Paleogenomics of the Neolithic Transition in North Africa

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1 Introduction

The Neolithic revolution, which is the transition from hunting and gathering to farming, started in the Near East around 13,000 BCE. How human populations acquired agricultural and herding technologies has historically been the focus of a heated debate. Two opposing models can be applied to explain the Neolithic transition: the demic diffusion model and the cultural diffusion model. The first one argues that the Neolithic revolution involved the movement of people, in such a way that the arrival of agricultural and herding techniques was the result of the migration of farmers, who would admix with or replace previous hunter-gatherer populations. The other model proposes that the Neolithic transition was the result of the movement of ideas. In this scenario, local hunter-gatherer populations would acquire agricultural and herding technologies from neighbouring populations, without genetic admixture.

Prehistoric research in North Africa has largely focused on the Lower and Middle Paleolithic periods as exemplified by the discovery of the oldest early modern human in Morocco (Richter et al., 2017) and the older stone artifacts and cut-marked bones in Algeria (Sahnouni et al., 2018). On the other hand, how populations transitioned to farming in North Africa has received less attention. Paleolithic North Africa is characterized by the Aterian culture that flourished about 30,000 BCE, and was later replaced by the Upper Paleolithic Iberomaurusian industry (~15,000 BCE). By ~12,000 BCE the Mesolithic Capsian culture appeared in the Maghreb, transitioning to farming communities in the 6th millennia BCE (Naylor 2015).

Based on archaeological data, it is thought that the Neolithic transition occurred simultaneously at both shores of the Western Mediterranean through a coastal diffusion (Zilhao 2001, Linstädter 2008). Two main diffusion waves have been evidenced in the Mediterranean basin: *i*) a first one defined by the spread of a specific lithic industry characterized by pressure and indirect percussion reduction *ii*) a later one related to the spread of Cardial pottery and domesticated plants and animals (Mulazzani et al., 2016). However, some

evidence points to the Neolithic revolution in North Africa as an *in situ* development from Iberomaurusian communities. For example, the Hattab II cave in Morocco produced a Late Paleolithic Iberomaurusian human burial dated 6,900 years BCE. This find questions the complete replacement of local hunter-gatherer communities in the Maghreb and evidences certain continuity in burial practices through the early Neolithic period (Barton et al., 2008). Also, elements usually associated with Neolithic economies, such as pottery fragments and an adze, have been found at two hunter-gatherer contexts dated 6,000 BCE in Tunisia (Mulazzani et al., 2016). These results point to a complex Neolithic transition scenario with both demic and cultural diffusion. Upon those layers, successive prehistoric and historical migrations reached North Africa. It is known that Bell-Beaker pottery, associated with the expansion of Bronze Age populations in Eurasia, is present in the North African region (Bokbot and Ben-Ncer 2008). In historical times, North Africa was settled by different populations including Romans, Phoenicians, Vandals, Byzantines and Arabs (Naylor 2015), leading to the complex genetic structure observed today.

2 What We Knew before Paleogenomics: Modern DNA Evidence on the Neolithic Transition in North Africa

Well before the emergence of the paleogenomics field, the analysis of classical markers on modern populations (e.g. blood groups, red cell enzymes and human leukocyte antigens (HLA)) produced the first genetic evidence of the impact of the Neolithic transition in human populations (Sokal, Oden, and Wilson 1991, Ammermann and Cavalli Sforza 1984, Cavalli-Sforza, Menozzi, and Piazza 1994). A major southeast to northwest cline was observed in Europe, coinciding with the advance of Neolithic farmers from the Fertile Crescent and supporting the demic diffusion model. As archaeological evidence indicates that North Africa and Western Mediterranean Europe mirrored each other during Neolithic times, it would be reasonable to expect a similar scenario in both regions. When analyzing classical markers data in North Africa, Bosch et al. (1997) observed a clear east-west pattern of variation, separating Berber and Arab populations in the Maghreb from the northeastern populations of Libya and Egypt. However, instead of the smooth cline found in Europe, the separation between East and West was rather abrupt. The authors concluded that, although a demic diffusion model for the Neolithic transition was possible for explaining differences between eastern and western North Africa, the presence of an older component was needed to account for the high level of differentiation of northwestern populations.

Additional evidence on the Neolithic transition in North Africa was obtained with the use of the two haploid, uniparental markers: the mitochondrial DNA (mtDNA) and the non-recombinant portion of the Y-chromosome. Many human population studies have been focused on these highly informative genetic markers, whose direct inheritance and absence of recombination allow the reconstruction of female and male genealogies. During the 90s and the 2000s, an increasing amount of mitochondrial DNA and Y-chromosome data was gathered from modern human populations worldwide and exhaustive phylogeographic analyses were performed both at global and regional levels. This allowed the building of more detailed phylogenetic trees for both genetic markers and led to a deeper knowledge of the times and routes of expansion of major maternal and paternal lineages around the globe. For example, Richards et al. (1996) reported that most of the mitochondrial DNA diversity in Europe had a Paleolithic origin. Contrary to conclusions drawn from classical markers, the European Neolithic transition from the mitochondrial DNA perspective was outlined as a cultural diffusion rather than the result of the migration of people. Accordingly, only a small set of modern European lineages was considered to have arrived from the Near East in Neolithic times, represented mainly by haplogroups J, K and T. Regarding North Africa's mitochondrial DNA composition, the most striking feature was the presence of two African-specific sub-lineages related to a back migration to Africa from Eurasia in Paleolithic times (Maca-Meyer et al., 2003, Olivieri et al., 2006, Gonzalez et al., 2007, Pereira et al., 2010): haplogroups U6 and M1. Both lineages are relatively frequent in modern-day North Africans indicating maternal continuity in the region since the Paleolithic (Secher et al., 2014, Pennarun et al., 2012). However, the presence in North Africa of mitochondrial DNA haplogroups linked to Neolithic expansions in Eurasia supported the existence of a farmer's migration into the area from the Middle East (Macaulay et al., 1999, Rando et al., 1998). Regarding the Y chromosome, current North Africans are characterized by the high frequency of haplogroup E-M81, a lineage considered to be autochthonous of this region. Interestingly, the frequency of E-M81 follows an east to west cline, with the highest frequencies in Morocco and the lowest in Egypt, similar to the results obtained for classical markers. Based on that evidence and contrary to the conclusions drawn from mitochondrial DNA, Arredi et al. (2004) proposed that North African paternal diversity was compatible with a demic expansion from the Middle East. Because the age of E-M81 and other common lineages in North Africa (E-M78 and J-304) were relatively recent, they proposed that the North African pattern of Y-chromosome variation was mostly of Neolithic origin.

With the contradictory evidence gathered from classical markers and the Y chromosome on one side and mitochondrial DNA on the other side, the advent of genome-wide technologies has been fundamental for obtaining a more nuanced picture of the complex genetic history of North Africa (Henn et al., 2012, Arauna et al., 2017, Fadhlou-Zid et al., 2013). Based on both genome-wide SNP data and whole-genome sequencing, it has been suggested that North Africans' ancestry can be explained as the result of four migration events. First, present-day ancestry in North Africans is characterized by an autochthonous Maghrebi component related to a Paleolithic back migration to Africa from Eurasia. Second, there is a recent admixture event with a Middle Eastern component, which can be the result of the Arab expansion in North Africa in the seventh century. Third, historical trans-Saharan migrations produced gene flow from sub-Saharan Africa. Finally, North Africans carry a European component that can be linked to historic movements, such as the Roman occupation.

Although useful to get a glimpse of the genetic history of a certain population, studies based on modern DNA variation are usually not suited for disentangling complex migration patterns. As several layers of migration have affected North Africa at different periods, recent human movements can erase or distort more ancient admixture signals. In these cases, obtaining ancient DNA from archaeological remains is more appropriate because it allows us to directly examining how the population changed through time. Because the two competing models proposed to explain the acquisition of farming technologies are based on either the existence or the absence of genetic admixture, ancient DNA analysis is a powerful tool to study the Neolithic transition. The reasoning behind this approach is quite simple: if the genetic composition of human remains from hunter-gatherers and farmers is similar, then the Neolithic transition happened through the movement of ideas rather than genes; on the other hand, if hunter-gatherers and early farmers are genetically different, then the spread of farming required the movement of people.

3 The Ancient DNA and Paleogenomics Revolutions

In 1986, the development of the polymerase chain reaction (PCR) technique allowed for the first time the amplification and analysis of trace amounts of DNA (Mullis et al., 1986). This methodology was immediately applied to obtaining DNA from archaeological and paleontological materials (Paabo, Gifford, and Wilson 1988) initiating the ancient DNA revolution. Between the late 80s and the early 2000s, researchers retrieved DNA from all sorts of ancient

materials and provided valuable information from the past of several species, including our own (Paabo et al., 2004). However, classic ancient DNA methodologies based on PCR amplification have important limitations. DNA extracted from archaeological remains is usually in very low concentration and highly degraded. For that reason, the vast majority of ancient DNA analyses had to be limited to specific regions such as the mitochondrial DNA. Each cell contains only one nucleus with one copy of the nuclear diploid genome. On the other hand, every cell has multiple mitochondria, with each one containing several copies of its mitochondrial DNA. For that reason, mitochondrial DNA is more abundant in ancient DNA extractions, and it is better suited to paleogenetic analyses based on PCR amplification. The limitation of using mitochondrial DNA is that this genetic material is maternally inherited and only carries information from the maternal side, thus it does not inform on the global ancestry of a population. Additionally, uniparental markers such as the mitochondrial DNA are more prone to suffer from the effects of genetic drift and the loss of genetic variants due to stochastic processes, complicating the interpretation of the genetic data. But maybe the biggest limitation of the classical techniques is the risk of contamination with modern DNA.

PCR was a revolution for ancient DNA studies because it can obtain amplified DNA from just a few molecules. But that same advantageous characteristic of PCR also represents its Achilles' heel: because PCR is extremely sensitive, any trace of modern DNA contamination can be potentially amplified, especially in highly degraded samples. The contamination problem is more dangerous when analyzing ancient human remains, as the sole manipulation of the samples by researchers is a potential source of modern DNA from the same species. To avoid and monitor contamination, several criteria were established to assure the authenticity of the ancient DNA results (Cooper and Poinar 2000). However, paleogenetic studies based on PCR have always been hindered by the risk of sample contamination, especially those involving extremely ancient human remains (Paabo et al., 2004).

As it occurred with the development of the PCR in the 80s, the implementation of next-generation sequencing techniques produced a huge revolution in the ancient DNA field. Instead of being limited to just one locus, this new methodology allows the recovery of complete genomes from archaeological materials. By providing information at the genomic level, ancient DNA analyses using next-generation sequencing are more appropriate to elucidate the demographic structure and history of ancient populations. Moreover, next-generation sequencing techniques overcome the problem of ancient DNA data authentication, solving the contamination conundrum. Due to post-mortem damage, two features characterize DNA recovered from ancient samples: high

fragmentation and chemical modification of the molecule's ends. Next-generation sequencing allows the identification of both damage patterns that are unique to old DNA and provides empirical verification of the authenticity of the results. The paleogenomics field has proved its value by illuminating the Prehistory of Eurasia (Haak et al., 2015, Lazaridis et al., 2016, Allentoft et al., 2015, Mathieson et al., 2015, Olalde et al., 2018, Mathieson et al., 2018, Olalde et al., 2019). For example, ancient DNA studies have provided a clear picture of the Neolithic transition in West Europe, establishing that early European farmers migrated from Anatolian and replaced previous hunter-gatherer populations with little genetic admixture. However, although this is the general picture, some West European Neolithic individuals exhibit considerable amounts of hunter-gatherer ancestry demonstrating the admixture of farmers with local populations. This evidence suggests a diverse genetic impact of the Neolithic transition in different regions of Europe, contradicting the more simplistic total acculturation or total replacement models.

4 Ancient Genomes from North Africa and What They Tell Us about the Neolithic Transition

The amount of detailed ancient DNA information obtained from Europe is not comparable to any other region worldwide, especially in the case of the African continent. One of the problems for developing ancient DNA studies in North Africa is the availability of well-conserved samples. Next-generation sequencing methodologies by themselves can obtain whole-genome information from ancient samples but only for archaeological remains with enough human endogenous DNA content. Human remains from warm areas such as North Africa usually contain low quantities of endogenous genetic material. As a consequence of their greater level of degradation, there is also a higher relative content of bacterial and fungal DNA from the soil. This limitation has hindered ancient DNA studies in the African continent, but recently it has been overcome with the development of enrichment techniques. For those samples with low human endogenous DNA, there are two methodologies available. One is directed to capture the whole human genome by using genomic DNA or RNA as baits (see Carpenter et al. (2013)). The other approach is directed at enriching ancient samples in DNA molecules covering sites that are known to be variable in human populations (see Lazaridis et al. (2016) or Fregel et al. (2018)). Both methodologies have been applied to the study of ancient populations in North Africa, producing valuable evidence on the Neolithic transition in the Maghreb.

4.1 *Taforalt (Morocco): A Eurasian Origin for North African Upper Paleolithic Populations*

The site of Taforalt, also known as Grotte des Pigeons, is located in eastern Morocco (Figure 7.1), in the Beni-Snassen Mountains. Taforalt's stratigraphic sequence evidences human occupation ranging from the Aterian to the Iberomaurusian period. The Iberomaurusian sequence contained the human remains of fourteen individuals, including eight adults and six children. Human remains were radiocarbon dated, yielding age estimates between 13,000 and 11,900 years BCE. Nine individuals were analyzed using paleogenomics techniques by van de Loosdrecht et al. (2018), but only seven produced enough endogenous DNA. Because human DNA content was low, the authors used two enrichment techniques: one directed to variants present in the Human Origins panel (Lazaridis et al., 2014) and the other aimed to capture the complete mitochondrial DNA sequence.

Mitochondrial DNA sequences from Taforalt individuals were all classified within the two autochthonous North African lineages associated with the back migration to Africa in Paleolithic times: haplogroups U6 and M1 (van de Loosdrecht et al., 2018). Phylogenetic analyses of the sequences indicated that the six individuals belong to different sub-lineages of haplogroup U6a, while the remaining one was classified as M1b (Fregel et al., 2018) (Figure 7.2). Congruently, all these lineages are believed to have originated in the Maghreb around the Upper Paleolithic period (Secher et al., 2014). The presence of U6a and M1b lineages in both Iberomaurusian and present-day populations confirmed temporal continuity in the Maghreb from Paleolithic times to the present, coinciding with results obtained using classical markers (Bosch et al., 1997) and genome-wide data (Henn et al., 2012). Interestingly, the mitochondrial DNA of

FIGURE 7.1 Locations (left panel) and radiocarbon dates (right panel) of the North African archaeological sites analyzed using paleogenomics techniques

FIGURE 7.2 Mitochondrial DNA phylogenetic tree including ancient samples from North Africa. In this phylogenetic tree, modified from Fregel et al. (2018), it is shown the different mitochondrial DNA composition of Early and Late Neolithic populations from North Africa. Upper Paleolithic (green) and Early Neolithic (violet) samples from North Africa belong to the North African autochthonous haplogroups U6 and M1. On the other hand, Moroccan Early Neolithic samples (orange) belong to haplogroups K1, T2 and X2, which are common in Anatolian and European Neolithic populations

additional Iberomaurusian remains from the Taforalt region had been previously studied using classical ancient DNA techniques (Kefi et al., 2005, Kefi et al., 2018). Some samples were assigned to the North African U6 mtDNA-haplogroup, but most of them were classified within Eurasian lineages such as H, U and Ro. Authenticating these results is challenging due to the high contamination risk associated with the PCR amplification of extremely degraded human samples. Future reanalysis of these individuals using paleogenomics techniques would be needed to confirm the presence of lineages different from U6 and M1 in Upper Paleolithic North Africa.

The obtaining of nuclear DNA from the seven Taforalt samples allowed the authors to identify their molecular sex. Six individuals were classified as males and one as a female, offering an excellent opportunity for understanding the Y-chromosome composition of Upper Paleolithic communities in the Maghreb. All male samples from Taforalt were classified within haplogroup E-M35 (van de Loosdrecht et al., 2018), a clade that is characterized by a broad geographic distribution, from northern and eastern Africa to Europe and western Asia (Trombetta et al., 2015). More concretely, Taforalt samples belong to haplogroup E-M78, a lineage that is also

widely distributed. E-M78 most probably originated in northeastern Africa (Cruciani et al., 2007, Trombetta et al., 2015, Sole-Morata et al., 2017) and dispersed from there approximately 20,300 - 14,800 years ago. As E-M78 is still frequent in North Africa (Arredi et al., 2004), this result also indicates a temporal continuity between Upper Paleolithic and current populations in the Maghreb.

Lazaridis et al. (2016) observed that Eurasian populations could be explained as a mixture of four sources of ancestry: Iranian Neolithic, Levantine Neolithic, East European Paleolithic and West European Paleolithic. When Taforalt people were compared to previously published ancient and modern DNA data, Upper Paleolithic North Africans can be modeled as a mixture of Natufians (Epipaleolithic populations from the Levant) and West Africans, without the contribution of Paleolithic Europe (van de Loosdrecht et al., 2018). This result suggests that Iberomaurusian populations in North Africa were related to Paleolithic people in the Levant, but also that migrations of sub-Saharan African origin reached the Maghreb during the Pleistocene. However, a pre-print from Lazaridis et al. (2018) has contested this conclusion based on new evidence from Paleolithic samples from the Dzudzuana site in Georgia (25,000 years BCE). When these samples are considered in the analysis, Taforalt can be better modeled as a mixture of a Dzudzuana component and a sub-Saharan African component. They also argue that it is the Taforalt people who contributed to the genetic composition of Natufians and not the other way around. More evidence will be needed to determine the specific origin of the North African Upper Paleolithic populations, but the presence of an ancestral U6 lineage in the Dzudzuana people is consistent with this population being related to the back migration to Africa.

4.2 *Ifri n'Amr o'Moussa (Morocco): Cultural Diffusion or in situ Development Explains the Early Phase of the Neolithic Transition in North Africa*

The Ifri n'Amr ou Moussa (IAM) site is located in central Morocco, on the banks of the river Beht. This cave has produced a wide stratigraphic sequence ranging from the Iberomaurusian to the Chalcolithic period (Martínez-Sánchez et al., 2017, Ben-Ncer et al., 2011, 2015, Laviano 2015). Human remains have been recovered from a sequence with archaeological evidence belonging to the Early Neolithic period, including the presence of cereal grains, Cardial pottery and possible domestic fauna (Martínez-Sánchez et al., 2017). In total, seven different individuals were excavated in anatomical connection. Direct radiocarbon dating of the human remains yielded dates pointing to the late 6th and early 5th millennia BCE (Figure 7.1). This result coincides with the

date obtained from a barley grain from the same stratigraphic level (Martínez-Sánchez et al., 2017). All individuals were sampled for obtaining ancient DNA by Fregel et al. (2018). Because of the low endogenous DNA content, Ifri n'Amr o'Moussa samples had to be subjected to enrichment methodologies directed both at the whole genome and to specific variants. Despite the application of capture methodologies, only five Ifri n'Amr o'Moussa samples produced enough DNA. Nuclear DNA analyses revealed that IAM.4 and IAM.5 shared a high proportion of relatedness and IAM.4 had to be excluded from analysis (Fregel et al., 2018).¹

Mitochondrial DNA analysis indicated that Early Neolithic samples in North Africa belong to the same haplogroups observed in Taforalt: individual 3 is classified within haplogroup M1b, while the remaining belong to U6a. In fact, Ifri n'Amr o'Moussa samples are classified within some of the same haplogroup sub-lineages present in Taforalt (Figure 7.2), which proves temporal continuity in North Africa from 13,000 to 5,000 years BCE. This first result is of great importance for addressing the Neolithic question in North Africa as it evidences that the first stages of this transition occurred through the movement of ideas rather than people.

From the four Ifri n'Amr o'Moussa samples that yielded DNA, two were classified as males but only one produced enough DNA to perform Y-chromosome phylogenetic analysis. This sample belongs to the E-M35 haplogroup, the same clade found in the Taforalt individuals. However, in this case, the sample clusters with the E-L19 clade, a lineage that is ancestral to the autochthonous North African E-M81 haplogroup. This result is expected because E-M81 is younger than Ifri n'Amr o'Moussa samples (2,000–3,000 years ago) (Fadhlaoui-Zid et al., 2015, Sole-Morata et al., 2017). As explained before, E-M81 is the most frequent Y-chromosome lineage within North Africa, with its frequency varying in a latitudinal fashion, with the highest frequencies in Morocco and the lowest in Egypt (Arredi et al., 2004). The observation of an ancestor of E-M81 in an Early Neolithic site in Morocco reinforces the idea of temporal continuity in the area from prehistoric to current times.

When analyzing genome-wide data, Fregel et al. (2018) reported that Early Neolithic samples from Morocco are similar to those from the Upper Paleolithic period in Taforalt (Figure 7.3). When compared using genetic ancestry inference, Early Neolithic and Upper Paleolithic samples from North Africa share the same ancestral components. In fact, both Taforalt and Ifri n'Amr o'Moussa share a component that is still retained in modern populations. This

¹ Later data analyses have proved that both samples belonged to the same individual.

FIGURE 7.3 Ancestry inference of ancient North African samples using principal component analysis (PCA). PCA is a statistical tool that can be used for exploring the relationship between individuals of different populations based on genome-wide data. (A) PCA including North African and other previously published ancient samples projected onto modern Eurasian populations (grey circles). (B) Upper Paleolithic samples from Taforalt (violet diamonds) are clustered close to North African populations. (C) Early Neolithic samples from Ifri n'Amr o'Moussa (dark green inverted triangles) are positioned close to Taforalt samples (violet diamonds), indicating temporal continuity during the early phases of Neolithic transition. (D) Late Neolithic samples from Kef el Baround (blue triangles) are clustered between ancient samples from North Africa (violet diamonds and dark green inverted triangles) and early farmers from Anatolia and Europe (light green and yellow inverted triangles), and close to samples from the indigenous population of the Canary Islands (light green squares)

is the same ancestral Maghrebi component reported by Henn et al. (2012) that follows an east to west cline in the North African region. Genetic distances also indicate that Early Neolithic from Ifri n'Amr o'Moussa and Upper Paleolithic samples from Taforalt are close between them and far from the rest, suggesting long-term isolation of North African between 13,000 - 5,000 years BCE. The similitude between Taforalt and Ifri n'Amr o'Moussa implies that early farming and herding acquisition in North Africa did not involve the migration of people and can be better explained either by the transmission of ideas or an *in situ* development of local populations.

5 Kef el Baroud (Morocco): Late Neolithic in North Africa is Explained by a Demic Diffusion Model

The Kef el Baroud (KEB) cave is located in central-western Morocco, close to the city of Casablanca (Figure 7.1). The stratigraphic sequence at Kef el Baroud shows human occupation ranging from the Epipaleolithic to the Bronze Age (Banerjee et al., 2011). Human remains were excavated in the Neolithic phase, which is characterized by undecorated ceramic fragments, flint tools and domestic fauna. Direct radiocarbon dating from the human material (Fregel et al., 2018) indicates the samples belonged to the Middle-Late Neolithic at the beginning of the 4th millennium BCE (Figure 7.1). Eight different teeth and phalanx samples were taken from Kef el Baroud to perform ancient DNA analyses (Fregel et al., 2018). Because the human remains were not in anatomical connection, it was possible that different samples could belong to the same individual. In fact, samples KEB.1 and KEB.8 were identified as being duplicates of the same individual. Due to low endogenous DNA content, samples were also captured using the same methodologies as in the Early Neolithic remains.

From the eight samples analyzed, only five produced complete mitochondrial DNA genomes. In sharp contrast to lineages observed in North African Upper Paleolithic and Early Neolithic samples, the mitochondrial DNA sequences obtained in Kef el Baroud belonged to typically Anatolian and European Neolithic haplogroups (Fregel et al., 2018). The five Kef el Baroud samples were classified within K1a1b1, K1a4a1, T2b3 and X2b (Figure 7.2). These haplogroups have been observed in Early and Middle Neolithic sites in the Near East and Europe. For example, an X2b sample was found in the Early Neolithic site of Garadna in Hungary (Gamba et al., 2014) and a K1a1b1 individual in the Middle Neolithic site of La Mina in Spain (Mathieson et al., 2015). The presence of these mitochondrial DNA lineages in Kef el Baroud suggests the migration of Near Eastern or European early farmers in North Africa ~3,000 years BCE and rules out the exclusive introduction of European ancestry from the Mediterranean during historical times.

Lineages associated with the Neolithic transition in the Near East and Europe have also been observed for the indigenous people of the Canary Islands (Maca-Meyer et al., 2004, Fregel et al., 2019), a population that can be considered an ancient North African isolate. The Canarian archipelago is located off the coast of Morocco (Figure 7.1) and its indigenous population is considered to be of North African origin based on multidisciplinary evidence. Radiocarbon dating indicates that the indigenous colonization of the islands happened around 100 years CE (Fregel et al., 2019). The conquest and colonization of the

islands by the Spanish Crown in the 15th century led to the complete admixture of the natives with the European population. However, ancient DNA analyses have allowed reconstructing the genetic composition of the Canarian indigenous people (Fregel et al., 2009, Maca-Meyer et al., 2004, Rodriguez-Varela et al., 2017). In line with the results observed in Kef el Baround, most of the lineages observed in the ancient population of the Canary Islands are related to Neolithic expansion in the Mediterranean (e.g. T2c1d, J1c3 or J2a2d) and attest the impact of this migration in North Africa (Fregel et al., 2019).

Three Late Neolithic samples from Morocco produced nuclear DNA and two were classified as males, with only one generating enough DNA to be assigned to a Y-chromosome haplogroup. Sample KEB.6 was classified within haplogroup T-M70, a result that is in agreement with the results observed for the mitochondrial DNA (Fregel et al., 2018). The presence of haplogroup T-M70 in Kef el Baround indicates a tight relationship of Late Neolithic North Africa with Near Eastern and European Neolithic populations. Haplogroup T-M70 has been found in Neolithic samples from Germany (Haak et al., 2015) and from Jordan (Lazaridis et al., 2016). In agreement again with the mitochondrial DNA, haplogroup T-M70 has also been observed in the indigenous population of the Canary Islands (Fregel et al., 2009). Interestingly, present-day haplogroup T-M70 frequencies in North Africa are higher in Egypt than in Morocco (Bekada et al., 2013), following an opposite distribution than E-M81 (Arredi et al., 2004). Although it is an exceedingly simplistic model that ignores later migrations, this result could be explained by a larger Paleolithic persistence in the western region and a larger Neolithic impact in eastern North Africa.

Uniparental markers show that Late Neolithic populations in North Africa had a genetic composition consistent with a demic diffusion from Europe or the Near East. This result was corroborated at a genome-wide level when Kef el Baround samples were found to be composed of both the same ancestral Maghrebi component observed in Ifri n'Amr o'Moussa and Taforalt, and an early Anatolian/European farmer's component (Figure 7.3). This is consistent with Late Neolithic Moroccans being a mixture of the local population and farmers coming from Europe or the Near East. Genetic distance estimates point to Kef el Baround people being closer to Early Neolithic populations in Iberia. In fact, Kef el Baround people can be modeled as a simple mixture of an Ifri n'Amr o'Moussa and an Early Iberian component. The existence of migrations through the Strait of Gibraltar has been already proposed based on archaeological evidence. Also, Olalde et al. (2019) have analyzed two Iberian sites from the Copper and the Bronze Age periods that show evidence of admixture with a component of North African origin, pointing to the existence of prehistoric exchanges between both sides of the strait.

A similar result is observed for the indigenous population of the Canary Islands (Fregel et al., 2018, Rodriguez-Varela et al., 2017). This North African isolate is similar to Kef el Baroud samples (Figure 7.3), but with additional evidence of Middle Neolithic/Chalcolithic Europe and sub-Saharan African gene flow. These two components could be the result of later migrations in North Africa, probably related to the Bell-Beaker expansion in the Mediterranean and trans-Saharan migrations. Also, the proportion of the Maghrebi component present in the indigenous population of the Canary Islands is higher than that observed in Late Neolithic Moroccans. This result is interesting as it indicates that the impact of the migration of early farmers from Europe in North Africa could have been heterogeneous, with some areas retaining a higher Paleolithic component.

6 Conclusions

Ancient DNA obtained directly from hunter-gatherer and early farmer human remains from Morocco has provided the first paleogenomic evidence on the Neolithic transition in North Africa (Fregel et al., 2018, van de Loosdrecht et al., 2018). The picture drawn from these studies points to a complex scenario where both cultural and demic diffusion led to the acquisition of farming and herding technologies. Upper Paleolithic and Early Neolithic populations in North Africa share the same genetic makeup, related to a back migration from Eurasia in Paleolithic times. This component is characterized by African autochthonous lineages U6 and M1 from the mitochondrial DNA and sublineages of E-M35 from the Y-chromosome. From a genome-wide perspective, Moroccan Upper Paleolithic and Early Neolithic populations are characterized by an autochthonous Maghrebi component still retained in present-day North Africans, following an east-to-west cline. Altogether, these results evidence that the early steps of farming and herding acquisition in North Africa happened through an *in situ* development process or mediated by the acculturation of local hunter-gatherer populations.

Late Neolithic individuals from Morocco are characterized by a mixture of both the autochthonous Maghrebi component and gene flow from early farmers in Europe. Mitochondrial DNA and Y-chromosome lineages in North African Late Neolithic are different from those in previous periods and have a clear affiliation to early farmers in the Near East and Europe. Genome-wide data indicate that Late Neolithic communities in Morocco had a Neolithic European component, most probably related to the migration of early farmers from

Iberia. Genetic evidence from the indigenous people of the Canary Islands suggests that the impact of the European Neolithic gene flow could have been heterogeneous and that additional European ancestry could have reached North Africa between the 4th millennium BCE and the 1st century CE, probably related to the expansion of the Bell-Beaker culture in the Mediterranean.

Compared to ancient DNA evidence in Europe, our understanding of the genetic composition of prehistoric populations of North Africa is just a crude draft. Our present knowledge is only based on two specific archaeological sites from Morocco that are not representative of the whole North African region. Additional paleogenomics evidence from different archaeological sites from both western and eastern North Africa will be needed to comprehend the nuances of Neolithic transition on this region and the human movements that shaped Berber populations.

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References

- Allentoft, M.E., Sikora, M., Sjogren, K.G., Rasmussen, S., Rasmussen, M., Stenderup, J., Damgaard, P.B., Schroeder, H., Ahlstrom, T., Vinner, L., Malaspinas, A.S., Margaryan, A., Higham, T., Chivall, D., Lynnerup, N., Harvig, L., Baron, J., Della Casa, P., Dabrowski, P., Duffy, P.R., Ebel, A.V., Epimakhov, A., Frei, K., Furmanek, M., Gralak, T., Gromov, A., Gronkiewicz, S., Grupe, G., Hajdu, T., Jarysz, R., Khartanovich, V., Khokhlov, A., Kiss, V., Kolar, J., Kriiska, A., Lasak, I., Longhi, C., McGlynn, G., Merkevicius, A., Merkyte, I., Metspalu, M., Mkrtchyan, R., Moiseyev, V., Paja, L., Palfi, G., Pokutta, D., Pospieszny, L., Price, T.D., Saag, L., Sablin, M., Shishlina, N., Smrcka, V., Soenov, V.I., Szeverenyi, V., Toth, G., Trifanova, S.V., Varul, L., Vicze, M., Yepiskoposyan, L., Zhitenev, V., Orlando, L., Sicheritz-Ponten, T., Brunak, S., Nielsen, R., Kristiansen, K., and Willerslev, E. 2015. Population genomics of Bronze Age Eurasia. *Nature* 522(7555): 167–172. doi: 10.1038/nature14507.
- Ammermann, A., and Cavalli Sforza, L.L. 1984. *The Neolithic transition and the genetics of populations in Europe*: Princeton University Press: Princeton, USA.

- Arauna, L.R., Mendoza-Revilla, J., Mas-Sandoval, A., Izaabel, H., Bekada, A., Benhamamouch, S., Fadhlouzi-Zid, K., Zalloua, P., Hellenthal, G., and Comas, D. 2017. Recent historical migrations have shaped the gene pool of Arabs and Berbers in North Africa. *Molecular Biology and Evolution* 34(2): 318–329. doi: 10.1093/molbev/msw218.
- Arredi, B., Poloni, E.S., Paracchini, S., Zerjal, T., Fathallah, D.M., Makrelouf, M., Pascali, V.L., Novelletto, A., and Tyler-Smith, C. 2004. A predominantly neolithic origin for Y-chromosomal DNA variation in North Africa. *American Journal of Human Genetics* 75(2): 338–345. doi: 10.1086/423147.
- Banerjee, A., Dindorf, W., Mikdad, A., Reischmann, T.H., and Schuhmacher, T.X. 2011. Die Elfenbeinfunde aus Kehf-el-Baroud (Ziaïda, Ben Slimane, Marokko) und die Frage des Nordafrikanischen Elefanten. *Madriider Mitteilungen* 52: 113–138.
- Barton, N., Bouzouggar, A., Humphrey, L., Berridge, P., Collcutt, S., Gale, R., Parfitt, S., Parker, A., Rhodes, E., and Schwenninger, J.L. 2008. Human burial evidence from Hattab II Cave and the question of continuity in Late Pleistocene–Holocene mortuary practices in Northwest Africa. *Cambridge Archaeological Journal* 18: 195–214. doi: 10.1017/S0959774308000255.
- Bekada, A., Fregel, R., Cabrera, V.M., Larruga, J.M., Pestano, J., Benhamamouch, S., and Gonzalez, A.M. 2013. Introducing the Algerian mitochondrial DNA and Y-chromosome profiles into the North African landscape. *PLoS One* 8(2): e56775. doi: 10.1371/journal.pone.0056775.
- Ben-Ncer, A., Bokbot, Y., Amani, F., and Ouachi, M. 2011. Study of the Chalcolithic Burial 2 and 3 of Ifri n'Amr ou Moussa (Morocco). In: Besse M. (ed) *Around the Petit-Chasseur Site in Sion (Valais, Switzerland) and New Approaches to the Bell Beaker Culture*. Archaeopress Archaeology: Oxford, UK, pp. 251–258.
- Ben-Ncer, A., Bokbot, Y., Amani, F., and Ouachi, M. 2015. La sépulture No 4 du site d'Ifri N'Amar Ou Moussa (Aït Khemisset, Maroc): Etude archéo-anthropologique. In: Sahnouni, M., Semaw, S., and Rios-Garaizar J (eds) *Proceedings of the II Meeting of African Prehistory*. Centro Nacional de Investigación sobre la Evolución Humana: Burgos: Spain.
- Bokbot, Y., and Ben-Ncer, A. 2008. Decouvertes campaniformes recentes dans les plateaux de Zemmour (Maroc). In: Baioni, M., Leonini, D., Lo Vetro, F., Martini, F., Poggiani Keller, R., and Sarti L. (eds) *Bell Beaker in Everyday Life*. Museo Fiorentino di Preistoria 'Paolo Graziosi': Firenze, Italy. pp. 327–330.
- Bosch, E., Calafell, F., Perez-Lezaun, A., Comas, D., Mateu, E., and Bertranpetit, J. 1997. Population history of north Africa: evidence from classical genetic markers. *Human Biology* 69(3): 295–311.
- Carpenter, M.L., Buenrostro, J.D., Valdiosera, C., Schroeder, H., Allentoft, M.E., Sikora, M., Rasmussen, M., Gravel, S., Guillen, S., Nekhrizov, G., Leshtakov, K., Dimitrova, D., Theodossiev, N., Pettener, D., Luiselli, D., Sandoval, K., Moreno-Estrada, A., Li,

- Y., Wang, J., Gilbert, M.T.P., Willerslev, E., Greenleaf, W.J., and Bustamante, C.D. 2013. Pulling out the 1%: Whole-Genome Capture for the Targeted Enrichment of Ancient DNA Sequencing Libraries. *American Journal of Human Genetics* 93(5): 852–864. doi: 10.1016/j.ajhg.2013.10.002.
- Cavalli-Sforza, L., Menozzi, P., and Piazza, A. 1994. *The History and Geography of Human Genes*. Princeton University Press: Princeton, USA.
- Cooper, A., and Poinar, H. N. 2000. Ancient DNA: do it right or not at all. *Science* 289(5482): 1139. doi: 10.1126/science.289.5482.1139b.
- Cruciani, F., La Fratta, R., Trombetta, B., Santolamazza, P., Sellitto, D., Colomb, E.B., Dugoujon, J.M., Crivellaro, F., Benincasa, T., Pascone, R., Moral, P., Watson, E., Melegh, B., Barbujani, G., Fuselli, S., Vona, G., Zagradsnik, B., Assum, G., Brdicka, R., Kozlov, A.I., Efremov, G.D., Coppa, A., Novelletto, A., and Scozzari, R. 2007. Tracing past human male movements in northern/eastern Africa and western Eurasia: new clues from Y-chromosomal haplogroups E-M78 and J-M12. *Molecular Biology and Evolution* 24(6): 1300–1311.
- Fadhlaoui-Zid, K., Garcia-Bertrand, R., Alfonso-Sanchez, M.A., Zemni, R., Benammar-Elgaaied, A., and Herrera, R.J. 2015. Sousse: extreme genetic heterogeneity in North Africa. *Journal of Human Genetics* 60(1): 41–49. doi: 10.1038/jhg.2014.99.
- Fadhlaoui-Zid, K., Haber, M., Martinez-Cruz, B., Zalloua, P., Benammar Elgaaied, A., and Comas, D. 2013. Genome-wide and paternal diversity reveal a recent origin of human populations in North Africa. *PLoS One* 8(11): e80293. doi: 10.1371/journal.pone.0080293.
- Fregel, R., Gomes, V., Gusmao, L., Gonzalez, A.M., Cabrera, V.M., Amorim, A., and Larruga, J.M. 2009. Demographic history of Canary Islands male gene-pool: replacement of native lineages by European. *BMC Evolutionary Biology* 9: 181. doi: 10.1186/1471-2148-9-181.
- Fregel, R., Mendez, F.L., Bokbot, Y., Martin-Socas, D., Camalich-Massieu, M.D., Santana, J., Morales, J., Avila-Arcos, M.C., Underhill, P.A., Shapiro, B., Wojcik, G., Rasmussen, M., Soares, A.E.R., Kapp, J., Sockell, A., Rodriguez-Santos, F.J., Mikdad, A., Trujillo-Mederos, A., and Bustamante, C.D. 2018. Ancient genomes from North Africa evidence prehistoric migrations to the Maghreb from both the Levant and Europe. *Proceedings of the National Academy of Sciences of the USA* 115(26): 6774–6779. doi: 10.1073/pnas.1800851115.
- Fregel, R., Ordonez, A.C., Santana-Cabrera, J., Cabrera, V.M., Velasco-Vazquez, J., Alberto, V., Moreno-Benitez, M.A., Delgado-Darias, T., Rodriguez-Rodriguez, A., Hernandez, J.C., Pais, J., Gonzalez-Montelongo, R., Lorenzo-Salazar, J.M., Flores, C., Cruz-de-Mercadal, M.C., Alvarez-Rodriguez, N., Shapiro, B., Arnay, M., and Bustamante, C.D. 2019. Mitogenomes illuminate the origin and migration patterns of the indigenous people of the Canary Islands. *PLoS One* 14(3): e0209125. doi: 10.1371/journal.pone.0209125.

- Gamba, C., Jones, E.R., Teasdale, M.D., McLaughlin, R.L., Gonzalez-Fortes, G., Matiangeli, V., Domboroczki, L., Kovari, I., Pap, I., Anders, A., Whittle, A., Dani, J., Raczký, P., Higham, T.F., Hofreiter, M., Bradley, D.G., and Pinhasi, R. 2014. Genome flux and stasis in a five millennium transect of European prehistory. *Nat Commun* 5:5257. doi: 10.1038/ncomms6257.
- Gonzalez, A.M., Larruga, J.M., Abu-Amero, K., Shi, Y., Pestano, J., and Cabrera, V.M. 2007. Mitochondrial lineage M1 traces an early human backflow to Africa. *BMC Genomics* 8:223. doi: 10.1186/1471-2164-8-223.
- Haak, W., Lazaridis, I., Patterson, N., Rohland, N., Mallick, S., Llamas, B., Brandt, G., Nordenfelt, S., Harney, E., Stewardson, K., Fu, Q., Mittnik, A., Banffy, E., Economou, C., Francken, M., Friederich, S., Pena, R.G., Hallgren, F., Khartanovich, V., Khokhlov, A., Kunst, M., Kuznetsov, P., Meller, H., Mochalov, O., Moiseyev, V., Nicklisch, N., Pichler, S.L., Risch, R., Rojo Guerra, M.A., Roth, C., Szecsenyi-Nagy, A., Wahl, J., Meyer, M., Krause, J., Brown, D., Anthony, D., Cooper, A., Alt, K.W., and Reich, D. 2015. Massive migration from the steppe was a source for Indo-European languages in Europe. *Nature* 522(7555): 207–211. doi: 10.1038/nature14317.
- Henn, B.M., Botigue, L.R., Gravel, S., Wang, W., Brisbin, A., Byrnes, J.K., Fadhlaoui-Zid, K., Zalloua, P.A., Moreno-Estrada, A., Bertranpetit, J., Bustamante, C.D., and Comas, D. 2012. Genomic ancestry of North Africans supports back-to-Africa migrations. *PloS Genetics* 8(1): e1002397. doi: 10.1371/journal.pgen.1002397.
- Kefi, R., Hechmi, M., Naouali, C., Jmel, H., Hsouna, S., Bouzaid, E., Abdelhak, S., Beraud-Colomb, E., and Stevanovitch, A. 2018. On the origin of Iberomaurusians: new data based on ancient mitochondrial DNA and phylogenetic analysis of Afalou and Taforalt populations. *Mitochondrial DNA. Part A, DNA Mapping, Sequencing, and Analysis* 29(1): 147–157. doi: 10.1080/24701394.2016.1258406.
- Kéfi, R., Stevanovitch, A., Bouzaid, E., and Colomb, B. 2005. Diversité mitochondriale de la population de Taforalt (12.000 ans bp - Maroc): une approche génétique à l'étude du peuplement de l'Afrique du Nord. *Anthropologie* 43(1): 1–11.
- Laviano, F. 2015. La faune néolithique du site d'Ifri n'Amr o'Moussa (Oued Beth, plateau de Zemmour, Maroc): méthodologie appliquée à une stratigraphie perturbée. Unpublished Master's Thesis. Université Paul Valéry Montpellier: Montpellier, France.
- Lazaridis, I., Nadel, D., Rollefson, G., Merrett, D.C., Rohland, N., Mallick, S., Fernandes, D., Novak, M., Gamarra, B., Sirak, K., Connell, S., Stewardson, K., Harney, E., Fu, Q., Gonzalez-Fortes, G., Jones, E.R., Roodenberg, S.A., Lengyel, G., Bocquentin, F., Gasparian, B., Monge, J.M., Gregg, M., Eshed, V., Mizrahi, A.S., Meiklejohn, C., Gerritsen, F., Bejenaru, L., Bluhner, M., Campbell, A., Cavalleri, G., Comas, D., Froguel, P., Gilbert, E., Kerr, S.M., Kovacs, P., Krause, J., McGettigan, D., Merrigan, M., Merriwether, D.A., O'Reilly, S., Richards, M.B., Semino, O., Shamoon-Pour, M., Stefanescu, G., Stumvoll, M., Tonjes, A., Torroni, A., Wilson, J.F., Yengo, L., Hovhannisyan, N.A., Patterson, N., Pinhasi, R., and Reich, D. 2016. Genomic insights into the origin of farming in the ancient Near East. *Nature* 536(7617): 419–424. doi: 10.1038/nature19310.

- Lazaridis, I., Patterson, N., Mittnik, A., Renaud, G., Mallick, S., Kirsanow, K., Sudmant, P.H., Schraiber, J.G., Castellano, S., Lipson, M., Berger, B., Economou, C., Bollongino, R., Fu, Q., Bos, K.I., Nordenfelt, S., Li, H., de Filippo, C., Prufer, K., Sawyer, S., Posth, C., Haak, W., Hallgren, F., Fornander, E., Rohland, N., Delsate, D., Francken, M., Guinet, J.M., Wahl, J., Ayodo, G., Babiker, H.A., Bailliet, G., Balanovska, E., Balanovsky, O., Barrantes, R., Bedoya, G., Ben-Ami, H., Bene, J., Berrada, F., Bravi, C.M., Brisighelli, F., Busby, G.B., Cali, F., Churnosov, M., Cole, D.E., Corach, D., Damba, L., van Driem, G., Dryomov, S., Dugoujon, J.M., Fedorova, S.A., Gallego Romero, I., Gubina, M., Hammer, M., Henn, B.M., Hervig, T., Hodoglugil, U., Jha, A.R., Karachanak-Yankova, S., Khusainova, R., Khusnutdinova, E., Kittles, R., Kivisild, T., Klitz, W., Kucinskas, V., Kushniarevich, A., Laredj, L., Litvinov, S., Loukidis, T., Mahley, R.W., Melegh, B., Metspalu, E., Molina, J., Mountain, J., Nakkalajarvi, K., Nesheva, D., Nyambo, T., Osipova, L., Parik, J., Platonov, F., Posukh, O., Romano, V., Rothhammer, F., Rudan, I., Ruizbakiev, R., Sahakyan, H., Sajantila, A., Salas, A., Starikovskaya, E.B., Tarekegn, A., Toncheva, D., Turdikulova, S., Uktveryte, I., Utevska, O., Vasquez, R., Villena, M., Voevoda, M., Winkler, C.A., Yepiskoposyan, L., Zalloua, P., Zemunik, T., Cooper, A., Capelli, C., Thomas, M.G., Ruiz-Linares, A., Tishkoff, S.A., Singh, L., Thangaraj, K., Villems, R., Comas, D., Sukernik, R., Metspalu, M., Meyer, M., Eichler, E.E., Burger, J., Slatkin, M., Paabo, S., Kelso, J., Reich, D., and Krause, J. 2014. Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature* 513(7518): 409–413. doi: 10.1038/nature13673.
- Lazaridis, I., Belfer-Cohen, A., Mallick, S., Patterson, N., Cheronet, O., Rohland, N., Bar-Oz, G., Bar-Yosef, O., Jakeli, N., Kvavadze, E., Lordkipanidze, D., Matzkevich, Z., Meshveliani, T., Culleton, B.J., Kennett, D.J., Pinhasi, R., and Reich, D. 2018. Paleolithic DNA from the Caucasus reveals core of West Eurasian ancestry. *bioRxiv*. doi: 10.1101/423079.
- Linstädter, J. 2008. Epipalaeolithic-Neolithic-Transition in the Mediterranean region of Northwest-Africa. *Quartär* 55: 41–62.
- Maca-Meyer, N., Armay, M., Rando, J.C., Flores, C., Gonzalez, A.M., Cabrera, V.M., and Larruga, J.M. 2004. Ancient mtDNA analysis and the origin of the Guanches. *European Journal of Human Genetics* 12(2): 155–162. doi: 10.1038/sj.ejhg.5201075.
- Maca-Meyer, N., Gonzalez, A.M., Pestano, J., Flores, C., Larruga, J.M., and Cabrera, V.M. 2003. Mitochondrial DNA transit between West Asia and North Africa inferred from U6 phylogeography. *BMC Genet* 4: 15. doi: 10.1186/1471-2156-4-15.
- Macauley, V., Richards, M., Hickey, E., Vega, E., Cruciani, F., Guida, V., Scozzari, R., Bonne-Tamir, B., Sykes, B., and Torroni, A. 1999. The emerging tree of West Eurasian mtDNAs: a synthesis of control-region sequences and RFLPs. *American Journal of Human Genetics* 64(1): 232–249. doi: 10.1086/302204.
- Martínez-Sánchez, R.M., Vera-Rodríguez, J.C., Pérez-Jordà, G., Peña-Chocarro, L., and Bokbot, Y. 2017. The beginning of the Neolithic in northwestern Morocco. *Quaternary International* 470: 485–496. doi: 10.1016/j.quaint.2017.05.052.

- Mathieson, I., Alpaslan-Roodenberg, S., Posth, C., Szecsenyi-Nagy, A., Rohland, N., Mallick, S., Olalde, I., Broomandkhoshbacht, N., Candilio, F., Cheronet, O., Fernandes, D., Ferry, M., Gamarra, B., Fortes, G.G., Haak, W., Harney, E., Jones, E., Keating, D., Krause-Kyora, B., Kucukkalipci, I., Michel, M., Mittnik, A., Nagele, K., Novak, M., Oppenheimer, J., Patterson, N., Pfengle, S., Sirak, K., Stewardson, K., Vai, S., Alexandrov, S., Alt, K.W., Andreescu, R., Antonovic, D., Ash, A., Atanassova, N., Bacvarov, K., Gusztav, M.B., Bocherens, H., Bolus, M., Boroneant, A., Boyadzhiev, Y., Budnik, A., Burmaz, J., Chohadzhiev, S., Conard, N.J., Cottiaux, R., Cuka, M., Cupillard, C., Drucker, D.G., Elenski, N., Francken, M., Galabova, B., Ganetsovski, G., Gely, B., Hajdu, T., Handzhyska, V., Harvati, K., Higham, T., Iliev, S., Jankovic, I., Karavanic, I., Kennett, D.J., Komso, D., Kozak, A., Labuda, D., Lari, M., Lazar, C., Leppek, M., Leshtakov, K., Vetro, D.L., Los, D., Lozanov, I., Malina, M., Martini, F., McSweeney, K., Meller, H., Mendusic, M., Mirea, P., Moiseyev, V., Petrova, V., Price, T.D., Simalcik, A., Sineo, L., Slaus, M., Slavchev, V., Stanev, P., Starovic, A., Szeniczey, T., Talamo, S., Teschler-Nicola, M., Thevenet, C., Valchev, I., Valentin, F., Vasilyev, S., Veljanovska, F., Venelinova, S., Veselovskaya, E., Viola, B., Virag, C., Zaninovic, J., Zauner, S., Stockhammer, P.W., Catalano, G., Krauss, R., Caramelli, D., Zarina, G., Gaydarska, B., Lillie, M., Nikitin, A.G., Potekhina, I., Papathanasiou, A., Boric, D., Bonsall, C., Krause, J., Pinhasi, R., and Reich, D. 2018. The genomic history of southeastern Europe. *Nature* 555(7695): 197–203. doi: 10.1038/nature25778.
- Mathieson, I., Lazaridis, I., Rohland, N., Mallick, S., Patterson, N., Roodenberg, S.A., Harney, E., Stewardson, K., Fernandes, D., Novak, M., Sirak, K., Gamba, C., Jones, E.R., Llamas, B., Dryomov, S., Pickrell, J., Arsuaga, J.L., de Castro, J.M., Carbonell, E., Gerritsen, F., Khokhlov, A., Kuznetsov, P., Lozano, M., Meller, H., Mochalov, O., Moiseyev, V., Guerra, M.A., Roodenberg, J., Verges, J.M., Krause, J., Cooper, A., Alt, K.W., Brown, D., Anthony, D., Lalueza-Fox, C., Haak, W., Pinhasi, R., and Reich, D. 2015. Genome-wide patterns of selection in 230 ancient Eurasians. *Nature* 528(7583): 499–503. doi: 10.1038/nature16152.
- Mulazzani, S., Belhouchet, L., Salanova, L., Aouadi, N., Dridi, Y., Eddargach, W., Morales, J., Tombret, O., Zazzo, A., and Zoughlami, J. 2016. The emergence of the Neolithic in North Africa: A new model for the Eastern Maghreb. *Quaternary International* 410: 123–143. doi: 10.1016/j.quaint.2015.11.089
- Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G., and Erlich, H. 1986. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. *Cold Spring Harbor Laboratory of Quantitative Biology* 51(Pt 1): 263–273.
- Naylor, P. C. 2015. *North Africa, Revised Edition: A History from Antiquity to the Present*. University of Texas Press: Texas, USA.
- Olalde, I., Brace, S., Allentoft, M.E., Armit, I., Kristiansen, K., Booth, T., Rohland, N., Mallick, S., Szecsenyi-Nagy, A., Mittnik, A., Altena, E., Lipson, M., Lazaridis, I., Harper, T.K., Patterson, N., Broomandkhoshbacht, N., Diekmann, Y., Faltyskova,

- Z., Fernandes, D., Ferry, M., Harney, E., de Knijff, P., Michel, M., Oppenheimer, J., Stewardson, K., Barclay, A., Alt, K.W., Liesau, C., Rios, P., Blasco, C., Miguel, J.V., Garcia, R.M., Fernandez, A.A., Banffy, E., Bernabo-Brea, M., Billoin, D., Bonsall, C., Bonsall, L., Allen, T., Buster, L., Carver, S., Navarro, L.C., Craig, O.E., Cook, G.T., Cunliffe, B., Denaire, A., Dinwiddy, K.E., Dodwell, N., Ernee, M., Evans, C., Kucharik, M., Farre, J.F., Fowler, C., Gazenbeek, M., Pena, R.G., Haber-Urriarte, M., Haduch, E., Hey, G., Jowett, N., Knowles, T., Massy, K., Pfrengle, S., Lefranc, P., Lemerrier, O., Lefebvre, A., Martinez, C.H., Olmo, V.G., Ramirez, A.B., Maurandi, J.L., Majó, T., McKinley, J.I., McSweeney, K., Mende, B.G., Modi, A., Kulcsar, G., Kiss, V., Czene, A., Patay, R., Endrodi, A., Kohler, K., Hajdu, T., Szeniczey, T., Dani, J., Bernert, Z., Hoole, M., Cheronet, O., Keating, D., Veleminsky, P., Dobes, M., Candilio, F., Brown, F., Fernandez, R.F., Herrero-Corral, A.M., Tusa, S., Carnieri, E., Lentini, L., Valenti, A., Zanini, A., Waddington, C., Delibes, G., Guerra-Doce, E., Neil, B., Brittain, M., Luke, M., Mortimer, R., Desideri, J., Besse, M., Brucken, G., Furmanek, M., Haluszko, A., Mackiewicz, M., Rapinski, A., Leach, S., Soriano, I., Lillios, K.T., Cardoso, J.L., Pearson, M.P., Włodarczak, P., Price, T.D., Prieto, P., Rey, P.J., Risch, R., Rojo Guerra, M.A., Schmitt, A., Serrallongue, J., Silva, A.M., Smrcka, V., Vergnaud, L., Zilhao, J., Caramelli, D., Higham, T., Thomas, M.G., Kennett, D.J., Fokkens, H., Heyd, V., Sheridan, A., Sjogren, K.G., Stockhammer, P.W., Krause, J., Pinhasi, R., Haak, W., Barnes, I., Lalueza-Fox, C., and Reich, D. 2018. The Beaker phenomenon and the genomic transformation of northwest Europe. *Nature* 555(7695): 190–196. doi: 10.1038/nature25738.
- Olalde, I., Mallick, S., Patterson, N., Rohland, N., Villalba-Mouco, V., Silva, M., Dulias, K., Edwards, C.J., Gandini, F., Pala, M., Soares, P., Ferrando-Bernal, M., Adamski, N., Broomandkhoshbacht, N., Cheronet, O., Culleton, B.J., Fernandes, D., Lawson, A.M., Mah, M., Oppenheimer, J., Stewardson, K., Zhang, Z., Jimenez Arenas, J.M., Toro Moyano, I.J., Salazar-Garcia, D.C., Castanyer, P., Santos, M., Tremoleda, J., Lozano, M., Garcia Borja, P., Fernandez-Eraso, J., Mujika-Alustiza, J.A., Barroso, C., Bermudez, F.J., Viguera Minguez, E., Burch, J., Coromina, N., Vivo, D., Cebria, A., Fullola, J.M., Garcia-Puchol, O., Morales, J.I., Oms, F.X., Majó, T., Verges, J.M., Diaz-Carvajal, A., Ollich-Castanyer, I., Lopez-Cachero, F.J., Silva, A.M., Alonso-Fernandez, C., Delibes de Castro, G., Jimenez Echevarria, J., Moreno-Marquez, A., Pascual Berlanga, G., Ramos-Garcia, P., Ramos-Munoz, J., Vijande Vila, E., Aguilera Arzo, G., Esparza Arroyo, A., Lillios, K.T., Mack, J., Velasco-Vazquez, J., Waterman, A., Benitez de Lugo Enrich, L., Benito Sanchez, M., Agusti, B., Codina, F., de Prado, G., Estalrich, A., Fernandez Flores, A., Finlayson, C., Finlayson, G., Finlayson, S., Giles-Guzman, F., Rosas, A., Barciela Gonzalez, V., Garcia Atienzar, G., Hernandez Perez, M.S., Llanos, A., Carrion Marco, Y., Collado Beneyto, I., Lopez-Serrano, D., Sanz Tormo, M., Valera, A.C., Blasco, C., Liesau, C., Rios, P., Daura, J., de Pedro Micho, M.J., Diez-Castillo, A.A., Flores Fernandez, R., Frances Farre, J., Garrido-Pena, R., Goncalves, V.S., Guerra-Doce,

- E., Herrero-Corral, A.M., Juan-Cabanilles, J., Lopez-Reyes, D., McClure, S.B., Merino Perez, M., Oliver Foix, A., Sanz Borrás, M., Sousa, A.C., Vidal Encinas, J.M., Kennett, D.J., Richards, M.B., Werner Alt, K., Haak, W., Pinhasi, R., Lalueza-Fox, C., and Reich, D. 2019. The genomic history of the Iberian Peninsula over the past 8000 years. *Science* 363(6432): 1230–1234. doi: 10.1126/science.aav4040.
- Olivieri, A., Achilli, A., Pala, M., Battaglia, V., Fornarino, S., Al-Zahery, N., Scozzari, R., Cruciani, F., Behar, D.M., Dugoujon, J.M., Coudray, C., Santachiara-Benerecetti, A., Semino, O., Bandelt, H.J., and Torroni, A. 2006. The mtDNA legacy of the Levantine early Upper Palaeolithic in Africa. *Science* 314(5806): 1767–1770. doi: 10.1126/science.1135566.
- Paabo, S., Gifford, J.A., and Wilson, A.C. 1988. Mitochondrial DNA sequences from a 7000-year old brain. *Nucleic Acids Research* 16(20): 9775–9787. doi: 10.1093/nar/16.20.9775.
- Paabo, S., Poinar, H., Serre, D., Jaenicke-Despres, V., Hebler, J., Rohland, N., Kuch, M., Krause, J., Vigilant, L., and Hofreiter, M. 2004. Genetic analyses from ancient DNA. *Annual Review of Genetics* 38: 645–679. doi: 10.1146/annurev.genet.37.110801.143214.
- Pennarun, E., Kivisild, T., Metspalu, E., Metspalu, M., Reisberg, T., Moisan, J.P., Behar, D.M., Jones, S.C., and Vilems, R. 2012. Divorcing the Late Upper Palaeolithic demographic histories of mtDNA haplogroups M1 and U6 in Africa. *BMC Evolutionary Biology* 12: 234. doi: 10.1186/1471-2148-12-234.
- Pereira, L., Silva, N.M., Franco-Duarte, R., Fernandes, V., Pereira, J.B., Costa, M.D., Martins, H., Soares, P., Behar, D.M., Richards, M.B., and Macaulay, V. 2010. Population expansion in the North African late Pleistocene signalled by mitochondrial DNA haplogroup U6. *BMC Evolutionary Biology* 10: 390. doi: 10.1186/1471-2148-10-390.
- Rando, J.C., Pinto, F., Gonzalez, A.M., Hernandez, M., Larruga, J.M., Cabrera, V.M., Bandelt, H.J. 1998. Mitochondrial DNA analysis of northwest African populations reveals genetic exchanges with European, near-eastern, and sub-Saharan populations. *Annals of Human Genetics* 62: 531–550. doi: 10.1046/j.1469-1809.1998.6260531.x.
- Richards, M., Corte-Real, H., Forster, P., Macaulay, V., Wilkinson-Herbots, H., Demaine, A., Papiha, S., Hedges, R., Bandelt, H.J., Sykes, B. 1996. Paleolithic and neolithic lineages in the European mitochondrial gene pool. *American Journal of Human Genetics* 59(1): 185–203.
- Richter, D., Grun, R., Joannes-Boyau, R., Steele, T.E., Amani, F., Rue, M., Fernandes, P., Raynal, J.P., Geraads, D., Ben-Ncer, A., Hublin, J.J., and McPherron, S.P. 2017. The age of the hominin fossils from Jebel Irhoud, Morocco, and the origins of the Middle Stone Age. *Nature* 546(7657): 293–296. doi: 10.1038/nature22335.
- Rodriguez-Varela, R., Gunther, T., Krzewinska, M., Stora, J., Gillingwater, T.H., MacCallum, M., Arsuaga, J.L., Dobney, K., Valdiosera, C., Jakobsson, M., Gotherstrom, A., and Girdland-Flink, L. 2017. Genomic analyses of pre-European conquest human remains from the Canary islands reveal close affinity to modern North Africans. *Current Biology* 27(21): 3396–3402 e5. doi: 10.1016/j.cub.2017.09.059.

- Sahnouni, M., Pares, J.M., Duval, M., Caceres, I., Harichane, Z., van der Made, J., Perez-Gonzalez, A., Abdessadok, S., Kandi, N., Derradji, A., Medig, M., Boulaghraif, K., and Semaw, S. 2018. 1.9-million- and 2.4-million-year-old artifacts and stone tool-cutmarked bones from Ain Boucherit, Algeria. *Science* 362(6420): 1297–1301. doi: 10.1126/science.aau0008.
- Secher, B., Fregel, R., Larruga, J.M., Cabrera, V.M., Endicott, P., Pestano, J.J., and Gonzalez, A.M. 2014. The history of the North African mitochondrial DNA haplogroup U6 gene flow into the African, Eurasian and American continents. *BMC Evolutionary Biology* 14(1): 109. doi: 1471-2148-14-109 [pii].
- Sokal, R.R., Oden, N.L., and Wilson, C. 1991. Genetic evidence for the spread of agriculture in Europe by demic diffusion. *Nature* 351(6322): 143–145. doi: 10.1038/351143a0.
- Sole-Morata, N., Garcia-Fernandez, C., Urasin, V., Bekada, A., Fadhlou-Zid, K., Zalloua, P., Comas, D., and Calafell, F. 2017. Whole Y-chromosome sequences reveal an extremely recent origin of the most common North African paternal lineage E-M183 (M81). *Scientific Reports* 7(1): 15941. doi: 10.1038/s41598-017-16271-y.
- Trombetta, B., D'Atanasio, E., Massaia, A., Ippoliti, M., Coppa, A., Candilio, F., Coia, V., Russo, G., Dugoujon, J.M., Moral, P., Akar, N., Sellitto, D., Valesini, G., Novelletto, A., Scozzari, R., and Cruciani, F. 2015. Phylogeographic Refinement and Large Scale Genotyping of Human Y Chromosome Haplogroup E Provide New Insights into the Dispersal of Early Pastoralists in the African Continent. *Genome Biology and Evolution* 7(7): 1940–1950. doi: 10.1093/gbe/evv118.
- van de Loosdrecht, M., Bouzouggar, A., Humphrey, L., Posth, C., Barton, N., Aximu-Petri, A., Nickel, B., Nagel, S., Talbi, E.H., El Hajraoui, M.A., Amzazi, S., Hublin, J.J., Paabo, S., Schiffels, S., Meyer, M., Haak, W., Jeong, C., and Krause, J. 2018. Pleistocene North African genomes link Near Eastern and sub-Saharan African human populations. *Science*. doi: 10.1126/science.aar8380.
- Zilhao, J. 2001. Radiocarbon evidence for maritime pioneer colonization at the origins of farming in west Mediterranean Europe. *Proceedings of the National Academy of Sciences of the USA* 98(24): 14180–14185. doi: 10.1073/pnas.241522898.