but also traced part of her ancestry to populations in the southern and eastern wings of the FC (i.e., Levant and Iran). Compared with recent genomic data from individuals at the northeast wing of the FC (Mardin, Nemrik 9, Shanidar, and Bestansur) (95), both Nevalı Çori and neighboring PPN Çayönü (113) are distinct since the presence of the Levantine-associated ancestry is not required to model the other eastern Mesopotamian sites. This pattern could reflect substructure within the FC owing to geography and could have been shaped even before the onset of the PPN. As more data are obtained, a consensus is reached, whereby gene flow after the HG in the PPN and the Neolithic populations from Anatolia cannot be explained under a uniform model with respect to the timing and its origin. When Nevalı Çori and the HG from Pınarbaşı were tested as source populations for the other PPN and Neolithic Anatolian sites, the model fit improved compared with the model with Neolithic Iran or Levant (instead of Nevalı Çori) only for the PPN Boncuklu. For the other groups (e.g., Barcın–Menteşe and Tepecik Çiftlik), the fit was less good (P value < 0.05, Dataset S7). Furthermore, in the Levant, both PPN 'Ain Ghazal and Ba'ja individuals saw an increase in their affinity to the Anatolian populations compared with the preceding population of "Natufians." Both the chronological alternations of Levantine and the Anatolian ancestral components indicated the increasingly intensive interaction network across the FC during the PPN periods. Considering the rich record of shared elements in the material culture, contacts between the Levant and SE Anatolia could have been established via bidirectional gene flow (1, 3), eventually reaching the farmers as north as Barcın and Menteşe in the Northwestern Anatolia, while such signatures were not traced in Southcentral Anatolia during the PPN period (Boncuklu site). Overall, these variegated connections in material culture and genetics point to different spheres of interactions between Anatolia, the Levant, and the Upper Mesopotamia. The genetic variability among populations from Mesopotamia suggests that the range from Southern to Eastern Anatolia and Northern Iraq contained different niches of blending ancestries that could have variably contributed to other populations in the Levant and the rest of Anatolia. Another genetic evidence, the ROH estimated on NEV009 and Çayönü individuals (113) indicate that these individuals belonged to a population of a larger effective population size compared with the coeval individuals from the Southern Levant, Western Iran, and Southcentral Anatolia. This corroborates different demographics in Upper Mesopotamia, which could have promoted long-range mobility resulting in many of the observed genetic signals in ancestry. Additional genetic data from the neighboring areas would be critical for investigating the overall dynamic transition that SE Anatolia experienced during the PPNB.

Disconnection and Collapse of the TSP Horizon. Our human and faunal ^{87}Sr/^{86}Sr from Nevalı Çori highlight the contrast between PPNB I and II, showing a dwindling mobility and hunting catchment, which might be interpreted as a transition in lifestyle, subsistence strategies, and social organization. Already published evidence suggests an increased reliance on cultivation at Nevalı Çori; wheats in all the PPNB layers are morphologically domestic (42, 114). Similarly, zooarchaeological research (10, 11, 115) has demonstrated that the biodiversity of faunal remains began to decrease toward the end of the ninth millennium cal. BC in SE Anatolia, which corresponds to the transition from PPNB I/II to III/IV at Nevalı Çori, showing a degradation of a broad-spectrum diet/economy and a gradual move away from the hunter-gatherer lifestyle. As the most common taxon recovered at Nevalı Çori, the fraction of gazelle in the assemblages of faunal remains decreased from PPNB I/II to IV/V, while the percentage of sheep and goats increased (11, 71). It is also noted that the proportion of female cattle at PPNB I/II Nevalı Çori was higher than that during the PPNA Göbekli Tepe (11, 115). *Ovis, Capra,* and *Sus* remains recovered from Nevalı Çori were, in part, from domestic caprines and pigs, which is indicated by diachronic changes in their frequencies during the site's occupation and their significantly smaller body sizes compared with their wild relatives at other sites (Göbekli Tepe, Cafer Höyük) in the region, and demographic profiles are consistent with early Neolithic husbandry systems rather than hunting regimes of foragers (116). Additional evidence for the domestic status of some *Ovis, Capra,* and *Sus* comes from the δ^{15}N analyses showing that the early stockkeepers of Nevalı Çori deliberately fed these animals with legumes (76).

In contrast to Nevalı Çori, there is no evidence for the development of agriculture at Göbekli Tepe during this key turning point in the PPNB, perhaps due to conflicting belief systems, e.g., that cultivation and domestication may have been taboo as they challenged the traditional hunting-gathering mentality or lifestyle—different societies sometimes may have radically different systems of value (117), or pressure from the environment and resources, e.g., water stress, which would restrict the development of agriculture without irrigation strategies, or simply used as different parts of the landscape and social system. An unstable animal-driven protein supply could have motivated people to practice a mixed subsistence pattern, with both hunting-gathering and farming acting as risk-buffering strategies, which might also suggest that the TSP ritual system did not collapse abruptly as reflected in the fact that the construction of monumental architecture continued at Nevalı Çori into PPNB II and III (71).

According to the strontium isotopic results, there are still some individuals (e.g., NEV008, NEV003, and NEV017) from post-PPNB I that fall into the $^{87}Sr/^{86}Sr$ range of Göbekli Tepe and other TSP communities, and these individuals may have continued to engage in the maintenance of the traditional belief system and social networks manifested by TSP, with other members at Nevalı Çori investing more in cultivation and domestication, becoming more sedentary.

Overall, however, the high mobility seen in PPNB I gradually declined at Nevalı Çori, with the proportion of hunting in the mixed subsistence model also declining until sedentary agriculture dominated, which marks the end of PPNB I as a key turning point. As subsistence farming increasingly overshadowed hunting and gathering, larger numbers of inhabitants could be fed within large, more sedentary communities. This perhaps further undermined ties among the TSP system, with Göbekli Tepe losing its prestige position as the main ritual center before becoming finally abandoned. The cult buildings were no longer prevalent at the end of PPNB Nevalı Çori, either (71). Meanwhile, the process of Neolithization led to a decentralized network among different socioeconomic communities. This brand-new social structure paved the way for the emergence of autonomous communities in the late Neolithic across a wider region (118–120). Another direction of research, that focuses on the familial relationships within communities (e.g., ref. 121), provides further insights into the inner-community organization during the late Neolithic in the Southern Levant. The presence of a consanguineous individual from Ba'ja—although too small a sample size to indicate a social practice—aligns with intrasite morphological analysis in the neighboring site of Basta in the late PPNB (7500 to 7000 cal. BC), which supports extensive social endogamy (122). If more genetic data in the future statistically support the fact that this endogamy phenomenon roots in a cultural preference, it would likely suggest that it was a way of strengthening the inner bonds within the relatively more autonomous and seclusive communities of the later Neolithic.

Conclusion

To contribute more insights into the dynamic process underlying the Neolithization across the FC, we have integrated bioarchaeological analyses on human and animal bones recovered from Nevalı Çori and Ba'ja during the PPNB. Based on the results of our isotopic data and the newly integrated timescale of Nevalı Çori, a decline in mobility and growing reliance on domesticates took place by ca. 8300 BC at the end of its first subphase of the PPNB, at a time of increasing apparent social detachment from Göbekli Tepe and its underlying system of living and associated world views. Göbekli Tepe's loss of centrality as a result of new modes of mobility and subsistence after the early phase of the PPNB appears to have resulted in the increasing independence of the sites formerly connected under the TSP phenomenon. In spite of local transformations in modes of mobility and societal organization, our genome-wide data for the PPNB human individuals from Nevalı Çori and Ba''ja provide evidence for long-distance connectedness within the FC during this time. The evidence of consanguinity in the late PPNB Ba'ja further raises the question of how the PPN societies were internally organized and calls for additional future analyses on potential endogamy in the sense of cultural behavior and social practice. These dual aspects of human mobility investigation via isotope and genetics of both inner/intersocieties in the FC and within-/trans-Neolithic time highlight the potential of tandem molecular analyses to yield nuanced insights into the process of the Neolithization in the Near East.

Materials and Methods

Excavation and Sampling. All human and animal individuals analyzed in this research were excavated and documented with their archaeological and osteological information from the sites of Nevalı Çori and Ba'ja, as well as the radiocarbon dates of the chronological phases of Nevalı Çori (Datasets S1, S4, S8, and S10). Access to the so-far mostly unpublished documentation of the excavations at Nevalı Çori under the direction of Harald Hauptmann is possible upon request in the archive of the Institute of Prehistory and Early History, Heidelberg University. The burial information of sampled individuals from both sites is provided in *SI Appendix*. The human remains from Nevalı Çori were exported in 1991 based on an export permit issued by the Museum Şanlıurfa, which permitted archaeometric analyses under the auspices of the University of Göttingen Anatomy Laboratory and subsequent permanent storage of the remains at Göttingen; the animal remains from the same site were collected at the LMU Institute of Palaeoanatomy archives. The Ba'ja human samples were provided by the Ba'ja Neolithic Project excavation team.

Regarding the human remains recovered from Nevalı Çori, there was no specific sex bias in the sampling of the various phases. Our contextual interpretation is based on the still-unpublished archaeological documentation of Harald Hauptmann and his team, which is accessible in the Hauptmann archive at the University of Heidelberg. No identifiable grave constructions in the PPNB phase were visible, and there are no distinguishable differentiations in burial practices for the PPNB samples. Stone cists were identified during the EBA and RI. When preservation was sufficient, we sampled multiple molars per individual to detect potential mobility during a single lifetime (Fig. 1*B* and Dataset S1). Regarding the sampling strategy for the enamel of both humans and other fauna, we took the bulk fragment samples across a large range along the buccal side of each tooth to get a more integrated and averaged isotopic signal to avoid the potential bias caused by partial sampling in which way the measured ratio/value only represents partial or seasonal duration. We selected the teeth of the ungulate animals (*Bos, Ovis, Capra,* and *Gazella*) with limited length to attenuate the amplitude of intratooth isotopic change along the time of enamel maturation. The "bulk" samples from these individuals provide an average assessment of their local dietary, hydrological, and geological context for comparison with the human material.

Isotope Analyses. The pretreatment of tooth enamel samples for both strontium and stable isotope analyses was conducted at the Stable Isotope Laboratory of the Department of Archaeology, Max Planck Institute of Geoanthropology (formerly the Max Planck Institute for the Science of Human History, MPI-SHH), Jena, Germany (MPI-GEA).

Stable carbon and oxygen isotopes were analyzed by a Thermo Scientific Gas Bench II connected to a Thermo Delta V Advantage Mass Spectrometer at the same laboratory. Stable carbon and oxygen isotope values were compared against the International Atomic Energy Agency standards (603, CO08, and NBS 18). The calibration process and analytical uncertainty are presented in the *SI Appendix*, Note S5.1.3. We applied the equations provided in Szpak et al. (123) to calculate overall uncertainty using the check and calibration standards measured in all analytical sessions producing data presented in this paper. Full details can be found in Datasets S11 and S12 and *SI Appendix*, Note S5.1.3.

Strontium isotope analysis was undertaken at the clean laboratory in the Department of Geological Sciences at the University of Cape Town. Following strontium elemental separation, the radiogenic $^{87}Sr/^{86}Sr$ ratios were measured using the Nu Instruments Nu Plasma HR MC-ICP-MS and referenced to a value of 0.710255 for NIST SRM987.

The full details of the protocols and rationales of the methods are described in *SI Appendix*, Note S5. Statistical analysis was carried out using JMP 11.0 and R 3.63; map illustration was carried out with QGIS 3.16.6.

Genetic Analyses. All the samples analyzed for aDNA were processed in the designated facilities of the MPI-SHH and the Max Planck Institute for Evolutionary Anthropology (MPI-EVA) in Germany. After the DNA extraction, genomic libraries were prepared with the single-stranded protocol, which efficiently immortalizes ultrashort chemically damaged (single-stranded) DNA with Illumina adapters (124). The metagenomic content of the libraries was analyzed after shallow sequencing on an Illumina HiSeq4000 platform (ca. 5 mio reads). The proportion of endogenous DNA (sequence reads mapping to the human reference genome sequence hs37d5) and the presence of deamination patterns were the main criteria to evaluate whether aDNA preservation was sufficient for employing the targeted enrichment of ca. 1.2 mio nuclear markers (SNPs) after Haak et al. (99), Mathieson et al. (125), and Fu et al. (126). The enriched libraries were then sequenced at 20 to 40 mio reads, and the sequencing data were processed through the pipeline Efficient Ancient Genome Reconstruction (EAGER) (127). The processed sequences in binary alignment map format (bam) were examined for contamination using three different methods (128–130), and pseudodiploid genotypes were called with the program pileupCaller (https://github.com/stschiff/sequenceTools) and the option "singleStrandMode." Libraries with qualified genotype data (i.e., low contamination and ≥ 40,000 SNPs) were checked for relatives or duplicate individuals by calculating the pairwise rate of mismatching alleles (131, 132) and were merged with the latest release of publicly available genotype datasets of ancient and modern individuals [v50.0] (https://reich.hms.harvard.edu/allen-ancient-dna-resource-aadr-downloadable-genotypes-present-day-and-ancient-dna-data) focusing on speficid published dataset relevant for our analyses [additional citations we've provided in the comment attached to this sentence].

We conducted an analysis of population structure with smartpca (133, 134) on the Human Origins (HO) SNP array data of 1,264 individuals from West Eurasia and projected all ancient individuals with the option lsqproject. We then used the package ADMIXTOOLS [v57.1] (135) and run the tools D-statistics and qpWave/qpAdm. With qpAdm, we modeled a target as a combination of coefficients from *n* source populations. The fit of the models and the admixture coefficients are estimated from a matrix of allele correlations (D/f_4-statistics) between the target, the sources, and a set of reference populations ("right pops" in the parameter file). As reference populations, we chose the modern Mbuti, Onge, Ami, Mixe, and the ancient Ust'Ishim hunter-gatherer from Russia, individuals MA1, and AfontovoGora3 grouped as "Ancestral North Eurasians" (ANE), Eastern European HG (EEHG), Kostenki14 hunter-gatherer from Russia, Balkan HG, Natufian HG from Israel, and CHG (Dataset S7).

Data, Materials, and Software Availability. Sources for all downloaded data are presented in Datasets S1–S12. The genome-wide data reported in this study can be accessible as fastq and BAM files through the European Nucleotide Archive (ENA) under the study name PRJEB58620.

ACKNOWLEDGMENTS. This research was funded by the CSC (China Scholarship Council) and the Max Planck-Harvard Research Center for the Archaeoscience of the Ancient Mediterranean (MHAAM). We thank Joseph Maran from the Institute of Prehistory and Early History, Heidelberg University, and Lee Clare from the German Archaeological Institute, Istanbul Department, who provided access to the original archive of fieldwork, and valuable information for this research. We thank Tara Ingman from the Koç University Research Center for Anatolian Civilizations (ANAMED) and Yufeng Sun from the Washington University in St. Louis for editing and revising the manuscript and their helpful comments. We thank Susan Klingner from the Department of Anatomy, University of Göttingen, for the osteological identification on the studied samples of Nevalı Çori human remains. Patrick Roberts would like to thank the Max Planck Society for funding. Analyses of the fauna from Nevalı Çori were funded by the Deutsche Forschungsgemeinschaft (DFG) in projects 46547580 and 165831460 to Joris Peters. We also wish to thank the late pioneering archaeologists Harald Hauptmann and Klaus Schmidt who led the excavation work at Nevalı Çori. This research has been conducted at the LMU (Ludwig Maximilian University Munich), MPI-SHH (Max Planck Institute for the Science of Human History), and MPI-EVA (Department of Archaeogenetics, Max Planck Institute for Evolutionary Anthropology); the relevant staff and members of all these institutions are thanked for their support. We are grateful to the handling editor and two anonymous reviewers for their helpful comments to improve this study.

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9. Manuscript B (the case study of Jericho)

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Isotopic and proteomic evidence for communal stability at Pre-Pottery Neolithic Jericho in the Southern Levant

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As one of the key, long-term occupied sites in the Southern Levant, Jericho was one of the most important early Neolithic centres to witness social and economic changes associated with the domestication of plants and animals. This study applies strontium ($^{87}Sr/^{86}Sr$), oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) isotope analyses to the enamel of 52 human teeth from Pre-Pottery Neolithic (PPN) layers of Jericho to directly study human diet and mobility and investigate the degree of consolidation and the flexibility of social organization of Jericho society in the PPN period. The results indicate only two non-local individuals out of the 44 sampled inhabitants identified by strontium isotope analysis and are consistent with the presence of a largely sedentary community at PPN Jericho with no evidence for large-scale migration. We also construct strontium spatial baselines ($^{87}Sr/^{86}Sr$ map) with local $^{87}Sr/^{86}Sr$ signatures for the sites across the Southern Levant based on systematic compilation and analysis of available data. In addition, we apply proteomic analysis of sex-specific amelogenin peptides in tooth enamel for sex estimation of the sampled individuals (n = 44), the results of which showed a sex-biased ratio (more male than female detected in this sample pool) in Jericho society during the PPN period, which may be due to the limited sample size or selective ritual practices like particular burial zones used for specific groups. We also pretreated a batch of human bone samples recovered from PPNB Jericho for stable carbon and nitrogen isotope analyses for dietary investigations. However, the extracted collagen showed poor preservation and no valid $\delta^{13}C$ or $\delta^{15}N$ data were obtained.

Neolithization, defined here as the process of a semi- or complete transition to agriculture with an expected concordant gradual move to sedentism[1–5], took place asynchronously in different communities in different regions of the Near East[6–8]. In contrast to initial ideas of a “Neolithic Revolution” emanating from a single region[9], multiple centers of experimentation with plant cultivation and animal herding have been identified across the Levant, southeastern Anatolia and the Zagros Mountains in the early Holocene[10,11], with the pace, pattern and mechanisms of domestication and spread of farming, as well as associated cultural changes, being highly variable[2,4,12–14]. It has been demonstrated that, from the Pre-Pottery Neolithic A (PPNA) onwards, people in the Southern Levant tended to live more sedentary lifestyles and that this change potentially started much earlier here in comparison to other regions of the Fertile Crescent (FC). This has been supported by recent strontium isotopic research in PPN Southeastern Anatolia at Nevali Çori which shows that the inhabitants did not become

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sedentary until the Middle Pre-Pottery Neolithic B (PPNB)[15]. To better understand the pace of sedentism in different regions of the FC and its relationship to economic and social changes associated with the domestication of plants and animals, it is essential to undertake multidisciplinary approaches to palaeodiet and palaeomobility in different parts of the FC.

Located at today's Tell es-Sultan, to the north of the Dead Sea in the Jordan Valley at N31°52′15″ E35°26′35″ (Fig. 1), ancient Jericho records over 10,000 years of human history, from the Late Natufian (10,500–8500 BC) to the Ottoman period (1516–1918 AD), without any apparent significant breaks in occupation[16–19]. The terminology of PPNA and PPNB, the transition between which is marked by changes in lithic industry, domestic architecture, and economic strategies, was first defined by Kenyon during her excavations at Jericho[20]. Since early excavations in the mid-twentieth century, Jericho has become one of the most emblematic sites of the PPN in the Southern Levant and has been a key site for understanding the Southern Levant during the PPNA (ca. 8500–7500 BC) and PPNB (ca. 7500–6000 BC) in particular[11,21,22]. Thus, Jericho provides a focal case study for testing the nature and timing of the social transition from highly mobile to more sedentary communities, as well as its relationship, or not, to plant cultivation and animal herding. So far, there is no clear evidence for animal domesticates during the PPNA, while the domestication of sheep and goats is evident in the succeeding PPNB levels[23–25]. From a frequently-used camp site for Natufian groups[20,26], the large scale of the site and the appearance of a stone tower that is over 8.5 m high and the walls dating to roughly 8300 BC in the PPNA make Jericho distinct from other contemporary sites in the Southern Levant in this period. However, despite decades of archaeological excavation, direct insights into ancient mobility and diet, as well as social structure, based on the biological materials recovered from Jericho is still lacking.

The function of the tower and the walls remains debated—they may have potentially served as defensive fortifications[27] or flood prevention barriers[28], as well as having possible communal or ritual significance according to some more recent research[29]. These large communal structures are potentially testimony to a society more sedentary than those that came before. Jericho has also yielded rare insights into PPN burial practices, where individuals were mostly interred under floors or in rubble fills, and collective burials were common[17,18,30]. A noteworthy mortuary custom was a ritual practice in the form of skull removal and skull plastering, with or without the mandibles included. Several skull caches have been uncovered at the site and are assumed to have served a kind of ancestral veneration or memorial function[31,32]. Although Jericho, to date, has preserved the highest numbers of plastered skulls in the region, this treatment of crania or statues of the dead is not unique to the site but is rather a common regional phenomenon during the PPN. Furthermore, unplastered skulls are

Figure 1. The location of Jericho and the relevant sites mentioned in the study (map illustration was carried out with QGIS 3.28 LTR, the URL link: https://qgis.org/downloads/macos/qgis-macos-ltr.dmg).

found even more widely across nearly the entire Southern Levant[32]. This shared ritual system across the PPN Levantine sphere, and the imported materials/products seem to indicate interaction and connections between Jericho and other communities, perhaps driven by human mobility. For example, the discovery of prestige items, such as obsidian from Anatolian sources, indicates a wide exchange network with spheres beyond the immediate environs that were already active since the Natufian period, continuing into the PPNB[33–35]. However, isotopic research of human remains from PPN periods that can provide direct evidence of human mobility and migration is still limited, in contrast with more focus on earlier materials dating to Natufian phases, e.g.,[36,37].

To shed more light on ancient mobility at PPN Jericho, we sampled human remains for multiple bio-archaeological analyses. ^{87}Sr/^{86}Sr ratios of tooth enamel derive from the geological context of an area and its contribution to an individual's water and food intake during enamel formation[38,39]. We sampled 52 human enamel samples from PPN Jericho for ^{87}Sr/^{86}Sr analysis, as well as one archaeological animal sample (JCH079.A, see Table 1 and Dataset S1) and modern plant samples collected from near Jericho (the coordinates are listed in the Dataset S4) to evaluate the local bioavailable ^{87}Sr/^{86}Sr range. To use strontium as a geochemical tracer for archaeological biomaterials, a baseline of bioavailable ^{87}Sr/^{86}Sr in the region must be determined[40] (SI Appendix Note S2.1). A growing number of isotope studies on materials recovered from the ancient Levant have been reported in previous publications. Therefore, we also compiled the available ^{87}Sr/^{86}Sr signatures published in relevant research to construct strontium spatial baselines (^{87}Sr/^{86}Sr map) across the Southern Levant, providing access to comparable data for future studies of archaeological, paleoenvironmental and paleoclimatic research in this area[41–45]. We also measured δ^{18}O values from the human tooth enamel samples as an independent record of an individual's water intake which, through their relationship to local temperatures, altitude, continentality and other environmental effects[46,47], allow them to also act as a geographical tracers. In addition, we applied δ^{13}C analysis to tooth enamel, to gain direct insights into diet (SI Appendix Note S2.2.1). Finally, because sex determination of the sampled individuals in the absence of both the complete skeletons for osteological identification and aDNA extraction for shotgun-genomic sex estimation was challenging, proteomics analysis was used, to determine biological sex for the sampled individuals from PPN Jericho[48,49].

Materials

We sampled 52 human teeth from 44 individuals from Jericho PPN, with three coming from the PPNA and the rest from the PPNB (for more details of sample information, see SI Appendix Dataset S1), for ^{87}Sr/^{86}Sr, δ^{13}C and δ^{18}O isotope analyses. Permanent 1st, 2nd and 3rd molars were the preferred dentition for sampling, representing, respectively, the ages of 0–3 years, 3–7 years, and adolescence or early adulthood, depending on individual differences in the formation time of M3[50,51]. Multiple teeth were sampled per individual, where preservation allowed, in order to evaluate possible variation reflecting mobility during the individual's lifetime. All samples were taken from the collection curated by the late anthropologist Olav Röhrer-Ertl and are now housed in the Anthropological Collection of the University of Göttingen, Germany (for more details on the history of the excavation and subsequent itinerary of the bone material, see SI Appendix Note S1). In addition, to determine the local ^{87}Sr/^{86}Sr baseline, we also sampled one archaeological animal (goat/sheep) tooth and four saltwort plants collected at different localities in the surrounding arid areas of Jericho where impacts of farming and fertilizer contamination could be ruled out. More details, including the coordinates of the plant samples, are presented in Dataset S4. We analyzed sex chromosome-linked isoforms of amelogenin by nanoflow liquid chromatography-tandem mass spectrometry (nanoLC-MS/MS) to determine the sex of the sampled individuals ($n = 44$). The full principles and methods are provided in SI Appendix Note S2.3.

A batch of samples ($n = 47$) of human bone recovered from PPNB Jericho were also pretreated for stable carbon and nitrogen isotope analyses of bone collagen to investigate possible dietary patterns (Dataset S3). Collagen was successfully extracted from all samples, however, the C/N ratios of all samples indicated that the extracted collagen did not qualify for further measurement because of poor preservation, which is a common situation in the similar studies in the Near East (also referring to Richards et al.[52]), which could be the result of the use of animal glues when the bones were re-articulated during past restauration efforts. Due to the same issue, ^{14}C dating was not possible on this batch of samples. Since the sampled material derived from the early excavations there was frequently a lack of correlation between the handwritten records and the archived samples. Due to the special mortuary practices performed *at situ*, some individuals were only represented by skulls[31,32] and the contextual information of the excavated individuals was limited in the original notes, thus these individuals were not always identifiable from the archive. Overall, as absolute dates of these samples are lacking, we have to rely on the records of the original excavations and relative chronological phasing, and association with ages determined from subsequent dating of these phases elsewhere in the site. Available archaeological contexts of the sampled individuals in this study are presented in Dataset S1. We also attempted aDNA analysis on the same batch of samples (targeting the petrous bones and dentine), but preservation levels again were insufficient, and the analysis was therefore not possible. The relevant pretreatment processes and methods and the data from the relevant measurements are included in the SI Appendix Note S2.4 and Dataset S3.

Results

Isotope analyses

The results of the multi-isotopic analyses of all samples from Jericho are presented in Dataset S1. To summarize, we report 52 ^{87}Sr/^{86}Sr ratios from 44 human individuals, and five ^{87}Sr/^{86}Sr ratios from the environmental samples (four modern plant samples and one dental enamel of archaeological animal) (see Fig. 2, Table 1, Datasets S1, S4), as well as 51 paired δ^{13}C and δ^{18}O values from 43 human individuals which were also measured for ^{87}Sr/^{86}Sr. Regarding the δ^{13}C and δ^{18}O data, the δ^{18}O values of all the 51 samples range from − 4.5 to − 0.5‰ with a mean

Sample ID	Material	Date	87Sr/86Sr	± 2SD internal
JCH003.C	Human dental enamel	PPNB	0.708004	0.000014
JCH004.C	Human dental enamel	PPNB	0.707998	0.000010
JCH011.C	Human dental enamel	PPNB	0.708069	0.000012
JCH015.B	Human dental enamel	PPNB	0.708011	0.000013
JCH019.B	Human dental enamel	PPNA	0.708014	0.000012
JCH042.B	Human dental enamel	PPNB	0.708145	0.000012
JCH042.C	Human dental enamel	PPNB	0.708064	0.000011
JCH053.B	Human dental enamel	PPNB	0.708058	0.000011
JCH054.C	Human dental enamel	PPNA	0.708163	0.000011
JCH055.B	Human dental enamel	PPNB	0.708037	0.000011
JCH056.B	Human dental enamel	PPNB	0.708065	0.000010
JCH057.B	Human dental enamel	PPNB	0.708062	0.000011
JCH058.B	Human dental enamel	PPNB	0.708089	0.000011
JCH058.C	Human dental enamel	PPNB	0.708050	0.000012
JCH059.B	Human dental enamel	PPNB	0.708060	0.000012
JCH060.A	Human dental enamel	PPNB	0.708011	0.000016
JCH060.B	Human dental enamel	PPNB	0.708018	0.000010
JCH060.C	Human dental enamel	PPNB	0.708020	0.000008
JCH061.B	Human dental enamel	PPNB	0.709341	0.000012
JCH061.D	Human dental enamel	PPNB	0.708497	0.000011
JCH062.A	Human dental enamel	PPNB	0.707972	0.000012
JCH062.C	Human dental enamel	PPNB	0.708006	0.000013
JCH063.A	Human dental enamel	PPNB	0.708043	0.000011
JCH063.E	Human dental enamel	PPNB	0.708077	0.000012
JCH064.A	Human dental enamel	PPNB	0.708015	0.000010
JCH064.B	Human dental enamel	PPNB	0.708017	0.000011
JCH065.B	Human dental enamel	PPNA	0.708025	0.000013
JCH066.C	Human dental enamel	PPNB	0.708003	0.000011
JCH067.A	Human dental enamel	PPNB	0.707954	0.000011
JCH068.B	Human dental enamel	PPNB	0.708064	0.000010
JCH069.A	Human dental enamel	PPNB	0.708034	0.000009
JCH070.B	Human dental enamel	PPNB	0.707980	0.000011
JCH071.B	Human dental enamel	PPNB	0.708027	0.000013
JCH072.B	Human dental enamel	PPNB	0.707974	0.000010
JCH074.A	Human dental enamel	PPNB	0.708102	0.000010
JCH075.A	Human dental enamel	PPNB	0.708146	0.000012
JCH076.B	Human dental enamel	PPNB	0.708160	0.000012
JCH079.A	**Fauna dental enamel**	**PPNB**	**0.707988**	**0.000010**
JCH084.B	Human dental enamel	PPNB	0.708407	0.000012
JCH086.B	Human dental enamel	PPNB	0.708061	0.000012
JCH087.C	Human dental enamel	PPNB	0.707999	0.000012
JCH089.A	Human dental enamel	PPNB	0.708104	0.000013
JCH092.A	Human dental enamel	PPNB	0.708087	0.000009
JCH093.C	Human dental enamel	PPNB	0.708073	0.000011
JCH096.A	Human dental enamel	PPNB	0.707905	0.000009
JCH097.A	Human dental enamel	PPNB	0.708153	0.000012
JCH099.A	Human dental enamel	PPNB	0.708132	0.000015
JCH100.B	Human dental enamel	PPNB	0.707974	0.000012
JCH103.B	Human dental enamel	PPNB	0.707978	0.000011
JCH104.C	Human dental enamel	PPNB	0.708007	0.000012
JCH105.B	Human dental enamel	PPNB	0.708126	0.000012
JCH106.B	Human dental enamel	PPNB	0.708114	0.000010
JCH107.B	Human dental enamel	PPNB	0.708062	0.000013
ZY-11156	**Saltworts**	**Modern**	**0.708069**	**0.000012**
ZY-11157	**Saltworts**	**Modern**	**0.708040**	**0.000010**
ZY-11158	**Saltworts**	**Modern**	**0.708073**	**0.000012**
ZY-11159	**Saltworts**	**Modern**	**0.707983**	**0.000012**

Table 1. The $^{87}Sr/^{86}Sr$ ratios of the samples from Jericho measured in this study. The bolded samples are used to build the local bioavailable strontium isotope baseline of Jericho, including 1 archaeological fauna enamel sample and 4 modern plants. More detailed contextual information is included in the Dataset S1.

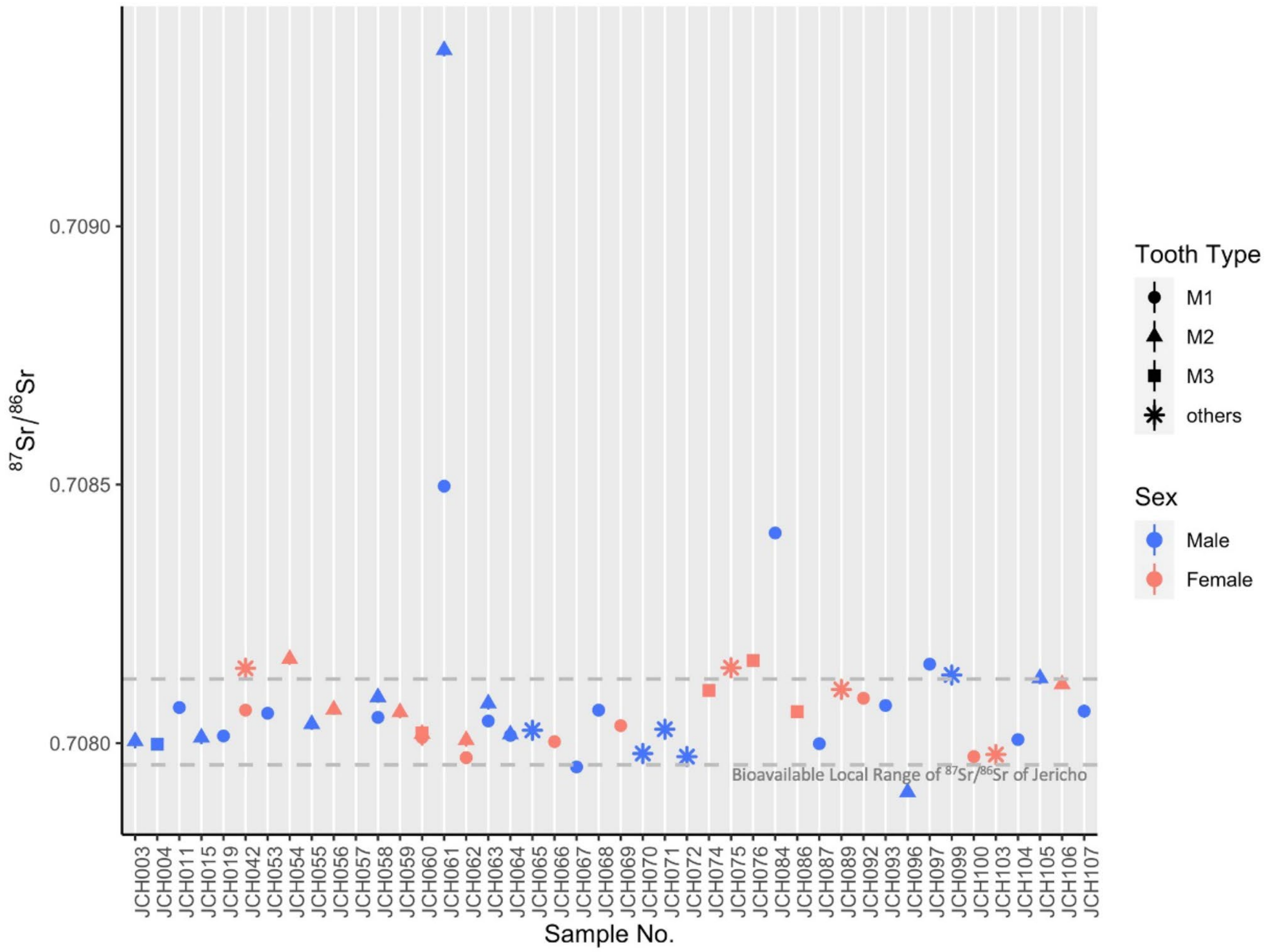

Figure 2. ^{87}Sr/^{86}Sr plot of the sampled human teeth excavated from PPNA and PPNB Jericho, with multiple samples from the same individual plotted on the same axis (Dataset S1).

of −2.8 ± 0.1‰ (2SD). Meanwhile, δ^{13}C values range from − 14.8 to − 11.3‰ with an average of − 12.8 ± 0.3‰ (2SD) (Datasets S1, S6, Fig. 3).

The results of ^{87}Sr/^{86}Sr analysis show that most human ^{87}Sr/^{86}Sr ratios fall within or close to local ^{87}Sr/^{86}Sr bioavailable values of Jericho based on the modern plant and archaeological animal samples, ranging from 0.707944 to 0.708117 (mean ± 2SD, mean = 0.708031) (more information of the environmental samples is included in Datasets S1, S4)[53]. The ^{87}Sr/^{86}Sr values of 42 individuals range from 0.707905 to 0.708163 with the exception of three teeth, coming from two male individuals (according to the sex determination results, see below and Table 2), JCH061 and JCH084, which had distinctively higher ^{87}Sr/^{86}Sr values and appear to show non-local residence at least during their childhood. Notably, JCH061 displays distinct values for both M1 (0.708497) and M2 (0.709341), which developed during his infancy and childhood respectively. Both values are much higher than the local range of Jericho, where he was buried, demonstrating potential evidence of mobility during his youth.

Geological context and ^{87}Sr/^{86}Sr baselines of the Southern Levant

The analysis of ^{87}Sr/^{86}Sr ratios depends largely upon the geological variability reflected in the bioavailable ^{87}Sr/^{86}Sr measured for the region under study[54,55]. The geological variation of the Southern Levant is distributed in north–south bands of different geological and lithological assemblages. However, it is not the case that one zone contains only a single type of bedrock or lithology; rather, all zones consist of multiple geological components of different ages[56–58]. Moffat et al. summarized and reviewed ^{87}Sr/^{86}Sr data based on geological materials and archaeological case studies from Israel/the Levant covering a period from 1993 to 2020[59]. Hartman and Richards studied the relative contributions of bedrock and atmospheric sources to bioavailable strontium pools in local soils in Northern Israel and the Golan regions and produced a map of bioavailable ^{87}Sr/^{86}Sr ratios based on modern plant and invertebrate samples[60].

To present the spatial distribution of ^{87}Sr/^{86}Sr values in the Southern Levant, we compiled and analyzed the published ^{87}Sr/^{86}Sr ratios from 36 sites and recalculated the mean values and standard deviations of data available for each site and used the maximum range within a given geological province defined to be of generally similar geological context (presented as distinctive legends in Fig. 4)[36,37,61–72]. For the sites with more than (and including) three datapoints available we applied mean ± 2SD to calculate the local bioavailable ^{87}Sr/^{86}Sr range

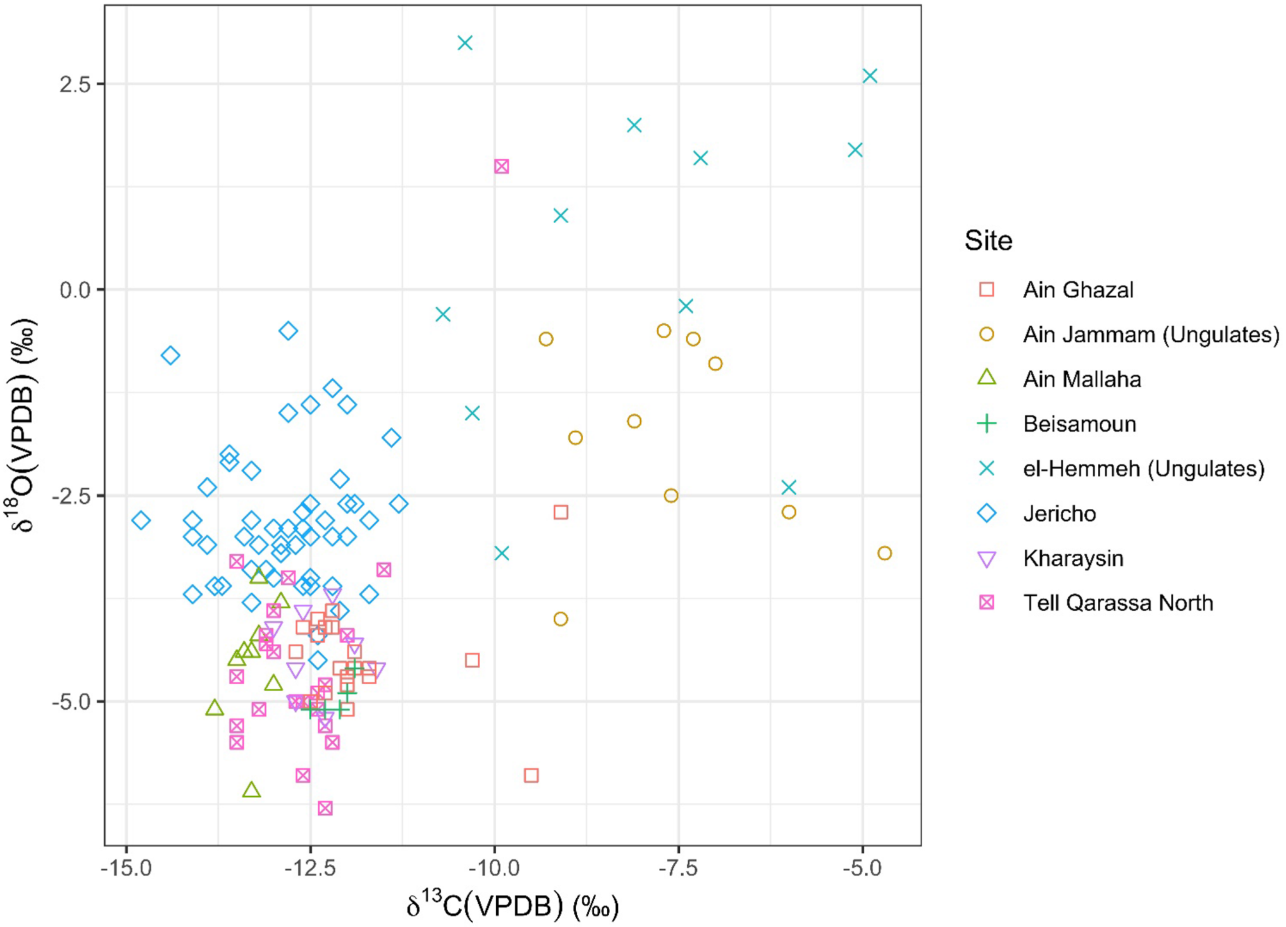

Figure 3. Scatter plot with $\delta^{13}C_{VPDB}$ and $\delta^{18}O_{VPDB}$ values ($n=51$) of the individuals ($n=44$) sampled from Jericho, grouping with published data from some other sites in the prehistoric (either of Natufian or PPN periods) Southern Levant (the details and references of the materials and the data included are presented in Dataset S6).

of each site. Otherwise, we use a single ratio or simply a range for the two ratios available. For bioavailable local $^{87}Sr/^{86}Sr$, ranges of the included sites corresponding to each legend are shown in in Fig. 4 (see also Dataset S2). For more details of the principles of calculation, the details of the type and chronological context of the material used for each dataset and the coordinates of the sites see Dataset S4. The information for the other sites with published data available, but beyond the scope of this map, are presented in Dataset S5. The data included here mainly derive from materials appropriate for bioavailable strontium assessments of multiple types, including modern or archaeological fauna or plant remains, while excluding data from geological materials, soil and rock which could reflect larger variability ratios compared to biological materials[73]. We tried to avoid using bone apatite ratios due to the risk of diagenesis unless there was no other option, in which case they are marked with asterisk. The distribution and potential sources of bioavailable strontium in northern Israel and the Golan have been thoroughly studied[60]. We, therefore, do not include these in Fig. 4. Notably, our bioavailable strontium 'map' may not strictly follow geological distinctions, and we must consider the potential endmembers in practice that served as the bioenvironment for local inhabitants. For instance, some bioavailable local strontium signatures represent eroded or alluvial sediments much younger than the local bedrock[61].

The geological context of the Southern Levant can be simplified to five[72] or seven[37] main zones: Coastal areas (Legend B), Western Highlands (Legends F, H, I), Jordan Rift Valley (Legend E), Eastern Highlands (Legends M, H), Golan Heights (Legend D), Hula Basin (Legend G), Basalt area (Legend D), Azraq Basin (Legend C) (Fig. 4). Jericho is located in the Jordan Rift Valley, the geological depression of which consists predominantly of Quaternary, sandstone, mudstone, and gravels, formed during the Miocene[56,72]. The geological context near Jericho consists of Lisan Marl diluvium which is dominated by limestone and gravel[56,74]. According to our spatial assessment of existing and measured $^{87}Sr/^{86}Sr$ data, sampling densities are unevenly available for the different zones. The most intensively investigated zone is Legend H including the Eastern Highlands and part of the Western Highlands (N (number) $_{sample}=51$, N (number) $_{site}=10$), followed by Legends M ($N_{sample}=47$, $N_{site}=4$), D ($N_{sample}=28$, $N_{site}=5$), I ($N_{sample}=28$, $N_{site}=5$) and F ($N_{sample}=24$, $N_{site}=3$). The southern-west and the northern-east of the Southern Levant have not been adequately investigated, and Legends A, J, K L and N are still lacking data to date. Our mapping of the data shows that the Jordan Valley fillings where Jericho is located present the widest distribution of $^{87}Sr/^{86}Sr$ ratios, spanning from 0.707924 to 0.7092260 (Legend E), with higher values in

Sample number	Sex	Number of total peptides	Number of AMELY unique peptides	Deamidation N (%)	Deamidation Q (%)
JCH003	M	152	13	99.49	100
JCH004	M	107	7	100	100
JCH011	M	92	3	100	100
JCH015	M	38	4	100	100
JCH019	M	93	4	100	100
JCH042	F	146	0	99.78	100
JCH053	M	67	2	100	100
JCH054	F	206	0	97.51	99.81
JCH055	M	87	9	99.11	100
JCH056	F	80	0	100	100
JCH057	M	127	8	100	97.92
JCH058	M	158	12	99.85	100
JCH059	F	40	0	82.71	92.58
JCH060	F	90	0	100	100
JCH061	M	102	4	91.09	99.35
JCH062	F	6	0	100	100
JCH063	M	133	7	100	100
JCH064	M	112	6	100	100
JCH065	M	76	4	97.65	100
JCH066	F	155	0	94.91	100
JCH067	M	53	4	100	100
JCH068	M	112	3	100	96.37
JCH069	F	197	0	100	99.46
JCH070	M	67	2	99.12	100
JCH071	M	125	16	100	100
JCH072	M	144	10	100	100
JCH074	F	99	0	98.06	100
JCH075	F	147	0	99.69	100
JCH076	F	144	0	100	100
JCH084	M	130	6	100	100
JCH086	F	82	0	100	100
JCH087	M	199	13	100	98.83
JCH089	F	227	0	100	100
JCH092	F	119	0	100	100
JCH093	M	192	14	100	99.41
JCH096	M	139	8	99.83	99.33
JCH097	M	172	12	97.83	99.89
JCH099	M	69	5	96.76	100
JCH100	F	200	0	99.77	100
JCH103	F	113	0	100	100
JCH104	M	88	4	97.50	100
JCH105	M	176	8	92.16	100.00
JCH106	F	131	0	100.00	100.00
JCH107	M	177	13	100.00	99.90

Table 2. Metadata from the proteomic analysis of sex-specific amelogenin peptides on the sampled individuals (n = 44) in this study.

the north and lower values in the south. The other sites in the same zone of the Jordan Valley are Pella, with a lower range (0.707924–0.707967, 2SD)[61] than Jericho, and Wadi Hammeh 27 (0.708020–0.709260, 2SD)[36], which is generally higher, as well as Wadi Fidan with values (0.707929–0.708144, 2SD)[61] that are close to the Jericho range (Dataset S2); there may be other possibilities not yet excavated or identified in surveys, as well.

The local bioavailable ^{87}Sr/^{86}Sr signature of Jericho (0.707944–0.708117, mean ± 2SD, based on the modern plant and archaeological animal samples as mentioned above) is consistent (or partly consistent) with almost all the geological zones, except for those represented by Legend B and Legend G, as well as the values documented for the region where the site sits previously. Therefore, interpretations of ^{87}Sr/^{86}Sr variability are challenging given the homogeneity of certain values across hundreds of kilometres as well as fine-grained variation within a matter of kilometres. This means that assessment of 'local' and 'non-local' using this approach is challenging, as

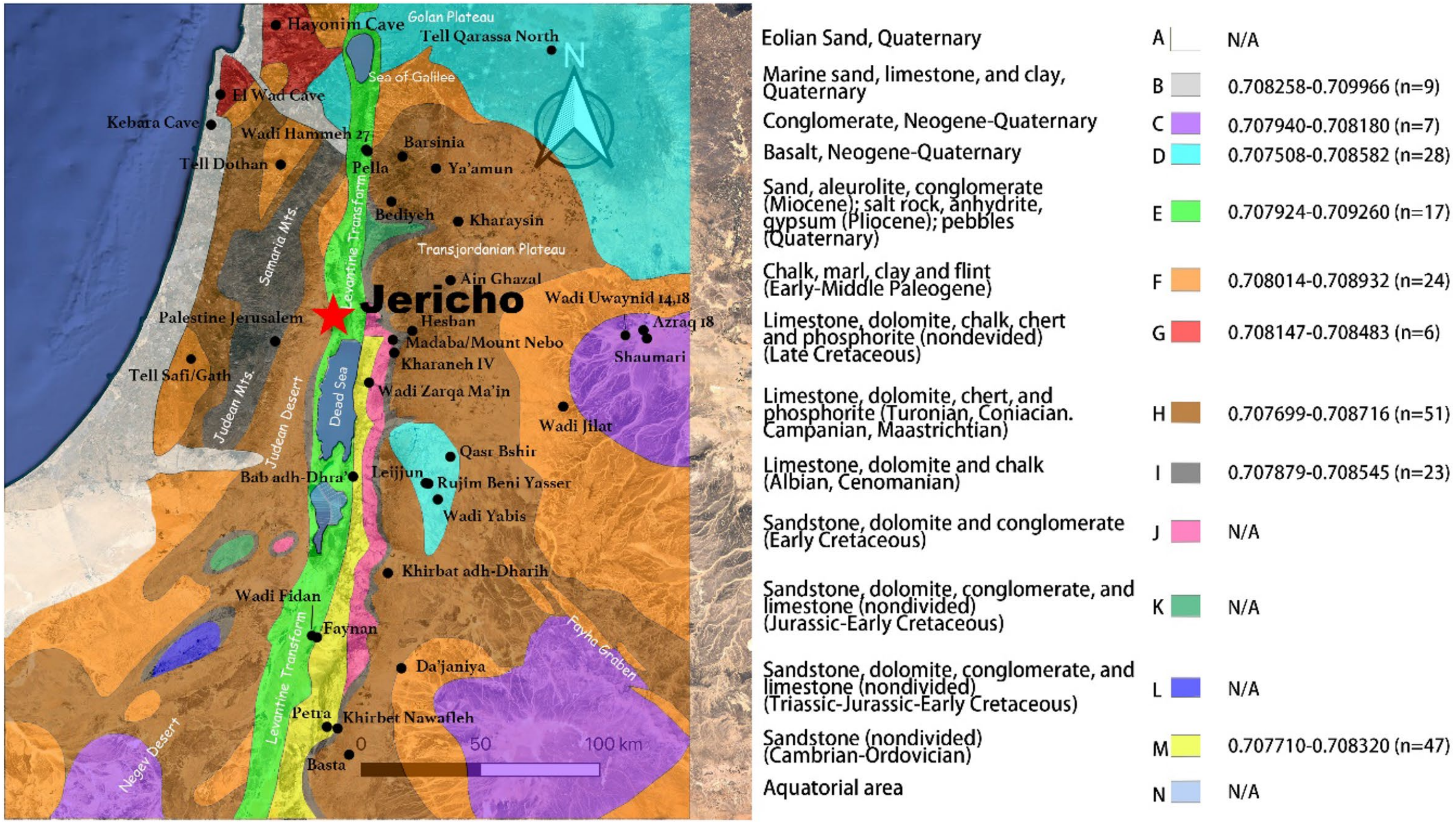

Figure 4. Integration of bioavailable $^{87}Sr/^{86}Sr$ intervals and geological formations across the Southern Levant, based on the published data to date and modified after[56,93]; N/A indicates that a local range could not be established due to the lack of data; detailed calculations of the included data and the corresponding references are presented in Dataset S2.

is any attempt to provenance individuals. As a result, in interpreting our human data, we focus more on relative difference and inner comparison amongst the measured samples from Jericho and assessment of intra-population patterns among our datasets.

Sex determination

Amelogenin peptides were successfully extracted from all samples. In this study, for the samples without AMELY unique peptides, the number of total peptides in all samples was larger than the reference value of 30. Therefore, we believe that the sex of the individuals identified in this study is secure. In addition, the deamidation percentage of glutamine (N) and asparagine (Q) in this study was higher than 80%, which was much higher than that of contaminants (N = 19.52%, Q = 26.49%). Moreover, no peptide related to amelogenin was found in the blank control group. Therefore, the identification of amelogenin peptides is considered reliable. Detailed results are presented in Table 2.

The sampling of individuals was randomized and the samples were from almost all the excavation units of the PPNB layers at Jericho (Dataset S1). The ratio of male to female individuals in the dataset was 27:17 (Table 2). There are several possible explanations for this bias toward male individuals. It could be a mere coincidence by which chance more males than females were selected for sampling or that the anthropological collection simply contained more males. Alternatively, the sampling accurately reflects differing burial practices for males and females leading to uneven obtaining of the samples in term of sex or that the population of Jericho in general comprised more males than females. Non-local $^{87}Sr/^{86}Sr$ ratios were found exclusively in two male individuals in this case. Irrespective of the intrinsic limitations of the strontium isotopic method, no isotopic evidence has been found indicating the mobility of any female individuals before their late adolescence in this study. There is no other obvious overall pattern according to sex in terms of burial type, or strontium, carbon and oxygen isotopic values.

Discussion

The results of $^{87}Sr/^{86}Sr$ analysis on the measured environmental samples from Jericho, i.e., the local bioavailable strontium isotope interval of Jericho, agree with the predictions from our spatial mapping of $^{87}Sr/^{86}Sr$ ratios for the region (Fig. 4) for the geological province in which Jericho located, i.e., Legend E ranging from 0.707924 to 0.709260 (Dataset S2). Comparison of our human data to both of these datasets suggests that most individuals analyzed likely spent their childhood locally. According to the results of our $^{87}Sr/^{86}Sr$ analysis, only two biologically male individuals showed clear 'non-local' signatures falling beyond the baseline range. They are either from a different area within the Jordan Valley (Fig. 4, Legend E), perhaps they occupied a different part of the local landscape, or they came from further afield. The documented variability in bioavailable $^{87}Sr/^{86}Sr$ makes any discussion of provenance problematic (Fig. 4). When comparing the three human $^{87}Sr/^{86}Sr$ values which fall beyond the estimated baseline, the ratios of the M1 of JCH084 (0.708407) and of JCH061 (0.708497) fall in the range of Rendzina soils (consisted of Chalk/Marle) distributed in the Judean foothills, extending from 0.7084 to 0.7085, lower than the ranges reflecting the areas dominated by coastal alluvium (~ 0.7089), calcareous sandstone (0.7087 ± 0.0001) and Terra rossa soils (consisted of limestone and dolomite, 0.7086 ± 0.0003)[67]. Alternatively, when comparing them at the scale of datasets from individual sites, the three most closely matching locales are the sites of Wadi Yabis (0.708398–0.708582, 2SD), Hayonim Cave (0.708147–0.708483, 2SD) and Palestine Jerusalem (0.707879–0.708545, 2SD) (Dataset S4), though they are not contemporaneous to Jericho. JCH061's M2 $^{87}Sr/^{86}Sr$ value of 0.709341 is the highest amongst all the data reported in our study, making it very possible that he came from the coastal region of the Eastern Mediterranean where marine sand and calcareous sandstone predominate (Fig. 4, Legend B, 0.708258–0.709966). To summarize the travel histories of the two non-local males recovered at PPN Jericho, they both may have been born somewhere outside Jericho possibly either within or beyond the Jordan Rift Valley. Later, individual JCH061 also lived in the coastal region based on the ratio of his M2. Nevertheless, we must be aware that there are still other possibilities for the variation in our data, including the potential of sourcing of food and water from different geologies by these individuals[75]. It is noteworthy that the M1 of individual JCH061 was heavily worn, seemingly due to certain crafting activities (SI Appendix Note S1). No special features of their burial contexts nor grave goods are distinctive from the other local inhabitants at PPN Jericho.

All other analyzed individuals generally fall within the local bioavailable strontium range for Jericho, despite some ratios being very close to the absolute limits of the defined local range (e.g., JCH042, 054, 075, 076, 097, Fig. 2). We take this to mean they were all raised locally (during the periods of life represented by the samples from the M1 and M2) within the Jericho area. However, the local expected $^{87}Sr/^{86}Sr$ range of Jericho spans the ranges of almost all the geological zones, not only the Southern Levant shown in Fig. 4, but also some areas in the Northern Levant such as the Amuq Valley[76]. As a result, movement between Jericho and areas with homogenous geological contexts cannot be excluded. Nevertheless, the extremely narrow distribution of $^{87}Sr/^{86}Sr$ ratios of the individuals from PPN Jericho, as shown in Fig. 2, may further support a high degree of homogeneity in the source region of their diet and water intake, which could be consistent with a local origin. The standard deviation among all the $^{87}Sr/^{86}Sr$ ratios reported, excluding the three outliers mentioned above, is 0.000058 (n = 49). Local human and faunal ratios typically vary by less than ± 0.0003[73]. Therefore, without evidence to the contrary, the data support the fact that no clear patterns of mobility were found in PPN Jericho, perhaps supporting the idea that sedentism had been achieved as early as the PPNA phase. This would be much earlier than at Nevali Çori, in Southeastern Anatolia, where the degree of mobility was still high in the early stages of the PPNB[15]. Nevertheless, this interpretation and comparison must remain cautious noting the limited sample size for each spatiotemporal group and requires higher resolution of the local strontium baseline and temporal changes in human $^{87}Sr/^{86}Sr$, as well as the application of other proxies like lead isotope analysis for further insights.

Based on this assumption, all of the tested female individuals at PPN Jericho seem to have spent their childhood at the site. This is evidenced by JCH060, for example, with the $^{87}Sr/^{86}Sr$ ratios of her M1 (0.7080110), M2 (0.708018) and M3 (0.708020) all showing that she likely lived at Jericho her entire childhood, adolescence, and early adulthood, though travelling after these life stages cannot be excluded due to the inherent limitations of this method which targets early life. The epigenetic traits of human teeth from the PPNB site Kfar HaHoresh in the Southern Levant showed biological relationships between females and subadults, which is not the case between any males and subadults, indicating a possible matrilocal residence pattern[77]. On top of the potential mobility pattern reflected by the $^{87}Sr/^{86}Sr$ values in this study, with no female and only two male immigrants at PPN Jericho, the assumption that a matrilocal social organization may have existed at Jericho should not be excluded, although we must note that the evidence for male mobility is also limited in terms of sample size and this is only an assumption awaits further more solid evidence to verify. The two non-local male individuals identified on the basis of their $^{87}Sr/^{86}Sr$ values, JCH061 and JCH084. In some cases of multi-sampled individuals (i.e., JCH062, 060, 058, 042), the $\delta^{13}C$ values of M1 are the highest which are supposed to result from weaning effect[78], with exceptions of JCH061, 063 and 064. It is noteworthy that for individual JCH061, the M2 had a $\delta^{13}C$ value 1.2‰ higher than its M1, which is a notably high intra-individual variability compared to other multi-sampled individuals measured that generally showed no detectable change or a variability of < 1‰ (i.e., JCH064, 063, 062, 060, 058, 042). This indicates that the diet of JCH061 during his late childhood, as represented by M2, might have incorporated more of a C_4 or C_3–C_4 intermediate input (in the form of vegetation or animals consuming these vegetation), perhaps suggesting movement to a drier environment[79]. Additionally, the $\delta^{18}O$ value of the M2 of JCH061 is lower than that of the M1, indicating that this individual likely moved somewhere further inland or at a higher altitude or latitude during the time reflected by the M2[80,81], which also corresponds to its highest $^{87}Sr/^{86}Sr$ ratio as an outlier mentioned above perhaps indicating a wider mobility.

We grouped the plots of paired $\delta^{13}C$ and $\delta^{18}O$ isotopes of Jericho human teeth with their human/animal counterparts from the other sites dating prior to (Ain Mallaha dating to Natufian period) or approximately coevally with (Tell Qarassa North, Kharaysin, 'Ain Ghazal, Beisamoun, el-Hemmeh and 'Ain Jamman of PPN periods) Jericho (Fig. 3, Dataset S6)[37,82]. The $\delta^{13}C$ values of Jericho are largely consistent with the corresponding values of human individuals from the other prehistoric sites in the Levant (i.e., 'Ain Mallaha, Tell Qarassa North, Kharaysin, Beisamoun, 'Ain Ghazal), reflecting a common dietary composition in terms of predominant consumption of C_3 resources in the early Holocene of the Southern Levant. The Jericho values are, however, more dispersed which potentially suggests a likely diversified and broader diet for Jericho inhabitants in PPN times (Fig. 3). However, Jericho human exhibit higher $\delta^{18}O$ values compared to those from 'Ain Mallaha, Tell Qarassa North, Kharaysin, Beisamoun, 'Ain Ghazal, sites whose ages range from the Natufian to PPNC periods. This is supported by a non-parametric Kruskal–Wallis test which shows significant differences between the $\delta^{18}O$ values of Jericho and the other five groups (Dataset S6, $p < 0.01$; a Shapiro–Wilk test showed that the data was not normally distributed, $W = 0.95$, $p < 0.01$). This difference could be linked to factors such as altitude, humidity and continental positioning that result in a wetter and warmer environment in Jericho compared to the other sites more inland or located at higher altitude (Figs. 1, 3). The $\delta^{13}C$ and $\delta^{18}O$ values of the plotted PPNB ungulates from el-Hemmeh and 'Ain Jamman are much more scattered as the livestock pastures were proven to be influenced by seasonally directed husbandry strategies (e.g., vertical transhumance) resulting to very different patterning of their diet access from human[82].

The results of sex determination on the randomly selected (meaning that the samples were not chosen with any special funerary context or obvious cultural/gender indication) individuals from PPN Jericho showed the human sex ratio at birth (SRB) is approximately equal to 1.59 (Male: Female = 27: 17), showing an imbalance. The natural ratio at birth between males and females in humans has been estimated to be within a narrow range of 1.07 to 1.03, slightly biased towards the male sex[83]. The sex ratio of a total ancient population can be affected by various factors[84] such as war, sex-selective abortions, infanticide, aging, gendercide and so on. In archaeological cases, the factor of potential sex-specific burial practices is also worth considering. The Neolithic Demographic Transition model (NDT) argues that the advent of a sedentary lifestyle led to a rise in female fertility, along with an upsurge in both female and infant mortality rates[85]. If this was the case at Jericho, at this early phase of sedentism, they may have also experienced an attendant explosive mortality rate of women of childbearing age. The mortuary system in the Southern Levant during the PPN was complex with, for instance, many of the human remains either missing or represented only by a skull[86,87]. However, without more information on potential special burial areas for specific groups at PPN Jericho, the sex bias found in this study suggests the possibility of a specialized burial locale for females or other potentially particular groups. Last but not least, it must be pointed out that the sample size in this study is limited. Moreover, the local range of Jericho is similar to other regions in the larger area of the southern Levant. More samples with detailed contextual information and more future work on baseline variability is needed.

The lack of aDNA preservation does not allow us to determine if cross-cousin marriage, which was practiced at Basta[65] and Ba'ja[15], was also practiced at Jericho in the later PPNB. However, all existing evidence from Jericho seems to indicate a stable community, as reflected by the apparent lack of large-scale and/or structured mobility during youth, which lines up with the settlement stability mirrored by parallel sites in the Southern Levant where consanguineous endogamy within local communities has been demonstrated. It has been argued that an increasing permanence and occupation area of communities, including but not limited to co-residence, are evident in the Natufian and into the PPN (certainly in the PPNA), as indicated by the nature of monumental architecture at that time at sites such as WF16, Göbekli Tepe and Jerf el Ahmar[88–90]. Community efforts and rituals embodied in both mortuary practices and monumental buildings like the walls and tower of Jericho would also have further contributed to establishing a hold over an area with favorable resources[34] and creating a local community with shared practices. Indeed, while a similar cultural framework can be identified across the whole Levant, distinctive characteristics of each regional locality and local community are emphasized in different areas. For example, skull rituals are commonly adopted in the PPN Levantine culture sphere, but they were executed differently in different sites/regions as reflected in the different use of plain skulls, plastered skulls and plaster statues at different sites[32].

To summarize, the results of the multi-isotope analyses conducted here do not contradict the existence of a sedentary community at PPN Jericho with no evidence for large-scale migration into the community or structural mobility, although it should be noted that local values could also represent other areas of the Levant beyond Jericho, and therefore this interpretation should remain tentative. Many elusive issues of Jericho society remain unsolved: for example, the relationship of the Jericho community to other sites in the Southern Levant, the political organization of society at the inter-group and intra-community levels[91], whether the concept of united community or/and households would have been even sharper for such a settlement with favorable living conditions and resources enclosed by the walls and how reproductive spheres were shaped and how they extended across space[92]. Considering the preservation issues for the collagen materials in the Levantine area, for example, we have failed in obtaining the $\delta^{13}C$ and $\delta^{15}N$ isotopic data from bone collagen and aDNA data which prevent us from further studies on the relevant diet and genomic history of populations during this time, researchers may have to rely more on approaches like elemental and isotopic analyses on excavated inorganic and bioapatite materials available and seek to undertake more tests on fresher samples from recent excavations. Nevertheless, this study provides a solid grounding for future multidisciplinary research that can shed further light on human societies across the PPNA and PPNB periods in the Southern Levant.

Data availability

Sources for all downloaded data are presented in SI Appendix, Datasets S1–S6. The mass spectrometry proteomics data have been deposited into the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the iProX partner repository[94] with the dataset identifier PXD037215.

Received: 30 May 2023; Accepted: 25 September 2023

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Acknowledgements

We thank Shlomit Bechar for collecting, recording, and delivering the environmental samples near the site of Jericho. We thank Gunnar Neumann, Căcilia Freund and Philomena Over for joining the sampling work on the human remains from the collection and Angela Mötsch for support with sample management. We appreciate the efforts by Anthi Tiliakou, Nelli-Johanna Saari, Eleftheria Orfanou and Ainash Childebayeva for identification of the tooth dentition. We thank Eleonora Röhrer-Ertl, the daughter of Olav Röhrer-Ertl (deceased 7th April 2019), for supporting our research efforts. Philipp W. Stockhammer and Patrick Roberts would like to thank the Max Planck Society for funding, whereby the research of Stockhammer was funded by the Max Planck-Harvard Research Center for the Archaeoscience of the Ancient Mediterranean at the Max Planck Institute for Evolutionary Anthropology, Leipzig. The research was also supported by the National Natural Science Foundation of China (Grant No. 41303080), USTC Youth Innovation Fund (2021, WK2110000018) and the USTC Research Funds of the Double First-Class Initiative (YD2110002027).

Author contributions

X.W. and P.W.S. designed research; X.W., S.E., B.Z., A.F., M.L., E.S., J.I., G.W., P.L.R., X.W. and X.Z. performed sample selection and took part in the laboratory analyses; X.W., B.Z., Y.S., P.R. and P.W.S. analyzed data; T.I. and S.E. and P.R. edited the manuscript; X.W., P.R., and P.W.S. wrote the manuscript with input from all co-authors.

Funding

Open Access funding enabled and organized by Projekt DEAL.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-023-43549-1.

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10. Appendix

10.1 Supplementary material for Manuscript A

PNAS

Supporting Information for

Stable isotopic and DNA analyses reveal multiscale prehistoric mobility and migration across Southeastern Anatolia and the Southern Levant

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This PDF file includes:

Figures S1 to S9e

SI References

Other supporting materials for this manuscript include the following:

Supplementary Tables (Datasets S1 to S12, in XLSX)

Supporting Information Text

Note S1. Archaeological background

S1.1 Nevalı Çori

Location. District Hilvan, Province Sanliurfa, Turkey.

Coordinates. 37° 31′ 6″ N, 38° 36′ 20″ E.

History of excavations. Two seasons of excavation during 1983–1987 and 1989–1991, organized by Prof. Dr. Hauptmann, University of Heidelberg, in cooperation with the Archaeological Museum of Sanliurfa.

Environmental Setting. The site of Nevalı Çori located in the Taurus foothills, along a side-valley of the Euphrates River, in the Southeastern Turkey. The formerly flat depression of the valley is deeply furrowed by the section of the perennial Kantara-creek, about 6m in depth. Several other tributary Wadis together with the mainstream destroyed some parts of the site. These erosional features do not antedate roman time. Therefore, the settlement is situated on both sides of the creek. The main and better-preserved part of the settlement is located on a triangular terrace on the river's eastern Bank underneath a steep hilltop. The Kantara-creek and two deep wadis separate the plateau from the rest of the valley bottom. The excavations were concentrated on the eastern Bank of the creek.

Subphases/stratigraphy.

- Roman Imperial Period (AD 1–375): only burials.
- Iron Age (1200–30 BC): only burials.
- Early Bronze Ib1: level VIIa–b (2900–2800 BC).
- Halaf Culture: level VI (5500–5000 BC).
- PPNB: level I–V (8800–6500 BC) (with Level I/II for the Early to Middle PPNB, Level III–IV for the Middle PPNB, a suspected Hiatus following, and Level V for the Late PPNB).

Overview. The PPNB settlement is an early farming village that already shows a clear differentiation of architecture such as public, residential and storage buildings in a context of a superordinate settlement pattern with common zones for working, storage, residency, and public activities. After a hiatus of ca. 2000 years the site was re-occupied by people of the Halaf Culture who built a single-phase homestead enclosure consisting of two round buildings connected by walls.

The next phase of occupation was in Early Bronze Age Ib1 when settlers built a hamlet of possibly 2–3 houses. The remains of a two-phased terraced building could be unearthed showing a small farming village community with hints to a pronounced household metallurgy (1). Around the EB house especially in the East and West lots of copper slags and some casts and melting pots were found as well as fragments of ovens that could have been used for metallurgy. Jörg Becker describes it as cottage metallurgy.

In the period of the Roman Empire, a part of the site was used as an isolated cemetery of a small settlement or a homestead in 300m distance on the west bank of the Kantara Creek where an intensive assemblage of contemporaneous pottery was found.

Materials. Considering that our sampling strategy aimed for teeth and petrous bones, the available material was reduced to 35 individuals for both isotopic and DNA analyses in total, among which 28 individuals were analyzed for the isotopic study, and six were only represented by petrous bones.

There are 15 individuals of Nevalı Çori in this study with effective radiocarbon dates based on three different batches measurement that were reported by different institutes/labs during the research history, among which there are four dates were newly reported by us (the MAMS series) and six individuals positive with genetic data (all the C14 dates available were presented in Dataset S4). Based on the combination of all the available radiocarbon dates so far and the original excavation archives of Nevalı Çori which showed that the recovered individuals were clearly stratified according to the archaeological phases of Nevalı Çori based on their context (e.g., shared archaeological unit/strata) enabling temporal differentiation even in cases where we had no possibility to radiocarbon date, we reported a reconstructed cultural-chronological framework of this site (Dataset S4).

Enamel preservation was monitored through the assessment of peak height, peak shape, and standard deviation between measurements of gas pulses. All enamel samples were considered as being well-preserved in a visual sense and, given the relatively young age of the material (such work has been successfully applied back millions of years in Africa, e.g., (2)), we did not apply further diagenetic assessment methods such as Fourier Transform Infrared Spectroscopy. Comparison of the human data with animal data, expected ecological variation, and other published data suggests that the patterns preserved are reflective of the in vivo situation.

Description of burials.

PPNB: early village settlement with intramural burials. A total of 93 burials were found dating from PPNB to the Roman Empire. Due to the soil conditions the bone material in general is poorly preserved and often fragmented. The 43 burials of the PPNB where mainly subfloor intramural burials in the basements of the buildings that can be distinguished into four types:

- depositions of single skulls without a special orientation
- depositions of skulls and long bones without a special orientation
- crouched burials in left-sided or right-sided with orientation of SE–NW or E–W
- group (collective) burial of three individuals in a room of house 21

Burial goods were exceedingly rare. Only a skull deposition was found together with a pounder made of nephrite and the group burial in house 21 contained flint tools. In some cases, a pestle, an adze, a blade, a sickle blade, or bones of a kestrel in one case were found within the context. The tools point to agricultural activities. The lithic inventory of Grave No. 84 cannot be classified as burial gifts with certainty. For Grave No. 84, a deposition of three skulls and long bones shows hints to violence because of the position of the flint dagger near Skull B.

The chert industries and other small finds and the architecture suggest dating the lower levels I–IV to the early to Middle PPNB while the uppermost level I can be dated to Late PPNB. This is sustained by the 14C dates covering a range from 8500 to 7600 cal. BC.

Almost all PPNB burials were found in the storage areas of the buildings except No. 78 that was found outside of any building under a pavement. No burials and almost no human bones could be detected in the “cult building” House 13 or its closer surrounding. Almost no complete skeleton was found. Very often only the skull and the long bones were deposited. Sometimes the bones of two or more individual were mixed. In the lower levels I–II, crouched burials seem to be common while in the upper-level depositions, single-skull or skulls with long bones are dominant. In two cases the head was taken out after a while for a particular treatment. Many of the bodies or body parts were laid down and tied together, some of which were in bags/baskets.

A very important group of human remains are the finds of ca. 90 human bone fragments were recovered amongst the animal bones in pits and open spaces (3). Even though some of them were found in houses and therefore might represent burials that have not been identified, this implies a different mortuary practice to deal with deceased by putting into garbage pits or scattering around.

Halaf Culture: single homestead with intramural burials. Only a single burial was reported for the Halaf Period, a left-sided crouched burial. The orientation is NE–SW. One copper ring was found, interpreted as costume component.

Early Bronze Age I: group of homesteads with intra- and extra-mural burials. Most of the 22 burials of the EBI period located around the EBI house were mainly in the west but there were also some subfloor intramural burials in the living area. They can be distinguished into 14 stone cist burials, five pithos burials and three simple earth burials. Burial goods are rare and mainly in the stone cist burials. The main orientation was NE–SW but also other orientations. Almost all burials were crouched left or right except one burial in supine position.

Iron Age and Roman Imperial Period: isolated cemeteries of a villa rustica or a group of homesteads. The 20 stone cist burials of the Iron age and Roman Empire represent an isolated cemetery of a small settlement or a homestead in 300m distance on the west bank of the Kantara Creek. The burials are oriented NE–SW with one exception in N–S. The dead were lying in supine position. Almost all burials were disturbed or robbed, only a few of which showed tools and costume components as burial goods.

S1.2 Ba`ja

Location. North of Petra, southern Jordan.

Coordinates. 30°24'55" N, 35°27'45" E.

History of excavations. 14 seasons of excavations at the Pre-Pottery Neolithic site of Ba`ja (ca. 7250 BC-6800 cal. BC) from 1997 to 2019 (to be continued), carried out at Free University of Berlin, continuously supported by ex oriente e.V., Berlin. A pilot study in 2016, and from 2018-2021 a three-year project "Household & Death in Ba`ja" has been sponsored by the German Research Foundation (4-7). The Ba`ja Neolithic Project continued under the co-directorships of Dr. Hans Georg K. Gebel, Dr. Marion Benz and Dr. Christoph Purschwitz from Free University of Berlin, coordinating three different subjects (Neolithic ethos, the dead and death, household production) and over 15 specialist researchers have been joining in this transdisciplinary enterprise.

Subphases/stratigraphy. According to ^{14}C dating results, a small burnt pistachio twig fragment from one of the lower deposits of the excavated sequence was radiocarbon dated to the first third of the 7th millennium BC (MAMS 42486: 7929 ± 29BP; 7027–6686 cal. BC, 2σ). It dates to a rather late phase of the late PPNB or even to the FPPNB/PPNC, similar as the radiocarbon dates of Burials CG8 and CG10 (4, 7).

There were at least three major burial phases with possibly several burial events in the southern and eastern part of the Room CR17. The so-far most ancient phase comprises a large pit in the eastern part of the room. It continues beneath the eastern Wall Loc. CR17:113. Since it is not fully excavated, comments on the burial goods are preliminary but it seems that there were only a few ornaments. The second and main phase of human deposits is above the leveling stone layer (Loc. CR17:116 [2018]/CR17:136). It is concentrated is the southwestern corner and extends far into the center. It seems to be delimited by the hump like feature (Loc. CR17:140) beneath and a double row of stones. Possibly all Loci CR17:131/133/135 and CR17:137 (central part) belong to this main phase. Most of the human remains come from this concentration including mainly bones of adults but also of subadults, and especially primary infant interments. It seems that most of the bones were sorted and/or pushed aside, but even for the well-articulated infant skeletons it cannot be excluded that they were buried secondarily. The stratigraphic sequence of burials will not only allow to recognize more precisely chronological sequences in the future, but also typological specificities may be identified. During the main phase, mother-of-pearl and shell ornaments were used intensively for the decoration of the dead. In contrast, in the upper layers, only two shells and two mother-of-pearl buttons were uncovered and stone objects such as the pendant and the white sandstone rings as well as one chrysocolla bead. Generally, the low number of ornaments in the latest burial phase is striking. The finds of the main phase are of the close typological relations to other burials. Bead and ornament types are remarkably standardized, this holds true especially for the pointed ovoid mother-of-pearl rings ("paillettes") which seem to be associated in most cases to infants.

It seems more and more probable that at least in the collective but possibly also in the double and multiple burials primary and secondary burials were combined and that corpses were possibly "stored" somewhere else or reburied later (see also CG1 and CG9). The use of fire during the burial rituals is very prominent in this burial sequence and matches several observations for other burials. The deliberate fragmentation and deposition of fragmented objects, especially of stone vessel fragments, is also typical and has been observed here for the two more recent phases. Besides, the covering of the burials with either a thick layer of stones and / or by reused terrazzo-like plaster floor was observed for the first burial phase (stones) and for the large heap of bones (Loc. CR17: 131/133/137) with finely crushed gravels to enhance the white appearance of the cover. Additionally, the use of red pigments which were intensively used in many other burials has also been found here, but in the more upper layers and the use of large lumps of yellow ochre seems to be increasingly frequent. However, the excavation of this room has not been terminated and only dates to the second major occupation phase. Further excavations are much waited for more information.

For more information on seasonal excavation reports of the LPPNB site of Ba`ja, see https://www.exoriente.org/baja/index.php.

Description of burials. Both BAJ020 and BAJ022 were spotted from Room CR17, collective burial CG11, the minimum number of individuals (MNI) recovered from which is 14. The original archaeological ID of BAJ020 (from an infant) is *Loc. CR17:127*. The original archaeological ID of BAJ022 (from an adult) is *Loc. CR17:130*. BAJ022 is slightly more ancient than BAJ020 according to the stratigraphic information.

Burial Settings.

CR17:127

Burial pit; touching CR17:123; length N–S: 80 cm; width E–W: 50 cm; containing human and animal bones; sediment ashes and loose silt; seems to be limited by a row of stones.

CR17:130

This locus comprises two layers: the upper layer is very ashy and contains many up to 10x10 cm large stones and a few bones; the lower layer of this locus has only a few charcoals, many disarticulated human and animal bones in the upper part and only human bones in the lower part. The layer is sloping down to the NE, thicker in the east (ca. 30 cm) and thinner in the west (ca. 5–10 cm) containing pieces of red and yellow ochre. In the center of the room, a zone of dark ashy soil contained a human mandibula with yellow ochre. This lower layer was part of the heap of human bones in the southwestern corner.

Note S2. Geological context in Southeastern Anatolia

The use of strontium isotope ratios ($^{87}Sr/^{86}Sr$) to investigate human and animal mobility based on the fact that rocks of different ages and Rb/Sr ratios have distinctive values. Therefore, different geological contexts lead to variations in the $^{87}Sr/^{86}Sr$ of soils and plants overlying the given lithological substrate thus the local bioavailable strontium baselines vary accordingly (73, 74). In many cases, ecosystem ratios are largely controlled by carbonate minerals, which weather rapidly and have total Sr concentrations ~2 to 6 times higher than most other rocks (8, 9). Nevertheless, the $^{87}Sr/^{86}Sr$ signatures were still subtly distinguishable in the Urfa region where the TSP sites, including Nevalı Çori, located, thanks to the contribution from occasional basalt formation, and different geological ages and rock types of the limestones, ranging from Eocene to Miocene across the region between Nevalı Çori and Göbekli Tepe, for example. The results of bioavailable strontium isotopic ranges provide informative distinctions between these two sites (Dataset S1, Fig. 1b). For 1:1 250 000 Scale geological maps of Turkey including Southeastern Anatolia with lithological distribution and geological substrate variations, one can refer to https://www.mta.gov.tr/en/maps/geological-1250000.

Note S3. Stable Isotope analysis of human remains

See Figs. S2 to S4.

Note S4. Stable isotope analysis of animal remains.

See Figs. S5, S6.

Note S5. Methods and Protocols

S5.1 Stable Isotope Analyses

S5.1.1 Background. In terrestrial environments, the primary source of stable carbon isotope variability is a result of fractionation during photosynthesis, where C_3-plants (Calvin cycle) discriminate significantly more against the heavy isotope ^{13}C than C_4-plants (Hatch-Slack cycle) (10). The carbon in the bone structural carbonate (bioapatite) is derived from blood bicarbonate and reflects carbon from all dietary components (protein, carbohydrates, fat) in its isotopic composition. Because of these links between environment, diet, and isotopic compositions, $\delta^{13}C$ in bioapatite has enjoyed extensive application in a variety of palaeoecological and paleodietary studies (11). C_3-plants have an average isotope composition of ~ –26‰, whereas C_4-plants cluster ~14‰ higher at ~ –12‰. This isotope difference is passed into herbivores which consume the plants: C_4 consumers have $\delta^{13}C$ values that cluster ~14‰ higher than C_3 consumers (0 to +2‰ vs. ~ –12‰). The 14‰ offset between plant and enamel $\delta^{13}C$ (e.g., C_3-plants vs. enamel of C_3-plants consumers) reflects two components: the bulk $\delta^{13}C$ enrichment of an animal over its food source (2–4‰, (12)) and a large isotopic fractionation between the bulk $\delta^{13}C$ of an animal and the $\delta^{13}C$ of the carbonate component of bioapatite. $\delta^{13}C$ values that fall between C_3 and C_4 diet endmembers probably reflect mixed feeding.

Oxygen naturally occurs, with decreasing abundance, as one of three stable isotopes: ^{16}O (99.755%), ^{18}O (0.206%) and ^{17}O (0.039%) (13, 14). Stable oxygen isotope analyses of skeletal remains ($^{18}O/^{16}O$, $\delta^{18}O$) are a powerful tool for exploring major themes in bioarcheology. The $\delta^{18}O$ value in mammal and human enamel bioapatite is expressed by either carbonate ($\delta^{18}O_{ca}$) or phosphate ($\delta^{18}O_p$) in which both $\delta^{18}O$ values are well correlated (15). The use of the VSMOW/SLAP scale is recommended and has been widely adopted; however, $\delta^{18}O$ values are often also reported on the VPDB (Vienna Pee Dee Belemnite) scale as the measurement for $\delta^{18}O_c$ is simpler than that for $\delta^{18}O_p$.

The two scales can be related to each other through VSMOW using conversion equation:

Thus,
$\delta^{18}O_{V\text{-}SMOW} = 1.03091\delta^{18}O_{PDB} + 30.91$
or
$\delta^{18}O_{PDB} = 0.97002\delta^{18}O_{V\text{-}SMOW} - 29.98$
(16)

At relatively constant body temperature, the $\delta^{18}O$ ratios in large mammal and human enamel form in isotopic equilibrium with body water which is systematically related to the isotope values of drinking water ($\delta^{18}O_{dw}$) and ultimately derived from precipitation ($\delta^{18}O_{precip}$) (17). The isotopic composition of precipitation depends on a variety of geographical factors such as temperature, latitude, altitude, and distance from the sea (18, 19). Numerous studies have established the connection of $\delta^{18}O$ values between drinking water and mammalian bioapatite phosphate or carbonate (20). A linear relationship between mean annual precipitation and human bone phosphate has been established (21). And a fractionation equation between the $\delta^{18}O$ value of drinking water and enamel phosphate ($\delta^{18}O_p/\delta^{18}O_{dw}$) using modern human tooth enamel has been proposed (22). By translating measured enamel $\delta^{18}O$ values into the corresponding drinking water values based on conversion equations and subsequently compared to the $\delta^{18}O$ local range could be of assistance in investigating the mobility of the inhabiting population (23, 24), although it can be notoriously difficult to be interpreted due to the result of climate change. Making use of the preserved animal and human tooth and bone commonly found at archaeological sites, applications include the reconstruction of paleo-climate and paleo-seasonality; animal husbandry and management practices; human and animal lifetime mobility and provenance; and cultural practices such as breastfeeding, weaning and even past culinary preparation techniques (25-27).

S5.1.2 Stable isotope analyses. Teeth were cleaned using a sandblaster to remove any external dirt. About 7mg enamel powder was taken along the full length of the buccal surface or fragment of the sampled teeth in order to maximize the period of formation represented by the resulting bulk samples. Enamel powder was pre-treated using a protocol to remove any organic or secondary carbonate contaminates. This consisted of the application of 1.5% sodium hypochlorite for 60 minutes, followed by three rinses in purified H_2O and centrifuging, before 0.1 M acetic acid was added for 10 minutes, followed by another three rinses in purified H_2O (28).

Following reaction with 100% phosphoric acid, gases evolved from the samples were analyzed for stable carbon and oxygen isotopic composition using a Thermo Gas Bench II connected to a Thermo Delta V Advantage Mass Spectrometer at the Max Planck Institute for Geoanthropology (MPI-GEA) (formerly the Science of Human History, MPI-SHH), Jena.

S5.1.3 Calibration and analytical uncertainty measurements. See Datasets S11 and S12 for more information on the materials and measured values for calibration and internal accuracy monitor. We compared the resulting values using a three-point calibration against International Standards IAEA-603 ($\delta^{13}C$ = 2.46‰; $\delta^{18}O$ = −2.37‰); IAEA-CO-8 ($\delta^{13}C$ = −5.8‰; $\delta^{18}O$ = −22.7‰); IAEA NBS 18 ($\delta^{13}C$ = 5.014‰, $\delta^{18}O$ = −23.2‰). USGS44 ($\delta^{13}C$ = −42.2‰) was run as an internal standard. Replicate analysis of internal bovid enamel standards suggest that the machine measurement errors are ±0.2‰ for $\delta^{13}C$ and ±0.2‰ for $\delta^{18}O$. An equid enamel standard was run to access systemic error (accuracy). Accuracy or systematic error (u(bias)) was determined to be 0.3‰ for $\delta^{13}C$ and $\delta^{18}O$ on the basis of the difference between the observed and known values of the check standards and the long-term standard deviations of these check standards. Precision ($u(R_w)$) was determined to be 0.2‰ for both $\delta^{13}C$ and $\delta^{18}O$ on the basis of repeated measurements of calibration standards, check standards, and sample replicates. Using the equations provided by Szpak et al. in 2017 (29), the total analytical uncertainty was estimated to be ±0.3‰ for $\delta^{13}C$ and ±0.4‰ for $\delta^{18}O$ (see Datasets S11 and S12).

S5.2 Strontium Isotope Analysis

S5.2.1 Background. Strontium (Sr) has four isotopes (^{84}Sr, ^{86}Sr, ^{87}Sr, and ^{88}Sr) in nature, among which additional ^{87}Sr forms from the decay of rubidium (^{87}Rb). The use of strontium isotope ratios (^{87}Sr/^{86}Sr) to determine human and animal mobility rests on the basic concept that rocks of different ages and compositions (with respect to Rb/Sr values) have distinctive values that do not fractionate from the bedrock into the biosphere (including the tissues of any individual measured) (30, 31). Older rocks have relatively high ^{87}Sr/^{86}Sr when compared to younger rocks, while different mineral contents with different ^{87}Sr/^{86}Sr ratios leads to characteristic ^{87}Sr/^{86}Sr based on rock 'type' (32). For example, limestones have lower Rb/Sr ratios than continental granites (30). While there is a lack of mass-dependent fractionation from the bedrock into the food chain, bedrock ^{87}Sr/^{86}Sr does not always correlate with bioavailable ^{87}Sr/^{86}Sr. Differential weathering of rocks with different ^{87}Sr/^{86}Sr ratios, as well as the geographic variability of hydrology and aeolian transport, lead to variations in the ^{87}Sr/^{86}Sr of soils and plants overlying a given distribution of rocks (30).

From plants, Sr^{2+} actively replaces Ca^{2+} in consumer tissues as part of the process of nutrient uptake and excretion (30, 33), with the amount of strontium incorporated into the skeleton believed to be directly reflective of that available from the diet (and environment) (34). In the context of tooth enamel from permanent teeth, Sr^{2+} (and ^{87}Sr/^{86}Sr ratios) will be incorporated during the process of mineralization which will vary depending on the tooth sampled anywhere between ten weeks before birth and 16 years of age (35). By sampling teeth that form during different periods of life and comparing the ^{87}Sr/^{86}Sr ratios of these teeth to bioavailable ^{87}Sr/^{86}Sr ratios in the burial locale of an individuals, it is possible to determine whether someone grew up in a different location to that in which they were buried, thus determining potential migration during the life of an individual (36-38). The robustness and detail of the bioavailable baseline created is important as the ^{87}Sr/^{86}Sr of an individual will be the combined consequence of all food consumed and all water drunk, as well as the extent of the range over which it obtains food and drink (significant in the context of trade in food or presence of long rivers) (30), in some cases with the special food consumption, the interpretation should be carefully made with the local context (39). In this case, we consider the pigs and fox excavated from *in situ* could be representatives for evaluating the local Sr isotope interval.

S5.2.2 Strontium isotope analysis. We sampled 44 human teeth of 28 individuals from Nevalı Çori spanning from PPNB to IA periods for strontium isotope analysis. The teeth sampled were permanent 1st, 2nd and 3rd molars, and one dentine sample with the value matching both the local baseline and the value of the enamel from the same tooth (only presented in the Dataset S1, but not statistically included in the study,) in this order, the different periods of life: 0–3 years, 3–7 years, early adult, adult or the burial environmental value are respectively (40) represented to see if there were any distinctions between their ^{87}Sr/^{86}Sr ratios during childhood and the bioavailable ^{87}Sr/^{86}Sr of their burial location. All teeth were first cleaned using a sandblaster and a groove was made along the buccal edge of the tooth, in order to average the signal for the period of enamel formation (40). We obtained powdered samples of 20 mg of each specimen using a Dremel drill at the Stable Isotope Laboratory of the Department of Archaeology, Max Planck Institute for the Science of Human History, Jena, Germany.

The samples were then shipped to the clean laboratory in the Department of Geological Sciences at the University of Cape Town. Samples were dissolved in 2 mL 65% 2B HNO3 in a closed Teflon beaker and placed for one hour on a hotplate at 140ºC. The samples were then dried down and re-dissolved in 1.5 mL 2M HNO_3. Strontium separation chemistry followed methods discussed in (41). After separation, the solutions for each sample were dried, dissolved in 2 mL 0.2% HNO_3 and diluted to 200 ppb Sr concentrations for strontium isotope analysis. Radiogenic $^{87}Sr/^{86}Sr$ ratios were measured using a Nu Instruments Nu Plasma HR MC-ICP-MS in the Department of Geological Sciences at the University of Cape Town. Sample values were corrected for instrumental mass fractionation using an $^{86}Sr/^{88}Sr$ ratio of 0.1194 (42) and isobaric ^{87}Rb interference using the measured ^{85}Rb signal and the natural Rb isotope ratio. All data presented are referenced to bracketing analyses of NIST SRM987 ($^{87}Sr/^{86}Sr$ reference value of 0.710255). Results for repeat analyses of an in-house carbonate reference material (NM95) processed and measured with the batches of unknown samples in this study gave an $^{87}Sr/^{86}Sr$ ratio of 0.708911 (2s = 0.000012; n = 7) agree with long-term results for this in-house reference material having an average $^{87}Sr/^{86}Sr$ ratio of 0.708911 (2s = 0.000040; n = 414).

S5.3 Genetic Analyses

S5.3.1 Sampling and data production. The aDNA analysis of the Nevalı Çori and Ba`ja individuals was conducted following a genome-scale approach. The sampling was targeted to the petrous portions of the temporal bone and the teeth, as these skeletal elements were shown to preserve DNA statistically better than others (43-45). The sampling process was based on standardized minimally invasive protocols, and all the steps followed can be found as archived protocols.io: (https://www.protocols.io/workspaces/mpieva-archaeogenetics). The bone powder lysates were converted into single-stranded libraries for Next Generation Sequencing (NGS) on an Illumina platform with a semi-automated liquid handling system (46-48). Following the targeted enrichment of ca. 1.2mio nuclear markers (SNPs; ‘1240K capture’) (49-51), a few of the libraries generated a very low amount of genetic information and were excluded from the downstream analyses (Datasets S5 and S6). These were typically libraries with very low portions of endogenous after the metagenomic shotgun screening (ca. 0.1% or more) but exhibited characteristic deamination patterns (52). To increase the resolution of genetic data, in some cases, more than one genomic libraries were prepared from the lysate of the sampled bone powder. The sequencing data from all the enriched libraries were processed using the EAGER pipeline (53), which included the clipping of remaining adaptor sequences (AdapterRemoval [v2.2.0] (54)), mapping of the sequenced reads longer than 30bp against the human genome reference (hs37d5) with Borrows wheeler aligner (BWA) [v0.7.12] (55), and removal of duplicate reads with the embedded tool (dedup [v0.12.2]). Bam files were merged across libraries from the same sample, and genotypes were extracted with pileupCaller from the pileups generated with samtools (56) at the targeted 1240K SNPs that were covered by at least one sequencing read. For the three PPNB individuals the mitochondrial genome and the Y-chromosome were enriched with in-solution hybridization protocols (57, 58).

S5.3.2 Estimation of contamination rates. Rates of contamination by external modern human DNA sources were estimated with three methods that differ in the information they harness: 1) schmutzi (59) estimates mitochondrial contamination based on the divergence from haploidy in the mitochondrial DNA and the presence of deamination at the ends of the fragments, 2) Xcont from ANGSD (60) looks into the divergence from haploidy in the X-chromosome in male individuals, and 3) AuthentiCT (61) leverages the non-random distribution of aDNA damage (C > T deaminations) across the fragments.

S5.3.3 Determination of genetic sex, relatives and uniparental haplogroups. Genetic sex could be unambiguously determined in all six individuals as the ratio of the coverage on X-chromosome and the Y-chromosome, both normalized by the overage on the autosomal chromosomes (62). The results from all the three methods are presented in Dataset S6.

Possible sample mix-ups and presence of genetic relatives were checked by calculating the rate of mismatching genotypes between a pair of individuals (63, 64), which indicated uniquely assigned individuals and no relatives within the present and the published dataset from Ba`ja (65).

Uniparental haplogroups were assigned on the sequencing data obtained after the1240K (for NEV020, NEV021, NEV030), or the mitochondrial e and the Y-chromosome enrichments (for BAJ020, BAJ022 and NEV009). For the mitochondrial genome, consensus sequences were first generated with Schmutzi (fasta format) after mapping with the CircularMapper (53), and the haplogroups were assigned with HaploGrep (66). To determine the Y-haplogroups, first we generated pileups with Rsamtools (56), and called the Y-chromosome SNPs while creating a list of ancestral and derived alleles for the ISOGG SNP index. We manually inspected whether the presence of diagnostic SNPs for a given Y-haplogroup were following a root-to-tip presence of derived alleles, without spurious jumps in the phylogeny owing to residual DNA damage (cytosine deaminations leading to C-T or transitions).

S5.3.4 Population genetics. To visualize genetic structure among the PPNB individuals, and the NEV individuals of later periods, we performed a principal component analysis (PCA), using the *smartpca* program of the EIGENSOFT software (67, 68). A usually preferred setting for this analysis is to compute the PCs from modern populations in order to account for the effect of ancient samples' missing data. Therefore, *smartpca* was run on present-day West Eurasian populations (ca. 1800 individuals) and all the ancient data from the present study and relevant published datasets were projected with the embedded function of the software *lsqproject*.

We formally tested visual remarks from the PCA with f_4 /*D*-statistics (69). In brief, these are four-population tests that calculate the allele correlations between the populations/individuals [e.g., f_4 (A, B; C, D)], and should not deviate from 0 if C and D form a distinct clade with respect to A and B. However, if the tested topology is not true, or gene-flow between A/B with C/D occurred after their divergence, the statistic takes a non-zero value, with ±3 SE being the commonly applied significance threshold. In the case A is outgroup to B, C and D, the f_4 /*D*-statistic is a direct measure of excessive allele sharing between B and either C (negative value of statistic), or D (positive value of statistic).

Furthermore, multiple f_4-statistics can be combined in a framework for estimating the independent streams of ancestry between a set of target and reference populations (*qpWave*), and for modeling a target as a linear combination from specific source populations (*qpAdm;* Fig. S7). For valid results with *qpAdm*, the target should relate with the references through the source populations, and no subsequent direct gene flow between the references and the target must have occurred. Therefore, the power of this tool to resolve admixture scenarios relies on the availability and the choice of reference populations. Here, the choice of the 11 reference populations ('right pops') represents diverse lineages that are expected to be outgroups to all sources (e.g., Mbuti), as well as ancestral Early Holocene groups that could relate differentially to the targets and references (e.g., the ‘Natufian’ and Caucasian hunter-gatherers). In this method the proposed admixture model of a linear combination between *x* sources (null model) is compared with a likelihood test with the full model in which the target population can vary freely (70). In the case the null model has a low p-value it gets rejected (typically p-value ≤ 0.01 or ≤ 0.05), and a more complex model is required to explain the data.

S5.3.5 Close-kin unions. Compared to the methods used above, haplotype-based methods harness information from chunks of markers across the chromosomes that are linked and thus are co-inherited from a single parent to the offspring. Within an individual, the paternal and maternal haplotype might be identical. These homozygous stretches are called Runs of Homozygosity (ROH), and their length and distribution disclose information related to both demography (i.e., population sizes, bottlenecks), and/or inbreeding/consanguinity. The recently developed method *hapROH* (71) can effectively estimate ROH from genome-wide data like those generated from 1240K capture data using a reference panel of haplotypes from modern populations. Following the recommendations of the authors, the method was tested on the Nevalı Çori and Ba`ja as well as other Early Holocene individuals with a coverage of at least 300,000 SNPs. We used the embedded functions of the program for plotting the ROH as individual histograms with expected distributions under specific scenarios of parental relatedness (Fig. S8).

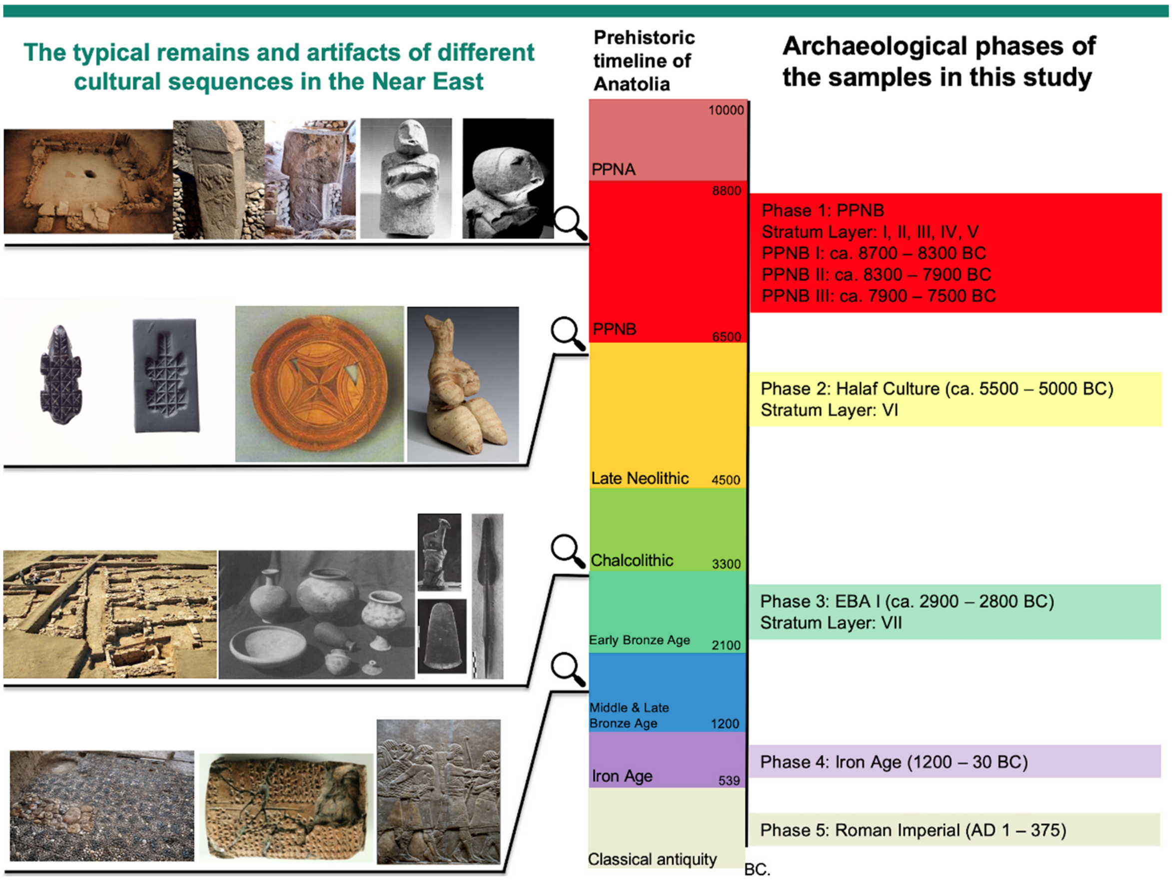

Fig. S1. Cultural Sequence of Nevalı Çori. The sequence of archeological cultures featured with typical remaining of buildings and articles of respective periods. The right column indicated the corresponding phases from which the samples in this study were excavated.

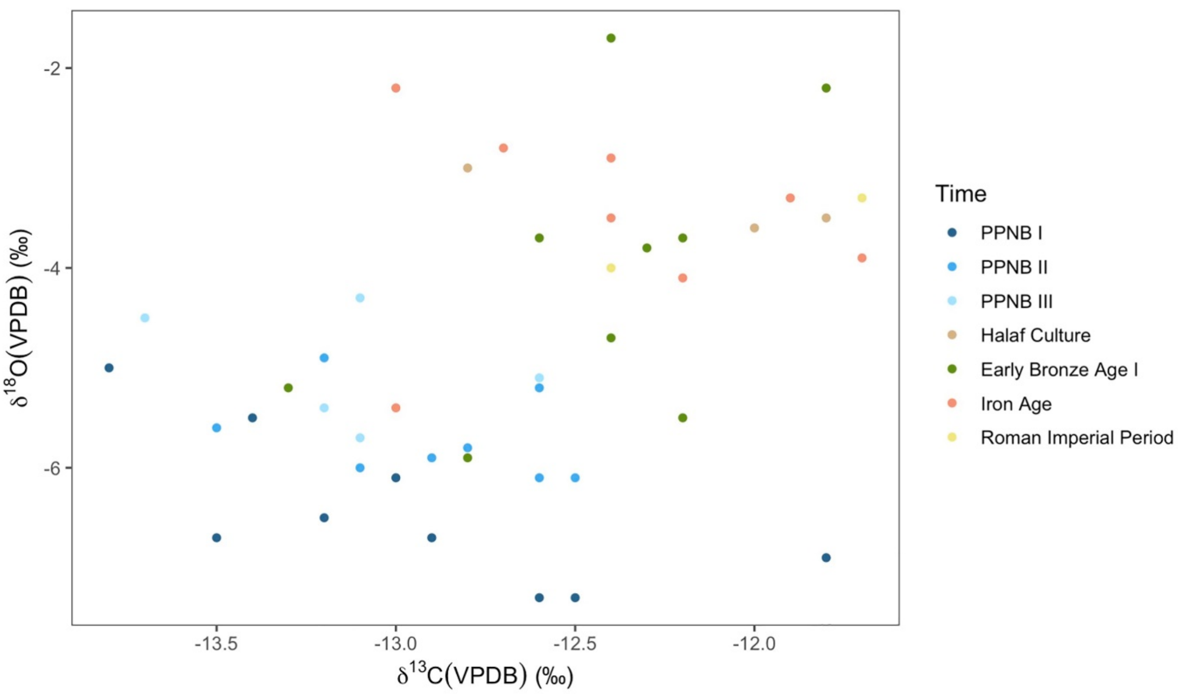

Fig. S2. Scatter plot of $\delta^{18}O$ and $\delta^{13}C$ values of the individuals from Nevalı Çori.

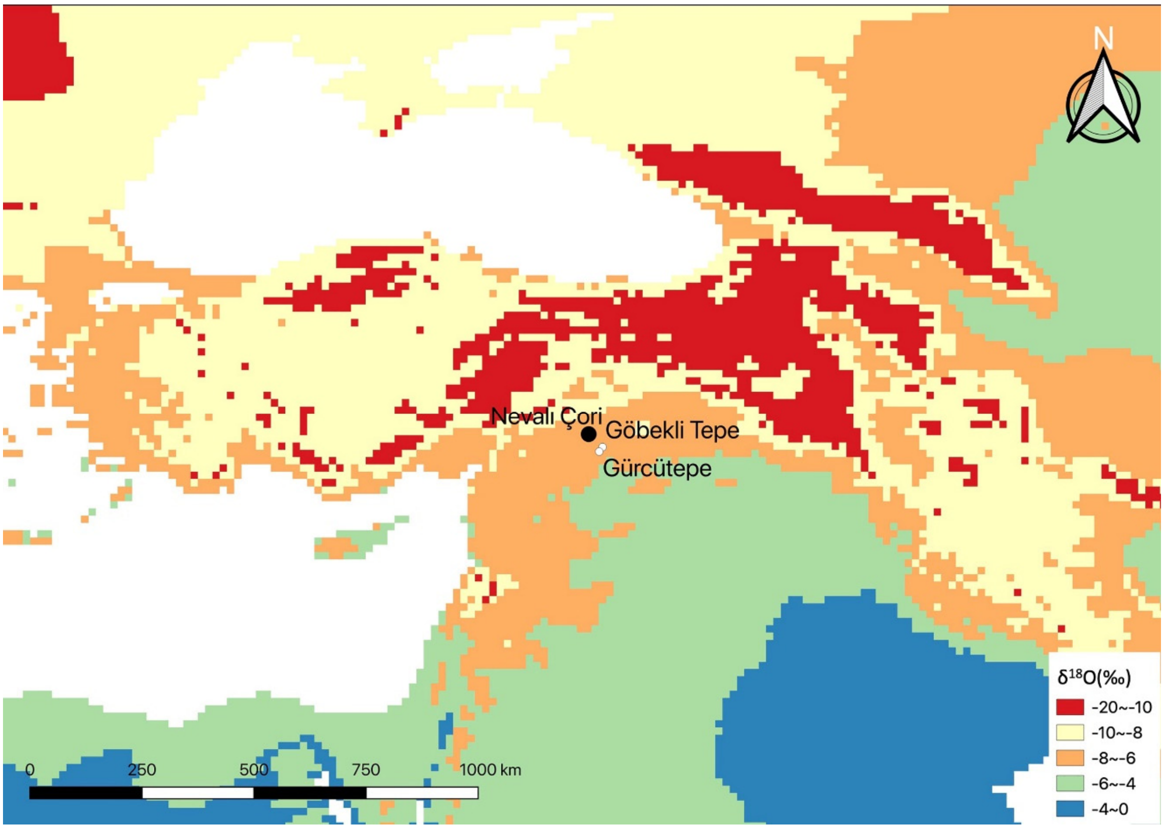

Fig. S3. The map showing relevant sites in the spatial distribution of annual mean precipitation δ^{18}O values in extensive Anatolian area using RCWIP data products (modified after 2013 Water Resources Programme International Atomic Energy Agency; Data sources: GNIP, Natural Earth 2, colorbrewer.org). Modern precipitation for this region, based on predictive modeling demonstrates δ^{18}O values in Urfa region with yearly averages ranging from –8‰ to –6‰, while the Göbekli Tepe and Nevalı Çori are too closed in the same zone to be distinguished.

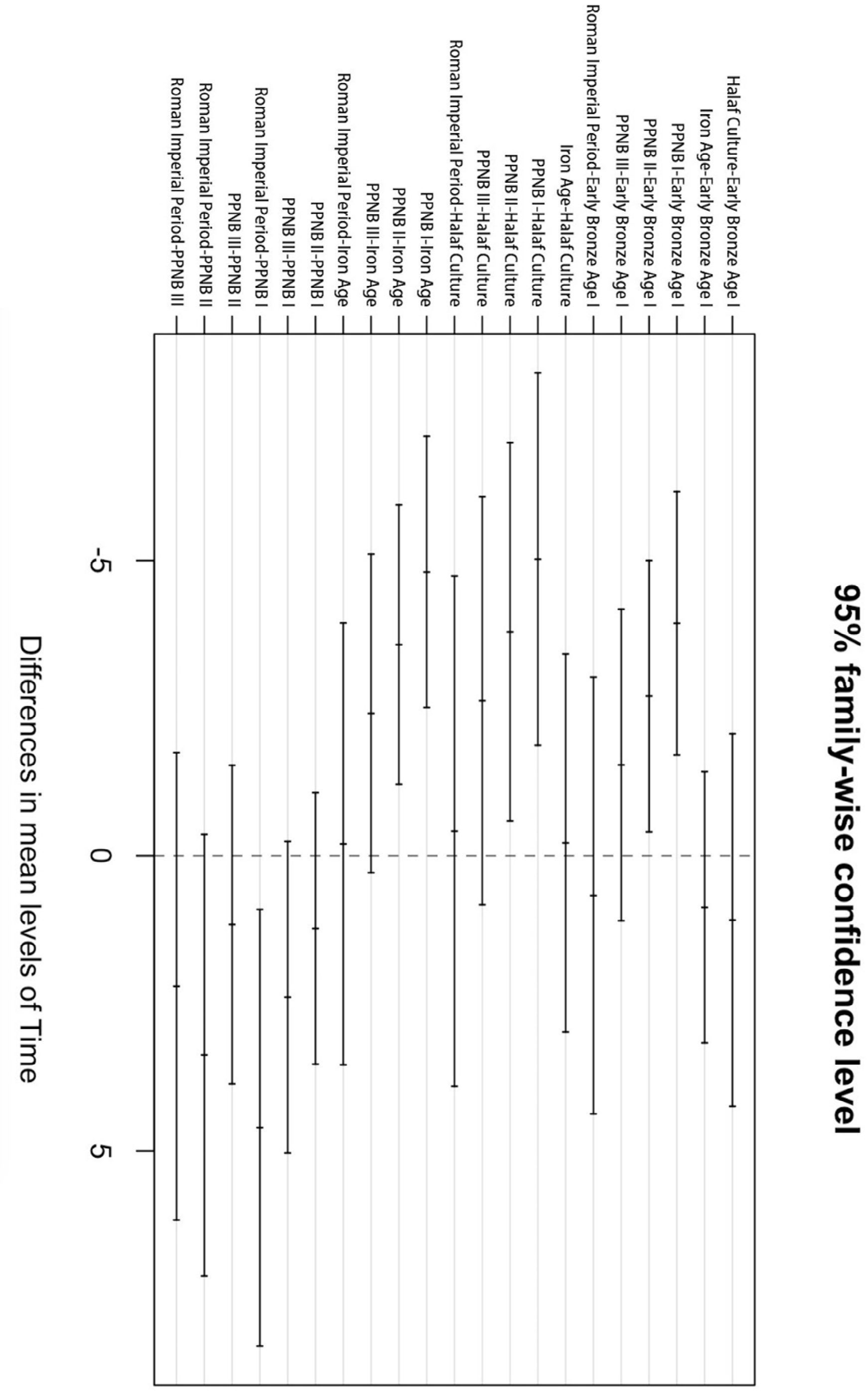

Fig. S4. Results of Tukey HSD comparison between the δ^{18}O value of human remains from different archaeological phases at Nevalı Çori.

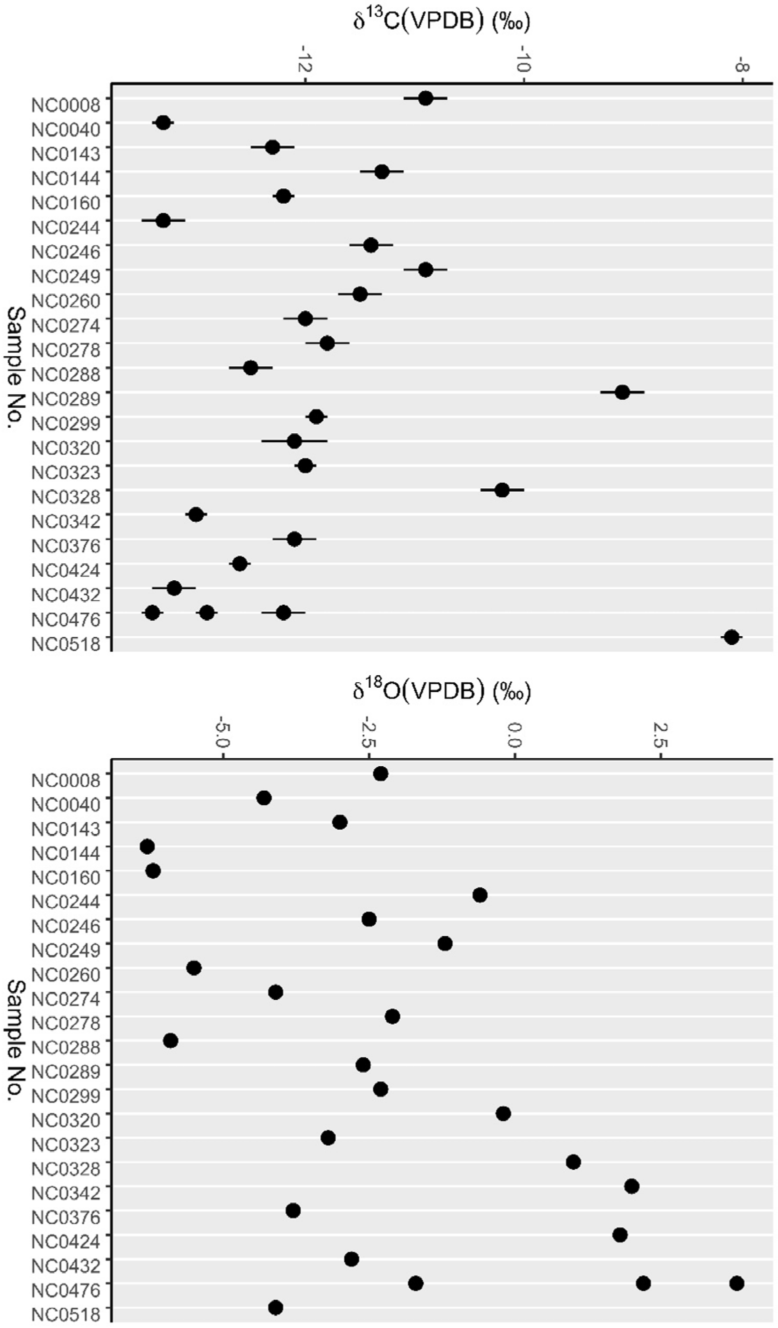

Fig. S5. The plot of δ^{13}C and δ^{18}O values of fauna samples recovered from Nevalı Çori.

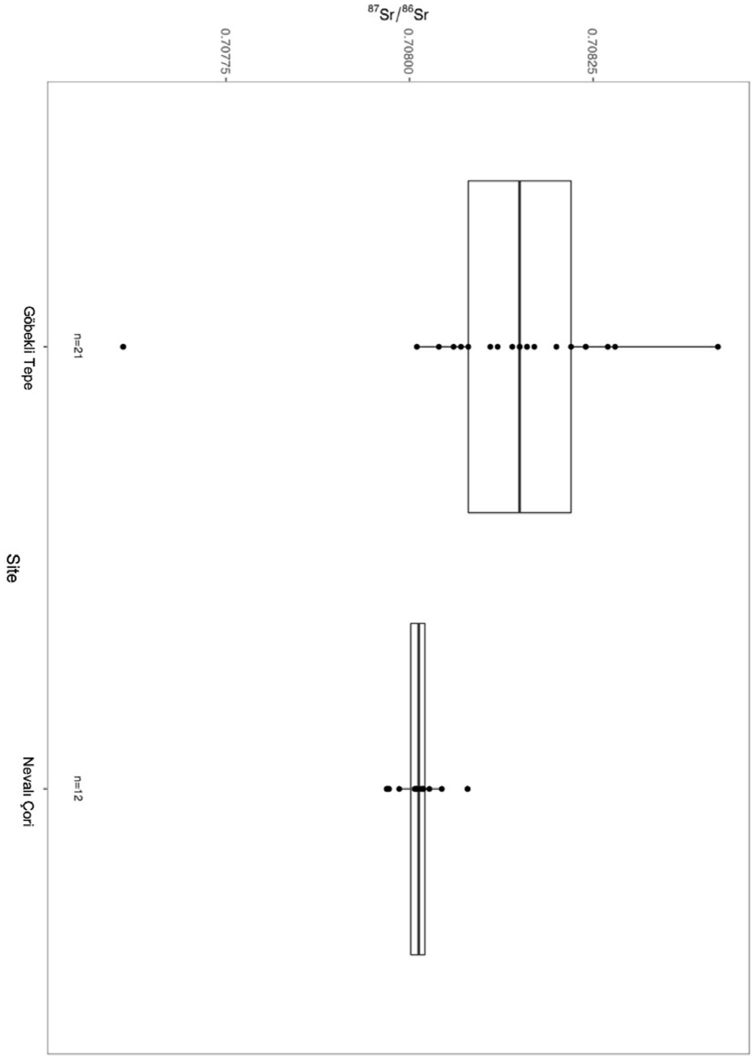

Fig. S6. The ^{87}Sr/^{86}Sr ratios of 12 gazelle individuals of Nevalı Çori from PPNB I and II, and 21 gazelle individuals from Göbekli Tepe. The data of Göbekli Tepe referred to Lang, et al., 2013 (72).

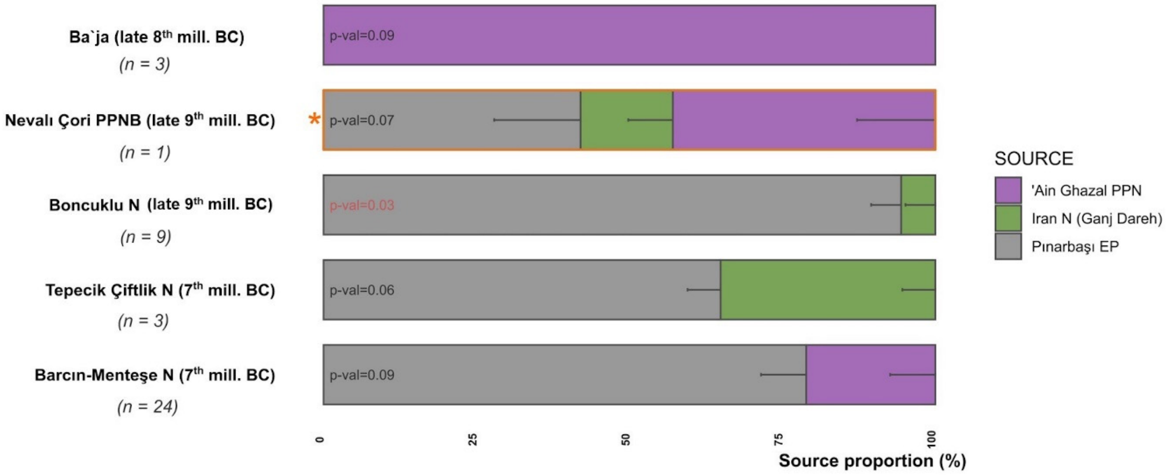

Fig. S7. Ancestry admixture with *qpAdm*. All Anatolian populations including Nevalı Çori carry predominantly ancestry descending from the Anatolian HG. However, for Nevalı Çori a model of three-way contribution including a S. Levantine (‘Ain Ghazal), and a W. Iranian (Ganj Dareh) source is adequate, suffice that CHG is not included in the reference (right) populations (**). Levantine or Iranian-like ancestries are fitted in the models of the other farming Anatolian groups with the complete list of right pops. Standard errors are plotted as –1SE. P-values that larger than 0.05 are typically interpreted as a good fit of the *qpAdm* model.

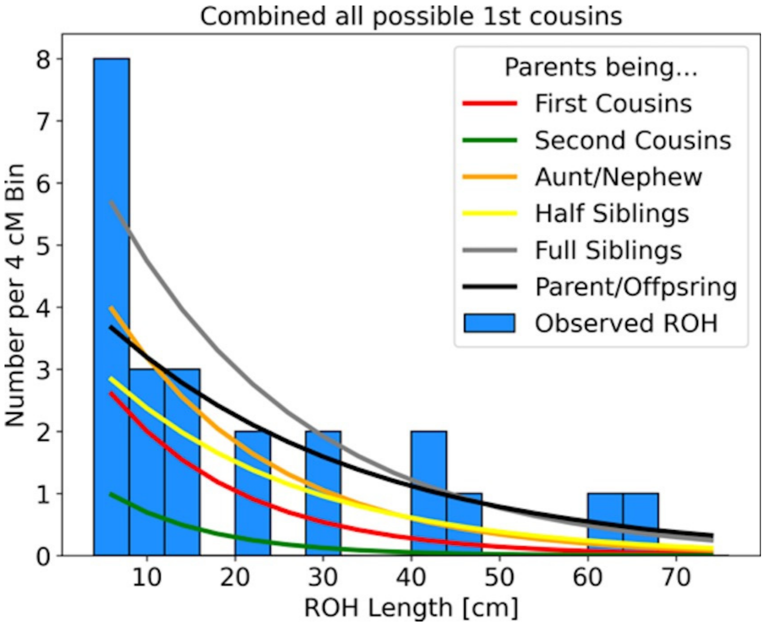

Fig. S8. Histogram of ROH (>4 cM) distribution within NEV020 in bins of 4 cM, produced from the HapROH software. Different scenarios of parental unions lead to different degrees of relatedness and/or meiotic events which determine distinct ROH length distributions.

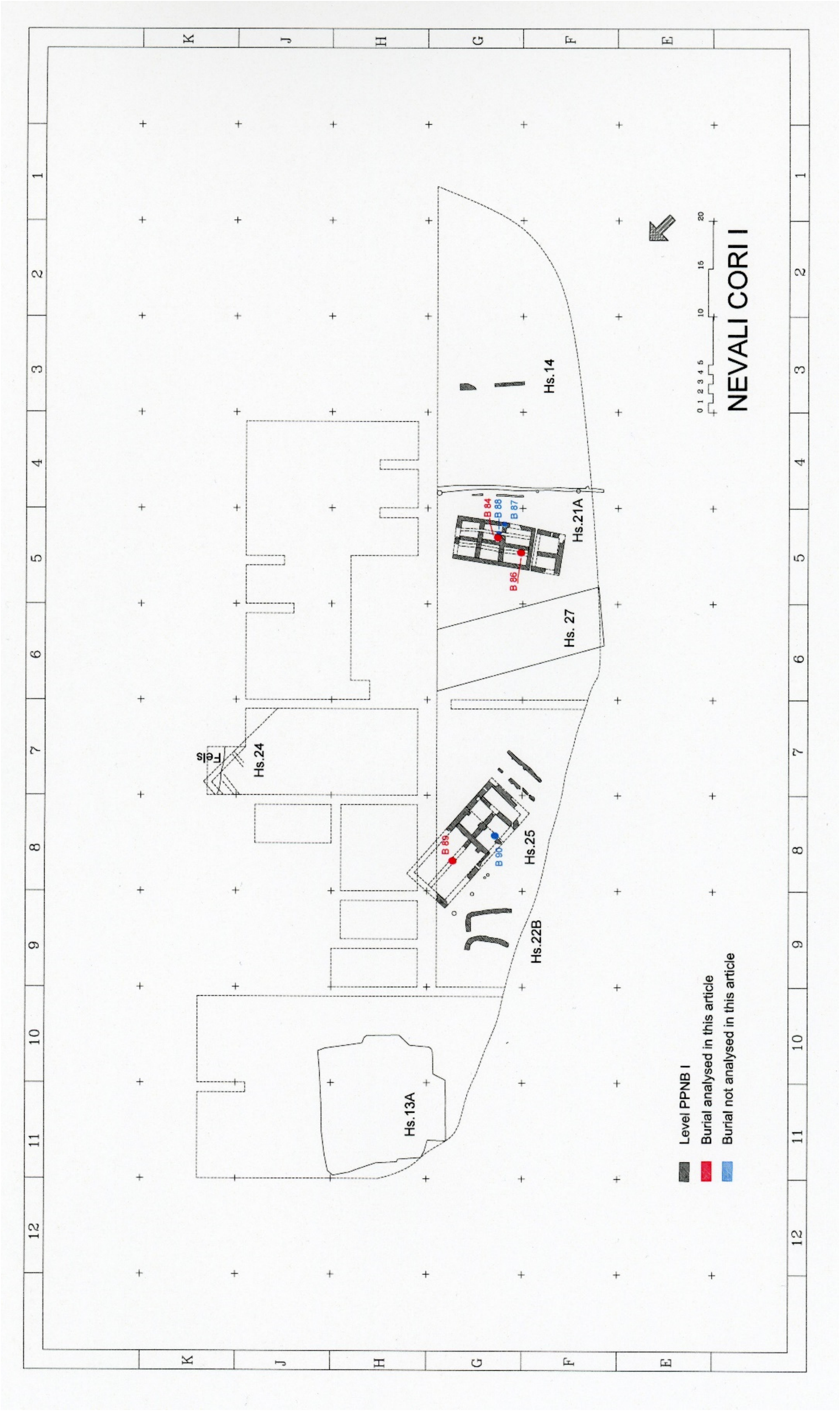

Fig. S9a. Ground plan of Nevalı Çori site with the burials of sampled individuals of the PPNB I.

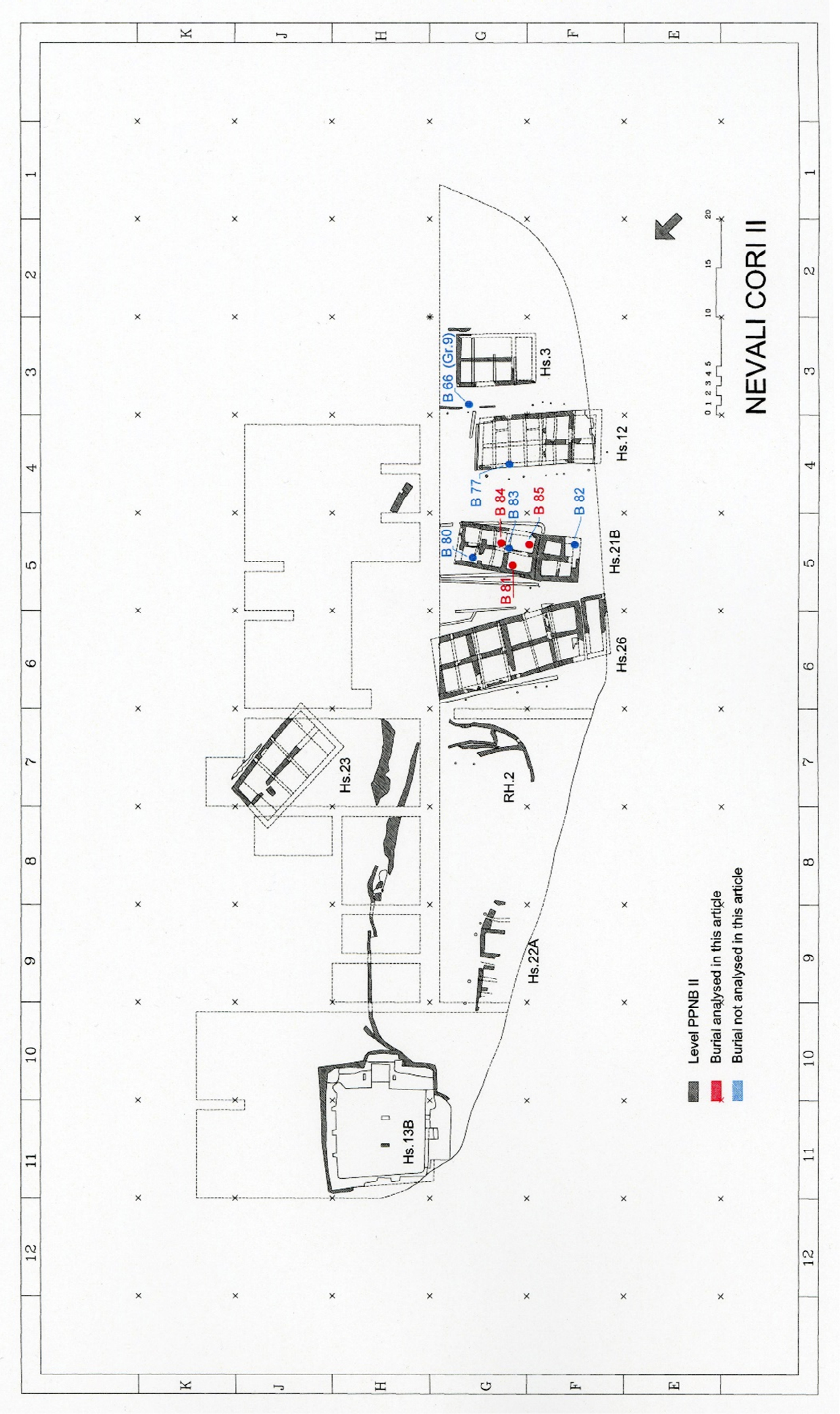

Fig. S9b. Ground plan of Nevalı Çori site with the burials of sampled individuals of the PPNB II.

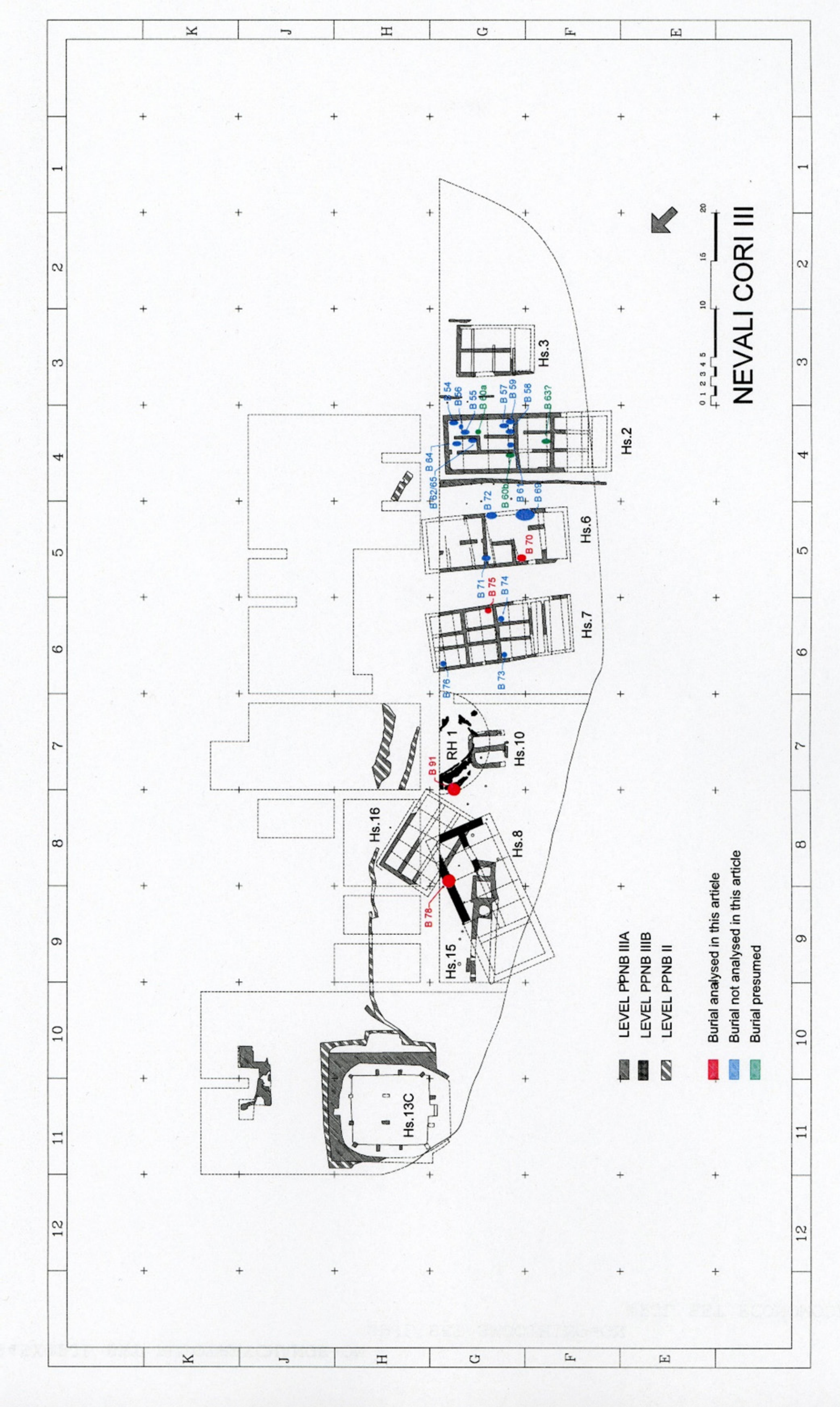

Fig. S9c. Ground plan of Nevalı Çori site with the burials of sampled individuals of the PPNB III.

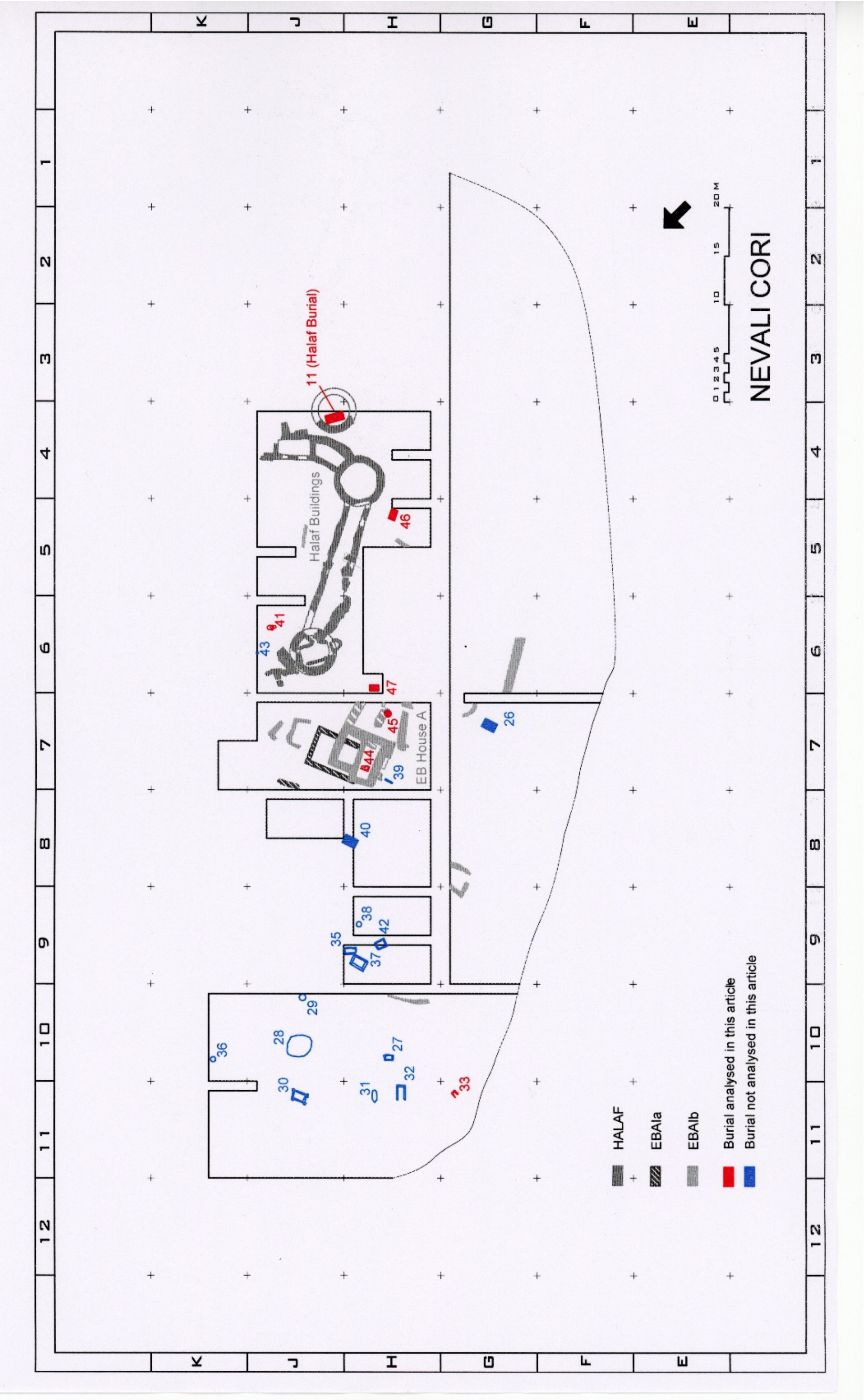

Fig. S9d. Ground plan of Nevalı Çori site with the burials of sampled individuals of Halaf period to the Bronze Age.

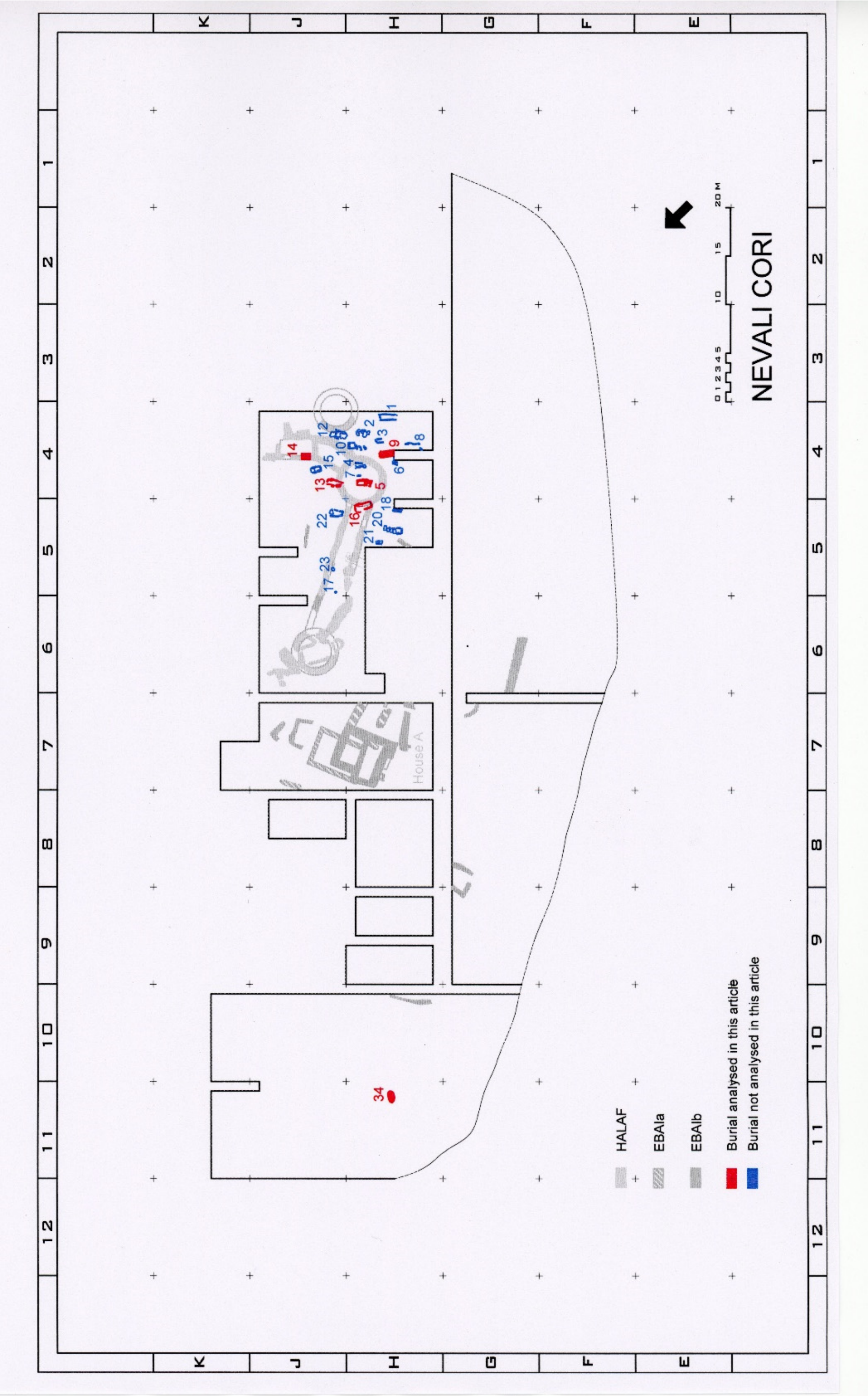

Fig. S9e. Ground plan of Nevalı Çori site with the burials of sampled individuals of the Iron Age.

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10.2 Supplementary material for Manuscript B

Isotopic and proteomic evidence for communal stability at Pre-Pottery Neolithic Jericho in the Southern Levant

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Supplementary Material

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S1 Materials

The detailed contextual information of the samples is listed in the Appendix Table S1. All samples were recovered from the site of Jericho, where the first excavations at the site were conducted by Charles Warren in 1868 [1]. From 1952 to 1958, extensive excavations and research were led by Kathleen M. Kenyon [2-4]. Subsequently, the Italian-Palestinian archaeological expedition carried out 13 seasons of excavation in 20 years (1997–2017) [5,6].

All skeletal material included in this study was excavated during the fieldwork under the direction of Kathleen Kenyon. During Kenyon's fieldwork, Jericho was part of the territory of Jordan and all fieldwork followed the rules and permissions of the Jordanian state. As part of her team, she included the young German physical anthropologist Gottfried Kurth (*1912–†1990), who was responsible for the excavation and documentation of all human bone material found during the fieldwork. He worked closely together with Kenyon as well as the workmen, who gave him the nickname "Uncle Bone". Including a physical anthropologist in the field in order to guarantee the best possible preservation and study of human bone material was novel and ground-breaking at that time. All bone material found during the excavation was kept - irrespective of the size of the fragment - and Kurth was to study this material as his contribution to the Jericho excavations. This treatment of the human bone material was rather unusual for that time, as many excavations of that time and later in many parts of the world usually discarded most of the bone material and only kept complete human skulls.

At the end of her work at Jericho, Kathleen Kenyon decided to hand over the human bone material to Kurth for further study. Consequently, the material was exported legally according to the rule of find division and brought to Germany where it should form the basis of Kurth's anthropological collection at the University of Braunschweig, where Kurth had just accepted an academic position ("Hochschuldozent") in 1965. During his life, Kurth tried to acquire funding for the study of the Jericho bone material, but he was unsuccessful, as reviewers considered the material too fragmented to be relevant for further study. When Kurth retired in 1977, the University of Braunschweig decided not to replace his position and wanted to discard Kurth's bone collection. In order to preserve his collection, he handed it over to one of his last Ph.D. students, Olaf Röhrer-Ertl (*1940–†2019), whom he told to continue the analyses of the Jericho material. Shortly afterwards, Röhrer-Ertl received a position at the Bavarian Anthropological State Collection in Munich and, consequently, took the Jericho bones with him. The bone material was then stored in the rooms of his private apartment until his death in 2019. Then, the heirs' community decided that the bones should be moved out of the apartment and rented a cellar for their storage. In the same year, Stockhammer was contacted by the heirs' community via a lawyer. He was asked to help in the search for a future and sustainable storage place for human bones and to continue their scientific evaluation following the bequest and wishes of Kurth and the late Olaf Röhrer-Ertl. The permit for sampling was, therefore, issued by Eleonora Röhrer-Ertl, one of the daughters of Olaf Röhrer-Ertl as representative of the heirs' community. Subsequently, an agreement was made with the anthropological collection of the University of Göttingen, where the bones were then brought for future storage after they had been sampled for bioarchaeological analyses in Munich. Since 2020, the bones have been kept under the custody of the University of Göttingen.

All information on the history and itinerary of the human bone material is based on the written estate of Röhrer-Ertl (private letters, notes, diaries etc.), which was scanned by the heirs' and all scans were kindly forwarded to us. However, a comprehensive publication of the detailed contextual and anthropological analyses of Kurth and Röhrer-Ertl, which are preserved in this legacy, are beyond the scope of this publication.

Among the bone material selected by us for bioarchaeological study, it was interesting to note that among the teeth the M1 of individual JCH061 was heavily worn (Fig. S1), which is caused by different kinds of long-term habitual practice, such as subsistence or crafting activities.

In addition, to determine a baseline interval for the local $^{87}Sr/^{86}Sr$ signature at Jericho, we also sampled 1 archaeological animal tooth and 4 modern plant samples from different places in the vicinity of Jericho, with the coordinates all around 31.8 N, 35.4 E.

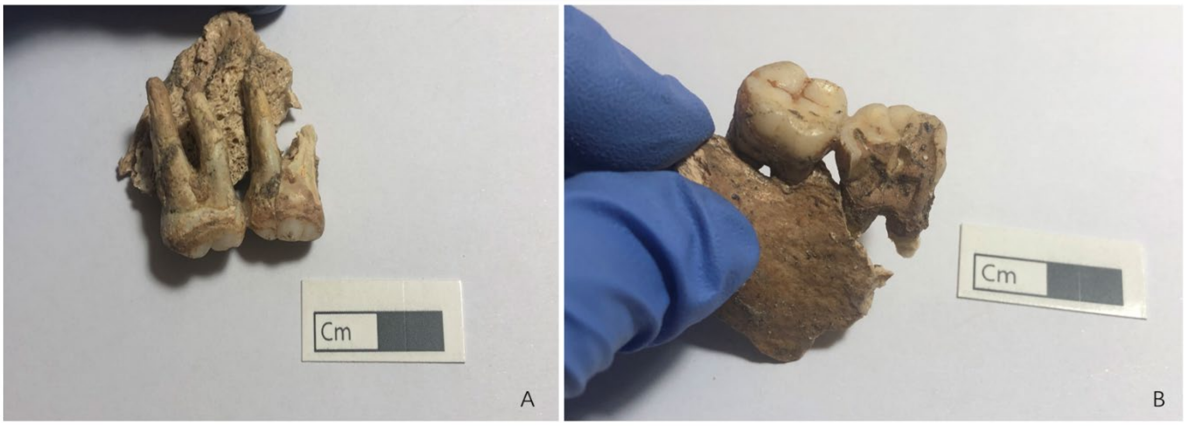

Fig. S1. The osteological morphology of the M1 of individual JCH061.

Fig. S2. The environmental contexts where the modern plant samples were collected.

S2 Methods

S2.1. Strontium isotope analysis

Analysis of $^{87}Sr/^{86}Sr$ of various bio- and geo-materials has been reported in many published studies from geographically and geologically varied regions across the Levant, both in comprehensive regional studies [7,8] and in local case studies where small batches of environmental materials from the target area serve as local base intervals (e.g., [9-13]). These studies have enabled the construction of a reliable $^{87}Sr/^{86}Sr$ map for the southern Levantine area comparable with those have been built in other regions of the world, e.g., [14-17].

^{87}Sr is a product of rubidium (^{87}Rb) decay and varies in abundance within different types of bedrocks. The fact that rocks of different ages and Rb/Sr ratios have distinctive values provides the foundation for the use of strontium isotope ratios ($^{87}Sr/^{86}Sr$) to investigate human and animal mobility [18,19]. The ratio of $^{87}Sr/^{86}Sr$ does not biologically fractionate as strontium moves through the food chain from the bedrock into the biosphere [20-23]. $^{87}Sr/^{86}Sr$ in bone and teeth should thus reflect the bio-available strontium in the area where the human and animal lived during tissue formation [21,24]. Tooth enamel is not remodeled after the formation of the crown, unlike bone which remodels throughout the life of individual with a turnover of strontium of ca. 26% per year [25]. Thus, the $^{87}Sr/^{86}Sr$ ratio of dental enamel can serve as a good proxy for evaluating the locale where the individual was born and grew up [21,26-

[29]. To establish local ranges (i.e., bioavailable local $^{87}Sr/^{86}Sr$ signature), different materials have been tested and evaluated such as bedrock, soil, underground water, spring water, and biological materials like plant, snails, modern and archaeological faunal teeth. This is due to the ways in which differential weathering of bedrock, different rooting heights, and other environmental factors can influence bio-available $^{87}Sr/^{86}Sr$ [30-32]. It is essential to create a robust and reliable bioavailable base range for a regional iso-scape [7,30,32-37]. Archaeological fauna, especially dental enamel from small mammals, provide an important material for constructing bioavailable local $^{87}Sr/^{86}Sr$ signature for an archaeological site [26,35].

All teeth in this study were sampled at the Stable Isotope Laboratory, Max Planck Institute of Geoanthropology (formerly the Max Planck Institute for the Science of Human History), Jena, Germany before being analyzed at the clean laboratory in the Department of Geological Sciences at the University of Cape Town. They were first cleaned using a sandblaster, and a groove was made along the buccal edge of the tooth in order to average the signal for the period of enamel formation [26]. We obtained powdered samples of 20 mg from each specimen in Jena using a Dremel drill, and the samples were then shipped to Cape Town. Samples were dissolved in 2 mL 65% HNO_3 in a closed Teflon beaker and placed for one hour on a hotplate at 140ºC. The samples were then dried down and re-dissolved in 1.5 mL 2M HNO_3. Strontium separation chemistry followed methods discussed in [38]. After separation, the solutions for each sample were dried, dissolved in 2 mL 0.2% HNO_3 and diluted to 200 ppb Sr concentrations for strontium isotope analysis. Radiogenic $^{87}Sr/^{86}Sr$ ratios were measured using a Nu Instruments Nu Plasma HR MC-ICP-MS in the Department of Geological Sciences at the University of Cape Town. Sample values were corrected for instrumental mass fractionation using the exponential law and an $^{86}Sr/^{88}Sr$ ratio of 0.1194 [39], and isobaric ^{87}Rb interference using the measured ^{85}Rb signal and the natural Rb isotope ratio. All data presented here are referenced to bracketing analyses of NIST SRM987 ($^{87}Sr/^{86}Sr$ reference value of 0.710255). Results for repeat analyses of an in-house carbonate reference material (NM95) processed and measured with the batches of unknown samples in this study gave an average $^{87}Sr/^{86}Sr$ ratio of 0.708911 (2s = 0.000012; n = 7) and are in agreement with long-term results for this in-house reference material having an average $^{87}Sr/^{86}Sr$ ratio of 0.708911 (2s = 0.000040; n = 414). Total procedural elemental Sr blanks agreed with typical values of < 250 pg in this facility, and therefore negligible.

The $^{87}Sr/^{86}Sr$ measurement of the plant samples was conducted at the Archaeometry Laboratory of the University of Science and Technology of China, Hefei. Approximately 10–25 mg of each lyophilized and chalked plant sample was weighed, and then 8M and 3M HNO_3 were added for secondary purification, followed by the samples being dissolved at 200°C. Then, the solutions were centrifugalized, and the supernatant liquor was taken. In addition, the Teflon columns filled with Eichrom Sr-specific resin (mesh 100–150 μm) were rinsed with MilliQ water and 3M HNO_3 multiple times to eliminate any Sr produced in the process of resin manufacturing. The purified Sr was extracted using cation exchange chromatography with a 3M HNO_3 medium in the mobile phase. Then the Sr solutions were heated to dry and sealed after cooling. Part of the purified Sr was taken and diluted with 2% HNO_3. The $^{87}Sr/^{86}Sr$ ratios were measured on a Neptune Plus MC-ICP-MS at the CAS Key Laboratory of Crust-Mantle Materials and Environments, School of Earth and Space Sciences, University of Science and Technology of China. The Sr carbonate standard NBS 987 yielded a value of $^{87}Sr/^{86}Sr = 0.710248 \pm 0.000012$ (2SD, n = 99).

S2.2 Stable carbon and oxygen isotope analyses

S2.2.1 Methods of $\delta^{18}O$ and $\delta^{13}C$ isotope analyses

The carbon in mammalian bioapatite is derived from blood bicarbonate and reflects carbon from all dietary components (protein, carbohydrates, fat) of the diet. Stable carbon isotope analysis of human tooth enamel carbonate, expressed as $\delta^{13}C$ values relative to the V-PDB standard, has been applied extensively in palaeoecological and paleodietary studies [40]. The patterning of carbon isotopic fractionation in plants is driven by the photosynthetic pathway (C_4, C_3 or CAM) through which the plant assimilate carbon [41,42], where C_3-plants (Calvin cycle) discriminate more strongly against the heavier isotope of ^{13}C than C_4-plants (Hatch-Slack cycle) [43]. Carbon isotope values can be impacted by water status, salinity, altitude, and more complicated environmental effects [44-50]. On average, C_3-plants have stable carbon isotope values of ca. –26‰ and C_4-plants have values ~14‰ higher at ca. –12‰ [42,51]. The $\delta^{13}C$ of tooth bioapatite results from the whole diet and the consumption of various plant types during childhood and adolescence thus provides a good proxy for reconstructing the proportion of the C_3/C_4 plants utilized in diets of ancient inhabitant, e.g., [52,53], as well as animal dietary change associated with seasonal changes in pasture and human interference, e.g., [54-56]. Absolute C_4 consumers in pre-industrial contexts are demonstrated to have $\delta^{13}C$ values that present ~14‰ higher than absolute C_3 consumers (0 to +2‰ vs. ca. –12‰) [57]. $\delta^{13}C$ values that fall between C_3 and C_4 diet endmembers probably reflect mixed feeding.

Stable oxygen isotope ($\delta^{18}O$) analysis of skeletal remains has become a complementary tool for exploring human mobility and migration in bioarcheology. $\delta^{18}O$ values are often utilized as a support for $^{87}Sr/^{86}Sr$ ratios when investigating human/animal mobility. These values indicate an individual's water intake and differ according to local temperatures, altitude, seasonality, continentality and other environmental effects [54,58]. The application of both $^{87}Sr/^{86}Sr$ and $\delta^{18}O$ is often used to explore mobility patterns, including the observation of people buried in a different location to where they grew up. In order to explore these questions of mobility, we sampled both human remains from PPN Jericho and modern environmental contexts for $^{87}Sr/^{86}Sr$ analysis, allowing us to provide a new bioavailable $^{87}Sr/^{86}Sr$ range to fill a gap in existing $^{87}Sr/^{86}Sr$ baselines around this critical PPN site. $\delta^{18}O$ values of mammal and human enamel bioapatite can be measured in carbonate ($\delta^{18}O_{ca}$) or phosphate ($\delta^{18}O_p$) which are derived from imbibed water and dietary water [59,60], which are in turn related to local environmental water [61,62]. Given that dental enamel does not turnover after mineralization, $\delta^{18}O$ in human dental enamel provides insight into the water source an individual relied on during tooth formation [63]. $\delta^{18}O$ measured in meteoric water varies across different geographic regions according to multiple variables such as distance from the ocean, precipitation, altitude, humidity, and latitude [61,64-66]. $\delta^{18}O$ values can therefore be applied to reconstruction of paleoclimate and paleoseasonality, human and animal lifetime provenance and cultural practices such as breastfeeding, animal husbandry and management practices, weaning behavior and culinary techniques, e.g., [55,56,67].

The sampled teeth were cleaned using a sandblaster to remove any external dirt. 7mg of enamel powder was taken and pre-treated using a protocol to remove any organic or secondary carbonate contaminates as followings. First we applied 1% sodium hypochlorite soaking the sample for 60 minutes, which was then rinsed by purified H_2O three times and centrifuge, before 0.1 M acetic acid was added for 10 minutes, and the sampled was rinsed again in purified H_2O for another three times [68]. After reaction of the sample with 100% phosphoric acid, gases evolved from the samples were analyzed for stable carbon and oxygen isotopic composition using a Thermo Gas Bench II connected to a Thermo Delta V Advantage Mass Spectrometer at the Max Planck Institute of Geoanthropology (formerly the Max Planck Institute for the Science of Human History), Jena, Germany.

S2.2.2 Stable isotope calibration and analytical uncertainty

We compared the resulting values using a three-point calibration against International Standards (IAEA-603 ($\delta^{13}C$=2.46‰; $\delta^{18}O$=−2.37‰); IAEA-CO-8 ($\delta^{13}C$=−5.8‰; $\delta^{18}O$=−22.7‰); IAEA NBS 18 ($\delta^{13}C$ =5.014‰, $\delta^{18}O$= -23.2‰). USGS44 ($\delta^{13}C$=−42.2‰) was run as an internal standard. The data from these standards suggest that the machine measurement error is c. ± 0.18‰ for $\delta^{13}C$ and ± 0.38‰ for $\delta^{18}O$. An equid enamel standard was run to access systemic error (accuracy). Measurement bias due to systematic error was determined to be ±0.2 ‰ for $\delta^{13}C$ and ±0.2 ‰ for $\delta^{18}O$.

S2.3 Proteomic Analysis

Because sex determination of the sampled individuals in the absence of both the complete skeletons for osteological identification and aDNA extraction for shotgun-genomic sex estimation is challenging, proteomics analysis was used, a technique that samples the amelogenin peptides on the enamel, which is the most accurate (and, indeed, the only) option to determine biological sex for the sampled individuals from PPN Jericho [69,70].

Recently, proteomic analysis of sex-specific amelogenin peptides in tooth enamel has been shown to provide reliable new approach to sex determination in archaeological studies [69]. Amelogenin (AMEL) is the main protein for enamel formation, accounting for 90wt% of enamel matrix proteins (EMPs) in mature enamel [71,72]. It is encoded by both chromosomes X and Y, with slight differences in amino acid sequences between the X and Y-derived AMEL, which allows sex estimation that enabled discussions of mobility along sex-based lines. Furthermore, dental enamel is the hardest, densest, and most calcified tissue in toothed vertebrates, which can protect proteins from external physical and chemical damage [73]. A number of studies have shown the reliability of this method, especially in minors and poorly preserved individuals [70,73-79].

S2.3.1 Protein extraction

Most of the samples come from the residual enamel fragments of strontium isotope experiments, and a few samples directly etch the surface of the dental crown. The tooth surface was abraded using a dental burr to remove obvious surface contaminants. The enamel was washed with 3% H_2O_2 for 30 s and then rinsed with ultrapure water. 100 µL of 1.5M HCl was used to etch tooth enamel for 15 min, and the etching solution was collected in 1.5ml Eppendorf tubes. Desalting and elution were carried out according to Stewart et al [69]. Finally, a 10 µL 60% acetonitrile/0.1% formic acid elution buffer with peptides was dried and centrifuged in a drying centrifuge. At the same time, a blank control was added to monitor possible contamination during the experiment.

S2.3.2 nanoLC-MS/MS analysis

The dried peptides were resuspended with 10 µl 0.1% formic acid solution and analysed by Easy-nLC1200 nanoflow liquid chromatography system (Thermo Fisher Scientific) coupled to a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific). 3µL of the samples were loaded on a pre-column (Acclaim PepMapTM 100, 100µm×2cm, nanoViper 2PK, C18, 5µm, 100Å) at a flow rate of 300nL/min. After desalting, peptides were separated on an analytical column (Acclaim PepMapTM RSLC, 50µm×15cm, nanoViper, C18, 2µm, 100Å) with a linear gradient program: 3%−8% B in 3min，8%−28% B in 36min，28%−45% B in 10min，45%−90% B in 1min，90%−90% B in 10min (solvent A: 0.1% FA, solvent B: 80%CAN/0.1%FA).

The Q Exactive Plus mass spectrometer was operated in positive ion mode with a nanospray voltage of 1.8 kV and a source temperature of 275 °C. Mass spectrometry data were acquired by automatic switching between MS1 scanning to up to 20 MS/MS scans (topN method). The target value of MS1 scanning was set as 1e6 in the m/z 350−2000 range with the maximum ion injection time of 50 ms and a resolution of 70000. Ion precursors were isolated with a window width of 1.6 m/z and MS/MS fixed first mass of m/z 110. Ion precursors were fragmented using the high-energy collision dissociation technique with the normalized collision energy of 27%. MS/MS scans were obtained with the resolution of 17500 and target ion value of 1e5 with the maximum ion injection time of

50ms.

S2.3.3 Database search

The AMELX gene encodes three amelogenin isoforms formed by alternative splicing: Q99217-1, Q99217-2 and Q99217-3 (Uniprot ID). The Q99217-1 isoform is considered the canonical amino acid sequence. The Q99217-2 isoform differs from the canonical sequence by deletion of the 19-34 fragment, and the isoform Q99217-3 by insertion of the ENSHAQAINVDRTAL sequence replacing Glu34 residue. As a result of alternative splicing, the AMELY gene produces two protein isoforms: Q99218-1 and Q99218-2 (Uniprot ID). The Q99218-2 isoform corresponds to the canonical amino acid sequence. The Q99218-1 isoform differs from the canonical sequence by deletion of the 19-34 fragment. Amelogenin isoforms Q99217-3 and Q99218-2 have the highest similarity. This study only focused on the sex of individuals: as such, two amelogenin isoforms Q99217-3 and Q99218-2, from UniProtKB were selected as retrieval databases, which could greatly improve the retrieval efficiency.

MS/MS data were analyzed with MaxQuant (version 1.6.0.1). Searches were conducted with unspecific digestion. Oxidation (M) and Deamidation (NQ) were set as variable modifications. The minimum peptide length was set to 6. Precursor mass tolerance was set to 10 ppm and fragment mass tolerance to 0.05 Da. The false-discovery rate of peptide spectrum matches was set to 0.01. Protein identifications were accepted if at least two unique peptide sequences were registered.

S2.3.4 Deamidation analysis

Python command line script provided by Mackie et al. 2018 [80] was used to calculate the deamidation percentage of glutamine (N) and asparagine (Q) in each sample to evaluate the reliability of ancient protein identification (https://github.com/dblyon/deamidation). The calculations were done separately for potentially original peptides and potential contaminants (human keratin). Briefly, the Maxquant "evidence. txt" file was used as the original file of the calculation. For each peptide containing N and Q residues, the ratio between the number of residues in the modified form and the total number of the residues was calculated, and this value was then multiplied for the intensity of the peptide. The values obtained were summed. The result was then divided by the total sum of all intensity values of the peptides in the modified and unmodified forms. 1000 bootstrap iterations were applied to calculate mean, standard deviation, and 95% lower and upper confidence intervals and to estimate the calculation error.

2.4 Carbon and Nitrogen Stable Isotope Analysis of Bone (failed to get successful results)

The human bone samples utilized in this study were prepared and analyzed at the Max Planck Institute for Geoanthropology (formerly the Max Planck Institute for the Science of Human History), Jena. Samples were taken from either long bones or ribs and prepared following the method described in Richards and Hedges [81]. Samples were soaked in 0.5 M HCl to demineralize the collagen samples for two weeks with the HCl solution changed every 48 hours. Samples were rinsed three times with purified H_2O then heated for 48 h at 70°C in water acidified to pH 3, then removed from heat and immediately filtered through EZEE Filters. Finally, samples were freeze dried and weighed into tin capsules for analysis. Samples were analyzed in duplicate when sample size allowed, and results reflect their mean values. Mass spectrometry analysis for all new samples was conducted at the MPI-GEA Stable Isotope Laboratory using a Thermo Fisher Scientific FLASH 2000 HT Elemental Analyzer via a Thermo Fisher Scientific Conflo IV continuous flow interface coupled to a Thermo Fisher Scientific Delta V Advantage Isotope Ratio Mass Spectrometer. Collagen was successfully extracted in most cases, however, the results revealed that none of the samples fell within the required C/N ratio range or had a suitable standard deviation between runs (Table S3).

Table S6. Results of Kruskal-Wallis test comparison between the δ^{18}O value of Jericho human teeth and the other parallel sites in the southern Levant

Level	- Level	Score Mean Difference	Std Err	Z	p-Value	Hodges-Lehmann	Lower CL	Upper CL
Jericho	Ain Ghazal	33.0192	5.406954	6.10680	<.0001*	1.50000	1.20000	1.80000
Jericho	Beisamoun	27.8902	7.630860	3.65492	0.0003*	2.00000	1.50000	2.50000
Jericho	Ain Mallaha	27.3856	6.305786	4.34294	<.0001*	1.50000	1.00000	2.20000
Kharaysin	Beisamoun	4.3556	2.310141	1.88541	0.0594	0.50000	-0.10000	1.20000
Tell Qarassa North	Beisamoun	2.4545	3.917350	0.62658	0.5309	0.15000	-0.40000	1.10000
Kharaysin	Ain Ghazal	0.7828	3.575779	0.21893	0.8267	0.00000	-0.40000	0.50000
Kharaysin	Ain Mallaha	0.4444	2.512713	0.17688	0.8596	0.10000	-0.60000	0.80000
Ain Mallaha	Ain Ghazal	-0.3914	3.583433	-0.10923	0.9130	0.00000	-0.50000	0.50000
Tell Qarassa North	Ain Mallaha	-1.9571	3.591434	-0.54493	0.5858	-0.20000	-0.90000	0.60000
Tell Qarassa North	Kharaysin	-3.6010	3.592524	-1.00236	0.3162	-0.30000	-0.90000	0.40000
Beisamoun	Ain Mallaha	-3.8889	2.304956	-1.68719	0.0916	-0.60000	-1.30000	0.20000
Tell Qarassa North	Ain Ghazal	-5.2273	3.866154	-1.35206	0.1764	-0.30000	-0.70000	0.20000
Beisamoun	Ain Ghazal	-9.3273	3.906490	-2.38764	0.0170*	-0.50000	-1.00000	-0.10000
Kharaysin	Jericho	-28.4314	6.305874	-4.50871	<.0001*	-1.50000	-2.00000	-1.00000
Tell Qarassa North	Jericho	-29.9938	5.407581	-5.54661	<.0001*	-1.80000	-2.20000	-1.40000

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