up a representation of the whole social companion [10]. A sensitivity to ordered transitional probabilities of different images that are rapidly alternated might thus lie at the foundation of such ability.

SUPPLEMENTAL INFORMATION

Supplemental Information including experimental procedures can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.10.011.

ACKNOWLEDGMENTS

We are grateful to Jenny Saffran for comments. The experiments comply with current EC laws for the ethical treatment of animals and were approved by the Univ. of Padua Ethical Committee (N. 100845; 23/11/2013). The study was supported to G.V. by an ERC Advanced Grant (PREMESOR - 2011-ADG20110406).

REFERENCES

1. Saffran, J.R. (2009). What is statistical learning, and what statistical learning is not. In Neoconstructivism: The New Science of Cognitive Development, S.P. Johnson, ed. (New York, NY: Oxford University Press), pp. 180–194.
2. Dehaene, S., Meyniel, F., Wacongne, C., Wang, L., and Pallier, C. (2015). The neural representation of sequences: from transition probabilities to algebraic patterns and linguistic trees. Neuron. *88*, 2–19.
3. Goujon, A., and Fagot, J. (2013). Learning of spatial statistics in nonhuman primates: contextual cueing in baboons (Papio papio). Behav. Brain Res. *247*, 101–109.
4. Bateson, P. (1973). Preferences for familiarity and novelty: a model for the simultaneous development of both. J. Theor. Biol. *41*, 249–259.
5. Bulf, H., Johnson, S.P., and Valenza, E. (2011). Visual statistical learning in the newborn infant. Cogn. *121*, 127–132.
6. Hinton, G.E., and Sejnowski, T.J. (1999). Unsupervised Learning: Foundations of Neural Computation (MIT: MIT Press).
7. Versace, E., Regolin, L., and Vallortigara, G. (2006). Emergence of Grammar as Revealed by Visual Imprinting in Newly-hatched Chicks. The Evolution of Language. Proceedings of the 6th International Conference, pp. 457–458.
8. Martinho, A., and Kacelnik, A. (2016). Ducklings imprint on the relational concept of "same or different". Science *353*, 286–288.
9. Santolin, C., Rosa-Salva, O., Regolin, L., and Vallortigara, G. (2016). Generalization of visual regularities in newly hatched chicks (*Gallus gallus*). Anim. Cogn. *19*, 1007–1017.
10. Bateson, P. (1990). Is imprinting such a special case? Phil. Trans. R. Soc. Lond. B *329*, 125–131.

[1]Department of General Psychology, University of Padova, Italy. [2]Waisman Center, University of Wisconsin-Madison, 1500 Highland Avenue, Madison, WI 53705, USA. [3]Department of Psychology and Cognitive Sciences, University of Trento, Rovereto, Italy. [4]CIMeC, Center for Mind-Brain Sciences, University of Trento, Rovereto, Italy.
*E-mail: giorgio.vallortigara@unitn.it (G.V.), santolin@wisc.edu (C.S.)

Correspondence

Plasmodium falciparum malaria in 1st–2nd century CE southern Italy

Stephanie Marciniak[1,2,*], Tracy L. Prowse[2], D. Ann Herring[2], Jennifer Klunk[1,3], Melanie Kuch[1], Ana T. Duggan[1,2], Luca Bondioli[4], Edward C. Holmes[5], and Hendrik N. Poinar[1,2,3,6,7,8,*]

The historical record attests to the devastation malaria exacted on ancient civilizations, particularly the Roman Empire [1]. However, evidence for the presence of malaria during the Imperial period in Italy (1st–5th century CE) is based on indirect sources, such as historical, epigraphic, or skeletal evidence. Although these sources are crucial for revealing the context of this disease, they cannot establish the causative species of *Plasmodium*. Importantly, definitive evidence for the presence of malaria is now possible through the implementation of ancient DNA technology. As malaria is presumed to have been at its zenith during the Imperial period [1], we selected first or second molars from 58 adults from three cemeteries from this time: Isola Sacra (associated with Portus Romae, 1st–3rd century CE), Velia (1st–2nd century CE), and Vagnari (1st–4th century CE). We performed hybridization capture using baits designed from the mitochondrial (mtDNA) genomes of *Plasmodium* spp. on a prioritized subset of 11 adults (informed by metagenomic sequencing). The mtDNA sequences generated provided compelling phylogenetic evidence for the presence of *P. falciparum* in two individuals. This is the first genomic data directly implicating *P. falciparum* in Imperial period southern Italy in adults.

The story of malaria in Imperial Italy is drawn from a rich historical narrative; see, for example, Hippocrates' *On Epidemics*, Celsus' *De Medicina*, or Galen's *De Morborum Temporibus*. These texts describe the classic fever periodicity — tertian, semi-tertian, quartan or quotidian — that have been documented since the 5th century BCE. Despite this, the timing and geographical range of malaria remains uncertain, as only a broad northward spread is thought to have occurred across Italy from 500 BCE to 1000 CE [2]. The inability to connect malaria to a precise historical and geographical space in antiquity is further complicated by its pathogenesis, as this infection does not cause distinct pathological changes to the human skeleton, although non-specific skeletal indicators of physiological stress are prevalent in malarious environments [3]. Furthermore, the existing molecular evidence for malaria in Imperial Italy currently consists of a single PCR product corresponding to *P. falciparum* from an infant from 5th century CE Lugnano in Umbria [4]. The prevalence and influence of malaria among adults in southern Italy therefore remain unknown.

Malaria is responsive to climate, topography, human activity, and ecology on a local scale, so there is likely no single mortality profile that is applicable to all of Imperial-period Italy [1,2]. Accordingly, we used ecologically diverse coastal and rural localities to determine the presence of *Plasmodium* through ancient DNA technology. We focused on Velia (a coastal promontory between alluvial plains) [5], Portus Romae (a low-lying basin of woodlands near the Tiber River alongside marshes and lagoons) [6], and inland Vagnari (a wooded river valley with lowland hills) [7] (Figure S1A).

We detected *P. falciparum* mtDNA fragments from two individuals, LV13 (Velia) and LG20 (Vagnari), dating to the 1st–2nd century CE (Figure 1A), with no positive results from the Portus Romae samples, although this does not preclude the presence of malaria in this locality (Table S1). Through an RNA-bait set designed from four human and two non-human *Plasmodium* species, we were able to enrich a total of 3,033 bp (120 reads total), or 50.8% of the 5,967 bp *P. falciparum* mitochondrial genome, when reads from LG20 and LV13 were combined. Separately, LG20 yielded 300 bp (7 reads) and LV13 yielded 2,901 bp (113 reads). Although our baits will enrich for *P. vivax* and *P. malariae*, which may have co-circulated with *P. falciparum*, we did not detect any reads matching these species. Importantly, *Plasmodium*

CrossMark

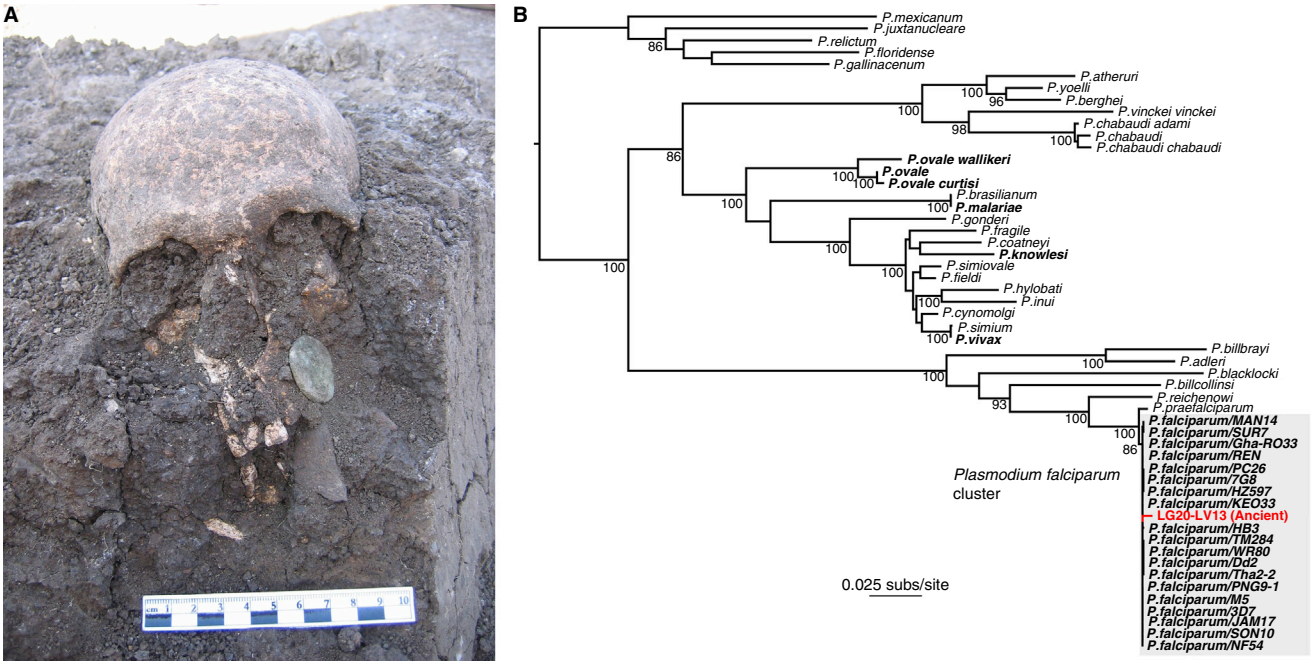

Figure 1. Skeletal remains of an individual from Vagnari and a phylogenetic tree of ancient malarial mitochondrial sequences.
(A) The burial of individual F234 at Vagnari (adult male, approximately 35.2 ± 9.4 years of age). Velia individual 186 (not pictured) is also an adult male, approximately 20–25 years of age. (B) Maximum likelihood phylogenetic tree of 54 *Plasmodium* spp. including the Imperial period Italian sequence obtained here shown in red (the ancient LG20 and LV13 sequences were combined for this analysis). The phylogeny was estimated using a 4,570 bp sequence alignment inferred after multiple rounds of alignment followed by the removal of all ambiguously aligned positions (see Supplemental Information). The tree was rooted on non-mammalian *Plasmodium* sequences and all horizontal branch lengths are drawn to a scale of nucleotide substitutions per site. *Plasmodium* species that infect humans are shown in bold. Bootstrap support values (>80%) are shown for key nodes.

species rely on host (mosquito and human) cellular machinery for survival and are species-specific, with parasites restricted to particular hosts [8]. These factors preclude environmental reservoirs of infection and hence contamination of the skeletal remains from surrounding sediments.

The sequence reads mapping to *P. falciparum* averaged 51 bp, with damage patterns characteristic of ancient DNA (C>T at the 3' and 5' termini; Figure S1B, S1C). Low-coverage data required using human mitochondrial reads from individuals LG20 and LV13 as a proxy for quantitative assessments of ancient DNA damage (Figure S1D, S1E). The positive, but low, *P. falciparum* signal is perhaps unsurprising considering the idiosyncrasies of recovering pathogen DNA and the nature of parasite pathogenesis that includes differential expression of the parasite's mtDNA that varies with infective stage [9], the recurrence of infection (that is, whether re-infection or recrudescence), and the spectrum of host–parasite interactions (asymptomatic to lethal).

Phylogenetic analyses of the LG20–LV13 sequence, combined with mtDNA genomes from diverse *Plasmodium* spp. (n = 53), place our Imperial-period strain within a clade of exclusively *P. falciparum* sequences (n = 19) with strong support (86%; 100% bootstrap support linking the *P. falciparum* cluster with its closest non-human relative, *P. praefalciparum* from gorillas; Figures 1B, S1F). However, due to the fragmentary nature of the LG20–LV13 sequence, it was not possible to resolve evolutionary relationships within the *P. falciparum* cluster nor determine the time scale of *P. falciparum* evolution through molecular-clock dating.

Our results are compatible with Sallares and Gomzi's [4] identification of *P. falciparum* from central Italy, but predate their detection of the parasite by several centuries. Detecting signatures of *P. falciparum* in individuals from Vagnari and Velia, but not Portus Romae, as well as the recovery of *P. falciparum* among adults at these disparate localities, and in infants [4], means that the nature of malaria (endemic, epidemic, or sporadically imported) in Imperial Italy remains unclear. Indeed, it is possible that malaria exhibited complex population dynamics at the localities studied here, reflecting the variable impact of demography (population movement, migration), economic activities (trade, resource use), social circumstances (urbanization, land use patterns), and parasite biology (regional variation among strains or vectors) [10]. Our results underscore the tremendous challenges inherent in the recovery of organic signatures of ancient pathogens; further improvements in ancient biomolecule methodology and/or detection may enhance the search for infectious diseases of our past.

In sum, these data provide a key reference point for the antiquity of *P. falciparum* in humans. Ancient DNA therefore lends a materiality to the existence of malaria in Imperial Italy, complementing the multi-faceted narrative of 'malarial fevers' told by authors thousands of years ago.

SUPPLEMENTAL INFORMATION

Supplemental Information includes experimental procedures and acknowledgements, one figure and one table and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.10.016.

REFERENCES

1. Sallares, R. (2002). Malaria and Rome: A History of Malaria in Ancient Italy (Oxford: Oxford University Press).
2. Sallares, R., Bouwman, A., and Anderung, C. (2004). The spread of malaria to southern Europe in antiquity: new approaches to old problems. Med. Hist. *48*, 311–328.
3. Gowland, R., and Garnsey, P. (2010). Skeletal evidence for health, nutritional status and malaria in Rome and the Empire. In Roman Diasporas: Archaeological Approaches to Mobility and Diversity in the Roman Empire, H. Eckardt, ed. (Portsmouth: Journal of Roman Archaeology, Supplement 78), pp. 131–156.
4. Sallares, R., and Gomzi, S. (2001). Biomolecular archaeology of malaria. Anc. Biomol. *3*, 195–213.
5. Amato, L., Bisogno, G., Cicala, L., Cinque, A., Romano, P., Ruello, M.R., and Ermolli, E.R. (2010). Palaeo-environmental changes in the archaeological settlement of Elea-Velia: climatic and/or human impact signatures? In Scienze naturali e archeologia. Il paesaggio antico: interazione uomo ambiente ed eventi catastrofici, A. Ciarallo, M.R. Senatore, eds. (Rome: Aracne Editrice), pp. 13–16.
6. Keay, S.J., and Paroli, L., eds. (2011). Portus and its Hinterland: Recent Archaeological Research (London: British School at Rome).
7. Small, A.M., ed. (2011). Vagnari. The Village, the Industries, the Imperial Property (Italy: Edipuglia).
8. Paul, A.S., Egan, E.S., and Duraisingh, M.T. (2015). Host-parasite interactions that guide red blood cell invasion by malaria parasites. Curr. Opin. Hematol. *22*, 220–226.
9. Krungkrai, J. (2004). The multiple roles of the mitochondrion of the malarial parasite. Parasitol. *129*, 511–524.
10. Martens, P. (2002). Of malaria and models: challenges in modeling global climate change and malaria risk. In The Contextual Determinants of Malaria, E.A. Casman, H. Dowlatabadi, eds. (Washington: RFF Press), pp. 14–26.

[1]McMaster Ancient DNA Centre, Department of Anthropology, McMaster University, Hamilton, Ontario L8S 4L9, Canada. [2]Department of Anthropology, McMaster University, Hamilton, Ontario L8S 4L9, Canada. [3]Department of Biology, McMaster University, Hamilton, Ontario L8S 4L9, Canada. [4]Museo delle Civiltà, Museo Nazionale Preistorico Etnografico 'Luigi Pigorini', Sezione di Bioarcheologia, P. le G. Marconi 14, 00144, Rome, Italy. [5]Marie Bashir Institute for Infectious Diseases and Biosecurity, Charles Perkins Centre, School of Life and Environmental Sciences and Sydney Medical School, The University of Sydney, Sydney, NSW 2006, Australia. [6]DeGroote Institute for Infectious Disease Research, McMaster University, Hamilton, Ontario L8S 4L9, Canada. [7]Humans & the Microbiome Program, Canadian Institute for Advanced Research, Toronto, Ontario M5G 1Z8, Canada. [8]Lead Contact.
*E-mail: marcis@mcmaster.ca (S.M.), poinarh@mcmaster.ca (H.N.P.)

Correspondence

Auditory fovea in the ear of a duetting katydid shows male-specific adaptation to the female call

Jan Scherberich[1,3], Jennifer Hummel[1,3], Stefan Schöneich[2,4], and Manuela Nowotny[1,4,5,*]

Convergent evolution has led to surprising functional and mechanistic similarities between the vertebrate cochlea and some katydid ears [1,2]. Here we report on an 'auditory fovea' (Figure 1A) in the duetting katydid *Ancylecha fenestrata* (Tettigoniidae). The auditory fovea is a specialized inner-ear region with a disproportionate number of receptor cells tuned to a narrow frequency range, and has been described in the cochlea of some vertebrates, such as bats and mole rats [3,4]. In tonotopically organized ears, the location in the hearing organ of the optimal neuronal response to a tone changes gradually with the frequency of the stimulation tone. However, in the ears of *A. fenestrata*, the sensory cells in the auditory fovea are tuned to the dominant frequency of the female call; this area of the hearing organ is extensively expanded in males to provide an overrepresentation of this behaviorally important auditory input. Vertebrates developed an auditory fovea for improved prey or predator detection. In *A. fenestrata*, however, the foveal region facilitates acoustic pair finding, and the sexual dimorphism of sound-producing and hearing organs reflects the asymmetry in the mutual communication system between the sexes (Figures 1B, S1).

At nighttime, male *A. fenestrata* produced mating calls with a dominant frequency of about 30 kHz; these calls consisted of a median sound pulse of 67 ms (interquartile range (IQR) = 59–83 ms; N = 7 animals and n = 38 total measurements) repeated every 5.5 s (median) (IQR = 5.0–7.2 s; N = 4 animals, n = 207 measurements). Nearby females occasionally responded to the male calls (Figures 1B, S1B) by a single sound pulse with a dominant frequency of about 10 kHz and 41 ms in length (median) (IQR = 32–54 ms; n = 21 measurements). Female replies strictly occurred within 148–164 ms (IQR) (median = 155 ms; n = 11 measurements) after a male call (Figures 1B, S1B). These extremely sparse female signals make acoustic mate recognition and localization very challenging for the male [5]; therefore, the precise timing of the female reply is often the crucial feature for species recognition that initiates male phonotaxis [6].

For sound perception, male and female *A. fenestrata* have a linear array of auditory sensors embedded in the so-called *crista acustica* located in the tibia of their forelegs. The *crista acustica* is the part of a hearing-organ complex that is most sensitive to airborne sounds. Here, dendrite tips of bipolar receptor cells lay in a row, held and packed by supporting cells (such as scolopale and cap cells, respectively). *In vivo* recordings from individual receptor cells revealed that the systematic, stepwise sequence of frequency coding along the *crista acustica* (Figure 1C, 1D) is disrupted in the middle half of the hearing organ, where all auditory receptors are tuned to a narrow frequency range at about 10 kHz (Figure 1C, 1E). A similar irregularity in tonotopy has only been described for five adjacent receptor cells in the *crista acustica* of *Mygalopsis marki* [7], an Australian cone-head katydid that belongs to the Tettigoniidae subfamily of Copiphorinae and has a rather short hearing organ with only about 20 sensory receptors. In *A. fenestrata* (subfamily Phaneroterinae) males, although there was a clear position-dependent change of frequency tuning in the proximal (2 kHz per 10% organ length) and distal (6.6 kHz per 10% organ length) part of the *crista acustica*, a large area of the medial part (about 55 sensory cells and 50% of the total organ length) features only minor changes (0.6 kHz per 10% organ length). Such disproportionate frequency representation is a main characteristic of an auditory fovea (Figure 1A).

Compared to other katydids, *A. fenestrata* were found to have a notably long *crista acustica* with an exceptionally large number of sensory cells [8] that differed significantly ($p < 0.001$) between males (median = 115 cells; IQR = 113–116 cells; N = 9 animals) and females