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# Temperature monitoring in archaeological animal bone samples in the Near East arid area, before, during and after excavation

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## Summary

In order to estimate experimentally the intensity and amplitude of thermal shocks during and after excavation, we monitored temperatures of archaeological bones on the field at three Syrian sites of the arid steppe, Qaramel, Dja'de and Aswad. Water cleaning and sun drying appear to be the most damaging steps, with temperature variations of ca. 11,000 °C/h and 84 °C/h, respectively. Ancient DNA (aDNA) bone samples kept between –7 and +12 °C from their extraction to the lab suffered much lower thermal variations (6 °C/h). Estimation of the temperature variations at different depths in the soil suggests that aDNA has suffered negative thermal conditions shortly after burial and again during excavation, before their extraction by archaeologists.

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## 1. Introduction

Ancient DNA (aDNA) can bring important contributions to the understanding of the origins of early domesticates, and to their spread throughout the world (Bailey et al., 1996; Edwards et al., 2004, 2007; Bollongino et al., 2006; Fernández et al., 2006; Zeder et al., 2006). This is especially true for the Near East, where the great majority of domestic ungulates namely sheep, goat and cattle originated. However, as for collagen (Weiner and Bar-Yosef, 1990), the preservation of DNA in animal bones of the Preneolithic and Neolithic sites in the Near East is very inadequate. As an example, in the paper by Edwards et al. (2004) on cattle, only two samples out of 70 yielded good, replicable ancient DNA. Two samples from the Bronze Age Tell Brak (Syria), 23 samples of Çatal Höyük and 23 other ones from five sites in Israel yielded no results. In

a more recent paper (Edwards et al., 2007), eight samples from Jerf El Ahmar failed to produce results, and only one out of five from Dja'de produced a result. Taken together, those two papers give an estimate of 3.4% success ( $N = 83$ ).

The main causes for ancient DNA degradation are hydrolysis, oxidation, autolysis and bacterial attacks (Lindahl, 1993; Burger et al., 1999). Acidity of the soil, humidity, pressure, biological activity of the soil and airflow can exacerbate one or the other of these four main processes. However, only high temperatures intensify all of them, especially DNA depurination, which is the principal mechanism of molecular degradation, so that some palaeogeneticists suggested calculating the thermal age of the samples (Smith et al., 2003). In addition, high-speed temperature variations in the bone (thermal shocks) likely provoke fragmentation of the organic molecules. In the arid zones of the Near East, high as well as contrasting temperatures are probably the main factors of aDNA degradation. Higher temperatures in storerooms can also cause aDNA to decay, as recently evidenced by Pruvost et al. (2007) comparing the aDNA yield of two refitting bones of French

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Neolithic aurochs found at two different sessions of the excavation of the same site, the second one being 20 years later (see also Burger et al., 1999).

In order to contribute, via a macroscopic (i.e. not molecular) experimental way, to the understanding of what temperature conditions and variations archaeological bones undergo before, during and after the excavation in the arid zones of the Near East, we organized a preliminary field experiment on three Pre-ceramic Neolithic sites in Syria, in September 2005. The aims were: (i) to estimate experimentally the intensity and amplitude of the thermal shocks that archaeological animal bones undergo under usual archaeological practices of excavation, water cleaning, sun drying and storage under differing conditions of sun and shadow; (ii) to compare those samples to what happened to about 100 samples that we excavated ourselves under the conditions of aDNA sampling and kept at low temperatures from the field to the lab, with the goal of extracting aDNA from them. In parallel, using current models for estimating the soil temperature at different depth, we attempted (iii) to estimate temperatures and seasonal variations that archaeological bones have suffered in the soil, from burial to excavation.

## 2. The sites

We obtained sampling authorizations and help from excavators for three Syrian sites (Fig. 1), dating from the Khiamian to the Recent PPNB, i.e. from ca. 9500 to 7000 B.C. These are Tell Qaramel, Dja'de El Mughara and Tell Aswad, all situated in the arid area, with average annual precipitation ranging from 100 to 400 mm (Besançon and Geyer, 2006).

Tell Qaramel is a large Bronze Age tell located 20 km north of Aleppo (Mazurowski, 2004). It partially covers a lower tell dated to the Late Khiamian–PPNA (ca. 10,000–9000 cal. B.C.). The latter is being excavated under the direction of Krysiak Mazurowski (University of Warsaw). The layers from which we collected the bone samples were situated 4–6 m under the present day ground level. Today, it is situated at the boundary of the upper arid steppe (average annual precipitation = 400 mm; Besançon and Geyer, 2006); it is the least arid of the three sites sampled.

Tell Dja'de El Mughara is situated on the left Middle Euphrates bank. It is now located a few meters from the dam, but was at several tens of meters from the Euphrates river at the time of its occupation. However, the modern artificial

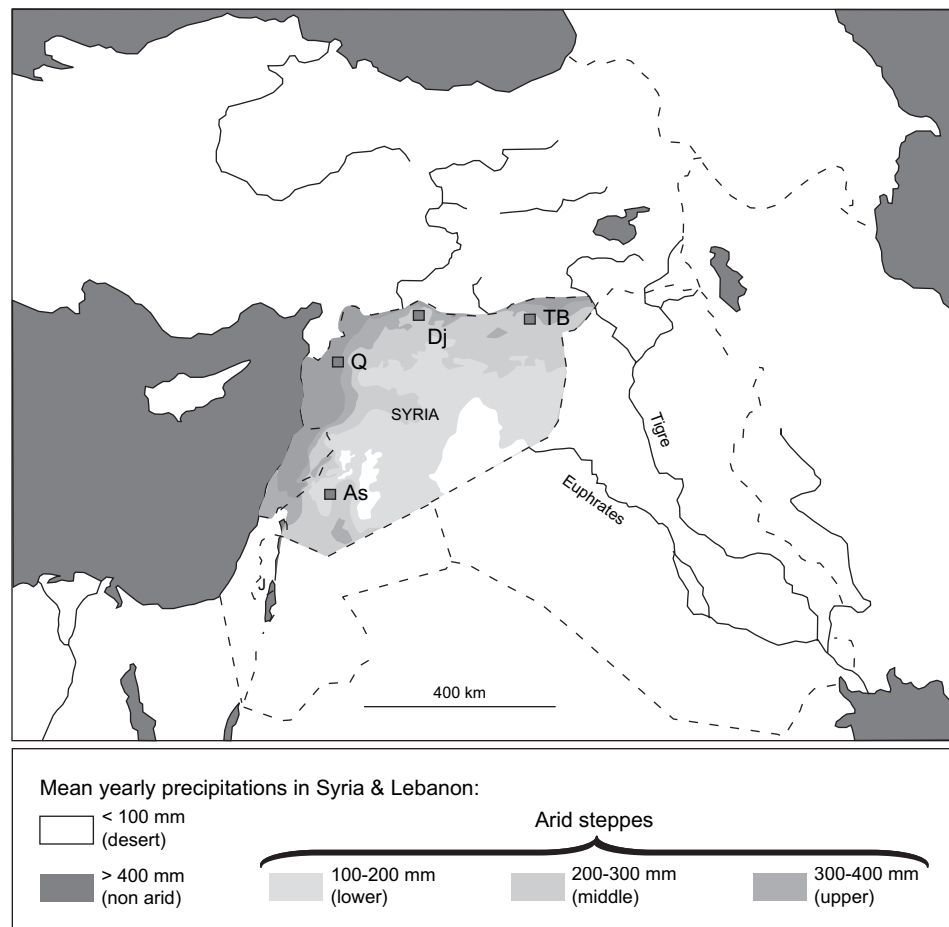


Fig. 1. Location of the three sampled sites (As, Tell Aswad; Q, Tell Qaramel; Dj, Tell Dja'de) and of Tell Brak (TB) in Syria, and borders of the arid steppes, according to Besançon and Geyer (2006).

increase in the lake level was far enough to flood even the basal part of the tell. It has been under excavation since 1991 under the supervision of Eric Coqueugniot (CNRS, Maison de l'Orient, Lyon). This is a long but shallow tell. It is 6 m high with reference to the present day level of the plain, but it was eight at the time of its occupations. These are all dated to the Early PPNB (ca. 8700–8300 cal. B.C.; Coqueugniot, 2000). Dja'de is located in the medium arid steppe (250–300 mm precipitation per year; Besançon and Geyer, 2006).

Tell Aswad is located very close to the Damascus airport. It is a very large tell located near two palaeolakes, which completely dried out during the 20th century. Danielle Stordeur (CNRS, Maison de l'Orient, Lyon) conducts excavations on the eastern extremity of the tell, near the lake area. However, this part of the site has never been flooded by the lake waters. In this area, the Early PPNB Horizons are still under study, but Middle and Recent PPNB (ca. 8500?–7200 cal. B.C.) are well characterized (Stordeur, 2003a,b; Stordeur et al., in press). The bone samples were extracted from layers 2–4 m under the soil surface. Today, this location is the most arid of the three, with 100–200 mm annual precipitation (lower arid steppe; Besançon and Geyer, 2006).

### 3. Methods

#### 3.1. Bone sampling

Bone samples for aDNA were collected with all the usual precautions: masks, gloves and sterile plastic bags. Tools were decontaminated with bleach. We excavated all the bones ourselves, at different locations on the sites. In order to reduce the risk of contamination by aerosols, we processed as quickly as possible from the time when the bone became visible to the time when it was put in the plastic bag, even if this speed extraction in some cases necessitated breaking the bone in several fragments. Samples of soil were also collected in order to monitor possible DNA contaminations through the environment.

All the samples were immediately stored in electric coolers, with the temperature monitored in real time. The three sites were less than 5 min from a freezer, either walking or driving, so all samples in the coolers were brought to a temperature of  $-8$  to  $-2$  °C within 2 h of their extraction, and kept at that temperature constantly until they reached the lab. However, they were re-warmed briefly thereafter, from two to four times depending on the sample series, for measurements, transportation and administrative export formalities. The temperature during these short re-warming phases remained low, however, owing to the fact that bones remained in electric coolers, which generated low temperatures in buildings and during car journeys. Temperatures of the bones were monitored the entire time. Additional re-warming of the bones was necessary for sample preparation in the lab (altogether 2 h of UV-radiation, bone cutting and grinding).

#### 3.2. Temperature monitoring

Temperature monitoring was processed with digital thermometers ( $\pm 0.1$  °C), which gave both the air temperature

and the internal bone temperature owing to a small sensor that could be driven into the sample. These thermometers were used inside the coolers and the freezers, but also in the field, for monitoring bones that were not sampled for ancient DNA, but only used for temperature investigations. In Dja'de and Aswad, we monitored bones lying on the soil, still set in the earth, in the plastic bags, either in the sun or in the shade, during water cleaning and sun drying (Fig. 2), on and inside the bone, and in the storerooms (Dja'de), over both days and nights.

Since it was not possible to drill the aDNA samples, their internal temperature was considered equal to that of the soil, cooler or freezer where they were temporarily stored.

#### 3.3. Estimating internal soil temperatures

As it would have been dangerous for the archaeological remains and stratigraphies, it was not possible to drill the tells to measure temperatures deep in the soil. We estimated these temperatures for Aswad, using Hillel's formula (1982, cited by Nofziger, 2000):

$$T(z, t) = T_a + (A_0 e^{-z/d}) \sin \left[ \frac{2\pi(t - t_0)}{365} - \frac{z}{d} - \frac{\pi}{2} \right],$$

which gives the soil temperature at time  $t$  (d) and depth  $z$  (m) with reference to:

- $T_a$ , average soil temperature (°C),
- $A_0$ , annual amplitude of the surface soil temperature (°C),
- $d$ , the damping depth (m) of annual fluctuation ( $d = (2D_h/w)^{1/2}$ , where  $D_h$  is the thermal diffusivity (TD) and  $w = 2\pi/365(d^{-1})$ ), and
- $t_0$ , the time lag from an arbitrary starting date to the occurrence of the minimum temperature in a year.

For  $A_0$ , we used the air temperature  $+2$  °C, according to the field experiments of Wu and Nofziger (1999). We used the air temperature given by the World Meteorological Organization



Fig. 2. Temperature monitoring of the air and bone's interior during the sun drying in Dja'de.

for Damascus ([www.worldweather.org/099/c00213.htm](http://www.worldweather.org/099/c00213.htm)) that we compared and finally validated with the daily meteorological data of the Damascus airport, considered a local reference for the Aswad excavations (web site of the Damascus airport).

The thermal diffusivity varies between 0 and 0.5. It depends on the porosity, humidity and argillite richness of the soil.

We simulated different situations using software from the Department of Plant and Soil Sciences of the University of Oklahoma (Nofziger and Wu), which is available on the web (<http://soilphysics.okstate.edu/toolkit/temperature/index0.html>) and used the Hillel's formula. We assumed Tell Aswad has always been under bare soil conditions.

The Nofziger's model can be considered reliable for our example in Damascus, since Wu and Nofziger (1999) demonstrated that except for daily variations, which are not taken into account in the model, it does not differ from experimental conditions observed during 1080 days beginning January 1, 1986 in Hebei Province, China, i.e. at the same latitude and temperature variations as Damascus.

### 3.4. Ancient DNA extraction and amplification

In order to find out if aDNA fragments could be extracted from our samples, aDNA extractions were processed in the lab of the Institute for Anthropology at the University of Mainz. It is a dedicated lab for ancient DNA analyses. Each sample was extracted once or twice, according to a phenol–chloroform extraction protocol with subsequent purification and concentration with filter columns, and amplified as described in Burger et al. (2004). The extracted soil and bone samples were amplified in a 50  $\mu$ l-PCR reaction volume. Each extract was tested for presence of inhibitors. Primers were designed to amplify *Bos* and *Bison* but might also fit other ungulate species. The primers flank a 92 bp long fragment (including primers) in order to detect even highly degraded and fragmented DNA.

## 4. Results

### 4.1. Estimation of temperatures at different depths in the soil

As we did not know the porosity and argillite proportions in the soil at Tell Aswad, we could not know the thermal diffusivity (TD). We simulated three different situations where TD was 0.1, 0.25 and 0.4. Fig. 3 gives the results of these simulations, based upon Nofziger and Wu's software.

The three diagrams on the left show the temperature variation throughout the year, starting from January 1st at the surface of the soil, and at 1, 4 and 10 m deep in the soil. At 1 m deep under current top level of the tell, the temperature increases up to 33 °C in summer, and the total variation between winter and summer is between 26 and 28 °C. At 4 m deep, the summer maximum is 26–29 °C, and the annual thermal amplitude ranges between 11 and 18 °C, depending on the TD. At 10 m, archaeological animal bones would still have suffered annual temperature variations of 8–10 °C if the TD is

high, and their temperature would have varied from 16 to 25 °C.

The three diagrams on the right of Fig. 3 show the modeled increase or decrease of temperature from the surface to 20 m deep, respectively, on January 1st and August 1st. The point where the two lines cross gives the depth under which the temperature remains constant (thermal stability) and equal to ca. 20 °C all year (except very deep, where the geothermal energy begins to take effect and increases the temperature again). Of course, this model is simplistic because it assumes that the soil is geologically homogeneous up to 20 m deep, either in terms of mineralogical components or quantity of water. However, it appears that thermal stability occurs between 6 and 12 m, depending on the thermal diffusivity. This means that thermal variations are still noticeable for most of the bones in the Pre-ceramic Neolithic tells that we studied.

The experiments led by Wu and Nofziger (1999) in 1986 in Hebei Province, China, indicate that daily variations range from 5 to 10 °C at 5 cm under the surface of the soil, but that they drop to less than 2 °C below 1 m, and can therefore be considered negligible.

### 4.2. Temperature variations at the surface during the excavation: sun and shade

Over the course of the day on September 25, 2005, in Tell Aswad, we monitored the inside temperature of two bones still set in a few centimeters of sediment (but visible from the surface), one in the sun (# B197), the other in the shade (# F144); we also monitored over the same time period their respective local air temperatures. In the sun (Fig. 4), the air temperature increased from 18 °C at 6:30 am to 45 °C at 12:30 pm. The bone temperature increased with a delay of 1 or 2 h in relation to the air temperature, and reached 40 °C at ca. 1 pm. The temperature variation in the bone from 6:30 am to 1 pm was 35 °C, and again from that time to the next morning. There was no place on the excavated surface that remained in the shade all along the day. Consequently, the second bone was chosen from a location where the shade lasted as long as possible, i.e. until noon. Here, the air temperature slowly increased from 18 to 28 °C in the morning then became very high. In the bone, it went up to 25 °C during the same time period, i.e. a 6 °C increase; during the afternoon, the bone temperature slowly increased to 38 °C. The difference in the speed of temperature increase at different times and different bones is probably related to the quantity of water they contained, which might be important. We did not measure this parameter.

### 4.3. Temperature variations from excavation to the storage room

On September 21st and 22nd at Dja'de, we monitored bones from the excavation to the storeroom, together with local external temperature at the different places they passed through. We selected two different bones, the first found in the shade and kept in the shade until water cleaning, the

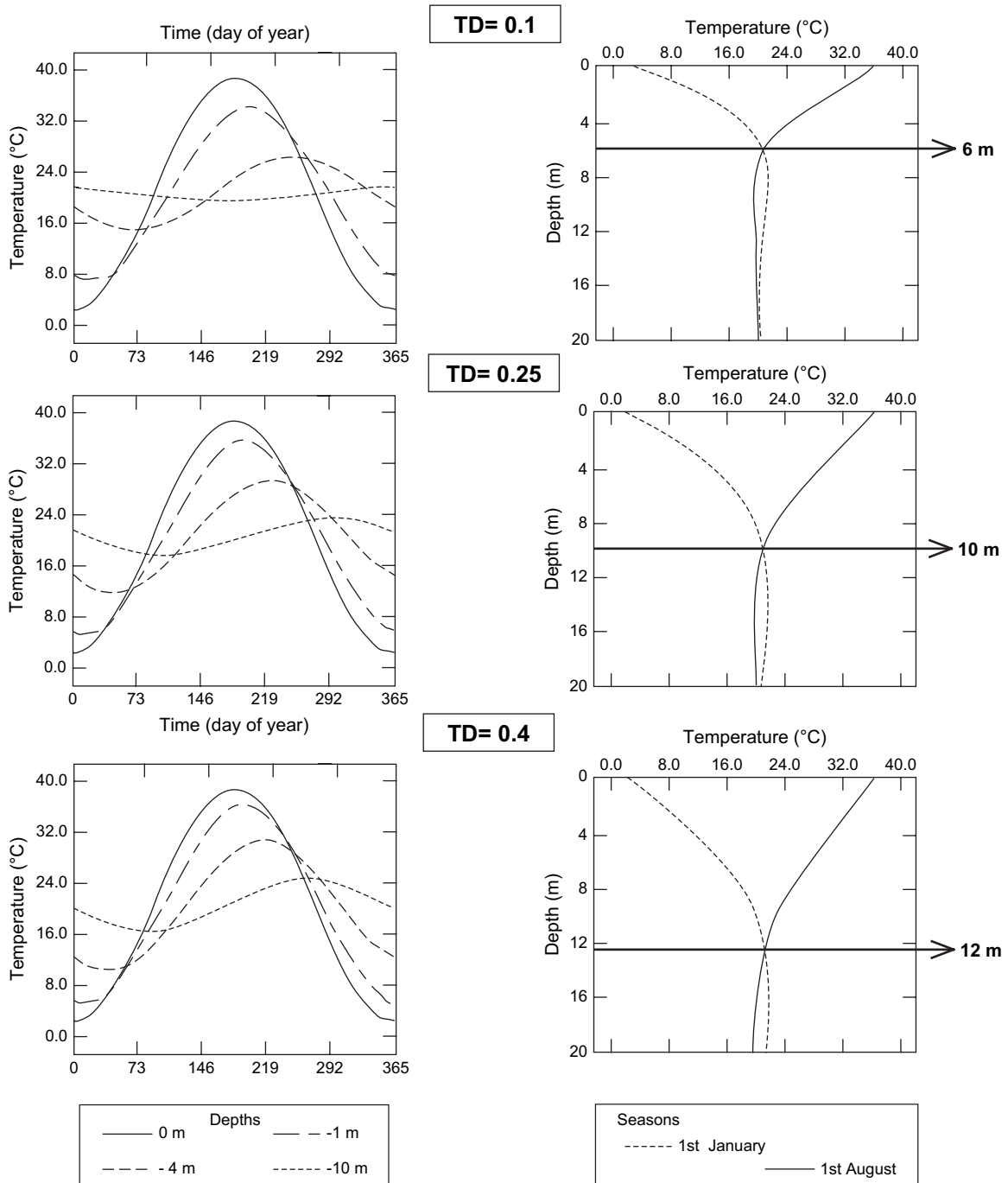


Fig. 3. Simulation of temperature variations in the soil at Damascus, according to seasons at different depth (left column) and according to depth during winter and summer (right column), with three different thermal diffusivities (TD), using Nofziger and Wu's software (<http://soilphysics.okstate.edu/toolkit/temperature/index0.html>) and meteorological data for Damascus from the World Meteorological Organization ([www.worldweather.org/099/c00213.htm](http://www.worldweather.org/099/c00213.htm)). The three depths on the extreme right of the chart are the depth under which there is no further temperature variation in the soil throughout the year, according to the three values of TD.

second found in a sunny place and kept in plastic bags in the sun all morning, until water cleaning.

The local environmental temperature for the first bone (shade: # 22/09) increased slightly from 24 to 28 °C in the plastic bag, while the temperature in the bone remained ca. 21–22 °C (Fig. 5). Therefore, water cleaning at 20.6 °C did not bring very strong variations. But the first step, drying in

the sun (Fig. 2) brought a strong increase of 7 °C in the bone, up to 27.8 °C after a few minutes. However, this increased rapidly but temporarily stopped (around 9 am), probably because of the strong evaporation of water out of the bone, which creates a drastic loss of energy and compensates for the temperature increase over nearly 1 h. Then, in the drying place where the bone remained for the rest of the day, the

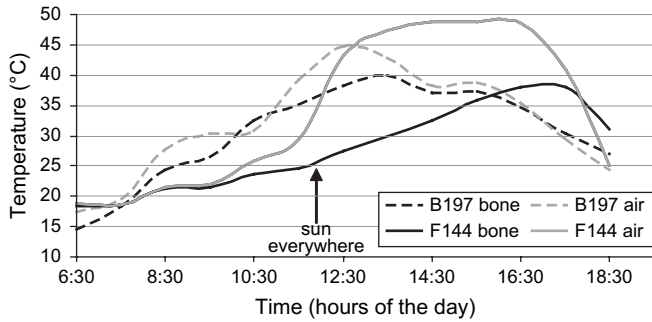


Fig. 4. Temperature monitoring of two bone samples in the surface sediment at Aswad over the course of September 25th, one in the sun (# B197); the other in the shade (# F144), together with the ambient temperature of each.

temperature increased to 48.8 °C at 12:30, then suddenly decreased because of wind, before the progressive afternoon decrease down to 28 °C. During this time, the internal temperature of the bone also increased, but it reached only 34.6 °C and then decreased during the afternoon. The effect of the cool wind is, however, slightly noticeable inside the bone. During the drying time, the bone suffered a 14 °C increase of temperature, then a 12 °C decrease. The temperature in the reserve was 33.6 °C at 5 pm, and decreased to 31 °C at 9 pm and 21.7 °C early in the morning. The daily bone temperature variation in the storage room is estimated at ca. 7 °C at this time of the year.

For the bone # 21/09, which was found in the sun and kept in a plastic bag in the sun, the variation of temperature during the 2 h after soil extraction was 28 °C. The temperature of the bone in the plastic bag was 49 °C, i.e. water cleaning (21 °C) provoked a decrease of nearly 30 °C in a few seconds. Then, the bone suffered the same temperature variations as # 22/09 during sun drying and storage.

Fig. 6 shows a summary of variations in bone temperature inside the two Dja'de samples (sun, # 21/09 and shade, # 22/09) as described above. They can be considered examples of what can occur to aDNA bone samples that were sampled a long time after excavation. It appears that the maximal temperature variations (20–48 °C) occurred during excavation, but that excavation in the shade lowered those variations substantially. Conversely, variations in the storeroom are very slight in comparison with the ones of excavation, although, beginning at 50 h, our diagram gives a rough extrapolation,

which does not take into account seasonal variations during the year.

#### 4.4. Keeping the aDNA bone samples at low temperature

In the same diagram (Fig. 6), we add the temperature variations of our aDNA bones sampled in the field and immediately placed in a cooler and subsequently in a freezer. We present here only six examples out of more than 100 samples. We selected two samples from Aswad, two from Tell Qaramel and two from Dja'de, as representative of the variety of the different sampling sessions. We observed that we succeeded in keeping all these samples constantly below 10 °C from the time of excavation. This is a very different situation in comparison to a bone sampled out of the bulk, long after the excavation. However, measurement sessions, transportation and administrative formalities (although very kindly expedited by the Syrian authorities) caused frozen bones to thaw to temperature of up to +5 to +10 °C two to four times, depending on the series, each time causing variations of 10–18 °C in the space of 1–3 h.

#### 4.5. Ancient DNA results

None of the bones that we sampled during these experiments fulfilled the criteria for a suitable sample. Most of them showed severe microbial degradation. Additionally we could observe the presence of inhibitors in some of the samples. None of the 25 cattle samples and none of the three soil samples that have been processed so far have yielded any aDNA.

### 5. Comparisons and conclusions

Table 1 gives the temperature range, thermal amplitude and speed of temperature variations per hour in bone samples under different conditions that can be considered typical: at 10, 4 and 1 m under the soil surface, on the ground, during extraction from the soil, during water cleaning, during drying, during storage and for our aDNA samples. We can see that the highest temperature ranges, up to 40 and 50 °C, occur on the ground and at the time of extraction, especially in plastic bags, which are exposed for several hours to the sun. But the bones also suffer high temperatures, up to 30–35 °C, at less than 4 m

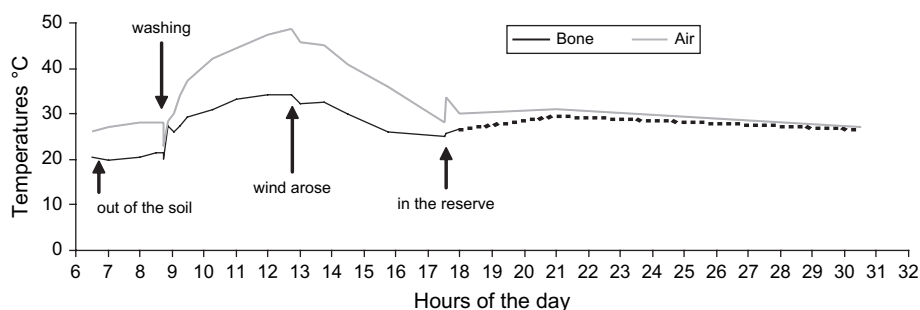


Fig. 5. Temperature monitoring of a bone sample (# 22/09) and of the surrounding air at Dja'de, from excavation to storage in the reserve.

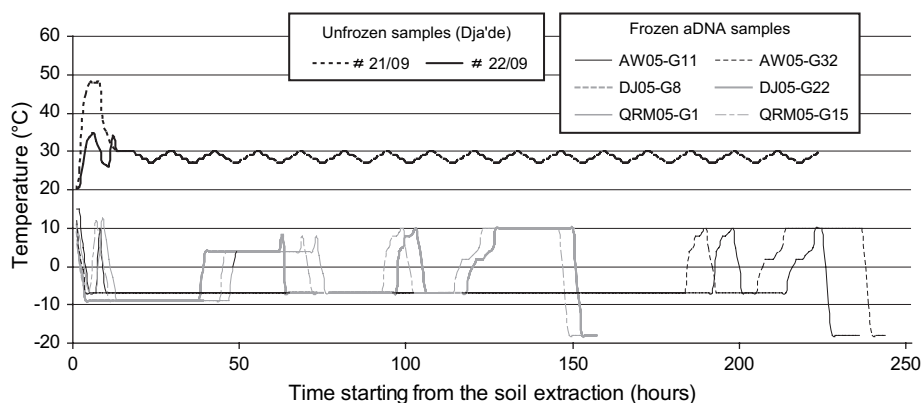


Fig. 6. Temperature monitoring of two unfrozen reference bone samples and of six frozen aDNA bone samples during the 250 h following the beginning of the experiment. Among the six frozen samples, two came from Aswad (AW), two from Dja'de (DJ) and two from Qaramel (QRM).

deep and during drying and storage. With respect to thermal range, our cooling and freezing system is by far better than the current situation.

However, Table 1 also shows that water cleaning provokes by far the most significant thermal shock, which is equivalent to 11,000 °C/h. The first steps of sun drying and, secondarily, extraction from the soil must also be considered extreme thermal shocks with, respectively, 84 and 9.3 °C/h. The temperature variations between the freezer and the coolers provoked only a slight thermal shock of ca. 6 °C/h. Thus we recommend not to wash any samples that are envisaged for aDNA analyses, particularly as we observed that washing is a major source of irreversible contamination of the inner bone structure (Pruvost et al., 2007).

In tell villages, domestic refuse would have first been covered by a thicker and thicker layer of sediment due to the increase of the tell. Then the upper layer would have slowly decreased with the subsequent erosion of the tell, and finally disappeared fast due to excavation. Consequently, in the arid zones of the Near East, animal bones would have suffered temperatures between 20 and 40 °C, with variations of 3.4 °C/h each day, on the ground before being buried, and then temperatures between 5 and 28–34 °C over several

centuries inside the upper part of the sediment, before being covered by at least 4 m of sediment. In most of the open air sites of the arid Near East, aDNA would have been destroyed before and during these pre-burial and burial phases, i.e. during the first decades or centuries after their rejection, before the tell became thick enough (if it did) to protect bones from high temperatures and thermal shocks. If any aDNA survived these hard conditions, the process of excavation again puts the bones in bad conditions. The unavoidable decrease in the sediment above the bone sample following each excavation session would provoke new seasonal increases in temperature, becoming greater each year until the time of the extraction.

However, the literature reports three successful aDNA samples in the same climatic conditions (medium arid steppe; Besançon and Geyer, 2006): two are from Tell Brak (Edwards et al., 2004) and one from early excavations in Dja'de (Edwards et al., 2007). They may bring some confirmation and precision to the present observations. Compared to the relatively small Pre-ceramic tells at Dja'de, Jerf, Qaramel or Aswad, Tell Brak is a huge tell more than 100 m high and 110 ha wide; in addition, it dates from the Bronze Age to more recent classical occupations (Matthews, 1996) by much

Table 1

Summary of the temperature range, thermal amplitude and speed of temperature variation in bone samples under different conditions, before, during and after excavation, basing on both theoretical and experimental observations collected in the present paper

		Level of temperatures (°C)		Temperature variations			
				Amplitude (delta °C)		Intensity with ref to time	
		Mini	Maxi	Mini	Maxi	Observation	Calibration (°C/h)
In the soil (theoretical estimations)	At 10 m depth	18	23	5		0.8 °C/month	0.001
	At 4 m depth	12	28	16		2.6 °C/month	0.004
	At 1 m depth	5	34	29		2 °C/month	0.8
During the archaeological process (experiments)	Just under the soil surface	20	40	6	24	Max: 3.4 °C/h	3.4
	During the extraction	20	50	14	28	Max: 9.3 °C/h	9.3
	During the water cleaning	21		0	30	Max: 30 °C/10 s	10,800
	During the sun drying	26	34	14		7 °C/5 min, then 2.3 °C/h	84 then 2.3
	During the storage	27	34	7		Max: 1.75 °C/h	1.75
	aDNA samples	-7	10	10	18	Max: 6 °C/h	6

more important and active human groups than the small ones of the Pre-ceramic periods. The increase in the tell, i.e. in the protection of the bones against high and contrasted temperatures, must have been much more rapid in Tell Brak than in Tell Qaramel, for example. According to our observations, it seems that it has been possible to get positive aDNA samples in Tell Brak, not primarily because it is more recent, but because the protective layer of sediment has grown quicker and thicker. In Dja'de, the only sample which produced any result comes from the sounding B, excavated at the beginning of the archaeological exploration of the tell, in 1992 (Edwards et al., 2007). The bone was situated between 2 and 3 m under the present day top surface of the tell, which is not very deep, but the excavation was very rapid (two annual sessions only) and the sounding was so narrow that the sun never entered it (Coqueugniot pers. comm.). In addition, the sample never suffered high temperatures, since it was immediately brought to France and stored in the cool (and really cold in winter!) collection room at Jalès (Ardèche, France). If the aDNA extracted from this sample does not come from a contaminant in the washing water (not excluded) or in the storage room, this example would suggest that some DNA could have been preserved in the deep parts of the Pre-ceramic tells of the Near Eastern arid regions.

Consequently, we recommend avoiding aDNA sampling in the archaeological areas which have been under excavation for several years: in such areas, if any aDNA had so far been preserved, it will have been destroyed during the last few years prior to sampling.

Of course, we have not yet developed the experimental protocols that would have allowed us to evaluate the effects of the thermal shocks that we inflicted on the aDNA samples in the freezers and coolers. Assuming that any aDNA had survived in the bones, to do that, it would have been necessary to cut each fragment into two sub-samples, one being kept in the same low temperature conditions the entire time, which is not possible in Syria due to obligatory administrative export formalities. One might propose that we could have avoided such severe thermal shocks if we had not frozen the samples immediately after soil extraction. With regard to possible shearing forces that might additionally damage the DNA in the freezer, it might have been better to keep the samples at cool temperatures of ca. 5–15 °C, for example, which we could have done with ice from the freezer and our electric coolers, and only to freeze them at the very end of the process, once they had arrived in the Paris lab. However, taking into account that many bones were rather wet, freezing may have been the best way to stop bacterial activity. New experiments are necessary in order to better estimate which is the less damaging procedure.

In conclusion, like for bone collagen (Weiner and Bar-Yosef, 1990), it seems that it would be very difficult to get large series of good aDNA samples from the open air sites in the arid zones and low elevation plains of the Near East. It would be more useful to concentrate efforts on northern zones (Anatolia) and on cave sites, where thermal conditions may be much better.

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