DENTAL CALCULUS

MUNI SCI

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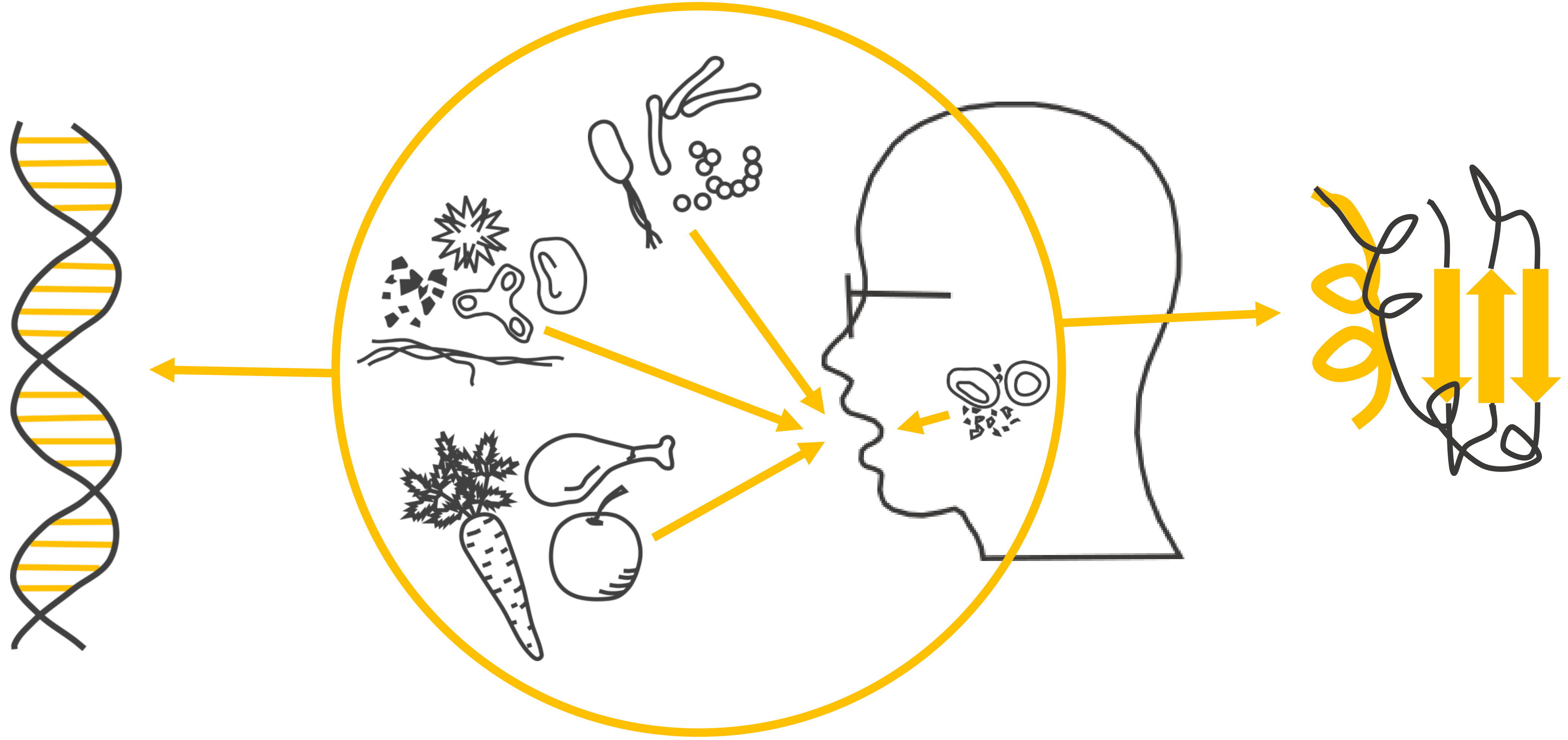
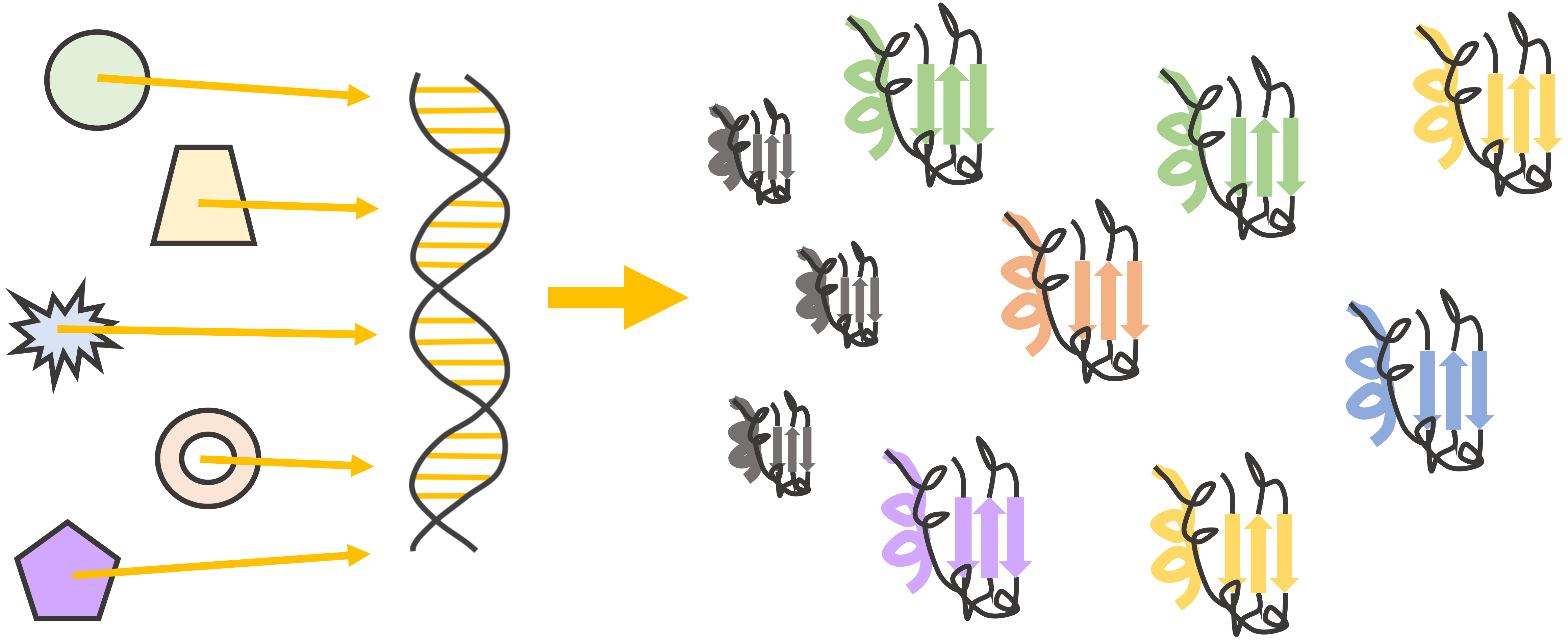
LABORATORY OF BIOLOGICAL AND MOLECULAR ANTHROPOLOGY
DEPARTMENT OF EXPERIMENTAL BIOLOGY

DENTAL CALCULUS

CONTENT



SAME DNA, DIFFERENT PROTEINS



DNA VS PROTEINS

AMPLIFICATION MISTAKES
AND CONTAMINATION

NO AMPLIFICATION

DNA VS PROTEINS

AMPLIFICATION MISTAKES
AND CONTAMINATION

CAN DISTIGUISH CLOSER SPECIES

NO AMPLIFICATION

OFTEN CONSERVED

DNA VS PROTEINS

AMPLIFICATION MISTAKES AND CONTAMINATION

CAN DISTIGUISH CLOSER SPECIES

WORSE PRESERVATION

NO AMPLIFICATION

OFTEN CONSERVED

BETTER PRESERVATION

DNA VS PROTEINS

AMPLIFICATION MISTAKES AND CONTAMINATION

CAN DISTIGUISH CLOSER SPECIES

WORSE PRESERVATION

THE SAME FOR ALL CELLS

NO AMPLIFICATION

OFTEN CONSERVED

BETTER PRESERVATION

SHOWS FUNCTION AND ACTIVE PROCESES – TISSUE DIFFERENCES

CHALLENGES

LIMITED AMOUNT

UNIFIED PROTOCOLS

MORE SENSITIVE METHODS

OMICS

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A unified protocol for simultaneous extraction of DNA and proteins from archaeological dental calculus

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Article

Extraction Protocol for Parallel Analysis of Proteins and DNA from Ancient Teeth and Dental Calculus

Eva Chocholova, Pavel Roudnicky, David Potesil, Dana Fialova, Karolina Krystofova, Eva Drozdova,* and Zbynek Zdrahal*

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ABSTRACT: Dental calculus is becoming a crucial material in the study of past populations with increasing interest in its proteomic and genomic content. Here, we suggest further development of a protocol for analysis of ancient proteins and a combined approach for subsequent ancient DNA extraction. We tested the protocol on recent teeth, and the optimized protocol was applied to ancient tooth to limit the destruction of calculus as it is a precious and irreplaceable source of dietary, microbiological, and ecological information in the archeological context. Finally, the applicability of the protocol was demonstrated on samples of the ancient calculus.

KEYWORDS: *bioarcheology, paleoproteomics, ancient DNA, ancient proteins, dental calculus*

CHALLENGES

LIMITED AMOUNT

CONTAMINATION

- LIMITATION OF PRE-LABORATORY CONTAMINATION
- DECONTAMINATION
- SPECIALISED LABORATORY
- PROCESSING OF NEGATIVE CONTROLS AND SOIL
- BIOINFORMATICS
- INTERPRETATION

CHALLENGES

LIMITED AMOUNT

CONTAMINATION

SHORT TIME PERIOD → COMBINATION WITH OTHER METHODS

INTERPRETATION

CHALLENGES

LIMITED AMOUNT

CONTAMINATION

SHORT TIME PERIOD

FRAGILITY, LOSS

EARLIER SAMPLING

CARE IN HANDLING (E.G. WASHING SKELETAL MATERIAL)

MODIFICATION OF DECONTAMINATION PROTOCOLS

CHALLENGES

LIMITED AMOUNT

CONTAMINATION

SHORT TIME PERIOD

FRAGILITY, LOSS

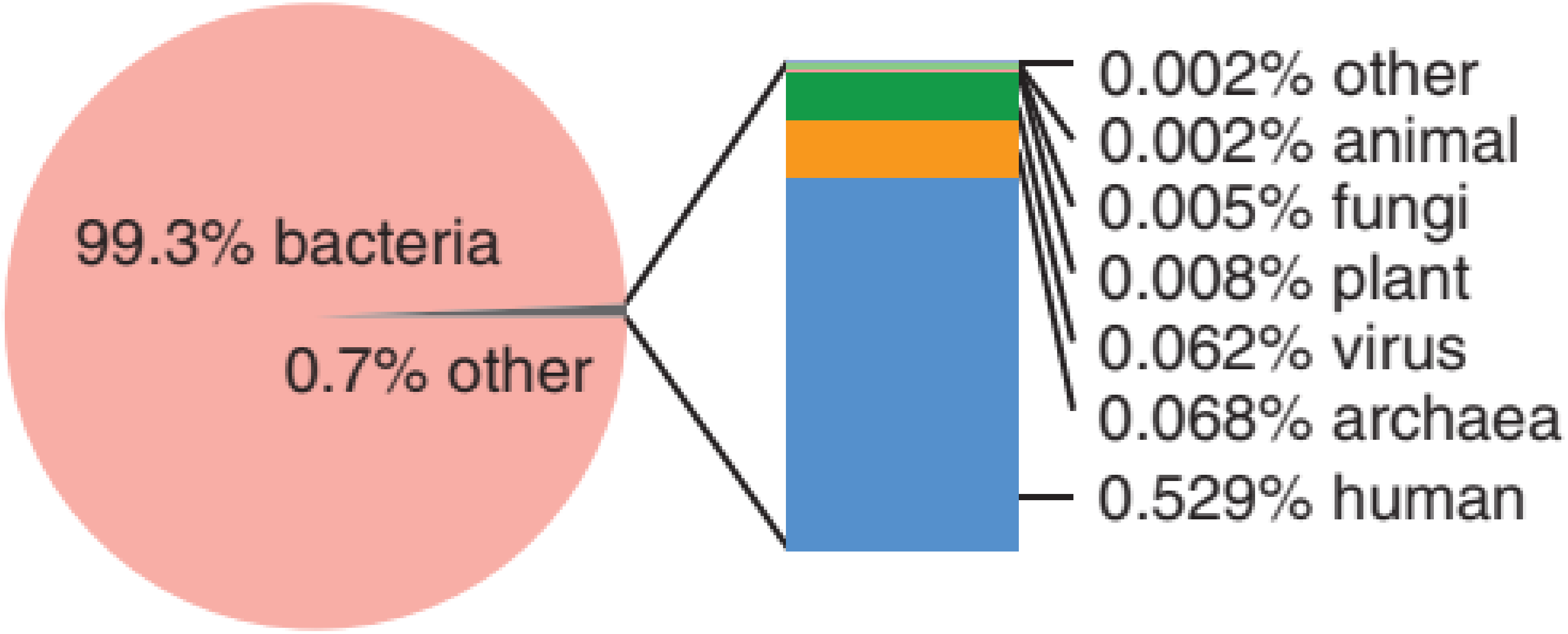
UNPREDICTABILITY OF CONTENT, PREDOMINANCE OF MICROORGANISMS

RESEARCH DESIGN AND OBJECTIVES

COMBINATION WITH OTHER METHODS

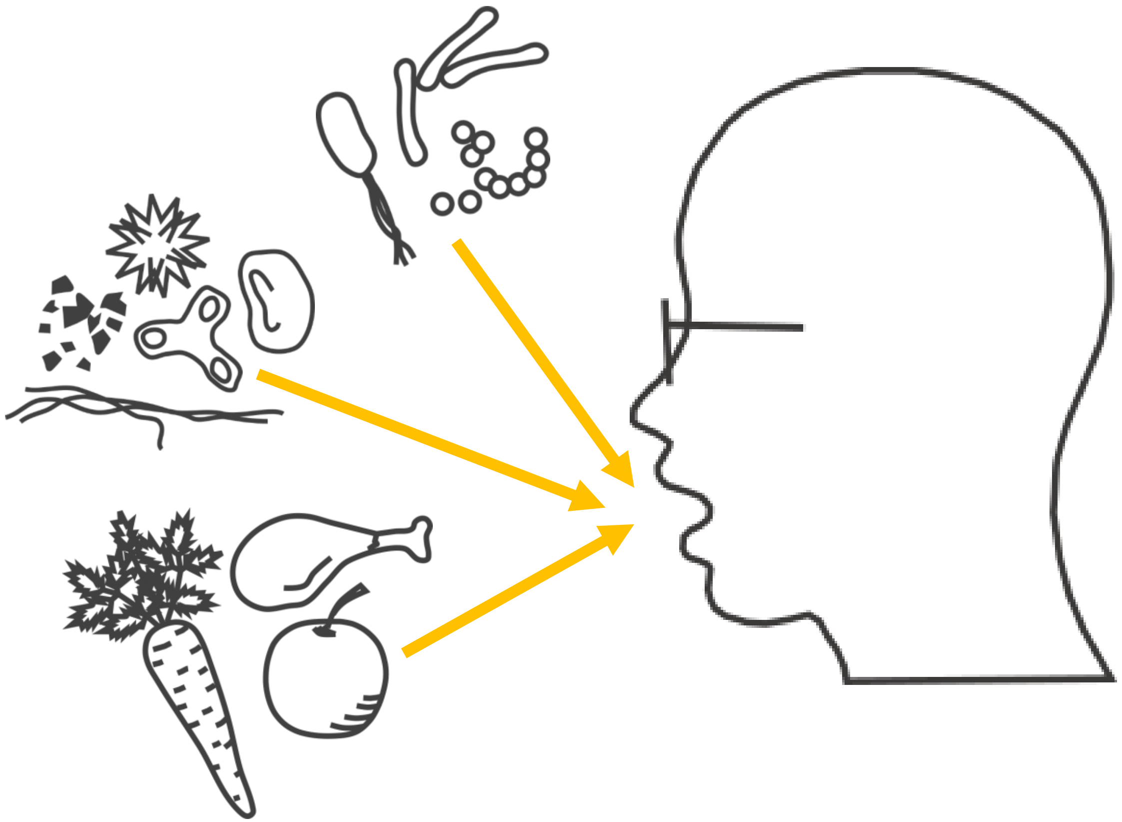
PATIENCE

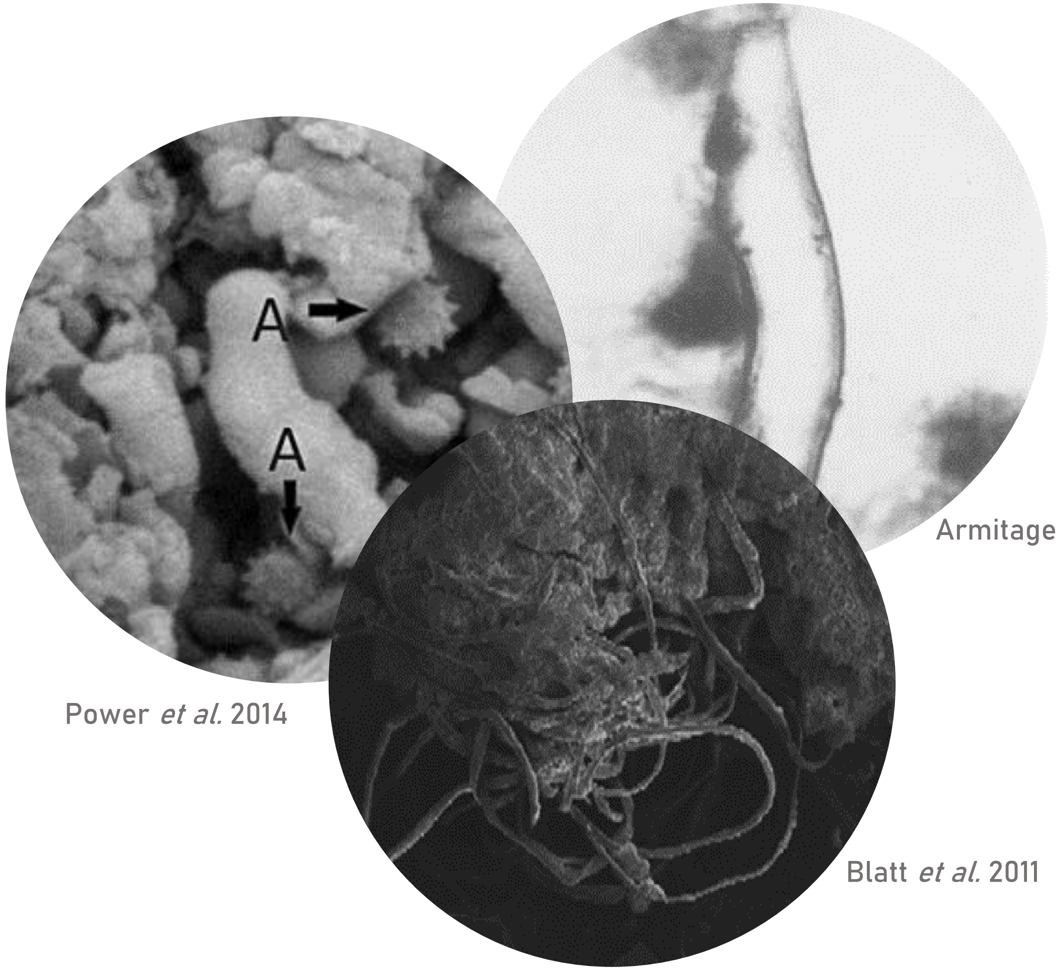
UNPREDICTABILITY OF CONTENT



Warinner *et al.*, 2014

CONTENT



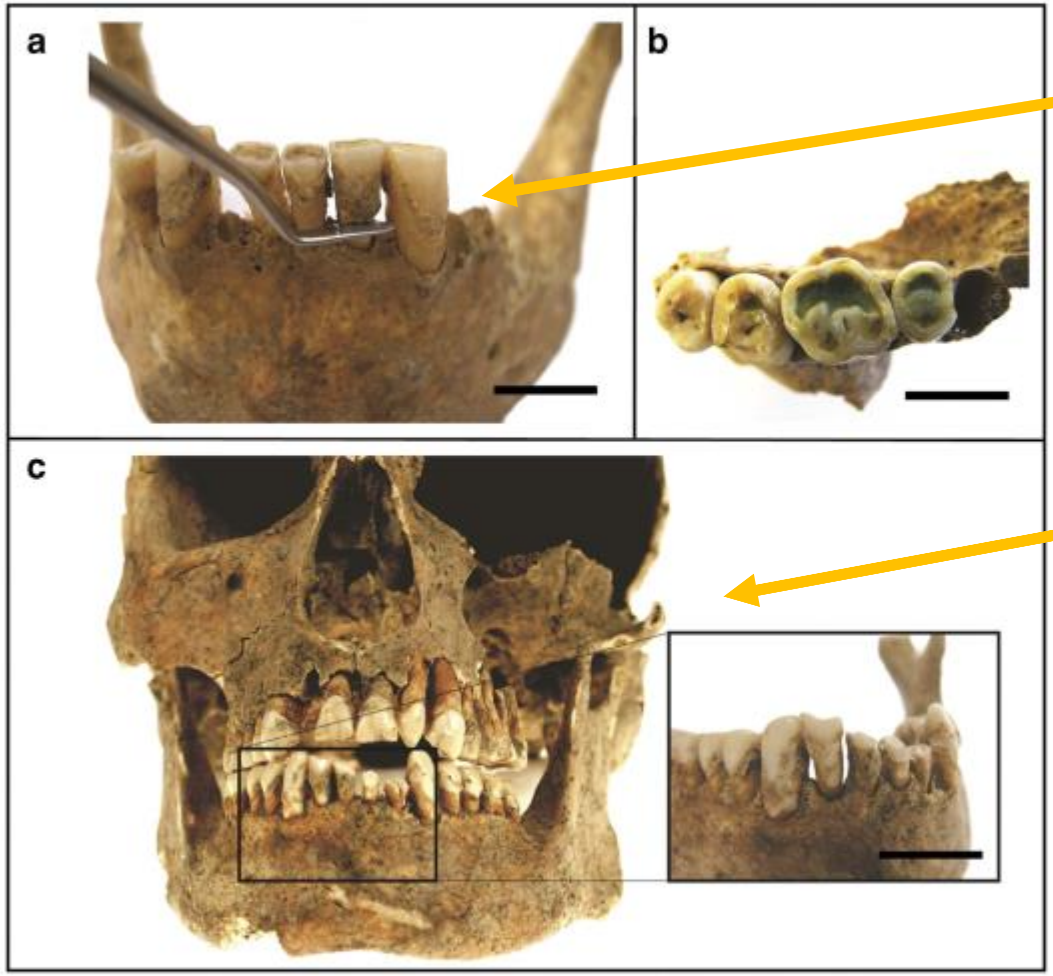


Power *et al.* 2014

Armitage 1975

Blatt *et al.* 2011

HABITS



USED GRINDING STONES

BRONZE GRAVE GOODS

OPENED PAPER CARTRIDGES
(GUNPOWDER + BULLET)

Figure 2. Images of the samples. Bar = 10 mm. **a:** The sampling of the ancient human dental calculus from a grave in Znojmo-Hradiště No. 464 in the Czech Republic. **b:** Green-coloured teeth of the man No. 53 from Devín-Za kostolom in the Slovak Republic. **c:** Man No. 801 from the site Majetín in the Czech Republic. Incisors of his lower jaw showed traces of a trauma with a military origin.

Fialová et al., 2017

HUMAN DNA

Dental Calculus as an Alternate Source of Mitochondrial DNA for Analysis of Skeletal Remains

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Central Washington University, Ellensburg, Washington

Mitochondrial DNA (mtDNA) is widely used in studies of affinities among living peoples and prehistoric populations represented by skeletal remains excavated at archaeological sites. Although many Indian groups see the utility of using mtDNA analysis as a means of connecting past and present, cultural norms regarding treatment of human remains prevent the use of destructive techniques in obtaining DNA. In this paper we discuss the utility of using dental calculus collected from a number of individuals comprising a pre-contact burial site (CA-SOL-357; A.D. 600-1000) as a possible source of mtDNA.

NEANDERTHALS

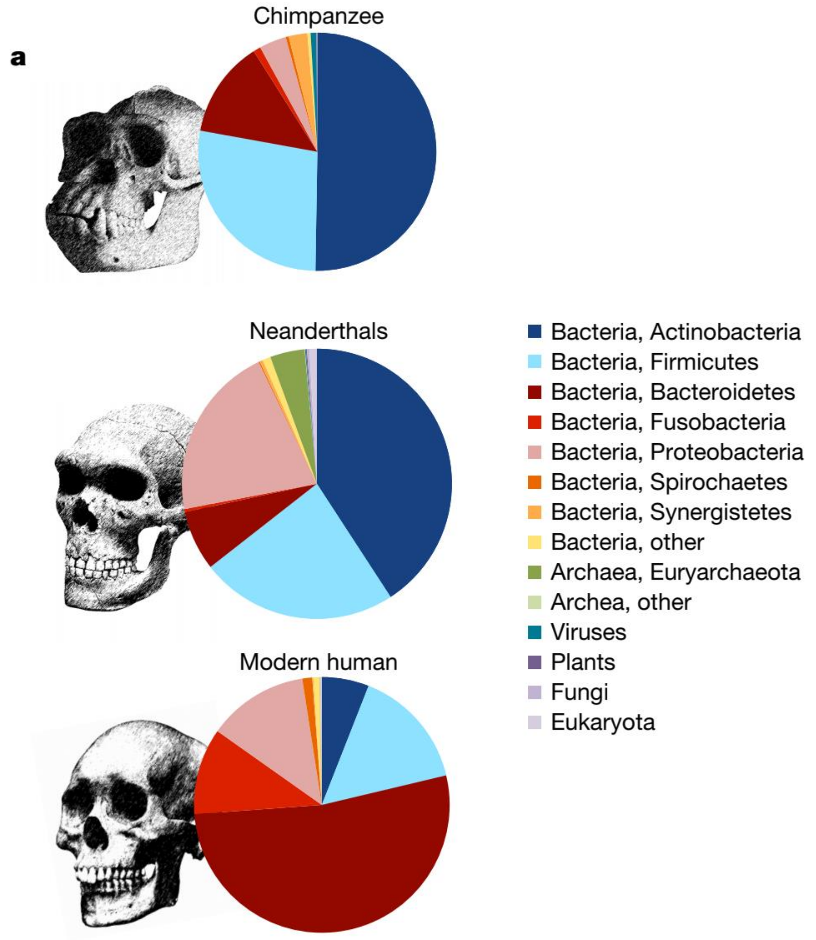
Neanderthal behaviour, diet, and disease inferred from ancient DNA in dental calculus

Laura S. Weyrich[1], Sebastian Duchene[2], Julien Soubrier[1], Luis Arriola[1], Bastien Llamas[1], James Breen[1], Alan G. Morris[3], Kurt W. Alt[4,5,6,7], David Caramelli[8], Veit Dresely[5,6], Milly Farrell[9], Andrew G. Farrer[1], Michael Francken[10], Neville Gully[11], Wolfgang Haak[1], Karen Hardy[12,13], Katerina Harvati[10], Petra Held[14], Edward C. Holmes[2], John Kaidonis[11], Carles Lalueza-Fox[15], Marco de la Rasilla[16], Antonio Rosas[17], Patrick Semal[18], Arkadiusz Soltysiak[19], Grant Townsend[11], Donatella Usai[20], Joachim Wahl[21], Daniel H. Huson[22], Keith Dobney[23,24,25] & Alan Cooper[1]

Table 1 | Dietary information preserved in calculus

Scientific name	Common name of probable source	Hominid pathogen or medicinal use	El Sidrón 1	El Sidrón 2	Spy II	Chimpanzee	Modern human	Laboratory control (EBC)	Spy I*
Zymoseptoria tritici	Plant (wheat) pathogen		4.13%	0	0	0	0	0	2.87%
Phaeosphaeria nodorum	Plant (wheat) pathogen		12.22%	0	0	3.98%	0	0	0.45%
Penicillium rubens	Food fungus	MU	3.97%	0	0	0	0	0	1.35%
Myceliophthora thermophila	Cellulose fungus		0	0	0.56%	0	0	0	0.13%
Coprinopsis cinerea	Edible mushroom (grey shag)		0	0	2.44%	0	0	0	0
Schizophyllum commune	Edible mushroom (split gill)		3.65%	0	0	0	0	0	0.10%
Malassezia globosa	Human fungal commensal		3.65%	8.89%	0	0	19.92%	0	5.49%
Enterocytozoon bieneusi	Intracellular parasite (microsporidia)	HP	8.10%	0	0	0	0	0	0
Ovis aries	Sheep (wild mouflon)		0	0	62.03%	0	0	0	1.17%
Ceratotherium simum	White rhinoceros (woolly rhinoceros)		0	0	34.40%	0	0	0	0.11%
*Ixodes scapularis**	Tick		0	0	0	0	2.15%	0	0.15%
Physcomitrella patens	Moss		2.06%	0	0	0	0	0	0.09%
Pinus koraiensis	Pine tree		13.49%	19.60%	0	4.45%	0	0	0.40%
Populus trichocarpa	Poplar tree	MU	2.86%	0	0	0	0	0	0.44%
Total eukaryotic reads			630	551	532	427	3,760	5	25,294

DNA sequences mapping to eukaryotic species are shown as a proportion of the total eukaryotic reads identified within each sample. Eukaryotic sequences were also identified in the extraction blank controls (EBCs) and the Spy I Neanderthal, which is heavily contaminated with modern DNA; these samples are shown to the right.
HP, human pathogen; MU, medicinal use.
*Samples or taxa that are probably the results of contamination, as they do not represent biological processes (see Supplementary Information).

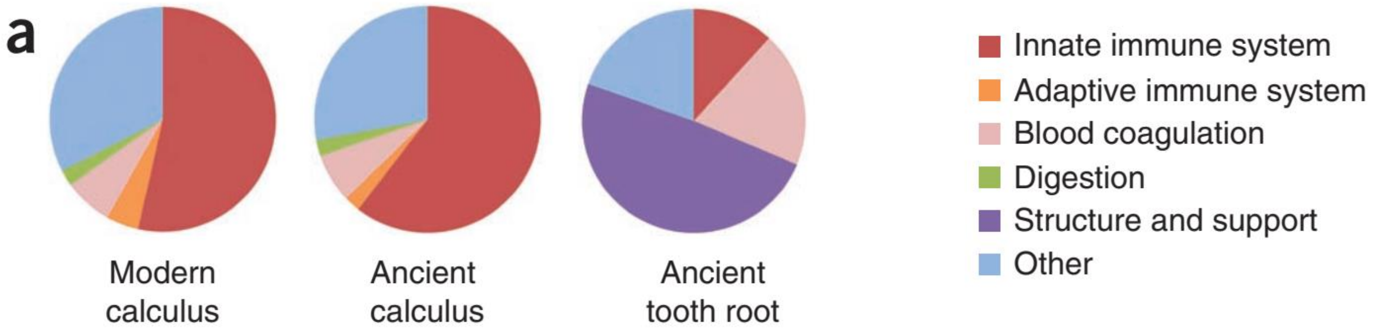


Weyrich et al. 2017; DOI: 10.1038/nature21674

IMMUNE REACTION

Pathogens and host immunity in the ancient human oral cavity

Christina Warinner[1,2], João F Matias Rodrigues[3,4], Rounak Vyas[3,4], Christian Trachsel[5], Natallia Shved[1], Jonas Grossmann[5], Anita Radini[6,7], Y Hancock[8], Raul Y Tito[2], Sarah Fiddyment[6], Camilla Speller[6], Jessica Hendy[6], Sophy Charlton[6], Hans Ulrich Luder[9], Domingo C Salazar-García[10–12], Elisabeth Eppler[13,14], Roger Seiler[1], Lars H Hansen[15,16], José Alfredo Samaniego Castruita[17], Simon Barkow-Oesterreicher[5], Kai Yik Teoh[6], Christian D Kelstrup[18], Jesper V Olsen[18], Paolo Nanni[5], Toshihisa Kawai[19,20], Eske Willerslev[17], Christian von Mering[3,4], Cecil M Lewis Jr[2], Matthew J Collins[6], M Thomas P Gilbert[17,21], Frank Rühli[1,22] & Enrico Cappellini[17,22]



Warinner et al. 2014; DOI: 10.1038/ng.2906

Exotic foods reveal contact between South Asia and the Near East during the second millennium BCE

Ashley Scott[a,b], Robert C. Power[b,c], Victoria Altmann-Wendling[b,d], Michal Artzy[e], Mario A. S. Martin[f], Stefanie Eisenmann[a,b], Richard Hagan[a,b], Domingo C. Salazar-García[g,h], Yossi Salmon[e], Dmitry Yegorov[i], Ianir Milevski[i], Israel Finkelstein[f], Philipp W. Stockhammer[a,b,1], and Christina Warinner[a,j,1]

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Edited by Dolores R. Piperno, Smithsonian Institution, Washington, DC, and approved November 10, 2020 (received for review July 21, 2020)

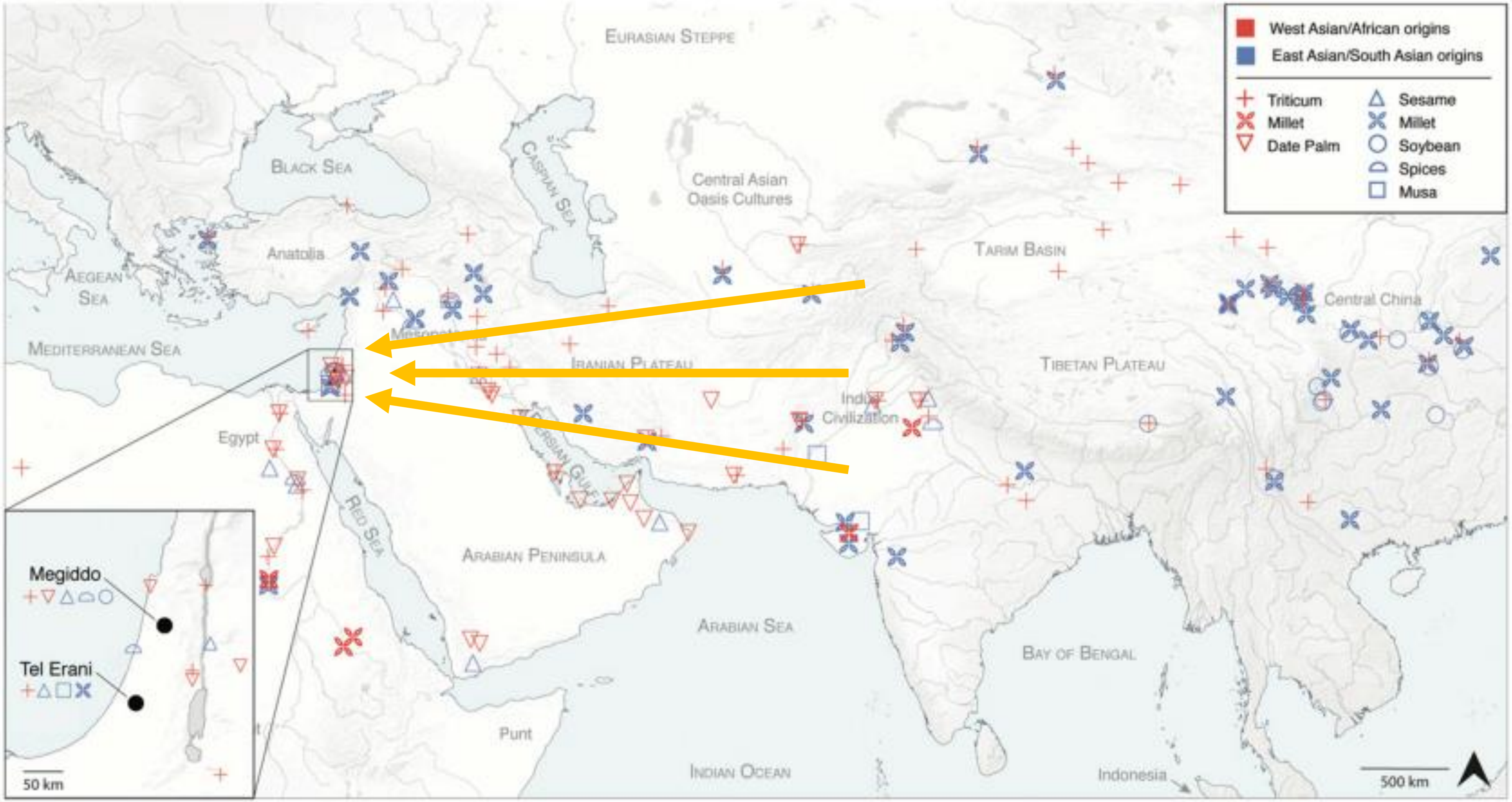


Fig. 1. Map of representative archaeobotanical evidence for the spread and trade of food crops prior to 500 BCE. See *SI Appendix* for data sources. Map *Inset* shows the location of the sites of Megiddo and Tel Erani on the southern Levantine coast; new dietary finds reported in this study are indicated for each site.

Scott et al. 2020; DOI: 10.1073/pnas.201495611

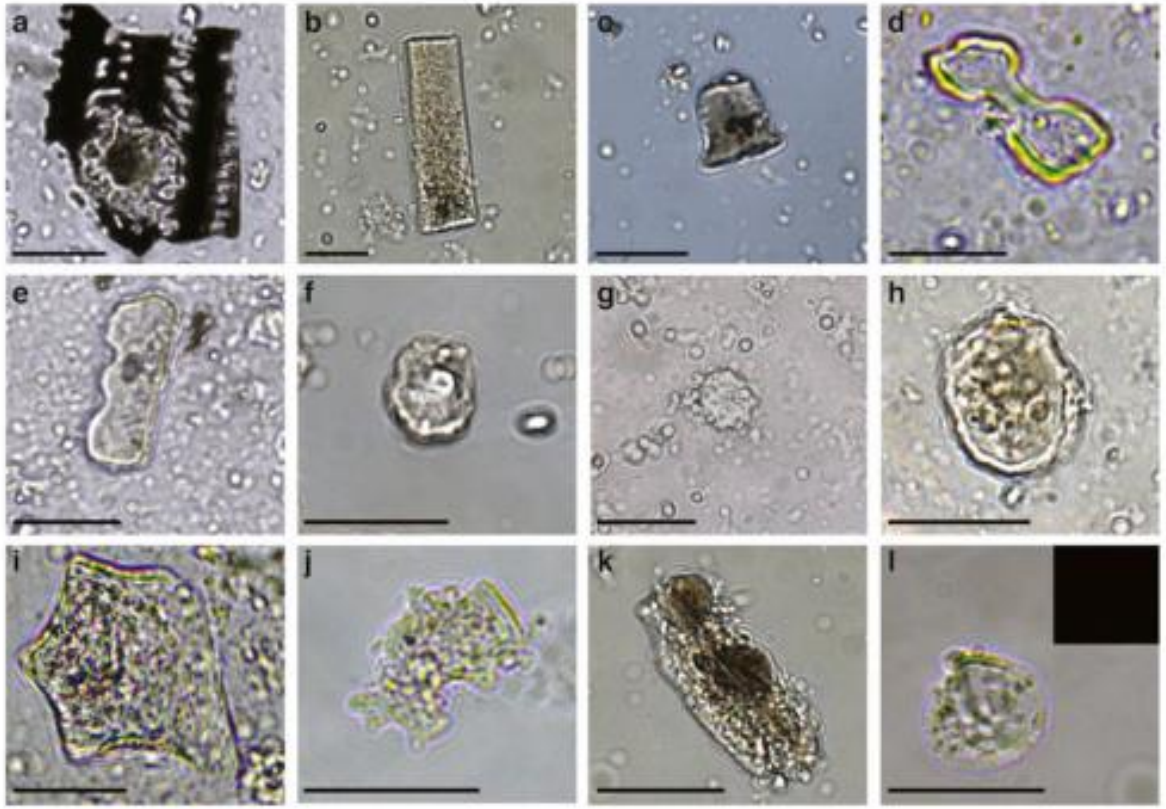
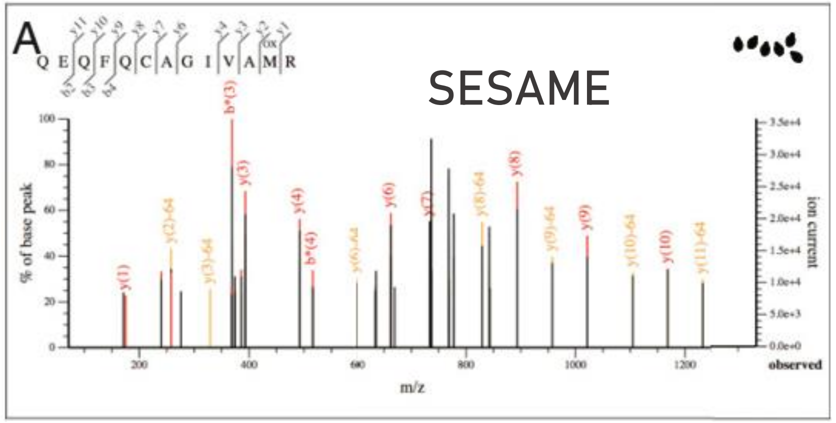
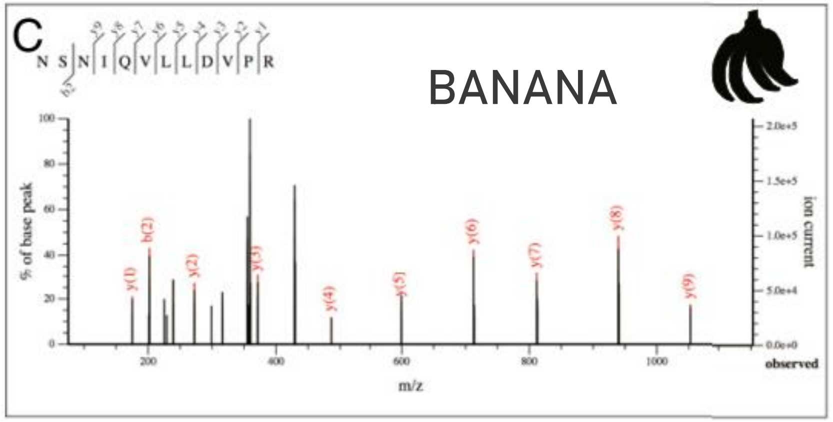


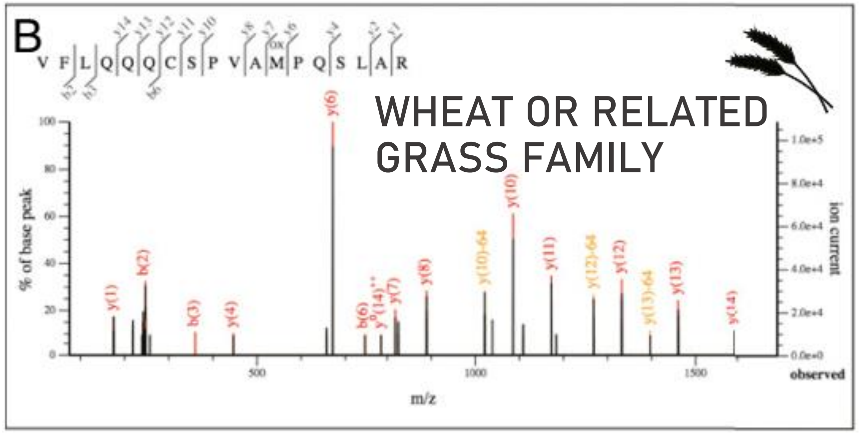
Fig. 3. Microremains in Megiddo and Tel Erani dental calculus. (*A*) Articulated Poaceae husk phytolith, identified as wheat (MGD001). (*B*) Poaceae stem/leaf phytolith (MGD001). (*C*) Poaceae short cell rondel (MGD001). (*D*) Wide-lobed bilobate short cell identified as panicoid (ERA017). (*E*) Poaceae polylobate short cell (MGD001). (*F*) Cone phytolith identified as sedge leaf (MGD018). (*G*) Spheroid echinate identified as date palm (MGD001). (*H*) Spheroid echinate phytolith, identified as nondiagnostic palm (MGD001). (*I*) Polyhedral plate phytolith, identified as eudicot (MGD011). (*J*) Decorated jigsaw phytolith, likely from fruit (MGD001). (*K*) Spheroid psilate phytolith, identified as bark type (MGD001). (*L*) Damaged Triticeae starch in brightfield, with *Inset* showing an absence of birefringence in cross-polarized light (MGD010). (Scale bars, 20 µm.)

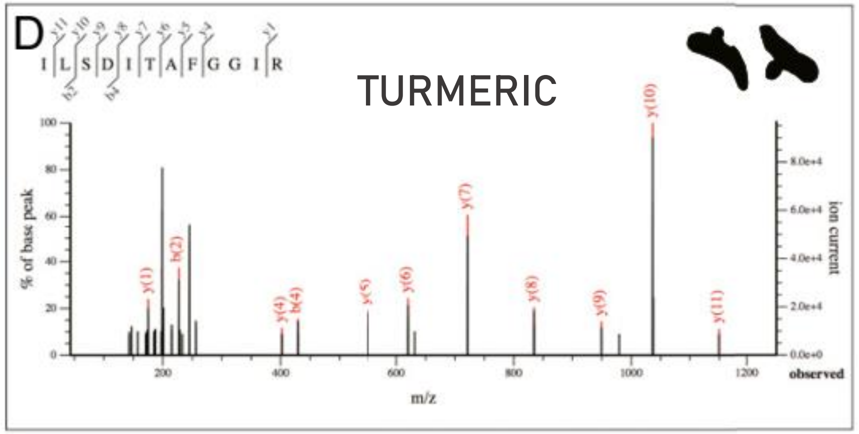
Scott et al. 2020; DOI: 10.1073/pnas.201495611

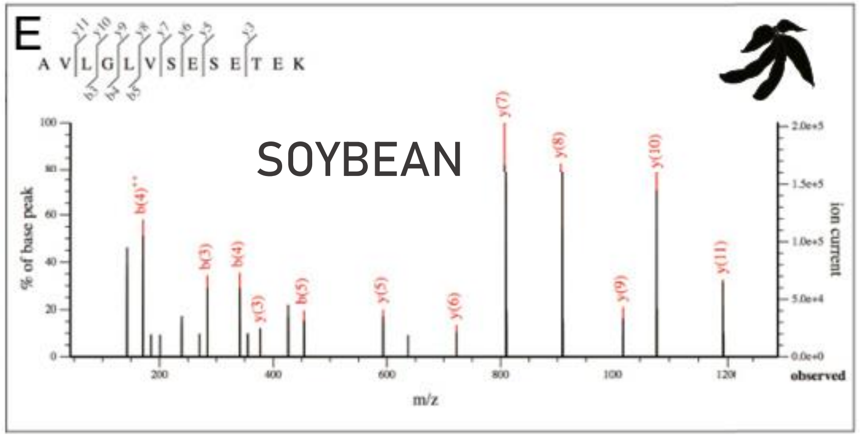
EXOTIC FOODS











Scott et al. 2020; DOI: 10.1073/pnas.201495611

FAMINE

Relief food subsistence revealed by microparticle and proteomic analyses of dental calculus from victims of the Great Irish Famine

Jonny Geber[a,1,2], Monica Tromp[b,c,1], Ashley Scott[d,1], Abigail Bouwman[e], Paolo Nanni[f], Jonas Grossmann[f], Jessica Hendy[d,g], and Christina Warinner[d,h]

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Fig. 1. "Destitution in Ireland. Failure of the potato crop" illustration was published in *The Pictorial Times* on 22 August 1846 (52). Image courtesy of the National Library of Ireland.

Geber et al. 2019; DOI: 10.1073/pnas.1908839116

MILK CONSUMPTION

Ancient proteins provide evidence of dairy consumption in eastern Africa

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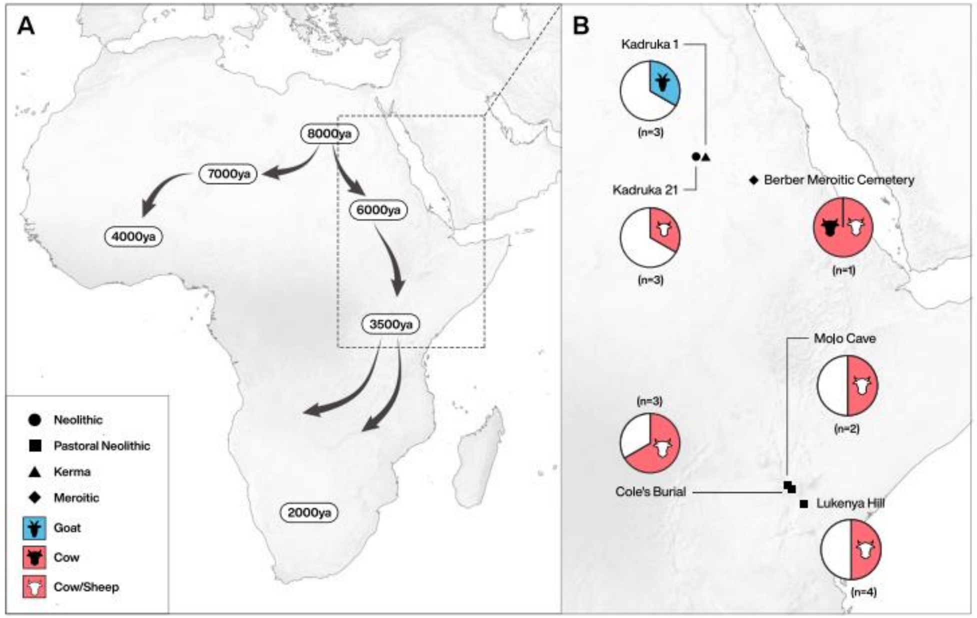

Fig. 2 Map of sites with calculus containing milk proteins. A Area of study in relation to the spread of cattle-based pastoralism across Africa (after Marshall and Hildebrand[35]). **B** Pie charts showing the number of individuals per site with milk proteins (shaded) proportionate to the total number of individuals that passed screening with Oral Signature Screening Database (see "Methods" and Supplementary Note 3 for full details). Neolithic: ~8000–5500 cal. BP; Kerma: ~4450–3450 cal. BP; Pastoral Neolithic: ~3500–1200 cal. BP; Meroitic: ~2300–1600 cal. BP. The maps were created for this study by Michelle O'Reilly (Graphic Designer for the Max Planck Institute for the Science of Human History, Jena, Germany) using QGIS 3.12 [https://qgis.org/en/site/] and the Natural Earth Database from [https://www.naturalearthdata.com/downloads/]. Additional edits were made using Adobe Illustrator CC.

CONCLUSION

COMPLEX MATERIAL

CONCLUSION

COMPLEX MATERIAL

WORTH STUDYING

CONCLUSION

COMPLEX MATERIAL

WORTH STUDYING

INTERDISCIPLINARY APPROACH,
COMBINATION OF METHODS

CONCLUSION

COMPLEX MATERIAL

WORTH STUDYING

INTERDISCIPLINARY APPROACH, COMBINATION OF METHODS

INTERPRETATION IS CRUCIAL

- What we can find
- Better preservation