The Archaeogenetic Evidence

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Introduction

The Levant has been considered as one of the core areas where sedentism and farming started. Many studies, including the new evidence of the *Household and Death Project* have highlighted the interconnectedness and close cultural and economic ties of these early farming communities with other areas of the so-called Fertile Crescent (*e.g.*, Cauvin 1997; Simmons 2007; Goring-Morris and Belfer Cohen 2020). Diffusionist models have been suggested to explain the steep increase in the number of sites, their size, and in supposed population densities during the Late Pre-Pottery Neolithic B (Late PPNB) in the southern Levant (*e.g.,* Rollefson 2000; Gebel 2004). To understand the social organisation within these so-called *mega-sites*, various models of familial and supra-household structures were surmised (Flannery 2002; Byrd 2005; Benz 2010; Benz *et al.* 2017; Gebel 2017). However, genetic data to support one of these scenarios – either on the macro- or on the micro-level – have been lacking for many years and are still scarce in the Levant. Whereas genetic data on the expansion of farming communities to Europe have increased enormously during the last 20 years, the poor preservation of ancient DNA (aDNA) due to the hot climate in the Levant hampered large scale testing. Though single-locus approaches such as mitochondrial DNA amplification have been successful in PPNB sites such as Tell Halula, Tell Ramad, and Dja'de El Mughara (Fernández *et al.* 2014), the information that can be gained from such approaches is limited. Data spanning entire human genomes from the prehistoric Levant could only be retrieved over the last six years due to recent advancements in technology and methodology. Some of the earlier data was recovered from the Natufian site of Raqefet Cave as well as from the Pre-Pottery Neolithic sites of Motza and 'Ain Ghazal, but the majority of analysed individuals originated in post-Neolithic periods (Lazaridis *et al.* 2016; Haber *et al.* 2017, 2019, 2020; Harney *et al.* 2018; Feldman *et al.* 2019a, 2019b; Agranat-Tamir *et al.* 2020). Analyses concerning prehistoric populations of the Levant have mostly focused on multi-regional population genetics, due to the poor preservation, which so far hardly allowed for a large enough sample size to generalise on familial biological relations in an individual site (*cf.* for a first tentative approach on the micro-level see Fernández *et al.* 2008; for Neolithic Anatolia see Yaka *et al.* 2021). Ba`ja is thus among the handful of sites from which Levantine early Holocene genomes have been successfully reconstructed and despite the low number of individuals that yielded valid results, important insights on biological relationships are possible (Feldman *et al.* 2019a; Wang *et al.* 2023).

In this chapter we aim to describe the genetic findings from the late PPNB site of Ba`ja in the context of the known Near Eastern genetic diversity and to outline the potential interpretations of these findings when contextualised with the archaeological and anthropological evidence. In addition, we discuss potential future avenues for this interdisciplinary effort considering the ongoing increase in the availability of ancient reference datasets and in prospect of higher sampling densities in Ba`ja itself. The further accumulation of such data will potentially expand the scope of the current archaeological research to go beyond macro-regional aspects and address local questions regarding genetic relatedness as well as social structure, such as some of the core questions steering the *Household and Death Project* (see Gebel *et al.* 2020 with further references).

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Over the last decade, human genomic data recovered from archaeological remains has proven to be an important resource in recording the past, complementing traditional archaeological approaches as well as other biological and molecular methods. Although aDNA has been studied from as early as the 1980's, early methodologies were difficult to reproduce (Cano *et al.* 1994; Cano and Borucki 1995; Austin *et al.* 1997). Only with the advent of new sequencing technologies, throughputs dramatically increased and the quantification of contamination became feasible allowing for a reliable authentication of aDNA (Margulies *et al.* 2005; Bentley *et al.* 2008; Mardis 2008). This advancement was followed by the publication of dozens of ancient genomes, mostly from European sites, however at that point, archaeogenetic research in the Levant was lagging. This was because DNA degrades faster in environments where temperature and humidity are high (Lindahl 1993). Thus, a subtropical region such as the southern Levant is less likely to preserve DNA over time in comparison with temperate regions. In fact, the idea of obtaining genome-wide data from the southern Levant seemed improbable up until 2016 when Lazaridis and colleagues reported the first Epipalaeolithic and early Neolithic genomes from the region (Lazaridis *et al.* 2016). This breakthrough was achieved mainly due to two developments in methodology: 1) the design of new DNA enrichment methods that accommodate the unique features of aDNA (Fu *et al.* 2013; Haak *et al.* 2015; Mathieson *et al.* 2015), and 2) a shift in sampling strategy to one targeting the inner ear (Pinhasi *et al.* 2015) (see *Sampling and Laboratory Procedures* from the *Materials and Methods* Section). In the following years, additional Near Eastern prehistoric genomes were reported, providing the first clues to the genetic landscape of the prehistoric Near East (Broushaki *et al.* 2016; Hofmanová *et al.* 2016; Kılınç *et al.* 2016; Omrak *et al.* 2016). However, to this date, success rates in the southern Levant remain low compared to more temperate areas and extensive sampling efforts are required. Currently, there are still only a few southern Levantine prehistoric sites from which genome-wide data is available (Lazaridis *et al.* 2016; Feldman *et al.* 2019a).

Naturally, much of the genetic research in the Near East has been focused on detecting demographic changes associated with Neolithisation, both in global and local contexts. For this purpose, an approach comparing genomic profiles of hunter-gatherers and early farmers has been commonly used. In contrast to central Europe, where the advent of early farming was accompanied by an expansion of people from west Anatolia (Bramanti *et al.* 2009; Haak *et al.* 2010; Brandt *et al.* 2013; Lazaridis *et al.* 2014; Mathieson *et al.* 2015; Hofmanová *et al.* 2016; Omrak *et al.* 2016), early farming centres including the southern Levant, the Zagros Mountains of Iran, and central Anatolia, show a high degree of genetic continuity between the hunter-gatherers and early farmers within each of these regions. Interestingly, the Iranian, Levantine, and Anatolian early farmer groups could be clearly differentiated by their genetic affinities, indicating that the groups were relatively isolated from each other for long periods (Larzaridis *et al*. 2016). Overall, these findings suggest that across the described sites in the Near East, farming either developed locally or was mainly driven by the spread of culture. Moreover, it suggests that long distance trade networks between these communities, evident in the archaeological record *e.g*., obsidian exported from central Anatolia to the Levant (Carter *et al*. 2005) was not facilitated by large-scale movements of people (Broushaki *et al.* 2016; Kılınç *et al.* 2016; Lazaridis *et al.* 2016; Feldman *et al*. 2019a).

In the southern Levant, the currently available late Pleistocene/ early Holocene genome-wide data comes solely from present-day Jordan and Israel. The bulk of the data comes from the site of 'Ain Ghazal (Jordan) from which 12 genomes were reconstructed (Lazaridis *et al.* 2016). In addition, one genome from the site of Motza Tachtit (Israel) was reported. These individuals were assigned to the PPNB/ PPNC periods mostly based on non-human radiocarbon dating of the stratigraphic layers in which the remains were found. The same publication reported the genomic data of six individuals excavated in the Raqefet Cave, in Israel associated with the Epipalaeolithic Natufian culture. By comparing these groups, Lazaridis and colleagues found that the Levantine early farmers were mostly descended from a gene pool related to that of the "Natufians" from Raqefet (Lazaridis *et al.* 2016). Nonetheless, they harboured an additional ancestral component related to a Neolithic Anatolian ancestry that was not present in the "Natufians", suggesting gene flow occurred between these regions during the early Neolithic period (Lazaridis *et al.*

2016; Feldman *et al.* 2019a). Similarly, when the genetic makeup of Anatolian hunter-gatherers and early farmers was compared, a Levantine component that was not present in the hunter-gatherers was detected in the early farmers of western Anatolia. Thus, the genetic link between the Neolithic Levant and Anatolia was likely bidirectional. Later, genomic data were retrieved from a female infant that was excavated at the site of Kfar Hahoresh in present day northern Israel and directly radiocarbon-dated to the Early PPNB (Feldman *et al.* 2019a). This infant showed a similar genetic profile as the 'Ain Ghazal and Motza individuals, suggesting a wide geographic prevalence of this gene pool.

Beyond the supra-regional questions of human mobility, archaeogenetic studies can be instrumental in identifying biological relatedness and therefore can help in understanding local and regional social structures and kinship practices. Such studies already proved feasible in Europe (*e.g.*, Knipper *et al.* 2017; Cassidy *et al.* 2020) and even in more temperate regions of the Near East such as in central Anatolia (Yaka *et al.* 2021). In the southern Levant, one study that investigated global patterns of consanguinity in the genomes of *c.* 2,000 ancient individuals detected relatively low parental relatedness in Chalcolithic and Bronze Age Levantine groups compared with present-day ones (Ringbauer *et al.* 2021). However, a local "zoomed-in" approach which would require both well-defined archaeological context and a relatively high local sampling density is still challenging for early Holocene southern Levantine sites due to the limited biomolecular preservation. Some clues to biological relatedness within archaeological sites come from other biological approaches such as non-morphometric trait analysis that has been implemented in several Near Eastern early Holocene sites. So far, the body of evidence points to diverse patterns of kinship practices. In the site of Kfar HaHoresh for instance, a comprehensive systematic study of non-metric traits on teeth supported a pattern of burial in family plots and pointed to some features of matrilocality such as a closer biological relatedness between female sub-adults and adults compared to male ones. However, the authors note that the poor morphological preservation did not allow for a robust identification of matrilocality (Alt *et al.* 2015). An investigation at the Late PPNB site of Basta in present-day Jordan also used a non-morphometric approach coupled with strontium isotope analysis and found a clear signal of within-group inbreeding that could imply the routine practice of endogamous kin unions (Alt *et al.* 2013). Finally, in the site of Abu Hureyra in present-day Syria, a study that examined some characteristic dental and cranial traits reported the co-burial of biologically related individuals (Moore *et al.* 2000).

In central Anatolia, genomic data from the early Neolithic sites of Boncuklu and Aşıklı Höyük in present-day Turkey, revealed co-burials of first-degree related individuals (4 out of 5 individuals in Aşıklı Höyük and 4 out of 9 in Boncuklu). In contrast, evidence for co-burials of first-degree relatives was more limited in the close-by later sites of Çatalhöyük and Barcın Höyük (2 and 4, respectively of 10 individuals at each site). In addition, a lower level of inbreeding is reported in the later sites (Yaka *et al.* 2021). Furthermore, an investigation of mitochondrial genomes (genomes inherited via the maternal line) at Çatalhöyük measured a degree of heterogeneity within individuals buried under the same house that might support a large patrilocal or non-genetically kin-based society (Chyleński *et al.* 2019). These results are supported by former investigations on phenotypic dental data (Pilloud and Larsen 2011). Potentially, similar data types, if recovered from Ba`ja, could shed light on biological familial relationships among the burials and might help address known cultural parallels/ differences between Ba`ja and the above sites.

In the following sections we describe our findings from the analysis of genome-wide data reconstructed from the three Late PPNB individuals excavated in Ba`ja. The genetic results have been reported in two studies: in 2019, Feldman and colleagues reconstructed the genome-wide data of Individual BAJ001 and analysed it in the context of other southwest Asian groups (Feldman *et al.* 2019a). Recently, Wang and colleagues could reconstruct genome-wide data of two additional individuals, BAJ020 and BAJ022 which they analysed as part of an interdisciplinary effort combining isotopic and genetic analysis from both the Levant and Anatolia (Wang *et al.* 2023).

Materials and Methods

Sampling and Laboratory Procedures

The genetic analysis of the Ba`ja individuals was conducted following a genome-scale approach.

The sample collection was designed to address questions on population history and on relatedness between households. For this purpose, 36 skeletal elements were sampled from 27 individuals that were buried in various contexts (Table 1). Mostly the petrous portions of the temporal bone and teeth were targeted as they are known to preserve DNA well (Gamba *et al.* 2014; Pinhasi *et al.* 2015; Parker *et al.* 2020). These were collected in two different phases of the Ba`ja excavations (Table 1) and analysed as part of two genetic studies where they were co-analysed with contemporaneous individuals from west Eurasia (Feldman *et al.* 2019a; Wang *et al.* 2023). The processing of the samples took place in the designated facilities of the Max-Planck Institute for the Science of Human History (MPI-SHH) and the Max-Planck Institute for Evolutionary Anthropology (MPI-EVA) in Germany. The sampling process followed standardised minimally invasive protocols. The extraction of the DNA from the bone powder was followed by preparation of genomic libraries for Next Generation Sequencing (NGS) on an *Illumina* platform. For the earlier-phase samples, a protocol

Table 1 Summary information of the genetic screening from Ba`ja. Human DNA is the percentage of sequences that map against the human reference genome. A threshold of 0.1% human DNA was used in screening for the DNA enrichment (annotated in grey); l.=left, r.=right; *Teeth are annotated according to the FDI system; ** Differentiation between the three individuals in the field was difficult. *** NB: another bone of the same individual No 18 was not analysed.

Individual ID	Archaeological ID	Skeletal Element	Human DNA %	Presence of DNA Damage (y/n)	DNA Enrichment (1240K) (y/n)
BAJ001	Loc. C10:405-Individual I	Pars petrosa r.	5.51	y	y
BAJ002	Loc. C10:405-Individual II	Pars petrosa r.	0.04	y	n
BAJ003	Loc. C10:408	Tooth (27)*	0.02	n	n
BAJ004	Loc. C1:46	Pars petrosa l.	0.02	n	n
BAJ005	Loc. CR17:117, Bone No 12	Tooth (37)*	0.03	n	n
BAJ006	Loc. CR17:117, Bone No 46	Tooth (46)*	0.02	n	n
BAJ007	Loc. DR19:110	Pars petrosa r.	0.11	y	y
BAJ008	Loc. CR6:40	Pars petrosa r.	0.32	n	n
BAJ009	Loc. CR6:48	Pars petrosa r.	1.17	n	n
BAJ010	Loc. CR6:23a	Pars petrosa l.	0.21	n	n
BAJ011	Loc. CR5:53	Pars petrosa r.	0.24	y	y
BAJ012	Loc. CR5:54	Pars petrosa l.	0.24	n	n
BAJ013	Loc. CR 28,2:122a/ 122b/ 123a**	Pars petrosa l.	0.13	y	y
BAJ014	Loc. CR 28,2:122a/ 122b/ 123a**	Pars petrosa l.	0.10	n	n
BAJ015	Loc. CR 28,2:122a/ 122b/ 123a**	Pars petrosa l.	0.10	n	n
BAJ016	Loc. CR 17:130, No 21	Pars petrosa l.	0.48	n	n
BAJ017	Loc. CR 17:133/135, No 101	Pars petrosa r.	0.39	n	n
BAJ018	Loc. CR 17:137, No 110	Pars petrosa r.	0.11	n	n
BAJ019	CR 17, No 100	Pars petrosa r.	0.34	n	n
BAJ020	Loc. CR 17:127, No 13***	Pars petrosa l.	0.63	y	y
BAJ021	CR 17, No 102	Pars petrosa l.	0.19	n	n
BAJ022	Loc. CR 17:130, skull beneath E-wall	Pars petrosa l.	4.35	y	y
BAJ023	CR 17, infans beneath W-wall	Pars petrosa r.	0.21	n	n
BAJ024	CR 17, No 91a	Pars petrosa l.	0.12	n	n
BAJ025	Loc. CR 28,2:122a/ 122b/ 123a**	Pars petrosa r.	0.11	y	y
BAJ026	Loc. CR 28,2:122a/ 122b/ 123a**	Pars petrosa r.	0.06	n	n
BAJ027	Loc. CR 28,2:122a/ 122b/ 123a**	Pars petrosa r.	0.07	y	n

immortalising double-stranded DNA fragments was used (Kircher *et al.* 2012). Instead, in the second phase an improved protocol immortalising single-stranded DNA fragments, that can recover highly degraded short DNA, was implemented (Gansauge and Meyer 2013; Gansauge *et al.* 2017). The detailed protocols can be found online: (https://www.protocols.io/workspaces/mpieva-archaeogenetics) as well as in Feldman *et al.* (2019a) and Wang *et al.* (2023). In order to estimate the proportion of human aDNA in the libraries, they were directly sequenced at a low depth (*c.* 5 million reads). As expected, due to the poor DNA preservation in southern Levantine skeletal material, the proportion of human DNA was not sufficient for genome-scale analysis. Therefore, selected samples that fulfilled thresholds for human DNA content and displayed DNA damage patterns typical of aDNA, were subjected to a commonly used DNA enrichment assay that targets *c.* 1.24 million ancestry-informative single nucleotide polymorphisms (SNP) across the genome, called "1240K capture" (Fu *et al.* 2013; Haak *et al.* 2015; Mathieson *et al.* 2015; Table 1). Finally, three libraries, recovered from three different individuals, yielded sufficient and validated data to be used for genome-scale analysis. These three libraries were also enriched for the mitochondrial DNA and diagnostic Y-chr positions (Table 2; see details in the next section).

Archaeological and Anthropological Context of Analysed Individuals

BAJ001 was a complete skeleton recovered from a double burial, Loc. C10:405. BAJ001 was estimated by morphological analysis to be an infant aged 6-12 months and was co-buried with another infant aged 3-4 years (BAJ002). BAJ002 was also screened for aDNA, however, aDNA was not sufficiently preserved. A charcoal sample from the upper filling of the double infant burial was dated to 7027-6685 BCE (2 sigma range, see Purschwitz and Benz forthcoming).

Full archaeological ID of interment: Room number CR35, Loc. C10:405, Grave CG8 (Benz *et al.* this volume).

The two sub-adults were buried in a crouched/sitting position, in a rather small pit, facing each other. BAJ001 was facing E and BAJ002 facing W. This burial is one of the four interments in which subadults of the age of 3-4 years were buried with another infant of 0-2 years. It is also one of the two rare child burials in which no grave goods were uncovered at all.

BAJ020 and ***BAJ022***

Full archaeological ID: Room CR17, multiple burial CG11, most recent phase of burial events: BAJ020: Loc. CR17:127; BAJ022: Loc. CR17:130, with BAJ022 being slightly more ancient than BAJ020 (Gebel *et al*. 2020; Benz *et al.* this volume).

In Silico Analyses

Processing of the sequenced data: The enriched sequencing data were processed using the *EAGER* pipeline (Peltzer *et al.* 2016), which included mapping of the sequenced reads against the human genome reference, removal of duplicate reads, and assessment of damage patterns typical of aDNA (Li and Durbin 2009; Jónsson *et al.* 2013).

Sex determination and contamination estimates: Levels of contamination by external human DNA sources were estimated using three different methods based on: 1) divergence from haploidy in the mitochondrial DNA as described in Renaud *et al.* (2015), 2) divergence from

Table 2 Summary of genetic sexing, contamination, genome-wide SNP coverage, and uniparental haplogroup assignment. Estimates of mitochondrial contamination are provided for both 1240K- and mitochondrial (mt) -captured libraries. When only one value is presented, the analysis was conclusive only on the mt capture (BAJ020).

ID	Genetic Sex	Contamination (mito)	Contamination (aDNA Damage)	Nº1240K SNPs	Mt-Haplogroup	Y-Haplogroup
BAJ001	F	0.01±0.02/ 0.01±0.01		440,642	N1b1a	
BAJ020	F	0.01±0.01	0.001±0.016	47,393		
BAJ022	M	0.02±0.02/ 0.02±0.01	0.001±0.02	333,935	N1a1a	E1b1b1b2a1

haploidy in the X-chromosome in males as described in Korneliussen *et al.* (2014), and 3) the distribution of aDNA damage across the fragments as described in (Peyrégne and Peter 2020). Genetic sex was determined by calculating the ratio between the DNA fragments assigned to the X-chromosome and those assigned to the Y-chromosome and normalised by those assigned to the autosomal chromosomes.

Dataset: For every individual, genotypes (*i.e.*, the allele states at each SNP) were assigned at the targeted SNPs that were covered by at least one sequencing read. Subsequently, these data were compiled with publicly available datasets of present-day and ancient individuals, and used for population genetic analyses.

Genetic relatedness: Biological relatedness was examined with a method that is conceptually described in Jeong *et al.* (2018) and Kennett *et al.* (2017), whereby the coefficient of relatedness [0,1] between two individuals was estimated from their rate of allele mismatching corrected with the average of pairwise allele differences from the population (PMR).

Population history: An initial evaluation of the genetic relation among the Ba`ja individuals, as well as to other contemporaneous populations, was conducted by principal component analysis (PCA), using the *smartpca* program of the *EIGENSOFT* software (Patterson *et al.* 2006; Price *et al.* 2006). The PCA was computed on present-day west Eurasian populations (*c.* 1,800 individuals), whereas the ancient individuals were projected onto the PCs with an embedded function of the program. This adjustment is usually opted in aDNA studies as it accounts for the effect of ancient samples' missing data.

While PCA can effectively visualise genetic structure, as a non-parametric method it is less informative with respect to the underlying population history. This is why visual observations derived from a PCA need to be explicitly tested with formal tests. A powerful test that can delineate admixture signals is the test of cladality, or f_4/ *D*-statistics (Patterson *et al.* 2012). In a nutshell, this test calculates allele correlation patterns between the four populations/ individuals included in the test, *e.g.*, f_4(A, B; C, D). Under the null hypothesis, C and D form a distinct clade with respect to A and B, hence the test is expected to be zero. If the test is significantly different from zero, either the evolutionary topology of the populations is not correct, or gene-flow between A/ B with C/ D occurred after their divergence. In the case population A is an outgroup with respect to B, C and D, the test directly measures excessive allele sharing between B and either C (negative test value), or D (positive test value). In this way, f_4/ *D*-statistics can provide evidence for admixture and indicate proxies of the possible real sources.

In addition to this, information from multiple f_4-statistics can be combined in a framework of admixture modelling (*qpWave*/ *qpAdm*), whereby ancestries from source populations can be attempted to fit to a target population, and the admixture coefficients are estimated. The power to resolve admixture scenarios with this method depends on the availability of reference populations that relate differentially to the targets and sources. Importantly, an adequate model is not the only model that could describe the data, nor should it be literally interpreted as direct admixture from the populations used as sources.

The power of the aforementioned genetic methods relies on harnessing information from common variation between human populations, in the form of single-nucleotide polymorphisms located across the nuclear genome (SNPs). Recombination between homologous autosomal chromosomes, by which the paternal chromosome exchanges chunks with the maternal one, occurs before one of them is inherited by the offspring. Due to this process, markers which are spread across the genome are mostly unlinked, meaning that their heredity to the next generation would be independent from each other. By consequence, the genetic diversity represented by such markers in one human actually contains information from multitudes of ancestors. Another kind of information can be gained from chunks of markers that are linked and thus are co-inherited from a single parent (known as haplotypes). For some of these chunks, the maternal and paternal copies are identical. The length and distribution of these haplotypes called Runs of Homozygosity (ROH) discloses information related to both demography (*i.e.*, population sizes), and/ or inbreeding/ consanguinity. The method *HapROH* (Ringbauer *et al.* 2021), that was implemented in Wang *et al.* (2023), can effectively estimate ROH from genome-wide data of a certain coverage using a reference panel of haplotypes from modern populations. Following the recommendations of the authors, the method was tested on the Ba`ja individuals and other relevant individuals with a coverage of ≥300,000 SNPs.

In Silico Analyses (Uniparental Haplogroups)

Uniparental haplogroups were assigned on the sequencing data obtained from the genomic libraries enriched for the mitochondrion and the Y-chromosome. For the former, consensus sequences were first generated with *Schmutzi* (Renaud *et al.* 2015) and the haplogroups were assigned with *Haplosearch* and *Haplogrep*. For the latter, haplogroups were designated with *yhaplo*. In addition, the presence of diagnostic SNPs for a given Y-chr haplogroup were manually inspected for spurious jumps in the phylogeny because of accumulated DNA damage (C-T or G-A transitions).

Results and Discussion

Quality Assessment of the Recovered Genomes

All three individuals that yielded sufficient markers for genome-wide analysis (BAJ001, BAJ0020, and BAJ022) measured low external DNA contamination rates (below 4%) (an overview of the evaluation statistics is presented in Table 2). The number of targeted SNPs recovered from the male Individual BAJ022 (also referred to as "coverage") was lower compared to those recovered from the other two individuals from Ba`ja. On average, the DNA preservation in Ba`ja (if measured by the coverage) was much higher than in the other published late Pleistocene/ early Holocene southern Levantine sites such as the "Natufians" of Raqefet Cave and the PPN individuals from 'Ain Ghazal and Kfar HaHoresh (Lazaridis *et al.* 2016; Feldman *et al.* 2019a). Currently, only one individual from Motza has surpassed the average Ba`ja coverage. Such variability in preservation can be common for sites of that age and can be attributed either to different taphonomic processes, or the treatment of the samples after the excavation. The fact that the skeletal material was recently excavated and not subjected to any special chemical treatment or long storage periods might have contributed to the higher DNA preservation. Although preservation of the bones was overall poor, the subadult skeletons were better preserved than the adult ones. From an initial visual inspection larger bones appeared more eroded (Benz *et al*. 2019). As it seems, for the double infant burial, the burying process was rather quick and the corpses were covered with sand. Histological taphonomic studies on the bones also confirm a primary burial and decomposition of the corpse, at least for the infant (BAJ001) inside the burial (Haddow this volume). In contrast, the adult skull (BAJ022) and the infant (BAJ020) were deposited during the last use phase of the collective Burial CG11. The bones of the infant were scattered in front of the southern wall of Room CR17, whereas the male skull was very close to the southeastern corner of that room, beneath a more recent wall. While current data suggest that retrieving more than half of the targeted markers is challenging for the Levant, a range of some hundreds of thousands of SNPs is feasible. Achieving this coverage is very encouraging, as it can provide statistical resolution to most of the applied analyses.

Population History

To understand the position of the Ba`ja individuals in relation to the known genetic diversity in west Eurasia, they were projected, together with other published ancient individuals, onto a PCA calculated from present-day Eurasians (Fig. 1). The plotted first two components represent a relatively small percentage of the total genetic variation in west Eurasia, but nonetheless they effectively visualise the genetic structure in west Eurasia during the early Holocene. As previously reported, this structure was characterised by a high differentiation between the different regions. In fact, until the early stages of the Neolithic the genetic differentiation within Southwest Asia approximated the levels of the genetic diversity between modern Europeans and east Asians (Broushaki *et al.* 2016; Lazaridis *et al.* 2016). This genetic feature makes the identification of genetic shifts or detection of individuals with different genetic affinities compared to the local gene-pool more straight-forward. In the case of the Ba`ja individuals, their PC1-PC2 coordinates overlap with other contemporaneous individuals from the southern Levant and are all shifted from the Natufian individuals towards the direction of Anatolian Neolithic groups. The range of PC1-PC2 space occupied by the Ba`ja individuals is more restricted than the other individuals from *e.g.*, 'Ain Ghazal, although this might reflect differences in sample sizes between the two groups.

To test whether these visual remarks derive from statistically significant differences in ancestry, the Ba`ja individuals were compared with other contemporaneous southwest Asian groups using $f_4$-statistics (Table 3). Similarly as previously reported for earlier PPN groups, the Ba`ja individuals exhibit a higher genetic affinity to Neolithic populations from Anatolia

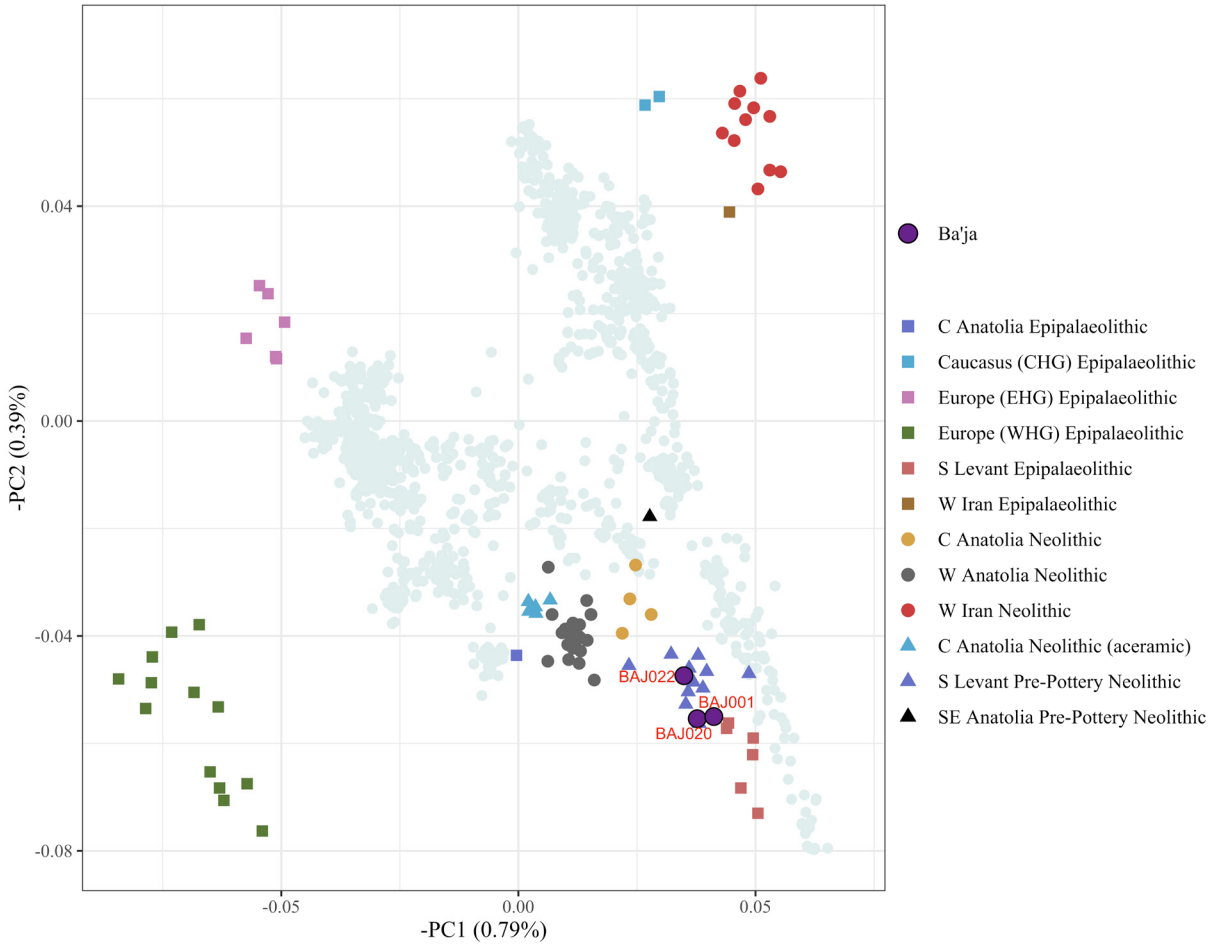

Fig. 1 Scatterplot of the first two PCs from PCA performed on modern west Eurasian populations (light grey points). Early Holocene individuals from west Eurasia (coloured symbols) exhibit higher inter-regional differentiation with respect to their present-day counterparts (grey-coloured circles). The three Ba`ja individuals are overlapping with contemporaneous individuals from present-day Jordan and Israel and are distinguishable from Anatolian and Iranian individuals. Details on groupings: S Levant Epipalaeolithic= "Natufian" (Raqefet Cave, Israel); S Levant Pre-Pottery Neolithic = 'Ain Ghazal (Jordan), Kfar HaHoresh and Motza (Israel); W Iran Neolithic = Ganj Dareh and Tepe Abdul Hosein; C Anatolia Neolithic = Tepecik Çiftlik; W Anatolia Neolithic = Barcın and Menteşe; SE Anatolia PPN = Nevalı Çori; C Anatolia Neolithic (aceramic) = Boncuklu; C Anatolia Epipalaeolithic = Pınarbaşı.

than the preceding "Natufians" do (Lazaridis *et al.* 2016; Feldman *et al.* 2019a). In addition, the 'Ain Ghazal group shows higher affinity to Anatolian populations than the Ba`ja one, although this signal is slightly under the common threshold of Z-score ≥|3| used to determine significance, as measured in the statistics $f_4$ (Mbuti, C Anatolia Epipal or W Anatolia N; 'Ain Ghazal, Ba`ja). The 'Ain Ghazal and Ba`ja groups could be modelled using the Natufian individuals, as the main source representing the preceding local ancestry, and Anatolian groups, like Boncuklu as the additional source (Fig. 2). In agreement with the f_4 estimates, the Anatolian-related component was estimated to be higher in 'Ain Ghazal compared to Ba`ja. Overall, this corroborates to the genetic affinity of the two groups which mainly derive their ancestry from Natufian-related populations. Quantitative differences regarding the proportion of the genetic variation related to Anatolian groups can be discerned when the data from all three Ba`ja individuals are combined but are very subtle and their significance should be tested by further increasing the sample sizes from either group. In addition, some qualitative remarks can be drawn with respect to the affinity to Anatolia. Besides the Boncuklu group, other Anatolian sources like western Anatolian groups, or the individual from the PPN Nevalı Çori in southeastern Anatolia could fit the admixture models of Ba`ja and 'Ain Ghazal while Iranian and European sources did not fit. All the aforementioned Anatolian groups are

Table 3 Summary with the most informative f_4-statistics for Pre-Pottery Neolithic (PPN) Ba`ja individuals. Populations that share a significantly higher proportion of alleles with each other (|Z-score| higher than ~3) are marked in bold.

Pop A (Outgroup)	Pop B	Pop C	Pop D	f_4	Z-score	Significant (y/n)
Mbuti	**C Anatolia_N (aceramic)**	S Levant_Epipal.	**Ba`ja_PPN**	0.0302	6.51	Y
Mbuti	**W Anatolia_N**	S Levant_Epipal.	**Ba`ja_PPN**	0.0265	6.96	Y
Mbuti	**C Anatolia_N (aceramic)**	S Levant_Epipal.	**'Ain Ghazal_PPN**	0.0313	7.52	Y
Mbuti	**W Anatolia_N**	S Levant_Epipal.	**'Ain Ghazal_PPN**	0.0346	10.23	Y
Mbuti	**W Iran_N (Ganj Dareh)**	**SE Anatolia_PPN**	Ba`ja_PPN	-0.0187	-3.77	y
Mbuti	**W Iran_N (Ganj Dareh)**	**C Anatolia_N (aceramic)**	Ba`ja_PPN	-0.0182	-5.79	y
Mbuti	**W Iran_N (Ganj Dareh)**	**W Anatolia_N**	Ba`ja_PPN	-0.0212	-8.09	y
Mbuti	S Levant_Epipal.	'Ain Ghazal_PPN	Ba`ja_PPN	0.0007	1.29	n
Mbuti	W Iran_N (Ganj Dareh)	'Ain Ghazal_PPN	Ba`ja_PPN	-0.0003	-0.88	n
Mbuti	SE Anatolia_PPN	'Ain Ghazal_PPN	Ba`ja_PPN	0.0000	-0.03	n
Mbuti	**C Anatolia_Epipal.**	**'Ain Ghazal_PPN**	Ba`ja_PPN	-0.0016	-2.97	y
Mbuti	C Anatolia_N (aceramic)	'Ain Ghazal_PPN	Ba`ja_PPN	-0.0002	-0.51	n
Mbuti	W Anatolia_N	'Ain Ghazal_PPN	Ba`ja_PPN	-0.0008	-2.57	n

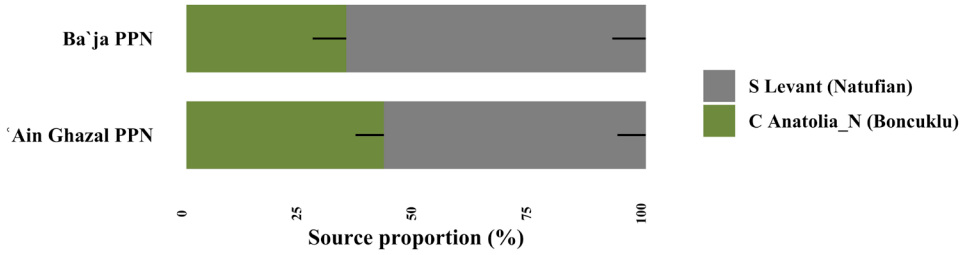

Fig. 2 The *qpAdm* modelling performed separately on Ba`ja and 'Ain Ghazal individuals shows deviations in the estimation of ancestry coefficient from Epipalaeolithic Levantine ("Natufians") and Anatolian_N-related individuals (-1SE).

genetically similar but can be differentiated (Feldman *et al.* 2019a; Wang *et al.* 2023). In particular, the individual from Nevalı Çori was shown to trace a part of her ancestry to both Iran and the Levant, indicating that the ancestries prevailing in these areas had admixed, and reached southeastern Anatolia by the PPNB. Besides these differences, the fit of the various Anatolian groups in the admixture models suggests that the gene-flow in the Levant was likely related to Anatolia, but a specific area of origin cannot be pinpointed by the present data. More importantly, the direct implication of these results is that population interactions were carried at a broad geographical scale. In this respect, the smaller Anatolian-like genetic component at Ba`ja might result from a gradual genetic blending of populations, whereby among the sampled sites, Ba`ja represents the southernmost end of this gradient. In addition, while distance from Anatolia might play a role, other factors affecting human mobility, such as the distance from the coast or topography, could also account for such genetic differences. Future data from Ba`ja and other Levantine sites can shed further light on the genetic structure and diversity across the area during the PPN.

Sex Determination, Genetic Kinship, and Consanguinity

The genetic sex of the three Ba`ja individuals, could be unambiguously assigned. BAJ001 and BAJ020 were determined as biological females. BAJ022 was determined as a biological male.

The estimation of biological relatedness showed that the three individuals were not closely related to each other. More distant relatedness (third degree or beyond) between BAJ020 and BAJ022 who were buried in the same collective burial, cannot be excluded, but cannot be robustly tested under the resolution provided by the presently available data. This is because the accuracy of the methods that can infer more distant degrees of relatedness (*e.g.*, *lcMLkin*) depends on high rates of data coverage as well as on estimations of the genotype frequencies of the population that can only be drawn from a larger sample of individuals. The analysis of the mitochondrial genomes revealed that Individual BAJ001 carried most of the diagnostic SNPs for the N1b1a mitochondrial (mt) haplogroup while Individual BAJ022 was assigned to the N1a1a haplogroup. The N1b haplogroup was extremely rare in Neolithic and post-Neolithic Europe, whereas branches of the N1a haplogroups saw an increase in frequency during the Neolithic (Haak *et al.* 2005, 2010; Bramanti *et al.* 2009; Mathieson *et al.* 2015, 2018; Lipson *et al.* 2017; Rivollat *et al.* 2020). However, in Southwest Asia both N1a and N1b branches have been reported in individuals from the southern Levant to eastern Anatolia and as early as the 12th millennium BCE "Natufians" (Mathieson *et al.* 2015; Kılınç *et al.* 2016; Lazaridis *et al.* 2016; Harney *et al.* 2018; Feldman *et al.* 2019a; Skourtanioti *et al.* 2020; Yaka *et al.* 2021). Furthermore, BAJ022 was found to carry the E1b1b1b2a1 Y-chromosome haplotype. This haplotype belongs to the same lineage as individuals from 'Ain Ghazal, while the more basal form of the haplogroup (*i.e.*, E1b1b) was already found during the Epipaleolithic Natufian period (Lazaridis *et al.* 2016), as well as in northern Africa (van de Loosdrecht *et al.* 2018). The fact that in Anatolia, Iran and the Caucasus this haplogroup has been sampled, so far, only in post-Neolithic individuals (*e.g.*, Narasimhan *et al.* 2019; Skourtanioti *et al.* 2020) is in agreement with evidence from nuclear DNA suggesting that genetic links intensified between the Levant and these areas after the Neolithic. Beyond observations on broad-scale connectivity in Southwest Asia, we note that a larger sample size of mitochondrial genomes and Y-chromosome data from the site could help address the diversity of the uniparental lineages at Ba`ja and its different burial contexts.

Further insights on biological relatedness in Ba`ja were gained through the analysis of Runs of Homozygosity (ROH). ROH are contiguous genome stretches within the genome of an individual in which the paternal and maternal copies are identical. In a large population without any inbreeding (consanguinity), individuals are expected to have ROH in very low frequency. However, individuals tend to accumulate short ROH when they come from a small population, and this information can be used to estimate (effective) population sizes. For example, the elevated sum of short ROH in most of the aceramic individuals from the site of Boncuklu in Anatolia led to the conclusion of small-sized communities compared to the later ceramic farmers (Kılınç *et al.* 2016; Ceballos *et al.* 2021; Ringbauer *et al.* 2021). In contrast, a high frequency of longer ROH, *i.e.*, 20 centimorgans (cM) in an individual genome suggests that the parents were closely related to each other. Currently, there are very few late Pleistocene and early Holocene individuals from Southwest Asia that meet the coverage criteria required for such analysis (Fig. 3). In the southern Levant, one Natufian (from the Raqefet Cave) and one Neolithic individual (from 'Ain Ghazal) harbour a high frequency of short ROH (*i.e.*, 4-8 cM) that is typical for smaller population sizes. BAJ001 also displays a similar pattern. However, additional individuals from these sites are needed to infer effective population sizes. Interestingly, BAJ022 contains a high frequency of long ROH, which are typical in offspring of closely-related parents. In particular, the ROH distribution on BAJ022 is equivalent to parents related at the second to fourth degree (*e.g.*, first to second cousins).

Close-kin unions have been previously identified in genome-wide data from prehistoric contexts, but were shown to be occasional (Ringbauer *et al.* 2021). In this respect, BAJ022 represents the first genetically identified case of consanguinity within the PPN southern Levantine populations. Further evidence comes from intra-site morphological analysis that has shown that endogamy might have been practiced by some communities, such as the PPN inhabitants of Basta, at about 20km southeast of Ba`ja (Alt *et al.* 2013). Both sites were culturally closely related (Nissen *et al.* 2004; Gebel *et al.* 2006, 2020; Kinzel 2013; Purschwitz 2017). It is possible that BAJ022 does not represent an isolated case of consanguinity, but instead a social feature of the Ba`ja community. Such a scenario could potentially be addressed by future investigations that would include tandem genetic and morphological studies on larger skeletal assemblages. It should be recalled here too that none of the three individuals were genetically related, although two of them were

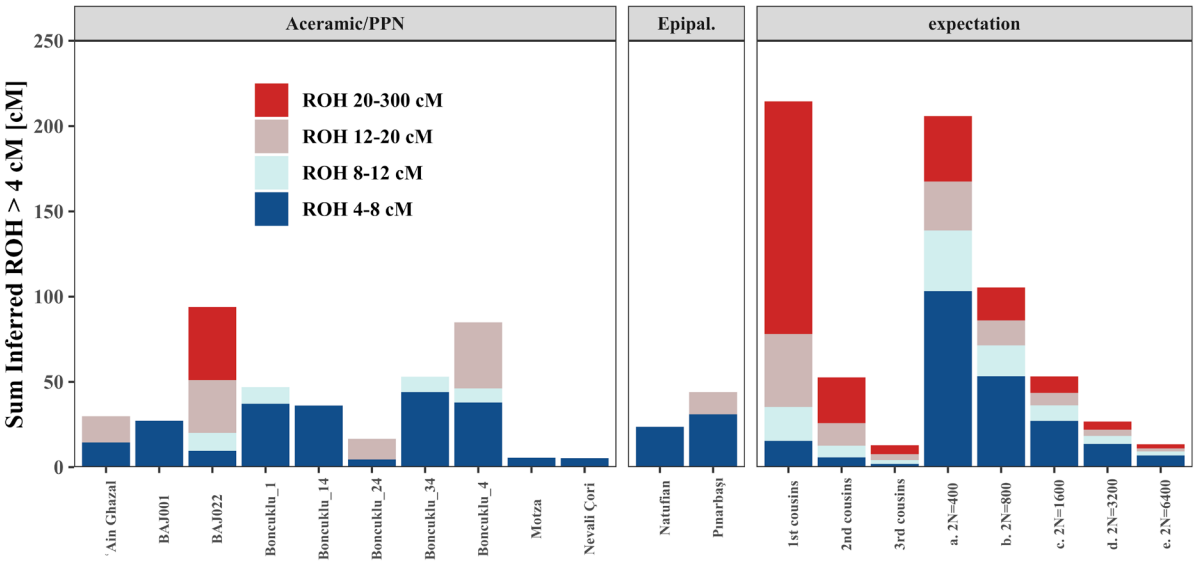

Fig. 3 Distribution or Runs of Homozygosity (ROH) for Ba`ja and other contemporaneous individuals from Anatolia and the Levant that fulfil the coverage criteria (*c.* ≥300,000 SNPs covered). ROH are classified in four length bins, and the distribution of the total sum within each bin is compared with expectations under certain demographic scenarios and close-kin unions (right panel). Each bar represents the ROH estimated within one individual. The ROH are measured in Morgan [M] units or genetic distance.

buried in the same collective burial, and the double infant burial was just in a room next to Room CR17 (see Benz *et al.* this volume). According to these first tentative insights on genetic relations within only a few communities (see also Yaka *et al.* 2021), we rather should expect variable social constructions of kinship (Brück 2021) and corporate identities (Benz *et al.* 2017) with different importance of genetic relations. Prehistoric research on this topic, beyond ethnographic models and architecture, is only at its beginnings. To assess familial and household relations in a more valid way, more integrative scientific investigations on individuals from Ba`ja and from other contemporary sites are indispensable. Additionally, sites that were sampled before the Next Generation Sequencing was available should be re-examined with this new method (*e.g.*, Ramad and Halula). The different types of contexts – open secondary collective burial in Room CR17 where intensive handling of bones is attested (BAJ020 and BAJ022) and a firmly closed primary double burial (BAJ001) – do not allow any generalisation on which factors may have promoted a good preservation in the given climatic conditions with very hot summers. Even well protected individuals in firmly covered stone cists do not guarantee a sufficient preservation (*e.g.*, BAJ004, BAJ008). However, if aDNA is preserved, the rather good coverage is promising for future analyses.

Conclusions

The genetic analysis of the Neolithic inhabitants of Ba`ja was designed to address genetic relationships at both the macro- and the micro-level. On the macro-level, a supra-regional comparison of the Ba`ja population with other groups within the Levant and, more broadly, within Southwest Asia was attempted. At a micro-level, we asked to what extent biological relatedness in Ba`ja correlated to social structures and social practices, a question placed at the heart of the *Household and Death Project*. In line with previous reports for archaeological sites in the southern Levant, only a small fraction of the sampled individuals finally yielded genome-wide data (Feldman 2020), limiting mostly the intra-site approach. However, the relatively high quality of data obtained from the Ba`ja individuals, surpassing that of most contemporaneous Levantine sites, is encouraging in prospect of future efforts to gain a larger sample size from the Ba`ja skeletal assemblage. These genomes also constitute an important reference for future work in the region, not only expanding the geographic range of available human genomic data further south in the Levant, but also extending the temporal coverage, as the Late PPN was so far poorly represented in the Levantine genetic record. This extended range was instrumental to our observation that the southern Levantine earlier Neolithic gene pool persisted at least until the

Late Pre-Pottery Neolithic and extended further south than previously known. In the broader context, the pattern of inter-regional homogenisation in west Eurasia, in which the clear genetic differentiation of the late Pleistocene and early Holocene groups gradually reduced as a result of complex processes of population movement and admixture (reviewed in Feldman *et al.* 2021) is evident in Ba`ja, mainly due to the described genetic link with the Anatolian gene pool. Prehistoric population movements are known to have created long range genetic gradients *e.g.*, between western Anatolia to the southern Caucasus/ Iran in the late Neolithic (Narasimhan *et al.* 2019; Skourtanioti *et al.* 2020). The detected differences in Anatolian ancestry between Ba`ja and 'Ain Ghazal might reflect such a gradient between Anatolia and the Levant, however, this scenario could be better addressed when more Neolithic genomes along this trajectory become available. Additionally, earlier genomes from the southern Levant and neighbouring regions (*e.g.*, the Arabian Peninsula and present-day Iraq) might also help in addressing this issue and in general, aid in a better understanding of the Levantine late Pleistocene/ early Holocene genetic diversity. Finally, the finding of consanguinity in BAJ022 calls for genetic characterisation of additional individuals. Combining this level of genetic information with archaeological and palaeo-anthropological evidence enhances the prospect to shed light on the extent in which biological relatedness played a role in defining the local social groups in Ba`ja and to address questions on local social organisation.

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