Sessione

Antropologia Molecolare

poster

Piazza d'Armi of Spoleto (PG, 720 – 580 BCE): a bioarcheological research to understand the Umbrians origins and their complex social relationships

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Among the pre-Roman populations of Central Italy, research on the Umbrians is limited when compared to the coeval and geographically close Etruscans and Picentes. The Iron age necropolis of Piazza d'Armi at Spoleto (PG), dated to the Orientalizing period (720 – 580 BCE) represents an ideal context to try to fill this gap in bioarchaeological studies. This site is unique for the extraordinary richness of its burials. Weapons and sceptres associated with infants and perinatal individuals, both male and female, have been interpreted as a proof of the first attempt to transfer social status and power across generations. Furthermore, some grave goods found in female burials may indicate their involvement in priestly roles. This hypothesis opens new perspectives on the role of women and children in that community, an issue that remains often obscure in reconstructions of early Italic societies.

The Spoleto necropolis is a complex context not only for the luxury of the grave goods but also for the structure of its society, rising questions on whether the elite was formed by a singular aristocratic nucleus or more, as suggested by the numerous round barrows, and whether it was formed by local people or also foreigners, considering the presence of non-local objects.

To test these hypotheses and to further investigate Umbrians' society through genetic ancestry, mobility pattern, and diet, a multidisciplinary approach was adopted focusing on the analysis of ancient biomolecules (DNA and stable isotopes). Skeletal remains were collected and studied from 38 individuals. DNA was extracted from teeth and petrous bones and then converted into double-stranded genomic libraries. Whole-genome shotgun sequencing and DNA capture with "Twist Ancient DNA" assay targeting over 1.2M SNPs were performed. Specific bioinformatics aDNA pipelines were employed to authenticate sequences, assess individual ancestry, and reconstruct genetic kinship.

Migration pathways were investigated by comparing Sr isotopic signature from tooth enamel, bones and soil. Dietary reconstruction was based on C and N isotopic ratios from collagen which reflect the isotopic signature of foods consumed in the last years of life. Both analyses were carried out using mass spectrometry.

These data provide preliminary information about the origins of ancient Umbrians, their social structure and relationships with the other pre-Roman populations.

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Preliminary paleogenomic analysis on individuals associated with the Villanovan culture in Northern Italy

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The origin of Etruscan civilization is still the subject of much debate today. Two main hypotheses have been debated over time: one suggesting an eastern origin (Anatolian or Aegean) and a second supporting an allochthonous origin from the Villanovan culture, which flourished in central Italy between the 9th and 8th centuries BC. Even if recent studies pointed to a local origin of the Etruscans, no direct genetic comparison between Villanovan and Etruscan individuals has been carried out to date, mainly due to the poor preservation of genetic material in cremated skeletal remains, a common funerary practice in Villanovan culture. In this context, the analysis of ancient DNA from individuals of the Villanovan period is crucial to better understand how and where the Etruscan civilization developed.
Here we present the preliminary paleogenetic results on a set of 24 individuals from three necropolis in the area of Bologna archaeologically dated between the 9th and the 8th–7th centuries BCE. The necropolis of San Vitale, Benacci, e Arnoaldi were excavated between the end of the 19th and the beginning of 20th centuries and the rich funerary materials recovered became part of the collections of the recently established Museo Civico Archeologico di Bologna. For this work we selected petrous bones from inhumated individuals that were analyzed using laboratory techniques specifically made for the study of ancient DNA. Subsequently, specific bioinformatics tools were used to assess DNA preservation, estimate modern DNA contamination, and to perform preliminary genetic analyses.
The samples exhibited high molecular preservation with endogenous DNA percentage ranging between 20 and 88%. Molecular sex determination, reconstruction of uniparental markers and the determination of kinship relationships among individuals were performed. In order to confirm the archaeological chronology, a subset of samples were radiocarbon dated. The analyzed individuals carry a genetic profile which seems to resemble the one previously found in Etruria and, overall, in the rest of the Italian Peninsula during the Iron Age (and the Bronze Age),
Considering the informative potential of the samples here analyzed, additional work will involve more informative population genetic analyses in order explore more in-depth the ancient phases of the development of the Etruscan Population, as well as their relationships with contemporary and earlier groups from central Italy.

High-Resolution Kinship Inference with SNPs: A Forensic Tool for Complex and Distant Relationships

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Kinship recognition between anonymous DNA samples is becoming increasingly relevant in forensic science, particularly as national and international DNA databases continue to expand. While Short Tandem Repeats (STRs) remain the gold standard for close kinship analysis, their limitations in detecting distant relationships—due to high mutation rates and a restricted number of loci—necessitate more advanced approaches.

Next-generation sequencing (NGS) has emerged as a powerful tool for personal identification, enabling the simultaneous analysis of a wide range of genetic markers. In this context, we previously developed and evaluated a novel panel of 4,849 Single Nucleotide Polymorphisms (SNPs) specifically designed for high-resolution kinship inference on 150,000 simulated individual pairs, ranging from unrelated to fifth-degree relatives using a combination of the Forrel package in R and supervised machine learning algorithms.

The panel demonstrated strong performance in identifying kinships up to the third and fourth degrees, with recall values exceeding 0.6 even for fifth-degree relationships. Machine learning integration further enhanced accuracy, improving F1 scores by approximately 12.25% and 20% for fourth- and fifth-degree relationships, respectively. Notably, the method achieved over 99% accuracy in distinguishing related from unrelated individuals.

To assess real-world applicability, we evaluated the panel's performance using sequencing data from 2,386 individuals with documented kinship ties up to the third degree, available through the 1000 Genomes Project. Specifically, we processed 1,206 confirmed parent-child pairs, 15 sibling pairs, 36 grandparent-grandchild or avuncular pairs, and 29 third-degree relatives using a Python- and R-based pipelines.

Overall, this study highlights the potential of SNP-based panels, combined with advanced computational methods, to significantly improve kinship inference—especially in complex or distant relationships—offering a robust alternative to traditional STR-based approaches in modern forensic investigations. This approach is particularly valuable in mass disaster scenarios and missing person investigations, where establishing biological relationships is often the only viable path to identification.

APOE DNA methylation variability in human populations and its implication for human health

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Apolipoprotein E (APOE) haplotypes are among the most extensively studied genetic factors associated with age-related diseases, human longevity and fertility in natural fertility populations. APOE isoforms present variability across different populations as well as high pleiotropic effects. However, despite the strong associations with phenotype identified, the geographic distribution of the genetic variants does not always reflect the distribution of these traits in human populations, suggesting complex gene-environment interactions. Among the many biological mechanisms beyond, DNA methylation may certainly play a major role. Despite the high number of studies on genetic variations, few studies that mainly focused on populations of European ancestry have investigated the role of DNA methylation at APOE CpG sites. Moreover, the interactions between APOE genetic variants and DNA methylation variation in non-European populations and in natural fertility populations are still missing.

In this framework, the aim of this study is to investigate:

1. patterns of natural variations of 11 CpGs sites located in the APOE in non-European populations;
2. the interactions between APOE genetic variants and DNA methylation variation in non-European populations

To this aim we considered individuals from different ethnic groups of global distribution whose methylation data are publicly available, including those of indigenous Latin Americans, as well as native and admixed populations of Argentina, such as the Criollos and Wichí. The latter populations represent a unique context for studying genetic diversity and epigenetic variability for which genotypic data further allows to investigate genotype-specific methylation patterns. We used data from the Infinium Human Methylation 450 array and the Infinium MethylationEPIC BeadChip, for public data, those from online databases, mainly the Gene Expression Omnibus (GEO). The results showed natural variations among human groups in DNA methylation patterns in a subset of CpGs sites. Moreover, the study provides new information and results on the association between DNA methylation and APOE isoforms.

In conclusion, we present preliminary insights into the DNA methylation variability associated with the APOE gene and its isoforms, which is crucial for further investigations into the role of methylation in modulating the effects of APOE genetic variants across different environmental, ecological, and anthropological contexts.

Gene Polymorphisms for Strength and Sprint Performance among Female Volleyball Players.

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Background. Volleyball is a high-performance intermittent sport that requires alternating aerobic and anaerobic phases, with anaerobic alactic metabolism used for explosive efforts.
The ACTN3 rs1815739, MCT1 rs1049434 and ACE I/D polymorphism are the most studied genetic markers in sport sciences, and they have been associated with sprint and strength performance. ACTN3 codes for the -actinin-3 protein, which is a decisive factor in the ability to produce high-velocity muscular contractions. MCT1 codes for monocarboxylate transporters 1 that plays a relevant role in the intracellular pH homeostasis. ACE code for angiotensin converting enzyme, that has a relevant role in fluid homeostasis.
Aim of the study. This study examined the distribution of common genetic variants in the ACTN3 (rs1815739), MCT1 (rs1049434) and ACE (I/D) gene and their influence on short linear sprint and strength performance in female volleyball players.
Methods. A total of 99 female volleyball players (Elite, sub-elite, and amatorial) participated in the study. In a sub-sample of 66 athletes (sub-elite and amateurs) some anthropometric variables and motor tests were measured. 3 SNPs in ACTN3, MCT1, and ACE were performed.
Results. No significant differences have been found in allelic frequency distribution among the 3 groups of athletes. The sample met the Hardy Weinberg equilibrium for the 3 polymorphisms. The allelic frequencies of ACTN3 and MCT1 (ACTN3*R= 0.5885, ACTN3*X= 0.4115; MCT1*A= 0.3594, MCT1*T= 0.6406) are in the range of worldwide population variability, on the contrary ACE I/D (ACE*I= 0.3670, ACE*D= 0.6330) showed an increase of D frequency.
The association analysis, carried out through SNPSTAT program, gave back significant results only for ACTN3/5m sprint. Precisely, the players harboring a copy of the R allele (RR+RX genotypes) showed a better sprint time than players with XX genotype (RR+RX= 1.27±0.01 sec vs XX=1.36±0.04 sec, p=0.023). Differences were also found among positions on the field, with liberos that were significantly fastest than setters (p = 0.021) and middle blockers (p = 0.010).
Conclusion. We found, for the first time, an association between the ACTN3 polymorphism and liner running speed in youth female volleyball players. If our findings are replicated in larger research, it could be used in future to adapt the training protocols to enhance volleyball performance.

Urban Kinship and Genetic Diversity in Thessaloniki: A Genomic Perspective

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Urbanization has long fostered interaction and interdependence among individuals beyond close biological kin. Yet, we still know little about how familial, religious, and economic networks shaped everyday life in Roman and post-Roman urban centers. This study employs ancient DNA analysis to investigate such dynamics in the city of Thessaloniki — a major urban and cultural hub throughout European history. Our dataset spans the entire Byzantine period (324–1453 CE), with a particular emphasis on the Late Byzantine period (1204–1453 CE).

We explore how the formation of social, religious, and neighborhood-based subgroups may have encouraged endogamy, thereby reducing genetic diversity within subgroups. At the same time, factors such as trade, migration, and exogamous marriage practices likely contributed to increased genetic diversity at the population level.

In addition to standard approaches for inferring ancestry and biological relatedness, we applied population genetic metrics including inbreeding coefficients (F), Wright's FST and nucleotide diversity estimates (and) to assess genetic structure and heterogeneity. We integrated these genomic findings with historical and archaeological sources to better understand how cultural norms and social organization influenced patterns of diversity in the city.

Our results indicate clear signs of population structure especially in Byzantine Thessaloniki, which we interpret as a probable reflection of localized family systems and broader socio-cultural frameworks.

This research is part of an ERC-Consolidator Grant entitled CityLife: A bioarchaeological study of 1,800 years of resilience and adaptation to urbanity (Project: 101126337).

Untangling the complex hybridization history of true lemurs (genus Eulemur)

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The true lemurs (genus Eulemur) are a genetically diverse and geographically widespread group inhabiting most of Madagascar's forests. With 12 recognized species, the genus comprises four major clades: the Brown Lemur Species Complex (BLSC), the coronatus-macaco-flavifrons complex (CMFC), E. mongoz, and E. rubriventer, although monophyly for the CMFC is not always supported. Recent genomic studies revealed topological and temporal discordances between nuclear and mitochondrial phylogenies in Eulemur, likely due to past hybridization events. To investigate the evolutionary history of the genus, we compiled a dataset of previously published whole-genome sequences and reconstructed both nuclear and mitochondrial phylogenies. Using this framework, we applied a population genomics approach (ABBA-BABA tests, hPSMC, and admixture graphs) to test for gene flow among clades. We detected multiple hybridization events, most notably between E. rubriventer and the BLSC, helping to explain mito-nuclear phylogenetic discordance. To further explore this, we examined nuclear-encoded mitochondrial proteins in E. rubriventer and found an enrichment of topologies matching mitochondrial phylogenies, suggesting mito-nuclear co-adaptation following introgression.By directly testing for hybridization across the genus, our study presents a refined evolutionary model for Eulemur, highlighting the role of gene flow in shaping its genomic landscape, while also agreeing with the most supported ecological model for the rapid speciation of the genus during the last 5 million years. These findings contribute to a deeper understanding of this emblematic Malagasy lineage and lay the groundwork for future research.

Kinship Reconstruction of the Velimna Family from the Hypogeum of the Volumnis (3rd Century BCE, Umbria)

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The genetic history of central Italy remains only partially explored. While several genomic studies have shed light on ancient groups such as the Etruscans in Tuscany and the Picenes in Marche, the region of Umbria and the ancient Umbrian population has yet to be fully investigated.
In this study, we aim to investigate the genetic history of the Volumnia family buried in the Hypogeum of the Volumnis, within the context of the Necropolis of Palazzone (III BCE), a monumental tomb that preserves the urns of seven members of the Velimna family. Inscriptions at the site suggest close familial relationships among six of them, offering a unique opportunity to reconstruct biological relatedness using ancient DNA.
The samples analyzed included three cremated individuals and one inhumed individual (Velia Velimnei).
DNA was extracted from highly degraded and carbonized petrous bone fragments in a dedicated clean lab following strict aDNA procedures.
Genomic libraries were prepared using methods optimized for highly fragmented molecules. Shotgun sequencing in combination with a target enrichment approach were used to reconstruct mitochondrial and nuclear genome of the analyzed individuals.
The shallow shotgun sequencing enabled assessment of endogenous DNA content and contamination levels, guiding the application of whole-genome deep sequencing for well-preserved samples and targeted enrichment for those with lower endogenous DNA content.
The authenticity of aDNA is evaluated through the damage patterns analysis and by examining the heterozygosity of the X chromosome in the male individuals.
Biological sex was inferred using the ratio of reads aligning to the sex chromosomes.
Finally, the phenotypically and functional informative SNPs will be analyzed for well-preserved individuals.
This study will thus allow for a deeper investigation of the genetic relationships among the members of the Velimna family.

Uncovering Genetic Signatures of an Iron Age Umbrian Population from Montecchio, Necropoli del Vallone di San Lorenzo (Umbria, Italy)

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The complex demographic and cultural transitions that occurred during the Iron Age which shaped pre-Roman Italy still remain a central topic in molecular anthropology. During the Iron Age (c. 1000–350 BCE), before the unification under the Roman control, the Italian Peninsula is populated by several regional groups with different cultural identities and different languages which are traditionally called "The Italic peoples". In this study, we present preliminary genetic data from eight human skeletal remains excavated at the necropoli del Vallone di San Lorenzo, Montecchio (Umbria, Central Italy), dated to the late Iron Age (ca. 6th to 5th centuries BCE). The archaeological context, including artifactual evidence and tomb typology, points to an Umbrian site with strong Etruscan cultural influence and even possible Picene connections.
Recent ancient DNA studies on Italic people such as the Etruscans and Picenes have revealed a heterogeneous genetic landscape, reflecting both continuity from Bronze Age populations and interactions with incoming groups during the Iron Age.
All eight individuals were processed following the standard ancient DNA laboratory workflow, and the obtained libraries were screened by NextSeq 550 (Illumina) at ISIS@MACH facility, Tor Vergata University. Three individuals were excluded from further analyses due to low endogenous DNA content. The remaining five samples exhibited typical aDNA features, including cytosine deamination (C¿T) at read ends, negligible mitochondrial contamination, and, for males, low X-chromosome contamination as assessed using an optimized custom pipeline. Notably, two samples yielded exceptionally high endogenous content (¿60%). These findings indicate favorable preservation conditions at the site, despite the typically warm climate of central Italy. They also demonstrate the site's strong potential for generating high-quality ancient DNA suitable for low coverage whole-genome sequencing analyses.
Our long-term objective is to increase genome sequencing coverage at least 0.1 X to enable robust placement of these individuals within the genetic variability of prehistoric Italian populations. Comparative analyses with published data on Etruscan and Picene groups will help to clarify whether the Montecchio individuals share affinities with known Etruscan gene pools, supporting the hypothesis of cultural and genetic continuity in the region during the final stages of the Iron Age, on the eve of Roman expansion.

The origin of social inequalities in Northern Italy: clues from ancient genomes

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Understanding the dynamics that promoted the onset and perpetuation of social inequalities in prehistoric societies is a major challenge. Thanks to the increase in genomic data from ancient remains, the development of kinship estimation methods, and computational approaches for reconstructing past evolutionary dynamics from low coverage data, we now have a unique opportunity to exploit genomic data to describe past population structures and shed light on the socioeconomic processes and sociocultural dynamics underlying the emergence of inequalities. Archaeogenetic has revealed that two major population dynamics in the last 10,000 years significantly impacted the genomic composition of Europeans: the Neolithic expansion and the Bronze-Age migration from the Steppe. Despite the importance of these events, our genetic understanding is mainly built upon pan-European sampling strategies, resulting in limited knowledge of how these migrations affected individual societies. This project proposes a high-resolution multidisciplinary study of approximately 120 individuals from three necropolises in north-eastern Italy dating back to the Neolithic (Valdaro Paganella), Eneolithic (Corna Nibbia di Bione) and Bronze Age (Arano) periods, with the specific aim to infer the social and genetic structures and their potential changes over this time span. We are analysing whole genomes to reconstruct biological relatedness within each necropolis, infer population structure and admixture proportions, and model past demographic dynamics. Currently, we have an almost exhaustive paleogenomic overview of the Eneolithic site of Corna Nibbia. Analyses of the Arano site are providing extensive data, allowing us to draft a preliminary pedigree. Meanwhile, genomic analyses of the Neolithic site are in progress. The genomic data, combined with a high-resolution chronology, archaeological evidence, and ongoing stable isotopes analyses to investigate dietary patterns and individual mobility, will allow us to obtain information about potential inequalities between members of each burial site and to recognise possible patterns behind the transmission of social status and wealth. This multidisciplinary strategy will help us to better understand social inequality in north-eastern Italy, as well as the cultural and biological mechanisms that promoted its development.

Tracing Genetic Heritage in the South Caucasus: Ancient DNA from Bronze Age Armenians

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Armenia has been a key geographical region with intense migratory flows since the Upper Palaeolithic. Due to its proximity to Anatolia, it was among the first regions to adopt agriculture. Another major event was the migration from the Caucasus to the steppe, which contributed to the formation of Steppe ancestry. During the Bronze Age, Armenia as a crossroads between the Middle East, the Caucasus, Central Asia and Iran, become a hub of cultural and linguistic exchange. This complex historical background makes the study of Armenian demographic history essential for understanding regional population dynamics and migration patterns.
Our aim is to genetically analyze 51 individuals from the western and southern shores of Lake Sevan, at altitudes of 1,800–2,000 meters, from five collective burials dating from 2000–1400 BCE. Our principal goals is to characterize this population at genetic point of view and assess potential kinship relationships among individuals buried in collective graves.
Specifically, teeth and petrous bones from five sites: Kanageh, Noratus, Nerkin Getashen, Lchashen and Zorats Karer were analysed. From each sample, a minimum of 50 mg of bone powder was used for DNA extraction, double-stranded libraries were constructed, indexed and screened using the Illumina NextSeq550 at ISIS@MACH facility, Tor Vergata University.
Initial results show good DNA preservation, with endogenous DNA content ranging from 20% to 75%, except in a few cases. Deamination rates (C to T transition) at the 5' end, confirming the ancient origin of the material. The data was authenticated by the mtDNA contamination test using contamMix, and in males the X chromosome contamination rate using ANGSD.
Our screening results indicate good preservation of the analyzed samples, likely due to the low temperatures at the lake's altitude. Notably, this preservation is particularly impressive given that most of the processed samples were teeth, which are generally not considered the most optimal material for ancient DNA analysis in prehistoric contexts. Next step will be achieve a coverage more than 0.5X, sufficient for genotype imputation. These imputed data will be analyzed alongside previously published genomes to enhance our understanding of genetic variability in the Armenian population during the Bronze Age. Furthermore, our analysis aims to elucidate the role of Lake Sevan in the migration routes connecting the Caucasus, northern Levant, Iran, and Anatolia.

PRIN2020 "Crossing the Sea": Depicting the adaptive evolutionary history of Sicilian ancestors through the analysis of modern genomes

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The Mediterranean Sea played a crucial role during the principal phases of the peopling of European territories and in the major cultural changes associated with them. In this context, Sicily has long represented a pivotal crossroad, acting as a fundamental meeting place for several populations during all the migration processes that interested the continent through time. In addition to such a complex demographic history, the genetic background of present-day inhabitants of the island might have been shaped also by the evolution of biological adaptations in response to local selective pressures, which were plausibly different with respect to those experienced by human groups distributed along the peninsula. While previous studies already addressed the genetic history of Sicilian people, they relied solely on the analysis of uniparental markers or autosomal loci typed with SNP-chip approaches, thus failing to investigate its full spectrum of variation. This also prevented exhaustive identification of the genetic determinants of possible adaptive traits. Within the framework of the PRIN2020 "Crossing the Sea" project, which is aimed at investigating the evolutionary history of populations from the main Western Mediterranean islands, we generated high-coverage whole-genome sequence data for 41 individuals three generations native of different areas of Sicily. Population structure and haplotypes sharing-based analyses setting the examined samples into the context of a large panel of Euro-Mediterranean genomes made up of nearly 900 individuals from 17 populations, revealed that Sicilian individuals grouped within the same genetic cluster and showed proportions of ancestry components appreciably distinct with respect to those observed in peninsular Italians. We thus considered this homogeneous population sample to infer the adaptive evolution of Sicilian ancestors. For this purpose, we applied likelihood- and network-based methods suitable to detect both strong and weak selective events and we searched for biological functions enriched for putative adaptive loci. This approach enabled to test different models of adaptive evolution, ranging from classical selective sweeps to polygenic adaptations, thus providing an unprecedented overview of the environmental factors having represented remarkable selective pressures during the evolutionary history of Sicilian peoples.